Structure and functions of chromosomes and chromatin

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SRB, GAL, SNF, SWI, RPB, RSC, TRAC...
SOS!

Chromatin

For *Chroma* (ancient Greek): color

Chromosome

1889, from German Chromosom, coined 1888 by German anatomist Wilhelm von Waldeyer-Hartz (1836-1921), from Latinized form of Greek khroma "color" (see chroma) + soma "body" (see somato-). So called because the structures contain a substance that stains readily with basic dyes (Hoechst). Online Etymology Dictionary

Chromatin (modern definition):

The combination of DNA and proteins that make up the contents of the nucleus of a cell

- Compacts DNA so that it fits in the nucleus (10-15 μM in diameter)
  (human DNA is 2 meter-long!)

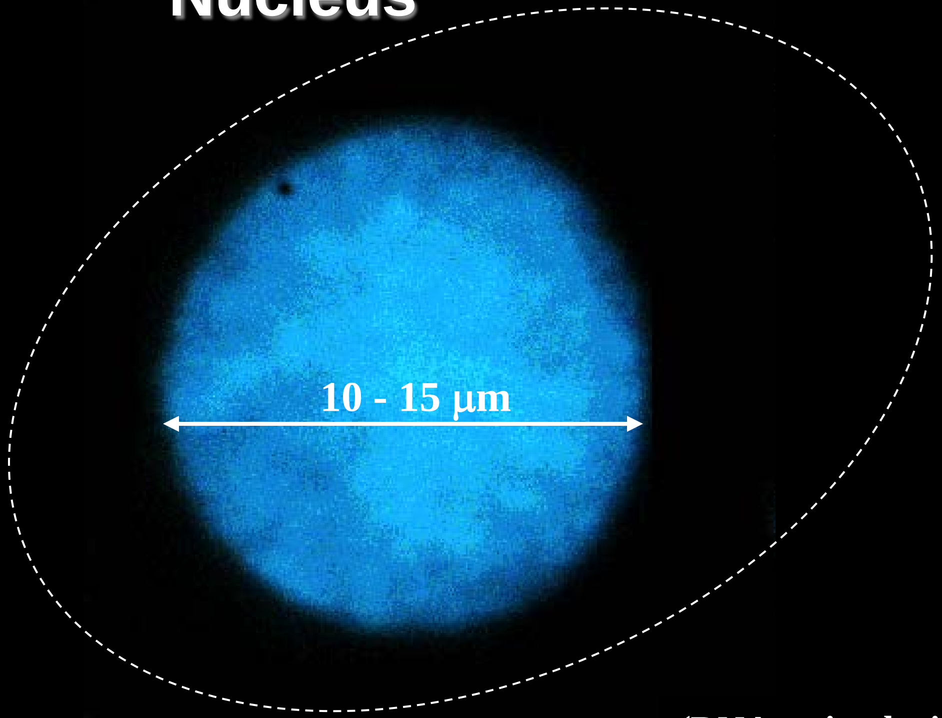
- Allows for the segregation of chromosomes during mitosis

- Protects DNA from damage

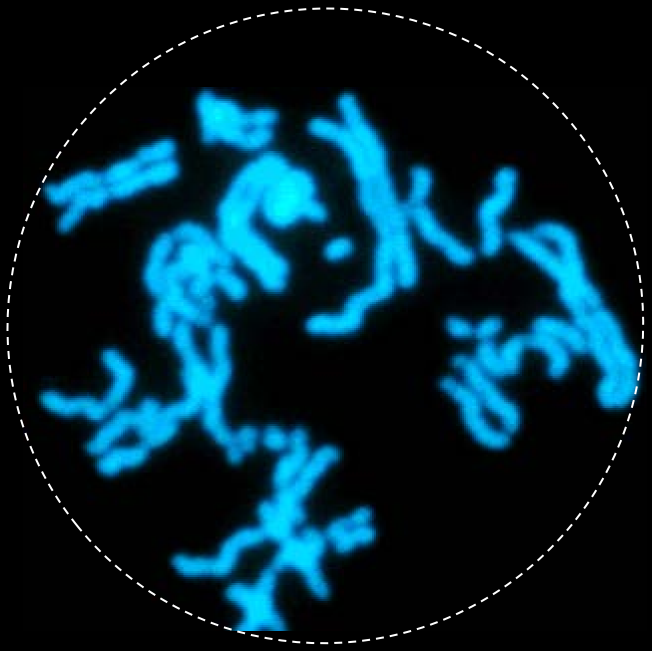
- Controls DNA replication during S phase

- Controls gene expression

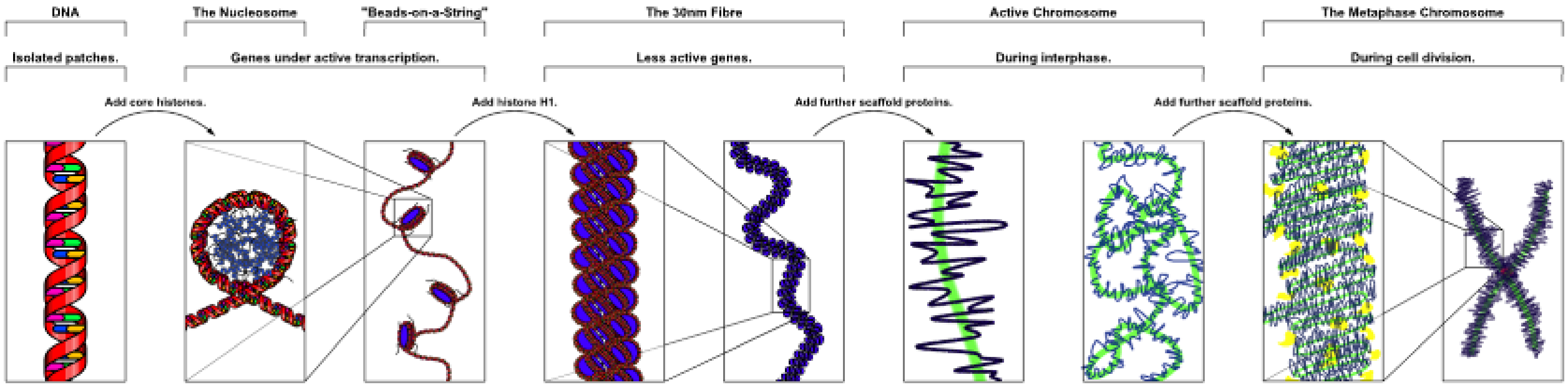
Nucleus

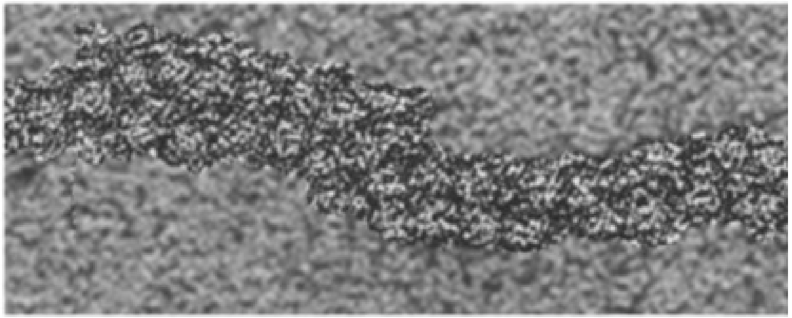


Mitotic chromosomes

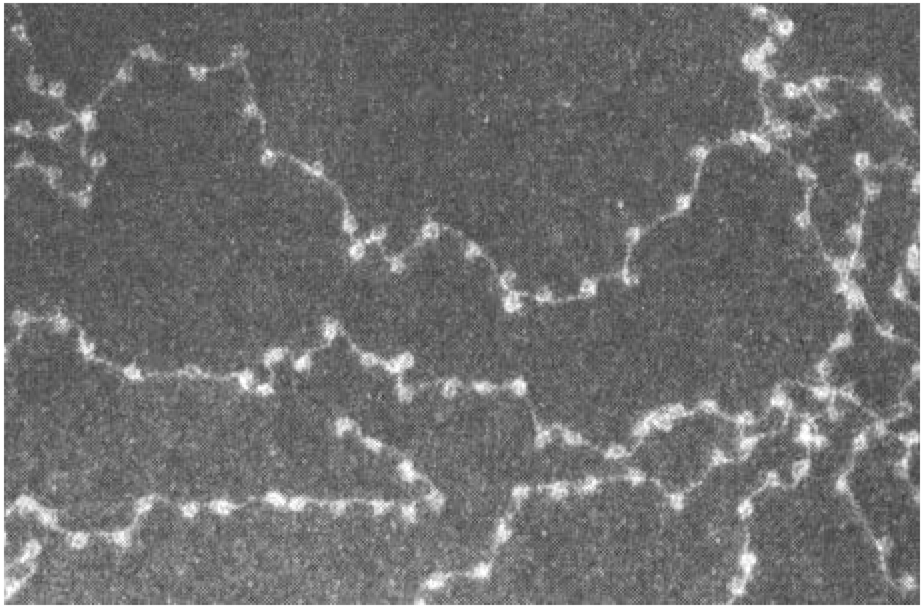


(DNA stained with
Hoechst 33258)

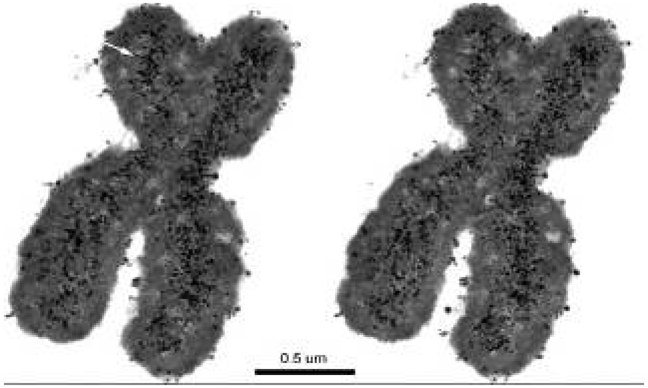




30nm fibre



Beads-on-a-string



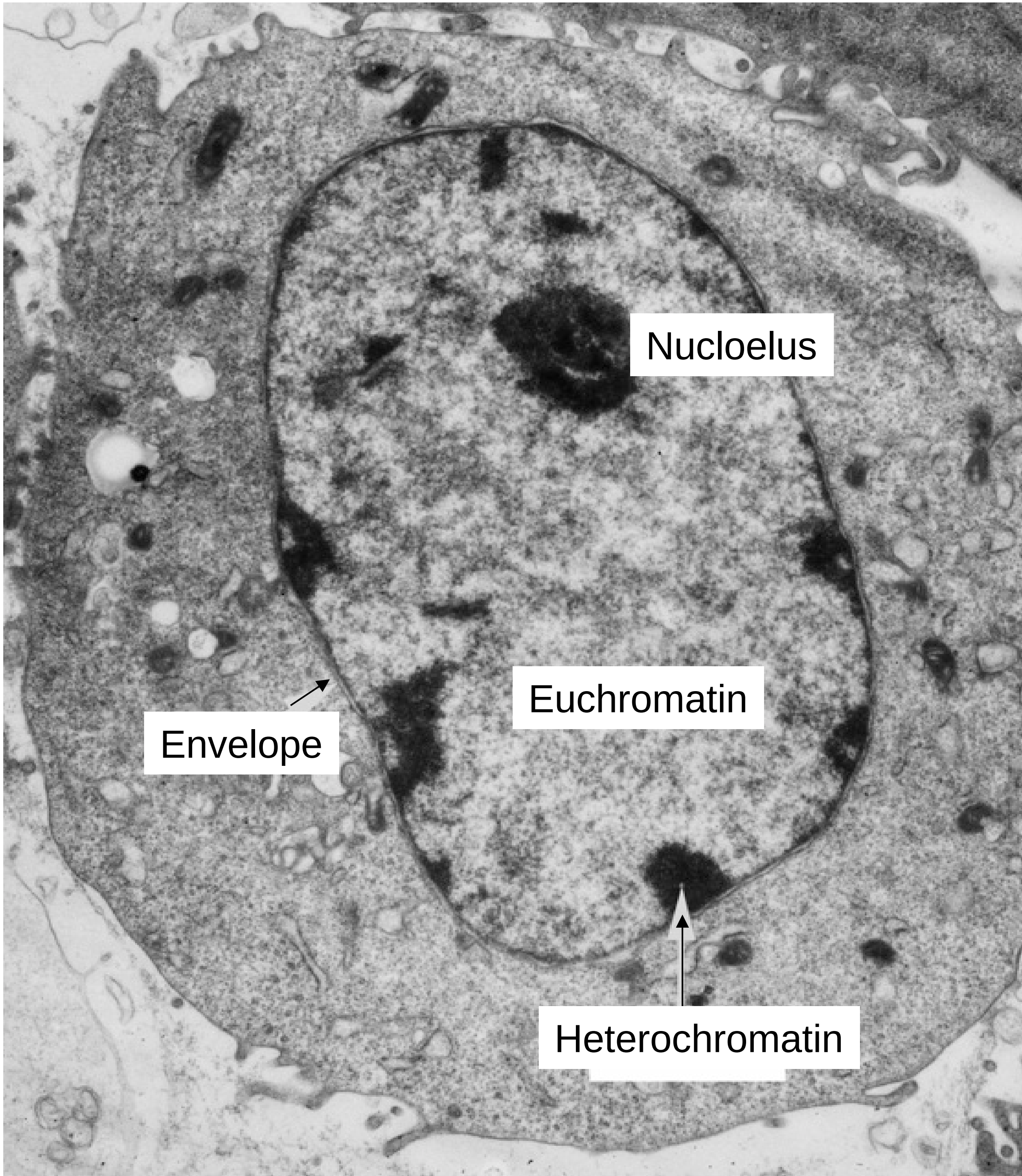
Metaphase chromosome

“*In 1928 the German botanist Emil Heitz visualised in moss nuclei chromosomal regions that do not undergo postmitotic decondensation [Heitz E (1928) Das Heterochromatin der Moose. Jahrb Wiss Botanik 69: 762–818.].*

He termed these parts of the chromosomes *__heterochromatin__*, *whereas fractions of the chromosome that decondense and spread out diffusely in the interphase nucleus are referred to as* *__euchromatin__*

Heitz proposed that heterochromatin reflects a functionally inactive state of the genome, and we now know that DNA in heterochromatic regions is less accessible to nucleases and less susceptible to recombination events.”

quote from Straub T (2003) PLoS Biol 1(1): e14.



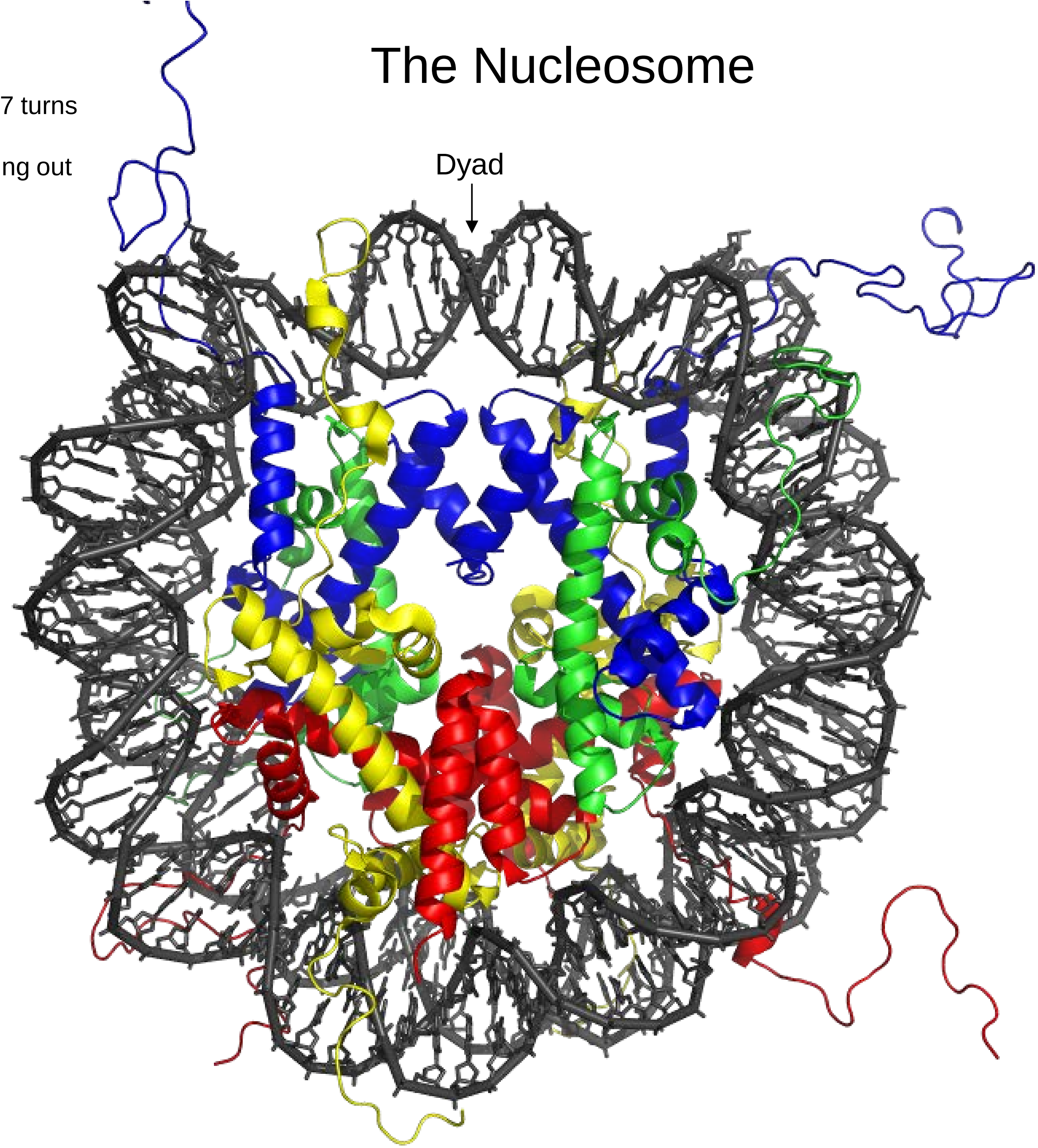
Two types of heterochromatin

Constitutive heterochromatin	**Facultative heterochromatin**
Sequences associated with heterochromatin in all cell types	Sequences associated with heterochromatin in some but not all cell types (dynamic heterochromatin)
Often repetitive sequences	Contains genes involved in differentiation and development

The basic unit of chromatin

The Nucleosome

- 2 copies of each core histone
- 146pb of DNA wrapped around 1.67 turns (left-handed superhelical)
- N-terminal tails of histones protruding out
- DNA bent at several places

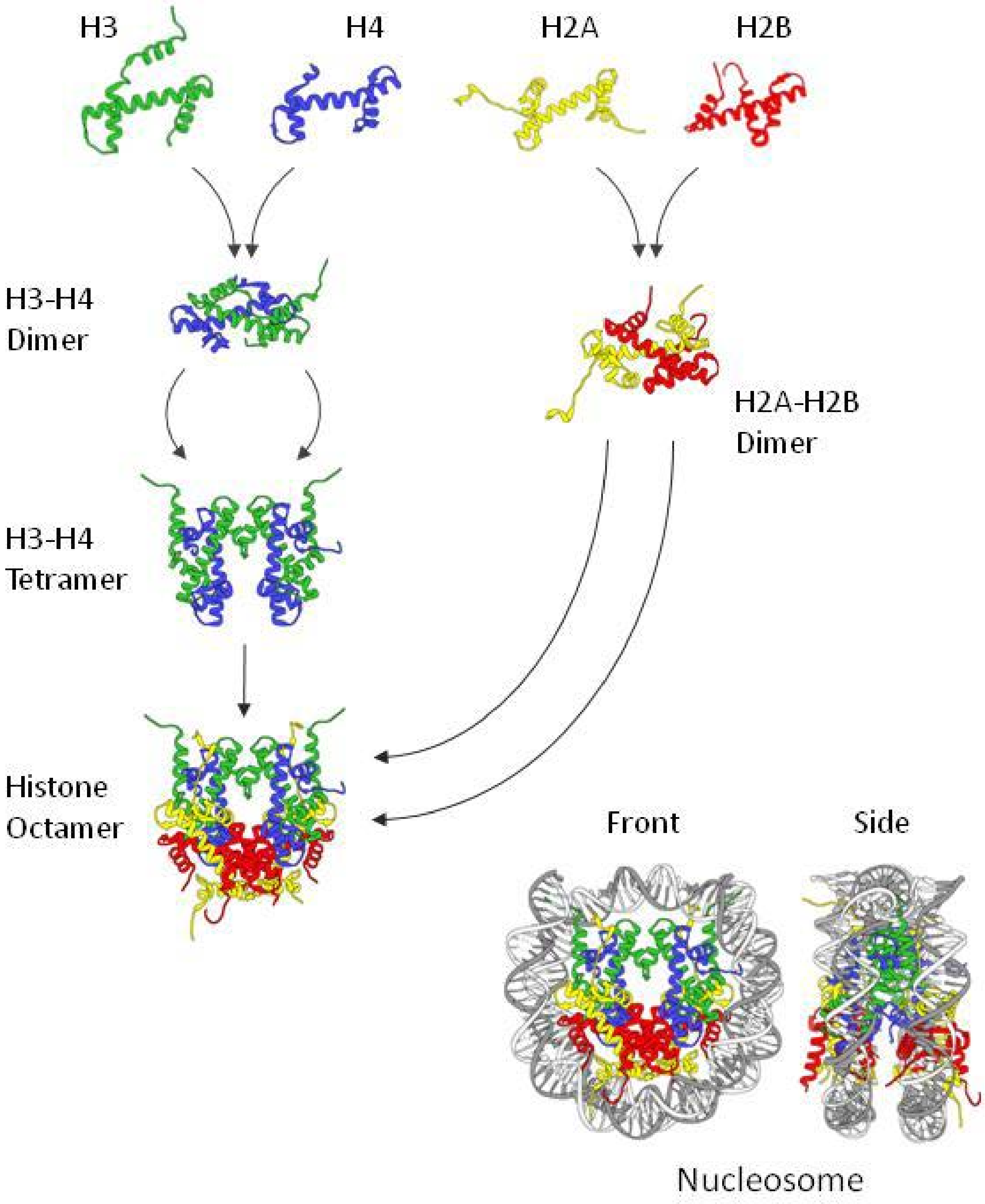


H3

H4

H2A

H2B


H3
H4
H2A
H2B
H3-H4 Dimer
H2A-H2B Dimer
H3-H4 Tetramer
Histone Octamer
Front
Side
Nucleosome

