Mitotic inheritance of cell identity and epigenetic memory

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"UE non-coding RNAs and epigenetics"

universite PARIS-SACLAY
GRADUATE SCHOOL Life Sciences and Health
MASTER Biologie-Sante Life Sciences and Health
Genetics Genomics Epigenetics Evolution GenE2
Master Biologie Santé universite PARIS-SACLAY

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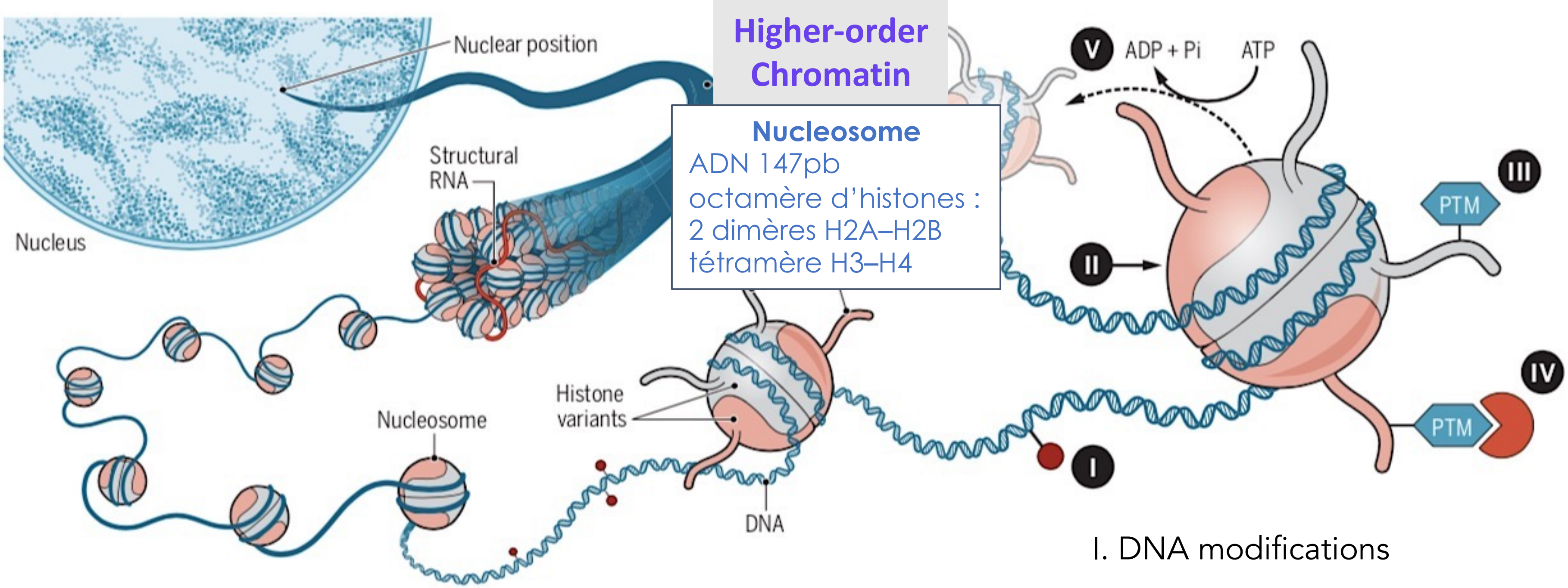
Epigenetics : definitions and mechanisms

Epigenetic mechanisms



Higher-order Chromatin

Nucleosome
ADN 147pb
octamère d'histones :
2 dimères H2A–H2B
tétramère H3–H4

I. DNA modifications

II. Histone variants

III. Histone modifications

IV. Histone readers

V. Remodeling factor

VI. Non coding RNAs

(Yadav, Quivy & Almouzni, 2018)

Histone modifications
Methylation

Acetylation
Phosphorylation
Sumoylation…

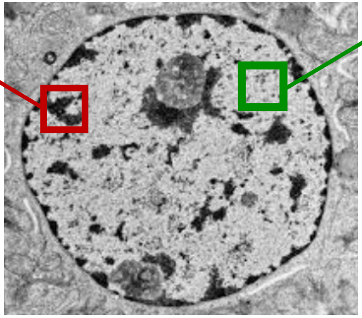
▶ Chromatin opening or compaction >> determining gene expression

Chromatin structure in interphase

Heterochromatin
Highly compacted
Mostly transcriptionally inactive

Signatures
Hypo-acetylation
DNA methylation
H3K27me3, H4K20me3, **H3K9me3…**

Interphase nucleus

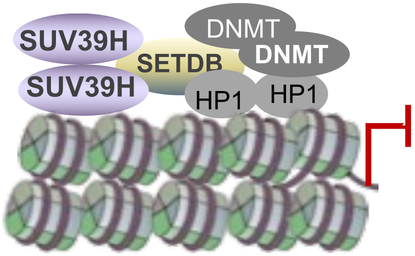


gene expression
genomic stability
nuclear organisation

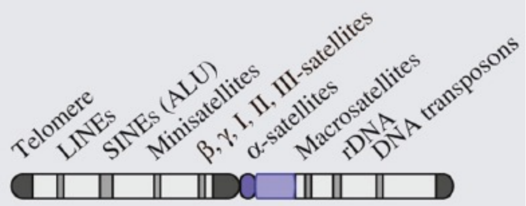
Euchromatin
Relatively decondensed Transcriptionally active during interphase

Signatures
Acetylation des Histones H3 et H4
H3K4me2/3, H3K36me3
H3K9me1, H3K9me2…

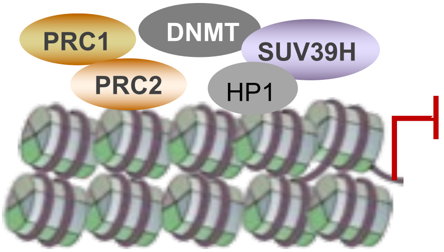
Constitutive heterochromatin



Stable repression of genomic repeat elements (satellites, retroviral)



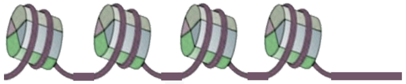
Facultative heterochromatin



Histone methyltransferases

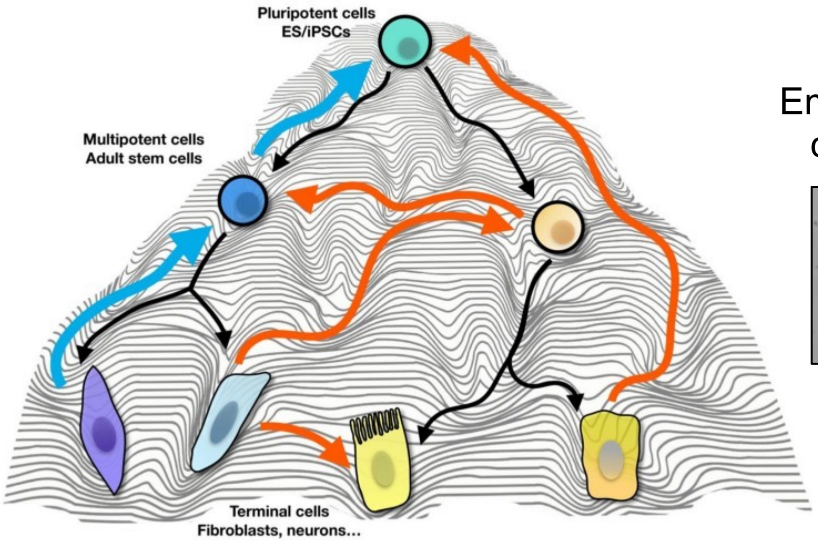
Histone demethylases

Euchromatin



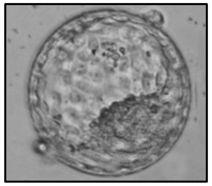
Regulation of lineage-restricted genes
>> Development and Differentiation

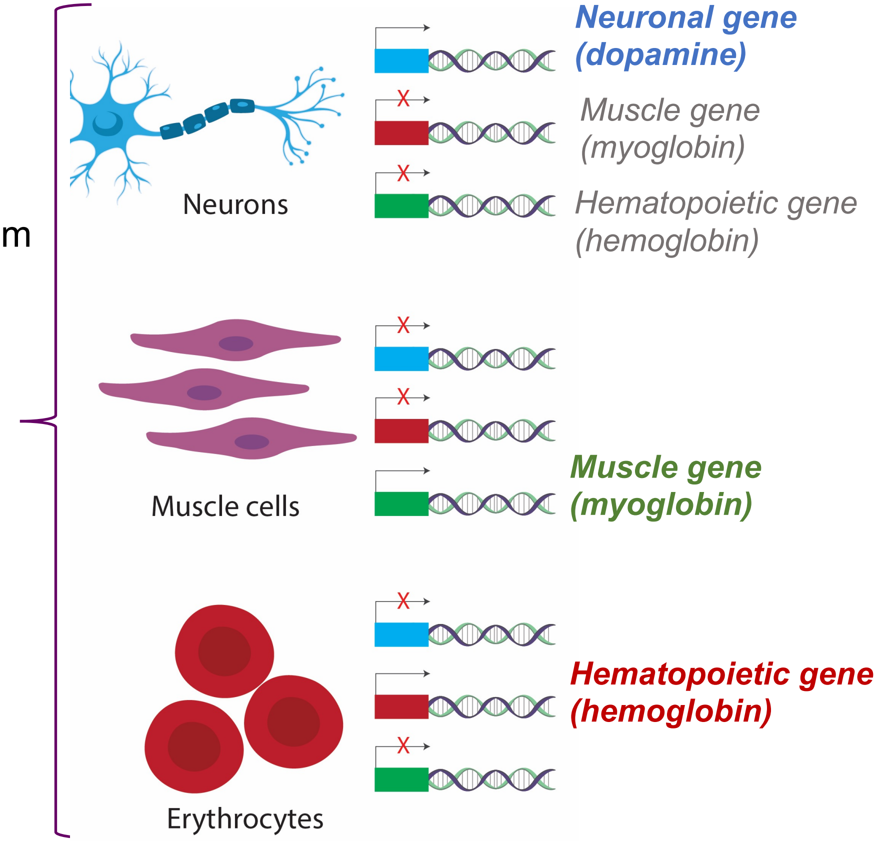
Epigenetics shape cell identity



The epigenetic landscape model
by Conrad Waddington in 1957

Embryonic stem
cells (ESCs)





Neurons

Neuronal gene (dopamine)

Muscle gene (myoglobin)

Hematopoietic gene (hemoglobin)

Muscle cells

Muscle gene (myoglobin)

Erythrocytes

Hematopoietic gene (hemoglobin)

Cell type-specific transcription factors
Chromatin structure and modifications

Cell identity

Epigenetics : from old to more recent definitions …

Waddington in 1942, the term epigenetics define the causal mechanisms by which the genes of a genotype bring about a phenotype.
Revisited definition in 1987, Holliday applied the term epigenetic to situations in which changes in DNA methylation result in changes in gene activity.

Today, the most widely accepted definition designates epigenetics as the study of heritable changes in genome function that occur without alterations to the DNA sequence. This definition implies that particular states that define cell identity are attained by heritable instructions. **Geneviève Almouzni 2009**

Almost three-quarters of a century later, we know that epigenetic mechanisms transduce the inheritance of gene expression patterns without altering the underlying DNA sequence but by adapting chromatin, which is the physiological form of our genetic information. ***David Allis & Thomas Jenuwein, 2016***

We define ‘epigenetics’ as mitotically inherited chromatin states. It is broadly accepted that chromatin states convey gene expression and repression information between cell divisions; the specific contribution of individual chromatin components, such as histone post-translational modifications (PTMs), DNA methylation, or histone variants, is less clear. ***Anja Groth, 2020***

With our current understanding of how chromatin impacts gene expression, epigenetics now encompasses the stable transmission through cell divisions of distinct gene expression programmes that are established independently of changes in the DNA sequence. These epigenetic programmes reflect changes in gene expression as a consequence of an initiating signal, yet are fully heritable after this signal subsides. ***Danny Reinberg, 2021***

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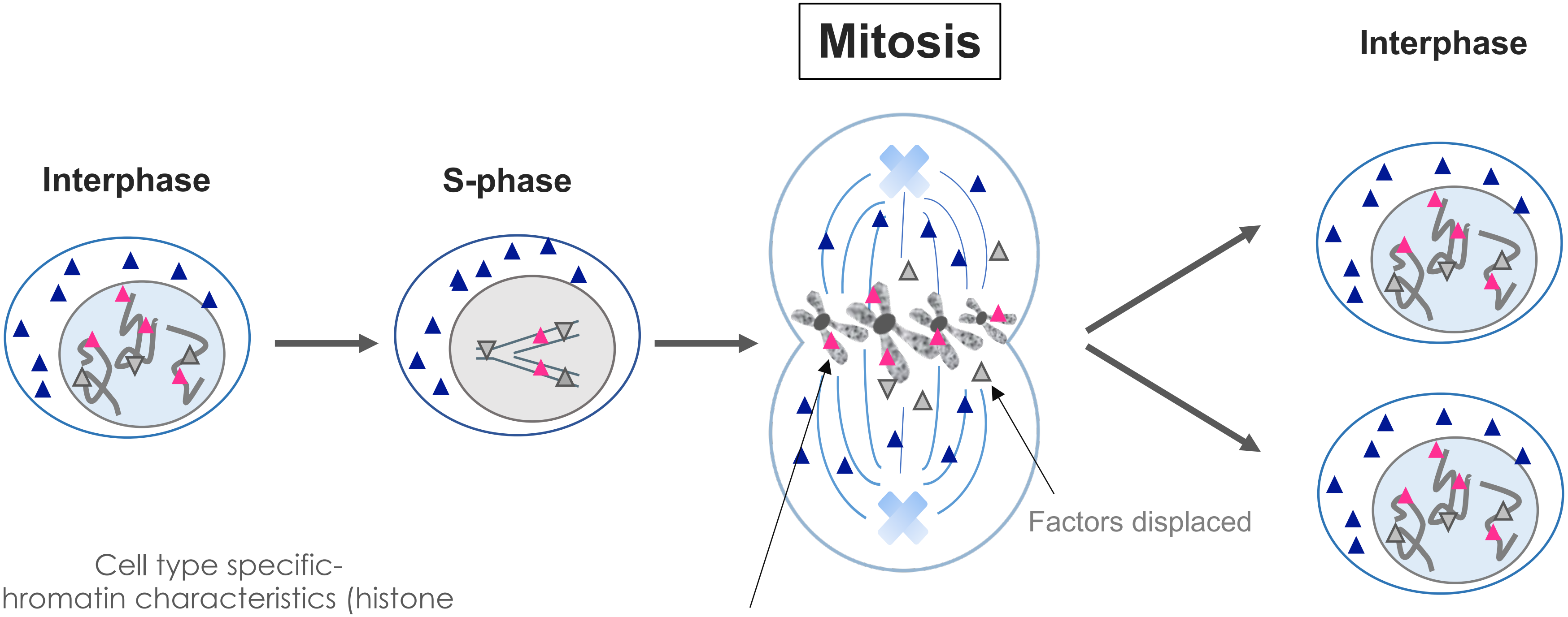
Epigenetic memory through cell division

How are cell identity and epigenetic memory preserved through cell division ?



