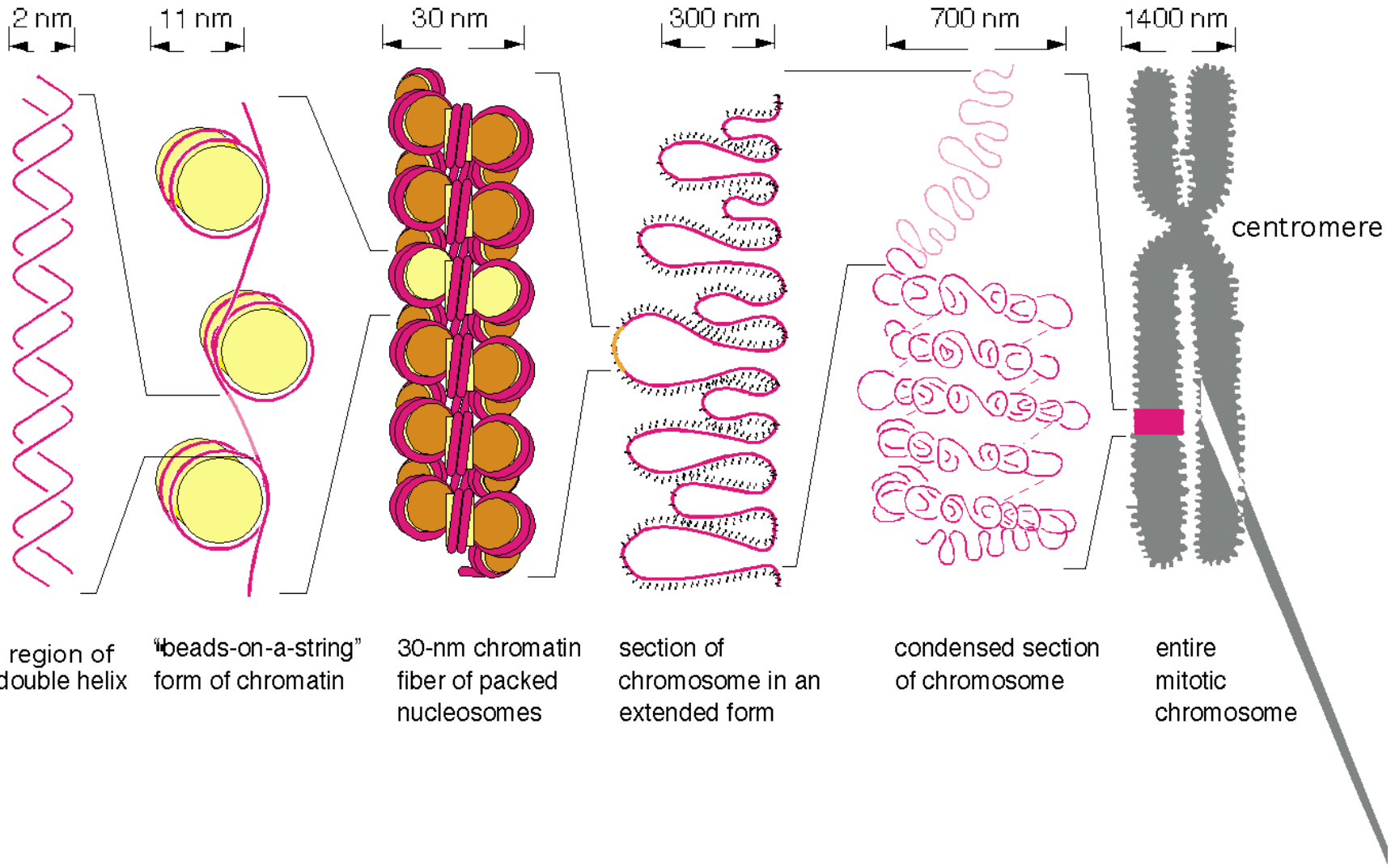
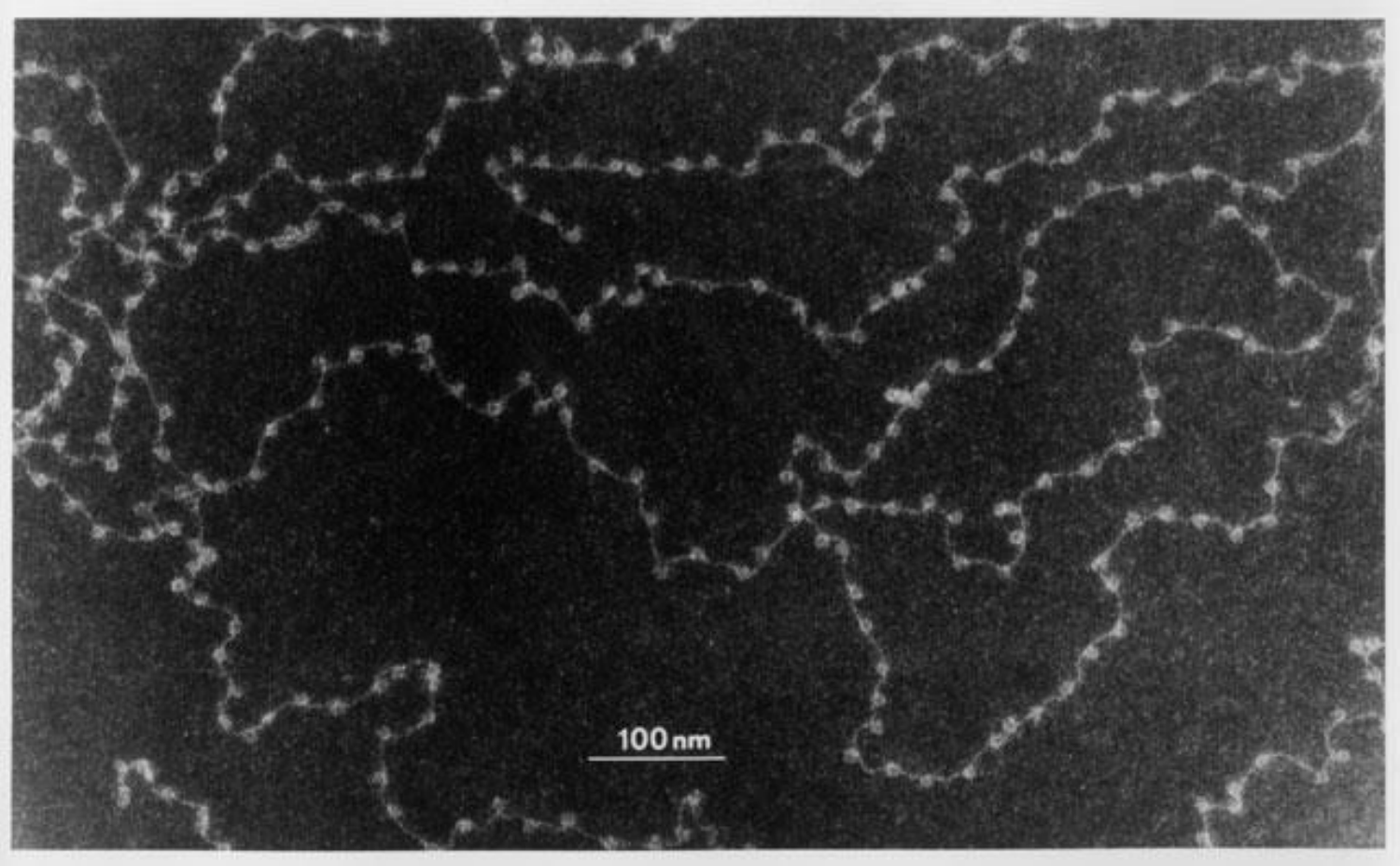
Chromatin structure

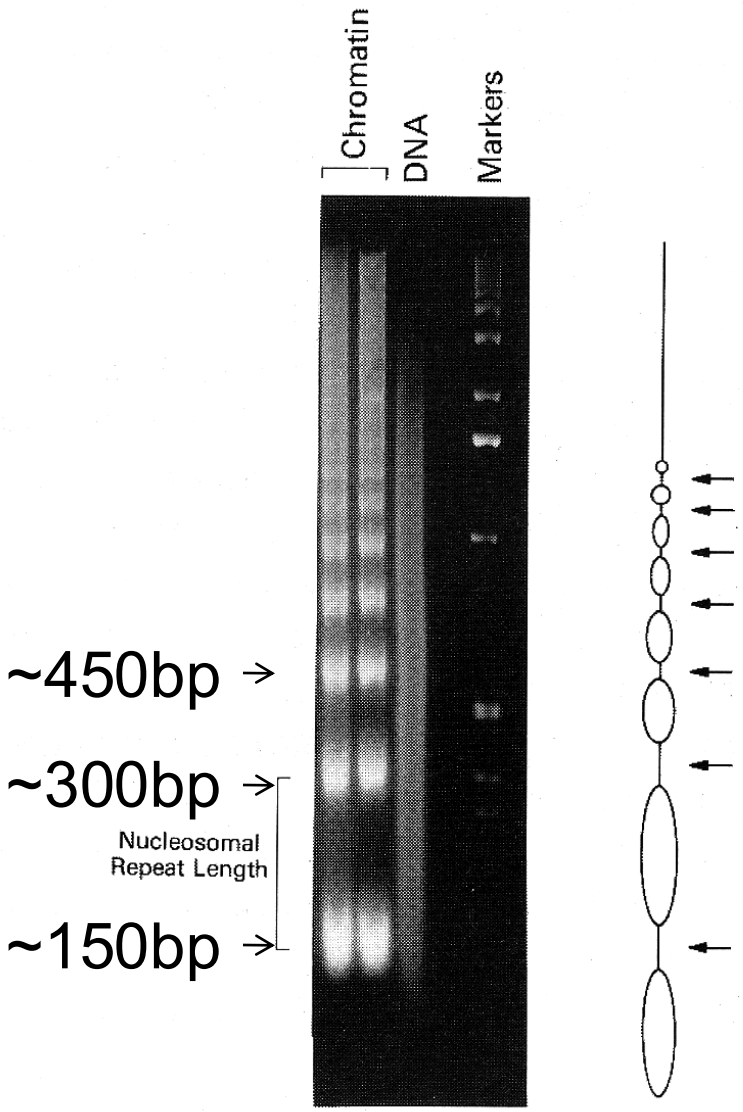
Levels of Chromatin Packing



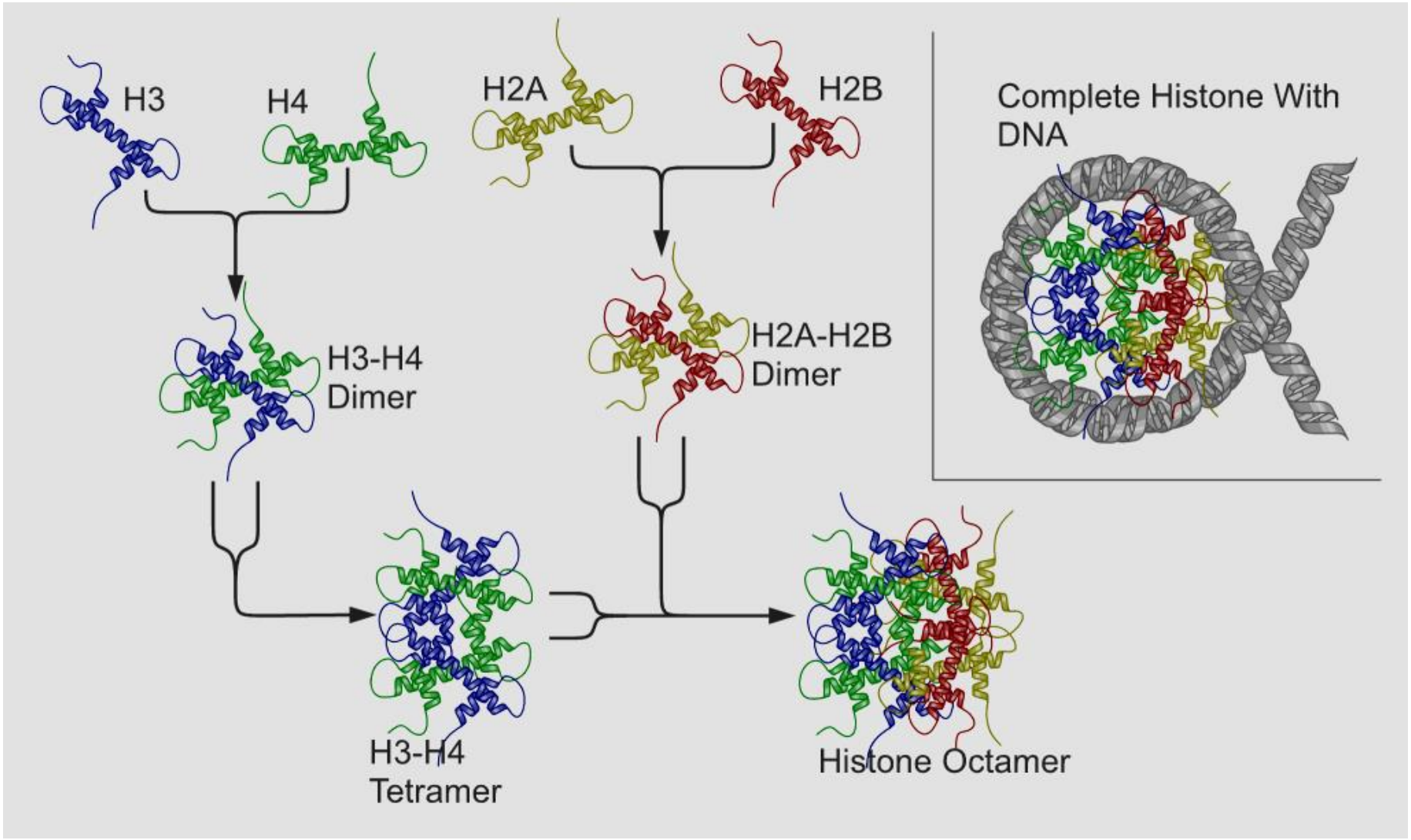
Beads on a string



Micrococcal nuclease (MNase) digestion of chromatin



Chromatin assembly



Histone chaperones

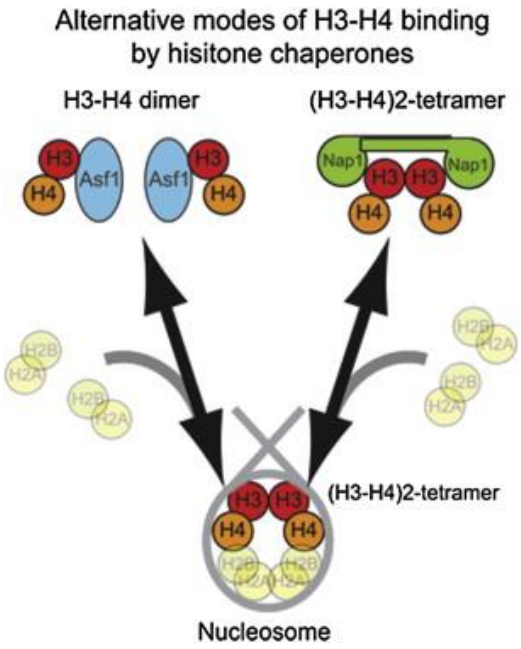
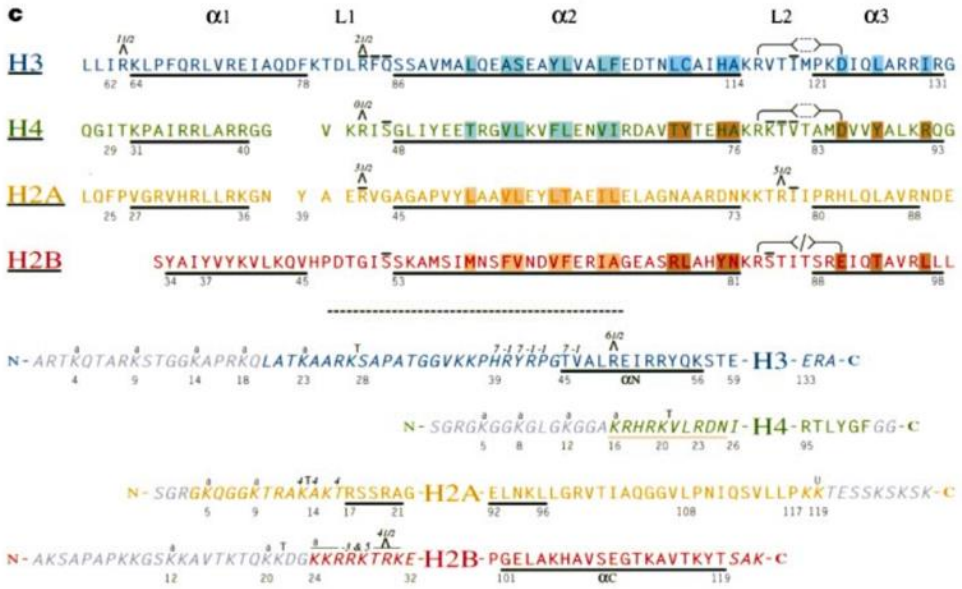


Table 1. Histone Chaperones that Modulate Chromatin Marks

Histones	Chaperone	Nuclear process(es)	Histone PTMs
H2A-H2B	FACT	Replication, Repair, Transcription	+H2B K123ub
	Nap1	Import, Transcription	–
	Chz1	Transcription	+H2B K123ub
H2AX-H2B	FACT	Repair	–H2AX S139 (129) phos
H2AZ-H2B	Nap1	Transcription	–
	Chz1	Transcription	–
H3-H4	Asf1	Import, Replication, Repair, Transcription	+H3 K56ac
			+H3 K9ac
			+H4 K5ac
			+H4 K12ac
			+H3 K36me3
			+Parental histone PTMs during replication
			–H3 K4me3
	CAF-1	Import, Replication, Repair	+H3 K56ac
			+H4 K5/12ac in import
	Vps75	Replication	+H3 K9ac
			+H3 K23/27ac
	Rtt106	Replication	+H3 K56ac
	Spt6	Transcription	+H3 K36me2/3
	Nap1	Transcription	–H3ac
			–H3 K4me3
H3.3-H4	HIRA	Transcription	–H3ac
	Daxx	Transcription	–

HCs with known links to establishment, maintenance and propagation (+) or removal (–) of histone marks. "Histone PTMs" refers to posttranslational modifications with a clear connection to the chaperone indicated.

The Core Histones



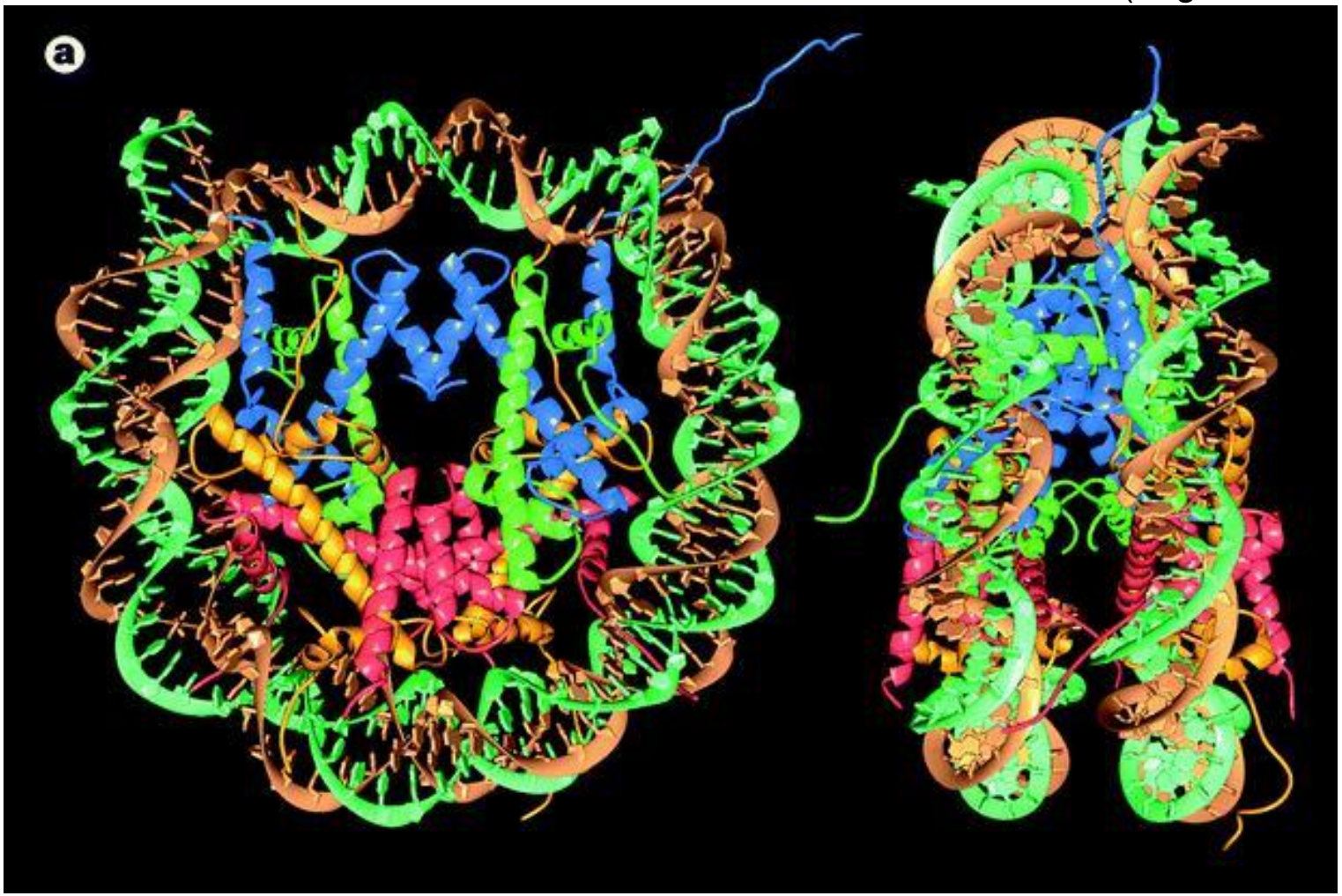
c, Core histone sequences. The histone fold regions for H3, H4, H2A and H2B are aligned on the basis of their structures and labelled on the left (top). The α-helix (α1, α2, α3) and loop (L1, L2) secondary structural elements are labelled; α-helices are underlined. Λ designates an arginine side chain that is inserted into the DNA minor groove at the indicated SHL. The overlying lines indicate β-strand hydrogen bonds between L1 and L2 loops. The side chains indicate the positions of buried arginine–aspartate pairs for H3–H4 and the lack of a homologous pair for H2A–H2B. The histone-fold extensions and tail (italics) regions are shown with the core histone name representing the histone fold (bottom). Grey-shaded sequences were not included in the atomic model. Sites of *in vivo* acetylation are indicated with a letter 'a'. Trypsin cleavage sites that produce a 'tailless' core particle are denoted by T (ref. 50). The site of ubiquitination is marked U (ref. 51). SHL labels above the sequence indicate contacts with DNA at those points. The H4 sequence at amino acids 16–25 and underlined in orange makes a clear inter-particle contact with a H2A–H2B dimer. Some interaction sites are highlighted: (1) H3–H4 α2-α2 (blue-green); (2) H2A–H2B α2-α2 (orange); (3) H3 4-helix bundle (blue); (4) H4–H2B 4-helix bundle (brown).

Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F. & Richmond, T. J. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* **389**, 251-260 (1997).

X-ray crystal structure of the nucleosome

(Luger et al., 97)

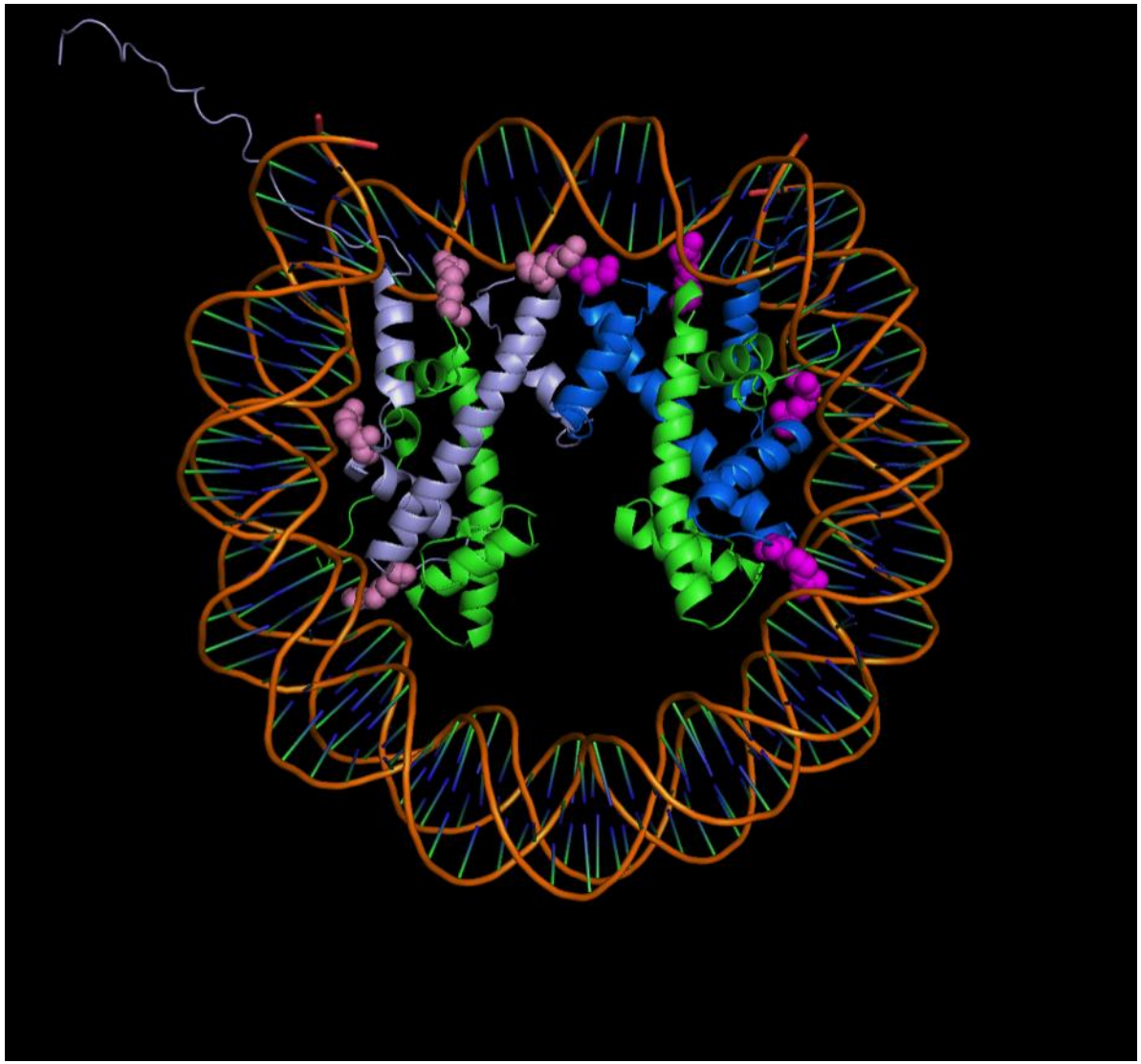
H3
H4
H2A
H2B



X-ray crystal structure of the nucleosome

(Luger et al., 97)

H3
H4



X-ray crystal structure of the nucleosome

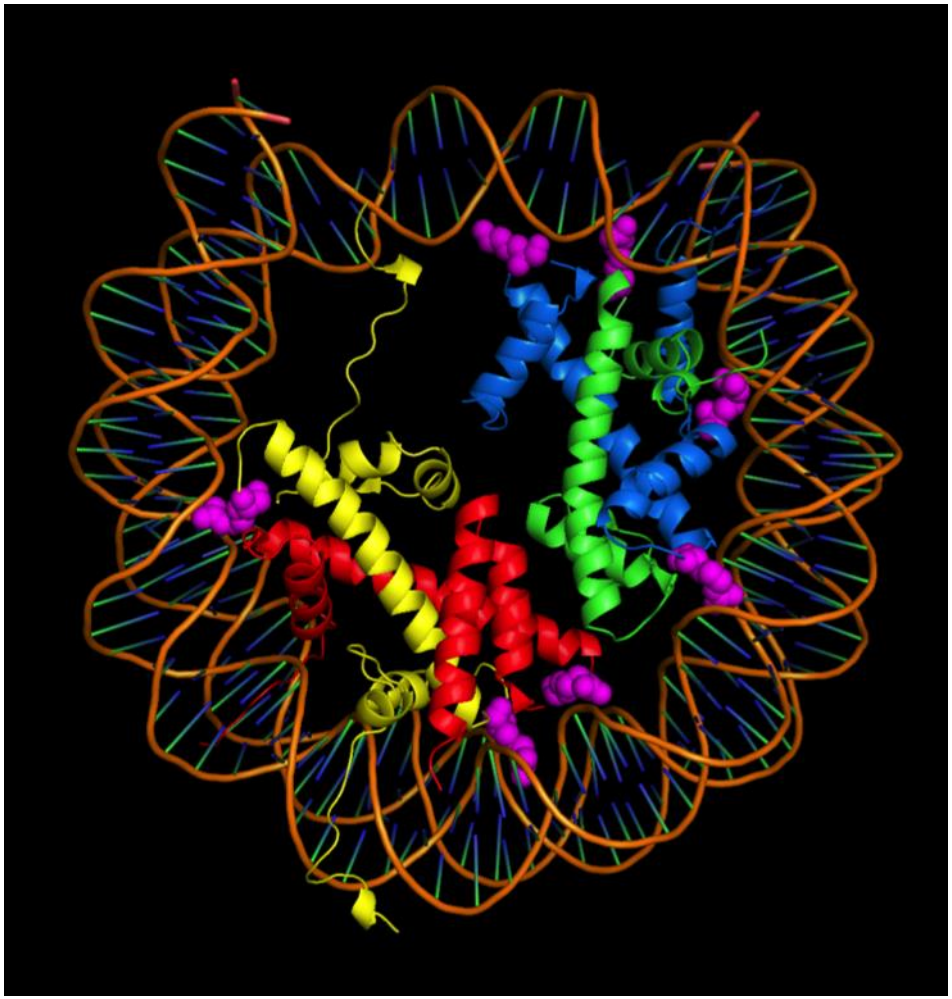
(Luger et al., 97)

H3
H4

X-ray crystal structure of the nucleosome

(Luger et al., 97)