Histones are highly conserved, small, basic proteins

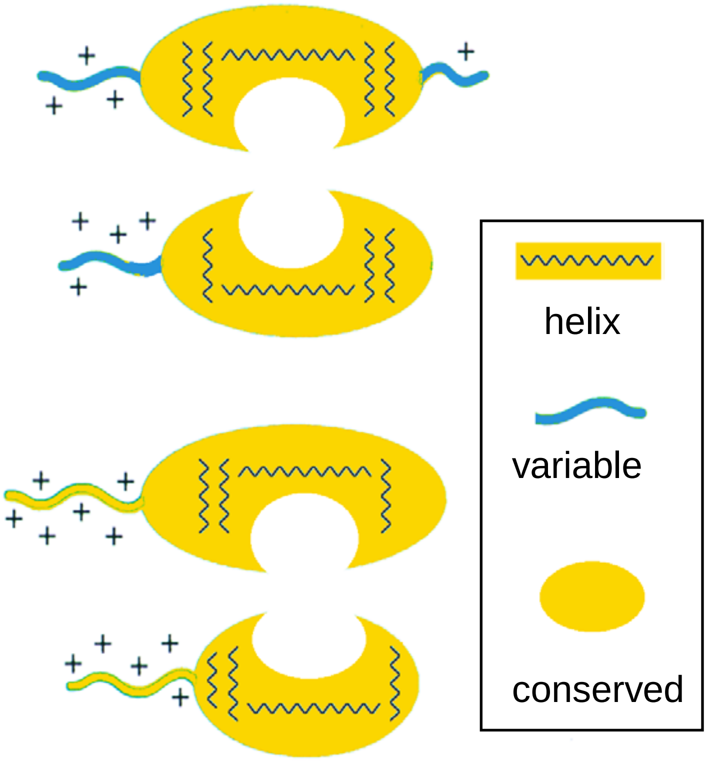
Core histones

H2A

H2B

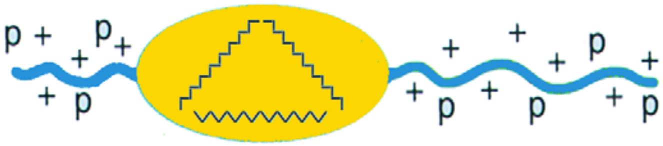
H3

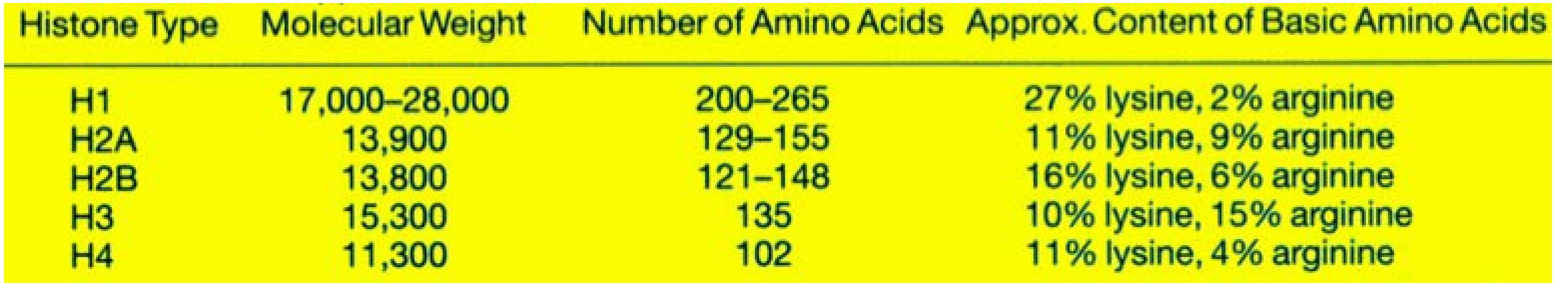
H4



Linker histone

H1

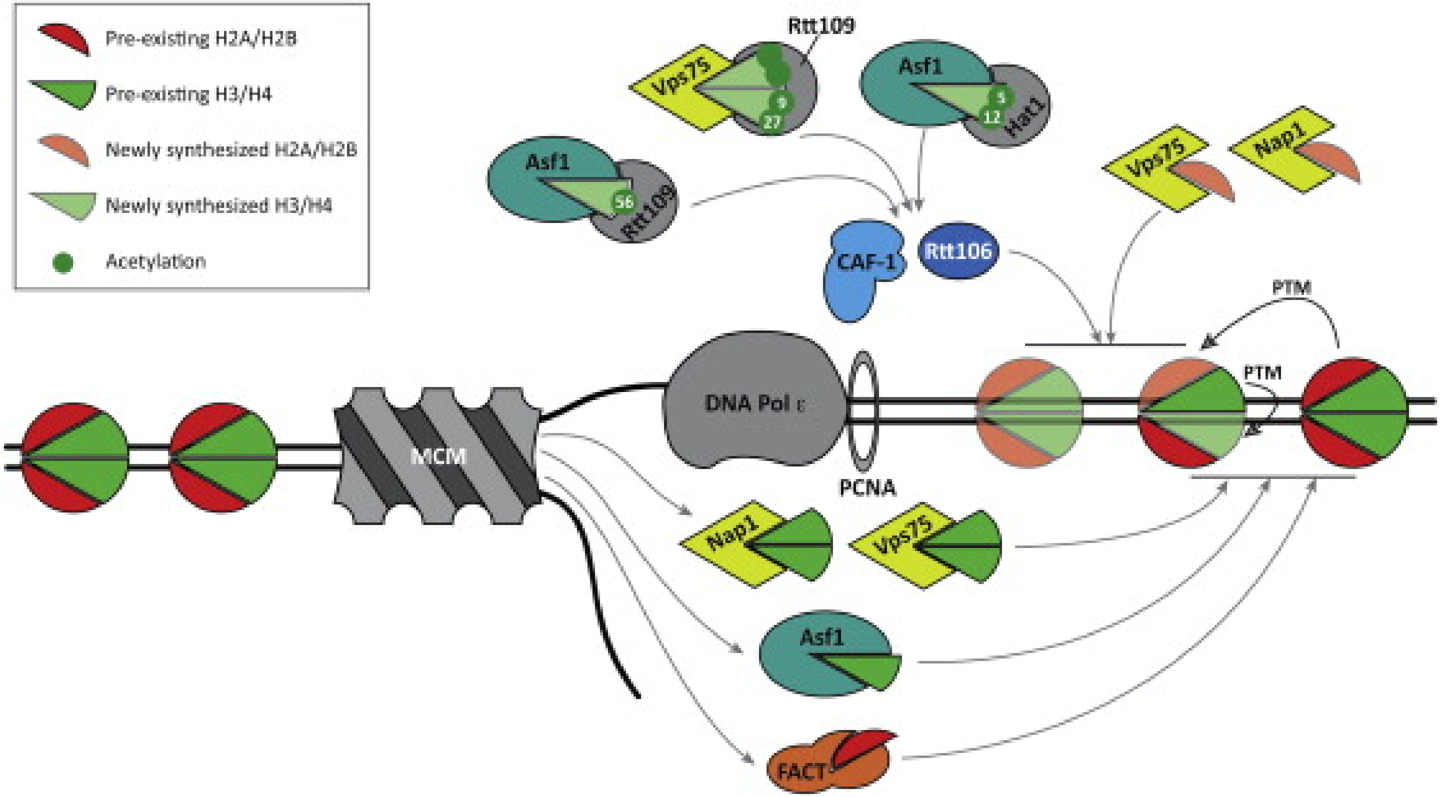




Histone Type	Molecular Weight	Number of Amino Acids	Approx. Content of Basic Amino Acids
H1	17,000–28,000	200–265	27% lysine, 2% arginine
H2A	13,900	129–155	11% lysine, 9% arginine
H2B	13,800	121–148	16% lysine, 6% arginine
H3	15,300	135	10% lysine, 15% arginine
H4	11,300	102	11% lysine, 4% arginine

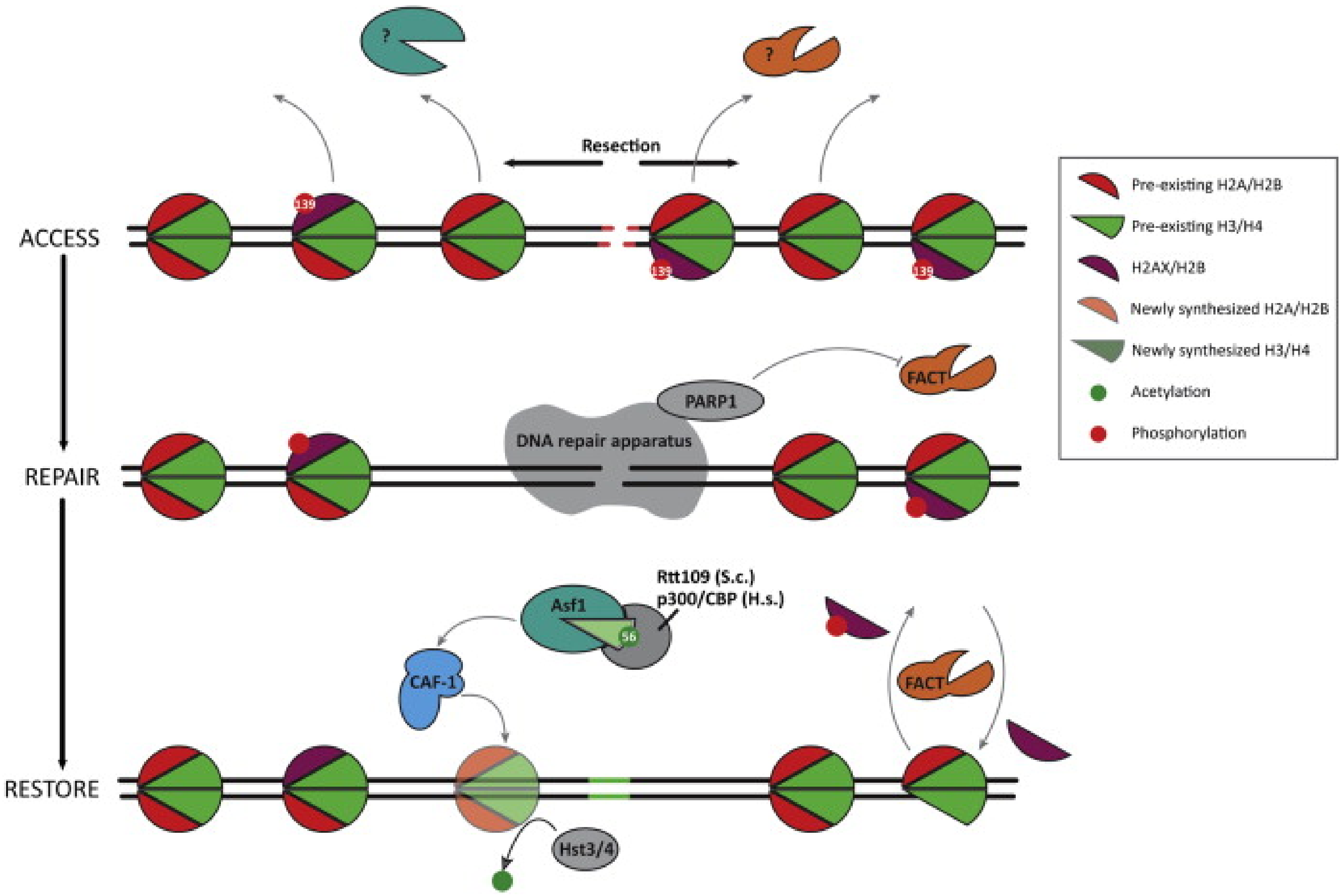
Chromatin assembly

Replication-dependent chromatin assembly requires several histone chaperones



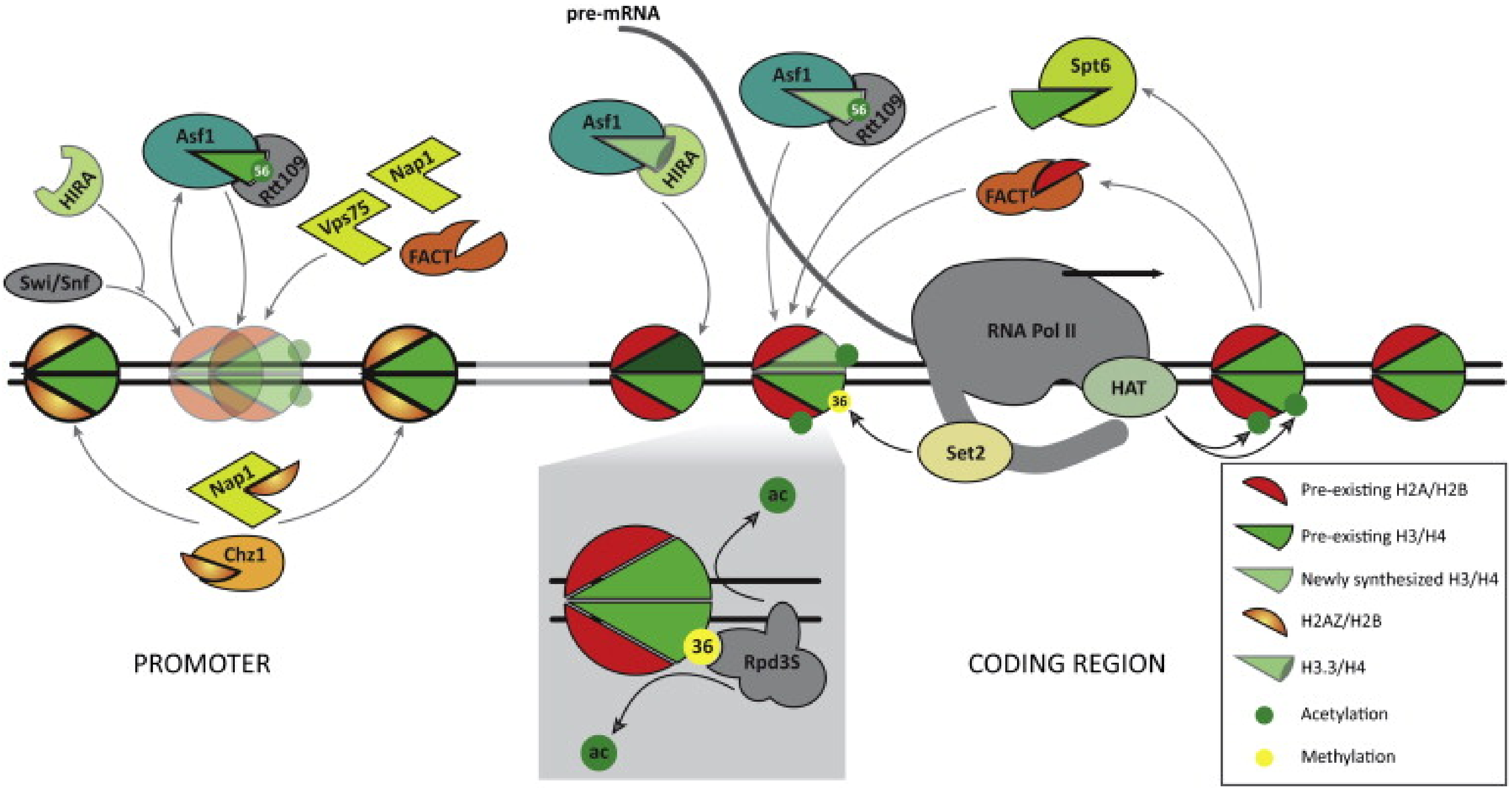
Mol Cell (2011) 41(5):502-14

Several histone chaperones are also required to reassemble chromatin after DNA repair



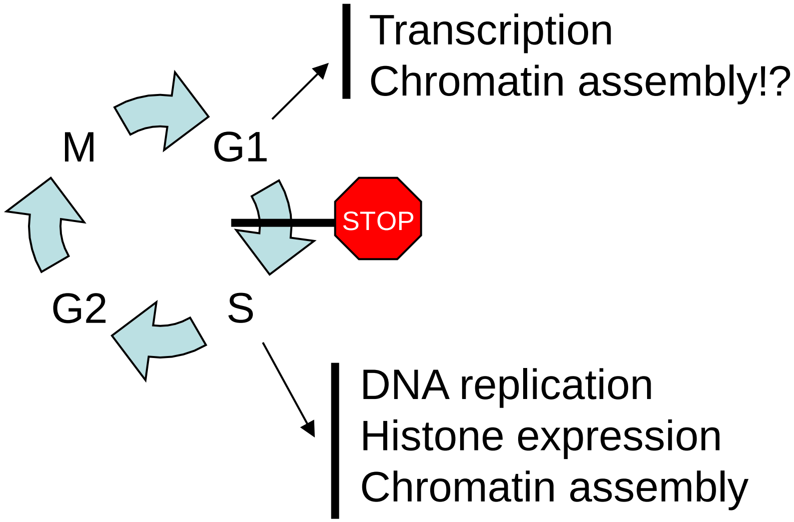
Mol Cell (2011) 41(5):502-14

Transcription-dependent and –independent pathways mediate nucleosome assembly outside of S phase



Mol Cell (2011) 41(5):502-14

A system to measure histone exchange in vivo

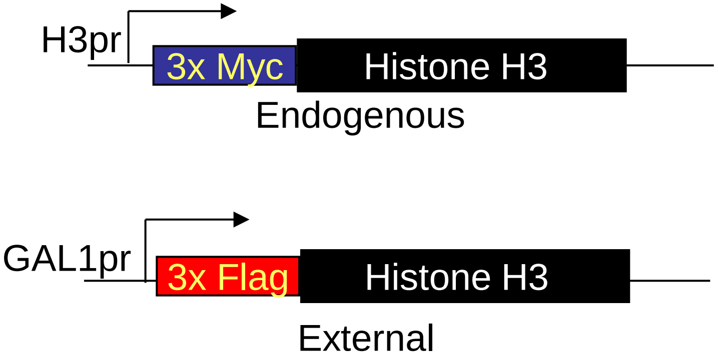


Transcription
Chromatin assembly!?

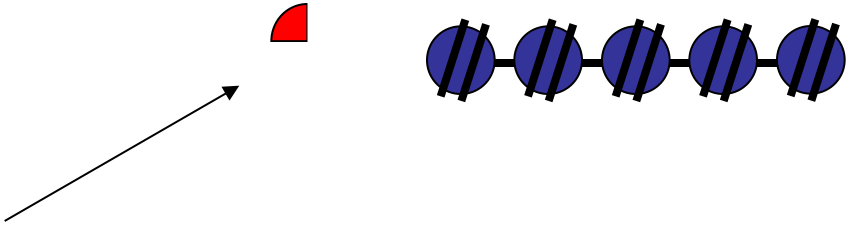
DNA replication
Histone expression
Chromatin assembly

1) Block cells in G1 with alpha factor

2) Switch to galactose medium


H3pr
3x Myc
Histone H3
Endogenous

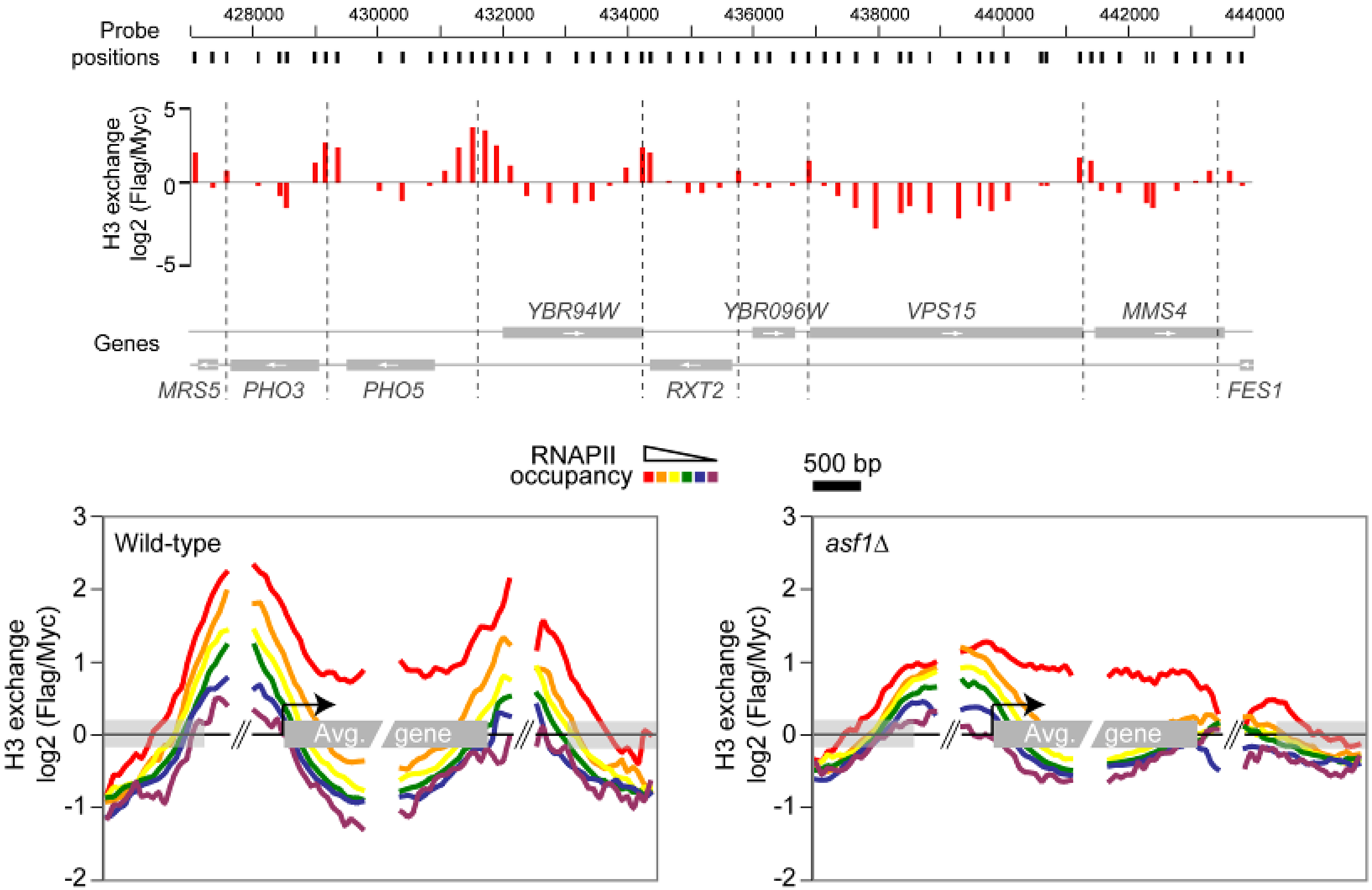
GAL1pr
3x Flag
Histone H3
External



A) Is Flag-H3 incorporated in chromatin during G1?
B) Where?

