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**Role de DAXX et de CAF-1 dans le ciblage de l'histone H3.3 au
chromosome X et aux elements répétés**

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This Thesis is dedicated to my Parents...!

«A **scientific truth** does not triumph by convincing its opponents and making them see the light, but rather because its opponents eventually die and a new generation grows up that is familiar with it »

Max Planck

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List of Abbreviations

5caC	5-carboxylcytosine
5fC	5-formylcytosine
5hmC	5-hydroxymethylcytosine
5mC	5-methylcytosine
ATP	Adenosine TriPhosphate
ATRX	Alpha-Thalassemia/mental Retardation syndrome, X-linked
BER	Base Excision Repair
Cabin1	Calcineurin-binding protein 1
CENP-A	CENtromeric Protein A
CAF-1	Chromatin Assembly Factor 1
ChIP-seq	Chromatin Immunoprecipitation - sequencing
CHD	CHromodomain-helicase-DNA-binding protein
cds	Coding DNA sequence
cDNA	Complementary DeoxyRibonucleic Acid
cKI	Conditional Knock-In
DAXX	Death domain-Associated protein 6
DNA	DeoxyRibonucleic Acid
DIPG	Diffuse Intrinsic Pontine Glioma
DBD	DNA-Binding Domain
DNMT	DNA-MethylTransferase
ES	Embryonic Stem Cell
ESC	Embryonic Stem Cell
ERV	Endogenous RetroVirus
EZH1/2	Enhancer of Zeste Homolog 1 or 2
EGFR	Epidermal Growth Factor Receptor
EMT	Epithelial-Mesenchymal Transition
FBS	Fetal Bovine Serum
FCS	Fetal Calf Serum
FGF	Fibroblast Growth Factor
HAS	Helicase/SANT-Associated
HP1	Heterochromatin Protein 1
HGG	High-Grade Glioma
HAT	Histone AcetylTransferase
HIRA	Histone cell cycle RegulAtor 1
HDAC	Histone DeAcetylTransferase
HKDM	Histone Lysine DeMethylase
KDM	Histone Lysine DeMethylase

HKMT	Histone Lysine MethylTransferase
HRP	HorseRadish Peroxidase
ISWI	Imitation SWItch
IgG	Immunoglobulin G
IP	ImmunoPrecipitation
INO80	Inositol requiring 80
IAP	Intracisternal A-Particle
KAP1	KRAB-Associated Protein 1
LTR	Log Terminal Repeats
LINE	Long Interspersed Nuclear Elements
mRNA	Messenger RiboNucleic Acid
MBD	Methyl-CpG-binding domain protein
MMR	MisMatch Repair
MEF	Mouse Embryonic Fibroblasts
mESC	Mouse Embryonic Stem Cell
MYCN	N-myc proto-oncogene protein
NuRD/HDAC	Nucleosome Remodeling and Deacetylase/Histone DeACetylase
ORF	Open Reading Frame
PHGG	Pediatric High-Grade Glioma
RD/RI	Replication Dependent/Replication Independent
RD/RI	Replication Dependent/Replication Independent

Summary in french

Rôle de DAXX et de CAF-1 dans le ciblage de l'histone H3.3 au chromosome X et aux éléments répétés

Résumé

L'histone chaperonne DAXX cible H3.3 à l'hétérochromatine péricentrique et télomérique de manière indépendante de la réplication. Le mécanisme qui assure un recrutement approprié de DAXX dans la région hétérochromatique est largement inconnu. En utilisant des approches protéomiques et biochimiques, nous montrons qu'en plus du complexe ATRX/H3.3 bien connu, DAXX forme un complexe alternatif avec CAF-1, ADNP, HP1 et Trim28. DAXX interagit physiquement avec l'extrémité C-terminale de la sous-unité CAF1-p150 via son domaine N-terminal. Le domaine SIM de DAXX s'est avéré essentiel pour cibler DAXX sur les PML-NB, pour le recrutement de CAF-1 à l'hétérochromatine. Nos données génomiques ont également révélé que DAXX cible les éléments répétitifs et le chromosomes X. L'inactivation de DAXX entraîne un appauvrissement majeur de CAF-1 des SINEs et LINEs du chromosome X. Nos données indiquent une nouvelle fonction de DAXX et de CAF-1 dans le ciblage de H3.3 sur le chromosome X.

Introduction

Les organismes eucaryotes sont fréquemment pluricellulaires et différenciés, ainsi les cellules sont spécialisées et n'expriment qu'une partie de l'information dont elles disposent. Pour assurer le contrôle de l'information génétique, la cellule a développé des moyens d'organisation spatiale de l'ADN très sophistiqués. Il est généralement admis que cette organisation sous forme de chromatine détermine les compétences de l'ADN à toutes les fonctions biologiques. Le nucléosome constitue l'unité structurale de base de la chromatine. Il est composé d'un fragment d'ADN de 147 paires de bases enrouler autour d'un octamère des quatre histones (H2A, H2B, H3 et H4) (Luger et al, 1997). D'un point de vue fonctionnel, les nucléosomes peuvent réguler de nombreux processus cellulaires en contrôlant l'accessibilité de certaines protéines à leurs séquences cibles sur l'ADN (Beato et Eisefeld, 1997). Pour moduler cette fonction, la cellule utilise différentes stratégies incluant la méthylation de l'ADN, les modifications d'histones, le remodelage dépendant de l'ATP de la chromatine, les chaperons d'histones et enfin les variants d'histones (Henikoff et Ahmad, 2005 ; Narlika et al, 2002 ; Polo et Almouzni, 2006).

Le remplacement des histones canoniques par des variants est aujourd'hui considéré comme un des mécanismes fondamentaux de l'organisation et la régulation du génome. Les variants

sont des isoformes non allélique des histones conventionnels capables de les substituer au sein du nucléosome, ce qui génère un nouveau nucléosome aux propriétés structurales et à la stabilité modifiée. Ainsi, des analyses biophysiques ont montré par exemple qu'un nucléosome contenant les variants H3.3 et H2A.Z est significativement moins stable qu'un nucléosome contenant H2A ou H3 (Jin et Felsenfeld, 2007). Cette déstabilisation engendrée par les variants pourrait être à l'origine de changements locaux de la structure chromatinienne, ce qui pourrait avoir des répercussions transcriptionnelles. De plus, les variations de séquences introduites chez les variants pourraient favoriser le recrutement de partenaires protéiques spécifiques capables de modifier également la structure chromatinienne.

A ce jour, il a été mis en évidence des variants pour les histones H2A, H2B et H3. Au nombre de quatre, les variants de H3 présentent une diversité de fonction tout à fait remarquable : trois fortement similaires H3.1, H3.2 et H3.3, et un isoforme plus divergent CENPA (Sarma et Reinberg, 2005).

Le variant H3.3 est exprimée chez les métazoaires et diffère de la forme canonique par quatre acides aminés dont trois sont localisés au sein du domaine globulaire. Ces trois résidus semblent suffisants pour induire un mécanisme différent de déposition au cours du cycle cellulaire. En effet, H3.1 et H3.2 sont incorporés spécifiquement au cours de la phase S alors que H3.3 peut aussi être incorporé durant la phase G1 (Ahmad et Henikoff, 2002). H3.3 peut être déposé hors de la phase S par une voie d'assemblage indépendante de la réplication au sein de région génomique où l'activité induit une perte de nucléosome (Ahmad et Henikoff, 2002 ; Mito et al, 2005 et 2007 ; Schwartz et Ahmad, 2005). Les machineries de déposition impliquées dans l'assemblage de la chromatine indépendante ou dépendante de la réplication ne sont pas encore complètement caractérisées. La machinerie principalement identifiée chez l'homme pour la déposition dépendante de la réplication de H3.1 est le complexe CAF-1, alors que pour H3.3 le complexe impliqué dans la déposition indépendante de la réplication est composé soit de la protéine HirA (Tagami et al, 2004) ou de la protéine Daxx (Drané et al, 2010).

Résultats

Purification du complexe e-DAXX et identification de ses partenaires protéiques associés

Des travaux antérieurs du laboratoire ont montré que l'histone chaperonne DAXX forme un complexe stable avec le facteur de remodelage de la chromatine ATRX qui cible H3.3 vers des foyers hétérochromatiques tels que les péricentromes et les télomères. Cependant, il reste à déterminer comment DAXX exerce sa fonction au niveau de ces régions. Pour mieux comprendre la fonction de DAXX, nous avons sauvé les lignées de fibroblastes embryonnaires

(MEF) de souris knock-out (*Daxx*^{-/-}) avec DAXX marqué par une étiquette en C-terminal contenant les épitopes Flag et HA (e-DAXX) en utilisant la transduction rétrovirale (Drane et al., 2010). Comme attendu, les modèles d'expression e-DAXX montrent une coloration spécifique dans tout le nucléoplasme, formant de nombreux points correspondant à la coloration DAPI des foyers hétérochromatiques et des corps de la PML (PML-NBs) (Fig. 1A). Le complexe DAXX marqué par les épitopes Flag et HA a ensuite été purifié par double immunoaffinité à partir d'un extrait nucléaire soluble de MEF par immunoprécipitations séquentielles avec un anticorps anti-Flag suivi d'un anticorps anti-HA (Drane et al., 2010). Les protéines associées à DAXX ont été séparées sur des gels à gradient de polyacrylamide contenant 4 à 12 % de SDS, puis colorées à l'argent (Fig. 1B, panneau supérieur). Les analyses par immuno-blotting et spectrométrie de masse ont permis d'identifier ATRX et H3.3 comme des composants spécifiques du complexe DAXX (Figs. 1B panneau inférieur, 1C) comme nous l'avons montré précédemment (Drane et al., 2010). De manière surprenante, d'autres partenaires inattendus ont été trouvés dans ce complexe, tels que le complexe spécifique H3.1 CAF-1 (p150, p60 et p48), le marqueur spécifique de l'hétérochromatine HP1, l'ubiquitine ligase Trim28, l'ubiquitine hydrolase USP7 et le facteur de transcription potentiel ADNP (Figs. 1B, 1C). Pour éliminer la possibilité que nous ayons purifié un mélange de complexes de prédéposition H3.3 et H3.1, nous avons analysé davantage la teneur en H3.1 de la bande H3.3 par spectrométrie de masse. Les analyses par spectrométrie de masse ont révélé que le complexe nucléaire associé à DAXX contient principalement du H3.3 puisque le rapport H3.3/H3.1 a été trouvé égal à 20/1 (Fig 1C). Pour mieux comprendre la composition du complexe, nous avons soumis le complexe e-DAXX à une séparation sur gradient de glycérol (15% à 35%, Fig. 2A). Les expériences d'immunoblotting ont montré que les protéines associées à DAXX se fractionnaient en au moins deux complexes macromoléculaires. Dans le complexe de poids moléculaire inférieur (LMWC), DAXX est associé à ATRX et H3.3 (Fig. 2A, B). Dans le complexe de haut poids moléculaire (HMWC), DAXX et H3,3 sont associés au complexe d'assemblage de la chromatine CAF-1, à HP1 et à ADNP et à d'autres protéines (Fig. 2B). Ces expériences montrent que DAXX appartient à au moins deux complexes macromoléculaires différents spécifiques de H3.3. Dans notre étude précédente, réalisée sur des cellules HeLa, nous avons observé que deux des trois sous-unités CAF-1 (p150 et p60) étaient hautement spécifiques du complexe e-H3.1, alors que la troisième sous-unité (p46/48) était un composant commun aux complexes e-H3.1 et e-H3.3 (Drane et al., 2010). Dans la présente étude, réalisée sur des cellules MEF, nous avons trouvé de façon surprenante que CAF-1 fait partie du complexe DAXX-ATRX-H3.3.

DAXX interagit physiquement in vitro avec CAF-1

DAXX est une protéine acide (pI 4.6) contenant un domaine riche en résidus acides glutamiques et d'acide aspartiques incorporé dans un domaine homologue au chaperonne d'histone de levure Rtt106, deux domaines d'HAP et un domaine riche en S/P/T (Fig. 2C). Nous avons ensuite émis l'hypothèse que DAXX interagit physiquement avec la sous-unité CAF1-p150, et nous avons tenté d'évaluer ces interactions par des essais pull down. FLAG-DAXX et HA-CAF1-p150 ont été co-exprimés dans des cellules d'insectes SF9 et ont été immunoprécipité à l'aide de billes d'agarose anti-FLAG M2. Les protéines liées aux billes ont été largement lavées et éluées à l'aide du peptide FLAG. Les protéines éluées ont été analysées sur un gel SDS-PAGE et colorées au bleu colloïdal (Fig. 2D). Les données obtenues ont montré que DAXX interagit physiquement avec CAF1-p150. Pour déterminer plus précisément la région de DAXX impliquée dans l'interaction avec CAF1-p150, nous avons généré des mutants de délétion incluant le domaine N-terminal (1-302), central (302-495) ou C-terminal (495-740) de DAXX. Ces mutants ont été exprimés sous forme de protéines de fusion avec la GST et ont été étudiés pour leur liaison à CAF1-p150 ou ATRX (Fig. 2E). L'immunoblotting à l'aide d'un anticorps dirigé contre CAF1-p150 et ATRX a révélé que ni le domaine semblable à Rtt106 ni le domaine riche en S/P/T ne se liaient à CAF1-p150 ou à ATRX. En revanche, la région (1-302) englobant les deux domaines PAH (Paired Amphipathic Helix) (PAH1 & PAH2), a montré une forte interaction avec CAF1-p150 et ATRX à 0,15 ou 0,5 M de NaCl.

Nous avons ensuite déterminé la région de CAF1-p150 impliquée dans l'interaction avec DAXX. Nous avons généré des mutants de délétion de CAF1-p150 comprenant le domaine N-terminal (1-296), central (297-641) ou C-terminal (642-938) de CAF1-p150 (Fig. 2F). Ces mutants ont été exprimés dans les bactéries sous forme de protéines de fusion 6xHis et ont été testés pour leur interaction avec GST-DAXX (1-302) (Fig. 2G). L'immunoblotting à l'aide d'un anticorps anti-His dirigé contre les domaines His-CAF1-p150 a révélé que DAXX interagit exclusivement avec la région C-terminale de CAF1-p150 (642-938).

Ces données démontrent que la partie N-terminale de DAXX est le médiateur de l'interaction avec ATRX et la partie C-terminale de CAF1-p150. Le domaine de type Rtt106 étant impliqué dans une forte interaction avec l'histone H3.3 (Drane et al., 2010), nos résultats suggèrent que DAXX assure le lien entre ATRX ou CAF-1 et H3.3 et que les deux protéines pourraient probablement entrer en compétition pour le même site de liaison sur DAXX.

Le passage transitoire du DAXX à travers les PML-NBs est essentiel pour son association avec le CAF-1 mais pas avec ATRX.

La protéine DAXX est un des composants majeurs des PML-NBs et interagit directement avec ATRX (Drane et al., 2010). La somoylation de DAXX est essentielle pour son accumulation au niveau des PML-NBs. Le ciblage de DAXX sur les PML-NBs est un événement clé dans la régulation de la transcription hétérochromatique péricentrique (Lin, Lucia, Ishov).

Le mécanisme de répression de l'hétérochromatine par DAXX est inconnu et la chronologie de sa transition séquentielle des PML-NBs vers l'hétérochromatine reste à déterminer. Nous avons donc évalué les effets de la délétion du domaine Sumo-Interacting Motif (SIM) C-terminal de DAXX, correspondant aux sept derniers résidus d'acides aminés de la protéine (Fig. 3A). Par conséquent, la protéine DAXX- Δ S, dépourvue du domaine SIM, a été exprimée de façon stable dans les cellules MEF *Daxx*^{-/-}. Contrairement à e-DAXX de type sauvage, e-DAXX- Δ S n'a pas réussi à se co-localiser avec les PML-NBs (Fig. 3B), ce qui suggère que le domaine SIM C-terminal de DAXX est impliqué dans le ciblage de DAXX vers les PML-NBs. De même, nous avons étudié la localisation de CAF1-p150 par rapport aux PML-NBs. Nous avons observé que la majorité du signal correspondant à CAF1-p150 étaient co-localisés avec les PML-NBs (Fig. 3B).

Cette hypothèse a été confirmée par l'absence de cette forme modifiée de DAXX dans le complexe e-DAXX- Δ S (Fig. 3D). Il est intéressant de noter que nous n'avons pas pu détecter le complexe CAF1 parmi les protéines associées à e-DAXX- Δ S, ni par immunoblotting (Fig. 3D), ni par spectrométrie de masse (Fig. 3E).

Ce résultat est assez surprenant, compte tenu du fait que CAF1-p150 interagit directement avec la partie N-terminale de DAXX, mais suggère néanmoins un rôle possible du domaine SIM dans le recrutement de CAF-1 dans le complexe DAXX. Pour exclure une interaction possible de CAF1-p150 avec le domaine SIM C-terminal de DAXX, une immunopurification a été réalisée avec un extrait soluble de cellules SF9 qui co-expriment FLAG-DAXX- Δ S et HA-CAF1p150. En utilisant un anticorps anti-FLAG, les protéines liées aux billes ont été éluées et fractionnées sur SDS-PAGE et colorées au bleu colloïdal (Fig. 3F). Les données obtenues ont montré que la suppression du domaine SIM n'a pas pu affecter l'interaction entre DAXX et CAF1-p150.

A la lumière des données obtenues par l'analyse du complexe nucléaire e-DAXX- Δ S, nous avons étudié l'effet de la suppression du domaine C-terminal SIM de DAXX sur la transcription

MJS par RT-PCR (Fig. 3G). Il est intéressant de noter que l'expression DAXX-ΔS dans les cellules MEF Daxx^{-/-} n'a pas pu sauver le niveau d'ARNm des MJS, démontrant ainsi que le domaine SIM de DAXX est nécessaire à la transcription des MJS, probablement par le recrutement de la sous-unité CAF1-p150. Pris ensemble, ces résultats montrent clairement que le domaine SIM C-terminal de DAXX n'est pas nécessaire pour l'interaction avec CAF1-p150 mais est indispensable pour son recrutement par les PML-NBs, et donc pour la régulation de la transcription des MJS. Nous avons ensuite étudié le rôle du CAF-1 dans le contrôle de l'expression des MJS. Nous avons émis l'hypothèse que DAXX, ATRX et CAF1-p150 présentent une interdépendance dans le contrôle de l'expression des MJS. En utilisant la RT-PCR quantitative, nous avons observé que la déplétion de CAF1-p150 à l'aide de deux shARN spécifiques entraînait une réduction drastique de l'expression des transcrits des MJS (Fig. 3H). En revanche, lorsque l'on a épuisé ATRX avec deux shARN différents, la transcription des MJS a été considérablement augmentée (Fig. 3I), ce qui suggère que ATRX est impliqué dans la répression de la transcription des MJS. Les taux relatifs de CAF1-p150 et d'ARNm d'ATRX ont été évalués par RT-PCR pour s'assurer de l'efficacité de la déplétion (Fig. 3J, K). Ces résultats appuient l'idée que les deux protéines, à savoir CAF1-p150 et ATRX, agissent de façon antagoniste pour réguler la transcription des répétitions d'ADN au niveau des régions péri-centromériques.

Conséquences de la perte de DAXX sur la distribution génomique de CAF1-p150

La protéine CAF-1 de mammifère se co-localise avec l'hétérochromatine en cours de réplication où elle favorise l'assemblage de l'histone H3.1, et la sous-unité CAF1-p150 interagit avec la protéine HP1 de l'hétérochromatine. Il a donc été suggéré que CAF-1 est impliquée dans la formation et la régulation de l'hétérochromatine, mais il manque encore des preuves fonctionnelles directes pour étayer ce modèle. L'association de CAF1-p150 avec DAXX nous a incité à analyser sa distribution génomique en relation avec DAXX. Dans ce but, nous avons effectué une analyse ChIP-seq de la sous-unité CAF1-p150 en utilisant des anticorps disponibles dans le commerce dans des cellules WT et DAXX KO MEF. L'identification des pics CAF-1 dans le génome à l'aide d'une lecture cartographiée unique a permis d'identifier 41 146 pics spécifiques aux cellules WT, 63 931 pics spécifiques aux cellules DAXX KO et 23 207 pics CAF1-p150 qui sont communs entre les cellules WT et DAXX KO (Fig. 4A). Les cartes thermiques et les courbes de densité normalisées correspondantes ont révélé que l'inactivation de DAXX entraînait une déplétion et un déplacement d'environ deux fois plus

importants de plusieurs pics de CAF1-p150 vers des régions chromatinienne où il était déjà faiblement présent dans les cellules WT (Fig. 4B, C). Une analyse complète des données a montré un enrichissement de CAF1-p150 au niveau des éléments répétitifs de l'ADN du génome de la souris, sans différences majeures entre WT et KO (Fig. 4D). CAF1-p150 a été trouvé spécifiquement enrichi au niveau des LINES L1Md (Fig. 4E, F) et SINEs B1m (Fig. 4G, H). De façon surprenante, on a constaté que la CAF1-p150 était appauvrie en promoteurs (figure 4I) et en activateurs (figure 4J) dans les cellules WT et KO.

L'association du CAF-1 avec des répétitions d'ADN telles que les SINEs et les LINES nous a incité à effectuer une analyse plus approfondie des données ChIP-seq en tenant compte des lectures avec des occurrences multiples, qui représentent des éléments répétitifs non cartographiables (Dreszer et al., 2012). En effet, la majorité des lectures de CAF1-p150 ont eu des occurrences multiples et ont été mises en correspondance avec les différents éléments répétitifs du génome de la souris (Fig. 4D). CAF1-p150 a été trouvé enrichi au niveau des satellites mineurs (famille SYNREP_MM) et majeurs (famille GSAT_MM), aux répétitions télomériques (TTAGGGn/CCCTAA_n), aux L1Md LINES et aux SINEs spécifiques à la souris (Fig. 4K) avec quelques différences importantes entre les cellules WT et les cellules MEF DAXX knock-out (Fig. 4K). Cette dernière observation nous a incité à analyser la distribution de CAF1-p150 chromosome par chromosome. Cette analyse a révélé une forte déplétion de CAF1-p150 du chromosome X dans les cellules KO (Fig. 4L) avec un effet important sur les SINEs (Fig. 4M, N) et les LINES L1Md (Fig. 4O, P).

Nous concluons cela : (i) l'association de CAF-1 avec DAXX affecte le chromosome X de manière distincte par rapport aux autosomes et, (ii) DAXX est essentiel pour cibler CAF1 sur le chromosome X.

Conséquences de la perte de DAXX sur la distribution génomique de H3.3

Après avoir montré que DAXX cible CAF-1 sur le chromosome X, nous avons ensuite étudié l'effet de l'inactivation de DAXX sur la déposition de H3.3. Dans ce but, des lignées cellulaires WT et Knock-out DAXX ont été transduites avec un H3.3 marqué avec les épitopes Flag et HA pour générer des lignées cellulaires stables exprimant ectopiquement Flag-HA-H3.3. Comme témoin, nous avons utilisé une lignée cellulaire MEF exprimant le Flag-HA endogène marqué H3.3 (Ors et al., 2017). Les lignées cellulaires endogènes et ectopiques marquées H3.3 ont ensuite été soumises à une analyse ChIP-seq. L'assignation des pics en utilisant une lecture cartographiée unique a révélé que 81% des pics H3.3 endogènes cartographiés aux activateurs

et 19% aux promoteurs (Sup_Fig. 1A). Les cartes thermiques et les courbes de densité normalisées (Fig. 5A) ont révélé qu'en l'absence de DAXX, le H3.3 surexprimé s'accumule au niveau des promoteurs, tandis que la présence de DAXX empêche cette accumulation ectopique (Fig. 5A). L'absence de DAXX a eu un effet moins prononcé sur l'accumulation ectopique de H3.3 au niveau des activateurs, n'affectant que les activateurs situés à distance des TSS (Fig. 5B). Cependant, l'absence de DAXX a fortement altéré le recrutement de H3.3 au niveau des activateurs situés sur le chromosome X (Fig. 5C). Nous avons ensuite étudié la présence de H3.3 sur les éléments répétitifs. Contrairement à CAF1-p150, H3.3 n'a pas été trouvé sur les éléments répétitifs tels que les LINES et les SINEs (Sup_Fig. 1B, C). Nous avons conclu que le dépôt de H3.3 est étroitement contrôlé par DAXX, qui empêche son accumulation ectopique sur les promoteurs et cible spécifiquement H3.3 sur les éléments répétés du chromosome X.

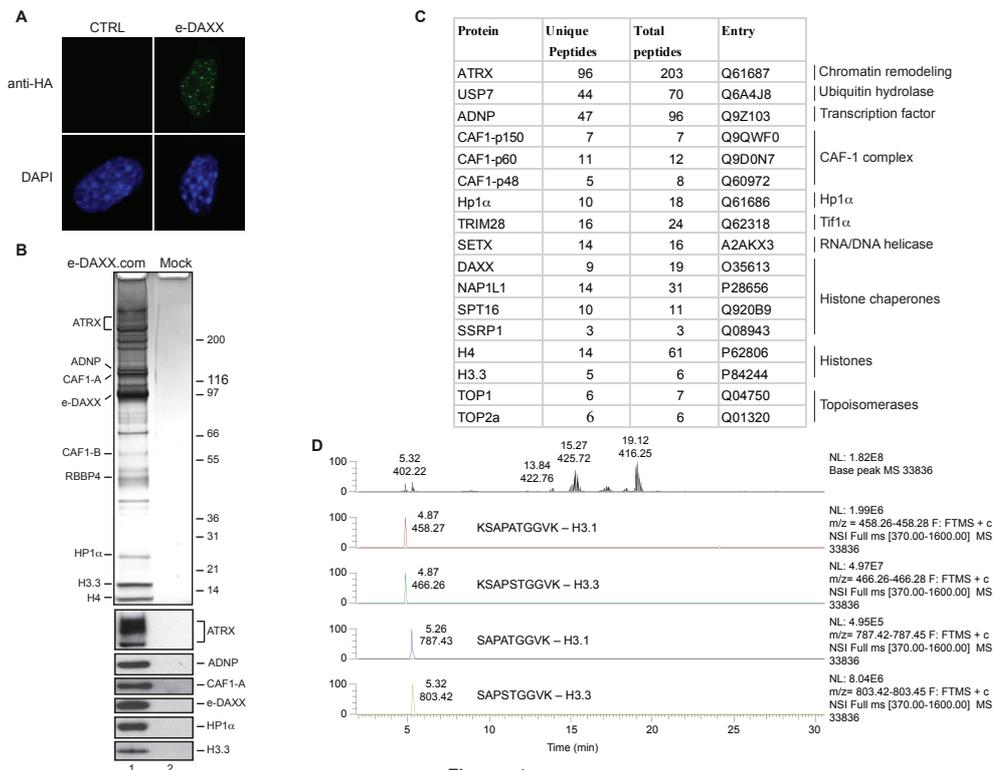


Figure 1

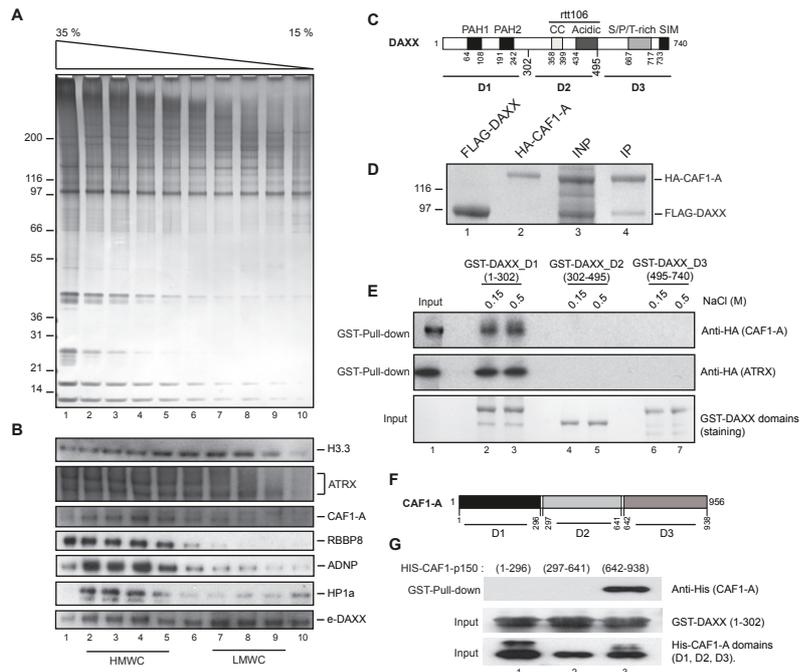


Figure 2

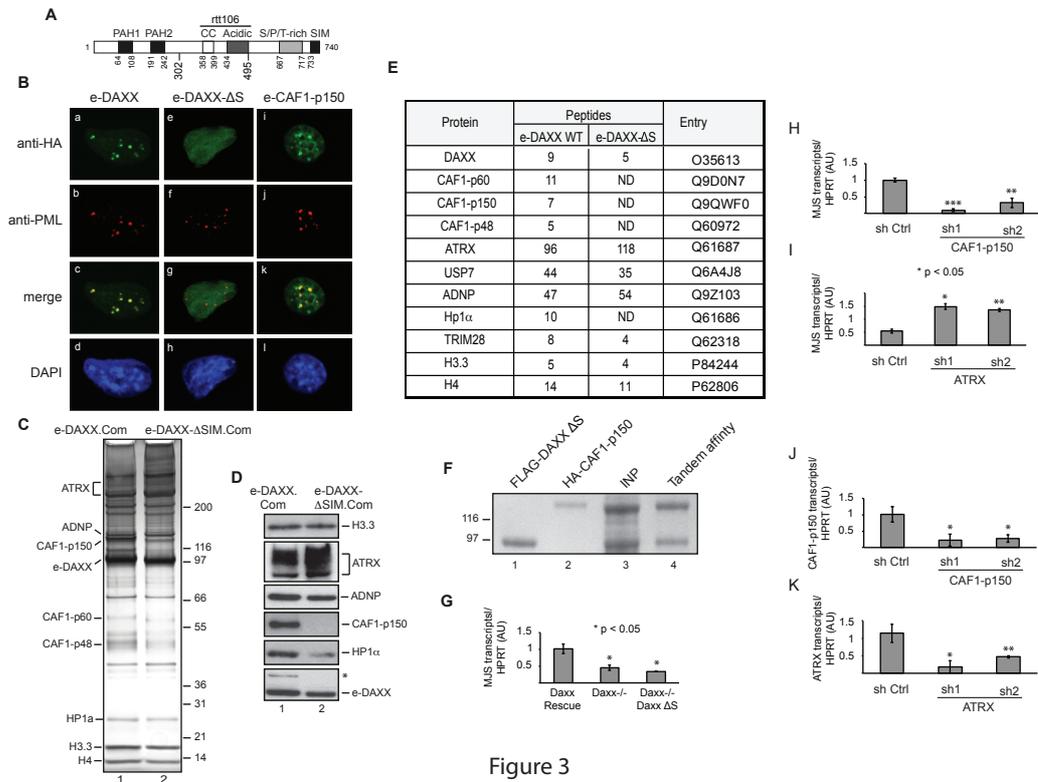


Figure 3

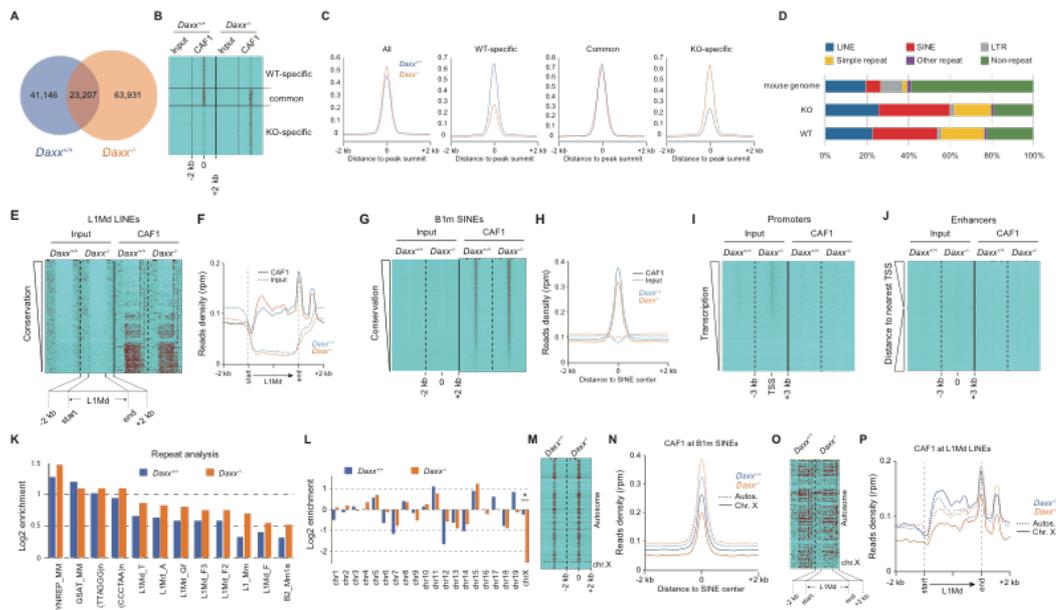


Figure 4

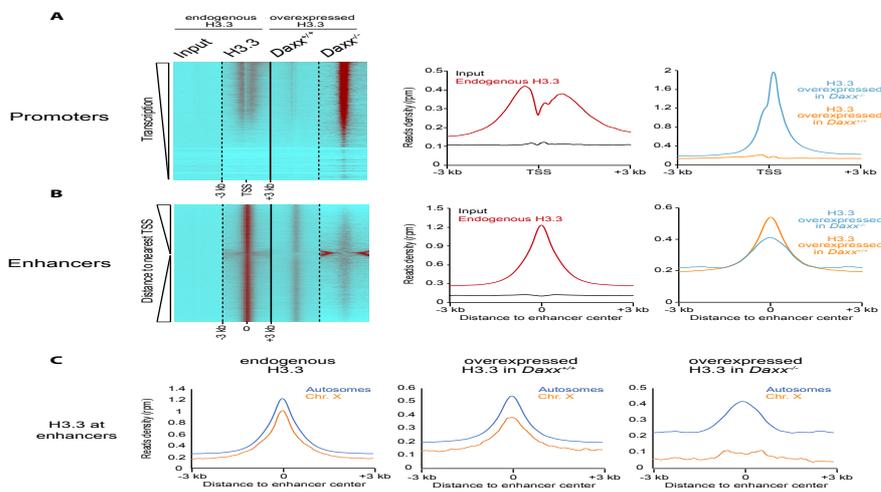


Figure 5

Figure 1. Immunopurification du complexe de déposition e-DAXX spécifique à H3.3 à partir de fractions nucléaires solubles dans le MEF.