H3
H4
H2A
H2B



Conservation of histone proteins

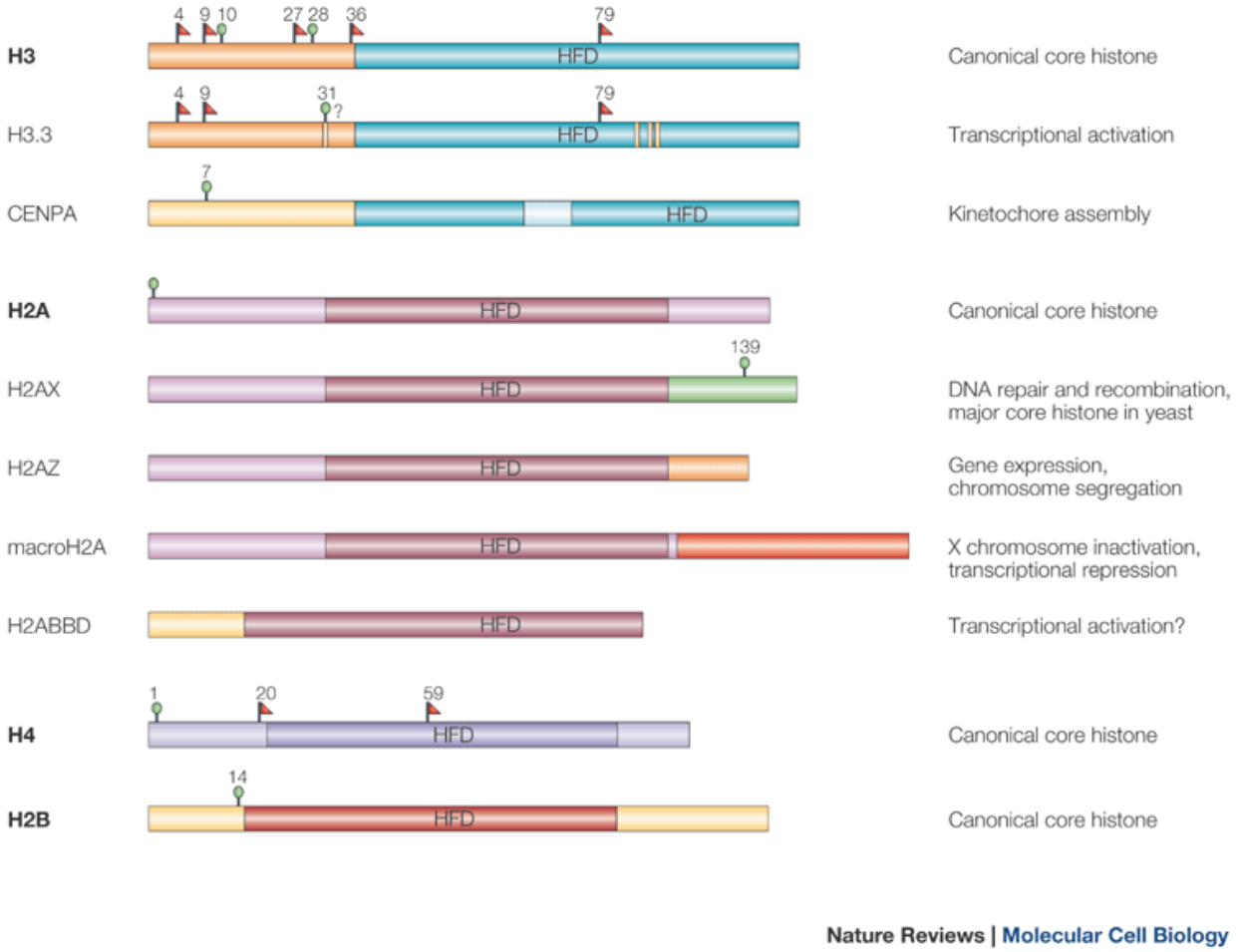
Query: **Budding yeast Histone H3**
Subject: **histone H3.3 [Homo sapiens]**

```
 Score =   250 bits (639),  Expect = 9e-86, Method: Compositional matrix adjust.
 Identities = 123/136 (90%), Positives = 131/136 (96%), Gaps = 0/136 (0%)

Query  1    MARTKQTARKSTGGKAPRKQLASKAARKSAPSTGGVKKPHRYKPGTVALREIRRFQKSTE  60
            MARTKQTARKSTGGKAPRKQLA+KAARKSAPSTGGVKKPHRY+PGTVALREIRR+QKSTE
Sbjct  1    MARTKQTARKSTGGKAPRKQLATKAARKSAPSTGGVKKPHRYRPGTVALREIRRYQKSTE  60

Query  61   LLIRKLPFQRLVREIAQDFKTDLRFQSSAIGALQESVEAYLVSLFEDTNLAAIHAKRVTI  120
            LLIRKLPFQRLVREIAQDFKTDLRFQS+AIGALQE+ EAYLV LFEDTNL AIHAKRVTI
Sbjct  61   LLIRKLPFQRLVREIAQDFKTDLRFQSAAIGALQEASEAYLVGLFEDTNLCAIHAKRVTI  120

Query  121  QKKDIKLARRLRGERS  136
              KDI+LARR+RGER+
Sbjct  121  MPKDIQLARRIRGERA  136
```

Query: **Budding yeast Histone H3**
Subject: **histone H3.1 [Homo sapiens]**

```
 Score =   248 bits (633),  Expect = 8e-85, Method: Compositional matrix adjust.
 Identities = 121/136 (89%), Positives = 130/136 (96%), Gaps = 0/136 (0%)

Query  1    MARTKQTARKSTGGKAPRKQLASKAARKSAPSTGGVKKPHRYKPGTVALREIRRFQKSTE  60
            MARTKQTARKSTGGKAPRKQLA+KAARKSAP+TGGVKKPHRY+PGTVALREIRR+QKSTE
Sbjct  1    MARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALREIRRYQKSTE  60

Query  61   LLIRKLPFQRLVREIAQDFKTDLRFQSSAIGALQESVEAYLVSLFEDTNLAAIHAKRVTI  120
            LLIRKLPFQRLVREIAQDFKTDLRFQSSA+ ALQE+ EAYLV LFEDTNL AIHAKRVTI
Sbjct  61   LLIRKLPFQRLVREIAQDFKTDLRFQSSAVMALQEACEAYLVGLFEDTNLCAIHAKRVTI  120

Query  121  QKKDIKLARRLRGERS  136
              KDI+LARR+RGER+
Sbjct  121  MPKDIQLARRIRGERA  136
```

Histone variants



Kavitha Sarma and Danny Reinberg
Nature Reviews Molecular Cell Biology 6, 139-149 (February 2005)

Conservation of histone proteins

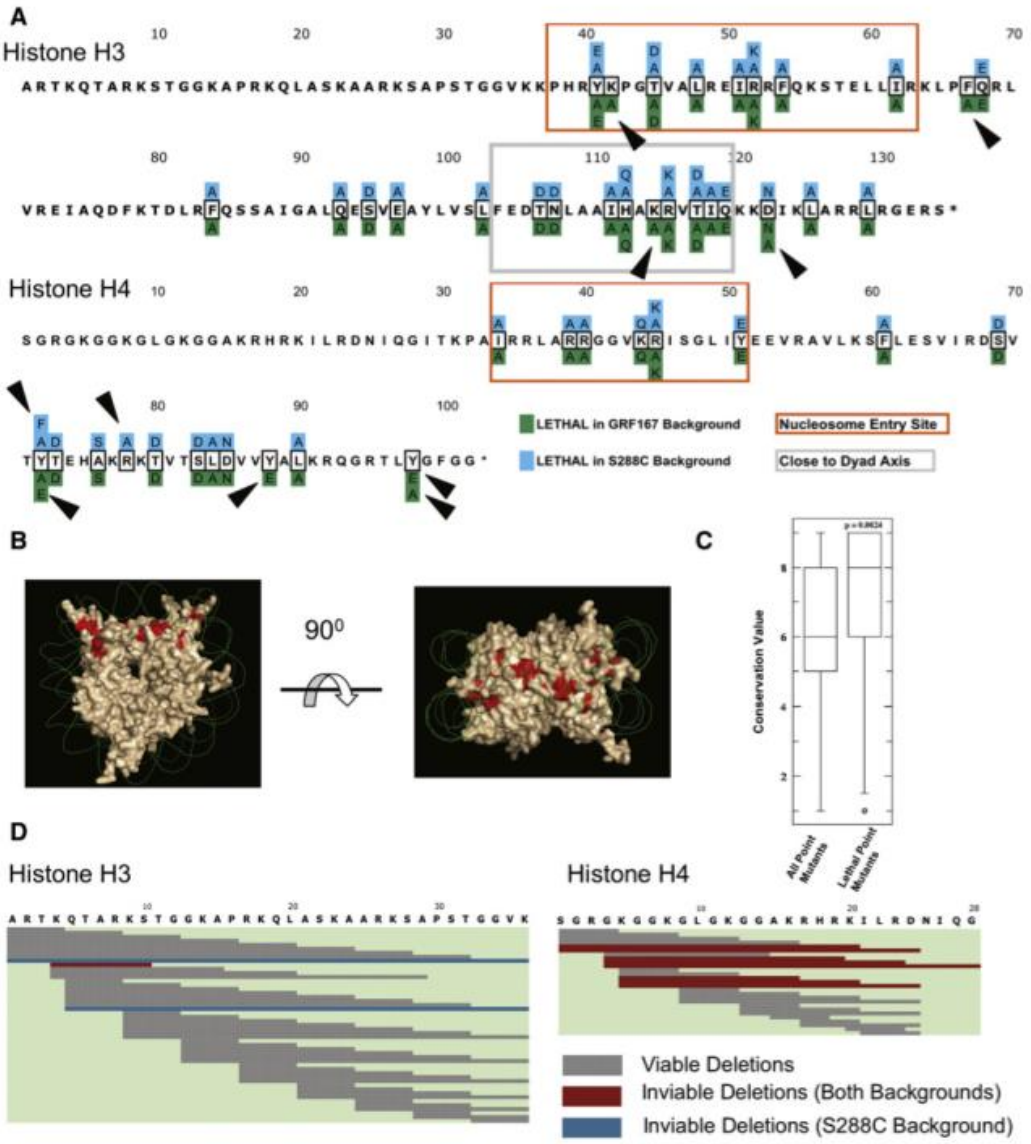
Query: **Budding yeast Histone H3**
Subject: **histone H3.3 [Homo sapiens]**

```
 Score =   250 bits (639),  Expect = 9e-86, Method: Compositional matrix adjust.
 Identities = 123/136 (90%), Positives = 131/136 (96%), Gaps = 0/136 (0%)

Query  1    MARTKQTARKSTGGKAPRKQLASKAARKSAPSTGGVKKPHRYKPGTVALREIRRFQKSTE  60
            MARTKQTARKSTGGKAPRKQLA+KAARKSAPSTGGVKKPHRY+PGTVALREIRR+QKSTE
Sbjct  1    MARTKQTARKSTGGKAPRKQLATKAARKSAPSTGGVKKPHRYRPGTVALREIRRYQKSTE  60

Query  61   LLIRKLPFQRLVREIAQDFKTDLRFQSSAIGALQESVEAYLVSLFEDTNLAAIHAKRVTI  120
            LLIRKLPFQRLVREIAQDFKTDLRFQS+AIGALQE+ EAYLV LFEDTNL AIHAKRVTI
Sbjct  61   LLIRKLPFQRLVREIAQDFKTDLRFQSAAIGALQEASEAYLVGLFEDTNLCAIHAKRVTI  120

Query  121  QKKDIKLARRLRGERS  136
              KDI+LARR+RGER+
Sbjct  121  MPKDIQLARRIRGERA  136
```

Query: **Budding yeast Histone H3**
Subject: **histone H3.1 [Homo sapiens]**

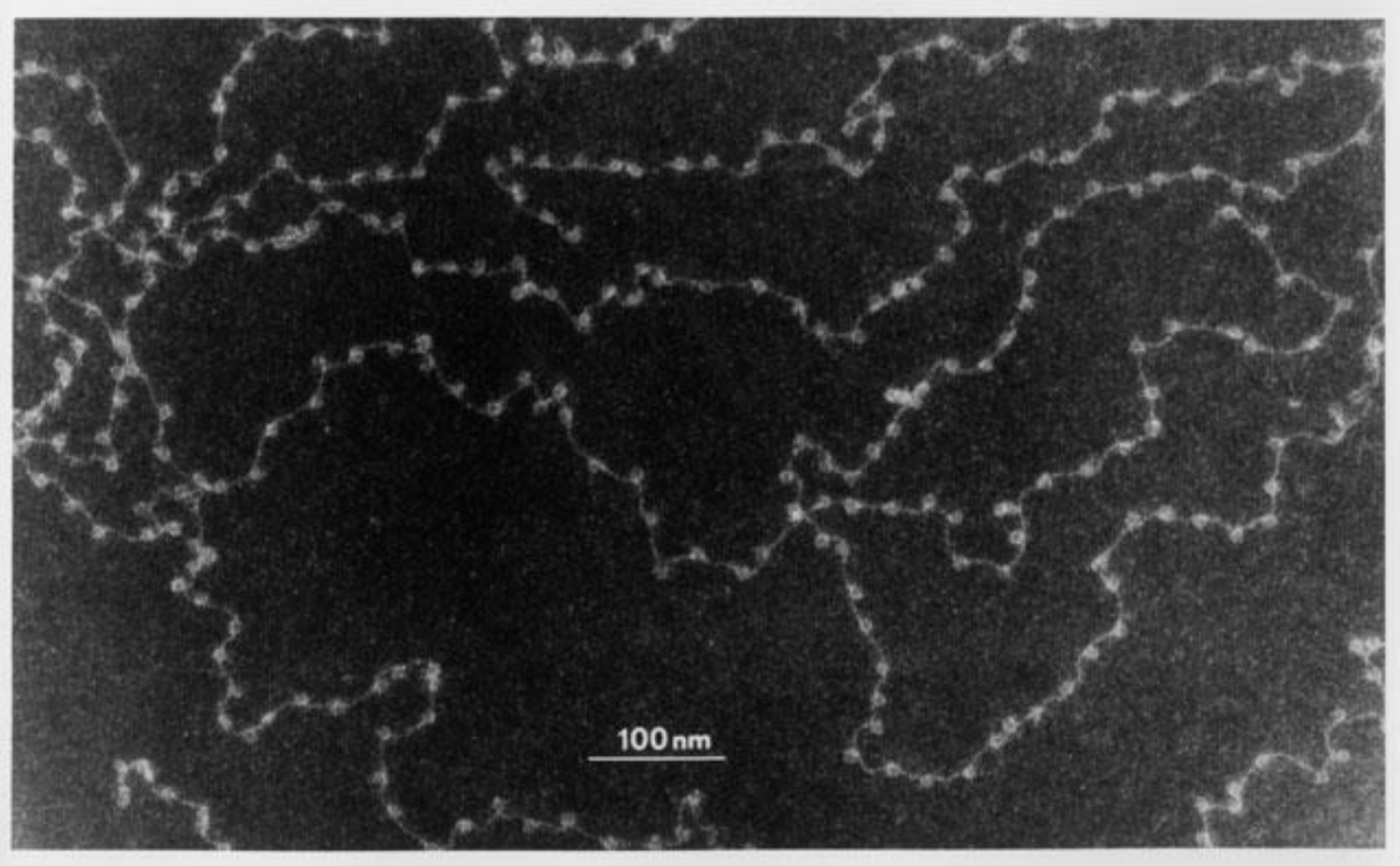
```
 Score =   248 bits (633),  Expect = 8e-85, Method: Compositional matrix adjust.
 Identities = 121/136 (89%), Positives = 130/136 (96%), Gaps = 0/136 (0%)

Query  1    MARTKQTARKSTGGKAPRKQLASKAARKSAPSTGGVKKPHRYKPGTVALREIRRFQKSTE  60
            MARTKQTARKSTGGKAPRKQLA+KAARKSAP+TGGVKKPHRY+PGTVALREIRR+QKSTE
Sbjct  1    MARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALREIRRYQKSTE  60

Query  61   LLIRKLPFQRLVREIAQDFKTDLRFQSSAIGALQESVEAYLVSLFEDTNLAAIHAKRVTI  120
            LLIRKLPFQRLVREIAQDFKTDLRFQSSA+ ALQE+ EAYLV LFEDTNL AIHAKRVTI
Sbjct  61   LLIRKLPFQRLVREIAQDFKTDLRFQSSAVMALQEACEAYLVGLFEDTNLCAIHAKRVTI  120

Query  121  QKKDIKLARRLRGERS  136
              KDI+LARR+RGER+
Sbjct  121  MPKDIQLARRIRGERA  136
```

Histone point mutants in budding yeast



Cell 134, 1066–1078,
September 19, 2008

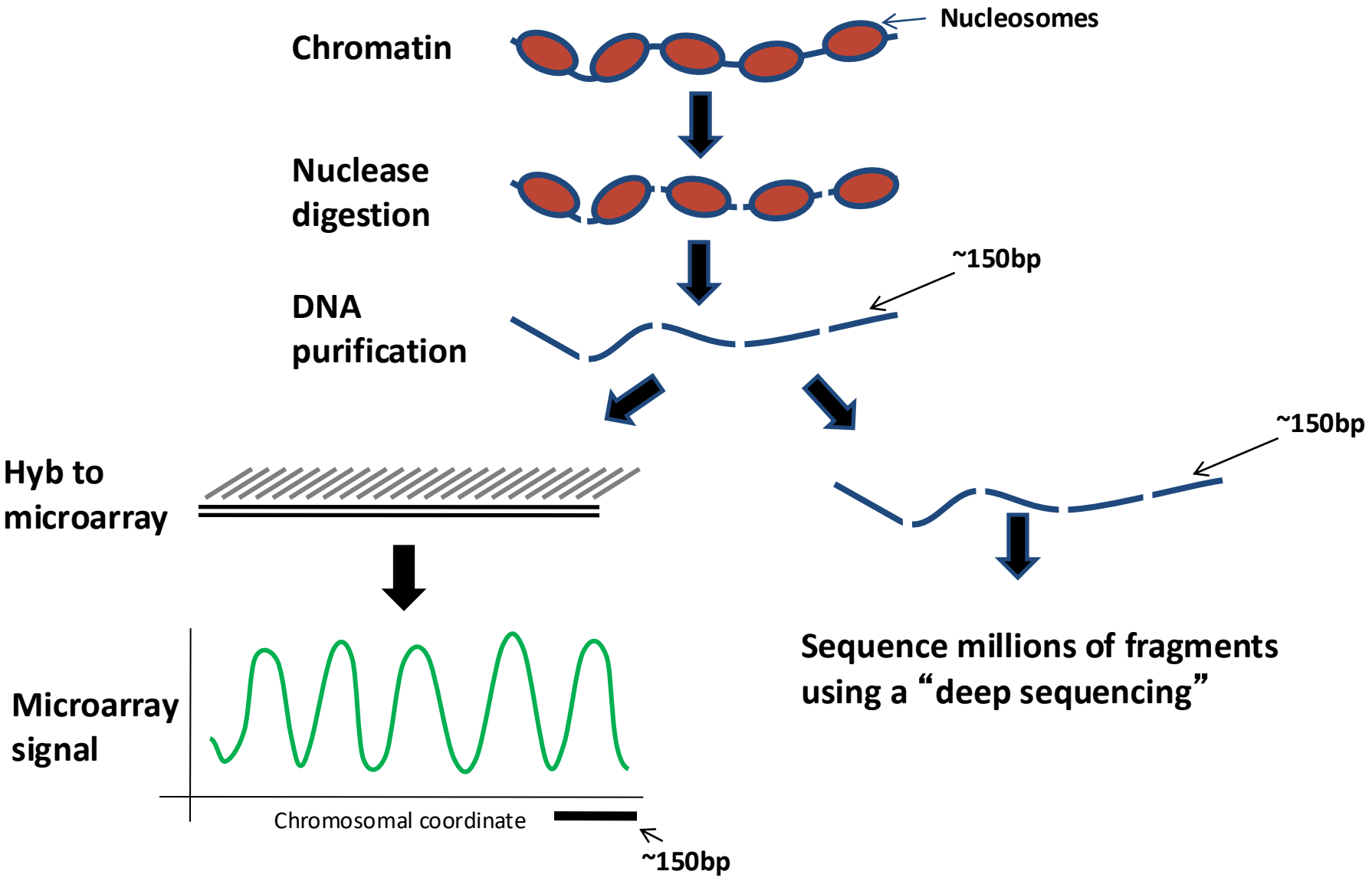
Where are nucleosomes located ?



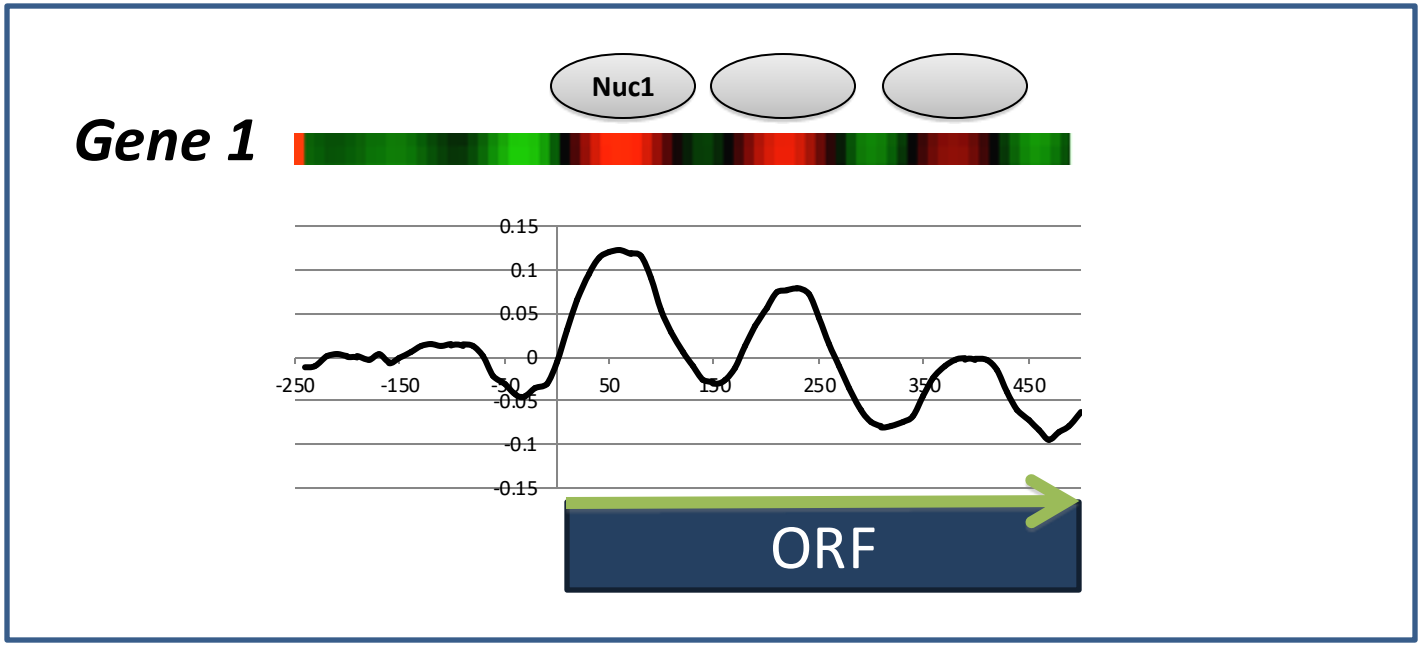
Meta-data

Annotating the genome

Nucleosome mapping using microarrays



Nucleosome positions relative to the Transcription Start Site

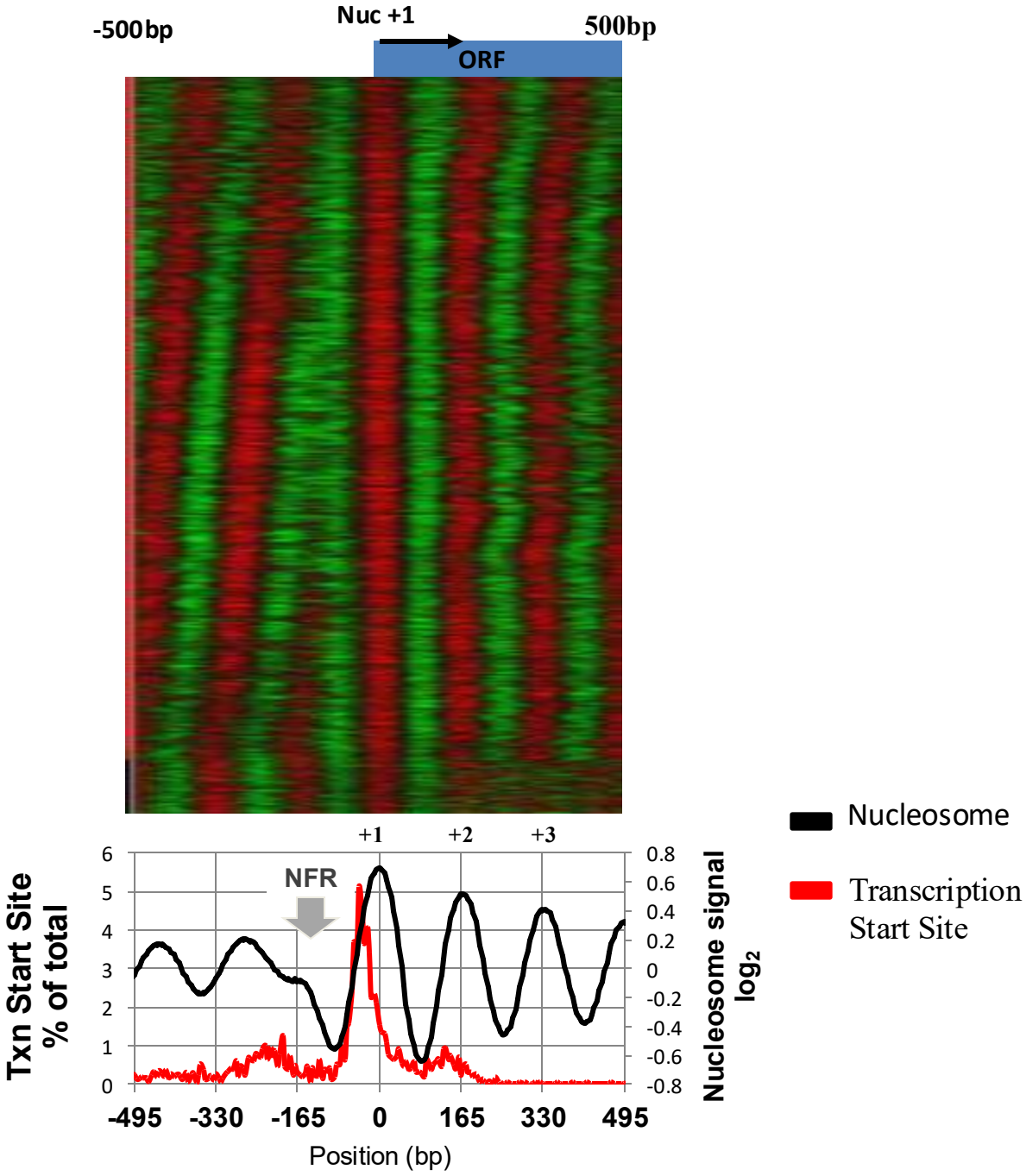


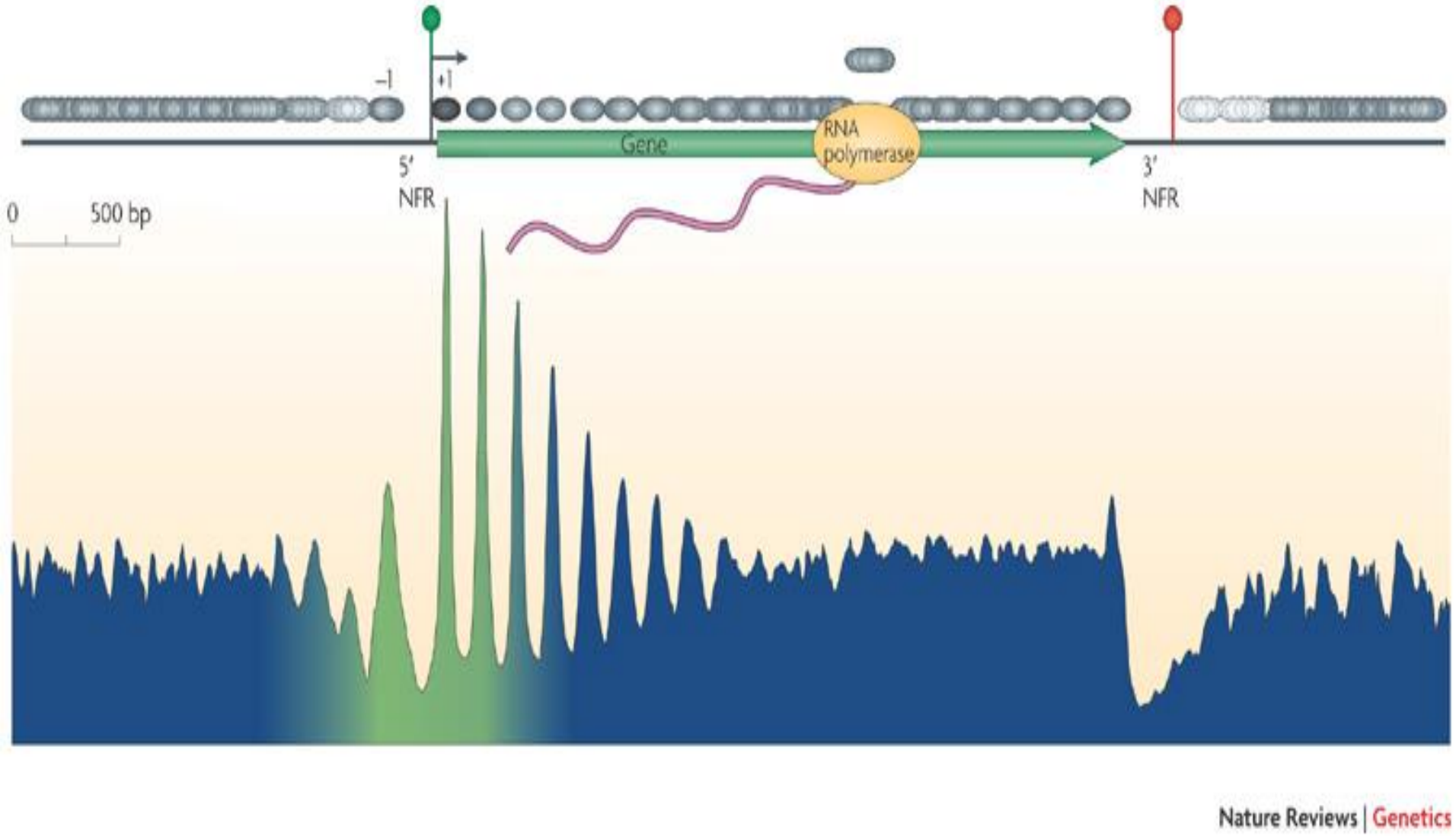
Nucleosome positions relative the +1 Nucleosome

