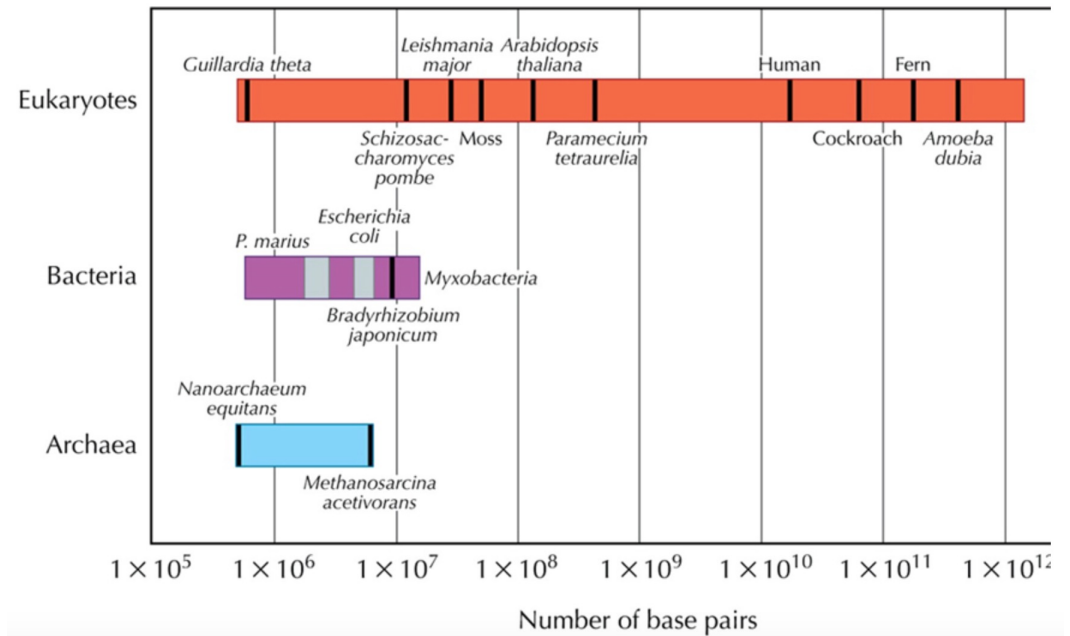
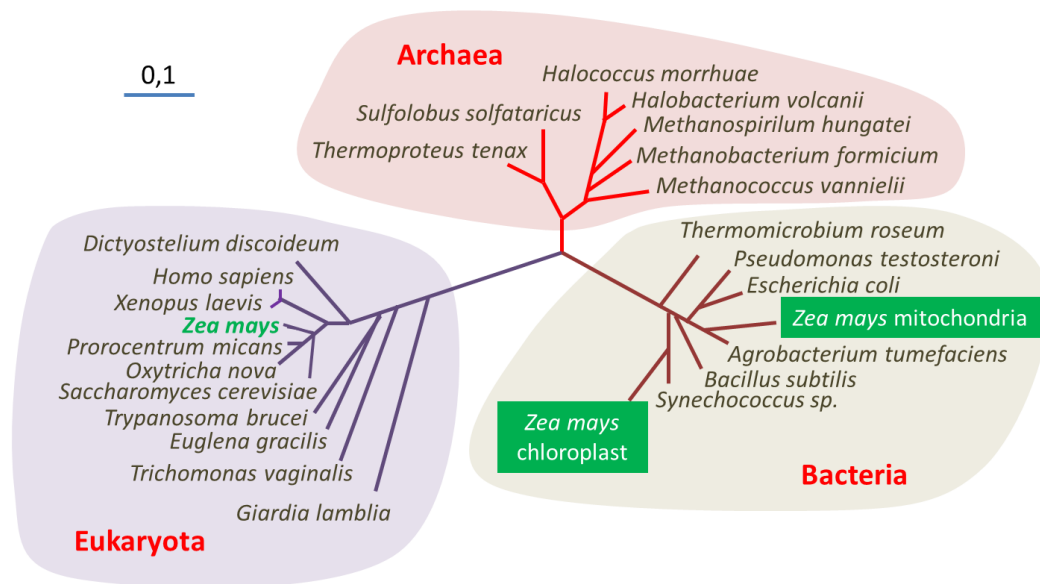


Organization of chromatin - Histones

Genomes in the 3 domains of living organisms

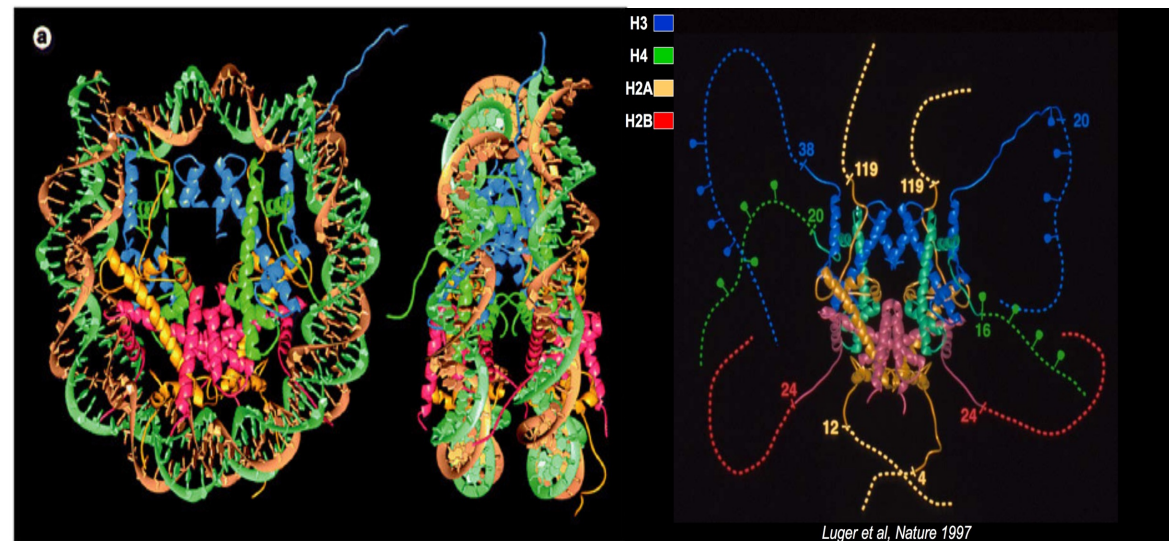


The unrooted phylogenetic tree of the three domains of living organisms, produced using a gene from the small ribosomal subunit

organism	genome size (base pairs)	protein coding genes	number of chromosomes
model organisms			
model bacteria <i>E. coli</i>	4.6 Mbp	4,300	1
budding yeast <i>S. cerevisiae</i>	12 Mbp	6,600	16
fission yeast <i>S. pombe</i>	13 Mbp	4,800	3
amoeba <i>D. discoideum</i>	34 Mbp	13,000	6
nematode <i>C. elegans</i>	100 Mbp	20,000	12 (2n)
fruit fly <i>D. melanogaster</i>	140 Mbp	14,000	8 (2n)
model plant <i>A. thaliana</i>	140 Mbp	27,000	10 (2n)
moss <i>P. patens</i>	510 Mbp	28,000	27
mouse <i>M. musculus</i>	2.8 Gbp	20,000	40 (2n)
human <i>H. sapiens</i>	3.2 Gbp	21,000	46 (2n)
viruses			
hepatitis D virus (smallest known animal RNA virus)	1.7 Kb	1	ssRNA
HIV-1	9.7 kbp	9	2 ssRNA (2n)
influenza A	14 kbp	11	8 ssRNA
bacteriophage λ	49 kbp	66	1 dsDNA
Pandoravirus salinus (largest known viral genome)	2.8 Mbp	2500	1 dsDNA
organelles			
mitochondria - <i>H. sapiens</i>	16.8 kbp	13 (+22 tRNA +2 rRNA)	1
mitochondria - <i>S. cerevisiae</i>	86 kbp	8	1
chloroplast - <i>A. thaliana</i>	150 kbp	100	1
bacteria			
<i>C. ruddii</i> (smallest genome of an endosymbiont bacteria)	160 kbp	182	1
<i>M. genitalium</i> (smallest genome of a free living bacteria)	580 kbp	470	1
<i>H. pylori</i>	1.7 Mbp	1,600	1
Cyanobacteria <i>S. elongatus</i>	2.7 Mbp	3,000	1
methicillin-resistant <i>S. aureus</i> (MRSA)	2.9 Mbp	2,700	1
<i>B. subtilis</i>	4.3 Mbp	4,100	1
<i>S. cellulorum</i> (largest known bacterial genome)	13 Mbp	9,400	1
archaea			
<i>Nanoarchaeum equitans</i> (smallest parasitic archaeal genome)	490 kbp	550	1
<i>Thermoplasma acidophilum</i> (flourishes in pH<1)	1.6 Mbp	1,500	1
<i>Methanocaldococcus (Methanococcus) jannaschii</i> (from ocean bottom hydrothermal vents; pressure >200 atm)	1.7 Mbp	1,700	1
<i>Pyrococcus furiosus</i> (optimal temp 100°C)	1.9 Mbp	2,000	1
eukaryotes - multicellular			
pufferfish <i>Fugu rubripes</i> (smallest known vertebrate genome)	400 Mbp	19,000	22
poplar <i>P. trichocarpa</i> (first tree genome sequenced)	500 Mbp	46,000	19
corn <i>Z. mays</i>	2.3 Gbp	33,000	20 (2n)
dog <i>C. familiaris</i>	2.4 Gbp	19,000	40
chimpanzee <i>P. troglodytes</i>	3.3 Gbp	19,000	48 (2n)
wheat <i>T. aestivum</i> (hexaploid)	16.8 Gbp	95,000	42 (2n=6x)
marbled lungfish <i>P. aethiopicus</i> (largest known animal genome)	130 Gbp	unknown	34 (2n)
herb plant <i>Paris japonica</i> (largest known genome)	150 Gbp	unknown	40 (2n)

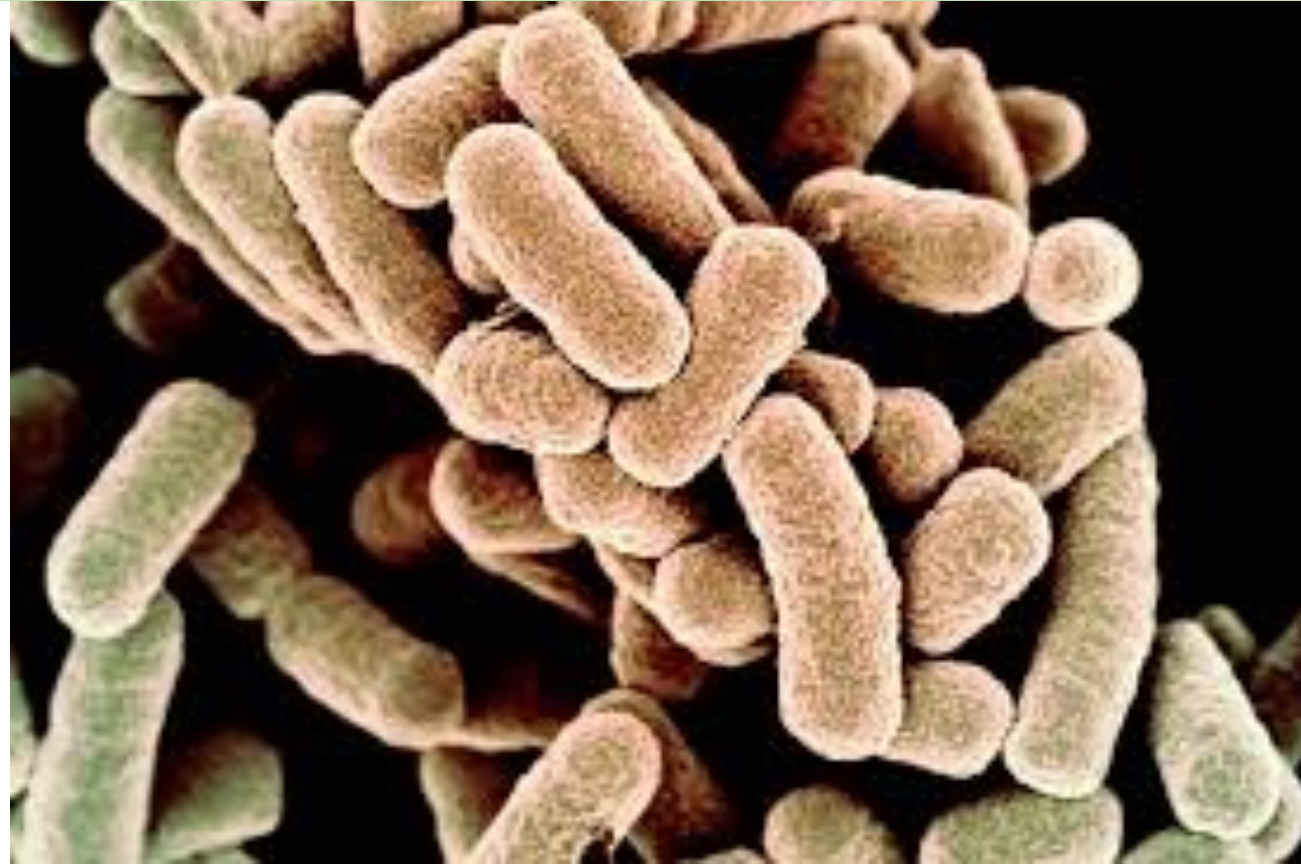
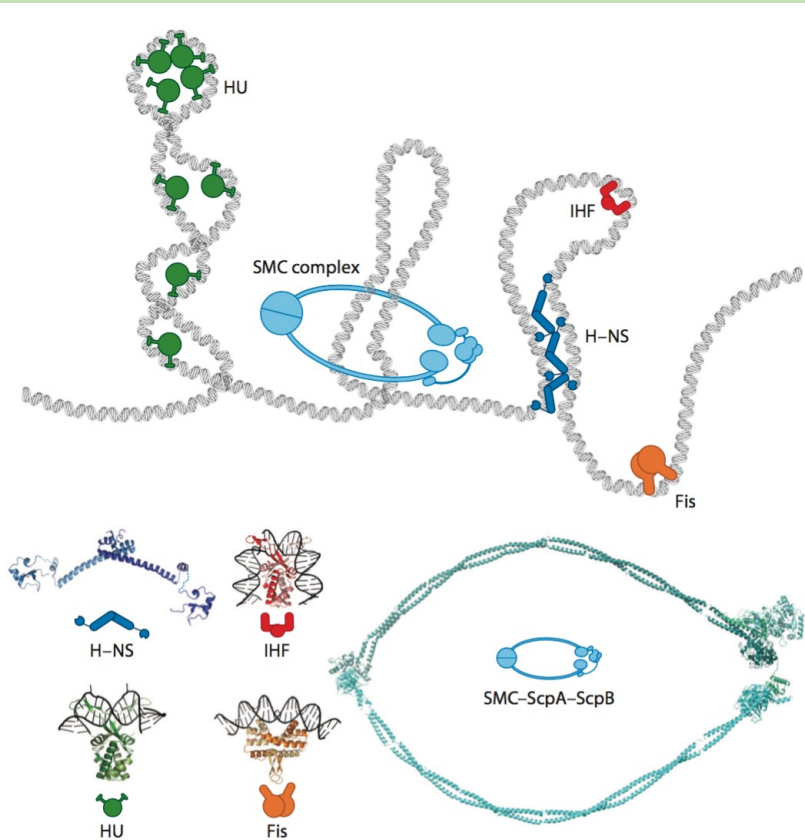
Genomes are organized

- Bacteria
- Archaeobacteria
- Eukaryotes



Luger et al., 1997

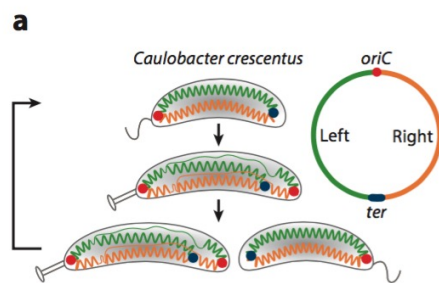
The bacterial nucleoid - Genome organization in bacteria



Helical fold of DNA in bacteria

The bacterial genome is organized on the macroscopic level

A helix-like conformation of DNA has been observed in replicating bacteria. The biological significance of a helical fold is unknown but may represent an energy-minimal configuration for fitting chromosomes within rod-shaped cells.

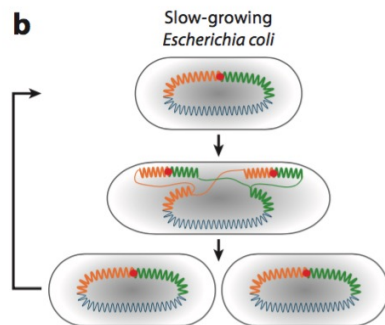


Caulobacter crescentus

- The spatial positions of loci within the cell recapitulated the genetic map, with the origin of replication (*oriC*) at one cell pole (**polar localization**), the replication terminus (*ter*) at the opposite pole, and the left and right chromosomal arms likely running in parallel down the long axis of the cell, a pattern referred to as the **ori-ter configuration**.

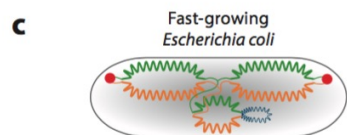
- Found by DNA-FISH using specific DNA probes that map along the chromosome

- In replicating cells, one new copy of *oriC* is rapidly segregated to the opposite pole. As replication proceeds, the newly generated DNA moves to its respective position, again with loci arranged relative to the origin in a manner that reflects the genetic map



Escherichia coli

The origin resides near midcell, with the two chromosomal arms on opposite sides of the cell and the terminus typically near midcell, in a so-called **left-ori-right configuration** (Nielsen et al. 2006b, Wang et al. 2006). DNA replication and segregation of the origins to cell quarter positions regenerates a **left-ori-right organization** for each chromosome.

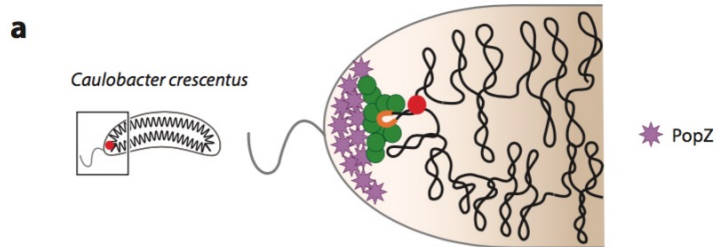


Chromosome consists of 4 macrodomains – organisational units (found by DNA-FISH)

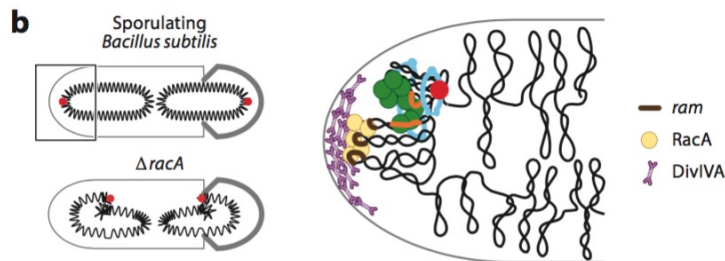
By contrast, fast-growing *E. coli* cells adopt an **ori-ter configuration** of the chromosome with polarly localized origins

Anchoring of genomic DNA in bacteria by chromatin

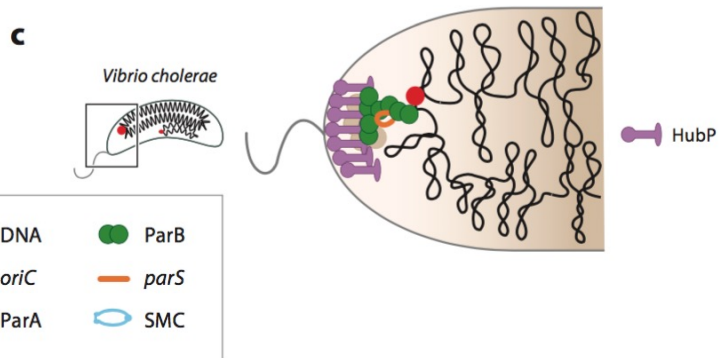
Polar anchoring of origins of chromosomes in ***ori-ter* configuration**, is thought to enforces the *ori-ter* pattern, and may help to ensure that each daughter cell inherits a full copy of the genome after DNA replication.



In *C. crescentus*, a *parS* site that is critical for chromosome segregation is located ~13 kb from the origin and is bound by ParB which also binds PopZ, a cytoplasmic protein that self-aggregates into a proteinaceous matrix at cell poles



A protein called RacA accumulates prior to sporulation and concentrates near the cell pole (Ben-Yehuda et al. 2003, Wu & Errington 2003). RacA binds 25 RacA-binding motif (*ram*) sites near *oriC*, helping tether *ori*-proximal regions of the chromosome to the pole. Polar localization of RacA requires a small peripheral membrane protein called DivIVA, which recognizes the concave curvature of the polar membrane



In *Vibrio cholerae*, a membrane-associated protein called HubP anchors the origin of the large chromosome, *ChrI*, to the pole. HubP interacts with ParAI, which likely interacts with ParBI, which in turn binds a *parS* site near the *ChrI* origin. HubP has a peptidoglycan-binding LysM domain, which is required for polar localization.

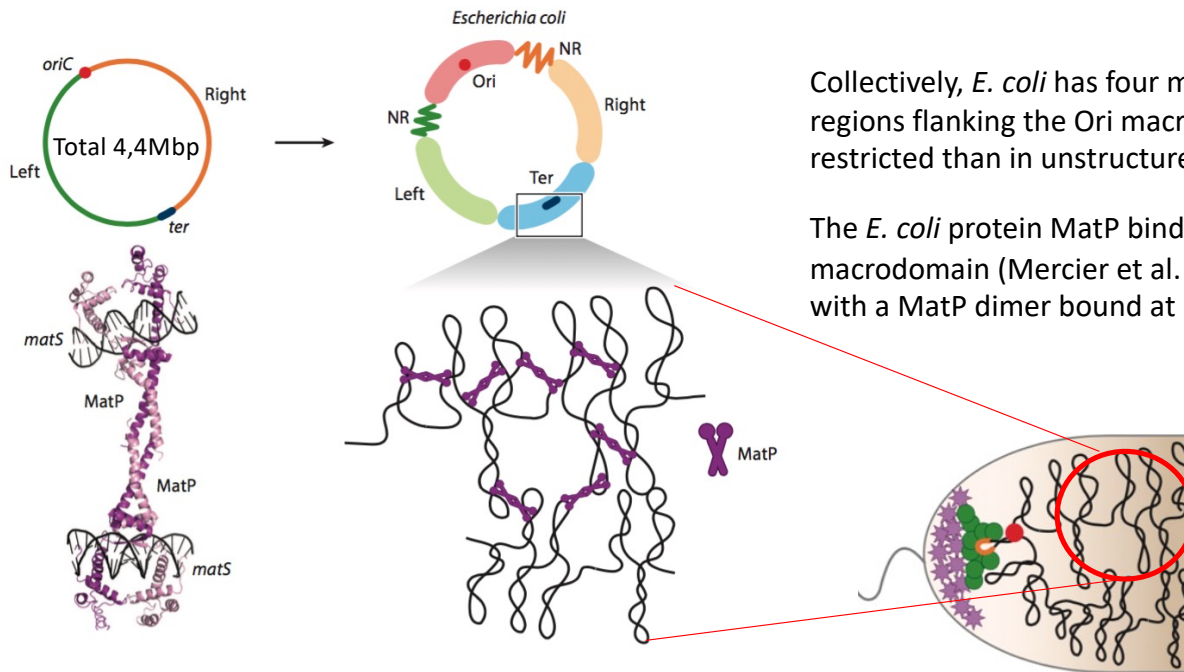
Note: Although PopZ, RacA, and HubP each anchor chromosomes to a cell pole, these proteins bear no sequence similarity, suggesting they arose independently

In *E. coli*, no polar anchoring complex has been identified. If one exists, it may function only during fast growth, when chromosomes exhibit an *ori-ter* pattern.

Macrodomains in bacteria

Bacterial chromosomes are further organized into Mb-sized domains called **macrodomains**, which were first suggested by FISH studies in *E. coli* that demonstrated certain loci frequently co-occupy the same restricted cytoplasmic space

a



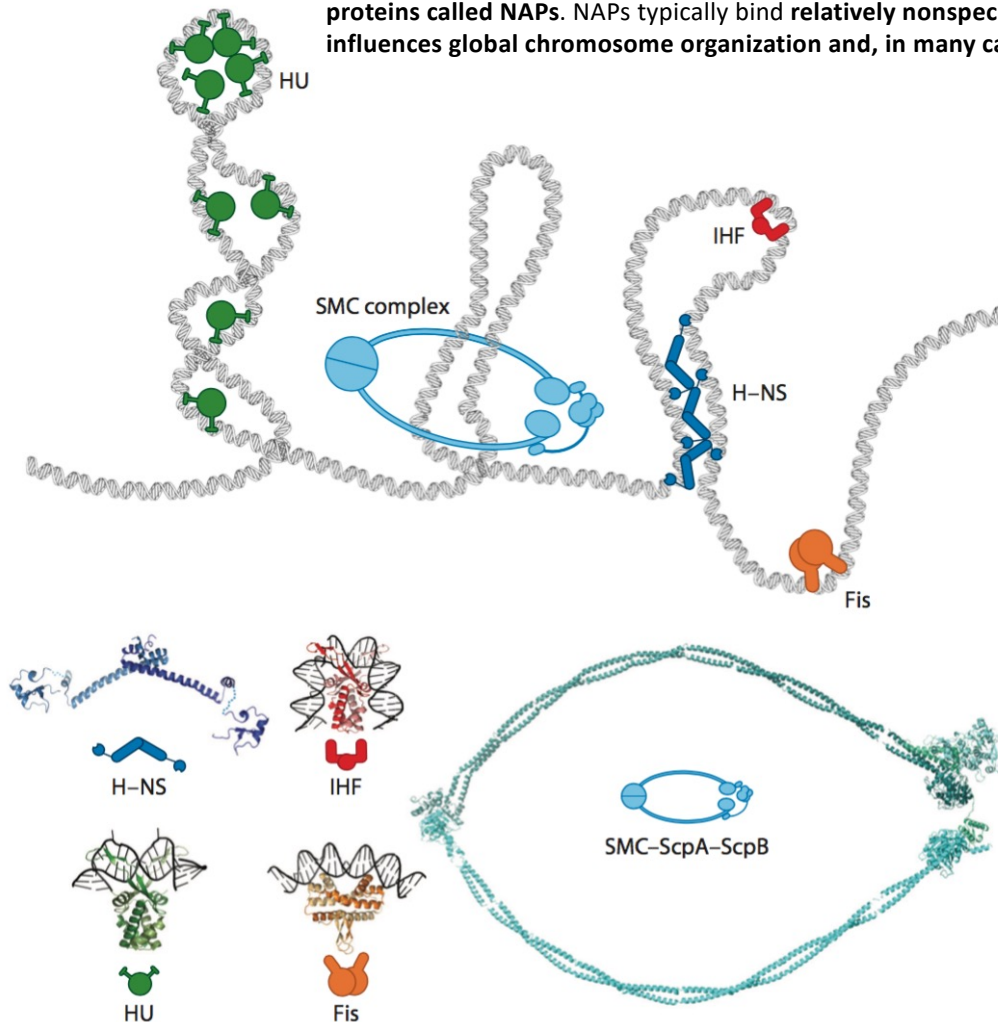
Collectively, *E. coli* has four macrodomains, Ori, Ter, Left, and Right, with two less-structured DNA regions flanking the Ori macrodomain (a). DNA movement within macrodomains is more restricted than in unstructured regions (NR)

The *E. coli* protein MatP binds to 13-bp *matS* sites present exclusively in the ~800-kb Ter macrodomain (Mercier et al. 2008). A MatP dimer bound to one *matS* site can form a tetramer with a MatP dimer bound at another site bringing different sites in the genome together.

Macrodomains and chromosomal interaction domains. (a) Macrodomain organization of the *Escherichia coli* chromosome, (left) or with the four macrodomains, Ori, Ter, Left, and Right, The crystal structure of two MatP dimers, each bound to a *matS* recognition site, is shown.

Nucleoid-Associated Proteins (NAP) in bacteria

The organization of bacterial chromosomes is profoundly influenced by DNA-binding proteins and in particular by a **heterogeneous class of abundant proteins called NAPs**. NAPs typically bind **relatively nonspecifically across bacterial genomes, wrapping, bending, or bridging DNA**, ultimately **influences global chromosome organization and, in many cases, transcriptional patterns**.



E. coli **H-NS** is a small (15.5 kDa) protein that can bridge DNA, **bringing loci** separated on the primary sequence level into close physical proximity, **constrains supercoiling**, can also **oligomerize and spread** along DNA to occlude binding sites for RNA polymerase or transcription activators (**repression**)

HU is another small (18 kDa), abundant (~30,000 copies/cell) NAP found in many bacteria that coats and **wraps chromosomal DNA around itself in a fashion grossly similar to that of histones**. Can coat 10% of DNA, aggregates, has proposed role in DNA **compaction**, **promotes supercoiling**,

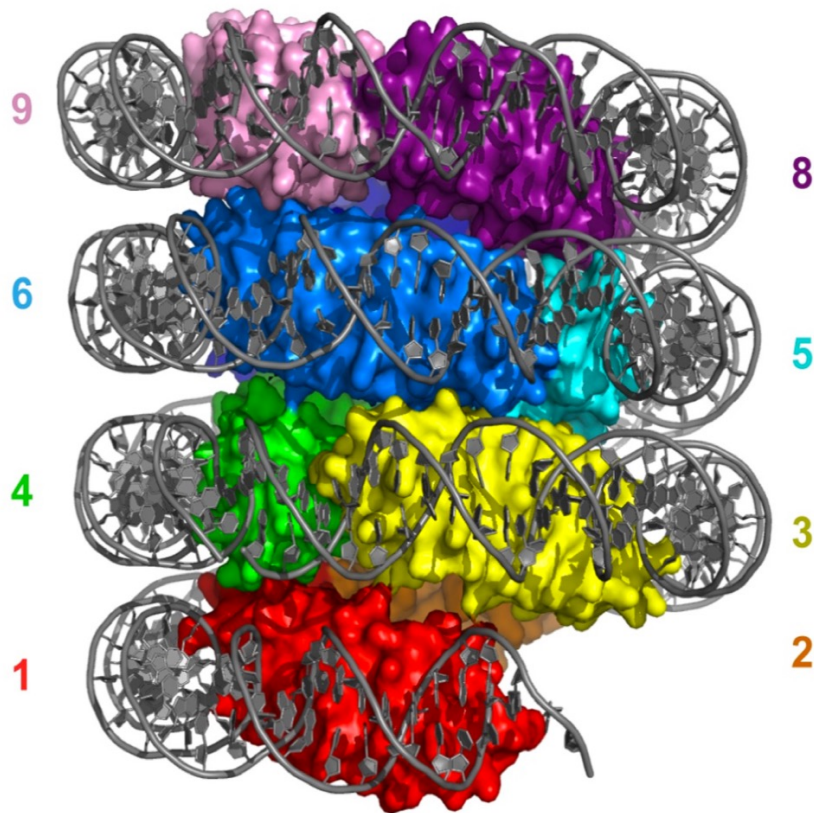
Integration host factor (**IHF**) and factor for inversion stimulation (**Fis**). Composed of two subunits, binds DNA more specifically and **introduces dramatic ~160° bends**. IHF alters DNA shape and facilitates **the formation of loops, frequently bringing RNA polymerase together with distant regulatory proteins**. IHF also impacts a range of other DNA-based processes, including **replication initiation and recombination**. **Fis** binds throughout the genome (Kahramanoglou et al. 2011), **impacting transcription, replication, and recombination. Modulates supercoiling**

SMC (125kDa) forms an extended, antiparallel coiled coil with a hinge domain at one end and an ATPase domain at the other. Homodimerization via the hinge domains creates a ring-like structure that **may encircle DNA**. SMC associates with two regulatory proteins, ScpA and ScpB, which likely modulate its ATPase activity, thereby affecting the opening and closing of the homodimeric ring. Mutations in SMC produce a range of chromosomal defects in different bacteria, often including an increase in anucleate cells. SMC likely contributes to both **chromosome segregation and chromosome compaction**.

HOMOLOG IN EUKARYOTES: CONDENSIN

Note: the function of these proteins was found by introducing mutations and study DNA topology/gene expression/recombination...

