

REGULATION OF HISTONE GENE EXPRESSION AND HISTONE CHAPERONS

Histone gene clusters in vertebrates

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

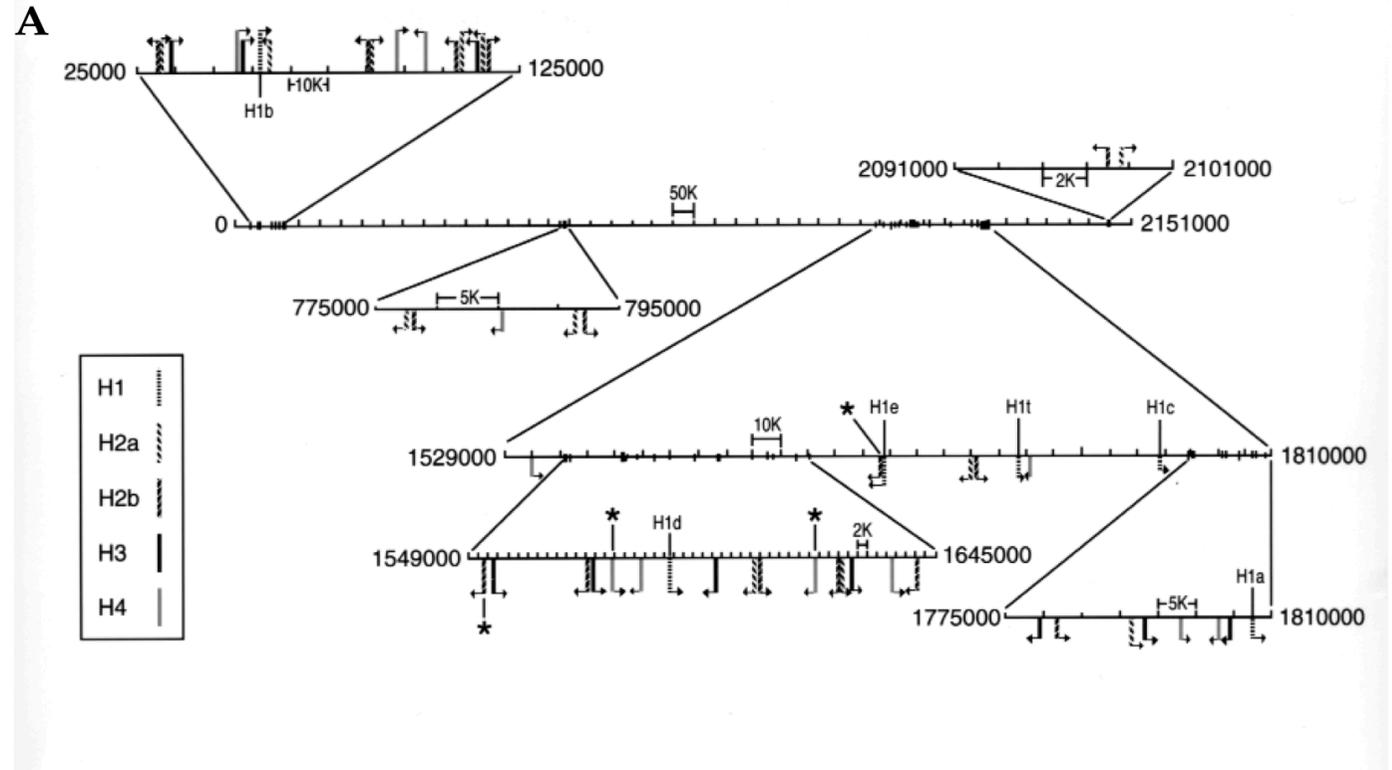
Chr.1: minor histone gene locus HIST3 cluster: 3 histone genes

Yeast:

2 copies for each core histone

Sea Urchin:

More than 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 histone 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.

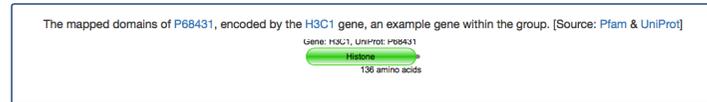
Histone gene clusters in vertebrates

Gene group: H3 histones (H3) ?

A subgroup of ? "Histones"



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]



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.

All the histone **H4 genes encode the same protein**, **histone H3 genes** encodes variants with aa changes, there **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.f	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

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 is lethal → precise control of histone gene expression required → cell cycle

2. Controlled production of nucleosomes:

A. REPLICATION DEPENDENT REGULATION: Canonical histone proteins: Histone mRNA levels increase ca 15-fold increase in 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