Nucleosome

Free DNA

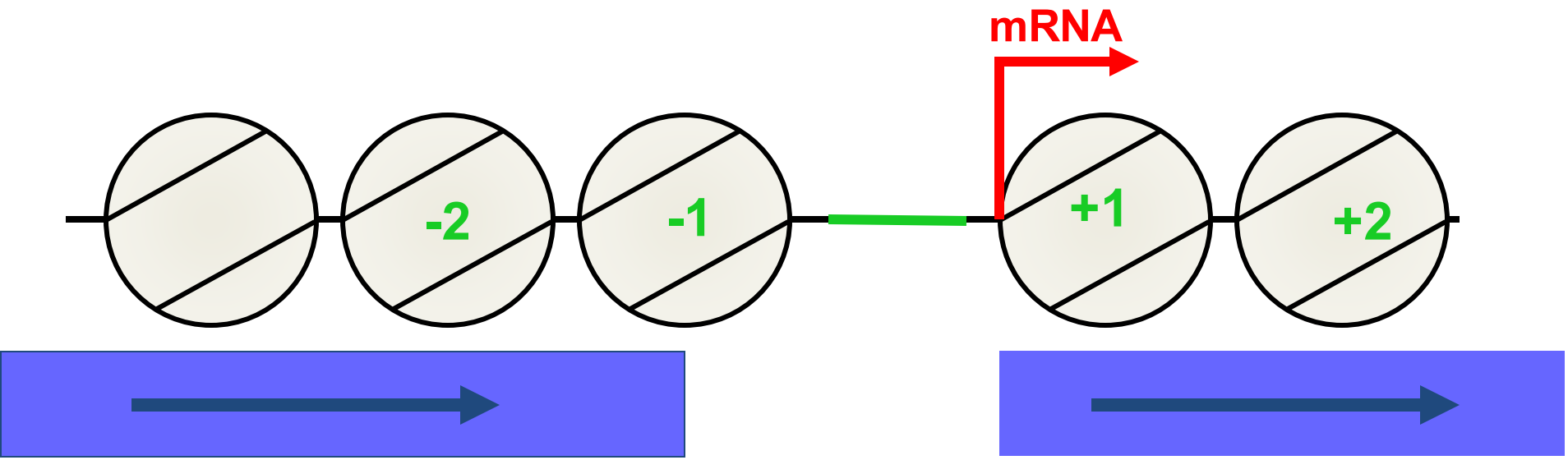
n=5767


-500bp
Nuc +1
500bp
ORF
+1
+2
+3
NFR
Txn Start Site
% of total
Nucleosome signal
log2
Position (bp)
-495
-330
-165
0
165
330
495
Nucleosome
Transcription
Start Site

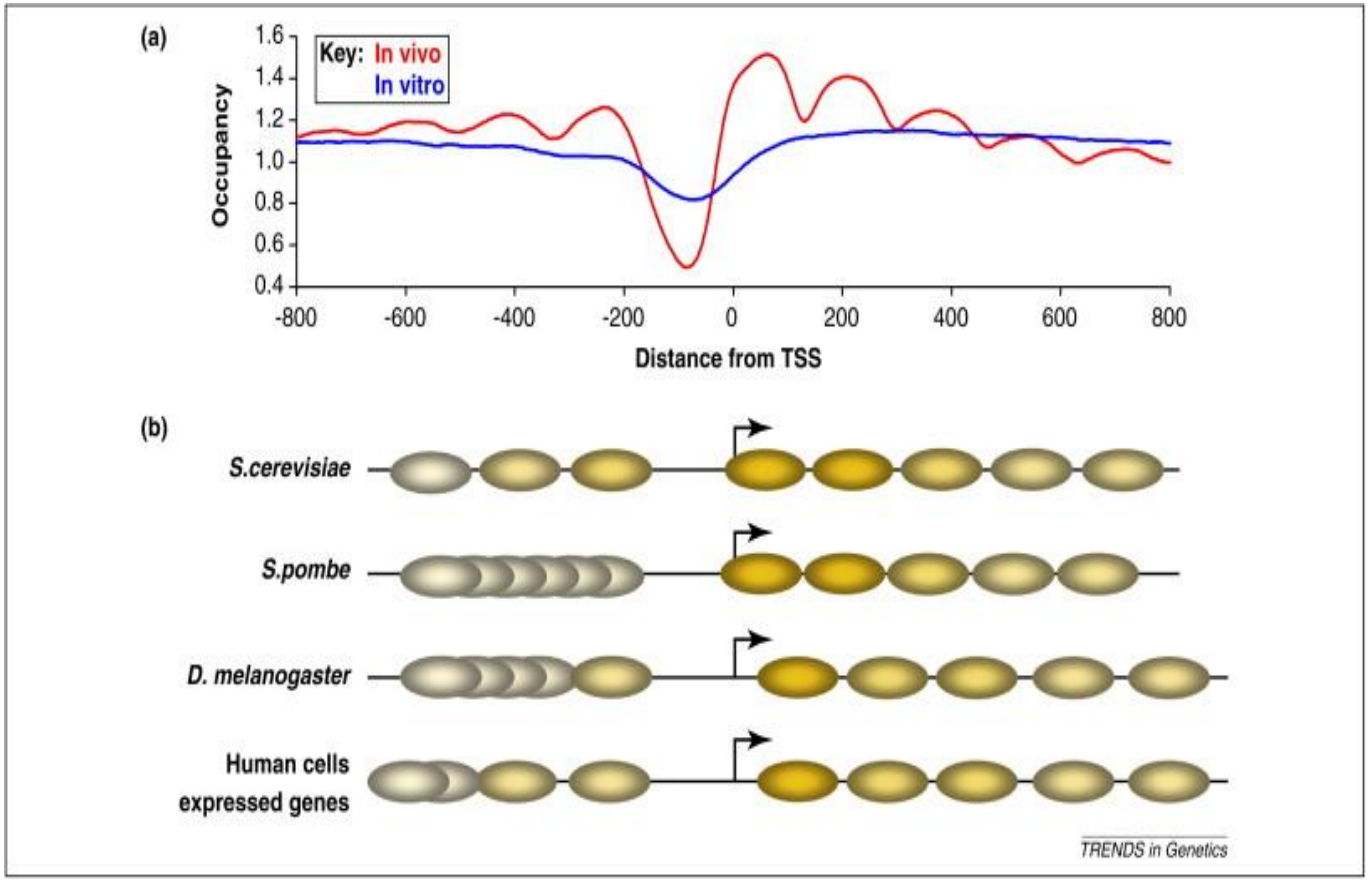


Jiang and Pugh 2009

Typical chromatin structure around gene promoters



Nucleosomes are organized at the 5′ end of genes

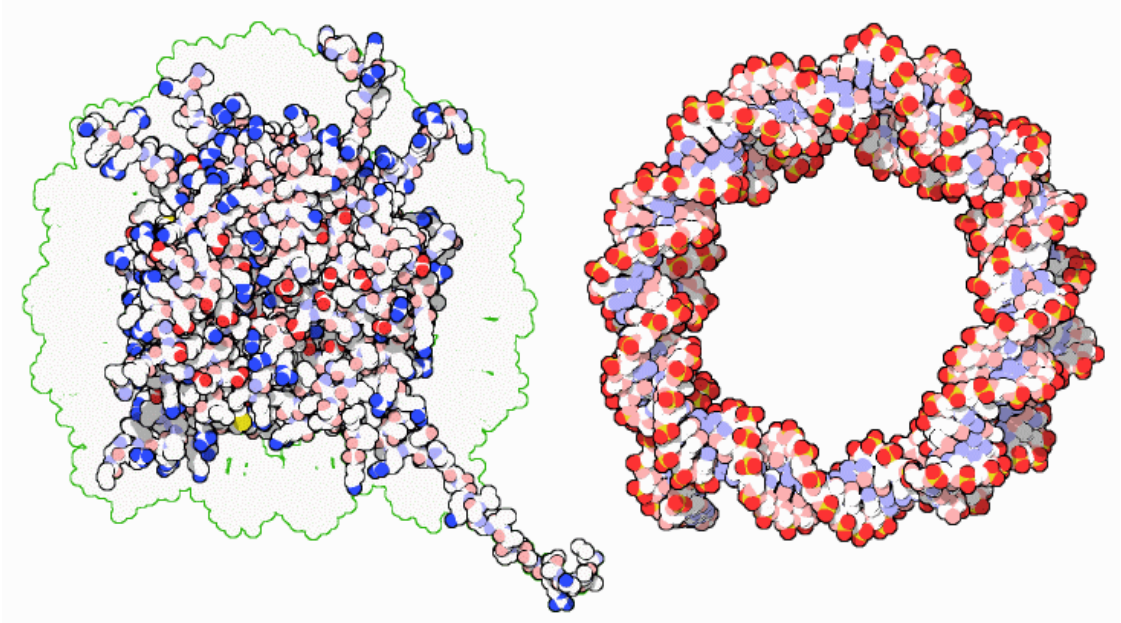


{Bai and Morozov, 2010, Trends Genet, 26,

What positions nucleosomes?

1. DNA sequence

DNA in the Nucleosome



Histone Octamer *Nucleosomal DNA*

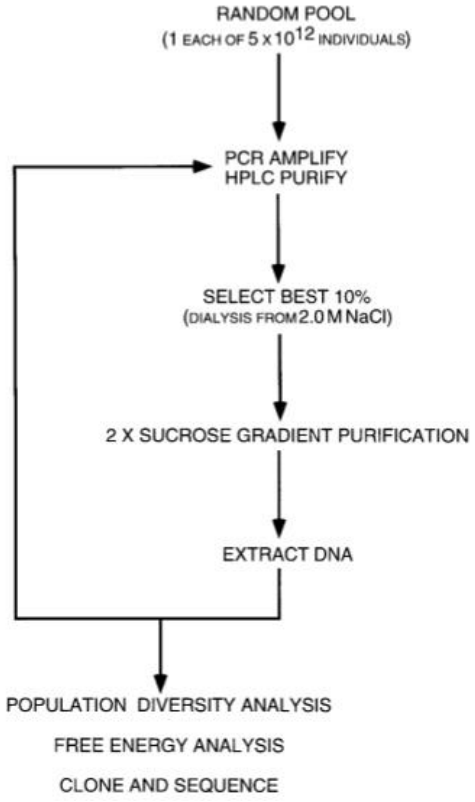
Structure: Luger et al., 1997
Image: Protein data bank

J. Mol. Biol. (1998) 276, 19–42

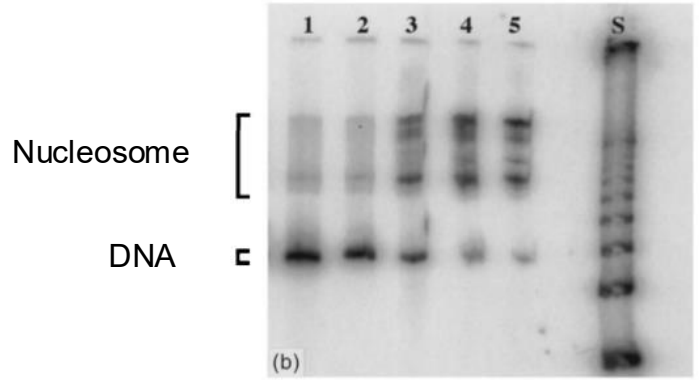
JMB

New DNA Sequence Rules for High Affinity Binding to Histone Octamer and Sequence-directed Nucleosome Positioning

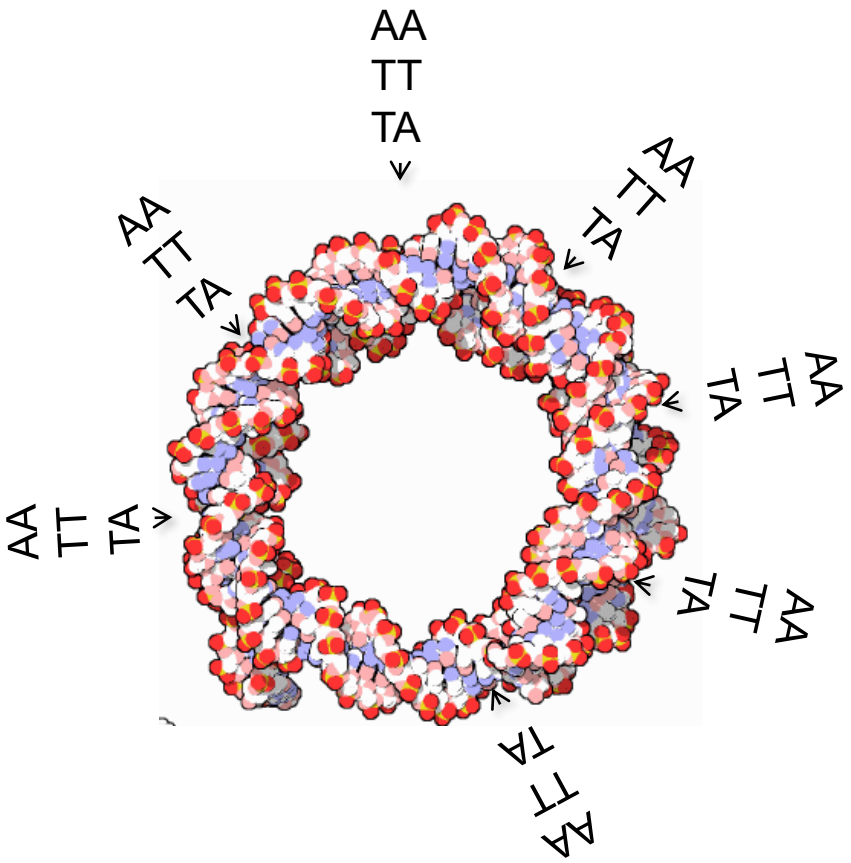
P. T. Lowary[1] and J. Widom[1,2*]

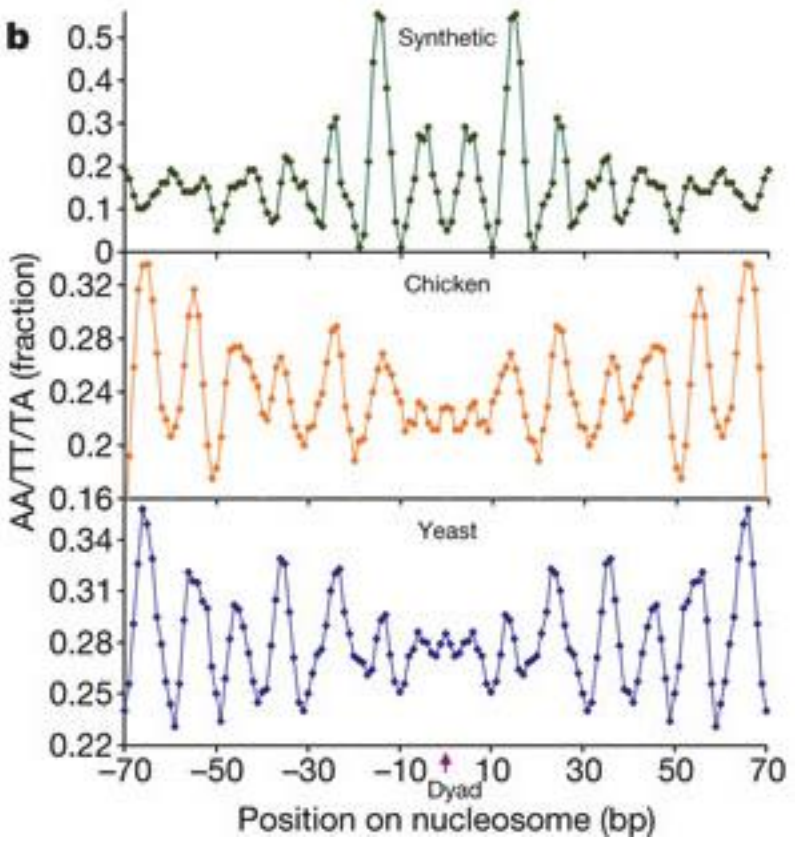


Native PAGE



Flexible DNA is preferred





Rigid DNA is NOT!

eg AAAAAAAAAAAAAAAAAAAAA

How do DNA sequences affect nucleosome positioning *in vivo?*

A genomic code for nucleosome positioning.
Segal et al., Nature 442:772-778, 2006.

Sequence directed nucleosome positioning

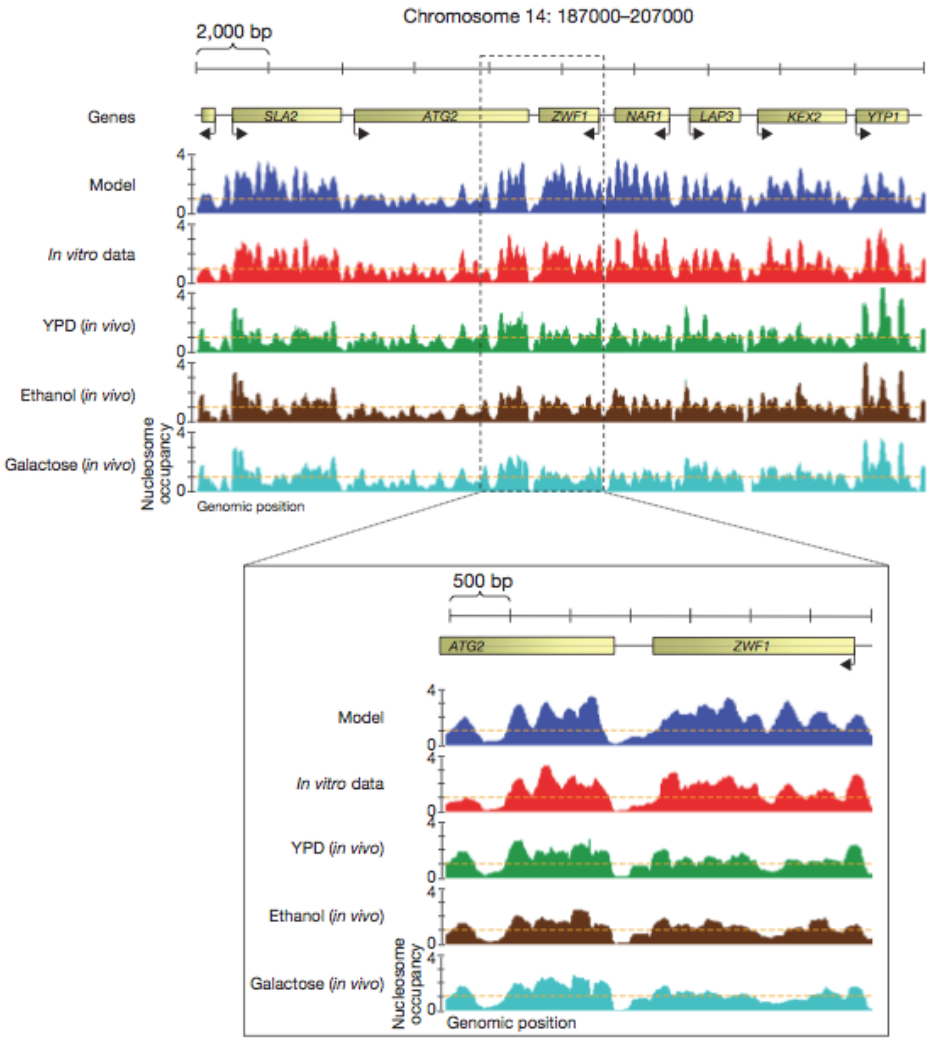
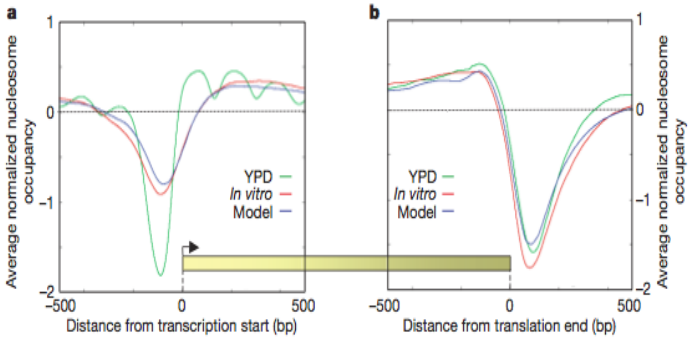
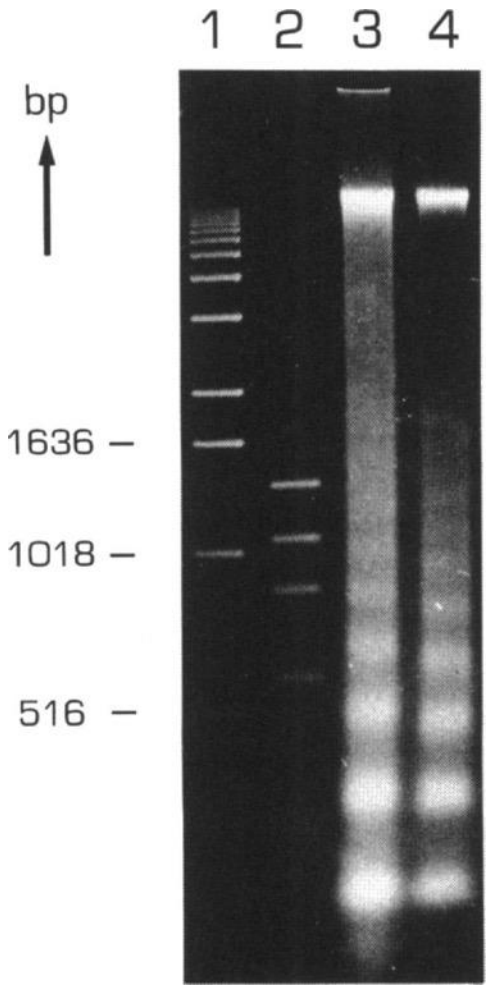


Figure 1 | The intrinsic DNA-encoded nucleosome organization at a typical genomic region. Shown are the four different maps of nucleosome occupancy measured in this study for a typical 20,000-bp-long genomic region: the *in vitro* map, which reflects only the intrinsic nucleosome sequence preferences, and *in vivo* yeast maps for three different growth conditions (YPD, ethanol and galactose). Each track plots the measured nucleosome occupancy per base pair, computed by summing all of the nucleosome reads obtained in that experiment, and dividing that number by the average number of reads per base pair across the genome. The line of $y = 1$ thus represents the genome-wide average and is shown as a dashed orange line. The average nucleosome occupancy predictions from our model are shown in blue.

Average gene profiles

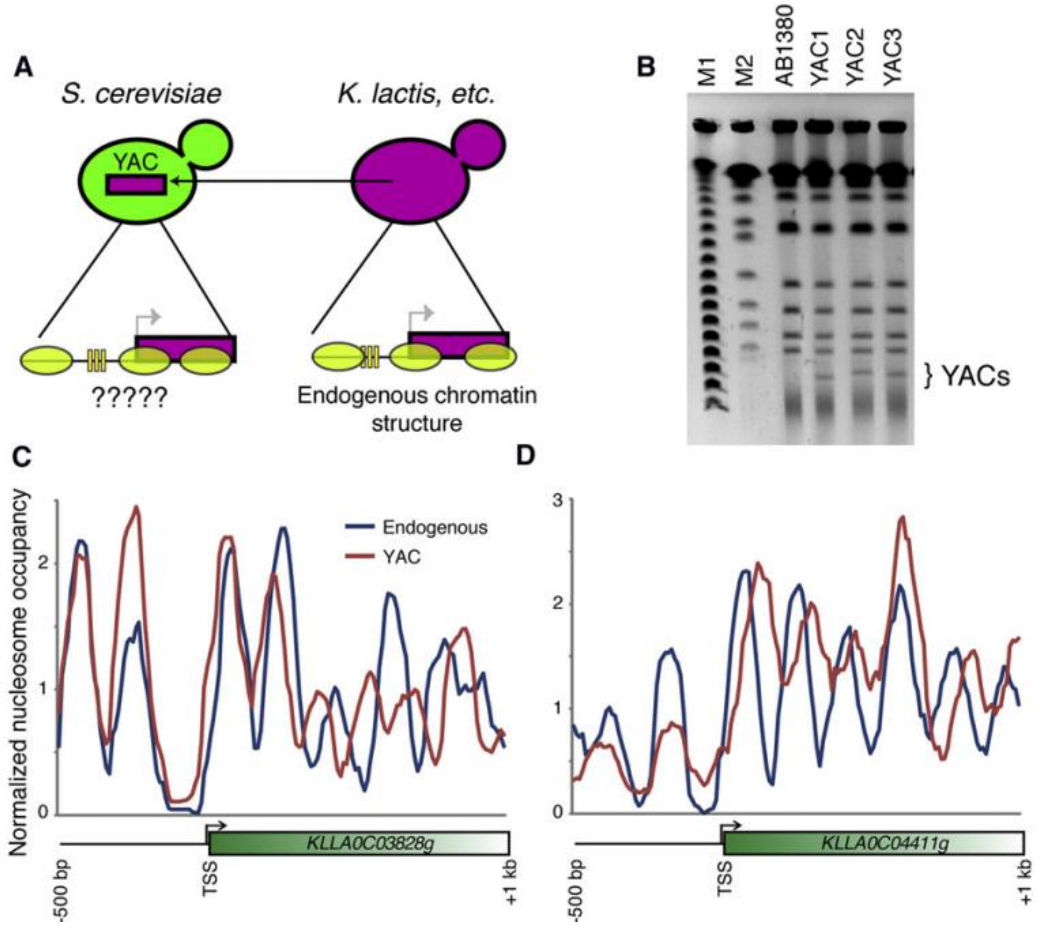


Nucleosomes are arranged differently in different organisms and cell types.



Lane 3 = K lactis
Lane 4 = S cerevisiae

Is nucleosome positioning conserved?



Nucleosome positions are determined by the host organism

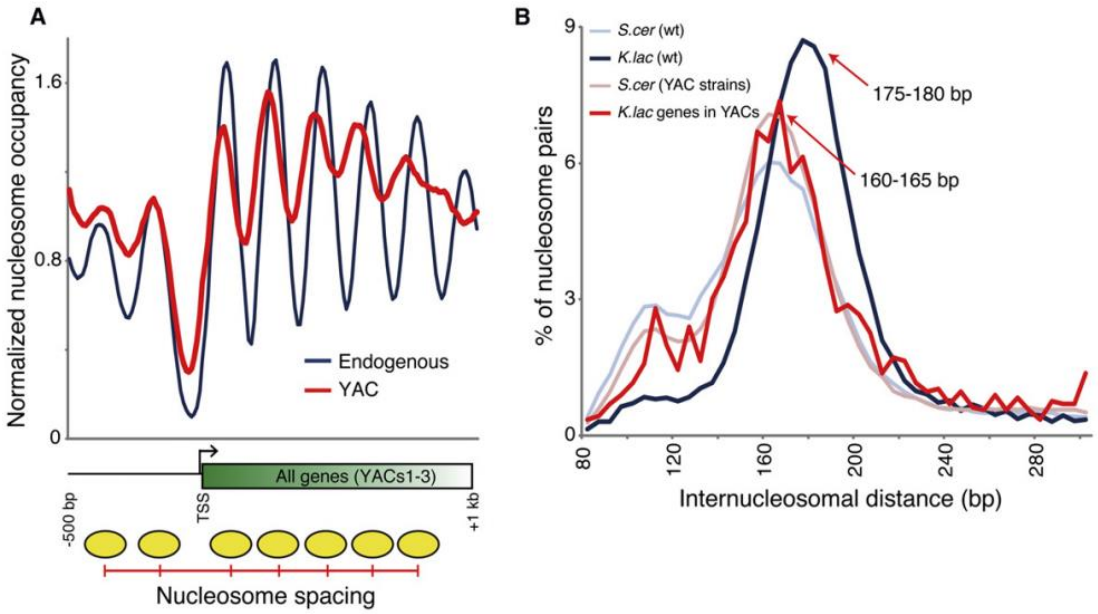


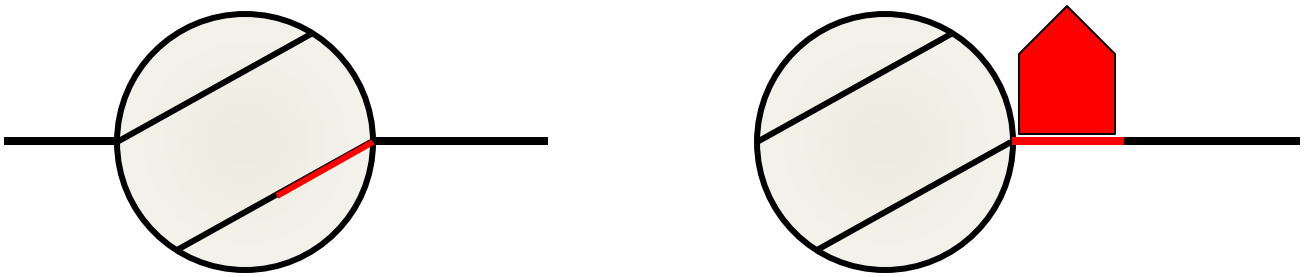
Figure 3. Nucleosome Spacing Is Set In *trans*

(A) Averaged data for all *K. lactis* genes on YACs 1–3. Genes are aligned by the +1 nucleosome position as defined in Tsankov et al. (2010), and data from either wild-type *K. lactis* or from the YAC strains are averaged for 184 genes, as indicated.

(B) *K. lactis* sequences adopt *S. cerevisiae* spacing when carried in *S. cerevisiae*. Nucleosome positions were called, and the distribution of all internucleosomal distances (center to center) is shown for 184 *K. lactis* genes from wild-type or in the YACs. Similar distributions for *S. cerevisiae* nucleosome positioning from wild-type and YAC-containing strains indicate that YACs do not perturb host chromatin state (see also Figure S2).

What positions nucleosomes?

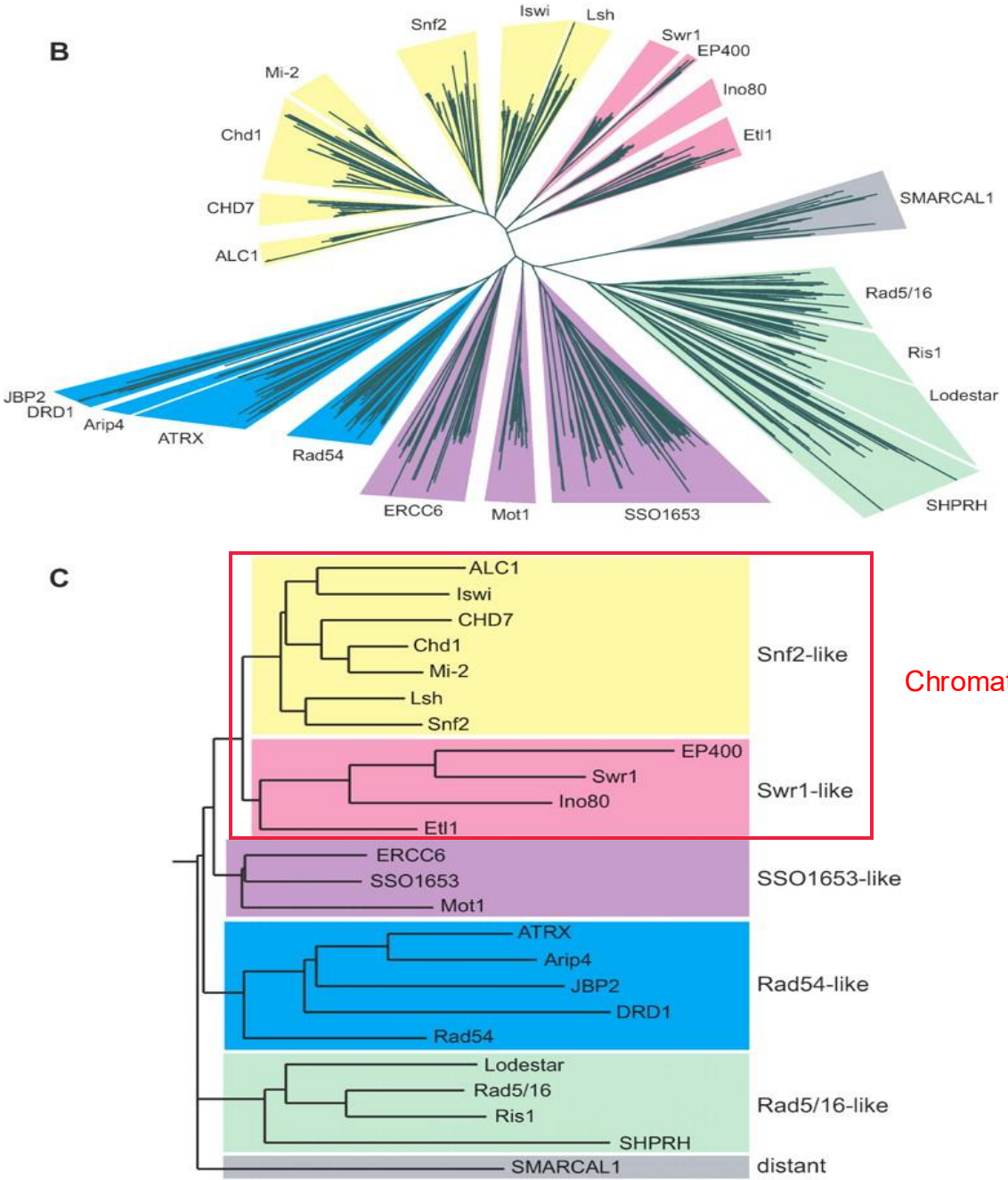
2- DNA binding factors



What positions nucleosomes?