(A) Expression stable de e-DAXX dans les cellules MEF Daxx^{-/-}. Les cellules exprimant e-DAXX et les cellules témoins (CTRL) ont été colorées avec l'anti-HA (en haut) et du DAPI (en bas). (B) Panneau supérieur : Coloration à l'argent des protéines associées à e-DAXX (piste 1). Le complexe contenant e-DAXX (e-DAXX.com) a été purifié par double immunoaffinité à partir d'un extrait nucléaire soluble de MEF. Les polypeptides identifiés par analyse de spectrométrie de masse et les positions des marqueurs moléculaires de tailles sont indiqués. (piste 2) Maquette ; panneau inférieur : CAF1-p150, ADNP et HP1 sont des composants de e-DAXX. Le complexe e-DAXX (piste 1) a été analysé par immunoblotting avec les anticorps indiqués. (piste 2). (C) Analyses par spectrométrie de masse du complexe e-DAXX. Tableau résumant les polypeptides détectés par l'analyse par spectrométrie de masse du complexe e-DAXX, présentés sur la figure 1B et purifiés par double immunoaffinité. (D) Analyse par spectrométrie de masse des sous-types de variantes H3 associés à e-DAXX. Analyse comparative du rapport histone H3.3/H3.1 dans le complexe e-DAXX. Les abondances des peptides pour les différents variants H3 sont indiquées. Il est à noter que H3.3 est au moins 20 fois plus abondant que H3.1.

Figure 2. DAXX et CAF1-p150 se trouvent dans le même complexe nucléaire in vivo.

(A) Coloration à l'argent du complexe nucléaire e-DAXX fractionné sur un gradient de glycérol. Le complexe e-DAXX purifié par double affinité a été séparé sur un gradient de glycérol. Les fractions ont été collectées comme indiqué au sommet du gel : la fraction 1 correspond à la fraction de poids moléculaire le plus élevé et la fraction 10 à la plus faible. (B) Immunoblotting des fractions de glycérol contenant des sous-complexes nucléaires de e-DAXX avec les anticorps indiqués. (C) Structure primaire de DAXX. DAXX contient plusieurs domaines putatifs : deux hélices amphipathiques appariées (PAH1 et PAH2), un domaine Coiled Coil (CC), un domaine acide (acide), un domaine riche en Ser/Pro/Thr (riche en S/P/T) et un domaine semblable à Rtt106 (Rtt106). (D) DAXX interagit avec CAF1-P150 in vitro. Une purification d'affinité en tandem a été effectuée avec des cellules SF9, qui co-expriment FLAG-DAXX et HA-CAF1-P150. Les protéines liées aux billes ont été lavées avec un tampon TGEN 150 mM. Les protéines éluées ont été fractionnées sur SDS-PAGE et colorées au bleu colloïdal. FLAG-DAXX et HA-CAF1-P150 ont été immunoprécipitées seules comme contrôle (pistes 1, 2). La colonne INP (piste 3) représente la quantité de protéines utilisée pour le pull-down. L'immunoprécipitation en tandem (IP) est indiquée dans la colonne 4. (E) La partie N-terminale de DAXX contient un domaine interagissant avec CAF1-p150 et ATRX. Les régions N-terminale (1-302), centrale (302-495) et C-terminale (495-740) de DAXX ont été produites sous forme de protéines de fusion GST. Les protéines de fusion (pistes 2-7), immobilisées sur du glutathion-agarose, ont été incubées avec du CAF1-p150 marqué par un épitope HA ou du ATRX marqué par un épitope HA. Après lavage avec du NaCl 0,15 ou 0,5 M, le CAF1-p150

ou le ATRX lié à la résine ont été sondés avec un anticorps anti-HA. Pour comparer les niveaux des fusions GST utilisées pour le pull-down, le blot a d'abord été coloré avec du bleu colloïdal. La ligne (INP) représente 20 % de la quantité de CAF1-p150 ou d'ATRX utilisée pour le pull-down. (F) Structure primaire de CAF1-p150. (G) La partie C-terminale de CAF1-p150 contient un domaine d'interaction DAXX. La région N-terminale de DAXX (1-302) produite comme protéine de fusion GST a été immobilisée sur du glutathion-agarose et incubée avec les régions N-terminale (1-296), centrale (297-641) et C-terminale (642-938) de 6XHIS CAF1-p150. Après lavage avec du NaCl 0,5 M, les domaines CAF1-p150 liés à la résine ont été sondés avec l'anticorps anti-His.

Figure 3. Le motif d'interaction sumo DAXX est essentiel pour cibler DAXX sur les PML-NB et pour le recrutement de CAF1-p150.

(A) La structure primaire de DAXX contient un domaine sumo-interactif-motif (SIM) situé dans la partie C-terminale. (B) Le SIM cible DAXX vers les PML-NBs. Distribution des cellules HA-DAXX (a,e), HA-CAF1-p150 (i) ou de PML endogène (b,f,i) dans les cellules MEF e-DAXX, e-DAXX- Δ S ou e-CAF1-p150 analysées par coloration par immunofluorescence à l'aide d'anticorps anti-HA ou anti-PML, respectivement. (d,h,l) L'ADN a été coloré par DAPI. (c,g,k) Les images fusionnées correspondent à la superposition de la fluorescence rouge (PML) et verte (DAXX) ou CAF1-p150. (C, D) CAF1-p150 est absent de l'e-DAXX- Δ S. (C) Coloration à l'argent des protéines associées à e-DAXX (piste 1) et e-DAXX- Δ S (piste 2). Les complexes contenant e-DAXX (e-DAXX.com) et e-DAXX- Δ S (e-DAXX- Δ S.com) ont été purifiés par double immunoaffinité à partir d'extraits nucléaires (NC) solubles de cellules MEF. Les polypeptides identifiés par analyse de spectrométrie de masse et les positions des marqueurs de taille moléculaire sont indiqués. (D) Les complexes e-DAXX (piste 1) et e-DAXX- Δ S (piste 2) ont été analysés par immunoblotting avec les anticorps indiqués. Le DAXX sumoylé (*) est spécifique du e-DAXX. (E) Analyses par spectrométrie de masse des complexes e-DAXX WT et e-DAXX- Δ S.com. Tableau résumant les polypeptides détectés par l'analyse par spectrométrie de masse des complexes e-DAXX WT et e-DAXX- Δ S.com, présentés dans la figure 3C et purifiés par double immunoaffinité. (F) DAXX Δ S interagissent avec CAF1-p150 in vitro. La purification par double affinité a été réalisée à partir de cellules SF9 co-exprimant FLAG-DAXX Δ S et HA-CAF1-p150. Les protéines liées aux billes ont été lavées avec du tampon TGEN 150 mM. Les protéines éluées ont été fractionnées sur SDS-PAGE et colorées au bleu colloïdal. FLAG-DAXX Δ S et HA-CAF1-p150 ont été immunoprécipités seuls comme contrôle (voie 1, 2). La ligne 3 (INP) représente la quantité de protéines utilisée pour le pull-down. La purification d'affinité (IP) en tandem est indiquée sur la ligne 4. (G) Le niveau relatif d'ARNm pour les répétitions d'ADN péri-centromériques dans les DAXX rescue, DAXX-/- et DAXX- Δ S MEF a été déterminé par RT-PCR quantitative. Les résultats sont représentés sous forme de niveau d'expression relatif des répétitions d'ADN péri-centromérique par rapport à l'HPRT. Moyenne \pm écart-type de trois expériences indépendantes. * : $p < 0,05$ comparer avec le type sauvage. Le niveau de transcriptions des répétitions d'ADN péri-centromériques est réduit dans les cellules MEF DAXX-/- et DAXX- Δ S. (H) La déplétion de p150CAF-1 a entraîné une diminution de la transcription des répétitions d'ADN péri-centromériques. Les MEF ont été transfectés avec l'ARN messager témoin (sh Ctrl)

ou deux ARN messagers différents de CAF1-p150 (sh-1 et sh-2). Le niveau relatif d'ARNm pour les répétitions d'ADN péricentromériques a été déterminé par RT-PCR quantitative.

Figure 4 : Impact de l'inactivation de DAXX sur la distribution génomique de CAF1 dans les fibroblastes embryonnaires de souris.

(A) Diagrammes de Venn montrant le chevauchement entre les pics de CAF1 dans le contrôle (Daxx+/+) et les MEF déficients en Daxx (Daxx-/-). (B, C). Cartes thermiques (B) et quantification (C) des densités des tags des pics de CAF1 spécifiques au WT, communs et spécifiques au KO. (D) Pourcentages de pics chevauchant des éléments répétitifs en utilisant la base de données UCSC RepeatMasker. Nous avons observé une accumulation de CAF1 à tous les éléments répétitifs à l'exception des rétrotransposons LTR. (E, F). Cartes thermiques (E) et quantification (F) de CAF1 au niveau des LINES L1Md pleine longueur (longueur > 5 kb, n = 12 916), classées selon le score de conservation. Les densités des tags ont été recueillies dans des fenêtres coulissantes de 50 pb couvrant 2 kb (divisées en 20 bins) de la L1Md normalisé en longueur (divisée en 40 bins). (G, H). Cartes thermiques (G) et quantification (H) de CAF1 au niveau des SINEs B1m (+/- 2 kb) classées par score de conservation. (I, J). Cartes thermiques de CAF1 au niveau des promoteurs (I) classés selon leur niveau d'expression, et au niveau des enhancers (J) classés selon leur distance par rapport aux TSS les plus proches. (K) Enrichissement relatif de CAF1 au niveau des familles répétées d'ADN en présence (Daxx+/+) ou en l'absence de DAXX (Daxx-/-). Les calculs ont été effectués en incluant les lectures de plusieurs résultats " non cartographiables " en les attribuant à leur famille répétée unique (voir Méthodes). (L) Histogramme (construit à partir des données ChIP sur l'ensemble du génome) montrant l'enrichissement relatif de CAF1 (\log_2 IP/Input) au niveau de chaque chromosome témoin et des MEF déficients en Daxx. On constate que la CAF1 est significativement appauvrie au niveau du chromosome X en l'absence de DAXX. (M-P). Cartes thermiques (M, O) et quantification (N, P) de CAF1 dans des cellules DAXX WT et KO, à des SINE B1m (M, N) ou LINE L1Md (O, P), triées selon leur distribution chromosomique.

Figure 5 : Conséquences de la perte de DAXX sur la distribution génomique de H3.3. Les mononucléosomes natifs étiquetés FLAG-HA ont été isolés par double purification par immuno-affinité à partir de cellules MEF exprimant un H3.3 endogène étiqueté FLAG-HA, et à partir de cellules MEF témoins (Daxx+/+) ou de Daxx knockout (Daxx-/-) surexprimant un H3.3 marqué par un épitope Flag-HA. (A, B) Cartes thermiques (panneaux de gauche) et quantification (panneaux de droite) des signaux H3.3 moyens au niveau des promoteurs (A) classés selon leur niveau d'expression, et au niveau des enhancers (B) classés selon leur distance par rapport au TSS le plus proche. Alors que les amplificateurs cibles H3.3 surexprimés dans les cellules WT et KO, une accumulation de H3.3 marqué par un épitope a été trouvée spécifiquement au niveau des promoteurs en l'absence de DAXX. (C) Signaux H3.3 moyens au niveau des enhancers triés selon leur distribution chromosomique. L'absence de DAXX a entraîné une déplétion de H3,3 au niveau des enhancers du chromosome X.

Table of Contents

ACKNOWLEDGMENTS	4
I. INTRODUCTION.....	17
INTRODUCTION	18
1. BACKGROUND	18
1.1 ORGANISATION OF CHROMATIN	20
<i>TRANSCRIPTIONAL ACTIVE FORM</i>	21
1.1.1. HETEROCHROMATIN.....	22
1.1.2. EUCHROMATIN	24
1.2 THE NUCLEOSOME	25
1.2.1. STRUCTURE OF THE NUCLEOSOME	26
1.2.2. NUCLEOSOME ASSEMBLY	28
1.3 HISTONES	29
1.3.1. CORE HISTONES	30
1.3.2. LINKER HISTONES	31
2. DNA METHYLATION AND CHROMATIN REMODELLING FACTORS	32
2.1 DNA METHYLATION	32
2.2 CHROMATIN REMODELING COMPLEXES	35
2.2.1 THE SWI/SNF FAMILY	36
2.2.2. THE ISWI FAMILY	38
2.2.3. THE CHD OR MI2 FAMILY.....	40
2.2.4. THE INO FAMILY	41
3. HISTONE MODIFICATIONS.....	44
3.1 ROLE OF THESE MODIFICATIONS	44
3.1.1. ACETYLATION OF HISTONES	47
3.1.2. HISTONE METHYLATION	48
3.1.3. PHOSPHORYLATION OF HISTONES.....	50

3.1.4. UBIQUITINYLATION OF HISTONES	51
3.1.5. SUMOYLATION OF HISTONES	52
4. HISTONE VARIANTS.....	52
4.1. VARIANTS OF THE H1 HISTONE.....	54
4.2. VARIANTS OF THE HISTONE H2A	55
4.2.1. H2A.Z	55
4.2.2. H2A.X	56
4.2.3. MACROH2A	57
4.2.4. H2A.BBD	58
4.3. VARIANTS OF THE HISTONE H2B	59
4.4. VARIANTS OF THE HISTONE H3.....	60
4.4.1. THE CANONICAL HISTONES H3.1 AND H3.2.....	61
4.4.2. H3T	61
4.4.3. H3.3	62
4.4.5. OTHER VARIANTS OF HISTONE H3 (H3.X, H3.Y AND H3.5).....	65
5. DEPOSITION OF CORE HISTONES	65
5.1. DEPOSITION OF HISTONES H3-H4.....	66
5.1.1. FROM THE CYTOPLASM TO THE NUCLEUS	66
5.1.2. DEPOSITION OF HISTONES ON CHROMATIN	68
5.1.3. <i>REPLICATION-DEPENDENT DEPOSITION</i>	68
5.1.4. <i>REPLICATION INDEPENDENT DEPOSITION OF H3.3</i>	69
5.1.5. <i>Deposition of H3.3 by HIRA</i>	69
5.1.6. <i>DEPOSITION OF H3.3 BY DAXX</i>	71
5.1.7. <i>DEPOSITION OF H2A-H2B HISTONES</i>	74
6. <i>Histone H3.3 biological functions</i>	74
II. RESULTS	82
III. DISCUSSION	113

IV. REFERENCES.....121

I. INTRODUCTION

INTRODUCTION

1. Background

In each cell, DNA strands must be compacted, in an organized manner, into a structure that respects the size constraints of the nucleus. With a diameter of about 10 μm , the nucleus implies the need for DNA compaction of an order of between 10^4 and 10^5 , but the negatively charged phosphate skeleton of the DNA induces electrostatic repulsion between adjacent regions, making it difficult to fold the double helix on itself (Maeshima et al., 2010). Therefore, this compaction is achieved by the binding of DNA with histone and non-histone proteins to form a very compact superstructure called chromatin. The packaging of the genome within the complex chromatin organization plays a key role in the control of its activity. The basic unit of chromatin, the nucleosome, is composed of a histone octamer (two each of H2A, H2B, H3 and H4) around which two superhelical turns of DNA are wrapped (*Luger et al., 1997*). All the conventional histones, except H4, have histone variants. Histone variants are non-allelic forms of the conventional histones (van Holde, 1988). Incorporation of histone variants into the nucleosome is involved in the regulation of several epigenetics phenomena (reviewed in Ahmad and Henikoff, 2002; Malik and Henikoff 2003). For instance, CENP-A, the centromeric specific H3 variant, was demonstrated to be selectively retained in discrete foci in mammalian sperm nuclei (Palmer et al., 1990).

Nucleosomes, separated by DNA, forms a structure known as a 10nm chromatin filament or "pearl necklace", and is the first level of chromatin organization (Figure 1). The 10nm filament is then compacted into a 30nm fiber through interaction with the linker histones, thus constituting the second level of chromatin organization (Li and

Reinberg, 2011). The complex folding of the 30 nm fiber allows access to higher levels of organization (Woodcock and Ghosh, 2010). Nucleosomes are the layer of epigenetic information (Brahma, 2019 ;Kale, 2019 ;Klemm, 2019).

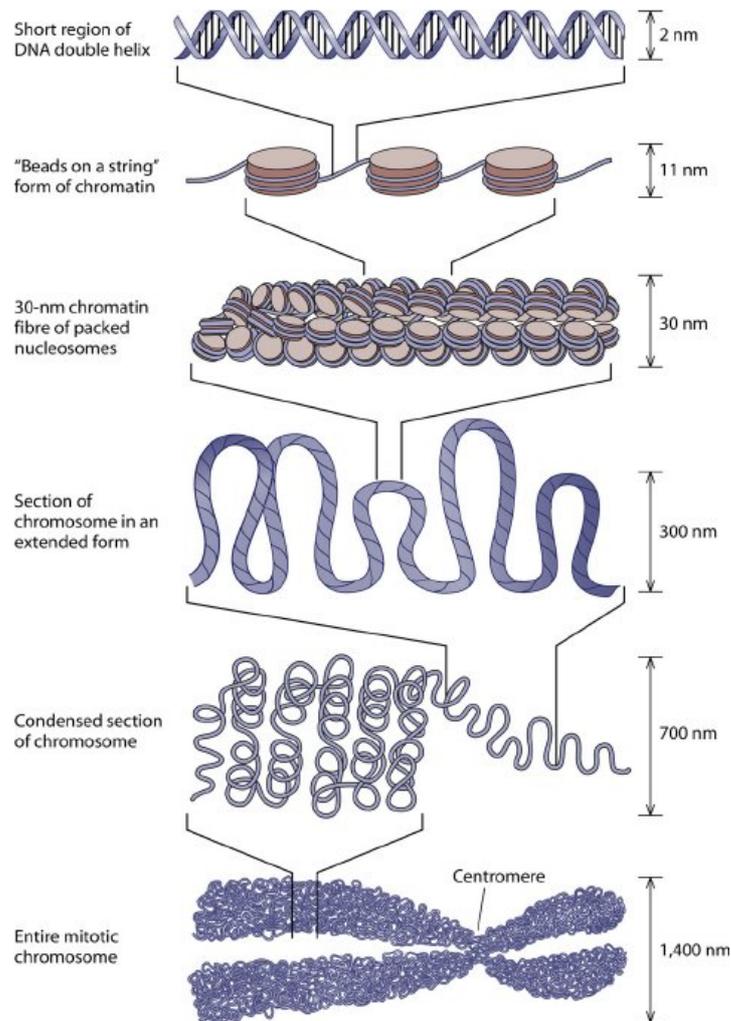


Figure 1: Chromatin structure (Adapted from Felsenfeld and Groudine, Nature 2003). DNA is wrapped around a histone octamer to form nucleosomes. Nucleosomes are connected by stretches of linker DNA. This basic nucleosome structure is folded into a fiber-like structure of about 30 nm in diameter. These 30-nm fibers are further compacted into higher-order chromatin structures.

The term "epigenetics" defines changes in gene expression that do not depend on changes in the nucleotide sequence itself and are transmitted by mitosis and meiosis (Jablonka and Lamb, 2002). Within a multicellular organism represented by a single

genome, there are a multitude of phenotypes that can be a function of the individual, the stage of development or the cell type for example. The transmission and maintenance of this specific gene expression profile is mainly based on epigenetic mechanisms (Probst et al., 2009). Epigenetic processes modulate gene activity through local modifications of DNA, histones and chromatin structure. In addition, these modifications make it possible to maintain the specific function of certain regions such as the centromeric, pericentromeric and telomeric regions. The major mechanisms involved are DNA methylation, post-translational histone modifications, non-coding RNA, chromatin remodelling and incorporation of histone variants. Profound alterations in this epigenetic information are responsible for the onset of various diseases, including, cancer, neurodegenerative diseases, inflammatory pathologies (Fardi, 2018 ;Rosa-Garrido, 2018).

1.1 Organisation of chromatin

Eukaryotic chromatin is a highly dynamic structure, allowing both a high level of compaction and access to the DNA matrix during various essential cellular processes such as DNA replication, DNA repair, transcription and recombination. The plasticity of the chromatin structure is regulated by many effectors such as histone chaperones, ATP-dependent chromatin remodelling factors, histone variants, post-translational histone modifications and many other factors (Campos and Reinberg, 2009; Cavalli, 2019). In higher eukaryotes, chromatin is divided into several domains that differ in structure and function (Cremer et al., 2006). These domains correspond to heterochromatin and euchromatin, which determine the density of expressed genes, respectively (Figure 2)

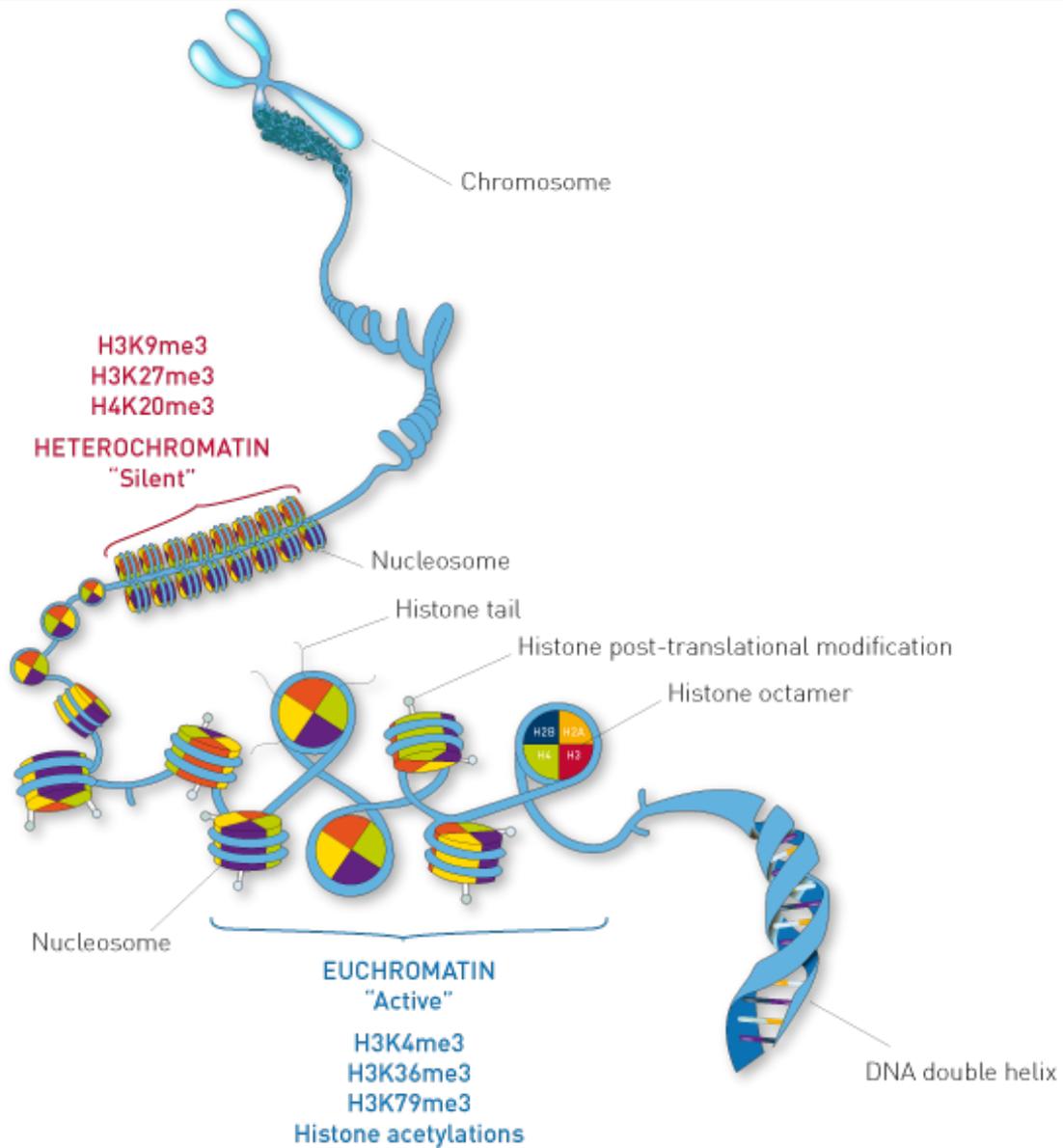


Figure 2. chromatin composition and levels of organization (from PngFans, free source). The diagram shows the fundamental unit of chromatin, the nucleosome, where the DNA wrapped around an octamer of Histone proteins (H3, H4, H2A, and H2B). and both states of chromatin heterochromatin, the inactive form and euchromatin, the transcriptional active form.

1.1.1. Heterochromatin

Heterochromatin is associated with an inactive transcription and are replicated at the end of phase S. At the microscopic level, heterochromatin regions are characterized by Giemsa chromosome labelling. At the molecular level, these regions are generally associated with post-translational modifications of histones specific for inactive transcription, including trimethylation of the lysine residue 9 of histone H3 (H3K9me) and lysine 27 of histone H3 (H3K27me3), which allows recruitment of the specific heterochromatin factor HP1 (HuisGrewal and Jia, 2007; Nishibuchi, 2014) (Figure 3). Nevertheless, the relationship between heterochromatin and completely inactive transcription has been qualified in recent years.

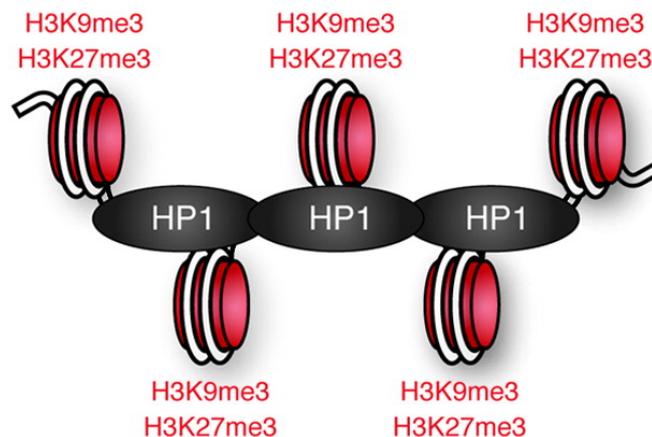


Figure 3. Silent, repressive heterochromatin (Adapted from Tollervey and Lunyak, 2012). Silent heterochromatin is characterized by methylation of histone H3 lysine 9 (H3K9Me3) and lysine 27 (H3K27Me3).

In eukaryotes, the centromeric regions allow chromosome separation during mitosis and meiosis. The establishment and maintenance of these regions is therefore essential for genome stability. Despite this vital function, centromeric and pericentromeric DNA have a low conservation rate, in terms of sequence and length, during evolution. From yeast to humans, these regions extend over a few kilobases to

several megabases. Centromeric and pericentromeric heterochromatin are characterized by the presence of tandem repeated sequences called respectively α and I, II and III satellites in humans; and minor and major satellites in mice (Eymery et al., 2009).

Traditionally, the centromeric and pericentromeric regions are considered as a heterochromatin region and therefore not transcribed, although transcripts from these regions were detected forty years ago (Harel et al., 1968; Cohen et al., 1973). In *S. pombe*, it has been shown that pericentromeric transcripts are involved in the establishment and maintenance of pericentromeric heterochromatin via the RNA interference pathway (Volpe et al., 2002; Zofall and Grewal, 2006; Grewal and Jia, 2007). In mice, it has been shown that centromeric and pericentromeric repeated sequences are transcribed under stress, differentiation or cell confluence (Terranova et al., 2005; Bouzinba-Segard et al., 2002). Finally, it has also been shown that the centromeric and pericentromeric regions are respectively enriched with CENP-A and H3.3 histone variants (Smith, 2002; Drané et al., 2010).

Unlike bacterial chromosomes, eukaryotic chromosomes are linear, meaning that they have ends. These ends pose a problem for DNA replication. The DNA at the very end of the chromosome cannot be fully copied in each round of replication, resulting in a slow, gradual shortening of eukaryotic chromosomes. In addition, it is also necessary to ensure the protection of these DNA extremities against inadequate and harmful repair mechanisms that can lead to genome instability (van Steensel et al., 1998; McEachern et al., 2000; de Lange, 2005). In humans, in order to maintain genome stability, the ends of the chromosomes are therefore made up of repeated sequences called "TTAGGG" or telomeric sequences. There are two situations: (i)

part of the telomeric region is not replicated with each cell cycle and therefore lost or (ii) the length of this region is preserved under the action of telomerase.

Telomerase is a ribonucleoparticle, in particular composed of a reverse transcriptase (TElomerase Reverse Transcriptase, TERT) and an RNA matrix (TElomerase RNA Component, TERC), which allows the addition of telomeric repeat sequences, and thus the preservation of the length of this region. In somatic cells, telomerase activity is variable, particularly depending on the stage of development and cell type. It has been shown that the regulation of telomerase activity is involved in cell aging, senescence mechanisms and loss of control of the cell cycle, characteristic of cancer cells (Blasco, 2007; Tümpel and Rudolph, 2012).

Telomeric regions have the classic epigenetic markings of heterochromatin, such as trimethylation of lysine residues 9 and 20 of histones H3 and H4 respectively (García-Cao et al., 2004; Gonzalo et al., 2005; 2006), as well as a low level of acetylation of these histones (Benetti et al., 2007; Galvani, 2015). Nevertheless, there is a transcriptional activity emanating from telomeric heterochromatin, allowing the synthesis of Tel RNA. It has been shown, *in vitro*, that an accumulation of this telomere transcript directly and specifically inhibits telomerase activity suggesting the formation of an RNA duplex with the TERC matrix of the telomerase complex (Schoeftner and Blasco, 2008). In addition, it has also been shown that the telomeric region is a "centre" for the repression of genes located in its vicinity (Baur et al., 2001; Koering et al., 2002).

1.1.2. Euchromatin

The term euchromatin is used to define a decondensed and accessible state of chromatin. These regions are traditionally associated with high gene density, active transcription and are replicated at the beginning of S phase. At the microscopic level,

the euchromatin regions are characterized by weak Giemsa chromosome labelling. At the molecular level, these regions are generally associated with post-translational modifications of histones specific to active transcription, including a high level of acetylation and methylation of histone H3 (H3K9ac, H3K14ac, H3K4me3 and H3K79me3) (Figure 4), and a low level of post-translational modifications of histones specific to inactive transcription.

