Treating Cellular Stress and Damage : Use of Healthy Mitochondria Isolated from Donor Cells in the Artificial Mitochondria Transfer / Transplant (Amt/T) to Repair Mitochondrial Disfunction in Differentiated (Peripheral Blood Mononuclear Cells) and Germinal Cells (Oocytes)

Francisco Javier Cabrera Aulestia

THÈSE POUR OBTENIR LE GRADE DE DOCTEUR DE L'UNIVERSITÉ DE MONTPELLIER

En Biologie et Chimie pour la Santé

École doctorale CBS2

Unité de recherche : Institute of Regenerative Medicine and Biotherapy

En partenariat international avec Universidad San Francisco de Quito, EQUATEUR

TREATING CELLULAR STRESS AND DAMAGE:

Use of Healthy Mitochondria Isolated from Donor Cells in the Artificial Mitochondria Transfer / Transplant (AMT/T) to Repair Mitochondrial Disfunction in Differentiated (Peripheral Blood Mononuclear Cells) and Germinal Cells (Oocytes)

Présentée par Francisco Javier CABRERA AULESTIA

Le 13 DECEMBRE 2019

Sous la direction de Christian JØRGENSEN
et Andrés B. CAICEDO P.

Devant le jury composé de

John DE VOS	Directeur de Recherche	Institute of Regenerative Medicine and Biotherapy-UM	Président du Jury
Marbel TORRES	Chercheuse Principal	Universidad de las Fuerzas Armadas-ESPE, Equateur	Examinatrice
Vanessa ROMERO	Chercheuse Principal	Universidad San Francisco de Quito, Equateur	Examinatrice
Iván MOYA	Chercheur Principal	Universidad de las Américas, Equateur	Rapporteur/Examinateur
Luz María GRANADOS V	Directrice de Recherche	Universidad UTE, Equateur	Rapporteur/Examinateur

UNIVERSITÉ DE MONTPELLIER

Acknowledgments

To my wife, for her patience, her strength and for taking care of me during all these years of duplicate efforts.

To the Universidad "San Francisco de Quito" for trusting my abilities and giving me a chance.

To Dr Germán Romo for his intentional advice: "Don't let anyone stop you."

To the entire team of the Molecular Biology laboratory of the COCSA-USFQ School of Medicine: Lina, Gaby, Mayra, Francesca, Emilia, Veronica, etc., etc. Thank you for staying with me in so many experiments.

And my sincere thanks to all those who doubted me. They motivated me to prove myself more than they imagine.

Abstract

According to the endosymbiotic theory, mitochondria is an organelle derived from an ancient alpha-proteobacteria that developed a symbiosis with a eukaryotic ancestor. Mitochondrial DNA (mtDNA) exists in hundreds to thousands of copies in each cell and encodes for 13 structural proteins which are subunits of respiratory chain. Mitochondria generate energy for cellular processes by producing ATP through oxidative phosphorylation. Also, they control other processes as nucleotide and heme syntheses, redox balance, calcium metabolism, waste management (urea and ROS) and apoptosis. mtDNA deletions, point mutations, thymine dimers and mtDNA depletions are strongly related with disease in humans and other mammals. Some mtDNA alterations can arise spontaneously during life spam, other can be inherited by maternal lineage as specific mutations. So, nuclear DNA mutations can produce mitochondrial disorders because while mtDNA encodes 13 proteins, mitochondria need almost 2000 proteins with structural and functional roles. In these cases, a mendelian inheritance pattern can be observed. mtDNA alterations can be produced by exposure to toxic substances or UV and high-energy radiations. mtDNA mutations are cumulative because mitochondria lack reparative mechanisms. Normal and mutant mtDNA can coexist in the same cell, a condition known as heteroplasmy. Heteroplasmy allows the persistence of an otherwise lethal mutation through generations. Mitochondrial disorders can appear as myopathies, cardiomyopathies, lactic acidosis diabetes mellitus, female's subfertility, lipodystrophy, neuropathies as autism or Alzheimer's diseases or haematological and renal disorders. Due to heteroplasmy, these disorders can appear with a wide range of intensities, because the mutant mtDNA needed to cause a disorder varies among organisms, among organ systems and within a given tissue, and depends on a delicate balance between ATP supply and demand. Another kind of problem surges at tissues under hypoxemic-related damage, where mitochondria play an important role in cell survival and recovery. Finally, the role played by mitochondria in cancer survival and treatment is focused in many researches.

Mitochondrial disorders have not a single treatment. In the most serious cases of inherited mitochondrial diseases, the supportive treatment only improves the life quality slightly. Nowadays, the most of experimental approaches search prevents the clinical manifestations of these diseases by reducing the mutant mtDNA percentage into the oocyte or the early embryo via nuclear transfer. Artificial Mitochondrial Transfer/Transplant (AMT/T) rises as an alternative to many acquired or inherited mitochondrial disorders, both *ex vivo*, *in vitro* and *in vivo* conditions**. For this reason, our main objective is the updating of Mitoception to two cell models: PBMC's and murine oocyte.** The present work shows the variation of an AMT/T method -MitoCeption- in a cellular model for in vitro treatment of acquired mtDNA disorder caused by UV Radiation by using Peripheral Blood Mononuclear Cells (PBMCs) and the feasibility of the same method for ex vivo AMT/T to murine oocytes and early embryos**. In this model, we had found that irradiated PMBC's shown a reduction of mitochondrial mass, metabolic activity, viable mtDNA and cell survivance, detected by Flow Cytometry, MTT Assay, qPCR and Trypan Blue Stain, respectively. After Mitoception, PBMC's shown a restoration of mitochondrial mass, metabolic activity, viable mtDNA and cell survivance, detected by the same methods.** In this *in vitro* model of cell damage by UV radiation, the main results represent an upgrading in the applications of AMT/T. We showed that PBMCs could be used as a primary allogeneic mixed source of mitochondria. We also showed that these mitochondria can be transferred in a mix from different donors (PAMM) to UVR-damaged, non-adherent primary cells. Additionally, the duration of the MitoCeption protocol was reduced. On the other hand, Mitoception used on murine oocytes and early embryos probed to be a safe method for AMT/T by using human

mitochondrial mix (PAMM). Murine 0ocytes' and embryos' exogenous mitochondrial content was observed by fluorescence microscopy and exogenous mtDNA was quantified by qPCR and $2^{\Delta CT}$ method. Finally, healthy murine new-borns were obtained by embryo transfer, without clear evidence that human mitochondria remain in murine cells during embryos development after implantation.

Résumé

Selon la théorie endosymbiotique, la mitochondrie est un organite dérivé d'une ancienne alpha protéobactérie qui a développé une symbiose avec un ancêtre eucaryote. L'ADN mitochondrial (ADNmt) existe dans des centaines à des milliers d'exemplaires dans chaque cellule et code pour 13 protéines structurelles qui sont des sous-unités de la chaîne respiratoire. Les mitochondries génèrent de l'énergie pour les processus cellulaires en produisant de l'ATP par phosphorylation oxydative. Ils contrôlent également d'autres processus tels que la synthèse de nucléotide et d'hème, l'équilibre rédox, le métabolisme du calcium, la gestion des déchets (urée et ROS) et l'apoptose. Les délétions d'ADNmt, les mutations ponctuelles, les dimères de thymine et les déplétions d'ADNmt sont fortement liés à la maladie chez l'homme et d'autres mammifères. Certaines altérations de l'ADNmt peuvent survenir spontanément pendant le spam de la vie, d'autres peuvent être héritées de la lignée maternelle sous forme de mutations spécifiques. Ainsi, les mutations de l'ADN nucléaire peuvent produire des troubles mitochondriaux car, alors que l'ADNmt code pour 13 protéines, les mitochondries ont besoin de près de 2000 protéines dotées de rôles structurels et fonctionnels. Dans ces cas, un modèle d'héritage mendélien peut être observé. Des altérations de l'ADNmt peuvent être produites par l'exposition à des substances toxiques ou à des rayonnements UV et à haute énergie. Les mutations de l'ADNmt sont cumulatives car les mitochondries sont dépourvues de mécanismes de réparation. Les ADNmt normaux et mutants peuvent coexister dans la même cellule, une condition appelée l'hétéroplasmie. L'hétéroplasmie permet la persistance d'une mutation par ailleurs mortelle à travers les générations. Les troubles mitochondriaux peuvent apparaître sous forme de myopathies, cardiomyopathies, diabète sucré, l'acidose lactique, hypofertilité féminine, lipodystrophie, neuropathies comme l'autisme ou la maladie d'Alzheimer ou des troubles hématologiques et rénaux. En raison de l'hétéroplasmie, ces troubles peuvent apparaître avec une vaste gamme d'intensités, car l'ADNmt mutant nécessaire pour provoquer un trouble varie selon les organismes, les systèmes organiques et au sein d'un tissu donné, et dépend d'un équilibre délicat entre l'offre et la demande en ATP. Un autre type de problème survient au niveau des tissus soumis à une lésion liée à l'hypoxémie, où les mitochondries jouent un rôle important dans la survie et le rétablissement des cellules. Enfin, le rôle joué par les mitochondries dans la survie et le traitement du cancer est concentré dans de nombreuses recherches.

Les troubles mitochondriaux semblent sous la forme d'une large gamme de signes cliniques. Dans la plupart des cas de maladies mitochondriales, le traitement symptomatique améliore légèrement la qualité de vie. La plupart des abords expérimentales cherchent à prévenir ces maladies en réduisant le pourcentage d'ADNmt mutant dans l'embryon par transfert nucléaire. Le transfert/transplantation artificiel mitochondrial (AMT/T) parait comme traitement alternatif. Pour cette raison, notre objectif principal est la mise à jour de Mitoception en deux modèles cellulaires : les **cellules sanguines mononucléaires périphériques** et l'ovocyte murin. Nous montrons une méthode AMT/T -MitoCeption- dans un modèle cellulaire pour le traitement du trouble acquis de l'ADNmt défié par la radiation UV en usant des leucocytes plus la viabilité de Mitoception pour AMT/T sur des embryons murins. **Dans ce modèle, nous avons constaté que les cellules sanguines mononucléaires périphériques irradiés présentaient une réduction de la masse mitochondriale, de l'activité métabolique, de l'ADNmt viable et de la survie cellulaire, détectées respectivement par cytométrie en flux, test MTT, qPCR et coloration bleue au trypan. Après la Mitoception, les cellules sanguines mononucléaires périphériques ont montré une restauration de la masse mitochondriale, de l'activité**

métabolique, de l'ADNmt viable et de la survie cellulaire, détectées par les mêmes méthodes. Ces résultats représentent une mise à niveau des applications AMT/T. Nous montrâmes que les leucocytes pouvaient être utilisées comme source de mitochondries et le transfert mitochondrial de multiples donneurs vers des cellules altérées. La MitoCeption usée sur des embryons semble être une méthode sûre pour la AMT/T en utilisant des mitochondries humaines. Des chiots murins saines furent obtenus, sans preuve claire que les mitochondries humaines restent dans les cellules murines pendant le développement des embryons après l'implantation.

RÉSUMÉ SUBSTANCIAL

La cellule est considérée comme l'unité de base de la vie, où se produisent toutes les réactions biochimiques indispensables au maintien de la vie. Elle se caractérise par la membrane plasmique, qui est une frontière complexe et active lui permettant d'interagir avec son environnement. Les composants membranaires existent dans une diversité d'états fluctuant d'immobilisés à complètement mobiles transversalement et rotatifs autour d'un axe de rotation perpendiculaire au niveau de la membrane, comme cela a été souligné dans le modèle de mosaïque fluide, dans lequel il était proposé de fixer les protéines membranaires intégrales à la bicouche lipidique. La membrane plasmique et les membranes limitantes des organites servent à délimiter et à coordonner les fonctions cellulaires, permettant ainsi la séparation des métabolites et des réactions enzymatiques. La subdivision de l'espace intracellulaire en compartiments fonctionnels est importante pour cette coordination. La compartimentation peut être atteinte par les membranes intracellulaires, qui entourent les organites et agissent comme des barrières physiques. De plus, les cellules ont développé des mécanismes complexes pour diviser leur cytosol de manière profondément régulée.

Le plan général d'organisation cellulaire eucaryote varie d'un organisme à l'autre, mais malgré ces modifications, toutes les cellules se ressemblent de manière fondamentale et elles partagent les mêmes composants : (1) une membrane plasmique, (2) un cytoplasme, (3) des ribosomes pour la synthèse de protéines, (4) des mitochondries et (5) un noyau contenant du matériel génétique. Comme décrit couramment, les mitochondries sont des organites cellulaires issues de l'intégration d'un endosymbionte alpha protéo-bactérique lié à Rickettsies dans une cellule hôte lié à Archaea. Les mitochondries jouent un rôle fondamental dans diverses fonctions au sein de la cellule : (1) la synthèse de l'ATP, (2) l'homéostasie du Ca^{2+}, (3) la synthèse des nucléotides et de l'hème, (4) la régulation de l'apoptose (dans le cadre de la croissance, de la différenciation et du devenir cellulaires), (5) de la balance rédox et 6) gestion des déchets. Cependant, les mitochondries ne fonctionnent pas de la même manière dans toutes les cellules. Comme différents tissus ont des besoins physiologiques spécifiques, les mitochondries répondent à ces besoins en étant métaboliquement actives. Des recherches récentes ont mis en évidence des différences entre la réplication, l'expression génique et la topologie de l'ADNmt du tissu adipeux brun, du cerveau, du cœur, des muscles squelettiques, du foie et des reins. Par exemple, les cellules hépatiques et rénales répliquent leur ADNmt en utilisant le mécanisme asynchrone connu à partir de cellules cultivées, tandis que les tissus à forte activité OXPHOS, tels que le cœur, le cerveau, les muscles squelettiques et la graisse brune, utilisent un mode de réplication couplé à un brin, associé à des niveaux accrus de recombinaison.

Depuis les études pionnières de Palade, il est bien établi que les mitochondries sont des organites à membrane, avec une membrane mitochondriale externe (MME) et une membrane mitochondriale interne (MMI) qui est enroulée et pliée dans des mitochondriales cristallines. La présence de ces plis ou crêtes augmente la surface de l'MMI. MMME et MMI définissent deux compartiments internes, l'espace intermembranaire (EIM) entre les deux membranes et la matrice mitochondriale - un compartiment dans lequel se trouvent l'ADNmt, les enzymes métaboliques et les agents de signalisation - à l'intérieur de la membrane interne. L'espace intra cristal au sein des plis des crêtes est continu, l'espace intermembranaire communiquant par de petites jonctions circulaires également appelées jonctions des crêtes (CJ). La formation des CJs est médiée par le complexe de système d'organisation de crêtes (MICOS) et de site de contact mitochondrial. Cette formation évite la diffusion entre l'espace intra cristal et l'IMS en séquestrant le contenu des crêtes et en régulant son transfert. Les sous-unités codées au niveau

mitochondrial du système OXPHOS s'assemblent avec des sous-unités à codage nucléaire dans des complexes enzymatiques. L'expression du génome mitochondrial implique la réplication, la transcription et la traduction intra mitochondriale. La traduction mitochondriale est associée à d'autres fonctions mitochondriales et cellulaires. La synthèse des protéines codées par les mitochondries est coordonnée par la connexion de la synthèse des protéines mitochondriales avec un assemblage de complexes de la chaîne respiratoire. La traduction mitochondriale contrôle la prolifération cellulaire et le cycle cellulaire, et les sous-unités ribosomales mitochondriales jouent un rôle dans la réponse au stress cytoplasmique. Ainsi, la traduction dans les mitochondries est intégrée dans les processus cellulaires. De plus, les mitochondries sont connues pour réguler les processus cellulaires clés, notamment la signalisation du calcium, le métabolisme et la mort cellulaire.

Les mitochondries contiennent leur propre ADN, hérité par voie matrilinéaire, avec son propre code génétique codant pour 13 composants de la chaîne de transport d'électrons (sous-unités ND1 à ND6 du complexe CTE I; sous-unités COX1 à COX3 du complexe CTE IV. ; les sous-unités 6 et 8 de l'ATPase mitochondriale (complexe V) et du complexe CTE III), 2 ARN ribosomiques et 22 ARN de transfert. Les protéines histones peuvent protéger le génome nucléaire de divers types d'agents endommageant l'ADN, mais le génome mitochondrial en manque, donc l'ADN mitochondrial est désormais plus susceptible de cumuler les dommages que l'ADN nucléaire. L'ADN mitochondrial est exposé à divers types d'agents endogènes et exogènes d'endommagement de l'ADN, dont certains peuvent entraîner divers types de maladies, notamment la forme neurodégénérative, le cancer, la cardiomyopathie, le diabète et plusieurs troubles liés au vieillissement. La qualité des mitochondries est assurée par l'équilibre entre le nombre de mitochondries fonctionnelles et les besoins énergétiques de la cellule. Les mitochondries sont des organites très dynamiques qui se répliquent par fission lorsque la cellule a besoin de plus d'énergie. Le processus de fission est déclenché via l'activation de Drp1. Lorsque les mitochondries sont surchargées, leurs composants moléculaires peuvent être endommagés par les ROS et ces dommages peuvent être réparés par fusion, qui est médiée par Opa1, Mfn1 et Mnf2. Les mitochondries inutiles et non fonctionnelles sont éliminées par la mitophagie.

Les lésions aux tissus et aux organes commencent au niveau cellulaire. Le développement de modifications structurelles est précédé de modifications biochimiques qui perturbent l'homéostasie cellulaire. Dans certains cas, les modifications biochimiques sont les seuls changements détectables. Il existe des milliers d'agents étiologiques, d'origine interne ou externe, mais ils activent tous un ou plusieurs des quatre mécanismes biochimiques communs qui sont les ultimes responsables des dommages cellulaires : (1) épuisement de l'ATP, (2) perméabilisation des membranes cellulaires, (3) perturbation du voies biochimiques et (4) dommages à l'ADN. La plupart des médiateurs primaires de la réponse au stress, notamment les hormones (glucocorticoïdes et catécholamines), les facteurs immunitaires (cytokines) et les protéines de choc thermique, exercent de nombreux effets sur la réponse mitochondriale. Cette réponse est modulée dans un rapport proportionnel aux besoins en énergie des tissus affectés et en fonction de la nature de l'agent de stress: (1) augmentation du nombre mitochondrial par biogenèse et / ou augmentation de leur volume; (2) l'amélioration de l'expression et de l'activité des sous-unités de phosphorylation oxydative (OXPHOS); (3) réguler le découplage de la chaîne respiratoire, résultant pour conséquence une libération d'énergie sous forme de chaleur; (4) permettre la transduction du signal entre les mitochondries et le noyau et d'autres organites intracellulaires; (5) production de ROS pour la signalisation ou la défense; et (6) induire la cascade apoptotique. La différence entre adaptation et blessure dépend de l'intensité et de la durée des stimuli.

Le dysfonctionnement mitochondrial a été impliqué dans divers troubles, notamment les maladies mitochondriales primaires et d'autres affections courantes telles que le diabète, les

maladies neurodégénératives, le cancer et les maladies liées au vieillissement. Les causes pour lesquelles les mitochondries peuvent devenir sous-fonctionnelles sont variées. Par exemple, les mitochondries du muscle cardiaque, après un événement d'hypoxie et / ou d'ischémie, subissent des modifications métaboliques qui persistent après le rétablissement de l'apport d'oxygène au myocarde. Quelque chose de similaire se produit dans les tissus affectés par des processus inflammatoires, même après la résolution de ces processus. La détérioration mitochondriale peut montrer une large gamme de dysfonctionnements, liés aux maladies acquises. Beaucoup de ces maladies sont liées au vieillissement ou partagent sa physiopathologie avec le processus de vieillissement normal. Entre les anomalies mitochondriales, la production de ROS a été considérée comme l'une des conséquences majeures du dysfonctionnement mitochondrial, en plus du déficit en ATP, des mutations dans l'ADNmt, des pores de transition de la perméabilité mitochondriale, de l'apoptose, de la dérégulation du Ca2 +, de l'inflammation et de la dynamique de fusion / fission altérée. Toutes les caractéristiques mitochondriales qui, bien qu'elles n'agissent pas nécessairement indépendamment, deviennent perturbées par le processus de vieillissement et de nombreux troubles connexes comme le diabète sucré de type 2, la maladie de Parkinson, la maladie d'Alzheimer, l'athérosclérose, une susceptibilité accrue aux infections et le cancer. Enfin, tant chez les mammifères domestiques que chez l'homme, des mutations spécifiques et héréditaires du génome mitochondrial ont été identifiées, liées à des anomalies génétiques mitochondriales héritées de la mère ou à des troubles mitochondriaux secondaires dus à des anomalies du gène nucléaire.

Les signes et symptômes cliniques courants de la maladie mitochondriale comprennent l'ophtalmoplégie externe, le ptosis, la myopathie proximale et l'intolérance à l'exercice, la cardiomyopathie, la surdité sensoriel neurale, l'atrophie optique, la rétinopathie pigmentaire et le diabète sucré. Les caractéristiques communes du système nerveux central sont l'encéphalopathie fluctuante, les convulsions, la démence, la migraine, les épisodes ressemblant à un accident cérébrovasculaire, l'ataxie et la spasticité. Une incidence élevée de avortements en milieu et en fin de grossesse est un phénomène fréquent qui est souvent méconnu. Ces caractéristiques peuvent être présentes dans les maladies nucléaires ou codées par la mitochondrie, mais les formes mitochondriales sont plus fréquentes. Les défauts des gènes nucléaires peuvent être hérités de manière autosomique récessive ou dominante. Les anomalies de l'ADN mitochondrial ne sont héritées que de manière maternelle. De nombreuses maladies, lésions tissulaires et vieillissement mettent au défi la cellule et sa mitochondrie, perturbant ainsi leur intégrité, leur fonctionnement et leur homéostasie.

Les cellules ont naturellement la capacité d'échanger des composants intracellulaires, en particulier les mitochondries, par le biais de différents processus tels que le contact cellule à cellule, les microvésicules, les structures nano tubulaires et autres mécanismes. Clark et Shay (1985) ont lancé le transfert artificiel de mitochondries (TMA), qui impliquait le transfert de mitochondries contenant des gènes résistants aux antibiotiques dans des cellules sensibles, leur permettant ainsi de survivre dans un milieu sélectif et d'ouvrir ce nouveau champ de recherche. À la suite de ces travaux, le développement de transfert artificiel continue de reproduire certains aspects du transport cellulaire naturel, en particulier des mécanismes que les cellules utilisent naturellement pour sauver d'autres cellules endommagées.

Nous réfléchirons aux progrès nécessaires pour élargir les connaissances actuelles sur le transfert artificiel des mitochondries et sur la manière dont ces techniques pourraient être utilisées de manière thérapeutique. Nous fournirons un aperçu des caractéristiques de la structure mitochondriale qui sont importantes pour maintenir son intégrité tout au long du transfert artificiel. Actuellement, il existe une certaine probabilité de réparation du dysfonctionnement mitochondrial dans les tissus humains matures qui se rétablissent de différentes formes de dommages cellulaires en transférant des mitochondries obtenues du

patient lui-même, d'un donneur humain ou même d'une autre espèce. Des alternatives pharmacologiques pour la réparation des mitochondries dysfonctionnelles ont été également étudiées. Dans le domaine de la reproduction, différentes méthodes de remplacement des mitochondries dysfonctionnelles par des mitochondries saines provenant d'un donneur ont été étudiées. Ces techniques comprennent : le transfert du noyau de l'ovocyte dysfonctionnel sur un ovocyte sain énucléé, le transfert du cytoplasme riche en mitochondries d'un ovocyte sain à un ovocyte dysfonctionnel et le transfert direct de mitochondries purifiées, prélevées sur quelque tissu d'un donneur sain à une cellule dysfonctionnelle.

L'accord médical sur le diagnostic et les traitements des maladies mitochondriales est décevant. Dans la plupart des cas, ils présentent une hétérogénéité remarquable, à la fois dans leur présentation clinique et leur étiologie génétique, posant des défis pour le diagnostic, la gestion clinique et la clarification du mécanisme moléculaire. Le traitement est limité à l'exercice et aux compléments alimentaires sans base statistique suffisante prouvant leur efficacité. Les maladies mitochondriales acquises et certaines maladies à implication centrale comme les maladies de Parkinson, d'Alzheimer, de Diabète de type II ou d'athérosclérose n'ont pas de traitement visant à améliorer le comportement mitochondrial ou l'approvisionnement en énergie. D'autre part, des recherches récentes ont prouvé que les troubles acquis tels que la dégénérescence musculaire ou neurale post-traumatique et hypoxémique peuvent être traités avec le Transfert/ Transplant Artificiel du Mitochondrie. De nos jours, des efforts ont été faits pour modifier l'ADN mitochondrial dans les maladies mitochondriales en utilisant des modèles *in vitro* : certains chercheurs avaient obtenu des cellules saines d'un patient atteint de LHON après avoir modifié leur ADNmt mutant en utilisant la technologie de cybrides.

La communication cellule à cellule est un processus essentiel pour le développement et le maintien d'organismes multicellulaires. Divers mécanismes d'échange d'informations moléculaires entre cellules ont été décrits, par exemple : sécrétion de ligands, synapsis, transfert de composants cytosoliques par des jonctions lacunaires ou des exosomes. Parmi ceux-ci, le transfert mitochondrial horizontal (cellule à cellule, ou TMH) entre cellules de mammifères a été observé pour la première fois il y a quelques décennies. Cette découverte a inspiré de nouveaux concepts sur la ségrégation et l'héritage des mitochondries et de l'ADNmt. Différents modes de TMH ont été rapportés dans diverses conditions physiopathologiques. Cependant, la base de la sélection cellulaire d'un mode de TMH reste incertaine.

TMH favorise l'intégration des mitochondries dans le réseau mitochondrial original de cellules receveuses, contribuant ainsi à modifier la compétence bioénergétique et leurs autres propriétés fonctionnelles, non seulement in vitro mais également in vivo. En outre, TMH comprend également le transfert de gènes mitochondriaux, ce qui a des conséquences importantes sur la physiopathologie des dysfonctionnements mitochondriaux. Le transfert de cellule à cellule des mitochondries ou de leurs composants peut également entraîner l'initiation de la différenciation des cellules souches, la reprogrammation de cellules différenciées ou la stimulation des voies de signalisation inflammatoires. Divers mécanismes facilitent le TMH, notamment les nanotubes à effet tunnel, les exosomes, les microvésicules, la libération mitochondriale ou la fusion cytoplasmique, entre autres. Ces découvertes ont ouvert de nouvelles pistes de recherche pour le traitement des troubles liés aux mitochondries par transfert mitochondrial sain.

Inspirés par l'origine symbiotique des mitochondries et par la capacité de la cellule à transférer ces organites vers des voisins endommagés, de nombreux chercheurs ont mis au point des procédures permettant de transférer artificiellement les mitochondries d'une cellule à l'autre. Les techniques actuellement utilisées vont des simples cocultures de mitochondries isolées et de cellules receveuses à l'utilisation d'approches physiques et biochimiques pour

induire l'intégration. Ces méthodes imitent le transfert naturel des mitochondries. Pour pouvoir utiliser le transfert mitochondrial en médecine, nous devons répondre aux questions clés sur la manière de reproduire certains aspects des processus de transport naturel afin d'améliorer les méthodes de transfert artificielles actuelles. Une autre priorité est de déterminer la quantité optimale et la source de cellules / tissus de la mitochondrie afin d'induire une reprogrammation cellulaire ou une réparation des tissus, dans des applications in vitro et in vivo. De plus, il est important que le domaine explore de quelle manière les techniques de transfert artificiel de mitochondries peuvent être utilisées pour traiter différentes maladies et comment gérer les problèmes éthiques inhérents à de telles procédures. Il ne fait aucun doute que les mitochondries sont plus que de simples centrales à cellules, car nous continuons de découvrir leur potentiel d'utilisation en médecine.

Dans l'avenir immédiat, les techniques T/TAM, et en particulier MitoCeption, pourrait être utilisées pour améliorer la survie et la réparation des tissus, réduisant ainsi les signes et les symptômes de ces maladies. Par exemple, un article récent affirme que le transfert direct de mitochondries humaines peut améliorer les déficits cognitifs, la perte neuronale et la gliose dans un modèle murin d'Alzheimer. Nous avons prouvé que MitoCeption peut réparer la diminution de l'activité métabolique, de la masse mitochondriale et de la stabilité de la séquence de l'ADNmt, diminuer l'expression du gène p53 et diminuer le pourcentage de cellules mononucléées du sang périphérique (CMSP) mortes, toutes causées par les rayons UV. Nous avons utilisé une adaptation de la technique MitoCeption dans les CMSP, qui permet non seulement une absorption efficace et proportionnelle dans les cellules primaires, mais réduit également le temps du protocole pour une meilleure efficacité dans les applications en aval. Cette étude ouvre la possibilité d'utiliser des mitochondries de différents donneurs (PAMM) pour traiter le stress dû aux rayons UV et éventuellement d'autres maladies ou dysfonctionnements métaboliques dans les cellules dans des applications *ex-vivo*.

Nous proposons que ces maladies soient éligibles pour un traitement préventif par MitoCeption au lieu du transfert pronucléaire ou par fuseau. Récemment, nous avons prouvé avec succès que Mitoception peut être utilisé avec un modèle de transfert xénogénique avec mélange mitochondrial humain sur des ovocytes et des zygotes de femelles de jeunes rongeurs en bonne santé, sans affecter leur capacité de développement ni leur implantation (données non encore publiées, voir document n ° 3 ci-dessous). Ce modèle peut répondre à des préoccupations éthiques et médicales en évitant l'utilisation de l'ovocyte du donneuse, l'application de médicaments anticancéreux comme la cytochalasine B ou des lésions plasmiques sur l'ovocyte.

Les troubles mitochondriaux acquis et hérités sont des problèmes centraux pour la sous-fertilité maternelle, les maladies liées à l'âge, certains troubles métaboliques et dégénératifs et les maladies maternelles héréditaires telles que LHON, MERRF, MELAS, le syndrome de Leigh, etc. Les modèles du animaux domestiques comme la vache et le porc et les modèles murins ont prouvé l'importance de la masse mitochondriale et de la quantité normale d'ADNmt dans l'ovocyte en relation avec la qualité du matériel génétique et la capacité de développement de l'embryon. Dans certains cas de sous-fertilité maternelle, le transfert mitochondrial, et spécialement la MitoCeption aux ovocytes ou aux zygotes, pourrait rétablir la masse et la qualité des mitochondries, résolvant ainsi le problème des femmes subfertiles.

Dans le cas des maladies mitochondriales héritées de la mère, les approches thérapeutiques pour les personnes affectées incluent des traitements de soutien avec des médicaments mitogènes, une supplémentation alimentaire mitochondriale et des exercices physiques. Aujourd'hui, les approches préventives médicales se limitent à la micro-injection du cytoplasme de l'ovocyte et au transfert pronucléaire des ovocytes ou zygotes de la mère

affectée à des cellules similaires énucléées d'une donneuse en bonne santé, dans le but de réduire le pourcentage d'ADNmt mutant et de réduire la probabilité d'apparition de signes chez le nouveau-né. Ces techniques ont créé des problèmes médicaux et éthiques qui entravent le développement et la propagation de ces thérapies. Les techniques de transfert / transplantation mitochondriales, et spécialement MitoCeption, pourraient être utilisées pour prévenir les maladies mitochondriales héréditaires en augmentant le pourcentage de mitochondries normales dans le matériel génétique féminin sans sacrifier le matériel génétique du donneur. Notre dernier travail visait à déterminer si MitoCeption avait introduit avec succès les mitochondries humaines dans les ovocytes et les zygotes murins, sans affecter leur capacité de développement. Nous concluons que MitoCeption pourrait être un outil puissant dans le traitement et / ou la prévention de la sous fertilité chez les femmes et des animaux domestiques d'élevage et dans le traitement et la prévention des maladies mitochondriales héritées de l'homme, par le biais du don de mitochondries saines à des cellules présentant des anomalies génétiques mitochondriales.

TABLE OF CONTENTS

List of Figures

List of Tables

List of Abbreviations

adPEO: autosomal-dominant Progressive External Ophthalmoplegia

AHS: Alpers-Huttenlocher syndrome

AMT: Artificial Mitochondrial Transfer/Transplant

ANS: Ataxia neuropathy spectrum

Apaf1: Apoptotic protease activating factor-1

arPEO: autosomal-recessive Progressive External Ophthalmoplegia

ATP: Adenosine triphosphate

BCAAs: Branched-Chain Amino Acids

CJs: Cristae Junctions

CPEO: Chronic Progressive External Ophthalmoplegia

Cyt-c: cytochrome c

DISC: Death-Inducing Signalling Complex

DNA: Deoxyribonucleic Acid

DOA: Dominant Optic Atrophy

ER: Endoplasmic reticulum

GA: Golgi Apparatus

IMM: Inner Mitochondrial Membrane

IMS: Intermembrane Space

KSS: Kearns-Sayre Syndrome

LHON: Leber Hereditary Optic Neuropathy

MAMs: ER-mitochondrial protein connections

MCHS: Childhood myocerebrohepatopathy spectrum

MELAS: Mitochondrial Encephalomyopathy with Lactic Acidosis and Stroke-like episodes

MEMSA: Myoclonic epilepsy Myopathy Sensory Ataxia

MERRF: Myoclonic Epilepsy with Ragged-Red Fibers

MICOS: Mitochondrial Contact Site and Cristae Organizing System Complex

MiDAS: Mitochondrial Dysfunction-Associated Senescence

MIDD; Maternally inherited Deafness and Diabetes

MILS: Maternally inherited Leigh Syndrome

MIRAS: Mitochondrial Recessive Ataxia Syndrome

MNGIE: Mitochondrial Neurogastrintestinal Encephalopathy

mRNA: messenger Ribonucleic Acid

mtDNA: Mitochondrial DNA

NARP: Neurogenic Muscle Weakness, Ataxia, and Retinitis Pigmentosa

nm: nanometres

NPCs: Nuclear Pore Complexes

NPCs: Nuclear Pore Complexes

OMM: outer mitochondrial membrane

OXPHOS: Oxidative Phosphorylation

PBMCs: Peripheral Blood Mononuclear cells

PMPs: Peroxisomal Membrane Proteins

POLG: Polymerase Gamma-related Disease

RNA: Ribonucleic Acid

ROS: Reactive Oxygen species

SANDO: Sensory Ataxia Neuropathy, Dysarthria, Ophthalmoplegia

SCAE: Spinocerebellar Ataxia with Epilepsy

TCA: Tricarboxylic Acid

TEM: Transmission Electronic Microscopy

TNF: Tumour Necrosis Factor

tRNA: transfer Ribonucleic Acid

VF: viral factory

Brief Thesis Overview

This manuscript begins with introductory chapters (1-5) which provide the necessary theoretical background and synthesize parts of the growing body of knowledge about mitochondrial function, mitochondrial disorders and treatment approaches.

Chapter 6 presents the thesis results, methodology and discussion in the form of 3 articles: a published literature review, one published research article and one research article, recently summited. All the articles are self-contained and include their own list of references. The references cited in the introductory chapters are listed in Chapter 7.

Chapter 1. The human cell and the mitochondria

a. THE HUMAN CELL

Biology studies life, how different organisms and its structures maintain homeostasis, adapt to change and die. To facilitate its study, life is organized in levels of complexity such as cells, tissues, organs, organ systems, organisms, populations, communities, ecosystem, and biosphere. Among organisms, they can be categorized in two broad types: unicellular or multicellular. The latter includes all the more complex living beings including humans, with multiple cell to cell interactions that even today are not fully understood (Mason, Losos, Singer, & Raven, 2017).

The cell is considered the basic unit of life, where all life-sustaining biochemical reactions happen. It is characterized by having a complex and active boundary allowing it to interact with its surroundings, known as the plasma membrane (Pollard, Earnshaw, Lippincot-Schwartz, & Johnson, 2017). Membrane components are in a diversity of states fluctuating from immobilized to completely crosswise and rotationally mobile (with axis of rotation perpendicular to the membrane plane), as was emphasized in the fluid mosaic model, in which integral membrane proteins were proposed to be fixed in a lipid bilayer plain (Jacobson, Liu, & Lagerholm, 2019). The plasma membrane and limiting membranes of organelles function to demarcate and coordinate cellular functions, allowing the separation of metabolites and enzymatic reactions. Significant to such coordination is the subdivision of intracellular space into functional compartments. Compartmentalization can be reached by intracellular membranes, which surround organelles and act as physical barriers. In addition, cells have developed complex mechanisms to divide their cytosol in a closely regulated manner (Aguzzi & Altmeyer, 2016).

Some structures and the molecular pathways involved are shared among life forms, as they all descended from a common progenitor that lived 3 to 4 billion years ago. Even though this progenitor no longer exists, its descendants had spread into three **domains**: *Bacteria, Archaea,* and *Eukarya* (J. Xiao et al., 2019). The last one, *Eukarya*, is characterized by the presence of the nucleus, a membrane-bound compartment that contains the genetic information of a cell, the DNA. The genetic material inside the nucleus can be found as a coiled structure with linear bundles called chromosomes. Furthermore, eukaryotic cells have also others internal structures called organelles (Golgi apparatus, lysosomes, endoplasmic reticulum, mitochondria, cilia, etc) and a cytoskeleton composed of microtubules, microfilaments, and intermediate filaments, which play an important role in defining the cell's organization and shape (Figure 1). These features are neither present in *Bacteria* nor *Archaea* domains (Dacks et al., 2016). Additionally, eukaryotic cells are typically much larger than prokaryotes with an average volume of 10,000 times greater than prokaryotic cells (Yamaguchi & Worman, 2014) (Figure 1).

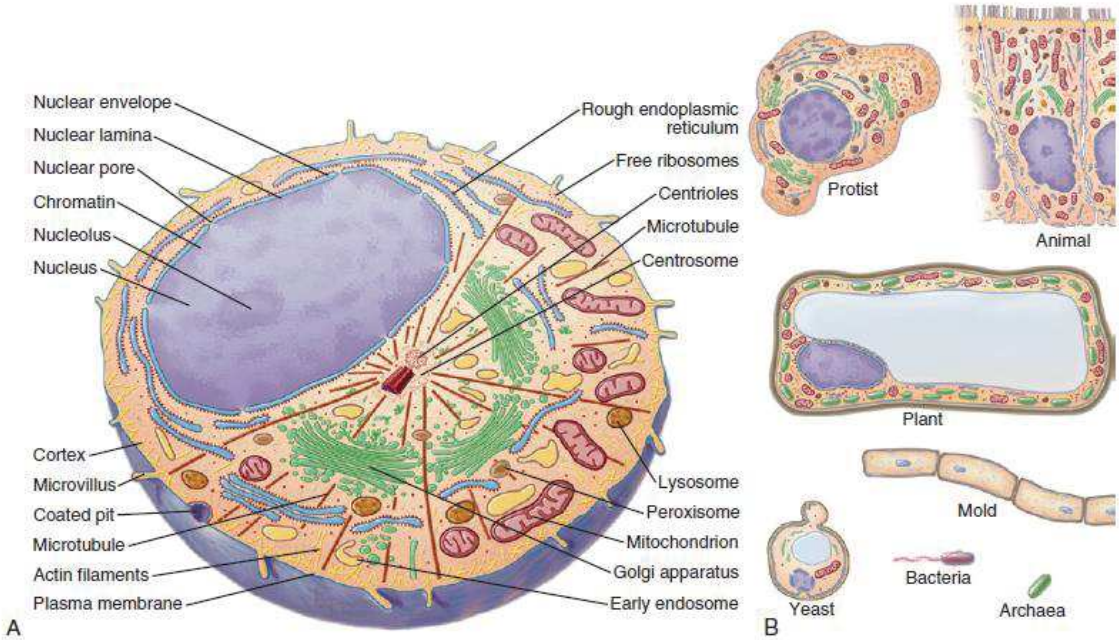


Figure 1. Basic Cellular Architecture. A, Section of a eukaryotic cell showing its internal and general components: Structures as cell wall and chloroplast are exclusive of plants, while cilia and flagella are exclusive of protist and animal cells. **B,** Comparison of cells from the mayor branches of the phylogenetic tree (Pollard et al., 2017).

Among the *Eukarya* domain*,* we find four kingdoms: *Animalia, Plantae, Fungi* and *Protista*. All Eukaryotic cells differ among them, for example, animal cells lack an exterior wall and chloroplasts and obtain energy by ingesting nutrients and even other living cells (Mason et al., 2017) (see Figure 1) whereas plant cells can obtain energy from inorganic compounds and possess the aforementioned cellular wall and chloroplasts . As an individual unit, the cell can absorbs and metabolizes its own nutrients in order to obtain energy (catalysis) and components, it also can produce other types of molecules (synthesis), eliminates metabolic waste (excretion), interacts with the surrounding environment (irritability and secretion) and proliferate in order to produce succeeding generations (reproduction). In fact, most unicellular organisms reproduce by binary fission, an asexual process where there are no differences between the progeny and the progenitor cell.

On the other hand, some multicellular organisms reproduce via sexual reproduction, in which new organisms are created, by combining the genetic information from two individuals of different sexes. The genetic material is carried on chromosomes within the nucleus of specialized sex cells called gametes. In males, these gametes are named spermatozoa and in females the gametes are called eggs or oocytes. During sexual reproduction the two gametes join in a fusion process known as fertilization, to create a zygote. The zygote is the precursor to an embryo offspring, which DNA is inherited half from each of its parents.

The genetic information contained in cells is subject to errors or changes, called mutations that cumulate within DNA over time, through cell mitosis. In unicellular organisms, asexual reproduction simply passes these mutations on to their offspring, while organisms which combine their DNA through sexual reproduction allow only a portion of their mutations to pass to their offspring, increasing their probabilities of survival. This effect is increased through

natural selection, where individuals with exceptionally harmful mutations are unable to pass on their genes through sexual reproduction (Cavalier-Smith, 2010).

Cell differentiation implies the specialization to one or more of the functions mentioned above. During differentiation and specialization, the cell adapt its own structures to perform an specific function or role (Alberts, 2015). For instance, to obtain a cell specialized in irritability (the capacity to react to an external stimuli with an electric membrane potential), a neuroblast -a non-specialized round and small cell- generates thousands of input connections (dendrites), an unique output connection (axon), a cytoplasm with a large endoplasmic reticulum and an active cytoskeleton. All these features allow the neuroblast to become a cell capable of generating and transporting electric potential along its axonal plasma membrane with the sole purpose of liberating a special secretory substance, called neurotransmitter (Hyttel, 2010; Ji & Tang, 2019).

Normally, a fully developed cell enters in a non-proliferative mode (stable or terminal differentiation). However, some events as inflammation, trauma and others that lead to necrosis and loss of functional cells, can start a natural regenerative process (Zachary, 2017) that implies stem-cells' activation or mature cell's dedifferentiation. Cell's dedifferentiation involves a terminally differentiated cell returning to a less differentiated stage from within its own cell line. This process allows the cell to multiply again before re-differentiating, leading to the replacement of those cells that have been lost. In some cases, the dedifferentiated cell can switch their lineage, a process called trans-differentiation or metaplasia (Jopling, Boue, & Belmonte, 2011). Dedifferentiation implies a temporal elimination of functional specific structures. For instance, zebra fish's heart regeneration includes a lack of sarcomeres (by contractile apparatus disassembling) in cardiomyocytes as a step prior to cardiomyocytes' proliferation. This is a required step, because sarcomere maintenance is resource-demandant to the cell and physically impairs cell division. However, dedifferentiation must not be confused with a "lack of differentiation", a similar process in which the cell loss resource-demandant functional structures to survive in low energy conditions as nutrient depriving, hypoxia, ischaemia, etc.

Cell structure

Metazoans, a member of the *Animalia* kingdom, is characterized by having the body composed of cells differentiated into tissues and organs and usually a digestive cavity lined with specialized cells. For instance, cellular shape correlates with its function, and this is determined during embryogenesis and cell differentiation (Pollard et al., 2017). Embryogenesis is the process by which the embryo forms and develops being characteristic of many multicellular organisms with sexual reproduction. During the early cellular differentiation, three embryonic layers are developed that give origin to all the mature types of cells and tissues: ectoderm, mesoderm and endoderm (Hyttel, 2010). All the neural cells, epidermal structures, teeth, cochlear sensory cells, corneal external epithelium and eye's lens are examples of ectoderm's derivates. Fibroblasts, osteocytes, fat cells, chondrocytes, myocytes, nephrons, reproductive tracts and blood cells are derivates from mesoderm and finally, urinary digestive tract and glands, respiratory system and germ cells come from endoderm (Gartner, 2017).

The general plan of eukaryotic cellular organization varies between different organisms, but despite these modifications, all cells resemble one another in certain fundamental ways. Before we begin a detailed examination of cell structure, let's first summarize four major

features all cells have in common: (1) a plasma membrane, (2) cytoplasm, (3) ribosomes to synthesize proteins, and (4) a nucleus where genetic material is located.

Plasma Membrane is the interphase between the cell and its environment. It is made of a phospholipid bilayer, integral proteins, cholesterol and other components (Nicolson, 2014). The lipid components are hydrophobic; thus, the plasma membrane is impermeable to ions and most of water-soluble compounds. Subsequently, these compounds can cross the membrane only through specialized transmembrane proteins, that form specific **channels, carriers and pumps** (Ariño, Ramos, & Sychrova, 2019). By means of these integral proteins, the cell can obtain nutrients, release its metabolic final products, control internal ion concentrations and create a transmembrane electrical potential. A second group of integral proteins are the **membrane receptors**, that facilitate the interaction between the cell and their immediate environment. These receptors convert the binding with extracellular signals (i.e. hormones, growth factors, death factors, etc) into chemical and/or electrical signals that modify the metabolic activity of the cell (Ma, He, & Berkowitz, 2019). A third group of integral proteins is formed by **adhesion proteins** which allow the binding between cells or between the cell and extracellular matrix. Cell to cell adhesions allow a strong association, commonly seen in epithelia and cardiomyocytes. Similar adhesion proteins play a significant role in leukocytes capability to bind and phagocyte bacteria and other microscopic pathogens (Pollard et al., 2017).

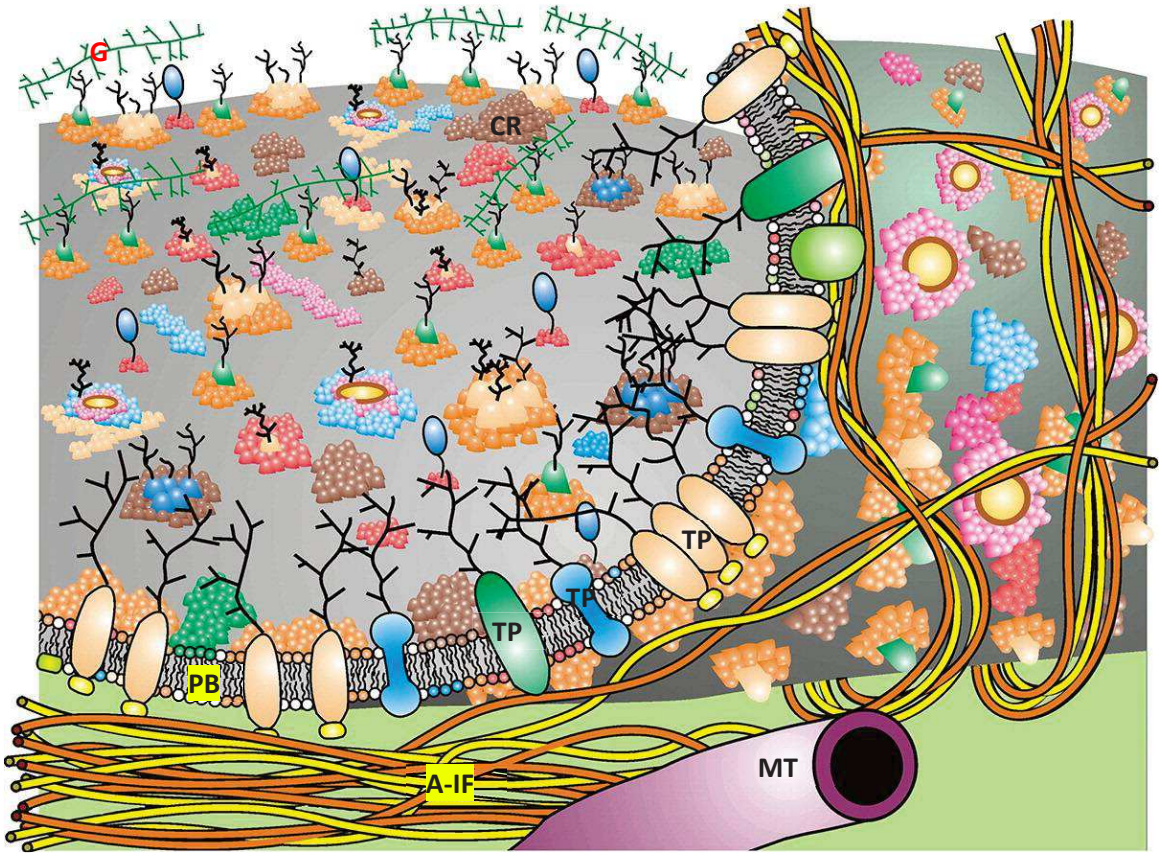


Figure 2. General Components of Plasma Membrane: G. Glycocalix, **CR.** Cholesterol Raft, **TP.** Transmembrane proteins, **PB**. Phospholipidic Bilayer, **MT.** Microtubule (cytoplasmic component), **A-IF** Actin and Intermediate Filaments (cytoplasmic component). Transmembrane proteins in pink represent receptors or carriers with enzymatic intracellular domains (as ATPase-dependant Na-K pump) (Modified from (Jacobson et al., 2019)).

The cytoskeleton is a cytoplasmic dynamic network formed by three protein polymers: **actin filaments, intermediate filaments and microtubules**. This structure not only maintains the shape of a cell but allows its modification, intra cellular movements and organelle traffic. Cellular locomotion, muscle contraction, organelles transport, mitosis and cilia and flagella beating are issued by **ATP-powered motor proteins (myosin, kinesins, dynein)** that use the actin filaments and microtubules as roads to perform them (VanDelinder, Imam, & Bachand, 2019). In fact, these motor proteins not only move the plasma membrane and organelles but the cytoskeleton itself. Organelle traffic is an essential part of organelles' metabolic integration that depends on the interaction between organelles and motor proteins by means of a specific connector molecule -called adaptor complex- (Mason et al., 2017) The crosslink between cytoskeleton and plasma membrane reinforce the cell's surface against external forces and allows microvilli development and balance, increasing the surface area of the plasma membrane for transporting nutrients, enabling substances interchange between spermatozoa and epididymis' epithelium and a large number of other processes (Gartner, 2017; Pollard et al., 2017).

The nucleus is a membrane-bound organelle found in eukaryotic cells. The nucleus is the repository of the genetic information that enables the synthesis of nearly all proteins of a living eukaryotic cell. It is deeply integrated in the metabolism itself modulating the transcription of the genetic information stored in specific genes in response to genetic, developmental, and environmental signals. It is separated from the cytoplasm by its own **double membrane** and all the traffic into and out of the nucleus pass through **nuclear pore complexes** (NPCs), because, unlike the cytoplasmic membrane, the nuclear membrane lacks other transport channels except the NPCs (Figure 3) (Caramori, Ruggeri, Mumby, Atzeni, & Adcock, 2019; Hampoelz, Andres-Pons, Kastritis, & Beck, 2019). The nuclear membranes develop since the interaction between chromatin and endoplasmic reticulum. They are separated by the **nuclear cistern**, the same one that is connected to the endoplasmic reticulum thanks to the continuity between the outer nuclear membrane and the membranes of this organelle. This continuity allows to ER connect with the *lamina* attachment site in the inner face of the nuclear membrane and also participate in NPC formation (Dawson, Lazarus, Hetzer, & Wente, 2009). The nuclear ***lamina*** is a network of intermediate filaments (lamins) that supports the DNA and provides support to nuclear envelope. The lamina also acts as an anchoring site for chromosomes and the cytoplasmic cytoskeleton (via protein complexes that span the nuclear envelope). The (Ho & Hegele, 2019).

Chromatin is a complex of DNA and protein found in eukaryotic cells (Mondal, Rasmussen, Pandey, Isaksson, & Kanduri, 2010). Chromatin's primary function is packaging very long DNA molecules into a more compact, denser shape, which prevents the strands from becoming tangled and plays important roles in reinforcing the DNA during cell division, preventing DNA damage, and regulating gene expression and DNA replication. During mitosis and meiosis, chromatin facilitates proper segregation of the chromosomes in anaphase; the characteristic shapes of chromosomes visible during this stage are the result of DNA being coiled into highly condensed networks of chromatin. In the case of human DNA, there are almost 3 billion bp in each single nucleus from which only 2% codes for genes and structural RNA (International Human Genome Sequencing Consortium, 2004). DNA is associated with proteins called histones. Interactions between DNA, histones and other proteins fold each chromosome densely, allowing the DNA to fit into the nucleus' small volume (Delage & Dashwood, 2008): In this packing process, the firs level is represented by each histone and its associated DNA (146 bp long) forming a **nucleosome**, separate form the next by a 200 bp long DNA chain. The nucleosomes' filament, known as a 10-nm fiber, condenses DNA approximately sevenfold

relative to naked DNA. In a second packing level, thousands of nucleosomes organized in tetranucleosomes, form **a solenoid fiber with 30 nm diameter**. At third level, this solenoid is coiled in a mayor solenoid, the **100-nm chromonema fiber**. Finally, the chromonema fiber form the central core of the **chromatid** (chromosome arm) (Pollard et al., 2017).

The chromatin has been categorized into two main classes based on structural and functional criteria. **Euchromatin** contains almost all genes, equally actively transcribed and quiescent. **Heterochromatin** is transcriptionally repressed and is typically more condensed than euchromatin. However, the term heterochromatin involves several distinct modes of chromatin compaction that have different implications for gene expression. Accordingly, heterochromatin should not be supposed of as just condensing inactive DNA, but as a descriptor for compact chromatin domains that share the common characteristic of being remarkably resilient to gene expression (Alberts, 2015).

According to the Viral Eukaryogenesis hypothesis, the nucleus -the main characteristic of eukaryotic cells- is a descendant of the viral factory of a DNA phage -from NucleoCytoplasmic Large DNA Viruses group- that infected the eukaryotes' archaeal ancestor (Bell, 2019; Forterre & Gaïa, 2016). Since analogies between VFs and eukaryote's nuclei had been found, there are some central issues for understanding the origin of the nucleus: (1) nuclear assembly of endomembranes around chromatin are comparable to VFs from viruses than replicate into the cytoplasm; (2) many cytoplasmic VFs recruit part of the nucleus and/or ER membrane to build their own membrane; (3) origin of centromeres and mitotic spindle, without which nuclear chromosomes cannot be inherited (Cavalier-Smith, 2010). For some researchers, the nucleus can be a relatively late acquisition in the evolution of eukaryotes, since remains unclear if this structure emerged before, after or in a parallel process with other organelles in transitional eukaryotes. A central topic of this problem is the evolution of the nuclear pore complex (NPC), which allows a critical channel between nucleoplasm and cytoplasm (Field & Rout, 2019)**.**

Endoplasmic Reticulum and Ribosomes: ribosomes catalyse the synthesis of proteins, using the nucleotide sequences of mRNA to specify the order of amino acids in a polypeptide that is going to be produce (process called "translation of the genetic code") (Arranz-Gibert, Patel, & Isaacs, 2019; Chatterjee & Yadav, 2019). Some ribosomes are found free in the cytoplasm and their function is to synthetize proteins for the cytoplasm itself (enzymes, carriers, cytoskeleton components, etc). In the other hand, ribosomes that synthetize proteins destined for insertion into cellular membranes, for lysosomes' content or for exportation are associated to the external face of **endoplasmic reticulum (ER)**, in a special region called **rough ER**. This region forms a continuous pattern of sacks and tubules called the **reticulum's cisternae** (Voeltz, Rolls, & Rapoport, 2002) (Figure 3). Proteins synthetized on the reticulum's external surface include amino acids' signal sequences that target their ribosomes to the rough ER. The amino acid sequence of polypeptides synthetized on the rough ER determines if the protein folds up into the ER membrane or translocates across the lipid bilayer into the lumen of the reticulum's cisternae. In some cases, enzymes undergo post-translational protein modifications by adding carbohydrate's chains.

ER membranes and cisternae move across the cytoplasm thanks to motor proteins and cytoskeleton's attachments (Guardia et al., 2019). There is continuous bidirectional traffic of small vesicles between ER and Golgi apparatus transporting soluble proteins and membrane lipids and proteins (Y. Yang et al., 2005). These vesicles carry protein receptors and ligands to identify the proper membrane to which they will join (Peotter, Kasberg, Pustova, & Audhya,

2019). A special region of the ER which lacks ribosomes is called **smooth ER**. Enzymes found in this region synthesize many cellular lipids and metabolize drugs, while ER pumps and channels regulate the cytoplasmic Ca2+ concentration (Yu et al., 2019).

Golgi Apparatus: The GA is constituted by a stack of flattened, membrane-limited sacks with many associated vesicles. It is typically located in the cell's centre, near to the nucleus and the centrosome. The face near the nucleus and ER (Cis face) is convex and receives the transfer vesicles from the ER, full of newly synthetized proteins, to which the GA will performs post translational modifications by adding lateral carbohydrates chains (Pothukuchi et al., 2019). This process occurs both to transmembrane and secreted proteins. The face oriented to the cellular apical side (Trans face) releases vesicles to the cytoplasm (lysosomes) or to the plasma membrane (secreted proteins). As proteins pass through the stacked Golgi membranes from one side to the other, enzymes in specific stacks modify the lateral carbohydrates chains of these proteins. Nowadays, it is established that GA has distinct functional zones that regulate specific functions: glycosylphosphatidylinositol-anchored proteins; proteoglycan, mucin, and lipid glycosylation; transport of cholesterol and ceramides; protein degradation (Golgi membrane-associated degradation); and signalling for apoptosis (Sasaki & Yoshida, 2019). Many components of the plasma membrane including receptors for extracellular molecules are recycled from the plasma membrane to endosomes and back to the cell surface many times before they are degraded (Simonetti & Cullen, 2019).

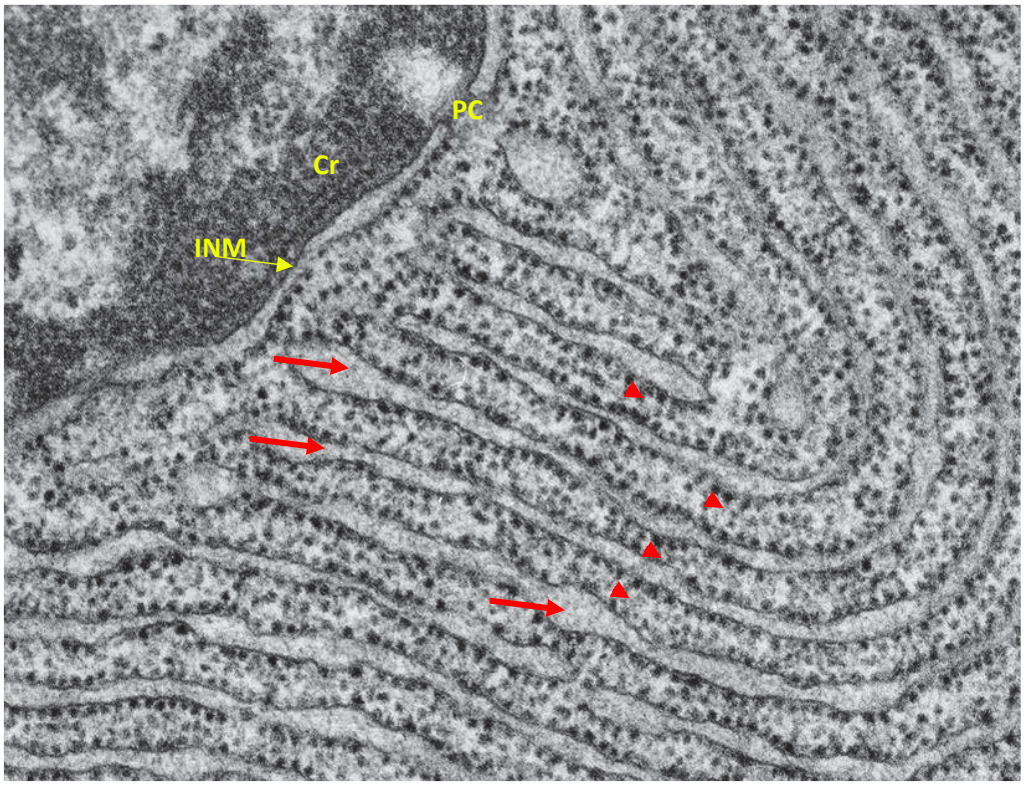


Figure 3. Nucleus and Rough Endoplasmic Reticulum. **Cr**, cromatin. **PC**, pore complex. **INM**, internal nuclear membrane. **Red Arrows**, ER cisternae**. Arrows' heads**, ribosomes. Modified from: (Schekman, 2004)

Lysosomes: these organelles have an impermeable membrane separating degradative enzymes from other cellular components. After synthesis by rough ER, lysosomal enzymes move through the Golgi apparatus, which add the modified carbohydrate, phosphorylated mannose. Vesicular transport, guided by phosphomannose receptors, carries lysosomal proteins to the lumen of lysosomes (Čaval et al., 2019). Cells eliminate afunctional organelles and macromolecules by surrounding them into a membrane vesicle (the phagophore), which later fuses with a lysosome, becoming an autophagosome (C. O. Davis et al., 2014; Parkinson-Lawrence et al., 2010). Recognition of afunctional organelles depends of both physical and chemical interactions between organelles and lysosomes (i.e. mitochondrial ROS production and mitochondrial surface's Pink1 retention; ER mis-folded protein production, etc) (Boga et al., 2019; Raimundo, Fernández-Mosquera, Yambire, & Diogo, 2016), Additionally, cells ingest microorganisms and other materials in endocytic vesicles derived from the plasma membrane, becoming phagosomes after fusion between lysosomes and endocytic vesicles. The contents of these autophagosomes and phagosomes are then degraded by lysosomal enzymes (Klionsky et al., 2016).

Peroxisomes: Peroxisomes are ubiquitous single membrane-bound organelles containing a fine granular matrix composed by enzymes that play a crucial role in lipids and reactive oxygen species (ROS) metabolism (Baboota et al., 2019) and synthesis of ether phospholipids and bile acids. Most cells have spherical or spheroidal peroxisomes with a diameter of 0.1–1 µm. Nonetheless, the peroxisomes can vary in shape and size in different tissues (Imanaka, 2019). Like mitochondria, peroxisomal enzymes oxidize fatty acids, but the energy is not used to synthesize ATP. Biosynthesis of peroxisomes in mammals involves three different processes: (1) the pre-peroxisomes budded from ER, (2) the import of peroxisomal membrane proteins (PMPs) and matrix proteins to the pre-peroxisomes, that will allow the peroxisome to grow and mature, (3) peroxisomal fission (Sugiura, Mattie, Prudent, & McBride, 2017). Peroxisomal proteins are synthesized in the cytoplasm and imported into the organelle using the same strategy as mitochondria but with different targeting sequences and transport machinery. Due to their central metabolic role, peroxisomes must cooperate with many organelles involved in cellular lipid metabolism such as the ER, mitochondria, lipid droplets, lysosomes, and phagosomes (Schrader, Kamoshita, & Islinger, 2019).

Among all cell structures, the mitochondria play an essential role. From an energy production plant to its transfer from one cell to another, this organelle continues challenging research as it has an important role in health.

b. **MITOCHONDRIA**

Mitochondria plays a fundamental role in various functions within the cell. It is responsible for the synthesis of most of the ATP required to cover the energy needs. It also acts as an important mediator of the use and production of organic molecules, such as lipids, nucleotides, proteins and carbohydrates (Scheffler, 2008). Similarly, they participate in the control of intracellular calcium levels, the process of cellular apoptosis, heme synthesis, heat generation and participate in growth, differentiation, and survival of the cell (Hsu, Wu, Yu, & Wei, 2016; Mishra & Chan, 2014; Picard, Wallace, & Burelle, 2016).

Origin: The endosymbiont theory for the origin of mitochondria is now widely accepted. As usually described, mitochondria are cell organelles descended from the integration of an alphaproteobacterial endosymbiont related with *Ricketsiales* (Emelyanov, 2001, 2003; Kannan, Rogozin, & Koonin, 2014) into an host cell related to Archaea. Their monophyletic origin and

close connexion to the order *Rickettsiales* arose from multiple phylogenetic reconstructions based on conserved proteins, the small subunit (12S) of rRNA and comparisons between bacterial and Mitochondrial DNA sequences (Dyall, 2004; Emelyanov, 2001; Karlberg, Canbäck, Kurland, & Andersson, 2000). The evolutionary transition from the original bacterium to a permanent organelle implied three mayor phenomena: **(1) Genetic integration as** a extensive lack of their genome, transferred to the nucleus or simply lost; **(2) Metabolic integration or mutual dependency of the metabolisms** by addition of carrier proteins to its inner membrane, outer membrane modifications, cristae specialization, evolution of contact sites between membranous organelles and mitochondria, connexions with cytoskeleton, host cell's proteins retargeting, among other mechanisms, and **(3) Cellular integration** in the form of synchronization of cellular and mitochondrial cycles (Bock, 2017; Poole & Gribaldo, 2014; Zimorski, Ku, Martin, & Gould, 2014).

Structure: Since Palade's pioneer studies, it has been well stablished that mitochondria are membrane-bound organelles, with **an outer mitochondrial membrane (OMM)** and an **inner mitochondrial membrane (IMM)** which is convoluted and folded into ***cristae mitochondriales*** (Palade, 1953). The presence of these folds or cristae increases the surface area of the IMM. OMM and IMM define two internal compartments, the **intermembrane space (IMS)** between the two membranes and the **mitochondrial matrix** - a compartment in which Mitochondrial DNA, metabolic enzymes and signalling agents are found- within the inner membrane. The intracristal space within the cristae folds is continuous with the intermembrane space communicating through small circular junctions also called c**ristae junctions (CJs)** (T. G Frey, Renken, & Perkins, 2002). CJs formation is mediated by mitochondrial contact site and cristae organizing system (MICOS) complex (van der Laan, Horvath, & Pfanner, 2016) and their formation avoids diffusion between the intracristae space and the IMS by sequestering cristae contents and regulating its transfer (Terrence G. Frey & Mannella, 2000).

In most mitochondria, the IMS is small and electron-lucid, whereas the mitochondrial matrix is electron-dense, not homogeneous and exhibit a fine granularity with a frequently distinct crystalline inclusions with high electron density (Fig 4). The mitochondria cristae exhibit a variable appearance according to the cell type as well as the developmental and metabolic states of the cell. For instance, they are often lamellar in shape, but can also be found in a tubular conformation in adrenal cortex and Leydig's cells or as triangular prims in some neural cells (Scheffler, 2008; van der Laan et al., 2016). The mitochondrial shape is also variable, depending of the cell's metabolic needs, these organelles can merge with each other (fusion), forming giant mitochondria or even a mitochondrial network. Furthermore, through fission, these organelles can separate into smaller mitochondria (Pernas & Scorrano, 2016).

Mitochondrial Function in different cell types

Mitochondria play a main role in **(1)** ATP synthesis, **(2)** Ca^{2+} homeostasis, **(3)** Nucleotide and heme synthesis, **(4)** apoptosis regulation (as part of cellular growth, differentiation and fate), **(5)** redox balance and **(6)** waste management. However, mitochondria don't work the same in all cells. As different tissues have specific physiological requirements, the mitochondria respond to these needs being metabolically active. Recent research (Herbers, Kekäläinen, Hangas, Pohjoismäki, & Goffart, 2019) found differences between brain, heart, skeletal muscle, liver, kidney and brown fat tissue's mtDNA replication, gene expression and topology. For example, both liver and kidney cells replicate their mtDNA using the asynchronous mechanism known from cultured cells; while tissues with high OXPHOS activity, such as heart, brain, skeletal muscle

and brown fat, employ a strand-coupled replication mode, combined with increased levels of recombination.

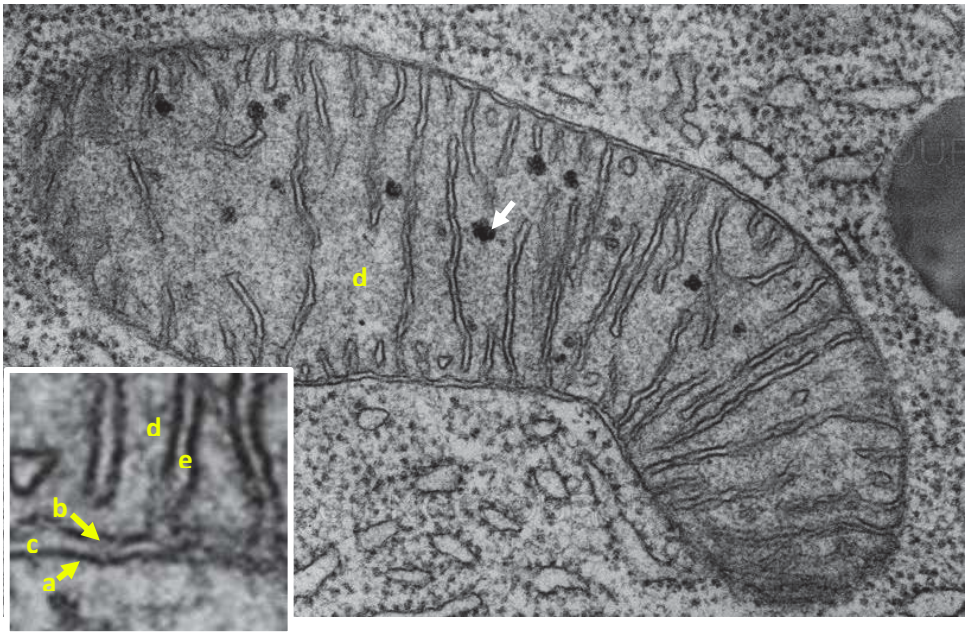


Fig. 4 Mitochondrion in Bat Pancreas Cell. a. Outer Mitochondrial Membrane, **b.** Inner Mitochondrial Membrane, **c.** Intermembrane space, **d.** Mitochondrial Matrix, **e.** Cristae, **White Arrow:** calcium granule. **Inset:** amplification of OMM and IMM. TEM micrography modified from (Science Source, n.d.)

Further differences between cells regarding mitochondrial shape and activation are more obvious at a functional level. Muscle cells responds fast to external stressors as physical activity, improving mitochondrial biogenesis, OXPHOS activity and mitophagy (Favaro et al., 2019). In these cells, mitochondrial network is divided spatially in two mitochondrial types: the first type is the subsarcolemmal mitochondria, that provides ATP for membrane active transport, as well as for gene transcription. The second type, the intermyofibrillar mitochondria, regulates ATP synthesis for contractile filaments function and controls Ca^{2+} release from sarcoplasmic reticulum (Hood, Memme, Oliveira, & Triolo, 2019).

1. **ATP synthesis:** cells consume energy sources (called fuels) as carbohydrates, amino acids and fatty acids to generate energy-storing molecules as ATP and GTP. Each fuel has its own catabolic pathway that starts in the cytosol and ends in the OXPHOS chain.

    a. **Carbohydrates catabolism** starts with the glycolytic pathway, where carbohydrates as glucose are hydrolysed to pyruvate yielding 2 mol of ATP and 2 mol of NADH per mol of glucose. In aerobic conditions, pyruvate is transferred to the mitochondrial matrix and integrated to the Tricarboxylic Acid (TCA) cycle, where 8 mol of NADH and 2 mol of FADH are produced. Then, NADH (including the NADH obtained in glycolysis, transferred to the mitochondria) and FADH are oxidized through the respiratory chain to obtain a theoretical yield of 30 mol of ATP glucose (Bender et al., 2018; Spinelli & Haigis, 2018; Vanderperre, Bender, Kunji, & Martinou, 2015).

b. **Amino Acids catabolism**: Mitochondria can metabolize (or synthetize) glutamate, glutamine, leucine, isoleucine and valine. Glutamate is transformed in Glutamine, which is transformed in alfa-ketoglutarate that enters in TCA cycle**.** The others mentioned amino acids (branched-chain amino acids or BCAAs) must be transformed in acetyl-CoA or succinyl CoA before entering in TCA cycle. Although the BCAAs mitochondrial pathway is well characterized, their import mechanism remains unknown (Spinelli & Haigis, 2018)

c. **Fatty acids catabolism:** Fatty acids are hydrolysed in the mitochondria by oxidation to acetyl-CoA in a process that produces large quantities of energy. When this pathway is proceeding at a high rate, three compounds, acetoacetate, D-3-hydroxybutyrate, and acetone, known as the ketone bodies, are produced by the liver. Acetoacetate and D-3-hydroxybutyrate are used as fuels by extrahepatic tissues in normal metabolism (specially in neurons and striated muscle), but overproduction of ketone bodies causes neural toxicity, a pathology called ketosis (Houten, Violante, Ventura, & Wanders, 2016).

d. **Respiratory Chain:** Even though the TCA cycle that takes place in the mitochondrial matrix is part of aerobic metabolism, it does not use oxygen itself (Alberts, 2015). Only the final step of oxidative metabolism uses molecular oxygen (O_2) directly. Almost all the energy obtainable from metabolizing carbohydrates, fats, and other "fuels" in previous steps is stored in the form of energy-rich compounds that transfer electrons into the respiratory chain in the IMM. These electrons, most of which are carried by NADH, at last term combine with O_2 and H^+ at the end of the respiratory chain to form water. The energy released during the complex sequence of electron transfers from NADH to O_2 is diffused along the respiratory complexes I and III, and III and IV in the IMM to produce an electrochemical gradient that energies the conversion of ADP + Pi to ATP at Complex V (Chaban, Boekema, & Dudkina, 2014). For this reason, the term **oxidative phosphorylation (OXPHOS)** describe this final series of reactions. The total quantity of energy released by biological oxidation in the respiratory chain is equal to that released by the explosive combustion of hydrogen when it combines with oxygen in a single step to form water. However, the combustion of hydrogen in a single-step chemical reaction, which has a strongly negative ΔG, releases this large amount of energy unproductively as heat. In the respiratory chain, the same energetically favourable reaction $H_2 + ½\ O_2 \rightarrow H_2O$ is divided into small steps, allowing the cell to store almost half of the total energy that is released in the ATP molecule.

2. **Ca^{2+} homeostasis**: Adequate cytosolic Ca^{2+} levels depend on a delicate equilibrium between cellular Ca^{2+} intake (transmembrane Ca^{2+} channels), cellular Ca^{2+} release (ATP-dependant Ca^{2+} pumps) and Ca^{2+} mobilization from intracellular stores as ER. However, ER's Ca^{2+} intake can be too slow to allow a rapid ionic equilibrium. Mitochondria can uptake the cytosolic Ca^{2+}, released by ER previously, by similar or even larger cycles. Mitochondrial Ca^{2+} storing is due to the large mitochondrial membrane potential (usually from -150 to -180 mV) and by the ER-mitochondrial protein connections (**MAMs**) that allow direct ion transfer between organelles (Naia, Ferreira, Ferreiro, & Rego, 2016; Patergnani et al., 2011).

3. **Heme and Nucleotide synthesis**: The biosynthesis of **heme groups** play a central part in electron transfer and it is another main process that is shared between the mitochondria and the cytoplasm. Iron–sulphur clusters, which are crucial not only for electron transfer in the respiratory chain, but also for the conservation and stability of the nuclear genome, are produced in mitochondria. Nuclear genome instability, a hallmark of cancer, can sometimes be linked to the decreased function of cellular proteins that contain iron–sulphur clusters.
   Nucleotide synthesis: Nucleotides can be produced through recover pathways, via the recycling of pre-existing nucleosides and nucleobases, or through the *de novo* synthesis pathways, using amino acids and small molecules to synthetize the purine and pyrimidine rings. Healthy mature cells use the recover pathways for nucleotides' synthesis but proliferating and cancer cells prefer *de novo* synthesis pathway. Mitochondria is responsible for one step in pyrimidine synthesis, although purine synthesis develops only in the cytosol, however, mitochondria play a primary role controlling both pathways, because de-novo synthesis of pyrimidine nucleotides is coupled to the mitochondrial respiratory chain and other mitochondrial pathways. Oncogenic activation of RAS and lack of tumour suppressors as p53 promote pyrimidine synthesis by activating urea cycle. Cytosolic purine synthesis is stimulated by mTORC1, also stimulates the formation and assembly on the mitochondrial surface of the purinosome complex, which carries out de novo purine synthesis (Gattermann et al., 2004; Spinelli & Haigis, 2018; Villa, Ali, Sahu, & Ben-Sahra, 2019).

4. **Apoptosis regulation**: Apoptosis is a key mechanism that mediates cell replacement, body growth embryogenesis and defence: During morphogenesis or epithelial replacement, tissues must have an equilibrium between cellular production and death. In the other hand, damaged, infected, abnormal, misplaced, non-functional, or potentially dangerous cells must be eliminated by apoptosis before they threaten the health of the organism (Alberts, 2015). There are two mechanisms that induce apoptosis: the extrinsic and the intrinsic (mitochondrial) pathways. In the first one, cells respond to external signals (hormones, cytokines, etc) called **Fas-ligands** that bind to cell-surface death receptors. These receptors are homotrimers and belong to the **tumour necrosis factor (TNF) receptor** family, which includes a receptor for TNF itself and the **Fas death receptor** (Steller, 2015). When triggered by the binding of Fas-ligand, the death domains on the cytosolic extremities of the Fas death receptors bind intracellular adaptor proteins, which bind initiator caspases (primarily **caspase-8 and 10**), forming a **death-inducing signalling complex (DISC)**. After dimerized and activated in the DISC, the initiator caspases cleave their associate proteins and then activate downstream executioner caspases to induce apoptosis (Reed & Green, 2011).

   Apoptosis as response to cellular or DNA damage or development signals are governed by the **intrinsic pathway**, which depends on the release into the cytosol of mitochondrial proteins as **cytochrome c (Cyt-c)**, a water-soluble component of the mitochondrial electron-transport chain. After hypoxia or ischemia, there are a depletion of cellular ATP and phosphocreatine. These disturbances in energy metabolism start pathophysiological responses that convergence on mitochondria such as cellular uptake of calcium. The increase of cytosolic calcium is compensated with mitochondrial calcium uptake and swelling, impairment of respiration and increased production of ROS that trigger mitochondrial permeabilization and Cyt-c release (Thornton & Hagberg, 2015). When released into the cytosol, it binds to an adaptor protein called **apoptotic protease activating factor-1 (Apaf1)**, causing the Apaf1 to oligomerize into a heptamer named an

apoptosome (Yuan & Akey, 2013). The Apaf1 proteins in the apoptosome then recruit initiator **caspase-9** proteins, which are supposed to be triggered by proximity in the apoptosome, just as caspase-8 is triggered in the DISC. The activated caspase-9 molecules then activate downstream executioner caspases to induce apoptosis (Dorstyn, Akey, & Kumar, 2018).

During embryogenesis, programmed cell death is also controlled by the apoptosis intrinsic pathway, which is regulated by **Bcl-2 family proteins**. This family includes both anti-apoptosis and pro-apoptosis members. The pro- and antiapoptotic molecules can directly or indirectly antagonize each other. In the absence of an apoptotic stimulus, anti-apoptotic Bcl2 members bind to and constrain the effector Bcl2 members on the OMM and in the cytosol. In the presence of an apoptotic stimulus, BH3-only proteins are activated and bind to the anti-apoptotic Bcl2's so that they can no longer constrain the effector Bcl2 family proteins; the latter then become triggered, aggregate in the OMM, and promote the release of intermembrane mitochondrial proteins as Cyt-c into the cytosol (Gross, 2016; Yin & Dong, 2003).

