Forming the functional structure of the cell nucleus

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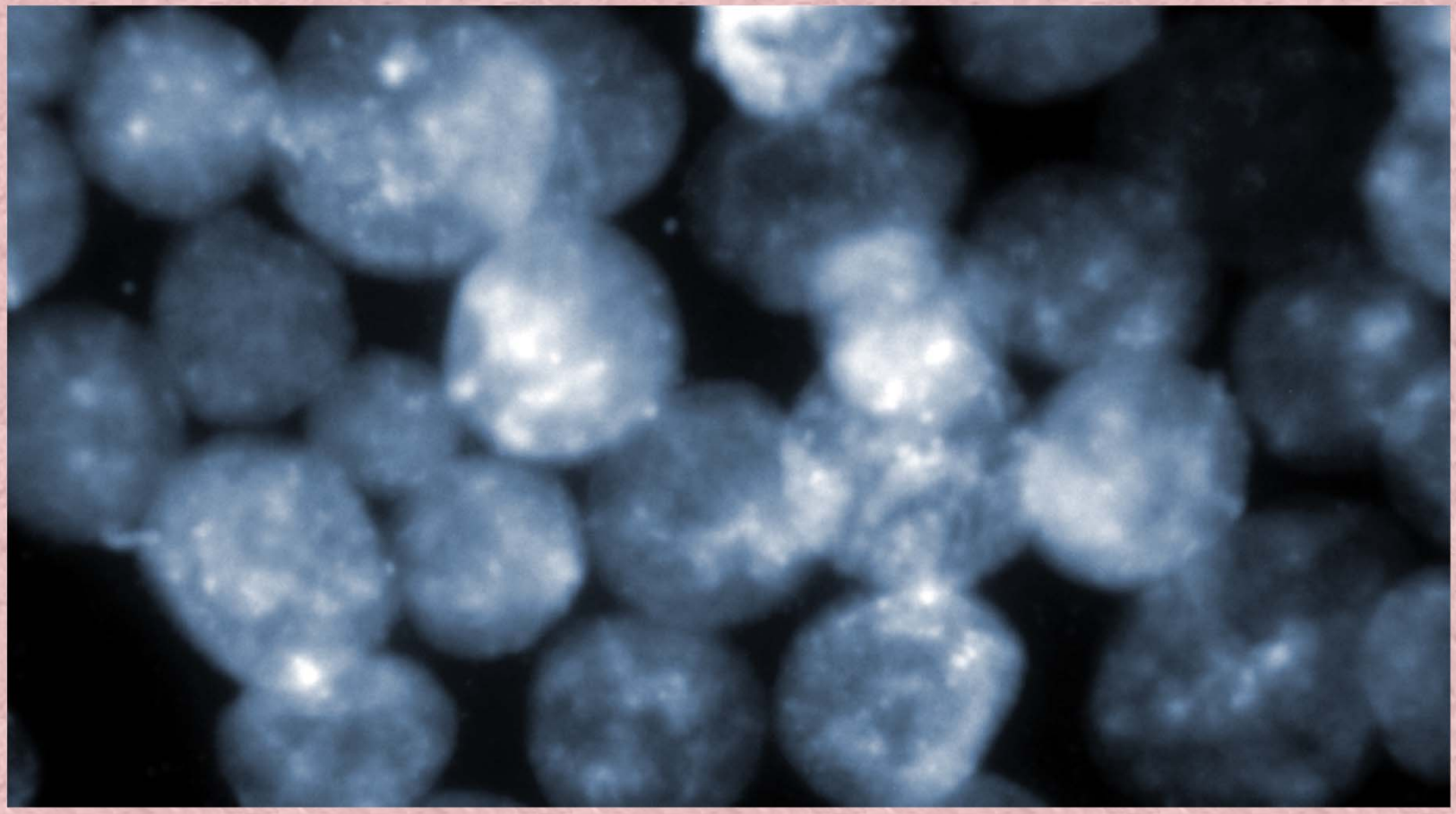
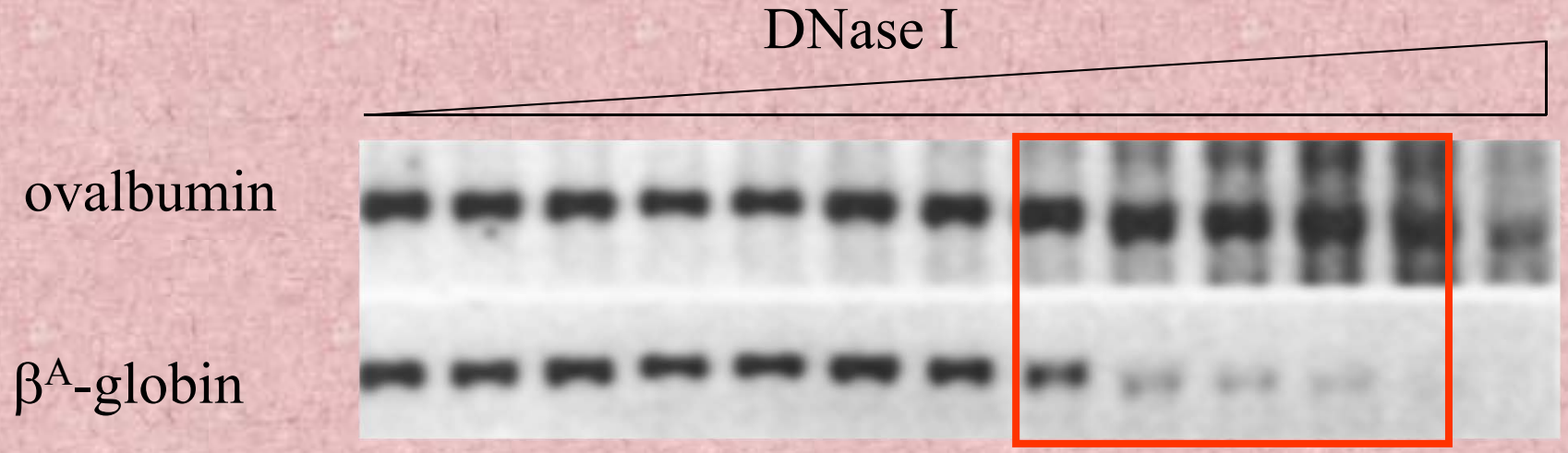
In interphase nucleus the DNA is not packed uniformly. There are more compact regions (heterochromatin) and less compact regions (euchromatin).