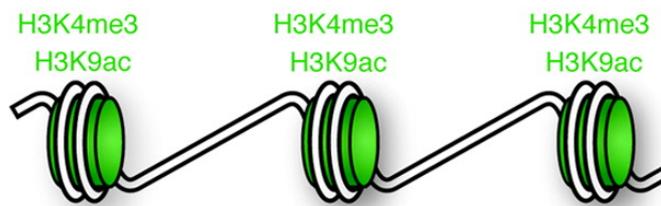


Figure 4. Open, active euchromatin (Adapted from Tollervey and Lunyak, 2012). Euchromatin is characterized by histone H3 lysine 4 methylation (H3K4Me3) and lysine 9 acetylation (H3K9Ac).

1.2 The nucleosome

The nucleosome is the basic fundamental unit of chromatin. The discovery of the nucleosome emerged from the observation of a "pearl necklace" nuclear structure on DNA by electron microscopy (Olins and Olins, 1974; Oudet et al., 1975) (Figure 1), as well as biochemical and structural data suggesting the existence of a repeated unity on DNA (Kornberg, 1974). All of these data were subsequently confirmed by the atomic resolution of the crystallographic structure of the nucleosome (Luger et al., 1997; Davey et al., 2002; Ohno, 2018).

The nucleosome consists of a core histone octamer wrapped around 147 base pairs DNA in about 1.7 turns. The histone octamer is composed of the four core histones (H2A, H2B, H3 and H4). The nucleosome core is highly conserved between species, however, the length of the linker DNA separating two nucleosomes varies between species and cell type. Thus, the total length of the nucleosome DNA can vary

from 160 to 241 base pairs depending on the species. Nucleosomes interact with each other to form the 30 nm fiber and higher levels of organization that are less well characterized.

1.2.1. Structure of the nucleosome

The first data on nucleosome structure were published in 1977 from a low-resolution image obtained by combining X-ray diffraction and electron microscopy techniques (Finch et al., 1977). Since then, structural data has continued to improve. In 1984, the nucleosome structure was first determined at 7Å resolution (Richmond et al., 1984), and in 1991, the histone octamer structure, in the absence of DNA and with a high salt concentration, was determined at 3.1Å (Arents et al., 1991). These data revealed for the first time the tripartite assembly of the octamer composed of two heterodimers H2A-H2B and a tetramer H3-H4. Within this tetramer, H3 and H4 interact through their "histone fold" (HFD) domain, which forms a "handshake" to form two dimers, just as in the H2A-H2B dimers (Widom, 1998).

All of these data were confirmed and detailed by determining the crystallographic structure of the nucleosome at 2.8 Å in 1997 (Luger et al., 1997, Figure 5).

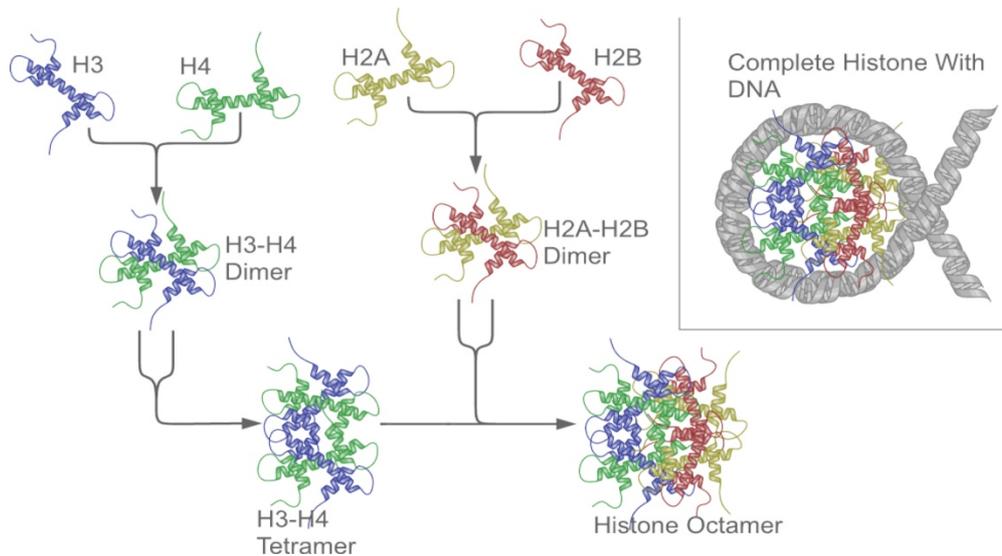


Figure 5. Schematic representation of the assembly of the core histones into the nucleosome (Richard Wheeler (Zephyris – Wikipedia, 2019). Histone H2A (yellow) dimerize with histone H2B (red) to form the H2A/H2B dimer. Histone H3 (purple) dimerize with histone H4 (green) to form the (H3-H4)₂ tetramer which then assemble into an octamer by incorporating the two H2A-H2B dimers. The histone octamer can wrap 147 bp of DNA to form the nucleosome.

This work has highlighted the importance of HFD within the nucleosomal structure, particularly in histone-histone and histone-DNA interactions. HFDs allow the organization of 121 base pairs of DNA. Each histone dimer in the nucleosome core provides 3 binding sites for the DNA molecule. These 12 binding sites are responsible for wrapping of 121 base pairs of the nucleosomal DNA. In addition, the N-terminal end of the HFD of each H3 histone interacts with 13 base pairs at each end of the nucleosomal DNA. These data therefore show that the nucleosome consists of 146 base pairs of DNA wrapped around the histone octamer in 1.65 turns. The crystallographic structure also revealed that the N-terminal end of histones H3 and H2B are located in the alignment of the minor groove of the DNA double helix. It has also been shown that residues 16 to 25 of histone H4 interact with the adjacent nucleosome at a negatively charged domain of the H2A-H2B dimer.

Within the nucleosome, each H2A-H2B dimer interacts at two contact points with the H3-H4 tetramer. On the one hand, a pattern similar to that formed by the two histones H3 within the tetramer H3-H4, called "bundle of four helices" or "four-helix bundle", allows the interaction between the histones H2B and H4. On the other hand, the H2A anchor domain also interacts with the H3-H4 tetramer (Figure 5).

Subsequent work has identified and refined the crystallographic structure of the nucleosome to a resolution of 1.9 Å (Davey et al., 2002). In 2005, the structure of a tetranucleosome was determined at a resolution of 9 Å, hence opening the door to understand inter-nucleosomal interactions (Schalch et al., 2005). Finally, the structure of an hexanucleosome was also determined at a resolution of 9.7 Å, thus providing a better understanding of the inter-nucleosomal interactions that govern the formation of compact chromatin structure, particularly the 30nm fibre (Garcia-Saez et al., 2018).

1.2.2. Nucleosome assembly

In vivo, nucleosome assembly is a sequential process initiated by the deposition of two H3-H4 dimers or an H3-H4 tetramer on DNA followed by the addition of two H2A-H2B dimers (Hamiche and Shuaib, 2012). Wrapped in 146 base pairs of DNA, the structure formed is called a nucleosome (Figure 6). This assembly is an orderly process that is assisted by histone chaperone proteins and chromatin assembly factors.

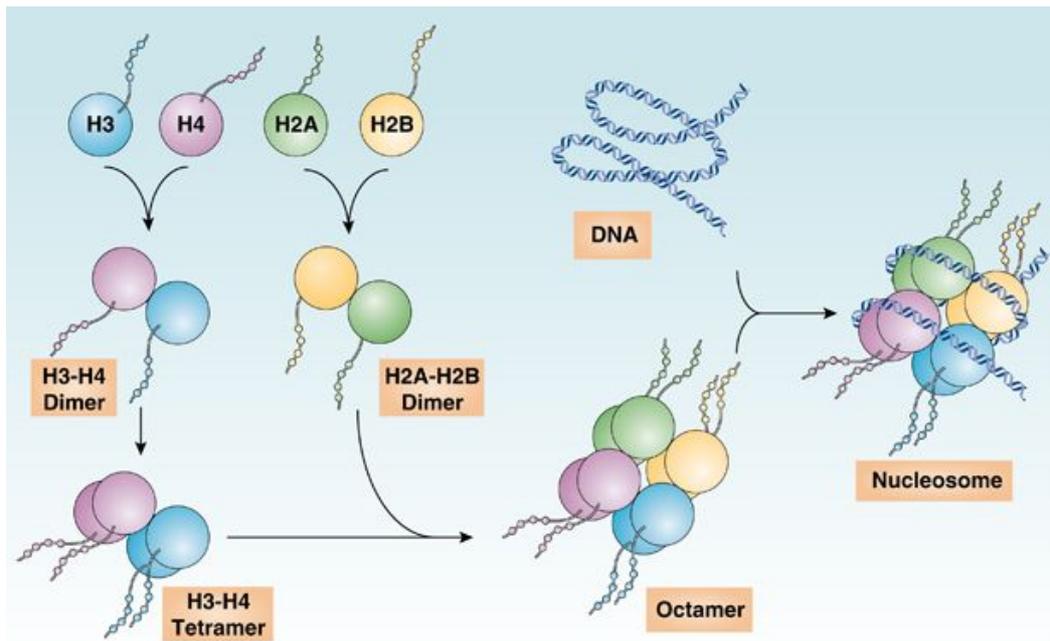


Figure 6. Schematic illustration of histones assembly into Nucleosome (R. Chen, Kang, Fan, & Tang, 2014). Two molecules of each of the four core histone proteins (H2A, H2B, H3 and H4) form the histone octamer via formation of one tetramer of H3 and H4 and two dimers of H2A and H2B. The histone octamer is wrapped by 146bp of DNA to complete nucleosome formation.

1.3 Histones

There are five families of histones that can be grouped into two categories: the core histone families (H2A, H2B, H3 and H4) and the linker histone family (H1). Histones are proteins with a strong basic property and low molecular weight that are among the most conserved eukaryotic proteins during evolution, which reflects the importance of their biological function. The histone sequences are rich in positively charged lysine and arginine residues, thus allowing the cohesion of the nucleosome, in particular through hydrogen bonds between these residues and the negatively charged phosphate skeleton of the DNA.

The genes encoding the canonical histones are devoid of introns and organized into a cluster containing several copies of histone genes. Canonical histone mRNAs

have a unique 3' stem-loop structure recognized by specific factors and allowing the regulation of their transcription, translation and degradation (Sullivan et al., 2009). Thus, a high level of expression is ensured in S phase, when a large quantity of histones is required for replication, and rapid degradation can be induced outside this phase.

1.3.1. Core histones

Despite a low sequence conservation between core histones, each canonical histone has similar structural characteristics that are conserved during evolution. Indeed, HFD, formed by a large helix α flanked at each end by a loop and a shorter helix α (Arents and Moudrianakis, 1995), is found in the structure of all canonical core histones of eukaryotes, and constitutes the structure of most euryarcheota histones (Sandman et al., 1998). This structure allows a "handshake" interaction within the H3-H4 and H2A-H2B dimers, provided by hydrophobic interactions and hydrogen bonds (Wood et al., 2005).

Unlike HFDs, the N-terminal end of histones are extremely variable in sequence and length, depending on the type of histone. These regions, rich in basic lysine and arginine residues, are the site of numerous post-translational modifications that can modify the overall charge of these residues, recruit specific regulatory factors and modulate protein-protein and protein-DNA interactions (Jenuwein and Allis, 2001). Many results show that the N-terminal ends of histones are involved in intra-nucleosomal and inter-nucleosomal interactions, suggesting a key role in establishing different degrees of chromatin organization (Li and Reinberg, 2011). For example, it has been shown that the N-terminal end of H3 and H2B are located in the alignment of the minor grooves of the DNA double helix, thus contributing to the compaction of

chromatin by sealing the binding to DNA on each side of the nucleosome (Hill and Thomas, 1990; Kan et al., 2007). The H2A and H2B proteins also have a basic C-terminal end. In the case of H2A, this region binds to DNA around the dyad axis, while the N-terminal end interacts with DNA at the periphery of the nucleosome (Arya and Schlick, 2006).

1.3.2. Linker histones

Linker histones are associated with the portion of DNA that separates two adjacent nucleosomes. Unlike core histones, linker histones have a lower conservation rate between species. The unstructured N-terminal and C-terminal ends have high variability in sequence and length. As with core histones, these regions are highly basic and are the target of post-translational modifications (Allan et al., 1980). In addition, linker histones have a central globular domain that is essential for interaction with DNA (Figure 7). This domain adopting a conformation called "winged helix", composed of three helices α , is characteristic of some families of DNA binding factors (Cerf et al., 1993; Ramakrishnan et al., 1993). Some studies suggest that linker histones play a key role in the formation of different levels of chromatin compaction, particularly the 30 nm fibre, but these results need to be clarified (Woodcock and Ghosh, 2010). Linker histones, such as histone H1 have important biological functions. For instance, Histone H1 plays a role in hormonal gene regulation, both as a structural component of chromatin and as a dynamic modulator of transcription (Vicent, 2016).

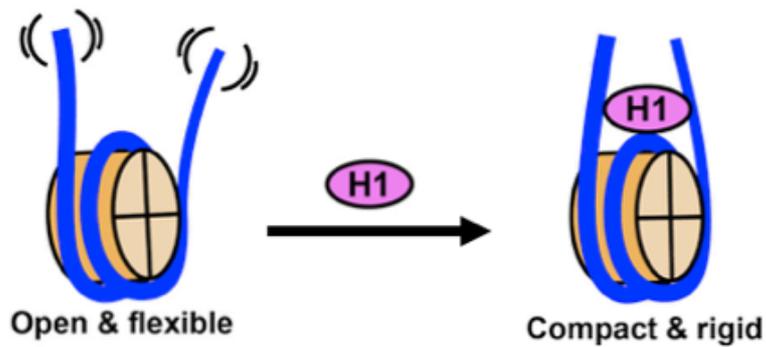


Figure 7. Histone 1 binding to the nucleosome (adapted from Bednar et al., 2017). Histone H1 binding to the nucleosome induces a more compact and rigid conformation.

2. DNA methylation and chromatin remodelling factors

2.1 DNA methylation

DNA methylation corresponds to the addition of a methyl group, derived from the S-adenosyl methionine cofactor, to the carbon 5 of a cytosine (5mC) (Figure 8).

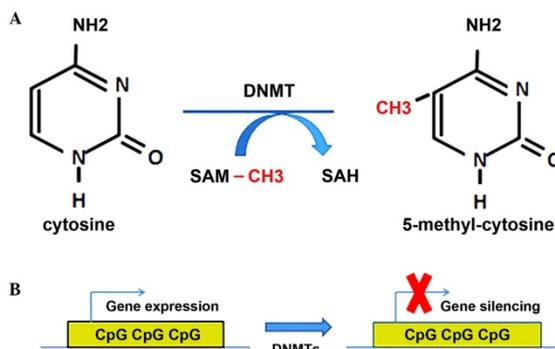


Figure 8. Characteristics of DNA methylation (Cui et al., 2016). (A) Cytosine is converted to 5'-methylcytosine by the action of DNMTs. (B) Cytosine is typically methylated in the context of CpG dinucleotides, and the methylation of constitutive heterochromatic and promoter regions is generally associated with gene silencing. SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; DNMT, DNA methyltransferase.

This epigenetic modification is catalyzed by enzymes of the DNA methyltransferase (DNMT) family. DNA methylation is an epigenetic modification recognized by specific effectors classified into two families in vertebrates: the MBD (Methyl-Binding Domain) family and the Kaiso family (Clouaire and Stancheva, 2008). In contrast to plants, 5mC in mammals covers most of the genome with exceptions in CpG islands. The 5mC patterns are established during early embryogenesis thanks to the activities of *de novo* DNA methyltransferases DNMT3A and DNMT3B (Cedar, 2009 ;Hermann, 2004 ;Jeltsch, 2016 ;Jeltsch, 2006). In highly differentiated tissues, mC is mainly found in a CpG context which is a condition that allows the appearance of a symmetric methylation pattern as bi-methylated DNA strand. In differentiated cells, DNA methylation is symmetric, *i.e.*, occurs on both DNA strands; this represents the basis for mitotic duplication. Approximately 45% of CpGs are within repetitive elements and presumed constitutively methylated (Das, 2006). Considering the 29 million CpGs in the haploid genome, DNA methylation patterns in normal human cells are largely unexplored. Several general methylation patterns are recognized. First, 7% of all CpGs are within CpG islands (CGIs), a majority of which are unmethylated(Rollins, 2006) . Most 5mCs are found in repetitive sequences, gene bodies, and intergenic regions(Law, 2010 ;Yang, 2014 ;Felsenfeld, 2014).

In mammalian somatic cells, methylation is classically associated with inactive transcription and mainly targets CpG dinucleotides that are sporadically found or grouped as islets located near the promoter region, the untranslated region or the first exon of many genes (Espada and Esteller, 2010). The condensed state of the chromatin allowing inactivation of transcription is allowed by the complementarity between DNA methylation and other epigenetic markings. For example, the effector protein MBD2 is found in the NuRD chromatin remodelling complex, which has histone

deacetylation activity, characteristic of condensed and non-transcribed chromatin (Wade et al., 1999; Zhang et al., 1999).

DNA methylation also plays a major role in other cellular processes such as X chromosome inactivation, transposon and centromeric sequence repression. It has also been shown that changes in the methylation profile are involved in cancerogenesis and aging processes (Heyn et al., 2012; Sandoval and Esteller, 2012). During mammalian embryogenesis, methylation plays a key role in gene regulation and chromatin organization through the parental genomic fingerprinting mechanism. Indeed, at the beginning of development, the genome undergoes first a massive demethylation and then a de novo establishment of the methylation profile, specific to the maternal and paternal genome (Messerschmidt, 2012). In somatic cells, DNA methylation is then transmitted after each cycle of DNA replication by the DNMT1 enzyme, allowing the methylation profile to be maintained (Espada and Esteller, 2010; Jeltsch, 2019; Gowher, 2018). The Ten eleven translocation (TET1, 2 and 3) enzymes can oxidize the 5mC into 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) (Ito et al., 2010 and 2011). Moreover, 5fC and 5caC can further be excised to regenerate unmodified cytosines by the action of thymine DNA glycosylase (TDG) together with the base excision repair (BER) enzymes (Cortellino et al, 2011; He et al., 2011; Maiti and Drohat, 2011) (see Figure 9).

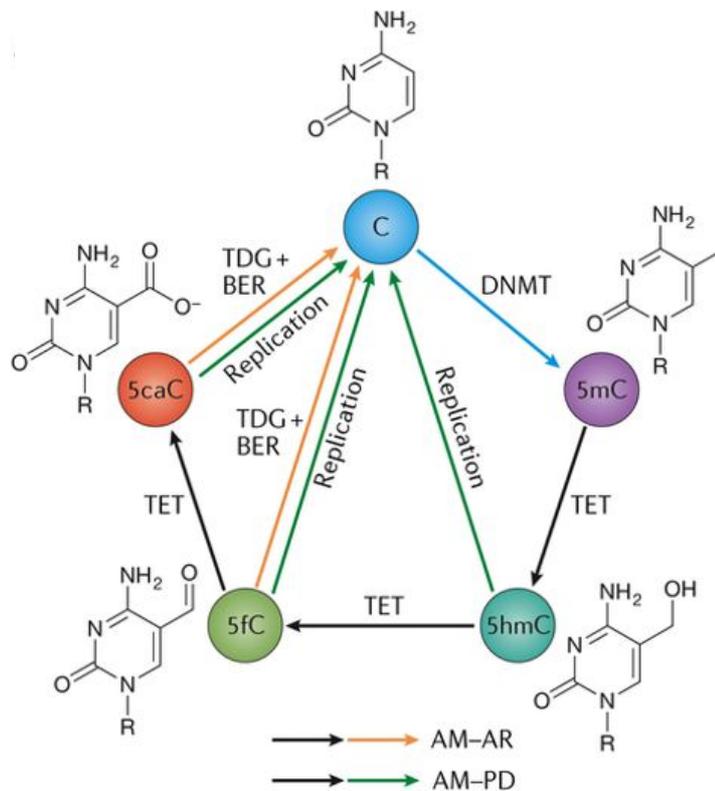


Figure 9. The cycle of active DNA demethylation (from Wu and Zhang, 2017).

DNA methyltransferases convert unmodified cytosine to 5mC (5-methylcytosine). 5mC can further be oxidized by TET proteins to 5hmC (5-hydroxymethylcytosine), 5fC (5-formylcytosine) and 5caC (5-carboxylcytosine). 5fC or 5caC are further excised by thymidine DNA glycosylase (TDG) coupled with base excision repair (BER). AM-AR, active modification-removal; AM-PD, active modification-passive dilution.

2.2 Chromatin remodeling complexes

The plasticity of chromatin, a dynamic balance between compaction and accessibility, is essential to ensure all the functions of the genome such as transcription regulation, replication, repair and condensation of chromosomes. This plasticity is regulated by the combination of epigenetic marks, discussed above, and ATP-dependent chromatin remodelling factors. These factors physically modify the structure or position of nucleosomes using the energy provided by the hydrolysis of ATP.

In vitro, chromatin remodelling factors can facilitate viral DNA replication (Alexiadis et al., 1998) and the mechanism of repair by nucleotide excision (Ura et al.,

2001). *In vivo*, chromatin remodelling factors have various actions such as the exchange of H2A variants (Krogan et al., 2003; Mizuguchi et al., 2004), in nucleosome positioning (Lomvardas and Thanos, 2001; Fazzio and Tsukiyama, 2003), the increase in DNA accessibility on the surface of a nucleosome (Truss et al., 1995), or the absence of nucleosomes in regulatory sequences (Reinke and Hörz, 2003, Figure 10)

Chromatin remodelling factors are found in protein complexes containing an enzyme with ATPase activity. These enzymes belong to the super-family of helicases 2 (SF2 helicase) and are conserved from yeast to humans (Côté et al., 1994; Imbalzano et al., 1994; Kwon et al., 1994; Papoulas et al., 1998). Chromatin remodelling factors have a high affinity for nucleosomes, domains for recognition of post-translational histone modifications, ATPase activity and domains for interaction with regulatory proteins (Clapier and Cairns, 2009). Chromatin remodelling complexes are divided into four families based on different ATPase activity: the SWI/SNF family, the ISWI family, the CHD1 family and the INO80 family (Figure 10).

2.2.1 The SWI/SNF family

The SWI/SNF complex (mating type SWItching and Sucrose Non-Fermentable) is the first ATP-dependent chromatin remodelling complex that has been identified in *Saccharomyces cerevisiae* (Winston and Carlson, 1992). The function of the SWI/SNF complex is highly conserved throughout evolution from yeast to man. The SWI/SNF family contains ySWI/SNF in yeast (Côté et al., 1994), the RSC complex (Cairns et al., 1996), BRM complexes in *Drosophila* (Dingwall et al., 1995) and hSWI/SNF complexes containing the catalytic subunit BRG1 or hBRM in humans (Figure 10) (Imbalzano et al., 1994; Kwon et al., 1994). Each catalytic subunit of the SWI/SNF family has a strong homology of the ATPase domain and a C-terminal bromodomain that can recognize

acetylated histones (Marmorstein and Berger, 2001). Each of the enzymes in the SWI/SNF family is present in a protein complex containing 8 to 15 partners. In yeast, the SWI/SNF complex contains 11 subunits (Winston and Carlson, 1992) (Figure 10).

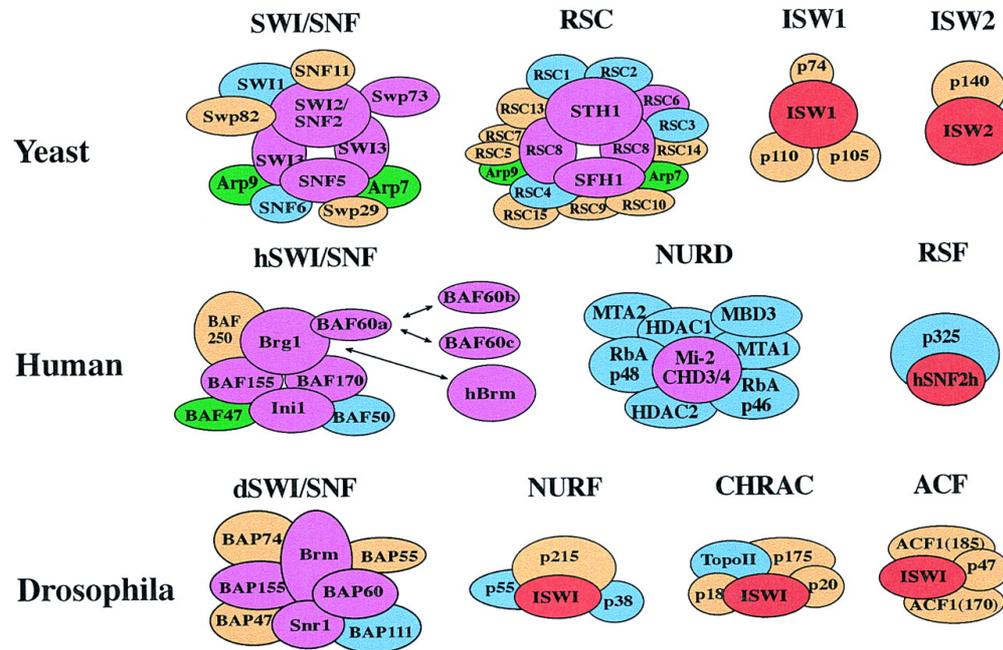


Figure 10. Composition of different ATP-dependent chromatin remodeling complexes (from Kingston and Narlikar., 2009). Conserved proteins in SWI/SNF family complexes are indicated in purple; ISWI homologs are indicated in red. Blue indicates proteins with known sequence, and green depicts actin-related proteins. Stoichiometries and direct contacts between proteins are speculative.

In yeast, members of the SWI/SNF family play an essential role in controlling the transcription of specific genes (Holstege et al., 1997; Sudarsanam and Winston, 2000) and can even cooperate with HAT complexes to activate transcription. The specificity of action of SWI/SNF complexes is enabled by interaction with transcription factors that specifically target certain genes. The RSC complex is involved in chromosome segregation (Hsu et al., 2003; Huang et al., 2004).

In humans, SWI/SNF complexes are essential for cell viability and play a role in regulating nuclear receptor function and cell growth. It has also been shown that mutations of BRG1, hBRM and hSNF5/INI1 are involved in the development of certain cancers (Roberts et al., 2002; Shain et al., 2012; Wu, 2012). Finally, the remodelling

protein ATRX (α -Thalassemia Mental Retardation X-linked), a specific partner of the histone H3.3 deposition complex, is involved in the regulation of many genes and is highly enriched in pericentromeric and telomeric regions.

2.2.2. The ISWI family

The first member of the ISWI family (SWItch Imitation) was identified in *Drosophila* based on the homology of its ATPase domain with the SWI/SNF family (Goodwin, 2018). Two other domains characterize the ISWI family remodelers: a SANT domain (Switching-defective protein 3, Adaptor 2, Nuclear receptor co-repressor, Transcription factor TF-IIIB) essential for histone binding, and a SLIDE domain (SANT-Like ISWI Domain) required for DNA binding and ATPase activity (Hamiche et al., 1999; Grüne et al., 2003; Fazzio et al., 2005). Other ISWI complexes were then discovered in yeast, fruit fly, mouse and humans (Dirscherl and Krebs, 2004). In *Drosophila*, there are three ISWI complexes: the ACF complex (ATP-dependent Chromatin assembly and remodelling Factor), the NURF complex (NUcleosome Remodelling Factor) and the CHRAC complex (CHRomatin Accessibility Complex) (Figure 10), which differ in the different subunits that compose them. The NURF complex is composed of ISWI, the NURF301 regulatory subunit, NURF38 pyrophosphatase and the WD40 domain protein NURF55 (Tsukiyama and Wu, 1995; Tsukiyama et al., 1995). The CHRAC and ACF complexes are composed of ISWI and ACF1 but CHRAC also contains two proteins with HFD, CHRAC14 and CHRAC16. In yeast, there are two ISWI genes, ISWI1 and ISWI2, which encode the catalytic subunits of four complexes: ISWI1a, ISWI1b, ISWI2 and yCHRAC. In humans, the ATPases of the ISWI family are SNF2H and SNF2L contained in the hACF, WICH (WSTF-ISWI CHromatin remodelling

complex), hCHRAC, RSF and SNF2H/NuRD/cohesion complexes (Dirscherl and Krebs, 2004).

ISWI complexes are involved in many biological processes such as the activation and suppression of transcription, replication and assembly of chromatin (Flaus et al., 2006). In yeast, ISWI complexes are mainly involved in the suppression of transcription. The ISWI1a complex appears to suppress transcription at the initiation stage, while ISWI1b could control elongation and termination of transcription by delaying the release of RNA polymerase II (Morillon et al., 2003; 2005). In *Drosophila*, the NURF complex is involved in transcription regulation in the Hsp70 promoter region (Heat Shock Protein 70) and NURF301 is essential for the expression of homeostasis genes (Tsukiyama et al., 1995; Tsukiyama and Wu, 1995). In addition, ISWI is also essential for maintaining the structure of the X chromosome (Deuring et al., 2000). In humans, the WICH complex facilitate DNA replication by decondensing chromatin and facilitating the recruitment of PCNA (Proliferating Cell Nuclear Antigen) to replication forks. In addition, the depletion of WSTF or SNF2H induces the aberrant formation of heterochromatin in neosynthesized DNA strands (Poot et al., 2004). It has also been shown that SNF2H forms a nucleolar remodelling complex (NoRC, Nucleolar-Remodelling Complex) with the Tip5 protein (TTF-I-Interacting Protein 5), possessing a bromodomain, and plays a key role in the suppression of promoters of genes encoding ribosomal RNA by promoting heterochromatin formation (Strohner et al., 2001; Zhou and Grummt, 2005). Finally, the NURF complex is involved in the activation of transcription, particularly for genes involved in neural development (Barak et al., 2003).

2.2.3. The CHD or Mi2 family

In 1998, several laboratories identified a complex containing a protein from the Mi2 family and having an ATPase domain from the SNF2 family. Mi2-containing complexes allow nucleosomes to slide along the DNA (Brehm et al., 2000). The NuRD complex (Nucleosome remodelling and histone deacetylation) includes a domain of chromatin remodelling and deacetylase activity that is often associated with transcriptional repression (Bowen et al., 2004). It consists of seven subunits, Mi-2 β , HDAC1, HDAC2, RbAp48, RbAp46, NURD70 and MBD3 (Figure 11).

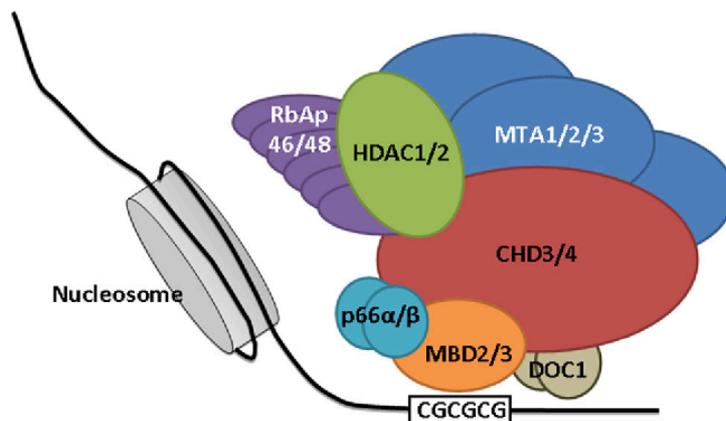


Figure 11. Schematic description of the NuRD complex (from Torchy et al., 2015). The three-dimensional structure of the complex has not been determined yet and precise subunit interactions are unknown.

The Mi-2 β subunit is an ATPase/helicase ortholog of Swi2/Snf2, with DNA binding zinc finger domains and two chromodomains. The MBD3 subunit belongs to the MePC2 family, proteins with a domain of binding to methylated CpG islands (Wade, 2001). Mi2 facilitates access of HDACs to histones (Bowen et al., 2004). It is therefore likely that MBD3 targets methylated DNA regions so that this complex can reshape the chromatin region and thus facilitate histone deacetylation (Xue et al., 2000).