5. **REDOX balance**: Mitochondria are critical for buffering the redox potential in the cytosol. Cells need a continuous amount of the electron acceptor NAD^+ for the conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate during glycolysis. This NAD+ is transformed to NADH in the process, and the NAD^+ needs to be renewed by transferring the high-energy NADH electrons somewhere (Bender et al., 2018). The NADH electrons will finally be used to help drive oxidative phosphorylation inside the mitochondria. However, the inner mitochondrial membrane is impermeable to NADH. The electrons are therefore passed from the NADH to smaller molecules in the cytosol that can move through the inner mitochondrial membrane. Once in the matrix, these smaller molecules transfer their electrons to NAD^+ to form mitochondrial NADH, after which they are returned to the cytosol for recharging—creating a shuttle system for the NADH electrons (Alberts, 2015). However, the cytosol is a more oxidizing location in which the NAD+/NADH proportion ranges between 60 and 700. On the contrary, mitochondria employ more reductive metabolic reactions, and the NAD+/NADH proportion is approximately 7–8. To sustain the imbalanced distribution of NAD, mammalian cells use indirect pathways: malate–aspartate shuttle, citrate–malate shuttle, α-Glycerophosphate shuttle and one carbon metabolism pathway (Spinelli & Haigis, 2018)

6. **Waste management**: the final products of catabolism have poor energy content, or they are toxic. However, compounds depicted as waste (ammonia and ROS) have a functional role into the mitochondria.

    a. **Urea cycle:** The urea cycle is a central metabolic pathway in mammals that converts the ammonia (NH_4^+) produced by the catabolism of amino acids and nucleotides to the non-metabolizable (by mammals) urea excreted in urine. As part of their fate, amino acids are imported into mitochondria and oxidized to produce NADH for ATP production (see up). Additionally, mitochondria can process exceeding ammonia transferring it to alfa-ketoglutarate to produce glutamate and glutamine in bidirectional reactions.

The urea cycle consists of the action of three mitochondrial enzymes -**N-acetylglutamate synthetase**, **Carbamoyl phosphate synthetase 1** and **Ornithine transcarbamylase**- that transform glutamate to carbamoyl phosphate. Then, by binding carbamoyl phosphate to ornithine to create citrulline, three cytosolic enzymes **- Argininosuccinate synthetase, Argininosuccinate lyase and Arginase-** transfer ammonia from citrulline to arginine, which is divided in urea and ornithine. Finally, a small group of transporting proteins transfers ornithine from cytosol to mitochondrial matrix and citrulline from mitochondria to cytosol (Foschi et al., 2015).

b. **Reactive Oxygen Species:** It is worldwide admitted that mitochondrial produced **ROS** can damage mitochondrial DNA (mtDNA), and ROS-induced lesions in mtDNA can lead to somatic mutations that accumulate, affect the integrity of respiratory chain, and cause mitochondria-dependent aging (D.-X. Tan, 2019). Mitochondria produce ROS in response to stressors such as hypoxia, starvation, cytokine stimulation and changes in mitochondrial membrane potential (Zsurka, Peeva, Kotlyar, & Kunz, 2018). To evade ROS production's consequences, mitochondria does ROS clearance through enzymes as superoxide dismutase. This enzyme transforms ROS in Hydrogen peroxide (H_2O_2) that is reduced to water and O_2 due to the combined activities of peroxiredoxins 3 and 5, thioredoxin 2 and thioredoxin reductase 2 (Spinelli & Haigis, 2018). However, mitochondrial ROS are also implied in some critical process as signalling agents: they promote cellular proliferation, differentiation and migration. Likewise, they stimulate glycolysis in cancer cells -The Warburg effect- (Liberti & Locasale, 2016). Finally, mitochondrial ROS have a role in immune defence by stimulating macrophages' antibacterial immune response and B cell and T cell activation (Dan Dunn, Alvarez, Zhang, & Soldati, 2015; Müllebner, Dorighello, Kozlov, & Duvigneau, 2018).

Mitochondrial Integration with the cell

Mitochondrially encoded subunits of the OXPHOS system assemble with nuclear-encoded subunits into enzymatic complexes. Expression of the mitochondrial genome involves intramitochondrial replication, transcription, and translation. Mitochondrial translation is associated to other mitochondrial and cellular functions. The synthesis of mitochondrial-encoded proteins is coordinated by the connection of mitochondrial protein synthesis with assemblage of respiratory chain complexes. Mitochondrial translation controls cellular proliferation and cell cycle, and mitochondrial ribosomal subunits play a role in the cytoplasmic stress response. Thus, translation in mitochondria is integrated into cellular processes. Additionally, mitochondria are known to regulate key cellular processes, including calcium signalling, cell metabolism and cell death, among others.

a. **Genetic integration:** Mitochondria retains 63 proteins or less encoded in its genome, but it harbour almost 2000 proteins involved in its ancestrally prokaryotic biochemistry (Zimorski et al., 2014). This discrepancy is explained inside the endosymbiotic theory as the Endosymbiotic Gene Transfer Phenomenon, in other words, the mitochondrial gene transfer to the nucleus. The transferred genes became pseudogenes, or their transcripts became cytosolic proteins until mitochondria developed the protein import machinery. Then, the transferred genes obtained the expression and targeting signals needed. Al

last, mitochondria retained only the genes for the electron transport chain proteins, its own ribosome and the transport RNAs (Bock, 2017).

b. **Metabolic integration: Mitochondria distribution** in the cytoplasm of the cell is not random. Their distribution responds to the cell's metabolic needs. For instance, in a secretory cell, mitochondria are located near the ER-Golgi complex. Their position is different as in a kidney's proximal tubule's cell, where mitochondria are located near the apical surface, close to ATP-dependant membrane's transport mechanisms. The needs of ATP can change along the cell's life; therefore, mitochondrial position, number, shape and size should change in correspondence. These changes are mediated by mitochondria-cytoskeleton interactions. For instance, when mitochondria are observed in live cells, they seem to be coarsely ranged along their long axes. However, when they colocalise with cytoskeletal structures, their association with microtubules becomes immediately apparent. Motor proteins as kinesin-1 and some adaptor complexes (**as kinectins, Miro, syntabulin** and others) that link kinesin to mitochondria have been identified but their participation in mitochondria's motility, morphology and anchorage remains poorly understood (Anesti & Scorrano, 2006; Boldogh & Pon, 2007; Sukhorukov & Meyer-Hermann, 2015).

Additionally, there is a correlation between ATP cell's needs and the **number, shape and size of mitochondria** (Scheffler, 2008). From cristae modifications to the formation of a mitochondrial networks, these morphological changes warrant the functionality required to fit the roles needed to promote cellular survival (Pernas & Scorrano, 2016). There is a relation between cristae shape and oxidative phosphorylation (OXPHOS) function, suggesting that membrane morphology modulates the organization and function of the OXPHOS system, with a direct impact on cellular metabolism (Chaban et al., 2014) . A rise in cristae number follows exposure to a substrate rich in oxygen (non-glycolytic). This rise is accompanied by higher levels of respiratory chain proteins and supercomplexes, which together increase oxidative phosphorylation activity (Cogliati, Enriquez, & Scorrano, 2016). In cell culture starvation models, a decrease in cristae thickness supports increased ATPase dimerization and activity, improving respiratory chain supercomplex assembly and mitochondria-dependent cellular growth. Independently of the role of cristae in metabolism, a reduction in cristae thickness decreases the release of apoptotic molecules following a cell death stimulus (Pernas & Scorrano, 2016).

Another main issue in mitochondrial metabolic integration is the presence of close contacts between mitochondria and other organelles (**mitochondrial MCS**) (Elbaz & Schuldiner, 2011). Contact sites are regions where two membranes are closely apposed but the membranes do not fuse maintaining the organelles' identities. The contact sites among the ER and mitochondria have been measured to be 10–30 nm wide (Rowland & Voeltz, 2012; Stacchiotti et al., 2019). Contacts between mitochondria and Golgi Complex and/or ER can serve to recycle and repair mitochondrial membranes, calcium homeostasis, lipid transport, ion exchange and formation of specialized protein microdomains that allow to coordinate diverse signalling pathways. Additionally, ER-mitochondrial contacts play fundamental roles in mitochondrial biogenesis (fission) and mitochondrial proteome transfer. As in mitochondrial traffic, there are a growing set of protein connectors in the contact sites: **a voltage-dependent anion channel (VDAC1)**

on the outer mitochondrial membrane related to calcium transfer**, GRAMD1A–GRAMD1C** (in metazoans) related to sterol transfer from ER to mitochondria, **ERMES complex** related to phospholipid transport from ER to mitochondria and **mitochondrial-vacuole contacts**, termed **vCLAMPs,** also related to phospholipid transport, in coordination with ER or bypassing it (Lahiri, Toulmay, & Prinz, 2015; Murley & Nunnari, 2016).

c. **Cell cycle integration:** The cell cycle is an essential cellular mechanism that regulates the fate of cells and typically consists of four phases: **S-phase**, during which DNA replication occurs; **M-phase**, where cell division, or mitosis, takes place, and the gap phases that separate the two; **G1 and G2**, respectively (Sen, 2019)**.** As mentioned previously, mitochondria can change their shape, number and internal structure as part of their metabolic integration (Scheffler, 2008). Additionally, these changes, expressed by means of biogenesis and mitophagy, are correlated with the cell cycle within the healthy cells. Several cellular signalling pathways, as well as those that drive cell division, interact closely with the mechanisms that regulate mitochondrial function, specifically mechanisms that control mitochondrial fission and fusion, mitochondrial biogenesis, mitochondrial activity, and mitochondrial apoptosis by intrinsic pathway (Lopez-Mejia & Fajas, 2015). Mitochondrial biogenesis is controlled through coordinated transcriptional regulation of nuclear and mitochondrial genes. The activity of these transcription factors is controlled by the energetic demands of the cells (Andreux, Houtkooper, & Auwerx, 2013). During cell cycle development, the 'structure' of the mitochondrial network within the cell can change from an inter-connected network in G1, to a hyperpolarized, giant single tubular network in the G1/S transition, and to a very fragmented network in mitosis (Margineantu et al., 2002; Pernas & Scorrano, 2016).

Quality control

Mitochondrial quality is granted by the balance between the number of functional mitochondria and the cell's energy needs. Mitochondria are very dynamic organelles that replicate by fission when the cell needs more energy. The fission process is triggered via **Drp1** activation. When mitochondria become overloaded, their molecular components can be damaged by ROS and these damages can be repaired by fusion, which is mediated by **Opa1, Mfn1, and Mnf2**. The unnecessary and non-functional mitochondria are removed by mitophagy.

a. **Mitochondrial fission** and concomitant mtDNA replication are the assumed mechanisms for mitochondrial proliferation that guarantee that growing and dividing cells hold an adequate number of mitochondria to sustain their cellular functions. This process produces not only new organelles but offers a quality control mechanism by segregating and targeting damaged mitochondria for elimination via selective autophagy (Bess, Crocker, Ryde, & Meyer, 2012). The key transcription factors controlling mitochondrial biogenesis are **peroxisome proliferator-activated receptors (PPARs), PGC1 coactivators (PGC1a and PGC1b), and nuclear respiratory factors 1 and 2 (NRF1 and NRF2)** (Khan, Roberts, & Wu, 2019; Lopez-Mejia & Fajas, 2015). Fission involves the coordination of cytoplasmic, cytoskeletal, and organellar elements and consists of three key steps: (a) marking of a fission site, (b) assemblage of cytosolic **dynamin-related protein 1 (DRP1)** dimers and oligomers into a spiral-shaped superstructure around the marked fission site, and (c) GTP hydrolysis and DRP1 helix

constriction that separate the mitochondrion in two daughter mitochondria (Pernas & Scorrano, 2016).

b. **Mitochondrial fusion** is a mechanism to repair damaged mtDNA and mitochondria. Healthy mitochondria with wild-type (normal) mtDNA can fuse with abnormal mitochondria to compensate for defects by sharing components (Youle & Van Der Bliek, 2012). Because nucleoids do not seem to exchange DNA, mitochondria in heteroplasmic cells balance one another by sharing RNA or proteins (L. Yang et al., 2015). Fusion between mitochondria can also rescue two mitochondria with mutations in different genes by cross-complementation to one another, and it can alleviate the effects of environmental damage through the exchange of proteins and lipids with other mitochondria. Mitochondrial fusion can therefore maximize oxidative capacity in response to toxic stress, if the stress is under a critical threshold. In mammals, fusion between OMMs is facilitated by membrane-anchored dynamin family proteins named **Mfn1 and Mfn2**, whereas fusion between IMMs is facilitated by a single dynamin family protein called **Opa1** (Roy, Reddy, Iijima, & Sesaki, 2015).

c. **Mitophagy:** Mitochondria are very active organelles that are continuously being synthesized and recycled. This turnover promotes the preservation of an optimally operative pool of mitochondria. Some types of mitochondrial damage can trigger mitochondrial DNA destruction, either via direct degradation or through mitophagy. Mitochondrial membrane depolarization, increased ROS production or loss of DNA repair enzymes can increase mtDNA damage and mitophagy of dysfunctional mitochondrial fragments (Van Houten, Hunter, & Meyer, 2016). In the **PINK1-Parking mitophagy pathway**, non-functional mitochondria can suffer OMM proteins' covalent modification (i.e. phosphorylation of **Translocase of Outer Membrane (TOM)** components). Once the mitochondrion loses its membrane potential**, PINK1**, which is usually imported and degraded within the matrix, cannot pass in the matrix, and it stabilizes on the OMM where it recruits **Parkin,** which then ubiquitinates OMM proteins, and attracts the adapter protein **SQSTM1/p62** to the ubiquitin chains to flag the organelle for mitophagy by inducing contact with the phagophore's membrane (Hattori, Saiki, & Imai, 2014; Hood et al., 2019; Lionaki, Markaki, Palikaras, & Tavernarakis, 2015)

Chapter 2. Cellular damage and mitochondria

A clear understanding of cellular structure and physiology is essential to study its response to injury. The cell has an external boundary -the plasma membrane- and a cytosol functionally divided in interconnected compartments called organelles. The organelles' function depends in great manner on the chemistry of their membranes and intracellular matrix. Cell membranes and organelles are target of many types of etiologic agents (from biological, chemical or physical origin) and the cellular reaction to the pathogens' aggression is in the core of the physiopathology of many diseases.

1. Types of cellular stress or damage (Differences, exogenous, endogenous, UVR stress or damage)

Injury to tissues and organs starts at a cellular level. The development of structural alterations is preceded by biochemical modifications. In some cases, the biochemical modifications are the only detectable changes. Cell damage disrupts cellular homeostasis. There are thousands of etiologic agents, from internal or external origin, but all of them activate one or more of four final common biochemical mechanisms to cell damage: (1) ATP depletion, (2) cell membranes' permeabilization, (3) biochemical pathways' disruption and (4) DNA damage.

Etiologic agents: The more common etiologic agents of cellular injury are: (1) hypoxia, (2) physical agents (trauma, temperature extremes, radiant energy and electric shock), (3) biological agents (viruses, microbes, parasites), (4) nutritional imbalances, (5) genetic derangement, (6) chemical (and pharmacological) agents, (7) immunologic dysfunction and (8) aging.

a. **Hypoxia:** Hypoxia is the insufficient oxygen supply for the cell's metabolic needs and its value is different according to cell's metabolic rate. Hypoxia can be a consequence from insufficient oxygenation of blood as result of cardiac or respiratory failure, decrease of vascular perfusion (ischaemia), reduced O2 transport by erythrocytes (as in anaemia), or inhibition of cellular respiratory enzymes (i.e. cyanide toxicosis) which results in an adaptive response that comprises a temporary cell-cycle arrest and reduced respiratory chain activity energy availability (Qian, Gelens, & Bollen, 2019).
   Hypoxia is one of the most common and most significant causes of injury; indeed, it is habitually the final cause of cell injury (F. Xiao, Li, Wang, & Cao, 2019). Interestingly, hypoxia can be a physiological condition for mesenchymal stem cells. Hypoxia (as low as 1-5% Oxygen concentration) can stimulate the cell differentiation in mesenchymal cells and permits cell proliferation because this concentration is enough to warrant ATP synthesis by the stem cell's mitochondrial respiration at certain developmental stages. Additionally, while mitochondrial ROS production trigger replication factors expression under hypoxic conditions, ROS repression under the same conditions leads to preservation of stem cells quiescence and function (Ivanovic, 2009; Shang et al., 2019; Yoshida, Takahashi, Okita, Ichisaka, & Yamanaka, 2009).

b. **Physical agents**: trauma can damage cells directly by crushing or slashing or indirectly by blood supply interruption. Hyperthermia can hurt blood vessel (at low intensity) or denatures enzymes and proteins (Munir et al., 2019; Raff et al., 2019; Tenorio et al., 2019). Cold can affects blood supply by causing severe vascular constriction or literally

freezes cells, forming intracytoplasmic ice crystals that disrupt cell membranes (Gómez & Khan, 2019). Radiant energy (Ionizing and ultraviolet radiation) are the main forms of radiation triggering cellular damage. Radiant energy affects atoms and molecules, UV radiation can disrupt cellular bonds with the formation of reactive oxygen species (ROS) (X. Xiao, Huang, Fan, & Zuo, 2018). Ionizing radiation causes direct cell membrane or organelle damage or the production of ROS, that reacts with other cellular components, as DNA (Baselet, Sonveaux, Baatout, & Aerts, 2019). Electric shock generates heat as it passes through tissues, burning them. Additionally, electricity disrupts the nervous impulses in respiratory centres, cardiac conduction system or neuromuscular junctions (D. H. Lee, Desai, & Gauger, 2018).

c. **Biological agents**: biological agents range from prions, to microbes and to macroscopic parasites. The main difference with another etiologic agents is that biological agents can replicates once they gain access to cells and tissues (Westman, Hube, & Fairn, 2019). Biological agents can sequestrate DNA synthesis machinery (viruses), produce toxins (bacteria and fungi), bargain nutrients or produce inflammation and immune response (all of them).

d. **Nutritional imbalances**: Nutritional imbalances predispose the cell to damage. Animals can adapt to short-term dietary deficiencies in protein or calories through glycolysis, lipolysis, and catabolism of muscle protein. Nevertheless, long-term malnourishment leads to atrophy of cells and tissues. On the other hand, caloric excess can overload cells with glycogen and lipids and lead to obesity with metabolic disorders that predispose the obese animal to a variety of diseases, as arteriosclerosis (Hennig, Toborek, McClain, & Diana, 1996; Martins, 2018). Some dietary deficiencies of essential amino acids, fatty acids, vitamins, or minerals can lead to muscle degeneration, reduced growing, augmented vulnerability to infection, metabolic disorders, and many other diseases, depending on which nutritional factors are missing from or disproportionate in the diet (Zachary, 2017).

e. **Genetic instability**: selective reproduction and/or endogamy in closed populations can result in declined genetic diversity and increased prevalence of hereditary diseases as metabolic abnormalities, neoplasia, autoimmune diseases or increased susceptibility to infections. Both animals and humans, many of these inherited disorders show a familial phenotype, i.e. human Tay-Sachs disease (Solovyeva et al., 2018) or Dobermann Pincher dog's granulocyte deficiency.

f. **Chemical (and pharmacological) agents**: chemicals, drugs and toxins can change cellular homeostasis. The therapeutic effect of pharmacological agents is due to the modification of the homeostasis of certain groups of cells, within acceptable parameters. All chemicals must be considered toxins if they modify cells' homeostasis out of their limits without benefits. Chemicals disturb cells by binding receptors, inhibiting or inducing enzymes, altering metabolic pathways, increasing membrane permeability, producing free radicals, damaging chromosomes or cell's structural components (Chen, Patwari, & Liu, 2019; Ore & Olayinka, 2019; Tousson, El-Atrsh, Mansour, & Abdallah, 2019). The susceptibility of a cell to chemical injury depends on such factors as its mitotic rate and its ability to bind, take up, concentrate, or metabolize the chemical.

g. **Immunologic dysfunction:** immunologic dysfunction can produce cell damage indirectly, by allowing microbial colonization (immunodeficiency) or directly, through an excessive response (hyper-sensibility, i.e. allergies) to a foreign antigen or by attacking same organism's antigens (autoimmunity).

h. **Aging**: aging is related to the cumulative damage on cell's proteins, membrane lipids and DNA. Much of these damages are attributed to ROS production, DNA mutations and cellular senescence. Cumulated DNA damage predisposes to malignant cell transformation. Senescence is related with telomeres shortening, a process that occurs with each mitosis until cell stop dividing and die. In cell with terminal differentiation, as neurons and muscular fibres, there are not mitosis along the organism life's spam, but these types of cell cumulate lipofuscin and others metabolic wastes by a reduction in cellular exocytic capacity, contributing to cellular degeneration and death (Giorgi et al., 2018; D.-X. Tan, 2019; Woods, Khrapko, & Tilly, 2018).

2. Cellular response to stress and damage (regulatory cascade, p53 and others, cell-to-cell communication): Cells have a restricted range of responses to injury, depending on the cell type and the nature of the injury. These responses can be classified as (1) adaptation, (2) degeneration, or (3) death (Zachary, 2017). Adaptation and degeneration are considered reversible responses: A cell may adapt to a stimulus or sublethal injury positively, with increased efficiency or productivity (i.e. muscular hypertrophy under training) or suffer degeneration with reduced functional capacity. This means that a stressor doesn't necessarily cause damage or that the risk of adverse effects increases linearly as the total dose of the stressor. Indeed, a stressor can have a strong stimulatory effect on the cell at an appropriate dose and causes damage at other one. This cell behaviour is named **hormesis** and explain the not-linear cell response to many stimuli (Costantini, 2019). For instance, low to mild intensity muscular training cause muscular hypertrophy, a desirable adaptative effect for athletes and fitness' fans, but excessive training without appropriate rest can cause muscular necrosis, atrophy and myoglobinuria. On the other hand, opioids show an opposite type of hormesis: they are temporally strong analgesics at higher doses, before lost their analgesic (Dudekula, Arora, Callaerts-Vegh, & Bond, 2005). The response to injury can be reversible, with eventual restoration of the previous normal cellular structure and function (healing) or irreversible with evolution from degeneration to cell's death. Irreversible DNA damage can result in growth arrest (senescence), cell death, or cancer.

a. **Reversible cell damage**: The most important manifestations of cell damage are the metabolic disorders following intracellular accumulation of abnormal amounts of several substances. These intracellular accumulations occur as a result of not only sublethal injury sustained by cells but also normal (but inefficient) cell function. Two groups of substances can cause accumulations: (1) a normal cellular substance (such as water, protein, lipid, and carbohydrate excesses); or (2) an abnormal substance, either endogenous (such as a product of abnormal metabolism or synthesis) or exogenous (e.g., infectious agents or a mineral) (McCance & Huether, 2019). These products can accumulate momentarily or permanently and can be toxic or innocuous.

A primary cell response to injury is acute water accumulation or cell swelling (hydropic degeneration). In a general way, hypoxia, hypotonic environment and ATP synthesis reduction cause Na^+/ K^+-ATPase pump dysfunction, affecting the cellular capacity to conserve the appropriate membrane's electrochemical gradient and hydric equilibrium. Then, Na^+ cumulates in the cytosol and later into the membranous organelles as ER and mitochondria, with water movement from external to internal membranes' face and functional decay. The membranous organelles take a vesiculate shape and their internal cavity lacks density by reduction of metabolites' concentration. In the same way toxic substances can produce the same initial lesions if they interfere with Na^+/ K^+-ATPase pump or with respiratory chain (Coleman & Tsongalis, 2018). As seen previously, if these interferences with cellular homeostasis are sublethal or they have short duration, the cell recovers their homeostasis, producing enough ATP to maintain Na^+/ K^+-ATPase pump, expulse Na+ and water until reach the physiologic level and restore cell structure and function.

Metabolic disturbances as alcoholic toxicity or exceeding dietary calories can cause muscular and liver ***steatosis*** (intracellular fatty acids cumulation, or fatty degeneration). Dietary steatosis is related with cell need for storage exceeding caloric molecules, transforming all of them (carbohydrates, amino acids and fats) in intracytoplasmic fat droplets (Zachary, 2017). Normally, each cell can storage a certain quantity of fat droplets without metabolic disturbances. However, if dietary offer is excessive, fat droplets cumulate and merge, forming giant droplets, displacing and compressing organelles. In muscular fibres, this displacement can damage and atrophy the contractile apparatus. In liver, both dietary and hepatotoxic-derived chronic steatosis can lead a toxic and inflammatory response (steatohepatitis), with hepatic insufficiency, cirrhosis and neoplasia as probable terminal consequences. For example, alcohol consumption is the most frequent cause of steatohepatitis (**Alcoholic Liver Disease**) in humans (McCance & Huether, 2019).

The pathogenesis of ALD is not entirely characterized, and new studies reveal a main role of mitochondrial participation: ethanol is transformed in acetaldehyde at cytosolic level, with participation of cytochrome P450 (placed in smooth ER surface) (Guengerich, Wilkey, & Phan, 2019) and transported to mitochondrial matrix. Acetaldehyde is transformed in acetate and delivered to TCA cycle, fatty acids, ketone bodies and amino acids synthesis. Animal studies have shown that alcohol causes Cyt-P_{450} and mitochondrial DNA damage, lipid accumulation and oxidative stress, leading to steatohepatitis (McCance & Huether, 2019).

b. Irreversible cell damage and cell death: The cellular response to injury depends on the type of cell damaged, its vulnerability and/or resistance to hypoxia and direct membrane damage and the nature, severity, and extent of the injury. I.e. neurons, cardiac myocytes, endothelium, and kidney's proximal tubule epithelium are cells that are very vulnerable to hypoxia. Whereas fibroblasts, adipocytes, and other mesenchymal structural cells are fewer vulnerable. While many stresses and stimuli cause cell death, the type of cell death follows one of two pathways. The first is necrosis, a pathological term denoting to areas of dead cells within a tissue or organ. Necrosis is the result of an acute and usually deep metabolic disturbance, such as ischemia and severe toxicant-induced

damage, where cell death occurs with abrupt onset after adenosine triphosphate (ATP) depletion (Zachary, 2017).

Since necrosis as detected in tissue sections is a consequence rather than a process, the term **oncosis** describe the process leading to necrotic cell death. The second pattern is programmed cell death, most commonly manifested as apoptosis. In this case, specific stimuli start execution of well-defined process leading to orderly cells' resorption with slight escape of cellular components into the extracellular space and little inflammation. Unlike the abrupt necrosis' event, apoptosis can take hours to conclude and is an ATP-requiring process without an evident point of no return.

I. **Necrosis:** There are a common set of events that occur in necrotic cell death: failure of respiratory chain leads to ATP depletion, which causes cytoskeletal alterations and cellular ion transport disruption. These changes at molecular level cause cellular swelling and organelle vacuolization. Following, mitochondrial depolarization, lysosomal breakdown, bidirectional leakiness of the plasma membrane to organic anions, intracellular Ca^{2+} and pH dysregulation, and accelerated vacuoles formation with more rapid swelling occurs. Finally, the process culminates with the rupture of vacuolized organelles' membrane. Vacuoles' rupture leads to loss of metabolic intermediates, leakage of cytosolic and lysosomal enzymes, and collapse of all electrical and ion gradients across the membrane. This all-or-nothing collapse of the plasma membrane permeability barrier is long-lasting, irreversible, and incompatible with cell's life (Zachary, 2017).

II. **Apoptosis:** Apoptosis is a mechanism to regulate cell number and is vital throughout the life of all metazoans. Different to oncotic necrosis, in which the dying cell swells until it literally explodes, apoptotic cell death is a process of condensation, shrinkage and fragmentation followed by phagocytosis. As explained above, there are two apoptosis pathways, the intrinsic and the extrinsic pathway. While some different types of biochemical events have been recognized as important in apoptosis, maybe the core role belongs to the **caspases.** Remarkably, inhibition of caspases, rather than defending the cell from death, could readdress it from apoptosis to necrotic cell death. Their enzymatic properties are ruled by a dominant specificity for protein substrates containing Asp and using a Cysteine side chain for catalysing peptide bond cleavage. This specificity allows caspases to do a small number of peptide cleavages on a specific protein, normally only one, but this only cleavage can activate or deactivate (but don't degrade) this protein. Three caspases act as **initiators**: **caspase-8 and 10** in extrinsic pathway, **caspase-2** (activated by p53 following DNA damage) and **caspase-9** in the intrinsic (mitochondrial) pathway. Then, initiator caspases activate the apoptosis executioner **caspase-3, caspase-6, and caspase-7** (Steller, 2015).

In the apoptosis extrinsic pathway, Death Factors (DFs) as Tumoral Necrosis Factor, act as ligands binding to cell surface Death Receptors (DRs). Once activated by their ligands, DRs as **FAS** and **TRAILR** (TNF-related apoptosis-inducing ligand receptor) recruit and trigger its associated death domains (**FAD** and **TRADD**, respectively) and **procaspase-8** to form the cytoplasmic **DISC** (Reed & Green, 2011).

TRADD cooperates with FADD, which activates procaspase-8. Enough active caspase-8 then activates executioner caspases 3 and 7 to execute apoptosis. Caspase-8 can also cleaves Bid, a proapoptotic Bcl-2 protein, which translocates to mitochondria to activate intrinsic apoptosis. However, if caspase-8 activity is deficient, DR-mediated apoptosis can be increased by mitochondria, usually through Bcl-2 proteins, such as the proapoptotic **Bak** (Bcl-2 antagonist) and **Bax** (Bcl-2–associated X protein).

In the apoptosis intrinsic pathway, no ligation between DFs and DRs are required. The triggers are toxins as ROS, starvation, lacking growth factors or hormones and DNA damage that leads to stimulation of **p53-upregulated modulator of apoptosis (PUMA)** (Sung et al., 2018). Here, the key event is **MOMP** (a lethal **mitochondrial outer membrane permeabilization**), triggered by activation, posttranslational modification, and upregulation of proapoptotic BH3-only proteins as the mentioned PUMA. For instance, PUMA induce MOMP via oligomerization of Bax and Bak to form channels in the OMM (Beyfuss & Hood, 2018). This permeabilization of OMM releases cytochrome c from the intermembrane space into the cytosol. Cytochrome c promotes the assembly of the caspase-activating complex or apoptosome, which involves to **caspase-9** and **apoptotic protease activating factor 1** (**Apaf-1**) (Dorstyn et al., 2018). MOMP also releases to cytosol the **second mitochondrial activator of caspases** (**SMAC/Diablo**), as well as the catabolic hydrolases, **apoptosis inducing factor (AIF),** and **endonuclease G** (Thornton & Hagberg, 2015). In the execution phase of apoptosis, the activated executioner (caspases 3, 6 and 7) cleave cell proteins after Asp residues. In a similar way, **Granzyme B** from cytotoxic T lymphocytes and NK cells can also trigger apoptosis by activating caspase-3 and caspase-7 (Martinvalet, 2019). Executioner caspases cleave nuclear and cytoplasmic proteins, leading to disintegration of the nucleus, disruption of the cytoskeleton and cell fragmentation.

3. Mitochondria response to stress and damage (Adaptation, fission, fusion, senescence, apoptosis)

Most of the primary mediators of the stress response, including hormones (Glucocorticoids and catecholamines), immune factors (cytokines) and heat-shock proteins, exert numerous effects on mitochondrial response. This response is modulated in a proportional ratio with the energy needs of tissue affected (Herbers et al., 2019) and depending on the nature of the stressor (Klionsky et al., 2016; Manoli et al., 2007): (1) growing mitochondrial number by biogenesis and/or increasing their volume; (2) improving the expression and activity of oxidative phosphorylation (OXPHOS) subunits; (3) regulating the uncoupling of the respiratory chain, with consequent release of energy in the form of heat; (4) enabling signal transduction between mitochondria and nucleus and other intracellular organelles; (5) producing ROS for signalling or defence; and (6) inducing the apoptotic cascade. As explained previously, **difference between adaptation and injury** depends on the intensity and duration of stimuli.

a. As shown earlier, **mtDNA replication and mitochondrial fission** are the mechanism for mitochondrial proliferation that guarantees that growing and dividing cells hold an adequate number of mitochondria to sustain their cellular functions. Both in a physiological response and in a response to deleterious stimuli, these processes are stimulated by stressors that

increase the Ca^{2+} ER release and/or Ca^{2+} transmembrane influx from intercellular matrix to cytosol. As a result, **Mid49, Mid51, and Mff** are activated and they recruit **Drp1** to mitochondria (Youle & Van Der Bliek, 2012), whereas intracellular Ca2+ increase activates Drp1 through **calcineurin** (Baba, Shinmura, Tada, Amo, & Tsukamoto, 2019). Drp1 is a **cytosolic dynamin-related GTPase**, which is translocated to the mitochondrial outer membrane where itself assembles by using GTP as energy source. Drp1 is recruited from the cytosol to form spirals that include ER tubules and actomyosin filaments around a single mitochondria to constrict and separate it in two (Roy et al., 2015). On the other hand, stress-increased energy needs activate the **nuclear respiration factor 1** (**NRF-1**) that triggers a structural protein of mitochondrial nucleoid, the mitochondrial transcription factor A (**TFAM**) which, in conjunction with **nuclear-DNA polymerase γ (POLγA)** activate mtDNA replication (Herbers et al., 2019).

b. **Mitochondrial fusion** is essential for mixing of mitochondrial contents and preserving electrical conductivity throughout the mitochondria. When mtDNA is damaged (mutations, deletions, oxidation by ROS, or UV-induced pyrimidine-dimers formation, etc) or membrane integrity and electric potential are compromised, mitochondria respond by fusion between them. This process allows mtDNA reparation by recombination (cross-complementation) and through the exchange of lipids and proteins. Mitochondrial fusion is activated by **dynamin-related GTPases** (**Mfn1 and 2, Opa1**). Mfn1 and 2 have transmembrane domains fixed to OMM that tether OMM with other organelles' membrane, including other mitochondria. Normally, **Opa1** is involved in maintain cristae morphology and the assembly of the pro-apoptotic factor cytochrome c and other components of electron transport chain complexes. The function of Opa1 is controlled by the mitochondrial energetic status. In healthy cells, Opa1 is present in two main forms: **an IMM-anchored form, responsible for mitochondrial fusion at IMM level** and a soluble form in the intermembrane space. The soluble form is produced by proteolytic cleavage of Opa1 transmembrane domain by some IMM- located ATP-dependent proteases. Opa1 proteolysis is also optionally triggered by decreased mitochondrial function and membrane potential, that activate a metalloprotease**, Oma1**. Then, **Oma 1 cleaves Opa1 and mitochondria can become uncapable to perform fusion**.

c. In a final step, mixed mitochondria separate all the damaged components (mtDNA, matrix proteins and membranes) from the healthy or functional ones, by using the fission process, and then, the non-functional mitochondria can be eliminated by **autophagy**, as described previously.

d. **Cellular senescence** denotes a stress response targeting to preserve cellular homeostasis. The senescent cell is a stressed or damaged, yet viable, cell that has entered an irreversible, non-proliferative state while remaining metabolically active (Wiley et al., 2016). The first condition described to lead cellular senescence has telomeres' shortening (de Magalhães & Passos, 2018). Additionally, there are a wide range of other stimuli documented as main inducers of premature cellular senescence, such as **oxidative stress, activated oncogenes** (oncogene induced senescence/OIS), **failure to repair DNA damage** by irradiation or genotoxic drugs, **cell–cell fusion, epigenetic modifiers**, or **disturbed protein functions and regulation (proteostasis)** (Vasileiou et al., 2019).

There are two conflicting points of view about **mitochondrial dysfunction-associated senescence (MiDAS)**: on the one hand, dysregulation of mitochondrial homeostatic mechanisms, such as impaired mitochondrial biogenesis, metabolism and dynamics, **has arose as a hallmark of cellular senescence**. On the other, impaired mechanisms in mitochondrial function (**mitostasis**) have been suggested **to induce cellular senescence**. As in a vicious circle, senescent cells are characterized by increased production of reactive oxygen species (ROS), mainly attributed to dysfunctional OXPHOS. Mitochondrial ROS can exacerbate cellular senescence by increasing the mtDNA damage and its response signalling pathway (DDR) (Vasileiou et al., 2019). So, the greater the mtDNA damage, the greater the degree of OXPHOS dysfunction and greater ROS production.

In **MiDAS,** perturbation of mitochondrial homeostasis produces various results that act as triggers of senescence molecular axes involving the tumour suppressor **p53** and **Rb-p16^{INK4A}**. p53 acts as the starter, whereas Rb-p16^{INK4A} continue the cell-cycle arrest. Mitochondrial dysfunction includes a **decreased NAD$^+$: NADH** and/or **increased ADP: ATP or AMP: ATP** cytosolic ratios. These metabolic imbalances activate the energy sensor 5'AMP-activated protein kinase (**AMPK**). AMPK leads p53 activation, Rb-p16^{INK4A} stabilization and **transcriptional promotor NF-*κ*B** inhibition, leading to IL-1 inhibition, IL-10, TNF-α and CCL_{27} expression and **secretion**. This secretory phenotype drive to senescence acting as cytocrine and paracrine stimuli (Beyfuss & Hood, 2018; Wiley et al., 2016). Additionally, chronic upregulation of p53 is a major stimulus to intrinsic (mitochondrial) **apoptosis pathway**, as explained previously.

Chapter 3. Mitochondrial diseases

Mitochondrial dysfunction has been implicated in a variety of disorders, including primary mitochondrial diseases and other common conditions, such as diabetes, neurodegenerative diseases, cancer and aging-related diseases. The causes why mitochondria can become sub functional are varied. For example, the mitochondria of cardiac muscle, after an event of hypoxia and / or ischemia, undergo metabolic changes that persist after the oxygen supply to the myocardium has been re-established. Something similar occurs in tissues affected by inflammatory processes, even after the resolution of these processes (James D. McCully, Levitsky, del Nido, & Cowan, 2016; Wu et al., 2016). Acquired disorders involving mitochondria include Type II diabetes mellitus, some neurodegenerative diseases, atherosclerosis, osteoporosis, increased infections susceptibility and cancer. These disorders are described below.

In mammalian females, age correlates with the accumulation of damage in the mitochondrial DNA of the oocytes and even with the decrease in the number of mitochondria present in these cells, causing problems of decreased female fertility, due to the infeasibility of oocytes, impediment of embryo implantation or early embryonic death (Bentov, Yavorska, Esfandiari, Jurisicova, & Casper, 2011; Ferreira et al., 2016; Hammond et al., 2016; Shourbagy, Spikings, Freitas, & John, 2006). On the other hand, if a female with this condition has a live birth, the new-born will have a high probability of suffering diseases characterized by mitochondrial dysfunction, whether of reproductive or metabolic type.

Finally, both in domestic mammals and in humans, specific and inherited mutations in the mitochondrial genome have been identified, related to maternally inherited mitochondrial genetic defects or secondary mitochondrial disorders due to nuclear gene defects. A list of these disorders is included in table 1 (see below) (Claiborne, English, & Kahn, 2016; Levinger, Oestreich, Florentz, & Mörl, 2004; Park & Larsson, 2011; Seppet et al., 2009; Tachibana et al., 2009).

1. **Acquired dysfunction (Chronic diseases, description, types of treatment)**

   Nowadays, our comprehension of the aging process remains incomplete, but most gerontology's researchers would agree that aging starts with molecular damage, leading to cell, tissue and eventually organ dysfunction. According to this viewpoint, the inherent process of aging would create a platform upon which the age-related diseases appear (Payne & Chinnery, 2015). The best-known hypothesis to explicate aging is the free radical theory, which proposes a central role for mitochondria as the primary source of intracellular reactive oxygen species (ROS) that leads to mtDNA mutations (Sun, Youle, & Finkel, 2016). Acquired mtDNA mutations have been widely described in normal human aging, mostly in tissues with terminal differentiation such as muscular fibres and neurons, but also in cells with high mitotic rates such as enterocytes, and somatic mtDNA mutations are also well-described in age-associated neurodegenerative diseases (Giorgi et al., 2018).

Table 1.
Mitochondrial diseases and associated genes

Primary mitochondrial diseases due to mitochondrial gene defects

Maternally inherited diseases	**Genes affected**	**Observations**
Hypertrophic cardiomyopathy, neuropathy, ATP deficiency	MT-ATP8	
LHON	MT-ND1, MT-ND4, MT-NDL, MT-ND6, MT-CYTB	
Leigh syndrome	MT-ND2, MT-ND3	
Leigh syndrome (late onset)	MT-ATP6	
MELAS (MIDD)	MT-ND1, MTND5, MT tRNA (Leu (UUR))	
MELAS (atypical)	MT-RNR2	
MERFF	MT-ND1, MT-ND5, MT tRNA (Lys)	
Mitochondrial Myopathy	MT-CYTB, MTCO1, MTCO2, MTCO3, MT tRNA (Ala, Asn, Leu (CUN), Phe, Ser (AGY), Ser (UCN), Tyr)	
Myopathy and Diabetes	MT tRNA (Glu)	
NARP (MILS if homoplasmic)	MT-ATP6	
Reversible COX deficiency	MT tRNA (Glu)	
Sensorineural deafness	MT-RNR1, MT tRNA (Ser (UCN))	
Sporadic disorders		
Exercise intolerance	MT-CYTB	
Fatal infantile encephaopathy (Leigh-like syndrome	MT-ND3	
CPEO	mtDNA deletion/depletion	
Diabetes and deafness	mtDNA deletion	
KSS	mtDNA deletion/depletion	
Pearson syndrome	mtDNA deletion	
Secondary mitochondrial disorders due to nuclear gene defects		
adPEO	POLG1, POLG2, ANT1, TWINKLE, TK2, DGUOK, RRM2B, DNA2, MGME1, MPV17, OPA1	Autosomal dominant
arPEO	POLG1	Autosomal recessive
DOA/DOA "plus"	OPA1 Autosomal dominant or recessive, MFN2 Autosomal recessive	
MNGIE	TYMP	Autosomal recessive
POLG (including ANS, AHS, MCHS, MIRAS, SANDO, SCAE and MEMSA)	POLG1	Autosomal dominant or recessive

Disease acronyms: **ANS:** Ataxia neuropathy spectrum; **AHS:** Alpers-Huttenlocher syndrome; **adPEO**: autosomal-dominant Progressive External Ophthalmoplegia; **arPEO**: autosomal-recessive Progressive External Ophthalmoplegia; **CPEO**: Chronic Progressive External Ophthalmoplegia; **DOA**: Dominant Optic Atrophy; **KSS**: Kearns-Sayre Syndrome; **LHON**: Leber Hereditary Optic Neuropathy; **MCHS**: Childhood myocerebrohepatopathy spectrum; **MELAS**: Mitochondrial Encephalomyopathy with Lactic Acidosis and Stroke-like episodes; **MEMSA:** Myoclonic epilepsy Myopathy Sensory Ataxia; **MERRF**: Myoclonic Epilepsy with Ragged-Red Fibers; **MIDD**; Maternally inherited Deafness and Diabetes; **MILS:** Maternally inherited Leigh Syndrome; **MIRAS**: Mitochondrial Recessive Ataxia Syndrome **MNGIE**: Mitochondrial Neurogastrintestinal Encephalopathy; **NARP**: Neurogenic Muscle Weakness, Ataxia, and Retinitis Pigmentosa; **POLG:** Polymerase Gamma-related Disease. **SANDO**: Sensory Ataxia Neuropathy, Dysarthria, Ophthalmoplegia; **SCAE:** Spinocerebellar Ataxia with Epilepsy. Modified from (B. H. Cohen, Chinnery, & Copeland, 2018) and (R. L. Davis, Liang, & Sue, 2018)

Mitochondrial deterioration can show a wide range of dysfunctions, related with acquired diseases. Many of these diseases are aging-related or share its physiopathology with normal aging process. Between mitochondrial abnormalities, ROS production have been considered one of the major consequences of mitochondrial dysfunction, additionally, ATP deficiency, mutations in mtDNA, mitochondrial permeability transition pore (mPTP) opening, apoptosis, Ca2+ deregulation, inflammation, and altered fusion/fission dynamics are all mitochondrial features that, whereas not necessarily acting independently, become

disrupted in aging process and many related disorders as Type 2 diabetes mellitus, Parkinson's Disease, Alzheimer's Disease, Atherosclerosis, Increased infections susceptibility and cancer (Lane, Hilsabeck, & Rea, 2015).

a. **Type 2 Diabetes:** Diabetes mellitus (DM) is one of the most common metabolic diseases worldwide, and its prevalence has continued to rise in recent years. It was estimate that there are around 451 million people with DM (in 2017) and 4-5 million deaths worldwide each year (Cho et al., 2018; Roglic & Unwin, 2010). Whereas Type 1 DM is a low-prevalence autoimmune disease characterized by β-cell destruction and deficient insulin secretion, Type 2 DM (**T2DM**) is characterized by an impairment of insulin action and account almost the 90% of diabetes cases (Fujimaki & Kuwabara, 2017). T2DM includes insulin resistance in peripheral tissues and increased levels of blood glucose due to overnutrition accompanied by insulin secretion deficiency-related to β-cell exhaustion. T2DM is commonly related with the development of associated comorbidities, affecting muscular tissues, nervous system, liver, kidney and endocrine glands.

   For instance**,** in **skeletal muscle,** T2DM shows increased ROS production, with a reduction in mitochondrial biogenesis and content (Martin & McGee, 2014). Mitochondrial content reduction is the main cause of the muscular impaired energy metabolism (Lai, Kummitha, & Hoppel, 2017). Additionally, T2DM shows decreased mitochondrial fusion and increased mitophagy, that induces the accumulation of damaged mitochondria in skeletal muscle, leading to the disturbance of energy metabolism (Rieusset, 2015). **Neurons** doesn't depend of GLUT4 -the insulin-dependent glucose carrier- therefore, insulin resistance affects neurons indirectly. However, T2DM affects neurons by triggering mitochondrial dysfunction: reduced respiratory chain activity, increased neurons' ROS production, ER and mitochondrial calcium release to cytosol and apoptosis by intrinsic pathway. In fact, individuals with T2DM have twofold risk to develop dementia, the most common type of Alzheimer's Disease (**T2DM-related AD**) (Silzer & Phillips, 2018)

b. **Neurodegenerative diseases:** there are a list of neurodegenerative diseases with participation of mitochondrial dysfunction: Parkinson's disease (**PD**), Alzheimer's disease **(AD)**, Huntington´s disease, amyotrophic lateral sclerosis (**ALS**) and Friedreich's ataxia, among others. There are cumulative evidence that diseases as PD, stroke, ALS and AD are more common in some human groups, linking these diseases with specific mitochondrial haplogroups (Coskun et al., 2012; Keogh & Chinnery, 2015). Probably, these haplogroups have an increased predisposition to pre-mitochondrial aetiological factors.

   For example, the mitochondrial role in **Parkinson's disease** (**PD**) was found by studying the Parkinson-like toxic syndromes related with heroine consumption and paraquat or rotenone poisoning. The development of Parkinson-like toxic models -by using the mentioned toxins- revealed a reduction of complex I activity and a rise in ROS production leading the accumulation of mtDNA mutations in dopaminergic neurons at *substancia nigra* as direct effect of these toxins (Guillet-Pichon & Verny, 2016). Sporadic form of PD shows similar mitochondrial dysfunction at complex I, related with

a set of primary aetiological factors as neuronal autotoxicity and previous mitochondrial damages. Otherwise, in the **familial form of PD**, there are heritable mutations affecting a set of **nuclear DNA genes** as α-synuclein, LRRK2, Ubiquitin hydrolase and ligase 1, Parkin, DJ-1 and Phosphatase and Tensin homologue-induced kinase (PINK 1). These mutations affect a wide range of brain neurons, including dopaminergic and cholinergic ones (Das & Sharma, 2016; Villeneuve, Purnell, Boska, & Fox, 2016): PINK1, Parkin, LRRK2 and α-synuclein mutations alter mitochondrial ultrastructure and morphology, DJ-1 mutation is accompanied by mitochondrial fragmentation plus reduced mitochondrial membrane potential and the failure of ubiquitin-proteasomal system alters mitostasis by failing in the non-functional mitochondrial proteome elimination (Faustini et al., 2017; Rango & Bresolin, 2018).

A second illustration of mitochondrial dysfunction associated with neurodegenerative disease is **Alzheimer's Disease.** In **AD**, is widely recognized that intercellular Amiloid-β peptide (Aβ) cumulation -that leads amyloid plaque formation- and intraneuronal protein-Tau cumulation is responsible of neurofibrillary degeneration, which are the central lesions in AD. However, it is unclear if mitochondrial dysfunction is the cause or a consequence of these lesions (J. Wang & Chen, 2016). Nowadays, the MERCs/MAMs hypothesis for AD theorises that Mitochondrial-ER contacts (MERCs) may be the places at which all the AD-related mitochondrial dysfunctions converge: a proteomic data analysis revealed that Mitochondria Associated ER Membranes (MAMs) are enriched with compounds associated with many neurodegenerative diseases. This protein cumulation could induce MAMs' functional failure, leading to mitochondrial dysfunction (De Mario, Quintana-Cabrera, Martinvalet, & Giacomello, 2016; Naia et al., 2016; Paillusson et al., 2016).

c. Atherosclerosis: atherosclerosis is a chronic inflammatory disease of the vessel wall characterized by the chronic inflammatory cell infiltrate of macrophages. The macrophages' conversion into pathologic foam cells filled with lipidic vacuoles, suggests that the macrophage is exposed to a devastating load of lipid and cell debris that might paralyze any previous defensive role. Evidence indicates that atherosclerotic lesion development is intensely compromised if monocytes are prevented from entering the blood vessel wall and thus a pathologic contribution is indicated. Atherosclerosis starts with lipids as triglycerides and cholesterol are deposited under the vessels' intima, forming the atheromatous plaques. Theses plaques growth by the proliferation and cumulation of fibrous tissue and smooth muscle, protruding in the vessel's lumen and consequently reducing the vessel's diameter and the blood flow (Victor, Apostolova, Herance, Hernandez-Mijares, & Rocha, 2009; Zachary, 2017).

The lipids deposited under the intima bind to the proteoglycans of intercellular matrix and suffer spontaneous oxidation that provokes intima's inflammation and monocytes and lymphocytes infiltration. Then, macrophages phagocyte the oxidized lipids, but they are incapable to degrade these lipids into their phagosomes, which becomes vacuoles permanently stored into macrophages' cytosol (foam cells). On the other hand, T lymphocytes are activated by oxidized lipids acting as antigens. T

Lymphocytes release cytokines and inflammatory mediators, leading to vasculitis (Rafieian-Kopaei, Setorki, Doudi, Baradan, & Nasri, 2014).

Mitochondrial dysfunction and cardiovascular disease are closely related to primary cardiomyopathy, hypertension, atrial fibrillation, heart failure, ROS production, and atherosclerosis by regulating systemic metabolism inflammation, proliferation, and apoptosis. mtDNA damage arises before atherosclerotic lesions. Atherosclerotic fibrous cap and core area exhibit significant mitochondrial dysfunction characterized by a reduction in mtDNA copy number, oxygen consumption and reduced mtDNA-encoded subunit complexes I, III, IV, and V, which lead to abnormal proliferation of vascular smooth muscle cells (VSMCs) and augmented apoptosis and release of inflammatory factors. The mitochondrial dysfunction causes an opening of mitochondrial permeability transition pore (mPTP) and reduction of mitochondrial membrane potential. The continued opening of the mPTP leads to the swelling of mitochondrial matrix and rupture of OMM. This process results in the release of pro-apoptotic factors into the cytosol and cell apoptosis. Vessel's wall inflammation activates the dendritic cells, neutrophils, and endothelial cells through Toll-like receptor 9 **(TLR9**) signalling and aggravates atherosclerotic lesion. These phenomena promote the development of atherosclerosis and plaque vulnerability (J. Lee et al., 2009; Peng et al., 2019; Victor et al., 2009).

d. Osteoporosis: Osteoporosis is characterized by decreased bone mass and increased fracture susceptibility. Bone loss in elderly is the result of complex interactions between endocrine-induced metabolism reduction, changes in activity level, changes in nutrients intake, obesity and age-related disorders, including mitochondrial dysfunction (Greco, Pietschmann, & Migliaccio, 2019). Most of mentioned osteoporosis factors can act on bone metabolism and structure indirectly, through age-related muscular metabolism impairment (sarcopenia), where bone loss is understood as consequence of reduced muscular-bone physic and metabolic interactions that control bone remodelling. Also, direct effect of the mentioned factors can act on bone metabolism and structure. However, effective therapeutic strategies for preventing and treating osteoporosis are unavailable because of the limited understanding of mechanisms underlying this disease.

New evidence has confirmed that oxygen consumption rate and intracellular ATP content are significantly upregulated during osteoblastogenesis, indicating contribution of mitochondrial energy metabolism in bone stem cells' differentiation (Zheng, Sui, Qiu, Hu, & Jin, 2019). Mitochondrial role in osteoporosis is connected to oxidative stress (**OS**) that leads to osteoblasts apoptosis, osteoblastogenesis downregulation and bone remodelling impairment (Zheng et al., 2019). The mechanism involves an initial disturbance of mitochondrial fission and fusion mechanisms by OPA-1 ROS-induced alteration. However, it is unclear whether OPA1-mediated mitochondrial events regulate OS-induced osteoblast apoptosis (Cai et al., 2019). Recently, researchers have found that ROS activate **FoxOs** (maybe impairing FoxOS deactivation by sirtuins), causing **Wnt** signalling inhibition in bone stems cells, causing a reduction in osteoblastogenesis and osteoclastogenesis activation. FoxOs is a group of proteins that performs a key role in the cells' adaptation to a variety of

stressors such as oxidative stress and growth factor deprivation by promoting cell cycle arrest, DNA damage repair, autophagy, and free radicals cleaning (Almeida & Porter, 2019). Additionally, increased ROS production and ATP depletion following mitochondrial transcription factor A (**Tfam**) deficiency lead to increased osteoclast differentiation and activity (bone-resorbing) even with augmented apoptosis (Suh, Chon, Jung, & Choi, 2019).

e. **Increased infections susceptibility**: In elderly, increased prevalence and severity of infections is caused by a decline in bone marrow (BM) myelopoiesis and reduced leukocytes' activity. However, aging causes a paradoxical behaviour on immune system. On one hand, there are the mentioned impairment of defensive reaction to infections. On the other hand, an increased and diffuse inflammatory response and a rise of autoimmunity disorders can be observed.

Age-related impairment of mitochondrial function can impair immune response. Mitochondrial ROS production is a crucial antimicrobial function of macrophages, dendritic cells, and neutrophils (Pinegin, Vorobjeva, Pashenkov, & Chernyak, 2018). Aging is characteristically accompanied by reduced mitochondrial production of antimicrobial ROS and a simultaneous rise of phagocytes' oxidative stress. Reduction in phagocytes' activity impair antigen's presentation and lymphocyte activation. Additionally, elderly's phagocytes show a DNA release from damaged mitochondria to cytosol that is a main promoter of ROS production and inflammation and may consequently promote host immunosenescence by different mechanisms. (Jose, Bendickova, Kepak, Krenova, & Fric, 2017; Picca et al., 2019).

Some researchers explore the relation between a widespread asymptomatic β-herpesvirus HHV5 (**Cytomegalovirus, CMV**) infection with the mentioned inflammatory response ("inflamm-aging") (Franceschi, Garagnani, Parini, Giuliani, & Santoro, 2018; Pawelec & Gupta, 2019). However, the possibility of mitochondrial origin of inflamm-aging remains on the table since mitochondrial "danger signals" released by cells during stress, apoptosis or necrosis (e.g. cardiolipin, n-formyl peptides (e.g., fMet), TFAM, ATP, mitochondrial ROS and mtDNA) can be recognized **as mitochondrial damage associated molecular patterns (mtDAMPs)** by phagocyte's **pattern recognition receptors (PRRs)** as Toll-like receptors (TLR) and others. Mitochondrial release of DAMPs may induce inflammation via recognition by PRRs that leads to caspase-1 and **NLRP3** inflammasome activation that leads the release of pro-inflammatory cytokines (McGuire, 2019). Other factor to be considered is that an **unusual proinflammatory macrophage's phenotype (M2 type)** and its cumulation into tissues can be triggered by age-related macrophage's endoplasmic reticulum stress and mitochondrial dysfunction (van Beek, Van den Bossche, Mastroberardino, de Winther, & Leenen, 2019)

f. **Cancer**: Most of cancer cells have somatic mutations in mtDNA and/or the mtDNA depletion, leading to mitochondrial dysfunction. Mitochondrial dysfunction is associated with aggressive malignancy and poor prognosis in some types of cancer (Fakouri, Hansen, Desler, Anugula, & Rasmussen, 2019). Reduced mtDNA content is equally detected in tumour-initiating cells. Despite mutations in mitochondrial genes

are common in cancer cells, they don't eliminate entirely the mitochondrial energy metabolism and functionality. Instead, they promote redirecting of cancer cell's bioenergetics and biosynthetic phenotype through a mitochondria-to-nucleus signalling triggered by "dysfunctional" mitochondria (Chakrabarty et al., 2018) that changes transcription and/or activity of cancer-related genes and signalling pathways. Different cancer cell types may undergo different bioenergetic changes, some to more glycolytic and some to more oxidative. These different metabolic profiles may coincide within the same tumour mass (intratumor heterogeneity), including some similar to mesenchymal stem cells' profiles that can explain tumour chemoresistance (Aponte & Caicedo, 2017; Guerra, Arbini, & Moro, 2017).

Cancer cells reinforce their metabolism to promote growth, survival, proliferation, and long-term preservation. The common characteristic of this reformed metabolism is the improved glucose uptake and a preference for lactic glycolysis. This behaviour is detected even in the presence of adequate oxygen concentration and totally operational mitochondria and, together, is known as the 'Warburg Effect' (Liberti & Locasale, 2016). Recently, some studies showed that the Warburg Effect is required for tumour growth. In one hand, mesenchymal stem cells-derived stanniocalcin-1 (**STC1)** promotes survival of cancer cells by uncoupling oxidative phosphorylation, reducing intracellular ROS, and shifting metabolism towards a more glycolytic metabolic profile. Additionally, it has been exposed that neither hypoxia nor OXPHOS defects imply a stopping of mitochondrial metabolism, and in any case the tricarboxylic acid (TCA) cycle can alter metabolic fluxes to promote a glutamine dependent biosynthetic pathway that sustains tumour progression. Hence, the TCA cycle represents a metabolic pivot that drives substrate use upon changes in resources accessibility (Vatrinet et al., 2017). These changes can promote cell survival by regulating mitochondrial respiration and avoiding ROS-induced apoptosis (Ohkouchi et al., 2012).

g. Subfertility: in many species, ovarian (female reproductive) aging and subfertility arise long before animals are considered chronologically aged. Because the ovaries suffer this relatively early aging process, maternal aging is a main cause of subfertility in women and domestic animal females. The following features are common in ovarian aging:

- the ovarian oocyte pool remains quiescent **since the foetal life until their recruitment in a menstrual or estral cycle**. In women, this recruitment can be delayed by more than 30 years, augmenting the aging consequences (Albu & Albu, 2017). In domestic animals, specially murine, bovine and porcine models, the species' metabolic rate and/or the pressure for zootechnic yields causing obesity and metabolic imbalance accelerate the animals' ovarian aging rate (Aardema, van Tol, & Vos, 2019; D'Occhio, Baruselli, & Campanile, 2019; Iwata et al., 2011; Marei, Raemdonck, Baggerman, Bols, & Leroy, 2019).

- During the quiescent period, oocyte's DNA (including mtDNA) and cytoplasmic structures are very vulnerable to damage by radiation, ROS, toxic compounds and more. Chromosomal aberrations are recurrently found in human gametes (21% of

oocytes and 9% of spermatozoa), with a predominance of aneuploidies in oocytes, while structural chromosomal abnormalities predominate in sperm cells. Chromosomes 21, 22, and 16 are frequently overrepresented in aneuploid gametes (Albu & Albu, 2017). The influence of age on ovary is owed to both reduced number and quality of the oocytes, consequence of the high rate of chromosomal aneuploidy in the embryo and mitochondria dysfunction.

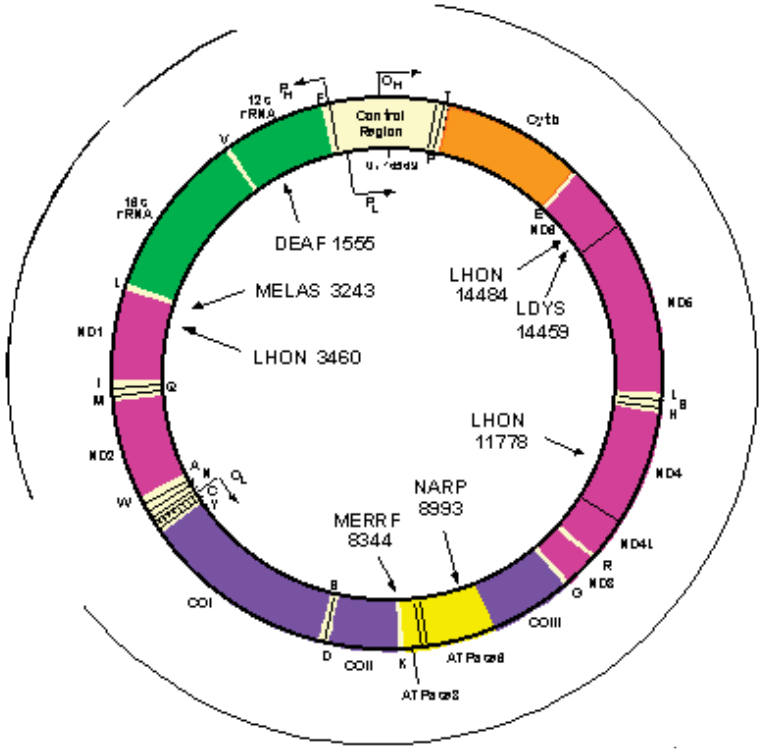
- **mtDNA damage** plays a main role in reduced oocyte and embryo quality and developmental capacity because it lacks repair systems, including the opportunity of recombination with a healthy and fresh mtDNA pool from the sperm after fertilization. In most animal species, sperm's mitochondria are ubiquitinated and destroyed by autophagy before the zygote's first division. In humans, the sperm's elimination mechanism remains poorly understood, but its existence is worldwide accepted and human paternal mtDNA inheritance is a very polemical find and reason for immediate report and debate in the literature (Balciuniene & Balciunas, 2019; Luo et al., 2018; McWilliams & Suomalainen, 2019; Rius et al., 2019; Salas, Schönherr, Bandelt, Gómez-Carballa, & Weissensteiner, 2019).

Aged ovaries show an oocyte's lesser response to normal pituitary's stimuli, leading to a reduction in oocyte recruitment. Additionally, oocytes from aged ovaries show cytoplasm and mitochondrial structure's alteration and reduced mitochondrial and mtDNA amount (Bianchi et al., 2015; Kushnir et al., 2012). Age-related cumulative mitochondrial damage may be attributed to severe ATP synthesis reduction and oxidative stress generated by ROS from the mitochondria's own basal metabolism, affecting preovulatory oocyte's mitochondrial biogenesis, and postovulatory calcium homeostasis and ATP availability (Babayev et al., 2016; Roth, 2018; Simsek-Duran et al., 2013). These alterations reduce oocyte's fertilization rate, embryo development and implantation success (Mohammadzadeh, Fesahat, Khoradmehr, & Khalili, 2018; Payton et al., 2018; T. Wang, Zhang, Jiang, & Seli, 2017; Woods et al., 2018)

2. Mitochondrial Hereditary diseases (Description, Types of treatment)

Mitochondria contain their own DNA, inherited by matrilineal route (Liu et al., 2014), with its own genetic code that encodes 13 components of electron transport chain (ND1 to ND6 subunits of ETC complex I; COX1 to COX3 subunits of ETC complex IV; subunit 6 and 8 of mitochondrial ATPase (complex V) and ETC complex III), 2 ribosomal RNAs and 22 transfer RNAs (Mishra & Chan, 2014). The histone proteins can protect the nuclear genome from various types of DNA damaging agents, but mitochondrial genome lacks them, hereafter, mitochondrial DNA is more susceptible to cumulate damage than nuclear DNA. The mitochondrial DNA is exposed to various kinds of endogenous and exogenous DNA damaging agents, some of which can lead to various kinds of diseases including of neuro degenerative form, cancer, cardiomyopathy, diabetes and several aging-related disorders.

Low frequency deletion zone



High frequency deletion zone

Figure 5: Map of Human Mitochondrial DNA and main mutations and deletions causing inherited mitochondrial diseases. Descriptions in the text ("WebHome < MITOMAP < Foswiki," n.d.)

The fact that mitochondria are inherited only through the mother's way means that mitochondrial DNA does not benefit from the possibility of genetic recombination that the nuclear genome possesses thanks to sexual reproduction. Animal cells depend on the mitochondria because they only possess the enzymes required to perform oxidative phosphorylation, so the damage or mitochondrial DNA mutation (mtDNA) translates into the probability that the cells cannot maintain their balance bioenergetic, and in the appearance of clinical signs based on tissue dysfunction with high energy consumption (Mishra & Chan, 2014), such as muscle tissue, neurons and many tissues with endocrine function.

The developing eggs in the female embryo go through an "mtDNA bottleneck", in which the number of mtDNA copies is first reduced to less than 50 copies and then amplified to more than 100,000 copies (Marlow, 2017; Shoubridge & Wai, 2007; Woods et al., 2018). Therefore, unpredictable quantities of mutant and normal mtDNA are present in the mature eggs of an individual woman, and, therefore, in the cells of her offspring. This phenomenon influences the severity of diseases caused by mtDNA mutations and can lead to very different manifestations between individuals from the same family.

Mitochondrial diseases are one of the most common forms of inherited metabolic syndrome, which show unique variability in clinical presentation and can manifest at any age in any organ. Even with dramatic advances in the genetic and metabolic diagnosis of these severe progressive diseases, there are still no curative treatments (Suomalainen & Battersby, 2018). Mitochondrial diseases are a heterogeneous group of disorders that results of dysfunction of the mitochondrial respiratory chain. They have a very variable spam of clinical presentations and

they can be triggered by mutation both nuclear and mitochondrial DNA (mtDNA). Whereas some mitochondrial disorders only disturb a single organ (e.g., the ear in in mitochondrial deafness), most involve multiple organ systems, frequently accompanied with by manifest neurologic and myopathic symptoms. Mitochondrial disorders may present at any age. **We focused this work only on mtDNA-related diseases.**

Many persons with a mtDNA mutation show a set of clinical features that are grouped within a distinct clinical syndrome, such as mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (**MELAS**), the Kearns-Sayre syndrome (**KSS**), chronic progressive external ophthalmoplegia (**cPEO**), myoclonic epilepsy with ragged-red fibers (**MERRF**) and neurogenic weakness with ataxia and retinitis pigmentosa (**NARP**). However, there are a substantial clinical variability, and many individuals do not enclose precisely into one of the syndromes already mentioned, which is demonstrated by the overlapping range of disease phenotypes resulting from mutation of the nuclear gene *POLG*, which has arisen as a major cause of mitochondrial disease (Chinnery, 2014) in MIRAS and Leigh´s Syndrome.

Common clinical signs and symptoms of mitochondrial disease include external ophthalmoplegia, ptosis, proximal myopathy and exercise intolerance, cardiomyopathy, sensorineural deafness, optic atrophy, pigmentary retinopathy, and diabetes mellitus. Common central nervous system features are fluctuating encephalopathy, seizures, dementia, migraine, stroke-like episodes, ataxia, and spasticity. A high incidence of mid- and late pregnancy loss is a common occurrence that often goes unrecognized. These features can be present in nuclear or mitochondria- encoded diseases, but the mitochondrial forms are more frequent. Nuclear gene defects may be inherited in a recessive or dominant autosomal manner. Mitochondrial DNA defects are inherited in a maternal way only (Chinnery, 2014).

a. **MELAS: Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes**, better known as **MELAS,** is a mitochondrial disease linked-in most the cases- to a point mutation in the **MT-TL 1** gene -known as **m.3243A>G mutation-** on position 3243 of the mtDNA, where an adenine (A) is substituted with a guanine (G). This gene codes for **mitochondrial tRNA^{Leu}** (Esterhuizen et al., 2019; Meseguer et al., 2019). The mutation alters protein synthesis at Complexes I, III, IV and V of the oxidative phosphorylation (OXPHOS) system. MELAS is a multisystem disorder and its patients develop symptoms between ages two and 40 years. Most frequent symptoms include stroke-like episodes, encephalopathy with seizures and/or dementia, muscle weakness and exercise intolerance, repeated headaches, repeated vomiting, hearing loss, peripheral neuropathy, learning disability, and short stature. Lactic acidaemia is very frequent and show ragged red fibres are typically found in muscle histopathologic samples (El-Hattab, Almannai, & Scaglia, 2018).

b. MERRF: Myoclonic Epilepsy with Ragged Red Fibers is another multisystem mitocho0ndrial disorder characterised by myoclonus, combined to epilepsy, ataxia, weakness and dementia. MERRF manifestations appear in childhood. As in MELAS, common findings include ragged red fibres, hearing loss and short stature. Additionally, optic atrophy, cardiomyopathy with Wolff-Parkinson-White (WPW) syndrome and pigmentary retinopathy and lipomatosis are occasionally observed (DiMauro & Hirano, 2015). The MERRF's most commonly associated mitochondrial mutation (80% of the cases) affects **the gene MT-TK encoding tRNA^{Lys}**, by presenting an A-to-G transition at nucleotide 8344 **(m.8344A>G**) which is associated with severe defects in protein

synthesis, leading to fragmentation of mitochondria -correlated with altered processing of OPA1- and impaired OXPHOS (Chuang et al., 2017)**.** Others pathogenic variations have also been reported in a subgroup of persons with MERRF.

c. **LHON: Leber hereditary optic neuropathy** develops during young adult life, around 15-35 years old. This disorder affects four times more men than women. It is characterised a sudden bilateral subacute failure of the central visual field. in 75% of the cases, an eye is affected first, followed by the other in a period of two or three months. In the rest of the cases, both eyes are affected simultaneously. Most patients qualify for registration as legally blind (visual acuity ≤20/200). Neurologic syndromes such as postural tremor, peripheral neuropathy, myopathy, and movement disorders are more common in individuals with LHON than in the general population. Some persons with LHON, usually women, may also develop a multiple sclerosis-like disease (Yu-Wai-Man & Chinnery, 2016). LHON is caused by one of three possible mutations that affect Complex 1 ND1, 4 or 6 subunits synthesis: **m.3460G>A in MT-ND1, m.11778G>A in MT-ND4, or m.14484T>C in MT-ND6** (Wong et al., 2017; Yu-Wai-Man & Chinnery, 2016).

d. **Maternally-inherited Leigh's syndrome** (**MILS**) and **Neurogenic Muscle Weakness, Ataxia, and Retinitis Pigmentosa** (**NARP**) are part of a range of progressive neurodegenerative disorders caused by anomalies of mitochondrial energy generation**. MILS** is a subacute necrotizing encephalomyelopathy characterized by typical symptoms beginning of between ages three and 12 months, habitually subsequent to a viral infection. Mitochondrial most common variant (**m.8993 T>G)** affects mtDNA that encodes for the ATP6 subunit of complex V (mtATP6 gene) (Guy & Yuan, 2019). Another variants include **m.8993 T>C, m.9035 T>C, m.9176 T>C and m.9185 T>C** mutations in the same mtATP6 gene (Ng et al., 2019).

In MILS, decompensation with elevated lactate levels in blood and/or cerebrospinal fluid during an intercurrent infection is characteristically related to psychomotor delay or deterioration. Neurologic features include hypotonia, spasticity, movement disorders (including chorea), cerebellar ataxia, and peripheral neuropathy. Extra neurologic features may include hypertrophic cardiomyopathy. About 50% of affected individuals die by age three years, most often as a result of respiratory or cardiac failure (Thorburn, Rahman, & Rahman, 2017).

NARP is characterized by proximal neurogenic muscle weakness with sensory neuropathy, ataxia, and pigmentary retinopathy. Onset of symptoms, particularly ataxia and learning difficulties, is often in early childhood. Individuals with NARP can be relatively stable for many years, but may suffer episodic deterioration, often in association with viral illnesses. Most cases of NARP are caused by mutations in the same genes related to MILS**:** mtATP6 (**m.8993 T>G,**) or mtND6 genes (**m.14484T>C, m.14459G>A and m.14487T>C**) (Su et al., 2019; Thorburn et al., 2017)

e. **Pearson syndrome (PS):** this is a large-scale single mtDNA deletion syndrome, along with Kearns–Sayre syndrome and chronic progressive external ophthalmoplegia. In PS first cases reported, specific probes showed that the deletions spanned the genes coding for **4 subunits of NADH dehydrogenase, 1 subunit of cytochrome oxidase, and**

1 subunit of ATPase. The deletion spanned 4,977 bp (Rotig et al., 1989). Other cases range single mtDNA deletions between 3.5 to 8 kb (Jacobs et al., 2004).

PS is a fatal syndrome that manifests bone marrow failure, recurrent sideroblastic anaemia, and inconstant exocrine pancreatic insufficiency with organ's fibrotic atrophy and steatorrhea. PS features are variable and progressive. Anaemia typically starts into the first year of life and can be accompanied by pancytopenia and multisystem participation, including failure to thrive, hypotonia, and metabolic imbalances including lactic acidosis. Other features may include hydrops fetalis, hepatic failure with elevated transaminases and steatosis, microcephaly, renal Fanconi syndrome, endocrine hyposecretion (growth hormone deficiency, hypothyroidism, hypoparathyroidism, diabetes mellitus, and adrenal insufficiency), splenic atrophy, impaired cardiac function, refractory diarrhoea related to chronic colitis, and acute metabolic decompensations during intercurrent sickness. Death may happen in early childhood due to metabolic decompensation, liver failure, or sepsis due to neutropenia. Survival and spontaneous recovery from bone marrow dysfunction after several years is possible, with a transition to clinical manifestations of KSS (Goldstein & Falk, 2019).

f. **Kearns–Sayre syndrome:** KSS is usually due to a single mtDNA deletion of 4,977 bp known as m.8470_13446del4977 -involving lack of several mitochondrial genes -e.g. ATPase subunits, COIII, Complex I subunits and some mitochondrial tRNAs- (Goldstein & Falk, 2019; Saldaña-Martínez et al., 2019). Though KSS is due to a single mtDNA deletion in the majority of the cases, there are a number of cases in which KSS is due to mtDNA point mutations, such as **m.3249G>A, m.3255G>A or m.3243A>G**, all of them in the $tRNA^{Leu}$ gene (Finsterer, 2018). Patients present a core set of symptoms as **weakness, onset <20 years old, myopathy, ptosis, ophthalmoplegia and retinal pigmentary abnormalities as main symptoms**, frequently accompanied by hearing loss, elevated CSF protein, endocrine abnormalities and short stature (Finsterer, 2019). Cardiac conduction defects may be present or develop later. Diagnosis is easily made by recognizing the patient's phenotype and screening for mtDNA deletions in leukocytes. Early recognition and diagnosis are beneficial to allow for symptomatic management and routine cardiac monitoring (Goldstein & Falk, 2019).

g. **CPEO: Chronic Progressive External Ophthalmoplegia** is the third syndrome caused mainly by the same large-scale mtDNA depletion that KSS, involving striated muscles only. However, numerous pathogenic variants in either mtDNA or nuclear genes also cause PEO that is often associated with other clinical manifestations, suggesting an underlying nuclear gene aetiology (Montano et al., 2019; Nogueira et al., 2014). cPEO is characterized by ptosis, impaired ocular movements due to paralysis of the extraocular muscles (ophthalmoplegia), oropharyngeal weakness, and intermittent severe proximal limb weakness with exercise intolerance (Goldstein & Falk, 2019).

Treatments: MELAS's treatment lacks a specific consensus approach. Symptomatic management can include cochlear implants for sensorineural hearing loss, anticonvulsants, standard analgesics, dietary modifications and hypoglycaemic agents for diabetes, regular exercise and psychiatric treatments. Some dietary supplementations as antioxidants, L-carnitine and coenzyme Q10 (CoQ10) can improve mitochondrial dysfunction. Intravenous l-arginine

and/or citrulline infusion during stroke-like episodes helps to recovery and prevention of these manifestations by improving blood supply. Medications that affect negatively mitochondrial function as anticonvulsant valproic acid must be avoided (A. W. El-Hattab, Adesina, Jones, & Scaglia, 2015). No cure exists for MERRF syndrome. As in MELAS, treatment with antiepileptic drugs, regular exercise, high doses of CoQ10, L-carnitine, and vitamins' composite has been tried to recover mitochondrial function and reduce mitochondria-generated oxidative stress; however, success has been limited (Chang et al., 2013; DiMauro & Hirano, 2015). In the same way, treatment of MILS, NARP, KSS and CPEO are all symptomatic: endocrine replacement, prisms and lenses to correct ptosis, acidosis control with sodium bicarbonate, anticonvulsants, physical therapy, cardiac pacemakers, etc.

Nowadays, only LHON treatment with **idebenone** has been approved by the European Medicine Agency**.** This compound is a synthetic, short-chain analogue of ubiquinone, which is responsible for transporting electrons from complexes I and II directly to complex III. Also, idebenone shows a better bioavailability than CoQ10 at mitochondrial matrix. Other neuroprotective agents, improving of mitochondrial biogenesis, near-infrared therapy and more approaches are being studied (Jurkute, Harvey, & Yu-Wai-Man, 2019)

Chapter 4. Artificial Mitochondrial Transfer / Transplant (AMT/T)

1. From cell to cell communication to the AMT/T

Cell-to-cell communication is an essential process for the development and maintenance of multicellular organisms. Various mechanisms for the exchange of molecular information between cells have been reported, e.g. ligands secretion, synapsis, cytosolic components transfer through gap junctions or exosomes (Gerdes, Bukoreshtliev, & Barroso, 2007). Among them, **Horizontal** (cell-to-cell) **mitochondrial transfer** (**HMT**) between mammalian cells was first observed some decades ago (Clark & Shay, 1982). This find has inspired new concepts about mitochondria and mtDNA segregation and inheritance. Different modes of HMT have been reported under various pathophysiological conditions. However, the basis for cellular selection of a HMT mode remains unclear (Paliwal, Chaudhuri, Agrawal, & Mohanty, 2018).