Genome organization in archaea bacteria



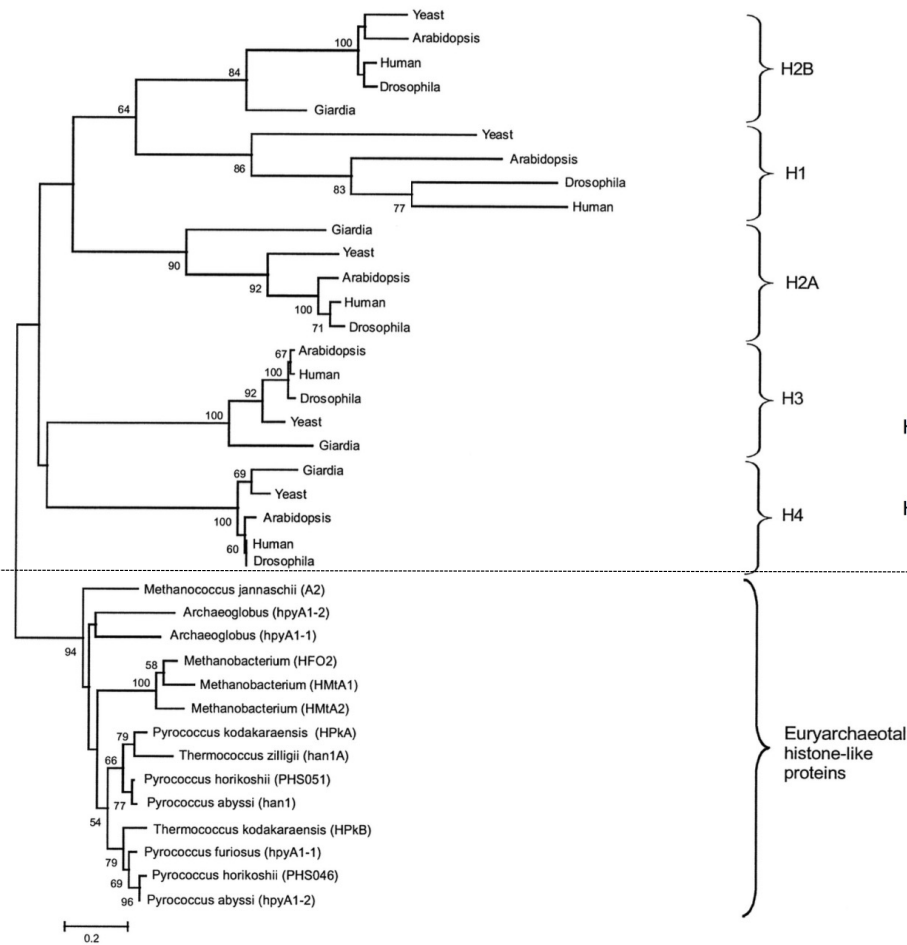
Halobacterium

A phylogenetic view on histone proteins

Eukaryotic and Archaeal histones have a common ancestor

Organization of bacterial genomes are unrelated to eukaryotes and archaee bacteria

Histones are highly conserved among eukaryotes

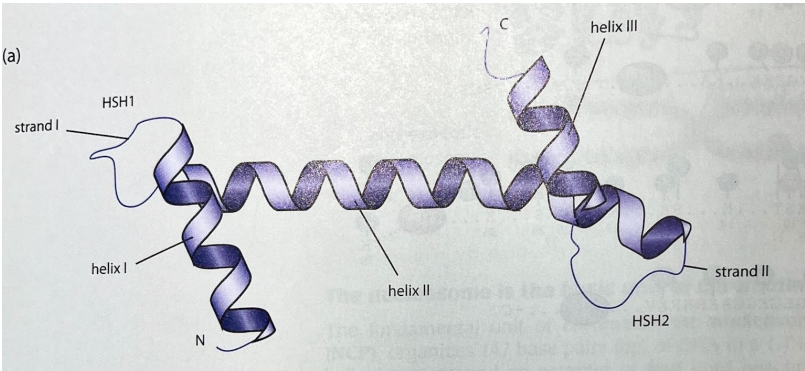


H4: very highly conserved between species
(98% identical between cows and peas)
~ 1% change in 600 million years (2 changes, Val to Ile, Lys to Arg)

H3: also very conserved (97% identical)
~ 1% change in 300 million years

Histone-like proteins
In archaeobacteria

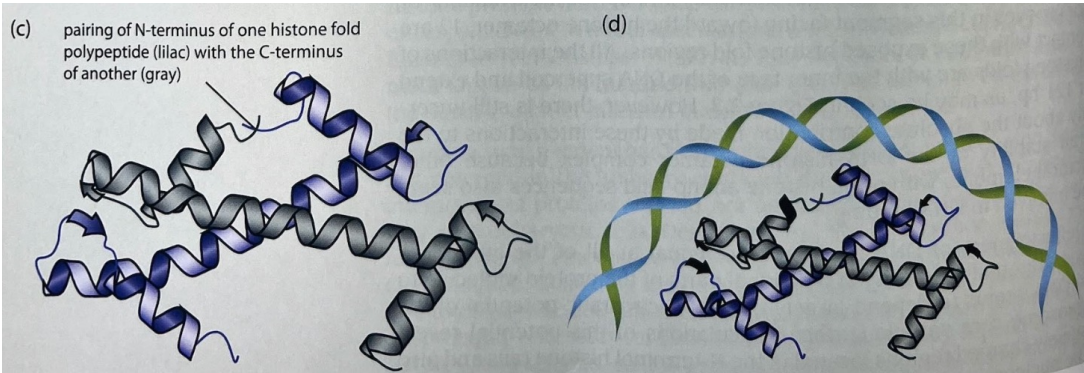
The histone fold domain (HFD)



	11aa	27aa	11aa		
(b)					
	helix I	loop/ strand I	helix II	loop/ strand II	helix III
H2A (25)	PVGRVHRLLRKGN	YAERVGAGAPVYLA	AVLEYLTA	EILELAGNPARDN	KKTRIIPRHLQLAIRND (38)
H2B (36)	YSIYVYKVLKQV	HPDTGISSKAMG	IMNSFVNDI	FERIAGEASRLA	HNKRSTITSREIQTAVRLL (24)
H3 (66)	FQRLVREIAQ	DFKTDLRFQSSA	VMALQWASEA	YLVGLFEDTN	LCAIHAKRVTIMPKDIQLARRIR (4)
H4 (29)	TKPAIRRLARR	GGV-KRISGLI	YEETRGVLK	VFLENVIRDA	VTYTEHAKRKTVTAMDVVYALKRQ (9)

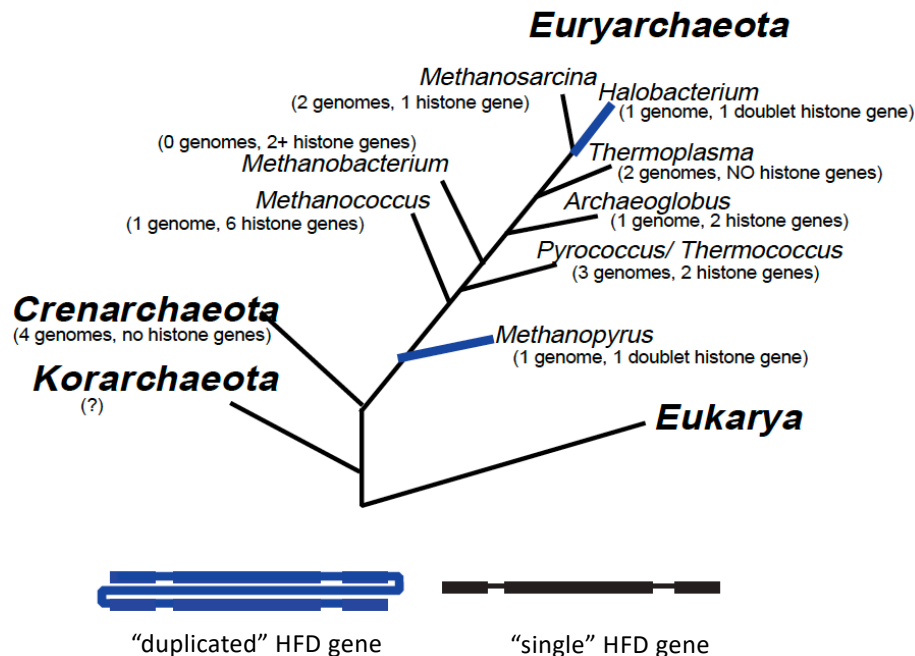
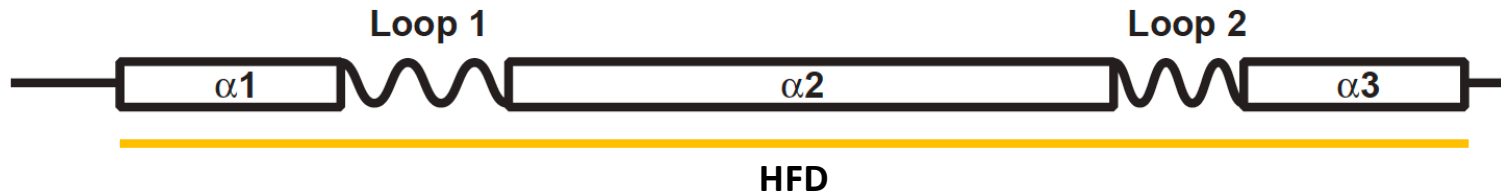
mammals

Highly basic proteins



Dimerization
Tetramerization
Octamers

The origin of histone proteins: Archaeobacteria



Histone proteins first evolved in Archaeobacteria

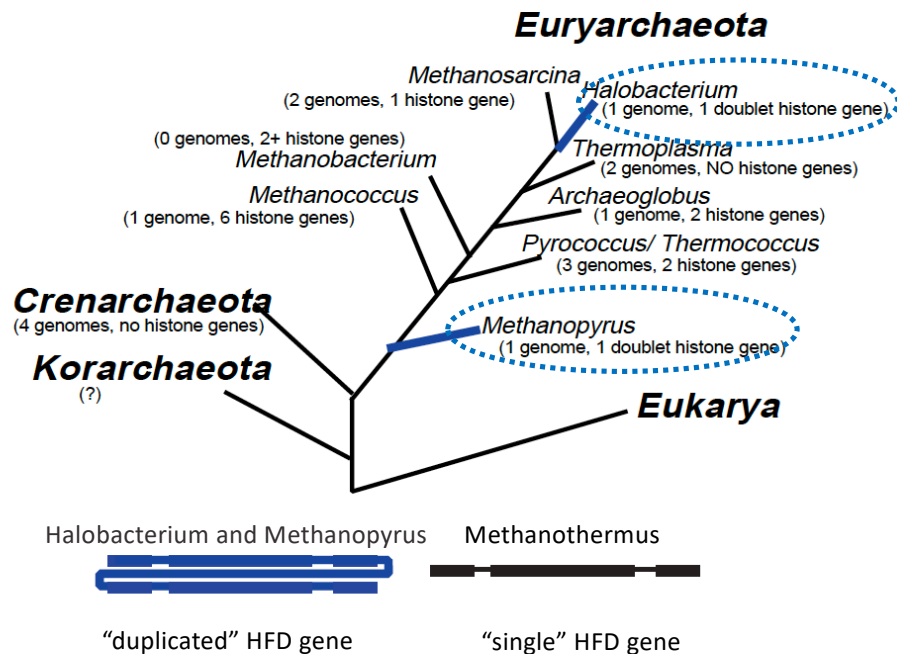
→ «Histone like proteins»

→ «Archeal nucleosomes»

Histone fold (HFD) domain: dimerization and DNA binding

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’.

The origin of histone proteins: Archaeobacteria



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.

HMfA and HMfB form both, homodimers and heterodimers.

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

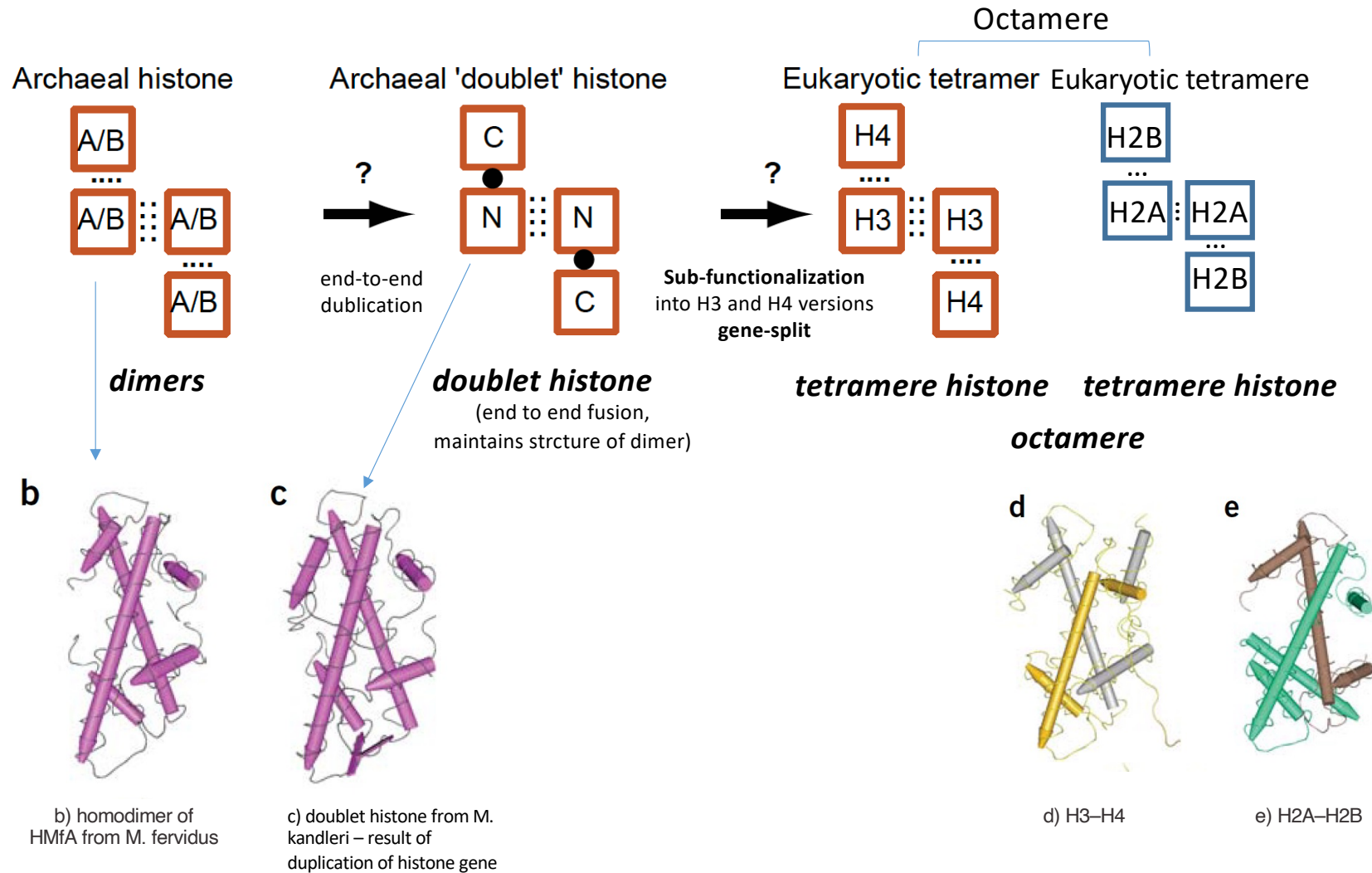
NOTE: Not all euryarchaeal lineages have a similar complement of two histones. The histone gene complement in completely sequenced euryarchaeal genomes varies from one to six genes

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: duplication requires less protein-protein interaction to form a «tetramere». N and C terminal portions can subfunctionalize

From Archaeobacteria to Eukaryote histone tetramers

A model for the evolution of 4 different eukaryotic histones octamers from 1 or 2 areacheal histone-like proteins



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

Archaeal and eukaryotic histones

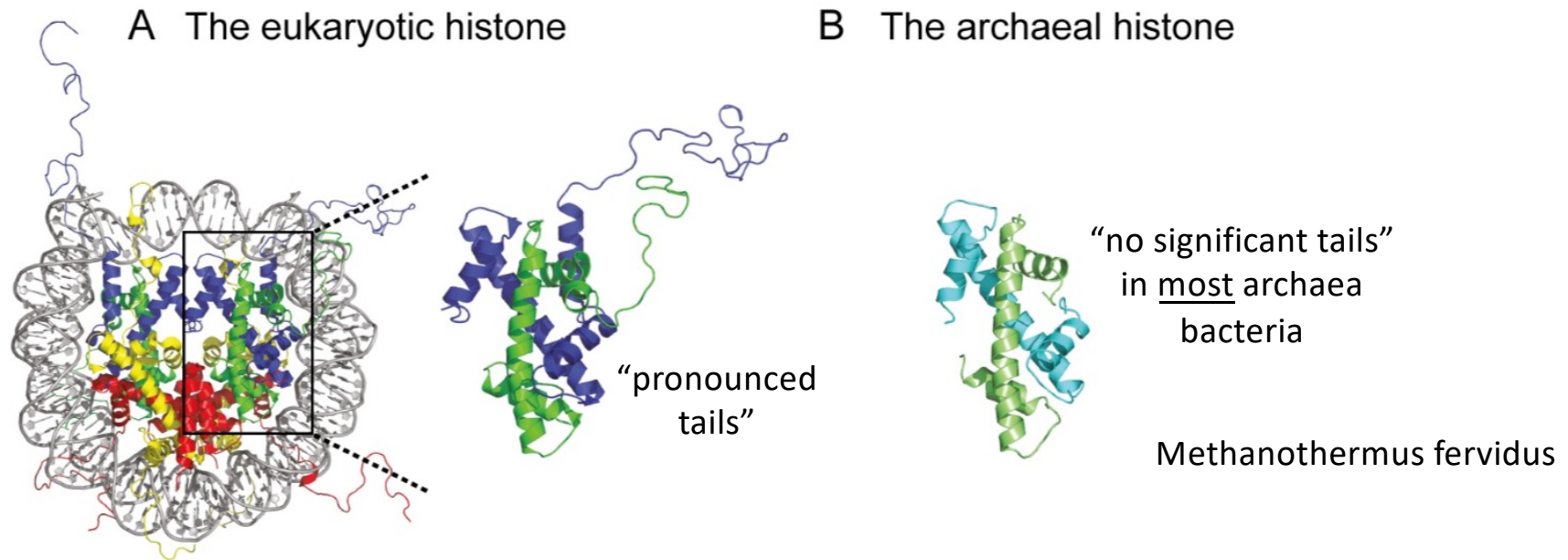


Fig 1. Eukaryotic and archaeal histones. (A) Eukaryotic nucleosome consisting of DNA wrapped around a core of a (H3-H4)₂ tetramer and two H2A-H2B dimers. Yellow, H2A; red, H2B; blue, H3; green, H4. (B) Archaeal histone homodimer of HMfB. HMfB, Histone B from *Methanothermobacter fervidus*.

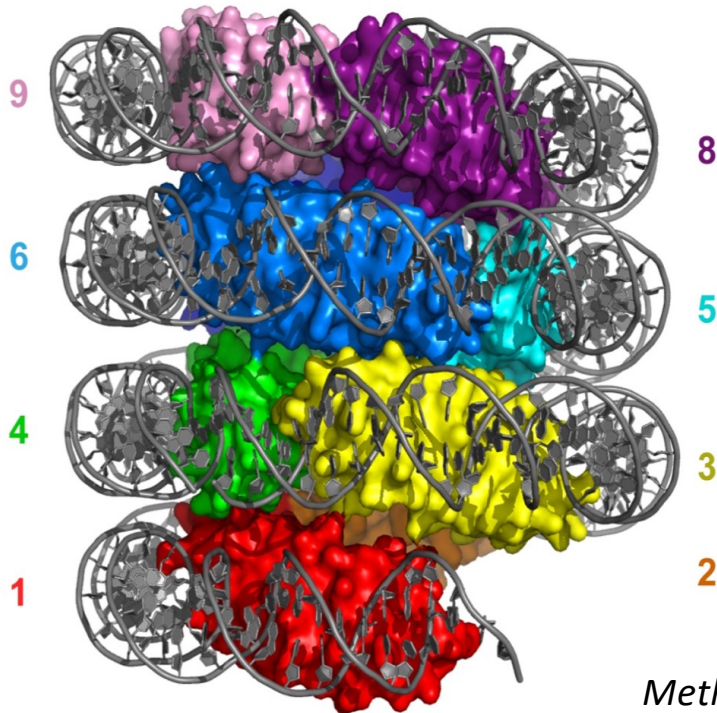
<https://doi.org/10.1371/journal.pgen.1007582.g001>

Although Archaea and Bacteria have common features, such as a circular genome and the absence of a nucleus, at the genetic level, Archaea seem to be more related to eukaryotes.

Amongst others, archaeal RNA polymerase, a key component of cellular life in all domains, is more similar to RNA polymerase from eukaryotes than bacterial RNA polymerase

The **hypernucleosome** in Archaea bacteria

Rod with 9 dimers



Methanothermobacter fervidus

Fig 2. Overview of the hypernucleosome structure. HMfB dimers stack to form a continuous, central protein core that wraps the DNA in a left-handed superhelix. Nine HMfB dimers are shown, each dimer in surface mode and in rainbow colors. Numbering indicates position of the nine histone dimers; note that dimer 5 and 6 occlude the view of dimer 7. DNA is in gray and shown as cartoon. *Image generated using PDB entry 5T5K [64].* HMfB, Histone B from *Methanothermobacter fervidus*; PDB, Protein Data Bank.

<https://doi.org/10.1371/journal.pgen.1007582.g002>

Histones (HMfB) assemble into an endless left-handed rod in vitro that bind DNA = “hypernucleosome”

DNA is wrapped around the protein rod

Histone–DNA complexes consist of discrete **multiples of a dimeric histone subunit that are not limited to dimers and tetramers** in vivo; without obvious dependence on the DNA sequence (no sequence specific binding)

10-fold compaction

Histones from Archaea and eukaryotes are similar in tertiary but **not in quaternary** structure when bound to DNA.

Eukaryotic histones: form octamers

Archaeal histones: form filaments of variable size = hypernucleosomes

Histone tails in Archaea bacteria hyper-nucleosomes

Most archaea bacteria do not show “no significant tails”

Exceptions exist: The tails of the two histones from *Heimdallarchaeota* and *Huberarchaea* are of roughly the same length and sequence composition as eukaryotic H4 tails

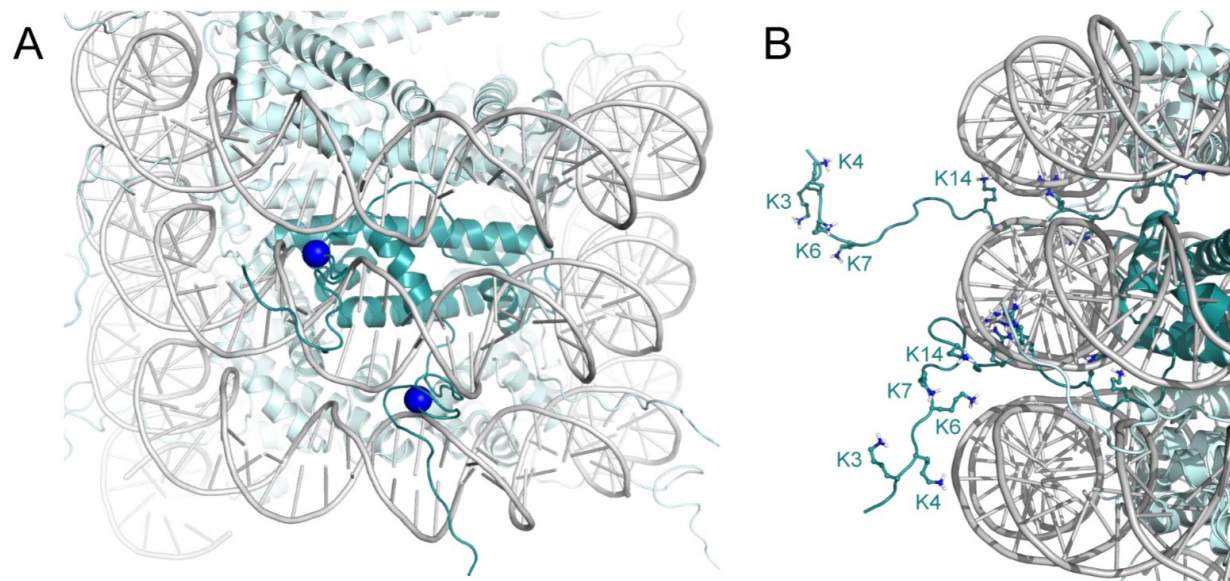


Fig 4. Model of a Heimdall LC₃ hypernucleosome with N-terminal tails. (A) View showing histone tails protruding through the DNA minor grooves. The R17 C α -atom is shown as a blue sphere to mark the exit point of the tail. (B) Close up of the histone tails with lysine and arginine residues shown as sticks, and N-terminal lysines are labeled. Homodimers of Heimdall LC₃ histone HA are shown in teal; one dimer is highlighted in darker colors. Models are based on the structure of HMfB (PDB entry 5T5K); the tail in the top (bottom) of panel B is modeled in the H3 (H4) tail conformation (PDB entry 1KX5). HA, Histone A; HMfB, Histone B from *Methanothermus fervidus*; PDB, Protein Data Bank.

<https://doi.org/10.1371/journal.pgen.1007582.g004>

Three subsequent arginines (R17–R19) could facilitate passing of the tails through the DNA gyres.

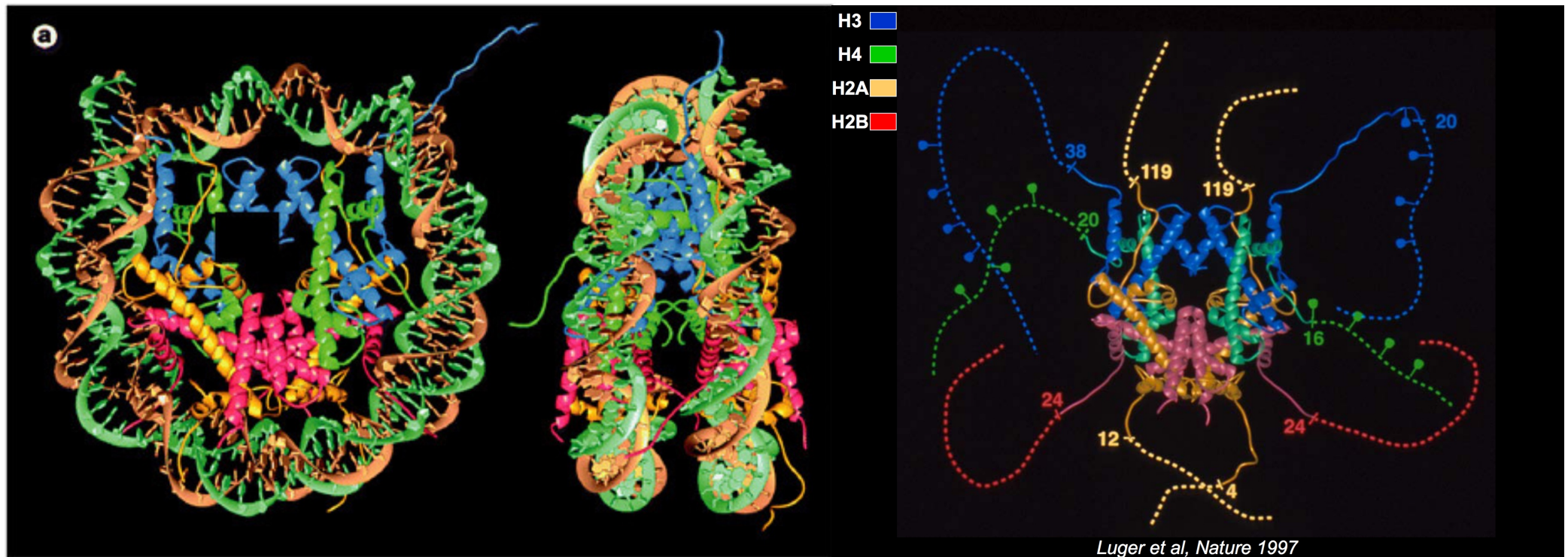
The **tails exit the hypernucleosome through DNA minor grooves**, similar to eukaryotic histone tails, and might position their lysine side **chains to bind to the hypernucleosomal DNA or to other DNA close by**, facilitating (long-range) genomic interactions *in trans*.

Like the H4 tail that is subject to acetylation of lysines K5, K8, K12, and K16, lysines in the Heimdallarchaeal histone tail may well be subject to acetylation.

Archaeal genomes are known to **have several candidate lysine acetyltransferase and deacetylase enzymes**.

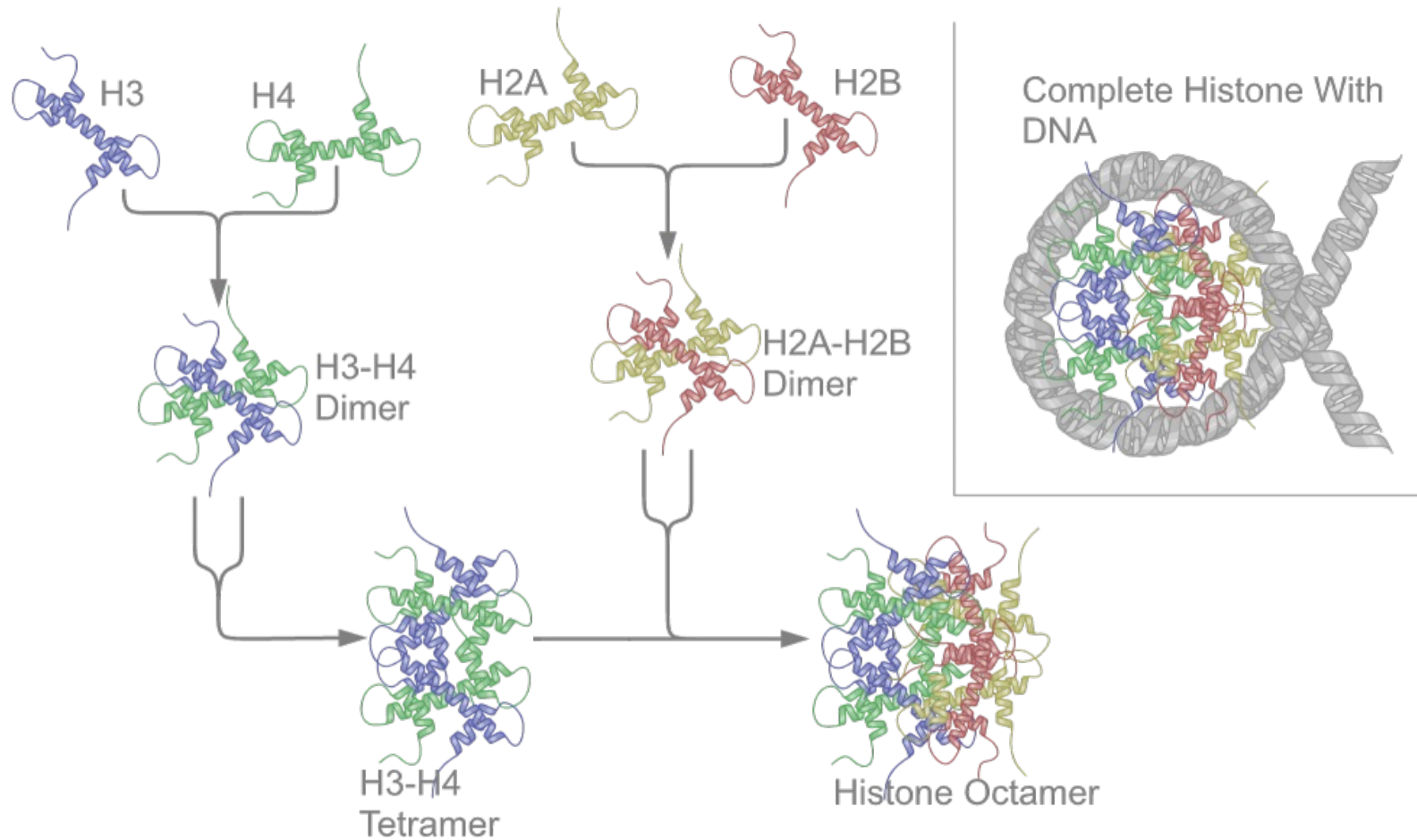
Not frequent, functional relevance not clear

The structure of the eukaryotic nucleosome



Luger et al., 1997

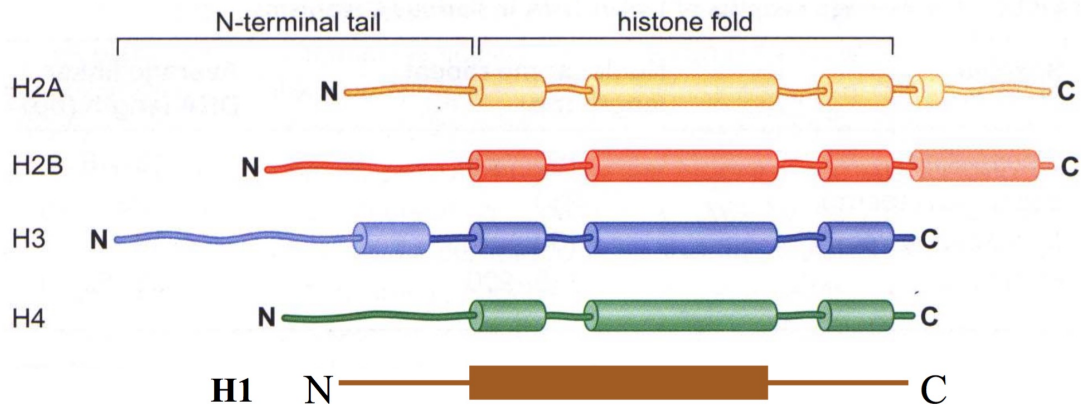
Heterodimerization + tetramerization = histone octamere formation



**Nucleosome
or
Nucleosome
Core Particle
(NPC):**

**2 wraps of DNA
around
Octamere: 147bp**

Eukaryotic histones have key structural domains



“Tail” domain

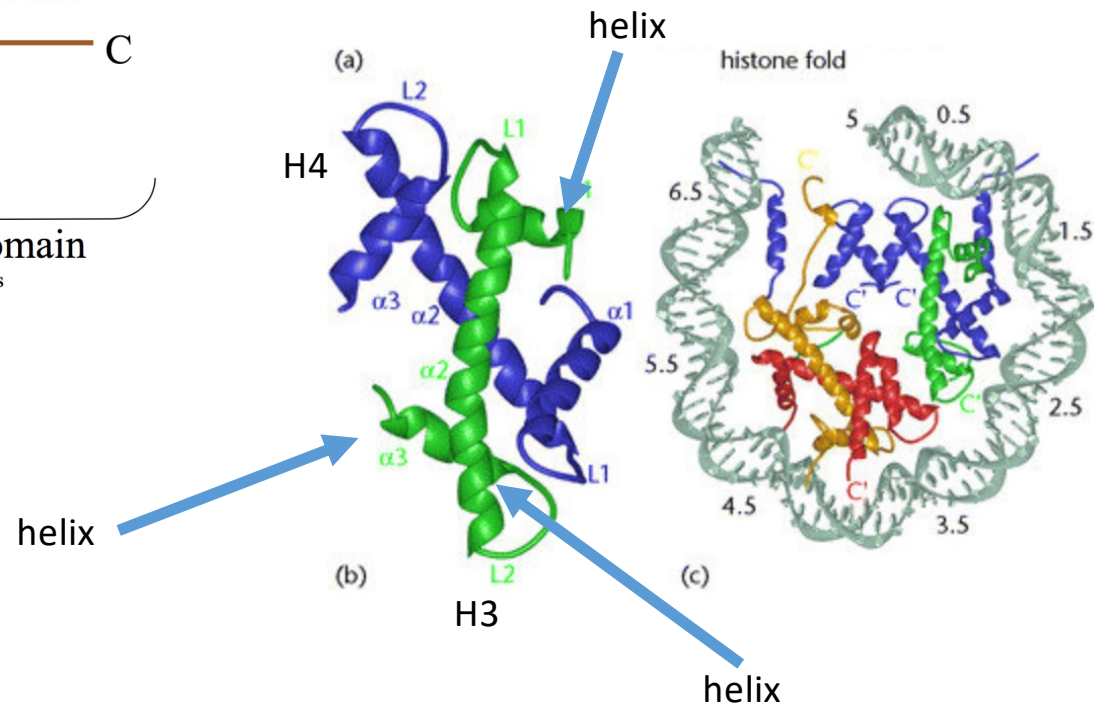
- Regulatory domain
- Involved in higher-order packing

“Globular” domain

- Histone-histone interactions
- DNA wrapping

The histone fold averages about 70 amino acids and consists of three alpha helices connected by two short, unstructured loops

‘helix turn helix turn helix’ motif (DNA-binding protein and protein interaction)



