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Benoit Maffei

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Sorbonne Université

Ecole doctorale Complexité du Vivant

Unité de Biologie Cellulaire de l'Infection Microbienne

Investigation of the role of tissue transglutaminase (TG2) in the infection by *Chlamydia trachomatis*: a key player in metabolic rewiring of the host cell.

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Thèse de doctorat de Microbiologie Cellulaire

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ABBREVIATIONS

BP	biotin pentylamine
CoA	coenzyme A
EB	elementary body
ECM	extracellular matrix
EMT	epithelial-to-mesenchymal transition
ER	endoplasmic reticulum
GFPT	glucosamine-fructose-6-phosphate amidotransferase
GlcNAc	N-acetylglucosamine
HBP	hexosamine biosynthesis pathway
hpi	hours post infection
HS	heparan sulfate
KO	knock-out
L2	<i>C. trachomatis</i> LGV L2
LGV	lymphogranuloma venereum
MEF	mouse embryonic fibroblast
MOI	multiplicity of infection
MOMP	major outer membrane protein
NO	nitric oxide
O-GlcNAcylation	O-N-acetylglucosaminylation
OxPhos	oxidative phosphorylation
P	phosphate
PDI	protein disulfide isomerase
PPP	pentose phosphate pathway
RB	reticulate body
ROS	reactive oxygen species
STI	sexually transmitted infection
T3SS	type 3 secretion system
TCA	tri-carboxylic acid cycle
TG2	transglutaminase 2
UDP-GlcNAc	UDP-N-acetylglucosamine
WHO	Worldwide Health Organization
WT	wild type

ABSTRACT

Chlamydia trachomatis is an obligate intracellular pathogenic bacterium that can only infect humans. It is the leading cause of sexually transmitted infections of bacterial origin and can also infect the eye conjunctiva, causing acquired blindness (trachoma).

During its biphasic developmental cycle, *C. trachomatis* remains in a membrane-bound compartment called inclusion, separated from the cytoplasm. The bacteria have acquired several strategies to hijack cellular functions to secure their niche and meet their metabolic needs.

In this thesis we focused on the impact of a cellular enzyme, the tissue transglutaminase (TG2), on *C. trachomatis* development. The main activity of this multifunctional enzyme is a transamidating activity, i.e. the catalysis of a covalent bond between two proteins or with a small amine present in the cell.

We have shown that TG2 expression and transamidating activity increased upon *C. trachomatis* infection. This increase is necessary for optimal bacterial growth, a conclusion supported by experiments using mice knocked-out for the expression of TG2 infected with *C. muridarum*.

At the cellular level, we focused our work on the impact of TG2 on cellular metabolism modification during *C. trachomatis* infection. We showed that TG2 played a central role in the control of glucose import in infected cells by regulating *GLUT1* and *GLUT3* transcription. In addition, we identified Gln328 on the enzyme GFPT1 (glucosamine-fructose-6-phosphate amidotransferase 1), as a target of TG2. GFPT1 is the first and rate-limiting enzyme of the hexosamine biosynthetic pathway, regulating the synthesis of UDP-N-acetylglucosamine. This metabolite is used for post-translational modification of proteins by *O*-GlcNAcylation. Our work uncovered an unsuspected link between TG2 transamidating activity and *O*-GlcNAcylation. This link was disrupted in infected cells, likely because UDP-N-acetylglucosamine is rapidly consumed by the bacteria, in particular to assist division.

In conclusion, our work established TG2 as a key player in controlling glucose-derived metabolic pathways in mammalian cells, themselves hijacked by *C. trachomatis* to sustain its own metabolic needs.

RÉSUMÉ

Chlamydia trachomatis est une bactérie pathogène intracellulaire obligatoire dont l'Homme est l'hôte exclusif. Elle représente la première cause mondiale d'infections sexuellement transmissibles d'origine bactérienne mais peut également infecter la conjonctive de l'œil et causer des cécités (trachome).

Au cours de son cycle infectieux, biphasique, *C. trachomatis* réside au sein d'un compartiment appelé inclusion, séparé du cytoplasme par une membrane. Afin de sécuriser leur niche écologique et d'assurer leurs besoins métaboliques, ces bactéries ont développés des stratégies variées leur permettant de détourner des fonctions cellulaires.

Dans cette thèse nous avons étudié l'impact d'une enzyme cellulaire, la transglutaminase 2 (TG2), sur le développement de *C. trachomatis*. L'activité principale de cette enzyme multifonctionnelle est une activité transamidase, consistant en la catalyse d'un lien covalent entre deux protéines ou entre une protéine et une petite amine.

Nous avons pu montrer que l'activité transglutaminase de TG2 ainsi que la quantité de cette enzyme augmentaient au cours de l'infection par *C. trachomatis*. Cette augmentation est nécessaire pour permettre une croissance bactérienne optimale. Cette conclusion est appuyée par des expériences sur des souris n'exprimant pas TG2, infectées par *Chlamydia muridarum*.

Au niveau cellulaire, nous nous sommes intéressés à l'impact de TG2 sur la modification du métabolisme cellulaire lors de l'infection par *C. trachomatis*. Nous avons pu mettre en évidence un rôle clef de TG2 dans le contrôle de l'import de glucose dans les cellules infectées par une régulation transcriptionnelle des transporteurs de glucose *GLUT1* et *GLUT3*. De plus, nous avons identifié la Gln328 de l'enzyme GFPT1 (glucosamine-fructose-6-phosphate amidotransférase 1) comme cible de TG2. GFPT1 est la première et cinétiquement limitante enzyme de la voie de biosynthèse des hexosamines, régulant la synthèse d'UDP-N-acétylglucosamine. Ce métabolite est utilisé pour la modification post-traductionnelle de protéines par *O*-GlcNAcylation. Notre travail a révélé un lien inattendu entre l'activité transamidase de TG2 et l'*O*-GlcNAcylation. Ce lien est interrompu dans des cellules infectées, probablement parce que l'UDP-N-acétylglucosamine est consommé par les bactéries, en particulier pour permettre leur division.

En conclusion, ce travail présente TG2 comme un effecteur clef dans le contrôle de voies métaboliques dérivées du glucose dans des cellules de mammifère, elles-mêmes détournées par *C. trachomatis* afin de subvenir à ses propres besoins métaboliques.

RÉSUMÉ DÉTAILLÉ

Le phylum des *Chlamydiae* regroupe un ensemble de bactéries intracellulaires obligatoires à Gram négatif. Ces bactéries présentent un cycle de développement biphasique très particulier se déroulant au sein d'un hôte eucaryote, pouvant être un protozoaire (amibes par exemple) ou un métazoaire. La famille des *Chlamydiaceae* regroupe un ensemble de *Chlamydiae* pathogènes d'animaux (mammifères, insectes, poissons, marsupiaux...) qui ont un impact en santé vétérinaire et humaine. Deux espèces de *Chlamydiaceae* infectent l'Homme de manière régulière : *Chlamydia trachomatis* et *Chlamydia pneumoniae*. A la différence de *C. pneumoniae* capable d'infecter des hôtes variés, et causant des infections de l'appareil respiratoire chez l'Homme, *C. trachomatis* a pour hôte exclusif l'Homme. Certaines souches (serovars A à C) infectent la conjonctive de l'œil, pouvant conduire au trachome lorsqu'elles ne sont pas traitées à temps : c'est la première cause de cécité acquise au monde. Les autres serovars infectent la région uro-génitale de manière souvent asymptomatique, mais dont les conséquences peuvent être lourdes sur le long terme chez la femme (grossesse extra-utérine, inflammation pelvienne chronique, infertilité) : elle représente la première infection sexuellement transmissible d'origine bactérienne au monde avec plus de 100 millions de nouveaux cas par an.

Le cycle biphasique des *Chlamydiaceae* implique une alternance entre deux formes de la bactérie : le corps élémentaire (EB), infectieux, non répliatif et métaboliquement peu actif et le corps réticulé (RB), plus gros, répliatif, et osmotiquement fragile. Un EB adhère à la surface d'une cellule-hôte puis est internalisé au sein d'un compartiment appelé inclusion, une vacuole parasitophore qui le sépare du cytosol par une membrane. Une fois au sein de l'inclusion, le EB se différencie en RB, qui se multiplie par fission binaire. Dès 24 h après entrée dans la cellule, certains RB présents dans l'inclusion se redifférencient en EB, de manière asynchrone, de sorte qu'au bout de 48 h environ, l'inclusion contient très majoritairement des EB. A ce moment-là, les EB sont libérés de la cellule-hôte soit par lyse de celle-ci, soit par un processus d'extrusion où l'inclusion est libérée dans le milieu extracellulaire entourée d'une membrane supplémentaire. Les EB libérés peuvent infecter les cellules adjacentes et commencer un nouveau cycle.

En lien avec le mode de répllication de ces bactéries, intracellulaire obligatoire, les *Chlamydiae* ont subi une réduction génique conduisant à la perte d'un certain nombre de gènes et à une dépendance à certaines fonctions cellulaires, notamment en ce qui concerne l'acquisition de métabolites. Tout au long du cycle, la bactérie, protégée au sein de l'inclusion, interagit avec sa cellule-hôte grâce à la sécrétion de protéines effectrices. Le principal mécanisme de sécrétion est appelé système de sécrétion de type 3, il est retrouvé dans de nombreuses bactéries à Gram négatif pathogènes. Les protéines sécrétées jouent un rôle clef dans la manipulation de l'hôte et le détournement des fonctions cellulaires au profit de la bactérie. Un des éléments cruciaux pour la multiplication bactérienne est le glucose, métabolite central pour la synthèse d'énergie sous forme d'ATP mais aussi pour la biosynthèse de nombreuses autres molécules organiques. Divers travaux ont montré que *C. trachomatis* augmentait l'import de glucose dans la cellule hôte. Il existe des arguments pour penser que le métabolisme de la cellule hôte est détourné vers un métabolisme de type glycolyse aérobie, caractérisé par une très forte consommation de glucose dans la glycolyse et une forte production de lactate reflétant une respiration mitochondriale relativement faible comparée au fonctionnement glycolytique de ces cellules. Ce métabolisme particulier, aussi appelé effet Warburg, dont l'intérêt pour les cellules le réalisant reste toujours débattu, est retrouvé chez les cellules cancéreuses entre autres. Dans le cas de cellules épithéliales mammaires, il a été montré qu'une enzyme cellulaire, la transglutaminase 2 (TG2), jouait un rôle important dans l'orientation du métabolisme vers la glycolyse aérobie.

Cette enzyme multifonctionnelle tient son nom de sa capacité à catalyser la réaction de transamination qui correspond à la création d'une liaison covalente entre le résidu glutamine d'une protéine et le résidu lysine d'une autre protéine (ou de la même protéine), ou une petite amine présente dans la cellule comme l'histamine ou la sérotonine par exemple. L'activité transaminase de TG2 est fortement contrôlée et nécessite une forte élévation de la concentration en calcium, au moins locale. Par des actions impliquant cette activité transaminase ainsi que d'autres indépendantes de celle-ci – liées aux interactions que TG2 réalise avec d'autres protéines dont le facteur de transcription NFκB – TG2 constitue, dans certains cancers comme le cancer ovarien, un moteur des changements transcriptionnels aboutissant au détournement du métabolisme cellulaire vers un métabolisme de type glycolyse aérobie, notamment en induisant l'expression ou la stabilisation du facteur de transcription HIF-1. Par ailleurs, l'expression de TG2 est induite par plusieurs cytokines et une activation de l'enzyme est observée dans de nombreux processus inflammatoires tels que des fibroses rénales,

l'athérosclérose ou la transformation tumorale. Curieusement la question de l'activation de TG2 lors d'une réaction inflammatoire classique que constitue une infection microbienne a été l'objet de très peu d'études. Nous avons cherché dans cette thèse à déterminer si TG2 était sollicitée lors de l'infection de cellules épithéliales par *C. trachomatis* ainsi que le rôle que pouvait avoir cette enzyme, dans le cycle de développement bactérien.

Résultats

Nous avons dans un premier temps cherché à déterminer si l'activité transaminase de TG2 était augmentée au cours de l'infection par *C. trachomatis*, en utilisant une sonde incorporée dans les protéines cibles de la réaction de transamination. Nous avons observé une augmentation de cette activité sur des temps plutôt tardif du cycle, après 24 h d'infection et jusqu'à la fin du cycle vers 48 h. Cette augmentation est bien liée à TG2 car lorsque l'on inhibe spécifiquement cette enzyme à l'aide d'un inhibiteur compétitif, le CP4d, on perd cette augmentation du signal transaminase cellulaire global. Lorsque l'on traite les cellules avec de la cycloheximide, un inhibiteur de la traduction cellulaire, le signal transaminase est largement diminué, indiquant que l'augmentation de l'activité de TG2 lors de l'infection passe en partie par une augmentation de l'expression de la protéine. Celle-ci corrèle avec une augmentation de la transcription du gène correspondant *TGM2*. On perd également une grande partie de l'activation transcriptionnelle de TG2 en traitant les cellules avec du CP4d, indiquant une boucle de rétroaction positive : l'activité transaminase de TG2 est impliquée dans l'augmentation de l'expression de l'enzyme.

Etant donné l'augmentation d'activité transaminase par TG2 lors de l'infection, nous avons cherché à comprendre son impact sur le cycle de développement de *C. trachomatis*. S'intéressant dans un premier temps au cycle dans son ensemble, nous avons pu mettre en évidence l'importance de l'activité de TG2 pour la bactérie qui produit moins de EB par cellule lorsqu'elle se multiplie au sein de cellules traitées au CP4d, ou ne présentant pas du tout d'enzyme TG2 (fibroblastes murins issus de lignées KO pour TG2). Plus précisément, nous avons montré que TG2 n'était pas importante pour l'étape d'attachement des bactéries à la surface des cellules mais qu'elle l'était dans l'entrée des bactéries. De plus, TG2 est également importante pour le développement de la bactérie une fois celle-ci entrée, puisque la taille des inclusions contenant les bactéries dans des cellules traitées au CP4d était inférieure à celle dans les cellules contrôle. Ces résultats ont été confirmés sur des cellules primaires humaines issues d'épithélia dérivés des trompes, et avec différents sérovars uro-génitaux de *C. trachomatis*.

En utilisant un modèle murin d'infection par *C. muridarum*, espèce adaptée à la souris génétiquement très proche de *C. trachomatis*, nous avons confirmé l'importance de TG2 sur le développement bactérien. Des souris KO pour TG2 présentent, 25 jours après infection, moins de symptômes (hydrosalpinx) liés à l'inflammation provoquée par l'infection. De plus, chez les souris TG2 KO, la charge bactérienne tend à être inférieure au niveau des voies génitales supérieures à celle mesurée dans des souris contrôle.

Du fait de l'importance de TG2 pour rediriger le métabolisme cellulaire vers la glycolyse aérobie dans certaines cellules cancéreuses, ainsi que la forte demande en glucose créée par la multiplication bactérienne dans les cellules infectées, nous avons alors entrepris de tester l'implication de TG2 dans les modifications du métabolisme cellulaire au cours de l'infection par *C. trachomatis*. Dans un premier temps nous avons montré que les bactéries infectant des cellules KO pour TG2 étaient plus sensibles à la concentration en glucose dans le milieu que les bactéries infectant des cellules contrôle. En effet, dans des conditions où la concentration en glucose devient limitante (à partir de 1 mg.mL⁻¹ de glucose dans le milieu), nous avons observé une diminution plus importante du nombre de bactéries produites par cycle infectieux dans les cellules KO pour TG2, indiquant effectivement une plus forte dépendance à la concentration en glucose dans le milieu lorsque TG2 est absente. Etant donné que la glycolyse aérobie se traduit par une augmentation de la présence à la membrane plasmique des transporteurs du glucose GLUT et qu'au cours de l'infection par *C. trachomatis* une augmentation des transporteurs GLUT-1 et GLUT-3 avait été décrite dans la littérature, nous avons cherché à savoir s'il y avait un lien entre l'augmentation de transcription des gènes codant GLUT-1 et GLUT-3 et TG2 dans le cas de l'infection. Par RT-qPCR, nous avons démontré qu'en présence de CP4d inhibant TG2, les cellules infectées 46 h ne présentaient plus d'augmentation de l'expression des gènes codant GLUT-1 et GLUT-3 et revenaient à un niveau d'expression équivalent à des cellules non infectées. Tous ces résultats semblent indiquer que dans les cellules infectées, l'élévation de l'expression et de l'activité de TG2 permettent une modification du métabolisme cellulaire, en particulier de l'expression des gènes codant GLUT-1 et GLUT-3. Cette augmentation est nécessaire à un import suffisant de glucose au sein des cellules infectées, pour le bon développement et la bonne multiplication des bactéries.

Par ailleurs, nous avons entrepris d'identifier des cibles de TG2 au cours de l'infection par *C. trachomatis*. Pour ce faire, nous avons incubé des cellules infectées ou non avec une sonde de l'activité de TG2, la biotine pentyamine, et infecté ces cellules pendant 48 h. Cette

sonde, biotinylée, est incorporée par liaison covalente dans les substrats de TG2, que nous avons ensuite purifiés à l'aide de billes couplés à la streptavidine puis identifiés par spectrométrie de masse. Sur les soixante-deux protéines identifiées qui étaient significativement enrichies dans les échantillons infectés par rapport aux échantillons non infectés, certaines protéines étaient des cibles déjà décrites comme cibles de TG2, notamment la fibronectine, RhoA ou la galectine-3. De plus, les deux isoformes de GFPT (glucosamine-6-phosphate amidotransférase), GFPT1 et GFPT2 ont été identifiées. GFPT est la première enzyme, catalysant l'étape cinétiquement limitante, de la voie de biosynthèse des hexosamines (VBH). Cette enzyme convertit le fructose-6-phosphate, produit à partir de glucose dans la glycolyse, en glucosamine-6-phosphate et est donc directement en lien avec le métabolisme du glucose. Le produit final de cette voie métabolique, l'UDP-N-acétylglucosamine (UDP-GlcNAc), est utilisé dans les cellules pour réaliser les *O*- et *N*-glycosylations des protéines dans la voie sécrétoire dans le réticulum endoplasmique et l'appareil de Golgi, ainsi que l'*O*-N-acétylglycosylation (*O*-GlcNAcylation), une modification post-traductionnelle de protéines principalement cytosoliques et nucléaires. Afin de tester si une des isoformes de GFPT avait un impact sur le développement de *C. trachomatis* et pouvait représenter une cible intéressante de TG2, nous avons testé l'effet d'une diminution de la quantité de GFPT1, au cours de l'infection. Cet ARNsi dirigé contre GFPT1 induit un effet sur l'infection, et cet effet est assez similaire à celui de l'inhibition de TG2 par le CP4d une fois que les bactéries sont entrées dans la cellule : on observe des inclusions de taille réduite, avec moins de bactéries produites à l'issue d'un cycle de développement.

Fort de cette observation, nous avons émis l'hypothèse que la modification de GFPT1 par TG2 devait avoir un effet stabilisant voire activateur sur cette protéine, puisqu'en absence de GFPT1 le phénotype sur l'infection était similaire à une absence de TG2. GFPT est l'enzyme limitante de la VBH, l'augmentation de son activité entraîne une augmentation du produit final de la VBH, l'UDP-GlcNAc. Ce métabolite est lui-même le substrat de l'*O*-GlcNAc transferase (OGT), et, de fait, la mesure du niveau global d'*O*-GlcNAcylation sert communément de révélateur du niveau d'activité de GFPT. Dans un premier temps, nous avons étudié le niveau d'*O*-GlcNAcylation après une activation de TG2 par le traitement des cellules avec un ionophore du calcium, pour s'affranchir de la complexité du système infectieux. Des cellules traitées pendant 6 h avec l'ionomycine présentent une forte augmentation de l'activité transaminase de TG2, ainsi qu'une augmentation du signal lié à l'*O*-GlcNAcylation détecté par immunoblot. Cette augmentation est perdue si les cellules sont traitées avec un inhibiteur de

TG2, ou si l'expression de l'enzyme est éteinte par ARNsi. La quantité de protéine de GFPT1 restant sensiblement identique suite à la stimulation par l'ionomycine, cela confirme que TG2 influe positivement sur l'activité de GFPT1. Dans un second temps, nous avons suivi le niveau d'*O*-GlcNAcylation au cours de l'infection. Nous avons été surpris de ne pas observer d'augmentation de l'*O*-GlcNAcylation dans ce contexte, alors même que l'augmentation de l'activité de TG2 est patente. Nous avons alors postulé que l'UDP-GlcNAc produit par la VBH était peut-être importé dans l'inclusion et utilisé par la bactérie, notamment pour synthétiser son peptidoglycane. Soutenant cette hypothèse, nous avons observé que l'activation de TG2 avec de l'ionomycine entraîne une augmentation moindre de l'*O*-GlcNAcylation dans les cellules infectées que dans les cellules non infectées. L'hypothèse d'une utilisation de l'UDP-GlcNAc par les bactéries est également étayée par la présence à la membrane de l'inclusion d'un transporteur normalement présent au niveau du réticulum endoplasmique, SLC35D2, capable de transporter entre autres l'UDP-GlcNAc.

Conclusion

Au cours de ce travail nous avons pu mettre en évidence le rôle clef de TG2 dans la manipulation de la cellule hôte par la bactérie *C. trachomatis*. La question de l'acquisition des nutriments au sein d'une cellule pour les pathogènes intracellulaires, qu'ils soient bactériens, viraux ou parasitaires, tout en maintenant cette cellule en vie suffisamment longtemps, est capitale. Nous avons pu montrer ici l'importance de TG2 pour l'augmentation de l'import de glucose dans la cellule infectée ainsi que pour la production d'un métabolite utilisé par la bactérie pour sa division ; il se pourrait que la stratégie démontrée ici chez *Chlamydia* ait été adoptée indépendamment au cours de l'évolution par d'autres pathogènes ou symbiontes.

Par ailleurs, cette étude ne se cantonne pas au modèle d'infection et identifie une nouvelle cible de TG2 hors contexte infectieux : GFPT1. L'origine de l'augmentation de l'activité de GFPT1 permise par sa modification par TG2 reste incomprise, cependant nous avons initié des études biochimiques et identifié une glutamine de GFPT1 modifiée, ouvrant la voie à une explication moléculaire de ce phénomène.

Plusieurs hypothèses peuvent expliquer l'augmentation de TG2 et de son activité durant l'infection par *C. trachomatis*. La production par les cellules infectées d'IL-6, une cytokine pro-inflammatoire pouvant agir sur le promoteur de *TGM2* et induire sa transcription pourrait être à la base de l'élévation du niveau de TG2, en partie responsable de l'élévation de l'activité

transaminase dans ces cellules. Par ailleurs, une élévation locale à proximité de la membrane de l'inclusion de la teneur en Ca^{2+} cytosolique, liée probablement à la présence de réticulum endoplasmique à cet endroit, décrite précédemment, pourrait être à la base de l'activation de TG2. De plus, en permettant une activation uniquement locale de TG2, ceci ne perturberait pas l'homéostasie cellulaire par une activation massive et généralisée dans la cellule de cette enzyme.

Dans le contexte plus complexe de l'infection d'un organisme animal par *C. trachomatis*, il est probable que le rôle de TG2 se complexifie du fait de ses différentes localisations cellulaires et des fonctions variées que cette enzyme peut exercer dans les différents compartiments qu'elle occupe ainsi que suivant le type cellulaire l'exprimant. De plus, les rôles déjà décrits de TG2 dans l'établissement de phénomènes de fibrose tissulaire au cours de processus de cicatrisation mal régulés laissent à penser que l'activation, au cours de l'infection, de cette enzyme puisse être reliée avec les symptômes se développant des suites d'infections chroniques à *Chlamydia*, à la base de la stérilité pouvant être induite chez la femme.

INTRODUCTION

I. *Chlamydia trachomatis*, a human-adapted obligate intracellular pathogen.

A. *C. trachomatis* phylogeny and associated pathologies.

1. The *Chlamydiae*: a phylum of its own.

Chlamydia trachomatis is a bacterium belonging to the phylum “*Chlamydiae*”. This phylum of Gram-negative bacteria branched out very early in evolution, several hundreds of millions of years ago, and one key feature of its members is that they are all obligate intracellular organisms, unable to multiply outside a host cell (Horn and Wagner, 2004). The second characteristic feature of all *Chlamydiae* is that these bacteria undergo a very peculiar biphasic developmental cycle that is discussed in the next part (Horn, 2008).

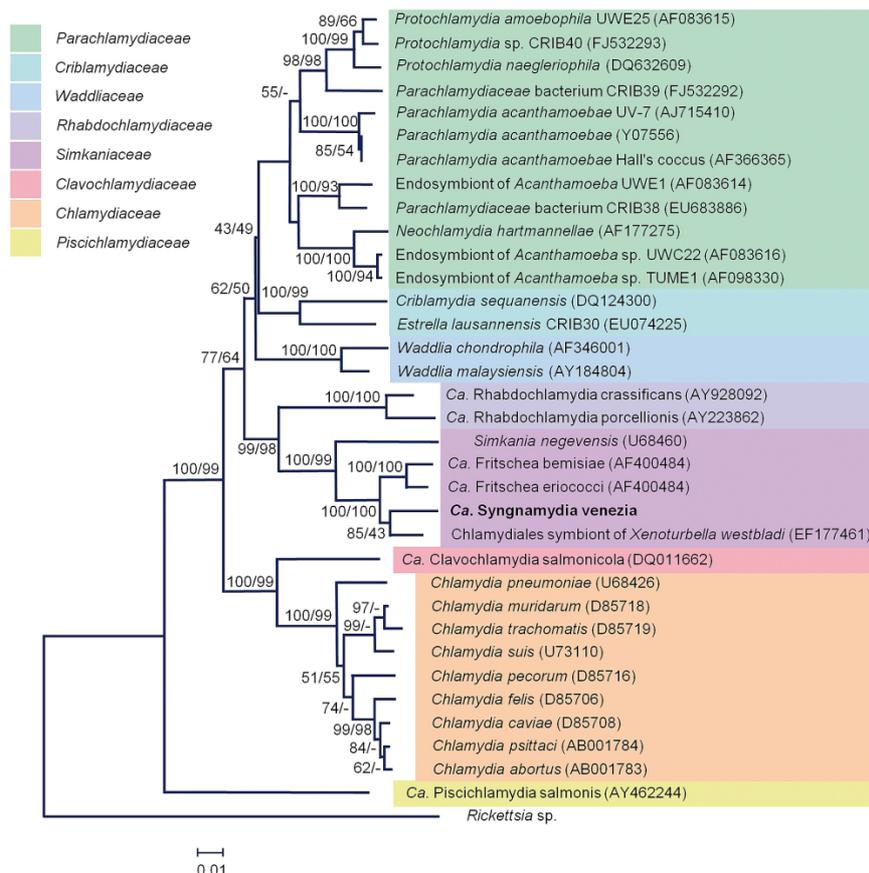


Figure 1: Phylogenetic tree of the *Chlamydiae*

This phylogenetic representation of the *Chlamydiae* phylum is based on the complete 16S rRNA sequence, using *Rickettsia* sp. as outgroup. *Ca.* stands for *Candidatus*, species discovered at the time of the publication and proposed as new species. From Fehr *et al*, 2013.

The *Chlamydiae* phylum counts only one Class (*Chlamydiia*) and one order, the *Chlamydiales*. Increasing numbers of families are being described thanks to deep sequencing techniques applied to various environmental samples such as sediments, soils or aquatic environments (figure 1) (Bavoil *et al*, 2013; Horn, 2008; Salamin *et al*, 2015).

2. The *Chlamydiaceae*, a family adapted to animal hosts.

The species belonging to the family *Chlamydiaceae* have a very condensed genome for bacteria (around 1Mb). In contrast to members of other chlamydial families that can develop in unicellular organisms, *Chlamydiaceae* only infect animals. Co-evolution with these hosts has led to a massive reduction in the size of their genome (in comparison, the genome of *Protochlamydia amoebophila* makes 2.4 Mb), and to an increased dependency on metabolic functions of their host (Brian *et al*, 2004; Stephens, 1998; Omsland *et al*, 2014).

Infections by *Chlamydiaceae* lead to the development of diverse pathologies depending on the host and the organs infected. The history of the *Chlamydiae* started with the discovery of organisms developing in the conjunctival epithelial cells of humans that were later ascribed to the genus *Chlamydia*. It is only during the 1960s that they were classified as bacteria and not viruses or protozoa given their small size and their unique developmental cycle. All *Chlamydia* species infect animals including mammals. The range of animals possibly infected varies with the species, and most of the available information deals with species that have an impact on human health or on domesticated animals (Nunes and Gomes, 2014). Five out of the nine *Chlamydia* species are able to infect humans. *C. trachomatis* and *C. pneumoniae* are the two most important species considering the number of cases of infection and the associated symptoms. *C. abortus*, *C. psittaci*, and to a lesser extent *C. felis*, are also able to infect man through zoonotic transmission from infected domesticated or wild animals. For the remaining species, no zoonotic transmission has been reported yet (Everett and Bush, 2001; Nunes and Gomes, 2014).

C. pneumoniae develops in the respiratory tract. Often asymptomatic, it can cause acute respiratory infections in humans and its prevalence in the population is high. It has also been associated to chronic diseases such as atherosclerosis or obstructive pulmonary disease (Choroszy-Król, 2014). It has a large spectrum of hosts: marsupials, frogs, horses, etc (Nunes and Gomes, 2014). *C. trachomatis* on the other hand only infects humans and is the *Chlamydia* species with the strongest impact on human health.

3. *C. trachomatis*: a species strictly adapted to humans.

C. trachomatis is the first species of the *Chlamydiae* that has been discovered in the beginning of the 20th century from conjunctival epithelial cells of patients suffering from trachoma. Given the shape and the Giemsa coloration of the vacuole-containing bacteria and the bacteria within cells, researchers at that time thought they had found a new protozoan surrounded by a cloak, hence its original name of “Chlamydozoa”, coming from the ancient Greek “χλαμυσο” meaning cloak.

This species is the causative agent of different pathologies, depending on the site of infection. Due to a diversity in the immunoreactivity of the major outer membrane protein (MOMP), 15 serovars have been identified so far from clinical samples, and those serovars have been grouped into three different biovars associated with pathologies (figure 2) (Nunes *et al*, 2008).

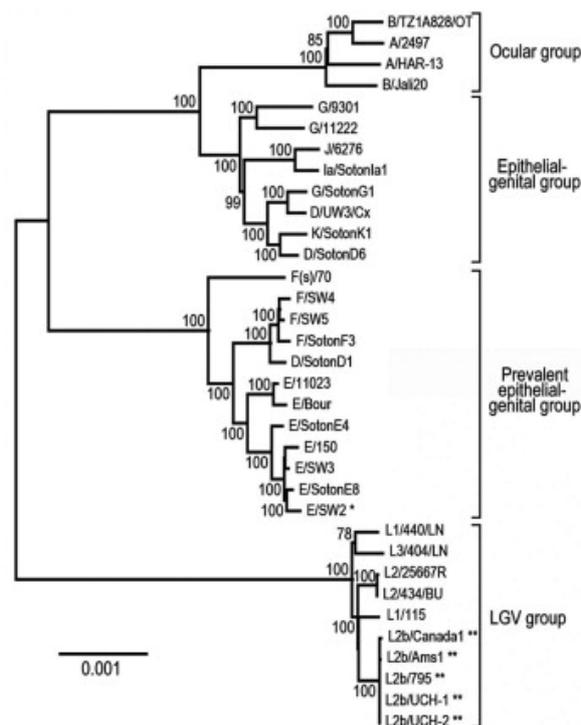


Figure 2: Phylogenetic reconstruction of *C. trachomatis*

The tree was made by comparing 819 genes conserved from the sequenced genomes of different serovars of *C. trachomatis*, showing a genetic correlation between tissue tropism and infection symptoms, and serovar. From Nunes and Gomes, 2014.

a. Serovars A to C and ocular infections.

Serovars A, B, Ba and C have a tropism for the eye conjunctiva. These serovars are mainly endemic in developing countries, where they transmit from contact between infected

people or through flies that have contacted infected fluids. They are the causative agents of trachoma, an eye disease leading to blindness in long term and non-treated infections. The infection causes an inflammation of the eye conjunctiva and the eyelid, resulting progressively in an entropion, where the eyelid folds inward, and the scratching of the cornea by the eyelashes. This phenomenon called trichiasis means the beginning of an opacification of the cornea leading to blindness if left untreated (Mohammadpour *et al*, 2016).

Trachoma is the leading cause of acquired blindness worldwide with 1.8 million people affected and endemic in 51 countries according to the Worldwide Health Organization (WHO). In 2015, 85 million people were diagnosed with an ocular infection by *C. trachomatis* (Taylor *et al*, 2014).

b. Serovars D to K and uro-genital infections.

C. trachomatis is also the leading cause of sexually transmitted infections (STIs) of bacterial origin worldwide, with an estimation of 130.9 million new infections in 2012 (figure 3) (Newman *et al*, 2012).

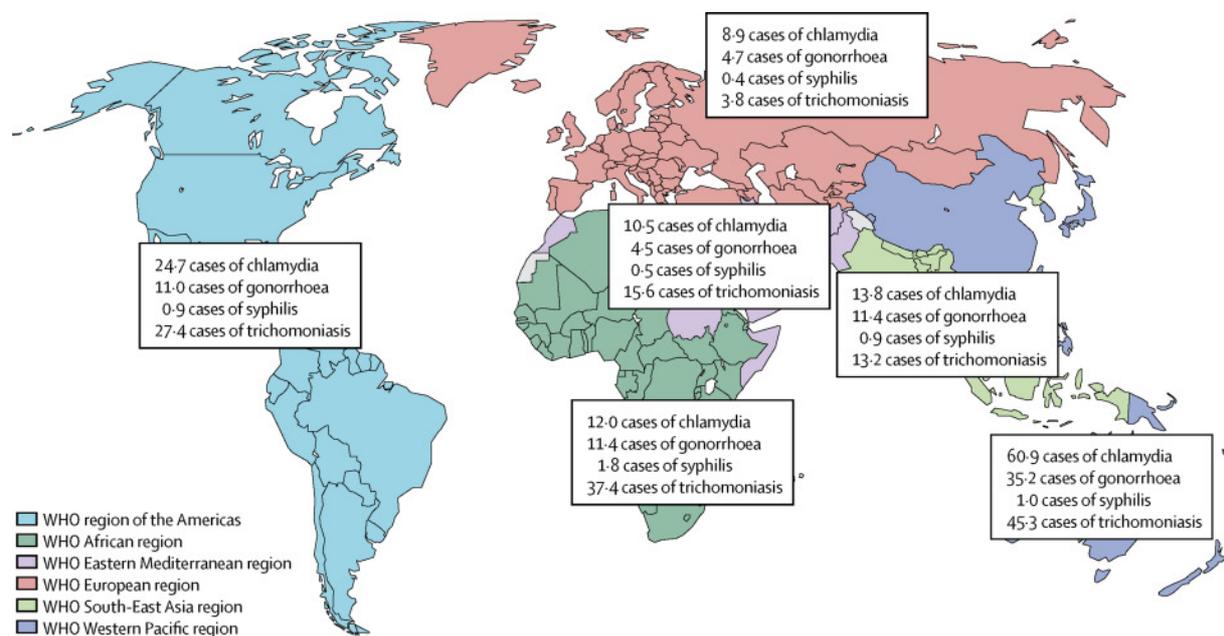


Figure 3: WHO estimates of new cases of four curable STI including *C. trachomatis* in 2012

The estimates are made per region and expressed in millions of new cases per year, data from 2012. From Unemo *et al*, 2017.

The urogenital tract colonization by *C. trachomatis* is quite common for both men and women and often asymptomatic – for one man out of two and three women out of four. The

infection can remain in the lower parts of the genital tract causing cervicitis in woman and urethritis in men. It can also ascend and generate inflammation of the fallopian tubes (salpingitis), the inner lining of the uterus (endometritis) or the epididymis in men. Tissue scarring, especially in long-term or recurring infections in women, can lead to serious sequelae such as chronic pelvic pain, ectopic pregnancies or even infertility (Mackern-Oberti *et al*, 2013; Unemo *et al*, 2017).

c. Serovars L1 to L3 and *lymphogranuloma venereum*.

The serovars L1, L2, L2a and L3 are more invasive and are responsible for a STI called *lymphogranuloma venereum* or LGV. This disease starts with the infection of the genital or colonic epithelium and spreads rapidly towards the blood circulation, disseminating to the draining lymph node by infected circulating monocytes. Once the lymph nodes become infected (10 to 30 days after the initial contamination), the pathology evolves quickly with different symptoms like lymph node necrosis, abscesses or buboes formation, and results in permanent tissue damages (Ceovic and Gulin, 2015; Mabey and Peeling, 2002; Unemo *et al*, 2017).

4. Treatments.

Currently, *C. trachomatis* infections are treated efficiently with antibiotics of the macrolide family such as azithromycin for non LGV serovars, or of the tetracycline family such as doxycycline. No resistance has been reported yet, very likely due to the particular lifestyle of the bacterium, isolated in its intracellular niche (the inclusion, see below). However, the development of a vaccine remains a priority, because many asymptomatic infections are left untreated until the damages are irreversible. Also, infections treated with antibiotics can be followed with reinfections and no efficient immune memory and immunity seems to develop (Unemo *et al*, 2017; WHO strategy, 2016).

Regarding ocular infections, the WHO has launched a program called SAFE (Surgery – for trichiasis, Antibiotics, Facial cleanliness and Environmental improvement) to eradicate the blinding disease in developing countries in 1996. In 1998 based on the SAFE strategy, a resolution passed for the Global Elimination of Trachoma by 2020 (GET2020). This strategy aimed particularly at increasing the health conditions in the developing countries suffering from trachoma. The WHO report in 2014 pointed out that the situation reported in the 30 countries that participated actively in the survey has improved significantly but major work is still to be done, especially on the F and E components of the SAFE strategy (WHO report, 2014).

5. Animal models of infection.

Since *C. trachomatis* only infects humans, the *in vivo* research on this bacterium has suffered from the lack of suitable animal model. *C. muridarum* is a species adapted to mice and is genetically very close to *C. trachomatis*. It has thus been extensively used as an animal model of *Chlamydia* infection, although important immunological differences between the human and mouse physiology in the reproductive tract exist, and in particular in the innate immune response (De Clercq *et al*, 2013; Haldar *et al*, 2016). It is possible to infect mice with *C. trachomatis* but a higher dose of bacteria is needed and the bacteria are cleared in less than 2 weeks, even in nude mice, implying that the murine innate immune system is sufficient to eliminate *C. trachomatis* (Tuffrey *et al*, 1982).

Guinea pig infection with *C. caviae* was one of the first animal model implemented, and the immune response seems closer to the one happening in human (Miyairi *et al*, 2010; Shaw *et al*, 2017). But the paucity of tools in guinea pigs compared to mice, and its higher cost have limited the use of this alternative animal model.

Finally, nonhuman primate models have also been tested. The pig-tailed macaque is arguably the closest to human in terms of physiology (menstrual cycle of 28 to 30 days, anatomy similar) and physiopathology (no need for pre-treating the animals with hormones for example to be susceptible to *C. trachomatis* genital infections) and is more convenient for breeding (Bell *et al*, 2011). However, it is scarcely used, due to the practical disadvantages and ethical concerns inherent to macaque studies.

Finally, the last few years have seen the emergence of a new concept, supported by many epidemiological observations, clinical samples and experiments in mice: the possibility that at least some *Chlamydia* species are commensal bacteria colonizing our gastrointestinal tract. Data from mice experiment using *C. muridarum* support this hypothesis by revealing a possible gastrointestinal tract long-lasting colonization from a genital tract initial infection (Bavoil *et al*, 2017; Wang *et al*, 2018; Zhong, 2017). There is currently no data supporting a gastrointestinal tract colonization by *C. trachomatis* in humans, but such a scenario would explain among others the reinfection of certain women without any sexual relation after treatment and may account for a large number of chronic infections causing long-term sequelae. This is thus certainly an interesting hypothesis to follow on.

B. A bacterium with a peculiar biphasic developmental cycle.

1. Overview of the developmental cycle.

C. trachomatis, along with the other *Chlamydiae*, undergo an intracellular developmental cycle characterized by the alternation of two different developmental forms, a small (around 0.3 μm of diameter) and infectious form called elementary body (EB) and a dividing form (around 1 μm of diameter) called reticulate body (RB).

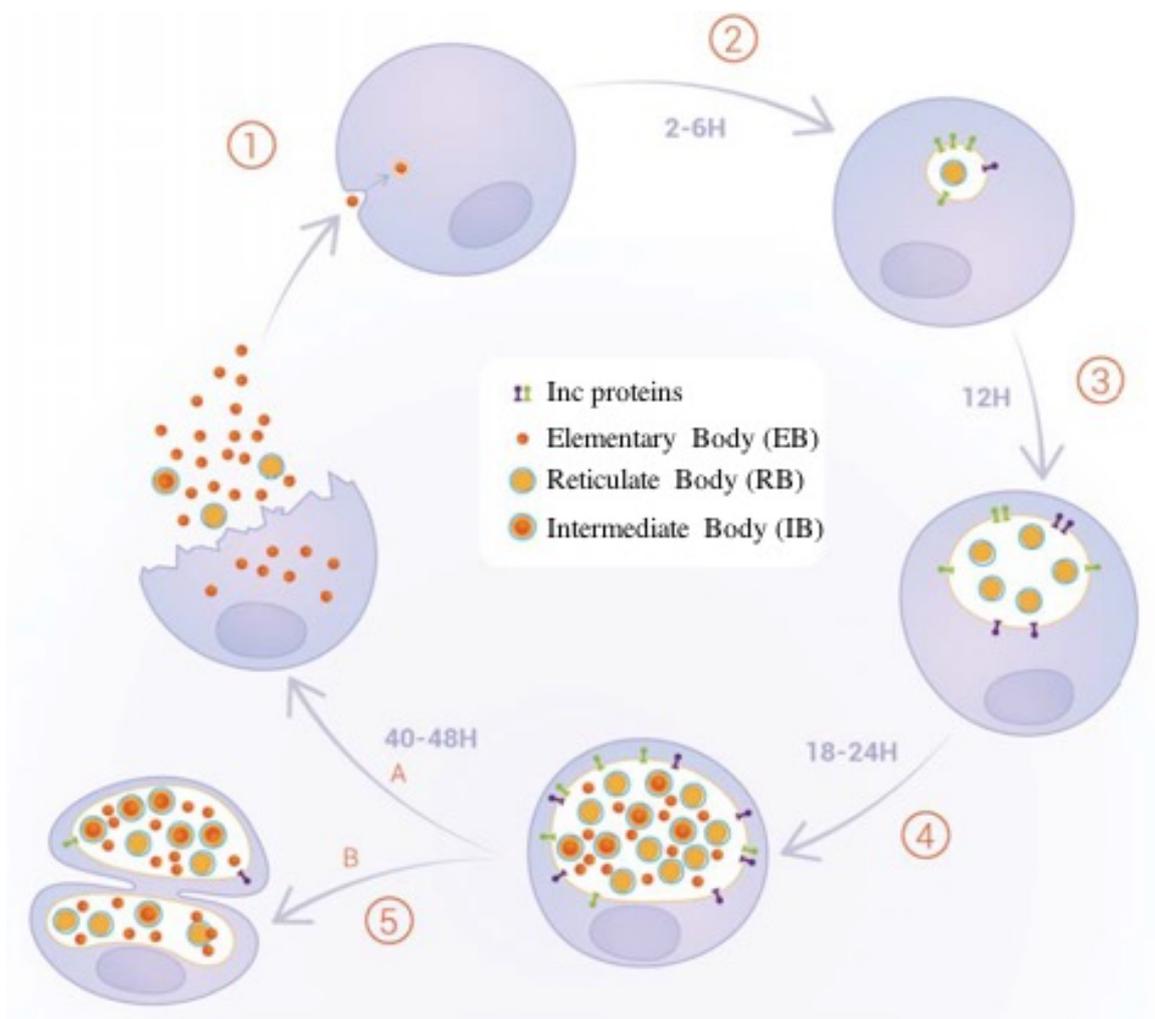


Figure 4: *C. trachomatis* developmental cycle

After adhesion at the cell surface, the EB gets quickly internalized in a vacuole called “inclusion” (1). The inclusion migrates towards the microtubule organizing center. The EB differentiates into an RB (2), that is the only replicative form of the bacteria (3). After about 24 h, some RBs differentiate back into EBs in an asynchronous manner (4) and ultimately the bacteria, mainly EBs, exit the cell either by lysis (5A) or extrusion (5B). Figure kindly provided by Dr. Mathilde Cossé.

The cycle starts with the attachment of an EB at the surface of a cell, followed by its internalization (figure 4). Once inside the cellular cytoplasm, the bacterium remains in a membrane-bound compartment called inclusion all along the cycle. Within a few hours following entry, the EB gradually differentiates into an RB. The RB divides inside the growing inclusion until, asynchronously, some RBs differentiate back into EBs starting 24 h post-infection (hpi) and until the end of the cycle about 48 h after entry of the bacterium. An intermediate form called intermediate body (IB) corresponding to a transition from RB to EB has been described based on electron microscopy pictures (Kajima *et al*, 1964). At the end of the developmental cycle, the bacteria, mostly EBs, are released from the cell either by lysis of the cell after breakage of the inclusion membrane and the plasma membrane, or by a so-called extrusion with the release of bacteria still wrapped into an inclusion surrounded itself by another membrane.

2. Adhesion and invasion of the host cell.

Although *C. trachomatis* is able to infect mostly epithelial cells *in vivo*, many different cell types can be infected *in vitro*, even cells of non-human origin such as mouse embryonic fibroblasts (MEFs). This ability *in vitro* indicates a diversity of receptors involved in the bacterial adhesion and entry (Guseva *et al*, 2007). Indeed, several surface proteins have been implicated as potential bacterial receptors as detailed below. The adhesion process proceeds in two steps, a first step which is a loose adhesion based on electrostatic interactions, followed by a firm and irreversible adhesion preceding the invasion step (Dautry-Varsat *et al*, 2004).

The heparan sulfate (HS) present on the proteoglycan secreted by the cells and on their surface have been shown to play an important role in the adhesion: bacteria no longer attached to cells treated with an HS lyase (Zhang and Stephens, 1992). Other cellular receptors have been identified such as the protein disulfide isomerase (PDI), the platelet-derived growth factor receptor beta (PDGFR β) or the EphrinA2 receptor, using naturally KO cell lines or siRNA screening (Abromaitis and Stephens, 2009; Elwell *et al*, 2008; Hegemann and Moelleken, 2012; Subbarayal *et al*, 2015).

On the bacterial side, it has been shown that MOMP exposed at the surface of the EBs was able to interact with HS present on cellular glycosaminoglycans, and cells incubated with recombinant MOMP were resistant to infection by *C. trachomatis* LGV-L2 (Su *et al*, 1996; Stephens *et al*, 2001). Other bacterial determinants have been shown to be important such as

the outer membrane complex B (OmcB) and polymorphic membrane proteins (Pmps) (Becker and Hegemann 2014; Fadel and Eley, 2008).

It is important to point out that the requirements in one or another receptor at the cell surface vary in importance depending on the serovars used and may account for the difference in tropism *in vivo* (Carabeo and Hackstadt, 2001; Fadel and Eley, 2008). Moreover, the diversity of proteins identified on both sides of the interaction reflects probably, at the evolution scale, the absolute requirement for the bacterium to enter a cell in order to complete its developmental cycle.

During this firm adhesion step, the bacterial type 3 secretion systems (T3SSs) present in the EBs, preformed and polarized, get in contact with the cellular plasma membrane, allowing the injection of bacterial effectors such as CT694 or the translocated actin recruiting phosphoprotein (TarP) (figure 5) (Clifton *et al*, 2004; Fields *et al*, 2003; Hower *et al*, 2009; Nans *et al*, 2014).