HMT promotes the integration of mitochondria into the original mitochondrial network of recipient cells, contributing to changes in the bioenergetic competence and in other functional properties of them, not only *in vitro* but also *in vivo* (Berridge, McConnell, et al., 2016). Additionally, HMT comprises also the transfer of mitochondrial genes, which has significant consequences in the physiopathology of mitochondrial dysfunction. The cell-to-cell transfer of mitochondria or their components, may also result in the initiation of stem cell differentiation, reprogramming of differentiated cells, or stimulation of inflammatory signalling pathways. Various mechanisms facilitate HMT, including tunnelling nanotubes, exosomes, microvesicles, mitochondrial release or cytoplasmic fusion, among others (Sinha, Islam, Bhattacharya, & Bhattacharya, 2016a; Torralba, Baixauli, & Sánchez-Madrid, 2016). These finds have opened new research lines for the treatment of mitochondrial related disorders by healthy mitochondrial transfer.

a. **HMT by cellular contacts: TNTs** were first reported as large, ultrafine intercellular structures derived from filopodia-like protrusions, connecting cultured cells between them and forming complex cellular networks (Rustom, 2004). TNTs have a diameter of 50 to 1500 nm and an average length of 30 to 140 µm. TNTs possess a prominent F-actin pack crossing the whole length of the nanotube and, in case of the thick ones, cytokeratin and microtubules. Through TNTs, cells can share cytosolic components, endosomes and mitochondria (Gerdes et al., 2007). TNTs had probed to an important communication way between mesenchymal stem cells (MSC), immune cells and cancer cells (Jorgensen, 2019). HMT by nanotubes was probed to be capable for restore aerobic respiration (Ahmad et al., 2014; Spees, Olson, Whitney, & Prockop, 2006), improve phagocytic activity (Jackson et al., 2016), prevent inflammation damage and apoptosis (X. Li et al., 2014a; X. Wang & Gerdes, 2015) and improve chemoresistance in tumoral cells (Pasquier et al., 2013). **Gap junctional channels** had been reported as a HMT way in an acute lung damage model (Islam et al., 2012a)**,** but more research is necessary to stablish if this is a main way.

b. **HMT through exosomes and microvesicles**: Cells release diverse types of membrane vesicles of endosomal (exosomes) or plasma membrane origin (microvesicles) into the extracellular environment. These extracellular vesicles (**EVs**) represent a significant method of intercellular communication by serving as vehicles for transfer of membrane and

cytosolic proteins, lipids, and RNA between cells. The **exosomes** are released by a diversity of cells in vivo or in vitro conditions and this term is exclusive for vesicles released from cytosolic multivesicular bodies, whereas microvesicles are shed from plasma membrane (Raposo & Stoorvogel, 2013). These EVs contain a wide range of bioactive molecules, including membrane receptors, adhesion proteins, cytokines, chemokines, growth factors, and even different RNA species, including functional mRNA and miRNAs (Sluijter, Verhage, Deddens, van den Akker, & Doevendans, 2014). EVs transfer is involved in cell differentiation, paracrine regulation, disease signalling and progression and inflammatory modulation. EVs related HMT had been observed in response to acute lung injury models (Islam et al., 2012a), also in astrocyte response to neuronal damage (Berridge, Schneider, & McConnell, 2016)

c. **Internalization of isolated mitochondria in HMT**: some studies have found that stress signals released by damaged or inflamed cells, including damaged mitochondria, released mtDNA and mitochondrial metabolites as ROS, are categorized as damage associated molecular patterns (DAMPs) by surrounding mesenchymal cells (MSCs) and immune system cells (Unuma, Aki, Funakoshi, Hashimoto, & Uemura, 2015). mtDNA released by damaged cells are internalized by MSCs that then triggers the cytoprotective function of MSCs and improved mitochondrial biogenesis through retrograde signalling, thus preparing MSCs for HMT (Hayakawa et al., 2016) (Moschoi et al., 2016) (Tomoya Kitani, Kami, Matoba, & Gojo, 2014). Although some reports of cellular competence for mitochondrial internalization by macropinocytosis was reported (Kitani et al., 2014), but there is not enough evidence that this is a frequent process (Rogers & Bhattacharya, 2013)

2. History and development of the Artificial Mitochondrial Transfer/Transplant techniques

As seen before, many diseases, tissue damage, and aging challenge the cell and its mitochondrion, thus disturbing their integrity, function, and homeostasis (Lane et al., 2015; Sinha et al., 2016a). Cells naturally have the capacity to exchange intracellular components and especially mitochondria through different processes such as cell-to-cell contact, microvesicles, nanotubular structures, and other mechanisms (Islam et al., 2012a; Rustom, 2004; Spees et al., 2006). Clark and Shay initiated the artificial mitochondria transfer (AMT), which involved transferring mitochondria with antibiotic-resistant genes into sensitive cells, thereby enabling them to survive in a selective medium (Clark & Shay, 1982) and opening this new field of research. Subsequently to this work, the development of artificial transfer has and continues to mimic aspects of naturally occurring cell transport, especially in the mechanisms cells naturally use to rescue other damaged cells.

We will ponder key advances needed to expand the current knowledge about the artificial transfer of mitochondria and how these techniques could be used therapeutically. We will provide an overview of the features of the mitochondrial structure that are important in maintaining its integrity throughout artificial transfer (Huang et al., 2016; Onfelt et al., 2006; Sluijter et al., 2014). Currently, there is a certain probability of achieving repair of mitochondrial dysfunction in mature human tissues that recover from different forms of cell damage (Islam et al., 2012; McCully et al., 2016) by transferring mitochondria obtained from the patient himself, from a human donor or even from another species. Pharmacological alternatives for the repair of dysfunctional mitochondria are also studied (Andreux et al., 2013). In the reproductive area,

diverse methods for the replacement of dysfunctional mitochondria by healthy mitochondria from a donor are studied (Wolf, Mitalipov, & Mitalipov, 2015a). These techniques include: the transfer of the nucleus of the dysfunctional oocyte to a healthy enucleated oocyte (Tachibana et al., 2009), the transfer of cytoplasm rich in mitochondria from a healthy oocyte to a dysfunctional oocyte (Wu et al., 2016) and the direct transfer of purified mitochondria, taken from any tissue from a healthy donor to a dysfunctional cell (Andrés Caicedo et al., 2015).

a. Non- invasive AMT/T *in vitro*: In vitro AMT/T have a range of methodological approaches, from isolated mitochondrial transfer until cell-to-cell mitochondrial transfer. The first and simplest approach is the **Mitochondrial Transformation (MT)**, developed by Clark and Shay in 1982. In this research, they used the antibiotics chloramphenicol (CAP) and efrapeptin (EF), which inhibit the mitochondria's protein synthesis and ATPase function to kill sensitive mammalian cells. They demonstrated that purified mitochondria obtained from **(CAP/EF)-resistant cells** are taken by mutant **(CAP/EF)-sensitive cells** by using a presumed endocytic pathway after 12 hours coculture. Then, the mutant (CAP/EF)-sensitive cells can obtain resistance to these antibiotics by mtDNA recombination. Subsequently, the cellular internalization of mitochondria by macropinocytosis was demonstrated (Kitani et al., 2014a; Kitani et al., 2014b). However, the same research by Clark and Shay showed that the mitochondria of modified cells, transferred to new cells, did not confer resistance to the recipient cells. This offered evidence that mitochondria can only survive by having the genes for antibiotic resistance, i.e. with a healthy pool of genes.

The developing of Clark and Shay's Mitochondrial Transformation continued until recent years. In the way, some efforts failed, possibly by methodological errors (Spees et al., 2006). The Katrangi et al research (2007) demonstrated that isolated mitochondria are very sensitive to management conditions, specially temperature, and that recipient cells need specific conditions to show successful mitochondrial internalization capability. For instance, cell with mitochondrial disfunction as A549 ρ^0 cells must be cultured in medium enriched with uridine and pyruvate to facilitate energy supply before performing mitochondrial intake (Spees et al., 2006). On the other hand, mitochondrial transformation can be blocked by the presence in the medium of some substances as pentosan polysulphate and heparin, which indicate critical participation of cell surface's heparan sulphate proteoglycans in the mitochondrial transformation process (Kesner, Saada-Reich, & Lorberboum-Galski, 2016).

While the previous paragraphs are oriented to describe the use of Mitochondrial Transformation as a strategy to improve the synthesis of energy and the survival of recipient cells, some researches show that MT can be used to unbalance and eliminate cancer cells. Mitochondrial enzymes succinate dehydrogenase (SDH) and fumarate hydratase had been reported as tumour suppressors (R. L. Elliott, Jiang, & Head, 2012). Additionally, the improving of mitochondrial respiration, ROS production and cell apoptosis by increasing the nuclear translocation of apoptosis-inducing factor had been reported as consequence of Mitochondrial transfer to cancer cells (Chang et al., 2019). On the other hand, some studies demonstrated the protective role of mitochondrial transfer from MSCs to cancer cells on cancer cells viability and survivance (Ohkouchi et al., 2012; Sansone et al., 2017). These researches show that different types of cancer must be confronted by different cancer therapy strategies with mitochondria in mind: some types of cancer are damaged by healthy mitochondrial transfer, other kind of cancer could be treated with

damaged mitochondria and finally, mitochondria can be a target for pharmacological attack, avoiding cancer cells proliferation or inducing apoptosis in them.

Mitoception, a new approach for AMT/T in cultured or isolated cells, has been developed by Caicedo et al (2015). This approach allows the reduction of mitochondria-cell coculture time. Its main advantage is the augmentation of mitochondrial cell intake, avoiding the mitochondrial decay as consequence of large coculture time lapse. The Mitoception protocol has been designed originally to be used on *in vitro* cultured cancer cells, attached to a culture substrate (MDA-MB-231 target cancer cells), or in neurospheres (glioblastoma stem cells) (Andrés Caicedo et al., 2015; Mombo et al., 2017). Recently, Mitoception was adapted to be used on suspended cells as PBMCs or gametes (Parra et al 2019, Cabrera et al 2019). This research shows that AMT/T in vitro, by using isolated mitochondria, can improve some cellular indicators as metabolic activity, mitochondrial mass, mtDNA sequence stability, p53 gene expression, and percentage of dead cells.

Berridge et al (2016) have assembled an important research corpus about allogenic and exogenic **AMT/T under cell coculture conditions**. In these researches, there is a manifest preference for the use of mesenchymal cells, both human and animal, as mitochondria's donor cells by cell-to-cell communication. Other cells that can act as mitochondria donors are epithelial cells, cardiomyocytes and some nervous system's cells. However, mitochondrial transfer between cells in vivo had little evidence until recent years. Both approaches investigate the therapeutic use of mitochondria in a set of physiopatological conditions as immunosuppression (Luz-Crawford et al., 2019a), hypoxemia, bacterial infection, trauma (H. Li et al., 2019) and antibiotic toxicity, among others. In these researches, the critical role of tunnelling nanotubules (TNTs) over another types of cell-to-cell mitochondrial transfer leads to the development of a new type of therapeutic approaches.

b. **Non- invasive AMT/T *in vivo*: Direct mitochondrial injection to damaged tissues** (McCully et al., 2016) of mitochondria isolated from the same patient is the main technic in development. Mitochondrial injection to damaged tissues had demonstrated an improvement of high energy synthesis and cellular ATP storage along with an altered myocardial proteome. Once internalized the transplanted mitochondria rescued cellular function and replaced damaged mitochondrial DNA. No immune or auto-immune reaction and no pro-arrhythmia as a result of the transplanted mitochondria was detected in these cases, opening a new field in the treatment research of hypoxia-ischemia lesions. This cell-to-cell communication approach has been studied in lung-diseases models, proving that mesenchymal cells introduced at damaged tissues can restore epithelial function and regenerative abilities (Islam et al., 2012; Li et al., 2014; Morrison et al., 2017; Paliwal et al., 2018). Other models study mitochondrial transfer as treatment for ischaemic cardiomyopathy (Blitzer et al., 2019; Masuzawa et al., 2013; J. D. McCully et al., 2009; Moskowitzova et al., 2019), muscular (Orfany et al., 2019) and brain injury (Zhang et al., 2019) and mitochondrial-related behavioural disorders (Y. Wang et al., 2019)

c. **Invasive techniques for AMT/T: The Ooplasm Transfer (OT)** by microinjection to the oocyte is a technic originally developed by Cohen et al (1998) in order to treat the recurrent implantation failure in women. In OT, from 5 to 15% of the ooplasm from healthy and fertile

donor oocytes is injected into each developmentally compromised oocyte to improve its fertilizability and development's capability (Barritt, Brenner, Malter, & Cohen, 2001; J. Cohen, 1998). Also, this procedure had been successfully applied in experimental animal models as bovine, fish and another species (Labarta, de los Santos, Escribá, Pellicer, & Herraiz, 2019). **However, the impossibility to develop glass injection needles of required size (<1 µm in diameter) caused that cytosolic microinjection fails with smaller cells as somatic ones, limiting its practical applicability to oocytes**.

Nowadays, **OT is a standard procedure for subfertility cases in women** and remains as an experimental procedure in animal models as rodents, cows and sows (Brenner, Barritt, Willadsen, & Cohen, 2000; Landry et al., 2016; Z.-B. Wang et al., 2017). In this procedure, mitochondria and messenger RNAs (mRNAs), proteins, and other factors are transferred, but the specific manner as ooplasmic transfer affects the physiology of the early human embryo was not initially understood. Earlier studies probe that aged females carry some acquired damage in oocyte's mtDNA, calcium metabolism, cytoskeleton structure, cytosolic proteins function and more (Duran, Simsek-Duran, Oehninger, Jones, & Castora, 2011; Igarashi, Takahashi, Hiroi, & Doi, 1997; Igarashi et al., 2016; Iwata et al., 2011; Narita, Niimura, & Ishida, 1992; Reynier et al., 2001; Simsek-Duran et al., 2013; Takeo, Goto, Kuwayama, Monji, & Iwata, 2013). However, more and more efforts have been directed to understand the mitochondrial role in the aged female oocyte, because most of the afore-mentioned damages are related to mitochondrial dysfunction (Boudoures Anna, 2017; Darbandi et al., 2016; Hammond et al., 2016; F. Li et al., 2017; Pawlak, Chabowska, Malyszka, & Lechniak, 2016). On the other hand, this procedure has been suggested as non-appropriate for mitochondrial inherited diseases' prevention. Some researchers consider that the large ooplasm volume needed to reduce oocyte's mutant mtDNA percentage needs more invasive manipulations that invoke serious ethical concerns (Yabuuchi et al., 2012).

Between invasive AMT/T technics developed, **Mitochondrial Supplementation (MS)** is characterized by use isolated mitochondrial transfer, in this case, by microinjection (Cagnone et al., 2016; Pinkert, Irwin, Johnson, & Moffatt, 1997). As OT, MS is not available for small somatic cells. Neither for subfertility treatment yet, but it is a useful tool for the study of mitochondrial fate during early embryo development. From these researches, some findings of interest are: mitochondrial MitoTracker toxicity, xenogeneic mitochondria elimination during blastocyst stage, unviable offspring after xenogeneic MS because embryo and foetus's inadequate capacity for oxidative phosphorylation and possibly increased oxidative stress (Cannon et al., 2011; Ingraham, 2003; Trounce et al., 2004) or mitochondria fate in intraspecific MS determined by mitochondrial origin's tissue (Takeda et al., 2005; Takeda, 2019).

Photothermal Nanoblade (PN) utilizes a metallic nanostructure to produce a short laser pulse energy and transform it into a highly confined and precisely shaped explosive vapor bubble. Rapid bubble expansion and collapse perforates a lightly-contacting cell membrane via high-speed fluidic flows and induced transient shear stress. Developed in 2010, PN has been used by an single research team in some cellular transfer experiments (Wu, Teslaa, Teitell, & Chiou, 2010; Wu et al., 2011; French et al., 2011). In a recent study, this technic thtawas used by AMT/T in somatic cell lineage (143BTK-ρ0 cells). Mitochondrial transfer by

PN is reported as more efficient than cell fusion. Additionally, as other AMT/T technics, PN restores metabolic profile in recipient cells. However, PN works cell by cell prejudicing the obtention of enough cell quantities for statistical analysis in somatic cell models (Wu et al., 2016). A similar new twchnic, the Biophotonic Laser-Assisted Surgery Tool (BLAST) could deliver large cargo elements into 100,000 cells in 1 min, but mitochondrial transfer results with this technic has not been published yet (Patananan, Wu, Chiou, & Teitell, 2016; Wu et al., 2016)

Magnetomitotransfer (MMT) is a novel technic that use antibodies anti-TOM22 bonded to magnetic microbeads. The antibodies bond to mitochondria, allowing their movement and transfer by using magnetic fields to move mitochondria. Interestingly, MMT can be used to transfer mitochondria inward or outward cells, making theoretically possible both rise the healthy mitochondria or reduce the damaged or mutated mitochondria amounts into the cultured cells (Hubbard et al., 2019; Macheiner et al., 2016). An additional advantage reported by the researchers is that MMT has a better mitochondrial yield than differential centrifugation (DC) and density gradient high speed/ultracentrifugation (UC), the basis of most the mitochondrial isolation protocols. However, it is possible that additional experiments with lower microbead concentrations are needed to test possible toxic effects of microbeads in AMT/T to cultured cells.

Chapter 5. Use of MitoCeption® for the Treatment of Mitochondrial disorders and diseases

Medical consensus on mitochondrial diseases diagnosis and treatments is disappointing. In the most cases, they have remarkable heterogeneity, both in their clinical presentation and genetic aetiology, offering challenges for diagnosis, clinical management and clarification of molecular mechanism. treatment is restricted to exercise and dietary supplements without enough statistical basis proving their effectivity (Hirano, Emmanuele, & Quinzii, 2018). Acquired mitochondrial disorders and some diseases with central mitochondrial involvement as Parkinson's, Alzheimer's, Diabetes type II or Atherosclerosis have no treatment focused on improving mitochondrial behaviour or energy supply. On the other hand, recent researches proved that acquired disorders as post traumatic and hypoxemic muscular or neural degeneration can be treated with AMT/T (Masuzawa et al., 2013). Nowadays, some efforts for mtDNA modification in mitochondrial diseases have been done by using *in vitro* models: some researchers had obtained healthy cells from a LHON patient after modify their mutant mtDNA by using cybrid's technology (Wong et al., 2017). In the immediate future, AMT/T, and specially MitoCeption, could be used to improve tissues´ survival and reparation, reducing signs and symptoms of these diseases. For instance, a recent paper claims that direct human mitochondria transferred can ameliorates cognitive deficits, neuronal loss and gliosis in an Alzheimer murine model (Nitzan et al., 2019).

MitoCeption was originally developed as an in vitro AMT/T technic for cell metabolism and mitochondria-cell interactions research by using substrate-attached cells (Andrés Caicedo et al., 2015). However, it is possible that MitoCeption can be used in mitochondrial disorders therapies. MitoCeption could be used to add healthy mitochondria in aged cells and thus allow the renewal of the mitochondrial pool in cells *ex-vivo* before cell transplantation to patients. Mitochondrial acquired damage is due to many causes: hypoxia, toxic metabolites, drugs, nutritional imbalance, UV or gamma radiation, etc. Damage by UV radiation is a main concern in dermatology and skin oncology. Additionally, there is a participation of UV radiation causing immune suppression behind skin cancer progression. UV-related immune suppression involves myeloid tissue and leukocytes' mtDNA alterations (Hart & Norval, 2017). To study if AMT/T can be used in order to treat UV-related mtDNA damage, we developed a UVR mtDNA damage model by using UV-C irradiated Peripheral Blood Mononuclear Cells (PBMCs) as study objects and PBMCs from healthy donors to obtain high amounts of Mitochondria needed for Mitoception as AMT/T treatment approach. Our results demonstrated that UV-C cause mtDNA depletion, loss of cell viability, increase of mitochondrial ROS and P53 expression and Mitoception can restore UV-irradiated PBMCs' mtDNA account, mitochondrial mass and normal metabolic profile *in vitro*.

The same procedure can be used in cell therapy. In some cases of age-related immunodeficiency due to phagocytic inability, Mitoception could renovate the mitochondrial pool of myeloid stem cells, improving phagocytes' capacities. This approach mimics the observations made on normal MSC and phagocytes behaviour in vitro (Gerdes et al., 2007; Jackson et al., 2016; Morrison et al., 2017). In the same way, MitoCeption could be used to cancer treatment. *In vitro* models of AMT/T proved that cancer cells can be affected by mitochondria transferred into the cells (Andrés Caicedo et al., 2015; Chang et al., 2019; Mombo

et al., 2017). These *in vivo* and *ex vivo* models have proved that mesenchymal stem cells (MSC) can transfer mitochondria to cancer cells naturally. On the other hand, some researches proved that horizontally transferred normal mitochondria can enhance tumoral growth (Dong et al., 2017; A. S. Tan et al., 2015). However, it is possible to use Mitoception to produce autologous MSC loaded with defective or damaged mitochondria (as Trojan Horses) and transplant these MSC to the tumour boundaries. The horizontal transfer of damaged mitochondria to the tumour cells could impair the tumour growth and even starts an apoptotic process inside it.

On the other hand, elder women and domestic females suffer from low or loss of fertility since oocyte quality decreases as maternal aging (Kansaku et al., 2017; Mohammadzadeh et al., 2018; Rambags et al., 2014; Woods et al., 2018). As energy source, mitochondria play essential roles in oocyte development, determining pre-fertilization oocyte quality. With advanced maternal age, increased dysfunctions emerge in oocyte mitochondria, which decrease oocyte quality and its developmental potential (Schatten, Sun, & Prather, 2014; Z.-B. Wang et al., 2017). Nowadays, autologous and exogenous AMT/T by microinjection to oocytes was proved by some research teams, showing an improvement of oocyte's developmental potential (Kujjo et al., 2013; Liu, Yang, Lu, & Qiu, 2008; Takeda et al., 2005; Woods & Tilly, 2015). We suggest that autologous MitoCeption by using PBMCs as source of mitochondria, can be an easier, faster and less expensive method to improve oocyte's developmental potential, by improving the oocyte's fertilization yield and embryo's implantation probability in aged women and domestic females as cows and female pigs.

Nowadays, pronuclear or spindle transfer are the only technics used to prevent the transmission of maternally inherited mitochondrial diseases (Hyslop et al., 2016; Mykytenko et al., 2019; Rai, Craven, Hoogewijs, Russell, & Lightowlers, 2018), with the aim of reducing the mutant mtDNA percentage and reducing the probability of signs appearance in the new-born. We propose that these diseases are eligible for preventive treatment by MitoCeption instead of pronuclear or spindle transfer. Recently, we have successfully proved that Mitoception can be used with a xenotransfer model with Human Mitochondrial Mix on oocytes and zygotes from healthy, young rodent females, without affect their developmental capacity and implantation (data not published yet, see paper N° 3 below). This model can satisfy both ethical and medical concerns by avoiding the use of donor's germplasm, cancer drugs application as cytochalasin B or ooplasm damage.

Chapter 6. Results

a. Published paper #1

Hindawi
Stem Cells International
Volume 2017, Article ID 7610414, 23 pages
https://doi.org/10.1155/2017/7610414

Review Article

Artificial Mitochondria Transfer: Current Challenges, Advances, and Future Applications

Andrés Caicedo,[1,2,3] Pedro M. Aponte,[3,4] Francisco Cabrera,[3,5,6] Carmen Hidalgo,[3,7] and Maroun Khoury[3,7,8,9]

[1]*Colegio de Ciencias de la Salud, Escuela de Medicina, Universidad San Francisco de Quito (USFQ), 170901 Quito, Ecuador*
[2]*Colegio de Ciencias Biológicas y Ambientales, Instituto de Microbiología, Universidad San Francisco de Quito (USFQ), 170901 Quito, Ecuador*
[3]*Mito-Act Research Consortium, Quito, Ecuador*
[4]*Colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito (USFQ), 170901 Quito, Ecuador*
[5]*Colegio de Ciencias de la Salud, Escuela de Medicina Veterinaria, Universidad San Francisco de Quito (USFQ), 170901 Quito, Ecuador*
[6]*Institute for Regenerative Medicine and Biotherapy (IRMB), INSERM U1183, 2 Montpellier University, Montpellier, France*
[7]*Laboratory of Nano-Regenerative Medicine, Faculty of Medicine, Universidad de Los Andes, Santiago, Chile*
[8]*Consorcio Regenero, Chilean Consortium for Regenerative Medicine, Santiago, Chile*
[9]*Cells for Cells, Santiago, Chile*

Correspondence should be addressed to Andrés Caicedo; acaicedo@usfq.edu.ec

Received 21 February 2017; Revised 30 April 2017; Accepted 15 May 2017; Published 2 July 2017

Academic Editor: Pascal May-Panloup

The objective of this review is to outline existing artificial mitochondria transfer techniques and to describe the future steps necessary to develop new therapeutic applications in medicine. Inspired by the symbiotic origin of mitochondria and by the cell's capacity to transfer these organelles to damaged neighbors, many researchers have developed procedures to artificially transfer mitochondria from one cell to another. The techniques currently in use today range from simple coincubations of isolated mitochondria and recipient cells to the use of physical approaches to induce integration. These methods mimic natural mitochondria transfer. In order to use mitochondrial transfer in medicine, we must answer key questions about how to replicate aspects of natural transport processes to improve current artificial transfer methods. Another priority is to determine the optimum quantity and cell/tissue source of the mitochondria in order to induce cell reprogramming or tissue repair, in both in vitro and in vivo applications. Additionally, it is important that the field explores how artificial mitochondria transfer techniques can be used to treat different diseases and how to navigate the ethical issues in such procedures. Without a doubt, mitochondria are more than mere cell power plants, as we continue to discover their potential to be used in medicine.

1. Introduction

Mitochondria are cell organelles descended from an alpha-proteobacterial endosymbiont [1] and play a fundamental role in growth, differentiation, and survival beyond sustaining the energetics of the cell [2, 3]. Diseases, tissue damage, and aging challenge the cell and its mitochondria, thereby affecting their integrity, function, and homeostasis [4, 5]. Cells naturally have the capacity to exchange intracellular material and especially mitochondria through different processes such as cell-to-cell contact, microvesicles, nanotubular structures, and other mechanisms [6–8]. Clark and Shay pioneered the artificial mitochondria transfer (AMT), which involved transferring mitochondria with antibiotic-resistant genes into sensitive cells, thereby enabling them to survive in a selective medium [9] and opening this new field of research. Since the work of Clark and Shay, the process of artificial transfer has and continues to mimic aspects of naturally occurring cell transport, especially in the mechanisms cells naturally use to rescue other damaged cells. The

AMT restores and increases respiration and proliferation and completes other cellular processes [5, 10–16].

This review will consider key advances necessary to improve the current knowledge about the artificial transfer of mitochondria and how these techniques could be used therapeutically. We will provide an overview of the features of the mitochondrial structure that are important in maintaining its integrity throughout artificial transfer [13, 14]. Next, we will discuss how a cell naturally protects the mitochondria during their transport by using intercellular bridges or microvesicles and the effects of the transferred mitochondria in the receiver cell [6, 17, 18]. The in vivo artificial transfer of mitochondria was carried out at the same time as many in vitro assays [5, 7, 12, 13, 16, 19]. These approaches will be covered in the third section. For example, those assays performed by McCully in 2009 [16] and recently by Huang et al. in 2016 [19] raised questions about the best source of mitochondria, what kinds of stress during their transfer could affect mitochondrial function or prevent their arrival to the target tissue, among other questions. The key to developing new lines of research in this field is determining the diseases in which AMT could be effective as well as the potential advantages of such therapeutic treatments over others. Taking this into account, it is essential that we further study the effectiveness of different donor sources of mitochondria in repairing recipient cells and determine how such findings can help to establish ethical guidelines that will facilitate future safety research and enable the development of new medical applications of AMT. Without a doubt, more advances are needed to better understand and improve AMT and lay the foundation for its safe use in treating mitochondrial damage and related diseases.

2. Structural and Functional Characteristics of Mitochondria for a Successful Artificial Transfer

The mitochondrion is an organelle present in most of eukaryotic cells; it is in charge of ATP synthesis via oxidative phosphorylation (OX-PHOS), calcium metabolism, and the control of the apoptotic intrinsic pathway, among other functions. At present, the mitochondrion is recognized as an endosymbiotic organism, whose noneukaryotic origin could facilitate its ability to be transferred from one cell to another. It has a double protective membrane and partial transcriptional independence from the nucleus, thereby making the mitochondria an item which can naturally be exchanged by microvesicles and nanotubes between cells [20–22]. Given that there is no cellular protection when performing AMT, it is important to conserve mitochondrial integrity after isolation when exposed to an extracellular environment. The isolation procedure and stressors present outside the cell or organism like temperature change and surrounding media would greatly modified the structural stability, function, and potential effects of the mitochondria in the receiver cell [23]. In this section, we will focus on key biological aspects that should be taken into consideration when the AMT to other cells is sought.

The mitochondria evolved from a prokaryotic organism, and when it colonized the first protoeukaryotic cell, it developed a system of close communication with the nucleus by exchanging its own mtDNA sequences with it [24, 25]. It is estimated that mitochondria need almost 2000 proteins to work properly, but in many species, mtDNA encodes barely 63 proteins or less [26, 27] and most of these proteins are synthesized in the cytoplasm by means of ribosomes encoded in the nucleus and not by those of the mitochondria, thereby making them partially independent [28]. The interaction between nuclear and mitochondrial genes is essential for the organelle transcription, translation of proteins, and respiration [29]. Considering this close relationship, the compatibility between the mitochondria of one cell or species interacting with the nucleus of another could potentially affect their crosstalk, thereby inhibiting cell respiration and function [29–32]. These specific differences in the nuclear and mitochondria genome between cells or species could cause incompatibility if the auto, allo, and xenogenic AMT is pursued [13].

The mitochondria's small size as well as its capacity to change its shape and length allows it to be transported by subcellular transporting mechanisms such as tunneling nanotubes (TNTs) and microvesicles (MVs) [33, 34]. Its diameter varies between 0.5 and 1.0 μm, and its length shows great variability, from 0.5 to 10 μm. Although its shape is defined as rounded or elongated, mitochondria can be very pleomorphic, or in other words, they may exhibit great morphological variations. Some mitochondria could be fused and interconnected in networks, in contrast to the classic bean shape that appears in most illustrations [35, 36]. This organelle is characterized by a double lipoprotein membrane, each of which are about 7 nm thick. The outer mitochondrial membrane is smooth, biochemically identical to the membranes of eukaryotic cells, and rich in cholesterol (possibly contributing to the cell capacity to internalize this organelle when it is free in external medium) [11].

Guaranteeing the integrity of the outer and inner membranes during any process of AMT between one cell to another is key to protecting this organelle's function and the effects on the receiver cell after transfer. The outer mitochondria membrane (OMM) serves as a barrier and a platform to exchange products between that cytoplasm and the intermembrane space [37, 38]. The OMM also protects the cell from any harmful product, like free radicals from the active metabolic processes carried out by the mitochondria [37, 39]. OMM permeabilization can be induced by toxins, *gamma* and/or UV irradiation, hypoxia, and growth factor deprivation causing irreparable mitochondria DNA (mtDNA) damage. These factors can lead to the activation of proapoptotic multidomain Bcl-2 proteins, such as Bax or Bak [40–43]. A permeabilized or fragile OMM would not be effectively internalized after AMT by the receiver cell or even could activate apoptotic processes instead of repairing or increasing cellular functions [11]. Further studies should be completed in order to fully understand the interactions between the OMM and the receiver cell membrane and to understand the process of uptake.

The inner mitochondrial membrane (IMM) is chemically similar to bacterial cell membranes and rich in cardiolipin, a

phospholipid made of 4 fatty acids that decreases this membrane's permeability to protons. The IMM's lack of proton permeability is essential because it allows the existence of differential concentrations between the mitochondrial compartments (intermembranous space and mitochondrial matrix). The IMM is composed of the inner boundary membrane (IBM) and cristae membrane (CM), where the IBM is opposed to the OMM and the CMs are extended protrusions of the IBM inside the matrix [44]. The CM's shape is created by multiple folds in the membrane. This allows more IMM to be packed into the organelle and thereby provides scaffolding for the electron transport chain complexes and ATP synthase which represent 80% of the protein mass of the inner membrane [45, 46]. The disruption of the IMM architecture could result in the alteration of the cristae dynamics in the mitochondria, consequentially affecting its capacity to fuse with other mitochondria and to produce ATP [37, 47, 48]. One of the therapeutic possibilities of AMT is enabling the exchange of mtDNA from exogenous healthy mitochondria to damaged receiver mitochondria thereby contributing to the ATP production in which maintaining the integrity of IMM could favor the process.

Mitochondrial fitness is essential to maintain the integrity and functioning of the cell. Many reactions take place inside the mitochondria and are the consequence of its good condition, among fatty acids β-oxidation, Kreb's cycle, urea cycle, heme biosynthesis, and part of the steroid, cardiolipin, and ubiquinone biosynthesis pathways. Genetic variations in mitochondria and the presence of deleterious mutations in their DNA can alter their structure, function, and integrity. Many crucial aspects of their physiology are still not fully understood which are necessary to understand how physiological changes or stressors, like subproducts of the electron transport chain (i.e., reactive oxygen species (ROS)) and others induced by the environment (contamination or age), can damage components of the mitochondria. In order to develop more efficient mechanisms and succeed the AMT, we must find ways to maintain their structural integrity during AMT, guaranteeing that the outer and inner membrane structures will be conserved and also that the mitochondria does not lose its function during the transfer, thus assuring the beneficial effects of the procedure [9, 14]. Previous work about the AMT evaluates mitochondrial function by fluorescent probes and electron microscopy being a key aspect of the transfer procedure [14, 16, 49, 50]. Picard et al. observed in 2011 that the isolation procedure of the mitochondria from cells or tissues induces the fragmentation of the organelle, modulates the permeability of the transition pore sensitivity to calcium, alters the respiration rates of oxygen consumption, and increases the mitochondrial stress-producing free radicals [23]. There is still no information about the absolute or relative number of damaged versus healthy mitochondria during the AMT process. Obtaining this information could contribute to a better evaluation and comparison of the different AMT methods discussed in this review.

Cells and mitochondria change during the process of differentiation. It has been described that stem cell mitochondria are in a dormant and immature state: they are small and favor anaerobic metabolism. Through the process of differentiation and loss of their pluripotency, mitochondria proliferate and the quantity of DNA, the rate of respiration, and the generation of ATP synthase increase. These changes cause the mitochondria to develop an elongated morphology and swollen cristae. Its matrix also becomes more dense, being relocated to a wider extent in the cells [51–54]. It has not been studied whether the isolated mitochondria show variations on their effects on the recipient cells depending on the differentiation states as mitochondria show strong differences on their structure and metabolic profiles. Questions like whether the cristae distribution change, ROS produced during the transfer, need to be answered and incorporated to the isolation and transfer protocols.

In the next section, we will describe key aspects of natural intercellular mitochondria transfer, a cellular function which protects other cells from damage or stress [7, 8, 33, 55]. During transport, mitochondria are enclosed and secured by membranes, thus protecting them from external damage. In order to achieve successful artificial transfer, these mechanisms will need to be recreated in order to protect the mitochondria.

3. Natural Intercellular Mitochondrial Transfer

To date, several groups have reported the horizontal transfer of mitochondria in different cell types in vitro and in vivo, describing a new cellular property [7, 8, 21, 56, 57]. Most of the work about mitochondrial delivery from one cell to another deals with the rescue of damaged cells by healthy ones, such mesenchymal stem cells (hMSCs) [8, 56, 58]. Additionally, other studies have linked this transfer process to MSCs' enhanced immune response to macrophages; this is just one example of the diverse effects this mechanism has on cells involved in the transfer [33]. Recently, this process was also observed occurring between astrocytes and neurons during focal cerebral ischemia [21]. Interestingly enough, in such cases, mitochondria from the retinal ganglion cell are transferred to astrocytes of the optic nerve head to be broken down and recycled [57]. From the first description of the transfer of intracellular material between cells in 2004 by Rustom et al. [6], the work of Spees et al. in 2006 [7] to the in vivo assays performed by Islam et al. in 2012 [8] and Jackson et al. in 2016 [33], most studies show that MSCs are the best cells to transfer mitochondria. Considering the potential benefits of natural mitochondria transfer, there is great urgency to better comprehend, facilitate, and artificially replicate this process.

The transport of mitochondria from one cell to another is part of the dialogue necessary to the development and maintenance of homeostasis in multicellular organisms (Figure 1) [59]. Mitochondria can travel from one cell to another by intercellular structures such as tunneling nanotubes (TNTs) and secreted cellular bodies, such as microvesicles [5, 20, 33, 60]. In 2004, Rustom et al. described TNTs as a structure that enables cell-to-cell interaction. Since then, a number of groups have studied the cells that produce TNTs and receive mitochondria and other intracellular cargo [5, 6, 33, 61]. Other reviews in this special issue and

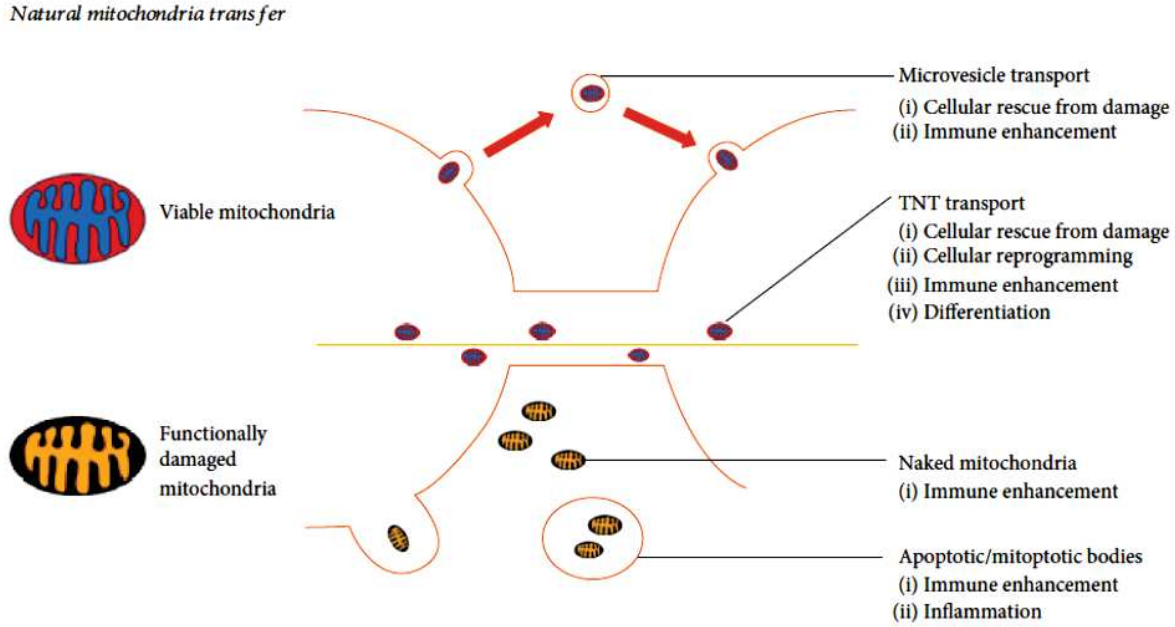


FIGURE 1: Natural mitochondria transfer. Viable and nonfunctional mitochondria can be shared by the cell inducing different cellular responses from cellular rescue to promoting inflammation. The first transfer mechanism is the microvesicle transport of mitochondria, it has been observed specially in MSCs in which the secreted microvesicles carrying mitochondria, once internalized by the recipient cells, induce its rescue from cellular damage and enhance the phagocytic properties of immune cells [182, 183]. The second way of transfer is by TNTs; many cells share the ability to produce them and transport mitochondria with proven effects in the rescue from cellular damage, metabolic reprogramming, and immune enhancement and it was also associated with its differentiation [65, 184]. During cellular stress, defective mitochondria can be released without being covered like in apoptotic or mitoapoptotic bodies and being naked promoting the immune response and inflammation [17, 77, 185].

recently published work recapitulate the details of TNT structure generation, characteristics, and mitochondria transfer [60, 62–64].

TNTs are produced by the outgrowth of filopodia-like cell membrane protrusions that connect with the target cell. The membrane from each cell extends to fuse together, thereby forming a tightly connected bridge which is independent from any substrate [22]. TNTs contain a skeleton mainly composed of F-actin and transport proteins like MIRO1 that facilitate the active transfer of cargo and mitochondria along these structures [58]. TNTs were first described in rat-cultured pheochromocytoma PC12 cells [6], and subsequent studies have shown that they connect a wide variety of cell types. These studies provide more evidence that TNTs are involved in mitochondrial transport between cells, the repair of cell damage, the activation of enhanced immune responses, and cell metabolic reprogramming [5, 8, 33, 61, 65].

The directionality of the transport of intracellular material and mitochondria through the TNTs is not fully understood. It is important to define what factors promote the donation of material and their effects on the recipient cells. Sun et al. observed that TNTs' growth is guided by the extracellular protein S100A4 and its putative receptor RAGE (receptor for advanced glycation end product). Stressed hippocampal neurons and astrocytes initiated the formation of TNTs after p53 activation. This signaling pathway triggered caspase 3, which decreased S100A4 in injured cells and caused cells with a high level of S100A4 to become receptor cells [66]. By these results, the authors proposed that damaged cells need to transfer cellular contents to healthy ones, in a process related to the spread of danger signals but no insights about mitochondrial participation were given. In contrast, Spees et al. in 2006 observed that MSCs transferred mitochondria to respiration-deficient cancer cells, but the direction of the transport or bidirectionality mode were difficult to determine due to the fact that the recipient cells were depleted of mitochondria, and it was not described whether the MSCs received any intracellular material from the cancer cells [7]. Koyanagi et al. in 2005 have shown that mitochondria are exclusively transported by TNTs from human endothelial progenitor cells to neonatal undifferentiated cardiomyocytes in a process intended to sustain their maturation [67]. Gao's team in 2016 used microfluidic channels while tracking TNTs' formation and exchange of material in coculture assays. Gao's team observed that MSCs were responsible of the TNT formation and of mitochondria transfer to cardiomyocytes, as opposite to fibroblasts (negative control of the interaction) [68]. Bidirectional transport of mitochondria is also plausible as it was observed between malignant mesothelioma cells. These cells produce more TNTs than normal mesothelioma cells, but interestingly, their proliferation was inversely correlated with TNT formation during their culture in low serum, hyperglycemic, acidic growth medium [69]. These represent just a few examples of the extensive literature about the exchange of intracellular material and mitochondria and its directionality. Yet, mitochondrial transport is not fully understood. For example, the field still needs to define the cell types that

produce TNTs and deliver cargo to recipient cells [68]. The determination of the directionality of and conditions necessary for mitochondria transfer between different cells is essential to understanding the potential role of this process in helping cells exposed to stress or during the transmission of danger warning signals among cells. Another important question that still remains unanswered is the reason why MSCs have a greater propensity to form TNTs compared with other cells.

Many groups of researchers that use lung disease models have corroborated that mitochondria can be transferred to other cells in vivo. Islam et al. in 2012 reported that bone marrow-derived stem cells (BMSCs) could be used to supply healthy mitochondria to alveolar epithelial cells in a mouse model of *E. coli* LPS-induced acute lung injury [8]. The delivery of mitochondria into injured cells increased ATP levels which in turn maintained cellular bioenergetics and recovered epithelium functions. A follow-up study in lung disease models (rotenone-induced lung injury and allergen-induced asthma) contributed to the understanding of the mechanisms involved in mitochondrial transfer through nanotubes, confirmed the protective effect of mitochondrial donation, and revealed a Miro1-regulated mitochondrial movement from MSC to damaged recipient epithelial lung cells [58]. All these data corroborate that mitochondrial delivery can rescue damaging cells. Furthermore, in a mouse model of *E. coli*-induced pneumonia and acute respiratory distress syndrome (ARDS), transfer of mitochondria from MSC toward innate immune cells by TNTs enhanced macrophage bacterial phagocytosis in the harmed tissue, thus improving the process of repair [33].

Extracellular vesicles (EVs) are also involved in the transport of intracellular cargo to other cells. EVs are spheroid structures surrounded by a lipid bilayer membrane [70] and are capable of transporting proteins, lipids, carbohydrates, metabolites, small RNAs [71], and mtDNA [17]. EVs are classified depending on their size and biogenesis. This classification includes exosomes (30 to 100 nm in diameter), microvesicles (100 nm to 1 μm in diameter), and apoptotic bodies (1 to 2 μm in diameter) [72]. Apoptotic bodies have been less studied due to their rapid elimination by phagocytic cells [70]. Lastly, exosomes and microvesicles are released by diverse cell types including, platelets, endothelial cells, and breast cancer cells [34]. Both mRNA and microRNA have been found in exosomes and could be transported to target cells [73]. Guescini et al. in 2010 also observed the delivery of mtDNA by exosomes. mtDNA can also be delivered via exosomes, as it was detected in glioblastoma cells and astrocytes [74]. The full understanding of the mechanisms of mitochondrial transfer by EVs and effects in receiver cells are still unclear.

It has been observed that the nervous system benefits from the transfer of mitochondria for different purposes. For example, the transfer of mitochondria allows cells to breakdown nonfunctional mitochondria and to transfer healthy mitochondria to stressed neurons. The process of mitochondrial transfer is not always meant to protect damaged cells but also to recycle these organelles in other cells in a process called transcellular degradation of mitochondria or transmitophagy [57]. The transmitophagy process is mediated by cellular evulsions containing mitochondria from neurons, in which these structures are embraced by astrocytes and then recycled [57]. The reason why transmitophagy takes place is still unknown, but it has been hypothesized that focal axon damage stimulates the process. Another theory posits that transporting damaged mitochondria back to the neuron soma is energetically disadvantageous and that there are specialized astrocytes to perform this task and clearance of unfunctional mitochondria [57]. Astrocytes are responsible for protecting and repairing damaged neurons through several mechanisms in which the transfer of mitochondria by extracellular MVs containing vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF-2), and mitochondria is vital to support cell recovery after stroke or cellular stress [21, 75]. Understanding transmitophagy and the natural transfer of mitochondria by microvesicles in the nervous system will allow us to find new therapeutic options in which this processes could mediate the recovery of neurons' homeostasis and function in degenerative diseases.

One of the multiple mechanisms by which MSCs exert their natural therapeutic effects is via EVs. In 2012, Lee et al. isolated the exosomes from mouse and human MSCs. Subsequently, they injected the MSC-derived exosomes into the murine model of hypoxic pulmonary hypertension (HPH) and observed the therapeutic effects of MSC action in the tissue [76]. The same study found that MSCs prompt depolarized mitochondria to move to the outer limits of the plasma membrane in response to a higher concentration of oxygen (21%). This movement is mediated by the arrestin domain with protein 1-mediated MVs larger than 100 nm. Finally, these MVs are secreted and fuse with macrophages, thus enhancing their oxygen consumption rate and most likely improving their therapeutic properties as well.

MSCs secrete exosomes with microRNAs, thus inhibiting the activation of the macrophages and repressing the TLR signaling. Phinney et al. in 2015 found an association between this response and a mechanism in which MSCs make macrophages more susceptible to acquiring exogenous vesicles and mitochondria [17]. In 2012, Cho et al. [56] replicated the assays performed by Spees in 2006 [7] in which he cultured MSCs with human osteosarcoma 143B cells, subsequently causing their mitochondria to become compromised or depleted [7]. Cho et al. observed that MSCs actively transferred healthy mitochondria by nanotubular structures to the 143B mitochondria-depleted cells [56]. Cho et al. treated the MSCs with rhodamine 6G in order to alter mitochondria activity but not mtDNA. The authors observed that fully functional mitochondria were needed to recover the loss of respiration of the 143B mitochondria free cells. In a crucial part of the experiment, they cocultured MSCs with cells carrying mtDNA mutations (A3243G mutation or 4977 bp deletion) and saw no recovery of function [56].

Cells tend to dispose of their mitochondria when they are unfit after exposure to stress conditions or when keeping them becomes harmful as mitochondria can produce large quantities of ROS [77]. Around 30 to 50% of the highly glycolytic HeLa cells were able to survive after their

mitochondria were damaged by ejecting them through selective elimination or mitoptosis [77]. During this process, the mitochondria were degraded by their inclusion in membrane vesicles and exocytosis. The presence of degraded mitochondria and especially of mtDNA in the extracellular space has been associated with a proinflammatory response and the presence of antimitochondria antibodies such as anticardiolipin and antisarcosine dehydrogenase, which are characteristic of sepsis and associated with negative patient outcomes [77].

The mtDNA and ROS released by eosinophils have been shown to provide antimicrobial protection; they also represent a key component of the innate immune response [78]. Stimulated LPS hepatocytes and mouse embryonic fibroblasts extrude mitochondrial material through autolysosomal exocytosis, thereby activating polymorphonuclear leukocytes [79]. The release of mitochondrial contents activated inflammatory responses [79]. Lastly, intact mitochondria from necroptotic cells may play a role in hazard signaling when they are ejected from cells during tumor necrosis factor alpha (TNF α) induced necroptosis. These mitochondria are engulfed by macrophages and dendritic cells, resulting in the secretion of proinflammatory cytokines by macrophages and dendritic cell maturation [80].

A handful of in vivo studies have shown that mitochondria can be released either naked or encapsulated by a membrane bilayer. Nakajima et al. in 2008 used a mouse model to confirm that naked mitochondria are released into the intercellular space after an anti-Fas antibody injection. In response to this treatment, cytoplasmic vacuoles engulfed fragmented mitochondria and extruded them from apoptotic hepatocytes [81]. Likewise, activated platelets released respiratory-competent mitochondria, both as free organelles and encapsulated within the microparticles. These extracellular mitochondria mediate inflammatory responses [82]. Elucidating the mechanisms involved in mitochondrial extrusion will lead to a better comprehension of the diseases produced by dysfunctional mitochondria and inflammatory disorders.

Apparently, cells, especially MSCs, use mitochondria as a direct reprogramming agent because the mitochondria are independent from receptors or coupled proteins to induce their effects. Cytokines, miRNAs, transcription factors, and other cell components require the activation of specific signal pathways in order to induce a response of proliferation, growth, or other in cells [83, 84]. However, we can speculate that the exogenous mitochondria, once inside the cell, start to breathe and fuse with other mitochondria. These characteristics or mechanisms make the transport of mitochondria through TNTs or vesicles important to their protection and ensure their integrity and stability. We do not know if mitochondria free in circulation or inside microvesicles have the same effects or which one could better induce proliferation, cell repair, or other [5, 8, 33, 67, 68]. Another issue is if the isolating protocol of mitochondria when applied to cells or tissues could damage its function and effects in cells [85].

The quest for the most efficient method to deliver mitochondria in vitro and in vivo remains ongoing. Based on currently available literature, it appears that achieving effective AMT will require us to preserve the integrity and effectiveness of the mitochondria by protecting them within membrane structures, such as microvesicles. Since the first description of the mitochondrial transfer from MSCs to mitochondria-depleted cells [7], numerous studies have been conducted in vivo and in vitro. The results of these studies have provided more evidence to this novel field of research. Understanding these cell properties opens a new avenue for the development of therapeutic strategies like AMT to the treatment of mitochondrial-related disorders.

4. Artificial Mitochondria Transfer (AMT)

Without a doubt, the mitochondrion is the master organelle of cell energetics, fueling multiple processes like proliferation, migration, differentiation, and stress resistance [86–89]. The transfer of mitochondria between cells through nanotubes or microvesicles stimulates these processes and also protects the recipient cells from stress-related injury. Several research teams are currently working on AMT in order to understand how to promote cellular repair in this context [5, 8, 10, 55]. Since the first formal mitochondrial transfer from one xenogeneic cell to another was completed by Clark and Shay in 1982 through coincubation [9, 19], this rapidly growing field has developed new AMT methods in order to observe its effects in recipient cell types and imagine new possible applications (Figure 2).

In 1982, Clark and Shay developed "Mitochondrial Transformation," the very first technique to transfer mitochondria from one cell to another [9]. In their model, they were able to transform around 30,000 recipient cells in just one procedure, making it a highly efficient method. They used the antibiotics chloramphenicol (CAP) and efrapeptin (EF), which inhibit the mitochondria's protein synthesis and ATPase function in order to kill sensitive mammalian cells. Cells resistant to CAP have mutations in their mtDNA located in one region of the mitochondrial large subunit rRNA gene [9]. They observed that the transfer of mitochondria from CAP and EF-resistant fibroblasts increased the survival of the recipient cells, which were sensitive to these antibiotics. Interestingly, they observed that when the mitochondria of sensitive cells are transferred to new cells, they did not confer resistance to the recipient cells. This provides evidence that a higher concentration of mitochondria in and of itself is not sufficient to protect cells from CAP or EF; rather, mitochondria can only survive by having the genes for antibiotic resistance. It was also apparent that mitochondria from murine fibroblasts which were resistant to CAP and EF did not increase the survival of sensitive human cells. This indicates that mixing endogenous and transferred mitochondria across different species could potentially be restricted. A crucial observation of this article is that the failure of AMT into murine cells by simple coincubation suggests that this process is not equally efficient among different cell types and that some cells may be more receptive than others [9]. Clark and Shay's observations and questions regarding the mechanism of mitochondrial transfer have opened up the path for further advances in the field.

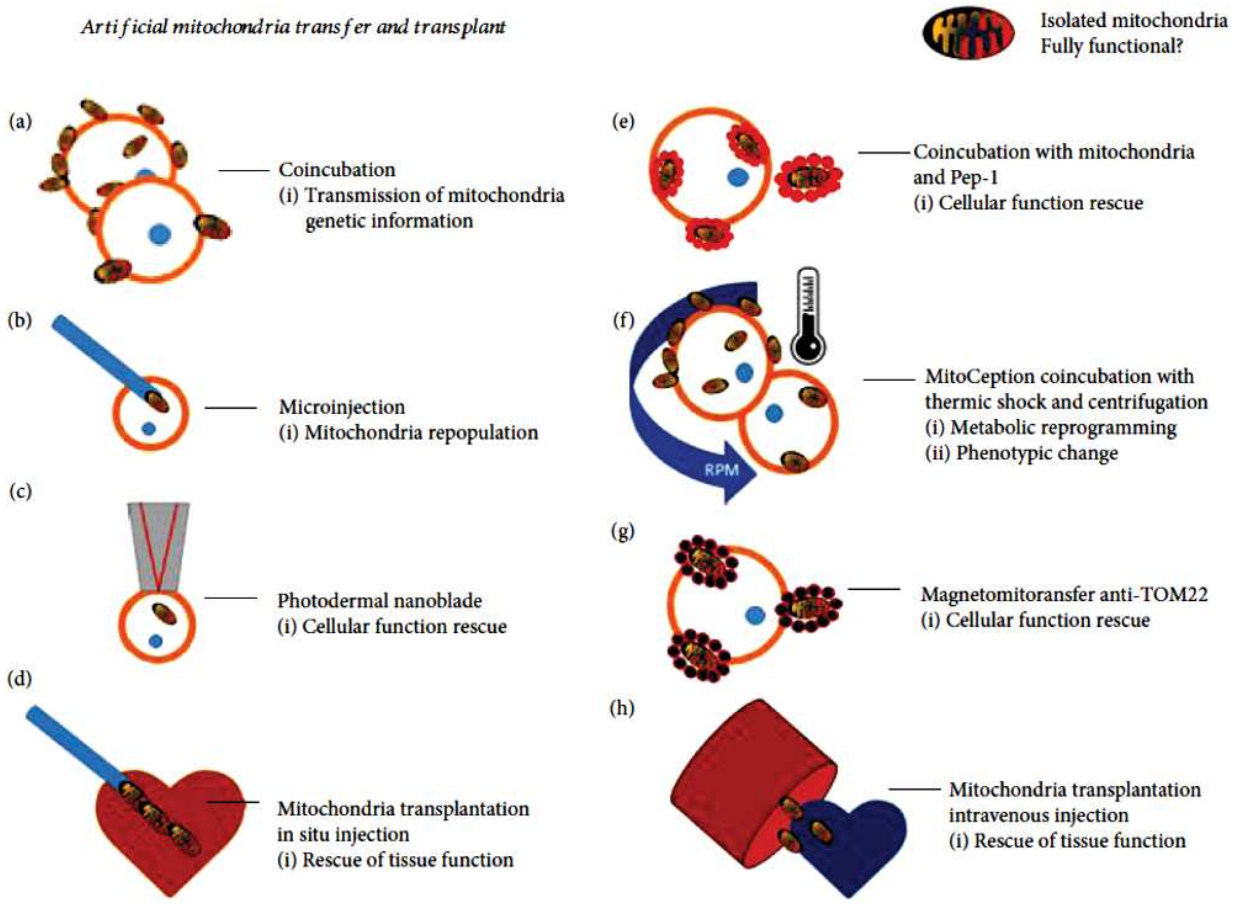


FIGURE 2: Artificial mitochondria transfer (AMT) and transplant. Different techniques emerged to mimic the natural transfer or mitochondria on its in vivo and in vitro applications. The coincubation technique was the first proposed in which the antibiotic resistance carried in the mitochondrial DNA was passed to sensitive cells [9], later after the technique was used to rescue respiratory deficient cells among other damaged cells [10, 11, 14, 63]. Microinjection of exogenous mitochondria was applied in assays to eliminate the endogenous copies of oocytes carrying mitochondrial diseases [90, 93]. The photothermal nanoblade effectively transferred isolated mitochondria inside the cell; even if they showed great effectiveness, its application is limited to small cell numbers [12]. Two different approaches were developed to facilitate the mitochondria internalization in the recipient cells, the first is by using Pep-1 and the other with magnetic beads (Magnetomitotransfer) designed to bind to TOM22 a receptor complex in the mitochondrial membrane. The MitoCeption technique uses a thermic shock and a centrifugation to improve the process of mitochondria uptake; first applied in cancer cells, this technique induces the metabolic reprogramming of these cells. The in vivo application of the mitochondria transfer applies two approaches: the first is to directly inject mitochondria to the harmed tissue and the other in the circulatory system close to the area of interest. Both of them have shown to restore tissue function but the in situ injection showed better results [16, 49, 50].

In 1988, King and Attardi then developed the first AMT technique using invasive instruments; they injected exogenous mitochondria isolated from CAP resistant cells into sensitive human cells [90]. Their method was less efficient than Clark and Shay's coincubation protocol because the technique limited the number of cells that could be transformed in each procedure and caused harm to the recipient cell. However, this study demonstrated that the injection of just one mitochondrion could very quickly repopulate a cell depleted of its endogenous mitochondria in just six to ten weeks. Additional techniques to perform AMT involving nanoblades and other invasive instruments have been developed, but all of them are less efficient than coincubation [12, 91].

Mitochondria carrying genetic mutations can cause diseases that can be transmitted to offspring through the oocyte [92]. To prevent and treat such diseases, experiments have utilized a variety of different AMT approaches, from microinjecting healthy mitochondria into oocytes [93] to transferring the nucleus of an unfertilized, mutation-carrying oocyte to a healthy enucleated ovule. Using King and Attardi's AMT technique, Pinkert et al. used microinjection to transfer mitochondria isolated from the livers of *Mus spretus* to fertilized oocytes taken from *Mus musculus* [93]. After 4.5 days in culture, Pinkert et al. detected xenogeneic mitochondria DNA sequences in the recipient cells, thereby demonstrating that xenogeneic mitochondria from closely related species are able to survive in recipient oocytes for at least a limited time

[93]. In 2007, Yi's team observed that zygotes that had received mitochondria transferred from the livers of young mice developed better through the blastocyst stage, as compared to old zygotes that did not undergo AMT [94]. In 2010, Takeda et al. used the mitochondria from bovine fibroblasts cultured in 10% and 0.5% serum [95]. Interestingly, the oocytes that received mitochondria isolated from cells at 0.5% serum showed a lower rate of development [95]. Recently, the transfer of mitochondria by microinjection has been replaced by enucleating the ovule of a healthy donor and adding the zygote nuclear material from a carrier of mitochondrial mutations in a pronuclear state and in metaphase II [96]. Although these techniques have been successful [97], they are still ethically controversial due to the amount of germ cells sacrificed in the procedure; these issues will be discussed in a later section of this review.

Clark and Shay's assay [9, 14] coincubates isolated mitochondria with the recipient cell, a technique which can be easily applied to many different types of cells. This procedure provides an opportunity to study the behavior and effects of artificially transferred exogenous mitochondria inside recipient cells. Despite other successes using this technique, in 2005, AMT unexpectedly failed when Spees et al. coincubated mitochondria isolated from hMSCs with human lung carcinoma recipient cells (A549) [7]. Before the procedure, A549 cells were pretreated with ethidium bromide in order to deplete their mtDNA and to make the cells unable to perform aerobic respiration, just as King and Attardi had done before [90]. When Prockop's group cocultured the depleted A549 cells with hMSCs, they observed the natural transfer of mitochondria between them. This transfer appeared to rescue respiration of the dysfunctional A549 cells. After their coincubation assay failed, they hypothesized that the transfer of mitochondria is mediated by active mechanisms such as the formation of nanotubular structures like TNTs or vesicles which transport these organelles to the interior of recipient cells [6]. Unknown details about temperature changes during the mitochondria isolation may have been instrumental to understanding the lack of passive transfer. Two years later, in a xenogeneic model, Weissig successfully transferred isolated mitochondria from mouse livers into human cancer cells, MDA-MB-231 and MCF-7. Katrangi et al. tested this technique in four models, using each type of cancer cell with and without mitochondria depletion by ethidium bromide [13]. The success of each of these models can be attributed to the methodology they used to isolate the mitochondria. The key of their methodology was maintaining the mitochondria at 4°C at all times in order to preserve their structure and function. They also maintained the cells in normal culture medium with uridine and pyruvate. The success of this protocol provides insights into the specific conditions that recipient cells may need in order to successfully internalize exogenous mitochondria and to prevent changes in cellular function and metabolism due to exposure to temperature variability. Another possibility is that some cells may be more receptive to accepting mitochondria. For instance, cancer cell lines such as MDA-MB-231 naturally incorporate more material from their surroundings in a process described as entosis or cell cannibalism [98].

Supporting Weissig's work (2007), the same year Yoon et al. observed that mitochondria from different species have the ability to fuse together. Their study did not use the mitochondria transfer technique, but instead they fused the cells with polyethylene glycol (PEG). They also labeled human and mice mitochondria differently (mtGFP and mtDsRed, resp.) and observed a mix of the two types of mitochondria 45 min after adding the PGE and fusion of the mitochondria of all hybrids at 4 h. The fusion of both human and mice mitochondria apparently occurs because of the homology of the sequence between the proteins responsible for this process, the mitofusins proteins (Mfn1 and Mfn2). Mfn1 and Mfn2 share a 90.7% and 94.8% homology between humans and mice. The formation of the mitofusin homodimers between the membranes of both types of mitochondria initiates the tethering and the fusion of the inner membrane [99]. However, even when mitochondria from both species fused, Yoon et al. were not able to create long-term cybrids from the mouse-human fusion, although they were able to achieve it from mouse-mouse fusions. This can be explained by the accumulation of differences between species, especially within the nuclear-coded mitochondrial genes and the dialogue between mitochondria and nuclei; it appears that long-term crosstalk between the nucleus and the xenomitochondria cannot be established [99, 100]. Yoon's work on the compatibility of xenomitochondria with human cells demonstrates that successful mitochondrial transfer may only be possible between cells and/or tissues of the same species.

In 2012, Elliott et al. coincubated mitochondria isolated from immortalized breast epithelial cells with their malignant counterparts MCF7, MDA-MB-231, and ADR-Res. They observed a decrease in their proliferative potential and a higher sensitivity to chemotherapeutic drugs such as doxorubicin, abraxane, and carboplatin [101]. Interestingly, they observed that only isolated mitochondria from the immortalized breast epithelial cells were able to enter the breast cancer cells, but not the original immortalized breast epithelial cells [101]. It was not further discussed in the article whether the characteristics of the immortalized cells' mitochondria are different from those of normal epithelial cells or if such differences could affect the process of their integration into the immortalized and cancer cells. To sustain the transfer of isolated mitochondria into recipient cells by coincubation, Kitani et al. demonstrated that this process can be performed autogeneically and xenogeneically. They documented the results of the transfer through real-time PCR and fluorescent imaging [11]. Nevertheless, although they obtained functional cells with integrated xenomitochondria, Yoon was unable to show the permanence of the exogenous mtDNA for longer than two weeks [99].

Assays to optimize transfers involving the use of chemical compounds and physical methods have been performed since 1988. In 2013, Liu and colleagues conjugated isolated mitochondria with penetrating peptides to foster their internalization. They used Pep-1, a cell-penetrating peptide that was originally developed to induce pores in the membrane to facilitate the delivery of molecules like oligonucleotides into the cell. The authors adapted Pep-1 to conjugate it with isolated mitochondria of human osteosarcoma 143B cells

[15]. The mix of Pep-1 and the isolated mitochondria promoted their internalization by fibroblasts involved in a model of the mitochondrial disease myoclonic epilepsy with ragged red fiber (MERRF) syndrome. They observed that the Pep-1-mediated transfer was more successful in facilitating the internalization of mitochondria than mitochondria alone [102]. Unfortunately, the authors did not describe the efficacy of or the rationale behind conjugating Pep-1 and mitochondria. They also did not describe the unexpected lack of internalization of the exogenous mitochondria not conjugated with Pep-1. Interestingly, in 2016, Liu's team used the Pep-1-conjugated allogeneic and xenogeneic mitochondria in an in vivo assay of a Parkinson disease model (PDM) [103]. They observed an increase of neuron survival and movement recovery in the animals of the experimental group as compared with the control. Yet, questions arise from Lui's technique, such as whether the Pep-1 peptide acts as a protective agent from environmental damage or whether it acts as an internalizing agent that facilitates the transfer of mitochondria to the affected tissues. Finally, defining the optimal quantity of the mitochondria administered to the PDM in rats will be important in studying the possibility of applying this technique to treat neurodegenerative disorders or any other disease.

In 2015, our group standardized the transfer mechanism of isolated mitochondria to cultured cells (MDA-MB-231, human breast cancer cells), adding two extra steps to the coincubation procedure: centrifugation and a thermic shock. We named the protocol *MitoCeption*. Our technique allowed the constant and reproducible increase of mitochondrial uptake by the recipient cells proportionally to the material added. Following these observations, we could prove an equivalent increase of respiration and ATP production in accordance with the supplementation of mitochondria. We witnessed a functional change in the mitocepted cancer cells: their proliferative and invasive potential increased. Interestingly, we also observed that a cancer cell cannot constantly incorporate mitochondria without harming their functional properties; the proliferation and invasive capacities of the cells diminished after increasing their mitochondria concentrations. Our work showed that it is useless to constantly improve the mitochondria transfer mechanism if there is a functional threshold of the internalized mitochondria. It is clear that this technique cannot be used for the in vivo transfer of mitochondria to organs or tissues, but cells can be mitocepted before being introduced into in a living organism with the purpose of reprogramming or repairing its metabolism and function [10].

The transfer of mitochondria by coincubation seems to depend on the viability and metabolic activity of the donor mitochondria and particularly on the integrity of the outer membrane, as this is the first structure organelle to interact with the receiver cell. Kesner et al. in 2016 further studied the coincubation transfer mechanism using isolated mitochondria from Hela cells and transferring them to other cancerous cell lines, healthy fibroblasts, and cells carrying mitochondrial mutations. They noted that the uptake was fast, with recipient cells uptaking the exogenous mitochondria just 10 minutes after the coincubation began. Kesner et al. also established that the mitochondrial transfer is principally mediated by macropinocytosis [14], which is a regulated form of endocytosis mainly involved in the uptake of molecules, nutrients, and other materials from the extracellular space [104, 105]. Furthermore, they found that perturbing the outer membrane of mitochondria with digitonin or other mitochondria-damaging molecules inhibits the uptake process. Their key observations provided insights about how mitochondria integrity is important to the success of the transferring process. They also importantly noted that membrane characteristics of the recipient cells may play a major role in the macropinocytosis of mitochondria.

Wu et al. developed a photothermal nanoblade to deliver cargo including mitochondria to the interior of mammalian cells, bypassing cell fusion and endocytosis [12]. Progressive resistance BTK-143 osteosarcoma and MDA-MB-453 p0 cells lacking mtDNA were treated with the photodermal nanoblade in order to deliver HEK293T-expressing mitochondria labeled with DsRed. The goal of this procedure was to rescue the metabolic function of the receiving cells. This technique effectively transferred exogenous mitochondria, but due to the technical expertise and equipment required to carry out this procedure, the results obtained in the experiment are difficult to replicate. Furthermore, as the authors mention, the transfer of mitochondria by the photothermal nanoblade is low throughput, meaning that the technique must be adapted in order to achieve the same efficiency as coincubation or MitoCeption techniques. Later the same year, Macheiner and colleagues [91] developed the use of anti-TOM22 magnetic beads to improve the purity of mitochondria isolates. They used a magnet to transfer the mitochondria coupled with the magnetic beads into host cells, naming this technique Magnetomitotransfer [91]. TOM22 is a multisubunit translocase embedded in the outer membrane of the mitochondria [106]. Coupling the mitochondria with anti-TOM22 beads increased the quantity of viable mitochondria able to be transferred. However, because the beads can also bind with nonfunctional mitochondria fragments, they may also inadvertently transfer them into recipient cells. According to the authors, a greater ratio of transfer was achieved using this technique after one to three days of culture as compared to passive transfer. By the same token, Kesner had already observed that the process of internalization of mitochondria via coincubation can occur in as little as 10 minutes [14]. This observation could put into question the need of the magnetic beads to accelerate the process. The authors also did not show whether the fusion of exogenous and endogenous mitochondria is affected in some way by the anti-TOM22 beads. Fusion is an essential process for the exchange of mtDNA between mitochondria; therefore, this method may not actually be effective if fusion is not facilitated by the anti-TOM22 beads. As they mentioned, further studies are needed to learn about the toxicity and changes in cell physiology after magnetic mitochondrial transfer, especially those associated with respiration and metabolic reprogramming. Both the photothermal nanoblades and Magnetomitotransfer are still limited in terms of the number of cells that they can reach, the damage they may cause, and the greater technical challenges involved in executing each procedure [10].

In 2009, McCully et al. proved that mitochondria can be used in vivo to repair damaged tissues [16]. Because ischemic damage affects the mitochondria in tissues, these authors hypothesized that the replacement of affected mitochondria with healthy ones would significantly improve the postischemic recovery. They induced ischemia in the heart of rabbits by occluding the left coronary artery using the Langendorff perfusion allowing to test the contractile strength and heart rate. Then, they injected either a vehicle, vehicle with mitochondria, or mitochondria alone which had been thawed after an overnight period at −20°C in the presence of the vehicle. These were injected directly into the ischemic zone just before reperfusion. Interestingly, they observed that the infarcted area was reduced and the functional recovery increased after injecting mitochondria combined with the vehicle. This was not observed in the tissue only injected with mitochondria isolates, meaning that mitochondria must be active in order to serve therapeutic functions, as described in a previous work in vitro [13]. The authors used healthy heart tissue from rabbits as a supply of mitochondria, which limited the impact of the study. This strategy has many translational limitations. The use of other sources, like unharmed tissue from the same donor or other nonvital tissue from other rabbits, would have provided greater evidence for further applications.

Later in 2013, Masuzawa et al. added further assays to sustain the efficacy of the transfer of mitochondria in the ischemic heart model in rabbits [49]. This time, they isolated mitochondria from the pectoral muscle of the same rabbit used for the ischemic shock (autologous transfer). After a follow-up of 28 days, the authors observed that the autologous transplantation of mitochondria was not proarrhythmic: infarct marker levels decreased and the generation of precursor metabolites for energy and cellular respiration increased. Interestingly, they also found that mitochondria were internalized by the cardiomyocytes 2 hours after transplantation, with cardioprotective effects after 28 days. The proposal of a mechanism of mitochondria transfer and their internalization in vitro based on macropinocytosis by Kitani et al. and Kesner et al. [11, 14] was not conclusive. In their latest contribution, McCully's team (2015) observed that the transfer of mitochondria and their in vivo internalization was mediated by actin-dependent endocytosis and not by macropinocytosis. The authors used different inhibitors to prevent the internalization of mitochondria. They used cytochalasin D to inhibit actin polymerization, methyl-β-cyclodextrin (MβCD) to stop endocytosis, and nocadozole to block tunneling nanotubes. They observed that the use of (MβCD) greatly inhibited the uptake process, inferring that internalization is mainly mediated by actin-dependent endocytosis [50]. Further assays need to be developed in vivo to fully understand the process of internalization related to AMT, possible heterogeneity across different tissues, and the effects of the transfer of mitochondria to harmed tissue. Such assays may also help to illuminate ways to improve AMT and address its limitations.

After McCully's experiments in 2013, Lin et al. applied the same procedure to replace damaged mitochondria with healthy ones to mitigate symptoms in the rat model of hepatic ischemia-reperfusion [107]. Both McCully and I-Rue Lai observed that the introduction of mitochondria to diseased ischemic tissue decreased damage and oxidative stress and improved recovery. Despite this success, these experiments were not able to make any conclusions about three factors which may be key to the success of mitochondria therapies: extract concentration, mitochondria viability, and organ-to-mitochondria ratio. With respect to the first factor, neither experiment demonstrated a dose response related to the concentration of isolated mitochondria introduced to the damaged tissue. McCully used $9.7 \times 10^6 \pm 1.5 \times 10^6$/ml of mitochondria isolated from healthy hearts in injections of 0.1 ml, eight times into the affected zone of the ischemic hearts [49]. In his study, I-Rue Lai used a concentration of $7.7 \times 10^6 \pm 1.5 \times 10^6$/ml of mitochondria isolated from healthy livers in one injection of 0.1 ml into the subcapsular region of the spleen poles. Although each concentration yielded therapeutic benefits, neither was established as the optimal injection concentration. Additionally, each study verified the viability of the isolated mitochondria before injecting them into the given tissue using fluorescent probes dependent on membrane potential, including CMTMRos [107], JC1, and respirometry [49]. However, it remains unclear how the mitochondria's state at the moment of injection affects the success of the therapy. We cannot assume that the mitochondria's optimal injection state is simply indicated by activity, because activity is not related to the coupling of the electron transport chain or ROS production, which can damage the mitochondria and the cell [108]. Additionally, these studies did not consider the impact of organ-to-mitochondria ratio, although this factor may have important implications in the successes of mitochondria therapy. McCully and I-Rue Lai's studies are highly important because they demonstrate that AMT in vivo can have therapeutic benefits to the damaged renal and cardiac tissues; perhaps even more impactfully, they have also opened new lines of investigation to help us understand how to optimize these procedures for clinical applications.

In 2014, Sun et al. transferred mitochondria to the damaged lung tissue of adult male rats affected by acute respiratory distress syndrome (ARDS) [109]. In this experiment, Hon-Yap Yip and colleagues transferred mitochondria, melatonin, and mitochondria in combination with melatonin to diseased lung tissue. Melatonin was used because it is a known anti-inflammatory molecule which is protective against lung injury disease [110]. Islam et al.'s previous experiment in which they observed the transfer of mitochondria from bone marrow-derived stromal cells (BMSCs) to harmed lung tissue served as the foundation for Hon-Yap Yip's work [8]. Islam et al. demonstrated that BMSC mitochondria transferred to the affected alveolar epithelia resulted in an increase of cell bioenergetics and had additional protective effects [8]. Taking into consideration the work of Islam et al. [8], Masuzawa et al. [49], and Lin et al. [107], Sun et al. [109] completed onetime intravenous injections of two different mitochondria concentrations (not coupled with melatonin) 750 μg and 1500 μg diluted in IBc buffer in rats 6 hours after inducing ARDS. They did not specify the total volume they injected. In other assays, the authors injected mitochondria in combination with melatonin and melatonin

alone. In both cases, the melatonin was injected in a concentration of 50 mg/kg, 6 and 24 hours after ARDS was induced. Sun et al. [109] observed that the treatment with just mitochondria and mitochondria plus melatonin decreased DNA damage, ROS generation, apoptosis, and the quantity of albumin in bronchoalveolar lavage (BAL), an indicator of capillary leakage in proteins and proinflammatory cytokines such as MMP-9, TNF-α, and NF-κB, [109]. The fact that this study requires a great quantity of mitochondria makes it less likely that it could be successfully applied in larger organisms. However, this transfer method could be better used in localized injections, as put into practice by McCully et al. in their studies [16, 49].

Huang et al. transferred mitochondria isolated from the kidney of young hamsters to rats that had suffered a cerebral stroke induced by middle cerebral artery occlusion (MCAO) [19, 109]. The authors discovered that the administration of the xenogenic mitochondria had protective effects and were associated with a faster recovery of motor performance in the rats. They injected 75 μg of mitochondria diluted in 10 μl of SEH solution (0.25 M sucrose, 0.5 mM ethylene glycol tetraacetic acid (EGTA), and 3 mM N-(2-hydroxyethyl) piperazine-N¢-ethanesulfonic acid (HEPES), pH 7.2) into the ischemic stratum and infused 750 μg in 100 μl into the femoral artery. Interestingly, the authors showed that the direct in situ injection of the isolated mitochondria were more effective in rescuing motor activity than the mitochondria injected through the artery. They also showed that the exogenous mitochondria have a low percentage of internalization in the harmed neural cells, but even so, the low internalization rate seems to be sufficient to exert their protective properties.

The application of the AMT in vivo should be further developed because all investigations to date have only used one or two mitochondria doses [16, 19, 107, 109]; the similarity of this aspect of research protocols limits our understanding of the effects and therapeutic applications of mitochondria. Hon-Yap Yip [109] injected isolated mitochondria intravenously and Hong Lin-Sun [19] injected mitochondria intra-arterially; they compared the limitations of the therapeutic effects of these methods with those of the in situ infusion. It will be important to further study if the regenerative properties of mitochondria injected into the circulatory system are comparable with direct infusion into the damaged site. Some possible limitations of the systemic infusion may include the loss of integrity of the mitochondrial membrane, the delay in arriving to the damaged site, and the possibility that others cell in the circulatory system endocytose the mitochondria, thus restricting the quantity of mitochondria that ultimately arrive to the harmed tissue.

Two trends in the study and application of the AMT between cells have emerged in the last 30 years. In 1982, Clark and Shay created the mitochondrial transformation technique, based on the simple coincubation of isolated mitochondria and cultured cells [9]. In 2009, McCully et al. innovated the direct in vivo approach [16]. These techniques established important questions to guide the future development of AMT. For example, they revealed that mitochondrial integration may not occur equally between cells and that it may be possible for cells with different membrane properties or tissue organization to be similarly transformed. Additionally, they brought into question how isolation techniques affect the mitochondria's functioning and integrity, and if these changes could influence the transfer itself. Lastly, they raised questions about how the genetic patrimony of donor mitochondria could influence the effects of the transferred mitochondria in the host [9]. These questions have been addressed by authors like Spees et al. [7], Katrangi et al. [13], Kitani et al. [11], Kesner et al. [14], Wu et al. [12], and Caicedo et al. [10]; however, work is still necessary in order to be able to apply AMT in clinical settings.

Many relevant questions in the field remain to be answered. For example, it is still unknown how mitochondria from different cells are able to improve or decrease cellular processes. Similarly, the field must also determine whether transferred mitochondria are able to fuse with endogenous ones and communicate correctly with the nucleus, which is essential to their long-term effectiveness. Caicedo et al. previously observed that the transfer of mitochondria from MSCs to cancer cells by MitoCeption in concentrations higher than 1.25 μg (measured in protein) was deleterious for cell proliferation and that even higher concentrations (2.5 μg>) were restrictive for invasion [10]. Recently, Kitani et al. [11] and Kesner et al. [14] observed that the coincubation of isolated mitochondria and cultured cells was sufficient to prompt the cells to internalize the mitochondria, thereby resulting in the repair of cellular function. However, more research is necessary to understand if additional procedures like thermic shock, centrifugation [10], cell penetration by peptides [15] with mitochondria-conjugated beads [91], or introduction by nanoblades [12] can optimize the AMT and induce the desired effects in the recipient cells.

To develop AMT procedures that are easily replicable and effective, it is important to define a mitochondria-isolating procedure standard that allows scientists to obtain pure mitochondria and analyze the effects of transfer. The isolation of mitochondria is based on the conditions and speed of centrifugation, the concentration of the sucrose solution, and some other factors which, when managed incorrectly, can contaminate the mitochondria concentrate and therefore negatively impact the therapeutic effectiveness of the AMT procedure. Contamination can happen because of the structural closeness and functional connections of the mitochondria with other organelles [85]. For example, mitochondria closely interact with the endoplasmic reticulum (ER), together forming the mitochondria-associated membranes (MAMs) which play a crucial role in calcium homeostasis, regulation of lipid metabolism, and autophagy [111, 112]. Due to the tight contact between the MAMs, contamination with ER may be common in most AMT protocols. Similarly, in other cell structures, mitochondria isolation may be contaminated with other organelles with which the mitochondria interact, including the nucleus, lysosomes, and peroxisomes [111, 113–116]. Clark and Shay [9], Katrangi et al. [13], Kitani et al. [11], Kesner et al. [14], Sun et al. [109], and Huang et al. [19] used the classic sucrose gradient with differential centrifugation, and then, Elliot et al.

