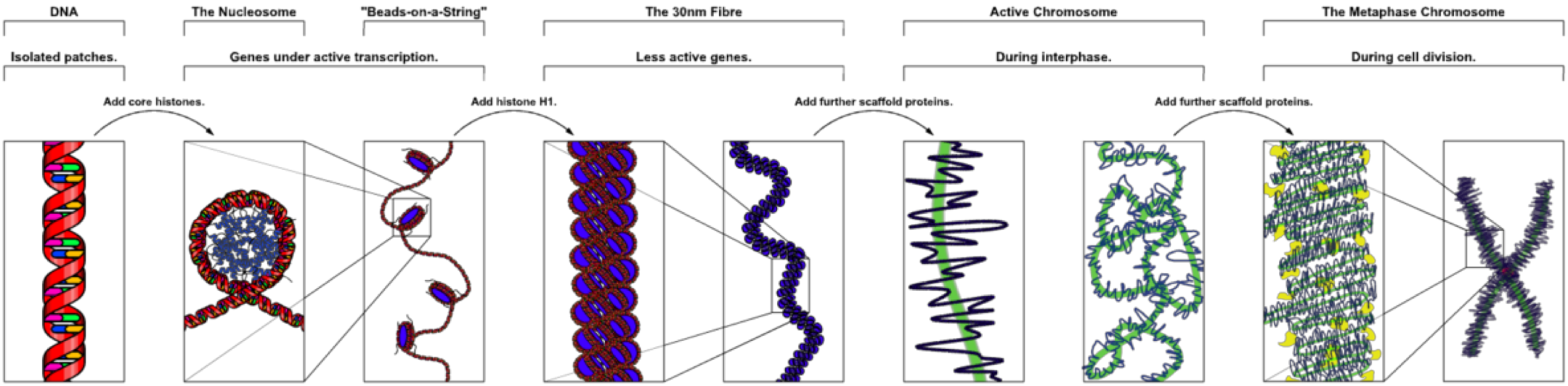
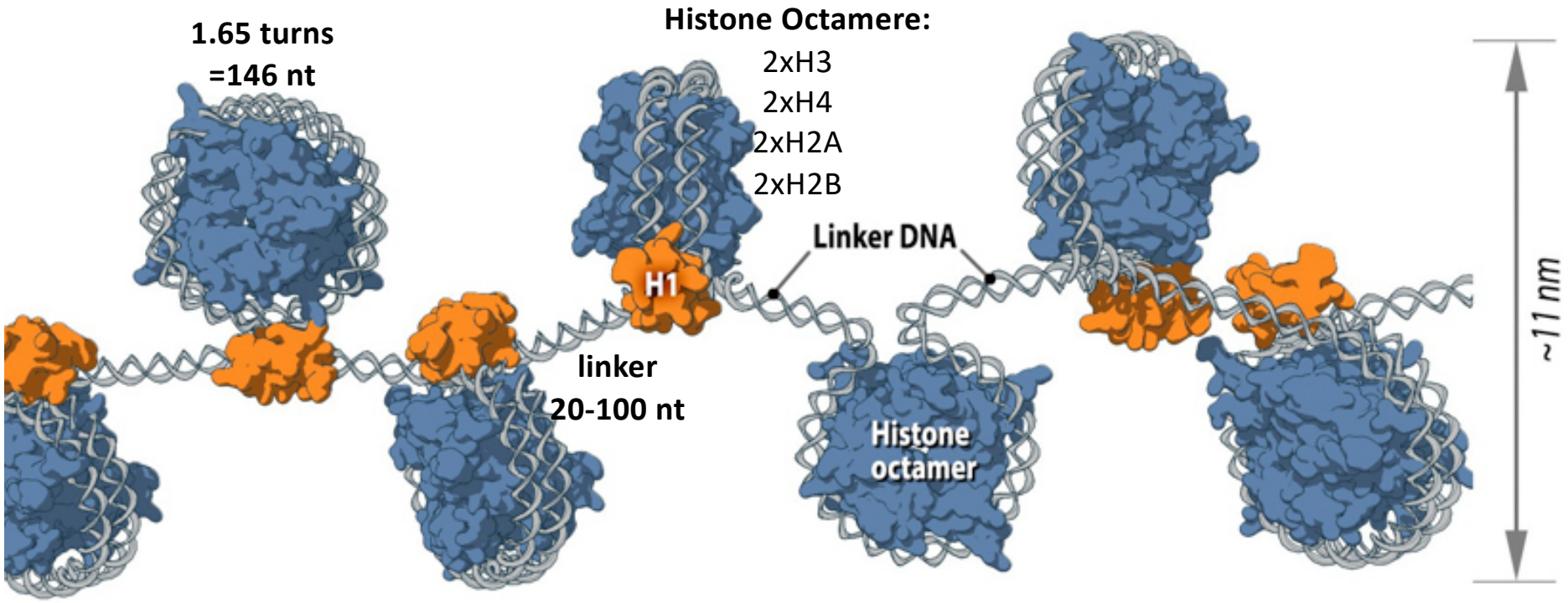
Histone variants and epigenetics

The major structures in DNA compaction



THE EURKARYOTIC NUCLEOSOME



7-fold shortening of DNA

Nucleosome core particle: 146nt DNA + 2xH3, 2xH4, 2xH2A, 2xH2B

Histone Octamerne: 2xH3, 2xH4, 2xH2A, 2xH2B

The origin of histone proteins: Archaebacteria

Bacteria

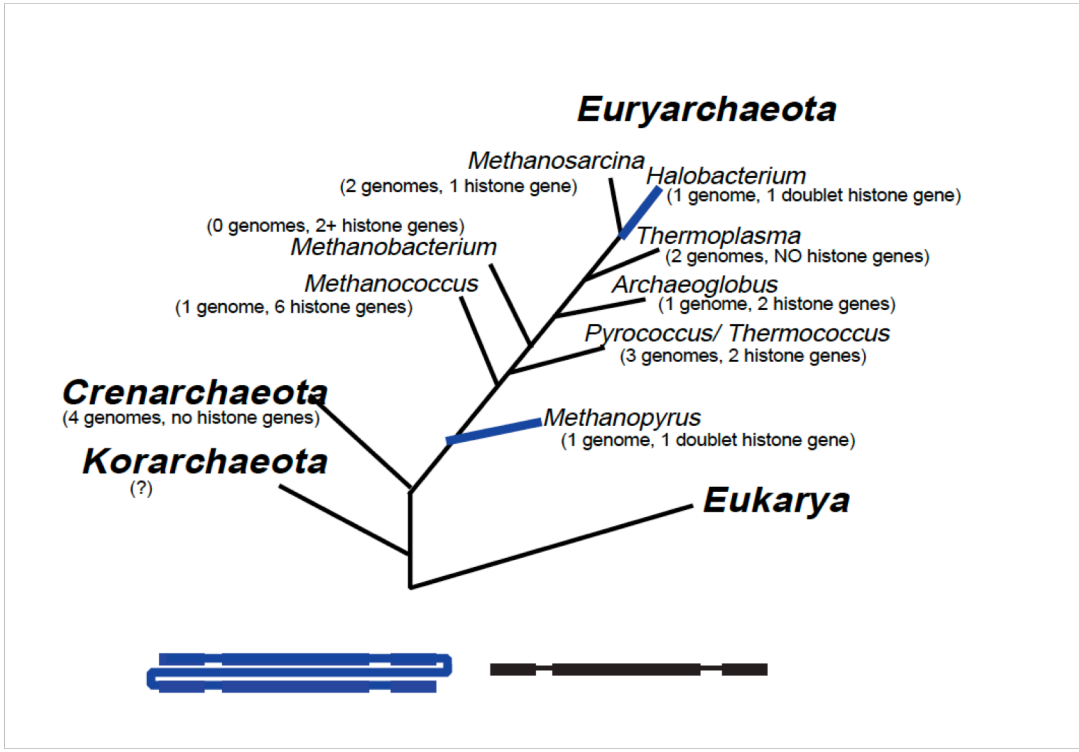
NO HISTONES,
Few structural proteins

Archeabacteria

SOME ARCHEABACTERIA SPECIES ENCODE
HISTONE PROTEINS

The origin of histone proteins: Archaebacteria

HFD



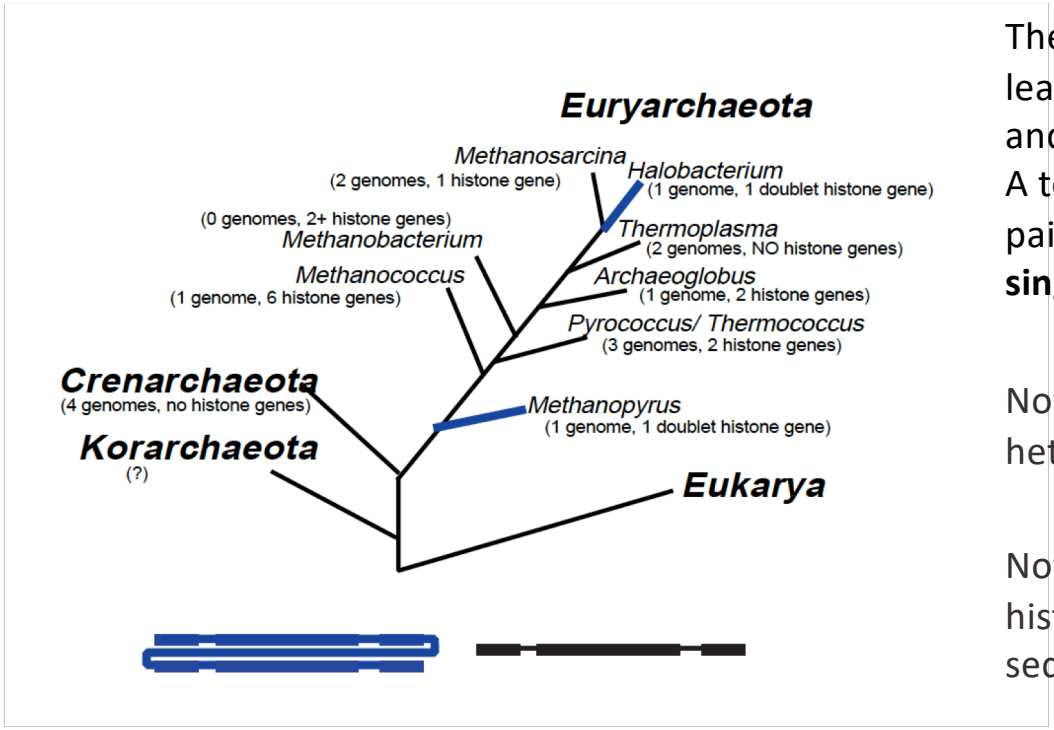
Histone proteins first evolved in Archaebacteria
→ Form «Archeal nucleosomes»

Histone fold (HFD) domain: dimerization

Most archaeal histones comprise a single histone fold domain (HFD), characterized by three α-helices, and two intervening loops, with no N-terminal or C-terminal ‘tails’.

Malik 2013

The origin of histone proteins: Archaebacteria



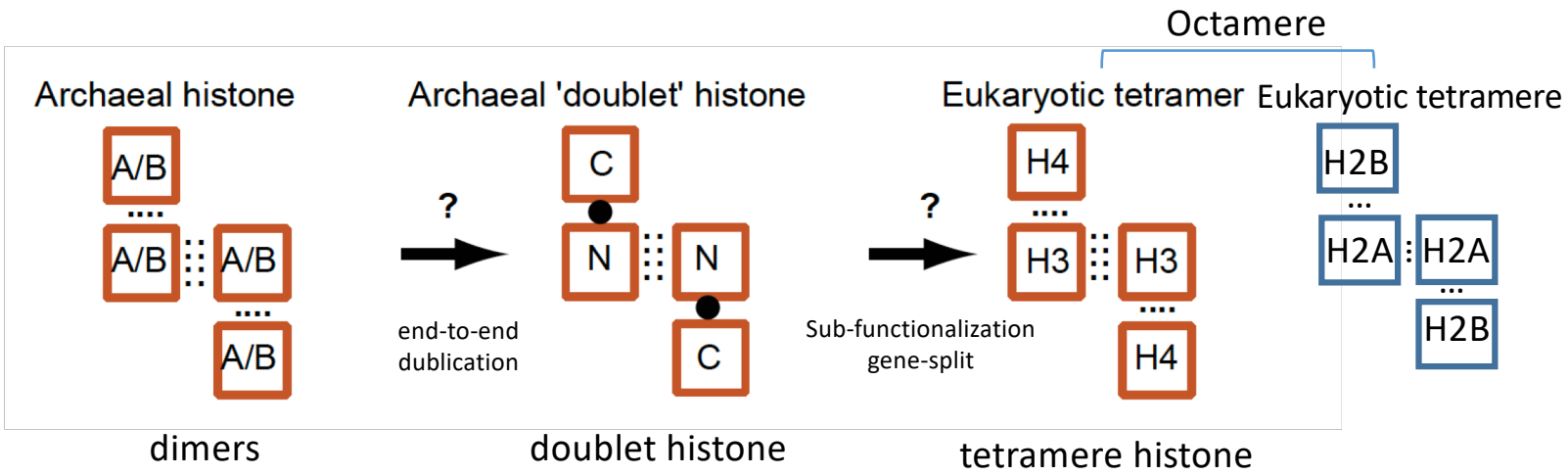
Halobacterium and Methanopyrus kandleri encode histones that are twice as long as typical archaeal histones and consist of an end-to-end duplication of the histone fold.
Evolutionary relevant: dublication requires less protein-protein interaction to form a «tetramere». N and C terminal portions can subfunctionalize

The genome of Methanothermus fervidus encodes at least two distinct histones, HMfA (for histone M. fervidus A) and HMfB, which have been shown to compact DNA.
A tetramer of these histone proteins is able to protect 60 base pairs of packaged DNA from nuclease digestion, suggesting a **single wrap** of DNA around the tetramer

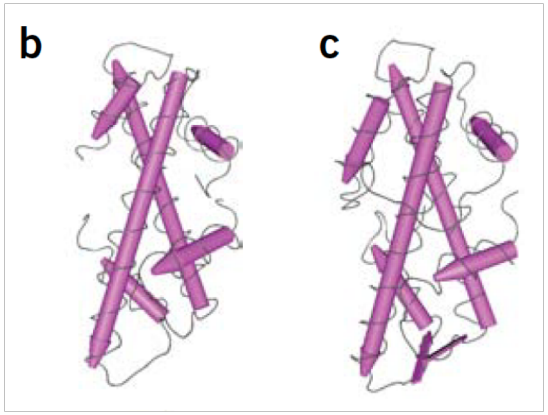
Note: HMfA and HMfB form both, homodimers and heterodimers.

Not all euryarchaeal lineages have a similar complement of two histones. The histone gene complement in completely sequenced euryarchaeal genomes varies from one to six genes

From Archaebacteria to Eukaryote histone tetramers

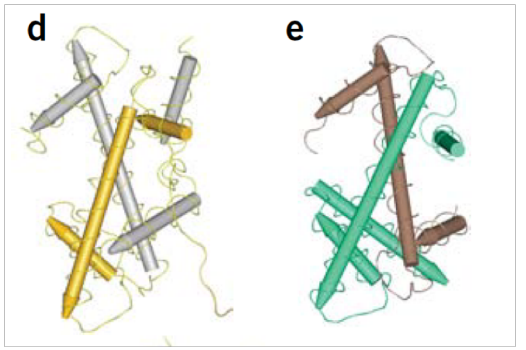


The formation of the doublet histone directly resulted in an asymmetric (subfunctionalized N and C terminus) dimer that could have preceded the actual separation of the H3-H4 and H2A-H2B genes



b) homodimer of HMfA from M. fervidus

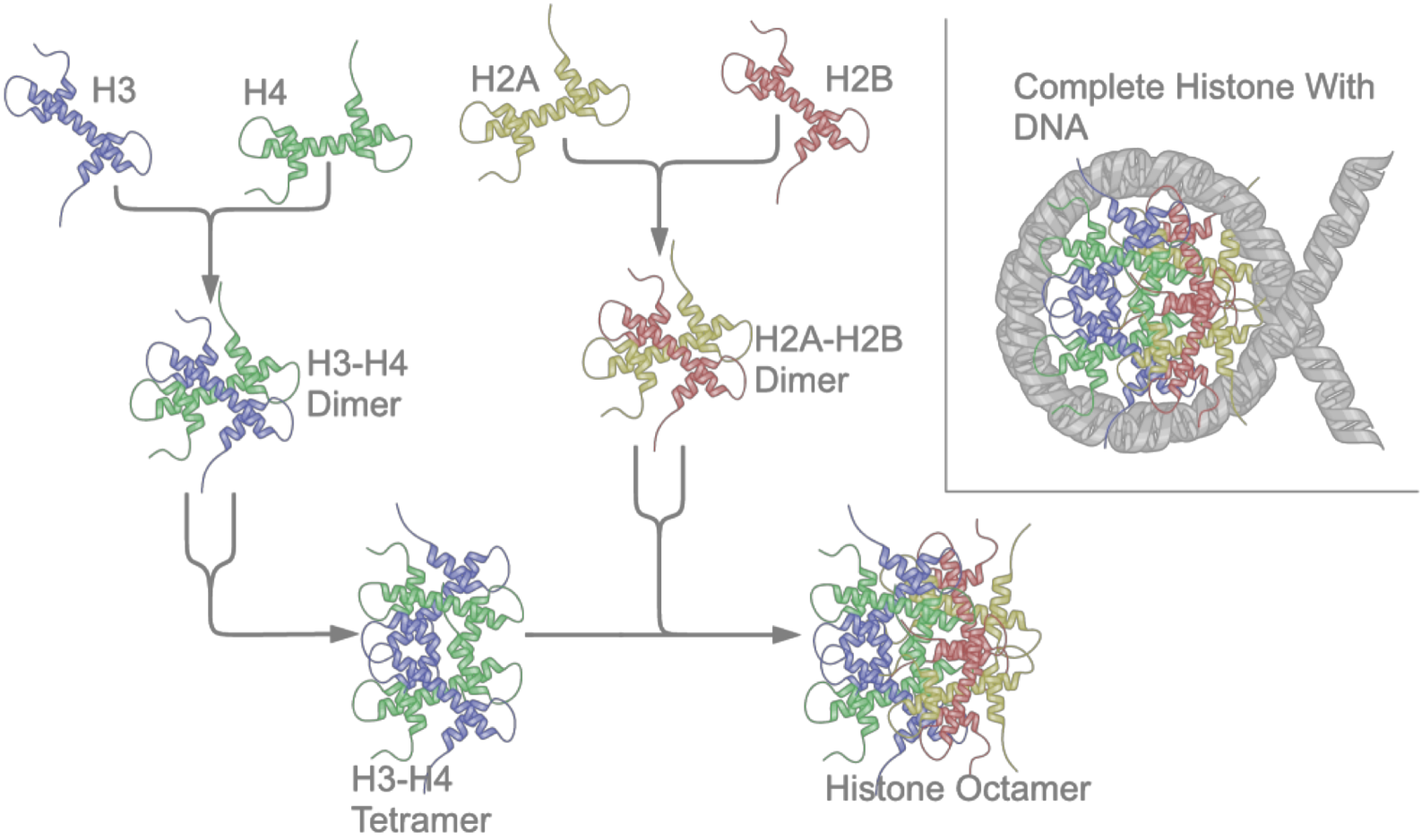
c) doublet histone from M. kandleri



d) H3–H4

e) H2A–H2B

Heterodimerization + tetramerization = eukaryotic octamere formation



Nucleosome
2 wraps of DNA
around
Octamere:

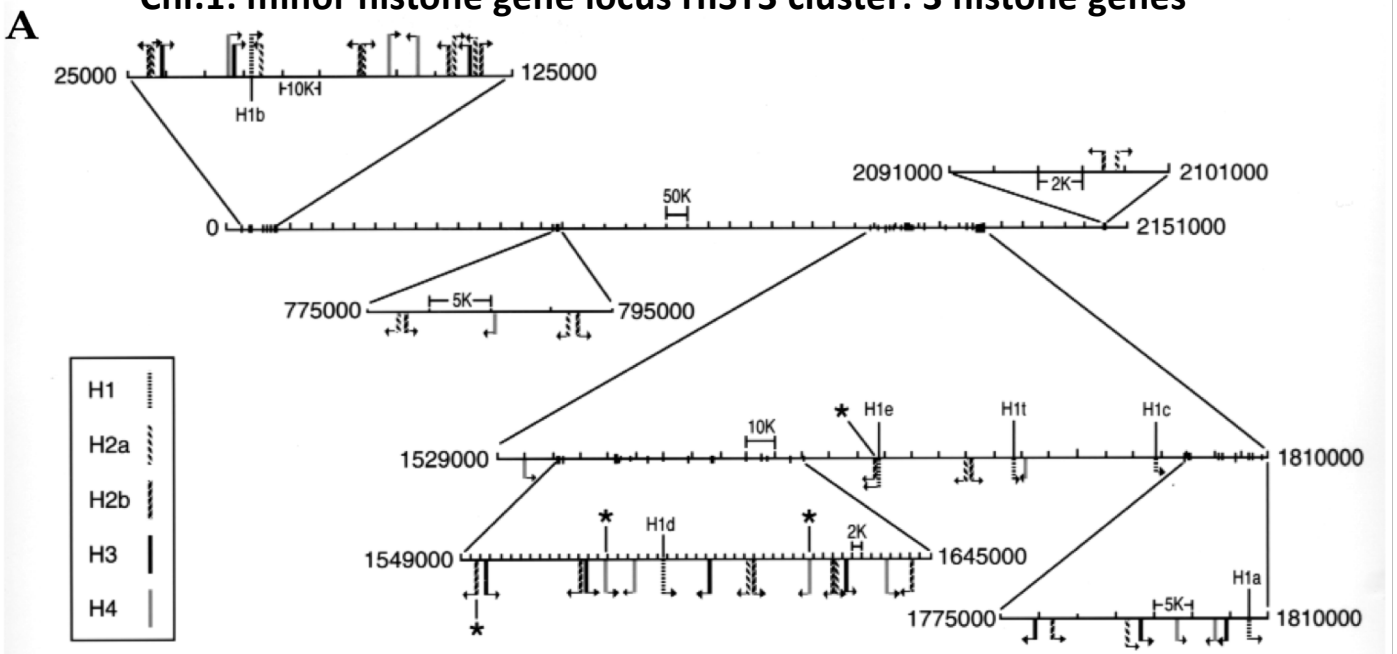
Histones and DNA replication

Histone genes are organized in histone gene clusters

Human: Chr.6: major histone gene locus: HIST1 cluster: 45 core histone genes ; 6 Histone H1 genes

Chr.1: minor histone gene locus HIST2 cluster: 6 histone genes

Chr.1: minor histone gene locus HIST3 cluster: 3 histone genes



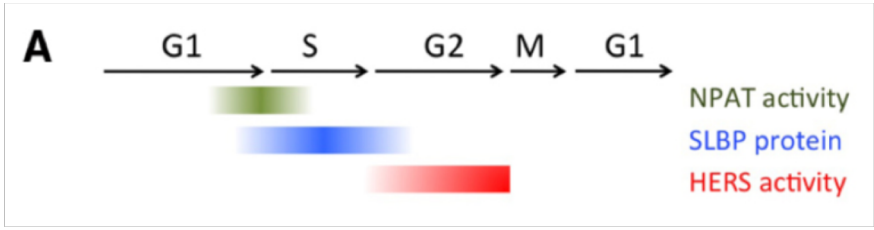
The human and mouse major histone gene cluster. (A) The histone gene cluster on human chromosome 6p21–p22 is shown. The position and direction of transcription of the 55 histon genes in this region are indicated, with the genes for the five histone pro- teins indicated in the box. Only "real" genes are shown (defined as genes that contain the expected 3 end of histone mRNA). The portion of chromosome 6 is going (left to right) from the cen- tromere to telomere. HISTH4A is the first H4 gene starting from the right and the same is true for the other core histone genes. The numbers are nucleotides from the arbitrary start of the cluster at 0. The regions where there are tightly grouped clusters of histone genes have been expanded. The scale of each section is indicated in kilobases (kb). The position of each of the histone H1 histone gene is indi- cated with the nomenclature H1a–e, H1t, and the symbols for the core his- tone genes are in the inset. The aster- isks indicate the position of genes pres- ent in human and not in mouse.

In each species, there are a total of 10–20 genes encoding each of the core histone proteins. Each of these genes encodes a unique mRNA, with a distinct 5 - and 3 -UTR and promoter, as well as nucleotide changes in the coding region. Although all the histone **H4 genes encode the same protein** and the **histone H3 genes encodes** variants previously described , there are **10–12 different H2a and H2b** proteins.

https://www.genenames.org/cgi-bin/genefamilies/set/864

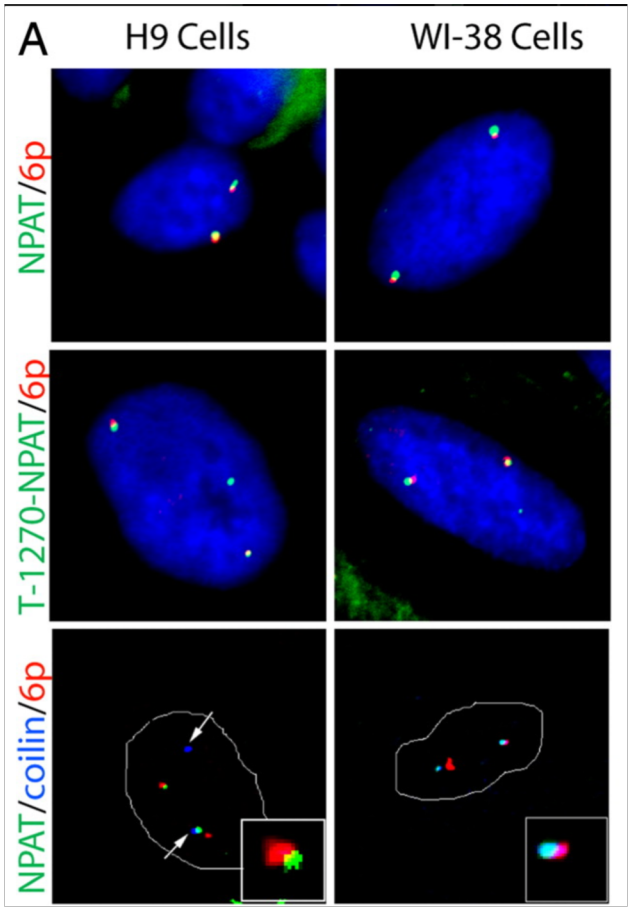
Histones and DNA replication

Histone synthesis is limited to S-Phase



The transcription of histone gene takes place in a subnuclear organelle termed the **histone locus body (HLB)**, containing factors required for the processing of histone pre-mRNAs which have an **unusual mRNA structure, with a 3'UTR that forms a stem-loop structure instead of a polyA tail** (White et al., 2007; Nizami et al., 2010).