Cell type specific-
chromatin characteristics (histone
modifications, DNA methylation…)
and transcription program
= cell identity

Bookmarking factors
- chromosome-bound factors
- epigenetic marks

▶ **Inheritance of cell identity**

How epigenetic information is copied during S-phase at newly synthesised DNA

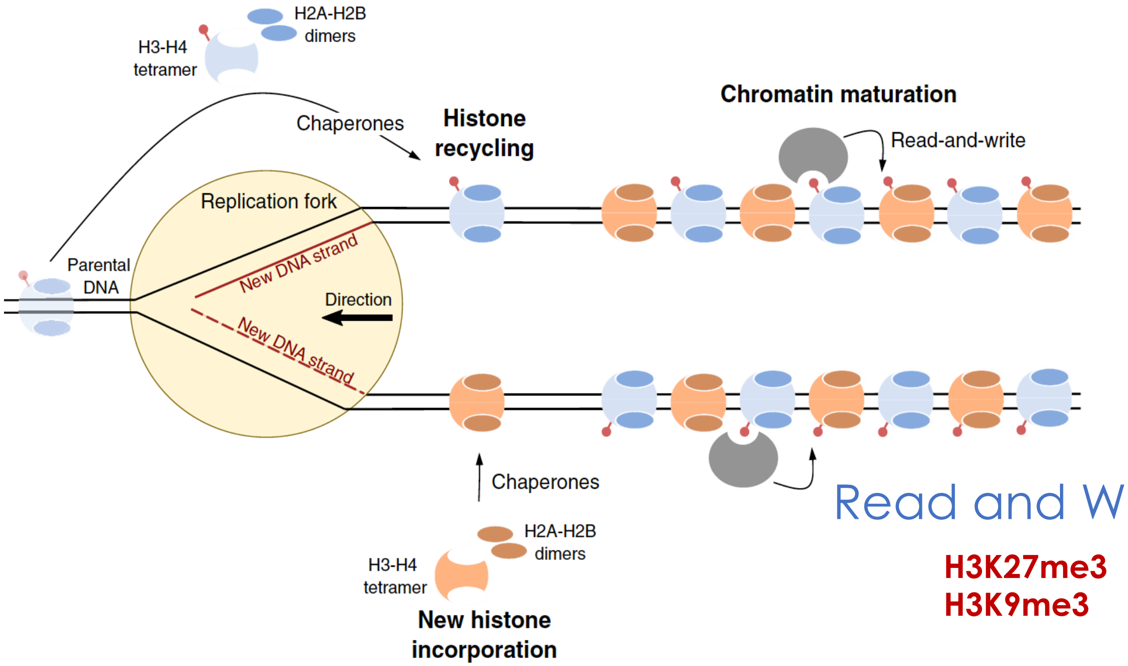
DNA methylation is appropriately copied on newly synthesised DNA.

histone chaperones, such as minichromosome maintenance complex MCM2, ensure that parental histones are evenly allocated to the leading and lagging strands

Some Histone modifications are appropriately copied on newly synthesised DNA by “Read and Write” mechanisms :

- PRC2 histone modification H3K27me3 : (PRC2) can both methylate histone H3 at lysine 27 (through Ezh2) and recognise this mark (through Eed)

- H3K9me3 through stimulation of the activity of the seT domain of SUV39H1/H2

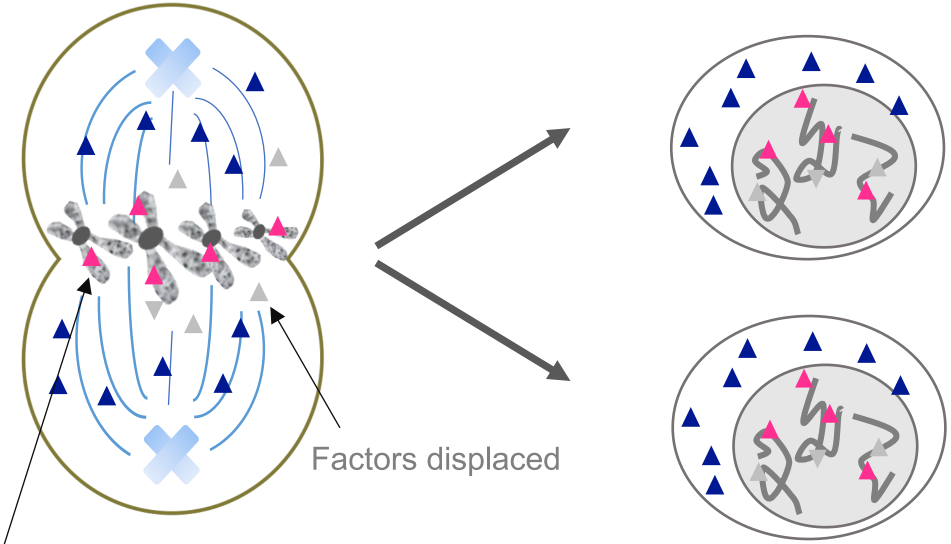


Read and Write

H3K27me3
H3K9me3

(Alabert et al., 2014; Hansen et al., 2008; Hermann et al., 2004; Margueron et al., 2009; Petryk et al., 2018; Probst et al., 2009; Reveron-Gomez et al., 2018; Stewart-Morgan et al., 2020; Yu et al., 2018)

How are cell identity and epigenetic memory preserved through mitosis?



Mitosis

Interphase

Factors displaced

Bookmarking factors
- chromosome-bound factors
- epigenetic marks

Inheritance of cell identity

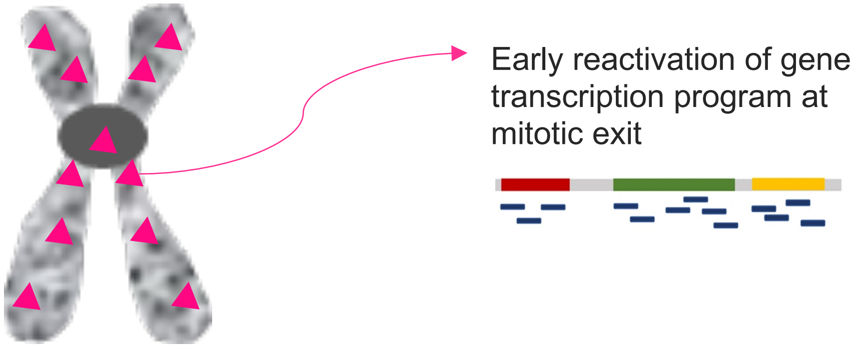
During mitosis

- chromosomes highly condense following progressive activation of cyclin B1-Cdk1 kinase pathway
- transcription is largely reduced
- nuclear membrane breaks down and
- many DNA-binding factors are evicted from mitotic condensed chromosomes.

mitosis = very challenging chromatin environment to sustain cell identity memory.

How are cell identity and epigenetic memory preserved through mitosis?

Transcription factors : Gata1, Foxal and Esrrb

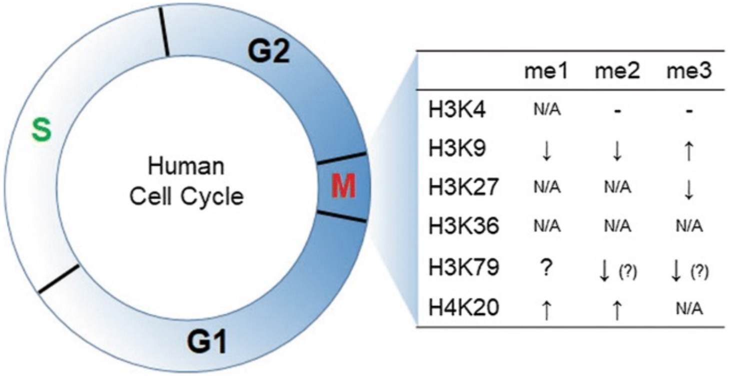


Early reactivation of gene transcription program at mitotic exit

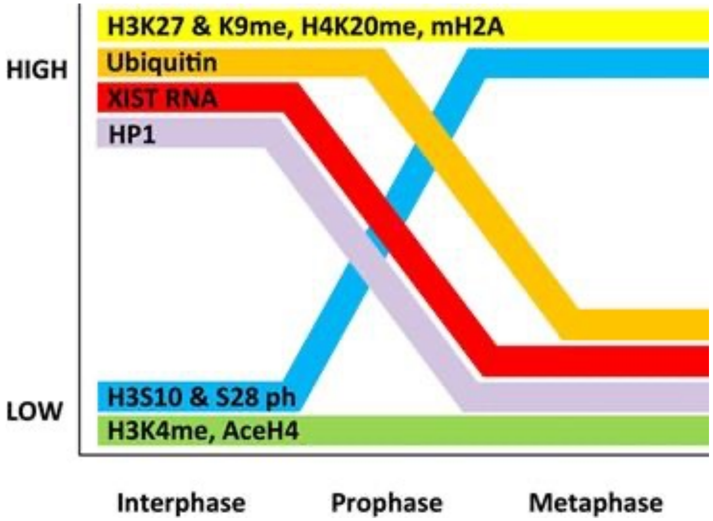
Factors that :
- remain chromosome-bound throughout mitosis, and are able to occupy (at least) a subset of the genomic sites bound during interphase, so called “mitotic bookmarking”

- And are able to reinstate the gene transcription programs at mitotic exist in the daughter cells

Epigenetic marks :

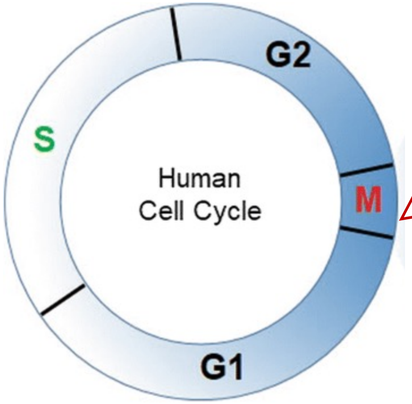


	me1	me2	me3
H3K4	N/A	-	-
H3K9	↓	↓	↑
H3K27	N/A	N/A	↓
H3K36	N/A	N/A	N/A
H3K79	?	↓ (?)	↓ (?)
H4K20	↑	↑	N/A



Technical challenges to study mitotic memory

Synchronization difficulties



Nocodazole
Colcemid
Mitotic shake off
Cdk1 inh +
Mitotic release

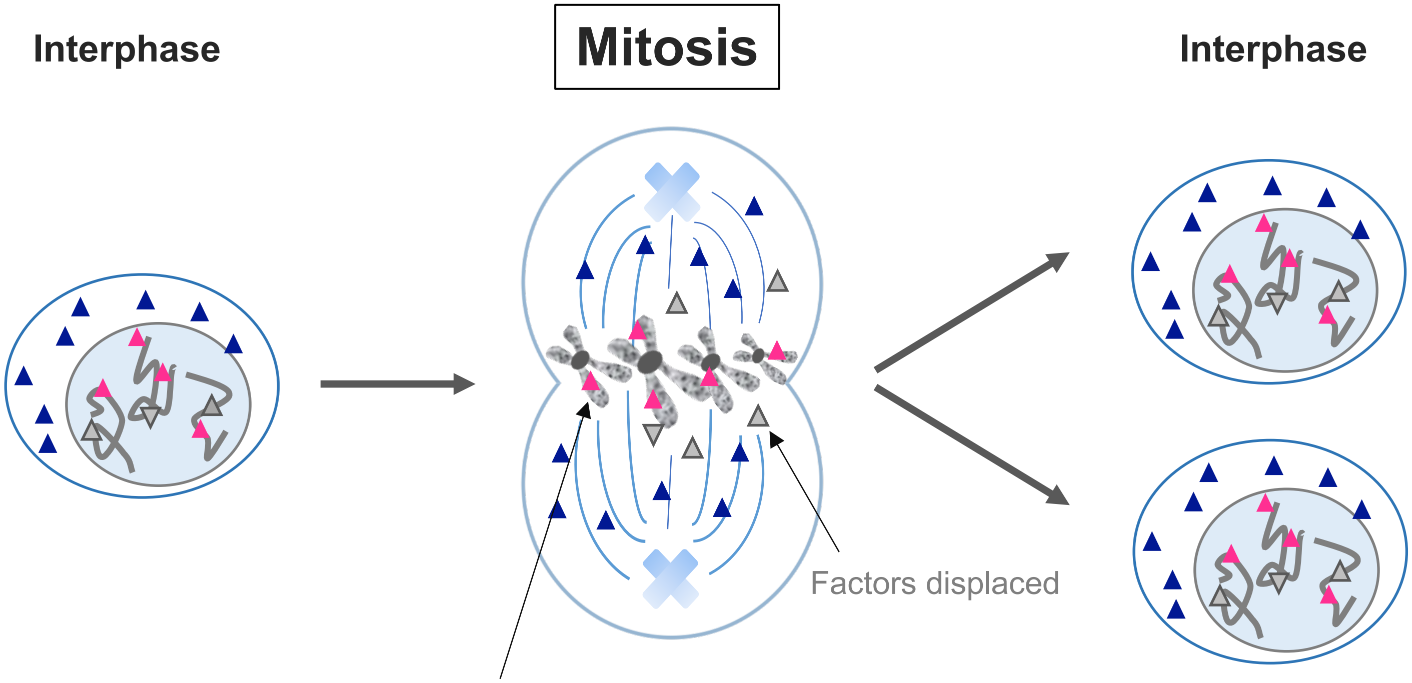
Purity of mitotic samples compromised

Fixation artefacts

Cross-linking (fixation by formaldehyde), artificially displace TFs from chromatin by reducing binding affinity, specifically in mitosis

Underestimation of the number of potential bookmarking factors
(Lerner et al., 2016; Teves et al., 2016)

How are cell identity and epigenetic memory preserved through cell division ?