[101], Chang et al. [102], Macheiner et al. [91], and Lin et al. [107] used experimental kits to guarantee the purity and viability of mitochondria for downstream applications. McCully et al. did not clearly define the type of protocol used for mitochondria isolation for his in vivo procedures [16, 49, 50], but in his latest review [117], he suggested two protocols by Gostimskaya and Galkin [118] and Claude [119] which can rapidly isolate mitochondria. In 2006, Spees et al. briefly mentioned a mitochondria isolation protocol that involves differential centrifugation; however, the article may not have mentioned important details of this protocol because the experiment did not yield any successful internalization of mitochondria in incubation with the recipient cells [7]. It is possible that this protocol unexpectedly failed because of contamination with MAMs, which may influence the internalization of isolated mitochondria, although no work to date has addressed this potential problem.

Mitochondrial tissue specificity or differentiation state should be taken into account when choosing the donor mitochondria. Mitochondria differ in their shape, size, energy production, and metabolic processes among cell types and states of differentiation [120, 121]. During cell proliferation, the mitochondria modifies its dynamics, segregates from others, and fuses with other mitochondria in a process mediated by the expression of Mfn1 and Mfn2 or dynamin-related protein 1 (DRP1) in the OM [122]. Interestingly, the growth factor erv1-like (Gfer) plays an important role in regulating DRP1 in embryonic stem cells (ESCs). When Gfer is not present, DRP1 is highly expressed driving mitochondrial network fragmentation and a decrease of pluripotency in ESCs [123, 124]. Isolating mitochondria from a cell in a specific state like proliferation could prime the mitochondria and influence their impact in recipient cells' mitochondria networks or even their capacity to fuse with endogenous mitochondria. Most of the studies regarding the transfer procedure were performed using mitochondria from differentiated cells like fibroblasts, liver cells, MSCs, and others [7, 9, 13, 90, 93]. Testing different cell states will be important to understand how exogenous mitochondria interact with the endogenous organelles, how the cell's phenotype changes after transfer, and if metabolic reprogramming is possible.

AMT has shown promising results in healing damaged or stressed cells in vitro and in vivo. Understanding their mechanisms of action inside the cell will allow us to explore and mix AMT with other techniques to repair dysfunctional mitochondria. The use of AMT could potentially repair endogenous and damaged mitochondria by introducing healthy copies to recipient cells and inducing a state of heteroplasmy. In heteroplasmy, altered or pathogenic mtDNA exists together with healthy or wild-type mtDNA [125]. A cell eliminates damaged mitochondria carrying altered mtDNA by mitophagy, a key process in maintaining the mitochondria pool quality [126]. Mitochondria pass through fission in which unhealthy copies carrying altered mtDNA, those with low membrane potential, and those with excessive ROS are eliminated mainly by the PTEN-induced kinase 1 (PINK1) and Parkin pathway [127]. In recent years, the field has gained interest in mitophagy because of its implications in maintaining cell viability and stemness [125, 126, 128–130]. The inhibition of rapamycin (mTOR) kinase activity activates mitophagy, thereby enhancing the selection against dysfunctional mitochondria. However, but healthy mitochondria must also be present to induce this process [126]. Using mitophagy inducers could improve mitochondrial function in mitochondrial diseases or other conditions in which the mitochondria metabolism may be altered [131]. Understanding how AMT may induce heteroplasmy in cells carrying mitochondria mutations and encourage the clearance of unhealthy mitochondria copies in order to support the quality control of mitochondria by mitophagy may reveal new therapeutic possibilities [126].

More studies are needed to understand the possible applications and challenges of AMT. Solid preclinical assays must be designed in order to select the best mitochondria donor cells to treat a specific disease. With this approach, tuning the delivery methods of AMT will further facilitate the reconceptualization of mitochondria not only as the powerhouse of the cell but also as active therapeutic agents.

5. Mitochondrial Diseases and Their Potential Treatment by AMT

The pathophysiology of mitochondrial diseases is complex considering that they can be caused by mitochondrial or nuclear genes involved in the correct biogenesis and function of this organelle. At present, medical approaches for the treatment of mitochondrial disease are only palliative. For this reason, mitochondrial transfer techniques could potentially play a curative role in the care of individuals at risk for or those already suffering from mitochondrial disease caused by mutations in their DNA. Primary mitochondria diseases (PMDs) are caused by mitochondrial mutations that are inherited and transmitted by one's maternal lineage. The transmission of these mutations to successive generations causes most of the known mitochondrial disorders such as Leigh syndrome. This syndrome can affect mtDNA and nuclear DNA (nuDNA), which are considered mitochondria-associated genes [132]. Mutations in the mtDNA can occur during life, and the expression of the disease depends on the quantity of mitochondria that are damaged in comparison with healthy copies in the cell. These diseases are secondary mitochondria diseases [47]. Once an individual's cells cross a certain threshold of damaged mitochondria, the disease will manifest [133]. It is in these cases that the artificial transfer of healthy mitochondria to damaged cells or tissue may help to treat the disease.

Although usually considered rare, mitochondrial diseases appear to be more common than ever thought. Recent work establishes population frequencies of at least 1 : 5000 [134, 135]. The first links between mtDNA alterations and mitochondrial disease were established in the year 1988 [136–138], and today, more than 260 disease-causing mutations and 120 mitochondrial genome rearrangements have been classified [139]. Furthermore, carriers of asymptomatic mtDNA mutations are estimated to be 1 : 200 [134], a frequency 25 times higher than that of people actually suffering from mitochondrial disease. Because healthy carriers of genes that cause mitochondrial

diseases likely do not know that they have these mutations, there is an increased risk of them passing on their potentially deleterious genes to their children.

Mitochondrial disorders are extremely difficult to diagnose because they appear with signs and symptoms common in many other non-mitochondrial-related diseases [140]. Therefore, they should be considered as syndromes. Clinical manifestations may affect any system in the body, but since mitochondria act as power stations, the most affected tissues or organs are those that manage and consume a great deal of energy. Accordingly, most common mitochondrial disorders affect the nervous system (including the sensory organs) and the musculoskeletal system. Because the reproductive system also has organs that require a lot of energy, it can also be affected by mitochondrial disease. However, such diseases have not received as much research attention as mitochondrial diseases of other systems, perhaps because they are not life-threatening. The severity of mitochondrial diseases and their negative impact on patients' quality of life emphasize the need for innovative management of these disorders.

With new therapeutic techniques, the manipulation of mitochondrial biology can allow mothers carrying mutations in their mtDNA to have healthy offspring. Used for the first time in humans in 2016, this set of technique is called mitochondria replacement (MRTs) and gives rise to "three-parent babies" [97, 141]. MRT techniques eliminate the majority of mutant mitochondria in the female germ line during very early developmental periods before the baby is even born, thereby preventing mitochondria-related morbidity entirely. One method, pronuclear transfer, transplants the nucleus from a zygote carrying mutant mitochondria to a donor-derived enucleated oocyte with healthy mitochondria [96]. The offspring receives its nuclear genome from the sperm and the nucleus from the original oocyte, which are transplanted into the donor-derived enucleated oocyte which contains cytoplasm and healthy mitochondria. Despite a few successful applications of MRT, these highly invasive techniques can cause difficulties in fertilization because of the high levels of manipulation involved. For example, maternal spindle transfer (MST) transplants the microtubular spindle system with all chromosomes attached (before pronucleus formation) into a healthy oocyte that has had its own spindle fibers removed during the same developmental stage; this technique has proven to be very effective in monkeys [142] but fertilization issues have been observed in some human spindle-transferred oocytes [143]. This technique requires that the embryo be pierced with micropipettes, which not only ruptures the cell membrane, but probably also disrupts cytoplasmic organelles and the cytoskeleton. This invasive procedure is required in all MRT techniques involving nuclear material transfer [143, 144]. Moreover, oocytes must undergo additional manipulation during the necessary ablation of the *zona pellucida* [142, 144]. During spindle transfer, several chemicals including the cancer drugs cytochalasin B and nocodazole are also applied to the cells involved, possibly damaging genetic material in the cells [142, 144]. Additionally, the use of MRTs is ethically controversial because embryos have to be destroyed during the procedure [145]. AMT offers a less invasive alternative to allow mothers with mitochondrial mutations to have healthy children, especially in cases in which there are only palliative treatments available for the given mitochondrial disease [146].

In the future, AMT could be used in conjunction with induced pluripotent stem cells (iPSCs) to model mitochondrial diseases or to generate healthy cells to be reintroduced into the patient. It is thought that diseased somatic cells could be used to generate iPSCs cells, which would then be forced to differentiate into lineage restricted adult stem cells of the specific diseased tissues. Once obtained, these cells would be inoculated with healthy mitochondria by AMT and then injected into the diseased tissue. This combinatory technique may have applications in diseases like mitochondrial retinopathy, which currently has no known curative treatments [147, 148]. It may be possible to combine AMT with currently available techniques used to derive specific retinal progenitors in order to introduce healthy mitochondria copies into diseased cells, thereby repairing the damaged mitochondrial pool and curing the disease [149].

Patients with skeletal muscular syndromes caused by mtDNA damage may also benefit from AMT. One therapeutic technique that may be able to treat such diseases involves the systemic injection of mitochondria to allow them to arrive to the target tissue. However, because the injection could become diluted in the circulatory system, a sufficient number of mitochondria probably would not reach the muscle tissue in order to heal it. The Magnetomitotransfer could offer an alternative to better guide mitochondria into the target tissue by using magnets [91]. Yet another option may be to inject a greater concentration of healthy mitochondria into small local arteries feeding specific muscles or directly into the muscle mass. This approach has been attempted in the heart, a muscle which suffers from mitochondrial damage after ischemic episodes during myocardial infarction [16, 117]. McCully et al. directly injected mitochondria into the infarcted areas and subsequently discovered that the procedure was beneficial to the recovery of the cardiac tissue [16].

The theoretical AMT procedures outlined here may have great therapeutic potential in a range of applications, from curing mitochondrial retinopathies to treating muscular skeletal syndromes [150]. However, in order to unlock the therapeutic potential of AMT, we must address a number of ethical concerns and technical challenges to safely use this techniques in humans. Significant preclinical experimentation must be performed before adapting AMT for clinical trials.

6. Ethical Issues

The UK has become the first country in the world to formally approve the use of mitochondrial donation, both MST and PTN [151]. This regulation was created in October 2015, under the licensing and regulation of the UK Human Fertilization and Embryology Authority (HFEA) [152]. This medical and legal advance gave families with serious mitochondrial diseases a range of possibilities to allow them to have their own genetically healthy children. In 2016, MRTs were deemed ethical by a panel of U.S. experts, provided that the procedures

adhered to certain guidelines. The panel further recommended that MRT only be used to produce male babies, thereby avoiding the transmission of the surrogate donor mitochondria to future generations [153].

Prior to the approval of the UK statutory instrument, there were several round table discussions involving the public which focused on the scientific and ethical implications of MRT. These well-scrutinized debates have engendered both widespread support and significant dismay. Ultimately, the common ground that helped pass this regulation was the understanding that mitochondrial donation can prevent a child from inheriting metabolic disease, thereby offering parents with mitochondrial disease an opportunity to have healthy children. As demonstrated in this case, good regulation helps science to advance, avoid setbacks, and, ultimately, reach patients within a reasonable time frame.

On the other hand, some constituents voiced great disagreement following this debate before and even after the final approval of MRT. The responsible regulatory agencies are now obligated to develop a robust case-by-case licensing protocol which takes into account the technical challenges and ethical complexities of this procedure. This is a crucial component of regulation to avoid setbacks, to facilitate the further development of the MRT, and to continue to provide families affected by mitochondrial disease a way to ensure the health of their babies. In practical terms, for a clinic to be able to carry out mitochondrial donation, it will need to follow a two-stage licensing process: first, it will need to apply for a license and then seek additional authorization to initiate the treatment in a particular case. Having recently concluded this lengthy legalization process, the first babies conceived using MRT following this protocol are expected to be born this year in the UK.

Many experts have recommended that families that use mitochondrial donation should be encouraged but not obligated to take part in long-term follow-up studies in order to monitor any possible effects on children conceived through this technique and on future generations. This post-procedure follow-up has been deemed crucial to ensuring that the development of this technique keeps pace with ethical advancements and the evolving sociopolitical climate of the UK and the wider world. However, by the same token, there are potential pitfalls looming on the horizon. For example, "medical tourism" from countries that lack the technology or the legal approval for such procedures may attract patients to the UK. This may significantly limit clinical follow-up of children conceived with MRT and the eventual identification of any related safety issues [154].

Most of the arguments against MRT are either scientific or ethical in nature. One question that dominates this debate is about the classification of MRT as a medical procedure. Should MRT be considered more similar to egg/sperm donation or tissue/organ donation? Given that mitochondrial donation involves the transfer of genetic but not nuclear material, this has led to uncertainty as to whether it should be regulated as egg or as tissue donation [155]. Many studies have concluded that the genes that contribute to personal characteristics and traits come solely from nuclear DNA [155–157]. In other words, traits arise from a child's mother and father, not the mitochondrial donor. Although interactions between mtDNA and cellular entities including nuclear DNA do exist, there is no evidence that nuclear DNA can be altered through epigenetic or translocation mechanisms. Considering the limited genetic contribution of mitochondrial donors, MRT is more likely to be classified as a procedure similar to tissue donation. The only confirmed traits that could arise from the donor mtDNA are related to energy production; these traits are considered minor in their overall impact on the organism. For example, variations in the mitochondrial genome have been associated with subtle differences in energy metabolism, such as the ability to cope at high altitudes.

Other studies consider the contribution of mtDNA to bioenergetics [158] to be highly important given that mitochondria-related metabolic processes in the brain play an essential role in neurotransmitter release and synaptic plasticity [159]. For this reason, the great metabolic demands of normal brain function make human cognition dependent upon mitochondrial function. Impaired mitochondrial function caused by mtDNA damage may render neurons more susceptible to oxidative injury [160] and thereby allow systemic or environmental factors to exert a noticeable effect on the brain. Certain allelic variants in mtDNA genes often lead to cognitive impairments, and it has even been hypothesized that "mitochondrial dementia" may exist [161]. Supporting this argument, a great deal of other evidence suggests that mitochondrial dysfunction may play a role in psychiatric disorders such as schizophrenia, bipolar disorder, and major depressive disorder. A study of mitochondrial dysfunction in a small cohort found that increased common deletions and decreased gene expression in mitochondria was associated with increased prevalence of psychiatric illnesses [162]. The role of mtDNA in human cognition and in the onset of degenerative diseases requires further clinical investigation in large cohorts.

In many countries, ethical review committees allow parents to decide if they wish to undergo the MRT technique. Although MRT has been successfully applied, there are ongoing questions about the cost benefits of this procedure, especially related to the stress and invasiveness of the technique on the potential mother [163]. An expert UK panel commissioned on evaluating the safety of MRT concluded that the techniques were "not unsafe" based on successful trials completed in mice and monkeys [142]. An important question remains as to whether additional rigorous preclinical safety testing is still required, although the technology has already been approved for use in humans. There is emerging evidence in recent literature which raises concerns regarding MRT. For example, a recent publication highlighted the gradual loss of donor mtDNA in embryonic stem cells (ES cells) derived from MRT embryos and a reversal to the maternal haplotype. The group identified a polymorphism within the conserved sequence box II region of the D-loop as a plausible cause behind the preferential replication of specific mtDNA haplotypes. In addition, they demonstrated that some haplotypes confer proliferative and growth advantages to cells [164]. Another group provides direct evidence of mtDNA involvement in cognitive functioning. In fact, the

Table 1: A comparative description of MST and PNT techniques and ethical concerns.

	MST	PNT
Technical approach	Transfer of nuclear DNA before fertilization	Transfer of nuclear DNA postfertilization
mtDNA carry-over	Lower (1%)	Higher (1-2%)
Risk of chromosomal abnormalities	Higher	Lower
Operator dependent	Yes	Yes
Proof-of-concept In macaque	Yes ($n = 4$)	No
Ethical issues		
Approval (UK)	Yes	Yes
Approach	Selective reproduction	Therapy based on embryo modification
Manipulation and destruction of	Oocytes	Fertilized eggs
Occurs	Preconception	Postconception

association between mtDNA and the functioning of the nervous system during neural development and synaptic activity involves mitochondrial genes. The total substitution of mtDNA modified learning, exploration, and sensory development as well as the anatomy of the brain; all of these changes persisted with age. These findings demonstrate that mitochondrial polymorphisms are not as insignificant as previously believed [165].

Additionally, new evidence has emerged that shows that even low levels of heteroplasmy introduced into human oocytes by mitochondrial carry-over during nuclear transfer often vanish. Low levels of heteroplasmy can sometimes instead result in mtDNA genotypic drift and reversion to the original genotype [166]. It is important not to overinterpret these results, as most of the corresponding experiments were performed in mice. Furthermore, this evidence should not be used as an argument to rethink the approval of MRT, but rather to be considered when performing the recommended follow-up of children born following MRT intervention [164].

It is important to mention that both PNT (pronuclear transfer) and MST were approved by the Human Fertilization and Embryology Authority based on successful studies completed with rodents and nonhuman primates. However, they may not be equally safe because only MST has been tested in large animals. When MST was tested in mature nonhuman primate oocytes (*Macaca mulatta*), it showed normal fertilization, embryo development, and production healthy offspring [142]. A point-by-point comparison between MST and PNT is detailed in Table 1 [167]. The chart details a lower carry-over of mtDNA but higher potential risk of chromosomal abnormalities for MST [168].

AMT which does not involve any kind of nuclear transfer has great potential to satisfy both ethical and safety concerns. Mitoception involves the transferring of mitochondria from a donor to a recipient cell or tissue and does not involve the passing on of nuclear material. Nevertheless, this technique requires a thorough ethical analysis before considering clinical implementation. One central issue is the origin of donor mitochondria. This requires individual analyses of autotransfer, allotransfer, and xenotransfer because each donor source may have unique ethical and biological implications. In autotransfer, the mitochondria from a tissue with a low mtDNA mutation risk could be used to treat a highly compromised organ of the same person. This donor source poses few ethical concerns but entails great biological challenges that require further complex experimentation and the use of animal models to develop. Allotransfer would use mitochondria donations from genetically close family members. Ideally, the human donor and recipient should share the same haplotype [169]. Alternatively, if no close relatives are available, haplotype matching could be considered [170]. Another ethically tricky option for allotransfer is the potential to use the still-viable mitochondria from a dead human relative in treatment [171, 172]. The final donor source option, xenotransfer, involves the transfer of mitochondria from another species to humans [173]. As unorthodox as it may appear, experiments involving this variation of the AMT did not show apparent mismatch effects in the animal-to-animal models [174]. If xenotransfer of mitochondria between animals and humans were to be successfully executed in vitro or in vivo, numerous ethical concerns would need to be addressed before considering any potential clinical applications.

The use of AMT techniques not involving the transfer of nuclear material raises a number of ethical and safety concerns but may also provide new therapeutic options. One of the key ethical debates related to MST involves the birth of babies with "three parents"; AMT may provide a way to solve this debate by allowing the father's mitochondria and not the donor's to populate the zygote. Another relevant concern in biomedical sciences today deals with the donation of organs and tissues from other people or animals for therapeutic purposes. AMT may allow us to bypass these debates by transferring only microscopic organelles rather than the entire cells or organs to deal and treat certain conditions. The applications of AMT ought to be further explored given these possibilities; however, it is crucial to note that moving from in vitro to in vivo and later to clinical applications AMT implies even greater ethical and biosafety hurdles.

Adequately framing the ethical challenges of cutting edge biomedical procedures like AMT is extremely difficult because these debates are, at least in part, anchored in the sociocultural contexts in which they arise [175]. For example,

the UK recently took a major step forward in permitting and regulating the therapeutic applications of MRT by developing a guideline for its use [151]. It should be noted that the UK has been fertile ground for scientific development and that many other countries have not even begun to consider these techniques even for experimentation because of localized ethical and safety concerns [176]. The only way to appropriately address the myriad of ethical and safety issues that impede the global development of this technology is by gathering further evidence on these procedures and facilitating national and international dialogues on these subjects.

7. Conclusions

This review analyzes all current AMT techniques and describes the future steps necessary to develop better in vitro, in vivo, and clinical applications. We focus in providing the first academic work known to globally analyze and summarize the field of AMT. To our knowledge, no other publication has compared and contrasted investigators working in in vitro and in vivo applications of AMT. Our review provides a comprehensive summary of the impact of the mitochondria in the cell, the mechanisms through which it is naturally and artificially transferred, and the ethical implications related to its potential clinical applications. This review gives a concise yet detailed overview of the past, present, and future of AMT, which we hope will orient scientists within and outside of this field and to help them contribute to the progress of this technology.

Keeping in mind our goal to orient other scientists, the authors would like to point out three final observations important to the advancement of this field. With respect to the development of AMT techniques, in vitro and in vivo procedures have evolved in a parallel, rather than in a sequential manner [177, 178]. This is unusual in the biomedical field in which techniques are usually first perfected in vitro and then later further developed in in vivo and clinical applications. While this somewhat unorthodox trajectory has certainly produced valuable knowledge, it is also important to recognize that by "skipping steps" scientists may have left important gaps in our knowledge about AMT.

Another potential avenue for scientific investigation not covered in this review is the study of genetic modification of mitochondria before artificially transferring them. Such modifications could range from slight alterations of the mtDNA in order to better facilitate specific cellular processes to the creation of completely artificial "super" mitochondria [179, 180]. As is the case with other genetically modified organisms, this line of investigation not only raises a whole host of ethical, legal, and biosafety questions but also has the potential to greatly benefit humanity if developed correctly.

Finally, the authors would like to critique the terminology used within the field of AMT in service of better elucidating the uniqueness of AMT and its possible therapeutic applications. Investigators currently use the terms mitochondria transformation [9], transfer [93], and transplant [117, 181] interchangeably to designate the artificial transport of mitochondria from one cell to another via diverse methodologies. According to our criteria, the term that best defines this process is *transfer*. Mitochondria can be effectively transferred in vitro and in vivo via a variety of processes such as MitoCeption [10] and Magnetomitotransfer [91]. Mitochondrial "transformation" engenders misunderstandings because the cell is transformed, not the mitochondria. Additionally, because the term "transplant" has come to be associated with tissue and organ transport between donors and recipients, transfer should be used to denote the transport of subcellular components such as mitochondria from one cell to another in order to distinguish the ethical, legal, and biomedical nuances associated with such procedures and to facilitate more exact discussion of these issues.

In order to continue to advance the development of AMT, it is essential that scientists answer questions key to the functioning of these techniques, troubleshoot the challenges for clinical applications, and resolve the ethical, legal, and biosecurity concerns that will determine if this technology is logistically applicable around the world. Addressing these hurdles will enable our generation to unlock the transformative potential of an organelle that was once merely considered the cell power plant.

Abbreviations

AMT:	Artificial mitochondria transfer
AIF:	Apoptosis-inducing factor
ARDS:	Acute respiratory distress syndrome
BAL:	Bronchoalveolar lavage
BBB:	Blood-brain barrier
BMSCs:	Bone marrow-derived stem cells
CAP:	Chloramphenicol
CM:	Cristae membrane
DRP1:	Dynamin-related protein 1
EF:	Efrapeptin
EGTA:	Ethylene glycol tetraacetic acid
ER:	Endoplasmic reticulum
ESCs:	Embryonic stem cells
EVs:	Extracellular vesicles
FGF-2:	Fibroblast growth factor 2
Gfer:	Growth factor erv1-like
HEPES:	N-(2-hydroxyethyl) piperazine-N′-ethanesulfonic acid
HFEA:	Human Fertilization and Embryology Authority
hMSCs:	Human mesenchymal stem cells
HPH:	Hypoxic pulmonary hypertension
IBM:	Inner boundary membrane
IMM:	Inner mitochondrial membrane
iPSCs:	Induce pluripotent stem cells
LPS:	Lipopolysaccharide
MAMs:	Mitochondria-associated membranes
MCAO:	Middle cerebral artery occlusion
MERRF:	Myoclonic epilepsy with ragged red fiber syndrome
Mfn1:	Mitofusin 1
Mfn2:	Mitofusin 2
MMP-9:	Matrix metalloproteinase-9
MRTs:	Mitochondria replacements

MSCs: Mesenchymal stem cells
MST: Maternal spindle transfer
mtDNA: Mitochondrial DNA
mTOR: Mammalian target of rapamycin
MVs: Microvesicles
MβCD: Methyl-β-cyclodextrin
NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells
nuDNA: Nuclear DNA
OM: Outer membrane
OMM: Outer mitochondria membrane
OX-PHOS: Oxidative phosphorylation
PEG: Polyethylene glycol
PDM: Parkinson disease model
PINK1: PTEN-induced kinase 1
PMDS: Primary mitochondria diseases
PNT: Pronuclear transfer
RAGE: Receptor for advanced glycation end product
ROS: Reactive oxygen species
TNF α: Tumor necrosis factor alpha
TNTs: Tunneling nanotubes
UV: Ultraviolet
VEGF: Vascular endothelial growth factor.

Conflicts of Interest

The authors declare no conflict of interests.

Acknowledgments

The authors acknowledge the support of Caley Mikesell. This work was funded by the Universidad San Francisco, USFQ Collaboration, USFQ Chancellor, USFQ Medical School Grant for research and the USFQ Publication Fund, and the Chilean FONDECYT—1170852, a research grant from CONICYT, the Chilean commission for scientific and technological investigation.

References

[1] A. A. Pittis and T. Gabaldón, "Late acquisition of mitochondria by a host with chimaeric prokaryotic ancestry," *Nature*, vol. 531, no. 7592, pp. 101–104, 2016.

[2] R. S. Rogers and J. Bhattacharya, "When cells become organelle donors," *Physiology*, vol. 28, no. 6, pp. 414–422, 2013.

[3] Y.-C. Hsu, Y.-T. Wu, T.-H. Yu, and Y.-H. Wei, "Mitochondria in mesenchymal stem cell biology and cell therapy: from cellular differentiation to mitochondrial transfer," *Seminars in Cell & Developmental Biology*, vol. 52, pp. 119–131, 2016.

[4] L. Guarente, "Mitochondria—a nexus for aging, calorie restriction, and sirtuins?" *Cell*, vol. 132, no. 2, pp. 171–176, 2008.

[5] P. Sinha, M. N. Islam, S. Bhattacharya, and J. Bhattacharya, "Intercellular mitochondrial transfer: bioenergetic crosstalk between cells," *Current Opinion in Genetics & Development*, vol. 38, pp. 97–101, 2016.

[6] A. Rustom, R. Saffrich, I. Markovic, P. Walther, and H.-H. Gerdes, "Nanotubular highways for intercellular organelle transport," *Science*, vol. 303, no. 5660, pp. 1007–1010, 2004.

[7] J. L. Spees, S. D. Olson, M. J. Whitney, and D. J. Prockop, "Mitochondrial transfer between cells can rescue aerobic respiration," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 5, pp. 1283–1288, 2006.

[8] M. N. Islam, S. R. Das, M. T. Emin et al., "Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury," *Nature Medicine*, vol. 18, no. 5, pp. 759–765, 2012.

[9] M. A. Clark and J. W. Shay, "Mitochondrial transformation of mammalian cells," *Nature*, vol. 295, no. 5850, pp. 605–607, 1982.

[10] A. Caicedo, V. Fritz, J. M. Brondello et al., "MitoCeption as a new tool to assess the effects of mesenchymal stem/stromal cell mitochondria on cancer cell metabolism and function," *Scientific Reports*, vol. 5, p. 9073, 2015.

[11] T. Kitani, D. Kami, S. Matoba, and S. Gojo, "Internalization of isolated functional mitochondria: involvement of macropinocytosis," *Journal of Cellular and Molecular Medicine*, vol. 18, no. 8, pp. 1694–1703, 2014.

[12] T.-H. Wu, E. Sagullo, D. Case et al., "Mitochondrial transfer by photothermal nanoblade restores metabolite profile in mammalian cells," *Cell Metabolism*, vol. 23, no. 5, pp. 921–929, 2016.

[13] E. Katrangi, G. D'Souza, S. V. Boddapati et al., "Xenogenic transfer of isolated murine mitochondria into human ρ0 cells can improve respiratory function," *Rejuvenation Research*, vol. 10, no. 4, pp. 561–570, 2007.

[14] E. E. Kesner, A. Saada-Reich, and H. Lorberboum-Galski, "Characteristics of mitochondrial transformation into human cells," *Scientific Reports*, vol. 6, article 26057, 2016.

[15] H. Margus, K. Padari, and M. Pooga, "Cell-penetrating peptides as versatile vehicles for oligonucleotide delivery," *Molecular Therapy*, vol. 20, no. 3, pp. 525–533, 2012.

[16] J. D. McCully, D. B. Cowan, C. A. Pacak, I. K. Toumpoulis, H. Dayalan, and S. Levitsky, "Injection of isolated mitochondria during early reperfusion for cardioprotection," *American Journal of Physiology. Heart and Circulatory Physiology*, vol. 296, no. 1, pp. H94–H105, 2009.

[17] D. G. Phinney, M. Di Giuseppe, J. Njah et al., "Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs," *Nature Communications*, vol. 6, p. 8472, 2015.

[18] B. Onfelt, S. Nedvetzki, R. K. Benninger et al., "Structurally distinct membrane nanotubes between human macrophages support long-distance vesicular traffic or surfing of bacteria," *The Journal of Immunology*, vol. 177, no. 12, pp. 8476–8483, 2006.

[19] P.-J. Huang, C. C. Kuo, H. C. Lee et al., "Transferring xenogenic mitochondria provides neural protection against ischemic stress in ischemic rat brains," *Cell Transplantation*, vol. 25, no. 5, pp. 913–927, 2016.

[20] J. P. G. Sluijter, V. Verhage, J. C. Deddens, F. van den Akker, and P. A. Doevendans, "Microvesicles and exosomes for intracardiac communication," *Cardiovascular Research*, vol. 102, no. 2, pp. 302–311, 2014.

[21] K. Hayakawa, E. Esposito, X. Wang et al., "Transfer of mitochondria from astrocytes to neurons after stroke," *Nature*, vol. 535, no. 7613, pp. 551–555, 2016.

[22] H.-H. Gerdes, N. V. Bukoreshtliev, and J. F. V. Barroso, "Tunneling nanotubes: a new route for the exchange of components between animal cells," *FEBS Letters*, vol. 581, no. 11, pp. 2194–2201, 2007.

[23] M. Picard, T. Taivassalo, D. Ritchie et al., "Mitochondrial structure and function are disrupted by standard isolation methods," *PLoS One*, vol. 6, no. 3, article e18317, 2011.

[24] I. E. Scheffler, *Mitochondria*, Wiley-Liss, Hoboken, N.J, 2nd edition, 2008.

[25] R. Obar and J. Green, "Molecular archaeology of the mitochondrial geonome," *Journal of Molecular Evolution*, vol. 22, no. 3, pp. 243–251, 1985.

[26] V. Zimorski, C. Ku, W. F. Martin, and S. B. Gould, "Endosymbiotic theory for organelle origins," *Current Opinion in Microbiology*, vol. 22, pp. 38–48, 2014.

[27] G. Burger, M. W. Gray, L. Forget, and B. F. Lang, "Strikingly bacteria-like and gene-rich mitochondrial genomes throughout jakobid protists," *Genome Biology and Evolution*, vol. 5, no. 2, pp. 418–438, 2013.

[28] D. Mokranjac and W. Neupert, "Protein import into mitochondria," *Biochemical Society Transactions*, vol. 33, no. 5, pp. 1019–1023, 2005.

[29] C. D. Meiklejohn, M. A. Holmbeck, M. A. Siddiq, D. N. Abt, D. M. Rand, and K. L. Montooth, "An incompatibility between a mitochondrial tRNA and its nuclear-encoded tRNA synthetase compromises development and fitness in drosophila," *PLoS Genetics*, vol. 9, no. 1, article e1003238, 2013.

[30] C. N. Trier, J. S. Hermansen, G.-P. Sætre, and R. I. Bailey, "Evidence for mito-nuclear and sex-linked reproductive barriers between the hybrid Italian sparrow and its parent species," *PLoS Genetics*, vol. 10, no. 1, article e1004075, 2014.

[31] T. D. Kocher, W. K. Thomas, A. Meyer et al., "Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers," *Proceedings of the National Academy of Sciences*, vol. 86, no. 16, pp. 6196–6200, 1989.

[32] S. D. Ferris, R. D. Sage, C. M. Huang, J. T. Nielsen, U. Ritte, and A. C. Wilson, "Flow of mitochondrial DNA across a species boundary," *Proceedings of the National Academy of Sciences*, vol. 80, no. 8, pp. 2290–2294, 1983.

[33] M. V. Jackson, T. J. Morrison, D. F. Doherty et al., "Mitochondrial transfer via tunneling nanotubes is an important mechanism by which mesenchymal stem cells enhance macrophage phagocytosis in the in vitro and in vivo models of ARDS," *Stem Cells*, vol. 34, no. 8, pp. 2210–2223, 2016.

[34] G. Raposo and W. Stoorvogel, "Extracellular vesicles: exosomes, microvesicles, and friends," *The Journal of Cell Biology*, vol. 200, no. 4, pp. 373–383, 2013.

[35] S. Cogliati, J. A. Enriquez, and L. Scorrano, "Mitochondrial cristae: where beauty meets functionality," *Trends in Biochemical Sciences*, vol. 41, no. 3, pp. 261–273, 2016.

[36] C. R. Hackenbrock, "Ultrastructural bases for metabolically linked mechanical activity in mitochondria," *The Journal of Cell Biology*, vol. 30, no. 2, pp. 269–297, 1966.

[37] L. Pernas and L. Scorrano, "Mito-morphosis: mitochondrial fusion, fission, and cristae remodeling as key mediators of cellular function," *Annual Review of Physiology*, vol. 78, no. 1, pp. 505–531, 2016.

[38] Q. Zhang, M. Raoof, Y. Chen et al., "Circulating mitochondrial DAMPs cause inflammatory responses to injury," *Nature*, vol. 464, no. 7285, pp. 104–107, 2010.

[39] N. N. Danial and S. J. Korsmeyer, "Cell death," *Cell*, vol. 116, no. 2, pp. 205–219, 2004.

[40] T. F. Reubold and S. Eschenburg, "A molecular view on signal transduction by the apoptosome," *Cellular Signalling*, vol. 24, no. 7, pp. 1420–1425, 2012.

[41] S. Yuan and C. W. Akey, "Apoptosome structure, assembly, and procaspase activation," *Structure*, vol. 21, no. 4, pp. 501–515, 2013.

[42] C. Thornton and H. Hagberg, "Role of mitochondria in apoptotic and necroptotic cell death in the developing brain," *Clinica Chimica Acta*, vol. 451, Part A, pp. 35–38, 2015.

[43] S. Yuan, X. Yu, M. Topf, S. J. Ludtke, X. Wang, and C. W. Akey, "Structure of an apoptosome-procaspase-9 CARD complex," *Structure*, vol. 18, no. 5, pp. 571–583, 2010.

[44] R. Rabl, V. Soubannier, R. Scholz et al., "Formation of cristae and crista junctions in mitochondria depends on antagonism between Fcj1 and Su e/g," *The Journal of Cell Biology*, vol. 185, no. 6, pp. 1047–1063, 2009.

[45] T. G. Frey and C. A. Mannella, "The internal structure of mitochondria," *Trends in Biochemical Sciences*, vol. 25, no. 7, pp. 319–324, 2000.

[46] P. Paumard, J. Vaillier, B. Coulary et al., "The ATP synthase is involved in generating mitochondrial cristae morphology," *The EMBO Journal*, vol. 21, no. 3, pp. 221–230, 2002.

[47] J. B. Stewart and P. F. Chinnery, "The dynamics of mitochondrial DNA heteroplasmy: implications for human health and disease," *Nature Reviews Genetics*, vol. 16, no. 9, pp. 530–542, 2015.

[48] S. Meeusen, R. DeVay, J. Block et al., "Mitochondrial inner-membrane fusion and crista maintenance requires the dynamin-related GTPase Mgm1," *Cell*, vol. 127, no. 2, pp. 383–395, 2006.

[49] A. Masuzawa, K. M. Black, C. A. Pacak et al., "Transplantation of autologously derived mitochondria protects the heart from ischemia-reperfusion injury," *American Journal of Physiology. Heart and Circulatory Physiology*, vol. 304, no. 7, pp. H966–H982, 2013.

[50] C. A. Pacak, J. M. Preble, H. Kondo et al., "Actin-dependent mitochondrial internalization in cardiomyocytes: evidence for rescue of mitochondrial function," *Biology Open*, vol. 4, no. 5, pp. 622–626, 2015.

[51] C. D. L. Folmes, P. P. Dzeja, T. J. Nelson, and A. Terzic, "Metabolic plasticity in stem cell homeostasis and differentiation," *Cell Stem Cell*, vol. 11, no. 5, pp. 596–606, 2012.

[52] J. M. Facucho-Oliveira and J. C. St John, "The relationship between pluripotency and mitochondrial DNA proliferation during early embryo development and embryonic stem cell differentiation," *Stem Cell Reviews*, vol. 5, no. 2, pp. 140–158, 2009.

[53] A. Vazquez-Martin, B. Corominas-Faja, S. Cufi et al., "The mitochondrial H+-ATP synthase and the lipogenic switch," *Cell Cycle*, vol. 12, no. 2, pp. 207–218, 2013.

[54] Y.-C. Hsu, C.-T. Chen, and Y.-H. Wei, "Mitochondrial resetting and metabolic reprogramming in induced pluripotent stem cells and mitochondrial disease modeling," *Biochimica et Biophysica Acta*, vol. 1860, no. 4, pp. 686–693, 2016.

[55] M. V. Berridge, R. T. Schneider, and M. J. McConnell, "Mitochondrial transfer from astrocytes to neurons following ischemic insult: guilt by association?" *Cell Metabolism*, vol. 24, no. 3, pp. 376–378, 2016.

[56] Y. M. Cho, H. W. Jeong, J. H. Sohn, D. B. Seo, W. G. Kim, and S. J. Lee, "Mesenchymal stem cells transfer mitochondria to

the cells with virtually no mitochondrial function but not with pathogenic mtDNA mutations," *PloS One*, vol. 7, no. 3, article e32778, 2012.

[57] C. H. Davis, K. Y. Kim, E. A. Bushong et al., "Transcellular degradation of axonal mitochondria," *Proceedings of the National Academy of Sciences*, vol. 111, no. 26, pp. 9633–9638, 2014.

[58] T. Ahmad, S. Mukherjee, B. Pattnaik et al., "Miro1 regulates intercellular mitochondrial transport & enhances mesenchymal stem cell rescue efficacy," *The EMBO Journal*, vol. 33, no. 9, pp. 994–1010, 2014.

[59] S. Domhan, L. Ma, A. Tai et al., "Intercellular communication by exchange of cytoplasmic material via tunneling nano-tube like structures in primary human renal epithelial cells," *PloS One*, vol. 6, no. 6, article e21283, 2011.

[60] D. Torralba, F. Baixauli, and F. Sánchez-Madrid, "Mitochondria know no boundaries: mechanisms and functions of intercellular mitochondrial transfer," *Frontiers in Cell and Development Biology*, vol. 4, p. 107, 2016.

[61] K. Gousset, L. Marzo, P.-H. Commere, and C. Zurzolo, "Myo10 is a key regulator of TNT formation in neuronal cells," *Journal of Cell Science*, vol. 126, Part 19, pp. 4424–4435, 2013.

[62] V. Sanchez and A. Brusco, "Mitochondrial Transfer by Intercellular Nanotubes," in *Biochemistry of Oxidative Stress*, R. J. Gelpi, A. Boveris and J. J. Poderoso, Eds., pp. 95–108, Springer International Publishing, 2016, http://link.springer.com/chapter/10.1007/978-3-319-45865-6_7.

[63] E. Y. Plotnikov, V. A. Babenko, D. N. Silachev et al., "Intercellular transfer of mitochondria," *Biochemistry (Moscow)*, vol. 80, no. 5, pp. 542–548, 2015.

[64] M.-L. Vignais, A. Caicedo, J.-M. Brondello, and C. Jorgensen, "Cell Connections by Tunneling Nanotubes: Effects of Mitochondrial Trafficking on Target Cell Metabolism, Homeostasis, and Response to Therapy," *Stem Cells International*, vol. 2017, Article ID 6917941, 14 pages, 2017.

[65] N. V. Bukoreshtliev, X. Wang, E. Hodneland, S. Gurke, J. F. V. Barroso, and H.-H. Gerdes, "Selective block of tunneling nanotube (TNT) formation inhibits intercellular organelle transfer between PC12 cells," *FEBS Letters*, vol. 583, no. 9, pp. 1481–1488, 2009.

[66] X. Sun, Y. Wang, J. Zhang et al., "Tunneling-nanotube direction determination in neurons and astrocytes," *Cell Death & Disease*, vol. 3, article e438, 2012.

[67] M. Koyanagi, R. P. Brandes, J. Haendeler, A. M. Zeiher, and S. Dimmeler, "Cell-to-cell connection of endothelial progenitor cells with cardiac myocytes by nanotubes: a novel mechanism for cell fate changes?" *Circulation Research*, vol. 96, no. 10, pp. 1039–1041, 2005.

[68] H. Yang, T. K. Borg, Z. Ma et al., "Biochip-based study of unidirectional mitochondrial transfer from stem cells to myocytes via tunneling nanotubes," *Biofabrication*, vol. 8, no. 1, article 15012, 2016.

[69] J. W. Ady, S. Desir, V. Thayanithy et al., "Intercellular communication in malignant pleural mesothelioma: properties of tunneling nanotubes," *Frontiers in Physiology*, vol. 5, p. 400, 2014.

[70] J. M. Pitt, G. Kroemer, and L. Zitvogel, "Extracellular vesicles: masters of intercellular communication and potential clinical interventions," *The Journal of Clinical Investigation*, vol. 126, no. 4, pp. 1139–1143, 2016.

[71] P. D. Robbins and A. E. Morelli, "Regulation of immune responses by extracellular vesicles," *Nature Reviews Immunology*, vol. 14, no. 3, pp. 195–208, 2014.

[72] V. Zappulli, K. P. Friis, Z. Fitzpatrick, C. A. Maguire, and X. O. Breakefield, "Extracellular vesicles and intercellular communication within the nervous system," *The Journal of Clinical Investigation*, vol. 126, no. 4, pp. 1198–1207, 2016.

[73] H. Valadi, K. Ekström, A. Bossios, M. Sjöstrand, J. J. Lee, and J. O. Lötvall, "Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells," *Nature Cell Biology*, vol. 9, no. 6, pp. 654–659, 2007.

[74] M. Guescini, S. Genedani, V. Stocchi, and L. F. Agnati, "Astrocytes and glioblastoma cells release exosomes carrying mtDNA," *Journal of Neural Transmission*, vol. 117, no. 1, pp. 1–4, 2010.

[75] V. Budnik, C. Ruiz-Cañada, and F. Wendler, "Extracellular vesicles round off communication in the nervous system," *Nature Reviews Neuroscience*, vol. 17, no. 3, pp. 160–172, 2016.

[76] C. Lee, S. A. Mitsialis, M. Aslam et al., "Exosomes mediate the cytoprotective action of mesenchymal stromal cells on hypoxia-induced pulmonary hypertension," *Circulation*, vol. 126, no. 22, pp. 2601–2611, 2012.

[77] K. G. Lyamzaev, O. K. Nepryakhina, V. B. Saprunova et al., "Novel mechanism of elimination of malfunctioning mitochondria (mitoptosis): formation of mitoptotic bodies and extrusion of mitochondrial material from the cell," *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, vol. 1777, no. 7-8, pp. 817–825, 2008.

[78] S. Yousefi, J. A. Gold, N. Andina et al., "Catapult-like release of mitochondrial DNA by eosinophils contributes to antibacterial defense," *Nature Medicine*, vol. 14, no. 9, pp. 949–953, 2008.

[79] K. Unuma, T. Aki, T. Funakoshi, K. Hashimoto, and K. Uemura, "Extrusion of mitochondrial contents from lipopolysaccharide-stimulated cells: involvement of autophagy," *Autophagy*, vol. 11, no. 9, pp. 1520–1536, 2015.

[80] A. Maeda and B. Fadeel, "Mitochondria released by cells undergoing TNF-α-induced necroptosis act as danger signals," *Cell Death & Disease*, vol. 5, article e1312, 2014.

[81] A. Nakajima, H. Kurihara, H. Yagita, K. Okumura, and H. Nakano, "Mitochondrial extrusion through the cytoplasmic vacuoles during cell death," *The Journal of Biological Chemistry*, vol. 283, no. 35, pp. 24128–24135, 2008.

[82] L. H. Boudreau, A. C. Duchez, N. Cloutier et al., "Platelets release mitochondria serving as substrate for bactericidal group IIA-secreted phospholipase A2 to promote inflammation," *Blood*, vol. 124, no. 14, pp. 2173–2183, 2014.

[83] J. Godlewski, A. M. Krichevsky, M. D. Johnson, E. A. Chiocca, and A. Bronisz, "Belonging to a network—microRNAs, extracellular vesicles, and the glioblastoma microenvironment," *Neuro-Oncology*, vol. 17, no. 5, pp. 652–662, 2015.

[84] I. V.-S. Louis and P. R. Bohjanen, "Post-transcriptional Regulation of Cytokine Signaling During Inflammatory Responses," in *Post-transcriptional Mechanisms in Endocrine Regulation*, K. M. J. Menon and A. Goldstrohm, Eds., pp. 55–70, Springer International Publishing, 2016, http://link.springer.com/chapter/10.1007/978-3-319-25124-0_3.

[85] P. Azimzadeh, H. Asadzadeh Aghdaei, P. Tarban, M. M. Akhondi, A. Shirazi, and H. R. Khorram Khorshid,

"Comparison of three methods for mitochondria isolation from the human liver cell line (HepG2)," *Gastroenterology and Hepatology from Bed to Bench*, vol. 9, no. 2, pp. 105–113, 2016.

[86] M. Picard, D. C. Wallace, and Y. Burelle, "The rise of mitochondria in medicine," *Mitochondrion*, vol. 30, pp. 105–116, 2016.

[87] K. Birsoy, T. Wang, W. W. Chen, E. Freinkman, M. Abu-Remaileh, and D. M. Sabatini, "An essential role of the mitochondrial electron transport chain in cell proliferation is to enable aspartate synthesis," *Cell*, vol. 162, no. 3, pp. 540–551, 2015.

[88] F. K. Teixeira, C. G. Sanchez, T. R. Hurd et al., "ATP synthase promotes germ cell differentiation independent of oxidative phosphorylation," *Nature Cell Biology*, vol. 17, no. 5, pp. 689–696, 2015.

[89] A. Koziel, I. Sobieraj, and W. Jarmuszkiewicz, "Increased activity of mitochondrial uncoupling protein 2 improves stress resistance in cultured endothelial cells exposed in vitro to high glucose levels," *American Journal of Physiology Heart and Circulatory Physiology*, vol. 309, no. 1, pp. H147–H156, 2015.

[90] M. P. King and G. Attardi, "Injection of mitochondria into human cells leads to a rapid replacement of the endogenous mitochondrial DNA," *Cell*, vol. 52, no. 6, pp. 811–819, 1988.

[91] T. Macheiner, V. H. Fengler, M. Agreiter et al., "Magnetomitotransfer: an efficient way for direct mitochondria transfer into cultured human cells," *Scientific Reports*, vol. 6, p. 35571, 2016.

[92] A. Diot, E. Dombi, T. Lodge et al., "Modulating mitochondrial quality in disease transmission: towards enabling mitochondrial DNA disease carriers to have healthy children," *Biochemical Society Transactions*, vol. 44, no. 4, pp. 1091–1100, 2016.

[93] C. A. Pinkert, M. H. Irwin, L. W. Johnson, and R. J. Moffatt, "Mitochondria transfer into mouse ova by microinjection," *Transgenic Research*, vol. 6, no. 6, pp. 379–383, 1997.

[94] Y.-C. Yi, M.-J. Chen, J. Y.-P. Ho, H.-F. Guu, and E. S.-C. Ho, "Mitochondria transfer can enhance the murine embryo development," *Journal of Assisted Reproduction and Genetics*, vol. 24, no. 10, pp. 445–449, 2007.

[95] K. Takeda, M. Tasai, S. Akagi et al., "Microinjection of serum-starved mitochondria derived from somatic cells affects parthenogenetic development of bovine and murine oocytes," *Mitochondrion*, vol. 10, no. 2, pp. 137–142, 2010.

[96] P. Amato, M. Tachibana, M. Sparman, and S. Mitalipov, "Three-parent in vitro fertilization: gene replacement for the prevention of inherited mitochondrial diseases," *Fertility and Sterility*, vol. 101, no. 1, pp. 31–35, 2014.

[97] X. Zhang and S. Wang, "From the first human gene-editing to the birth of three-parent baby," *Science China Life Sciences*, vol. 59, no. 12, pp. 1341-1342, 2016.

[98] Q. Sun, E. S. Cibas, H. Huang, L. Hodgson, and M. Overholtzer, "Induction of entosis by epithelial cadherin expression," *Cell Research*, vol. 24, no. 11, pp. 1288–1298, 2014.

[99] Y. G. Yoon, C. L. Haug, and M. D. Koob, "Interspecies mitochondrial fusion between mouse and human mitochondria is rapid and efficient," *Mitochondrion*, vol. 7, no. 3, pp. 223–229, 2007.

[100] L. Kenyon and C. T. Moraes, "Expanding the functional human mitochondrial DNA database by the establishment of primate xenomitochondrial cybrids," *Proceedings of the National Academy of Sciences*, vol. 94, no. 17, pp. 9131–9135, 1997.

[101] R. L. Elliott, X. P. Jiang, and J. F. Head, "Mitochondria organelle transplantation: introduction of normal epithelial mitochondria into human cancer cells inhibits proliferation and increases drug sensitivity," *Breast Cancer Research and Treatment*, vol. 136, no. 2, pp. 347–354, 2012.

[102] J.-C. Chang, K. H. Liu, Y. C. Li et al., "Functional recovery of human cells harbouring the mitochondrial DNA mutation MERRF A8344G via peptide-mediated mitochondrial delivery," *Neurosignals*, vol. 21, no. 3-4, pp. 160–173, 2013.

[103] J.-C. Chang, S. L. Wu, K. H. Liu et al., "Allogeneic/xenogeneic transplantation of peptide-labeled mitochondria in Parkinson's disease: restoration of mitochondria functions and attenuation of 6-hydroxydopamine-induced neurotoxicity," *Translational Research*, vol. 170, pp. 40–56, 2016, e3.

[104] J. P. Lim and P. A. Gleeson, "Macropinocytosis: an endocytic pathway for internalising large gulps," *Immunology and Cell Biology*, vol. 89, no. 8, pp. 836–843, 2011.

[105] M. Bohdanowicz and S. Grinstein, "Role of phospholipids in endocytosis, phagocytosis, and macropinocytosis," *Physiological Reviews*, vol. 93, no. 1, pp. 69–106, 2013.

[106] S. van Wilpe, M. T. Ryan, K. Hill et al., "Tom22 is a multifunctional organizer of the mitochondrial preprotein translocase," *Nature*, vol. 401, no. 6752, pp. 485–489, 1999.

[107] H.-C. Lin, S.-Y. Liu, H.-S. Lai, and I.-R. Lai, "Isolated mitochondria infusion mitigates ischemia-reperfusion injury of the liver in rats," *Shock*, vol. 39, no. 3, pp. 304–310, 2013.

[108] M. K. C. Hesselink, V. Schrauwen-Hinderling, and P. Schrauwen, "Skeletal muscle mitochondria as a target to prevent or treat type 2 diabetes mellitus," *Nature Reviews Endocrinology*, vol. 12, no. 11, pp. 633–645, 2016.

[109] C.-K. Sun, F. Y. Lee, Y. H. Kao et al., "Systemic combined melatonin-mitochondria treatment improves acute respiratory distress syndrome in the rat," *Journal of Pineal Research*, vol. 58, no. 2, pp. 137–150, 2015.

[110] S. Honiden and M. N. Gong, "Diabetes, insulin, and development of acute lung injury," *Critical Care Medicine*, vol. 37, no. 8, pp. 2455–2464, 2009.

[111] M. R. Wieckowski, C. Giorgi, M. Lebiedzinska, J. Duszynski, and P. Pinton, "Isolation of mitochondria-associated membranes and mitochondria from animal tissues and cells," *Nature Protocols*, vol. 4, no. 11, pp. 1582–1590, 2009.

[112] A. Danese, S. Patergnani, M. Bonora et al., "Calcium regulates cell death in cancer: roles of the mitochondria and mitochondria-associated membranes (MAMs)," *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 2017, in Press, http://www.sciencedirect.com/science/article/pii/S0005272 81730004X.

[113] K. Diekert, A. I. P. M. de Kroon, G. Kispal, and R. Lill, "Chapter 2 isolation and subfractionation of mitochondria from the yeast *Saccharomyces cerevisiae*," *Methods in Cell Biology*, vol. 65, pp. 37–51, 2001.

[114] J. Avruch and D. F. Hoelzl Wallach, "Preparation and properties of plasma membrane and endoplasmic reticulum fragments from isolated rat fat cells," *Biochimica et Biophysica Acta (BBA) - Biomembranes*, vol. 233, no. 2, pp. 334–347, 1971.

[115] H.-T. Hornig-Do, G. Günther, M. Bust, P. Lehnartz, A. Bosio, and R. J. Wiesner, "Isolation of functional pure mitochondria by superparamagnetic microbeads," *Analytical Biochemistry*, vol. 389, no. 1, pp. 1–5, 2009.

[116] P. Bozidis, C. D. Williamson, and A. M. Colberg-Poley, "Isolation of Endoplasmic Reticulum, Mitochondria, and Mitochondria-Associated Membrane Fractions from Transfected Cells and from Human Cytomegalovirus-Infected Primary Fibroblasts," in *Current Protocols in Cell Biology*, vol. 37, no. 3.27, pp. 3.27.1–3.27.23, 2007.

[117] J. D. McCully, S. Levitsky, P. J. del Nido, and D. B. Cowan, "Mitochondrial transplantation for therapeutic use," *Clinical and Translational Medicine*, vol. 5, 2016.

[118] I. Gostimskaya and A. Galkin, "Preparation of highly coupled rat heart mitochondria," *JoVE (Journal of Visualized Experiments)*, no. 43, article e2202, 2010.

[119] A. Claude, "Fractionation of mammalian liver cells by differential centrifugation: II. Experimental procedures and results," *The Journal of Experimental Medicine*, vol. 84, no. 1, pp. 61–89, 1946.

[120] S. Hoppins, "The regulation of mitochondrial dynamics," *Current Opinion in Cell Biology*, vol. 29, pp. 46–52, 2014.

[121] X. Xu, S. Duan, F. Yi, A. Ocampo, G.-H. Liu, and J. C. Izpisua Belmonte, "Mitochondrial regulation in pluripotent stem cells," *Cell Metabolism*, vol. 18, no. 3, pp. 325–332, 2013.

[122] P. Mishra and D. C. Chan, "Mitochondrial dynamics and inheritance during cell division, development and disease," *Nature Reviews. Molecular Cell Biology*, vol. 15, no. 10, pp. 634–646, 2014.

[123] L. R. Todd, M. N. Damin, R. Gomathinayagam, S. R. Horn, A. R. Means, and U. Sankar, "Growth factor ero1-like modulates Drp1 to preserve mitochondrial dynamics and function in mouse embryonic stem cells," *Molecular Biology of the Cell*, vol. 21, no. 7, pp. 1225–1236, 2010.

[124] C. Hu, Y. Huang, and L. Li, "Drp1-dependent mitochondrial fission plays critical roles in physiological and pathological progresses in mammals," *International Journal of Molecular Sciences*, vol. 18, no. 1, 2017.

[125] N. P. Kandul, T. Zhang, B. A. Hay, and M. Guo, "Selective removal of deletion-bearing mitochondrial DNA in heteroplasmic drosophila," *Nature Communications*, vol. 7, 2016.

[126] Y. Dai, K. Zheng, J. Clark et al., "Rapamycin drives selection against a pathogenic heteroplasmic mitochondrial DNA mutation," *Human Molecular Genetics*, vol. 23, no. 3, pp. 637–647, 2014.

[127] S. Geisler, K. M. Holmström, D. Skujat et al., "PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1," *Nature Cell Biology*, vol. 12, no. 2, pp. 119–131, 2010.

[128] P. M. Aponte and A. Caicedo, "Stemness in cancer: stem cells, cancer stem cells, and their microenvironment," *Stem Cells International*, vol. 2017, Article ID 5619472, 17 pages, 2017.

[129] T. E. S. Kauppila, J. H. K. Kauppila, and N.-G. Larsson, "Mammalian mitochondria and aging: an update," *Cell Metabolism*, vol. 25, no. 1, pp. 57–71, 2017.

[130] M. A. Teitell, "Adult stem-like cells exclude 'older' mitochondria," *Cell Metabolism*, vol. 21, no. 5, pp. 658-659, 2015.

[131] M. Villanueva Paz, D. Cotán, J. Garrido-Maraver et al., "Targeting autophagy and mitophagy for mitochondrial diseases treatment," *Expert Opinion on Therapeutic Targets*, vol. 20, no. 4, pp. 487–500, 2016.

[132] D. M. Niyazov, S. G. Kahler, and R. E. Frye, "Primary mitochondrial disease and secondary mitochondrial dysfunction: importance of distinction for diagnosis and treatment," *Molecular Syndromology*, vol. 7, no. 3, pp. 122–137, 2016.

[133] G. J. Farrar, N. Chadderton, P. F. Kenna, and S. Millington-Ward, "Mitochondrial disorders: aetiologies, models systems, and candidate therapies," *Trends in Genetics*, vol. 29, no. 8, pp. 488–497, 2013.

[134] H. R. Elliott, D. C. Samuels, J. A. Eden, C. L. Relton, and P. F. Chinnery, "Pathogenic mitochondrial DNA mutations are common in the general population," *American Journal of Human Genetics*, vol. 83, no. 2, pp. 254–260, 2008.

[135] A. M. Schaefer, R. McFarland, E. L. Blakely et al., "Prevalence of mitochondrial DNA disease in adults," *Annals of Neurology*, vol. 63, no. 1, pp. 35–39, 2008.

[136] I. J. Holt, A. E. Harding, and J. A. Morgan-Hughes, "Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies," *Nature*, vol. 331, no. 6158, pp. 717–719, 1988.

[137] D. C. Wallace, G. Singh, M. T. Lott et al., "Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy," *Science*, vol. 242, no. 4884, pp. 1427–1430, 1988.

[138] D. C. Wallace, X. X. Zheng, M. T. Lott et al., "Familial mitochondrial encephalomyopathy (MERRF): genetic, pathophysiological, and biochemical characterization of a mitochondrial DNA disease," *Cell*, vol. 55, no. 4, pp. 601–610, 1988.

[139] A. Herrera, I. Garcia, N. Gaytan, E. Jones, A. Maldonado, and R. Gilkerson, "Endangered species: mitochondrial DNA loss as a mechanism of human disease," *Frontiers in Bioscience (Scholar Edition)*, vol. 7, pp. 109–124, 2015.

[140] N. A. Khan, P. Govindaraj, A. K. Meena, and K. Thangaraj, "Mitochondrial disorders: challenges in diagnosis & treatment," *The Indian Journal of Medical Research*, vol. 141, no. 1, pp. 13–26, 2015.

[141] J. Couzin-Frankel, "Unanswered questions surround baby born to three parents," Science/AAAS, 2016, February 2017, http://www.sciencemag.org/news/2016/09/unanswered-questions-surround-baby-born-three-parents.

[142] M. Tachibana, M. Sparman, H. Sritanaudomchai et al., "Mitochondrial gene replacement in primate offspring and embryonic stem cells," *Nature*, vol. 461, no. 7262, pp. 367–372, 2009.

[143] M. Tachibana, P. Amato, M. Sparman et al., "Towards germline gene therapy of inherited mitochondrial diseases," *Nature*, vol. 493, no. 7434, pp. 627–631, 2013.

[144] L. Craven, H. A. Tuppen, G. D. Greggains et al., "Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease," *Nature*, vol. 465, no. 7294, pp. 82–85, 2010.

[145] J. Lyon, "Sanctioned UK trial of mitochondrial transfer nears," *JAMA*, vol. 317, no. 5, pp. 462–464, 2017.

[146] A. Suomalainen, "Therapy for mitochondrial disorders: little proof, high research activity, some promise," *Seminars in Fetal & Neonatal Medicine*, vol. 16, no. 4, pp. 236–240, 2011.

[147] C. M. Ramsden, M. B. Powner, A.-J. Carr, M. J. Smart, L. da Cruz, and P. J. Coffey, "Stem cells in retinal regeneration: past, present and future," *Development (Cambridge, England)*, vol. 140, no. 12, pp. 2576–2585, 2013.

[148] M. Takahashi, "Retinal cell therapy using iPS cells," *Nippon Ganka Gakkai Zasshi*, vol. 120, no. 3, pp. 210–225, 2016.

[149] J. Reynolds and D. A. Lamba, "Human embryonic stem cell applications for retinal degenerations," *Experimental Eye Research*, vol. 123, pp. 151–160, 2014.

[150] G. K. Sakellariou, T. Pearson, A. P. Lightfoot et al., "Mitochondrial ROS regulate oxidative damage and mitophagy but not age-related muscle fiber atrophy," *Scientific Reports*, vol. 6, 2016.

[151] M. Herbert and D. Turnbull, "Mitochondrial donation—clearing the final regulatory hurdle in the United Kingdom," *The New England Journal of Medicine*, vol. 376, no. 2, pp. 171–173, 2017.

[152] M. J. Falk, A. Dechemey, and J. P. Kahn, "Mitochondrial replacement techniques—implications for the clinical community," *The New England Journal of Medicine*, vol. 374, no. 12, pp. 1103–1106, 2016.

[153] A. B. Claiborne, R. A. English, and J. P. Kahn, "Ethics of new technologies. Finding an ethical path forward for mitochondrial replacement," *Science*, vol. 351, no. 6274, pp. 668–670, 2016.

[154] J. Schandera and T. K. Mackey, "Mitochondrial replacement techniques: divergence in global policy," *Trends in Genetics*, vol. 32, no. 7, pp. 385–390, 2016.

[155] R. Dimond, "Social and ethical issues in mitochondrial donation," *British Medical Bulletin*, vol. 115, no. 1, pp. 173–182, 2015.

[156] R. T. K. Pang and P. C. Ho, "Designer babies," *Obstetrics, Gynaecology and Reproductive Medicine*, vol. 26, no. 2, pp. 59-60, 2016.

[157] F. Baylis, "The ethics of creating children with three genetic parents," *Reproductive Biomedicine Online*, vol. 26, no. 6, pp. 531–534, 2013.

[158] M. Grazina, J. Pratas, F. Silva, S. Oliveira, I. Santana, and C. Oliveira, "Genetic basis of Alzheimer's dementia: role of mtDNA mutations," *Genes, Brain, and Behavior*, vol. 5, pp. 92–107, 2006.

[159] F. M. Menzies, M. R. Cookson, R. W. Taylor et al., "Mitochondrial dysfunction in a cell culture model of familial amyotrophic lateral sclerosis," *Brain*, vol. 125, no. 7, pp. 1522–1533, 2002.

[160] S. M. de la Monte, T. Luong, T. R. Neely, D. Robinson, and J. R. Wands, "Mitochondrial DNA damage as a mechanism of cell loss in Alzheimer's disease," *Laboratory Investigation*, vol. 80, no. 8, pp. 1323–1335, 2000.

[161] J. Finsterer, "Cognitive decline as a manifestation of mitochondrial disorders (mitochondrial dementia)," *Journal of the Neurological Sciences*, vol. 272, no. 1-2, pp. 20–33, 2008.

[162] L. Shao, M. V. Martin, S. J. Watson et al., "Mitochondrial involvement in psychiatric disorders," *Annals of Medicine*, vol. 40, no. 4, pp. 281–295, 2008.

[163] Mitochondrial replacement: no need for a rethink," New Scientist, April 2017, https://www.newscientist.com/article/dn26400-mitochondrial-replacement-no-need-for-a-rethink/.

[164] E. Kang, J. Wu, N. M. Gutierrez et al., "Mitochondrial replacement in human oocytes carrying pathogenic mitochondrial DNA mutations," *Nature*, vol. 540, no. 7632, pp. 270–275, 2016.

[165] P. L. Roubertoux, F. Sluyter, M. Carlier et al., "Mitochondrial DNA modifies cognition in interaction with the nuclear genome and age in mice," *Nature Genetics*, vol. 35, no. 1, pp. 65–69, 2003.

[166] M. Yamada, V. Emmanuele, M. J. Sanchez-Quintero et al., "Genetic drift can compromise mitochondrial replacement by nuclear transfer in human oocytes," *Cell Stem Cell*, vol. 18, no. 6, pp. 749–754, 2016.

[167] K. Reinhardt, D. K. Dowling, and E. H. Morrow, "Medicine. Mitochondrial replacement, evolution, and the clinic," *Science*, vol. 341, no. 6152, pp. 1345-1346, 2013.

[168] D. P. Wolf, N. Mitalipov, and S. Mitalipov, "Mitochondrial replacement therapy in reproductive medicine," *Trends in Molecular Medicine*, vol. 21, no. 2, pp. 68–76, 2015.

[169] J. P. Burgstaller, I. G. Johnston, and J. Poulton, "Mitochondrial DNA disease and developmental implications for reproductive strategies," *Molecular Human Reproduction*, vol. 21, no. 1, pp. 11–22, 2015.

[170] P. F. Chinnery, L. Craven, S. Mitalipov, J. B. Stewart, M. Herbert, and D. M. Turnbull, "The challenges of mitochondrial replacement," *PLoS Genetics*, vol. 10, no. 4, article e1004315, 2014.

[171] R. Steinbrook, "Organ donation after cardiac death," *The New England Journal of Medicine*, vol. 357, no. 3, pp. 209–213, 2007.

[172] S. Stevens, "Synthetic biology in cell and organ transplantation," *Cold Spring Harbor Perspectives in Biology*, vol. 9, no. 2, 2017.

[173] R. E. Michler, "Xenotransplantation: risks, clinical potential, and future prospects," *Emerging Infectious Diseases*, vol. 2, no. 1, pp. 64–70, 1996.

[174] M. V. Cannon, D. A. Dunn, M. H. Irwin et al., "Xenomitochondrial mice: investigation into mitochondrial compensatory mechanisms," *Mitochondrion*, vol. 11, no. 1, pp. 33–39, 2011.

[175] W. H. Organization and C. for I. O. of M. Sciences, *International Ethical Guidelines for Health-Related Research Involving Humans*, Council for International Organizations of Medical Sciences, Geneva, 2016.

[176] A. Hedgecoe, "Scandals, ethics, and regulatory change in biomedical research," *Science, Technology & Human Values*, vol. 42, no. 4, pp. 577–599, 2016.

[177] D. F. Horrobin, "Modern biomedical research: an internally self-consistent universe with little contact with medical reality?" *Nature Reviews Drug Discovery*, vol. 2, no. 2, pp. 151–154, 2003.

[178] M. Abou-El-Enein, G. N. Duda, E. A. Gruskin, and D. W. Grainger, "Strategies for derisking translational processes for biomedical technologies," *Trends in Biotechnology*, vol. 35, no. 2, pp. 100–108, 2017.

[179] S. Fogleman, C. Santana, C. Bishop, A. Miller, and D. G. Capco, "CRISPR/Cas9 and mitochondrial gene replacement therapy: promising techniques and ethical considerations," *American Journal of Stem Cells*, vol. 5, no. 2, pp. 39–52, 2016.

[180] H. Marx, S. Pflügl, D. Mattanovich, and M. Sauer, "Synthetic Biology Assisting Metabolic Pathway Engineering," in *Synthetic Biology*, A. Glieder, C. P. Kubicek, D. Mattanovich, B. Wiltschi and M. Sauer, Eds., pp. 255–280, Springer International Publishing, 2016, http://link.springer.com/chapter/10.1007/978-3-319-22708-5_7.

[181] J. D. McCully, D. B. Cowan, S. M. Emani, and P. J. del Nido, "Mitochondrial transplantation: from animal models to clinical use in humans," *Mitochondrion*, vol. 34, pp. 127–134, 2017.

[182] A. Fierabracci, A. Del Fattore, R. Luciano, M. Muraca, A. Teti, and M. Muraca, "Recent advances in mesenchymal stem cell

immunomodulation: the role of microvesicles," *Cell Transplantation*, vol. 24, no. 2, pp. 133–149, 2015.

[183] E. Favaro, A. Carpanetto, C. Caorsi et al., "Human mesenchymal stem cells and derived extracellular vesicles induce regulatory dendritic cells in type 1 diabetic patients," *Diabetologia*, vol. 59, no. 2, pp. 325–333, 2015.

[184] P. J. Quesenberry, J. Aliotta, M. C. Deregibus, and G. Camussi, "Role of extracellular RNA-carrying vesicles in cell differentiation and reprogramming," *Stem Cell Research & Therapy*, vol. 6, no. 1, 2015.

[185] D. G. Phinney, "Functional heterogeneity of mesenchymal stem cells: implications for cell therapy," *Journal of Cellular Biochemistry*, vol. 113, no. 9, pp. 2806–2812, 2012.

b. Published paper # 2

Cabrera et al. BMC Biotechnology (2019) 19:42
https://doi.org/10.1186/s12896-019-0534-6

BMC Biotechnology

RESEARCH ARTICLE Open Access

Primary allogeneic mitochondrial mix (PAMM) transfer/transplant by MitoCeption to address damage in PBMCs caused by ultraviolet radiation

Francisco Cabrera[1,5,9,10], Mayra Ortega[2,9,10†], Francesca Velarde[9,10†], Eliseo Parra[6,7,8], Stephany Gallardo[2], Diego Barba[3], Lina Soto[3], Gabriela Peña[3], Luis Alberto Pedroza[3,4], Christian Jorgensen[5,10], Maroun Khoury[6,7,8,10] and Andrés Caicedo[3,9,10,11*]

Abstract

Background: Artificial Mitochondrial Transfer or Transplant (AMT/T) can be used to reduce the stress and loss of viability of damaged cells. In MitoCeption, a type of AMT/T, the isolated mitochondria and recipient cells are centrifuged together at 4 °C and then co-incubated at 37 °C in normal culture conditions, inducing the transfer. Ultraviolet radiation (UVR) can affect mitochondria and other cell structures, resulting in tissue stress, aging, and immunosuppression. AMT/T could be used to repair UVR cellular and mitochondrial damage. We studied if a mitochondrial mix from different donors (Primary Allogeneic Mitochondrial Mix, PAMM) can repair UVR damage and promote cell survival.

Results: Using a simplified adaption of the MitoCeption protocol, we used peripheral blood mononuclear cells (PBMCs) as the recipient cell model of the PAMM in order to determine if this protocol could repair UVR damage. Our results showed that when PBMCs are exposed to UVR, there is a decrease in metabolic activity, mitochondrial mass, and mtDNA sequence stability as well as an increase in p53 expression and the percentage of dead cells. When PAMM MitoCeption was used on UVR-damaged cells, it successfully transferred mitochondria from different donors to distinct PBMCs populations and repaired the observed UVR damage.

Conclusion: Our results represent an advancement in the applications of MitoCeption and other AMT/T. We showed that PBMCs could be used as a PAMM source of mitochondria. We also showed that these mitochondria can be transferred in a mix from different donors (PAMM) to UVR-damaged, non-adherent primary cells. Additionally, we decreased the duration of the MitoCeption protocol.

Keywords: Mitochondria, MitoCeption, Artificial mitochondria transfer / transplant (AMTT), Primary allogeneic mitochondrial mix (PAMM), Ultraviolet radiation (UVR), Cellular damage, p53, Primary immune cells, Cell repair

* Correspondence: acaicedo@usfq.edu.ec
†Mayra Ortega and Francesca Velarde contributed equally to this work.
[3]Colegio de Ciencias de la Salud, Escuela de Medicina, Universidad San Francisco de Quito, 17-12-841 Quito, Ecuador
[9]Instituto de Investigaciones en Biomedicina, Universidad San Francisco de Quito, 17-12-841 Quito, Ecuador
Full list of author information is available at the end of the article

Background

A substantial number of in vitro and in vivo assays have demonstrated the natural ability of cells to transfer mitochondria amongst each other [1]. This phenomenon is most commonly observed in mitochondrial transfer from healthy mesenchymal stem/stromal cells (MSCs) to damaged cells [2–7]. The transfer replaces or repairs damaged mitochondria and thereby reduces the percentage of dead cells and restores normal functions [3, 4, 8]. In 1982, Clark and Shay introduced a type of AMT/T model using a co-incubation step between the recipient cell and exogenous mitochondria [9]. Their pioneering study demonstrated for the first time that the mitochondrial DNA (mtDNA) of donor cells could be integrated into recipient cells and subsequently transmit hereditary traits and induce functional changes. AMT/T mimics the natural process of mitochondrial transfer, reprograms cellular metabolism, and induces proliferation [10–13]. The introduction of this model elucidated the possible use of mitochondria as an active therapeutic agent.

Since 1982, numerous adaptations of AMT/T have been developed for in vitro and in vivo applications [10–12]. Among all available methods, the use of a centrifugation during co-incubation seems to reduce the quantity of mitochondria needed to facilitate successful mitochondrial internalization by the recipient cells [11, 14, 15]. In-vitro cultured cells, especially MSCs, have been used as one of the most common sources of mitochondria for AMT/T [11, 12, 14]. However, using stem cells or other cultured cells, which require an extensive time to proliferate, increases the cost and reduces time-effectiveness of the process. Furthermore, a large number of cells are needed to successfully obtain high yields of mitochondria for transfer. As an advancement in AMT/T, McCully et al. successfully transplanted autologous mitochondria from skeletal muscle and injected them into damaged myocardium after ischemic injury, which lead to an improvement in ventricular function in humans [16].

Our study tests a modification of the original MitoCeption protocol which reduces the time and complexity of the protocol. We sought to determine if primary allogenic mitochondrial mix (PAMM) MitoCeption could be used to repair peripheral blood mononuclear cells (PBMCs) damaged by ultraviolet radiation (UVR) (UVC-UVR wavelength of 254 nm). PAMM is composed of the PBMCs of at least three donors. A secondary objective was to provide further evidence as to how UVR affects mitochondria and cell viability. To first determine the effects of UVR on cells and mitochondria, we created a cellular model in which human PBMCs were irradiated with UVR. Mitochondrial damage was assessed according to changes in mitochondrial mass, metabolic activity estimated by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and percentage of dead cells; these indicators were examined 30 min to 120 min after (early time point) and 18 h after (late time point) exposure to radiation. Then, we selected a standard exposure time of 3 min for the protocol, because this level of UVR exposure resulted in harm but not complete cell death. Irradiated cells were rescued with varying doses of mitochondria isolated from different PBMC donors (PAMM) using the updated MitoCeption protocol. Using this approach, we showed that PAMM transfer from PBMC donors can repair UVR damage in recipient PBMCs. PBMCs can internalize PAMM and decrease the percentage of dead cells together with the repairing effect of immune cells' respiratory burst (RB) after 18 h. Here, we describe a new method to repair UVR-damaged mitochondria using PAMM MitoCeption; 1 h to 2 h after PAMM MitoCeption, we determined that exogenous mitochondria had been internalized by recipient cells and normal cell viability and mitochondrial mass, metabolic function, DNA patrimony, p53 expression, and RB had been recuperated.

Results

UVR exposure decreases PBMC mitochondrial mass and metabolic function and increases the percentage of dead cells

To understand the effects of UVR on cell physiology, we developed an in vitro model that used fresh PBMCs and exposed them to a gradient of increasing doses of UVR during different lengths of time. For these assays, we isolated PBMCs from healthy donors of different ages and genders. PBMCs were exposed to increasing doses of UVC from 2 min (48 mJ/cm^2) to 6 min (144 mJ/cm^2). The effects of UVR on mitochondrial mass, function, and percentage of dead cells were measured at two separate times: immediately after exposure (early time point) and 18 h after culture (late time point). To test the effects of UVR on mitochondrial mass, we labeled the mitochondria with MitoTracker Green® and measured changes using flow cytometry with mean fluorescence intensity values (MFI). The shape, size, and granularity of treated and untreated PBMCs were analyzed (Additional file 1: Figure S1). The metabolic activity of PBMCs was measured with spectrophotometry using the MTT assay. Mitochondrial mass data was analyzed using flow cytometry and MTT assay for metabolic activity and subsequently normalized using sample/control-average transformation [17]. Finally, we stained PBMCs with Trypan Blue to determine the percentage of dead cells.

We observed a statistically significant reduction (ANOVA test, $*p < 0.05$) in the mitochondrial mass of the lymphocytes (PBMCs) immediately after UVR

exposure especially from 3 min (72 mJ/cm^2) to 6 min (144 mJ/cm^2) (Fig. 1a). Other specific PBMC populations differed in their mitochondrial masses and varied responses to UVR damage were observed (data not shown). After 18 h of culture, lymphocytes in the PBMCs that survived the UVR exposure did not recover their mitochondrial mass in most of the conditions tested (ANOVA test, ***$p < 0.001$) (Fig. 1b). The MTT

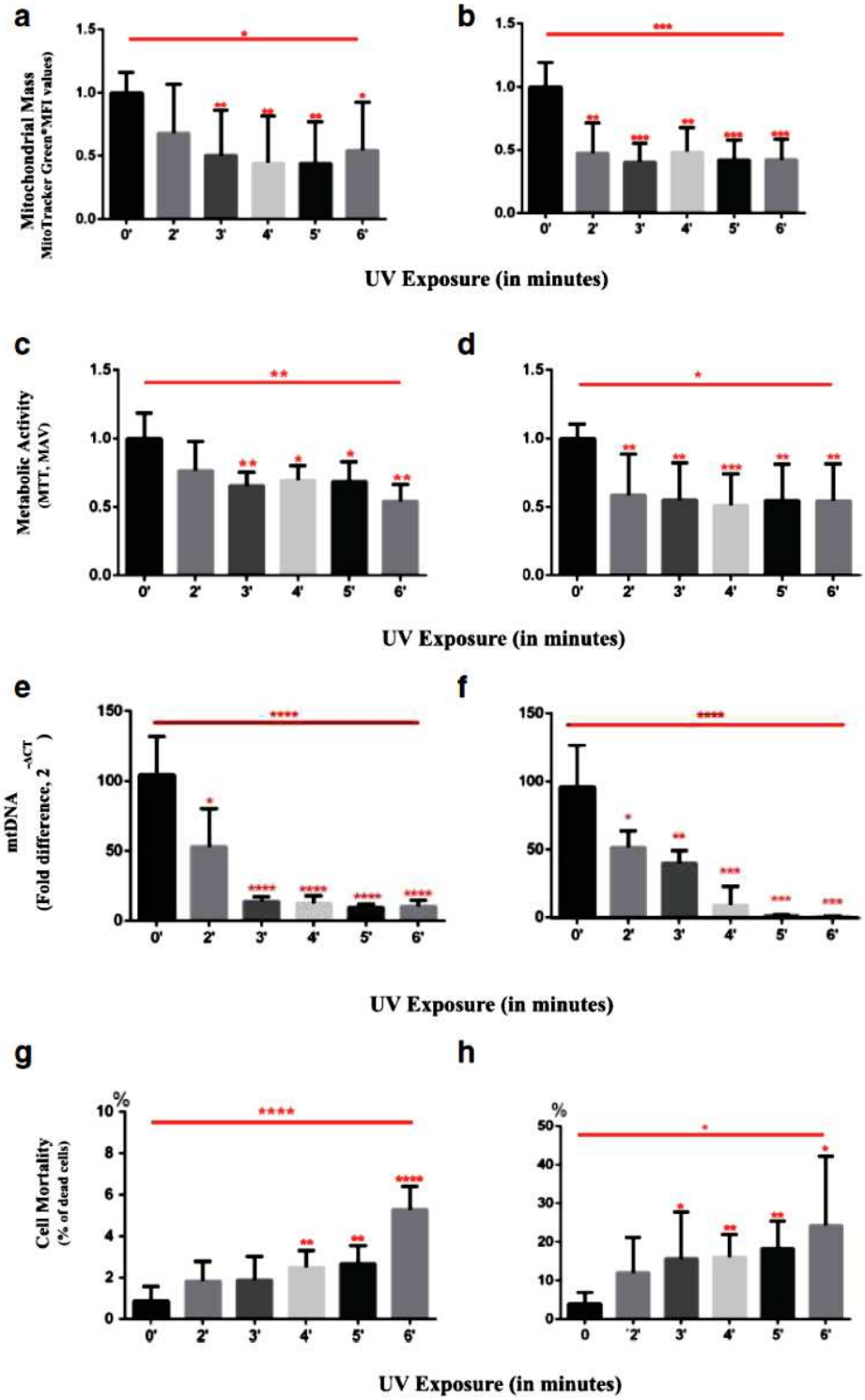


Fig. 1 (See legend on next page.)