3. ATP dependent chromatin remodelling enzymes

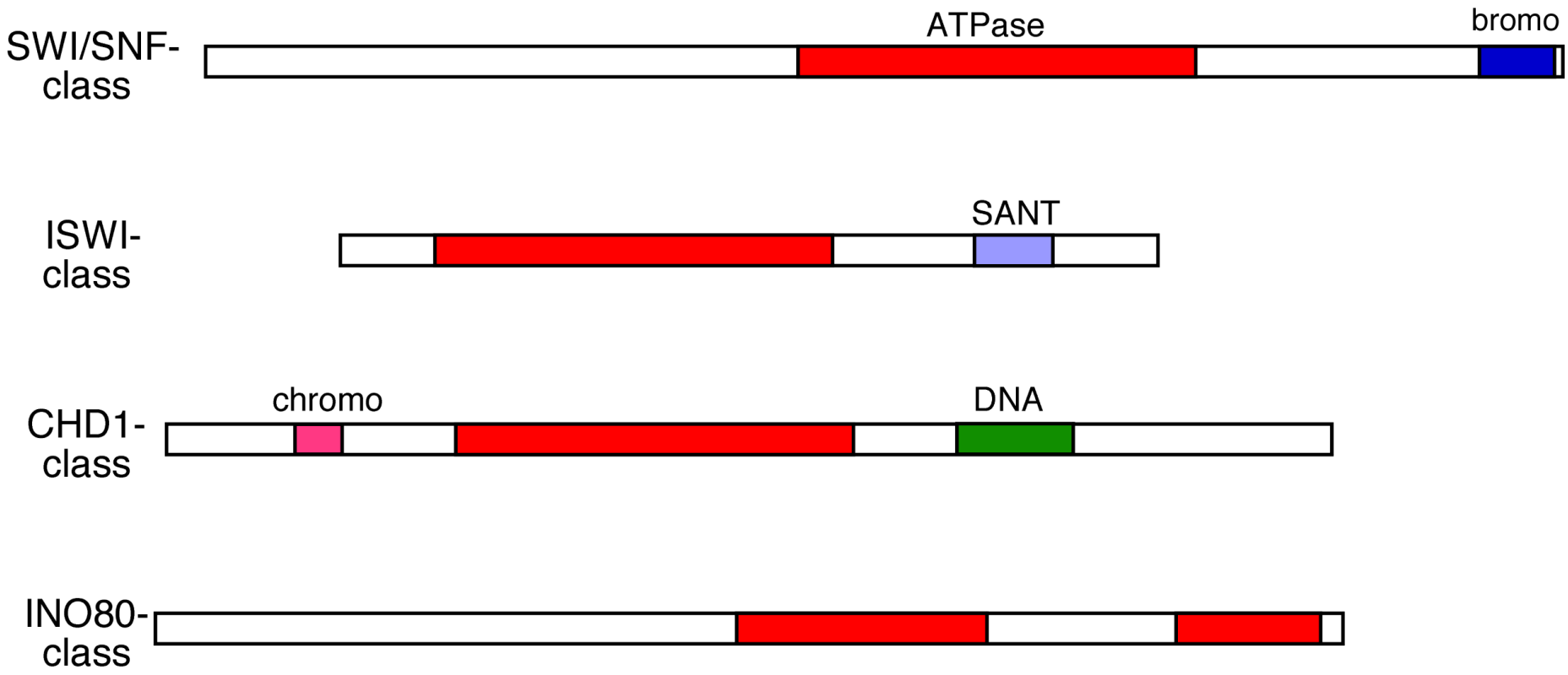
Tree view of SNF2-like ATPase superfamily members



Chromatin associated

Flaus et al., 2005

ATP-dependent chromatin remodeling factors are classified by their ATPase subunits

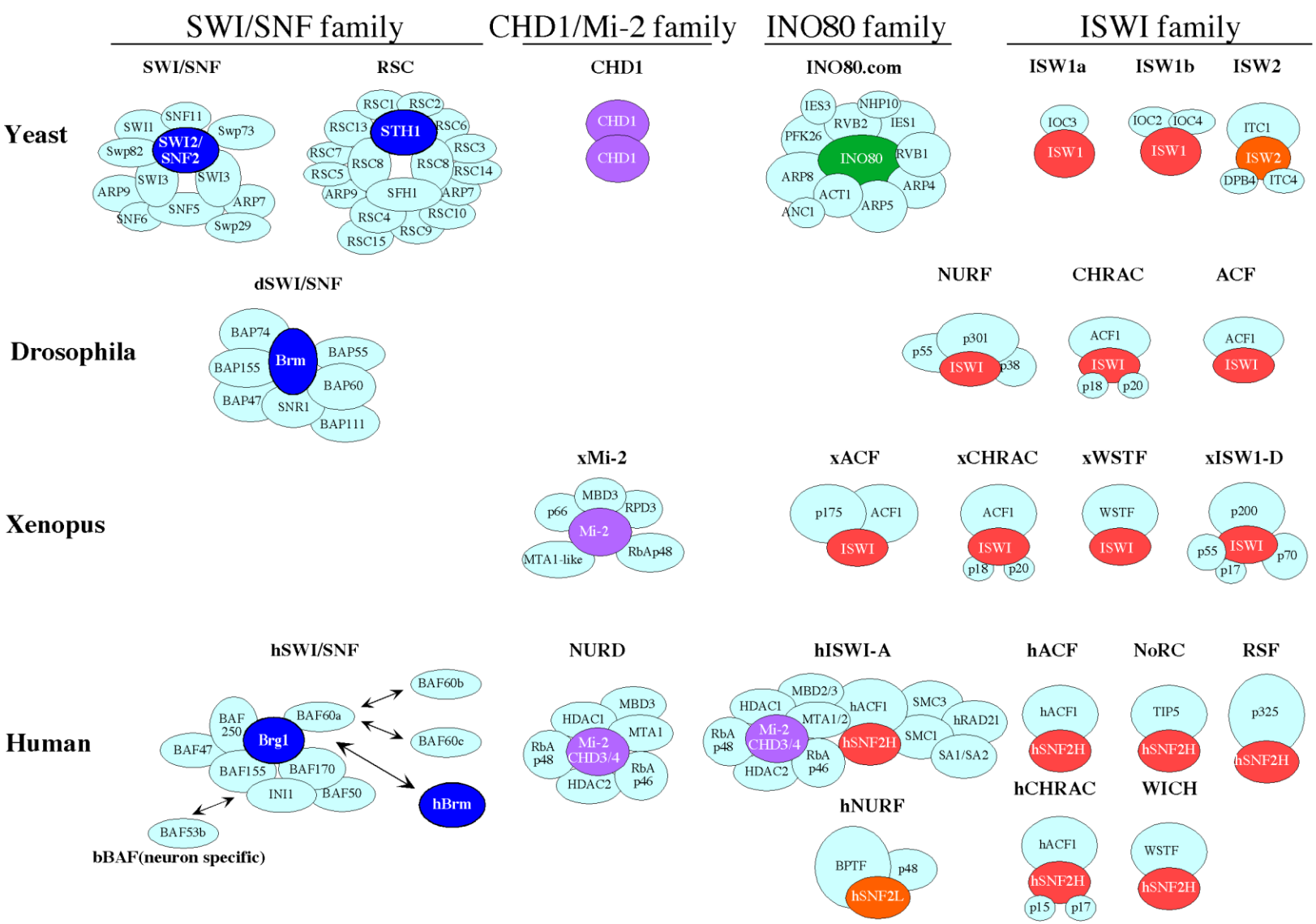


Subunit composition of various ATP-dependent chromatin remodeling factors

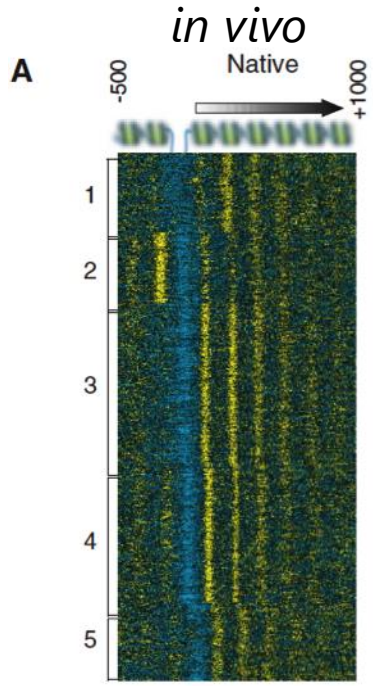
Table 2. Subfamily occurrences in selected complete eukaryotic genomes

	Snf2	Iswi	Lsh	ALC1	Chd1	Mi-2	CHD7	Swr1	EP400	Ino80	Etl1	Rad54	ATRX	Arip4	DRD1	JBP2	Rad5/16	Ris1	Lodestar	SHPRH	Mot1	ERCC6	SSO1653	SMARCAL1	rapA group
(A) Predicted proteins																									
Fungi																									
S.cerevisiae	2	2	1	0	1	0	0	1	0	1	1	2	0	0	0	0	2	1	0	1	1	1	0	0	0
S.pombe	2	0	0	0	2	1	0	1	0	1	3	2	0	0	0	0	2	3	0	1	1	1	0	0	0
Neurospora crassa	1	1	1	0	1	1	0	1	0	1	1	1	0	1	0	0	3	3	3	3	1	2	0	0	0
Plant																									
A.thaliana	6	3	1	2	1	3	0	1	0	1	1	1	1	0	6	0	5	5	0	2	1	3	0	2	0
Invertebrates																									
Ciona intestinalis	10	10	6	0	7	5	2	2	0	0	2	3	2	1	0	0	0	0	0	0	2	4	0	5	0
Caenorhabditis elegans	3	1	0	0	1	2	1	1	0	0	1	2	1	1	0	0	0	0	6	2	1	1	0	4	0
Apis mellifera	5	2	1	0	2	5	1	0	0	1	1	3	8	2	0	0	0	0	4	0	4	0	0	6	0
Anopholes gambiae	1	1	1	0	1	1	1	1	0	1	1	2	3	2	0	0	0	0	2	1	1	0	0	1	0
D.melanogaster	4	3	0	0	1	3	1	3	0	1	1	1	2	1	0	0	0	0	2	1	1	0	0	1	0
Vertebrates																									
Danio rerio	6	5	0	2	7	16	13	1	0	6	4	1	0	1	0	0	2	0	1	1	1	6	0	1	0
Tetraodon nigroviridis	3	1	1	1	2	4	3	1	2	1	1	3	2	2	0	0	1	0	1	1	2	1	0	1	0
Fugu rubripes	16	2	1	1	9	15	10	4	3	1	1	3	7	2	0	0	3	0	2	1	2	4	0	3	0
Xenopus tropicalis	14	11	4	0	15	13	11	1	3	5	1	6	4	2	0	0	4	0	2	3	2	4	1	4	0
Gallus gallus	2	4	3	1	7	6	11	0	3	2	2	2	2	1	0	0	0	0	2	1	1	5	0	5	0
Mammals																									
Monodelphis domestica	2	2	1	1	3	3	4	1	3	1	1	2	1	1	0	0	1	0	1	1	1	4	0	2	0
Canis familiaris	7	2	1	4	5	13	8	2	4	2	1	5	1	1	0	0	2	0	1	2	2	3	0	3	0
Bos taurus	4	3	1	2	2	5	4	2	1	1	1	2	1	1	0	0	1	0	1	1	1	2	0	2	0
Rattus norvegicus	3	3	1	1	2	2	4	1	0	2	1	1	1	1	0	0	1	0	1	1	1	4	0	3	0
M.musculus	2	7	1	1	3	2	6	0	4	2	1	4	2	1	0	0	1	0	2	3	1	4	0	6	0
Pan troglodytes	4	2	1	3	2	5	4	2	1	2	3	2	1	1	0	0	1	0	1	1	2	4	0	4	0
H.sapiens	5	3	1	3	2	6	4	2	1	3	3	2	4	1	0	0	2	0	1	1	1	4	0	4	0

Subunit composition of various ATP-dependent chromatin remodeling factors



In vitro reconstitution of nucleosomes on genomic DNA

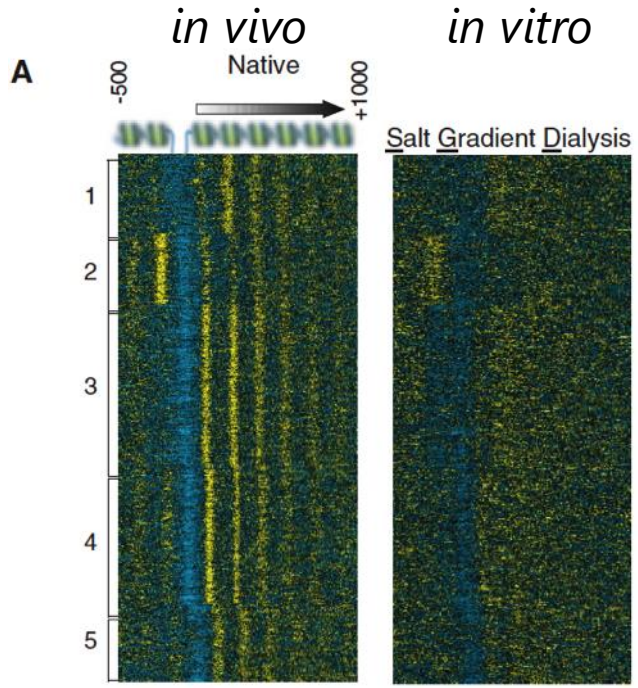


Nucleosome

Free DNA

Zhang, Z. et al. A packing mechanism for nucleosome organization reconstituted across a eukaryotic genome. *Science* **332**, 977-980 (2011).

In vitro reconstitution of nucleosomes on genomic DNA

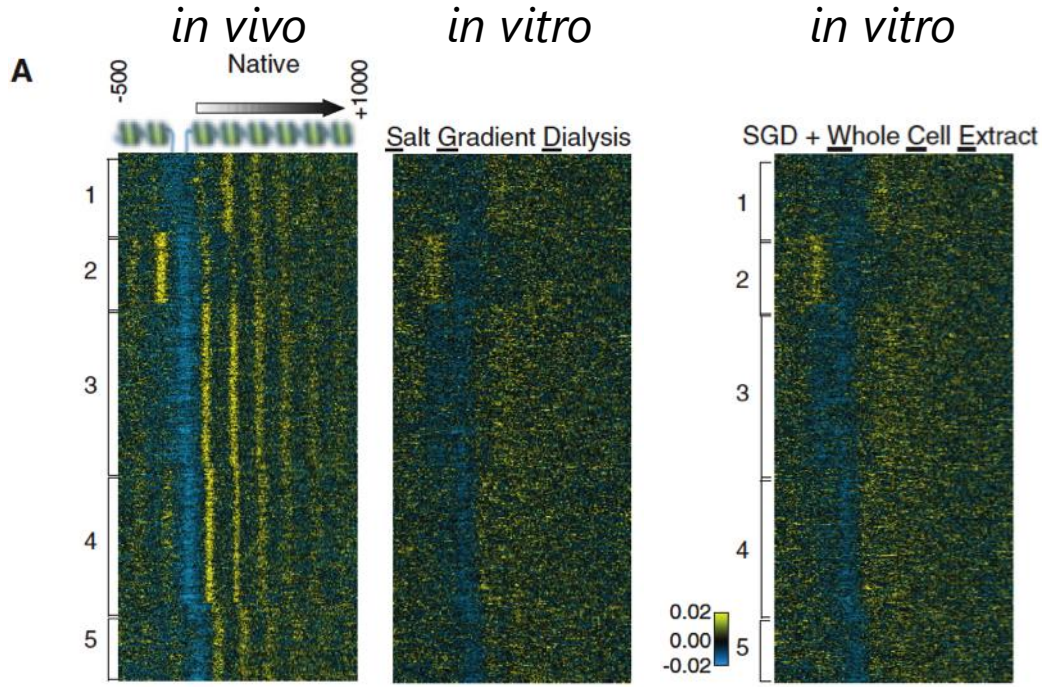


Nucleosome

Free DNA

Zhang, Z. et al. A packing mechanism for nucleosome organization reconstituted across a eukaryotic genome. *Science* **332**, 977-980 (2011).

In vitro reconstitution requires whole cell extract and ATP.

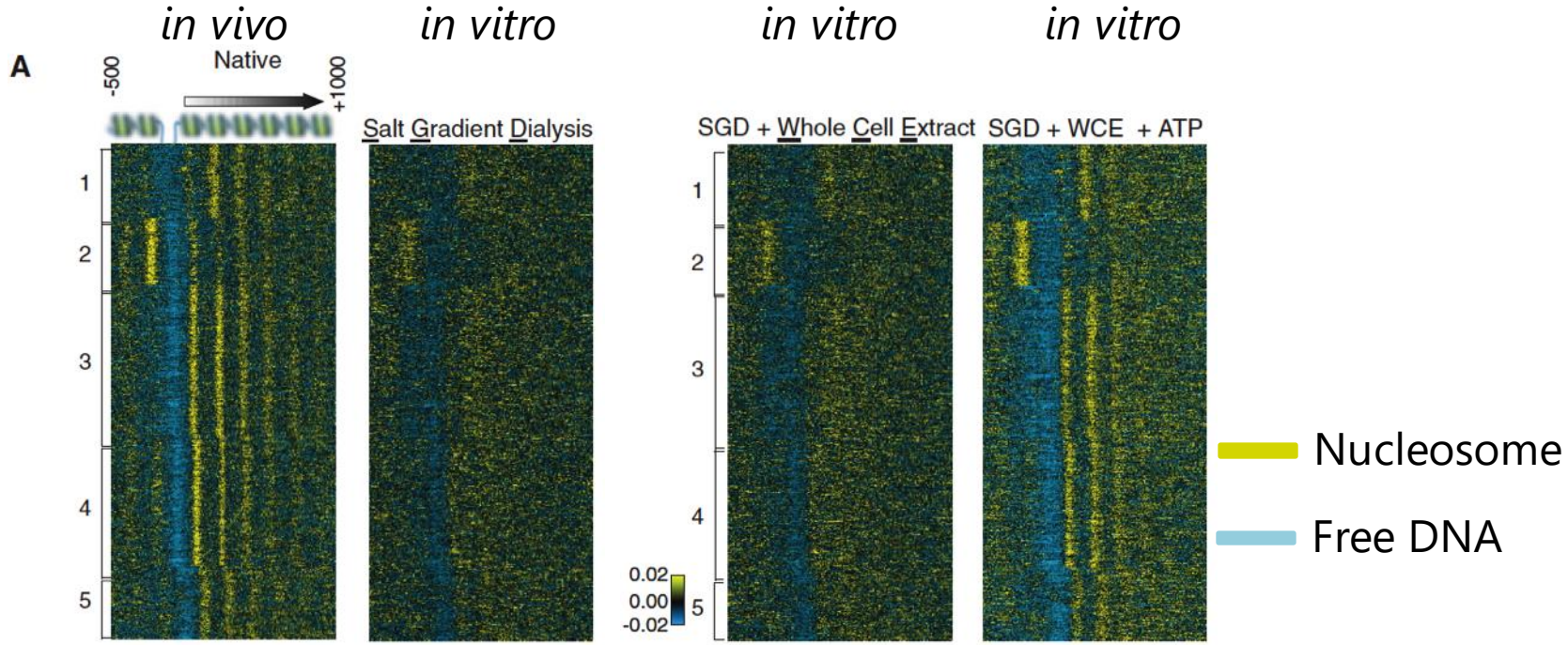


Nucleosome

Free DNA

Zhang, Z. et al. A packing mechanism for nucleosome organization reconstituted across a eukaryotic genome. *Science* **332**, 977-980 (2011).

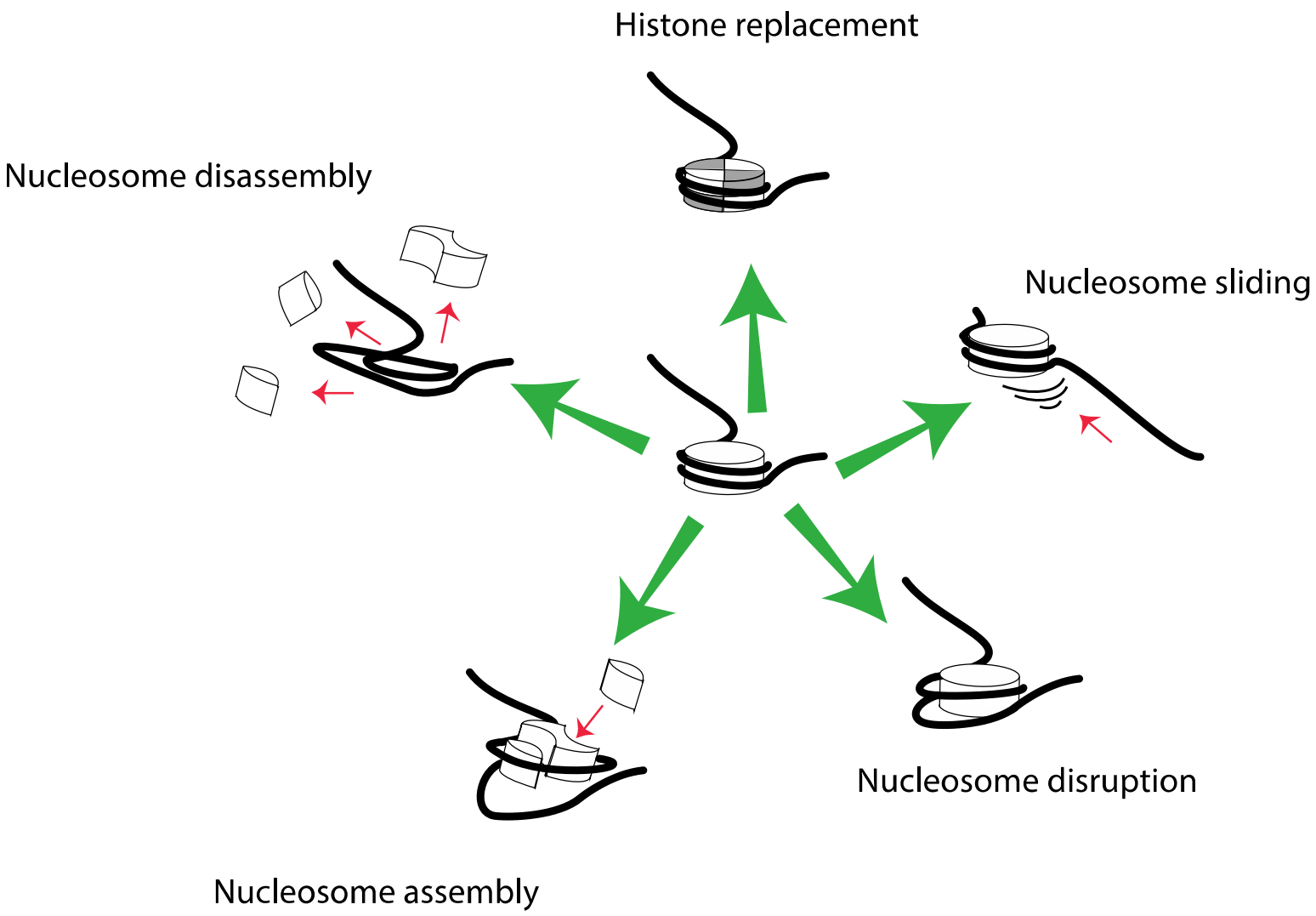
In vitro reconstitution requires whole cell extract and ATP.



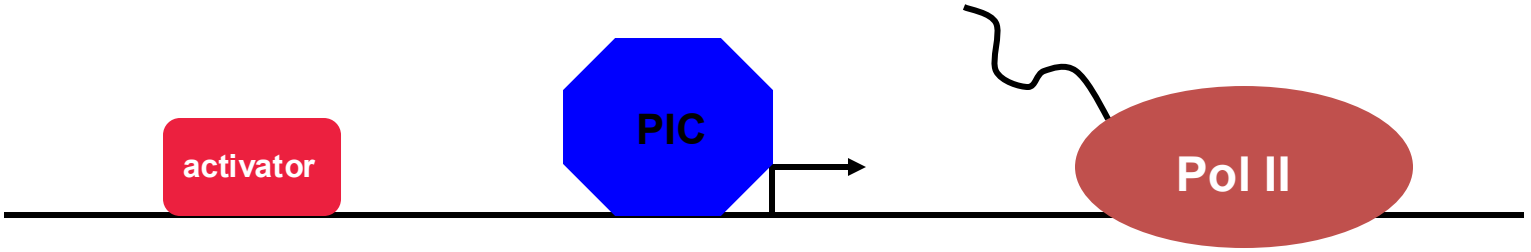
Zhang, Z. et al. A packing mechanism for nucleosome organization reconstituted across a eukaryotic genome. *Science* **332**, 977-980 (2011).

Altering chromatin structure

Chromatin remodeling with ATP hydrolysis



Nucleosomes can inhibit multiple steps in transcription



Functions of Swi/Snf complexes

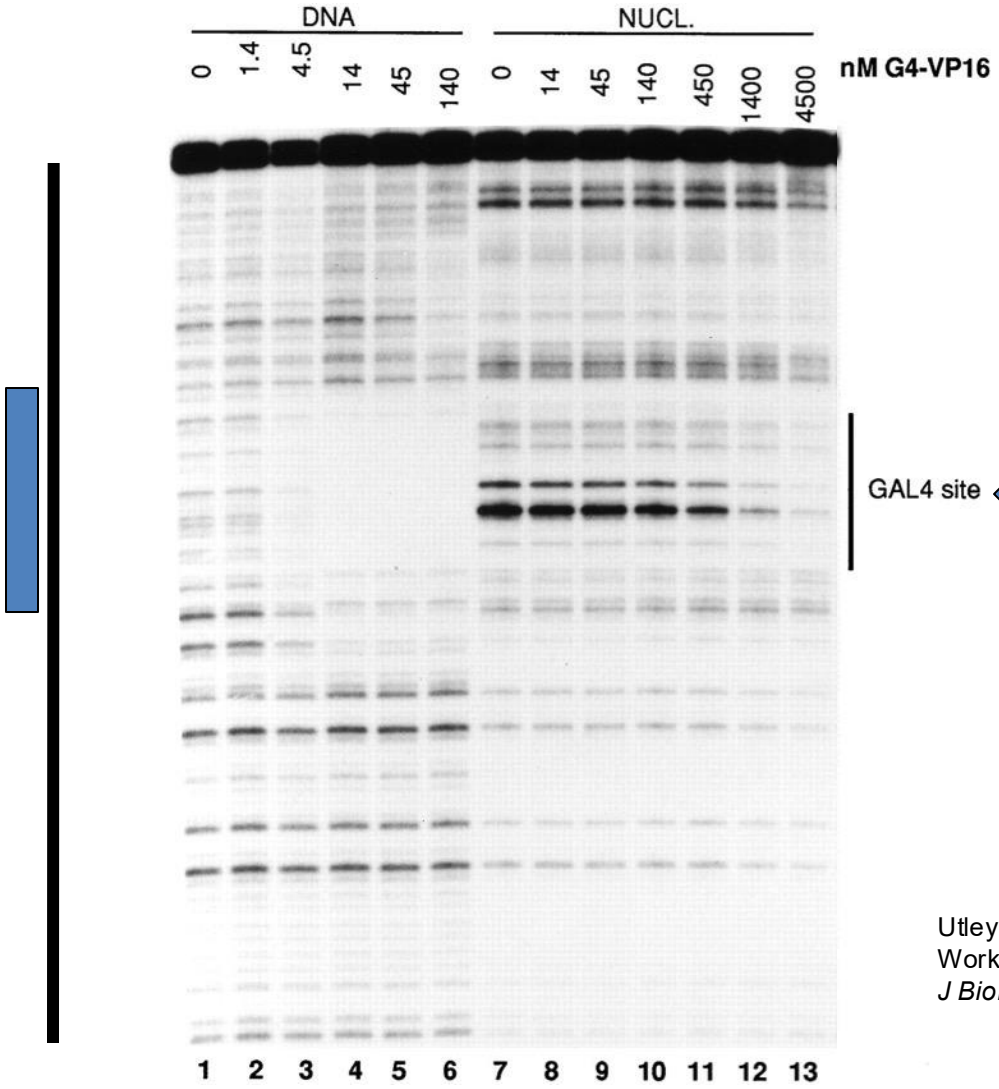
Characterized in budding yeast as a transcriptional co-activator.