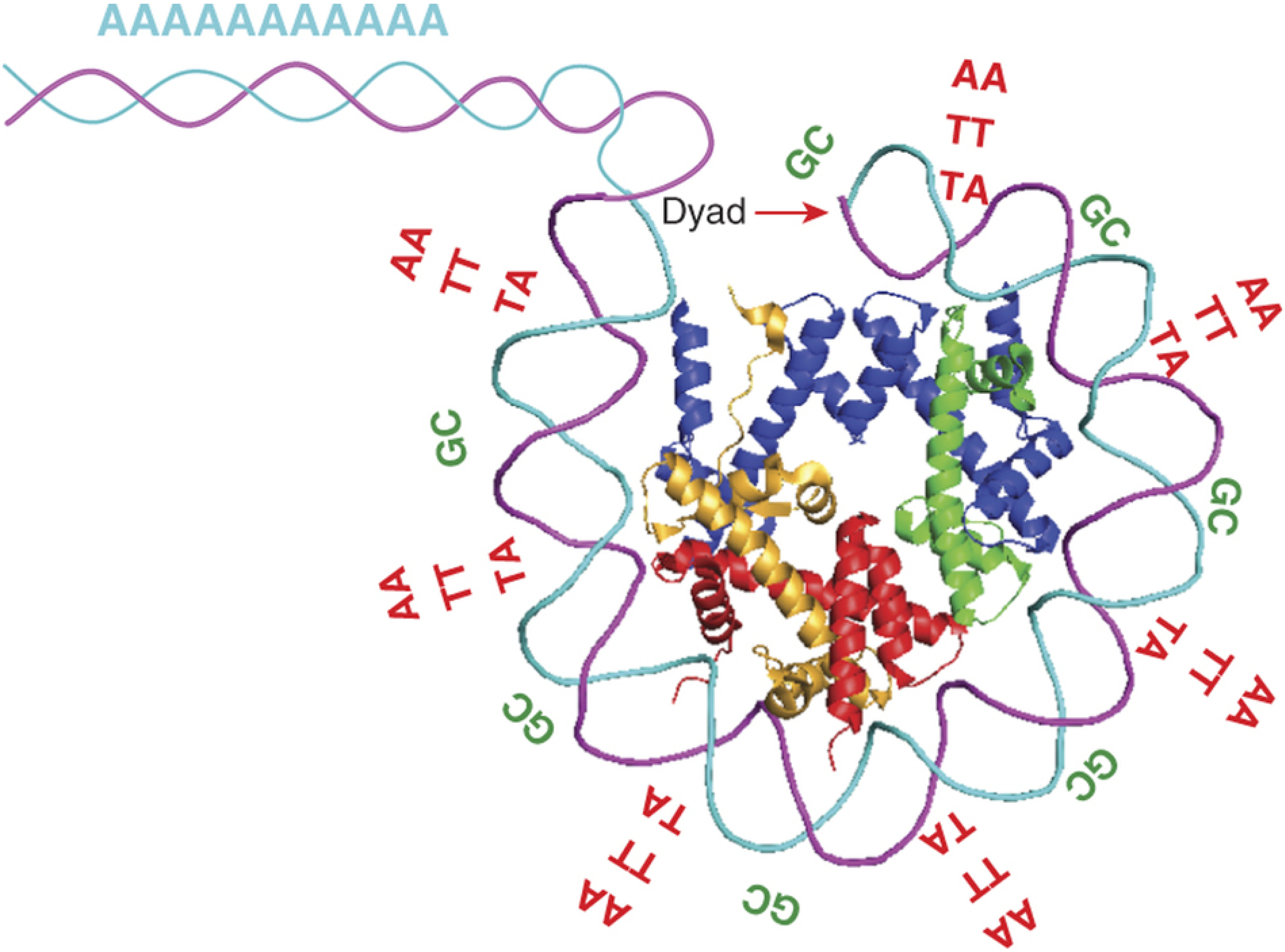
ChIP Myc
ChIP Flag → Hyb on Whole genome tiling array

Nucleosomes in promoter regions are highly dynamic



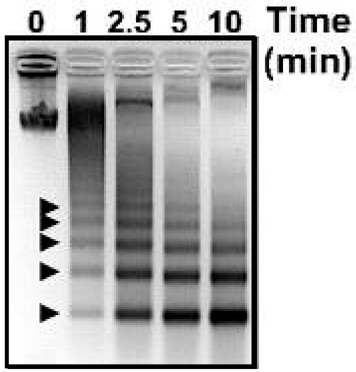
From Molecular Cell (2007) 7(3)393–405

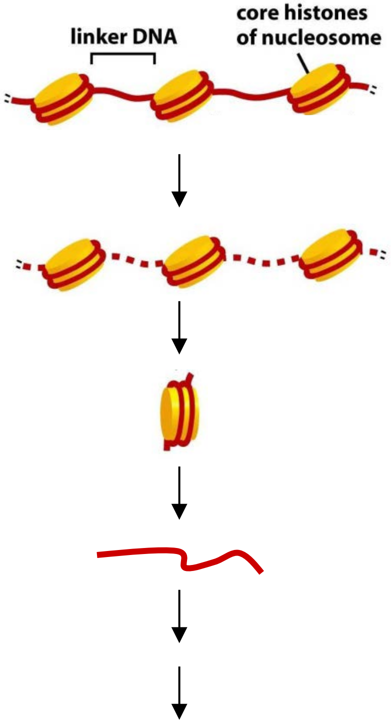
Nucleosome positionning


AAAAAAAAAAAA
AA
TT
TA
GC
Dyad
GC
AA
TT
TA
AA
TT
TA
GC
GC
AA
TT
TA
TA
TT
AA
GC
GC
GC
TA
TT
AA
TA
TT
AA

MNase-Seq, a method to map nucleosomes *in vivo*

MNase preferentially digests the linker DNA





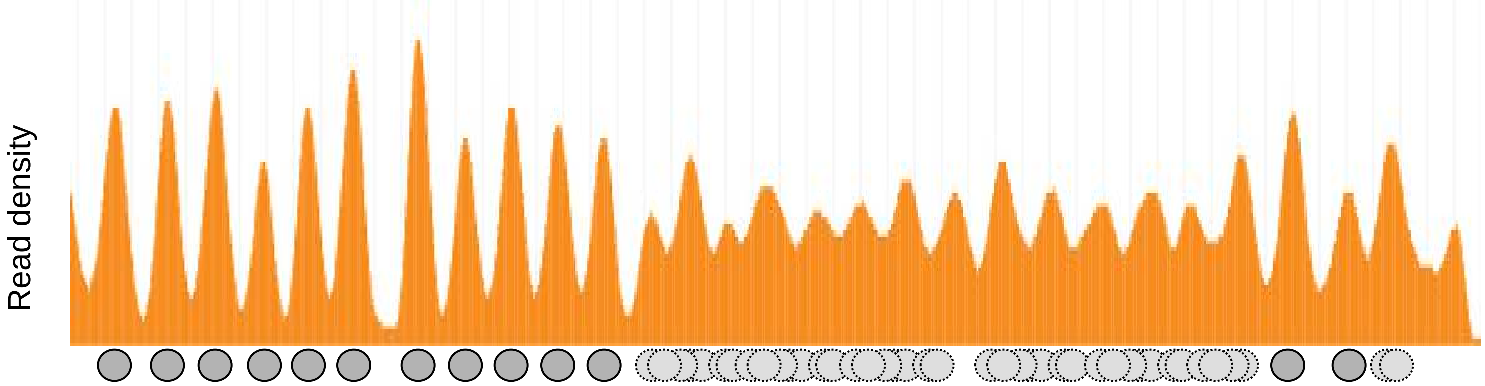
Isolate chromatin or nuclei

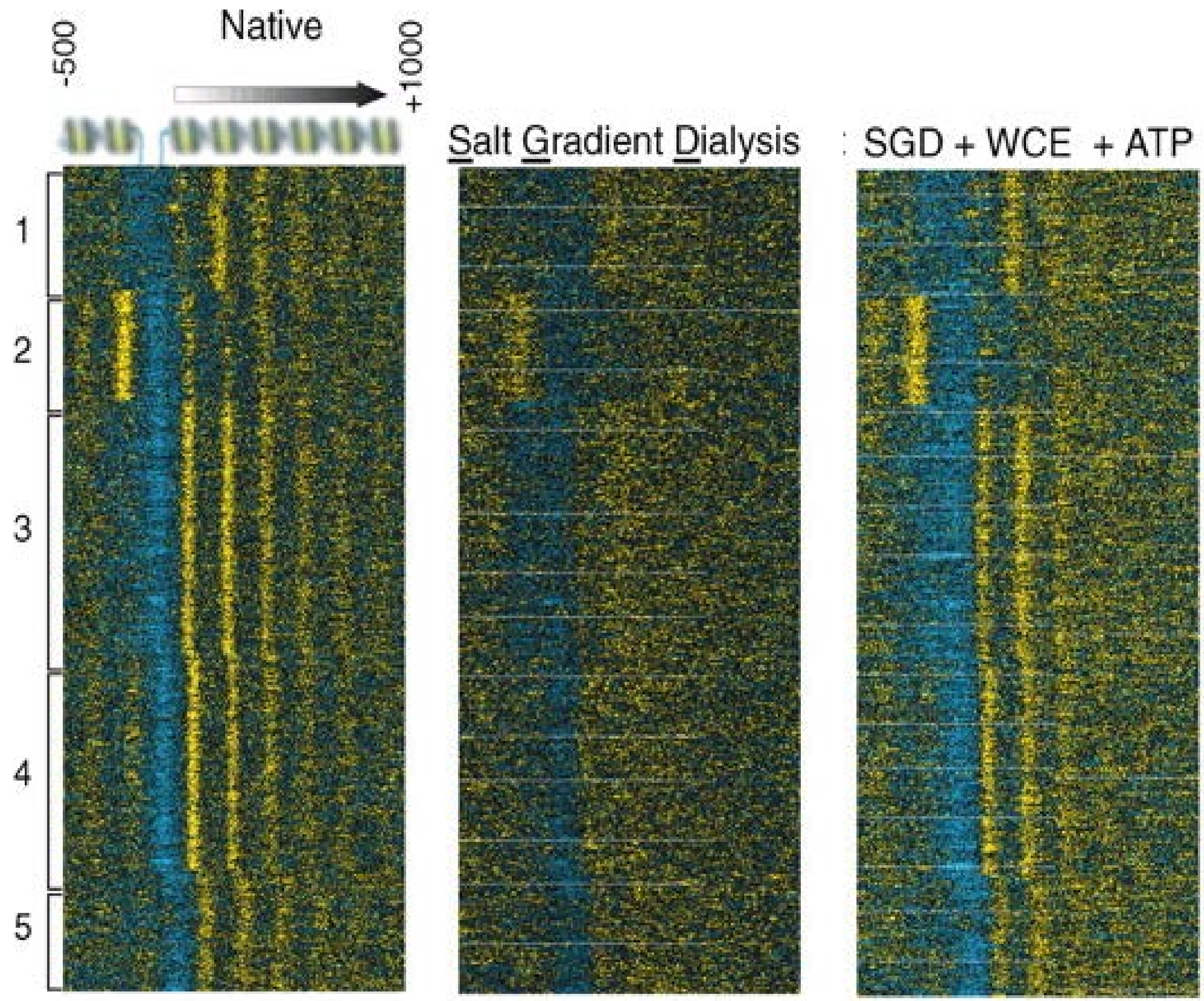
Digest chromatin with micrococcal nuclease (MNase)

Isolate MNase-resistent DNA

Deep sequencing

Map reads on the genome

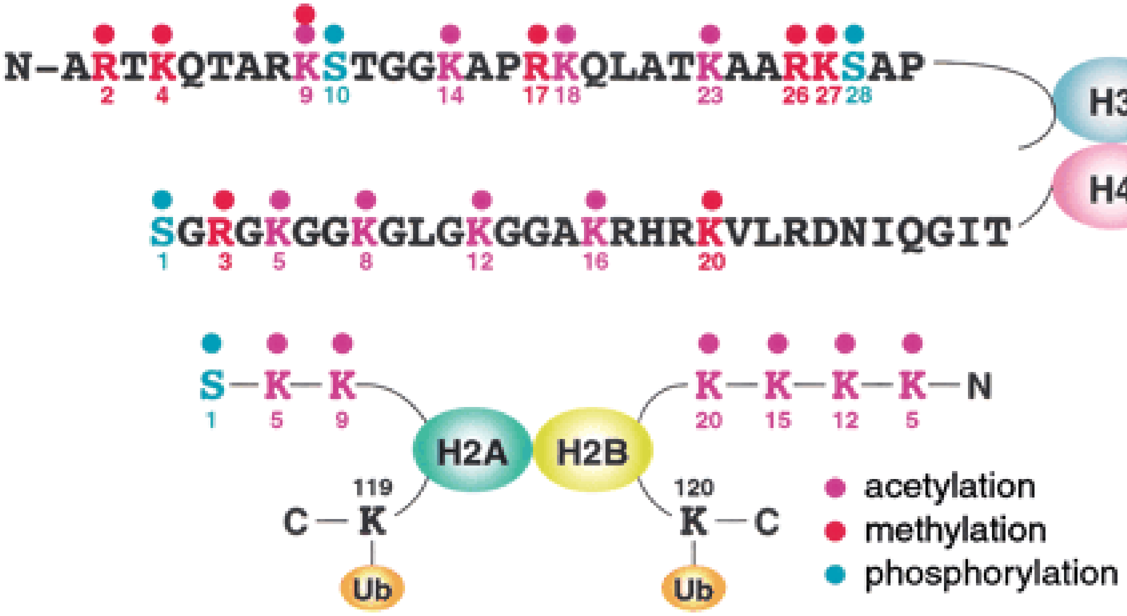


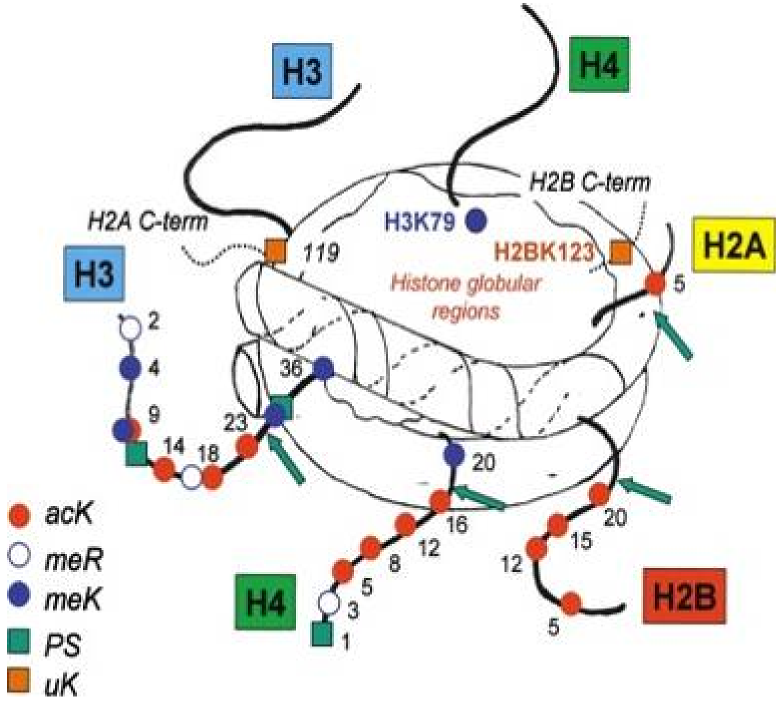


From Zhang et al. (2011) Science 332(6032):977-80

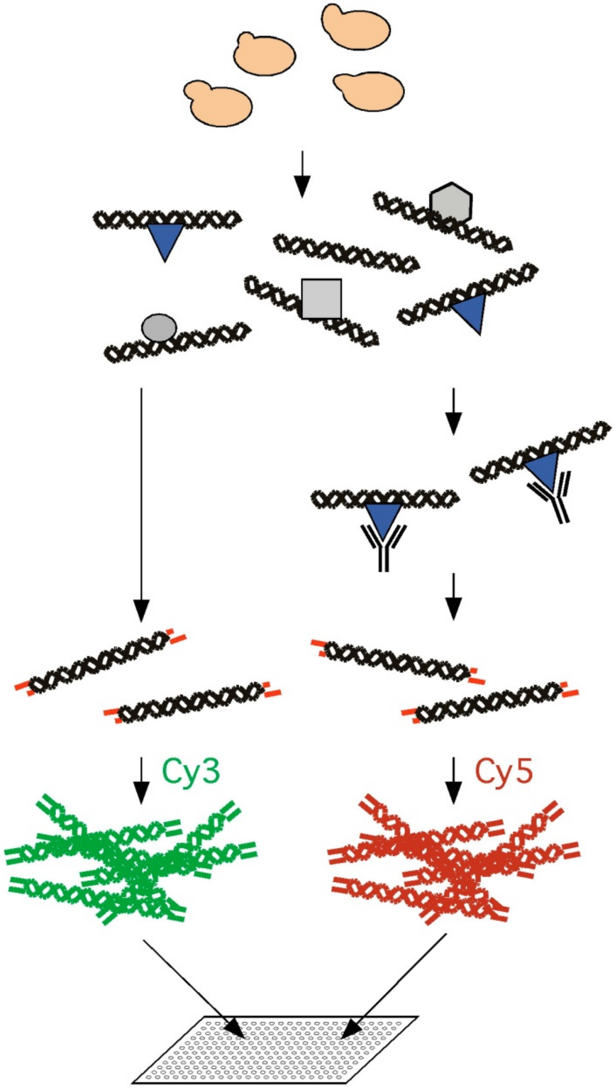
Post-translational modifications of histones

Histones, especially their N-terminal tails, are subject to massive post-translational modifications





ChIP-chip



Crosslink protein to DNA
in vivo with formaldehyde

Break open cells and
shear DNA

Immunoprecipitate

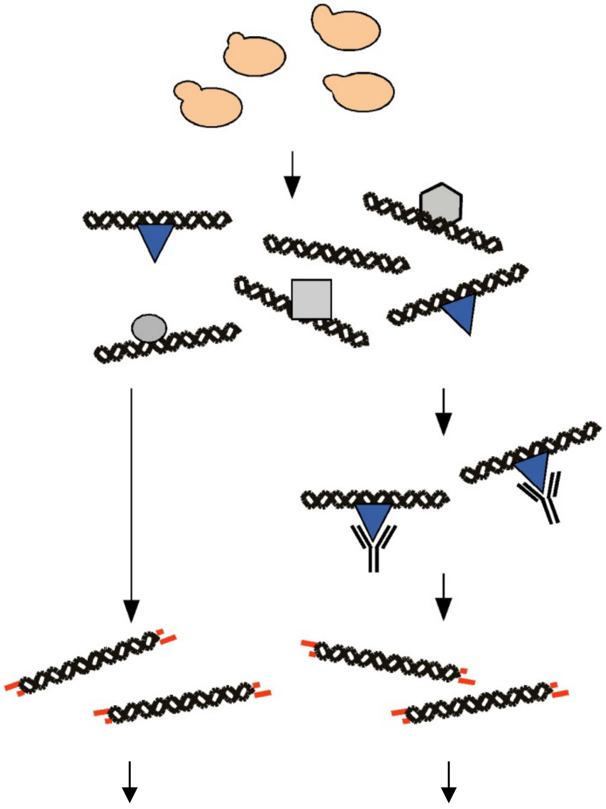
Reverse-crosslinks,
blunt DNA and ligate
to unidirectional linkers

LM-PCR

Hybridize to array

Science, 290, 2306-9

ChIP-seq



Crosslink protein to DNA
in vivo with formaldehyde

Break open cells and
shear DNA

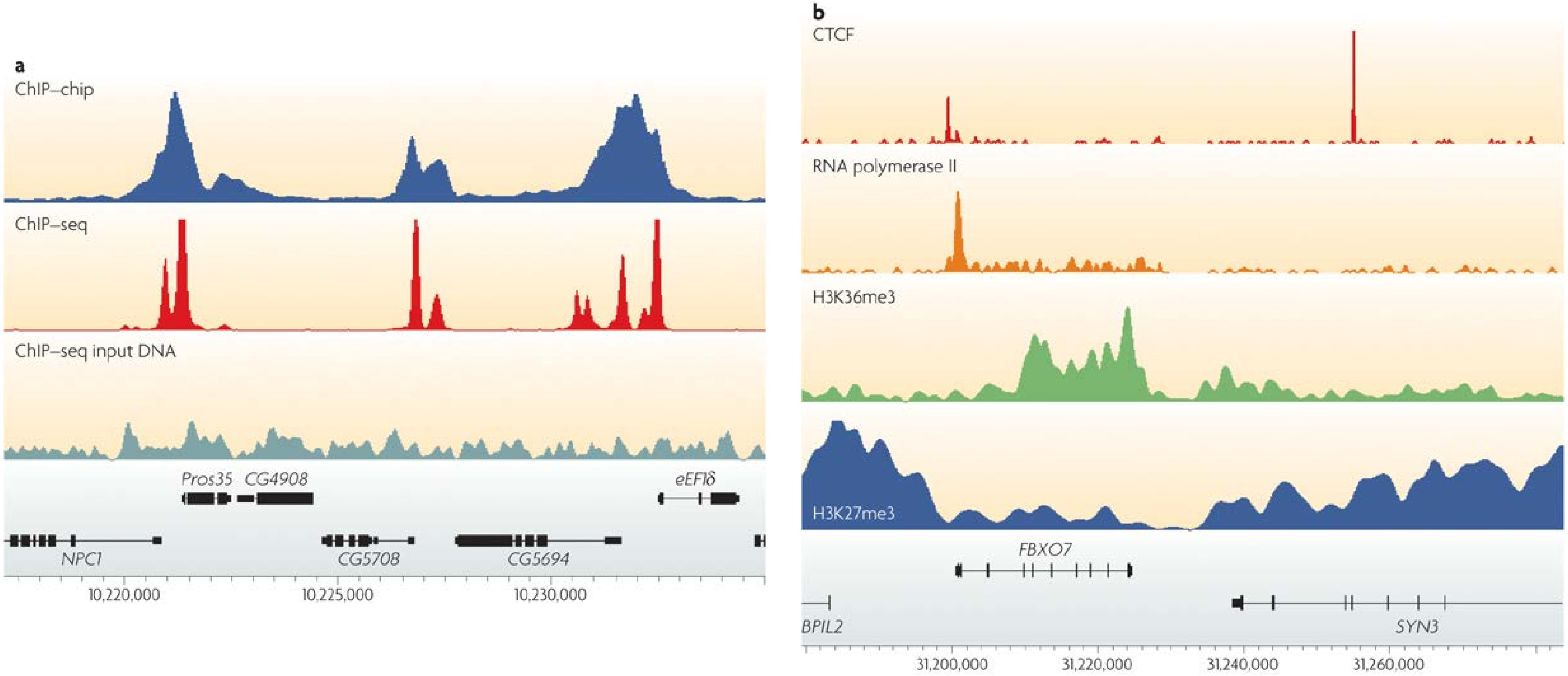
Immunoprecipitate

Reverse-crosslinks,
blunt DNA and ligate
to unidirectional linkers

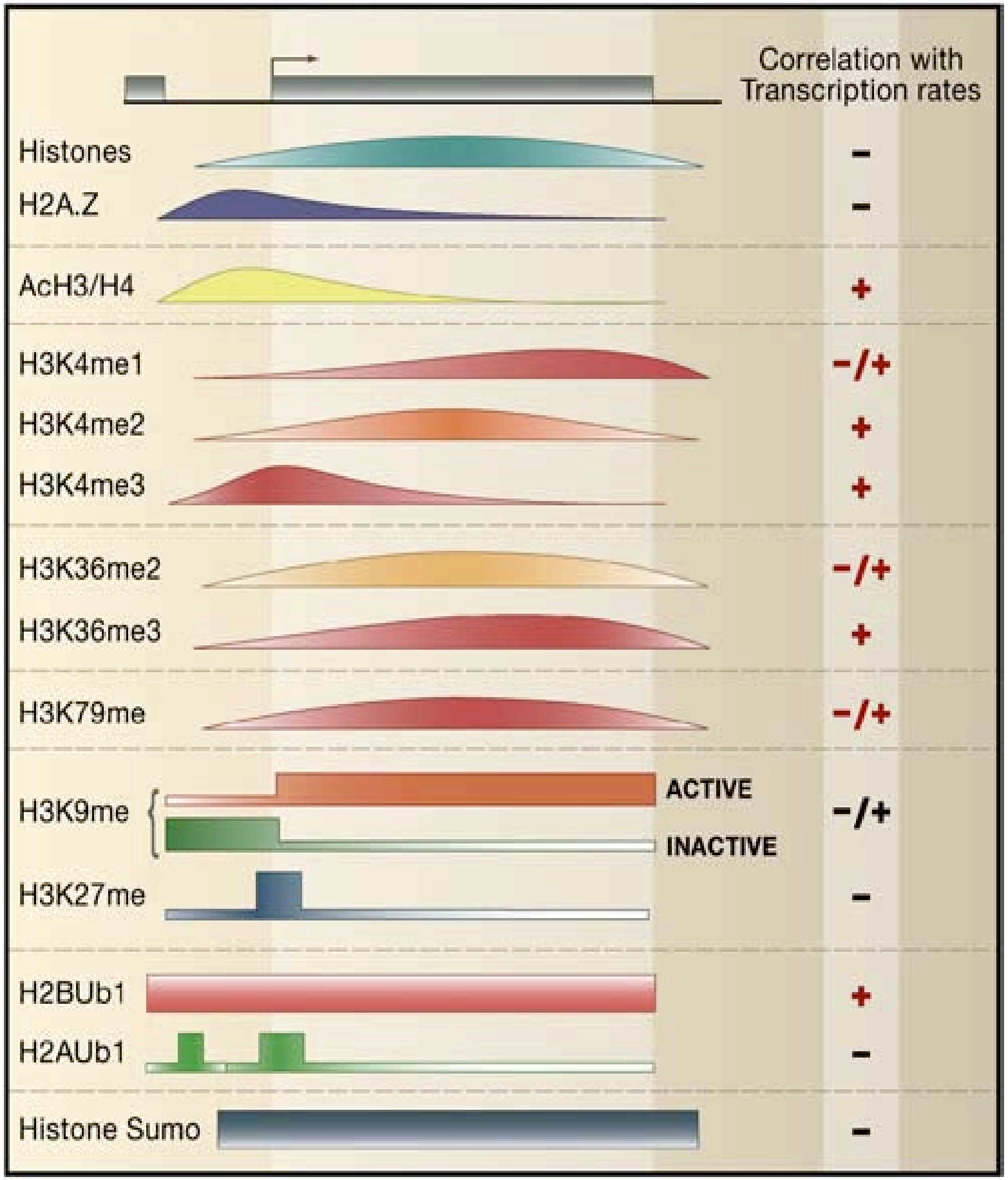
Deep sequencing
+ read mapping

Deep sequencing
+ read mapping

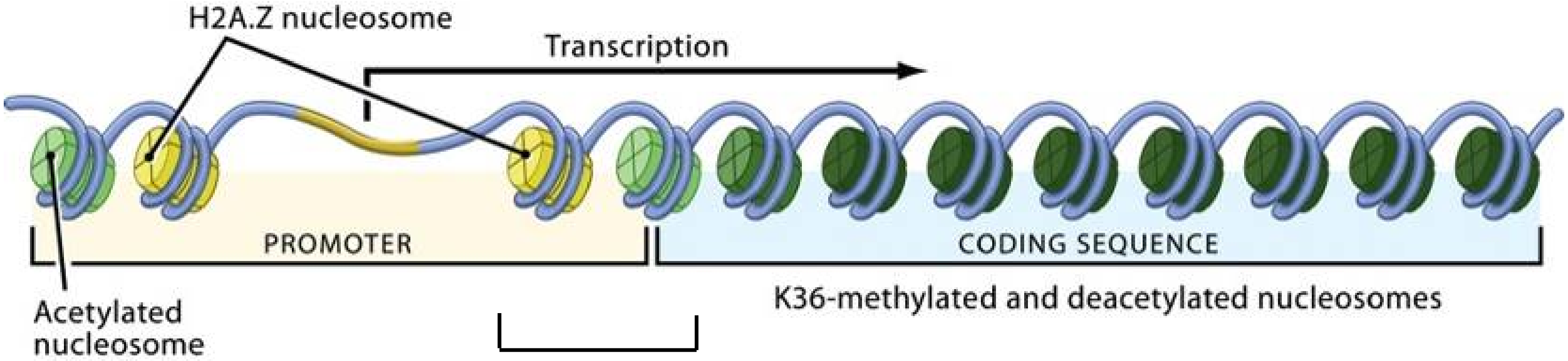
Outputs from ChIP-chip and ChIP-Seq experiments