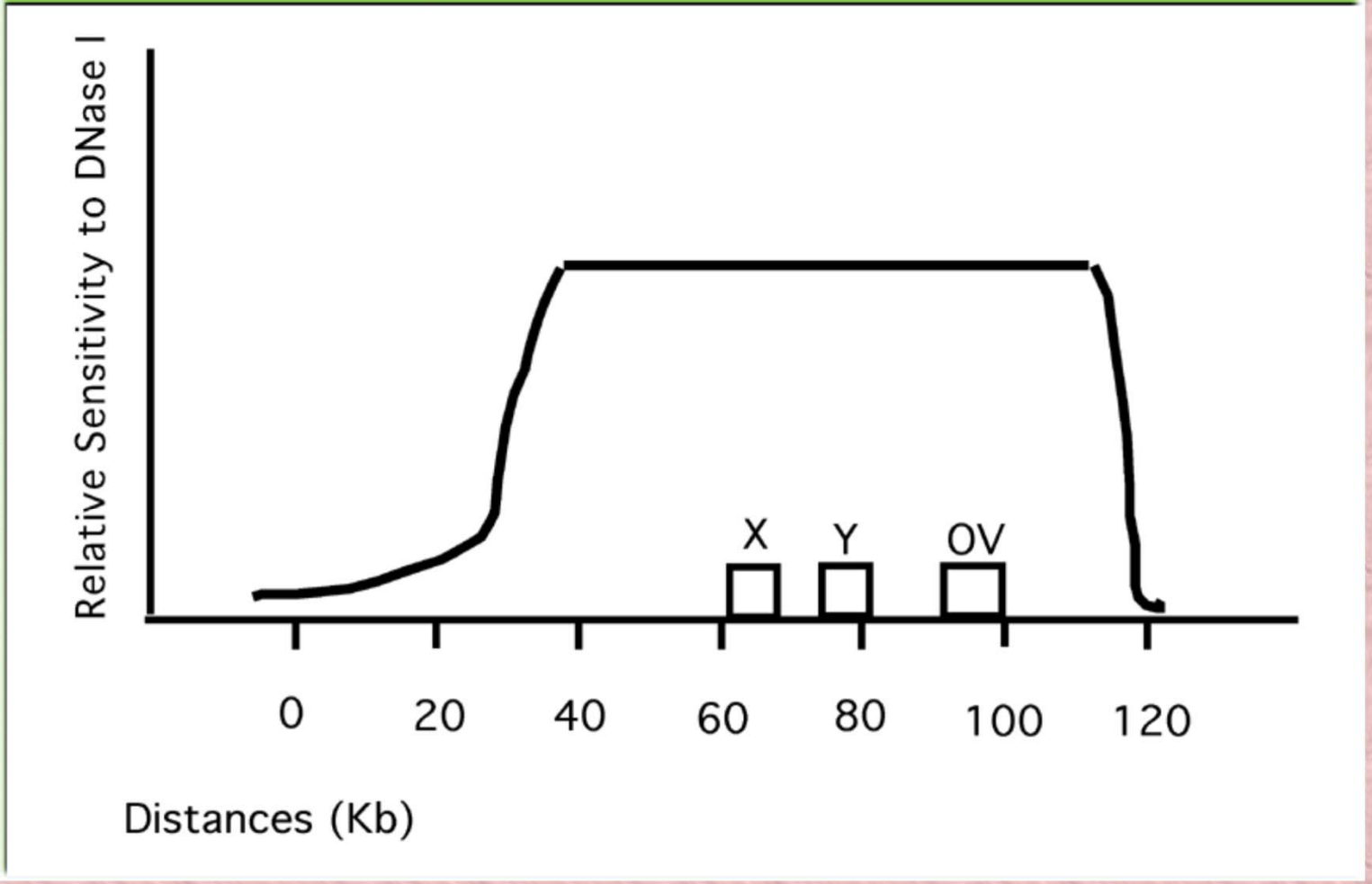


Active genes are preferentially sensitive to DNase I

In erythroid cells the globin genes are preferentially sensitive to DNase I



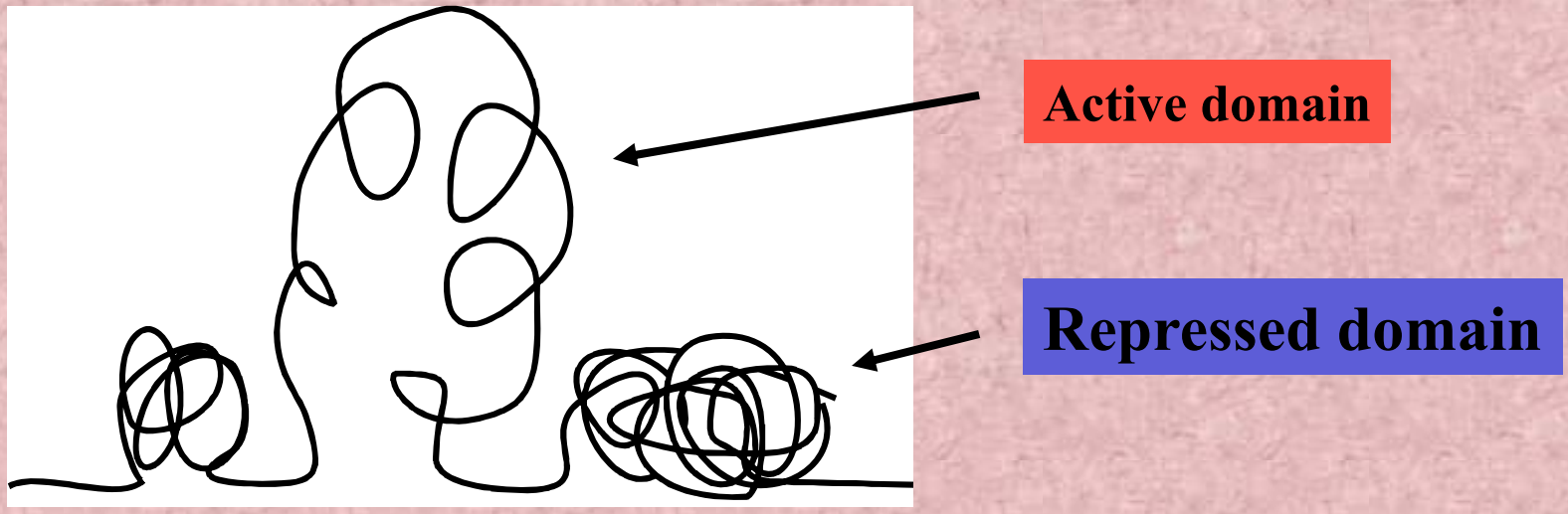
Chicken ovalbumin genes and flanking DNA sequences are located within 100 kb long DNaze - sensitive domain that has relatively sharp borders (Lawson et al., 1982, J. Biol. Chem. 257, 1501)