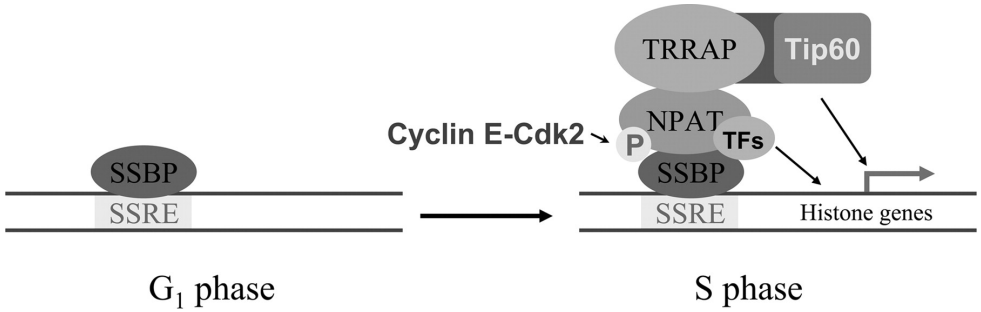
It has been suggested that excess free histones may be toxic to cells, explaining the evolutionary pressure for their conserved, yet peculiar regulation (De Koning et al., 2007).



Human cells in S-phase
IF staining for NPAT combined with
DNA FISH for the HIST1 cluster on Chr.6

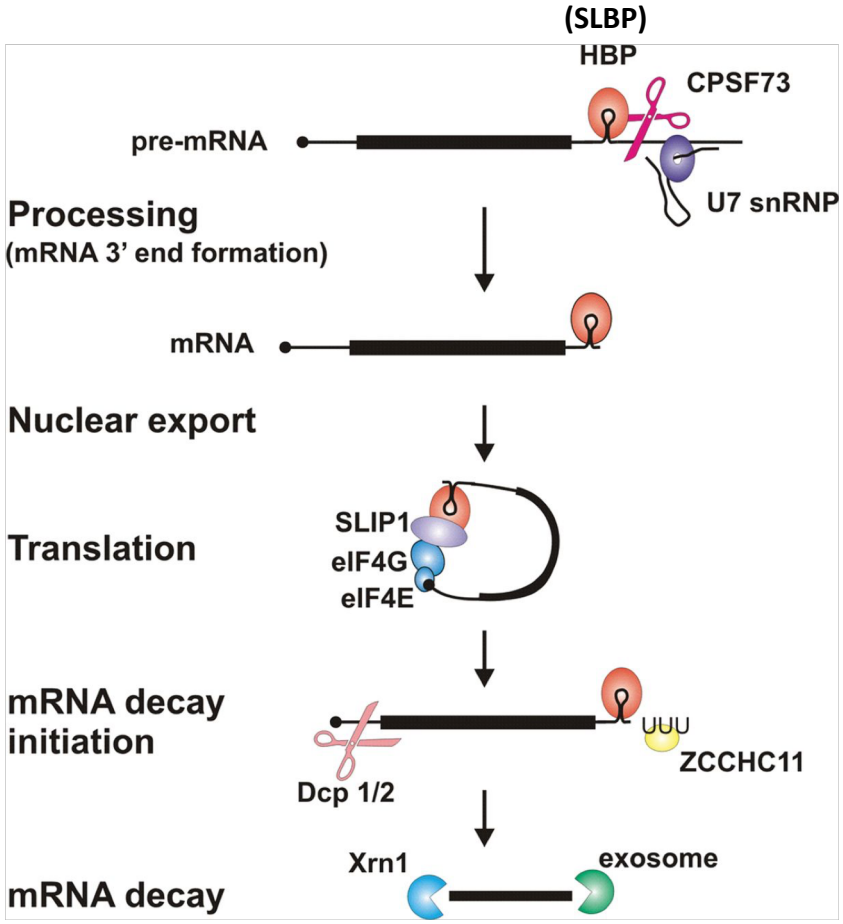
High expression in S-Phase

Entry into S-phase is triggered by the activity of the G1-S Cyclin complex, CyclinE/Cdk2. In addition to phosphorylating targets to initiate DNA replication, CyclinE/Cdk2 also phos-phorylates **nuclear protein ataxia-telangiectasia locus (NPAT**), to initiate transcription of the histone genes (Ma et al., 2000; Zhao et al., 2000; Ye et al., 2003). After CyclinE/Cdk2 activity has reached its peak in early S-phase, CyclinE/Cdk2 activity drops due to the degradation of the essential CyclinE component



SSRE, subtype–specific regulator element; SSBP, proteins, such as Oct–1 and HiNF–P that directly bind SSRE elements within the promoters of a histone subtype; TFs, transcription factors, P, phosphorylation

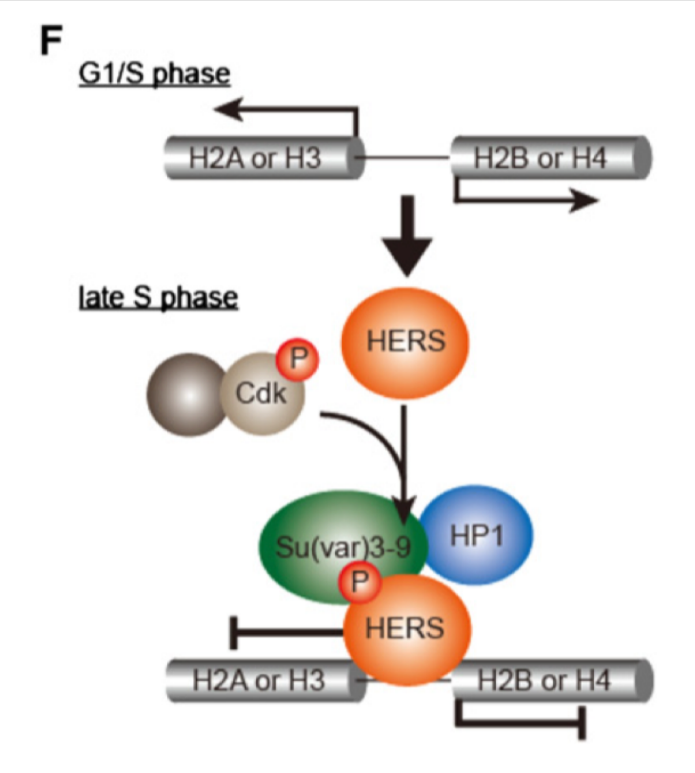
Histones and DNA replication



SLBP protein itself is cell cycle regulated. SLBP mRNA is synthesized constantly throughout the cell cycle, but **SLBP becomes translated just prior to S-phase entry and the protein is degraded at the end of S-phase**

Histone mRNA 3'-end processing requires the RNA-binding protein **HBP (also called SLBP)** , which binds to the conserved hairpin structure in histone pre-mRNA, and the U7snRNP, which binds to a sequence element downstream of the cleavage site. Together with other factors they position the nuclease CPSF73 for cleavage to produce histone mRNA ending immediately after the. After nuclear export, HBP interacts with SLIP1 and other translation initiation factors to form a closed-loop structure for efficient translation. This structure is disrupted, presumably when histone mRNA decay is initiated, for example at the end of S-phase. Addition of an oligo(U) tail by the terminal uridylyl transferase ZCCHC11 is an early step in decay, which involves decapping followed by 5'→3' degradation by Xrn1 or 3'→5' decay by the exosome.

Histones and DNA replication



Drosophila: The histone gene-specific epigenetic repressor in late S-phase (**HERS**) protein becomes phos- phorylated by the late S-G2 Cyclin complex CyclinA/Cdk1, which localizes it to the histone genes where it acts to silence histone genes after S-phase

Cdk-activated HERS silence histone gene expression in late S phase through recruitment of Su(var)3-9/HP1 repressor complex.

Ito et al. 2012 Mol Cell. Epigenetic Silencing of CoreHistone Genes by HERS in Drosophila

REPLICATION COUPLED HISTONES

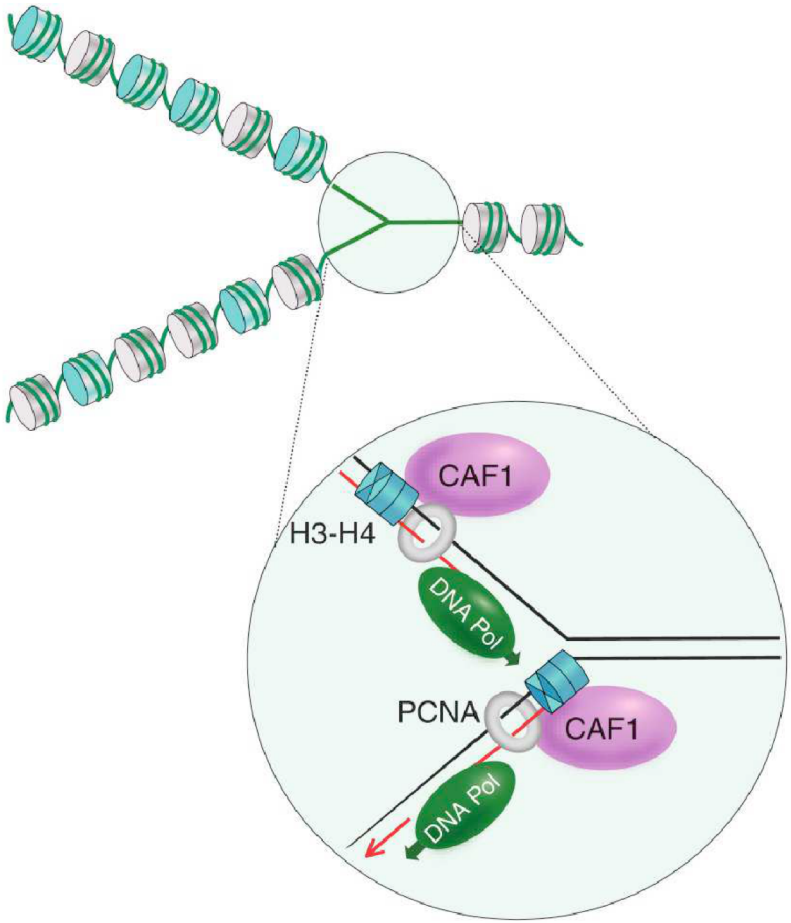


Figure 4. Distribution of old and new nucleosomes at a replication fork. Old nucleosomes (gray disks) are randomly distributed behind the replication fork and new nucleosomes (cyan disks) are deposited in the gaps. CAF-1-mediated nucleosome assembly is depicted on the leading and lagging strand in magnification. DNA polymerase (green); replication processivity clamp, PCNA (gray ring); histone H3-H4 tetramers (cyan); newly synthesized DNA (red lines).

REPLICATION COUPLED (RCs) HISTONES:
H2A, H2B, H3, H4
Are incorporated into new and old DNA strand during DNA replication

Chromatin assembly factor 1 (CAF-1) is a HISTONE CHAPERON that is associated with PCNA. → Facilitates the formation of new nuclosomes
The assembly of a nucleosome consists of the loading of an (H3-H4)2 tetramer (tetrasome) that is followed by the addition of 2 H2A-H2B dimers.

REPLICATION INDEPENDENT (RIs) HISTONES:
Are incorporated independently of DNA replication

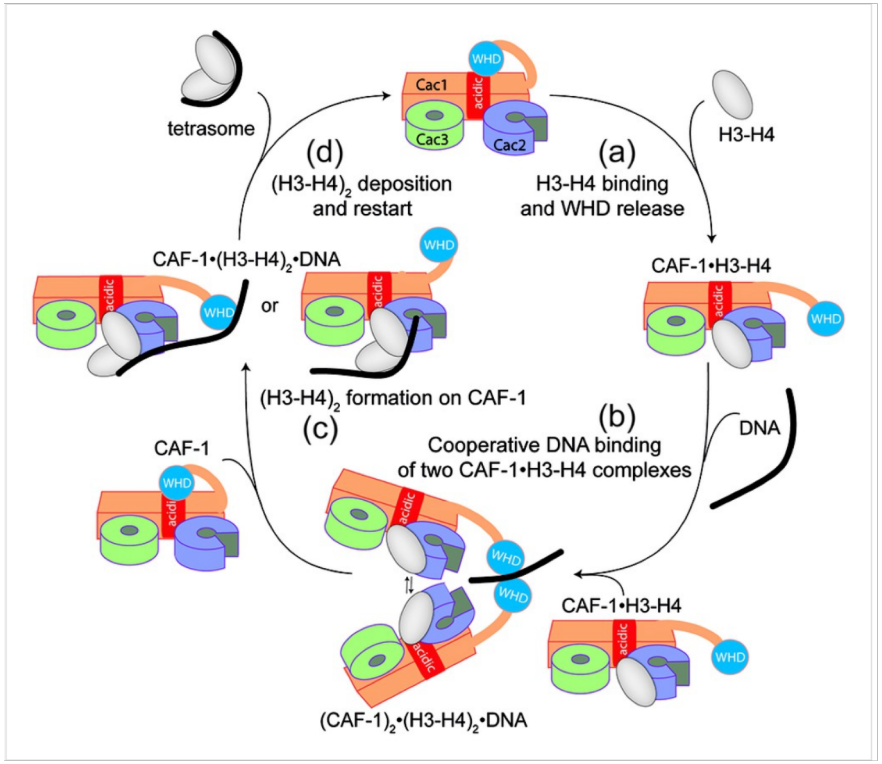
RI histones require the displacement of a preexisting nucleosome unit (active displacement or “loss”)

RI histones can **reset the epigenetic state of a pre-exising nucelosome**

Histone variants with high similarity to ”normal” histone are also incorporated into cannonical octamers during replication. However concentration of variants is very low → no big effect on chromatin structure

HOWEVER: HISTONE CHAPERONS EXIST THAT ENSURE CONCENTRATED INCORPORATION OF HISTONE VARIANTS AT DEFINED SITES → CONTROLLED LOCAL CONCENTRATION → ALTERATION OF CHROMATIN STRUCTURE

Incorporation of replication coupled histones by CAF-1 histone chaperone



Humans CAF-1 subunits: p150, p60, and p48,
Budding yeast CAF-1 subunits: Cac1, Cac2, and Cac3

CAF-1 p150 interacts with PCNA

(a) The nucleosome assembly mechanism of CAF-1 is activated by H3-H4 binding, which releases the WHD domain from an intramolecular interaction with the acidic region on Cac1.
(b) DNA binding promotes the association of two CAF-1•H3-H4 complexes to join the histones into a (H3–H4)2 tetramer
(c) In the presence of DNA of sufficient length, the (H3–H4)2 histones are directly sequestered from CAF-1.
(d) (H3–H4)2 are transferred to the DNA to form the **tetrasome**, and the WHD rebinds to the now free acidic region, resulting in its dissociation from DNA.

H2A-H2B can spontaneously associate with tetrasomes in vitro and because CAF-1 itself has significantly lower affinity for H2A-H2B compared to H3-H4, it appears that the primary role of CAF-1 is to promote the formation of an ordered $(H3\text{-}H4)_2$•DNA complex, the tetrasome

Mattiroli F; Elife. 2017; PMID: 28315523

REPLICATION INDEPENDENT (RIs) HISTONES: - HISTONE VARIANTS

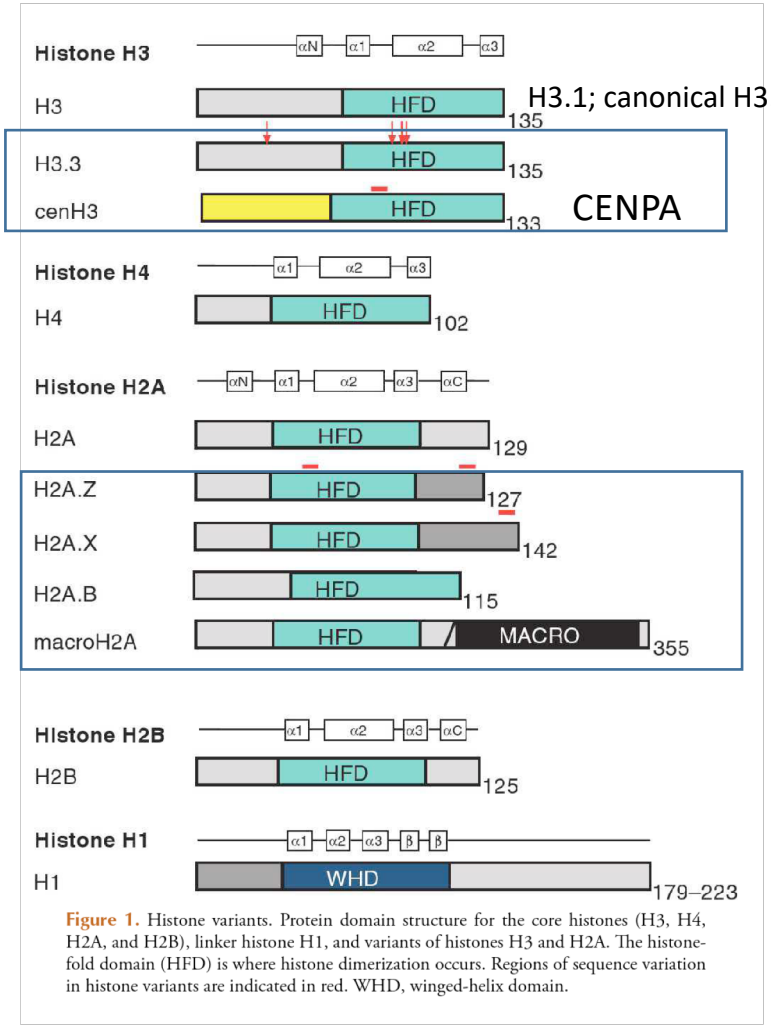


Figure 1. Histone variants. Protein domain structure for the core histones (H3, H4, H2A, and H2B), linker histone H1, and variants of histones H3 and H2A. The histone-fold domain (HFD) is where histone dimerization occurs. Regions of sequence variation in histone variants are indicated in red. WHD, winged-helix domain.

REPLICATION INDEPENDENT (RIs) HISTONES:
Are incorporated throughout the cells cycle and independently of DNA replication

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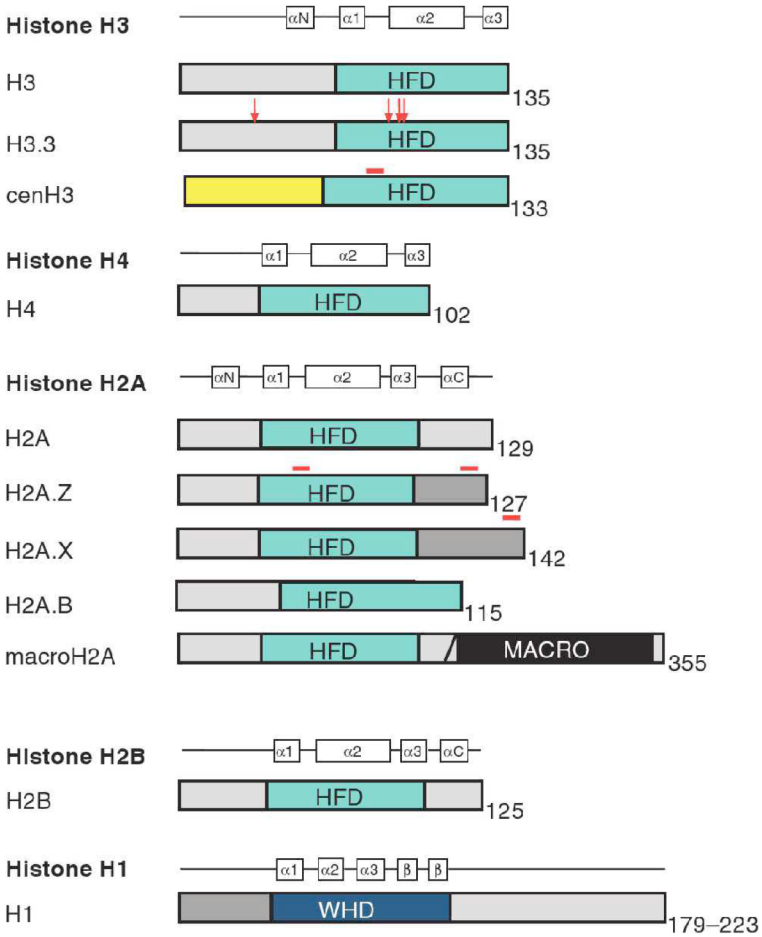

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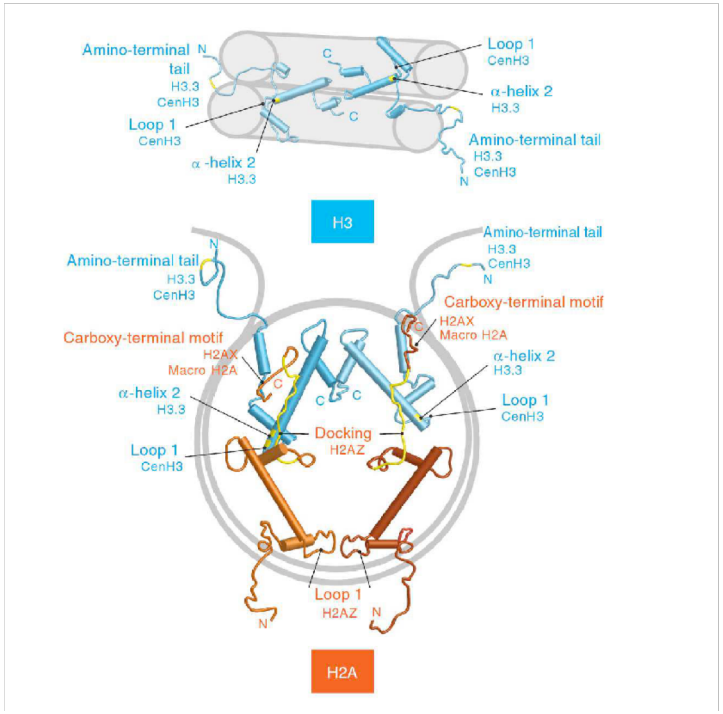
Humans:
Many isoforms of H2A and H3 exist; H2B and H4 have not diversified

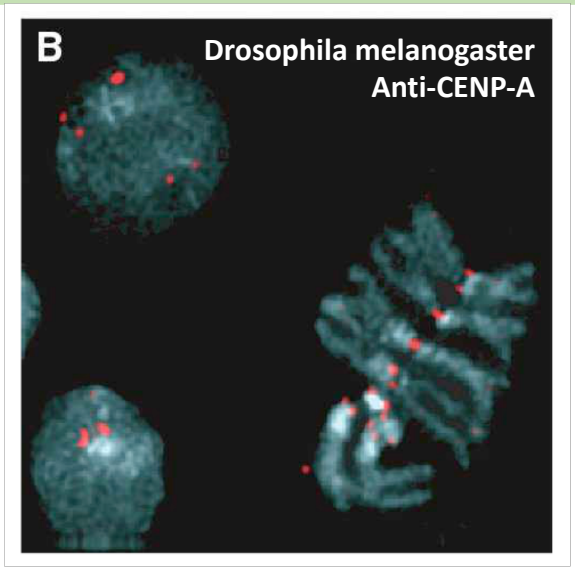
Incorporation of variants of histones into nucleosomes dramatically altered chromatin structure

Some histone variants are deposited by specialized nucleosome assembly complexes

Variation to classic histone is very small (except macroH2A/CENPA)

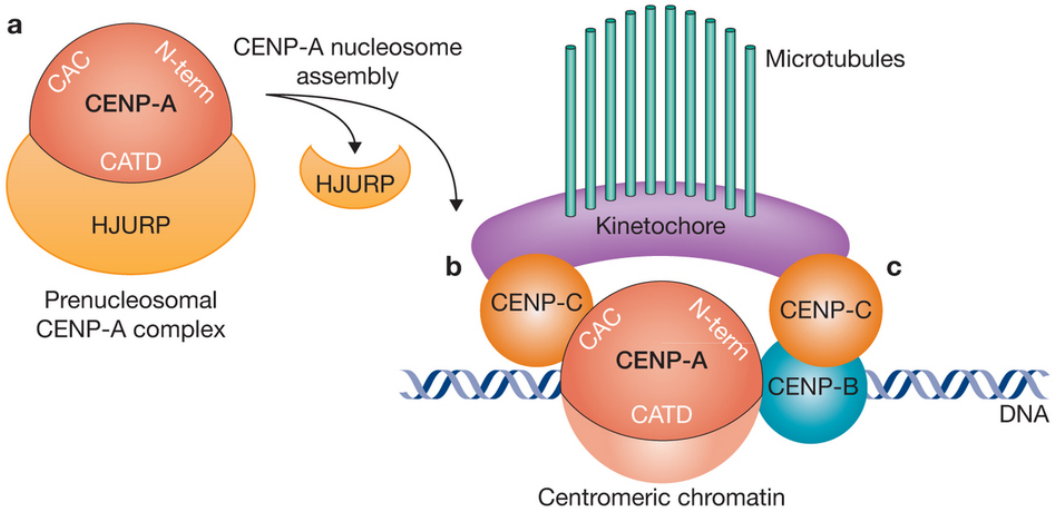


H3 VARIANTS: CENP-A: A HISTON VARIANT THAT DEFINES EUKARYOTIC CENTROMERES



Centromere protein **C** 1 is a centromere autoantigen and a component of the inner kinetochore plate. The protein is required for maintaining proper kinetochore size and a timely transition to anaphase.

