



Dynamics of H3.1 and H3.3 histone variants at mouse chromocenters during cell cycle and differentiation

Stefano Arfè

► To cite this version:

Stefano Arfè. Dynamics of H3.1 and H3.3 histone variants at mouse chromocenters during cell cycle and differentiation. Cellular Biology. Université Paris sciences et lettres, 2022. English. NNT : 2022UPSL022 . tel-04209304

HAL Id: tel-04209304

<https://theses.hal.science/tel-04209304>

Submitted on 17 Sep 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



THÈSE DE DOCTORAT
DE L'UNIVERSITÉ PSL

Préparée à l'Institut Curie

Dynamics of H3.1 and H3.3 histone variants at mouse chromocenters during cell cycle and differentiation

Dynamique des variants d'histones H3.1 et H3.3 dans leur localisation au niveau des chromocentres au cours du cycle cellulaire et de la différenciation dans le modèle murin

Soutenue par

Stefano ARFÈ

Le 16 Septembre 2022

Ecole doctorale n° 515

Complexité du vivant

Spécialité

Biologie Cellulaire et

Développement

Composition du jury :

Emmanuelle FABRE, DR
CNRS Hôpital Saint-Louis, Paris *Présidente du jury*

Saadi KHOCHBIN, DR
Institut for Advanced Bioscience, Grenoble *Rapporteur*

Aline PROBST, DR
GReD, Clermont-Ferrand *Rapporteuse*

Daniele FACHINETTI, DR
CNRS - Institut Curie, Paris *Examineur*

Geneviève ALMOUZNI, DR
CNRS - Institut Curie, Paris *Directrice de thèse*

Jean-Pierre QUIVY, DR
CNRS - Institut Curie, Paris *Co-directeur de thèse*

*Ai miei genitori,
To my parents.*

*“To understand things, I first travel an uphill road, a difficult road,
among thorns.
Precisely because I realize the difficulty to my readers,
I try to make them take this road downhill,
among the roses.”*

Piero Angela (1928-2022)

Acknowledgements

As the first Ph.D. graduate in my family, I think it is worth spending a couple of words to celebrate this moment and show my appreciation with whom I shared this path. In fact, this thesis is somehow the collective effort of all the people who got involved directly and indirectly in my Ph.D. project. Nobody prepares you for the next steps of your career path, even less when facing a Ph.D. Despite all the challenges and failures, I feel proud to have spent some time standing on the shoulder of the giants.

I would like to start by thanking my two Ph.D. supervisors, Geneviève Almouzni and Jean-Pierre Quivy, two complementary personalities who with their work spirit helped me in balancing my work approaches and ideas. It has been a privilege and a great opportunity for me to be hosted in your lab and to work under your supervision.

Geneviève, I thank you for all the encouragement you gave me and for always pushing me to make a step forward to do better science. During these years, I admired your passion and patience in sharing your enormous knowledge with me, and this motivation helped me get through my work. I am grateful to have shared my career path with you and I will always be thankful for reminding me of the duties and the honors of being a good scientist.

Jean-Pierre, thank you for guiding me with your unbeatable patience and sarcasm. I am sure we could have saved a good 50% of our time if only I would have spoken less during our discussions, both on a scientific and personal base. For this and more, I will always be grateful for your support and for teaching me in being meticulous even in the small things to prepare for an excellent job. I hope that this *pipiant* will make the best out of your advice in the future.

I want to express my gratitude to all the people from the scientific community who helped me in building insightful perspectives in this work. I thank all the people involved in my Ph.D. jury, in particular, Dr. Aline Probst and Dr. Saadi Khochbin for critically reading and evaluating my manuscript, Dr. Emmanuelle Fabbre, and Dr. Daniele Fachinetti, for kindly taking part in my Ph.D. jury. I also want to thank the members of my thesis committee Dr. Ignacio Izeddin, Dr. Chun-long Chen, Dr. Jack-Christophe Cossec, along with the team leaders of the UMR3664 at Institut Curie Angela Taddei, Antoine Coulon, Nathalie Dostatni and Ines Drinnenberg, for their insightful views throughout my Ph.D. course. I also thank all the people involved in the SiMoNa meetings, including Dr. Bassam Hajj, for their out-of-the-box perspectives.

I am grateful to the entire unit UMR3664 and Pavillon Pasteur of the Institut Curie for sharing great scientific discussions and moments between the labs and the meetings. Un grand merci à Caroline, Marie, Marion, Anifa et Prisca pour tous les bonjours et votre aide quotidienne. Thanks to the PICT-IBISA Imagerie Platform

including Patricia, David, and Mickael. There are so many beautiful people that I am afraid I will miss some of them, so please be kind if I am forgetful.

I am grateful to the doctoral school Complexité du Vivant, Institut Curie, and the Université Paris Sciences et Lettres having given me the training and the facilities to work in a such successful environment. A special thanks to the Horizon 2020 – MSCA-ITN “EpiSyStem” program, successfully run by Dr. Eran Meshorer and managed by Dr. Eitan Segev, and to Fondation pour la Recherche Medicale (FRM) for giving me access to the funding that has enabled me to pursue a Ph.D.. I am equally grateful to the LabEX DEEP and the Training unit for successful collaborations and for organizing the different editions of the stimulating Epigenetic courses.

As part of our name, I want to thank all the present and past members of the Chromatin Dynamics lab (a.k.a. Frogteam) that, for several reasons, have been part of my formation and stepped into my professional life. In the current team, I want to thank Christèle, Dominique, Delphine, Iva, Audrey, Sebastien, Weitao, Valentyna, and Nicole. Special thanks to Emma for helping with logistic organization and for the amazing brunch places. Thank you Kamila for the exuberant fresh air you brought into the lab. We suddenly became the “old guard” of the lab and, despite that, I feel touched every single time you want a scientific suggestion from my side. Keep rocking and do not dare to bring more modified Italian recipes to the lab ! Thank you Marina, we found a friendship in a place full of spritz and misplaced “H” sounds, and I wish to keep going like this. Your spirit gave me the spring to face all the little down moments that only a great person, like you are, could cheer me up. Grazie Alberto for being my first unofficial chaperone. Despite our language barrier, I found in you a great daily and witty source of inspiration. Our lunch routines and pavlovian coffee times, with a sprinkle of local Italian curses, were a good valid reason to feel at home during work time. Thank you Shweta for always being nice to me, you are the best gossip and chatty girl of our drinking time. Thank you Daniel DJ for all your patience, for reading several times my manuscripts, and sorry (not sorry) for my English. Thank you Katia and Laura for being supportive when I needed your advice during critical times. Merci à David to be the first who I bothered for all the Ph.D. advice and French stuff. Your savoir-faire and your work-life conversations still make me laugh. Thank you Julia for being a good friend in a SNAP of the fingers and for all the animated moments we shared during coffee times. Merci à ma petite famille française, Hatem et Magali. Votre aide avec SFR, la Sécurité Sociale et la bureaucratie française en general était aussi important qui m’a rendu un petit rituel moin perdu. To all of you guys, I wish you all the best of luck wherever you are.

A special word is needed for the current Ph.D. fellows who have been more supportive than ever. Thank you Charlene for accepting my daily vents about n’importe quoi and laughing about it. Thank you for your kindness and be strong, I am sure that you will be more than successful. *Dulcis in fundo*, I wish to thank my Bulgarian/Neapolitan partner in crime Tina. Beyond being an amazing colleague, a supportive friend, and a true pug hater, I want to express my gratitude for how much I cherished this lifetime with you. I can genuinely think that half of this thesis could have been worse if I did not meet you with your inspiring hard work commitment and your unconditioned genuine support. I am also quite sure you could have been a more

successful scientist if I did not delay you with my bad influence in terms of memes and rants. Despite being a small off-track to your career path, I am looking forward to putting my proud moment flag when the time will come, just to shout: “I told you, Malko Tsveete!”. Thank you for always being there for me, for listening to my no-sense Latin speeches, for your apartment without a TV, for all our dinners, and for always managing to find the best in me.

Finally, I want to thank the EpiSyStem group, including Patrick, Juliane, Helena, Adrianos, Marlene, Joe, Pinar, Priya, and Xue. We got a lot of fun and shared memories around the world and I got lucky to find you as a group of friends. I wish you guys all the success you deserve. To my Ritals group of friends Alessandro Manzo, Arianna, Syd, Flaminia, and Paolo for sharing and preserving our little Italian moments. Special thanks to my fabulous annoying Antonio that really blows my nerves, but I know we can always have each other's back. Special thanks also to Gonçalo, Manuela, Gertjan, and Anouk, not only for discovering you as deep friends but especially for accepting my weird and dorky passions.

It is not an easy task to have been far from home during these past 4 years, with a pandemic in the meanwhile, but, luckily, I have a great family of relatives and friends that never let me feel down. With a conclusive transition in Italian, vorrei ringraziare tutti quelli che mi hanno supportato e sopportato mentre sono stato a Parigi. Ringrazio i miei amici Anna, Dario, Simona, Lello per essermi stati vicini durante questi anni. Grazie Clelia per essere la mia gemellina incondizionata e per le tue opere di convincimento ad ogni vacanza. Grazie Vanni per avermi salvato dalla pandemia e aver trovato un amico vero con cui condividere le stesse passioni nerd e per i videogiochi. A Franca e Salvatore, grazie per aver controllato i miei tempi di volo e atterraggio, e soprattutto per trattarmi come parte integrante della famiglia. A tutti, spero di non essere stato più antipatico di quanto non lo sia normalmente. Ho amato voi e ogni singolo momento passato insieme appena sono tornato a casa.

Martina, tu sei tutte le parole che ti ho dedicato, tutte le attenzioni che ti ho regalato e tutto l'affetto che mi hai ricambiato. Sei tutta la bellezza che mi hai donato, sei tutti gli insegnamenti che abbiamo imparato. Mi hai insegnato che la distanza e la sofferenza in questi anni, purtroppo, sono parte della nostra esistenza e che, nonostante queste si prendano prepotentemente pezzi di vita, bisogna farle spazio accanto alla gioia, alla tenacia, alle vittorie e all'amore. Quell'amore immenso che sa arrivare fin dove la materia si dissolve, in un luogo o non-luogo in cui c'è già e che ci sta solo aspettando, come lo stato di gioia ricorrente di quando alzo lo sguardo e riesco a trovare la tua testa riccioluta in mezzo ai corridoi dei centri commerciali. Anche se non conosciamo ancora la nostra strada, tanto mi basterà averti accanto per illuminare il nostro futuro. Sei la mia casa e il mio presente. So sempre che sarai lì per me come io lo sarò per te.

A Erika, mia Sorella. Grazie per essere l'instancabile colonna portante della famiglia e scusami per essere stato così distante. Ti ringrazio per Ilaria e Serena, siete voi tutte la gioia dello zio. Ricordati che sei il mio punto di riferimento di dove e come vorrei essere nella vita.

A Marina e Roberto, Mamma e Papà. Vi ringrazio per essermi stati vicini come sapete farlo solo voi. Grazie per tutte le domande che mi avete fatto, chiedendomi se ho mangiato, di come stanno le cellule e di cosa mi dice Marie Curie dal suo balcone. Onestamente, credo che ormai siete diventati sempre più ferrati sul metodo scientifico, molto più di quanto io abbia fatto durante questi anni. So che avete patito più di me nel vedermi crescere lontano da casa e per questo ringrazio la vostra discrezione nell'accettare questo percorso della mia vita per assicurarmi un futuro dignitoso. Quando conclusi la laurea triennale, nei ringraziamenti scrissi "*La condotta dei genitori è la guida dei figli*" e non credo ci sia insegnamento migliore nel crescere con i valori che mi avete trasmesso. Ora siete liberi di esprimere senza remore il vostro orgoglio come lo sono io per voi.

There are places I'll remember all my life
Though some have changed
Some forever, not for better
Some have gone and some remain
All these places have their moments
With lovers and friends I still can recall
Some are dead and some are living
In my life, I've loved them all

Beatles – In My Life (1965)

Preface

It is fascinating to consider how a complete adult organism develops from a single cell. For development to proceed, at fertilization, two highly specialized differentiated cells, the parental gametes fuse to generate a totipotent zygote constituting the basis for all cells in the organism (Evans and Kaufman, 1981; Martin, 1981). Understanding how cells with identical DNA content can adopt different cell identities while coordinating biological functions is a major challenge addressed in the epigenetic field.

Efforts to understand the establishment and maintenance of non-genetic information during development have mainly focused on the process of transcription. Changes in gene expression programs result in specific protein translation for dedicated cellular and tissue functions. However, before understanding what genes are encoded in a cell, biologists were interested in why and how cells assume different morphologies along with their structural components. For example, adipocyte cells are largely shaped by the amount of lipid droplets that can contain in their cytoplasm, whereas the nucleus of resting lymphocytes and stem cells accommodate the majority of cellular space. Inside the nucleus of the mouse cell model, inactive transcribed regions of chromatin tend to self-interact, forming nuclear domains, such as the chromocenters, that are, fundamental for nuclear organization and correct establishment of cell identities during differentiation (Terranova *et al.*, 2005; Solovei *et al.*, 2009).

To date, understanding how the building blocks of chromatin, the histones, can influence the packaging and unwrapping of DNA in response to external cues, and how this translates into reshaping higher-order nuclear compartments, remains to be addressed.

In my Ph.D. thesis, I will address these knowledge gaps by exploring the composition of the “dark matter” – non-coding regions of chromatin, with a particular focus on chromocenters. My objective was to reveal whether such nuclear domains displayed a choice of histones and relative variants and to evaluate how the impact of differentiation events may affect this status.

I begin my introduction by breaking down the key components of the eukaryotic genome and compiling knowledge concerning their contribution to the nuclear organization. Then, I present the main technologies

and methodologies to study chromatin dynamics in the context of cell differentiation that I have capitalized on during my Ph.D. studies. Finally, I will discuss my results in the context of the current literature.

Table of Contents

INTRODUCTION.....	12
CHAPTER 1: NUCLEAR ORGANIZATION IN EUKARYOTIC GENOMES.....	13
1.1. DNA.....	13
1.2. EPIGENETICS.....	14
1.3. CHROMATIN.....	15
1.4. HISTONES.....	16
1.4.1 Histone post-translational modifications.....	18
1.5. HISTONE VARIANTS.....	19
1.5.1. H2A variants.....	21
1.5.2. H2B variants.....	22
1.5.3. H4 variants.....	22
1.5.4. H3 variants.....	23
1.5.5. H3.1 and H3.3 variants and cancer.....	26
1.5.6. Centromeric histone variant: CenH3.....	27
1.6. CENTROMERES.....	28
1.6.1. Transposable elements: ancient inhabitants of the genome.....	29
1.6.2. Satellite DNA sequences: heterochromatin structural components.....	30
1.6.3. Chromocenters: lessons from plants.....	32
1.6.4. Mouse chromocenters.....	34
1.6.5. Phase separation: solid-like or liquid-like heterochromatin?.....	37
1.7. CHROMATIN ORGANIZATION AT FINER SCALES.....	39
1.7.1. Sequencing methods.....	40
1.7.2. Proteomics methods.....	42
1.7.3. Imaging methods.....	42
CHAPTER 2: GENOME REGULATION AND HETEROCHROMATIN MAINTENANCE.....	45
2.1. DNA METHYLATION.....	45
2.2. HISTONE WRITERS AND READERS OF HETEROCHROMATIN.....	49
2.3. MOLECULAR CHARACTERIZATION OF CHROMOCENTERS.....	50
2.4. ADDITIONAL FACTORS INVOLVED IN CHROMOCENTER FORMATION.....	52
2.5. HISTONE CHAPERONES.....	53
2.5.1. CAF-1 complex.....	55
2.5.2. HIRA complex.....	55
2.5.3. DAXX-ATR complex.....	56
2.6. INTERPLAY BETWEEN HISTONE CHAPERONES AND HISTONE VARIANTS.....	58
2.7 DNA REPLICATION AND CHROMOCENTER ASSEMBLY.....	59
CHAPTER 3: ONE, NO ONE, ONE HUNDRED THOUSAND: THE MULTIFACETED ROLE OF HETEROCHROMATIN DURING DEVELOPMENT.....	61
3.1. EMBRYONIC STEM CELLS.....	61
3.2. PLURIPOTENT PATHWAYS & MECHANISMS.....	62
3.3. CELL CYCLE CONTROL DURING PLURIPOTENCY.....	64
3.4. HETEROCHROMATIN ESTABLISHMENT DURING DEVELOPMENT AND REPROGRAMMING.....	65
3.5. HISTONE VARIANTS AND CHAPERONES DURING DEVELOPMENT.....	66
QUESTIONS.....	70
RESULTS.....	72
APPROACH & METHODOLOGY.....	73
MANUSCRIPT.....	76
Abstract.....	77
Introduction.....	78
Results.....	82
Discussion.....	107
Materials and Methods.....	111

<i>References</i>	116
THE SHOW MUST GO ON: ADDITIONAL RESULTS	123
<i>H3.1 and H3.3 enrichment in ES and NPC at repetitive sequences</i>	125
<i>Single-molecule visualization of H3 variants reveals their mutual occupancy at chromocenters</i>	129
<i>Suv39h2, but not Suv39h1, supports H3.1 enrichment at chromocenters</i>	133
DISCUSSION	140
CONCLUSION	154
REFERENCES	158

Abbreviation List

2C	(Two cells)	IAP	(Intracisternal A-particle)
2i	(Two Inhibitors)	ICM	(Inner cell mass)
5hmC	(5-hydroxymethylcytosine)	iPSCs	(induced pluripotent stem cells)
5mC	(5-methylcytosine)	kDa	(kiloDalton)
Ab	Antibody	KDM	(Lysine demethylase)
ADD	(ATRX-DNMT3-DNMT3L)	KMT	(Lysine methyltransferase)
ALT	(Alternative lengthening of telomeres)	KO	(Knock-out)
ASF1	(Anti silencing factor 1)	LIF	(Leukemia inhibitory factor)
AT	(Adenine-Thymine)	LINE	(Long Interspersed Elements)
ATRX	(Alpha thalassemia/mental retardation syndrome X-linked)	LLPS	(Liquid-Liquid Phase Separation)
bp	(Base pair)	LTR	(Long Terminal Repeat)
BrdU	(5-bromo-2-deoxyuridine)	MajSat / MS	(Major Satellite)
CABIN1	(Calcineurin binding protein 1)	Mb	(Megabase)
CAF-1	(Chromatin Assembly Factor 1)	MeCP2	(Methyl-CpG-binding protein 2)
CENP-A	(Centromeric protein A)	MEF	(Mouse embryonic fibroblast)
ChIP-seq	(Chromatin Immunoprecipitation followed by Sequencing)	mRNA	(Messenger RNA)
DMEM	(Dulbecco's Modified Eagle Medium)	ncRNA	(Non-coding RNA)
IF	(Immunofluorescence)	NPC	(Neuronal precursor cell)
CHK1	(Checkpoint Kinase 1)	PCNA	(Proliferating Cell Nuclear Antigen)
CpG	(CG dinucleotide)	PHC	(Pericentric heterochromatin)
DAPI	(4',6-diamidino-2-phenylindole)	Pol	(Polymerase)
DAXX	(Death domain-associated protein)	PRC2	(Polycomb Repressive Complex 2)
DNMT	(DNA methyltransferase)	PTM	(Post-translational modification)
dn	(double null)	PWWP	(Pro-Trp-Trp-Pro)
DNA	(Deoxyribonucleic acid)	rDNA	(Ribosomal DNA)
DSC	(DNA-synthesis coupled)	SET	(Su(var), Enhancer of Zeste, and Trithorax)
DSI	(DNA-synthesis independent)	SINE	(Short Interspersed Elements)
EdU	(5-ethynyl-2-deoxyuridine)	STORM	(Stochastic Optical Reconstruction Microscopy)
ERV	(Endogenous Retrovirus)	Suv39	(Suppressor of Var(iegation) 3-9)
ES cells	(Embryonic stem cells)	TALE	(Transcription Activator-Like Effector)
ETN	(Early Trasponson)	TE	(Transposable element)
FISH	(Fluorescence in situ hybridization)	TMR	(Tetramethylrhodamine)
FRAP	(Fluorescence recovery after photobleaching)	UBN	(Ubinuclein)
GFP	(Green fluorescent protein)	YFP	(Yellow Fluorescent Protein)
H3K27me1,2,3	(Mono-, Di-, Tri- Methylation on the lysine 27 of the histone H3)		
H3K4me1,2,3	(Mono-, Di-, Tri- Methylation on the lysine 4 of the histone H3)		
H3K9ac	(Acetylation of the lysine 9 of the histone H3)		
H3K9me1,2,3	(Mono-, Di-, Tri-methylation on the lysine 9 of the histone H3)		
HeLa	(Henrietta Lacks)		
HFD	(Histone Fold Domain)		
HIRA	(Histone regulator A)		
HJURP	(Holliday junction recognition protein)		
HMGA	(High Mobility Group AT-Hook)		
HP1	(Heterochromatin Protein 1)		

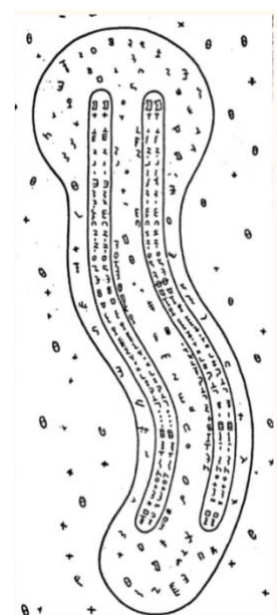
Introduction

Chapter 1: Nuclear organization in eukaryotic genomes

1.1. DNA

All of the discoveries helping to understand our world fuel new questions. For example, two of the fundamental questions that define the field of cell biology are: “what is inside a cell? What is its purpose?”. Based on Virchow's idea that all cells came from a single cell (“*omnia cellula ex cellula*” (Virchow, 1860)), in 1927 Koltsov proposed that the transmission of hereditary traits, including genes, consists in the propagation of macromolecules deriving from other molecules (“*omnis molecula ex molecula*”). Without any particular evidence supporting his hypothesis, he intuited that: « Each chromatid would consist of one giant hereditary molecule made up of two mirror strands that would replicate in a semi-conservative fashion using each strand as a template; each gene would be a segment within this molecule » (Morange, 2011) (Fig. 1). These statements anticipated all the pioneering work that followed for the discovery of a polymer, in the form of deoxyribonucleic acid (DNA), later shown containing the heritable genetic traits, including genes (Hershey and Chase, 1952). DNA is made of two antiparallel polynucleotide filaments with a sugar-phosphate backbone forming a double helix structure (Franklin and Gosling, 1953; Watson and Crick, 1953; Wilkins, Stokes and Wilson, 1953). In humans, each nucleotide is regularly separated from the next by 0.34 nm and each cell accommodates at least 3 billion base pairs (bp) of nucleotides, almost 2 meters in length of DNA, in a micron nuclear complex defined as chromatin.

Figure 1. Giant hereditary molecules. According to Koltsov's hypothesis, each chromosome is made up of two chromatids, each of them composed of one double-stranded giant hereditary molecule, with each gene represented by its own symbol. Each strand is used as a template for the synthesis of its replica during cell division so that the lineage can be preserved in hereditary records "through exact positioning (with the help of Van der Waals' forces, or crystallization forces) on points in which similar side groups exist in the molecule that serves as a template." Adapted from (Soyfer, 2001)



1.2. Epigenetics

The term epigenetics was first coined by Waddington (Waddington, 1942) to establish the link between genotype and phenotype with the influence of the surrounding environment. It has its conceptual roots in the theory of “Epigenesis” going back to the antiquity with Aristotle that was using it to explain how to develop a complex structure from the inform. In this broad view of epigenetics a zygote with its own identity can give rise to different cell types through canalization processes during embryonic development, as illustrated with a rolling marble committed through different trajectories while reaching its lowest height point among different valleys (Fig. 2) (Waddington, 1956).

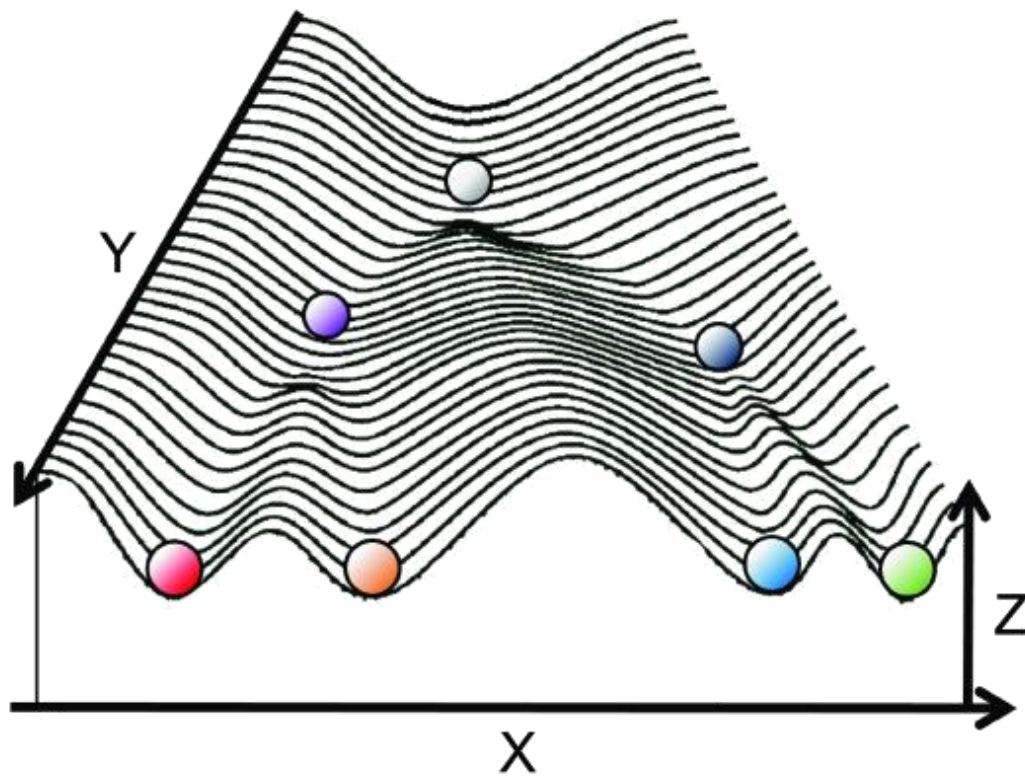


Figure 2. Waddington's epigenetic landscape. The cell differentiation process is conceptually explained as the motion of a marble along the landscape in which valleys correspond to differentiated cell types. The X horizontal axis represents a cellular state, the height (Z-axis) represents the probability that a cell takes state X, and the Y-axis represents slow developmental change. Adapted from (Matsushita and Kaneko, 2020) inspired by (Waddington, 1956)

Remarkably, the term was coined before DNA structure was known. DNA molecules transmit and convey genetic information through cell division. Clearly, the association of DNA with related proteins provides an

additional layer of information through chemical modifications and/or via the nature of the protein itself. These features can be used to refer to the term “epigenetics” which revolves “around” or “on top of” the genetic code providing an additional layer of information for chromatin.

As the molecular biology and genetics fields rapidly evolved, Holliday proposed a new definition for epigenetics as the consequence of heritable phenotypes without altering the DNA sequences (Holliday, 1994). This definition was in line with the current knowledge observed from DNA methylation studies. However, according to Riggs, it was more important to distinguish as true epigenetic events the transmission of long-term changes in phenotype occurring through mitotic or meiotic events, therefore over cell generations, rather than the short-term variations for example taking place on protein motifs, defined as signaling events (Russo, Martienssen and Riggs, 1996). Finally, a more recent point of view defines epigenetics as those events that perpetuate modifications on chromosomal structure, but also DNA, RNA, or histones, so that variations are registered and maintained in altered states (Bird, 2007).

1.3. Chromatin

Chromatin, previously known as nuclein (Miescher, 1871), was named after the cytological preparation of plant nuclei and its coloration by different dyes (Flemming, 1879, 1882). It was unknown however where genes were located in the nucleus. With the improvement of microscopy technologies, Sutton and Boveri independently proved and formalized the “chromosomal theory of inheritance” where chromosomes, like Mendelian genes, follow non-random distributions during cell divisions with individual genes found at specific locations on linear chromosomes (Sutton, 1903; Boveri, 1904). Finally, pioneering work by Heitz showed that the moss’ condensing chromatin could be differentially stained highlighting different chromosomal regions. Regions lightly stained and located in the inner part of the nucleus were called euchromatin, whereas the ones strongly stained and positioned at the periphery of the nucleus or nucleoli were called heterochromatin (Heitz, 1928). This evidence provided the basis for the presence of different regions and modular units for chromatin composition.

From a molecular viewpoint, DNA forms a nucleoprotein complex along with basic polar proteins, the histones, neutralizing the negative charges that are present on the phosphate backbone. The association of DNA and histones forms the fundamental repetitive basic unit of chromatin defined as the nucleosome (Fig.

3). The first evidence showing a conserved repetitive modular unit in eukaryotes came from experiments showing that chromatin digestion with nucleases results in DNA fragments of ~200 bp (Hewish and Burgoyne, 1973). This was later demonstrated by electron microscopy visualization of chromatin as a 30 nm structure appearing as “beads on a string”, with linker DNA connecting consecutive nucleosomes (Olins and Olins, 1974). Following these discoveries, euchromatin has been longstanding considered to be transcriptionally active for the presence of genes whereas heterochromatin, generally devoid of these elements, is transcriptionally inactive. The presence of distinct compartments within the nucleus was attributed to play a central role in genome function. However, a complex regulatory network regulating physical access to DNA contributes to defining genome function, and vice versa. I will now introduce how genetic information is organized at different scales, from a molecular perspective up to higher-order chromatin organization in the nucleus.

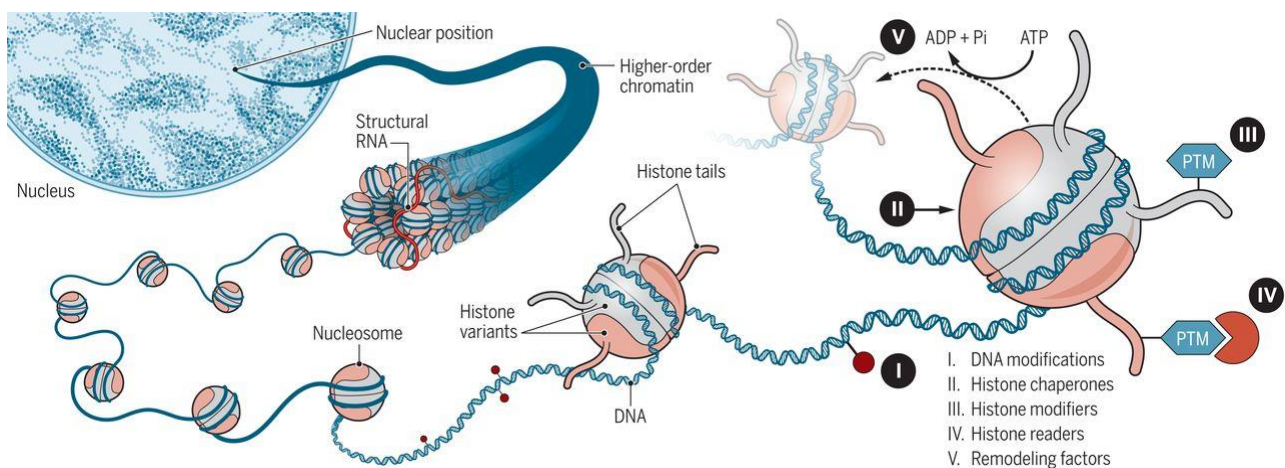


Figure 3. The nucleosome, the most basic unit of chromatin. The hierarchy of chromatin organization in a nucleus is shown along with factors acting at each level. Nucleosomes are adaptable units that can contain a variety of histone variants with numerous post-translational modifications. Nucleosomal DNA can also carry modifications, which together will influence chromatin structure. Adapted from (Yadav et al., 2018).

1.4. Histones

Each nucleosome core particle contains approximately 1.7 turns of DNA, with 147 bp wrapped around a histone octamer (Oudet, Gross-Bellard and Chambon, 1975; Finch and Klug, 1976; Kornberg, 1977; Noll and Kornberg, 1977) (Fig. 4), with the exception of the centromeric CENP-A-containing nucleosomes (see [1.5.6.](#)

[Centromeric histone variant: CenH3](#) paragraph). At the next level of DNA packaging, the chromatosome contains 166 bp of DNA and it comprises a nucleosome, a protruding DNA linker that connects consecutive nucleosomes, and a histone linker H1 mediating chromatin compaction (Simpson, 1978). Histones are relatively small proteins (~20 kDa) and they are highly evolutionary conserved proteins, with the exception of the linker H1 which is not part of the core particle. The linker histone H1 is quite distinct with a globular domain and binds at dyad axis of the nucleosome along with a recognition of an additional ~20 bp of linker DNA. In contrast, the core histones (H2A, H2B, H3 and H4) all show a histone fold domain (HFD), a domain important for histone dimerization which comprises 3 alpha-helices, in addition they all harbor a C-terminus tail, and a less-structured tail at the N-terminus. Many histone modifications target this accessible N terminal tail, but additional modifications are also observed in the more internal part of the histones, including in the HFD domain. (Arents et al., 1991; Arents and Moudrianakis, 1995; Luger et al., 1997; Davey and Richmond, 2002). A tetramer of (H3-H4)₂ resides at the center of the nucleosome flanked by two dimers of H2A-H2B with the surrounding DNA assembled around this core complex. The protruding N-terminal tails are responsible not only for interacting with other nucleosomes but also, can be decorated by chemical post-translational modifications. These modifications are not exclusive to the tails, as they are also found in the HFD domain (Cosgrove, Boeke and Wolberger, 2004; Mersfelder and Parthun, 2006). Together, they can act as a potential “histone code” that could be read and interpreted by regulatory proteins as well as creating a local chromatin environment to regulate genome function and/or compaction (Jenuwein and Allis, 2001).

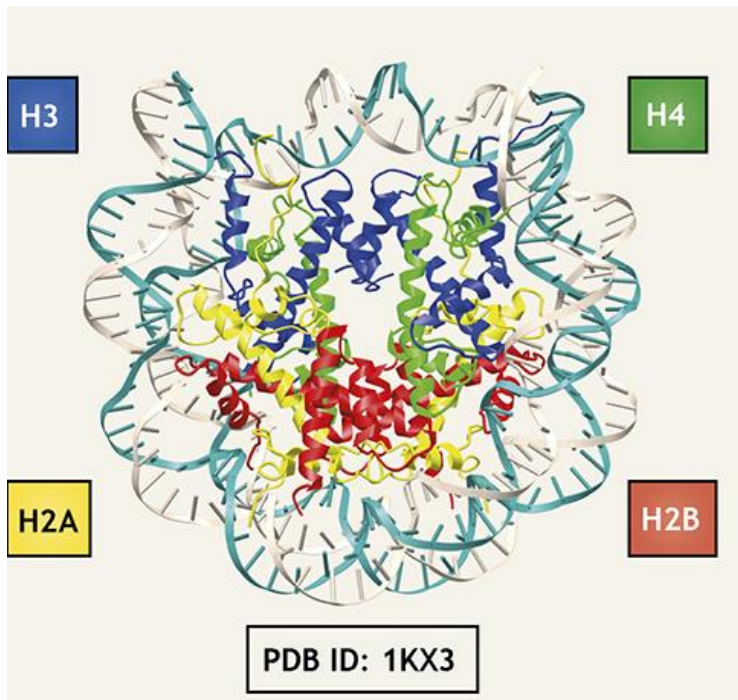


Figure 4. Nucleosome core particle.

Ribbon traces for the 146-bp DNA phosphodiester backbones (grey and turquoise) and eight histone protein main chains (blue: H3; green: H4; yellow: H2A; red: H2B). Visualization from down the DNA superhelix axis. The pseudo-twofold axis is aligned vertically with the DNA centre at the top. Crystal structure from Protein Database (PDB) 1KX3. Adapted from (Luger et al. 1997)

1.4.1 Histone post-translational modifications

Post-translational modifications (PTMs) are an additional layer of epigenetic control that can be found on histones. The approximately 40 amino acid long amino-terminal tails of histones, which extend out from the nucleosome core, are subjected to PTMs. PTMs can be dynamically added and removed enzymatically at any point during the histone's lifespan, from its production to its degradation (Loyola *et al.*, 2006). These modifications have the potential to change the interactions between histones-DNA, histones-histones, and histones-proteins. As a result, they offer a way to temporarily alter nucleosome conformation, which in turn affects chaperone binding, histone deposition, and the final chromatin state (Cosgrove, Boeke and Wolberger, 2004). Therefore, PTMs can either alter nucleosome physical properties affecting chromatin structure or they can alter protein partner binding and influence the recruitment of alternative proteins that can impact chromatin states.

The best-studied PTMs include acetylation, methylation, phosphorylation, ubiquitylation, and ribosylation. Several enzymes have been identified to add amino acid modifications (writers), to remove them (erasers), or recognize and bind these modifications to interpret a specific function (readers). In principle, chromatin states that are defined as “open” or “closed” can be influenced by histone PTMs. The first indication that histone alterations may be connected to transcription came from the discovery that hyperacetylated histones

corresponded with gene expression (Allfrey, Faulkner and Mirsky, 1964). If the histone tail is unmodified, the positive charge of the histones balances the negative charge of the DNA to allow for compaction. Lysine acetylation, in contrast, neutralizes the histones' positive charge, which reduces their affinity for negatively charged DNA. Consequently, the targeted region is more accessible to transcription factors and chromatin compaction is decreased (Shahbazian and Grunstein, 2007). For instance, in terms of histone H3 modifications, the H3K4me3 is one of the most prevalent mark at active promoters (Bernstein *et al.*, 2005; Pokholok *et al.*, 2005), H3K36me3 at gene bodies (Barski *et al.*, 2007), H3K27ac and H3K4me1 for active enhancers (Heintzman *et al.*, 2009; Creyghton *et al.*, 2010; Rada-Iglesias *et al.*, 2011). Conversely, other PTMs are characteristic markers of heterochromatin such as H3K27me3 and H3K9me3 marking facultative and constitutive heterochromatin, respectively (Peters *et al.*, 2001, 2003; Bernstein *et al.*, 2006; Lee *et al.*, 2006; Mikkelsen *et al.*, 2007). The main differences occurring between these two types of heterochromatin are discussed thoroughly in the following sections (see [2.2. Histone writers and readers of heterochromatin](#)).

The distribution and function of histone PTMs, however, are extremely complex and cannot be simply divided into active or repressive modifications that favor open or closed chromatin. Additionally, many PTMs can co-exist and resulting in a variety of combinations. In order to create bivalent domains around poised promoters, nucleosomes can combine both activating and suppressing histone marks at the same time, including for example the co-enrichment of H3K27me3 and H3K4me3. Genes for transcription factors that are expressed at low levels frequently have bivalent domains (Bernstein *et al.* 2006). Additionally, these domains are essential for modulating developmental gene programs during development.

In summary, the first scale of chromatin is an array of nucleosomes, containing different histones with relative PTMs which influence chromatin structure and function. This organization can be further diversified depending on the structural variations present on histones.

1.5. Histone variants

Beyond the histone code, nucleosomes present tremendous potential for versatility by substituting diverse histone variants, defined according to differences in their amino acid sequence content, expression patterns during the cell cycle, or pathways of chromatin incorporation (Mendiratta, Gatto and Almouzni, 2019).

Such variation correlates not only to specific biological functions but is also fundamental for chromatin organization. Histone variants are present across distantly related eukaryotic species, including mammals and plants. Despite encoding similar proteins, there are significant differences in coding and amino acid sequences. Most remarkable is the large variation in terms of their mode of regulation, processing and histone chaperone recognition. In the following section, I will illustrate briefly the currently known histone variants that contribute to diversify nucleosome composition, with a particular focus on the H3 variants. Mammalian histone variants and relative [histone chaperones](#) are summarized in Table 1.

Table 1 – Mammalian histone variants, histone chaperones, and their functions.

Core histone	Chaperones or chromatin remodeling complexes	General function
<i>Histone variants with specific chaperones or remodeling complexes</i>		
<i>H2A family</i>		
H2A.Z.1	p400, SRCAP (deposition); INO80, ANP32E (eviction)	Binding of regulatory complexes and chromatin dynamics
H2A.Z.2.1 and H2A.Z.2.2	p400, SRCAP (deposition); ANP32E (eviction)	Binding of regulatory complexes and chromatin dynamics
<i>H3 family</i>		
H3.3	HIRA–UBN–CABIN1	Transcriptional activation and chromatin dynamics
	ATRX–DAXX	Heterochromatin formation and telomere stabilization
H3.Y.1 and H3.Y.2 (also known as H3.X) ^a	HIRA–UBN–CABIN1	Transcriptional activation
CENP-A	HJURP	Centromere identity and genome stability
<i>Histone variants that, to date, rely on general histone deposition machinery</i>		
<i>H2A and H2B family</i>		
macroH2A1.1	FACT (eviction);	Gene silencing and higher-order chromatin compaction
macroH2A1.2	ATRX (antagonizes deposition);	
macroH2A2	ND (deposition)	
H2A.X	FACT	DNA damage response and chromatin remodeling

H2A.B	NAP1	Nucleosome destabilization, active transcription and mRNA splicing
H2A.L.2	NAP2L4	Histone-to-protamine transition
TH2B (also known as TS H2B.1)	NAP2L4	Histone-to-protamine transition
<i>H3 family</i>		
H3.4 (also known as H3T)	NAP2	Histone-to-protamine transition
<i>H4 family</i>		
H4G	Nucleophosmin	Upregulation of rDNA transcription
<i>Histone variants with unknown or undetermined deposition machinery</i>		
<i>H2A family</i>		
H2A.L (with several possible splice isoforms) ^b	NAP2L4 (H2A.L.2)	Histone-to-protamine transition shown for H2A.L.2
<i>H2B family</i>		
H2B.W (also known as H2BFWT)	ND	ND
<i>H3 family</i>		
H3.5	ND	Histone-to-protamine transition

ATRX, α -thalassaemia mental retardation syndrome X-linked; CABIN1, calcineurin-binding protein cabin 1; CENP-A, centromeric protein A; DAXX, death domain-associated protein; FACT, facilitates chromatin transcription; HIRA, histone regulator A; HJURP, Holliday junction recognition protein; NAP, nucleosome assembly protein; ND, not determined; rDNA, ribosomal DNA; SRCAP, Snf2-related CREBBP activator protein; UBN, ubinuclein. ^aEncoded by two highly related genes. ^bPresent in various mammals, including mice, but not humans. Adapted from (Martire and Banaszynski, 2020).

1.5.1. H2A variants

H2A variants are positioned at the entry and exit sites of DNA in the nucleosome, making them ideal candidates to control differential access. In mammals and plants, at least three main variants, including spliced isoforms, have been identified. H2AX is mainly known for its phosphorylated isoform γ H2A.X in response to DNA damage to recruit DNA repair factors (Talbert and Henikoff, 2014). H2A.W in plants and macroH2A in mammals possess an extended C-terminal that protects an extra 10 bp of the DNA linker and is found to be enriched in heterochromatin (Yelagandula *et al.*, 2014). In most animals, macroH2A showed reduced interactions with chromatin remodelers of the SWI/SNF complex, which proved inhibitory for acetylation by

p300 (Doyen *et al.*, 2006; Chang *et al.*, 2008) and when examined behaved as a barrier to reprogramming (Pliatska *et al.*, 2018). In sharp contrast, in plants, H2A.W deposition on mobile transposable elements depends on the SWI/SNF2 chromatin remodeler DDM1 (Osakabe *et al.*, 2021). Thus, there are clearly distinct ways for chromatin remodelers to interact with distinct histone variants. H2A.Z enrichment is often associated with transcription as its extended acid patch stimulates ATP-remodelers (Goldman, Garlick and Kingston, 2010; Dann *et al.*, 2017) and promotes RNAPII recruitment (Adam *et al.*, 2001). H2A.Z occupies, together with H3.3, the +1 nucleosome position, which is thought to lower the transcription barrier (Jin and Felsenfeld, 2007; Jin *et al.*, 2009; Bagchi *et al.*, 2020). However, in plants, H2A.Z is also found within gene bodies where it is rather linked to a poised state (Coleman-Derr and Zilberman, 2012; Sura *et al.*, 2017; Gómez-Zambrano, Merini and Calonje, 2019). Finally, the characterization of the spermatid-specific histone variants and chaperones required for nucleosome to protamine assembly in male germ cells, including H2A.L and possible isoforms (Barral *et al.*, 2017), further indicates the wide range of functions ensured by H2A variants.

1.5.2. H2B variants

H2B variants are less characterized compared to their H2A counterparts. For example, in *Arabidopsis* (*Arabidopsis thaliana*), eleven known H2B variants exist. They vary mainly in amino acid content at their N-terminal and are expressed in a tissue-specific manner (Bergmüller, Gehrig and Gruissem, 2007). Although their deposition is largely operating in a DNA synthesis-coupled (DSC) manner, enrichment of H2B.3 over gene bodies in mature leaves, does rather resemble to H3.3 patterns which are deposited independently of DNA synthesis in mammals (Jiang *et al.*, 2020). Other H2B variants present in mammals, including H2B.E and H2B.21, are similarly restricted to tissue-specific roles (Reviewed in (Talbert and Henikoff, 2021)).

1.5.3. H4 variants

Although less frequent, an H4 variant, H4.G, has been discovered in breast and colon human cancer cell lines and its deposition at ribosomal RNA (rRNA) genes correlates with low chromatin compaction and increased rRNA transcription (Long *et al.*, 2019; Pang *et al.*, 2020).

1.5.4. H3 variants

H3 variants can be categorized as either replicative or non-replicative histones (Fig. 5). In mammals, replicative histones have a peak of expression during S-phase and their genes are organized in multi-copy co-regulated clusters. Additionally, a peculiarity of replicative histone genes in mammals is their lack of introns and produce transcripts harboring a stem-loop structure instead of a poly-A tail for their stabilization (Marzluff, 2005). This is not observed in plant histone genes. H3.1 and H3.2, often referred to as “canonical” H3, are the primary replicative variants. The only amino acid difference between the two replicative variants is at residue 96, a cysteine (C) in H3.1 that is a serine (S) in H3.2. Replicative histones are deposited into chromatin genome-wide in a DNA-synthesis coupled (DSC) manner, mainly during DNA replication in S-phase. Outside of S-phase, replicative histones are incorporated during the DNA synthesis associated with DNA repair (Polo, Roche and Almouzni, 2006). In contrast, non-replicative (or replacement) H3 variants, such as H3.3, are organized as single copies in the genome with conventional transcriptional processing via splicing and polyadenylation steps and their expression is not cell-cycle regulated. Notably, some organisms, including yeast, have only one H3 protein, with characteristics from both the H3.3 and H3.1 variants (Ahmad and Henikoff, 2002). Furthermore, non-replicative variants are incorporated at specific loci in a DNA synthesis independent (DSI) manner. This choice in deposition relies on the differences in the histone fold domain that mediates interactions with different histone chaperones. Currently, as the name suggests and discussed in more detail in the following sections, a histone chaperone is defined as “a histone associating protein involved in their transfer without being part of the final product” (De Koning *et al.*, 2007). In mammals, the specific incorporation of H3.1 and H3.2, but not of H3.3, is mediated by the histone chaperone complex CAF-1 via the DSC pathway (Tagami *et al.*, 2004). Importantly, DSI histone chaperones discriminate H3.3 from H3.1 depending on the residues A87-I89-G90 (AIG) in the HFD in replacement variant(s) (Liu *et al.*, 2012; Ricketts *et al.*, 2015; Elsässer *et al.*, 2015), as they differ from residues S87-V89-M90 (SVM) present in replicative variants (Sitbon *et al.*, 2020).

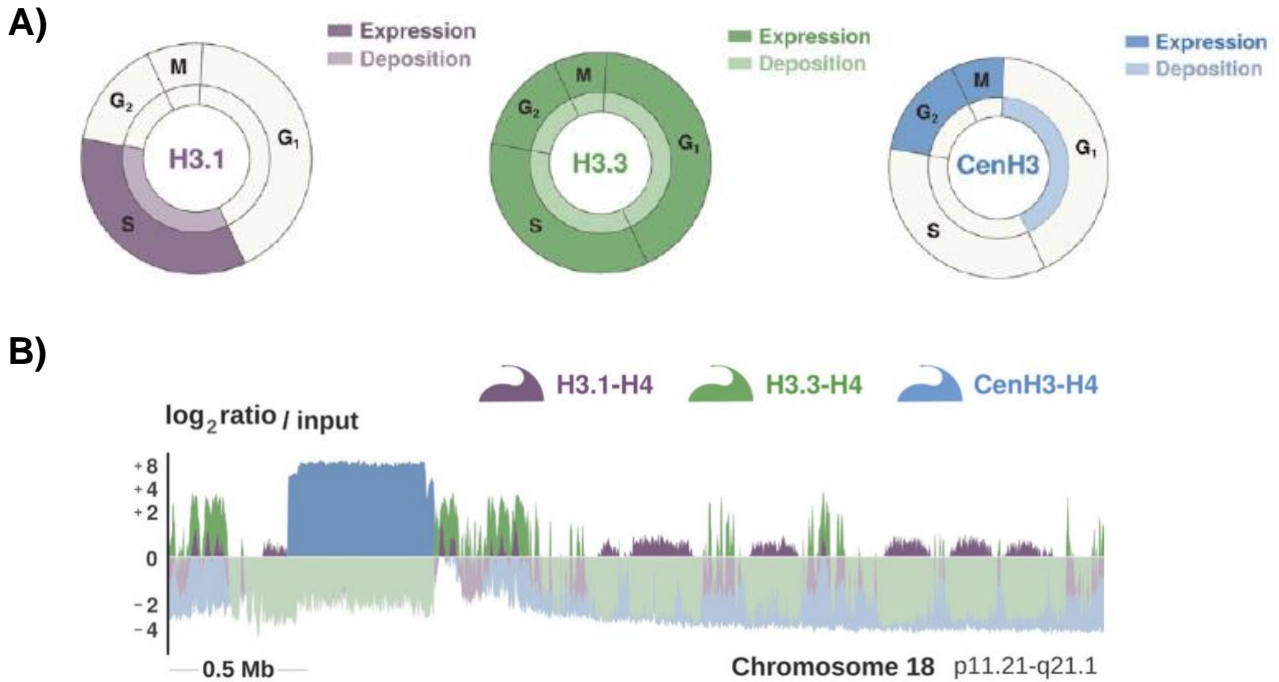


Figure 5. H3 variants are deposited at different timings and locations. A) Schematic representation of H3.1, H3.3 and CenH3 expression and timing of deposition. B) Genomic distribution (ChIP-seq) of H3.1, H3.3, and CenH3 in HeLa cells. The plot shows the enrichment relative to input for all variants at a representative region spanning the centromere and the proximal short and long arms of chromosome 18 (p11.21-q21.1). Adapted from (Mendiratta et al., 2019).

Histone H3.3 is the predominant non-replicative H3 variant in non-dividing cells, including post-mitotic or quiescent cells (Wu, Tsai and Bonner, 1982; Piña and Suau, 1987; Rogakou and Sekeri-Pataryas, 1999). It is also required in the fertilized zygote to replace the paternal protamines before the first round of replication during embryo development (Loppin *et al.*, 2005; van der Heijden *et al.*, 2005; Torres-Padilla *et al.*, 2006). In mammals, there are two copies of the H3.3 gene: *H3F3A* and *H3F3B*. Double knockout (KO) of these genes results in embryonic lethality in mice (Jang *et al.*, 2015), and morpholino depletion for H3.3 mRNA in *Xenopus* (*Xenopus Laevis*) results in gastrulation defects (Szenker, Lacoste and Almouzni, 2012; Sitbon *et al.*, 2020). In cycling cells, H3.3 is enriched at promoters and gene bodies in active regions of the genome and at telomeric and pericentric heterochromatin, where it maintains chromosome integrity by preventing spurious reactivation of retrotransposons or telomeric sequences (Drané *et al.*, 2010; Goldberg *et al.*, 2010; Elsässer *et al.*, 2015; He *et al.*, 2015). The histone fold domain of H3.3 contains AIG at residues 87-90. This domain is recognized by

the histone chaperone complex HIRA for the deposition of H3.3 in active regions, while the histone chaperone responsible for its deposition in heterochromatin is DAXX-ATRX (reviewed in (Mendiratta, Gatto and Almouzni, 2019)). Apart from the histone fold domain, the only amino acid residues that differ H3.3 from the replicative variants (apart from S96 instead of C96 in H3.1) is the presence of S31 instead of A31 on the N-tail. Importantly, this S31 is phosphorylated during mitosis (Hake *et al.*, 2005; Wong *et al.*, 2008), transcription (Armache *et al.*, 2020) and important at gastrulation (Sitbon *et al.*, 2020), and promotes acetylation of nearby residues (Martire *et al.*, 2019). The reader protein ZMYND11 that binds H3.3K36me3 and influences RNA PolIII elongation specifically recognizes H3.3S31, illustrating a specific recognition of H3.3 exploiting a distinct amino acid that can be modified (Wen *et al.*, 2014). Interestingly, in Arabidopsis, H3.3 contains a Threonine instead of a Serine, at residue 31. This threonine inhibits ATXR5/6 H3K27me1 methyltransferase activity (Jacob *et al.*, 2014) which thus prefers to use H3.1 as a substrate. ATXR5/6 recognizes newly deposited H3.1 at the replication fork and stimulate K27me1 decoration within genes, promoting further methylation to K27me3 mediated by PRC2 in inactive chromatin regions (Jiang and Berger, 2017). However, unmethylated H3.1 is recognized by the A31-specific reader TONSOKU (TSK) to mediate DNA repair at DNA double strand breaks (Davarinejad *et al.*, 2022). Conversely, H3.1K27me1 imposed by ATXR5/6 inhibits TSK recognition of H3.1 and in turn promotes genomic instability and over-replication of large tandem duplications in heterochromatin regions (Jacob *et al.*, 2010). Similarly, the N-tail amino acid sequence in other H3 histone variants can modulate their recognition by KMTs. A striking example is the sperm-specific H3.10 in plants that is largely divergent around K27 in amino acid residues and thus a poor substrate for the different methyltransferase acting on this amino-acid residue, this in turn favors the expression of spermatogenesis genes (Borg *et al.*, 2020). These observations underline how histone variants presence, in concert with their modifications, can act as important regulators of key biological functions. Given their differences in expression and chromatin incorporation, H3.1 and H3.3 are extensively investigated for their potential role during differentiation and, potentially, for tumorigenesis (Ray-Gallet and Almouzni, 2022).

Other H3 variants in mammals have been reported, in particular during spermatogenesis (Rousseaux *et al.*, 2005). For example, H3t, a testis-specific variant differs by 4 amino acids from H3.1 (Witt, Albig and Doenecke, 1996; Govin *et al.*, 2005) and H3.5, an evolutionarily derivative of H3.3, is equally found in testis, (Urahama *et al.*, 2016). Finally, additional H3 variants previously annotated as pseudogenes in mouse are

mainly present in euchromatin and regulated in a tissue-specific manner (Maehara *et al.*, 2015). Further work will help to better understand how these variants provide specific nucleosome functions in different tissues, as well as how they may influence cell differentiation or development.

1.5.5. H3.1 and H3.3 variants and cancer

Several H3 mutants, referred to as “oncohistones”, are associated with cancer. Pediatric high-grade gliomas show a high frequency of modifications in the histone H3 genes *H3F3A* and *H3C2*, encoding for H3.3 and H3.1, respectively (Khuong-Quang *et al.*, 2012; Schwartzentruber *et al.*, 2012; Behjati *et al.*, 2013; Taylor *et al.*, 2014). The amino acid substitutions often occur in the conserved N-terminus of the H3 tail, including K27, K36, and G34. The loss of H3K27me3 in H3K27M mutants is associated with inhibition of Polycomb repressor complex 2 (PRC2) activity, which in turn leads to upregulation of genes involved in neurodevelopmental progression (Bender *et al.*, 2013; Lewis *et al.*, 2013; Mohammad *et al.*, 2017). H3G34R gives rise to recurrent cerebral hemispheric-specific tumors through inhibition of the KDM4 demethylase and upregulation of the MYCN oncogene (Behjati *et al.*, 2013; Bjerke *et al.*, 2013). H3K36M promotes sarcomagenesis by inhibition of H3K36 methyltransferases, thereafter impeding mesenchymal differentiation processes (Lu *et al.*, 2016). Such specificity of mutations towards particular tissues might be explained by the different gene expression dynamics of H3 variants across different cell types. Mutations occurring on replicative variants, rather than non-replicative, affect predominantly chromatin landscapes of proliferating cells, including stem cells, whereas mutated non-replicative variants will be progressively incorporated in long-lived cells. In addition, replicative variants are incorporated globally across the genome, leading to more severe phenotypes, while H3.3 is incorporated at specific genomic targets. Finally, a recent report suggests that oncohistones also occur in other underappreciated cancers. However, it is unclear if they are a driver or passenger mutations in these tumor types. Understanding how mutations on histone variants will impinge on signaling pathways, chromatin remodeling, and histone PTMs crosstalk remains of utmost interest for uncovering new therapeutic targets in precision medicine treatments.

1.5.6. Centromeric histone variant: CenH3

The centromeric histone H3 variant, CenH3, is known by different names depending on the species: for example, Cse4 in budding yeast, CENP-A in mammals, and CENH3 in plants. It was first identified as a kinetochore protein and was discovered from autoantibodies targeting centromeres of patients with CREST syndrome (calcinosis, Reynaud syndrome, esophageal, dysmotility, sclerodactyly, telangiectasia) (Earnshaw and Rothfield, 1985). This histone variant exists as a single copy gene, is highly divergent across species, and shares <50% amino acid content with the other H3 variants (Palmer *et al.*, 1991; Wolffe, 1995). In fact, centromeric DNA sequences are also poorly conserved given the high frequency of recombination events happening during meiosis for inclusion in the female eggs, translating into a low level of conservations for both DNA and relative binding protein. Such divergent centromere composition could be explained by the “centromere drive hypothesis”, where centromeres expand their adenine/thymine (A/T) content for stably wrapping nucleosomes, while both DNA and protein components rapidly co-evolve to maintain their centromere function (Henikoff, Ahmad and Malik, 2001; Drinnenberg, Henikoff and Malik, 2016). CENP-A, at least in mammals, epigenetically defines centromeres (Barnhart *et al.*, 2011; Fachinetti *et al.*, 2013). Compared to H3.1 and H3.3, CENP-A nucleosomes contain 121 bp of DNA that might contribute to both the identity and function of centromeres (Tachiwana *et al.*, 2011; Geiss *et al.*, 2014; Lacoste *et al.*, 2014). Centromeres are the sites where kinetochores form and direct chromosome segregation during mitosis. Apart from this function during cell division, centromeres contribute to genome architecture as robust nuclear domains in interphase nuclei of mammalian cells (Dunleavy *et al.*, 2009; Lacoste *et al.*, 2014). The synthesis of CENP-A in late S/G2 allows a delayed incorporation in late mitosis/G1 in somatic mammalian cells (Shelby, Vafa and Sullivan, 1997; Jansen *et al.*, 2007). Thus, there is a dilution of CENP-A in S phase at centromeres. Lack of this variant results in disruption of kinetochore formation during mitosis (Hoffmann *et al.*, 2016; Müller and Almouzni, 2017) and CENP-A KO mice don’t develop beyond stage E8.5 due to accumulation of mitotic defects and chromosome mis-segregations (Howman *et al.*, 2000). To ensure its loading at the centromere, the Holliday Junction recognition protein (HJURP) is the histone chaperone dedicated to CENP-A (Dunleavy *et al.*, 2009; Foltz *et al.*, 2009; Dunleavy, Almouzni and Karpen, 2011). HJURP handles a dimer of CENP-A-H4, but its dimerization facilitates the formation of CENP-A-H4 tetramers (Zasadzińska *et al.*, 2013). Since both H3.1 and H3.3 are deposited at centromeric regions in S-phase, it was remarkable to note

that only H3.3 is reduced at centromeres in G1 phase compared to S phase. This observation has led to the hypothesis according which H3.3 could represent a “placeholder” to allow new CENP-A deposition in G1 (Dunleavy, Almouzni and Karpen, 2011). Therefore, the regulation of CENP-A transcription, stability, and chromatin deposition, together with other H3 variants is critical to ensuring genome stability.