Properties of histone proteins; Interaction Histones - DNA

Types and Properties of Histones

Histone	Molecular weight (Da)	Number of amino acid residues	Content of basic amino acids (% of total)	
			Lys	Arg
H1*	21,130	223	29.5	1.3
H2A*	13,960	129	10.9	9.3
H2B*	13,774	125	16.0	6.4
H3	15,273	135	9.6	13.3
H4	11,236	102	10.8	13.7

core histones

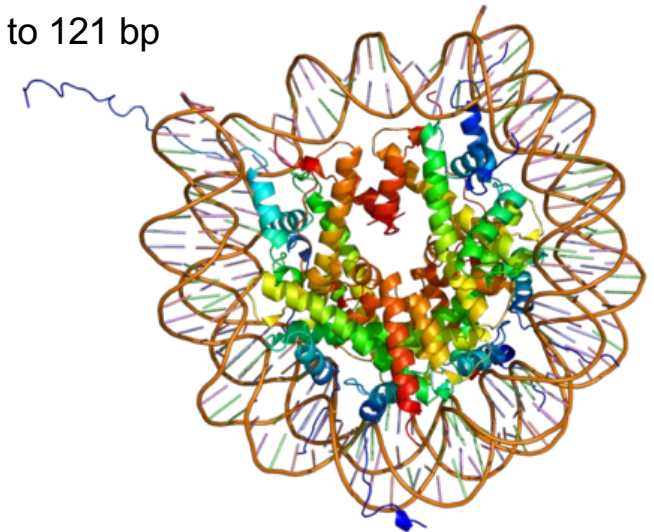
- **Helix-dipoles form alpha-helices in H2B, H3, and H4 cause a net positive charge to accumulate at the point of interaction with negatively charged phosphate groups on DNA**
- Hydrogen bonds between the DNA backbone and the amide group on the main chain of histone proteins
- Non-polar interactions between the histone and deoxyribose sugars on DNA
- Salt bridges and hydrogen bonds between side chains of basic amino acids (especially lysine and arginine) and phosphate oxygens on DNA
- Non-specific minor groove insertions of the H3 and H2B N-terminal tails into two minor grooves each on the DNA molecule

Histone proteins are basic

- They contain many **positively-charged amino acids Lysine and arginine**
- These bind with the phosphates along the DNA backbone (not sequence specific)

Contact histones - DNA

- Segment of DNA on nucleosome: 147bp
- 12 out of 14 possible contacts between minor groove and DNA
- Direct contact to 121 bp



Properties of histone proteins; Interaction Histones - DNA

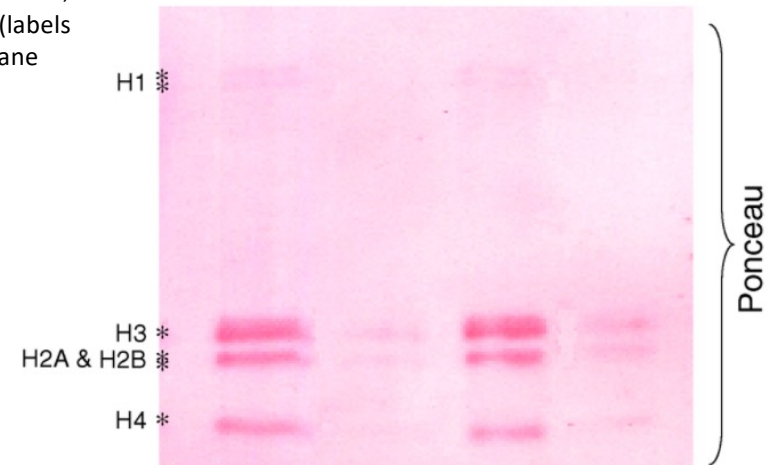
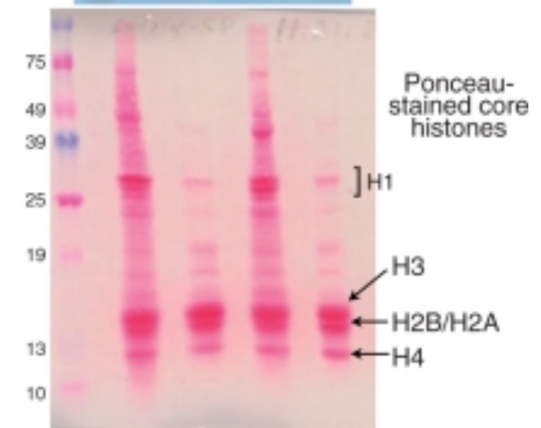
Meeting histones in lab → western blotting

Types and Properties of Histones

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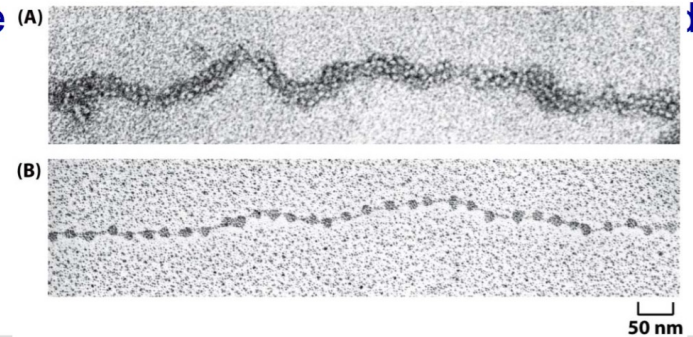
Da

High % PAA gel; run for a long time; proteins transferred to western blotting membrane; Stained with ponceau (labels proteins on membrane)

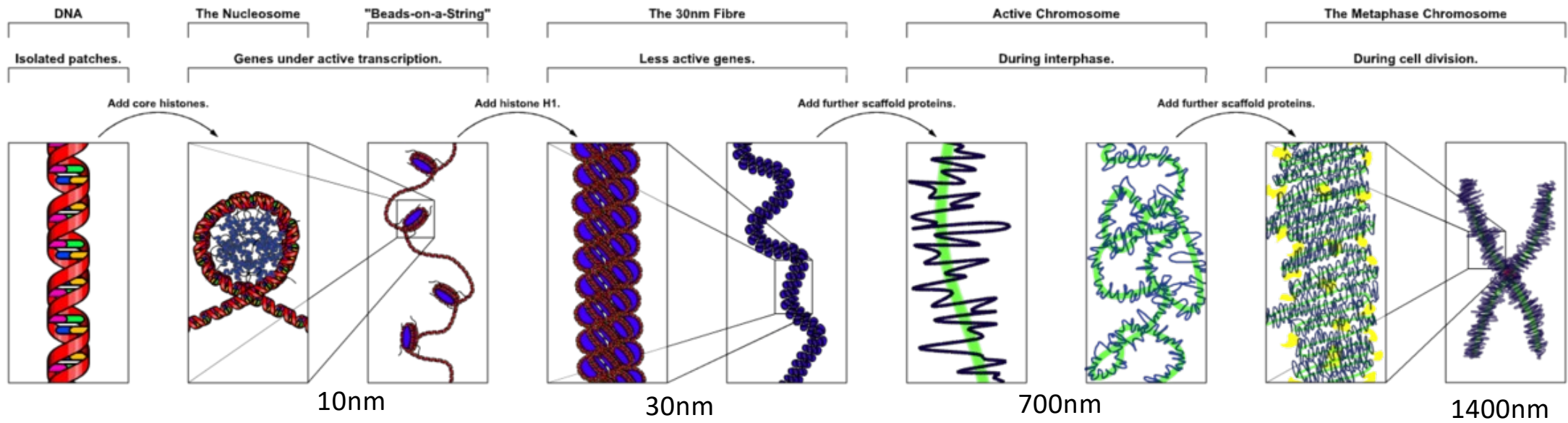


DNA Packaging by histones

- Long sequence of DNA must be stored within the geometry of a nucleus
 - Example: human chromosome 22, 48 million bp
 - Extends to length of ~ 1.5 cm
 - Measures $2\text{ }\mu\text{m}$ in mitosis
 - Packaging ratio on the level of 10^4 in mitosis
 - Packaging ratio ~ 500 in interphase
- Packaged DNA must provide for gene expression.

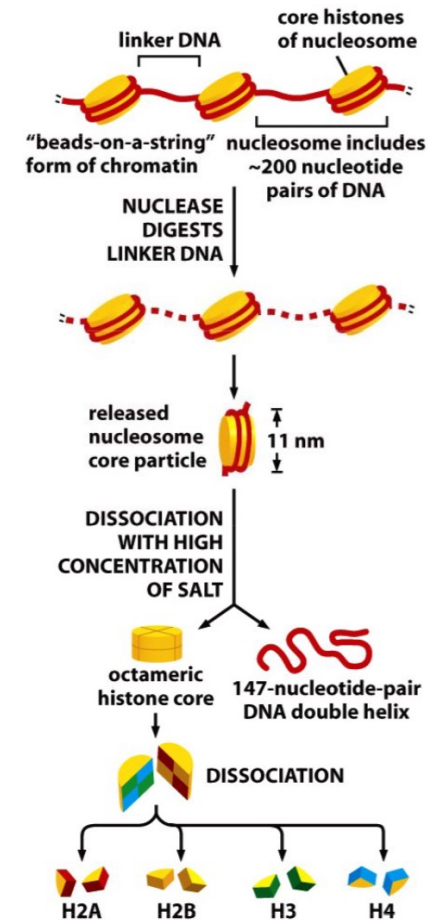


The major structures in DNA compaction



The 10nm fibre – Nucleosomes

- DNA is coiled around a protein core to form nucleosomes
- ~7 folds in packaging.
- Histone H2A, H2B, H3, H4 with 147 bp DNA.
- Nucleosomes repeat at every 200 bp. So ~30 million nucleosomes in a human cell.
- Total mass of histones approximately equal to that of DNA.



Nucleosomes are repeating units

Figure 19.7 Micrococcal nuclease digests chromatin in nuclei into a multimeric series of DNA bands that can be separated by gel electrophoresis. Photograph kindly provided by Markus Noll.

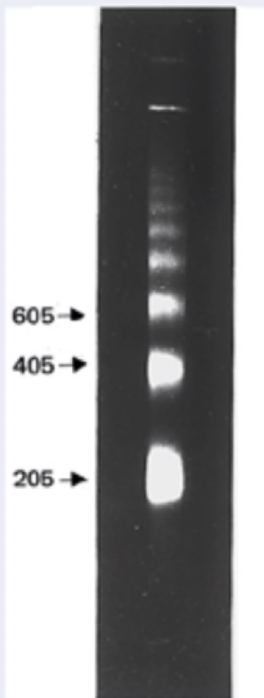
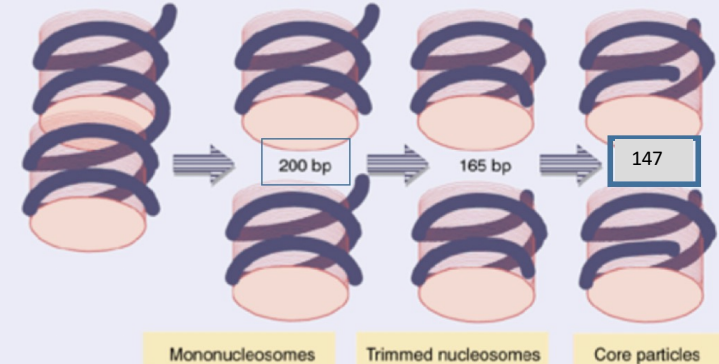


Figure 19.10 Micrococcal nuclease initially cleaves between nucleosomes. Mononucleosomes typically have ~200 bp DNA. End-trimming reduces the length of DNA first to ~165 bp, and then generates core particles with 146 bp.

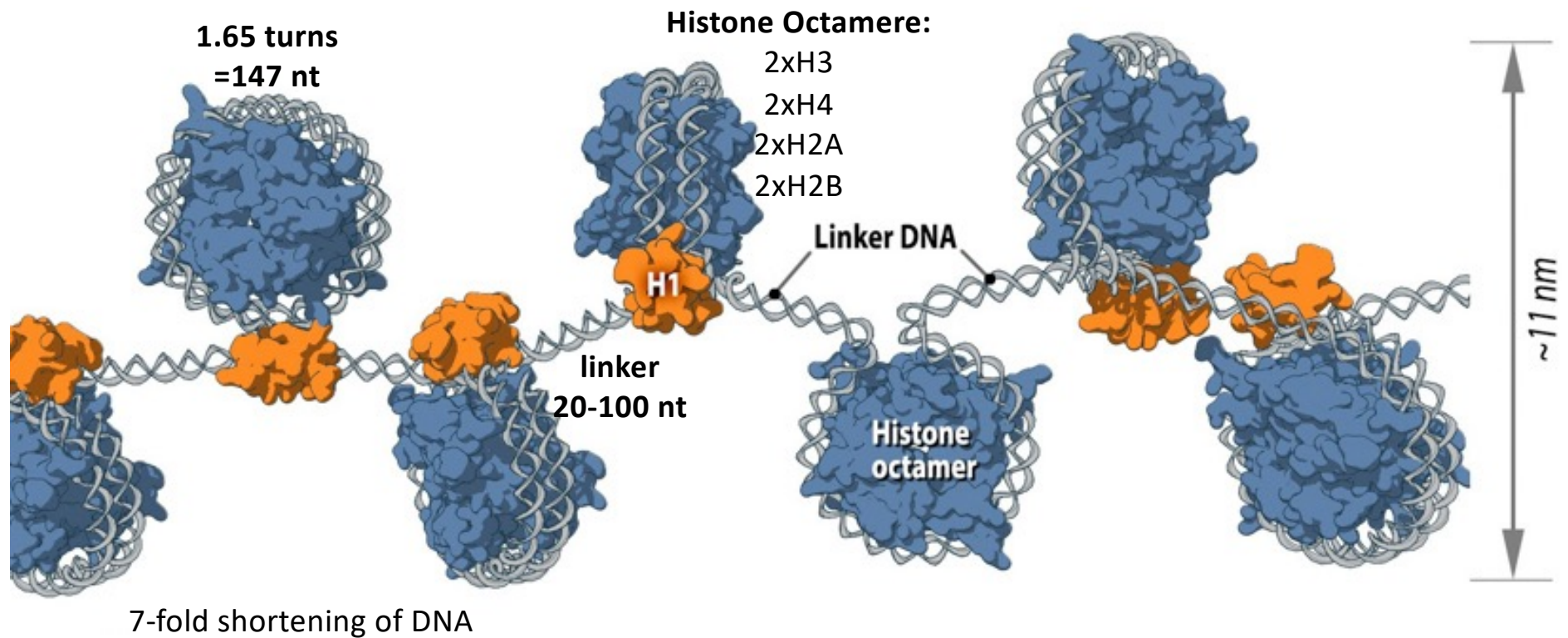


DNA at nucleosomes that is accessible can be digested by DNases

1. Prepare chromatin
2. Digest with Micrococcal nuclease (DNase) at concentration that does not allow full digestion of available DNA
3. Gel electrophoresis
→ Fragment size: 206, 405, 605 (repeating units)
4. Proceed with end trimming using exonucleases:
→ Fragment size: 147bp

Strong contacts between histones and DNA: inaccessible to DNases

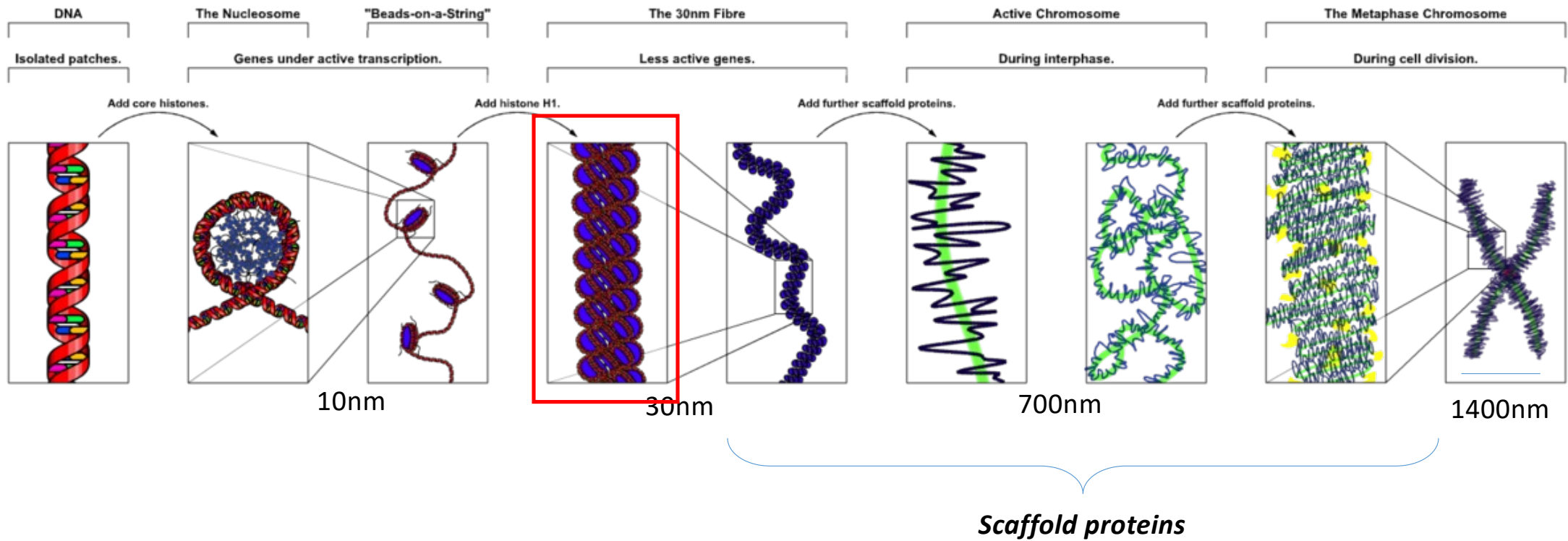
Histone H1 compacts chromatin to the 30nm fibre



Nucleosome core particle: 147nt dsDNA + CORE HISTONE PROTEINS 2xH3, 2xH4, 2xH2A, 2xH2B

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

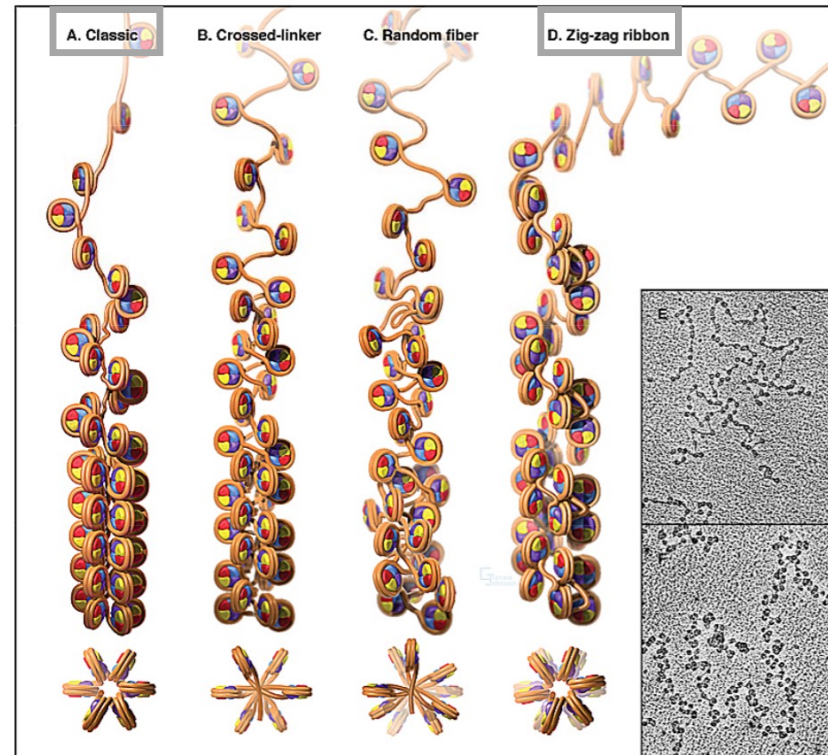
The major structures in DNA compaction



Histone H1 compacts chromatin to the 30nm fibre

The structure of the 30-nm chromatin fiber remains unclear

- Nucleosomes are further packaged into 30-nm fibers.
- The precise structure of the 30-nm fiber is not yet known.
- Chromatin structure beyond nucleosomes is poorly understood.

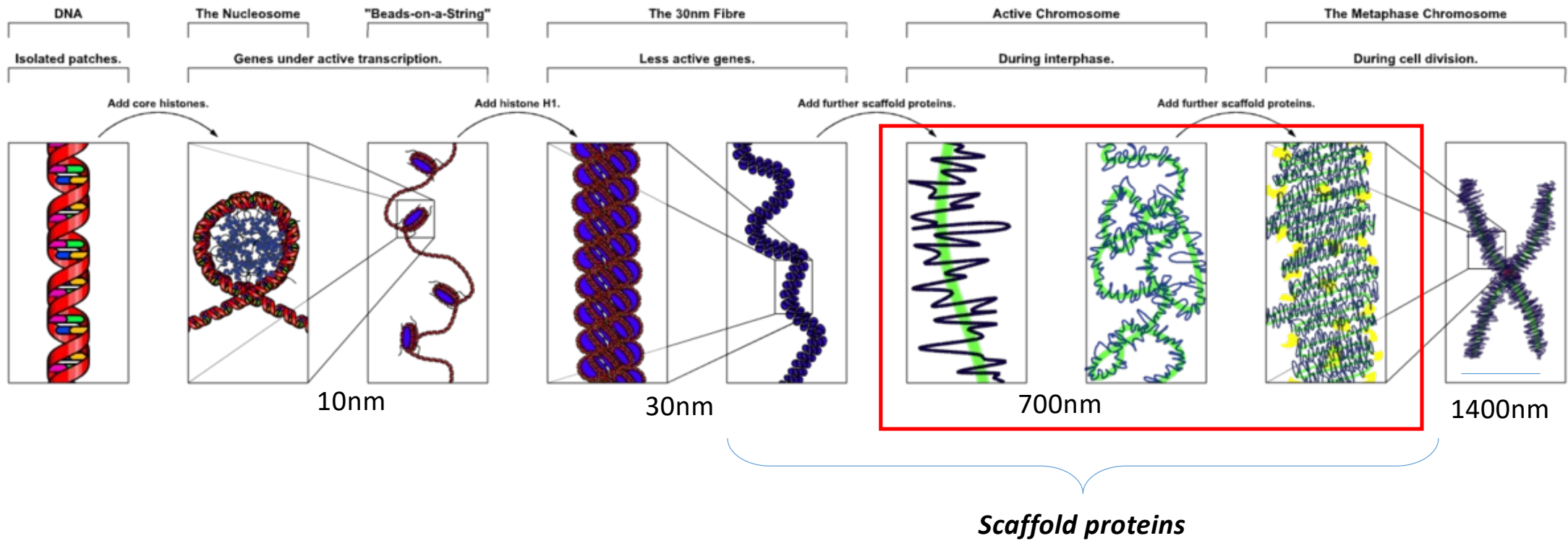


Different models of the 30-nm fiber

- ~40 folds in packaging.

30-nm fiber most likely represents a delicate balance of different configurations that can be modified by a number of factors, including but not limited to, deposition of linker histones and high mobility group (HMG) proteins, incorporation of variant histones, modifications of histone tails, activity of chromatin remodeling factors, presence of phased nucleosome arrays

The major structures in DNA compaction

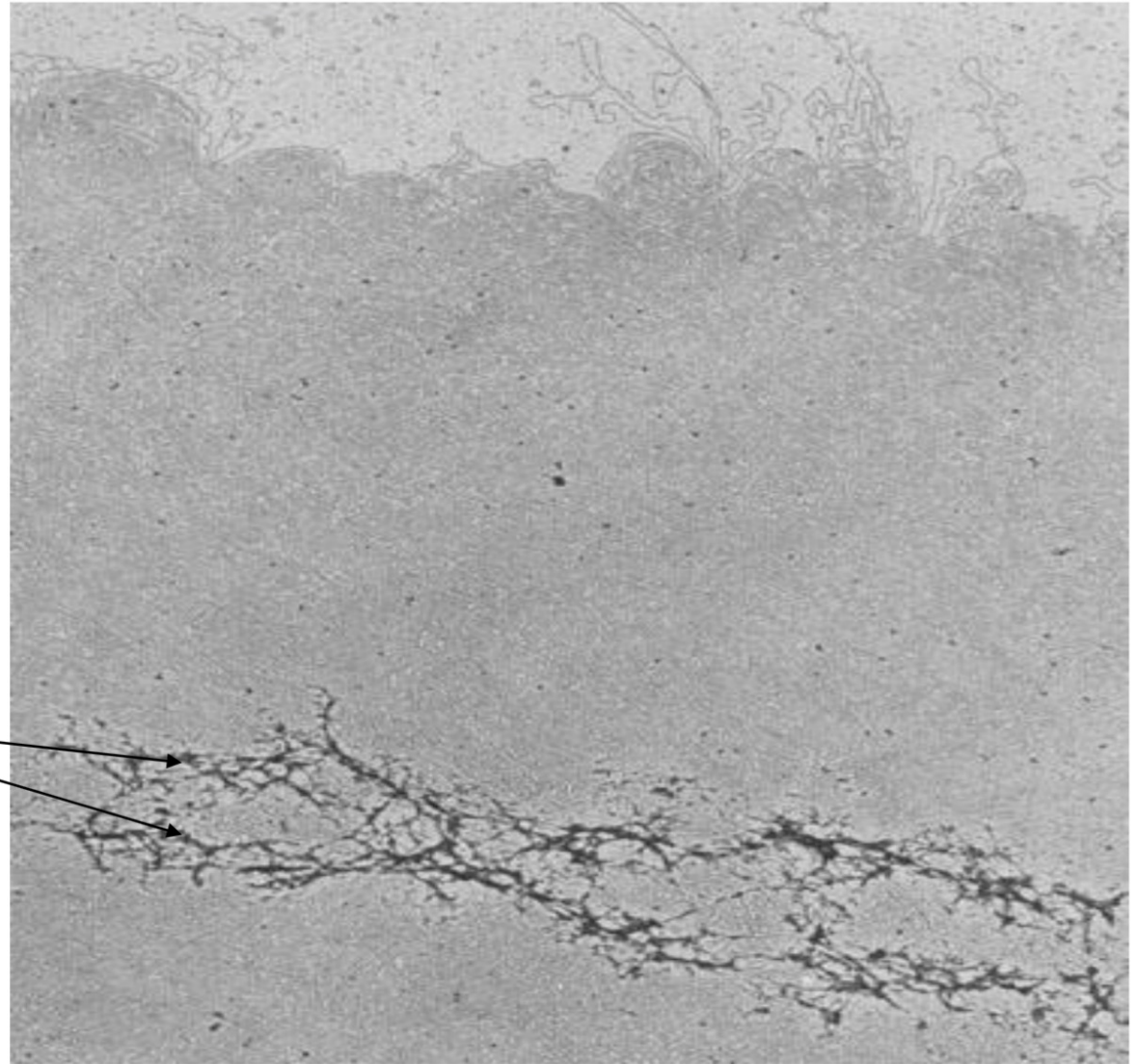


A protein scaffold is required to further compact the genome towards metaphase chromosome

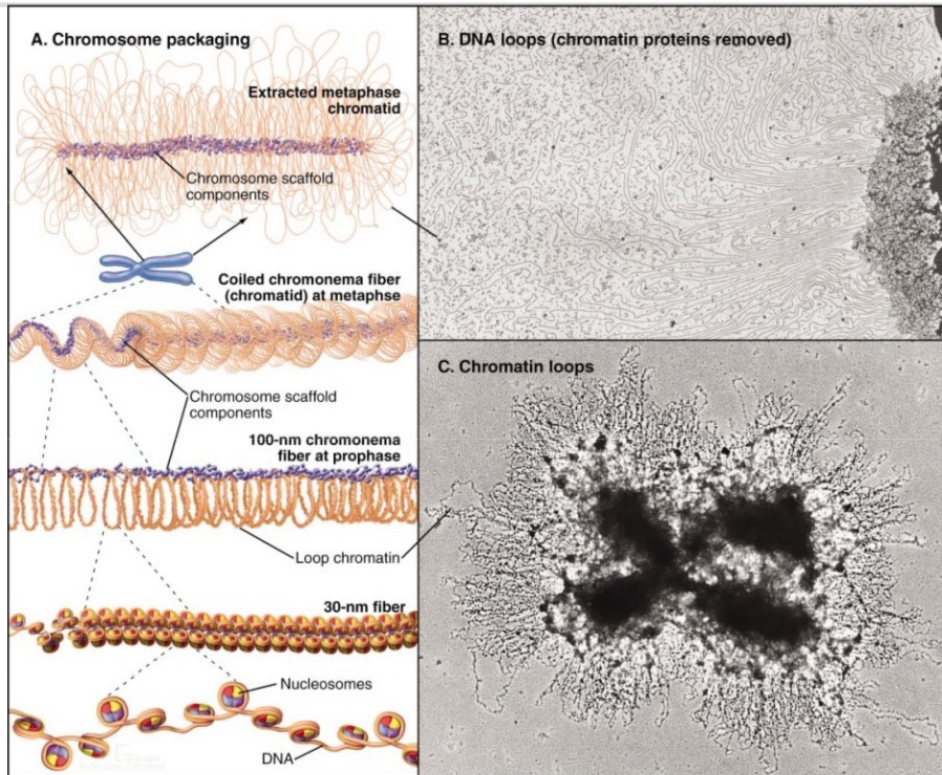
Histone depleted chromosomes

Loops of DNA

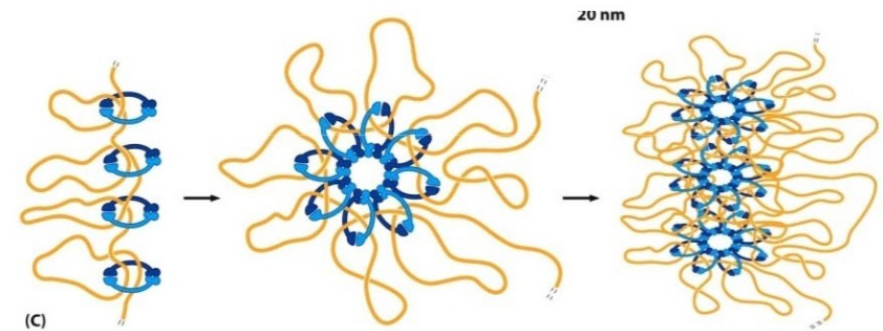
Protein scaffold



A protein scaffold is required to further compact the genome

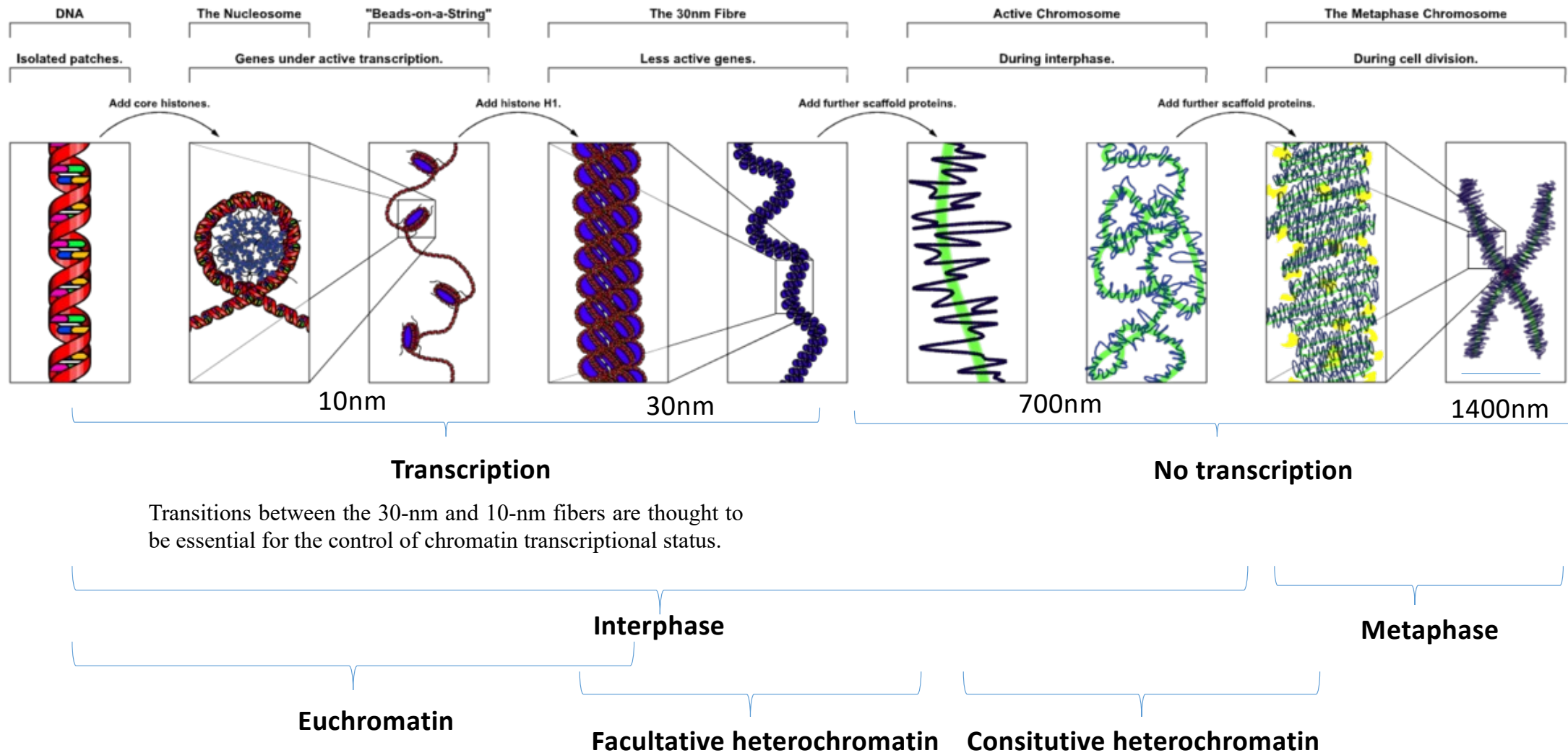


Model of mitotic chromosomes



The axially-positioned chromosome scaffold of both chromatids mainly comprises non-histone proteins: so-called scaffold proteins, including **condensin** (remember bacteria), **Topoisomerase II α** (Topo II α) and Kinesin family member 4

The major structures in DNA compaction



Histones

Replication dependent, canonical histones

- Gene organization
- Gene expression control mechanisms in S-phase
- Histone chaperons

Replication independent histones – histone variants

- A list of histone variants
- Histone variant at centromere
- Histone H3.3 a variant with multiple function
- Histone gammaH2AX and DNA damage
- Histone variants with chromosome specific accumulation

Coordinated control of histone gene expression and incorporation into DNA

1. Histone and cell cycle regulation:

Maintaining a stable and balanced histone pool is of vital importance for appropriate gene regulation, cell cycle progression and genome stability. Excess of free histones has damaging effect → precise control of histone gene expression required → connected to cell cycle regulation

2. Controlled production of nucleosomes:

A. REPLICATION DEPENDENT REGULATION: Canonical histone proteins: mRNA non-polyadenylated; defined RNA processing mechanisms; expression regulated in histone locus body. Histone mRNA levels increase ca 40-fold increase at the level of histone mRNAs during S phase. At the end of S phase or DNA synthesis is interrupted, cells turned off histone transcription and histone mRNA levels declined rapidly. Replication dependent histone chaperons are linked to DNA polymerase and generate nucleosomes in S-Phase

B. REPLICATION INDEPENDENT REGULATION: histone variants: Classic polyadenylylated RNAs Nucleosome need also be formed in G1, G2 phase when nucleosome arrays get disturbed (transcription, DNA damage, eviction, etc...). The second class of histones (non canonical histones) is composed of **histone variants that are expressed at a relatively low level throughout the cell cycle, and are therefore regulated in a DNA replication-independent manner. Histone variants have specialized functions!!**

Histones

Replication dependent, canonical histones

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Replication independent histones – histone variants

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- Histone variants with chromosome specific accumulation

Histone gene clusters in vertebrates – Canonical histones

Histone genes are organized in histone gene clusters

Human:

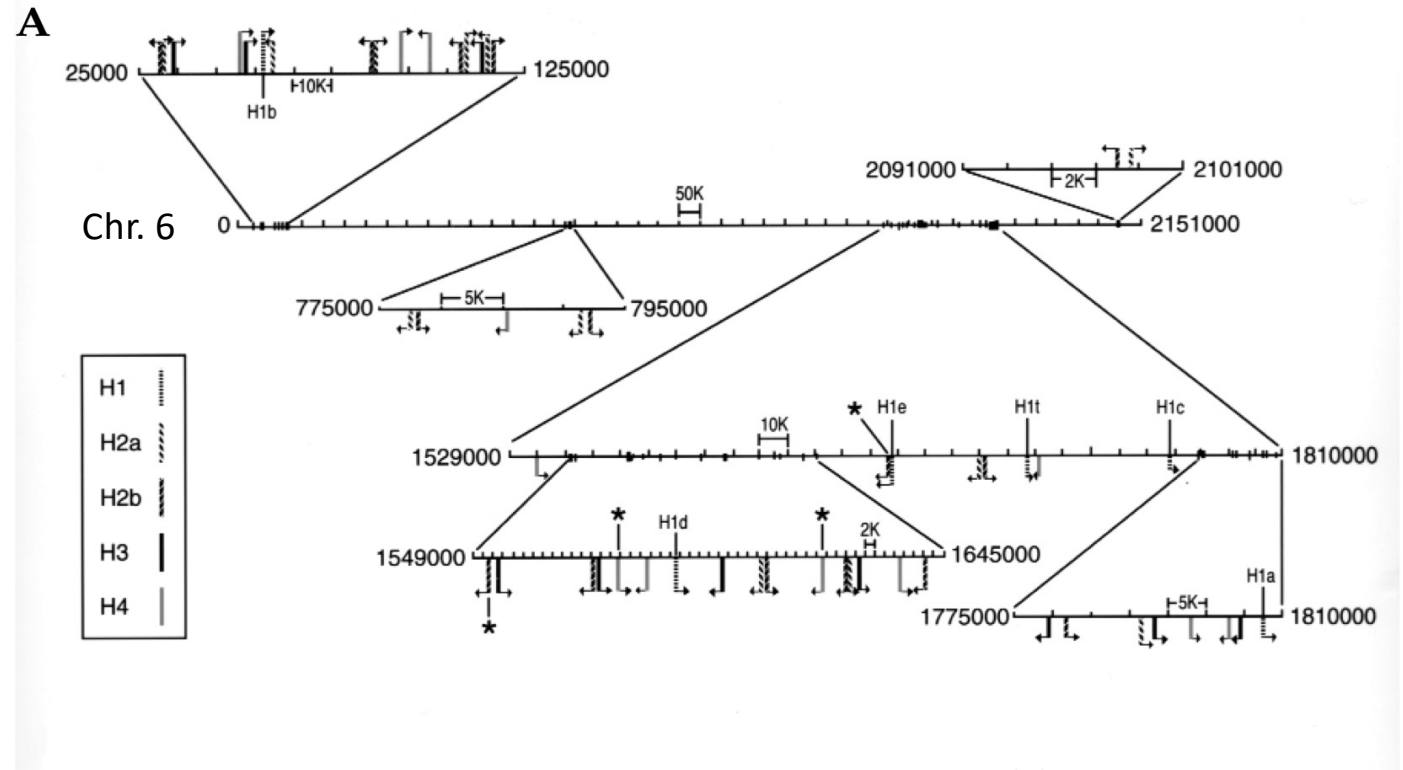
***Chr.6*: major histone gene locus: HIST1 cluster: 45 core histone genes ; 6 Histone H1 genes**

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

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

Yeast:

2 copies for each core histone



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 proteins 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 centromere 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 indicated with the nomenclature H1a–e, H1t, and the symbols for the core histone genes are in the inset. The asterisks indicate the position of genes present in human and not in mouse.