2.2.4. The INO family

ATPase INO80 has been identified in the search for ISWI protein homologues (Shen et al., 2000) and in genetic screening of mutants affecting inositol biosynthesis in *S. cerevisiae* (Ebbert et al., 1999). This protein is similar to Isw1 and Isw2, but it does not have the SANT domain. INO80 has been purified in a complex containing 11 other proteins, including actin (Act1) and three actin-associated proteins, Arp4, 5 and 8 (Figure 12). This composition is similar to those of the Swi/Snf and RSC complexes in *S. cerevisiae* which contain Arp7 and 9. The INO80 complex also contains two proteins homologous to RuvB, a DNA helicase involved in bacterial repair (Kanemaki et al., 1997), called Rvb1 and Rvb2. This could explain the ATP-dependent helicase DNA activity associated with the INO80 complex in the 3'-5' direction (Shen et al., 2000) and the fact that a mutation in the INO80 gene leads to increased sensitivity to ionizing radiation, ultraviolet, alkylating agents and DNA synthesis inhibitors (Shen et al., 2000; van Attikum et al., 2004). Thus, this complex could have a role in repairing DNA damage. In humans, the homologues of Rvb1 and Rvb2 are called Tip49a and Tip49b respectively. These two proteins are part of an ATP-dependent chromatin remodeling complex, containing eight subunits homologous to those of the INO80 complex in *S. cerevisiae*, as well as at least five other human-specific subunits (hINO80) (Jin et al., 2005).

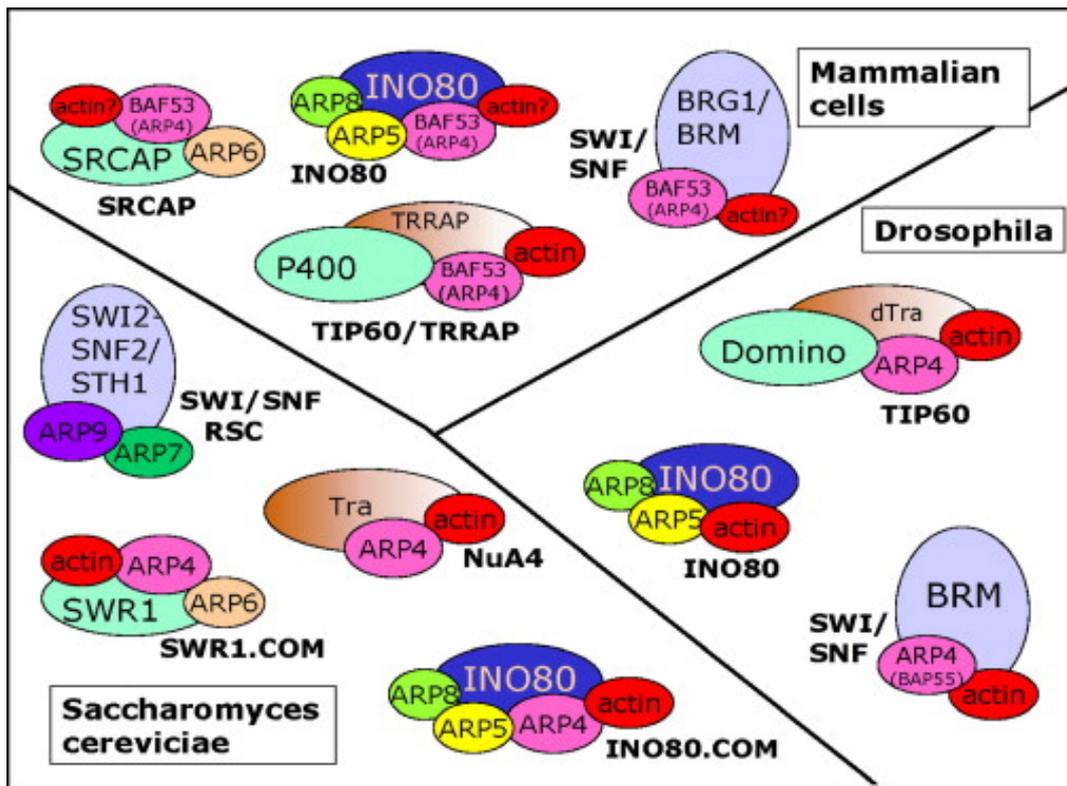


Figure 12. Comparison of the different of chromatin remodeling complexes in mammalian cells, *Drosophila*, and *Saccharomyces cerevisiae* (Östlund Farrants, 2008). In mammalian cells and *Drosophila*, Tip60/TRRAP have both activities corresponding to the yeast SWR1 and NuA4, and the ATPase is indicated by a turquoise color (SWR1, SRCAP, p400 or Domino). The ATM-like protein is indicated by Tra/TRRAP.

There is a second complex composed of 13 subunits in yeast, called the Swr1 complex. Several subunits (Act1, Arp4, Rvb1 and Rvb2) are common to the INO80 complex (Shen et al., 2000) (Figure 12). This complex is capable of remodelling chromatin by exchanging H2A-H2B dimers for H2AZ-H2B dimers (Krogan et al., 2003; Mizuguchi et al., 2004). Recently, a Swr1 ortholog has been identified in humans and is called SRCAP (Snf-2-related CREB-binding protein activator protein). SRCAP exchanges H2AZ-H2B dimers in reconstituted mono-nucleosomes with recombinant histones H2A, H2B, H3 and H4 (Ruhl et al., 2006).

2.2.5. ATRX/DAXX and PML-NBs

ATRX chromatin remodelling factor was discovered through the aim to identify the gene that contributes to the ψ -thalassemia, mental retardation, X-linked (ATRX) syndrome (Gibbons et al. 2003). The ATP/helicase domain was proposed to be involved in nucleosome repositioning and increasing DNA accessibility while the ADD domain has been shown to bind histone H3 tails containing the H3K9me3 mark, but not the active H3K4me3/2 mark (Gibbons et al. 2003). ATRX has been shown to localize in the PML-NBs, heterochromatin and telomeric/subtelomeric chromosomal regions. ATRX forms a stable complex with DAXX that colocalize to PML-NBs and heterochromatin. The domain composition of ATRX/DAXX is shown in figure 13.

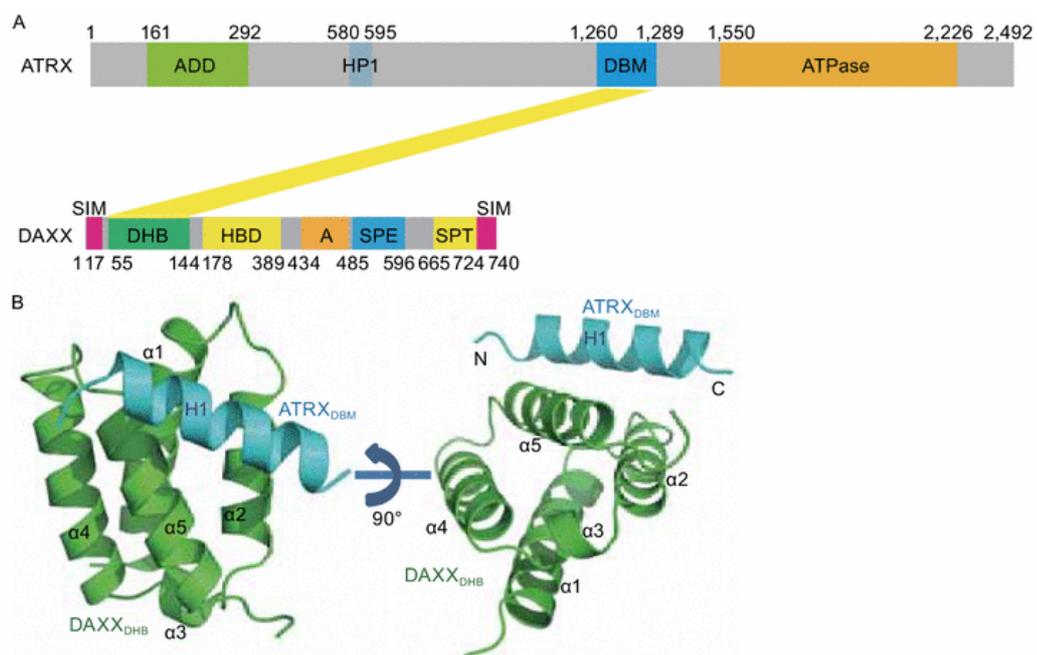


Figure 13. The structure of the DAXX/ATRX complex (from Wang et al., 2017). (A) Structure and domain organization of the ATRX and DAXX. ADD, ATRX-DNMT1-DNMT1L domain; HP1, HP1-binding motif; DBM, DAXX binding motif; ATPase, ATPase domain; SIM, Sumo-interaction motif; DHB, DAXX helical bundle; HBD, histone binding domain; Acidic, segment rich in Glu/Asp residues; SPE, segment rich in Ser/Pro/Glu residues; SPT, segment rich in Ser/Pro/Thr residues. (B) Two orthogonal views of the DAXX_{DHB}-ATRX_{DBM} complex. DAXX_{DHB} is colored in green and ATRX_{DBM} is colored in cyan.

Numerous studies have shown that DAXX protein is a major component of PML nuclear bodies (PML-NBs) (Negorev and Maul, 2001; Weidtkamp-Peters et al., 2008; Geng et al., 2012). It has been shown that PML is the main component of PML-NBs and that the sumoylation of this protein is a post-translational modification essential to the formation of PML-NBs (Ishov et al., 1999; Zhong et al., 2000; Brand et al., 2010). It has also been shown that the DAXX protein has a SIM domain (SUMO-Interacting Motif (Song et al., 2004)) on its C-terminal end which is involved in the interaction with PML (Lin et al., 2006; Escobar-Cabrera et al., 2011). PML-NBs are involved in the progression of the cell cycle, the DNA damage response, transcription regulation, viral infection, heterochromatin formation, and apoptosis, however, to date, the precise function of PML-NBs in these mechanisms remains poorly understood (Bernardi and Pandolfi, 2007; Borden and Culjkovic, 2009).

3. Histone modifications

3.1 Role of these modifications

Histones are the target of many post-translational modifications that can modify the structure of chromatin and therefore influence major genomic processes such as gene expression regulation, DNA repair and replication. The different histone modifications are illustrated in figure 14.

Numerous studies have shown the importance of the N-terminal ends of H3 and H4 as well as the C-terminal ends of H2A and H2B in regulating genes and chromatin structure (Rodríguez-Paredes and Esteller, 2011). In *Saccharomyces cerevisiae*, it has been shown that the N-terminal end of histones H3 and H4 is essential for the suppression of mating genes and telomers (Thompson et al., 1994). In addition, the deletion of the N-terminal end of histones H3 and H4 is lethal in yeast (Ling et al., 1996)

and point mutations of the N-terminal end of H4 have been shown to inhibit the suppression of certain genes (Hecht et al., 1995).

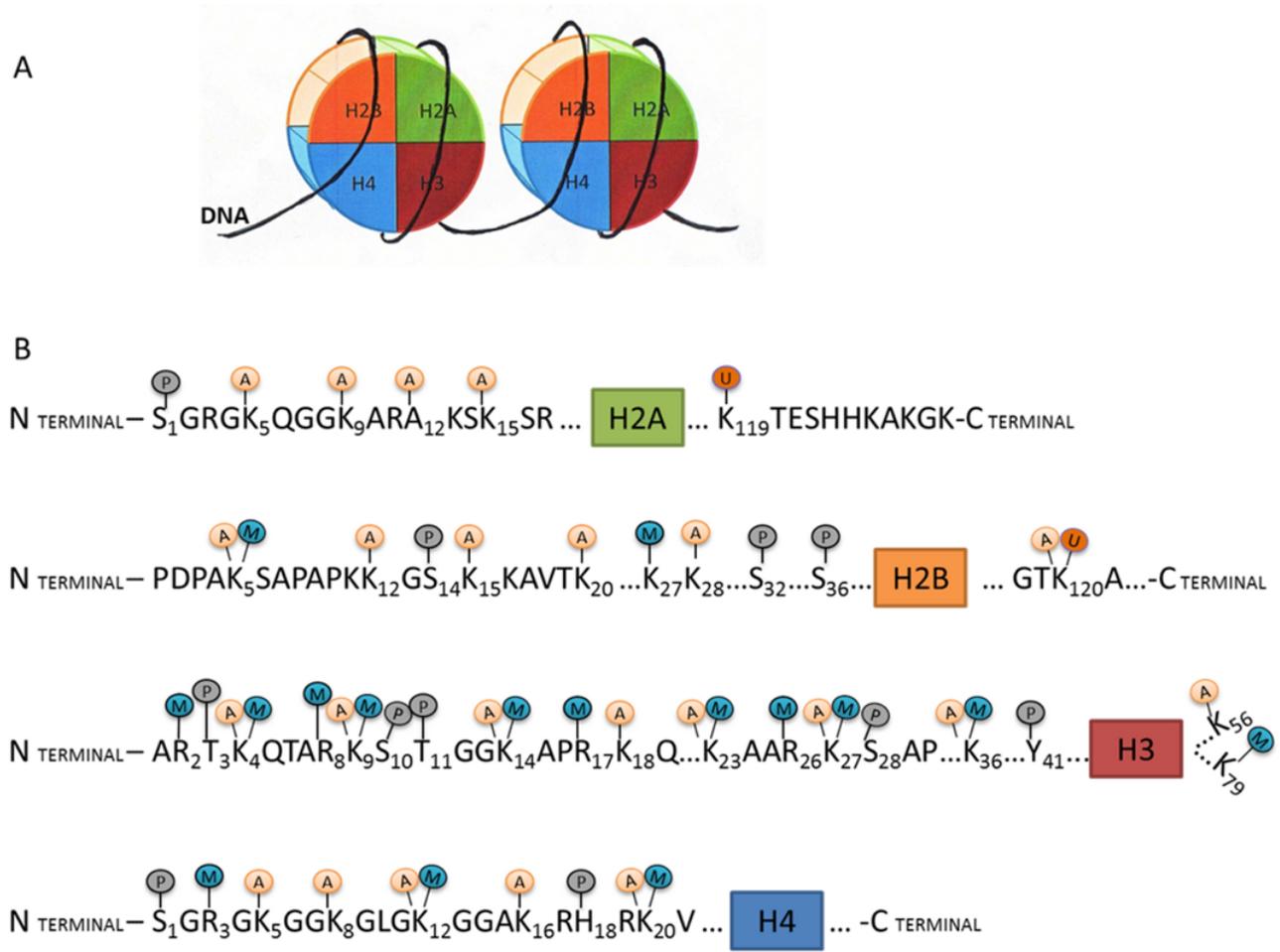


Figure 14. Schematic representation of the different histone modifications occurring on the nucleosome (Adopted from Zhong Xu et al., 2013). (A) Structure of the nucleosome (B). The major histone modifications shown include acetylation (A), methylation (M), phosphorylation (P) and ubiquitination (U). Histone modifications mainly occur on the N-terminal tails of histones but also on the C-terminal tails and globular domains.

To date, several types of post-translational modifications of histone N-terminal tails are described (Figure 14). The best characterized are acetylation, methylation, phosphorylation (Campos and Reinberg, 2009) but there are other types such as ADP-ribosylation, ubiquitinylation, sumoylation, glycosylation, citrullination, biotinylation, carbonylation, butyrylation and propionylation (Chen et al, 2007; Bártoová et al., 2008;

Hassan and Zempleni, 2008; Garcia-Dominguez and Reyes, 2009; Bergmann, 2010; Fujiki et al., 2010). On the one hand, there are factors that specifically recognize these changes and then contribute, directly or indirectly, to the regulation of gene expression. These factors have a specific structure such as chromodomains (CHRomatin Organization MODifier) and bromodomains that allow to recognize, methylated and acetylated lysine residues respectively, in a specific way. On the other hand, some of these modifications may act by changing the charge of a histone residue, thus modulating their interaction with DNA or adjacent nucleosomes. Some of these post-translational changes occur directly after histone synthesis in the cytoplasm, such as acetylation of lysine residues 5 and 12 of H4 (Sobel et al., 1995), while the majority occur in the nucleus. The specific post-translational modification profile of histones gives complex combinations that are involved in a wide spectrum of cellular processes, from regulating gene expression to maintaining chromosome structures. For example, many studies have shown that acetylation is associated with transcription activation (Koch et al., 2007) while phosphorylation is associated with chromosome formation or DNA repair (Thiriet and Hayes, 2005).

In addition, there is a relationship between the different post-translational modifications of histones. For example, it has been shown that phosphorylation of the serine residue 10 of histone H3 plays a major role in regulating the modification of other residues of the N-terminal tail of H3, including methylation of lysine residue 9, acetylation of lysine residue 14, and phosphorylation of threonine residues 6 and 11 (Noma K et al., 2001; Liokatis et al., 2012). The diversity and relationships between post-translational histone modifications have led to the emergence of the concept of "histone code". According to this hypothesis, combinations of post-translational

modifications are transmitted during cell divisions and contain information on the expression status of genes (Jenuwein and Allis, 2001).

3.1.1. Acetylation of histones

The acetylation of histones has been the subject of much work to determine the mechanisms for implementing this modification and its role in regulating gene expression.

Acetylation consists of adding one or more acetyl groups to the amino groups α or ϵ of the lysine residues of histones and other proteins. This reaction is catalyzed by enzymes of the acetyltransferase category, which are divided into four families (Table 1). The GNAT family (GCN5-related N-terminal AcetylTransferases); the MYST family including enzymes that catalyse the transfer of an acetyl group from the acetyl-CoA cofactor to the primary amine group of non-histone proteins and small molecules (Dyda et al, 2000); the p300/CBP family which is distinct but related to the p270 family; and the general transcription factors HAT (Histone AcetylTransferase) which include TAF250, the largest of the TAF (TATA binding protein-Associated Factor) factors in the TFIID transcription complex, which has in vitro acetyltransferase activity specific to the N-terminus of histones H3 and H4 (Mizzen et al., 1996).

Histone acetylation is reversible and highly dynamic. Enzymes of the histone deacetylase (HDAC) class catalyze the removal of acetyl groups from histones and are divided into several families. The SIR2 family (Silent Information Regulator 2), the HD2 family, and the RPD3/HDA1 family. Histone deacetylases of the RPD3/HDA1 family have a high conservation rate across all eukaryotic genomes and are subdivided into three classes: HDAC Class I (HDAC-1, -2, -3 and -8) which are linked to the yeast

RPD3 protein; HDAC Class II (HDAC-4, -5, -6 and -7) which are homologous to the yeast protein HDAC1; and HDAC Class III called sirtuins (SIRT1 to 7 in mammals).

The molecular mechanisms that govern the action of acetylation on expression remain poorly understood to this day. Nevertheless, there are two non-exclusive theories that explain how histone acetylation contributes to the formation of an open chromatin environment conducive to active transcription by allowing access to transcription factors in gene promoter regions. On the one hand, acetylation of lysine residues neutralizes the positive charge of histone tails, thus destabilizing interactions with DNA but also intra- and inter-nucleosomal interactions and thus contributing to the formation of an open and permissive local environment for transcription (Ausió and van Holde, 1986). On the other hand, histone acetylation allows the specific recruitment at modified sites of protein factors with a bromodomain (Taverna et al., 2007). Unlike acetylation, histone deacetylation contributes to the formation of a closed chromatin environment that is unfavourable to transcription. Indeed, it is accepted that heterochromatin regions are generally hypoacetylated while euchromatin regions are generally associated with histone hyperacetylation (Schübeler et al., 2000).

3.1.2. Histone methylation

Methylation is a post-translational modification of the lysine and arginine residues particularly well characterized for histones H3 and H4. The lysine residue side chain is the target for mono, di or trimethylation while the arginine residue side chain can be mono or dimethylated. All these modifications provide significant functional diversity at each methylation site (Bannister et al., 2002; Margueron et al., 2005).

The addition of methyl group on histone residues is catalyzed by enzymes of the category of histone methyltransferases (HMT), which use the S-adenosyl

methionine cofactor. These enzymes have a high substrate specificity that allows methylation of lysine residues at the level of free histones or in a nucleosomal context. For example, it has been shown that methylation of histone tails by Dot1, Set2 and PR-Set7/Set8 is only possible within the nucleosome (Strahl and Allis, 2000; Nishioka et al., 2002; van Leeuwen et al., 2002). To date, histone methylation has been reported at 24 known sites (17 on lysine residues and 7 on arginine residues). The best characterized methylation sites are lysine residues 4, 9, 27, 36, 79 for histone H3 and lysine residue 20 for histone H4 (Sims et al., 2003; Lee et al., 2005). The histone methyltransferases specific to lysine residues have a SET (Suppressor of variegation - Enhancer of zest - Trithorax) catalytic domain. Methylation does not modify the load of lysine residues but increases their hydrophobicity, which disrupts hydrogen bonds and can also create recognition sites for specific factors. Arginine residues 2, 8, 17, 26 of histone H3 and arginine residue 3 of histone H4 are mono or dimethylation sites. Histone methyltransferases CARM1/PRMT are responsible for the modification of arginine residues (Schurter et al., 2001).

Methylation of arginine residues is traditionally associated with transcription activation, while the role of methylation of lysine residues is more variable. Indeed, it has been shown, for example, that the trimethylation of H3 lysine residue 27 has a repressive effect on transcription, that the trimethylation of H3 lysine residue 4 has an activating effect on transcription and that these two post-translational modifications can coexist in embryonic stem cells, at the same gene promoter involved in cell differentiation (Vastenhouw and Schier, 2012). Histone methylation has long been considered irreversible in view of the high thermodynamic stability of the N-CH₃ bond. Nevertheless, recent studies have highlighted the demethylation activity, without

histone exchange, of the JmjC-domain enzyme family, as well as its role in controlling transcriptional activity (Chen et al., 2012; Mansour et al., 2012).

3.1.3. Phosphorylation of histones

The discovery of histone phosphorylation in 1967 (Gutierrez and Hnilica, 1967) paved the way for numerous studies that led to the characterization of a multitude of kinases responsible for histone phosphorylation (Langan, 1968; Prigent and Dimitrov, 2003). This post-translational modification affects the lateral chain of serine, threonine and sometimes tyrosine residues of core histones and linker histones.

Phosphorylation consists of the addition of a phosphate group, derived from triphosphate nucleotides (ATP, GTP, AMPc), to the hydroxyl group in the side chain of the residues concerned. This reaction, catalyzed by specific enzymes of the kinase class, is also reversible by the action of enzymes of the phosphatase class.

Histone phosphorylation plays a key role in many cellular processes such as transcription regulation, apoptosis, cell cycle, DNA repair, chromosome condensation, gene regulation, protein degradation and enzyme activity regulation (Johansen and Johansen, 2006; Kouzarides, 2007; Loomis et al., 2009).

Overall, phosphorylation of histone tails at the serine and threonine residues is involved in chromatin condensation during mitosis and meiosis. For example, it has been shown that phosphorylation of the threonine residue 119 of the C-terminal end of H2A plays a role in regulating the structure of chromatin during mitosis (Banerjee and Chakravarti, 2011). Histone H3 variants have a high retention of N-terminal residues, including threonine residues 3 and 11, and serine residues 10 and 28 which are phosphorylated in many organisms during mitosis. Nevertheless, phosphorylation of serine residues 10 and 28 of histone H3 also appears to have a role in activating gene

transcription. The kinases involved in the phosphorylation of the serine 10 residue of H3 are IpL1 and Snf-1 in yeast (Glover et al., 1995; Lo et al., 2001) while in mammals, the Aurora B, IKK, Rsk2 and AKT kinases are responsible for this post-translational modification (Sassone-Corsi et al., 1999; Prigent and Dimitrov, 2003; Yamamoto et al., 2003; Teperek-Tkacz et al., 2010).

3.1.4. Ubiquitylation of histones

Ubiquitylation of histones was first observed in 1970. Since then, it has been shown that the histones of the core H2A, H2B, H3 and their respective variants are the targets of this post-translational modification. Ubiquitin is a low molecular weight protein (8 kDa) that is classically attached as a polymer to the amine group ϵ of the lysine residues of proteins intended for proteasome degradation (Wilkinson, 2000). Ubiquitylation is generally a sequential reaction involving enzymes of the E1, E2 and E3 families. On the other hand, the withdrawal of one or more ubiquitin groups is achieved by a single enzyme from the isopeptidase class (Wilkinson, 2000).

In many eukaryotes, ubiquitylation affects about 5 to 15% of the H2A histone pool, the majority of which is mono-ubiquitylated. However, poly-ubiquitylated histone H2A has also been detected in many tissues (Nickel and Davie, 1989). For example, it has been shown that the lysine residue 119 of histone H2A is a highly conserved site of ubiquitylation (Nickel and Davie, 1989). It should be noted that in yeast *Saccharomyces cerevisiae*, no ubiquitylation of H2A was observed (Robzyk et al., 2000). Ubiquitylation of H2B has also been detected in many eukaryotic organisms, with the exception of *S. pombe* and *Arabidopsis thaliana*. Finally, another

work has shown that histones H3 and H1 are also targets of this post-translational modification (Chen et al., 1998; Pham and Sauer, 2000).

3.1.5. Sumoylation of histones

Sumoylation is a specific post-translational modification of lysine residues that consists of the addition of one or more SUMO (Small Ubiquitin-like MOdifier) proteins. In mammals, there are three SUMO paralogues, SUMO-1, -2 and -3; while in yeast *Saccharomyces cerevisiae* there is only one SUMO paralogue gene, Smt3p (Wang and Dasso, 2009). Overall, sumoylation is a sequential and reversible reaction similar to ubiquitylation (Wohlschlegel, 2004).

4. Histone variants

Histone variants are non-allelic isoforms of canonical histones. With the exception of H4, the histones of the core (H2A, H2B and H3) and the linker histone H1 have variants that show structural and functional differences. Indeed, histone variants differ from their corresponding canonical histones in gene organization, expression, protein sequence, and deposition mechanism. These histone variants can replace canonical histones and thus locally modify the composition and functions of the nucleosome (Table 1). Indeed, it has been shown that the incorporation of these variants plays an essential role in gene expression regulation, heterochromatin formation and maintenance of epigenetic chromatin markings (Ahmad and Henikoff, 2002a; Ausió and Abbott, 2002; Smith, 2002; Henikoff et al., 2004; Kamakaka and Biggins, 2005; Sarma and Reinberg, 2005; Hamiche and Shuaib, 2012).

Histone	Number of gene copies	Cell-cycle expression	Location	Function	Knockout phenotype	Refs
H2A	15	RD	Throughout the genome	Core histone	ND	153,154
H2A.X	1	RI	Throughout the genome	DNA repair (mediated by the phosphorylated form γ H2A.X) and genome integrity	Male infertility (that is, defects in sperm meiosis) in mice	27
H2A.Z	2	RI	Throughout the genome	Gene activation, gene silencing and chromosome segregation	Embryonic lethality in H2A.Z.1-knockout mice at E4.5–E7.5	48,50,52
MacroH2A	2	Possibly RI	Inactive X chromosome	Gene silencing	Brain malformation in zebrafish	57–59
H2A.Bbd	3	RI	Active X chromosome and euchromatin	Active transcription	ND	18,22
H2B	17	RD	Throughout the genome	Core histone	ND	153,154
TSH2B	1	Possibly RI	Throughout the sperm genome and in telomeres of somatic cells	Chromatin-to-nucleoprotamine transition	ND	19,21
H2BFWT	1	Possibly RI	Primate telomeres in sperm	ND	ND, as there is no gene product in mice	153,154
H2BE	1	RI	Throughout the genome of olfactory neurons	ND	Overexpression of olfactory receptor in mice	79
H3.1	10	RD	Throughout the genome	Core histone	ND	87,153,154
H3.2	3	RD	Throughout the genome	Core histone	ND	87,153,154
H3.3	2	RD and RI	Throughout the genome	Gene activation, silencing and chromosome segregation	ND; adult infertility in H3.3A-gene-trap and H3.3B-knockout mice	42–44
H3.4	1	RI	Sperm genome and nucleolus of somatic cells	ND	ND	20
H3.5	1	Possibly RI	Euchromatin in hominid testis	ND	ND, as there is no gene product in mice	15
H3.X and H3.Y	1 of each	Possibly RI	Euchromatin in primates	ND	ND, as there is no gene product in mice	53
CENP-A	1	RI	Centromeres	Chromosome segregation	Embryonic lethality at E3.5–E8.5 in mice	113
H4	14	RD	Throughout the genome	Core histone	ND	153,154

CENP-A, histone H3-like centromeric protein A; E, embryonic day; H2BFWT, histone H2B type W-T; ND, not determined; RD, replication dependent; RI, replication independent.

Table 1. Histone variants in vertebrates (Maze et al., 2014). structural and functional differences between vertebrate histone variants.

Histones can be divided into two groups: canonical histones and variant histones. In humans, genes encoding canonical histones are located on chromosomes 6 and 1, organized in clusters, devoid of introns and transcribed into non-polyadenylated mRNAs (Marzluff et al., 2002). In contrast, genes encoding histone variants are found outside these clusters, in small numbers of copies containing introns and transcribed into polyadenylated mRNAs (Woodland, 1984). Canonical histones are specifically expressed during S phase to ensure chromatin assembly after the passage of the replication fork (Krude, 1999) while variant histones are expressed and incorporated into the chromatin throughout the cell cycle. Histones can therefore also

be divided according to their mode of incorporation into chromatin, replication-dependent (RD) or replication-independent (RI).

There are many variants of histones in different species, which will now be reviewed.

4.1. Variants of the H1 histone

The H1 histone family has a high variability according to species, tissues and stage of development. Many variants of histone H1 have been identified in mammals and plants. In mammals, at least eleven variants of H1 have been described, while in *Drosophila* there is only one H1 histone. Variants of the linker histone show differences in mode of expression, post-translational modification status, DNA compaction capacity and localization (Th'ng et al., 2005; Clausell et al., 2009).

Variants of histone H1 can be classified into three groups based on their mode of expression: H1 histones expressed in phase S (H1.1 to H1.5); H1 histones expressed independently of phase S (H1.0 and H1x); and germ-line specific H1 histones (H1t, H1T2, H1LS1 and H1oo). H1 histones are characterized by their ability to facilitate chromatin condensation, which can also be used to classify these variants (Happel and Doenecke, 2009; Öberg et al., 2012).

Variants H1.2 to H1.5 are expressed in most somatic cells while H1.1 is limited to certain tissues and H1.0 is expressed in cells at the end of differentiation. H1.2 and H1.4 are the dominant variants in most of the tissues studied (Meergans et al., 1997; Franke et al., 1998). To date, H1x is the least well characterized variant of the H1 family. Nevertheless, it has been shown, in some cell types, that H1x is expressed independently of replication and is non-randomly located in poorly accessible regions of the genome (Happel et al., 2005). The other four variants of H1 are germ cell

specific, including H1oo which is expressed in oocytes, and H1t, H1T2 and H1LS1 in the testis (Izzo et al., 2008; Happel and Doenecke, 2009).

In order to determine the biological functions of the H1 histone variants, gene invalidation experiments were performed in mice. It has been shown that the inactivation of genes encoding H1.2, H1.3 or H1.4 does not induce a specific phenotype (Sirotkin et al., 1995) whereas the simultaneous invalidation of these genes causes a specific phenotype with embryonic death occurring in mid-gestation (Fan et al., 2003). These results suggest that histone H1 variants may have partially redundant functions (Izzo et al., 2008). Variants of histone H1 are specifically associated with active or inactive regions of chromatin and are involved in transcription regulation. In addition, H1 variants could be involved in other biological processes such as DNA repair (Hashimoto et al., 2007).

4.2. Variants of the histone H2A

The H2A histone family contains the largest number of variants and is highly heterogeneous in terms of sequence and size. To date, 265 members have been identified among different species and many isoforms have no known function. In mammals, four major variants of histone H2A have been characterized: H2A.Z, macroH2A, H2ABbd and H2A.X.

4.2.1. H2A.Z

The histone variant H2A.Z has a high conservation during evolution, and a percentage of identity of about 60% with the canonical histone H2A. H2A.Z is essential for viability in fruit fly, xenopus and mice but not in yeast (Clarkson et al., 1999; Faast et al., 2001; Ridgway et al., 2004). This observation suggests that the role of H2A.Z is variable

depending on the species, and to date its exact function remains controversial. Nevertheless, research shows that H2A.Z is involved in many biological processes such as transcription activation, chromosome segregation, cell cycle control and heterochromatin formation (Zlatanova and Thakar, 2008).