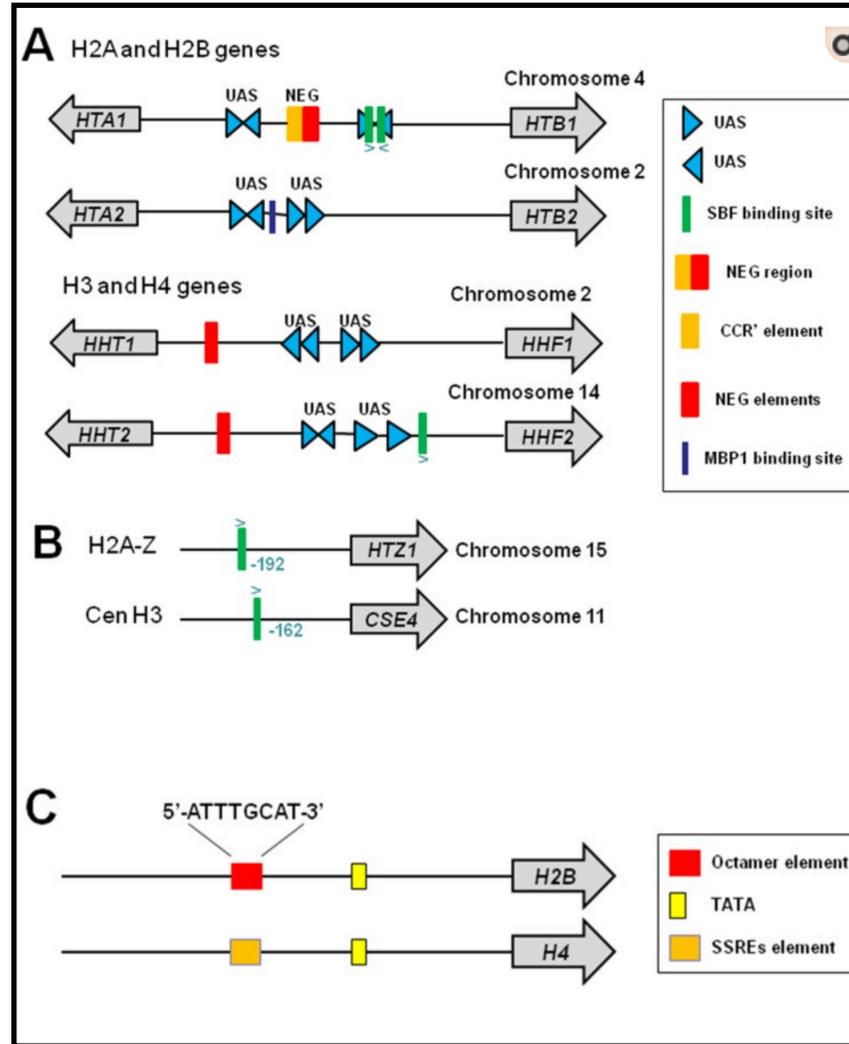
B. REPLICATION INDEPENDENT REGULATION: histone variants: Nucleosome need also be formed in G1, G2 phase when nucleosome arrays get disturbed (transcription, DNA damage, etc...). The second class of histones is composed of **histone variants that are expressed at a relatively low level throughout the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

Coordinated control of histone gene expression

Structure of mammalian canonical histone genes
 Structure of yeast histone variants H2AZ and CenH3.
 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.

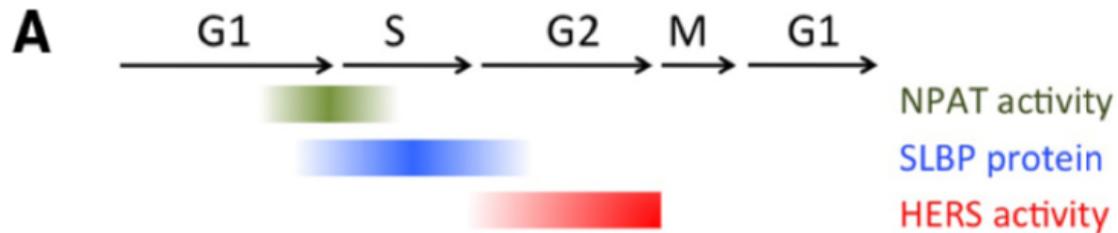
Histone promoters also contain specialized cis elements that required for histone gene expression. Histone H2B promoter contains an octamer element (5'-ATTTGCAT-3'), which is bound by transcription activator Oct-1 (octamer-binding factor 1). Histone H4 promoter contains subtype-specific regulatory elements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



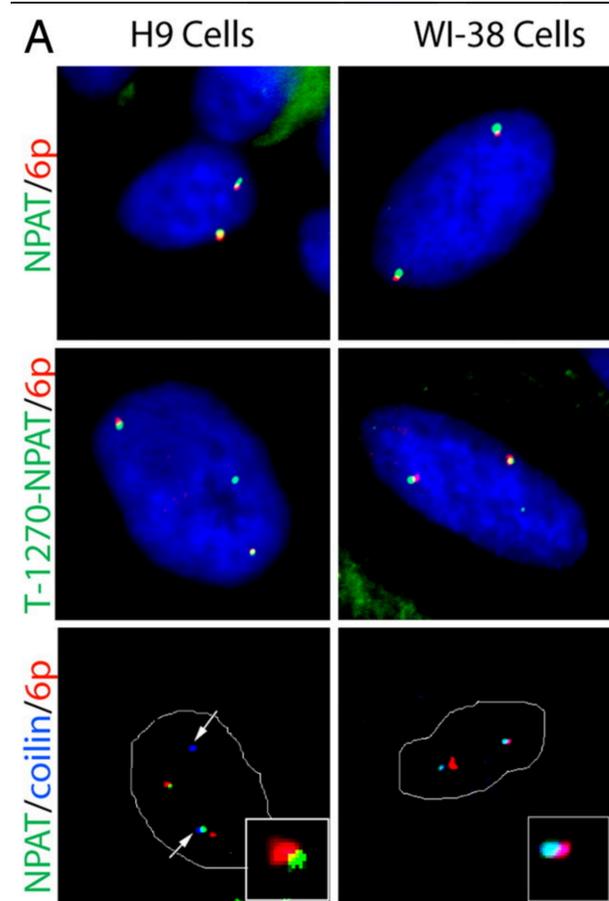
HTA1-HTB1 and *HTA2-HTB2*: encode H2A-H2B pairs
HHT1-HHF1 and *HHT2-HHF2*: encode H3-H4 pairs;