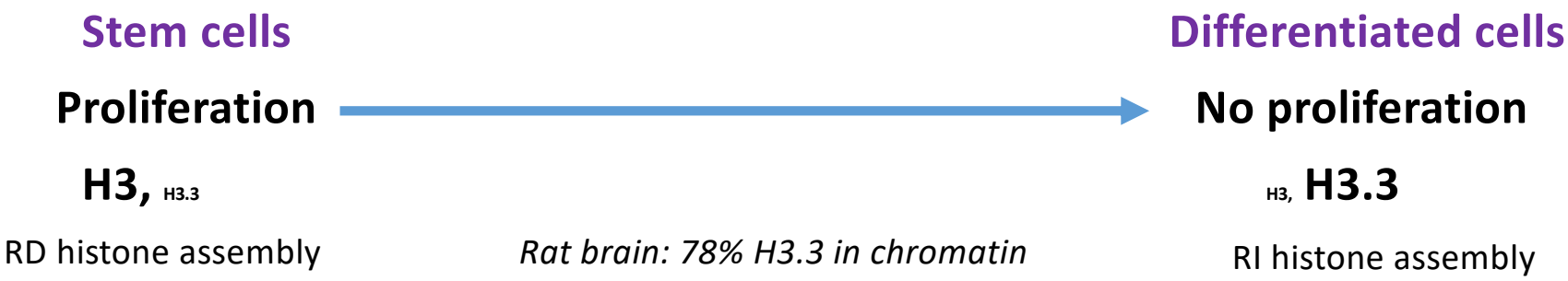
Centromere protein B is a highly conserved protein that facilitates centromere formation. It is a DNA-binding protein that is derived from transposases of the pogo DNA transposon family. It contains a helix-loop-helix DNA binding motif at the N-terminus, and a dimerization domain at the C-terminus. The DNA binding domain recognizes and binds a 17-bp sequence (CENP-B box) in the centromeric alpha satellite DNA. This protein is proposed to play an important role in the assembly of specific centromere structures in interphase nuclei and on mitotic chromosomes. It is also considered a major centromere autoantigen recognized by sera from patients with anti-centromere antibodies.



CENP-A: RI histone variants, incorporated by the cenH3 Specific histone HJURP chaperones

Kinetochore assembles on CENP-A (Mechanism unknown) CENP-C and CENP-B are non histone centromere founder proteins

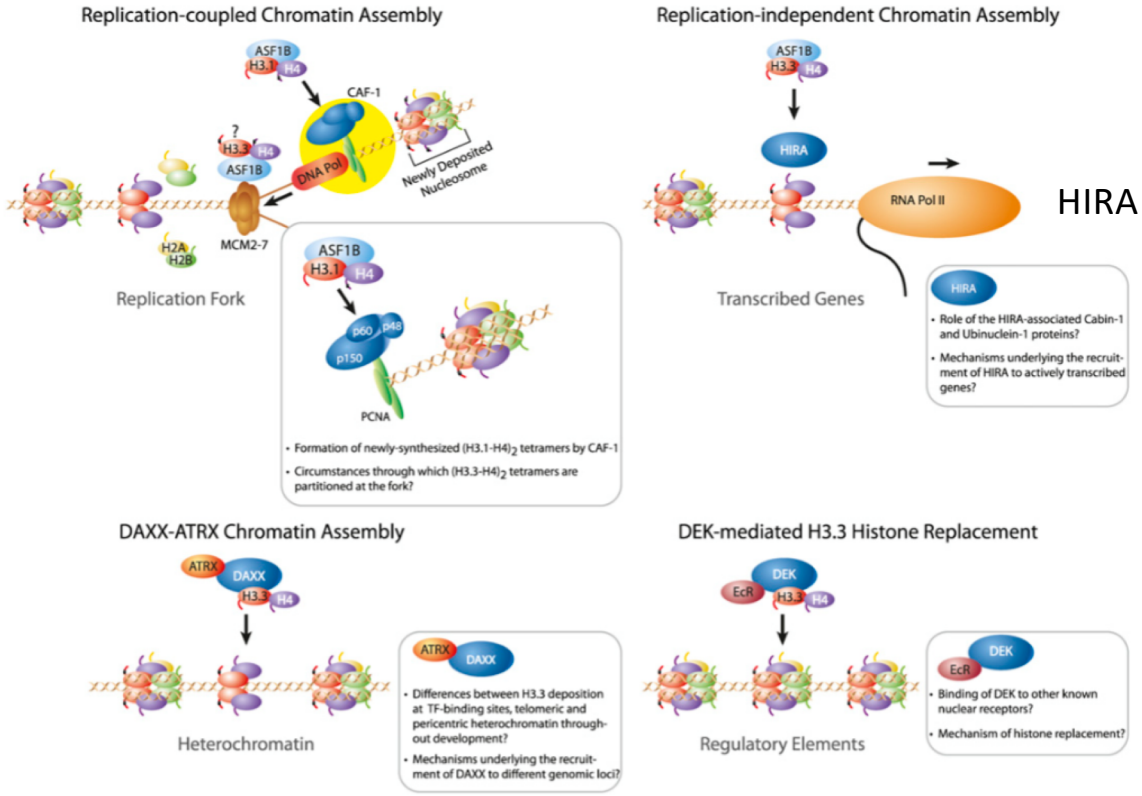
H3.3 INCORPORATION INTO GENOME IS HIGHLY DYNAMIC: AN EXAMPLE



H3.3 is essential to fill nucleosome gaps in terminally differentiated cells

Gene expression is not altered → H3.3 compensates for H3

Pathways if RI incorporation of H3.3 into chromatin



HIRA → H3.3 → active genes

DAXX/ATRX →
H3.3 → repetetive elements

Figure 2. During RC assembly, the ASF1 chaperone is thought to transfer newly synthesized soluble H3.1–H4 dimers to CAF-1 through direct interactions with its p60 subunit (Tyler et al. 2001; Mello et al. 2002). CAF-1 would then facilitate the assembly of a central $(H3.1–H4)_2$ tetramer to which two H2A–H2B dimers are juxtaposed by other chaperones to complete a core nucleosomal unit. Similarly, during RI chromatin assembly, ASF1-bound H3.3–H4 would be transferred to HIRA for tetramer formation (Green et al. 2005). Note, however, that the deposition of H3.3 in the *Drosophila* male pronucleus is HIRA-dependent, but ASF1-independent (Bonnefoy et al. 2007) in the histone-rich fertilized egg. Novel alternate pathways for H3.3–H4 deposition include the DAXX chaperone coupled to the ATRX ATP-dependent chromatin remodeler (Drané et al. 2010; Goldberg et al. 2010), as well as targeted H3.3 deposition to regulatory elements by DEK (Sawatsubashi et al. 2010). Boxes highlight queries to consider in future studies.

H3 VARIANTS: H3.3 A HISTONE VARIANT MARKING ACTIVE CHROMATIN

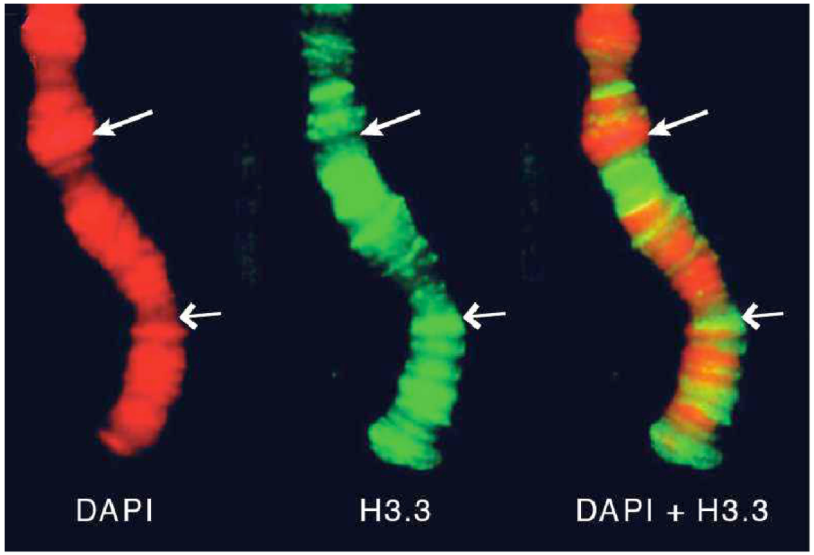
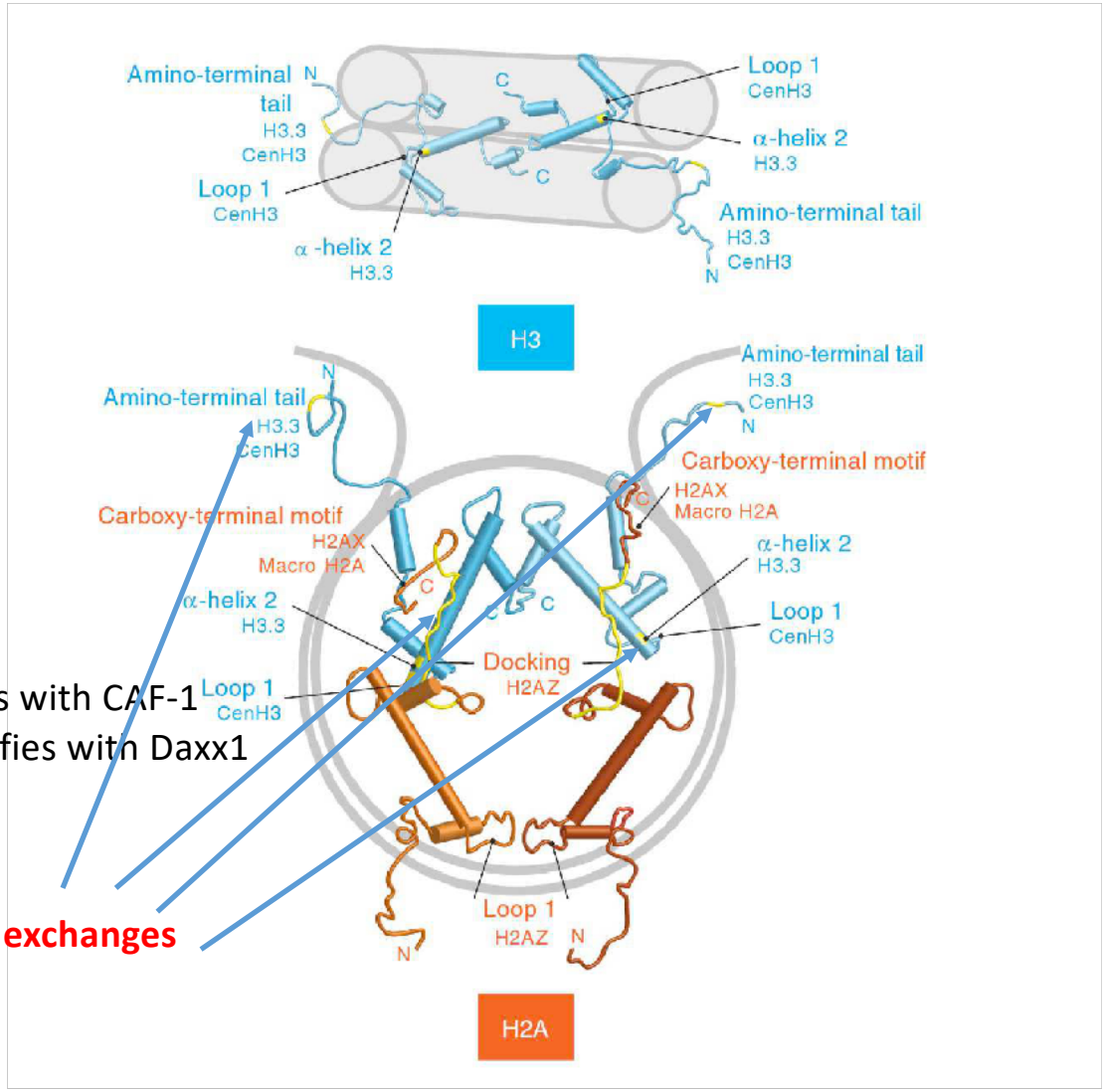


Figure 7. H3.3 preferentially localizes to actively transcribed regions of *Drosophila* polytene chromosomes. DAPI staining (red) shows the DNA banding pattern (*left*), and H3.3-GFP (green) localizes to interbands (*middle*), which are sites of RNA Pol II localization. The merge (Schwartz and Ahmad 2005) is shown on the *right*. In each image, the shorter arrow points to a decondensed interband that is enriched in H3.3, and the longer arrow points to a condensed band that lacks H3.3.



H3: incorporated during replication (RC histone); co-purifies with CAF-1

H3.3.: incorporated during replication (RC histone); Co-purifies with Daxx1
also incorporated by histone chaperones (RI histone)

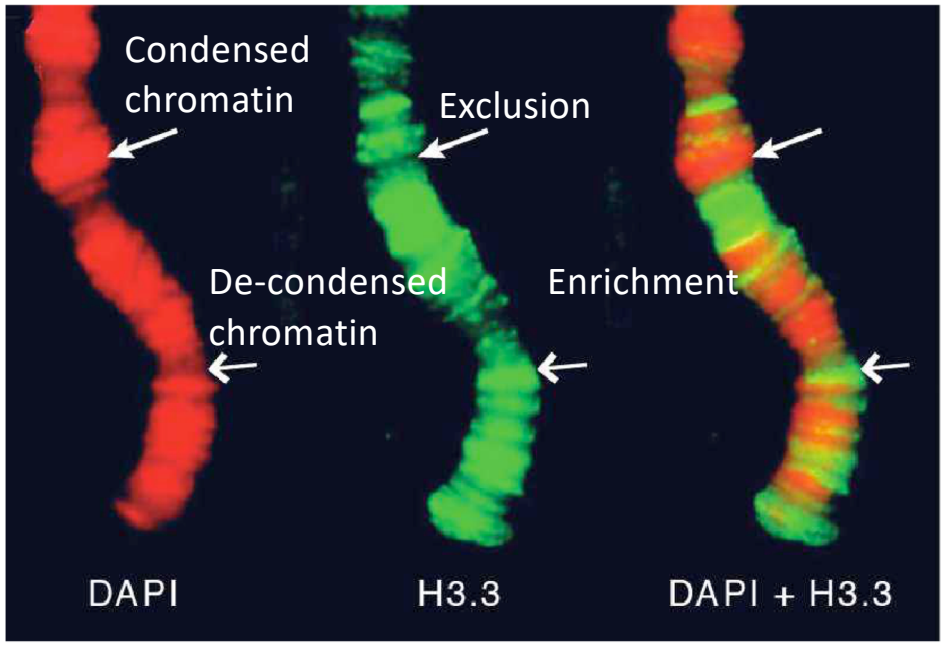
H3.2: canonical H3 (CAF-1, S-Phase)

H3.1: canonical H3 1aa exchange to H3.2 (CAF1, S-Phase)

H3.3: isoform, 4 aa exchange (DAXX, RI histone)

Only 4 aa exchanges

DOI: https://doi.org/10.1016/j.tibs.2007.08.004



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H3.3 can form heterodimer with H4 → H3.3-H4 is incorporated by RI assembly

Chromatin is in flux during in G1/G2 and DNA is transcribed → frequent RI chromatin reassembly → enrichment of H3.3 chromatin **in active genes**

Active genes are defined by epigenetic writers (i.e. H3K4 HMTse) that impose active histone marks on H3.3.

Additional positive effect: eventual repressive modifications (H3K9me3) are eliminated by exchanging H3-mod for H3.3 unmodified

H3.3 carries same modification like classic H3

Gene induction triggers histone replacement.

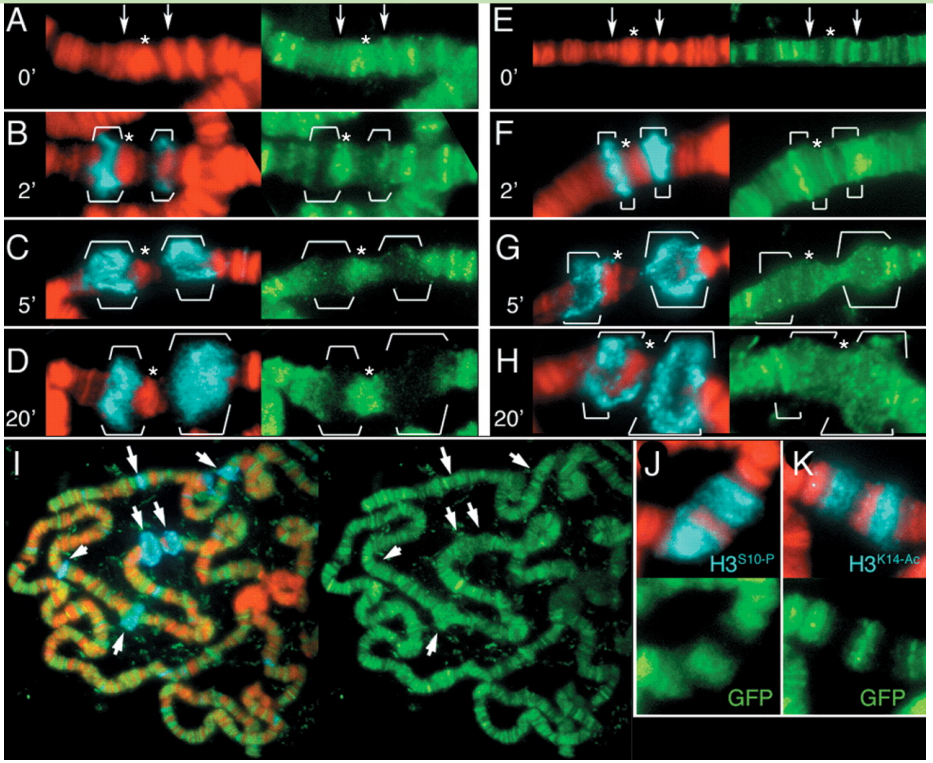
Polytene chromosome

Heat shock

Heatshock response genes activated

Formation of “Puffs”
(aligned genes are activated
Opening of chromatin visible)

Green: GFP-H3
Blue: RNA Pol II active
Red: DNA



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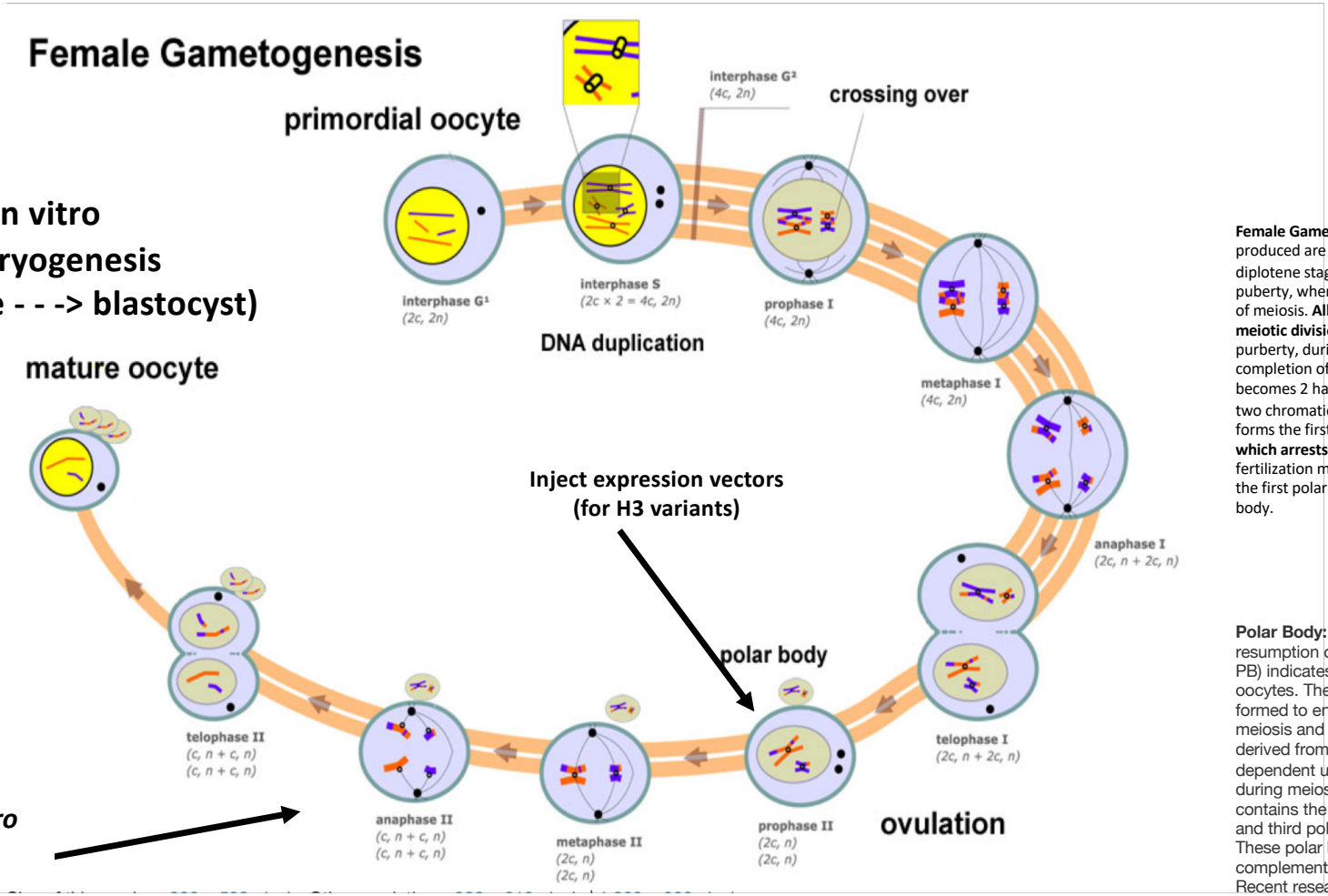
Blue: RNA Pol II active
Red: DNA

Brian E. Schwartz, and Kami Ahmad Genes Dev. 2005;19:804-814

Gene induction triggers histone replacement. Heat-shock induction triggers rapid puffing and transcription of the HSP70 genes at polytene bands 87A and 87C. Polytene chromosomes from larvae that contain a constitutively expressed H3-GFP construct (A–D,J,K), or a constitutively expressed H3.3core-GFP construct (E–I) were induced for the indicated times (in minutes) and stained with antibodies against **phosphorylated RNA polymerase II (blue**), which marks activated puffs. Arrows in A and E indicate the positions of the HSP70 loci at 87A and 87C before induction, while the puffs (B–D,F–H) are bracketed. Asterisks indicate chromosome bands that were used as internal standards for quantifying the summed intensities of histone-GFP signals in puffs. (B,C) **Puffs contain some H3 in the first 5 min of induction, but have much less by the time puffs reach their maximal size (D). (F–H) H3.3core-GFP in expanding puffs rapidly increases. (I) After 20 min of heat-shock induction, many active HSP puffs (arrows) have large amounts of H3.3core-GFP, while staining throughout the arms is undiminished.** (J,K) Histone H3 modifications that mark heat-shock puffs do not overlap with H3-GFP**. DNA is in red, and histone-GFP in green.**

Reprogramming of epigenetic marks by dynamic replacement of histone H3 variants

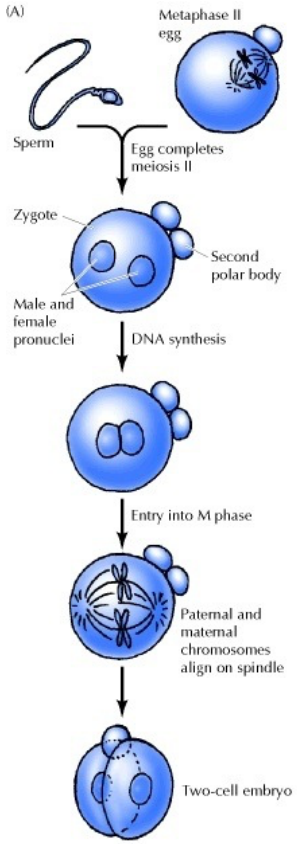


Female Gametogensis: In females, the total number of eggs ever to be produced are present in the newborn female initially arrested at the diplotene stage of the meiosis I from fetal life through childhood until puberty, when the lutenizing hormone (LH) surges stimulate the resumption of meiosis. **All eggs are arrested at an early stage (prophase I) of the first meiotic division as a primary oocyte (primordial follicle**). Following purberty, during each menstrual cycle, pituitary gonadotrophin stimulates completion of meiosis 1 the day before ovulation. In meiosis 1, a diploid cell becomes 2 haploid (23 chromosomes) daughter cells, each chromosome has two chromatids. One cell becomes the secondary oocyte the other cell forms the first polar **body.The secondary oocyte then commences meiosis 2 which arrests at metaphase and will not continue without fertilization**.At fertilization meiosis 2 completes, forming a second polar body. Note that the first polar body may also undergo this process forming a third polar body.