Interphase

Mitosis

Interphase

Factors displaced

Bookmarking factors
- chromosome-bound factors
- epigenetic marks

▶ **Inheritance of cell identity**

Main research questions

- Mechanisms underlying cell identity inheritance through mitosis ?
- Chromatin characteristics of mitotic chromosomes ?
- Impact of chromatin changes on cellular memory and lineage stability ?

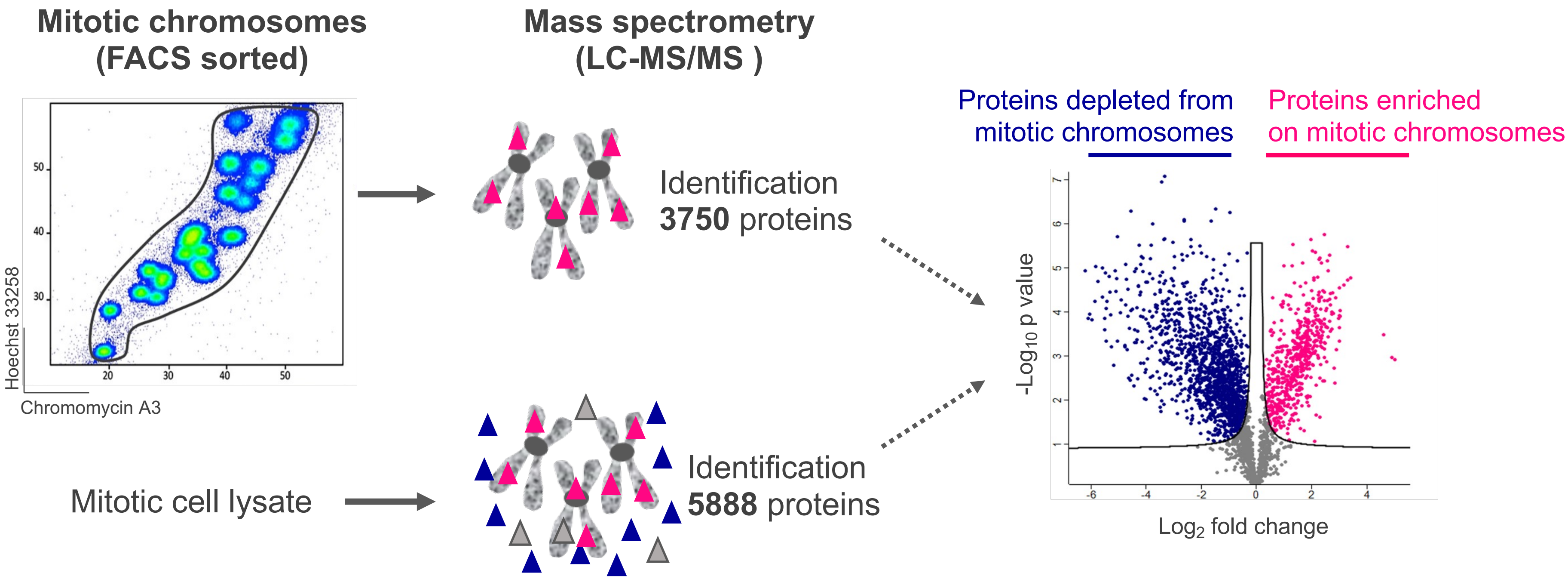
I. **Experimental approach : Identification of proteins bound to native mitotic chromosomes and functional role**

II. Role of repressive heterochromatin mark H3K9me3 in sustaining cell identity

III. Epigenetic memory of XCI in B lymphoid cells

Identification of proteins bound to native mitotic mESC chromosomes

Experimental approach :



Mitotic chromosomes (FACS sorted)

Mass spectrometry (LC-MS/MS)

Identification
3750 proteins

Mitotic cell lysate

Identification
5888 proteins

Proteins depleted from mitotic chromosomes

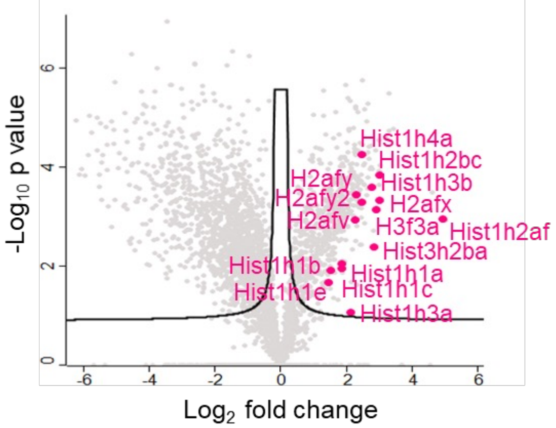
Proteins enriched on mitotic chromosomes

(Djeghloul et al., Nat Commun 2020)

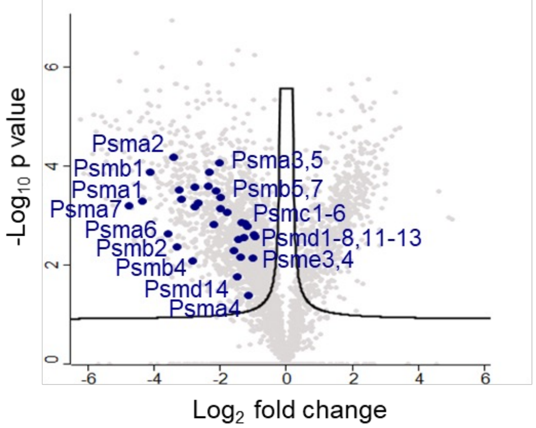
Analysis of proteins bound to isolated metaphase ESC chromosomes

- Examples of proteins depleted or enriched on mitotic ESC chromosomes -

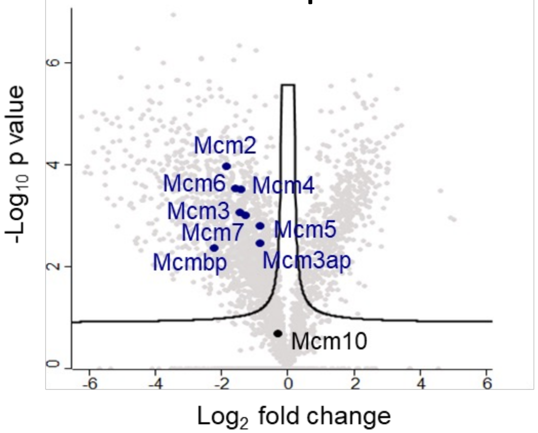
Histones



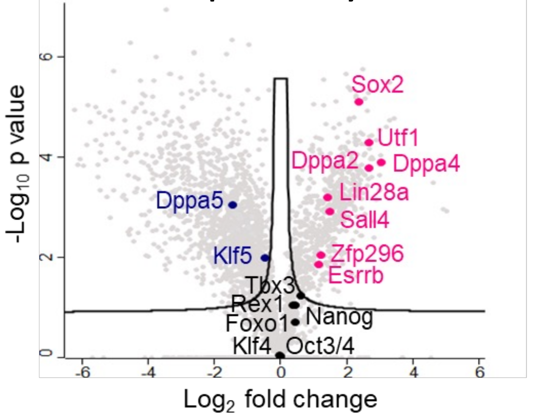
Proteasome



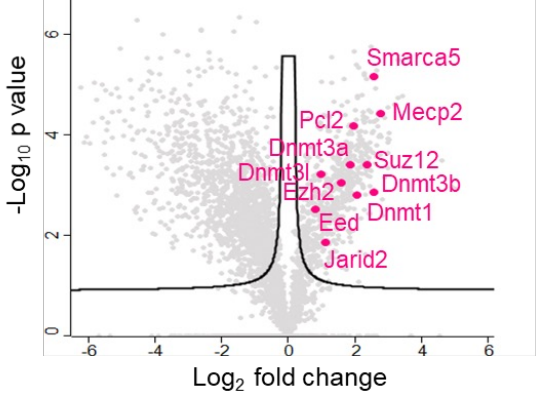
DNA replication



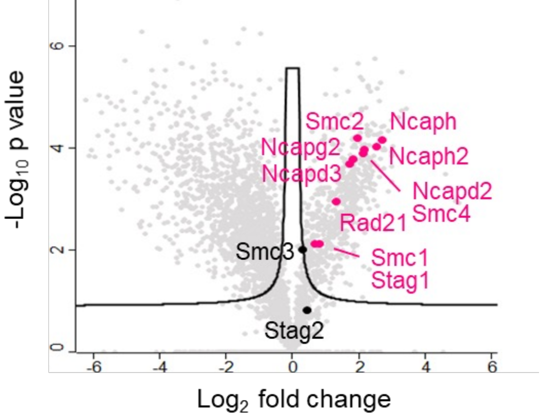
Pluripotency TFs



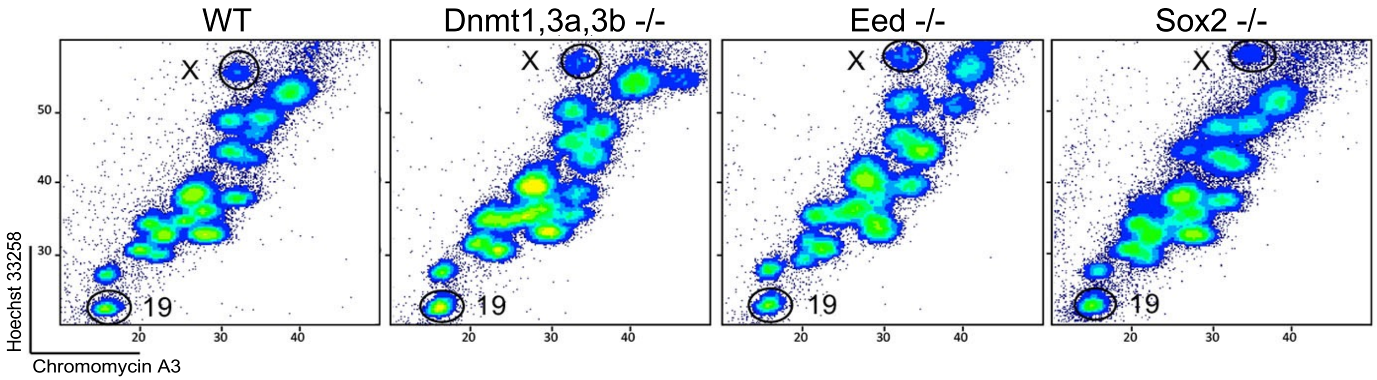
Chromatin repressors



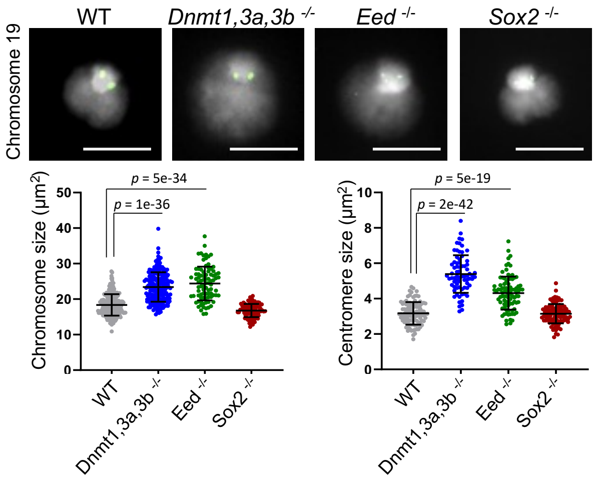
SMC associate



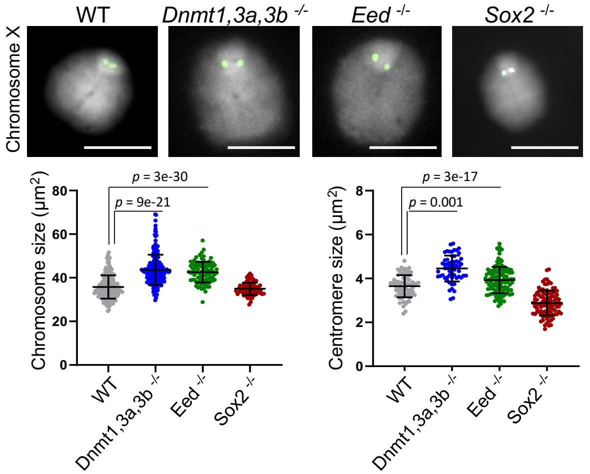
DNMTs and PRC2 activity keep mitotic chromosomes compact