1.6. Centromeres

Centromeres are regions of chromosomes that serve as the foundation for the assembly and attachment of the kinetochore complex proteins in mitosis to ensure correct chromosome segregations during cell divisions. While the essential function of centromeres is evolutionary conserved, the proteins binding these regions and the underlying DNA content are not (Karpen and Allshire, 1997). Often referred to as the “dark matter” of the genome, centromeric sequences are highly variable among species in DNA content and size, ranging from only 120 bp in *S. cerevisia* to megabase (Mb) long regions in higher eukaryotes (Muller, Gil and Drinnenberg, 2019) (Fig. 6). The Mb-long centromeres in humans are made up of satellite repeats interspersed with transposable elements. Satellite DNA carries different ratios of nucleotides as compared to genomic DNA, resulting in a distinct (or satellite) DNA band when separated from bulk DNA through a density gradient (Kit, 1961). They consist of arrays of repetitive, non-coding, short DNA sequences of up to 200 bp. For this reason, centromeric domains pose a major hurdle for completing genomic maps. Apart from recent efforts to assemble entire human chromosomes with long-read sequencing (Nurk *et al.*, 2022), mapping short reads in non-unique elements gave rise to enormous ambiguity when assigning their positions along a chromosome. Human centromeres, for example, are mainly composed of long tracts of a-satellite monomer repeats enriched with CENP-A and reduced CpG methylation regions. Compared to the densely CpG methylated pericentromeric repeats, containing b- and y- satellites, centromeres are highly divergent between chromosomes, as each can vary in content of a- satellite variants, as well for the presence of duplication, inversions and TEs insertion events (Altemose *et al.*, 2022; Gershman *et al.*, 2022; Hoyt *et al.*, 2022).

One important aspect is to distinguish centromeric from pericentromeric features. The situation can vary in different model organisms. In fact, while centromeric and pericentromeric repeats can be nearly identical in some cases (e.g., alpha satellites in human), they can differ not only in terms of sequence composition (e.g.,

minor and major satellites in *Mus musculus* (Guenatri *et al.*, 2004)), but also in terms of epigenetic marks (e.g., 180-bp repeats methylation status in plants (Zhang *et al.*, 2008)).

In this section, I will briefly introduce the main components of centromeres and their regulation in a nuclear organization context.

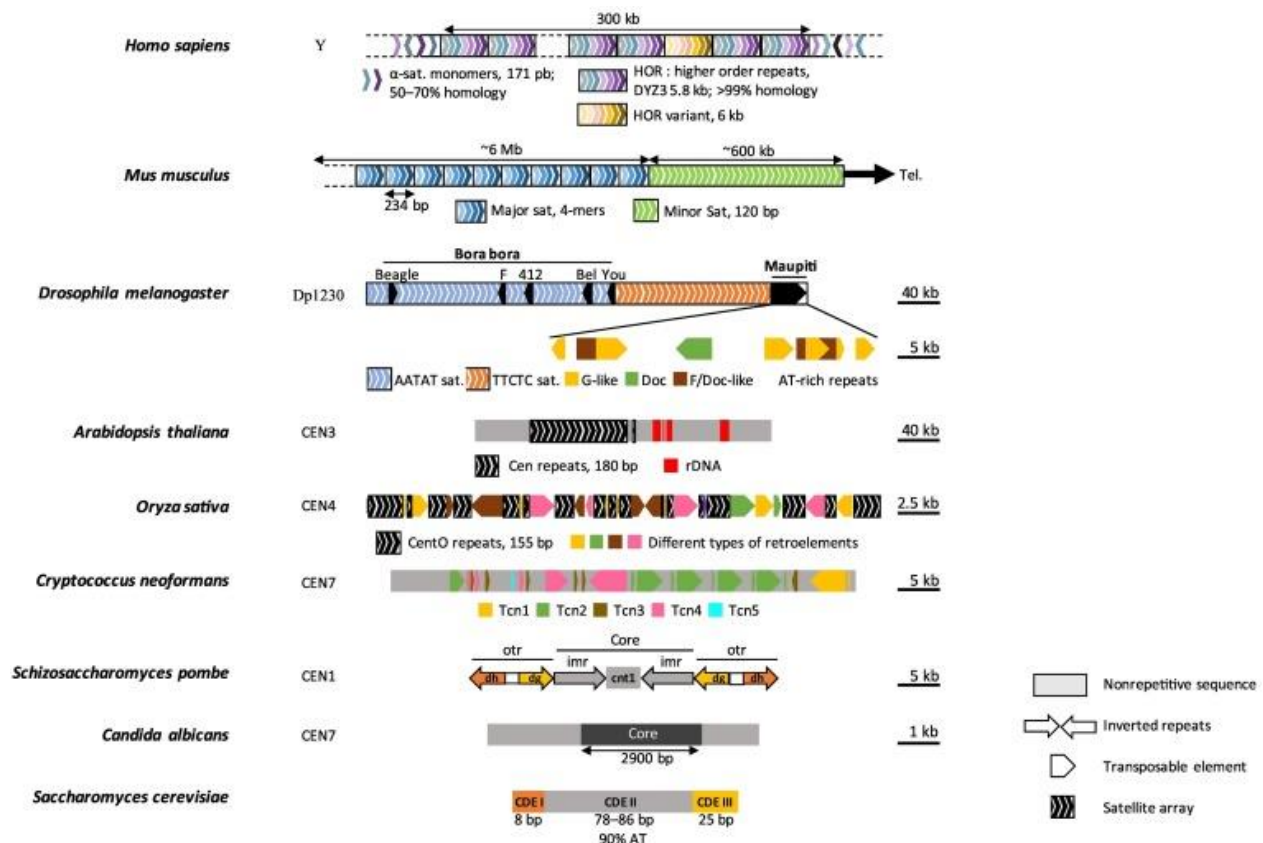


Figure 6. Centromere Sequence Organization in eukaryotes. The typical centromere organization for each species is shown, along with the size range for features of interest. When a centromere of a chromosome is depicted, its number is shown after the species name, and an approximate scale is shown on the right. Adapted from (Muller *et al.*, 2019)

1.6.1. Transposable elements: ancient inhabitants of the genome

Pioneer work from Barbara McClintock in the 1950s, studying changes of color pigmentation in maize kernels, led to the groundbreaking discovery of transposable elements (TEs). She observed that certain color patterns were gained or lost from one generation to another. This was mainly attributed to chromosomal insertion, deletion, or translocations that were present in the daughter cells but not in the parental line (McClintock,

1953). She hypothesized that these phenotypes were caused by a “position-effect” of some genetic material, later defined as “controlling, or transposable, elements”. TEs are able to move (or transpose) along the genome causing different phenotypes, sometimes even deleterious. In humans, the majority of genetic information consists of non-coding sequences, mostly shared by TEs, while less than 2% of genome encodes proteins (Lander *et al.*, 2001). As changes in gene expression rely on non-coding regions, in particular regulatory regions, increasing evidence displays the relevance of TEs in shaping genome complexity in the light of evolution. . Broadly, TEs can be divided into two main classes based on their translocation mechanism. DNA transposons, the least abundant class, excise and insert their DNA. Retrotransposons transcribe RNA intermediates before genomic integration. Retrotransposons can be further classified if they possess long terminal repeats (LTR), or not (Non-LTR or solo-LTR). In mammals, among LTR retrotransposons we include endogenous retrovirus elements (ERVs), such as Intracisternal A-particle (IAP), and early transposons (ETns) that can mobilize autonomously. Among non-LTR, we have long interspersed elements (LINEs) and non-autonomous short interspersed elements (SINEs). Some of these elements carry regulatory regions enabling their expression in the host germline causing *de novo* mutations in the offspring through vertical transmission (reviewed in (Fueyo *et al.*, 2022)). The peak in TE expression occurs in totipotent and pluripotent embryonic cells, where clashes between the binding of transcription factors and changes in heterochromatin compartments occur. As a result, the host genome takes advantage (or co-opts) of TE sequences for genome regulatory functions and genome organization. Several epigenetic mechanisms co-evolved to modulate TE repression and maintenance while simultaneously tuning lineage-committed gene expression of chromatin modifiers, including transcription factors, histone writers, readers, and erasers to ultimately develop the diverse cell types of an entire organism.

1.6.2. Satellite DNA sequences: heterochromatin structural components

Most of the eukaryotic centromeres, including humans and mice, are organized by satellite sequences. However, compared to humans, a typical mouse chromosome is acrocentric, implying that one centromere is closer to one telomeric region than the other (Fig. 7). In the mouse centromere, we can distinguish two chromatin domains consisting of different properties. Minor satellites are present in the centric regions, where CenH3-containing nucleosomes form the foundation for kinetochore assembly. The basic minor satellite repeat

unit is 121 bp long and it is repeated >2500 times per chromosome. The pericentric heterochromatin (PHC) regions border the centric regions and are enriched in major satellite repeats and constitutive heterochromatin, including H3K9me3, as well as its reader Heterochromatin Protein 1 (HP1). The major satellite repetitive unit is 234 bp long and is repeated up to 6Mb, comprising up to 10% of the mouse genome (Wong and Rattner, 1988; Guenatri *et al.*, 2004). Albeit present in heterochromatin, it was subsequently noted that these sequences can produce low levels of non-coding transcripts (Saksouk, Simboeck and Déjardin, 2015). In *Pombe* (*Schizosaccharomyces pombe*), RNA Pol II transcribes satellite sequences and these transcripts are processed by the RNA interference machinery (RNAi) to trigger heterochromatin formation (Volpe *et al.*, 2002). In mice, an RNA component is involved in structuring the three-dimensional organization of heterochromatin at pericentromeric regions, as treatment with RNase disrupts foci of H3K9me3 and HP1 (Maison *et al.*, 2002).

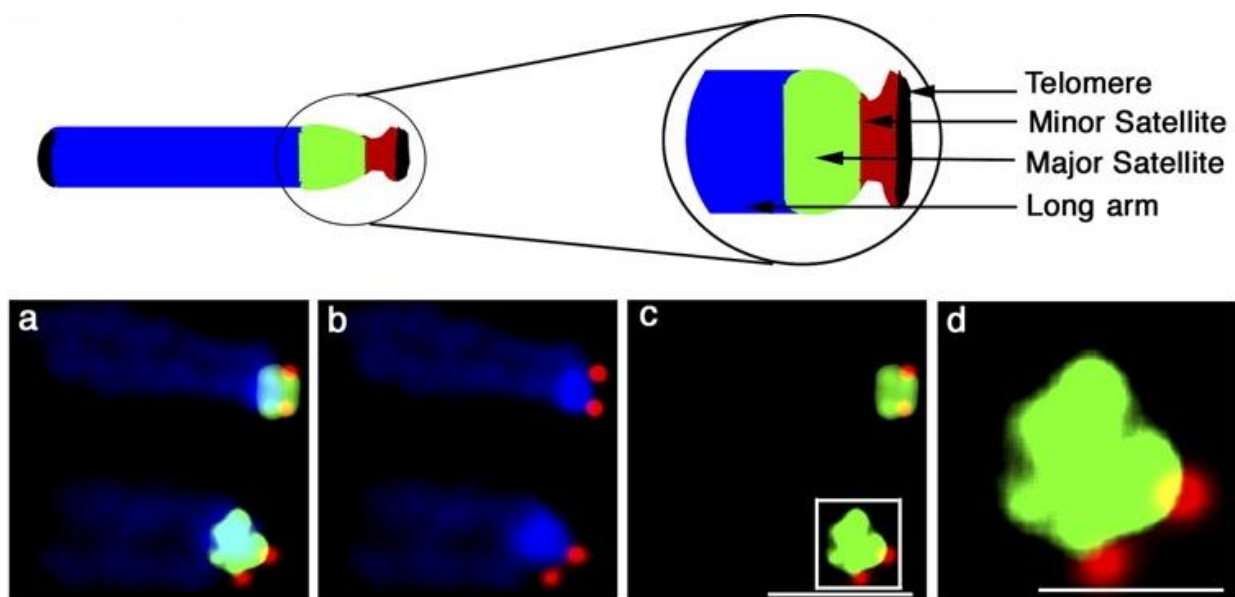


Figure 7. Major and minor satellite DNA localization on mouse chromosomes. Top panel: Schematic of a typical acrocentric mouse chromosome highlighting the inset with corresponding centromeric region around the telomere (black). Bottom panel: Fluorescence in situ hybridization (FISH) localize minor satellite (red) and major satellite (green) probes, as well as DNA (DAPI staining; blue). (A) is merged of major, minor satellites and DNA; (B) same but with no major satellites; (C) and (D) (inset) same but with no DNA. Adapted from (Guenatri *et al.*, 2004).

1.6.3. Chromocenters: lessons from plants

In the longstanding discussion to assess the best staining procedure for visualizing cell nuclei, it was widely postulated that the presence of darker areas in the nucleus usually corresponded to the accumulation of chromosomes (Rosenberg 1903). The Italian biologist Baccarini reported the presence of recurrent chromatin masses accumulating inside the *nuclear juice* that he defined as *cromocentri* given that they came in frequent and regular rounded shapes (Fig. 8) (Baccarini, 1908).

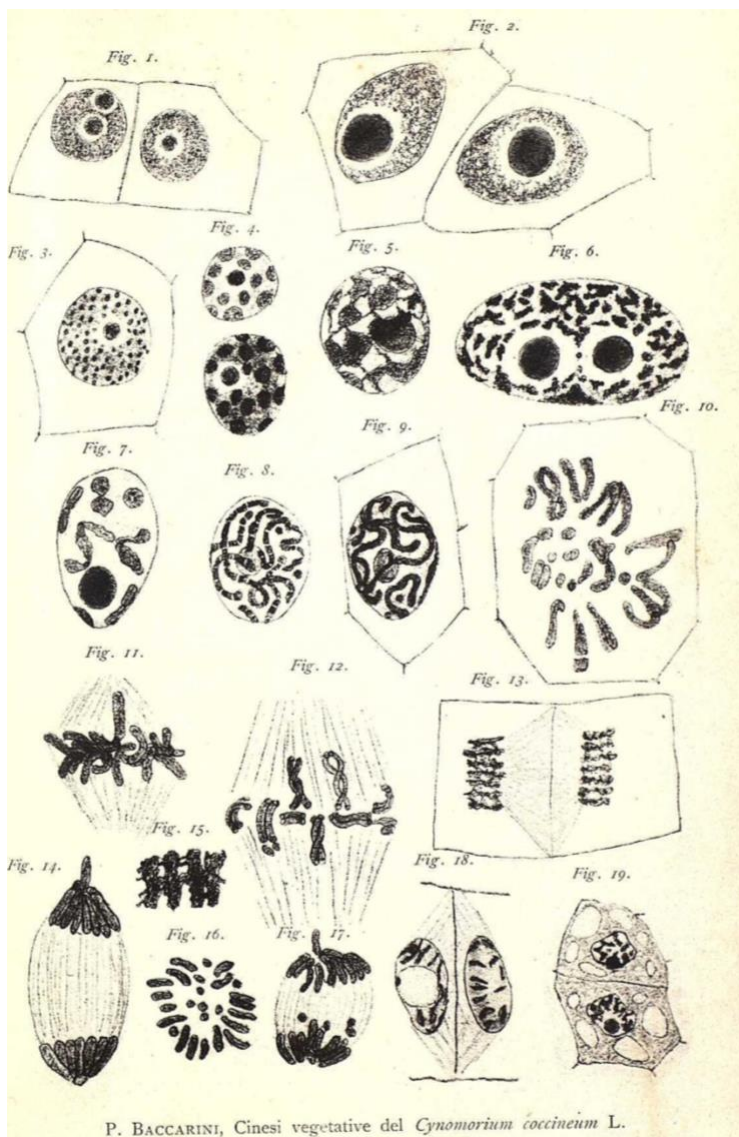


Figure 8. Chromocenters in plants.

Original illustrations from *Sulle Cinesi Vegetative del Cynomorium coccineum* (Baccarini, 1908). Presence of chromocenters in Fig. 1-6.

Spirem/chromosome condensation starts in Fig 7-10 followed by mitosis from Fig 11-19.

Chromocenters emerged as a paradigm for constitutive heterochromatin and a hub for centromere organization in several species. However, all organisms possess centromeres, but not necessarily visible chromocenters. By assessing by microscopy the properties of these regions, 3 main categories of centromeres emerge: point or

monocentric, where the kinetochore assembly machinery is restricted to a single nucleosomal region (i.e., CSE4 in *S. cerevisiae*, reviewed in (Henikoff and Henikoff, 2012); holocentric, where the spindle microtubules attach along the entire chromosome (i.e., in diptera insects, reviewed in (Drinnenberg, Henikoff and Malik, 2016)); and regional, commonly found in mammals and plants, identified by the presence of centromeric-specific nucleosomes distinguishing centromeric from pericentromeric regions. All these configurations ensure correct chromosome segregation into two daughter cells. Centromeres allow the assembly of kinetochore protein complexes and microtubule spindle attachment, although a lack of consensus regarding centromere architecture still persists (Kursel and Malik, 2018).

In plants, two main configurations of centromeric organization exist: a Rabl configuration characterized by centromeres organized in one pole of the nucleus and telomeres at the opposite site, and a non-Rabl configuration, where centromeres and telomeres intermingle in the nucleus (Dong and Jiang, 1998). Among these, some species possess chromocenters to feature. *Arabidopsis* possesses a non-Rabl configuration and often shows less than ten chromocenters in the nucleus ($2n=10$) (Fransz *et al.*, 2002). Centromeric regions in *Arabidopsis* are mostly made up of a 178-bp sequence known as CEN180, AtCon, pAL1 or 180 bp that is arranged in head-to-tail tandem repeats (Kumekawa *et al.*, 2000, 2001). At the nucleosomal level, they are enriched in CenH3 (Nagaki *et al.*, 2003; Shibata and Murata, 2004), which, as in mammals, defines the centromeric regions and distinguishes them from the flanking pericentromeric regions enriched in repressive marks (reviewed in (Simon *et al.*, 2015)). The histone-methyltransferases ATXR5/6 preferentially mark heterochromatin via mono-methylation on lysine 27 of the canonical histone H3.1 (Jacob *et al.*, 2014), which is highly enriched in pericentromeric regions (Benoit *et al.*, 2019), but not of H3.3, mostly present at telomeres (Vaquero-Sedas and Vega-Palas, 2013) as well euchromatin regions (Shu *et al.*, 2012). Despite the challenge of precisely assigning the relative enrichment of nucleosomes in interspersed regions, the establishment of a correct reference sequence map will help us to better decipher how specific variants are positioned along these regions. For example, a recent study using long-reads sequencing approaches provided a reference map of *Arabidopsis* centromeres. Centromeres mainly consist of CEN180 repeats, but present different rearrangements of repeats and variants on each chromosome as a result of genetic and epigenetic adaptation during evolution (Naish *et al.*, 2021). Interestingly, CENH3 nucleosomes occupancy correlates with the least divergent CEN180 satellite repeats and regions with high DNA methylation, which may act to limit DNA

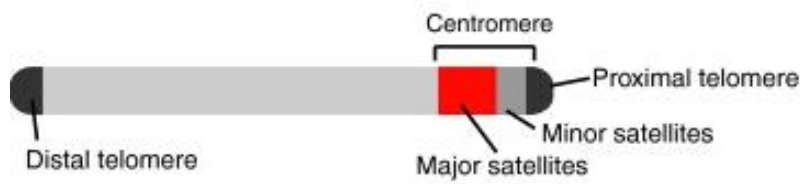
recombination events and invasion of retrotransposons, such as ATHILA elements (Naish *et al.*, 2021). Thus, exploiting long-read sequencing will help to pinpoint how sequences corresponding to centromeric and pericentromeric regions can display distinct chromatin composition in terms of histone variants in a very refined manner.

Of note, chromocenters, and relative organizations, are stable and evolutionary conserved structures present in higher eukaryotes, including in the mouse model organism *Mus Musculus*, and can provide information about the epigenetic status of constitutive heterochromatin in mammals.

1.6.4. Mouse chromocenters

Mouse PHC possesses an AT high content making them easily identifiable by DNA markers, including the well-exploited Hoechst and 4-6-diamidino-2-phenylindole (DAPI) stains, which intercalate into the DNA minor groove of AT-rich regions. The clustering of major satellite repeats from different chromosomes results in the formation of chromocenters, with minor satellites at the periphery of these clusters (Guenatri *et al.*, 2004) (Fig. 9). This organization is fundamental to relocate euchromatin in the nuclear interior while heterochromatin borders the nuclear periphery or the surrounding nucleoli. Not only being organized in distinct spatial domains, centric and pericentric heterochromatin replicate at different time windows during S-phase. While centric repeats replicate throughout S-phase, major satellite repeats replicate specifically during mid S-phase, coincidentally with a reduction in transcription at these sites (Lu and Gilbert, 2007). This difference in replication timing may be a critical factor in establishing the nuclear pattern and organization of these two domains in the nucleus (Gilbert, 2010).

A) Typical mouse acrocentric chromosome



B) Chromocenter organization

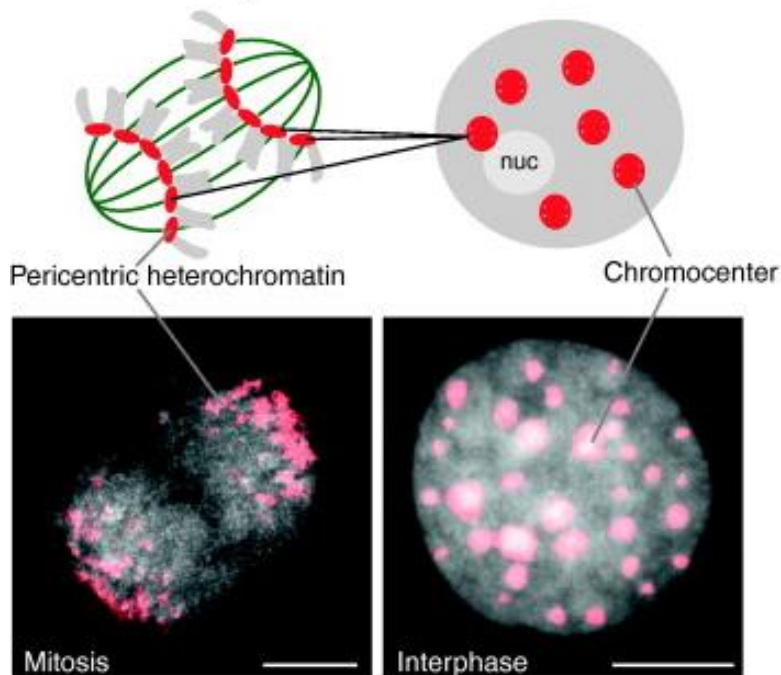


Figure 9. Organization of pericentric heterochromatin in *Mus Musculus*.

A) Mouse centromeres are usually localized closer to one telomere (black) compared to the other and are composed of major (red) and minor (dark grey) satellites. Major satellites mediate sister chromatid cohesion whereas minor satellites are important for kinetochore formation.

B) In interphase, pericentric heterochromatin domains (red) of each individual chromosome (left) cluster together with corresponding regions from other chromosomes to form chromocenters (right). The images below show DNA fluorescence in situ hybridization (FISH) for major satellite repeats of mouse 3T3 cell in anaphase and interphase nuclei. DAPI (grey) is used to stain the DNA; nuc, nucleolus; scale bar, 10 μ M. Adapted from (Probst and Almouzni, 2011)

The non-random organization of chromocenters in the nucleus might account for their role in preventing transcription factors from scanning underlying DNA content, including transposable elements. However, until recently, it was still not clear the type of DNA for which they are enriched, apart from major satellite repeats. Chromocentric DNA purification followed by high-throughput sequencing (Ostromyshenskii *et al.*, 2018) revealed the presence of LINE elements as the second most abundant class at chromocenters, followed by ERVs, namely IAP elements. Despite their relative abundance in the genome, however, it is still unclear why some elements are present and incorporated in these regions while others are not. Furthermore, the mechanism governing the formation of such distinct domains is still poorly understood. The clustering relies on homotypic interaction between similar DNA sequences, as well with nuclear scaffold proteins present on the nuclear lamina or at nucleoli, which are constantly associated with chromocenters (Gibcus and Dekker, 2013; Cournac,

Koszul and Mozziconacci, 2016; Solovei, Thanisch and Feodorova, 2016; Werken *et al.*, 2017). Early investigations using fluorescence in situ hybridization (FISH) revealed that G-bands of mitotic chromosomes, corresponding to heterochromatin and enriched in LINE elements, are located in the nuclear and nucleoli periphery, while the gene-rich R-bands, enriched in SINEs, preferentially reside in the nuclear interior (Fig. 10). A notable exception to this nuclear organization are rod receptor cells of nocturnal mammals, where the heterochromatin translocates to the center of the nucleus in maturing rod cells (Solovei *et al.*, 2009). Heterochromatin positioning to the nuclear periphery is mediated by two different nuclear membrane proteins, the Lamin B receptor (LBR) and Lamin A/C, as their depletion in differentiated cells phenocopy this distinct pattern (Solovei *et al.*, 2013). The atypical nuclear organization facilitates gene positioning and light transmission in rod nuclei but does not show a major effect on overall gene expression (Solovei *et al.*, 2013). Another example of unconventional nuclear organization occurs in olfactory sensory neurons. Nearly all types of olfactory receptor cells express a single active olfactory receptor, whereas the other inactive ~2800 receptors, grouped in 92 gene clusters, are grouped around a single chromocenter (Clowney *et al.*, 2012). This clustering results in a position-effect silencing, as ectopic expression of LBR, results in chromosomal dispersal and loss of cell-specific olfactory receptor expression. The significance of why chromocenter clustering, as well as satellite rearrangements, vary in different cell types (Mayer *et al.*, 2005) and cell states (Terranova *et al.*, 2005) is unclear, but it may serve as a platform for epigenetic factors and maintenance of heterochromatin status (Probst and Almouzni, 2011) as well for genome stability (García Fernández and Fabre, 2022). Further studies are required to address how chromocenter clustering is linked to position-effect expression in cell-type specific contexts.

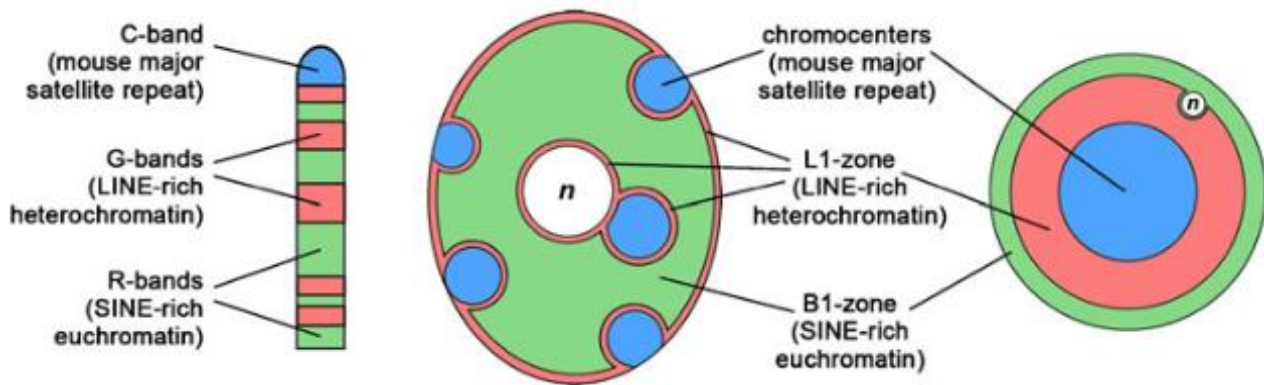


Figure 10. Conventional and inverted nuclear architecture. Schematic of the positions of the main chromatin classes in mouse mitotic chromosome bands (left), conventional (middle), and inverted (right) nuclei. n, nucleolus. Adapted from (Eberhart *et al.*, 2013)

1.6.5. Phase separation: solid-like or liquid-like heterochromatin?

As membrane-less nuclear domains play a central role in nuclear organization and dynamics, the possibility that chromocenters are compartmentalized through liquid-liquid phase separation (LLPS) is intriguing. In this view, a single homogenous mixture (of chromatin) possesses different phases (or compartments) with liquid-like features so that macromolecules in a given space, upon reaching a critical concentration, interact more frequently between each other than with the rest of the molecules in a surrounding environment. Given this, several biomolecules are proposed to form separate compartments, or condensates, in the nucleus, in particular heterochromatin regions. This was supported by the ability of HP1 proteins to 1) promote multivalent interactions *in vitro* and *in vivo*; 2) oligomerize due to the presence of intrinsically disordered regions; 3) induce heterochromatin silencing by promoting steric hindrance of large protein complexes, e.g., RNA polymerase. For these reasons, it was proposed that chromocenters mediate local molecular exchanges through HP1 oligomerization, promoting in turn LLPS (Larson *et al.*, 2017; Strom *et al.*, 2017). Furthermore, histones promote HP1 binding (via H3K9me3) and nucleosome–nucleosome interactions, mediated by their unstructured N-terminal tails, favors condensate formation. Histone acetylation instead promotes chromatin decompaction, but favors local recruitment of bromodomain-containing factors, leading to condensates rich in transcription factors (Gibson *et al.*, 2019) (Fig. 11). However, the hypothesis is still heavily debated, given the lack of robust hallmarks for LLPS, exclusive to other biological mechanisms (reviewed in (McSwiggen *et al.*, 2019)). For example, the use of fluorescence recovery after photobleaching (FRAP) that is used to estimate

the recovery time of a macromolecule to self-aggregate as a condensate inside the nucleus. This method is prone to high variability across different biological samples and makes it difficult to benchmark thresholds for which a molecule is considered more mobile (or “liquid-like”) than others. Similarly, the use of aliphatic alcohols interfering with weak hydrophobic interactions, such as 1,6-hexanediol, has been exploited for dissolving LLPS condensates. However, its use at higher concentrations (1-10%) inhibits enzymatic activities including kinases, phosphatases, and DNA polymerases (Düster *et al.*, 2021). Rather than liquid droplets, chromocenters were proposed to behave as collapsed chromatin globules (Erdel *et al.*, 2020). In this view, chromocenters do not increase proportionally in size or density with the accumulation of heterochromatin proteins, as in LLPS; rather they require a critical concentration of clustered heterochromatin elements to be formed. Consistently, chromocenters can be abruptly dissolved in response to variations of the surrounding chromatin environment, including transcription of underlying DNA sequences. In agreement with this, a recent study found that chromocenters-associated factors, as well as DNA, possess solid-like properties showing very limited molecular exchanges on both nucleosome arrays as well as chromocenters in various physiological conditions (Strickfaden *et al.*, 2020). This model also reconciles the behavior of proteins that bind transiently (or are “liquid-separated”) on chromocenters compared to the rest of the surrounding chromatin. In fact, the absence of HP1 recruitment to chromatin as a result of a decrease of H3K9me3 in cells lacking the methyltransferase Suv39 does not compromise the formation of chromocenters or their dimensions (Peters *et al.*, 2001), but an acute depletion of HP1 does reflect a general and rapid change in nuclear rigidity and stiffness (Strom *et al.*, 2021). In conclusion, chromatin may adopt a solid-like (or gel-like) phase, which functions as a scaffold for heterochromatin proteins, while the heterochromatin proteins possess liquid-like properties that can eventually coalesce around chromocenters.

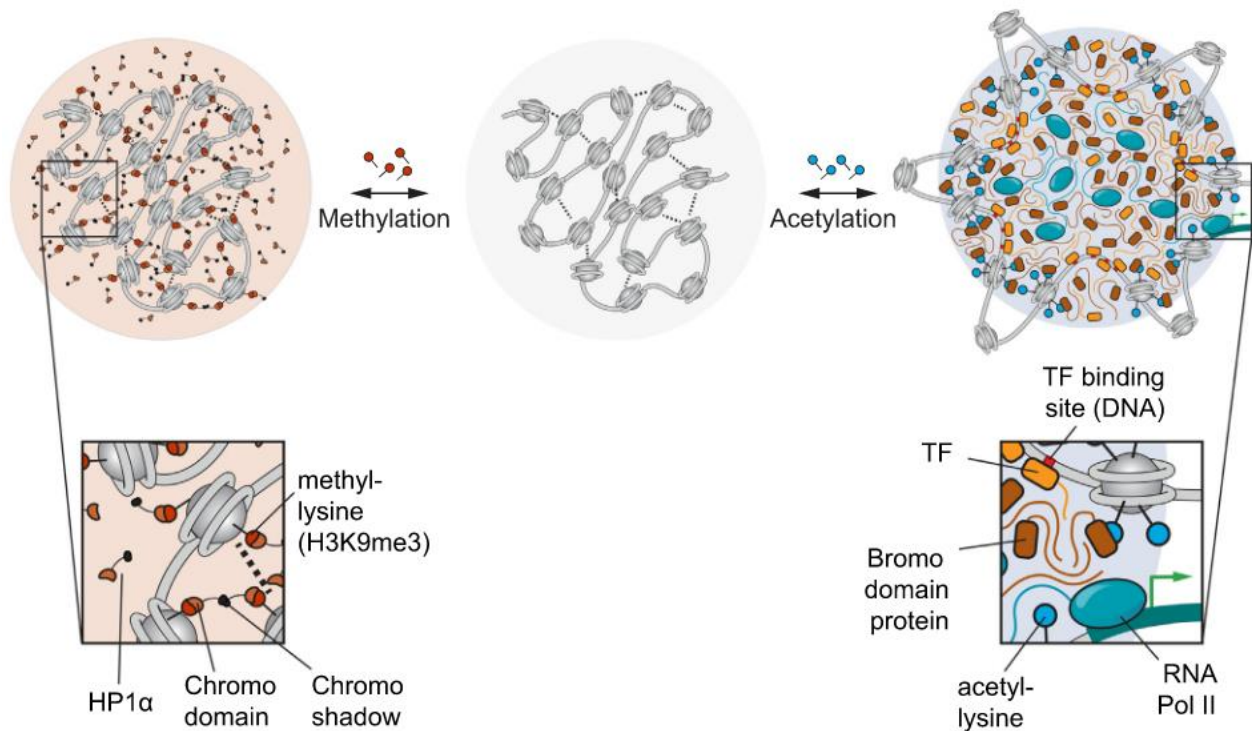


Figure 11. Biomolecular condensates can be regulated by modifying the underlying multivalent interactions. Reversible modifications of nucleosomal histones can result in changes in chromatin states. Inter-nucleosomal interactions (dotted lines) mediate the formation of chromatin condensate (central, gray). HP1 can be recruited to histone H3K9me3 via chromodomain binding, resulting in a condensate rich in HP1 and other heterochromatin components (left). Histone acetylation at several lysine residues, on the other hand, can diminish inter-nucleosomal contacts, exposing TF-binding sites on DNA and recruiting bromodomain-containing proteins, resulting in a condensate rich in transcriptional machinery components (right). Adapted from (Sabari et al., 2020).

1.7. Chromatin organization at finer scales

How is chromatin organized at the next scale level? Because of the existence of different domains and technological approaches, the eukaryotic genome is structured in space (from pm to μm size) and sequence (bp) at numerous levels. Furthermore, each level of organization is linked to genomic functions. In this section, I will present briefly the state-of-art of chromatin organization from both a technological and molecular perspective.

1.7.1. Sequencing methods

While microscopy studies are useful to depict the DNA in its chromosomal configuration, arising interest revolved around the proteins that bind the intermingled DNA during interphase and in euchromatin., Gilmour and Lis developed the first key assay, later called chromatin immunoprecipitation (ChIP), to detect DNA regions associated with heat-shock proteins (Gilmour and Lis, 1985). This method helps determine whether selected proteins are involved in DNA interaction at specific genomic sites. The method relies on obtaining a DNA-protein complex from a sample (typically after crosslinking), shearing it into small fragments, immunoprecipitating the protein of interest, and isolating the eluted DNA for analysis. With the improvement of high throughput sequencing technologies, ChIP followed by sequencing (ChIP-seq) allows to identify protein contacts genome-wide and therefore provides a map of genome occupancy for proteins of interest, and histone PTMs, but from the linear point of view of genome reference (Fig. 12).

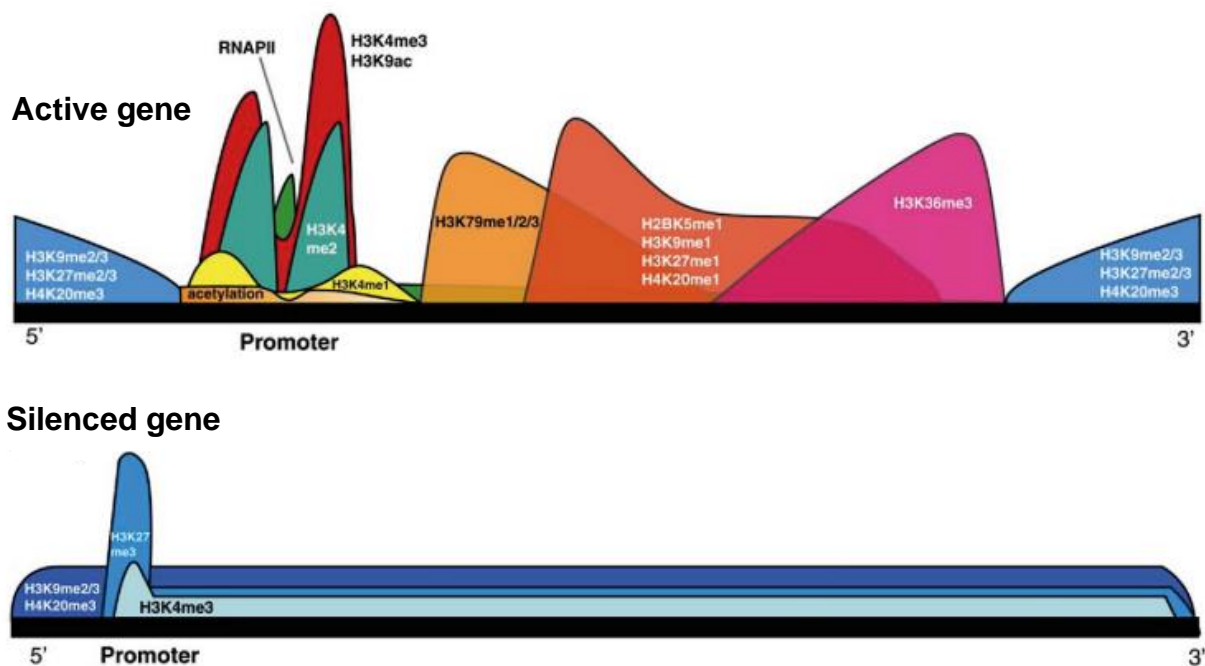


Figure 12. Representative distribution of histone H3 PTMs. Top and bottom panel corresponds to hPTMs present on active and inactive genes, respectively. Adapted from (Barth and Imhof, 2010).

However, given that DNA is bundled in 3D in a confined space, genomic loci that are linearly located at a great genomic distance along a chromosome (or even on different ones), can be found nearby in the nucleus. In the past decade, chromosome conformation capture techniques (i.e, 3C techniques) combining proximity

ligation and sequencing, allowed the establishment of contact maps discriminating differentially interacting genomic regions (Lieberman-Aiden *et al.*, 2009). On a large scale from 1-10 Mb, the genome is organized into two main distinct compartments, showing a higher frequency of contacts within each compartment and lower contacts between compartments. Among these, compartments associated with active transcription and open chromatin were referred to as A compartment, whereas those associated with inactive genes and close chromatin were called B compartment. On a smaller scale of 0.4-1 Mb, finer structures harboring local features, such as promotor-enhancer interactions, can be detected as topologically associating domains (TADs) (Dixon *et al.*, 2012; Nora *et al.*, 2012). Although recognized as a highly conserved feature, the mechanism and the functional relevance of bridging together genomic regions at relatively short distances is still actively debated. Although these methods proved useful to understand 3D genome organization rewiring during differentiation and diseases (reviewed in (Szabo *et al.*, 2019)), such observations of hierarchically folded domains rely on the relative frequency interactions between genomic loci coming from millions of cells, which are not necessarily indicative of physical interactions occurring in the nucleus of each cell at the same time. Emerging methods to improve the resolution of Hi-C techniques, such as Micro-C (Hsieh *et al.*, 2016; Krietenstein *et al.*, 2020), or by coupling these with super-resolution microscopy (Boettiger *et al.*, 2016; Bintu *et al.*, 2018; Szabo *et al.*, 2020) will help future studies to gain more insight into how TADs and compartments organize in the nucleus at the single-cell level.

Finally, a novel biophysical approach relies on the cross-linking and isolation from a sucrose gradient of sonication-sensitive and sonication-resistant heterochromatin (srHC) regions, without bias for the histone PTMs that are enriched (Becker, Nicetto and Zaret, 2016; Nicetto *et al.*, 2019). Using srHC as a proxy of chromatin compaction, the authors identified H3K9me3-decorated and sonication-resistant genes that, upon hepatocyte differentiation, lose this modification to allow their transcription (Becker, Nicetto and Zaret, 2016; Grindheim *et al.*, 2019; Nicetto *et al.*, 2019). Intriguingly, several srHC regions were kept transcriptionally silent and not covered in repressive histone PTMs, questioning the mechanism underlying chromatin compaction and repression at these regions.

1.7.2. Proteomics methods

An orthogonal approach is to interrogate the interacting chromatin proteome by selecting the DNA sequence as a means of purification of the Protein-DNA complexes. Indeed, a defined sequence would be independent of antibody or genetic tag biases. Proteomics of isolated chromatin (PICh) (Déjardin and Kingston, 2009) combines chromatin-DNA in situ hybridization with pull-down and mass-spectrometry to detect all proteins interacting with a specific probe. Given the abundance of repetitive sequences at constitutive heterochromatin, including telomeres and major satellites, this method proved useful to identify novel interactors of chromocenters. For example, in the absence of DNA methylation, the PRC2 complex is tethered at pericentric heterochromatin via BEND3 recruitment that in turn recruits the NuRD histone deacetylation complex promoting heterochromatin formation (Saksouk *et al.*, 2014). However, this method is limited by the relative genomic abundance of a given target sequence, making it virtually impossible to purify the interactome of a single-copy locus. For example, a relatively long gene of 3kb represents only 0,0001% of the 3 Gb-long human genome, whereas telomeres are 300 kb long and thus have a genome coverage of 0.01%. Strategies to obtain purer material and isolate single loci are being developed, including a strategy using RNA-guided d-Cas9 harboring affinity purification moieties (Vermeulen and Déjardin, 2020). Using these approaches, previously unappreciated proteins associated with ribosomal RNA genes, despite being less abundant than satellites, but still in high-copy numbers, have been identified (Ide and Dejardin, 2015). Nevertheless, a comprehensive determination of the specific protein composition at smaller unique loci, including genes, that are distinct from transient interactors or background proteins is still a major hurdle due to technology limitations.

1.7.3. Imaging methods

Currently, one of the best approaches to depict DNA organization in interphase nuclei relies on microscopy methodologies. With the use of a probe for specific DNA sequences, fluorescence in situ hybridization (FISH) allowed the visualization of human chromosomes in organized nuclear compartments, named thereafter “chromosome territories” (Lichter *et al.*, 1988; Bolzer *et al.*, 2005). Chromatin compartmentalization can be observed via electron microscopy showing electron-dense regions corresponding to heterochromatin permeated by electron-poor regions of euchromatin (Davies, 1967). So far, epifluorescent microscopy is

constrained by the light-diffraction resolution limit (Abbe, 1873), limiting the possibility of visualizing structures smaller than 200 nm, for example, the organized 30-nm chromatin fiber, directly *in cellulo*. New technologies are defined collectively under the umbrella of “Super-Resolution” microscopy, including Photo-Activated Localization Microscopy (PALM) (Betzig *et al.*, 2006), Stochastic Optical Reconstruction Microscopy (STORM) (Rust, Bates and Zhuang, 2006) and Structured Illumination Microscopy (SIM) (Scheremelleh *et al.*, 2008), provided a new 3D dimension of nuclear and chromatin visualization down to 10 nm resolution. Recent studies demonstrated that chromatin, visualized both by DNA and/or histones, is not organized in ordered structures but rather in heterogeneous groups of clutches and/or conglomerates in a transcription- or replication-associated manner, as well as cell fate related (Fig. 13) (Ricci *et al.*, 2015; Nozaki *et al.*, 2017; Clément *et al.*, 2018; Xu *et al.*, 2018).

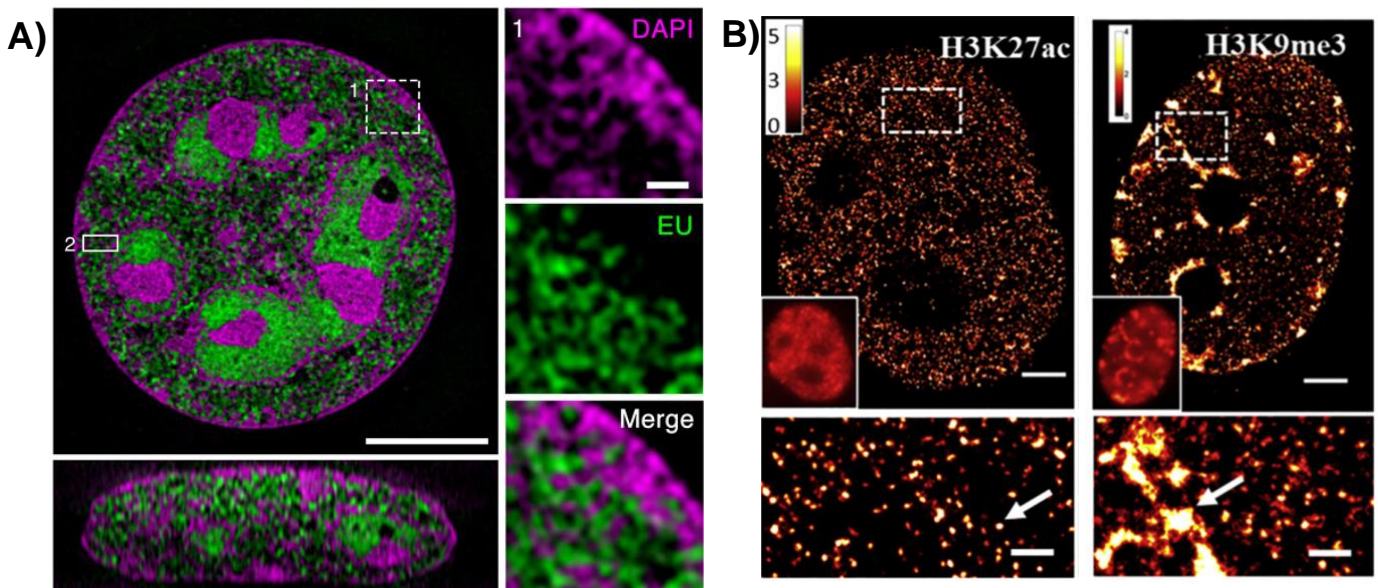


Figure 13. Chromatin regions in super-resolution microscopy. A) Structured illuminated microscopy (SIM) image of mouse C127 epithelial cells from (Miron *et al.*, 2020). Regions in magenta are DNA; regions in green are RNA. Lateral (top) and orthogonal (bottom) cross-sections of 3D image stacks. Right: Inset magnifications of region 1. Scale bars 5 and 0.5 μm (inset) B) Super-resolution images (STORM) of H3K27Ac (euchromatin) and of H3K9me3 (constitutive heterochromatin) in HeLa cells with corresponding wide-field images in inset (bottom right) from (Xu *et al.*, 2018). Enlargements highlight the featured structure of histone marks with arrows pointing to specific conglomerates/clutches organization. Heatmap represent relative local density of localizations. Scale bars, 2 μm and 500 nm in the original and magnified images, respectively.

In euchromatin of human nuclei, nucleosomes organize in small clusters regularly interspersed in the interior nucleus. Heterochromatin is stably condensed as coalesced, compact nanostructures at the periphery of nucleoli and the nuclear periphery. This organization at sub-nanometer scales is consistent with a dynamic arrangement where chromatin continuously adapts in response to external cues to exploit its genome function. In summary, chromatin in the nucleus is divided into compartments sharing functional biological properties. How chromatin is mechanistically regulated at the molecular level is depicted in the next chapter.

Chapter 2:

Genome regulation and heterochromatin maintenance

2.1. DNA methylation

DNA methylation is the most prevalent DNA modification. In mammals, this modification refers to the addition of a methyl group on the fifth carbon of cytosine referred to as 5-methylcytosine, or 5mC. 5mC is concentrated in genomic areas defined as CpG islands, which have a higher content of GC dinucleotides (Bird *et al.*, 1985). CpG islands are located at the transcriptional start sites of genes and their methylation status inversely correlates with their expression. The ubiquitous presence of this mark is essential for different biological functions based on genomic context. For example, hypermethylation is associated with the silencing of genes (Boyes and Bird, 1991), DNA repeats (Lehnertz *et al.*, 2003), or inactivation of the X-chromosome (Mohandas, Sparkes and Shapiro, 1981). However, while it generally correlates with silencing at transposons and gene promoters, DNA methylation is also found at gene bodies and is linked to gene expression regulation, although its precise function still remains elusive (reviewed in (Greenberg and Bourc'his, 2019).

Mechanistically, DNA methylation takes place in three phases: *de novo* methylation, maintenance, and demethylation.

In mammals, *de novo* methylation is mediated by DNMT3A and DNMT3B that are responsible for the methylation of unmethylated CpG islands (Okano, Xie and Li, 1998; Okano *et al.*, 1999). DNMT3A/B possess the ATRX-DNMT3-DNMT3L (ADD) and PWWP domains, which engage specifically with unmethylated H3K4 and H3K36me2/3, respectively, to promote DNA methylase activity on promoters and euchromatin (Fig 14). A catalytically inactive DNMT, DNMT3L, interacts and stimulates DNMT3A/B activity specifically in the germline (Bourc'his *et al.*, 2001; Ooi *et al.*, 2007). The presence of H3K4me3, especially at promoters, outcompetes DNMT preventing irreversible gene repression (Ooi *et al.*, 2007). In a similar way, H3K36me3 attracts DNMTs at active regions by inducing methylation of intragenic regions (Dhayalan *et al.*, 2010; Baubec *et al.*, 2015; Weinberg *et al.*, 2019). This puzzling interplay between DNA methylation and H3K36me3 might

function either to facilitate transcription (Bender *et al.*, 1999; Lister *et al.*, 2009; Varley *et al.*, 2013) as well to inhibit intragenic and cryptic promoters (Neri *et al.*, 2017; Teissandier and Bourc'his, 2017).

Moreover, DNMT3A possesses a putative interacting motif for H2AK119Ub, which is shared by PRC1 targets (Weinberg *et al.*, 2019). H2AK119Ub deposition at CpG islands is dependent on KDM2B, a CXXC motif subunit of PRC1, hence modulating H3K27me3 deposition in a DNA methylation sensitive manner (Brinkman *et al.*, 2012; Farcas *et al.*, 2012). Given that PRC1 and PRC2 act in a positive-feedback loop, H3K27me3 and DNA methylation are likely to be mutually exclusive in cell-specific contexts. A recent DNMT3C class has been uncovered that, unlike DNMT3A/B, lacks the PWWP domain but maintains its *de novo* methylation activity to specifically silence retrotransposons in mouse male fetal germ cells (Barau *et al.*, 2016).

DNMT1 is responsible for the maintenance of DNA methylation during DNA replication, cooperating with UHRF1 that recognizes hemimethylated DNA strands. Mechanistically, UHRF1 recognizes both DNA methylation and H3K9me3 resulting in the recruitment and targeting of additional DNMT1 molecules at the replication fork (Karagianni *et al.*, 2008; Bartke *et al.*, 2010; Rothbart *et al.*, 2012). This positive relationship between H3K9me3 and DNA methylation subsists during embryo development where H3K9me3-marked regions are resistant to DNA methylation turnover in cells with hypomethylated conditions, including blastocyst (Leung *et al.*, 2014). If methylation is not maintained by DNMT1, methylated DNA can be diluted progressively through DNA replication and cell division or actively via TET proteins that convert a methylated cytosine to 5-hydroxymethylcytosine (5hmC) (Kriaucionis and Heintz, 2009; Tahiliani *et al.*, 2009; Ito *et al.*, 2010, 2011; He *et al.*, 2011). Despite waves of global demethylation throughout mammalian embryonic development and reprogramming, a substantial proportion (~20%) of CpG retains DNA methylation in the inner cell mass of mice and humans to constrain the repetitive genome (Fig. 14) (Wang *et al.*, 2014; Zhu *et al.*, 2018). TEs are one of the main targets of the DNA methylation machinery (Dél  ris, Berger and Duharcourt, 2021). TEs can disrupt protein function or gene regulation simply by insertion within genes. In fact, DNA methylation supports genome stability by preventing TEs from transposing and promoting DNA recombination, thus limiting chromosomal rearrangements (Zamudio *et al.*, 2015).

Importantly, DNA methylation has been lost in several eukaryotic lineages, including common model organisms such as *Drosophila* (*Drosophila melanogaster*), *Pombe*, and *Caenorhabditis elegans*. Yet, it remains

unknown if alternative mechanisms have evolved to compensate for the absence of DNA methylation in such organisms.

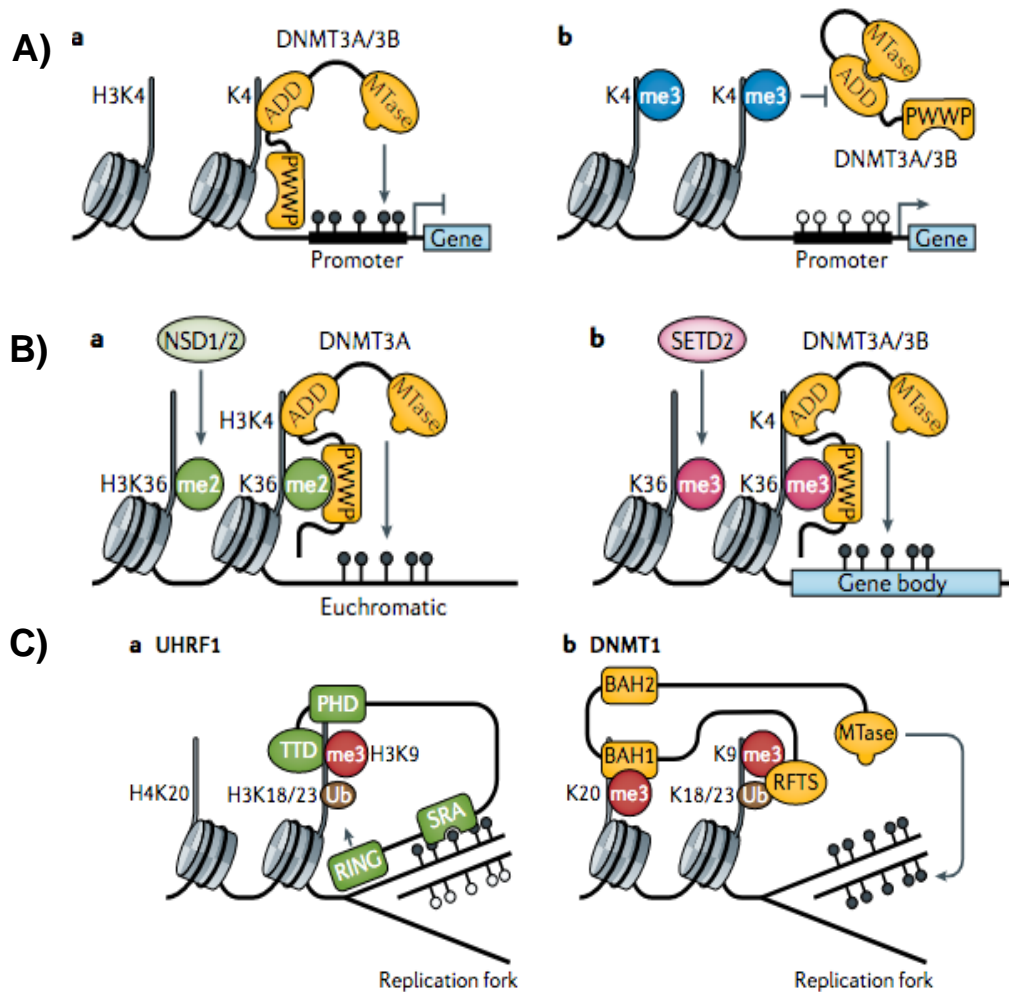


Figure 14. DNA methylation machinery and mechanism. A) Mechanism of promoter methylation: Left, a): in absence of H3K4me3, the ADD domain binds to H3K4, the auto-inhibition on DNMT3A/B is relieved and it allows the methyltransferase (MTase) domain to methylate the DNA. Right, b): H3K4me3, which marks active and poised promoters, prevents binding to chromatin of the ADD domain of DNMT3A/B, thereby causing it to bind MTase domain and autoinhibit DNMT3 enzymes. B) Mechanism of euchromatin methylation: In euchromatic regions, both at inter- and intragenic regions, the ADD domain in DNMT3A binds unmethylated H3K4 as in (A). In addition, the PWWP domain of DNMT3A recognizes the H3K36me2/3 as a module to promote de novo DNA methylation at euchromatin. Left, a) DNMT3A preferentially recognizes the dimethylation modification in H3K36 (H3K36me2) imposed by Nuclear Set Domain 1/2 (NSD1/2) in euchromatin, both at intra- and intergenic regions. Right, b) DNMT3B preferentially recognizes trimethylation modification in H3K36 (H3K36me3) imposed by SETD2 specifically in gene bodies, promoting DNA methylation at intragenic regions. In a redundant manner, DNMT3A can still recognize H3K36me3 rich-regions in absence of DNMT3B. C) Maintenance of DNA

methylation during DNA replication. Left, a): UHRF1 interacts with hemimethylated DNA via its SRA domain, H3K9me3 via its TTD, and the histone H3 tail via its PHD domain at the replication fork. The catalytic RING domain of UHRF1 enhances E3 ligase activity and promotes H3K18Ub and H3K23Ub. Right, b): The RFTS domain and ubiquitylated H3 interact to recruit DNMT1. The UBL domain of UHRF1 interacts with DNMT1 (not shown). RFTS domain can connect directly with H3K9me3, whereas the BAH1 domain interacts with H4K20me3 that generally co-marks sites enriched for H3K9me3. These interactions allosterically stimulate catalytic activity of the MTase domain of DNMT1, resulting in high-fidelity maintenance of DNA methylation in heterochromatic regions, including transposable elements. Adapted from (Janssen and Lorincz, 2021).