Histone genes are organized in clusters in vertebrates

Gene group: H3 histones (H3) ?

A subgroup of ? "Histones"

Gene group hierarchy map ?

Histone H3: Histone H3 is one of the five main histone proteins involved in the structure of chromatin in eukaryotic cells. Featuring a main globular domain and a long N-terminal tail, H3 is involved with the structure of the nucleosomes of the 'beads on a string' structure. Histone proteins are highly post-translationally modified however Histone H3 is the most extensively modified of the five histones. The term "Histone H3" alone is purposely ambiguous in that it does not distinguish between sequence variants or modification state. Histone H3 is an important protein in the emerging field of epigenetics, where its sequence variants and variable modification states are thought to play a role in the dynamic and long term regulation of genes. [Source: Wikipedia]

The mapped domains of P68431, encoded by the **H3C1** gene, an example gene within the group. [Source: Pfam & UniProt]

In vertebrates, 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**, as well as **nucleotide changes** in the coding region (but same AA sequence; silent!)

All histone **H4** genes **encode the same protein** (no variants),
Some histone H3 genes **can** encodes variant histones with aa changes (i.e. H3.3,...)

10–12 different H2A and H2B proteins are known

HGNC ID (gene)	Approved symbol	Approved name	Previous symbols	Aliases	Chromosome
HGNC:4766	H3C1	H3 clustered histone 1	H3FA, HIST1H3A	H3/A	6p22.2
HGNC:4776	H3C2	H3 clustered histone 2	H3FL, HIST1H3B	H3/I	6p22.2
HGNC:4768	H3C3	H3 clustered histone 3	H3FC, HIST1H3C	H3/c, H3.1	6p22.2
HGNC:4767	H3C4	H3 clustered histone 4	H3FB, HIST1H3D	H3/b	6p22.2
HGNC:54427	H3C5P	H3 clustered histone 5, pseudogene			6p22.2
HGNC:4769	H3C6	H3 clustered histone 6	H3FD, HIST1H3E	H3/d, H3.1	6p22.2
HGNC:4773	H3C7	H3 clustered histone 7	H3FI, HIST1H3F	H3/i	6p22.2
HGNC:4772	H3C8	H3 clustered histone 8	H3FH, HIST1H3G	H3/h	6p22.2
HGNC:18982	H3C9P	H3 clustered histone 9, pseudogene	HIST1H3PS1	dJ45P21.6, H3F3AP1, p36	6p22.2
HGNC:4775	H3C10	H3 clustered histone 10	H3FK, HIST1H3H	H3/k, H3F1K	6p22.1
HGNC:4771	H3C11	H3 clustered histone 11	H3FF, HIST1H3I	H3/f, H3.1	6p22.1
HGNC:4774	H3C12	H3 clustered histone 12	H3FJ, HIST1H3J	H3/j	6p22.1
HGNC:25311	H3C13	H3 clustered histone 13	HIST2H3D		1q21.2
HGNC:20503	H3C14	H3 clustered histone 14	H3F2, H3FM, HIST2H3C	MGC9629, H3/m, H3, H3.2, H3/M	1q21.2
HGNC:20505	H3C15	H3 clustered histone 15	HIST2H3A	H3/n, H3/o	1q21.2
HGNC:43735	H3Y1	H3.Y histone 1		H3.Y, H3.Y.1	5p15.1
HGNC:43734	H3Y2	H3.Y histone 2		H3.X, H3.Y.2	5p15.1
HGNC:1851	CENPA	centromere protein A		CENP-A, CenH3	2p23.3
HGNC:32060	H3-2	H3.2 histone (putative)	HIST2H3PS2	p06	1q21.1
HGNC:4764	H3-3A	H3.3 histone A	H3F3, H3F3A	H3.3A	1q42.12
HGNC:4765	H3-3B	H3.3 histone B	H3F3B	H3.3B	17q25.1
HGNC:4778	H3-4	H3.4 histone	H3FT, HIST3H3	H3t, H3/g, H3.4	1q42.13
HGNC:33164	H3-5	H3.5 histone	H3F3C	H3.5	12p11.21

Canonical H3

Histone H3 variants with special function

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

Coordinated control expression of replication dependent histones

S. cerevisiae

Structure of yeast canonical histone genes

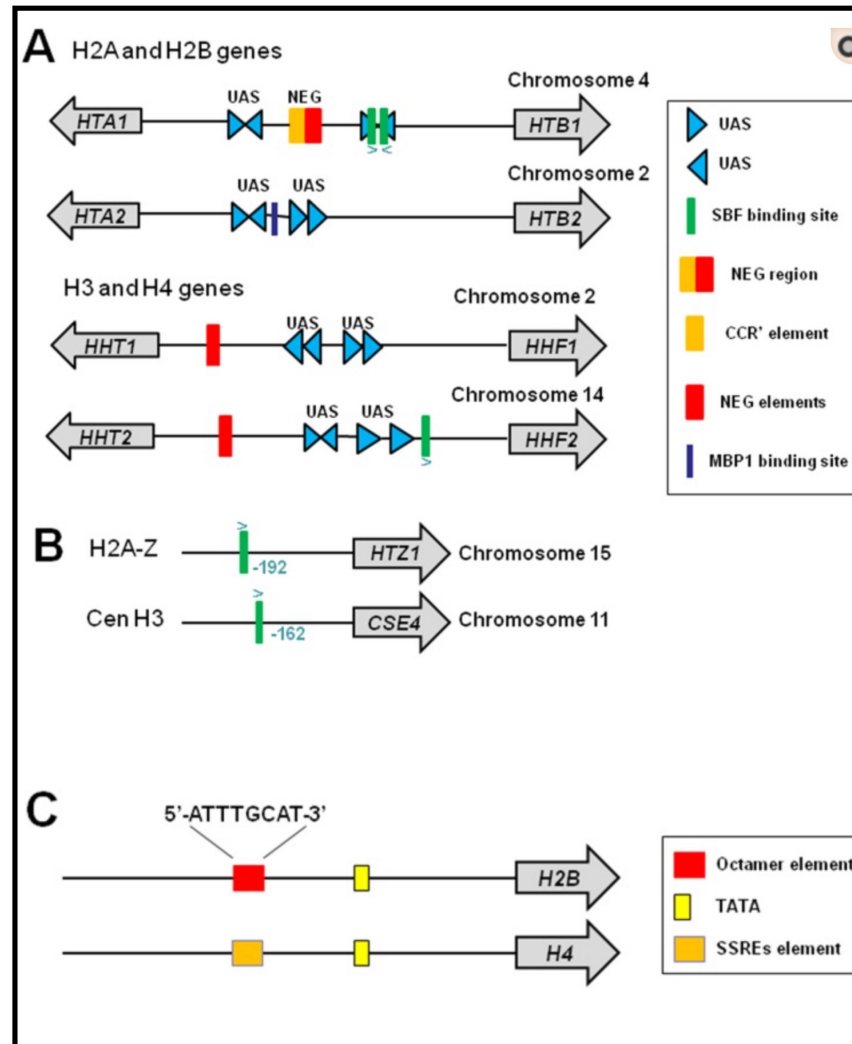
The core histone gene promoters contain specialized DNA elements that enable cis-regulation of histone gene expression: UAS (upstream activating sequence) and NEG (negative regulation of expression). **Divergent arrangement** of histone promoters allows coordinated gene expression to get equal amount of all four core histones.

Structure of yeast histone variants H2AZ and CenH3.

H. sapiens

Structure of mammalian canonical histone genes

Histone promoters also contain specialized cis elements that required for histone gene expression. Histone H2B promoter contains an octamer element (5'-ATTGTCAT-3'), which is bound by transcription activator Oct-1 (octamer-binding factor 1). Histone H4 promoter contains subtype-specific regulatory elements (SSREs)



HTA1-HTB1 and HTA2-HTB2: encode H2A-H2B pairs

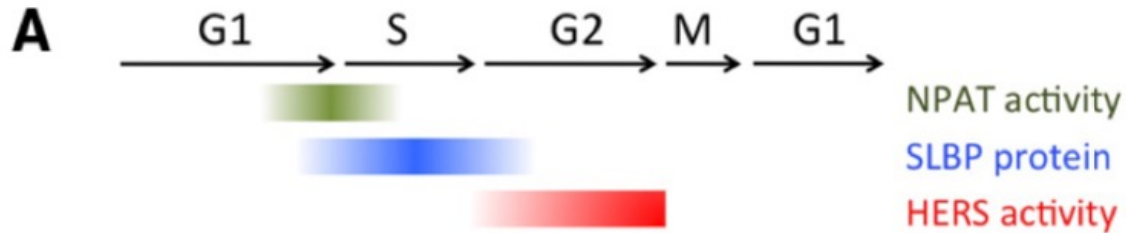
HHT1-HHF1 and HHT2-HHF2: encode H3-H4 pairs;

SBF: transcription factor that drives histone gene expression at G1/S transition

MBF is required for histone gene activation

CONTROL OF HISTONE EXPRESSION IN S-PHASE (replication dependent histones)

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**

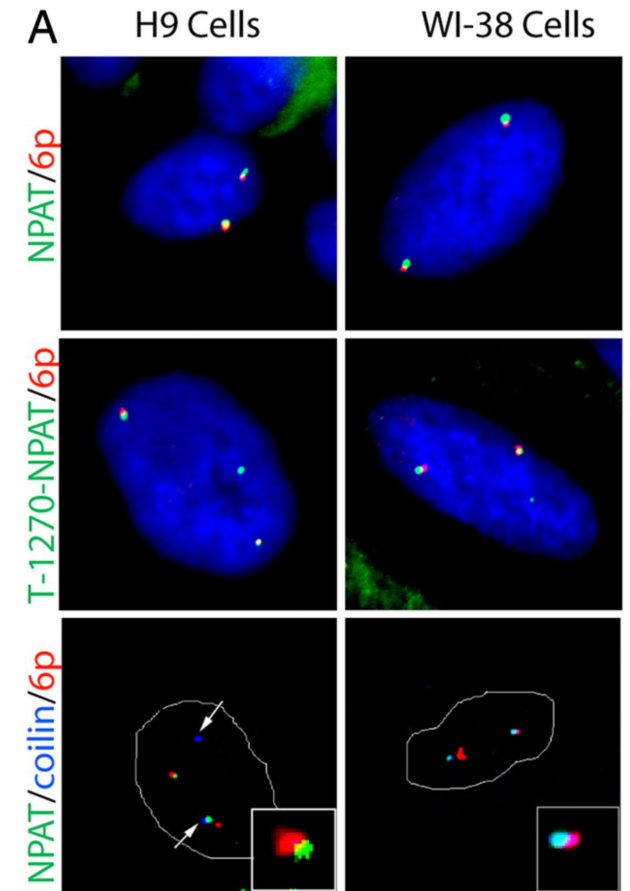
G1 → S transition:

- Transcription increases 5-fold during the G1 to S phase transition
- RNA processing enhanced RNA levels 8-fold
- Total increase: 30–40-fold increase of mature histone mRNAs.
- Production of 400 mio histone proteins during S-phase (ca. 8 hrs)

S → G2 transition:

At the end of S phase, the half-life of these histone mRNAs drops dramatically in response to a destabilization of the specific 3' ends.

Coilin = most abundant proteins in Cajal bodies = site for chemical RNA modifications and RNA:Protein complex assembly)



Human cells in S-phase

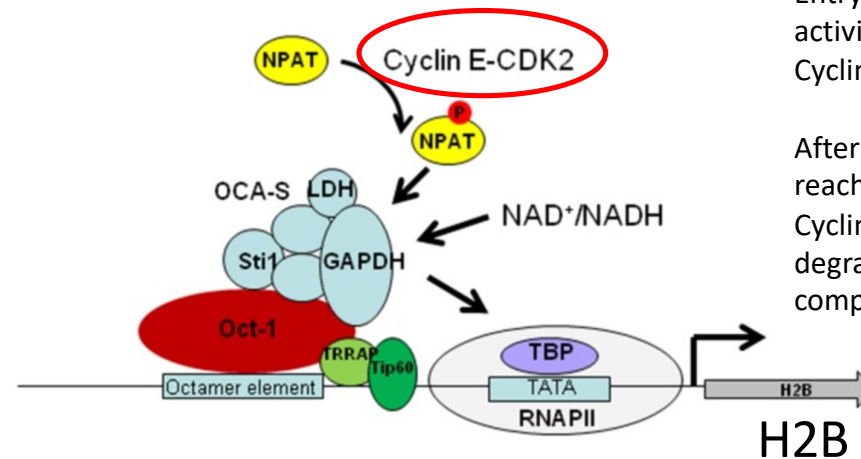
IF staining for NPAT (**GREEN**) combined with DNA FISH for the HIST1 cluster on Chr.6: **RED**

CONTROL OF HISTONE EXPRESSION IN S-PHASE – TRANSCRIPTIONAL REGULATION

NPAT – TRANSCRIPTIONAL ACTIVATION

Histone synthesis is limited to S-Phase

Activation of histone H2B. Oct-1 binds to octamer elements in H2B promoter. During S phase, activated cyclin E/CDK2 complex phosphorylates NPAT. In combination with NPAT, Oct-1 recruits OCA-S to H2B promoter to activate the expression of H2B.

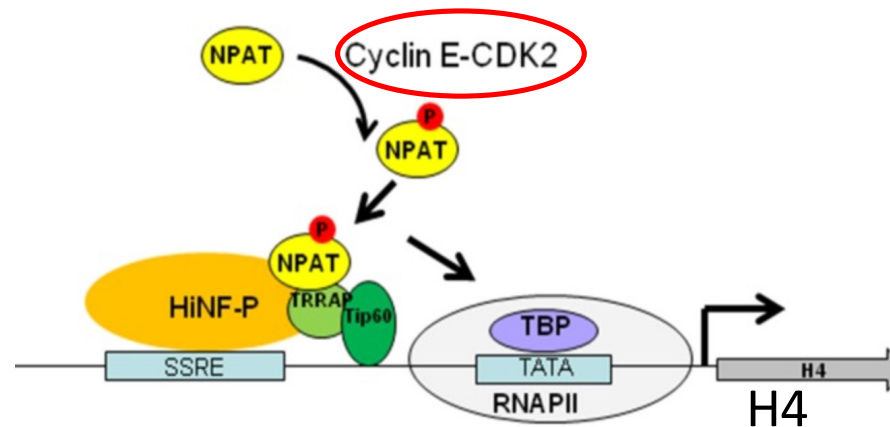


CDK2 and the cell cycle:

Entry into S-phase is triggered by the activity of the G1-S Cyclin complex, CyclinE/Cdk2.

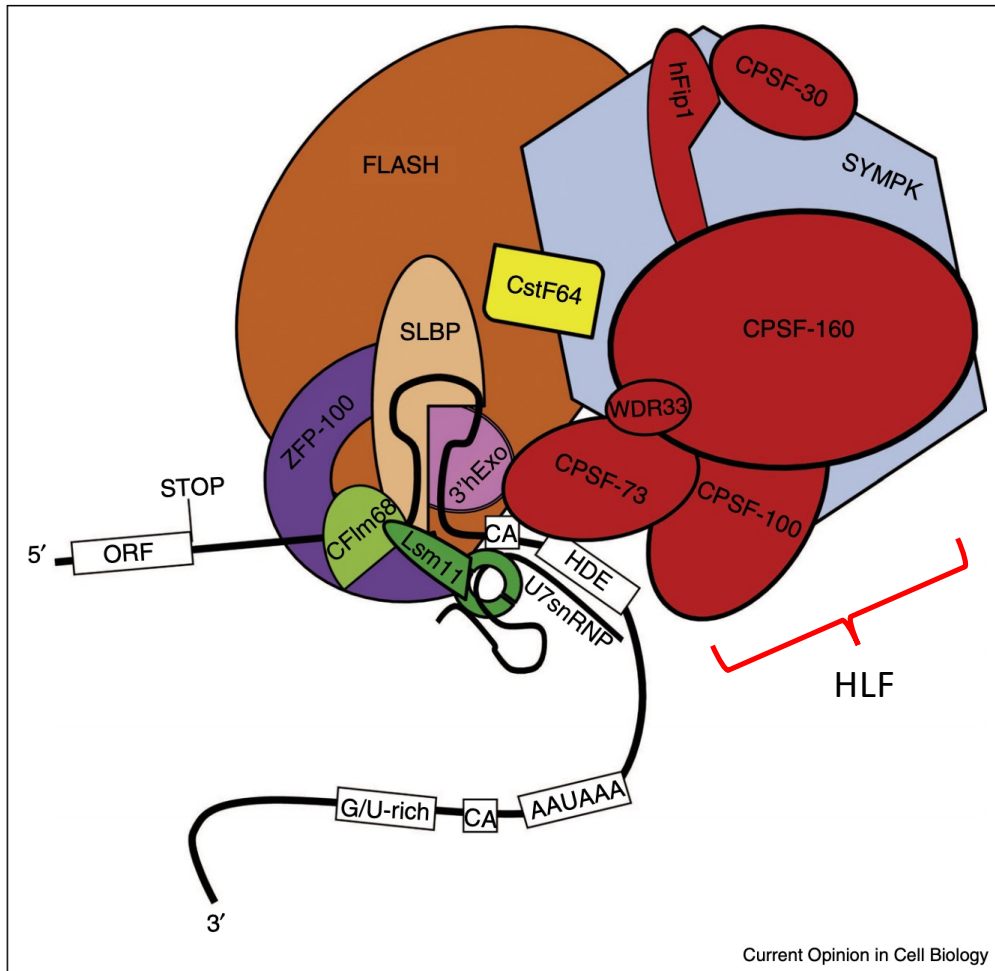
After CyclinE/Cdk2 activity has reached its peak in early S-phase, CyclinE/Cdk2 activity drops due to the degradation of the essential CyclinE component

Activation of histone H4. HiNF-P binds to SSRE within H4 promoters and recruits NPAT and RNA polymerase II to activate gene transcription. NPAT recruits the Tip60 histone acetyltransferase complex to acetylate histone H4 at the G1/S-phase transition. At the end of S phase, the tyrosine kinase WEE1 is recruited to histone promoters to phosphorylate H2B tyrosine 37, which evicts NPAT and RNA polymerase II and instead recruits HIRA to repress histone gene expression.



NPAT, Nuclear Protein Ataxia-Telangiectasia Locus; RNAPII, RNA polymerase II; TRRAP, transformation/transactivation domain-associated protein; SSRE, subtype-specific regulatory elements; OCA-S, Oct-1 co-activator in S-phase; HiNF-P, histone nuclear factor P; TBP, TATA-box binding protein.

CONTROL OF HISTONE EXPRESSION IN S-PHASE – REPLICATION DEPENDENT HISTONE RNA PROCESSING (RHP)



Schematic view of the replication-dependent histone RNA processing complex

2 conserved mRNA processing signals 50 nucleotides downstream of the histone ORF stop codon:

- stem-loop element: recognized by stem-loop binding protein (**SLBP**)
- purine-rich histone downstream element (HDE): paired by the RNA component of the **U7 snRNP** (RNA-Protein complex)

A 100 kDa zinc finger protein (ZFP-100) forms a connection between these two factors and stabilizes the complex.

The U7 snRNP contains a ring-like structure composed of five Sm proteins and the U7-specific Sm-like proteins, Lsm10 and Lsm11.

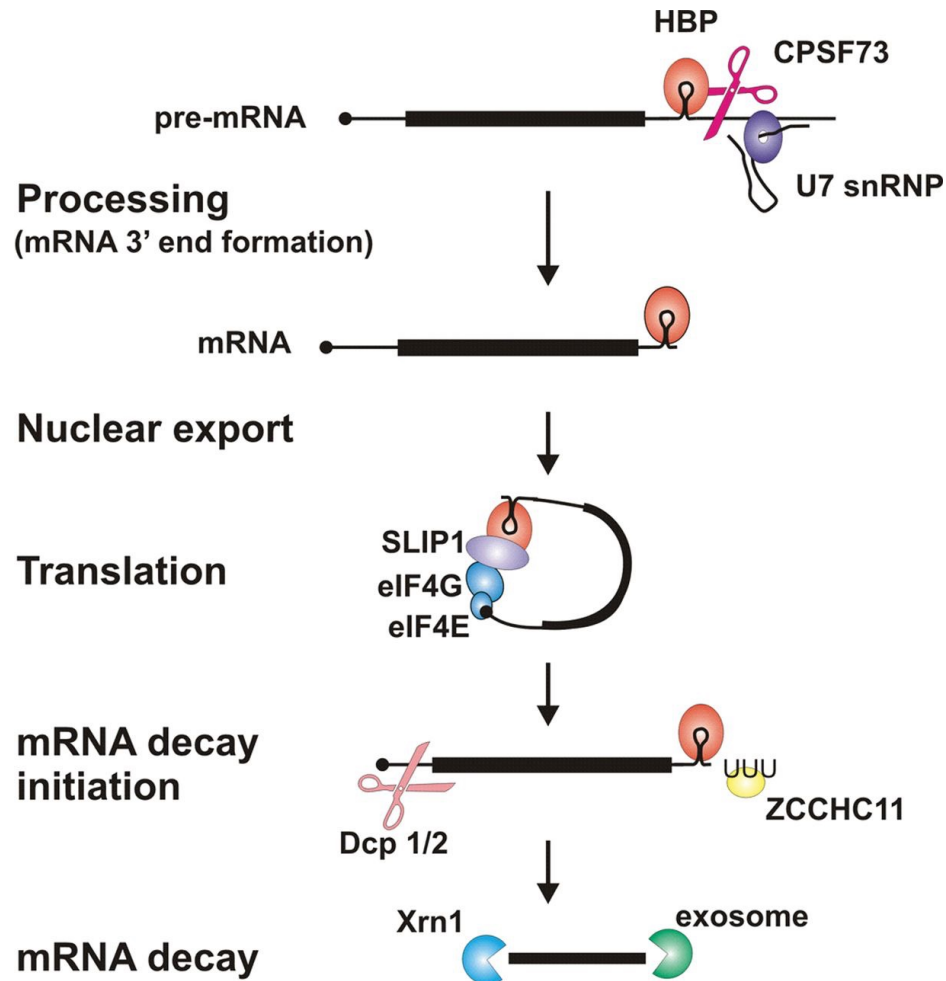
A N-terminal extension of Lsm11 additionally binds the 68 kDa subunit of mammalian cleavage factor I (CFIm68) and FLASH. Together, Lsm11 and FLASH provide a docking platform for the **Heat Labile Factor (HLF)** which consists of proteins that are also involved in cleavage/polyadenylation (CPA): symplekin (SYMPK), Cleavage Stimulation Factor 64 kDa subunit (CstF64) and all six subunits of Cleavage and Polyadenylation Factor (CPSF). **CPSF-73 is the endonuclease that cleaves the pre-mRNA after a CA dinucleotide.**

While SLBP binds to the 5' side of the hairpin, the 3' side is occupied by **hExo**, an exonuclease that trims the 3' end of the mRNA after cleavage and may also be involved in histone mRNA degradation.

If the canonical histone processing site is not recognized, a downstream **polyadenylation signal** can be used by the CPA machinery, resulting in polyadenylated histone transcripts. Poly-adenylated transcripts remain nuclear and get rapidly degraded by the nuclear exosome. Only a small fraction can be detected on polysomes.

CONTROL OF HISTONE EXPRESSION IN S-PHASE – REPLICATION DEPENDENT HISTONE RNA PROCESSING (RHP)

HBP (SLBP) - REGULATION OF RNA METABOLISM



HBP (histone RNA binding protein; also called SLBP) protein itself is cell cycle regulated.

SLBP mRNA is synthesized constantly throughout the cell cycle, but HBP 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.

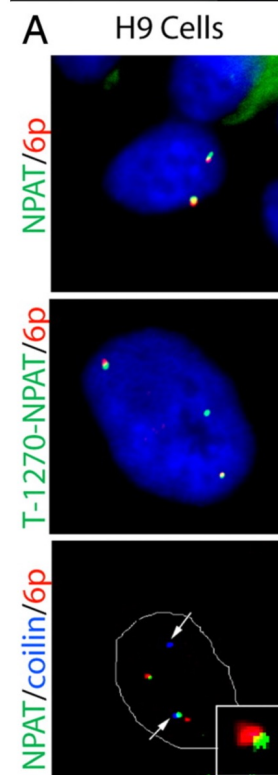
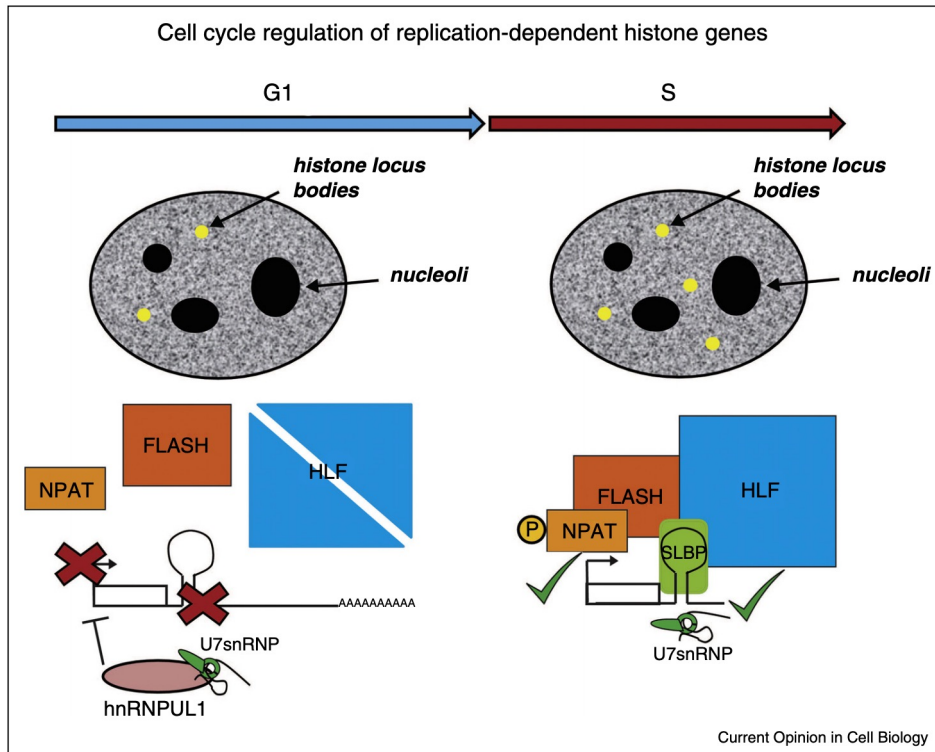
Together with other factors they position the nuclease CPSF73 for cleavage to produce histone mRNA ending immediately after the stem loop.

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.

FORMATION OF SUBNUCLEAR STRUCTURE = HISTONE LOCUS BODY



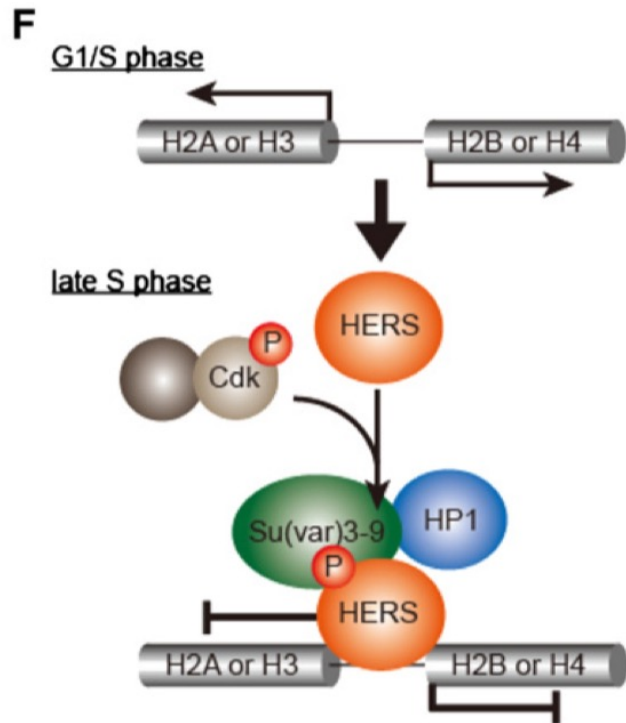
Histone locus bodies (HLBs) are subnuclear sites containing the clustered RD histone genes and components of the corresponding gene expression and processing machinery.

A. In G1, only two HLBs corresponding to the major histone cluster on chromosome 6 can be stained by antibodies against HLB marker proteins such as FLASH. As NPAT is not phosphorylated, the interaction with FLASH is weak. The level of SLBP is low, and the U7 snRNP, interacting with hnRNP UL1, inhibits histone transcription. Transcription is minimal and RNA 3' processing is inefficient so that most transcripts from the Replication dependent histone mRNAs become polyadenylated at cryptic polyA signals downstream from their regular cleavage sites.

B. In S phase, NPAT gets phosphorylated by cyclinE/Cdk2 which stabilizes the interaction with FLASH. The concentration of factors in HLBs increases, and two more HLBs corresponding to the minor cluster on chromosome 1 can be detected. SLBP is up-regulated, and FLASH interacts with the U7 snRNP, hence providing a platform for the recruitment of the now activated HLF.

CONTROL OF HISTONE EXPRESSION IN S-PHASE

DROSOPHILA: HERS and Su(var)3-9



Drosophila: The histone gene-specific epigenetic repressor in late S-phase (HERS) protein becomes phosphorylated 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.

Drosophila: also presence of HLB

REPLICATION COUPLED HISTONES

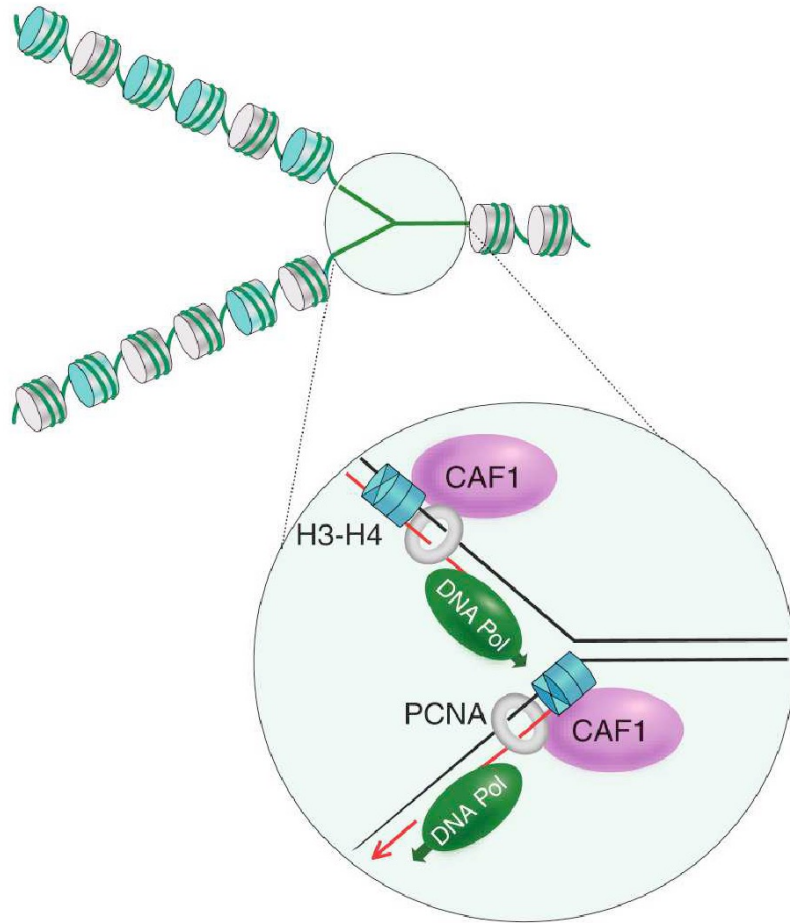


Figure 4. Distribution of old and new nucleosomes at a replication fork. Old nucleosomes (gray disks) are randomly distributed behind the replication fork and new nucleosomes (cyan disks) are deposited in the gaps. CAF-1-mediated nucleosome assembly is depicted on the leading and lagging strand in magnification. 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 nucleosomes
The assembly of a nucleosome consists of the loading of an (H3-H4)₂ tetramer to DNA (tetrasome) that is followed by the addition of 2 H2A-H2B dimers by the **FACT** (H2A/H2B dimer chaperone).