Indeed, it has been shown that H2A.Z is associated with repression and activation of transcription (Santisteban et al., 2000; Meneghini et al., 2003). Recent studies on the entire genome have also shown that H2A.Z deposition is involved in the establishment and maintenance of boundaries that define promoter regions and genes (Jin et al., 2009). In humans, an enrichment of H2A.Z in promoter regions has been shown (Zlatanova and Thakar, 2008) and the function of H2A.Z is regulated by post-translational modifications. Indeed, the acetylation level of H2A.Z is higher in euchromatin regions than in heterochromatin regions and H2A.Z mono-ubiquitylation seems important in the suppression of X chromosome transcription (Talbert and Henikoff, 2010).

4.2.2. H2A.X

The histone variant H2A.X also has a high degree of evolutionary conservation and differs from the canonical histone H2A by its conserved C-terminal domain containing an SQ motif. The H2A.X variant plays a key role in the repair mechanisms of double-stranded DNA breaks, thus helping to maintain the integrity of the genome. In response to a double-stranded break, the specific DNA damage kinases, ATM (Ataxia Telangiectasia Mutated), ATR (ATM and Rad53- Related) and DNA-PK (DNA-dependent Protein Kinase) phosphorylate and bind the serine residue 139 of the H2A.X SQ motif. The modified form is known as γ -H2A.X (Rogakou et al., 1998). The modified γ -H2A.X is present at double-stranded DNA break sites during repair but also

during meiotic recombination (Mahadevaiah et al., 2001), apoptosis (Rogakou et al., 2000) and V(D)J recombination (Chen et al., 2000). In addition, the invalidation of the gene encoding H2A.X induces infertility in male mice due to a mismatch between the X and Y chromosomes during the meiosis prophase (Fernandez-Capetillo et al., 2003).

4.2.3. MacroH2A

The macroH2A histone variant was discovered and characterized by Pehrson and Costanzi (Pehrson and Fried, 1992; Costanzi and Pehrson, 2001). The macroH2A variant is the largest member of the H2A family with 327 amino acids and 40 kDA molecular weight. Indeed, macroH2A have a characteristic hybrid structure composed of an N-terminal domain similar to that of the canonical histone H2A and a novel C-terminal domain NHR (Non-Histone Region) containing a "leucine zipper" motif and a macrodomain (Karras et al., 2005).

In the human genome, there are two genes encoding macroH2A. The first gene, H2AFY, is located on chromosome 5 and codes for two isoforms produced by alternative splicing, macroH2A1.1 and macroH2A1.2 (Rasmussen et al., 1999). The second gene on chromosome 10 codes for the macroH2A2 variant (Chadwick and Willard, 2001b).

The macroH2A histone variant is involved in transcriptional repression. For example, it has been shown that the inactivated X chromosome is enriched with macroH2A1.2 and macroH2A2, suggesting a role in the inactivation of the X chromosome (Costanzi and Pehrson, 1998; Rasmussen et al., 1999; Chadwick and Willard, 2001b). The role of macroH2A in transcription repression has also been described and characterized for the inducible *Hsp 70* (Heat-Shock Protein) genes. Indeed, the promoter region of the inducible genes Hsp70.1 and Hsp70.2 are strongly

enriched in macroH2A1.1 (Ouararhni et al., 2006). It has been shown that the macrodomain (Karras et al., 2005) of macroH2A1.1 interacts with the PARP-1 protein (Poly-ADP-Ribose Polymerase 1) and inactivates its enzymatic activity. During a thermal shock, macroH2A1.1 and PARP-1 are released from the promoter region of Hsp70.1, allowing the self-activation of PARP-1. Thanks to its ADP-ribosylation activity, PARP-1 can then modify the other proteins associated with the Hsp70.1 promoter and thus activate transcription (Ouararhni et al., 2006).

4.2.4. H2A.Bbd

The H2A.Bbd variant (Barr Body-Deficient) was discovered by Chadwick and Willard in a study showing its exclusion from the inactive X chromosome (Chadwick and Willard, 2001a). It has been shown that this variant is highly variable within mammals unlike all other histones described to date (Malik and Henikoff, 2003; Eirín-López et al., 2008). In humans, three genes devoid of introns and located on the X chromosome code for two histones H2A.Bbd, H2A.Bbd1 and H2A.Bbd2, which differ from only one amino acid (Chadwick and Willard, 2001a). From a structural point of view, H2A.Bbd differs first of all from H2A by the absence of a C-terminal acid domain that interacts with H4 (Luger et al., 1997). In addition, the retained residues carrying post-translational modifications at the N-terminal tail of H2A are also absent from the structure of H2A.Bbd which, on the other hand, has 6 characteristic arginine residues in this region.

In vitro, nucleosomes containing H2A.Bbd are highly unstable and contain only 118 to 130 base pairs (Bao et al., 2004; Doyen et al., 2006). *In vivo*, recent data suggest that H2A.Bbd plays a role in spermatogenesis, particularly in the exchange between histones and protamines (Ishibashi et al., 2010). In addition, it has been

shown that H2A.Bbd is involved in activating transcription and regulating mRNA splicing (Tolstorukov et al., 2012).

4.3. Variants of the histone H2B

To date, the H2B histone family has 214 different isoforms described in various species, and in humans, there are 23 genes that code for 19 H2B isoforms. However, H2B variants have only been observed in the male germ line and in plant pollen, their function remaining poorly characterized to date. In humans, two variants of H2B, TSH2B (Testis-Specific H2B) and H2BFWT (H2B Family Member W, Testis-specific), have been described in testis.

The TSH2B variant is conserved in mice, rats and humans and has 85% identity with the canonical histone H2B (Zalensky et al., 2002). In humans, TSH2B is specifically expressed in about 30% of sperm cells. This variant is associated with faster chromatin decondensation, suggesting a role in pronucleus formation and subsequent paternal gene activation (Singleton et al., 2007).

The histone H2BFWT has been described as a variant of H2B expressed from the X chromosome, specific to primate testicles, and potentially associated with telomeric DNA (Churikov et al., 2004a). Indeed, it has been shown that H2BFWT is partially localized between telomeric repetitions (Churikov et al., 2004b). Other studies suggest that H2BFWT is also involved in male infertility. Indeed, a point mutation in the 5' untranslated region of H2BFWT induces a decrease in the translation of the variant causing a decrease in sperm count and vitality (Lee et al., 2009).

4.4. Variants of the histone H3

The H3 family of histones includes 8 isoforms: the canonical histones H3.1 and H3.2; and the variant histones H3t, H3.3, CENP-A, H3.X, H3. Y and H3.5 (Figure 15). These histones can be divided according to their mode of incorporation into chromatin, replication-dependent (H3.1 and H3.2) or replication-independent (H3t, H3.3, CENP-A, H3.X, H3. Y and H3.5). In addition, variants H3.3, CENP-A, H3.X and H3. Y are specifically found in somatic cells, while variants H3t and H3.5 are specific to the germ line. In contrast, in yeast, there is only one H3 isoform that is equivalent to the H3.3 isoform found in mammals. In fact, the gene encoding H3.3 is the common ancestor from which the three major isoforms H3.1, H3.2 and H3t emerged during evolution (Postberg et al., 2010). The yeast H3 histone can be incorporated in a replication-dependent and replication-independent manner.

A. Sequence alignment of human H3 variants

H3.1	-ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALREIRRYQKSTE	59
H3.2	-ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALREIRRYQKSTE	59
H3t	-ARTKQTARKSTGGKAPRKQLATK V ARKSAPATGGVKKPHRYRPGTVALREIRRYQKSTE	59
H3.3	-ARTKQTARKSTGGKAPRKQLATKAARKSAP S TGGVKKPHRYRPGTVALREIRRYQKSTE	59
H3.5	-ARTKQTARKSTGGKAPRKQLATKAARKS TPST CGVK-PHRYRPGTVALREIRRYQKSTE	58
H3.X	-ARTKQTARK ATAWQ APRK PL ATKAARK RASPT GGI KK PHRY KPGT LALREIR KY QKST Q	59
H3.Y	-ARTKQTARK ATAWQ APRK PL ATKAAG KRAP PTGGI KK PHRY KPGT LALREIR KY QKST Q	59
CENP-A	GP RRRS RKPE APRRRS P-SPT TP GGPS RRGP SLG ASS HQHS RRR -QG WL KEIR K I QKST H	58
H3.1	LLIRKLPFQRLVREIAQDFKT--DLRFQSSAVMALQEACEAYLVGLFEDTNLCAIHAKRV	117
H3.2	LLIRKLPFQRLVREIAQDFKT--DLRFQSSAVMALQEASEAYLVGLFEDTNLCAIHAKRV	117
H3t	LLIRKLPFQRL M REIAQDFKT--DLRFQSSAVMALQEACE S YLVGLFEDTNLCVIHAKRV	117
H3.3	LLIRKLPFQRLVREIAQDFKT--DLRFQ SAA IGALQEASEAYLVGLFEDTNLCAIHAKRV	117
H3.5	LLIRKLPFQRLVREIAQDF NT --DLRFQ S AVV G ALQEASEAYLVGLLEDTNLCAIHAKRV	116
H3.X	LLL R KLPFQRLVREIAQ AI SP--DLRFQ SAA IGALQEASEAYLV Q L F EDTNLCAIHARRV	117
H3.Y	LLL R KLPFQRLVREIAQ AI SP--DLRFQ SAA IGALQEASEAYLV Q L F EDTNLCAIHARRV	117
CENP-A	LLIRKLPF S RL A REI CVK F TR GV D FN W Q A Q LL ALQE A EA EL V L FED A Y L L T I H AG R V	118
H3.1	TIMPKDIQLARRIGERA-----	135
H3.2	TIMPKDIQLARRIGERA-----	135
H3t	TIMPKDIQLARRIGERA-----	135
H3.3	TIMPKDIQLARRIGERA-----	135
H3.5	TIMPKDIQLARRIGERA-----	134
H3.X	TIM PR D M QLARR L R G E G A G E P T L L G N L A L	146
H3.Y	TIM PR D M QLARR L R R E G P-----	135
CENP-A	T L P K D V QLARRIG L E E GL G -----	139

Figure 15. Amino acid sequence alignment of human H3 variants (adapted from Hamiche and Shuaib, 2012). The variation in amino acid residues between H3.3, H3.1, H3.2, H3t and CENP-A are highlighted in red. Despite the high sequence

homology between H3.3, H3t and H3.1/2, H3.3 is specifically recognized and escorted by dedicated chaperones leading to its enrichment at particular genome sites.

4.4.1. The canonical histones H3.1 and H3.2

Canonical histone H3.1 is only found in mammals while H3.2 is present in all eukaryotic organisms except yeast (Bernstein and Hake, 2006). Histones H3.2 and H3.1 differ from only one amino acid substitution at position 96 (S96C), which is why these two proteins are often confused. However, mass spectrometric analyses have clearly shown that the expression level and post-translational change profile of histones H3.1 and H3.2 are different and specific (Hake et al., 2006; Garcia et al., 2008). Indeed, it has been shown that H3.1 is associated with post-translational modifications of the constitutive heterochromatin such as the dimethylation of the lysine residue 9 but also the monomethylation of the lysine residue 64 and the acetylation of the lysine residue 14. In contrast, H3.2 is associated with post-translational modifications such as dimethylation and trimethylation of the lysine residue 27, characteristic of optional heterochromatin (Hake et al., 2006). These functional differences can be explained by a hypothesis involving the cysteine residue 96. Indeed, it has been proposed that the cysteine residue 96 of H3.1 may be involved in the formation of disulfide bridges with neighbouring nucleosomes, lamin B receptors and other factors, thus promoting chromatin condensation (Hake et al., 2006).

4.4.2. H3t

The histone variant H3t, specifically found in mammalian testicles, has four amino acid substitutions compared to H3.1 (A24V, V71M, A98S and A111V) (Witt et al., 1996) (Figure 15). A recent study showed that substitutions at positions 71 and 111 cause instability of nucleosomes containing histone H3t, which could play a role in

spermatogenesis by promoting the replacement of histones by protamines (Tachiwana et al., 2010). Although H3t is predominantly expressed in the testis, this variant has also been detected in small amounts in the HeLa cell line (Andersen et al., 2005), mouse embryos, and in the brain and spleen of adult mice (Govin et al., 2007). To date, the function of the somatic fraction of the histone H3t remains to be determined.

4.4.3. H3.3

In humans, the histone variant H3.3 is encoded by the genes H3F3A and H3F3B, located on chromosomes 1 and 17 respectively. This variant has a 96% identity percentage with H3.1, *i.e.* five amino acid substitutions (A31S, S87A, V89I, M90G and C96S) (Figure 15). Despite these small differences, it has been shown that H3.3 behaves in a very specific way. Indeed, H3.3 is expressed throughout the cell cycle and can be incorporated into chromatin in a way that is dependent and independent of DNA replication, unlike H3.1 (Ahmad and Henikoff, 2002b). Mutagenesis experiments in *Drosophila* have been carried out to better understand these differences. The S31A, A87S, I89V, G90M mutations of histone H3.3 do not affect its behaviour, however, only one of the S87A, V89I, M90G mutations on histone H3.1 causes its partial incorporation, independent of replication (Ahmad and Henikoff, 2002c).

Classically, H3.3 is considered to be an active transcription marker (Shi, 2017). This is supported by results demonstrating a specific enrichment of H3.3 in post-translational modifications associated with transcription activation, such as lysine residue 4 methylation and lysine residue 9 acetylation (McKittrick et al., 2004; Hake et al., 2006). Indeed, in embryonic stem cells, it has been shown that histone H3.3, associated with the trimethylation of lysine residues 4 and 27, is enriched at the site of initiation of transcription of active and non-active genes (Goldberg et al., 2010). These

observations highlight the notion of bivalent promoter regions, where activating (such as H3K4me3) and repressive (such as H3K27me3) post-translational modifications coexist to allow a fine and rapid regulation of transcription (Vastenhouw and Schier, 2012) (Figure 16). However, it is possible that one allele of the gene is associated with heterochromatin, and the other one with euchromatin. This would give the appearance of bivalent chromatin (Figure 16).

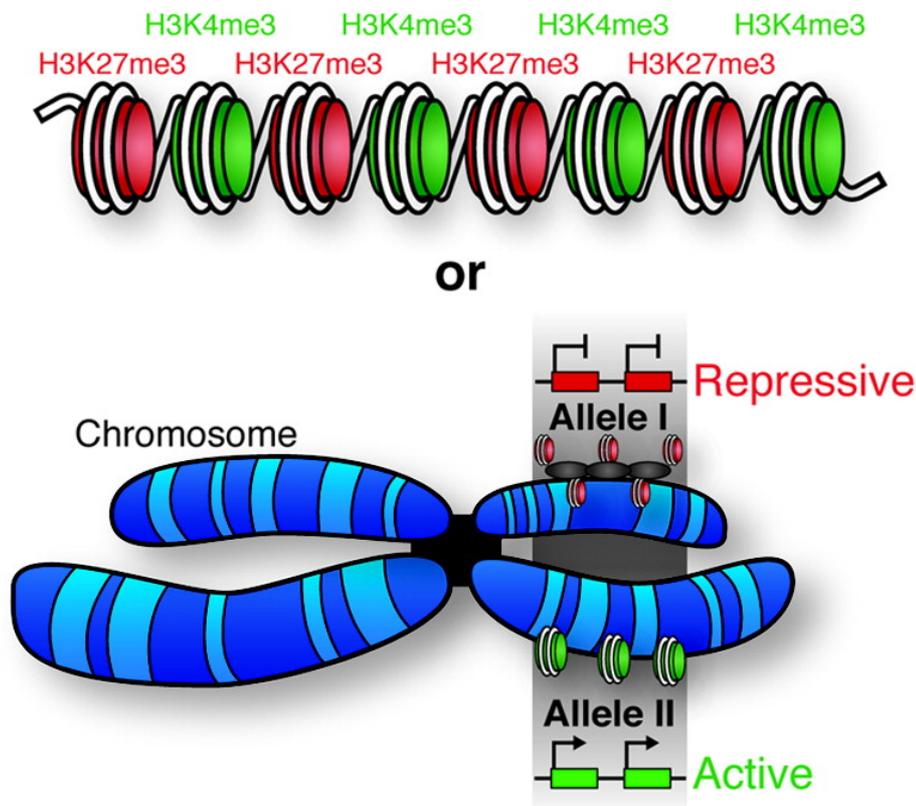


Figure 16. Bivalent promoter regions (Adapted from Tollervey and Lunyak, 2012). Some chromatin regions have been identified with both activating (H3K4me3) and repressive (H3K27Me3) histone marks; termed bivalent chromatin. It is possible that these PTMs occur on neighboring nucleosomes, however, it is also possible that one allele of the gene is associated with heterochromatin, and the other euchromatin. This would give the appearance of bivalent chromatin.

Other studies have highlighted the importance of H3.3 in the regulation of several types of repeated sequences. In embryonic stem cells, it has been shown that the incorporation of histone H3.3 is involved in regulating the transcription of telomeric

repeat sequences (Goldberg et al., 2010) and that maintaining the integrity of these regions is involved in the development of certain cancers (Wong et al., 2009; 2010). Histone variant H3.3 also plays a major role in regulating the transcription of pericentric chromatin repeat sequences. In mice, recent studies have shown that a lack of incorporation of H3.3 in these regions leads to a collapse of the transcription of pericentric repeat sequences (Drané et al., 2010). Nevertheless, in humans, the functional role of pericentromeric transcripts remains largely misunderstood to this day.

Several studies have shown the involvement of H3.3 in cancer processes. In particular, it has been shown that mutations in the N-terminal region of H3.3, including lysine 27 and glycine 34 residues, are found in several types of very aggressive brain tumours (Schwartzentruber et al., 2012; Wu et al., 2012). In addition, it has been shown that some proteins involved in the incorporation of H3.3 into chromatin, such as ATRX, are implicated in other diseases. These data highlight the importance of the deposition mechanisms that will be addressed below.

4.4.4 CENP-A

The CENP-A histone variant is specifically incorporated at the centromere in all eukaryotes (Smith, 2002). The centromere is a specialized region of eukaryotic chromosomes required for the assembly of kinetochores. This region is therefore of vital importance for genome stability. Indeed, it has been shown that a lack of chromosome segregation can lead to aneuploidy and tumor formation (Lv et al., 2012). Numerous studies have shown that CENP-A is required for the assembly and maintenance of active centromeres (Vafa and Sullivan, 1997; Ahmad and Henikoff, 2001; Shuaib et al., 2010). Unlike the other H3 variants, CENP-A has a low percentage of identity with H3.1, or 42%. In addition, during evolution, the N-terminal end of CENP-

A has low conservation in sequence and length. In order to ensure the specific incorporation of CENP-A at the centromere level, some work suggests that this variability would be the result of an adaptation to the inter-species variability that exists at the level of centromeric repeat sequences (Cooper and Henikoff, 2004).

To date, the only known post-translational modification of CENP-A are the phosphorylation of the serine 7 residue by Aurora B kinase during mitosis prophase (Zeitlin et al., 2001) and the ubiquitination of lysine 124 which is involved in its deposition (Niikura, 2017).

4.4.5. Other variants of histone H3 (H3.X, H3.Y and H3.5)

Two variants of the histone H3, H3.X and H3.Y, encoded by genes located on chromosome 5, have been identified (Wiedemann et al., 2010). These variants, found only in primates, have amino acid substitutions preserved and modified in the sequence of histones H3.1, H3.2 and H3.3; in humans, variants H3.X and H3.Y are expressed in small amounts in bone tissue, breasts, lungs, testicles, certain areas of the brain, ovarian tumor tissue and the U2OS cell line (Wiedemann et al., 2010). Another new histone H3 variant, H3.5, has been identified in the testicles, in association with regions of active transcription (Schenk et al., 2011). However, its function is still unknown.

5. Deposition of core histones

The deposition of histones during chromatin assembly is an ordered process that is assisted by histone chaperone proteins. In the context of chromatin assembly, histone chaperones are defined as proteins that bind to histones to ensure their incorporation into DNA without being present in the final product. Following the discovery of the first

histone chaperone in 1978, then called nucleoplasmin (Laskey et al., 1978), many similar proteins were identified and characterized. Histone chaperones are involved in the deposition, dependent or independent of replication, of histones on DNA but also in their storage and transfer within the cell, their exchange with other histones, and their removal from the nucleosome. In addition, chaperones prevent non-specific interactions that could occur between histones, DNA and other proteins. Finally, chaperones are most likely to be involved, directly or indirectly, in post-translational modifications of histones.

5.1. Deposition of histones H3-H4

Classically, there are two main ways of depositing H3-H4 histones on chromatin: replication-coupled deposition (RC) and replication-independent deposition (RI). The RC and RI deposition processes have been described from yeast to humans, although yeast has only one H3 histone, similar to the H3.3 histone found in mammals. *In vivo*, nucleosome assembly is preceded by several steps: synthesis and folding of histones in the cytoplasm, transfer to the nucleus and recruitment on DNA at specific deposition sites. This flow must be regulated and guided to ensure the essential functions of histones.

5.1.1. From the cytoplasm to the nucleus

Following histone synthesis, transfer from the cytoplasm to the nucleus is the first step in the deposition process involving histone chaperones. The protein Asf1 (Anti-Silencing Function 1), the first chaperone identified, supports histones in the cytoplasm to transfer them to the nucleus and other chaperones such as CAF-1 (Chromatin

Assembly Factor 1) and HIRA (Histone Regulatory Homolog A) (Tyler et al., 1999; Groth et al., 2005). Biochemical and structural studies have shown that Asf1 specifically binds to the dimer H3-H4 (English et al., 2005; 2006), thus preventing the formation of the tetramer H3-H4. It has been shown that the synthesis and transport of newly synthesized histones are related processes that involve multi-protein chaperone complexes and specific post-translational modifications (Jasencakova et al., 2010). For example, acetylation of lysine residues 5 and 12 of histone H4, conserved in many eukaryotes and catalyzed by holoenzyme HAT1-RbAp46, is characteristic of newly synthesized histones (Verreault et al., 1998).

The purification of proteins associated with histone H3 has led to a better understanding of the assembly and transfer mechanisms of H3-H4 dimers. In the cytoplasm, histones H3 and H4 are associated with HSC70, HSP90, tNASP, sNASP, RbAp46, Asf1a/b, importin 4 and HAT1 proteins (Campos et al., 2010; Alvarez et al., 2011). After their synthesis, histones H3 and H4 interact with HSP90 and HSC70 to ensure functional folding and prevent aggregation phenomena (Campos et al., 2010; Alvarez et al., 2011). Before transfer to the nucleus and deposition, the proteins HSP90 and tNASP facilitate the formation of the H3-H4 dimer (Campos et al., 2010). Once the H3-H4 dimer is assembled, RbAp46 associates with the C-terminal end of H4 and induces HAT1 recruitment which allows acetylation of lysine residues 5 and 12 of histone H4 (Campos et al., 2010). The acetylated H3-H4 dimer is then transferred to Asf1a/b and importin 4 to provide transport to the nucleus (Campos et al., 2010; Alvarez et al., 2011).

5.1.2. Deposition of histones on chromatin

The analysis of soluble nuclear complexes, known as "pre-assembly complexes", associated with H3 histones allowed to identify the proteins HJURP, CAF-1, Asf1a/b, HIRA and DAXX as major chaperones involved in the specific deposition of H3 histones. The CAF-1 chaperone provides RC deposition of the canonical histones H3, while the RI deposition of variant H3.3 is provided by the proteins HIRA and DAXX. Asf1a/b proteins play a role in both deposition modes. To date, the chaperones involved in the deposition of H3t, H3.X, H3. Y and H3.5 are unknown.

5.1.3. *Replication-dependent deposition*

Canonical histones H3.1 and H3.2 are specifically expressed and deposited during the replication phase of the cell cycle. In S phase, the amount of DNA is doubled, which implies the need to form new nucleosomes. Two sources of histones are then available within the cell: the "old" histones, and the newly synthesized histones. Classically, it is accepted that nucleosomes are disassembled into two dimers H2A-H2B and a H3-H4 tetramer during replication (Annunziato, 2005). The nucleosomes are then reassembled on the strands of daughter DNA with the "old" histones and the "new" histones. There are three models to explain the distribution of these histones after replication: (i) the random model, in which the old and new histones are randomly incorporated; (ii) the semi-conservative model, in which the old histones are equally distributed over the strands of daughter DNA which are then completed by the new histones; (Mori, 2002) the asymmetric model, in which all the old histones are incorporated into a single strand of son DNA, the other strand being the new histones. These models are not necessarily exclusive, but rather context-specific, such as cell type or nuclear domain (Probst et al., 2009). Recent study coming from the Groth

laboratory showed that parental histones H3-H4 segregate to both daughter DNA strands with a weak leading-strand bias (Petryk, 2018).

It has been shown that CAF-1 provides RC deposition of the canonical histones H3 (Figure 17). In vitro, CAF-1 can also promote the assembly of chromatin on SV40 virus replicative DNA (Smith and Stillman, 1989). In mammals, the CAF-1 deposition complex is strongly conserved and consists of the subunits p150, p60 and p48. It has been shown that the p150 subunit is recruited at DNA replication sites via direct interaction with PCNA (Proliferating Cell Nuclear Antigen) (Krude, 1995; Marheineke and Krude, 1998; Shibahara and Stillman, 1999; Moggs et al., 2000; Gérard et al., 2006). In vivo, CAF-1 complex specifically interacts with canonical histones H3.1 and H3.2 but not with variant histone H3.3 These observations clearly demonstrate CAF-1's direct involvement in RC deposition (Tagami et al., 2004).

In the nucleus, the newly synthesized H3-H4 dimers are first associated with the Asf1 protein and then transmitted to the CAF-1 complex. This transfer is facilitated by the interaction between Asf1 and p60 which has been demonstrated in vitro and in vivo (Tyler et al., 2001) although the exact transfer mechanism of H3-H4 dimers remains poorly characterized.

5.1.4. *Replication independent deposition of H3.3*

Unlike canonical H3 histones, the expression and deposition of the histone H3.3 variant takes place throughout the cell cycle. Histone H3.3 is incorporated into DNA in specific regions of the genome, suggesting specific mechanisms and deposition factors. Indeed, it has been shown that the incorporation of the histone variant H3.3 into chromatin is ensured by the specific chaperones HIRA and DAXX.

5.1.5. Deposition of H3.3 by HIRA

Historically, HIRA has been the first specific chaperone of the histone variant H3.3. It was shown *in vitro* that the RI assembly of chromatin is facilitated by the HIRA protein in *Xenopus* egg extracts (Ray-Gallet et al., 2002) and that histones interact specifically with this HIRA (Lorain et al., 1998). Subsequently, the purification of the pre-assembly complexes of H3.1 and H3.3 made it possible to identify, in HeLa cells, the specificity of the CAF-1 and HIRA chaperones (Tagami et al., 2004). It has also been shown that the enrichment of H3.3 in many genes, transcribed or repressed, is dependent on the HIRA chaperone in embryonic stem cells (Goldberg et al., 2010) (Figure 17). However, the interaction of HIRA with H3.3 was recently challenged by two recent studies showing that it is Ubinuclein which confers H3.3 recognition and selectivity to the HIRA complex (Ricketts, 2015; Xiong, 2018). These data suggest that Ubinuclein is the H3.3 bona fide histone chaperone.

In *Drosophila*, HIRA is involved in the deposition of H3.3 during male pronucleus decondensation, but is not required in embryonic and adult cells (Loppin et al., 2005), suggesting other specific mechanisms for deposition of the H3.3 variant histone. The protein Asf1, also present in the H3.3 complex, is most probably also involved in the transfer of H3.3-H4 dimers to chromatin.

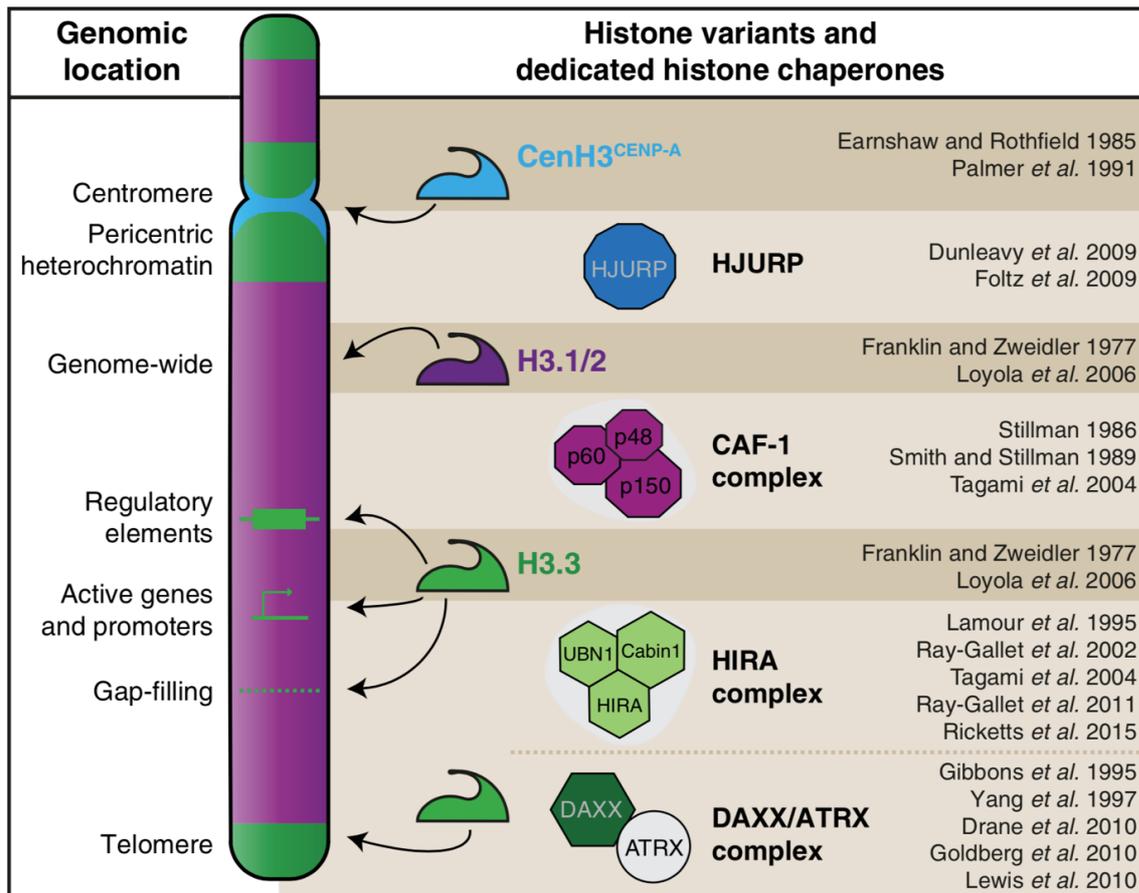


Figure 17. Histone variants and their dedicated chaperones for H3 family (from Sitbon *et al.*, 2017). *CenH3* is incorporated at centromeres by *HJURP*. *H3.3* is incorporated at regulatory elements by the *HIRA* complex and at telomeres and by the *DAXX/ATR* complex at pericentric heterochromatin. Canonical *H3.1* is incorporated genome-wide by the *CAF-1* complex.

5.1.6. Deposition of H3.3 by DAXX

Initially, DAXX (Death domain-associated protein 6) was described as an apoptosis regulatory protein, linked to the FAS receptor and the JNK signalling pathway (Jun N-terminal Kinase) (Yang *et al.*, 1997). Other data have also suggested that DAXX protein is involved in stabilizing ubiquitin ligase Mdm2, which is involved in the degradation of p53 (Tang *et al.*, 2006). In addition, a role in controlling transcription through the suppression of certain transcription factors, including Pax3 (Hollenbach *et al.*, 1999) and ETS1 (Li *et al.*, 2000), has been described.