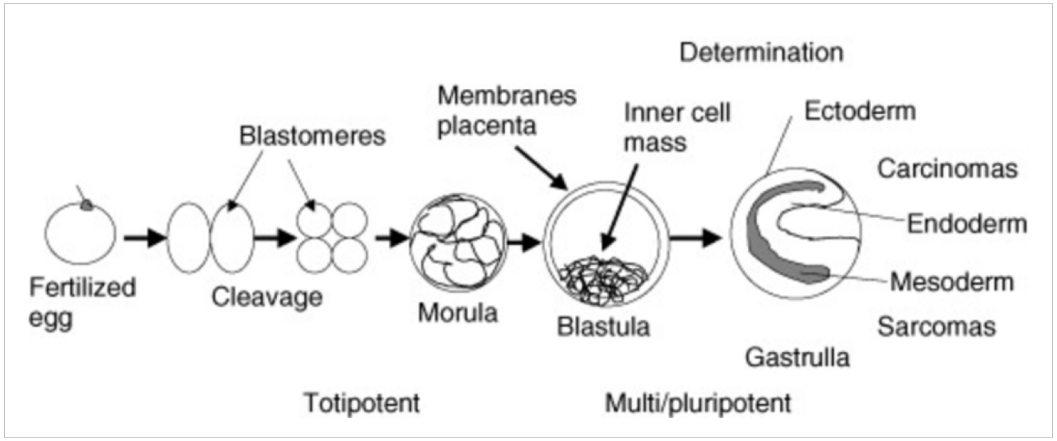
Polar Body: The breakdown of the germinal vesicle indicates a resumption of meiosis and the extrusion of the first polar body (1 PB) indicates completion of the first meiotic division in human oocytes. The polar body is a small cytoplasmic exclusion body formed to enclose the excess DNA formed during the oocyte (egg) meiosis and following sperm fertilization. There are 2-3 polar bodies derived from the oocyte present in the zygote, the number is dependent upon whether polar body 1 (the first polar body formed during meiosis 1) divides during meiosis 2. This exclusion body contains the excess DNA from the reductive division (the second and third polar bodies are formed from meiosis 2 at fertilization). These polar bodies do not contribute to the future genetic complement of the zygote, embryo or fetus.
Recent research in some species suggest that the space formed by the peripheral polar body (between the oocyte and the zona pellucia) can influence the site of spermatozoa fertilization.

https://embryology.med.unsw.edu.au/embryology/index.php/Oocyte_Development

Reprogramming of epigenetic marks by dynamic replacement of histone H3 variants

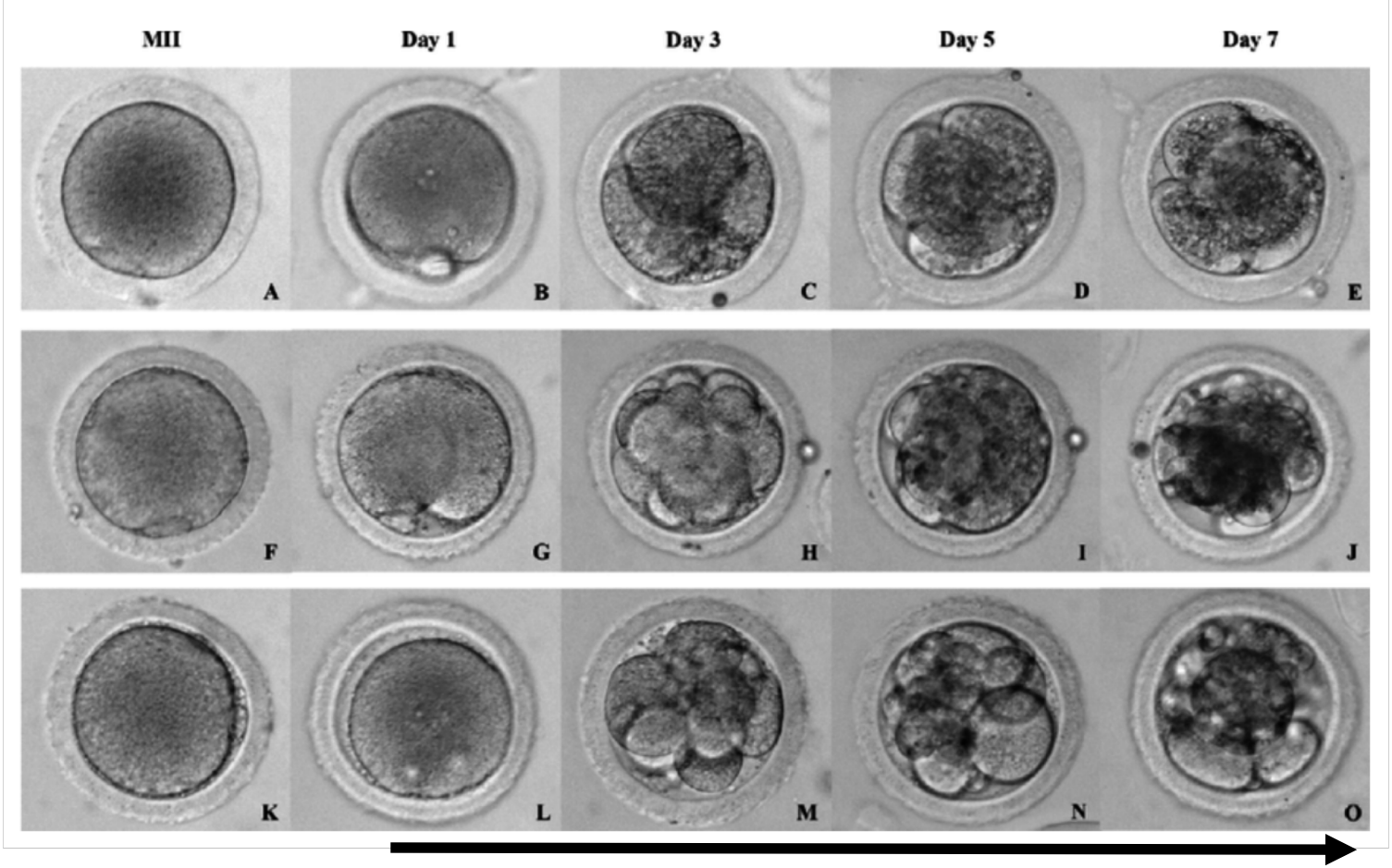


(A) Fertilization induces the transition from metaphase II to anaphase II, leading to completion of oocyte meiosis and emission of a second polar body (which usually degenerates). The sperm nucleus decondenses, so the fertilized egg (zygote) contains two haploid nuclei (male and female pronuclei). In mammals, the pronuclei replicate DNA as they migrate toward each other. They then initiate mitosis, with male and female chromosomes aligning on a common spindle. Completion of mitosis and cytokinesis thus gives rise to a two-cell embryo, with each cell containing a diploid genome.



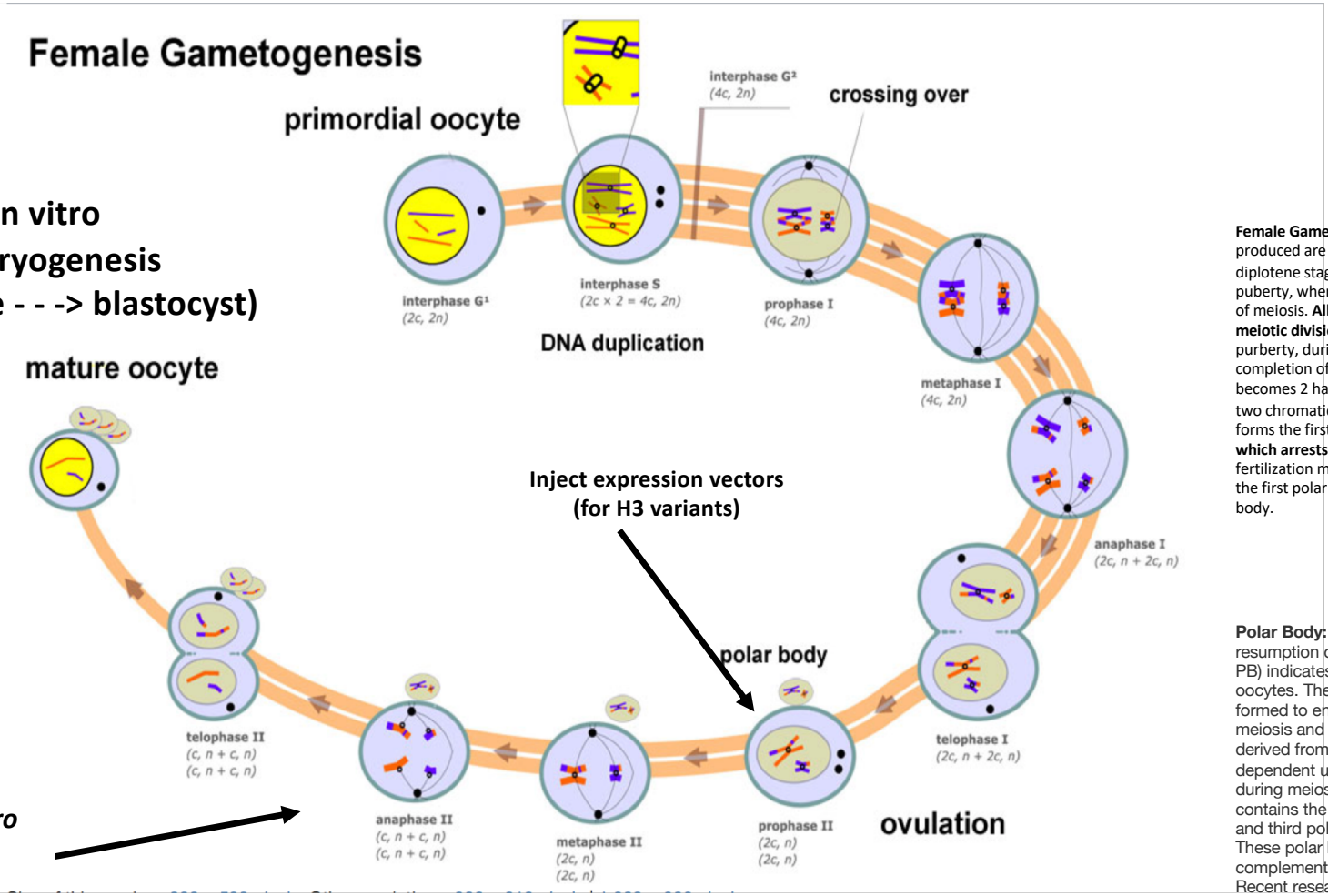
Early development of the embryo. Division of the fertilized egg results in the formation of daughter cells called blastomeres, which form a ball of cells called a morula. After six or so divisions, a cavity appears at one end of the morula to form the blastocyst. Until this stage, each of the cells divides evenly to produce two daughter cells, each of which retains the same characteristics, that is, they are totipotent. The inner cell mass of the blastula is the source of ESCs (see below). Invagination of one pole of the blastula leads to formation of the primitive germ cell layers, with formation of the gastrula. During gastrulation and later formation of the fetus, the daughter cells lose potential as they gain specialized function. This process is known as determination

Reprogramming of epigenetic marks by dynamic replacement of histone H3 variants



Representative pictures of MII oocytes prior to (A , F , and K) and after insemination using ICSI (Day 1: B , G , and L ; Day 3: C , H , and M ; Day 5: D , I , and N ; Day 7: E , J , and O). After insemination, the presence of two pronuclei (PN) and two polar bodies (PB) was checked to confirm fertilization (B , G , and L). Some embryos arrested at very early stages (e.g., four cell; D and E), whereas others continued cleavaging until the morula stage (J and O), when they arrested.

Reprogramming of epigenetic marks by dynamic replacement of histone H3 variants



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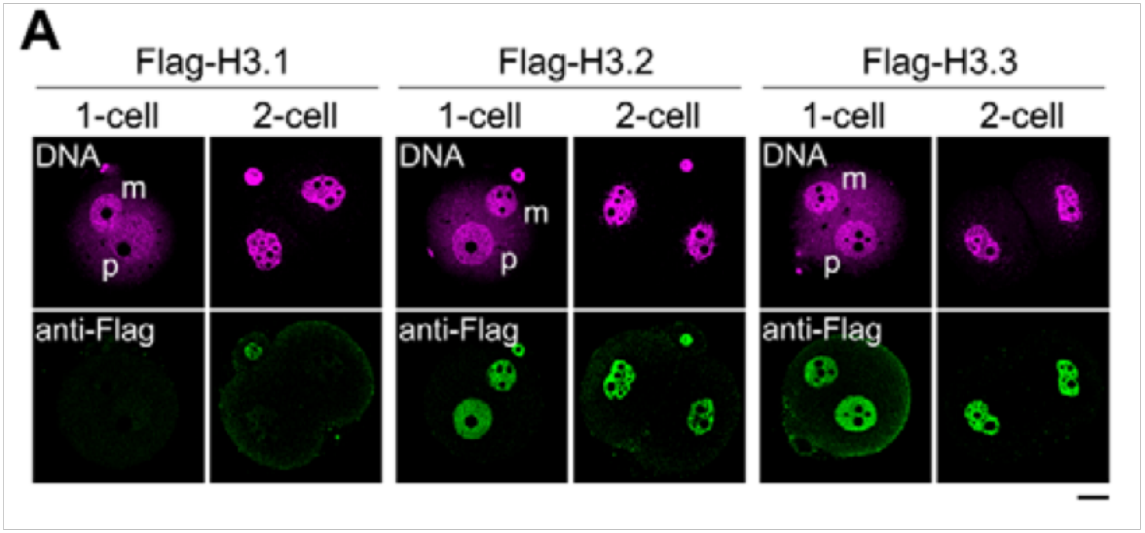
Reprogramming of epigenetic marks by dynamic replacement of histone H3 variants

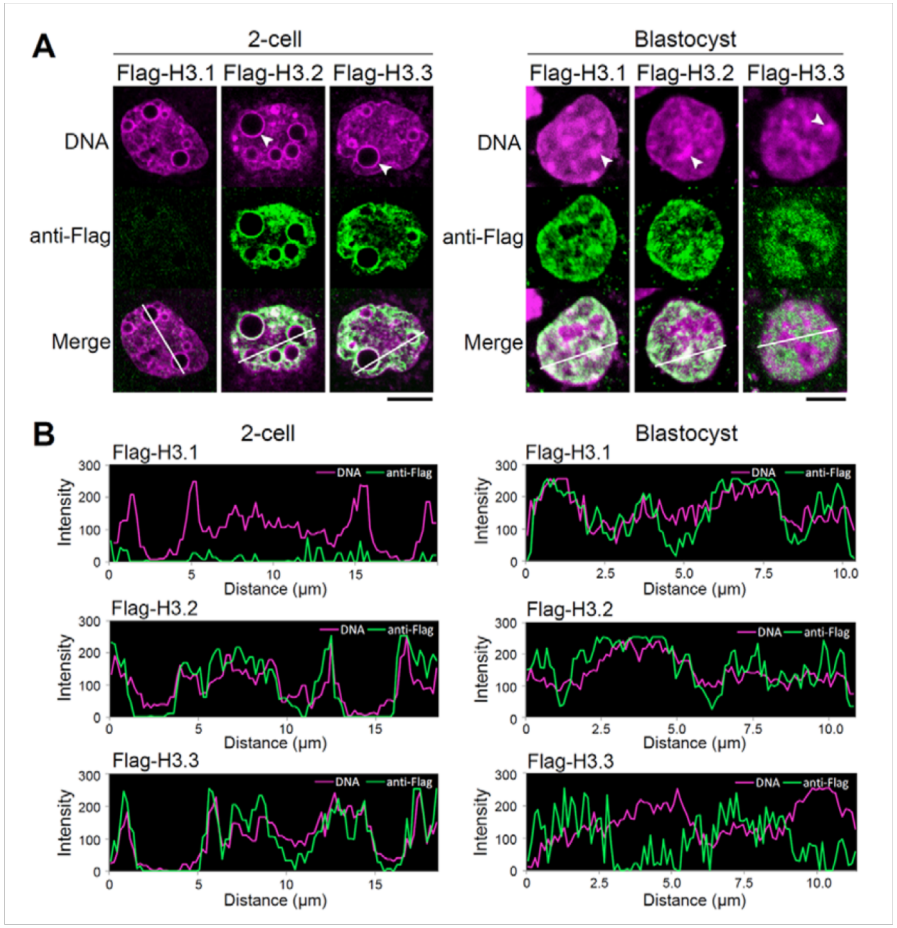


MII oocytes injected with flag-tagged H3.1 or H3.2 or H3.3 variants →
In vitro fertilization →
Allow 1-cell and 2 cell development→
Anti-flag IF

P: paternal pronucleus
M: maternal pronucleus

H3.2: canonical H3 inserted into pronuclei + 2 cell embryo
H3.3 RI histone: inserted into into pronuclei + 2 cell embryo

Reprogramming of epigenetic marks by dynamic replacement of histone H3 variants

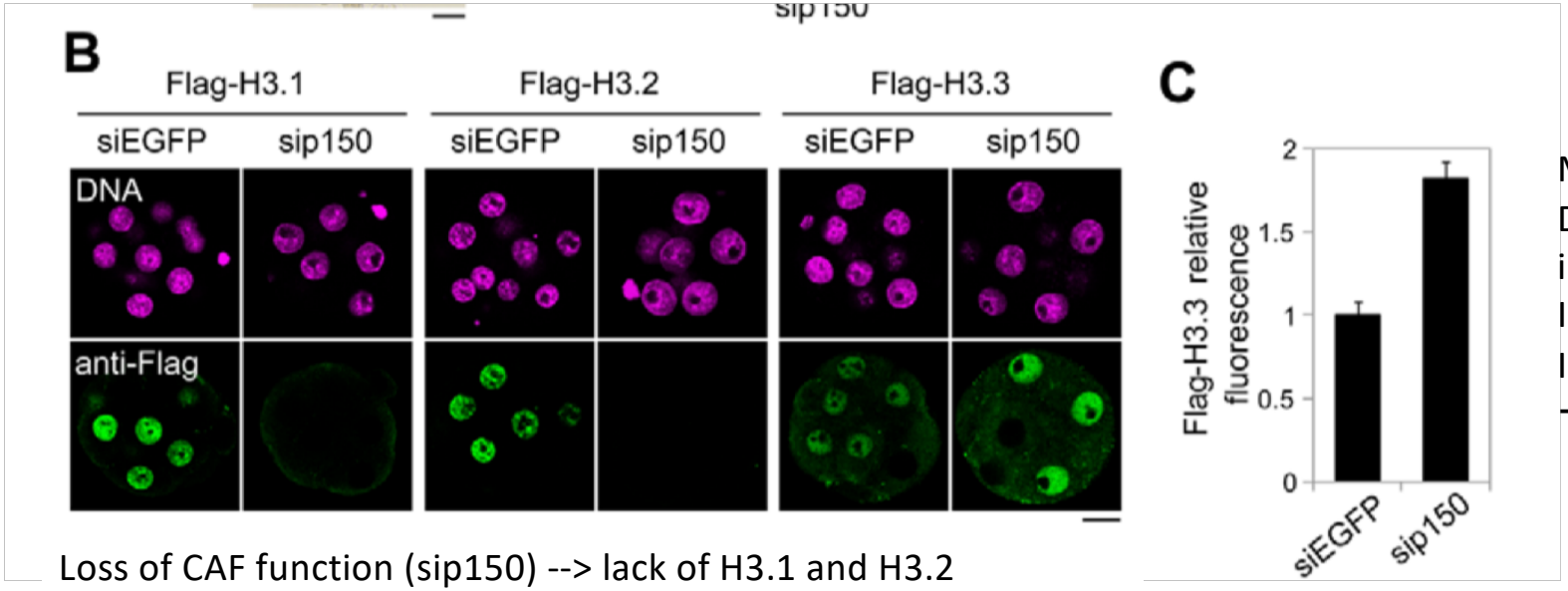


2 cell stage → blastocyst

H3.1: incorporation into blastocyst chromatin (eu+heterochromatin)
H3.2: incorporation in 2 cell and blastocyst chromatin (eu+heterochr.)
H3.3: incorporation in 2 cell embryos (hetero + euchromatin)
incorporation in blastocyst euchromatin (DNA not condensed)

→ ACTIVATION OF GENE EXPRESSION

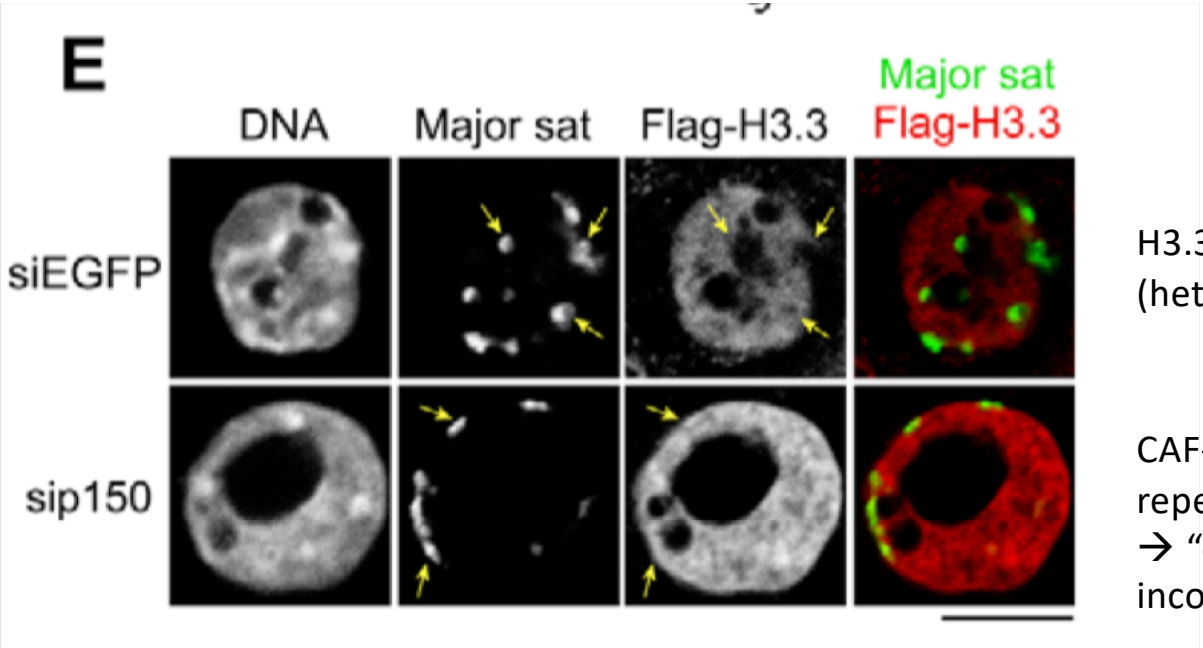
Reprogramming of epigenetic marks by dynamic replacement of histone H3 variants



Loss of CAF function (sip150) --> lack of H3.1 and H3.2 incorporation
H3.3 incorporation not affected (increases)
→ H3.3 is an RI histone

MII oocytes fertilized →
Develop to 1 cell stage →
injected with siRNAs →
Injection of Flag tagged variants
In 1 blastomere of 2 cell embryo
→ analysis of morula cells

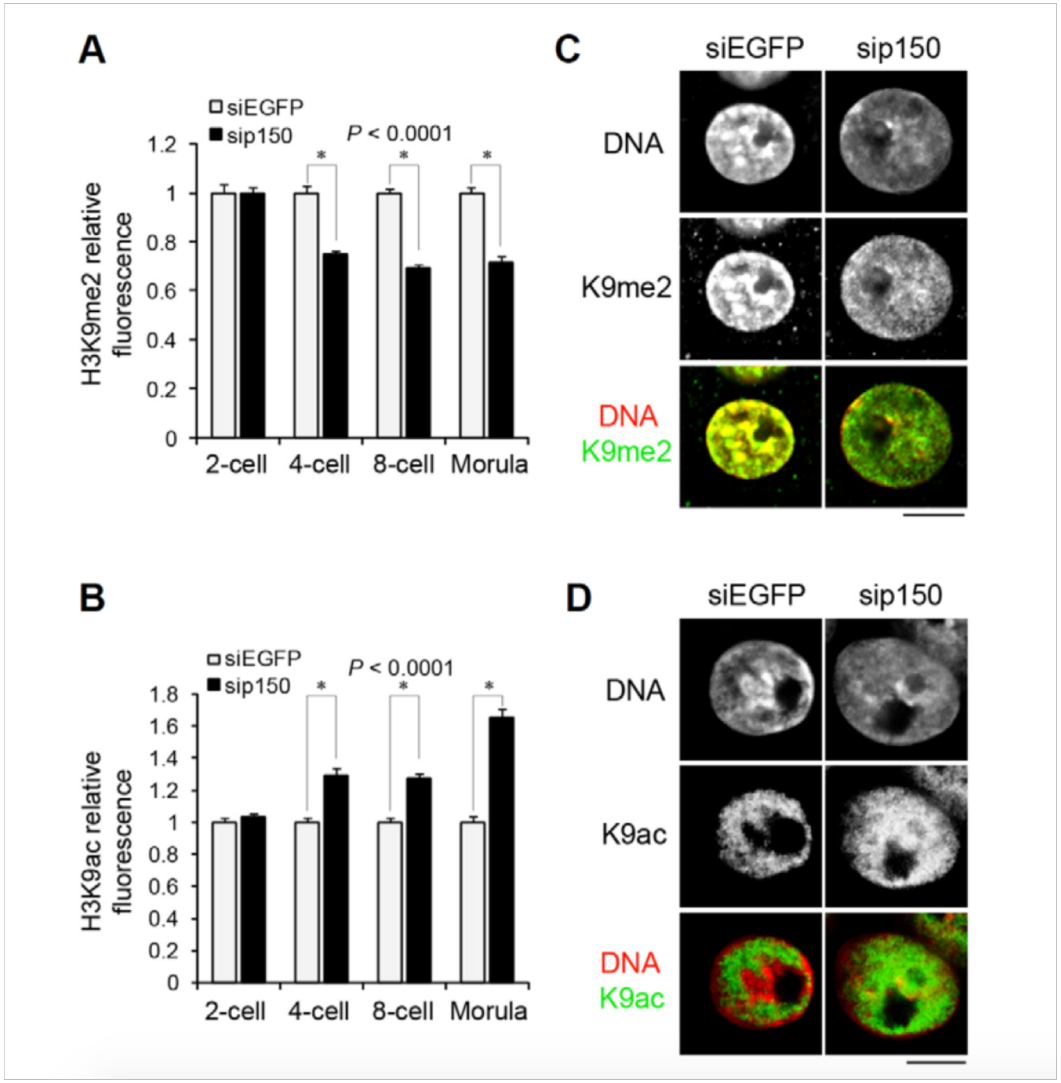
Reprogramming of epigenetic marks by dynamic replacement of histone H3 variants