REPLICATION INDEPENDENT (RIs) HISTONES:

Are incorporated independently of DNA replication using specific histone chaperons

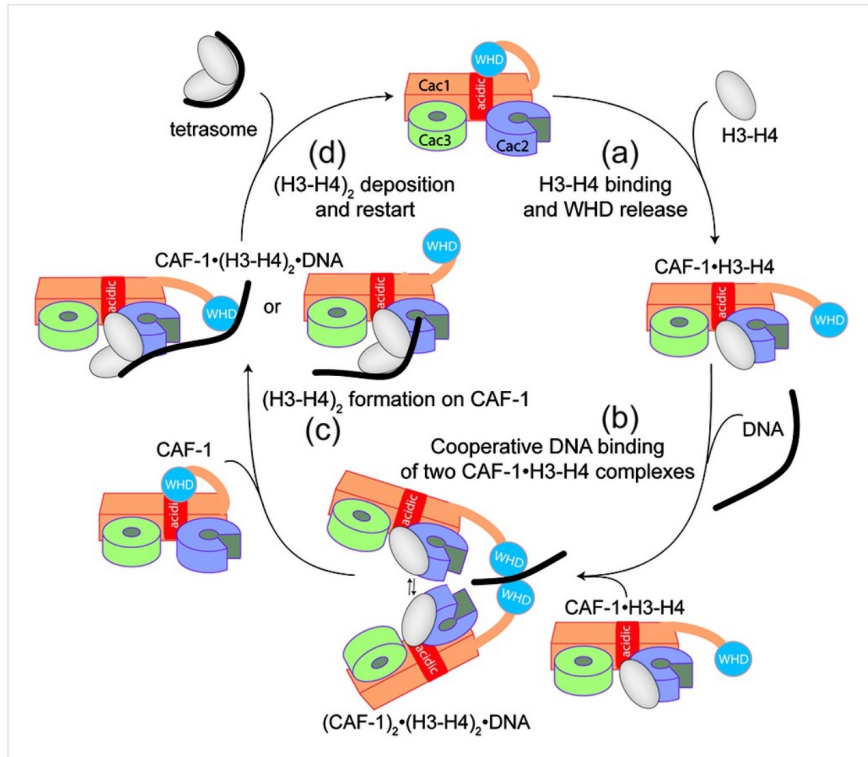
RI histones require the displacement of a preexisting nucleosome unit (active displacement or “loss”)

RI histones can **reset the epigenetic state of a pre-existing nucleosome**

Histone variants with high similarity to “normal” histone are also incorporated into canonical 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 winged helix domain (WHD) 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-H4)₂•DNA complex, the tetrasome**

Histones

Replication dependent, canonical histones

- Gene organization
- Gene expression control mechanisms in S-phase
- Histone chaperons

Replication independent histones – histone variants

- A list of histone variants
- Histone variant at centromere
- Histone H3.3 a variant with multiple function
- Histone H2AX and DNA damage
- Histone variants with chromosome specific accumulation

REPLICATION INDEPENDENT (RIs) HISTONES: - HISTONE VARIANTS

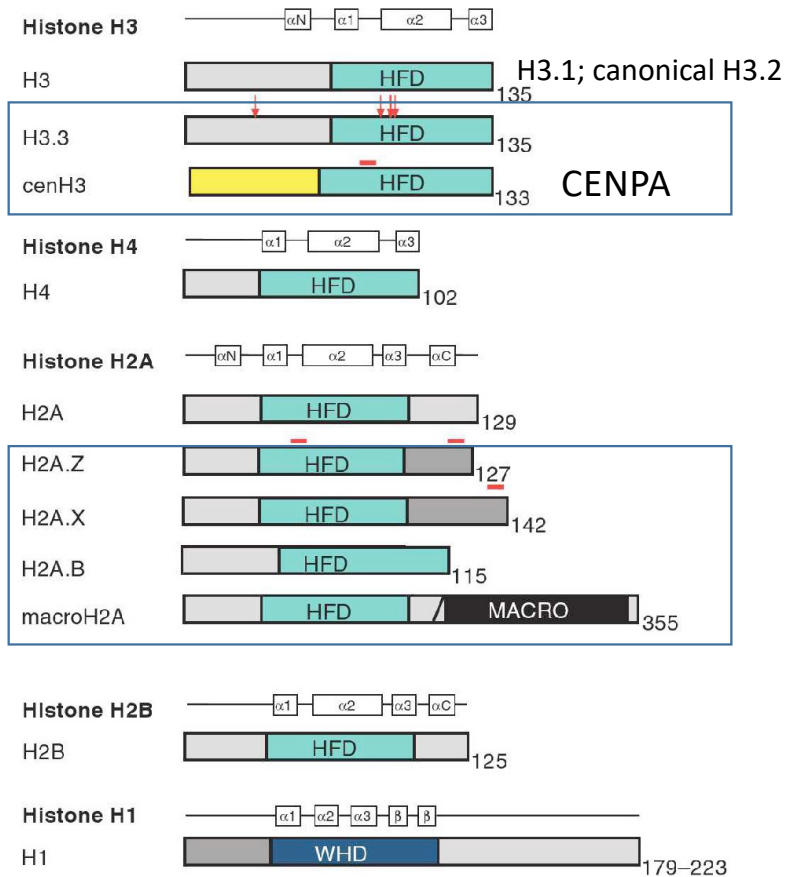


Figure 1. Histone variants. Protein domain structure for the core histones (H3, H4, H2A, and H2B), linker histone H1, and variants of histones H3 and H2A. The histone-fold domain (HFD) is where histone dimerization occurs. Regions of sequence variation in histone variants are indicated in red. WHD, winged-helix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

REPLICATION INDEPENDENT (RIs) HISTONES:

Are expressed and incorporated throughout the cell cycle and independently of DNA replication

RI histones require the displacement of a pre-existing nucleosome unit

RI histones can reset the epigenetic state of a pre-existing nucleosome

Assure nucleosome organization in interphase cells or terminally differentiated cells (transcription, remodeling)

Used in defined biological process

Histone variants with high similarity to "normal" histone can also be incorporated into canonical 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

REPLICATION INDEPENDENT (RIs) HISTONES: - HISTONE VARIANTS

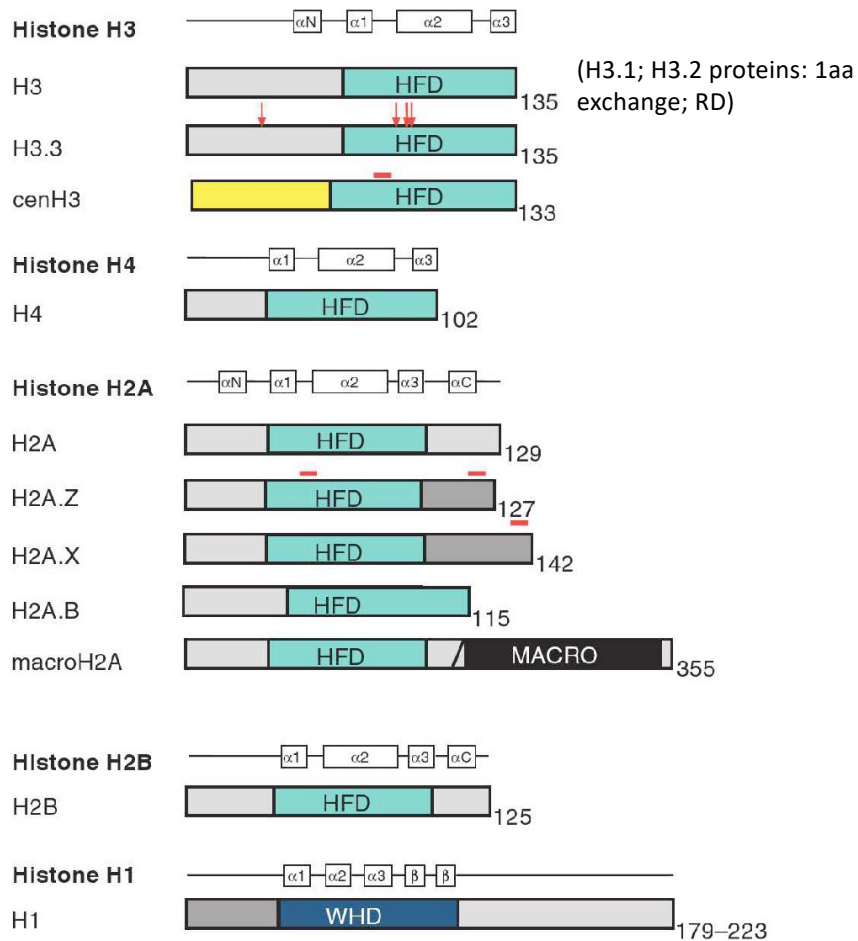


Figure 1. Histone variants. Protein domain structure for the core histones (H3, H4, H2A, and H2B), linker histone H1, and variants of histones H3 and H2A. The histone-fold domain (HFD) is where histone dimerization occurs. Regions of sequence variation in histone variants are indicated in red. WHD, winged-helix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

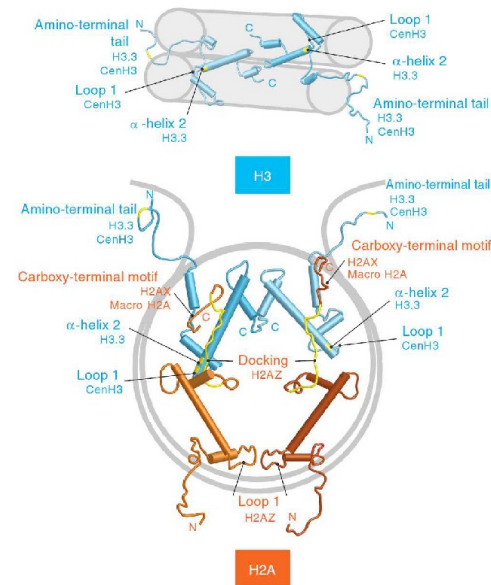
Humans:

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)



Histone Variants

1. A H3 variant at the centromere: CENP-A: a histone variant that defines eukaryotic centromeres

2. Histone H3.3 maintains chromatin structure and correct nucleosome organization

2a. Histone H3.3: maintains euchromatin structure

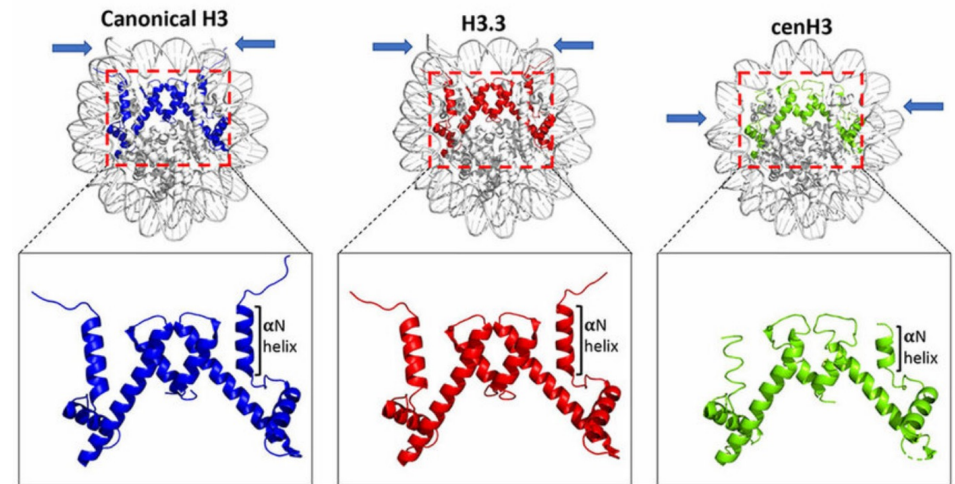
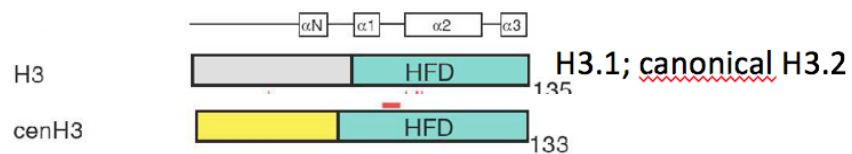
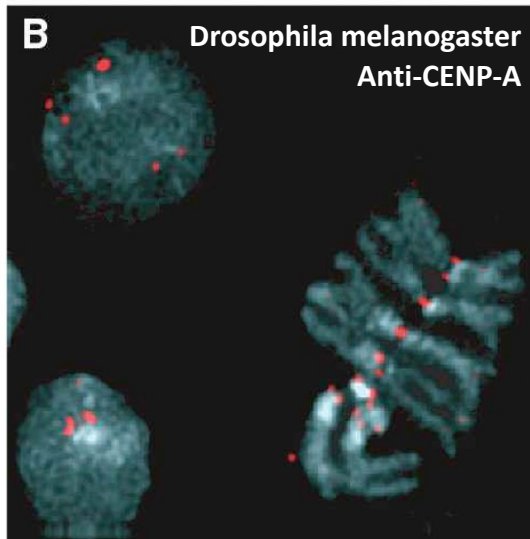
2b. H3.3 incorporation in nucleosomes at repeats contributes to genome stability

3. Role of H2AX in DNA damage response

4. A role for macroH2A and H2A.B on the X-chromosome

5. A role for H2A.Z at transcribed genes

1. A H3 VARIANT AT THE CENTROMERE: CENP-A: A HISTONE VARIANT THAT DEFINES EUKARYOTIC CENTROMERES

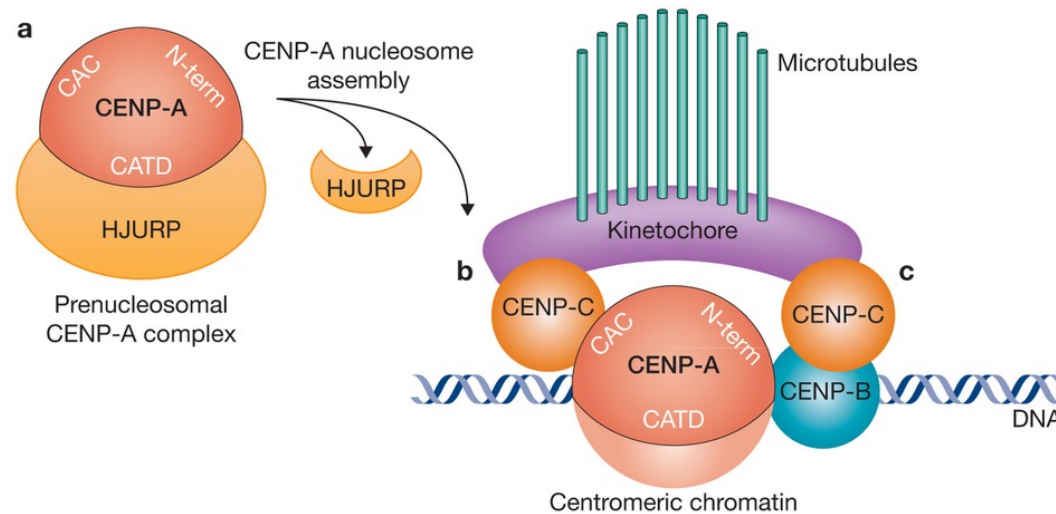


Structural diversity of histone H3 variants within the nucleosome core particle. Crystal structures of human nucleosome core particles containing either canonical histone H3.1 (PDB ID: 5Y0C) [16], histone variant H3.3 (PDB ID: 3AV2) [174] or histone variant cenH3 (PDB ID: 3AN2) [156] are shown. A close-up view of the histone H3 variants highlighting the α -N helix in cenH3 is shown. Note the shorter α -N helix in cenH3 compared to H3.1 and H3.3. The blue arrows point to the DNA ends visible in the crystal structures of the nucleosome core particles corresponding to the different H3 variants. Note the shorter tract of DNA associated with the histone octamer within the cenH3 nucleosome core particle. Nucleosome core particle figures were made using PyMol software.

N-terminal Tail: CENP-A has a unique N-terminal tail that is distinct from canonical H3. This region is crucial for its centromere-specific function and interaction with other centromeric proteins.

Histone Fold Domain: While CENP-A shares structural similarities with H3, it has specific amino acid substitutions in the histone fold domain. These substitutions contribute to the stability and unique structure of CENP-A-containing nucleosomes

1. A H3 VARIANT AT THE CENTROMERE: CENP-A: A HISTONE VARIANT THAT DEFINES EUKARYOTIC CENTROMERES



CENP-A: RI histone variant, incorporated by the cenH3 specific histone HJURP histone chaperone

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

Centromere protein C: 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.

Histone Variants

1. A H3 variant at the centromere: CENP-A: a histone variant that defines eukaryotic centromeres

2. Histone H3.3 maintains chromatin structure and correct nucleosome organization

2a. Histone H3.3: maintains euchromatin structure in expressed genes

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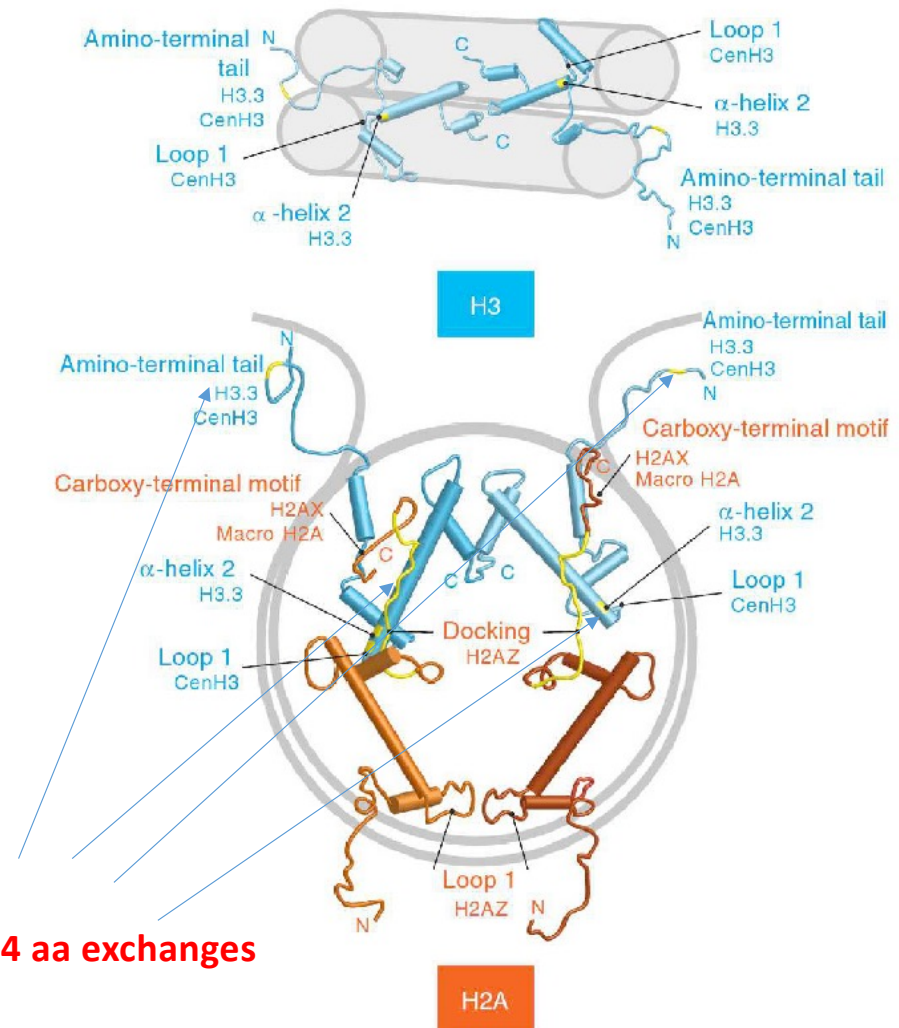
2. Histone H3.3 maintains chromatin structure and correct nucleosome organization

Problem for maintenance of the epigenetic code and chromatin conformation in G1 and G2:

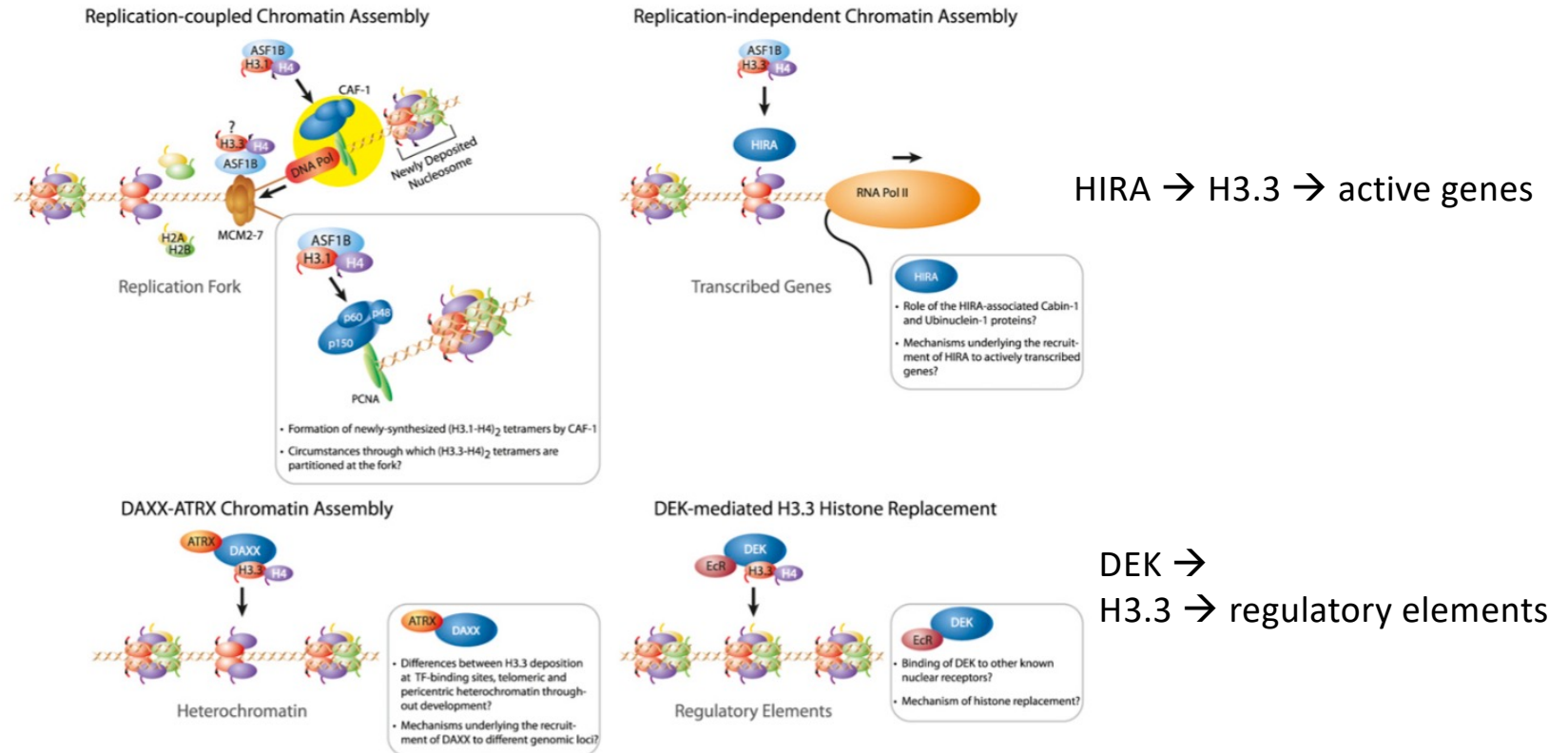
- Euchromatic Regions: Transcription, DNA repair, Nucleosome eviction: loss of nucleosomes, loss of epigenetic code
- Regulatory elements: Dynamic binding of transcriptional regulators; nucleosome dynamics
- Constitutive heterochromatin: Repressive chromatin essential for genome stability and to avoid recombination

H3.3 can be modified on the post-translational level like canonical H3 → maintenance of epigenetic code

Only 4 aa exchanges



Histone H3.3 maintains chromatin structure and correct nucleosome organization



DAXX/ATRX →
H3.3 → repetitive
elements/constitutive
heterochromatin

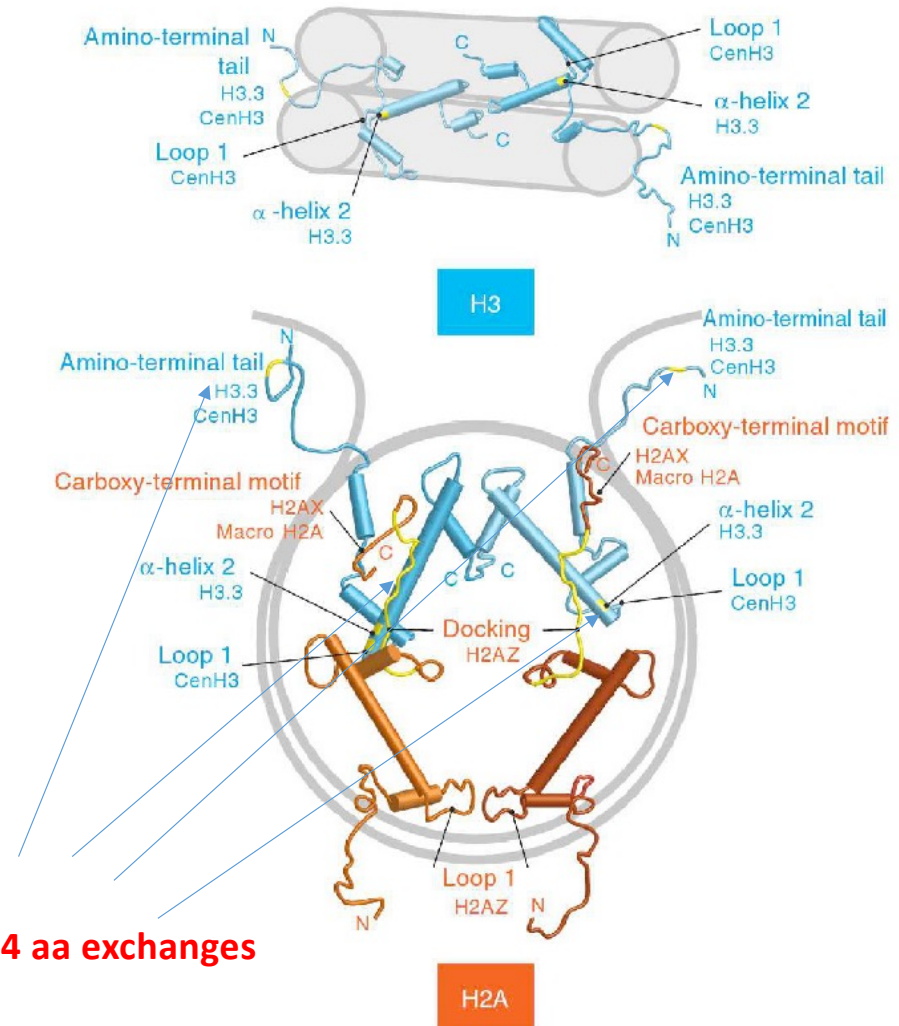
Figure 2. During RC assembly, the ASF1 chaperone is thought to transfer newly synthesized soluble H3.1-H4 dimers to CAF-1 through direct interactions with its p60 subunit (Tyler et al. 2001; Mello et al. 2002). CAF-1 would then facilitate the assembly of a central (H3.1-H4)₂ tetramer to which two H2A-H2B 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.

2. Histone H3.3 maintains chromatin structure and correct nucleosome organization

Problem for maintenance of the epigenetic code and chromatin conformation in G1 and G2:

- **2 a :Euchromatic Regions: Transcription, DNA repair, Nucleosome eviction: loss of nucleosomes, loss of epigenetic code**
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- Constitutive heterochromatin: Repressive chromatin essential for genome stability and to avoid recombination

H3.3 can be modified on the post-translational level like canonical H3 → maintenance of epigenetic code



Only 4 aa exchanges

CRITICAL OBSERVATION ON THE USAGE OF H3.3: MAINTAINS CHROMATIN STATE INDEPENDENTLY ON REPLICATION

- POST-MITOTIC CELLS

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

Stem cells
Proliferation
H3, H3.3
 RD histone assembly

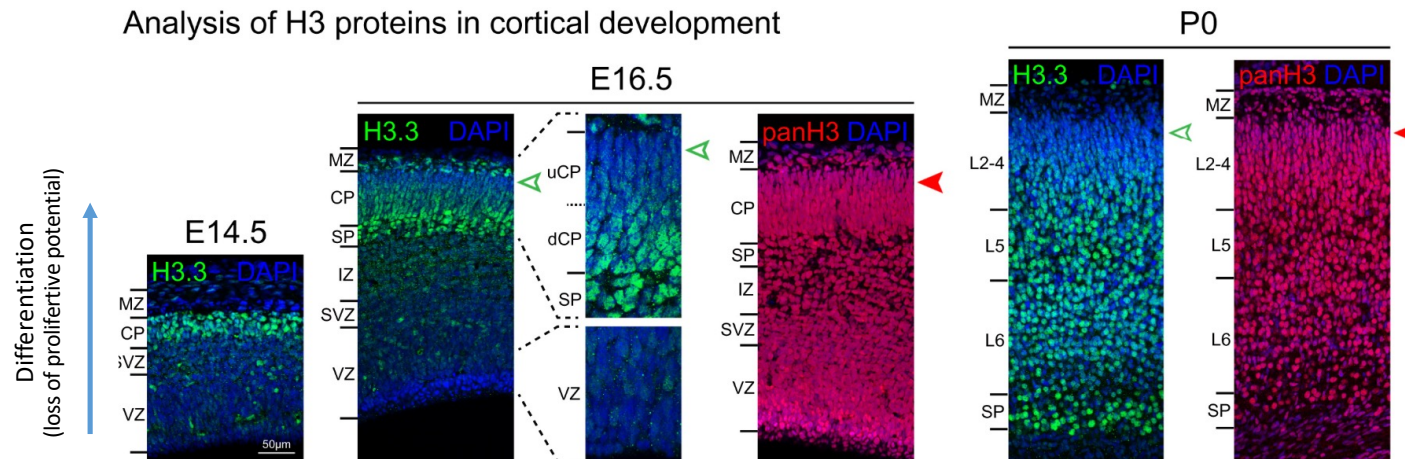
Rat brain: 78% H3.3 in chromatin
H3.3 is essential to fill nucleosome gaps in terminally differentiated cells
Gene expression is not altered → H3.3 compensates for H3

Differentiated cells
No proliferation
H3, H3.3
 RI histone assembly

Histone modifications maintained by H3.3

Analysis of H3 proteins in cortical development

New data from mouse recapitulate data from rat:



VZ: ventricular zone
 SVZ: sub-ventricular zone
 CP: cortical plate
 dCP: deep cortical plate
 uCP: upper cortical plate
 SP: subplate
 MZ: marginal zone

Analysis of H3.3 and pan-H3 protein in cortical development by immunostaining.

*On E14.5 and E16.5, low levels of H3.3 (green) were present in the VZ and SVZ, composed largely of **cycling NPCs**. High levels of H3.3 were present in **the CP, composed largely of postmitotic neurons**. In E16.5 CP and P0 cortex, higher levels of H3.3 were present in the early-born neurons of the SP and deep layers, compared to the late-born neurons of the upper layers (open arrowheads). Pan-H3 (red) was broadly present and showed no preference for early-born neurons; high levels were present in late-born upper-layer neurons (solid arrowhead).*

<https://doi.org/10.1073/pnas.2116956119>

2a. Histone H3.3: MAINTAINS EUCHROMATIN STRUCTURE

- PROLIFERATING CELLS

POLYTHENE CHROMOSOMES *D. Melanogaster*

Decondensed regions map with H3.3

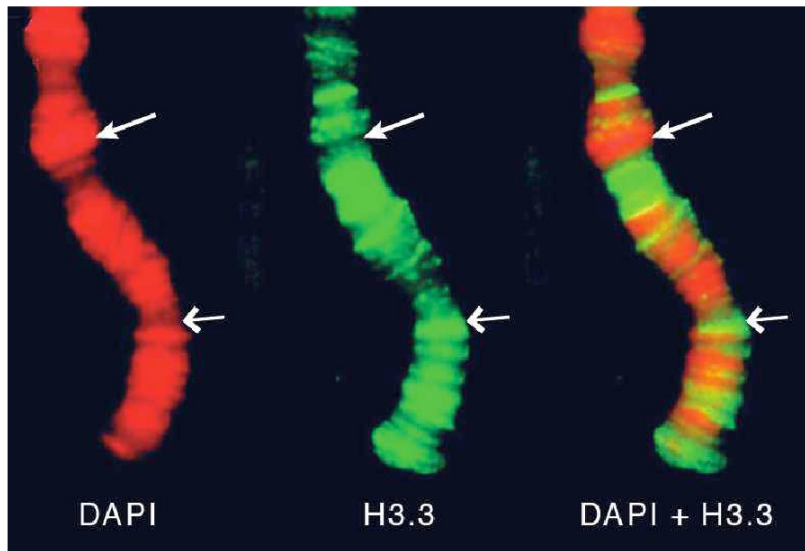
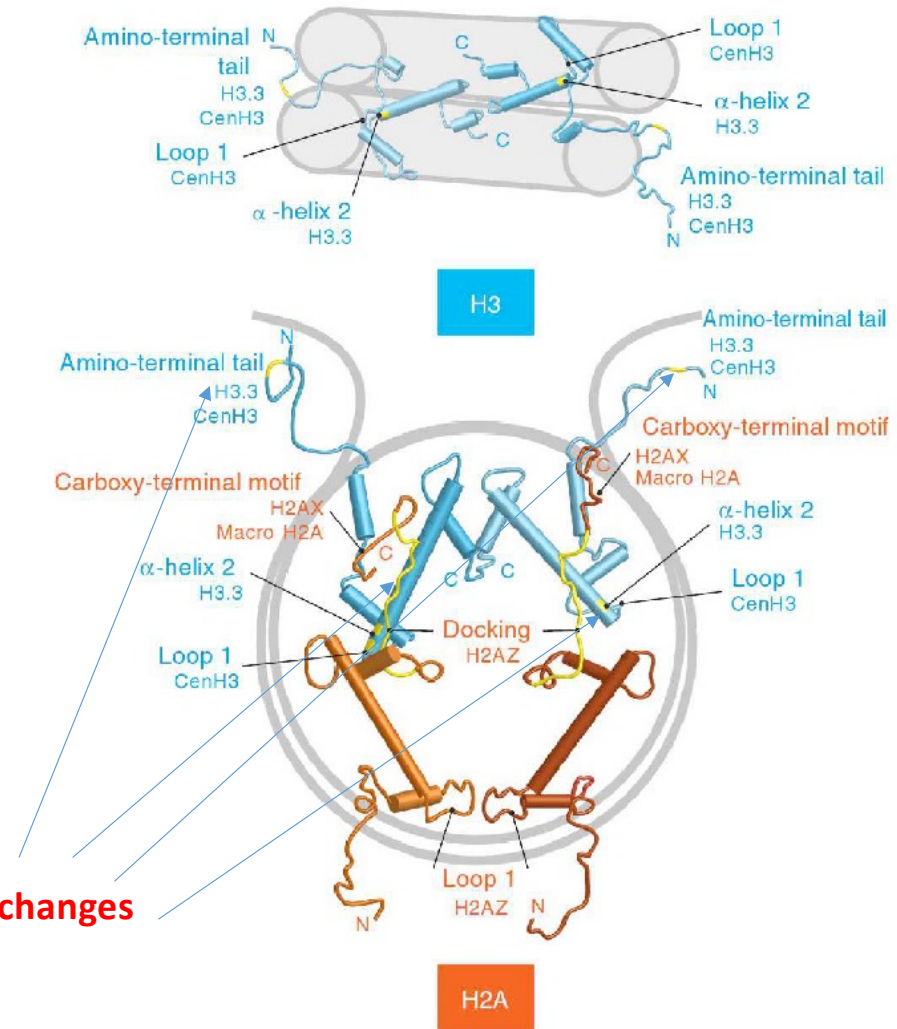


Figure 7. H3.3 preferentially localizes to actively transcribed regions of *Drosophila* polytene chromosomes. DAPI staining (red) shows the DNA banding pattern (left), and H3.3-GFP (green) localizes to interbands (middle), which are sites of RNA Pol II localization. The merge (Schwartz and Ahmad 2005) 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.