Patterns of histone modifications can predict functional elements and states



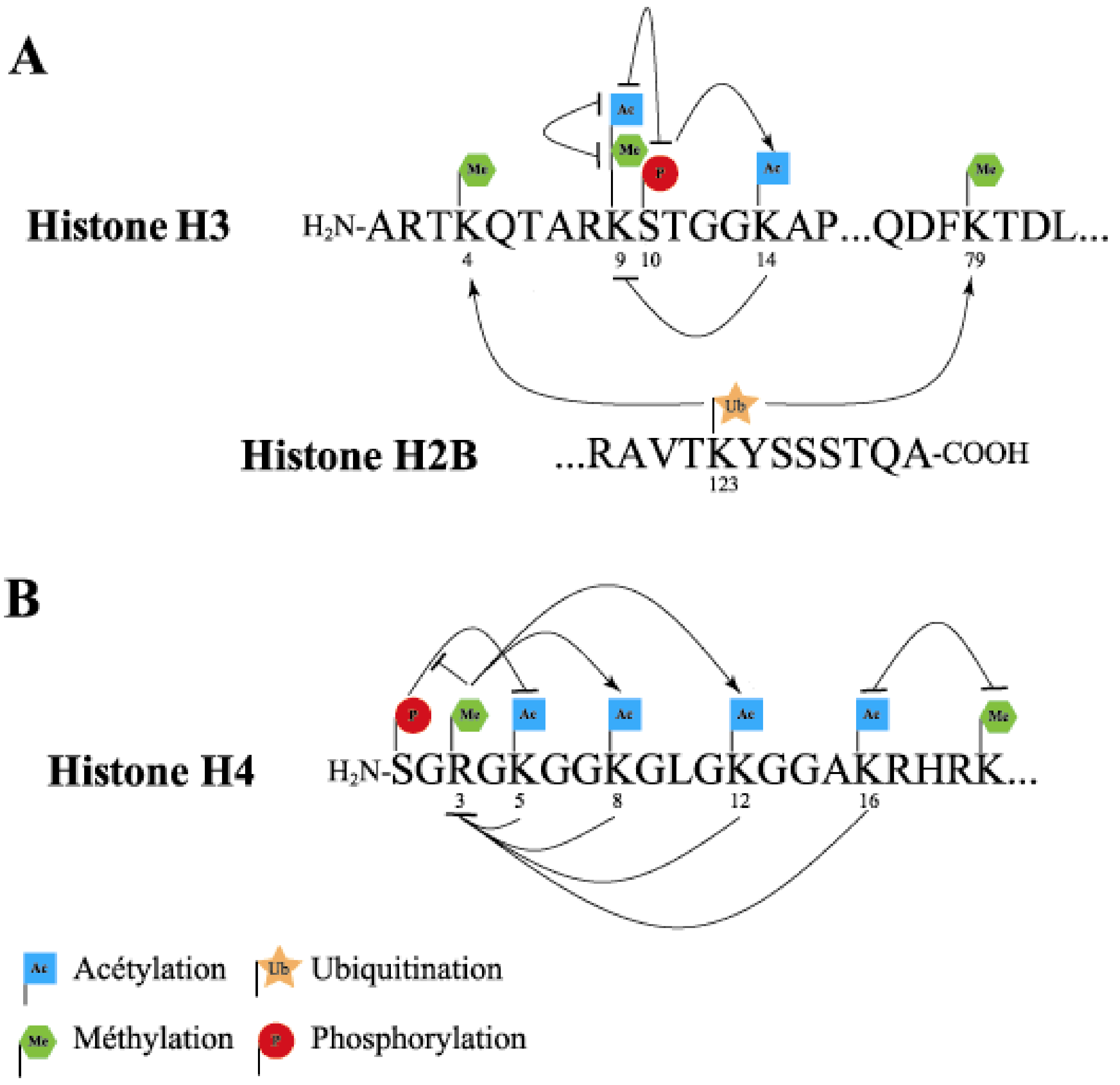
Chromatin domains along a transcription unit



K4-trimethylated nucleosomes

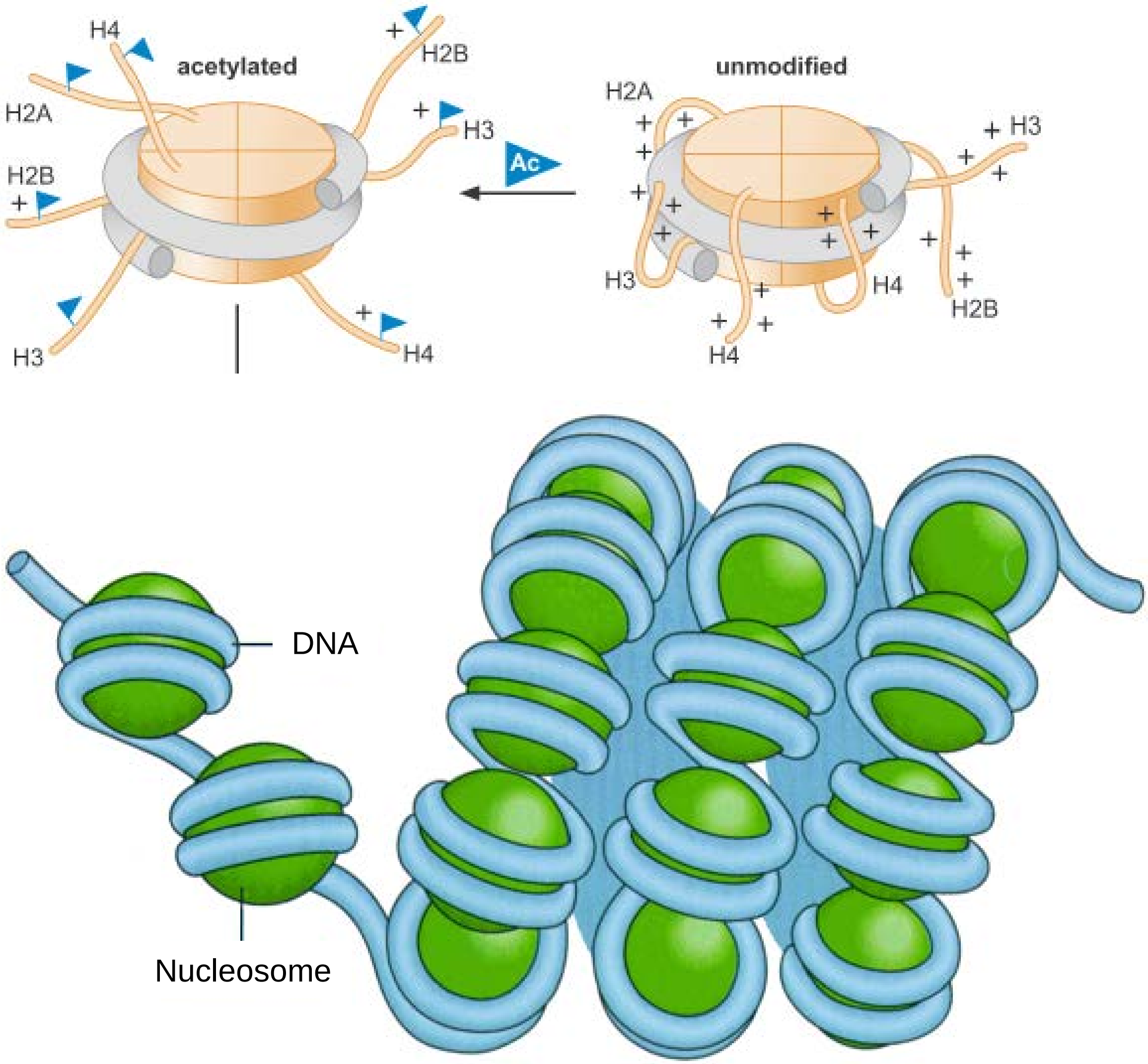
Lieb and Clarke, Cell 2005

Crosstalks between the different histone marks

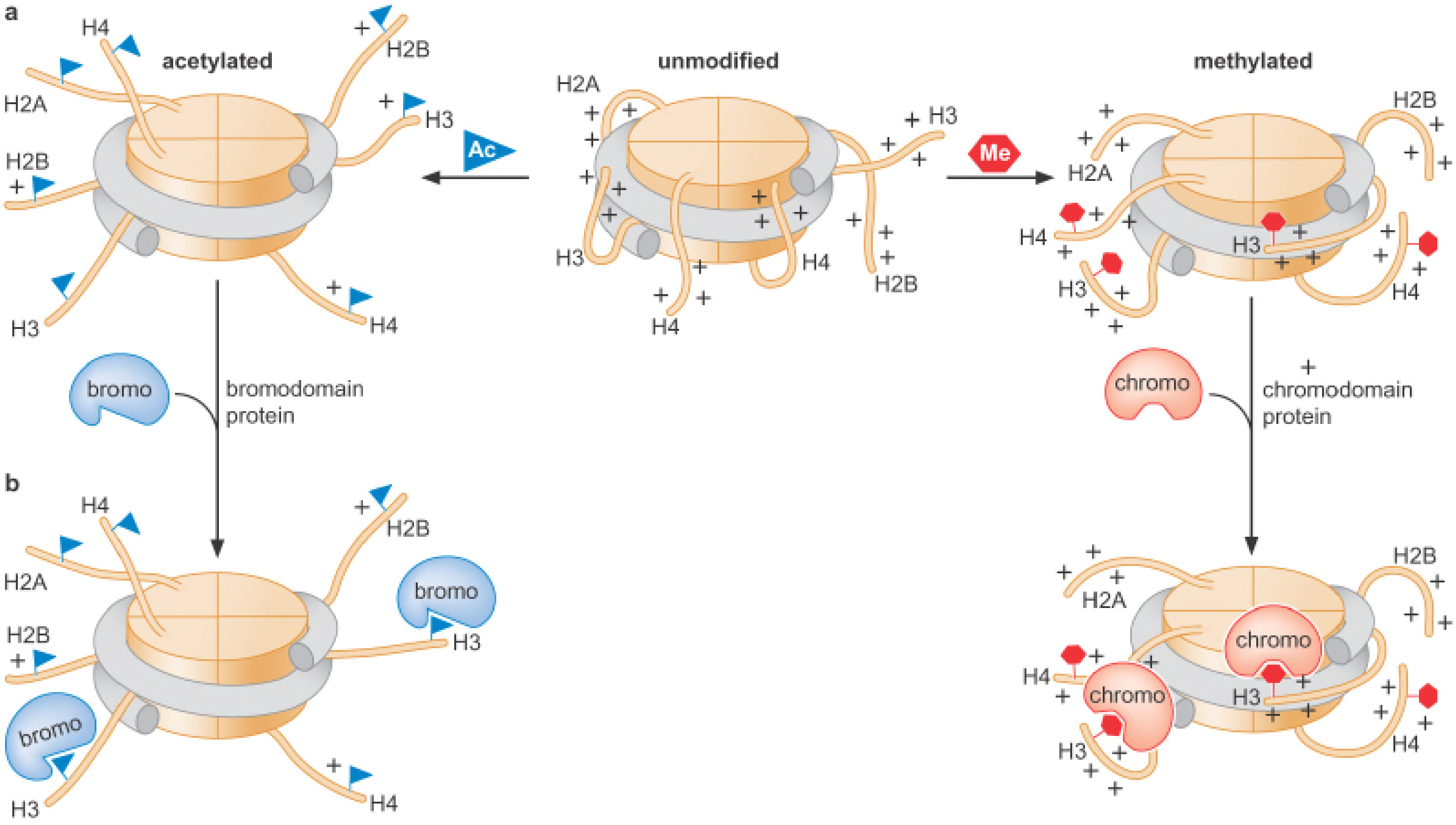


What are the functional consequences of histone modifications?

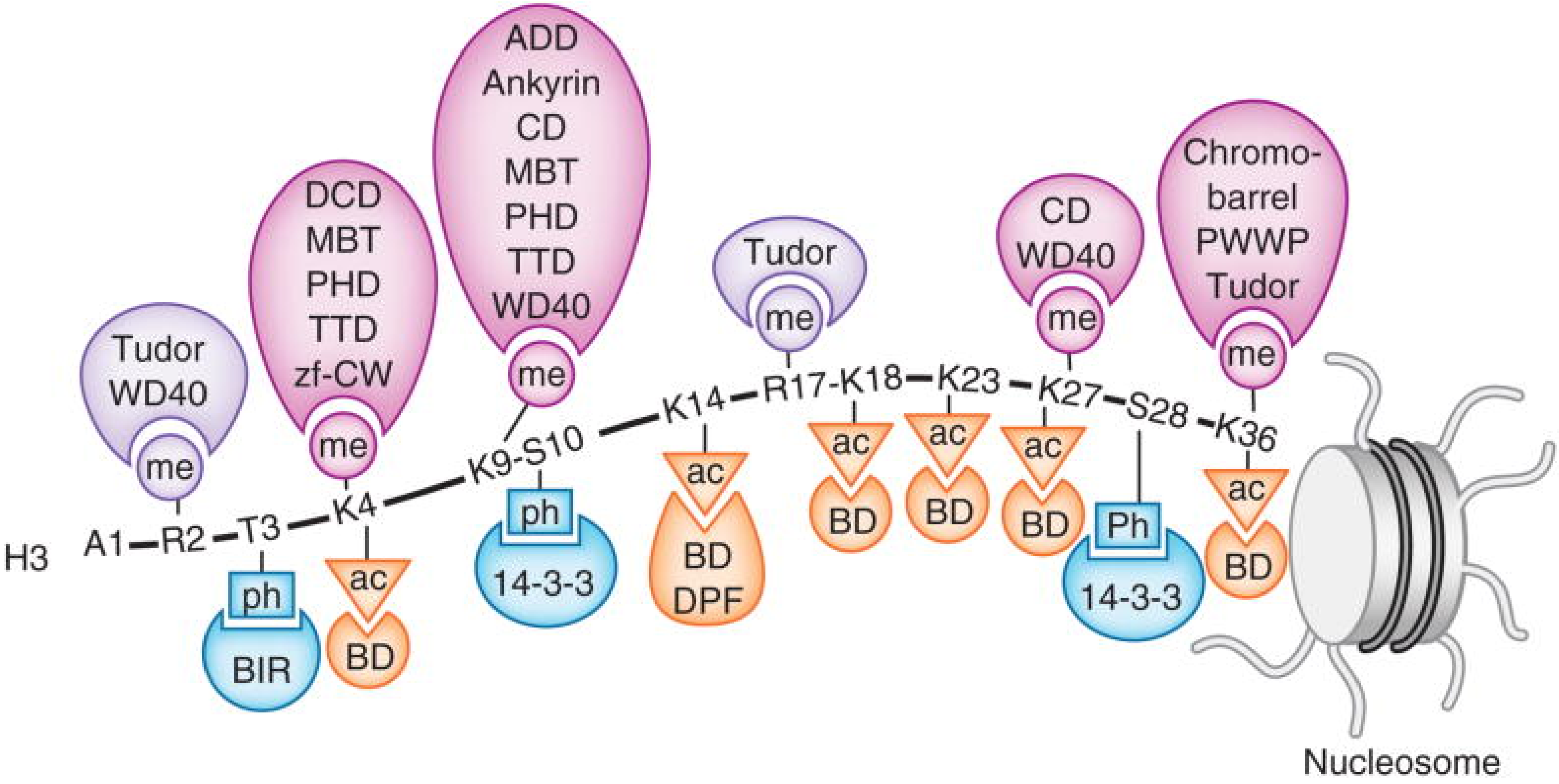
Histone acetylation favors more “relaxed” chromatin conformations



Specific protein domains recognize different epigenetic marks (The histone code hypothesis)



Several protein domains can recognize different histone modifications



From NSMB (2012)19(12):1218-27

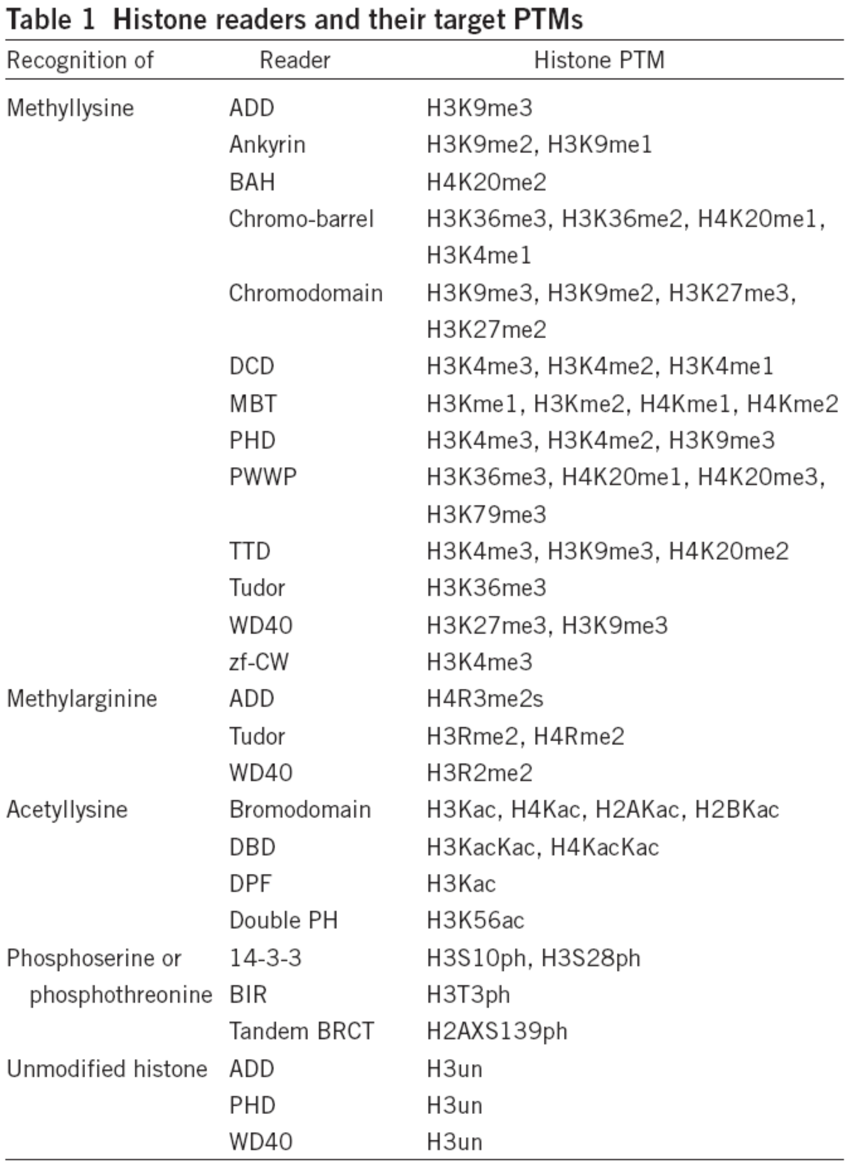
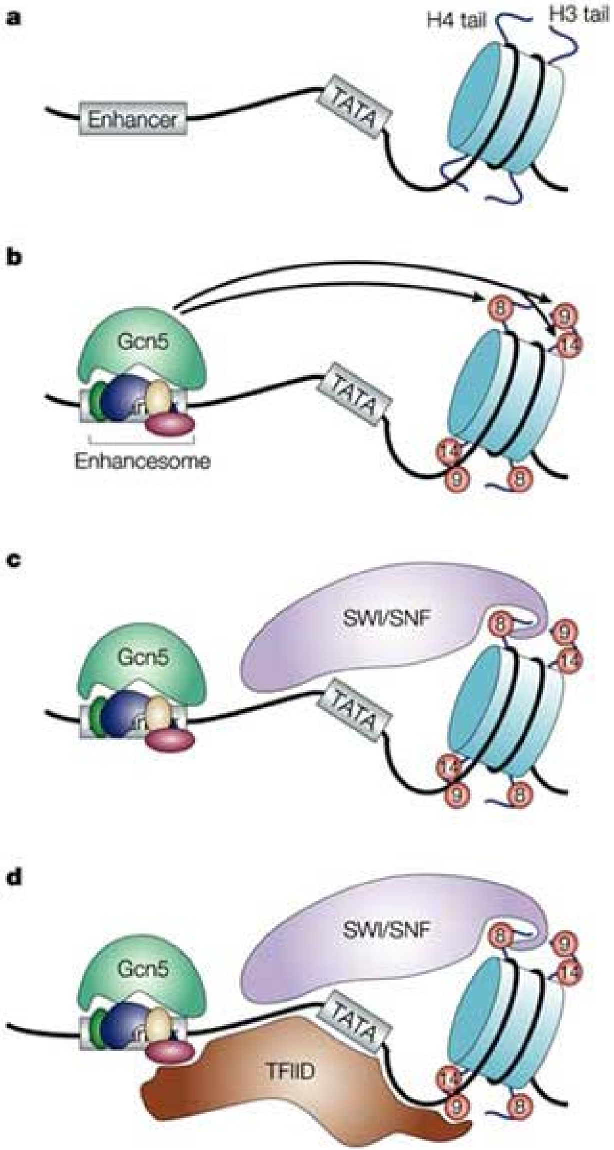


Table 1 Histone readers and their target PTMs

Recognition of	Reader	Histone PTM
Methyllysine	ADD	H3K9me3
	Ankyrin	H3K9me2, H3K9me1
	BAH	H4K20me2
	Chromo-barrel	H3K36me3, H3K36me2, H4K20me1, H3K4me1
	Chromodomain	H3K9me3, H3K9me2, H3K27me3, H3K27me2
	DCD	H3K4me3, H3K4me2, H3K4me1
	MBT	H3Kme1, H3Kme2, H4Kme1, H4Kme2
	PHD	H3K4me3, H3K4me2, H3K9me3
	PWWP	H3K36me3, H4K20me1, H4K20me3, H3K79me3
	TTD	H3K4me3, H3K9me3, H4K20me2
	Tudor	H3K36me3
	WD40	H3K27me3, H3K9me3
	zf-CW	H3K4me3
Methylarginine	ADD	H4R3me2s
	Tudor	H3Rme2, H4Rme2
	WD40	H3R2me2
Acetyllysine	Bromodomain	H3Kac, H4Kac, H2AKac, H2BKac
	DBD	H3KacKac, H4KacKac
	DPF	H3Kac
	Double PH	H3K56ac
Phosphoserine or phosphothreonine	14-3-3	H3S10ph, H3S28ph
	BIR	H3T3ph
	Tandem BRCT	H2AXS139ph
Unmodified histone	ADD	H3un
	PHD	H3un
	WD40	H3un