Required for mating type Switching and Sucrose fermentation

The catalytic subunit is also known as (depending on the organism):

Snf2p, Sth1p, snf21, SMARCA4, BRG1, BAF190, hSNF2beta, SNF2L4, SMARCA2, hBRM, hSNF2a, SNF2L2, SNF2LA, SYD, splayed, psa-4, brahma

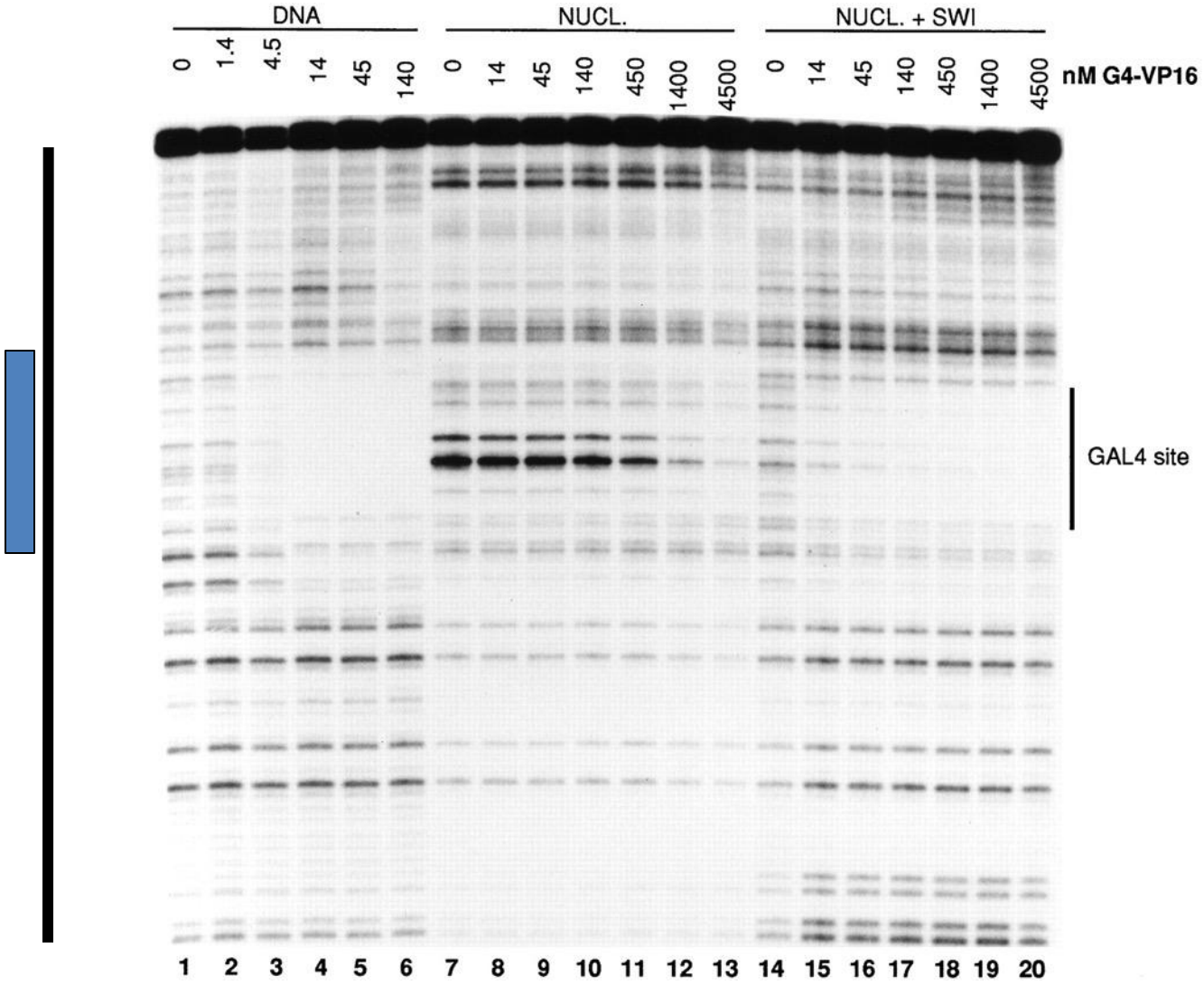
Inhibitory effect of nucleosomes on DNA binding proteins



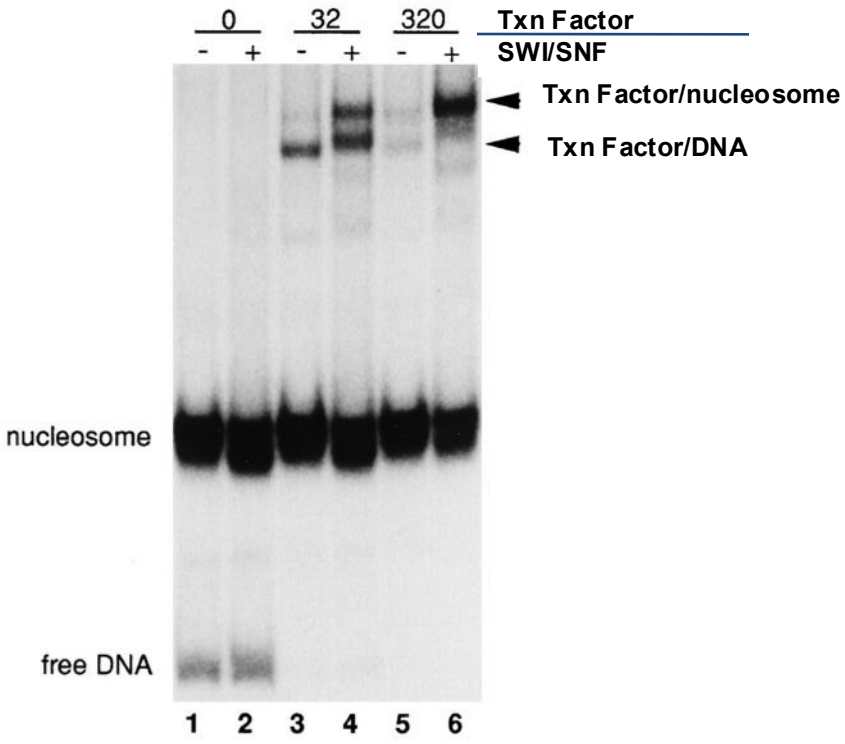
Activator

Utley, R. T., Cote, J., Owen-Hughes, T. & Workman, J. L.
J Biol Chem **272**, 12642-12649 (1997).

Inhibitory effect of nucleosomes on DNA binding proteins

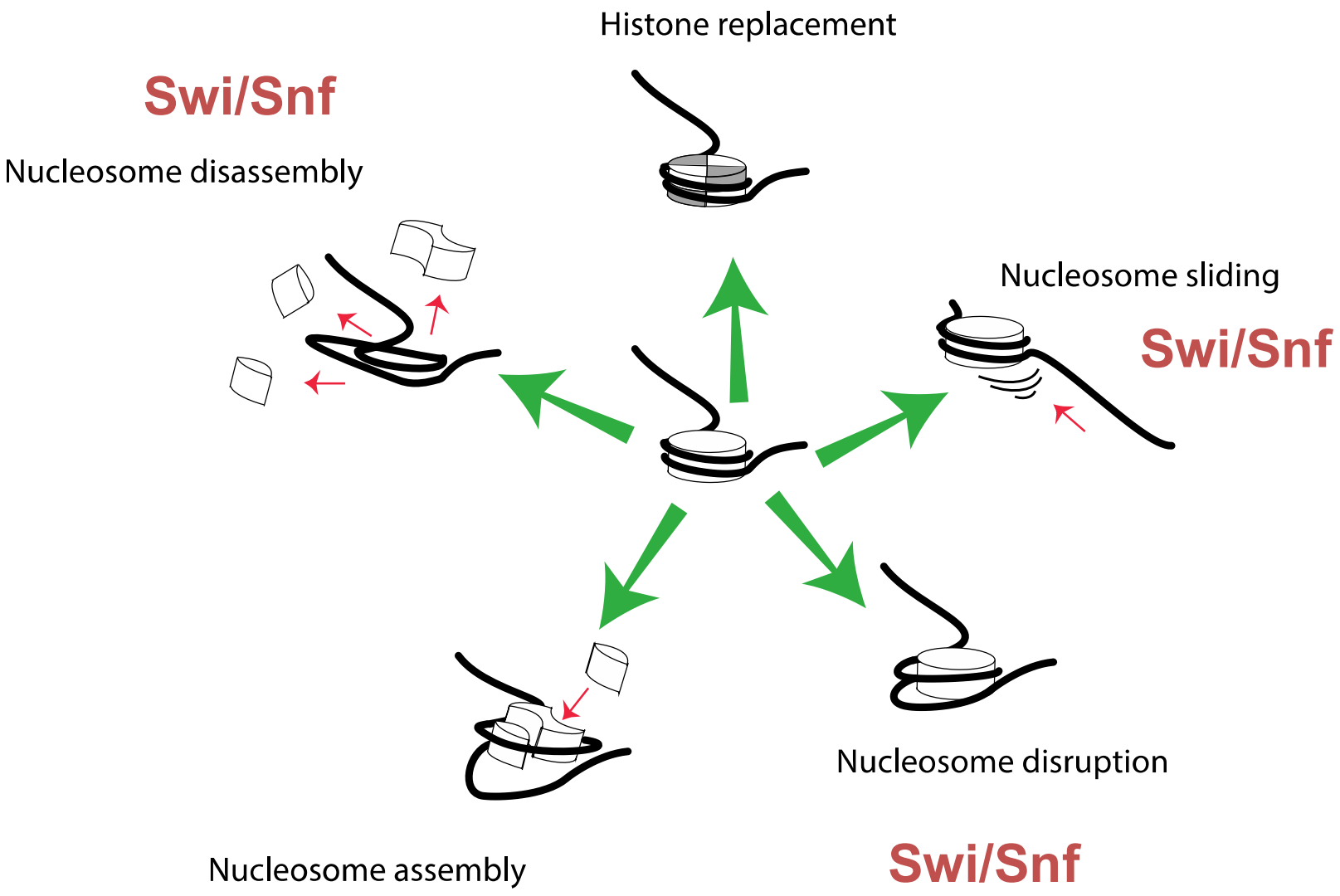


SWI/SNF overcomes the Inhibitory effect of nucleosomes on DNA binding proteins



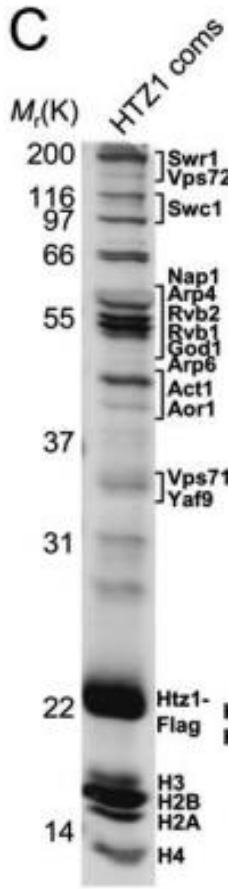
{Utley et al., 1997, J Biol Chem, 272, 12642-9}

Chromatin remodeling with ATP hydrolysis



Functions of Swr1 class complexes

SWR 1 and Htz1



Science vol 303 16 january 2004

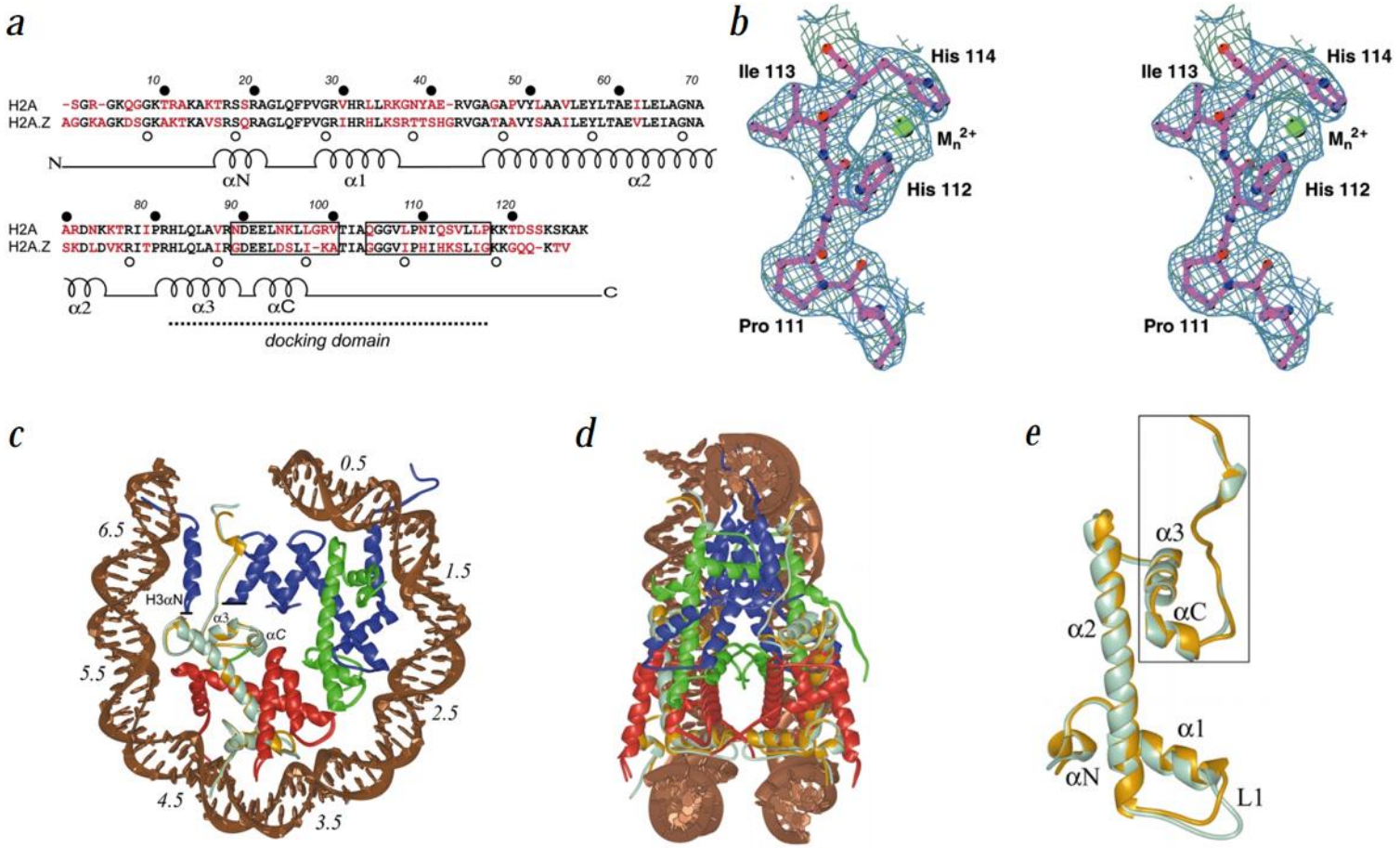
Some histone variants……

Histone	Description/functions	Escort and/or chaperone complex
H3, H4	Canonical core histones encoded by replication-coupled genes	CAF1, Asf1
CenH3	Centromere-specific histone 3 variant	RbAp48
H3.3	Replacement histone 3 variant found in active chromatin	HirA, Asf1
H2A, H2B	Canonical core histones encoded by replication-coupled genes	FACT
H2A.X	H2A form with a C-terminal motif that becomes serine phosphorylated at sites of double-strand breaks	INO80?
H2A.Z	Diverged form of H2A enriched around gene promoters	SWR1
H2Av	A single Drosophila melanogaster variant that is both H2A.X and H2A.Z	Tip60
MacroH2A	Vertebrate specific H2A variant associated with silent chromatin that has an additional globular domain	Unknown
H2ABbd	Vertebrate-specific H2A variant associated with active chromatin	Unknown
Sperm histones	Variants that have evolved to tightly package sperm or pollen	Unknown

Typically only H2A and H3 are found as variants

Not strictly coupled to replication

Htz1



nature structural biology • volume 7 number 12 • December 2000

Htz1 Nucleosomes in yeast

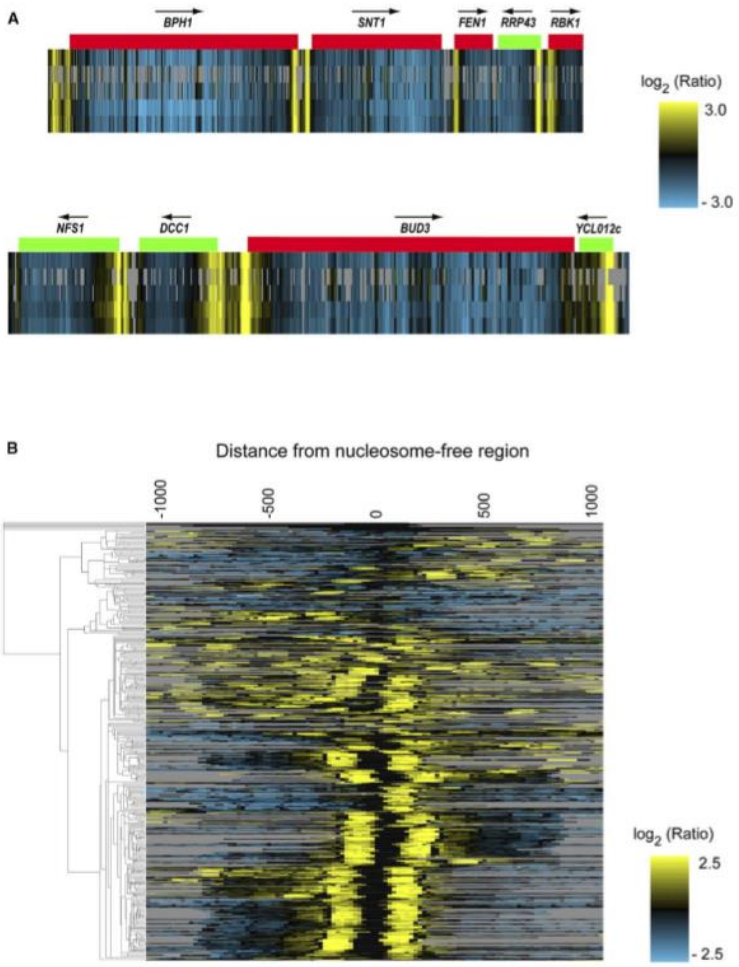
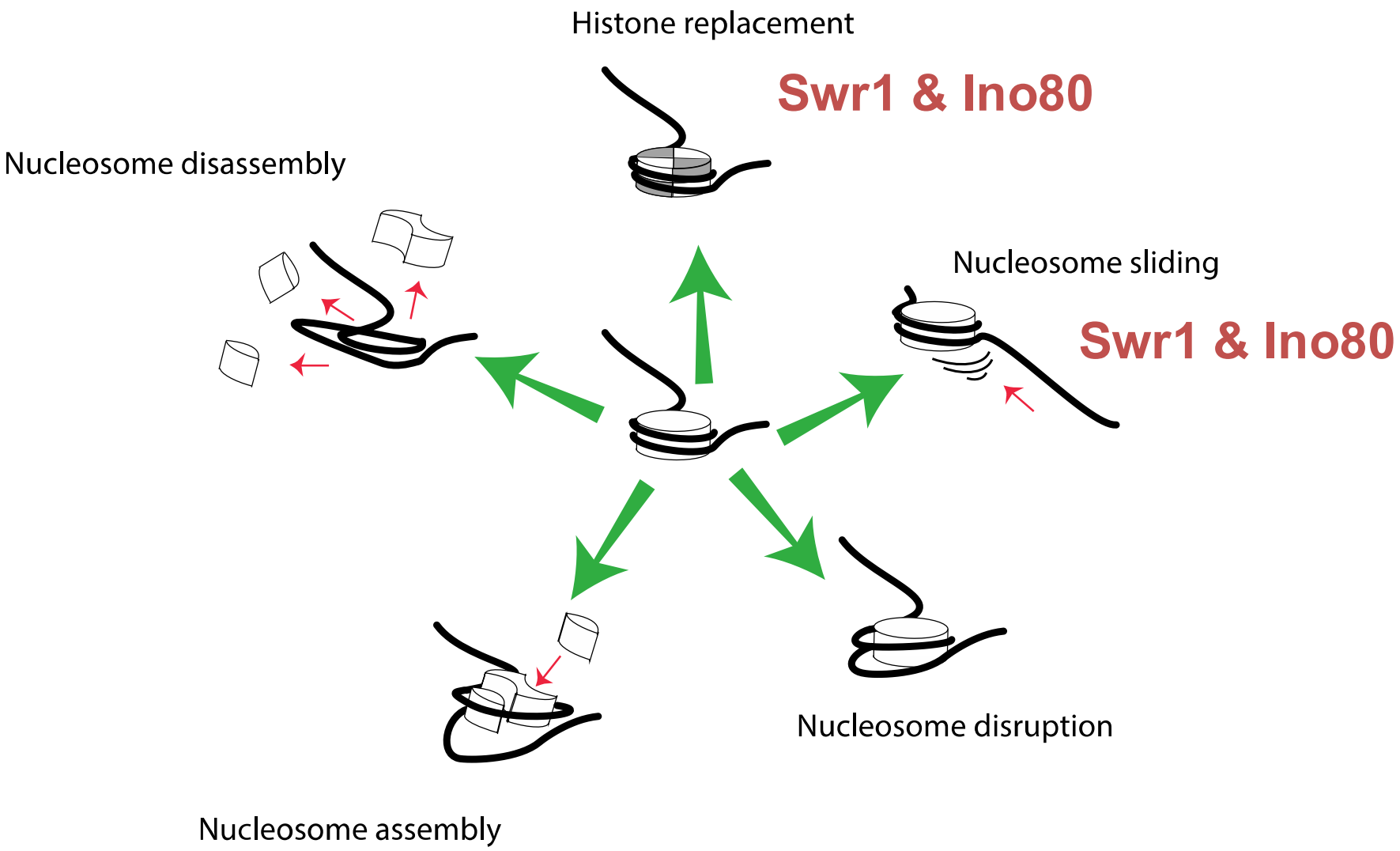


Figure 2. High-Resolution Mapping of H2A.Z Nucleosomes

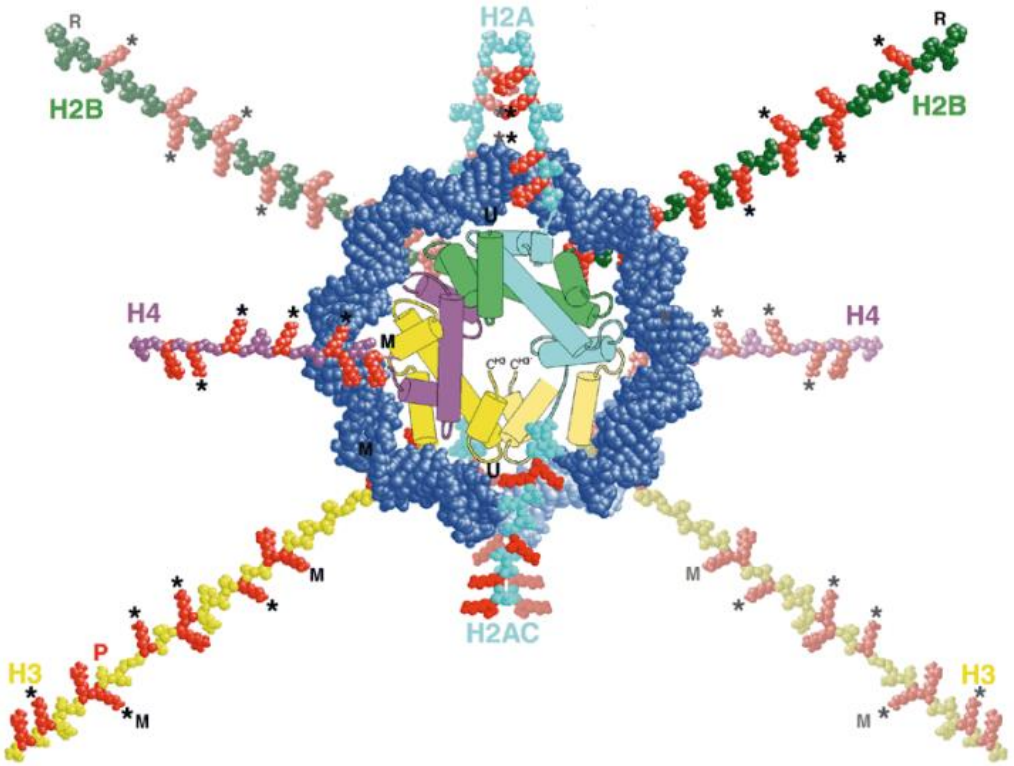
Shown is a color depiction of ratio of the ChIP signal for mononucleosomal DNA immunoprecipitated using anti-Htz1 antibodies divided by those for DNA extracted from mononucleosomes for regions covered by a high-resolution oligonucleotide tiling microarray.
(A) H2A.Z enrichment in representative euchromatic regions analyzed in Figures 1C and 1D. Shown are the data for five replicate microarray hybridizations. Yellow represents a positive relative enrichment for H2A.Z over the median enrichment versus blue for negative enrichment.
(B) Clustered array dataset centered on nucleosome-free regions (NFRs) of gene promoters. Shown are data from probes from up to 1 kb upstream and 1 kb downstream of the position of the NFR estimated from previous studies (Yuan et al., 2005). Each row represents a single promoter region, and columns correspond to data from microarray oligonucleotides at a given position with respect to the NFR.

Cell, Vol. 123, 233–248, October 21, 2005,

Chromatin remodeling with ATP hydrolysis



Histone modifications



How do modifications work?

“Writers”

Protein/domain	Mark
HAT histone acetyl transferase	Acetylation
SET Suppressor of variegation, Enhancer of zeste and Trithorax	Lysine Methylation
Dot1 (methylase fold)	Lysine methylation
PRMT	Arginine Methylation
Kinase	Phosphorylation
Ubiquitin ligase	Ubiquitin

How do modifications work?

“Readers”

Protein domain	Mark
Bromodomain	Acetylation
Chromodomain	Methylation
PHD (zinc finger) Plant Homeodomain	Methylation
WD40 (after tryptophan and aspartic acid residues and length of approximately 40 amino acids)	Methylation
Tudor domain	Methylation Arginine
14-3-3	Phosphorylation

Domains are not exclusively involved in these functions eg WD40 of Cdc4 recognize phosphorylated serine and threonine containing peptides

How do modifications work?

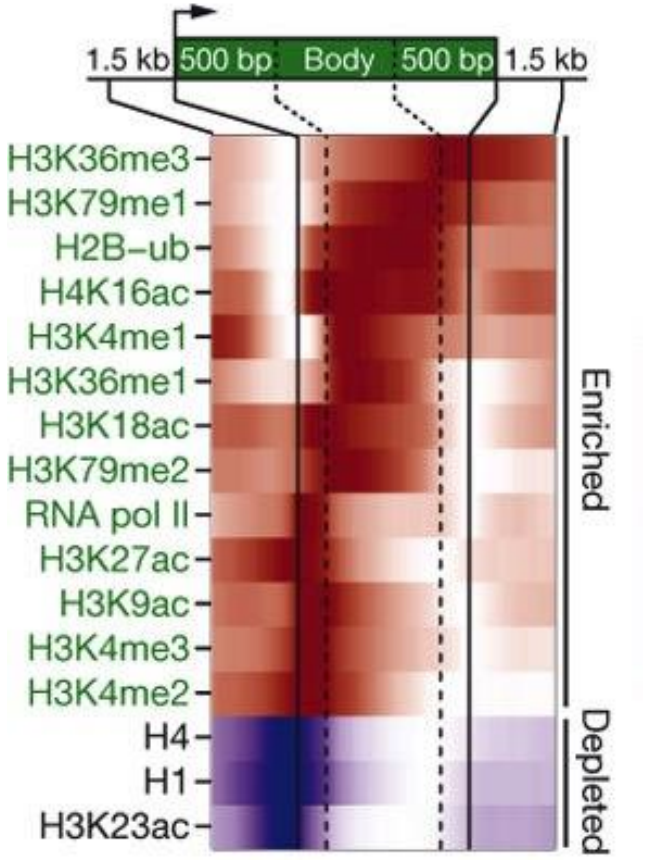
“Erasers”

Protein or domain	Function
HDAC histone deacetylase	Deacetylation
Sirtuin	Deacetylation
Jmj Jumanji	Demethylation
LSD SWIRM	Demethylation
Phosphatase	Dephosphorylation
Deubiquitylation Ubp8+10	Deubiquitylation

How do modifications work?

Genome annotation of histone modifications

Chromatin patterns associated with transcriptionally active genes.



PV Kharchenko *et al. Nature* (2010)

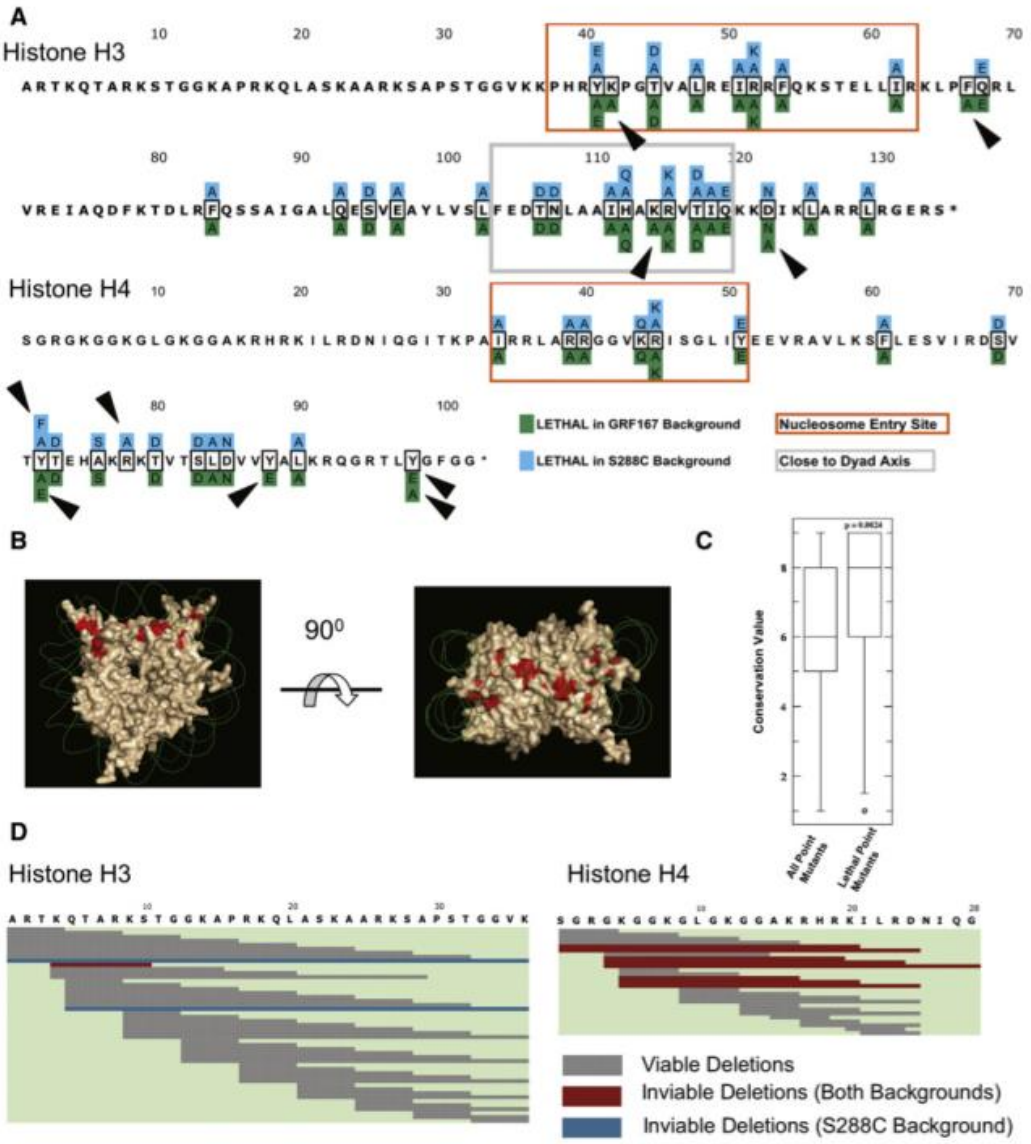
How do modifications work?

Massive potential combinatorial complexity

Correlation?

Causation?