CONTROL OF HISTONE EXPRESSION IN S-PHASE

Histone synthesis is limited to S-Phase



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



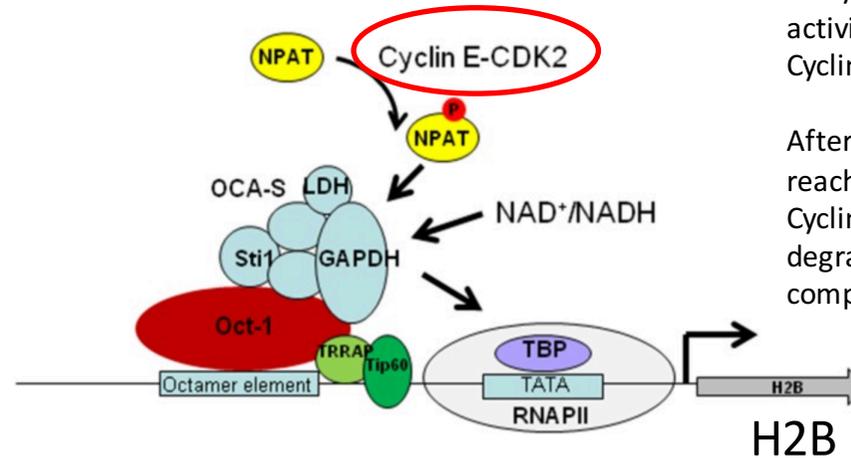
Human cells in S-phase
IF staining for NPAT combined with
DNA FISH for the HIST1 cluster on Chr.6

CONTROL OF HISTONE EXPRESSION IN S-PHASE

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.

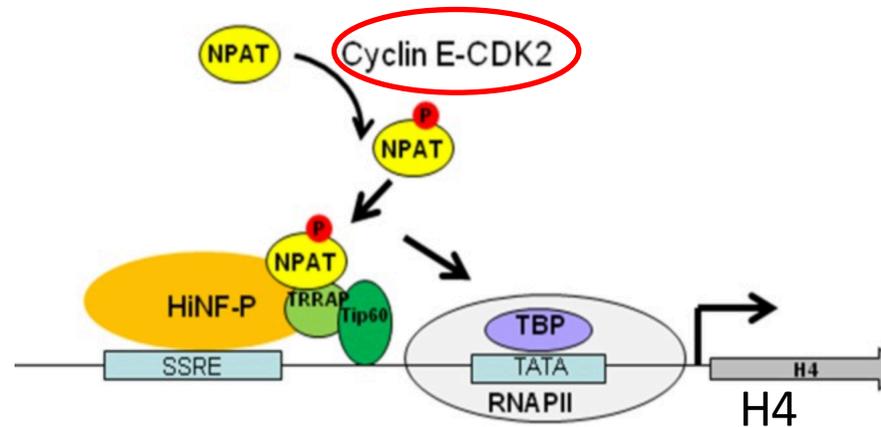


CDK2 and the cell cycle:

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

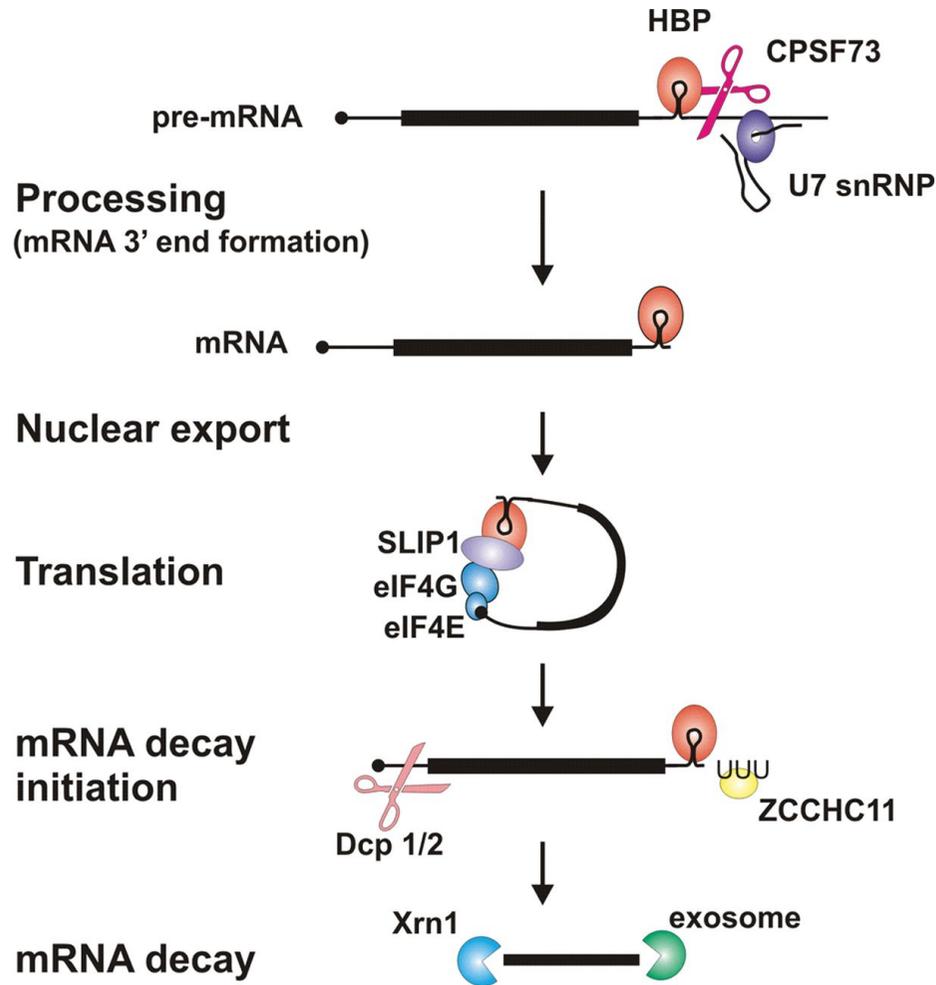
Activation of histone H4. HiNF-P binds to SSRE within H4 promoters and recruits NPAT and RNA polymerase II to activate gene transcription. NPAT recruits the Tip60 histone acetyltransferase complex to acetylate histone H4 at the G1/S-phase transition. At the end of S phase, the tyrosine kinase WEE1 is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