Our group and David Allis' group defined DAXX as a specific chaperone for the histone variant H3.3 following the accumulation of clear data from different study models. Indeed, DAXX is an acid protein (pI 4,6) which has a domain rich in glutamic and aspartic acid contained in a domain homologous to the Rtt106 domain found in yeast. This type of domain is a characteristic shared by many histone chaperones such as yeast protein Rtt106 (Huang et al., 2005), nucleoplasmin (Dutta et al., 2001) and the FACT complex (Belotserkovskaya et al., 2003). In view of these data, our work and that of David Allis' team sought to highlight DAXX's chaperoning activity. First, it was shown, in HeLa cells, that the protein DAXX, associated with the ATRX chromatin remodeler, is a major and specific component of the preassembly complex of the histone variant H3.3 (Drané et al., 2010), absent from the preassembly complex of the canonical histone H3.1. In addition, the acid domain of DAXX is involved in the direct and specific interaction with H3.3 (Drané et al., 2010), and the "AAIG" motif, containing three of the substitutions of H3.3 with respect to H3.1, carries this specificity (Lewis et al., 2010). Finally, in vitro, the deposition of the recombinant H3.3-H4 tetramer on bacterial DNA is facilitated by the presence of DAXX (Drané et al., 2010). All these data clearly show that the DAXX protein is a specific chaperone of the histone variant H3.3 (Figure 18).

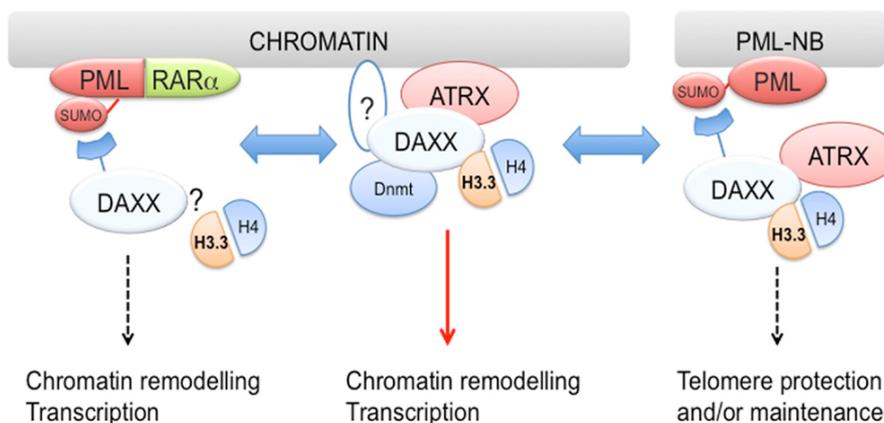


Figure 18. DAXX and H3.3 associate with both PML-NBs (Salomoni 2013). DAXX and H3.3 are found at PML-NBs, suggesting that PML may regulate H3.3 loading to

heterochromatin. DAXX association with PML-RAR α is required for transformation in vitro. Although it is presently unknown whether H3.3 also associates with PML-RAR α , it is conceivable that PML-RAR α via interaction with DAXX could modulate H3.3 loading.

The existence of two deposition complexes (DAXX and HIRA) specific for histone H3.3 has been partially explained by several studies. Indeed, it has been shown that DAXX-ATRAX and HIRA complexes incorporate H3.3 in different regions of the genome. In embryonic stem cells, the enrichment of histone H3.3 in many genes, transcribed or repressed, is dependent on the HIRA chaperone. Nevertheless, the invalidation of HIRA does not lead to significant changes in the overall genome expression profile (Goldberg et al., 2010), suggesting the existence of compensatory mechanisms. In contrast, the incorporation of histone H3.3 in telomeric and pericentromeric regions is independent of the HIRA chaperone and is provided by the DAXX-ATRAX complex in embryonic stem cells (Drané et al., 2010; Goldberg et al., 2010).

It is important to note that DAXX protein is also, together with SP100 protein, a major component of PML nuclear bodies (ProMyelocytic Leukemia) which have been involved in alternative telomere extension mechanisms characteristic of certain cancers (Heaphy et al., 2011b; Chung et al., 2012), as well as in the formation of heterochromatin foci associated with senescence (Ye et al., 2007; Rai and Adams, 2012). In addition, it has been shown that the deposition of histone H3.3 in pericentromeric repeat sequences is specifically ensured by the DAXX-ATRAX complex in murine embryonic fibroblasts through the PML-NBs (Drané et al., 2010).

5.1.7. Deposition of H2A-H2B histones

The incorporation of H2A-H2B dimers into the chromatin also involves several proteins. In the cytoplasm, it has been shown that the binding and transfer to the nucleus of H2A-H2B dimers is ensured by the NAP1 protein (Nucleosome Assembly Protein 1) (Mosammaparast et al., 2002). In vitro, it has also been shown that NAP-1 allows nucleosome assembly (Ishimi et al., 1987; Chang et al., 1997). However, biochemical studies indicate that NAP-1 is preferentially associated with histones H3 and H4 when all histones of the core are present (McBryant et al., 2003; Mazurkiewicz et al., 2006; Eckey et al., 2007).

In yeast, the deposition of the histone variant Htz (homologous to H2A.Z) involves the Chz1 protein and the SWR1 complex (Korber and Hörz, 2004; Luk et al., 2007). The CHZ pattern, found in the human protein HIRIP3 (HIRA-Interacting Protein 3), is characteristic of certain factors that interact with histones (Lorain et al., 1998). In addition, p400 and SRCAP complexes (homologues of the SWR1 complex) can facilitate the exchange between the H2A-H2B and H2A.Z-H2B dimers within the nucleosome (Gévry et al., 2007). As a result, HIRIP3, p400 and SRCAP could be involved in the specific deposition of H2A.Z, however, to date, these results have yet to be specified. Finally, it has been shown that the FACT complex is involved in the exchange between the dimers γ -H2A.X-H2B and H2A.X-H2B (Heo et al., 2008), although no specific H2A.X deposition activity has been identified. The macroH2A and H2A.Bbd variant histones do not have specific chaperones identified to date.

6. Histone H3.3 biological functions

The histone variant H3.3 diverge from the canonical H3.1 histone by only 5 amino acids. Despite this high homology, H3.1 cannot rescue H3.3 loss and the two

histones play different biological functions (Couldrey et al., 1999; Bush et al., 2013; Tang et al., 2015). The histone variant H3.3 has been implicated in several important nuclear processes, including gene regulation, DNA repair and chromosome segregation. However, its exact biological functions remain unknown. Below, we will describe some of the current knowledges about histone H3.3 biological functions.

6.1. *Role of H3.3 in transcription activation*

The histone H3.3 variant was initially found associated with transcriptionally active chromatin enriched in active post-translational modifications (Ahmad and Henikoff, 2002; McKittrick et al., 2004). The deposition of H3.3 was shown to be uncoupled from replication (Schwartz and Ahmad, 2005). In contrast to the canonical histone, H3.3 was found to play an active role in the maintenance of an accessible chromatin structure at enhancer and actively transcribed genes. In addition, nucleosomes harboring the hybrid H3.3/H2A.Z histones have been shown to be intrinsically unstable and to promote gene activation (Jin and Felsenfeld 2007; Jin et al. 2009; Chen et al. 2013). Accordingly, the histone H3.3 variant was found enriched at transcription start sites (TSS) of both active and repressed CG-rich promoters, and in the gene bodies and transcriptional end site of active genes (Goldberg et al., 2010). Histone H3.3 has been shown to promote transcriptional recovery after a genotoxic stress in a HIRA-dependent manner (Adam et al., 2013). The deposition of H3.3 by HIRA has been shown to be stimulated by interferon to regulate interferon stimulated genes (Bachu et al., 2019).

However, despite mounting evidence about the association of H3.3 with active chromatin (Goldberg et al. 2010) its role in transcription activation is still debated and whether H3.3 deposition indeed drives transcription or simply reflects the need to

recruit a replacement histone during transcription. Accordingly, several studies reported that the absence of H3.3 has only a mild effect on basal or activated transcription (Bush et al., 2013; Jang et al., 2015, Ors et al., 2017). This leads to the conclusion that H3.3 might not play a pivotal role in transcriptional activation despite its importance during development.

6.2. H3.3 function during development

One of the most important function of H3.3 is its role in the early step of development. After oocyte activation, the canonical histone H3.1 is replaced by maternal-derived H3.3 in the donor nucleus through the HIRA-dependent pathway. Immediately after fertilization, maternal H3.3 invades paternal chromatin by replacing protamines (Loppin et al., 2005; Torres-Padilla et al., 2006). In addition, HIRA-mediated H3.3 deposition is also required for rRNA transcription in addition to its role in parental genome reprogramming (Lin et al., 2014).

Loss or inactivation of the two H3.3 genes in flies has no effect on embryonic and postnatal development but leads to infertility (Hodl and Basler 2009; Sakai et al. 2009). Surprisingly, the fertility problem can be rescued by overexpressing H3.2 (Hodl and Basler, 2012). Conversely, overexpression of H3.3 in S-phase can also rescue growth defects in H3.2-null flies (Hodl and Basler, 2012). In flies, it appears that only the overall level of H3 histones is important for fly development and the H3.3 variant is interchangeable with canonical histones. In *Caenorhabditis elegans*, the complete loss of H3.3 has not effect on viability or fertility (Piazzesi et al., 2016). However, in more developed organism such as *Xenopus*, H3.3 loss leads to gastrulation defects that cannot be rescued by canonical histones (Szenker et al., 2012). The histone H3.3 was

found important for the proper cranial neural crest cell differentiation in zebrafish, (Cox et al., 2012).

Inactivation of both *H3f3a* and *H3f3b* genes in mice led to early embryonic lethality and double *H3f3a*^{KO/WT} / *H3f3b*^{KO} mutant males are totally infertile, while double *H3f3a*^{KO/WT} / *H3f3b*^{KO} females are fertile, revealing a specific role of H3.3 in spermatogenesis (Jang et al. 2015). Another study showed that H3.3B depletion is alone sufficient to generate infertility in mice (Yuen et al. 2014). Other reports claimed that *H3f3b* heterozygotes were growth-deficient with males being sterile because of problems at the level of round spermatids, while H3.3A depleted males were found subfertile with dysmorphic spermatozoa (Daniel Ricketts et al. 2015). A hypomorphic gene-trap mutation in *H3f3a* led to high mortality by weaning and the surviving animals exhibited severe infertility (Couldrey et al. 1999). Taken as a whole, these data demonstrate that H3.3 is an important player in spermatogenesis. The various genetic background of the animals used could explain the observed different impact of H3.3A and H3.3B on the progress of spermatogenesis in the different studies. The molecular mechanism of H3.3 function during spermatogenesis and in particular, the impact of the H3.3 deficiency on transcription at genome-wide level, are, however, only superficially addressed.

H3.3 was also found to play an important role in early development. H3.3 mutations such as H3.3R26 and H3.3K27 have been shown to be essential for proper oogenesis and good partitioning of the cells to the inner cell mass of the early embryo (Zhou et al., 2017). H3.3 also plays a key role in cell fate transition by maintaining the parental cell identities during reprogramming and by regulating genes implicated in the lineage reprogramming (Fang et al., 2018b). In embryonic stem cells (ESC) H3.3 was found to be required for the developmentally regulated bivalent promoters harboring

both by the activation-associated H3K4me3 mark and the repression-associated H3K27me3 mark (Bernstein et al., 2006). H3.3 was shown involved in the recruitment of the Polycomb-repressive complex 2 (PRC2) (Banaszynski et al. 2013).

6.3. H3.3 and mitosis

Inactivation of H3.3 in mouse embryonic stem cells or mouse embryonic fibroblasts caused several important mitotic defects such as lagging chromosomes and chromosome bridges during anaphase (Jang et al., 2015) (Ors et al., 2017). H3.3 is also involved in DNA replication by maintaining the replication fork and in the transcription restart after UV damage (Adam et al., 2013; Frey et al., 2014). These effects are likely to be mediated by histone H3.3 N-terminal tail. Deletion of H3.3 tail was shown to lock the cells in the senescent process (Duarte et al., 2014).

6.4. Heterochromatin

One of the most important function of H3.3 is its role in telomere function, heterochromatin formation and mobile elements silencing. H3.3 is deposited at pericentromeric heterochromatin and telomeres by the DAXX/ATRX complex (Drané et al., 2010; Goldberg et al., 2010). Mouse embryonic fibroblast lacking H3.3 have a decondensed chromatin at telomeres, centromeres and pericentromeres (Jang et al., 2015).

H3.3 deposition at telomeric (TTAGGG)_n repeats is dependent on ATRX in mESC (Wong et al., 2009; Goldberg et al., 2010). H3.3 deposition at telomeres involve other factors such as DEK and HP1 γ (Ivanauskiene et al., 2014). The interaction between ATRX, HP1 γ and H3.3 is important in the maintenance of telomere structural

integrity (Wong et al., 2010). The deposition of the repressive H3.3K9me3 methylation was shown to serve as an ATRX docking site and is essential for telomere repression (Udugama et al., 2015).

H3.3 also plays a key role at pericentric heterochromatin where it is deposited by the ATRX/DAXX complex in a PML-dependent manner (Delbarre et al., 2017) (Drané et al., 2010). The recruitment of H3.3 by DAXX into the PML nuclear bodies has been shown to be essential for the transcriptional regulation of pericentromeric satellite repeats in both mouse and human (Morozov et al., 2012).

H3.3 was also found enriched at other repeats such as endogenous retroviral elements (Elsässer et al., 2015) containing long terminal repeats (LTRs). In mouse embryonic stem cells, ERVs form a subset of transposable elements repressed by the KAP1 complex in a H3K9me3 dependent manner (Rowe et al., 2010; Rowe et al., 2013b). Accordingly, depletion of H3.3 in ESCs resulted in ERVs de-repression and reactivation (Elsässer et al., 2015). Thus, H3.3 seems to play a critical role in ERVs silencing.

In conclusion, H3.3 plays an important role yet underexplored in promoting H3K9me3 formation, heterochromatin propagation and mobile elements silencing.

6.5. Histone H3.3 mutations in cancer

Recently, sequencing of tumor cells revealed that histone H3.3 is frequently mutated in various tumors including pediatric high-grade glioma (pHGG), with up to 80 % of diffuse intrinsic pontine gliomas (DIPGs) carrying K27M mutation and 36 % of non-brainstem gliomas carrying either K27M or G34R/V mutations (Schwartzentruber et al. 2012; Wu et al. 2012). Both groups reported recurrent somatic heterozygous mutations in the gene encoding the histone variant H3.3A (i.e., *H3F3A*). This was the

first demonstration that histone mutations may be drivers of disease (Vogelstein et al. 2013) (Figure 19).

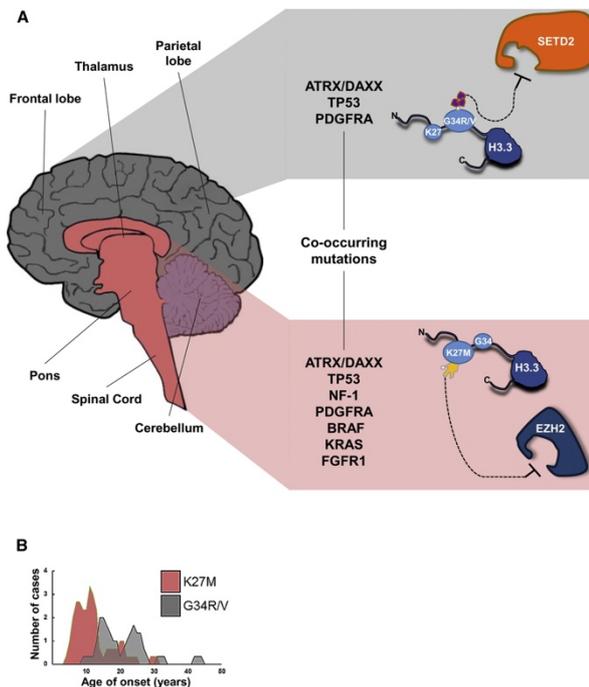


Figure 19. Histone H3.3 mutations in cancer (Yuen and Knöpfner, 2013). K27M and G34R/V mutations of H3.3 (*H3F3A*) display distinct characteristics from one another.

(A) G34R/V mutations (gray, top) in *H3F3A* localize primarily to cerebral/cortical hemispheres. K27M mutations (pink, bottom) in *H3F3A* localize primarily to midline locations, including the spinal cord, thalamus, pons, and brainstem. G34R/V mutations overlap with mutations in *TP53* and *ATRX/DAXX* and *PDGFRA*. K27M mutations overlap with mutations in *TP53* and *ATRX/DAXX*, *NF-1*, *PDGFRA*, *BRAF*, *KRAS*, and *FGFR1*.

(B) Age of onset of H3.3-mutated tumors. K27M mutations are more prevalent in younger patients (median age, 12 years) while G34R/V mutations are more prevalent in older patients (median age, 20 years).

Intriguingly, H3.3 mutations are also found to simultaneously overlap with other specific mutations within the same tumor. Approximately 30% of K27M mutations are associated with mutations in *ATRX/DAXX* and 60% with mutations in the tumor suppressor gene *TP53* (coding for p53)(Schwartzentruber et al. 2012). K27M mutations have also been found at much lower frequency alongside mutations in *NF-1*, *PDGFRA*, *BRAF*, *KRAS*, and *FGFR1* in gliomas (Khuong-Quang et al. 2012; Schwartzentruber et al. 2012). Meanwhile, G34R/V mutations completely overlap with

tumors containing mutations in TP53 and ATRX/DAXX, but have also been found together with mutations in PDGFRA (Schwartzentruber et al. 2012). Tumors with mutations in H3F3A, TP53, and ATRX/DAXX contain a higher abundance of copy number alterations (Schwartzentruber et al. 2012), although there was not a clear association between increases in copy number alterations and H3F3A mutations alone. Given that H3F3A mutations are found in heterozygous form and that these gliomas exhibit similar rates of TP53 mutation with other glioma types, H3.3K27M and H3.3G34R/V may act as driver mutations with TP53 mutations occurring as a second hit (Khuong-Quang et al. 2012; Schwartzentruber et al. 2012), although it is important to note that G34R/V mutations display much higher association with mutations in ATRX/DAXX and TP53.

Despite these scientific advances, the mechanistic aspects of H3.3 mutation functions are largely unknown. It is tempting to speculate that H3.3 mutations affect heterochromatin formation and mobile elements silencing.

II. RESULTS

An alternative histone H3.3 deposition pathway using DAXX and CAF-1 targets X-chromosome and DNA repeats

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Abbreviations: DAXX, death domain-associated protein; CAF-1, chromatin assembly factor 1; ATRX, α -thalassemia mental retardation X-linked; MJS, major satellite repeats; PML-NBs, promyelocytic leukemia nuclear bodies

Keywords: DAXX, CAF-1, histone H3.3, X-chromosome, Repetitive elements.

Abstract

The histone chaperone DAXX targets H3.3 to pericentric and telomeric heterochromatin in a replication independent manner. The mechanism that ensures appropriate recruitment of DAXX to heterochromatic region is largely unknown. Using proteomic and biochemical approaches, we show that in addition to the well-known ATRX/H3.3 complex, DAXX forms an alternative complex with CAF-1, ADNP, HP1 and Trim28. DAXX physically interact with the C-terminus of CAF1-p150 subunit through its N-terminal domain. The DAXX SIM domain was found to be essential for targeting DAXX to PML-NBs, for the recruitment of CAF-1 to heterochromatin. Our genomic data further revealed that DAXX targets CAF-1 to X-chromosome and repetitive elements. Inactivation of DAXX results in major depletion of CAF-1 from X-chromosome SINEs and LINEs. Our data point to a novel function of DAXX and CAF-1 in targeting H3.3 to X-chromosome.

Introduction

The packaging of the genome within the complex chromatin organization plays a key role in the control of its activity. The basic unit of chromatin, the nucleosome, is composed of a histone octamer (two each of H2A, H2B, H3 and H4) around which two superhelical turns of DNA are wrapped (Luger et al., 1997). All the conventional histones, except H4, have histone variants. Histone variants are non-allelic forms of the conventional histones (van Holde, 1988). The incorporation of histone variants into the nucleosome is implicated in the regulation of several epigenetics phenomena (reviewed in (Ahmad and Henikoff, 2002a; Malik and Henikoff, 2003)). Indeed, H2A and H3 variants have diversified to assume roles in epigenetic silencing and centromere and telomere functions (Amor et al., 2004; Grewal and Jia, 2007; Henikoff and Dalal, 2005; Van Hooser et al., 2001). The Histone variant H3.3 marks active chromatin by replacing the conventional histone H3.1 but its exact function is remains elusive (Ahmad and Henikoff, 2002b, c; Hamiche and Shuaib, 2012). The histone variant H3.3 is implicated in several important nuclear events, including transcription and mitosis (Goldberg et al., 2010; Jang et al., 2015; Jin and Felsenfeld, 2007; Ors et al., 2017). In mice, H3.3 becomes enriched at paternal chromosomes, thus being involved in an important role of *de novo* heterochromatin formation at pericentromeric regions (Akiyama et al., 2011; Sugimura et al., 2010). It has also been shown that interfering with major satellite transcription prevents the formation of chromocenter during early stage of mammalian development (Probst et al., 2010). H3.3-containing nucleosomes are considered as of lower stability than nucleosomes with other variants of H3 (Jin and Felsenfeld, 2007) thus this property may mediate the promotion of pericentromeric DNA repeats transcription (Boyarchuk et al., 2011). This might come from the fact that their transcription is facilitated by reducing the energy required to evict nucleosomes from active genes.

Genome-wide analysis of the H3.3 distribution has shown that H3.3 is located on promoters, enhancers, gene bodies and both pericentromeric and telomeric heterochromatin (Goldberg et al., 2010; Jang et al., 2015; Jin and Felsenfeld, 2007; Ors et al., 2017), but how does H3.3 act on these elements is not clear. The turnover of H3.3 depends on its localization, suggesting that the residence time of H3.3 association with specific chromatin loci is linked to its function (Huang and Zhu, 2014).

H3.3 is also involved in spermatogenesis. P element transposition-deletion of both

H3.3A and *H3.3B* in *Drosophila* resulted in severe infertility and reduced viability (Sakai et al., 2009). Similarly, inactivation of both *H3f3a* and *H3f3b* genes in mice led to early embryonic lethality and double *H3f3a*^{KO/WT} / *H3f3b*^{KO} mutant males are totally infertile, while double *H3f3a*^{KO/WT} / *H3f3b*^{KO} females are fertile, revealing a specific role of H3.3 in spermatogenesis (Jang et al., 2015). Another study revealed that H3.3B depletion alone is sufficient to generate mice infertility (Yuen et al., 2014).

H3.1 and H3.3 variants share 96% identity but they exhibit important differences in behavior such as during cell cycle regulation. For instance, H3.1 is synthesized in S phase and is deposited during DNA replication, whereas H3.3 is expressed throughout the complete cell cycle and is incorporated at all phases of the cell cycle (Ahmad and Henikoff, 2002b, c; Schwartz and Ahmad, 2005). H3.1 and H3.3 are histone variants that associate with different chromatin assembly complexes (Drane et al., 2010; Lewis et al., 2010; Tagami et al., 2004). H3.1 was found to be mainly belonging to a complex containing the replication-dependent Chromatin Assembly Factor 1 (CAF-1), whereas H3.3 co-purified with different complexes containing either the HIRA or the DAXX histone chaperones (Drane et al., 2010; Lewis et al., 2010; Tagami et al., 2004). In mouse ESC cells, HIRA was found to be required for H3.3 deposition at promoters and active gene bodies, while DAXX was found essential for H3.3 deposition at telomeres (Goldberg et al., 2010; Wong et al., 2010). DAXX was also found to be required for H3.3 deposition at pericentric heterochromatin in mouse MEF cells (Drane et al., 2010). Interestingly, DAXX was found in a complex with the chromatin-remodeling factor ATRX (α-thalassemia/mental retardation syndrome protein) (Drane et al., 2010; Goldberg et al., 2010; Lewis et al., 2010). In this complex, DAXX cooperates with ATRX for H3.3 deposition at telomeres and pericentromeres (Drane et al., 2010; Goldberg et al., 2010; Lewis et al., 2010; Wong et al., 2010). Previously, it has been shown that DAXX and ATRX colocalize with human pericentromeric DNA repeats within promyelocytic nuclear bodies (PML-NBs) (Luciani et al., 2006). In addition, we found that DAXX colocalizes with H3.3 within PML-NBs and regulates the expression of mouse pericentric DNA repeats (Drane et al., 2010).

In this study, we investigated the function of the histone chaperone DAXX and identified several novel interacting partners. Among the DAXX partners we identified the replication-coupled chromatin assembly factor CAF-1 as one of the main interacting partners. DAXX physically interacted with CAF1-p150 subunit. DAXX was

found to reside into two different H3.3 sub-complexes. A low molecular weight complex containing ATRX and a high molecular weight complex containing CAF-1, ADNP, HP1 α , ADNP, and Trim28. Interestingly, we observed that the Sumo-Interacting Motif (SIM) is required for DAXX proper sumoylation, localization to PML-NBs and association with CAF-1. We observed that CAF1-p150, DAXX and PML-NBs are all indispensable for MJS transcription and ATRX has a repressive role of this latter. Our genomic data further revealed that DAXX targets CAF-1 to X-chromosome and repetitive elements. Inactivation of DAXX results in major depletion of CAF-1 from X-chromosome SINEs and LINEs. Our data point to a novel function of DAXX and CAF-1 in targeting H3.3 to X-chromosome.

Results

Purification of e-DAXX complex and identification of its associated protein partners

The histone chaperone DAXX was previously found to form a stable complex with the chromatin remodeling factor ATRX that targets H3.3 to heterochromatic foci such as pericentromeres and telomeres. However, how DAXX exerts its function at these regions remains to be determined. To get more insight into the function of DAXX, we rescued the DAXX knock-out (*Daxx*^{-/-}) mouse embryonic fibroblast cell lines (MEF) with C-terminal Flag- and HA-epitope-tagged DAXX (e-DAXX) using retroviral transduction (Drane et al., 2010). As expected, e-DAXX expression patterns show a specific staining throughout the nucleoplasm, forming numerous dots corresponding to the DAPI staining of heterochromatic foci and PML bodies (Fig. 1A). Epitope-tagged DAXX complex was then purified by double immunoprecipitation from MEF soluble nuclear extract by sequential immunoprecipitations with anti-Flag antibody followed by anti-HA antibody (Drane et al., 2010). Proteins associated with DAXX were separated by SDS-containing 4%-12% polyacrylamide gradient gels and subsequently silver-stained (Fig. 1B, upper panel). Immunoblotting and mass spectrometry analyses allowed the identification of ATRX and H3.3 as specific components of the DAXX complex (Figs. 1B lower panel, 1C) as we have shown previously (Drane et al., 2010). Surprisingly, additional unexpected partners have been found in this complex, such as the H3.1 specific complex CAF-1 (p150, p60 and p48), the specific heterochromatin marker HP1 α , the ubiquitin ligase Trim28, the ubiquitin hydrolase USP7 and the potential transcription factor ADNP (Figs. 1B, 1C). To eliminate the possibility that we purified a mixture of H3.3 and H3.1 predeposition complexes, we further analyzed the H3.1 content of the H3.3 band by mass spectrometry. Mass spectrometry analyses revealed that the DAXX-associated nuclear complex contains mainly H3.3 since the H3.3/H3.1 ratio was found equal to 20/1 (Fig 1D).

To gain more insight on the composition of the complex, we subjected the e-DAXX complex to separation on glycerol gradient (15%-35%, Fig. 2A). Immunoblotting experiments showed that DAXX-associated proteins fractionated into at least two macro-molecular complexes. In the lower molecular weight complex (LMWC), DAXX is associated with ATRX and H3.3 (Fig. 2A, B). In the high molecular weight complex (HMWC), DAXX and H3.3 were found associated with the CAF-1 chromatin assembly

complex, HP1 α and ADNP and other proteins (Fig. 2B). These experiments show that DAXX belongs to at least two different H3.3 specific macro-molecular complexes. In our previous study, performed on HeLa cells, we observed that two of the three CAF-1 subunits (p150 and p60) were highly specific to the e-H3.1 complex, whereas the third subunit (p46/48) was a component common to e-H3.1 and e-H3.3 complexes (Drane et al., 2010). In the present study, performed on MEF cells, surprisingly we found CAF-1 as part of the DAXX-ATR-X-H3.3 complex.

DAXX physically interacts in vitro with CAF-1

DAXX is an acidic protein (pI 4.6) containing a stretch rich in glutamic and aspartic acid residues embedded within a yeast histone chaperone, Rtt106-like domain, two PAH domains and a S/P/T-rich domain (Fig. 2C). We next hypothesized that DAXX physically interacts with CAF1-p150 subunit, and tried to assess these interactions by pull down assays. FLAG-DAXX and HA-CAF1-p150 were co-expressed in SF9 insect cells and pulled down using anti-FLAG M2 agarose beads. Bead-bound proteins were extensively washed and eluted using FLAG peptide. Eluted proteins were analyzed on SDS-PAGE and stained with colloidal blue (Fig. 2D). The data obtained showed that DAXX physically interacts with CAF1-p150. To further determine the region of DAXX involved in the interaction with CAF1-p150, we generated deletion mutants including the N-terminal (1-302), central (302-495), or C-terminal (495-740) domain of DAXX. These mutants were expressed as fusion proteins with GST and investigated for their binding to either CAF1-p150 or ATRX (Fig. 2E). Immunoblotting using an antibody directed against CAF1-p150 and ATRX revealed that neither the Rtt106-like domain nor the S/P/T-rich domain exhibited binding to either CAF1-p150 or ATRX. In contrast, the region (1-302) encompassing the two PAH (Paired Amphipathic Helix) domains (PAH1 & PAH2), showed strong interaction with CAF1-p150 and ATRX at either 0.15 or 0.5M of NaCl.

We next determined the region of CAF1-p150 involved in the interaction with DAXX. We generated CAF1-p150 deletion mutants including the N-terminal (1-296), central (297-641), or C-terminal (642-938) domain of CAF1-p150 (Fig. 2F). These mutants were expressed in bacteria as 6xHis fusion proteins and tested for their interaction with GST-DAXX (1-302) (Fig. 2G). Immunoblotting using an anti-His

antibody directed against His-CAF1-p150 domains revealed that DAXX interacts exclusively with the C-terminal region of CAF1-p150 (642-938).

These data demonstrate that the N-terminal part of DAXX mediates the interaction with ATRX and the C-terminal part of CAF1-p150. The Rtt106-like domain being involved in a strong interaction with e-H3.3-H4 (Drane et al., 2010), our results suggest that DAXX ensures the link between ATRX or CAF-1 and H3.3 and the two proteins might likely compete for the same binding site on DAXX.

Transient passage of DAXX through PML-NBs is essential for its association with, CAF-1 but not ATRX.

DAXX is a major component of the PML-NBs and directly interacts with ATRX (Drane et al., 2010). Sumoylation of DAXX is essential for its accumulation at PML-NBs. DAXX targeting to PML-NBs is a key event in the regulation of pericentric heterochromatin transcription (Lin, Lucia, Ishov).

The mechanism for heterochromatin repression by DAXX is unknown and the chronology of its sequential transition from PML-NBs to heterochromatin remains to be determined. Hence, we assessed effects of a deletion of the DAXX C-terminal Sumo-Interacting Motif (SIM), corresponding to the last seven amino acid residues of the protein (Fig. 3A). Therefore, DAXX- Δ S protein, lacking the SIM domain, was stably expressed in MEF *Daxx*^{-/-} cells. Unlike e-DAXX wild type, e-DAXX- Δ S failed to co-localize with PML-NBs (Fig. 3B) suggesting that the C-terminal SIM domain of DAXX is involved in the targeting of DAXX to PML-NBs. Similarly, we investigated the localization of CAF1-p150 versus PML-NBs. We observed that the most prominent dots of epitope tagged CAF1-p150 co-localized with PML-NBs (Fig. 3B).

In order to understand the consequences of the deletion of the SIM domain of DAXX, proteins associated with DAXX- Δ S were purified by the double-immunoaffinity method from soluble nuclear extracts of MEF cells and the composition of the complex compared to that of the WT DAXX complex by SDS-PAGE (Fig. 3C). Immunoblotting analysis revealed that DAXX showed a modified form (Fig. 3D) that, according to the literature, we assume to be the result of a SUMOylation process. This assumption was confirmed by the absence of this modified form of DAXX in the e-DAXX- Δ S complex (Fig. 3D). Interestingly, we were unable to detect the CAF1 complex among the e-

DAXX- Δ S-associated proteins either by immunoblotting (Fig. 3D) or by mass spectrometry analysis (Fig. 3E). This result was rather surprising, given the fact that CAF1-p150 directly interacts with the N-terminal part of DAXX, but nevertheless suggests a possible role of the SIM domain in the recruitment of CAF-1 to the DAXX complex. To rule out a possible interaction of CAF1-p150 with the DAXX C-terminal SIM domain, an affinity immunopurification was made with soluble extract from SF9 cells which co-expressed FLAG-DAXX- Δ S and HA-CAF1p150. Using a FLAG-pull down assays, bead-bound proteins were eluted and fractioned on SDS-PAGE and stained with colloidal blue (Fig. 3F). The obtained data showed that the deletion of the SIM domain was unable to affect the interaction between DAXX and CAF1-p150.