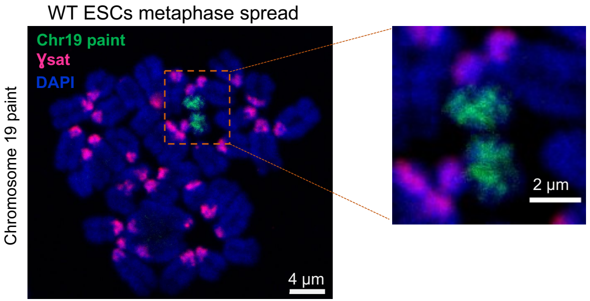


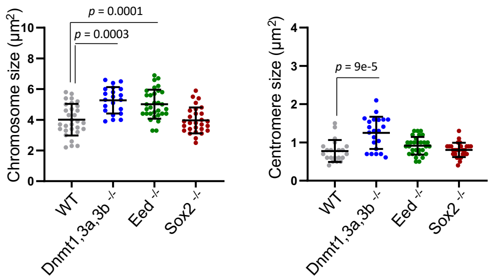
Chromosome 19



Chromosome X







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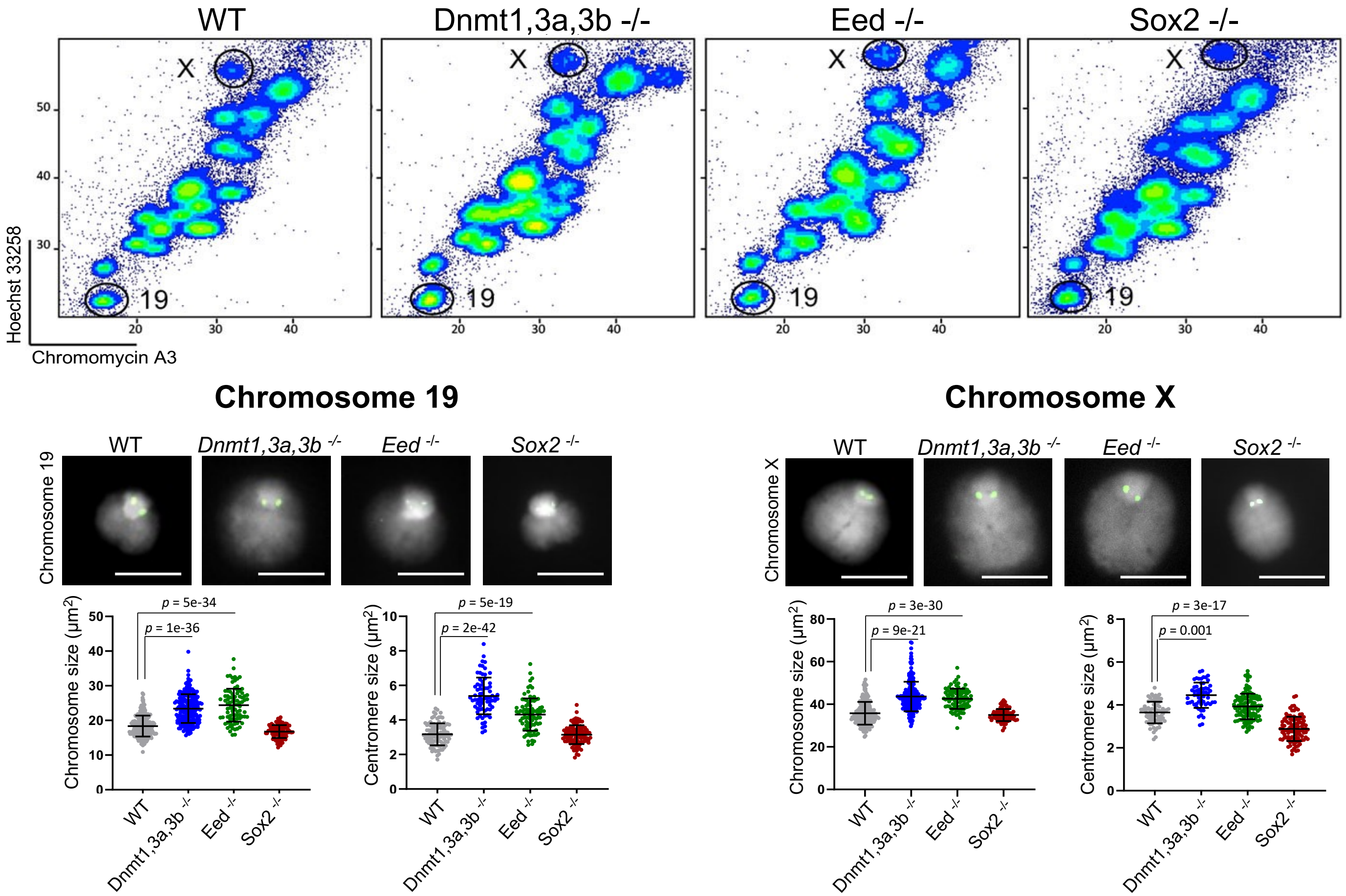


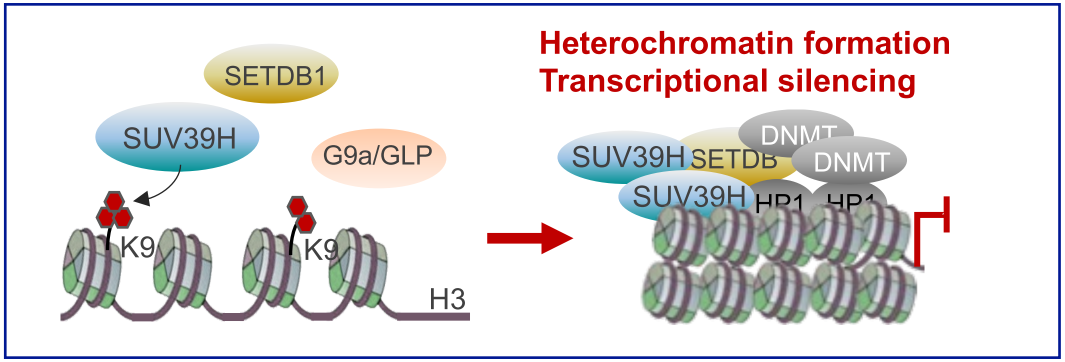
Only exception
Cells deficient in H3K9 trimethylation

I. Experimental approach : Identification of proteins bound to native mitotic chromosomes and functional role

II. Role of repressive heterochromatin mark H3K9me3 in sustaining cell identity

III. Epigenetic memory of XCI in B lymphoid cells

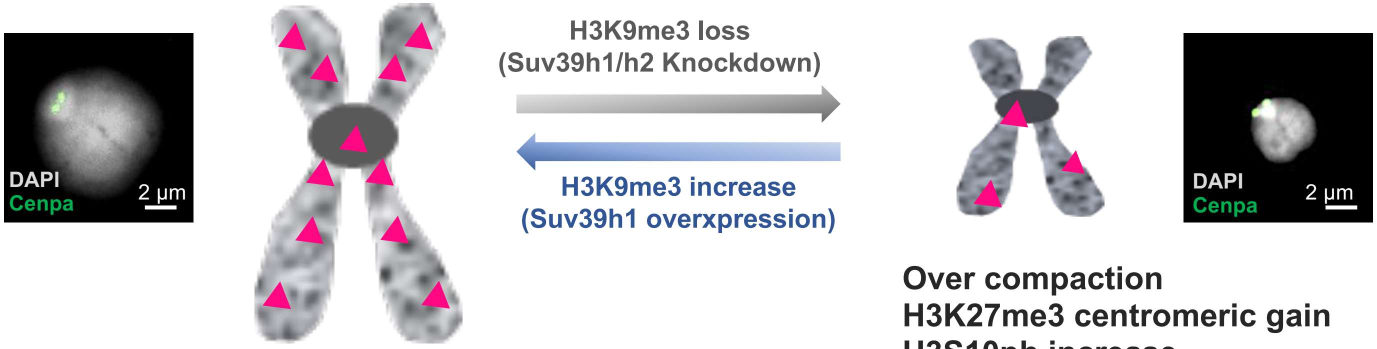
H3K9me3 is critical for sustaining mitotic chromosome structure and bookmarking



Heterochromatin formation
Transcriptional silencing

WT
mitotic chromosome

H3K9me3 deficient
mitotic chromosome



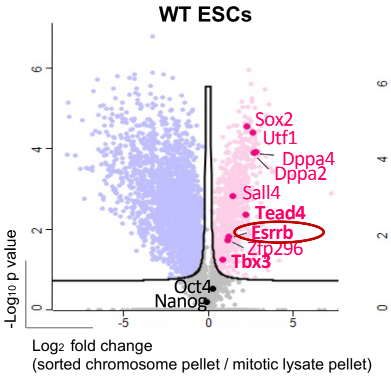
H3K9me3 loss
(Suv39h1/h2 Knockdown)

H3K9me3 increase
(Suv39h1 overxpression)

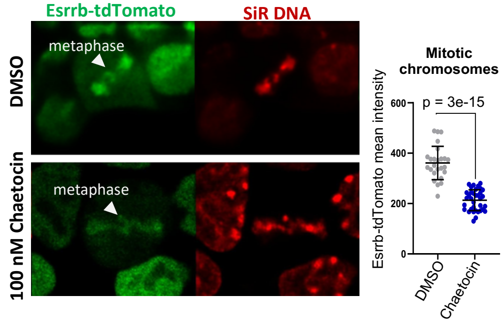
Over compaction
H3K27me3 centromeric gain
H3S10ph increase
Loss of retention of TFs (potential mitotic bookmarkers)

Djeghloul et al., Nat Struct Mol Biol 2023

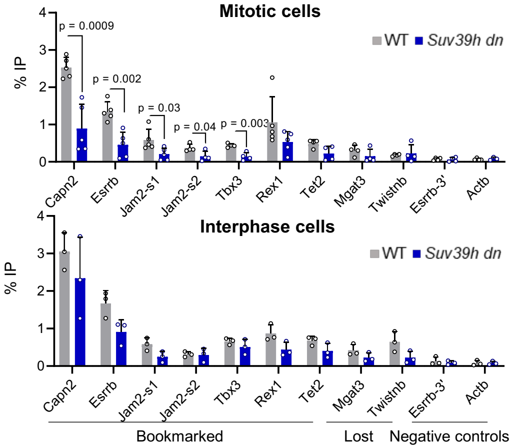
Proteomic analysis



Esrrb-tdTomato live cell imaging

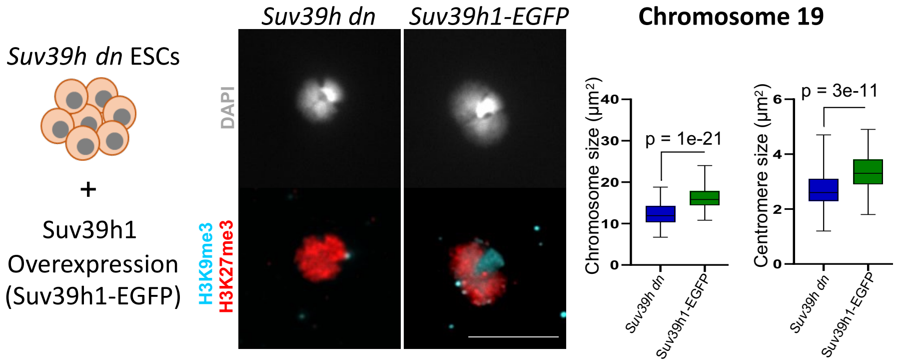


Esrrb ChIP



- ➢ **Mitotic binding of Essrb is altered during mitosis in *Suv39h dn* ESCs**

Chromosome size analysis



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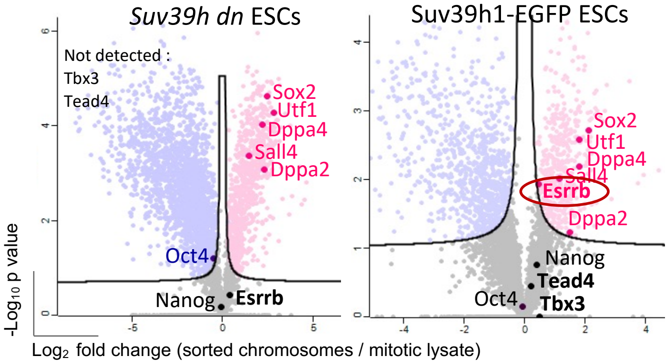
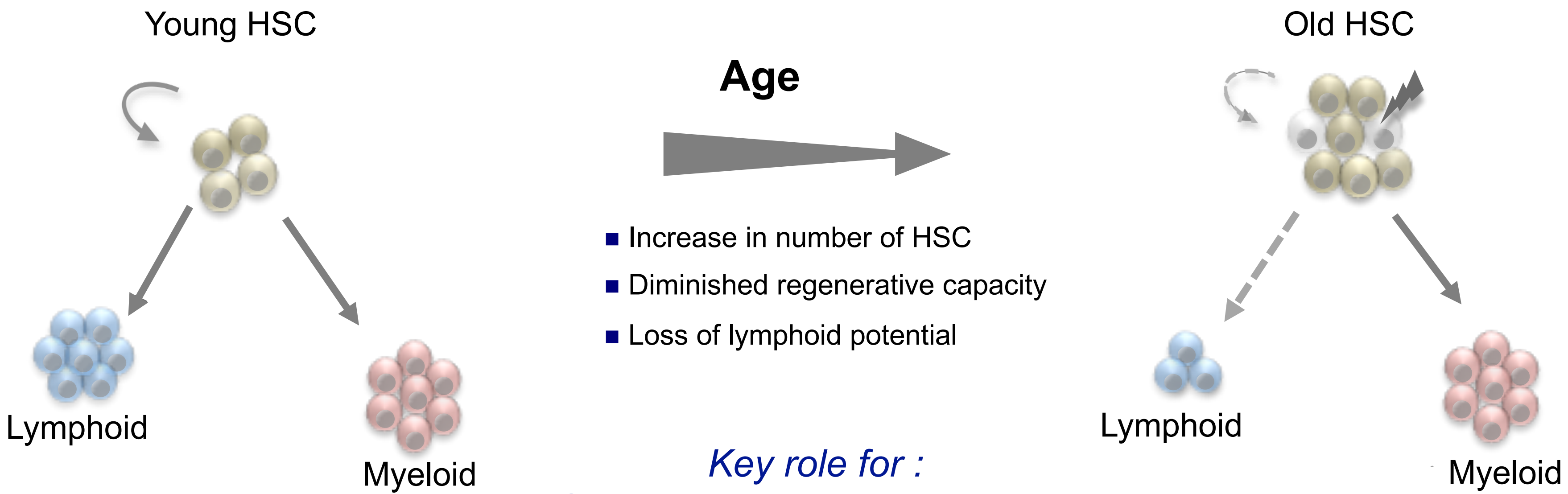