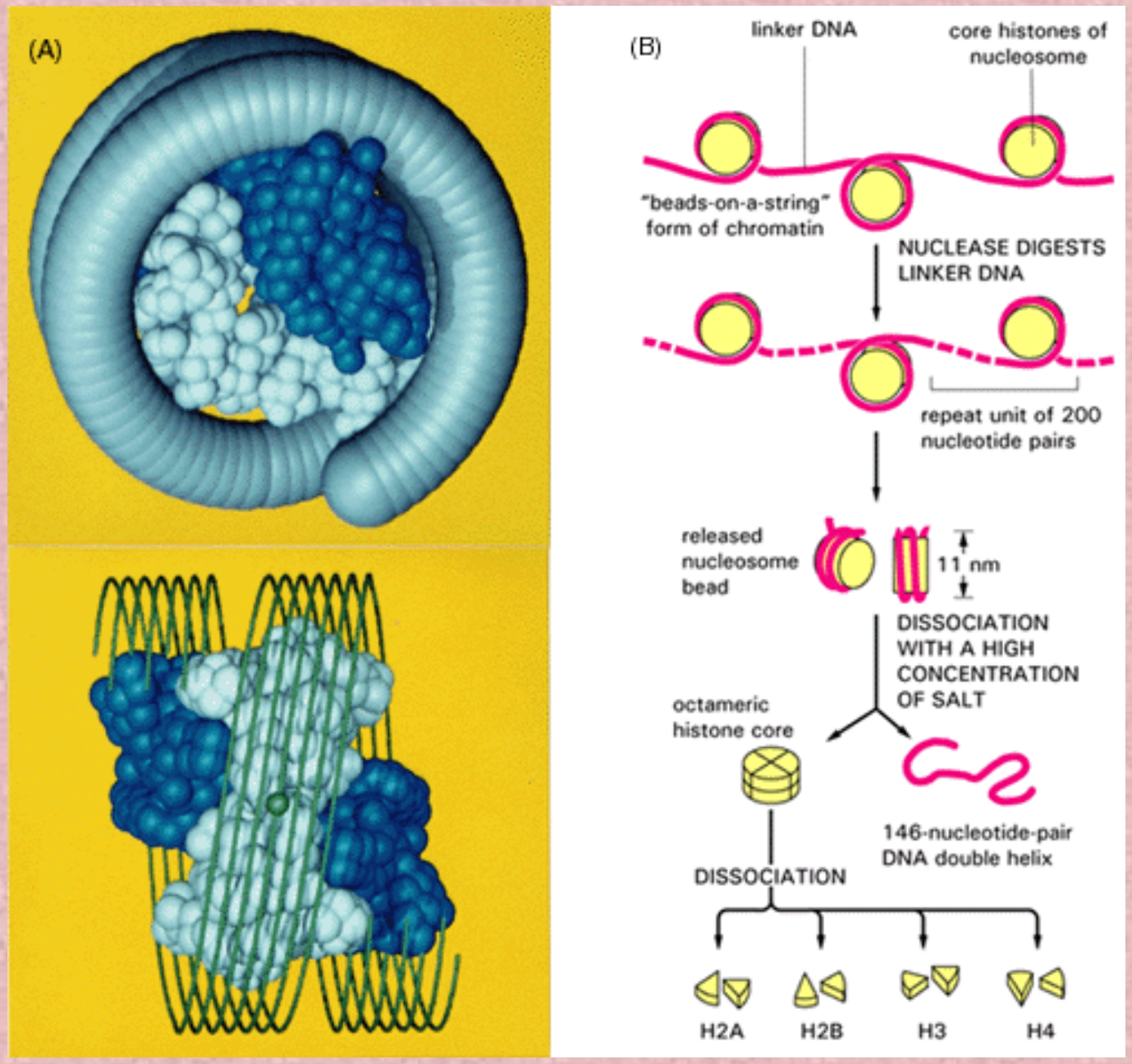


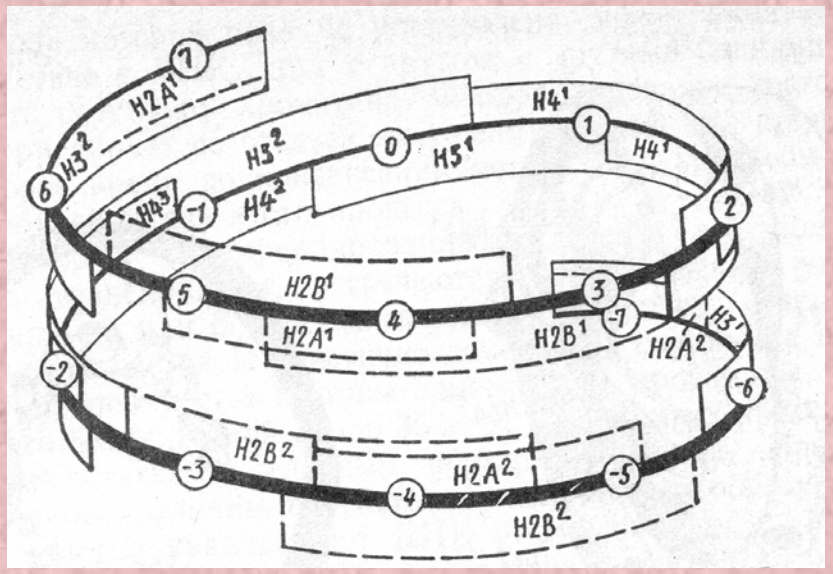
Domain hypothesis of eukaryotic genome organization

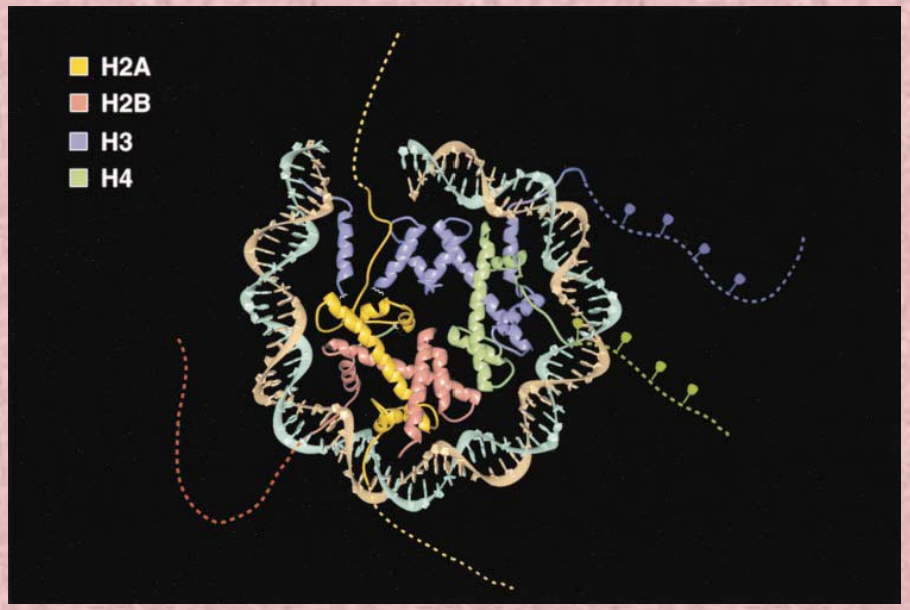
The whole genome is built from similar blocks – domains that can include one or several genes

The domains are the targets for special control mechanisms that regulate transcriptional status of the domains by changing the mode of DNA packaging within domains




(A)
(B)
linker DNA
core histones of nucleosome
"beads-on-a-string" form of chromatin
NUCLEASE DIGESTS LINKER DNA
repeat unit of 200 nucleotide pairs
released nucleosome bead
11 nm
DISSOCIATION WITH A HIGH CONCENTRATION OF SALT
octameric histone core
146-nucleotide-pair DNA double helix
DISSOCIATION
H2A
H2B
H3
H4