Cooperation between host cell proteins and bacterial effectors allow the entry of the bacteria into the cell mediated by a local actin remodeling (Cossé *et al*, 2016). TarP is able to interact with actin monomers and nucleate the polymerization of filaments (Clifton *et al*, 2004; Jewett *et al*, 2006). CT694 interacts with AHNAK, a host cell protein involved in the formation of actin stress fibers and inhibits its F-actin bundling activity. However, the analysis of CT694 KO strains recently suggested that the role of CT694 in entry was not mediated by its interaction with AHNAK (Hower *et al*, 2009; McKuen *et al*, 2017). On the cellular side, it has been shown that the small GTPase Rac was activated locally, leading to a recruitment and activation of Arp2/3 complexes through a signaling cascade, also contributing to a local actin remodeling (Carabeo *et al*, 2004; Carabeo *et al*, 2007; Elwell *et al*, 2008). This modification in the actin cytoskeleton allows for the formation of membrane projections that surrounds the bacterium, leading eventually to its internalization in a membrane bound compartment: the inclusion (Carabeo *et al*, 2002; Nans *et al*, 2014). More recently, syntaxin-9 (SNX9), a cellular protein involved in filipodia formation and micropinocytosis, joined the growing list of proteins involved in actin remodeling to allow bacterial entry (Ford *et al*, 2018).

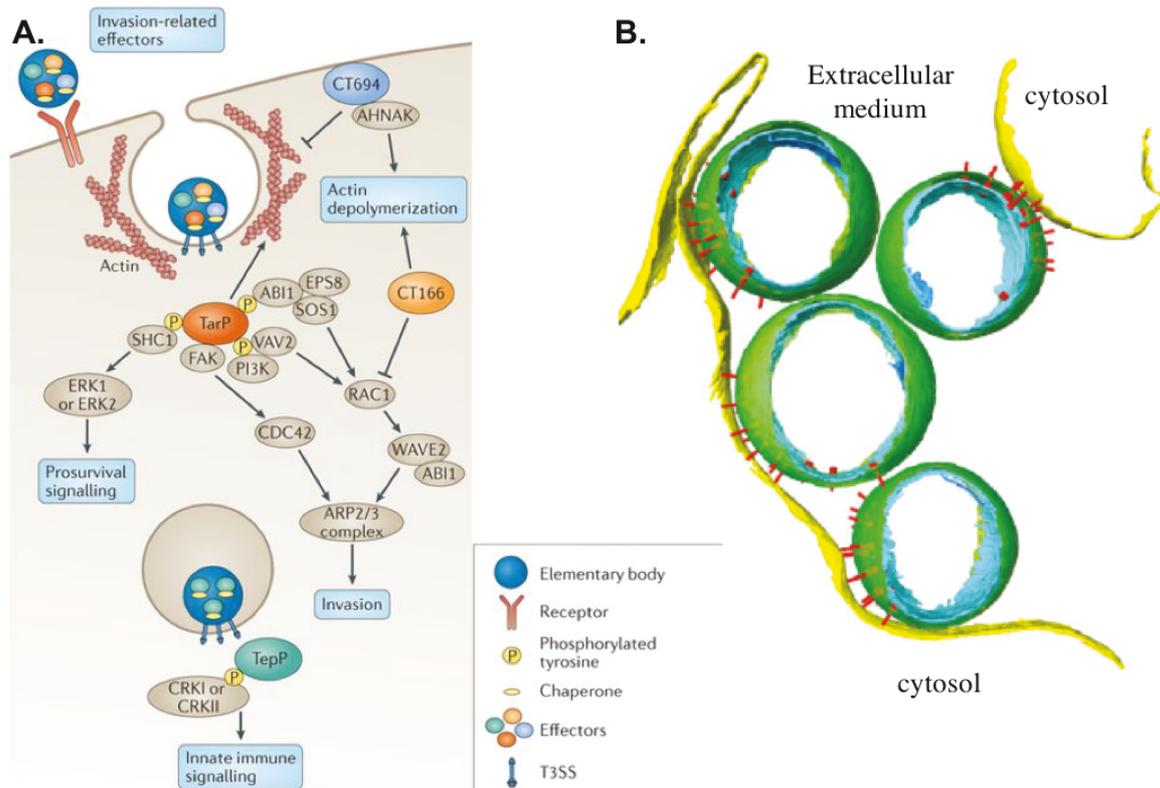


Figure 5: *Chlamydia* effectors secreted at the entry of the bacteria into its host cell

A. The secretion of TarP and its phosphorylation by cellular tyrosine kinases leads to the polymerization of actin cytoskeleton, forming protrusions around the bacteria. CT166 and CT694 by stimulating local actin depolymerization favor cytoskeleton rearrangements beneficial for bacterial entry. TarP contributes also to stimulating pro-survival signaling pathways while TepP, secreted and phosphorylated a few minutes after invasion, is involved in the downregulation of the cellular immune response. From Elwell *et al.*, 2016.

B. Three-dimensional reconstruction from cryo-electron tomogram of *C. trachomatis* adhering at the surface of U2OS cells, with T3SSs polarized towards the plasma membrane of the cells. Yellow: cellular plasma membrane; red: T3SSs; green: bacterial outer membrane; cyan: bacterial inner membrane. From Nans *et al.*, 2014.

3. The EB to RB conversion.

After its entry in the host cell, the differentiation program from EB to RB is initiated. Transcription of a specific subset of genes is turned on less than an hour after entry of the bacterium, allowing the expression of 29 immediate-early genes involved in nutrient acquisition and constitution of the identity of the inclusion membrane (Belland *et al.*, 2003; Shaw *et al.*, 2000). This second feature is achieved by the production of so-called Inc proteins, proteins secreted by *Chlamydia* T3SS and inserted into the inclusion membrane (Bannantine *et al.*, 2000). *C. trachomatis* genome encodes for about fifty Inc proteins, which are transcribed and secreted at different times during the cycle of the bacterium and largely contribute to define the interactions between the new cellular compartment – the inclusion – and the rest of the

organelles and vesicles (Mirrashidi *et al*, 2015; Weber *et al*, 2015). This is mediated by interactions between the Inc proteins and host proteins such as various proteins of the Rab family, SNAREs constituting the vesicle-fusion cell machinery, cytoskeletal proteins like CEP170 (Damiani *et al*, 2014; Delevoye *et al*, 2008; Dumoux *et al*, 2015; Kabeiseman *et al*, 2013; Mirrashidi *et al*, 2015; Moore *et al*, 2011; Pokrovskaya *et al*, 2012)...

The signal(s) inducing the transition from EB to RB is not known to date, but this transition occurs between 2 and 6 hours after entry. Two main morphological modifications happen to the EB, necessary for the RB functions:

- a reduction of the disulfide bounds cross-linking the proteins from the chlamydial outer membrane complex (COMC), mainly MOMP and the cysteine rich proteins OmcA and OmcB, leading to a relaxation of the EB (Hackstadt *et al*, 1985; Hatch *et al*, 1986).
- a decondensation of the nucleoid allowed by the dissociation of the histone-like proteins Hc1 and Hc2 from the bacterial chromosome (Barry *et al*, 1992; Brickman *et al*, 1993; Christiansen *et al*, 1993). A small metabolite from the methylerythritol 4-phosphate pathway, produced after the immediate-early transcription, is involved in this process (Grieshaber *et al*, 2004).

The differentiation from EB to RB is characterized by a drastic change in the size of the bacterium (0.3 μm of diameter for the EB compared to 1 μm for the RB) accompanied by the transcription of many genes allowing an active metabolism and the multiplication of the RB (Belland *et al*, 2003; Nicholson *et al*, 2003). RBs are non-infectious and osmotically much more fragile than EBs, mainly due to the absence of the disulfide bounds crosslinking proteins from the COMC. The bacterial actin-homologue protein MreB has been shown as crucial to allow this transition, maybe by ordering and stabilizing the change in shape (Liechti *et al*, 2016).

A few hours after bacterial entry, the inclusion is transported along microtubules by the dynein minus-end-directed molecular motor (Clausen *et al*, 1997; Grieshaber *et al*, 2003). The interaction between dynein and the inclusion is likely mediated by the Inc protein CT850, shown to interact with the dynein light chain DYNLT1 (Mital *et al*, 2015). This transport ends with the localization of the inclusion in close proximity with the nuclear membrane, the endoplasmic reticulum (ER) and the Golgi apparatus, at the microtubule organization center (MTOC) (Dumoux *et al*, 2015; Hackstadt *et al*, 1996; Stanhope *et al*, 2017).

4. The proliferation phase.

When considering the multiplication of RBs inside the inclusion, it is important to realize that no multiplication can take place without a supply in energy and nutrients coming partially from the host cell, and without an expansion and stabilization of the inclusion that needs an increase in size to accommodate the increasing number of bacteria. All the elements of the cytoskeleton have been shown to play an important role in the stability of the inclusion. A stable microtubule network (detyrosinated microtubules) surrounds the inclusion at already 12 hours post infection and stays until the end (Al-Zeer *et al*, 2014). The organization of this network is achieved by the interaction between an Inc protein called IPAM (inclusion protein acting on microtubules) and the centrosomal protein of 170 kDa (CEP170) (figure 6) (Dumoux *et al*, 2015). An actin “cage” has also been described, forming a nest around the inclusion along with septin fibers. If the actin network or the septin is disorganized by using respectively a drug (cytochalasin D) or siRNAs, the inclusion tends to rupture (Kumar and Valdivia, 2008; Zuck *et al*, 2017). Intermediate filaments contribute ultimately to the stability of this large stabilizing scaffold with vimentin for which the modification of certain residues by addition of *O*-linked β -N-acetylglucosamine is essential (Tarbet *et al*, 2018). A disruption of any of these different cytoskeletal elements results in a decrease in the progeny produced by the bacteria after an infectious cycle.

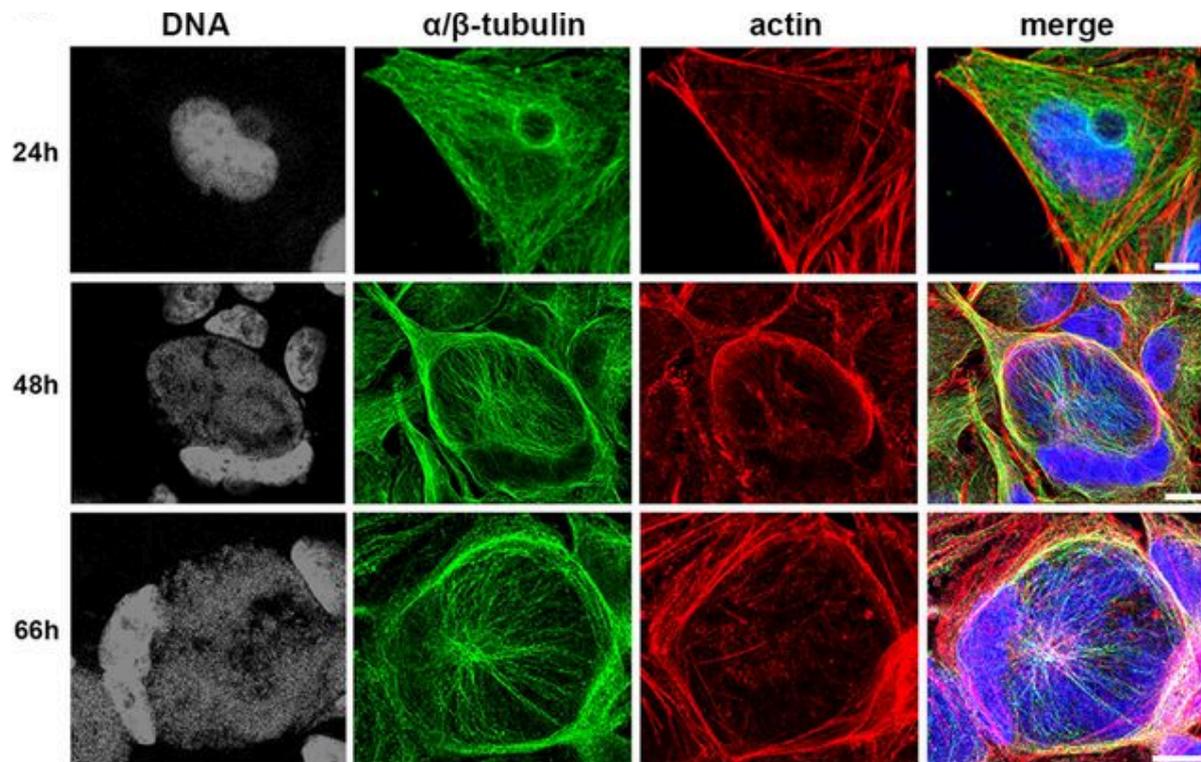


Figure 6: actin and microtubule filaments organization around *C. trachomatis* inclusion

HeLa cells were infected with *C. trachomatis* for 24 to 66 h (indicated on the left), then fixed and stained for confocal microscopy revealing tubulins (green), actin (red) or DNA (grey, blue on merge pictures). A maximum intensity projection of z-stacks is represented. Scale bar: 10 μ m. From Dumoux *et al*, 2015.

Inside the inclusion, RBs divide actively and even though a recent study was suggesting that they divided by some sort of budding mechanism, the last data acquired using quantitative three-dimensional electron microscopy confirmed that they divide by binary fission as assumed initially (Abdelrahman *et al*, 2016; Lee *et al*, 2018). This division is allowed by a subversion of host cell metabolites such as glucose (more detailed in part II), or lipids. Indeed, eukaryotic lipids such as cholesterol or phosphatidylcholine seem to be present in purified bacteria, for example (Cox *et al*, 2012; Cox *et al*, 2015; Hatch and McClarty, 1998; Yao *et al*, 2015).

During a long period, the division mechanism of *Chlamydia* was a mystery, since the bacterial genome does not encode a homologue of FtsZ, a protein commonly regulating division along with peptidoglycan biogenesis (Stephens, 1998). Moreover, no detection of any peptidoglycan was achieved by common techniques, even though the bacterial growth was sensitive to penicillin and the genome encoded all the enzymes for peptidoglycan biosynthesis (Barbour *et al*, 1982; Fox *et al*, 1990; Moulder *et al*, 1963; Stephens, 1998). Recent studies have unraveled this so-called “chlamydial anomaly”, by detecting the peptidoglycan at the septum of division (Packiam *et al*, 2015). This peptidoglycan is composed of glycan chains of

N-acetylmuramic acid linked to *N*-acetylglucosamine (GlcNAc) saccharides cross-linked to peptide chains and seems to be produced only at the division septum of the bacteria along with MreB. The low abundance of peptidoglycan might be part of the adaptation to Vertebrates, to avoid a stimulation of pathogen recognition receptors (PRRs) present in the cell such as NOD2 or TLR4 (Liechti *et al*, 2014; Liechti *et al*, 2016; Packiam *et al*, 2015).

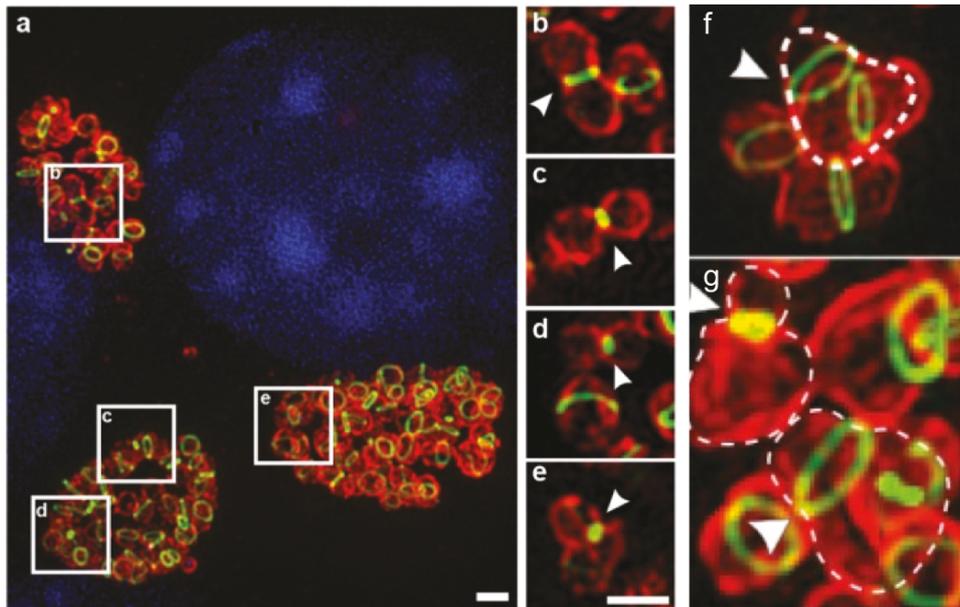


Figure 7: *C. trachomatis* mid-cell peptidoglycan ring

HeLa cells infected with *C. trachomatis* were incubated with ethynyl-D-Alanine-D-Alanine (green in the pictures), a fluorescent probe incorporated into the peptidoglycan during its biosynthesis, from 2 to 18 hpi. Cells were fixed and stained for MOMP (red) to delimit the bacterial outer membrane and for the cell nuclei with DAPI (blue). Scale bar: 1 μ m. Images a. to e. show a maximum intensity projection of confocal microscopy images revealing the presence of a peptidoglycan ring. Magnifications b. to e. present with arrowheads division septa with peptidoglycan puncta. Images f. and g. were acquired with structured illumination microscopy and present with arrowheads asymmetric divisions of the bacterium. From Liechti *et al*, 2016.

5. Retro-conversion and cell exit.

The signals involved in the conversion from RB back to EB are not fully understood. This differentiation happens asynchronously, with the first EB observed in the inclusion about 24 hours post infection while other RB continue to replicate, so that mature inclusions contain of a mixture of EBs and RBs. This year, an elegant study based on quantitative three-dimensional electron microscopy at different times of infection allowed Lee and collaborators to formulate a new hypothesis to explain the asynchrony in RB to EB differentiation. Based on the observation that RBs differ in size, the authors proposed that size reduction occurs because division takes place before bacterial size doubles, and that under a certain size threshold, the

transition RB to EB is initiated. This hypothesis is supported by a mathematical model, predicting the number of EBs and RBs in an inclusion at a certain time of infection (Lee *et al*, 2018). However, the signal that initiates the conversion *per se* remains to be determined.

After 36 to 48 hours of infection, the newly formed EBs are freed in the extracellular medium by two alternative mechanisms: lysis of the host cell or extrusion of the inclusion, which can potentially leave the host cell intact (Hybiske and Stephens, 2007). The cell lysis relies on the action of proteases permeabilizing the inclusion membrane first, followed with other organelles. Ultimately, a calcium-dependent permeabilization of the plasma membrane allows the release of thousands of EBs in the extracellular medium (figure 8) (Hybiske and Stephens, 2007). This process takes about 15 min for *C. trachomatis* LGV-L2 serovar. The extrusion mechanism on the other hand requires the activation of the actin-based motor myosin II by phosphorylation. Work by the Hackstadt's lab unraveled a complex Ca²⁺ based control of myosin II phosphorylation, involving the recruitment, by Inc proteins, of several host kinases and phosphatases, and calcium channels, at specific sites of contacts between the endoplasmic reticulum (ER) and the inclusion membrane (Lutter *et al*, 2013; Nguyen *et al*, 2018). Eventually, local increase of calcium, released from endoplasmic reticulum, promotes myosin motor activity, which drives the extrusion process and the release of part of an inclusion or an entire inclusion surrounded by a membrane in the medium. This exit strategy has proven to be protective for the bacteria, increasing their survival in the extracellular medium, and, even more interestingly, after being phagocytosed by macrophages and dendritic cells, allowing the exit of still infectious particles from those cells (Sherrid and Hybiske, 2017; Zuck *et al*, 2017). Very recently *in vivo* evidence for the existence of bacteria enclosed within an extrusion membrane has been documented in mice (Shaw *et al*, 2017).

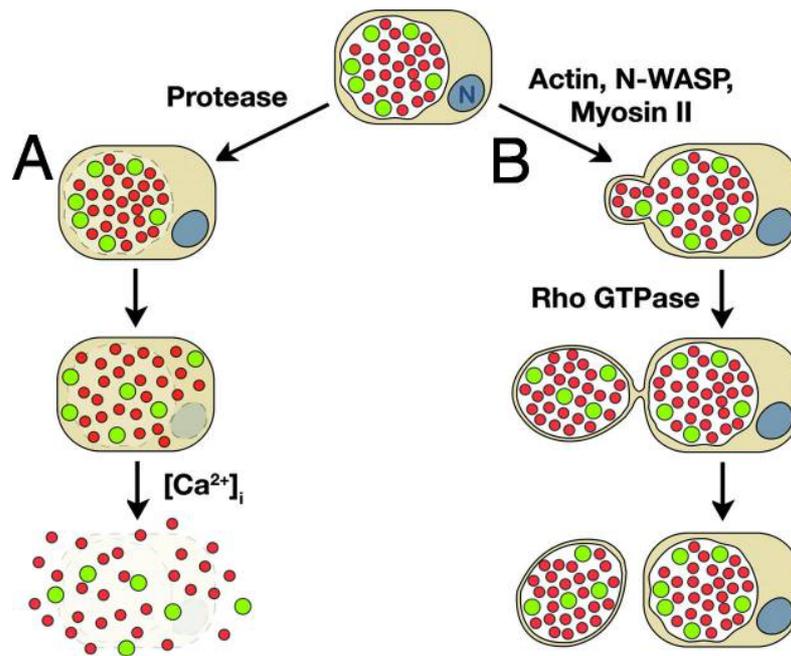


Figure 8: Exit mechanisms of *C. trachomatis*

Schematic representation of the two mechanisms for *Chlamydia* exit from its host cell: a cell lysis after rupture of the vacuole membrane, dependent on calcium influx (A) or the extrusion mechanism controlled by actin cytoskeleton remodeling (B). Red dots represent EBs and green dots represent RBs. From Hybiske and Stephens, 2007.

C. Host-bacterium interactions.

1. A fragile equilibrium until the completion of the developmental cycle.

Eukaryotic cells have developed a number of defense strategies to eliminate intruders. To survive in this environment, *Chlamydiae* have developed molecular strategies to interfere with host defense, in particular with the ultimate host response that is cell death. It is particularly important for these bacteria that the host cell survives as long as possible after the beginning of infection because premature cell death releases mostly RBs, which are not able to initiate a new cycle. Also, very important for the bacteria is to ensure access to host resources in spite of their separation from the cytoplasm. Overall, interactions between the host and the bacteria are omnipresent during the whole developmental cycle, from the entry step to the extracellular release. Most of these interactions are mediated by bacterial proteins called effectors, which evolved to utilize host proteins to their advantage, and to neutralize host defenses, after being secreted in the cytoplasm of the cell.

2. Secretion of chlamydial proteins in the host cytoplasm.

Secretion refers to the capacity, shared by all cells, to release a selected subset of the proteins they produce beyond the membrane that defines them as individual entities. Bacterial secretion systems are often complex multiproteic structures (Green and Mecsas, 2016). Since the pathogenicity of many bacteria is linked to their ability to secrete proteins, these machineries constitute important targets for specific antibacterial drug development.

Among the secretion systems identified so far, the genome of *C. trachomatis* encodes the proteins constituting only three different secretion systems: the type 2 secretion system (T2SS), type 3 secretion system (T3SS) and the type 5 secretion system (T5SS) (Stephens, 1998). When it comes to protein secretion beyond the inclusion membrane, the T3SS is the main machinery involved. However, a few secreted proteins, including the extensively studied chlamydial protease-like activity factor (CPAF), do not use T3SS but possess a signal peptide for translocation into the periplasm (Chen *et al*, 2010). The mechanism allowing translocation of these proteins from the periplasm to the host cell cytoplasm remains unclear, possibly thanks to vesicles released into the inclusion lumen that would fuse with the inclusion membrane (Giles *et al.*, 2006; Heuer *et al*, 2003; Zhong *et al*, 2001).

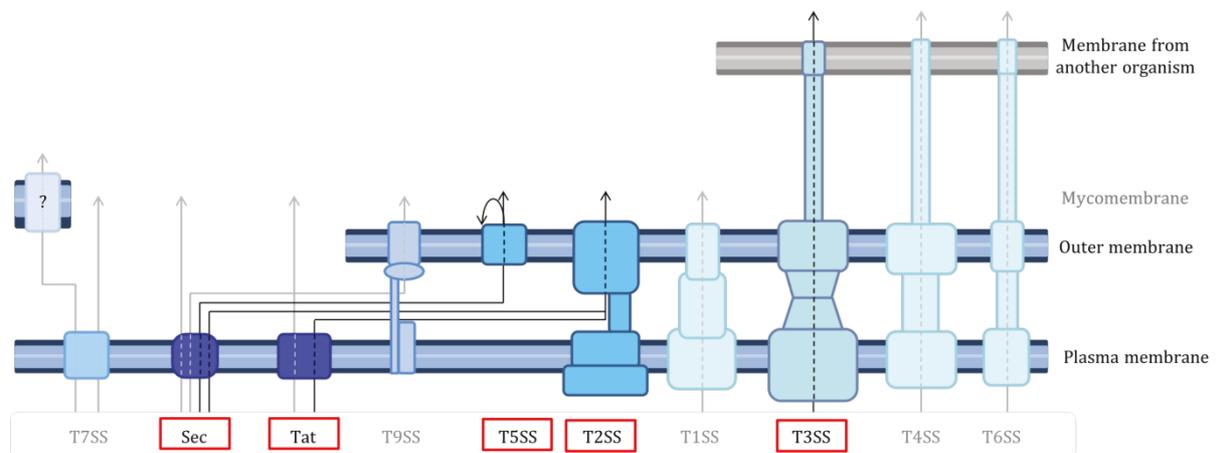


Figure 9: Diversity of the bacterial secretion systems

Schematic representation of the discovered secretion systems in the bacterial kingdom. The secretion systems highlighted (dark writing squared with red) are the one encoded in *C. trachomatis* genome. Adapted from Maffei *et al*, 2017.

The T3SS, also named injectisome, forms a needle-like structure that can be observed by electron microscopy: a basal core called translocon links the inner and outer membrane where it contacts the polymer of needle-constituting proteins, that ends with a tip that can be inserted and a eukaryotic membrane. The translocation of proteins from the cytoplasm of the

bacterium to the cytoplasm of the eukaryotic cell is activated upon contact between the tip of the structure and the eukaryotic membrane (Abrusci *et al*, 2014; Nans *et al*, 2015). The ATP-dependent translocation of a protein through the T3SS requires the protein to be still unfolded, which is generally allowed by the presence of chaperone proteins that maintain this unfolded state and can also determine a time during infection when a protein is secreted (Chen *et al*, 2014; Dai and Li, 2014; Diepold *et al*, 2017; Pais *et al*, 2013; Spaeth *et al*, 2009).

T3S machineries are assembled both at the surface of EBs and RBs, and allow for the secretion of an estimated ~ 100 different effectors along the developmental cycle, half of which being Inc proteins, inserted in the inclusion membrane (Betts-Hampikian and Fields, 2011; Nans *et al*, 2014).

3. A variety of T3S effectors to meet multiple needs.

The effectors are secreted during the entire cycle and are essential for the establishment of the bacterial niche and for bacterial growth. The identification of *Chlamydia* T3SS effectors has been based for many years on the use of heterologous secretion assays in genetically tractable bacteria possessing a T3SS, either in *Yersinia pseudotuberculosis* or *Shigella flexneri* (Clifton *et al*, 2004; Subtil *et al*, 2005).

The study of *Chlamydia* effector proteins revealed a targeting strategy of a variety of cellular functions depicted in Figure 10:

- Reorganization of the cytoskeleton at the entry site and around the inclusion (Clifton *et al* 2005; Dumoux *et al*, 2015; Jiwani *et al*, 2012; McKuen *et al*, 2017).
- Interactions with and highjacking of cellular trafficking (Delevoye *et al*, 2008; Mital *et al*, 2015; Paul *et al*, 2017; Stanhope *et al*, 2017; Wesolowski *et al*, 2017)...
- Apoptosis inhibition (Sixt *et al*, 2017; Weber *et al*, 2017; Fischer *et al*, 2017).
- Modification of the immune response (Carpenter *et al*, 2017; Chen *et al*, 2014; Patton *et al*, 2016; Rajeeve *et al*, 2018; Tang *et al*, 2015).

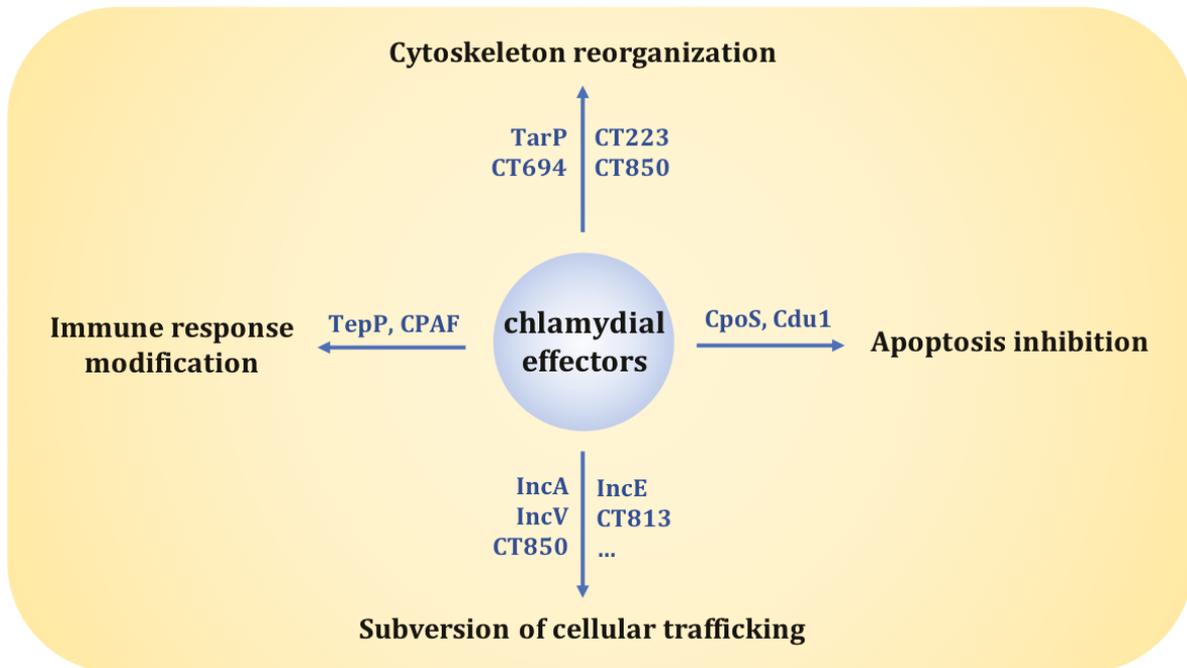


Figure 10: *Chlamydia* effectors and cellular manipulation

Along its cycle, *Chlamydia* secretes effector proteins that hijack different cellular functions, allowing optimal conditions for bacterial multiplication to happen. The different functions targeted are represented in bold dark and the effectors associated in blue.

So far only a handful of effector proteins have been the subject of functional studies. Many host functions targeted by these proteins likely remain to be discovered.

4. Host-pathogen interactions at the metabolic level

Since *C. trachomatis* remains in the inclusion along its developmental cycle, this bacterium has evolved mechanisms to acquire metabolites, essential for its growth and multiplication. The principal nutrient required is glucose, the main source of energy for the bacteria, however in this part we will focus on the other metabolites known, since glucose metabolism will be developed in the next part.

Generally, the molecular mechanisms involving metabolites import in the inclusion lumen are not well characterized. However, some questions have been unraveled in regard of nutrient acquisition by *Chlamydia*. The transport of biotin, a cofactor of certain enzymes involved in lipid metabolism, involves the cellular sodium multivitamin transporter (SMVT) present at the inclusion membrane, that allows the import of this vitamin into the inclusion lumen. Then the bacterial transporter of biotin (BioY), present in the membrane of the RBs, imports it from the inclusion lumen to the bacterial cytoplasm: it is a two-step mechanism (Fisher *et al.*, 2012). Moreover, since SMVT is also important for the cellular import of

pantothenic acid and lipoic acid, two other cofactors essential for the catalysis of metabolic enzyme, this transporter might be involved in the acquisition of those metabolites, essential for *Chlamydia*.

The import of amino acids and nucleotides is, to date, still a mystery. However, quite a lot of data have been compiled regarding lipid acquisition. *C. trachomatis* is able to acquire lipids from different cellular sources:

- Lipid droplets which are translocated inside the inclusion lumen (Cocchiari *et al*, 2008). Since they do not accumulate in the inclusion lumen, they must be degraded and the lipids constituting them used by the bacteria either to produce energy or to synthesize their membrane.
- Peroxisomes are also translocated in the inclusion lumen and the plasmalogens, particular lipids synthesized in these organelles, are used by the bacteria and incorporated in their membrane (Boncompain *et al*, 2014).
- Sphingomyelin is imported by vesicular transport from the Golgi by hijacking a secretory route (Hackstadt *et al*, 1996).
- Sphingomyelin is also directly translocated from the ER to the inclusion membrane by the ceramide transfer protein (CERT) at membrane contact sites between those two organelles (Derré *et al*, 2011; Elwell *et al*, 2011). The IncD protein seems to be involved in this recruitment by interacting with the pleckstrin homology (PH) domain of CERT.
- Scavenging of the host high density lipoprotein (HDL) biogenesis machinery, relocated at the inclusion membrane, allows for the acquisition of certain lipids such as phosphatidylcholine (Cox *et al*, 2012).

Understanding glucose metabolism in *Chlamydia*-infected cells is a central and wide topic regarding host-pathogen metabolic interactions. It will be the main focus of the next part.

II. Glucose metabolism during *Chlamydia* infection.

A. Glucose in mammalian cells, an energy source and a biosynthesis precursor.

Glucose is a small molecule belonging to the carbohydrate also called sugar superfamily. All mammalian cells harness the energy residing in the chemical bond between the carbon atoms of glucose and other organic molecules and oxidize it in order to get the energy they need to fulfill their function in the organism: they are chemoorganotrophs. In addition to this energetic role, glucose is a key precursor metabolite for many biosynthetic pathways and a central molecule of the mammalian metabolism.

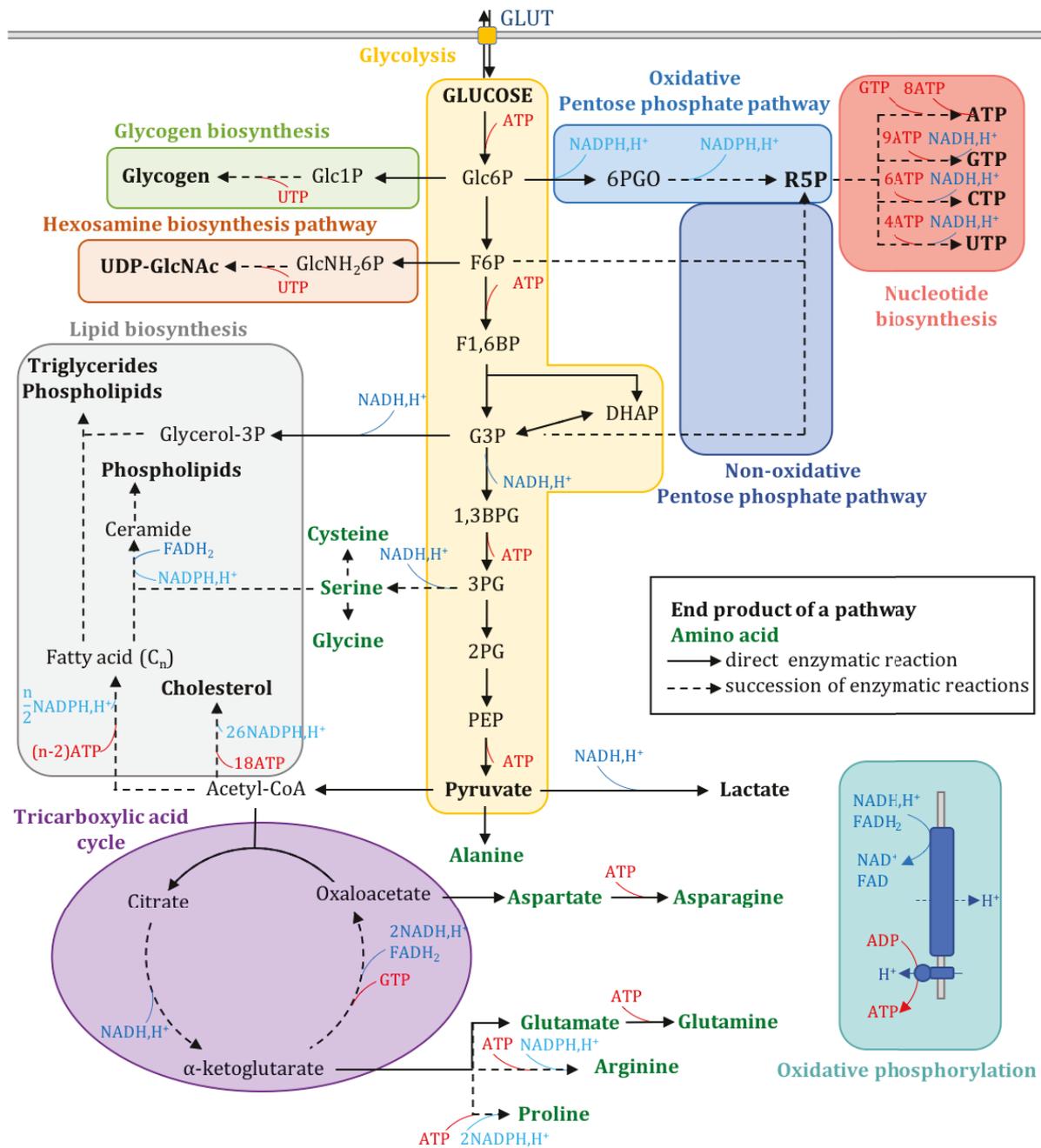


Figure 11: Glucose metabolism in eukaryotic cells

1,3BP, 1,3-bisphosphoglycerate; 2PG, 2-phosphoglycerate; 3PG, 3-phosphoglycerate; 6PGO, 6-phosphogluconolactone; DHAP, dihydroxyacetone-phosphate; F6P, fructose-6-phosphate; F1,6BP, fructose-1,6-bisphosphate; G3P, glyceraldehyde-3-phosphate; Glc1P, Glucose-1-phosphate; Glc6P, Glucose-6-phosphate; GlcNH₂6P, glucosamine-6-phosphate; PEP, phosphoenolpyruvate; R5P, ribose-5-phosphate; UDP-GlcNAc, uridine diphosphate-N-acetylglucosamine.

1. A metabolite used for energy production.

Glucose is one of the major sources of energy of mammalian cells, together with fatty acids. The oxidation of one glucose molecule in aerobic conditions goes through the glycolysis to give rise to pyruvate which is then fully oxidized in the mitochondria in the tri-carboxylic acid (TCA) cycle to release carbon dioxide. All along this process, energetic potential is transferred to redox cofactors, nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD), or directly ATP, the molecule used in living cells as the “molecular unit of currency” of intracellular energy transfer. While the direct generation of ATP only takes place with substrate-level phosphorylation during glycolysis, the generation of reduction power, NADH,H^+ and FADH_2 , involves both glycolysis and TCA cycle, even though the main source is the TCA cycle. This reduction power is then converted into ATP by a complex process occurring in the inner membrane of the mitochondria called oxidative phosphorylation (OxPhos).

This OxPhos couples an electron transfer from the reduced NADH,H^+ or FADH_2 to a dioxygen molecule (producing a water molecule), with the creation of a proton gradient across the mitochondrial inner membrane (figure 12). This gradient creates a proton-motive force which is dissipated by the functioning of the ATP-synthase complex that couples it to an ATP production from ADP and inorganic phosphate. It has been calculated that the complete oxidation of one molecule of glucose by this process – glycolysis followed by TCA cycle and OxPhos – generated 36 molecules of ATP: 2 during substrate level phosphorylation in the glycolysis, 2 equivalent ATP during the TCA cycle (2 GTP produced) and 32 during the OxPhos. Thus, OxPhos is the major source of ATP production during glucose complete oxidation.

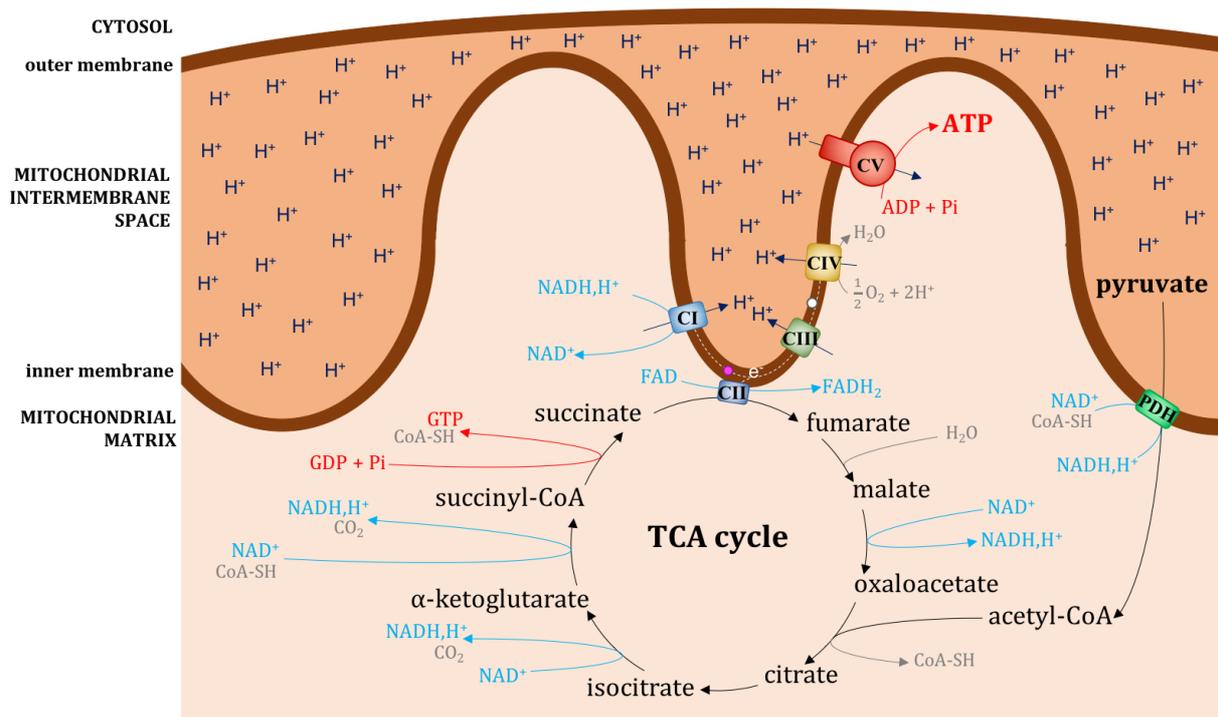


Figure 12: TCA cycle and oxidative phosphorylation in the mitochondria

Pyruvate, coming from glucose degradation in the glycolysis for example, fuels the TCA cycle in the mitochondria after its activation by the pyruvate dehydrogenase complex (PDH) into acetyl-coenzyme A (acetyl-CoA). All the reduction power produced under NADH, H^+ or FADH_2 forms is reoxidized by the respiratory complex I (CI) or II (CII) respectively. The electrons are then transferred to different molecules (pink and white dots represent ubiquinone and ferredoxin respectively) and membrane complexes III (CIII) and IV (CIV) to the final acceptor, dioxygen. Along this process, protons are transferred from the mitochondrial matrix to the intermembrane space, creating a gradient. Ultimately, this gradient is dispersed by the ATP-synthase (CV) that uses the proton-motive force to ATP synthesis.

Glucose oxidation can be complete under aerobic conditions or incomplete, usually under dioxygen limiting concentration or particular conditions (aerobic glycolysis is described in part C). However, the incomplete oxidation of glucose into pyruvate and then lactate allowing the reoxidation of reduced cofactors NADH, H^+ generates way less ATP molecules per glucose molecule than its complete oxidative phosphorylation: 2 ATP generated during partial oxidation versus 36 with complete oxidation.

Glycolysis is tightly regulated through the three enzymes catalyzing its irreversible steps: the hexokinase, the phosphofructokinase-1 and the pyruvate kinase. They are generally activated by an increase in AMP or ADP indicating a need of energy in the cell or by upstream products revealing a need of this pathway to be more active. Their inhibition can be due to an increase of ATP or downstream products accumulation indicating a saturation of the pathway (figure 13).

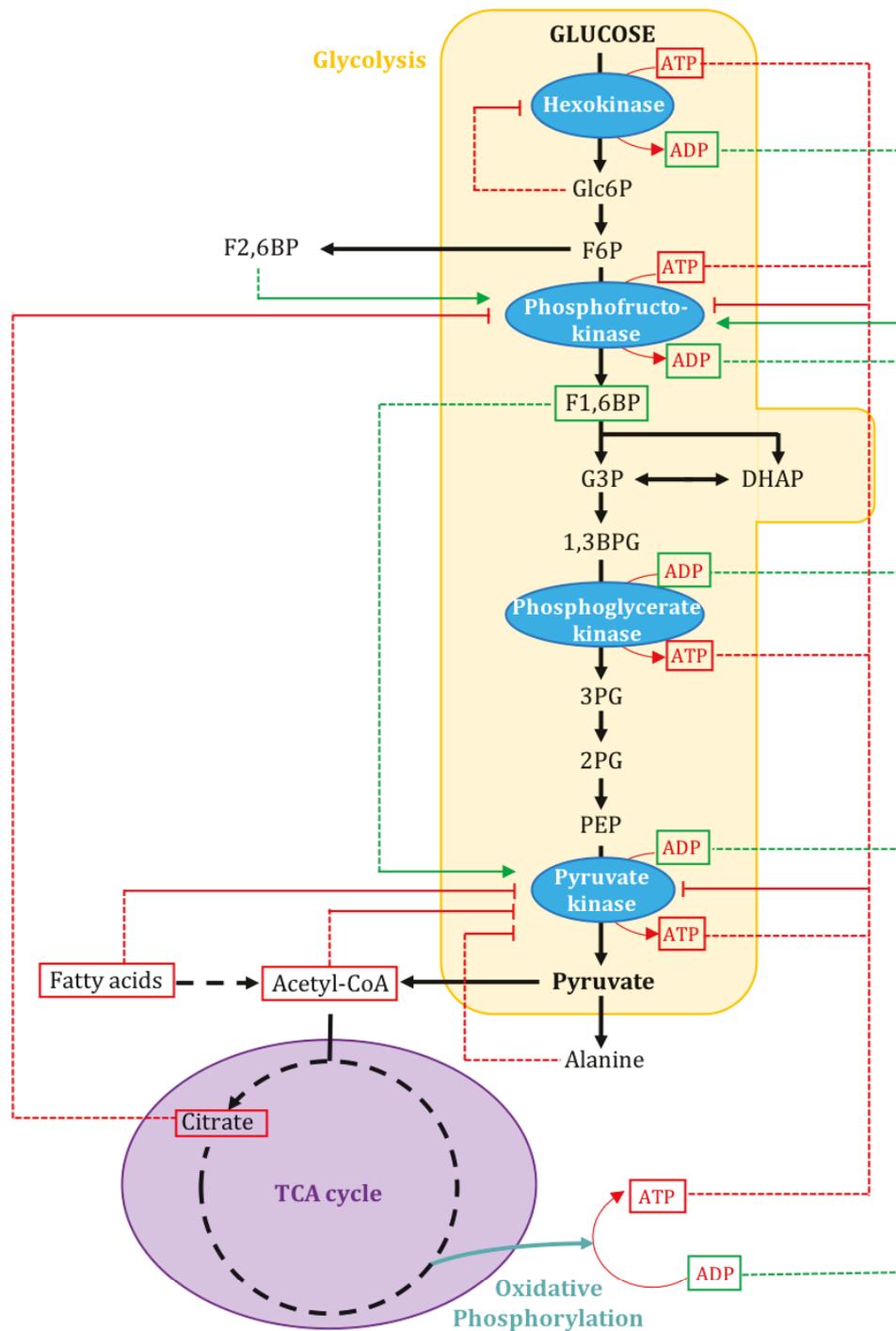


Figure 13: Rapid regulation of glycolytic flux by metabolic products

An accumulation of initial glycolytic substrate (fructose-1,6-bisphosphate (F1,6BP) and fructose-2,6-bisphosphate (F2,6BP)) or a lack of energy in the cell traduced by elevated concentrations of AMP and ADP activate key enzymes of this metabolic pathway (green arrows). On the contrary, an accumulation of downstream products (citrate, acetyl-CoA), other cellular sources of energy (fatty acids, alanine) or an accumulation of energy in the cell (ATP) leads to an inhibition of glycolysis on the same key enzymes (red lines). 1,3BP, 1,3-bisphosphoglycerate; 2PG, 2-phosphoglycerate; 3PG, 3-phosphoglycerate; DHAP, dihydroxyacetone-phosphate; F6P, fructose-6-phosphate; G3P, glyceraldehyde-3-phosphate; Glc6P, Glucose-6-phosphate; PEP, phosphoenolpyruvate.