- ➢ **Chromosome architecture and mitotic binding of Essrb is resorted following Suv39h1 re-expression**

Importance H3K9me3 in sustaining mitotic chromosome structure and cell memory during cell division

H3K9me3 is important for maintaining B lymphoid lineage during aging both in mouse and human

Hematopoietic Stem Cell (HSC) aging



Young HSC

Lymphoid

Myeloid

Age

- Increase in number of HSC
- Diminished regenerative capacity
- Loss of lymphoid potential

Old HSC

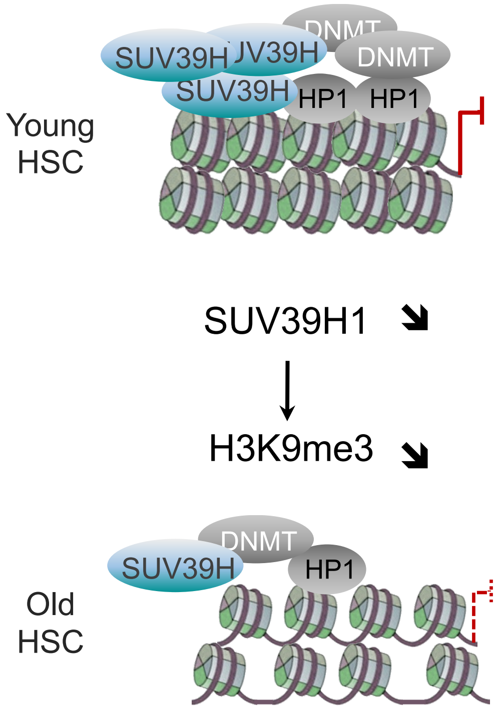
Lymphoid

Myeloid

Key role for :
Suv39H1 – H3K9me3 pathway

(Rossi et al PNAS 2005, Chambers et al PLoS Biol 2007, Lescale et al Aging Cell 2010, Dykstra et al J. Exp. Med. 2011, Florian et al Nature 2013, Flash et al Nature 2014)

H3K9me3 is important for maintaining B lymphoid lineage during aging both in mouse and human



Young HSC

SUV39H1 ↘

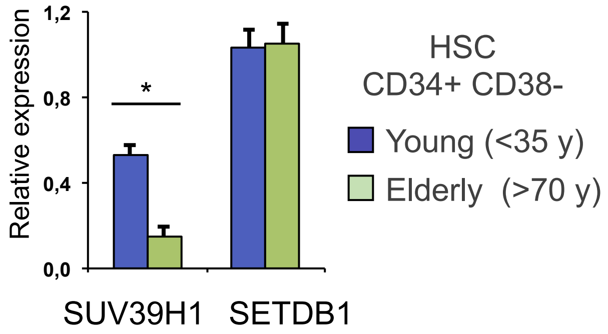
↓

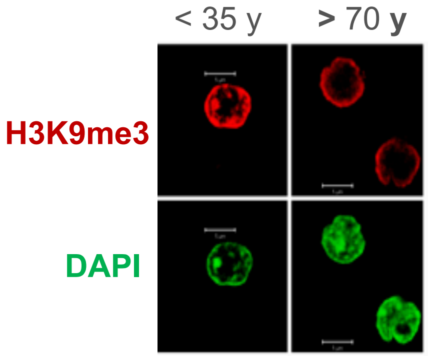
H3K9me3 ↘

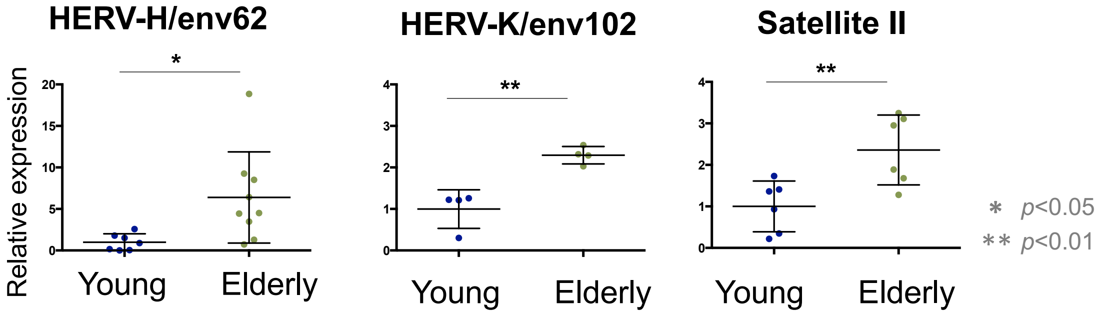
Old HSC

Global decrease in H3K9me3 heterochromatin

Derepression of repetitive sequences
Deregulation of lineage specific genes
Impaired HSC function

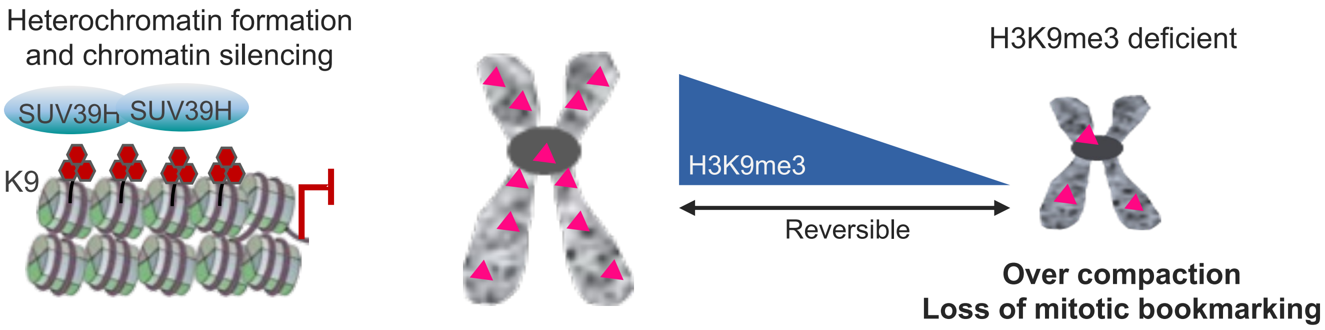






Conclusions
- H3K9me3 and cell identity -

H3K9me3 is critical for sustaining mitotic chromosome structure and TF retention



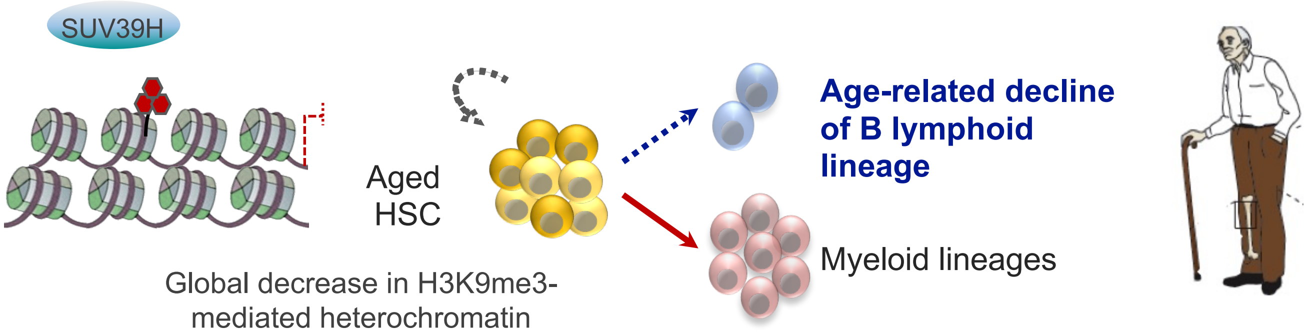
Heterochromatin formation
and chromatin silencing

H3K9me3 deficient

Reversible

Over compaction
Loss of mitotic bookmarking

H3K9me3 is important for maintaining B lymphoid lineage identity and function during aging



Aged
HSC

Global decrease in H3K9me3-
mediated heterochromatin

Age-related decline
of B lymphoid
lineage

Myeloid lineages

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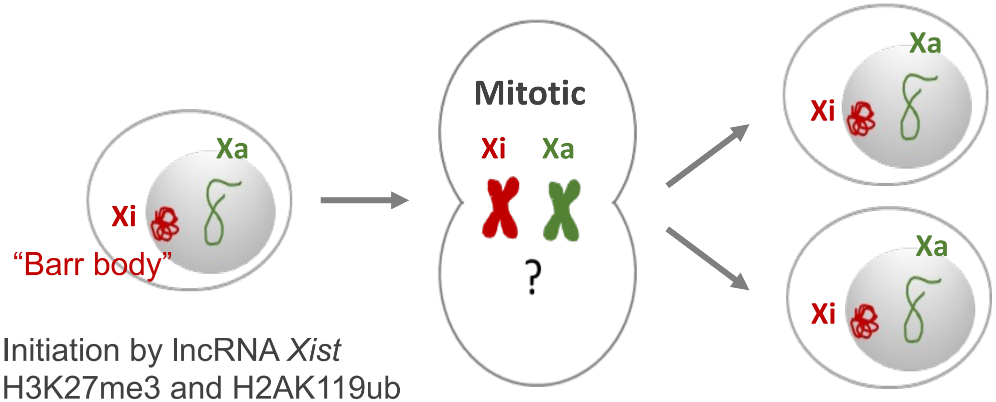
II. Role of repressive heterochromatin mark H3K9me3 in sustaining cell identity

III. **Epigenetic memory of XCI in B lymphoid cells**

Studying epigenetic memory of XCI in B cells

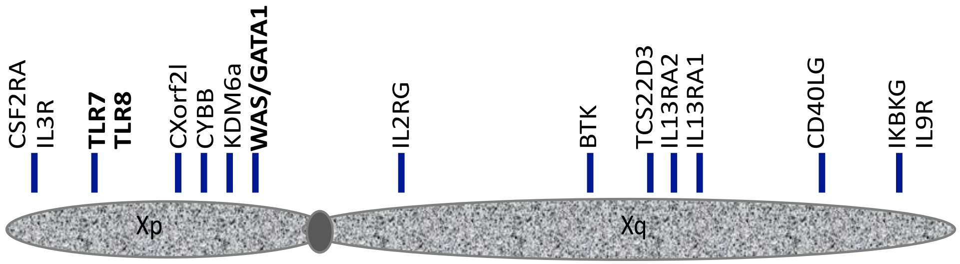
X chromosome inactivation (XCI), a paradigm of the epigenetic memory



- Initiation by lncRNA *Xist*
- H3K27me3 and H2AK119ub
- MacroH2A and DNA methylation

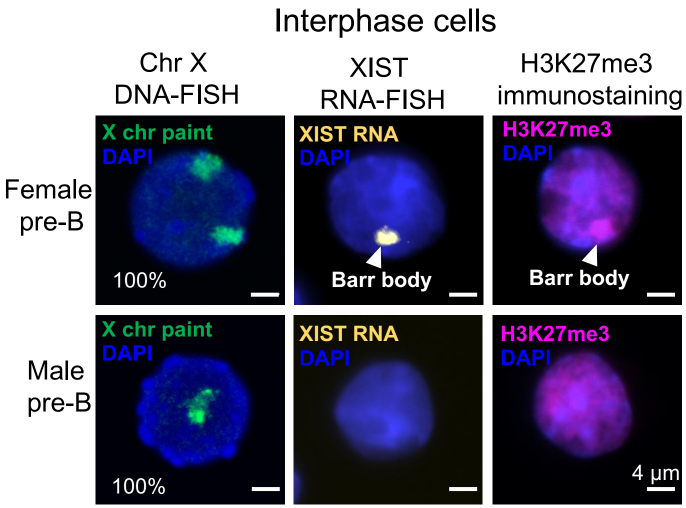
Stable maintenance of XCI through cell divisions

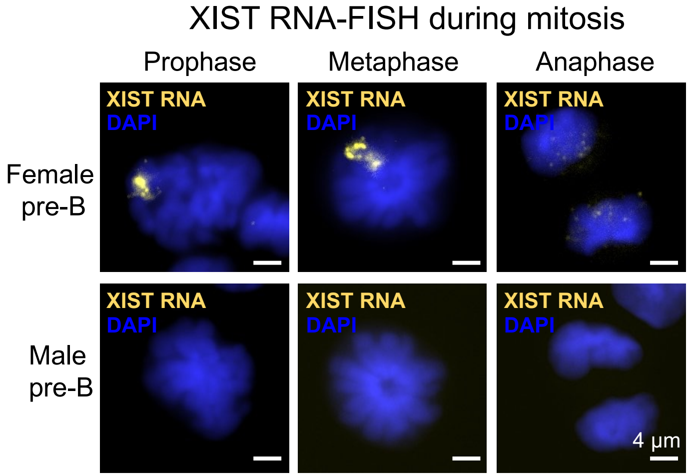
X chromosomes

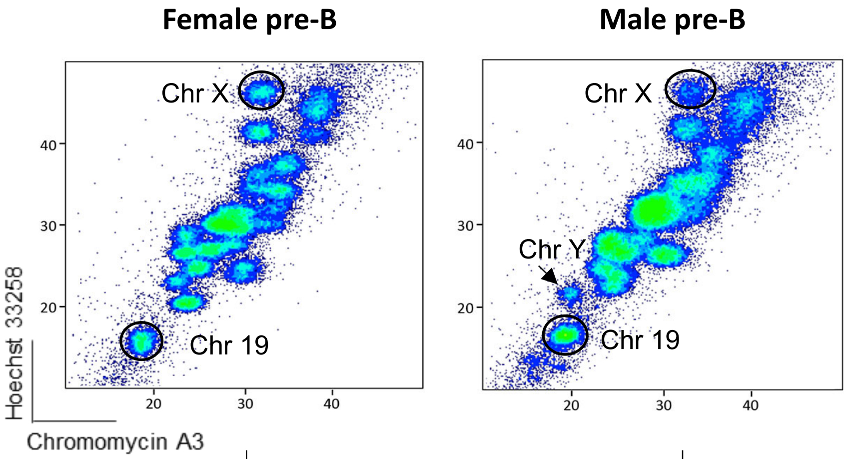


Numerous immune-related genes

Identifying proteins bound to mitotic female vs male X chromosomes isolated from mouse pre-B cell lines