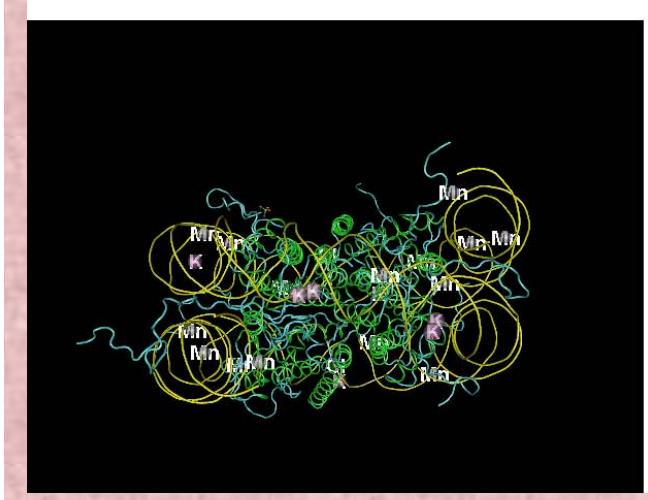


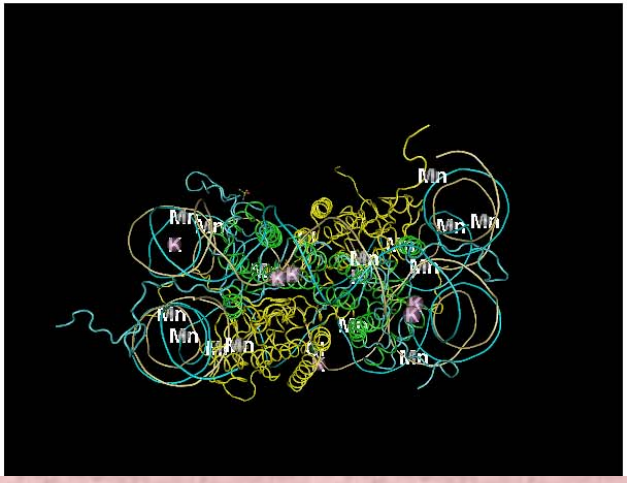


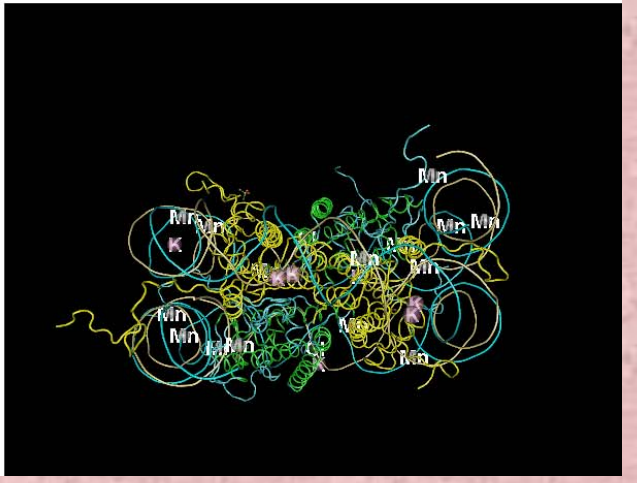
ADN

H2A-H2B

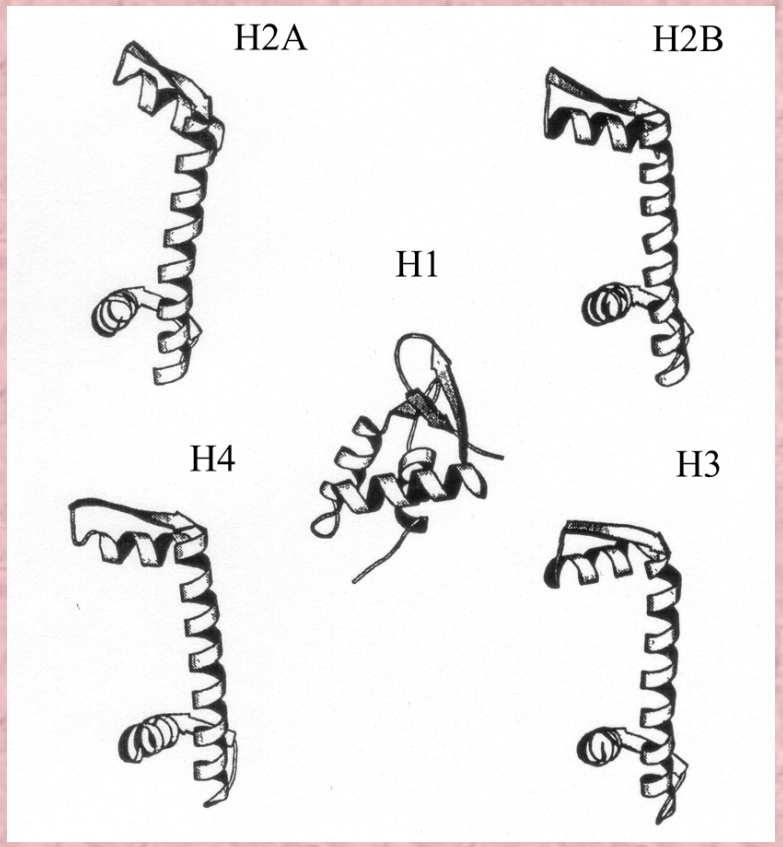
H3-H4

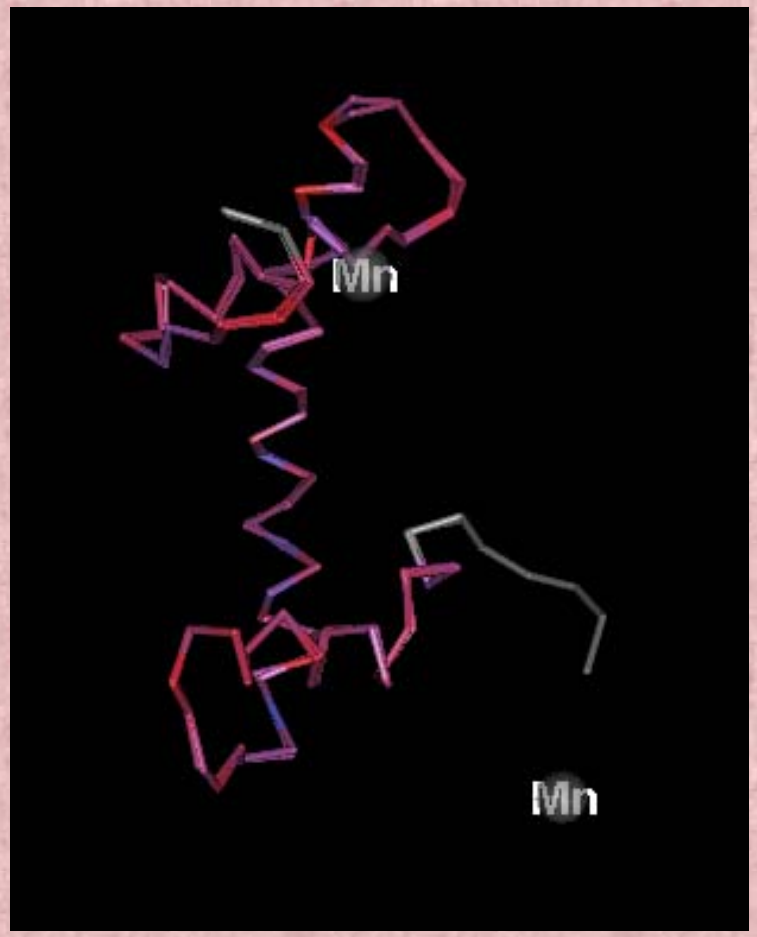




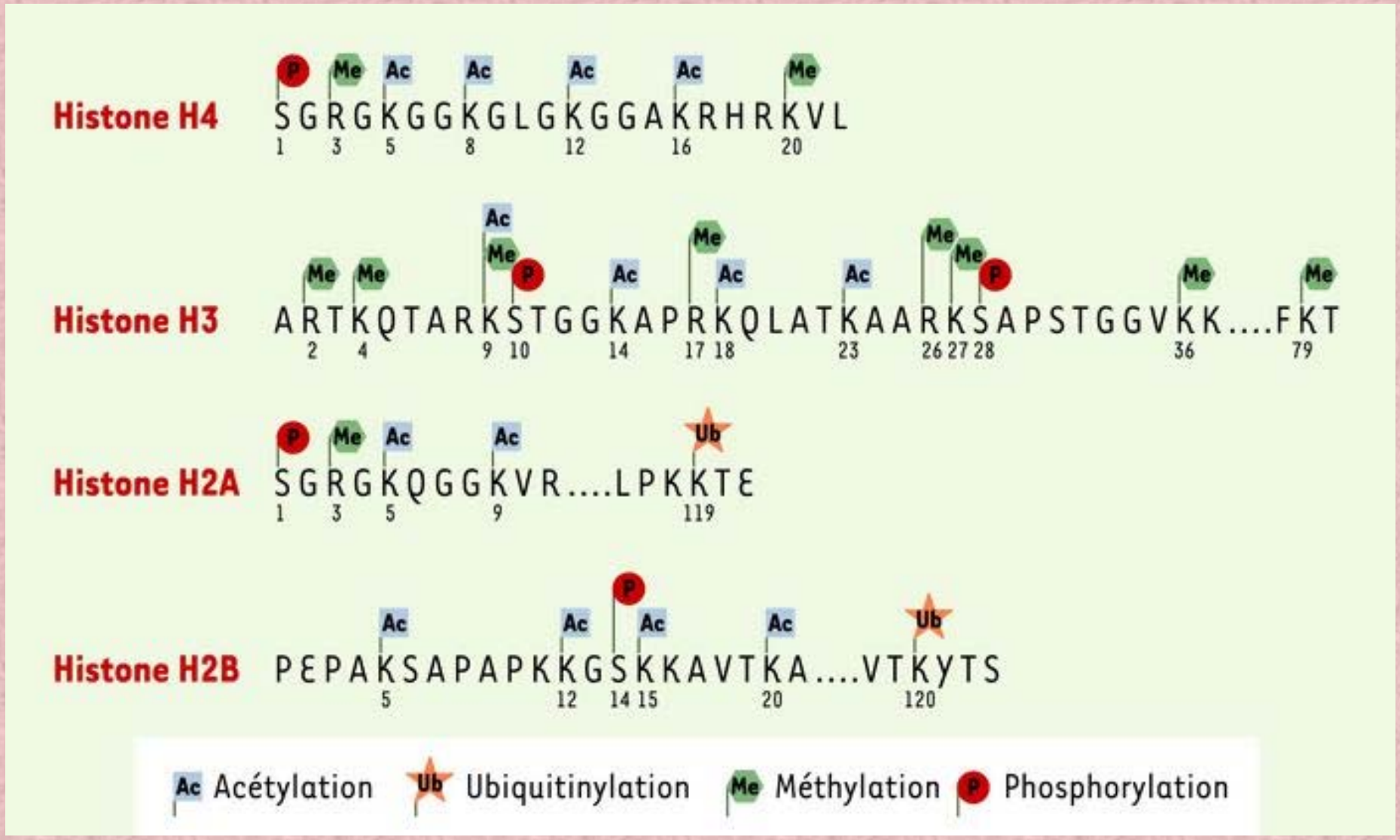


Secondary structure of all nucleosomal histones is characterized by the presence be the presence of long central alpha-heix domain (**histone fold**) which is flanked from both sides by domains containing short alpha-helixes and loops. 15-30 amino-acid residues at the N-terminal ends of all nucleosomal histones are not organized in any structures. They are frequently referred to as N-terminal “tails”.