H3.3 not asociated with major satallite repeats (heterochromatin)

CAF-1 LOF: H3.3 localizes with major satellite repeats
→ “competetition of CAF-1 with H3.3 incorporation (HIRA/DAXX)

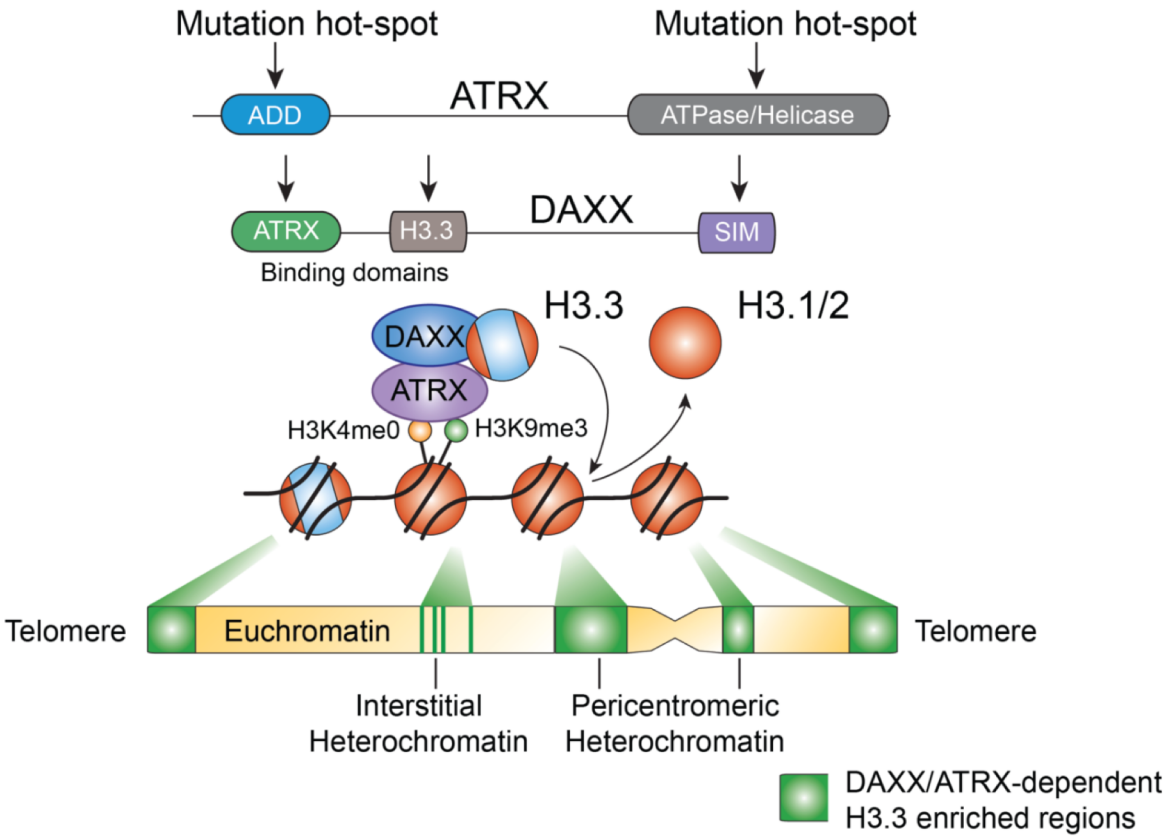
Reprogramming of epigenetic marks by dynamic replacement of histone H3 variants



CAF-1 LOF:
H3.3 incorporation into chromatin
→ Less H3K9me3
→ More H3K9Ac

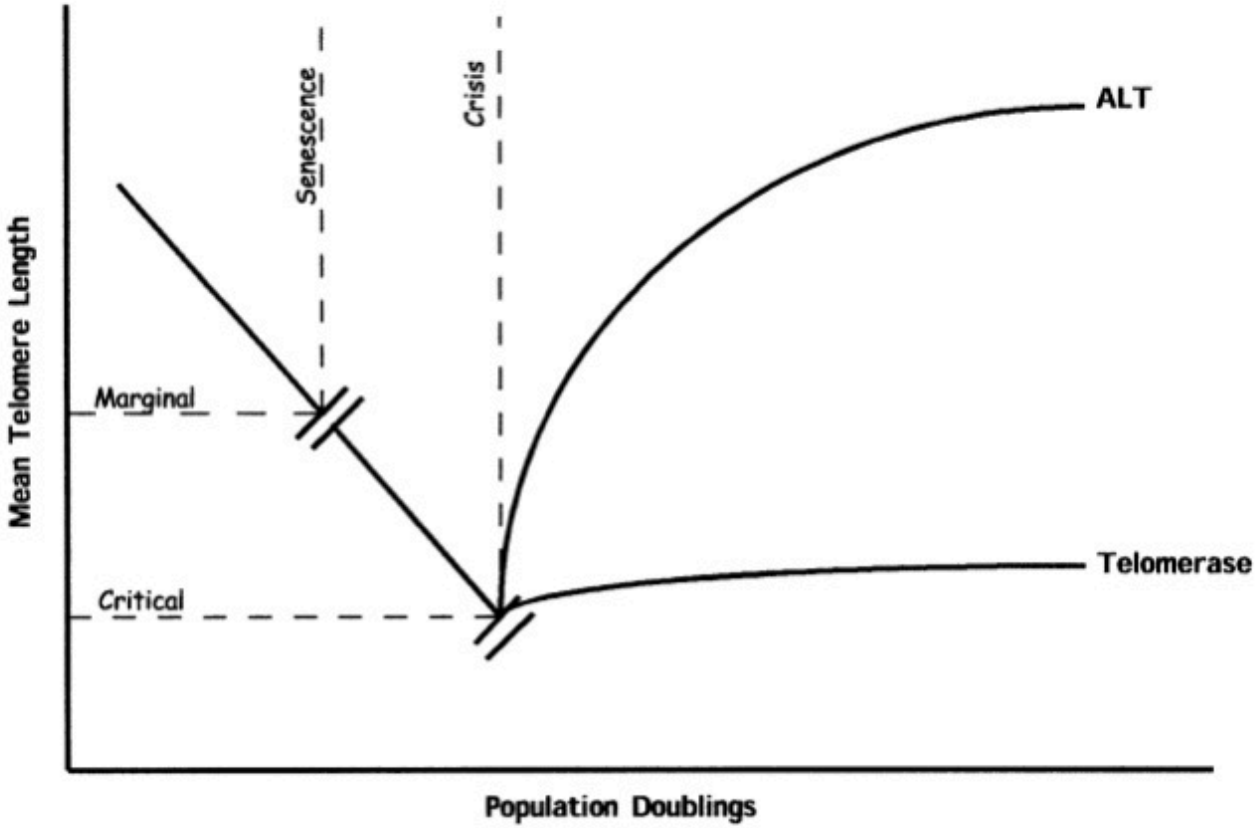
→ → **gene activation**

H3.3 is enriched at repeat elements contributing to genomic stability



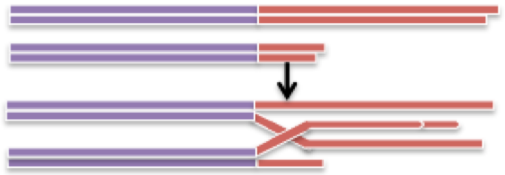
In addition to genes, recent studies have shown that H3.3 is enriched at repetitive regions such as **telomeres, pericentric repeats and endogenous retroelements**. Deposition at these regions is mediated by the **ATRX/DAXX complex**. This complex is mutated in many human cancers, with links to a process known as alternative lengthening of telomeres, or ALT.

ROLE OF H3.3 IN TUMORFORMATION



10% of tumors

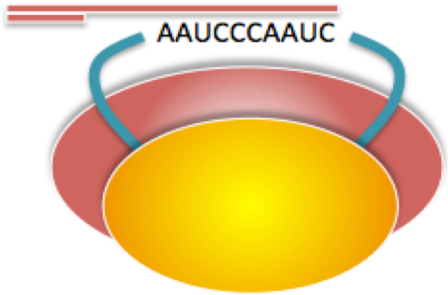
Alternative lengthening of Telomeres (ALT)



Homologous Recombination and Extension

90% of tumors

Telomerase



Reverse Transcription from Template

DAXX – ATRX insert H3.3 at repeat elements: at heterochromatic telomeres and centromeric repeats

Daxx histone chaperon complex

ATRX ATP nucleosome remodelling complex

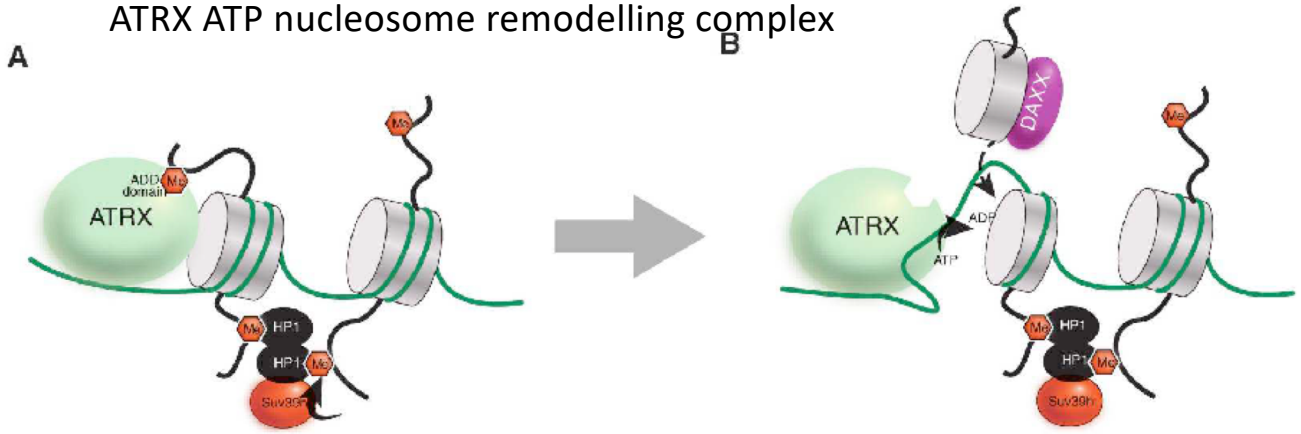


Figure 8. Model for maintenance of histone modifications by the concerted action of multiple chromatin regulators via RI replacement with H3.3. We address the question of how a histone modification can be inherited when a nucleosome is lost and replaced. (*A*) The Suv39h H3K9 methyltransferase (an ortholog of fly Su(var)3-9) is recruited by HP-1 protein, which binds specifically to methylated H3K9. To perpetuate this mark when the nucleosome turns over, we speculate that the ATRX ATPase is recruited to the site via its ATRX-DNMT3-DNMT3L (ADD) domain, which binds with high specificity to methylated H3K9 on tails that entirely lack H3K4 methylation (because there are no H3K4 methyltransferases in this region of the genome). (*B*) ATRX provides the energy of ATP and works together with the H3.3-specific DAXX histone chaperone complex to incorporate the new nucleosome (Goldberg et al. 2010), or half-nucleosome in the case of partial eviction (Xu et al. 2010). The high local concentration of Suv39h results in a new nucleosome with the same H3K9 methylation as the nucleosome that was lost.

H3.3 also enriched at tandem repeat containing sequences (i.e telomere or centromere, …)

ATRX binds to H3-tails that are H3K4me0 and H3K9me3

ATRX is enriched at telomeres and centromeric repeats

ATRX brings in DAXX → H3.3 RI assembly

There is transcription at telomeres an centromeres → nucleosome turnover

A model for DAXX-ATRX mediated suppression genomic instability: telomeres as an example

The MRN complex (MRX complex in yeast) is a protein complex consisting of Mre11, Rad50 and Nbs1 (also known as Nibrin in humans and as Xrs2 in yeast). In eukaryotes, the MRN/X complex plays an important role in the initial processing of double-strand DNA breaks prior to repair by homologous recombination or non-homologous end joining.

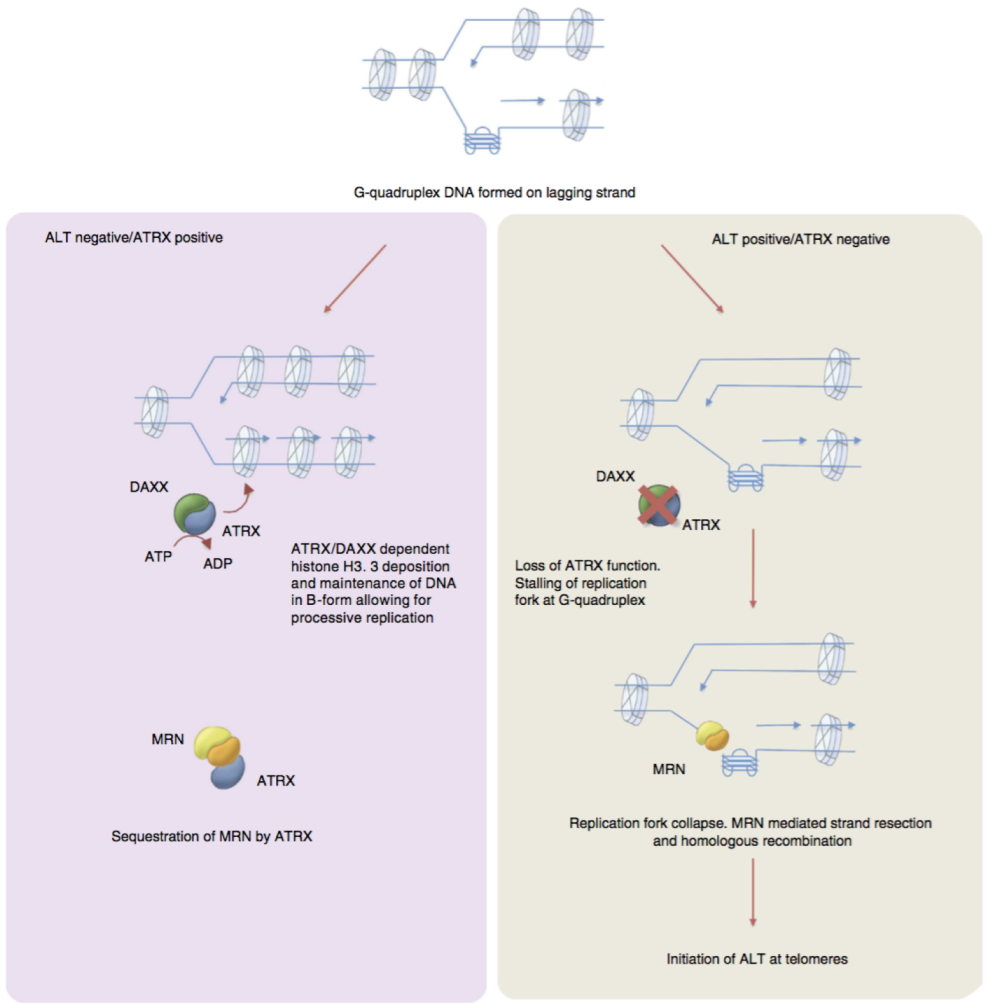
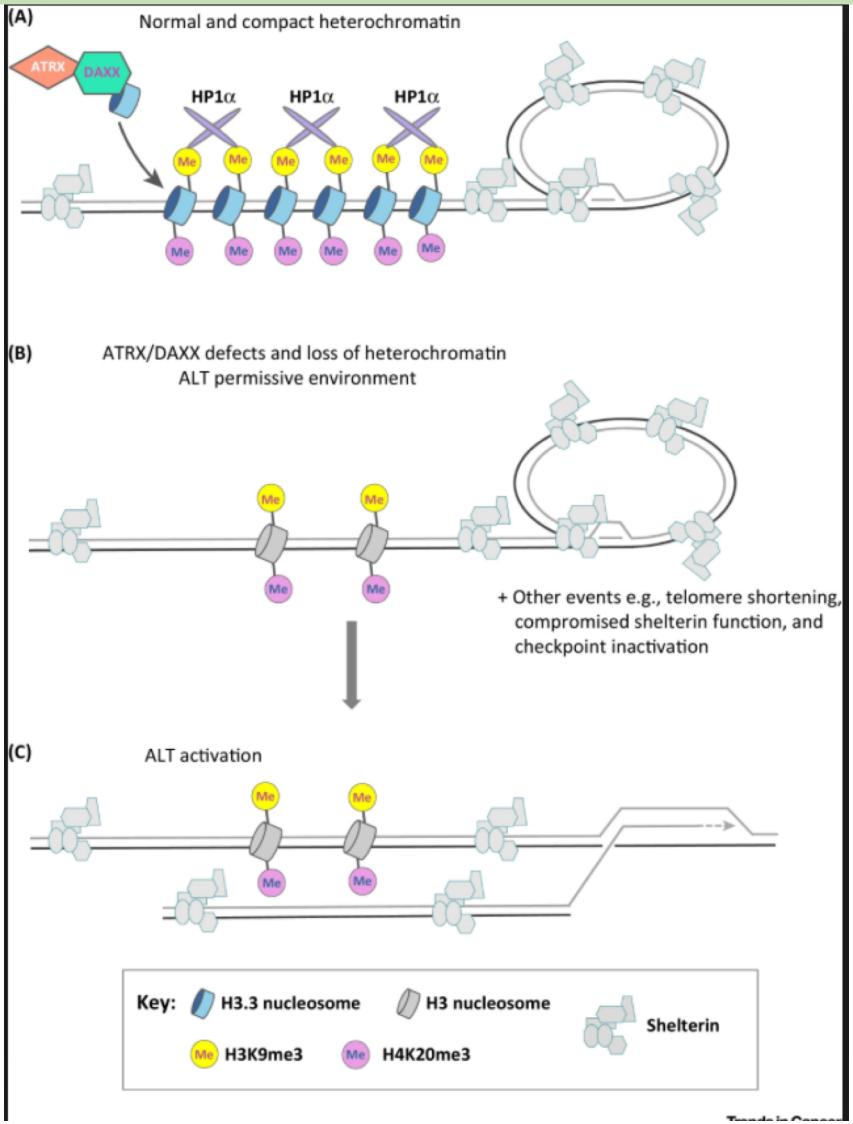


Figure 6 | Model for ATRX-mediated suppression of the ALT pathway. ATRX together with DAXX deposits histone H3.3 at telomeres, which in turn may facilitate DNA replication through G-quadruplex sequences. The presence of G-quadruplex structures in an ATRX null tumour cell leads to replication fork stalling and collapse, providing a substrate for MRN-dependent homologous recombination and maintenance of telomere length through ALT. ATRX additionally interacts with the MRN complex, facilitating its distribution away from PML bodies and telomeres, further limiting HR.

A model for DAXX-ATRX mediated suppression genomic instability: telomeres as an example



Loss of ATRX/DAXX function → lack of H3.3 incorporation → abnormal chromatin → breaks → induction of homologous recombination

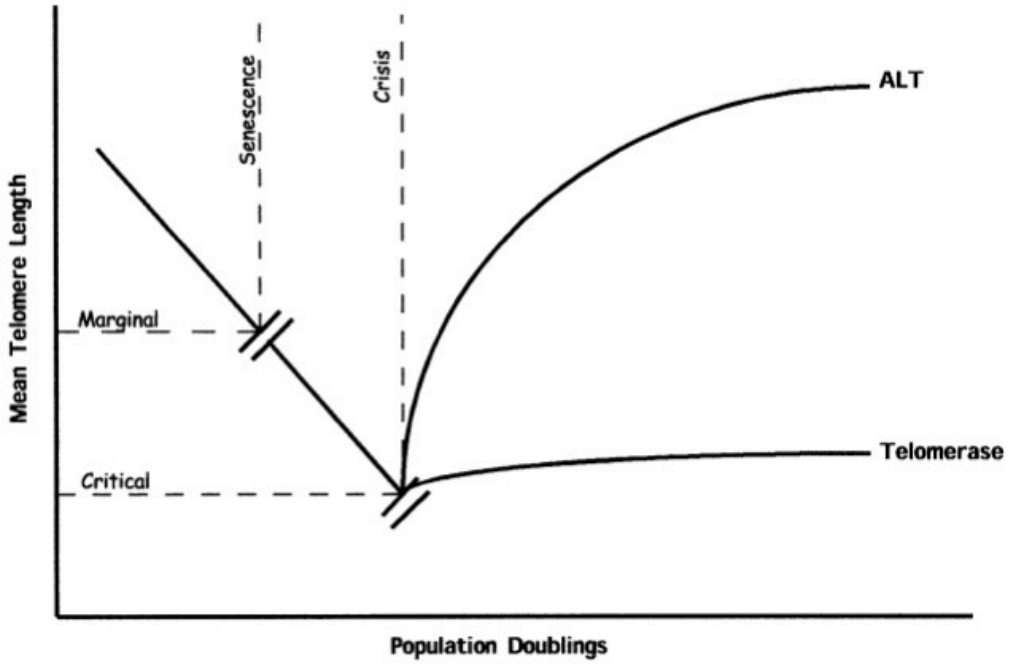
= ALT (alternative lengthening of telomeres)

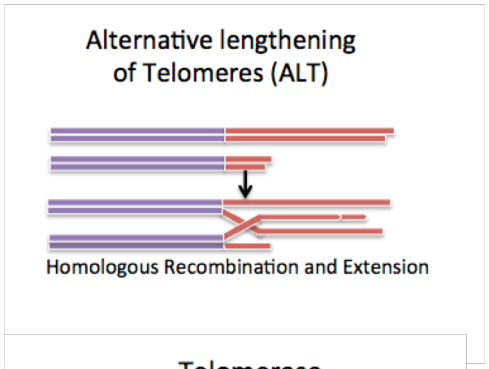
ROLE OF H3.3 IN TUMORFORMATION

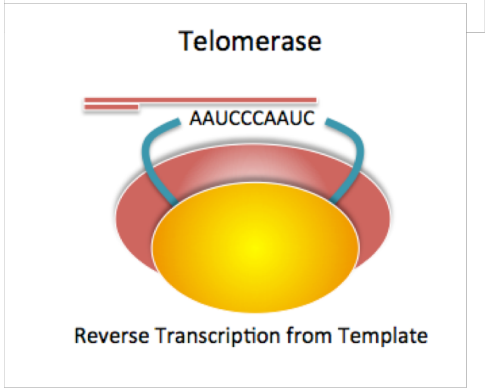
PanNETs: human pancreatic neuroendocrine tumors: 40% have loss of function mutations in ATRX or Daxx

Loss of ATRX or Daxx is associated with the induction of recombination at telomeres
→ telomerase independent telomere elongation

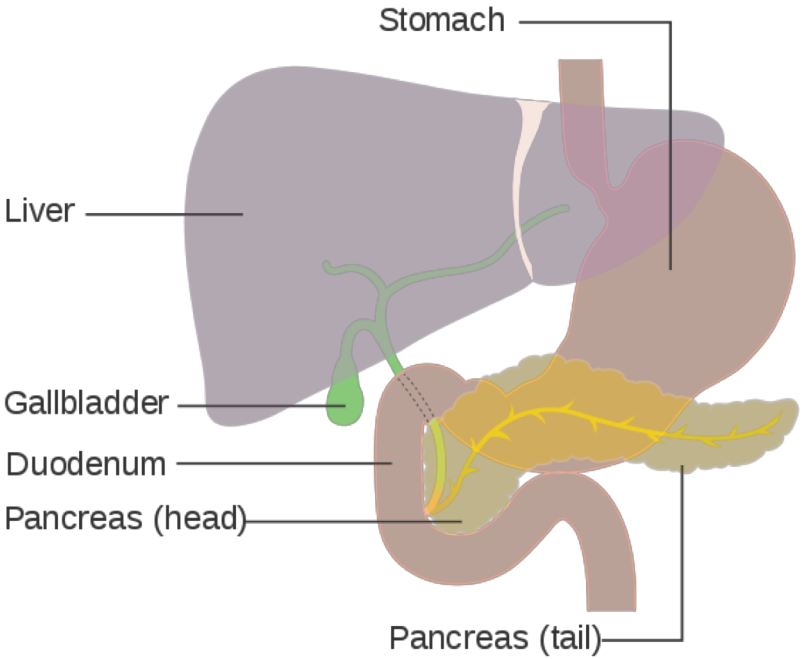
Pediatric glioblastoma; Mutations in H3.3 G34R/V associated with ATRX loss → tumor formation







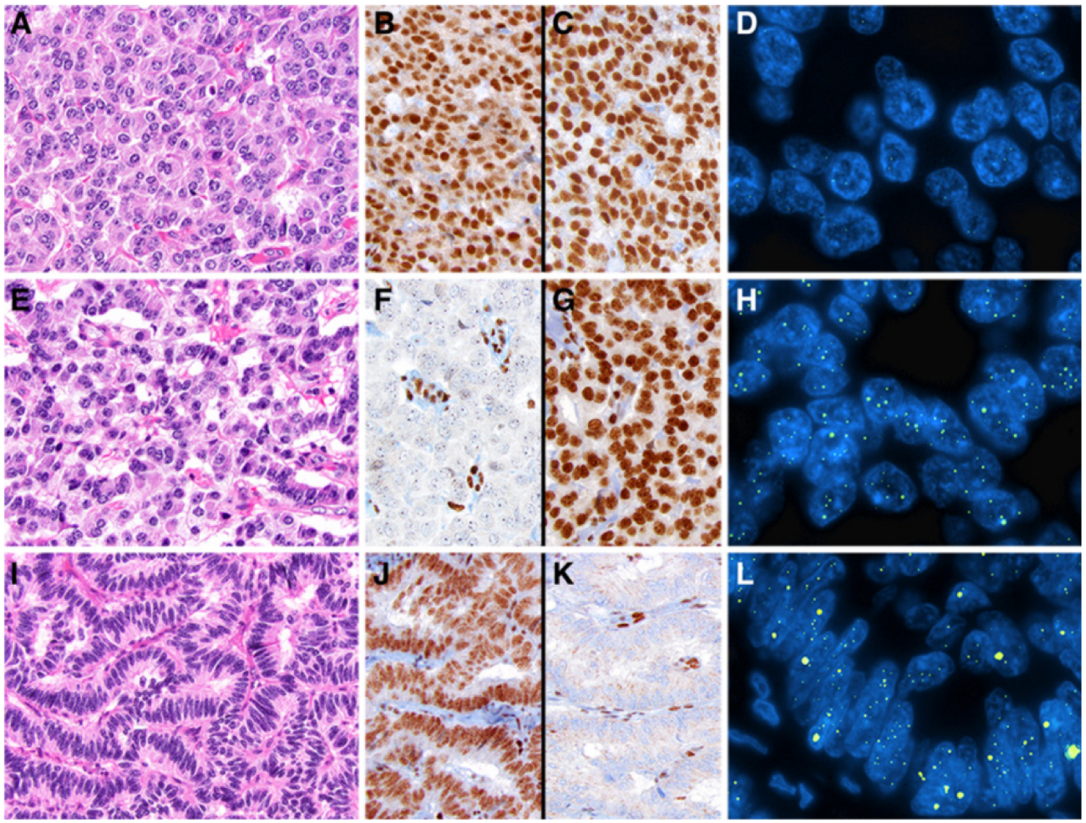
ROLE OF ATRX/DAXX AND H3.3 IN Pancreatic neuroendocrine tumors (PanNETs)



Pancreatic neuroendocrine tumors (PanNETs, PETs, or PNETs), often referred to as "islet cell tumors", or "pancreatic endocrine tumors" are neuroendocrine neoplasms that arise from cells of the endocrine (hormonal) and nervous system within the pancreas. PanNETs are a type of neuroendocrine tumor, representing about one third of gastroenteropancreatic neuroendocrine tumors (GEP-NETs). Many PanNETs are benign, while some are malignant. Aggressive PanNET tumors have traditionally been termed "islet cell carcinoma".PanNETs are quite distinct from the usual form of pancreatic cancer, the majority of which are adenocarcinomas, which arises in the exocrine pancreas. Only 1 or 2% of clinically significant pancreas neoplasms are PanNETs.