HBP (SLBP) - REGULATION OF RNA METABOLISM



HBP (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.

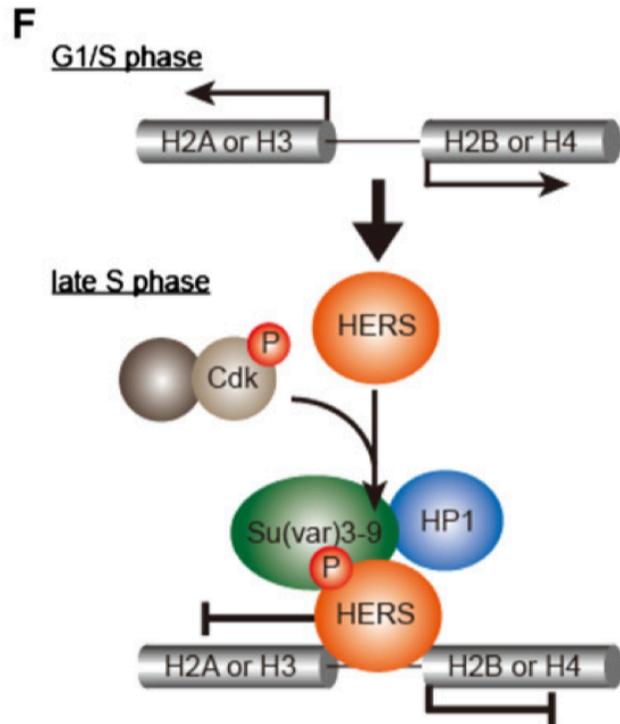
After nuclear export, HBP interacts with SLIP1 and other translation initiation factors to form a closed-loop structure for efficient translation.

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.

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

REPLICATION COUPLED HISTONES

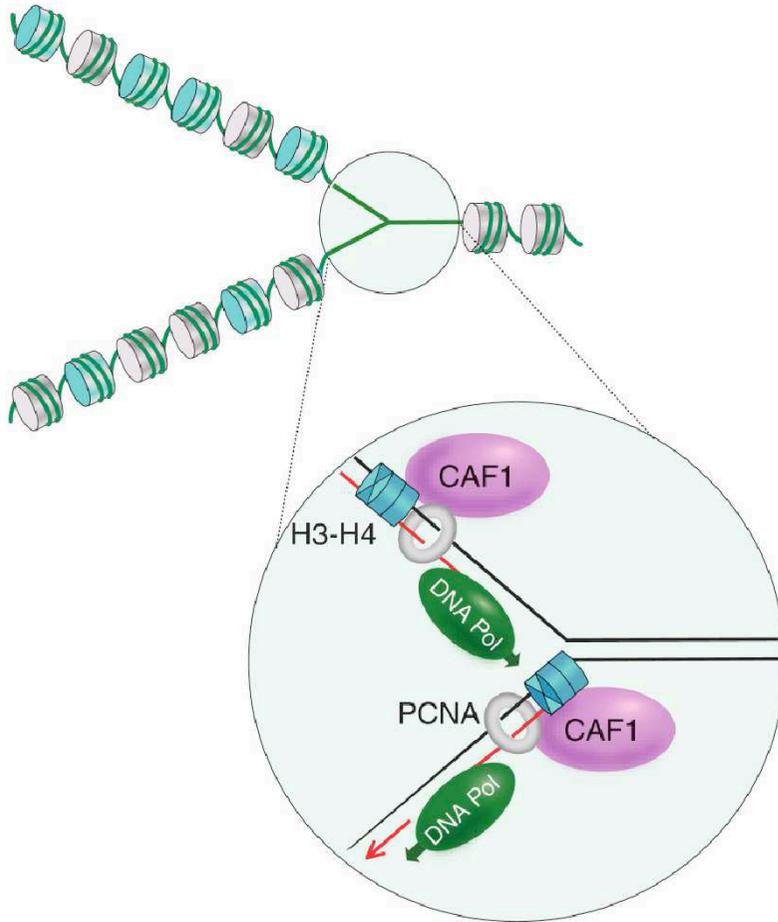


Figure 4. Distribution of old and new nucleosomes at a replication fork. Old nucleosomes (gray disks) are randomly distributed behind the replication fork and new nucleosomes (cyan disks) are deposited in the gaps. CAF-1-mediated nucleosome assembly is depicted on the leading and lagging strand in magnification. 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 (tetrasome) that is followed by the addition of 2 H2A-H2B dimers.