In addition to this fast adaptation to the intracellular conditions, a long-term adaptation is allowed by genetic regulation exerted by the surrounding conditions, particularly the oxygen level, hormones, cytokines, growth factors, ... These mechanisms modulate the quantity of enzyme catalyzing the different reactions of the pathway, as well as the isoforms expressed, some more affine for their substrate, more catalytically efficient, or less subjected to inhibition. As an example, the hypoxia inducible factor-1 (HIF-1), a transcription factor stabilized under hypoxia among other conditions, induces an increase in the expression of the glucose transporter GLUT1 and the hexokinase 2 (HK2), leading to an increase in glucose uptake and its phosphorylation – a process that traps glucose inside the cell. HIF-1 induces also the expression of the isoform A of the lactate dehydrogenase (LDHA also known as LDH5) resulting in more pyruvate to be converted into lactate and faster regeneration of the cofactor NAD⁺. This isoform is also more affine for pyruvate than lactate, favoring the reaction towards the generation of lactate, whereas the LDHB has opposite affinities (figure 14) (Doherty *et al*, 2013). Other signaling pathways contribute to the regulation of glucose metabolism such as pathways involving Myc, Akt, p53 or the AMP-activated protein kinase (AMPK) (Mulukutla *et al*, 2010).

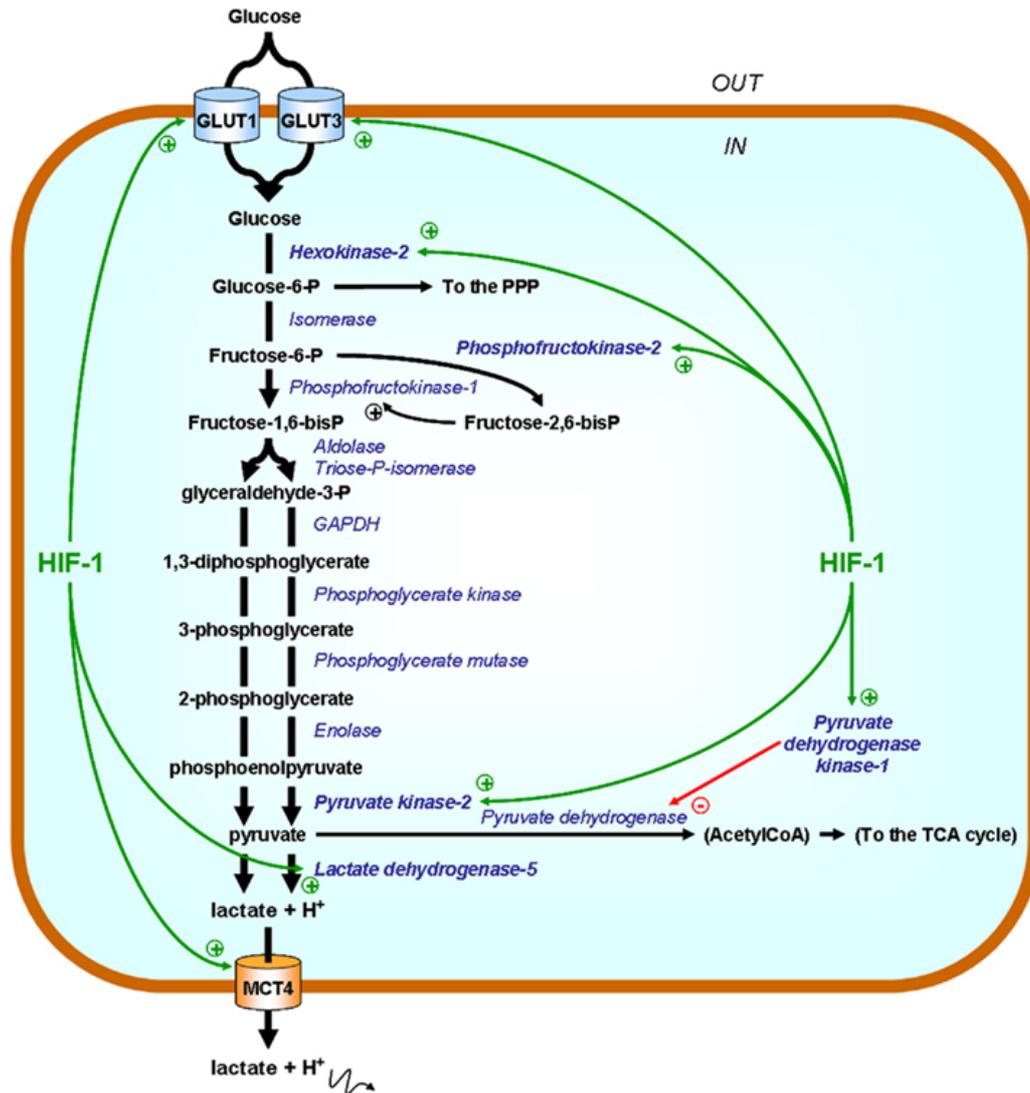


Figure 14: HIF-1 regulation of glycolysis at the transcription level

The activation of the transcription factor HIF-1, in anoxic conditions particularly, leads to the upregulation of various genes encoding glycolytic enzymes (green arrows). This allows an increase in the glycolytic flux and the production of lactate secreted in the extracellular medium by monocarboxylate transporter 4 (MTA4). Phosphorylation of the pyruvate dehydrogenase closes pyruvate entry into the TCA cycle and mitochondrial OxPhos, in which are not compatible with anoxic conditions. From Porporato *et al*, 2011.

2. A precursor used for many biosynthesis pathways.

The glucose metabolism is not only an energy source but also an essential starting point for many anabolic pathways. Most of these pathways do not use glucose as starting point but products of the glucose catabolism, such as glycolysis intermediates, except for the glycogen biosynthesis.

Glycogen is a storage polysaccharide polymer made of glucose molecules linked by an α -1,4 glycosidic bond with α -1,6 bonds at branching points. After the conversion of glucose

into glucose-6-P in the cell, a phosphoglucomutase converts it into glucose-1-P, which in turns serves as substrate to produce UDP-glucose. UDP-glucose serves as the activated glucose unit for glycogen biogenesis, catalyzed by the glycogen synthase (figure 11). Molecules of glycogen allow for an osmotic inert storage of numerous molecules of glucose that can be used as energy source during starvation or a sudden activity, or for anabolic reactions in actively multiplying cells. The balance between glycogen accumulation and degradation is hormonally tightly controlled for the organism function, especially in the liver where it is mainly stored, but not exclusively: for example, an effect of sex hormones was shown for glycogen accumulation in the rat uterus (Poteat and Bo, 1977).

Glucose-6-P can also be oxidized to enter the pentose phosphate pathway (PPP), generating NADPH from NADP, a reduced molecule used in many anabolic reactions such as lipid, nucleotide or some amino acid biosynthesis. Moreover, the PPP allows for the production of ribose-5-P, the precursor for nucleotide biosynthesis. This ribose-5-P can also be produced by a so-called “non-reductive branch” of the PPP, using fructose-6-P and glyceraldehyde-3-P as starting point, two products of glucose degradation during glycolysis (figure 11).

Other metabolites produced during glucose oxidation in the glycolysis (3-P-glycerate or pyruvate) or the TCA cycle (α -ketoglutarate or oxaloacetate) serve as starting point for the biosynthesis of non-essential amino acids such as alanine, serine, glutamate, aspartate (figure 11).

Lipid biosynthesis also requires glucose degradation: acetyl-CoA is used for fatty acid elongation or cholesterol biogenesis, and the glycerol-3-P or the ceramide from glycolysis products (glyceraldehyde-3-P and 3-P-glycerate) are used for fatty acid, and sphingolipid biosynthesis respectively (figure 11).

Finally, the fructose-6-P intermediate is the starting point of the hexosamine biosynthesis pathway (HBP), converted by the glucosamine-fructose-6-phosphate amidotransferase (GFPT) into glucosamine-6-P (figure 11). This pathway leads to the production of UDP-N-acetylglucosamine (UDP-GlcNAc) that can be converted into other nucleotide sugars which are the building blocks used for *O*- and *N*-linked glycosylation of proteins in the Golgi and the ER. UDP-GlcNAc is also the substrate for *O*-N-acetylglucosylation (*O*-GlcNAcylation), a post-translational modification consisting in the addition of a GlcNAc onto a serine or threonine residue of a cytosolic or nuclear protein. This modification is controlled by two enzymes: *O*-GlcNAc transferase (OGT) catalyzes the addition of *O*-GlcNAc whereas *O*-GlcNAcase (OGA) removes it. In cellular or *in vivo* models this

modification has been shown to impact histone remodeling, transcription, proliferation or aging (Denzel *et al*, 2014; Love and Hanover, 2005).

The entry of glucose into these different pathways can be controlled by the presence and activity of glycolytic enzymes, leading to the accumulation of substrates which are used in biosynthesis pathways. For example, under an oxidative stress, the production of reactive oxygen species (ROS) leads to the oxidation of cysteine 258 of the pyruvate kinase isoform M2 (PKM2), thereby inhibiting its activity. As a consequence, the concentration of the glycolysis products from the downstream steps increases (Anastasiou *et al*, 2011). The glyceraldehyde phosphate dehydrogenase (GAPDH), upstream in the glycolysis, is also inhibited by oxidative conditions. This results in a net increase in the concentration of glucose-6-P, increasing the flux of the PPP and thus the generation of NADPH, cofactor used for the regeneration of glutathione involved in the antioxidant response in eukaryotic cells (Anastasiou *et al*, 2011).

The quantity of enzymes catalyzing the different reactions of these biosynthesis pathways is also a powerful mechanism to control the entry into these pathways. For instance, the two key enzymes of respectively the oxidative and non-oxidative branch of the PPP, the glucose-6-P dehydrogenase (G6PD) and the transketolase, have an increased expression during DNA replication at the S phase, probably necessary for production of the nucleotides used for DNA synthesis (Vizán *et al*, 2009). GFPT, that controls the entry of fructose-6-P into the HBP, has a short half-life time (less than 2 h). Products of the HBP have been proposed to have a negative effect on GFPT transcription, constituting a genetic negative feedback-loop (Marshall *et al*, 1991). In addition, GFPT is inhibited by UDP-GlcNAc, the end product of the biochemical pathway (Assrir *et al* 2014).

B. *C. trachomatis* and glucose: a long story.

Given the peculiar lifestyle of *C. trachomatis* and the gene reduction that happened during the coevolution between this bacterium and its host, it is not surprising that it relies on host glucose as an energy source and as a precursor, but also as a source for many other metabolites to compensate for gene loss along several biosynthetic pathways.

1. Acquisition of glucose by the bacteria.

The idea that *C. trachomatis* was relying on glucose for its development was approached quite a long time ago, with the description of glycogen accumulation within the inclusion (Gordon and Quan, 1965).

a. Different sources of cellular glucose.

The sequencing of *C. trachomatis* genome revealed that the bacteria must rely on an activated molecule of glucose (such as glucose-6-P) for its glycolysis to happen, since it lacked a hexokinase. The nature of this activated glucose and the exchangers allowing its transport from the host cytoplasm to the bacterial lumen, were unraveled only recently (Gehre *et al*, 2016).

Glycogen granules coming from the host cytoplasm are imported directly into the inclusion lumen, by a mechanism that remains to be determined. Complementary to this bulk import of glycogen, UDP-glucose is translocated into the inclusion lumen. At least one host transporter, SLC35D2, a sugar transporter normally present in the ER and the Golgi and able to transport also GDP-Mannose and UDP-GlcNAc, has been implicated in UDP-glucose import (Gehre *et al*, 2016). Once in the inclusion lumen, UDP-glucose serves as a substrate for bacterial glycogen synthesis enzymes GlgA and GlgB, which are secreted in the inclusion lumen for synthesizing glycogen in this enclosed space (figure 15).

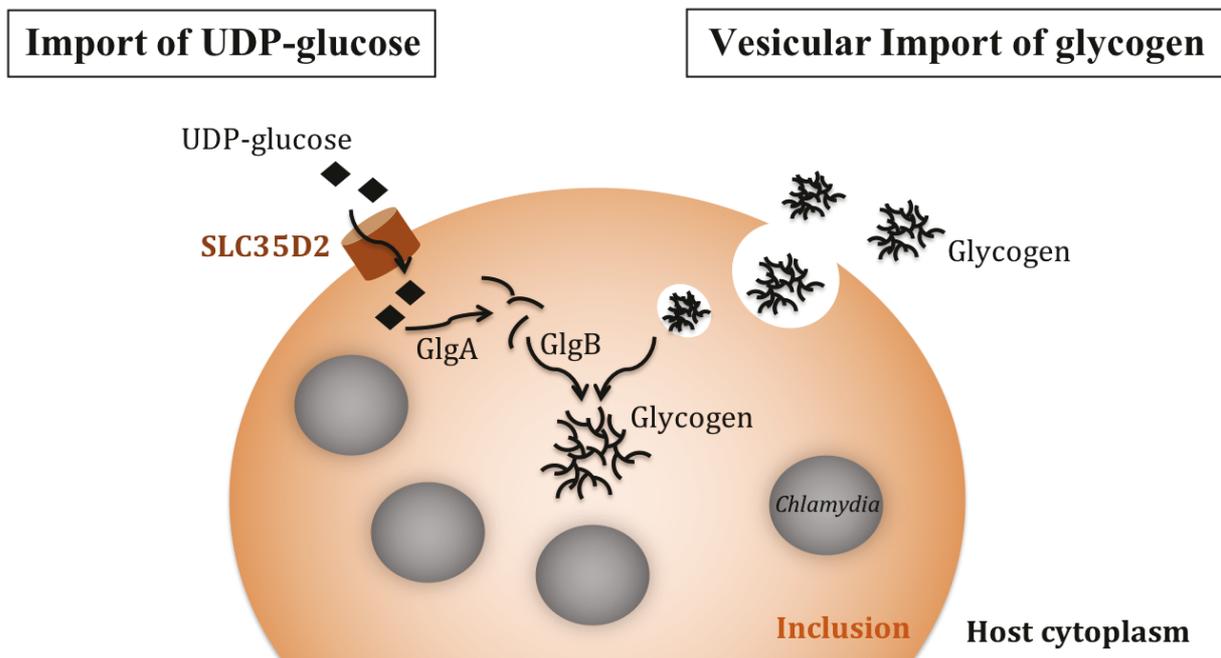


Figure 15: Glucose import into the inclusion lumen by *C. trachomatis*

C. trachomatis imports glucose from its host cell under two different forms: a bulk import of glycogen that can be degraded then into the inclusion lumen into glucose-1-phosphate, or an import of UDP-glucose through the transporter SLC35D2. This UDP-glucose can be used by *Chlamydia* glycogen synthases GlgA and GlgB to synthesize new glycogen into the inclusion lumen. Adapted from Gehre *et al*, 2016.

b. Glucose uptake by the bacteria.

The degradation of the glycogen by secreted bacterial proteins leads to the release of glucose-1-P within the lumen of the inclusion. An experiment using radio-labeled glucose sources (glucose, glucose-1-P or glucose-6-P) and competition with cold glucose revealed that only glucose-6-P was imported by the bacteria probably by the UhpC transporter (Gehre *et al*, 2016; Schwoppe *et al*, 2002). The conversion of glucose-1-P into glucose-6-P requires the secretion in the inclusion lumen of a chlamydial phosphoglucomutase (Triboulet *et al*, in preparation). The glucose-6-P can then be translocated in the bacteria and used as energy source and biosynthetic substrate.

2. The glucose, a source of energy.

The axenic culture of EB and RB forms of *Chlamydia* in medium containing different sources of energy (glucose-6-P or ATP) in the presence of radiolabeled methionine revealed that EBs were able to actively synthesize proteins using glucose-6-P as the main source of energy. In contrast, RBs required ATP (Omsland *et al*, 2012). RBs are able to produce ATP at their membrane by a sodium-dependent ATP synthase, and it appears that *Chlamydia* dependency on ATP generation by the host is more important in early stages of infection (Liang *et al*, 2018). It is thus likely that glucose-6-P is also used by RBs in order to generate energy, at least in the later stages of infection. The enzymes catalyzing the first two steps of the TCA cycle are missing in *C. trachomatis*. This implies that the ATP produced from glucose-6-P by OxPhos consumes only the NADH,H⁺ produced during glycolysis, strongly diminishing the yield of ATP production per glucose molecule (Kubo and Stephens, 2001).

3. Glucose as a precursor.

Chlamydia possesses a functional PPP along with all the enzymes for the synthesis of the building blocks of the peptidoglycan (hexosamines and isoprenoids). The bacterium synthesizes also the cofactors necessary for the electron transport chain in the futasine pathway. It possesses the required enzymes for the synthesis of several amino acids but many need to be imported from the host, including glutamate, which can enter the incomplete TCA cycle in order to produce ATP *in fine* (Stephens, 1998).

In addition, the bacteria rely completely on the host cell for biosynthesis of nucleotides and a drug blocking the guanine nucleotide biosynthesis inhibits drastically bacterial multiplication (Rother *et al*, 2018).

Even though the initial concept of “energy parasite” has been revised with the demonstration that *Chlamydia* was able to produce ATP, the inability of this bacterium to generate all the biomolecules essential for its multiplication and growth makes it a metabolic parasite for its host (Ilyffe-Lee and McClarty, 1999; Moulder, 1962). In order to supply all the metabolites required by the bacterium, the infected cell undergoes major changes in its glucose metabolism.

C. Glucose metabolism modifications in *Chlamydia*-infected cell.

1. Increase in the uptake of glucose from the culture medium.

Given the central part that glucose plays in mammalian cells and in *Chlamydia* biology for both energy production and metabolites biosynthesis, a critical point that infected cells need to control is their glucose uptake from the extracellular medium.

The entry of glucose in cells is mediated by different glucose transporter proteins (GLUTs), whose expression depends on the cell type and the membrane domains: GLUT-2 for example is mainly expressed at the basal membrane of intestinal epithelial cells whereas GLUT-1 is present at the apical membrane of the same cells.

An early study revealed an increase in the quantity of GLUT-1 in cells infected by the guinea pig adapted strain *C. caviae* (Ojcius *et al*, 1998). In the context of *C. trachomatis* infection, GLUT-1 increase was demonstrated initially by Siegl and collaborators and recently confirmed, with an expression dependent on HIF-1 α presence, a transcription factor known to regulate *GLUT-1* transcription (Siegl *et al*, 2014; Shima *et al*, 2018; Wang *et al*, 2017). Wang *et al* revealed that only GLUT-1 and GLUT-3 were upregulated at the transcriptional level in HeLa cells, but none of the other 11 potential glucose transporters. This upregulation was confirmed at the protein level by Western Blot. When bacteria were inoculated on cells in which either *GLUT-1* or *GLUT-3* had been silenced with siRNA, a two-fold reduction in bacterial progeny was observed, supporting the hypothesis that increased glucose import is important to sustain bacterial development.

GLUT transporters have a reversible activity. Once the glucose is imported in the cell, a phosphorylation step is needed to trap it intracellularly. The expression of hexokinase II increases during *C. trachomatis* infection through the activation of a PDK1-MYC axis, and active hexokinase II remains associated to mitochondria (Al-Zeer *et al*, 2017). Glucose-6-P is thus efficiently trapped inside the cell and can be used by the cell itself or by the bacteria.

2. Modification of the use of glucose.

A drastic change in the infected-cell metabolism is required to meet the needs of multiplying *Chlamydia* and supply the growing inclusion with many metabolites including lipids, amino acids, sugars, and more importantly nucleotides. As discussed above, several features of *Chlamydia* infected cells suggest that these cells undergo a switch to aerobic glycolysis, similar to what is observed in cancer cells.

a. Aerobic glycolysis.

Cells undergoing rapid proliferation such as activated lymphocytes or cancer cells use a different metabolism called aerobic glycolysis, also referred to as the Warburg effect as it was described for the first time in the 1920s by Otto Warburg (Hedekov, 1968; Warburg, 1956). Classically, cells in aerobic conditions mainly produce ATP through OxPhos in the mitochondria with pyruvate, produced after glycolysis, that is converted into acetyl-CoA and enters the TCA cycle (figure 11 and 12). In aerobic glycolysis, cells mainly produce ATP by substrate level phosphorylation during glycolysis and then produce lactate from pyruvate by fermentation in order to regenerate the redox coenzymes. This aerobic glycolysis functions with an increase in the expression on GLUT transporters and key enzymes of the glycolysis and lactate production in order to generate enough ATP. It is important to note that this metabolism is not accompanied by defective mitochondria or mitochondrial respiration: proliferating cells have a normal or even a bit higher mitochondria generation of ATP, but the main part of ATP comes from glycolysis which is highly upregulated (figure 16) (Zu and Guppy, 2004).

This shift in metabolism may appear to have some advantages, but none is completely obvious (figure 16):

- The incomplete oxidation of glucose into lactate occurs much faster than the complete oxidation of glucose (10 to 100 times faster) allowing for a greater production of ATP in conditions where glucose is non-limiting (Shestov *et al*, 2014). This could be required to meet a higher energy demand in fast dividing cells. However, it can be argued that the amount of ATP required for cell growth and division is not different (if not less important) than that of non-dividing cells for their maintenance (Locasale and Cantley, 2011).
- The increased uptake of glucose is used for an increased biosynthesis of metabolites such as nucleotides, necessary for cell replication. But lactate excretion in the medium is a hallmark of

this metabolism, suggesting that biosynthesis of metabolites is not so prominent (Lunt and Vander Heiden, 2011).

- The active glycolysis and secretion of lactate induces an acidification of the surrounding media that may be an advantage for cancer cells and helps defining the tumor microenvironment along with orientating the immune response towards a pro-tumor immunity. This advantage is irrelevant in non-tumorigenic contexts in which high glycolysis is observed such as lymphocytes or yeast multiplication (Colegio *et al*, 2014). However, in the context of an epithelial infection, it may regain relevancy considering the competition for metabolites with the surrounding microbiota.

- The aerobic glycolysis induces modifications in cell signaling particularly in the redox homeostasis and histone acetylation since acetyl-CoA is the substrate for this modification (Cai *et al*, 2011; Cai and Tu, 2011; Hamanaka and Chandel, 2011). Histone acetylation for example may in return be enough to drive cells into the growth phase (Lu and Thompson, 2012).

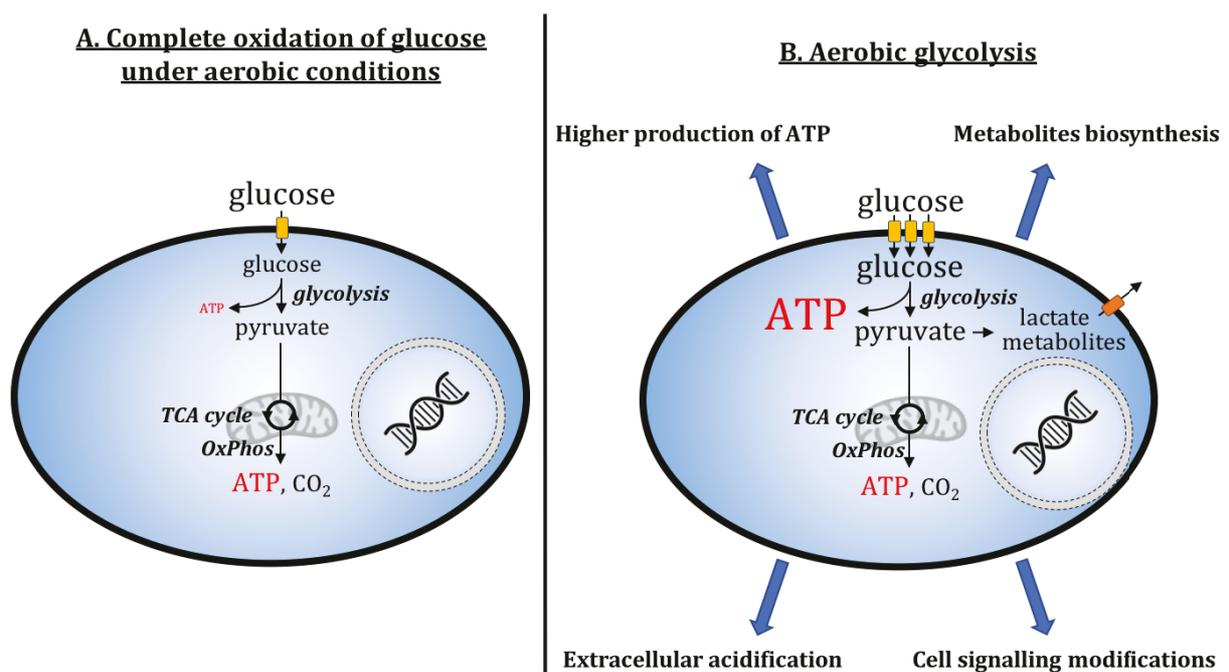


Figure 16: Representation of the main differences between aerobic glycolysis and normal complete oxidation of glucose in mammalian cells

Under normal conditions (A), glucose is imported inside the cell and goes through glycolysis, TCA cycle and OxPhos, producing ATP mainly by mitochondrial respiration. Cells undergoing aerobic glycolysis (B) have an increased uptake of glucose that is mainly partially oxidized in the glycolysis, producing a lot of ATP through phosphorylation substrate-level. TCA cycle and OxPhos are still functioning but not enough to regenerate the cofactors and oxidize the pyruvate produced, which is converted into lactate and can serve for metabolite anabolism, along with other glycolytic intermediates.

b. Aerobic glycolysis in *Chlamydia*-infected cells.

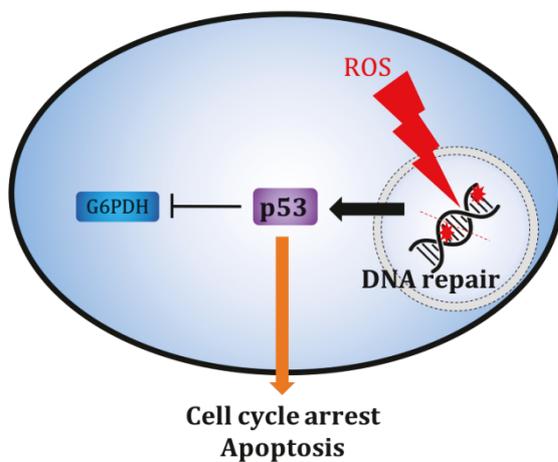
The question of the metabolic modifications occurring on the host cell during *Chlamydia* infection has been addressed very recently with an siRNA screen against the host transcriptome. Several of the selected siRNAs that impacted *Chlamydia* development targeted genes involved in energetic metabolism. Using gas chromatography coupled with mass spectrometry, Rother and collaborators performed a profiling of many metabolites and their results supported the hypothesis that infected cells had increased aerobic glycolysis (Rother *et al*, 2018). Notably, the levels of pyruvate and lactate were strongly elevated during infection, indicating a strong glycolysis along with less TCA cycle in the mitochondria. However, one important limit of this study is that metabolites of host and bacterial origin were not separated. Even at the earliest point examined, 18 hours post infection, the bacterial metabolism likely makes a significant contribution to the generation of these metabolites. Interestingly, the elevated levels of TCA cycle intermediates and glutamate revealed that mitochondrial function and ATP production were still very active. This cycle may be fed by other metabolic sources than the acetyl-CoA produced from pyruvate, such as glutamate taken up from the extracellular environment. Along with these changes, an increase in the import of essential amino acids and the production of metabolites from nucleotide biosynthesis, particularly purine biosynthesis, were observed, suggesting a full rerouting of the host cell metabolite production and transport towards aerobic glycolysis, in order to meet bacterial multiplication requirements.

Given that *C. trachomatis* depends entirely on its host cell for nucleotide production, it was probably expected that a blockage of either the pentose phosphate pathway by siRNA against G6PD (first and rate-limiting enzyme of this pathway that produces ribose-6-P, the precursor for purine biogenesis), or the purine *de novo* biogenesis by siRNA or chemical agents induced a strong decrease in the bacterial progeny both *in vitro* and *in vivo* (Prusty *et al*, 2012; Rother *et al*, 2018; Siegl *et al*, 2014).

An essential question raised by those observations is how this profound modification is orchestrated. Regulation occurs both at the transcriptional and enzymatic level. The protein p53 was identified as one of the factors involved in this drastic change. In normal conditions, p53 presence inhibits the G6PD activity by direct binding to this protein, preventing its activating dimerization (Jiang *et la*, 2011). *C. trachomatis* infection of human cells induces a downregulation of p53 by activation of the phosphoinositide-3-kinase (PI3K)/Akt/HDM2 (human form of murine double minute 2) leading to p53 proteasomal degradation. This process

is essential for the bacterial development since p53 stabilization results in a loss of bacterial infectivity and a persistent state (Siegl *et al*, 2014). Moreover, the effect of p53 stabilization on *Chlamydia* development was reverted by overexpressing G6PD, indicating a major role of the PPP in p53 effect (figure 17). In addition to providing metabolites and precursors for nucleotide biogenesis and reduced cofactor NADPH used for lipid synthesis and other biosynthetic pathways, the NADPH is also important for handling the oxidative stress and ROS production happening during *C. trachomatis* infection (Chumduri *et al*, 2013).

Cell submitted to an oxidative stress



Chlamydia-infected cell

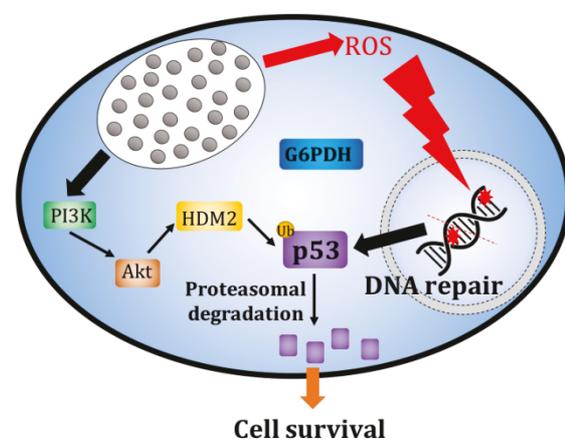


Figure 17: p53 degradation induced by *Chlamydia* infection modifies the normal cellular response against oxidative stress and favors bacterial growth through expression of the glucose-6-phosphate dehydrogenase (G6PDH)

The physiological reaction of a cell against ROS formation under oxidative stress leads to the stabilization of p53. This stabilization among others results in a downregulation of the expression of the G6PDH and cell cycle arrest, to allow DNA repair. *Chlamydia trachomatis* infection leads to the formation of ROS inside the cell but not the accumulation of p53. The activation of the PI3K pathway and the p53-E3 ligase HDM2 leads to p53 ubiquitination and proteasomal degradation. The continuous expression of the G6PDH, along with an active pentose phosphate pathway, allows for the production of NADPH, H⁺ essential for the reduction of antioxidants molecules such as thioredoxin and biogenesis of lipids and nucleotides, essential for the bacteria. Inspired by Siegl *et al*, 2014.

Other factors than p53 must be involved in the reprogramming of cellular metabolism during *C. trachomatis* infection towards an aerobic glycolysis metabolism but are yet to be found. In cancer cells, different pathways have been discovered at the basis of the Warburg effect, depending on the cancer type, for example Myc, Akt, Kras or HIF-1 (Hay, 2016;). We focused during this work on one enzyme that has been implicated in metabolic reprogramming in mammary epithelial cells, the transglutaminase 2 (Kumar *et al* 2014).

III. The Tissue Transglutaminase, a cellular enzyme.

In 1957, Sarkar and collaborators demonstrated that the soluble protein fraction of livers isolated from different rodents triggered the incorporation of ^{14}C radiolabeled amines into proteins. This enzymatic activity was only detected in presence of the cofactor Ca^{2+} . This study provided the first description of the transglutaminase activity catalyzed by the tissue transglutaminase, also known as transglutaminase 2 (TG2) (Sarkar *et al*, 1957).

A. TG2, a member of the transglutaminase family.

1. The transglutaminase family.

Transglutaminases are widespread in nature and are found in bacteria such as *Streptovorticillium mobaraense* (Ando *et al*, 1989), plant tissues (Falcone *et al*, 1993), invertebrates (Tokunaga *et al*, 1993) and vertebrates (Yasueda *et al*, 1994). Mammalian transglutaminases, and more recently microbial and plant transglutaminases, are being studied a lot in the agroindustry for food production, including conservation as well as rheological properties of the goods (Kieliszek and Misiewicz, 2014). Their name arises from their enzymatic activity catalyzing the reaction between a specific glutamine residue of a protein and the primary amine residue of another molecule (detailed below) or water (less often).

The mammalian transglutaminase family encompasses nine members, with one member catalytically inactive, presented in Table 1. All the enzymatically active transglutaminases share a conserved catalytic triad of Cys-His-Asp (Cys277-His335-Asp358 for TG2) (Lorand and Graham, 2003). The tissue-specific expression of the transglutaminases and their subcellular localization vary depending on the enzyme. TG2 is the only known transglutaminase to be expressed ubiquitously in all cell types and localized in many cellular compartments: cytosol, nucleus, mitochondria, extracellular matrix (ECM), plasma membrane (on the inner and outer sides), recycling vesicles, and within the endoplasmic reticulum (Balklava *et al*, 2002; Janiak *et al*, 2006; Krasnikov *et al*, 2005; Lesort *et al*, 1998; Piacentini *et al*, 2014; Zemskov *et al*, 2011). Functions and targets have been identified in the different compartments and will be discussed in details below.

Table 1: The mammalian transglutaminase family

Transglutaminase	Other Name	Gene	Function	Size aa (kDa)	Location
Factor XIII	Fibrin Stabilizing Factor	F13A1	Blood clotting and wound healing	732 (83)	Cytosol, extracellular
Band 4.2	Erythrocyte Membrane Protein	EPB42	Structural protein in erythrocyte, no enzymatic activity	690 (72)	Plasma membrane
TG1	Keratinocyte Transglutaminase	TGM1	Cornified envelope assembly in surface epithelia	814 (90)	Cytosol, plasma membrane
TG2	Tissue Transglutaminase	TGM2	Cell death/differentiation, adhesion, matrix assembly, cell signaling	686 (80)	Cytosol, nucleus, plasma membrane, cell surface, extracellular
TG3	Epidermal Transglutaminase	TGM3	Cornified envelope assembly in surface epithelia	692 (77)	Cytosol
TG4	Prostate Transglutaminase	TGM4	Semen coagulation in rodents	683 (77)	Unknown
TG5	TG X	TGM5	epidermal differentiation	719 (81)	Nuclear matrix, cytoskeleton
TG6	TG Y	TGM6	unknown	625 (71)	Unknown
TG7	TG Z	TGM7	unknown	710 (80)	Unknown

Mice knockout for TG2 have been obtained and are viable with no major developmental or organ abnormalities (De Laurenzi and Melino, 2001). However, it is very likely that other transglutaminases are expressed to compensate for TG2 absence. Even with this limitation, knockout mice have been very valuable over the years to assess the importance of TG2 in the development of diseases such as neurodegenerative diseases, celiac disease, cancer, fibrosis, etc.

2. The transaminase reaction.

The reaction catalyzed by transglutaminases always involves the glutamine residue of a protein called acceptor, but the donor part can vary, resulting in different modifications of the glutamine. The reaction takes place in two steps. The first one consists in the nucleophilic addition of the sulfur atom present on the active cysteine residue of the catalytic site of the enzyme to the carbon on the amide group of the γ -glutaminy residue from the acceptor protein. This step results in the formation of a thioester bond linking covalently the enzyme and the substrate protein in an acyl-enzyme intermediate, with the release of ammonium. In a second step, the acylated enzyme can react either with an amine donor or with a water molecule if no suitable donor is present. The latter case results in the conversion of a glutamine to a glutamic acid residue (deamidation). The amine donor reacting on the acyl-enzyme intermediate can be the ϵ -lysine from the same protein bearing the γ -glutaminy residue, thus creating an intra-protein covalent bound, or from another protein, creating an inter-protein covalent bound. The amine donor can also be a small molecule amine present in the cell such as serotonin, histamine, spermine, etc, resulting in a post-translational modification of the protein (Lorand and Graham, 2003). This last possibility has been exploited since many years to track the transglutaminase

activity with artificial biotinylated or radiolabeled polyamine substrates (Folk, 1980; Slaughter *et al*, 1992). It was initially thought that small amines, which are abundant in cells, acted solely as natural inhibitors of transglutaminases. However, the beginning of the twenty-first century brought convincing evidence that the serotonylation of small GTPases by TG2 was essential for cytoskeletal rearrangements and exocytosis of platelet granules, implicating that the reaction with small amines could be functionally relevant (Walther *et al*, 2003).

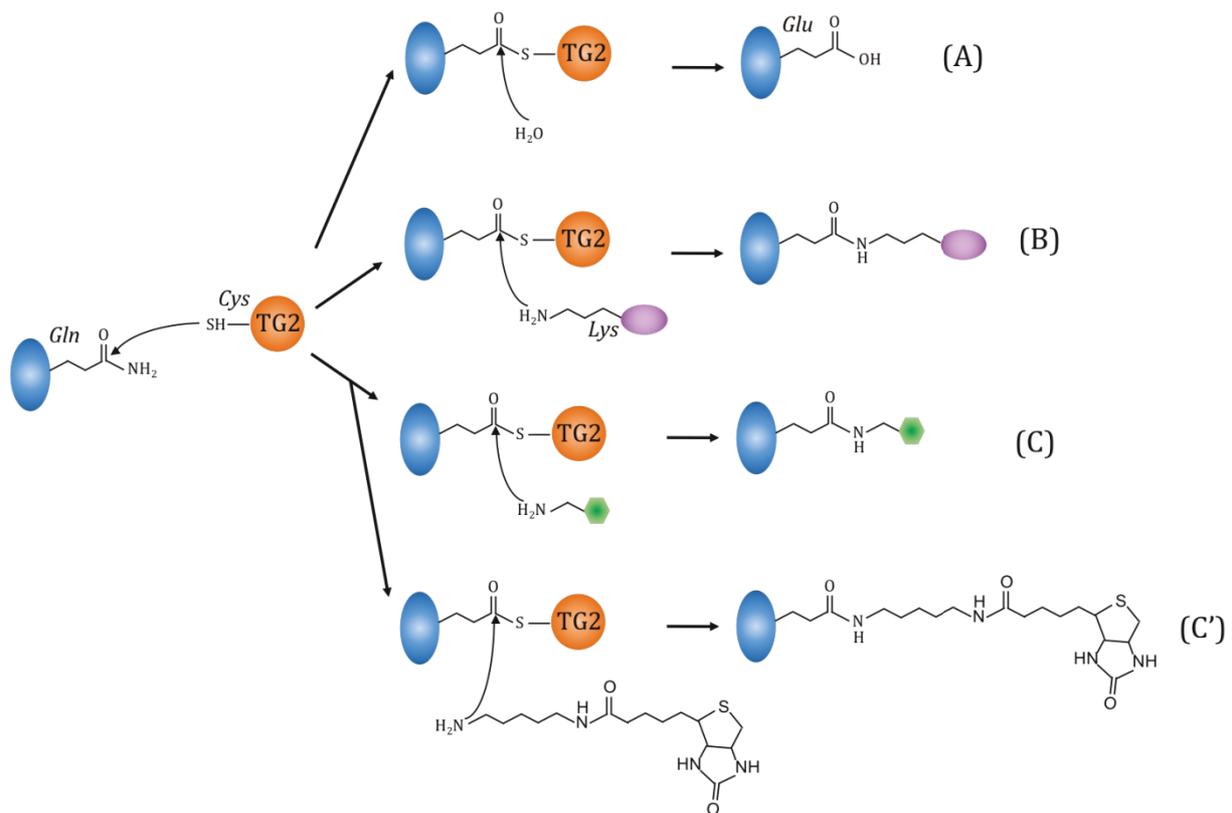


Figure 18: The transaminase reactions catalyzed by TG2

First TG2 reacts with the glutamine (Gln) residue of a protein (blue oval) that leads to the formation of an acyl-enzyme intermediate. In presence of water, the acyl-enzyme is hydrolyzed leading to the formation of a glutamic acid residue (Glu) and a deamidation of the initial glutamine (A). The reaction can also happen between the acyl enzyme and a lysine residue (Lys) of another protein or the same protein (purple oval), generating an inter or intra-protein cross-link respectively (B). Finally, small amines (green hexagone) present in the cell can also react on the acyl-enzyme intermediate, leading to the post-translational modification of the target protein (C). This last reaction can be used as a readout of TG2 activity in the cell by adding in the culture medium compounds such as the biotin pentylamine (BP), that covalently tag the glutamine residue(s) of the proteins targeted by TG2 (C').

Over the years, many substrates have been developed to track the transamidating activity of transglutaminases, and to detect either the glutamine donor substrate, using for instance biotin pentylamine (BP) as primary amine, or the lysine donor substrate using the T26 peptide (see figure 18) (Lee *et al*, 1988; Sugimora *et al*, 2006). The specificity for the γ -glutaminy and ϵ -lysine residue is primarily dictated by secondary and tertiary structure (accessibility of the residue to the enzyme). Phage display and bioinformatics approaches have identified primary sequences around the γ -glutaminy residue that are favored by TG2 or the Factor XIIIa (Coussons *et al*, 1992; Keresztessy *et al*, 2006; Sugimura *et al*, 2006). Many substrates have been identified and are listed in the TRANSDAB database (Csöszet *al*, 2009). For TG2 transamidating activity alone, more than 150 substrates have been identified. In most cases the identified substrate is the glutamine-donor, but a few substrates providing the ϵ -lysine were also identified.

The deamidation reaction using water as a nucleophile is favored in conditions with low enzyme concentration, low amine concentrations, and low pH (Stamnaes *et al*, 2008). In the case of TG2, pathological consequences of deamidation have been identified in a few cases such as the modification of the gliadin from wheat, which constitutes the ground of the pathogenesis in celiac disease, or the deamidation of the Gln6 in the neuropeptide substance P, increasing its agonist potency towards its natural receptor (Fornelli *et al*, 2011; Shan *et al*, 2002). More recently a deamidation of several glutamine residues of GAPDH was shown to promote trophoblastic carcinoma cell fusion. GAPDH deamidation might modify its electrostatic interaction with the actin cytoskeleton, thereby promoting the formation of the syncytiotrophoblast in the placenta (Iwai *et al*, 2014).

Although the transamidating activity of TG2 is the best described and studied, this enzyme possesses also other enzymatic and non-enzymatic functions.

3. Other enzymatic functions of TG2.

TG2 presents four other enzymatic activities in addition to the transamidating activity: a GTPase activity, an ATPase activity, a protein disulfide isomerase activity and a protein kinase activity.

a. GTPase activity.

The GTPase and GTP binding ability of TG2 was discovered in 1987. At that time, the non-classical G protein identified, called Gh α because of its unusually heavy molecular weight, was thought to be a new protein. It is only later on that it was realized that Gh α and TG2 were

the same protein (Achyuthan and Greenberg, 1987; Nakaoka *et al*, 1994). Like classical heterotrimeric G proteins, TG2 can be associated with a G-protein coupled receptor (GPCR) like the α_{1D} adrenergic receptor or the thromboxane A2 receptor (Baek *et al*, 1992; Vezza *et al*, 1999). Agonist binding to its receptor induces both a replacement of the GDP bound to TG2 to a GTP and the dissociation of TG2 from its inhibitory Gh β also known as calreticulin, activating downstream signaling pathway through phospholipase C δ 1 (PLC δ 1) (Feng *et al*, 1999; Mhaouty-Kodja, 2004). Deactivation occurs when the intrinsic but low GTPase activity of TG2 hydrolyzes GTP into GDP, leading to the re-association with calreticulin and with the GPCR. Despite significant progresses in understanding TG2 GTPase activity, the physiological roles of the intracellular signaling downstream of this activity are poorly understood.

b. ATPase activity.

The ATPase activity of TG2 is localized in the same domain as the GTPase activity (Iismaa *et al*, 1997). The demonstration that ATP was a source of phosphate for mineralization of the bone matrix in an osteoblast culture, and that the ATPase activity of extracellular TG2 was probably involved since cysteine, an inhibitor of TG2, diminished mineralization brought the first, and so far only, physiological function for this activity (Nakano *et al*, 2007). The same group later confirmed the involvement of TG2 by showing that TG2 ATPase activity was enhanced after a cleavage of the protein in the ECM, controlled by the membrane-type matrix metalloproteinase-1 (MT1-MMP). The activation of MT1-MMP would lead to the cleavage of TG2, activation of its ATPase activity and increased mineralization of the bone matrix (Nakano *et al*, 2010).

c. Protein disulfide isomerase (PDI) activity.

The PDI activity consists in catalyzing the formation, breakup and exchange of disulfide bounds on cysteine residues of proteins. TG2 has a relatively low PDI activity, initially described *in vitro* (Hasegawa *et al*, 2003). Evidences for the importance of this activity *in vivo* came from the comparison of TG2 knock-out (KO) mice with wild type (WT) mice. TG2 KO mice have defaults in the mitochondrial respiratory chain, resulting in lower ATP production, potentially due to lacks in disulfide bonds in several complexes of the respiratory chain (Mastroberardino *et al*, 2006). This activity would also be important for regulating the activity of the mitochondrial ADP/ATP transporter adenine nucleotide translocator 1 (ANT1) by regulating its oligomerization state (Malorni *et al*, 2009). Very recently, the first evidence of the existence of the PDI activity outside from the mitochondria was published, with TG2

exerting the PDI activity in the nucleus of cells on heat shock factor 1 (HSF1), triggering the trimerization and activation of this transcription factor (Rossin *et al*, 2018). A mutant lacking only this enzymatic activity would be important to dig further into the functions of this PDI activity.

d. Protein kinase activity.

The ability of TG2 to add a phosphate from ATP onto serine or threonine residues of proteins was identified first in 2004 with the phosphorylation of the insulin-like growth factor-binding protein-3 (IGFBP-3). Enzymatic characteristics were similar to those of other cellular kinases (Mishra and Murphy, 2004). The same group later presented evidences for a phosphorylation of p53, RB1 and the histones H1 and H3, in the nucleus (Mishra and Murphy, 2006; Mishra *et al*, 2006; Mishra *et al*, 2007). This phosphorylation would impact gene expression and the anti-apoptotic balance (for p53). Recently, using an on-ship kinase assay, several potential targets of TG2 kinase activity in the cytoplasm, nucleus and ECM, were identified. Confirmation that these events occur and have a function, *in vivo*, is still needed (Jung *et al*, 2016).

B. Regulation of TG2 transglutaminase activity.

A lot of work has been devoted to the study of the regulation of TG2 activity. We will focus in this part on the regulations that are likely to happen in epithelial cells, which is the cellular model used in our study.

1. Genetic regulation.

Although TG2 is expressed in all tissues, its abundance varies a lot in the different cell types that constitute the human body. The greatest part of the regulation is done at the transcriptional level. The transcription of *TGM2* gene encoding TG2 is regulated both epigenetically and transcriptionally.