(See figure on previous page.)
Fig. 1 PBMCs' mitochondrial mass, metabolic activity, mortality, and mtDNA quantification after UVR exposure. **a**, **b** Mitochondria mass ($n = 8$, 8 PBMC donors) determined by the mean fluorescence intensity values (MFI) of mitochondria labelled with MitoTracker® Green. **a** 1 h culture after exposure and 30'of MitoTracker® Green incubation. **b** 18 h culture and 30'of MitoTracker® Green incubation. Analysis, the sample/control-average transformation was used, Anderson-Darling normality test was applied. Un-paired, ANOVA test and Student's t-test are shown in the figure with an alpha-value of 0.05 (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$) to observe statistically significant differences. **c**, **d** Metabolic activity measured by MTT ($n = 5$, 5 PBMC donors). Mean absorbance values (MAV) were measured by spectrophotometry of PBMCs treated with MTT after UVR exposure. **c** After 2 h incubation with MTT. **d** After 18 h in culture and 2 h incubation with MTT. Analysis: the sample/control-average transformation was used, Anderson-Darling normality test was applied. Un-paired, ANOVA test and Student's t-test are shown in the figure with an alpha-value of 0.05 (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$) to observe statistically significant differences. **e**, **f** Determination of the effects on the mtDNA sequence of PBMCs after the exposure to UVR by qPCR ($n = 8$, 8 PBMC donors). The qPCR $2^{-\Delta CT}$ fold method was used. Primers: HB2M as the housekeeping gene sequence (Human B2M Beta-2-microglobulin, NCBI AH002619.1.), and HMito (designed for the mitochondrial genome, between positions 241 and 390, NCBI NC_012920.1) from Ajaz et al.(2015) [18] **e** 1 h culture after exposure. **f** After 18 h culture. Analysis: Un-paired, ANOVA test and Student's t-test are shown in the figure with an alpha-value of 0.05 (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$) to observe statistically significant differences. **g**, **h** Mortality of PBMCs induced by UVR ($n = 7$, 7 PBMC donors). The percentage of dead cells was estimated by counting those positive for Trypan Blue staining and then dividing them by the total; non-labelled viable cells were considered as well. **g** 1 h culture after exposure. **h** After 18 h culture. Statistical analysis: Un-paired, ANOVA test and Student's t-test are shown in the figure with an alpha-value of 0.05 (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$) to observe statistically significant differences

assay showed a reduction of metabolic activity of the PBMCs in all conditions tested when exposed to increasing UVR exposure of (ANOVA test, **$p < 0.01$) (Fig. 1c). After 18 h of culture, viable cells still exhibited reduced metabolic activity (ANOVA test, *$p < 0.05$) (Fig. 1d). mtDNA damage data was analyzed using the qPCR $2^{-\Delta CT}$ fold method; the level of damage was estimated by hMito primers' failure to recognize the mtDNA sequence using qPCR with a nuclear housekeeping gene (hB2M). hMito primers bind to a unique region of the mtDNA (between positions 241 and 390), which has been shown to be less similar to nuclear DNA sequences, thereby ensuring more accurate identification by the primers [18, 19]. hB2M is the nuclear human β-2 microglobulin gene [18, 19]. We observed a statistically significant and proportional reduction in the number of copies of mtDNA after all UVR dosages at both time measurements (ANOVA test, ****$p < 0.0001$) (Fig. 1e, f). Following UVR exposure, measurement at the early time point revealed that 10% of the total number of cells were dead (Fig. 1g). After 18 h of culture, PBMCs showed a statistically significant (ANOVA test, *$p < 0.05$) increase in the percentage of dead cells following exposure to a higher dose of UVR (Fig. 1h).

MitoCeption protocol update to facilitate the AMT/T using PAMM donors, unattached recipient cells, and alternative cell support in less time

In MitoCeption, the isolated mitochondria and recipient cells are centrifuged together at 4 °C and then co-incubated at 37 °C at normal culture conditions, inducing the transfer. The centrifugation and thermic shock are key steps in the protocol which successfully transfer small quantities of mitochondria to recipient cells [11]. The protocol was modified and adapted to transfer mitochondria to unattached PBMCs without using a plate or coating of a well surface as cell support. The cell support was changed from plates to 1.5 mL micro-centrifuge tubes (Fig. 2.1). We reduced centrifugation force (500 x g for 5 min at 4 °C) and exposure time to exogenous mitochondria (1 h); statistically significant (ANOVA test, ***$p < 0.001$) exogenous mitochondria up-take was still observed at 1 h and at 18 h (Fig. 2.2; 2.3).

Using flow cytometry, we observed that the patterns of exogenous mitochondria internalization by recipient PBMCs subjected to the MitoCeption protocol varied according to cell type and population (Fig. 2.2; 2.3; Additional file 1: Figure S1). In CD3+ lymphocytes, two uptake patterns were observed. In one population, cells incorporated between 5 and 40% of exogenous mitochondria in proportion to the increasing dose of PAMM. Another population of CD3+ lymphocytes did not internalize mitochondria (Fig. 2.2.a, 2.2c-d). In contrast, after one hour, 60 to 100% of CD14+ monocytes had successfully internalized exogenous mitochondria from the lowest (1.25 ng/μl) to the highest (5 ng/μl) mitochondrial concentration; the mitochondrial uptake rate in CD14+ monocytes was proportional to increasing exogenous mitochondrial doses, although the observed effect was not as substantial as in the case of CD3+ lymphocytes (Fig. 2.3.a, 2.3c-d). In both CD3+ lymphocytes and CD14+ monocytes, a better definition of the fluorescence peak and a stabilization of the population that internalized the exogenous mitochondria was observed independently of the concentration, 30 to 40% for the lymphocytes and 100% for the monocytes (Fig. 2.2.b – 2.2.e-f; 2.3.b-2.3.e-f). In both CD3 + lymphocytes and CD14+ monocytes recipient cells, we observed an increase in fluorescence and MFI proportional to the concentration of mitochondria and successful mitochondria internalization after 1 h and 18 h (Fig. 2.2a-b; 2.2.d; 2.2.f; 2.3a-b; 2.3.d; 2.3.f).

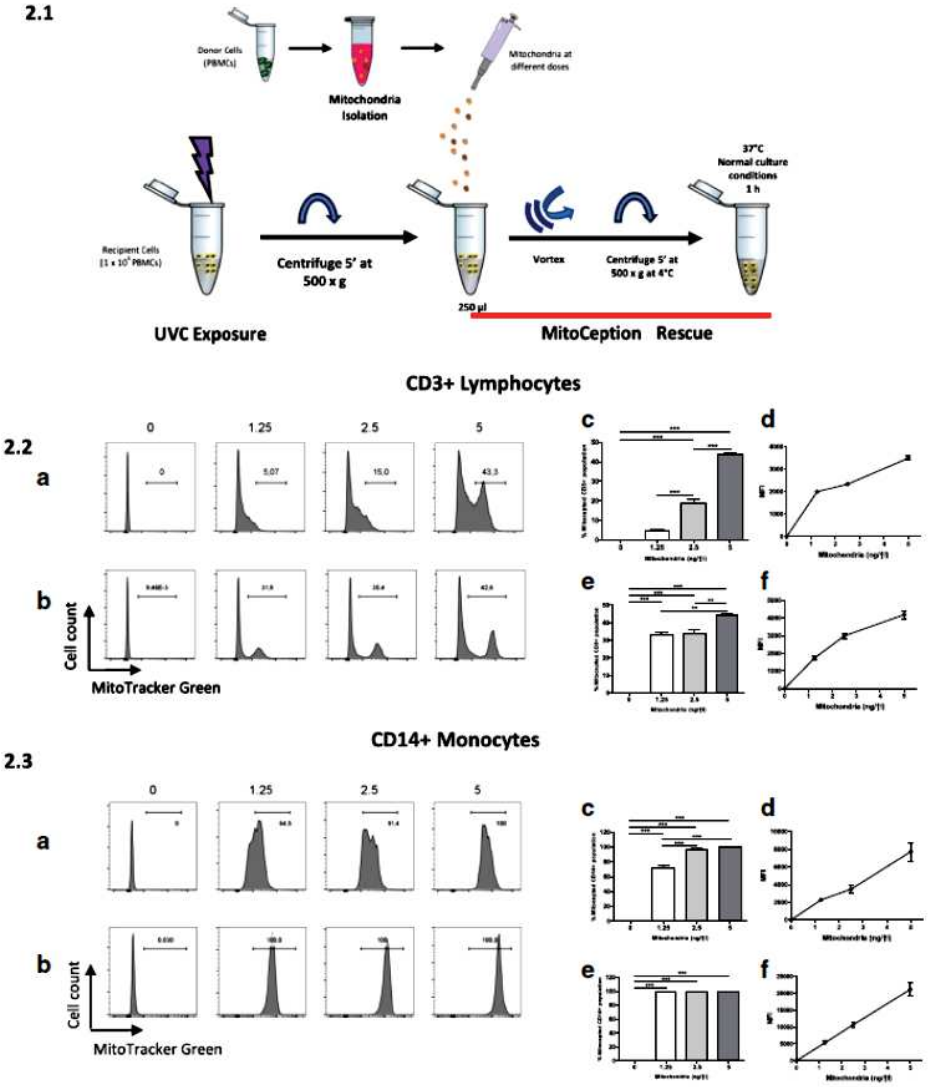


Fig. 2 MitoCeption protocol update. (1) Schema of the updated protocol. Unattached recipient cells can be MitoCepted in a microcentrifuge tube of 1.5 ml and then centrifuged at 500 x g at during 5 min and immediately put in incubation for at least 1 h with mitochondria. Then, cells can be washed of excess mitochondria and used for downstream applications. Graphs show the proportional increase of the transfer of fluorescent mitochondria in percentages and MFIs due to the uptake in relation to the control of non-MitoCepted mitochondria. PAMM is composed of the PBMCs of at least three donors. (2, 3) MitoCepted CD3+ Lymphocytes and CD14+ Monocytes ($n = 3$, 3 PBMC donors, 3 PBMC donors for PAMM). Fresh PBMCs were MitoCepted with isolated mitochondria labeled with MitoTracker® Green from PBMCs; lymphocytes and monocytes were selected by their size, granularity, singles, alive cells (– for Annexin and 7AAD), and CD3+ and CD14+ identification. (2.2a, 2.2c, 2.2d) Flow cytometry of CD3+ cells after 1 h of MitoCeption. (2.2b, 2.2e, 2.2f) CD3+ after wash of excess mitochondria and 18 h of MitoCeption. (2.2c) CD3+ percentage of MitoCeption cells after 1 h. (2.2d) CD3+ MFI of the MitoCepted cells after 1 h. (2.2e) CD3+ Percentage of MitoCeption cells after 18 h. (2.2f) CD3+ MFI of the MitoCepted cells after 18 h. (2.3a, 2.3c, 2.3d) Flow cytometry of CD14+ cells after 1 h of MitoCeption. (2.3b, 2.3e, 2.3f) Flow cytometry of CD14+ cells after 18 h of MitoCeption. (2.3c) CD14+ percentage of MitoCeption cells after 1 h. (2.3d) CD14+ MFI of the MitoCeption cells after 1 h. (2.3e) CD14+ Percentage of MitoCeption cells after 18 h. (2.3f) CD14+ MFI of the MitoCepted cells after 18 h. Statistical analysis for all conditions: Mean ± SEM one-way ANOVA and Tuckey's pot-test. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

PAMM transfer of mitochondria by MitoCeption rescues mitochondrial mass, function, and viability in PBMCs damaged by UVR

We demonstrated that UVR exposure in PBMCs cause a reduction of mitochondrial mass and function, loss of or changes in mtDNA, and an increase in the percentage of dead cells (Fig. 1a-h). Among all UVR doses tested, we determined that 3 min (72 mJ/cm^2) was the minimal dose required to induce statistically significant cell and mitochondrial damage (ANOVA test, **$p < 0.01$); we used this UVR dose in all of the tested conditions. PBMCs damaged by 3 min of UVR exposure (72 mJ/cm^2) were MitoCepted during 1 h with allogeneic mitochondria from different PBMCs donors (PAMM) and

subsequently washed to remove excess exogenous organelles that were not successfully internalized (Fig. 3). Isolated mitochondria were MitoCepted in doses of 1.25, 2.5, and 5 ng/μl to 1×10^6 UVR-damaged PBMCs.

Additionally, we used 5 ng/μl of isolated mitochondria to MitoCept PBMCs that had not been exposed to UVR in order to observe cytotoxicity. As expected, controls irradiated with a 3 min dose of UVR (72 mJ/cm^2) exhibited a statistically significant decrease in their mitochondrial mass and function and increase in percentage of dead cells (Fig. 3a-f). Treatment with PAMM induced a complete recovery of mitochondrial mass and function and reduced percentage of dead cells at the early and late time points in the lymphocytes population (PBMCs) (Fig. 3a-f). In both the non-exposed PBMCs and those exposed to 3 min (72 mJ/cm^2) of UVR which were then MitoCepted with 5 ng/μl of mitochondria, an increase of dead cells and mitochondrial mass was observed at the early time point; mitochondrial mass increase was more substantial in the UVR-damaged PBMCs. Additionally, this finding may indicate that internalization of excessive of mitochondria could be harmful to the cell (Fig. 3e) . We did not observe adverse effects associated with the use of allogeneic mitochondria in doses ranging from 1.25 to 2.5 ng; there was no increase in the percentage of dead cells associated with these dosages (Fig. 3e-f). Additionally, 1 h exposure to exogenous mitochondria was enough to recover the loss of mitochondrial mass, metabolic activity, and percentage of dead cells in UVR-damaged recipient cells.

Rescue of the loss of mtDNA hMito sequence and normalization p53 mRNA expression in UVR-damaged PBMCs using PAMM MitoCeption

After determining the appropriate UVR dose (3 min) in our PBMC model and the quantity of exogenous mitochondria (2.5 ng / μl by 1×10^6 cells) needed to achieve statistically significant improvements in damaged cells, we assessed if the UVR damage of mtDNA could induce changes in p53 gene expression and if this could be repaired by PAMM MitoCeption. UVR caused a statistically significant loss of the mtDNA recognition sequence for hMito primers by qPCR, which was reversed by PAMM MitoCeption at both the early and late time points (Fig. 4a-b). UVR exposure caused a statistically significant increase (ANOVA test, $^{***}p < 0.001$) in p53 gene expression, as measured by qRT-PCR at 2 h after exposure and subsequent MitoCeption; this increase was then reversed by MitoCeption (Fig. 4c). We chose to measure the mRNA of p53 at 2 h because in preliminary assays the p53 expression at 1 h or after 18 h was minimally detected.

Rescue of RB (ROS production) of damaged PBMCs by MitoCeption

We used flow cytometry analysis of lymphocyte and monocyte size and granularity (Additional file 1:Figure S1) in order to determine the level of ROS production by Phorbol 12-myristate 13-acetate (PMA); we detected ROS production using Dihydro-rhodamine-123 (DHR-123) in all conditions tested. PBMCs monocytes responded to PMA by increasing ROS production (also known as RB) in all tested conditions (Fig. 5a-b). Monocytes that were MitoCepted increased their ROS production quantity at the early and late time points. After the UVR damage, monocytes decreased their ROS production and recovered it after MitoCeption; the statistical significance of these results was confirmed using ANOVA and Student's t-test (ANOVA test, $^{*}p < 0.05$). The lymphocytic population did not exhibit statistically significant change in ROS production at the early time point. However, after 18 h, the lymphocyte population exposed to 3 min of UVR damage showed a statistically significant reduction (ANOVA test, $^{**}p < 0.01$) in ROS production (Fig. 5c-d). After MitoCeption, PMA-stimulated lymphocytes showed a slight increase in ROS production (Fig. 5a-b). After 18 h, the UVR-damaged lymphocytes partially recovered their ROS production following MitoCeption.

Discussion

MitoCeption has been shown to reprogram cell metabolism and increase cancer cell proliferation and invasion 24 h after the transfer of mitochondria isolated from MSCs [11]. Our results showed that MitoCeption rescues the loss of mitochondrial mass, function, and viability as soon as 1 h after cells were exposed to UVR. Here, we adapted the MitoCeption protocol to be used with fresh non-adherent immune cells such as PBMCs and changed the exogenous mitochondria source, centrifugation time and force, co-incubation time, and cell support. Some protocols use MSCs as a mitochondria source; however, the reduced proliferation capacity of MSCs makes obtaining sufficient cells impractical and expensive [14, 20, 21]. Therefore, we tested the suitability of PBMCs as the mitochondria source, which constitutes a novel approach in the field and is a more accessible mitochondria source because a greater number of mitochondria donor cells can be obtained from multiple donors. We decreased the force and time of centrifugation (500 x g for 5 min at 4 °C) and changed the original protocol's volume of medium from 1 mL to 250 μL in order to maximize recipient cell and mitochondria interaction. Finally, we used a different supporting material by changing the culture plate to 1.5 mL microcentrifuge tubes, thereby adapting this protocol for use with unattached cells like PBMCs.

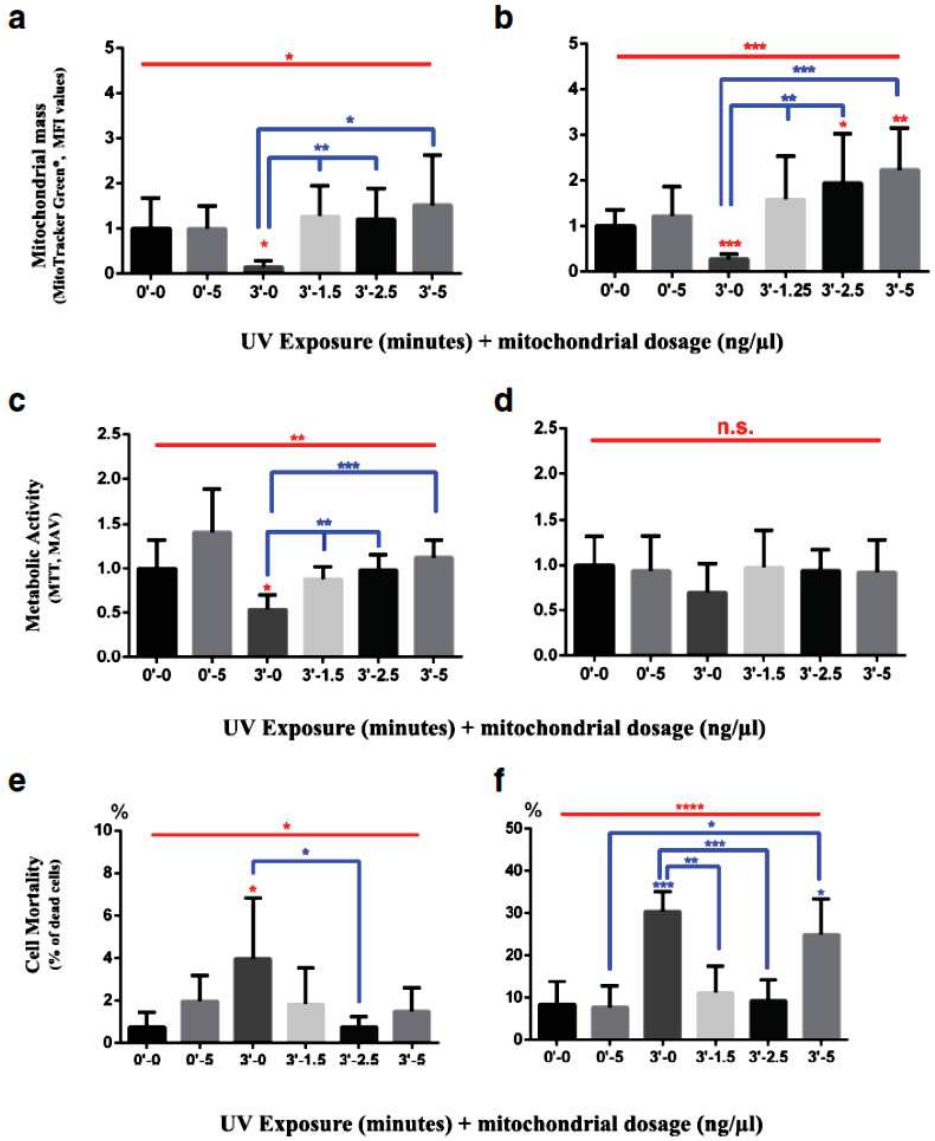


Fig. 3 Estimation of the rescue of mitochondrial mass, metabolic activity, and mortality of damaged PBMCs using PAMM MitoCeption. PBMCs were isolated, exposed to UVR (3 min = 72 mJ/cm^2), and treated with 3 allogeneic mitochondria doses (1,25-2,5–5,0 µg of mitochondria). **a**, **b** Mitochondria mass (n = 7, 7 PBMC donors exposed to UVR and PAMM of 3 donors). MFI of PBMCs labelled with MitoTracker Green® was analyzed by flow cytometry. **a** 1 h after exposure to UVR, 1 h after MitoCeption, wash, and 30` incubation with MitoTracker® Green. **b** After 1 h exposure to UVR, 1 h MitoCeption, wash and 18 h in culture. Analysis, the sample/control-average transformation was used, Anderson-Darling normality test was applied. Un-paired, ANOVA test and Student's t-test are shown in the figure with an alpha-value of 0.05 (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$) to observe statistically significant differences. **c**, **d** Metabolic activity measured by MTT ($n = 5$, 5 PBMC donors exposed to UVR and PAMM of 3 donors). MAV were measured by spectrophotometry of PBMCs treated with MTT after UVR exposure and MitoCeption at the early and late time points. **c** 1 h after exposure to UVR, 1 h of MitoCeption, wash and 2 h of incubation with MTT. **d** 1 h after exposure to UVR, 1 h of MitoCeption, wash, 18 h in culture and 2 h of incubation with MTT. Analysis: the sample/control-average transformation was used, Anderson-Darling normality test was applied. Un-paired, ANOVA test and Student's t-test are shown in the figure with an alpha-value of 0.05 (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$) to observe statistically significant differences. **e**, **f** Mortality of PBMCs caused by UVR (n = 5, 5 PBMC donors exposed to UVR and PAMM of 3 donors), the percentage was estimated by counting dead cells positive for Trypan Blue staining and dividing that number by viable cells. **e** Early time point measured 1 h after exposure to UVR, 1 h MitoCeption and wash **f** Late time point 1 h after exposure to UVR, 1 h MitoCeption, wash and 18 h in culture. Analysis: Anderson-Darling normality test was applied to the resulting data. Un-paired, ANOVA test and Student's t-test are shown in the figure with an alpha-value of 0.05 (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001) to observe statistically significant differences

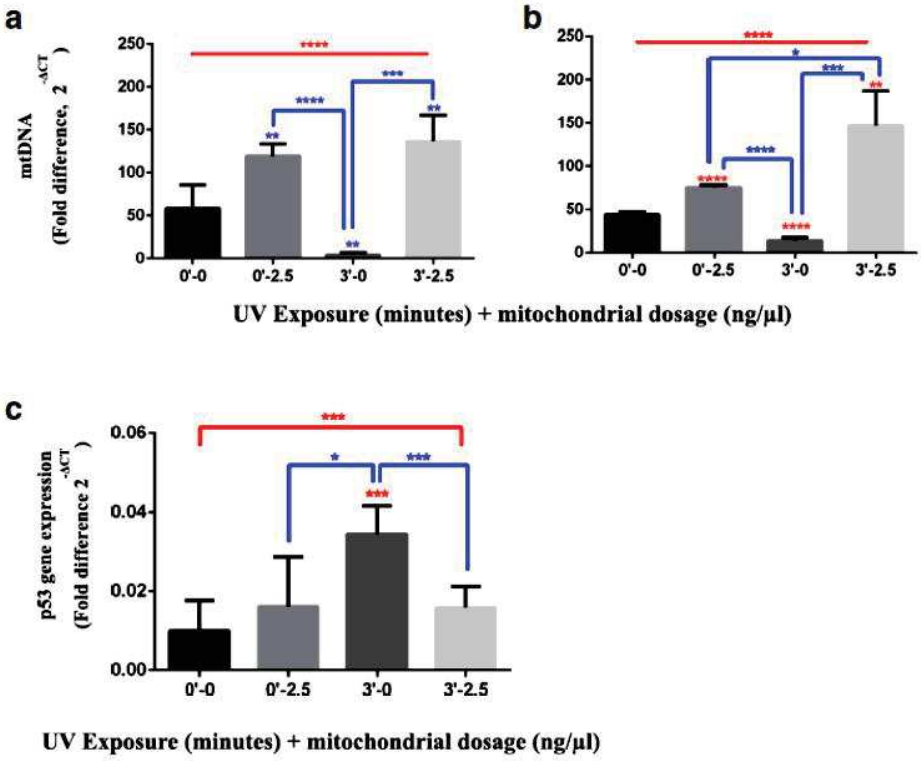


Fig. 4 Determination of the effects on the mtDNA sequence, changes in the expression of the p53 gene induced by UVR, and rescue by adding exogenous mitochondria using PAMM MitoCeption in the PBMC model. **a**, **b** mtDNA damage quantification by the $2^{\Delta CT}$ fold by qPCR after 3 min UVR exposure and rescue by PAMM Mitoception (n = 5, 5 PBMC donors exposed to UVR and PAMM of 3 donors). The qPCR $2^{\Delta CT}$ fold method was used. Primers: HB2M as the housekeeping gene sequence (Human B2M Beta-2-microglobulin, NCBI AH002619.1.), and HMito (designed for the mitochondrial genome, between positions 241 and 390, NCBI NC_012920.1) from Ajaz et al.(2015) [18] **a** 1 h culture after UVR exposure and PAMM MitoCeption. **b** 18 h culture after UVR exposure and PAMM MitoCeption. Analysis: Un-paired, ANOVA test and Student's t-test are shown in the figure with an alpha-value of 0.05 (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001) to establish statistically significant differences. **c** Analysis of the p53 gene expression after UVR damage and PAMM MitoCeption ($n = 6$, 6 PBMC donors exposed to UVR and PAMM of 3 donors). Primers for detecting p53 mRNA and for the nuclear PUM (housekeeping) were used to estimate p53 levels after UVR exposure and PAM MitoCeption in receptor cells with or without UVR damage. p53 gene expression was measured by qRT-PCR at 2 h after exposure and subsequent MitoCeption. Statistical analysis: Un-paired, ANOVA test and Student's t-test are shown in the figure with an alpha-value of 0.05 (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001) to establish statistically significant differences. Un-paired, ANOVA test and Student's t-test ($p < 0.05$)

The MitoCeption protocol uses centrifugation to put recipient cells in close contact with the exogenous mitochondria and a thermic shock to attempt to facilitate mitochondrial uptake. It has been reported that MitoCeption requires 1.25 to 20 µg/ml of mitochondria (concentration measured using Bradford protein quantification assay) per 100,000 cancer cells to achieve phenotypical changes in the recipient cells [11]. MitoCeption allows minimal quantities of mitochondria to be used. The use of additional steps in co-incubation may depend on the user needs, cell type, and application. Studies performed by Lorberboum-Galski and her team showed that simple incubation between receiver cells and exogenous mitochondria was sufficient to facilitate transfer in a process called "Mitochondrial Transformation". In most assays, her team used 300 µg / mL of mitochondria with 50,000 HepG2 cells, MCF-7, 30,000 HEK-293, or 75,000 fibroblasts, did not use centrifugation, and completed data collection 1 h and 24 h after combining the recipient cells and exogenous motochondria [15]. In our study, we found that we were able to obtain yields of 50 µg / mL of mitochondria using only 16×10^6 PBMCs (4 donors), which was sufficient for all conditions tested. Interestingly, other groups have also found that performing a centrifugation step prior to the co-incubation of the isolated mitochondria with the recipient cells greatly improves the mitochondria uptake; for example, Mi Jin Kim et al. found that 0.05, 0.5 and 5 µg of exogenous mitochondria were successfully transferred to 10,000 recipient cells after 24 h [14].

To our knowledge, this study is the first to demonstrate in-vitro that MitoCeption can be used to re-establish mitochondrial function loss caused by UVR exposure. Additionally, we successfully transferred a mix of different PBMC donors to one PAMM that was used to repair damaged cells. Other research groups have

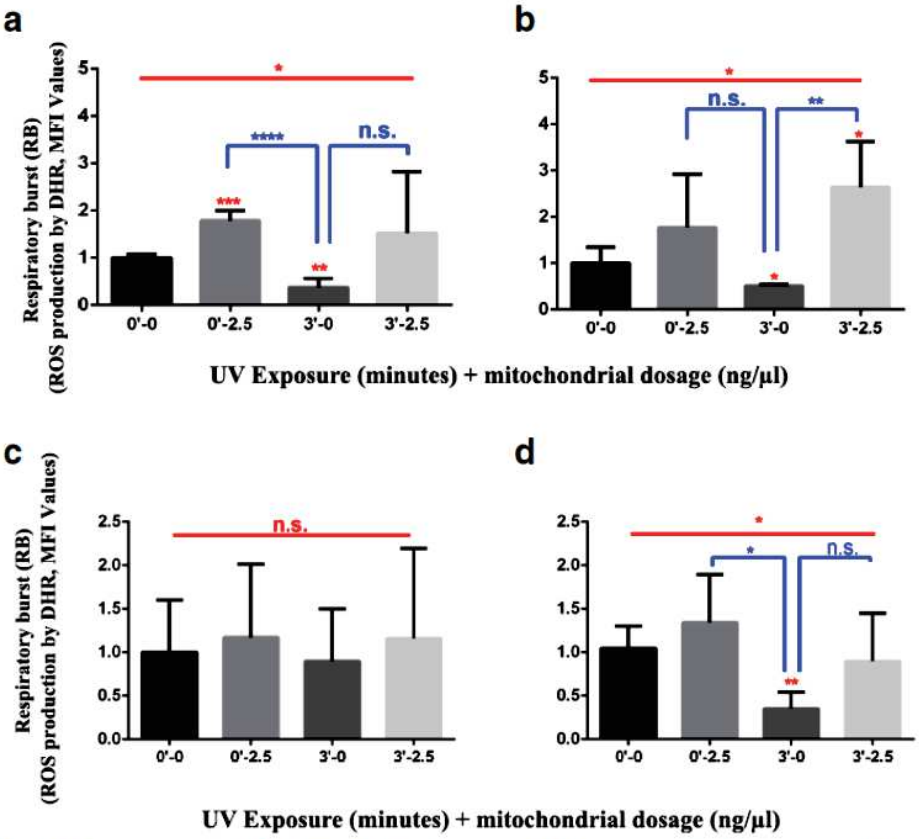


Fig. 5 Estimation of the RB (ROS production) of UVR-exposed PBMCs (monocytes and lymphocytes) and assessment of PBMC rescue by PAMM MitoCeption. **a**, **b**, **c**, **d** ROS production measured with DHR-PMA treatment and flow cytometry (n = 6, 6 PBMC donors exposed to UVR and PAMM of 3 donors) **a**. Monocytes behavior 1 h after 3 min exposure to UVR and rescue by PAMM MitoCeption. **b**. Monocytes behavior after 18 h culture and rescue by PAMM MitoCeption. **c**. Lymphocytes behavior 1 h after 3 min exposure to UVR and rescue by PAMM MitoCeption. **d**. Lymphocytes behavior after 18 h culture and rescue by PAMM MitoCeption. Analysis: the sample/control-average transformation was used, Anderson-Darling normality test was applied. Un-paired, ANOVA test and Student's t-test are shown in the figure with an alpha-value of 0.05 (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$) to observe statistically significant differences

successfully transferred mitochondria from one cell donor type to others [10, 11, 20]; however, none of them have mixed mitochondria isolated from different donors for the transfer/transplant. This study elucidates the potential to use mitochondria from different donors (PAMM) to treat UVR stress and possibly other types of damage or metabolic malfunctions in cells, resulting in not only in-vitro but also ex-vivo applications. The repairing capacity of the PAMM and MitoCeption protocols require further investigation because the effects beyond 18 h of culture, the long-term effects of multiple mitochondria patrimonies inside the recipient cells, and the capacity to re-use them in-vivo for distinct applications is unknown.

In our study, assessment of mitochondrial state in the PAMM was limited, although the PAMM demonstrated reparative properties. Our results demonstrate that PAMM MitoCeption can repair some cell damage inflicted by UVR exposure. In order to promote mitochondrial stability during co-culture, in our protocol, we reduced the centrifugation and co-incubation time and the possible cellular stress induced by the long centrifugation and thermic shock included in the original MitoCeption methodology. Nonetheless, it is crucial that further assays be performed to determine if the mitochondria in PAMM are active, energetically coupled or uncoupled, and structurally complete in order to fully understand their repairing properties.

We observed that UVR-exposed PBMCs exhibited a decrease in mitochondrial mass and metabolic activity as measured by MTT. Likewise, UVR exposure induced changes or loss of mtDNA and increased the percentage of dead cells. Furthermore, increased p53 gene expression was associated with changes or loss in the mtDNA target sequence of hMito primers. UVR exposure also caused a decrease in ROS production (especially in monocytes), thus affecting the RB. The RB is part of the innate response to infection and host defense based on ROS production by the NADPH oxidase enzyme, which is situated in the membranes of monocytes and which

may be sensitive to UVR [22]. We provided further insight into how mitochondria are affected by UVR in PBMCs in order to validate the use of MitoCeption to rescue cells exposed to UVR-induced stress (Additional file 2: Figure S2).

UVC was used to generate a standard damaging procedure for PBMCs to validate the use of the MitoCeption of PAMM to repair this damage. UVC greatly disturbs DNA and particularly mtDNA; damage especially accumulates in the latter molecule due to the lack of mtDNA repairing systems as compared to the nucleus [23]. Moreover, when mtDNA reaches a threshold limit for damage, it causes mitochondrial malfunction, increased ROS production, and induces apoptosis [23–26]. By using UVC, our study showed that increasing doses of radiation causes proportional loss of mtDNA, mitochondrial mass integrity, and metabolic function as soon as 1 h after exposure.

Measurement of the mitochondrial mass by MitoTracker Green® has been proven to be an accurate method to distinguish differences among cells and experimental conditions [27, 28]. Once we successfully used MitoCeption to transfer isolated mitochondria to undamaged PBMCs and UVR-damaged PBMCs, flow cytometry was used to demonstrate that recipient cells exhibited an increase in mitochondrial mass proportional to the dose of transferred mitochondrial. Thus, a statistically significant increase in mitochondrial mass was observed primarily in recipient cells that had been co-cultured with exogenous mitochondria in concentrations greater than or equal to 2.5 ng / μl. After UVR damage, the decrease in percentage of dead cells was completely recovered by the adapted PAMM MitoCeption protocol.

In the MTT technique, the metabolic activity of living cells transforms the MTT chemical compound into formazan crystals, which appear blueish to violet in color. Once living cells have transformed the MTT in formazan crystals, dimethyl sulfoxide (DMSO) lysates the cells and dilutes the crystals. The viability and metabolic activity of the cells can be estimated by analyzing this bluish to violet solution with spectrophotometry [29]. The Trypan Blue dye exclusion assay is a widely-used technique to determine living cells (non-blue) from dead cells (blue); however, it has been reported that it overestimates cell viability and underestimates non-viable cell density [30]. In Fig. 3, at the early time point, the MTT results (c panel) and the percentage of dead cells (e panel) showed a congruent response: a decrease in MTT and an increase in the number of dead cells at the 3 min measurement. Exposure to 5 ng of mitochondria increased the MTT labeling, although it induced a slight increase in dead cells. This effect could be related to cytotoxicity. A recovery of the MTT staining, which reflects metabolic activity, was observed in the 3 min exposure conditions and repaired by PAMM MitoCeption. Cells exposed to 3 min of UVR and which were MitoCepted showed a greater decrease in the percentage of dead cells as compared with cells exposed to 3 min of UVR but which were not MitoCepted. At the early time point, even Trypan Blue differentiates living from dead cells, it does not provide information about cells that could have been weakened by UVR and MitoCeption and which could die after 18 h.

After 18 h (Fig. 3), cells continued to die and accumulated from the early time point in the conditions of 3 min UVR exposure and 3 min UVR plus MitoCeption, resulting in the increased percentage of positive Trypan Blue cells at the late time point (non-viable) (panel f). This result demonstrated that the number of dead cells increased after 18 h under the most stressful conditions. These conditions were cells exposed to 3 min of UVR. It seems that the initial Trypan Blue staining could have underestimated the number of weakened cells. The MTT conversion to formazan crystals in the cells at 18 h can only be performed by living cells under all the conditions (panel d); this reaction occurs independently of the increase in the percentage of dead cells (panel f), which did not affect the overall MTT assay result.

mRNA was isolated to measure p53 gene expression levels after the PBMCs were exposed to UVR and cultured for 2 h. Results showed that PBMCs exposed to radiation increased their p53 mRNA levels in a response that could be associated with the induction of cell arrest or repair of mtDNA [31]. The decreased recognition of mtDNA sequence by hMito primers in cells exposed to UVR and the increased p53 mRNA expression could imply that these effects are linked. However, when PBMCs were rescued by adding 2.5 ng of exogenous mitochondria, mtDNA was recovered and p53 expression levels were decreased. The mechanism by which exogenous mitochondria transfer interacts with p53 gene expression is unknown and could influence future research on how MitoCeption could inhibit damage or senescence response [31].

Our article showed that the in-vitro use of PAMM MitoCeption could repair UVR damage in our cellular model. Even if the MitoCeption technique is limited to an in-vitro AMT/T, our protocol allows an effective and proportional exogenous mitochondrial uptake in cell lines and primary cells in proportion to their dose. This technique and properties could be used for ex-vivo experimentation.

It is still a challenge to standardize and predict proportional uptake in-vivo AMT/T by damaged tissue; this is true whether exogenous mitochondria are delivered to the tissue by direct or systemic injection. Nevertheless, the therapeutic value of exogenous mitochondria for patients is supported by medical evidence [10, 16].

MitoCeption in combination with PAMM could have clinical applications in the ex-vivo transfer of mitochondria to target cells in order to repair or modify them prior to reintroducing them to a patient's body. In the future, the MitoCeption technique could be used as part of a protocol to isolate primary cells with direct or indirect mitochondrial malfunctions, transfer/transplant exogenous mitochondria, and reintroduce them into the source organism. Today, the ex-vivo modification of cells for therapeutic purposes (such as in CAR-T cell generation and others) has been applied in anti-cancer medicine [32–36]. MitoCeption or other mitochondria transfer/transplant techniques could be used as in CAR-T cell therapy design and clinically applied to patients in future studies.

Conclusion

In conclusion, we showed for the first time that PAMM MitoCeption can repair the decrease in metabolic activity, mitochondrial mass, and mtDNA sequence stability, decrease p53 gene expression, and decrease percentage of dead PBMCs, all of which are caused by UVR damage. We used an adaptation of the MitoCeption technique in PBMCs, which not only allows an effective and proportional uptake in primary cells, but also reduces the time of the protocol for better efficiency in downstream applications. This study opens the possibility to use mitochondria from different donors (PAMM) to treat UVR stress and possibly other metabolic diseases or malfunctions in cells in ex-vivo applications.

Methods

PBMCs isolation

Blood was collected from healthy female and male donors between 20 and 30 years old, each of whom provided written informed consent in accordance with relevant guidelines and regulations for mitochondria studies approved by the Bioethics Committee (the Institutional Review Board) of the Universidad San Francisco de Quito (study code: 2017-026IN). After blood collection, PBMCs were isolated using Ficoll-Paque Premium (GE Healthcare Life Sciences; MA, U.S) and centrifuged at 400 x g for 30 min without break or acceleration in order to create a density gradient. PBMCs were washed by suspension with 1X phosphate buffered saline (PBS) (Gibco by Life Technologies, ThermoFisher Scientific; MA, U.S) in a standardized volume of 10 mL and centrifuged at 1500 x g for 20 min at 4 °C. Supernatant was discarded and the pellet was re-suspended in 10 mL of PBS. Cells were counted in a Neubauer cell counting chamber and diluted to 1×10^6 cell/mL in 1% FCS RPMI (Gibco by Life Technologies, ThermoFisher Scientific, MA, U.S.), resulting in a final volume of 2 mL in an Eppendorf tube; one such tube was prepared for each condition. Cells were stored for a maximum of 2 h at 4 °C until further assays were performed.

UVR exposure

Isolated cells were incubated at room temperature for 20 min prior to UVR exposure. Cells stored at low temperature seemed to resist irradiation better, thus creating variability in the results (data not shown). PBMCs were exposed to a UVR wavelength of 254 nm (UVC) emitted by a BS-02 UVR system (Purifier Logic Class II, KS, U.S). The UVC exposure dosages were: 2 min (48 mJ/cm^2), 3 min (72 mJ/cm^2), 4 min (96 mJ/cm^2), 5 min (120 mJ/cm^2), and 6 min (144 mJ/cm^2).

Mitochondrial isolation

Mitochondria was isolated from 10 to 20×10^6 of fresh PBMCs using the Mitochondria Isolation Kit for Tissue (ThermoFisher Scientific, MA, U.S) following manufacturer's instructions with a final recovery step using a centrifugation at 3000 x g for 15 min to obtain highly purified mitochondria isolates. A second wash was performed using the same centrifugation conditions in order to discard residue of Reactive C from the kit. 1 mL of DMEM High Glucose (Gibco by Life Technologies, ThermoFisher Scientific, MA, U.S) without serum (DMEM pure) was added to re-suspend the mitochondrial pellet. Mitochondrial concentration was determined using a Pierce™ Coomassie Plus (Bradford) Assay Kit (ThermoFisher Scientific, MA, U.S). A typical mitochondria yield was 30 to 50 μg/mL. The mitochondrial solution was stored at 4 °C until MitoCeption of PBMCs was performed.

Adapted MitoCeption protocol

PBMCs from all conditions were centrifuged at 500 x g at 4 °C for 5 min in 2 mL of RPMI 1% FCS in a microcentrifuge tube. The supernatant was discarded and 250 μL of the isolated mitochondrial solution in pure DMEM was added at different concentrations. 1.5 mL Eppendorf tubes were vortexed at maximum speed for 5 s with the 250 μL of mitochondria and PBMCs. A final centrifugation was performed at 500 x g for 5 min at 4 °C. Finally, cells were co-incubated at 37 °C for 1 h, washed with 1 mL of PBS, and left to rest until downstream assays were performed. PAMM is composed of the PBMCs of at least three donors (Fig. 2.1).

Percentage of dead cells assessment

After UVR exposure, MitoCeption, and after the early and late time points, cells were washed and re-suspended in 250 μL of 1% FCS RPMI. A 10 μl aliquot of each exposed, non-exposed, treated, and un-treated cells with mitochondria was mixed through resuspension, with 10 μL of 0.02% Trypan Blue staining (Gibco by Life

Technologies, ThermoFisher Scientific, MA, U.S). Two different operators counted the cells in a Neubauer cell counting chamber using an inverted Olympus microscope. Percentage of dead cells was calculated by dividing the number of dead cells (positive for Trypan Blue) by the total number of cells in the chamber.

Mitochondria labeling with MitoTracker green®

PBMCs were incubated with 250 nm/mL of MitoTracker Green® (Molecular Probes by Life Technologies, ThermoFisher Scientific, MA, U.S) solution in 1% FCS RPMI at 37 °C during 30 min. Two washes were performed to eliminate excess dye and cells were re-suspended in 1 mL of PBS.

Determination of the mitochondria transfer

PBMCs tested in all conditions were washed with 1 mL of PBS and centrifuged at 500 x g for 5 min prior to the assay to eliminate un-transferred mitochondria. Mito-Cepted PBMCs passed directly to flow cytometry analysis to determine the transfer success. Cell viability was verified (selection of cells - for Annexin and 7AAD). During the flow cytometry analysis, we focused on the lymphocyte or monocyte population using size, granularity, and CD3+ or CD14+ labeling. PBMCs were pelleted by centrifugation at 500 x g for 5 min, supernatant was discarded, and fresh RPMI + 1% FCS was added. Cells were analyzed 1 h or 18 h after MitoCeption by flow cytometry, focusing on the CD3+ lymphocyte and CD14+ monocyte populations. Cells were stained with APC mouse anti-human CD3 (555,335; BD biosciences) and PE mouse anti-human CD14 (555,398; BD Biosciences) antibodies. To determine the transfer success, a Flow Cell Cytometer Canto™ II (BD Biosciences, NJ, U.S) was used and, for the mitochondrial mass quantitation, the assays were performed using a Flow Cell Cytometer BD Accuri™ C6 Plus (BD Biosciences, NJ, U.S.). Data was analyzed by the FlowJo™, BD Accuri™ CSampler and flow cytometry Diva BD Bioscience software.

Metabolic activity

A solution of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (M6494; ThermoFisher Scientific, MA, U.S) was prepared at a concentration of 20 μg/mL and filtered through a 0.45 μm filter. 100 μL of the MTT solution was added to each tested condition in 1 mL of PBMCs in 1% FCS RPMI (final MTT concentration was 200 nmol/mL), followed by a 2 h incubation. Centrifugation at 500 x g for 5 min was performed to concentrate the viable cells containing dark violet crystals. After centrifugation, we discarded RPMI plus MTT and allowed 50 μL of the medium mix to settle so as not disturb the pellet. Then, 200 μL of DMSO (sc-358,801; Santa Cruz Biotechnology, TX, U.S) was added to each condition and mixed carefully to dissolve the dark violet crystals. To perform the measurement in the microplate spectrophotometer (Epoch; BioTek Instruments), we used a 96-well ELISA plate with a flat bottom with 100 μL of each condition. The plate was read using a wavelength of 570 nm.

Mitochondrial DNA damage quantitation by qPCR

PBMCs tested in all conditions were washed with 1 mL PBS and centrifuged at 500 x g for 5 min prior to the assay to eliminate any kind of debris or un-transferred mitochondria. DNA was extracted from PBMCs using MagMax™-96 Mutli-Sample Kit (Applied Biosystems by ThermoFisher Scientific, MA, U.S). Real time quantitative PCR was performed in a StepOne Real-Time PCR System (Applied Biosystems by ThermoFisher Scientific, MA, U.S). Fast SYBR® Green Master Mix (4,385,610; Applied Biosystems by ThermoFisher Scientific, MA, U.S) was used according to manufacturer's instructions, resulting in a final DNA concentration of 10 ng/μL in a 10 μL reaction. mtDNA was identified and quantified using primers (hMitoF3 and hMitoR3), manufactured by Invitrogen™: hMitoF3 5′- CACTTTCCACACAGACATCA – 3′; hMitoR3 5′- TGGTTAGGCTGGTGTTAGGG – 3′ corresponding to a unique region of the mitochondria DNA between positions 241 and 390, which has been shown to be less similar to nuclear DNA sequences, thereby ensuring more accurate identification by the primers [18, 19]. The nuclear human β-2 microglobulin gene was used as a housekeeping gene with the primers: hB2MF1 5′- TGT TCC TGC TGG GTA GCT CT – 3′; and hB2MR1 5′- CCT CCA TGA TGC TGC TTA CA – 3′, as suggested by Ajaz et al.(2015) [18]. The qPCR reaction was based on the Ajaz et al. (2015) protocol with a minor modification: we included pre-incubation at 95 °C for 5 min (1 cycle); denaturation at 95 °C for 10 min; annealing and extension at 63 °C for 30 min (denaturation and extension steps were repeated for 40 cycles); and melting at 95 °C for 5 min, 63 °C for 60 min, 95 °C for 5 min, and 40 °C for 30 min. Data were collected by estimating the $2^{-\Delta CT}$; for statistical analysis, we used the Anderson-Darling test for normality to determine the use of parametric or non-parametric analysis. After statistical normality assumptions, we used un-paired, parametric ANOVA and Student's t-test to analyze the differences among conditions, using an alpha-value of 0.05 ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$) to establish significant differences.

p53 gene expression

RNA was isolated from PBMCs using the PureLink™ RNA Mini Kit (12,183,025; Ambio by Life Technologies, ThermoFisher Scientific, MA, U.S). Real time quantitative PCR was performed in a StepOne Real-Time PCR

System. SuperScript® III Platinum® SYBER® Green One-Step qRT-PCR Kit with Rox (11,745,500; Invitrogen by Life Technologies, ThermoFisher Scientific, MA, U.S) was used according to the manufacturer's instructions with a final DNA concentration of 10 ng/μL in a 10 μL reaction. mtDNA was quantified using primers manufactured by Invitrogen: Tp53F 5′ - CCT CAG CAT CTT ATC CGA GTG G – 3′; Tp53R 5′ – TGG ATG GTG GTA CAG TCA GAG C – 3′; and the nuclear PUM gene was used as the housekeeping gene with the primers: PUM1F 5′- AGT GGG GGA CTA GGC GTT AG – 3; and PUM1R 5′- GTT TTC ATC ACT GTC TGC ATC C – 3′. The qPCR reaction was performed under the following conditions: pre-incubation at 95 °C for 5 min (1 cycle); denaturation at 95 °C for 15 min; annealing and extension at 60 °C for 30 min (repeat denaturation and extension steps for 45 cycles); melting at 95 °C for 15 min, 60 °C for 60 min, and 95 °C for holding. Data were collected by estimating the $2^{-\Delta CT}$.

ROS quantification

Aliquots of 1 million PBMCs in 2 mL of RPMI medium with 1% FCS were exposed to 6 min UVR (dosage: 72 mJ/cm^2) in 2 mL Eppendorf tubes. After 1 h culture at 37 °C in a 5% CO2/humidified air atmosphere, exposed and non-exposed samples (controls) were MitoCepted with 2.5 ng of mitochondria, according to the method explained above. 10 μL of Dihydro-rhodamine-123 (DHR-123) (D23806; ThermoFisher Scientific, MA, U.S) was added to 400 μL of each sample (final concentration: 1 mmol/L) followed by 1 μL Phorbol 12-myristate 13-acetate (PMA) (final concentration of 1 mmol/L) (79, 346; SIGMA, Merck, U.S) to induce cellular stress. Cells were cultured in the dark for 2 h. Flow cytometry was performed with a BD AccuriTM C6 Plus device (BD Biosciences, NJ, U.S) with 488 nm laser excitation and analyzed with BD AccuriTM CSampler software. Five thousand cells were collected from each sample. Results were calculated as the mean fluorescence intensity (MFI) (arbitrary units) and normalized by folding. Our data are shown with mean fluorescence intensity values.

Statistical analyses

Data were analyzed using Minitab 17 and GraphPad Prism 6 software. The results for the mitochondrial mass and ROS production (estimated by flow cytometry), and metabolic activity (estimated with MTT assay) were normalized by using sample/control-average transformation. The percentage of dead cells was estimated using the Trypan Blue stain. The qPCR $2^{-\Delta CT}$ fold method was applied to mtDNA quantification and expression of the p53 gene. Then, we performed an Anderson-Darling normality tests on all data sets to determine the use of parametric or non-parametric analysis. After statistical normality assumptions, we used un-paired, parametric ANOVA and Student's t-test to analyze the differences among conditions, using an alpha-value of 0.05 ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$) to observe significant differences. Red headlines with asterisks represent the overall ANOVA significance; blue headlines with asterisks represent Student's t-test significance between conditions and red asterisks over columns represent Student's t-test significance between the control and the individual condition as stated in each figure.

Additional files

Additional file 1: Figure S1. PBMCs lymphocytes and monocytes populations. Representative images. (PPTX 81 kb)

Additional file 2: Figure S2. Schematic representation of the UVR damage and PAMM MitoCeption rescue of PBMCs. (PPTX 209 kb)

Abbreviations

AMT/T: Artificial Mitochondrial Transfer or Transplant; CD14: Cluster of differentiation- co-receptor detection Lipopolysaccharide (LPS); CD3: Cluster of differentiation- T-cell co-receptor; DHR-123: Dihydro-rhodamine-123; DMEM: Dulbecco's Modified Eagle Medium; DMSO: Dimethyl sulfoxide; ELISA: Enzyme-linked immunosorbent assay; FCS: Fetal calf serum; MAV: Mean absorbance values; MFI: Mean fluorescence intensity; MSCs: Mesenchymal stem/stromal cells; mtDNA: Mitochondrial DNA; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NADPH: Nicotinamide adenine dinucleotide phosphate; p53: Tumor suppressor gene 53; PAMM: Primary allogeneic mitochondrial mix; PBMCs: Peripheral blood mononuclear cells; PBS: Phosphate buffered saline; PMA: Phorbol 12-myristate 13-acetate; qPCR: Real-time quantitative polymerase chain reaction; RB: Respiratory burst; ROS: Reactive oxygen species; RPMI: Roswell Park Memorial Institute; UVC: UVR wavelength of 254 nm; UVR: Ultraviolet radiation

Acknowledgements

We would like to thank Marisol Llerena, Veronica Castañeda and Emilia Morales, biotechnology students from the Universidad San Francisco de Quito (USFQ) for their assistance during the assays of the article. We are thankful to Dr. Marcela Bovera, Anita Franco, Nancy Tigasi, and Yolanda Calderón, members of the "Laboratorio de Análisis Clínicos" of the Hospital de los Valles in Quito for their help during blood collection from donors. We would like to thank Danny Navarrete, a USFQ colleague, for his help in the statistical analysis. The authors would also like to thank Esther L. Wisdom and Caley Mikesell for assistance in revising the manuscript.

Authors' contributions

FC, MO, FV, EP and AC planned and performed the experiments, wrote the manuscript, analyzed data and created figures. SG set-up the MTT assays and contributed to the manuscript. DB performed the qPCR experiments regarding p53 expression levels. LN, GP, LAP, and EP performed flow cytometry assays and contributed to the manuscript. ChJ and MK advised on data analysis and contributed to the manuscript. F.V. drew the images of Fig. 2.1. AC conceptualized the article and developed PAMM. All authors have read and approved the manuscript.

Funding

This research was funded by USFQ Collaboration Grant (institutional codes: HUBi 5501, USFQ Veterinary Medicine School Grants for Research; # 3281 and #10098) and SIME R&D. The funding bodies evaluated this project before its submission to BMC Biotechnology through grant proposals and bioethics review processes according to institutional criteria. Funding bodies did not play any role in the design, data collection, analysis, and interpretations of data or in writing the manuscript.

Availability of data and materials
All data generated in this article and derived data supporting the findings of this study are available from the corresponding author upon request.

Ethics approval and consent to participate
Blood was collected from healthy female and male donors between 20 and 30 years old, each of whom provided written informed consent in accordance with relevant guidelines and regulations for mitochondria studies approved by the Bioethics Committee (the Institutional Review Board) of the Universidad San Francisco de Quito (study code: 2017-026IN).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Colegio de Ciencias de la Salud, Escuela de Medicina Veterinaria, Universidad San Francisco de Quito, 17-12-841 Quito, Ecuador. [2]Colegio de Ciencias Biológicas y Ambientales, Escuela de Biotecnología, Universidad San Francisco de Quito, 17-12-841 Quito, Ecuador. [3]Colegio de Ciencias de la Salud, Escuela de Medicina, Universidad San Francisco de Quito, 17-12-841 Quito, Ecuador. [4]Baylor College of Medicine: Department of Pediatrics, Section of Immunology, Allergy, and Rheumatology, Baylor College of Medicine, Houston, TX, USA. [5]IRMB CHU Saint Eloi, University of Montpellier, France, 80 rue Augustin Fliche, 34295 Montpellier, cedex 5, France. [6]Laboratory of Nano-Regenerative Medicine, Faculty of Medicine, Universidad de Los Andes, Santiago, Chile. [7]Consorcio Regenero, Chilean Consortium for Regenerative Medicine, Santiago, Chile. [8]Cells for Cells, Santiago, Chile. [9]Instituto de Investigaciones en Biomedicina, Universidad San Francisco de Quito, 17-12-841 Quito, Ecuador. [10]Mito-Act Research Consortium, Quito, Ecuador. [11]Sistemas Médicos, SIME, Universidad San Francisco de Quito, 17-12-841 Quito, Ecuador.

Received: 14 November 2018 Accepted: 14 June 2019
Published online: 28 June 2019

References

1. Miliotis S, Nicolalde B, Ortega M, Yepez J, Caicedo A. Forms of extracellular mitochondria and their impact in health. Mitochondrion. 2019. https://doi.org/10.1016/j.mito.2019.02.002.
2. Liu C-S, et al. Delivering healthy mitochondria for the therapy of mitochondrial diseases and beyond. Int J Biochem Cell Biol. 2014;53:141–6.
3. Jackson MV, et al. Mitochondrial transfer via tunneling nanotubes is an important mechanism by which mesenchymal stem cells enhance macrophage phagocytosis in the in vitro and in vivo models of ARDS: mitochondrial transfer from MSC to macrophages. Stem Cells. 2016;34:2210–23.
4. Wang X, Gerdes H-H. Transfer of mitochondria via tunneling nanotubes rescues apoptotic PC12 cells. Cell Death Differ. 2015;22:1181.
5. Spees JL, Olson SD, Whitney MJ, Prockop DJ. Mitochondrial transfer between cells can rescue aerobic respiration. Proc Natl Acad Sci U S A. 2006;103:1283–8.
6. Islam MN, et al. Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. Nat Med. 2012;18:759–65.
7. Acquistapace A, et al. Human mesenchymal stem cells reprogram adult cardiomyocytes toward a progenitor-like state through partial cell fusion and mitochondria transfer. Stem Cells. 2011;29:812–24.
8. Berridge MV, Schneider RT, McConnell MJ. Mitochondrial transfer from astrocytes to neurons following ischemic insult: guilt by association? Cell Metab. 2016;24:376–8.
9. Clark MA, Shay JW. Mitochondrial transformation of mammalian cells. Nature. 1982;295:605–7.
10. McCully JD, Levitsky S, del Nido PJ, Cowan DB. Mitochondrial transplantation for therapeutic use. Clin Transl Med. 2016;5(1):16.
11. Caicedo A, et al. MitoCeption as a new tool to assess the effects of mesenchymal stem/stromal cell mitochondria on cancer cell metabolism and function. Sci Rep. 2015;5:9073.
12. Caicedo A, et al. Artificial mitochondria transfer: current challenges, advances, and future applications. Stem Cells Int. 2017. https://doi.org/10.1155/2017/7610414.
13. Hsu Y-C, Wu Y-T, Yu T-H, Wei Y-H. Mitochondria in mesenchymal stem cell biology and cell therapy: from cellular differentiation to mitochondrial transfer. Semin Cell Dev Biol. 2016;52:119–31.
14. Kim MJ, Hwang JW, Yun C-K, Lee Y, Choi Y-S. Delivery of exogenous mitochondria via centrifugation enhances cellular metabolic function. Sci Rep. 2018;8:3330.
15. Kesner EE, Saada-Reich A, Lorberboum-Galski H. Characteristics of mitochondrial transformation into human cells. Sci Rep. 2016;6:26057.
16. Emani SM, Piekarski BL, Harrild D, del Nido PJ, McCully JD. Autologous mitochondrial transplantation for dysfunction after ischemia-reperfusion injury. J Thorac Cardiovasc Surg. 2017;154:286–9.
17. Dahlmanns M, et al. Chemotherapeutic xCT inhibitors sorafenib and erastin unraveled with the synaptic optogenetic function analysis tool. Cell Death Discov. 2017;3:17030.
18. Ajaz S, Czajka A, Malik A. Accurate measurement of circulating mitochondrial DNA content from human blood samples using real-time quantitative PCR. Methods Mol Biol Clifton NJ. 2015;1264:117–31.
19. Malik AN, Shahni R, Rodriguez-de-Ledesma A, Laftah A, Cunningham P. Mitochondrial DNA as a non-invasive biomarker: accurate quantification using real time quantitative PCR without co-amplification of pseudogenes and dilution bias. Biochem Biophys Res Commun. 2011;412:1–7.
20. Kitani T, Kami D, Matoba S, Gojo S. Internalization of isolated functional mitochondria: involvement of macropinocytosis. J Cell Mol Med. 2014;18:1694–703.
21. Wu M, et al. Comparison of the biological characteristics of mesenchymal stem cells derived from the human placenta and umbilical cord. Sci Rep. 2018;8:5014.
22. Thomas DC, et al. Eros is a novel transmembrane protein that controls the phagocyte respiratory burst and is essential for innate immunity. J Exp Med. 2017;214:1111–28.
23. Birch-Machin M a, Russell E v, Latimer J a. Mitochondrial DNA damage as a biomarker for ultraviolet radiation exposure and oxidative stress. Br J Dermatol. 2013;169:9–14.
24. Gniadecki R, Thorn T, Vicanova J, Petersen A, Wulf HC. Role of mitochondria in ultraviolet-induced oxidative stress. J Cell Biochem. 2001;80:216–22.
25. Torregrosa-Muñumer R, Goffart S, Haikonen JA, Pohjoismäki JLO. Low doses of ultraviolet radiation and oxidative damage induce dramatic accumulation of mitochondrial DNA replication intermediates, fork regression, and replication initiation shift. Mol Biol Cell. 2015;26:4197–208.
26. Takai D, et al. UV-irradiation induces oxidative damage to mitochondrial DNA primarily through hydrogen peroxide: analysis of 8-oxodGuo by HPLC. Free Radic Res. 2006;40:1138–48.
27. Agnello M, Morici G, Rinaldi AM. A method for measuring mitochondrial mass and activity. Cytotechnology. 2008;56:145–9.
28. Marlein CR, et al. PGC-1α driven mitochondrial biogenesis in stromal cells underpins mitochondrial trafficking to leukemic blasts. Leukemia. 2018;1. https://doi.org/10.1038/s41375-018-0221-y.
29. van Meerloo J, Kaspers GJL, Cloos J. Cell Sensitivity Assays: The MTT Assay. In: Cree IA, editor. Cancer Cell Culture: Methods and Protocols: Humana Press; 2011. p. 237–45. https://doi.org/10.1007/978-1-61779-080-5_20.
30. Altman SA, Randers L, Rao G. Comparison of trypan blue dye exclusion and fluorometric assays for mammalian cell viability determinations. Biotechnol Prog. 1993;9:671–4.
31. Rufini A, Tucci P, Celardo I, Melino G. Senescence and aging: the critical roles of p53. Oncogene. 2013;32:5129–43.
32. Olweus J. Manufacture of CAR-T cells in the body. Nat Biotechnol. 2017;35:520.
33. Srivastava S, Riddell SR. Engineering CAR-T cells: design concepts. Trends Immunol. 2015;36:494–502.
34. Ren J, et al. A versatile system for rapid multiplex genome-edited CAR T cell generation. Oncotarget. 2017;8:17002–11.
35. Stewart MP, et al. *In vitro* and *ex vivo* strategies for intracellular delivery. Nature. 2016;538:183–92.
36. Schumann K, et al. Generation of knock-in primary human T cells using Cas9 ribonucleoproteins. Proc Natl Acad Sci. 2015;112:10437–42.

Publisher's Note

c. Submitted paper # 3 (DRAFT)

Mitochondrial replacement therapy of oocytes and embryos by MitoCeption, as a proof of concept to repair hereditary mitochondrial damage

Francisco Cabrera[1,3], Emilia Morales[+] & Verónica Castañeda[+], Franchesca Velarde, Mayra Ortega, Ariana Leon, Christian Jorgensen[3,4] and Andrés Caicedo[2,4]*

[1]Universidad San Francisco de Quito, COCSA, Escuela de Medicina Veterinaria, Quito, 17-12-841, Ecuador

[2]Universidad San Francisco de Quito, COCSA, Escuela de Medicina, Quito, 17-12-841, Ecuador

[3]IRMB CHU Saint Eloi, 80 rue Augustin Fliche, 34295 Montpellier Cedex 5, University of Montpellier, France Montpellier University, Montpellier, France

[4]Mito-Act Research Consortium, Quito, Ecuador

[5]Universidad San Francisco de Quito, Instituto de Investigaciones en Biomedicina, Quito 17-12-841,Ecuador

+Authors equally contributed to this work

*corresponding author: acaicedo@usfq.edu.ec

Key words:

Abstract:

Introduction

Mitochondrial health is essential for maintaining the cellular metabolism, growth, survival and differentiation(Guillet-Pichon & Verny, 2016; Keogh & Chinnery, 2015). This organelle contains an independent circular genome (mtDNA), conserved from its bacterial origin, with 16 569 base pairs (bp) encoding 37 genes; 2rRNA, 22 tRNAs and 13 protein subunits part of the respiratory chain complexes responsible for energy production(Boengler, Heusch, & Schulz, 2011; Falkenberg, Larsson, & Gustafsson, 2007). The mtDNA and nuclear DNA (nDNA) co-evolved to coordinate cell functions and maintain homeostasis(K. H. Kim, Son, Benayoun, & Lee, 2018). For example, complexes are ensemble with subunits encoded by nDNA and the mtDNA in the mitochondria, mutations in any of their sequences could cause a failure and loss of cell homeostasis. Additionally, mutual regulatory mechanisms between both genomes to control mitochondria dynamics, biogenesis and cell homeostasis are in place to survive stress and aging(Boengler et al., 2011; Gammage, Moraes, & Minczuk, 2018; K. H. Kim et al., 2018). mtDNA is very different from nDNA as it lacks a chromatin packing and repairing mechanisms, make it particularly vulnerable to cellular and environmental mutagens. 700 mutations of mtDNA have been described and some of them directly associated to infertility, myopathies, neurodegenerative disorders, among others diseases(Wolf, Mitalipov, & Mitalipov, 2015b). Prevalence of mtDNA diseases has been estimated of 1 in 5000 individuals with a frequency of 1 in 200 new-borns(H. R. Elliott, Samuels, Eden, Relton, & Chinnery, 2008; Gorman et al., 2016). As mtDNA is maternally inherited, it has been observed that 150 to 780 women per year are in risk of transmitting an mtDNA disease in the UK and US(Herbert & Turnbull, 2018). The mitochondrial susceptibility to harmful mutations and the frequency observed emphasize the importance of developing new therapeutic options to prevent the transmission or cure of mitochondrial diseases early in development(Herbert & Turnbull, 2018).

The oocyte can carry inherited mitochondrial diseases or develop de novo mutations during its development causing mitochondrial dysfunction that would reflect disease later in growth. The oocyte issued from an aged mother can accumulated mutations with time causing infertility. Patients with inherited or de novo mtDNA mutations have a variety of pathological phenotypes as cells can have coexisting healthy and mutated mtDNA genomes, this mix is called heteroplasmy. An imbalance towards unhealthy mtDNA increases the possibility of cellular dysfunction resulting in cells and tissues more affected than others in an organism(Gorman et al., 2016). Replacing or rebalance the heteroplasmy in the oocyte towards healthy mitochondria could be a viable therapy to mutated mtDNA related disorders and oocyte aging.

Nowadays, a few options are available before and after conception for women carrying mtDNA hereditary mutations when desiring to have a healthy child. First, is necessary to estimate the levels of heteroplamsy and homoplasmy (were all the mtDNA is affected) by observing the patient symptoms and by DNA screening through tissue biopsy. If low penetration of the mtDNA mutations is observed, it is possible to continue to in vitro fertilisation (IVF) and select embryos carrying the less of mtDNA mutations. In the case of having a high percentage to tissues affected with mtDNA mutations or homoplasmy, egg donation and adoption are the most common choices. As an emerging option, mitochondrial replacement therapy (MRT) or mitochondrial donation offers the possibility to have offspring genetically related and a reduction of the risk of transmitting an mtDNA related disorders. So far, MRT has shown positive effects in clinical applications(Wolf et al., 2015b). As a possibility, gene therapy could be plausible, however more evidence regarding the safety of this technology is needed before human tests(Nightingale, Pfeffer, Bargiela, Horvath, & Chinnery, 2016).

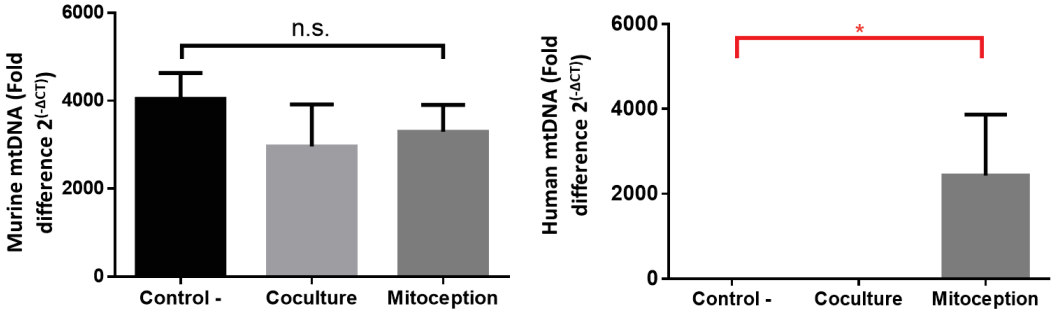
MRT uses different methods of transplanting the nuclear genome from an unfertilized or fertilized egg of an affected patient or couple to an enucleated egg coming from a healthy donor. MRT techniques applied before oocyte fertilization are spindle - chromosomal complexes (karyoplast) transfer (ST) and polar body pronuclear transfer (PNT). Pronuclear transfer (PNT), is an MRT performed after fertilization. Even if these procedures have resulted successful during in vivo and in clinical procedures, invasive manipulation, chemicals, unhealthy mitochondria residuals and recipient ovule loss due to enucleation are still causes of concern. Nevertheless, MRT is difficult to put in practice due the need of specialized equipment and extensive manipulation of germinal cells(Sato et al., 2005). Techniques based in the cytoplasmic microinjection of mitochondria in oocytes represent a harm to its physiology as there is the risk of cell's destruction and a pronounced decrease of the amount of developing embryos, independently of the content of the needle or the developmental stage treated (oocyte or zygote) (Liu, Yang, Lu, & Qiu, 2008; Sokolova et al., 2004). Less invasive procedures to increase the quantity of healthy or eliminate unhealthy mitochondria are needed. Better mitochondrial therapies, less complex to apply and with less manipulation could increase the possibility to have healthy offspring for women with mitochondrial disorders or oocyte fertility problems due to age.

It has been reported that Mesenchymal Stem / Stromal Cells have the capacity to transfer functional mitochondria to stressed or damaged cells and different cell types(Andr&#x00E9 Caicedo et al., 2017; Islam et al., 2012b; Jackson Megan V. et al., 2016; X. Li et al., 2014b; Luz-Crawford et al., 2019b; Miliotis, Nicolalde, Ortega, Yepez, & Caicedo, 2019;

Sinha, Islam, Bhattacharya, & Bhattacharya, 2016b). Inspired by this process we develop the MitoCeption technique based in the artificial transfer / transplant of healthy mitochondria (AMT/T) to a recipient cell using a thermic shock and a centrifugation. We previously observed that the transfer of healthy mitochondria to UVR stressed recipient cells repair the endogenous mitochondrial mass, mtDNA damage, metabolic activity and reduced the expression of stress markers such as p53(Cabrera et al., 2019). As oocytes and embryos can carry defective mitochondria, by mutations on its mtDNA, we sought to apply the MitoCeption (AMT/T) technique to transfer effectively increasing amounts of healthy exogenous mitochondria, increasing heteroplasmy and the possibility to reduce the diseased phenotype. For this reason, we propose the use of the Mitoception technique as an alternative of MRT for mitochondrial transfer to oocytes or early embryos.

Results

AMT by Coincubation and MitoCeption to oocytes.



To determine if murine oocytes can receive exogenous mitochondria by Mitoception, we develop a model in which we detect them by fluorescence microscopy and real time quantitative polymerase chain reaction (qPCR) in murine oocytes. Mitochondria was isolated from human peripheral Blood Mononuclear Cells (PBMCs) labelled with MitoTracker Red, combined in a three-donors mix and quantified. Mix was made in order to minimize the quantity of blood required from the donor without affecting the total amount of recovered mitochondria (50 to 60 ng/µL). Oocytes were collected from 8-12 weeks old female mice. MitoCeption and coincubation between murine oocytes (25-30) and human mitochondria (25 ng/µL) was performed following the modified protocol for non-attached cells (MitoCeption, in a 1.5 ml microcentrifuge tube, 500 µL of RPMI at 5% FCS at 4°C, 500 g during 5 min, then placed at to 37°C, 5% CO2 during 1h)(Cabrera et al., 2019). Coincubation, in a microcentrifuge tube, murine oocytes were incubated with human mitochondria during 1h at 37°C, 5% CO2). After 1h of MitoCeption and coincubation, oocytes were washed and used for fluorescence and quantitative real-time qPCR detection of internalized human exogenous mitochondria.

Qualitatively, we observed an increase of red mitochondria detected in the green murine oocytes that were mitocepted in comparison with the controls (Fig. 1b). DNA was isolated from the control and Mitocepted oocytes. Human mtDNA, was detected by using primers that specifically bind to a unique region between positions 241 and 390 previously used in literature(Ajaz, Czajka, & Malik, 2015; Malik, Shahni, Rodriguez-de-Ledesma, Laftah, & Cunningham, 2011). Mouse specific mitochondria primers (mMito) were designed by Malik et. al. 2016 at position 1321-1447(Malik, Czajka, & Cunningham, 2016). Nuclear Beta-2 microgobulin gene (mB2M) were used for quantifying murine mitochondria and as a housekeeping gene respectively. We observed a statistically significant increase in the number of copies of human mtDNA in the murine mitocepted oocytes (ANOVA test, $*p < 0.01$). No changes were observed in the quantity of mMito.

AMT by MitoCeption to oocytes

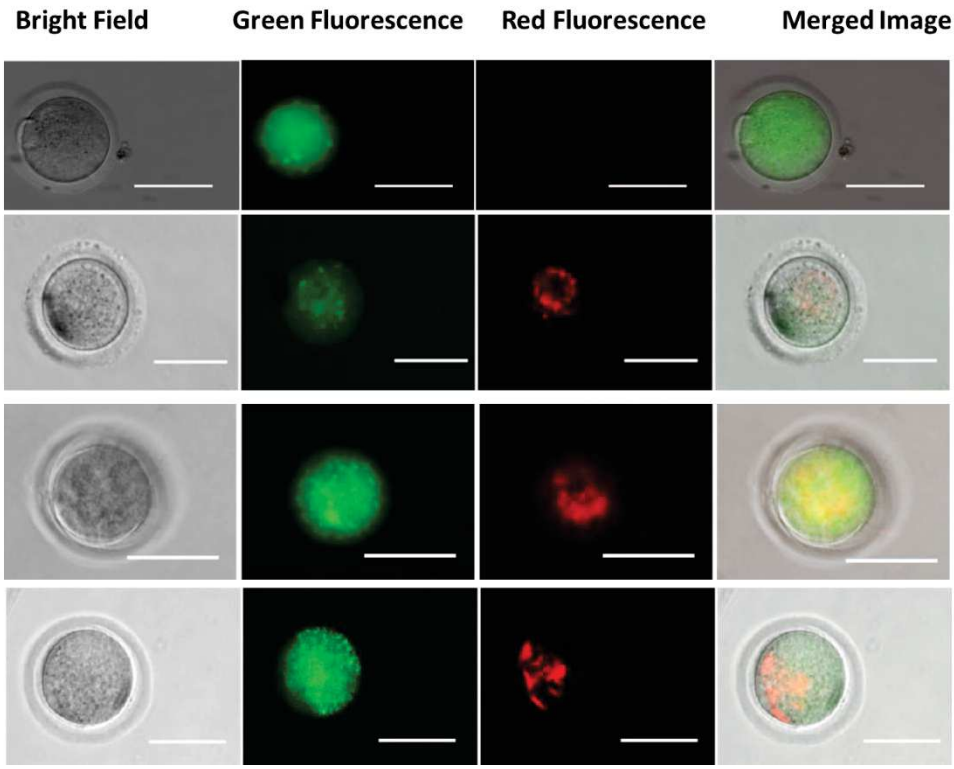


Figure 2. Artificial Mitochondrial Transfer by MitoCeption to murine Oocytes. Mature oocytes were stained with CytoTracker Green; HPMM isolated from PBMCs was marked with MitoTracker Red. MitoCeption of oocytes with HPMM was performed after isolation of the oocytes and washing. Micrographs were taken after 1h culture of the MitoCepted oocytes. Columns: first, bright field; second, green fluorescence micrograph; third, red fluorescence micrograph; fourth, merged of bright field and fluorescence in green and red. **a.** Control oocyte without exogenous mitochondria **b.** Oocyte with 12.5 ng / µL of HPMM **c.** Oocyte with 25 ng / µL of HPMM. **d.** Oocyte with 50 ng / µL of HPMM. Scale bar 100 µm

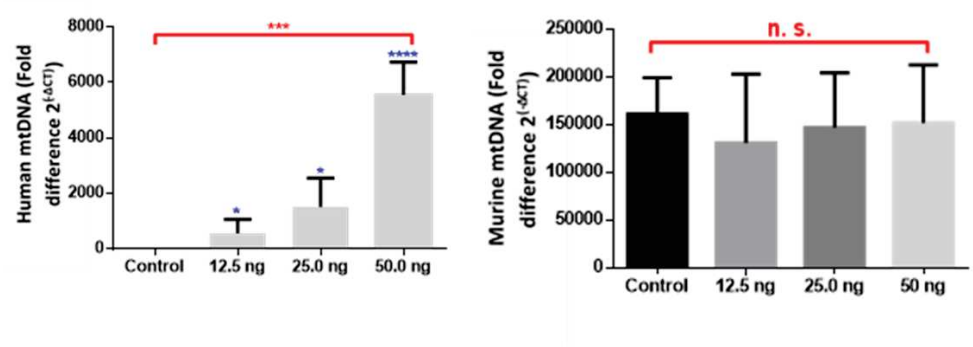


Figure 3: Detection of human and murine mitochondrial genes in mitocepted murine oocytes calculated by $\mathbf{2^{(-\Delta CT)}}$ method at 3 dosages: 12.5, 25 and 50 ng/ul of mitochondrial protein. Housekeeper gene: Murine B_2M (MB_2M). a: Human Mito (HMito) b: Murine Mito (MMito)

12.5, 25 and 50 ng/µL of human mitochondria labelled with MitoTracker Red mix were transferred to 25 to 30 oocytes dyed with CellTracker Green by condition following the previously detailed conditions. Qualitatively, we observed an increase of red mitochondria detected in the green murine oocytes in proportion to the amount transferred, representative images of the oocytes are shown (Fig. 3a). Later, DNA was isolated from the control and Mitocepted oocytes. Human mtDNA, was detected observing a statistically significant and proportional increase in the number of copies of human mtDNA in the murine mitocepted oocytes (ANOVA test, ****$p < 0.0001$). No changes were observed in the quantity of mMito (Fig. 3b).