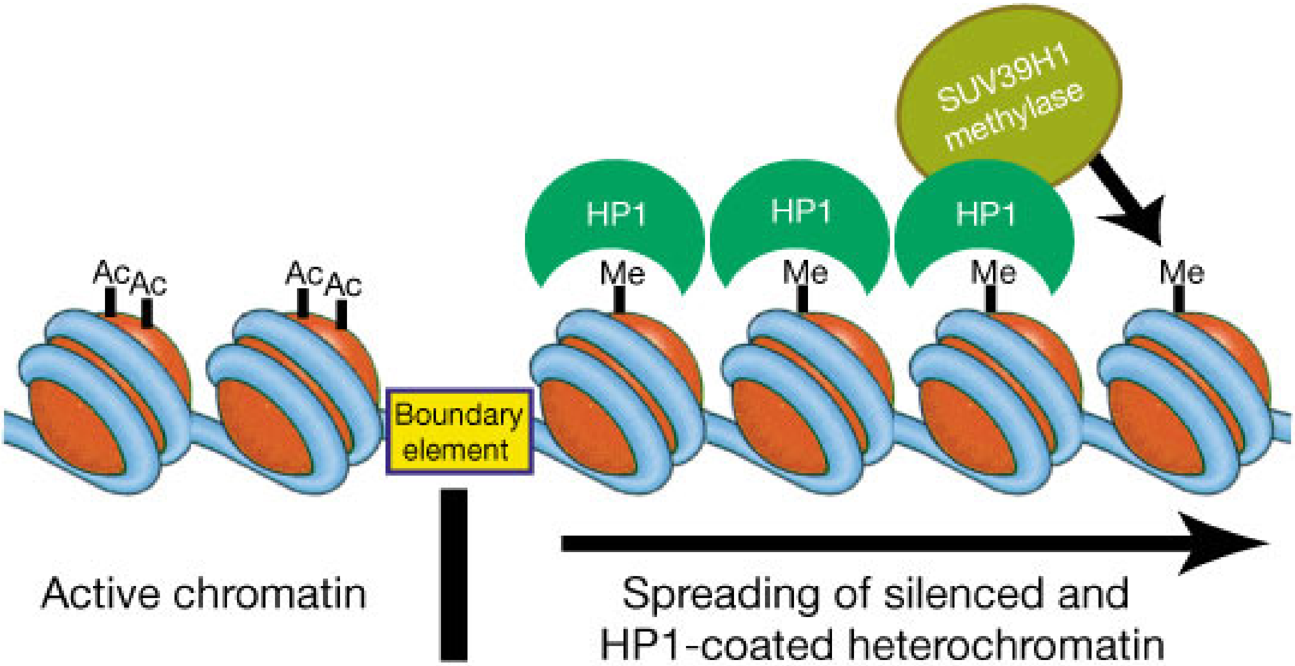
From NSMB (2012)19(12):1218-27

The ability to read a mark allows for the recruitment of transcription co-activators

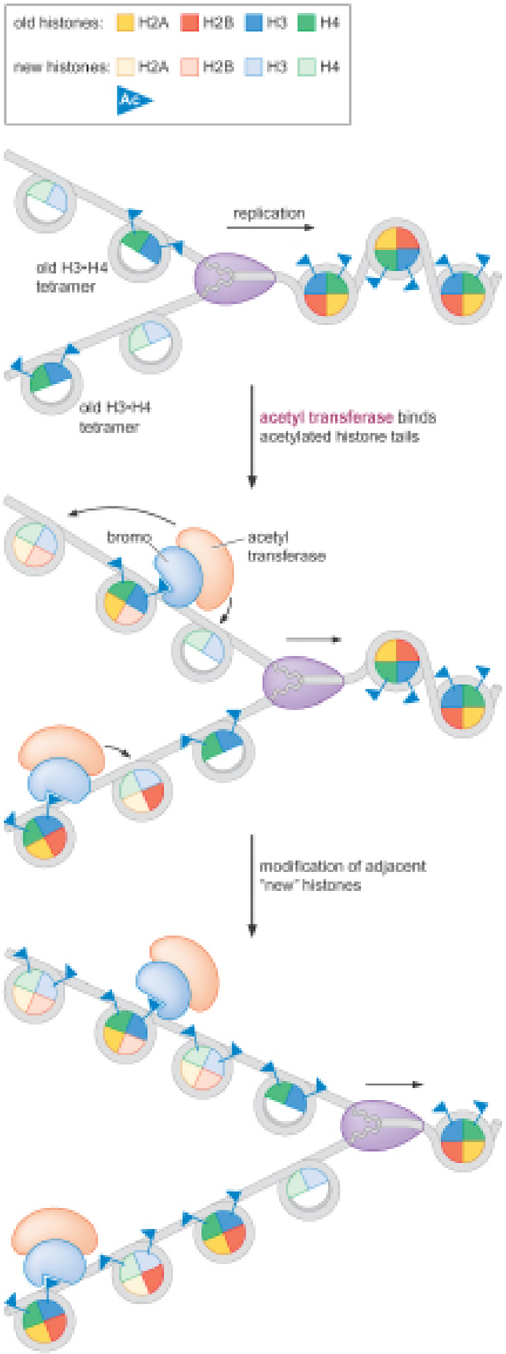


SWI/SNF and TFIID contain bromodomains that allow them to recognize acetylated histones

The ability to read and write a mark allows for the spreading of that mark

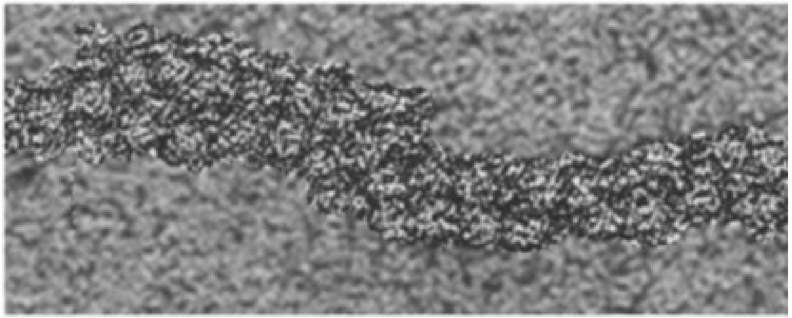


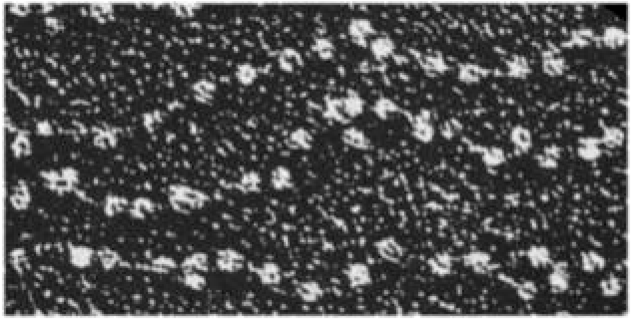
The ability to read and write a mark allows for its inheritance through DNA replication

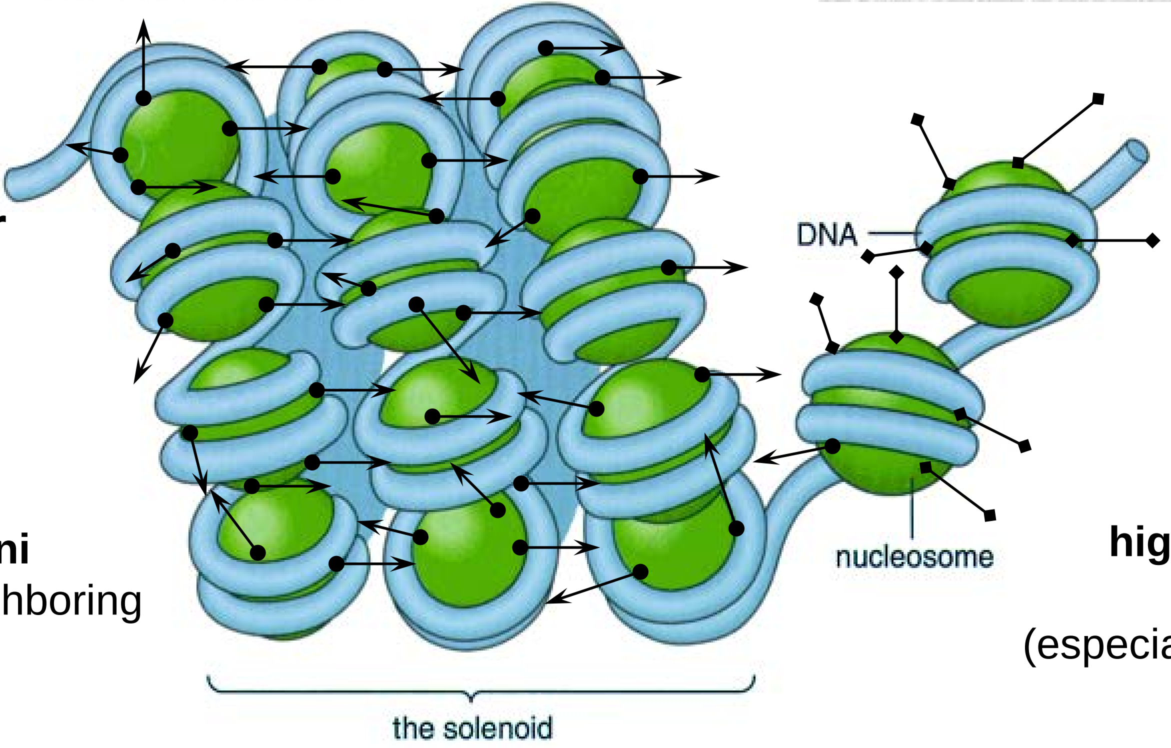


Linker histones

Chromatin fibers







30 nm chromatin fiber

11 nm (beads)

●⟶

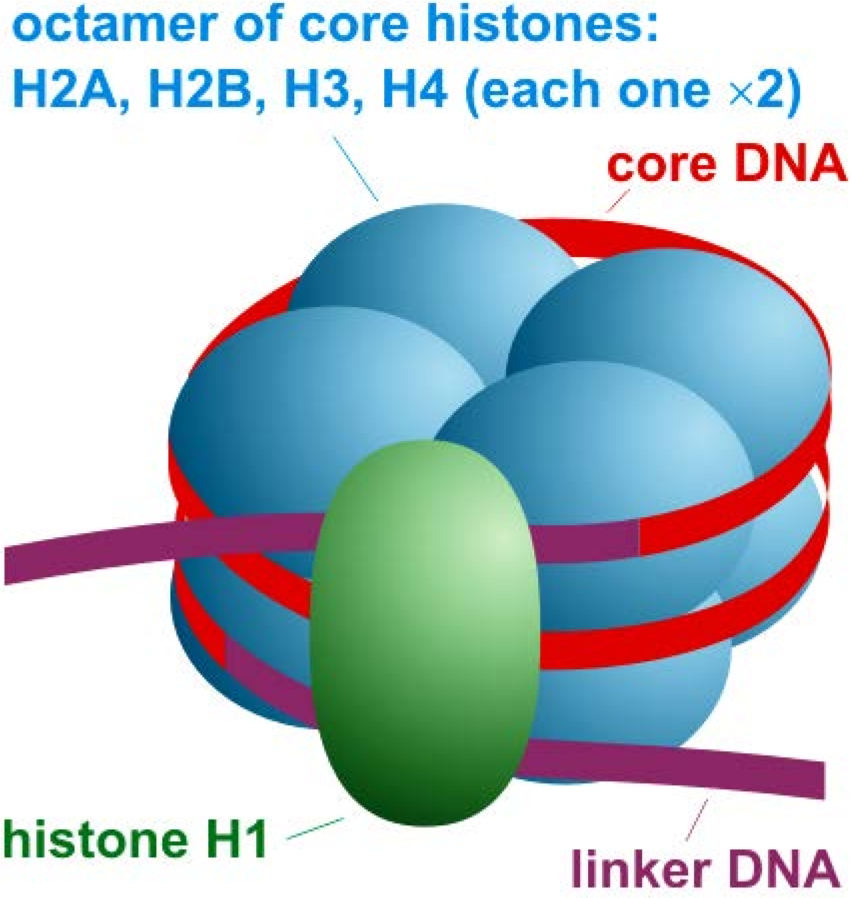
+ charged N termini
(bind DNA and neighboring nucleosomes)

◆—◆

highly acetylated core histones
(especially H3 and H4)

- High level of histone H1
- No gene transcription

- Reduced level of histone H1
- Gene transcription possible



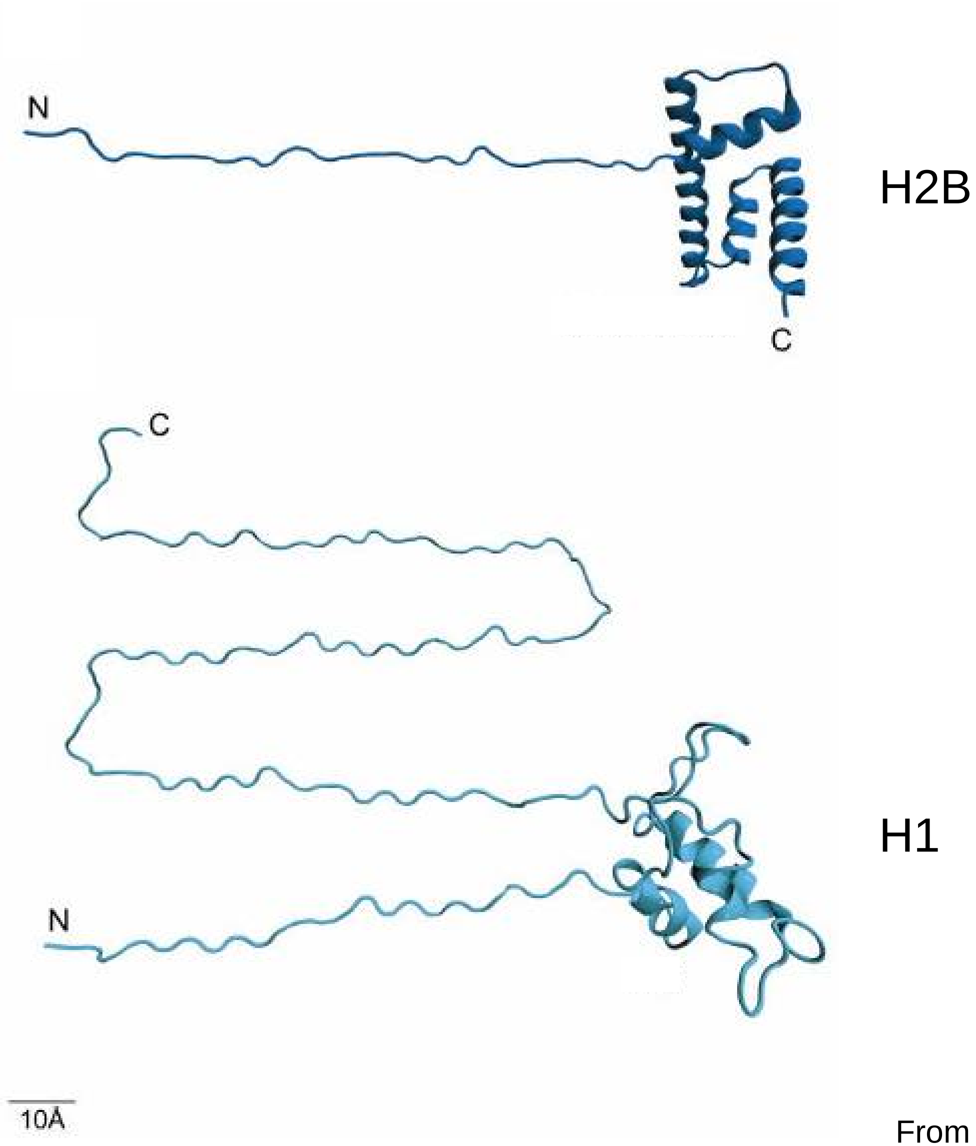
octamer of core histones:
H2A, H2B, H3, H4 (each one ×2)

core DNA

histone H1

linker DNA

Histone H1 has a very long C-terminal tail



From Cell Res. (2010) 20(5):519-28

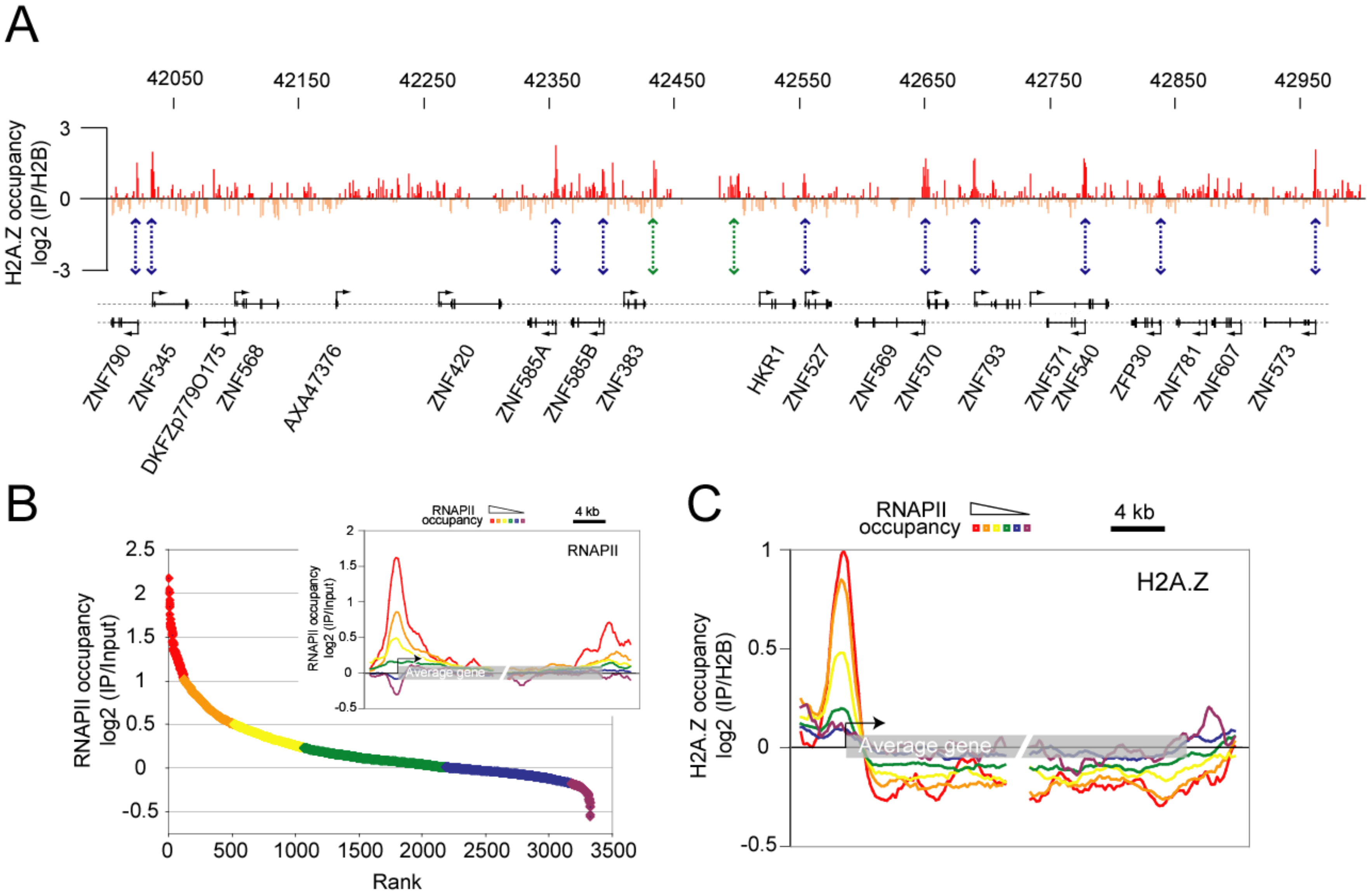
Histone variants

Histone variants

H2A variants	
H2A.Z	Gene expression, DNA repair
H2A.X	DNA repair
macroH2A	Silencing
H2A.Bbd	unknown

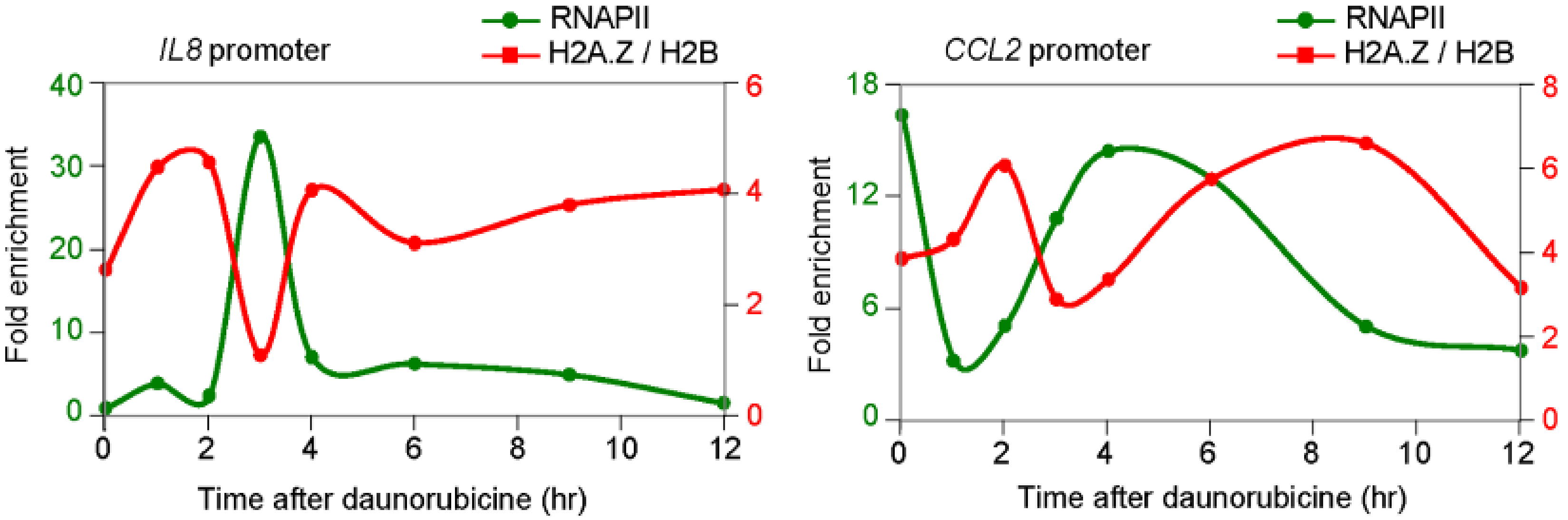
H3 variants	
H3.3	Replaces H3 outside of S phase
cenH3	Centromeric H3

Human H2A.Z localizes to promoters occupied by RNAPII



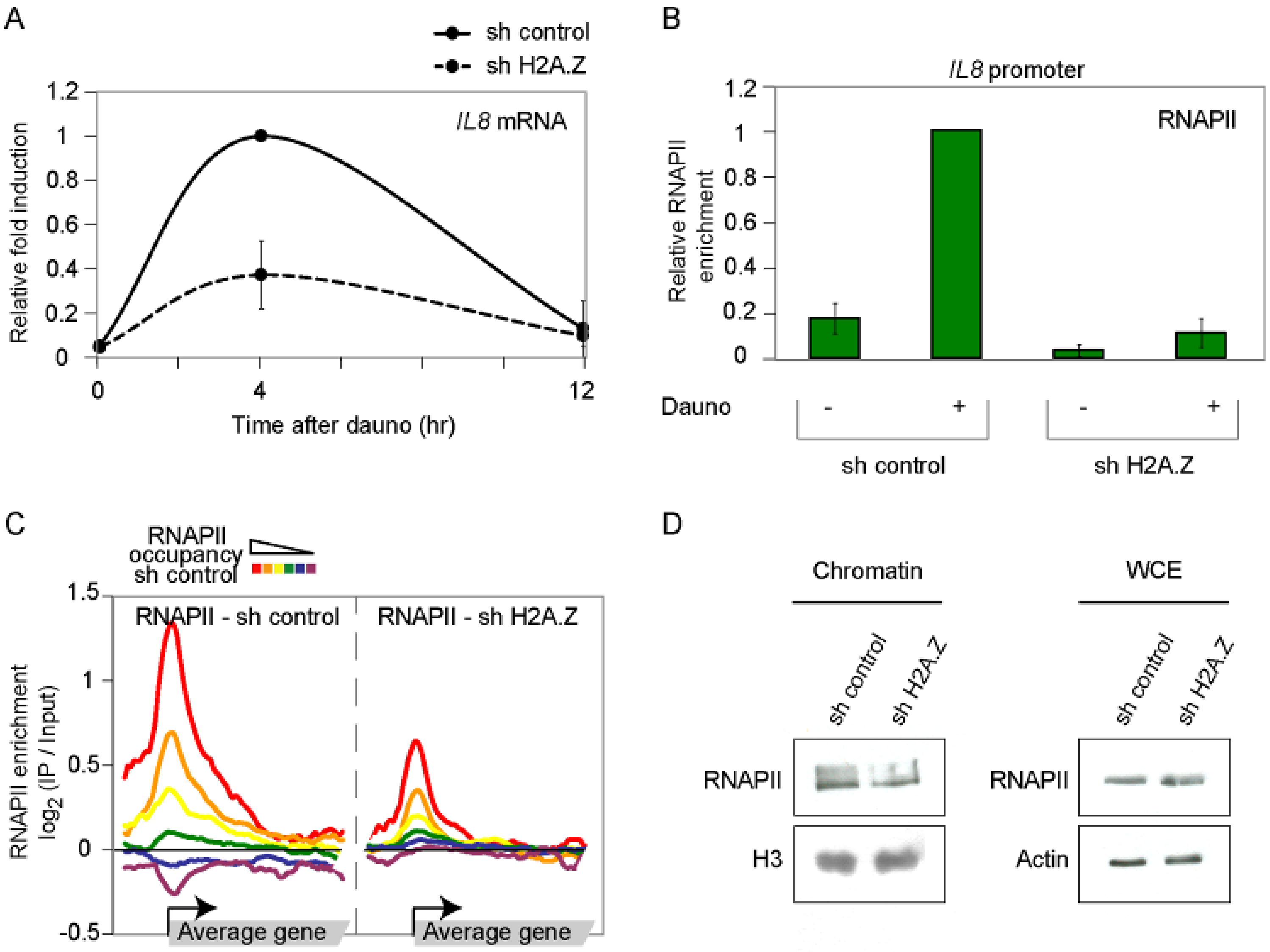
Hardy et al PLoS Genetics 5(10):e1000687