ROLE OF ATRX/DAXX AND H3.3 IN PanNETs

Hematoxylin and eosin stain | DAXX | ATRX | TTAGGG DNA-FISH Telomeres



DAXX+/ATRX+

DAXX-/ATRX+ ALT

DAXX+/ATRX- ALT

Figure 1.
Representative examples of PanNETs assessed by DAXX and ATRX IHC and telomere-specific FISH. **A,** PanNET with preserved nuclear expression for both DAXX (**B**) and ATRX (**C**) and absence of the ALT phenotype (**D**). **E,** PanNET with DAXX loss (**F**), but preserved expression for ATRX (**G**). The loss of DAXX expression correlated with the presence of large, ultrabright intranuclear foci by telomere-specific FISH, consistent with ALT (**H**). **I,** PanNET with preserved expression for DAXX (**J**), but ATRX loss (**K**) and ALT positive (**L**) by telomere-specific FISH.

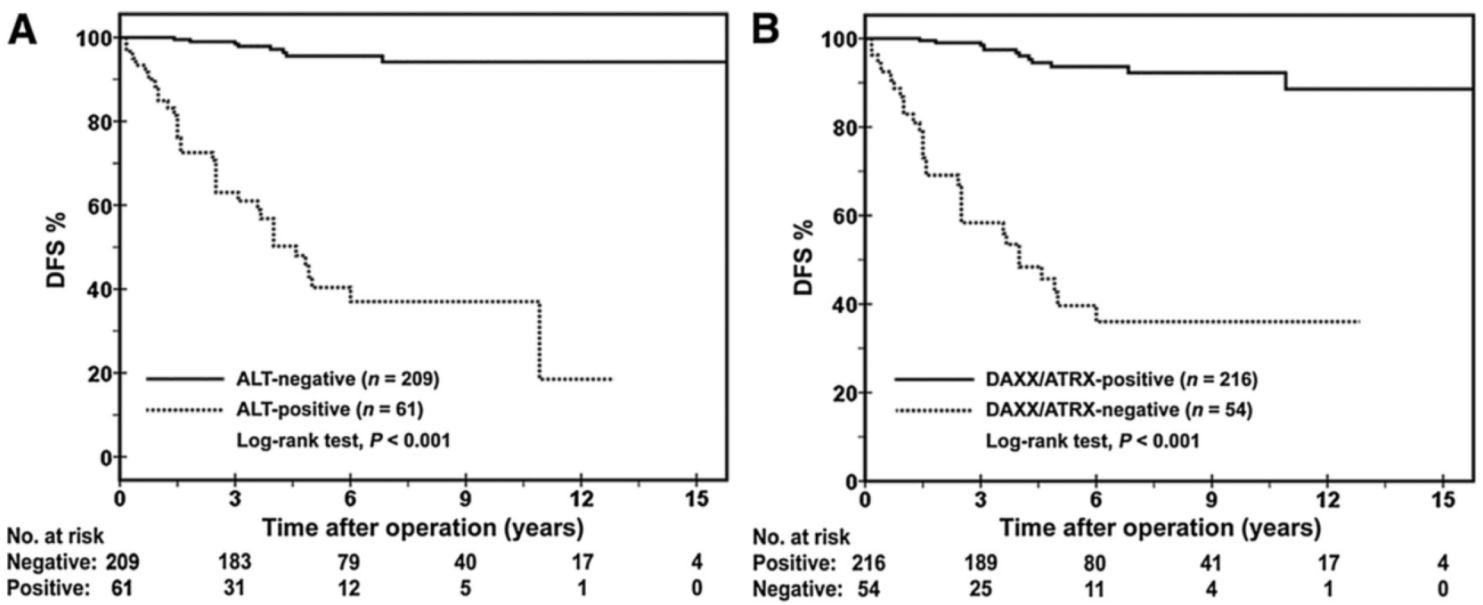
ROLE OF ATRX/DAXX AND H3.3 IN PanNETs

Table 1. Clinical and pathologic comparison of ALT and DAXX/ATRX status in PanNETs

Patient or tumor characteristics	ALT-positive, *n* = 98 (31%)	ALT-negative, *n* = 223 (69%)	*P*	DAXX/ATRX-negative, *n* = 84 (26%)	DAXX/ATRX-positive, *n* = 237 (74%)	*P*
Gender						
Female	35 (35%)	108 (51%)	0.011[a]	29 (35%)	121 (51%)	0.011[a]
Male	63 (65%)	115 (49%)		55 (65%)	116 (49%)	
Mean age (range), years	60.5 (31–85)	58.6 (29–83)	0.195	61.4 (31–85)	58.4 (29–83)	0.050
Mean tumor size (range), cm	5.0 (1.0–15.0)	2.8 (0.6–18.0)	<0.001[a]	5.0 (1.0–15.0)	2.8 (0.6–18.0)	<0.001[a]
Functional						
No	95 (97%)	190 (85%)	0.002[a]	81 (96%)	204 (86%)	0.008[a]
Yes	3 (3%)	33 (15%)		3 (4%)	33 (14%)	
Location						
Head and uncinate	37 (38%)	90 (40%)	0.300	29 (35%)	98 (41%)	0.300
Body and tail	61 (62%)	133 (60%)		55 (65%)	139 (59%)	
WHO grade						
Low (G1)	28 (29%)	157 (70%)	<0.001[a]	25 (30%)	160 (68%)	<0.001[a]
Intermediate (G2)	66 (67%)	66 (30%)		56 (66%)	76 (32%)	
High (G3)	4 (4%)	0 (0%)		3 (4%)	1	
Lymphovascular invasion						
Absent	22 (22%)	163 (73%)	<0.001[a]	20 (24%)	165 (70%)	<0.001[a]
Present	76 (78%)	60 (27%)		64 (76%)	72 (30%)	
Perineural invasion						
Absent	55 (56%)	185 (83%)	<0.001[a]	35 (42%)	191 (81%)	<0.001[a]
Present	43 (44%)	38 (17%)		49 (58%)	46 (19%)	
Primary tumor (pT) stage						
T1	6 (6%)	110 (49%)	<0.001[a]	6 (7%)	110 (46%)	<0.001[a]
T2	28 (29%)	71 (32%)		26 (31%)	73 (31%)	
T3	64 (65%)	42 (19%)		52 (62%)	54 (23%)	
Regional node (pN) stage	*n* = 96	*n* = 172		*n* = 83	*n* = 185	
N0	39 (41%)	129 (75%)	<0.001[a]	32 (39%)	136 (74%)	<0.001[a]
N1	57 (59%)	43 (25%)		51 (61%)	49 (26%)	
Synchronous metastases						
Absent	61 (62%)	209 (94%)	<0.001[a]	54 (64%)	216 (91%)	<0.001[a]
Present	37 (38%)	14 (6%)		30 (36%)	21 (9%)	
Metachronous metastases	*n* = 61	*n* = 209		*n* = 54	*n* = 216	
Absent	28 (46%)	200 (96%)	<0.001[a]	25 (46%)	203 (94%)	<0.001[a]
Present	33 (54%)	9 (4%)		29 (54%)	13 (6%)	
ALT						
Negative				0 (0%)	223 (94%)	<0.001[a]
Positive				84 (100%)	14 (6%)	

[a]Indicates that the value in question is statistically significantly better than the relevant control, where significance is defined by $P < 0.05$.

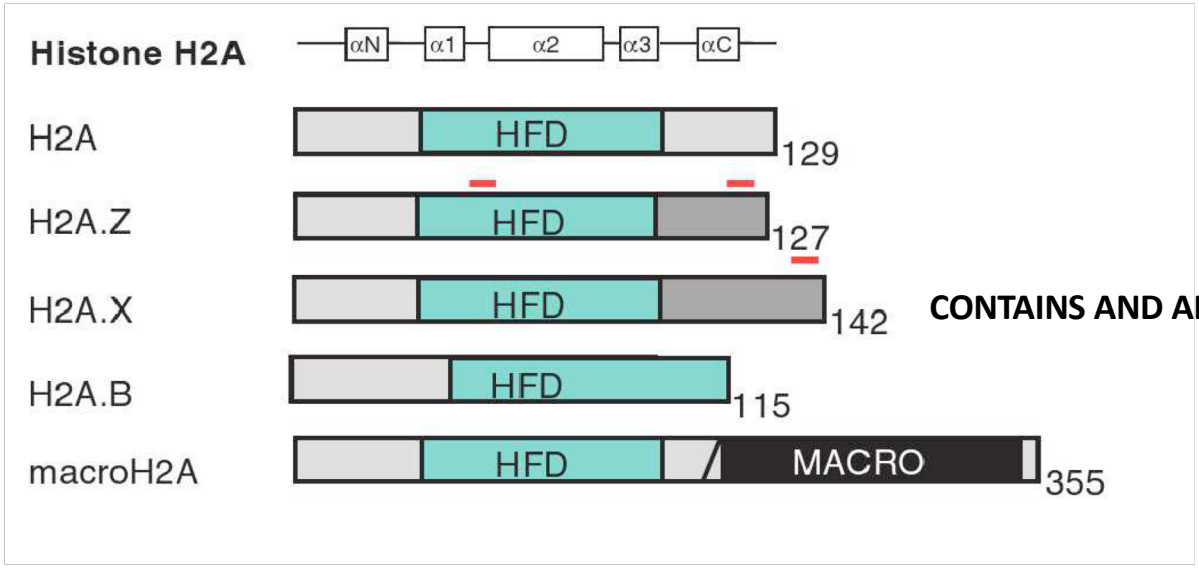
ALT and LOSS OF DAXX/ATRX mediates PanNET aggressivenss



disease-free survival
In cancer, the length of time after primary treatment for a cancer ends that the patient survives without any signs or symptoms of that cancer. In a clinical trial, measuring the disease-free survival is one way to see how well a new treatment works. Also called DFS, relapse-free survival, and RFS.

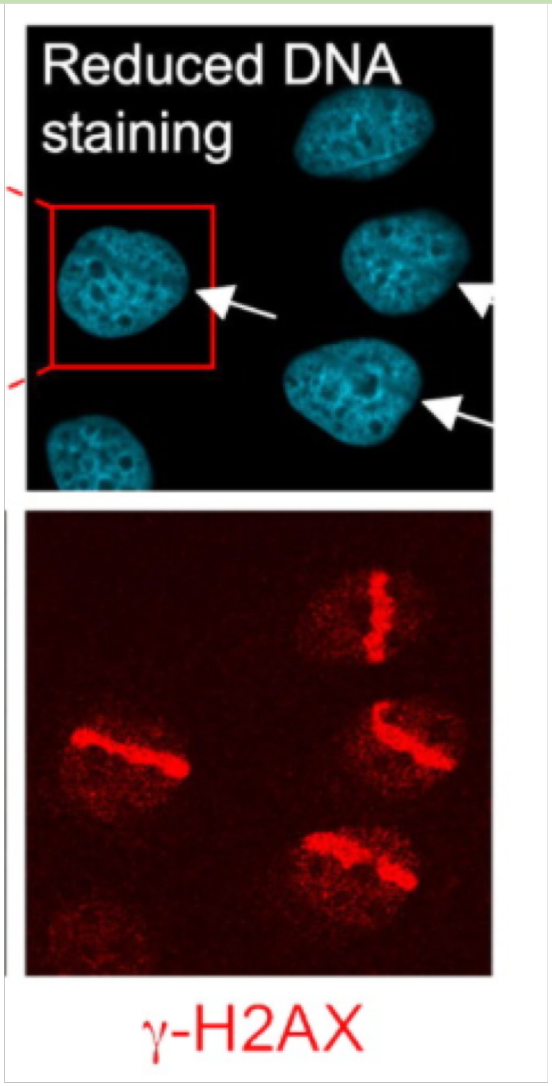
ROLE OF H2A.X IN DNA DAMAGE RESPONSE

HISTONE H2A VARIANTS



CONTAINS AND ADDITIONAL COARBOXY TERMINAL SEQUENCE MOTIF

Epigenetics and Histone Variants meet to organize DNA DAMAGE RESPONSE



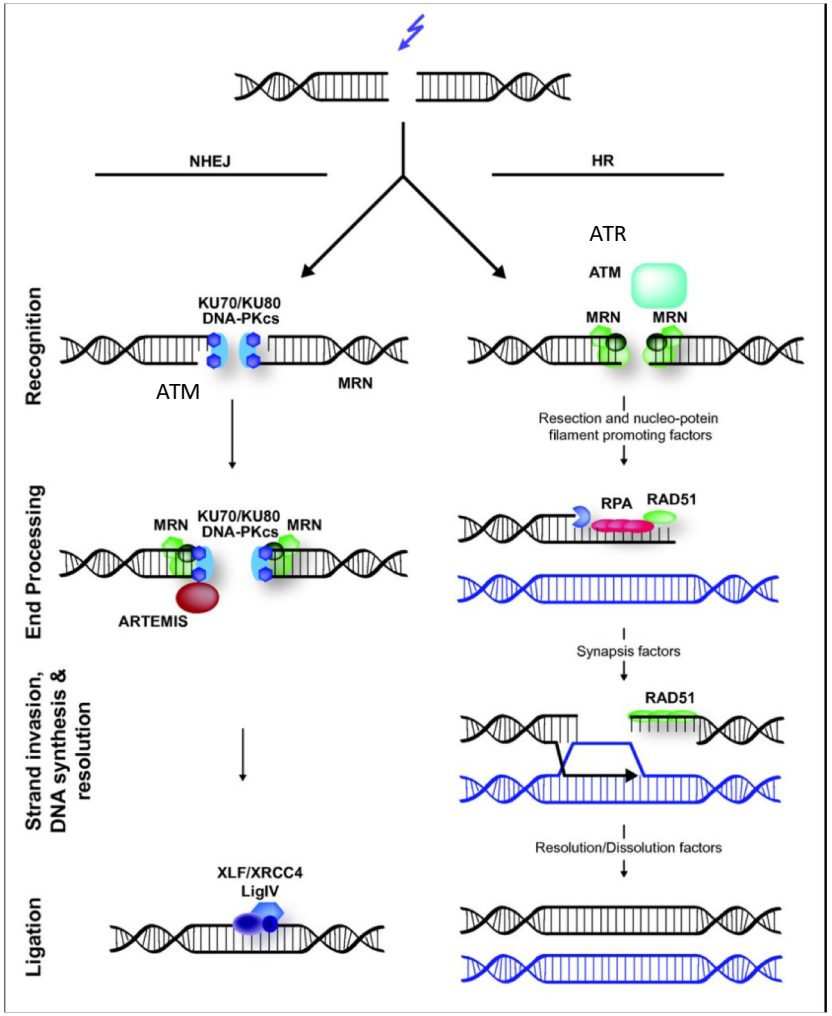
Cells cut with laser →
DNA breaks across nucleus →
phosphorylation of H2AX at Ser 139 = γH2AX

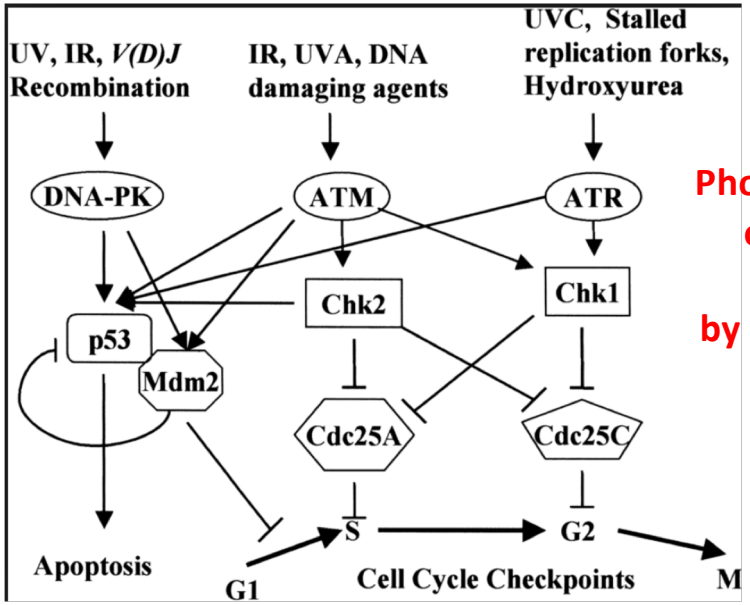
ACTIVATION OF DNA DAMAGE RESPONSE

H2AX is distributed throughout the genome. DNA damage -> phosphorylation of H2AX by ATM/ATR/DNA PK that spreads max 1 Mb from DNA damage site

=DNA DAMGE FOCI

Epigenetics and Histone Variants meet to organize DNA DAMAGE RESPONSE





Phosphorylation of H2AX at Ser139 by DNA-PK, ATR or ATM

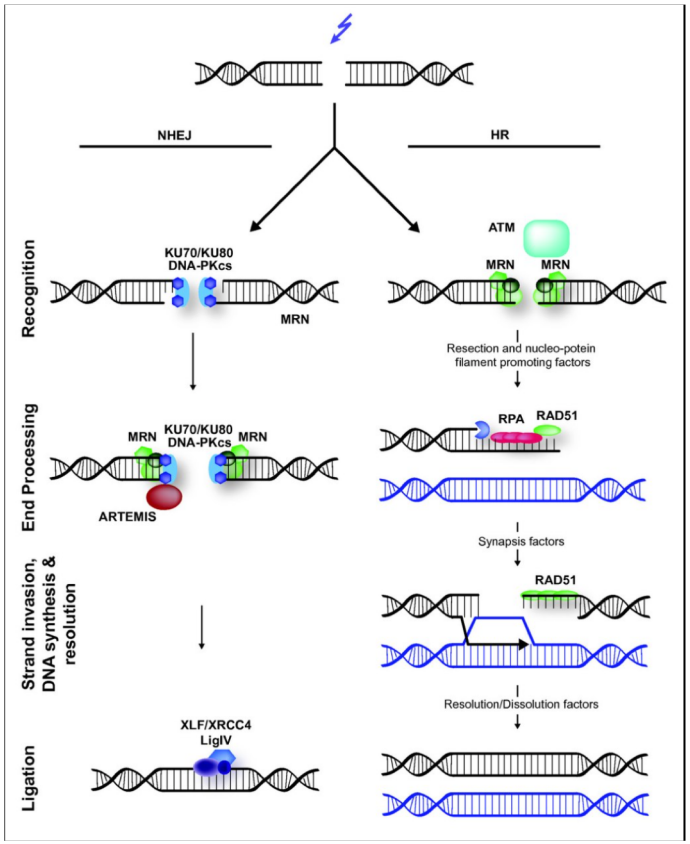
NHEJ NHEJ (HR) HR

DNA double-strand breaks (DSBs) are cytotoxic damages that can be repaired either by the homologous recombinational repair (HR) pathway or by the non-homologous end-joining (NHEJ) pathway. NHEJ, although faster than HR, is less accurate. The early divergent step between the two pathways is end resection, and this step is regulated by numerous factors. In particular, BRCA1 and 53BP1 play a role in determining the balance between the two pathways. 53BP1 restricts resection and promotes NHEJ.

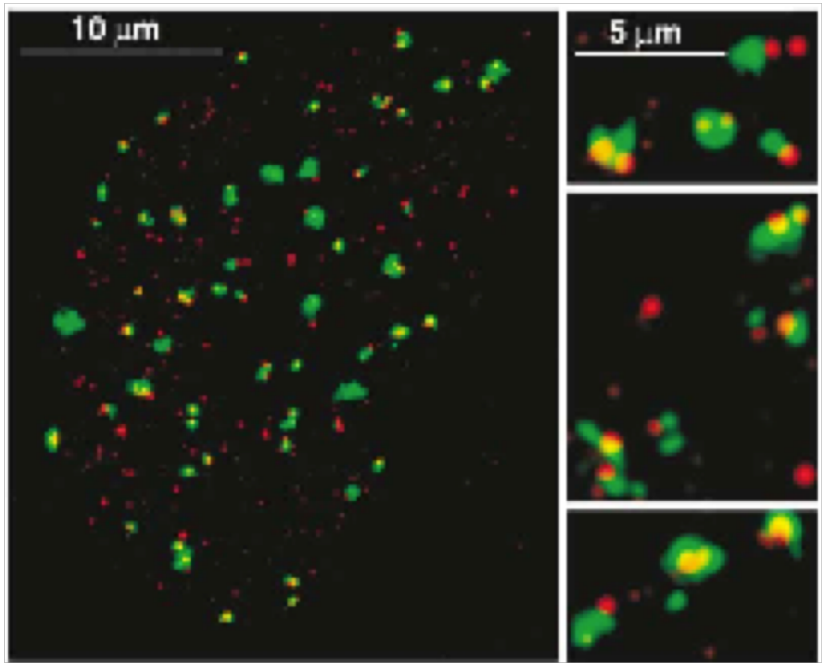
The MRN complex binds avidly to double-strand breaks both in vitro and in vivo and may serve to tether broken ends prior to repair by non-homologous end joining or to initiate resection prior to repair by homologous recombination. Recruits ATM. The MRN complex also participates in activating the checkpoint kinase ATM in response to DNA damage.