Proteomic analysis (LC-MS/MS)
- Female chr X (Xi+Xa)
- Female chr 19 (autosome)

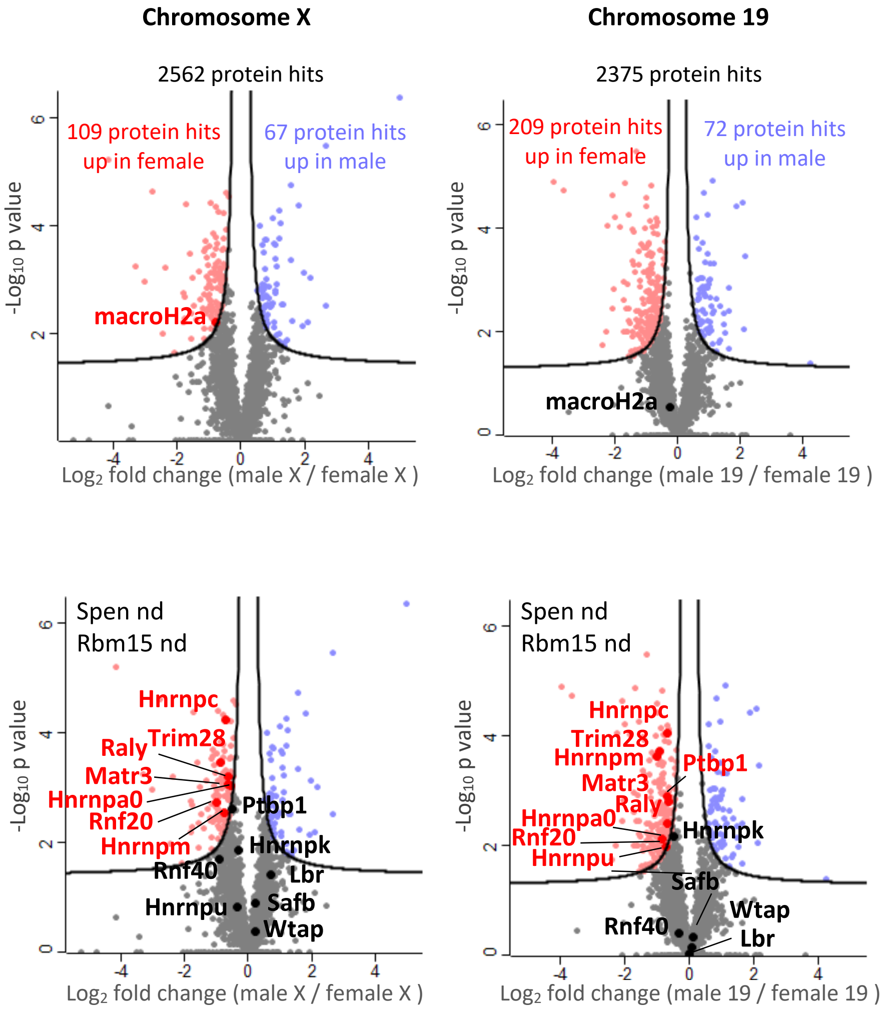
Proteomic analysis (LC-MS/MS)
- Male chr X (Xa)
- Male chr 19 (autosome)

Identify factors enriched on female X chromosome

Identifying proteins bound to mitotic female vs male X chromosomes

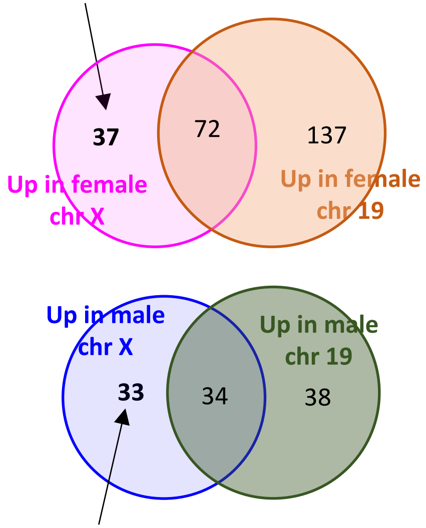
MacroH2A

XIST interacting factors


Chromosome X

Chromosome 19

proteins enriched only on female X chromosomes (37)
excluding those also enriched on chr19

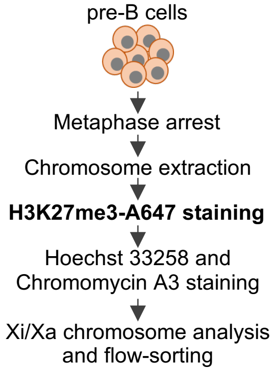


proteins enriched only on male X chromosomes (33)
excluding those also enriched on chr19

The heterogeneity of female X and the mpre-B cell lines acts as a confounder in the detection of proteomic differences between mitotic Xi and Xa

Biphasic distribution of H3K27me3 on mitotic X chromosomes enables isolation of Xa and Xi

Experimental strategy to FACS sort mitotic Xi and Xa



pre-B cells

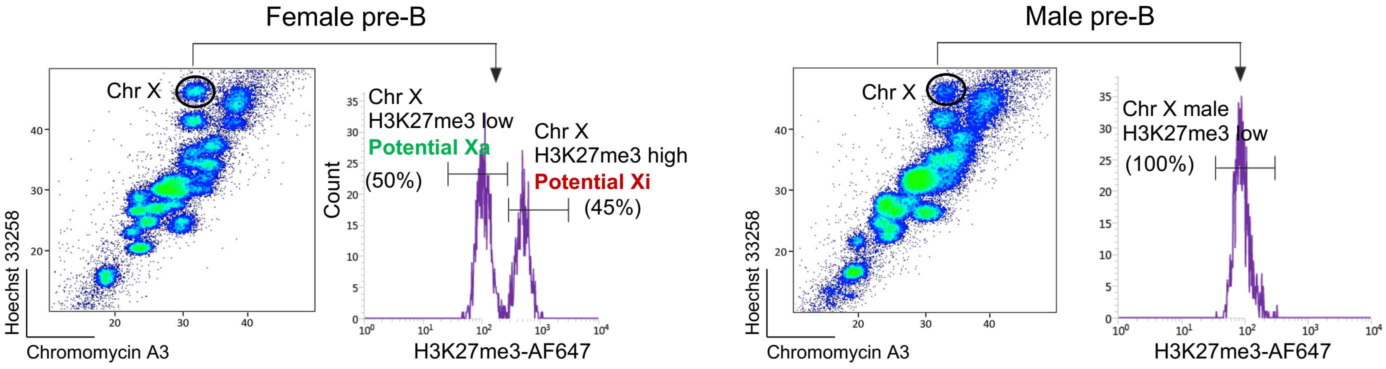
Metaphase arrest

Chromosome extraction

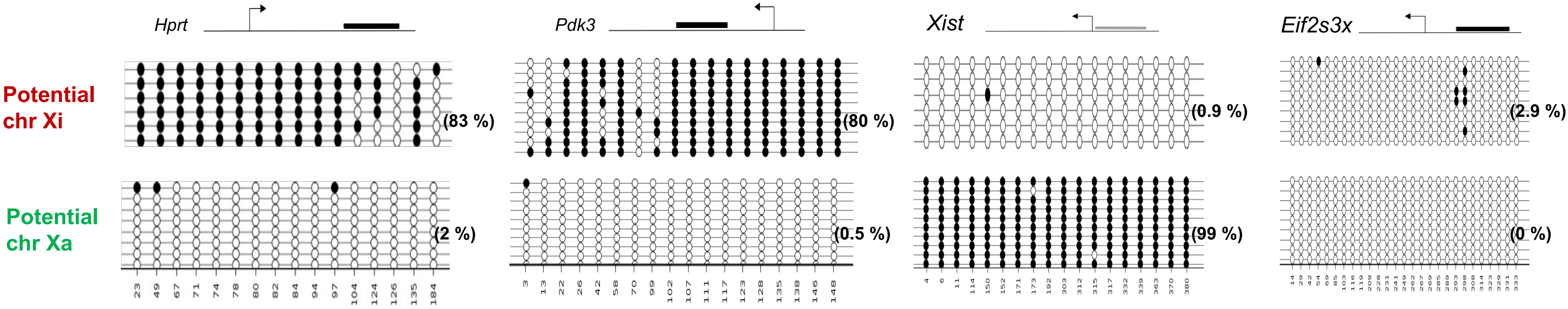
H3K27me3-A647 staining

Hoechst 33258 and Chromomycin A3 staining

Xi/Xa chromosome analysis and flow-sorting



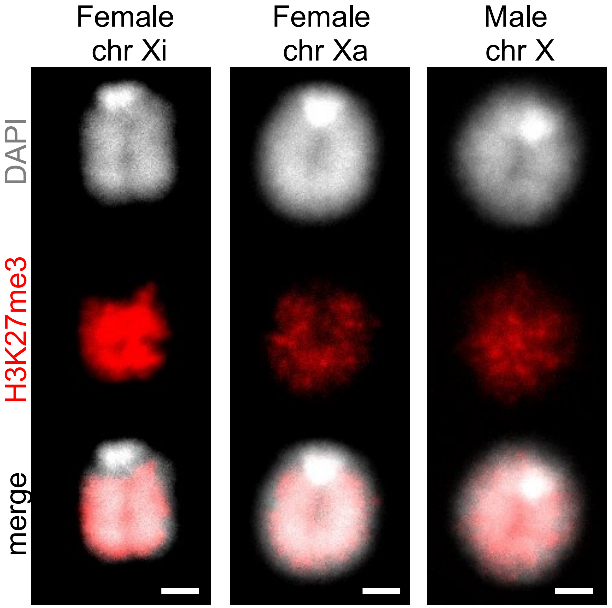
Validation of Xi and Xa FACS sort by DNA methylation assay

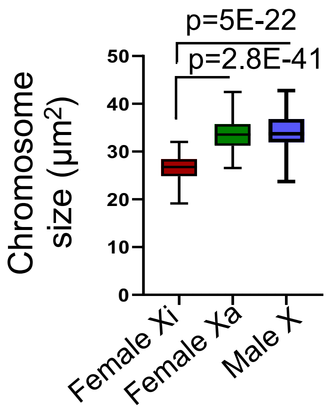


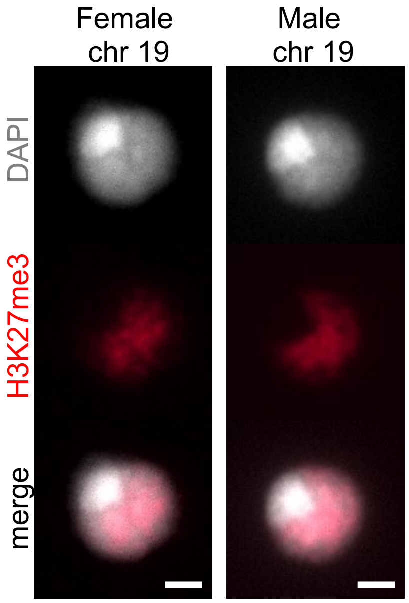
● methylated CpG

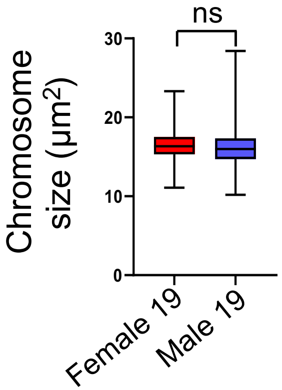
○ non methylated CpG

Flow-sorted mitotic Xa and Xi show differential size/compaction





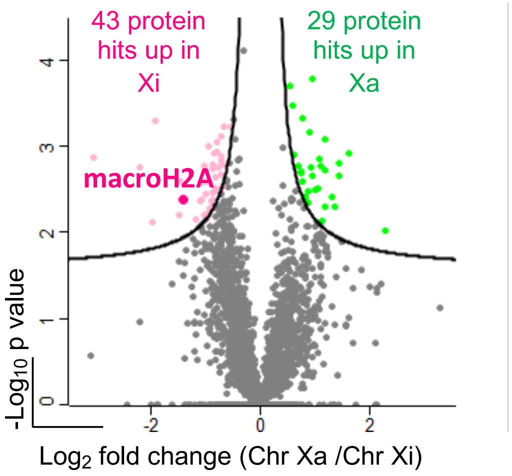


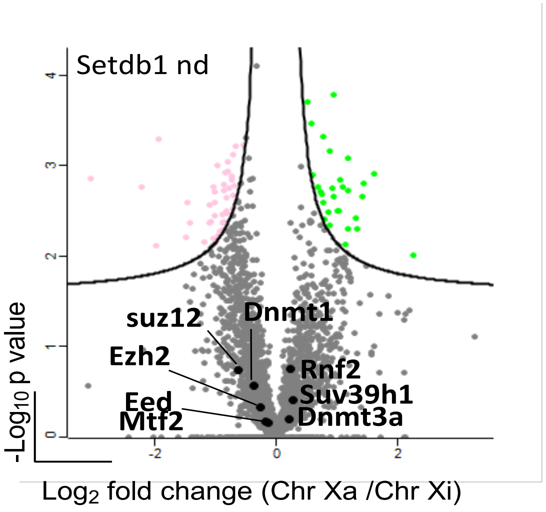


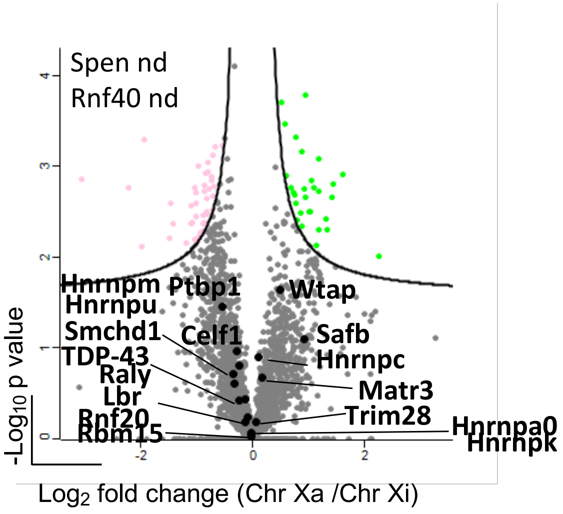
Histone acetyl transferases (HATs) are selectively enriched on mitotic Xa