2.2. Histone writers and readers of heterochromatin

In mouse cells, H3 lysine methyltransferases (KMTs) mediate K9 methylation on histone H3 through their SET domain. Depending on their properties and cofactors, SET domain-containing proteins can mono-, di- or tri- methylate Lys substrates by using S-adenosyl-Met (SAM) as a methyl group donor. Based on their sequence homology, KMTs can be divided into six subfamilies based on the SET sequence similarity: 1) the Suppressor of variegation 3-9 (or SUV39) family; 2) the EZH family; 3) the SET-domain containing 1 (SET1) family of activating KMTs; 4) the SET2 family; 5) the PR domain-containing (PRDM); and 6) SET and MYND domain-containing (SMYD) families (reviewed in (Mozzetta, 2015)).

Suv39 (or Clr4 in *Pombe*) is a KMT that recognizes lysine-methylated residues through its chromatin-organization modified domain (chromodomain). It contains a pre-SET and a post-SET domain to stabilize its KMT activity. In mammals, Suv39 exists as two isoforms, Suv39h1 and Suv39h2 and promotes di- and tri-methylation by recognizing methylated H3K9 with higher affinity over unmethylated ones (Loyola *et al.*, 2006). SET domain bifurcated 1 (or SETDB1) can promote mono-, di-, tri-methylation that elicits Suv39h1 activity (Wang *et al.*, 2003). Once targeted at the correct place, Suv39h1 and Suv39h2 propagate H3K9me2 and H3K9me3 to neighboring regions serving as a docking site for HP1 proteins (Rea *et al.*, 2000; Bannister *et al.*, 2001; Lachner *et al.*, 2001). This H3K9me3 enrichment serves not only to repress major satellite sequences (Peters *et al.*, 2003), but also TEs that are marked by H3K9me3-SetDB1 and 5mC, including ERVs and LINEs, respectively (Bulut-Karslioglu *et al.*, 2014).

Additionally, G9a/GLP enzymes mediate gene repression by imposing mono/di-methylation (Tachibana *et al.*, 2002, 2005). They are found to be mainly enriched in euchromatin regions, but also to repress retrotransposon elements including MERV1 which are co-expressed with 2C-specific genes (Maksakova *et al.*, 2013). Despite mediating combinatorial methylation status, KMTs display some redundancy in their role. Nevertheless, Suv39h1 and Suv39h2 are mainly enriched in heterochromatin and their loss results in general transcription deregulation (Peters *et al.*, 2001; García-Cao *et al.*, 2004). H3K9me3 KMTs interact with many transcription co-repressors, including tripartite motif-containing protein 28 (TRIM28, or KAP1) (Schultz *et al.*, 2002), as well as readers of heterochromatin, such as heterochromatin proteins 1 (HP1), which is a reader of H3K9me2 and H3K9me3.

HP1 was first discovered in *Drosophila* as a dominant effector of Su(var)3-9 (James and Elgin, 1986; Eissenberg *et al.*, 1990). It mediates its silencing function by multiple domains: a chromodomain interacting with H3K9me2/3 (Bannister *et al.*, 2001; Lachner *et al.*, 2001); a chromo-shadow domain forming a dimerization interface to interact with other ligands (Cowieson *et al.*, 2000; Smothers and Henikoff, 2000); and finally, a connecting hinge domain, which interacts with RNA and DNA (Muchardt *et al.*, 2002; Meehan, Kao and Pennings, 2003). Mammalian HP1 proteins are shown to oligomerize with other isoforms, besides dimers (Yamada *et al.*, 1999; Yamamoto and Sonoda, 2003). The three mammalian HP1 isoforms (α , β , γ) can interact with constitutive heterochromatin (Maison and Almouzni, 2004). HP1 α in particular is a reader specific for pericentric heterochromatin that helps in recruiting Suv39h1 to spread the H3K9me2/3 marks on neighboring nucleosomes (Melcher *et al.*, 2000; Loyola *et al.*, 2006; Sampath *et al.*, 2007). Initiation of H3K9me3 at pericentric domains (which is then propagated) involves SUMO-modified HP1 α which shows an increased binding affinity for major satellite transcripts (Maison *et al.*, 2016). Finally, this positive-feedback loop could be regulated by the activity of other heterochromatin components including MeCP2 (Methyl-CpG binding protein 2) and sentrin-specific protease 7 (SEN7) (Müller-Ott *et al.*, 2014; Romeo *et al.*, 2015).

2.3. Molecular characterization of chromocenters

At the molecular level, chromocenters are enriched in constitutive heterochromatin marks rather than facultative heterochromatin, such as H3K27me3, which is required to maintain gene silencing. Among the hallmarks of constitutive heterochromatin are the enrichment in DNA methylation, H3K9me3, H4K20me3, H3K27me1, HP1 proteins, and ncRNA including satellite transcripts.

It is still unclear how chromocenter formations are initiated in mammals. In *Pombe*, the heterochromatin is initiated by ncRNAs that are expressed and processed by the RNAi machinery in centromeric regions (Volpe 2002). This process helps in recruiting and targeting heterochromatin modifiers at pericentric regions by performing a seeding step, which promotes methylation of H3K9 residues and deacetylation of nearby residues. In the developing mouse embryo, heterochromatin establishment is acquired by a complex interplay of epigenetic features (Probst and Almouzni, 2011). Before fertilization, maternal and paternal pronuclei carry different histone PTMs, with the maternal chromatin decorated at pericentric heterochromatin with H3K9me3, H4K20me3, H3K64me3, and H3K36me3 reviewed in (Fadloun, Eid and Torres-Padilla, 2013). Meanwhile,

the paternal chromatin lacks such marks and is rather decorated in H3K27me3 (Puschendorf *et al.*, 2008; Santenard *et al.*, 2010). Notably, chromocenters are not detected at this stage and major satellite repeats form a ring-like structure surrounding nucleoli in the zygote (Fig. 15). At the 2-cell state, a burst in major satellite transcription occurs at the time of chromocenter reorganization from a ring shape towards the dots typical of chromocenters (Probst *et al.*, 2010; Probst *et al.*, 2007). Depletion of these transcripts is sufficient to cause defects in chromocenter formation and impair development beyond the 2-cell stage, regardless of the chromatin marks associated with PHC (Casanova *et al.*, 2013). After the first cell divisions, these chromatin features are reset and heterochromatin features, including H3K9me3, are progressively acquired over lineage-committed genomic elements to bookmark regions undergoing heterochromatin compaction or transcriptional silencing (Wang *et al.*, 2018).

This process promotes a favorable environment for the KMTs of the SET family, including Suv39. Lack of H3K9me3 impairs HP1 recruitment, as demonstrated in mouse MEF cells lacking Suv39h1/h2 (Peters *et al.*, 2001). H3K9me3 maintenance by Suv39h1 is crucial for lineage commitment in terminally differentiated cells, including T-cells (Allan *et al.*, 2012; Pace *et al.*, 2018). However, the double KO of Suv39h1/h2 did not affect chromocenters formation in mouse cells (Peters *et al.*, 2001), while a progressive loss of Suv39 along with Setdb1 dramatically reduced chromocenters formation (Pinheiro *et al.*, 2012; Montavon *et al.*, 2021). Interestingly, Triple KO of Suv39h1, h2, and Setdb1 failed in activating markers for adult hepatocytes without affecting aneuploidy (Nicetto *et al.*, 2019). Together these data confirm the role of H3K9me3 and chromocenters formation in the early steps of embryogenesis rather than later differentiated cells.

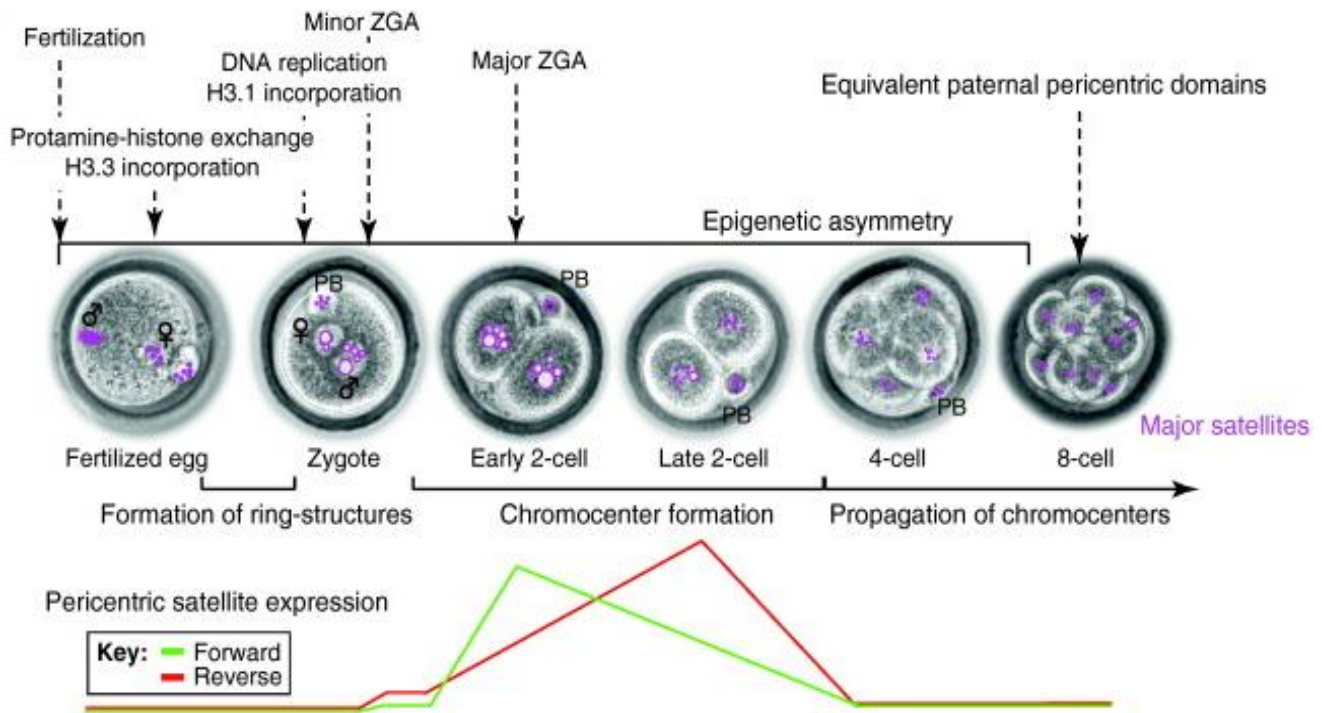


Figure 15. Pericentric satellite reorganization and expression during preimplantation development. Pericentric heterochromatin dynamically reorganizes and undergoes a transcriptional burst during which expression is regulated in a strand-specific manner during the first cleavage stages. Images of cultured embryos are superimposed on schematics illustrating the organization of major satellites (pink) at various stages. Pericentric satellites form ring structures around nucleolus-like bodies after fertilization. The formation of chromocenters occurs during the two-cell stage and is then propagated during subsequent cleavage stages. Main events including histone variant incorporation and transcriptional activation are mentioned. Transcription dynamics of the Forward (green) and Reverse (red) major satellite transcripts during early preimplantation development are depicted below. Transcription in two-cell embryos peaks during chromocenter formation and is downregulated in four-cell embryos. PB: polar body. Adapted from (Probst and Almouzni, 2011).

2.4. Additional factors involved in chromocenter formation

Chromocenter condensation does not only occur through multivalent recognition of histone PTMs but also thanks to its underlying DNA content. In fact, proteins containing multiple DNA-binding motifs known as AT-hooks can readily mediate the clustering of PHC coming from different chromosomes after cell division. HMGA1 in mammals and D1 in *Drosophila* possess 3 AT-hooks and 11 AT-hooks, respectively. Subsequently, the proliferation disruptor (Prod) protein from *Drosophila* was recognized to specifically bind the abundant 10bp AATAACATAG repeat (Jagannathan, Cummings and Yamashita, 2018). Abrogation in one of these

factors led mainly to disruption of chromocenters and defects in genome compaction, including the increase of micronuclei frequency without the formation of mitotic errors (Jagannathan, Cummings and Yamashita, 2018, 2019). Interestingly, overexpression of D1 in mouse cells, despite being evolutionarily divergent, led to the targeting and formation of bigger chromocenters (Jagannathan 2018), suggesting a conserved function for AT-hook proteins in actively encapsulating genomic compartments. Another set of proteins that might be involved in chromocenter clustering is the 5mC reader MeCP2. The strongest evidence linking MeCP2 to chromocenters was observed when ectopic expression of this protein, even in absence of H3K9me, was sufficient to increase satellite DNA clustering resulting in fewer and fused chromocenters (Brero et al., 2005; Georgel et al., 2003).

2.5. Histone chaperones

Histones are basic polar proteins that are extremely toxic for the cells because of a predisposition to form aggregates. For this reason, histone chaperones escort non-nucleosomal (free) histones before nucleosome assembly on DNA, or after their eviction. The first definition of a histone chaperone comes from the observed properties of Nucleoplasmin promoting nucleosome assembly, the term was coined by Ron Laskey (Laskey *et al.*, 1978). Histone chaperones are currently defined as histone associating protein involved in their transfer without being part of the final product (De Koning *et al.*, 2007). Their fundamental activity is to escort histones from their sites of synthesis until their deposition on naked DNA to form nucleosomes. *In vivo*, histone chaperones make sure that histones are never free in the cell from the moment they are synthesized, while performing different functions including histone transport, buffering, storage, modification, remodeling, recycling, and deposition. During the last decade, several studies shed light on the role of key histone chaperones that are specific (dedicated) to a given histone variant, having distinct modes of action and pathways to deposit histones on DNA (Mendiratta, Gatto and Almouzni, 2019). The picture that emerges is thus that the histone chaperone, rather than the variant itself, dictates where, how, and when deposition occurs (Fig. 16). In this section, I will summarize the state-of-the-art for the key histone chaperones dedicated to H3 variants.

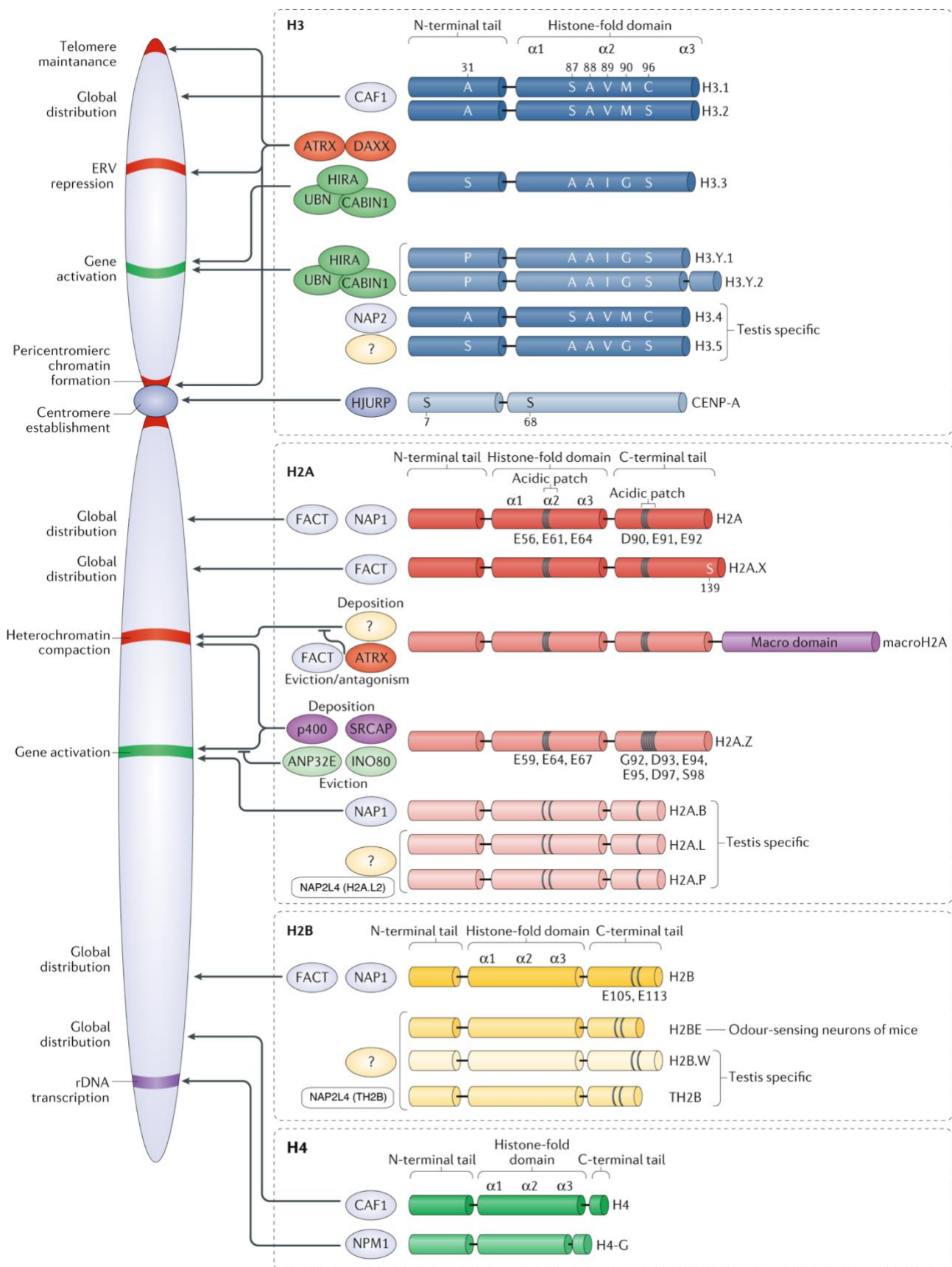


Figure 16. Core histones, their variants and associated chaperone/remodeler machineries. Replication-coupled and variant core histones with respective chaperones, genomic distribution and

functional output are shown. Specific amino acid residues are illustrated. Different shades of color in the histone's structures are used to indicate differences within the domains compared with the replication-coupled histone. ANP32E, acidic leucine-rich nuclear phosphoprotein 32 family member E; ATRX, α -thalassaemia mental retardation syndrome X-linked; CABIN1, calcineurin-binding protein cabin 1; CAF1, chromatin assembly factor 1; DAXX, death domain-associated protein; ERV, endogenous retroviral element; FACT, facilitates chromatin transcription; HIRA, histone regulator A; HJURP, Holliday junction recognition protein; NAP, nucleosome assembly protein; NPM1, nucleophosmin; rDNA, ribosomal DNA; UBN, ubinuclein; SRCAP, Snf2-related CREBBP CBP activator protein. Adapted from (Martire and Banaszynski, 2020).

2.5.1. CAF-1 complex

The evolutionarily conserved chromatin assembly factor 1 (CAF-1) promotes the deposition of replicative histones coupled to DNA synthesis during DNA replication and DNA repair. This complex contains three subunits that are named according to their molecular weight in kDa: p150, p60, and p48, in mammals. P150 is the largest subunit and is the platform of interaction with other proteins including p60 and p48 via multiple domains. The N-terminal portions possess a PCNA interacting peptide (PIP) motif (Shibahara and Stillman, 1999; Moggs *et al.*, 2000; Gérard *et al.*, 2006) providing the molecular link between CAF-1 and DNA synthesis. It also interacts with HP1 proteins participating therefore to their dynamics of deposition when chromocenters duplicate and reassemble (Murzina *et al.*, 1999; Quivy *et al.*, 2004). The mid subunit p60 interacts with the histone chaperone Asf1 (Tagami *et al.*, 2004; Groth *et al.*, 2005) which may be involved in recycling parental histones for CAF-1 during fork progression (Mello *et al.*, 2002). P60 possesses a WD40 motif involved in the binding and loading of H3-H4 dimers. The small subunit p48 is less characterized and can act as an accessory protein involved in chromatin-regulating processes. The current model of histone deposition proposed using *S. cerevisiae* CAF1 (CAC1), describes a transient interaction between two CAC complexes containing each an H3-H4 dimer, which deposit in concert with an H3-H4 tetramer (Sauer *et al.*, 2018).

2.5.2. HIRA complex

H3.3 binds selective histone chaperones through its AIG motif present in the HFD: the histone regulator A (HIRA) complex and the Death Domain Associated Protein - Alpha-Thalassemia/Mental Retardation

Syndrome X-Linked (DAXX-ATRX) complex. HIRA shares amino acid sequence homology with the *S. cerevisiae* proteins Hir1p and Hir2p (Histone Regulating Protein) and was first found deleted in a developmental disorder, the Di George Syndrome (Lamour *et al.*, 1995). The identification of HIRA nucleosome activity in *Xenopus* extracts revealed, first, its chaperone function in assembling chromatin in a DSI manner (Ray-Gallet *et al.*, 2002), and later it was revealed to be in complex with H3.3 specifically (Tagami *et al.*, 2004). HIRA histone chaperone complex includes three main components: HIRA, ubinuclein 1 or 2 (UBN1/2) (Banumathy *et al.*, 2009) and calcineurin-binding protein 1 (CABIN1) (Rai *et al.*, 2011). HIRA acts as the central platform of the HIRA complex, forming a trimeric structure required to interact with a dimer of CABIN1 subunits, forming a functional complex with UBN1 (Ray-Gallet *et al.*, 2018). UBN1 interacts with H3.3 thanks to a stronger affinity for the AIG motif present in the histone-fold domain, where Gly90 mediates the specificity of binding of H3.3 over H3.1/H3.2 (Fig.17) (Daniel Ricketts *et al.*, 2015). In particular, UBN1, first discovered as a nuclear protein interacting with transcription factors (Aho *et al.*, 2000), is specifically involved in depositing new histones during transcription, but not recycling old histones (Torné *et al.*, 2020). Little is known about its paralog UBN2. In mouse ES cells, UBN1 and UBN2 can potentially form two different complexes acting cooperatively on cis-regulatory elements and retrotransposons silencing (Xiong *et al.*, 2018; Zhang *et al.*, 2022).

2.5.3. DAXX-ATRX complex

Although DAXX-ATRX also binds H3.3, it does not form a complex with HIRA. ATRX was discovered from mutations of the corresponding gene in young males with X-linked mental retardation syndrome (Weatherall *et al.*, 1981; Gibbons *et al.*, 1995). DAXX was described as a Fas death-receptor binding protein via the JNK pathway (Yang *et al.*, 1997). In this complex, ATRX targets H3K9me3-heterochromatin through its ATRX-Dnmt3-Dnmt3L (ADD) domain (Xue *et al.*, 2003), whereas DAXX is the actual chaperone binding to H3.3 for a stronger affinity of the H3.3G90 over the hydrophobic Met90 in H3.1, as for UBN1 (Fig.17) (Elsässer *et al.*, 2012; Liu *et al.*, 2012). Given the preferential recognition of heterochromatin regions over euchromatin, DAXX-ATRX complex deposits H3.3 at sites where transcriptional repression might be required, including transposable elements and telomeres (Law *et al.*, 2010; Lewis *et al.*, 2010; Elsässer *et al.*, 2015). Specificity

for H3.3 is altered when CENP-A is overexpressed in the context of tumoral cell lines, leading to mislocalization of CENP-A to chromosome arms by this complex (Lacoste *et al.*, 2014).

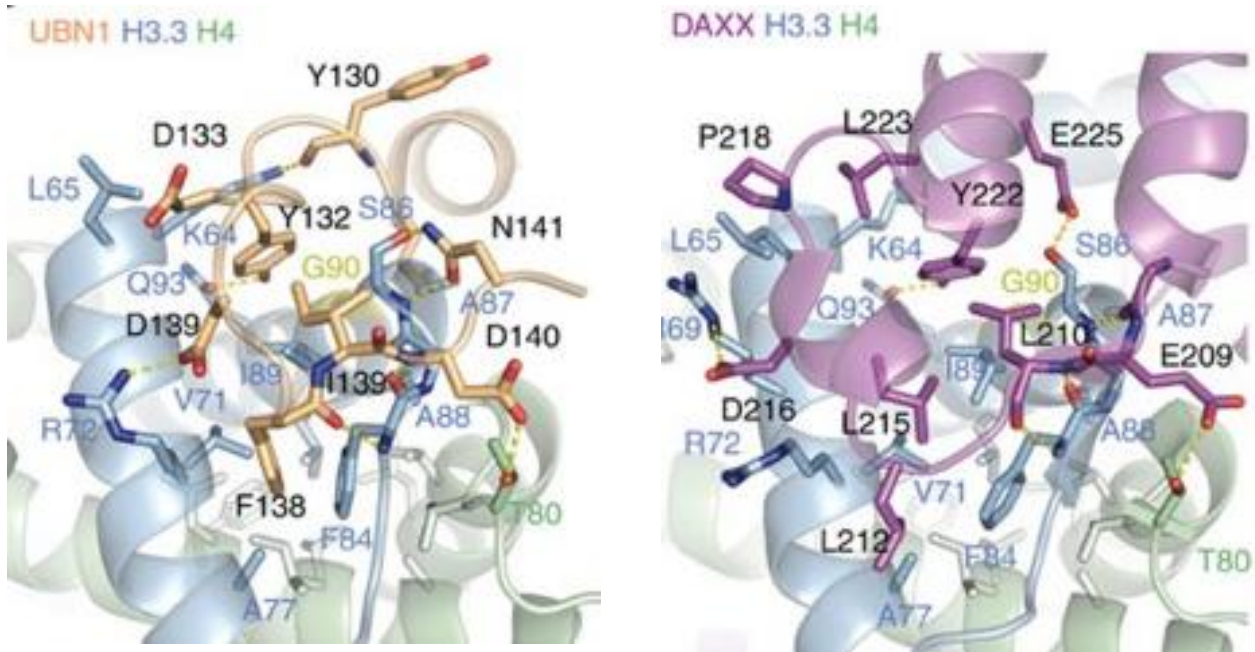


Figure 17. Crystal structure of the UBN1/H3.3/H4 and DAXX/H3.3/H4 complex. Detailed stereo view of UBN1 (orange) and DAXX (purple). Both UBN1 and DAXX have an intimate association with the H3.3 (light blue)/H4 (green) surface with close proximity to H3.3 G90 (yellow). UBN1 residues with sensitivity to alanine mutation are labeled. Adapted from (Daniel Ricketts *et al.*, 2015)

2.6. Interplay between histone chaperones and histone variants

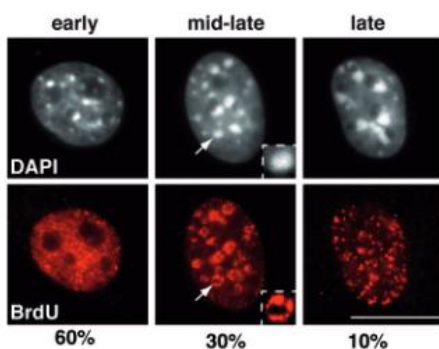
During replication or transcription, nucleosomes are transiently disrupted ahead of the replication or transcription machinery to give access to the DNA and then reassembled after. During these processes, histones used for re-assembly may be recycled from the evicted nucleosomes (referred to as parental histones), potentially contributing to the preservation of the positional information of histone PTMs and variants (Delaney and Almouzni, 2022). They can either be newly synthesized histones available at this time and place in the nucleus, but they might be different in the terms of PTMs and nature of the variant, favoring therefore a loss of the parental marking. CAF-1 is involved in both DNA replication and repair synthesis (Smith and Stillman, 1989; Gaillard *et al.*, 1996) and it deposits H3.1/H3.2 genome-wide during S-phase thanks to the SVM recognition motif (Goldberg *et al.*, 2010). It is currently unknown what and if there are other histone chaperones depositing H3.1 outside of the S phase/DNA repair synthesis. Interestingly, in the case of transient CAF-1 mediated deposition failure, HIRA compensates for H3.1 absence by H3.3 deposition at the same sites, as an alternative mechanism to prevent the exposure of naked DNA regions and maintain genome integrity (Ray-Gallet *et al.*, 2011).

While H3.3 deposition was observed outside of replication early on (Ahmad and Henikoff, 2002), its preferred genome deposition sites and mechanism were discovered later. The HIRA complex is involved in the DSI deposition of new H3.3 mainly at gene bodies of active genes, promoters, and genic and intergenic regions (Goldberg *et al.*, 2010). This co-occurrence with transcription was later confirmed by co-immunoprecipitation assays of HIRA with RNA Pol II subunits (Ray-Gallet *et al.*, 2011), along with lineage-committed genes that are transcribed during differentiation (Banaszynski *et al.*, 2013; Yang *et al.*, 2016). Of note, the HIRA complex mediates H3.3 deposition independently of transcription at UV-damaged sites (Polo, Roche and Almouzni, 2006; Adam *et al.*, 2016) and in paternal chromatin of fertilized embryos before the first cell division (Torres-Padilla *et al.*, 2006). In contrast, the DAXX-ATRX complex deposits H3.3 at pericentric and telomeric regions (Goldberg *et al.*, 2010; Elsässer *et al.*, 2015) by resolving G-quadruplexes and/or silencing transposable elements that are formed and transcribed at these regions (Clynes *et al.*, 2015).

2.7 DNA replication and chromocenter assembly

During DNA synthesis, PHC domains and corresponding DNA sequences reproduce stereotypical nuclear patterns compared to other chromatin landmarks. Because chromocenters in mouse cells are maintained throughout the S-phase, it is possible to identify at least three S-phase patterns that can be visualized by detecting ongoing DNA synthesis with thymidine analogs such as BrdU or EdU or with replication fork factors like PCNA. First, an Early-S pattern shows a punctate signal throughout the nucleus corresponding to ‘euchromatin’; then, a Mid-S pattern displays a signal around and at the chromocenters, and; finally, a late-S pattern, displays a signal mostly at the nuclear periphery (Fig. 18). Careful examination of replication of chromocenters in Mid-S shows a horse-shoe or ring-like pattern surrounding chromocenters (Quivy *et al.*, 2004), suggesting that replication and heterochromatin assembly occur outside the chromocenters and that once duplicated chromatin is pulled inside the chromocenters (Quivy *et al.*, 2004).

A



B

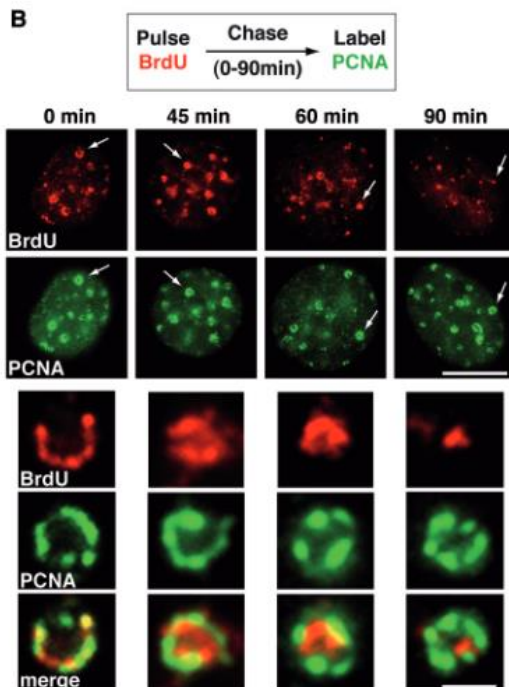
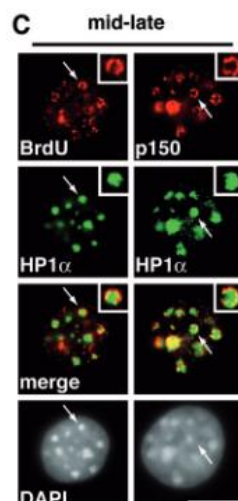


Figure 18. Replication in mouse cells is spatially and temporally regulated.

(A) Typical frequency of S-phase patterns in NIH-3T3 cells stained with DAPI after being pulsed with BrdU (red, bottom). (B) When NIH-3T3 cells were chased for the indicated times after being pulsed with BrdU for 10 minutes, newly replicated DNA (PCNA, green) is found at the DAPI-dense region's periphery and then internalized. Merged images and corresponding DAPI staining are indicated (arrows) and magnified (below). (C) In mid-late S-phase, mouse p150CAF-1 and BrdU incorporation (red) were observed together with HP1 (green). Adapted from (Maison *et al.*, 2010) and (Quivy *et al.*, 2004)

C



During chromocenter replication, HP1-p150/CAF1 interaction, which is not critical for histone deposition, is important for S phase progression (Quivy *et al.*, 2008). Within the centromeric regions - comprising respectively centric and pericentric DNA - each part shows a distinct replication time. This is clear for minor satellites which are located in the inner (most centric) regions and are typically replicated before the flanking major satellites that replicate during Mid-Late S phase (Guenatri *et al.*, 2004). However, this is not the case for centromeric sequences in *Drosophila* and *S. pombe* (Ahmad and Henikoff, 2001; Kim, Dubey and Huberman, 2003) or minor satellites in mouse ES cells (Jørgensen *et al.*, 2007; Rausch *et al.*, 2020) that, compared to MEF cells, replicate in early-Mid S. These features might be linked with their genome organization status depending on their cell state (Pope *et al.*, 2014; Sima *et al.*, 2019). Sequestering replication and heterochromatin assembly at the periphery of the chromocenters and therefore disrupting chromatin only locally might explain why these nuclear domains do not display obvious changes in higher-order structure when they replicate. So why do these domains tend to replicate from the periphery to the center? One reason could be that peripheral regions at the euchromatin interface are more accessible to DNA replication factor machineries than the interior (Chagin *et al.*, 2016). In this way, the proposed “domino-effect” explains how consecutive DNA units are replicated in a linear mode through consecutive chromosome segments. However, this model does not take into account the replication processivity in regions that are less accessible than others, for which, they need to be eventually replicated. Recent reports show that local heterochromatin regions can be partially de-compacted without altering their overall architecture in response to DNA replication. With the improvement of imaging technologies, replicating DNA was shown to remodel constitutive heterochromatin domains by forming tunnel-void-like structures as a consequence of the replication fork passage (Chagin *et al.*, 2019; Miron *et al.*, 2020). This destabilization might provide a time window for additional components of replication factors machineries to access and start replication in previously inaccessible regions. It will be therefore important to determine how these dynamic events of chromatin compaction occur in contexts where heterochromatin establishment is impaired or delayed.

Taken together, these data show that an orchestrated complex of histone modifications and histone modifiers is crucial to faithfully reproduce the chromatin landscape. In the next chapter, I will describe how this interplay occurs during mouse embryo development.

Chapter 3: One, No One, One Hundred Thousand: The multifaceted role of heterochromatin during development

3.1. Embryonic stem cells

The concept of potency derives from Latin “potentia” meaning “power, authority” and also “able to”. In the development context, totipotency reflects the capability of a single cell to develop into any other cell. This fascinating feature holds for a spore or a plant callus, but also for a fertilized zygote that ultimately develops, and therefore differentiates, into a full organism. In the developing mouse embryo, the zygote (1-cell stage) and the blastomere (2-cell up to 4-cell) are totipotent cells (Condic, 2014). After the 8-cell stage or morula stage, cells cannot revert their potency ability and they can contribute either to the trophectoderm, including extraembryonic tissues such as future placenta and yolk sac, or the inner cell mass (ICM) of the blastocyst. A pluripotent cell from the ICM, in opposition to the totipotent cell, possesses the ability to give rise to the three germ layers (ectoderm, endoderm, and mesoderm), contributing to the development of a full embryo and then an adult organism (Smith, 2017). Mouse embryonic stem (ES) cells are cell lines derived from the inner cell mass (ICMs) of the blastocyst at E4.5 before implantation of the mouse embryo (Evans and Kaufman, 1981; Martin, 1981). They are considered pluripotent, as opposed to totipotent, but have proven to be a powerful model, as they possess the capacity to 1) give rise to all specialized cells except for extra-embryonic tissues, 2) divide indefinitely and generate cells of the same type (self-renewal), and 3) when re-implanted, contribute to chimeras and colonize the germline (Bradley et al., 1984). Given their high plasticity, they led to the “open chromatin” hypothesis (Meshorer and Misteli, 2006; Giadrossi, Dvorkina and Fisher, 2007; Gaspar-Maia *et al.*, 2009), where ES cells possess a less compact but more dynamic chromatin state that enhances their potential to differentiate in other cell types (Fig. 19).

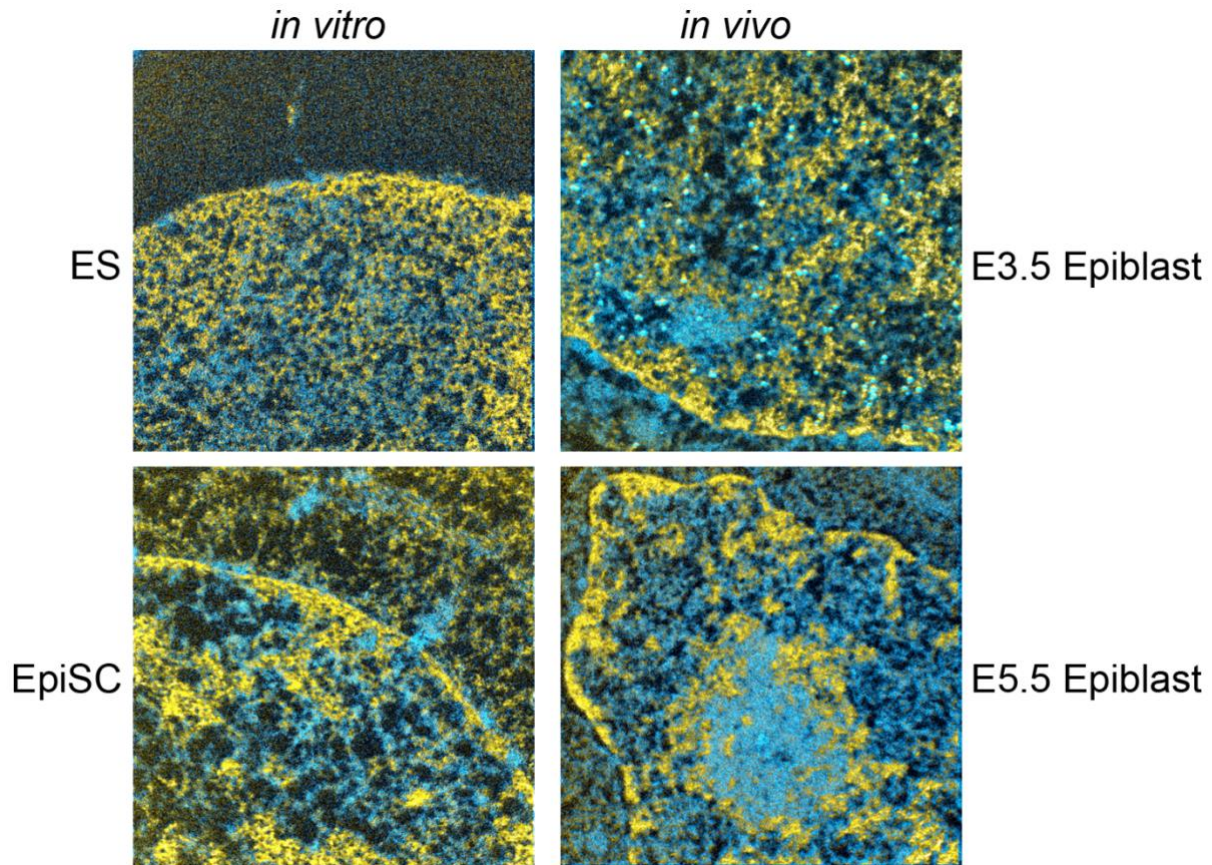


Figure 19. The global chromatin architecture of *in vitro* and *in vivo* stem cell populations. The left column contains electro-spectroscopic images (ESI) images of embryonic stem (ES) and epiblast stem cells (EpiSCs). The right column displays nuclei from embryo stages E3.5 epiblast and E5.5 epiblast. Chromatin (yellow) and protein and RNPs (shades of blue) are determined from nitrogen and phosphorus maps. As cells develop, chromatin becomes more compact. The scale bar represents 500 nanometers. Adapted from (Ahmed et al., 2010)

3.2. Pluripotent pathways & mechanisms

A network of transcription factors cooperates to preserve stem cell identity and regulate pluripotency by controlling the expression of various downstream target genes. Among them, Oct4, Sox2, and Nanog are the best characterized in ES cells (Jaenisch and Young, 2008).

Oct4 expression levels are crucial to determine the stem features of ESCs, as misregulation of its expression correlates with the loss of pluripotency and the acquisition of a differentiation phenotype (Pan *et al.*, 2002). Oct4 binds to an octamer motif of DNA (5'-ATGCAAAT-3') to control the expression of many pluripotency-associated genes, and, in many cases, works together with Sox2 (Nichols *et al.*, 1998). Sox2 expression is widely distributed in the developing embryo, including ICM, epiblast, neural tissues, and extra-embryonic

ectoderm. Sox2 often acts as a heterodimer with Oct4 to regulate the transcription of important genes such as Fgf4, Nanog, and Lefty1, as well as Oct4 and Sox2 themselves (Masui *et al.*, 2007). Nanog acts in coordination with Oct4 and Sox2 to establish the ES cells' identity (Huang *et al.*, 2015), but differently from the other two, its overexpression alone confers the ability to preserve the undifferentiated state even in the absence of LIF (Chambers *et al.*, 2007). Its mechanism has been recently demonstrated by stimulating a plethora of effects in the presence or absence of LIF, for which Nanog, would either promotes chromatin accessibility for pluripotent factors or block differentiation by sustaining H3K27me3 at developmental genes, respectively (Heurtier *et al.*, 2019).

While a complex interaction of transcription factors and chromatin modifications maintains the pluripotent state of ES cells, the mechanisms ruling these processes are far from clear. In particular, some of the ES features, including poised transcription, bivalent domains, and DNA methylation status, are largely confounded by the adoption of different experimental approaches to maintain ES cells in culture (Marks *et al.*, 2012; Ficiz *et al.*, 2013; Habibi *et al.*, 2013; Leitch *et al.*, 2013). Although possessing the same differentiation potential, a first distinction exists between “confused” and “ground” state, with the former generally referring to pluripotent ES cells exposed to a high concentration of serum, and the latter to cells cultured in 2i conditioned media (containing 2-inhibitors of GSK3/ERK pathways), as reviewed in (Schlesinger and Meshorer, 2019). 2i-cultured cells are hypomethylated and less spurious in transcription, as compared to serum-grown cells (Marks *et al.*, 2012). Serum-grown ES cells are subjected to uncontrolled perturbations and thus characterized by heterogenous expression of pluripotency factors and poised transcription of developmental genes (Toyooka *et al.*, 2008; Kalkan *et al.*, 2017). This fluctuation is demonstrated by the presence of cells in Nanog-high and Nanog-low states, resulting in higher transcription of repetitive elements (major and minor satellites, LINEs, SINEs, IAPs), as compared to ground state cells (Efroni *et al.*, 2008). This does not occur in 2i-grown ES cells where the highest variability in transcription occurs in genes related to the cell cycle (Kolodziejczyk *et al.*, 2015). Besides transcriptomics, epigenetic features are distinct between the two different cell culture approaches. Serum-grown cells have characteristic bivalent domains, regions that are decorated in both H2K27me3 and H3K4me3, whereas these features are drastically reduced in the 2i condition. Although present in every cell type, including differentiated and cancer cells, bivalent domains were proposed in ES cells to demarcate a set of poised lineage-committed genomic regions, usually, promoters, which are

resolved in later developmental stages. Therefore, they have been used as a hallmark of developmental ability (Azuara, 2006; Bernstein *et al.*, 2006; Mikkelsen *et al.*, 2007). In fact, increasing evidence shows that these marks, rather than promoting transcription, protect from irreversible DNA methylation (Kumar *et al.*, 2021; Shah *et al.*, 2021). While the differentiation capacity might be largely unaffected, modifications on the chromatin level impinge on the higher-order organization. This results, at least in ground state cells, in the lowest number of nucleosomes present for “clutches” in chromatin (Ricci *et al.*, 2015) and the highest number of long-distance interactions at the 3D genome level (Joshi *et al.*, 2015). While these properties seem to have a more indirect effect rather than a causative role, it is worth exploring how such chromatin “openness” is a predictor of pluripotency function.

3.3. Cell cycle control during pluripotency

The cell cycle control of an early embryo changes dramatically compared to a somatic equivalent. In fact, a rapid cell cycle is associated with greater efficiency in reprogramming (Guo *et al.*, 2014). A somatic cell cycle is approximately 24h long, with extended gap phases and an S-phase lasting ~8h. However, in early mouse embryos and ES cells, the cell cycle is more rapid, lasting on average ~12h by reducing the gap phases, in particular G1 (Palmer and Kaldis, 2016). Such short G1 might be instrumental to reduce chromatin modifications that are imposed after their dilution in DNA replication (Ma, Kanakousaki and Buttitta, 2015). Therefore, it will be intriguing to understand how epigenetic and transcription factors are re-established at the onset of mitosis. Mitotic bookmarking factors are proposed to reestablish self-renewal and maintain pluripotency, in contrast to the historical view of mitotic chromosomes as inaccessible regions for transcription. Esrrb was one of the first transcription factors found to bind active promoters and enhancers of ES cells during mitosis and interphase (Festuccia *et al.*, 2016). Its binding correlates with H3K27ac enrichment and transcription of genes mainly present in the epiblast lineage. In concert with Esrrb, specific degradation of Oct4 and Sox2 during mitosis alters self-renewal while inducing spontaneous differentiation of ES cells (Deluz *et al.*, 2016; Liu *et al.*, 2017). Oct4 binding to chromatin is also shown to be regulated by Aurora B kinase and Protein Phosphatase 1 (PP1) during G2/M and M/G1 transitions, respectively (Shin *et al.*, 2016). All these events are promoted to enhance the transcription of key pluripotent factors so that a stable pool will be sufficiently expressed and further available throughout the S phase and later in G2. In fact, ground state cells

also possess a longer G1 phase and a shorter G2 phase as a consequence of the inactivation of the ERK signaling pathway and hypo-phosphorylation of Rb (ter Huurne *et al.*, 2017). Together these studies display a link between the pluripotency network and cell cycle control, pointing to a fundamental role of mitotic bookmarking as an epigenetic mechanism to ensure chromatin states while transitioning from one cell state to another.

3.4. Heterochromatin establishment during development and reprogramming

Pericentromeric heterochromatin is typically associated with high levels of H3K9me3 and DNA methylation, giving rise to a highly repressive environment. Many studies, however, reveal that the composition of constitutive heterochromatin can be highly dynamic (Déjardin, 2015). For example, ES cells depleted in Suv39h enzymes show a dramatic loss in H3K9me3 with a gain in H3K27me3 at chromocenters (Peters *et al.*, 2003). As a consequence of a compromised chromatin state, a cross-talk between constitutive and facultative heterochromatin is required to rescue the transcriptional repression and organization in pericentromeric regions. A perturbation of the chromatin dynamics leads to more severe defects, as pluripotent cells cannot cope with CAF-1 absence to reform chromocenters (Houlard *et al.*, 2006).

Interestingly, ES cells express repetitive elements at low levels that are instead silenced in differentiated cells, as a cause (or a consequence) of their decondensed chromatin (Martens *et al.*, 2005). In this picture, KMTs are engaged in a complex interplay with transcription factors to ensure self-renewal and heterochromatin maintenance. Among these, SETDB1 functions as a co-repressor with Oct4 to silence differentiation genes (Bilodeau *et al.*, 2009). Similarly, Oct4 expression promotes DNA demethylation mediated by KMD3A and KMD4C on Nanog and Tcf1 genes (Loh *et al.*, 2007; Ginno *et al.*, 2020). Not only that, germline ablation of SETDB1 led to decreased levels of H3K9me3 at ERVs and LINE1 (Dodge *et al.*, 2004) and it functions in concert with CAF-1 to silence proviruses and ERVs (Yang *et al.*, 2015). While H3K9me3 at these elements might be required in the post-implantation stages to prevent spurious transcription, their progressive loss in expression in differentiated cells might be explained by the erasure of activating mark H3K4me3 rather than the acquisition of new H3K9me3 (Fadloun, Eid and Torres-Padilla, 2013).

Finally, heterochromatinization functions also as a barrier to reprogramming. Yamanaka factors (ie, Oct4, Sox2, Klf4), were the first transcriptional factors identified in reprogramming fibroblasts to induced

pluripotent stem cells (iPSCs). Overexpression of Yamanaka factors led to the discovery of H3K9me3 mega-base scale regions covering pluripotent genes (Takahashi and Yamanaka, 2006; Takahashi *et al.*, 2007). Depletion of the KMTs responsible for H3K9me3 deposition boosts Oct4 and Sox2 binding as well as the number of iPSCs colonies (Soufi, Donahue and Zaret, 2012). Overall, H3K9me3-controlled deposition underpinning cellular identity implies a key role for heterochromatin maintenance in preventing cell conversion.

3.5. Histone variants and chaperones during development

In mammals, histone variant expression is highly dynamic (Buschbeck and Hake, 2017) except that H3.3 protein levels are stably maintained from the zygote to the blastocyst stage (Torres-Padilla *et al.*, 2006). H3.3 is essential for mouse embryonic development *in vivo* (Jang *et al.*, 2015). Single ablation of H3F3A and H3F3B genes gives rise to offspring with inconsistent phenotypes, from growth rate to copulatory activity, likely due to different KO approaches, mouse backgrounds, and differences in the 3'-UTR of the two genes (Couldrey *et al.*, 1999; Bush *et al.*, 2013; Tang *et al.*, 2013, 2015).

Paternal and maternal chromatin is marked by different chromatin modifications from the zygote until at least the 2-cell stage (Mayer *et al.*, 2000). H3.3 preferentially localizes to paternal chromatin during the zygote stage, as a possible consequence of protamine-to-histone transition (Loppin *et al.*, 2005; Torres-Padilla *et al.*, 2006). In this time window, early H3.3 deposition ensures a chromatin assembly in absence of cell division. This incorporation is also crucial for providing, after the first cell division, its canonical genome-wide pattern of enrichment in the genome (Ishiuchi *et al.*, 2021).

Compared to replicative variants, H3.3, in particular Ser 31, is fundamental for correct gastrulation events in *Xenopus*, regardless of the chromatin incorporation pathway (Sitbon *et al.*, 2020). In ES cells, this promotes the histone methyltransferase SET domain-containing 2 (SETD2) activity by enhancing H3.3K36me3 deposition that, in turn, recruits p300 to deposit H3K27ac (Martire *et al.*, 2019). Additionally, the absence or loss of histones over time can be used as a proxy for indicating active transcription. Despite showing a higher turnover at the +1 nucleosome position in active genomic regions (Deaton *et al.*, 2016; Schlesinger *et al.*, 2017), H3.3 presence is not necessarily causative of transcription, given that a similar rate of turnover occurs at human-centric repeat regions acting as a placeholder for CENP-A (Dunleavy, Almouzni and Karpen, 2011).

In ES cells, the HIRA and DAXX-ATRAX complexes mediate H3.3 deposition at active and heterochromatin regions, respectively (Goldberg *et al.*, 2010). In the first case, HIRA mediates H3.3 deposition at developmental loci and is required to maintain repressive epigenetic marker H3K27me3 at bivalent domains (Banaszynski *et al.*, 2013). In fact, depletion of the HIRA complex, but not of DAXX-ATRAX, is crucial for correct differentiation and reprogramming specification (Banaszynski *et al.*, 2013; Fang *et al.*, 2018). However, in the absence of DNA methylation, H3.3-DAXX-ATRAX deposition is linked to H3K9me3-repression at retrotransposons, including IAP/ETn elements and telomeres (Elsässer *et al.*, 2015; He *et al.*, 2015).

Currently, there are no models of H3.1 KO given its organization in multiple gene clusters and its strong interplay with DNA replication processes. However, its activity during embryo development can be inferred by tracking its dynamic presence by FRAP. Early work in mouse ES cells showed “hyperdynamic chromatin” based on a fast signal recovery of structural chromatin proteins, including H3.1, H3.2, and HP1 (Meshorer *et al.*, 2006). Similar results were observed *in vivo* during embryo development, showing a slower recovery of replicative variants at the implantation stage, as compared to totipotent and pluripotent cells (Boskovic *et al.*, 2014). Interestingly, the H3.3 signal recovered slower than the replicative variants in both studies. Despite being deposited genome-wide, H3.1 (and predicted variants based on aminoacidic similarities) tend to accumulate in heterochromatin regions (Maehara *et al.*, 2015). This accumulation, together with H2A.W, occurs mainly in chromocenters during Arabidopsis seedling development and is linked to CAF-1 activity (Benoit *et al.*, 2019). Additionally, the coordinated deposition of H3K9me3-H3.1/H3.2 mediated by CAF-1 helps in establishing a barrier for reprogramming in the trophoblast lineage (Hada *et al.*, 2022). Compared to other histone chaperones, impairment of CAF-1 results in stronger phenotypes, impacting the nuclear organization and reprogramming events. CAF-1 KO is embryonic lethal and the derived embryos contained only 8 instead of 32 cells at the E4.5 stage (Houlard *et al.*, 2006). p150 depletion in ES cells, but not in MEFs, dissolves chromocenters (Houlard *et al.*, 2006). Recent studies by ultra-low input ChIP-seq analysis observed that CAF1 activity contributes to the establishment of H3K9me3, required to repress LTR transcription, from mature oocytes to pre-implantation stages (Wang *et al.*, 2018; Ishiuchi *et al.*, 2021). After the first cell division, H3.1-CAF-1 deposition redistributes H3.3 in active chromatin regions, given that H3.3 counterbalances H3.1/H3.2 heterochromatin absence in oocyte and zygote cells (Ishiuchi *et al.*, 2021). Moreover, CAF-1 acts

as a master regulator of chromatin accessibility, as its depletion contributes to loss of transcription factor targeting at lineage-committed genomic regions, enhanced reprogramming ability, and promotion of a totipotent state (Cheloufi *et al.*, 2015; Ishiuchi *et al.*, 2015; Franklin *et al.*, 2022). These studies highlight the importance of nucleosome assembly and histone modifications in the orchestration of heterochromatin formation during cell fate decisions (Fig. 20).

Understanding how this balance of histone variants and histone chaperones is linked to the nuclear organization, and how this will eventually impact euchromatin organization, remains thus of the utmost interest.

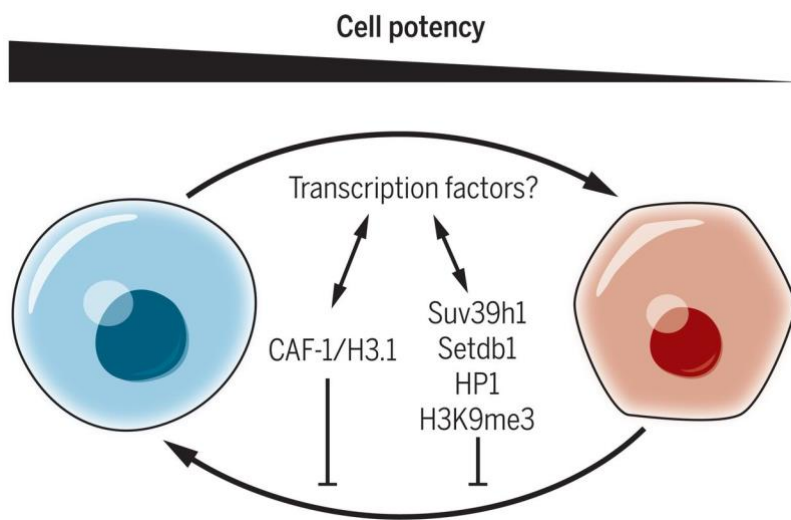


Figure 20. CAF-1 and heterochromatin as cell plasticity regulators. A schematic model emphasizes the role of the histone chaperone CAF-1, the replicative histone H3 variant H3.1, and the presence of the heterochromatin-specific H3K9me3 mark. Chromatin assembled with the replicative histone variant, as well as the imposition of the H3K9me3 mark, acts as a barrier to stemness, participating cells in nonreversible cell fates. Adapted from (Yadav et al., 2018)

Questions

Chromocenters are membrane-less nuclear domains that act as hub for heterochromatin establishment throughout embryo development. A complex interplay of DNA methylation, transcription factors and chromatin remodelers balance what must be transmitted to the following cell generations and what has to be transcribed for exploiting genome function with preventing expression of possibly detrimental non-coding regions. Histone variant function are often inferred from their PTMs yet it is unclear how their deposition, as well as their mode of transmission, influences these regions as well as their higher order structures. During the course of my PhD, I tried to address these fundamental questions:

How are histone variants H3.1 and H3.3 distributed at mouse chromocenters of different cell potential?

To this end, I monitored H3.1 and H3.3 nuclear distribution throughout cell differentiation and cell cycle. I exploited and combined high-resolution imaging with high-throughput sequencing approaches revealing a unique signature of the replicative and replacement histone variants at chromocenter-associated elements.

What is the mechanism regulating the accumulation and maintenance of H3 variants at chromocenters?

For this, I tested in more details whether the deposition mechanism or the histone variants' ability to carry particular PTMs is essential for their enrichment at chromocenters.

Results

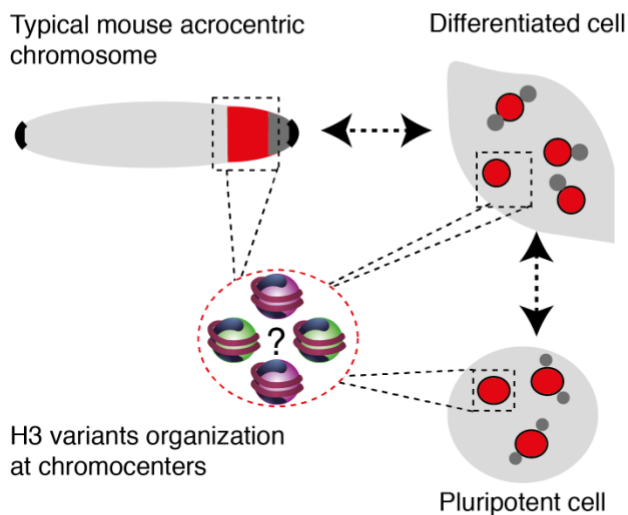
During my Ph.D., I sought to understand the interplay between the histone H3 variants, their dedicated histone chaperones, and chromocenters during mouse cell differentiation and throughout the cell cycle. I have summarized my general approach and methodology in the section below. Next, I have prepared a manuscript ready for submission summarizing my key results, entitled “H3.3 deposition counteracts the replication-dependent enrichment of H3.1 at pericentric heterochromatin in the cell cycle of pluripotent cells”. Furthermore, to gain a better understanding of how histone variants are enriched and distributed on the mouse genome during differentiation, I performed additional experiments, including super-resolution single-molecule imaging by STORM and further ChIP-seq analysis, which I have summarized in the section titled “Additional Results”.

Approach & Methodology

Here is a brief overview of the technical approach (summarized in Figure 21).