REPLICATION INDEPENDENT (RIs) HISTONES:

Are incorporated independently of DNA replication

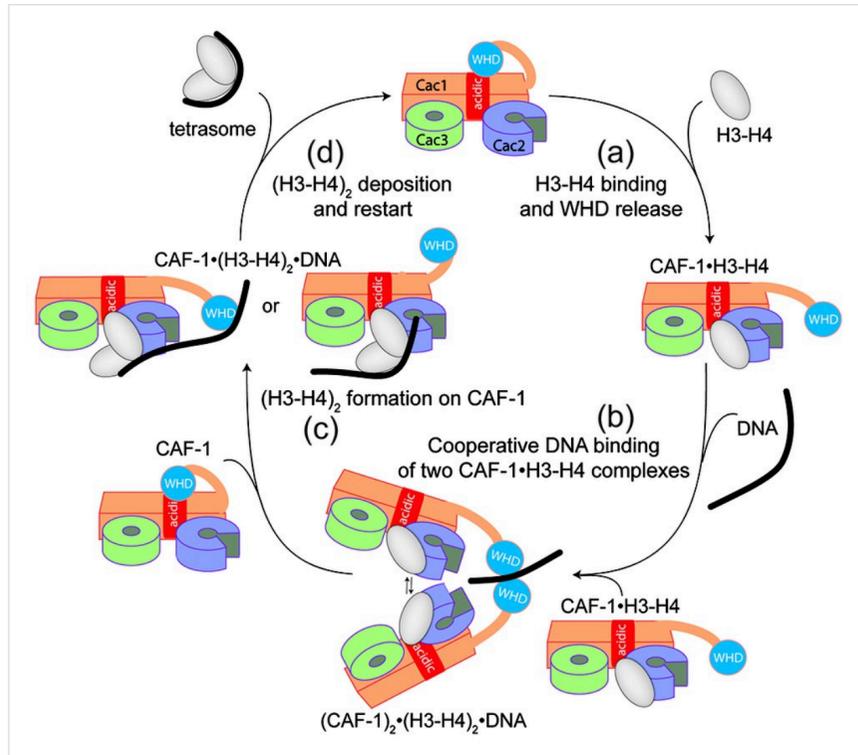
RI histones require the displacement of a preexisting nucleosome unit (active displacement or “loss”)

RI histones can **reset the epigenetic state of a pre-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 WHD domain from an intramolecular interaction with the acidic region on Cac1.
- (b) DNA binding promotes the association of two CAF-1•H3-H4 complexes to join the histones into a (H3-H4)₂ tetramer
- (c) In the presence of DNA of sufficient length, the (H3-H4)₂ histones are directly sequestered from CAF-1.
- (d) (H3-H4)₂ are transferred to the DNA to form the **tetrasome**, and the WHD rebinds to the now free acidic region, resulting in its dissociation from DNA.

H2A-H2B can spontaneously associate with tetrasomes in vitro and because CAF-1 itself has significantly lower affinity for H2A-H2B compared to H3-H4, it appears that the primary role of CAF-1 is to promote the formation of an ordered (H3-H4)₂•DNA complex, the tetrasome