H2A.Z dynamically associates with its target genes



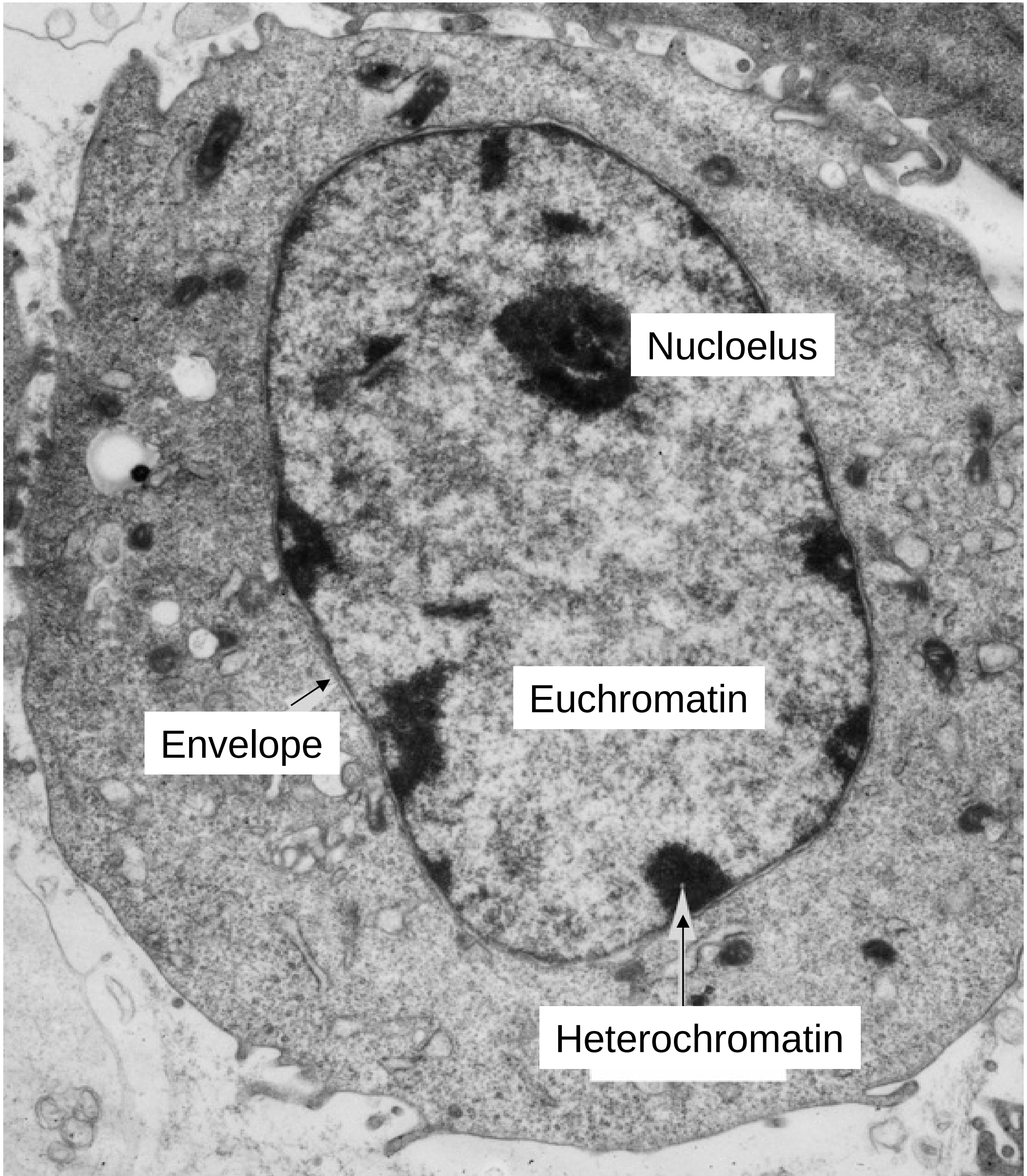
Hardy et al PLoS Genetics 5(10):e1000687

H2A.Z helps in the recruitment of RNAPII

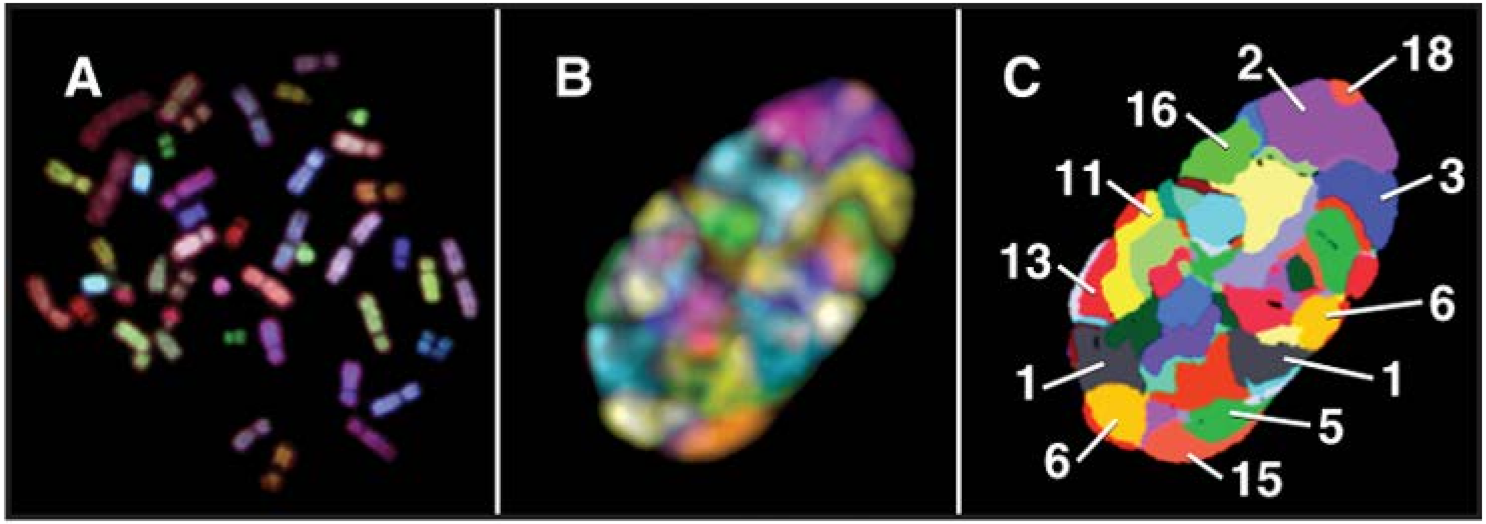


Hardy et al PLoS Genetics 5(10):e1000687

Higher order structure


Nucloelus
Euchromatin
Envelope
Heterochromatin

Chromosomal Territories

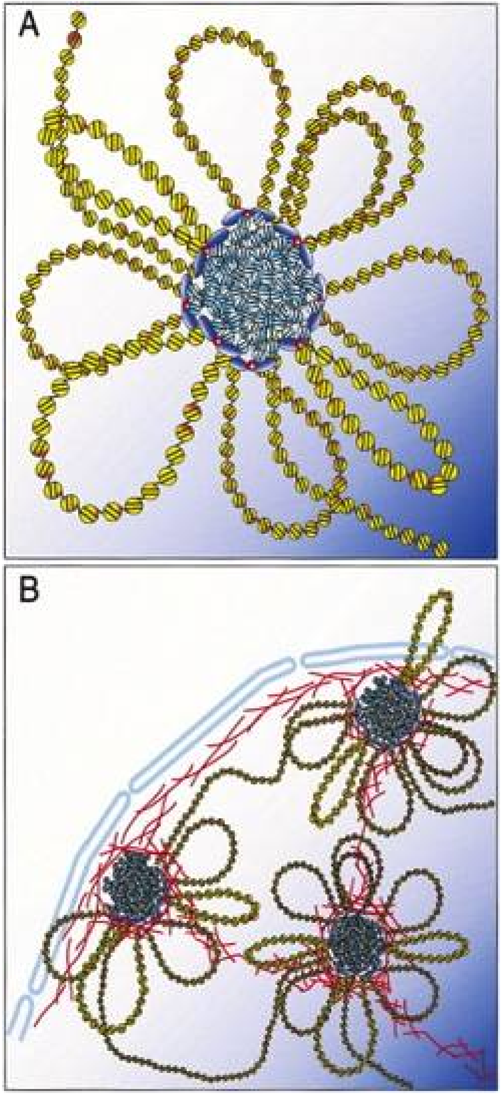


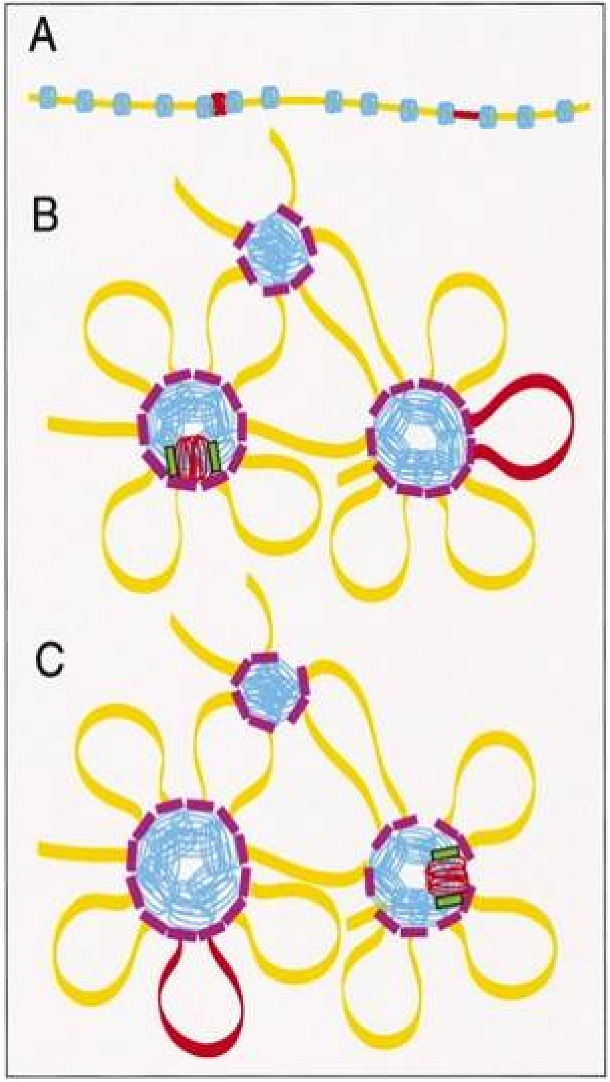
© Elsevier. Pollard et al: Cell Biology 2e - www.studentconsult.com

Chromosomes painting by FISH

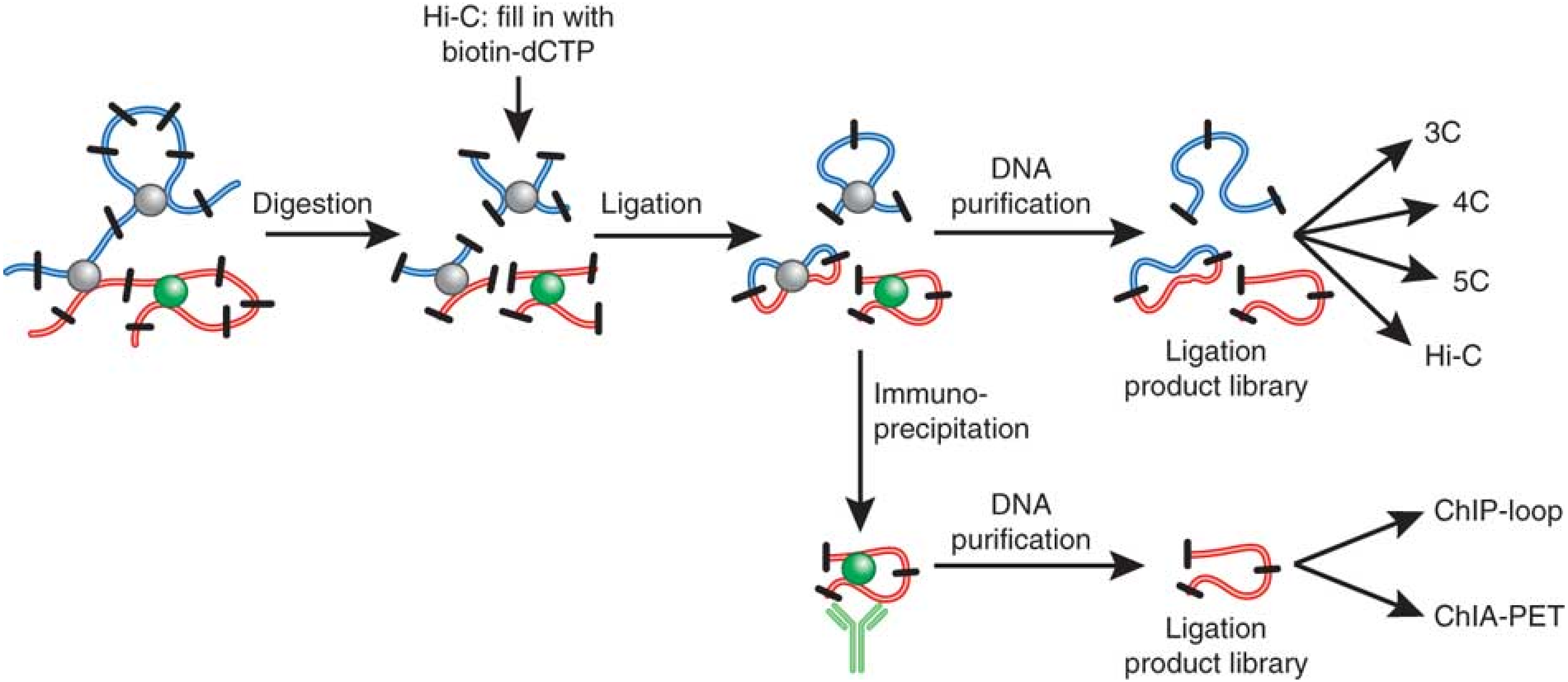
-Chromosomes generally occupy well defined territories and rarely mix with each other.
-Active genes generally localize to the surface of those territories.

Chromatin Domains



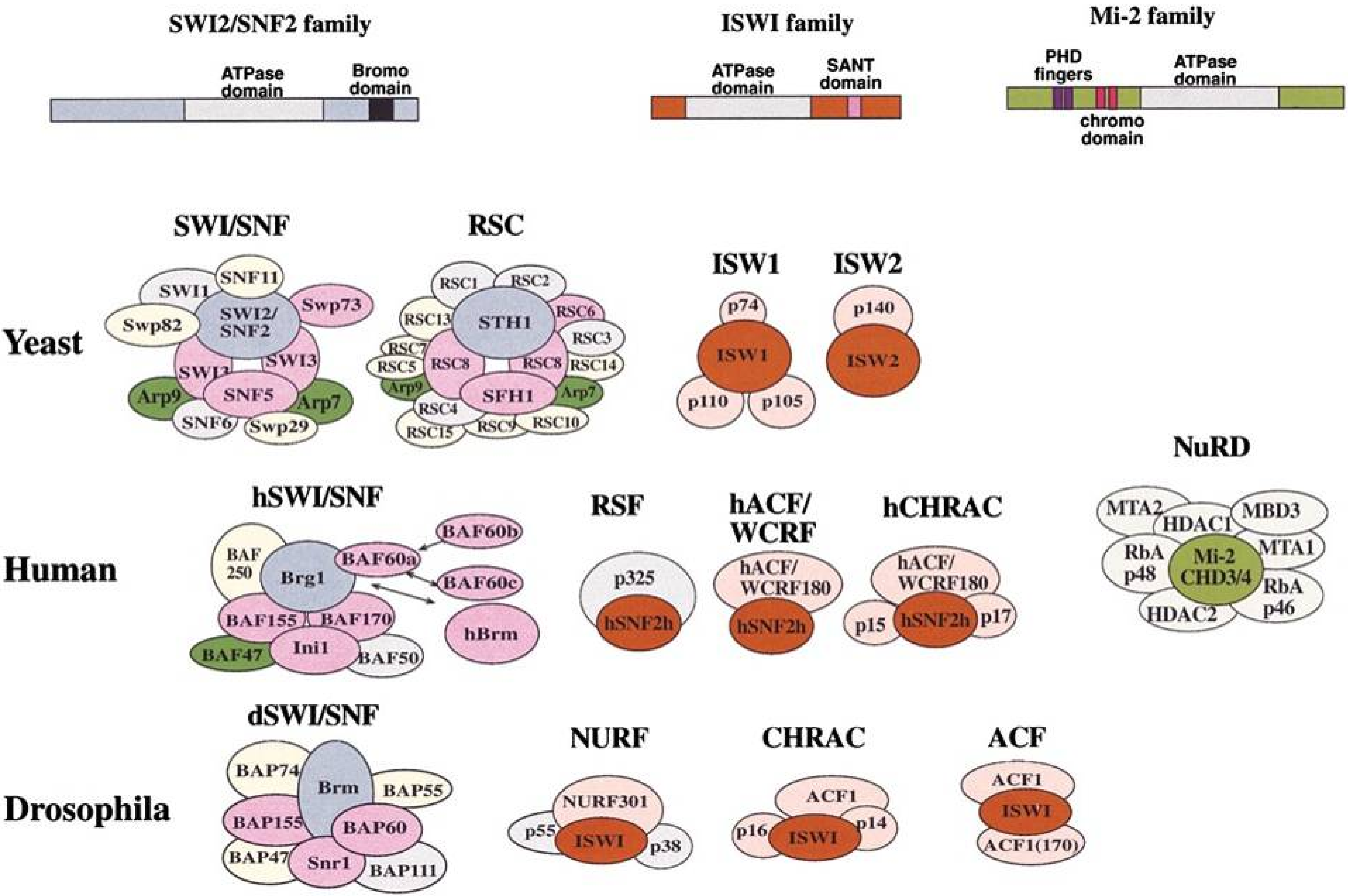


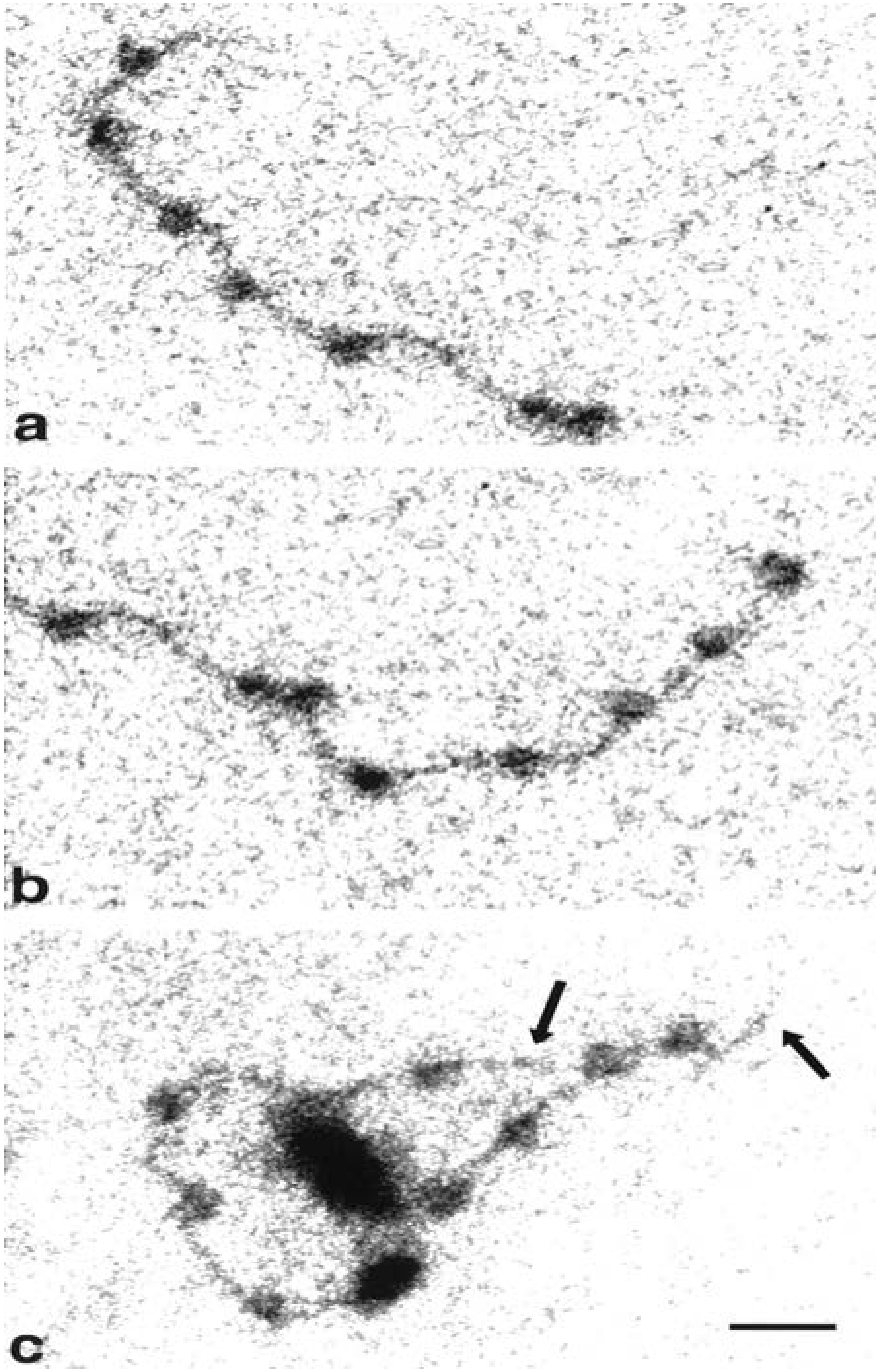
3C(Chromatin Conformation Capture)-Based Methods to study high order chromatin structures

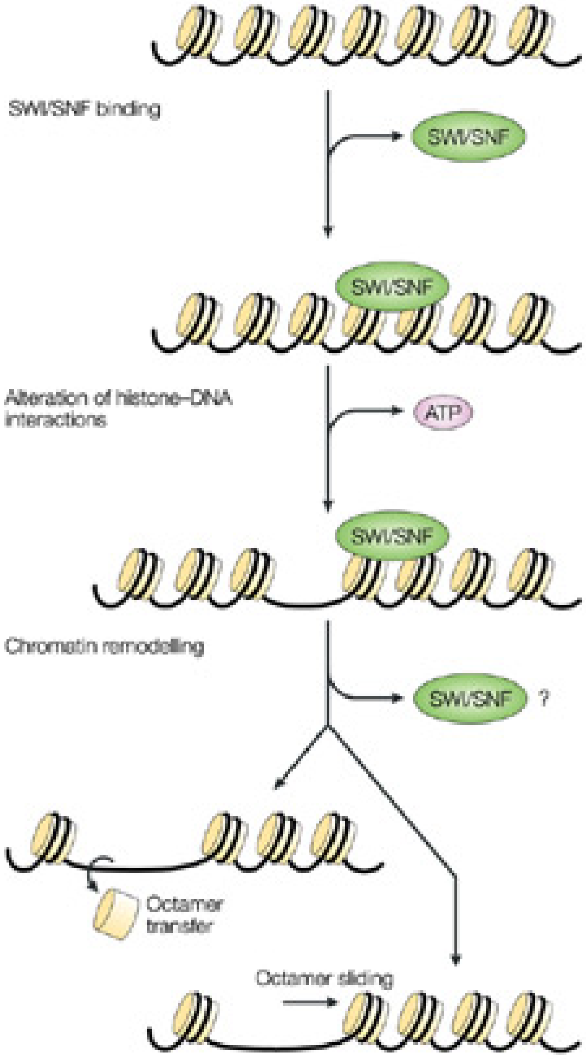


How is chromatin dynamics achieved?