Histone point mutants in budding yeast



Cell 134, 1066–1078,
September 19, 2008

Histone genetics is now possible in *drosophila:*

Transcription in the Absence of Histone H3.2 and H3K4 Methylation

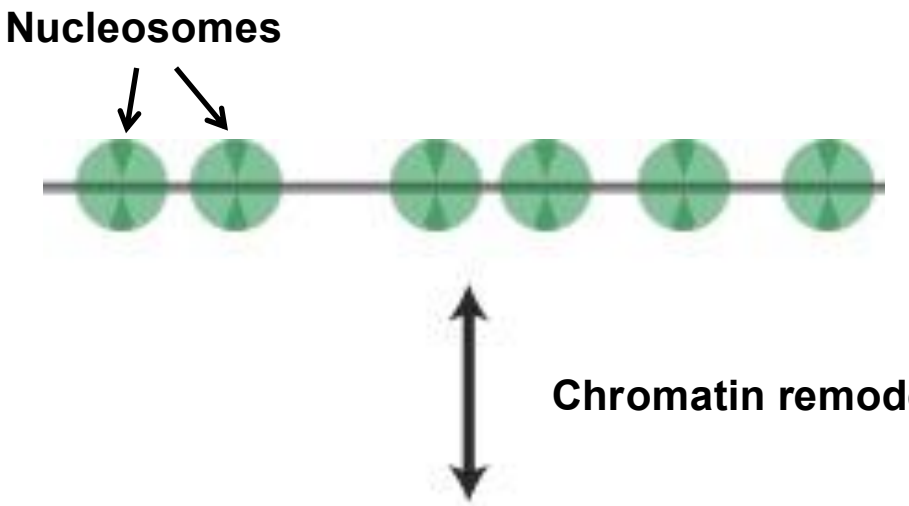
Martina Hödl[1] and Konrad Basler[1,*]
[1]Institute of Molecular Life Sciences, University of Zurich,
Winterthurerstrasse 190, 8057 Zurich, Switzerland

Summary

Histone H3 proteins play fundamental roles in DNA packaging, gene transcription, and the transmission of epigenetic states. In addition to posttranslational modifications of their N termini, the use of H3 variants contributes to their regulatory repertoire. Canonical histone H3.2 is expressed during S phase and differs by four amino acid residues from the variant histone H3.3, which is synthesized in a cell-cycle-independent manner [1]. Because H3.3 is enriched within actively transcribed loci [1–3], and because di- and trimethylation of H3 lysine 4 are hallmarks of chromatin at such sites in the genome [4], the H3.3K4 residue is considered to serve as the major regulatory determinant for the transcriptional state of a gene. Here we use genetic approaches in *Drosophila* to replace all 46 gene copies of *His3.2* with mutant derivatives and thereby demonstrate that canonical and variant H3 can functionally replace each other. Cells are able to divide and differentiate when H3.2 is entirely absent but replaced by S phase-expressed H3.3. Moreover, although slowed down in their proliferative capacity, cells that code for a nonmethylatable residue instead of K4 in all canonical and variant H3 genes are competent to respond to major developmental signaling pathways by activating target gene expression. Hence, the presence of different H3 protein species is not essential in *Drosophila* and transcriptional regulation can occur in the complete absence of H3K4 methylation.

Most histone modifications associated with gene activation have a modest, positive effect on transcription.

Chromatin states

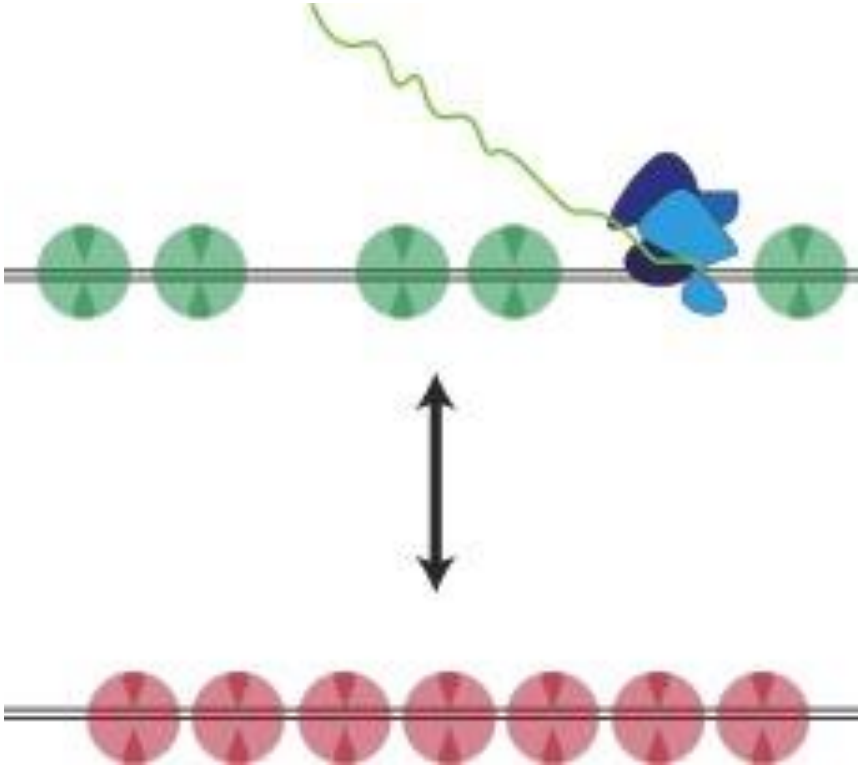


State A
(Histone acetylation,
H3 K4 methylation)

Chromatin remodeling factors

State B
(Histone deacetylation,
H3 K9 methylation)

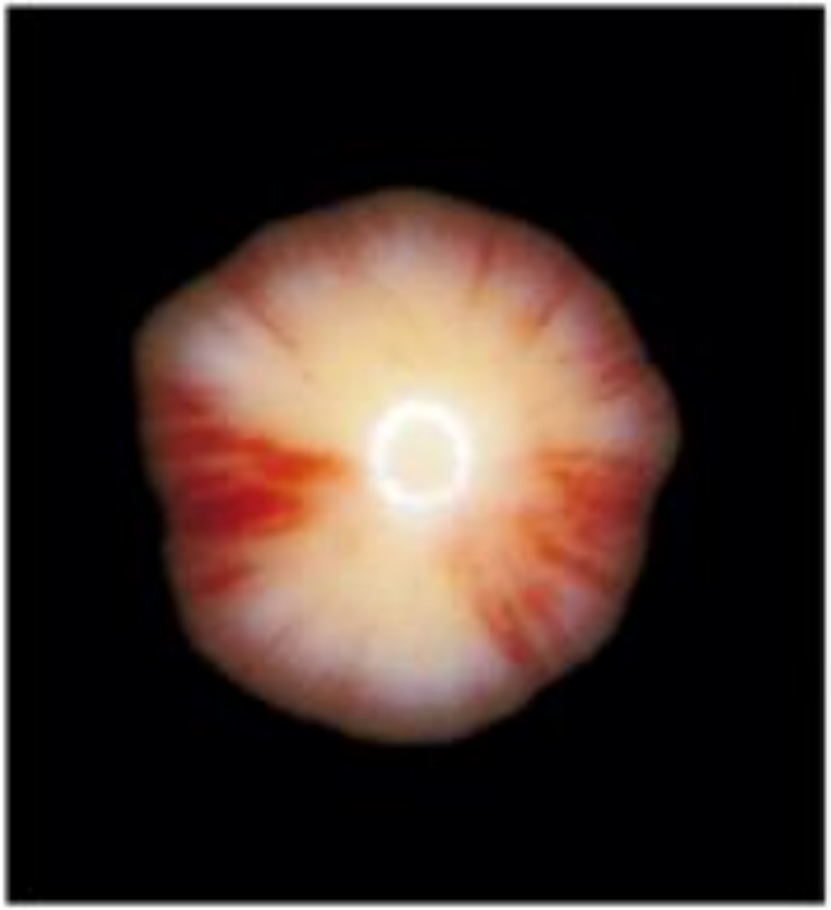
Chromatin states

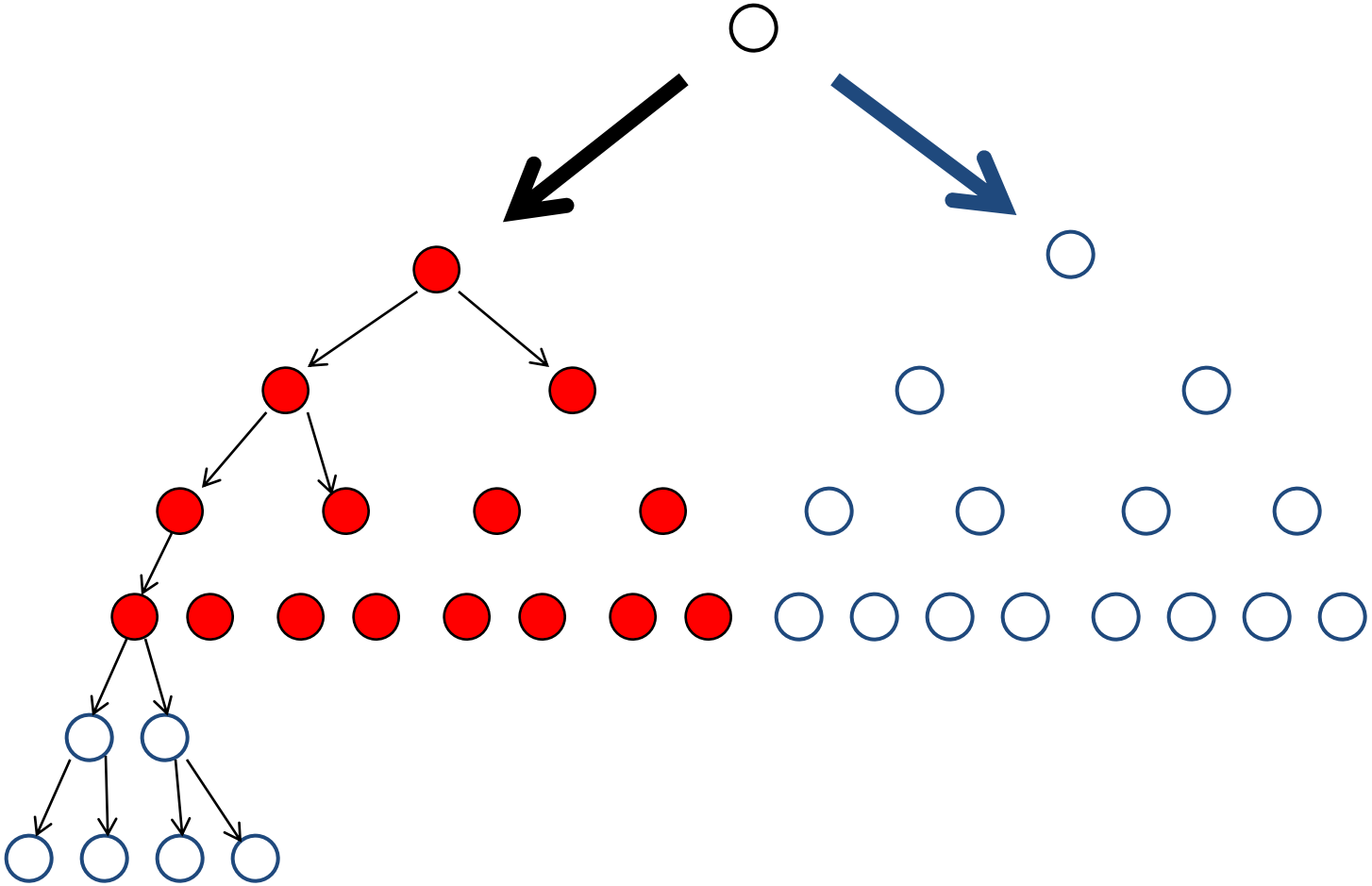


Gene ON

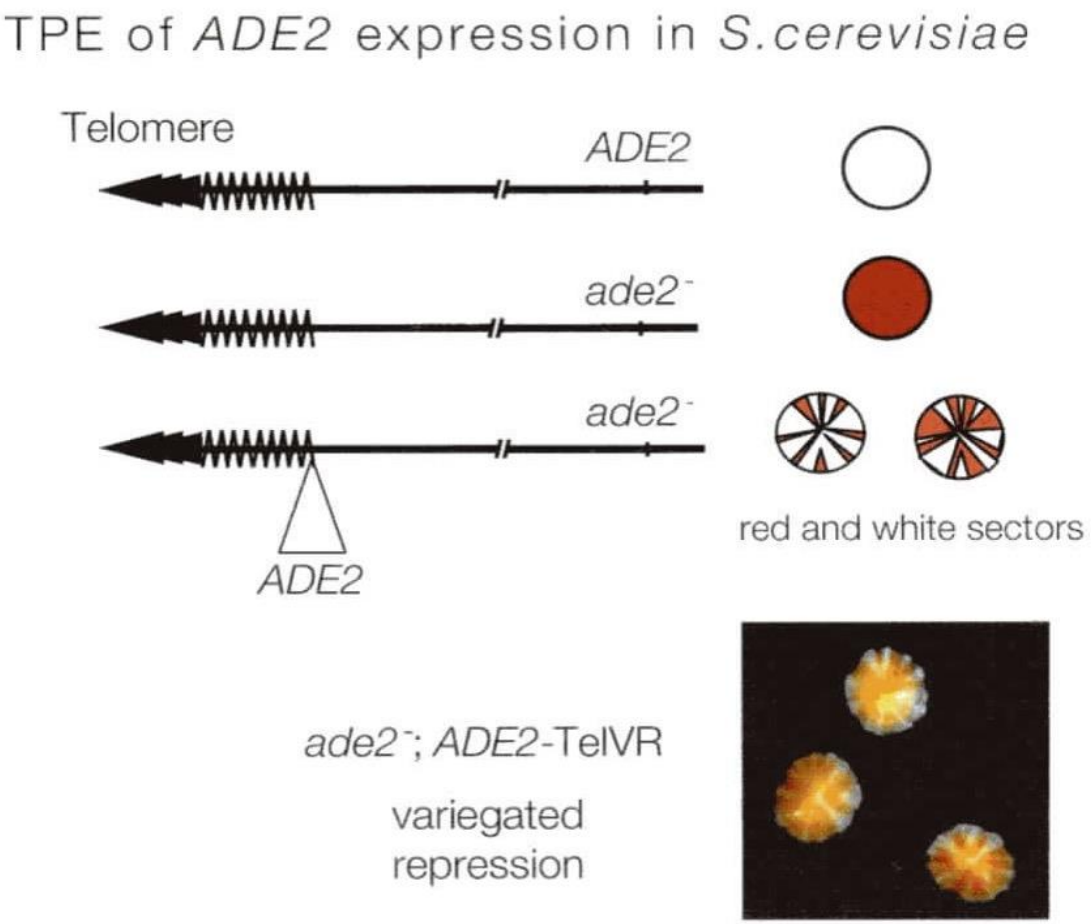
Gene OFF

Epigenetics in Yeast





Telomere Position Effect



Telomere Position Effect

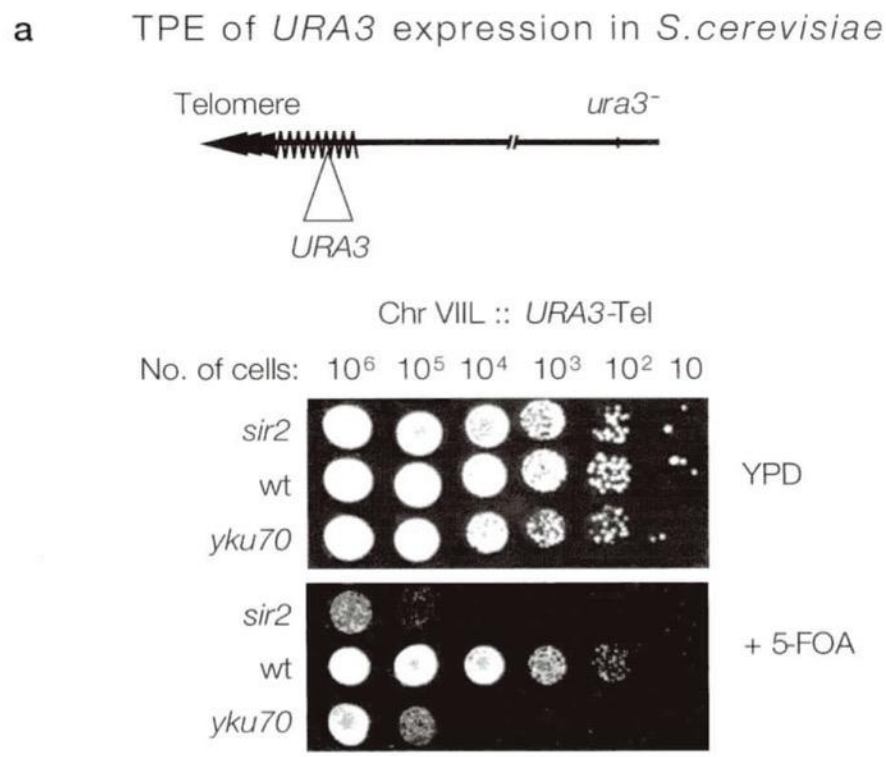
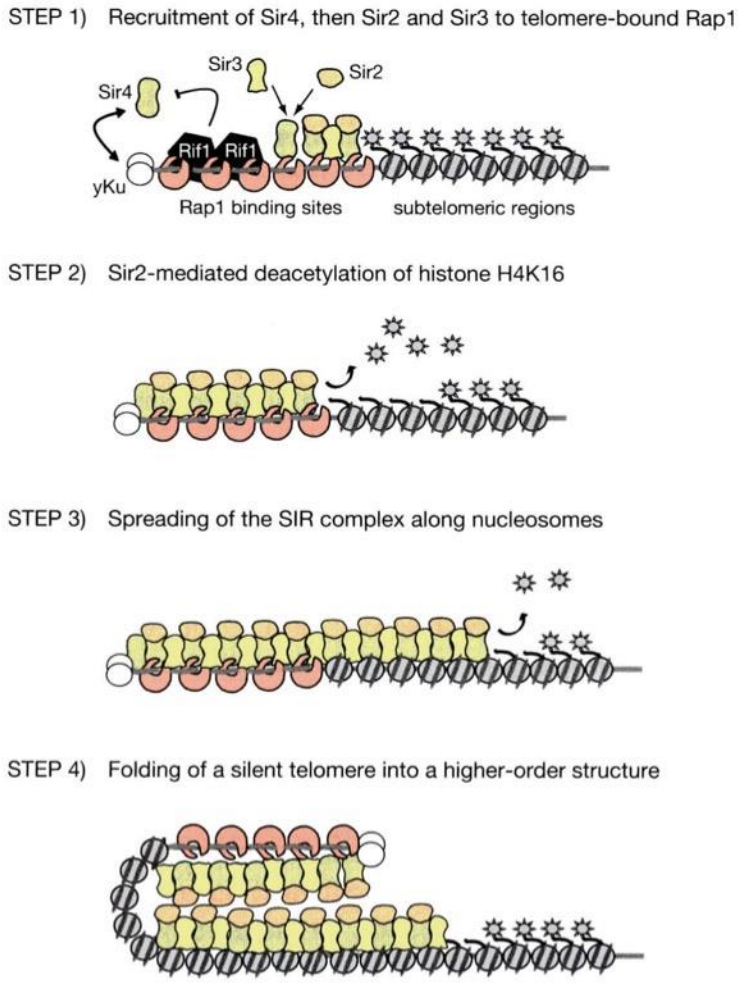


Figure 1. Silencing and TPE in Yeast

(*a*) The *Ura3* gene, inserted near the telomeric simple TG-rich repeat at the left arm of chromosome VII, is silenced by telomeric heterochromatin in this yeast strain. In normal rich medium (YPD), no growth difference can be detected between wild-type (wt) cells that repress the subtelomeric *Ura3* gene and silencing mutants that lose telomeric heterochromatin and express *Ura3*. In media containing 5-FOA (*lower panel*), on the other hand, cells that repress *Ura3* (e.g., wt cells) can grow, whereas cells that express it (*sir2* and *yku70* mutants) cannot. This is because the *Ura3* gene product converts 5-FOA to the toxic intermediate 5-fluorouracil. The serial dilution/drop assay allows detection of silencing in as few as 1 in 10^6 cells. (*b*) Cells containing the wt *Ade2* gene produce a colony that is "white," whereas those containing mutant ade2 appear red, due to the accumulation of a reddish intermediate in adenine biosynthesis. When the *Ade2* gene is inserted near the telomere at the right arm of chromosome V, it is silenced in an epigenetic manner. The silent *Ade2* state and the active *Ade2* state in genetically identical cells are both inherited, creating red and white sectors in a colony (much like PEV).

5-FOA is toxic to cells expressing URA3

Sir complexes



Sir3 and Sir2/4
bind deacetylated histone tails (H4 K16)

Sir3 forms high order oligomers

Figure 6. Steps in the Assembly of Telomeric Heterochromatin

(*Step 1*) At telomeres, Rap1 and yKu recruit Sir4 even in the absence of Sir2 or Sir3. Only Sir4 can be recruited, in the absence of the other Sir proteins, and its binding is antagonized by Rif1 and Rif2 (Mishra and Shore 1999). (*Step 2*) Sir4-Sir2 and Sir4-Sir3 interact strongly, creating Sir complexes along the TG repeats. Sir2 NAD-dependent histone deacetylase activity is stimulated by complex formation, and Sir2 deacetylates the acetylated histone H4 K16 residue in nearby nucleosomes. (*Step 3*) SIR complexes spread along the nucleosomes, perhaps making use of the O-acetyl ADP ribose intermediate produced by NAD hydrolysis (Liou et al. 2005). Sir3 and Sir4 bind the deacetylated histone H4 tails. Although the deacetylated histone H3 amino-terminal tail also binds Sir3 and Sir4 proteins, it is not shown here. (*Step 4*) The silent chromatin "matures" at the end of M phase to create an inaccessible structure. This may entail higher-order folding and sequestering at the nuclear envelope.

Stopping the spread

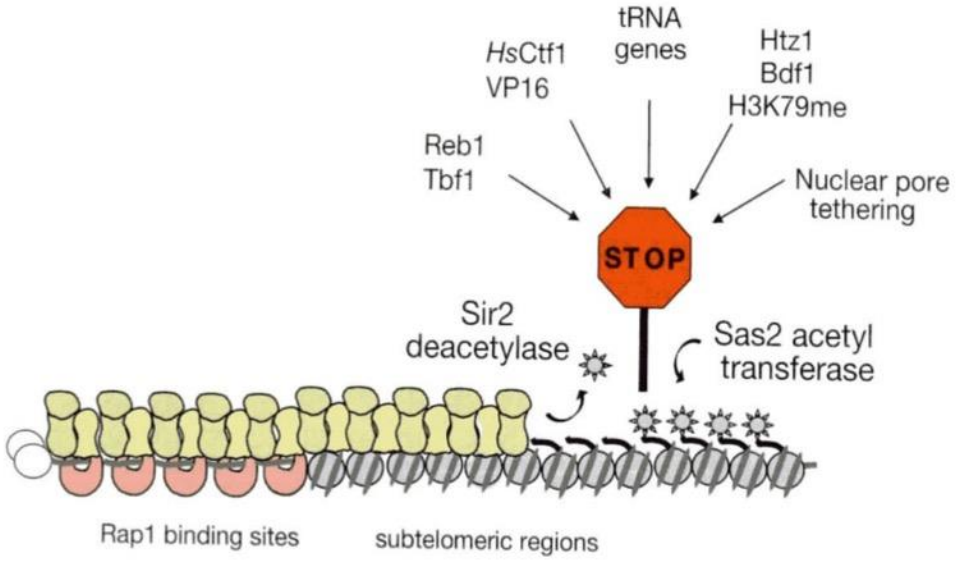
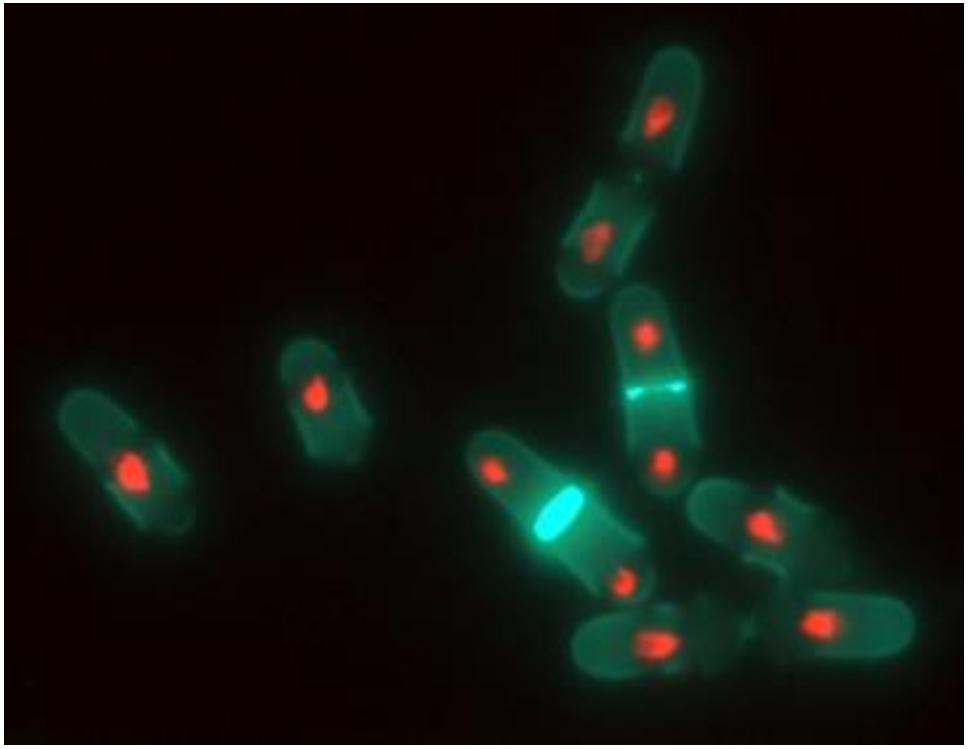


Figure 7. Heterochromatin Boundary Function in Budding Yeast

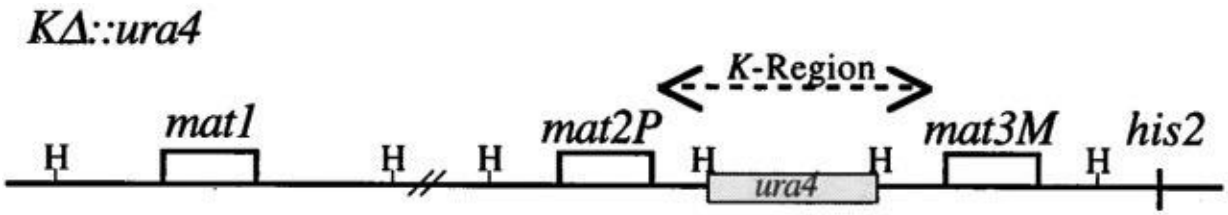
Spreading of heterochromatin through deacetylation of histone H4 K16 by Sir2 is limited by the competing activity of Sas2 histone acetyltransferase which acetylates H4K16 in adjacent euchromatin, thus preventing Sir3 binding. Methylation of H3K79 in adjacent euchromatin also affects the spreading of heterochromatin. In addition, factors such as Reb1, Tbf1, and mammalian or viral factors Ctf1 or VP16, nuclear pore tethering, and the presence of tRNA genes may also mediate boundary function. It is conceivable that several of these factors function through the recruitment of histone acetyltransferases.

Schizosaccharomyces Pombe



Differs from budding yeast (*S. cerevisiae*) in that it has no Sir complex, but does have H3K9 methylation which is a hallmark of heterochromatin in metazoa. Also has RNAi pathways.

Epigenetics in Pombe



K region is replaced by the *ura4* gene.

KΔ:: ura4 cells show interesting covariegation of ura4 expression: most cells are capable of growing on -URA media (ura4 expressed), but a small proportion grow on FOA plates (ura4 repressed).

Expression states were stable for many generations but switched at low frequency.

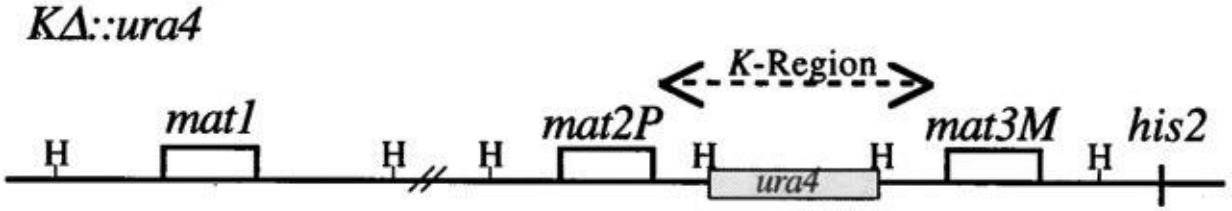
Grewal, S. I. & Klar, A. J. Chromosomal inheritance of epigenetic states in fission yeast during mitosis and meiosis. *Cell* **86**, 95-101 (1996).

Epigenetics in Pombe

Identical cells have different expression of the Ura4 gene: ON or OFF

What would happen if you mated “expressing” and “non-expressing” haploids?