Fertilization and development of MitoCepted oocytes to zygotes

12.5, 25 and 50 ng/µL of human mitochondria were transferred to 25 to 30 oocytes by condition following the previously detailed conditions. Sperm collection was performed from a mature male mouse (3 to 6 months old). After the 30 minutes for sperms' capacitation, we evaluate the sperms' motility with a microscope before taking 20-30 µl of sperms' suspension (Number of Sperm) and putting it in the medium drop of fertilization dish. We place the fertilization dish in an incubator (37°C, 5% CO_2 in air) by 3 hours. Fertilization was estimated by counting oocytes polar body II formation or first division between controls and mitocepted oocytes in different conditions showing no significant differences except for the 50 ng/µL conditions were polar body formation decreased (Fig. 3a).

AMT by MitoCeption to zygotes

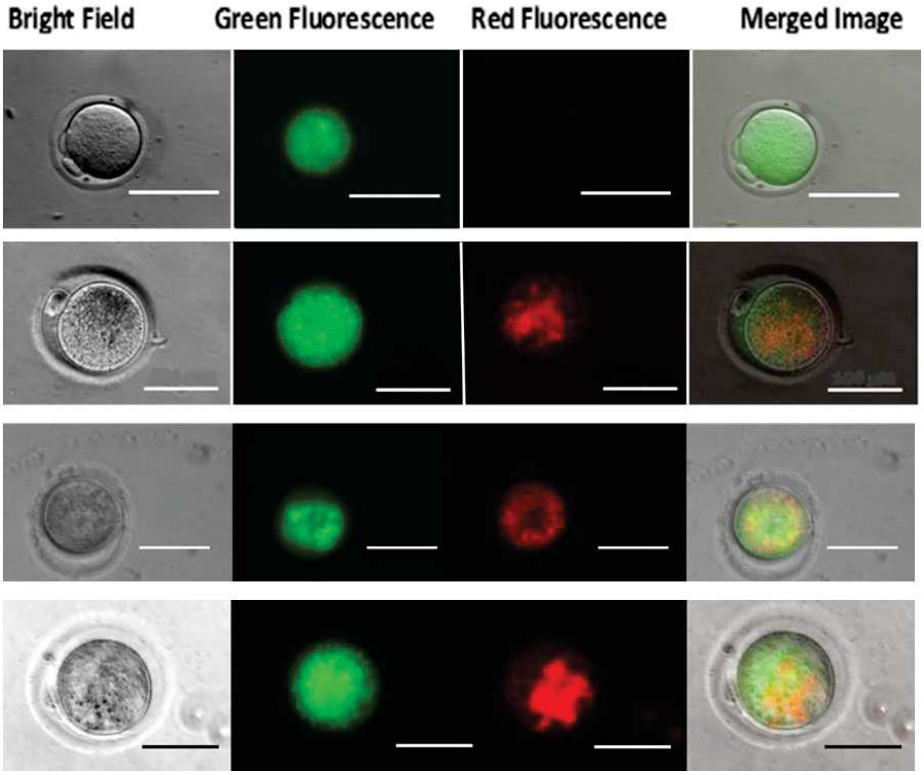


Figure 4. Artificial Mitochondrial Transfer by MitoCeption after IVF to murine zygotes. Mature oocytes were fertilized, stained with CytoTracker Green, and immediately after MitoCepted with HPMM isolated from PBMCs labelled with MitoTracker Red. Micrographs were taken after 1h culture of the MitoCepted zygotes. Columns: first, bright field; second, green fluorescence micrograph; third, red fluorescence micrograph; fourth, merged of bright field and fluorescence in green and red. **a.** Control zygote without exogenous mitochondria **b.** Zygote with 12.5 ng / µL of HPMM **c.** Zygote with 25 ng / µL of HPMM. **d.** Zygote with 50 ng / µL of HPMM. Scale bar 100 µm

100 to 120 cell tracker green dyed oocytes were fertilized accordingly to previously detailed conditions. 25 to 30 zygotes were recovered and mitocepted with 12.5, 25 and 50 ng/µL of human mitochondria labelled with MitoTracker Red mix. Qualitatively, we observed an increase of red mitochondria detected in the green murine zygotes in proportion to the amount transferred, representative images of the oocytes are shown (Fig. 4a). Later, DNA was isolated from the control and mitocepted oocytes. Human mtDNA, was detected observing a statistically significant and proportional increase in the number of copies of human mtDNA in the murine mitocepted zygotes (ANOVA test, ****$p < 0.0001$). No changes were observed in the quantity of mMito (Fig. 4b).

Development of MitoCepted zygotes to embryos

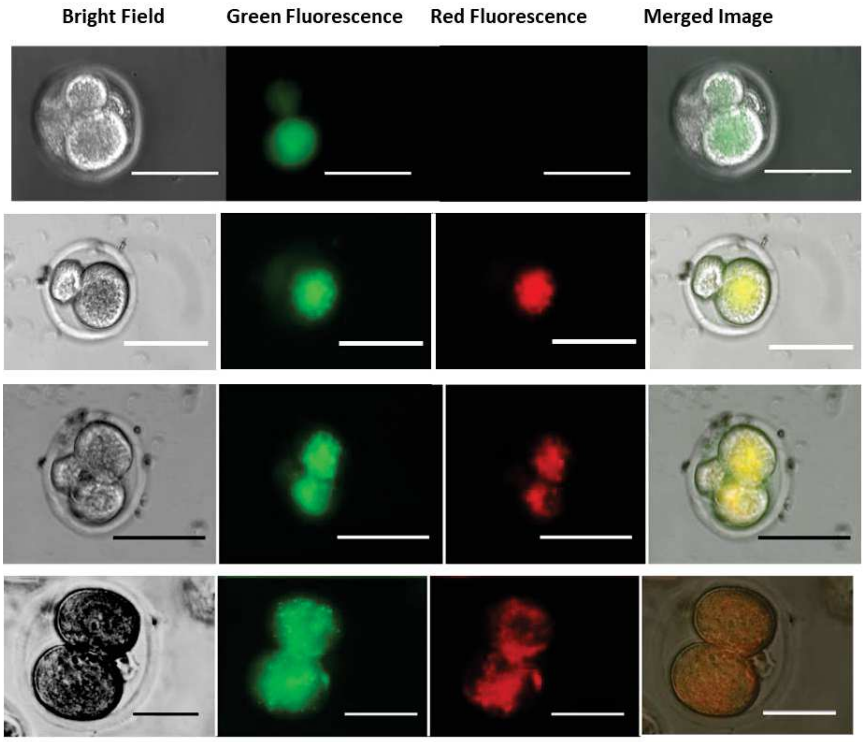


Figure 3. 2-cell development of Mitocepted embryos with HPMM. Green zygotes after MitoCeption with HPMM (in red) were maintained in culture during 6h (2-cell embryos) and micrographs were performed to follow up its development and survival. Columns: first, bright field; second, green fluorescence micrograph; third, red fluorescence micrograph; fourth, merged of bright field and fluorescence in green and red. **a.** Control 2-cell embryos without exogenous mitochondria **b.** 2-cell embryos with 12.5 ng / µL of HPMM **c.** 2-cell embryos with 25 ng / µL of HPMM. **d.** 2-cell embryos with 50 ng / µL of HPMM. Scale bar 100 µm

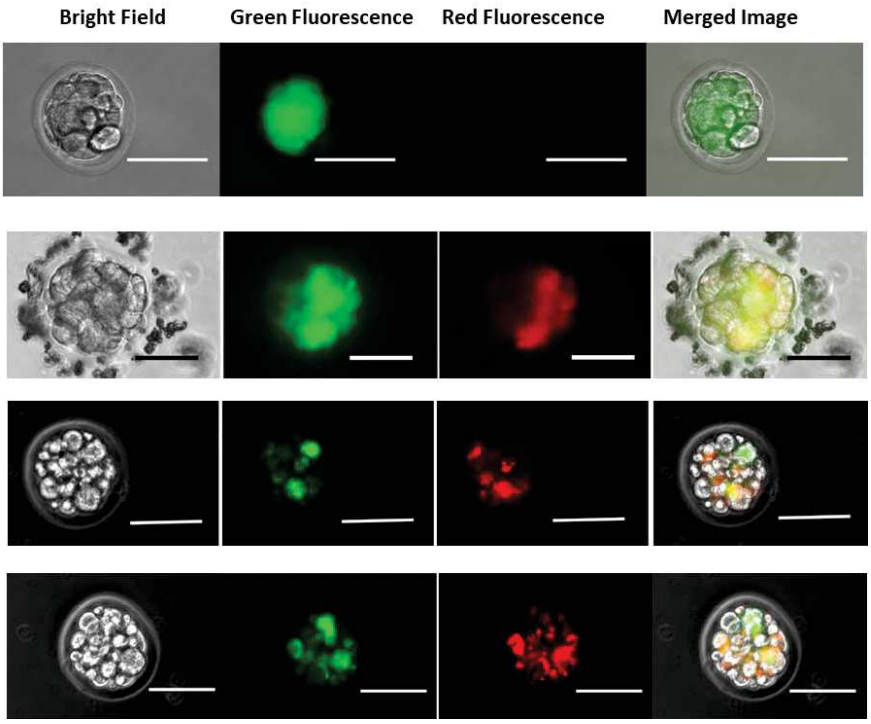


Figure 4. Morula development of Mitocepted embryos with HPMM. Green zygotes after MitoCeption with HPMM (in red) were maintained in culture during 10h (Morula stage) and

micrographs were performed to follow up its development and survival. Columns: first, bright field; second, green fluorescence micrograph; third, red fluorescence micrograph; fourth, merged of bright field and fluorescence in green and red. **a.** Control morula without exogenous mitochondria **b.** morula with 12.5 ng / µL of HPMM **c.** morula with 25 ng / µL of HPMM. **d.** morula with 50 ng / µL of HPMM. Scale bar 100 µm

25 to 30 control and mitocepted zygotes with 12.5, 25 and 50 ng/µL of human mitochondria labelled with MitoTracker Red mix were maintained in culture during 6h (2-cell embryos) and 10h (morula stage) (Fig. 5b). Qualitatively, we observed an increase of red mitochondria detected in the green murine 2-cell embryos and in the morulae in proportion to the amount transferred, representative images are shown (Fig. 5a & 5b). Later, DNA was isolated from the control and mitocepted morulae. Human mtDNA, was detected observing a statistically significant and proportional increase in the number of copies of human mtDNA in the murine morulae (ANOVA test, ****$p < 0.0001$). No changes were observed in the quantity of mMito (Fig. 5c).

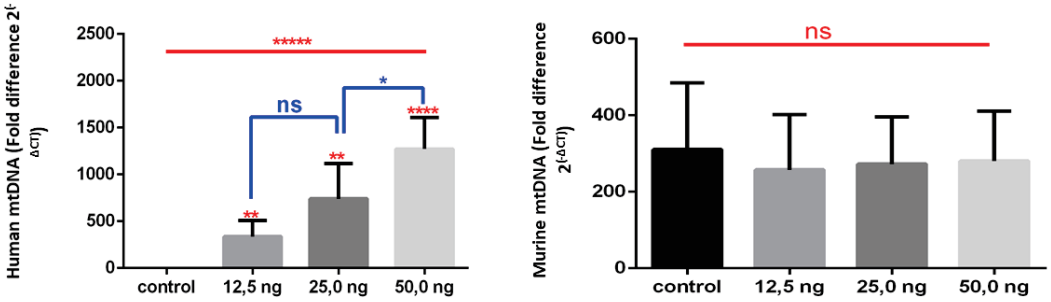


Figure xx: Detection of murine and human mitochondrial genes in mitocepted murine embryos (morulae) calculated by Delta CT method at 3 dosages: 12.5, 25 and 50 ng/ul of mitochondrial protein. a. expression of Human nuclear gene HB2M b. Expression of Murine Mitochondrial gene (Mmito). c. Expression of Human Mitochondrial gene (Hmito). Un-paired ANOVA and t-student with alpha-value of 0.05 (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

Development of the MitoCepted embryos in pups

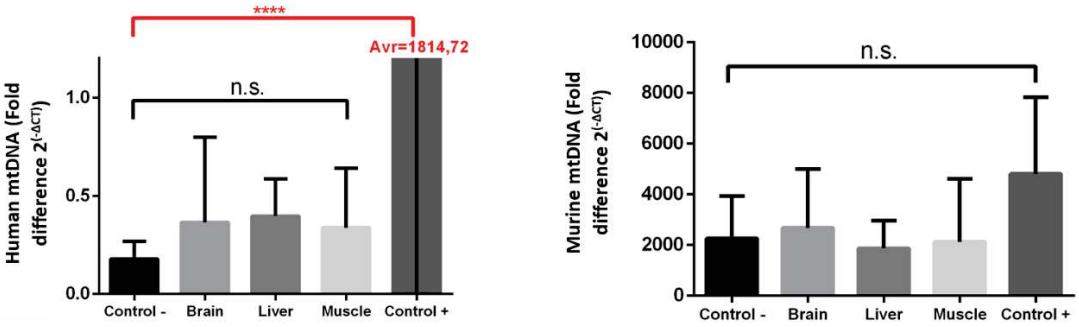


Figure xx: Detection of murine and human mitochondrial genes in mitocepted murine new-borns calculated by Delta CT method at 25 ng/ul of mitochondrial protein in three tissues: Brain, liver and muscle and using mitocepted morulae genomic DNA as positive control. a. expression

of Human nuclear gene mtDNA b. Expression of Murine Mitochondrial gene (Mmito). Un-paired ANOVA and t-student with alpha-value of 0.0001 (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

25 to 30 control and MitoCepted embryos with 25 and 50 ng/µL of human mitochondria mix were maintained in culture 10h (morula stage) and 12-15 were transferred into a subrogate mice female oviduct. After 20-21 days we observed 5 to 6 new-born pups (Fig. Isolation of DNA from the different body tissues. Human mtDNA, was absent observing a statistically significant difference in the number of copies of human mtDNA in the different body tissues (ANOVA test, ****$p < 0.0001$). No changes were observed in the quantity of mMito (Fig. 4b).

Discussion:

Methods:

Superovulation: We induce superovulation by injecting 7.0 IU of pregnant mare's serum gonadotropin (PMSG) i.p. into each mature female mouse (8-12 weeks old), between the hours of 17:00 and 18:00. Follow this up 48- hours later with a 7.0 IU i.p. injection of human chorionic gonadotropin (hCG). The next day, at 07:00, we prepare two Petri's dishes with Liquid paraffin: one for sperm capacitation (sperms' dish) and the second for oocytes' collection and IVF (fertilization dish). In both dishes, we put a drop (100 µl) of RPMI medium enriched with 1300 mg/L of sodium pyruvate and 2000 mg/L of sodium lactate and we place the dishes on a hot plate at 37°C.

Oocytes' collection: We sacrifice the female mice and dissect them to expose the abdominal cavity. Then, we move forward the digestive tract from inside the abdomen and expose the uteruses, oviducts and ovaries. Remove the uteruses, oviducts and ovaries, and place them on sterile filter paper. By carefully dissection, we remove the oviducts (ampullae) only, avoiding as much fat, blood and tissue fluid as possible. We immerse the removed oviducts in liquid paraffin contained within fertilization dish. Later, using forceps to hold the oviduct against the base of the fertilization dish, we use a 30G injection's needle to tear open the ampulla of the oviduct and release the cumulus-oocyte-complexes (COCs) from within. Then, we drag them into the drop of RPMI enriched medium. We cultivate the COCs at 37°C for the time needed for sperms' collection and capacitation. During this time (50 minutes average), the cumuli's cells partially come off the oocytes' surface (note: we did not sacrifice multiple mice at once; instead, we did sacrifice one mouse and swiftly remove its oviducts before moving on to the next mouse).

Sperm collection and capacitation: We sacrifice one mature male mouse (3 to 6 months old) and remove his *cauda epididymis*, avoiding as much fat, blood and tissue fluid as possible. We place the tissue on sterile filter paper to blot away any blood and fluid. Then, we place the removed *cauda epididymis* in a sperm dish containing liquid paraffin. Then, we cut the duct of

each cauda epididymis using a 30G injection's needle and we use a dissecting needle to gently press the surface of the cauda epididymis and release the sperm within. Using a dissecting needle, we introduce the clots of spermatozoa released from the *cauda epididymis* into the drop of RPMI enriched medium at 37°C. We store the sperms' suspension for 30 minutes on the hot plate to perform the immediate sperms' capacitation thanks to the normal contain of PBS of the medium.

In vitro fertilization (IVF): After the 30 minutes for sperms' capacitation, we evaluate the sperms' motility with a microscope before taking 20-30 µl of sperms' suspension and putting it in the medium drop of fertilization dish. We place the fertilization dish in an incubator (37°C, 5% CO_2 in air) by 3 hours.

Embryo wash and selection: after IVF, wash the embryos 3 times in fresh KSOM M16 Mouse Embryo Medium in a washing dish filled with liquid paraffin, avoiding the transfer of RPMI medium. The wash procedure consists in the individual oocyte transfer from one medium drop (100 µl) to the follow, using a capillary pipette (modified Pasteur's pipette). We discard any non-fertilized oocyte and parthenogenic embryos before continuing any procedure.

Embryo culture: In a 12 well plate, we put 250 µl of KSOM M2 Mouse Embryo Medium for well and assay's condition, and we place 20 embryos (2-cell stage and forward) each. This medium allows culture until blastocyst stage (3-5 days) in an incubator at 37°C with 5% CO_2 in air.

Embryo Transfer into subrogate mother's oviduct: While females selected and treated as oocytes' donors, synchronize an additional female of the same age with the same procedure (see up). The second day of embryo culture select 20 embryos of 2-6 cell stage and put them in a drop of 100 µl of KSOM M2 Mouse Embryo Medium in a Petri's dish filled with liquid paraffin and incubate at 37°C with 5% CO_2 in air until their transfer. Anesthetize the synchronized female with Ketamine 80-120mg/kg i.p. + Xilacine 5-mg/kg i.p. Disinfect each paralumbar groove with 70% isopropanol and shave the hair from both zones. Put the female on the hot plate at 37°C. Cut the skin and abdominal wall of **one side** and pull out the ovary, oviduct and part of the uterine horn. Clip a "mosquito" clamp onto the fat pat attached to the cranial side of ovarian bursa. Charge the capillary pipette with 2-3 bubbles of air, followed by **10 embryos**. With a 30-gauge needle, make a hole in the external surface of the oviduct, just forward the ampulla and insert the capillary pipette into the hole. Expel the embryos and the air bubbles. Cover the oviduct with the fat pad, push the ovary, oviduct and uterine horn into the abdomen and close the wound using 3-0 Blue polypropylene suture or wound clips**. Repeat the procedure in the other side of the body**. Disinfect the chirurgical wounds with iodine and maintain the female over the hot plate at 37°C until her fully recovery from anaesthesia effects. If the embryo transfer was successful, a minimum of 3-4 new-borns must be obtained 21 days later.

PBMCs Isolation. Blood was collected from healthy adult donors, women and men between 20-30 years old, each of whom provided informed consent in accordance with relevant guidelines and regulations approved by the Ethics Committee of the Universidad San Francisco de Quito for mitochondria studies (2017-026IN). After blood collection, PBMCs were isolated using Ficoll-Paque Premium (GE Healthcare Life Sciences; MA, U.S) and centrifuged at 400 x g for 30 min without break or acceleration to create a density gradient. PBMCs were washed by suspension with 1X PBS (Gibco by Life Technologies, ThermoFisher Scientific; MA, U.S) in a standardized volume of 10 mL and centrifuged at 1500 x g for 20 min at 4ºC. Supernatant was discarded, and the pellet was re-suspended in 10 mL of PBS. Cells were counted in a Neubauer Cell Chamber and diluted to 1x106 cell/mL in 1% FCS RPMI (Gibco by Life Technologies, ThermoFisher Scientific, MA, U.S.) with a final volume of 2 mL in an Eppendorf tube per condition. Cells were stored for a maximum of 2 h at 4^{o}C until further assays were performed.

Mitochondria labelling with MitoTracker Red. PBMCs were incubated with 250 nm/mL of MitoTracker Red (Molecular Probes by Life Technologies, ThermoFisher Scientific, MA, U.S) solution in 1% FCS RPMI at 37°C during 30 min. Two washes were performed to eliminate excess dye and cells were re-suspended in 1 mL of PBS. Once the mitochondria of PBMCs were labelled they were processed for isolation.

Mitochondria isolation. Stained mitochondria were isolated from 10-20 million of fresh PBMCs using the Mitochondria Isolation Kit for Tissue (ThermoFisher Scientific, MA, U.S) following manufacturer instructions with a final recovery step using a centrifugation at 3,000 x g for 15 min to obtain highly pure mitochondria isolates. A second wash was performed using the same centrifugation conditions to discard residues of Reactive C of the Kit. 1 mL of DMEM High Glucose (Gibco by Life Technologies, ThermoFisher Scientific, MA, U.S.) without serum (DMEM pure) was added to re-suspend the mitochondrial pellet. Mitochondrial concentration was determined using Pierce TM Coomassie Plus (Bradford) Assay Kit (ThermoFisher Scientific, MA, U.S). A typical mitochondria yield was 30 to 50 ug/mL. The mitochondrial solution was stored at 4°C until MitoCeption of embryos was performed.

Adapted MitoCeption Protocol. Zygotes from all conditions were centrifuged at 400 x g at 4°C during 5’ in 2 mL of RPMI 1% FCS in a microcentrifuge tube, the supernatant was discarded and 250 µL of the isolated mitochondrial solution, in DMEM pure, was added at different concentrations. 1.5 mL Eppendorf tubes were centrifuged at 500 x g for 5’ with the 250 µL of stained mitochondria and zygotes at 4ºC. Finally, zygotes were co-incubated at 37°C for 1 and washed by collecting each embryo individually with a capillary pipette and placed in 250 µl of KSOM M2 Mouse Embryo Medium for well and assay’s condition before left to culture until downstream assays.

Mitochondrial DNA transfer quantitation by qPCR. Embryos (zygotes and morulae) tested in all conditions were washed with 1mL PBS and centrifuged at 1200 x g for 5 min prior to the assay to eliminate un-transferred mitochondria. DNA was extracted from embryos using MagMax™-96 Mutli-Sample Kit (Applied Biosystems by ThermoFisher Scientific, MA, U.S). Real Time quantitative PCR was performed in a StepOne Real-Time PCR System (Applied Biosystems by ThermoFisher Scientific, MA, U.S). Fast Syber Green Master Mix (4385610; Applied Biosystems by ThermoFisher Scientific, MA, U.S) was used accordingly to manufacturer's instructions with a final DNA concentration of 10 ng/µL in a 10µL reaction. Murine and human DNA was quantified using primers, manufactured by InvitrogenTM: (**MMito F3; MMito R3**); and the murine nuclear β-2 microglobulin gene was used as a standard with the primers: (**MB2M F1 ; and MB2M R1),** (**HMito F3; HMito R3**) hMitoF3 5'- CAC TTT CCA CAC AGA CAT CA – 3'; hMitoR3 5'- TGG TTA GGC TGG TGT TAG GGC AC – 3', (**HB2M F1 ; and HB2M R1)** hB2MF1 5'- TGT TCC TGC TGG GTA GCT CT – 3'; and hB2MR1 5'- CCT CCA TGA TGC TGC TTA CA – 3'. The qPCR reaction was based on Ajaz et al., 2015 protocol with a minor modification: a pre-incubation at 95°C for 5 min (1 cycle); denaturation at 95°C for 10 seconds; annealing and extension at 63°C for 30 s (denaturation and extension steps were repeated for 40 cycles); melting at 95°C for 5 seconds, 63°C for 1 min, 95°C for 5 seconds and 40°C for 30 seconds. Data were collected by estimating the 2-ΔCT.

Statistical analyses. Our data was analysed using GraphPad Prism 6. We performed Anderson-Darling Normality tests to determine the use of parametric or non-parametric analysis. After, we used un-paired ANOVA and t-Student's tests to analyse the differences among conditions, using an alpha-value of 0.05 (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, *****p<0,00001) to establish significant differences.

Conclusion and discussion

Mitochondrial diseases are a group of inherited sicknesses characterized for consistently disturb mitochondrial respiratory chain function and cellular ATP synthesis. Nowadays, it could be considered as a main cause of inherited metabolic disease in humans (R. L. Davis et al., 2018), with a prevalence of 1 of 2000-5000 individuals in…. (Gorman et al., 2015; Suomalainen & Battersby, 2018). However, instead of their high prevalence, since mitochondrial diseases have a wide range of clinical presentations and intensities, their correct diagnosis and treatment remains difficult (Tranchant & Anheim, 2016).

A little set of therapies for mitochondrial diseases' patients had been developed. Pharmacological, nutritional and sporting approaches are the most commons, but all of them with ambiguous benefits. Emerging therapies include (1) stimulation of mitochondrial

biogenesis; (2) regulation of mitophagy and mitochondrial dynamics; (3) evade of OXPHOS defects; (4) mitochondrial replacement therapy (MRT); and (5) chronic hypoxia. (Hirano et al., 2018). Among the emerging therapies mentioned, only MRT could be applied on the oocyte or embryo in order of prevent the development of these diseases (Rai et al., 2018).

MRT was developed in somatic cells since the basic co-culture (Clark & Shay, 1982) until microinjection (Liu et al., 2008). In the middle, another technics as peptide-mediated mitochondrial delivery (Chang et al., 2013) magnetomitotransfer (Macheiner et al., 2016), Photothermal Nanoblade (Wu et al., 2016) and centrifugation (Andrés Caicedo et al., 2015; M. J. Kim, Hwang, Yun, Lee, & Choi, 2018) were studied. Remarkably, only the mentioned MTR by microinjection to oocytes or embryos was performed in experimental assays. Nowadays, the effort for prevent the mitochondrial diseases is focused in ooplasm, spindle or nuclear transfer from one oocyte or zygote to another, always by using microinjection technics (Amato, Tachibana, Sparman, & Mitalipov, 2014; Ishii & Hibino, 2018). However, countries as China or USA had declared illegal the treatments and researches that involve spindle or nuclear transfer, and this theme becomes very controversial (Appleby, 2015; Baylis, 2013). To our knowledge, this is the first attempt to perform MTR in mouse embryos by Mitoception, a non-invasive technic developed originally for cultured cells by Caicedo et al, (2015).

MRT were developed by using different methods of transplanting the nuclear genome from an unfertilized or fertilized egg of an affected patient or couple to an enucleated egg coming from a healthy donor. The MRT is performed by transfer spindle - chromosomal complexes (karyoplast) (ST) between mature metaphase II-arrested oocytes. The Karyoplast is introduced into an enucleated recipient egg (cytoplast) and then fertilized by intracytoplasmic sperm injection(Tachibana, Sparman, & Mitalipov, 2010). Pronuclear transfer (PNT) MRT is performed after fertilization; the haploid separated and packed maternal and paternal genetic material pronuclei are transplanted to an enucleated donor egg. Polar body transfer (PBT), uses a oocyte from a patient during its maturation, were it passes through two reductive divisions characterized by an uneven segregation of the cytoplasm obtaining two small bodies (PB1 and PB2, respectively) containing a complement of chromosomes. PB1 has a diploid and PB2 has a haploid set, both have shown to continue to meiosis and full-term development of viable offspring when transferred to a donor oocyte. When used for MRT, PBT has shown to carry little or undetectable diseased mitochondria, however PB1 and PB2 suffer in a brief time DNA fragmentation which lower the success of this technique(Wolf et al., 2015b). Even if these procedures have resulted successful, invasive manipulation, chemicals, unhealthy mitochondria residuals and ovule loss to enucleate are still evidenced during the protocol which are of concern

when performed in human cells, being difficult to achieve due the need of specialized equipment(Sato et al., 2005).

Techniques based in the cytoplasmic microinjection of mitochondria in oocytes represent a harm to its physiology as there is the risk of cell's destruction and a pronounced decrease of the amount of developing embryos, independently of the content of the needle or the developmental stage treated (oocyte or zygote) (Liu, Yang, Lu, & Qiu, 2008; Sokolova et al., 2004). For this reason, we propose the use of the Mitoception technique as a better alternative for mitochondrial transfer to oocytes or early embryos.

It has been reported that Mesenchymal Stem / Stromal Cells have the capacity to transfer functional mitochondria to stressed or damaged cells and different cell types(Andr&#x00E9 Caicedo et al., 2017; Islam et al., 2012b; Jackson Megan V. et al., 2016; X. Li et al., 2014b; Luz-Crawford et al., 2019b; Miliotis et al., 2019; Sinha et al., 2016b). Inspired by this process we develop the MitoCeption technique based in the artificial transfer / transplant of healthy mitochondria (AMT/T) to a recipient cell using a thermic shock and a centrifugation. We previously observed that the transfer of healthy mitochondria to UVR stressed recipient cells repair the endogenous mitochondrial mass, mtDNA damage, metabolic activity and reduced the expression of stress markers such as p53(Cabrera et al., 2019). As oocytes and embryos can carry defective mitochondria, by mutations in their DNA, we sought to apply the MitoCeption (AMT/T) technique to transfer effectively increasing amounts of healthy exogenous mitochondria, increasing heteroplasmy and the possibility to reduce the diseased phenotype.

Mitochondria transfer history until Mitoception 1 paragraph

In this study we seek to artificially transfer mitochondria to oocytes and zygotes and followed its normal development to later embryos by the updated MitoCeption protocol. In order to observe its survival and implantation capacities in vitro and under an embryo's transfer protocol to subrogated mothers.

Variability in mtDNA or nDNA sequences encoding for mitochondrial proteins are common and some of them could influence its protostasis leading to a higher susceptibility to disease and hereditary disorders(Latorre-Pellicer et al., 2016).

Bibliography:

1. Aardema, H., van Tol, H. T. A., & Vos, P. L. A. M. (2019). An overview on how cumulus cells interact with the oocyte in a condition with elevated NEFA levels in dairy cows. *Animal Reproduction Science*, *207*, 131–137. https://doi.org/10.1016/j.anireprosci.2019.06.003
2. Aguzzi, A., & Altmeyer, M. (2016). Phase Separation: Linking Cellular Compartmentalization to Disease. *Trends in Cell Biology*, *26*(7), 547–558. https://doi.org/10.1016/j.tcb.2016.03.004
3. Ahmad, T., Mukherjee, S., Pattnaik, B., Kumar, M., Singh, S., Kumar, M., … Agrawal, A. (2014). Miro1 regulates intercellular mitochondrial transport & enhances mesenchymal stem cell rescue efficacy. *The EMBO Journal*, *30*(9), 994–1010. https://doi.org/10.1002/embj.201386030
4. Ajaz, S., Czajka, A., & Malik, A. (2015). Accurate Measurement of Circulating Mitochondrial DNA Content from Human Blood Samples Using Real-Time Quantitative PCR. In V. Weissig & M. Edeas (Eds.), *Mitochondrial Medicine: Volume I, Probing Mitochondrial Function* (pp. 117–131). https://doi.org/10.1007/978-1-4939-2257-4_12
5. Alberts, B. (2015). *Molecular biology of the cell* (Sixth edition). New York, NY: Garland Science, Taylor and Francis Group.
6. Albu, A. I., & Albu, D. (2017). The Impact of Aging on Fertility: Similarities and Differences between Ovaries and Testes. In A. M. Darwish (Ed.), *Testes and Ovaries—Functional and Clinical Differences and Similarities*. https://doi.org/10.5772/intechopen.68905
7. Almeida, M., & Porter, R. M. (2019). Sirtuins and FoxOs in osteoporosis and osteoarthritis. *Bone*, *121*, 284–292. https://doi.org/10.1016/j.bone.2019.01.018
8. Amato, P., Tachibana, M., Sparman, M., & Mitalipov, S. (2014). Three-parent in vitro fertilization: Gene replacement for the prevention of inherited mitochondrial diseases. *Fertility and Sterility*, *101*(1), 31–35. https://doi.org/10.1016/j.fertnstert.2013.11.030
9. Andreux, P. A., Houtkooper, R. H., & Auwerx, J. (2013). Pharmacological approaches to restore mitochondrial function. *Nature Reviews Drug Discovery*, *12*(6), 465–483. https://doi.org/10.1038/nrd4023
10. Anesti, V., & Scorrano, L. (2006). The relationship between mitochondrial shape and function and the cytoskeleton. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1757*(5), 692–699. https://doi.org/10.1016/j.bbabio.2006.04.013
11. Aponte, P. M., & Caicedo, A. (2017). Stemness in Cancer: Stem Cells, Cancer Stem Cells, and Their Microenvironment. *Stem Cells International*, *2017*, 1–17. https://doi.org/10.1155/2017/5619472
12. Appleby, J. B. (2015). The ethical challenges of the clinical introduction of mitochondrial replacement techniques. *Medicine, Health Care and Philosophy*, *18*(4), 501–514. https://doi.org/10.1007/s11019-015-9656-3
13. Ariño, J., Ramos, J., & Sychrova, H. (2019). Monovalent cation transporters at the plasma membrane in yeasts. *Yeast*, *36*(4), 177–193. https://doi.org/10.1002/yea.3355
14. Arranz-Gibert, P., Patel, J. R., & Isaacs, F. J. (2019). The Role of Orthogonality in Genetic Code Expansion. *Life*, *9*(3), 58. https://doi.org/10.3390/life9030058
15. Baba, M., Shinmura, A., Tada, S., Amo, T., & Tsukamoto, A. (2019). Mitochondrial Remodeling in Endothelial Cells under Cyclic Stretch Is Independent of Drp1 Activation. *Molecular & Cellular Biomechanics*, *16*(1). Retrieved from http://confsubmission.org/index.php/mcb/article/view/5199
16. Babayev, E., Wang, T., Szigeti-Buck, K., Lowther, K., Taylor, H. S., Horvath, T., & Seli, E. (2016). Reproductive aging is associated with changes in oocyte mitochondrial dynamics, function, and mtDNA quantity. *Maturitas*, *93*, 121–130. https://doi.org/10.1016/j.maturitas.2016.06.015
17. Baboota, R. K., Shinde, A. B., Lemaire, K., Fransen, M., Vinckier, S., Van Veldhoven, P. P., … Baes, M. (2019). Functional peroxisomes are required for β-cell integrity in mice. *Molecular Metabolism*, *22*, 71–83. https://doi.org/10.1016/j.molmet.2019.02.001
18. Balciuniene, J., & Balciunas, D. (2019). A Nuclear mtDNA Concatemer (Mega-NUMT) Could Mimic Paternal Inheritance of Mitochondrial Genome. *Frontiers in Genetics*, *10*. https://doi.org/10.3389/fgene.2019.00518
19. Barritt, J. A., Brenner, C. A., Malter, H. E., & Cohen, J. (2001). Mitochondria in human offspring derived from ooplasmic transplantation: Brief communication. *Human Reproduction*, *16*(3), 513–516. https://doi.org/10.1093/humrep/16.3.513
20. Baselet, B., Sonveaux, P., Baatout, S., & Aerts, A. (2019). Pathological effects of ionizing radiation: Endothelial activation and dysfunction. *Cellular and Molecular Life Sciences*, *76*(4), 699–728. https://doi.org/10.1007/s00018-018-2956-z
21. Baylis, F. (2013). The ethics of creating children with three genetic parents. *Reproductive BioMedicine Online*, *26*(6), 531–534. https://doi.org/10.1016/j.rbmo.2013.03.006

22. Bell, P. J. (2019). Evidence supporting a viral origin of the eukaryotic nucleus. *BioRxiv*. https://doi.org/10.1101/679175
23. Bender, D. A., Botham, K. M., Boyle, P. J., Kennelly, P. J., Rodwell, V. W., Weil, P. A., & Weitz, M. (2018). *Harper's Illustrated Biochemistry* (31st ed.).
24. Bentov, Y., Yavorska, T., Esfandiari, N., Jurisicova, A., & Casper, R. F. (2011). The contribution of mitochondrial function to reproductive aging. *Journal of Assisted Reproduction and Genetics*, *28*(9), 773–783. https://doi.org/10.1007/s10815-011-9588-7
25. Berridge, M. V., McConnell, M. J., Grasso, C., Bajzikova, M., Kovarova, J., & Neuzil, J. (2016). Horizontal transfer of mitochondria between mammalian cells: Beyond co-culture approaches. *Current Opinion in Genetics & Development*, *38*, 75–82. https://doi.org/10.1016/j.gde.2016.04.003
26. Berridge, M. V., Schneider, R. T., & McConnell, M. J. (2016). Mitochondrial Transfer from Astrocytes to Neurons following Ischemic Insult: Guilt by Association? *Cell Metabolism*, *24*(3), 376–378. https://doi.org/10.1016/j.cmet.2016.08.023
27. Bess, A. S., Crocker, T. L., Ryde, I. T., & Meyer, J. N. (2012). Mitochondrial dynamics and autophagy aid in removal of persistent mitochondrial DNA damage in Caenorhabditis elegans. *Nucleic Acids Research*, *40*(16), 7916–7931. https://doi.org/10.1093/nar/gks532
28. Beyfuss, K., & Hood, D. A. (2018). A systematic review of p53 regulation of oxidative stress in skeletal muscle. *Redox Report : Communications in Free Radical Research*, *23*(1), 100–117. https://doi.org/10.1080/13510002.2017.1416773
29. Bianchi, S., Macchiarelli, G., Micara, G., Linari, A., Boninsegna, C., Aragona, C., … Nottola, S. A. (2015). Ultrastructural markers of quality are impaired in human metaphase II aged oocytes: A comparison between reproductive and in vitro aging. *Journal of Assisted Reproduction and Genetics*, *32*(9), 1343–1358. https://doi.org/10.1007/s10815-015-0552-9
30. Blitzer, D., Guariento, A., Doulamis, I. P., Shin, B., Moskowitzova, K., Barbieri, G. R., … McCully, J. D. (2019). Delayed Transplantation of Autologous Mitochondria for Cardioprotection in a Porcine Model. *The Annals of Thoracic Surgery*. https://doi.org/10.1016/j.athoracsur.2019.06.075
31. Bock, R. (2017). Witnessing Genome Evolution: Experimental Reconstruction of Endosymbiotic and Horizontal Gene Transfer. *Annual Review of Genetics*, *51*(1). Retrieved from http://www.annualreviews.org/doi/abs/10.1146/annurev-genet-120215-035329
32. Boengler, K., Heusch, G., & Schulz, R. (2011). Nuclear-encoded mitochondrial proteins and their role in cardioprotection. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1813*(7), 1286–1294. https://doi.org/10.1016/j.bbamcr.2011.01.009
33. Boga, J. A., Caballero, B., Potes, Y., Perez-Martinez, Z., Reiter, R. J., Vega-Naredo, I., & Coto-Montes, A. (2019). Therapeutic potential of melatonin related to its role as an autophagy regulator: A review. *Journal of Pineal Research*, *66*(1), e12534. https://doi.org/10.1111/jpi.12534
34. Boldogh, I. R., & Pon, L. A. (2007). Mitochondria on the move. *Trends in Cell Biology*, *17*(10), 502–510. https://doi.org/10.1016/j.tcb.2007.07.008
35. Boudoures Anna. (2017). Mitochondrial Damage Accumulation in Oocytes—A Potential Link Between Maternal Obesity and Increased Cardiometabolic Disease Risk in Offspring—ProQuest. Retrieved June 20, 2018, from https://search.proquest.com/openview/d82353b3a7a7a5ab43e180fbf542ca1c/1?pq-origsite=gscholar&cbl=18750&diss=y
36. Brenner, C. A., Barritt, J. A., Willadsen, S., & Cohen, J. (2000). Mitochondrial DNA heteroplasmy after human ooplasmic transplantation. *Fertility and Sterility*, *74*(3), 573–578.
37. Cabrera, F., Ortega, M., Velarde, F., Parra, E., Gallardo, S., Barba, D., … Caicedo, A. (2019). Primary allogeneic mitochondrial mix (PAMM) transfer/transplant by MitoCeption to address damage in PBMCs caused by ultraviolet radiation. *BMC Biotechnology*, *19*(1), 42. https://doi.org/10.1186/s12896-019-0534-6
38. Cai, W. J., Chen, Y., Shi, L. X., Cheng, H. R., Banda, I., Ji, Y. H., … Zhao, S. F. (2019). AKT-GSK3β Signaling Pathway Regulates Mitochondrial Dysfunction-Associated OPA1 Cleavage Contributing to Osteoblast Apoptosis: Preventative Effects of Hydroxytyrosol [Research article]. https://doi.org/10.1155/2019/4101738
39. Caicedo, Andrés, Fritz, V., Brondello, J.-M., Ayala, M., Dennemont, I., Abdellaoui, N., … Vignais, M.-L. (2015). MitoCeption as a new tool to assess the effects of mesenchymal stem/stromal cell mitochondria on cancer cell metabolism and function. *Scientific Reports*, *5*, 9073. https://doi.org/10.1038/srep09073
40. Caicedo, Andr&#x00E9, s, Aponte, P. M., Cabrera, F., Hidalgo, C., & Khoury, M. (2017). Artificial Mitochondria Transfer: Current Challenges, Advances, and Future Applications [Research article]. https://doi.org/10.1155/2017/7610414

41. Cannon, M. V., Dunn, D. A., Irwin, M. H., Brooks, A. I., Bartol, F. F., Trounce, I. A., & Pinkert, C. A. (2011). Xenomitochondrial mice: Investigation into mitochondrial compensatory mechanisms. *Mitochondrion*, *11*(1), 33–39. https://doi.org/10.1016/j.mito.2010.07.003
42. Caramori, G., Ruggeri, P., Mumby, S., Atzeni, F., & Adcock, I. M. (2019). Transcription Factors. In *ELS* (pp. 1–8). https://doi.org/10.1002/9780470015902.a0005278.pub3
43. Čaval, T., Zhu, J., Tian, W., Remmelzwaal, S., Yang, Z., Clausen, H., & Heck, A. J. R. (2019). Targeted Analysis of Lysosomal Directed Proteins and Their Sites of Mannose-6-phosphate Modification. *Molecular & Cellular Proteomics*, *18*(1), 16–27. https://doi.org/10.1074/mcp.RA118.000967
44. Cavalier-Smith, T. (2010). Origin of the cell nucleus, mitosis and sex: Roles of intracellular coevolution. *Biology Direct*, *5*(1), 7. https://doi.org/10.1186/1745-6150-5-7
45. Chaban, Y., Boekema, E. J., & Dudkina, N. V. (2014). Structures of mitochondrial oxidative phosphorylation supercomplexes and mechanisms for their stabilisation. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1837*(4), 418–426. https://doi.org/10.1016/j.bbabio.2013.10.004
46. Chakrabarty, S., Kabekkodu, S. P., Singh, R. P., Thangaraj, K., Singh, K. K., & Satyamoorthy, K. (2018). Mitochondria in health and disease. *Mitochondrion*, *43*, 25–29. https://doi.org/10.1016/j.mito.2018.06.006
47. Chang, J.-C., Chang, H.-S., Wu, Y.-C., Cheng, W.-L., Lin, T.-T., Chang, H.-J., … Liu, C.-S. (2019). Mitochondrial transplantation regulates antitumour activity, chemoresistance and mitochondrial dynamics in breast cancer. *Journal of Experimental & Clinical Cancer Research*, *38*(1). https://doi.org/10.1186/s13046-019-1028-z
48. Chang, J.-C., Liu, K.-H., Chuang, C.-S., Su, H.-L., Wei, Y.-H., Kuo, S.-J., & Liu, C.-S. (2013). Treatment of human cells derived from MERRF syndrome by peptide-mediated mitochondrial delivery. *Cytotherapy*, *15*(12), 1580–1596. https://doi.org/10.1016/j.jcyt.2013.06.008
49. Chatterjee, S., & Yadav, S. (2019). The Origin of Prebiotic Information System in the Peptide/RNA World: A Simulation Model of the Evolution of Translation and the Genetic Code. *Life*, *9*(1), 25. https://doi.org/10.3390/life9010025
50. Chen, Z., Patwari, M., & Liu, D. (2019). Cytotoxicity of orthodontic temporary anchorage devices on human periodontal ligament fibroblasts in vitro. *Clinical and Experimental Dental Research*, *0*(0), 1–7. https://doi.org/10.1002/cre2.230
51. Chinnery, P. F. (2014). Mitochondrial Disorders Overview. In M. Adam, H. Ardinger, & R. Pagon et al. ,. (Eds.), *GeneReviews* (p. 21). Seattle (WA): University of Washington, Seattle.
52. Cho, N. H., Shaw, J. E., Karuranga, S., Huang, Y., da Rocha Fernandes, J. D., Ohlrogge, A. W., & Malanda, B. (2018). IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Research and Clinical Practice*, *138*, 271–281. https://doi.org/10.1016/j.diabres.2018.02.023
53. Chuang, Y.-C., Liou, C.-W., Chen, S.-D., Wang, P.-W., Chuang, J.-H., Tiao, M.-M., … Lin, T.-K. (2017). Mitochondrial Transfer from Wharton's Jelly Mesenchymal Stem Cell to MERRF Cybrid Reduces Oxidative Stress and Improves Mitochondrial Bioenergetics. *Oxidative Medicine and Cellular Longevity*, *2017*, 1–22. https://doi.org/10.1155/2017/5691215
54. Claiborne, A. B., English, R. A., & Kahn, J. P. (2016). Finding an ethical path forward for mitochondrial replacement. *Science*, *351*(6274), 668–670.
55. Clark, M. A., & Shay, J. W. (1982). Mitochondrial transformation of mammalian cells. *Nature*, *295*, 605.
56. Cohen, B. H., Chinnery, P. F., & Copeland, W. C. (2018). POLG-Related Disorders. In M. Adam, H. Ardinger, & R. Pagon et al. ,. (Eds.), *GeneReviews* (p. 32). Retrieved from https://www.ncbi.nlm.nih.gov/books/
57. Cohen, J. (1998). Ooplasmic transfer in mature human oocytes. *Molecular Human Reproduction*, *4*(3), 269–280. https://doi.org/10.1093/molehr/4.3.269
58. Coleman, W., & Tsongalis, G. (Eds.). (2018). *Molecular Pathology: The Molecular Basis of Human Disease* (2nd ed.). London: Academic Press.
59. Coskun, P., Wyrembak, J., Schriner, S. E., Chen, H.-W., Marciniack, C., LaFerla, F., & Wallace, D. C. (2012). A mitochondrial etiology of Alzheimer and Parkinson disease. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1820*(5), 553–564. https://doi.org/10.1016/j.bbagen.2011.08.008
60. Costantini, D. (2019). Hormesis Promotes Evolutionary Change. *Dose-Response*, *17*(2), 1559325819843376. https://doi.org/10.1177/1559325819843376
61. Dacks, J. B., Field, M. C., Buick, R., Eme, L., Gribaldo, S., Roger, A. J., … Devos, D. P. (2016). The changing view of eukaryogenesis – fossils, cells, lineages and how they all come together. *Journal of Cell Science*, *129*(20), 3695–3703. https://doi.org/10.1242/jcs.178566

62. Dan Dunn, J., Alvarez, L. A., Zhang, X., & Soldati, T. (2015). Reactive oxygen species and mitochondria: A nexus of cellular homeostasis. *Redox Biology*, *6*, 472–485. https://doi.org/10.1016/j.redox.2015.09.005
63. Darbandi, S., Darbandi, M., khorshid, H. R. K., Sadeghi, M. R., Al-Hasani, S., Agarwal, A., … Akhondi, M. M. (2016). Experimental strategies towards increasing intracellular mitochondrial activity in oocytes: A systematic review. *Mitochondrion*, *30*, 8–17. https://doi.org/10.1016/j.mito.2016.05.006
64. Das, N. R., & Sharma, S. S. (2016). Cognitive Impairment Associated with Parkinson's Disease: Role of Mitochondria. *Current Neuropharmacology*, *14*(6), 584–592. https://doi.org/10.2174/1570159X14666160104142349
65. Davis, C. O., Kim, K.-Y., Bushong, E. A., Mills, E. A., Boassa, D., Shih, T., … Marsh-Armstrong, N. (2014). Transcellular degradation of axonal mitochondria. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(26), 9633–9638. https://doi.org/10.1073/pnas.1404651111
66. Davis, R. L., Liang, C., & Sue, C. M. (2018). Mitochondrial diseases. In D. Geschwind, H. Paulson, & C. Kein (Eds.), *Handbook of Clinical Neurology* (pp. 125–141). https://doi.org/10.1016/B978-0-444-63233-3.00010-5
67. Dawson, T. R., Lazarus, M. D., Hetzer, M. W., & Wente, S. R. (2009). ER membrane–bending proteins are necessary for de novo nuclear pore formation. *The Journal of Cell Biology*, *184*(5), 659–675. https://doi.org/10.1083/jcb.200806174
68. de Magalhães, J. P., & Passos, J. F. (2018). Stress, cell senescence and organismal ageing. *Mechanisms of Ageing and Development*, *170*, 2–9. https://doi.org/10.1016/j.mad.2017.07.001
69. De Mario, A., Quintana-Cabrera, R., Martinvalet, D., & Giacomello, M. (2016). (Neuro)degenerated Mitochondria-ER contacts. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1016/j.bbrc.2016.07.056
70. Delage, B., & Dashwood, R. H. (2008). Dietary Manipulation of Histone Structure and Function. *Annual Review of Nutrition*, *28*(1), 347–366. https://doi.org/10.1146/annurev.nutr.28.061807.155354
71. DiMauro, S., & Hirano, M. (2015). MERRF. In M. Adam, H. Ardinger, & R. Pagon et al. ,. (Eds.), *GeneReviews* (p. 21). Retrieved from https://www.ncbi.nlm.nih.gov/books/
72. D'Occhio, M. J., Baruselli, P. S., & Campanile, G. (2019). Metabolic health, the metabolome and reproduction in female cattle: A review. *Italian Journal of Animal Science*, *18*(1), 858–867. https://doi.org/10.1080/1828051X.2019.1600385
73. Dong, L.-F., Kovarova, J., Bajzikova, M., Bezawork-Geleta, A., Svec, D., Endaya, B., … Neuzil, J. (2017). Horizontal transfer of whole mitochondria restores tumorigenic potential in mitochondrial DNA-deficient cancer cells. *ELife*, *6*. https://doi.org/10.7554/eLife.22187
74. Dorstyn, L., Akey, C. W., & Kumar, S. (2018). New insights into apoptosome structure and function. *Cell Death & Differentiation*, 1. https://doi.org/10.1038/s41418-017-0025-z
75. Dudekula, N., Arora, V., Callaerts-Vegh, Z., & Bond, R. A. (2005). The Temporal Hormesis of Drug Therapies. *Dose-Response*, *3*(3), dose-response.003.03.009. https://doi.org/10.2203/dose-response.003.03.009
76. Duran, H. E., Simsek-Duran, F., Oehninger, S. C., Jones, H. W., & Castora, F. J. (2011). The association of reproductive senescence with mitochondrial quantity, function, and DNA integrity in human oocytes at different stages of maturation. *Fertility and Sterility*, *96*(2), 384–388. https://doi.org/10.1016/j.fertnstert.2011.05.056
77. Dyall, S. D. (2004). Ancient Invasions: From Endosymbionts to Organelles. *Science*, *304*(5668), 253–257. https://doi.org/10.1126/science.1094884
78. Elbaz, Y., & Schuldiner, M. (2011). Staying in touch: The molecular era of organelle contact sites. *Trends in Biochemical Sciences*, *36*(11), 616–623. https://doi.org/10.1016/j.tibs.2011.08.004
79. El-Hattab, A., Almannai, M., & Scaglia, F. (2018). MELAS. In M. Adam, H. Ardinger, & R. Pagon et al. ,. (Eds.), *GeneReviews* (p. 25). Retrieved from : https://www.ncbi.nlm.nih.gov/books/
80. Elliott, H. R., Samuels, D. C., Eden, J. A., Relton, C. L., & Chinnery, P. F. (2008). Pathogenic mitochondrial DNA mutations are common in the general population. *American Journal of Human Genetics*, *83*(2), 254–260. https://doi.org/10.1016/j.ajhg.2008.07.004
81. Elliott, R. L., Jiang, X. P., & Head, J. F. (2012). Mitochondria organelle transplantation: Introduction of normal epithelial mitochondria into human cancer cells inhibits proliferation and increases drug sensitivity. *Breast Cancer Research and Treatment*, *136*(2), 347–354. https://doi.org/10.1007/s10549-012-2283-2
82. Emelyanov, V. V. (2001). Evolutionary relationship of Rickettsiae and mitochondria. *FEBS Letters*, *501*(1), 11–18. https://doi.org/10.1016/S0014-5793(01)02618-7
83. Emelyanov, V. V. (2003). Mitochondrial connection to the origin of the eukaryotic cell. *European Journal of Biochemistry*, *270*(8), 1599–1618. https://doi.org/10.1046/j.1432-1033.2003.03499.x

84. Esterhuizen, K., Lindeque, J. Z., Mason, S., van der Westhuizen, F. H., Suomalainen, A., Hakonen, A. H., … Louw, R. (2019). A urinary biosignature for mitochondrial myopathy, encephalopathy, lactic acidosis and stroke like episodes (MELAS). *Mitochondrion*, *45*, 38–45. https://doi.org/10.1016/j.mito.2018.02.003
85. Fakouri, N. B., Hansen, T. L., Desler, C., Anugula, S., & Rasmussen, L. J. (2019). From Powerhouse to Perpetrator—Mitochondria in Health and Disease. *Biology*, *8*(2), 35. https://doi.org/10.3390/biology8020035
86. Falkenberg, M., Larsson, N.-G., & Gustafsson, C. M. (2007). DNA Replication and Transcription in Mammalian Mitochondria. *Annual Review of Biochemistry*, *76*(1), 679–699. https://doi.org/10.1146/annurev.biochem.76.060305.152028
87. Faustini, G., Bono, F., Valerio, A., Pizzi, M., Spano, P., & Bellucci, A. (2017). Mitochondria and α-Synuclein: Friends or Foes in the Pathogenesis of Parkinson's Disease? *Genes*, *8*(12), 377. https://doi.org/10.3390/genes8120377
88. Favaro, G., Romanello, V., Varanita, T., Andrea Desbats, M., Morbidoni, V., Tezze, C., … Sandri, M. (2019). DRP1-mediated mitochondrial shape controls calcium homeostasis and muscle mass. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-10226-9
89. Ferreira, R. M., Chiaratti, M. R., Macabelli, C. H., Rodrigues, C. A., Ferraz, M. L., Watanabe, Y. F., … Baruselli, P. S. (2016). The Infertility of Repeat-Breeder Cows During Summer Is Associated with Decreased Mitochondrial DNA and Increased Expression of Mitochondrial and Apoptotic Genes in Oocytes. *Biology of Reproduction*, *94*(3), 66–66. https://doi.org/10.1095/biolreprod.115.133017
90. Field, M. C., & Rout, M. P. (2019). Pore timing: The evolutionary origins of the nucleus and nuclear pore complex. *F1000Research*, *8*. https://doi.org/10.12688/f1000research.16402.1
91. Finsterer, J. (2018). The Genetic Cause of Kearns-Sayre Syndrome Determines Counselling and Outcome of These Patients. *Canadian Journal of Cardiology*, *34*(9), 1234.e7. https://doi.org/10.1016/j.cjca.2018.05.027
92. Finsterer, J. (2019). Mitochondrial Ophthalmoplegia Is Not Only due to mtDNA Deletions. *Yonsei Medical Journal*, *60*(2), 230–231. https://doi.org/10.3349/ymj.2019.60.2.230
93. Forterre, P., & Gaïa, M. (2016). Giant viruses and the origin of modern eukaryotes. *Current Opinion in Microbiology*, *31*, 44–49. https://doi.org/10.1016/j.mib.2016.02.001
94. Foschi, F. G., Morelli, M. C., Savini, S., Dall'Aglio, A. C., Lanzi, A., Cescon, M., … Stefanini, G. F. (2015). Urea cycle disorders: A case report of a successful treatment with liver transplant and a literature review. *World Journal of Gastroenterology : WJG*, *21*(13), 4063–4068. https://doi.org/10.3748/wjg.v21.i13.4063
95. Franceschi, C., Garagnani, P., Parini, P., Giuliani, C., & Santoro, A. (2018). Inflammaging: A new immune–metabolic viewpoint for age-related diseases. *Nature Reviews Endocrinology*, *14*(10), 576–590. https://doi.org/10.1038/s41574-018-0059-4
96. French, C. T., Toesca, I. J., Wu, T.-H., Teslaa, T., Beaty, S. M., Wong, W., … Miller, J. F. (2011). Dissection of the Burkholderia intracellular life cycle using a photothermal nanoblade. *Proceedings of the National Academy of Sciences*, *108*(29), 12095–12100. https://doi.org/10.1073/pnas.1107183108
97. Frey, T. G, Renken, C. W., & Perkins, G. A. (2002). Insight into mitochondrial structure and function from electron tomography. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1555*(1–3), 196–203. https://doi.org/10.1016/S0005-2728(02)00278-5
98. Frey, Terrence G., & Mannella, C. A. (2000). The internal structure of mitochondria. *Trends in Biochemical Sciences*, *25*(7), 319–324.
99. Fujimaki, S., & Kuwabara, T. (2017). Diabetes-Induced Dysfunction of Mitochondria and Stem Cells in Skeletal Muscle and the Nervous System. *International Journal of Molecular Sciences*, *18*(10). https://doi.org/10.3390/ijms18102147
100. Gammage, P. A., Moraes, C. T., & Minczuk, M. (2018). Mitochondrial Genome Engineering: The Revolution May Not Be CRISPR-Ized. *Trends in Genetics*, *34*(2), 101–110. https://doi.org/10.1016/j.tig.2017.11.001
101. Gartner, L. P. (2017). *Textbook of Histology* (4th ed., Vols. 1–1). Philadelphia: Elsevier.
102. Gattermann, N., Dadak, M., Hofhaus, G., Wulfert, M., Berneburg, M., Loeffler, M. L., & Simmonds, H. A. (2004). Severe Impairment of Nucleotide Synthesis Through Inhibition of Mitochondrial Respiration. *Nucleosides, Nucleotides and Nucleic Acids*, *23*(8–9), 1275–1279. https://doi.org/10.1081/NCN-200027545
103. Gerdes, H.-H., Bukoreshtliev, N. V., & Barroso, J. F. V. (2007). Tunneling nanotubes: A new route for the exchange of components between animal cells. *FEBS Letters*, *581*(11), 2194–2201. https://doi.org/10.1016/j.febslet.2007.03.071

104. Giorgi, C., Marchi, S., Simoes, I. C. M., Ren, Z., Morciano, G., Perrone, M., … Wieckowski, M. R. (2018). Mitochondria and Reactive Oxygen Species in Aging and Age-Related Diseases. *International Review of Cell and Molecular Biology*, *340*, 209–344. https://doi.org/10.1016/bs.ircmb.2018.05.006
105. Goldstein, A., & Falk, M. J. (2019). Mitochondrial DNA Deletion Syndromes. In M. Adam, H. Ardinger, & R. Pagon et al. ,. (Eds.), *GeneReviews* (p. 21). Retrieved from https://www.ncbi.nlm.nih.gov/books/
106. Gómez, L. J. M.-, & Khan, T. H. (2019). Hypothermia in trauma. *Anaesthesia, Pain & Intensive Care*, 232–240.
107. Gorman, G. S., Chinnery, P. F., DiMauro, S., Hirano, M., Koga, Y., McFarland, R., … Turnbull, D. M. (2016). Mitochondrial diseases. *Nature Reviews Disease Primers*, *2*, 16080. https://doi.org/10.1038/nrdp.2016.80
108. Gorman, G. S., Schaefer, A. M., Ng, Y., Gomez, N., Blakely, E. L., Alston, C. L., … McFarland, R. (2015). Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease. *Annals of Neurology*, *77*(5), 753–759. https://doi.org/10.1002/ana.24362
109. Greco, E. A., Pietschmann, P., & Migliaccio, S. (2019). Osteoporosis and Sarcopenia Increase Frailty Syndrome in the Elderly. *Frontiers in Endocrinology*, *10*. https://doi.org/10.3389/fendo.2019.00255
110. Gross, A. (2016). BCL-2 family proteins as regulators of mitochondria metabolism. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1857*(8), 1243–1246. https://doi.org/10.1016/j.bbabio.2016.01.017
111. Guardia, C. M., Pace, R. D., Sen, A., Saric, A., Jarnik, M., Kolin, D. A., … Bonifacino, J. S. (2019). Reversible association with motor proteins (RAMP): A streptavidin-based method to manipulate organelle positioning. *PLOS Biology*, *17*(5), e3000279. https://doi.org/10.1371/journal.pbio.3000279
112. Guengerich, F. P., Wilkey, C. J., & Phan, T. T. N. (2019). Human cytochrome P450 enzymes bind drugs and other substrates mainly through conformational-selection modes. *Journal of Biological Chemistry*, jbc.RA119.009305. https://doi.org/10.1074/jbc.RA119.009305
113. Guerra, F., Arbini, A. A., & Moro, L. (2017). Mitochondria and cancer chemoresistance. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*. https://doi.org/10.1016/j.bbabio.2017.01.012
114. Guillet-Pichon, V., & Verny, C. (2016). Mitochondrie et maladies neurodégénératives. *Pratique Neurologique - FMC*, *7*(2), 117–122. https://doi.org/10.1016/j.praneu.2016.01.024
115. Guy, J., & Yuan, H. (2019). REVERSAL OF PARALYSIS AND PREVENTION OF PREMATURE DEATH IN A MOUSE GERMLINE MODEL OF LEIGH SYNDROME CAUSED BY A MUTATION IN MITOCHONDRIAL ATP SYNTHASE (S51.003). *Neurology*, *92*(15 Supplement), S51.003.
116. Hammond, E. R., Green, M. P., Shelling, A. N., Berg, M. C., Peek, J. C., & Cree, L. M. (2016). Oocyte mitochondrial deletions and heteroplasmy in a bovine model of ageing and ovarian stimulation. *Molecular Human Reproduction*, *22*(4), 261–271. https://doi.org/10.1093/molehr/gaw003
117. Hampoelz, B., Andres-Pons, A., Kastritis, P., & Beck, M. (2019). Structure and Assembly of the Nuclear Pore Complex. *Annual Review of Biophysics*, *48*(1), 515–536. https://doi.org/10.1146/annurev-biophys-052118-115308
118. Hart, P. H., & Norval, M. (2017). Ultraviolet radiation-induced immunosuppression and its relevance for skin carcinogenesis. *Photochemical & Photobiological Sciences*. https://doi.org/10.1039/C7PP00312A
119. Hattori, N., Saiki, S., & Imai, Y. (2014). Regulation by mitophagy. *The International Journal of Biochemistry & Cell Biology*, *53*, 147–150. https://doi.org/10.1016/j.biocel.2014.05.012
120. Hayakawa, K., Esposito, E., Wang, X., Terasaki, Y., Liu, Y., Xing, C., … Lo, E. H. (2016). Transfer of mitochondria from astrocytes to neurons after stroke. *Nature*, *535*(7613), 551–555. https://doi.org/10.1038/nature18928
121. Hennig, B., Toborek, M., McClain, C. J., & Diana, J. N. (1996). Nutritional implications in vascular endothelial cell metabolism. *Journal of the American College of Nutrition*, *15*(4), 345–358. https://doi.org/10.1080/07315724.1996.10718609
122. Herbers, E., Kekäläinen, N. J., Hangas, A., Pohjoismäki, J. L., & Goffart, S. (2019). Tissue specific differences in mitochondrial DNA maintenance and expression. *Mitochondrion*, *44*, 85–92. https://doi.org/10.1016/j.mito.2018.01.004
123. Herbert, M., & Turnbull, D. (2018). Progress in mitochondrial replacement therapies. *Nature Reviews Molecular Cell Biology*, *19*, 71–72. https://doi.org/10.1038/nrm.2018.3
124. Hirano, M., Emmanuele, V., & Quinzii, C. M. (2018). Emerging therapies for mitochondrial diseases. *Essays In Biochemistry*, EBC20170114. https://doi.org/10.1042/EBC20170114
125. Ho, R., & Hegele, R. A. (2019). Complex effects of laminopathy mutations on nuclear structure and function. *Clinical Genetics*, *95*(2), 199–209. https://doi.org/10.1111/cge.13455
126. Hood, D. A., Memme, J. M., Oliveira, A. N., & Triolo, M. (2019). Maintenance of Skeletal Muscle Mitochondria in Health, Exercise, and Aging. *Annual Review of Physiology*, *81*(1), 19–41. https://doi.org/10.1146/annurev-physiol-020518-114310

127. Houten, S. M., Violante, S., Ventura, F. V., & Wanders, R. J. A. (2016). The Biochemistry and Physiology of Mitochondrial Fatty Acid β-Oxidation and Its Genetic Disorders. *Annual Review of Physiology*, *78*(1), 23–44. https://doi.org/10.1146/annurev-physiol-021115-105045
128. Hsu, Y.-C., Wu, Y.-T., Yu, T.-H., & Wei, Y.-H. (2016). Mitochondria in mesenchymal stem cell biology and cell therapy: From cellular differentiation to mitochondrial transfer. *Seminars in Cell & Developmental Biology*, *52*, 119–131. https://doi.org/10.1016/j.semcdb.2016.02.011
129. Huang, P.-J., Kuo, C.-C., Lee, H.-C., Shen, C.-I., Cheng, F.-C., Wu, S.-F., … Su, H.-L. (2016). Transferring Xenogenic Mitochondria Provides Neural Protection Against Ischemic Stress in Ischemic Rat Brains. *Cell Transplantation*, *25*(5), 913–927. https://doi.org/10.3727/096368915X689785
130. Hubbard, W. B., Harwood, C. L., Prajapati, P., Springer, J. E., Saatman, K. E., & Sullivan, P. G. (2019). Fractionated mitochondrial magnetic separation for isolation of synaptic mitochondria from brain tissue. *Scientific Reports*, *9*(1), 1–13. https://doi.org/10.1038/s41598-019-45568-3
131. Hyslop, L. A., Blakeley, P., Craven, L., Richardson, J., Fogarty, N. M. E., Fragouli, E., … Herbert, M. (2016). Towards clinical application of pronuclear transfer to prevent mitochondrial DNA disease. *Nature*, *534*(7607), 383–386. https://doi.org/10.1038/nature18303
132. Hyttel, P. (Ed.). (2010). *Essentials of domestic animal embryology*. Edinburgh ; New York: Saunders/Elsevier.
133. Igarashi, H., Takahashi, E., Hiroi, M., & Doi, K. (1997). Aging-related changes in calcium oscillations in fertilized mouse oocytes. *Molecular Reproduction and Development*, *48*(3), 383–390. https://doi.org/10.1002/(SICI)1098-2795(199711)48:3<383::AID-MRD12>3.0.CO;2-X
134. Igarashi, H., Takahashi, T., Abe, H., Nakano, H., Nakajima, O., & Nagase, S. (2016). Poor embryo development in post-ovulatory in vivo-aged mouse oocytes is associated with mitochondrial dysfunction, but mitochondrial transfer from somatic cells is not sufficient for rejuvenation. *Human Reproduction*, *31*(10), 2331–2338. https://doi.org/10.1093/humrep/dew203
135. Imanaka, T. (2019). Biogenesis and Function of Peroxisomes in Human Disease with a Focus on the ABC Transporter. *Biological and Pharmaceutical Bulletin*, *42*(5), 649–665. https://doi.org/10.1248/bpb.b18-00723
136. Ingraham, C. (2003). Developmental fate of mitochondria microinjected into murine zygotes. *Mitochondrion*, *3*(1), 39–46. https://doi.org/10.1016/S1567-7249(03)00075-8
137. International Human Genome Sequencing Consortium. (2004). Finishing the euchromatic sequence of the human genome. *Nature*, *431*(7011), 931–945. https://doi.org/10.1038/nature03001
138. Ishii, T., & Hibino, Y. (2018). Mitochondrial manipulation in fertility clinics: Regulation and responsibility. *Reproductive Biomedicine & Society Online*, *5*, 93–109. https://doi.org/10.1016/j.rbms.2018.01.002
139. Islam, M. N., Das, S. R., Emin, M. T., Wei, M., Sun, L., Westphalen, K., … Bhattacharya, J. (2012a). Mitochondrial transfer from bone-marrow–derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nature Medicine*, *18*(5), 759–765. https://doi.org/10.1038/nm.2736
140. Islam, M. N., Das, S. R., Emin, M. T., Wei, M., Sun, L., Westphalen, K., … Bhattacharya, J. (2012b). Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nature Medicine*, *18*(5), 759–765. https://doi.org/10.1038/nm.2736
141. Ivanovic, Z. (2009). Hypoxia or in situ normoxia: The stem cell paradigm. *Journal of Cellular Physiology*, *219*(2), 271–275. https://doi.org/10.1002/jcp.21690
142. Iwata, H., Goto, H., Tanaka, H., Sakaguchi, Y., Kimura, K., Kuwayama, T., & Monji, Y. (2011). Effect of maternal age on mitochondrial DNA copy number, ATP content and IVF outcome of bovine oocytes. *Reproduction, Fertility and Development*, *23*(3), 424–432. https://doi.org/10.1071/RD10133
143. Jackson, M. V., Morrison, T. J., Doherty, D. F., McAuley, D. F., Matthay, M. A., Kissenpfennig, A., … Krasnodembskaya, A. D. (2016). Mitochondrial Transfer via Tunneling Nanotubes is an Important Mechanism by Which Mesenchymal Stem Cells Enhance Macrophage Phagocytosis in the In Vitro and In Vivo Models of ARDS: Mitochondrial Transfer from MSC to Macrophages. *STEM CELLS*, *34*(8), 2210–2223. https://doi.org/10.1002/stem.2372
144. Jackson Megan V., Morrison Thomas J., Doherty Declan F., McAuley Daniel F., Matthay Michael A., Kissenpfennig Adrien, … Krasnodembskaya Anna D. (2016). Mitochondrial Transfer via Tunneling Nanotubes is an Important Mechanism by Which Mesenchymal Stem Cells Enhance Macrophage Phagocytosis in the In Vitro and In Vivo Models of ARDS. *STEM CELLS*, *34*(8), 2210–2223. https://doi.org/10.1002/stem.2372
145. Jacobs, L. J. a. M., Jongbloed, R. J. E., Wijburg, F. A., Klerk, J. B. C. de, Geraedts, J. P. M., Nijland, J. G., … Smeets, H. J. M. (2004). Pearson syndrome and the role of deletion dimers and duplications in the mtDNA. *Journal of Inherited Metabolic Disease*, *27*(1), 47–55. https://doi.org/10.1023/B:BOLI.0000016601.49372.18

146. Jacobson, K., Liu, P., & Lagerholm, B. C. (2019). The Lateral Organization and Mobility of Plasma Membrane Components. *Cell*, *177*(4), 806–819. https://doi.org/10.1016/j.cell.2019.04.018
147. Ji, S.-L., & Tang, S.-B. (2019). Differentiation of retinal ganglion cells from induced pluripotent stem cells: A review. *International Journal of Ophthalmology*, *12*(1), 152–160. https://doi.org/10.18240/ijo.2019.01.22
148. Jopling, C., Boue, S., & Belmonte, J. C. I. (2011). Dedifferentiation, transdifferentiation and reprogramming: Three routes to regeneration. *Nature Reviews Molecular Cell Biology*, *12*(2), 79–89. https://doi.org/10.1038/nrm3043
149. Jorgensen, C. (2019). Role of Tunneling Nanotubules in the Cross-Talk Between Mesenchymal Stem Cells and Their Target Cells. *Current Stem Cell Reports*, *5*(2), 53–56. https://doi.org/10.1007/s40778-019-00154-1
150. Jose, S. S., Bendickova, K., Kepak, T., Krenova, Z., & Fric, J. (2017). Chronic Inflammation in Immune Aging: Role of Pattern Recognition Receptor Crosstalk with the Telomere Complex? *Frontiers in Immunology*, *8*. https://doi.org/10.3389/fimmu.2017.01078
151. Jurkute, N., Harvey, J., & Yu-Wai-Man, P. (2019). Treatment strategies for Leber hereditary optic neuropathy. *Current Opinion in Neurology*, *32*(1), 99. https://doi.org/10.1097/WCO.0000000000000646
152. Kannan, S., Rogozin, I. B., & Koonin, E. V. (2014). MitoCOGs: Clusters of orthologous genes from mitochondria and implications for the evolution of eukaryotes. *BMC Evolutionary Biology*, *14*, 237. https://doi.org/10.1186/s12862-014-0237-5
153. Kansaku, K., Takeo, S., Itami, N., Kin, A., Shirasuna, K., Kuwayama, T., & Iwata, H. (2017). Maternal aging affects oocyte resilience to carbonyl cyanide-m-chlorophenylhydrazone -induced mitochondrial dysfunction in cows. *PLOS ONE*, *12*(11), e0188099. https://doi.org/10.1371/journal.pone.0188099
154. Karlberg, O., Canbäck, B., Kurland, C. G., & Andersson, S. G. E. (2000). The dual origin of the yeast mitochondrial proteome. *Yeast*, *17*(3), 170–187. https://doi.org/10.1002/1097-0061(20000930)17:3<170::AID-YEA25>3.0.CO;2-V
155. Keogh, M. J., & Chinnery, P. F. (2015). Mitochondrial DNA mutations in neurodegeneration. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1847*(11), 1401–1411. https://doi.org/10.1016/j.bbabio.2015.05.015
156. Kesner, E. E., Saada-Reich, A., & Lorberboum-Galski, H. (2016). Characteristics of Mitochondrial Transformation into Human Cells. *Scientific Reports*, *6*, 26057. https://doi.org/10.1038/srep26057
157. Khan, S., Roberts, J., & Wu, S.-B. (2019). Genes involved in mitochondrial biogenesis and function may not show synchronised responses to mitochondria in shell gland of laying chickens under infectious bronchitis virus challenge. *BMC Molecular and Cell Biology*, *20*(1), 3. https://doi.org/10.1186/s12860-019-0190-7
158. Kim, K. H., Son, J. M., Benayoun, B. A., & Lee, C. (2018). The Mitochondrial-Encoded Peptide MOTS-c Translocates to the Nucleus to Regulate Nuclear Gene Expression in Response to Metabolic Stress. *Cell Metabolism*, *28*(3), 516-524.e7. https://doi.org/10.1016/j.cmet.2018.06.008
159. Kim, M. J., Hwang, J. W., Yun, C.-K., Lee, Y., & Choi, Y.-S. (2018). Delivery of exogenous mitochondria via centrifugation enhances cellular metabolic function. *Scientific Reports*, *8*(1), 3330. https://doi.org/10.1038/s41598-018-21539-y
160. Kitani, T., Kami, D., Kawasaki, T., Nakata, M., Matoba, S., & Gojo, S. (2014). Direct Human Mitochondrial Transfer: A Novel Concept Based on the Endosymbiotic Theory. *Transplantation Proceedings*, *46*(4), 1233–1236. https://doi.org/10.1016/j.transproceed.2013.11.133
161. Kitani, Tomoya, Kami, D., Matoba, S., & Gojo, S. (2014). Internalization of isolated functional mitochondria: Involvement of macropinocytosis. *Journal of Cellular and Molecular Medicine*, *18*(8), 1694–1703. https://doi.org/10.1111/jcmm.12316
162. Klionsky, D. J., Abdelmohsen, K., Abe, A., Abedin, M. J., Abeliovich, H., Arozena, A. A., … Zughaier, S. M. (2016). Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). *Autophagy*, *12*(1), 1–222. https://doi.org/10.1080/15548627.2015.1100356
163. Kujjo, L. L., Acton, B. M., Perkins, G. A., Ellisman, M. H., D'Estaing, S. G., Casper, R. F., … Perez, G. I. (2013). Ceramide and its transport protein (CERT) contribute to deterioration of mitochondrial structure and function in aging oocytes. *Mechanisms of Ageing and Development*, *134*(1–2), 43–52. https://doi.org/10.1016/j.mad.2012.12.001
164. Kushnir, V. A., Ludaway, T., Russ, R. B., Fields, E. J., Koczor, C., & Lewis, W. (2012). Reproductive aging is associated with decreased mitochondrial abundance and altered structure in murine oocytes. *Journal of Assisted Reproduction and Genetics*, *29*(7), 637–642. https://doi.org/10.1007/s10815-012-9771-5