The proximal promoter of *TGM2* is composed of three GpC-rich domains that can be methylated. The methylation status of these regions controls the expression of *TGM2*: a hypermethylation observed in lymphocytes or monocytes correlates with constitutive absence of TG2 expression whereas a lack of methylation or a demethylation of these regions correlates with an expression of the gene (Cacciamani *et al*, 2002; Lu and Davis, 1997).

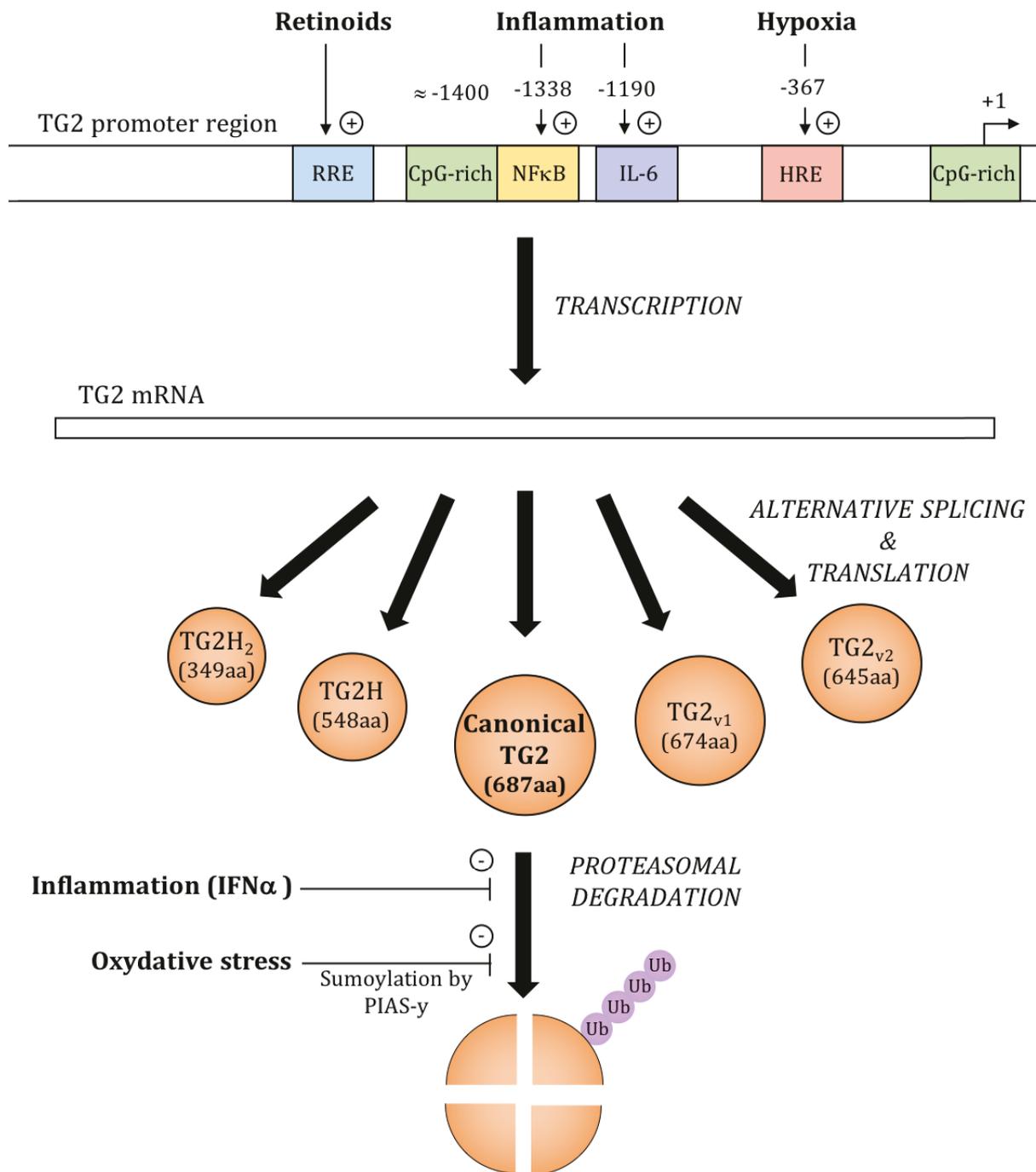


Figure 19: schematic view of TG2 protein level regulation

TG2 protein level is modulated at different levels: transcription (regulated by several external stimuli), mRNA (alternative splicing) and protein stability. This regulation is also cell-type dependent (not represented here).

Retinoic acid has been the first factor shown to control *TGM2* expression, initially in myeloid cells, but also in neuroblastoma cells, and epithelial cells such as HeLa and bronchial cells (Mehta *et al*, 1985; Moore *et al*, 1984; Piacentini *et al*, 1991; Vollberg *et al*, 1992). This transcription control is mediated by a retinoid response element (RRE) present upstream from

the *TGM2* promoter, around 1.7 kb (Nagy *et al*, 1996). Linked to the retinoic acid control of *TGM2* transcription in myeloid cells, an epigenetic priming of the chromatin upstream from the *TGM2* promoter was shown by Balint and collaborators, increasing the accessibility of the RRE. The factors triggering the increase in methylation of Arg3 in histone H4 and the decrease in methylation of Lys4 in histone H3, which constitute this priming step, are unknown (Balint *et al*, 2005).

Inflammatory cytokines have also been reported as inducers of *TGM2* transcription: interleukin-1 β (IL-1 β) in astrocytes, tumor necrosis factor- α (TNF- α) in liver cells, interferon- γ (IFN- γ) in intestinal cells, IL-8 in chondrocytes, etc (Kim *et al*, 2002; Kuncio *et al*, 1998; Merz *et al*, 2003; Monsonego *et al*, 1997; Suto *et al*, 1993). Other inflammatory stimuli such as bacterial lipopolysaccharide (LPS) have the same effect (Ghanta *et al*, 2011). This induction is controlled by the activation of the nuclear factor kappa B (NF κ B) that directly binds an NF κ B motif around 1.3 kb upstream from the transcription start site (Mirza *et al*, 1997). Apart from the activation of NF κ B during an inflammatory response, studies have demonstrated the importance of this pathway for activating *TGM2* transcription in a number of cancer cells (Cao and Huang, 2016; Mehta *et al*, 2010). This activation may require the interaction of NF κ B with the metastatic tumor antigen 1 (MTA1), a master chromatin modifier, at least in LPS-stimulated macrophages (Ghanta *et al*, 2011).

HIF-1 is another inducer of *TGM2* expression under hypoxic conditions, due to a hypoxia response element (HRE) in *TGM2* promoter. An inhibition of TG2 in hypoxic cells sensitized them to apoptosis, indicating a protective role for the induction of *TGM2* in this context (Jang *et al*, 2010). The epidermal growth factor (EGF) also strongly increases *TGM2* transcription in various cell types and in pathological contexts such as tumorigenesis and metastasis through a yet unknown pathway (Antonyak *et al*, 2009; Zemskov *et al*, 2012).

Other modulators of *TGM2* expression have been identified with a more complex role, depending on the cell type. For example, the transforming growth factor β (TGF β) pathway identified thirty years ago acting on a TGF β response element activated by TGF β 1, TGF β 2 or bone morphogenetic proteins 2 or 4 (BMP2 or 4) can either activate or inhibit *TGM2* transcription (George *et al*, 1990; Ritter and Davies, 1998). TGF β 1 increases *TGM2* transcription in fibroblasts and other cell types whereas it inhibits it in non-transformed epithelial cells (Kumar *et al*, 2010; Quang *et al*, 2005; Welge-Lüssen *et al*, 2000). In contrast, the oncogenic protein H-Ras induces the expression of *TGM2* in epithelium-derived cells but blocks it in fibroblasts (Akimov and Belkin, 2003; Antonyak *et al*, 2009).

The regulation of *TGM2* gene expression is thus very complex, integrating several external signals, in a cell type-dependent manner. The dysregulation of this transcriptional control is often involved in the development of pathologies such as cancer or tissue fibrosis.

2. Protein isoforms and stability control.

Variants of the canonical TG2 (≈ 75 kDa) have been observed in certain cell types such as neurons, leukocytes or endothelial cells, arising from alternative splicing of the mRNA (Citron *et al*, 2002; Lai *et al*, 2007). Those variants (four identified so far) are shorter versions of the canonical TG2 and show a generally higher transamidating capacity (Fraij *et al*, 1992; Fraij and Gonzales, 1996; Lai *et al*, 2007). The pathways controlling this alternative splicing are completely unknown, however it is becoming clear that the isoforms play different roles and some hints on the regulation of their expression are beginning to emerge. For example, a shorter isoform of TG2 is induced in rat brain astrocytes by cytokine treatments in culture (Monsonogo *et al*, 1996). Relevance of this short isoform was confirmed *in vivo* after spinal cord injury in rat brains. In this model, the transient appearance of a short isoform of TG2 with an increased transamidating activity correlated with an induction of apoptosis (Festoff *et al*, 2002). The direct involvement of the short isoform in the induction of apoptosis was demonstrated four years later (Antonyak *et al*, 2006). This same shorter isoform was reported to be expressed and correlating with neuronal loss in neurodegenerative diseases such as Alzheimer's or Parkinson's disease (Citron *et al*, 2002). More recently, a team tried to gain a more in-depth comprehension of the alternative splicing phenomenon by reverse transcription followed by a quantitative polymerase chain reaction (RT-qPCR) on RNA extracts from different normal tissue cells and cancer cell lines. This study allowed to firmly confirm that *TGM2* splicing is cell type-dependent. In cancer cells, splicing can differ from the one observed in the original, non-transformed, organ, indicating an adaptive process in this pathological condition (Phatak *et al*, 2013).

Little is known regarding the stability of TG2. As for many proteins ubiquitination of TG2 leads to its proteasomal degradation. Ubiquitination of TG2 is down-regulated by interferon alpha ($\text{IFN}\alpha$) in lung carcinoma cells and up-regulated in cells exposed to a high level of oxidative stress in the presence of high calcium concentration (Esposito *et al*, 2003; Jeong *et al*, 2009). One E3 ligase, carboxy-terminus of Hsp70-interacting protein (CHIP) was recently shown to promote polyubiquitination of TG2 and its subsequent proteasomal degradation in renal cancer cells (Min *et al*, 2016). This TG2-mediated degradation decreases

the angiogenesis at the renal tumor site and the tumor growth itself. In addition, TG2 phosphorylation by phosphatase and tensin homolog (PTEN)-induced putative serine-threonine kinase 1 (PINK1) has been described as preventing TG2 from ubiquitination and subsequent proteasomal degradation, complexifying further the regulation of TG2 stability (Min *et al*, 2015).

The SUMOylation (SUMO – small ubiquitin-like modifier) of TG2 by protein inhibitor of activated STAT- γ (PIAS- γ) in bronchial epithelial cells expressing a deficient cystic fibrosis transmembrane conductance regulator (CFTR), and in mice with cystic fibrosis, was able to reduce TG2 ubiquitination thus stabilizing the protein and increasing the oxidative stress and inflammation in this respiratory disease (Luciani *et al*, 2009).

3. Allosteric and conformational regulation.

At steady state TG2 is thought to be in a closed conformation with the catalytic transamidating site hidden by two β -barrel domains, preventing any transamidation activity (Casadio *et al*, 1999; Pinkas *et al*, 2007). The close state is stabilized by the cytosolic concentration of free GDP/GTP that binds the enzyme, and the low Ca^{2+} concentration (Mariani *et al*, 2000). Increase in Ca^{2+} concentration and its binding to TG2 elicits a strong conformational change in the enzyme leading to its open conformation in which the active site catalyzing transamidation is accessible to substrates (see figure 20). Given the Ca^{2+} concentrations required to induce this conformational change, it is quite clear that without external stimuli or stresses, the transamidating function of TG2 is inactive inside the cytoplasm (Király *et al*, 2011). In the ECM the high concentration of calcium ions and the really low concentration of GDP/GTP bring TG2 in its open conformation. However, the transamidating activity is normally inhibited, because of the oxidative conditions, by the formation of a disulfide bond (Stamnaes *et al*, 2010). An extracellular reducing enzyme such as thioredoxin-1 secreted by macrophages upon pro-inflammatory stimuli for example can reduce this disulfide bond and activate extracellular pools of TG2 (Plugis *et al*, 2017). Thus, at steady state, both intracellular and extracellular TG2 are inactive for the transamidating activity. Of note, recent, biophysics experiments pointed towards a dimerization of TG2 mediated by its C-terminal domain, that would be necessary for the protein to gain its open conformation (Kim *et al*, 2017; Stamnaes *et al*, 2015).

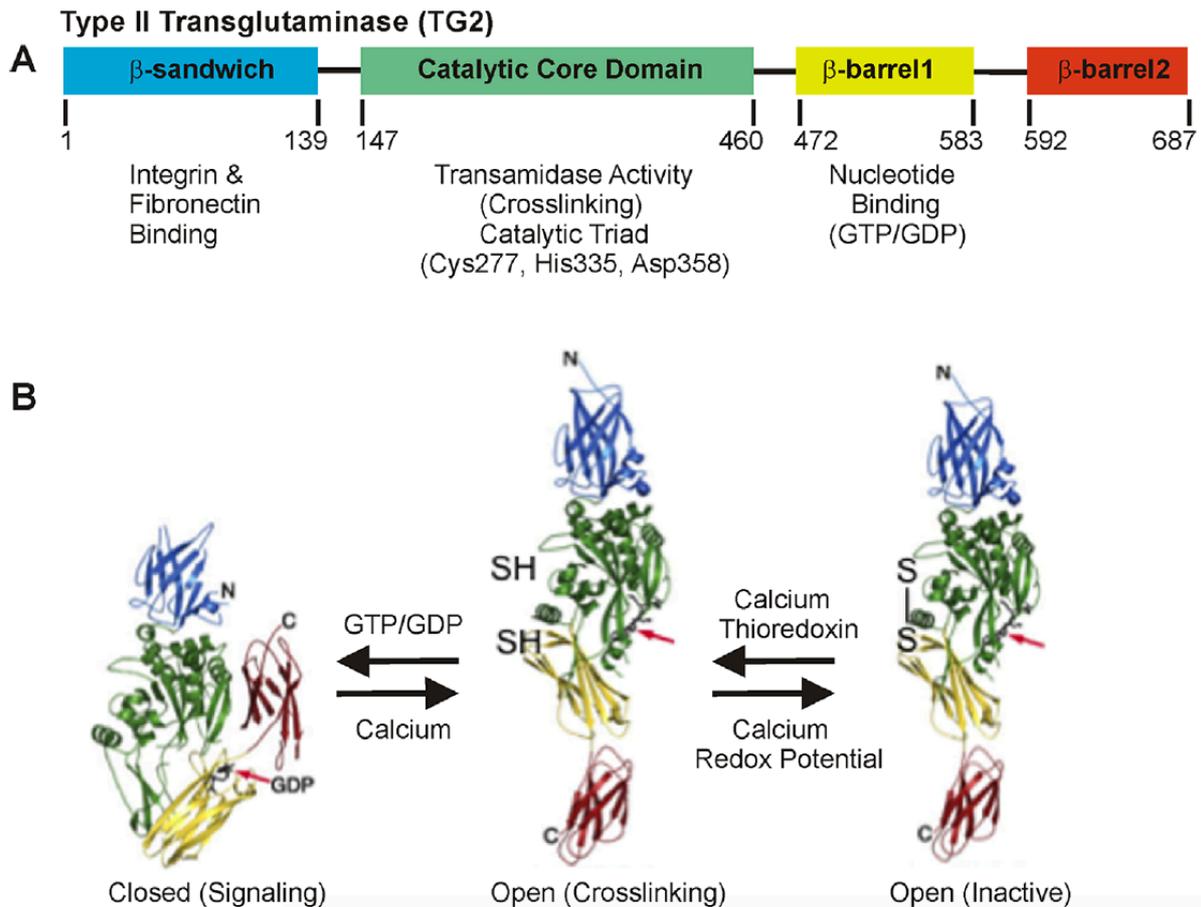


Figure 20: Conformational regulation of TG2 transamidating activity

(A) TG2 is composed of 4 domains with different functions.

(B) At steady-state, TG2 inside the cell is in a closed, catalytically inactive, conformation, because of the strong concentration of GTP/GDP and the low concentration of calcium. In this conformation TG2 can interact with partners and play a part in regulating cell signaling. Upon a strong influx of calcium, TG2 binds calcium ions instead of GTP and moves to its opened, catalytically active, conformation. However, under oxidative conditions such as the ones happening in the extracellular medium, even in presence of calcium (open conformation), TG2 is catalytically inactive because of intracellular disulfide bonds created between cysteine residues. TG2-inactivation by NO is not depicted here. From Eckert *et al*, 2015.

Other modifications and molecules have been identified that modulate the transamidating activity of TG2. *S*-nitrosylation of several cysteine residues by nitric oxide (NO) inhibits TG2 activity. This is particularly relevant in the vasculature where the diminution of NO bioavailability leads to an increase of TG2 crosslinking activity on the ECM components and thereby to a stiffening of blood vessels during the aging process (Jung *et al*, 2013; Santhanam *et al*, 2010). Recently this modification was shown to also happen intracellularly, in cardiomyocytes, regulating their contractility through mitochondrial ATP production control in normal and pathological conditions (Jeong *et al*, 2018). Phosphorylation is another posttranslational modification known to modulate TG2 activity: phosphorylation by the protein

kinase A (PKA) on the Ser216 inhibits the transamidating activity of TG2 while increasing its kinase activity, in response to different cellular stimulations (Mishra and Murphy, 2006; Mishra *et al*, 2007).

The interaction between TG2 and certain lipids has been shown to favor TG2 transamidating activity, particularly with the sphingosine-phosphorylcholine, a sphingophospholipid present in very scarce amounts in biological membranes (Lai *et al*, 1997, Nemes *et al*, 2009). The mechanism of TG2 increased activity in such conditions remains unknown, along with its biological relevance. One might speculate that TG2 recruitment at membranes (plasma membrane or endomembranes) may provoke a conformational change of the protein, or bring it in close proximity to calcium-rich compartments allowing for its local activation.

Finally, the interaction between TG2 and other proteins is also able to modulate its transamidating activity. For instance, in the ECM an interaction between TG2 and syndecan-4 enhances its transamidating activity (Scarpellini *et al*, 2009). However, even though many evidences indicate that TG2 transamidating activity can be regulated by other proteins, following EGF or retinoids stimulation in particular, no clear binding partner has been identified yet (Antonyak *et al*, 2009; Singh and Cerione, 1996).

Given the importance of TG2 in the development of many pathologies, inhibitors of the transamidating activity of TG2 have been developed and tested *in vitro*, *in vivo*, in cell culture, but also in animals for some of them. Historically, cystamine, spermine and other small amines that are competitive inhibitors of the reaction catalyzed by transglutaminases have been used but given their non-specificity for TG2 and their other possible biological effects on treated cells, more specific inhibitors have been designed (Birckbichler *et al*, 1981; Folk *et al*, 1980; Keillor *et al*, 2015). For example, CP4d, a reversible inhibitor, was shown to trap TG2 in a closed conformation. It is specific of TG2 since it doesn't inhibit TG1, TG3 nor Factor XIIIa (Pardin *et al*, 2008; Caron *et al*, 2012).

4. Spatial distribution and its regulation.

TG2 is classically mostly present in the cytosol where the activation of its transamidating activity is caused upon an external stimulus (stress, chemokine, growth factor, etc) as described above (figure 21).

External stimuli such as retinoids or a strong calcium influx in the cytosol can also lead to the translocation of the protein into the nucleus (Balajthy *et al*, 2006; Lesort *et al*, 1998). A

nuclear localization signal (NLS) and a nuclear export signal (NES) have been identified in TG2 (Shrestha *et al*, 2015). Importin- α 3 seems to be involved in its transfer towards the nuclear compartment and exportin-1 in its export (Peng *et al*, 1999; Shrestha *et al*, 2015). A compound, phenosafranin, has been recently identified as a specific inhibitor of TG2 nuclear localization (Furutani *et al*, 2017). Understanding how this compound works may shed light on the mechanisms of TG2 cytosol-nucleus shuttling and its control. It will also be an important tool to understand the physiological roles of TG2 activity in the nucleus.

Secretion of TG2 by cells is thought to be a general and constitutive mechanism, allowing the association of this protein to the outer leaflet of the plasma membrane or to the ECM, where it plays crucial physiological and pathological roles especially in ECM stability, cell migration, and adhesion. Since TG2 lacks a classical signal peptide, its secretion falls into the “unconventional secretion” mechanisms -a phenomenon also observed for other proteins such as IL-1 β (Malhotra, 2013). This mechanism has remained a mystery for a long time and only started being unraveled in the last decade. Instead of going through the classic secretory pathway involving the ER and the Golgi apparatus and accompanied by *N*- and/or *O*-linked glycosylation, TG2 is loaded, by an unknown, phospholipid-dependent mechanism, in perinuclear recycling endosomes also called multivesicular bodies (MVB) (Zemskov *et al*, 2011). Once in recycling endosomes, TG2 interacts with endocytosed integrins and is secreted via a Rab11-dependent pathway at the plasma membrane (Zemskov *et al*, 2011). More recently, TG2 was found to be associated with material known to be enriched in extracellular vesicles called exosomes, whose extracellular release was stimulated by the TGF-1 β in tubular kidney epithelial cells (Furini *et al*, 2018). A similar phenomenon might occur in smooth muscle cells, in which case the association of TG2 to exosomes appears to require TG2 transamidating activity (van den Akker *et al*, 2012). In contrast, NO has an inhibitory effect on TG2 secretion by endothelial cells by inducing the *S*-nitrosylation of *N*-ethylmaleimide sensitive factor (NSF), a protein involved in the fusion of secretory vesicles with the plasma membrane (Santhanam *et al*, 2011).

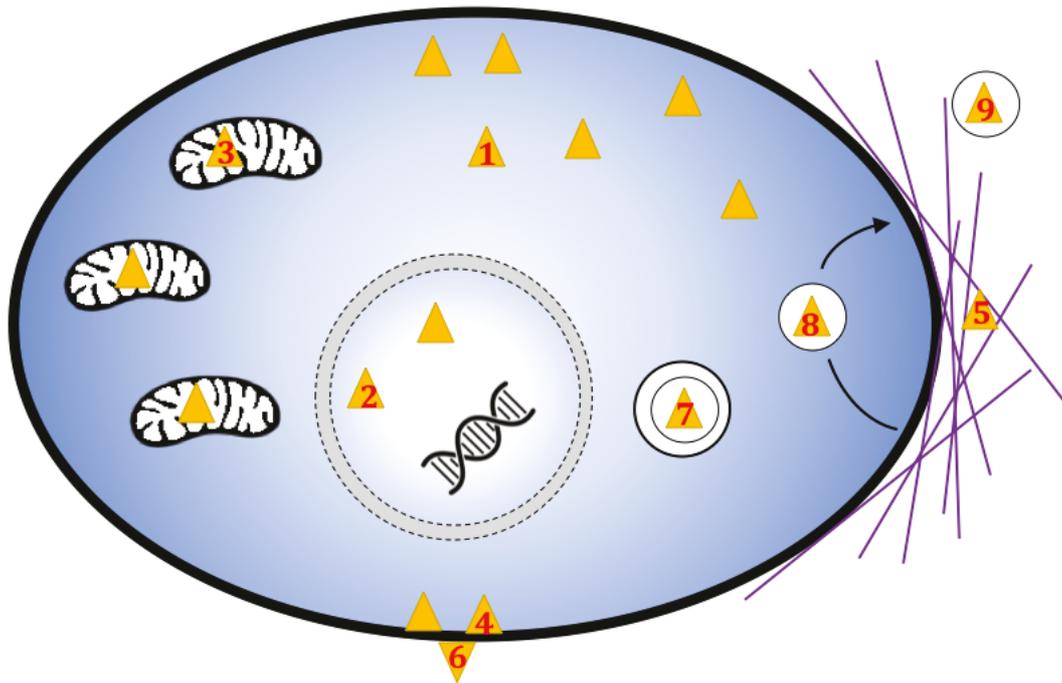


Figure 21: Spatial distribution of TG2

TG2 is mainly present in the cytosol (1) but can shuttle towards the cell nucleus (2), the mitochondria (3) or be associated with the plasma membrane (4). It is secreted and can be present associated to the extracellular matrix (5) or the external leaflet of the plasma membrane (6). The mechanism of secretion involves TG2 loading into multivesicular bodies (7) and TG2 turnover in the extracellular medium is controlled by its recycling in endosomes (8). More recently, TG2 has been described in exosomes (9).

TG2 extracellular turn-over is controlled by its secretion but also by its internalization and by its proteolysis in the extracellular space. The internalization of TG2 has been shown to depend on its interaction with the low-density lipoprotein receptor-related protein 1. It is to some extent regulated by TG2 interaction with components of the ECM that anchor the protein in that environment and prevent its internalization (Zemskov *et al*, 2007). Once internalized, TG2 is degraded in the acidic endo-lysosomal compartment (Zemskov *et al*, 2007). Membrane-type-matrix metalloproteinases (MT-MMPs) such as MT1-MMP, and their soluble counterparts MMPs such as MMP2, have been described to degrade very efficiently TG2 in the extracellular compartment, either associated with the plasma membrane or the ECM (Belkin *et al*, 2001 Belkin *et al*, 2004). Thrombospondin, an ECM protein, can prevent TG2 proteolytic degradation by MMP2 (Agah *et al*, 2005). The activation of those proteases depends on external stimuli such as pro-inflammatory stimuli, cytokines, proliferating or migrating factors and are dysregulated in many cases of cancer especially during the epithelium-to-mesenchyme transition (EMT) (Axelrad *et al*, 2004; Kim *et al*, 2007; Liu *et al*, 2017).

TG2 has been shown to be present in the mitochondria by electron microscopy and cell fractionation, associated with the external membrane or present in the inter-membrane space mainly, but also present in the inner membrane and the matrix to a lesser extent (5 to 10 %) even though no classical N-terminal mitochondria-targeting signal is present on TG2 primary sequence (Park *et al*, 2010; Piacentini *et al*, 2014). TG2 contains a BH3 domain, which might be involved in TG2 tropism for mitochondria. The particular lipid composition of the inner membrane, especially the presence of cardiolipin, may also contribute to this subcellular localization (Zemskov *et al*, 2011). Localization of TG2 to mitochondria was proposed to contribute to the control of apoptosis by interacting with and post-translationally modifying pro-apoptotic members of the Bcl-2 family (Rodolfo *et al*, 2004).

Given its multiple enzymatic activities and localizations, TG2 is a strongly regulated enzyme that plays critical roles in physiological processes but also many pathological conditions.

C. Physiopathological importance of TG2.

1. TG2, a critical regulator of cell proliferation, migration and differentiation.

a. Extracellular TG2, ECM regulation and outside-in signaling.

TG2 transamidating activity in the ECM has different functions that are important for cell adhesion and migration. First, by generating highly ordered polymers with a longer half-life time, TG2-modified ECM molecules such as collagen, fibronectin, or fibrillin-1 promote integrin clustering at the cell surface and subsequent outside-in signaling (Belkin *et al*, 2005; Bowness *et al*, 1987; Fesus *et al*, 1986; Khew *et al*, 2010, Raghunath *et al*, 1996). Together with the direct ability of TG2 to interact with integrins and syndecan-4 at the cell surface, and fibronectin from the ECM, reinforcing this naturally weak interaction, the extracellular TG2 pull is crucially involved in the activation of the outside-in signaling cascade downstream of the integrin receptors, stimulating cell migration and adhesion to the matrix (Akimov *et al*, 2000; Telci *et al*, 2008). Integrin clustering in interaction with fibronectin causes the activation of focal adhesion kinase (FAK) and Src, increasing the intracellular pool of active GTP-bound RhoA, leading to Rho-associated protein kinase (ROCK) activation and the formation of stress fibers and focal adhesions (Janiak *et al*, 2006). In a similar way, TG2 can also contribute to

stimulating cell migration and proliferation by interacting and bringing together integrins and growth factor receptors such as the platelet-derived growth factor receptor (PDGFR) (Zemskov *et al.*, 2009). Downstream signaling pathways also involve RhoA, activated by Shp2, and, additionally, other pro-proliferative pathways such as the mitogen-activated protein kinase (MAPK) pathway involving extracellular signal-regulated kinase (ERK) and Akt1 pathway. More generally, TG2 interaction with growth hormone receptors is important for pro-survival and proliferation signal enhancement. This interaction can be non-covalent like with the PDGFR, or it can involve TG2 transamidating activity, creating a high molecular weight complex for example between TG2 and the vascular endothelial growth factor receptor (VEGFR) (Dardik and Inbal, 2006).

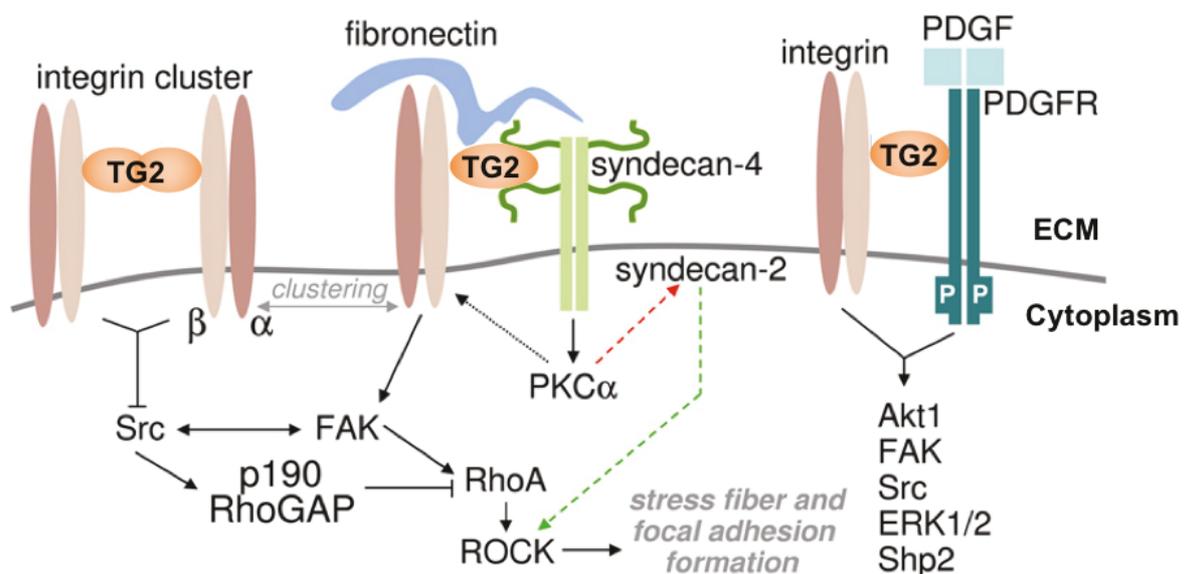


Figure 22: TG2-mediated adhesion and signaling at the cell surface

TG2 promotes the clustering of integrins, syndecans and elements from the extracellular matrix such as fibronectin, resulting in an activation or a reinforcement of the integrin outside-in signaling, activating cellular adhesion and migration through actin cytoskeleton remodeling by ROCK. Integrin and PDGFR clustering by TG2 leads to cell migration and proliferation by activation of the MAPK pathway. Adapted from Belkin, 2011.

In a more mechanical manner, the cross-linking of fibronectin, collagen or osteopontin molecules contribute to the ECM stiffness and stability and thereby to better adhesion and migration properties (Forsprecher *et al.*, 2009; Higashikawa *et al.*, 2007; Spurlin *et al.*, 2009).

Finally, the cross-linking of osteopontin has been described as essential for demasking cryptic interaction sites between osteopontin and the $\alpha 9\beta 1$ integrins expressed by neutrophils, process required for neutrophil migration *in vitro* and *in vivo* (Nishimichi *et al.*, 2009, 2011).

b. Intracellular TG2 also participates in migration and proliferation.

More directly, TG2 can be part of signaling cascades involved in cell migration and proliferation. Intracellular TG2 transamidating activity induces the expression of MMP2 gene transcription in ovarian cancer cells by activation of cAMP-response element-binding protein (CREB) (Satpathy *et al*, 2009). MMP9 expression is also induced in lung cancer cells by active TG2, under EGF stimulation, enhancing cell migration (Antonyak *et al*, 2009; Li *et al*, 2011). More generally TG2 seems to be an effector of the EGF signaling pathway since it has been described as located and active at the leading edge of migrating cells where it interacts with the chaperone heat shock protein 70 (Hsp70) and is essential for migration (Boroughs *et al*, 2011).

c. TG2 and differentiation.

TG2 activity is important for the differentiation of many cell types such as keratinocytes, neutrophils, neuron, and a key factor involved in the differentiation of mesenchymal stem cells into chondrocytes, osteoblasts, myoblasts through its activity on cytoskeletal proteins like actin, tubulin, vimentin, myosin (Balajthy *et al*, 2006; Bersten *et al*, 1983; Eckert *et al*, 2005; Esposito and Caputo, 2005; Tucholski *et al*, 2001).

A particularly important pathway involved in the differentiation of intestinal epithelium among others is the TGF- β 1 pathway, which is intrinsically linked with the activity of TG2. TG2 is essential for the deposition of latent TGF- β binding protein-1 (LTBP-1) in the ECM, thereby controlling the local concentration of TGF- β 1 (Verderio *et al*, 1999). Moreover, in some cell types like fibroblasts, TG2 can increase the transcription of the TGF- β 1 gene by NF κ B signaling (Szondy *et al*, 2003; Telci *et al*, 2009). In return, TGF- β 1 also activates TG2 transcription (see section III.B.1.), resulting in a positive feedback loop. TGF- β 1 secreted by the pericryptal fibroblasts and neurons from the enteric nervous system is one of the most critical growth factors involved in the differentiation of enterocytes, from the mesenchymal phenotype in the crypt, towards an epithelial one while progressing along the crypt-villus axis (Liao *et al*, 2013; Rathor *et al*, 2011; Puzan *et al*, 2018). TG2 plays thus a very important role in the morphogenesis of the intestinal epithelium.

d. TG2 and epithelial-to-mesenchymal transition (EMT) in cancer.

Given the factors controlled by TG2 in many different cell types, it is not surprising that dysregulations of its expression and/or transamidating activity are often described in metastatic cancer cells (Kumar *et al*, 2011; Satpathy *et al*, 2007; Verma *et al*, 2006). The EMT is a

hallmark of epithelial cell transformation, leading to a loss of adherence, migration and metastasis formation. TG2 can activate signaling pathways in cancer cells leading to NF κ B or FAK/Akt activation which are able to drive the EMT (Kumar *et al*, 2010; Shao *et al*, 2009; Verma *et al*, 2008). For example, in ovarian tumor cells, the activation of TG2 transglutaminase activity leads to the activation of NF κ B that activates the transcription of zinc finger E-box-binding homeobox 1 (Zeb1), a transcriptional repressor that in turn represses the expression of E-cadherin, a key step in the EMT (Shao *et al*, 2009). The activation of TG2 is caused at least partially by the TGF- β 1 signaling in those cells (Cao *et al*, 2012).

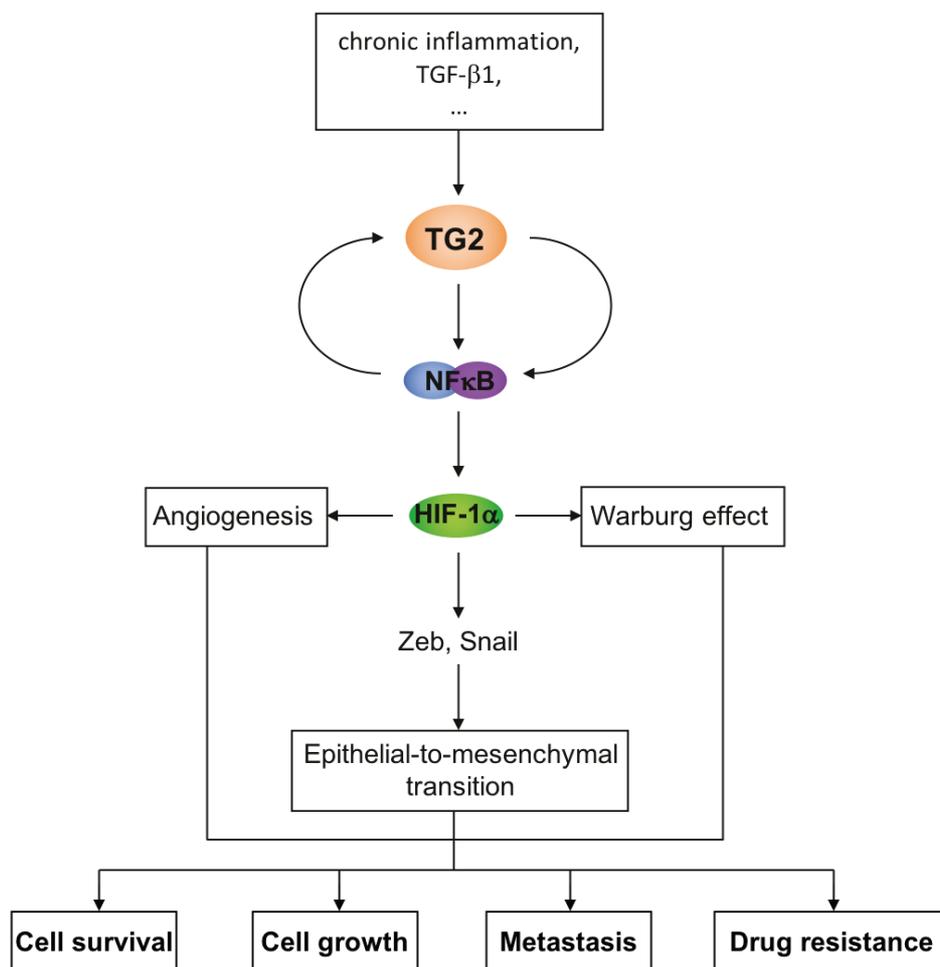


Figure 23: Model for TG2-induced EMT and cancer evolution

Under different stimuli, the expression of TG2 is elevated, leading to the activation of NF κ B and an increased HIF-1 α transcription. HIF-1 α -expression modifies the cell metabolism (see part II) towards a Warburg metabolism, induces angiogenesis and EMT. These modifications are cancer-driving events, allowing for cell survival and growth, metastatic capacity and drug resistance.

Moreover, it was demonstrated that microvesicles containing TG2 (but not TG2 alone) derived from cancer cells *in vitro* were able to confer a transformed phenotype to epithelial cells and fibroblasts, indicating the importance of TG2 in this phenotype (Antonyak *et al*, 2011).

Finally, the overexpression and activation of TG2 in cancer cells is often associated with resistance against treatments. For example glioblastoma, lung, breast cancer cells overexpressing TG2 are resistant to anthracyclines (inhibitors of the type II topoisomerase activity) used in cancer therapy such as doxorubicin (Dyer *et al*, 2011; Han and Park, 1999; Mehta, 1994). This deregulation of TG2 expression can happen through an epigenetic modification (hypermethylation of its promoter silencing its expression, or the opposite) but can also be mediated by external stimuli present at the tumor site, particularly inflammatory stimuli secreted locally (Dyer *et al*, 2011).

2. TG2 and cellular response against several stresses.

a. Inflammation.

Many inflammatory cytokines such as IL-1, IL-6, TNF- α or IFN- γ are able to induce TG2 expression (see section III.B.1.). Moreover, in an inflammatory context created for example by LPS-stimulation, *TGM2* is more transcribed (Ghanta *et al*, 2011). The increase in *TGM2* expression in those cases relies mostly on the activation of the NF κ B pathway.

TG2 plays several roles during acute inflammation, depending on the cell type in which it is expressed. It is important for leukocyte adhesion, extravasation and migration towards the inflammation site, role mediated mainly by extracellular TG2 in interaction with integrins, as described above. Monocytes differentiating into macrophages for example undergo increased *TGM2* expression, leading to an enhanced activation of integrin signaling, actin cytoskeleton rearrangements, and cell diapedesis and migration (Schnoor, 2015; Seiving *et al*, 1991; van Strien *et al*, 2015). Extracellular TG2 is also important for macrophages efferocytosis – clearance of apoptotic bodies – by regulating integrin outside-in signaling and leading to cytoskeletal remodeling (Toth *et al*, 2009). This role is involved in the limitation of the atherosclerosis plaque progression by allowing their partial removal (Boisvert *et al*, 2006).

Associated with the inflammatory process, TG2 implication in ECM remodeling is also important in wound healing and tissue repair, which tend to restore tissue stability and end the inflammatory process. In injured tissues, TG2 production and secretion is induced by pro-inflammatory stimuli and leads to ECM remodeling as described above. This in turns enhances cell-adhesion through integrin-mediated signaling for tissue-resident cells and inhibits cell

apoptosis (Vederio *et al*, 2003). TG2 implication in tissue repair can lead to TG2-mediated tissue fibrosis, deleterious for the tissue function under chronic inflammation in the kidney, cardiac muscle, lung, etc (Johnson *et al*, 2007; Olsen *et al*, 2011; Shinde *et al*, 2018).

The role of intracellular TG2 during the inflammatory process might not be limited to its transamidating activity. TG2 interaction with NF κ B has been described as an inflammatory regulator. During acute inflammation, the canonical pathway for NF κ B activation shows I κ B kinase (IKK) activation, that phosphorylates inhibitor of κ B α (I κ B α), leading to its degradation and the release of NF κ B that then migrates towards the cell nucleus and activates the transcription of a number of pro-inflammatory genes (see figure 24) (Yamamoto and Gaynor, 2004). During chronic inflammation, a non-canonical way of activating NF κ B involving TG2-mediated I κ B α proteasomal degradation allowed by the interaction between these two proteins, independent of TG2 transamidating activity, has been described (Kumar and Mehta, 2012). Since NF κ B is an inducer of TG2 expression, a positive feedback loop regulating TG2 and NF κ B activation seems to establish during chronic inflammation, allowing for the persistence of NF κ B activation. This finding might also be relevant for tumor transformation, in which it was proposed that a TG2/NF κ B/HIF-1 α axis promoted the expression of glucose transporter GLUT1 (Kumar *et al*, 2014). The hypothesis that this axis was independent of the transamidating activity of TG2 was not formally tested.

Among the pro-inflammatory activities of TG2 that require its transamidating activity lies its ability to create an intramolecular cross-link in the secretory isoform of the phospholipase A2 (sPLA2), increasing its catalytic activity and thereby enhancing the production of pro-inflammatory eicosanoids (Currò *et al*, 2014; Hallstrand *et al*, 2010). The pro-inflammatory action of TG2 has also been revealed by an *in vitro* model of cystic fibrosis in which lung cells expressing a functionally deficient cystic fibrosis transmembrane conductance regulator (CFTR) were expressing high levels of TG2, creating cross-linked peroxisome proliferator-activated receptor γ (PPAR γ). Polyubiquitination and degradation of PPAR γ induced an inflammatory response (Maiuri *et al*, 2008; Pascual *et al*, 2005).

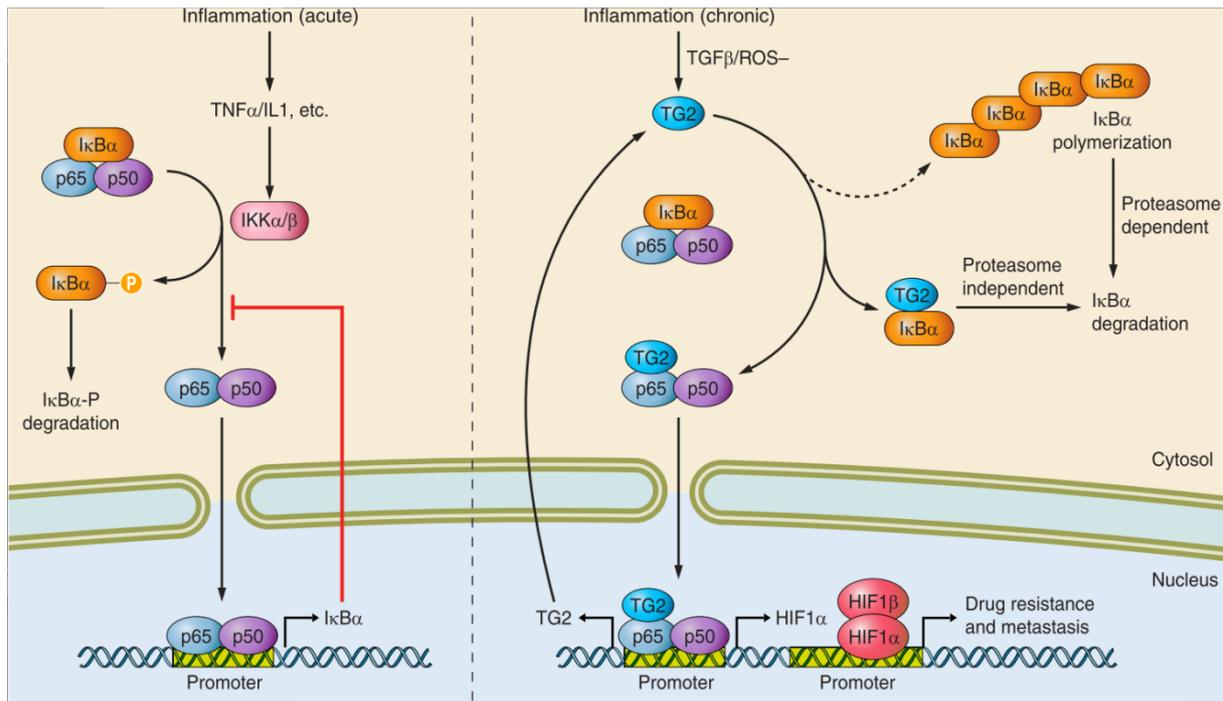


Figure 24: NFκB activation under acute and chronic inflammation: TG2 as a key factor

Under acute inflammation, NFκB (p65-p50) is activated by a canonical pathway downstream of pathogen recognition receptors (PRRs) or interleukin receptor signaling. This pathway involves the phosphorylation then degradation of IκBα, allowing for the translocation of NFκB to the cell nucleus and the activation of the transcription of target genes, including IκBα gene, in a negative feedback loop (left panel).

Under chronic inflammation, the levels of intracellular TG2 allow for its interaction with IκBα and the subsequent degradation of IκBα in a pathway independent from the proteasome. There might also be a cross-linking of IκBα by TG2-transamidating activity resulting in IκBα polymer proteasomal degradation. TG2 binds to free NFκB both in the cytosolic and the nuclear compartment. TG2/NF-κB complexes activate the transcription of a different subset of genes, including HIF-1α (right panel).

b. Other stresses.

Related to its contribution to inflammation during cystic fibrosis, TG2 transamidating activity is an inhibitor of cellular autophagy under certain stress conditions. In the case of cystic fibrosis, oxidative stress-related TG2 activation through HIF-1α stabilization induces cross-linking and subsequent sequestration of beclin-1 in aggresomes, inhibiting its function of autophagy inducer (Luciana *et al*, 2010). This mechanism was also described in cancer cells where activation of TG2 by protein kinase C delta (PKCδ) led to beclin-1 degradation and autophagy diminution (Ozpolat *et al*, 2007). However, TG2 activity is also involved in the maturation of autophagosomes under stressful conditions, indicating a complex role for this enzyme in this particular cellular process (Altuntas *et al*, 2015; D'Eletto *et al*, 2009).

In addition to its role in stress-induced autophagy, TG2 is involved in controlling apoptosis, and was thus proposed to play a central role in orienting cells to survival or death (Rossin *et al*, 2012). Depending on the cell type, the kind of stress, and the localization of the enzyme (cytoplasm, nucleus or mitochondria) TG2 was shown to induce or prevent cell-death by apoptosis. Just taking epithelial cells as an example, under DNA-stress caused by cisplatin, an anti-cancer drug, TG2 was shown to activate NF κ B signaling pathway and to block cell apoptosis in ovarian cancer epithelial cells, whereas under ultraviolet B light stimulus, corneal epithelial cells were undergoing apoptosis induced by TG2 (Cao *et al*, 2008; Tong *et al*, 2006). The very complex interplay between TG2 activation and apoptosis- or autophagy-induction upon stress is not clear. Further experimental work is required to understand the role of TG2 in different diseases, particularly neurodegenerative diseases such as Huntington's or Parkinson's disease, and cancer, in order to be able to build new and more efficient treatment strategies.