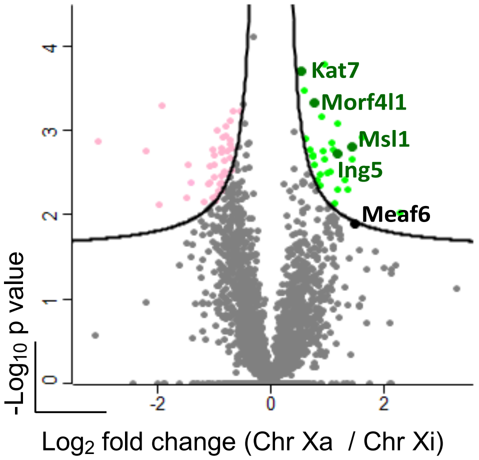
Proteomic comparison of Xa and Xi metaphase chromosomes

2418 protein hits

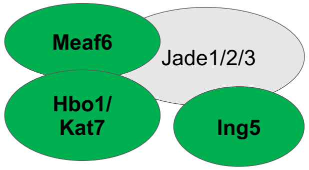




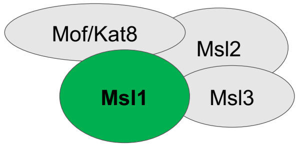


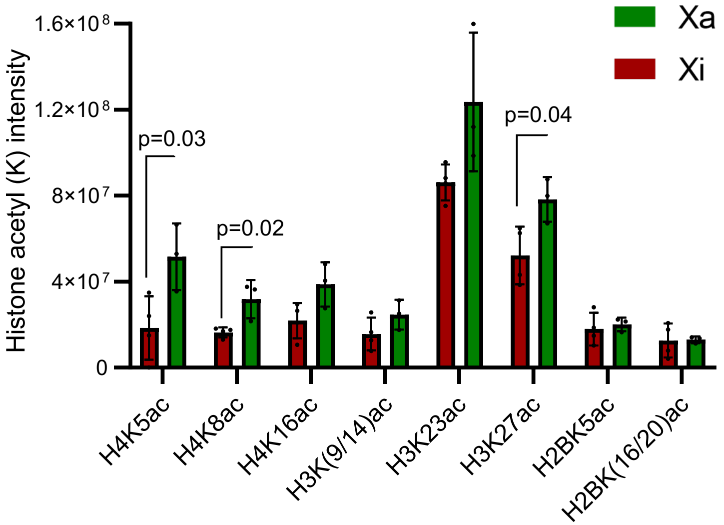


Hbo1 complex →
H3K(9/14)ac, H4K(5/8/)ac



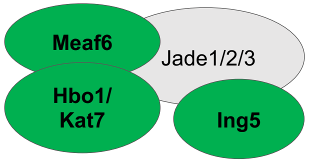
Mof-Msl complex → H4K16ac



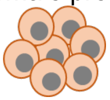


Increased size of the mitotic Xa is dependent on H3K14ac

Hbo1 complex
H4K(5/8/12)ac
H3K(9/14)ac

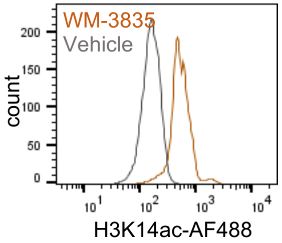


Female pre-B

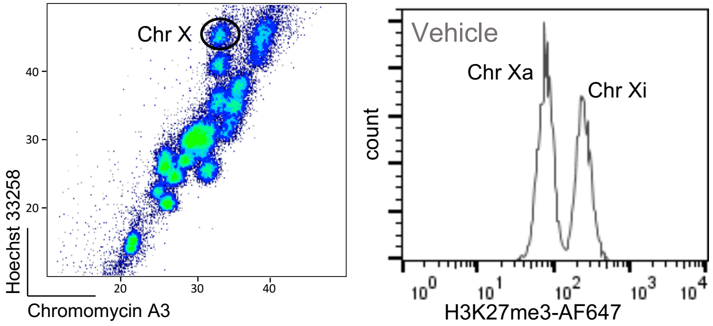


+
WM-3835
(Kat7 inhibitor)
or DMSO (Vehicle)
↓
Metaphase arrest
↓
Chromosome extraction
↓
H3K27me3-A647 staining
↓
Hoechst 33258 and Chromomycin A3 staining
↓
Xi/Xa chromosome flow-sorting and imaging

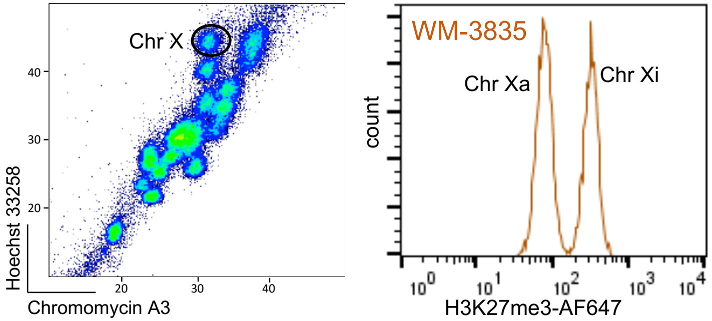
→ Histone acetylation staining on cells

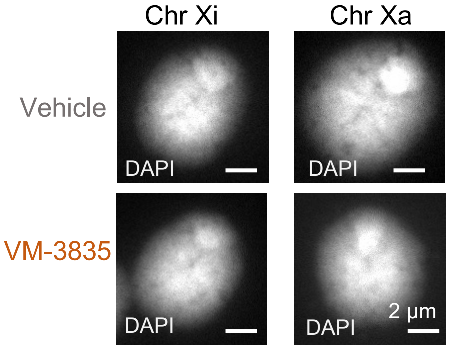


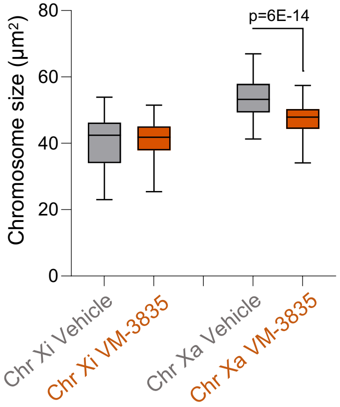
Pre-B female + Vehicle



Pre-B female + WM-3835



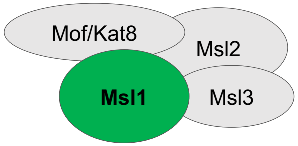




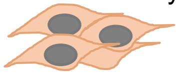
Loss of H3K14ac increases Xa chromosome compaction, so that it resembles the Xi in mitosis

Mof-Msl sustain differential size and H3K27me3 profiles of mitotic Xi and Xa

Mof-Msl complex
H4K16ac



Female MEFs
Msl2 WT and KO
from E13.5 embryos



Culture
(4 to 6 passages)

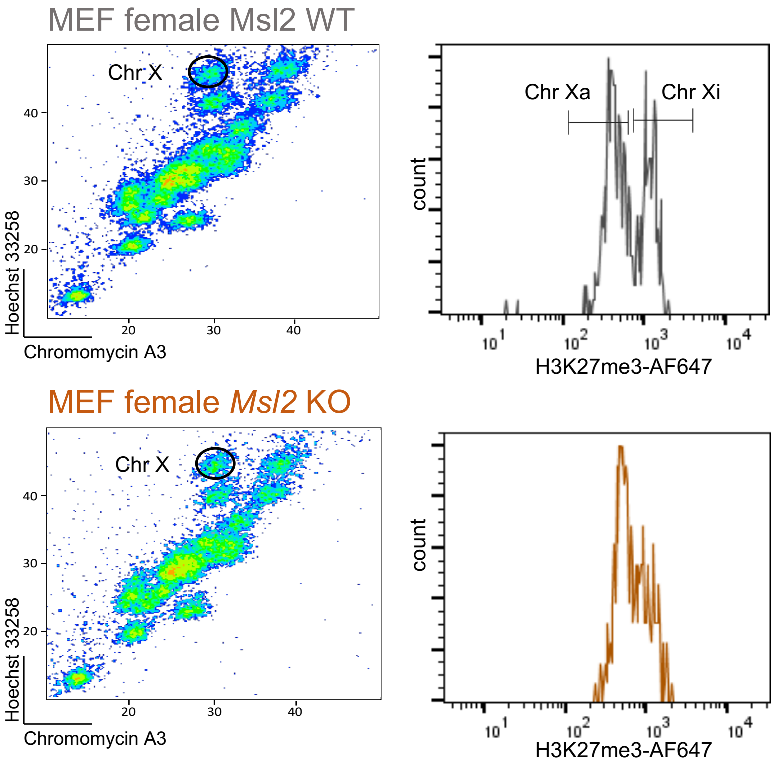
Metaphase
arrest

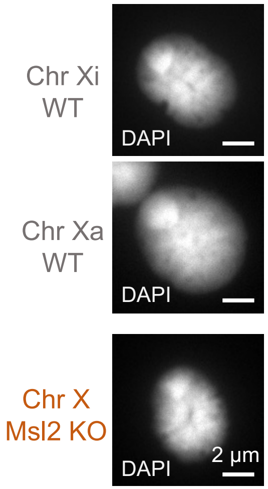
Chromosome
isolation

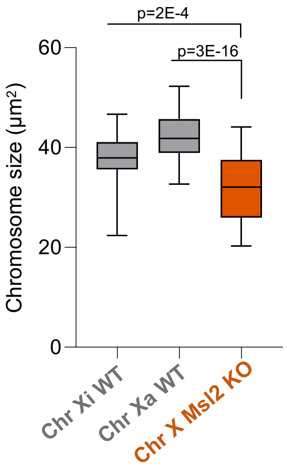
H3K27me3-A647
staining

Hoechst 33258
Chromomycin A3
staining

Chromosome
analysis

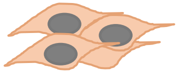






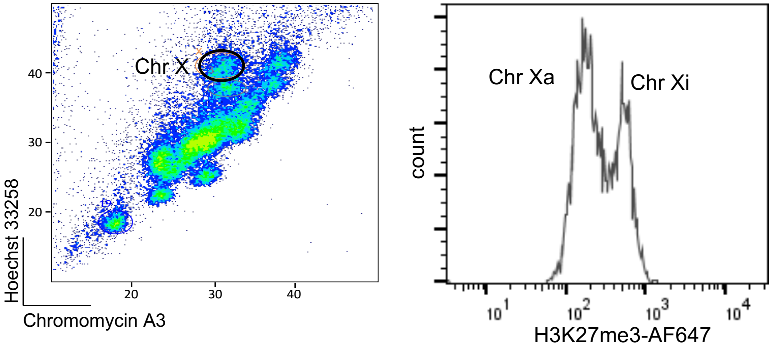
Mof-Msl sustain differential size and H3K27me3 profiles of mitotic Xi and Xa

MEF female
Mof$^{fl/fl}$ *Cre-ERT2*$^{T/+}$
from E13.5 embryos

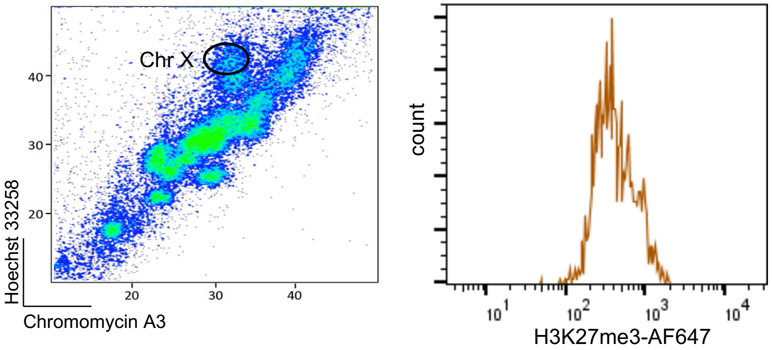


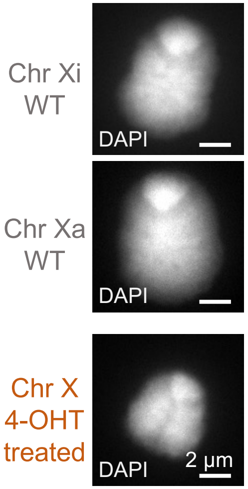
\+
4-OHT treated
or EtOH Vehicle
↓
Culture
(4 to 6 passages)
↓
Metaphase
arrest
↓
Chromosome
isolation
↓
H3K27me3-A647
staining
↓
Hoechst 33258
Chromomycin A3
staining
↓
Chromosome
analysis