165. Labarta, E., de los Santos, M. J., Escribá, M. J., Pellicer, A., & Herraiz, S. (2019). Mitochondria as a tool for oocyte rejuvenation. *Fertility and Sterility*, *111*(2), 219–226. https://doi.org/10.1016/j.fertnstert.2018.10.036
166. Lahiri, S., Toulmay, A., & Prinz, W. A. (2015). Membrane contact sites, gateways for lipid homeostasis. *Current Opinion in Cell Biology*, *33*, 82–87. https://doi.org/10.1016/j.ceb.2014.12.004
167. Lai, N., Kummitha, C., & Hoppel, C. (2017). Defects in skeletal muscle subsarcolemmal mitochondria in a non-obese model of type 2 diabetes mellitus. *PLoS ONE*, *12*(8). https://doi.org/10.1371/journal.pone.0183978
168. Landry, D. A., Bellefleur, A.-M., Labrecque, R., Grand, F.-X., Vigneault, C., Blondin, P., & Sirard, M.-A. (2016). Effect of cow age on the in vitro developmental competence of oocytes obtained after FSH stimulation and coasting treatments. *Theriogenology*, *86*(5), 1240–1246. https://doi.org/10.1016/j.theriogenology.2016.04.064
169. Lane, R. K., Hilsabeck, T., & Rea, S. L. (2015). The role of mitochondrial dysfunction in age-related diseases. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1847*(11), 1387–1400. https://doi.org/10.1016/j.bbabio.2015.05.021
170. Latorre-Pellicer, A., Moreno-Loshuertos, R., Lechuga-Vieco, A. V., Sánchez-Cabo, F., Torroja, C., Acín-Pérez, R., … Enríquez, J. A. (2016). Mitochondrial and nuclear DNA matching shapes metabolism and healthy ageing. *Nature*, *535*(7613), 561–565. https://doi.org/10.1038/nature18618
171. Lee, D. H., Desai, M. J., & Gauger, E. M. (2018). Electrical Injuries of the Hand and Upper Extremity: *Journal of the American Academy of Orthopaedic Surgeons*, 1. https://doi.org/10.5435/JAAOS-D-17-00833
172. Lee, J., Zhuang, Y., Wei, X., Shang, F., Wang, J., Zhang, Y., … Zheng, Q. (2009). Contributions of PD-1/PD-L1 pathway to interactions of myeloid DCs with T cells in atherosclerosis. *Journal of Molecular and Cellular Cardiology*, *46*(2), 169–176. https://doi.org/10.1016/j.yjmcc.2008.10.028
173. Levinger, L., Oestreich, I., Florentz, C., & Mörl, M. (2004). A Pathogenesis-associated Mutation in Human Mitochondrial tRNALeu(UUR) Leads to Reduced 3'-End Processing and CCA Addition. *Journal of Molecular Biology*, *337*(3), 535–544. https://doi.org/10.1016/j.jmb.2004.02.008
174. Li, F., Castora, F. J., Ford, W., Alarid, K., Jones, H. W., & Swanson, R. J. (2017). Reproductive competency and mitochondrial variation in aged Syrian hamster oocytes. *Reproduction, Fertility and Development*, *29*(7), 1384–1391. https://doi.org/10.1071/RD15404
175. Li, H., Wang, C., He, T., Zhao, T., Chen, Y., Shen, Y., … Wang, L. (2019). Mitochondrial Transfer from Bone Marrow Mesenchymal Stem Cells to Motor Neurons in Spinal Cord Injury Rats via Gap Junction. *Theranostics*, *9*(7), 2017–2035. https://doi.org/10.7150/thno.29400
176. Li, X., Zhang, Y., Yeung, S. C., Liang, Y., Liang, X., Ding, Y., … Lian, Q. (2014a). Mitochondrial Transfer of Induced Pluripotent Stem Cell–Derived Mesenchymal Stem Cells to Airway Epithelial Cells Attenuates Cigarette Smoke–Induced Damage. *American Journal of Respiratory Cell and Molecular Biology*, *51*(3), 455–465. https://doi.org/10.1165/rcmb.2013-0529OC
177. Li, X., Zhang, Y., Yeung, S. C., Liang, Y., Liang, X., Ding, Y., … Lian, Q. (2014b). Mitochondrial Transfer of Induced Pluripotent Stem Cell–Derived Mesenchymal Stem Cells to Airway Epithelial Cells Attenuates Cigarette Smoke–Induced Damage. *American Journal of Respiratory Cell and Molecular Biology*, *51*(3), 455–465. https://doi.org/10.1165/rcmb.2013-0529OC
178. Liberti, M. V., & Locasale, J. W. (2016). The Warburg Effect: How Does it Benefit Cancer Cells? *Trends in Biochemical Sciences*, *41*(3), 211–218. https://doi.org/10.1016/j.tibs.2015.12.001
179. Lionaki, E., Markaki, M., Palikaras, K., & Tavernarakis, N. (2015). Mitochondria, autophagy and age-associated neurodegenerative diseases: New insights into a complex interplay. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1847*(11), 1412–1423. https://doi.org/10.1016/j.bbabio.2015.04.010
180. Liu, F., Yang, H., Lu, K., & Qiu, Y. (2008). Effect of Mitochondrial Transplantation from Cumulus Granular Cells to the Early Embryos of Aged Mice. *Journal of Reproduction and Contraception*, *19*(4), 211–218. https://doi.org/10.1016/S1001-7844(08)60023-1
181. Lopez-Mejia, I. C., & Fajas, L. (2015). Cell cycle regulation of mitochondrial function. *Current Opinion in Cell Biology*, *33*, 19–25. https://doi.org/10.1016/j.ceb.2014.10.006
182. Luo, S., Valencia, C. A., Zhang, J., Lee, N.-C., Slone, J., Gui, B., … Huang, T. (2018). Biparental Inheritance of Mitochondrial DNA in Humans. *Proceedings of the National Academy of Sciences*, *115*(51), 13039–13044. https://doi.org/10.1073/pnas.1810946115
183. Luz-Crawford, P., Hernandez, J., Djouad, F., Luque-Campos, N., Caicedo, A., Carrère-Kremer, S., … Jorgensen, C. (2019a). Mesenchymal stem cell repression of Th17 cells is triggered by mitochondrial transfer. *Stem Cell Research & Therapy*, *10*(1), 232. https://doi.org/10.1186/s13287-019-1307-9

184. Luz-Crawford, P., Hernandez, J., Djouad, F., Luque-Campos, N., Caicedo, A., Carrère-Kremer, S., … Jorgensen, C. (2019b). Mesenchymal stem cell repression of Th17 cells is triggered by mitochondrial transfer. *Stem Cell Research & Therapy*, *10*(1), 232. https://doi.org/10.1186/s13287-019-1307-9
185. Ma, Y., He, K., & Berkowitz, G. A. (2019). Editorial: From Structure to Signalsomes: New Perspectives About Membrane Receptors and Channels. *Frontiers in Plant Science*, *10*. https://doi.org/10.3389/fpls.2019.00682
186. Macheiner, T., Fengler, V. H. I., Agreiter, M., Eisenberg, T., Madeo, F., Kolb, D., … Sargsyan, K. (2016). Magnetomitotransfer: An efficient way for direct mitochondria transfer into cultured human cells. *Scientific Reports*, *6*, srep35571. https://doi.org/10.1038/srep35571
187. Malik, A. N., Czajka, A., & Cunningham, P. (2016). Accurate quantification of mouse mitochondrial DNA without co-amplification of nuclear mitochondrial insertion sequences. *Mitochondrion*, *29*, 59–64. https://doi.org/10.1016/j.mito.2016.05.003
188. Malik, A. N., Shahni, R., Rodriguez-de-Ledesma, A., Laftah, A., & Cunningham, P. (2011). Mitochondrial DNA as a non-invasive biomarker: Accurate quantification using real time quantitative PCR without co-amplification of pseudogenes and dilution bias. *Biochemical and Biophysical Research Communications*, *412*(1), 1–7. https://doi.org/10.1016/j.bbrc.2011.06.067
189. Manoli, I., Alesci, S., Blackman, M. R., Su, Y. A., Rennert, O. M., & Chrousos, G. P. (2007). Mitochondria as key components of the stress response. *Trends in Endocrinology & Metabolism*, *18*(5), 190–198. https://doi.org/10.1016/j.tem.2007.04.004
190. Marei, W. F. A., Raemdonck, G. V., Baggerman, G., Bols, P. E. J., & Leroy, J. L. M. R. (2019). 166 Mitochondrial stress responses in bovine cumulus cells and oocytes matured under lipotoxic conditions: A proteomic insight. *Reproduction, Fertility and Development*, *31*(1), 208–208. https://doi.org/10.1071/RDv31n1Ab166
191. Margineantu, D. H., Gregory Cox, W., Sundell, L., Sherwood, S. W., Beechem, J. M., & Capaldi, R. A. (2002). Cell cycle dependent morphology changes and associated mitochondrial DNA redistribution in mitochondria of human cell lines. *Mitochondrion*, *1*(5), 425–435. https://doi.org/10.1016/S1567-7249(02)00006-5
192. Martin, S. D., & McGee, S. L. (2014). The role of mitochondria in the aetiology of insulin resistance and type 2 diabetes. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1840*(4), 1303–1312. https://doi.org/10.1016/j.bbagen.2013.09.019
193. Martins, I. (2018). Anti-Aging Gene linked to Appetite Regulation Determines Longevity in Humans and Animals. *International Journal of Aging Research*, *1*(6). https://doi.org/10.28933/ijoar-2018-05-1101
194. Martinvalet, D. (2019). Mitochondrial Entry of Cytotoxic Proteases: A New Insight into the Granzyme B Cell Death Pathway [Research article]. https://doi.org/10.1155/2019/9165214
195. Mason, K. A., Losos, J. B., Singer, S. R., & Raven, P. H. (2017). *Biology* (Eleventh edition). New York, NY: McGraw-Hill Education.
196. Masuzawa, A., Black, K. M., Pacak, C. A., Ericsson, M., Barnett, R. J., Drumm, C., … McCully, J. D. (2013). Transplantation of autologously derived mitochondria protects the heart from ischemia-reperfusion injury. *AJP: Heart and Circulatory Physiology*, *304*(7), H966–H982. https://doi.org/10.1152/ajpheart.00883.2012
197. McCance, K., & Huether, S. (Eds.). (2019). *Pathophysiology- THE BIOLOGIC BASIS FOR DISEASE IN ADULTS AND CHILDREN* (8th ed.). St. Louis, Missouri: Elsevier.
198. McCully, J. D., Cowan, D. B., Pacak, C. A., Toumpoulis, I. K., Dayalan, H., & Levitsky, S. (2009). Injection of isolated mitochondria during early reperfusion for cardioprotection. *AJP: Heart and Circulatory Physiology*, *296*(1), H94–H105. https://doi.org/10.1152/ajpheart.00567.2008
199. McCully, James D., Levitsky, S., del Nido, P. J., & Cowan, D. B. (2016). Mitochondrial transplantation for therapeutic use. *Clinical and Translational Medicine*, *5*(1). https://doi.org/10.1186/s40169-016-0095-4
200. McGuire, P. J. (2019). Mitochondrial Dysfunction and the Aging Immune System. *Biology*, *8*(2), 26. https://doi.org/10.3390/biology8020026
201. McWilliams, T. G., & Suomalainen, A. (2019). Mitochondrial DNA can be inherited from fathers, not just mothers. *Nature*, *565*(7739), 296–297. https://doi.org/10.1038/d41586-019-00093-1
202. Meseguer, S., Navarro-González, C., Panadero, J., Villarroya, M., Boutoual, R., Sánchez-Alcázar, J. A., & Armengod, M.-E. (2019). The MELAS mutation m.3243A>G alters the expression of mitochondrial tRNA fragments. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1866*(9), 1433–1449. https://doi.org/10.1016/j.bbamcr.2019.06.004
203. Miliotis, S., Nicolalde, B., Ortega, M., Yepez, J., & Caicedo, A. (2019). Forms of extracellular mitochondria and their impact in health. *Mitochondrion*. https://doi.org/10.1016/j.mito.2019.02.002

204. Mishra, P., & Chan, D. C. (2014). Mitochondrial dynamics and inheritance during cell division, development and disease. *Nature Reviews Molecular Cell Biology*, *15*(10), 634–646. https://doi.org/10.1038/nrm3877
205. Mohammadzadeh, M., Fesahat, F., Khoradmehr, A., & Khalili, M. A. (2018). Influential effect of age on oocyte morphometry, fertilization rate and embryo development following IVF in mice. *Middle East Fertility Society Journal*, *23*(2), 117–120. https://doi.org/10.1016/j.mefs.2017.09.006
206. Mombo, B. N., Gerbal-Chaloin, S., Bokus, A., Daujat-Chavanieu, M., Jorgensen, C., Hugnot, J.-P., & Vignais, M.-L. (2017). MitoCeption: Transferring Isolated Human MSC Mitochondria to Glioblastoma Stem Cells. *JoVE (Journal of Visualized Experiments)*, (120), e55245. https://doi.org/10.3791/55245
207. Mondal, T., Rasmussen, M., Pandey, G. K., Isaksson, A., & Kanduri, C. (2010). Characterization of the RNA content of chromatin. *Genome Research*, *20*(7), 899–907. https://doi.org/10.1101/gr.103473.109
208. Montano, V., Simoncini, C., Calì, C. L., Legati, A., Siciliano, G., & Mancuso, M. (2019). CPEO and Mitochondrial Myopathy in a Patient with DGUOK Compound Heterozygous Pathogenetic Variant and mtDNA Multiple Deletions [Research article]. https://doi.org/10.1155/2019/5918632
209. Morrison, T. J., Jackson, M. V., Cunningham, E. K., Kissenpfennig, A., McAuley, D. F., O'Kane, C. M., & Krasnodembskaya, A. D. (2017). Mesenchymal Stromal Cells Modulate Macrophages in Clinically Relevant Lung Injury Models by Extracellular Vesicle Mitochondrial Transfer. *American Journal of Respiratory and Critical Care Medicine*, *196*(10), 1275–1286. https://doi.org/10.1164/rccm.201701-0170OC
210. Moschoi, R., Imbert, V., Nebout, M., Chiche, J., Mary, D., Prebet, T., … Griessinger, E. (2016). Protective mitochondrial transfer from bone marrow stromal cells to acute myeloid leukemic cells during chemotherapy. *Blood*, *128*(2), 253–264. https://doi.org/10.1182/blood-2015-07-655860
211. Moskowitzova, K., Shin, B., Liu, K., Ramirez-Barbieri, G., Guariento, A., Blitzer, D., … McCully, J. D. (2019). Mitochondrial transplantation prolongs cold ischemia time in murine heart transplantation. *The Journal of Heart and Lung Transplantation*, *38*(1), 92–99. https://doi.org/10.1016/j.healun.2018.09.025
212. Müllebner, A., Dorighello, G. G., Kozlov, A. V., & Duvigneau, J. C. (2018). Interaction between Mitochondrial Reactive Oxygen Species, Heme Oxygenase, and Nitric Oxide Synthase Stimulates Phagocytosis in Macrophages. *Frontiers in Medicine*, *4*. https://doi.org/10.3389/fmed.2017.00252
213. Munir, T., Mahmood, A., Fakhar-e-Alam, M., Imran, M., Sohail, A., Amin, N., … Mahmood, K. (2019). Treatment of breast cancer with capped magnetic-NPs induced hyperthermia therapy. *Journal of Molecular Structure*, *1196*, 88–95. https://doi.org/10.1016/j.molstruc.2019.06.067
214. Murley, A., & Nunnari, J. (2016). The Emerging Network of Mitochondria-Organelle Contacts. *Molecular Cell*, *61*(5), 648–653. https://doi.org/10.1016/j.molcel.2016.01.031
215. Mykytenko, D., Mazur, P., Dyachenko, L., Borisov, M., Masliy, Y., Lamtyeva, V., … Zukin, V. (2019). 55. EFFICACY OF NUCLEAR TRANSFER TO PREVENT THE INHERITED MITOCHONDRIAL PATHOLOGY. *Reproductive BioMedicine Online*, *39*, e60. https://doi.org/10.1016/j.rbmo.2019.04.108
216. Naia, L., Ferreira, I. L., Ferreiro, E., & Rego, A. C. (2016). Mitochondrial Ca2+ handling in Huntington's and Alzheimer's diseases – Role of ER-mitochondria crosstalk. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1016/j.bbrc.2016.07.122
217. Narita, A., Niimura, S., & Ishida, K. (1992). Age-Related Changes in Adenylate Cyclase Activity in Mouse Oocytes. *Journal of Reproduction and Development*, *38*(1), 1–3. https://doi.org/10.1262/jrd.38.1
218. Ng, Y. S., Martikainen, M. H., Gorman, G. S., Blain, A., Bugiardini, E., Bunting, A., … McFarland, R. (2019). Pathogenic variants in MT-ATP6: A United Kingdom–based mitochondrial disease cohort study. *Annals of Neurology*, *86*(2), 310–315. https://doi.org/10.1002/ana.25525
219. Nicolson, G. L. (2014). The Fluid—Mosaic Model of Membrane Structure: Still relevant to understanding the structure, function and dynamics of biological membranes after more than 40years. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1838*(6), 1451–1466. https://doi.org/10.1016/j.bbamem.2013.10.019
220. Nightingale, H., Pfeffer, G., Bargiela, D., Horvath, R., & Chinnery, P. F. (2016). Emerging therapies for mitochondrial disorders. *Brain*, *139*(6), 1633–1648. https://doi.org/10.1093/brain/aww081
221. Nitzan, K., Benhamron, S., Valitsky, M., Kesner, E., Lichtenstein, M., Ben-Zvi, A., … Rosenmann, H. (2019). Mitochondrial Transfer Ameliorates Cognitive Deficits, Neuronal Loss, and Gliosis in Alzheimer's Disease Mice. *Journal of Alzheimer's Disease*, *Preprint*(Preprint), 1–18. https://doi.org/10.3233/JAD-190853
222. Nogueira, C., Almeida, L. S., Nesti, C., Pezzini, I., Videira, A., Vilarinho, L., & Santorelli, F. M. (2014). Syndromes associated with mitochondrial DNA depletion. *Italian Journal of Pediatrics*, *40*(1), 34. https://doi.org/10.1186/1824-7288-40-34

223. Ohkouchi, S., Block, G. J., Katsha, A. M., Kanehira, M., Ebina, M., Kikuchi, T., … Prockop, D. J. (2012). Mesenchymal Stromal Cells Protect Cancer Cells From ROS-induced Apoptosis and Enhance the Warburg Effect by Secreting STC1. *Molecular Therapy*, *20*(2), 417–423. https://doi.org/10.1038/mt.2011.259
224. Onfelt, B., Nedvetzki, S., Benninger, R. K. P., Purbhoo, M. A., Sowinski, S., Hume, A. N., … Davis, D. M. (2006). Structurally Distinct Membrane Nanotubes between Human Macrophages Support Long-Distance Vesicular Traffic or Surfing of Bacteria. *The Journal of Immunology*, *177*(12), 8476–8483. https://doi.org/10.4049/jimmunol.177.12.8476
225. Ore, A., & Olayinka, E. T. (2019). Toxic Mechanisms of Aryloxyphenoxypropionates in Target and Non-target Organisms. *International Journal of Biochemistry Research & Review*, 1–11. https://doi.org/10.9734/ijbcrr/2019/v27i130109
226. Orfany, A., Arriola, C. G., Doulamis, I. P., Guariento, A., Ramirez-Barbieri, G., Moskowitzova, K., … McCully, J. D. (2019). Mitochondrial transplantation ameliorates acute limb ischemia. *Journal of Vascular Surgery*. https://doi.org/10.1016/j.jvs.2019.03.079
227. Paillusson, S., Stoica, R., Gomez-Suaga, P., Lau, D. H. W., Mueller, S., Miller, T., & Miller, C. C. J. (2016). There's Something Wrong with my MAM; the ER–Mitochondria Axis and Neurodegenerative Diseases. *Trends in Neurosciences*, *39*(3), 146–157. https://doi.org/10.1016/j.tins.2016.01.008
228. Palade, G. (1953). AN ELECTRON MICROSCOPE STUDY OF THE MITOCHONDRIAL STRUCTURE. *Journal of Histochemistry & Cytochemistry*, *1*(4), 188–211. https://doi.org/10.1177/1.4.188
229. Paliwal, S., Chaudhuri, R., Agrawal, A., & Mohanty, S. (2018). Regenerative abilities of mesenchymal stem cells through mitochondrial transfer. *Journal of Biomedical Science*, *25*(1). https://doi.org/10.1186/s12929-018-0429-1
230. Park, C. B., & Larsson, N.-G. (2011). Mitochondrial DNA mutations in disease and aging. *The Journal of Cell Biology*, *193*(5), 809–818.
231. Parkinson-Lawrence, E. J., Shandala, T., Prodoehl, M., Plew, R., Borlace, G. N., & Brooks, D. A. (2010). Lysosomal Storage Disease: Revealing Lysosomal Function and Physiology. *Physiology*, *25*(2), 102–115. https://doi.org/10.1152/physiol.00041.2009
232. Pasquier, J., Guerrouahen, B. S., Al Thawadi, H., Ghiabi, P., Maleki, M., Abu-Kaoud, N., … Rafii, A. (2013). Preferential transfer of mitochondria from endothelial to cancer cells through tunneling nanotubes modulates chemoresistance. *Journal of Translational Medicine*, *11*, 94. https://doi.org/10.1186/1479-5876-11-94
233. Patananan, A. N., Wu, T.-H., Chiou, P.-Y., & Teitell, M. A. (2016). Modifying the Mitochondrial Genome. *Cell Metabolism*, *23*(5), 785–796. https://doi.org/10.1016/j.cmet.2016.04.004
234. Patergnani, S., Suski, J. M., Agnoletto, C., Bononi, A., Bonora, M., De Marchi, E., … Pinton, P. (2011). Calcium signaling around Mitochondria Associated Membranes (MAMs). *Cell Communication and Signaling*, *9*, 19. https://doi.org/10.1186/1478-811X-9-19
235. Pawelec, G., & Gupta, S. (2019). Editorial: Immunology of Aging. *Frontiers in Immunology*, *10*. https://doi.org/10.3389/fimmu.2019.01614
236. Pawlak, P., Chabowska, A., Malyszka, N., & Lechniak, D. (2016). Mitochondria and mitochondrial DNA in porcine oocytes and cumulus cells—A search for developmental competence marker. *Mitochondrion*, *27*, 48–55. https://doi.org/10.1016/j.mito.2015.12.008
237. Payne, B. A. I., & Chinnery, P. F. (2015). Mitochondrial dysfunction in aging: Much progress but many unresolved questions. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1847*(11), 1347–1353. https://doi.org/10.1016/j.bbabio.2015.05.022
238. Payton, R. R., Rispoli, L. A., Nagle, K. A., Gondro, C., Saxton, A. M., Voy, B. H., & Edwards, J. L. (2018). Mitochondrial-related consequences of heat stress exposure during bovine oocyte maturation persist in early embryo development. *Journal of Reproduction and Development*, 2017–2160. https://doi.org/10.1262/jrd.2017-160
239. Peng, W., Cai, G., Xia, Y., Chen, J., Wu, P., Wang, Z., … Wei, D. (2019). Mitochondrial Dysfunction in Atherosclerosis. *DNA and Cell Biology*, *38*(7), 597–606. https://doi.org/10.1089/dna.2018.4552
240. Peotter, J., Kasberg, W., Pustova, I., & Audhya, A. (2019). COPII-mediated trafficking at the ER/ERGIC interface. *Traffic*, *20*(7), 491–503. https://doi.org/10.1111/tra.12654
241. Pernas, L., & Scorrano, L. (2016). Mito-Morphosis: Mitochondrial Fusion, Fission, and Cristae Remodeling as Key Mediators of Cellular Function. *Annual Review of Physiology*, *78*(1), 505–531. https://doi.org/10.1146/annurev-physiol-021115-105011
242. Picard, M., Wallace, D. C., & Burelle, Y. (2016). The rise of mitochondria in medicine. *Mitochondrion*, *30*, 105–116. https://doi.org/10.1016/j.mito.2016.07.003
243. Picca, A., Guerra, F., Calvani, R., Bucci, C., Lo Monaco, M. R., Bentivoglio, A. R., … Marzetti, E. (2019). Mitochondrial Dysfunction and Aging: Insights from the Analysis of Extracellular Vesicles. *International Journal of Molecular Sciences*, *20*(4), 805. https://doi.org/10.3390/ijms20040805

244. Pinegin, B., Vorobjeva, N., Pashenkov, M., & Chernyak, B. (2018). The role of mitochondrial ROS in antibacterial immunity. *Journal of Cellular Physiology*, *233*(5), 3745–3754.
245. Pollard, T., Earnshaw, W., Lippincot-Schwartz, J., & Johnson, G. (Eds.). (2017). *Cell Biology* (Third, Vols. 1–1). Philadelphia.
246. Poole, A. M., & Gribaldo, S. (2014). Eukaryotic origins: How and when was the mitochondrion acquired? *Cold Spring Harbor Perspectives in Biology*, a015990.
247. Qian, J., Gelens, L., & Bollen, M. (2019). Coordination of Timers and Sensors in Cell Signaling. *BioEssays*, *41*(3), 1800217. https://doi.org/10.1002/bies.201800217
248. Raff, A. B., Thomas, C. N., Chuang, G. S., Avram, M. M., Le, M. H., Anderson, R. R., & Purschke, M. (2019). Lidocaine-induced potentiation of thermal damage in skin and carcinoma cells. *Lasers in Surgery and Medicine*, *51*(1), 88–94. https://doi.org/10.1002/lsm.23027
249. Rafieian-Kopaei, M., Setorki, M., Doudi, M., Baradan, A., & Nasri, H. (2014). Atherosclerosis: Process, Indicators, Risk Factors and New Hopes. *International Journal of Preventive Medicine*, *5*(8), 927–946.
250. Rai, P. K., Craven, L., Hoogewijs, K., Russell, O. M., & Lightowlers, R. N. (2018). Advances in methods for reducing mitochondrial DNA disease by replacing or manipulating the mitochondrial genome. *Essays In Biochemistry*, *62*(3), 455–465. https://doi.org/10.1042/EBC20170113
251. Raimundo, N., Fernández-Mosquera, L., Yambire, K. F., & Diogo, C. V. (2016). Mechanisms of communication between mitochondria and lysosomes. *The International Journal of Biochemistry & Cell Biology*, *79*, 345–349. https://doi.org/10.1016/j.biocel.2016.08.020
252. Rambags, B. P. B., van Boxtel, D. C. J., Tharasanit, T., Lenstra, J. A., Colenbrander, B., & Stout, T. A. E. (2014). Advancing maternal age predisposes to mitochondrial damage and loss during maturation of equine oocytes in vitro. *Theriogenology*, *81*(7), 959–965. https://doi.org/10.1016/j.theriogenology.2014.01.020
253. Rango, M., & Bresolin, N. (2018). Brain Mitochondria, Aging, and Parkinson's Disease. *Genes*, *9*(5), 250. https://doi.org/10.3390/genes9050250
254. Raposo, G., & Stoorvogel, W. (2013). Extracellular vesicles: Exosomes, microvesicles, and friends. *The Journal of Cell Biology*, *200*(4), 373–383. https://doi.org/10.1083/jcb.201211138
255. Reed, J. C., & Green, D. R. (Eds.). (2011). *Apoptosis: Physiology and pathology*. Cambridge: Cambridge University Press.
256. Reynier, P., May-Panloup, P., Chrétien, M.-F., Morgan, C. J., Jean, M., Savagner, F., … Malthièry, Y. (2001). Mitochondrial DNA content affects the fertilizability of human oocytes. *Molecular Human Reproduction*, *7*(5), 425–429. https://doi.org/10.1093/molehr/7.5.425
257. Rieusset, J. (2015). Contribution of mitochondria and endoplasmic reticulum dysfunction in insulin resistance: Distinct or interrelated roles? *Diabetes & Metabolism*, *41*(5), 358–368. https://doi.org/10.1016/j.diabet.2015.02.006
258. Rius, R., Cowley, M. J., Riley, L., Puttick, C., Thorburn, D. R., & Christodoulou, J. (2019). Biparental inheritance of mitochondrial DNA in humans is not a common phenomenon. *Genetics in Medicine*, 1–4. https://doi.org/10.1038/s41436-019-0568-0
259. Rogers, R. S., & Bhattacharya, J. (2013). When Cells Become Organelle Donors. *Physiology*, *28*(6), 414–422. https://doi.org/10.1152/physiol.00032.2013
260. Roglic, G., & Unwin, N. (2010). Mortality attributable to diabetes: Estimates for the year 2010. *Diabetes Research and Clinical Practice*, *87*(1), 15–19. https://doi.org/10.1016/j.diabres.2009.10.006
261. Roth, Z. (2018). Symposium review: Reduction in oocyte developmental competence by stress is associated with alterations in mitochondrial function1. *Journal of Dairy Science*, *101*(4), 3642–3654. https://doi.org/10.3168/jds.2017-13389
262. Rotig, A., Colonna, M., Bonnefont, J. P., Blanche, S., Fischer, A., Saudubray, J. M., & Munnich, A. (1989). MITOCHONDRIAL DNA DELETION IN PEARSON'S MARROW/PANCREAS SYNDROME. *The Lancet*, *333*(8643), 902–903. https://doi.org/10.1016/S0140-6736(89)92897-3
263. Rowland, A. A., & Voeltz, G. K. (2012). Endoplasmic reticulum–mitochondria contacts: Function of the junction. *Nature Reviews Molecular Cell Biology*, *13*(10), 607–615. https://doi.org/10.1038/nrm3440
264. Roy, M., Reddy, P. H., Iijima, M., & Sesaki, H. (2015). Mitochondrial division and fusion in metabolism. *Current Opinion in Cell Biology*, *33*, 111–118. https://doi.org/10.1016/j.ceb.2015.02.001
265. Rustom, A. (2004). Nanotubular Highways for Intercellular Organelle Transport. *Science*, *303*(5660), 1007–1010. https://doi.org/10.1126/science.1093133
266. Salas, A., Schönherr, S., Bandelt, H.-J., Gómez-Carballa, A., & Weissensteiner, H. (2019). Extraordinary claims require extraordinary evidence in the case of asserted mtDNA biparental inheritance. *BioRxiv*, 585752. https://doi.org/10.1101/585752

267. Saldaña-Martínez, A., Muñoz, M. de L., Pérez-Ramírez, G., Montiel-Sosa, J. F., Montoya, J., Emperador, S., … Ramírez, R. G. (2019). Whole sequence of the mitochondrial DNA genome of Kearns Sayre Syndrome patients: Identification of deletions and variants. *Gene*, *688*, 171–181. https://doi.org/10.1016/j.gene.2018.11.085
268. Sansone, P., Savini, C., Kurelac, I., Chang, Q., Amato, L. B., Strillacci, A., … Bromberg, J. (2017). Packaging and transfer of mitochondrial DNA via exosomes regulate escape from dormancy in hormonal therapy-resistant breast cancer. *Proceedings of the National Academy of Sciences*, *114*(43), E9066–E9075. https://doi.org/10.1073/pnas.1704862114
269. Sato, A., Kono, T., Nakada, K., Ishikawa, K., Inoue, S.-I., Yonekawa, H., & Hayashi, J.-I. (2005). Gene therapy for progeny of mito-mice carrying pathogenic mtDNA by nuclear transplantation. *Proceedings of the National Academy of Sciences*, *102*(46), 16765–16770. https://doi.org/10.1073/pnas.0506197102
270. Schatten, H., Sun, Q.-Y., & Prather, R. (2014). The impact of mitochondrial function/dysfunction on IVF and new treatment possibilities for infertility. *Reproductive Biology and Endocrinology*, *12*, 111. https://doi.org/10.1186/1477-7827-12-111
271. Scheffler, I. E. (2008). *Mitochondria* (2nd ed). Hoboken, N.J: Wiley-Liss.
272. Schekman, R. (2004). Merging cultures in the study of membrane traffic. *Nature Cell Biology*, *6*(6), 483–486. https://doi.org/10.1038/ncb0604-483
273. Schrader, M., Kamoshita, M., & Islinger, M. (2019). Organelle interplay—Peroxisome interactions in health and disease. *Journal of Inherited Metabolic Disease*, *0*(0), 1–19. https://doi.org/10.1002/jimd.12083
274. Science Source—Mitochondrion in Bat Pancreas Cell, TEM. (n.d.). Retrieved July 30, 2019, from https://www.sciencesource.com/archive/Mitochondrion-in-Bat-Pancreas-Cell--TEM-SS2624953.html
275. Sen, N. (2019). Aberrant cell cycle induction is pivotal for mitochondrial biogenesis after traumatic brain injury. *Neural Regeneration Research*, *14*(7), 1215–1216. https://doi.org/10.4103/1673-5374.251305
276. Seppet, E., Gruno, M., Peetsalu, A., Gizatullina, Z., Nguyen, H. P., Vielhaber, S., … Gellerich, F. N. (2009). Mitochondria and Energetic Depression in Cell Pathophysiology. *International Journal of Molecular Sciences*, *10*(5), 2252–2303. https://doi.org/10.3390/ijms10052252
277. Shang, T., Li, S., Zhang, Y., Lu, L., Cui, L., & Guo, F. F. (2019). Hypoxia promotes differentiation of adipose-derived stem cells into endothelial cells through demethylation of ephrinB2. *Stem Cell Research & Therapy*, *10*(1), 133. https://doi.org/10.1186/s13287-019-1233-x
278. Shourbagy, S. H. E., Spikings, E. C., Freitas, M., & John, J. C. S. (2006). Mitochondria directly influence fertilisation outcome in the pig. *Reproduction*, *131*(2), 233–245. https://doi.org/10.1530/rep.1.00551
279. Silzer, T. K., & Phillips, N. R. (2018). Etiology of type 2 diabetes and Alzheimer's disease: Exploring the mitochondria. *Mitochondrion*, *43*, 16–24. https://doi.org/10.1016/j.mito.2018.04.004
280. Simsek-Duran, F., Li, F., Ford, W., Swanson, R. J., Jones, H. W., & Castora, F. J. (2013). Age-Associated Metabolic and Morphologic Changes in Mitochondria of Individual Mouse and Hamster Oocytes. *PLoS ONE*, *8*(5). https://doi.org/10.1371/journal.pone.0064955
281. Sinha, P., Islam, M. N., Bhattacharya, S., & Bhattacharya, J. (2016a). Intercellular mitochondrial transfer: Bioenergetic crosstalk between cells. *Current Opinion in Genetics & Development*, *38*, 97–101. https://doi.org/10.1016/j.gde.2016.05.002
282. Sinha, P., Islam, M. N., Bhattacharya, S., & Bhattacharya, J. (2016b). Intercellular mitochondrial transfer: Bioenergetic crosstalk between cells. *Current Opinion in Genetics & Development*, *38*, 97–101. https://doi.org/10.1016/j.gde.2016.05.002
283. Sluijter, J. P. G., Verhage, V., Deddens, J. C., van den Akker, F., & Doevendans, P. A. (2014). Microvesicles and exosomes for intracardiac communication. *Cardiovascular Research*, *102*(2), 302–311. https://doi.org/10.1093/cvr/cvu022
284. Sokolova, V. A., Kustova, M. E., Arbuzova, N. I., Sorokin, A. V., Moskaliova, O. S., Bass, M. G., & Vasilyev, V. B. (2004). Obtaining mice that carry human mitochondrial DNA transmitted to the progeny. *Molecular Reproduction and Development*, *68*(3), 299–307. https://doi.org/10.1002/mrd.20075
285. Solovyeva, V. V., Shaimardanova, A. A., Chulpanova, D. S., Kitaeva, K. V., Chakrabarti, L., & Rizvanov, A. A. (2018). New Approaches to Tay-Sachs Disease Therapy. *Frontiers in Physiology*, *9*. https://doi.org/10.3389/fphys.2018.01663
286. Spees, J. L., Olson, S. D., Whitney, M. J., & Prockop, D. J. (2006). Mitochondrial transfer between cells can rescue aerobic respiration. *Proceedings of the National Academy of Sciences*, *103*(5), 1283–1288. https://doi.org/10.1073/pnas.0510511103

287. Spinelli, J. B., & Haigis, M. C. (2018). The multifaceted contributions of mitochondria to cellular metabolism. *Nature Cell Biology*, *20*(7), 745–754. https://doi.org/10.1038/s41556-018-0124-1
288. Stacchiotti, A., Favero, G., Lavazza, A., Garcia-Gomez, R., Monsalve, M., & Rezzani, R. (2019). Perspective: Mitochondria-ER Contacts in Metabolic Cellular Stress Assessed by Microscopy. *Cells*, *8*(1), 5. https://doi.org/10.3390/cells8010005
289. Steller, H. (Ed.). (2015). *Apoptosis and development* (1. ed). Amsterdam: Elsevier, Acad. Press.
290. Su, X., Rak, M., Tetaud, E., Godard, F., Sardin, E., Bouhier, M., … Tribouillard-Tanvier, D. (2019). Deregulating mitochondrial metabolite and ion transport has beneficial effects in yeast and human cellular models for NARP syndrome. *Human Molecular Genetics*. https://doi.org/10.1093/hmg/ddz160
291. Sugiura, A., Mattie, S., Prudent, J., & McBride, H. M. (2017). Newly born peroxisomes are a hybrid of mitochondrial and ER-derived pre-peroxisomes. *Nature*, *542*(7640), 251–254. https://doi.org/10.1038/nature21375
292. Suh, K. S., Chon, S., Jung, W.-W., & Choi, E. M. (2019). Crocin attenuates methylglyoxal-induced osteoclast dysfunction by regulating glyoxalase, oxidative stress, and mitochondrial function. *Food and Chemical Toxicology*, *124*, 367–373. https://doi.org/10.1016/j.fct.2018.12.031
293. Sukhorukov, V. M., & Meyer-Hermann, M. (2015). Structural Heterogeneity of Mitochondria Induced by the Microtubule Cytoskeleton. *Scientific Reports*, *5*(1). https://doi.org/10.1038/srep13924
294. Sun, N., Youle, R. J., & Finkel, T. (2016). The Mitochondrial Basis of Aging. *Molecular Cell*, *61*(5), 654–666. https://doi.org/10.1016/j.molcel.2016.01.028
295. Sung, Y.-J., Kao, T.-Y., Kuo, C.-L., Fan, C.-C., Cheng, A. N., Fang, W.-C., … Lee, A. Y.-L. (2018). Mitochondrial Lon sequesters and stabilizes p53 in the matrix to restrain apoptosis under oxidative stress via its chaperone activity. *Cell Death & Disease*, *9*(6). https://doi.org/10.1038/s41419-018-0730-7
296. Suomalainen, A., & Battersby, B. J. (2018). Mitochondrial diseases: The contribution of organelle stress responses to pathology. *Nature Reviews Molecular Cell Biology*, *19*(2), 77–92. https://doi.org/10.1038/nrm.2017.66
297. Tachibana, M., Sparman, M., & Mitalipov, S. (2010). Chromosome transfer in mature oocytes. *Nature Protocols*, *5*(6), 1138–1147. https://doi.org/10.1038/nprot.2010.75
298. Tachibana, M., Sparman, M., Sritanaudomchai, H., Ma, H., Clepper, L., Woodward, J., … Mitalipov, S. (2009). Mitochondrial gene replacement in primate offspring and embryonic stem cells. *Nature*, *461*(7262), 367–372. https://doi.org/10.1038/nature08368
299. Takeda, K. (2019). Functional consequences of mitochondrial mismatch in reconstituted embryos and offspring. *Journal of Reproduction and Development*. https://doi.org/10.1262/jrd.2019-089
300. Takeda, K., Tasai, M., Iwamoto, M., Onishi, A., Tagami, T., Nirasawa, K., … Pinkert, C. A. (2005). Microinjection of Cytoplasm or Mitochondria Derived from Somatic Cells Affects Parthenogenetic Development of Murine Oocytes. *Biology of Reproduction*, *72*(6), 1397–1404. https://doi.org/10.1095/biolreprod.104.036129
301. Takeo, S., Goto, H., Kuwayama, T., Monji, Y., & Iwata, H. (2013). Effect of Maternal Age on the Ratio of Cleavage and Mitochondrial DNA Copy Number in Early Developmental Stage Bovine Embryos. *Journal of Reproduction and Development*, *59*(2), 174–179. https://doi.org/10.1262/jrd.2012-148
302. Tan, A. S., Baty, J. W., Dong, L.-F., Bezawork-Geleta, A., Endaya, B., Goodwin, J., … Berridge, M. V. (2015). Mitochondrial Genome Acquisition Restores Respiratory Function and Tumorigenic Potential of Cancer Cells without Mitochondrial DNA. *Cell Metabolism*, *21*(1), 81–94. https://doi.org/10.1016/j.cmet.2014.12.003
303. Tan, D.-X. (2019). Aging: An evolutionary competition between host cells and mitochondria. *Medical Hypotheses*, *127*, 120–128. https://doi.org/10.1016/j.mehy.2019.04.007
304. Tenorio, B. M., Pereira da Silva, R., Tenorio, F. das C. A. M., Costa Rosales, R. R., Amaro da Silva Junior, V., & de Albuquerque Nogueira, R. (2019). Effect of heat stress and Hsp90 inhibition on T-type calcium currents and voltage-dependent potassium currents in leydig cells. *Journal of Thermal Biology*, *84*, 1–7. https://doi.org/10.1016/j.jtherbio.2019.05.022
305. Thorburn, D. R., Rahman, J., & Rahman, S. (2017). Mitochondrial DNA-Associated Leigh Syndrome and NARP. In M. Adam, H. Ardinger, & R. Pagon et al. ,. (Eds.), *GeneReviews* (p. 48). Retrieved from https://www.ncbi.nlm.nih.gov/books/
306. Thornton, C., & Hagberg, H. (2015). Role of mitochondria in apoptotic and necroptotic cell death in the developing brain. *Clinica Chimica Acta*, *451, Part A*, 35–38. https://doi.org/10.1016/j.cca.2015.01.026
307. Torralba, D., Baixauli, F., & Sánchez-Madrid, F. (2016). Mitochondria Know No Boundaries: Mechanisms and Functions of Intercellular Mitochondrial Transfer. *Frontiers in Cell and Developmental Biology*, *4*. https://doi.org/10.3389/fcell.2016.00107

308. Tousson, E., El-Atrsh, A., Mansour, M., & Abdallah, A. (2019). Modulatory effects of Saussurea lappa root aqueous extract against ethephon-induced kidney toxicity in male rats. *Environmental Toxicology*, *0*(0), 1–8. https://doi.org/10.1002/tox.22828
309. Tranchant, C., & Anheim, M. (2016). Movement disorders in mitochondrial diseases. *Revue Neurologique*, *172*(8–9), 524–529. https://doi.org/10.1016/j.neurol.2016.07.003
310. Trounce, I. A., McKenzie, M., Cassar, C. A., Ingraham, C. A., Lerner, C. A., Dunn, D. A., … Pinkert, C. A. (2004). Development and Initial Characterization of Xenomitochondrial Mice. *Journal of Bioenergetics and Biomembranes*, *36*(4), 421–427. https://doi.org/10.1023/B:JOBB.0000041778.84464.16
311. Unuma, K., Aki, T., Funakoshi, T., Hashimoto, K., & Uemura, K. (2015). Extrusion of mitochondrial contents from lipopolysaccharide-stimulated cells: Involvement of autophagy. *Autophagy*, *11*(9), 1520–1536. https://doi.org/10.1080/15548627.2015.1063765
312. van Beek, A. A., Van den Bossche, J., Mastroberardino, P. G., de Winther, M. P. J., & Leenen, P. J. M. (2019). Metabolic Alterations in Aging Macrophages: Ingredients for Inflammaging? *Trends in Immunology*, *40*(2), 113–127. https://doi.org/10.1016/j.it.2018.12.007
313. van der Laan, M., Horvath, S. E., & Pfanner, N. (2016). Mitochondrial contact site and cristae organizing system. *Current Opinion in Cell Biology*, *41*, 33–42. https://doi.org/10.1016/j.ceb.2016.03.013
314. Van Houten, B., Hunter, S. E., & Meyer, J. N. (2016). Mitochondrial DNA damage induced autophagy, cell death, and disease. *Frontiers in Bioscience (Landmark Edition)*, *21*, 42–54.
315. VanDelinder, V., Imam, Z. I., & Bachand, G. (2019). Kinesin motor density and dynamics in gliding microtubule motility. *Scientific Reports*, *9*(1), 1–9. https://doi.org/10.1038/s41598-019-43749-8
316. Vasileiou, P. V. S., Evangelou, K., Vlasis, K., Fildisis, G., Panayiotidis, M. I., Chronopoulos, E., … Havaki, S. (2019). Mitochondrial Homeostasis and Cellular Senescence. *Cells*, *8*(7), 686. https://doi.org/10.3390/cells8070686
317. Vatrinet, R., Leone, G., De Luise, M., Girolimetti, G., Vidone, M., Gasparre, G., & Porcelli, A. M. (2017). The α-ketoglutarate dehydrogenase complex in cancer metabolic plasticity. *Cancer & Metabolism*, *5*, 3. https://doi.org/10.1186/s40170-017-0165-0
318. Victor, V., Apostolova, N., Herance, R., Hernandez-Mijares, A., & Rocha, M. (2009). Oxidative Stress and Mitochondrial Dysfunction in Atherosclerosis: Mitochondria-Targeted Antioxidants as Potential Therapy. *Current Medicinal Chemistry*, *16*(35), 4654–4667. https://doi.org/10.2174/092986709789878265
319. Villa, E., Ali, E. S., Sahu, U., & Ben-Sahra, I. (2019). Cancer Cells Tune the Signaling Pathways to Empower de Novo Synthesis of Nucleotides. *Cancers*, *11*(5), 688. https://doi.org/10.3390/cancers11050688
320. Villeneuve, L. M., Purnell, P. R., Boska, M. D., & Fox, H. S. (2016). Early expression of Parkinson's disease-related mitochondrial abnormalities in PINK1 knockout rats. *Molecular Neurobiology*, *53*(1), 171–186. https://doi.org/10.1007/s12035-014-8927-y
321. Voeltz, G. K., Rolls, M. M., & Rapoport, T. A. (2002). Structural organization of the endoplasmic reticulum. *EMBO Reports*, *3*(10), 944–950. https://doi.org/10.1093/embo-reports/kvf202
322. Wang, J., & Chen, G.-J. (2016). Mitochondria as a therapeutic target in Alzheimer's disease. *Genes & Diseases*, *3*(3), 220–227. https://doi.org/10.1016/j.gendis.2016.05.001
323. Wang, T., Zhang, M., Jiang, Z., & Seli, E. (2017). Mitochondrial dysfunction and ovarian aging. *American Journal of Reproductive Immunology*, *77*(5), n/a-n/a. https://doi.org/10.1111/aji.12651
324. Wang, X., & Gerdes, H.-H. (2015). Transfer of mitochondria via tunneling nanotubes rescues apoptotic PC12 cells. *Cell Death and Differentiation*, *22*(7), 1181.
325. Wang, Y., Ni, J., Gao, C., Xie, L., Zhai, L., Cui, G., & Yin, X. (2019). Mitochondrial transplantation attenuates lipopolysaccharide- induced depression-like behaviors. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *93*, 240–249. https://doi.org/10.1016/j.pnpbp.2019.04.010
326. Wang, Z.-B., Hao, J.-X., Meng, T.-G., Guo, L., Dong, M.-Z., Fan, L.-H., … Yao, Y.-Q. (2017). Transfer of autologous mitochondria from adipose tissue-derived stem cells rescues oocyte quality and infertility in aged mice. *Aging (Albany NY)*, *9*(12), 2480–2488. https://doi.org/10.18632/aging.101332
327. WebHome < MITOMAP < Foswiki. (n.d.). Retrieved September 1, 2019, from https://www.mitomap.org/MITOMAP
328. Westman, J., Hube, B., & Fairn, G. D. (2019). Integrity under stress: Host membrane remodelling and damage by fungal pathogens. *Cellular Microbiology*, *21*(4), e13016. https://doi.org/10.1111/cmi.13016

329. Wiley, C. D., Velarde, M. C., Lecot, P., Liu, S., Sarnoski, E. A., Freund, A., … Campisi, J. (2016). Mitochondrial Dysfunction Induces Senescence with a Distinct Secretory Phenotype. *Cell Metabolism*, *23*(2), 303–314. https://doi.org/10.1016/j.cmet.2015.11.011
330. Wolf, D. P., Mitalipov, N., & Mitalipov, S. (2015a). Mitochondrial replacement therapy in reproductive medicine. *Trends in Molecular Medicine*, *21*(2), 68–76. https://doi.org/10.1016/j.molmed.2014.12.001
331. Wolf, D. P., Mitalipov, N., & Mitalipov, S. (2015b). Mitochondrial replacement therapy in reproductive medicine. *Trends in Molecular Medicine*, *21*(2), 68–76. https://doi.org/10.1016/j.molmed.2014.12.001
332. Wong, R. C. B., Lim, S. Y., Hung, S. S. C., Jackson, S., Khan, S., Van Bergen, N. J., … Pébay, A. (2017). Mitochondrial replacement in an iPSC model of Leber's hereditary optic neuropathy. *Aging (Albany NY)*, *9*(4), 1341–1347. https://doi.org/10.18632/aging.101231
333. Woods, D. C., Khrapko, K., & Tilly, J. L. (2018). Influence of Maternal Aging on Mitochondrial Heterogeneity, Inheritance, and Function in Oocytes and Preimplantation Embryos. *Genes*, *9*(5), 265. https://doi.org/10.3390/genes9050265
334. Woods, D. C., & Tilly, J. L. (2015). Autologous Germline Mitochondrial Energy Transfer (AUGMENT) in Human Assisted Reproduction. *Seminars in Reproductive Medicine*, *33*(6), 410–421. https://doi.org/10.1055/s-0035-1567826
335. Wu, T.-H., Sagullo, E., Case, D., Zheng, X., Li, Y., Hong, J. S., … Teitell, M. A. (2016). Mitochondrial Transfer by Photothermal Nanoblade Restores Metabolite Profile in Mammalian Cells. *Cell Metabolism*, *23*(5), 921–929. https://doi.org/10.1016/j.cmet.2016.04.007
336. Wu, T.-H., Teslaa, T., Kalim, S., French, C. T., Moghadam, S., Wall, R., … Chiou, P.-Y. (2011). Photothermal Nanoblade for Large Cargo Delivery into Mammalian Cells. *Analytical Chemistry*, *83*(4), 1321–1327. https://doi.org/10.1021/ac102532w
337. Wu, T.-H., Teslaa, T., Teitell, M. A., & Chiou, P.-Y. (2010). Photothermal nanoblade for patterned cell membrane cutting. *Optics Express*, *18*(22), 23153–23160. https://doi.org/10.1364/OE.18.023153
338. Xiao, F., Li, X., Wang, J., & Cao, J. (2019). Mechanisms of vascular endothelial cell injury in response to intermittent and/or continuous hypoxia exposure and protective effects of anti-inflammatory and anti-oxidant agents. *Sleep and Breathing*, *23*(2), 515–522. https://doi.org/10.1007/s11325-019-01803-9
339. Xiao, J., Fan, L., Wu, D., Xu, Y., Lai, D., Martin, W. F., … Zhang, C. (2019). Archaea, the tree of life, and cellular evolution in eukaryotes. *Science China Earth Sciences*, *62*(3), 489–506. https://doi.org/10.1007/s11430-018-9326-1
340. Xiao, X., Huang, M., Fan, C., & Zuo, F. (2018). Research on the senescence of human skin fibroblasts induced by ultraviolet b and its mechanism. *Biomedical Research*, *29*(2). Retrieved from http://www.alliedacademies.org/abstract/research-on-the-senescence-of-human-skin-fibroblasts-induced-by-ultraviolet-b-and-its-mechanism-9591.html
341. Yabuuchi, A., Beyhan, Z., Kagawa, N., Mori, C., Ezoe, K., Kato, K., … Kato, O. (2012). Prevention of mitochondrial disease inheritance by assisted reproductive technologies: Prospects and challenges. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1820*(5), 637–642. https://doi.org/10.1016/j.bbagen.2011.10.014
342. Yamaguchi, M., & Worman, C. O. (2014). *Deep-sea microorganisms and the origin of the eukaryotic cell*. *47*(1), 20.
343. Yang, L., Long, Q., Liu, J., Tang, H., Li, Y., Bao, F., … Liu, X. (2015). Mitochondrial fusion provides an 'initial metabolic complementation' controlled by mtDNA. *Cellular and Molecular Life Sciences*, *72*(13), 2585–2598. https://doi.org/10.1007/s00018-015-1863-9
344. Yang, Y., Elamawi, R., Bubeck, J., Pepperkok, R., Ritzenthaler, C., & Robinson, D. G. (2005). Dynamics of COPII Vesicles and the Golgi Apparatus in Cultured Nicotiana tabacum BY-2 Cells Provides Evidence for Transient Association of Golgi Stacks with Endoplasmic Reticulum Exit Sites. *The Plant Cell*, *17*(5), 1513–1531. https://doi.org/10.1105/tpc.104.026757
345. Yin, X.-M., & Dong, Z. (Eds.). (2003). *Essentials of apoptosis: A guide for basic and clinical research*. Totowa, N.J: Humana Press.
346. Yoshida, Y., Takahashi, K., Okita, K., Ichisaka, T., & Yamanaka, S. (2009). Hypoxia Enhances the Generation of Induced Pluripotent Stem Cells. *Cell Stem Cell*, *5*(3), 237–241. https://doi.org/10.1016/j.stem.2009.08.001
347. Youle, R. J., & Van Der Bliek, A. M. (2012). Mitochondrial fission, fusion, and stress. *Science*, *337*(6098), 1062–1065.
348. Yu, C., Jiang, F., Zhang, M., Luo, D., Shao, S., Zhao, J., … Guan, Q. (2019). HC diet inhibited testosterone synthesis by activating endoplasmic reticulum stress in testicular Leydig cells. *Journal of Cellular and Molecular Medicine*, *23*(5), 3140–3150. https://doi.org/10.1111/jcmm.14143

349. Yuan, S., & Akey, C. W. (2013). Apoptosome Structure, Assembly, and Procaspase Activation. *Structure*, *21*(4), 501–515. https://doi.org/10.1016/j.str.2013.02.024
350. Yu-Wai-Man, P., & Chinnery, P. F. (2016). Leber Hereditary Optic Neuropathy. In M. Adam, H. Ardinger, & R. Pagon et al. ,. (Eds.), *GeneReviews* (p. 19). Retrieved from https://www.ncbi.nlm.nih.gov/books/ Leber
351. Zachary, J. F. (Ed.). (2017). *Pathologic basis of veterinary disease* (Sixth edition). St. Louis, Missouri: Elsevier.
352. Zhang, Z., Ma, Z., Yan, C., Pu, K., Wu, M., Bai, J., … Wang, Q. (2019). Muscle-derived autologous mitochondrial transplantation: A novel strategy for treating cerebral ischemic injury. *Behavioural Brain Research*, *356*, 322–331. https://doi.org/10.1016/j.bbr.2018.09.005
353. Zheng, C.-X., Sui, B.-D., Qiu, X.-Y., Hu, C.-H., & Jin, Y. (2019). Mitochondrial Regulation of Stem Cells in Bone Homeostasis. *Trends in Molecular Medicine*. https://doi.org/10.1016/j.molmed.2019.04.008
354. Zimorski, V., Ku, C., Martin, W. F., & Gould, S. B. (2014). Endosymbiotic theory for organelle origins. *Current Opinion in Microbiology*, *22*, 38–48. https://doi.org/10.1016/j.mib.2014.09.008
355. Zsurka, G., Peeva, V., Kotlyar, A., & Kunz, W. (2018). Is There Still Any Role for Oxidative Stress in Mitochondrial DNA-Dependent Aging? *Genes*, *9*(4), 175. https://doi.org/10.3390/genes9040175

Chapter 7. Conclusions and Perspectives

Mitochondria are very important organelles. They are the cell powerhouse and, they control central metabolic process as cell differentiation and fate, calcium and urea homeostasis, and heme and nucleotides synthesis, among others. Additionally, mitochondria play a role in inflammation, tissues' recovery after hypoxemic conditions and cancer progression. Strict control of mitochondrial mtDNA structure, mitochondrial number and function is essential to normal cell metabolism. Cellular energy needs, hormones, cytokines and other stressors and signals exert influence on mitochondrial activity.

Because of the wide range of functions and process where mitochondria are involved and by their deficiency in mtDNA repair mechanisms, these organelles are very sensitive to suffer damage in their membrane proteins and DNA. These organelles that are crucial for life can also be main players in contributing to cell death and disease. Mitochondrial repair mechanisms include mitochondrial fusion, fission and mitophagy. These internal mechanisms can repair only acquired damage and fail in repair inherited mtDNA mutations or deletions. Only mitochondrial transfer can aid to replace the mutant mitochondria by collaborating with mitochondrial fusion, fission and mitophagy to eliminate abnormal mitochondria and improve the number of normal mitochondria. Because of this, horizontal (natural) mitochondrial transfer from mesenchymal stem cells to compromised cells can repair only acquired disorders related to inflammation and hypoxemic damage.

Acquired disorders can be treated with some mitochondrial therapies for tissue damage repair are being developed, including pharmacological treatments, mesenchymal stem cell transplant and direct autologous mitochondrial injection to the tissues. Some artificial mitochondrial transfer or transplant are being developed to improve mitochondrial load into the cells in vitro, searching to increase the mitochondrial repair rate in damaged cells and tissues. Among the alternatives, MitoCeption surges as a therapeutic approach suitable to be used as therapy for acquired mitochondrial disorders. Our published work demonstrated that MitoCeption can repair mitochondrial mass loss due to UV radiation mtDNA damage *in vitro*. There is no evidence that Mitoception alters mitochondrial fusion, fission or mitophagy in UV-irradiated cells in a negative way. In consequence, MitoCeption help reset normal mitochondrial mass and mtDNA quantity and reduce the expression of cell fate markers and deleterious metabolites as p53 and ROS, reducing cell mortality and restoring cellular metabolic profile.

Acquired and inherited mitochondrial disorders are central issues in maternal subfertility, age-related diseases, some metabolic and degenerative disorders, and maternally inherited diseases as LHON, MERRF, MELAS, Leigh Syndrome and others. Farm animal models and murine models have proved the importance of mitochondrial mass and normal mtDNA quantity into the oocyte in relation with germplasm quality and embryo developmental capability. In some cases of maternal subfertility, mitochondrial transfer, and specially MitoCeption to oocytes or zygotes, could restore mitochondrial mass and quality, solving the subfertility problem. In the case of maternally inherited mitochondrial diseases, the treatment approaches for affected people include supportive treatments with mitogenic drugs, mitochondrial dietary supplementation and exercitation. Preventive approaches are limited to ooplasm micro injection and spindle or pronuclear transfer from affected mother's oocytes or

zygotes to enucleated similar cells from a healthy donor woman, creating medical and ethical concerns that impair the developing and spreading of these therapies. Mitochondrial transfer/transplant technics, and specially MitoCeption, could be used to prevent inherited mitochondrial diseases by augmenting the percentage of normal mitochondria into the female germplasm without sacrifice donor's germplasm. Our last work probed that MitoCeption successfully introduced human mitochondria in murine oocytes and zygotes, without affecting their developmental capability. We conclude that MitoCeption could be a powerful tool in the human and animal female's subfertility and human inherited mitochondrial diseases treatment and/or prevention, through the donation of healthy mitochondria to cells with genetic mitochondrial defects.

References

1. Aardema, H., van Tol, H. T. A., & Vos, P. L. A. M. (2019). An overview on how cumulus cells interact with the oocyte in a condition with elevated NEFA levels in dairy cows. *Animal Reproduction Science*, *207*, 131–137. https://doi.org/10.1016/j.anireprosci.2019.06.003
2. Aguzzi, A., & Altmeyer, M. (2016). Phase Separation: Linking Cellular Compartmentalization to Disease. *Trends in Cell Biology*, *26*(7), 547–558. https://doi.org/10.1016/j.tcb.2016.03.004
3. Ahmad, T., Mukherjee, S., Pattnaik, B., Kumar, M., Singh, S., Kumar, M., … Agrawal, A. (2014). Miro1 regulates intercellular mitochondrial transport & enhances mesenchymal stem cell rescue efficacy. *The EMBO Journal*, *30*(9), 994–1010. https://doi.org/10.1002/embj.201386030
4. Ajaz, S., Czajka, A., & Malik, A. (2015). Accurate Measurement of Circulating Mitochondrial DNA Content from Human Blood Samples Using Real-Time Quantitative PCR. In V. Weissig & M. Edeas (Eds.), *Mitochondrial Medicine: Volume I, Probing Mitochondrial Function* (pp. 117–131). https://doi.org/10.1007/978-1-4939-2257-4_12
5. Alberts, B. (2015). *Molecular biology of the cell* (Sixth edition). New York, NY: Garland Science, Taylor and Francis Group.
6. Albu, A. I., & Albu, D. (2017). The Impact of Aging on Fertility: Similarities and Differences between Ovaries and Testes. In A. M. Darwish (Ed.), *Testes and Ovaries—Functional and Clinical Differences and Similarities*. https://doi.org/10.5772/intechopen.68905
7. Almeida, M., & Porter, R. M. (2019). Sirtuins and FoxOs in osteoporosis and osteoarthritis. *Bone*, *121*, 284–292. https://doi.org/10.1016/j.bone.2019.01.018
8. Amato, P., Tachibana, M., Sparman, M., & Mitalipov, S. (2014). Three-parent in vitro fertilization: Gene replacement for the prevention of inherited mitochondrial diseases. *Fertility and Sterility*, *101*(1), 31–35. https://doi.org/10.1016/j.fertnstert.2013.11.030
9. Andreux, P. A., Houtkooper, R. H., & Auwerx, J. (2013). Pharmacological approaches to restore mitochondrial function. *Nature Reviews Drug Discovery*, *12*(6), 465–483. https://doi.org/10.1038/nrd4023
10. Anesti, V., & Scorrano, L. (2006). The relationship between mitochondrial shape and function and the cytoskeleton. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1757*(5), 692–699. https://doi.org/10.1016/j.bbabio.2006.04.013
11. Aponte, P. M., & Caicedo, A. (2017). Stemness in Cancer: Stem Cells, Cancer Stem Cells, and Their Microenvironment. *Stem Cells International*, *2017*, 1–17. https://doi.org/10.1155/2017/5619472
12. Appleby, J. B. (2015). The ethical challenges of the clinical introduction of mitochondrial replacement techniques. *Medicine, Health Care and Philosophy*, *18*(4), 501–514. https://doi.org/10.1007/s11019-015-9656-3
13. Ariño, J., Ramos, J., & Sychrova, H. (2019). Monovalent cation transporters at the plasma membrane in yeasts. *Yeast*, *36*(4), 177–193. https://doi.org/10.1002/yea.3355
14. Arranz-Gibert, P., Patel, J. R., & Isaacs, F. J. (2019). The Role of Orthogonality in Genetic Code Expansion. *Life*, *9*(3), 58. https://doi.org/10.3390/life9030058
15. Baba, M., Shinmura, A., Tada, S., Amo, T., & Tsukamoto, A. (2019). Mitochondrial Remodeling in Endothelial Cells under Cyclic Stretch Is Independent of Drp1 Activation.

Molecular & Cellular Biomechanics, *16*(1). Retrieved from http://confsubmission.org/index.php/mcb/article/view/5199
16. Babayev, E., Wang, T., Szigeti-Buck, K., Lowther, K., Taylor, H. S., Horvath, T., & Seli, E. (2016). Reproductive aging is associated with changes in oocyte mitochondrial dynamics, function, and mtDNA quantity. *Maturitas*, *93*, 121–130. https://doi.org/10.1016/j.maturitas.2016.06.015
17. Baboota, R. K., Shinde, A. B., Lemaire, K., Fransen, M., Vinckier, S., Van Veldhoven, P. P., … Baes, M. (2019). Functional peroxisomes are required for β-cell integrity in mice. *Molecular Metabolism*, *22*, 71–83. https://doi.org/10.1016/j.molmet.2019.02.001
18. Balciuniene, J., & Balciunas, D. (2019). A Nuclear mtDNA Concatemer (Mega-NUMT) Could Mimic Paternal Inheritance of Mitochondrial Genome. *Frontiers in Genetics*, *10*. https://doi.org/10.3389/fgene.2019.00518
19. Barritt, J. A., Brenner, C. A., Malter, H. E., & Cohen, J. (2001). Mitochondria in human offspring derived from ooplasmic transplantation: Brief communication. *Human Reproduction*, *16*(3), 513–516. https://doi.org/10.1093/humrep/16.3.513
20. Baselet, B., Sonveaux, P., Baatout, S., & Aerts, A. (2019). Pathological effects of ionizing radiation: Endothelial activation and dysfunction. *Cellular and Molecular Life Sciences*, *76*(4), 699–728. https://doi.org/10.1007/s00018-018-2956-z
21. Baylis, F. (2013). The ethics of creating children with three genetic parents. *Reproductive BioMedicine Online*, *26*(6), 531–534. https://doi.org/10.1016/j.rbmo.2013.03.006
22. Bell, P. J. (2019). Evidence supporting a viral origin of the eukaryotic nucleus. *BioRxiv*. https://doi.org/10.1101/679175
23. Bender, D. A., Botham, K. M., Boyle, P. J., Kennelly, P. J., Rodwell, V. W., Weil, P. A., & Weitz, M. (2018). *Harper's Illustrated Biochemistry* (31st ed.).
24. Bentov, Y., Yavorska, T., Esfandiari, N., Jurisicova, A., & Casper, R. F. (2011). The contribution of mitochondrial function to reproductive aging. *Journal of Assisted Reproduction and Genetics*, *28*(9), 773–783. https://doi.org/10.1007/s10815-011-9588-7
25. Berridge, M. V., McConnell, M. J., Grasso, C., Bajzikova, M., Kovarova, J., & Neuzil, J. (2016). Horizontal transfer of mitochondria between mammalian cells: Beyond co-culture approaches. *Current Opinion in Genetics & Development*, *38*, 75–82. https://doi.org/10.1016/j.gde.2016.04.003
26. Berridge, M. V., Schneider, R. T., & McConnell, M. J. (2016). Mitochondrial Transfer from Astrocytes to Neurons following Ischemic Insult: Guilt by Association? *Cell Metabolism*, *24*(3), 376–378. https://doi.org/10.1016/j.cmet.2016.08.023
27. Bess, A. S., Crocker, T. L., Ryde, I. T., & Meyer, J. N. (2012). Mitochondrial dynamics and autophagy aid in removal of persistent mitochondrial DNA damage in Caenorhabditis elegans. *Nucleic Acids Research*, *40*(16), 7916–7931. https://doi.org/10.1093/nar/gks532
28. Beyfuss, K., & Hood, D. A. (2018). A systematic review of p53 regulation of oxidative stress in skeletal muscle. *Redox Report : Communications in Free Radical Research*, *23*(1), 100–117. https://doi.org/10.1080/13510002.2017.1416773
29. Bianchi, S., Macchiarelli, G., Micara, G., Linari, A., Boninsegna, C., Aragona, C., … Nottola, S. A. (2015). Ultrastructural markers of quality are impaired in human metaphase II aged oocytes: A comparison between reproductive and in vitro aging. *Journal of Assisted Reproduction and Genetics*, *32*(9), 1343–1358. https://doi.org/10.1007/s10815-015-0552-9
30. Blitzer, D., Guariento, A., Doulamis, I. P., Shin, B., Moskowitzova, K., Barbieri, G. R., … McCully, J. D. (2019). Delayed Transplantation of Autologous Mitochondria for

Cardioprotection in a Porcine Model. *The Annals of Thoracic Surgery*. https://doi.org/10.1016/j.athoracsur.2019.06.075
31. Bock, R. (2017). Witnessing Genome Evolution: Experimental Reconstruction of Endosymbiotic and Horizontal Gene Transfer. *Annual Review of Genetics*, *51*(1). Retrieved from http://www.annualreviews.org/doi/abs/10.1146/annurev-genet-120215-035329
32. Boengler, K., Heusch, G., & Schulz, R. (2011). Nuclear-encoded mitochondrial proteins and their role in cardioprotection. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1813*(7), 1286–1294. https://doi.org/10.1016/j.bbamcr.2011.01.009
33. Boga, J. A., Caballero, B., Potes, Y., Perez-Martinez, Z., Reiter, R. J., Vega-Naredo, I., & Coto-Montes, A. (2019). Therapeutic potential of melatonin related to its role as an autophagy regulator: A review. *Journal of Pineal Research*, *66*(1), e12534. https://doi.org/10.1111/jpi.12534
34. Boldogh, I. R., & Pon, L. A. (2007). Mitochondria on the move. *Trends in Cell Biology*, *17*(10), 502–510. https://doi.org/10.1016/j.tcb.2007.07.008
35. Boudoures Anna. (2017). Mitochondrial Damage Accumulation in Oocytes—A Potential Link Between Maternal Obesity and Increased Cardiometabolic Disease Risk in Offspring—ProQuest. Retrieved June 20, 2018, from https://search.proquest.com/openview/d82353b3a7a7a5ab43e180fbf542ca1c/1?pq-origsite=gscholar&cbl=18750&diss=y
36. Brenner, C. A., Barritt, J. A., Willadsen, S., & Cohen, J. (2000). Mitochondrial DNA heteroplasmy after human ooplasmic transplantation. *Fertility and Sterility*, *74*(3), 573–578.
37. Cabrera, F., Ortega, M., Velarde, F., Parra, E., Gallardo, S., Barba, D., … Caicedo, A. (2019). Primary allogeneic mitochondrial mix (PAMM) transfer/transplant by MitoCeption to address damage in PBMCs caused by ultraviolet radiation. *BMC Biotechnology*, *19*(1), 42. https://doi.org/10.1186/s12896-019-0534-6
38. Cai, W. J., Chen, Y., Shi, L. X., Cheng, H. R., Banda, I., Ji, Y. H., … Zhao, S. F. (2019). AKT-GSK3β Signaling Pathway Regulates Mitochondrial Dysfunction-Associated OPA1 Cleavage Contributing to Osteoblast Apoptosis: Preventative Effects of Hydroxytyrosol [Research article]. https://doi.org/10.1155/2019/4101738
39. Caicedo, Andrés, Fritz, V., Brondello, J.-M., Ayala, M., Dennemont, I., Abdellaoui, N., … Vignais, M.-L. (2015). MitoCeption as a new tool to assess the effects of mesenchymal stem/stromal cell mitochondria on cancer cell metabolism and function. *Scientific Reports*, *5*, 9073. https://doi.org/10.1038/srep09073
40. Caicedo, Andr&#x00E9, s, Aponte, P. M., Cabrera, F., Hidalgo, C., & Khoury, M. (2017). Artificial Mitochondria Transfer: Current Challenges, Advances, and Future Applications [Research article]. https://doi.org/10.1155/2017/7610414
41. Cannon, M. V., Dunn, D. A., Irwin, M. H., Brooks, A. I., Bartol, F. F., Trounce, I. A., & Pinkert, C. A. (2011). Xenomitochondrial mice: Investigation into mitochondrial compensatory mechanisms. *Mitochondrion*, *11*(1), 33–39. https://doi.org/10.1016/j.mito.2010.07.003
42. Caramori, G., Ruggeri, P., Mumby, S., Atzeni, F., & Adcock, I. M. (2019). Transcription Factors. In *ELS* (pp. 1–8). https://doi.org/10.1002/9780470015902.a0005278.pub3
43. Čaval, T., Zhu, J., Tian, W., Remmelzwaal, S., Yang, Z., Clausen, H., & Heck, A. J. R. (2019). Targeted Analysis of Lysosomal Directed Proteins and Their Sites of Mannose-6-phosphate Modification. *Molecular & Cellular Proteomics*, *18*(1), 16–27. https://doi.org/10.1074/mcp.RA118.000967

44. Cavalier-Smith, T. (2010). Origin of the cell nucleus, mitosis and sex: Roles of intracellular coevolution. *Biology Direct*, *5*(1), 7. https://doi.org/10.1186/1745-6150-5-7
45. Chaban, Y., Boekema, E. J., & Dudkina, N. V. (2014). Structures of mitochondrial oxidative phosphorylation supercomplexes and mechanisms for their stabilisation. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1837*(4), 418–426. https://doi.org/10.1016/j.bbabio.2013.10.004
46. Chakrabarty, S., Kabekkodu, S. P., Singh, R. P., Thangaraj, K., Singh, K. K., & Satyamoorthy, K. (2018). Mitochondria in health and disease. *Mitochondrion*, *43*, 25–29. https://doi.org/10.1016/j.mito.2018.06.006
47. Chang, J.-C., Chang, H.-S., Wu, Y.-C., Cheng, W.-L., Lin, T.-T., Chang, H.-J., … Liu, C.-S. (2019). Mitochondrial transplantation regulates antitumour activity, chemoresistance and mitochondrial dynamics in breast cancer. *Journal of Experimental & Clinical Cancer Research*, *38*(1). https://doi.org/10.1186/s13046-019-1028-z
48. Chang, J.-C., Liu, K.-H., Chuang, C.-S., Su, H.-L., Wei, Y.-H., Kuo, S.-J., & Liu, C.-S. (2013). Treatment of human cells derived from MERRF syndrome by peptide-mediated mitochondrial delivery. *Cytotherapy*, *15*(12), 1580–1596. https://doi.org/10.1016/j.jcyt.2013.06.008
49. Chatterjee, S., & Yadav, S. (2019). The Origin of Prebiotic Information System in the Peptide/RNA World: A Simulation Model of the Evolution of Translation and the Genetic Code. *Life*, *9*(1), 25. https://doi.org/10.3390/life9010025
50. Chen, Z., Patwari, M., & Liu, D. (2019). Cytotoxicity of orthodontic temporary anchorage devices on human periodontal ligament fibroblasts in vitro. *Clinical and Experimental Dental Research*, *0*(0), 1–7. https://doi.org/10.1002/cre2.230
51. Chinnery, P. F. (2014). Mitochondrial Disorders Overview. In M. Adam, H. Ardinger, & R. Pagon et al. ,. (Eds.), *GeneReviews* (p. 21). Seattle (WA): University of Washington, Seattle.
52. Cho, N. H., Shaw, J. E., Karuranga, S., Huang, Y., da Rocha Fernandes, J. D., Ohlrogge, A. W., & Malanda, B. (2018). IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Research and Clinical Practice*, *138*, 271–281. https://doi.org/10.1016/j.diabres.2018.02.023
53. Chuang, Y.-C., Liou, C.-W., Chen, S.-D., Wang, P.-W., Chuang, J.-H., Tiao, M.-M., … Lin, T.-K. (2017). Mitochondrial Transfer from Wharton's Jelly Mesenchymal Stem Cell to MERRF Cybrid Reduces Oxidative Stress and Improves Mitochondrial Bioenergetics. *Oxidative Medicine and Cellular Longevity*, *2017*, 1–22. https://doi.org/10.1155/2017/5691215
54. Claiborne, A. B., English, R. A., & Kahn, J. P. (2016). Finding an ethical path forward for mitochondrial replacement. *Science*, *351*(6274), 668–670.
55. Clark, M. A., & Shay, J. W. (1982). Mitochondrial transformation of mammalian cells. *Nature*, *295*, 605.
56. Cohen, B. H., Chinnery, P. F., & Copeland, W. C. (2018). POLG-Related Disorders. In M. Adam, H. Ardinger, & R. Pagon et al. ,. (Eds.), *GeneReviews* (p. 32). Retrieved from https://www.ncbi.nlm.nih.gov/books/
57. Cohen, J. (1998). Ooplasmic transfer in mature human oocytes. *Molecular Human Reproduction*, *4*(3), 269–280. https://doi.org/10.1093/molehr/4.3.269
58. Coleman, W., & Tsongalis, G. (Eds.). (2018). *Molecular Pathology: The Molecular Basis of Human Disease* (2nd ed.). London: Academic Press.
59. Coskun, P., Wyrembak, J., Schriner, S. E., Chen, H.-W., Marciniack, C., LaFerla, F., & Wallace, D. C. (2012). A mitochondrial etiology of Alzheimer and Parkinson disease.