3. Other physiological roles for TG2.

Considering the diversity of TG2 substrates, and the technical difficulty to identify these substrates, and their subsequent modification, it is clear that only the tip of the iceberg of TG2-induced regulation is starting to emerge. Serotonylation of proteins by TG2 illustrates how TG2 is likely involved in the fine tuning of many cellular processes.

Cytoplasmic TG2 is able to induce serotonylation of cytoskeletal-regulatory proteins such as RhoA, Rac1, Rab4A or cytoskeletal-related proteins like the smooth muscle α -, β - and γ -actin, the myosin heavy chain or filamin (Watts *et al*, 2009). These modifications allow an adaptation of the contractibility of the smooth muscle cells and a modification of the vessels smooth muscle tone. In the pancreatic β cells, serotonylation of Rab proteins is involved in the release of insulin by exocytosis upon glucose stimulation and in platelets it is involved in degranulation (Paulmann *et al*, 2009; Walther *et al*, 2003, 2011). TG2-induced serotonylation is thus an important mechanism controlling cytoskeleton structure and exocytosis under cell-type specific stimulations, accounting for the effects of the neuromodulator serotonin, independent from its receptor activation.

IV. Questions addressed in this doctoral work

Surprisingly, while the association between TG2 activity and inflammatory situations has been studied in several normal and pathological situations, the implication of this enzyme in a very classical inflammatory process, e.g. the defense response of a tissue to the invasion by a microorganism (bacterium, virus or parasite), has remained very poorly investigated. Furthermore, TG2 has been implicated in several cellular functions that are disturbed during *C. trachomatis* infection, such as the regulation of apoptosis, and glucose homeostasis. Preliminary data rapidly pointed to a strong increase in TG2 activity during *C. trachomatis* infection. This doctoral work:

- describes the consequences of an inhibition of TG2 activity on *C. trachomatis* infection in epithelial cells.
- dissects the steps, along the *C. trachomatis* developmental cycle, that are affected by the inhibition of TG2 transamidating activity or expression.
- shows that TG2 is required for the increase in glucose import in infected cells.
- identifies novel substrates of TG2 using a proteomic approach.
- demonstrates that the enzyme glucosamine-fructose-6P amidotransferase (GFPT) is one of these substrates and identifies glutamine 328 (Gln328) as a target of TG2.
- uncovers an unsuspected relationship between TG2 activity and *O*-GlcNacylation of proteins, through the activation of GFPT.
- shows that *C. trachomatis* disrupts this link, likely by hijacking the end product of the biosynthesis pathway controlled by GFPT to feed peptidoglycan synthesis: UDP-N-acetylglucosamine.

MATERIAL AND METHODS

Cells and bacteria

HeLa cells (ATCC) and mouse embryonic fibroblasts (MEFs) isolated from KO (TG2^{-/-}) or WT (TG2^{+/+}) C57B6 mice were grown in Dulbecco's modified Eagle's medium with Glutamax (DMEM, Invitrogen), supplemented with 10 % (v/v) heat-inactivated fetal bovine serum (FBS) (D'Eletto *et al*, 2009).

Primary cells used for experiments displayed in Figure 22 were isolated from human fallopian tubes and maintained in culture as previously described (Roth *et al*, 2010). Other primary cells were isolated from endocervix biopsies of female patients and were cultivated in keratinocyte-SFM medium (Thermo Fisher Scientific) containing 50 mg.L⁻¹ of bovine pituitary extract (Thermo Fisher Scientific) and 5µg.L⁻¹ of epidermal growth factor (EGF) human recombinant (Thermo Fisher Scientific) (Wu *et al*, in preparation). All cell cultures were maintained at 37 °C, in 5 % CO₂ atmosphere.

C. trachomatis serovar LGV L2 strain 434 and serovar D/UW-3/CX (ATCC), GFP-expressing L2 (L2^{IncD}GFP) or *C. muridarum* Nigg (for *in vivo* experiments) were propagated on HeLa cells, purified on density gradients as previously described and stored at -80 °C (Scidmore *et al*, 2005; Vromman *et al*, 2014).

***In vivo* infections and dissections**

Female TG2^{-/-} and TG2^{+/+} TIFA-KO mice were kindly provided by Dr. C. Papista (INSERM UMR970, Centre de Recherche Cardiovasculaire, Paris) and maintained in the animal facility of the Institut Pasteur, Paris. All animals are treated with 2.5 mg of medroxyprogesterone (Depo-provera-SC®, Pfizer) 7 days prior to infection to synchronize the menstrual cycle.

Mice were intravaginally inoculated with *C. muridarum*, 10⁵ IFU per animal. Twenty-five days after infection, animals were sacrificed and the organs excised including cervix, uterine horn and oviduct. The bacterial burden in the excised organs, in the right part of the upper genital tract was measured by qPCR. The left part of the upper genital tract was excised and rinsed into PBS for the morphological observation.

siRNA treatment

For siRNA experiments, 50 000 cells were plated in a 24 well-plate and immediately mixed with Lipofectamine RNAiMAX (Invitrogen) following the manufacturer's recommendation, using 10 nM of siRNA (Table 2). For electron microscopy experiments, 1.5 million cells were

plated in a 25 cm² flask. The culture medium was changed the next day and experiments (infection or treatment with Ionomycin, Sigma) were performed two days post treatment with the siRNA.

Table 2: Primer sequences used for siRNA, qPCR and mutagenesis

Sequences are given from 5'- end to 3'- end.

Experiment	Target	Sequence
siRNA	TG2	GGGCGAACCACCUGAACAAAdTdT
	GFPT1.1	GACAGAUUGUGGAGUUCAUdTdT
	GFPT1.2	CCUUGGUGGAGAGAGUUUdTdT
	GFPT1.3	GUGACUCCUGGACAGAAAAdTdT
	GFPT2.1	GUUCCAAGUUUGCGUAUAAAdTdT
	GFPT2.2	GACCGAAUUUCACUACAAAAdTdT
	GFPT2.3	CCAUCGCCAAGCUGAUUAAAdTdT
qPCR	α -actin	GGACTTCGAGCAAGAGATGG
		GCAGTGATCTCCTTCTGCATC
	<i>Chlamydia</i> 16S RNA	TGGATGAGGCATGCAAGTA
		TACTAACCCTTCCGCCACTAAA
	TG2	TAAGAGATGCTGTGGAGGAG
		CGAGCCCTGGTAGATAAA
	GLUT-1	AACTCTTCAGCCAGGGTCCAC
		CACAGTGAAGATGATGAAGAC
	GLUT-3	CACAGGTTTTGTGCCCATGTA
		ACCCCGCAGGGCAGTAG
Mutagenesis	Q58N	GGGAAGCCAATGCCTGCAAAATCAATCTTATTAAGAAGAAAGGAAAAGT
		ACTTTTCCTTTCTTCTTAATAAGTTGATTTTGCAGGCATTGGCTTCCC
	Q326N	CGAGCTGTGCAAACACTCAATATGGAAGTCCAGCAGATC
		GATCTGCTGGAGTTCCATATTGAGTGTTTGCACAGCTCG
	Q548N	CGAAATTCAGAACTAGCAACAGAAGCTTTATCATAATAAGTCAGTTCTGATAATG
		CATTATCAGAACTGACTTATTATGATAAAGTTCTGTTGCTAGTTTCTGAATTTCCG

RT-qPCR and qPCR

125 000 cells in 24-well plate were infected or not with L2 bacteria at a MOI = 1. Cells were treated with CP4d (40 μ M) or DMSO 2 hpi, or doxycycline (62.5 ng.mL⁻¹, Sigma) 24 hpi. Total RNAs were isolated 24 or 48 hpi with the RNeasy Mini Kit (Qiagen) with DNase treatment (DNase I, Roche). RNA concentrations were determined with a spectrophotometer NanoDrop

(Thermo Fisher Scientific) and normalized to equal contents. Reverse transcription (RT) was performed using the M-MLV Reverse Transcriptase (Promega) and quantitative PCR (qPCR) undertaken on the complementary DNA (cDNA) with LightCycler 480 system using LightCycler 480 SYBR Green Master I (Roche). For the experiments where bacterial load was measured to compare the infection between different conditions (experiment with doxycycline, CP4d or DMSO addition displayed in Figure 31) a duplicate well was used to extract genomic DNA (gDNA) of each time point using the DNeasy Blood and Tissue Kit (Qiagen), and the amounts of cells and bacteria in the samples determined by qPCR using actin and 16S RNA primers respectively. For *in vivo* experiments in mice, bacterial detection was performed by extracting DNA also using the DNeasy Blood and Tissue Kit (Qiagen). The amounts of cells and bacteria were determined by qPCR using actin and 16S RNA primers respectively. Primers used are listed in Table 2, their specificity was ensured through the analysis of melting curves and their efficiency tested using serial dilutions of samples encoding the genes of interest. For cDNA, data were analyzed using the Delta-Delta Ct method with the *actin* gene as a control gene (Schmittgen et al 2008). For gDNA, absolute quantitation was performed by analyzing as calibrator a serial dilution of DNA extracted from pure EBs (for bacterial 16S RNA) or pure actin cDNA obtained after a RT performed on RNA extracted from HeLa cells using specific primers followed by an amplification by PCR of this gDNA finally purified on gel.

Adhesion assay

Adhesion assays were performed as described previously (Vromman *et al*, 2014). In brief, MEFs cells plated in 24-well plate the day before (100 000 cells/well) were pre-cooled 30 min at 4 °C and then were incubated for 4 h at 4 °C with L2^{IncD}GFP bacteria at a MOI = 10, sonicated prior to infection in order to disrupt bacterial aggregates. Then cells were washed gently with PBS and detached using 0.5 mM EDTA in PBS. Samples were fixed 30 min in 2 % PFA, washed with PBS and analyzed using flow cytometry.

Entry assessment

Entry experiments were performed as described previously (Vromman *et al*, 2014). In brief, MEFs cells plated on coverslips in 24-well plate the day before (100 000 cells/well) were pre-cooled 30 min at 4 °C and then incubated for 45 min at 4 °C with L2^{IncD}GFP bacteria at a MOI

= 10, sonicated prior to infection in order to disrupt bacterial aggregates. Then pre-warmed medium was added and coverslips were incubated at 37 °C before being fixed at different time points in 4 % PFA for 20 min. Extracellular bacteria were stained with a mouse anti-MOMP-LPS (Argene # 11-114) antibody followed with Cy5-conjugated anti-mouse (Amersham Biosciences) secondary antibody. The dilutions were made in PBS containing 3 % of BSA. DNA was stained using 0.5 $\mu\text{g}\cdot\text{mL}^{-1}$ of Hoechst 33342 (Thermo Fisher Scientific) added in the secondary antibody solution. Images were acquired on an Axio observer Z1 microscope equipped with an ApoTomemodule (Zeiss, Germany) and a 63 \times Apochromat lens. Images were taken with an ORCAflash4.OLT camera (Hamamatsu, Japan) using the software Zen.

Progeny assay

For glucose privation tests on MEFs cells, 140 000 cells per well were seeded in a glucose-free DMEM (Invitrogen) supplemented with 10 % FBS. The following day, the medium was replaced with glucose-free DMEM supplemented with 10 % FBS and the indicated concentration of glucose (Sigma). The next day cells were infected with L2^{IncD}GFP bacteria at a MOI = 0.2.

For progeny assays on HeLa cells, primary cells or MEFs cells, 100 000 cells seeded in 24-well plate were infected with L2^{IncD}GFP bacteria at a MOI = 0.15 the next day, after 2 h of treatment with CP4d or DMSO or not. Cells treated with siRNA for 48 h were directly infected with L2^{IncD}GFP bacteria at a MOI = 0.2.

Thirty hpi, cells were detached and fixed in 2 % PFA in PBS prior to flow cytometry analysis in order to evaluate the first round of infection. In duplicate wells, cells were detached, lysed using glass beads and the supernatant was used to infect new untreated cells (or WT cells in the case of MEFs) plated the day before (100 000 cells/well in a 24-well plate), in serial dilution. The next day, 3 wells per condition with an infection lower than 30 % (checked by microscopy) were detached and fixed as described above, before analysis by flow cytometry and determination of the bacterial titer.

rhGFPT1 purification

Recombinant human GFPT1 (rhGFPT1) with an internal 6-His tag was produced from a plasmid pET28-rhGFPT1-6His in *E. coli* Rosetta (DE3) GlmS::Tc kindly given by Dr. Badet-Denisot (Centre de Recherche de Gif, France) (Li *et al.*, 2007). The mutated form rhGFPT1

Q58N, rhGFPT1 Q328N and rhGFPT1 Q546N were obtained using QuikChange technology (Agilent) on the plasmid pET28-rhGFPT1-6His, with primers listed in Table 2, following the manufacturer instructions and transformed in *E. coli* Rosetta (DE3) GlmS::Tc.

One liter of culture in 2YT medium supplemented with tetracycline (8 $\mu\text{g}\cdot\text{mL}^{-1}$, Sigma), kanamycin (50 $\mu\text{g}\cdot\text{mL}^{-1}$, Sigma), chloramphenicol (15 $\mu\text{g}\cdot\text{mL}^{-1}$, Sigma) and glucosamine (GlcNH₂, 2 $\text{mg}\cdot\text{mL}^{-1}$, Sigma) was grown with agitation at 37 °C until OD₆₀₀ reached 0.5. Protein expression was induced by addition of 0.5 mM of isopropyl β -D-thiogalactopyranoside (Sigma) at 25 °C for 24 h before being harvested. The cell pellets were resuspended in lysis buffer (20 mM NaP, pH 7.5, 200 mM NaCl, 20 mM imidazole, 2 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 10 % glycerol, 1 mM fructose-6-P, 10 mM phenylmethylsulfonyl fluoride (PMSF), Roche EDTA-free protease inhibitor cocktail) and disrupted by sonication. The recombinant protein was purified by incubation with Qiagen Ni-NTA agarose beads (Qiagen) for 1 h followed by three washing steps with the lysis buffer before elution with lysis buffer containing increasing concentrations of imidazole: 30 mM, 100 mM, 175 mM and finally 500 mM of imidazole. The fractions containing the protein, were dialyzed against lysis buffer before storage at -80 °C.

TG2 activity assay

In vivo:

Cells plated the day before (100 000 cells/well) were infected with L2 bacteria at a MOI of 1, and 0.5 μM biotin pentylamine (BP) (Thermo Fisher Scientific) was added after 24 h. In some experiments, cells were pre-incubated for 2 h with 40 μM CP4d or DMSO as control before addition of bacteria. CP4d keeps TG2 in a close conformation, inhibiting the transamidase activity (Caron *et al*, 2012). For experiments with Ionomycin (Sigma), cells pre-treated with siRNA (see siRNA treatment) for 48 h or infected as described above for 24 h were treated with Ionomycin and 0.5 μM BP was added concomitantly for 6 h.

At the end of the indicated incubation time, cells were lysed using 8 M urea buffer (30 mM Tris, 150 mM NaCl, 8 M urea, 1 % SDS, pH=8.0) and samples subjected to Western Blot.

In vitro:

1 mU of transglutaminase from guinea pig liver (Sigma) was incubated at 37 °C for 15 min or 3 h with 5 μg of rhGFPT1, rhGFPT1 Q58N, rhGFPT1 Q328N or rhGFPT1 Q546N and 1 mM of BP in 20 mM HEPES buffer pH 7.5, 200 mM NaCl, 2 mM TCEP, 10 % glycerol, 1 mM

Fructose-6-P, 10 mM CaCl₂. The reaction was stopped by adding ethylenebis(oxyethylenitrilo)tetraacetic acid (EGTA) at a final concentration of 20 mM and boiling the samples 5 min at 95 °C.

Streptavidin-precipitation of TG2 targets

20 millions of HeLa cells were seeded in a 163 cm² flask. One day later, the cells were infected or not with L2 bacteria at a MOI = 1. Two hours post infection the culture medium was changed and 40 mM CP4d or DMSO was added. BP was added to the culture medium at 0.5 mM 24 h post treatment (infection or not) and cells were lysed 46 hpi directly in the well using 8 M urea buffer. DNA was disrupted by sonication and a dialysis was performed against 2 M urea buffer (Tris 30 mM, NaCl 150 mM, urea 2 M, SDS 1%, pH=8.0) to reduce the urea concentration and enable biotin-streptavidin interaction. Samples were incubated overnight at 4 °C with streptavidin-agarose beads (Sigma). After 3 washes with 2 M urea buffer and 3 washes with phosphate-buffered saline (PBS), proteins precipitated on the beads were eluted using Laemmli's buffer containing dithiothreitol (Sigma) boiled 5 min at 95 °C. Samples were then analyzed by Western blot or by mass spectrometry.

SDS-PAGE and Western blot

Proteins were subjected to sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane, which was blocked with 1 x PBS containing 5 % bovine serum albumin (BSA, for biotin revelation only) or milk and 0.01 % Tween 20. The membranes were then immunoblotted with primary antibodies diluted in 1 x PBS containing 5 % milk and 0.01 % Tween 20. For analyzing the TG2 activity assay, biotin incorporation was revealed using streptavidin conjugated to horseradish peroxidase (HRP, Sigma). Primary antibodies used in the western blots were the mouse clone 7D2 against TG2 (#ABIN1109303, Covalab), rabbit anti-serum against GFPT (kindly given by Dr. C. Weigert, University of Tübingen, Germany), mouse clone RL2 against *O*-GlcNAcylation (#MA1-072 Thermo Fisher Scientific) and mouse clone AC-74 against β -actin (#A5441 Sigma). Secondary antibodies were anti-mouse-HRP (GE Healthcare) or anti-rabbit-HRP (Invitrogen) conjugated antibodies. Blots were developed using the Western Lightning Chemiluminescence Reagent (GE Healthcare). For quantification, signal obtained with the PXi (Thermo Fisher Scientific) revelator was analyzed using ImageJ software.

Mass Spectrometry

In solution protein digestion:

Samples were prepared in triplicate. For streptavidin-precipitation of TG2 targets samples, tryptic digestion was performed by enhanced filter-aided sample preparation (Erde *et al*, 2014). All steps were done in-filter. Briefly, samples were reduced (50 mM TCEP, 30 minutes at room temperature) and alkylated (50 mM iodoacetamide, 1 hat room temperature in the dark). Then, proteins were incubated overnight at 37 °C with 500ng trypsin (Trypsin Gold Mass Spectrometry Grade, Promega). Peptides were recovered by centrifugation.

After TG2/GFPT1 reactions *in vitro* samples were diluted in a large excess of 8 M urea / 100 mM Tris HCl pH 8.5 buffer and then, as previously described, reduced (5 mM TCEP, 30 minutes at room temperature) and alkylated (10 mM iodoacetamide, 30 minutes at room temperature in the dark). Proteins were first digested for 5 h at 37 °C with 500 ng rLys-C Mass Spec Grade (Promega, Madison, WI, USA) before being diluted 4-fold with 100 mM Tris HCl pH 8.5 to reach a concentration below 2 M urea. Samples were then incubated overnight at 37 °C with 500 ng Sequencing Grade Modified Trypsin (Promega, Madison, WI, USA). To achieve the complete digestion of the peptides, a second incubation with the same amount of trypsin (5 h at 37 °C) was performed. Digestion was stopped by adding formic acid to 5 % final concentration and peptides were desalted and concentrated on Sep-Pak C₁₈SPE cartridge (Waters, Milford, MA, USA) according to manufacturer instructions.

Mass spectrometry analysis:

Tryptic peptides were analyzed on a Q Exactive Plus instrument (Thermo Fisher Scientific, Bremen) coupled with an EASY nLC 1 000 or 1 200 chromatography system (Thermo Fisher Scientific, Bremen). Sample was loaded on an in-house packed 50 cm nano-HPLC column (75 µm inner diameter) with C₁₈ resin (1.9 µm particles, 100 Å pore size, Reprosil-Pur Basic C₁₈-HD resin, Dr. Maisch GmbH, Ammerbuch-Entringen, Germany) and equilibrated in 98 % solvent A (H₂O, 0.1 % FA) and 2 % solvent B (ACN, 0.1 % FA). 120 or 180 min gradient of solvent B at 250 nL.min⁻¹ flow rates were applied to separated peptides. The instrument method for the Q Exactive Plus was set up in DDA mode (Data Dependent Acquisition). After a survey scan in the Orbitrap (resolution 70 000), the 10 most intense precursor ions were selected for HCD fragmentation with a normalized collision energy set up to 28. Charge state screening was

enabled, and precursors with unknown charge state or a charge state of 1 and > 7 were excluded. Dynamic exclusion was enabled for 35 or 45 seconds respectively.

Data processing:

Data were searched using Andromeda with MaxQuant software 1.4.1.2 or 1.5.3.8 version against respectively a *Chlamydia trachomatis* Uniprot reference proteome database concatenated with Homo sapiens Uniprot reference proteome database. Data were also searched against usual known mass spectrometry contaminants and reversed sequences of all entries or an *Escherichia coli* K12 Uniprot reference proteome database concatenated with rhGFPT1 and gpTGase proteins (Cox *et al*, 2008; Cox *et al*, 2011; Tyanova *et al*, 2016). Andromeda searches were performed choosing trypsin as specific enzyme with a maximum number of two missed cleavages. Possible modifications included carbamidomethylation (Cys, fixed), oxidation (Met, variable), N-ter acetylation (variable) and BP (Gln, variable). The mass tolerance in MS was set to 20 ppm for the first search then 6 ppm for the main search and 10 ppm for the MS/MS. Maximum peptide charge was set to seven and five amino acids were required as minimum peptide length. The “match between runs” feature was applied between replicates with a maximal retention time window of 2 or 0.7 min. One unique peptide to the protein group was required for the protein identification. A false discovery rate (FDR) cutoff of 1 % was applied at the peptide and protein levels.

Data analysis:

To validate the identification of the BP on the glutamine of modified peptides, spectra were manually inspected (or fragment assignments).

For the global quantification, output files from MaxQuant were used for protein quantification. Quantification was performed using the XIC-based LFQ algorithm with the Fast LFQ mode as described previously (Cox *et al*, 2014). Unique and razor peptides, included modified peptides, with at least 2 ratio count were accepted for quantification.

For pairwise comparisons, proteins identified in the reverse and contaminant databases and proteins only identified by site were first discarded from the list. Then, proteins exhibiting fewer than 2 LFQ values in at least one condition were discarded from the list to avoid misidentified proteins. After log₂ transformation of the leftover proteins, LFQ values were normalized by median centering within conditions (normalized function of the R package DAPAR (Wieczorek *et al*, 2017)). Remaining proteins without any LFQ value in one of both conditions have been considered as proteins quantitatively present in a condition and absent in another.

They have therefore been set aside and considered as differentially abundant proteins. Next, missing values were imputed using the `imp.norm` function of the R package `norm` (Novo, 2013). Proteins with a foldchange under 2 have been considered not significantly differentially abundant. Statistical testing of the remaining proteins (having a foldchange over 2) was conducted using a `limma` t-test thanks to the R package `limma` (Ritchie *et al*, 2015; Smyth, 2005). An adaptive Benjamini-Hochberg procedure was applied on the resulting p-values thanks to the function `adjust.p` of R package `cp4p` using the robust method of Pounds and Cheng to estimate the proportion of true null hypotheses among the set of statistical tests (Giai *et al*, 2016; Pounds and Cheng, 2006). The proteins associated to an adjusted p-value inferior to an FDR level of 1% have been considered as significantly differentially abundant proteins.

Flow Cytometry

Acquisition was performed using a CytoFLEX S (Beckman Coulter) and 50 000 events per sample were acquired and then analyzed using FlowJo (version 10.0.7).

RESULTS

I. TG2 is expressed at high level and activated during *C. trachomatis* infection.

A widely-used technique to probe TG2 activation is to measure the incorporation of biotin pentylamine (BP) into proteins. When present in excess this membrane-permeable primary amine competes out other substrates for transamidase reaction catalyzed by TG2 and becomes covalently linked to glutamine residues of TG2 targets. The biotin group is then easily detectable by western blot using streptavidin coupled to horse radish peroxidase (Lee *et al*, 1992). This procedure was applied to HeLa cells either left uninfected, or infected for 48 h with *C. trachomatis*, in the presence or not of BP. In non-infected samples, BP incorporation was extremely low, as expected since in resting cells low Ca^{2+} concentration maintains TG2 in an inactive conformation. In contrast, infected cells showed a significant incorporation of BP, indicating that TG2 becomes activated during infection. This signal was due to the transamidase activity of TG2, since CP4d, a specific inhibitor of this activity, abolished BP incorporation in a dose-dependent manner (Figure 25A) (Caron *et al*, 2012). Live proliferating bacteria were needed for TG2 activation since filtered or heat-inactivated bacteria, or bacteria treated with the antibiotic doxycycline to prevent their proliferation, failed to induce BP incorporation (Figure 25B and Figure 26A). BP incorporation upon infection was also observed in WT mouse embryonic fibroblasts (MEFs) but not in MEFs isolated from *TGM2* KO animals ($\text{TG2}^{-/-}$), further supporting the implication of TG2 in this process (Figure 26B).

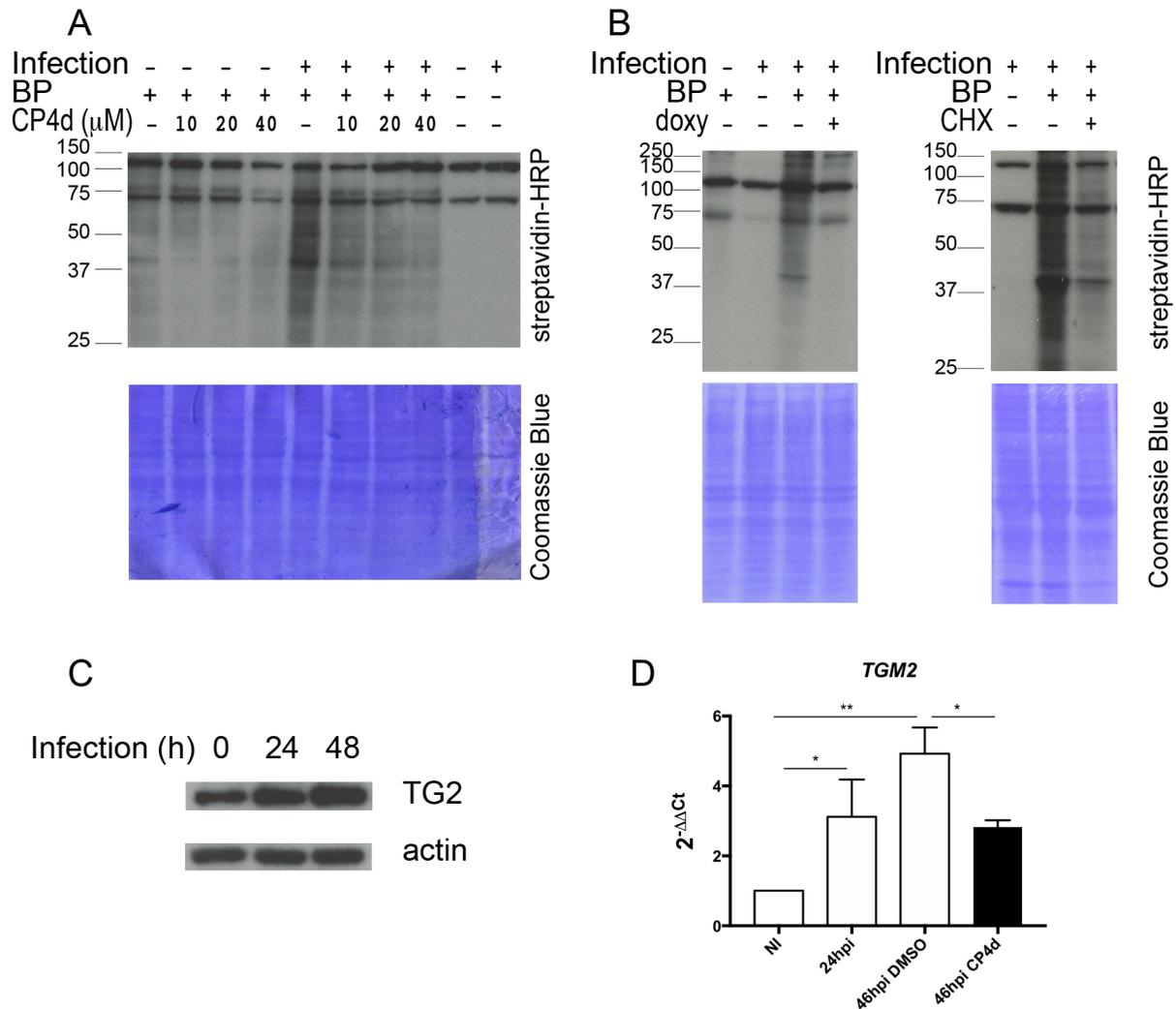


Figure 25: TG2 transamidase activity increases during *C. trachomatis* infection along with its expression

A- Whole cell lysates were prepared with HeLa cells infected or not for 48 h with *C. trachomatis* L2 (MOI =1) in the presence or not of BP. In the indicated samples CP4d was added 2 h before infection. Cell lysates were run on SDS-PAGE, proteins were transferred to a membrane and BP incorporation was revealed with HRP-conjugated streptavidin. The two main bands present in all samples correspond to naturally biotinylated host proteins. After blotting, the membrane was stained with Coomassie blue to control for equal loading of all lanes. **B-** Same as in A, except that where indicated 250 μM doxycycline (doxy, left) were added 24 hpi or 7 μM cycloheximide (CHX, right) were added 2 hpi. **C-** Western blot with anti-TG2 antibodies on total cell lysates infected or not with *C. trachomatis* L2. **D-** Cells were infected with *C. trachomatis* L2 (MOI =1) for 24 or 48 h. Where indicated, 40 μM CP4d were added 2 hpi. *TGM2* transcripts were measured by RT-qPCR and normalized to *actin* transcripts with the Delta-delta Ct method. The data are presented as relative mRNA levels compared to uninfected cells and shown as the mean \pm SD. Each experiment was performed in duplicate and repeated four times. The asterisk indicates two significantly different values ($P < 0.05$, Student's ratio-paired t-test).

Probing cellular lysates by western blot using anti-TG2 antibodies showed that activation of TG2 was accompanied with an increased expression of the enzyme (Figure 25C). Consistent with this observation, inhibition of protein synthesis with cycloheximide decreased infection-induced TG2 activation (Figure 25B). Reverse transcription followed by quantitative PCR (RT-qPCR) measurements revealed a 3 to 4-fold increase in TG2 transcripts in infected versus non-infected cells, demonstrating that the increase in TG2 amount during infection is at least partly controlled at the transcriptional level (Figure 25D). Interestingly, the transamidase activity of TG2 is required for the increase in TG2 expression since, when cells were treated with CP4d, TG2 transcription was no longer enhanced by infection (Figure 25D).

In conclusion, *C. trachomatis* infection increases TG2 levels and activates its transamidase activity.

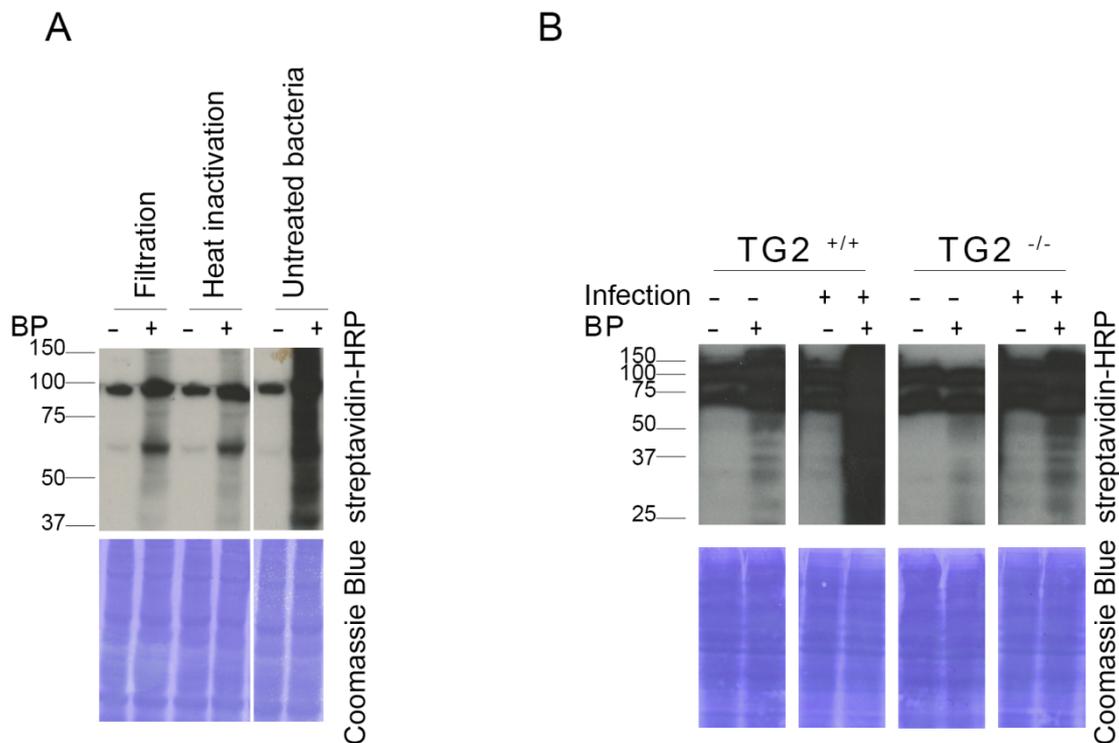


Figure 26: The increase in TG2 activity during infection is due to TG2 activation by live bacteria

A- Before addition to HeLa cells, the bacterial inoculum was either left untreated (MOI = 1), or filtered through a 0.22 μ m filter, or incubated for 30 min at 65 $^{\circ}$ C to kill the bacteria. The inoculum was applied to HeLa cells in the presence or not of BP and 48 h later whole cell lysates were prepared. After separation by SDS-PAGE, proteins were transferred to a membrane and BP incorporation was revealed with HRP-conjugated streptavidin. **B-** Whole cell lysates were prepared with WT or TG2 KO MEF cells infected or not for 48 h with *C. trachomatis* L2 in the presence or not of BP, and analyzed as in A.

II. TG2 activity sustains bacterial growth.

A. *In cellulo.*

To determine if TG2 activity affected bacterial development, we infected HeLa cells in the presence or not of the transamidase inhibitor CP4d. Thirty hours later, the progeny was collected and the number of infectious bacteria was determined by infecting fresh cells. Inhibition of TG2 activity with 40 μ M CP4d resulted in a 2-fold decrease in bacterial progeny (Figure 27A). Consistently, a similar reduction in bacterial progeny was observed when bacteria were grown on TG2^{-/-} MEFs compared to TG2^{+/+} (Figure 28A). To confirm these findings in primary cells, we used epithelial cells isolated from fallopian tubes (Roth *et al*, 2010). CP4d was even more potent at reducing the progeny after one developmental cycle in these cells than in HeLa cells as a ten-fold reduction was observed for 10 μ M CP4d (Figure 27B). The negative impact of TG2 inhibition on bacterial development was also observed in primary cells infected with *C. trachomatis* serovar D, showing that the effect is not restricted to the LGV biovar (Figure 27B).

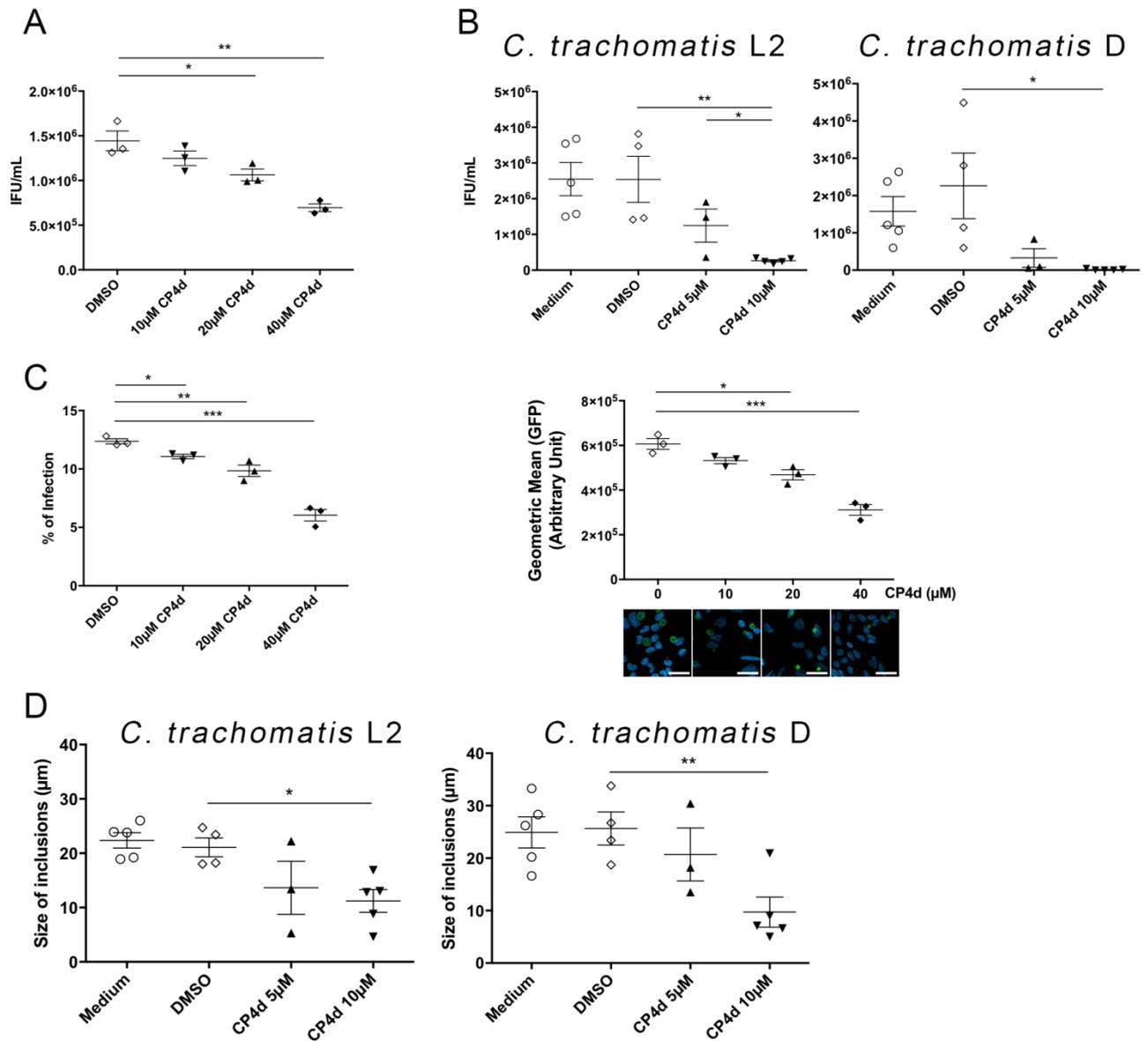


Figure 27: TG2 activity is important for *C. trachomatis* developmental cycle

A- HeLa cells were pre-treated with the indicated concentrations of CP4d (or DMSO alone) for 2 h before being infected with L2^{incD}GFPat MOI = 0.15. Thirty hours later, the cells were disrupted and bacterial titers were determined by re-infecting fresh HeLa cells as described in the methods. Three independent experiments are shown. **B-** Same as in A except that *C. trachomatis* L2 (left) or serovar D (right) were grown in primary cells isolated from fallopian tubes. For serovar D, cells for recovery were disrupted after 48 h. **C-** HeLa cells were pre-treated with the indicated concentrations of CP4d (or DMSO alone) for 2 h before being infected with L2^{incD}GFPat MOI = 0.15. Thirty hours later the cells were fixed and analyzed by flow cytometry. The percentage of infected cells (left) and the mean fluorescence of the infected population (right) are shown for three independent experiments. A representative field for each condition is shown, bar = 10 μm. **D-** Primary epithelial cells isolated from fallopian tubes were pre-treated with the indicated concentrations of CP4d (or DMSO alone) for 2 h before being infected with *C. trachomatis* L2 (left) or serovar D (right). Twenty-four hours later, the cells were fixed, bacteria were stained using FITC-labeled anti-Chlamydia-LPS antibodies, and the size of the inclusions manually determined using ImageJ, on twenty inclusions per experiment. Asterisks indicate two significantly different values (*: P < 0.05; **: P < 0.01, Student's paired t-test).

Reduced progeny could result from impairment of one or several of the steps of the chlamydial developmental cycle: adhesion, entry, differentiation into the replicative form, proliferation, and differentiation into the infectious form. We observed that inhibition of TG2 had no effect on bacterial adhesion (Figure 28B), but that 40 μ M CP4d decreased the percentage of infected cells by about 2-fold in HeLa cells (Figure 27C), consistent with a lower internalization efficiency (Figure 28C). In addition, inclusions were smaller in cells treated with CP4d, and contained less bacteria, indicating that bacterial growth and/or proliferation was slower in the absence of TG2 activity (Figure 27D). The reduction of inclusion size when TG2 was inhibited was also observed in primary cells infected with *C. trachomatis* L2 or serovar D (Figure 27D) confirming a default after the entry of the bacteria when TG2 was inhibited or absent.

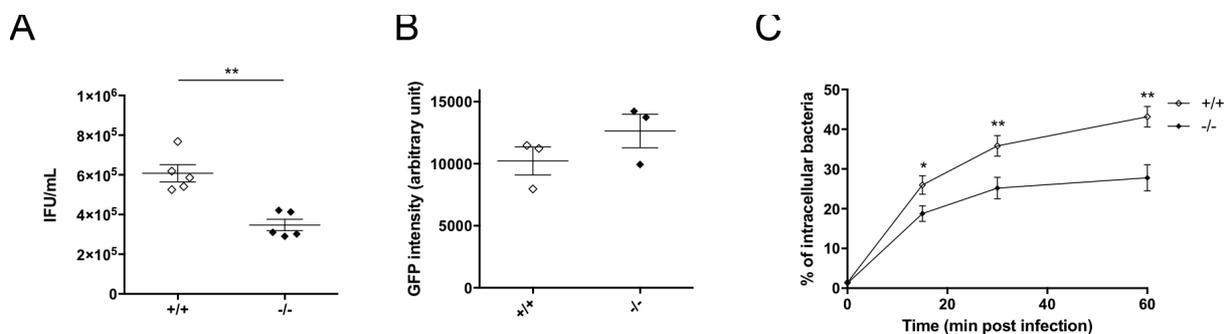


Figure 28: TG2 plays a role in bacterial entry

A- TG2^{+/+} and TG2^{-/-} MEFs were infected with L2^{incD}GFPat MOI = 0.15. Thirty hours later the cells were disrupted and bacterial titers were determined by re-infecting fresh TG2^{+/+} cells as described in the methods. Five independent experiments are shown. **B-** To measure bacterial adhesion TG2^{+/+} and TG2^{-/-} MEFs were incubated at 4 °C for 4 h with L2^{incD}GFPat MOI = 10 before being washed and fixed as described in the methods. The mean values of the cell associated fluorescence of three independent experiments are shown. **C-** TG2^{+/+} and TG2^{-/-} MEFs were infected with L2^{incD}GFPat MOI = 10 and fixed at the indicated time. Extracellular bacteria were differentially labeled as described in the methods. The mean \pm SD of three independent experiments are shown. Asterisks indicate two significantly different values (*: P < 0.05; **: P < 0.01, Student's paired t-test).

B. *In vivo*.

We next tested the incidence of the absence of TG2 on chlamydial development in a mouse model of infection. We infected TG2^{+/+} and TG2^{-/-} mice with *Chlamydia muridarum*, a mouse-adapted strain genetically very close to *C. trachomatis* (Read *et al*, 2000). Twenty-five days after infection, mice were sacrificed and the upper part of the genital tract was isolated from all of them (Figure 29 A). DNA was extracted from these tissues and bacterial load was determined by qPCR. A slightly higher number of mice retained detectable bacterial DNA in

III. TG2 plays a central role in metabolic rewiring during infection.

We have recently shown that *C. trachomatis* rewires the glucose metabolism in the infected cell (Gehre *et al*, 2016). By accumulating glycogen in the vacuole, it acts as a glucose sink, requiring the host to adapt its own metabolic fluxes. One of the host responses is to increase glucose uptake through overexpression of plasma membrane glucose transporters (Ojcius *et al*, 1998; Wang *et al*, 2017). In mammary epithelial cells, it was shown that TG2 overexpression led to profound changes in the cell metabolism, including the induction of the expression of the glucose transporter GLUT-1 (Kumar *et al*, 2014). As we have observed that TG2 level was increased during *C. trachomatis* infection, we wondered if this increase could control the concomitant rewiring of host metabolism in infected cells, as it does in mammary epithelial cells. If this hypothesis was correct, one prediction that we could make was that low glucose availability in the culture medium should be more detrimental to bacterial growth in TG2^{-/-} MEFs compared to the wild type MEFs, as TG2^{-/-} MEFs cell would be impaired in their ability to adjust their glucose uptake to sustain bacterial growth. To test this hypothesis, we grew MEFs in medium containing decreasing concentrations of glucose and measured the number of infectious bacteria collected 30 hpi. As expected we observed that decreasing glucose availability resulted in a sharp decrease in bacterial titers, in both cellular backgrounds. However, bacterial titers were more sensitive to glucose deprivation in the TG2^{-/-} MEFs than in the wild-type cells (Figure 30A). For instance, at 1 mg.ml⁻¹ glucose, the progeny was reduced by 82 % in the TG2^{-/-} MEFs, against only 35% in the TG2^{+/+} MEFs.

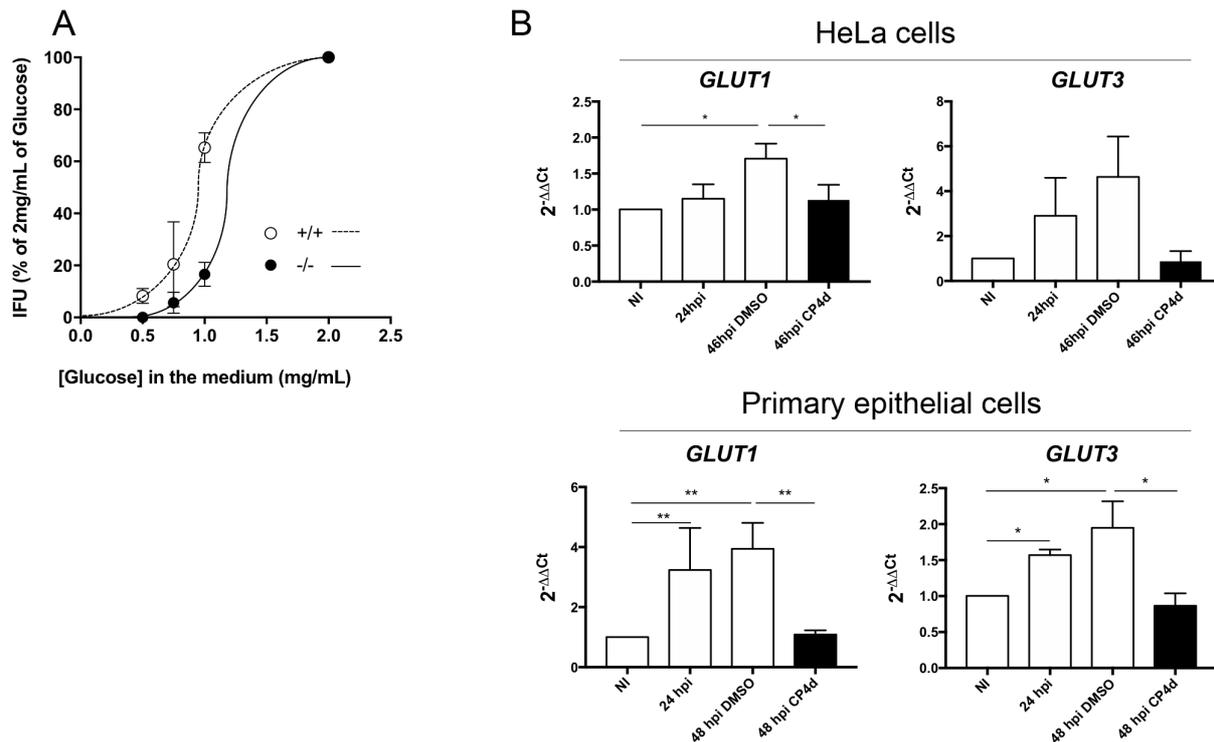


Figure 30: TG2 is required for enhanced glucose import in infected cells

A- MEFs were grown for 24 h in a culture medium complemented with the indicated concentration of glucose before being infected with $L2^{\text{IncD}}\text{GFP}$ bacteria (MOI = 0.2). Cells were disrupted 30 h later and the bacterial titer determined by re-infecting fresh wild type cells. The mean \pm SD of three independent experiments are shown. **B-** Cells were infected with *C. trachomatis* L2 (MOI = 1) for 24 or 48 h. Where indicated 40 μM CP4d was added 2 hpi. *GLUT1* and *GLUT3* transcripts were measured by RT-qPCR and normalized to *actin* with the Delta-delta Ct method. The data are presented as relative mRNA levels compared to uninfected cells and shown as the mean \pm SD. Each experiment was performed in duplicate and repeated four times. Asterisks indicate two significantly different values (*: $P < 0.05$; **: $P < 0.01$, Student's ratio-paired t-test).