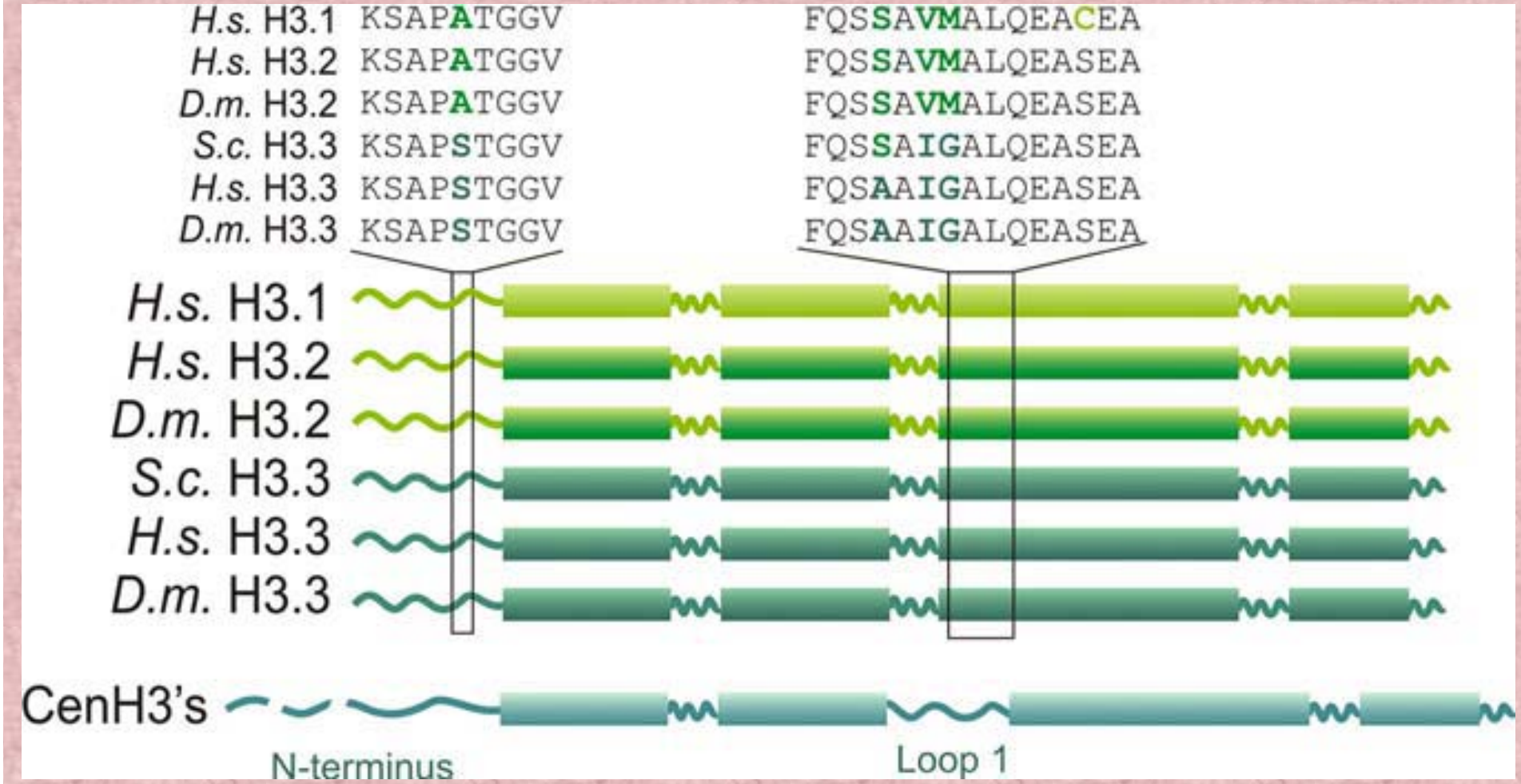


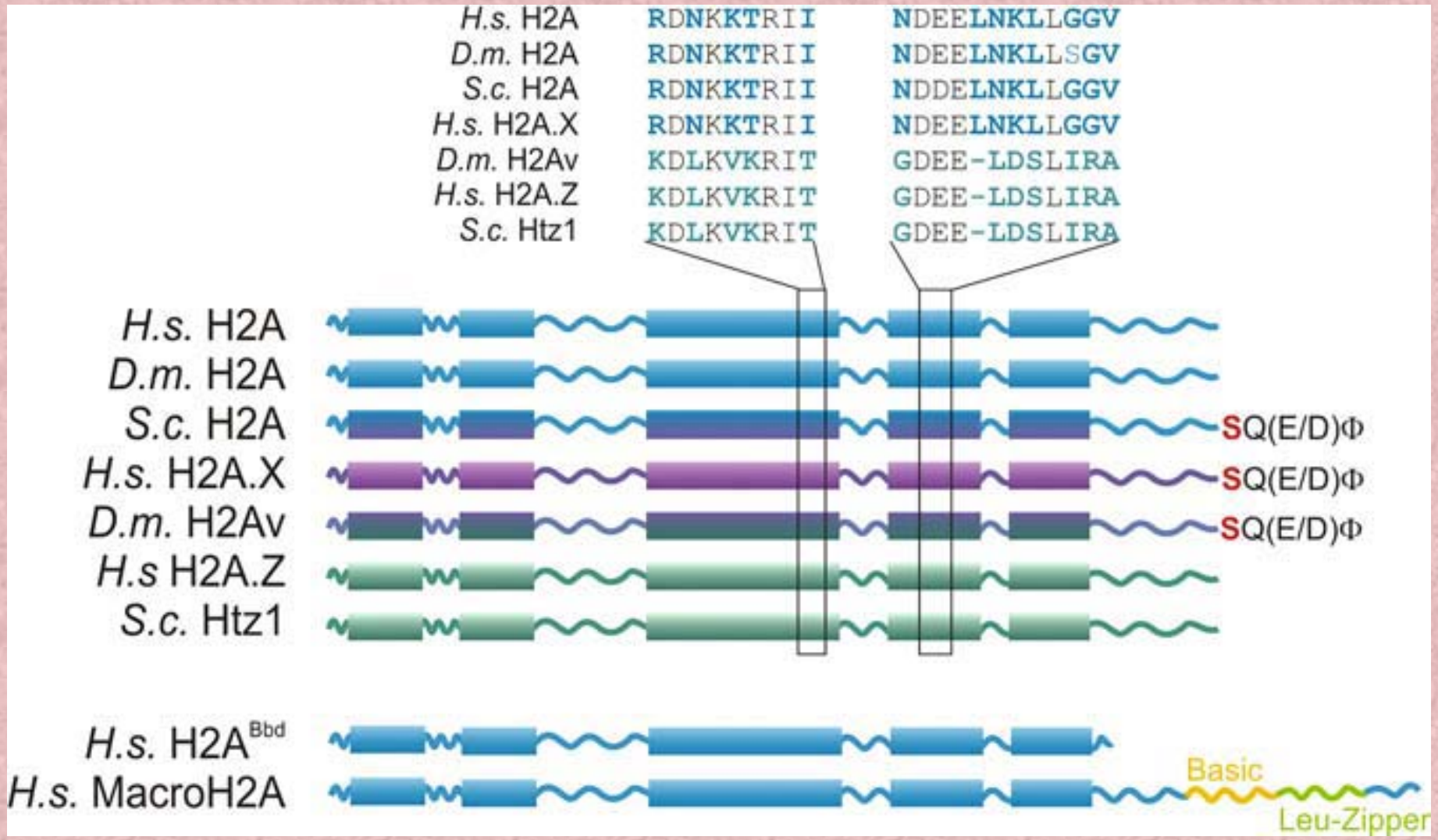
N-terminal domains of nucleosomal histones are frequently modified (acethylated, methylated, phosphorylated or ubiquitinilated)