Biochimica et Biophysica Acta (BBA) - General Subjects, *1820*(5), 553–564. https://doi.org/10.1016/j.bbagen.2011.08.008
60. Costantini, D. (2019). Hormesis Promotes Evolutionary Change. *Dose-Response*, *17*(2), 1559325819843376. https://doi.org/10.1177/1559325819843376
61. Dacks, J. B., Field, M. C., Buick, R., Eme, L., Gribaldo, S., Roger, A. J., … Devos, D. P. (2016). The changing view of eukaryogenesis – fossils, cells, lineages and how they all come together. *Journal of Cell Science*, *129*(20), 3695–3703. https://doi.org/10.1242/jcs.178566
62. Dan Dunn, J., Alvarez, L. A., Zhang, X., & Soldati, T. (2015). Reactive oxygen species and mitochondria: A nexus of cellular homeostasis. *Redox Biology*, *6*, 472–485. https://doi.org/10.1016/j.redox.2015.09.005
63. Darbandi, S., Darbandi, M., khorshid, H. R. K., Sadeghi, M. R., Al-Hasani, S., Agarwal, A., … Akhondi, M. M. (2016). Experimental strategies towards increasing intracellular mitochondrial activity in oocytes: A systematic review. *Mitochondrion*, *30*, 8–17. https://doi.org/10.1016/j.mito.2016.05.006
64. Das, N. R., & Sharma, S. S. (2016). Cognitive Impairment Associated with Parkinson's Disease: Role of Mitochondria. *Current Neuropharmacology*, *14*(6), 584–592. https://doi.org/10.2174/1570159X14666160104142349
65. Davis, C. O., Kim, K.-Y., Bushong, E. A., Mills, E. A., Boassa, D., Shih, T., … Marsh-Armstrong, N. (2014). Transcellular degradation of axonal mitochondria. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(26), 9633–9638. https://doi.org/10.1073/pnas.1404651111
66. Davis, R. L., Liang, C., & Sue, C. M. (2018). Mitochondrial diseases. In D. Geschwind, H. Paulson, & C. Kein (Eds.), *Handbook of Clinical Neurology* (pp. 125–141). https://doi.org/10.1016/B978-0-444-63233-3.00010-5
67. Dawson, T. R., Lazarus, M. D., Hetzer, M. W., & Wente, S. R. (2009). ER membrane–bending proteins are necessary for de novo nuclear pore formation. *The Journal of Cell Biology*, *184*(5), 659–675. https://doi.org/10.1083/jcb.200806174
68. de Magalhães, J. P., & Passos, J. F. (2018). Stress, cell senescence and organismal ageing. *Mechanisms of Ageing and Development*, *170*, 2–9. https://doi.org/10.1016/j.mad.2017.07.001
69. De Mario, A., Quintana-Cabrera, R., Martinvalet, D., & Giacomello, M. (2016). (Neuro)degenerated Mitochondria-ER contacts. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1016/j.bbrc.2016.07.056
70. Delage, B., & Dashwood, R. H. (2008). Dietary Manipulation of Histone Structure and Function. *Annual Review of Nutrition*, *28*(1), 347–366. https://doi.org/10.1146/annurev.nutr.28.061807.155354
71. DiMauro, S., & Hirano, M. (2015). MERRF. In M. Adam, H. Ardinger, & R. Pagon et al. ,. (Eds.), *GeneReviews* (p. 21). Retrieved from https://www.ncbi.nlm.nih.gov/books/
72. D'Occhio, M. J., Baruselli, P. S., & Campanile, G. (2019). Metabolic health, the metabolome and reproduction in female cattle: A review. *Italian Journal of Animal Science*, *18*(1), 858–867. https://doi.org/10.1080/1828051X.2019.1600385
73. Dong, L.-F., Kovarova, J., Bajzikova, M., Bezawork-Geleta, A., Svec, D., Endaya, B., … Neuzil, J. (2017). Horizontal transfer of whole mitochondria restores tumorigenic potential in mitochondrial DNA-deficient cancer cells. *ELife*, *6*. https://doi.org/10.7554/eLife.22187
74. Dorstyn, L., Akey, C. W., & Kumar, S. (2018). New insights into apoptosome structure and function. *Cell Death & Differentiation*, 1. https://doi.org/10.1038/s41418-017-0025-z

75. Dudekula, N., Arora, V., Callaerts-Vegh, Z., & Bond, R. A. (2005). The Temporal Hormesis of Drug Therapies. *Dose-Response*, *3*(3), dose-response.003.03.009. https://doi.org/10.2203/dose-response.003.03.009
76. Duran, H. E., Simsek-Duran, F., Oehninger, S. C., Jones, H. W., & Castora, F. J. (2011). The association of reproductive senescence with mitochondrial quantity, function, and DNA integrity in human oocytes at different stages of maturation. *Fertility and Sterility*, *96*(2), 384–388. https://doi.org/10.1016/j.fertnstert.2011.05.056
77. Dyall, S. D. (2004). Ancient Invasions: From Endosymbionts to Organelles. *Science*, *304*(5668), 253–257. https://doi.org/10.1126/science.1094884
78. Elbaz, Y., & Schuldiner, M. (2011). Staying in touch: The molecular era of organelle contact sites. *Trends in Biochemical Sciences*, *36*(11), 616–623. https://doi.org/10.1016/j.tibs.2011.08.004
79. El-Hattab, A., Almannai, M., & Scaglia, F. (2018). MELAS. In M. Adam, H. Ardinger, & R. Pagon et al. ,. (Eds.), *GeneReviews* (p. 25). Retrieved from : https://www.ncbi.nlm.nih.gov/books/
80. Elliott, H. R., Samuels, D. C., Eden, J. A., Relton, C. L., & Chinnery, P. F. (2008). Pathogenic mitochondrial DNA mutations are common in the general population. *American Journal of Human Genetics*, *83*(2), 254–260. https://doi.org/10.1016/j.ajhg.2008.07.004
81. Elliott, R. L., Jiang, X. P., & Head, J. F. (2012). Mitochondria organelle transplantation: Introduction of normal epithelial mitochondria into human cancer cells inhibits proliferation and increases drug sensitivity. *Breast Cancer Research and Treatment*, *136*(2), 347–354. https://doi.org/10.1007/s10549-012-2283-2
82. Emelyanov, V. V. (2001). Evolutionary relationship of Rickettsiae and mitochondria. *FEBS Letters*, *501*(1), 11–18. https://doi.org/10.1016/S0014-5793(01)02618-7
83. Emelyanov, V. V. (2003). Mitochondrial connection to the origin of the eukaryotic cell. *European Journal of Biochemistry*, *270*(8), 1599–1618. https://doi.org/10.1046/j.1432-1033.2003.03499.x
84. Esterhuizen, K., Lindeque, J. Z., Mason, S., van der Westhuizen, F. H., Suomalainen, A., Hakonen, A. H., … Louw, R. (2019). A urinary biosignature for mitochondrial myopathy, encephalopathy, lactic acidosis and stroke like episodes (MELAS). *Mitochondrion*, *45*, 38–45. https://doi.org/10.1016/j.mito.2018.02.003
85. Fakouri, N. B., Hansen, T. L., Desler, C., Anugula, S., & Rasmussen, L. J. (2019). From Powerhouse to Perpetrator—Mitochondria in Health and Disease. *Biology*, *8*(2), 35. https://doi.org/10.3390/biology8020035
86. Falkenberg, M., Larsson, N.-G., & Gustafsson, C. M. (2007). DNA Replication and Transcription in Mammalian Mitochondria. *Annual Review of Biochemistry*, *76*(1), 679–699. https://doi.org/10.1146/annurev.biochem.76.060305.152028
87. Faustini, G., Bono, F., Valerio, A., Pizzi, M., Spano, P., & Bellucci, A. (2017). Mitochondria and α-Synuclein: Friends or Foes in the Pathogenesis of Parkinson's Disease? *Genes*, *8*(12), 377. https://doi.org/10.3390/genes8120377
88. Favaro, G., Romanello, V., Varanita, T., Andrea Desbats, M., Morbidoni, V., Tezze, C., … Sandri, M. (2019). DRP1-mediated mitochondrial shape controls calcium homeostasis and muscle mass. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-10226-9
89. Ferreira, R. M., Chiaratti, M. R., Macabelli, C. H., Rodrigues, C. A., Ferraz, M. L., Watanabe, Y. F., … Baruselli, P. S. (2016). The Infertility of Repeat-Breeder Cows During Summer Is Associated with Decreased Mitochondrial DNA and Increased Expression of Mitochondrial

and Apoptotic Genes in Oocytes. *Biology of Reproduction*, *94*(3), 66–66. https://doi.org/10.1095/biolreprod.115.133017
90. Field, M. C., & Rout, M. P. (2019). Pore timing: The evolutionary origins of the nucleus and nuclear pore complex. *F1000Research*, *8*. https://doi.org/10.12688/f1000research.16402.1
91. Finsterer, J. (2018). The Genetic Cause of Kearns-Sayre Syndrome Determines Counselling and Outcome of These Patients. *Canadian Journal of Cardiology*, *34*(9), 1234.e7. https://doi.org/10.1016/j.cjca.2018.05.027
92. Finsterer, J. (2019). Mitochondrial Ophthalmoplegia Is Not Only due to mtDNA Deletions. *Yonsei Medical Journal*, *60*(2), 230–231. https://doi.org/10.3349/ymj.2019.60.2.230
93. Forterre, P., & Gaïa, M. (2016). Giant viruses and the origin of modern eukaryotes. *Current Opinion in Microbiology*, *31*, 44–49. https://doi.org/10.1016/j.mib.2016.02.001
94. Foschi, F. G., Morelli, M. C., Savini, S., Dall'Aglio, A. C., Lanzi, A., Cescon, M., … Stefanini, G. F. (2015). Urea cycle disorders: A case report of a successful treatment with liver transplant and a literature review. *World Journal of Gastroenterology : WJG*, *21*(13), 4063–4068. https://doi.org/10.3748/wjg.v21.i13.4063
95. Franceschi, C., Garagnani, P., Parini, P., Giuliani, C., & Santoro, A. (2018). Inflammaging: A new immune–metabolic viewpoint for age-related diseases. *Nature Reviews Endocrinology*, *14*(10), 576–590. https://doi.org/10.1038/s41574-018-0059-4
96. French, C. T., Toesca, I. J., Wu, T.-H., Teslaa, T., Beaty, S. M., Wong, W., … Miller, J. F. (2011). Dissection of the Burkholderia intracellular life cycle using a photothermal nanoblade. *Proceedings of the National Academy of Sciences*, *108*(29), 12095–12100. https://doi.org/10.1073/pnas.1107183108
97. Frey, T. G, Renken, C. W., & Perkins, G. A. (2002). Insight into mitochondrial structure and function from electron tomography. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1555*(1–3), 196–203. https://doi.org/10.1016/S0005-2728(02)00278-5
98. Frey, Terrence G., & Mannella, C. A. (2000). The internal structure of mitochondria. *Trends in Biochemical Sciences*, *25*(7), 319–324.
99. Fujimaki, S., & Kuwabara, T. (2017). Diabetes-Induced Dysfunction of Mitochondria and Stem Cells in Skeletal Muscle and the Nervous System. *International Journal of Molecular Sciences*, *18*(10). https://doi.org/10.3390/ijms18102147
100. Gammage, P. A., Moraes, C. T., & Minczuk, M. (2018). Mitochondrial Genome Engineering: The Revolution May Not Be CRISPR-Ized. *Trends in Genetics*, *34*(2), 101–110. https://doi.org/10.1016/j.tig.2017.11.001
101. Gartner, L. P. (2017). *Textbook of Histology* (4th ed., Vols. 1–1). Philadelphia: Elsevier.
102. Gattermann, N., Dadak, M., Hofhaus, G., Wulfert, M., Berneburg, M., Loeffler, M. L., & Simmonds, H. A. (2004). Severe Impairment of Nucleotide Synthesis Through Inhibition of Mitochondrial Respiration. *Nucleosides, Nucleotides and Nucleic Acids*, *23*(8–9), 1275–1279. https://doi.org/10.1081/NCN-200027545
103. Gerdes, H.-H., Bukoreshtliev, N. V., & Barroso, J. F. V. (2007). Tunneling nanotubes: A new route for the exchange of components between animal cells. *FEBS Letters*, *581*(11), 2194–2201. https://doi.org/10.1016/j.febslet.2007.03.071
104. Giorgi, C., Marchi, S., Simoes, I. C. M., Ren, Z., Morciano, G., Perrone, M., … Wieckowski, M. R. (2018). Mitochondria and Reactive Oxygen Species in Aging and Age-Related Diseases. *International Review of Cell and Molecular Biology*, *340*, 209–344. https://doi.org/10.1016/bs.ircmb.2018.05.006

105. Goldstein, A., & Falk, M. J. (2019). Mitochondrial DNA Deletion Syndromes. In M. Adam, H. Ardinger, & R. Pagon et al. ,. (Eds.), *GeneReviews* (p. 21). Retrieved from https://www.ncbi.nlm.nih.gov/books/
106. Gómez, L. J. M.-, & Khan, T. H. (2019). Hypothermia in trauma. *Anaesthesia, Pain & Intensive Care*, 232–240.
107. Gorman, G. S., Chinnery, P. F., DiMauro, S., Hirano, M., Koga, Y., McFarland, R., … Turnbull, D. M. (2016). Mitochondrial diseases. *Nature Reviews Disease Primers*, *2*, 16080. https://doi.org/10.1038/nrdp.2016.80
108. Gorman, G. S., Schaefer, A. M., Ng, Y., Gomez, N., Blakely, E. L., Alston, C. L., … McFarland, R. (2015). Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease. *Annals of Neurology*, *77*(5), 753–759. https://doi.org/10.1002/ana.24362
109. Greco, E. A., Pietschmann, P., & Migliaccio, S. (2019). Osteoporosis and Sarcopenia Increase Frailty Syndrome in the Elderly. *Frontiers in Endocrinology*, *10*. https://doi.org/10.3389/fendo.2019.00255
110. Gross, A. (2016). BCL-2 family proteins as regulators of mitochondria metabolism. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1857*(8), 1243–1246. https://doi.org/10.1016/j.bbabio.2016.01.017
111. Guardia, C. M., Pace, R. D., Sen, A., Saric, A., Jarnik, M., Kolin, D. A., … Bonifacino, J. S. (2019). Reversible association with motor proteins (RAMP): A streptavidin-based method to manipulate organelle positioning. *PLOS Biology*, *17*(5), e3000279. https://doi.org/10.1371/journal.pbio.3000279
112. Guengerich, F. P., Wilkey, C. J., & Phan, T. T. N. (2019). Human cytochrome P450 enzymes bind drugs and other substrates mainly through conformational-selection modes. *Journal of Biological Chemistry*, jbc.RA119.009305. https://doi.org/10.1074/jbc.RA119.009305
113. Guerra, F., Arbini, A. A., & Moro, L. (2017). Mitochondria and cancer chemoresistance. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*. https://doi.org/10.1016/j.bbabio.2017.01.012
114. Guillet-Pichon, V., & Verny, C. (2016). Mitochondrie et maladies neurodégénératives. *Pratique Neurologique - FMC*, *7*(2), 117–122. https://doi.org/10.1016/j.praneu.2016.01.024
115. Guy, J., & Yuan, H. (2019). REVERSAL OF PARALYSIS AND PREVENTION OF PREMATURE DEATH IN A MOUSE GERMLINE MODEL OF LEIGH SYNDROME CAUSED BY A MUTATION IN MITOCHONDRIAL ATP SYNTHASE (S51.003). *Neurology*, *92*(15 Supplement), S51.003.
116. Hammond, E. R., Green, M. P., Shelling, A. N., Berg, M. C., Peek, J. C., & Cree, L. M. (2016). Oocyte mitochondrial deletions and heteroplasmy in a bovine model of ageing and ovarian stimulation. *Molecular Human Reproduction*, *22*(4), 261–271. https://doi.org/10.1093/molehr/gaw003
117. Hampoelz, B., Andres-Pons, A., Kastritis, P., & Beck, M. (2019). Structure and Assembly of the Nuclear Pore Complex. *Annual Review of Biophysics*, *48*(1), 515–536. https://doi.org/10.1146/annurev-biophys-052118-115308
118. Hart, P. H., & Norval, M. (2017). Ultraviolet radiation-induced immunosuppression and its relevance for skin carcinogenesis. *Photochemical & Photobiological Sciences*. https://doi.org/10.1039/C7PP00312A

119. Hattori, N., Saiki, S., & Imai, Y. (2014). Regulation by mitophagy. *The International Journal of Biochemistry & Cell Biology*, *53*, 147–150. https://doi.org/10.1016/j.biocel.2014.05.012
120. Hayakawa, K., Esposito, E., Wang, X., Terasaki, Y., Liu, Y., Xing, C., … Lo, E. H. (2016). Transfer of mitochondria from astrocytes to neurons after stroke. *Nature*, *535*(7613), 551–555. https://doi.org/10.1038/nature18928
121. Hennig, B., Toborek, M., McClain, C. J., & Diana, J. N. (1996). Nutritional implications in vascular endothelial cell metabolism. *Journal of the American College of Nutrition*, *15*(4), 345–358. https://doi.org/10.1080/07315724.1996.10718609
122. Herbers, E., Kekäläinen, N. J., Hangas, A., Pohjoismäki, J. L., & Goffart, S. (2019). Tissue specific differences in mitochondrial DNA maintenance and expression. *Mitochondrion*, *44*, 85–92. https://doi.org/10.1016/j.mito.2018.01.004
123. Herbert, M., & Turnbull, D. (2018). Progress in mitochondrial replacement therapies. *Nature Reviews Molecular Cell Biology*, *19*, 71–72. https://doi.org/10.1038/nrm.2018.3
124. Hirano, M., Emmanuele, V., & Quinzii, C. M. (2018). Emerging therapies for mitochondrial diseases. *Essays In Biochemistry*, EBC20170114. https://doi.org/10.1042/EBC20170114
125. Ho, R., & Hegele, R. A. (2019). Complex effects of laminopathy mutations on nuclear structure and function. *Clinical Genetics*, *95*(2), 199–209. https://doi.org/10.1111/cge.13455
126. Hood, D. A., Memme, J. M., Oliveira, A. N., & Triolo, M. (2019). Maintenance of Skeletal Muscle Mitochondria in Health, Exercise, and Aging. *Annual Review of Physiology*, *81*(1), 19–41. https://doi.org/10.1146/annurev-physiol-020518-114310
127. Houten, S. M., Violante, S., Ventura, F. V., & Wanders, R. J. A. (2016). The Biochemistry and Physiology of Mitochondrial Fatty Acid β-Oxidation and Its Genetic Disorders. *Annual Review of Physiology*, *78*(1), 23–44. https://doi.org/10.1146/annurev-physiol-021115-105045
128. Hsu, Y.-C., Wu, Y.-T., Yu, T.-H., & Wei, Y.-H. (2016). Mitochondria in mesenchymal stem cell biology and cell therapy: From cellular differentiation to mitochondrial transfer. *Seminars in Cell & Developmental Biology*, *52*, 119–131. https://doi.org/10.1016/j.semcdb.2016.02.011
129. Huang, P.-J., Kuo, C.-C., Lee, H.-C., Shen, C.-I., Cheng, F.-C., Wu, S.-F., … Su, H.-L. (2016). Transferring Xenogenic Mitochondria Provides Neural Protection Against Ischemic Stress in Ischemic Rat Brains. *Cell Transplantation*, *25*(5), 913–927. https://doi.org/10.3727/096368915X689785
130. Hubbard, W. B., Harwood, C. L., Prajapati, P., Springer, J. E., Saatman, K. E., & Sullivan, P. G. (2019). Fractionated mitochondrial magnetic separation for isolation of synaptic mitochondria from brain tissue. *Scientific Reports*, *9*(1), 1–13. https://doi.org/10.1038/s41598-019-45568-3
131. Hyslop, L. A., Blakeley, P., Craven, L., Richardson, J., Fogarty, N. M. E., Fragouli, E., … Herbert, M. (2016). Towards clinical application of pronuclear transfer to prevent mitochondrial DNA disease. *Nature*, *534*(7607), 383–386. https://doi.org/10.1038/nature18303
132. Hyttel, P. (Ed.). (2010). *Essentials of domestic animal embryology*. Edinburgh ; New York: Saunders/Elsevier.
133. Igarashi, H., Takahashi, E., Hiroi, M., & Doi, K. (1997). Aging-related changes in calcium oscillations in fertilized mouse oocytes. *Molecular Reproduction and Development*, *48*(3),

383–390. https://doi.org/10.1002/(SICI)1098-2795(199711)48:3<383::AID-MRD12>3.0.CO;2-X

134. Igarashi, H., Takahashi, T., Abe, H., Nakano, H., Nakajima, O., & Nagase, S. (2016). Poor embryo development in post-ovulatory in vivo-aged mouse oocytes is associated with mitochondrial dysfunction, but mitochondrial transfer from somatic cells is not sufficient for rejuvenation. *Human Reproduction*, *31*(10), 2331–2338. https://doi.org/10.1093/humrep/dew203

135. Imanaka, T. (2019). Biogenesis and Function of Peroxisomes in Human Disease with a Focus on the ABC Transporter. *Biological and Pharmaceutical Bulletin*, *42*(5), 649–665. https://doi.org/10.1248/bpb.b18-00723

136. Ingraham, C. (2003). Developmental fate of mitochondria microinjected into murine zygotes. *Mitochondrion*, *3*(1), 39–46. https://doi.org/10.1016/S1567-7249(03)00075-8

137. International Human Genome Sequencing Consortium. (2004). Finishing the euchromatic sequence of the human genome. *Nature*, *431*(7011), 931–945. https://doi.org/10.1038/nature03001

138. Ishii, T., & Hibino, Y. (2018). Mitochondrial manipulation in fertility clinics: Regulation and responsibility. *Reproductive Biomedicine & Society Online*, *5*, 93–109. https://doi.org/10.1016/j.rbms.2018.01.002

139. Islam, M. N., Das, S. R., Emin, M. T., Wei, M., Sun, L., Westphalen, K., … Bhattacharya, J. (2012a). Mitochondrial transfer from bone-marrow–derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nature Medicine*, *18*(5), 759–765. https://doi.org/10.1038/nm.2736

140. Islam, M. N., Das, S. R., Emin, M. T., Wei, M., Sun, L., Westphalen, K., … Bhattacharya, J. (2012b). Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nature Medicine*, *18*(5), 759–765. https://doi.org/10.1038/nm.2736

141. Ivanovic, Z. (2009). Hypoxia or in situ normoxia: The stem cell paradigm. *Journal of Cellular Physiology*, *219*(2), 271–275. https://doi.org/10.1002/jcp.21690

142. Iwata, H., Goto, H., Tanaka, H., Sakaguchi, Y., Kimura, K., Kuwayama, T., & Monji, Y. (2011). Effect of maternal age on mitochondrial DNA copy number, ATP content and IVF outcome of bovine oocytes. *Reproduction, Fertility and Development*, *23*(3), 424–432. https://doi.org/10.1071/RD10133

143. Jackson, M. V., Morrison, T. J., Doherty, D. F., McAuley, D. F., Matthay, M. A., Kissenpfennig, A., … Krasnodembskaya, A. D. (2016). Mitochondrial Transfer via Tunneling Nanotubes is an Important Mechanism by Which Mesenchymal Stem Cells Enhance Macrophage Phagocytosis in the In Vitro and In Vivo Models of ARDS: Mitochondrial Transfer from MSC to Macrophages. *STEM CELLS*, *34*(8), 2210–2223. https://doi.org/10.1002/stem.2372

144. Jackson Megan V., Morrison Thomas J., Doherty Declan F., McAuley Daniel F., Matthay Michael A., Kissenpfennig Adrien, … Krasnodembskaya Anna D. (2016). Mitochondrial Transfer via Tunneling Nanotubes is an Important Mechanism by Which Mesenchymal Stem Cells Enhance Macrophage Phagocytosis in the In Vitro and In Vivo Models of ARDS. *STEM CELLS*, *34*(8), 2210–2223. https://doi.org/10.1002/stem.2372

145. Jacobs, L. J. a. M., Jongbloed, R. J. E., Wijburg, F. A., Klerk, J. B. C. de, Geraedts, J. P. M., Nijland, J. G., … Smeets, H. J. M. (2004). Pearson syndrome and the role of deletion dimers and duplications in the mtDNA. *Journal of Inherited Metabolic Disease*, *27*(1), 47–55. https://doi.org/10.1023/B:BOLI.0000016601.49372.18

146. Jacobson, K., Liu, P., & Lagerholm, B. C. (2019). The Lateral Organization and Mobility of Plasma Membrane Components. *Cell*, *177*(4), 806–819. https://doi.org/10.1016/j.cell.2019.04.018
147. Ji, S.-L., & Tang, S.-B. (2019). Differentiation of retinal ganglion cells from induced pluripotent stem cells: A review. *International Journal of Ophthalmology*, *12*(1), 152–160. https://doi.org/10.18240/ijo.2019.01.22
148. Jopling, C., Boue, S., & Belmonte, J. C. I. (2011). Dedifferentiation, transdifferentiation and reprogramming: Three routes to regeneration. *Nature Reviews Molecular Cell Biology*, *12*(2), 79–89. https://doi.org/10.1038/nrm3043
149. Jorgensen, C. (2019). Role of Tunneling Nanotubules in the Cross-Talk Between Mesenchymal Stem Cells and Their Target Cells. *Current Stem Cell Reports*, *5*(2), 53–56. https://doi.org/10.1007/s40778-019-00154-1
150. Jose, S. S., Bendickova, K., Kepak, T., Krenova, Z., & Fric, J. (2017). Chronic Inflammation in Immune Aging: Role of Pattern Recognition Receptor Crosstalk with the Telomere Complex? *Frontiers in Immunology*, *8*. https://doi.org/10.3389/fimmu.2017.01078
151. Jurkute, N., Harvey, J., & Yu-Wai-Man, P. (2019). Treatment strategies for Leber hereditary optic neuropathy. *Current Opinion in Neurology*, *32*(1), 99. https://doi.org/10.1097/WCO.0000000000000646
152. Kannan, S., Rogozin, I. B., & Koonin, E. V. (2014). MitoCOGs: Clusters of orthologous genes from mitochondria and implications for the evolution of eukaryotes. *BMC Evolutionary Biology*, *14*, 237. https://doi.org/10.1186/s12862-014-0237-5
153. Kansaku, K., Takeo, S., Itami, N., Kin, A., Shirasuna, K., Kuwayama, T., & Iwata, H. (2017). Maternal aging affects oocyte resilience to carbonyl cyanide-m-chlorophenylhydrazone -induced mitochondrial dysfunction in cows. *PLOS ONE*, *12*(11), e0188099. https://doi.org/10.1371/journal.pone.0188099
154. Karlberg, O., Canbäck, B., Kurland, C. G., & Andersson, S. G. E. (2000). The dual origin of the yeast mitochondrial proteome. *Yeast*, *17*(3), 170–187. https://doi.org/10.1002/1097-0061(20000930)17:3<170::AID-YEA25>3.0.CO;2-V
155. Keogh, M. J., & Chinnery, P. F. (2015). Mitochondrial DNA mutations in neurodegeneration. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1847*(11), 1401–1411. https://doi.org/10.1016/j.bbabio.2015.05.015
156. Kesner, E. E., Saada-Reich, A., & Lorberboum-Galski, H. (2016). Characteristics of Mitochondrial Transformation into Human Cells. *Scientific Reports*, *6*, 26057. https://doi.org/10.1038/srep26057
157. Khan, S., Roberts, J., & Wu, S.-B. (2019). Genes involved in mitochondrial biogenesis and function may not show synchronised responses to mitochondria in shell gland of laying chickens under infectious bronchitis virus challenge. *BMC Molecular and Cell Biology*, *20*(1), 3. https://doi.org/10.1186/s12860-019-0190-7
158. Kim, K. H., Son, J. M., Benayoun, B. A., & Lee, C. (2018). The Mitochondrial-Encoded Peptide MOTS-c Translocates to the Nucleus to Regulate Nuclear Gene Expression in Response to Metabolic Stress. *Cell Metabolism*, *28*(3), 516-524.e7. https://doi.org/10.1016/j.cmet.2018.06.008
159. Kim, M. J., Hwang, J. W., Yun, C.-K., Lee, Y., & Choi, Y.-S. (2018). Delivery of exogenous mitochondria via centrifugation enhances cellular metabolic function. *Scientific Reports*, *8*(1), 3330. https://doi.org/10.1038/s41598-018-21539-y

160. Kitani, T., Kami, D., Kawasaki, T., Nakata, M., Matoba, S., & Gojo, S. (2014). Direct Human Mitochondrial Transfer: A Novel Concept Based on the Endosymbiotic Theory. *Transplantation Proceedings*, *46*(4), 1233–1236. https://doi.org/10.1016/j.transproceed.2013.11.133
161. Kitani, Tomoya, Kami, D., Matoba, S., & Gojo, S. (2014). Internalization of isolated functional mitochondria: Involvement of macropinocytosis. *Journal of Cellular and Molecular Medicine*, *18*(8), 1694–1703. https://doi.org/10.1111/jcmm.12316
162. Klionsky, D. J., Abdelmohsen, K., Abe, A., Abedin, M. J., Abeliovich, H., Arozena, A. A., … Zughaier, S. M. (2016). Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). *Autophagy*, *12*(1), 1–222. https://doi.org/10.1080/15548627.2015.1100356
163. Kujjo, L. L., Acton, B. M., Perkins, G. A., Ellisman, M. H., D'Estaing, S. G., Casper, R. F., … Perez, G. I. (2013). Ceramide and its transport protein (CERT) contribute to deterioration of mitochondrial structure and function in aging oocytes. *Mechanisms of Ageing and Development*, *134*(1–2), 43–52. https://doi.org/10.1016/j.mad.2012.12.001
164. Kushnir, V. A., Ludaway, T., Russ, R. B., Fields, E. J., Koczor, C., & Lewis, W. (2012). Reproductive aging is associated with decreased mitochondrial abundance and altered structure in murine oocytes. *Journal of Assisted Reproduction and Genetics*, *29*(7), 637–642. https://doi.org/10.1007/s10815-012-9771-5
165. Labarta, E., de los Santos, M. J., Escribá, M. J., Pellicer, A., & Herraiz, S. (2019). Mitochondria as a tool for oocyte rejuvenation. *Fertility and Sterility*, *111*(2), 219–226. https://doi.org/10.1016/j.fertnstert.2018.10.036
166. Lahiri, S., Toulmay, A., & Prinz, W. A. (2015). Membrane contact sites, gateways for lipid homeostasis. *Current Opinion in Cell Biology*, *33*, 82–87. https://doi.org/10.1016/j.ceb.2014.12.004
167. Lai, N., Kummitha, C., & Hoppel, C. (2017). Defects in skeletal muscle subsarcolemmal mitochondria in a non-obese model of type 2 diabetes mellitus. *PLoS ONE*, *12*(8). https://doi.org/10.1371/journal.pone.0183978
168. Landry, D. A., Bellefleur, A.-M., Labrecque, R., Grand, F.-X., Vigneault, C., Blondin, P., & Sirard, M.-A. (2016). Effect of cow age on the in vitro developmental competence of oocytes obtained after FSH stimulation and coasting treatments. *Theriogenology*, *86*(5), 1240–1246. https://doi.org/10.1016/j.theriogenology.2016.04.064
169. Lane, R. K., Hilsabeck, T., & Rea, S. L. (2015). The role of mitochondrial dysfunction in age-related diseases. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1847*(11), 1387–1400. https://doi.org/10.1016/j.bbabio.2015.05.021
170. Latorre-Pellicer, A., Moreno-Loshuertos, R., Lechuga-Vieco, A. V., Sánchez-Cabo, F., Torroja, C., Acín-Pérez, R., … Enríquez, J. A. (2016). Mitochondrial and nuclear DNA matching shapes metabolism and healthy ageing. *Nature*, *535*(7613), 561–565. https://doi.org/10.1038/nature18618
171. Lee, D. H., Desai, M. J., & Gauger, E. M. (2018). Electrical Injuries of the Hand and Upper Extremity: *Journal of the American Academy of Orthopaedic Surgeons*, 1. https://doi.org/10.5435/JAAOS-D-17-00833
172. Lee, J., Zhuang, Y., Wei, X., Shang, F., Wang, J., Zhang, Y., … Zheng, Q. (2009). Contributions of PD-1/PD-L1 pathway to interactions of myeloid DCs with T cells in atherosclerosis. *Journal of Molecular and Cellular Cardiology*, *46*(2), 169–176. https://doi.org/10.1016/j.yjmcc.2008.10.028

173. Levinger, L., Oestreich, I., Florentz, C., & Mörl, M. (2004). A Pathogenesis-associated Mutation in Human Mitochondrial tRNALeu(UUR) Leads to Reduced 3'-End Processing and CCA Addition. *Journal of Molecular Biology*, *337*(3), 535–544. https://doi.org/10.1016/j.jmb.2004.02.008
174. Li, F., Castora, F. J., Ford, W., Alarid, K., Jones, H. W., & Swanson, R. J. (2017). Reproductive competency and mitochondrial variation in aged Syrian hamster oocytes. *Reproduction, Fertility and Development*, *29*(7), 1384–1391. https://doi.org/10.1071/RD15404
175. Li, H., Wang, C., He, T., Zhao, T., Chen, Y., Shen, Y., … Wang, L. (2019). Mitochondrial Transfer from Bone Marrow Mesenchymal Stem Cells to Motor Neurons in Spinal Cord Injury Rats via Gap Junction. *Theranostics*, *9*(7), 2017–2035. https://doi.org/10.7150/thno.29400
176. Li, X., Zhang, Y., Yeung, S. C., Liang, Y., Liang, X., Ding, Y., … Lian, Q. (2014a). Mitochondrial Transfer of Induced Pluripotent Stem Cell–Derived Mesenchymal Stem Cells to Airway Epithelial Cells Attenuates Cigarette Smoke–Induced Damage. *American Journal of Respiratory Cell and Molecular Biology*, *51*(3), 455–465. https://doi.org/10.1165/rcmb.2013-0529OC
177. Li, X., Zhang, Y., Yeung, S. C., Liang, Y., Liang, X., Ding, Y., … Lian, Q. (2014b). Mitochondrial Transfer of Induced Pluripotent Stem Cell–Derived Mesenchymal Stem Cells to Airway Epithelial Cells Attenuates Cigarette Smoke–Induced Damage. *American Journal of Respiratory Cell and Molecular Biology*, *51*(3), 455–465. https://doi.org/10.1165/rcmb.2013-0529OC
178. Liberti, M. V., & Locasale, J. W. (2016). The Warburg Effect: How Does it Benefit Cancer Cells? *Trends in Biochemical Sciences*, *41*(3), 211–218. https://doi.org/10.1016/j.tibs.2015.12.001
179. Lionaki, E., Markaki, M., Palikaras, K., & Tavernarakis, N. (2015). Mitochondria, autophagy and age-associated neurodegenerative diseases: New insights into a complex interplay. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1847*(11), 1412–1423. https://doi.org/10.1016/j.bbabio.2015.04.010
180. Liu, F., Yang, H., Lu, K., & Qiu, Y. (2008). Effect of Mitochondrial Transplantation from Cumulus Granular Cells to the Early Embryos of Aged Mice. *Journal of Reproduction and Contraception*, *19*(4), 211–218. https://doi.org/10.1016/S1001-7844(08)60023-1
181. Lopez-Mejia, I. C., & Fajas, L. (2015). Cell cycle regulation of mitochondrial function. *Current Opinion in Cell Biology*, *33*, 19–25. https://doi.org/10.1016/j.ceb.2014.10.006
182. Luo, S., Valencia, C. A., Zhang, J., Lee, N.-C., Slone, J., Gui, B., … Huang, T. (2018). Biparental Inheritance of Mitochondrial DNA in Humans. *Proceedings of the National Academy of Sciences*, *115*(51), 13039–13044. https://doi.org/10.1073/pnas.1810946115
183. Luz-Crawford, P., Hernandez, J., Djouad, F., Luque-Campos, N., Caicedo, A., Carrère-Kremer, S., … Jorgensen, C. (2019a). Mesenchymal stem cell repression of Th17 cells is triggered by mitochondrial transfer. *Stem Cell Research & Therapy*, *10*(1), 232. https://doi.org/10.1186/s13287-019-1307-9
184. Luz-Crawford, P., Hernandez, J., Djouad, F., Luque-Campos, N., Caicedo, A., Carrère-Kremer, S., … Jorgensen, C. (2019b). Mesenchymal stem cell repression of Th17 cells is triggered by mitochondrial transfer. *Stem Cell Research & Therapy*, *10*(1), 232. https://doi.org/10.1186/s13287-019-1307-9

185. Ma, Y., He, K., & Berkowitz, G. A. (2019). Editorial: From Structure to Signalsomes: New Perspectives About Membrane Receptors and Channels. *Frontiers in Plant Science*, *10*. https://doi.org/10.3389/fpls.2019.00682
186. Macheiner, T., Fengler, V. H. I., Agreiter, M., Eisenberg, T., Madeo, F., Kolb, D., … Sargsyan, K. (2016). Magnetomitotransfer: An efficient way for direct mitochondria transfer into cultured human cells. *Scientific Reports*, *6*, srep35571. https://doi.org/10.1038/srep35571
187. Malik, A. N., Czajka, A., & Cunningham, P. (2016). Accurate quantification of mouse mitochondrial DNA without co-amplification of nuclear mitochondrial insertion sequences. *Mitochondrion*, *29*, 59–64. https://doi.org/10.1016/j.mito.2016.05.003
188. Malik, A. N., Shahni, R., Rodriguez-de-Ledesma, A., Laftah, A., & Cunningham, P. (2011). Mitochondrial DNA as a non-invasive biomarker: Accurate quantification using real time quantitative PCR without co-amplification of pseudogenes and dilution bias. *Biochemical and Biophysical Research Communications*, *412*(1), 1–7. https://doi.org/10.1016/j.bbrc.2011.06.067
189. Manoli, I., Alesci, S., Blackman, M. R., Su, Y. A., Rennert, O. M., & Chrousos, G. P. (2007). Mitochondria as key components of the stress response. *Trends in Endocrinology & Metabolism*, *18*(5), 190–198. https://doi.org/10.1016/j.tem.2007.04.004
190. Marei, W. F. A., Raemdonck, G. V., Baggerman, G., Bols, P. E. J., & Leroy, J. L. M. R. (2019). 166 Mitochondrial stress responses in bovine cumulus cells and oocytes matured under lipotoxic conditions: A proteomic insight. *Reproduction, Fertility and Development*, *31*(1), 208–208. https://doi.org/10.1071/RDv31n1Ab166
191. Margineantu, D. H., Gregory Cox, W., Sundell, L., Sherwood, S. W., Beechem, J. M., & Capaldi, R. A. (2002). Cell cycle dependent morphology changes and associated mitochondrial DNA redistribution in mitochondria of human cell lines. *Mitochondrion*, *1*(5), 425–435. https://doi.org/10.1016/S1567-7249(02)00006-5
192. Martin, S. D., & McGee, S. L. (2014). The role of mitochondria in the aetiology of insulin resistance and type 2 diabetes. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1840*(4), 1303–1312. https://doi.org/10.1016/j.bbagen.2013.09.019
193. Martins, I. (2018). Anti-Aging Gene linked to Appetite Regulation Determines Longevity in Humans and Animals. *International Journal of Aging Research*, *1*(6). https://doi.org/10.28933/ijoar-2018-05-1101
194. Martinvalet, D. (2019). Mitochondrial Entry of Cytotoxic Proteases: A New Insight into the Granzyme B Cell Death Pathway [Research article]. https://doi.org/10.1155/2019/9165214
195. Mason, K. A., Losos, J. B., Singer, S. R., & Raven, P. H. (2017). *Biology* (Eleventh edition). New York, NY: McGraw-Hill Education.
196. Masuzawa, A., Black, K. M., Pacak, C. A., Ericsson, M., Barnett, R. J., Drumm, C., … McCully, J. D. (2013). Transplantation of autologously derived mitochondria protects the heart from ischemia-reperfusion injury. *AJP: Heart and Circulatory Physiology*, *304*(7), H966–H982. https://doi.org/10.1152/ajpheart.00883.2012
197. McCance, K., & Huether, S. (Eds.). (2019). *Pathophysiology- THE BIOLOGIC BASIS FOR DISEASE IN ADULTS AND CHILDREN* (8th ed.). St. Louis, Missouri: Elsevier.
198. McCully, J. D., Cowan, D. B., Pacak, C. A., Toumpoulis, I. K., Dayalan, H., & Levitsky, S. (2009). Injection of isolated mitochondria during early reperfusion for cardioprotection. *AJP: Heart and Circulatory Physiology*, *296*(1), H94–H105. https://doi.org/10.1152/ajpheart.00567.2008

199. McCully, James D., Levitsky, S., del Nido, P. J., & Cowan, D. B. (2016). Mitochondrial transplantation for therapeutic use. *Clinical and Translational Medicine*, *5*(1). https://doi.org/10.1186/s40169-016-0095-4
200. McGuire, P. J. (2019). Mitochondrial Dysfunction and the Aging Immune System. *Biology*, *8*(2), 26. https://doi.org/10.3390/biology8020026
201. McWilliams, T. G., & Suomalainen, A. (2019). Mitochondrial DNA can be inherited from fathers, not just mothers. *Nature*, *565*(7739), 296–297. https://doi.org/10.1038/d41586-019-00093-1
202. Meseguer, S., Navarro-González, C., Panadero, J., Villarroya, M., Boutoual, R., Sánchez-Alcázar, J. A., & Armengod, M.-E. (2019). The MELAS mutation m.3243A>G alters the expression of mitochondrial tRNA fragments. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1866*(9), 1433–1449. https://doi.org/10.1016/j.bbamcr.2019.06.004
203. Miliotis, S., Nicolalde, B., Ortega, M., Yepez, J., & Caicedo, A. (2019). Forms of extracellular mitochondria and their impact in health. *Mitochondrion*. https://doi.org/10.1016/j.mito.2019.02.002
204. Mishra, P., & Chan, D. C. (2014). Mitochondrial dynamics and inheritance during cell division, development and disease. *Nature Reviews Molecular Cell Biology*, *15*(10), 634–646. https://doi.org/10.1038/nrm3877
205. Mohammadzadeh, M., Fesahat, F., Khoradmehr, A., & Khalili, M. A. (2018). Influential effect of age on oocyte morphometry, fertilization rate and embryo development following IVF in mice. *Middle East Fertility Society Journal*, *23*(2), 117–120. https://doi.org/10.1016/j.mefs.2017.09.006
206. Mombo, B. N., Gerbal-Chaloin, S., Bokus, A., Daujat-Chavanieu, M., Jorgensen, C., Hugnot, J.-P., & Vignais, M.-L. (2017). MitoCeption: Transferring Isolated Human MSC Mitochondria to Glioblastoma Stem Cells. *JoVE (Journal of Visualized Experiments)*, (120), e55245. https://doi.org/10.3791/55245
207. Mondal, T., Rasmussen, M., Pandey, G. K., Isaksson, A., & Kanduri, C. (2010). Characterization of the RNA content of chromatin. *Genome Research*, *20*(7), 899–907. https://doi.org/10.1101/gr.103473.109
208. Montano, V., Simoncini, C., Calì, C. L., Legati, A., Siciliano, G., & Mancuso, M. (2019). CPEO and Mitochondrial Myopathy in a Patient with DGUOK Compound Heterozygous Pathogenetic Variant and mtDNA Multiple Deletions [Research article]. https://doi.org/10.1155/2019/5918632
209. Morrison, T. J., Jackson, M. V., Cunningham, E. K., Kissenpfennig, A., McAuley, D. F., O'Kane, C. M., & Krasnodembskaya, A. D. (2017). Mesenchymal Stromal Cells Modulate Macrophages in Clinically Relevant Lung Injury Models by Extracellular Vesicle Mitochondrial Transfer. *American Journal of Respiratory and Critical Care Medicine*, *196*(10), 1275–1286. https://doi.org/10.1164/rccm.201701-0170OC
210. Moschoi, R., Imbert, V., Nebout, M., Chiche, J., Mary, D., Prebet, T., … Griessinger, E. (2016). Protective mitochondrial transfer from bone marrow stromal cells to acute myeloid leukemic cells during chemotherapy. *Blood*, *128*(2), 253–264. https://doi.org/10.1182/blood-2015-07-655860
211. Moskowitzova, K., Shin, B., Liu, K., Ramirez-Barbieri, G., Guariento, A., Blitzer, D., … McCully, J. D. (2019). Mitochondrial transplantation prolongs cold ischemia time in murine heart transplantation. *The Journal of Heart and Lung Transplantation*, *38*(1), 92–99. https://doi.org/10.1016/j.healun.2018.09.025

212. Müllebner, A., Dorighello, G. G., Kozlov, A. V., & Duvigneau, J. C. (2018). Interaction between Mitochondrial Reactive Oxygen Species, Heme Oxygenase, and Nitric Oxide Synthase Stimulates Phagocytosis in Macrophages. *Frontiers in Medicine*, *4*. https://doi.org/10.3389/fmed.2017.00252
213. Munir, T., Mahmood, A., Fakhar-e-Alam, M., Imran, M., Sohail, A., Amin, N., … Mahmood, K. (2019). Treatment of breast cancer with capped magnetic-NPs induced hyperthermia therapy. *Journal of Molecular Structure*, *1196*, 88–95. https://doi.org/10.1016/j.molstruc.2019.06.067
214. Murley, A., & Nunnari, J. (2016). The Emerging Network of Mitochondria-Organelle Contacts. *Molecular Cell*, *61*(5), 648–653. https://doi.org/10.1016/j.molcel.2016.01.031
215. Mykytenko, D., Mazur, P., Dyachenko, L., Borisov, M., Masliy, Y., Lamtyeva, V., … Zukin, V. (2019). 55. EFFICACY OF NUCLEAR TRANSFER TO PREVENT THE INHERITED MITOCHONDRIAL PATHOLOGY. *Reproductive BioMedicine Online*, *39*, e60. https://doi.org/10.1016/j.rbmo.2019.04.108
216. Naia, L., Ferreira, I. L., Ferreiro, E., & Rego, A. C. (2016). Mitochondrial Ca2+ handling in Huntington's and Alzheimer's diseases – Role of ER-mitochondria crosstalk. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1016/j.bbrc.2016.07.122
217. Narita, A., Niimura, S., & Ishida, K. (1992). Age-Related Changes in Adenylate Cyclase Activity in Mouse Oocytes. *Journal of Reproduction and Development*, *38*(1), 1–3. https://doi.org/10.1262/jrd.38.1
218. Ng, Y. S., Martikainen, M. H., Gorman, G. S., Blain, A., Bugiardini, E., Bunting, A., … McFarland, R. (2019). Pathogenic variants in MT-ATP6: A United Kingdom–based mitochondrial disease cohort study. *Annals of Neurology*, *86*(2), 310–315. https://doi.org/10.1002/ana.25525
219. Nicolson, G. L. (2014). The Fluid—Mosaic Model of Membrane Structure: Still relevant to understanding the structure, function and dynamics of biological membranes after more than 40years. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1838*(6), 1451–1466. https://doi.org/10.1016/j.bbamem.2013.10.019
220. Nightingale, H., Pfeffer, G., Bargiela, D., Horvath, R., & Chinnery, P. F. (2016). Emerging therapies for mitochondrial disorders. *Brain*, *139*(6), 1633–1648. https://doi.org/10.1093/brain/aww081
221. Nitzan, K., Benhamron, S., Valitsky, M., Kesner, E., Lichtenstein, M., Ben-Zvi, A., … Rosenmann, H. (2019). Mitochondrial Transfer Ameliorates Cognitive Deficits, Neuronal Loss, and Gliosis in Alzheimer's Disease Mice. *Journal of Alzheimer's Disease*, *Preprint*(Preprint), 1–18. https://doi.org/10.3233/JAD-190853
222. Nogueira, C., Almeida, L. S., Nesti, C., Pezzini, I., Videira, A., Vilarinho, L., & Santorelli, F. M. (2014). Syndromes associated with mitochondrial DNA depletion. *Italian Journal of Pediatrics*, *40*(1), 34. https://doi.org/10.1186/1824-7288-40-34
223. Ohkouchi, S., Block, G. J., Katsha, A. M., Kanehira, M., Ebina, M., Kikuchi, T., … Prockop, D. J. (2012). Mesenchymal Stromal Cells Protect Cancer Cells From ROS-induced Apoptosis and Enhance the Warburg Effect by Secreting STC1. *Molecular Therapy*, *20*(2), 417–423. https://doi.org/10.1038/mt.2011.259
224. Onfelt, B., Nedvetzki, S., Benninger, R. K. P., Purbhoo, M. A., Sowinski, S., Hume, A. N., … Davis, D. M. (2006). Structurally Distinct Membrane Nanotubes between Human Macrophages Support Long-Distance Vesicular Traffic or Surfing of Bacteria. *The Journal of Immunology*, *177*(12), 8476–8483. https://doi.org/10.4049/jimmunol.177.12.8476

225. Ore, A., & Olayinka, E. T. (2019). Toxic Mechanisms of Aryloxyphenoxypropionates in Target and Non-target Organisms. *International Journal of Biochemistry Research & Review*, 1–11. https://doi.org/10.9734/ijbcrr/2019/v27i130109
226. Orfany, A., Arriola, C. G., Doulamis, I. P., Guariento, A., Ramirez-Barbieri, G., Moskowitzova, K., … McCully, J. D. (2019). Mitochondrial transplantation ameliorates acute limb ischemia. *Journal of Vascular Surgery*. https://doi.org/10.1016/j.jvs.2019.03.079
227. Paillusson, S., Stoica, R., Gomez-Suaga, P., Lau, D. H. W., Mueller, S., Miller, T., & Miller, C. C. J. (2016). There's Something Wrong with my MAM; the ER–Mitochondria Axis and Neurodegenerative Diseases. *Trends in Neurosciences*, *39*(3), 146–157. https://doi.org/10.1016/j.tins.2016.01.008
228. Palade, G. (1953). AN ELECTRON MICROSCOPE STUDY OF THE MITOCHONDRIAL STRUCTURE. *Journal of Histochemistry & Cytochemistry*, *1*(4), 188–211. https://doi.org/10.1177/1.4.188
229. Paliwal, S., Chaudhuri, R., Agrawal, A., & Mohanty, S. (2018). Regenerative abilities of mesenchymal stem cells through mitochondrial transfer. *Journal of Biomedical Science*, *25*(1). https://doi.org/10.1186/s12929-018-0429-1
230. Park, C. B., & Larsson, N.-G. (2011). Mitochondrial DNA mutations in disease and aging. *The Journal of Cell Biology*, *193*(5), 809–818.
231. Parkinson-Lawrence, E. J., Shandala, T., Prodoehl, M., Plew, R., Borlace, G. N., & Brooks, D. A. (2010). Lysosomal Storage Disease: Revealing Lysosomal Function and Physiology. *Physiology*, *25*(2), 102–115. https://doi.org/10.1152/physiol.00041.2009
232. Pasquier, J., Guerrouahen, B. S., Al Thawadi, H., Ghiabi, P., Maleki, M., Abu-Kaoud, N., … Rafii, A. (2013). Preferential transfer of mitochondria from endothelial to cancer cells through tunneling nanotubes modulates chemoresistance. *Journal of Translational Medicine*, *11*, 94. https://doi.org/10.1186/1479-5876-11-94
233. Patananan, A. N., Wu, T.-H., Chiou, P.-Y., & Teitell, M. A. (2016). Modifying the Mitochondrial Genome. *Cell Metabolism*, *23*(5), 785–796. https://doi.org/10.1016/j.cmet.2016.04.004
234. Patergnani, S., Suski, J. M., Agnoletto, C., Bononi, A., Bonora, M., De Marchi, E., … Pinton, P. (2011). Calcium signaling around Mitochondria Associated Membranes (MAMs). *Cell Communication and Signaling*, *9*, 19. https://doi.org/10.1186/1478-811X-9-19
235. Pawelec, G., & Gupta, S. (2019). Editorial: Immunology of Aging. *Frontiers in Immunology*, *10*. https://doi.org/10.3389/fimmu.2019.01614
236. Pawlak, P., Chabowska, A., Malyszka, N., & Lechniak, D. (2016). Mitochondria and mitochondrial DNA in porcine oocytes and cumulus cells—A search for developmental competence marker. *Mitochondrion*, *27*, 48–55. https://doi.org/10.1016/j.mito.2015.12.008
237. Payne, B. A. I., & Chinnery, P. F. (2015). Mitochondrial dysfunction in aging: Much progress but many unresolved questions. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1847*(11), 1347–1353. https://doi.org/10.1016/j.bbabio.2015.05.022
238. Payton, R. R., Rispoli, L. A., Nagle, K. A., Gondro, C., Saxton, A. M., Voy, B. H., & Edwards, J. L. (2018). Mitochondrial-related consequences of heat stress exposure during bovine oocyte maturation persist in early embryo development. *Journal of Reproduction and Development*, 2017–2160. https://doi.org/10.1262/jrd.2017-160

239. Peng, W., Cai, G., Xia, Y., Chen, J., Wu, P., Wang, Z., … Wei, D. (2019). Mitochondrial Dysfunction in Atherosclerosis. *DNA and Cell Biology*, *38*(7), 597–606. https://doi.org/10.1089/dna.2018.4552
240. Peotter, J., Kasberg, W., Pustova, I., & Audhya, A. (2019). COPII-mediated trafficking at the ER/ERGIC interface. *Traffic*, *20*(7), 491–503. https://doi.org/10.1111/tra.12654
241. Pernas, L., & Scorrano, L. (2016). Mito-Morphosis: Mitochondrial Fusion, Fission, and Cristae Remodeling as Key Mediators of Cellular Function. *Annual Review of Physiology*, *78*(1), 505–531. https://doi.org/10.1146/annurev-physiol-021115-105011
242. Picard, M., Wallace, D. C., & Burelle, Y. (2016). The rise of mitochondria in medicine. *Mitochondrion*, *30*, 105–116. https://doi.org/10.1016/j.mito.2016.07.003
243. Picca, A., Guerra, F., Calvani, R., Bucci, C., Lo Monaco, M. R., Bentivoglio, A. R., … Marzetti, E. (2019). Mitochondrial Dysfunction and Aging: Insights from the Analysis of Extracellular Vesicles. *International Journal of Molecular Sciences*, *20*(4), 805. https://doi.org/10.3390/ijms20040805
244. Pinegin, B., Vorobjeva, N., Pashenkov, M., & Chernyak, B. (2018). The role of mitochondrial ROS in antibacterial immunity. *Journal of Cellular Physiology*, *233*(5), 3745–3754.
245. Pollard, T., Earnshaw, W., Lippincot-Schwartz, J., & Johnson, G. (Eds.). (2017). *Cell Biology* (Third, Vols. 1–1). Philadelphia.
246. Poole, A. M., & Gribaldo, S. (2014). Eukaryotic origins: How and when was the mitochondrion acquired? *Cold Spring Harbor Perspectives in Biology*, a015990.
247. Qian, J., Gelens, L., & Bollen, M. (2019). Coordination of Timers and Sensors in Cell Signaling. *BioEssays*, *41*(3), 1800217. https://doi.org/10.1002/bies.201800217
248. Raff, A. B., Thomas, C. N., Chuang, G. S., Avram, M. M., Le, M. H., Anderson, R. R., & Purschke, M. (2019). Lidocaine-induced potentiation of thermal damage in skin and carcinoma cells. *Lasers in Surgery and Medicine*, *51*(1), 88–94. https://doi.org/10.1002/lsm.23027
249. Rafieian-Kopaei, M., Setorki, M., Doudi, M., Baradan, A., & Nasri, H. (2014). Atherosclerosis: Process, Indicators, Risk Factors and New Hopes. *International Journal of Preventive Medicine*, *5*(8), 927–946.
250. Rai, P. K., Craven, L., Hoogewijs, K., Russell, O. M., & Lightowlers, R. N. (2018). Advances in methods for reducing mitochondrial DNA disease by replacing or manipulating the mitochondrial genome. *Essays In Biochemistry*, *62*(3), 455–465. https://doi.org/10.1042/EBC20170113
251. Raimundo, N., Fernández-Mosquera, L., Yambire, K. F., & Diogo, C. V. (2016). Mechanisms of communication between mitochondria and lysosomes. *The International Journal of Biochemistry & Cell Biology*, *79*, 345–349. https://doi.org/10.1016/j.biocel.2016.08.020
252. Rambags, B. P. B., van Boxtel, D. C. J., Tharasanit, T., Lenstra, J. A., Colenbrander, B., & Stout, T. A. E. (2014). Advancing maternal age predisposes to mitochondrial damage and loss during maturation of equine oocytes in vitro. *Theriogenology*, *81*(7), 959–965. https://doi.org/10.1016/j.theriogenology.2014.01.020
253. Rango, M., & Bresolin, N. (2018). Brain Mitochondria, Aging, and Parkinson's Disease. *Genes*, *9*(5), 250. https://doi.org/10.3390/genes9050250
254. Raposo, G., & Stoorvogel, W. (2013). Extracellular vesicles: Exosomes, microvesicles, and friends. *The Journal of Cell Biology*, *200*(4), 373–383. https://doi.org/10.1083/jcb.201211138

255. Reed, J. C., & Green, D. R. (Eds.). (2011). *Apoptosis: Physiology and pathology*. Cambridge: Cambridge University Press.
256. Reynier, P., May-Panloup, P., Chrétien, M.-F., Morgan, C. J., Jean, M., Savagner, F., … Malthièry, Y. (2001). Mitochondrial DNA content affects the fertilizability of human oocytes. *Molecular Human Reproduction*, *7*(5), 425–429. https://doi.org/10.1093/molehr/7.5.425
257. Rieusset, J. (2015). Contribution of mitochondria and endoplasmic reticulum dysfunction in insulin resistance: Distinct or interrelated roles? *Diabetes & Metabolism*, *41*(5), 358–368. https://doi.org/10.1016/j.diabet.2015.02.006
258. Rius, R., Cowley, M. J., Riley, L., Puttick, C., Thorburn, D. R., & Christodoulou, J. (2019). Biparental inheritance of mitochondrial DNA in humans is not a common phenomenon. *Genetics in Medicine*, 1–4. https://doi.org/10.1038/s41436-019-0568-0
259. Rogers, R. S., & Bhattacharya, J. (2013). When Cells Become Organelle Donors. *Physiology*, *28*(6), 414–422. https://doi.org/10.1152/physiol.00032.2013
260. Roglic, G., & Unwin, N. (2010). Mortality attributable to diabetes: Estimates for the year 2010. *Diabetes Research and Clinical Practice*, *87*(1), 15–19. https://doi.org/10.1016/j.diabres.2009.10.006
261. Roth, Z. (2018). Symposium review: Reduction in oocyte developmental competence by stress is associated with alterations in mitochondrial function1. *Journal of Dairy Science*, *101*(4), 3642–3654. https://doi.org/10.3168/jds.2017-13389
262. Rotig, A., Colonna, M., Bonnefont, J. P., Blanche, S., Fischer, A., Saudubray, J. M., & Munnich, A. (1989). MITOCHONDRIAL DNA DELETION IN PEARSON'S MARROW/PANCREAS SYNDROME. *The Lancet*, *333*(8643), 902–903. https://doi.org/10.1016/S0140-6736(89)92897-3
263. Rowland, A. A., & Voeltz, G. K. (2012). Endoplasmic reticulum–mitochondria contacts: Function of the junction. *Nature Reviews Molecular Cell Biology*, *13*(10), 607–615. https://doi.org/10.1038/nrm3440
264. Roy, M., Reddy, P. H., Iijima, M., & Sesaki, H. (2015). Mitochondrial division and fusion in metabolism. *Current Opinion in Cell Biology*, *33*, 111–118. https://doi.org/10.1016/j.ceb.2015.02.001
265. Rustom, A. (2004). Nanotubular Highways for Intercellular Organelle Transport. *Science*, *303*(5660), 1007–1010. https://doi.org/10.1126/science.1093133
266. Salas, A., Schönherr, S., Bandelt, H.-J., Gómez-Carballa, A., & Weissensteiner, H. (2019). Extraordinary claims require extraordinary evidence in the case of asserted mtDNA biparental inheritance. *BioRxiv*, 585752. https://doi.org/10.1101/585752
267. Saldaña-Martínez, A., Muñoz, M. de L., Pérez-Ramírez, G., Montiel-Sosa, J. F., Montoya, J., Emperador, S., … Ramírez, R. G. (2019). Whole sequence of the mitochondrial DNA genome of Kearns Sayre Syndrome patients: Identification of deletions and variants. *Gene*, *688*, 171–181. https://doi.org/10.1016/j.gene.2018.11.085
268. Sansone, P., Savini, C., Kurelac, I., Chang, Q., Amato, L. B., Strillacci, A., … Bromberg, J. (2017). Packaging and transfer of mitochondrial DNA via exosomes regulate escape from dormancy in hormonal therapy-resistant breast cancer. *Proceedings of the National Academy of Sciences*, *114*(43), E9066–E9075. https://doi.org/10.1073/pnas.1704862114
269. Sato, A., Kono, T., Nakada, K., Ishikawa, K., Inoue, S.-I., Yonekawa, H., & Hayashi, J.-I. (2005). Gene therapy for progeny of mito-mice carrying pathogenic mtDNA by nuclear transplantation. *Proceedings of the National Academy of Sciences*, *102*(46), 16765–16770. https://doi.org/10.1073/pnas.0506197102

270. Schatten, H., Sun, Q.-Y., & Prather, R. (2014). The impact of mitochondrial function/dysfunction on IVF and new treatment possibilities for infertility. *Reproductive Biology and Endocrinology*, *12*, 111. https://doi.org/10.1186/1477-7827-12-111
271. Scheffler, I. E. (2008). *Mitochondria* (2nd ed). Hoboken, N.J: Wiley-Liss.
272. Schekman, R. (2004). Merging cultures in the study of membrane traffic. *Nature Cell Biology*, *6*(6), 483–486. https://doi.org/10.1038/ncb0604-483
273. Schrader, M., Kamoshita, M., & Islinger, M. (2019). Organelle interplay—Peroxisome interactions in health and disease. *Journal of Inherited Metabolic Disease*, *0*(0), 1–19. https://doi.org/10.1002/jimd.12083
274. Science Source—Mitochondrion in Bat Pancreas Cell, TEM. (n.d.). Retrieved July 30, 2019, from https://www.sciencesource.com/archive/Mitochondrion-in-Bat-Pancreas-Cell--TEM-SS2624953.html
275. Sen, N. (2019). Aberrant cell cycle induction is pivotal for mitochondrial biogenesis after traumatic brain injury. *Neural Regeneration Research*, *14*(7), 1215–1216. https://doi.org/10.4103/1673-5374.251305
276. Seppet, E., Gruno, M., Peetsalu, A., Gizatullina, Z., Nguyen, H. P., Vielhaber, S., … Gellerich, F. N. (2009). Mitochondria and Energetic Depression in Cell Pathophysiology. *International Journal of Molecular Sciences*, *10*(5), 2252–2303. https://doi.org/10.3390/ijms10052252
277. Shang, T., Li, S., Zhang, Y., Lu, L., Cui, L., & Guo, F. F. (2019). Hypoxia promotes differentiation of adipose-derived stem cells into endothelial cells through demethylation of ephrinB2. *Stem Cell Research & Therapy*, *10*(1), 133. https://doi.org/10.1186/s13287-019-1233-x
278. Shourbagy, S. H. E., Spikings, E. C., Freitas, M., & John, J. C. S. (2006). Mitochondria directly influence fertilisation outcome in the pig. *Reproduction*, *131*(2), 233–245. https://doi.org/10.1530/rep.1.00551
279. Silzer, T. K., & Phillips, N. R. (2018). Etiology of type 2 diabetes and Alzheimer's disease: Exploring the mitochondria. *Mitochondrion*, *43*, 16–24. https://doi.org/10.1016/j.mito.2018.04.004
280. Simsek-Duran, F., Li, F., Ford, W., Swanson, R. J., Jones, H. W., & Castora, F. J. (2013). Age-Associated Metabolic and Morphologic Changes in Mitochondria of Individual Mouse and Hamster Oocytes. *PLoS ONE*, *8*(5). https://doi.org/10.1371/journal.pone.0064955
281. Sinha, P., Islam, M. N., Bhattacharya, S., & Bhattacharya, J. (2016a). Intercellular mitochondrial transfer: Bioenergetic crosstalk between cells. *Current Opinion in Genetics & Development*, *38*, 97–101. https://doi.org/10.1016/j.gde.2016.05.002
282. Sinha, P., Islam, M. N., Bhattacharya, S., & Bhattacharya, J. (2016b). Intercellular mitochondrial transfer: Bioenergetic crosstalk between cells. *Current Opinion in Genetics & Development*, *38*, 97–101. https://doi.org/10.1016/j.gde.2016.05.002
283. Sluijter, J. P. G., Verhage, V., Deddens, J. C., van den Akker, F., & Doevendans, P. A. (2014). Microvesicles and exosomes for intracardiac communication. *Cardiovascular Research*, *102*(2), 302–311. https://doi.org/10.1093/cvr/cvu022
284. Sokolova, V. A., Kustova, M. E., Arbuzova, N. I., Sorokin, A. V., Moskaliova, O. S., Bass, M. G., & Vasilyev, V. B. (2004). Obtaining mice that carry human mitochondrial DNA transmitted to the progeny. *Molecular Reproduction and Development*, *68*(3), 299–307. https://doi.org/10.1002/mrd.20075

285. Solovyeva, V. V., Shaimardanova, A. A., Chulpanova, D. S., Kitaeva, K. V., Chakrabarti, L., & Rizvanov, A. A. (2018). New Approaches to Tay-Sachs Disease Therapy. *Frontiers in Physiology*, *9*. https://doi.org/10.3389/fphys.2018.01663
286. Spees, J. L., Olson, S. D., Whitney, M. J., & Prockop, D. J. (2006). Mitochondrial transfer between cells can rescue aerobic respiration. *Proceedings of the National Academy of Sciences*, *103*(5), 1283–1288. https://doi.org/10.1073/pnas.0510511103
287. Spinelli, J. B., & Haigis, M. C. (2018). The multifaceted contributions of mitochondria to cellular metabolism. *Nature Cell Biology*, *20*(7), 745–754. https://doi.org/10.1038/s41556-018-0124-1
288. Stacchiotti, A., Favero, G., Lavazza, A., Garcia-Gomez, R., Monsalve, M., & Rezzani, R. (2019). Perspective: Mitochondria-ER Contacts in Metabolic Cellular Stress Assessed by Microscopy. *Cells*, *8*(1), 5. https://doi.org/10.3390/cells8010005
289. Steller, H. (Ed.). (2015). *Apoptosis and development* (1. ed). Amsterdam: Elsevier, Acad. Press.
290. Su, X., Rak, M., Tetaud, E., Godard, F., Sardin, E., Bouhier, M., … Tribouillard-Tanvier, D. (2019). Deregulating mitochondrial metabolite and ion transport has beneficial effects in yeast and human cellular models for NARP syndrome. *Human Molecular Genetics*. https://doi.org/10.1093/hmg/ddz160
291. Sugiura, A., Mattie, S., Prudent, J., & McBride, H. M. (2017). Newly born peroxisomes are a hybrid of mitochondrial and ER-derived pre-peroxisomes. *Nature*, *542*(7640), 251–254. https://doi.org/10.1038/nature21375
292. Suh, K. S., Chon, S., Jung, W.-W., & Choi, E. M. (2019). Crocin attenuates methylglyoxal-induced osteoclast dysfunction by regulating glyoxalase, oxidative stress, and mitochondrial function. *Food and Chemical Toxicology*, *124*, 367–373. https://doi.org/10.1016/j.fct.2018.12.031
293. Sukhorukov, V. M., & Meyer-Hermann, M. (2015). Structural Heterogeneity of Mitochondria Induced by the Microtubule Cytoskeleton. *Scientific Reports*, *5*(1). https://doi.org/10.1038/srep13924
294. Sun, N., Youle, R. J., & Finkel, T. (2016). The Mitochondrial Basis of Aging. *Molecular Cell*, *61*(5), 654–666. https://doi.org/10.1016/j.molcel.2016.01.028
295. Sung, Y.-J., Kao, T.-Y., Kuo, C.-L., Fan, C.-C., Cheng, A. N., Fang, W.-C., … Lee, A. Y.-L. (2018). Mitochondrial Lon sequesters and stabilizes p53 in the matrix to restrain apoptosis under oxidative stress via its chaperone activity. *Cell Death & Disease*, *9*(6). https://doi.org/10.1038/s41419-018-0730-7
296. Suomalainen, A., & Battersby, B. J. (2018). Mitochondrial diseases: The contribution of organelle stress responses to pathology. *Nature Reviews Molecular Cell Biology*, *19*(2), 77–92. https://doi.org/10.1038/nrm.2017.66
297. Tachibana, M., Sparman, M., & Mitalipov, S. (2010). Chromosome transfer in mature oocytes. *Nature Protocols*, *5*(6), 1138–1147. https://doi.org/10.1038/nprot.2010.75
298. Tachibana, M., Sparman, M., Sritanaudomchai, H., Ma, H., Clepper, L., Woodward, J., … Mitalipov, S. (2009). Mitochondrial gene replacement in primate offspring and embryonic stem cells. *Nature*, *461*(7262), 367–372. https://doi.org/10.1038/nature08368
299. Takeda, K. (2019). Functional consequences of mitochondrial mismatch in reconstituted embryos and offspring. *Journal of Reproduction and Development*. https://doi.org/10.1262/jrd.2019-089
300. Takeda, K., Tasai, M., Iwamoto, M., Onishi, A., Tagami, T., Nirasawa, K., … Pinkert, C. A. (2005). Microinjection of Cytoplasm or Mitochondria Derived from Somatic Cells Affects

Parthenogenetic Development of Murine Oocytes. *Biology of Reproduction*, *72*(6), 1397–1404. https://doi.org/10.1095/biolreprod.104.036129

301. Takeo, S., Goto, H., Kuwayama, T., Monji, Y., & Iwata, H. (2013). Effect of Maternal Age on the Ratio of Cleavage and Mitochondrial DNA Copy Number in Early Developmental Stage Bovine Embryos. *Journal of Reproduction and Development*, *59*(2), 174–179. https://doi.org/10.1262/jrd.2012-148
302. Tan, A. S., Baty, J. W., Dong, L.-F., Bezawork-Geleta, A., Endaya, B., Goodwin, J., … Berridge, M. V. (2015). Mitochondrial Genome Acquisition Restores Respiratory Function and Tumorigenic Potential of Cancer Cells without Mitochondrial DNA. *Cell Metabolism*, *21*(1), 81–94. https://doi.org/10.1016/j.cmet.2014.12.003
303. Tan, D.-X. (2019). Aging: An evolutionary competition between host cells and mitochondria. *Medical Hypotheses*, *127*, 120–128. https://doi.org/10.1016/j.mehy.2019.04.007
304. Tenorio, B. M., Pereira da Silva, R., Tenorio, F. das C. A. M., Costa Rosales, R. R., Amaro da Silva Junior, V., & de Albuquerque Nogueira, R. (2019). Effect of heat stress and Hsp90 inhibition on T-type calcium currents and voltage-dependent potassium currents in leydig cells. *Journal of Thermal Biology*, *84*, 1–7. https://doi.org/10.1016/j.jtherbio.2019.05.022
305. Thorburn, D. R., Rahman, J., & Rahman, S. (2017). Mitochondrial DNA-Associated Leigh Syndrome and NARP. In M. Adam, H. Ardinger, & R. Pagon et al. ,. (Eds.), *GeneReviews* (p. 48). Retrieved from https://www.ncbi.nlm.nih.gov/books/
306. Thornton, C., & Hagberg, H. (2015). Role of mitochondria in apoptotic and necroptotic cell death in the developing brain. *Clinica Chimica Acta*, *451, Part A*, 35–38. https://doi.org/10.1016/j.cca.2015.01.026
307. Torralba, D., Baixauli, F., & Sánchez-Madrid, F. (2016). Mitochondria Know No Boundaries: Mechanisms and Functions of Intercellular Mitochondrial Transfer. *Frontiers in Cell and Developmental Biology*, *4*. https://doi.org/10.3389/fcell.2016.00107
308. Tousson, E., El-Atrsh, A., Mansour, M., & Abdallah, A. (2019). Modulatory effects of Saussurea lappa root aqueous extract against ethephon-induced kidney toxicity in male rats. *Environmental Toxicology*, *0*(0), 1–8. https://doi.org/10.1002/tox.22828
309. Tranchant, C., & Anheim, M. (2016). Movement disorders in mitochondrial diseases. *Revue Neurologique*, *172*(8–9), 524–529. https://doi.org/10.1016/j.neurol.2016.07.003
310. Trounce, I. A., McKenzie, M., Cassar, C. A., Ingraham, C. A., Lerner, C. A., Dunn, D. A., … Pinkert, C. A. (2004). Development and Initial Characterization of Xenomitochondrial Mice. *Journal of Bioenergetics and Biomembranes*, *36*(4), 421–427. https://doi.org/10.1023/B:JOBB.0000041778.84464.16
311. Unuma, K., Aki, T., Funakoshi, T., Hashimoto, K., & Uemura, K. (2015). Extrusion of mitochondrial contents from lipopolysaccharide-stimulated cells: Involvement of autophagy. *Autophagy*, *11*(9), 1520–1536. https://doi.org/10.1080/15548627.2015.1063765
312. van Beek, A. A., Van den Bossche, J., Mastroberardino, P. G., de Winther, M. P. J., & Leenen, P. J. M. (2019). Metabolic Alterations in Aging Macrophages: Ingredients for Inflammaging? *Trends in Immunology*, *40*(2), 113–127. https://doi.org/10.1016/j.it.2018.12.007
313. van der Laan, M., Horvath, S. E., & Pfanner, N. (2016). Mitochondrial contact site and cristae organizing system. *Current Opinion in Cell Biology*, *41*, 33–42. https://doi.org/10.1016/j.ceb.2016.03.013

314. Van Houten, B., Hunter, S. E., & Meyer, J. N. (2016). Mitochondrial DNA damage induced autophagy, cell death, and disease. *Frontiers in Bioscience (Landmark Edition)*, *21*, 42–54.
315. VanDelinder, V., Imam, Z. I., & Bachand, G. (2019). Kinesin motor density and dynamics in gliding microtubule motility. *Scientific Reports*, *9*(1), 1–9. https://doi.org/10.1038/s41598-019-43749-8
316. Vasileiou, P. V. S., Evangelou, K., Vlasis, K., Fildisis, G., Panayiotidis, M. I., Chronopoulos, E., … Havaki, S. (2019). Mitochondrial Homeostasis and Cellular Senescence. *Cells*, *8*(7), 686. https://doi.org/10.3390/cells8070686
317. Vatrinet, R., Leone, G., De Luise, M., Girolimetti, G., Vidone, M., Gasparre, G., & Porcelli, A. M. (2017). The α-ketoglutarate dehydrogenase complex in cancer metabolic plasticity. *Cancer & Metabolism*, *5*, 3. https://doi.org/10.1186/s40170-017-0165-0
318. Victor, V., Apostolova, N., Herance, R., Hernandez-Mijares, A., & Rocha, M. (2009). Oxidative Stress and Mitochondrial Dysfunction in Atherosclerosis: Mitochondria-Targeted Antioxidants as Potential Therapy. *Current Medicinal Chemistry*, *16*(35), 4654–4667. https://doi.org/10.2174/092986709789878265
319. Villa, E., Ali, E. S., Sahu, U., & Ben-Sahra, I. (2019). Cancer Cells Tune the Signaling Pathways to Empower de Novo Synthesis of Nucleotides. *Cancers*, *11*(5), 688. https://doi.org/10.3390/cancers11050688
320. Villeneuve, L. M., Purnell, P. R., Boska, M. D., & Fox, H. S. (2016). Early expression of Parkinson's disease-related mitochondrial abnormalities in PINK1 knockout rats. *Molecular Neurobiology*, *53*(1), 171–186. https://doi.org/10.1007/s12035-014-8927-y
321. Voeltz, G. K., Rolls, M. M., & Rapoport, T. A. (2002). Structural organization of the endoplasmic reticulum. *EMBO Reports*, *3*(10), 944–950. https://doi.org/10.1093/embo-reports/kvf202
322. Wang, J., & Chen, G.-J. (2016). Mitochondria as a therapeutic target in Alzheimer's disease. *Genes & Diseases*, *3*(3), 220–227. https://doi.org/10.1016/j.gendis.2016.05.001
323. Wang, T., Zhang, M., Jiang, Z., & Seli, E. (2017). Mitochondrial dysfunction and ovarian aging. *American Journal of Reproductive Immunology*, *77*(5), n/a-n/a. https://doi.org/10.1111/aji.12651
324. Wang, X., & Gerdes, H.-H. (2015). Transfer of mitochondria via tunneling nanotubes rescues apoptotic PC12 cells. *Cell Death and Differentiation*, *22*(7), 1181.
325. Wang, Y., Ni, J., Gao, C., Xie, L., Zhai, L., Cui, G., & Yin, X. (2019). Mitochondrial transplantation attenuates lipopolysaccharide- induced depression-like behaviors. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *93*, 240–249. https://doi.org/10.1016/j.pnpbp.2019.04.010
326. Wang, Z.-B., Hao, J.-X., Meng, T.-G., Guo, L., Dong, M.-Z., Fan, L.-H., … Yao, Y.-Q. (2017). Transfer of autologous mitochondria from adipose tissue-derived stem cells rescues oocyte quality and infertility in aged mice. *Aging (Albany NY)*, *9*(12), 2480–2488. https://doi.org/10.18632/aging.101332
327. WebHome < MITOMAP < Foswiki. (n.d.). Retrieved September 1, 2019, from https://www.mitomap.org/MITOMAP
328. Westman, J., Hube, B., & Fairn, G. D. (2019). Integrity under stress: Host membrane remodelling and damage by fungal pathogens. *Cellular Microbiology*, *21*(4), e13016. https://doi.org/10.1111/cmi.13016

329. Wiley, C. D., Velarde, M. C., Lecot, P., Liu, S., Sarnoski, E. A., Freund, A., … Campisi, J. (2016). Mitochondrial Dysfunction Induces Senescence with a Distinct Secretory Phenotype. *Cell Metabolism*, *23*(2), 303–314. https://doi.org/10.1016/j.cmet.2015.11.011
330. Wolf, D. P., Mitalipov, N., & Mitalipov, S. (2015a). Mitochondrial replacement therapy in reproductive medicine. *Trends in Molecular Medicine*, *21*(2), 68–76. https://doi.org/10.1016/j.molmed.2014.12.001
331. Wolf, D. P., Mitalipov, N., & Mitalipov, S. (2015b). Mitochondrial replacement therapy in reproductive medicine. *Trends in Molecular Medicine*, *21*(2), 68–76. https://doi.org/10.1016/j.molmed.2014.12.001
332. Wong, R. C. B., Lim, S. Y., Hung, S. S. C., Jackson, S., Khan, S., Van Bergen, N. J., … Pébay, A. (2017). Mitochondrial replacement in an iPSC model of Leber's hereditary optic neuropathy. *Aging (Albany NY)*, *9*(4), 1341–1347. https://doi.org/10.18632/aging.101231
333. Woods, D. C., Khrapko, K., & Tilly, J. L. (2018). Influence of Maternal Aging on Mitochondrial Heterogeneity, Inheritance, and Function in Oocytes and Preimplantation Embryos. *Genes*, *9*(5), 265. https://doi.org/10.3390/genes9050265
334. Woods, D. C., & Tilly, J. L. (2015). Autologous Germline Mitochondrial Energy Transfer (AUGMENT) in Human Assisted Reproduction. *Seminars in Reproductive Medicine*, *33*(6), 410–421. https://doi.org/10.1055/s-0035-1567826
335. Wu, T.-H., Sagullo, E., Case, D., Zheng, X., Li, Y., Hong, J. S., … Teitell, M. A. (2016). Mitochondrial Transfer by Photothermal Nanoblade Restores Metabolite Profile in Mammalian Cells. *Cell Metabolism*, *23*(5), 921–929. https://doi.org/10.1016/j.cmet.2016.04.007
336. Wu, T.-H., Teslaa, T., Kalim, S., French, C. T., Moghadam, S., Wall, R., … Chiou, P.-Y. (2011). Photothermal Nanoblade for Large Cargo Delivery into Mammalian Cells. *Analytical Chemistry*, *83*(4), 1321–1327. https://doi.org/10.1021/ac102532w
337. Wu, T.-H., Teslaa, T., Teitell, M. A., & Chiou, P.-Y. (2010). Photothermal nanoblade for patterned cell membrane cutting. *Optics Express*, *18*(22), 23153–23160. https://doi.org/10.1364/OE.18.023153
338. Xiao, F., Li, X., Wang, J., & Cao, J. (2019). Mechanisms of vascular endothelial cell injury in response to intermittent and/or continuous hypoxia exposure and protective effects of anti-inflammatory and anti-oxidant agents. *Sleep and Breathing*, *23*(2), 515–522. https://doi.org/10.1007/s11325-019-01803-9
339. Xiao, J., Fan, L., Wu, D., Xu, Y., Lai, D., Martin, W. F., … Zhang, C. (2019). Archaea, the tree of life, and cellular evolution in eukaryotes. *Science China Earth Sciences*, *62*(3), 489–506. https://doi.org/10.1007/s11430-018-9326-1
340. Xiao, X., Huang, M., Fan, C., & Zuo, F. (2018). Research on the senescence of human skin fibroblasts induced by ultraviolet b and its mechanism. *Biomedical Research*, *29*(2). Retrieved from http://www.alliedacademies.org/abstract/research-on-the-senescence-of-human-skin-fibroblasts-induced-by-ultraviolet-b-and-its-mechanism-9591.html
341. Yabuuchi, A., Beyhan, Z., Kagawa, N., Mori, C., Ezoe, K., Kato, K., … Kato, O. (2012). Prevention of mitochondrial disease inheritance by assisted reproductive technologies: Prospects and challenges. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1820*(5), 637–642. https://doi.org/10.1016/j.bbagen.2011.10.014
342. Yamaguchi, M., & Worman, C. O. (2014). *Deep-sea microorganisms and the origin of the eukaryotic cell*. *47*(1), 20.

343. Yang, L., Long, Q., Liu, J., Tang, H., Li, Y., Bao, F., … Liu, X. (2015). Mitochondrial fusion provides an 'initial metabolic complementation' controlled by mtDNA. *Cellular and Molecular Life Sciences*, *72*(13), 2585–2598. https://doi.org/10.1007/s00018-015-1863-9
344. Yang, Y., Elamawi, R., Bubeck, J., Pepperkok, R., Ritzenthaler, C., & Robinson, D. G. (2005). Dynamics of COPII Vesicles and the Golgi Apparatus in Cultured Nicotiana tabacum BY-2 Cells Provides Evidence for Transient Association of Golgi Stacks with Endoplasmic Reticulum Exit Sites. *The Plant Cell*, *17*(5), 1513–1531. https://doi.org/10.1105/tpc.104.026757
345. Yin, X.-M., & Dong, Z. (Eds.). (2003). *Essentials of apoptosis: A guide for basic and clinical research*. Totowa, N.J: Humana Press.
346. Yoshida, Y., Takahashi, K., Okita, K., Ichisaka, T., & Yamanaka, S. (2009). Hypoxia Enhances the Generation of Induced Pluripotent Stem Cells. *Cell Stem Cell*, *5*(3), 237–241. https://doi.org/10.1016/j.stem.2009.08.001
347. Youle, R. J., & Van Der Bliek, A. M. (2012). Mitochondrial fission, fusion, and stress. *Science*, *337*(6098), 1062–1065.
348. Yu, C., Jiang, F., Zhang, M., Luo, D., Shao, S., Zhao, J., … Guan, Q. (2019). HC diet inhibited testosterone synthesis by activating endoplasmic reticulum stress in testicular Leydig cells. *Journal of Cellular and Molecular Medicine*, *23*(5), 3140–3150. https://doi.org/10.1111/jcmm.14143
349. Yuan, S., & Akey, C. W. (2013). Apoptosome Structure, Assembly, and Procaspase Activation. *Structure*, *21*(4), 501–515. https://doi.org/10.1016/j.str.2013.02.024
350. Yu-Wai-Man, P., & Chinnery, P. F. (2016). Leber Hereditary Optic Neuropathy. In M. Adam, H. Ardinger, & R. Pagon et al. ,. (Eds.), *GeneReviews* (p. 19). Retrieved from https://www.ncbi.nlm.nih.gov/books/ Leber
351. Zachary, J. F. (Ed.). (2017). *Pathologic basis of veterinary disease* (Sixth edition). St. Louis, Missouri: Elsevier.
352. Zhang, Z., Ma, Z., Yan, C., Pu, K., Wu, M., Bai, J., … Wang, Q. (2019). Muscle-derived autologous mitochondrial transplantation: A novel strategy for treating cerebral ischemic injury. *Behavioural Brain Research*, *356*, 322–331. https://doi.org/10.1016/j.bbr.2018.09.005
353. Zheng, C.-X., Sui, B.-D., Qiu, X.-Y., Hu, C.-H., & Jin, Y. (2019). Mitochondrial Regulation of Stem Cells in Bone Homeostasis. *Trends in Molecular Medicine*. https://doi.org/10.1016/j.molmed.2019.04.008
354. Zimorski, V., Ku, C., Martin, W. F., & Gould, S. B. (2014). Endosymbiotic theory for organelle origins. *Current Opinion in Microbiology*, *22*, 38–48. https://doi.org/10.1016/j.mib.2014.09.008
355. Zsurka, G., Peeva, V., Kotlyar, A., & Kunz, W. (2018). Is There Still Any Role for Oxidative Stress in Mitochondrial DNA-Dependent Aging? *Genes*, *9*(4), 175. https://doi.org/10.3390/genes9040175