This result supports our hypothesis that one reason TG2 is needed for optimal bacterial development is that it is implicated in the rewiring of host metabolism to sustain bacterial growth. To test this more directly, we measured the incidence of TG2 inactivation on the cell capacity to uptake glucose. HeLa cells infected with *C. trachomatis* show increased transcription of *GLUT-1* and *GLUT-3*, which allows to increase glucose uptake and meet bacterial needs (Wang *et al*, 2017). We reproduced this result in HeLa cells, as well as in primary cells isolated from the endocervix (Figure 30B). In contrast, in the presence of the TG2 inhibitor CP4d, the transcription of the glucose transporter genes was no longer induced by infection, demonstrating that TG2 is necessary for the control of *GLUT-1* and *GLUT-3* transcription. Absence of increase in *GLUT-1* and *GLUT-3* transcripts 48 hpi in the presence of CP4d was not due to the lower bacterial burden because when bacterial proliferation was diminished 24 hpi by addition of doxycycline, we observed a comparable reduction in bacterial

load at 48 hpi as in cells treated with CP4d, but the transcription of the glucose transporter genes remained as high as in non-treated cells (Figure 31). Thus, similar to what was described in mammary epithelial cells, TG2 is instrumental during *C. trachomatis* infection to increase glucose uptake by enhancing the transcription of glucose transporter genes.

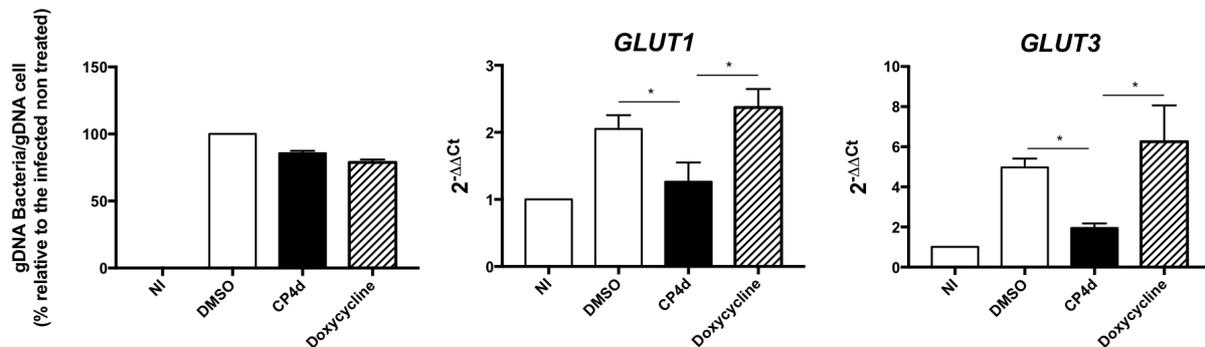


Figure 31: Differential effect of CP4d and doxycycline on *GLUT-1* and *GLUT-3* transcription

HeLa cells were infected or not with *C. trachomatis* (MOI = 1) in duplicate per condition. Twenty-four hours later, 62.5 ng.mL⁻¹ doxycycline or 40 μM CP4d were added to the culture medium. Forty-eight hpi DNA were extracted from one well to measure bacterial load, and RNA were extracted from the duplicate well. Bacterial gDNA (16S RNA) measured by qPCR was normalized to host gDNA (actin gene) and is expressed relative to the infected non-treated culture. *GLUT-1* and *GLUT-3* transcripts were measured by q RT-PCR and normalized to *actin* with the Delta-delta Ct method. The data are presented as relative mRNA levels compared to uninfected cells and shown as the mean ± SD. Each experiment was performed in duplicate and repeated four times. Asterisks indicate two significantly different values (P < 0.05, Student's ratio-paired t-test).

IV. Glucosamine-fructose-6-P amidotransferase is a substrate of TG2 transglutaminase activity.

To identify TG2 targets in the infectious process, HeLa cells were infected in the presence or absence of BP. Forty-eight hours later, the cells were lysed and biotinylated proteins were isolated on streptavidin-coated beads, and identified by mass-spectrometry. Sixty-two proteins were found to be significantly enriched in the infected cell lysates grown in the presence of BP (Table 3). RhoA, galectin 3, fibronectin, 40S ribosomal protein SA, hemoglobin beta and immunoglobulin κ chain C region were already identified as TG2 substrates (Guilluy *et al*, 2007; Mehul *et al*, 1995; Nelea *et al*, 2008; Orrù *et al*, 2003; Pincus and Waelsch, 1968; Sohn *et al*, 2010). BAG2 and several other mitochondrial proteins were also enriched in the samples prepared in the presence of BP, in agreement with TG2 being present and active in this compartment (Altuntas *et al*, 2015).

Table 3: Candidate TG2 substrates in *C. trachomatis* infected cells

The list displays streptavidin-bound proteins identified by mass spectrometry in the infected samples only in the presence of BP (first 37 entries) or more abundant in the presence of BP than in its absence (last 25 entries, $\text{Log}_2([\text{Mean intensity with BP}]/[\text{Mean intensity without BP}])$ and p-values are shown).

UniProt ID	Protein	Gene	Protein description	log2	P-value
Q8NE71-2	ABCF1	ABCF1	ATP-binding cassette sub-family F member 1	NA	NA
O95831-3	AIFM1	AIFM1	Apoptosis-inducing factor 1	NA	NA
P50995-2	ANX11	ANXA11	Annexin A11	NA	NA
P84077	ARF1	ARF1	ADP-ribosylation factor 1	NA	NA
Q13510	ASAH1	ASAH1	Acid ceramidase	NA	NA
Q12797-10	ASPH	ASPH	Aspartyl/asparaginyl beta-hydroxylase	NA	NA
P36542-2	ATPG	ATP5C1	ATP synthase subunit gamma	NA	NA
O95816	BAG2	BAG2	BAG family molecular chaperone regulator	NA	NA
Q13185	CBX3	CBX3	Chromobox protein homolog 3	NA	NA
Q9Y696	CLIC4	CLIC4	Chloride intracellular channel protein 4	NA	NA
Q16555-2	DPYL2	DPYSL2	Dihydropyrimidinase-related protein	NA	NA
P55884	EIF3B	EIF3B	Eukaryotic translation initiation factor 3 subunit B	NA	NA
P02751-4	FINC	FN1	Fibronectin	NA	NA
G3V1Q4	G3V1Q4	SEPT7	Septin 7	NA	NA
Q9HAV0	GBB4	GNB4	Guanine nucleotide-binding protein subunit beta	NA	NA
O94808	GFPT2	GFPT2	Glutamine--fructose-6-phosphate aminotransferase	NA	NA
P08754	GNAI3	GNAI3	Guanine nucleotide-binding protein G(k) subunit alpha	NA	NA
H3BSW3	H3BSW3	APRT	Adenine phosphoribosyltransferase	NA	NA
P68871	HBB	HBB	Hemoglobin subunit beta	NA	NA
AOA087WZW8	IGKV3-11	IGKV3-11	Ig kappa chain C region	NA	NA
Q15181	IPYR	PPA1	Inorganic pyrophosphatase	NA	NA
J3QLE5	J3QLE5	SNRPN	Small nuclear ribonucleoprotein-associated protein N	NA	NA
K7ENG2	K7ENG2	U2AF2	Splicing factor U2AF 65 kDa subunit	NA	NA
P17931	LEG3	LGALS3	Galectin-3	NA	NA
C9JIG9	OSR1	OSXR1	Serine/threonine-protein kinase OSR1	NA	NA
P17858	PFKAL	PFKL	ATP-dependent 6-phosphofructokinase	NA	NA
E9PQ98	PRMT1	PRMT1	Protein arginine N-methyltransferase 1	NA	NA
P62195-2	PRS8	PSMC5	26S proteasome regulatory subunit 8	NA	NA
O00487	PSDE	PSMD14	26S proteasome non-ATPase regulatory subunit	NA	NA
Q16401-2	PSMD5	PSMD5	26S proteasome non-ATPase regulatory subunit 5	NA	NA
Q06203	PUR1	PPAT	Amidophosphoribosyltransferase	NA	NA
P17812-2	PYRG1	CTPS1	CTP synthase 1	NA	NA
P43487-2	RANG	RANBP1	Ran-specific GTPase-activating protein	NA	NA
Q9P2E9-2	RRBP1	RRBP1	Ribosome-binding protein 1	NA	NA
P56192	SYMC	MARS	Methionine--tRNA ligase	NA	NA

O95497	VNN1	VNN1	Pantetheinase	NA	NA
E9PRD9	VNN2	VNN2	Vascular non-inflammatory molecule 2	NA	NA
P08243-2	ASNS	ASNS	Asparagine synthetase	7,62	1,81E-08
P30508	HLA-C	HLA-C	HLA class I histocompatibility antigen, Cw-12 alpha chain	9,05	4,07E-08
P35613-2	BASI	BSG	Basigin	2,33	1,85E-05
P10909-4	CLUS	CLU	Clusterin	2,13	3,91E-05
P49368	TCPG	CCT3	T-complex protein 1 subunit gamma	1,83	3,99E-04
O15427	MOT4	SLC16A3	Monocarboxylate transporter 4	1,93	6,21E-04
E9PLL6	RPL27A	RPL271	60S ribosomal protein L27a	1,36	9,12E-04
Q15233	NONO	NONO	Non-POU domain-containing octamer-binding protein	1,26	1,21E-03
P30837	AL1B1	ALDH1B1	Aldehyde dehydrogenase X	1,98	1,50E-03
E9PLA9	CAPRIN1	CAPRIN	Caprin-1	1,47	1,61E-03
P17987	TCPA	TCP1	T-complex protein 1 subunit alpha	1,91	2,84E-03
P39019	RS19	RPS19	40S ribosomal protein S19	1,44	3,74E-03
O43143	DHX15	DHX15	Putative pre-mRNA-splicing factor ATP-dependent RNA helicase	1,00	4,00E-03
P61586	RHOA	RHOA	Transforming protein RhoA	1,01	4,49E-03
C9J9K3	RPSA	RPSA	40S ribosomal protein SA	1,07	4,91E-03
A0A087WXM6	RPL17	RPL17	60S ribosomal protein L17	1,01	5,48E-03
P38919	EIF4A3	EIF4A3	Eukaryotic initiation factor 4A-III	1,08	7,23E-03
P00505	AATM	GOT2	Aspartate aminotransferase, mitochondrial	1,11	8,63E-03
P27105	STOM	STOM	Erythrocyte band 7 integral membrane protein	1,01	9,61E-03
A0A096LNZ9	ISG15	ISG15	Ubiquitin-like protein ISG15	1,25	1,10E-02
E9PEX6	DLD	DLD	Dihydrolipoyl dehydrogenase, mitochondrial	1,08	1,66E-02
Q10589-2	BST2	BST2	Bone marrow stromal antigen 2	1,15	2,37E-02
Q06210	GFPT1	GFPT1	Glutamine--fructose-6-phosphate aminotransferase	3,22	2,49E-02
A0A087WVM3	CYR61	CYR61	Protein CYR61	1,34	2,75E-02
Q13162	PRDX4	PRDX4	Peroxiredoxin-4	1,13	2,88E-02

Among these potential TG2 targets, the enzyme glucosamine-fructose-6-P amidotransferase (GFPT) caught our attention because it uses fructose-6-P as a substrate, which derives from glucose-6-P. We reasoned that targeting one key enzyme of sugar metabolism might contribute to the overall control of sugar metabolism that *C. trachomatis* exerts on its host (Gehre *et al*, 2016). Moreover, both isoforms of the enzyme, GFPT1 (also known as GFAT) and GFPT2, had been recovered from the proteomic approach, making it a very strong hit. We first confirmed that GFPT was recovered in the biotinylated fraction of cells infected with *C. trachomatis* in the presence of BP using anti-GFPT antibodies. GFPT was no longer found in the biotinylated fraction when infection had been performed in the presence of the

TG2 inhibitor CP4d, demonstrating that incorporation of the biotinylated probe in GFPT depended on the activity of TG2 (Figure 32A).

To further validate that GFPT is a novel substrate of TG2 we tested the ability for TG2 to cross-link a primary amine to GFPT *in vitro*. Purified TG2 and recombinant human GFPT1 (rhGFPT1) were incubated for 3 h at 37 °C in the presence of BP as primary amine donor. Proteins were then separated by SDS-PAGE and the incorporation of the biotinylated probe was analyzed by blotting with HRP-coupled streptavidin. The biotinylated probe was incorporated into rhGFPT1 in the presence and not in the absence of TG2. Furthermore, chelation of Ca²⁺ by EGTA inhibited the incorporation of the probe, as expected for a reaction dependent on the transamidase activity of TG2 (Figure 32B). We concluded from these experiments that GFPT is a novel substrate of TG2, that becomes modified by the transamidase activity of the enzyme during *C. trachomatis* infection.

In order to precisely determine which glutamine residue(s) of GFPT1 was being modified by TG2 *in vitro*, we performed the same experiment with purified TG2 and rhGFPT1 incubated for 3 h in the presence of BP and analyzed the products of the reaction by mass spectrometry. BP incorporation was identified in ten glutamine residues (out of twenty-eight, Figure 32C).

To determine which of these glutamine residues were preferred TG2 substrates, we introduced glutamine to asparagine point mutations to prevent modification by TG2 while minimizing a possible impact on protein folding. We started with the two glutamine residues that we identified as prone to modification by TG2 using bioinformatics tools designed to score peptidic environment favorable for TG2 activity, Q328 and Q546 (Keresztessy *et al*, 2006; Sugimura *et al*, 2006). As a control, we also tested Q58, another glutamine identified by mass spectrometry but not surrounded by a consensus sequence for TG2. Purified recombinant proteins were incubated with TG2 and BP for 30 min at 37 °C before stopping the reaction. BP incorporation was reduced in the rhGFPT1 Q328N but neither in rhGFPT1 Q546N nor rhGFPT1 Q58N, indicating that the glutamine 328 is a prominent glutamine for modification by TG2.

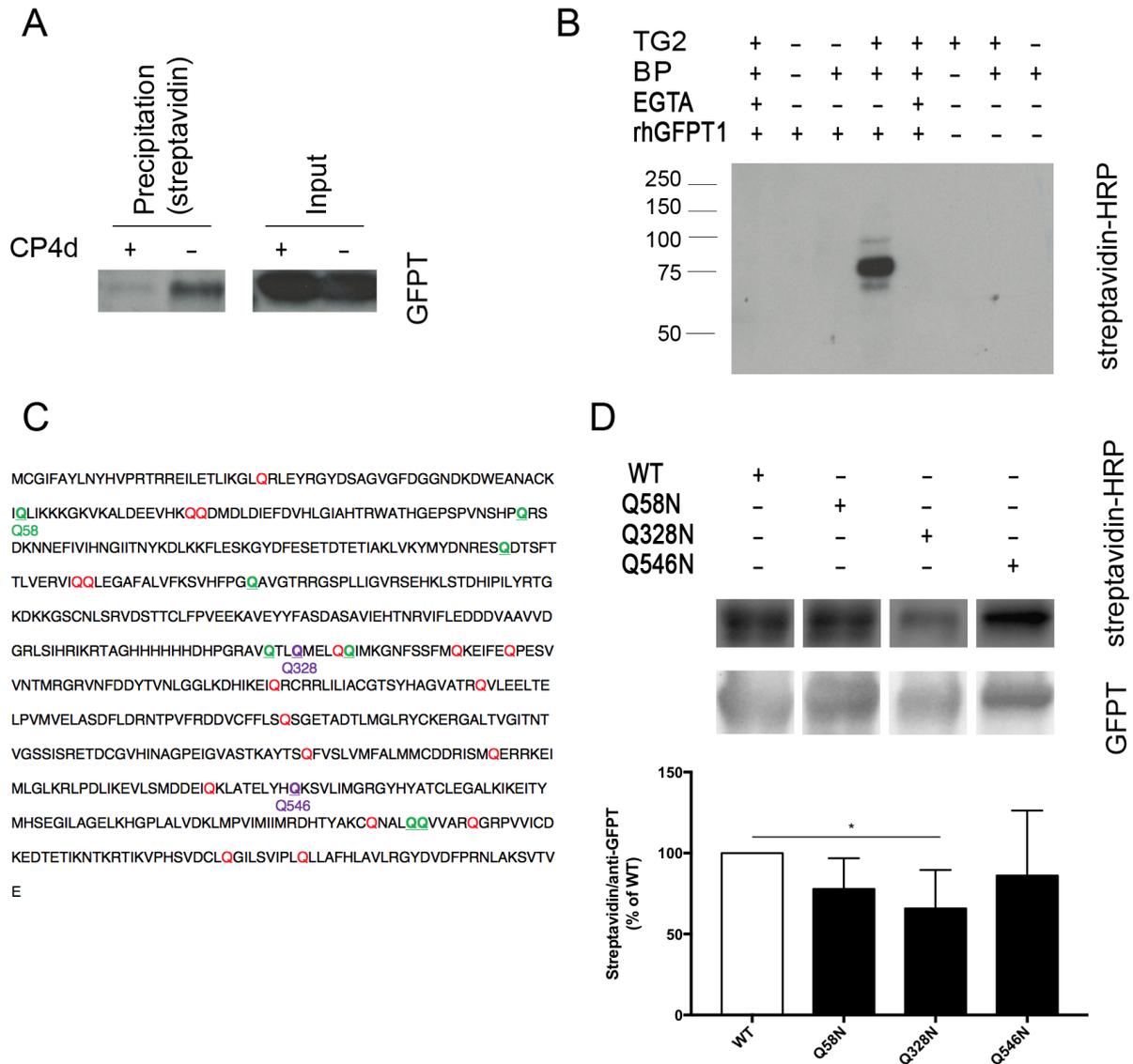


Figure 32: GFPT is a substrate of TG2 transamidase activity

A- HeLa cells were infected with *C. trachomatis* (MOI = 1) then treated or not after 2 h with 40 μ M CP4d. After 24 h 0.5 mM BP was added and cells were lysed at 48 hpi. Lysates were precipitated with streptavidin-coated beads to purify the proteins cross-linked to BP by TG2. After separation with SDS-PAGE, proteins were transferred to a membrane and GFPT was revealed in the purified fraction (precipitation streptavidin) or the fraction before precipitation on beads (input). **B-** *In vitro* assay testing the ability of purified guinea pig transglutaminase (TG2) to crosslink purified rhGFPT1 with BP. The addition of EGTA traps Ca^{2+} , inactivating TG2. Samples were incubated for 3 h at 37°C before separation by SDS-PAGE. Proteins were transferred to a membrane and BP was revealed using streptavidin coupled with HRP. rhGFPT1 is 77,5 kDa. **C-** Samples prepared as described in B were analyzed by mass spectrometry in order to identify the glutamine residues on GFPT1 where BP was incorporated. The sequence of rhGFPT1 is shown, glutamine residues in which no modification was identified are in red, the ones crosslinked to BP in green and the two crosslinked to BP and surrounded by a sequence already known as prone to TG2 transamidation in purple. **D-** *In vitro* assay was performed as described in B using wild type rhGFPT1 (WT), rhGFPT1 Q58N, rhGFPT1 Q328N or rhGFPT1 Q546N as substrate. The reaction was performed at 37 °C for 30 min. After probing with HRP-Streptavidin the membrane was washed and probed with anti-GFPT antibodies followed with HRP-conjugated secondary antibodies. The ratio of modified protein (streptavidin signal) compared to the unmodified (GFPT signal) is shown, normalized to its value with WT rhGFPT1. The mean of five independent experiments is shown, the asterisk indicates two significantly different values ($P < 0.05$, Student's ratio-paired t-test).

V. Modification of GFPT by TG2 enhances the hexosamine biosynthesis pathway.

GFPT catalyzes the first and rate limiting step of the *de novo* biosynthesis of hexosamines, i.e. the hexosamine biosynthesis pathway (HBP) (Figure 33A). The HBP leads to the formation of uridine 5'-diphospho-*N*-acetylglucosamine (UDP-GlcNAc), which is further used for *N*-glycosylation, *N*-glycan branching, and *O*-linked *N*-acetylglucosylation (*O*-GlcNAcylation) in the ER, Golgi, and cytosol and nucleus, respectively. Unlike *N*-glycosylation or *N*-glycan branching, which combine several UDP-GlcNAc moieties to other sugars, *O*-GlcNAcylation involves the transfer of a single UDP-GlcNAc moiety to the hydroxyl group of serines or threonines. It is dynamically regulated by *O*-GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA), which are respectively responsible for *O*-GlcNAc addition and removal. *O*-GlcNAc modification level of proteins is directly dependent on the concentration of UDP-GlcNAc, the donor substrate for OGT (Józwiak *et al*, 2014). To determine if GFPT modification by TG2 had an impact on the HBP, we measured the level of *O*-GlcNAcylation in primary epithelial cells. Ionomycin is a widely used TG2 activator, as this Ca²⁺ ionophore increases intracellular Ca²⁺ concentration, which opens TG2 in its active conformation. We observed an increase in *O*-GlcNAcylation in cells treated with ionomycin. This increase was dependent on TG2 activity since it was not observed in cells in the presence of the TG2 inhibitor CP4d (Figure 33B) or in cells in which *TGM2* expression had been silenced using siRNA (Figure 33C).

Altogether these experiments show that activation of TG2 transamidase activity by calcium influx enhances the HBP.

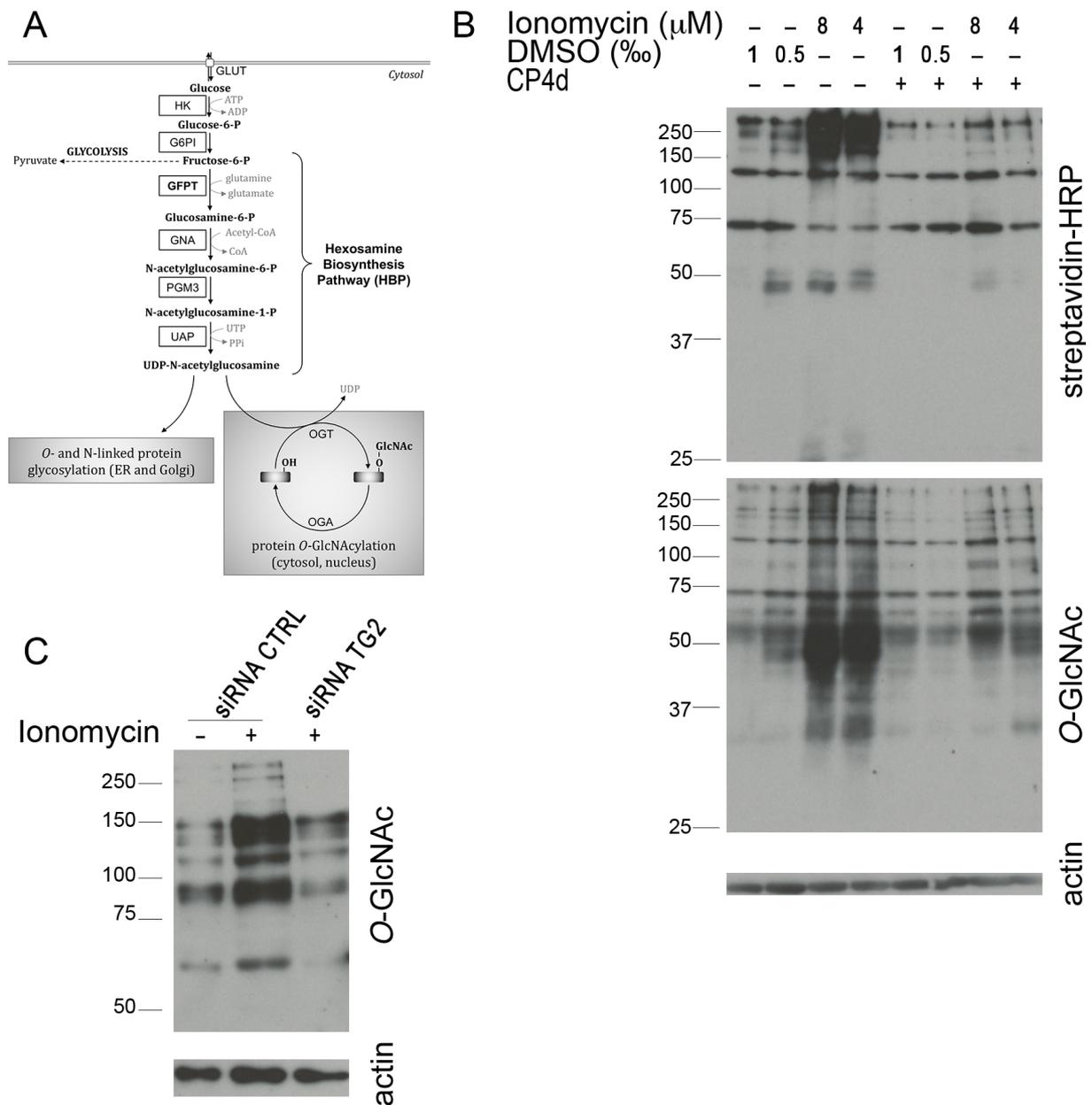


Figure 33: Modification of GFPT by TG2 leads to an increase in the HBP flux

A- Schematic view of the HBP. Production of glucosamine-6-P by GFPT is the first and rate-limiting step of the pathway that produces UDP-GlcNAc. HK: hexokinase; G6PI: glucose-6-P isomerase; GFPT: glutamine-fructose-6-P amidotransferase; GNA: glucosamine-6-P N-acetyltransferase; PGM3: phosphoglucomutase 3; UAP: UDP-N-acetylglucosamine pyrophosphorylase; OGT: O-GlcNAc transferase; OGA: O-GlcNAcase; GlcNAc: N-acetylglucosamine. **B-** Endocervical epithelial cells were pre-treated or not with 40 μM CP4d for 2 h before addition of the indicated concentration of ionomycin (or an equivalent volume of DMSO). 0.5 μM biotin pentylamine was added simultaneously as a parallel read-out of TG2 activity. Six hours after ionomycin treatment, whole cell lysates were analyzed by western blot. The membrane was first blotted with HRP-conjugated streptavidin to detect TG2 activity, then extensively washed and probed with anti-O-GlcNAcylation antibody followed with HRP conjugated secondary antibodies. Last the membrane was probed with anti-actin as a loading control. **C-** The same experimental procedure as described in B- was applied to HeLa cells treated for 48 h prior to Ionomycin treatment (8 μM) with siRNA control (siRNA CTRL) or siRNA directed against TG2 (siRNA TG2).

VI. TG2 activity controls the hexosamine biosynthetic pathway to provide the bacteria with UDP-GlcNAc.

Surprisingly, we did not observe an increase in *O*-GlcNAcylation in cells infected for 48 h by *C. trachomatis* (Figure 34A). Since *O*-GlcNAcylation directly depends on UDP-GlcNAc concentration, this observation suggested that UDP-GlcNAc levels in the cytoplasm were not significantly increased in infected cells. We have previously demonstrated that *C. trachomatis* co-opts SLC35D2, a host antiporter transporting UDP-GlcNAc, UDP-glucose and GDP-mannose to import these metabolites into the vacuole in which the bacteria develop (Gehre *et al*, 2016). We reasoned that UDP-GlcNAc might not accumulate in the cytoplasm in infected cells because it is rapidly taken up by the bacteria. Indeed, we observed that activation of TG2 by ionomycin elicited a lower increase in *O*-GlcNAcylation in infected cells compared to non-infected cells, indicating that less free UDP-GlcNAc is available for *O*-GlcNAcylation in the infected host cytoplasm (Figure 34B). Importantly GFPT expression was stable in all conditions, indicating that the decrease in *O*-GlcNAcylation in infected cells is not due to lower GFPT expression. This observation also implies that the increase in the HBP upon ionomycin treatment is not due to an increased expression of GFPT, but presumably to an increase in enzymatic activity upon modification by TG2.

If UDP-GlcNAc from the host was used by the bacteria, lowering the cellular levels in UDP-GlcNAc should negatively impact bacterial development. We tested this hypothesis by measuring the consequence of silencing GFPT, the rate limiting enzyme in UDP-GlcNAc biosynthesis, on infection. The mass spectrometry data showed that both isoforms GFPT1 and GFPT2 were expressed in HeLa cells (Table 3). However, none of the 3 siRNA against GFPT2 had any impact on the amount of GFPT detected by the antibody (that detects both isoforms) indicating that GFPT2 expression is very low. In contrast, silencing of GFPT1 with three different siRNAs greatly diminished the expression of the enzyme confirming that this is the main isoform expressed in HeLa cells (Figure 35A). We observed a reduction in the number of bacteria per inclusion 24 h post infection in the cells treated with siRNA against GFPT1, and the progeny collected was reduced 3-fold (Figure 35B). Of note, silencing of GFPT1 had no incidence on bacterial entry and the initiation of bacterial development, as the percentage of infected cells was identical to that in control cells (Figure 35B).

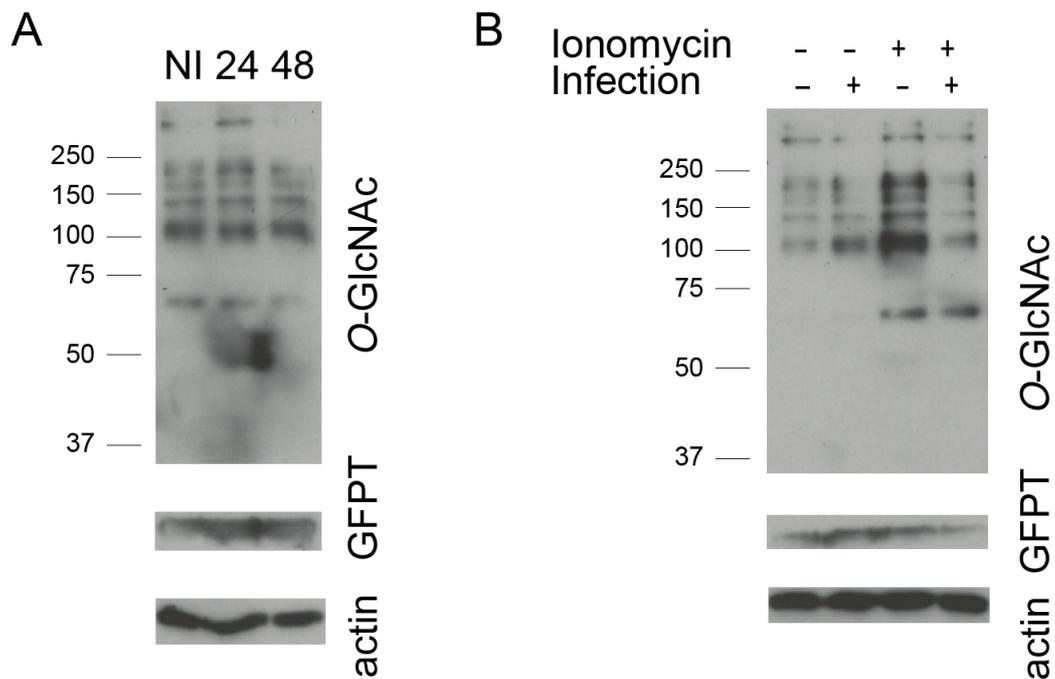


Figure 34: Infection dampens the increase in *O*-GlcNAcylation upon TG2 activation

A- HeLa cells were infected with *C. trachomatis* (MOI = 1), then lysed after 24 h or 48 h or left uninfected (NI). After separation with SDS-PAGE, proteins were transferred to a membrane, probed with anti-*O*-GlcNAcylation antibody followed with HRP conjugated secondary antibodies. Then the membrane was cut and probed with anti-GFPT and anti-actin antibody before revelation with HRP conjugated secondary antibodies. **B-** HeLa cells were infected with *C. trachomatis* (MOI = 1), then treated with 8 μ M Ionomycin (or the same volume of DMSO as control) and 0.5 mM BP after 24 h. After 6 h of treatment, cells were lysed, proteins revealed as in A.

Altogether, these data support the hypothesis that the increase in UDP-GlcNAc resulting from GFPT modification by TG2 is consumed by the bacteria. In bacteria, peptidoglycan biosynthesis requires high UDP-GlcNAc supply. *C. trachomatis* do not have a classical cell wall but uses peptidoglycan synthesis for bacterial division (Liechti *et al*, 2016). It is thus possible that *Chlamydia* takes advantage of the increase in UDP-GlcNAc production in the host, to assist bacterial division through peptidoglycan biosynthesis.

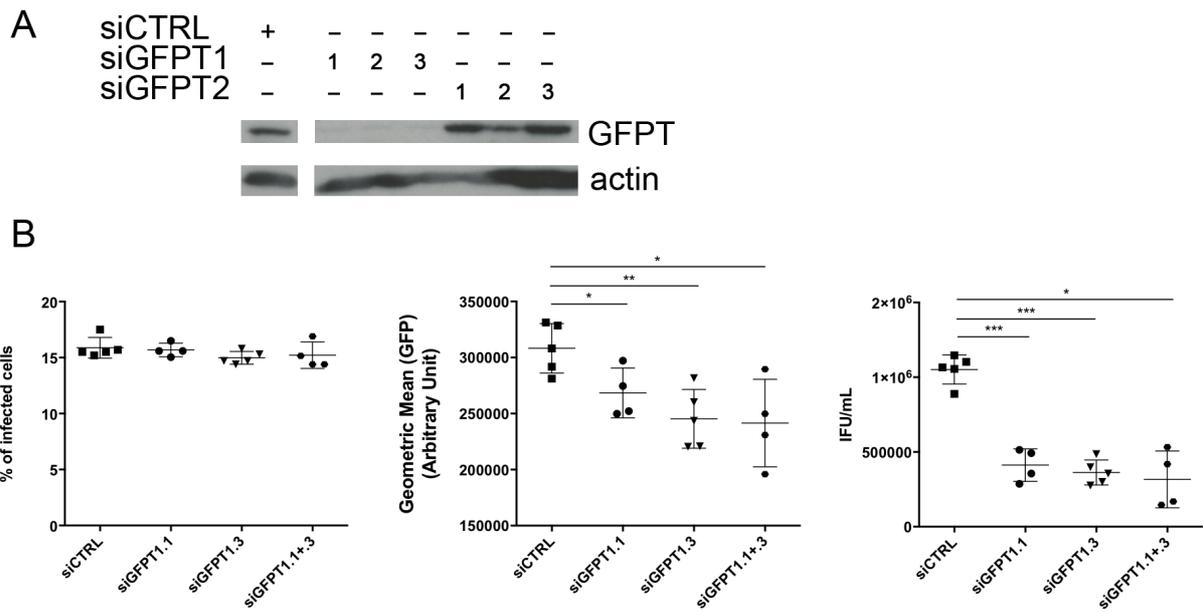


Figure 35: GFPT is required for optimal bacterial growth

A- HeLa cells treated for 72 h with siRNA targeting GFPT1 or GFPT2 were lysed. After separation with SDS-PAGE, proteins were transferred to a membrane, probed with anti-GFPT and anti-actin antibody before revelation with HRP-conjugated secondary antibodies. **B-** HeLa cells treated for 48 h with siRNA targeting GFPT1.3 or not (siCTRL) were infected with *C. trachomatis* (MOI = 0.15). Thirty hours later cells were fixed and analyzed by flow cytometry. The percentage of infected cells (top) and the mean fluorescence of the infected population (middle) are shown for at least four independent experiments. Duplicate wells were lysed and used to re-infect fresh HeLa cells to determine the bacterial titer (bottom). Asterisks indicate two significantly different values (**: $P < 0.01$, Student's paired t-test).

DISCUSSION

While TG2 has been known for a long time to be induced and have an important function during inflammation, the roles ascribed to this enzyme are often related to its secreted pool. Moreover, the inflammatory stimuli studied are often abiotic or rely on auto-immune response.

In this work, we aimed at unravelling the importance of TG2 during *C. trachomatis* infection in epithelial cells. It led to the identification of new targets of TG2 and we revealed an unsuspected link between this enzyme and the HBP.

I. *C. trachomatis* infection causes an increase in TG2 level and activity that benefits bacterial growth.

Given the importance of TG2 in rerouting cellular metabolism toward aerobic glycolysis in certain contexts such as cancer, and the importance of glucose supply in *C. trachomatis*-infected cells, we tested the levels of TG2 and of its transamidase activity during infection (Kumar *et al*, 2014; Rother *et al*, 2018). We observed a strong increase in TG2 transamidating activity (Figure 25A), mostly due to an increased transcription of the *TGM2* gene (Figure 25D) resulting in higher levels of TG2 (Figure 25B, C). This increase in activity and transcription starts about 24 h post infection and is dependent on TG2 activity. Thus, it seems that there is a positive feedback loop that amplifies TG2 transamidating activity by increasing the level of protein, which itself depends on this same activity. It could be that TG2 activates NFκB by a non-canonical pathway as previously described, that in return is able to activate *TGM2* transcription thanks to an element in the promoter of *TGM2* (Kumar and Mehta, 2012; Mirza *et al*, 1997). Also, in response to *C. trachomatis* infection, epithelial cells secrete cytokines such as IL-8 and IL-6, the latter being a known activator of *TGM2* transcription (Oh *et al*, 2011; Rasmussen *et al*, 1997). It is thus very plausible that TG2 expression increases as a result of cytokine stimulation.

The increase in TG2 activity requires the presence of live and proliferative bacteria (Figure 26A). The signal(s) activating TG2 transamidase activity is not clear. Inside a cell, Ca²⁺, the activator of TG2 transamidase activity, is mostly sequestered inside the ER, and this compartment is associated with the inclusion membrane at membrane contact sites (Derré *et al*,

2011). *C. trachomatis* infection activates the unfolded protein response pathway, a stress signaling pathway that was described as sufficient to activate TG2 (George *et al*, 2016; Lee *et al*, 2014). In line with the hypothesis that TG2 might be activated by local Ca^{2+} increase due to the infection, one study using Ca^{2+} sensitive probes reported an accumulation of cytoplasmic Ca^{2+} around the inclusion (Majeed *et al*, 1999). Thus, it is likely that the activation of TG2 transamidase activity is an indirect consequence of local Ca^{2+} increase caused by the infection. If it indeed happens in the vicinity of the inclusion, it provides the advantage of limiting TG2 activity to substrates that can benefit the bacteria close to the inclusion (see below for the generation of UDP-GlcNAc for instance), without compromising cellular homeostasis by a massive TG2 activation.

We provide strong evidence that TG2 expression is beneficial for the developmental cycle of *C. trachomatis*. Treating HeLa cells (Figure 27A) or primary cells (Figure 27B) isolated from human fallopian tube with a specific inhibitor of the transaminase activity CP4d resulted in a two to ten-fold decrease in the progeny produced after an infectious cycle, indicating an effect in favor of *Chlamydia* cycle of TG2 increase. The results were reproduced using the serovar D of *C. trachomatis*, a serovar causing different sexually transmitted infections than the serovar LGV L2, suggesting that TG2 importance is not serovar- or tissue-dependent. Moreover, infecting cells with *C. muridarum* or *C. caviae*, two other *Chlamydia* species, also resulted in a strong increase in TG2 activity after 48 h of infection (data not shown). Altogether, these data indicate an importance of TG2 for *Chlamydia* developmental cycle.

More precisely, our data indicate that TG2 is important at two different stages of the bacterial cycle: for the entry of the bacteria inside the host cell, and for its intracellular multiplication later on (Figure 27C, D). The effect observed at the entry step (Figure 28C) is probably related to functions of TG2 at the plasma membrane or in the extracellular matrix. TG2 is known to promote the clustering and the activation of PDGFRs, resulting in the activation of downstream signaling cascade including Akt1 kinase (Nurminskaya *et al*, 2014). Interestingly, PDGFR/Akt signaling are implicated in *C. trachomatis* entry (Elwell *et al*, 2008; Carpenter *et al*, 2017). PDGFR activation and its downstream signaling might be affected in the absence of functional TG2 and result in the entry defect observed here.

II. TG2 is required to meet glucose demand during infection.

Based on the description of TG2 as a master regulator of the aerobic glycolysis metabolism in mammary epithelial cells, we tested whether TG2 was implicated in the control of cellular metabolism in our infectious context (Kumar *et al*, 2014). Among the modifications occurring in *C. trachomatis* infected cells regarding glucose metabolism, we focused on the overexpression at the genetic and protein level of the glucose transporters GLUT1 and GLUT3 previously described (Wang *et al*, 2017). Indeed, GLUT1 was already shown to be upregulated by TG2 via NF κ B activation and with HIF-1 as intermediate transcription factor in a different context and since HIF-1, NF κ B and now TG2 are known to be activated upon *Chlamydia* infection, we aimed at identifying if this same pathway was involved during infection (Sellami *et al*, 2014; Sharma *et al*, 2011; Kumar *et al*, 2014). Using the chemical inhibitor CP4d, we demonstrated that the transcriptional increase in the genes encoding GLUT1 and GLUT3 during infection was controlled by TG2 (Figure 30B). Since CP4d treatment of infected cells induced a delay in bacterial development, it could be argued that the reduction in bacterial load accounts for the decrease in *GLUT1* and *GLUT3* transcription. We ruled this possibility out by showing that doxycycline, an antibiotic that delayed bacterial growth to the same extent as CP4d, did not impair the increase in *GLUT1* and *GLUT3* transcription. In a previous publication, the increase in *GLUT1* and *GLUT3* transcription during *C. trachomatis* infection was attributed to an activation of the MAPK p38 and JNK (Wang *et al*, 2017). This is not incompatible with our results since the activation of these kinases is an early phenomenon during infection, and may account for the increase in the transcription of those two genes in the 24 first hours of infection, when the activity and expression of TG2 have not yet increased. Based on our findings we propose that TG2 activation relays the MAPKs and becomes the principal activator of *GLUT1* and *GLUT3* transcription for the second half of the developmental cycle.

Supporting a role for TG2 in controlling glucose import we observed that bacteria grown on fibroblasts isolated from TG2^{-/-} mice were more susceptible to a decrease in glucose concentration in the medium, which is expected if they are not able to compensate for this decrease by increasing transporter amounts. In this experiment, *C. trachomatis* development in cells cultured in a medium containing 1 mg.mL⁻¹ of glucose was strongly weakened in TG2 KO cells compared to the wild type (Figure 30A). This is a relevant concentration since the normal glucose concentration in the human serum is between 0.6 and 1 mg.mL⁻¹ and in female vaginal fluid about 0.6 mg.mL⁻¹ (Exley *et al*, 2007).

III. TG2 exacerbates *C. muridarum* pathogenesis.

In order to test the importance of TG2 *in vivo*, we performed experiments with a murine model of infection, using *C. muridarum*, a mouse adapted strain genetically very close to *C. trachomatis*. In that model, after 25 days post infection, we analyzed the bacterial load present in the mice upper genital tract. No significant difference was obtained due to the limited number of TG2^{-/-} animals available, but TG2^{-/-} mice tended to be colonized by less bacteria than the control ones (Figure 29). At a higher scale, looking at the symptoms caused by *C. muridarum* infection, milder symptoms were observed in TG2^{-/-} mice. These results support the *in vitro* observations indicating that TG2 is important for an optimal bacterial infection. *In vivo*, another level of complexity is added due to the different functions played by TG2 in different cell types, and in particular the role of TG2 in supporting inflammation, which might account for the less severe phenotype of pathogenesis in the TG2^{-/-} mice. Another fact that must be considered is that mammalian genome encodes seven other enzymatically active members of the transglutaminase family. It is very likely that, in TG2^{-/-} mice, these enzymes compensate TG2 loss to some extent. This idea is supported by the fact that TG2^{-/-} mice live normally without any apparent major problems (De Laurenzi and Melino, 2001).

IV. GFPT1, a substrate of TG2 activity.

Given the increase in TG2 activity observed in *C. trachomatis* infected cells, we decided to get an in-depth view of the impact of TG2 activation by identifying proteins modified by the transaminase activity of the enzyme. Using mass spectrometry, we identified sixty-two candidate substrates. The list of known TG2 substrates for its transamidase activity contains already more than a hundred proteins so this number, for a particular cell-type in a given condition, does not seem unrealistic. Moreover, from the sixty-two proteins identified, six proteins were already described TG2 substrates, confirming the relevance of the approach used.

We focused our work on GFPT, whose two isoforms were recovered among TG2 substrate candidates. We confirmed that GFPT1 was a target of TG2 *in vitro* using purified proteins.

To determine whether GFPT is a substrate of TG2 even in a non-infectious context, we tested the effect of an artificial activation of TG2 using the calcium ionophore ionomycin on

the HBP. We used *O*-GlcNAcylation as a readout, as this reaction uses and is limited by the end product of the HBP (UDP-GlcNAc). The data confirmed the link between the activation of TG2 and the generation of UDP-GlcNAc (Figure 33). The effect of TG2 on GFPT activity could result from a stabilization of the protein, which has a short half-life time, or from an increase of its enzymatic activity (Marshall *et al*, 1991). Western Blot did not reveal any difference in the protein levels of GFPT, suggesting an effect of TG2 on GFPT catalytic activity (Figure 34A and B). This action could result from a modification of GFPT conformation, increasing the accessibility to one of its substrates, or releasing its inhibition by other molecules or modifications. For example, the phosphorylation of the Ser204 by the PKA was shown to decrease GFPT activity, so does the phosphorylation of Ser243 by AMPK (Chang *et al*, 2000; Zibrova *et al*, 2017). GFPT modification by TG2 may block the recognition of the enzyme by the AMPK or hide the Ser residue. GFPT activity is also inhibited by the end product of the HBP, the UDP-GlcNAc, with residues Gly445 and Gly461 crucial (Assrir *et al*, 2014). Its modification by TG2 could release this inhibition.

We were able to identify at least one of the glutamine residues on GFPT1 that is modified by TG2, Q329. Crosslinking of biotin pentylamine to GFPT1 by TG2 was impaired in a Q329N mutant of GFPT1 (Figure 33D). This residue is localized in the inter-domain region and is not part of the crystallized domain (the isomerase domain) (Nakaishi *et al*, 2009). Modification of the inter-domain region may impact the distance between the two domains of the protein or its oligomerization state. Identifying the amine donor that becomes cross-linked to GFPT1 remains a challenging project for the future. Having identified the modified glutamine is an important step in this direction. Once the cross-linked species identified, the modification could be reconstructed *in vitro* and lead to a detailed biochemical characterization of its consequences on GFPT1 activity.