Only 4 aa exchanges



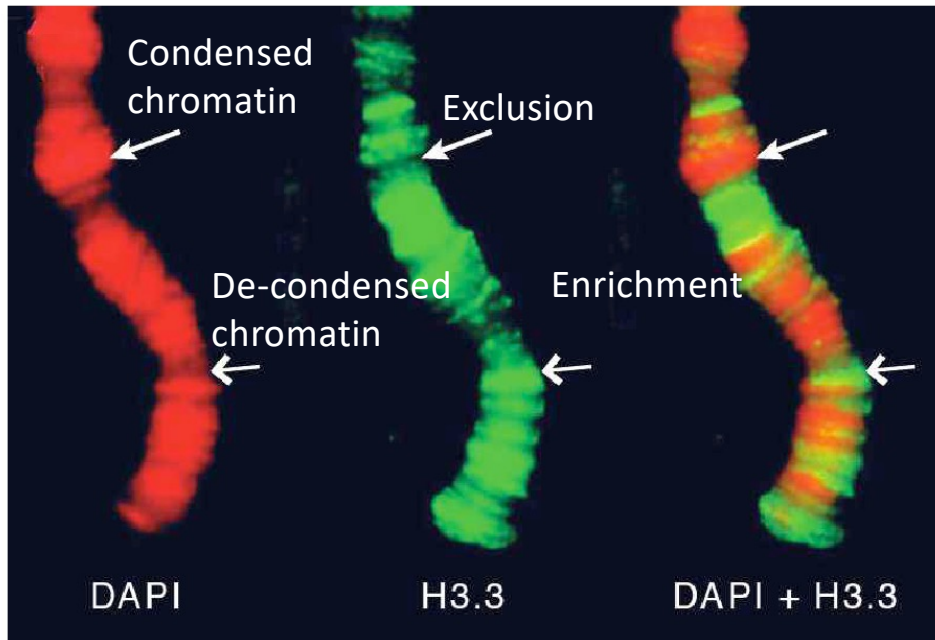


Figure 7. H3.3 preferentially localizes to actively transcribed regions of *Drosophila* polytene chromosomes. DAPI staining (red) shows the DNA banding pattern (*left*), and H3.3-GFP (green) localizes to interbands (*middle*), which are sites of RNA Pol II localization. The merge ([Schwartz and Ahmad 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.

H3.3 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.

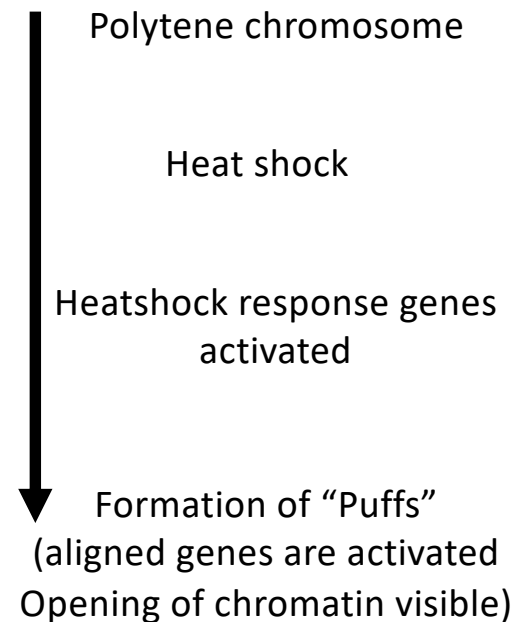
Additional positive effect: eventual repressive modifications (on canonical H3K9me3) are eliminated by exchanging H3-mod for H3.3 unmodified

H3.3 carries same modification like classic H3

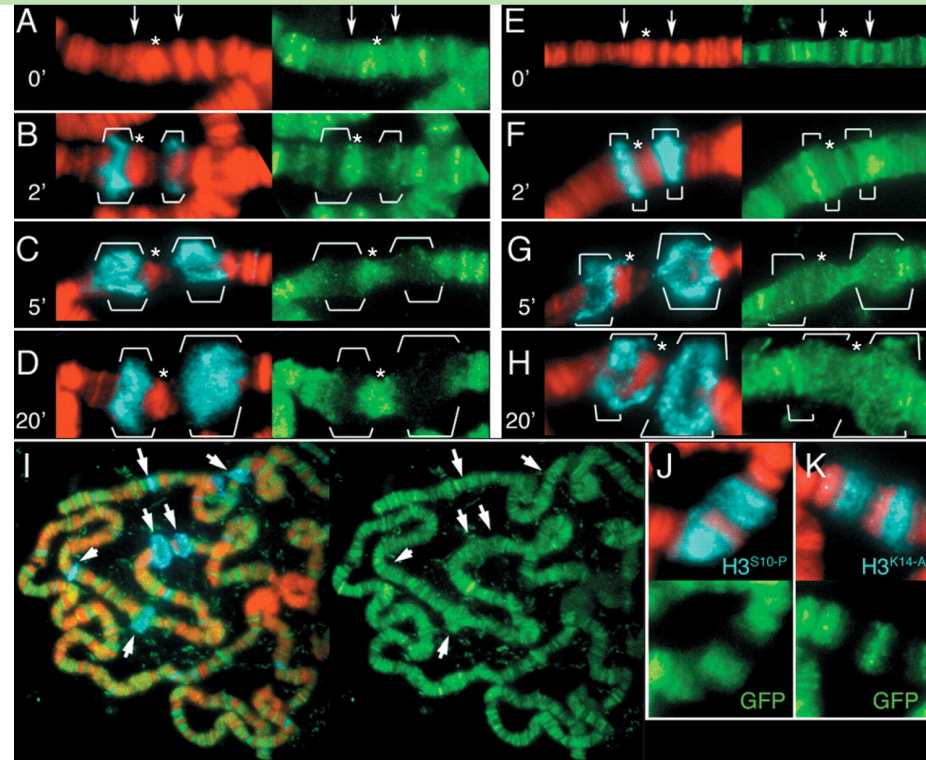
Drosophila: Gene expression in G1/G2 is paralleled histone replacement of H3 with H3.3

- PROLIFERATING CELLS

- CHROMATIN PUFFS



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



Green: **GFP-H3.3**
Blue: RNA Pol II active
Red: DNA

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

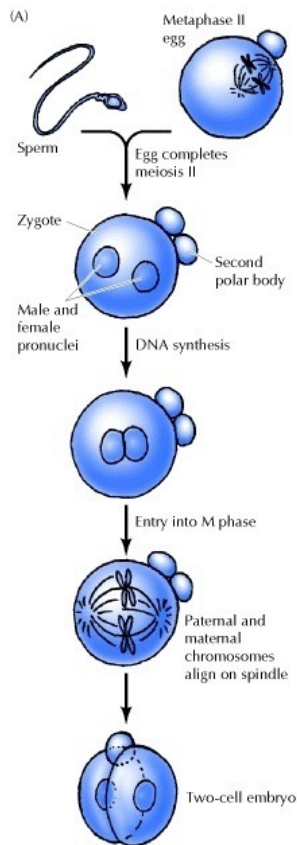
Gene induction triggers histone replacement. Heat-shock induction triggers rapid puffing and transcription of the HSP70 genes at polytene bands 87A and 87C. Polytene chromosomes from larvae that contain a constitutively expressed H3-GFP construct (A–D,J,K), or a constitutively expressed H3.3core-GFP 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.**

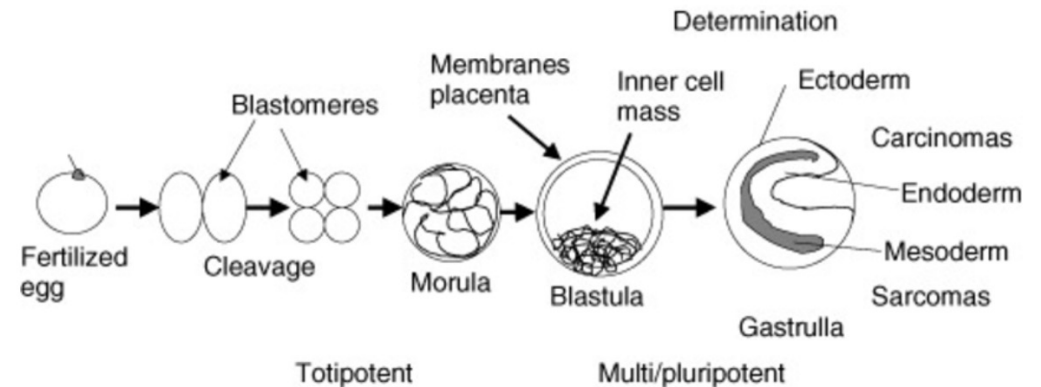
Vertebrates: Gene expression in G1/G2 is paralleled histone replacement of H3 with H3.3

- PROLIFERATING CELLS - EMBRYONIC DEVELOPMENT

Induction of gene expression programs of differentiation is linked with substitution of histone H3 with H3.3 variant



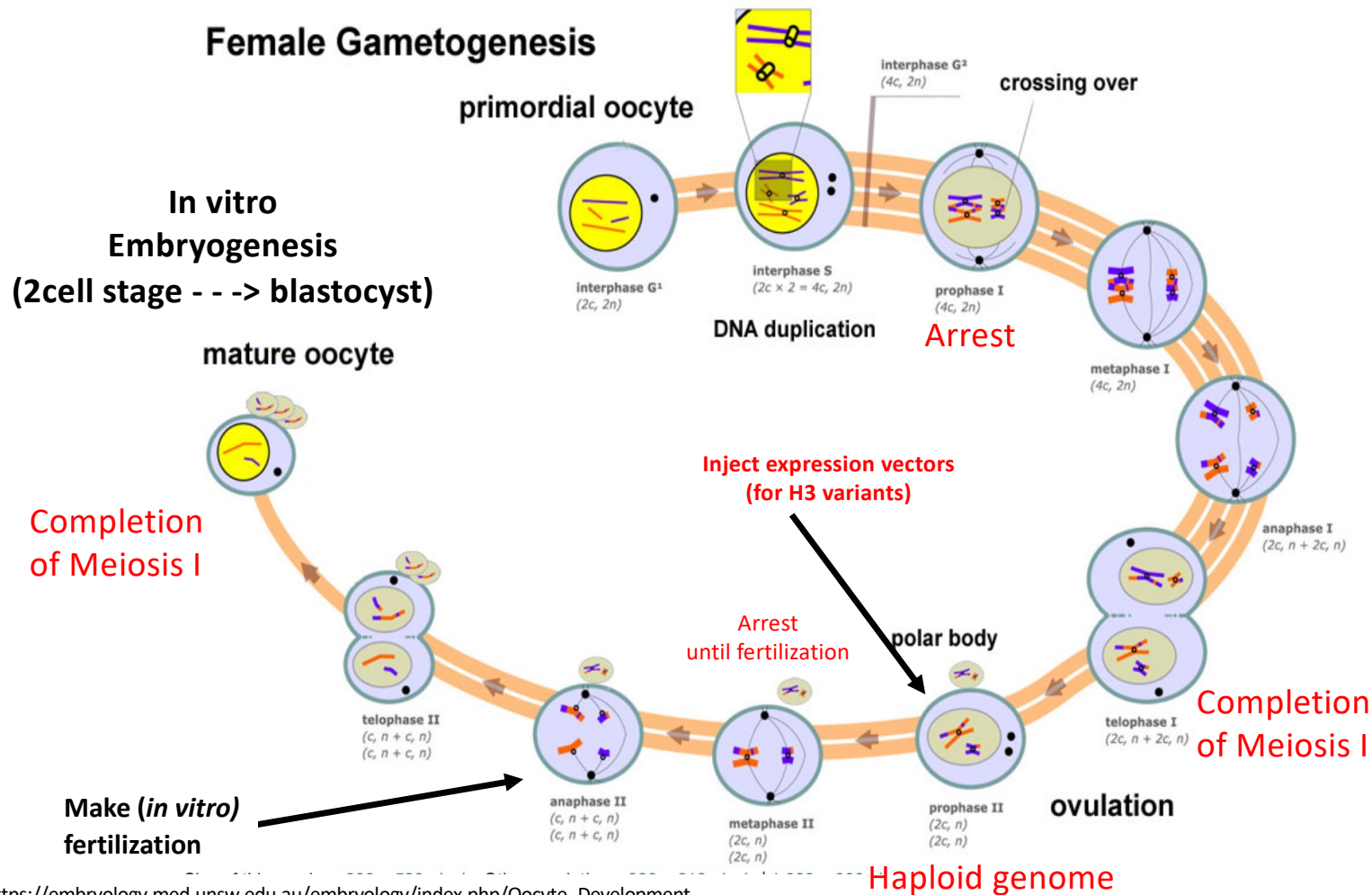
(A) Fertilization induces the transition from metaphase II to anaphase II, leading to completion of oocyte meiosis and emission of a second polar body (which usually degenerates). The sperm nucleus decondenses, so the fertilized egg (zygote) contains two haploid nuclei (male and female pronuclei). In 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.

Vertebrates: Gene expression in G1/G2 is paralleled histone replacement of H3 with H3.3

In vitro fertilization and differentiation as model system to study differentiation events



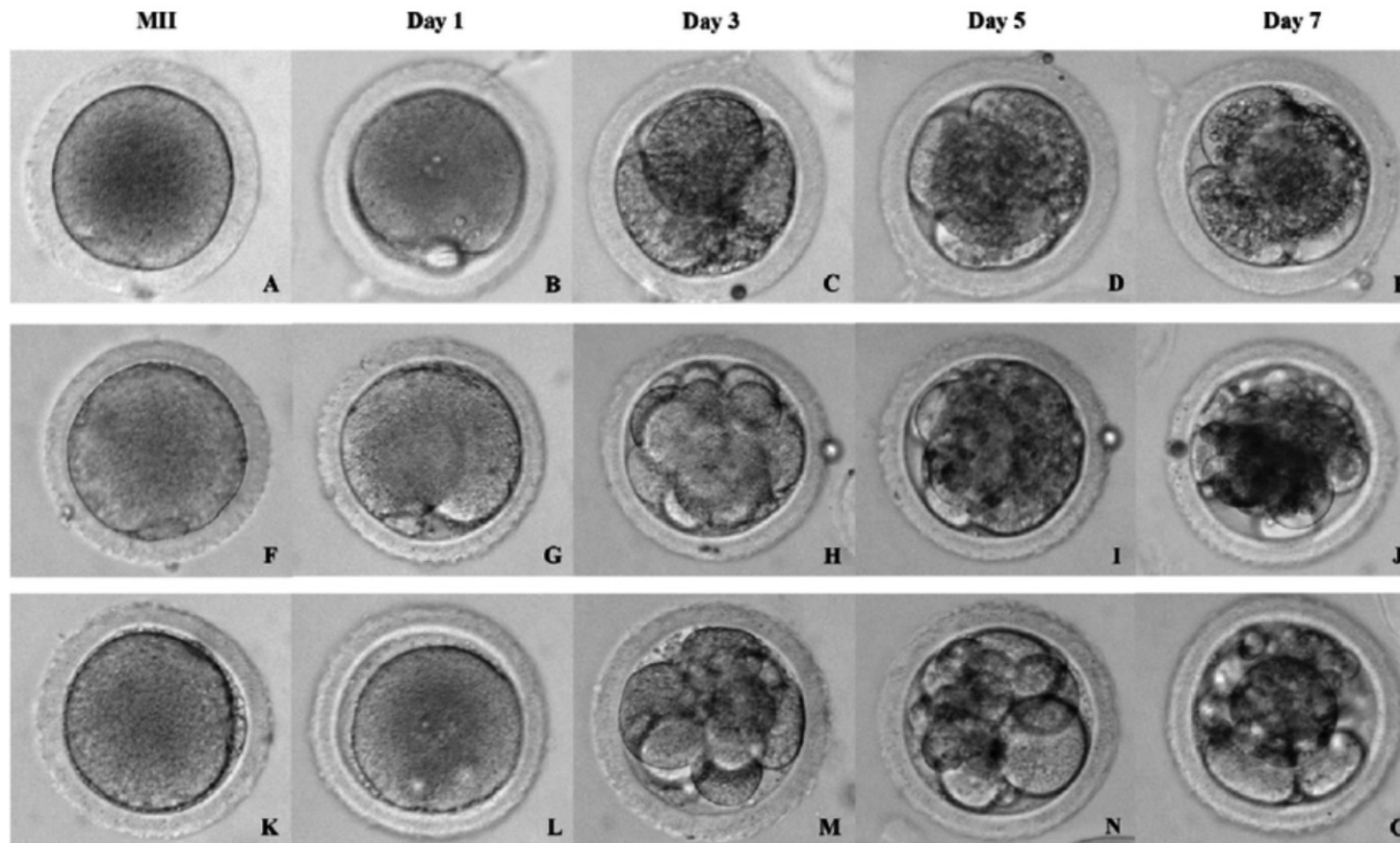
Female Gametogenesis: In females, the total number of eggs ever to be produced are present in the newborn female initially arrested at the diplotene stage of the meiosis I from fetal life through childhood until puberty, when the lutenizing hormone (LH) surges stimulate the resumption of meiosis. **All eggs are arrested at an early stage (prophase I) of the first meiotic division as a primary oocyte (primordial follicle).** Following puberty, 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 pellucida) can influence the site of spermatozoa fertilization.

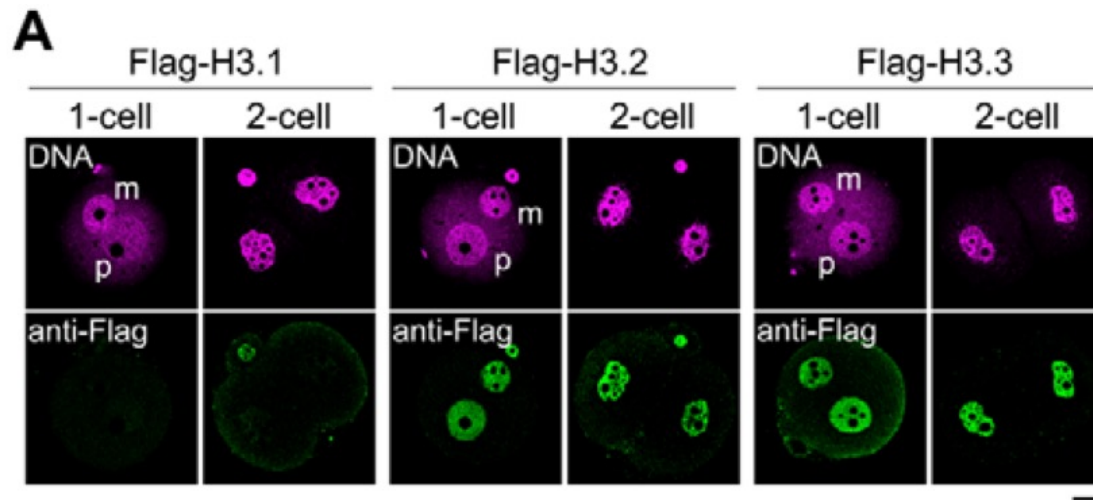
Vertebrates: Gene expression in G1/G2 is paralleled histone replacement of H3 with H3.3

In vitro fertilization and differentiation as model system to study differentiation events



**Activation of differentiation genes and
EPIGENETIC PROGRAMMING**

Vertebrates: Gene expression in G1/G2 is paralleled histone replacement of H3 with H3.3



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

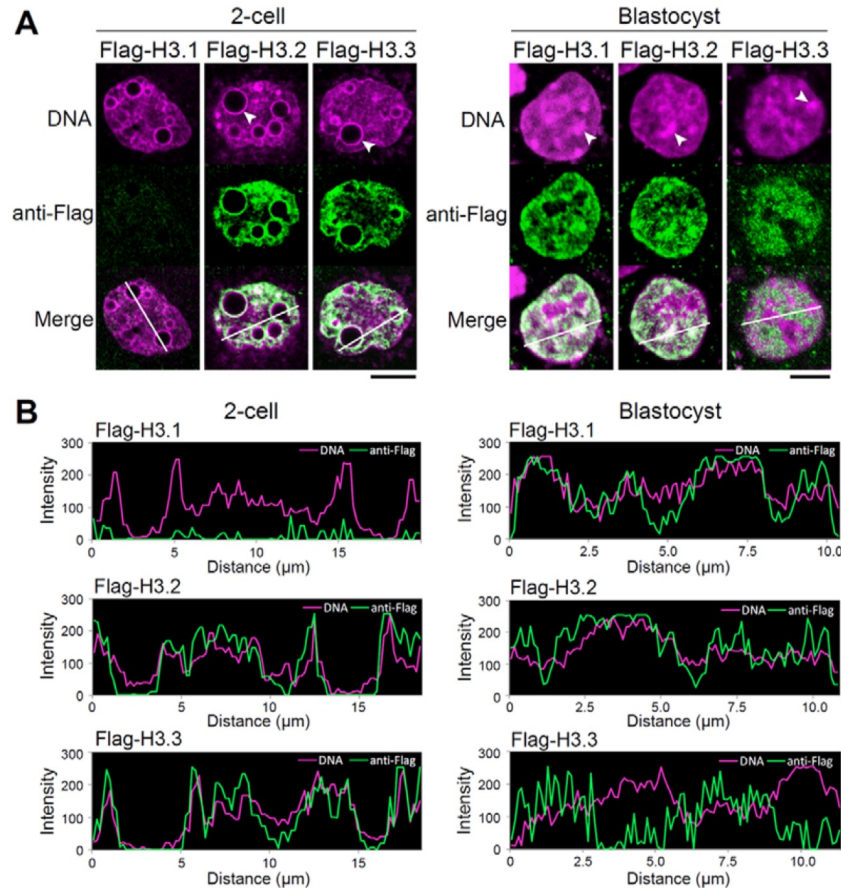
P: paternal pronucleus

M: maternal pronucleus

H3.2: canonical H3 inserted into pronuclei + 2 cell embryo

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

Vertebrates: Gene expression in G1/G2 is paralleled histone replacement of H3 with H3.3



2 cell stage → blastocyst

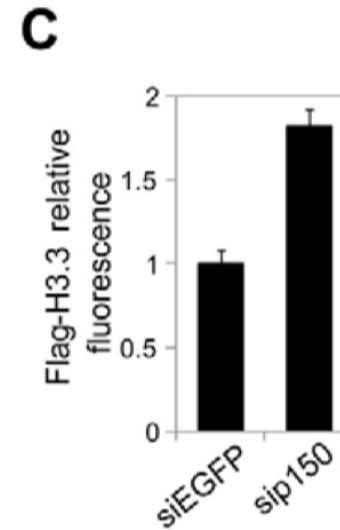
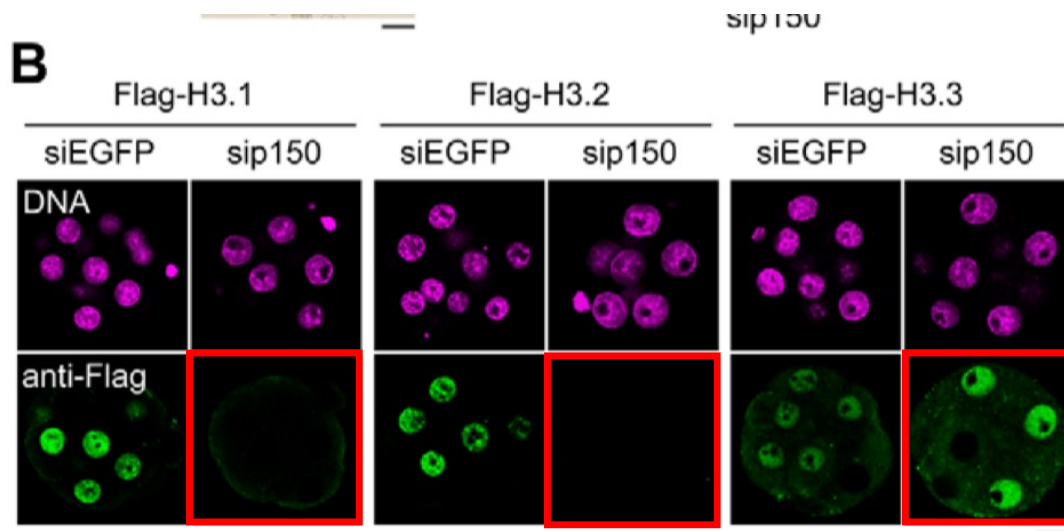
H3.1: incorporation into blastocyst chromatin (eu+heterochromatin)

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 PARALLELED BY H3.3
 INCORPORATION

Vertebrates: Gene expression in G1/G2 is paralleled histone replacement of H3 with H3.3



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

Loss of CAF function (sip150) --> lack of H3.1 and H3.2 incorporation

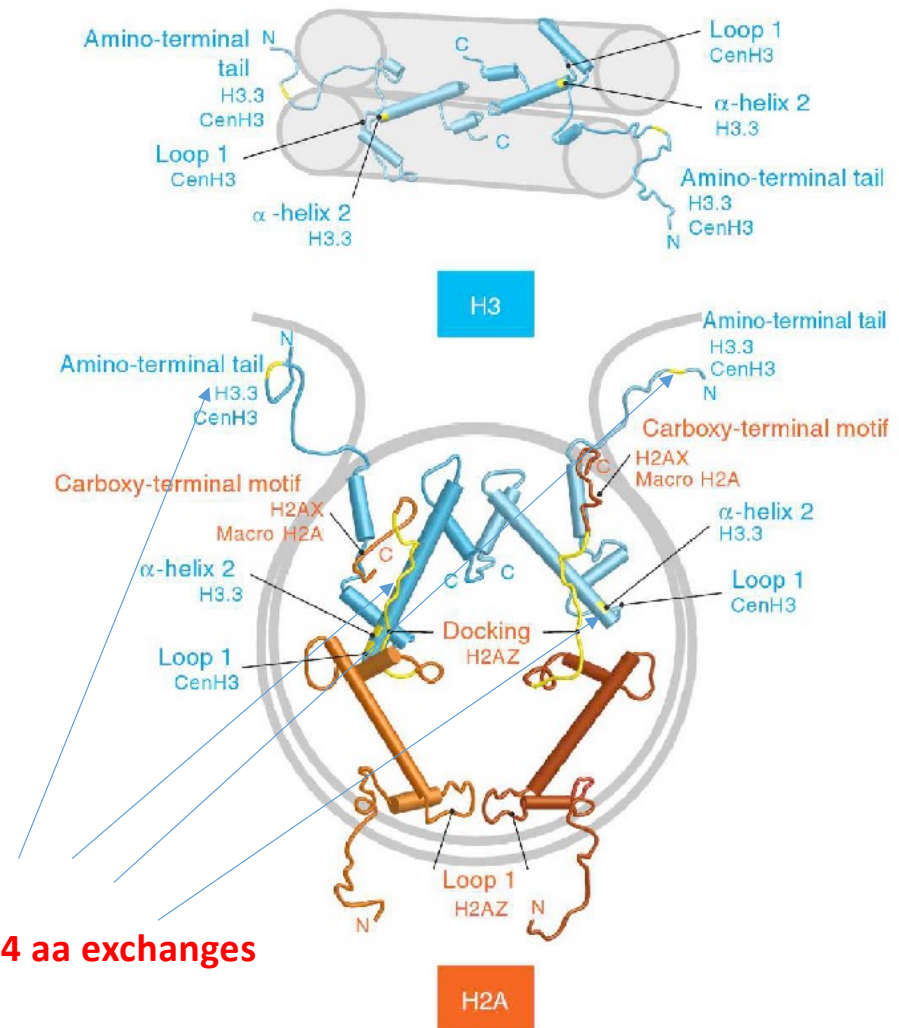
H3.3 incorporation not affected (increases)
 → H3.3 is an RI histone

2. Histone H3.3 maintains chromatin structure and correct nucleosome organization

Problem for maintenance of the epigenetic code and chromatin conformation in G1 and G2:

- **2 a :Euchromatic Regions: Transcription, DNA repair, Nucleosome eviction: loss of nucleosomes, loss of epigenetic code**
- Regulatory elements: Dynamic binding of transcriptional regulators; nucleosome dynamics
- **2 b :Constitutive heterochromatin: Repressive chromatin essential for genome stability and to avoid recombination**

H3.3 can be modified on the post-translational level like canonical H3 → maintenance of epigenetic code

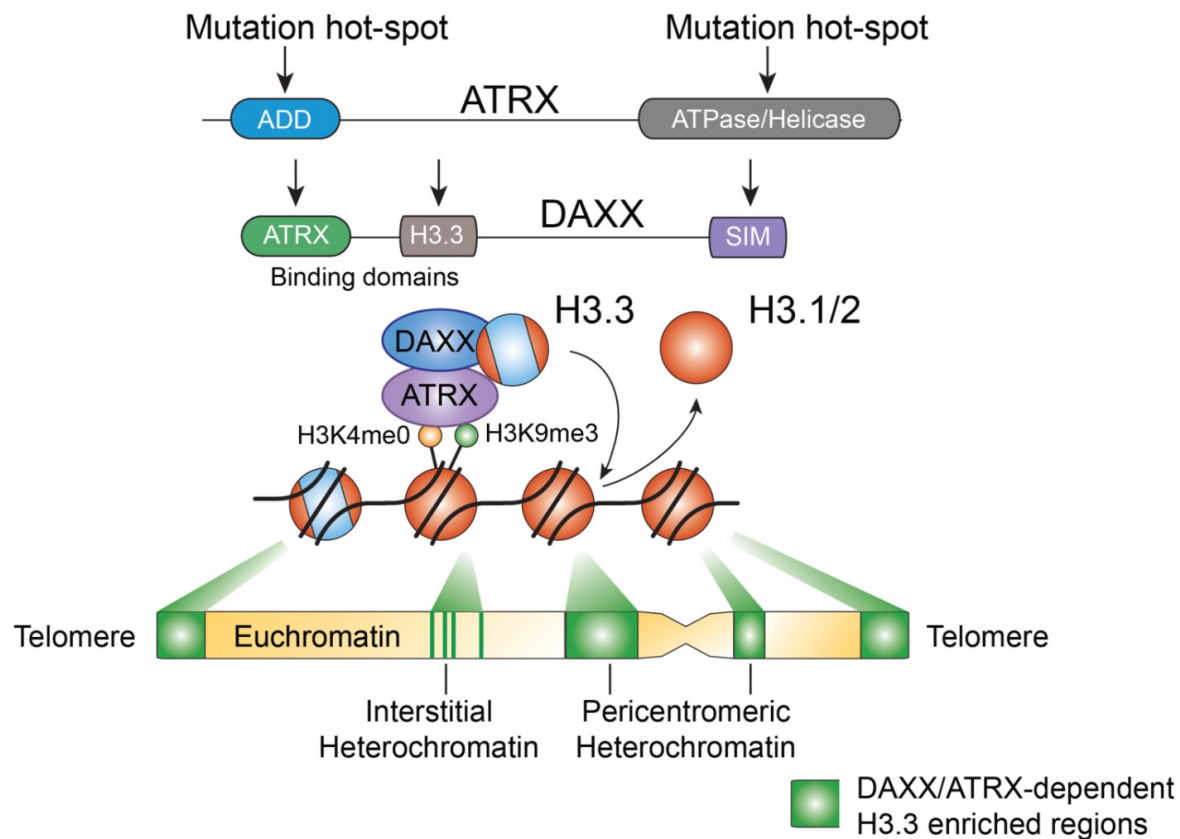


Only 4 aa exchanges

2b. H3.3 incorporation in nucleosomes at repeats contributes to genome stability

- MAINTENANCE OF EPIGENETIC CODE IN CONSTITUTIVE HETEROCHROMATIN

CHROMATIN REGULATION OF REPETITIVE DNA



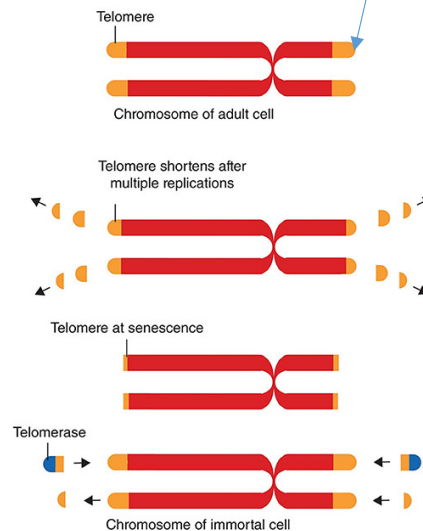
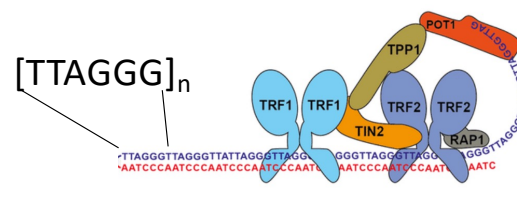
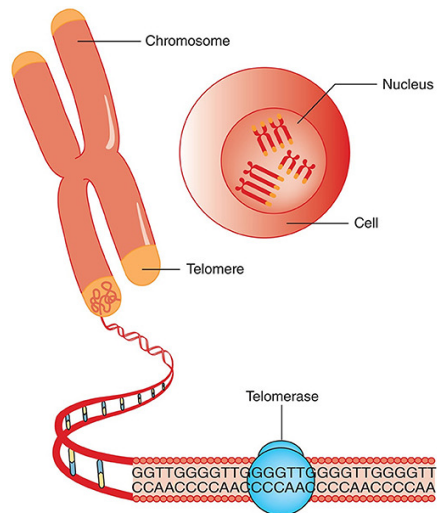
Repeats: enriched in H3K9me3 and HP1.