ATP-dependent chromatin remodeling


SWI2/SNF2 family
ATPase domain
Bromo domain
ISWI family
ATPase domain
SANT domain
Mi-2 family
PHD fingers
chromo domain
ATPase domain
Yeast
SWI/SNF
SNF11
SWI1
Swp73
Swp82
SWI2/ SNF2
SWI3
SWI3
Arp9
SNF5
Arp7
SNF6
Swp29
RSC
RSC1
RSC2
RSC13
STH1
RSC6
RSC3
RSC7
RSC5
RSC8
RSC8
RSC14
Arp9
SFH1
Arp7
RSC4
RSC15
RSC9
RSC10
ISW1
p74
ISW1
p110
p105
ISW2
p140
ISW2
NuRD
MTA2
HDAC1
MBD3
RbA p48
Mi-2 CHD3/4
MTA1
RbA p46
HDAC2
Human
hSWI/SNF
BAF 250
Brg1
BAF60a
BAF60b
BAF60c
hBrm
BAF155
BAF170
BAF47
Ini1
BAF50
RSF
p325
hSNF2h
hACF/ WCRF
hACF/ WCRF180
hSNF2h
hCHRAC
hACF/ WCRF180
p15
hSNF2h
p17
Drosophila
dSWI/SNF
BAP74
Brm
BAP55
BAP155
BAP60
BAP47
Snr1
BAP111
NURF
NURF301
p55
ISWI
p38
CHRAC
ACF1
p16
ISWI
p14
ACF
ACF1
ISWI
ACF1(170)


a
b
c



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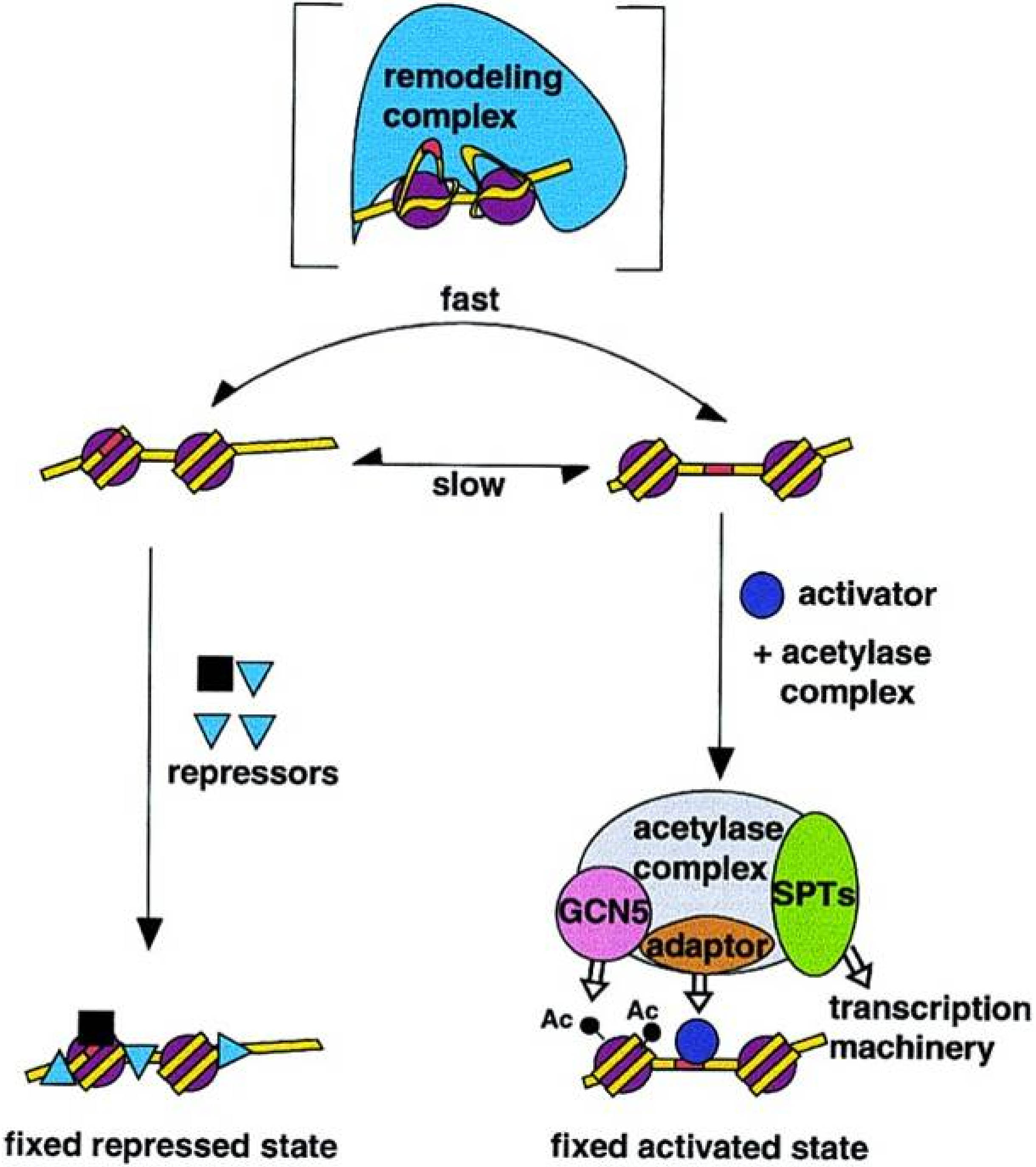


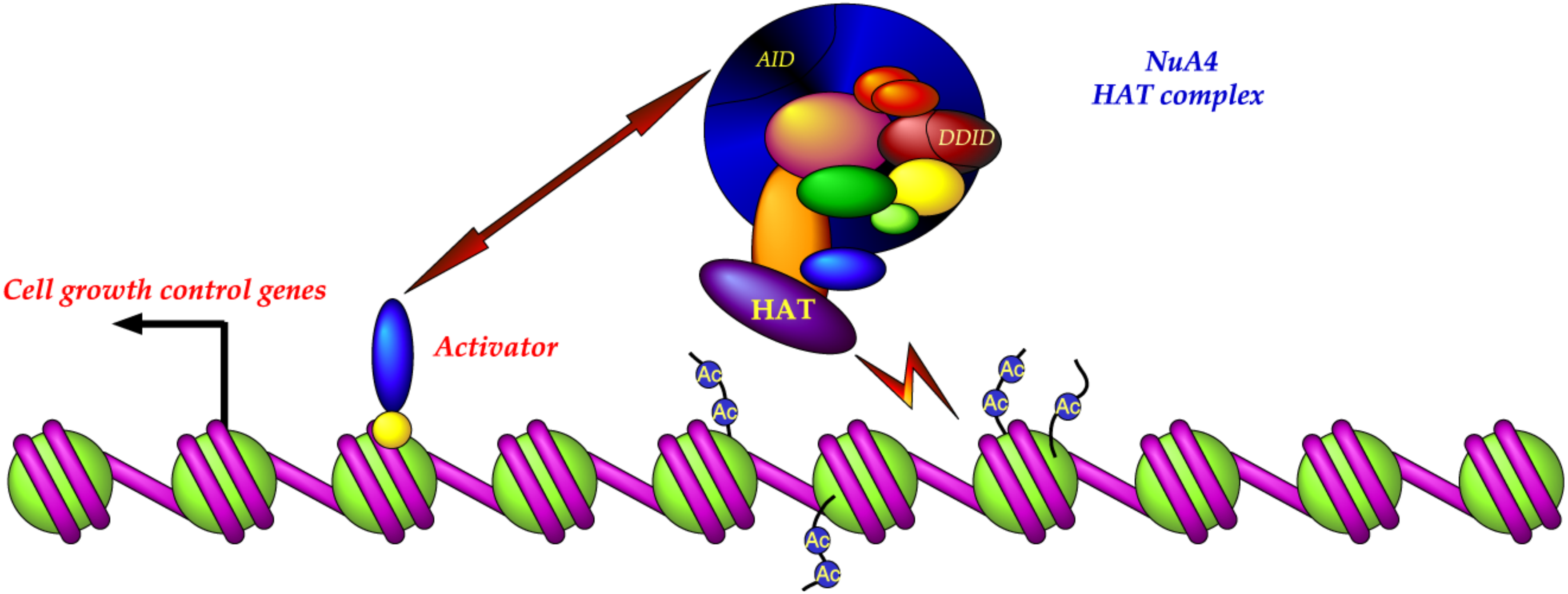
G&D 13:2339(1999)

Histone acetyltransferases (HAT)
or
Lysine acetyltransferases (KAT)

Table 2. K-Acetyltransferases (KATs; Formerly Acetyltransferases)

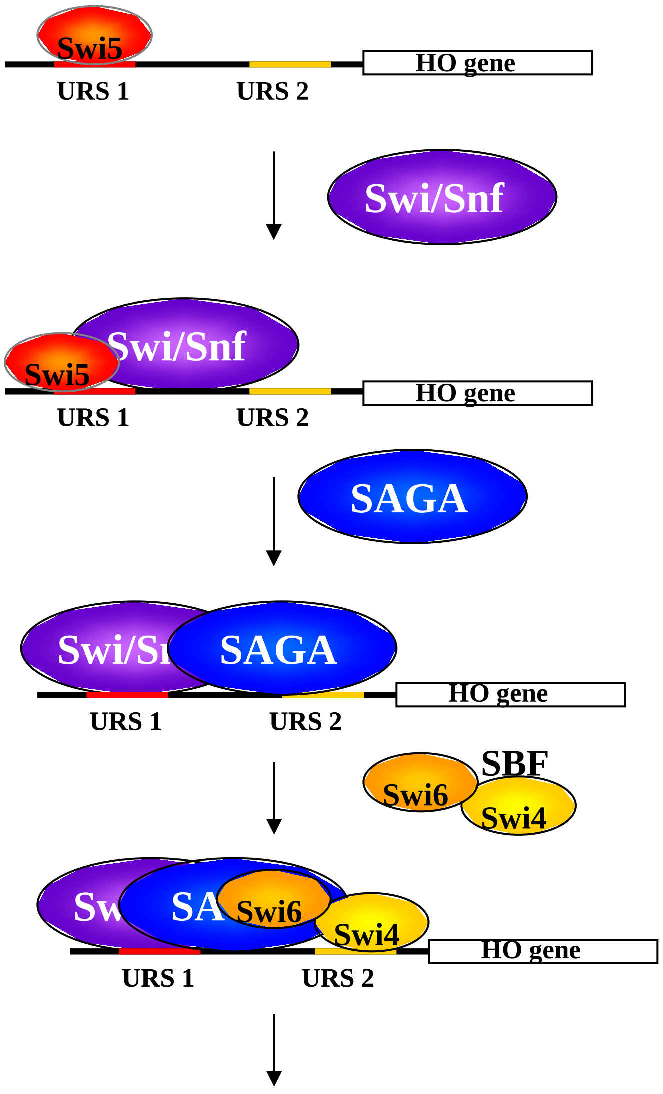
New Name	Human	*D. melanogaster*	*S. cerevisiae*	*S. pombe*	Substrate Specificity	Function
KAT1	HAT1	CG2051	Hat1	Hat1/ Hag603	H4 (5, 12)	Histone deposition, DNA repair
KAT2		dGCN5/PCAF	Gcn5	Gcn5	H3 (9, 14, 18, 23, 36)/ H2B; yHtzl (14)	Transcription activation, DNA repair
KAT2A	hGCN5				H3 (9, 14, 18)/H2B	Transcription activation
KAT2B	PCAF				H3 (9, 14, 18)/H2B	Transcription activation
KAT3		dCBP/NEJ			H4 (5, 8); H3 (14, 18)	Transcription activation, DNA repair
KAT3A	CBP				H2A (5); H2B (12, 15)	Transcription activation
KAT3B	P300				H2A (5); H2B (12, 15)	Transcription activation
KAT4	TAF1	dTAF1	Taf1	Taf1	H3 > H4	Transcription activation
KAT5	TIP60/PLIP	dTIP60	Esa1	Mst1	H4 (5, 8, 12, 16); H2A (yeast 4, 7; chicken 5, 9, 13, 15); dH2Av/yHtzl (14)	Transcription activation, DNA repair
KAT6		(CG1894)	Sas3	(Mst2)	H3 (14, 23)	Transcription activation and elongation, DNA replication
KAT6A	MOZ/MYST3	ENOK			H3 (14)	Transcription activation
KAT6B	MORF/MYST4				H3 (14)	Transcription activation
KAT7	HBO1/MYST2	CHM		(Mst2)	H4 (5, 8, 12) > H3	Transcription, DNA replication
KAT8	HMOF/MYST1	dMOF (CG1894)	Sas2	(Mst2)	H4 (16)	Chromatin boundaries, dosage compensation, DNA repair
KAT9	ELP3	dELP3/ CG15433	Elp3	Elp3	H3	
KAT10			Hap2		H3 (14); H4	
KAT11			Rtt109		H3 (56)	Genome stability, transcription elongation
KAT12	TFIIIC90				H3 (9, 14, 18)	Pol III transcription
KAT13A	SRC1				H3/H4	Transcription activation
KAT13B	ACTR				H3/H4	Transcription activation
KAT13C	P160				H3/H4	Transcription activation
KAT13D	CLOCK				H3/H4	Transcription activation

Recruitment of NuA4 in transcription activation

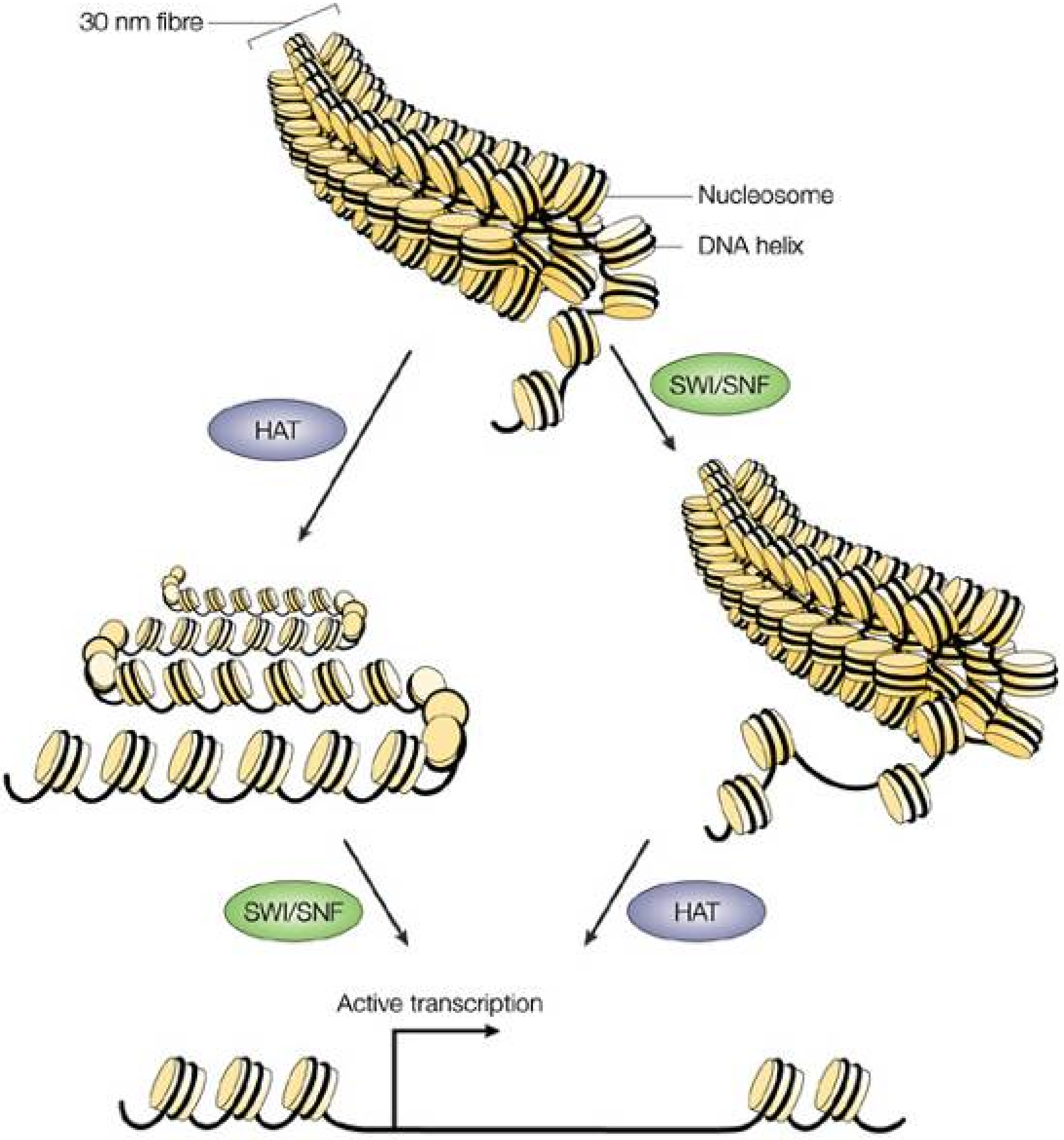


Cooperativity between Histone Acetylation and Chromatin Remodeling

Swi/Snf (ATP-dependent remodeler) and SAGA (HAT) cooperate to the activation of the HO gene in yeast



Transcription of HO



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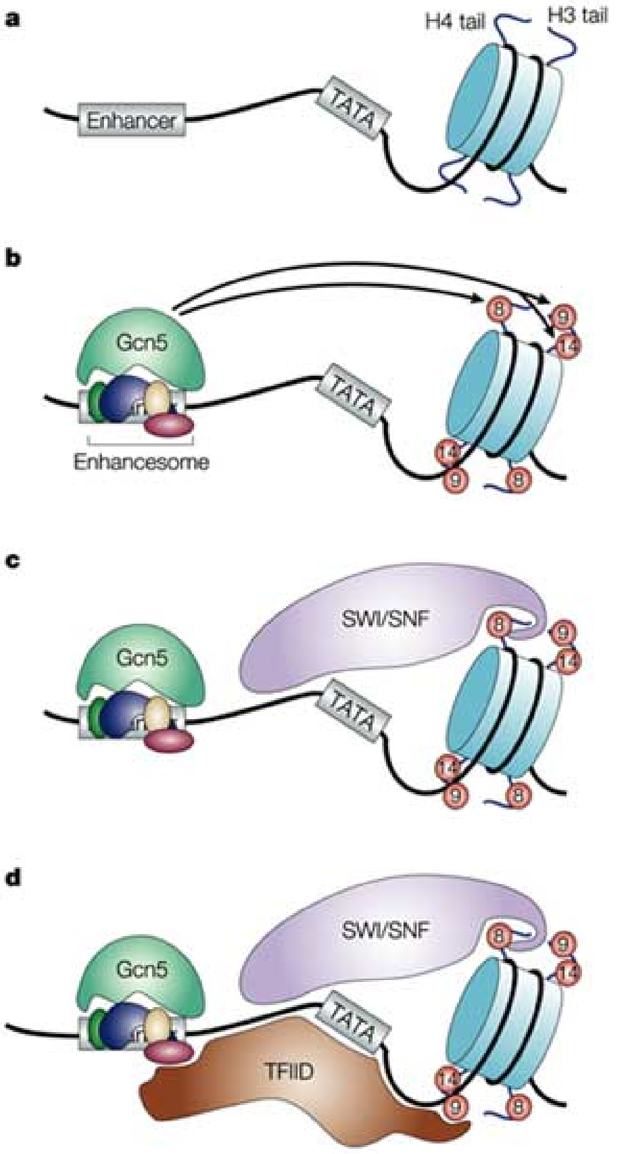

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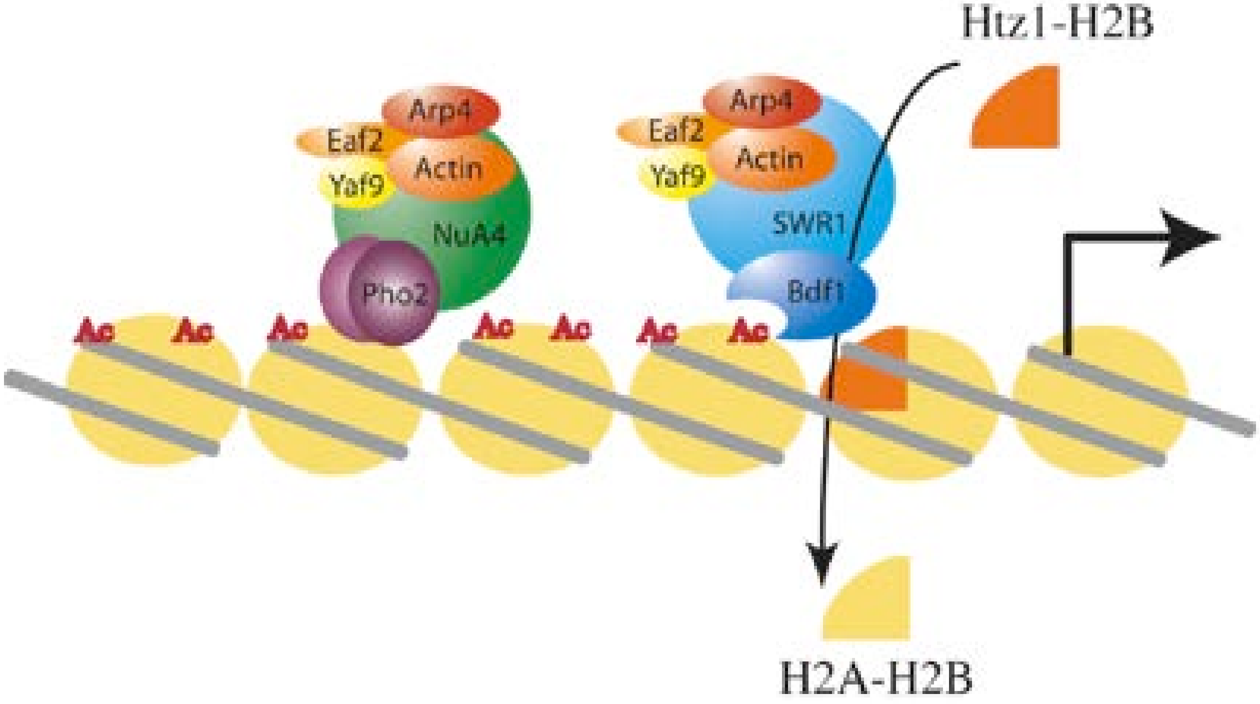
NuA4-dependent chromatin acetylation influences H2AZ deposition on chromatin