Strikingly, we did not detect a significant increase in the *O*-GlcNAcylation level during infection, in spite of the strong TG2 activation (Figure 34A). While we think that it is at least partly due to UDP-GlcNAc being efficiently retargeted to the bacteria (see below) it remains possible that some particular host proteins become *O*-GlcNAcyated during infection. For example, the transcriptional repressor Snail1 is stabilized by *O*-GlcNAcylation which prevents its phosphorylation and the subsequent degradation (Park *et al*, 2010). Increase in Snail1 is a known trigger of E-cadherin downregulation and E-cadherin is downregulated during *C. trachomatis* infection (Rajić *et al*, 2017). Thus, it would be interesting to see if an increase in

Snail *O*-GlcNAcylation is implicated in this process. An illustration of the importance of UDP-GlcNAc in infection came from the recent discovery that *O*-GlcNAcylation of vimentin was necessary for intermediate filament assembly and organization during *C. trachomatis* infection, creating a nest stabilization the inclusion during bacterial replication (Tarbet *et al*, 2018). As the vimentin network is quite substantial, this modification likely creates a local need for UDP-GlcNAc at the inclusion periphery. GFPT activation by TG2 might be necessary to provide sufficient UDP-GlcNAc.

V. UDP-GlcNAc production is rapidly consumed in *C. trachomatis* infected cells.

In *C. trachomatis* infected cells, *O*-GlcNAcylation levels remained unchanged in spite of TG2 activation. We hypothesized that the UDP-GlcNAc produced by the HBP was consumed rapidly after its production, so that its concentration did not increase, unlike what was observed in non-infected TG2-activated cells. Supporting this hypothesis, we observed that infected cells treated with ionomycin showed a lower increase in *O*-GlcNAcylation than non-infected cells (Figure 34B). Beside the fact that a fraction of UDP-GlcNAc might be consumed in vimentin modification, we considered the possibility that UDP-GlcNAc was imported into the inclusion lumen by the bacteria, where it would be used for peptidoglycan biosynthesis among others. Supporting this hypothesis, SLC35D2, a UDP-glucose transporter also known for transporting UDP-GlcNAc normally present in the ER, was recently described at the inclusion membrane (Gehre *et al*, 2016). Reinforcing this idea, we observed that silencing GFPT1 expression resulted in a 3-fold decrease in progeny (Figure 34B).

Concluding remarks.

Overall, our data place TG2 as a central regulator of host metabolism that is essential to sustain *C. trachomatis* infection (Figure 36). First, increase in TG2 during infection allows for an increase of GLUT1 and GLUT3 expression, resulting in an increased influx of glucose, which is essential for the bacterial developmental cycle. In parallel, TG2 activity leads to the modification of GFPT, resulting in an increased flux along the HBP. Thus, our work uncovered an unsuspected link between TG2 transamidating activity and protein modification by *O*-GlcNAcylation. Unlike what is observed in non-infected cells, the concentration of the product

of the HBP, UDP-GlcNAc, does not increase upon TG2 activation and our data suggest that this metabolite is being rapidly consumed, in particular during bacterial division.

While our data show that *C. trachomatis* biology exploit TG2 activation to meet its very specific needs in glucose and glucose derivative during infection, one obvious question raised by our results is whether TG2 is also implicated in other infections, and if so, whether it also has a role in metabolic control. Recently, it was reported that TG2 activity was also important for *Mycobacterium tuberculosis* intracellular survival and replication in isolated macrophages and host tissue (Palucci *et al*, 2018). In that study, TG2 activation was related to an impairment of autophagy homeostasis, a mechanism completely different from what we have described here. However, TG2 during *M. tuberculosis* infection of macrophages could also play an important role in modifying the glucose metabolism of these cells.

The results presented here in an infectious situation might be relevant in a non-infectious context. This is already demonstrated regarding the impact of an increase of TG2 on GLUT expression, previously demonstrated in normal epithelial cells and cancer cells, (Kumar *et al*, 2014). In the future we plan to test if activation of TG2, described in many cancer cell lines, correlate with an increase in the HBP, and whether drugs targeting TG2 and/or OGT alleviate some of the tumorigenic features of these cell lines. In *C. trachomatis* infection in humans, the role of this enzyme expressed not only by epithelial cells, but also by immune cells, is very likely more complex than what we have uncovered in epithelial cells. Still, targeting this enzyme in female patients suffering from long-term infections by the bacteria may help diminish the sequelae resulting from chronic infections, and alleviate the current burden they represent for the society.

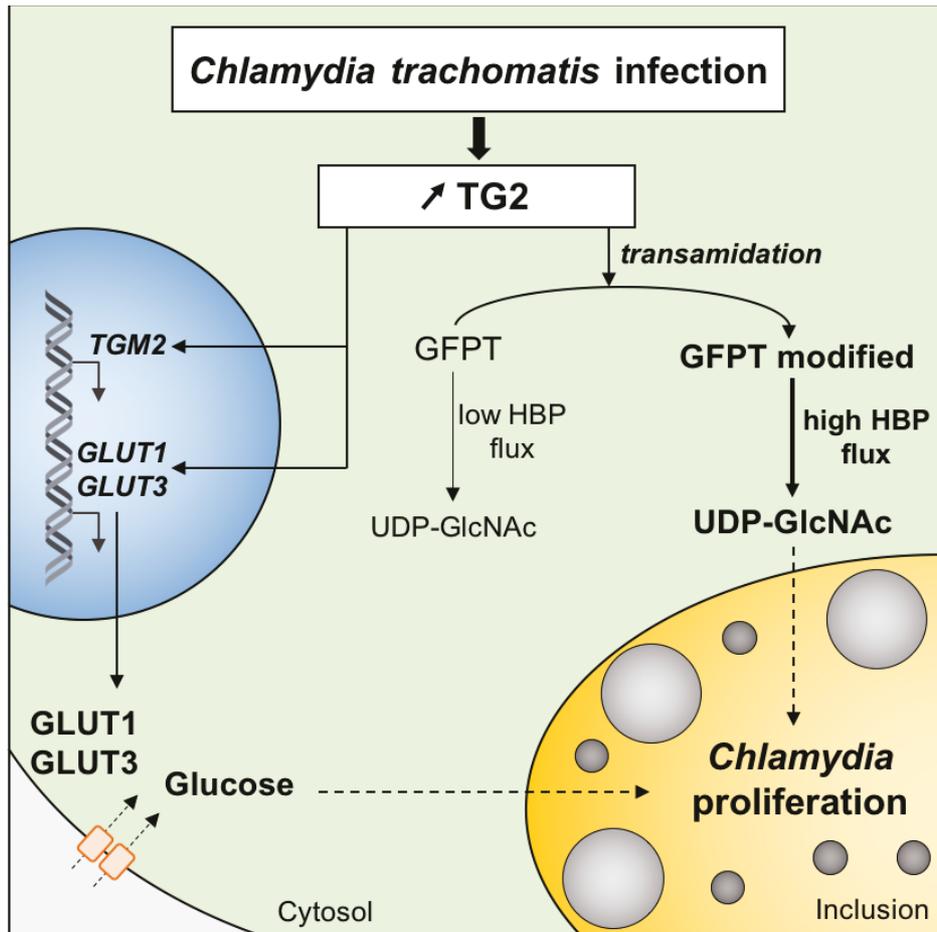


Figure 36 : TG2 has a central role in the control of glucose metabolism.

Infection activates TG2 mainly in the second half of the bacterial developmental cycle, when bacterial need for glucose and its derivatives is highest. TG2 synthesis is enhanced by a positive feedback loop on its own transcription, and drives the upregulation of expression of glucose transporters. Increase in glucose uptake is required for optimal bacterial growth: it fuels bacterial metabolism and serves as a precursor for the synthesis of UDP-GlcNAc, which is used in bacterial division. Activation of TG2 plays a second essential role in this context as the enzyme covalently modifies GFPT, the enzyme that controls the entry in the HBP and thereby the level of UDP-GlcNAc in the cell. UDP-GlcNAc is rapidly consumed in infected cells, in particular in bacterial division.

BIBLIOGRAPHY

1. Abdelrahman, Y., Ouellette, S. P., Belland, R. J. & Cox, J. V. Polarized Cell Division of *Chlamydia trachomatis*. *PLoS Pathog.* **12**, e1005822 (2016).
2. Abromaitis, S. & Stephens, R. S. Attachment and entry of *Chlamydia* have distinct requirements for host protein disulfide isomerase. *PLoS Pathog.* **5**, e1000357 (2009).
3. Abrusci, P., McDowell, M. A., Lea, S. M. & Johnson, S. Building a secreting nanomachine: a structural overview of the T3SS. *Curr. Opin. Struct. Biol.* **25**, 111–7 (2014).
4. Achyuthan, K. E. & Greenberg, C. S. Identification of a guanosine triphosphate-binding site on guinea pig liver transglutaminase. Role of GTP and calcium ions in modulating activity. *J. Biol. Chem.* **262**, 1901–6 (1987).
5. Agah, A., Kyriakides, T. R. & Bornstein, P. Proteolysis of cell-surface tissue transglutaminase by matrix metalloproteinase-2 contributes to the adhesive defect and matrix abnormalities in thrombospondin-2-null fibroblasts and mice. *Am. J. Pathol.* **167**, 81–8 (2005).
6. Akimov, S. S., Krylov, D., Fleischman, L. F. & Belkin, A. M. Tissue transglutaminase is an integrin-binding adhesion coreceptor for fibronectin. *J. Cell Biol.* **148**, 825–38 (2000).
7. Akimov, S. S. & Belkin, A. M. Opposing roles of Ras/Raf oncogenes and the MEK1/ERK signaling module in regulation of expression and adhesive function of surface transglutaminase. *J. Biol. Chem.* **278**, 35609–19 (2003).
8. Al-Zeer, M. A. *et al.* *Chlamydia trachomatis* remodels stable microtubules to coordinate Golgi stack recruitment to the chlamydial inclusion surface. *Mol. Microbiol.* **94**, 1285–97 (2014).
9. Al-Zeer, M. A. *et al.* *Chlamydia trachomatis* Prevents Apoptosis Via Activation of PDPK1-MYC and Enhanced Mitochondrial Binding of Hexokinase II. *EBioMedicine* **23**, 100–110 (2017).
10. Altuntas, S. *et al.* The transglutaminase type 2 and pyruvate kinase isoenzyme M2 interplay in autophagy regulation. *Oncotarget* **6**, 44941–54 (2015).
11. Anastasiou, D. *et al.* Inhibition of pyruvate kinase M2 by reactive oxygen species contributes to cellular antioxidant responses. *Science* **334**, 1278–83 (2011).
12. Ando, H. *et al.* Purification and Characteristics of a Novel Transglutaminase Derived from Microorganisms. *Agric. Biol. Chem.* **53**, 2613–2617 (1989).
13. Antonyak, M. A. *et al.* Cancer cell-derived microvesicles induce transformation by transferring tissue transglutaminase and fibronectin to recipient cells. *Proc. Natl. Acad. Sci.* **108**, 4852–4857 (2011).
14. Antonyak, M. A. *et al.* Tissue transglutaminase is an essential participant in the epidermal growth factor-stimulated signaling pathway leading to cancer cell migration and invasion. *J. Biol. Chem.* **284**, 17914–25 (2009).

15. Antonyak, M. A. *et al.* Two isoforms of tissue transglutaminase mediate opposing cellular fates. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 18609 (2006).
16. Assrir, N. *et al.* Mapping the UDP-N-acetylglucosamine regulatory site of human glucosamine-6P synthase by saturation-transfer difference NMR and site-directed mutagenesis. *Biochimie* **97**, 39–48 (2014).
17. Axelrad, T. W. *et al.* Platelet-activating factor (PAF) induces activation of matrix metalloproteinase 2 activity and vascular endothelial cell invasion and migration. *FASEB J.* **18**, 568–570 (2004).
18. Baek, K. J. *et al.* Evidence that the Gh protein is a signal mediator from alpha 1-adrenoceptor to a phospholipase C. I. Identification of alpha 1-adrenoceptor-coupled Gh family and purification of Gh7 from bovine heart. *J. Biol. Chem.* **268**, 27390–7 (1993).
19. Balajthy, Z. *et al.* Tissue-transglutaminase contributes to neutrophil granulocyte differentiation and functions. *Blood* **108**, 2045–2054 (2006).
20. Balint, B. L. *et al.* Arginine methylation provides epigenetic transcription memory for retinoid-induced differentiation in myeloid cells. *Mol. Cell. Biol.* **25**, 5648–63 (2005).
21. Balklava, Z. *et al.* Analysis of tissue transglutaminase function in the migration of Swiss 3T3 fibroblasts: the active-state conformation of the enzyme does not affect cell motility but is important for its secretion. *J. Biol. Chem.* **277**, 16567–75 (2002).
22. Bannantine, Griffiths, Viratyosin, Brown & Rockey. A secondary structure motif predictive of protein localization to the chlamydial inclusion membrane. *Cell. Microbiol.* **2**, 35–47 (2000).
23. Barbour, A. G., Amano, K., Hackstadt, T., Perry, L. & Caldwell, H. D. *Chlamydia trachomatis* has penicillin-binding proteins but not detectable muramic acid. *J. Bacteriol.* **151**, 420–8 (1982).
24. Bavoil, P. M., Marques, P. X., Brotman, R. & Ravel, J. Does Active Oral Sex Contribute to Female Infertility? *J. Infect. Dis.* **216**, 932–935 (2017).
25. Bavoil, P. M. & Byrne, G. I. Analysis of CPAF mutants: new functions, new questions (The ins and outs of a chlamydial protease). *Pathog. Dis.* **71**, 287–291 (2014).
26. Becker, E. & Hegemann, J. H. All subtypes of the Pmp adhesin family are implicated in chlamydial virulence and show species-specific function. *Microbiologyopen* **3**, 544–56 (2014).
27. Belkin, A. M. *et al.* Transglutaminase-mediated oligomerization of the fibrin(ogen) C domains promotes integrin-dependent cell adhesion and signaling. *Blood* **105**, 3561–3568 (2005).
28. Belkin, A. M. Extracellular TG2: emerging functions and regulation. *FEBS J.* **278**, 4704–16 (2011).

29. Belkin, A. M. *et al.* Matrix-dependent Proteolysis of Surface Transglutaminase by Membrane-type Metalloproteinase Regulates Cancer Cell Adhesion and Locomotion. *J. Biol. Chem.***276**, 18415–18422 (2001).
30. Belkin, A. M. *et al.* Cell-Surface-Associated Tissue Transglutaminase Is a Target of MMP-2 Proteolysis †. *Biochemistry***43**, 11760–11769 (2004).
31. Belland, R. J. *et al.* Transcriptome analysis of chlamydial growth during IFN-gamma-mediated persistence and reactivation. *Proc. Natl. Acad. Sci. U. S. A.***100**, 15971–6 (2003).
32. Belland, R. J. *et al.* Genomic transcriptional profiling of the developmental cycle of *Chlamydia trachomatis*. *Proc. Natl. Acad. Sci. U. S. A.***100**, 8478–83 (2003).
33. Berg, J. M. (Jeremy M., Tymoczko, J. L., Gatto, G. J. & Stryer, L.). *Biochemistry*. (2015).
34. Bersten, A. M., Ahkong, Q. F., Hallinan, T., Nelson, S. J. & Lucy, J. A. Inhibition of the formation of myotubes in vitro by inhibitors of transglutaminase. *Biochim. Biophys. Acta - Mol. Cell Res.***762**, 429–436 (1983).
35. Betts-Hampikian, H. J. & Fields, K. A. Disulfide bonding within components of the *Chlamydia* type III secretion apparatus correlates with development. *J. Bacteriol.***193**, 6950–9 (2011).
36. Betts-Hampikian, H. J. & Fields, K. A. The Chlamydial Type III Secretion Mechanism: Revealing Cracks in a Tough Nut. *Front. Microbiol.***1**, 114 (2010).
37. Birckbichler, P. J., Orr, G. R., Patterson, M. K., Conway, E. & Carter, H. A. Increase in proliferative markers after inhibition of transglutaminase. *Proc. Natl. Acad. Sci. U. S. A.***78**, 5005–8 (1981).
38. Boisvert, W. A. *et al.* Leukocyte transglutaminase 2 expression limits atherosclerotic lesion size. *Arterioscler. Thromb. Vasc. Biol.***26**, 563–9 (2006).
39. Boncompain, G. *et al.* The intracellular bacteria *Chlamydia* hijack peroxisomes and utilize their enzymatic capacity to produce bacteria-specific phospholipids. *PLoS One***9**, e86196 (2014).
40. Boroughs, L. K., Antonyak, M. A., Johnson, J. L. & Cerione, R. A. A unique role for heat shock protein 70 and its binding partner tissue transglutaminase in cancer cell migration. *J. Biol. Chem.***286**, 37094–107 (2011).
41. Bowness, J. M., Folk, J. E. & Timpl, R. Identification of a substrate site for liver transglutaminase on the aminopropeptide of type III collagen. *J. Biol. Chem.***262**, 1022–4 (1987).
42. Brickman, T. J., Barry, C. E., Hackstadt, T. & Hackstadt, T. Molecular cloning and expression of hctB encoding a strain-variant chlamydial histone-like protein with DNA-binding activity. *J. Bacteriol.***175**, 4274–81 (1993).
43. Cacciamani, T. *et al.* Specific methylation of the CpG-rich domains in the promoter of the human tissue transglutaminase gene. *Gene***297**, 103–112 (2002).

44. Cai, L., Sutter, B. M., Li, B. & Tu, B. P. Acetyl-CoA induces cell growth and proliferation by promoting the acetylation of histones at growth genes. *Mol. Cell***42**, 426–37 (2011).
45. Cai, L. & Tu, B. P. Acetyl-CoA drives the transcriptional growth program in yeast. *Cell Cycle***10**, 3045–6 (2011).
46. Cao, J. & Huang, W. Compensatory Increase of Transglutaminase 2 Is Responsible for Resistance to mTOR Inhibitor Treatment. *PLoS One***11**, e0149388 (2016).
47. Cao, L. *et al.* Tissue transglutaminase links TGF- β , epithelial to mesenchymal transition and a stem cell phenotype in ovarian cancer. *Oncogene***31**, 2521–2534 (2012).
48. Cao, L. *et al.* Tissue transglutaminase protects epithelial ovarian cancer cells from cisplatin-induced apoptosis by promoting cell survival signaling. *Carcinogenesis***29**, 1893–1900 (2008).
49. Carabeo, R. A. & Hackstadt, T. Isolation and characterization of a mutant Chinese hamster ovary cell line that is resistant to *Chlamydia trachomatis* infection at a novel step in the attachment process. *Infect. Immun.***69**, 5899–904 (2001).
50. Carabeo, R. A., Dooley, C. A., Grieshaber, S. S. & Hackstadt, T. Rac interacts with Abi-1 and WAVE2 to promote an Arp2/3-dependent actin recruitment during chlamydial invasion. *Cell. Microbiol.***9**, 2278–2288 (2007).
51. Carabeo, R. A., Grieshaber, S. S., Hasenkrug, A., Dooley, C. & Hackstadt, T. Requirement for the Rac GTPase in *Chlamydia trachomatis* Invasion of Non-phagocytic Cells. *Traffic***5**, 418–425 (2004).
52. Carabeo, R. A., Grieshaber, S. S., Fischer, E. & Hackstadt, T. *Chlamydia trachomatis* induces remodeling of the actin cytoskeleton during attachment and entry into HeLa cells. *Infect. Immun.***70**, 3793–803 (2002).
53. Caron, N. S., Munsie, L. N., Keillor, J. W. & Truant, R. Using FLIM-FRET to measure conformational changes of transglutaminase type 2 in live cells. *PLoS One***7**, e44159 (2012).
54. Carpenter, V., Chen, Y.-S., Dolat, L. & Valdivia, R. H. The Effector TepP Mediates Recruitment and Activation of Phosphoinositide 3-Kinase on Early *Chlamydia trachomatis* Vacuoles. *mSphere***2**, (2017).
55. Casadio, R. *et al.* The structural basis for the regulation of tissue transglutaminase by calcium ions. *Eur. J. Biochem.***262**, 672–9 (1999).
56. Ceovic, R. & Gulin, S. J. *Lymphogranuloma venereum*: diagnostic and treatment challenges. *Infect. Drug Resist.***8**, 39–47 (2015).
57. Chang, Q. *et al.* Phosphorylation of Human Glutamine:Fructose-6-phosphate Amidotransferase by cAMP-dependent Protein Kinase at Serine 205 Blocks the Enzyme Activity. *J. Biol. Chem.***275**, 21981–21987 (2000).

58. Chen, D. *et al.* Secretion of the chlamydial virulence factor CPAF requires the Sec-dependent pathway. *Microbiology***156**, 3031–40 (2010).
59. Chen, Y.-S. *et al.* The *Chlamydia trachomatis* type III secretion chaperone Slc1 engages multiple early effectors, including TepP, a tyrosine-phosphorylated protein required for the recruitment of CrkI-II to nascent inclusions and innate immune signaling. *PLoS Pathog.***10**, e1003954 (2014).
60. Choroszy-Król, I., Frej-Mądrzak, M., Hober, M., Sarowska, J. & Jama-Kmieciak, A. Infections caused by *Chlamydophila pneumoniae*. *Adv. Clin. Exp. Med.***23**, 123–6 (2014).
61. Christiansen, G., Pedersen, L. B., Koehler, J. E., Lundemose, A. G. & Birkelund, S. Interaction between the *Chlamydia trachomatis* histone H1-like protein (Hc1) and DNA. *J. Bacteriol.***175**, 1785–95 (1993).
62. Chumduri, C., Gurumurthy, R. K., Zadora, P. K., Mi, Y. & Meyer, T. F. *Chlamydia* infection promotes host DNA damage and proliferation but impairs the DNA damage response. *Cell Host Microbe***13**, 746–58 (2013).
63. Citron, B. A. *et al.* Protein crosslinking, tissue transglutaminase, alternative splicing and neurodegeneration. *Neurochem. Int.***40**, 69–78 (2002).
64. Clausen, J. D., Christiansen, G., Holst, H. U. & Birkelund, S. *Chlamydia trachomatis* utilizes the host cell microtubule network during early events of infection. *Mol. Microbiol.***25**, 441–449 (1997).
65. Clifton, D. R. *et al.* A chlamydial type III translocated protein is tyrosine-phosphorylated at the site of entry and associated with recruitment of actin. *Proc. Natl. Acad. Sci. U. S. A.***101**, 10166–71 (2004).
66. Clifton, D. R. *et al.* Tyrosine phosphorylation of the chlamydial effector protein Tarp is species specific and not required for recruitment of actin. *Infect. Immun.***73**, 3860–8 (2005).
67. Cocchiario, J. L., Kumar, Y., Fischer, E. R., Hackstadt, T. & Valdivia, R. H. Cytoplasmic lipid droplets are translocated into the lumen of the *Chlamydia trachomatis* parasitophorous vacuole. *Proc. Natl. Acad. Sci. U. S. A.***105**, 9379–84 (2008).
68. Colegio, O. R. *et al.* Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature***513**, 559–63 (2014).
69. Cossé, M. M., Hayward, R. D. & Subtil, A. One Face of *Chlamydia trachomatis*: The Infectious Elementary Body. (2016). doi:10.1007/82_2016_12
70. Coussons, P. J., Price, N. C., Kelly, S. M., Smith, B. & Sawyer, L. Factors that govern the specificity of transglutaminase-catalysed modification of proteins and peptides. *Biochem. J.***282 (Pt 3)** 929–30 (1992).
71. Cox, J. V, Naher, N., Abdelrahman, Y. M. & Belland, R. J. Host HDL biogenesis machinery is recruited to the inclusion of *Chlamydia trachomatis*-infected cells and regulates chlamydial growth. *Cell. Microbiol.***14**, 1497–512 (2012).

72. Cox, J. V., Abdelrahman, Y. M., Peters, J., Naher, N. & Belland, R. J. *Chlamydia trachomatis* utilizes the mammalian CLA1 lipid transporter to acquire host phosphatidylcholine essential for growth. *Cell. Microbiol.***18**, 305–318 (2016).
73. Cox, J. *et al.* Accurate proteome-wide label-free quantification by delayed normalization and maximal peptide ratio extraction, termed MaxLFQ. *Mol. Cell. Proteomics***13**, 2513–26 (2014).
74. Cox, J. & Mann, M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat. Biotechnol.***26**, 1367–1372 (2008).
75. Cox, J. *et al.* Andromeda: A Peptide Search Engine Integrated into the MaxQuant Environment. *J. Proteome Res.***10**, 1794–1805 (2011).
76. Csösz, É., Meskó, B. & Fésüs, L. Transdab wiki: the interactive transglutaminase substrate database on web 2.0 surface. *Amino Acids***36**, 615–617 (2009).
77. Currò, M. *et al.* Transglutaminase 2 and phospholipase A2 interactions in the inflammatory response in human Thp-1 monocytes. *Amino Acids***46**, 759–766 (2014).
78. D’Eletto, M. *et al.* Transglutaminase 2 is involved in autophagosome maturation. *Autophagy***5**, 1145–54 (2009).
79. Dai, W. & Li, Z. Conserved type III secretion system exerts important roles in *Chlamydia trachomatis*. *Int. J. Clin. Exp. Pathol.***7**, 5404–14 (2014).
80. Damiani, M. T., Gambarte Tudela, J. & Capmany, A. Targeting eukaryotic Rab proteins: a smart strategy for chlamydial survival and replication. *Cell. Microbiol.***16**, 1329–1338 (2014).
81. Dardik, R. & Inbal, A. Complex formation between tissue transglutaminase II (tTG) and vascular endothelial growth factor receptor 2 (VEGFR-2): Proposed mechanism for modulation of endothelial cell response to VEGF. *Exp. Cell Res.***312**, 2973–2982 (2006).
82. Darville, T. & Hiltke, T. J. Pathogenesis of Genital Tract Disease Due to *Chlamydia trachomatis*. *J. Infect. Dis.***201**, 114–125 (2010).
83. Dautry-Varsat, A., Balañá, M. E. & Wyplosz, B. *Chlamydia*-Host Cell Interactions: Recent Advances on Bacterial Entry and Intracellular Development. *Traffic***5**, 561–570 (2004).
84. De Clercq, E., Kalmar, I. & Vanrompay, D. Animal Models for Studying Female Genital Tract Infection with *Chlamydia trachomatis*. *Infect. Immun.***81**, 3060–3067 (2013).
85. De Laurenzi, V. & Melino, G. Gene disruption of tissue transglutaminase. *Mol. Cell. Biol.***21**, 148–55 (2001).
86. Delevoye, C. *et al.* SNARE protein mimicry by an intracellular bacterium. *PLoS Pathog.***4**, e1000022 (2008).

87. Denzel, M. S. *et al.* Hexosamine Pathway Metabolites Enhance Protein Quality Control and Prolong Life. *Cell***156**, 1167–1178 (2014).
88. Diepold, A. *et al.* A dynamic and adaptive network of cytosolic interactions governs protein export by the T3SS injectisome. *Nat. Commun.***8**, 15940 (2017).
89. Doherty, J. R. & Cleveland, J. L. Targeting lactate metabolism for cancer therapeutics. *J. Clin. Invest.***123**, 3685–92 (2013).
90. Dumoux, M., Menny, A., Delacour, D. & Hayward, R. D. A *Chlamydia* effector recruits CEP170 to reprogram host microtubule organization. *J. Cell Sci.***128**, 3420–3434 (2015).
91. Dyer, L. M. *et al.* The transglutaminase 2 gene is aberrantly hypermethylated in glioma. *J. Neurooncol.***101**, 429–40 (2011).
92. Eckert, R. L. *et al.* Transglutaminase is a tumor cell and cancer stem cell survival factor. *Mol. Carcinog.***54**, 947–58 (2015).
93. Eckert, R. L. *et al.* Transglutaminase regulation of cell function. *Physiol. Rev.***94**, 383–417 (2014).
94. Eckert, R. L., Sturniolo, M. T., Broome, A.-M., Ruse, M. & Rorke, E. A. Transglutaminase Function in Epidermis. *J. Invest. Dermatol.***124**, 481–492 (2005).
95. Elwell, C. A., Ceesay, A., Kim, J. H., Kalman, D. & Engel, J. N. RNA interference screen identifies Abl kinase and PDGFR signaling in *Chlamydia trachomatis* entry. *PLoS Pathog.***4**, e1000021 (2008).
96. Elwell, C., Mirrashidi, K. & Engel, J. *Chlamydia* cell biology and pathogenesis. *Nat. Rev. Microbiol.***14**, 385–400 (2016).
97. Erde, J., Loo, R. R. O. & Loo, J. A. Enhanced FASP (eFASP) to increase proteome coverage and sample recovery for quantitative proteomic experiments. *J. Proteome Res.***13**, 1885–95 (2014).
98. Esposito, C. & Caputo, I. Mammalian transglutaminases. *FEBS J.***272**, 615–631 (2005).
99. Esposito, C. *et al.* Ubiquitination of tissue transglutaminase is modulated by interferon alpha in human lung cancer cells. *Biochem. J.***370**, 205–12 (2003).
100. Everett, K. D. & Bush, R. M. Molecular evolution of the *Chlamydiaceae*. *Int. J. Syst. Evol. Microbiol.***51**, 203–220 (2001).
101. Exley, R. M. *et al.* Lactate acquisition promotes successful colonization of the murine genital tract by *Neisseria gonorrhoeae*. *Infect. Immun.***75**, 1318–24 (2007).
102. Fadel, S. & Eley, A. Differential glycosaminoglycan binding of *Chlamydia trachomatis* OmcB protein from serovars E and LGV. *J. Med. Microbiol.***57**, 1058–1061 (2008).
103. Falcone, P., Serafini-Fracassini, D. & Del Duca, S. Comparative Studies of Transglutaminase Activity and Substrates in Different Organs of *Helianthus tuberosus*. *J. Plant Physiol.***142**, 265–273 (1993).

104. Fehr, A. *et al.* *Candidatus Syngnamydia venezia*, a novel member of the phylum *Chlamydiae* from the broad nosed pipefish, *Syngnathus typhle*. *PLoS One***8**, e70853 (2013).
105. Feng, J.-F., Readon, M., Yadav, S. P. & Im, M.-J. Calreticulin Down-Regulates both GTP Binding and Transglutaminase Activities of Transglutaminase II[†]. *Biochemistry***38**, 10743–10749 (1999).
106. Festoff, B. W. *et al.* Injury-induced ‘switch’ from GTP-regulated to novel GTP-independent isoform of tissue transglutaminase in the rat spinal cord. *J. Neurochem.***81**, 708–718 (2002).
107. Fesus, L., Metsis, M. L., Muszbek, L. & Koteliansky, V. E. Transglutaminase-sensitive glutamine residues of human plasma fibronectin revealed by studying its proteolytic fragments. *Eur. J. Biochem.***154**, 371–4 (1986).
108. Fields, K. A., Mead, D. J., Dooley, C. A. & Hackstadt, T. *Chlamydia trachomatis* type III secretion: evidence for a functional apparatus during early-cycle development. *Mol. Microbiol.***48**, 671–683 (2003).
109. Folk, J. E. Transglutaminases. *Annu. Rev. Biochem.***49**, 517–531 (1980).
110. Folk, J. E. *et al.* Polyamines as physiological substrates for transglutaminases. *J. Biol. Chem.***255**, 3695–700 (1980).
111. Fornelli, L., Schmid, A. W., Grasso, L., Vogel, H. & Tsybin, Y. O. Deamidation and transamidation of substance P by tissue transglutaminase revealed by electron-capture dissociation fourier transform mass spectrometry. *Chemistry***17**, 486–97 (2011).
112. Forsprecher, J., Wang, Z., Nelea, V. & Kaartinen, M. T. Enhanced osteoblast adhesion on transglutaminase 2-crosslinked fibronectin. *Amino Acids***36**, 747–753 (2009).
113. Fox, A. *et al.* Muramic acid is not detectable in *Chlamydia psittaci* or *Chlamydia trachomatis* by gas chromatography-mass spectrometry. *Infect. Immun.***58**, 835–7 (1990).
114. Fraij, B. M., Birckbichler, P. J., Patterson, M. K., Lee, K. N. & Gonzales, R. A. A retinoic acid-inducible mRNA from human erythroleukemia cells encodes a novel tissue transglutaminase homologue. *J. Biol. Chem.***267**, 22616–23 (1992).
115. Fraij, B. M. & Gonzales, R. A. A third human tissue transglutaminase homologue as a result of alternative gene transcripts. *Biochim. Biophys. Acta***1306**, 63–74 (1996).
116. Furini, G. *et al.* Proteomic Profiling Reveals the Transglutaminase-2 Externalization Pathway in Kidneys after Unilateral Ureteric Obstruction. *J. Am. Soc. Nephrol.***29**, 880–905 (2018).
117. Furutani, Y., Toguchi, M., Shrestha, R. & Kojima, S. Phenosafranin inhibits nuclear localization of transglutaminase 2 without affecting its transamidase activity. *Amino Acids***49**, 483–488 (2017).

118. Gehre, L. *et al.* Sequestration of host metabolism by an intracellular pathogen. *Elife***5**, e12552 (2016).
119. Geisler, W. M., Suchland, R. J., Rockey, D. D. & Stamm, W. E. Epidemiology and Clinical Manifestations of Unique *Chlamydia trachomatis* Isolates That Occupy Nonfusogenic Inclusions. *J. Infect. Dis.***184**, 879–884 (2001).
120. George, M. D., Vollberg, T. M., Floyd, E. E., Stein, J. P. & Jetten, A. M. Regulation of transglutaminase type II by transforming growth factor-beta 1 in normal and transformed human epidermal keratinocytes. *J. Biol. Chem.***265**, 11098–104 (1990).
121. George, Z. *et al.* The roles of unfolded protein response pathways in *Chlamydia* pathogenesis. *J. Infect. Dis.* jiw569 (2016). doi:10.1093/infdis/jiw569
122. Ghanta, K. S. *et al.* MTA1 coregulation of transglutaminase 2 expression and function during inflammatory response. *J. Biol. Chem.***286**, 7132–8 (2011).
123. Giai Gianetto, Q. *et al.* Calibration plot for proteomics: A graphical tool to visually check the assumptions underlying FDR control in quantitative experiments. *Proteomics***16**, 29–32 (2016).
124. Giles, D. K., Whittimore, J. D., LaRue, R. W., Raulston, J. E. & Wyrick, P. B. Ultrastructural analysis of chlamydial antigen-containing vesicles everting from the *Chlamydia trachomatis* inclusion. *Microbes Infect.***8**, 1579–1591 (2006).
125. Gordon, F. B. & Quan, A. L. Occurrence Of Glycogen In Inclusions Of The Psittacosis-*Lymphogranuloma Venereum*-Trachoma Agents. *J. Infect. Dis.***115**, 186–96 (1965).
126. Grieshaber, N. A., Fischer, E. R., Mead, D. J., Dooley, C. A. & Hackstadt, T. Chlamydial histone-DNA interactions are disrupted by a metabolite in the methylerythritol phosphate pathway of isoprenoid biosynthesis. *Proc. Natl. Acad. Sci. U. S. A.***101**, 7451–6 (2004).
127. Grieshaber, S. S., Grieshaber, N. A., Hackstadt, T., Echeverri, C. J. & Vallee, R. B. *Chlamydia trachomatis* uses host cell dynein to traffic to the microtubule-organizing center in a p50 dynamitin-independent process. *J. Cell Sci.***116**, 3793–802 (2003).
128. Guilluy, C. *et al.* Transglutaminase-dependent RhoA activation and depletion by serotonin in vascular smooth muscle cells. *J. Biol. Chem.***282**, 2918–28 (2007).
129. Guseva, N. V, Dessus-Babus, S., Moore, C. G., Whittimore, J. D. & Wyrick, P. B. Differences in *Chlamydia trachomatis* serovar E growth rate in polarized endometrial and endocervical epithelial cells grown in three-dimensional culture. *Infect. Immun.***75**, 553–64 (2007).
130. Hackstadt, T., Rockey, D. D., Heinzen, R. A. & Scidmore, M. A. *Chlamydia trachomatis* interrupts an exocytic pathway to acquire endogenously synthesized sphingomyelin in transit from the Golgi apparatus to the plasma membrane. *EMBO J.***15**, 964–77 (1996).
131. Hackstadt, T., Todd, W. J. & Caldwell, H. D. Disulfide-mediated interactions of the chlamydial major outer membrane protein: role in the differentiation of *chlamydiae*? *J. Bacteriol.***161**, 25–31 (1985).

132. Hafner, L. M. Pathogenesis of fallopian tube damage caused by *Chlamydia trachomatis* infections. *Contraception***92**, 108–115 (2015).
133. Hallstrand, T. S. *et al.* Transglutaminase 2, a Novel Regulator of Eicosanoid Production in Asthma Revealed by Genome-Wide Expression Profiling of Distinct Asthma Phenotypes. *PLoS One***5**, e8583 (2010).
134. Hamanaka, R. B. & Chandel, N. S. Warburg effect and redox balance. *Science***334**, 1219–20 (2011).
135. Han, J. A. & Park, S. C. Reduction of transglutaminase 2 expression is associated with an induction of drug sensitivity in the PC-14 human lung cancer cell line. *J. Cancer Res. Clin. Oncol.***125**, 89–95 (1999).
136. Hasegawa, G. *et al.* A novel function of tissue-type transglutaminase: protein disulphide isomerase. *Biochem. J.***373**, 793–803 (2003).
137. Hatch, G. M. & McClarty, G. Phospholipid composition of purified *Chlamydia trachomatis* mimics that of the eucaryotic host cell. *Infect. Immun.***66**, 3727–35 (1998).
138. Hatch, T. P., Miceli, M. & Sublett, J. E. Synthesis of disulfide-bonded outer membrane proteins during the developmental cycle of *Chlamydia psittaci* and *Chlamydia trachomatis*. *J. Bacteriol.***165**, 379–85 (1986).
139. Hay, N. Reprogramming glucose metabolism in cancer: can it be exploited for cancer therapy? *Nat. Rev. Cancer***16**, 635–649 (2016).
140. Hedekov, C. J. Early effects of phytohaemagglutinin on glucose metabolism of normal human lymphocytes. *Biochem. J.***110**, 373–80 (1968).
141. Hernandez-Fernaud, J. R. *et al.* Secreted CLIC3 drives cancer progression through its glutathione-dependent oxidoreductase activity. *Nat. Commun.***8**, 14206 (2017).
142. Heuer, D., Brinkmann, V., Meyer, T. F. & Szczepek, A. J. Expression and translocation of chlamydial protease during acute and persistent infection of the epithelial HEp-2 cells with *Chlamydia (Chlamydia) pneumoniae*. *Cell. Microbiol.***5**, 315–22 (2003).
143. Higashikawa, F., Eboshida, A. & Yokosaki, Y. Enhanced biological activity of polymeric osteopontin. *FEBS Lett.***581**, 2697–2701 (2007).
144. Horn, M. *Chlamydiae* as symbionts in eukaryotes. *Annu. Rev. Microbiol.***62**, 113–31 (2008).
145. Horn, M. & Wagner, M. Bacterial Endosymbionts of Free-living Amoebae. *J. Eukaryot. Microbiol.***51**, 509–514 (2004).
146. Hower, S., Wolf, K. & Fields, K. A. Evidence that CT694 is a novel *Chlamydia trachomatis* T3S substrate capable of functioning during invasion or early cycle development. *Mol. Microbiol.***72**, 1423–1437 (2009).
147. Hybiske, K. & Stephens, R. S. Mechanisms of host cell exit by the intracellular bacterium *Chlamydia*. *Proc. Natl. Acad. Sci. U. S. A.***104**, 11430–5 (2007).

148. Iismaa, S. E. *et al.* The Core Domain of the Tissue Transglutaminase G_h Hydrolyzes GTP and ATP[†]. *Biochemistry***36**, 11655–11664 (1997).
149. Iismaa, S. E., Mearns, B. M., Lorand, L. & Graham, R. M. Transglutaminases and Disease: Lessons From Genetically Engineered Mouse Models and Inherited Disorders. *Physiol. Rev.***89**, 991–1023 (2009).
150. Iliffe-Lee, E. R. & McClarty, G. Glucose metabolism in *Chlamydia trachomatis*: the ‘energy parasite’ hypothesis revisited. *Mol. Microbiol.***33**, 177–87 (1999).
151. Iwai, K., Shibukawa, Y., Yamazaki, N. & Wada, Y. Transglutaminase 2-dependent Deamidation of Glyceraldehyde-3-phosphate Dehydrogenase Promotes Trophoblastic Cell Fusion. *J. Biol. Chem.***289**, 4989–4999 (2014).
152. Jang, G.-Y. *et al.* Transglutaminase 2 suppresses apoptosis by modulating caspase 3 and NF-κB activity in hypoxic tumor cells. *Oncogene***29**, 356–367 (2010).
153. Janiak, A., Zemskov, E. A. & Belkin, A. M. Cell surface transglutaminase promotes RhoA activation via integrin clustering and suppression of the Src-p190RhoGAP signaling pathway. *Mol. Biol. Cell***17**, 1606–19 (2006).
154. Jeong, E. M. *et al.* S-nitrosylation of transglutaminase 2 impairs fatty acid-stimulated contraction in hypertensive cardiomyocytes. *Exp. Mol. Med.***50**, 9 (2018).
155. Jeong, E. M. *et al.* Degradation of transglutaminase 2 by calcium-mediated ubiquitination responding to high oxidative stress. *FEBS Lett.***583**, 648–654 (2009).
156. Jewett, T. J., Fischer, E. R., Mead, D. J. & Hackstadt, T. Chlamydial TARP is a bacterial nucleator of actin. *Proc. Natl. Acad. Sci. U. S. A.***103**, 15599–604 (2006).
157. Jiang, P. *et al.* p53 regulates biosynthesis through direct inactivation of glucose-6-phosphate dehydrogenase. *Nat. Cell Biol.***13**, 310–6 (2011).
158. Jiwani, S. *et al.* *Chlamydia trachomatis* Tarp cooperates with the Arp2/3 complex to increase the rate of actin polymerization. *Biochem. Biophys. Res. Commun.***420**, 816–21 (2012).
159. Johnson, C. M. *et al.* Site-Specific, Insertional Inactivation of *incA* in *Chlamydia trachomatis* Using a Group II Intron. *PLoS One***8**, e83989 (2013).
160. Johnson, T. S. *et al.* Transglutaminase Inhibition Reduces Fibrosis and Preserves Function in Experimental Chronic Kidney Disease. *J. Am. Soc. Nephrol.***18**, 3078–3088 (2007).
161. Józwiak, P., Forma, E., Bryś, M. & Krześlak, A. O-GlcNAcylation and Metabolic Reprogramming in Cancer. *Front. Endocrinol. (Lausanne)***5**, 145 (2014).
162. Jung, S.-H. *et al.* Identification of transglutaminase 2 kinase substrates using a novel on-chip activity assay. *Biosens. Bioelectron.***82**, 40–48 (2016).

163. Jung, S. M. *et al.* Increased tissue transglutaminase activity contributes to central vascular stiffness in eNOS knockout mice. *Am. J. Physiol. Heart Circ. Physiol.***305**, H803-10 (2013).
164. Kabeiseman, E. J., Cichos, K., Hackstadt, T., Lucas, A. & Moore, E. R. Vesicle-associated membrane protein 4 and syntaxin 6 interactions at the chlamydial inclusion. *Infect. Immun.***81**, 3326–37 (2013).
165. Karyagina, A. S., Alexeevsky, A. V, Spirin, S. A., Zigangirova, N. A. & Gintsburg, A. L. Effector Proteins of *Chlamydiae*. *Mol. Biol.***43**, 26–8933 (2009).
166. Keillor, J. W., Apperley, K. Y. P. & Akbar, A. Inhibitors of tissue transglutaminase. *Trends Pharmacol. Sci.***36**, 32–40 (2015).
167. Keresztessy, Z. *et al.* Phage display selection of efficient glutamine-donor substrate peptides for transglutaminase 2. *Protein Sci.***15**, 2466–80 (2006).
168. Khew, S. T., Panengad, P. P., Raghunath, M. & Tong, Y. W. Characterization of amine donor and acceptor sites for tissue type transglutaminase using a sequence from the C-terminus of human fibrillin-1 and the N-terminus of osteonectin. *Biomaterials***31**, 4600–4608 (2010).
169. Kieliszek, M. & Misiewicz, A. Microbial transglutaminase and its application in the food industry. A review. *Folia Microbiol. (Praha)*.**59**, 241–250 (2014).
170. Kim, D. Y. *et al.* N-acetylcysteine prevents LPS-induced pro-inflammatory cytokines and MMP2 production in gingival fibroblasts. *Arch. Pharm. Res.***30**, 1283–92 (2007).
171. Kim, N. *et al.* Inter-molecular crosslinking activity is engendered by the dimeric form of transglutaminase 2. *Amino Acids***49**, 461–471 (2017).
172. Kim, S.-Y., Jeong, E.-J. & Steinert, P. M. IFN- γ Induces Transglutaminase 2 Expression in Rat Small Intestinal Cells. *J. Interf. Cytokine Res.***22**, 677–682 (2002).
173. Király, R., Demény, M. & Fésüs, L. Protein transamidation by transglutaminase 2 in cells: a disputed Ca²⁺-dependent action of a multifunctional protein. *FEBS J.***278**, 4717–4739 (2011).
174. Kleba, B. & Stephens, R. S. Chlamydial effector proteins localized to the host cell cytoplasmic compartment. *Infect. Immun.***76**, 4842–50 (2008).
175. Krasnikov, B. F. *et al.* Transglutaminase activity is present in highly purified nonsynaptosomal mouse brain and liver mitochondria. *Biochemistry***44**, 7830–43 (2005).
176. Kumar, A. *et al.* Evidence That Aberrant Expression of Tissue Transglutaminase Promotes Stem Cell Characteristics in Mammary Epithelial Cells. *PLoS One***6**, e20701 (2011).
177. Kumar, A. *et al.* Tissue Transglutaminase Promotes Drug Resistance and Invasion by Inducing Mesenchymal Transition in Mammary Epithelial Cells. *PLoS One***5**, e13390 (2010).

178. Kumar, S., Donti, T. R., Agnihotri, N. & Mehta, K. Transglutaminase 2 reprogramming of glucose metabolism in mammary epithelial cells via activation of inflammatory signaling pathways. *Int. J. Cancer***134**, 2798–2807 (2014).
179. Kumar, S. & Mehta, K. Tissue transglutaminase constitutively activates HIF-1 α promoter and nuclear factor- κ B via a non-canonical pathway. *PLoS One***7**, e49321 (2012).
180. Kumar, Y. & Valdivia, R. H. Actin and intermediate filaments stabilize the *Chlamydia trachomatis* vacuole by forming dynamic structural scaffolds. *Cell Host Microbe***4**, 159–69 (2008).
181. Kumar, Y. & Valdivia, R. H. Reorganization of the host cytoskeleton by the intracellular pathogen *Chlamydia trachomatis*. *Commun. Integr. Biol.***1**, 175–7 (2008).
182. Kuncio, G. S. *et al.* TNF-alpha modulates expression of the tissue transglutaminase gene in liver cells. *Am. J. Physiol.***274**, G240-5 (1998).
183. Lai, T. S., Davies, C. & Greenberg, C. S. Human tissue transglutaminase is inhibited by pharmacologic and chemical acetylation. *Protein Sci.***19**, 229–235 (2010).
184. Lai, T.-S., Liu, Y., Li, W. & Greenberg, C. S. Identification of two GTP-independent alternatively spliced forms of tissue transglutaminase in human leukocytes, vascular smooth muscle, and endothelial cells. *FASEB J.***21**, 4131 (2007).
185. Lee, J. K. *et al.* Replication-dependent size reduction precedes differentiation in *Chlamydia trachomatis*. *Nat. Commun.***9**, 45 (2018).
186. Lee, J.-H. *et al.* Endoplasmic reticulum stress activates transglutaminase 2 leading to protein aggregation. *Int. J. Mol. Med.***33**, 849–55 (2014).
187. Lee, K. N., Birckbichler, P. J. & Patterson, M. K. Colorimetric assay of blood coagulation factor XIII in plasma. *Clin. Chem.***34**, 906–10 (1988).
188. Lee, K. N., Maxwell, M. D., Patterson, M. K., Birckbichler, P. J. & Conway, E. Identification of transglutaminase substrates in HT29 colon cancer cells: use of 5-(biotinamido)pentylamine as a transglutaminase-specific probe. *Biochim. Biophys. Acta - Mol. Cell Res.***1136**, 12–16 (1992).
189. Lei, L., Dong, X., Li, Z. & Zhong, G. Identification of a novel nuclear localization signal sequence in *Chlamydia trachomatis*-secreted hypothetical protein CT311. *PLoS One***8**, e64529 (2013).
190. Leo, J. C., Grin, I. & Linke, D. Type V secretion: mechanism(s) of autotransport through the bacterial outer membrane. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.***367**, 1088–101 (2012).
191. Lesort, M., Attanavanich, K., Zhang, J. & Johnson, G. V. Distinct nuclear localization and activity of tissue transglutaminase. *J. Biol. Chem.***273**, 11991–4 (1998).