Epigenetics in Pombe

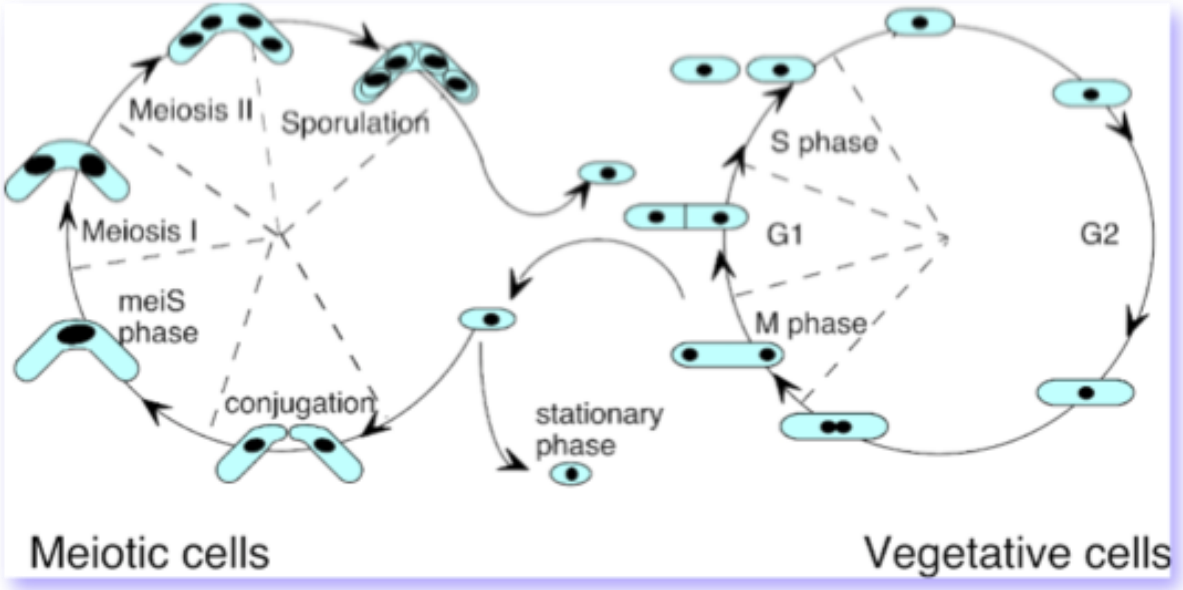


	ura4 expression	Iodine staining	
SPG27 (L) (*h*^{90}, *KΔ::ura4, his2, ade6-210*)	Strong	Light	*his-*
×			
SPG51 (D) (*h*^{90}, *KΔ::ura4, ade6-216*)	Weak	Dark	*his+*

haploids are genetically identical at KΔ:: ura4

Epigenetics in Pombe

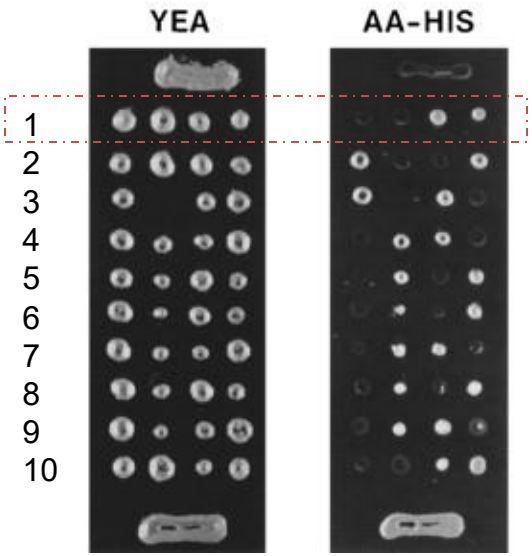
Sporulation and dissected of tetrads



	ura4 expression	Iodine staining
SPG27 (L) (*h*^{90}, *KΔ::ura4, his2, ade6-210*)	Strong	Light
×		
SPG51 (D) (*h*^{90}, *KΔ::ura4, ade6-216*)	Weak	Dark

Epigenetics in Pombe

	ura4 expression	Iodine staining
SPG27 (L) (*h*^{90}, *KΔ::ura4, his2, ade6-210*)	Strong	Light
×		
SPG51 (D) (*h*^{90}, *KΔ::ura4, ade6-216*)	Weak	Dark



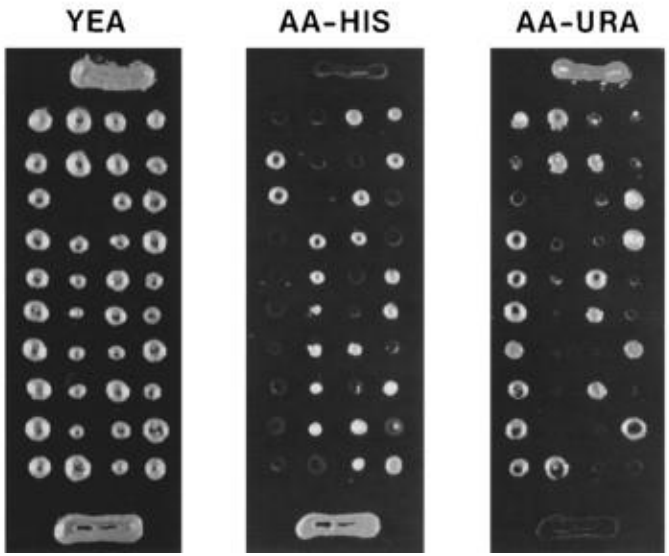
Tetrad #

Classic 2:2 segregation of his2

Light and Dark Variegated States Are Meiotically Stable and Linked to the mat-Region
A diploid was constructed by crossing the indicated strains, allowed to grow for at least 30 generations, sporulated, and subjected to tetrad analysis. The four spores from each ascus were placed on rich medium in a horizontal row by micromanipulation. After growth for 3 days, they were replicated onto plates containing the indicated medium. All plates were photographed directly after overnight growth at 33° C except for the PMA+ plate, which was incubated for 3 days at 25° C and stained with iodine vapors before photography. Each ascus produced two Ura+, His− light-staining, two Ura−, His+ dark-staining segregants. Patches at the top and bottom indicate the phenotype of the parental SPG27(L) and SPG51(D) strains, respectively, used in the cross.

Epigenetics in Pombe

	ura4 expression	Iodine staining
SPG27 (L) (*h*^{90}, *KΔ::ura4, his2, ade6-210*)	Strong	Light
×		
SPG51 (D) (*h*^{90}, *KΔ::ura4, ade6-216*)	Weak	Dark

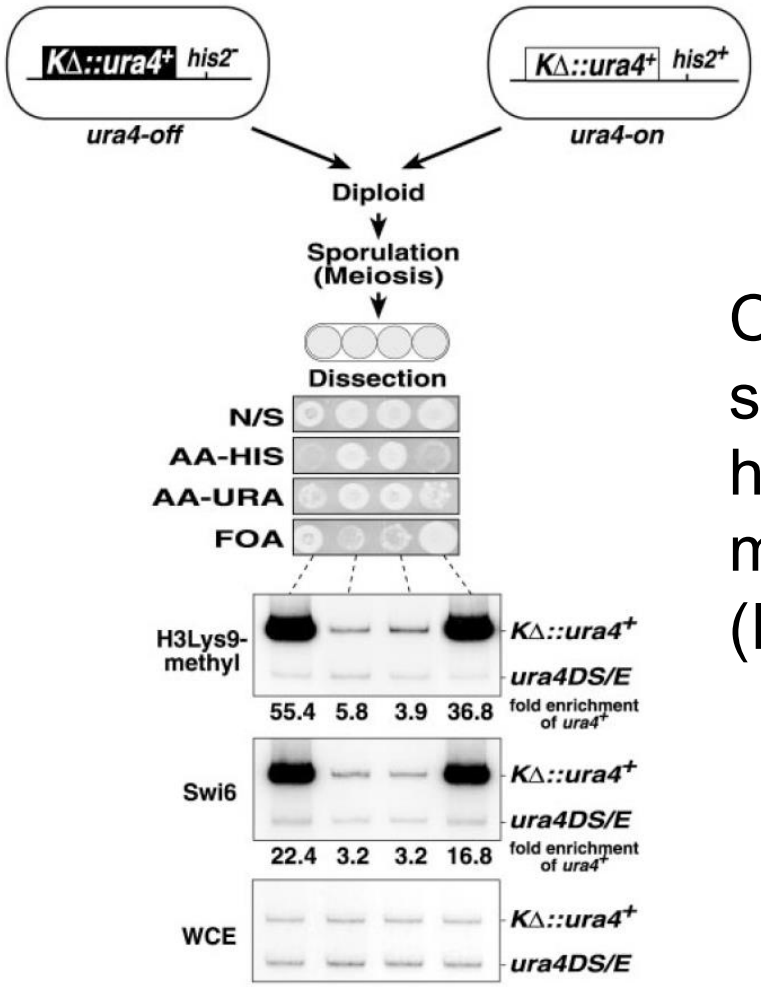


Classic 2:2 segregation of epi-alleles!

Light and Dark Variegated States Are Meiotically Stable and Linked to the mat-Region
A diploid was constructed by crossing the indicated strains, allowed to grow for at least 30 generations, sporulated, and subjected to tetrad analysis. The four spores from each ascus were placed on rich medium in a horizontal row by micromanipulation. After growth for 3 days, they were replicated onto plates containing the indicated medium. All plates were photographed directly after overnight growth at 33° C except for the PMA+ plate, which was incubated for 3 days at 25° C and stained with iodine vapors before photography. Each ascus produced two Ura+, His− light-staining, two Ura−, His+ dark-staining segregants. Patches at the top and bottom indicate the phenotype of the parental SPG27(L) and SPG51(D) strains, respectively, used in the cross.

Epigenetics in Pombe

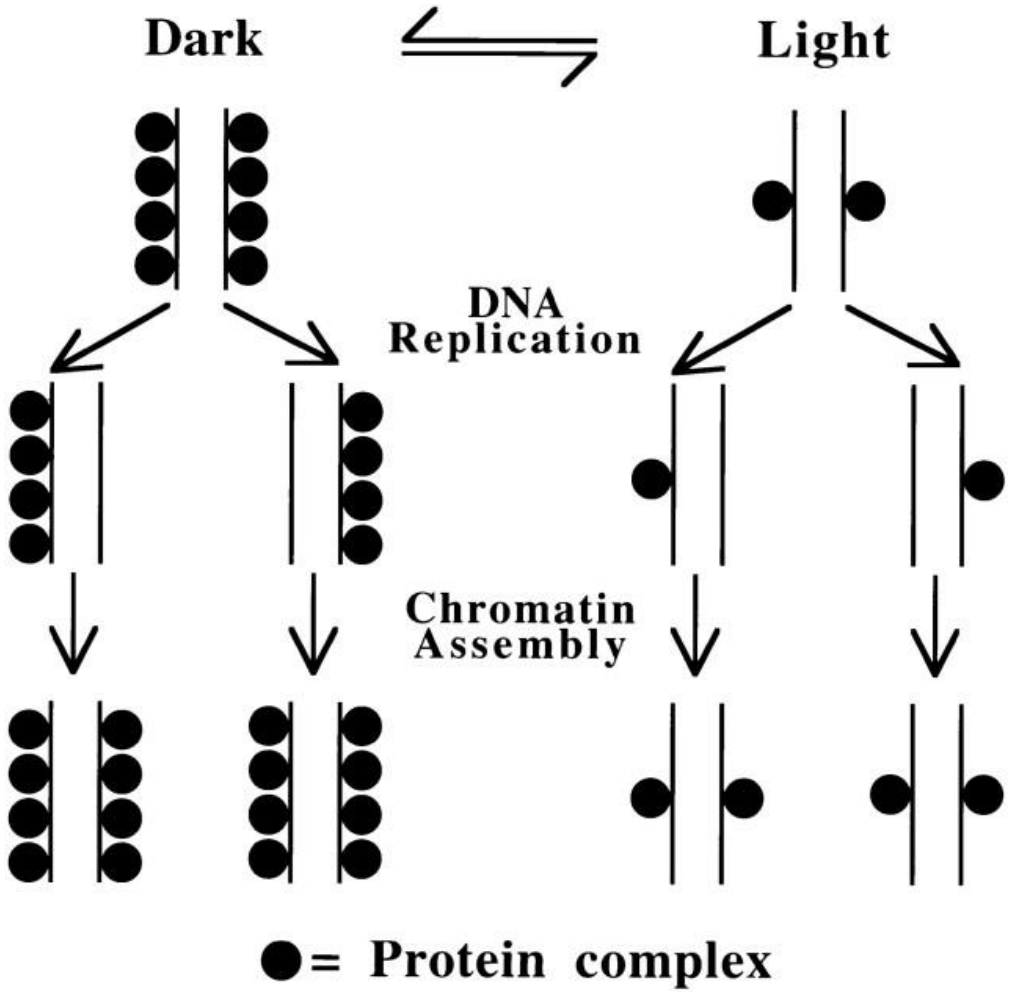
Fig. 2. Differential H3 Lys^9 methylation and Swi6 localization patterns shown by *ura4-off* and *ura4-on* epialleles are stably inherited in cis. Diagram of the cross is shown (**top**). *KΔ::ura4*$^+$ strains carrying *ura4-off* (*his2*$^-$) and *ura4-on* (*his2*$^+$) epialleles differing at the tightly linked *his2* marker were crossed, allowed at least 30 generations of diploid growth, sporulated, and subjected to tetrad analysis. Resulting colonies were replicated onto nonselective medium (N/S), medium lacking uracil (AA-URA), medium lacking histidine (AA-HIS), or medium containing 5-fluoroorotic acid (FOA), which selects for the growth of Ura$^-$ cells. Segregants from individual tetrads were subjected to ChIP analysis with Swi6 and H3 Lys^9-methyl antibodies (**bottom**).



Classic 2:2 segregation of histone methylation (H3K9)

Hall, I. M. et al. Establishment and maintenance of a heterochromatin domain. *Science* **297**, 2232-2237 (2002).

Epigenetics in Pombe



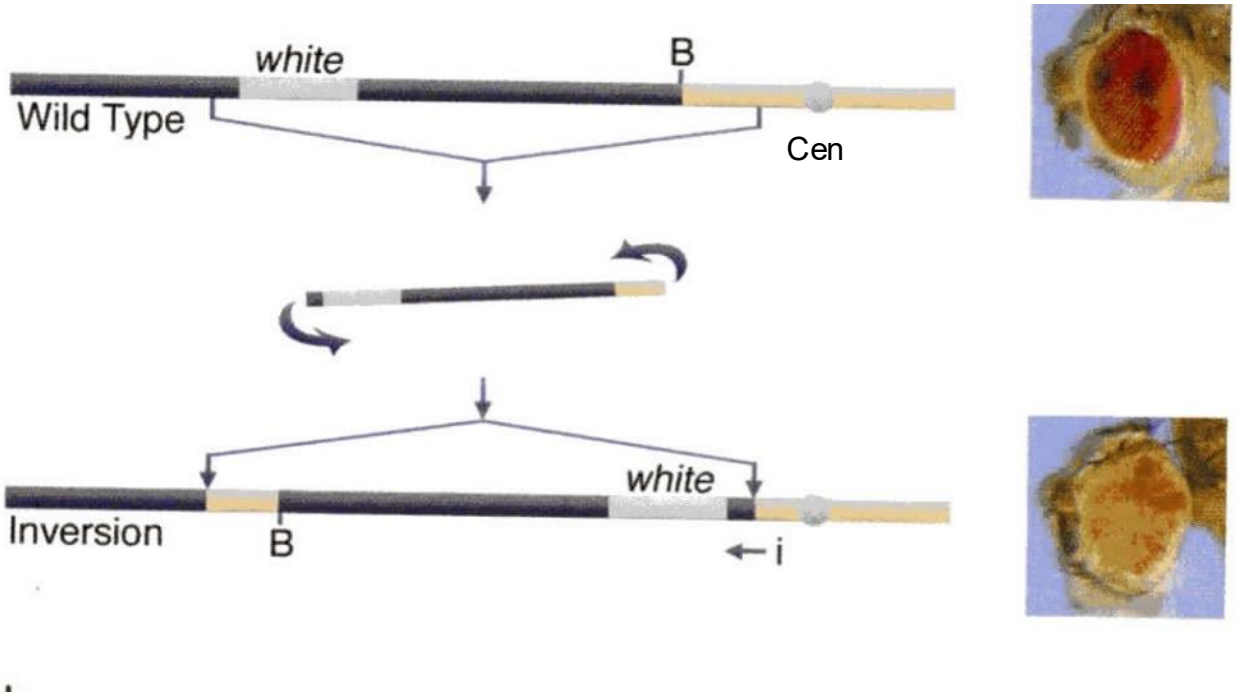
Epigenetics in Flies

1930's Muller zapped flies with X-rays
Identified a mutation called *"white"*

Variegation suggests that “white” gene is not damaged.
Further treatment with X-rays can rescue back to red

Position effect variegation (PEV)

Cytological examination of polytene chromosomes showed a large inversion



Position effect variegation PEV

Screen for second site mutations

Suppressors of PEV (*Suppressor of variegation*) Su(var)
Resulting in loss of silencing (red eye in this case)

Enhancers of PEV (*Enhancer of variegation*) E(var)
Increase in silencing (white eye in this case)

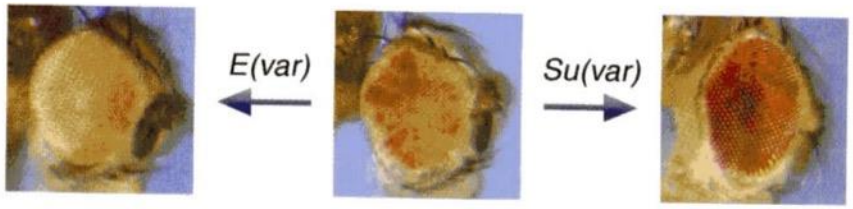


Table 1. Genetically defined *Su(var)* and *E(var)* genes and their molecular functions

Su(var)/ E(var) gene	Cytological position	Molecular function, protein distribution, and phenotypic effects
Suv4-20 [Su(var)]	X; 1B13-14	HKMT, histone H4K20 trimethylation
Su(z)5 [Su(var)]	2L; 21B2	*S*-adenosylmethionine synthetase
chm (chameau) [Su(var)]	2L; 27F3-4	Myst domain HAT; suppresses PEV but enhances Polycomb-group mutations
Su(var)2-5 (HP1)	2L; 28F2-3	heterochromatin protein HP1, binding of di- and trimethyl H3K9; binding of SU(VAR)3-9
Su(var)2-HP2	2R; 51B6	heterochromatin-associated protein, binds HP1
Su(var)2-10	2R; 45A8-9	PIAS protein, negative regulators of JAK/STAT pathway
Su(var)3-64B (HDAC1=RPD3)	3L; 64B12	histone deacetylase HDAC1, deacetylation of H3K9
E(z) [Su(var)]	3L; 67E5	HKMT, H3K27 mono-, di-, and trimethylation; extra gene copy enhances PEV; in null mutation, all euchromatic and heterochromatic H3K27 methylation lost, H3K9 methylation not affected
SuUR [Su(var)]	3L; 68A4	suppresses heterochromatin underreplication; heterochromatin-associated protein
Su(var)3-1 (JIL1)	3L; 68A5-6	antimorphic *JIL1* mutations, carboxy-terminal protein truncations do not affect kinase function; blocking of heterochromatin spreading
Dom (Domina) [Su(var)]	3R; 86B1-2	fork head winged-helix (FKH/WH) protein; heterochromatin-associated
Su(var)3-6	3R; 87B9-10	PP1 protein serine/threonine phosphatase
Su(var)3-7	3R; 87E3	zinc-finger protein, heterochromatin-associated; interacts with HP1 and SU(VAR)3-9
Su(var)3-9	3R; 89E6-8	HKMT, histone H3K9 methylation, heterochromatin-associated, interaction with HP1
mod (modulo) [Su(var)]	3R; 100E3	DNA- and RNA-binding protein, phosphorylated Mod binds rRNA
E(var)3-64E/ Ubp64^{Evar1}	3L; 64E5-6	putative ubiquitin-specific protease (Ubp46)
Trl (Trithorax-like) [E(var)]	3L; 70F4	GAGA factor, binding of repetitive DNA sequences
Mod(mdg4)/ E(var)3-93D	3R; 93D7	transcription regulator, more than 20 protein isoforms produced by *trans*-splicing
E(var)3-93E	3R; 93E9-F1	E2F transcription factor, haplo-enhancer and triplo-suppressor

See Flybase for original citations.

Stopping the spread

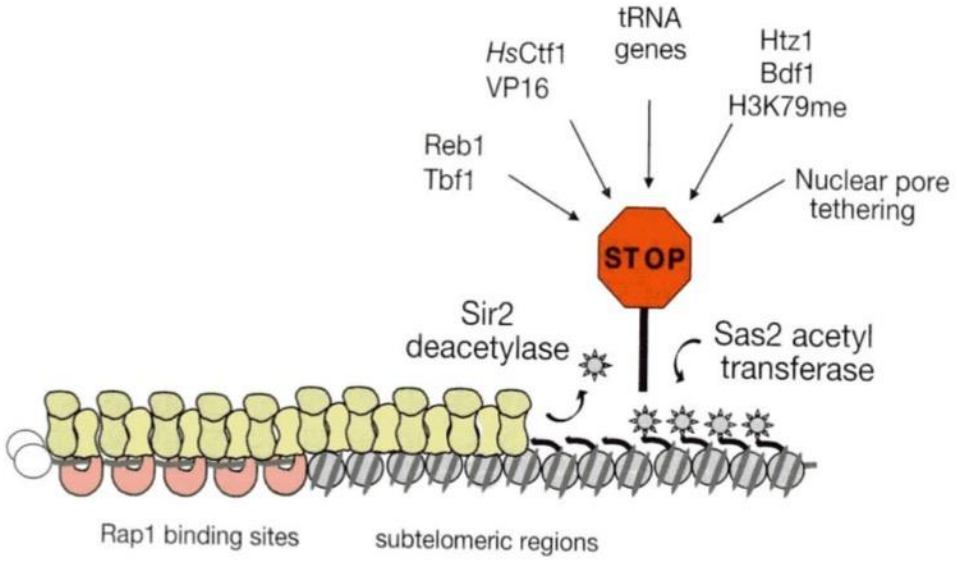


Figure 7. Heterochromatin Boundary Function in Budding Yeast

Spreading of heterochromatin through deacetylation of histone H4 K16 by Sir2 is limited by the competing activity of Sas2 histone acetyltransferase which acetylates H4K16 in adjacent euchromatin, thus preventing Sir3 binding. Methylation of H3K79 in adjacent euchromatin also affects the spreading of heterochromatin. In addition, factors such as Reb1, Tbf1, and mammalian or viral factors Ctf1 or VP16, nuclear pore tethering, and the presence of tRNA genes may also mediate boundary function. It is conceivable that several of these factors function through the recruitment of histone acetyltransferases.

Antagonism and dosage

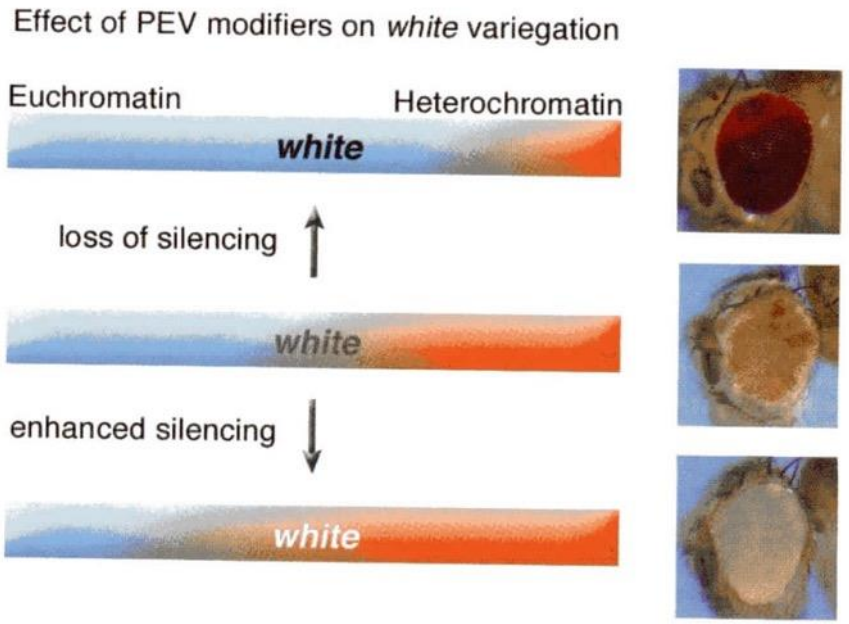


Figure 2. Dosage-dependent Effects of Some Modifiers of PEV

The modifiers of PEV that have a dosage-dependent effect are thought to be structural proteins of heterochromatin. Whereas a variegating phenotype (exhibited here by a *white* reporter gene) is seen when the wild-type modifier gene is present in two copies (*middle chromosome, middle fly eye*), the presence of three wild-type copies of the modifier gene will drive more extensive heterochromatin formation, resulting in an enhancement of reporter gene silencing (*lower chromosome, lower fly eye*). Conversely, the presence of only one wild-type copy of the modifier gene will result in less heterochromatin formation and more expression from the reporter gene (*upper chromosome, upper fly eye*).

How does Su(var)3-9 work?

in a complex…….

Su(var)2-5 (HP1) chromo and chromoshadow domains
Su(var)3-7 (Zn finger protein)
Su(var)3-9 (H3 K9 methyl transferase)

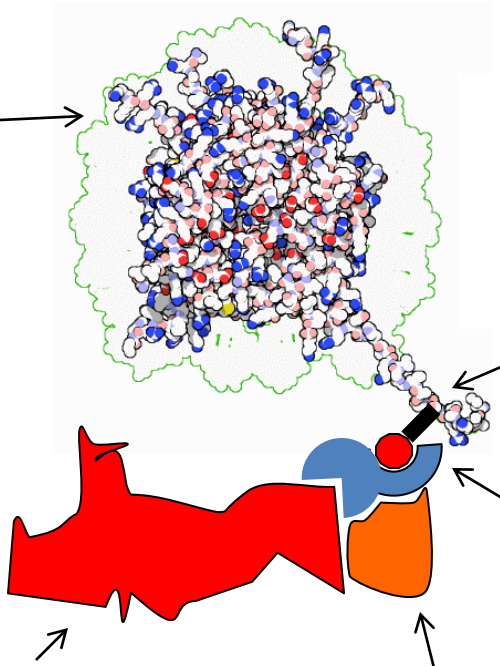
Su(var)3-9 methylates H3-K9 (2me)

The methylation is bound by the chromodomain of HP1. In su(var)3-9 null, binding of HP1 to pericentromeric heterochromatin is impaired.

Other sites of H3 K9 me are not dependent on Su(var)3-9 so HP1 still binds to these regions.

If you deplete HP1 then you lose Su(var)3-9 association with pericentromeric heterochromatin.

How does Su(var)3-9 work?



nucleosome

H3 K9 2/3me

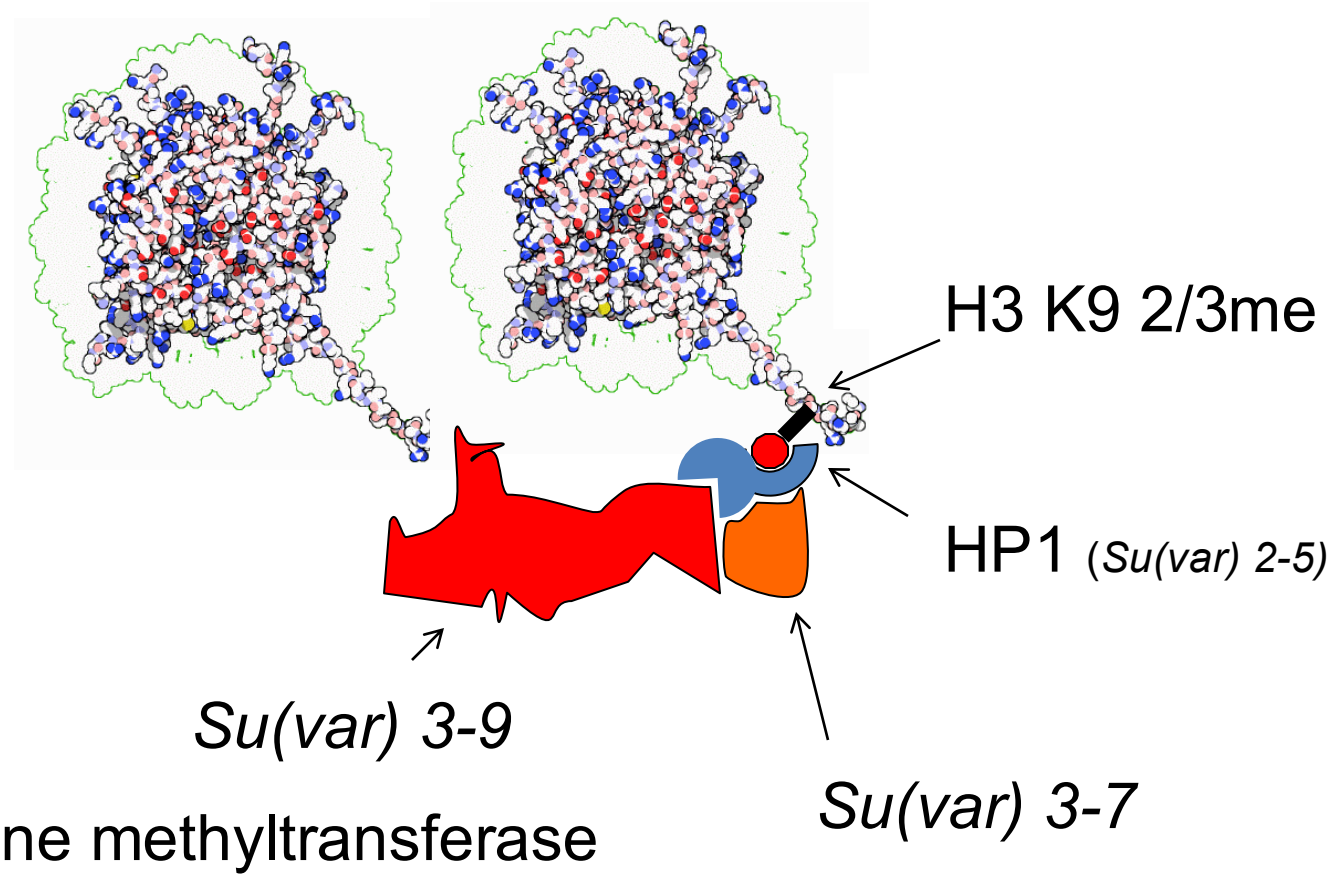
HP1 (*Su(var) 2-5)*

Su(var) 3-9

Histone methyltransferase

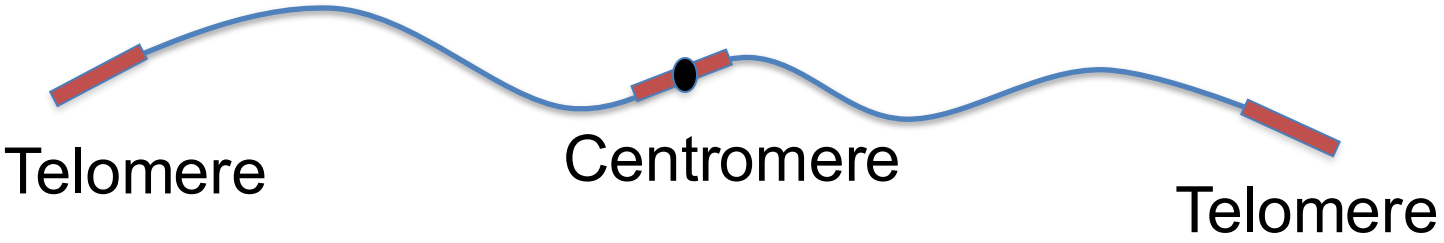
Su(var) 3-7

How does Su(var)3-9 work?



Histone methyltransferase

Heterochromatin domains



Large heterochromatin domains are typically found at centromere and telomeres.

Genes in or near these elements may have variegated expression.

Gene specific epigenetics

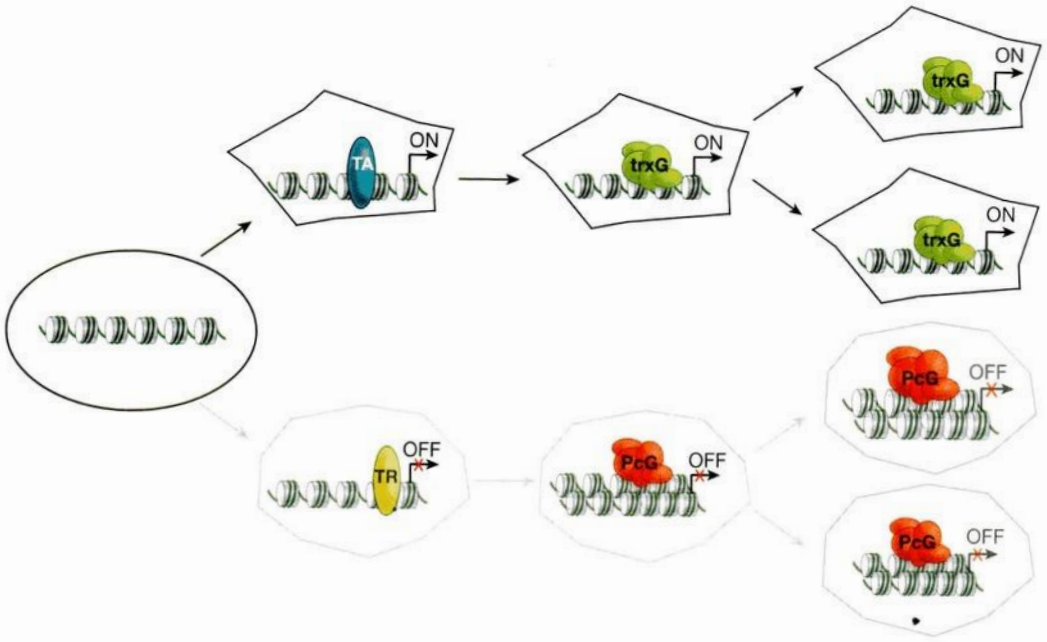


Figure 1. The Concept of Cellular Memory

Schematic illustration highlighting the role of trxG complexes in maintaining heritable states of active gene expression in contrast to heritable silencing by PcG complexes, as defined originally for the *Drosophila* Hox gene cluster.

trithorax

polycomb

Polycomb- *gene specific repression*

Identified a mutations that alter homeotic (HOX) gene expression.