Epigenetics and Histone Variants meet to organize DNA DAMAGE RESPONSE

DNA Damage → Exposure of DNA breaks → activation of PI3K family kinases (ATR/ATM/DNA PK) → phosphorylation cascade



DNA Damage Foci in genomic DNA of cell nucleus



γH2AX

53BP1

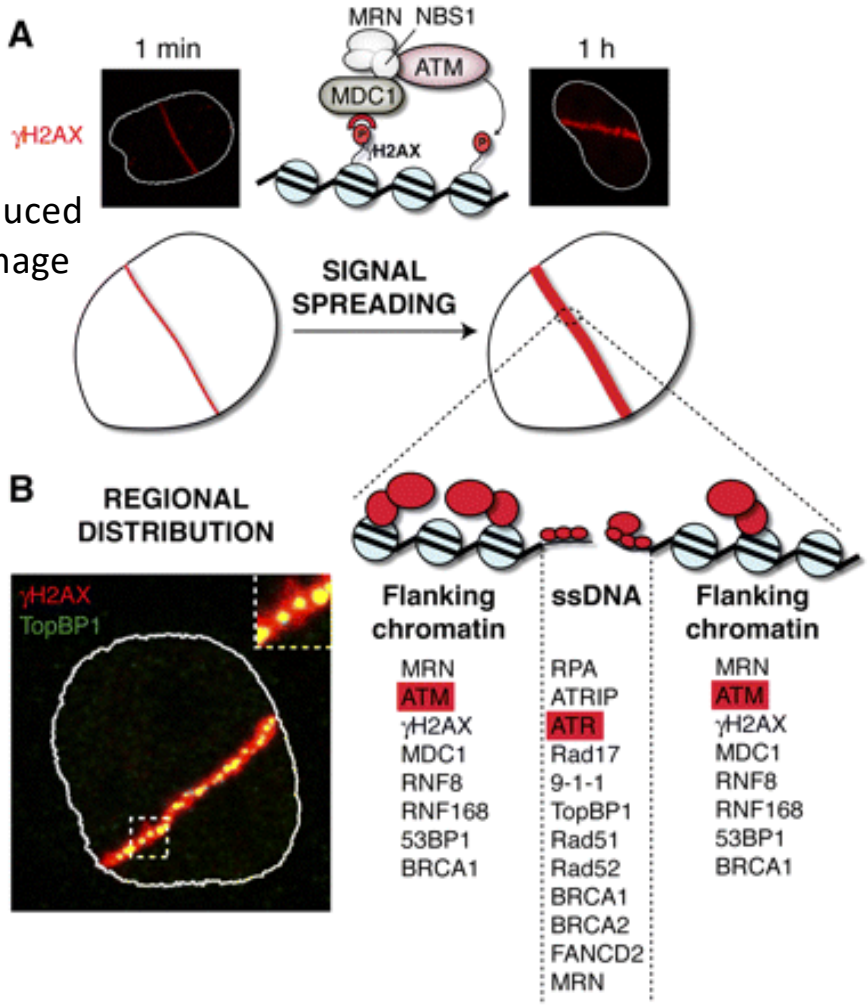
MRE11

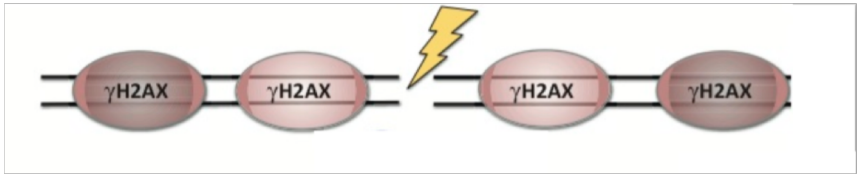
P-ATM

Histone H2AX is a substrate of several phosphoinositide 3-kinase-related protein kinases (PIKKs), such as ATM (ataxia teleangiectasia mutated), ATR (ATM and Rad3-related), or DNA-dependent protein kinase (DNA-PK).
Serves as a platform to recruit DNA damage response factors

ROLE OF H2A.X IN DNA DAMAGE RESPONSE

Laser induced DNA damage

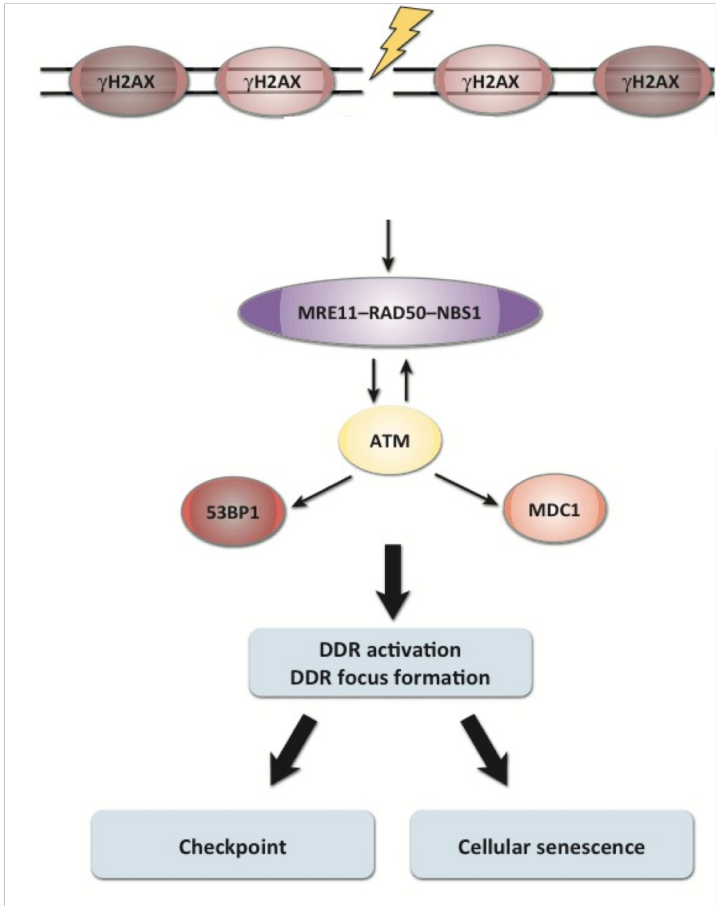


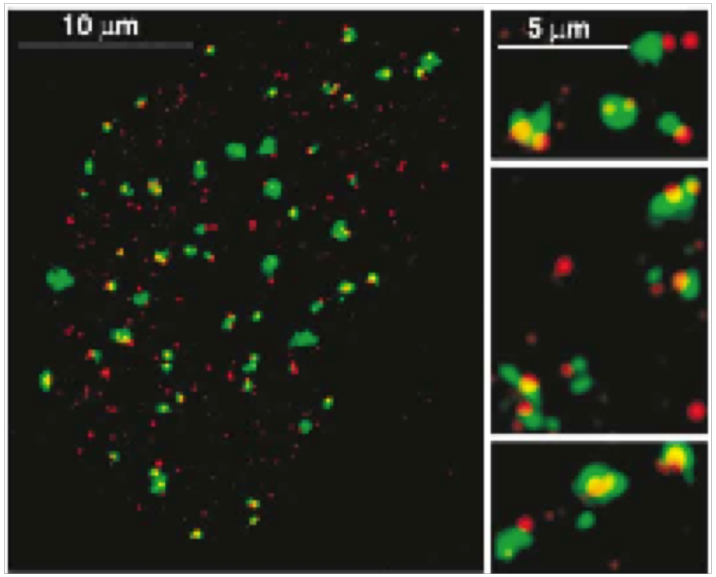


Spatial organization of DDR protein accumulation at DNA DSBs. (*A*) DDR signal spreading. DDR proteins initially accumulate at DSB sites and then spread at distance via a positive feedback loop involving MDC1, which binds γH2AX, the MRN complex, and ATM kinase, which phosphorylates additional H2AX molecules further away from the break site.

(*B*) Regional distribution of DDR proteins around DSBs. Factors involved in ATR signaling accumulate proximal to the break site on ssDNA generated by DNA end resection, while ATM signaling factors localize on flanking chromatin regions.

ROLE OF H2A.X IN DNA DAMAGE RESPONSE





γH2AX

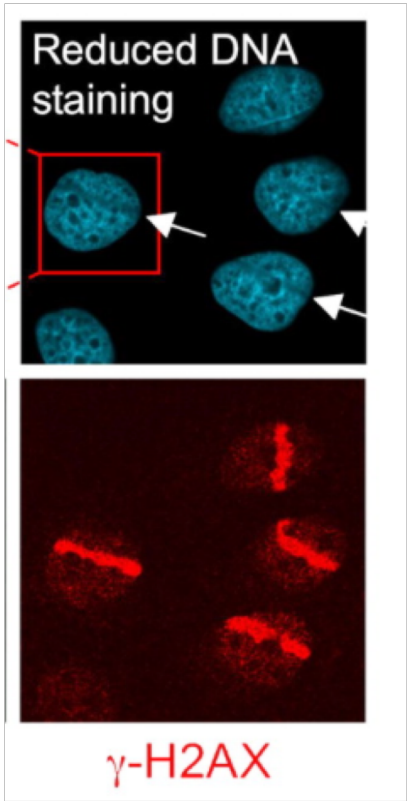
53BP1

MRE11

P-ATM

Epigenetics and Histone Variants meet to organize DNA DAMAGE RESPONSE

Several lines of evidence suggest the critical role of H2AX phosphorylation at DSB sites for nuclear foci formation and induction of DSB repair.



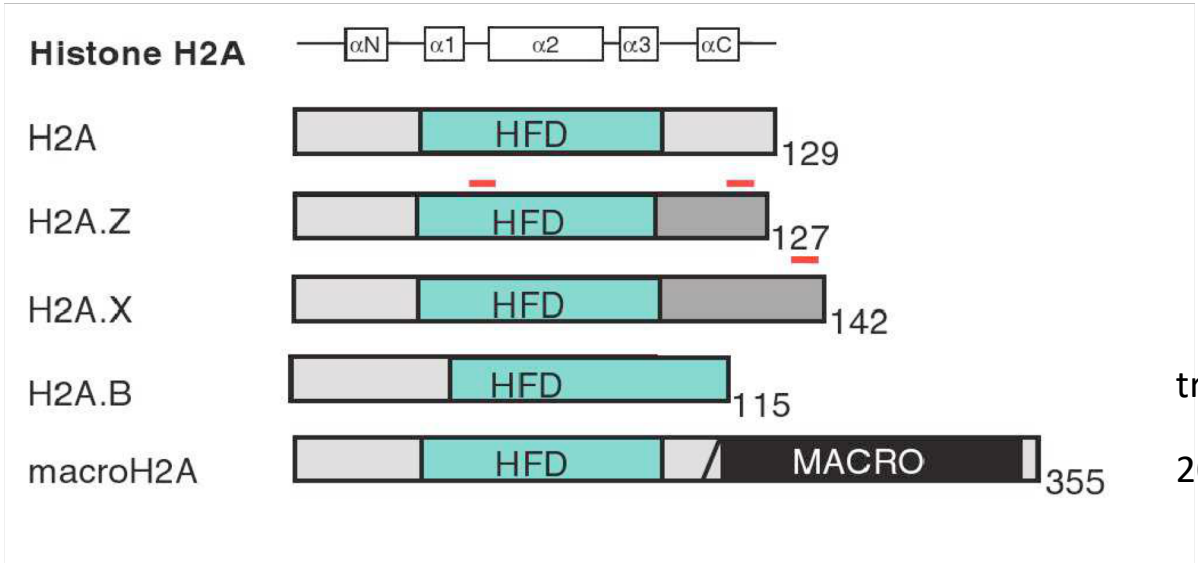
(i) H2AX-knockout cells manifested impaired recruitment of NBS1, 53BP1, and BRCA1 to irradiation-induced foci

(ii) Both H2AX and H2AX mouse thymocytes show an increase in chromosomal aberrations Mouse embryonic stem (ES) cells deficient in H2AX phosphorylation have alterations in efficiency of DNA repair by NHEJ or HR. As a result of these defects in DNA damage repair, cells showincreased sensitivity to DNA damage.

(iii) H2AX knock-out mice show male-specific infertility and reduced levels of secondary immunoglobulin isotypes, suggesting defects in class switch recombination (CSR). It was shown that efficient resolution of DSBs induced during CSR in lymphocytes requires H2AX, and its absence is associated with chromosome abnormalities involving the immunoglobulin locus.

These facts suggest that H2AX might serve as a docking site for DNA damage/repair proteins and functions to promote DSB repair and genome stability.

ROLE OF macroH2A AND H2A.B ON THE X-CHROMOSOME

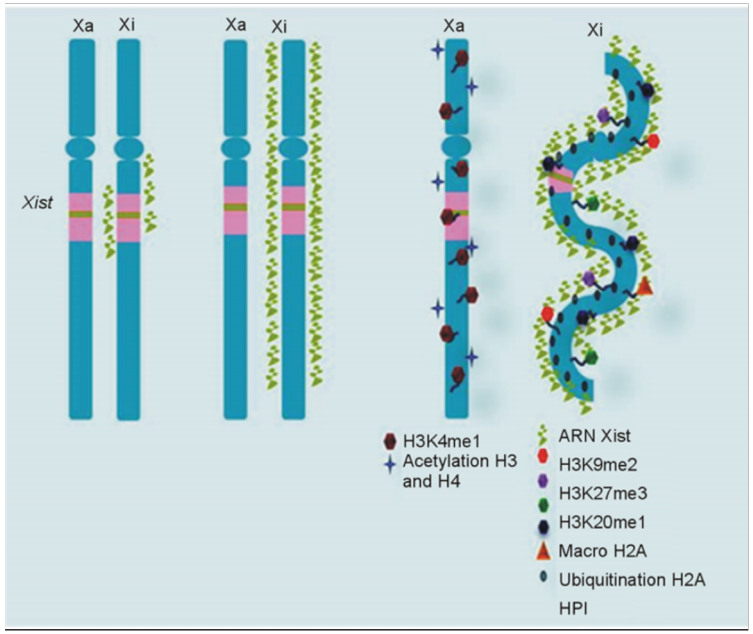


truncation

200 aa C-terminal domain

MacroH2A and H2A.B and X inactivation

X inactivation is associated with the exchange of H2A variants



macroH2A is enriched at the Xi

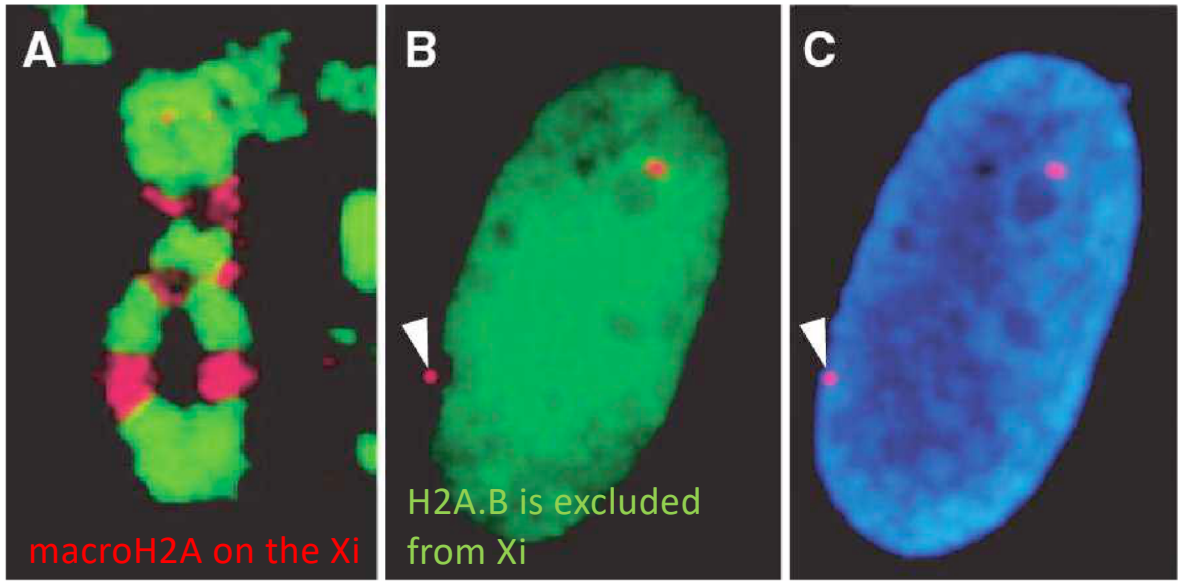
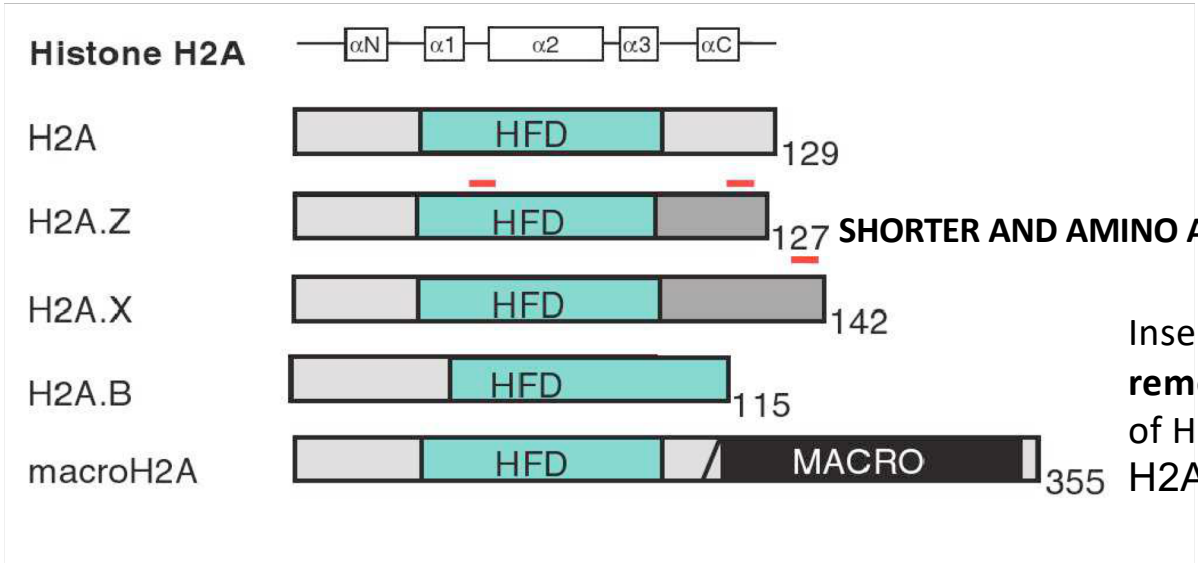


Figure 11. H2A variants and the inactive X chromosome of human females. (*A*) macroH2A (red) stains discrete regions of the inactive X chromosome that alternate with a marker for heterochromatin (histone H3K9me3). (*B*) H2A.B (green) is excluded from the inactive X chromosome (red dot with arrowhead pointing to it). (*C*) Same nucleus as in *B*, but stained with DAPI to show chromatin. (*A*, Reprinted, with permission, from Chadwick and Willard 2004, © National Academy of Sciences; *B,C*, reprinted, with permission, from Chadwick and Willard 2001, © 2001 The Rockefeller University Press. Originally published in *Journal of Cell Biology* **152:** 375–384. doi: 10.1083/jcb.152.2.375.)

ROLE OF H2A.Z IN CHROMATIN DYNAMICS



SHORTER AND AMINO ACID EXCHANGES

Different heterodimers possible:

1. H2A.Z/H2B + H2A.Z/H2B = ZZ
2. H2A.Z/H2B + H2A/H2B
3. H2A/H2B + H2A/H2B

Inserted by INO80 and SWR-C complexes (**chromatin remodelers with ATPase domain**); RI incorporation of H2AZ into chromatin.

H2AZ **Histone chaperon**: human YL1; yeast: Chz1

H2A.Z shows less adherence to DNA and is frequently localized **at the +1 nucleosome position** after the transcriptional start site:

Yeast: ZZ: 32%
ZA: 24%
AA: 44%

→ **>50% of genes have a H2A.Z at transcription start sites**

H2AZ Histone chaperon: human YL1; yeast: Chz1

NUCLEOSOME DEPLETED REGIONS (NDRs)

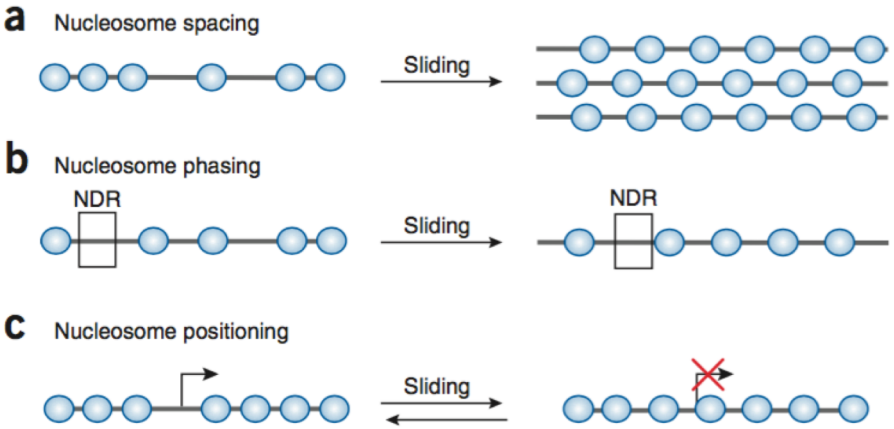
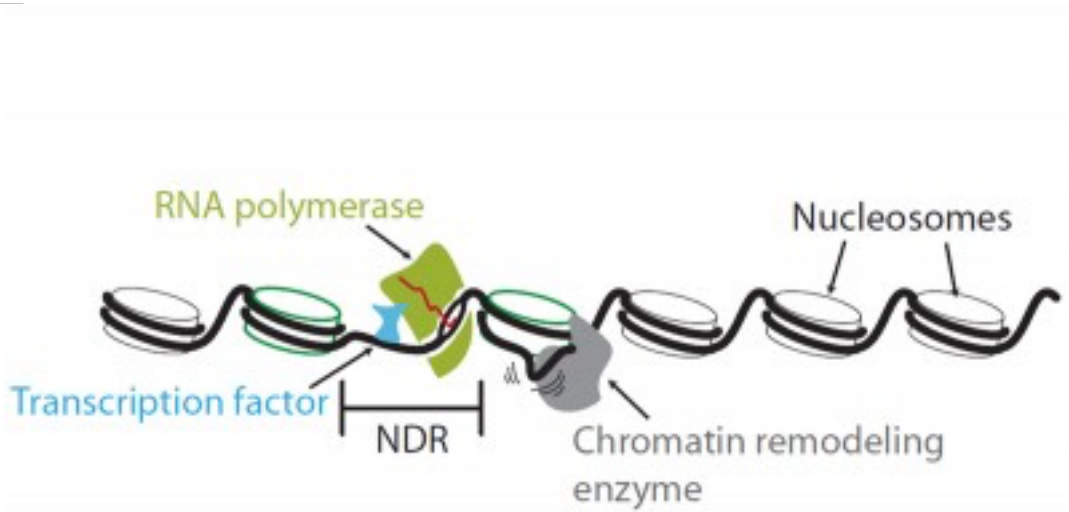
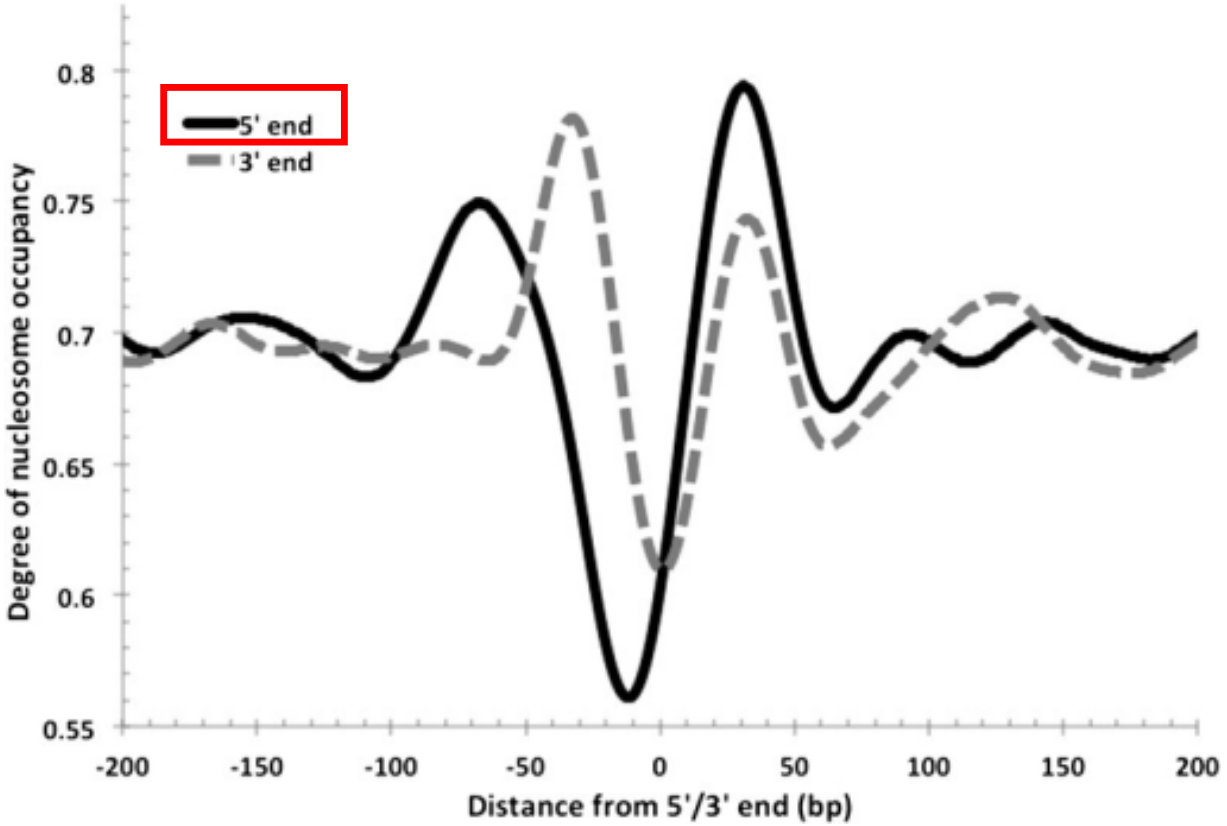


Figure 2 The different physiological outcomes of nucleosome sliding. (**a**) Nucleosome-remodeling enzymes can introduce and maintain regular spacing of nucleosomes. (**b**) The phasing of nucleosomal arrays with respect to a nucleosome-depleted region (NDR), a prominent feature of promoters, depends on nucleosome-remodeling enzymes. (**c**) Nucleosome-sliding activity regulates the accessibility of DNA sequences by positioning individual nucleosomes.