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.

H3.3 USAGE IS LINKED TO TELOMERE FUNCTION DURING IMMORTALIZATION - TUMORFORMATION

The shelterin complex binds telomere repeats and protects chromosome ends from eliciting a DNA damage response



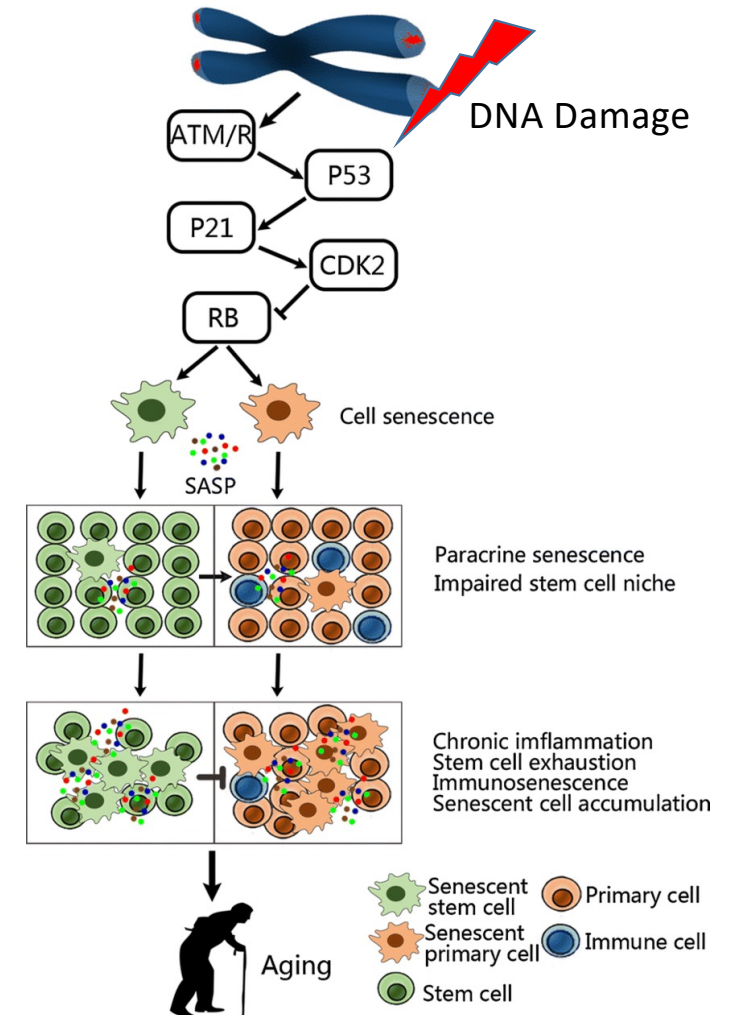
Telomeres shorten with every round of semiconservative replication

The reverse transcriptase telomerase replenishes telomere repeats in stem cells

Telomerase is down-regulated early after embryonic development; telomerase Activity in stem cells is not sufficient to prevent shortening (at slow rate)

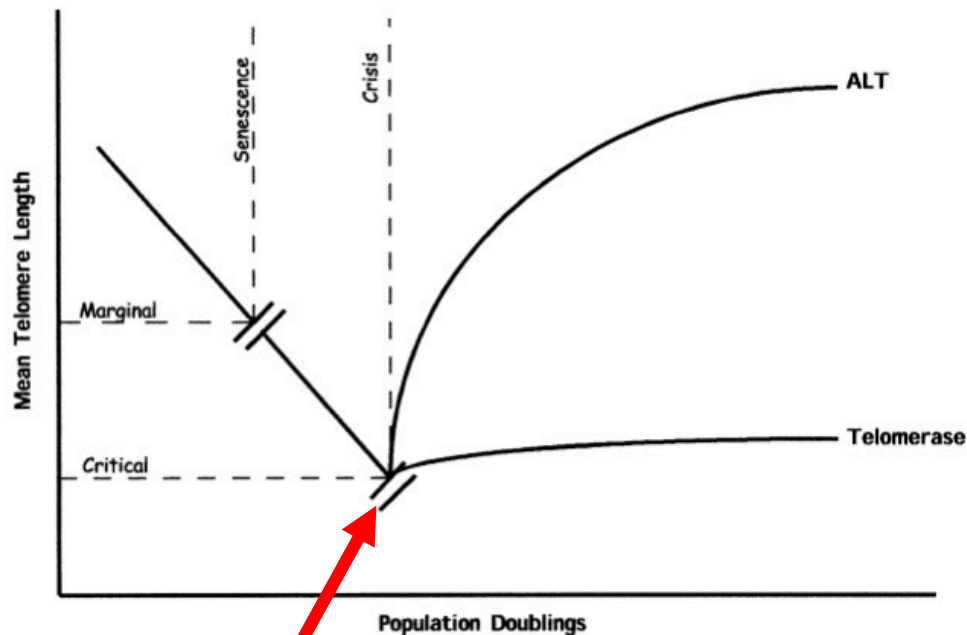
Loss of telomeres leads to the exposure of unprotected DNA ends →

DNA Damage → activation of cell cycle arrest → senescence → contributes to ageing

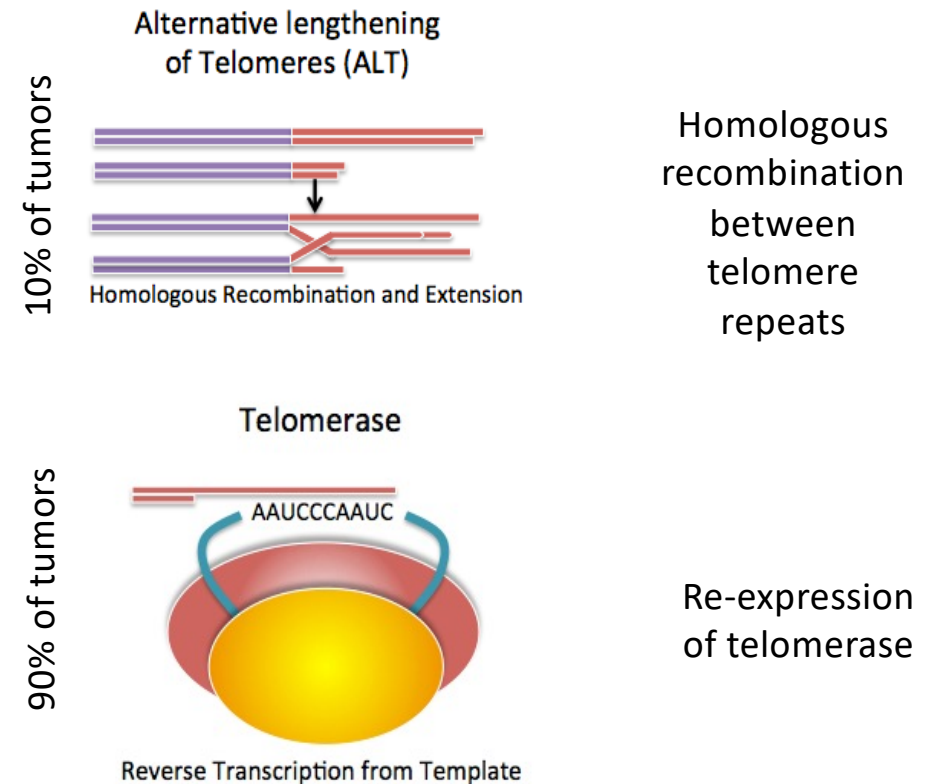


H3.3 USAGE IS LINKED TO TELOMERE FUNCTION DURING IMMORTALIZATION - TUMORFORMATION

Activation of telomere maintenance mechanisms is critical for tumor formation (replicative immortality)



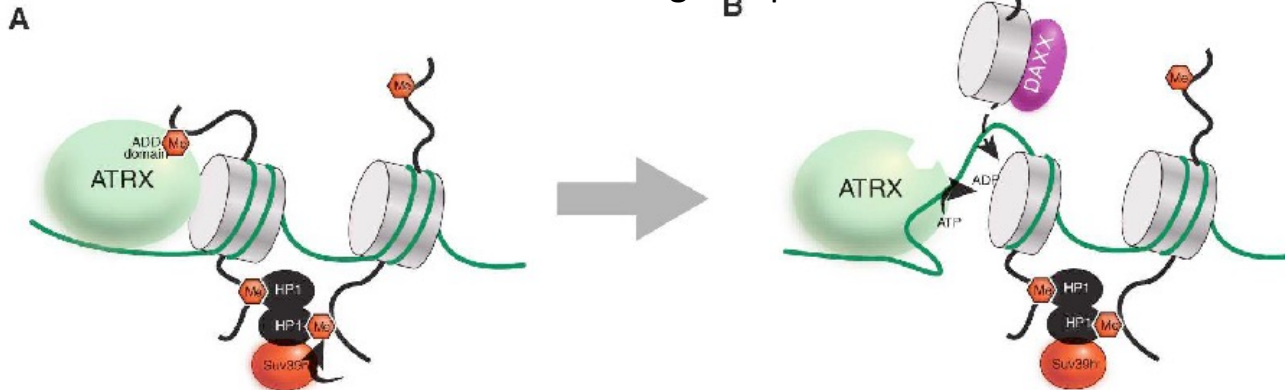
TUMORFORMATION



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

Daxx histone chaperon complex

ATRX ATP nucleosome remodelling complex



H3.3 is found to be present 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

Note: there is low transcription at telomeres and centromeres → nucleosome turnover in G1 and G2

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.

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.

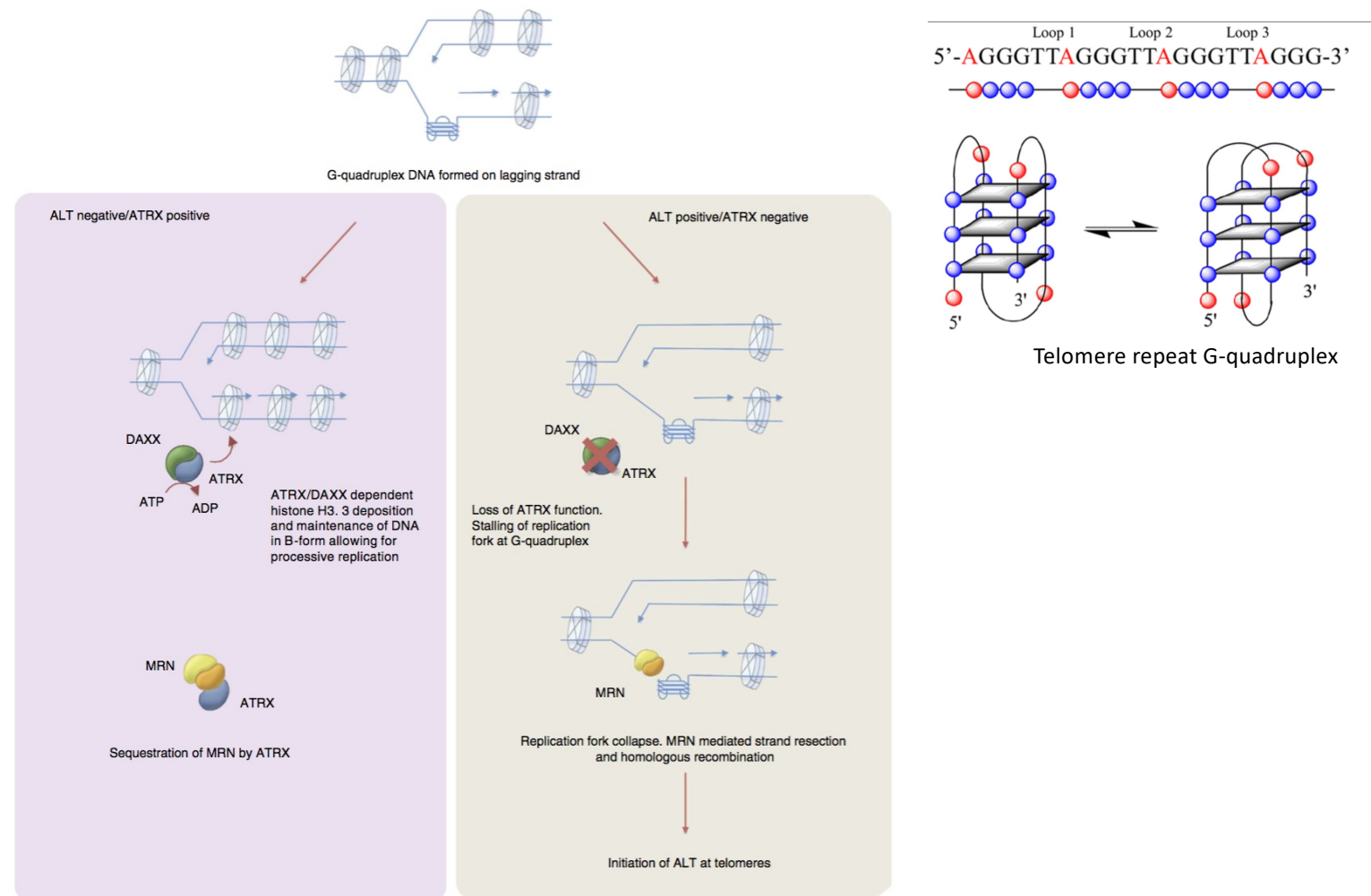
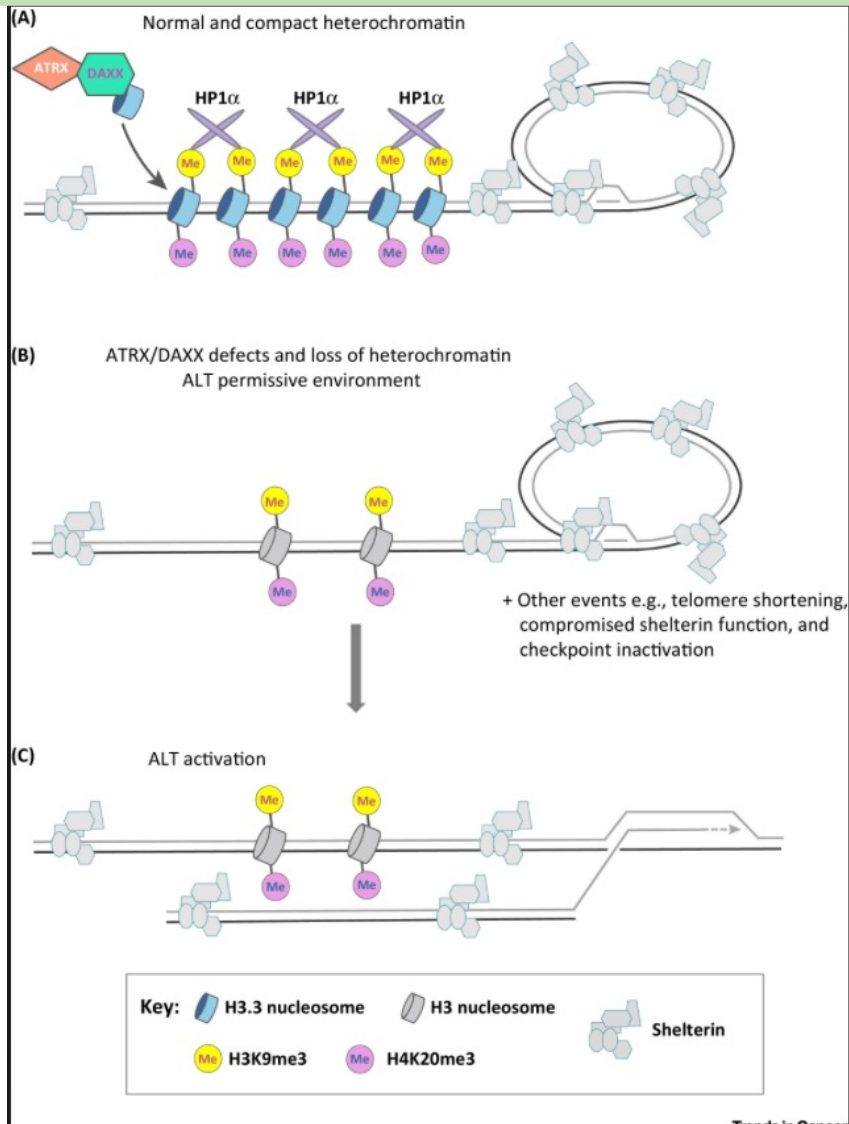


Figure 6 | Model for ATRX-mediated suppression of the ALT pathway. ATRX together with DAXX deposits histone H3.3 at telomeres, which in turn may facilitate DNA replication through G-quadruplex sequences. The presence of G-quadruplex structures in an ATRX null tumour cell leads to replication fork stalling 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



Observation: ATRX/DAXX/H3.3 is frequently mutated in a subset of tumors (osteosarcoma, PanNET). Tumors are telomerase negative

Loss of ATRX/DAXX function →

lack of H3.3 incorporation →

abnormal chromatin template →

frequent breaks →

induction of homologous recombination between repeat sequences such as telomeres →

Recombination involves DNA polymerase activity →

overall maintenance of telomeres

= ALT (alternative lengthening of telomeres)

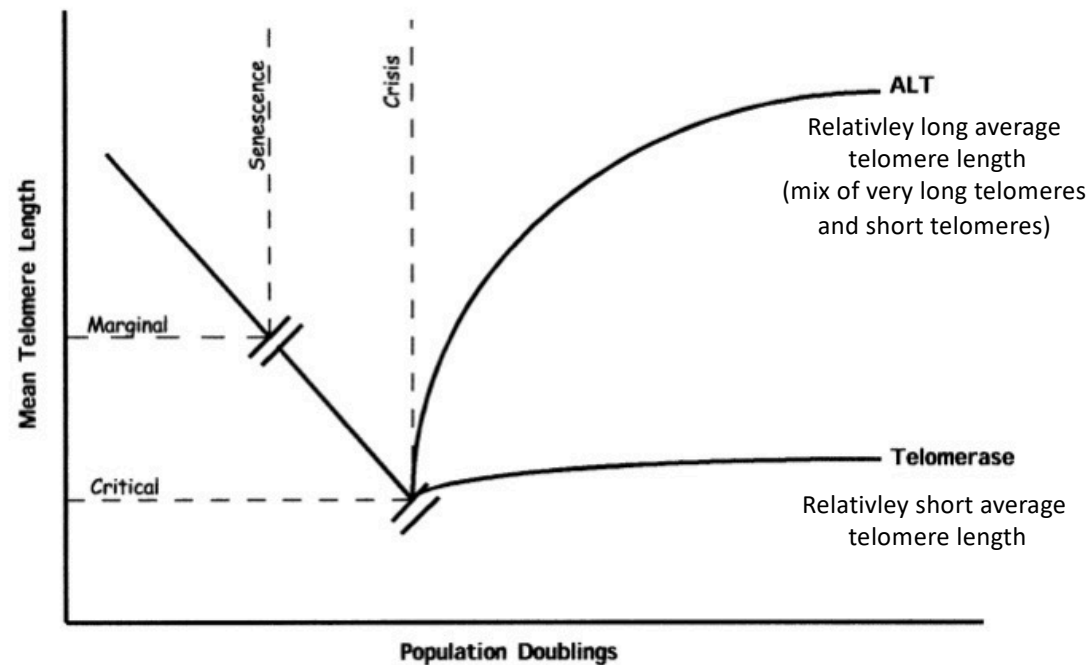
ROLE OF H3.3 IN TUMORFORMATION

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 (ALT)

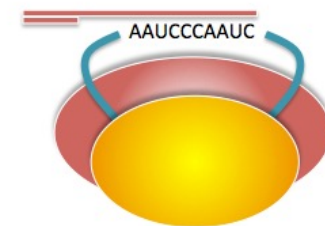
Osteosarcoma; Mutations in ATRX → tumor formation (ALT)



Alternative lengthening
of Telomeres (ALT)

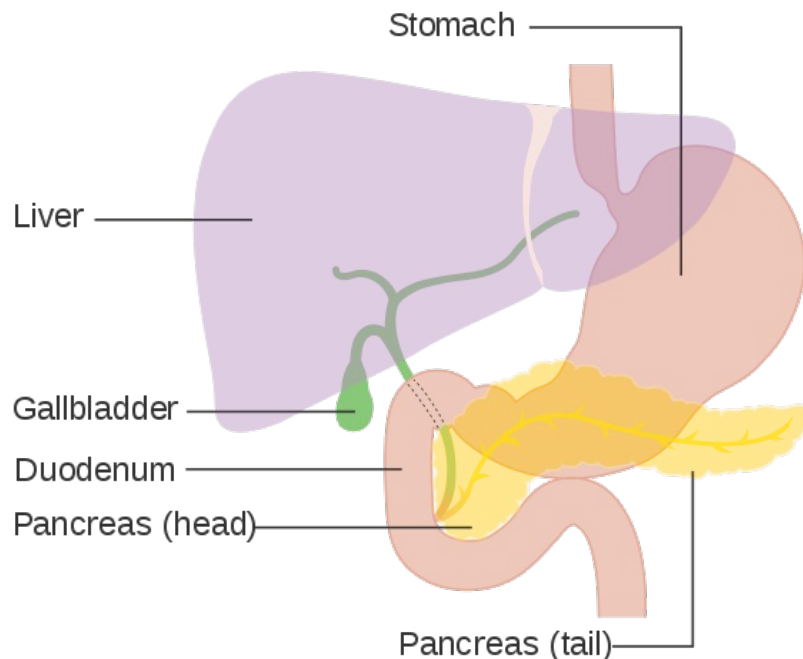


Telomerase



Reverse Transcription from Template

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).

Functional PanNETs produce excess hormones, and the symptoms depend entirely on which hormone is over-secreted. These effects can range from severe low blood sugar (insulinoma) to massive watery diarrhea (VIPoma) to recurrent ulcers (gastrinoma).

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

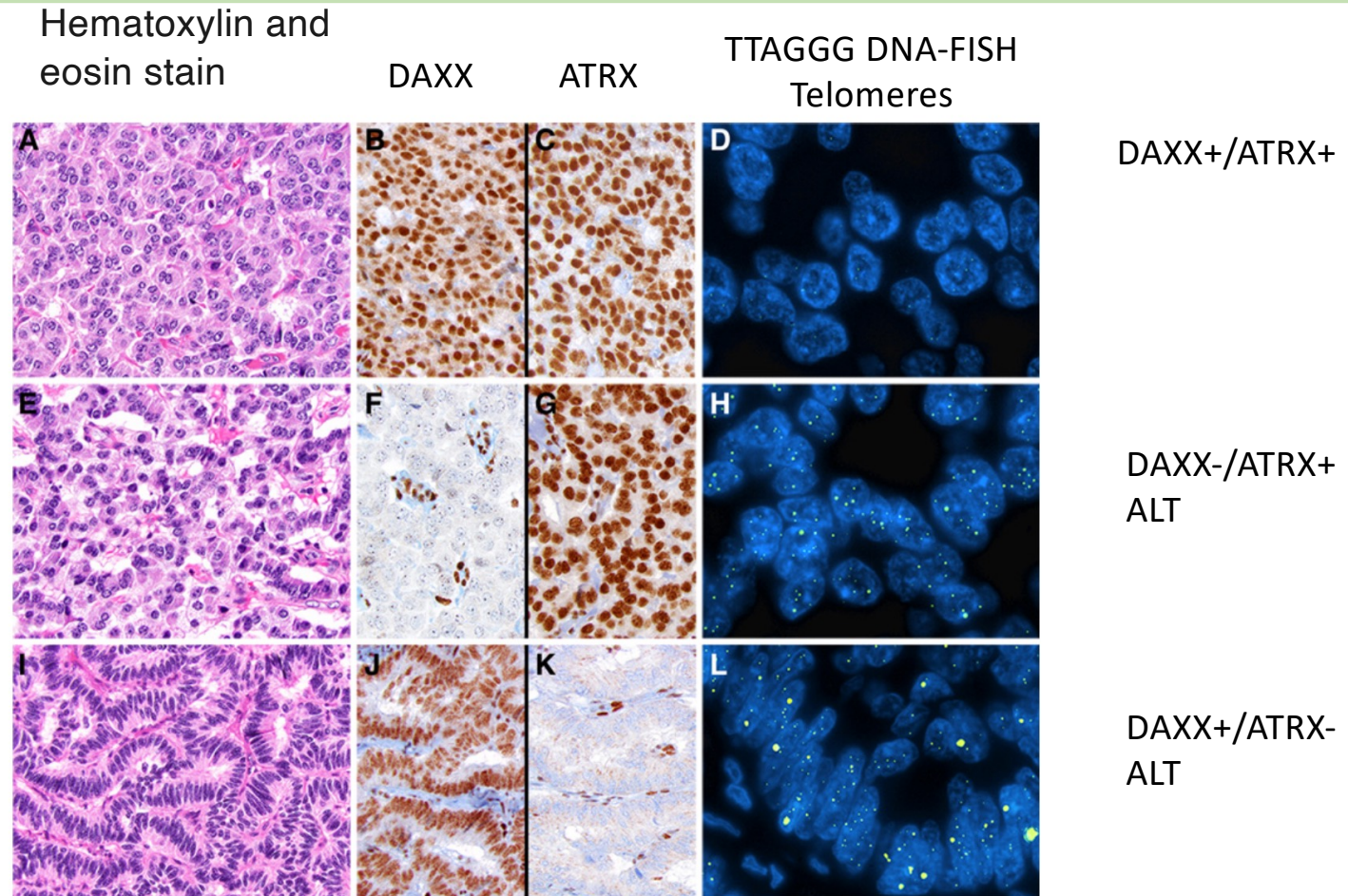


Figure 1.

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.

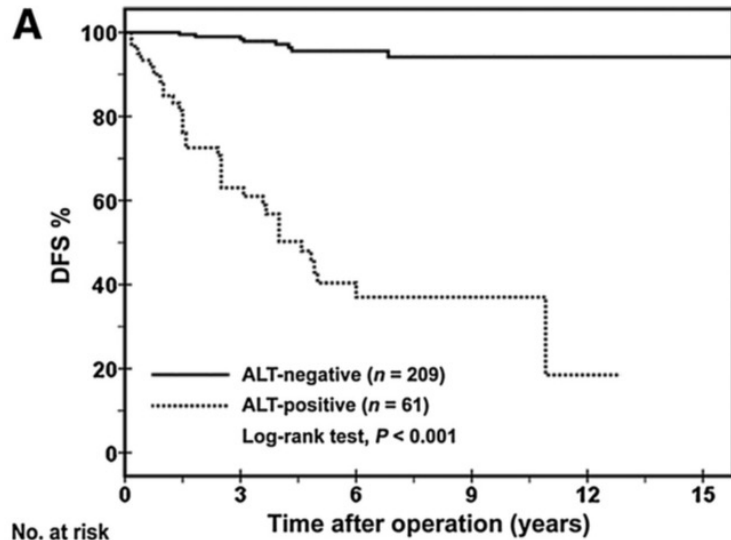
ROLE OF ATRX/DAXX AND H3.3 IN PanNETs

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

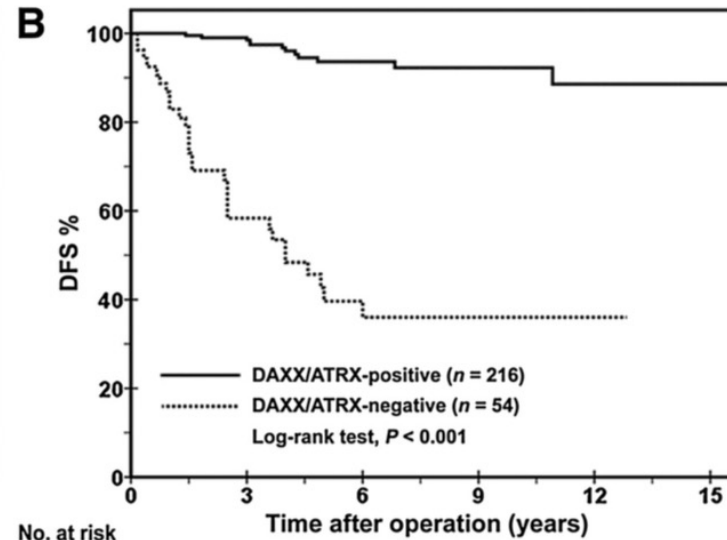
Patient or tumor characteristics	ALT-positive, n = 98 (31%)	ALT-negative, n = 223 (69%)	P	DAXX/ATRX-negative, n = 84 (26%)	DAXX/ATRX-positive, n = 237 (74%)	P
Gender						
Female	35 (35%)	108 (51%)	0.011 ^a	29 (35%)	121 (51%)	0.011 ^a
Male	63 (65%)	115 (49%)		55 (65%)	116 (49%)	
Mean age (range), years	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	
NO	39 (41%)	129 (75%)	<0.001 ^a	32 (39%)	136 (74%)	<0.001 ^a
NI	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%)	

^aIndicates 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 aggressiveness



No. at risk	0	3	6	9	12	15
Negative: 209	209	183	79	40	17	4
Positive: 61	61	31	12	5	1	0



No. at risk	0	3	6	9	12	15
Positive: 216	216	189	80	41	17	4
Negative: 54	54	25	11	4	1	0

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.

Histone Variants

1. A H3 variant at the centromere: CENP-A: a histone variant that defines eukaryotic centromeres

2. Histone H3.3 maintains chromatin structure and correct nucleosome organization

2a. Histone H3.3: maintains euchromatin structure

2b. H3.3 incorporation in nucleosomes at repeats contributes to genome stability

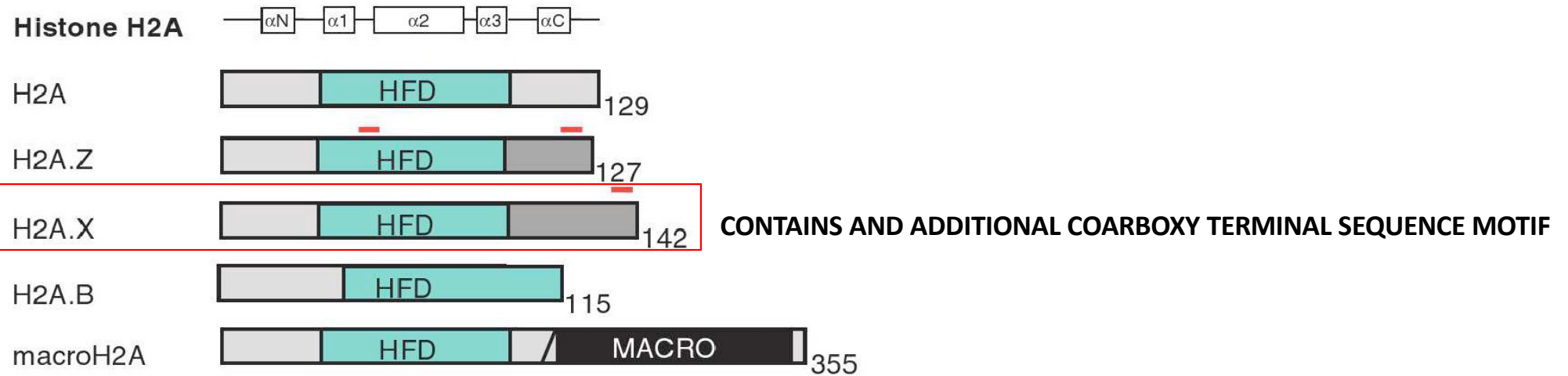
3. Role of H2AX in DNA damage response

4. A role for macroH2A and H2A.B on the X-chromosome

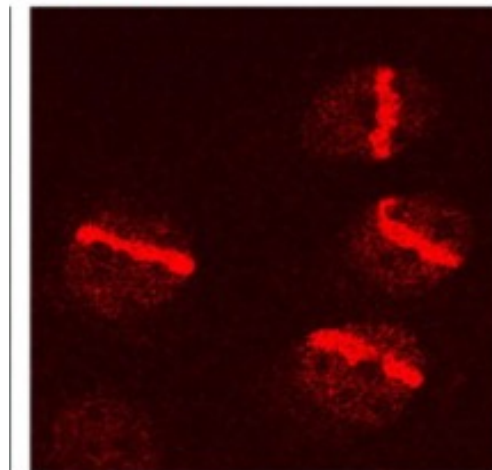
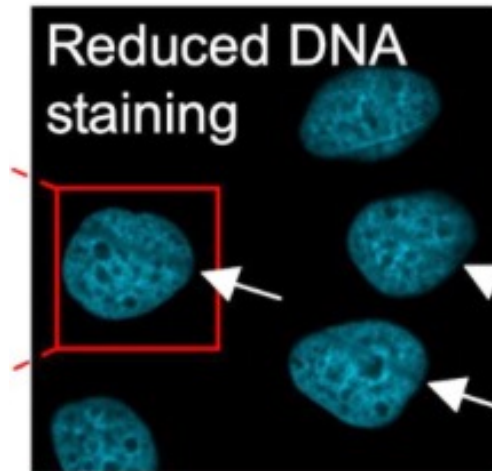
5. A role for H2A.Z at transcribed genes