Objective 1

Monitoring H3 histone variant at chromocenters relative to differentiation



Challenge

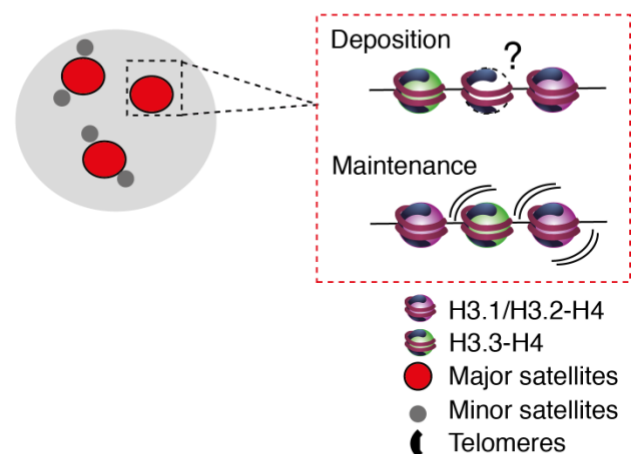
Define patterns of H3 enrichment at chromocenters
Effect of differentiation and cell cycle

Approach

Establish mouse ES cell lines with H3-SNAP-tag
Microscopy imaging (histones + DNA)
Genome-wide analysis at repetitive elements (SNAP-ChIP-seq)

Objective 2

Describing mechanisms of H3 histone variant enrichment



Challenge

Define dynamics of H3.1/H3.3:
Mechanisms of deposition and maintenance

Approach

Monitor H3 enrichment with cell cycle progression (replication markers)
Perform aa changes in H3 histone fold motif (histone chaperone) and N-terminal tail (hPTMs)
Targeting histone chaperones at chromocenters

Fig 21. Experimental approach

To elucidate if and how histones are deposited in different chromatin compartments, and how this is occurring, I needed to use a method allowing the tracking of histones both at the single-cell level, by imaging, and at the genomic level, by sequencing. In the laboratory, we have exploited a versatile tool to differentiate populations of histones in different cell cycle stages and cell states: the SNAP-Tag system (Jansen *et al.*, 2007; Ray-Gallet *et al.*, 2011; Clément *et al.*, 2018).

The SNAP-Tag is a self-labeling protein of 19.4 kDa that can be fused to any protein of interest. The SNAP-tag covalently reacts with benzylguanine derivatives of which permeable fluorophores such as tetramethylrhodamine (TMR) lead to fluorescent labeling of histone variants in living cells that can be further detected by microscopy (Ray-Gallet *et al.*, 2011; Clément *et al.*, 2018; Torné *et al.*, 2020). Given its high specificity and low interferences with chromatin dynamics processes compared with other tags, SNAP-tag has been recently and successfully used in our lab to obtain high-quality sequencing data of H3.1 and H3.3 domains that are specifically enriched at origin replications sites (Gatto *et al.*, 2022).

I first aimed to explore the dynamic distribution of total H3 histone variants in mouse cells throughout differentiation. To this aim, I generated two transgenic mouse ES cell lines expressing a single-inducible copy of H3.1- or H3.3- conjugated with SNAP-Tag-3xHA reporters under the control of a TetO promoter (Beard *et al.*, 2006). Exposure of cells to Doxycycline induces the exogenous histone variant expression, which can then be incorporated into chromatin. This system thus provided: 1) tunable histone variant expression, 2) live or fixed 'in cell' fluorescent labeling to follow by microscopy, and 3) the capacity to follow histone incorporation into chromatin and localization in the nucleus throughout differentiation. In addition to this system, I generated two additional mouse cell lines constitutively expressing H3.1- or H3.3- SNAP-Tag-3xHA in NIH-3T3 cells. These cells served as a model for H3 distribution in terminally differentiated fibroblasts and were used as a comparison with the Dox-inducible system from the ES cell lines.

In collaboration with Audrey Forest and Hatem Hmidan, research engineer and postdoc in our lab, respectively, we set up a differentiation protocol to improve the efficiency of differentiating cells, while obtaining enough material for downstream ChIP-seq. Then, in collaboration with Tina Karagyzova, Ph.D. student with bioinformatics expertise, we performed the analysis of sequencing data to determine which genomic loci are enriched with the H3.1 or H3.3 variants, with a particular focus on those that are present at chromocenters.

As discussed earlier, H3 histones are among the most evolutionarily conserved proteins. H3.1 and H3.3 histone variants share almost 96% of their amino acid content, except for the HFD, which is required for histone dimerization, and for a single residue at position 31 on the N terminal tail. For this reason, many of the studies in the literature rely on transgenic cell lines or tagged versions of the histones variants to uncover their dynamics in the nucleus. However, little is known about the behavior of endogenous H3 variants in the cells and it is unclear to which extent the use of genetic tags influences the nucleosome composition. To overcome this potential issue, I recently exploited variant-specific antibodies that can distinguish H3.1 from H3.3 based on the Ala 31 or a Ser 31. I then characterized their nuclear distribution during both cell cycle and cell fate differentiation by wide-field and single-molecule localization microscopy, showing consistent results with the data obtained using transgenic SNAP-tagged cell lines for the H3 variants.

To identify the H3-specific amino acid residues that are critical for nucleosome enrichment and maintenance at chromocenters, I produced a series of monoclonal mouse ES cell lines expressing variants of H3.1 and H3.3 histone by modifying the amino acid sequences in the histone fold domain or at residue 31 in the N-terminus. In the first case, a histone will be recognized by a histone chaperone and deposited independently of the type of histone PTMs that a nucleosome is carrying. In the second case, the presence of atypical H3-containing nucleosomes in canonical pathways of deposition could potentially impinge on the overall stability of the targeted local chromatin environment.

Finally, I took advantage of gene-editing tools to target chromocenters by a histone chaperone that is not commonly found enriched in these regions, such as HIRA. Tethering the HIRA complex at major satellite repeats provided an opportunity to increase H3.3 deposition at these sites and therefore impinge on H3 nucleosome stability at chromocenters.

In summary, most of my Ph.D. work was to dissect the chromocenter composition from the perspective of histone variants and chaperones. I did this by integrating high-resolution imaging with sequencing approaches, along with gene-editing and cell differentiation assays, providing a synthetic overview of how the equilibrium of histone variants is necessary to maintain nucleosome composition at nuclear domains. The results of this work are presented in the following section in a manuscript format.

Manuscript

H3.3 deposition counteracts the replication-dependent enrichment of H3.1 at pericentric heterochromatin in the cell cycle of pluripotent cells

Authors: S. Arfè ¹, T. Karagyozyova ¹, A. Forest ¹, H. Hmidan ¹, E. Meshorer ², J.-P. Quivy ¹ & G. Almouzni ¹

¹ Institut Curie, PSL University, Sorbonne Université, CNRS, Nuclear Dynamics, 75005 Paris, France

² Edmond and Lily Safra Center for Brain Sciences (ELSC), The Hebrew University of Jerusalem, Jerusalem, Israel 9190400.

Corresponding authors: genevieve.almouzni@curie.fr ; jean-pierre.quivy@curie.fr

Abstract

In mammalian cells, dynamics in chromatin organization during the cell cycle and cell fate contribute to changes in nuclear chromosomal domains. This is exemplified with membrane-less nuclear domains like chromocenters in mice. In interphase cells, chromocenters correspond to a clustering of pericentric heterochromatin (PHC) domains of distinct chromosomes which display hallmarks of constitutive heterochromatin. In this work, we explored which respective contribution the replicative H3.1/H3.2 and the replacement H3.3 histone variants make in building these domains. We focus on their dynamics during the cell cycle in ES cells before and after differentiation. For this, we combined microscopy imaging and genome-wide ChIP-seq analyses. We found that H3.1 enrichment at chromocenters with the exclusion of H3.3 is a fundamental property in embryonic stem (ES) cells and neuronal precursor cells (NPCs) as well as in differentiated immortalized mouse embryonic fibroblasts (MEF) and NIH-3T3 cells. We demonstrate that this selective enrichment is mechanistically linked to the replication-dependent H3.1 deposition pathway. Notably, in pluripotent ES cells, the cell cycle maintenance of H3.1 enrichment is less robust compared to differentiated cells. Importantly, when forcing H3.3 deposition at chromocenters using a targeted HIRA-dependent pathway, we challenge the H3.1 enrichment. We thus propose that the H3.1/H3.3-based feature of mouse chromocenters reflects opposing mechanisms with different enforcement according to the cycling of the cell, their potency, and their differentiation state.

Introduction

Chromatin states and their plasticity are associated with distinct cell fates (Yadav, Quivy and Almouzni, 2018). During mammalian development, starting from a fertilized egg, a series of cellular divisions, along with the acquisition of distinct specialized functions, provide the variety of cell types involved in the formation of a complete adult organism. Each of these cells displays distinct chromatin features using a versatile basic unit, the nucleosome. Indeed, the nucleosomal core particle, with 147 bp of genomic DNA wrapped around a histone octamer made of an H3-H4 tetramer flanked by two H2A-H2B dimers (Luger *et al.*, 1997), can display different flavors with the choice of histone variants and their histone post-translation modifications (PTMs) (Gurard-Levin, Quivy and Almouzni, 2014; Hammond *et al.*, 2017). Each variant escorted by histone chaperones and deposited along the genome can diversify the histone variant composition of nucleosomes. In mammals, two major non-centromeric histone H3 variants, only differing by 4 or 5 amino acids, display distinct deposition mechanisms reviewed (Mendiratta, Gatto and Almouzni, 2019). This is due to different recognition domains within the alpha-helix of H3. The replicative variants H3.1/H3.2, deposited mainly in S-phase, use a DNA synthesis coupled (DSC) pathway promoted by the CAF-1 complex and are broadly distributed throughout the genome. The replacement variant H3.3, deposited throughout the cell cycle, uses a DNA synthesis independent (DSI) pathway to accumulate mainly in transcribed regions under the control of the HIRA complex, as well as in a subset of heterochromatin regions, including telomeres and pericentromeres, involving the DAXX-ATRX complex reviewed in (Mendiratta, Gatto and Almouzni, 2019). Thus, the variants, as a means to index the genome with their dynamics and their associated chaperones, have emerged as important players in understanding euchromatin and heterochromatin establishment during development. For example, the maintenance of facultative heterochromatin marks (i.e., H3K27me3) at bivalent domains in developmental loci requires H3.3 deposition mediated by HIRA (Banaszynski *et al.*, 2013), in line with the defects in differentiation and reprogramming specification when impairing HIRA, but not of DAXX-ATRX (Banaszynski *et al.*, 2013; Fang *et al.*, 2018). Of note, H3.3 shows a unique amino-acid residue in its amino-terminal tail, S31, that can be phosphorylated (S31ph). The presence of S31ph on H3.3 proved key for the proper activation of transcriptional programs (Martire *et al.*, 2019), macrophage activation (Armache *et al.*,

2020), and, at gastrulation for embryonic developmental progression in *Xenopus*, regardless of its deposition mode (Sitbon *et al.*, 2020). Concerning another form of heterochromatin, constitutive heterochromatin, H3K9me3-repression at retrotransposons and telomeres has been linked to H3.3-DAXX-ATRX deposition for silencing in the absence of DNA methylation (Elsässer *et al.*, 2015; He *et al.*, 2015; Hoelper *et al.*, 2017). Finally, constitutive heterochromatin maintenance at pericentric domains relies on CAF-1 via the SETDB1-Suv39h-HP1 axis (Loyola *et al.*, 2006; Quivy *et al.*, 2008). Most remarkably, CAF-1 deficiency strongly impairs nuclear organization and results in the dispersion of chromocenters in ES cells (Houlard *et al.*, 2006). CAF-1 dosage control is critical to regulating chromatin accessibility, as shown by the loss of transcription factor targeting lineage-committed genomic regions, enhanced reprogramming ability, and promotion of a totipotent state (Cheloufi *et al.*, 2015; Ishiuchi *et al.*, 2015; Cheloufi and Hochedlinger, 2017; Franklin, Murn and Cheloufi, 2021; Franklin *et al.*, 2022).

A key issue is, thus, how do these distinct H3 variants selectively achieve this? How can a domain be enriched specifically with one of the variants? To explore this question, we focused on a particular nuclear region of mouse cells, the chromocenters. Indeed, chromocenters, visible as foci by DNA staining (DAPI) in interphase nuclei, represent tractable and well-characterized membrane-less subnuclear domains (Guenatri *et al.*, 2004). They correspond to pericentric heterochromatin domains (major satellites DNA repeats) (Guenatri *et al.*, 2004) located next to centric regions (minor satellites DNA repeats) of critical importance for kinetochore assembly. While centric regions contain nucleosomes with the centromeric histone variants CENP-A, a key mark for centromere function, pericentric domains do not contain CENP-A and harbor typical heterochromatin marks including H3K9me3, as well as HP1 proteins (Almouzni and Probst, 2011). Furthermore, each chromocenter brings together several chromosomes by clustering their pericentromeric regions. How the propagation of these heterochromatin signatures through cell generations operates has attracted much attention, since their presence proved important to prevent chromosome mis-segregation defects (Peters *et al.*, 2001). Notably, DNA replication with the fork passage causes transient nucleosome disruption. While the recycling of parental nucleosomes on nascent replicated DNA can contribute to preserving positional information of hPTMs, the addition of newly synthesized histones will dilute this information. This time and the potential balance offer a window of opportunity for chromatin remodelers and regulatory factors to access DNA. In the context of heterochromatin, enriched in repetitive non-coding DNA sequences, it is important to stress that in addition to

possible transcriptional switches, altering these specialized chromosomal domains can impact genome functions and genome stability including chromosome segregation (Almouzni and Probst, 2011; Sexton and Cavalli, 2015; García Fernández and Fabre, 2022). Furthermore, their dynamics during differentiation and in specialized cells (Terranova *et al.*, 2005; Solovei *et al.*, 2009) also underlines an important structural role to shape nuclear architecture. This is best exemplified during mouse pre-implantation development at the onset of zygote fertilization. Major satellite DNA repeats form a ring-like structure surrounding nucleoli in the zygote and, only at the 2-cell state transition, a burst in major satellites transcription is critical for chromocenter formation and next consolidation at the 4-cell stage (Probst *et al.*, 2007, 2010; Santenard *et al.*, 2010; Casanova *et al.*, 2013; Burton *et al.*, 2020). Heterochromatin features, including H3K9me3, are progressively acquired over lineage commitment at genome loci to bookmark regions undergoing heterochromatin compaction or to silence stemness genes until the pre-implantation stage (Wang *et al.*, 2018).

At a global level, H3.1/H3.2 are dynamically exchanged in the chromatin of totipotent and pluripotent cells compared to differentiated cells, suggesting different heterochromatin control at these developmental stages (Meshorer *et al.*, 2006; Boskovic *et al.*, 2014). Recent observations indicated that H3.1/H3.2 deposition and recycling occur in the same heterochromatin regions during replication and before differentiation, but not in active regions (Escobar *et al.*, 2019; Ishiuchi *et al.*, 2021). Interestingly, in plants, a specific enrichment in H3.1 has been characterized at chromocenters (Benoit *et al.*, 2019). Given that chromocenters are major candidates to act as a hub for heterochromatin factors throughout mouse development, H3.1 and H3.3 enrichment and their dynamics at chromocenters deserve to be formally addressed.

Here, we investigate H3.1 and H3.3 nuclear distribution with a focus on their relative enrichment at chromocenters throughout the cell cycle and in different cell states. For this, we exploited two distinct approaches (i) microscopy for visualization in individual cells within the nuclei and (ii) ChIP-seq for a mapping of the genomic loci. We carried out these analyses in pluripotent embryonic stem (ES) cells, and in their counterpart following differentiation in Neuronal Precursor cells (NPCs) using as reference differentiated fibroblast cells (MEF, NIH-3T3 cells). First, we observed that while both histone variants are detected in the whole nucleus, the replicative variants H3.1/H3.2, but not the replacement variant H3.3, systematically stand out at chromocenters, regardless of the cell potency state. Intriguingly, H3.1 enrichment is less prominent in ES cells where we observe a relative increase of H3.3. In line with these data by imaging, we also found H3.1

enrichment consistently in chromocenter-associated DNA sequences, namely major satellite repeats, that progressively increases as cells differentiate. Mechanistically, we find that DSC deposition is the major driver for the replicative H3 accumulation at chromocenters. Notably, this is ensured regardless of the capacity to be modified on the N-terminal tail of H3 and not altered in the presence of the S31 amino-acid specific of H3.3. Moreover, this accumulation can be challenged when forcing H3.3 deposition by its targeting via the HIRA deposition pathway. We thus discuss how the cell cycle regulated deposition of H3.1 and H3.3 variants can ensure a less stable accumulation of H3.1 at chromocenters in ES cells compared to differentiated cells with implications for the maintenance of epigenetics states and cell fates.

Results

Mouse pericentromeric heterochromatin is specifically enriched in replicative H3 variants.

To explore how the nuclear localization of the H3.1 and H3.3 variants differs in mouse cells with different cell potential and differentiation states by microscopy, we used pluripotent mouse embryonic stem (ES) cells. Additionally, we used NIH-3T3 fibroblasts as a reference for differentiated cells. To obtain neural progenitor cells (NPCs), we treated ES cells with retinoic acid (RA) conditioned media and retrieved ES cells exiting the pluripotent state after 4 days of differentiation (NPCs 4D). To obtain a more homogenous and differentiated population (NPCs 7D), we maintained NPCs 4D for three additional days (7 days total) in RA medium with additional growing factors (EGF+FGF). Loss of pluripotency potential occurred already after 4 days as attested by loss of Oct3/4 (Fig. S1a). To visualize endogenous H3 variants, we used antibodies specific for the replicative H3 variants (H3.1/H3.2) and for the replacement variant H3.3 (Fig. 1a, Fig. S1b). Given that the antibody against replicative variants does not discriminate between H3.1 and H3.2, we further generated ES cell lines that conditionally express SNAP-tagged H3.1 and H3.3 when induced with doxycycline for 48 hours (Fig. 1b, S1b, S1c). We verified that Dox treatment on ES cells (and consequently the expression of exogenous SNAP-tagged H3.1 and H3.3) did not interfere with their pluripotency potential as attested by the Oct3/4 detection. (Fig. S1a). In addition, as a control, we also generated NIH-3T3 cell lines constitutively expressing SNAP-tagged H3.1 and H3.3 with expression levels comparable to their endogenous counterparts. By Western blots analysis using total cell extracts, we observed that the SNAP-H3.1 represented less than 5% of the total histone pool (Fig. S1d). Importantly, using the same imaging system, detection by SNAP or using antibodies showed similar nuclear localization (Fig. S1b). Thus, with our assays, neither the expression level of the exogenous protein nor the presence of the tag leads to abnormal localization of the variants.

When visualizing endogenous H3 variants in ES, NPCs, and NIH-3T3, we found that irrespective of the differentiation status, the replicative H3.1/H3.2 histones localized in the whole nucleus with a marked enrichment at DAPI-dense regions corresponding to chromocenters (Fig. 1a, left panel). In contrast, we observed that H3.3 is broadly dispersed in the whole nucleus for every cell line. At chromocenters, H3.3 signal intensity was either equivalent to that of the overall nucleus or weakly depleted (Fig. 1a, right panel).

Consistently, exogenous H3.1- and H3.3-SNAP-tagged histones in ES, NPC, and NIH-3T3 cells displayed the same type of subnuclear distribution with H3.1 enriched at DAPI dense domains along with HP1-alpha (Fig. 1b). Given that chromocenters bring together distinct chromosomes by clustering their pericentric domains that are located next to centric regions (Guenatri *et al.*, 2004), we refined our analysis to consider the mouse centric regions that are marked by the centromeric histone variant CENP-A (Guenatri *et al.*, 2004; Maison *et al.*, 2010). Visualization of H3.1/H3.2 and H3.3 along with CENP-A detection confirmed that in ES cells CENP-A strictly marks the centric domains but not the pericentric region in both interphasic cells and mitotic chromosomes (Fig. 1c). Importantly, CENP-A enriched regions did not display any enrichment of H3.1/H3.2, while both the nearby pericentric regions of metaphase chromosomes or chromocenters of interphase cells showed strong enrichment in these replicative histone variants (Fig. 1c). Taken together, by imaging, these data show that in general from pluripotent to terminally differentiated cells, the pericentric regions of the mouse centromere show a preferential enrichment of H3.1.

Figure 1

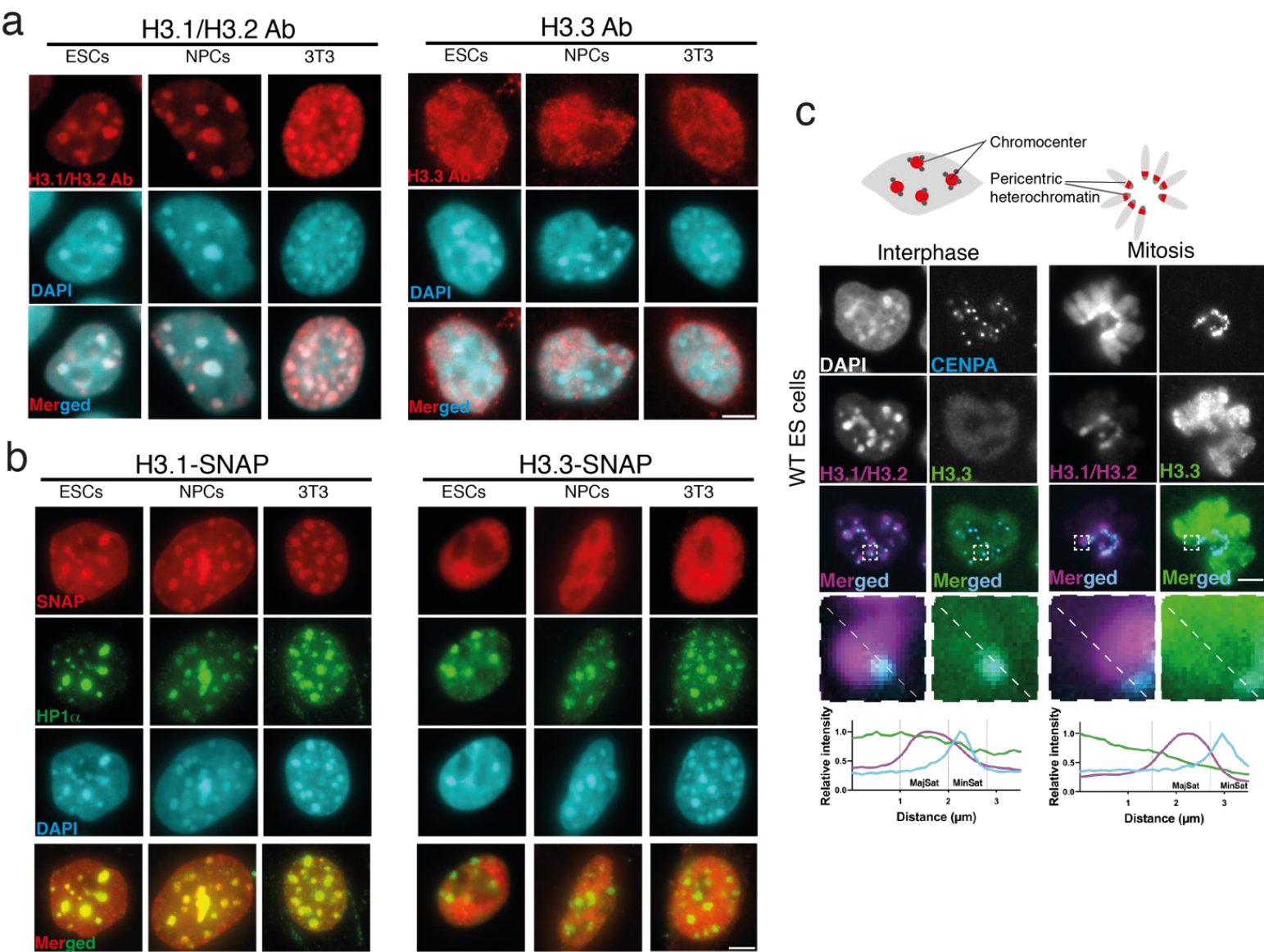


Fig.1: H3.1 histone variant is associated with PHC domains. (A) Endogenous total H3 histones are detected with H3 variant-specific antibody (Ab, red) as revealed by immunofluorescence in fixed mouse embryonic stem cells (ESCs) (left column), neuronal progenitor cells (NPCs) derived from ESCs after 4 days of differentiation (middle column) and NIH-3T3 cells (right column) followed by a DNA counterstaining (DAPI, cyan). (B) Representative epifluorescence images of H3.1/H3.3-SNAP (red) in mouse ESCs (left column), NPCs (middle) or NIH-3T3 (right) along with HP1a (green) and DNA (DAPI, cyan). Clusters of pericentric heterochromatin (PHC) were identified as DAPI-dense domains (C) Top panel: scheme for chromocenter organization in interphase and mitotic mouse cells. Chromocenter/pericentric heterochromatin regions are indicated in red and minor satellites marking the periphery of these domains in dark grey. Middle panel: representative epifluorescence images of endogenous H3 variants staining in ESCs along with DNA (DAPI) staining in grayscale. Merged images show CENP-A (cyan) with H3.1/H3.2 (magenta) or H3.3 (green). Bottom panel: graph displaying the relative intensities of H3.1/H3.2, H3.3, and CENP-A along a 3 μ m dashed line traced above a representative pericentric heterochromatin domain. Scale bars 5 μ m.

ES cells show a lower proportion of cells with H3.1 enrichment at chromocenters compared to differentiated cells

Since we noticed that every cell nucleus did not systematically display a clear enrichment in H3.1 at pericentromeric regions, we quantified the proportion of cells in three main categories: 1) ‘enriched’ when we detected preferential H3 variant localization at chromocenters; 2) ‘even’ with equivalent signals when comparing chromocenter and the whole nucleus; 3) ‘excluded’ for a lower signal at the chromocenters compared to the rest of the nucleus (Fig. 2a). For H3.3, ~75% of cells displayed ‘even’ and ~25% ‘exclusion’ patterns at PHC of exogenous H3.3-SNAP for every cell line analyzed (ES, NPC 4D, NPC 7D, 3T3) (Fig. 2b right panel). We obtained a similar distribution when detecting endogenous H3.3 with antibodies (Fig. 2c right panel). For H3.1, every cell line is distributed between ‘enriched’ and ‘even’ patterns, without any ‘exclusion’ pattern (Fig. 2b, 2c, left panels). However, in contrast to H3.3, we discovered that H3.1 showed different patterns in the four cell lines. While the three differentiated cell lines (NPCs 4D, NPCs 7D, and 3T3) displayed ‘enriched’ and ‘even’ patterns in ~85% and ~15%, respectively, for the pluripotent ES cell lines, we observed a reproducible lower frequency in cells with “enriched” patterns (~70%) by detection of either the endogenous and exogenously expressed SNAP-tagged H3.1 (Fig 2b, 2c, left panels). To further characterize this difference, we performed an automatic quantification of H3.1 and H3.3 signal enrichment at chromocenters in asynchronous ES and differentiated MEF cells, using a 3D-FIED (3 dimension-fluorescence intensity enrichment at domains) method (Cantaloube *et al.*, 2012). The 3D-FIED ImageJ macro provides, from 3D image acquisitions, the ratio between the mean signal intensity at the chromocenters and the mean signal intensity in the rest of the nucleus for a given protein of interest, in our case endogenous H3.1 (or H3.2) and H3.3 (Cantaloube *et al.*, 2012). A ratio above 1 indicates enrichment at the chromocenters, compared to the rest of the nucleus, and below 1 - exclusion. In line with a lower proportion of cells displaying an ‘enriched’ H3.1 pattern in ES compared to differentiated cell lines (Fig. 2b), the H3.1 enrichment ratio was lower in ES cells than WT MEF cells (Fig. 2d). Conversely, the H3.3 ratio tends to be higher in ES cells than in MEF cells. This reciprocal behavior suggests that there is a balance between H3.1 and H3.3 localization at chromocenters to ensure a proper H3 density.

Taken together, these data indicate that in ES cells, H3.1/H3.2 enrichment at chromocenters is less prominent than in differentiated cells and that a counterbalance with H3.3 is in place to ensure H3 variant density.

Figure 2

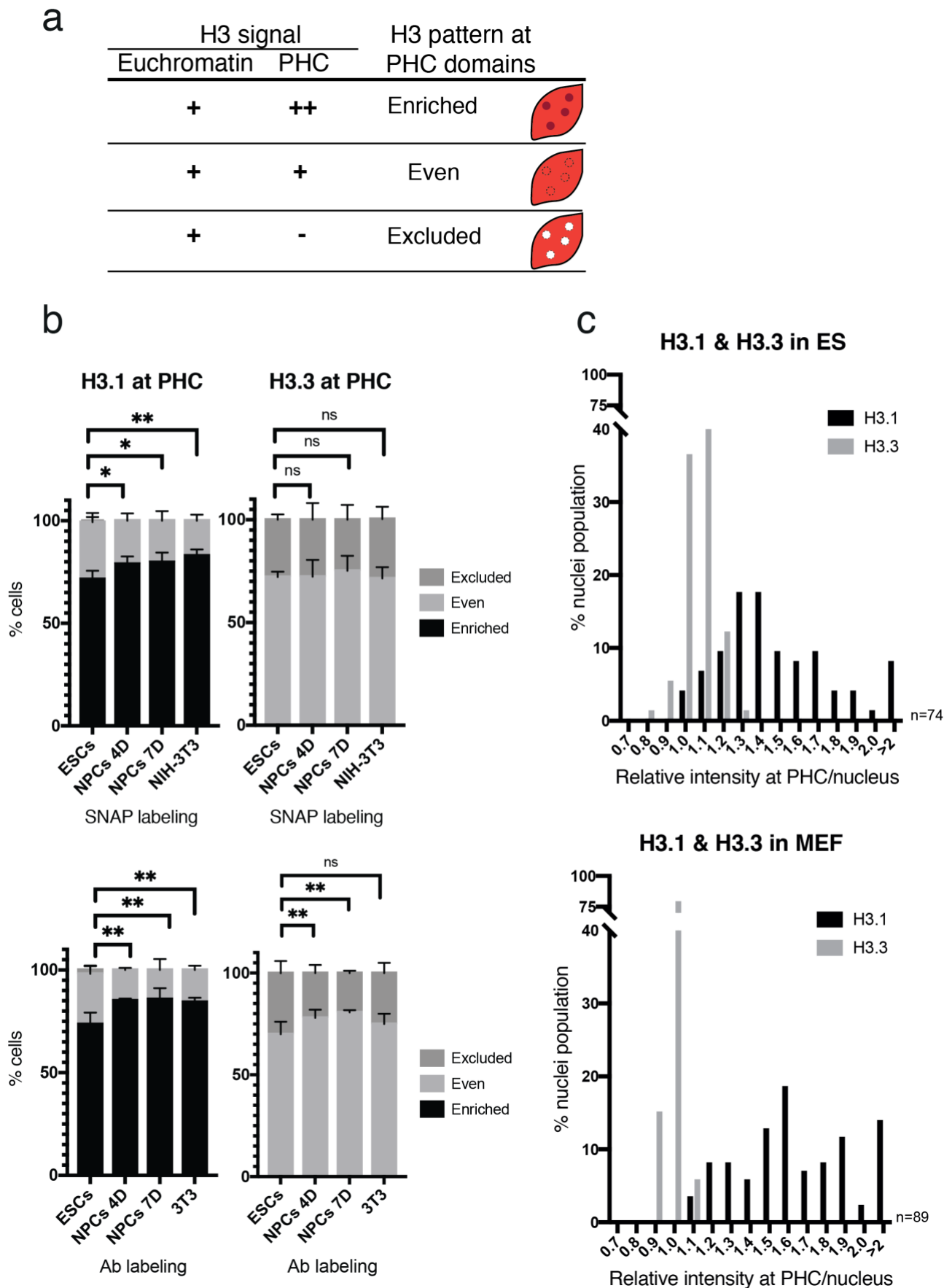


Fig. 2 H3.1 enriched at PHC is more frequent in differentiated compared to pluripotent cells. (A)

Schematic of observed patterns based on H3.1 and H3.3 fluorescence intensity in euchromatin and PHC. (B) and (C) Quantitative analysis of the percentage of cells exhibiting recurrent patterns of H3 variants at PHC in different cell backgrounds with SNAP-labeling or Ab labeling, respectively. Data show the mean and standard deviations (s.d.) from 3 biological independent experiments; >100 nuclei were quantified per condition. (D) Graphs showing the distribution of H3.1 and H3.3 enrichment values at PHC in percentages of the nuclei population from ESCs and iMEF. Number of nuclei analyzed is indicated as n. ANOVA tests were used for statistical analysis in B: ns ($p > 0.05$), * ($p < 0.05$), ** ($p < 0.01$).

Genomic H3.1 and H3.3 enrichment at centromeric repeats in ES and NPC differentiated cells

Our imaging approaches revealed the subnuclear distribution of the H3 variants at chromocenters, in individual cells without a resolution enabling us to identify the underlying DNA. Within the centromeric regions in *Mus Musculus*, major and minor satellite repeats correspond to pericentric and centric regions, respectively (Guenatri *et al.*, 2004). To map H3.1 and H3.3 at these regions, we exploited the H3.1- and H3.3-SNAP tagged ES cell lines and performed SNAP capture followed by high-throughput sequencing (SNAP capture-seq) as described (Gatto *et al.*, 2022). We carried out the experiments on ES cells and NPC at 4 days and 7 days of differentiation (Fig. 3a). We validated our SNAP capture-seq data by comparing it with previous datasets using a different H3-SNAP-Tag expression vector in ES cells (Deaton *et al.*, 2016) (Fig. S2). We next analyzed and quantified the distribution of H3.1 and H3.3 throughout the different differentiation steps at the set of elements corresponding to centromeres focusing on major satellite (GSAT_MM) and minor satellite (SYNREP_MM) repeats. Of note, additional repetitive elements are associated, but not exclusively present, in constitutive heterochromatin regions. In particular, ~10% of DNA at chromocenters likely corresponds to long interspersed elements (LINEs) L1 elements (Bulut-Karslioglu *et al.*, 2014, Ostromyshenskii *et al.*, 2018). The enrichment of H3.1 at major satellite repeats and L1 LINEs in ES, NPCs 4D, and NPCs 7D is systematically accompanied by H3.3 depletion (Fig. 3b). Importantly we observed an increased H3.1 enrichment from ES to NPC D7, confirming our imaging results. Interestingly, we also observed an increasing enrichment of H3.1 at minor satellites that occurred over differentiation, along with a mild enrichment of H3.3, consistent with the proposed role of H3.3 as a placeholder for CENP-A in centric domains (Dunleavy, Almouzni and Karpen, 2011). Overall the genomic results are consistent with our analyses by microscopy and confirm a specific H3.1 enrichment over chromocenter-associated repetitive elements conserved from pluripotent to differentiated cells. Importantly, they also support the fact that the H3.1 enrichment at chromocenters, specifically for major satellite sequences, becomes more prominent when comparing pluripotent to differentiated cells.

Figure 3

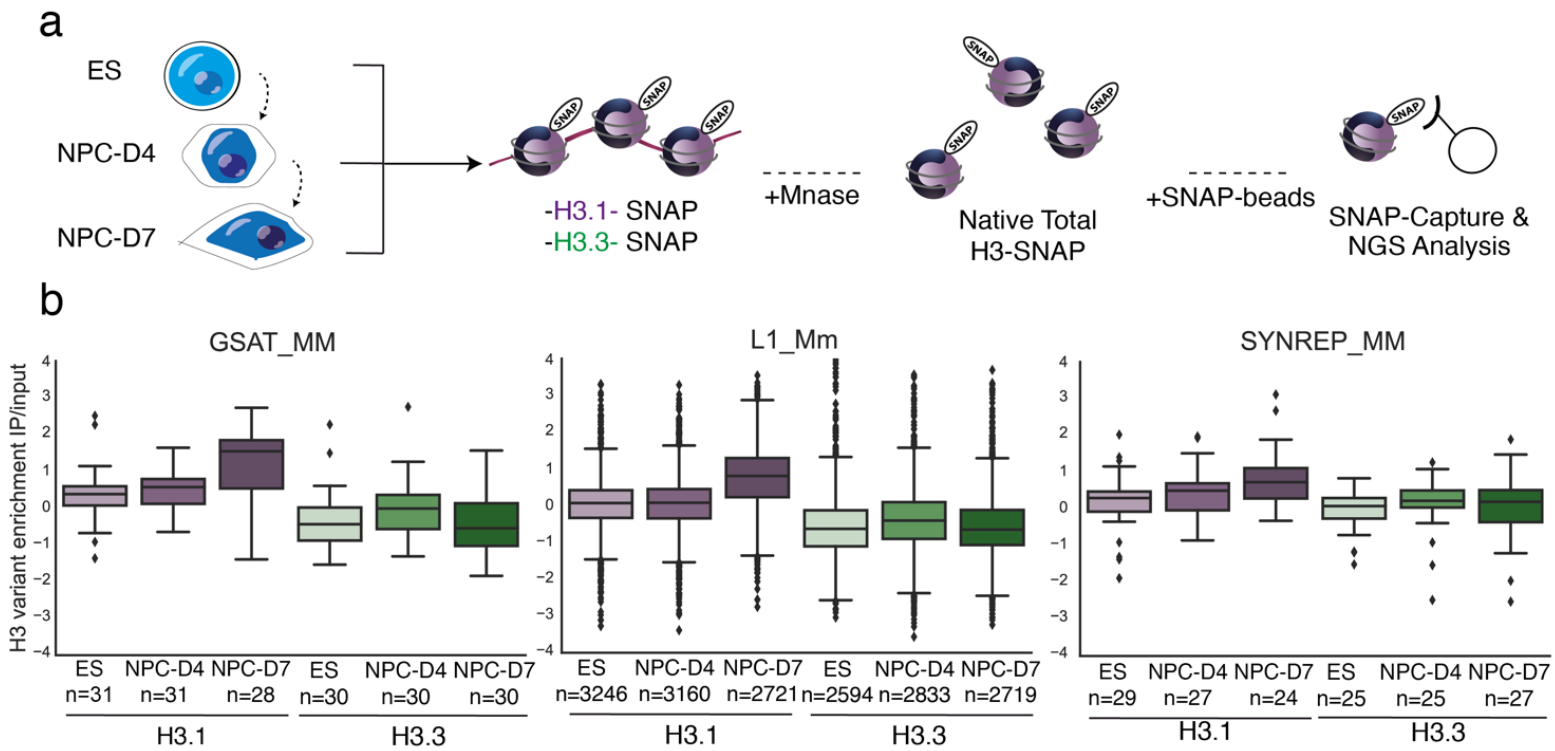


Fig.3 H3.1 and H3.3 enrichment at different repetitive sequences. (A) Scheme for SNAP-Seq in mESCs and in differentiating NPCs at two different points (D4 and D7) expressing H3.1 or H3.3- SNAP. MNase digestion optimized to obtain mononucleosomes enabled to produce samples subjected to capture and pull-down with SNAP beads, followed by sequencing. (B) Quantification of H3 variants at the major satellite, L1 LINEs, and minor satellite repeat elements throughout differentiation. Y-axis displays the H3 enrichment represented as a Z-score of \log_2 enrichment of IP over input. n displays the number of repeat elements at each differentiation step. The color palette from light to dark pattern reflects the differentiation conditions analyzed.

ES cells show cell cycle variability in H3.1 enrichment at chromocenters, compared to the steady distribution in differentiated cells

The fact that H3.1 enrichment at chromocenters is not detected in every cell in an asynchronous cell population suggests a possible dynamic change depending on the cell cycle. Thus, we monitored H3 patterns in parallel with cell cycle markers, including Aurora B and EdU labeling, to discriminate the G1 (Aurora B-/EdU-), S (Aurora B-/EdU+) and G2 cells (Aurora B+/EdU-) (Fig. 4a). The spatiotemporal organization of DNA replication foci (based on EdU pattern) also enables us to define S phase progression as (i) “Early S-phase” with a high density of small foci spread throughout the nucleus; (ii) “Mid S-phase” with typical ring-shaped staining around the chromocenters; and (iii) “Late S-phase” with a few large foci at the nuclear periphery (Dimitrova and Berezney, 2002; Quivy *et al.*, 2004; Rausch *et al.*, 2020). Additionally, within the S-phase cell population, we could distinguish cells that had not yet replicated chromocenters (‘early’) from those where chromocenters were undergoing replication (‘mid’) and/or had already experienced replication (‘late’) chromocenters. With this analysis, we found that while H3.3 is stable, the H3.1 nuclear signal changed with cell cycle progression (Fig. 4b). By estimating the proportion of cells displaying ‘enrichment’, ‘even’, and ‘exclusion’ patterns at chromocenters for H3.1, we found that NPCs and NIH-3T3 cells consistently presented a high proportion (>80%) of ‘Enriched’ patterns at all cell cycle stages (G1, Mid S-phase, Late S-phase, and G2) except for Early S-phase (~50% ‘Enriched’) (Fig. 4c). This early S-phase relative decrease may simply reflect the global increase in the H3.1 signal associated with early replication of the euchromatin as opposed to the mid-late replication of heterochromatin in chromocenters (see Fig. 4b, H3.1 signal in early S-phase). In line with this interpretation, this unbalance is overcome during mid-S and late-S when chromocenters replicate and heterochromatin doubles. This status is then maintained in subsequent G2 and propagated in G1. Surprisingly, the situation is quite different in ES cells. While >80% of ES cells in mid-S, late-S, and G2 phases displayed an ‘enriched’ pattern, similar to NPCs and NIH-3T3 cells, the proportion of cells with an ‘enriched’ pattern decreased to 50% already in G1 (Fig. 4c). Then, ES cells during the early S-phase showed the lowest proportion of H3.1 ‘enriched’ pattern (~10%) amongst all cell lines analyzed (Fig. 4c). When monitoring H3.3, a reciprocal picture emerged, with the highest proportion of cells showing H3.3 ‘exclusion’ corresponding precisely to those cells with the highest ‘enrichment’ of H3.1 (Fig. 4c). Conversely, when most of the cells did

not display H3.3 'exclusion', the situation corresponded to the lowest H3.1 'enriched' pattern. Importantly, ES cells in G1 displayed the lowest proportion of H3.3 'excluded' patterns. Taken together, these data further support that H3.1 and H3.3 at chromocenters dynamically compensate each other and that, in differentiated cells, H3.1 enrichment at chromocenters is maintained throughout the cell cycle. In contrast, in ES cells, H3.1 enrichment is lost during G1 and only re-established following chromocenter replication.

Figure 4

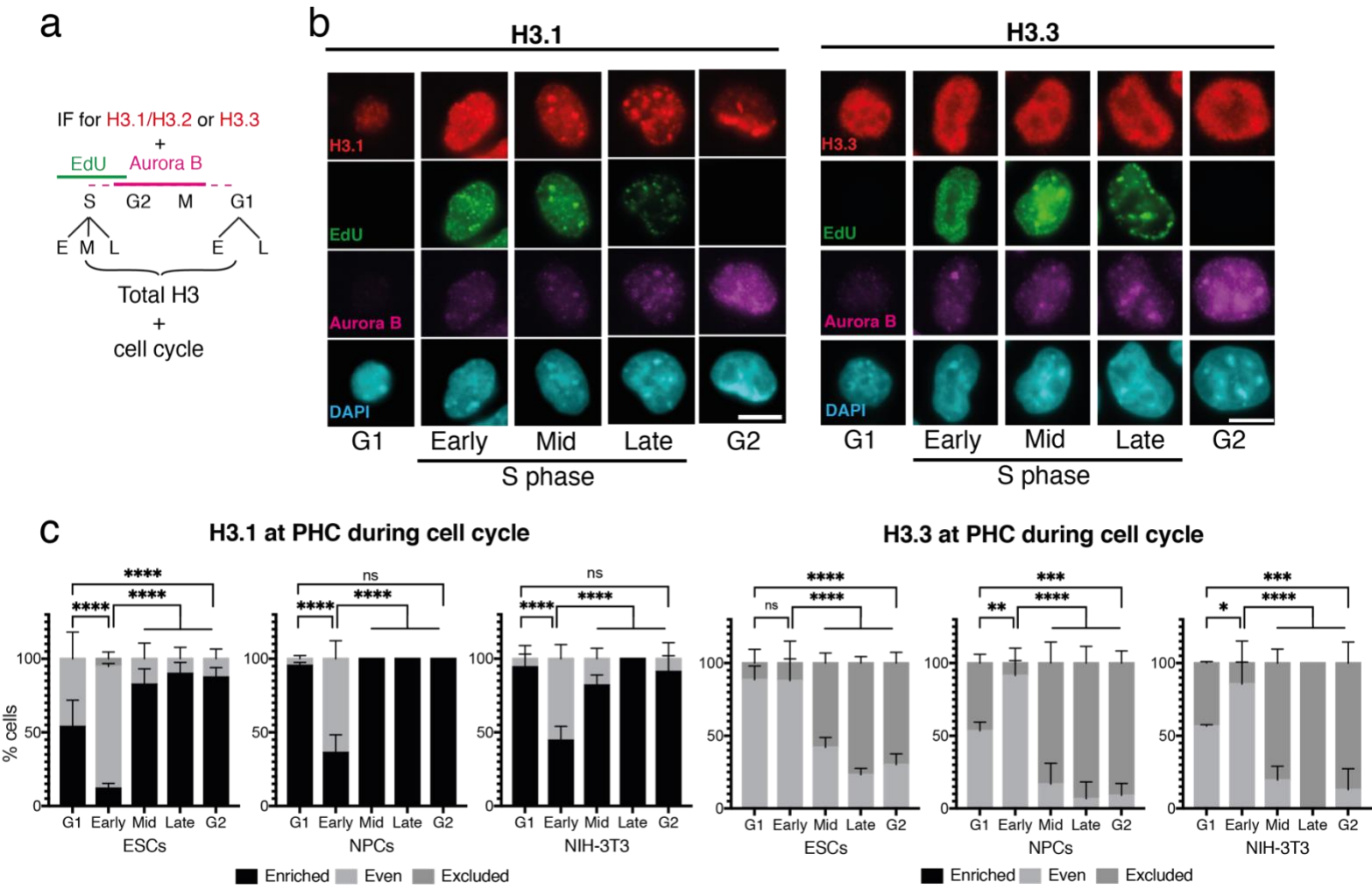


Fig.4 H3.1 enrichment follows PHC replication status. (A) Experimental scheme for visualizing endogenous H3.1/H3.2 or H3.3 during the S phase. Aurora B and EdU staining allowed to resolve the cell cycle stage of individual cells. Cells are scored as G1 (negative to Aurora B and EdU), S phase (EdU positive), or G2 (negative to EdU and positive to Aurora B). S-phase designations are based on EdU S phase patterns with the Early S phase defined by a diffused (euchromatin) staining; Mid S by a focused EdU labeling around DAPI foci; and Late by specific foci staining at the nuclear periphery. IF, immunofluorescence; E, Early; M, Mid; L, Late; M, mitosis. (B) Representative widefield images of ES cells after *in vivo* labeling with EdU (green), and immunofluorescent staining of H3.1/H3.2 (red), Aurora B (magenta), and DNA (DAPI, cyan). (C) Quantitative analysis of the percentage of cells exhibiting recurrent H3 variant patterns at PHC (as defined in Fig. 1c) in different cell lines (ES, NPCs, NIH-3T3) during the G1, S, and G2 phases. Data show the mean and sd (at least 3 biological independent experiments; >100 nuclei quantified per condition). Scale bars, 5 μ m. ANOVA tests were used for statistical analysis: ns ($p > 0.05$), * ($p < 0.05$), *** ($p < 0.001$), **** ($p < 0.0001$).

DNA synthesis promotes H3.1 enrichment at chromocenters

To investigate key parameters leading to the enrichment of H3.1 (but not H3.3) at chromocenters, we considered two aspects. These histone variants differ in their amino-acid sequence in two regions (Fig. 5a): (i) in the histone fold domain: a motif that allows distinct recognition by histone chaperones; (ii) in the amino-terminal tail at position 31: a serine in H3.3 instead of an alanine in H3.1, which phosphorylation proved critical to activate transcription during key cell fate transitions and development (Martire *et al.*, 2019; Armache *et al.*, 2020; Sitbon *et al.*, 2020). The DNA synthesis coupled (DSC) deposition involves the CAF-1 complex, that interacts with the SVM motif in H3.1/H3.2 (Ahmad and Henikoff, 2002; Ray-Gallet *et al.*, 2011; Latreille *et al.*, 2014). For DNA synthesis independent (DSI) deposition, other histone chaperones interact with the AIG motif in H3.3 (Tagami *et al.*, 2004; Goldberg *et al.*, 2010; Lewis *et al.*, 2010; Elsässer *et al.*, 2012) (Fig. 5a). Finally, H3.3 differs from H3.1 by a serine to cysteine substitution at position 96, equally present in H3.2 (Fig. 5a). We thus wondered whether the chromocenter enrichment with distinct histone variants depended on the deposition pathway and/or the potential modification of these variants or both. To this aim, we generated transgenic ES cell lines with wild-type H3.1, H3.2, and H3.3 SNAP-tag constructs, along with an H3.1 construct with an A31S substitution in its N-terminus (A31S) and an H3.3 with either a phosphomimic (S31D) or phospho-dead (S31A) substitution. We found that all constructs with the SVM motif (corresponding to DSC deposition) showed enrichment at the chromocenters (Fig. 5b). The presence of either a cysteine (H3.1) or serine (H3.2) at position 96 did not yield any significant difference, or the S31 residue (H3.3) did not prevent chromocenter enrichment (Fig. 5b). In contrast, constructs with the AIG motif (corresponding to DSI deposition), including the S31 phospho-mimic and phospho-dead mutants could not accumulate at chromocenters (Fig. 5b). Our quantification of the proportion of cells displaying ‘enriched’, ‘even’, and ‘excluded’ patterns at chromocenters with respect to S phase progression showed that constructs linked to DSC deposition led to ‘enriched’ patterns (Fig. 5c). Importantly, the proportion of cells displaying ‘enriched’ patterns during S phase progression was identical to endogenous H3.1, indicating that the SNAP-tag presence did not create any bias (compare Fig. 5c with Fig. 4c and Fig. S1b, ES cells). Importantly the presence of either S31 (from H3.3) or S96 (H3.2) leads to similar behavior to H3.1, suggesting that the possibility to modify or not this residue does not interfere with the stable enrichment. In contrast, the DSI constructs did not show

‘enrichment’ but led to ‘even’ and ‘excluded’ patterns during the S phase similarly to endogenous H3.3 (compare Fig. 5c with Fig. 4c and S1b, ES cells). Importantly, phospho-mimic D31 and phospho-dead S31 mutants led to identical proportions of ‘even’ and ‘excluded’ patterns compared to their WT counterparts. We conclude that the main parameter defining H3.1 enrichment at chromocenters is the DNA synthesis coupled deposition pathway as shown by the critical importance of the SVM motif.

Figure 5

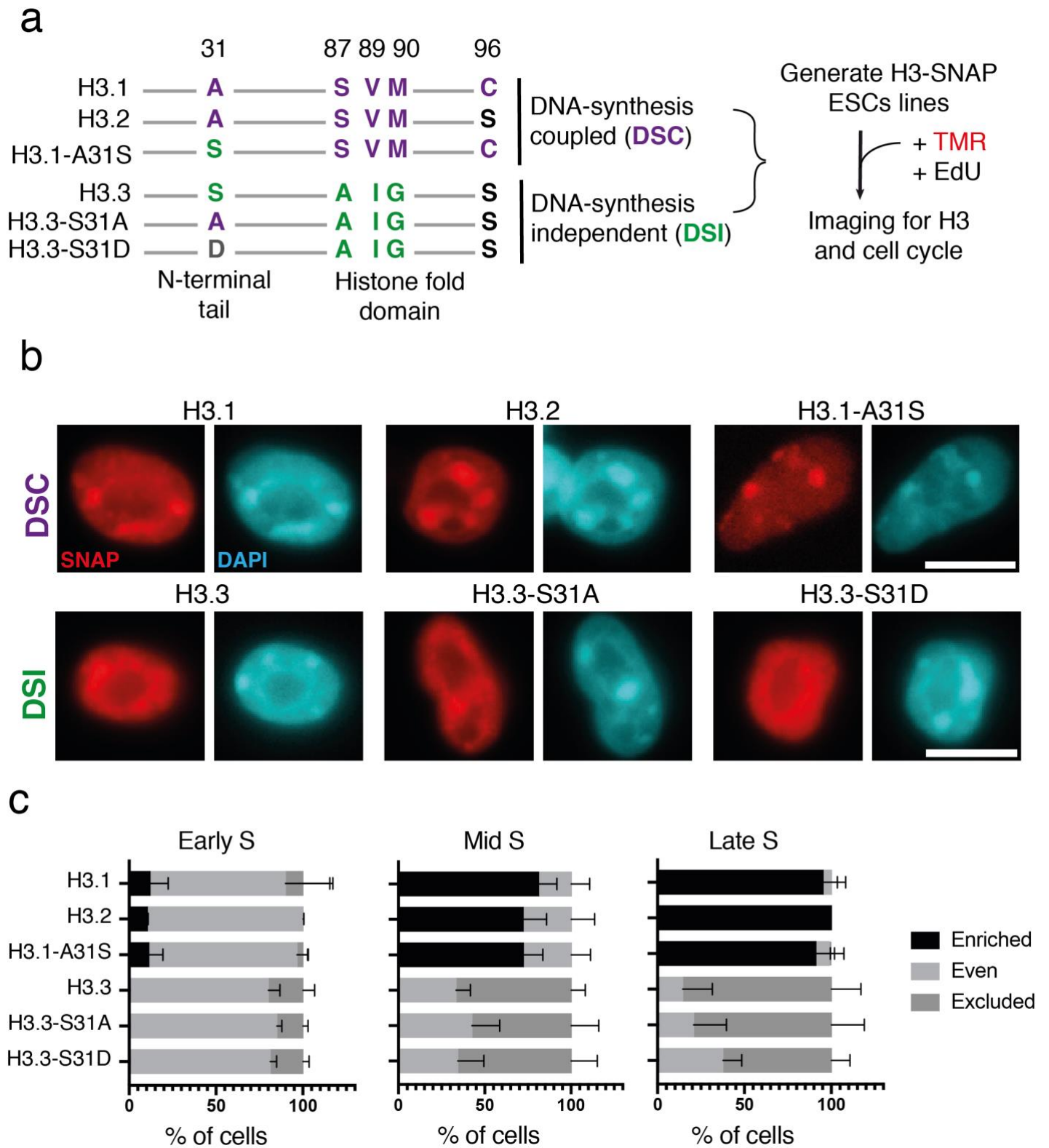


Fig. 5 SVM-CAF1 binding motif is sufficient for H3 enrichment at PHC domains, regardless of H3 variant-specific modifications on N-tail. (A) Left panel: Specific amino acidic residues are depicted and grouped based on their similarities with the histone chaperone recognition motif. Substitutions from original sequences of H3.1 or H3.3 are highlighted in purple, green, or gray. Right panel: Experimental scheme to obtain ES cell lines with point mutations on H3.1/H3.3-SNAP-Tag constructs and visualize total histone H3 patterns along with cell cycle (B) Representative epifluorescence images of H3-SNAP-Tagged histones (red) in ESCs upon 48h dox addition along with DNA (DAPI, cyan). (C) Quantification of cells exhibiting H3 patterns at PHC during Early, Mid, and Late S stages. Data show the mean and s.d. (at least 3 biologically independent experiments; >100 nuclei quantified per condition). Scale bars 10 μ m.

Targeting the HIRA H3.3 chaperone at chromocenters off competes for H3.1 enrichment in ES cells

The DSC deposition pathway is necessary for the enrichment of H3.1 at chromocenters, yet this enrichment is reduced during G1 in ES cells. We thus wondered how the replication-dependent enrichment could be counteracted. Since we observed reciprocal patterns of H3.1 enrichment mirrored by H3.3 depletion, we reasoned that H3.3 could compete with H3.1 at the chromocenters. To test this hypothesis, to force H3.3 deposition at chromocenters, we engineered a transcription activator-like effector (TALE) designed to bind specifically to the major satellite DNA repeats (Miyanari, Ziegler-Birling and Torres-Padilla, 2013; Maison *et al.*, 2016) in fusion with the H3.3 chaperone HIRA. We used both HIRA WT and a mutant (W700A-D800A) unable to interact with CABIN1 and thus unable to promote *de novo* H3.3 deposition (Ray-Gallet *et al.*, 2018). We also added a fluorescent YFP reporter tag, to directly visualize the exogenous HIRA (Fig. 6a). As a control, we used the TALE directly fused to the green fluorescent reporter mClover (TALE-mClover) (Miyanari, Ziegler-Birling and Torres-Padilla, 2013). We next transfected ES cells and visualized them by immunofluorescence endogenous H3.1/H3.2 and H3.3 along with the distinct TALE fusion proteins (Fig. 6b). Both WT and mutant TALE-HIRA-YFP fusion proteins readily localized at the DAPI dense chromocenters as we wished. In addition, cells transfected with the wild-type HIRA (TALE-HIRA-YFP WT) displayed a global H3.3 increase in the nucleus which was not observed for the mutant (TALE-HIRA-YFP mutant, Fig. 6b). Next, we quantified the proportion of cells displaying ‘enriched’, ‘even’, and ‘excluded’ patterns for H3.3 and H3.1/H3.2 in the transfected (YFP+) cells (Fig. 6c). For H3.3, TALE-HIRA-YFP WT transfection increased the proportion of cells with the ‘even’ pattern with a concomitant decrease of cells with the ‘excluded’ pattern. This was paralleled by a strong reduction in H3.1 enrichment at chromocenters and the proportion of cells with an ‘enriched’ H3.1 pattern dropped by half (from 60% to 30%). Importantly, neither the TALE-mClover nor the TALE HIRA-YFP mutant transfections showed any of these changes (Fig. 6c). Next, to compare the enrichment of H3.3 and H3.1/H3.2 at chromocenters targeted by TALE-HIRA-YFP WT or TALE-HIRA-YFP mutant, we analyzed the variations in the intensity of H3.3 and H3.1/H3.2 immunofluorescence signal from a line scan across chromocenters (Fig. 6d). Using this approach, H3.3 enrichment between the WT and HIRA mutant was even as expected because the global H3.3 signal in the whole nucleus is increasing upon TALE-HIRA WT expression (Fig. 6b). Notably though, while H3.1 enrichment was unaffected at chromocenters

targeted by the TALE-HIRA mutant (Fig. 6d), this H3.1 enrichment was lost with the TALE-HIRA-YFP WT targeted to chromocenters (Fig. 6d). These data strongly support the fact that HIRA-mediated H3.3 deposition targeted to chromocenters is dominant to off-competes H3.1 enrichment at chromocenters. Taken together, our results indicate that H3.1 enrichment at chromocenters depends on a fine-tuning between DSC and DSI deposition pathways where H3.3 DSI can be dominant to compete with H3.1.