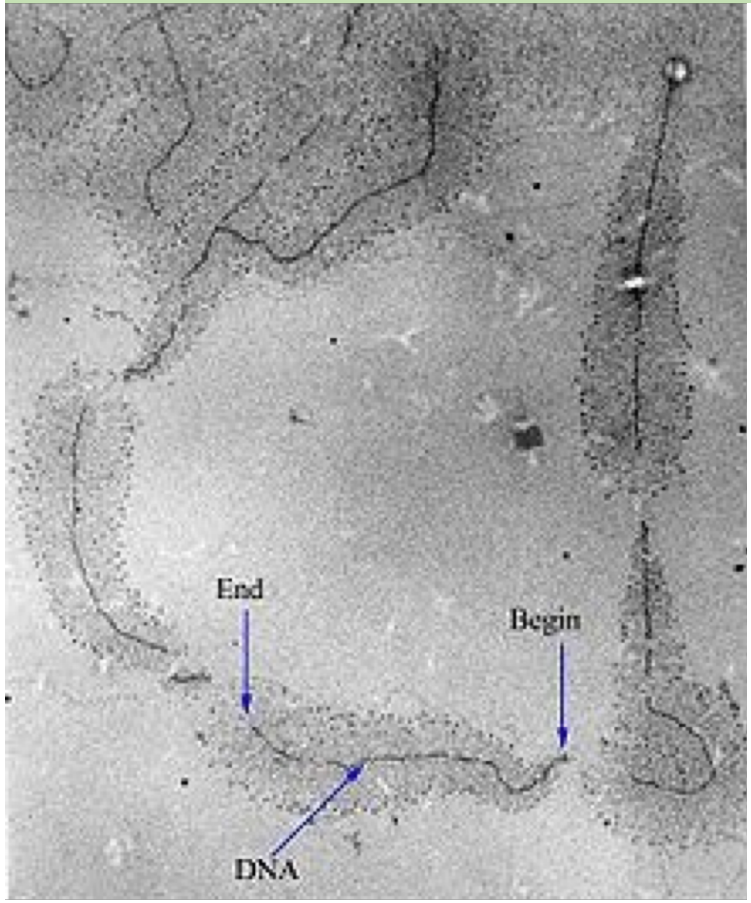
NUCLEOSOME DEPLETED REGIONS (NDRs)



Nucleosome-depleted regions at the 5′ and 3′ ends of transcripts. As observed in eukaryotes, Both 5′ and 3′ end profiles are overlaid in this figure for comparison. The 5′ NDR is, on average, more depleted and longer.

DOI:http://dx.doi.org/10.7554/eLife.00078.005

ROLE OF H2A.Z IN CHROMATIN DYNAMICS



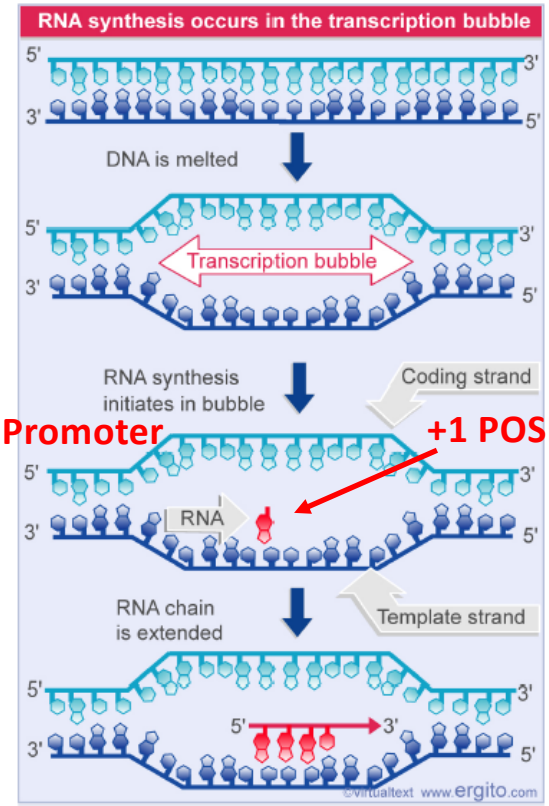


Figure 9.3 DNA strands separate to form a transcription bubble. RNA is synthesized by complementary base pairing with one of the DNA strands.

At promoter and +1 position high turn-over of nucleosomes

The initiation site of transcription is nucleosome-free

Nucleosomes containing H2A.Z (or H3.3) **are more instable**; leaving the promoter and +1 position more flexible

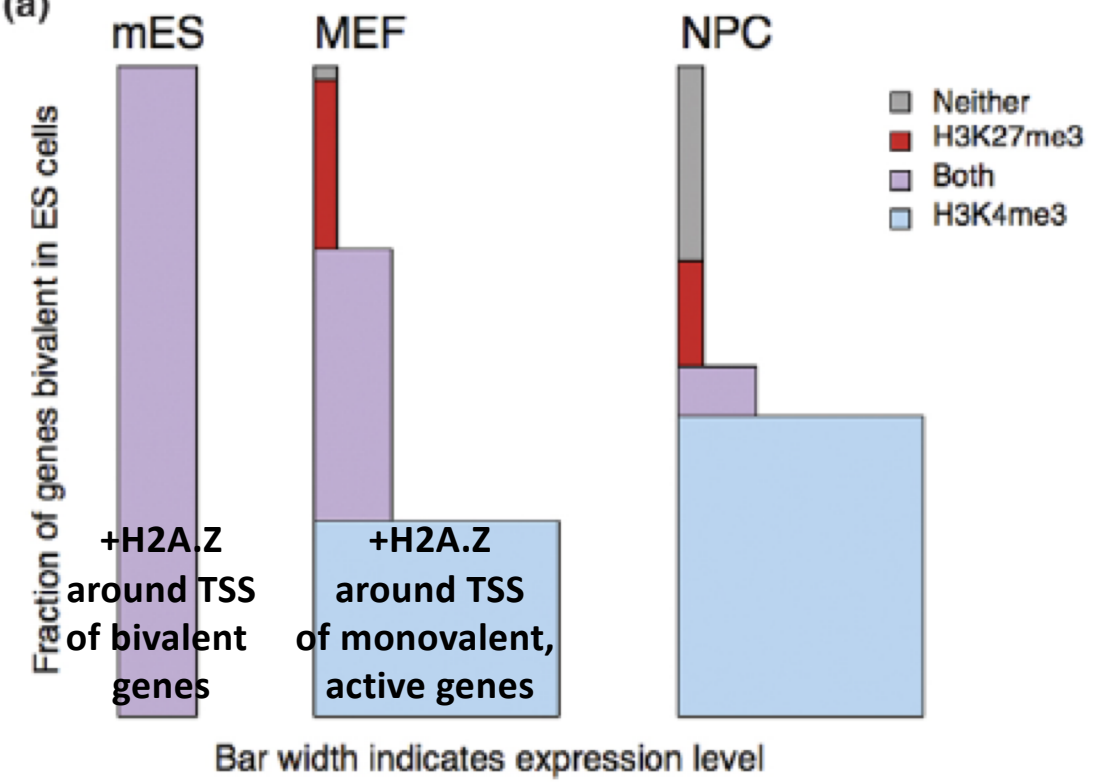
During G1, S, G2, M phase nucleosomes at +1 position (transcriptional start site) are **not present.**
Around these position H2A.Z is abundant!
In this way the TSS acquires an open configuration
- Also when cell pass trough M-Phase

=TRANSCRIPTIONAL MEMORY TO MARK START SITE
IN ACTIVE GENES!!!
"normal" histone modifications do not precisely mark the start site of transcription

MECHANSIMS THAT DECIDES HOW H2A.Z IS INCORPORATED (RI assembly) IS UNKNOWN

ROLE OF H2A.Z IN CHROMATIN AND EPIGENTIC INHERITANCE IN STEM CELLS

(a)



(b)

Genes involved in	ES	MEF	NPC
Haematopoiesis	Bivalent	K27 or neither	K27 or neither
Mesenchyme	Bivalent	K4	K27 or neither
Adipogenesis	Bivalent	Bivalent	K27 or neither
Neurogenesis	Bivalent	K27 or neither	Bivalent

EMBRYONIC STEM CELLS:
H2A.Z at bivalent promoters
(colocalization with PcG/Trx)

Depletion of H2A.Z:
- More stabile association of nucleosomes with promoters and enhancers
Reduced H3K4me3 and H3K27me3

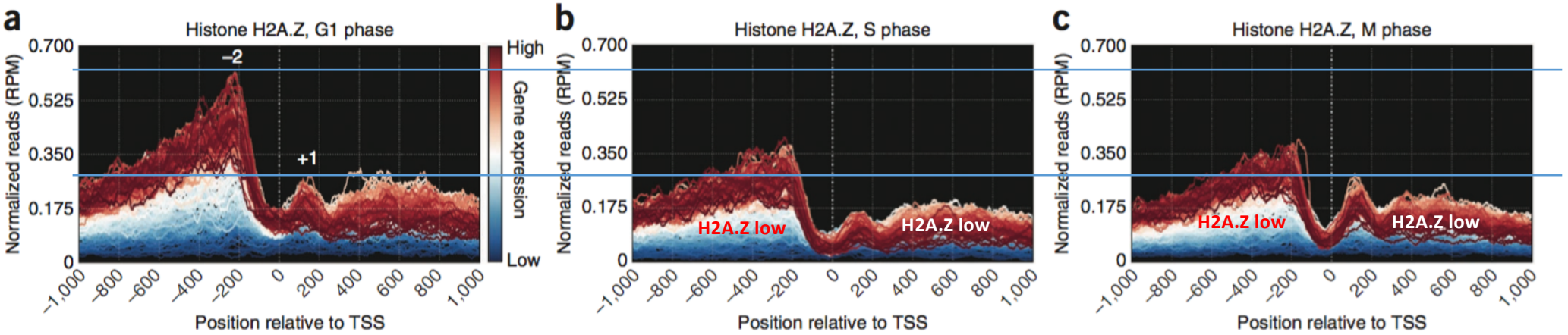
-**No precise shift from**
Bivalent to monovalent upon induction of differentiation → differentiation defects

NORMAL SITUATION:
After transition from bivalent inactive to monovalent active:
Concentration of H2A.Z at promoters of active genes

H2A.Z IS CELL CYCLE REGUALTED AT THE TSS

-1, 0 NDR: transcriptional start site

Brown color: highly expressed gene



Postion -2: high peak of H2A.Z

Postion -1,0: Nucleosome depleted region

Postion +1: peak of H2A.Z

Postion -2: low high peak of H2A.Z

Postion -1,0: Nucelosome depleted region

Postion +1: peak of H2A.Z

Postion -2: low high peak of H2A.Z

Postion -1,0: Nucelosome depleted region

Postion +1: peak of H2A.Z

EXPERIMENTS: Trophoblasts in G1, S, M Phase

→ gene expression array +

→ ChIP H2A.Z and ANALYSIS OF OCCUPANCY OF GENES THAT ARE ACTIVE IN G1, S, M PHASE

H2A.Z around transcriptional start site is cell cycle regulated

Nekrasov et al 2012, Nature Structural and Molecular Biology

HOW CAN WE ANALYSE H2A-H2A.Z HETEROTYPIC NUCLEOSOMES AT THE TSS DURING THE CELL CYCLE?

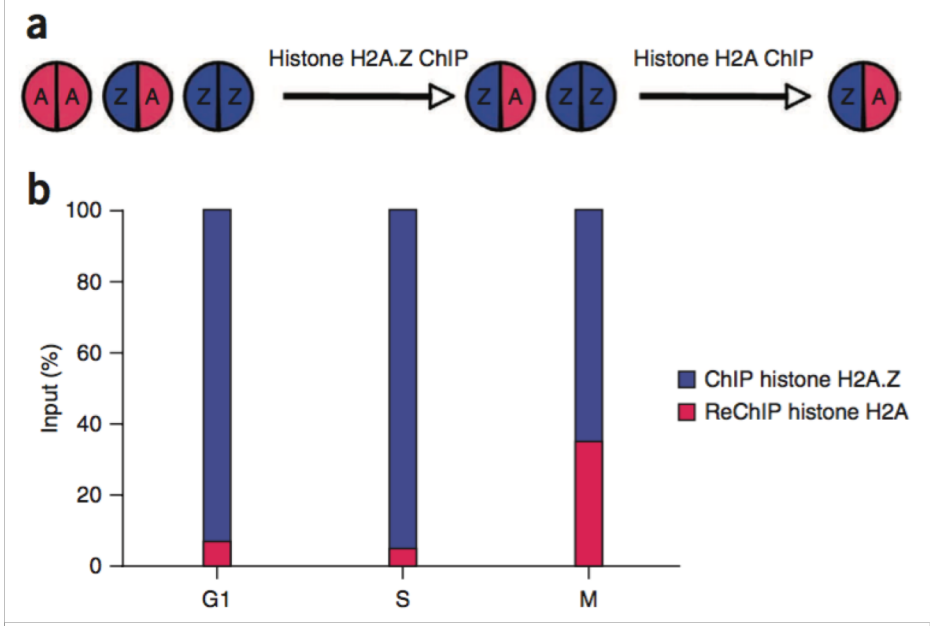


Figure 3 Heterotypic histone H2A.Z–H2A nucleosomes are assembled after S phase. (**a**) Schematic of the experiment: ChIP assays were first performed using affinity purified anti–histone H2A.Z antibodies to immunoprecipitate all histone H2A.Z-containing nucleosomes followed by a second ChIP using anti–histone H2A antibodies to pull down heterotypic nucleosomes. For the ChIP histone H2A.Z reChIP histone H2A experiments, the original ChIP H2A.Z material served as input DNA. A, histone H2A; Z, histone H2A.Z. (**b**) The amount of histone H2A.Z reChIP H2A DNA was determined relative to the original amount of histone H2A.Z ChIP DNA (blue), normalized to 100%, for each of the three stages of the cell cycle. Mean ± s.e.m. ($n = 6$) were: for G1 phase, 6.76 ± 1.15; for S phase, 5.11 ± 0.50; and for M phase, 35.2 ± 3.96.

A re-ChIP to quantify the proportion of H2A.Z heterotypic or H2A-Z homotypic nucelosomes
ChIP 1: anti-H2AZ
ChIP2: use IP products on ChIP 1 and re-ChIP with anti-H2A

G1: histone H2A.Z is predominantly **homotypic**
S: histone H2A.Z is predominantly **homotypic**
M: **heterotypic H2A-H2A.Z** nucleosomes are assembled after S phase

CELL CYCLE DEPENDENT ASSEMBLY OF NUCELOSOMES BY HISTONE CHAPERON

Postion -2: high peak of H2A.Z

Postion -1, 0: Nucelosome depleted region

Postion +1: peak of H2A.Z

Postion -2: low high peak of H2A.Z

Postion -1, 0: Nucelosome depleted region

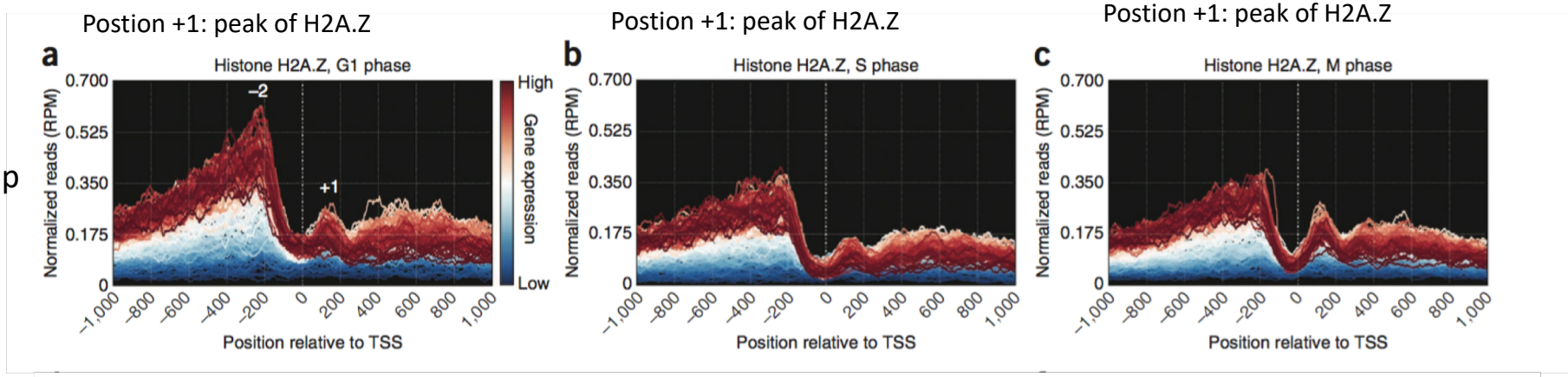
Postion +1: peak of H2A.Z

Postion -2: low high peak of H2A.Z

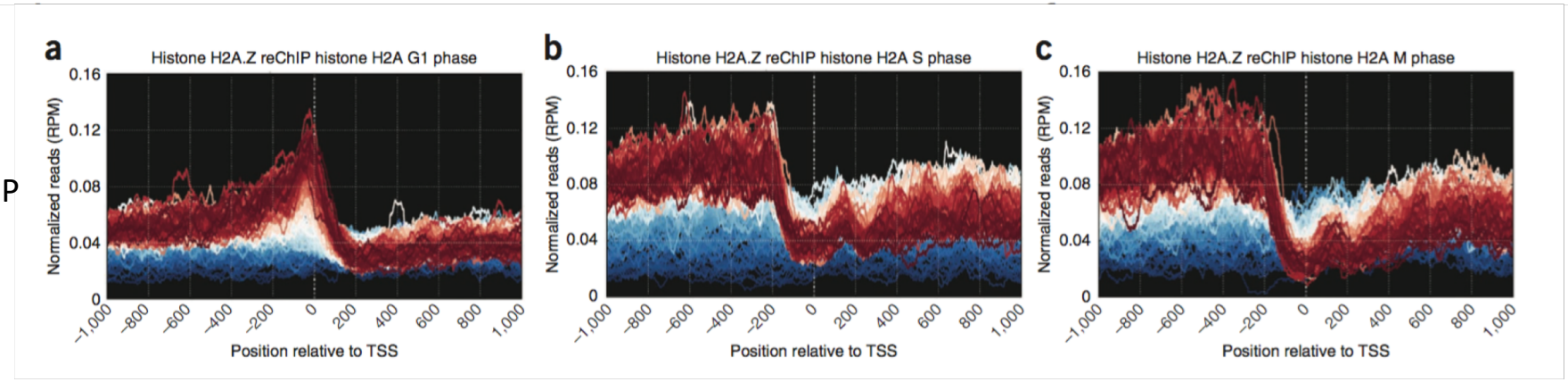
Postion -1, 0: Nucelosome depleted region

Postion +1: peak of H2A.Z

H2A.Z Chip



H2A.Z +
H2A re-ChIP

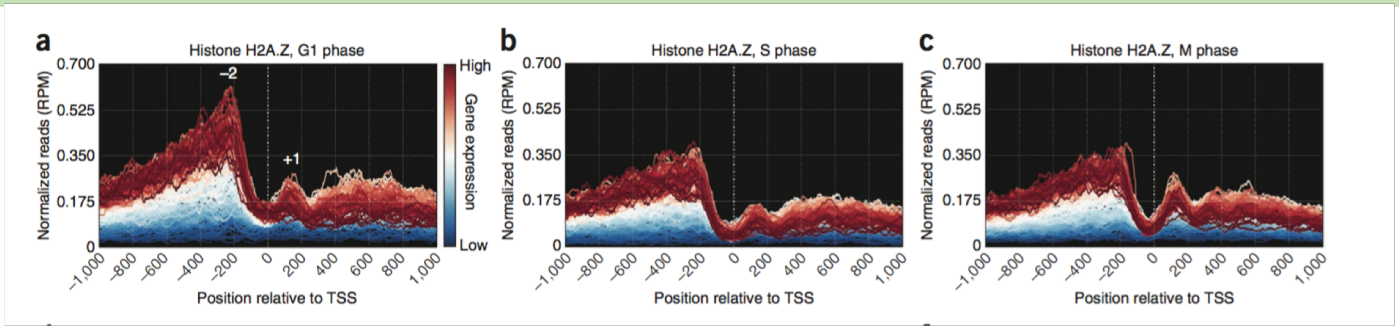


G1: Heterotypic
H2A/H2A.Z only at TSS
Lower of affinity of heterotypic H2AZ to DNA
Homotypic H2AZ downstream of TSS

S: Homotypic
H2A.Z/H2A.Z around TSS
more heterotypic upstream of TSS and lost downstream highly expressed genes
H2Az/H2A at +1 position

M: Homotypic
H2A.Z/H2A.Z around TSS
more heterotypic upstream of TSS and lost downstream highly expressed genes
H2Az/H2A at +1 position

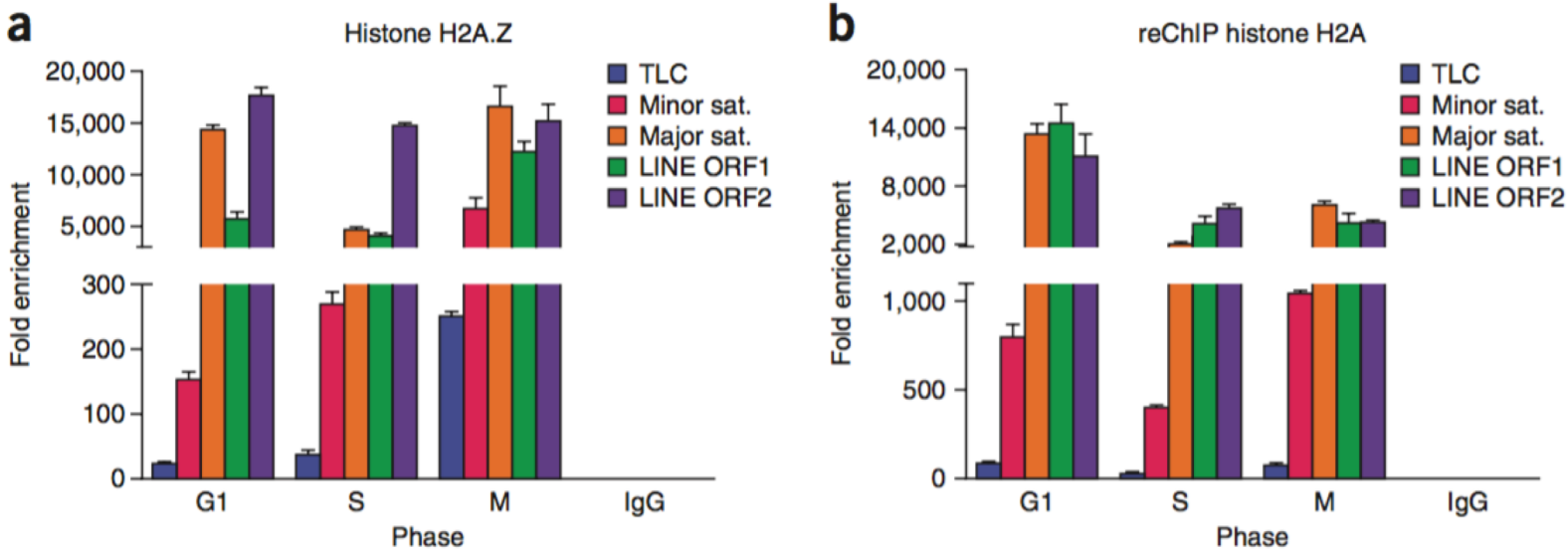
H2A.Z IS CELL CYCLE REGUALTED AT THE TSS AND SUBTELOMERES



H2A.Z is lost at promoters in M phase

H2A.Z is enriched at centromere and subtelomeres (TLC) in M phase

H2A is not re-chiped after H2A.Z ChIP
→ H2A.Z is homotypic



Homotypic H2A.Z accumulates in M Phase at TLCs; in M-Phase H2A.Z is sequestered at sub-telomeres, thus allowing the reduction of H2A.Z at gene promoters

DYNAMIC LOCATION OF H2A.Z AT TSS

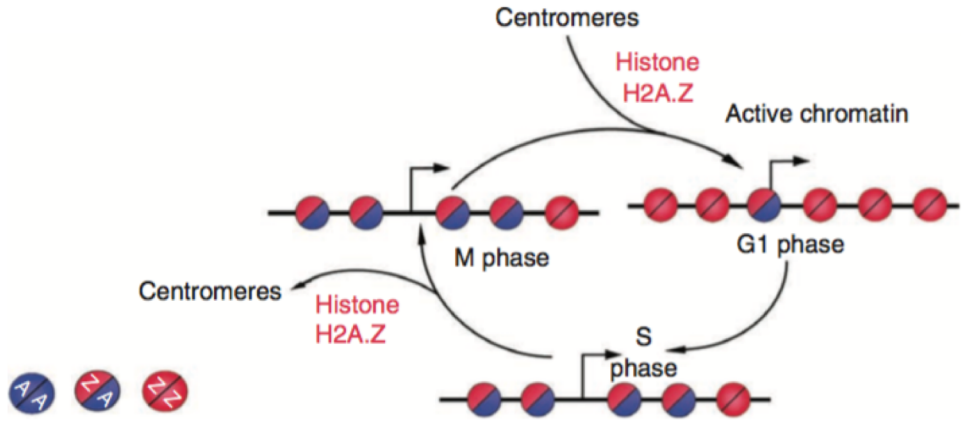


Figure 8 A model depicting the dynamic changes in histone H2A.Z at an active promoter throughout the cell cycle. Active genes in G1 phase have homotypic histone H2A.Z–containing nucleosomes immediately upstream and downstream of the TSS, with a heterotypic histone H2A.Z–H2A nucleosome located at the TSS. A, histone H2A; Z, histone H2A.Z. During DNA replication, homotypic histone H2A.Z nucleosomes become heterotypic and the heterotypic histone H2A.Z–H2A nucleosome at the TSS is lost. As cells progress to M phase, there is a net movement of histone H2A.Z to the centromere (and subtelomeric regions) preventing the reestablishment of homotypic nucleosomes at active promoters. Upon completion of mitosis, there is a redistribution of histone H2A.Z from the centromere back to active promoters to restore the original G1-phase active chromatin state.

Heterotypic H2A/H2AZ remains in M-Phase → potential memory mechanism to facilitate H2A/H2AZ at TSS in G1