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HISTONE H2A VARIANTS



Epigenetics and Histone Variants meet to organize DNA DAMAGE RESPONSE



γ -H2AX

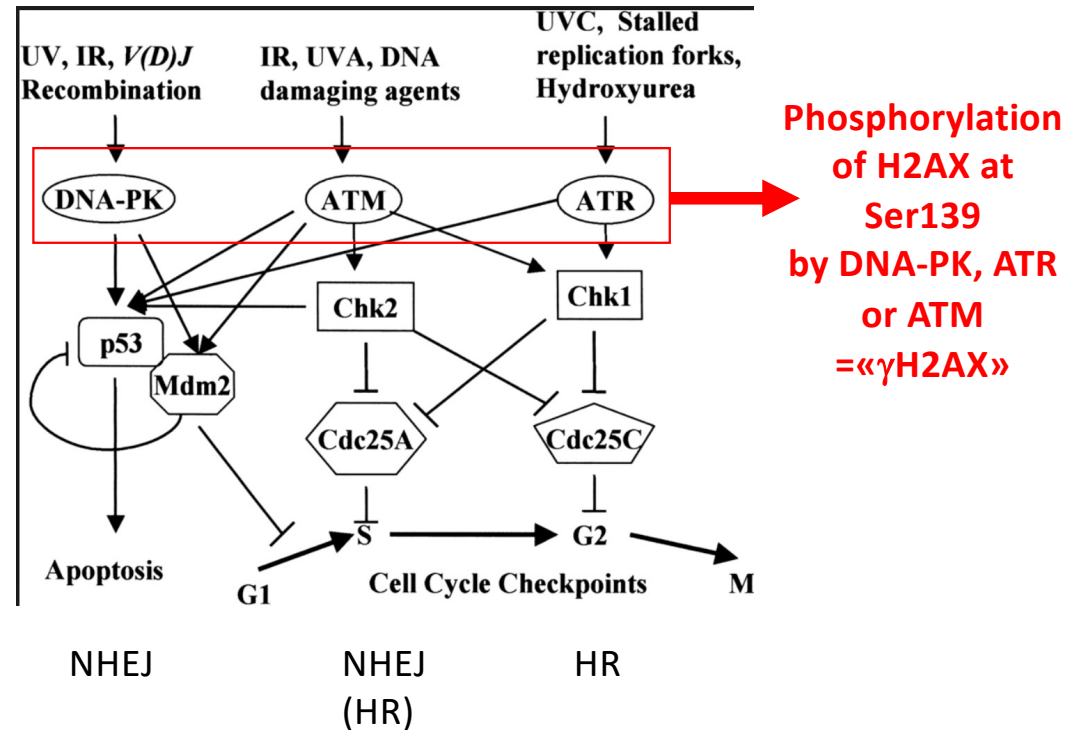
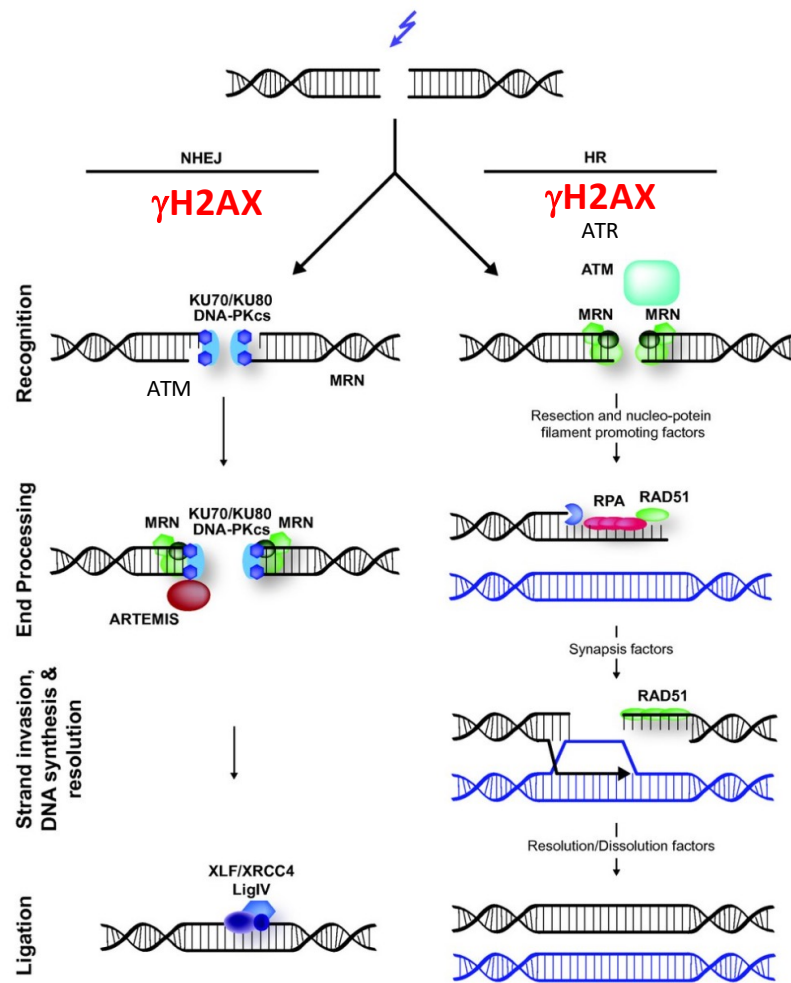
Model for damage:
cells cut with laser →
DNA breaks across nucleus →
phosphorylation of H2AX at Ser 139 = γ H2AX

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 DAMAGE FOCI

Epigenetics and Histone Variants meet to organize DNA DAMAGE RESPONSE

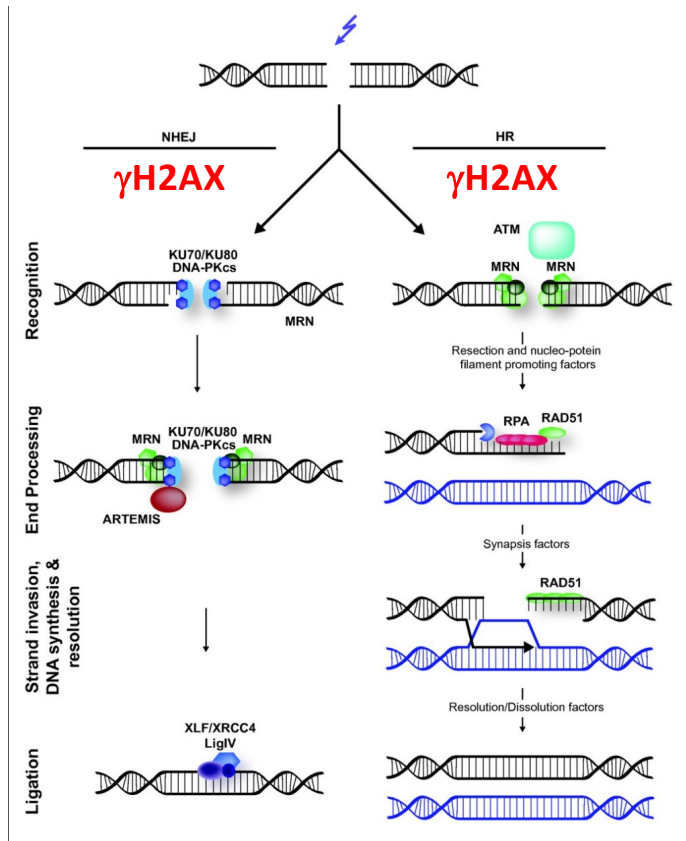


DNA double-strand breaks (DSBs) are cytotoxic damages that can be repaired either by the homologous recombinational repair (HR) pathway or by the non-homologous end-joining (NHEJ) pathway. NHEJ, although faster than HR, is less accurate. The early divergent step between the two pathways is end resection, 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.

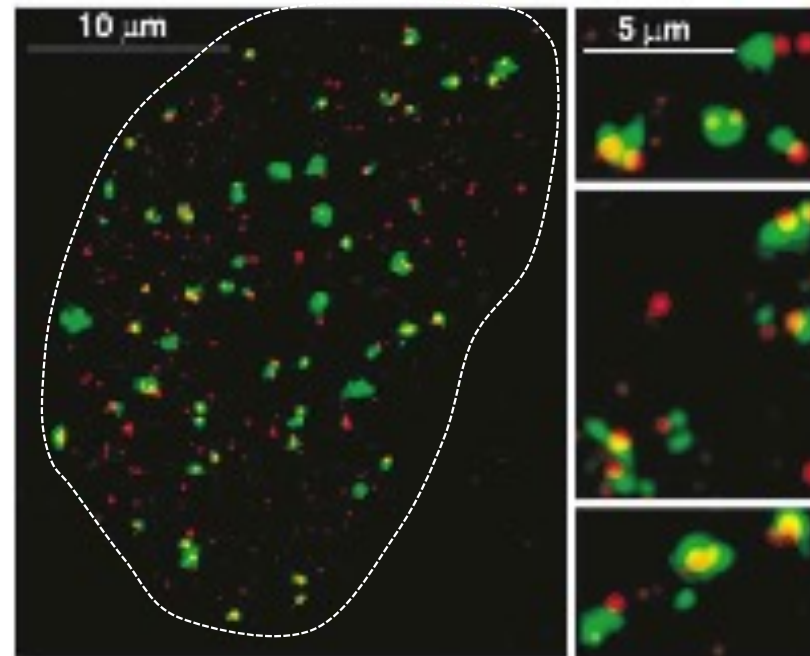
The MRN complex binds avidly to double-strand breaks both in vitro and in vivo and may serve to tether broken ends prior to repair by non-homologous end joining or to initiate resection prior to repair by homologous recombination. Recruits ATM. The MRN complex also participates in activating the checkpoint 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; H2AX and other targets



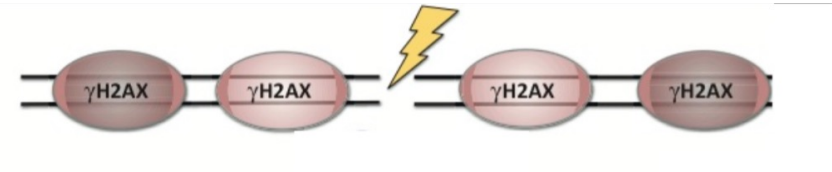
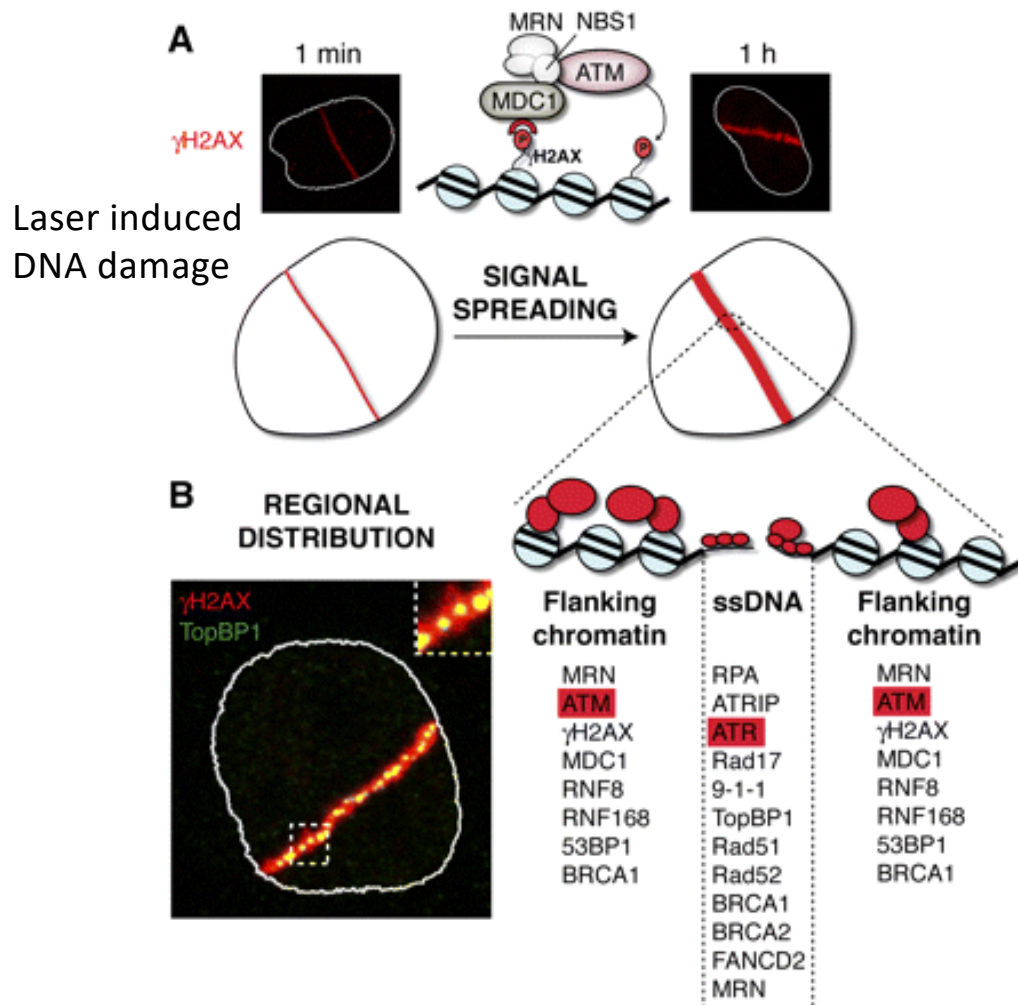
DNA Damage Foci in genomic DNA of cell nucleus



γ H2AX
53BP1
MRE11
P-ATM

Histone H2AX is a substrate of several phosphoinositide 3-kinase-related protein kinases (PIKKs), such as ATM (ataxia telangiectasia 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



H2AX is distributed across the genome
Incorporated mainly during S-Phase by the **FACT** (H2A/H2B dimer chaperone)

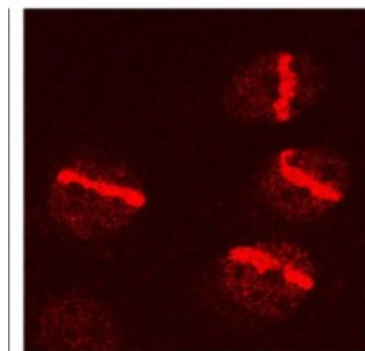
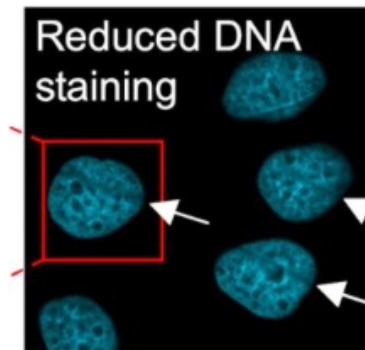
DAMAGE: γ H2AX typically spreads over a very large chromatin domain—on the order of hundreds of kilobases up to several megabases

Spatial organization of DDR protein accumulation at DNA DSBs. = **DNA DAMAGE REPAIR FOCI** formation (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 γ H2AX, 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.

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.



γ -H2AX

- (i) H2AX-knockout cells manifested impaired recruitment of NBS1, 53BP1, and BRCA1 to irradiation-induced foci
- (ii) Both, mutant human H2AX and H2AX mouse thymocytes show an increase in chromosomal aberrations.
- (iii) 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 show increased sensitivity to DNA damage.
- (iv) 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 indicate that γ H2AX serves as a docking site for DNA damage/repair proteins and functions to promote DSB repair and genome stability.

Loss of γ H2AX results in reduced DNA DAMAGE REPAIR FOCI → less efficient DNA damage repair

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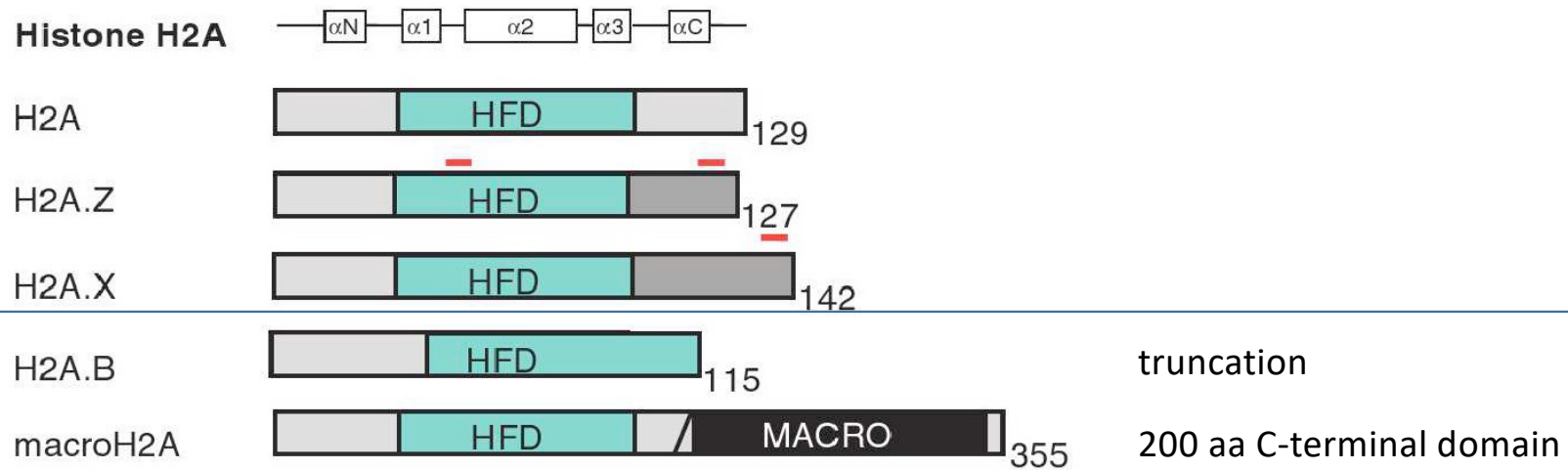
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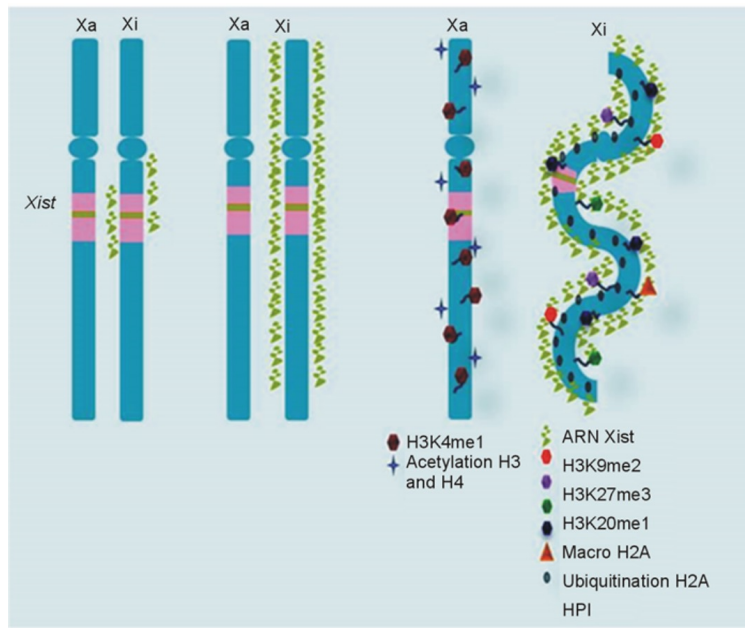
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MacroH2A and H2A.B and X inactivation

X inactivation is associated with the exchange of H2A variants



macroH2A is enriched at the Xi
histone chaperone: ANP32B

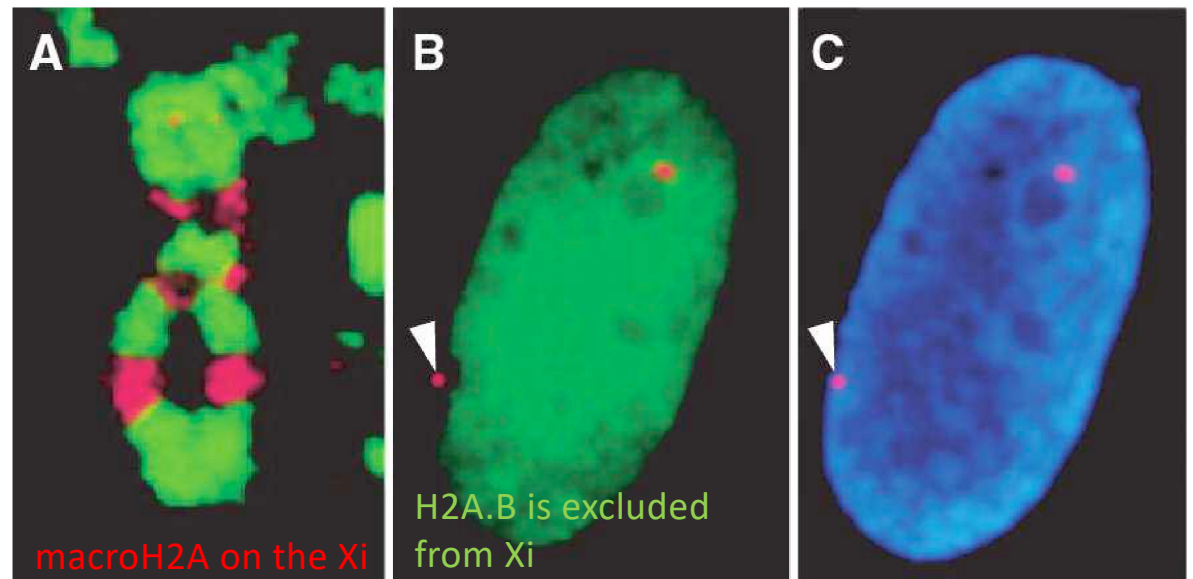


Figure 11. H2A variants and the inactive X chromosome of human females. (A) macroH2A (red) stains discrete regions of the inactive X chromosome that alternate with a marker for heterochromatin (histone H3K9me3). (B) H2A.B (green) is excluded from the inactive X chromosome (red dot with arrowhead pointing 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.)

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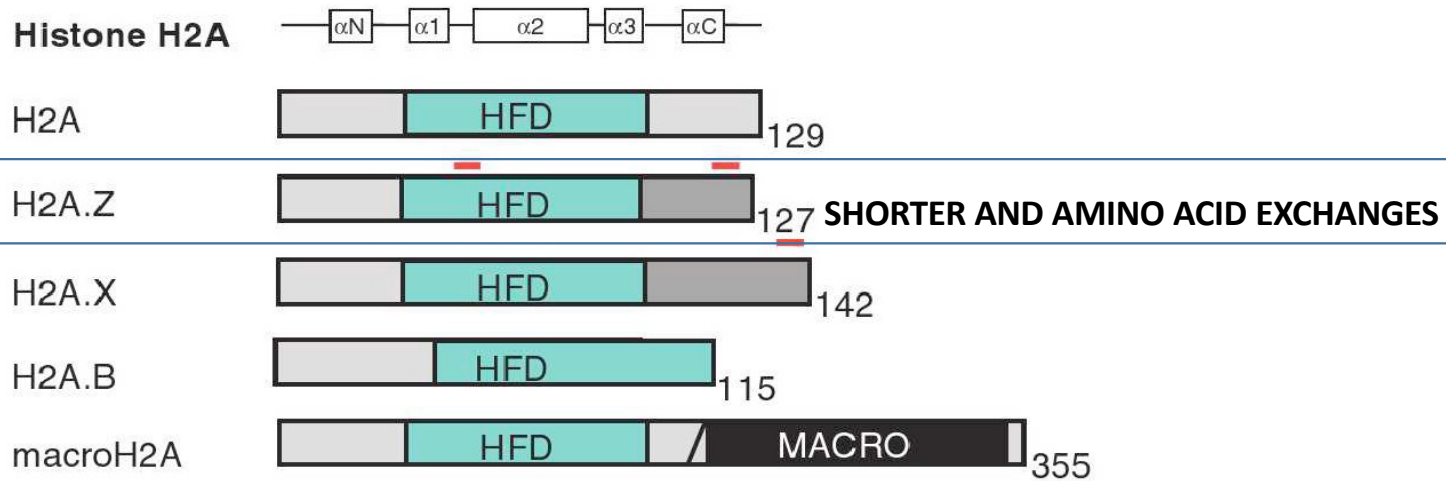
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Different heterodimers possible:

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

H2AZ: replication independent; Histone chaperon: human YL1; yeast: Chz1

H2A.Z shows less adherence to DNA and is frequently localized **at the -2 and +1 nucleosome position** after the transcriptional start site (TSS):

Yeast: ZZ: 32%

ZA: 24%

AA: 44%

Also H3.3 is localized in nucleosomes flanking the TSS

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

NUCLEOSOME DEPLETED REGIONS (NDRs)

Nucleosome remodelling

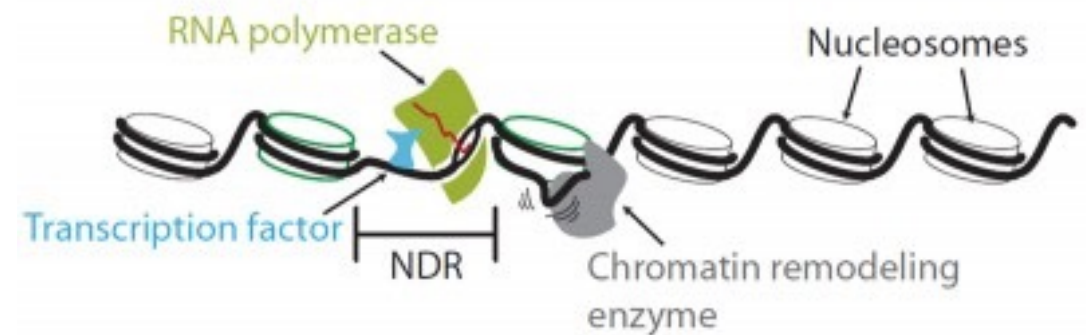
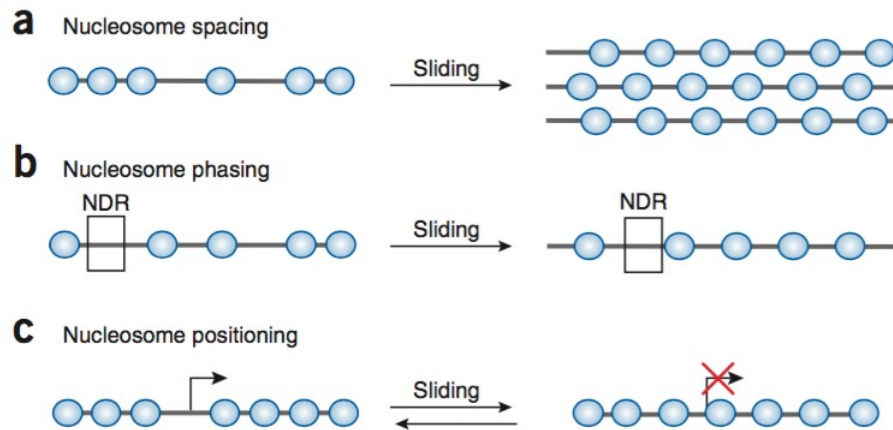
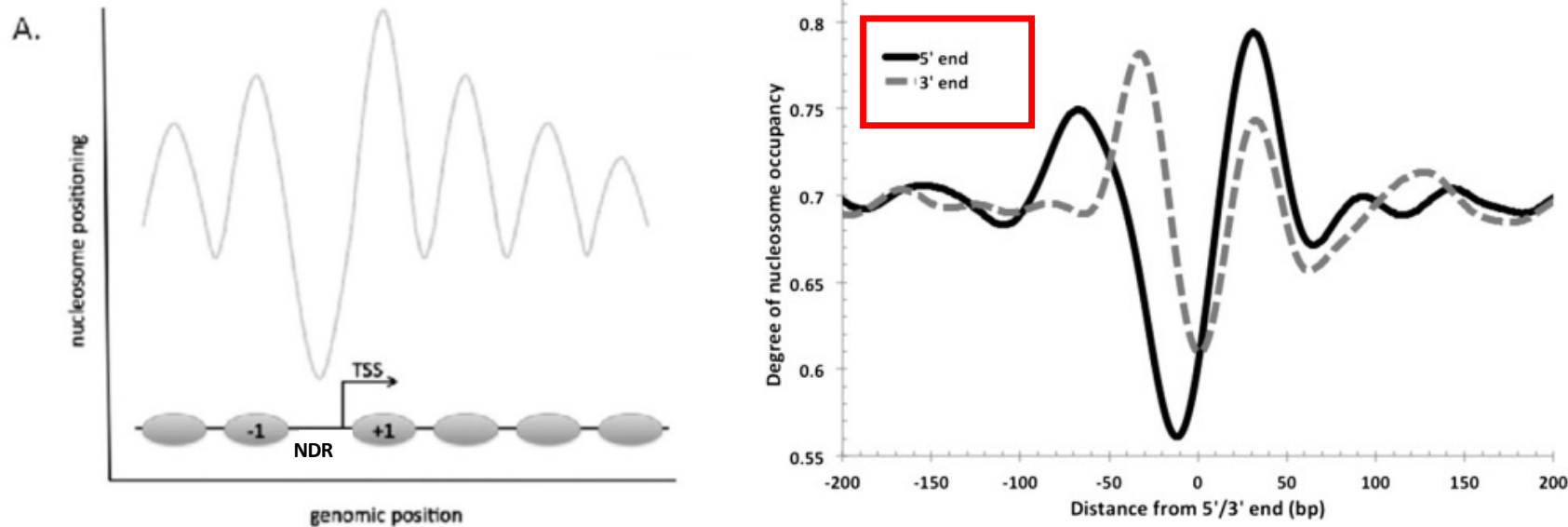


Figure 2 The different physiological outcomes of nucleosome sliding. (a) Nucleosome-remodeling enzymes can introduce and maintain regular spacing of nucleosomes. (b) The phasing of nucleosomal arrays with respect to a nucleosome-depleted region (NDR), a prominent feature of promoters, depends on nucleosome-remodeling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

NUCLEOSOME DEPLETED REGIONS AT TRANSCRIPTIONAL START SITES (TSS) AND TRANSCRIPTINAL TERMINATION SITES (TTS)



TSS and TTS are characterized by an absence of a nucleosome
→ Access of transcription machinery
→ Access of termination machinery

NDRs: Nucleosome-depleted regions at the 5' and 3' ends of transcripts as classic feature of transcribed genes.

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

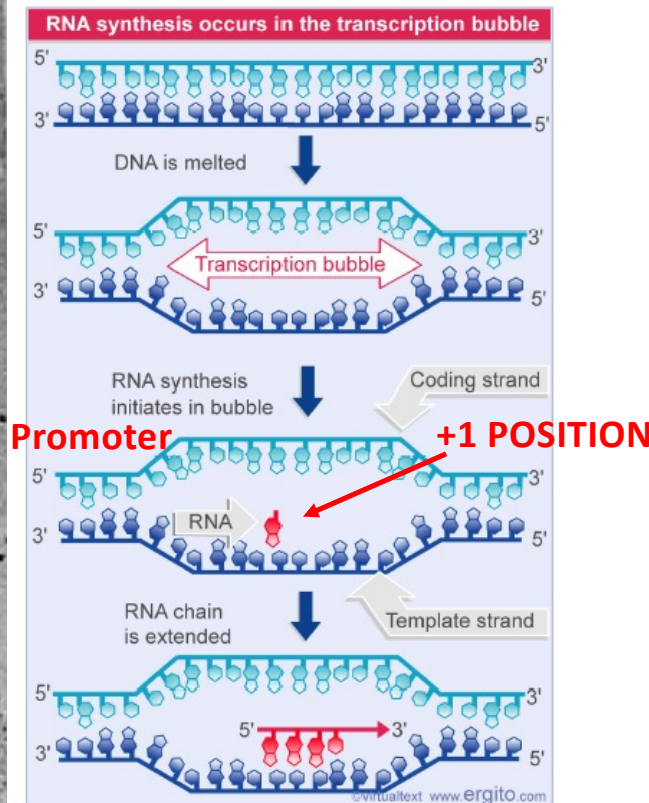
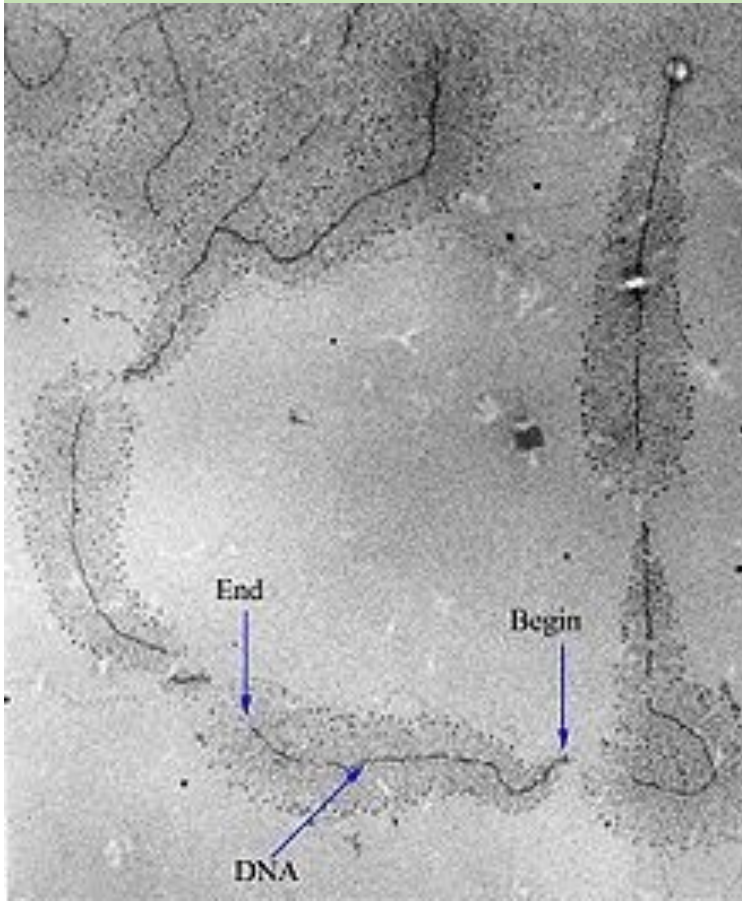


Figure 9.3 DNA strands separate to form a transcription bubble. RNA is synthesized by complementary base pairing with one of the DNA strands.

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

The initiation site of transcription is nucleosome-free

Nucleosomes containing H2A.Z and H3.3 are **more unstable**; 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 through M-Phase

=TRANSCRIPTIONAL MEMORY TO MARK START SITE IN ACTIVE GENES!!!

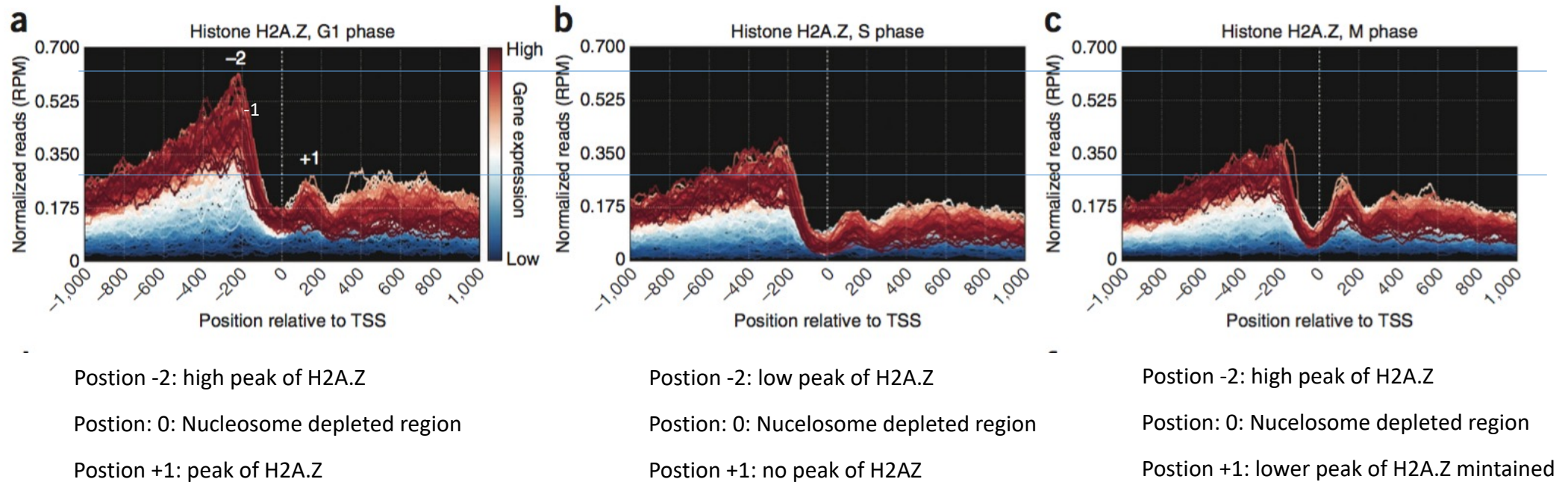
“normal” histone modifications do not precisely mark the start site of transcription

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

H2A.Z MARKS THE TSS DURING THE CELL CYCLE

0 - NDR: transcriptional start site

Brown color: highly expressed gene



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