Figure 6

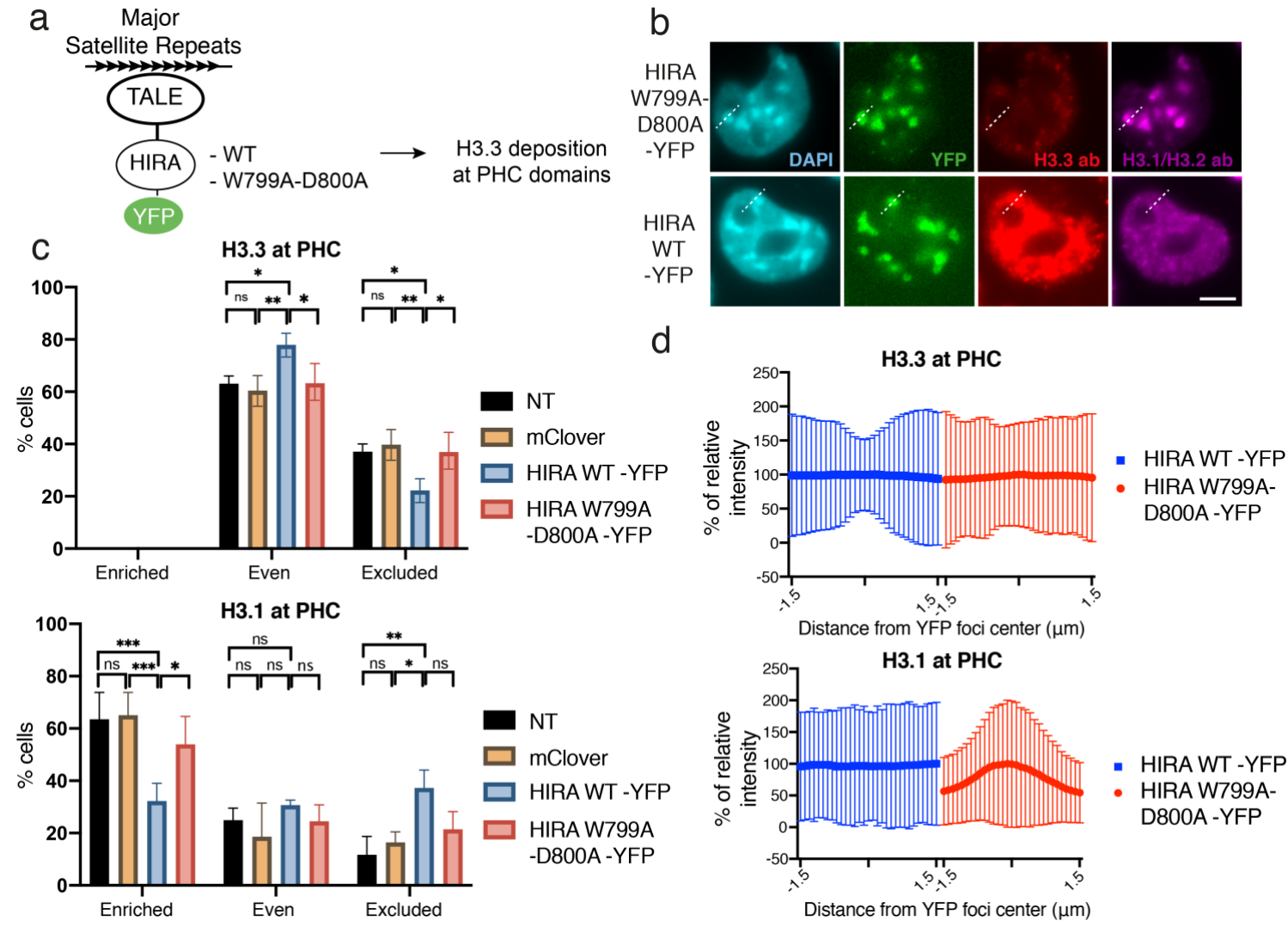
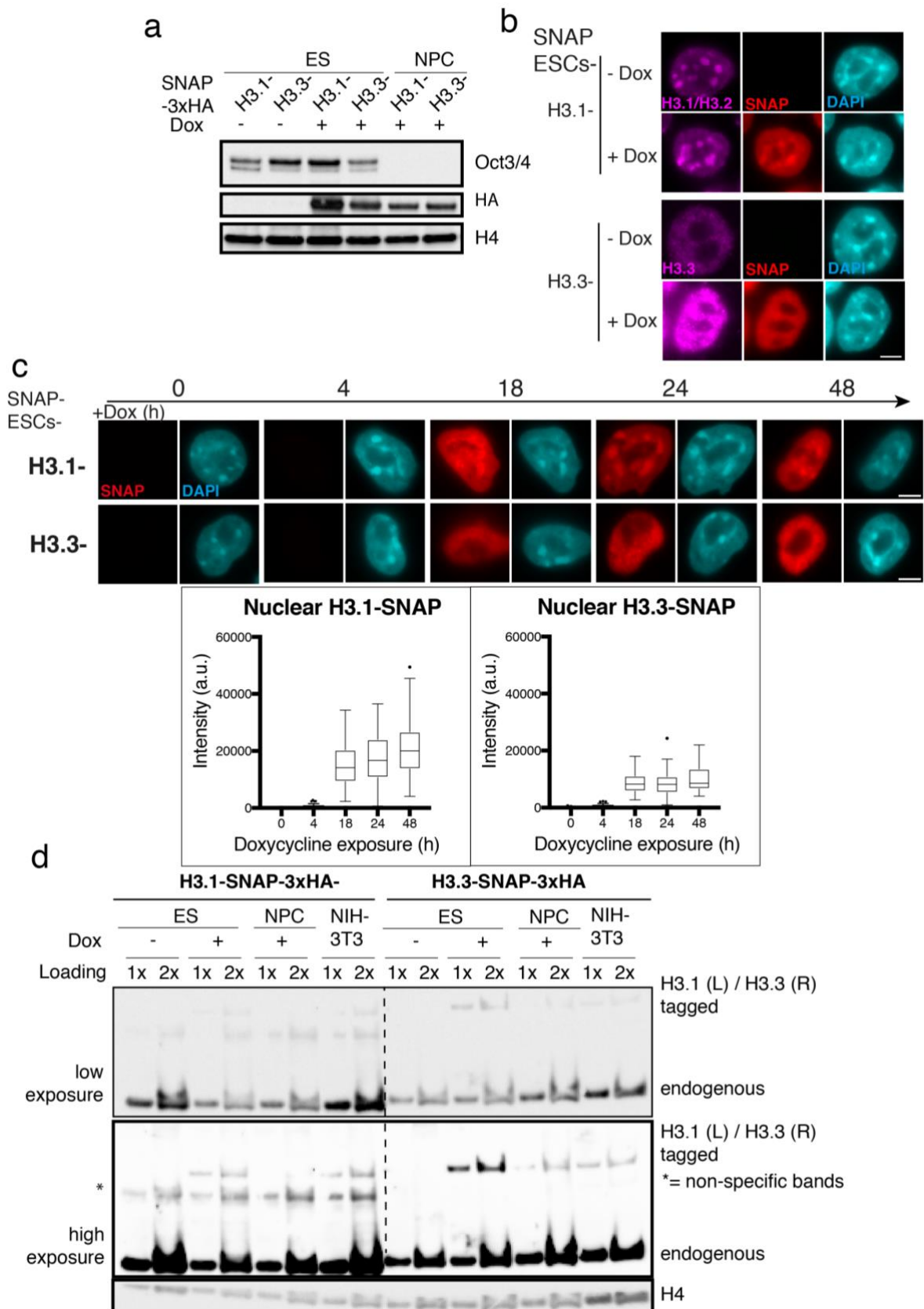


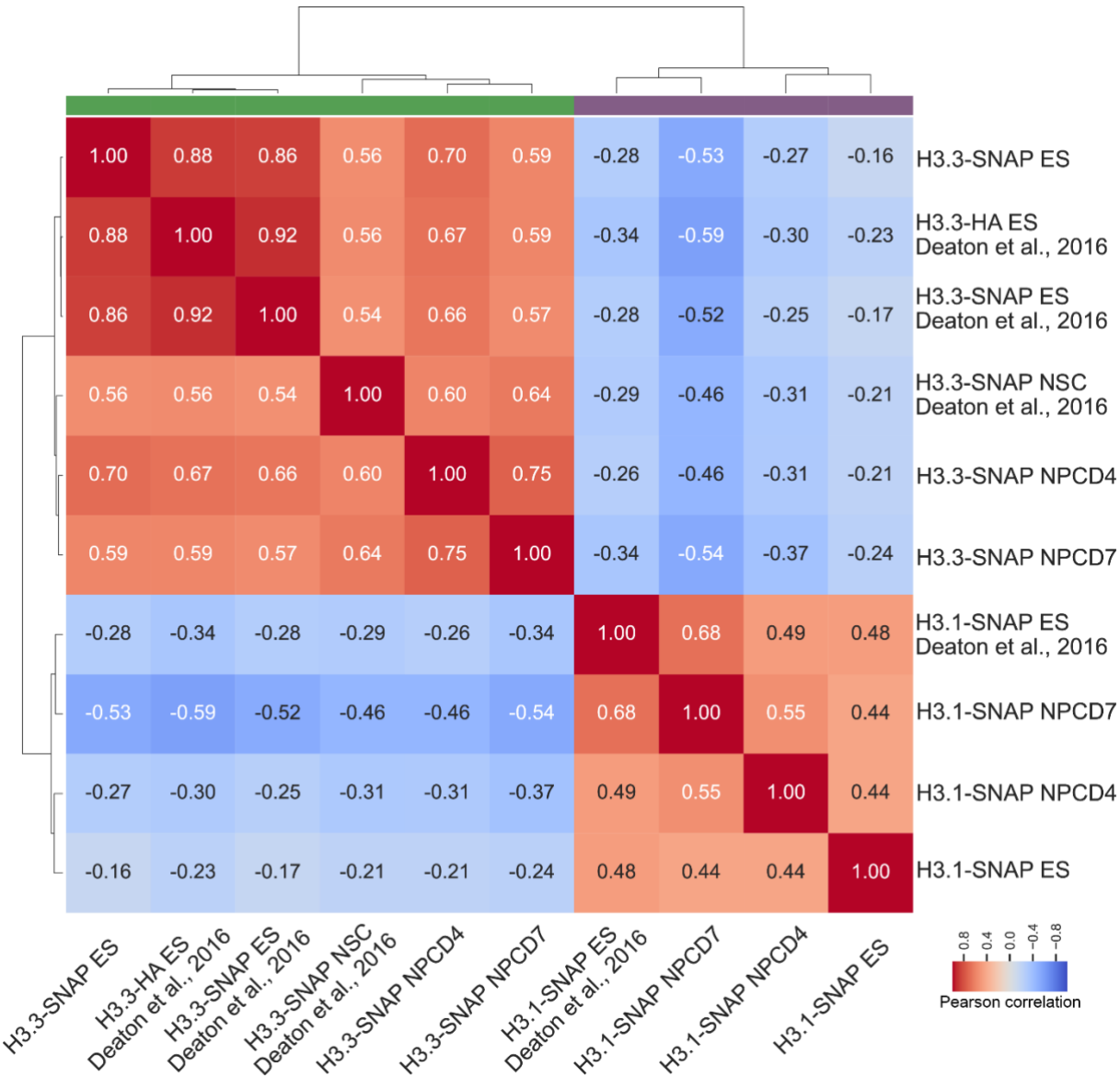
Fig.6 HIRA targeting at PHC alters local H3.1 enrichment. (A) Scheme depicting YFP-Tagged HIRA WT and W799A-D800A transgenic proteins fused to TALE protein recognizing major satellite sequences. (B) Representative images of ESCs transfected with TALE-Maj-Sat constructs. YFP (green) is detected at PHC, along with H3.1 or H3.2 (magenta), H3.3 (red) antibodies, and DNA (DAPI, cyan) staining. (C) Quantitative analysis of the percentage of cells exhibiting recurrent patterns of H3 variants at PHC upon transfection of either TALE-MajSat -mClover reporter, -HIRA-WT-YFP or -HIRA-W799A-D800A-YFP. Plots show averages and s.d. of at least 50 nuclei per condition from 3 biologically independent experiments. ANOVA tests were used for statistical analysis: ns ($p > 0.05$), * ($p < 0.05$), *** ($p < 0.001$). NT = Non-transfected cells control (D) Measurements in percentage of relative intensity profiles (bold lines) at individual PHC domains from 3 μm dotted line (white) scan in (B) for H3.3 or H3.1 signal upon TALE-Maj-Sat transfections of TALE-MajSat -HIRA-WT-YFP or -HIRA-W799A-D800A-YFP. Plots show average and s.d. error bars for at least 35 chromocenters per condition. Scale bars, 5 μm .

Supplementary Figure 1



Supplementary Fig 1. H3-SNAP-Tagged histones are all expressed and incorporated in a similar mode *in vivo*. (A) Expression of H3-SNAP-3xHA proteins by Western blot of total proteins in transgenic ESCs and NPCs. The presence of SNAP-tagged histones is verified with HA antibody in the presence (+) or absence (-) of Dox treatment. Differentiation from ESCs to NPCs is verified with Oct3/4 expression and detected with the corresponding antibody. (B) Representative fluorescence images of H3.1- or H3.3-SNAP (red) in cells \pm Dox along with H3.1/H3.2 or H3.3 staining (magenta) and DNA (DAPI, cyan). Scale bars 5 μ m. (C) Top panel: representative epifluorescence images of transgenic KH2 mESCs with H3.1- or H3.3-SNAP-Tag-3xHA constructs (red), along with DNA (DAPI, cyan) controlled by a TetON system inducing histone expression and further chromatin incorporation upon continuous exposure with doxycycline (Dox). Bottom panel: Quantification of H3-SNAP-Tagged signal intensity detected in DAPI-segmented nuclei at different time points upon dox addition. H3 expression detection starts after 4h of dox addition, whereas a specific total H3 histone pattern is achieved after a minimum of 18h. At least 100 nuclei were counted per condition and a single representative experiment is shown. (D) Comparison of endogenous vs exogenous expression of H3 histones in ESCs, NPCs, and NIH-3T3 cells by Western blot. Total extracts from 1×10^6 cells (= x) are loaded per lane. The presence of H3 variants is verified by H3.1/H3.2 and H3.3 antibodies on two separate membranes at different time exposures. Tagged and endogenous histones are reported at their corresponding heights. H4 histone is used as a loading control. * = non-specific bands.

Supplementary Figure 2



Supplementary Fig 2. Analysis of H3 variant ChIP-seq datasets over ES cells differentiation. To compare the H3 variant ChIP-seq data generated in this study with previous results, data from Deaton et al. (2016) was downloaded and pre-processed in the same way as the data generated in-house. Pearson correlation coefficients were computed for each pair of samples from genome-wide \log_2 IP/input signal binned at 10kb. Samples were then hierarchically clustered and organized in a heatmap based on the correlation between them. H3.1- and H3.3- ChIP-seq clustered samples are labeled in purple and green, respectively.

Discussion

In this study, we characterized how to define a specific enrichment at chromocenters for the replicative H3.1/H3.2 and the replacement H3.3 histone variants, by exploiting complementary approaches based on imaging and sequencing methods. First, we established that an H3.1/H3.2 enrichment, concomitant with H3.3 depletion, is a hallmark of chromocenters irrespective of the cell fate potential. The enrichment for replicative histone variants depends on the DSC deposition pathway mediated by the CAF-1 histone chaperone in the S phase. This replicative histone enrichment is robustly maintained throughout the cell cycle in differentiated cells compared to pluripotent ES cells. In the latter cells, this enrichment is challenged during the G1 phase where the loss of H3.1 enrichment occurs at the expense of a gain in H3.3 deposition. We could mimic experimentally such a challenge by forcing DSI deposition of H3.3 using an artificial HIRA targeting chromocenters. Taken together, our results establish a framework where replicative histone enrichment at chromocenters relies on balancing H3 histone deposition pathways in a manner that depends on the cell type and their cell cycle properties. Thus, the histone chaperones involved in distinct deposition pathways represent key regulatory means for shaping nucleosomal composition at nuclear domains in a manner that depends on the cell cycle. We discuss the implications of our findings for the specific cell cycles of pluripotent and differentiated cells.

H3.1 enrichment at chromocenters is a robust evolutionary conserved feature

Replicative and replacement variants show distinct genomic distributions, with H3.3 enriched at active sites, namely enhancers and promoters, but also proximally to telomeres and pericentric heterochromatin (Goldberg *et al.*, 2010). Conversely, H3.1 is mainly present in heterochromatin regions, visibly detected when discernable compartments form in the nuclear space. For example in plants, heterochromatin, along with H3.1, is mainly enriched in chromocenters, including the centromeric and pericentromeric chromatin regions (Stroud *et al.*, 2012; Wollmann *et al.*, 2012; Vaquero-Sedas and Vega-Palas, 2013). How the choice of histone variants in nuclear domains is conserved across various species and can dynamically change according to cell fate potential and during differentiation, remained to be established.

By first examining the pattern distribution of H3.1 and H3.3 at chromocenters in pluripotent (ES cells) to oligopotent (NPCs) and finally to terminally differentiated cells (MEF, NIH-3T3 cells), we detected a consistent H3.1 enrichment while H3.3 tended to be excluded (Fig. 1 and 2), in agreement with observations during Arabidopsis seedling development (Benoit *et al.*, 2019). Genome-wide analysis of this enrichment (Fig. 3) showed that H3.1 and H3.3 are both presents at minor satellite repeats, corresponding to the central regions of centromeric regions, as observed already in human and plants models (Nagaki *et al.*, 2003; Shibata and Murata, 2004; Dunleavy, Almouzni and Karpen, 2011). However, only H3.1, but not H3.3, is specifically enriched in elements associated with mouse chromocenters, including major satellites and L1 LINEs. This specific enrichment of H3.1 (and concomitant H3.3 depletion) at chromocenters was observed by detecting endogenous H3 variants and H3 variants expressed under a non-canonical promoter, independently of the cell background, the different transcriptional program, and the transcriptional regulation of the histone variant themselves. Furthermore, this feature is also conserved in Arabidopsis. These data thus underline a fundamental property whereby H3.1 enrichment represents a robust chromocenter and pericentric heterochromatin signature.

H3.1 enrichment at chromocenters is mediated by the DNA synthesis coupled deposition pathway and challenged with H3.3 deposition

Our analysis of the key amino acid residues in H3.1/H3.2, but not in H3.3, underlined the major importance of the SVM motif involved in the recognition by the CAF-1 histone chaperone to mediate H3 enrichment at chromocenters. In contrast, the S31 and S96 residues discriminating H3.1 from H3.3 did not prove critical. Thus, the mechanism contributing to replicative H3 enrichment is strictly dependent on the deposition pathway, but other features are dispensable, contrasting with the key role of S31ph involved in early *Xenopus* development at gastrulation (Sitbon *et al.*, 2020).

To maintain H3 enrichment at chromocenters, one has to consider not only the deposition but also the turnover rate at these sites. Analysis of H3.1 enrichment at chromocenters during cell cycle progression revealed that oscillations in H3.1 enrichment are mirrored by H3.3 presence indicating a compensatory mechanism and possibly systematic replacement. This thus suggests that H3.1 enrichment does not solely depend on

deposition, but also depends on the absence of H3.3 deposition at chromocenters, the two deposition mechanisms acting in a competing manner with H3.3 dominating over H3.1. Indeed, when H3.3 deposition is artificially targeted at chromocenters, we observed a decrease in H3.1 enrichment. Therefore, the loss of H3.1 enrichment in G1 in ES cells can be explained by HIRA-mediated H3.3 deposition at chromocenters. Transcription has been so far the main mechanism associated with H3.3 deposition. Thus, the fact that replicative H3 variants are maintained at chromocenters is consistent with a lower transcription in these regions compared to actively transcribed genomic regions. This is also in line with the observation that recycling of parental nucleosomes containing replicative histones correlates better with inactive over actively transcribed genomic regions before differentiation is engaged (Escobar *et al.*, 2019). This could take advantage of recycling mechanisms, including Mcm2-Pola1 and Pole3-Pole4, to participate in the transfer of both parental H3.1 and H3.3 on the same genomic regions (Xu *et al.*, 2022). Thus, a scheme emerges in which the replicative enrichment in a given region would require couple both the recycling of pre-existing variant along with new deposition of the same variant during replication.

H3.1 enrichment is challenged in G1 in ES cells

Analysis of the proportion of cells displaying H3.1 enrichment at chromocenters during the cell cycle revealed interesting fluctuations. We found that, for every cell line analyzed, except for ES cells, the peak of H3.1 enrichment consistently occurred during the G1, mid-S, late-S, and G2 phases whereas the early-S phase shows fewer cells with H3.1 enrichment. Since euchromatin regions, but not chromocenters, replicate during the early-S phase, the decrease of H3.1 enrichment at chromocenters likely results from the increased amounts of H3.1 deposited at euchromatin. Indeed, when chromocenters replicate in mid S and late S phase, and therefore the dosage of chromatin is restored between chromocenters and the rest of the nucleus, H3.1 enrichment is detected at identical levels for NPCs and NIH-3T3 cells.

Since ES cells proliferate and duplicate their genome faster than in differentiated cells, this could potentially provide more H3.1-CAF-1 mediated deposition at chromocenters. Surprisingly, in contrast, only ~50 % of G1 cells account for H3.1 enrichment at chromocenters (Fig.4). Furthermore, in asynchronous cells (Fig 2) and during mid-S, late-S, and G2 phases (Fig 4), the proportion of ES cells with H3.1 enrichment is consistently

lower than in the differentiated counterpart. In line with this, we detected a decrease in H3.3 exclusion at chromocenters in ES cells compared to differentiated cells. These findings thus suggest that, at least in the pluripotent context, the H3.1 maintenance at chromocenters is challenged by H3.3. The difference between ES and differentiated cells could result from the transcriptional shutdown of major satellite regions mediated both by DNA methylation and by histone PTMs. In pluripotent cells, the high expression of TET and KDM enzymes abundantly expressed promotes an “open” chromatin state (Loh *et al.*, 2007). This is in line with the fact that major satellite transcripts removal in ES cells triggers the formation of stable chromocenters (Novo *et al.*, 2022). An interesting candidate to promote this transcription could be Nanog since its overexpression is associated with low H3K9me3 and high major satellite transcription levels (Novo *et al.*, 2016). Thus, the constant replenishment of replicative histone variants is needed to ensure stable heterochromatin maintenance while preserving pluripotency ability (Meshorer *et al.*, 2006; Ishiuchi *et al.*, 2015; Nakatani *et al.*, 2022). Remarkably, structural chromatin proteins, including replicative histone variants and HP1, are dynamically incorporated in stem cells, but not H3.3, which is generally associated with active chromatin regions (Meshorer *et al.*, 2006; Boskovic *et al.*, 2014). Moreover, HIRA removal prevents specific H3.3 incorporation, thus, leading to heterochromatin foci formation and premature differentiation (Meshorer *et al.*, 2006).

The question that arises then is whether the more ‘labile’ H3.1 enrichment at chromocenters in ES reflects a general higher H3.1/H3.3 turn-over at chromocenters or if it could have a more direct role specifically at chromocenters. Indeed, chromocenters are less well-defined in size and shape in ES cells than in differentiated cells. Interestingly, when H3.1 deposition is impaired by knock-down of the p150 subunit of CAF-1, chromocenters are no longer maintained in ES cells, yet they are maintained in differentiated MEF cells (Houlard *et al.*, 2006). These differences underline distinct properties in histone chaperone function and possibly a more direct link with heterochromatin. Given the direct interaction of CAF-1 with HP1 proteins, it will be interesting to explore this further. Together, we have shown that replicative H3.1/H3.2 and the replacement H3.3 histone variants can be equivalently present at PHC and their accumulation is a matter of dosage balance, controlled by CAF-1 mediated replicative histone assembly and replacement by H3.3. We showed that while the setting of this balance is in normal favor of the replicative histones, it can be challenged and vary according to the cell potential.

Materials and Methods

Cell culture

We cultured all cells at 37°C in 5% CO₂: KH2 mESCs (Beard *et al.*, 2006) (a generous gift from E. Meshorer) on gelatinized feeder-free tissue culture plates in ESC media (Dulbecco's modified Eagle's medium supplemented with GlutaMax, Pyruvate and 4,5 g/L D-Glucose (Thermo Fisher Scientific), 15% calf foetal serum (Eurobio), 1000 U/ml penicillin/streptomycin, 1X MEM non-essential amino acids, 125 µM beta-mercaptoethanol supplemented with 1000 U/ml LIF (Millipore) and 2i inhibitors, which include 1 µM MEK1/2 inhibitor (PD0325901) and 3 µM GSK3 inhibitor (CHIR99021). To generate NPCs 4 days of differentiation (NPCs 4D), we cultured mESCs cells in NPC media (ESC media (without LIF and 2i) supplemented with 1 µM Retinoic Acid (RA), 1x N-2 Supplement (Gibco) and 1x B27 Supplement (Gibco). To generate NPCs 7D, we cultured NPCs 4D cells in the same NPC media supplemented with 10 ng/ml of FGF (Peprotech) and 20 ng/ml of EGF for three additional days. We cultured NIH-3T3 cells (ATCC #CRL-1658) and iMEF (provided by T. Jenuwein) in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% foetal calf serum (Eurobio), 1000 U/ml penicillin/streptomycin (Invitrogen).

Plasmid construction and generation of cell lines

We fused SNAP-3xHA coding sequences downstream H3.1 and H3.3 CDS (Ray-Gallet *et al.*, 2011) and inserted this fusion protein into the pBS31 vector (Beard *et al.*, 2006) using NEBuilder® HiFi DNA Assembly Master Mix (NEB). These expression vectors (pB31-H3.1- and pB31-H3.3- SNAP-3xHA) enabled the production of H3.1- and H3.3- proteins with the tag SNAP-3xHA at their C-terminus. To generate constructs with point mutations H3.2-, H3.1-A31S- and H3.3-S31A-, H3.3-S31D- SNAP-3xHA plasmids we used site-directed mutagenesis (GenScript) with pB31-H3.1- and pB31-H3.3- SNAP-3xHA vectors, respectively. We stably integrated the H3-SNAP-3xHA coding sequence in KH2 mES cells downstream of the Type I Collagen (Col1A1) locus containing an Frt site under the control of a TET-ON regulatory region, we co-transfected pB31-H3-SNAP-3xHA plasmids with the pCAGGS-FlpE Vector (Addgene #20733) using Nucleofector Kit 2 (Amaza) according to manufacturer's instructions. To obtain clones that stably integrated the H3-SNAP-3xHA

tag, we selected colonies on hygromycin for 14 days and isolated single clones screened by genotyping and sequencing. We induced histone expression by adding 1 µg/ml doxycycline at least 48h before analysis.

We generated NIH-3T3 cells stably expressing either H3.1- or H3.3- SNAP-3xHA as in (Ray-Gallet *et al.*, 2011) with selection using 10 µg/mL Blasticidin after retroviral transfection.

To generate TALE-HIRA-WT-YFP and TALE-HIRA-W799A-D800A-YFP, we replaced the mClover of the pTALYM3B15 plasmid (obtained from Addgene #47878) by HIRA-WT-YFP and HIRA-W799A-D800A-YFP (from Ray-Gallet 2018) using NEBuilder® HiFi DNA Assembly Master Mix (NEB) and verified all constructs by Sanger sequencing. We verified protein expression by Western blot and immunofluorescence analysis.

Total cell extract preparation and western blotting.

Total protein extracts were made by boiling cell pellet in SDS PAGE loading buffer (Invitrogen) complemented with NuPage reducing agent (Invitrogen) and Universal Nuclease (Pierce) and We performed Western blotting as described in (Sitbon *et al.*, 2020). We described our Primary antibodies in the Antibody list table (below). We acquired immunoblot images with ChemiDoc Imager (Biorad).

Immunofluorescence staining and epifluorescence microscopy acquisition.

Cells seeded and grown on fibronectin-coated glass coverslips were transferred into a four-well plate (ThermoFisher scientific) for labeling. We performed *in vivo* labeling of the SNAP-tag with 2 µM SNAP-Cell TMR-Star (New England Biolabs) and visualized the newly synthesized histones incorporated into chromatin by performing a pre-extraction of soluble histones before fixation with 2% paraformaldehyde for 20' as described in (Clement *et al.*, 2018). We revealed DNA synthesis by EdU incorporation and Click reaction (Click-iT EdU imaging kit, Invitrogen) as described in (Clément *et al.*, 2018). Immunofluorescence staining was performed after the Click reaction as described in (Clément *et al.*, 2018) with the primary antibodies described in the Antibody list table (below). For STORM acquisition, we used primary antibodies coupled with Alexa Fluor 647 secondary antibody. For wide-field epifluorescence imaging, we used an AxioImager Zeiss Z1 microscope with a 63x or 100x objective piloted with MetaMorph software (Molecular Devices). We acquired 5 µm stacks with the 100x objective for 3D images.

Antibody List and conditions

Target	Source of antibody	Specificity	Dilution
H3.1/3.2 mAb (Clone 1D4F2)	Active Motif, #61629	Monoclonal Mouse IgG2b	1:2000
H3.3	Active Motif, #91191	Monoclonal Mouse IgG2a	1:150
HA	Roche, #1867423	Monoclonal Rat	1:2000
Aurora B	BD Transduction Laboratories #611082	Monoclonal Mouse IgG1	1:1000
HP1alpha	Euromedex, 2HP-1H5-AS	Monoclonal Mouse	1:1000
HP1alpha	Sigma, #H2164	Rabbit Polyclonal	1:1000
Oct3/4	BD Biosciences 611203	Monoclonal Mouse IgG1	1:1000
H4	Abcam ab31830	Monoclonal Mouse IgG1	1:1000

Microscopy images visualization and analysis.

We used Fiji software for Microscopy image visualization. We quantified fluorescence intensity in the nucleus by normalizing the signal to the area of the nucleus segmented by DAPI using an automated custom Fiji macro. To quantify H3.1 or H3.3 enrichment at chromocenters, we used 3D-FIED Fiji macro (Cantaloube *et al.*, 2012). For quantification of cells displaying various enrichment of H3 variants at chromocenters, we defined cells as (i) ‘enriched’ when they displayed at least three chromocenters for which H3 signal intensity is higher than that of the nucleus; (ii) ‘even distribution’ when the H3 signal at the chromocenters was that of the nucleus; (iii) ‘excluded’ when the H3 signal at the chromocenters was lower than that of the nucleus. For quantification of cells within the cell cycle, we defined as S phase cells those positive for EdU detection, G2 those negative for EdU and positive for AuroraB detection, and as G1 those negative for EdU and AuroraB detection. Quantification of cells and fluorescence intensity was performed at least in 100 nuclei for at least three independent experiments unless otherwise indicated. All plots and data visualization are generated with Graphpad and Python.

H3.1- and H3.3- SNAP Capture-Seq

We performed H3 ChIP-Seq in ES and NPC cells by using the SNAP-capture procedure as described in (Gatto *et al.*, 2022). We induced synthesis of H3-SNAP-Tag in ESCs and NPCs by adding 1 µg/ml doxycycline before

cell collection. We prepared sequencing libraries at the Next Generation Sequencing (NGS) platform at Institut Curie (Illumina TruSeq ChIP kit) and performed PE100 sequencing on Illumina NovaSeq 6000. Publicly available H3.1 and H3.3 ChIP-seq data (Deaton *et al.*, 2016) obtained as raw reads in fastq format from ENA (<https://www.ebi.ac.uk/ena/browser/home>) were processed in the same way as the data generated here.

Sequencing reads were aligned to the mouse reference genome (GRCm38, soft-masked) as described in (Gatto *et al.*, 2022). Genome-wide Pearson correlation coefficients were computed for each pairwise combination of samples from 10kb-binned \log_2 IP/input normalized data and then plotted as a hierarchically-clustered heatmap.

We used repetitive element annotation from Ensembl (mus musculus core 102_38) and filtered out tandem ('trf') and low-complexity ('dust') repeat classes. For each sample, the number of reads at each repeat was calculated by overlapping the repeat annotation with the genomic coordinates of fragments mapped in pairs, excluding duplicates, extracted from bam files. Samples were normalized to the total number of reads mapped to all repeats (CPM), then by dividing to repeat length, and finally by dividing by the matching input sample. Then, IP to input ratio was \log_2 -transformed, and to allow comparison between conditions, the cross-sample normalized by computing z-scores.

Author contributions

G.A., J.P.Q. and S.A. conceived the overall strategy and wrote the paper. S.A. performed most of the experiments, generated the figures and analyzed data. G.A. and J.P.Q. supervised the work. A.F. and H.M. performed part of cell biology and chromatin immunoprecipitation experiments. T.K. analyzed the sequencing analysis and wrote corresponding parts in the papers. E.M. conceived cell biology experiments. Critical reading and discussion of data involved all authors.

Acknowledgments

We thank Ekaterina Boyarchuk for help at the initiation of the project. We thank Binyamin Kaffe for help in generating PBS31_H3.1-SNAP-3xHA plasmid. We also thank Dominique Ray-Gallet and Daniel Jeffery for critical reading, and members of UMR3664 and Almouzni team for helpful discussions. We acknowledge the Cell and Tissue Imaging Platform PICT-IBiSA (member of France-Bioimaging ANR-10-INBS-04) of the UMR3664 and ICGex NGS platform of the Institut Curie. This work was supported by the funding from La Ligue Nationale contre le Cancer (labellisation), Labex DEEP-PSL (ANR-11LABX-0044_DEEP, ANR-10-IDEX-0001-02), ERC-2015-ADG694694 ChromADICT. S.A. was supported by individual funding from H2020 MSCA-ITN – EpiSyStem (Grant No. 765966) and Fondation Recherche Medicale (FRM) (Grant No. FDT202106012804). Tina Karagyzova was supported by individual funding from H2020 MSCA-ITN – ChromDesign (Grant No. 813327) grant.

References

- Ahmad, K. and Henikoff, S. (2002) 'Histone H3 variants specify modes of chromatin assembly', *Proceedings of the National Academy of Sciences*, 99(suppl_4), pp. 16477–16484. Available at: <https://doi.org/10.1073/pnas.172403699>.
- Almouzni, G. and Probst, A.V. (2011) 'Heterochromatin maintenance and establishment: Lessons from the mouse pericentromere', *Nucleus*, 2(5), pp. 332–338. Available at: <https://doi.org/10.4161/nuc1.2.5.17707>.
- Armache, A. *et al.* (2020) 'Histone H3.3 phosphorylation amplifies stimulation-induced transcription', *Nature*, 583(7818), pp. 852–857. Available at: <https://doi.org/10.1038/s41586-020-2533-0>.
- Banaszynski, L.A. *et al.* (2013) 'Hira-Dependent Histone H3.3 Deposition Facilitates PRC2 Recruitment at Developmental Loci in ES Cells', *Cell*, 155(1), pp. 107–120. Available at: <https://doi.org/10.1016/j.cell.2013.08.061>.
- Beard, C. *et al.* (2006) 'Efficient method to generate single-copy transgenic mice by site-specific integration in embryonic stem cells', *genesis*, 44(1), pp. 23–28. Available at: <https://doi.org/10.1002/gene.20180>.
- Benoit, M. *et al.* (2019) 'Replication-coupled histone H3.1 deposition determines nucleosome composition and heterochromatin dynamics during Arabidopsis seedling development', *New Phytologist*, 221(1), pp. 385–398. Available at: <https://doi.org/10.1111/nph.15248>.
- Boskovic, A. *et al.* (2014) 'Higher chromatin mobility supports totipotency and precedes pluripotency in vivo', *Genes & Development*, 28(10), pp. 1042–1047. Available at: <https://doi.org/10.1101/gad.238881.114>.
- Burton, A. *et al.* (2020) 'Heterochromatin establishment during early mammalian development is regulated by pericentromeric RNA and characterized by non-repressive H3K9me3', *Nature Cell Biology*, 22(7), pp. 767–778. Available at: <https://doi.org/10.1038/s41556-020-0536-6>.
- Cantaloube, S. *et al.* (2012) 'Characterization of chromatin domains by 3D fluorescence microscopy: An automated methodology for quantitative analysis and nuclei screening', *BioEssays*, 34(6), pp. 509–517. Available at: <https://doi.org/10.1002/bies.201100188>.
- Casanova, M. *et al.* (2013) 'Heterochromatin Reorganization during Early Mouse Development Requires a Single-Stranded Noncoding Transcript', *Cell Reports*, 4(6), pp. 1156–1167. Available at: <https://doi.org/10.1016/j.celrep.2013.08.015>.

- Cheloufi, S. *et al.* (2015) ‘The histone chaperone CAF-1 safeguards somatic cell identity’, *Nature*, 528(7581), pp. 218–224. Available at: <https://doi.org/10.1038/nature15749>.
- Cheloufi, S. and Hochedlinger, K. (2017) ‘Emerging roles of the histone chaperone CAF-1 in cellular plasticity’, *Current opinion in genetics & development*, 46, pp. 83–94. Available at: <https://doi.org/10.1016/j.gde.2017.06.004>.
- Clément, C. *et al.* (2018) ‘High-resolution visualization of H3 variants during replication reveals their controlled recycling’, *Nature Communications*, 9(1), p. 3181. Available at: <https://doi.org/10.1038/s41467-018-05697-1>.
- Deaton, A.M. *et al.* (2016) ‘Enhancer regions show high histone H3.3 turnover that changes during differentiation’, *eLife*, 5, p. e15316. Available at: <https://doi.org/10.7554/eLife.15316>.
- Dimitrova, D.S. and Berezney, R. (2002) ‘The spatio-temporal organization of DNA replication sites is identical in primary, immortalized and transformed mammalian cells’, *Journal of Cell Science*, 115(Pt 21), pp. 4037–4051. Available at: <https://doi.org/10.1242/jcs.00087>.
- Dunleavy, E.M., Almouzni, G. and Karpen, G.H. (2011) ‘H3.3 is deposited at centromeres in S phase as a placeholder for newly assembled CENP-A in G1 phase’, *Nucleus*, 2(2), pp. 146–157. Available at: <https://doi.org/10.4161/nucl.2.2.15211>.
- Elsässer, S.J. *et al.* (2012) ‘DAXX envelops a histone H3.3–H4 dimer for H3.3-specific recognition’, *Nature*, 491(7425), pp. 560–565. Available at: <https://doi.org/10.1038/nature11608>.
- Elsässer, S.J. *et al.* (2015) ‘Histone H3.3 is required for endogenous retroviral element silencing in embryonic stem cells’, *Nature*, 522(7555), pp. 240–244. Available at: <https://doi.org/10.1038/nature14345>.
- Escobar, T.M. *et al.* (2019) ‘Active and Repressed Chromatin Domains Exhibit Distinct Nucleosome Segregation during DNA Replication’, *Cell*, 179(4), pp. 953–963.e11. Available at: <https://doi.org/10.1016/j.cell.2019.10.009>.
- Fang, H.-T. *et al.* (2018) ‘Global H3.3 dynamic deposition defines its bimodal role in cell fate transition’, *Nature Communications*, 9(1), p. 1537. Available at: <https://doi.org/10.1038/s41467-018-03904-7>.
- Franklin, R. *et al.* (2022) ‘Regulation of chromatin accessibility by the histone chaperone CAF-1 sustains lineage fidelity’, *Nature Communications*, 13(1), p. 2350. Available at: <https://doi.org/10.1038/s41467-022-29730-6>.

- Franklin, R., Murn, J. and Cheloufi, S. (2021) ‘Cell Fate Decisions in the Wake of Histone H3 Deposition’, *Frontiers in Cell and Developmental Biology*, 9. Available at: <https://www.frontiersin.org/article/10.3389/fcell.2021.654915> (Accessed: 20 June 2022).
- García Fernández, F. and Fabre, E. (2022) ‘The Dynamic Behavior of Chromatin in Response to DNA Double-Strand Breaks’, *Genes*, 13(2), p. 215. Available at: <https://doi.org/10.3390/genes13020215>.
- Gatto, A. *et al.* (2022) ‘HIRA-dependent boundaries between H3 variants shape early replication in mammals’, *Molecular Cell* [Preprint]. Available at: <https://doi.org/10.1016/j.molcel.2022.03.017>.
- Goldberg, A.D. *et al.* (2010) ‘Distinct Factors Control Histone Variant H3.3 Localization at Specific Genomic Regions’, *Cell*, 140(5), pp. 678–691. Available at: <https://doi.org/10.1016/j.cell.2010.01.003>.
- Guenatri, M. *et al.* (2004) ‘Mouse centric and pericentric satellite repeats form distinct functional heterochromatin’, *Journal of Cell Biology*, 166(4), pp. 493–505. Available at: <https://doi.org/10.1083/jcb.200403109>.
- Gurard-Levin, Z.A., Quivy, J.-P. and Almouzni, G. (2014) ‘Histone Chaperones: Assisting Histone Traffic and Nucleosome Dynamics’, *Annual Review of Biochemistry*, 83(1), pp. 487–517. Available at: <https://doi.org/10.1146/annurev-biochem-060713-035536>.
- Hammond, C.M. *et al.* (2017) ‘Histone chaperone networks shaping chromatin function’, *Nature Reviews Molecular Cell Biology*, 18(3), pp. 141–158. Available at: <https://doi.org/10.1038/nrm.2016.159>.
- He, Q. *et al.* (2015) ‘The Daxx/Atrx Complex Protects Tandem Repetitive Elements during DNA Hypomethylation by Promoting H3K9 Trimethylation’, *Cell Stem Cell*, 17(3), pp. 273–286. Available at: <https://doi.org/10.1016/j.stem.2015.07.022>.
- Hoelper, D. *et al.* (2017) ‘Structural and mechanistic insights into ATRX-dependent and -independent functions of the histone chaperone DAXX’, *Nature Communications*, 8(1), p. 1193. Available at: <https://doi.org/10.1038/s41467-017-01206-y>.
- Houlard, M. *et al.* (2006) ‘CAF-1 Is Essential for Heterochromatin Organization in Pluripotent Embryonic Cells’, *PLoS Genetics*, 2(11), p. 11.
- Ishiuchi, T. *et al.* (2015) ‘Early embryonic-like cells are induced by downregulating replication-dependent chromatin assembly’, *Nature Structural & Molecular Biology*, 22(9), pp. 662–671. Available at: <https://doi.org/10.1038/nsmb.3066>.

- Ishiuchi, T. *et al.* (2021) 'Reprogramming of the histone H3.3 landscape in the early mouse embryo', *Nature Structural & Molecular Biology*, 28(1), pp. 38–49. Available at: <https://doi.org/10.1038/s41594-020-00521-1>.
- Latreille, D. *et al.* (2014) 'Identification of histone 3 variant 2 interacting factors', *Nucleic Acids Research*, 42(6), pp. 3542–3550. Available at: <https://doi.org/10.1093/nar/gkt1355>.
- Lewis, P.W. *et al.* (2010) 'Daxx is an H3.3-specific histone chaperone and cooperates with ATRX in replication-independent chromatin assembly at telomeres', *Proceedings of the National Academy of Sciences*, 107(32), pp. 14075–14080. Available at: <https://doi.org/10.1073/pnas.1008850107>.
- Loh, Y.-H. *et al.* (2007) 'Jmjd1a and Jmjd2c histone H3 Lys 9 demethylases regulate self-renewal in embryonic stem cells', *Genes & Development*, 21(20), pp. 2545–2557. Available at: <https://doi.org/10.1101/gad.1588207>.
- Loyola, A. *et al.* (2006) 'PTMs on H3 Variants before Chromatin Assembly Potentiate Their Final Epigenetic State', *Molecular Cell*, 24(2), pp. 309–316. Available at: <https://doi.org/10.1016/j.molcel.2006.08.019>.
- Luger, K. *et al.* (1997) 'Crystal structure of the nucleosome core particle at 2.8 Å resolution', *Nature*, 389(6648), pp. 251–260. Available at: <https://doi.org/10.1038/38444>.
- Maison, C. *et al.* (2010) 'Heterochromatin at Mouse Pericentromeres A Model for De Novo Heterochromatin Formation and Duplication during Replication', *Cold Spring Harbor Symposia on Quantitative Biology*, 75, pp. 155–165. Available at: <https://doi.org/10.1101/sqb.2010.75.013>.
- Maison, C. *et al.* (2016) 'The methyltransferase Suv39h1 links the SUMO pathway to HP1α marking at pericentric heterochromatin', *Nature Communications*, 7(1), p. 12224. Available at: <https://doi.org/10.1038/ncomms12224>.
- Martire, S. *et al.* (2019) 'Phosphorylation of histone H3.3 at serine 31 promotes p300 activity and enhancer acetylation', *Nature Genetics*, 51(6), pp. 941–946. Available at: <https://doi.org/10.1038/s41588-019-0428-5>.
- Mendiratta, S., Gatto, A. and Almouzni, G. (2019) 'Histone supply: Multitiered regulation ensures chromatin dynamics throughout the cell cycle', *The Journal of Cell Biology*, 218(1), pp. 39–54. Available at: <https://doi.org/10.1083/jcb.201807179>.
- Meshorer, E. *et al.* (2006) 'Hyperdynamic Plasticity of Chromatin Proteins in Pluripotent Embryonic Stem Cells', *Developmental Cell*, 10(1), pp. 105–116. Available at: <https://doi.org/10.1016/j.devcel.2005.10.017>.

- Miyanari, Y., Ziegler-Birling, C. and Torres-Padilla, M.-E. (2013) ‘Live visualization of chromatin dynamics with fluorescent TALEs’, *Nature Structural & Molecular Biology*, 20(11), pp. 1321–1324. Available at: <https://doi.org/10.1038/nsmb.2680>.
- Nagaki, K. *et al.* (2003) ‘Chromatin immunoprecipitation reveals that the 180-bp satellite repeat is the key functional DNA element of Arabidopsis thaliana centromeres’, *Genetics*, 163(3), pp. 1221–1225. Available at: <https://doi.org/10.1093/genetics/163.3.1221>.
- Nakatani, T. *et al.* (2022) ‘DNA replication fork speed underlies cell fate changes and promotes reprogramming’, *Nature Genetics* [Preprint]. Available at: <https://doi.org/10.1038/s41588-022-01023-0>.
- Novo, C.L. *et al.* (2016) ‘The pluripotency factor Nanog regulates pericentromeric heterochromatin organization in mouse embryonic stem cells’, *Genes & Development*, 30(9), pp. 1101–1115. Available at: <https://doi.org/10.1101/gad.275685.115>.
- Novo, C.L. *et al.* (2022) ‘Satellite repeat transcripts modulate heterochromatin condensates and safeguard chromosome stability in mouse embryonic stem cells’, *Nature Communications*, 13(1), pp. 1–16. Available at: <https://doi.org/10.1038/s41467-022-31198-3>.
- Peters, A.H.F.M. *et al.* (2001) ‘Loss of the Suv39h Histone Methyltransferases Impairs Mammalian Heterochromatin and Genome Stability’, *Cell*, 107(3), pp. 323–337. Available at: [https://doi.org/10.1016/S0092-8674\(01\)00542-6](https://doi.org/10.1016/S0092-8674(01)00542-6).
- Probst *et al.* (2010) ‘A strand-specific burst in transcription of pericentric satellites is required for chromocenter formation and early mouse development.’, *Developmental Cell*, 19(4), pp. 625–638. Available at: <https://doi.org/10.1016/j.devcel.2010.09.002>.
- Probst, A.V. *et al.* (2007) ‘Structural differences in centromeric heterochromatin are spatially reconciled on fertilisation in the mouse zygote’, *Chromosoma*, 116(4), pp. 403–415. Available at: <https://doi.org/10.1007/s00412-007-0106-8>.
- Quivy, J.-P. *et al.* (2004) ‘A CAF-1 dependent pool of HP1 during heterochromatin duplication’, *The EMBO Journal*, 23(17), pp. 3516–3526. Available at: <https://doi.org/10.1038/sj.emboj.7600362>.
- Quivy, J.-P. *et al.* (2008) ‘The HP1–p150/CAF-1 interaction is required for pericentric heterochromatin replication and S-phase progression in mouse cells’, *Nature Structural & Molecular Biology*, 15(9), pp. 972–979. Available at: <https://doi.org/10.1038/nsmb.1470>.
- Rausch, C. *et al.* (2020) ‘Developmental differences in genome replication program and origin activation’, *Nucleic Acids Research*, 48(22), pp. 12751–12777. Available at: <https://doi.org/10.1093/nar/gkaa1124>.

- Ray-Gallet, D. *et al.* (2011) ‘Dynamics of Histone H3 Deposition In Vivo Reveal a Nucleosome Gap-Filling Mechanism for H3.3 to Maintain Chromatin Integrity’, *Molecular Cell*, 44(6), pp. 928–941. Available at: <https://doi.org/10.1016/j.molcel.2011.12.006>.
- Ray-Gallet, D. *et al.* (2018) ‘Functional activity of the H3.3 histone chaperone complex HIRA requires trimerization of the HIRA subunit’, *Nature Communications*, 9(1), p. 3103. Available at: <https://doi.org/10.1038/s41467-018-05581-y>.
- Santenard, A. *et al.* (2010) ‘Heterochromatin formation in the mouse embryo requires critical residues of the histone variant H3.3’, *Nature Cell Biology*, 12(9), pp. 853–862. Available at: <https://doi.org/10.1038/ncb2089>.
- Sexton, T. and Cavalli, G. (2015) ‘The Role of Chromosome Domains in Shaping the Functional Genome’, *Cell*, 160(6), pp. 1049–1059. Available at: <https://doi.org/10.1016/j.cell.2015.02.040>.
- Shibata, F. and Murata, M. (2004) ‘Differential localization of the centromere-specific proteins in the major centromeric satellite of *Arabidopsis thaliana*’, *Journal of Cell Science*, 117(Pt 14), pp. 2963–2970. Available at: <https://doi.org/10.1242/jcs.01144>.
- Sitbon, D. *et al.* (2020) ‘Histone variant H3.3 residue S31 is essential for *Xenopus* gastrulation regardless of the deposition pathway’, *Nature Communications*, 11(1), pp. 1–15. Available at: <https://doi.org/10.1038/s41467-020-15084-4>.
- Solovei, I. *et al.* (2009) ‘Nuclear architecture of rod photoreceptor cells adapts to vision in mammalian evolution’, *Cell*, 137(2), pp. 356–368. Available at: <https://doi.org/10.1016/j.cell.2009.01.052>.
- Stroud, H. *et al.* (2012) ‘Genome-wide analysis of histone H3.1 and H3.3 variants in *Arabidopsis thaliana*’, *Proceedings of the National Academy of Sciences*, 109(14), pp. 5370–5375. Available at: <https://doi.org/10.1073/pnas.1203145109>.
- Tagami, H. *et al.* (2004) ‘Histone H3.1 and H3.3 Complexes Mediate Nucleosome Assembly Pathways Dependent or Independent of DNA Synthesis’, *Cell*, 116(1), pp. 51–61. Available at: [https://doi.org/10.1016/S0092-8674\(03\)01064-X](https://doi.org/10.1016/S0092-8674(03)01064-X).
- Terranova, R. *et al.* (2005) ‘The reorganisation of constitutive heterochromatin in differentiating muscle requires HDAC activity’, *Experimental Cell Research*, 310(2), pp. 344–356. Available at: <https://doi.org/10.1016/j.yexcr.2005.07.031>.
- Vaquero-Sedas, M.I. and Vega-Palas, M.A. (2013) ‘Differential association of *Arabidopsis* telomeres and centromeres with Histone H3 variants’, *Scientific Reports*, 3(1), p. 1202. Available at: <https://doi.org/10.1038/srep01202>.

Wang, C. *et al.* (2018) ‘Reprogramming of H3K9me3-dependent heterochromatin during mammalian embryo development’, *Nature Cell Biology*, 20(5), pp. 620–631. Available at: <https://doi.org/10.1038/s41556-018-0093-4>.

Wollmann, H. *et al.* (2012) ‘Dynamic Deposition of Histone Variant H3.3 Accompanies Developmental Remodeling of the Arabidopsis Transcriptome’, *PLOS Genetics*, 8(5), p. e1002658. Available at: <https://doi.org/10.1371/journal.pgen.1002658>.

Xu, X. *et al.* (2022) ‘Stable inheritance of H3.3-containing nucleosomes during mitotic cell divisions’, *Nature Communications*, 13(1), p. 2514. Available at: <https://doi.org/10.1038/s41467-022-30298-4>.

Yadav, T., Quivy, J.-P. and Almouzni, G. (2018) ‘Chromatin plasticity: A versatile landscape that underlies cell fate and identity’, *Science*, 361(6409), pp. 1332–1336. Available at: <https://doi.org/10.1126/science.aat8950>.

The Show Must Go On: Additional Results

With the results included in the “[Manuscript](#)” section above, we answered a series of questions concerning the histone variant enrichment in mouse chromocenters. We have described how the DNA replication deposition mode is the main pathway mediating the accumulation of H3.1 and how H3.3 is consequently lost over time in these regions. We also observed the effect of histone chaperones competition over the same genomic regions, showing H3.3-HIRA forced deposition to counteract H3.1-CAF-1 mediated accumulation. This differential enrichment mediated by histone chaperones is key to understanding the dynamics occurring in these regions to preserve chromatin integrity and nuclear architecture.

In addition, I generated data in three areas that stemmed from “Manuscript” that are shown and discussed in the following sections.

1) H3.1 and H3.3 enrichment in ES and NPC at repetitive sequences

The fact that heterochromatin regions are highly adaptable to a choice of histone variants without disrupting genome organization suggests that PHC regions are highly dynamic. Moreover, the presence of additional repetitive elements characterized to be associated with biochemically purified chromocenters (Ostromyshenskii *et al.*, 2018) led us to reconsider on what is the relative abundance of H3 variants in these regions. For this, we further analyzed our H3.1- and H3.3- SNAP-ChIP-seq data produced to examine their differential enrichment throughout differentiation.

2) Single-molecule visualization of H3 variants reveals their mutual occupancy at PHCs

Next, the fact that those H3 histones are constantly present and in competition in the same regions over the cell cycle suggests us that they could be repositioned in space as DNA replication progresses. In order to dissect this, I needed a sufficient resolution to observe precisely the histone distribution by microscopy at chromocenters. In diffraction-limited epifluorescent microscopy experiments, two molecules that are close in space will emit broad fluorescence signals (~300 nm) overlapping each other and creating images that cannot be resolved. In collaboration with David Mazaud, research engineer specialist in super-resolution microscopy at Institut Curie, using Stochastic Optical Reconstruction Microscopy (STORM), we managed to achieve a

resolution of H3 variants at a resolution up to 10~20 nm on an XY focal plane. At a given time, a molecule will stochastically emit a fluorescent signal that is detected and the localization of the molecule is then recorded. By iterating this process for a sufficient amount of time, we can accumulate enough sparse localizations to reconstitute an entire image with a 10x fold increase in resolution compared to current diffraction-limited images.

3) Suv39h2, but not Suv39h1, supports H3.1 enrichment at chromocenters

Finally, we asked if the relative accumulation of H3.1 is a consequence of a lack of eviction in absence of functional transcription of major satellite repeats. To address this, we took advantage of mouse cell models of both ES and MEF cells lacking both isoforms of Suv39h KMTs (Peters *et al.*, 2001; Lehnertz *et al.*, 2003). In this way, we can follow the relative enrichment of both H3.1 and H3.3 in absence of active repression at PHC. These results add valuable information for discussion and bring insights for a better understanding of histone dynamics coupled to chromocenters organization.

H3.1 and H3.3 enrichment in ES and NPC at repetitive sequences

Our ChIP-seq results compare well with previously published data obtained using a different H3-SNAP-Tag expression vector in ES cells (Deaton et al. 2016) (See Manuscript, Fig Supp 2). Thus, we proceeded to use our datasets to study the distribution of H3.1 and H3.3 variants during differentiation from ES to NPC at repetitive elements. We were particularly interested in the dynamics of H3.1, for which little is currently known. After a comprehensive survey of the literature, we compiled a list of 50 repeat elements that have been associated with chromocenters (Bulut-Karslioglu *et al.*, 2014; Ostromyshenskii *et al.*, 2018) or have been studied in the context of repression by H3.3 incorporation (Elsässer *et al.*, 2015) as control. We quantified the enrichment of the two variants at these repeats and performed hierarchical clustering to evaluate the similarity of H3 variant dynamics throughout the different differentiation steps (Fig 21).

We found that H3.1 is enriched at both major and minor satellite repeats. H3.1 enrichment at major satellites was accompanied by H3.3 depletion, and it increased from ES to NPC D7, confirming the observations obtained by imaging. We also observed an increasing enrichment of H3.1 at minor satellites over differentiation, along with a mild enrichment of H3.3, consistent with their role in acting as placeholders for other histone variants, including CENP-A, at centromeric repeats (Dunleavy, Almouzni and Karpen, 2011). We also analyzed histone variant enrichment at additional repetitive elements being associated, but not exclusively present, in constitutive heterochromatin regions including chromocenters (Ostromyshenskii *et al.*, 2018). In particular, ~10% chromocenter DNA is estimated to be composed of LINE L1 elements (Ostromyshenskii *et al.*, 2018), likely decorated with constitutive heterochromatin marks, such as H3K9me3. Indeed, we found increasing H3.1 enrichment at L1 LINEs over differentiation, accompanied by depletion of H3.3.

In agreement with previous work (Elsässer *et al.*, 2015; He *et al.*, 2015), we confirmed the presence of H3.3 at short interspersed elements (SINEs), class I and class II endogenous retroviral elements (ERVs), namely early transposons (ETn) and intracisternal A-type particles (IAPs), in particular in ESCs. Notably, our data allowed us to concomitantly follow the dynamics of H3.1 at these elements. This revealed that with differentiation, H3.1 becomes enriched in the internal, but not LTR regions, of most IAP elements, and becomes depleted in SINEs, which are mainly enriched in euchromatin regions (Solovei *et al.*, 2009). Thus,

these results suggest that H3 histone variants distribution differs over the repetitive genome depending on the cell differentiation status. Together, the observed changes in H3.1 and H3.3 are concordant with their pathways of deposition and concomitant with the overall chromatin reorganization that takes place upon pluripotency exit.