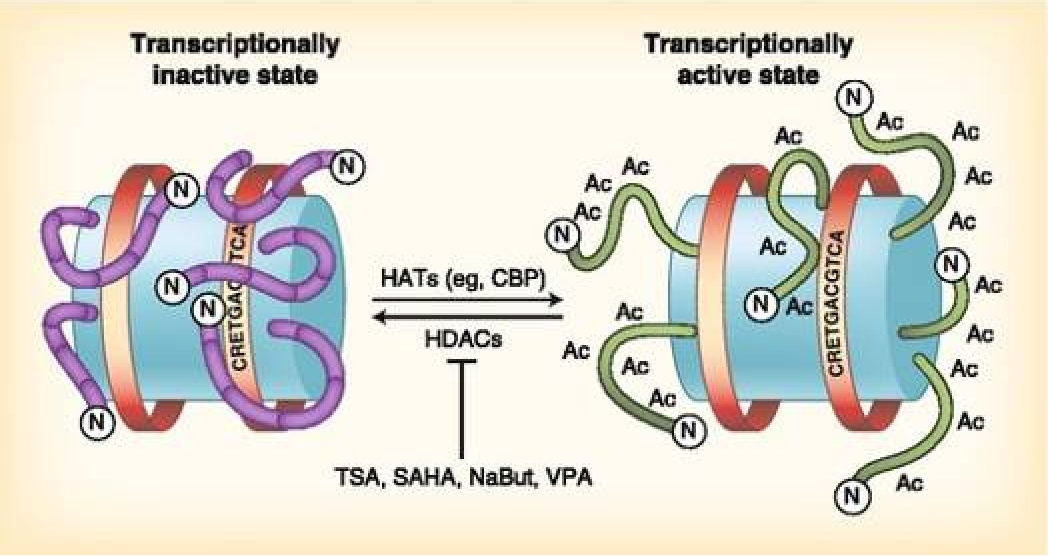


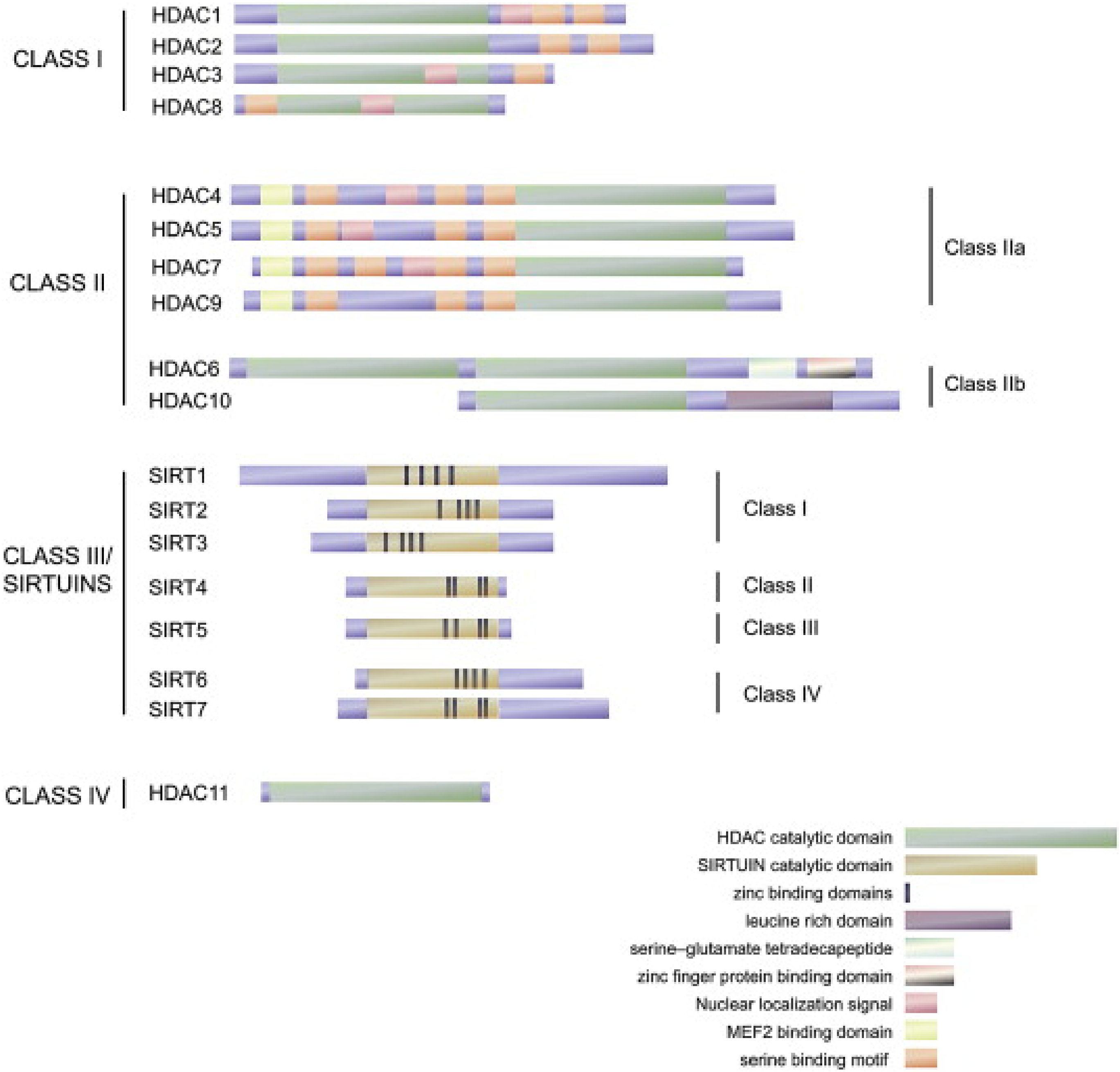
Histone deacetylases (HDAC)

or

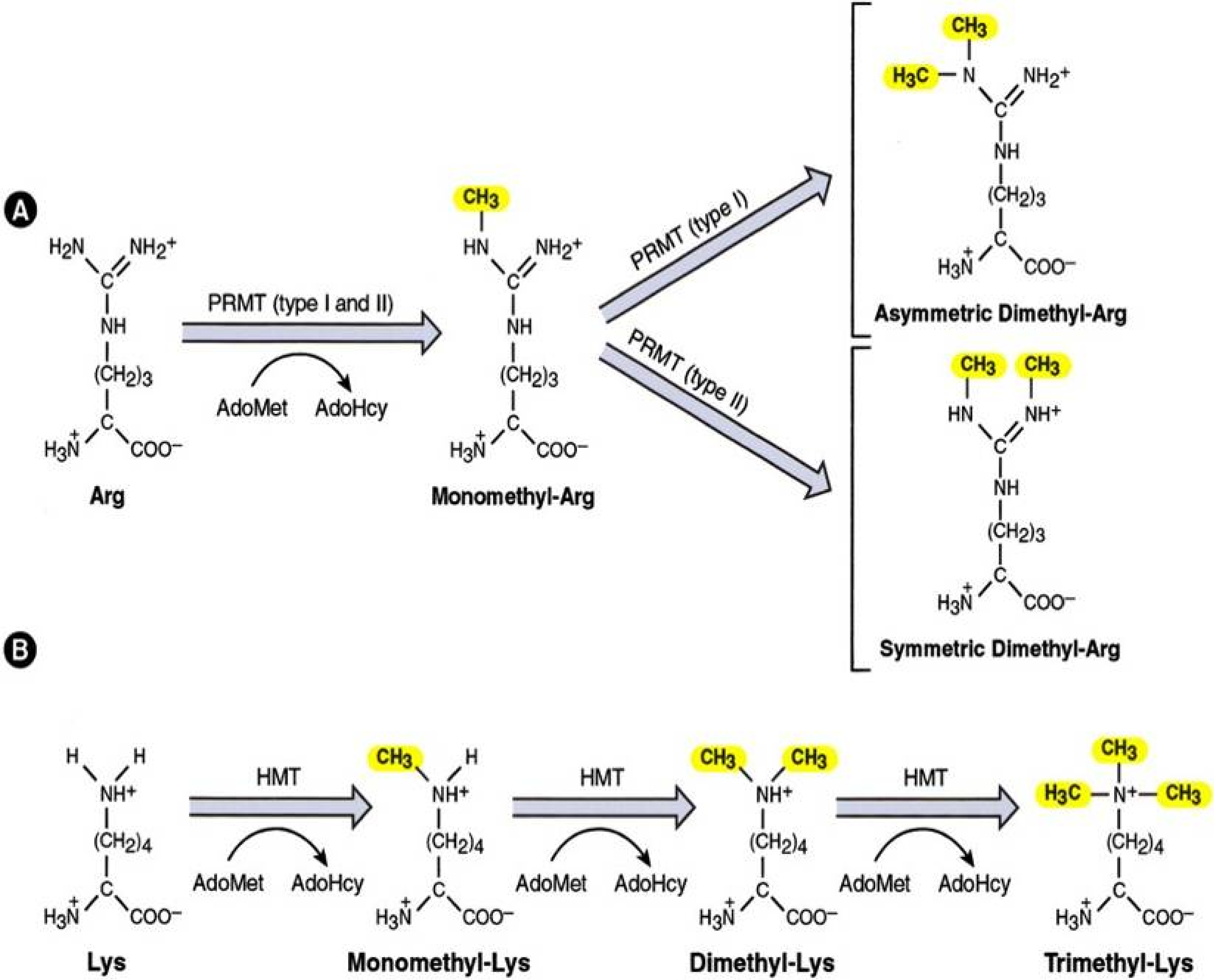
Lysine deacetylases (KDAC)

HDACs remove acetyl groups from acetylated histones



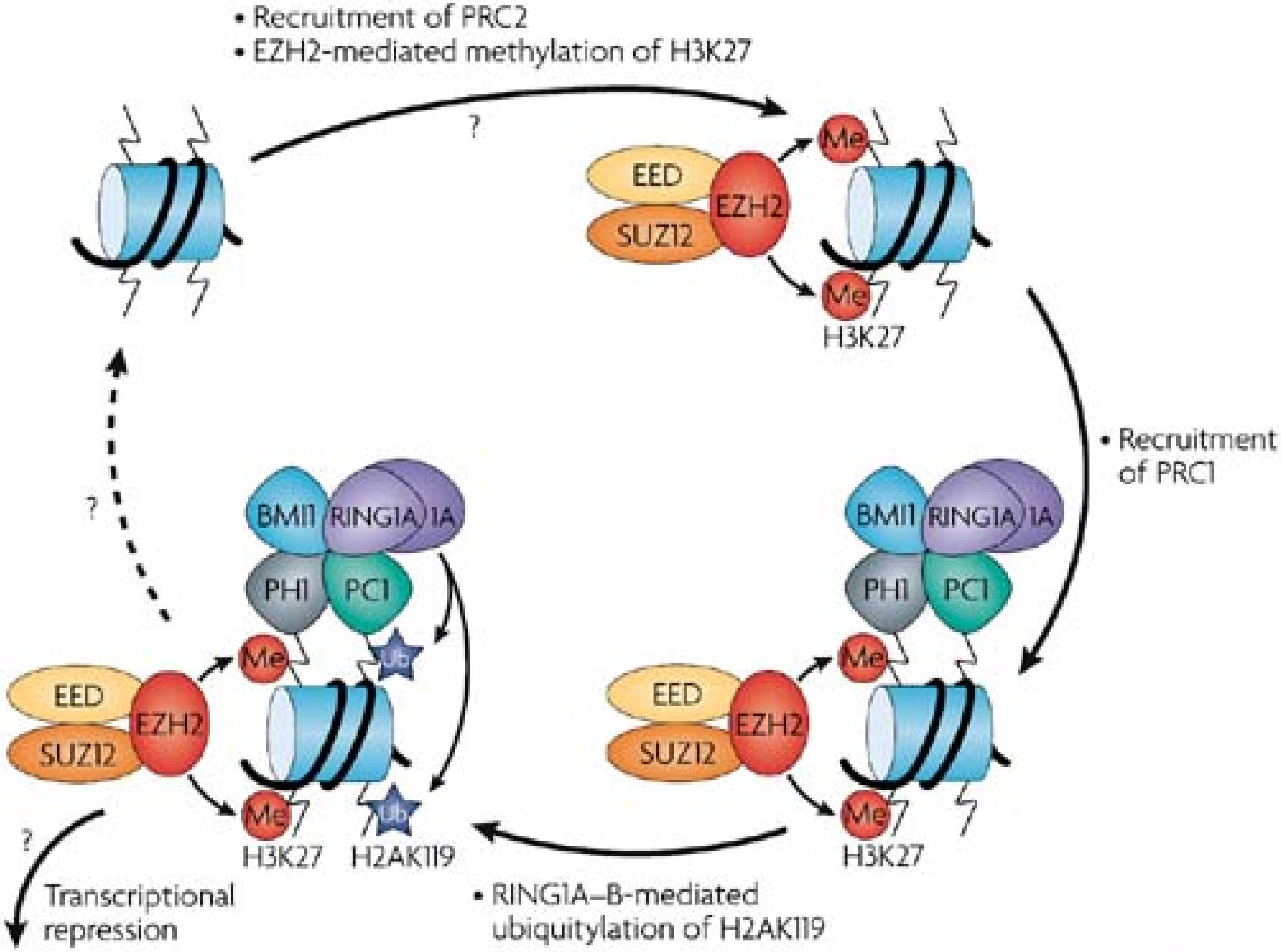

CLASS I
HDAC1
HDAC2
HDAC3
HDAC8
CLASS II
HDAC4
HDAC5
HDAC7
HDAC9
HDAC6
HDAC10
Class IIa
Class IIb
CLASS III/
SIRTUINS
SIRT1
SIRT2
SIRT3
SIRT4
SIRT5
SIRT6
SIRT7
Class I
Class II
Class III
Class IV
CLASS IV
HDAC11
HDAC catalytic domain
SIRTUIN catalytic domain
zinc binding domains
leucine rich domain
serine–glutamate tetradecapeptide
zinc finger protein binding domain
Nuclear localization signal
MEF2 binding domain
serine binding motif

Histone methyltransferase (HMT)
or
Lysine methyltransferase (KMT)


A
Arg
PRMT (type I and II)
AdoMet
AdoHcy
Monomethyl-Arg
PRMT (type I)
PRMT (type II)
Asymmetric Dimethyl-Arg
Symmetric Dimethyl-Arg
B
Lys
HMT
AdoMet
AdoHcy
Monomethyl-Lys
HMT
AdoMet
AdoHcy
Dimethyl-Lys
HMT
AdoMet
AdoHcy
Trimethyl-Lys

HMT group	HMT	Specificity	Function
Arginine	PRMT1	H4-R3 (non-histone proteins)	Transcriptional activation (signal transduction, etc)
	PRMT2	Unknown	Coactivator of estrogen receptor
	PRMT3	Unknown	Cytoplasmic (mitosis?)
	PRMT4/ CARM1	H3-R2, -R17, -R26. (Also at the C-terminal)	Transcriptional coactivator
	PRMT5	H2A, H4 (non-histone proteins)	Transcriptional repressor and spliceosome formation
Lysine SET domain	Suv39H1, Suv39H2	H3-K9	Heterochromatin formation, silencing
	G9a	H3-K9, H3-K27	Early embryogenesis role, transcriptional repression
	ASH1	H3-K4, -K9, H4-K20	Establishment of epigenetic, active transcription patterns
	Set1	H3-K4	Silencing
	Set2	H3-K36	Silencing, transcription
	Set7	H4-K20	Development, silent chromatin. Involved in aging
	Set9	H3-K4	Transcriptional activation
	ESC-E(z)	H3-K27	Polycomb-mediated silencing
	SETDB1	H3-K9	Silencing-mediated by the corepressor KAP-1
Dot	Dot1	H3-K79	Silencing by precluding Sir binding to bulk chromatin

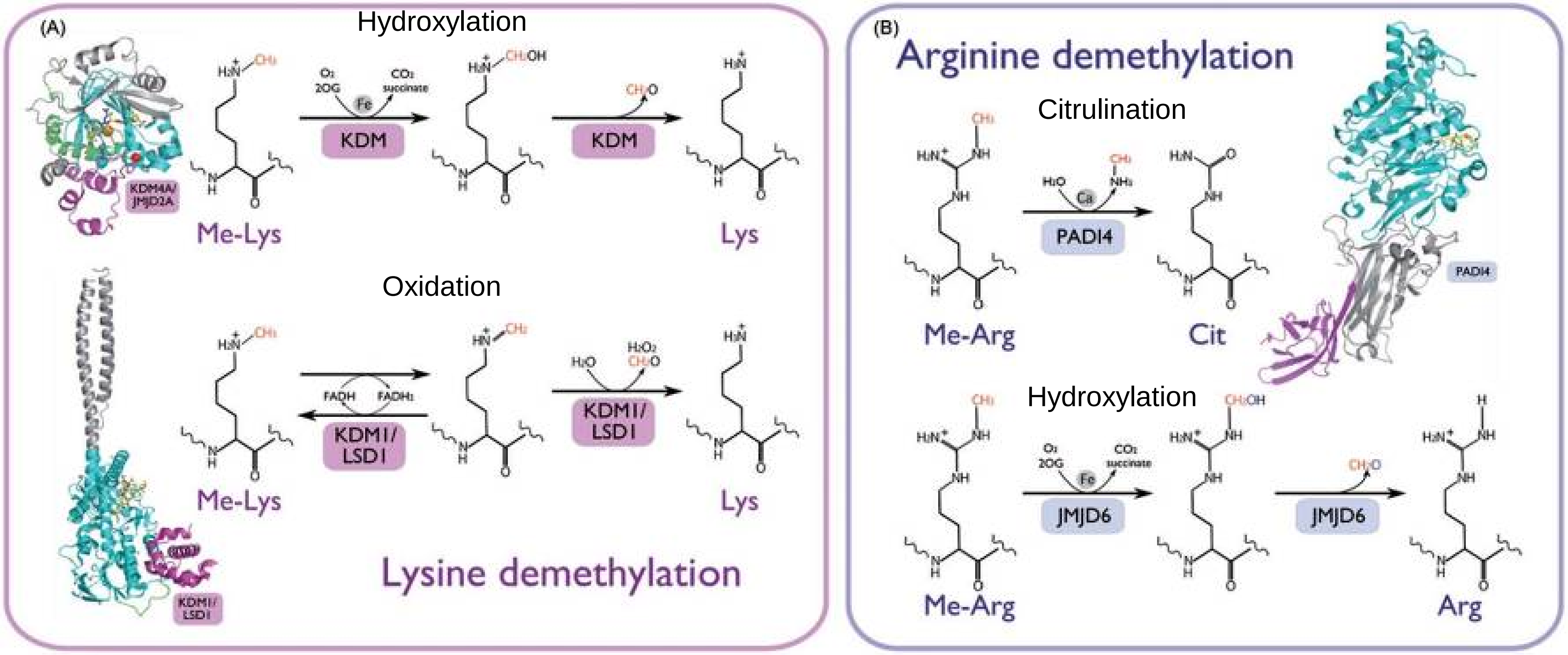
Repression by polycomb group proteins is a classical example of histone methylation-mediated repression



Nature Reviews | Genetics

Histone demethylases

Lysine and arginine demethylation can proceed via different mechanisms



Hydroxylation

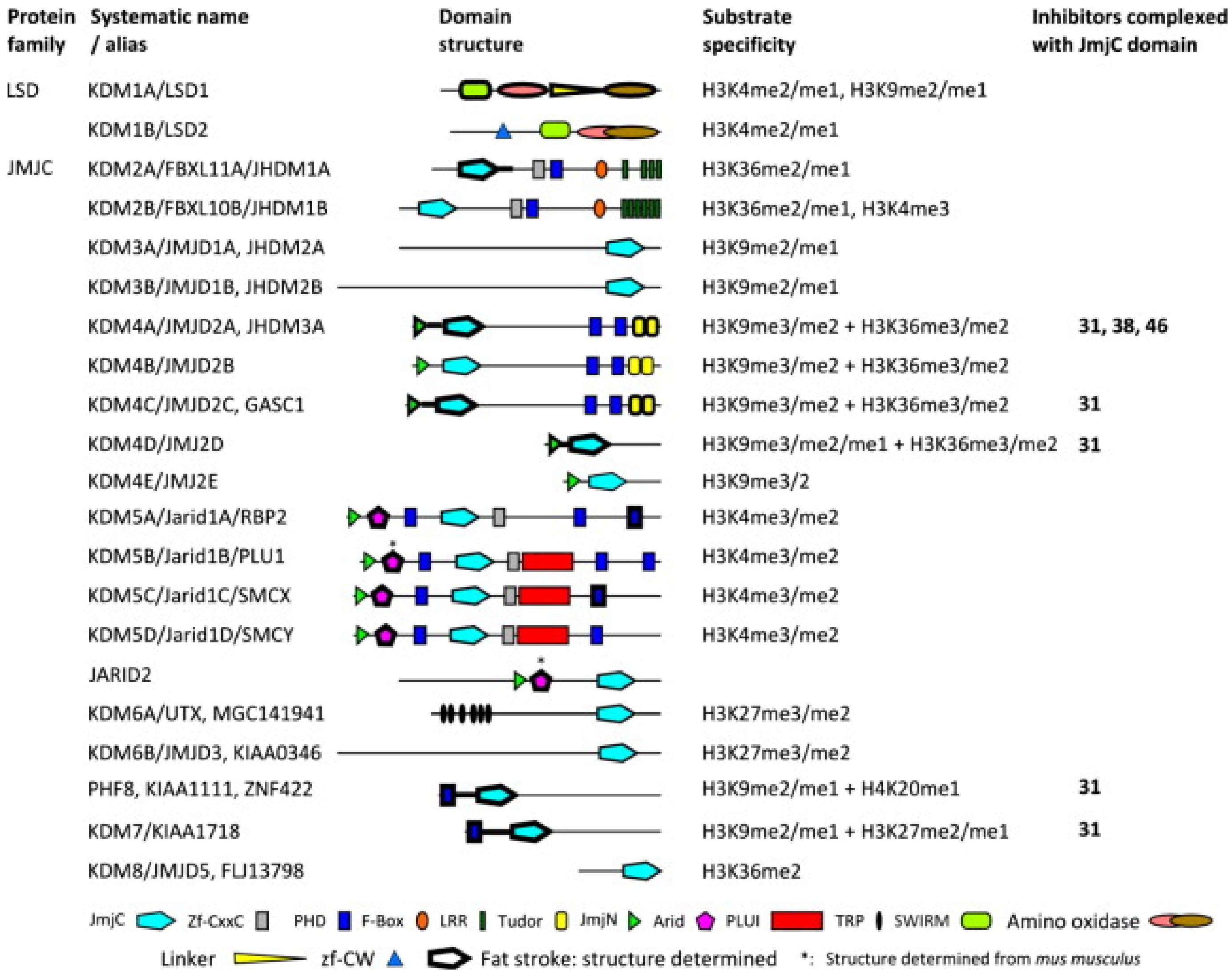
Oxidation

Citrulination

Hydroxylation

From Cell. Mol. Life Sci. 66 (2009) 407 – 422

Two families of lysine demethylases



Protein family	Systematic name / alias	Domain structure	Substrate specificity	Inhibitors complexed with JmjC domain
LSD	KDM1A/LSD1		H3K4me2/me1, H3K9me2/me1	
	KDM1B/LSD2		H3K4me2/me1	
JMJC	KDM2A/FBXL11A/JHDM1A		H3K36me2/me1	
	KDM2B/FBXL10B/JHDM1B		H3K36me2/me1, H3K4me3	
	KDM3A/JMJD1A, JHDM2A		H3K9me2/me1	
	KDM3B/JMJD1B, JHDM2B		H3K9me2/me1	
	KDM4A/JMJD2A, JHDM3A		H3K9me3/me2 + H3K36me3/me2	**31, 38, 46**
	KDM4B/JMJD2B		H3K9me3/me2 + H3K36me3/me2	
	KDM4C/JMJD2C, GASC1		H3K9me3/me2 + H3K36me3/me2	**31**
	KDM4D/JMJ2D		H3K9me3/me2/me1 + H3K36me3/me2	**31**
	KDM4E/JMJ2E		H3K9me3/2	
	KDM5A/Jarid1A/RBP2		H3K4me3/me2	
	KDM5B/Jarid1B/PLU1	*	H3K4me3/me2	
	KDM5C/Jarid1C/SMCX		H3K4me3/me2	
	KDM5D/Jarid1D/SMCY		H3K4me3/me2	
	JARID2	*		
	KDM6A/UTX, MGC141941		H3K27me3/me2	
	KDM6B/JMJD3, KIAA0346		H3K27me3/me2	
	PHF8, KIAA1111, ZNF422		H3K9me2/me1 + H4K20me1	**31**
	KDM7/KIAA1718		H3K9me2/me1 + H3K27me2/me1	**31**
	KDM8/JMJD5, FLJ13798		H3K36me2	

JmjC Zf-CxxC PHD F-Box LRR Tudor JmjN Arid PLUI TRP SWIRM Amino oxidase

Linker zf-CW Fat stroke: structure determined *: Structure determined from *mus musculus*

From Bioorganic & Medicinal Chemistry (2011)19(12), 3625–3636

Summary

- Chromatin is highly dynamic.
- It can me modified by:
  - Incorporation of H1, histone variants or non-histone proteins.
  - The action of ATP-dependent remodelers.
  - Various PTMs.
- Assembly of chromatin occurs at various extend all through the cell cycle and is assisted by various histone chaperones.
- Nucleosome positioning is regulated by cellular factors.
- Chromatin is an integrative aspect of all cellular processes involving DNA (transcription, DNA repair, DNA replication, etc.).
- Regulation of chromatin structure during these processes usually involve cooperation between several aspects of chromatin dynamics.

Other aspects not covered in this class

Non-coding RNAs are emerging as important players in chromatin structure and dynamics.

The structure and function of centromeric proteins during cell division.

Inheritance of chromatin states during DNA replication and cell division.

The role of chromatin in diseases.

Etc.