REPLICATION INDEPENDENT (RIs) HISTONES: - HISTONE VARIANTS

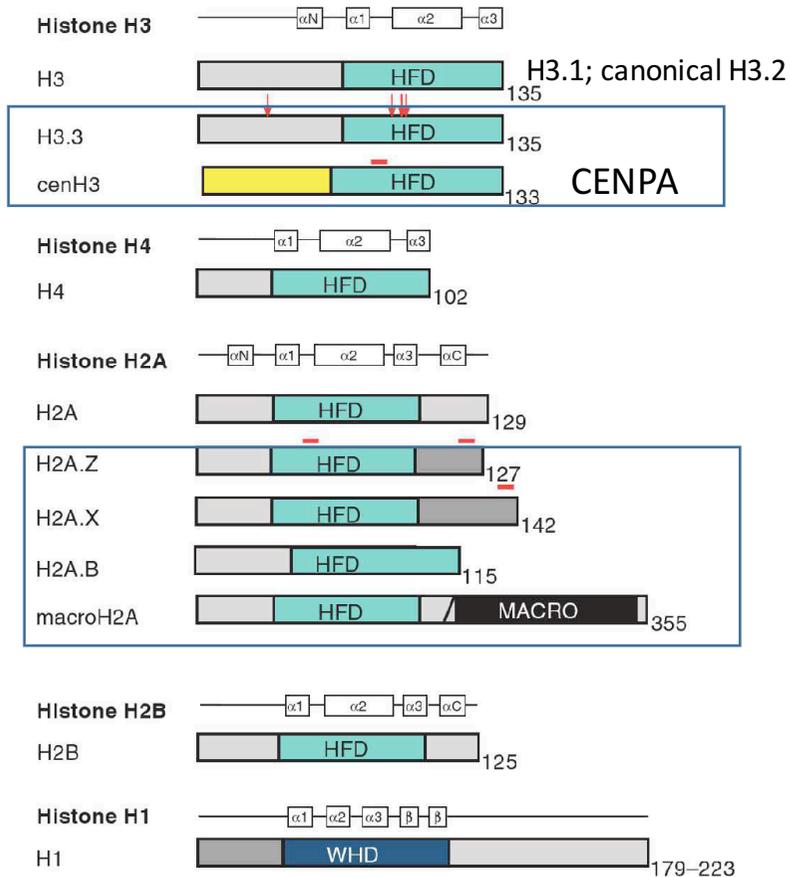


Figure 1. Histone variants. Protein domain structure for the core histones (H3, H4, H2A, and H2B), linker histone H1, and variants of histones H3 and H2A. The histone-fold domain (HFD) is where histone dimerization occurs. Regions of sequence variation in histone variants are indicated in red. WHD, winged-helix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

REPLICATION INDEPENDENT (RIs) HISTONES:

Are incorporated throughout the cells cycle and independently of DNA replication

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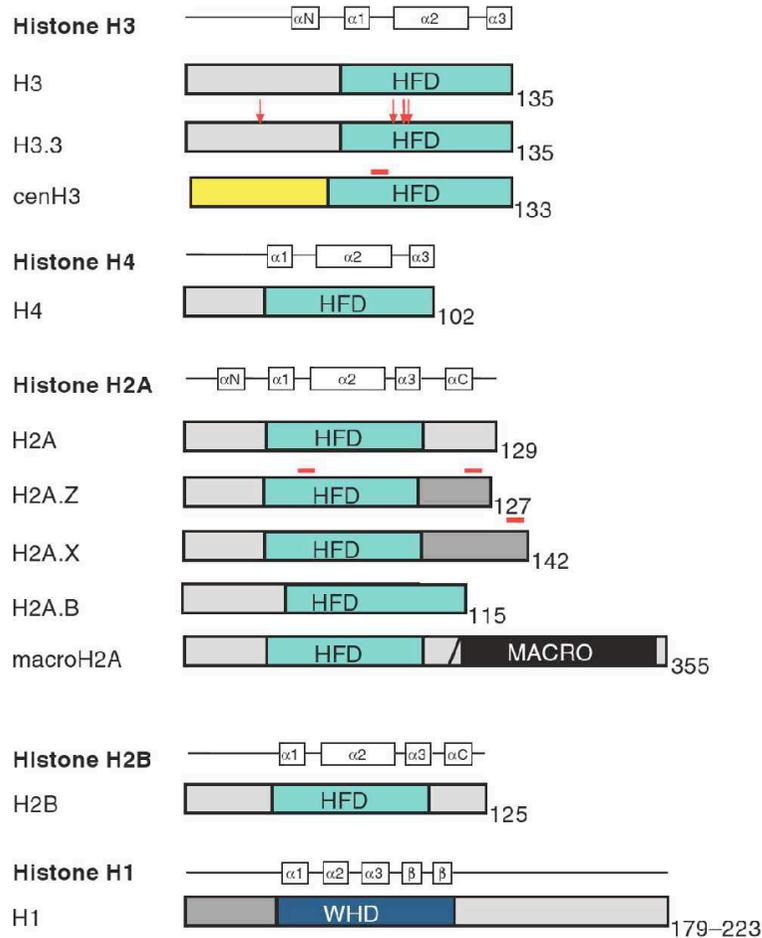


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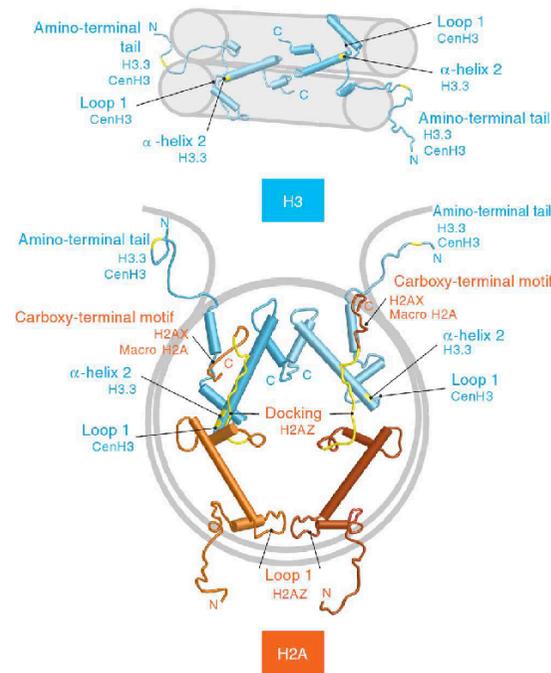
Humans:

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

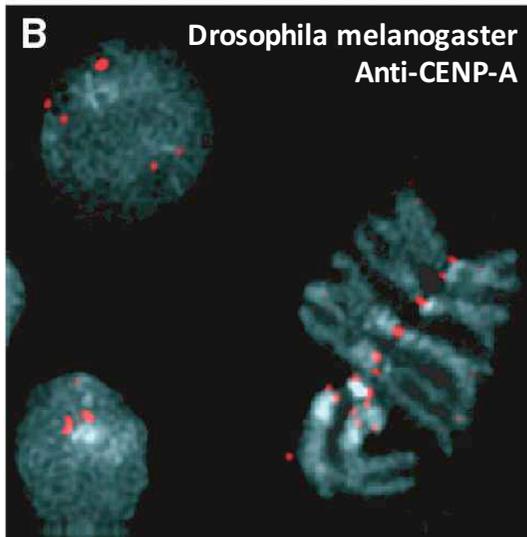
Incorporation of variants of histones into nucleosomes dramatically altered chromatin structure

Some histone variants are deposited by specialized nucleosome assembly complexes

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

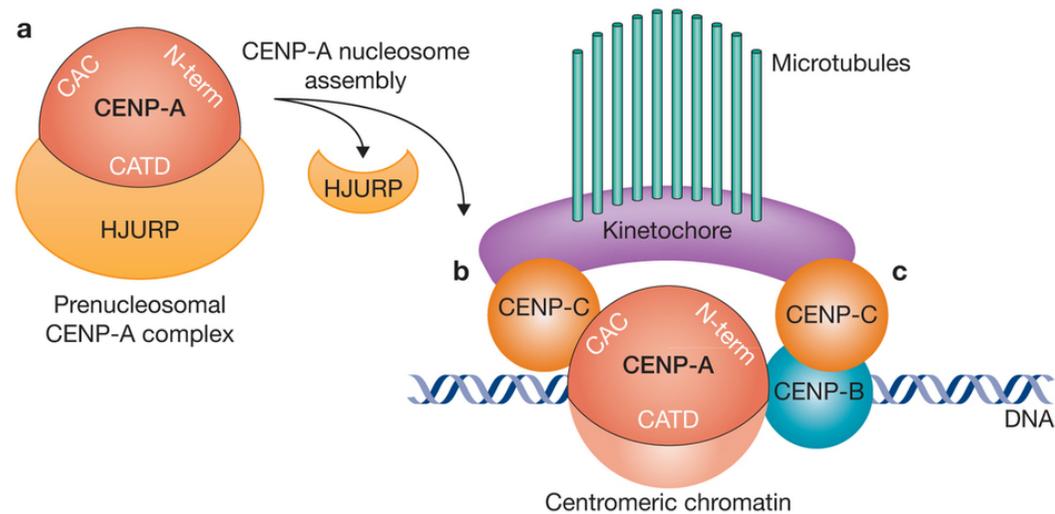


H3 VARIANTS: CENP-A: A HISTON VARIANT THAT DEFINES EUKARYOTIC CENTROMERES



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

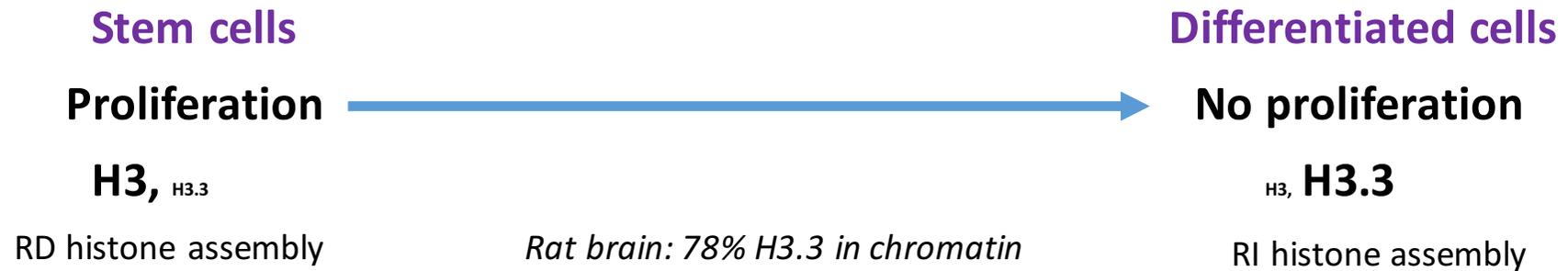
Centromere protein **B** is a highly conserved protein that facilitates centromere formation. It is a DNA-binding protein that is derived from transposases of the pogo DNA transposon family. It contains a helix-loop-helix DNA binding motif at the N-terminus, and a dimerization domain at the C-terminus.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