Figure 21

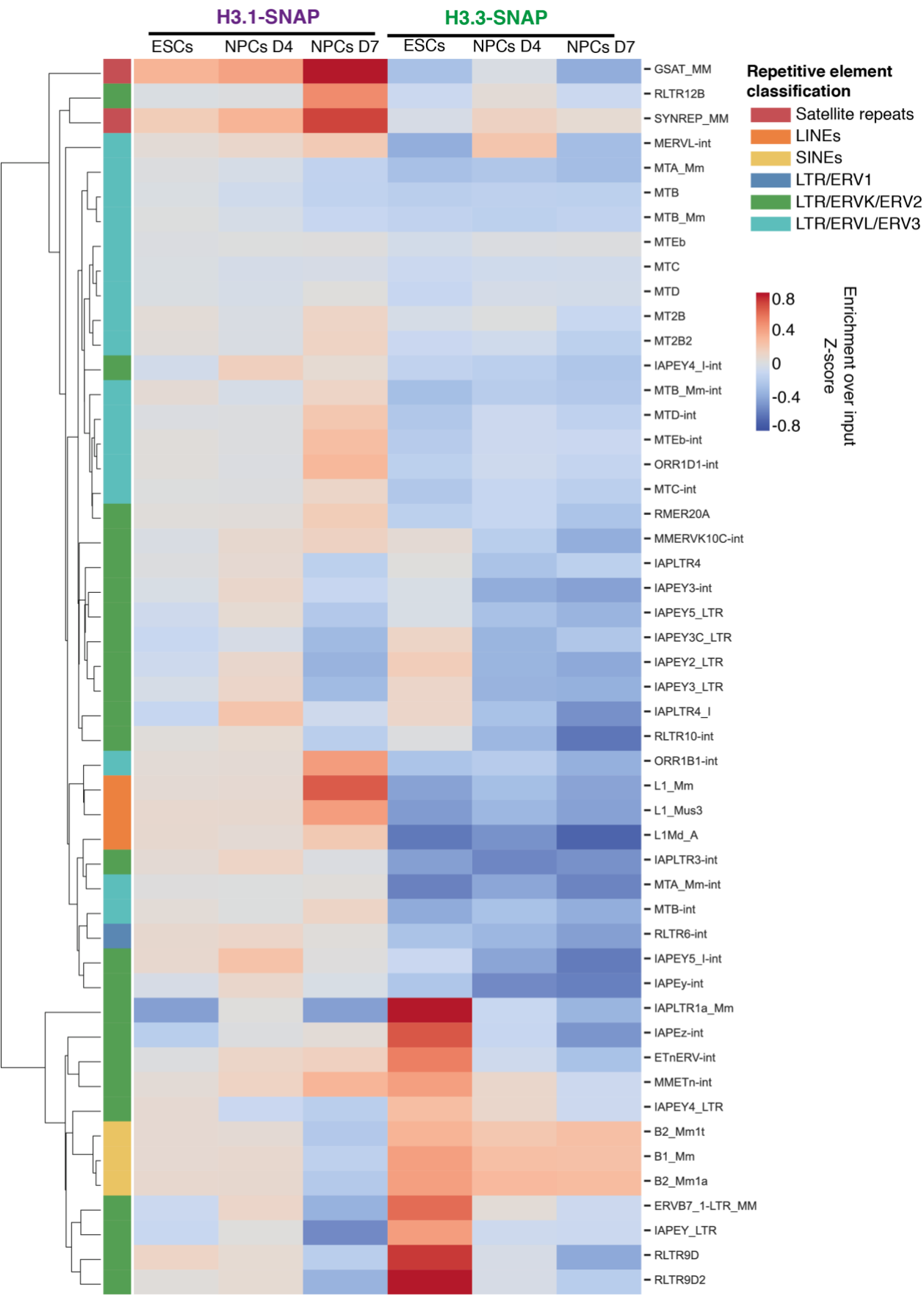


Fig 21. H3.1 and H3.3 enrichment at different repetitive sequences. Clustered heatmap of H3.1 and H3.3 enrichment in ES, NPC D4 and NPC D7 along a comprehensive list of mouse repetitive sequences present in constitutive heterochromatin. Data are obtained from H3.1- and H3.3- SNAP-ChIP-seq (as described in Fig. 3 of manuscript) and represented as a Z-score of \log_2 enrichment of IP over input.

Single-molecule visualization of H3 variants reveals their mutual occupancy at chromocenters

The observed differences of H3 variants distribution outside and at chromocenters offered a unique entry point to question the specific nucleosomal arrangements in the nucleus of the two variants. To perform quantitative analysis, we used super-resolution microscopy, including STORM (Betzig *et al.*, 2006; Rust, Bates and Zhuang, 2006), which allows the detection of sparse single molecules enhancing the spatial resolution of a protein of interest beyond the light-diffraction limit resolution (200 nm) in a crowded and compact environment such as PHC. We first verified our methodology in MEF cells in which endogenous H3.1/H3.2 were fluorescently labeled using antibodies. We then successfully reconstituted STORM images for global H3.1/H3.2 (Fig. 22). Consistent with epifluorescent widefield images, STORM images showed a broad distribution of H3.1 in the nucleus with specific enrichment at PHC while reaching a resolution in the range of ~30 nm in XY. At this resolution, we found that H3.1/H3.2 distributed heterogeneously at PHCs forming group of detections reminiscent of conglomerates (Clément *et al.*, 2018). We further performed STORM in MEF cells labeled for endogenous H3.1/H3.2 or H3.3 along with EdU staining to track H3 enrichment at PHC in and during S phase progression. Next, we analyzed the reconstituted localizations using a centroid-based algorithm called Voronoi Tessellation (Levet *et al.*, 2015). This clustering method is based on partitioning a space into polygons such that each polygon will contain a single localization and its size is inversely proportioned to the surrounding local density of the other localizations (Levet *et al.*, 2015). Using this approach, we observed that the areas occupied by H3.1 localizations were large and uniformly distributed at PHC of early S phase cells (Fig. 21). As cells progress from mid to late S phase, we observed a shift in disposition of smaller and denser polygons from the external periphery of PHCs to their internal side. In contrast, H3.3 density at PHC evolved throughout S phase, decreasing when progressing from early to mid/late S phase. This gradual H3.1 enrichment at PHC agrees with p150-CAF1 progression during PHC replication (Quivy *et al.*, 2004). Overall, our STORM analysis enables the first visualization of global and endogenous H3.1 and H3.3 at PHC revealing distinct configurations during cell cycle progression. H3.1, that is tightly linked to mid-late replicating regions, adopts a ring-like configuration that becomes denser and uniform at the end of S-phase. H3.3, despite being present at PHCs in early S-phase, is progressively replaced by H3.1 at the

same regions. Such mirrored dynamics is coherent with their global genomic distributions. Finally, these observations also indicate that S-phase diluted but did not erase H3.1/H3.3 distribution at PHC.

Figure 22

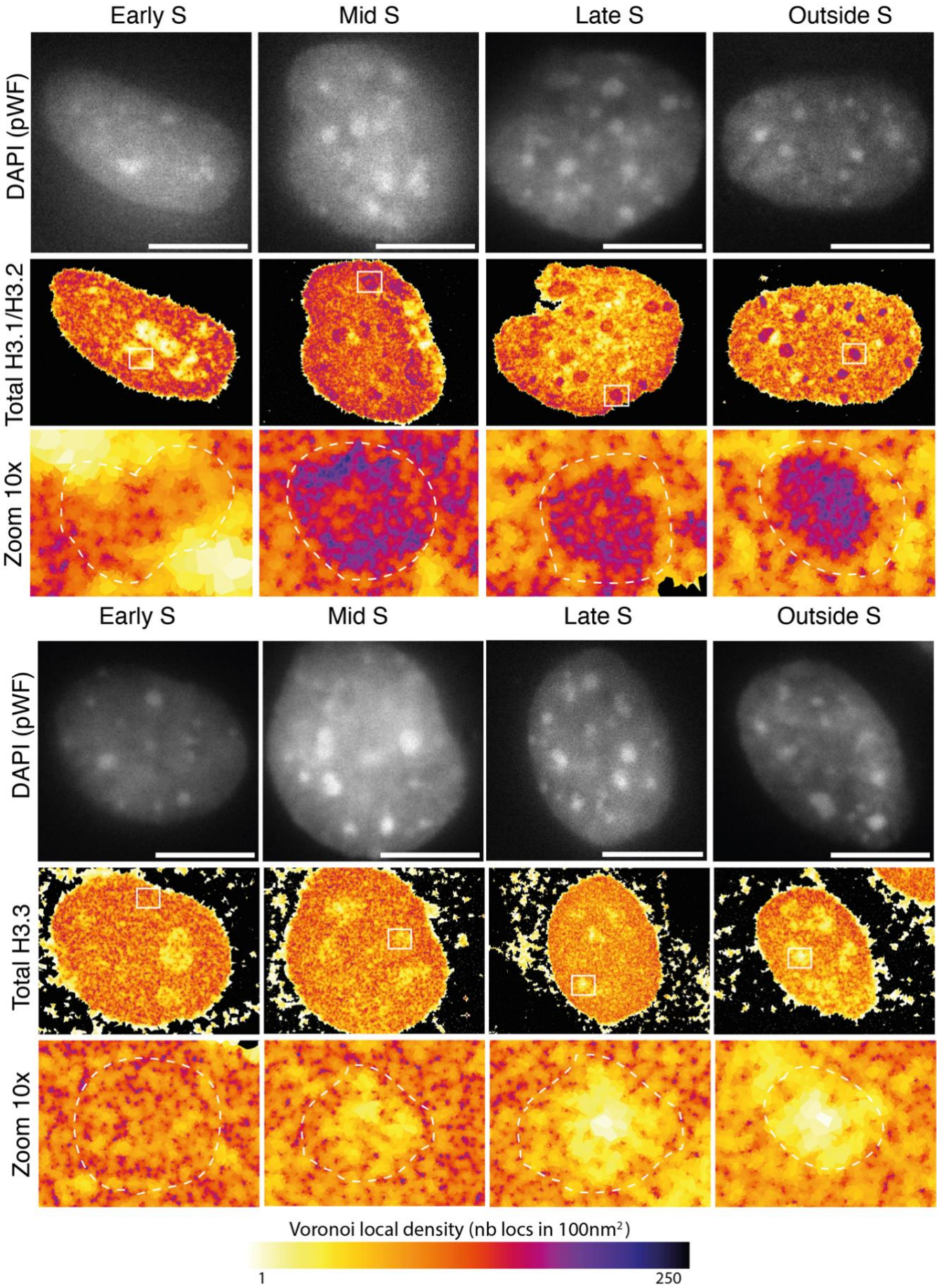


Fig. 22. Global distribution of H3.1 and H3.3 throughout S phase using STORM assay. A. Representative STORM images in MEF cells to visualize global H3.1/H3.2 (top panel) or H3.3 (bottom panel) throughout S phase progression. Single molecule localizations are rendered with Voronoi tessellations (Levet *et al.*, 2015) showing the density (defined as the inverse of the area that each localization occupies) in H3 variants in the whole nucleus (middle row) and at chromocenters (dashed lines, bottom row). Voronoi polygons are color-coded according to the local density of each localization in a neighboring area of 100 nm². The color gradient corresponds to the nb of localizations. Insets represent a 10-fold enlargement of selected chromocenters area based on corresponding DAPI pseudo-widefield (pWF) images acquired before STORM acquisitions of H3 variants. Scale bars represent 5 μ m.

Suv39h2, but not Suv39h1, supports H3.1 enrichment at chromocenters

Chromocenters are constitutive heterochromatin compartments enriched in repressive marks at DNA repetitive elements (Peters *et al.*, 2001). H3.3 presence in ES cells is associated with active turnover of pluripotent and early-lineage committed genomic regions (Deaton *et al.*, 2016; Schlesinger *et al.*, 2017), as well for silencing ERVs and telomeres in absence of DNA methylation (Elsässer *et al.*, 2015; He *et al.*, 2015). In our observations in ES cells, H3.3-HIRA mediated deposition or the presence of an H3.1 with an S31ph (H3.1-A31S) had no detectable impact on chromocenters numbers and shape, suggesting that either H3.3, once is incorporated into chromatin, mediates the same functions of H3.1 or that alteration of the H3.1/H3.3 balance does not lead to chromocenters' perturbations. Along the same line, I used another approach aimed at altering H3.1/H3.3 replacement mediated by major satellite transcription. For this, I took advantage of Suv39h1/2 KO (Suv39h dn) ES and MEF cell lines in which H3K9me3 repressive mark is not anymore enriched at chromocenters (Peters *et al.*, 2001; Lehnertz *et al.*, 2003). We performed IF for endogenous H3.1 and H3.3 in both cells and evaluated their nuclear distribution and enrichment at chromocenters. For MEF cells, we did not appreciate evident changes in patterns, except for a stronger enrichment of H3.1 at chromocenters and an increase in H3.3 signal in the rest of the nucleus (Fig 23a). We quantified the difference for H3.1/H3.3 enrichment at chromocenters in asynchronous WT and Suv39dn ES and MEF cells using the 3D-FIED ImageJ macro (Fig 23b). We found that H3.1 ratio was distributed more frequently at higher values in Suv39h dn cells compared to WT cells, confirming the increased contrast in the IF images, whereas H3.3 was largely unaffected. For ES cells, surprisingly, we found an almost complete loss of H3.1 enrichment at chromocenters in Suv39dn cells (Fig 23a). We applied the same automated quantification analysis and we observed a shift in H3.1 ratio distribution that resembled H3.3 distribution (Fig 23b).

In absence of Suv39h KMTs, transcriptional status at several elements, including satellite transcripts, is particularly impacted (Martens *et al.*, 2005). To test whether the changes in transcription at chromocenters follow a change in H3.1 pattern at these sites, we performed ImmunoRNA-FISH (as in (Probst *et al.*, 2010; Maison *et al.*, 2011; Casanova *et al.*, 2013)) for H3.1 and major satellite transcripts in ES cells WT or Suv39h dn (Fig 23c). In WT ES cells, H3.1 is clearly enriched at chromocenters while actively transcribing satellite transcripts are discernable as single foci at the periphery of these regions, as expected (Maison *et al.*, 2011).

In Suv39h dn ES cells, we observe a higher frequency of satellite transcripts inside single nuclei along with less defined chromocenters, probably due to a loss of PHCs clustering in absence of H3K9me3. Interestingly, H3.1 enrichment is also lost in cells preserving chromocenter shape (Fig. 23c), suggesting that H3.1 accumulation is not restored in the next rounds of replication at PHCs due to H3.3 replacement at the same sites where transcription likely occurred.

We finally tested the ability of Suv39h KMTs to restore H3.1 accumulation either by repressing satellite transcription via H3K9me3 deposition or to reform chromocenters by clustering PHCs regions. We transiently transfected Suv39h dn ES cells with vectors expressing Myc-tagged Suv39h isoforms (Suv39h1, Suv39h1, Suv39h1+Suv39h2) along with a catalytically dead mutant Suv39h1-H324K from (Maison *et al.*, 2016) and we used GFP construct as control. We monitored H3.1 localization in Myc+ cells and quantified their pattern in ‘enriched’, ‘even’ and ‘excluded’ at PHCs, as previously described (23d). We found that in Suv39dn ES cells, ~20% cells show an enrichment pattern that did not increase upon Suv39h1 KMTs isoforms transfection. Interestingly, Suv39h2 alone was sufficient to double H3.1 enrichment. The presence of Suv39h1+Suv39h2 slightly increases the proportion of ‘enriched’ patterns, with a concomitant reduction in ‘excluded’ patterns, but does not lead to a significant synergistic effect in this time window. Finally, the presence of a Suv39h1H324K, unable to promote a H3K9 KMT activity, gave the same pattern of GFP and Suv39h1, confirming the requirement of Suv39h2 to observe an H3.1 accumulation at these regions.

Together, our data support a model where a catalytically active Suv39h2 initiates heterochromatin restoration and thus H3.1 enrichment at chromocenters in pluripotent cells. Given that H3.1 accumulation is less robust and challenged by H3.3 in ES cells, the absence of Suv39 KMTs, and thus H3K9me3, leads to higher transcription of major satellites (Martens *et al.*, 2005) and H3.1 eviction at these sites. Suv39h1 alone is unable to restore H3.1, but the presence of Suv39h2 provides an opportunity to target and reinforce Suv39h1 activity. This is in line with the fact that Suv39h2 initiates H3K9me3 deposition at PHCs in the zygote-2-cell transition (Burton *et al.*, 2020) while CAF-1 activity reinforces H3K9me3 at constitutive heterochromatin regions throughout mouse development (Wang *et al.*, 2018). We conclude that H3.1 enrichment is a proxy of constitutive heterochromatin that, at least in ES cells, follows chromocenters transcriptional status and its local accumulation is supported by Suv39h KMTs activity.

Figure 23

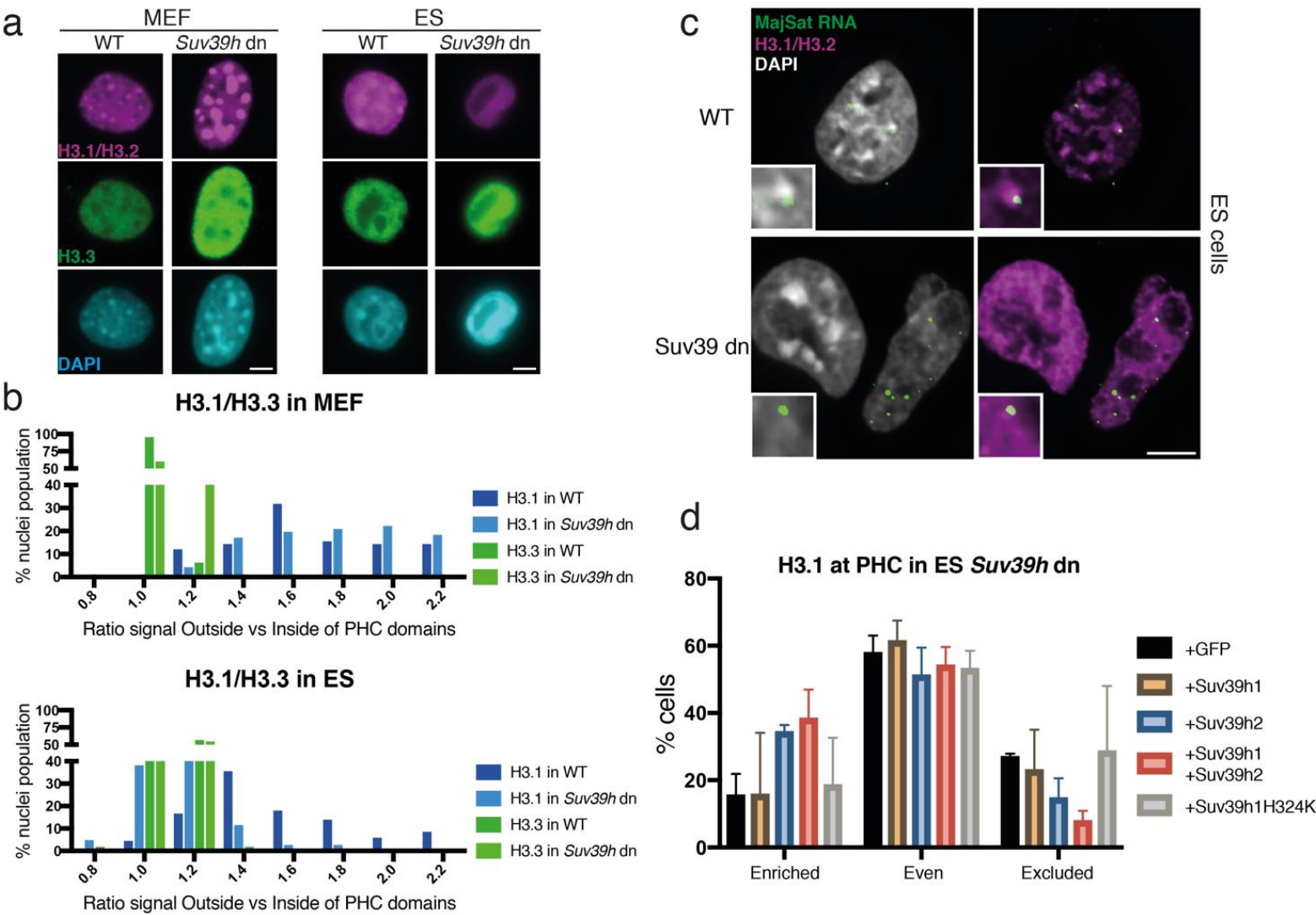


Fig 23. Suv39h KMTs increase H3.1 accumulation at chromocenters. A) Representative epifluorescence images of endogenous H3.1/H3.2 (magenta) and H3.3 (green) variants staining along with DNA (DAPI, cyan), from WT and *Suv39dn* cells in ES and MEF cell background. B) Graphs showing the quantification of H3.1 and H3.3 relative enrichment values at PHC in percentages of the nuclei population in MEF and ES cells. C) Immuno RNA-FISH for forward and reverse major satellite transcripts (MajSat, green) merged with H3.1/H3.2 antibody labeling (magenta) or DAPI (grey) in WT or *Suv39dn* ES cells. 4-fold magnifications as insets on the bottom right of the images show representative MajSat localizations. Scale bars 5 μ m. D) Quantitative analysis of the percentage of cells exhibiting recurrent patterns of H3.1 at PHCs upon Myc-*Suv39h* and GFP constructs transfection. Plots show averages and s.d. of at least 50 nuclei per condition from 2 independent experiments.

Materials and Methods

Cell Culture

We cultured feeder-free Suv39dn and corresponding control WT R1 ES cells (provided by T. Jenuwein, (Lehnertz et al., 2003) in ES cell media as we previously described in the manuscript. We cultured Suv39dn and corresponding control WT iMEF (provided by T. Jenuwein) in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% foetal calf serum (Eurobio), penicillin/streptomycin (Invitrogen) at 37 °C and 5% CO₂.

We performed Immuno RNA-FISH for forward and reverse major satellite transcripts, along with H3.1 detection, as in (Probst *et al.*, 2010; Casanova *et al.*, 2013; Maison *et al.*, 2016).

We transiently transfected Suv39h dn ES cells with vectors expressing Myc-tagged Suv39h isoforms (Suv39h1, Suv39h1, Suv39h1+Suv39h2) along with a catalytically dead mutant Suv39h1-H324K from (Maison *et al.*, 2016) using GFP construct as control.

Sequencing data analysis of repetitive elements

Repetitive element annotation was obtained from Ensembl (mus musculus core 102_38) and tandem ('trf') and low-complexity ('dust') repeat classes were filtered out. For each sample, the number of reads at each repeat were calculated by overlapping the repeat annotation with the genomic coordinates of fragments mapped in pair, excluding duplicates, extracted from bam files. Samples were normalized to the total number of reads mapped to all repeats (CPM), then by dividing to repeat length and finally by dividing by the matching input sample. Then, IP to input ratio was log₂-transformed and to allow comparison between conditions, cross-sample normalized by computing z-scores.

Stochastic Optical Reconstruction Microscopy (STORM)

For STORM imaging, we used adherent cells grown on 20mm diameter fibronectin-coated coverslips (Marienfeld, No. 1.5H). We incubated cells for 30 min with 0.1 mm diameter fluorescent beads (TetraSpeck Microspheres, ThermoFisher) to correct for sample drift during acquisition.

We mounted the coverslips in a home-made oxygen scavenger blinking buffer containing 100 mM MEA (Mercaptoethylamine, 30070, Sigma), 40 μ g/ml catalase (C3515, Sigma) and 50 μ g/ml Glucose oxidase (G2133, Sigma) on cavity slides (BR475505, Sigma) and sealed with Twinsil sealing medium (Rotec) before STORM imaging. We changed the mounting buffer between every acquisition session. We excited Alexa 647 with a 640 nm excitation laser in HiLo mode configuration with ASTER technology (Mau *et al.*, 2021) with a power of 3-4 kW/cm² at the sample. We imaged the fluorescence from the activated Alexa 647 with an Abbelight SAFe360 setup. The system is mounted on a Nikon TiE2-E microscope with sCMOS Hamamatsu ORCA-Fusion BT C15440-UP camera and using a 100X/1.49NA Nikon objective and controlled with Neo_acquisition (Abbelight) software. The image pixel size was 97 nm. We acquired at least 20000 frames at 40 ms time exposure. We detected the localizations in STORM movies with Neo_Analysis (Abbelight). We removed the first 500 frames because of overlapping single emitters' signals. We filtered out localizations with a too low photon count and merged localizations that were detected in consecutive frames within a 100 nm radius. For visualization, we performed Voronoi tessellation and rendered the localizations with SR-Tesseler (Levet et al 2015).

Discussion

During the course of my PhD, I aimed to gain a better understanding of how histone variant landscape is transmitted throughout differentiation and whether this occurs at specific chromatin landmarks. First, in the framework of an international collaboration, I developed new mouse embryonic stem cell lines expressing H3-SNAP-Tagged variants, which was a new toolbox for the lab. Second, I validated the observations made with transgenic lines by using specific antibodies for the detection of endogenous H3 variants, confirming the preliminary observations that I obtained with H3-SNAP-Tag labeling. This dual approach enabled me to establish confidently, for the first time, their physiological distribution and enrichment at particular nuclear domains, as well as during differentiation. In collaboration with colleagues from my team and at Institut Curie, by combining labeling approaches with sequencing, I identified the DNA elements with which the two histone variants are preferentially enriched in constitutive heterochromatin regions, particularly at mouse chromocenters. After setting up and validating optimal conditions for cell culture maintenance, image acquisitions and analysis of the results, I then determined how the global histones H3.1 and H3.3 differentially partition mouse nuclei for cells in states. Strikingly, the accumulation of replicative histone variants H3.1/H3.2, but not the replacement H3.3, proved to be a specific chromocenter feature of fundamental nature. These findings open new avenues to investigate H3.1 and H3.3 dynamics occurring at particular and unexplored chromatin landmarks. In the following sections, I will discuss the implications of having a constitutive pool of histones that replenish and reinforce epigenetic information in these domains.

H3.3 starts the match, but H3.1 maintains the balance

In the developing embryo, chromocenters start to be formed at 2- and 4- cell stages, when the major wave of zygotic genome activation occurs. After fertilization, the reset of (hetero)chromatin marks is an essential step for the correct reprogramming of the developing zygote. In mammals, the paternal genome is enriched in protamines that must be evicted and replaced by H3.3 to allow gamete fusion. Selective incorporation of H3.3 in the paternal genome results in asymmetrical H3 variants enrichment in the zygote that is later resolved at the 2-cell stage during DNA replication and H3.1 deposition mediated by CAF-1. In addition, repressive epigenetic signatures, including H3K9me3 and DNA methylation, are differentially distributed during development. While residual DNA methylation is progressively diluted during rapid cell divisions until the *de*

novo deposition after the blastocyst stage, H3K9me3 is readily established after the 2-cell stage and serves as a bookmark for correct gene expression during preimplantation events (Wang *et al.*, 2018; Nicetto and Zaret, 2019). Therefore, in a hypomethylated environment, such as the developing embryo and ES cells, H3K9me3 and DNA replication help together restore long-term transcriptional silencing. All these changes are accompanied by major chromatin rearrangements including the formation of chromocenters and heterochromatin establishment. In *Xenopus*, at the onset of gastrulation events, PTMs on H3 histones, in particular, acetylation and H1 incorporation are critical (Dimitrov *et al.*, 1993; Almouzni *et al.*, 1994). Furthermore, histone variants seem to play a key role in chromocenters' function during development progression. For example, the presence of K27 of H3.3, but not of H3.1, is fundamental for progression from zygote to the 2-cell stage in mouse cells (Santenard *et al.*, 2010). Early unbalanced incorporation of an H3.3 mutant on the paternal chromatin causes derepression of major satellite transcripts and dispersion of chromocenters (Santenard *et al.*, 2010). Different results have been obtained in other model organisms, including *Drosophila*, where replicative histone H3 variants can still compensate for the non-replicative histone H3.3 functions during early development (Hodl and Basler 2009; Sakai *et al.* 2009). Because histone variant H3.3 correlates with transcription and histone turnover (Deal, Henikoff and Henikoff, 2010; Kraushaar *et al.*, 2013; Deaton *et al.*, 2016), the ectopic presence of H3.3 (and active PTMs) may facilitate spurious recruitment of transcription factors at recurrent repressed regions. The maintenance of H3.1 at chromocenters instead might act as a barrier in preventing detrimental and excessive de-repression of inactive repetitive elements that are readily silenced as cells re-enter in S-phase.

In the “[Manuscript](#)” section, I discussed the interplay between the DNA replication machinery and histone chaperones and the consequent enrichment of histone variants at chromocenters. For this, I envisaged a model where both DNA synthesis coupled (DSC) and independent (DSI) deposition pathways participate in depositing histone variants at major satellite sequences (Fig. 24). In physiological conditions, the DSC pathway contributes to the bulk chromatin composition and particularly in constitutive heterochromatin regions shifting the balance in favor of replicative H3 variant enrichment at chromocenters. When interfering with this system by targeting HIRA to chromocenters, the DSI pathway counteracts such accumulation by depositing H3.3. I do not exclude that interfering with other DSI histone chaperones, including DAXX, may produce similar phenotypes given their role in protecting these regions from accumulating DNA damage (He *et al.*, 2015;

Canat *et al.*, 2021). In this context, the role of other histone chaperones, and in particular ASF1, given its role in preserving parental histone recycling (Clément *et al.*, 2018; Torné *et al.*, 2020) would deserve further investigation.

Figure 24

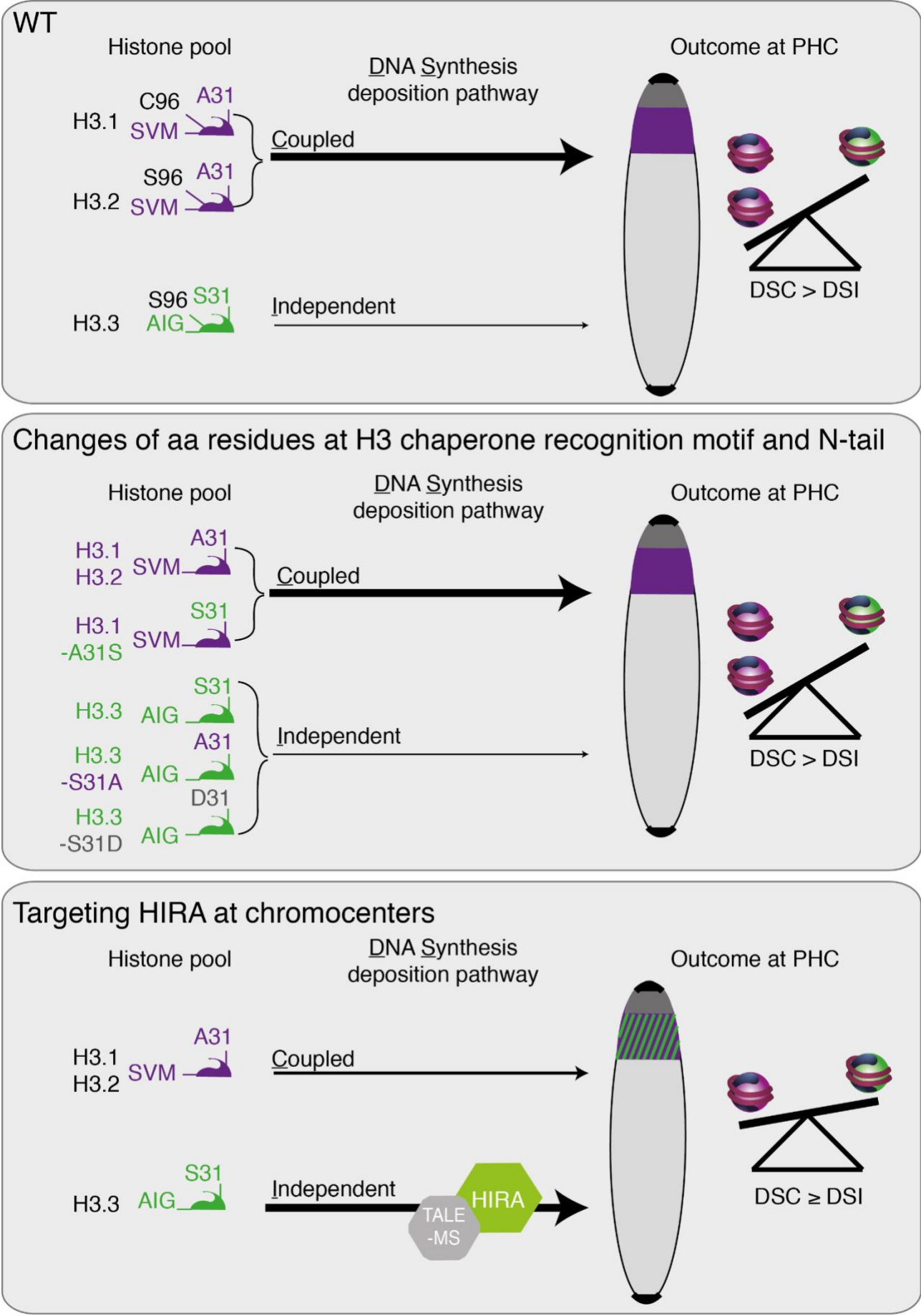


Figure 24. Schematic of the molecular mechanisms contributing to (un)balanced amounts of replicative H3 and replacement H3.3 histone variants at major satellites (chromocenters). Top panel shows H3.3 and H3.1/H3.2 deposition mediated by DNA synthesis coupled (DSC) or independent (DSI) pathways, respectively. H3.1/H3.2 are enriched at mouse pericentric heterochromatin (PHC) by CAF-1 histone chaperone. Middle panel shows H3 mutants and their respective pathways of deposition. Bottom panel shows the effect of HIRA targeting major satellites (MS) via TALE. In the first two panels, nucleosomes containing replicative H3 variants (in purple) are enriched at PHCs bordering the minor satellites (dark grey) close to one telomere (black) in the acrocentric mouse chromosome. This is further exemplified by the unbalanced amount of replicative variants (purple nucleosomes) compared to replacement variants (green). In the last scenario, H3.1/H3.2-containing nucleosomes presence is balanced at these sites due to H3.3-HIRA deposition at PHC (purple-green stripes). Specific amino acidic residues are highlighted, distinguishing the histone fold domain (SVM, AIG), from position 96 and residue 31 on the N-tail (S31, A31, D31).

H3.1 and H3.3 at repetitive regions of the genome

Chromocenters are an interesting model of heterochromatin domains. They are mainly composed, but not exclusively (Ostromyshenskii *et al.*, 2018), of repetitive elements, including satellite and ERV elements. Among the repetitive elements identified at chromocenters, L1 LINEs are extremely abundant in mammalian genomes making up to ~10% of chromocenters composition (Ostromyshenskii *et al.*, 2018). Together with major satellites, they form core-shell surrounding chromocenters as they are constitutively associated with heterochromatin (Solovei *et al.*, 2009; Solovei, Thanisch and Feodorova, 2016). The chromocenter enrichment in H3.1 that I observed is of particular interest, given the 5'-UTR composition of L1 LINEs in repetitive units. Indeed, this configuration in tandem arrays resembles the one of major satellite repeats (Bulut-Karslioglu *et al.*, 2014) and, importantly, H3.1 enrichment at these sites increases over differentiation, accompanied by H3.3 depletion. Additionally, future work with long-read sequencing approaches (Altemose *et al.*, 2022; Hoyt *et al.*, 2022) will help us to better decipher the exact nucleosome positioning of histone variants at these elements. Similar findings on H3.1 enrichment, along with H2A.W, has been reported in Arabidopsis chromocenters during seedling development (Benoit *et al.*, 2019). It is not surprising to observe a positive correlation between these two variants in heterochromatin given their genome-wide distribution and their relative absence in actively transcribed regions. Compared to mouse chromocenters, Arabidopsis 180-bp centromeric and Tsi pericentromeric repeats share the same chromocenter space, whereas mouse chromocenters are mainly composed of major satellites that do not overlap with minor satellites repeats. In fact, despite being close in space, mouse CENP-A and H3.1 are physically located in distinct regions with H3.3 that tends to be depleted from these regions. This absence would be likely explained by its role in being actively disrupted and for acting as a placeholder for CENP-A in centromeric regions (Dunleavy *et al.*, 2009). The recurrent H3.1 presence may be favored not only for higher stability of H3.1-containing nucleosomes over H3.3 equivalent (Jin and Felsenfeld, 2007) but also in preventing an excess of engagement from chromatin remodelers or other histone chaperones, namely HIRA, in depositing H3.3. An attractive speculation for such recurrent patterns of deposition would be that, during evolution, selective pressure on repetitive repeats helped determine more accessible gene-rich from gene-poor chromosomal regions. Therefore, chromatin rearrangement is instructed

first at the linear level both by DNA and histone contents and further reinforced in the nuclear space by scaffolding proteins by a choice of inactive over active marks.

In contrast to H3.1, H3.3 is enriched at ERVs elements, including IAP and ETn elements, only in ES cells (Elsässer *et al.*, 2015) and, in absence of DNA methylation mechanisms, H3.3-H3K9me3 deposition at these sites might be required for early stages of development (Buschbeck and Hake, 2017). Instead, H3.1 enrichment is sustained and reinforced over differentiation on the repetitive genome, including LTR and satellite repeats, given its intimate link with CAF-1 in preserving chromatin identities and acting as a barrier to cell fate change and rewiring (Cheloufi *et al.*, 2015; Ishiuchi *et al.*, 2015). In our observations, while there are relatively clear patterns for H3.1 and H3.3 enrichment in pluripotent ES and differentiated NPC D7, both at genomic and microscopy levels, the NPC D4 population reflects a transitioning state with few regions showing strong enrichment or depletion for either variant. Despite being often used as a proxy for exit of pluripotency, RA-induced differentiating ESCs possess intrinsic confounding agents that might influence the expected analysis and require further attention. First, ES cells and NPCs possess a different proliferation rate (~12h vs ~25h, respectively (Snow, 1977)) accounting for a differential genome-wide enrichment of replicative versus replacement variants. Then, RA-induced NPCs dramatically shift their expression program at the exit of pluripotency (Semrau *et al.*, 2017) and are reported to express high levels of major satellite repeats compared to ES and MEF cells (Martens *et al.*, 2005). Whether the burst in transcription at this time window reflects a need for recruiting additional transcriptional factors (Bulut-Karslioglu *et al.*, 2012) or induces further heterochromatin formation (Probst *et al.*, 2010) is an attractive hypothesis worth exploring.

Fine-tuning H3 balance at chromocenters

Mechanistically, the progressive enrichment of H3.1 at PHC is intimately linked with DNA replication and, specifically, with the recognition by the histone chaperone CAF-1. Conversely, H3.3 is enriched at active, nucleolar chromatin thanks to the presence of residues 90 and, more importantly, 87 in the histone fold domain, whereas residues 31 and 41 in the N-tail are critical for H3.3 disassembly from nucleolar rDNA arrays in *Arabidopsis* (Shi *et al.*, 2011).). We observed that a swap in the histone fold domain of H3.3 (from an AIG to an SVM amino acid motif) resulted in similar degrees of H3.3-SVM enrichment at PHCs, as compared to

H3.1/H3.2, whereas there were little to no changes in H3 variant enrichment frequency for amino acid substitutions at position 31. Therefore, we propose that the N-tail modifications on histone H3 at position 31 might be dispensable for H3 accumulation at PHC and, ultimately, its nucleosome composition. This aspect is interesting given that LLPS properties rely on multivalent interactions of hPTMs with their readers, including HP1 (Larson *et al.*, 2017; Strom *et al.*, 2017). The observations that histone chaperones might compete for the same sites in depositing histone variants provide a framework where chromatin remodelers would not necessarily work on a linear DNA but rather on a megabase scale over multi-fold domains. Several studies proposed that chromatin compartments may feature phase separation properties with dynamics that could regulate local transcription activity (Larson *et al.*, 2017; Strom *et al.*, 2017; Erdel *et al.*, 2020). Despite not being in a dynamic range of observations, our results by STORM unveiled previously unappreciated chromatin dynamics. Unlike H3.3, H3.1 nucleosomes form dense and large conglomerates that vary in size and density during the cell cycle. In Early S-phase, low-density H3.1 units organize homogenously between euchromatin and PHC. As cells progress from Mid to Late S-phase, H3.1 marks the periphery of PHC and gets progressively enriched in the inner core. (Fig. 25). Concomitantly, H3.3 is depleted in the same regions, indicating a mutually exclusive presence of these two variants depending on the replication status of PHC. If chromatin remodelers would compete for the same sites, it would be intriguing to explore how dynamically these local concentrations of histones vary at a single molecule event.

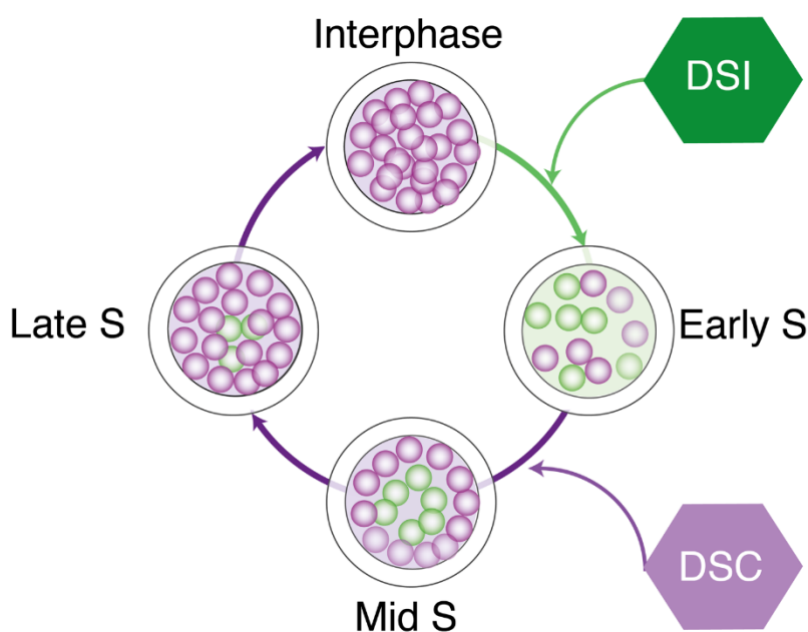


Figure. 25. H3.1/H3.2 enrichment at chromocenter during the cell cycle. Purple and green balloons correspond to H3.1/H3.2 and H3.3 nucleosomes, respectively. With DSI, equal amounts of H3.3 and H3.1/H3.2 are present in non-replicating PHCs during Early-S phase. With DSC, CAF-1 deposits H3.1/H3.2 at the periphery of PHCs during Mid-S phase gradually occupying the entirety of the domain in Late-S phase. After replication, H3.1/H3.2 can be replaced by H3.3 before re-entering in S-phase.

We observed a similar frequency of enrichment at chromocenters for histone variants containing an SVM motif and an S31 on H3.1 (H3.1-A31S), while histone variants with an AIG motif and an H3.3 that cannot be phosphorylated (H3.3-S31A) are not accumulated at the same regions. We also observed that this accumulation, once occurred, is maintained over cell cycle progression. Together, these data indicate that the H3 variants' contribution does not alter chromocenters formation given that, once they are formed, they are maintained and regulated by other factors, including residual DNA methylation activity and AT-hook proteins (Jagannathan, Cummings and Yamashita, 2018, 2019).

Despite the fact that H3.3S31ph can be present at PHC regions (Hake *et al.*, 2005), these sites do not preferentially preserve and accumulate such modification over cell generations, raising the question of its importance as a transient marking system. We recently discovered that, while the mode of incorporation is dispensable, the presence of S31ph in H3.3 is critical for *Xenopus* gastrulation, possibly acting as a signal module for hPTM crosstalk (Sitbon *et al.*, 2020). S31 can be phosphorylated during mitosis promoting activation of macrophage transcriptional programs (Hake *et al.*, 2005; Thorne, Ouboussad and Lefevre, 2012; Armache *et al.*, 2020). S31ph can also act as a docking site for different transcription factors, including p300, by promoting H3K27ac (Martire *et al.*, 2019) and this phosphorylation is enhanced by kinases, including CHK1 and AuroraB (Sitbon *et al.*, 2020). Moreover, AuroraB phosphorylation dissociates Oct4 from chromatin binding during mitotic transition in ES cells (Shin *et al.*, 2016). In recent work, S31ph prevents H3K9me3 demethylation from KMD4B at telomeres in ES cells maintaining a heterochromatic state from mitosis until the next G1/S phase (Udugama *et al.*, 2022).

In my data, H3.1 replaces H3.3 at chromocenters during replication progression, as observed with STORM, revealing gradual and denser chromatin compaction before and after replication at constitutive heterochromatin regions. However, while H3.1 deposition at chromocenters is restricted to S-phase progression in differentiated cells, H3.3 can replace H3.1 in G1 and Early S-phase of ES cells as shown by the lowest proportion of cells enriched in H3.1 (Fig. 26). This increased H3.3 deposition in both euchromatin and heterochromatin regions helps prevent unscheduled pluripotency exit, but also to ensure genome stability due to the lack of G1-S checkpoint in ES cells. In this sense, H3.3 supports genome integrity at specific loci including telomeres and pericentric heterochromatin (Wong *et al.*, 2008; Lewis *et al.*, 2010). Moreover, structural chromatin proteins, including replicative histone variants, are dynamically incorporated in stem cells, which is not the case for

H3.3 associating with actively transcribed regions (Meshorer *et al.*, 2006). Moreover, preventing H3.3 specific incorporation in HIRA KO ES cells led to heterochromatin formation and, thus, differentiation (Meshorer and Misteli, 2006). In this picture, H3.3 acts as a compensation mechanism in a hypomethylated context where spurious transcription of repetitive elements can also occur in heterochromatin regions including chromocenters. It will be interesting now to understand how the maintenance of H3.1 and H3.3 at other heterochromatin regions, including telomeres and minor satellites, can differ from the major satellites and how a choice of other histone variants, including CENP-A can challenge their stability.

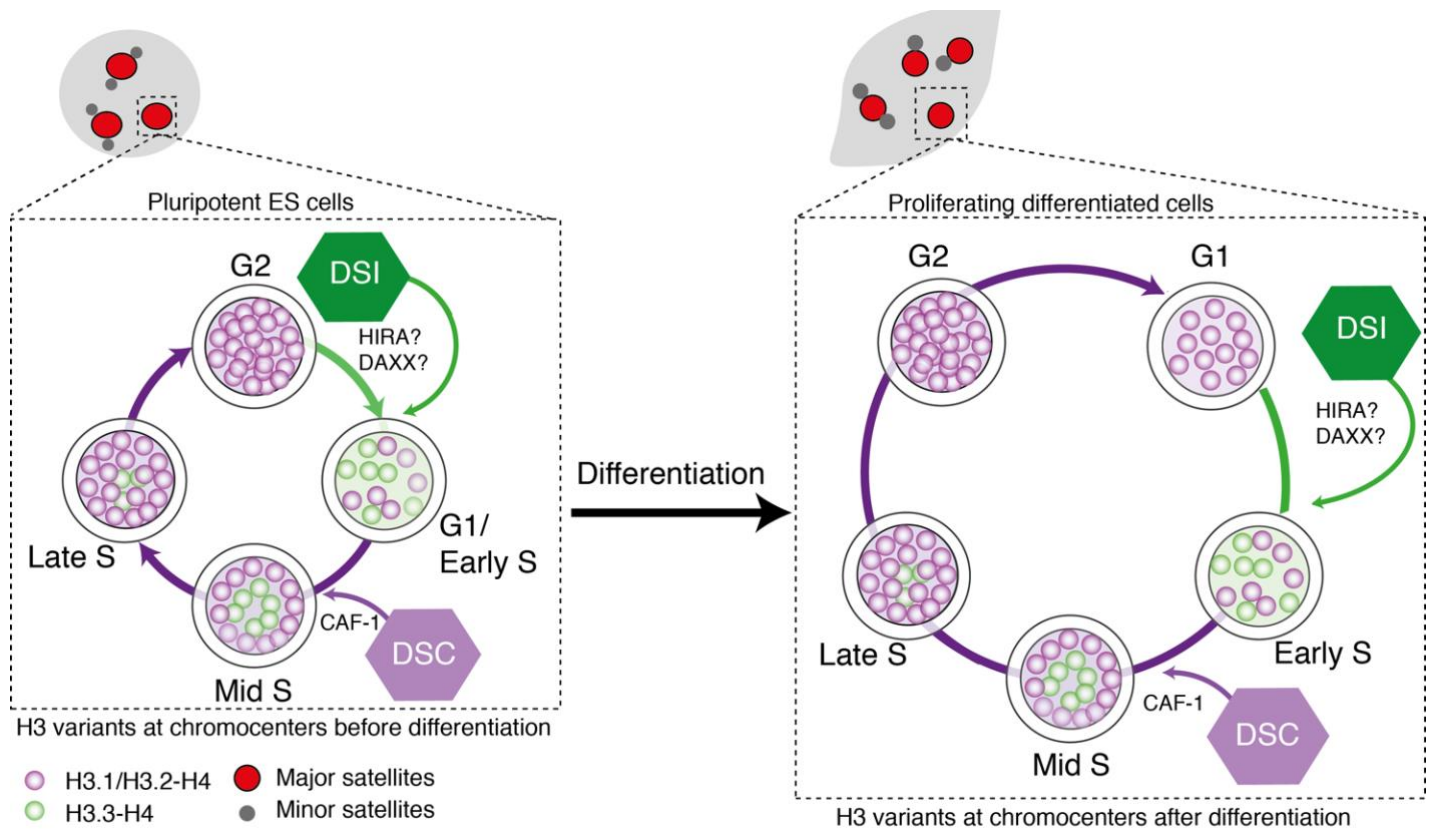


Figure. 26. Dynamic H3 variant enrichment at chromocenters during differentiation. Purple and green balloons correspond to H3.1/H3.2 and H3.3 nucleosomes, respectively, as in Fig.24. Red and dark grey spots indicate major and minor satellites, respectively. In pluripotent cells, gap phases are reduced and S-phase represents the majority of the pluripotent cell cycle. DSC mediates H3.1/H3.2 deposition and accumulation at PHCs from Mid S-phase up to the G2 phase. After mitosis, DSI deposits H3.3 both in G1 and in Early S-phase in absence of H3.1/H3.2-DSC deposition. In differentiated cells, the cell cycle is extended and gap phases' length is restored. H3.1/H3.2 enrichment is maintained from Mid S-phase up to the G1 phase and the H3.1/H3.2-H3.3 amount is equalized only during Early S-phase.

H3 variants clash at chromocenters

The effect of histone chaperones that are not commonly found in these regions will help us to decipher the role of H3.3 presence in heterochromatin regions in place of H3.1. In our experiments, we used HIRA considered as mainly present in actively transcribed regions, but not currently reported associated with heterochromatin regions apart when DNA damage occurs (Adam *et al.*, 2016; Bouvier *et al.*, 2021) or in ALT cells when ATRX is mutated (Heaphy *et al.*, 2011; Hoang *et al.*, 2020). In our setup, HIRA-mediated deposition is sufficient to displace (or prevent) H3.1 at chromocenters in ES cells. This indicates that the DSI pathway of deposition can prevail over DSC, in agreement with the fact that CAF-1 cannot compensate for H3.3 deposition in absence of HIRA (Ray-Gallet *et al.*, 2011). Interestingly, HIRA retargeting at PHCs did not lead to any obvious defects in chromocenters formation or numbers in ES cells. It is currently unknown if and how HIRA can distinguish between (un)methylated H3.3 histones. In this case, we could speculate that a proportion of soluble H3.3K9me1 and H3.3K9me2 might equally act as a substrate for SUV39H1/2 and SETDB1/G9a, respectively, when incorporated at chromocenters (Loyola *et al.*, 2006), thus preserving H3K9me3 maintenance at these sites (Peters *et al.*, 2001; Lehnertz *et al.*, 2003). It would be interesting to explore this approach in differentiated or differentiating cells and evaluate to which extent hPTMs status could affect chromocenter formation.

The absence of important chromatin modifiers, including Suv39h KMTs, reveal different histone dynamics depending on the cell background. In pluripotent ES cells lacking both Suv39h KMTs, H3.1 accumulation is lost, whereas we observed an, even more, exacerbated H3.1 accumulation in differentiated MEF cells with the same deletions. Why such discrepancy? In the case of differentiated cells, the DNA methylation status is not affected and the general transcription de-repression in absence of Suv39 does not affect major satellites. In the case of pluripotent cells, lacking both DNA methylation and repressive histone PTMs, including H3K9me2/3, H3.3-DAXX-ATRAX deposition could potentially compensate for the lack of H3.1 enrichment and thereby help in protecting these regions from DNA damage (He *et al.*, 2015; Canat *et al.*, 2021). It is important to stress that the absence of DNA methylation might give rise to chromosomal rearrangements of reactivated transposable elements in search of their homologous sequences (Zamudio *et al.*, 2015), for which a cell cannot tolerate, even in absence of all Suv39 isoforms (Montavon *et al.*, 2021). Recent reports observe that, in ES cells, major satellite repeats can recruit pioneer transcription factors, including Nanog, at PHCs to favor their local

decondensation (Novo *et al.*, 2016). Surprisingly, major satellite transcripts are not highly expressed in pluripotent as compared to differentiated cells (Martens *et al.*, 2005). If so then, it is unclear how a basal level of major satellites would be necessary for ES cells. One hypothesis could be due to the presence of m6A RNA modifications present on major satellite transcripts (Duda *et al.*, 2021) that favor the presence of RNA:DNA hybrids used as scaffolds for binding proteins (Camacho *et al.*, 2017). In line with this, our results show that Suv39h2, possessing an N-terminal domain with an additional affinity for major satellite repeats RNA, can help restore H3.1 accumulation at these sites. For this, H3.1 is preferentially decorated in H3K9me1 reinforcing Suv39h1 activity and promoting H3K9me3 and HP1 presence at PHCs (Loyola *et al.*, 2006). Collectively, steady incorporation of H3.1 might be required to bookmark these regions for further heterochromatin formation.

Histone variants & chaperones' effects on cancer and cell fate decision

Understanding the interplay between epigenome and tumorigenesis will help us to decipher the key factors involved in establishing nuclear domain organization.

Along with mutations on histones, many chromatin remodelers have been recently found altered and involved in cancer context (Vogelstein *et al.*, 2013). Among histone chaperones, ATRX absence is linked with alternative lengthening telomeres (ALT) cancers (Jiao *et al.*, 2011; Deeg *et al.*, 2017) and the spreading of H3.3S31ph on chromosome arms (Chang *et al.*, 2015). In a recent study, metastasis inducers have been found to lead to a reduction in CAF-1 expression with consequent H3.3-HIRA deposition at promoters and genes of upregulated pro-tumorigenic genes involved in epithelial-to-mesenchymal transition (EMT) (Gomes *et al.*, 2019). Indeed, CAF-1 acts as a master regulator of chromatin accessibility as its depletion increases cell state potential and cellular reprogramming (Cheloufi *et al.*, 2015; Ishiuchi *et al.*, 2015; Franklin *et al.*, 2022). Of note, CAF-1 and HIRA mutations have not been linked with pediatric cancer so far. The most parsimonious explanation is that lethal effects arising from both HIRA and CAF-1 mutations make any possible phenotype harder to be appreciated before development occurs.

Notably, centromeres are functional chromatin territories commonly found altered in cancer. Translating these observations into a human context can reveal new biomarkers of tumor cell states. For example, the clustering

and distribution of CENP-A labeled centromeres are drastically altered in human tumor samples compared to healthy tissues or benign lesions (Verrelle *et al.*, 2021). However, it is still unclear if it is CENP-A overexpression and its ectopic localization aside from centromeres that is one of the factors contributing to tumorigenesis (Lacoste *et al.*, 2014; Jeffery *et al.*, 2021). While CENP-A is generally associated with tumor aggressiveness depending on the genetic background and the anatomical context (Renaud-Pageot *et al.*, 2022), the balance in H3 variant dynamics provides means that can be followed according to cell fate and metastasis control (Schlesinger and Meshorer, 2019). For instance, new histone variants, and H3 mutants oncohistones, are discovered and found mutated in several cancers (Nacev *et al.*, 2019). It remains to be defined if these mutations are drivers or passengers of tumorigenesis.

Finally, the development and use of epigenetic drugs will be crucial to resolving previously incurable cancers (Mohammad *et al.*, 2017; Morel *et al.*, 2019). However, many limitations need to be overcome for efficient therapies and to better understand the biology controlling these processes. Deciphering the molecular mechanisms governing metastatic induction that, in turn, influences histone dynamics with severe impacts on nuclear architecture, will be a major challenge and a fundamental step to providing means of preventing tumorigenesis and unraveling novel prognosis or diagnostic markers.

Conclusion

My Ph.D. project aimed to deepen the interplay occurring between histone variants and mouse chromocenters in different cell fates. Several studies addressed already how histones and their variants, in particular H3.3, are enriched over genome-wide elements and how they correlate with an effect on transcriptional programs output. However, it is still unknown how the replicative variants' distribution and in particular their enrichment at constitutive heterochromatin regions occurs.

My first contribution was a technological development as I implemented a suitable mouse ES cells system in our lab to gain insight into how histone variants are differentially organized during mouse differentiation. This required adjustments in terms of experimental conditions, given the use of a different biological system compared to human equivalent ones (i.e., HeLa). I combined this system with the SNAP-tag technology and detection of endogenous histones which allowed me to monitor histone deposition over differentiation events. Given their genome-wide distribution and the challenges to establishing a correct pipeline of differentiation, this is to our knowledge the first complete roadmap of H3.1 and H3.3 distribution along with repetitive elements and at specific nuclear domains throughout differentiation.

The second contribution was to address biological issues providing a series of important results concerning the dynamics of H3 variants at a specific nuclear subdomain. First, I established how histone variant distribution is differentially organized in the nucleus of several mouse cells. By combining this methodology with high-resolution imaging and sequencing approaches I could provide a full overview of histone variants enrichment genome-wide and over repetitive elements. Secondly, I formally prove that the DNA replication coupled pathway is the main driver for H3.1 contribution to nucleosome composition at mouse chromocenters. Permutations of aminoacidic residues in the amino-terminal tail (to mimic the H3.3 variant) or allowing recognition by their respective histone-chaperones revealed that specific enrichment of the replicative histone variants at the chromocenter depended on his deposition mode. To my knowledge, this is the first work establishing a link between replicative variants and chromocenters in the mammalian system during differentiation events.

Importantly, the approach developed during my Ph.D. will establish the basis to help better understand how histone variant dynamics occur in other contexts. For example, a similar approach can be used to study other histone variant functions, i.e., CENP-A and its involvement in centromere composition by its chaperone(s). Ultimately, understanding how variants of histones, including oncohistones, reshuffle chromocenters' composition and impinge on higher-order nuclear structures will emerge. The complete characterization of histone variants, chaperones, and nuclear domains will define their exact epigenetic role in other contexts including disease and development.

References

- Adam, M. *et al.* (2001) 'H2A.Z Is Required for Global Chromatin Integrity and for Recruitment of RNA Polymerase II under Specific Conditions', *Molecular and Cellular Biology*, 21(18), pp. 6270–6279. Available at: <https://doi.org/10.1128/MCB.21.18.6270-6279.2001>.
- Adam, S. *et al.* (2016) 'Real-Time Tracking of Parental Histones Reveals Their Contribution to Chromatin Integrity Following DNA Damage', *Molecular Cell*, 64(1), pp. 65–78. Available at: <https://doi.org/10.1016/j.molcel.2016.08.019>.
- Ahmad, K. and Henikoff, S. (2001) 'Centromeres Are Specialized Replication Domains in Heterochromatin', *The Journal of Cell Biology*, 153(1), pp. 101–110. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2185517/> (Accessed: 2 May 2022).
- Ahmad, K. and Henikoff, S. (2002) 'Histone H3 variants specify modes of chromatin assembly', *Proceedings of the National Academy of Sciences*, 99(suppl_4), pp. 16477–16484. Available at: <https://doi.org/10.1073/pnas.172403699>.
- Aho, S. *et al.* (2000) 'Ubinuclein, a Novel Nuclear Protein Interacting with Cellular and Viral Transcription Factors', *Journal of Cell Biology*, 148(6), pp. 1165–1176. Available at: <https://doi.org/10.1083/jcb.148.6.1165>.
- Allan, R.S. *et al.* (2012) 'An epigenetic silencing pathway controlling T helper 2 cell lineage commitment', *Nature*, 487(7406), pp. 249–253. Available at: <https://doi.org/10.1038/nature11173>.
- Allfrey, V.G., Faulkner, R. and Mirsky, A.E. (1964) 'ACETYLATION AND METHYLATION OF HISTONES AND THEIR POSSIBLE ROLE IN THE REGULATION OF RNA SYNTHESIS', *Proceedings of the National Academy of Sciences of the United States of America*, 51, pp. 786–794. Available at: <https://doi.org/10.1073/pnas.51.5.786>.
- Almouzni, G. *et al.* (1994) 'Histone acetylation influences both gene expression and development of *Xenopus laevis*', *Developmental Biology*, 165(2), pp. 654–669. Available at: <https://doi.org/10.1006/dbio.1994.1283>.
- Almouzni, G. and Probst, A.V. (2011) 'Heterochromatin maintenance and establishment: Lessons from the mouse pericentromere', *Nucleus*, 2(5), pp. 332–338. Available at: <https://doi.org/10.4161/nucl.2.5.17707>.
- Altomose, N. *et al.* (2022) 'Complete genomic and epigenetic maps of human centromeres', *Science*, 376(6588), p. eabl4178. Available at: <https://doi.org/10.1126/science.abl4178>.
- Arents, G. *et al.* (1991) 'The nucleosomal core histone octamer at 3.1 Å resolution: a tripartite protein assembly and a left-handed superhelix', *Proceedings of the National Academy of Sciences of the United States of America*, 88(22), pp. 10148–10152. Available at: <https://doi.org/10.1073/pnas.88.22.10148>.
- Arents, G. and Moudrianakis, E.N. (1995) 'The histone fold: a ubiquitous architectural motif utilized in DNA compaction and protein dimerization', *Proceedings of the National Academy of Sciences of the United States of America*, 92(24), pp. 11170–11174. Available at: <https://doi.org/10.1073/pnas.92.24.11170>.
- Armache, A. *et al.* (2020) 'Histone H3.3 phosphorylation amplifies stimulation-induced transcription', *Nature*, 583(7818), pp. 852–857. Available at: <https://doi.org/10.1038/s41586-020-2533-0>.
- Azuara, V. (2006) 'Profiling of DNA replication timing in unsynchronized cell populations', *Nature Protocols*, 1(4), pp. 2171–2177. Available at: <https://doi.org/10.1038/nprot.2006.353>.
- Baccarini, P. (1908) *Sulle cinesi vegetative del "Cynomorium coccineum L."*. 15(2):189–203. (Ser 15(2):189–203).