In the light of the data obtained by the analysis of the e-DAXX- Δ S nuclear complex, we investigated the effect of deleting the C-terminal SIM domain of DAXX on the MJS transcription by RT-PCR (Fig. 3G). Interestingly, DAXX Δ S expression in the *Daxx*^{-/-} MEF cells was unable to rescue the mRNA level of MJS, thus demonstrating that the SIM domain of DAXX is required for the MJS transcription, probably through the recruitment of the CAF1-p150 subunit. Taken together, these results clearly show that the C-terminal SIM domain of DAXX is not required for the interaction with CAF1-p150 but is indispensable for its recruitment through the PML-NBs, and thus for the regulation of MJS transcription.

We next investigated the role of CAF-1 in the control of MJS expression. We hypothesized that DAXX, ATRX and CAF1-p150 present interdependence in the control of MJS expression. Using quantitative RT-PCR, we observed that depletion of CAF1-p150 using two specific shRNA resulted in a drastic reduction in MJS transcripts expression (Fig. 3H). In contrast, when depleting ATRX with two different shRNA, transcription of MJS was drastically enhanced (Fig. 3I), suggesting that ATRX is implicated in a repressive control on MJS transcription. Relative CAF1-p150 and ATRX mRNA levels were assessed by RT-PCR to ensure depletion effectiveness (Fig. 3J, K). These results support the notion that the two proteins, namely CAF1-p150 and ATRX, act antagonistically to regulate transcription of DNA repeats at the pericentromeric regions.

Consequences of DAXX loss on CAF1-p150 genomic distribution

Mammalian CAF-1 co-localizes with replicating heterochromatin where it promotes histone H3.1 assembly, and the CAF1-p150 subunit interacts with heterochromatin protein HP1 α . Therefore, it has been suggested that CAF-1 is involved in heterochromatin formation and regulation, but direct functional proof to support this model is still missing. The association of CAF1-p150 with DAXX prompted us to analyze its genomic distribution in relation to DAXX. Toward this goal, we performed a ChIP-seq analysis of the CAF1-p150 subunit using commercially available antibody in WT and DAXX KO MEF cells. Peak-calling using uniquely mapped read identified 41,146 peaks specific to the WT cells, 63,931 peaks specific to the DAXX KO cells and 23,207 CAF1-p150 peaks which are common between WT and DAXX KO cells (Fig. 4A). Heatmaps and corresponding normalized density curves revealed that inactivation of DAXX resulted in approximately two-fold depletion and relocation, of several CAF1-p150 peaks to chromatin regions where it was already weakly present in the WT cells (Fig. 4B, C). A comprehensive analysis of the data showed an enrichment of CAF1-p150 at DNA repetitive elements of the mouse genome with no major differences between WT and KO (Fig. 4D). CAF1-p150 was found specifically enriched at L1Md LINEs (Fig. 4E, F) and B1m SINEs (Fig. 4G, H). Surprisingly, CAF1-p150 was found depleted from promoters (Fig. 4I) and enhancers (Fig. 4J) in WT and KO cells.

The association of CAF-1 with DNA repeats such as SINEs and LINEs prompted us to perform a more thorough analysis of the ChIP-seq data by taking into account reads with multiple hits, which represent unmappable repetitive elements (Dreszer et al., 2012). Indeed, the majority of the CAF1-p150 reads had multiple hits and mapped to the different repetitive elements of the mouse genome (Fig. 4D). CAF1-p150 was found enriched at minor (SYNREP_MM family) and major satellites (GSAT_MM family), telomeric repeats (TTAGGGn/CCCTAA_n), L1Md LINEs and mouse-specific SINEs (Fig. 4K) with some important differences between WT and knock-out DAXX MEF cells (Fig. 4K). This later observation prompted us to analyze the distribution of CAF1-p150 chromosome by chromosome. This analysis revealed a strong depletion of CAF1-p150 from the X-chromosome in KO cells (Fig. 4L) with a prominent effect on SINEs (Fig. 4M, N) and L1Md LINEs (Fig. 4O, P).

We conclude that: (i) the association of CAF-1 with DAXX affects the X-chromosome in a distinct manner compared to the autosomes and, (ii) DAXX is essential for targeting CAF1 to the X-chromosome.

Consequences of DAXX loss on H3.3 genomic distribution

Having shown that DAXX targets CAF-1 to the X-chromosome, we next investigated the effect of DAXX inactivation on H3.3 deposition. Toward this goal, WT and Knock-out DAXX cell lines were transduced with an epitope tagged H3.3 to generate stable cell lines ectopically expressing Flag-HA-H3.3. As a control, we used a MEF cell line expressing endogenous Flag-HA tagged H3.3 (Ors et al., 2017). Endogenous and ectopic tagged H3.3 cell lines were then subjected to ChIP-seq analysis. Peak-calling using uniquely mapped read revealed that 81% of the endogenous H3.3 peaks mapped to enhancers and 19% to promoters (Sup_Fig. 1A). Heatmaps and normalized density curves (Fig. 5A) revealed that, in the absence of DAXX, the overexpressed H3.3 accumulates at promoters while the presence of DAXX prevented this ectopic accumulation (Fig. 5A). The absence of DAXX had less pronounced effect on ectopic H3.3 accumulation at enhancers, affecting only enhancers located at distance from TSS (Fig. 5B). However, the absence of DAXX strongly impaired H3.3 recruitment to enhancers located on the X-chromosome (Fig. 5C).

We next investigated the presence of H3.3 on repetitive elements. In contrast to CAF1-p150, H3.3 was not found at repetitive elements such as LINEs and SINEs (Sup_Fig. 1B, C).

We concluded that deposition of H3.3 is tightly controlled by DAXX, which prevents its ectopic accumulation on promoters and specifically targets H3.3 on X-chromosome enhancers.

Discussion

In this study, we purified the nuclear soluble DAXX complex from MEF cells and characterized its different partners. Among the DAXX partners, we found ATRX, CAF-1, ADNP, TRIM28, HP1 ✓ and H3.3. Many of these proteins represent novel DAXX binding partners and are components of multiprotein complexes involved in histone deposition, gene regulation and heterochromatin formation.

Glycerol gradient fractionation, showed that DAXX reside in at least two different complexes, the previously identified DAXX/ATRX complex and the newly identified DAXX/CAF1/ADNP/TRIM28/HP1 ✓/H3.3 complex. DAXX acts as the central player whose role is to recruit these regulatory proteins to chromatin to ensure a dynamic deposition/eviction of H3.3 at specific chromatin regulatory regions. Interestingly, DAXX was found to physically interact with the chromatin assembly factor 1 (CAF-1) to form a multi-subunit complex. The interaction of DAXX with the H3.1 deposition machinery suggests an implication of this new complex in the H3.1/H3.3 dynamic or exchange.

CAF1-p150 and ATRX were both found to bind to the N-terminus of DAXX and probably to compete for the same binding site. CAF-1 and ATRX were found to present interdependence in the control of pericentric DNA repeats expression. The two histone chaperones, DAXX and CAF-1, act in concert through the PML-NBs to positively regulate transcription of pericentric DNA repeats whereas DAXX/ATRX have opposite effect and negatively regulate the transcription of these repeats.

H3.3 was originally found associated with euchromatic sites of active transcription (Ahmad and Henikoff, 2002b, c; Schwartz and Ahmad, 2005) but has been also shown to associate with pericentric heterochromatin at telomeres (Drane et al., 2010; Goldberg et al., 2010; Lewis et al., 2010; Wong et al., 2010). We have previously shown that DAXX and ATRX form a complex with H3.3 and colocalize at PML-NBs and pericentric repeats (Drane et al., 2010). The PML-NBs are known to form multiprotein structures where proteins accumulate and undergo post-translational modifications, like SUMOylation (Ishov et al., 1999; Lin et al., 2006; Shen et al., 2006; Sternsdorf et al., 1999; Weidtkamp-Peters et al., 2008). PML-NBs are involved in diverse biological functions, including regulation of the different chromatin conformational states (Bernardi and Pandolfi, 2007; Weidtkamp-Peters et al., 2008). Human pericentric satellite repeats can enter the PML-NBs to associate with DAXX

and ATRX (Ishov et al., 1999; Ishov et al., 2004; Luciani et al., 2006). Our results suggest that in addition to its role as an H3.3 deposition factor, DAXX, through its C-terminal SUMO Interacting Motif (SIM), binds to PML and serves as a specific carrier that targets H3.3 to PML-NBs. Indeed, deletion of the 8 amino-acids SIM domains led to a complete delocalization of DAXX from PML-NBs and a strong decrease in pericentric heterochromatin transcription. Our results are in agreement with the previously published role of the SIM domain in targeting DAXX to PML-NBs (Lin et al., 2006). Our data also suggest that a transient passage of DAXX through the PML-NBs is a prerequisite for its association with the CAF-1 and for the maintenance of active heterochromatin transcription. Accordingly, CAF-1 was found to associate essentially with heterochromatic regions and retroelements such as SINEs and LINEs. The association of DAXX with CAF1-p150 subunit is interesting and suggests a cooperative action of these two histone chaperones in the regulation of repetitive elements and in targeting the X-chromosome in a distinct manner from the autosomes. DAXX was found essential for the proper localization of CAF-1 on X-chromosome mouse-specific SINEs and L1Md LINEs. DAXX was also found to tightly control the deposition of H3.3 at enhancers located on X-chromosome and to prevent ectopic H3.3 accumulation on promoters.

CAF1 could serve as a histone H3.1 donor/acceptor, which in coordination with the DAXX/ATRAX chromatin assembly machinery ensures a dynamic exchange of H3.1/H3.3 around the transcriptionally active DNA repeats. This function of CAF-1 in transcription activation is novel and distinct from the known property of the whole CAF1 complex to promote histone deposition during replication (Verreault et al., 1996). The CAF-1 complex should be considered not only as a histone-deposition complex, but also as a multitasking factor that integrates information via individual subunits. We propose that CAF-p150 provides a novel type of transcription control at pericentric regions enriched in HP1 α (Murzina et al., 1999). By efficiently coordinating the dynamic exchange of H3.1/H3.3 at pericentric regions, this mechanism would facilitate active transcription of pericentric repeats and mobile elements during G1 phase of the cell cycle and ensure reproduction of specialized chromatin structures present at key regions of the genome during S phase.

The association of DAXX with the replication-dependent deposition machinery CAF-1 is puzzling but could simply reflect a requirement for a dynamic exchange of H3.1/H3.3 at active transcription sites. Histone variant exchange is essential for regulated gene expression (Ooi and Henikoff, 2007). For instance, the yeast chromatin-remodeling complex SWR was shown to catalyze the ATP-dependent exchange of H2A.Z for nucleosomal H2A at active gene (Mizuguchi et al., 2003). Our data clearly show that the ATRX chromatin remodeling factor act in concert with DAXX to down-regulate transcription of pericentric DNA repeats and this could be achieved by regulating the level of H3.3 at these repeats. Indeed, ATRX was previously identified as a specific component of the H3.3 preassembly complex and required for *in vitro* deposition of H3.3 at pericentric and telomeric repeats (Drane et al., 2010; Lewis et al., 2010). Accordingly, diminished ATRX function also leads to an increase in TTAGGG repeat containing telomeric transcripts (referred to as TERRA), reduces telomeric loading of HP1 α , and causes modest levels of telomere dysfunction in mouse ES cells (Goldberg et al., 2010; Wong et al., 2010). The function of CAF1 in the H3.3 deposition reaction is not yet clear, but it could serve to maintain a dynamic active exchange of H3.1/H3.3 around pericentric chromatin and mobile elements.

We also questioned the role of ADNP and HP1 α in the DAXX complex. The targeting of ADNP to pericentromeric heterochromatin can be mediated by HP1 α (Mosch et al., 2011) and this was suggested being a key event for MJS repression. Pericentric heterochromatin is characterized by the presence of H3K9me₃, which is recognized by HP1 α to recruit ADNP (Mosch et al., 2011). According, to this study it would suggest that the DAXX complex, containing HP1 α and ADNP, is targeted to pericentric heterochromatin via HP1 α . However, we found that deleting the SIM domain of DAXX affected PML-NBs localization in spite of the presence of HP1 α albeit in less amount, and ADNP in the e-DAXX complex. This result shows that HP1 α and ADNP are not involved in the targeting of DAXX to PML-NBs.

Overall, our data suggests a cooperative action of two chromatin complexes in the repression of pericentric chromatin transcription. ATRX, CAF1 and DAXX might act together in a replication-independent chromatin assembly pathway that exchanges H3.3/H3.1 at pericentric chromatin. Their inactivation resulted in abnormal levels of repetitive elements transcription. It will be interesting to monitor the level of H3.3 at

those repeats in the absence of either CAF-1 or ATRX. Depletion of H3.3 from these repeats may affect centromere organization and genome integrity. Mutations in ATRX cause a mental-retardation syndrome and more recently ATRX, DAXX and H3.3 have been also identified as a potential tumor-suppressor in pediatric glioblastoma and pancreatic neuroendocrine tumors (Jiao et al., 2011; Schwartzentruber et al., 2012). The described physical and functional interactions between DAXX, H3.3, ATRX and CAF-1 make a unique contribution to pericentric heterochromatin organization and transcription and the misregulation of this pathway can certainly lead to cancer development.

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Materials and Methods

Cell lines

DAXX knock-out (*Daxx*^{-/-}) MEF line was a kind gift of Dr. Gerd G. Maul (The Wistar Institute) (Ishov et al. 2004). DAXX, DAXX Δ S and CAF1-p150 proteins fused with C-terminal Flag- and HA-epitope tags (e-DAXX / e-DAXX Δ S) were stably expressed in MEF cells by retroviral transduction (Ouararhni et al. 2006). Endogenous Flag-HA tagged H3.3 MEF cells were previously described in (Ors et al., 2017).

Antibodies

Antibodies used were as follows: Monoclonal anti-HP1 α was produced by the IGBMC facility; monoclonal antibody anti-Flag M2 (Sigma); monoclonal anti-HA 9E (Roche Diagnostics); antibodies from Santa Cruz Biotechnology anti-DAXX (sc-7152), anti-ATRX (sc-15408), anti-CAF-1 p150 (sc-10772) and anti-PML (sc-18425); antibodies from ProteinTech anti-ADNP (17987-1-AP), anti-CAF-1 p150 (17037-1-AP); anti-CAF-1 p48 (20364-1-AP); from abnova anti-H3.3 (H00003021-M01); from Millipore anti-H3 CT, pan (05-928, Upstate Biotechnologies).

Double-immunoaffinity purification

Extracts were prepared as described in (Drane et al., 2010) using a modification of the Dignam protocol (Dignam 1990). Briefly, cells were lysed in hypotonic buffer (10 mM Tris-HCl at pH 7.65, 1.5 mM MgCl₂, 10 mM KCl) and disrupted by Dounce homogenizer. The cytosolic fraction was separated from the pellet by centrifugation at 4°C. The nuclear-soluble fraction was obtained by incubation of the pellet in high-salt buffer (to get a final NaCl concentration of 300 mM). Tagged proteins were immunoprecipitated with anti-Flag M2-agarose (Sigma), eluted with Flag peptide (1 mg/mL), further affinity-purified with anti-HA antibody-conjugated agarose, and eluted with HA peptide (1 mg/mL). The HA and Flag peptides were first buffered with 50 mM Tris-HCl (pH 8.5), then diluted to 4 mg/mL in TGEN 150 buffer (20 mM Tris at pH 7.65, 150 mM NaCl, 3 mM MgCl₂, 0.1 mM EDTA, 10% glycerol, 0.01% NP40), and stored at -20°C until use. Between each step, beads were washed in TGEN 150 buffer. Complexes were resolved by SDS-PAGE and stained using the Silver Quest kit (Invitrogen).

For glycerol density gradient, samples were loaded onto a 4.5-mL glycerol gradient (15%–35%) and spun at 300,000g in a Beckman SW60 rotor for 18 h. Fractions were collected from the bottom of the tube and analyzed by SDS-PAGE.

Protein–protein interactions

DAXX GST fusion proteins immobilized on glutathione Sepharose were incubated for 1 h at room temperature with tagged HA-CAF1-p150 or HA-ATRX in TGN buffer (20 mM Tris at pH 7.65, 3 mM MgCl₂, 0.1 mM EDTA, 10% glycerol, 0.01% NP40) containing 0.15 M NaCl. Beads were then washed extensively in TGN containing 0.15 M or 0.5 M NaCl. Bound proteins were eluted in SDS sample buffer and fractionated on SDS-PAGE. HA-CAF1-p150 and HA-ATRX were probed with anti-HA antibody, while recombinant DAXX deletion mutants were stained by colloidal blue.

Mass spectrometry

Mass spectrometric analysis of proteins was accomplished using an ion-trap mass spectrometer (ThermoFinnigan LTQ-Orbitrap Velos) or by the Taplin Biological Mass Spectrometry Facility (Harvard Medical School, Boston, Massachusetts, USA) as described previously by Obri *et al.*, 2014

Immunofluorescence

Immunofluorescence used standard procedures. Rat anti-HA antibody (Roche) was used at 1/200 dilution; the secondary antibody used was a goat anti-Rat IgG coupled to Alexa Fluor 488 (Molecular Probes) at 1/400 dilution. Anti-PML antibody (SC-18425) was used at 1/200 dilution; the secondary antibody used was a rabbit anti-Goat IgG coupled to Alexa Fluor 647 (Molecular Probes) at 1/400 dilution.

Transfection, synchronization, and RNAi

Biologically active short hairpin RNAs (shRNAs) were generated from the pLKO.1-blast vector. HEK293T cells were seeded onto six-well plates and transfected with shRNA directed against ATRX and CAF1-p150 mRNA or an irrelevant shRNA using Effectene Reagent (Qiagen). Media containing viruses were collected 48 h after transfection. MEF cells were then infected for 24 h and then subjected to selection by 10 μ g/ml blasticidin for 72 h. Cells were then harvested for the assessment of the expression level of endogenous ATRX and CAF1-p150 mRNA, and of transcripts from pericentromeric repeats by real-time quantitative PCR analysis.

The shRNA target sequences were as follows: CAF1-p150 CCUGGACAGCUACUCCAAAU (TRCN0000109035), CGUAGGCUUGAGUACAAAGUU (TRCN0000109036) and CGGUGUGUCUGAAAGGAAGAA (TRCN0000109038); ATRX CCUGUCACUUUCACCUCUCA (TRCN0000081910), CCACUAACACUCCUGAGGAUU (TRCN0000081911); irrelevant shRNA CCUAAGGUUAAGUCGCCUCG.

Retrotranscription and real-time quantitative PCR

Total RNAs were purified using standard methods and cDNA was synthesized by random priming. Real-time quantitative PCR was done with the QuantiTect SYBR green PCR kit (Qiagen) and a Mastercycler ep Realplex apparatus (Eppendorf). Primer pairs used were CAGTCTTCAGGAGATGAAGC and ATTTTCAGGATCATTGTCGT for ATRX, GCTCTGGAGGCTAAACTGGA and TGCCTTAATGCGCTTCTCTT for CAF1-p150, GACGACTTGAAAAATGACGAAATC and CATATTCCAGGTCCTTCAGTGTGC for pericentromeric repeats (Lehnertz *et al.* 2003), and TGACACTGGCAAACAATGCA and GGTCCTTTTCACCAGCAAGCT for HPRT. Results were normalized to HPRT.

ChIP-seq

Micrococcal nuclease preparation and double-immunoaffinity purification of native FLAG-HA tagged mononucleosomes. Sub-confluent MEFs expressing FLAG-HA tagged H3.3 (endogenous or overexpressed) were lysed in hypotonic buffer (10 mM Tris-HCl at pH 7.65, 1.5 mM MgCl₂, 10 mM KCl) and disrupted by Dounce homogenizer. The cytosolic fraction was separated from the nuclei by centrifugation at 4°C. Nuclei were re-suspended in sucrose buffer (20 mM Tris-HCl at pH 7.65, 15 mM KCl 60 mM NaCl, 0.15 mM spermine, 0.5 mM spermidine) adjusted

with high-salt buffer (20 mM Tris-HCl at pH 7.65, 25 % glycerol, 1.5 mM MgCl₂, 0.2 mM EDTA, 900 mM NaCl) to get a final NaCl concentration of 300 mM. The nuclear-soluble fraction was recovered by centrifugation at 4°C. The pellet containing chromatin fraction was incubated in sucrose buffer containing 1mM CaCl₂, and MNase (2.5U/g of cells) 10 min at 37°C. Digestion was stopped with 4 mM EDTA and mononucleosomes were recovered by centrifugation at 4°C. Tagged nucleosomes were immunoprecipitated with anti-Flag M2-agarose (Sigma), eluted with Flag peptide (0.5 mg/mL), further affinity-purified with anti-HA antibody-conjugated agarose, and eluted with HA peptide (1 mg/mL). The HA and Flag peptides were first buffered with 50 mM Tris-Cl (pH 8.5), then diluted to 4 mg/mL in TGEN 150 buffer (20 mM Tris at pH 7.65, 150 mM NaCl, 3 mM MgCl₂, 0.1 mM EDTA, 10% glycerol, 0.01% NP40), and stored at -20°C until use. Between each step, beads were washed in TGEN 150 buffer. Nucleosomes were submitted to RNase and proteinase K digestion, and DNA was extracted by phenolchloroform.

Cross-linked ChIP. CAF-1 p150 cross-linked ChIP experiments were performed as previously described (Obri et al., 2014). Briefly, 50 µg of sonicated chromatin isolated from sub-confluent MEFs were immunoprecipitated using using 1 µl of antibody anti-CAF1-p150 (17037-1-AP). Five independent chromatin immunoprecipitations were pooled before sequencing analysis.

Library construction and sequencing. Libraries were prepared using the Diagenode MicroPlex Library Preparation kit v2, and sequenced on Illumina HiSeq 4000 sequencer as single-end 50 bp reads (for ChIP-seq analysis) or paired-end 50 bp reads (for nucleosome positioning analysis) following Illumina's instructions. Image analysis and base calling were performed using RTA 2.7.3 and bcl2fastq 2.17.1.14. Adapter dimer reads were removed using DimerRemover v0.9.2 (<https://sourceforge.net/projects/dimerremover/>). Reads were mapped to the mouse genome (mm9) using Bowtie (Langmead et al., 2009) v1.0.0 with the following arguments: -m 1 --strata --best -y -S -l 40 -p 2.

Computational analyses

Peak detection was performed running MACS (Zhang et al., 2008) (<http://liulab.dfci.harvard.edu/MACS/>) using datasets normalized to 10 millions uniquely mapped under settings where the input fraction was used as negative control. Detected peak summits were annotated using HOMER (<http://biowhat.ucsd.edu/homer/ngs/annotation.html>). Heatmaps, global clustering and quantitative comparisons of the ChIP-seq data were performed running seqMINER (Ye et al., 2011) (<http://bips.u-strasbg.fr/seqminer/>) using datasets normalized to 10 millions uniquely mapped reads. As reference coordinates, we used the annotated RepeatMasker (RMSK) database (for repetitive elements) or the Ensembl 67 database (for coding genes) of the mouse genome (mm9). Tag densities were collected in 50 bp sliding windows spanning 2 kb (for SINEs) of the repeat element center or in length-normalized L1Md. The L1Md sequences were divided in 40 bins and the adjacent 2 kb sequences into 20 bins. The average densities were normalized in reads per million mapped reads (rpm).

Repeat Analysis

Repeat analysis was performed as follow. Reads were aligned to repetitive elements in two passes. In the first pass, reads were aligned to the non-masked mouse reference genome (NCBI37/mm9) using BWA (Li and Durbin, 2009) v0.6.2. Positions of the reads mapped uniquely to the mouse genome were cross-compared with the positions of the repeats extracted from UCSC (RMSK table in UCSC database for mouse genome mm9) and reads overlapping a repeat sequence were annotated with the repeat family. In the second pass, reads not mapped or multi-mapped to the mouse genome in the previous pass were aligned to RepBase (Jurka et al., 2005) v18.07 repeat sequences for rodent. Reads mapped to a unique repeat family were annotated with their corresponding family name. Finally, we summed up the read counts per repeat family of the two annotation steps. Data were normalized based upon library size. For enrichment analysis, normalized read counts of ChIP samples per repeat family were divided by normalized read counts of matched input samples and expressed as \log_2 fold enrichment. Significance of the difference of repeat read counts between ChIP and input samples was assessed using the Bioconductor package DESeq.

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Legends of Figures

Figure 1. Immunopurification of the H3.3 specific e-DAXX deposition complex from MEF soluble nuclear fractions.

(A) Stable expression of e-DAXX in MEF *Daxx*^{-/-} cells. Cells expressing e-DAXX and control cells (CTRL) were stained with anti-HA (top) and DAPI (bottom). (B) Upper panel: Silver staining of proteins associated with e-DAXX NC (lane 1). The complex containing e-DAXX (e-DAXX.com) was purified by double immunoaffinity from MEF soluble nuclear extract (NC). Polypeptides identified by mass spectrometry analysis and the positions of molecular size markers are indicated. (lane 2) Mock; Lower panel: CAF1-p150, ADNP and HP1 ✓ are components of the e-DAXX NC. The e-DAXX (lane 1) complex was analyzed by immunoblotting with the indicated antibodies. (lane 2) Mock. (C) Mass spectrometry analyses of e-DAXX complex. Table summarizing polypeptides detected by mass spectrometry analysis of e-DAXX complex, presented in Figure 1B and purified by double immunoaffinity. (D) Mass spectrometry analysis of e-DAXX associated H3 variant subtypes. Comparative analysis of histone H3.3/H3.1 ratio in the e-DAXX complex. Peptide abundances for individual H3 variants are indicated. Note that H3.3 is at least 20 times more abundant than H3.1.

Figure 2. DAXX and CAF1-p150 are in the same nuclear complex *in vivo*.

(A) Silver staining of nuclear e-DAXX complex fractionated on a glycerol gradient. The e-DAXX NC purified by double affinity was separated on a glycerol gradient. Fractions were collected as indicated at the top of the gel : fraction 1 correspond to the higher molecular weight fraction and fraction 10 the lowest. (B) Immunoblotting of glycerol fractions containing e-DAXX nuclear subcomplexes with the indicated antibodies. (C) Primary structure of DAXX. DAXX contains several putative domains: two paired amphipathic helices (PAH1 and PAH2), a coiled-coil (CC), an acidic domain (acidic), a Ser/Pro/Thr rich domain (S/P/T-rich), and an Rtt106-like domain (Rtt106). (D) DAXX interacts with CAF1-P150 *in vitro*. Tandem affinity purification was made with SF9 cells, which coexpress FLAG-DAXX and HA-CAF1-P150. Bead-bound proteins were washed in TGEN 150 mM. Eluted proteins were fractionated on SDS-PAGE and stained with colloidal blue. FLAG-DAXX and HA-CAF1-P150 were immunoprecipitated alone as control (lane 1, 2). The input lane (lane 3) represents the amount of proteins used for the pull-down. Tandem immunoprecipitation (IP) is shown in lane 4. (E) The N-terminal part of DAXX contains a CAF1-p150-interacting and ATRX-interacting domain. N-terminal (1–302), central (302–495), and C-terminal (495–740) regions of DAXX were produced as GST fusion proteins. The fusion proteins (lanes 2–7), immobilized on glutathione-agarose, were incubated with epitope-tagged CAF1-p150 or epitope tagged ATRX. After washing with either 0.15 or 0.5 M NaCl, the resin-bound CAF1-p150 or ATRX were probed with anti-HA antibody. To compare the levels of the GST fusions used for the pull-down, the blot was first stained with colloidal blue. The input lane (INP) represents 20% of the amount of CAF1-p150 or ATRX used for the pull-down. (F) Primary structure of CAF1-p150. (G) The C-terminal part of CAF1-p150 contains a DAXX-interacting domain. The N-terminal region of DAXX (1–302) produced as GST fusion protein was immobilized on glutathione-agarose and incubated with either N-terminal (1–296),

central (297–641), and C-terminal (642–938) regions of 6XHIS CAF1-p150. After washing with 0.5 M NaCl, the resin-bound CAF1-p150 domains were probed with anti-His antibody.

Figure 3. DAXX Sumo-interacting motif is essential for targeting DAXX to PML-NBs and for the recruitment of CAF1-p150.

(A) Primary structure of DAXX contains a Sumo-Interacting Motif (SIM) located in the C-terminal part. **(B)** SIM targets DAXX to PML-NBs. Distribution of HA-DAXX (a,e), HA-CAF1-p150 (i) or endogenous PML (b,f,i) in e-DAXX, e-DAXX-ΔS or e-CAF1-p150 MEF cells analyzed by immunofluorescence staining using anti-HA or anti-PML antibodies, respectively. (d,h,l) DNA was stained with DAPI. (c,g,k) Merged images correspond to the overlay of red (PML) and green fluorescence (DAXX) or CAF1-p150. **(C, D)** CAF1-p150 is absent from the e-DAXX-ΔS. **(C)** Silver staining of proteins associated with e-DAXX (lane 1) and e-DAXX-ΔS (lane 2). The complexes containing e-DAXX (e-DAXX.com) and e-DAXX-ΔS (e-DAXX-ΔS.com) were purified by double immunoaffinity from MEF soluble nuclear extracts (NCs). Polypeptides identified by mass spectrometry analysis and the positions of molecular size markers are indicated. **(D)** The e-DAXX (lane 1) and e-DAXX-ΔS (lane 2) complexes were analyzed by immunoblotting with the indicated antibodies. Sumoylated DAXX (*) is specific of e-DAXX. **(E)** Mass spectrometry analyses of e-DAXX WT and e-DAXX-ΔS.com complexes. Table summarizing polypeptides detected by mass spectrometry analysis of e-DAXX WT and e-DAXX-ΔS.com complexes, presented in Figure 3C and purified by double immunoaffinity. **(F)** DAXX ΔS interact with CAF1-p150 *in vitro*. Tandem affinity purification was made from SF9 cells co-expressing FLAG-DAXX-ΔS and HA-CAF1-p150. Bead-bound proteins were washed in TGEN 150 mM. Eluted proteins were fractionated on SDS-PAGE and stained with colloidal blue. FLAG-DAXX ΔS and HA-CAF1-p150 were immunoprecipitated alone as control (lane 1, 2). The input lane 3 (INP) represents the amount of proteins used for the pull-down. The tandem affinity purification (IP) is shown in lane 4. **(G)** Relative mRNA level for pericentromeric DNA repeats in DAXX rescue, DAXX^{-/-} and DAXX-ΔS MEFs was determined by quantitative RT-PCR. Results are represented as relative expression level of pericentromeric DNA repeats versus *HPRT*. Mean ± standard deviation of three independent experiments. * : p < 0.05 compare to wild-type. The level of transcripts from pericentromeric DNA repeats is reduced in DAXX^{-/-} and DAXX-ΔS MEF cells. **(H)** Depletion of p150CAF-1 resulted in a decrease in transcription from pericentromeric DNA repeats. MEFs were transfected with control shRNA (sh Ctrl) or two different CAF1-p150 shRNA (sh-1 and sh-2). Relative mRNA level for pericentromeric DNA repeats was determined by quantitative RT-PCR. Results are represented as relative expression level of pericentromeric DNA repeats versus *HPRT*. **(I)** Depletion of *Atrx* resulted in an increase in transcription from pericentromeric DNA repeats. MEFs were transfected with control shRNA (sh Ctrl) or two different *Atrx* shRNA (sh-1 and sh-2). Relative mRNA level for pericentromeric DNA repeats was determined by quantitative RT-PCR. Results are represented as relative expression level of pericentromeric DNA repeats versus *HPRT*. **(J, K)** Controls were performed to assess depletion efficiencies of the respective shRNA, *i.e.*, shCAF1-p150 and shATRAX. Mean ± standard deviation of three independent experiments. * : p < 0.05 compare to control, ** : p < 0.01 compare to control, *** : p < 0.001 compare to control.

Figure 4: Impact of DAXX inactivation on the genomic distribution of CAF1 in mouse embryonic fibroblasts.