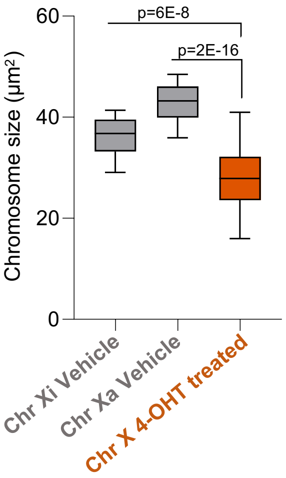
MEF female *Mof*$^{fl/fl}$ *Cre-ERT2*$^{T/+}$ (EtOH Vehicle)



MEF female *Mof*$^{fl/fl}$ *Cre-ERT2*$^{T/+}$ (4-OHT treated)







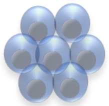
Critical role for acetylation pathways in preserving the chromatin properties of female X chromosomes during mitosis

Djeghloul* & Cheriyamkunnel et al., Nat Cell Biol, *2025*

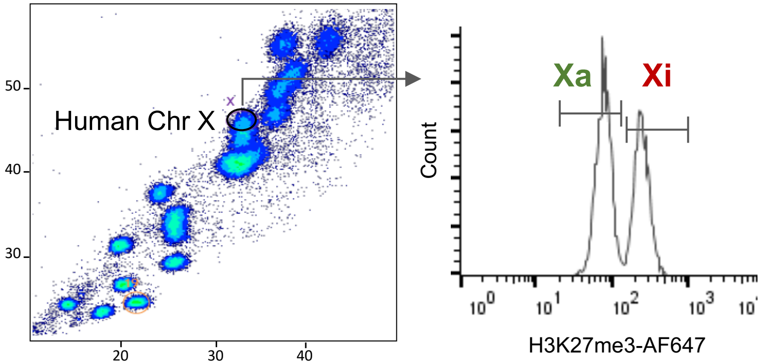
Studying epigenetic memory of XCI in human B cells

Next step: Epigenetic memory of XCI in human B lymphocytes
Role in X-linked immune gene dosage and sex-biased immune response

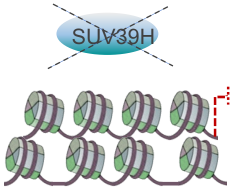
**Human female
B cell clones
with pairwise haplotypes**



FACS sorting of human Xi and Xa



- Xi and Xa- associated factors and chromatin features
- Functional role on immune gene dosage



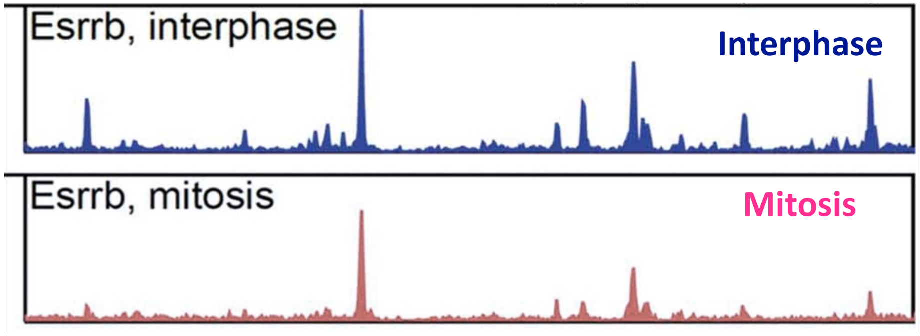
Impact of H3K9me3 loss
- Xi and Xa mitotic memory
- X-linked gene expression
  allele-specific nascent RNA-seq

What is **mitotic memory ?**

What is a mitotic **bookmarking factor ?**

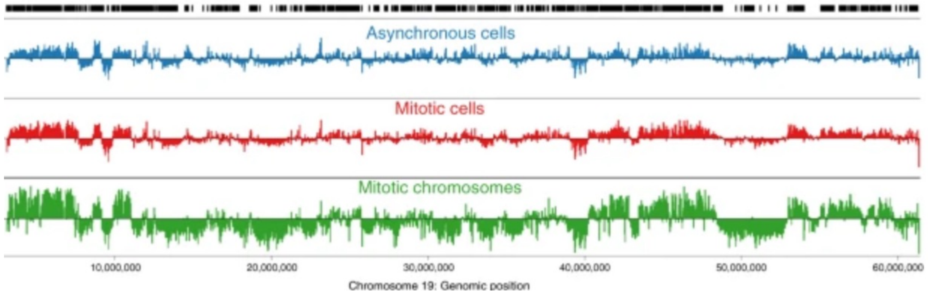
To deserve the title of a “bookmarking factors” you need :

① Occupy in during mitosis a subset of genomic site bound during interphase



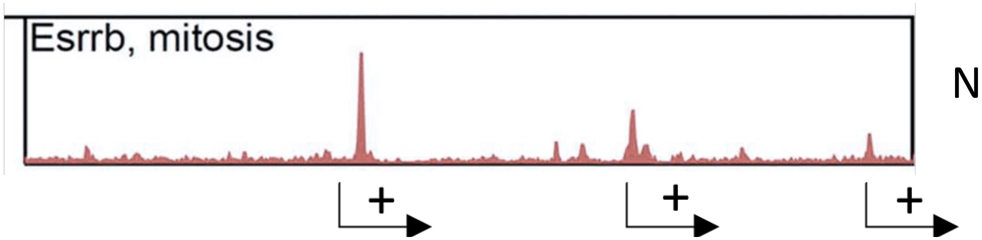
Native ChIP-seq

② Keep bookmarked sites accessible both in interphase and mitosis



ATAC-seq
Nucleosome positioning

③ Be able to reactivate (reinstate) the transcription of these bookmarked site (at lineage specific genes) at mitotic exit

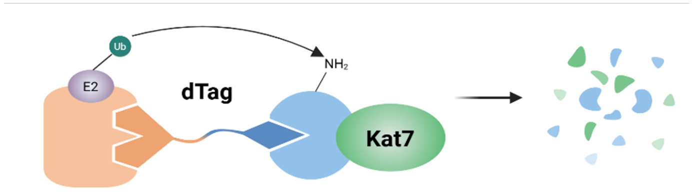


Early transcriptional reactivation early G1 at mitotic exit

Nascent RNA-seq

To keep in mind conveying a repressive state is as important ….

4 Deplete the Bookmarker candidate specifically in mitosis and test the impact on genes reactivation post mitosis



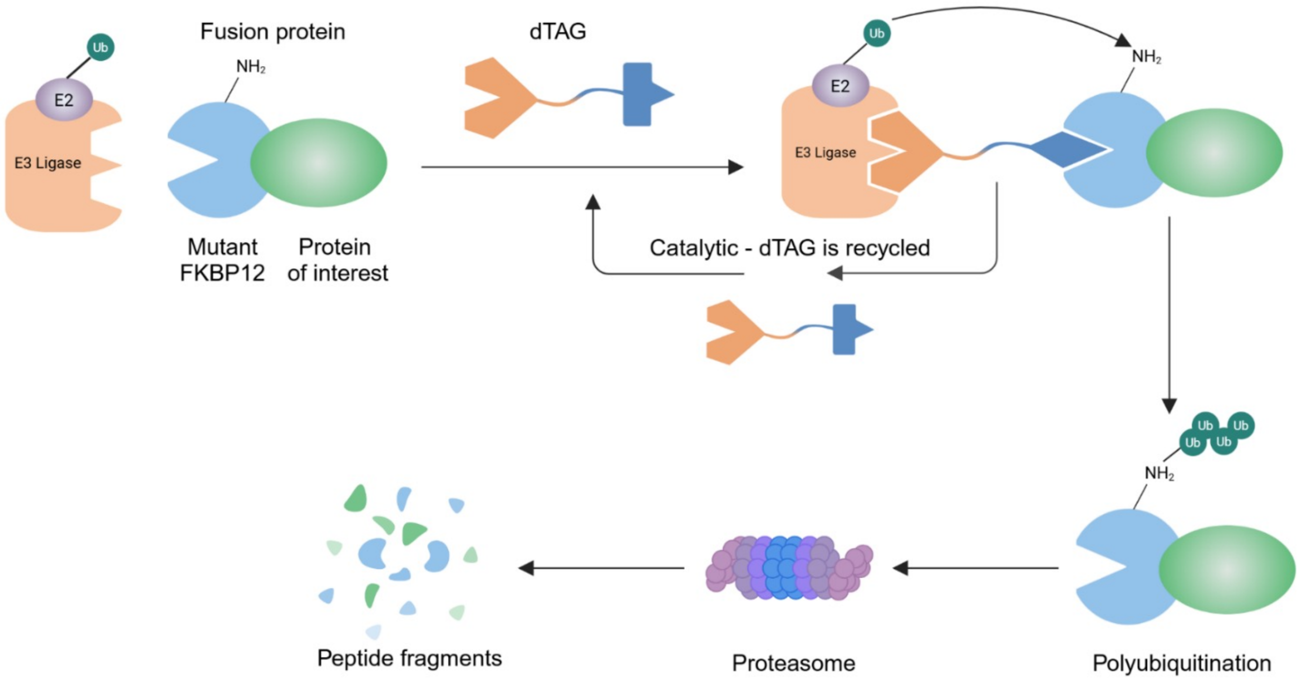
Degron systems to allow rapid/acute Degradation of candidate factors specifically in mitosis

+

Nascent RNA-seq

Reactivation of transcription program post mitosis

dTag degron system



Ub

E2

E3 Ligase

Fusion protein

NH₂

Mutant FKBP12

Protein of interest

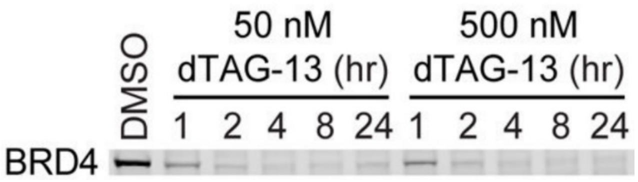
dTAG

Catalytic - dTAG is recycled

Polyubiquitination

Proteasome

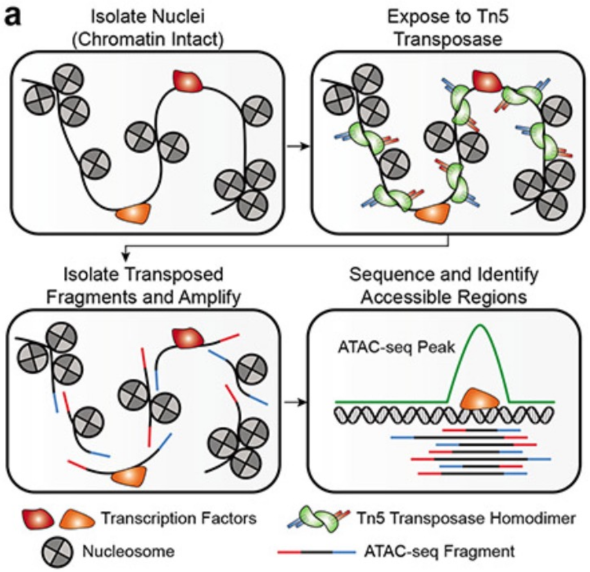
Peptide fragments

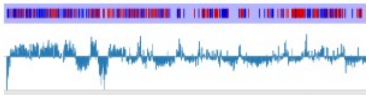


Nabet et al., 2018

Tools (techniques) to study mitotic bookmarking

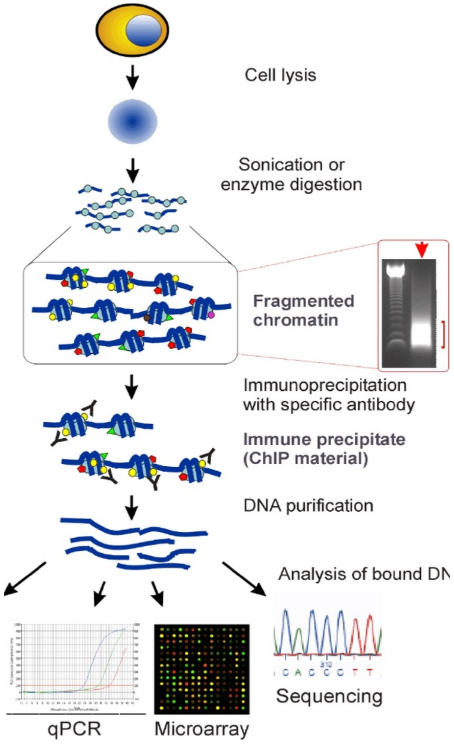
ATAC-seq





Chromatin accessibility
Globally maintained
throughout mitosis

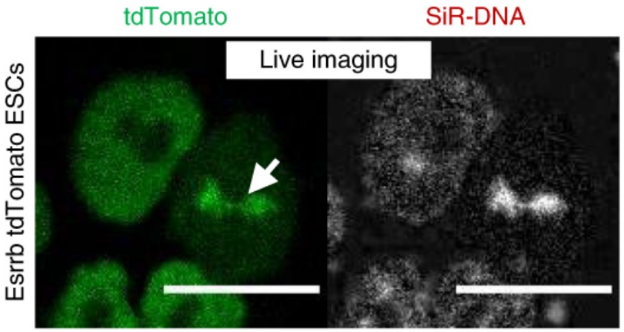
ChIP



Profiling genomic sites
bookmarked during mitosis

Tools (techniques) to study mitotic bookmarking

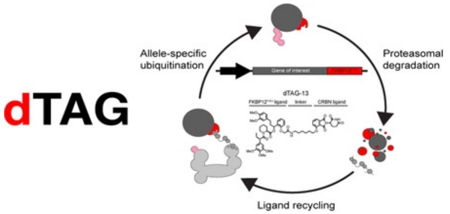
Endogenous tagging followed by Live cell imaging



Tracking chromatin biding thought cell division

Degron system coupled with Nascent RNA-seq

PROTAC
Auxin
dTag



+

Rapid degradation
Assess the reactivation of gene transcription program at mitotic exit (early G1)