HOX genes are transcription factors that control the identities of body segments

PcG mutants were identified as repressors of HOX genes.

PcG repression is established early in embryogenesis and will remain fixed for the remainder of an individuals lifespan.

Polycomb complexes

PRC1 complex :

contains structural proteins that bind chromatin (not found in *C. elegans* or *Arabidopsis*)

Polycomb	Pc	has a chromodomain and AT hook: binds H3K27 3me H3K9 me3 and RNA!
Posterior Sex Combs	Psc	Zinc finger
dRing	dRing	Ring finger: Ubiquitinates H2A K119

PRC2 complex:

Enhancer of Zeste -	(E(Z))	H3 K27 and/or H3 K9 methyltransferase
Extra sex combs -	ESC	binds methylated histone tails
Histone binding protein -	p55	
Suppressor of Zeste -	(Su(Z)12)	possible txn factor (binds RNA!)

YY1, PHO and potentially other DNA binding proteins…….

Polycomb

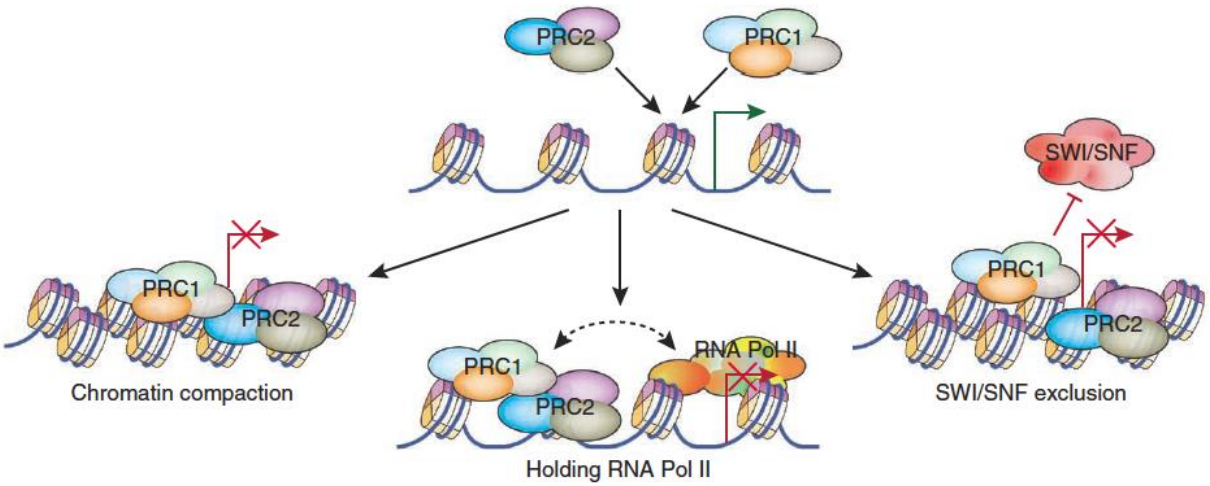
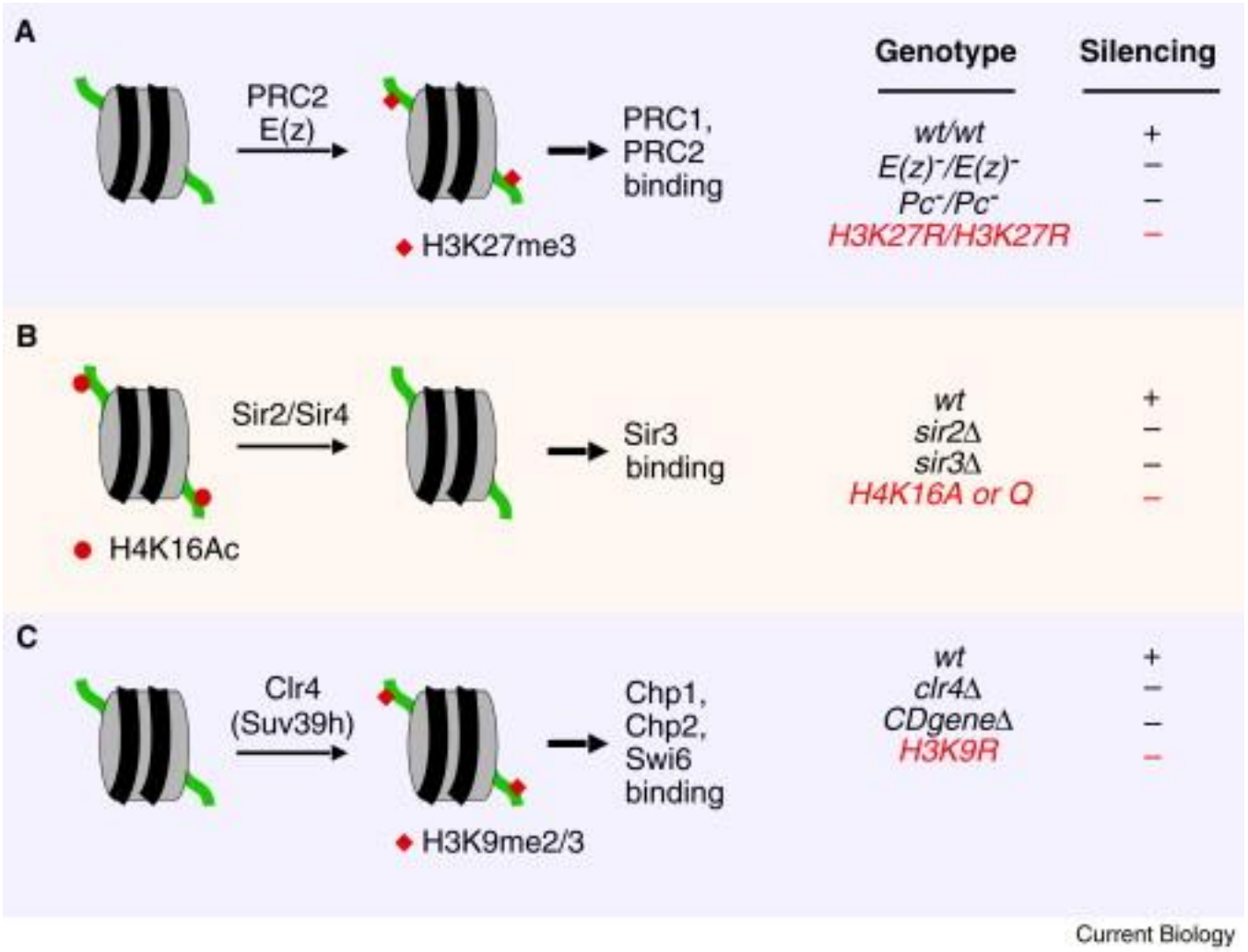


Figure 3 Polycomb-mediated gene repression is a multilayer process. Polycomb-complex binding contributes to gene silencing in numerous ways: it induces chromatin compaction, as observed both *in vitro* and *in vivo* (bottom left), and it interferes with transcription by preventing RNA Pol II activity (bottom middle) or SWI-SNF accessibility to promoters (bottom right).

Di Croce, L. & Helin, K. Transcriptional regulation by Polycomb group proteins. *Nat Struct Mol Biol* **20**, 1147-1155 (2013).

Histone modifications are important for silencing



Trithorax proteins

Trithorax proteins antagonize polycomb proteins

Trithorax proteins

Table 1. Biochemical functions of trxG proteins

Known function	Organism			Complexed with non-trxG proteins?
	Drosophila	human	yeast	
ATP-dependent chromatin remodeling	BRM	BRG1/HBRM	Swi2/Snf2, Sth1	yes (5–10)[a]
	OSA	BAF250	Swi1/Adr6	yes (5–10)
	MOR	BAF155, BAF170	Swi3, Rsc8	yes (5–10)
	SNR1	hSNF5/INI1	Snf5, Sfh1	yes (5–10)
	Kismet (KIS)	CHD7	–	not known
Histone methyltransferases	Trithorax (TRX)	MLL1, MLL2, hSET1	Set1	yes (5–20)
	Absent, small or homeotic 1(ASH1)	hASH1	–	not known
Mediator subunits	Kohtalo (KTO)	TRAP230	Srb8	yes (13–24)
	Skuld (SKD)	TRAP240	Srb9	yes (13–24)
Transcription factor	Trithorax-like (TRL)	BTBD14B	–	no
Growth factor receptor	Breathless (BTL)	FGFR3	–	not known
Other	Sallimus (SLS)	Titin		not known
	ASH2	hASH2L[b]	Bre2	yes (5–20)

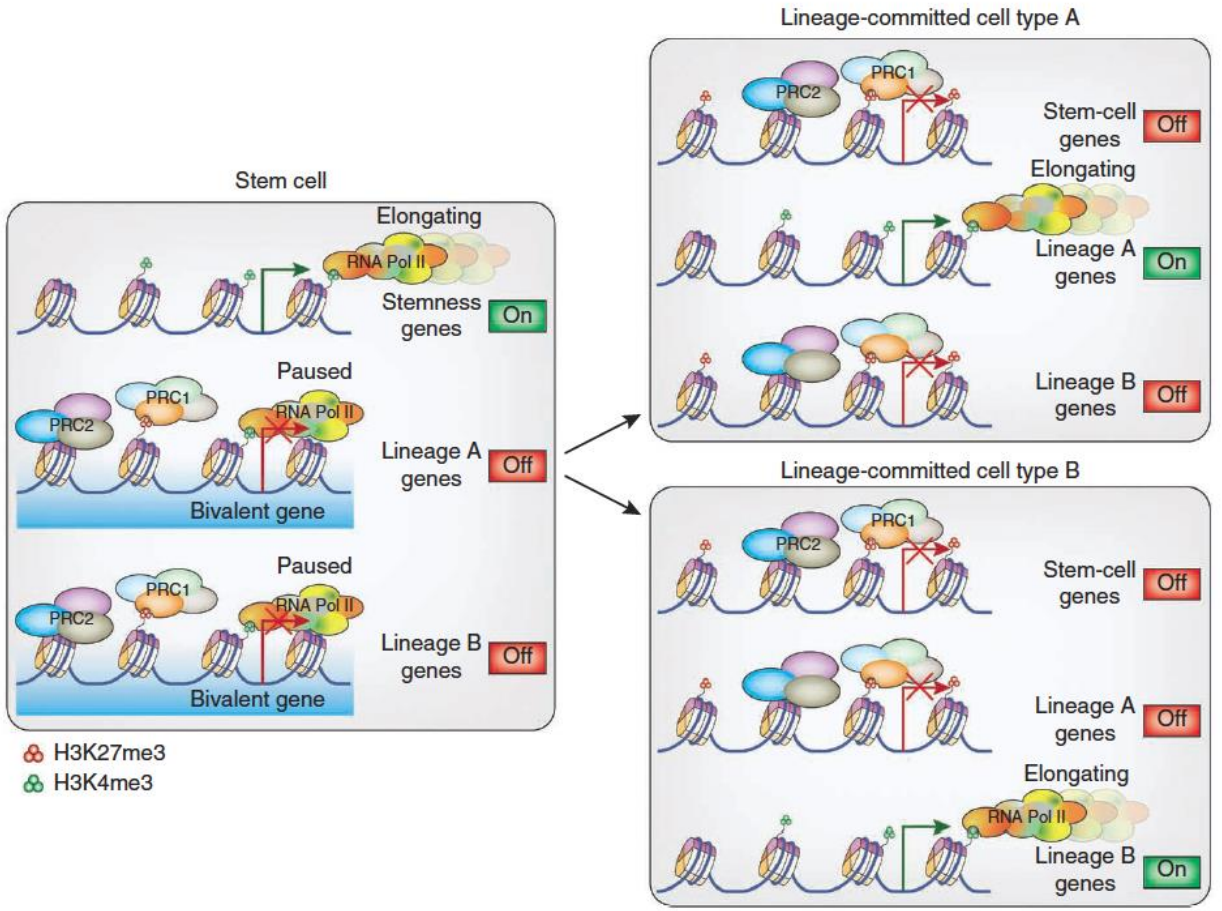
[a]BRM, OSA, MOR, and SNR1 can all be found in stable association with each other in a single complex.

[b]Relatively low sequence similarity to ASH2.

Associated with active transcription

Polycomb: Bivalent domains

Figure 2 Role of PcG proteins in determination of stem-cell fate. Stem cells and progenitor cells have the potential to differentiate into multiple lineages (here simplified as cell type A or B). Before differentiation stimuli act, genes that determine 'stemness' are transcribed by the RNA Pol II machinery, whereas bivalent genes implicated in lineage specification are silenced, in part also by PRCs. Of note, bivalent genes are also found in other cell types of restricted potency. During differentiation, PRCs are displaced from a specific set of lineage-specific genes, depending on the differentiation stimulus received. A switch in the composition of the PRCs then allows them to target and silence the stemness genes. In a differentiated cell, the bivalent domains are resolved into activated or repressed promoters, marked by RNA Pol II or PRCs, respectively.



Di Croce, L. & Helin, K. Transcriptional regulation by Polycomb group proteins. *Nat Struct Mol Biol* **20**, 1147-1155 (2013).

Epigenetics

1) The study of the changes in gene expression which occur in organisms with differentiated cells, and the mitotic *inheritance* of given patterns of gene expression.

2) Nuclear *inheritance* which is not based on changes in DNA sequence.

Holliday definition

epi- (Greek: over; above) -genetics

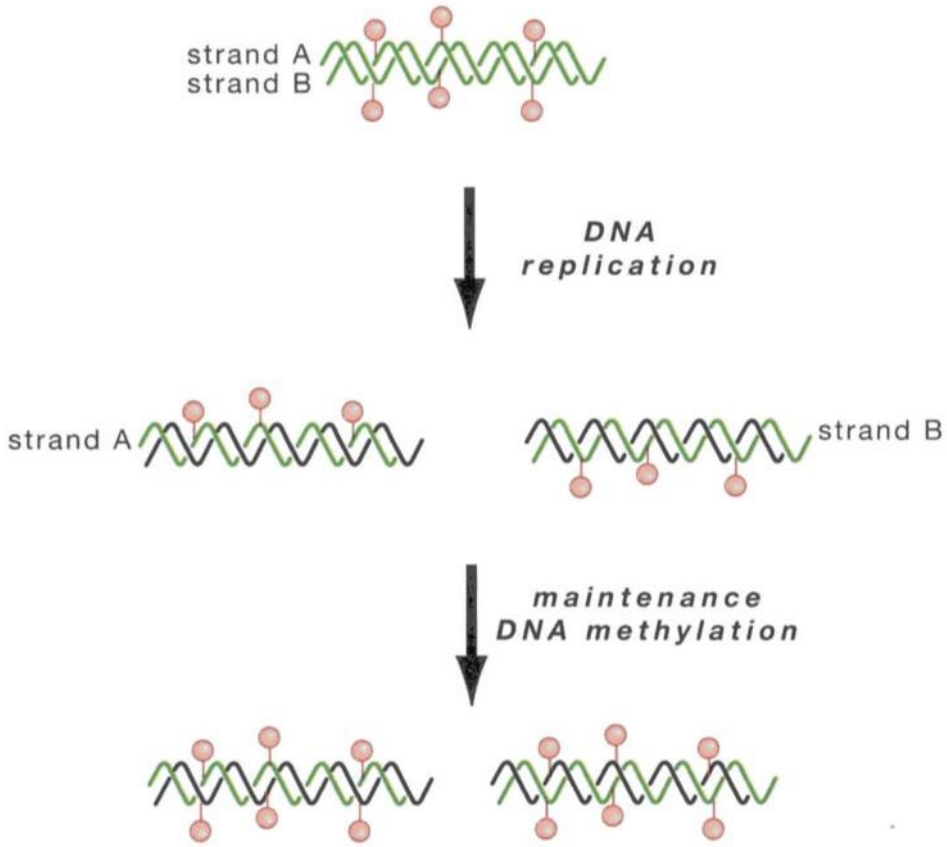
What makes something epigenetic?

How do you maintain a chromatin state through generations?

1, DNA methylation
2, Trans acting factors: ncRNA, DNA binding proteins
3, Inheritance of histones

What makes something epigenetic?

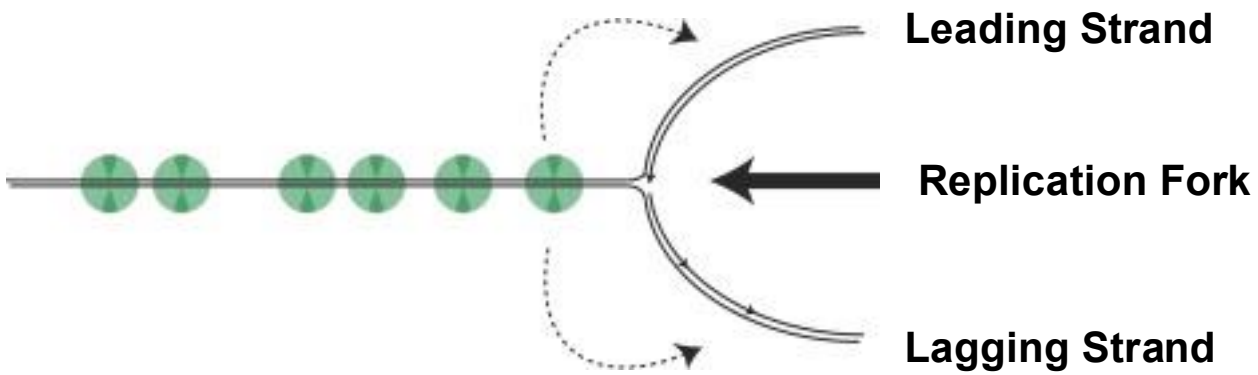
DNA methylation



Replicating Chromatin

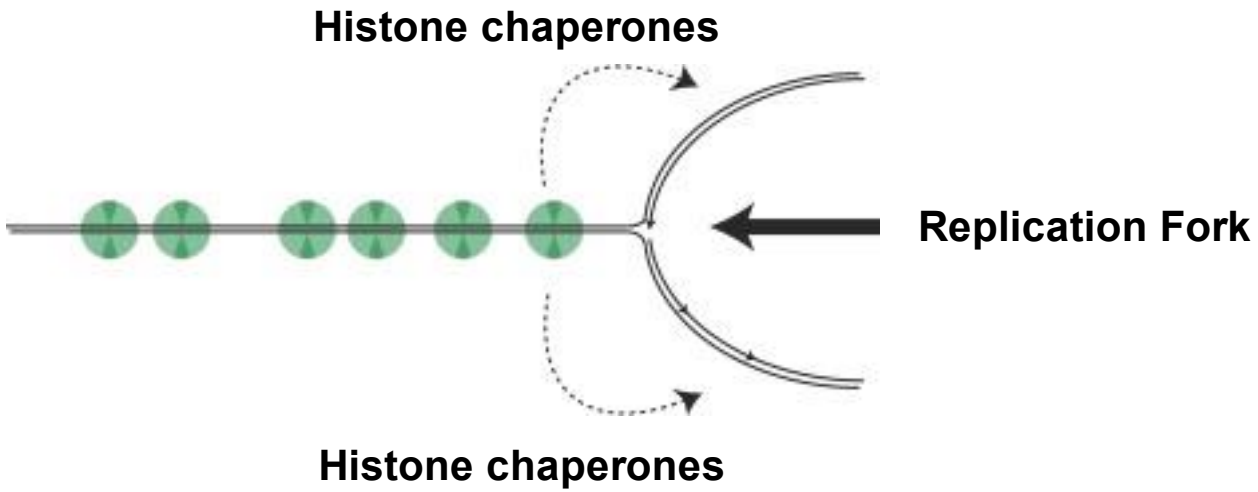
How is chromatin replicated?

Replicating Chromatin



A model

Replicating Chromatin



A model

Replicating Chromatin

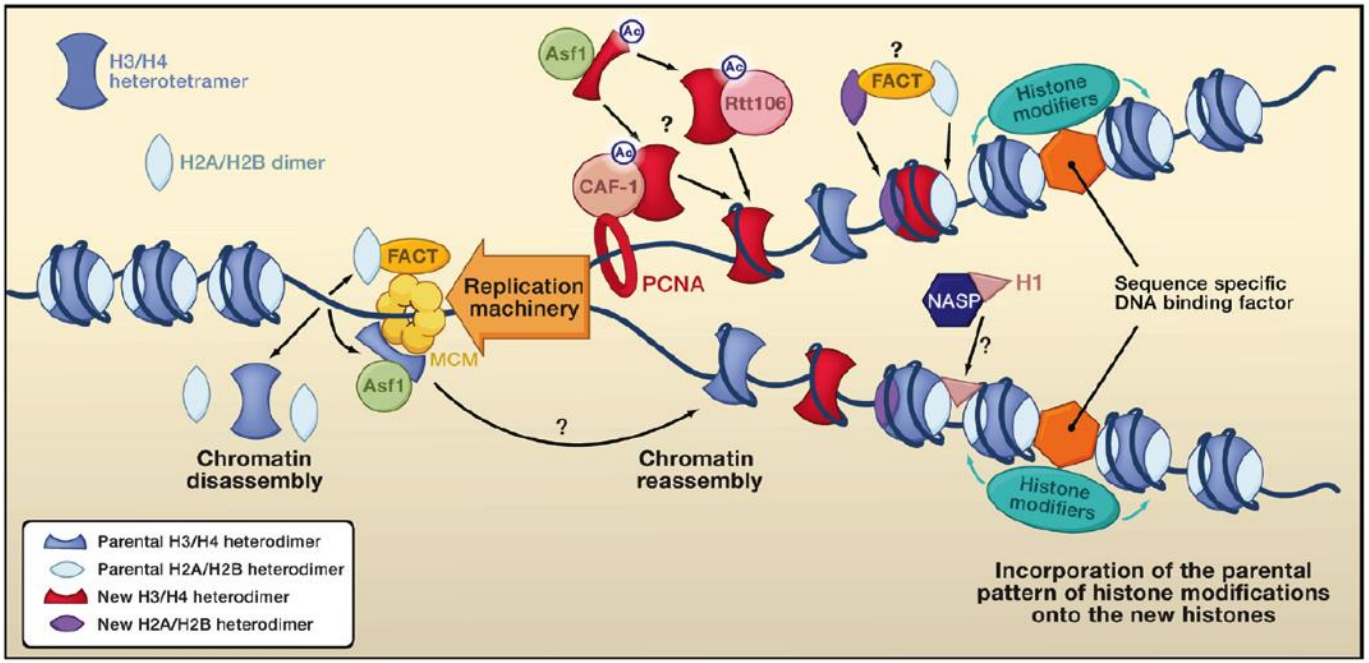
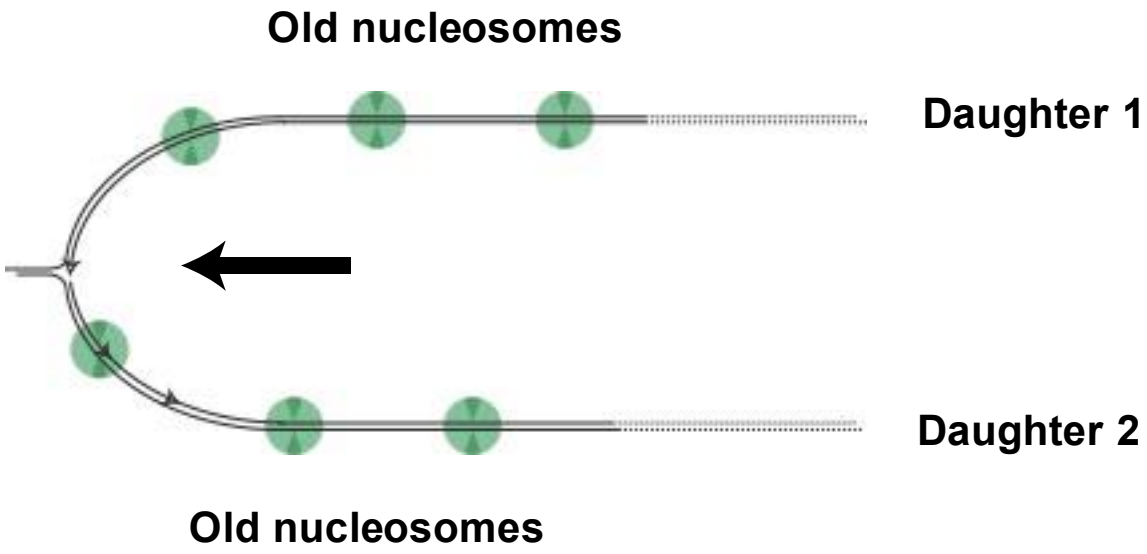


Figure 2. Replication-Coupled Chromatin Assembly and Disassembly
The histone chaperones, but not the ATP-dependent chromatin remodelers involved in these processes, are shown. Once the new histones have been post-translationally modified to adopt the pattern carried by the parental histones, they are considered parental histones and are colored accordingly. Question marks indicate steps that are somewhat speculative.

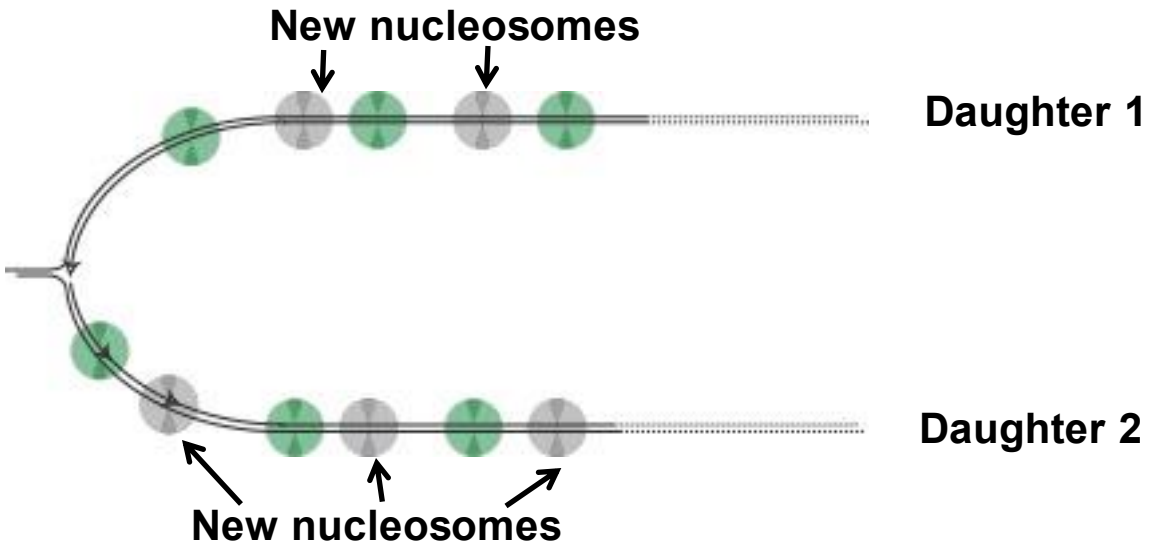
Ransom, M., Dennehey, B. K. & Tyler, J. K. Chaperoning histones during DNA replication and repair. *Cell* **140**, 183-195 (2010).

Replicating Chromatin



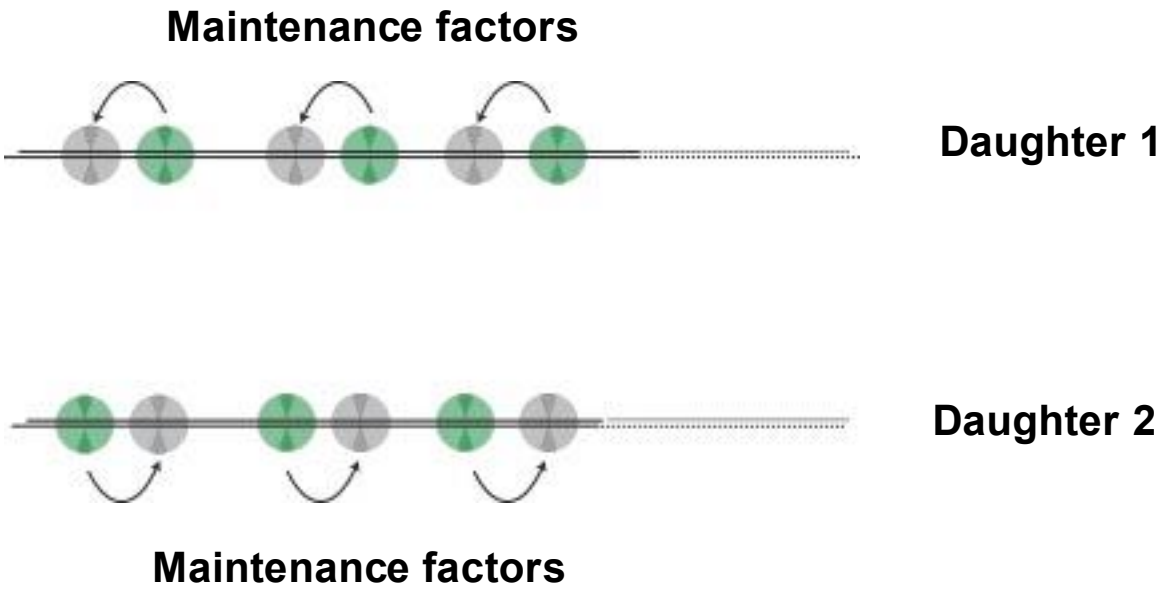
A model

Replicating Chromatin



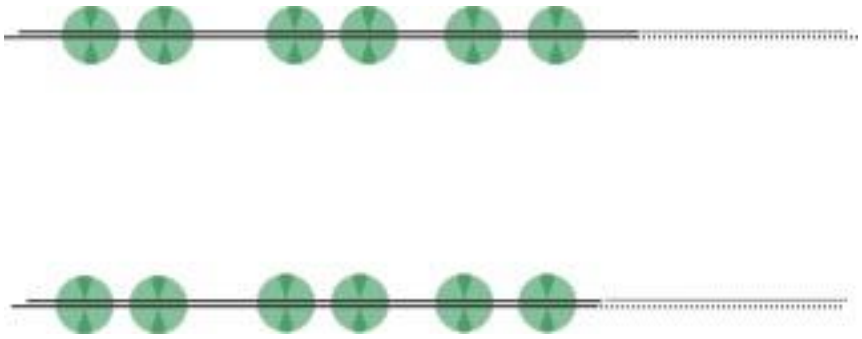
A model

Replicating Chromatin



A model

Replicating Chromatin



A model