192. Li, Y. *et al.* Identification of a Novel Serine Phosphorylation Site in Human Glutamine:Fructose-6-phosphate Amidotransferase Isoform 1. *Biochemistry***46**, 13163–9 (2007).
193. Li, Z., Xu, X., Bai, L., Chen, W. & Lin, Y. Epidermal growth factor receptor-mediated tissue transglutaminase overexpression couples acquired tumor necrosis factor-related apoptosis-inducing ligand resistance and migration through c-FLIP and MMP-9 proteins in lung cancer cells. *J. Biol. Chem.***286**, 21164–72 (2011).
194. Liang, P. *et al.* Dynamic energy dependency of *Chlamydia trachomatis* on host cell metabolism during intracellular growth: Role of sodium-based energetics in chlamydial ATP generation. *J. Biol. Chem.***293**, 510–522 (2018).
195. Liao, Y., Zhang, M. & Lönnnerdal, B. Growth factor TGF- β induces intestinal epithelial cell (IEC-6) differentiation: miR-146b as a regulatory component in the negative feedback loop. *Genes Nutr.***8**, 69–78 (2013).
196. Liechti, G. W. *et al.* A new metabolic cell-wall labelling method reveals peptidoglycan in *Chlamydia trachomatis*. *Nature***506**, 507–10 (2014).
197. Liechti, G. *et al.* Pathogenic *Chlamydia* Lack a Classical Sacculus but Synthesize a Narrow, Mid-cell Peptidoglycan Ring, Regulated by MreB, for Cell Division. *PLOS Pathog.***12**, e1005590 (2016).
198. Liu, D. *et al.* Interleukin-17A promotes esophageal adenocarcinoma cell invasiveness through ROS-dependent, NF- κ B-mediated MMP-2/9 activation. *Oncol. Rep.***37**, 1779–1785 (2017).
199. Locasale, J. W. & Cantley, L. C. Metabolic flux and the regulation of mammalian cell growth. *Cell Metab.***14**, 443–51 (2011).
200. Lorand, L. & Graham, R. M. Transglutaminases: crosslinking enzymes with pleiotropic functions. *Nat. Rev. Mol. Cell Biol.***4**, 140–156 (2003).
201. Love, D. C. & Hanover, J. A. The hexosamine signaling pathway: deciphering the “O-GlcNAc code”. *Science***2005**, re13 (2005).
202. Lu, C. & Thompson, C. B. Metabolic regulation of epigenetics. *Cell Metab.***16**, 9–17 (2012).
203. Lu, S. & Davies, P. J. Regulation of the expression of the tissue transglutaminase gene by DNA methylation. *Proc. Natl. Acad. Sci. U. S. A.***94**, 4692–7 (1997).
204. Luciani, A. *et al.* Defective CFTR induces aggresome formation and lung inflammation in cystic fibrosis through ROS-mediated autophagy inhibition. *Nat. Cell Biol.***12**, 863–75 (2010).
205. Lunt, S. Y. & Vander Heiden, M. G. Aerobic Glycolysis: Meeting the Metabolic Requirements of Cell Proliferation. *Annu. Rev. Cell Dev. Biol.***27**, 441–464 (2011).

206. Lutter, E. I., Barger, A. C., Nair, V. & Hackstadt, T. *Chlamydia trachomatis* inclusion membrane protein CT228 recruits elements of the myosin phosphatase pathway to regulate release mechanisms. *Cell Rep.***3**, 1921–31 (2013).
207. Mabey, D. & Peeling, R. W. *Lymphogranuloma venereum*. *Sex. Transm. Infect.***78**, 90–2 (2002).
208. Mackern-Oberti, J. P. *et al.* *Chlamydia trachomatis* infection of the male genital tract: An update. *J. Reprod. Immunol.***100**, 37–53 (2013).
209. Maffei, B., Francetic, O. & Subtil, A. Tracking Proteins Secreted by Bacteria: What's in the Toolbox? *Front. Cell. Infect. Microbiol.***7**, 1–17 (2017).
210. Maiuri, L. *et al.* Tissue transglutaminase activation modulates inflammation in cystic fibrosis via PPARgamma down-regulation. *J. Immunol.***180**, 7697–705 (2008).
211. Majeed, M., Krause, K. H., Clark, R. A., Kihlström, E. & Stendahl, O. Localization of intracellular Ca²⁺ stores in HeLa cells during infection with *Chlamydia trachomatis*. *J. Cell Sci.***112** (Pt 1), 35–44 (1998).
212. Malhotra, V. Unconventional protein secretion: an evolving mechanism. *EMBO J.***32**, 1660–4 (2013).
213. Malorni, W. *et al.* The adenine nucleotide translocator 1 acts as a type 2 transglutaminase substrate: implications for mitochondrial-dependent apoptosis. *Cell Death Differ.***16**, 1480–1492 (2009).
214. Mariani, P. *et al.* Ligand-Induced Conformational Changes in Tissue Transglutaminase: Monte Carlo Analysis of Small-Angle Scattering Data. *Biophys. J.***78**, 3240–3251 (2000).
215. Marshall, S., Bacote, V. & Traxinger, R. R. Complete inhibition of glucose-induced desensitization of the glucose transport system by inhibitors of mRNA synthesis. Evidence for rapid turnover of glutamine:fructose-6-phosphate amidotransferase. *J. Biol. Chem.***266**, 10155–61 (1991).
216. Mastroberardino, P. G. *et al.* “Tissue” transglutaminase contributes to the formation of disulphide bridges in proteins of mitochondrial respiratory complexes. *Biochim. Biophys. Acta - Bioenerg.***1757**, 1357–1365 (2006).
217. McKuen, M. J., Mueller, K. E., Bae, Y. S. & Fields, K. A. Fluorescence-Reported Allelic Exchange Mutagenesis Reveals a Role for *Chlamydia trachomatis* TmeA in Invasion That Is Independent of Host AHNAK. *Infect. Immun.***85**, e00640-17 (2017).
218. Mehta, K. High levels of transglutaminase expression in doxorubicin-resistant human breast carcinoma cells. *Int. J. cancer***58**, 400–6 (1994).
219. Mehta, K., Lopez-Berestein, G., Moore, W. T. & Davies, P. J. Interferon-gamma requires serum retinoids to promote the expression of tissue transglutaminase in cultured human blood monocytes. *J. Immunol.***134**, 2053–6 (1985).

220. Mehta, K., Kumar, A. & Kim, H. I. Transglutaminase 2: A multi-tasking protein in the complex circuitry of inflammation and cancer. *Biochem. Pharmacol.***80**, 1921–1929 (2010).
221. Mehul, B., Bawumia, S. & Colin Hughes, R. Cross-linking of galectin 3, a galactose-binding protein of mammalian cells, by tissue-type transglutaminase. *FEBS Lett.***360**, 160–164 (1995).
222. Melino, G. *et al.* Macrophages Engulfing Apoptotic Cells in Formation of an Efficient Phagocyte Portal Transglutaminase 2 Is Needed for the Transglutaminase 2 Is Needed for the Formation of an Efficient Phagocyte Portal in Macrophages Engulfing Apoptotic Cells 1. *J Immunol J. Immunol.***182**, 2084–2092 (2009).
223. Merz, D., Liu, R., Johnson, K. & Terkeltaub, R. IL-8/CXCL8 and growth-related oncogene alpha/CXCL1 induce chondrocyte hypertrophic differentiation. *J. Immunol.***171**, 4406–15 (2003).
224. Mhaoutykodja, S. Gh/tissue transglutaminase 2: an emerging G protein in signal transduction. *Biol. Cell***96**, 363–367 (2004).
225. Miller, J. F., Mekalanos, J. J. & Falkow, S. Coordinate regulation and sensory transduction in the control of bacterial virulence. *Science***243**, 916–22 (1989).
226. Min, B. *et al.* CHIP-mediated degradation of transglutaminase 2 negatively regulates tumor growth and angiogenesis in renal cancer. *Oncogene***35**, 3718–3728 (2016).
227. Min, B., Kwon, Y.-C., Choe, K.-M. & Chung, K. C. PINK1 phosphorylates transglutaminase 2 and blocks its proteasomal degradation. *J. Neurosci. Res.***93**, 722–735 (2015).
228. Mirrashidi, K. M. *et al.* Global Mapping of the Inc-Human Interactome Reveals that Retromer Restricts *Chlamydia* Infection. *Cell Host Microbe***18**, 109–21 (2015).
229. Mirza, A. *et al.* A role for tissue transglutaminase in hepatic injury and fibrogenesis, and its regulation by NF-kappaB. *Am. J. Physiol.***272**, G281-8 (1997).
230. Mishra, S., Melino, G. & Murphy, L. J. Transglutaminase 2 kinase activity facilitates protein kinase A-induced phosphorylation of retinoblastoma protein. *J. Biol. Chem.***282**, 18108–15 (2007).
231. Mishra, S. & Murphy, L. J. Tissue transglutaminase has intrinsic kinase activity: identification of transglutaminase 2 as an insulin-like growth factor-binding protein-3 kinase. *J. Biol. Chem.***279**, 23863–8 (2004).
232. Mishra, S. & Murphy, L. J. Phosphorylation of transglutaminase 2 by PKA at Ser216 creates 14-3-3 binding sites. *Biochem. Biophys. Res. Commun.***347**, 1166–1170 (2006).
233. Mishra, S. & Murphy, L. J. The p53 oncoprotein is a substrate for tissue transglutaminase kinase activity. *Biochem. Biophys. Res. Commun.***339**, 726–730 (2006).
234. Mishra, S., Saleh, A., Espino, P. S., Davie, J. R. & Murphy, L. J. Phosphorylation of histones by tissue transglutaminase. *J. Biol. Chem.***281**, 5532–8 (2006).

235. Mital, J., Lutter, E. I., Barger, A. C., Dooley, C. A. & Hackstadt, T. *Chlamydia trachomatis* inclusion membrane protein CT850 interacts with the dynein light chain DYNLT1 (Tctex1). *Biochem. Biophys. Res. Commun.***462**, 165–70 (2015).
236. Miyairi, I., Ramsey, K. H. & Patton, D. L. Duration of Untreated Chlamydial Genital Infection and Factors Associated with Clearance: Review of Animal Studies. *J. Infect. Dis.***201**, 96–103 (2010).
237. Mohammadpour, M., Abrishami, M., Masoumi, A. & Hashemi, H. Trachoma: Past, present and future. *J. Curr. Ophthalmol.***28**, 165–169 (2016).
238. Monsonogo, A. *et al.* Expression of GTP-dependent and GTP-independent tissue-type transglutaminase in cytokine-treated rat brain astrocytes. *J. Biol. Chem.***272**, 3724–32 (1997).
239. Moore, E. R., Mead, D. J., Dooley, C. A., Sager, J. & Hackstadt, T. The trans-Golgi SNARE syntaxin 6 is recruited to the chlamydial inclusion membrane. *Microbiology***157**, 830–8 (2011).
240. Moore, W. T., Murtaugh, M. P. & Davies, P. J. Retinoic acid-induced expression of tissue transglutaminase in mouse peritoneal macrophages. *J. Biol. Chem.***259**, 12794–802 (1984).
241. Moulder, J. W., Novosel, D. L. & Officier, J. E. Inhibition Of The Growth Of Agents Of The Psittacosis Group By D-Cycloserine And Its Specific Reversal By D-Alanine. *J. Bacteriol.***85**, 707–11 (1963).
242. Moulder, J. W. The Biochemistry of Intracellular Parasitism. *Science (80-.)***138**, 25–25 (1962).
243. Mueller, K. E., Wolf, K. & Fields, K. A. Gene Deletion by Fluorescence-Reported Allelic Exchange Mutagenesis in *Chlamydia trachomatis*. *MBio***7**, e01817-15- (2016).
244. Mulukutla, B. C., Khan, S., Lange, A. & Hu, W.-S. Glucose metabolism in mammalian cell culture: new insights for tweaking vintage pathways. *Trends Biotechnol.***28**, 476–484 (2010).
245. Nagy, L. *et al.* Identification and characterization of a versatile retinoid response element (retinoic acid receptor response element-retinoid X receptor response element) in the mouse tissue transglutaminase gene promoter. *J. Biol. Chem.***271**, 4355–65 (1996).
246. Nakaishi, Y. *et al.* Structural analysis of human glutamine:fructose-6-phosphate amidotransferase, a key regulator in type 2 diabetes. *FEBS Lett.***583**, 163–167 (2009).
247. Nakano, Y., Addison, W. N. & Kaartinen, M. T. ATP-mediated mineralization of MC3T3-E1 osteoblast cultures. *Bone***41**, 549–61 (2007).
248. Nakano, Y., Forsprecher, J. & Kaartinen, M. T. Regulation of ATPase activity of transglutaminase 2 by MT1-MMP: Implications for mineralization of MC3T3-E1 osteoblast cultures. *J. Cell. Physiol.***223**, n/a-n/a (2009).

249. Nakaoka, H. *et al.* Gh: a GTP-binding protein with transglutaminase activity and receptor signaling function. *Science***264**, 1593–6 (1994).
250. Nans, A., Kudryashev, M., Saibil, H. R. & Hayward, R. D. Structure of a bacterial type III secretion system in contact with a host membrane in situ. *Nat. Commun.***6**, 10114 (2015).
251. Nans, A., Saibil, H. R. & Hayward, R. D. Pathogen-host reorganization during *Chlamydia* invasion revealed by cryo-electron tomography. *Cell. Microbiol.***16**, 1457–72 (2014).
252. Nelea, V., Nakano, Y. & Kaartinen, M. T. Size Distribution and Molecular Associations of Plasma Fibronectin and Fibronectin Crosslinked by Transglutaminase 2. *Protein J.***27**, 223–233 (2008).
253. Nemes, Z. *et al.* Transglutaminase-mediated intramolecular cross-linking of membrane-bound alpha-synuclein promotes amyloid formation in Lewy bodies. *J. Biol. Chem.***284**, 27252–64 (2009).
254. Newman, L. *et al.* Global Estimates of the Prevalence and Incidence of Four Curable Sexually Transmitted Infections in 2012 Based on Systematic Review and Global Reporting. *PLoS One***10**, e0143304 (2015).
255. Nguyen, B. D. & Valdivia, R. H. Virulence determinants in the obligate intracellular pathogen *Chlamydia trachomatis* revealed by forward genetic approaches. *Proc. Natl. Acad. Sci. U. S. A.***109**, 1263–8 (2012).
256. Nguyen, P. H., Lutter, E. I. & Hackstadt, T. *Chlamydia trachomatis* inclusion membrane protein MrcA interacts with the inositol 1,4,5-trisphosphate receptor type 3 (ITPR3) to regulate extrusion formation. *PLOS Pathog.***14**, e1006911 (2018).
257. Nicholson, T. L., Olinger, L., Chong, K., Schoolnik, G. & Stephens, R. S. Global stage-specific gene regulation during the developmental cycle of *Chlamydia trachomatis*. *J. Bacteriol.***185**, 3179–89 (2003).
258. Novo, A. A. norm: Analysis of multivariate normal datasets with missing values. *R Packag. version 1.0-9.5* (2013).
259. Nunes, A. & Gomes, J. P. Evolution, phylogeny, and molecular epidemiology of *Chlamydia*. *Infect. Genet. Evol.***23**, 49–64 (2014).
260. Nunes, A., Nogueira, P. J., Borrego, M. J. & Gomes, J. P. *Chlamydia trachomatis* diversity viewed as a tissue-specific coevolutionary arms race. *Genome Biol.***9**, R153 (2008).
261. Nurminkaya, M., Beazley, K. E., Smith, E. P. & Belkin, A. M. Transglutaminase 2 promotes PDGF-mediated activation of PDGFR/Akt1 and β -catenin signaling in vascular smooth muscle cells and supports neointima formation. *J. Vasc. Res.***51**, 418–28 (2014).
262. Oh, K. *et al.* Transglutaminase 2 facilitates the distant hematogenous metastasis of breast cancer by modulating interleukin-6 in cancer cells. *Breast Cancer Res.***13**, R96 (2011).

263. Ojcius, D. M., Degani, H., Mispelter, J. & Dautry-Varsat, A. Enhancement of ATP levels and glucose metabolism during an infection by *Chlamydia*. NMR studies of living cells. *J. Biol. Chem.***273**, 7052–8 (1998).
264. Olsen, K. C. *et al.* Transglutaminase 2 and Its Role in Pulmonary Fibrosis. *Am. J. Respir. Crit. Care Med.***184**, 699–707 (2011).
265. Omsland, A., Sager, J., Nair, V., Sturdevant, D. E. & Hackstadt, T. Developmental stage-specific metabolic and transcriptional activity of *Chlamydia trachomatis* in an axenic medium. *Proc. Natl. Acad. Sci. U. S. A.***109**, 19781–5 (2012).
266. Omsland, A., Sixt, B. S., Horn, M. & Hackstadt, T. Chlamydial metabolism revisited: interspecies metabolic variability and developmental stage-specific physiologic activities. *FEMS Microbiol. Rev.***38**, 779–801 (2014).
267. Orrù, S., Caputo, I., D'Amato, A., Ruoppolo, M. & Esposito, C. Proteomics identification of acyl-acceptor and acyl-donor substrates for transglutaminase in a human intestinal epithelial cell line. Implications for celiac disease. *J. Biol. Chem.***278**, 31766–73 (2003).
268. Ouellette, S. P. Feasibility of a Conditional Knockout System for *Chlamydia*-Based on CRISPR Interference. *Front. Cell. Infect. Microbiol.***8**, 59 (2018).
269. Packiam, M., Weinrick, B., Jacobs, W. R., Maurelli, A. T. & Maurelli, A. T. Structural characterization of mucopeptides from *Chlamydia trachomatis* peptidoglycan by mass spectrometry resolves ‘chlamydial anomaly’. *Proc. Natl. Acad. Sci. U. S. A.***112**, 11660–5 (2015).
270. Pais, S. V., Milho, C., Almeida, F. & Mota, L. J. Identification of novel type III secretion chaperone-substrate complexes of *Chlamydia trachomatis*. *PLoS One***8**, e56292 (2013).
271. Palucci, I. *et al.* Transglutaminase type 2 plays a key role in the pathogenesis of Mycobacterium tuberculosis infection. *J. Intern. Med.***283**, 303–313 (2018).
272. Pardin, C., Roy, I., Lubell, W. D. & Keillor, J. W. Reversible and Competitive Cinnamoyl Triazole Inhibitors of Tissue Transglutaminase. *Chem. Biol. Drug Des.***72**, 189–196 (2008).
273. Park, D., Choi, S. S. & Ha, K.-S. Transglutaminase 2: a multi-functional protein in multiple subcellular compartments. *Amino Acids***39**, 619–631 (2010).
274. Park, S. Y. *et al.* Snail1 is stabilized by O-GlcNAc modification in hyperglycaemic condition. *EMBO J.***29**, 3787–96 (2010).
275. Pascual, G. *et al.* A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. *Nature***437**, 759–63 (2005).
276. Patton, M. J. *et al.* Chlamydial Protease-Like Activity Factor and Type III Secreted Effectors Cooperate in Inhibition of p65 Nuclear Translocation. *MBio***7**, (2016).
277. Paul, B. *et al.* Structural basis for the hijacking of endosomal sorting nexin proteins by *Chlamydia trachomatis*. *Elife***6**, (2017).

278. Paulmann, N. *et al.* Intracellular Serotonin Modulates Insulin Secretion from Pancreatic β -Cells by Protein Serotonylation. *PLoS Biol.***7**, e1000229 (2009).
279. Paumet, F. *et al.* Intracellular bacteria encode inhibitory SNARE-like proteins. *PLoS One***4**, e7375 (2009).
280. Peng, B. *et al.* Enhanced upper genital tract pathologies by blocking Tim-3 and PD-L1 signaling pathways in mice intravaginally infected with *Chlamydia muridarum*. *BMC Infect. Dis.***11**, 347 (2011).
281. Pennini, M. E., Perrinet, S., Dautry-Varsat, A. & Subtil, A. Histone methylation by NUE, a novel nuclear effector of the intracellular pathogen *Chlamydia trachomatis*. *PLoS Pathog.***6**, e1000995 (2010).
282. Phatak, V. M. *et al.* Expression of transglutaminase-2 isoforms in normal human tissues and cancer cell lines: dysregulation of alternative splicing in cancer. *Amino Acids***44**, 33–44 (2013).
283. Piacentini, M. *et al.* The expression of tissue transglutaminase in two human cancer cell lines is related with the programmed cell death (apoptosis). *Eur. J. Cell Biol.***54**, 246–54 (1991).
284. Piacentini, M. *et al.* Characterization of distinct sub-cellular location of transglutaminase type II: changes in intracellular distribution in physiological and pathological states. *Cell Tissue Res.***358**, 793–805 (2014).
285. Pincus, J. H. & Waelsch, H. The specificity of transglutaminase: I. Human hemoglobin as a substrate for the enzyme. *Arch. Biochem. Biophys.***126**, 34–43 (1968).
286. Pinkas, D. M., Strop, P., Brunger, A. T. & Khosla, C. Transglutaminase 2 undergoes a large conformational change upon activation. *PLoS Biol.***5**, e327 (2007).
287. Plugis, N. M., Palanski, B. A., Weng, C.-H., Albertelli, M. & Khosla, C. Thioredoxin-1 Selectively Activates Transglutaminase 2 in the Extracellular Matrix of the Small Intestine. *J. Biol. Chem.***292**, 2000–2008 (2017).
288. Pokrovskaya, I. D. *et al.* *Chlamydia trachomatis* hijacks intra-Golgi COG complex-dependent vesicle trafficking pathway. *Cell. Microbiol.***14**, 656–68 (2012).
289. Poteat, W. L. & Bo, W. J. The interaction of clomiphene, estradiol, and progesterone in the control of rat uterine glycogen metabolism. *Am. J. Anat.***149**, 153–163 (1977).
290. Pounds, S. & Cheng, C. Robust estimation of the false discovery rate. *Bioinformatics***22**, 1979–87 (2006).
291. Prusty, B. K. *et al.* Imbalanced oxidative stress causes chlamydial persistence during non-productive human herpes virus co-infection. *PLoS One***7**, e47427 (2012).
292. Puzan, M., Hosic, S., Ghio, C. & Koppes, A. Enteric Nervous System Regulation of Intestinal Stem Cell Differentiation and Epithelial Monolayer Function. *Sci. Rep.***8**, 6313 (2018).

293. Quan, G., Choi, J.-Y., Lee, D.-S. & Lee, S.-C. TGF- β 1 up-regulates transglutaminase two and fibronectin in dermal fibroblasts: a possible mechanism for the stabilization of tissue inflammation. *Arch. Dermatol. Res.* **297**, 84–90 (2005).
294. Raghunath, M. *et al.* Cross-linking of the dermo-epidermal junction of skin regenerating from keratinocyte autografts. Anchoring fibrils are a target for tissue transglutaminase. *J. Clin. Invest.* **98**, 1174–1184 (1996).
295. Rajeeve, K., Das, S., Prusty, B. K. & Rudel, T. *Chlamydia trachomatis* paralyzes neutrophils to evade the host innate immune response. *Nat. Microbiol.* **3**, 824–835 (2018).
296. Rajić, J. *et al.* *Chlamydia trachomatis* Infection Is Associated with E-Cadherin Promoter Methylation, Downregulation of E-Cadherin Expression, and Increased Expression of Fibronectin and α -SMA-Implications for Epithelial-Mesenchymal Transition. *Front. Cell. Infect. Microbiol.* **7**, 253 (2017).
297. Rasmussen, S. J. *et al.* Secretion of proinflammatory cytokines by epithelial cells in response to *Chlamydia* infection suggests a central role for epithelial cells in chlamydial pathogenesis. *J. Clin. Invest.* **99**, 77–87 (1997).
298. Rathor, N., Wang, S. R., Chang, E. T. & Rao, J. N. Differentiated intestinal epithelial cells express high levels of TGF- β receptors and exhibit increased sensitivity to growth inhibition. *Int. J. Clin. Exp. Med.* **4**, 299–308 (2011).
299. Read, T. D. *et al.* Genome sequences of *Chlamydia trachomatis* MoPn and *Chlamydia pneumoniae* AR39. *Nucleic Acids Res.* **28**, 1397–406 (2000).
300. Ritchie, M. E. *et al.* limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* **43**, e47 (2015).
301. Ritter, S. J. & Davies, P. J. Identification of a transforming growth factor-beta1/bone morphogenetic protein 4 (TGF-beta1/BMP4) response element within the mouse tissue transglutaminase gene promoter. *J. Biol. Chem.* **273**, 12798–806 (1998).
302. Robertson, D. K., Gu, L., Rowe, R. K. & Beatty, W. L. Inclusion biogenesis and reactivation of persistent *Chlamydia trachomatis* requires host cell sphingolipid biosynthesis. *PLoS Pathog.* **5**, e1000664 (2009).
303. Rodolfo, C. *et al.* Tissue transglutaminase is a multifunctional BH3-only protein. *J. Biol. Chem.* **279**, 54783–92 (2004).
304. Rossin, F., D’Eletto, M., Macdonald, D., Farrace, M. G. & Piacentini, M. TG2 transamidating activity acts as a reostat controlling the interplay between apoptosis and autophagy. *Amino Acids* **42**, 1793–1802 (2012).
305. Rossin, F. *et al.* TG2 regulates the heat-shock response by the post-translational modification of HSF1. *EMBO Rep.* **19**, e45067 (2018).
306. Roth, A. *et al.* Hypoxia abrogates antichlamydial properties of IFN- γ in human fallopian tube cells in vitro and ex vivo. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 19502–7 (2010).

307. Rother, M. *et al.* Combined Human Genome-wide RNAi and Metabolite Analyses Identify IMPDH as a Host-Directed Target against *Chlamydia* Infection. *Cell Host Microbe* (2018). doi:10.1016/j.chom.2018.04.002
308. Salamin, N., Bertelli, C., Greub, G. & Pillonel, T. Taxogenomics of the order *Chlamydiales*. *Int. J. Syst. Evol. Microbiol.***65**, 1381–1393 (2015).
309. Sandkvist, M. Type II secretion and pathogenesis. *Infect. Immun.***69**, 3523–35 (2001).
310. Santhanam, L., Berkowitz, D. E. & Belkin, A. M. Nitric oxide regulates non-classical secretion of tissue transglutaminase. *Commun. Integr. Biol.***4**, 584–6 (2011).
311. Santhanam, L. *et al.* Decreased S-nitrosylation of tissue transglutaminase contributes to age-related increases in vascular stiffness. *Circ. Res.***107**, 117–25 (2010).
312. Sarkar, N. K., Clarke, D. D. & Waelsch, H. An enzymically catalyzed incorporation of amines into proteins. *Biochim. Biophys. Acta***25**, 451–452 (1957).
313. Satpathy, M. *et al.* Enhanced peritoneal ovarian tumor dissemination by tissue transglutaminase. *Cancer Res.***67**, 7194–202 (2007).
314. Satpathy, M., Shao, M., Emerson, R., Donner, D. B. & Matei, D. Tissue transglutaminase regulates matrix metalloproteinase-2 in ovarian cancer by modulating cAMP-response element-binding protein activity. *J. Biol. Chem.***284**, 15390–9 (2009).
315. Scarpellini, A. *et al.* Heparan sulfate proteoglycans are receptors for the cell-surface trafficking and biological activity of transglutaminase-2. *J. Biol. Chem.***284**, 18411–23 (2009).
316. Schnoor, M. Endothelial actin-binding proteins and actin dynamics in leukocyte transendothelial migration. *J. Immunol.***194**, 3535–41 (2015).
317. Schwöppe, C., Winkler, H. H. & Neuhaus, H. E. Properties of the glucose-6-phosphate transporter from *Chlamydia pneumoniae* (HPTcp) and the glucose-6-phosphate sensor from *Escherichia coli* (UhpC). *J. Bacteriol.***184**, 2108–15 (2002).
318. Scidmore, M. A. Cultivation and Laboratory Maintenance of *Chlamydia trachomatis*. *Current Protocols in Microbiology* 11A11.11-11A11.25 (John Wiley & Sons, Inc., 2005). doi:10.1002/9780471729259.mc11a01s00
319. Scott, K. F., Meyskens, F. L. & Russell, D. H. Retinoids increase transglutaminase activity and inhibit ornithine decarboxylase activity in Chinese hamster ovary cells and in melanoma cells stimulated to differentiate. *Proc. Natl. Acad. Sci. U. S. A.***79**, 4093–7 (1982).
320. Seiving, B., Ohlsson, K., Linder, C. & Stenberg, P. Transglutaminase differentiation during maturation of human blood monocytes to macrophages. *Eur. J. Haematol.***46**, 263–71 (1991).
321. Sellami, H. *et al.* *Chlamydia trachomatis* infection increases the expression of inflammatory tumorigenic cytokines and chemokines as well as components of the Toll-

- like receptor and NF- κ B pathways in human prostate epithelial cells. *Mol. Cell. Probes***28**, 147–154 (2014).
322. Shan, L. *et al.* Structural basis for gluten intolerance in celiac sprue. *Science***297**, 2275–9 (2002).
 323. Shao, M. *et al.* Epithelial-to-Mesenchymal Transition and Ovarian Tumor Progression Induced by Tissue Transglutaminase. *Cancer Res.***69**, 9192–9201 (2009).
 324. Sharma, M. *et al.* HIF-1 α is involved in mediating apoptosis resistance to *Chlamydia trachomatis*-infected cells. *Cell. Microbiol.***13**, 1573–1585 (2011).
 325. Shaw, E. I. *et al.* Three temporal classes of gene expression during the *Chlamydia trachomatis* developmental cycle. *Mol. Microbiol.***37**, 913–925 (2000).
 326. Shaw, J. H., Behar, A. R., Snider, T. A., Allen, N. A. & Lutter, E. I. Comparison of Murine Cervicovaginal Infection by Chlamydial Strains: Identification of Extrusions Shed *In vivo*. *Front. Cell. Infect. Microbiol.***7**, 18 (2017).
 327. Sherrid, A. M. & Hybiske, K. *Chlamydia trachomatis* Cellular Exit Alters Interactions with Host Dendritic Cells. *Infect. Immun.***85**, (2017).
 328. Shestov, A. A. *et al.* Quantitative determinants of aerobic glycolysis identify flux through the enzyme GAPDH as a limiting step. *Elife***3**, (2014).
 329. Shima, K. *et al.* Interferon- γ interferes with host cell metabolism during intracellular *Chlamydia trachomatis* infection. *Cytokine* (2018). doi:10.1016/j.cyto.2018.05.039
 330. Shinde, A. V. *et al.* Pharmacologic inhibition of the enzymatic effects of tissue transglutaminase reduces cardiac fibrosis and attenuates cardiomyocyte hypertrophy following pressure overload. *J. Mol. Cell. Cardiol.***117**, 36–48 (2018).
 331. Shrestha, R. *et al.* Molecular mechanism by which acyclic retinoid induces nuclear localization of transglutaminase 2 in human hepatocellular carcinoma cells. *Cell Death Dis.***6**, e2002 (2015).
 332. Siegl, C., Prusty, B. K., Karunakaran, K., Wischhusen, J. & Rudel, T. Tumor Suppressor p53 Alters Host Cell Metabolism to Limit *Chlamydia trachomatis* Infection. *Cell Rep.***9**, 918–929 (2014).
 333. Singh, U. S. & Cerione, R. A. Biochemical effects of retinoic acid on GTP-binding Protein/Transglutaminases in HeLa cells. Stimulation of GTP-binding and transglutaminase activity, membrane association, and phosphatidylinositol lipid turnover. *J. Biol. Chem.***271**, 27292–8 (1996).
 334. Sixt, B. S. *et al.* The *Chlamydia trachomatis* Inclusion Membrane Protein CpoS Counteracts STING-Mediated Cellular Surveillance and Suicide Programs. *Cell Host Microbe***21**, 113–121 (2017).
 335. Slaughter, T. F., Achyuthan, K. E., Lai, T.-S. & Greenberg, C. S. A microtiter plate transglutaminase assay utilizing 5-(biotinamido)pentylamine as substrate. *Anal. Biochem.***205**, 166–171 (1992).

336. Smyth, G. K. *limma: Linear Models for Microarray Data. Bioinformatics and Computational Biology Solutions Using R and Bioconductor* 397–420 (Springer-Verlag, 2005). doi:10.1007/0-387-29362-0_23
337. Sohn, J., Chae, J. B., Lee, S. Y., Kim, S.-Y. & Kim, J.-G. A novel therapeutic target in inflammatory uveitis: transglutaminase 2 inhibitor. *Korean J. Ophthalmol.***24**, 29–34 (2010).
338. Spaeth, K. E., Chen, Y.-S. & Valdivia, R. H. The *Chlamydia* type III secretion system C-ring engages a chaperone-effector protein complex. *PLoS Pathog.***5**, e1000579 (2009).
339. Spurlin, T. A., Bhadriraju, K., Chung, K.-H., Tona, A. & Plant, A. L. The treatment of collagen fibrils by tissue transglutaminase to promote vascular smooth muscle cell contractile signaling. *Biomaterials***30**, 5486–5496 (2009).
340. Stammaes, J., Fleckenstein, B. & Sollid, L. M. The propensity for deamidation and transamidation of peptides by transglutaminase 2 is dependent on substrate affinity and reaction conditions. *Biochim. Biophys. Acta***1784**, 1804–11 (2008).
341. Stammaes, J., Iversen, R., du Pré, M. F., Chen, X. & Sollid, L. M. Enhanced B-Cell Receptor Recognition of the Autoantigen Transglutaminase 2 by Efficient Catalytic Self-Multimerization. *PLoS One***10**, e0134922 (2015).
342. Stammaes, J., Pinkas, D. M., Fleckenstein, B., Khosla, C. & Sollid, L. M. Redox regulation of transglutaminase 2 activity. *J. Biol. Chem.***285**, 25402–9 (2010).
343. Stanhope, R., Flora, E., Bayne, C. & Derré, I. IncV, a FFAT motif-containing *Chlamydia* protein, tethers the endoplasmic reticulum to the pathogen-containing vacuole. *Proc. Natl. Acad. Sci. U. S. A.***114**, 12039–12044 (2017).
344. Stephens, R. S., Koshiyama, K., Lewis, E. & Kubo, A. Heparin-binding outer membrane protein of *Chlamydiae*. *Mol. Microbiol.***40**, 691–9 (2001).
345. Stephens, R. S. Genome Sequence of an Obligate Intracellular Pathogen of Humans: *Chlamydia trachomatis*. *Science (80-.)***282**, 754–759 (1998).
346. Stephens, R. S. & Kubo, A. Substrate-specific diffusion of select dicarboxylates through *Chlamydia trachomatis* PorB. *Microbiology***147**, 3135–3140 (2001).
347. Su, H. *et al.* A recombinant *Chlamydia trachomatis* major outer membrane protein binds to heparan sulfate receptors on epithelial cells. *Proc. Natl. Acad. Sci. U. S. A.***93**, 11143–8 (1996).
348. Subtil, A. *et al.* A directed screen for chlamydial proteins secreted by a type III mechanism identifies a translocated protein and numerous other new candidates. *Mol. Microbiol.***56**, 1636–47 (2005).
349. Sugimura, Y. *et al.* Screening for the preferred substrate sequence of transglutaminase using a phage-displayed peptide library: identification of peptide substrates for TGASE 2 and Factor XIIIa. *J. Biol. Chem.***281**, 17699–706 (2006).

350. Szondy, Z. *et al.* Transglutaminase 2^{-/-} mice reveal a phagocytosis-associated crosstalk between macrophages and apoptotic cells. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 7812–7817 (2003).
351. Tarbet, H. J. *et al.* Site-specific glycosylation regulates the form and function of the intermediate filament cytoskeleton. *Elife***7**, e31807 (2018).
352. Taylor, H. R., Burton, M. J., Haddad, D., West, S. & Wright, H. Trachoma. *Lancet (London, England)***384**, 2142–52 (2014).
353. Telci, D., Collighan, R. J., Basaga, H. & Griffin, M. Increased TG2 expression can result in induction of transforming growth factor beta1, causing increased synthesis and deposition of matrix proteins, which can be regulated by nitric oxide. *J. Biol. Chem.***284**, 29547–58 (2009).
354. Telci, D. *et al.* Fibronectin-Tissue Transglutaminase Matrix Rescues RGD-impaired Cell Adhesion through Syndecan-4 and β_1 Integrin Co-signaling. *J. Biol. Chem.***283**, 20937–20947 (2008).
355. Tokunaga, F. *et al.* Limulus hemocyte transglutaminase. Its purification and characterization, and identification of the intracellular substrates. *J. Biol. Chem.***268**, 252–61 (1993).
356. Tucholski, J., Lesort, M. & Johnson, G. V. Tissue transglutaminase is essential for neurite outgrowth in human neuroblastoma SH-SY5Y cells. *Neuroscience***102**, 481–91 (2001).
357. Tuffrey, M., Falder, P. & Taylor-Robinson, D. Genital-tract infection and disease in nude and immunologically competent mice after inoculation of a human strain of *Chlamydia trachomatis*. *Br. J. Exp. Pathol.***63**, 539–46 (1982).
358. Tyanova, S., Temu, T. & Cox, J. The MaxQuant computational platform for mass spectrometry-based shotgun proteomics. *Nat. Protoc.***11**, 2301–2319 (2016).
359. Unemo, M. *et al.* Sexually transmitted infections: challenges ahead. *Lancet. Infect. Dis.***17**, e235–e279 (2017).
360. van den Akker, J. *et al.* Transglutaminase 2 is secreted from smooth muscle cells by transamidation-dependent microparticle formation. *Amino Acids***42**, 961–73 (2012).
361. van Strien, M. E. *et al.* Tissue Transglutaminase contributes to experimental multiple sclerosis pathogenesis and clinical outcome by promoting macrophage migration. *Brain. Behav. Immun.***50**, 141–154 (2015).
362. Vandahl, B. B. S., Birkelund, S. & Christiansen, G. Genome and proteome analysis of *Chlamydia*. *Proteomics***4**, 2831–42 (2004).
363. Verderio, E. A. M., Johnson, T. & Griffin, M. Tissue transglutaminase in normal and abnormal wound healing: Review article. *Amino Acids***26**, 387–404 (2004).

364. Verderio, E. A. M., Telci, D., Okoye, A., Melino, G. & Griffin, M. A Novel RGD-independent Cell Adhesion Pathway Mediated by Fibronectin-bound Tissue Transglutaminase Rescues Cells from Anoikis. *J. Biol. Chem.***278**, 42604–42614 (2003).
365. Verderio, E. *et al.* Regulation of Cell Surface Tissue Transglutaminase: Effects on Matrix Storage of Latent Transforming Growth Factor- β Binding Protein-1. *J. Histochem. Cytochem.***47**, 1417–1432 (1999).
366. Verma, A. *et al.* Tissue Transglutaminase Regulates Focal Adhesion Kinase/AKT Activation by Modulating PTEN Expression in Pancreatic Cancer Cells. *Clin. Cancer Res.***14**, 1997–2005 (2008).
367. Verma, A. *et al.* Increased Expression of Tissue Transglutaminase in Pancreatic Ductal Adenocarcinoma and Its Implications in Drug Resistance and Metastasis. *Cancer Res.***66**, 10525–10533 (2006).
368. Vizán, P. *et al.* Modulation of pentose phosphate pathway during cell cycle progression in human colon adenocarcinoma cell line HT29. *Int. J. Cancer***124**, 2789–2796 (2009).
369. Vollberg, T. M., George, M. D., Nervi, C. & Jetten, A. M. Regulation of Type I and Type II Transglutaminase in Normal Human Bronchial Epithelial and Lung Carcinoma Cells. *Am. J. Respir. Cell Mol. Biol.***7**, 10–18 (1992).
370. Vromman, F., Laverrière, M., Perrinet, S., Dufour, A. & Subtil, A. Quantitative monitoring of the *Chlamydia trachomatis* developmental cycle using GFP-expressing bacteria, microscopy and flow cytometry. *PLoS One***9**, e99197 (2014).
371. Walther, D. J. *et al.* Serotonylation of small GTPases is a signal transduction pathway that triggers platelet alpha-granule release. *Cell***115**, 851–62 (2003).
372. Walther, D. J., Stahlberg, S. & Vowinckel, J. Novel roles for biogenic monoamines: from monoamines in transglutaminase-mediated post-translational protein modification to monoaminylation deregulation diseases. *FEBS J.***278**, 4740–4755 (2011).
373. Wang, L. *et al.* Nonpathogenic Colonization with *Chlamydia* in the Gastrointestinal Tract as Oral Vaccination for Inducing Transmucosal Protection. *Infect. Immun.***86**, e00630-17 (2018).
374. Wang, X., Hybiske, K. & Stephens, R. S. Orchestration of the mammalian host cell glucose transporter proteins-1 and 3 by *Chlamydia* contributes to intracellular growth and infectivity. *Pathog. Dis.***75**, (2017).
375. Wang, Y. *et al.* Development of a transformation system for *Chlamydia trachomatis*: restoration of glycogen biosynthesis by acquisition of a plasmid shuttle vector. *PLoS Pathog.***7**, e1002258 (2011).
376. Warburg, O. On the origin of cancer cells. *Science***123**, 309–14 (1956).
377. Watts, S. W., Priestley, J. R. C. & Thompson, J. M. Serotonylation of vascular proteins important to contraction. *PLoS One***4**, e5682 (2009).

378. Weber, M. M., Bauler, L. D., Lam, J. & Hackstadt, T. Expression and localization of predicted inclusion membrane proteins in *Chlamydia trachomatis*. *Infect. Immun.* IAI.01075-15 (2015). doi:10.1128/IAI.01075-15
379. Weber, M. M. *et al.* Absence of Specific *Chlamydia trachomatis* Inclusion Membrane Proteins Triggers Premature Inclusion Membrane Lysis and Host Cell Death. *Cell Rep.***19**, 1406–1417 (2017).
380. Welge-Lüssen, U., May, C. A. & Lütjen-Drecoll, E. Induction of tissue transglutaminase in the trabecular meshwork by TGF-beta1 and TGF-beta2. *Invest. Ophthalmol. Vis. Sci.***41**, 2229–38 (2000).
381. Wesolowski, J. *et al.* Chlamydia Hijacks ARF GTPases To Coordinate Microtubule Posttranslational Modifications and Golgi Complex Positioning. *MBio***8**, (2017).
382. Wieczorek, S. *et al.* DAPAR & ProStaR: software to perform statistical analyses in quantitative discovery proteomics. *Bioinformatics***33**, 135–136 (2017).
383. Yamamoto, Y. & Gaynor, R. B. IκB kinases: key regulators of the NF-κB pathway. *Trends Biochem. Sci.***29**, 72–79 (2004).
384. Yasueda, H., Kumazawa, Y. & Motoki, M. Purification and Characterization of a Tissue-type Transglutaminase from Red Sea Bream *Pagrus major*. *Biosci. Biotechnol. Biochem.***58**, 2041–2045 (1994).
385. Zemskov, E. A. *et al.* Regulation of platelet-derived growth factor receptor function by integrin-associated cell surface transglutaminase. *J. Biol. Chem.***284**, 16693–703 (2009).
386. Zemskov, E. A., Mikhailenko, I., Strickland, D. K. & Belkin, A. M. Cell-surface transglutaminase undergoes internalization and lysosomal degradation: an essential role for LRP1. *J. Cell Sci.***120**, 3188–99 (2007).
387. Zemskov, E. A., Mikhailenko, I., Hsia, R.-C., Zaritskaya, L. & Belkin, A. M. Unconventional Secretion of Tissue Transglutaminase Involves Phospholipid-Dependent Delivery into Recycling Endosomes. *PLoS One***6**, e19414 (2011).
388. Zhang, J. P. & Stephens, R. S. Mechanism of *C. trachomatis* attachment to eukaryotic host cells. *Cell***69**, 861–869 (1992).
389. Zhong, G., Fan, P., Ji, H., Dong, F. & Huang, Y. Identification of a chlamydial protease-like activity factor responsible for the degradation of host transcription factors. *J. Exp. Med.***193**, 935–42 (2001).
390. Zhong, G. *Chlamydia* Spreading from the Genital Tract to the Gastrointestinal Tract – A Two-Hit Hypothesis. *Trends Microbiol.* (2017). doi:10.1016/J.TIM.2017.12.002
391. Zibrova, D. *et al.* GFAT1 phosphorylation by AMPK promotes VEGF-induced angiogenesis. *Biochem. J.***474**, 983–1001 (2017).
392. Zu, X. L. & Guppy, M. Cancer metabolism: facts, fantasy, and fiction. *Biochem. Biophys. Res. Commun.***313**, 459–65 (2004).

393. Zuck, M., Ellis, T., Venida, A. & Hybiske, K. Extrusions are phagocytosed and promote *Chlamydia* survival within macrophages. *Cell. Microbiol.* **19**, e12683 (2017).
394. WHO | Report Of The 18th Meeting Of The WHO Alliance For The Global Elimination Of Trachoma By 2020. *WHO* (2014).
395. WHO | Global health sector strategy on Sexually Transmitted Infections, 2016-2021. *WHO* (2016).

ABSTRACT

Chlamydia trachomatis is an obligate intracellular pathogenic bacterium that can only infect humans. It is the leading cause of sexually transmitted infections of bacterial origin and can also infect the eye conjunctiva, causing acquired blindness (trachoma).

During its biphasic developmental cycle, *C. trachomatis* remain in a membrane-bound compartment called inclusion, separated from the cytoplasm. The bacteria have acquired several strategies to hijack cellular functions to secure their niche and meet their metabolic needs.

In this thesis we focused on the impact of a cellular enzyme, the tissue transglutaminase (TG2), on *C. trachomatis* development. The main activity of this multifunctional enzyme is a transamidating activity, i.e. the catalysis of a covalent bond between two proteins or with a small amine present in the cell.

We have shown that TG2 expression and transamidating activity increased upon *C. trachomatis* infection. This increase is necessary for optimal bacterial growth, a conclusion supported by experiments using a mice knocked-out for the expression of TG2 infected with *C. muridarum*.

At the cellular level, we focused our work on the impact of TG2 on cellular metabolism modification during *C. trachomatis* infection. We showed that TG2 played a central role in the control of glucose import in infected cells by regulating *GLUT1* and *GLUT3* transcription. In addition, we identified Gln328 on the enzyme GFPT1 (glucosamine-fructose-6-phosphate amidotransferase 1), as a target of TG2. GFPT1 is the first and rate-limiting enzyme of the hexosamine biosynthetic pathway, regulating the synthesis of UDP-N-acetylglucosamine. This metabolite is used for post-translational modification of proteins by *O*-GlcNAcylation. Our work uncovered an unsuspected link between TG2 transamidating activity and *O*-GlcNAcylation. This link was disrupted in infected cells, likely because UDP-N-acetylglucosamine is rapidly consumed by the bacteria in particular to assist division. In conclusion, our work established TG2 as a key player in controlling glucose-derived metabolic pathways in mammalian cells, themselves hijacked by *C. trachomatis* to sustain its own metabolic needs.