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

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

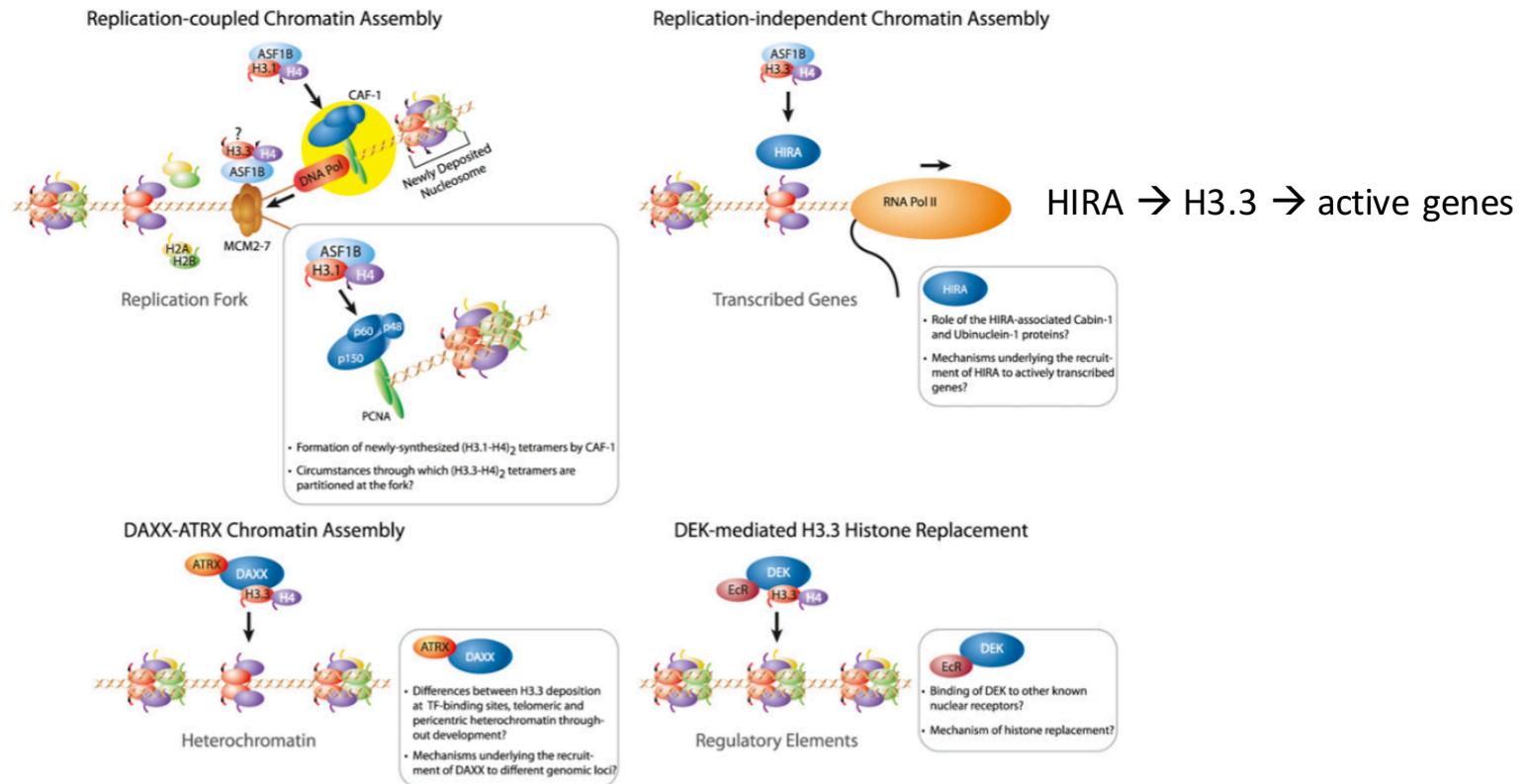
H3.3 INCORPORATION INTO GENOME IS HIGHLY DYNAMIC: AN EXAMPLE



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

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

Pathways if RI incorporation of H3.3 into chromatin



DAXX/ATRX →
H3.3 → repetitive
elements

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

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

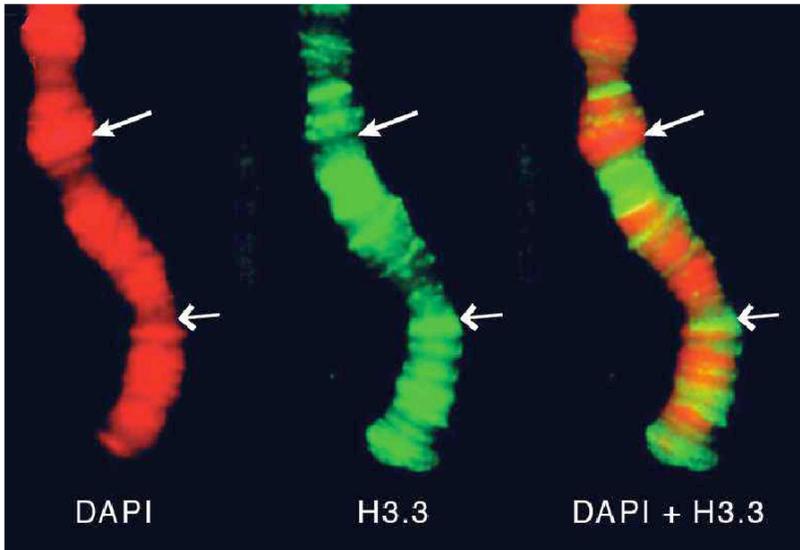


Figure 7. H3.3 preferentially localizes to actively transcribed regions of *Drosophila* polytene chromosomes. DAPI staining (red) shows the DNA banding pattern (*left*), and H3.3-GFP (green) localizes to interbands (*middle*), which are sites of RNA Pol II localization. The merge ([Schwartz and Ahmad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

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

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

also incorporated by histone chaperones (RI histone)

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

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

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

Only 4 aa exchanges