(A) Venn diagrams showing the overlap between CAF1 peaks in control (*Daxx*^{+/+}) and *Daxx*-deficient MEFs (*Daxx*^{-/-}). (B, C). Heatmaps (B) and quantifications (C) of tag densities at WT-specific, common and KO-specific CAF1 peaks. (D) Percentages of peaks overlapping with repetitive elements using the UCSC RepeatMasker database. We observed an accumulation of CAF1 at all repetitive elements with the exception of LTR retrotransposons. (E, F). Heatmaps (E) and quantification (F) of CAF1 at full-length L1Md LINEs (length > 5 kb, n = 12,916), ranked by conservation score. Tag densities were collected in 50-bp sliding windows spanning 2 kb (divided in 20 bins) of the length-normalized L1Md (divided in 40 bins). (G, H). Heatmaps (G) and quantification (H) of CAF1 at B1m SINEs (+/- 2 kb) ranked by conservation score. (I, J). Heatmaps of CAF1 at promoters (I) ranked according to their expression level, and at enhancers (J) sorted according to their distance to nearest TSS. (K) Relative enrichment of CAF1 at DNA repeat families in the presence (*Daxx*^{+/+}) or in the absence of DAXX (*Daxx*^{-/-}). Calculations were done including “unmappable” multihit reads by assigning them to their unique repeat family (see Methods). (L) Histogram (built using genome-wide ChIP data) showing the relative enrichment of CAF1 (log₂ IP/Input) at each chromosome in control and *Daxx*-deficient MEFs. CAF1 is found significantly depleted from the X chromosome in the absence of DAXX. (M-P). Heatmaps (M, O) and quantification (N, P) of CAF1 in DAXX WT and KO cells, at B1m SINEs (M, N) or L1Md LINEs (O, P), sorted according to their chromosomal distribution.

Figure 5: Consequences of DAXX loss on H3.3 genomic distribution. Native FLAG-HA tagged mononucleosomes were isolated by double immuno-affinity purification from MEFs expressing an endogenous FLAG-HA-tagged H3.3, and from control (*Daxx*^{+/+}) or *Daxx* knockout (*Daxx*^{-/-}) MEFs overexpressing an epitope-tagged H3.3. (A, B) Heatmaps (left panels) and quantifications (right panels) of average H3.3 signals at promoters (A) ranked according to their expression level, and at enhancers (B) sorted according to their distance to nearest TSS. Whereas, overexpressed H3.3 target enhancers in both WT and KO cells, an accumulation of epitope-tagged H3.3 were found specifically at promoters in the absence of DAXX. (C) Average H3.3 signals at enhancers sorted according to their chromosomal distribution. The absence of DAXX lead to a depletion of H3.3 from X-chromosome enhancers.

Sup. Figure 1: Histone H3.3 genomic distribution at coding and repetitive elements. (A) Pie chart of the genomic location distribution of H3.3 in MEFs using native ChIP-seq experiments. More than 80 % of the called peaks (n= 104,727) were found at enhancers. (B, C). Heatmaps of average H3.3 signals at full-length L1Md (B) ranked by conservation score, and at B1m SINEs (C) ranked by conservation score. H3.3 was not found at retroelements in MEFs.

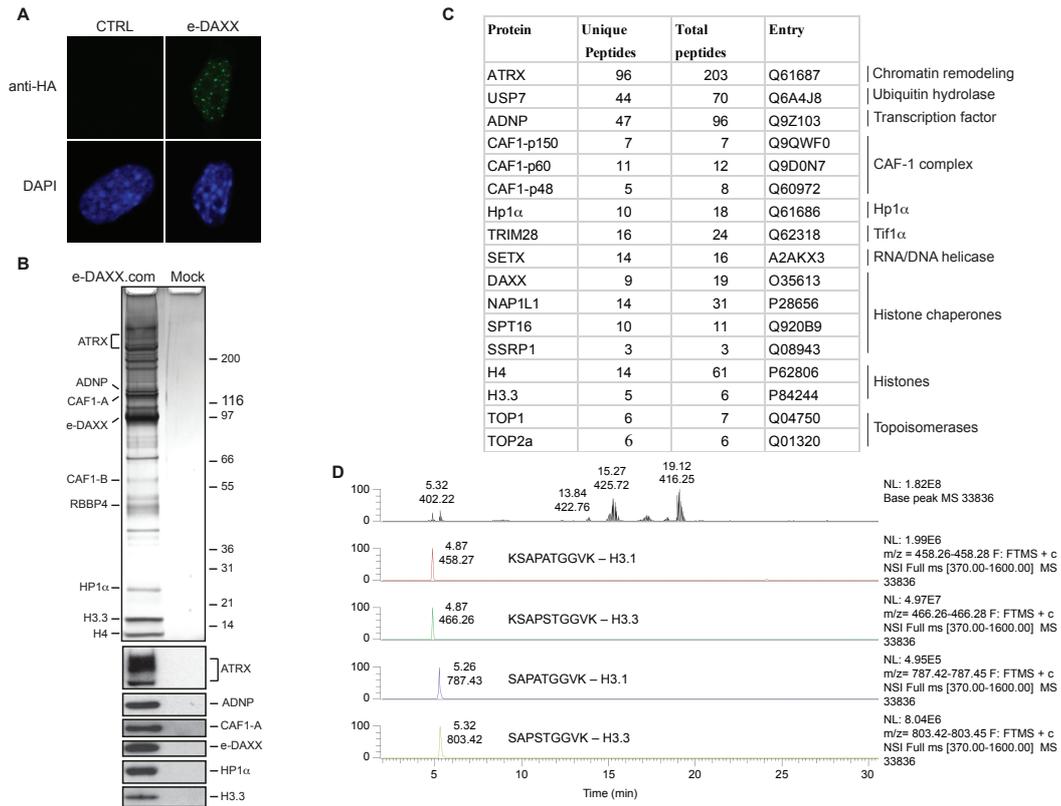


Figure 1

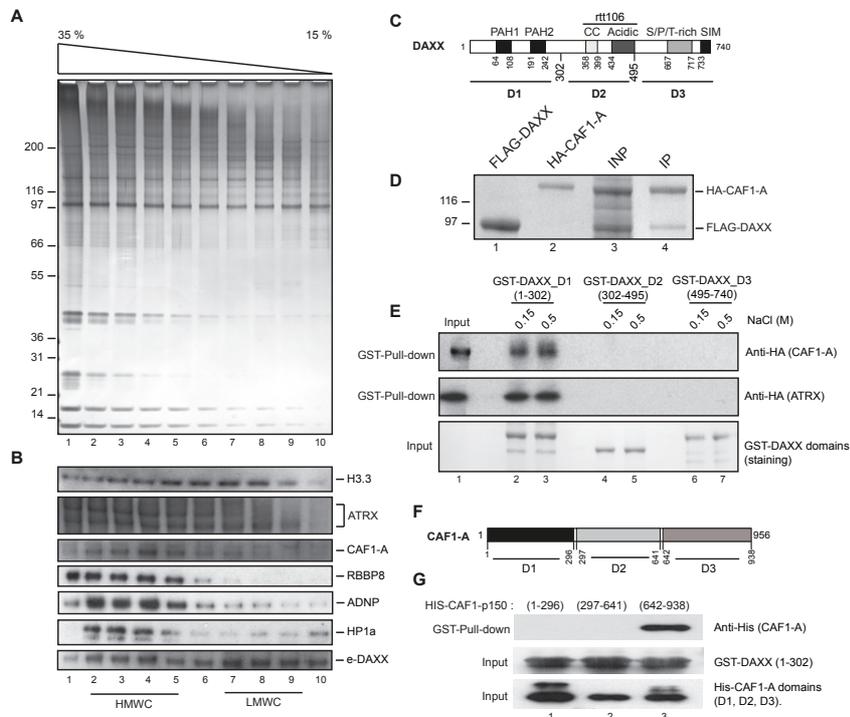


Figure 2

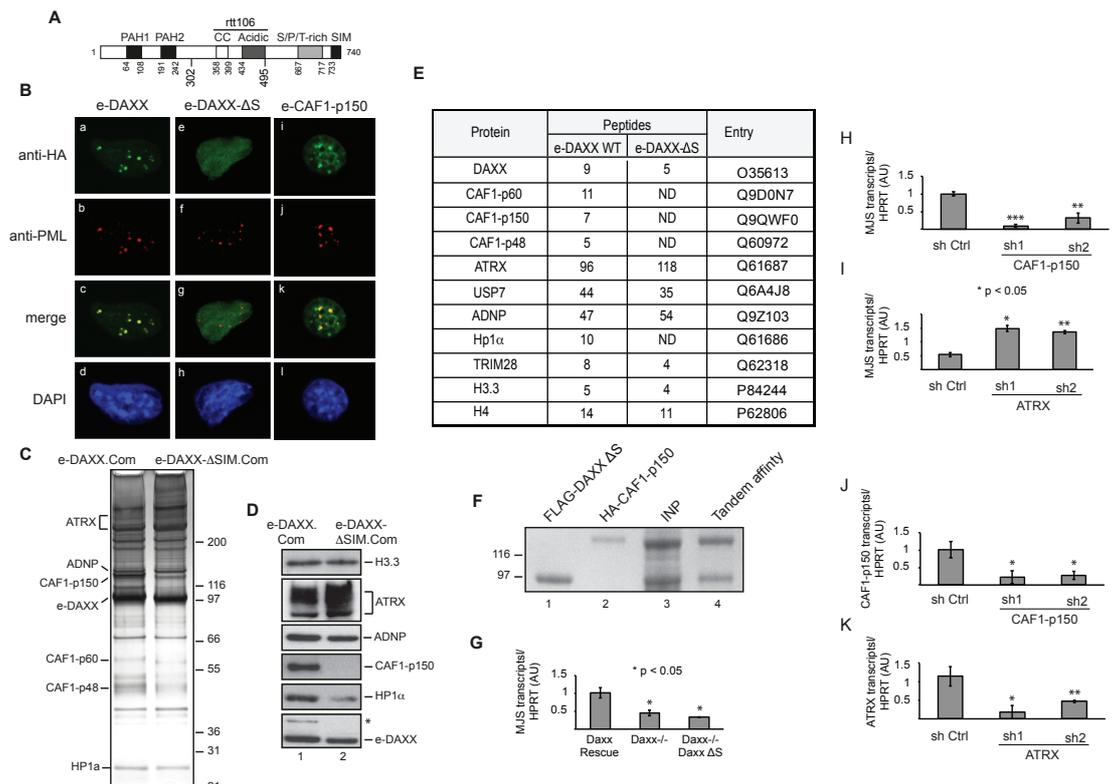


Figure 3

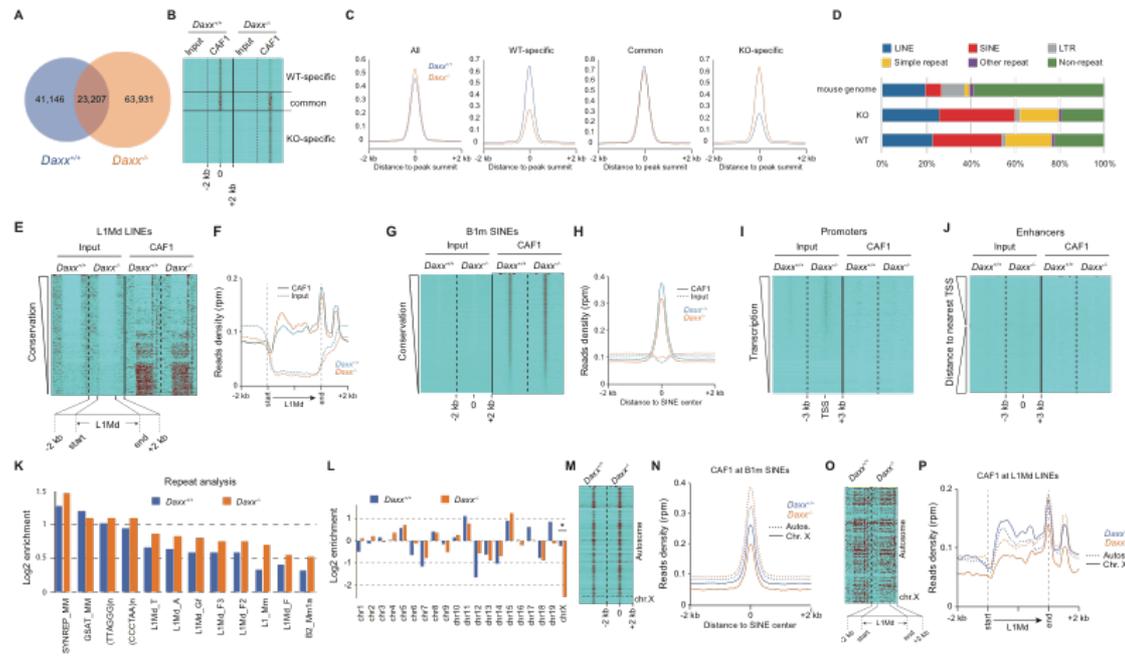


Figure 4

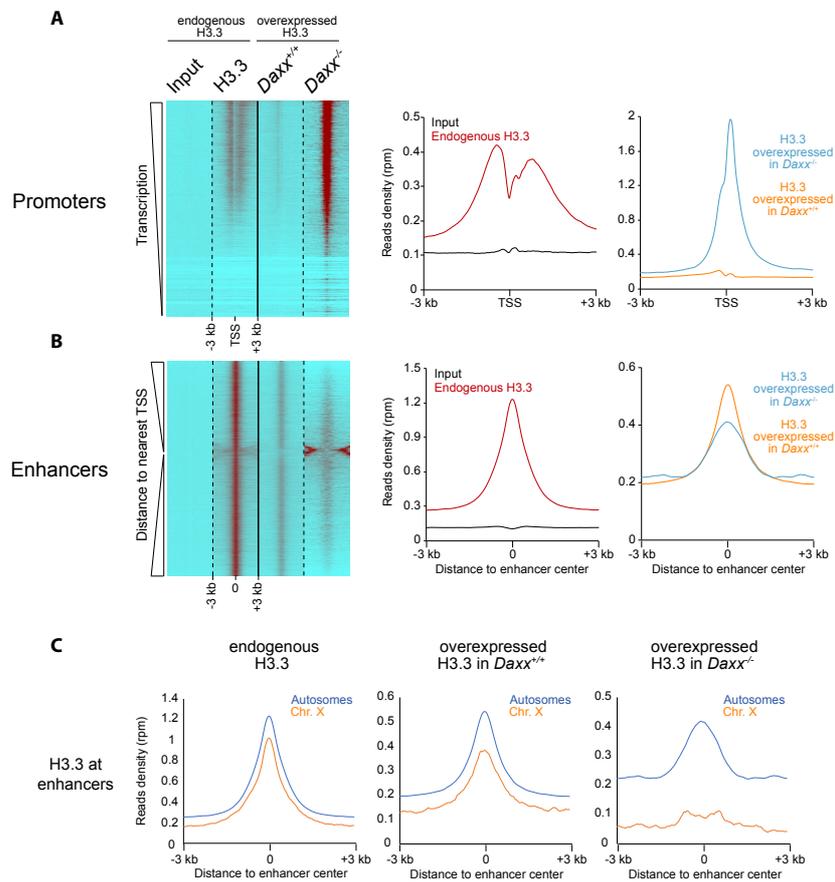
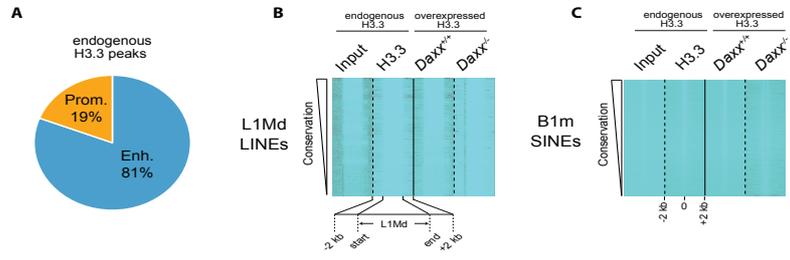


Figure. 5



Sup_Figure 1

III. DISCUSSION
and
CONCLUSION

III. Discussion and Conclusion

DAXX has been shown to mediate apoptosis through extrinsic death receptor pathway (Chang, 1998) as well as to control gene expression as a transcriptional co-repressor or co-activator by interacting with diverse DNA-binding transcription factors (TFs), epigenetic regulators, core histones and chromatin-associated proteins (Lehembre, 2001; Li, 2000). More recently, DAXX was identified as the histone H3.3 variant chaperone (Drané et al., 2010; Lewis et al., 2010).

The histone H3.3 variant can replace canonical histone H3.1 and thus locally modify nucleosome composition and chromatin function.

The study of histone H3.3 deposition complexes are an essential step in understanding and accurately characterizing the role of the histone H3.3 variant. To this end, the identification of the specific partners of the histone H3.3 chaperone, DAXX, was conducted. Toward this goal, murine embryonic fibroblasts knock-out for DAXX were used (MEF DAXX^{-/-}). An ectopic copy of DAXX bearing the FLAG and HA epitopes was then reintroduced to restore the expression of DAXX. This approach allowed the study of the DAXX macromolecular complexes in an almost physiological context, thus freeing itself from any artifacts induced by classical overexpression.

1. Identification and characterization of DAXX interacting partners and their role in histone H3.3 deposition

Tandem affinity purification of epitope tagged DAXX from mouse embryonic fibroblasts and further analysis of the complex by mass spectrometry and western-blotting confirmed the specificity of the complex for the histone H3.3 variant as well as for the chromatin remodeling factor ATRX. These results are fully in line with previous studies that have demonstrated the specificity of the DAXX-ATRX complex for H3.3 and its remodeling activity of chromatin containing H3.3 nucleosomes (Drané et al., 2010; Lewis et al., 2010). Surprisingly, this analysis also revealed the existence of a high molecular weight complex containing, in particular, the CAF-1 complex, the potential transcription factor ADNP, and the specific heterochromatin protein, HP1 ✓.

The CAF-1 complex, composed of the subunits p150, p60 and p46/48, is conventionally associated with the specific deposition of the canonical histones H3.1

and H3.2 (Smith and Stillman, 1989; Tagami et al., 2004). Although it has been shown that p46/48 subunits are also present within the H3.3 deposition complex (Drané et al., 2010), the presence of the entire CAF-1 complex associated with the DAXX-H3.3 complex is unexpected and puzzling. Nevertheless, the results obtained from the analysis of DAXX-associated proteins by mass spectrometry and western blotting clearly show the presence of CAF-1 and excluded the possibility of mixing between the H3.1 and H3.3 deposition complexes. All the data obtained during the identification of DAXX partners thus suggests a new role of CAF-1 in the mechanism of histone H3.3 deposition or exchange.

2. DAXX and CAF-1 physically interact with each other

In the light of the results obtained above, the presence of CAF-1 subunits and ATRX in the DAXX-H3.3 complex needed to be clarified. In vitro interaction experiments between DAXX, CAF1-p150 and ATRX were therefore performed using recombinant proteins produced in SF9 insect cells or bacteria. The results obtained clearly demonstrate a direct interaction between DAXX and CAF1-p150, and DAXX and ATRX. The histone chaperone DAXX can be divided into three distinct regions: The N-terminal region containing two PAH (Paired Amphipathic Helix) domains, the acidic central region containing an Rtt-106 like domain, and the serine, proline and threonine rich C-terminal domain. It has been shown previously that the central domain Rtt-106 is involved in the specific recognition of H3.3 (Drané et al., 2010; Lewis et al., 2010). It should be noted that CAF1-p150, as well as other histone chaperone proteins, also have an Rtt-106 domain. It has also been shown that DAXX interacts with ATRX via its N-terminal region containing the PAH domains (Tang et al., 2004). This study adds additional data to DAXX's interaction mapping. Indeed, the results obtained show that the N-terminal region containing the PAH domains of DAXX is also involved in the interaction with CAF1-p150. ATRX and CAF1-p150 are likely to compete for interacting with DAXX. Our data also showed that CAF1-p150 interact with DAXX through its C-terminal region encompassing amino-acids 642-938. These data confirm the results obtained during the identification of specific partners of DAXX and support the hypothesis of a role of the CAF-1 complex and the ATRX chromatin remodeling factor in the deposition/exchange mechanism of the histone variant H3.3.

3. Role of CAF-1 and ATRX in heterochromatin formation

Beyond the role of CAF-1 in the deposition of canonical histones H3.1 and H3.2, some work suggests that this complex, which has a high conservation rate during evolution, could be involved in other mechanisms. For example, CAF-1, as well as HP1, have been shown to be essential actors in the homologous recombination mechanism following DNA damage (Baldeyron et al., 2011). The analysis of the specific partners of the DAXX complex also allowed to identify the potential transcription factor ADNP (Activity-dependent neuroprotector homeobox protein). There is little data on the ADNP function, however, it has been shown that this protein is involved in the regulation of pericentromeric repeat sequences in a HP1-dependent manner (Mosch et al., 2011) but in a H3K9me3 independent manner (Ostapcuk et al., 2018). ADNP was also shown very recently to be part of the ChAHP complex which is involved in the maintenance of evolutionarily conserved spatial chromatin organization by buffering novel CTCF binding sites that emerged through SINE expansions (Kaaij et al., 2019). Accordingly, CAF1-p150 was found associated with mobile elements such as B2m SINEs and L1md LINEs. The connection between ADNP and H3.3 is interesting and needs to be addressed in more details by integrating the connection with CAF-1 and mobile elements. It has also been shown that inactivation of CAF-1 leads to a loss of maintenance and lack of replication of pericentromeric heterochromatin (Houlard et al., 2006; Dohke et al., 2008; Quivy et al., 2008; Huang et al., 2010). In view of these data, the role of CAF1-p150 in pericentromeric regions was studied by the RNA interference technique. The analysis of the results obtained by Q-PCR clearly shows a decrease in the level of transcription of pericentromeric repeat sequences in the absence of CAF1-p150, supporting the hypothesis of a role of the CAF-1 complex in the mechanisms regulating transcription of pericentromeric regions. In addition, it has been shown that CAF-1 and HP1 are essential for the replication of pericentromeric regions (Quivy et al., 2008) and are involved in the establishment and maintenance of these regions (Huang et al., 2010). It has also been shown that pericentromeric transcripts are finely regulated according to cell cycle in mice (Lu and Gilbert, 2007) and that the latter are also involved in the establishment and maintenance of pericentromeric heterochromatin in yeast (Volpe et al., 2002; Zofall and Grewal, 2006). Finally, it has been shown that the transcription of

pericentromeric repeat sequences is associated with the incorporation of H3.3 in the pericentromeric regions (Drané et al., 2010). In view of all these data, it seems reasonable to formulate the following hypothesis: CAF-1, in combination with HP1, and DAXX are present to allow the successive incorporation of H3.1/H3.2 and H3.3 thus ensuring a fine regulation of the transcription of pericentromeric heterochromatin.

In order to understand the interconnection between CAF-1 and ATRX in the DAXX complex, we also investigated the role of ATRX in the DAXX-H3.3 complex by RNA interference inactivation. Analysis of the results obtained by Q-PCR clearly shows an increase in the level of transcription of pericentromeric repeat sequences in the absence of ATRX.

From all these results, several hypotheses about the function of the DAXX complex can be made. Indeed, chaperone protein complexes are involved in the incorporation of histones into chromatin but also in eviction, a mechanism that allows the removal of histones from chromatin. It has been shown that ATRX is involved in the incorporation of H3.3 in pericentromeric repeat sequences (Goldberg et al., 2010). It is therefore reasonable to make the following assumptions: (i) the remodeling factor ATRX and the chromatin assembly complex CAF-1 have complementary functions within the DAXX complex, interfering with the incorporation and/or dynamic eviction of the histone variant H3.3; (ii) the presence of ATRX and CAF-1 reflects two different modes of management of H3.3 over time. In any case, in these hypotheses DAXX would therefore be the specific chaperone of H3.3 and ATRX and CAF-1 would be the factors regulating the eviction of H3.3 and replacement by H3.1 at pericentromeric chromatin, thus allowing a fine regulation of the transcription of pericentromeric repeat sequences.

4. Role of DAXX SIM domain in the regulation of pericentromeric chromatin

Several studies have shown that DAXX is a major component of PML nuclear bodies (PML-NBs) (Negorev and Maul, 2001; Weidtkamp-Peters et al., 2008; Geng et al., 2012). It has been shown that PML is the main component of PML-NBs and that sumoylation of this protein is a post-translational modification essential for the formation of PML-NBs (Ishov et al., 1999; Zhong et al., 2000; Brand et al., 2010). It

has also been shown that the DAXX protein has a SIM motif (SUMO-Interacting Motif (Song et al., 2004)) at its C-terminal end that is involved in the interaction with PML (Lin et al., 2006; Escobar-Cabrera et al., 2011).

PML-NBs are involved in cell cycle progression, DNA damage response, transcription regulation, viral infection and apoptosis, however, to date, the precise function of PML-NBs in these mechanisms remains poorly understood (Bernardi and Pandolfi, 2007; Borden and Culjkovic, 2009).

In order to better understand the existing relationships between PML-NBs and the DAXX complex, the DAXX- Δ S mutant, lacking the SIM motif, has been reintroduced into the MEF DAXX^{-/-} cells. Confocal microscopy confirmed the loss of interaction between DAXX- Δ S and PML-NBs. This result is consistent with other previous studies (Shen et al., 2006; Chang et al., 2011) and confirms the key role of the SIM domain in the localization of DAXX to PML-NBs. In addition, mass spectrometry and western-blotting analysis determined that the DAXX- Δ S complex is relatively similar to the wild-type DAXX complex. However, the CAF-1 complex, present in wild-type DAXX, is lost from the mutant complex. In order to better understand this result, we therefore performed an in vitro interaction experiment between DAXX- Δ S and CAF1-p150 using recombinant proteins produced in SF9 insect cells. The obtained results clearly demonstrate that the SIM domain is not mediating the interaction between DAXX- Δ S and CAF1-p150 subunit but necessary for the recruitment of CAF-1 to the DAXX complex. All these data suggest that the passage of DAXX through PML-NBs is necessary for its interaction with CAF-1-p150, and that this passage is dependent on the SIM domain of DAXX. Nevertheless, at this stage of the study, the biological mechanisms that occur at the level of PML-NBs remain to be determined.

5. Role of DAXX in recruiting CAF-1 to X-chromosome and mobile elements

In addition to its role in pericentric heterochromatin, CAF-1 was found to localize to mobile elements such as SINEs and LINEs. The association of DAXX with CAF1-p150 subunit is interesting and suggests a cooperative action of these two histone chaperones in the regulation of mobile elements and in targeting the X-chromosome

in a distinct manner from the autosomes. DAXX was found essential for the proper localization of CAF-1 on X-chromosome B2m SINEs and L1Md LINEs. DAXX was also found to tightly control the deposition of H3.3 at enhancers located on X-chromosome and to prevent ectopic H3.3 accumulation on promoters. These data revealed the close connection between DAXX and X-chromosome. It will be interesting to investigate whether DAXX is involved in X-chromosome inactivation and the connection between H3.3 and X-chromosome inactivation. Accordingly H3.3 was found to be involved in spermatogenesis in flies (Sakai et al., 2009) and mice (Jang et al., 2015). Inactivation of both *H3f3a* and *H3f3b* genes in mice led to early embryonic lethality and double *H3f3a*^{KO/WT} / *H3f3b*^{KO} mutant males are totally infertile, while double *H3f3a*^{KO/WT} / *H3f3b*^{KO} females are fertile, revealing a specific role of H3.3 in spermatogenesis (Jang et al., 2015). Another study revealed that H3.3B depletion alone is sufficient to generate mice infertility (Yuen et al., 2014). This mice infertility could be simply the failure to inactivate the X-chromosome in the absence of H3.3 or DAXX.

6. Conclusion

Our data suggests a cooperative action of ATRX and CAF1 in the repression of heterochromatin and repetitive elements. ATRX, CAF1 and DAXX might act together in a replication-independent chromatin assembly pathway that exchanges H3.3/H3.1 at repetitive elements and pericentric chromatin. Their inactivation resulted in abnormal levels of repetitive elements transcription. It will be interesting to monitor the level of H3.3 at those repeats in the absence of either CAF-1 or ATRX. Depletion of H3.3 from these repeats may affect centromere organization and genome integrity. Despite the results obtained, the role of DAXX complex in pericentromeric heterochromatin, mobile elements and X-inactivation remains to be clarified and some questions need to be answered.

What is the role of CAF-1 in the H3.3 deposition complex at repetitive elements and pericentromeric heterochromatin?

The most surprising result of this study is undoubtedly the presence of the CAF-1 complex within the DAXX-H3.3 complex, which does not question the role of CAF-1 in the deposition of canonical histones H3.1 and H3.2 during replication, but the data

provided suggest a more precise role for this complex in regulating the transcription of repetitive elements and pericentric heterochromatin.

In view of all available data, it is possible that the incorporation of H3.1/H3.2 and H3.3 by CAF-1 and DAXX complexes may be required to ensure fine regulation of heterochromatin transcription. In order to test this hypothesis, chromatin immunoprecipitation experiments directed against H3.3 or H3.1/H3.2, in the absence of CAF-1 and DAXX, could be considered.

What is the role of PML-NBs in regulating the transcription of heterochromatin by the DAXX complex?

Our study confirmed the importance of PML-NBs in regulating the transcription of pericentromeric repeat sequences. It seems reasonable to think that the PML-NBs play a role as a platform ensuring both the meeting of all the partners involved and their addressing to the pericentromeric regions. Nevertheless, in view of the studies conducted on PML-NBs and the highly regulated nature of the transcription of pericentromeric regions, it is highly likely that the events involved in this process are highly dynamic and difficult to tract biochemically. Based on this idea, it would be interesting to use super-resolution imaging technics to visualize DAXX, CAF1-p150, H3.3 and PML proteins dynamics around heterochromatin in living cells. This approach would allow to understand the regulation of the observed mechanisms throughout the cell cycle.

Finally, the results obtained strongly suggest that PML-NBs play a role as a platform where DAXX and CAF-1 meet to form a complex that interact to regulate specifically heterochromatin and X-chromosome formation or inactivation.

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Résumé

La chaperone d'histones DAXX cible H3.3 à l'hétérochromatine péricentrique et télomérique d'une manière indépendante de la réplication. Le mécanisme qui assure le recrutement de DAXX dans les régions hétérochromatiques est largement inconnu. En utilisant des approches protéomiques et biochimiques, nous montrons qu'en plus du complexe bien connu ATRX/H3.3, DAXX forme un complexe alternatif avec CAF-1, ADNP, HP1 et Trim28. DAXX interagit physiquement avec la partie C-terminal de la sous-unité CAF1-p150 via son domaine N-terminal. Le domaine SIM de DAXX est essentiel à son ciblage aux corps PML et pour le recrutement de CAF-1 à l'hétérochromatine. Nos données génomiques montrent que DAXX cible CAF-1 au chromosome X et aux éléments répétés. L'inactivation de DAXX entraîne une déplétion importante de CAF-1 des éléments répétés SINEs et LINEs du chromosome X. Nos données révèlent une nouvelle fonction de DAXX et de CAF-1 dans le ciblage de H3.3 au chromosome X.

Mots-clés : Histone variante H3.3, Histone chaperonne DAXX, CAF-1, Chromosome X, Eléments répétés SINEs et LINEs.

Summary

The histone chaperone DAXX targets H3.3 to pericentric and telomeric heterochromatin in a replication independent manner. The mechanism that ensures appropriate recruitment of DAXX to heterochromatic region is largely unknown. Using proteomic and biochemical approaches, we show that in addition to the well-known ATRX/H3.3 complex, DAXX forms an alternative complex with CAF-1, ADNP, HP1 and Trim28. DAXX physically interact with the C-terminus of CAF1-p150 subunit through its N-terminal domain. The DAXX SIM domain was found to be essential for targeting DAXX to PML-NBs, for the recruitment of CAF-1 to heterochromatin. Our genomic data further revealed that DAXX targets CAF-1 to X-chromosome and repetitive elements. Inactivation of DAXX results in major depletion of CAF-1 from X-chromosome SINEs and LINEs. Our data point to a novel function of DAXX and CAF-1 in targeting H3.3 to X-chromosome.

Keywords : Histone variant H3.3, Histone chaperone DAXX, CAF-1, X chromosome, repetitive elements SINEs and LINEs.