- Bagchi, D.N. *et al.* (2020) 'The histone variant H2A.Z in yeast is almost exclusively incorporated into the +1 nucleosome in the direction of transcription', *Nucleic Acids Research*, 48(1), pp. 157–170. Available at: <https://doi.org/10.1093/nar/gkz1075>.
- Banaszynski, L.A. *et al.* (2013) 'Hira-Dependent Histone H3.3 Deposition Facilitates PRC2 Recruitment at Developmental Loci in ES Cells', *Cell*, 155(1), pp. 107–120. Available at: <https://doi.org/10.1016/j.cell.2013.08.061>.
- Bannister, A.J. *et al.* (2001) 'Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain', *Nature*, 410(6824), pp. 120–124. Available at: <https://doi.org/10.1038/35065138>.
- Banumathy, G. *et al.* (2009) 'Human UBN1 Is an Ortholog of Yeast Hpc2p and Has an Essential Role in the HIRA/ASF1a Chromatin-Remodeling Pathway in Senescent Cells', *Molecular and Cellular Biology*, 29(3), pp. 758–770. Available at: <https://doi.org/10.1128/MCB.01047-08>.
- Barau, J. *et al.* (2016) 'The DNA methyltransferase DNMT3C protects male germ cells from transposon activity', *Science (New York, N.Y.)*, 354(6314), pp. 909–912. Available at: <https://doi.org/10.1126/science.aah5143>.
- Barnhart, M.C. *et al.* (2011) 'HJURP is a CENP-A chromatin assembly factor sufficient to form a functional de novo kinetochore', *Journal of Cell Biology*, 194(2), pp. 229–243. Available at: <https://doi.org/10.1083/jcb.201012017>.
- Barral, S. *et al.* (2017) 'Histone Variant H2A.L.2 Guides Transition Protein-Dependent Protamine Assembly in Male Germ Cells', *Molecular Cell*, 66(1), pp. 89–101.e8. Available at: <https://doi.org/10.1016/j.molcel.2017.02.025>.
- Barski, A. *et al.* (2007) 'High-Resolution Profiling of Histone Methylations in the Human Genome', *Cell*, 129(4), pp. 823–837. Available at: <https://doi.org/10.1016/j.cell.2007.05.009>.
- Bartke, T. *et al.* (2010) 'Nucleosome-interacting proteins regulated by DNA and histone methylation', *Cell*, 143(3), pp. 470–484. Available at: <https://doi.org/10.1016/j.cell.2010.10.012>.
- Baubec, T. *et al.* (2015) 'Genomic profiling of DNA methyltransferases reveals a role for DNMT3B in genic methylation', *Nature*, 520(7546), pp. 243–247. Available at: <https://doi.org/10.1038/nature14176>.
- Beard, C. *et al.* (2006) 'Efficient method to generate single-copy transgenic mice by site-specific integration in embryonic stem cells', *genesis*, 44(1), pp. 23–28. Available at: <https://doi.org/10.1002/gene.20180>.
- Becker, J.S., Nicetto, D. and Zaret, K.S. (2016) 'H3K9me3-Dependent Heterochromatin: Barrier to Cell Fate Changes', *Trends in Genetics*, 32(1), pp. 29–41. Available at: <https://doi.org/10.1016/j.tig.2015.11.001>.
- Behjati, S. *et al.* (2013) 'Distinct H3F3A and H3F3B driver mutations define chondroblastoma and giant cell tumor of bone', *Nature Genetics*, 45(12), pp. 1479–1482. Available at: <https://doi.org/10.1038/ng.2814>.
- Bender, C.M. *et al.* (1999) 'Roles of Cell Division and Gene Transcription in the Methylation of CpG Islands', *Molecular and Cellular Biology*, 19(10), pp. 6690–6698. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC84656/> (Accessed: 20 May 2022).
- Bender, S. *et al.* (2013) 'Reduced H3K27me3 and DNA hypomethylation are major drivers of gene expression in K27M mutant pediatric high-grade gliomas', *Cancer Cell*, 24(5), pp. 660–672. Available at: <https://doi.org/10.1016/j.ccr.2013.10.006>.
- Benoit, M. *et al.* (2019) 'Replication-coupled histone H3.1 deposition determines nucleosome composition and heterochromatin dynamics during Arabidopsis seedling development', *New Phytologist*, 221(1), pp. 385–398. Available at: <https://doi.org/10.1111/nph.15248>.

- Bergmüller, E., Gehrig, P.M. and Grussem, W. (2007) 'Characterization of Post-Translational Modifications of Histone H2B-Variants Isolated from *Arabidopsis thaliana*', *Journal of Proteome Research*, 6(9), pp. 3655–3668. Available at: <https://doi.org/10.1021/pr0702159>.
- Bernstein, B.E. *et al.* (2005) 'Genomic maps and comparative analysis of histone modifications in human and mouse', *Cell*, 120(2), pp. 169–181. Available at: <https://doi.org/10.1016/j.cell.2005.01.001>.
- Bernstein, B.E. *et al.* (2006) 'A Bivalent Chromatin Structure Marks Key Developmental Genes in Embryonic Stem Cells', *Cell*, 125(2), pp. 315–326. Available at: <https://doi.org/10.1016/j.cell.2006.02.041>.
- Betzig, E. *et al.* (2006) 'Imaging Intracellular Fluorescent Proteins at Nanometer Resolution', *Science*, 313(5793), pp. 1642–1645. Available at: <https://doi.org/10.1126/science.1127344>.
- Bilodeau, S. *et al.* (2009) 'SetDB1 contributes to repression of genes encoding developmental regulators and maintenance of ES cell state', *Genes & Development*, 23(21), pp. 2484–2489. Available at: <https://doi.org/10.1101/gad.1837309>.
- Bintu, B. *et al.* (2018) 'Super-resolution chromatin tracing reveals domains and cooperative interactions in single cells', *Science*, 362(6413). Available at: <https://doi.org/10.1126/science.aau1783>.
- Bird, A. *et al.* (1985) 'A fraction of the mouse genome that is derived from islands of nonmethylated, CpG-rich DNA', *Cell*, 40(1), pp. 91–99. Available at: [https://doi.org/10.1016/0092-8674\(85\)90312-5](https://doi.org/10.1016/0092-8674(85)90312-5).
- Bird, A. (2007) 'Perceptions of epigenetics', *Nature*, 447(7143), pp. 396–398. Available at: <https://doi.org/10.1038/nature05913>.
- Bjerke, L. *et al.* (2013) 'Histone H3.3. mutations drive pediatric glioblastoma through upregulation of MYCN', *Cancer Discovery*, 3(5), pp. 512–519. Available at: <https://doi.org/10.1158/2159-8290.CD-12-0426>.
- Boettiger, A.N. *et al.* (2016) 'Super-resolution imaging reveals distinct chromatin folding for different epigenetic states', *Nature*, 529(7586), pp. 418–422. Available at: <https://doi.org/10.1038/nature16496>.
- Bolzer, A. *et al.* (2005) 'Three-Dimensional Maps of All Chromosomes in Human Male Fibroblast Nuclei and Prometaphase Rosettes', *PLOS Biology*, 3(5), p. e157. Available at: <https://doi.org/10.1371/journal.pbio.0030157>.
- Borg, M. *et al.* (2020) 'Targeted reprogramming of H3K27me3 resets epigenetic memory in plant paternal chromatin', *Nature Cell Biology*, 22(6), pp. 621–629. Available at: <https://doi.org/10.1038/s41556-020-0515-y>.
- Boskovic, A. *et al.* (2014) 'Higher chromatin mobility supports totipotency and precedes pluripotency in vivo', *Genes & Development*, 28(10), pp. 1042–1047. Available at: <https://doi.org/10.1101/gad.238881.114>.
- Bourc'his, D. *et al.* (2001) 'Dnmt3L and the establishment of maternal genomic imprints', *Science (New York, N.Y.)*, 294(5551), pp. 2536–2539. Available at: <https://doi.org/10.1126/science.1065848>.
- Bouvier, D. *et al.* (2021) 'Dissecting regulatory pathways for transcription recovery following DNA damage reveals a non-canonical function of the histone chaperone HIRA', *Nature Communications*, 12(1), p. 3835. Available at: <https://doi.org/10.1038/s41467-021-24153-1>.
- Boveri, T. (1904) *Ergebnisse über die Konstitution der chromatischen Substanz des Zellkerns / von Theodor Boveri.*, *Wellcome Collection*. Available at: <https://wellcomecollection.org/works/an45kexq> (Accessed: 2 May 2022).

- Boyes, J. and Bird, A. (1991) 'DNA methylation inhibits transcription indirectly via a methyl-CpG binding protein', *Cell*, 64(6), pp. 1123–1134. Available at: [https://doi.org/10.1016/0092-8674\(91\)90267-3](https://doi.org/10.1016/0092-8674(91)90267-3).
- Bradley, A. *et al.* (1984) 'Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines', *Nature*, 309(5965), pp. 255–256. Available at: <https://doi.org/10.1038/309255a0>.
- Brinkman, A.B. *et al.* (2012) 'Sequential ChIP-bisulfite sequencing enables direct genome-scale investigation of chromatin and DNA methylation cross-talk', *Genome Research*, 22(6), pp. 1128–1138. Available at: <https://doi.org/10.1101/gr.133728.111>.
- Bulut-Karslioglu *et al.* (2014) 'Suv39h-Dependent H3K9me3 Marks Intact Retrotransposons and Silences LINE Elements in Mouse Embryonic Stem Cells', *Molecular Cell*, 55(2), pp. 277–290. Available at: <https://doi.org/10.1016/j.molcel.2014.05.029>.
- Bulut-Karslioglu, A. *et al.* (2012) 'A transcription factor-based mechanism for mouse heterochromatin formation', *Nature Structural & Molecular Biology*, 19(10), pp. 1023–1030. Available at: <https://doi.org/10.1038/nsmb.2382>.
- Burton, A. *et al.* (2020) 'Heterochromatin establishment during early mammalian development is regulated by pericentromeric RNA and characterized by non-repressive H3K9me3', *Nature Cell Biology*, 22(7), pp. 767–778. Available at: <https://doi.org/10.1038/s41556-020-0536-6>.
- Buschbeck, M. and Hake, S.B. (2017) 'Variants of core histones and their roles in cell fate decisions, development and cancer', *Nature Reviews Molecular Cell Biology*, 18(5), pp. 299–314. Available at: <https://doi.org/10.1038/nrm.2016.166>.
- Bush, K.M. *et al.* (2013) 'Endogenous mammalian histone H3.3 exhibits chromatin-related functions during development', *Epigenetics & Chromatin*, 6(1), p. 7. Available at: <https://doi.org/10.1186/1756-8935-6-7>.
- Canat, A. *et al.* (2021) *DAXX safeguards pericentromeric heterochromatin formation in embryonic stem cells*. preprint. Molecular Biology. Available at: <https://doi.org/10.1101/2021.04.28.441827>.
- Cantaloube, S. *et al.* (2012) 'Characterization of chromatin domains by 3D fluorescence microscopy: An automated methodology for quantitative analysis and nuclei screening', *BioEssays*, 34(6), pp. 509–517. Available at: <https://doi.org/10.1002/bies.201100188>.
- Casanova, M. *et al.* (2013) 'Heterochromatin Reorganization during Early Mouse Development Requires a Single-Stranded Noncoding Transcript', *Cell Reports*, 4(6), pp. 1156–1167. Available at: <https://doi.org/10.1016/j.celrep.2013.08.015>.
- Chagin, V.O. *et al.* (2016) '4D Visualization of replication foci in mammalian cells corresponding to individual replicons', *Nature Communications*, 7(1), pp. 1–12. Available at: <https://doi.org/10.1038/ncomms11231>.
- Chagin, V.O. *et al.* (2019) 'Processive DNA synthesis is associated with localized decompaction of constitutive heterochromatin at the sites of DNA replication and repair', *Nucleus*, 10(1), pp. 231–253. Available at: <https://doi.org/10.1080/19491034.2019.1688932>.
- Chambers, I. *et al.* (2007) 'Nanog safeguards pluripotency and mediates germline development', *Nature*, 450(7173), pp. 1230–1234. Available at: <https://doi.org/10.1038/nature06403>.
- Chang, E.Y. *et al.* (2008) 'MacroH2A Allows ATP-Dependent Chromatin Remodeling by SWI/SNF and ACF Complexes but Specifically Reduces Recruitment of SWI/SNF', *Biochemistry*, 47(51), pp. 13726–13732. Available at: <https://doi.org/10.1021/bi8016944>.

- Chang, F.T.M. *et al.* (2015) 'CHK1-driven histone H3.3 serine 31 phosphorylation is important for chromatin maintenance and cell survival in human ALT cancer cells', *Nucleic Acids Research*, 43(5), pp. 2603–2614. Available at: <https://doi.org/10.1093/nar/gkv104>.
- Cheloufi, S. *et al.* (2015) 'The histone chaperone CAF-1 safeguards somatic cell identity', *Nature*, 528(7581), pp. 218–224. Available at: <https://doi.org/10.1038/nature15749>.
- Cheloufi, S. and Hochedlinger, K. (2017) 'Emerging roles of the histone chaperone CAF-1 in cellular plasticity', *Current opinion in genetics & development*, 46, pp. 83–94. Available at: <https://doi.org/10.1016/j.gde.2017.06.004>.
- Clément, C. *et al.* (2018) 'High-resolution visualization of H3 variants during replication reveals their controlled recycling', *Nature Communications*, 9(1), p. 3181. Available at: <https://doi.org/10.1038/s41467-018-05697-1>.
- Clowney, E.J. *et al.* (2012) 'Nuclear Aggregation of Olfactory Receptor Genes Governs Their Monogenic Expression', *Cell*, 151(4), pp. 724–737. Available at: <https://doi.org/10.1016/j.cell.2012.09.043>.
- Clynes, D. *et al.* (2015) 'Suppression of the alternative lengthening of telomere pathway by the chromatin remodelling factor ATRX', *Nature Communications*, 6(1), p. 7538. Available at: <https://doi.org/10.1038/ncomms8538>.
- Coleman-Derr, D. and Zilberman, D. (2012) 'Deposition of histone variant H2A.Z within gene bodies regulates responsive genes', *PLoS genetics*, 8(10), p. e1002988. Available at: <https://doi.org/10.1371/journal.pgen.1002988>.
- Condic, M.L. (2014) 'Totipotency: What It Is and What It Is Not', *Stem Cells and Development*, 23(8), pp. 796–812. Available at: <https://doi.org/10.1089/scd.2013.0364>.
- Cosgrove, M.S., Boeke, J.D. and Wolberger, C. (2004) 'Regulated nucleosome mobility and the histone code', *Nature Structural & Molecular Biology*, 11(11), pp. 1037–1043. Available at: <https://doi.org/10.1038/nsmb851>.
- Couldrey, C. *et al.* (1999) 'A retroviral Gene Trap Insertion into the Histone 3.3A Gene Causes Partial Neonatal Lethality, Stunted Growth, Neuromuscular Deficits and Male Sub-fertility in Transgenic Mice', *Human Molecular Genetics*, 8(13), pp. 2489–2495. Available at: <https://doi.org/10.1093/hmg/8.13.2489>.
- Cournac, A., Koszul, R. and Mozziconacci, J. (2016) 'The 3D folding of metazoan genomes correlates with the association of similar repetitive elements', *Nucleic Acids Research*, 44(1), pp. 245–255. Available at: <https://doi.org/10.1093/nar/gkv1292>.
- Cowieson, N.P. *et al.* (2000) 'Dimerisation of a chromo shadow domain and distinctions from the chromodomain as revealed by structural analysis', *Current Biology*, 10(9), pp. 517–525. Available at: [https://doi.org/10.1016/S0960-9822\(00\)00467-X](https://doi.org/10.1016/S0960-9822(00)00467-X).
- Creyghton, M.P. *et al.* (2010) 'Histone H3K27ac separates active from poised enhancers and predicts developmental state', *Proceedings of the National Academy of Sciences of the United States of America*, 107(50), pp. 21931–21936. Available at: <https://doi.org/10.1073/pnas.1016071107>.
- Daniel Ricketts, M. *et al.* (2015) 'Ubinuclein-1 confers histone H3.3-specific-binding by the HIRA histone chaperone complex', *Nature Communications*, 6(1), p. 7711. Available at: <https://doi.org/10.1038/ncomms8711>.
- Dann, G.P. *et al.* (2017) 'ISWI chromatin remodellers sense nucleosome modifications to determine substrate preference', *Nature*, 548(7669), pp. 607–611. Available at: <https://doi.org/10.1038/nature23671>.

- Davarinejad, H. *et al.* (2022) ‘The histone H3.1 variant regulates TONSOKU-mediated DNA repair during replication’, *Science*, 375(6586), pp. 1281–1286. Available at: <https://doi.org/10.1126/science.abm5320>.
- Davey, C.A. and Richmond, T.J. (2002) ‘DNA-dependent divalent cation binding in the nucleosome core particle’, *Proceedings of the National Academy of Sciences of the United States of America*, 99(17), pp. 11169–11174. Available at: <https://doi.org/10.1073/pnas.172271399>.
- Davies, H.G. (1967) ‘Fine structure of heterochromatin in certain cell nuclei’, *Nature*, 214(5084), pp. 208–210. Available at: <https://doi.org/10.1038/214208a0>.
- De Koning, L. *et al.* (2007) ‘Histone chaperones: an escort network regulating histone traffic’, *Nature Structural & Molecular Biology*, 14(11), pp. 997–1007. Available at: <https://doi.org/10.1038/nsmb1318>.
- Deal, R.B., Henikoff, J.G. and Henikoff, S. (2010) ‘Genome-Wide Kinetics of Nucleosome Turnover Determined by Metabolic Labeling of Histones’, *Science*, 328(5982), pp. 1161–1164. Available at: <https://doi.org/10.1126/science.1186777>.
- Deaton, A.M. *et al.* (2016) ‘Enhancer regions show high histone H3.3 turnover that changes during differentiation’, *eLife*, 5, p. e15316. Available at: <https://doi.org/10.7554/eLife.15316>.
- Deeg, K.I. *et al.* (2017) ‘Dissecting telomere maintenance mechanisms in pediatric glioblastoma’. *bioRxiv*, p. 129106. Available at: <https://doi.org/10.1101/129106>.
- Déjardin, J. (2015) ‘Switching between Epigenetic States at Pericentromeric Heterochromatin’, *Trends in Genetics*, 31(11), pp. 661–672. Available at: <https://doi.org/10.1016/j.tig.2015.09.003>.
- Déjardin, J. and Kingston, R.E. (2009) ‘Purification of Proteins Associated with Specific Genomic Loci’, *Cell*, 136(1), pp. 175–186. Available at: <https://doi.org/10.1016/j.cell.2008.11.045>.
- Delaney, K. and Almouzni, G. (2022) ‘Transcription-coupled H3.3 recycling: A link with chromatin states’, *Seminars in Cell & Developmental Biology*, pp. S1084–9521(22)00159–8. Available at: <https://doi.org/10.1016/j.semcdb.2022.05.003>.
- Délérès, A., Berger, F. and Duhaucourt, S. (2021) ‘Role of Polycomb in the control of transposable elements’, *Trends in Genetics*, 37(10), pp. 882–889. Available at: <https://doi.org/10.1016/j.tig.2021.06.003>.
- Deluz, C. *et al.* (2016) ‘A role for mitotic bookmarking of SOX2 in pluripotency and differentiation’, *Genes & Development*, 30(22), pp. 2538–2550. Available at: <https://doi.org/10.1101/gad.289256.116>.
- Dhayalan, A. *et al.* (2010) ‘The Dnmt3a PWWP domain reads histone 3 lysine 36 trimethylation and guides DNA methylation’, *The Journal of Biological Chemistry*, 285(34), pp. 26114–26120. Available at: <https://doi.org/10.1074/jbc.M109.089433>.
- Dimitrov, S. *et al.* (1993) ‘Chromatin transitions during early *Xenopus* embryogenesis: changes in histone H4 acetylation and in linker histone type’, *Developmental Biology*, 160(1), pp. 214–227. Available at: <https://doi.org/10.1006/dbio.1993.1299>.
- Dimitrova, D.S. and Berezney, R. (2002) ‘The spatio-temporal organization of DNA replication sites is identical in primary, immortalized and transformed mammalian cells’, *Journal of Cell Science*, 115(Pt 21), pp. 4037–4051. Available at: <https://doi.org/10.1242/jcs.00087>.
- Dixon, J.R. *et al.* (2012) ‘Topological domains in mammalian genomes identified by analysis of chromatin interactions’, *Nature*, 485(7398), pp. 376–380. Available at: <https://doi.org/10.1038/nature11082>.

- Dodge, J.E. *et al.* (2004) 'Histone H3-K9 methyltransferase ESET is essential for early development', *Molecular and Cellular Biology*, 24(6), pp. 2478–2486. Available at: <https://doi.org/10.1128/MCB.24.6.2478-2486.2004>.
- Dong, F. and Jiang, J. (1998) 'Non-Rabl patterns of centromere and telomere distribution in the interphase nuclei of plant cells', *Chromosome Research: An International Journal on the Molecular, Supramolecular and Evolutionary Aspects of Chromosome Biology*, 6(7), pp. 551–558. Available at: <https://doi.org/10.1023/a:1009280425125>.
- Doyen, C.-M. *et al.* (2006) 'Mechanism of Polymerase II Transcription Repression by the Histone Variant macroH2A', *Molecular and Cellular Biology*, 26(3), pp. 1156–1164. Available at: <https://doi.org/10.1128/MCB.26.3.1156-1164.2006>.
- Drané, P. *et al.* (2010) 'The death-associated protein DAXX is a novel histone chaperone involved in the replication-independent deposition of H3.3', *Genes & Development*, 24(12), pp. 1253–1265. Available at: <https://doi.org/10.1101/gad.566910>.
- Drinnenberg, I.A., Henikoff, S. and Malik, H.S. (2016) 'Evolutionary Turnover of Kinetochore Proteins: A Ship of Theseus?', *Trends in Cell Biology*, 26(7), pp. 498–510. Available at: <https://doi.org/10.1016/j.tcb.2016.01.005>.
- Duda, K.J. *et al.* (2021) 'm6A RNA methylation of major satellite repeat transcripts facilitates chromatin association and RNA:DNA hybrid formation in mouse heterochromatin', *Nucleic Acids Research*, 49(10), pp. 5568–5587. Available at: <https://doi.org/10.1093/nar/gkab364>.
- Dunleavy, E.M. *et al.* (2009) 'HJURP Is a Cell-Cycle-Dependent Maintenance and Deposition Factor of CENP-A at Centromeres', *Cell*, 137(3), pp. 485–497. Available at: <https://doi.org/10.1016/j.cell.2009.02.040>.
- Dunleavy, E.M., Almouzni, G. and Karpen, G.H. (2011) 'H3.3 is deposited at centromeres in S phase as a placeholder for newly assembled CENP-A in G1 phase', *Nucleus*, 2(2), pp. 146–157. Available at: <https://doi.org/10.4161/nucl.2.2.15211>.
- Düster, R. *et al.* (2021) '1,6-Hexanediol, commonly used to dissolve liquid–liquid phase separated condensates, directly impairs kinase and phosphatase activities', *Journal of Biological Chemistry*, 296. Available at: <https://doi.org/10.1016/j.jbc.2021.100260>.
- Earnshaw, W.C. and Rothfield, N. (1985) 'Identification of a family of human centromere proteins using autoimmune sera from patients with scleroderma', *Chromosoma*, 91(3–4), pp. 313–321. Available at: <https://doi.org/10.1007/BF00328227>.
- Eberhart, A. *et al.* (2013) 'Epigenetics of eu- and heterochromatin in inverted and conventional nuclei from mouse retina', *Chromosome Research*, 21(5), pp. 535–554. Available at: <https://doi.org/10.1007/s10577-013-9375-7>.
- Efroni, S. *et al.* (2008) 'Global Transcription in Pluripotent Embryonic Stem Cells', *Cell Stem Cell*, 2(5), pp. 437–447. Available at: <https://doi.org/10.1016/j.stem.2008.03.021>.
- Eissenberg, J.C. *et al.* (1990) 'Mutation in a heterochromatin-specific chromosomal protein is associated with suppression of position-effect variegation in *Drosophila melanogaster*.', *Proceedings of the National Academy of Sciences*, 87(24), pp. 9923–9927. Available at: <https://doi.org/10.1073/pnas.87.24.9923>.
- Elsässer, S.J. *et al.* (2012) 'DAXX envelops a histone H3.3–H4 dimer for H3.3-specific recognition', *Nature*, 491(7425), pp. 560–565. Available at: <https://doi.org/10.1038/nature11608>.

- Elsässer, S.J. *et al.* (2015) ‘Histone H3.3 is required for endogenous retroviral element silencing in embryonic stem cells’, *Nature*, 522(7555), pp. 240–244. Available at: <https://doi.org/10.1038/nature14345>.
- Erdel, F. *et al.* (2020) ‘Mouse Heterochromatin Adopts Digital Compaction States without Showing Hallmarks of HP1-Driven Liquid-Liquid Phase Separation’, *Molecular Cell*, 78(2), pp. 236–249.e7. Available at: <https://doi.org/10.1016/j.molcel.2020.02.005>.
- Escobar, T.M. *et al.* (2019) ‘Active and Repressed Chromatin Domains Exhibit Distinct Nucleosome Segregation during DNA Replication’, *Cell*, 179(4), pp. 953–963.e11. Available at: <https://doi.org/10.1016/j.cell.2019.10.009>.
- Evans, M.J. and Kaufman, M.H. (1981) ‘Establishment in culture of pluripotential cells from mouse embryos’, *Nature*, 292(5819), pp. 154–156. Available at: <https://doi.org/10.1038/292154a0>.
- Fachinetti, D. *et al.* (2013) ‘A two-step mechanism for epigenetic specification of centromere identity and function’, *Nature Cell Biology*, 15(9), pp. 1056–1066. Available at: <https://doi.org/10.1038/ncb2805>.
- Fadloun, A., Eid, A. and Torres-Padilla, M.-E. (2013) ‘Chapter One - Mechanisms and Dynamics of Heterochromatin Formation During Mammalian Development: Closed Paths and Open Questions’, in E. Heard (ed.) *Current Topics in Developmental Biology*. Academic Press (Epigenetics and Development), pp. 1–45. Available at: <https://doi.org/10.1016/B978-0-12-416027-9.00001-2>.
- Fang, H.-T. *et al.* (2018) ‘Global H3.3 dynamic deposition defines its bimodal role in cell fate transition’, *Nature Communications*, 9(1), p. 1537. Available at: <https://doi.org/10.1038/s41467-018-03904-7>.
- Farcas, A.M. *et al.* (2012) ‘KDM2B links the Polycomb Repressive Complex 1 (PRC1) to recognition of CpG islands’, *eLife*. Edited by K. Struhl, 1, p. e00205. Available at: <https://doi.org/10.7554/eLife.00205>.
- Festuccia, N. *et al.* (2016) ‘Mitotic binding of Esrrb marks key regulatory regions of the pluripotency network’, *Nature Cell Biology*, 18(11), pp. 1139–1148. Available at: <https://doi.org/10.1038/ncb3418>.
- Ficz, G. *et al.* (2013) ‘FGF Signaling Inhibition in ESCs Drives Rapid Genome-wide Demethylation to the Epigenetic Ground State of Pluripotency’, *Cell Stem Cell*, 13(3), pp. 351–359. Available at: <https://doi.org/10.1016/j.stem.2013.06.004>.
- Finch, J.T. and Klug, A. (1976) ‘Solenoidal model for superstructure in chromatin’, *Proceedings of the National Academy of Sciences of the United States of America*, 73(6), pp. 1897–1901. Available at: <https://doi.org/10.1073/pnas.73.6.1897>.
- Flemming, W. (1879) ‘Ueber das Verhalten des Kerns bei der Zelltheilung, und über die Bedeutung mehrkerniger Zellen’, *Archiv für pathologische Anatomie und Physiologie und für klinische Medizin*, 77(1), pp. 1–29.
- Flemming, W. (1882) *Zellsubstanz, Kern und Zelltheilung*. Vogel.
- Foltz, D.R. *et al.* (2009) ‘Centromere-Specific Assembly of CENP-A Nucleosomes Is Mediated by HJURP’, *Cell*, 137(3), pp. 472–484. Available at: <https://doi.org/10.1016/j.cell.2009.02.039>.
- Franklin, R. *et al.* (2022) ‘Regulation of chromatin accessibility by the histone chaperone CAF-1 sustains lineage fidelity’, *Nature Communications*, 13(1), p. 2350. Available at: <https://doi.org/10.1038/s41467-022-29730-6>.
- Franklin, R., Murn, J. and Cheloufi, S. (2021) ‘Cell Fate Decisions in the Wake of Histone H3 Deposition’, *Frontiers in Cell and Developmental Biology*, 9. Available at: <https://www.frontiersin.org/article/10.3389/fcell.2021.654915> (Accessed: 20 June 2022).

- Franklin, R.E. and Gosling, R.G. (1953) 'Molecular Configuration in Sodium Thymonucleate', *Nature*, 171(4356), pp. 740–741. Available at: <https://doi.org/10.1038/171740a0>.
- Fransz, P. *et al.* (2002) 'Interphase chromosomes in Arabidopsis are organized as well defined chromocenters from which euchromatin loops emanate', *Proceedings of the National Academy of Sciences of the United States of America*, 99(22), pp. 14584–14589. Available at: <https://doi.org/10.1073/pnas.212325299>.
- Fueyo, R. *et al.* (2022) 'Roles of transposable elements in the regulation of mammalian transcription', *Nature Reviews Molecular Cell Biology*, pp. 1–17. Available at: <https://doi.org/10.1038/s41580-022-00457-y>.
- Gaillard, P.-H.L. *et al.* (1996) 'Chromatin assembly coupled to DNA repair: a new role for chromatin assembly factor I', *Cell*, 86(6), pp. 887–896. Available at: [https://doi.org/10.1016/s0092-8674\(00\)80164-6](https://doi.org/10.1016/s0092-8674(00)80164-6).
- García Fernández, F. and Fabre, E. (2022) 'The Dynamic Behavior of Chromatin in Response to DNA Double-Strand Breaks', *Genes*, 13(2), p. 215. Available at: <https://doi.org/10.3390/genes13020215>.
- García-Cao, M. *et al.* (2004) 'Epigenetic regulation of telomere length in mammalian cells by the Suv39h1 and Suv39h2 histone methyltransferases', *Nature Genetics*, 36(1), pp. 94–99. Available at: <https://doi.org/10.1038/ng1278>.
- Gaspar-Maia, A. *et al.* (2009) 'Chd1 regulates open chromatin and pluripotency of embryonic stem cells', *Nature*, 460(7257), pp. 863–868. Available at: <https://doi.org/10.1038/nature08212>.
- Gatto, A. *et al.* (2022) 'HIRA-dependent boundaries between H3 variants shape early replication in mammals', *Molecular Cell* [Preprint]. Available at: <https://doi.org/10.1016/j.molcel.2022.03.017>.
- Geiss, C.P. *et al.* (2014) 'CENP-A Arrays Are More Condensed than Canonical Arrays at Low Ionic Strength', *Biophysical Journal*, 106(4), pp. 875–882. Available at: <https://doi.org/10.1016/j.bpj.2014.01.005>.
- Gérard, A. *et al.* (2006) 'The replication kinase Cdc7-Dbf4 promotes the interaction of the p150 subunit of chromatin assembly factor 1 with proliferating cell nuclear antigen', *EMBO Reports*, 7(8), pp. 817–823. Available at: <https://doi.org/10.1038/sj.embor.7400750>.
- Gershman, A. *et al.* (2022) 'Epigenetic patterns in a complete human genome', *Science*, 376(6588), p. eabj5089. Available at: <https://doi.org/10.1126/science.abj5089>.
- Giadrossi, S., Dvorkina, M. and Fisher, A.G. (2007) 'Chromatin organization and differentiation in embryonic stem cell models', *Current Opinion in Genetics & Development*, 17(2), pp. 132–138. Available at: <https://doi.org/10.1016/j.gde.2007.02.012>.
- Gibbons, R.J. *et al.* (1995) 'Mutations in a putative global transcriptional regulator cause X-linked mental retardation with alpha-thalassemia (ATR-X syndrome)', *Cell*, 80(6), pp. 837–845. Available at: [https://doi.org/10.1016/0092-8674\(95\)90287-2](https://doi.org/10.1016/0092-8674(95)90287-2).
- Gibcus, J.H. and Dekker, J. (2013) 'The hierarchy of the 3D genome', *Molecular Cell*, 49(5), pp. 773–782. Available at: <https://doi.org/10.1016/j.molcel.2013.02.011>.
- Gibson, B.A. *et al.* (2019) 'Organization of Chromatin by Intrinsic and Regulated Phase Separation', *Cell*, 179(2), pp. 470–484.e21. Available at: <https://doi.org/10.1016/j.cell.2019.08.037>.
- Gilbert, D.M. (2010) 'Cell fate transitions and the replication timing decision point', *The Journal of Cell Biology*, 191(5), pp. 899–903. Available at: <https://doi.org/10.1083/jcb.201007125>.

- Gilmour, D.S. and Lis, J.T. (1985) 'In Vivo Interactions of RNA Polymerase II with Genes of *Drosophila melanogaster*', *MOL. CELL. BIOL.*, 5, p. 10.
- Gianno, P.A. *et al.* (2020) 'A genome-scale map of DNA methylation turnover identifies site-specific dependencies of DNMT and TET activity', *Nature Communications*, 11(1), p. 2680. Available at: <https://doi.org/10.1038/s41467-020-16354-x>.
- Goldberg, A.D. *et al.* (2010) 'Distinct Factors Control Histone Variant H3.3 Localization at Specific Genomic Regions', *Cell*, 140(5), pp. 678–691. Available at: <https://doi.org/10.1016/j.cell.2010.01.003>.
- Goldman, J.A., Garlick, J.D. and Kingston, R.E. (2010) 'Chromatin Remodeling by Imitation Switch (ISWI) Class ATP-dependent Remodelers Is Stimulated by Histone Variant H2A.Z', *Journal of Biological Chemistry*, 285(7), pp. 4645–4651. Available at: <https://doi.org/10.1074/jbc.M109.072348>.
- Gomes, A.P. *et al.* (2019) 'Dynamic Incorporation of Histone H3 Variants into Chromatin Is Essential for Acquisition of Aggressive Traits and Metastatic Colonization', *Cancer Cell* [Preprint]. Available at: <https://doi.org/10.1016/j.ccell.2019.08.006>.
- Gómez-Zambrano, Á., Merini, W. and Calonje, M. (2019) 'The repressive role of Arabidopsis H2A.Z in transcriptional regulation depends on AtBMI1 activity', *Nature Communications*, 10, p. 2828. Available at: <https://doi.org/10.1038/s41467-019-10773-1>.
- Govin, J. *et al.* (2005) 'Testis-specific histone H3 expression in somatic cells', *Trends in Biochemical Sciences*, 30(7), pp. 357–359. Available at: <https://doi.org/10.1016/j.tibs.2005.05.001>.
- Greenberg, M.V.C. and Bourc'his, D. (2019) 'The diverse roles of DNA methylation in mammalian development and disease', *Nature Reviews. Molecular Cell Biology*, 20(10), pp. 590–607. Available at: <https://doi.org/10.1038/s41580-019-0159-6>.
- Grindheim, J.M. *et al.* (2019) 'Polycomb Repressive Complex 2 Proteins EZH1 and EZH2 Regulate Timing of Postnatal Hepatocyte Maturation and Fibrosis by Repressing Genes With Euchromatic Promoters in Mice', *Gastroenterology*, 156(6), pp. 1834–1848. Available at: <https://doi.org/10.1053/j.gastro.2019.01.041>.
- Groth, A. *et al.* (2005) 'Human Asf1 regulates the flow of S phase histones during replicational stress', *Molecular Cell*, 17(2), pp. 301–311. Available at: <https://doi.org/10.1016/j.molcel.2004.12.018>.
- Guenatri, M. *et al.* (2004) 'Mouse centric and pericentric satellite repeats form distinct functional heterochromatin', *Journal of Cell Biology*, 166(4), pp. 493–505. Available at: <https://doi.org/10.1083/jcb.200403109>.
- Guo, S. *et al.* (2014) 'Nonstochastic Reprogramming from a Privileged Somatic Cell State', *Cell*, 156(4), pp. 649–662. Available at: <https://doi.org/10.1016/j.cell.2014.01.020>.
- Gurard-Levin, Z.A., Quivy, J.-P. and Almouzni, G. (2014) 'Histone Chaperones: Assisting Histone Traffic and Nucleosome Dynamics', *Annual Review of Biochemistry*, 83(1), pp. 487–517. Available at: <https://doi.org/10.1146/annurev-biochem-060713-035536>.
- Habibi, E. *et al.* (2013) 'Whole-Genome Bisulfite Sequencing of Two Distinct Interconvertible DNA Methylomes of Mouse Embryonic Stem Cells', *Cell Stem Cell*, 13(3), pp. 360–369. Available at: <https://doi.org/10.1016/j.stem.2013.06.002>.
- Hada, M. *et al.* (2022) 'Highly rigid H3.1/H3.2–H3K9me3 domains set a barrier for cell fate reprogramming in trophoblast stem cells', *Genes & Development*, p. genesdev;gad.348782.121v1. Available at: <https://doi.org/10.1101/gad.348782.121>.

- Hake, S.B. *et al.* (2005) ‘Serine 31 phosphorylation of histone variant H3.3 is specific to regions bordering centromeres in metaphase chromosomes’, *Proceedings of the National Academy of Sciences of the United States of America*, 102(18), pp. 6344–6349. Available at: <https://doi.org/10.1073/pnas.0502413102>.
- Hammond, C.M. *et al.* (2017) ‘Histone chaperone networks shaping chromatin function’, *Nature Reviews Molecular Cell Biology*, 18(3), pp. 141–158. Available at: <https://doi.org/10.1038/nrm.2016.159>.
- He, Q. *et al.* (2015) ‘The Daxx/Atrx Complex Protects Tandem Repetitive Elements during DNA Hypomethylation by Promoting H3K9 Trimethylation’, *Cell Stem Cell*, 17(3), pp. 273–286. Available at: <https://doi.org/10.1016/j.stem.2015.07.022>.
- He, Y.-F. *et al.* (2011) ‘Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA’, *Science (New York, N.Y.)*, 333(6047), pp. 1303–1307. Available at: <https://doi.org/10.1126/science.1210944>.
- Heaphy, C.M. *et al.* (2011) ‘Altered telomeres in tumors with ATRX and DAXX mutations’, *Science (New York, N.Y.)*, 333(6041), p. 425. Available at: <https://doi.org/10.1126/science.1207313>.
- van der Heijden, G.W. *et al.* (2005) ‘Asymmetry in Histone H3 variants and lysine methylation between paternal and maternal chromatin of the early mouse zygote’, *Mechanisms of Development*, 122(9), pp. 1008–1022. Available at: <https://doi.org/10.1016/j.mod.2005.04.009>.
- Heintzman, N.D. *et al.* (2009) ‘Histone modifications at human enhancers reflect global cell-type-specific gene expression’, *Nature*, 459(7243), pp. 108–112. Available at: <https://doi.org/10.1038/nature07829>.
- Heitz, E. (1928) *Das heterochromatin der moose*. Bornträger.
- Henikoff, S., Ahmad, K. and Malik, H.S. (2001) ‘The Centromere Paradox: Stable Inheritance with Rapidly Evolving DNA’, *Science*, 293(5532), pp. 1098–1102. Available at: <https://doi.org/10.1126/science.1062939>.
- Henikoff, S. and Henikoff, J.G. (2012) ‘“Point” Centromeres of *Saccharomyces* Harbor Single Centromere-Specific Nucleosomes’, *Genetics*, 190(4), pp. 1575–1577. Available at: <https://doi.org/10.1534/genetics.111.137711>.
- Hershey, A.D. and Chase, M. (1952) ‘INDEPENDENT FUNCTIONS OF VIRAL PROTEIN AND NUCLEIC ACID IN GROWTH OF BACTERIOPHAGE’, *Journal of General Physiology*, 36(1), pp. 39–56. Available at: <https://doi.org/10.1085/jgp.36.1.39>.
- Heurtier, V. *et al.* (2019) ‘The molecular logic of Nanog-induced self-renewal in mouse embryonic stem cells’, *Nature Communications*, 10(1), p. 1109. Available at: <https://doi.org/10.1038/s41467-019-09041-z>.
- Hewish, D.R. and Burgoyne, L.A. (1973) ‘Chromatin sub-structure. The digestion of chromatin DNA at regularly spaced sites by a nuclear deoxyribonuclease’, *Biochemical and Biophysical Research Communications*, 52(2), pp. 504–510. Available at: [https://doi.org/10.1016/0006-291x\(73\)90740-7](https://doi.org/10.1016/0006-291x(73)90740-7).
- Hoang, S.M. *et al.* (2020) ‘Regulation of ALT-associated homology-directed repair by polyADP-ribosylation’, *Nature Structural & Molecular Biology*, 27(12), pp. 1152–1164. Available at: <https://doi.org/10.1038/s41594-020-0512-7>.
- Hoelper, D. *et al.* (2017) ‘Structural and mechanistic insights into ATRX-dependent and -independent functions of the histone chaperone DAXX’, *Nature Communications*, 8(1), p. 1193. Available at: <https://doi.org/10.1038/s41467-017-01206-y>.
- Hoffmann, S. *et al.* (2016) ‘CENP-A Is Dispensable for Mitotic Centromere Function after Initial Centromere/Kinetochore Assembly’, *Cell Reports*, 17(9), pp. 2394–2404. Available at: <https://doi.org/10.1016/j.celrep.2016.10.084>.

- Holliday, R. (1994) 'Epigenetics: An overview', *Developmental Genetics*, 15(6), pp. 453–457. Available at: <https://doi.org/10.1002/dvg.1020150602>.
- Houlard, M. *et al.* (2006) 'CAF-1 Is Essential for Heterochromatin Organization in Pluripotent Embryonic Cells', *PLoS Genetics*, 2(11), p. 11.
- Howman, E.V. *et al.* (2000) 'Early disruption of centromeric chromatin organization in centromere protein A (Cenpa) null mice', *Proceedings of the National Academy of Sciences*, 97(3), pp. 1148–1153. Available at: <https://doi.org/10.1073/pnas.97.3.1148>.
- Hoyt, S.J. *et al.* (2022) 'From telomere to telomere: The transcriptional and epigenetic state of human repeat elements', *Science*, 376(6588), p. eabk3112. Available at: <https://doi.org/10.1126/science.abk3112>.
- Hsieh, T.-H.S. *et al.* (2016) 'Micro-C XL: assaying chromosome conformation from the nucleosome to the entire genome', *Nature Methods*, 13(12), pp. 1009–1011. Available at: <https://doi.org/10.1038/nmeth.4025>.
- Huang, G. *et al.* (2015) 'Molecular basis of embryonic stem cell self-renewal: from signaling pathways to pluripotency network', *Cellular and molecular life sciences: CMLS*, 72(9), pp. 1741–1757. Available at: <https://doi.org/10.1007/s00018-015-1833-2>.
- ter Huurne, M. *et al.* (2017) 'Distinct Cell-Cycle Control in Two Different States of Mouse Pluripotency', *Cell Stem Cell*, 21(4), pp. 449–455.e4. Available at: <https://doi.org/10.1016/j.stem.2017.09.004>.
- Ide, S. and DeJardin, J. (2015) 'End-targeting proteomics of isolated chromatin segments of a mammalian ribosomal RNA gene promoter', *Nature communications*, 6(1), pp. 1–10.
- Ishiuchi, T. *et al.* (2015) 'Early embryonic-like cells are induced by downregulating replication-dependent chromatin assembly', *Nature Structural & Molecular Biology*, 22(9), pp. 662–671. Available at: <https://doi.org/10.1038/nsmb.3066>.
- Ishiuchi, T. *et al.* (2021) 'Reprogramming of the histone H3.3 landscape in the early mouse embryo', *Nature Structural & Molecular Biology*, 28(1), pp. 38–49. Available at: <https://doi.org/10.1038/s41594-020-00521-1>.
- Ito, S. *et al.* (2010) 'Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification', *Nature*, 466(7310), pp. 1129–1133. Available at: <https://doi.org/10.1038/nature09303>.
- Ito, S. *et al.* (2011) 'Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine', *Science (New York, N.Y.)*, 333(6047), pp. 1300–1303. Available at: <https://doi.org/10.1126/science.1210597>.
- Jacob, Y. *et al.* (2010) 'Regulation of heterochromatic DNA replication by histone H3 lysine 27 methyltransferases', *Nature*, 466(7309), pp. 987–991. Available at: <https://doi.org/10.1038/nature09290>.
- Jacob, Y. *et al.* (2014) 'Selective methylation of histone H3 variant H3.1 regulates heterochromatin replication', *Science (New York, N.Y.)*, 343(6176), pp. 1249–1253. Available at: <https://doi.org/10.1126/science.1248357>.
- Jaenisch, R. and Young, R. (2008) 'Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming', *Cell*, 132(4), pp. 567–582. Available at: <https://doi.org/10.1016/j.cell.2008.01.015>.
- Jagannathan, M., Cummings, R. and Yamashita, Y.M. (2018) 'A conserved function for pericentromeric satellite DNA', *eLife*. Edited by K. VijayRaghavan, 7, p. e34122. Available at: <https://doi.org/10.7554/eLife.34122>.

- Jagannathan, M., Cummings, R. and Yamashita, Y.M. (2019) 'The modular mechanism of chromocenter formation in *Drosophila*', *eLife*. Edited by K. VijayRaghavan and S.A. Gerbi, 8, p. e43938. Available at: <https://doi.org/10.7554/eLife.43938>.
- James, T.C. and Elgin, S.C. (1986) 'Identification of a nonhistone chromosomal protein associated with heterochromatin in *Drosophila melanogaster* and its gene', *Molecular and Cellular Biology*, 6(11), pp. 3862–3872. Available at: <https://doi.org/10.1128/mcb.6.11.3862-3872.1986>.
- Jang, C.-W. *et al.* (2015) 'Histone H3.3 maintains genome integrity during mammalian development', *Genes & Development*, 29(13), pp. 1377–1392. Available at: <https://doi.org/10.1101/gad.264150.115>.
- Jansen, L.E.T. *et al.* (2007) 'Propagation of centromeric chromatin requires exit from mitosis', *The Journal of Cell Biology*, 176(6), pp. 795–805. Available at: <https://doi.org/10.1083/jcb.200701066>.
- Jeffery, D. *et al.* (2021) 'CENP-A overexpression promotes distinct fates in human cells, depending on p53 status', *Communications Biology*, 4(1), pp. 1–18. Available at: <https://doi.org/10.1038/s42003-021-01941-5>.
- Jenuwein, T. and Allis, C.D. (2001) 'Translating the histone code', *Science (New York, N.Y.)*, 293(5532), pp. 1074–1080. Available at: <https://doi.org/10.1126/science.1063127>.
- Jiang, D. *et al.* (2020) 'The evolution and functional divergence of the histone H2B family in plants', *PLOS Genetics*, 16(7), p. e1008964. Available at: <https://doi.org/10.1371/journal.pgen.1008964>.
- Jiang, D. and Berger, F. (2017) 'DNA replication-coupled histone modification maintains Polycomb gene silencing in plants', *Science (New York, N.Y.)*, 357(6356), pp. 1146–1149. Available at: <https://doi.org/10.1126/science.aan4965>.
- Jiao, Y. *et al.* (2011) 'DAXX/ATRAX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors', *Science (New York, N.Y.)*, 331(6021), pp. 1199–1203. Available at: <https://doi.org/10.1126/science.1200609>.
- Jin, C. *et al.* (2009) 'H3.3/H2A.Z double variant-containing nucleosomes mark “nucleosome-free regions” of active promoters and other regulatory regions', *Nature Genetics*, 41(8), pp. 941–945. Available at: <https://doi.org/10.1038/ng.409>.
- Jin, C. and Felsenfeld, G. (2007) 'Nucleosome stability mediated by histone variants H3.3 and H2A.Z', *Genes & Development*, 21(12), pp. 1519–1529. Available at: <https://doi.org/10.1101/gad.1547707>.
- Jørgensen, H.F. *et al.* (2007) 'The impact of chromatin modifiers on the timing of locus replication in mouse embryonic stem cells', *Genome Biology*, 8(8), p. R169. Available at: <https://doi.org/10.1186/gb-2007-8-8-r169>.
- Joshi, O. *et al.* (2015) 'Dynamic Reorganization of Extremely Long-Range Promoter-Promoter Interactions between Two States of Pluripotency', *Cell Stem Cell*, 17(6), pp. 748–757. Available at: <https://doi.org/10.1016/j.stem.2015.11.010>.
- Kalkan, T. *et al.* (2017) 'Tracking the embryonic stem cell transition from ground state pluripotency', *Development*, 144(7), pp. 1221–1234. Available at: <https://doi.org/10.1242/dev.142711>.
- Karagianni, P. *et al.* (2008) 'ICBP90, a novel methyl K9 H3 binding protein linking protein ubiquitination with heterochromatin formation', *Molecular and Cellular Biology*, 28(2), pp. 705–717. Available at: <https://doi.org/10.1128/MCB.01598-07>.
- Karpen, G.H. and Allshire, R.C. (1997) 'The case for epigenetic effects on centromere identity and function', *Trends in genetics: TIG*, 13(12), pp. 489–496. Available at: [https://doi.org/10.1016/s0168-9525\(97\)01298-5](https://doi.org/10.1016/s0168-9525(97)01298-5).

- Khuong-Quang, D.-A. *et al.* (2012) 'K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas', *Acta Neuropathologica*, 124(3), pp. 439–447. Available at: <https://doi.org/10.1007/s00401-012-0998-0>.
- Kim, S.-M., Dubey, D.D. and Huberman, J.A. (2003) 'Early-replicating heterochromatin', *Genes & Development*, 17(3), pp. 330–335. Available at: <https://doi.org/10.1101/gad.1046203>.
- Kit, S. (1961) 'Equilibrium sedimentation in density gradients of DNA preparations from animal tissues', *Journal of Molecular Biology*, 3, pp. 711–716. Available at: [https://doi.org/10.1016/s0022-2836\(61\)80075-2](https://doi.org/10.1016/s0022-2836(61)80075-2).
- Kolodziejczyk, A.A. *et al.* (2015) 'Single Cell RNA-Sequencing of Pluripotent States Unlocks Modular Transcriptional Variation', *Cell Stem Cell*, 17(4), pp. 471–485. Available at: <https://doi.org/10.1016/j.stem.2015.09.011>.
- Kornberg, R.D. (1977) 'Structure of Chromatin', *Annual Review of Biochemistry*, 46(1), pp. 931–954. Available at: <https://doi.org/10.1146/annurev.bi.46.070177.004435>.
- Kraushaar, D.C. *et al.* (2013) 'Genome-wide incorporation dynamics reveal distinct categories of turnover for the histone variant H3.3', *Genome Biology*, 14(10), p. R121. Available at: <https://doi.org/10.1186/gb-2013-14-10-r121>.
- Kriaucionis, S. and Heintz, N. (2009) 'The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain', *Science (New York, N.Y.)*, 324(5929), pp. 929–930. Available at: <https://doi.org/10.1126/science.1169786>.
- Krietenstein, N. *et al.* (2020) 'Ultrastructural Details of Mammalian Chromosome Architecture', *Molecular Cell*, 78(3), pp. 554–565.e7. Available at: <https://doi.org/10.1016/j.molcel.2020.03.003>.
- Kumar, D. *et al.* (2021) 'Decoding the function of bivalent chromatin in development and cancer', *Genome Research*, 31(12), pp. 2170–2184. Available at: <https://doi.org/10.1101/gr.275736.121>.
- Kumekawa, N. *et al.* (2000) 'The Size and Sequence Organization of the Centromeric Region of Arabidopsis thaliana Chromosome 5', *DNA Research*, 7(6), pp. 315–321. Available at: <https://doi.org/10.1093/dnares/7.6.315>.
- Kumekawa, N. *et al.* (2001) 'The Size and Sequence Organization of the Centromeric Region of Arabidopsis thaliana Chromosome 4', *DNA Research*, 8(6), pp. 285–290. Available at: <https://doi.org/10.1093/dnares/8.6.285>.
- Kursel, L.E. and Malik, H.S. (2018) 'The cellular mechanisms and consequences of centromere drive', *Current Opinion in Cell Biology*, 52, pp. 58–65. Available at: <https://doi.org/10.1016/j.ceb.2018.01.011>.
- Lachner, M. *et al.* (2001) 'Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins', *Nature*, 410(6824), pp. 116–120. Available at: <https://doi.org/10.1038/35065132>.
- Lacoste, N. *et al.* (2014) 'Mislocalization of the Centromeric Histone Variant CenH3/CENP-A in Human Cells Depends on the Chaperone DAXX', *Molecular Cell*, 53(4), pp. 631–644. Available at: <https://doi.org/10.1016/j.molcel.2014.01.018>.
- Lamour, V. *et al.* (1995) 'A human homolog of the *S. cerevisiae* HIR1 and HIR2 transcriptional repressors cloned from the DiGeorge syndrome critical region', *Human Molecular Genetics*, 4(5), pp. 791–799. Available at: <https://doi.org/10.1093/hmg/4.5.791>.
- Lander, E.S. *et al.* (2001) 'Initial sequencing and analysis of the human genome', *Nature*, 409(6822), pp. 860–921. Available at: <https://doi.org/10.1038/35057062>.

- Larson, A.G. *et al.* (2017) 'Liquid droplet formation by HP1 α suggests a role for phase separation in heterochromatin', *Nature*, 547(7662), pp. 236–240. Available at: <https://doi.org/10.1038/nature22822>.
- Laskey, R.A. *et al.* (1978) 'Nucleosomes are assembled by an acidic protein which binds histones and transfers them to DNA', *Nature*, 275(5679), pp. 416–420. Available at: <https://doi.org/10.1038/275416a0>.
- Latreille, D. *et al.* (2014) 'Identification of histone 3 variant 2 interacting factors', *Nucleic Acids Research*, 42(6), pp. 3542–3550. Available at: <https://doi.org/10.1093/nar/gkt1355>.
- Law, M.J. *et al.* (2010) 'ATR-X syndrome protein targets tandem repeats and influences allele-specific expression in a size-dependent manner', *Cell*, 143(3), pp. 367–378. Available at: <https://doi.org/10.1016/j.cell.2010.09.023>.
- Lee, T.I. *et al.* (2006) 'Control of developmental regulators by Polycomb in human embryonic stem cells', *Cell*, 125(2), pp. 301–313. Available at: <https://doi.org/10.1016/j.cell.2006.02.043>.
- Lehnertz, B. *et al.* (2003) 'Suv39h-Mediated Histone H3 Lysine 9 Methylation Directs DNA Methylation to Major Satellite Repeats at Pericentric Heterochromatin', *Current Biology*, 13(14), pp. 1192–1200. Available at: [https://doi.org/10.1016/S0960-9822\(03\)00432-9](https://doi.org/10.1016/S0960-9822(03)00432-9).
- Leitch, H.G. *et al.* (2013) 'Naive pluripotency is associated with global DNA hypomethylation', *Nature Structural & Molecular Biology*, 20(3), pp. 311–316. Available at: <https://doi.org/10.1038/nsmb.2510>.
- Leung, D. *et al.* (2014) 'Regulation of DNA methylation turnover at LTR retrotransposons and imprinted loci by the histone methyltransferase Setdb1', *Proceedings of the National Academy of Sciences of the United States of America*, 111(18), pp. 6690–6695. Available at: <https://doi.org/10.1073/pnas.1322273111>.
- Levet, F. *et al.* (2015) 'SR-Tesseler: a method to segment and quantify localization-based super-resolution microscopy data', *Nature Methods*, 12(11), pp. 1065–1071. Available at: <https://doi.org/10.1038/nmeth.3579>.
- Lewis, P.W. *et al.* (2010) 'Daxx is an H3.3-specific histone chaperone and cooperates with ATRX in replication-independent chromatin assembly at telomeres', *Proceedings of the National Academy of Sciences*, 107(32), pp. 14075–14080. Available at: <https://doi.org/10.1073/pnas.1008850107>.
- Lewis, P.W. *et al.* (2013) 'Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma', *Science (New York, N.Y.)*, 340(6134), pp. 857–861. Available at: <https://doi.org/10.1126/science.1232245>.
- Lichter, P. *et al.* (1988) 'Delineation of individual human chromosomes in metaphase and interphase cells by in situ suppression hybridization using recombinant DNA libraries', *Human Genetics*, 80(3), pp. 224–234. Available at: <https://doi.org/10.1007/BF01790090>.
- Lieberman-Aiden, E. *et al.* (2009) 'Comprehensive mapping of long range interactions reveals folding principles of the human genome', *Science (New York, N.Y.)*, 326(5950), pp. 289–293. Available at: <https://doi.org/10.1126/science.1181369>.
- Lister, R. *et al.* (2009) 'Human DNA methylomes at base resolution show widespread epigenomic differences', *Nature*, 462(7271), pp. 315–322. Available at: <https://doi.org/10.1038/nature08514>.
- Liu, C.-P. *et al.* (2012) 'Structure of the variant histone H3.3–H4 heterodimer in complex with its chaperone DAXX', *Nature Structural & Molecular Biology*, 19(12), pp. 1287–1292. Available at: <https://doi.org/10.1038/nsmb.2439>.

- Liu, Y. *et al.* (2017) 'Widespread Mitotic Bookmarking by Histone Marks and Transcription Factors in Pluripotent Stem Cells', *Cell Reports*, 19(7), pp. 1283–1293. Available at: <https://doi.org/10.1016/j.celrep.2017.04.067>.
- Loh, Y.-H. *et al.* (2007) 'Jmjd1a and Jmjd2c histone H3 Lys 9 demethylases regulate self-renewal in embryonic stem cells', *Genes & Development*, 21(20), pp. 2545–2557. Available at: <https://doi.org/10.1101/gad.1588207>.
- Long, M. *et al.* (2019) 'A novel histone H4 variant H4G regulates rDNA transcription in breast cancer', *Nucleic Acids Research*, 47(16), pp. 8399–8409. Available at: <https://doi.org/10.1093/nar/gkz547>.
- Loppin, B. *et al.* (2005) 'The histone H3.3 chaperone HIRA is essential for chromatin assembly in the male pronucleus', *Nature*, 437(7063), pp. 1386–1390. Available at: <https://doi.org/10.1038/nature04059>.
- Loyola, A. *et al.* (2006) 'PTMs on H3 Variants before Chromatin Assembly Potentiate Their Final Epigenetic State', *Molecular Cell*, 24(2), pp. 309–316. Available at: <https://doi.org/10.1016/j.molcel.2006.08.019>.
- Lu, C. *et al.* (2016) 'Histone H3K36 mutations promote sarcomagenesis through altered histone methylation landscape', *Science (New York, N.Y.)*, 352(6287), pp. 844–849. Available at: <https://doi.org/10.1126/science.aac7272>.
- Lu, J. and Gilbert, D.M. (2007) 'Proliferation-dependent and cell cycle-regulated transcription of mouse pericentric heterochromatin', *Journal of Cell Biology*, 179(3), pp. 411–421. Available at: <https://doi.org/10.1083/jcb.200706176>.
- Luger, K. *et al.* (1997) 'Crystal structure of the nucleosome core particle at 2.8 Å resolution', *Nature*, 389(6648), pp. 251–260. Available at: <https://doi.org/10.1038/38444>.
- Ma, Y., Kanakousaki, K. and Buttitta, L. (2015) 'How the cell cycle impacts chromatin architecture and influences cell fate', *Frontiers in Genetics*, 0. Available at: <https://doi.org/10.3389/fgene.2015.00019>.
- Maehara, K. *et al.* (2015) 'Tissue-specific expression of histone H3 variants diversified after species separation', *Epigenetics & Chromatin*, 8(1), p. 35. Available at: <https://doi.org/10.1186/s13072-015-0027-3>.
- Maison, C. *et al.* (2002) 'Higher-order structure in pericentric heterochromatin involves a distinct pattern of histone modification and an RNA component', *Nature Genetics*, 30(3), pp. 329–334. Available at: <https://doi.org/10.1038/ng843>.
- Maison, C. *et al.* (2010) 'Heterochromatin at Mouse Pericentromeres A Model for De Novo Heterochromatin Formation and Duplication during Replication', *Cold Spring Harbor Symposia on Quantitative Biology*, 75, pp. 155–165. Available at: <https://doi.org/10.1101/sqb.2010.75.013>.
- Maison, C. *et al.* (2011) 'SUMOylation promotes de novo targeting of HP1 α to pericentric heterochromatin', *Nature Genetics*, 43(3), pp. 220–227. Available at: <https://doi.org/10.1038/ng.765>.
- Maison, C. *et al.* (2016) 'The methyltransferase Suv39h1 links the SUMO pathway to HP1 α marking at pericentric heterochromatin', *Nature Communications*, 7(1), p. 12224. Available at: <https://doi.org/10.1038/ncomms12224>.
- Maison, C. and Almouzni, G. (2004) 'HP1 and the dynamics of heterochromatin maintenance', *Nature Reviews. Molecular Cell Biology*, 5(4), pp. 296–304. Available at: <https://doi.org/10.1038/nrm1355>.
- Maksakova, I.A. *et al.* (2013) 'Distinct roles of KAP1, HP1 and G9a/GLP in silencing of the two-cell-specific retrotransposon MERVL in mouse ES cells', *Epigenetics & Chromatin*, 6(1), p. 15. Available at: <https://doi.org/10.1186/1756-8935-6-15>.

- Marks, H. *et al.* (2012) ‘The Transcriptional and Epigenomic Foundations of Ground State Pluripotency’, *Cell*, 149(3), pp. 590–604. Available at: <https://doi.org/10.1016/j.cell.2012.03.026>.
- Martens, J.H.A. *et al.* (2005) ‘The profile of repeat-associated histone lysine methylation states in the mouse epigenome’, *The EMBO Journal*, 24(4), pp. 800–812. Available at: <https://doi.org/10.1038/sj.emboj.7600545>.
- Martin, G.R. (1981) ‘Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells.’, *Proceedings of the National Academy of Sciences*, 78(12), pp. 7634–7638. Available at: <https://doi.org/10.1073/pnas.78.12.7634>.
- Martire, S. *et al.* (2019) ‘Phosphorylation of histone H3.3 at serine 31 promotes p300 activity and enhancer acetylation’, *Nature Genetics*, 51(6), pp. 941–946. Available at: <https://doi.org/10.1038/s41588-019-0428-5>.
- Martire, S. and Banaszynski, L.A. (2020) ‘The roles of histone variants in fine-tuning chromatin organization and function’, *Nature Reviews Molecular Cell Biology*, pp. 1–20. Available at: <https://doi.org/10.1038/s41580-020-0262-8>.
- Marzluff, W.F. (2005) ‘Metazoan replication-dependent histone mRNAs: a distinct set of RNA polymerase II transcripts’, *Current Opinion in Cell Biology*, 17(3), pp. 274–280. Available at: <https://doi.org/10.1016/j.ceb.2005.04.010>.
- Masui, S. *et al.* (2007) ‘Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells’, *Nature Cell Biology*, 9(6), pp. 625–635. Available at: <https://doi.org/10.1038/ncb1589>.
- Matsushita, Y. and Kaneko, K. (2020) ‘Homeorhesis in Waddington’s Landscape by Epigenetic Feedback Regulation’, *Physical Review Research*, 2(2), p. 023083. Available at: <https://doi.org/10.1103/PhysRevResearch.2.023083>.
- Mau, A. *et al.* (2021) ‘Fast widefield scan provides tunable and uniform illumination optimizing super-resolution microscopy on large fields’, *Nature Communications*, 12(1), p. 3077. Available at: <https://doi.org/10.1038/s41467-021-23405-4>.
- Mayer, R. *et al.* (2005) ‘Common themes and cell type specific variations of higher order chromatin arrangements in the mouse’, *BMC Cell Biology*, 6(1), p. 44. Available at: <https://doi.org/10.1186/1471-2121-6-44>.
- Mayer, W. *et al.* (2000) ‘Demethylation of the zygotic paternal genome’, *Nature*, 403(6769), pp. 501–502. Available at: <https://doi.org/10.1038/35000656>.
- McClintock, B. (1953) ‘INDUCTION OF INSTABILITY AT SELECTED LOCI IN MAIZE’, *Genetics*, 38(6), pp. 579–599. Available at: <https://doi.org/10.1093/genetics/38.6.579>.
- McSwiggen, D.T. *et al.* (2019) ‘Evaluating phase separation in live cells: diagnosis, caveats, and functional consequences’, *Genes & Development* [Preprint]. Available at: <https://doi.org/10.1101/gad.331520.119>.
- Meehan, R.R., Kao, C.-F. and Pennings, S. (2003) ‘HP1 binding to native chromatin in vitro is determined by the hinge region and not by the chromodomain’, *The EMBO journal*, 22(12), pp. 3164–3174. Available at: <https://doi.org/10.1093/emboj/cdg306>.
- Melcher, M. *et al.* (2000) ‘Structure-Function Analysis of SUV39H1 Reveals a Dominant Role in Heterochromatin Organization, Chromosome Segregation, and Mitotic Progression’, *Molecular and Cellular Biology*, 20(10), pp. 3728–3741. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC85674/> (Accessed: 3 May 2022).

- Mello, J.A. *et al.* (2002) ‘Human Asf1 and CAF-1 interact and synergize in a repair-coupled nucleosome assembly pathway’, *EMBO Reports*, 3(4), pp. 329–334. Available at: <https://doi.org/10.1093/embo-reports/kvf068>.
- Mendiratta, S., Gatto, A. and Almouzni, G. (2019) ‘Histone supply: Multitiered regulation ensures chromatin dynamics throughout the cell cycle’, *The Journal of Cell Biology*, 218(1), pp. 39–54. Available at: <https://doi.org/10.1083/jcb.201807179>.
- Mersfelder, E.L. and Parthun, M.R. (2006) ‘The tale beyond the tail: histone core domain modifications and the regulation of chromatin structure’, *Nucleic Acids Research*, 34(9), pp. 2653–2662. Available at: <https://doi.org/10.1093/nar/gkl338>.
- Meshorer, E. *et al.* (2006) ‘Hyperdynamic Plasticity of Chromatin Proteins in Pluripotent Embryonic Stem Cells’, *Developmental Cell*, 10(1), pp. 105–116. Available at: <https://doi.org/10.1016/j.devcel.2005.10.017>.
- Meshorer, E. and Misteli, T. (2006) ‘Chromatin in pluripotent embryonic stem cells and differentiation’, *Nature Reviews Molecular Cell Biology*, 7(7), pp. 540–546. Available at: <https://doi.org/10.1038/nrm1938>.
- Miescher, F. (1871) ‘Hoppe-Seyler’s Med’, *chem. Unters*, 441, p. 502.
- Mikkelsen, T.S. *et al.* (2007) ‘Genome-wide maps of chromatin state in pluripotent and lineage-committed cells’, *Nature*, 448(7153), pp. 553–560. Available at: <https://doi.org/10.1038/nature06008>.
- Miron, E. *et al.* (2020) ‘Chromatin arranges in chains of mesoscale domains with nanoscale functional topography independent of cohesin’, *Science Advances*, 6(39), p. eaba8811. Available at: <https://doi.org/10.1126/sciadv.aba8811>.
- Miyanari, Y., Ziegler-Birling, C. and Torres-Padilla, M.-E. (2013) ‘Live visualization of chromatin dynamics with fluorescent TALEs’, *Nature Structural & Molecular Biology*, 20(11), pp. 1321–1324. Available at: <https://doi.org/10.1038/nsmb.2680>.
- Moggs, J.G. *et al.* (2000) ‘A CAF-1-PCNA-mediated chromatin assembly pathway triggered by sensing DNA damage’, *Molecular and Cellular Biology*, 20(4), pp. 1206–1218. Available at: <https://doi.org/10.1128/MCB.20.4.1206-1218.2000>.
- Mohammad, F. *et al.* (2017) ‘EZH2 is a potential therapeutic target for H3K27M-mutant pediatric gliomas’, *Nature Medicine*, 23(4), pp. 483–492. Available at: <https://doi.org/10.1038/nm.4293>.
- Mohandas, T., Sparkes, R.S. and Shapiro, L.J. (1981) ‘Reactivation of an inactive human X chromosome: evidence for X inactivation by DNA methylation’, *Science (New York, N.Y.)*, 211(4480), pp. 393–396. Available at: <https://doi.org/10.1126/science.6164095>.
- Montavon, T. *et al.* (2021) ‘Complete loss of H3K9 methylation dissolves mouse heterochromatin organization’, *Nature Communications*, 12(1), p. 4359. Available at: <https://doi.org/10.1038/s41467-021-24532-8>.
- Morange, M. (2011) ‘The attempt of Nikolai Koltzoff (Koltsov) to link genetics, embryology and physical chemistry’, *Journal of Biosciences*, 36, pp. 211–214. Available at: <https://doi.org/10.1007/s12038-011-9075-4>.
- Morel, D. *et al.* (2019) ‘Combining epigenetic drugs with other therapies for solid tumours — past lessons and future promise’, *Nature Reviews Clinical Oncology*, pp. 1–17. Available at: <https://doi.org/10.1038/s41571-019-0267-4>.
- Mozzetta, C. (2015) ‘Sound of silence: the properties and functions of repressive Lys methyltransferases’, p. 15.

- Muchardt, C. *et al.* (2002) 'Coordinated methyl and RNA binding is required for heterochromatin localization of mammalian HP1alpha', *EMBO reports*, 3(10), pp. 975–981. Available at: <https://doi.org/10.1093/embo-reports/kvf194>.
- Muller, H., Gil, J. and Drinnenberg, I.A. (2019) 'The Impact of Centromeres on Spatial Genome Architecture', *Trends in Genetics*, 35(8), pp. 565–578. Available at: <https://doi.org/10.1016/j.tig.2019.05.003>.
- Müller, S. and Almouzni, G. (2017) 'Chromatin dynamics during the cell cycle at centromeres', *Nature Reviews Genetics*, 18(3), pp. 192–208. Available at: <https://doi.org/10.1038/nrg.2016.157>.
- Müller-Ott, K. *et al.* (2014) 'Specificity, propagation, and memory of pericentric heterochromatin', *Molecular Systems Biology*, 10, p. 746. Available at: <https://doi.org/10.15252/msb.20145377>.
- Nacev, B.A. *et al.* (2019) 'The expanding landscape of "oncohistone" mutations in human cancers', *Nature*, 567(7749), pp. 473–478. Available at: <https://doi.org/10.1038/s41586-019-1038-1>.
- Nagaki, K. *et al.* (2003) 'Chromatin immunoprecipitation reveals that the 180-bp satellite repeat is the key functional DNA element of Arabidopsis thaliana centromeres', *Genetics*, 163(3), pp. 1221–1225. Available at: <https://doi.org/10.1093/genetics/163.3.1221>.
- Naish, M. *et al.* (2021) 'The genetic and epigenetic landscape of the Arabidopsis centromeres', *Science*, 374(6569), p. eabi7489. Available at: <https://doi.org/10.1126/science.abi7489>.
- Nakatani, T. *et al.* (2022) 'DNA replication fork speed underlies cell fate changes and promotes reprogramming', *Nature Genetics* [Preprint]. Available at: <https://doi.org/10.1038/s41588-022-01023-0>.
- Natalia V. Murzina *et al.* (1999) 'Heterochromatin dynamics in mouse cells: interaction between chromatin assembly factor 1 and HP1 proteins.', *Molecular Cell*, 4(4), pp. 529–540. Available at: [https://doi.org/10.1016/s1097-2765\(00\)80204-x](https://doi.org/10.1016/s1097-2765(00)80204-x).
- Neri, F. *et al.* (2017) 'Intragenic DNA methylation prevents spurious transcription initiation', *Nature*, 543(7643), pp. 72–77. Available at: <https://doi.org/10.1038/nature21373>.
- Nicetto, D. *et al.* (2019) 'H3K9me3-heterochromatin loss at protein-coding genes enables developmental lineage specification', *Science*, 363(6424), pp. 294–297. Available at: <https://doi.org/10.1126/science.aau0583>.
- Nicetto, D. and Zaret, K.S. (2019) 'Role of H3K9me3 heterochromatin in cell identity establishment and maintenance', *Current Opinion in Genetics & Development*, 55, pp. 1–10. Available at: <https://doi.org/10.1016/j.gde.2019.04.013>.
- Nichols, J. *et al.* (1998) 'Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4', *Cell*, 95(3), pp. 379–391. Available at: [https://doi.org/10.1016/s0092-8674\(00\)81769-9](https://doi.org/10.1016/s0092-8674(00)81769-9).
- Noll, M. and Kornberg, R.D. (1977) 'Action of micrococcal nuclease on chromatin and the location of histone H1', *Journal of Molecular Biology*, 109(3), pp. 393–404. Available at: [https://doi.org/10.1016/s0022-2836\(77\)80019-3](https://doi.org/10.1016/s0022-2836(77)80019-3).
- Nora, E.P. *et al.* (2012) 'Spatial partitioning of the regulatory landscape of the X-inactivation centre', *Nature*, 485(7398), pp. 381–385. Available at: <https://doi.org/10.1038/nature11049>.
- Novo, C.L. *et al.* (2016) 'The pluripotency factor Nanog regulates pericentromeric heterochromatin organization in mouse embryonic stem cells', *Genes & Development*, 30(9), pp. 1101–1115. Available at: <https://doi.org/10.1101/gad.275685.115>.

- Novo, C.L. *et al.* (2022) ‘Satellite repeat transcripts modulate heterochromatin condensates and safeguard chromosome stability in mouse embryonic stem cells’, *Nature Communications*, 13(1), pp. 1–16. Available at: <https://doi.org/10.1038/s41467-022-31198-3>.
- Nozaki, T. *et al.* (2017) ‘Dynamic Organization of Chromatin Domains Revealed by Super-Resolution Live-Cell Imaging’, *Molecular Cell*, 67(2), pp. 282–293.e7. Available at: <https://doi.org/10.1016/j.molcel.2017.06.018>.
- Nurk, S. *et al.* (2022) ‘The complete sequence of a human genome’, *Science*, 376(6588), pp. 44–53. Available at: <https://doi.org/10.1126/science.abj6987>.
- Okano, M. *et al.* (1999) ‘DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development’, *Cell*, 99(3), pp. 247–257. Available at: [https://doi.org/10.1016/s0092-8674\(00\)81656-6](https://doi.org/10.1016/s0092-8674(00)81656-6).
- Okano, M., Xie, S. and Li, E. (1998) ‘Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases’, *Nature Genetics*, 19(3), pp. 219–220. Available at: <https://doi.org/10.1038/890>.
- Olins, A.L. and Olins, D.E. (1974) ‘Spheroid chromatin units (v bodies)’, *Science (New York, N.Y.)*, 183(4122), pp. 330–332. Available at: <https://doi.org/10.1126/science.183.4122.330>.
- Ooi, S.K.T. *et al.* (2007) ‘DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA’, *Nature*, 448(7154), pp. 714–717. Available at: <https://doi.org/10.1038/nature05987>.
- Osakabe, A. *et al.* (2021) ‘The chromatin remodeler DDM1 prevents transposon mobility through deposition of histone variant H2A.W’, *Nature Cell Biology*, 23(4), pp. 391–400. Available at: <https://doi.org/10.1038/s41556-021-00658-1>.
- Oscar Velazquez Camacho *et al.* (2017) ‘Major satellite repeat RNA stabilize heterochromatin retention of Suv39h enzymes by RNA-nucleosome association and RNA:DNA hybrid formation’, *eLife*, 6. Available at: <https://doi.org/10.7554/elife.25293>.
- Ostromyshenskii, D.I. *et al.* (2018) ‘Mouse chromocenters DNA content: sequencing and in silico analysis’, *BMC Genomics*, 19(1), p. 151. Available at: <https://doi.org/10.1186/s12864-018-4534-z>.
- Oudet, P., Gross-Bellard, M. and Chambon, P. (1975) ‘Electron microscopic and biochemical evidence that chromatin structure is a repeating unit’, *Cell*, 4(4), pp. 281–300. Available at: [https://doi.org/10.1016/0092-8674\(75\)90149-x](https://doi.org/10.1016/0092-8674(75)90149-x).
- Pace, L. *et al.* (2018) ‘The epigenetic control of stemness in CD8+ T cell fate commitment’, *Science*, 359(6372), pp. 177–186. Available at: <https://doi.org/10.1126/science.aah6499>.
- Palmer, D.K. *et al.* (1991) ‘Purification of the centromere-specific protein CENP-A and demonstration that it is a distinctive histone.’, *Proceedings of the National Academy of Sciences of the United States of America*, 88(9), pp. 3734–3738. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC51527/> (Accessed: 2 May 2022).
- Palmer, N. and Kaldis, P. (2016) ‘Chapter One - Regulation of the Embryonic Cell Cycle During Mammalian Preimplantation Development’, in M.L. DePamphilis (ed.) *Current Topics in Developmental Biology*. Academic Press (Mammalian Preimplantation Development), pp. 1–53. Available at: <https://doi.org/10.1016/bs.ctdb.2016.05.001>.
- Pan, G.J. *et al.* (2002) ‘Stem cell pluripotency and transcription factor Oct4’, *Cell Research*, 12(5–6), pp. 321–329. Available at: <https://doi.org/10.1038/sj.cr.7290134>.

- Pang, M.Y.H. *et al.* (2020) 'Histone H4 variant, H4G, drives ribosomal RNA transcription and breast cancer cell proliferation by loosening nucleolar chromatin structure', *Journal of Cellular Physiology*, 235(12), pp. 9601–9608. Available at: <https://doi.org/10.1002/jcp.29770>.
- Peters, A.H.F.M. *et al.* (2001) 'Loss of the Suv39h Histone Methyltransferases Impairs Mammalian Heterochromatin and Genome Stability', *Cell*, 107(3), pp. 323–337. Available at: [https://doi.org/10.1016/S0092-8674\(01\)00542-6](https://doi.org/10.1016/S0092-8674(01)00542-6).
- Peters, A.H.F.M. *et al.* (2003) 'Partitioning and Plasticity of Repressive Histone Methylation States in Mammalian Chromatin', *Molecular Cell*, 12(6), pp. 1577–1589. Available at: [https://doi.org/10.1016/S1097-2765\(03\)00477-5](https://doi.org/10.1016/S1097-2765(03)00477-5).
- Piña, B. and Suau, P. (1987) 'Changes in the proportions of histone H1 subtypes in brain cortical neurons', *FEBS letters*, 210(2), pp. 161–164. Available at: [https://doi.org/10.1016/0014-5793\(87\)81328-5](https://doi.org/10.1016/0014-5793(87)81328-5).
- Pinheiro, I. *et al.* (2012) 'Prdm3 and Prdm16 are H3K9me1 Methyltransferases Required for Mammalian Heterochromatin Integrity', *Cell*, 150(5), pp. 948–960. Available at: <https://doi.org/10.1016/j.cell.2012.06.048>.
- Pliatska, M. *et al.* (2018) 'The Histone Variant MacroH2A Blocks Cellular Reprogramming by Inhibiting Mesenchymal-to-Epithelial Transition', *Molecular and Cellular Biology*, 38(10), pp. e00669-17. Available at: <https://doi.org/10.1128/MCB.00669-17>.
- Pokholok, D.K. *et al.* (2005) 'Genome-wide Map of Nucleosome Acetylation and Methylation in Yeast', *Cell*, 122(4), pp. 517–527. Available at: <https://doi.org/10.1016/j.cell.2005.06.026>.
- Polo, S.E., Roche, D. and Almouzni, G. (2006) 'New Histone Incorporation Marks Sites of UV Repair in Human Cells', *Cell*, 127(3), pp. 481–493. Available at: <https://doi.org/10.1016/j.cell.2006.08.049>.
- Pope, B.D. *et al.* (2014) 'Topologically associating domains are stable units of replication-timing regulation', *Nature*, 515(7527), pp. 402–405. Available at: <https://doi.org/10.1038/nature13986>.
- Probst *et al.* (2010) 'A strand-specific burst in transcription of pericentric satellites is required for chromocenter formation and early mouse development.', *Developmental Cell*, 19(4), pp. 625–638. Available at: <https://doi.org/10.1016/j.devcel.2010.09.002>.
- Probst, A.V. *et al.* (2007) 'Structural differences in centromeric heterochromatin are spatially reconciled on fertilisation in the mouse zygote', *Chromosoma*, 116(4), pp. 403–415. Available at: <https://doi.org/10.1007/s00412-007-0106-8>.
- Probst, A.V. and Almouzni, G. (2011) 'Heterochromatin establishment in the context of genome-wide epigenetic reprogramming', *Trends in Genetics*, 27(5), pp. 177–185. Available at: <https://doi.org/10.1016/j.tig.2011.02.002>.
- Puschendorf, M. *et al.* (2008) 'PRC1 and Suv39h specify parental asymmetry at constitutive heterochromatin in early mouse embryos', *Nature Genetics*, 40(4), pp. 411–420. Available at: <https://doi.org/10.1038/ng.99>.
- Quivy, J.-P. *et al.* (2004) 'A CAF-1 dependent pool of HP1 during heterochromatin duplication', *The EMBO Journal*, 23(17), pp. 3516–3526. Available at: <https://doi.org/10.1038/sj.emboj.7600362>.
- Quivy, J.-P. *et al.* (2008) 'The HP1–p150/CAF-1 interaction is required for pericentric heterochromatin replication and S-phase progression in mouse cells', *Nature Structural & Molecular Biology*, 15(9), pp. 972–979. Available at: <https://doi.org/10.1038/nsmb.1470>.
- Rada-Iglesias, A. *et al.* (2011) 'A unique chromatin signature uncovers early developmental enhancers in humans', *Nature*, 470(7333), pp. 279–283. Available at: <https://doi.org/10.1038/nature09692>.

- Rai, T.S. *et al.* (2011) 'Human CABIN1 Is a Functional Member of the Human HIRA/UBN1/ASF1a Histone H3.3 Chaperone Complex', *Molecular and Cellular Biology*, 31(19), pp. 4107–4118. Available at: <https://doi.org/10.1128/MCB.05546-11>.
- Rausch, C. *et al.* (2020) 'Developmental differences in genome replication program and origin activation', *Nucleic Acids Research*, 48(22), pp. 12751–12777. Available at: <https://doi.org/10.1093/nar/gkaa1124>.
- Ray-Gallet, D. *et al.* (2002) 'HIRA is critical for a nucleosome assembly pathway independent of DNA synthesis', *Molecular Cell*, 9(5), pp. 1091–1100. Available at: [https://doi.org/10.1016/s1097-2765\(02\)00526-9](https://doi.org/10.1016/s1097-2765(02)00526-9).
- Ray-Gallet, D. *et al.* (2011) 'Dynamics of Histone H3 Deposition In Vivo Reveal a Nucleosome Gap-Filling Mechanism for H3.3 to Maintain Chromatin Integrity', *Molecular Cell*, 44(6), pp. 928–941. Available at: <https://doi.org/10.1016/j.molcel.2011.12.006>.
- Ray-Gallet, D. *et al.* (2018) 'Functional activity of the H3.3 histone chaperone complex HIRA requires trimerization of the HIRA subunit', *Nature Communications*, 9(1), p. 3103. Available at: <https://doi.org/10.1038/s41467-018-05581-y>.
- Ray-Gallet, D. and Almouzni, G. (2022) 'H3-H4 histone chaperones and cancer', *Current Opinion in Genetics & Development*, 73, p. 101900. Available at: <https://doi.org/10.1016/j.gde.2022.101900>.
- Rea, S. *et al.* (2000) 'Regulation of chromatin structure by site-specific histone H3 methyltransferases', *Nature*, 406(6796), pp. 593–599. Available at: <https://doi.org/10.1038/35020506>.
- Renaud-Pageot, C. *et al.* (2022) 'CENP-A Regulation and Cancer', *Frontiers in Cell and Developmental Biology*, 10. Available at: <https://www.frontiersin.org/article/10.3389/fcell.2022.907120> (Accessed: 9 June 2022).
- Ricci, M.A. *et al.* (2015) 'Chromatin Fibers Are Formed by Heterogeneous Groups of Nucleosomes In Vivo', *Cell*, 160(6), pp. 1145–1158. Available at: <https://doi.org/10.1016/j.cell.2015.01.054>.
- Rogakou, E.P. and Sekeri-Pataryas, K.E. (1999) 'Histone variants of H2A and H3 families are regulated during in vitro aging in the same manner as during differentiation', *Experimental Gerontology*, 34(6), pp. 741–754. Available at: [https://doi.org/10.1016/s0531-5565\(99\)00046-7](https://doi.org/10.1016/s0531-5565(99)00046-7).
- Romeo, K. *et al.* (2015) 'The SENP7 SUMO-Protease Presents a Module of Two HP1 Interaction Motifs that Locks HP1 Protein at Pericentric Heterochromatin', *Cell Reports*, 10(5), pp. 771–782. Available at: <https://doi.org/10.1016/j.celrep.2015.01.004>.
- Rothbart, S.B. *et al.* (2012) 'Association of UHRF1 with methylated H3K9 directs the maintenance of DNA methylation', *Nature Structural & Molecular Biology*, 19(11), pp. 1155–1160. Available at: <https://doi.org/10.1038/nsmb.2391>.
- Rousseaux, S. *et al.* (2005) 'Establishment of male-specific epigenetic information', *Gene*, 345(2), pp. 139–153. Available at: <https://doi.org/10.1016/j.gene.2004.12.004>.
- Russo, V.E.A., Martienssen, R.A. and Riggs, A.D. (1996) *Epigenetic mechanisms of gene regulation*. Cold Spring Harbor Laboratory Press. Available at: https://scholar.google.com/scholar_lookup?title=Epigenetic+mechanisms+of+gene+regulation&author=Russo%2C+V.+E.+A.+%28Vincenzo+E.+A.%29&publication_year=1996 (Accessed: 19 May 2022).
- Rust, M.J., Bates, M. and Zhuang, X. (2006) 'Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM)', *Nature Methods*, 3(10), pp. 793–796. Available at: <https://doi.org/10.1038/nmeth929>.

- Saksouk, N. *et al.* (2014) ‘Redundant Mechanisms to Form Silent Chromatin at Pericentromeric Regions Rely on BEND3 and DNA Methylation’, *Molecular Cell*, 56(4), pp. 580–594. Available at: <https://doi.org/10.1016/j.molcel.2014.10.001>.
- Saksouk, N., Simboeck, E. and Déjardin, J. (2015) ‘Constitutive heterochromatin formation and transcription in mammals’, *Epigenetics & Chromatin*, 8(1), pp. 1–17. Available at: <https://doi.org/10.1186/1756-8935-8-3>.
- Sampath, Srihari C. *et al.* (2007) ‘Methylation of a histone mimic within the histone methyltransferase G9a regulates protein complex assembly’, *Molecular Cell*, 27(4), pp. 596–608. Available at: <https://doi.org/10.1016/j.molcel.2007.06.026>.
- Santenard, A. *et al.* (2010) ‘Heterochromatin formation in the mouse embryo requires critical residues of the histone variant H3.3’, *Nature Cell Biology*, 12(9), pp. 853–862. Available at: <https://doi.org/10.1038/ncb2089>.
- Sauer, P.V. *et al.* (2018) ‘Mechanistic insights into histone deposition and nucleosome assembly by the chromatin assembly factor-1’, *Nucleic Acids Research*, 46(19), pp. 9907–9917. Available at: <https://doi.org/10.1093/nar/gky823>.
- Schermelleh, L. *et al.* (2008) ‘Subdiffraction multicolor imaging of the nuclear periphery with 3D structured illumination microscopy’, *Science (New York, N.Y.)*, 320(5881), pp. 1332–1336. Available at: <https://doi.org/10.1126/science.1156947>.
- Schlesinger, S. *et al.* (2017) ‘A hyperdynamic H3.3 nucleosome marks promoter regions in pluripotent embryonic stem cells’, *Nucleic Acids Research*, 45(21), pp. 12181–12194. Available at: <https://doi.org/10.1093/nar/gkx817>.
- Schlesinger, S. and Meshorer, E. (2019) ‘Open Chromatin, Epigenetic Plasticity, and Nuclear Organization in Pluripotency’, *Developmental Cell*, 48(2), pp. 135–150. Available at: <https://doi.org/10.1016/j.devcel.2019.01.003>.
- Schwartzentruber, J. *et al.* (2012) ‘Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma’, *Nature*, 482(7384), pp. 226–231. Available at: <https://doi.org/10.1038/nature10833>.
- Semrau, S. *et al.* (2017) ‘Dynamics of lineage commitment revealed by single-cell transcriptomics of differentiating embryonic stem cells’, *Nature Communications*, 8(1), p. 1096. Available at: <https://doi.org/10.1038/s41467-017-01076-4>.
- Sexton, T. and Cavalli, G. (2015) ‘The Role of Chromosome Domains in Shaping the Functional Genome’, *Cell*, 160(6), pp. 1049–1059. Available at: <https://doi.org/10.1016/j.cell.2015.02.040>.
- Shah, R.N. *et al.* (2021) ‘Re-evaluating the role of nucleosomal bivalency in early development’. *bioRxiv*, p. 2021.09.09.458948. Available at: <https://doi.org/10.1101/2021.09.09.458948>.
- Shahbazian, M.D. and Grunstein, M. (2007) ‘Functions of site-specific histone acetylation and deacetylation’, *Annual Review of Biochemistry*, 76, pp. 75–100. Available at: <https://doi.org/10.1146/annurev.biochem.76.052705.162114>.
- Shelby, R.D., Vafa, O. and Sullivan, K.F. (1997) ‘Assembly of CENP-A into Centromeric Chromatin Requires a Cooperative Array of Nucleosomal DNA Contact Sites’, *Journal of Cell Biology*, 136(3), pp. 501–513. Available at: <https://doi.org/10.1083/jcb.136.3.501>.
- Shi, L. *et al.* (2011) ‘Four amino acids guide the assembly or disassembly of Arabidopsis histone H3.3-containing nucleosomes’, *Proceedings of the National Academy of Sciences*, 108(26), pp. 10574–10578. Available at: <https://doi.org/10.1073/pnas.1017882108>.

- Shibahara, K. and Stillman, B. (1999) 'Replication-dependent marking of DNA by PCNA facilitates CAF-1-coupled inheritance of chromatin', *Cell*, 96(4), pp. 575–585. Available at: [https://doi.org/10.1016/s0092-8674\(00\)80661-3](https://doi.org/10.1016/s0092-8674(00)80661-3).
- Shibata, F. and Murata, M. (2004) 'Differential localization of the centromere-specific proteins in the major centromeric satellite of *Arabidopsis thaliana*', *Journal of Cell Science*, 117(Pt 14), pp. 2963–2970. Available at: <https://doi.org/10.1242/jcs.01144>.
- Shin, J. *et al.* (2016) 'Aurkb/PP1-mediated resetting of Oct4 during the cell cycle determines the identity of embryonic stem cells', *eLife*, 5(FEBRUARY2016). Available at: <https://doi.org/10.7554/eLife.10877>.
- Shu, H. *et al.* (2012) 'Distinct modes of DNA accessibility in plant chromatin', *Nature Communications*, 3(1), p. 1281. Available at: <https://doi.org/10.1038/ncomms2259>.
- Sima, J. *et al.* (2019) 'Identifying cis Elements for Spatiotemporal Control of Mammalian DNA Replication', *Cell*, 176(4), pp. 816–830.e18. Available at: <https://doi.org/10.1016/j.cell.2018.11.036>.
- Simon, L. *et al.* (2015) 'Structure and Function of Centromeric and Pericentromeric Heterochromatin in *Arabidopsis thaliana*', *Frontiers in Plant Science*, 6. Available at: <https://www.frontiersin.org/article/10.3389/fpls.2015.01049> (Accessed: 8 March 2022).
- Simpson, R.T. (1978) 'Structure of the chromatosome, a chromatin particle containing 160 base pairs of DNA and all the histones', *Biochemistry*, 17(25), pp. 5524–5531. Available at: <https://doi.org/10.1021/bi00618a030>.
- Sitbon, D. *et al.* (2020) 'Histone variant H3.3 residue S31 is essential for *Xenopus* gastrulation regardless of the deposition pathway', *Nature Communications*, 11(1), pp. 1–15. Available at: <https://doi.org/10.1038/s41467-020-15084-4>.
- Smith, A. (2017) 'Formative pluripotency: the executive phase in a developmental continuum', *Development*, 144(3), pp. 365–373. Available at: <https://doi.org/10.1242/dev.142679>.
- Smith, S. and Stillman, B. (1989) 'Purification and characterization of CAF-I, a human cell factor required for chromatin assembly during DNA replication in vitro', *Cell*, 58(1), pp. 15–25. Available at: [https://doi.org/10.1016/0092-8674\(89\)90398-x](https://doi.org/10.1016/0092-8674(89)90398-x).
- Smothers, J.F. and Henikoff, S. (2000) 'The HP1 chromo shadow domain binds a consensus peptide pentamer', *Current Biology*, 10(1), pp. 27–30. Available at: [https://doi.org/10.1016/S0960-9822\(99\)00260-2](https://doi.org/10.1016/S0960-9822(99)00260-2).
- Snow, M.H.L. (1977) 'Gastrulation in the mouse: Growth and regionalization of the epiblast', *Development*, 42(1), pp. 293–303. Available at: <https://doi.org/10.1242/dev.42.1.293>.
- Solovei, I. *et al.* (2009) 'Nuclear architecture of rod photoreceptor cells adapts to vision in mammalian evolution', *Cell*, 137(2), pp. 356–368. Available at: <https://doi.org/10.1016/j.cell.2009.01.052>.
- Solovei, I. *et al.* (2013) 'LBR and lamin A/C sequentially tether peripheral heterochromatin and inversely regulate differentiation', *Cell*, 152(3), pp. 584–598. Available at: <https://doi.org/10.1016/j.cell.2013.01.009>.
- Solovei, I., Thanisch, K. and Feodorova, Y. (2016) 'How to rule the nucleus: divide et impera', *Current Opinion in Cell Biology*, 40, pp. 47–59. Available at: <https://doi.org/10.1016/j.ceb.2016.02.014>.
- Soufi, A., Donahue, G. and Zaret, K.S. (2012) 'Facilitators and Impediments of the Pluripotency Reprogramming Factors' Initial Engagement with the Genome', *Cell*, 151(5), pp. 994–1004. Available at: <https://doi.org/10.1016/j.cell.2012.09.045>.

- Soyfer, V.N. (2001) 'The consequences of political dictatorship for Russian science', *Nature Reviews Genetics*, 2(9), pp. 723–729. Available at: <https://doi.org/10.1038/35088598>.
- Strickfaden, H. *et al.* (2020) 'Condensed Chromatin Behaves like a Solid on the Mesoscale In Vitro and in Living Cells', *Cell*, 183(7), pp. 1772–1784.e13. Available at: <https://doi.org/10.1016/j.cell.2020.11.027>.
- Strom, A.R. *et al.* (2017) 'Phase separation drives heterochromatin domain formation', *Nature*, 547(7662), pp. 241–245. Available at: <https://doi.org/10.1038/nature22989>.
- Strom, A.R. *et al.* (2021) 'HP1 α is a chromatin crosslinker that controls nuclear and mitotic chromosome mechanics', *eLife*. Edited by G.J. Narlikar, K. Struhl, and S. Redding, 10, p. e63972. Available at: <https://doi.org/10.7554/eLife.63972>.
- Stroud, H. *et al.* (2012) 'Genome-wide analysis of histone H3.1 and H3.3 variants in *Arabidopsis thaliana*', *Proceedings of the National Academy of Sciences*, 109(14), pp. 5370–5375. Available at: <https://doi.org/10.1073/pnas.1203145109>.
- Sura, W. *et al.* (2017) 'Dual Role of the Histone Variant H2A.Z in Transcriptional Regulation of Stress-Response Genes', *The Plant Cell*, 29(4), pp. 791–807. Available at: <https://doi.org/10.1105/tpc.16.00573>.
- Sutton, W.S. (1903) *The chromosome in Heredity, The chromosome in heredity*. Available at: <https://web.archive.org/web/20160314091118/http://esp.org/foundations/genetics/classical/holdings/s/wss-03.pdf> (Accessed: 2 May 2022).
- Szabo, Q. *et al.* (2020) 'Regulation of single-cell genome organization into TADs and chromatin nanodomains', *Nature Genetics*, pp. 1–7. Available at: <https://doi.org/10.1038/s41588-020-00716-8>.
- Szenker, E., Lacoste, N. and Almouzni, G. (2012) 'A developmental requirement for HIRA-dependent H3.3 deposition revealed at gastrulation in *Xenopus*', *Cell Reports*, 1(6), pp. 730–740. Available at: <https://doi.org/10.1016/j.celrep.2012.05.006>.
- Tachibana, M. *et al.* (2002) 'G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis', *Genes & Development*, 16(14), pp. 1779–1791. Available at: <https://doi.org/10.1101/gad.989402>.
- Tachibana, M. *et al.* (2005) 'Histone methyltransferases G9a and GLP form heteromeric complexes and are both crucial for methylation of euchromatin at H3-K9', *Genes & Development*, 19(7), pp. 815–826. Available at: <https://doi.org/10.1101/gad.1284005>.
- Tachiwana, H. *et al.* (2011) 'Crystal structure of the human centromeric nucleosome containing CENP-A', *Nature*, 476(7359), pp. 232–235. Available at: <https://doi.org/10.1038/nature10258>.
- Tagami, H. *et al.* (2004) 'Histone H3.1 and H3.3 Complexes Mediate Nucleosome Assembly Pathways Dependent or Independent of DNA Synthesis', *Cell*, 116(1), pp. 51–61. Available at: [https://doi.org/10.1016/S0092-8674\(03\)01064-X](https://doi.org/10.1016/S0092-8674(03)01064-X).
- Tahiliani, M. *et al.* (2009) 'Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1', *Science (New York, N.Y.)*, 324(5929), pp. 930–935. Available at: <https://doi.org/10.1126/science.1170116>.
- Takahashi, K. *et al.* (2007) 'Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors', *Cell*, 131(5), pp. 861–872. Available at: <https://doi.org/10.1016/j.cell.2007.11.019>.
- Takahashi, K. and Yamanaka, S. (2006) 'Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors', *Cell*, 126(4), pp. 663–676. Available at: <https://doi.org/10.1016/j.cell.2006.07.024>.

- Talbert, P.B. and Henikoff, S. (2014) 'Environmental responses mediated by histone variants', *Trends in Cell Biology*, 24(11), pp. 642–650. Available at: <https://doi.org/10.1016/j.tcb.2014.07.006>.
- Talbert, P.B. and Henikoff, S. (2021) 'Histone variants at a glance', *Journal of Cell Science*, 134(6), p. jcs244749. Available at: <https://doi.org/10.1242/jcs.244749>.
- Tang, M.C.W. *et al.* (2013) 'Conditional allelic replacement applied to genes encoding the histone variant H3.3 in the mouse', *Genesis (New York, N.Y.: 2000)*, 51(2), pp. 142–146. Available at: <https://doi.org/10.1002/dvg.22366>.
- Tang, M.C.W. *et al.* (2015) 'Contribution of the Two Genes Encoding Histone Variant H3.3 to Viability and Fertility in Mice', *PLOS Genetics*, 11(2), p. e1004964. Available at: <https://doi.org/10.1371/journal.pgen.1004964>.
- Taylor, K.R. *et al.* (2014) 'Recurrent activating ACVR1 mutations in diffuse intrinsic pontine glioma', *Nature Genetics*, 46(5), pp. 457–461. Available at: <https://doi.org/10.1038/ng.2925>.
- Teissandier, A. and Bourc'h, D. (2017) 'Gene body DNA methylation conspires with H3K36me3 to preclude aberrant transcription', *The EMBO journal*, 36(11), pp. 1471–1473. Available at: <https://doi.org/10.15252/embj.201796812>.
- Terranova, R. *et al.* (2005) 'The reorganisation of constitutive heterochromatin in differentiating muscle requires HDAC activity', *Experimental Cell Research*, 310(2), pp. 344–356. Available at: <https://doi.org/10.1016/j.yexcr.2005.07.031>.
- Thorne, J.L., Ouboussad, L. and Lefevre, P.F. (2012) 'Heterochromatin protein 1 gamma and IκB kinase alpha interdependence during tumour necrosis factor gene transcription elongation in activated macrophages', *Nucleic Acids Research*, 40(16), pp. 7676–7689. Available at: <https://doi.org/10.1093/nar/gks509>.
- Torné, J. *et al.* (2020) 'Two HIRA-dependent pathways mediate H3.3 de novo deposition and recycling during transcription', *Nature Structural & Molecular Biology*, pp. 1–12. Available at: <https://doi.org/10.1038/s41594-020-0492-7>.
- Torres-Padilla, M.-E. *et al.* (2006) 'Dynamic distribution of the replacement histone variant H3.3 in the mouse oocyte and preimplantation embryos', *The International Journal of Developmental Biology*, 50(Next). Available at: <https://doi.org/10.1387/ijdb.052073mt>.
- Toyooka, Y. *et al.* (2008) 'Identification and characterization of subpopulations in undifferentiated ES cell culture', *Development*, 135(5), pp. 909–918. Available at: <https://doi.org/10.1242/dev.017400>.
- Udugama, M. *et al.* (2022) 'Histone H3.3 phosphorylation promotes heterochromatin formation by inhibiting H3K9/K36 histone demethylase', *Nucleic Acids Research*, p. gkac259. Available at: <https://doi.org/10.1093/nar/gkac259>.
- Urahama, T. *et al.* (2016) 'Histone H3.5 forms an unstable nucleosome and accumulates around transcription start sites in human testis', *Epigenetics & Chromatin*, 9(1), p. 2. Available at: <https://doi.org/10.1186/s13072-016-0051-y>.
- Vaquero-Sedas, M.I. and Vega-Palas, M.A. (2013) 'Differential association of Arabidopsis telomeres and centromeres with Histone H3 variants', *Scientific Reports*, 3(1), p. 1202. Available at: <https://doi.org/10.1038/srep01202>.
- Varley, K.E. *et al.* (2013) 'Dynamic DNA methylation across diverse human cell lines and tissues', *Genome Research*, 23(3), pp. 555–567. Available at: <https://doi.org/10.1101/gr.147942.112>.

- Vermeulen, M. and Déjardin, J. (2020) ‘Locus-specific chromatin isolation’, *Nature Reviews Molecular Cell Biology*, 21(5), pp. 249–250. Available at: <https://doi.org/10.1038/s41580-020-0217-0>.
- Verrelle, P. *et al.* (2021) ‘CENP-A Subnuclear Localization Pattern as Marker Predicting Curability by Chemoradiation Therapy for Locally Advanced Head and Neck Cancer Patients’, *Cancers*, 13(16), p. 3928. Available at: <https://doi.org/10.3390/cancers13163928>.
- Virchow, R. (1860) *Cellular Pathology as based upon physiological and pathological histology. Twenty lectures delivered in ... 1858. Translated from the second edition of the original by F. Chance. With notes and numerous emendations principally from MS. notes of the author, and illustrated by ... engravings on wood.*
- Vogelstein, B. *et al.* (2013) ‘Cancer genome landscapes’, *Science (New York, N.Y.)*, 339(6127), pp. 1546–1558. Available at: <https://doi.org/10.1126/science.1235122>.
- Volpe, T.A. *et al.* (2002) ‘Regulation of Heterochromatic Silencing and Histone H3 Lysine-9 Methylation by RNAi’, *Science*, 297(5588), pp. 1833–1837. Available at: <https://doi.org/10.1126/science.1074973>.
- Waddington, C.H. (1942) ‘Canalization of Development and the Inheritance of Acquired Characters’, *Nature*, 150(3811), pp. 563–565. Available at: <https://doi.org/10.1038/150563a0>.
- Waddington, C.H. (1956) ‘Genetic Assimilation of the Bithorax Phenotype’, *Evolution*, 10(1), pp. 1–13. Available at: <https://doi.org/10.2307/2406091>.
- Wang, C. *et al.* (2018) ‘Reprogramming of H3K9me3-dependent heterochromatin during mammalian embryo development’, *Nature Cell Biology*, 20(5), pp. 620–631. Available at: <https://doi.org/10.1038/s41556-018-0093-4>.
- Wang, H. *et al.* (2003) ‘mAM facilitates conversion by ESET of dimethyl to trimethyl lysine 9 of histone H3 to cause transcriptional repression’, *Molecular Cell*, 12(2), pp. 475–487. Available at: <https://doi.org/10.1016/j.molcel.2003.08.007>.
- Wang, L. *et al.* (2014) ‘Programming and inheritance of parental DNA methylomes in mammals’, *Cell*, 157(4), pp. 979–991. Available at: <https://doi.org/10.1016/j.cell.2014.04.017>.
- Watson, J.D. and Crick, F.H.C. (1953) ‘Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid’, *Nature*, 171(4356), pp. 737–738. Available at: <https://doi.org/10.1038/171737a0>.
- Weatherall, D.J. *et al.* (1981) ‘Hemoglobin H Disease and Mental Retardation’, *New England Journal of Medicine*, 305(11), pp. 607–612. Available at: <https://doi.org/10.1056/NEJM198109103051103>.
- Weinberg, D.N. *et al.* (2019) ‘The histone mark H3K36me2 recruits DNMT3A and shapes the intergenic DNA methylation landscape’, *Nature*, 573(7773), pp. 281–286. Available at: <https://doi.org/10.1038/s41586-019-1534-3>.
- Wen, H. *et al.* (2014) ‘ZMYND11 links histone H3K36me3 to transcription elongation and tumour suppression’, *Nature*, 508(7495), pp. 263–268. Available at: <https://doi.org/10.1038/nature13045>.
- Werken, H.J.G. van de *et al.* (2017) ‘Small chromosomal regions position themselves autonomously according to their chromatin class’, *Genome Research*, 27(6), pp. 922–933. Available at: <https://doi.org/10.1101/gr.213751.116>.
- Wilkins, M.H.F., Stokes, A.R. and Wilson, H.R. (1953) ‘Molecular Structure of Nucleic Acids: Molecular Structure of Deoxypentose Nucleic Acids’, *Nature*, 171(4356), pp. 738–740. Available at: <https://doi.org/10.1038/171738a0>.

- Witt, O., Albig, W. and Doenecke, D. (1996) 'Testis-specific expression of a novel human H3 histone gene', *Experimental Cell Research*, 229(2), pp. 301–306. Available at: <https://doi.org/10.1006/excr.1996.0375>.
- Wolffe, A.P. (1995) 'Centromeric Chromatin: Histone deviants', *Current Biology*, 5(5), pp. 452–454. Available at: [https://doi.org/10.1016/S0960-9822\(95\)00088-1](https://doi.org/10.1016/S0960-9822(95)00088-1).
- Wollmann, H. *et al.* (2012) 'Dynamic Deposition of Histone Variant H3.3 Accompanies Developmental Remodeling of the Arabidopsis Transcriptome', *PLOS Genetics*, 8(5), p. e1002658. Available at: <https://doi.org/10.1371/journal.pgen.1002658>.
- Wong, A.K. and Rattner, J.B. (1988) 'Sequence organization and cytological localization of the minor satellite of mouse', *Nucleic Acids Research*, 16(24), pp. 11645–11661. Available at: <https://doi.org/10.1093/nar/16.24.11645>.
- Wong, L.H. *et al.* (2008) 'Histone H3.3 incorporation provides a unique and functionally essential telomeric chromatin in embryonic stem cells', *Genome Research*, 19(3), pp. 404–414. Available at: <https://doi.org/10.1101/gr.084947.108>.
- Wu, R.S., Tsai, S. and Bonner, W.M. (1982) 'Patterns of histone variant synthesis can distinguish G0 from G1 cells', *Cell*, 31(2 Pt 1), pp. 367–374. Available at: [https://doi.org/10.1016/0092-8674\(82\)90130-1](https://doi.org/10.1016/0092-8674(82)90130-1).
- Xiong, C. *et al.* (2018) 'UBN1/2 of HIRA complex is responsible for recognition and deposition of H3.3 at cis-regulatory elements of genes in mouse ES cells', *BMC Biology*, 16(1), p. 110. Available at: <https://doi.org/10.1186/s12915-018-0573-9>.
- Xu, J. *et al.* (2018) 'Super-Resolution Imaging of Higher-Order Chromatin Structures at Different Epigenomic States in Single Mammalian Cells', *Cell Reports*, 24(4), pp. 873–882. Available at: <https://doi.org/10.1016/j.celrep.2018.06.085>.
- Xu, X. *et al.* (2022) 'Stable inheritance of H3.3-containing nucleosomes during mitotic cell divisions', *Nature Communications*, 13(1), p. 2514. Available at: <https://doi.org/10.1038/s41467-022-30298-4>.
- Xue, Y. *et al.* (2003) 'The ATRX syndrome protein forms a chromatin-remodeling complex with Daxx and localizes in promyelocytic leukemia nuclear bodies', *Proceedings of the National Academy of Sciences of the United States of America*, 100(19), pp. 10635–10640. Available at: <https://doi.org/10.1073/pnas.1937626100>.
- Yadav, T., Quivy, J.-P. and Almouzni, G. (2018) 'Chromatin plasticity: A versatile landscape that underlies cell fate and identity', *Science*, 361(6409), pp. 1332–1336. Available at: <https://doi.org/10.1126/science.aat8950>.
- Yamada, T. *et al.* (1999) 'Functional Domain Structure of Human Heterochromatin Protein HP1 Hsa: Involvement of Internal DNA-Binding and C-Terminal Self-Association Domains in the Formation of Discrete Dots in Interphase Nuclei', *The Journal of Biochemistry*, 125(4), pp. 832–837. Available at: <https://doi.org/10.1093/oxfordjournals.jbchem.a022356>.
- Yamamoto, K. and Sonoda, M. (2003) 'Self-interaction of heterochromatin protein 1 is required for direct binding to histone methyltransferase, SUV39H1', *Biochemical and Biophysical Research Communications*, 301(2), pp. 287–292. Available at: [https://doi.org/10.1016/S0006-291X\(02\)03021-8](https://doi.org/10.1016/S0006-291X(02)03021-8).
- Yang, B.X. *et al.* (2015) 'Systematic Identification of Factors for Provirus Silencing in Embryonic Stem Cells', *Cell*, 163(1), pp. 230–245. Available at: <https://doi.org/10.1016/j.cell.2015.08.037>.
- Yang, J.-H. *et al.* (2016) 'Differential regulation of the histone chaperone HIRA during muscle cell differentiation by a phosphorylation switch', *Experimental & Molecular Medicine*, 48(8), pp. e252–e252. Available at: <https://doi.org/10.1038/emm.2016.68>.

- Yang, X. *et al.* (1997) 'Daxx, a novel Fas-binding protein that activates JNK and apoptosis', *Cell*, 89(7), pp. 1067–1076. Available at: [https://doi.org/10.1016/s0092-8674\(00\)80294-9](https://doi.org/10.1016/s0092-8674(00)80294-9).
- Yelagandula, R. *et al.* (2014) 'The Histone Variant H2A.W Defines Heterochromatin and Promotes Chromatin Condensation in Arabidopsis', *Cell*, 158(1), pp. 98–109. Available at: <https://doi.org/10.1016/j.cell.2014.06.006>.
- Zamudio, N. *et al.* (2015) 'DNA methylation restrains transposons from adopting a chromatin signature permissive for meiotic recombination', *Genes & Development*, 29(12), pp. 1256–1270. Available at: <https://doi.org/10.1101/gad.257840.114>.
- Zasadzińska, E. *et al.* (2013) 'Dimerization of the CENP-A assembly factor HJURP is required for centromeric nucleosome deposition', *The EMBO Journal*, 32(15), pp. 2113–2124. Available at: <https://doi.org/10.1038/emboj.2013.142>.
- Zhang, M. *et al.* (2022) 'Histone chaperone HIRA complex regulates retrotransposons in embryonic stem cells', *Stem Cell Research & Therapy*, 13(1), p. 137. Available at: <https://doi.org/10.1186/s13287-022-02814-2>.
- Zhang, W. *et al.* (2008) 'Epigenetic modification of centromeric chromatin: hypomethylation of DNA sequences in the CENH3-associated chromatin in Arabidopsis thaliana and maize', *The Plant Cell*, 20(1), pp. 25–34. Available at: <https://doi.org/10.1105/tpc.107.057083>.
- Zhu, P. *et al.* (2018) 'Single-cell DNA methylome sequencing of human preimplantation embryos', *Nature Genetics*, 50(1), pp. 12–19. Available at: <https://doi.org/10.1038/s41588-017-0007-6>.

RÉSUMÉ

Dans les cellules eucaryotes, l'ADN est organisé en un complexe nucléo-protéique, la chromatine. Des changements dans l'organisation de la chromatine, de son unité de base, le nucléosome, jusqu'à des niveaux plus élevés dans des domaines nucléaires, ont été associés à des états distincts au cours du cycle cellulaire ainsi qu'au cours de transitions du destin cellulaire. Au cours de ma thèse de doctorat, j'ai exploré la présence de deux variants différents de l'histone H3, le variant réplcatif H3.1 et le variant de remplacement H3.3, aux domaines d'hétérochromatine péricentrique (PHC) et la manière dont leur équilibre est contrôlé tout au long de la différenciation des cellules souches embryonnaires (ES) de souris. Pour cela, j'ai combiné des approches d'imagerie et de séquençage à l'échelle du génome. Tout d'abord, j'ai observé par imagerie un enrichissement de H3.1, au détriment de H3.3, dans les domaines PHC. Cet enrichissement en H3.1 se révèle tout particulièrement pendant la réplication des domaines PHC et est plus fréquent dans les cellules différenciées que dans les cellules pluripotentes. En parallèle, H3.3 est exclu de ces sites pendant la réplication. En réalisant la cartographie de ces variants à l'échelle du génome par séquençage, j'ai identifié un enrichissement spécifique de H3.1 au niveau des répétitions satellites majeures, qui représentent les séquences d'ADN principales correspondant aux PHC tout au long de la différenciation. D'un point de vue mécanistique, j'ai démontré que des substitutions d'acides aminés dans le motif de H3.1, qui est clé pour l'interaction avec son chaperon d'histone dédié CAF-1, affectent l'enrichissement de H3.1 aux domaines PHC. Cependant, en ciblant le chaperon d'histone HIRA aux domaines PHC, j'ai pu forcer le dépôt de H3.3 dans ces domaines et ainsi interférer avec l'accumulation de H3.1 dépendante du cycle cellulaire. Sur la base de ces résultats, je propose un modèle dans lequel l'enrichissement local de H3.1 dans les domaines PHC résulte d'une accumulation par la voie d'incorporation dans la chromatine couplée à la réplication qui n'est pas contrecarrée par le dépôt de H3.3. Je discute de l'impact de ces voies d'assemblage des variants de l'histone H3 sur l'organisation des domaines subnucléaires PHC en lien avec le maintien du destin cellulaire et la stabilité des chromosomes.

MOTS CLÉS

Dynamique de la chromatine, Chaperones d'histone, Variants d'histone, Cellules souches embryonnaires, Chromocentre

ABSTRACT

In eukaryotic cells, DNA is organized in a nucleo-protein complex, named chromatin. Changes in chromatin states and organization, from its basic unit, the nucleosome, up to higher levels in nuclear domains, have been associated with distinct states during the cell cycle as well as during cell fate transitions. During my PhD thesis, I explored the presence of two different histone H3 variants, the replicative H3.1 and the replacement H3.3 variant, at pericentric heterochromatin (PHC) domains and how their balance is controlled throughout differentiation of mouse embryonic stem (ES) cells. For this, I combined genome-wide and imaging approaches. First by imaging, I observed a consistent enrichment of H3.1, at the expense of H3.3, at PHC domains. This H3.1 enrichment is highly prevalent as PHC domains replicate and is more pronounced in differentiated rather than in pluripotent cells. Using genome-wide mapping of the variants combined with sequencing, I could identify a specific enrichment of H3.1 at major satellite repeats, which represent the main DNA sequences corresponding to PHC, throughout differentiation. Mechanistically, I revealed that substitutions of amino acids within the histone fold domain of H3.1, that is key for its interaction with the dedicated histone chaperone CAF-1 (Chromatin Assembly Factor), impinge on the enrichment of H3.1 at PHC domains. However, by targeting the histone chaperone HIRA (Histone Regulator A) at PHC domains, I could force H3.3 deposition at these sites and thereby interfering with the cell-cycle dependent accumulation of H3.1. Based on these results, I propose a model in which the local enrichment of H3.1 at PHC domains results from an enhanced provision through the replication coupled deposition pathway that is not counteracted by H3.3 deposition via the non-replicative deposition pathways. I thus discuss how the deposition and maintenance of H3 variants can influence the higher-order chromatin structure at the PHC domains in the context of cell fate and chromosome stability.

KEYWORDS

Chromatin Dynamics, Histone chaperone, Histone variants, ES cells, Chromocenters.