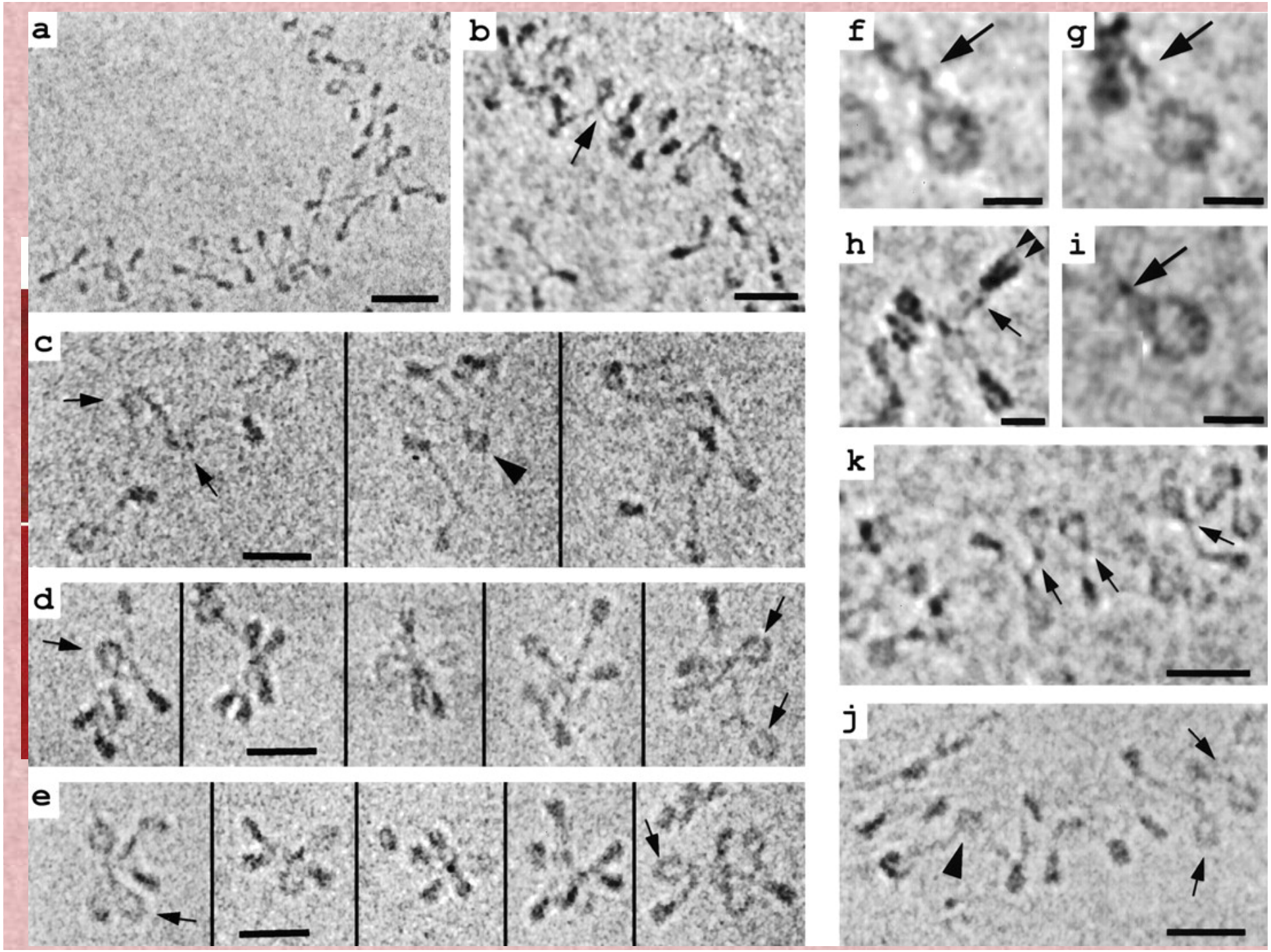
Different variant forms of histones encoded by separate genes were identified

Histones	Cell-cycle expression	Localization
H2A (15)	RD	TG
H2A.X (1)	RI	TG[a]
H2A.Z (1)	RI	TG[b]
H2A.Bbd (1)	ND	Xi exclusion
MacroH2A1.1 (1)	ND	Xi
MacroH2A1.2 (1#)	ND	Xi
MacroH2A2 (1)	ND	Xi
H2B (17)	RD	TG
spH2B (ND) (TS)	ND	Telomeres
H2BFWT (1) (TS)	ND	Telomeres?
hTSH2B (1) (TS)	ND	ND
H3.1 (10)	RD	ND
H3.2 (1)	RD	ND
H3.3 (2)	RI	Euchromatin
H3.1t (1) (TS)	RD?	ND
CENP-A (1)	RI	Centromeres
H4 (14)	RD	TG


H.s. H3.1 KSAPATGGV FQSSAVMALQEACEA
H.s. H3.2 KSAPATGGV FQSSAVMALQEASEA
D.m. H3.2 KSAPATGGV FQSSAVMALQEASEA
S.c. H3.3 KSAPSTGGV FQSSAIGALQEASEA
H.s. H3.3 KSAPSTGGV FQSAAIGALQEASEA
D.m. H3.3 KSAPSTGGV FQSAAIGALQEASEA
H.s. H3.1
H.s. H3.2
D.m. H3.2
S.c. H3.3
H.s. H3.3
D.m. H3.3
CenH3's
N-terminus
Loop 1


H.s. H2A RDNKKTRII NDEELNKLLGGV
D.m. H2A RDNKKTRII NDEELNKLLSGV
S.c. H2A RDNKKTRII NDDELNKLLGGV
H.s. H2A.X RDNKKTRII NDEELNKLLGGV
D.m. H2Av KDLKVKRIT GDEE-LDSLIRA
H.s. H2A.Z KDLKVKRIT GDEE-LDSLIRA
S.c. Htz1 KDLKVKRIT GDEE-LDSLIRA
H.s. H2A
D.m. H2A
S.c. H2A
H.s. H2A.X
D.m. H2Av
H.s H2A.Z
S.c. Htz1
SQ(E/D)Φ
SQ(E/D)Φ
SQ(E/D)Φ
H.s. H2A^Bbd
H.s. MacroH2A
Basic
Leu-Zipper

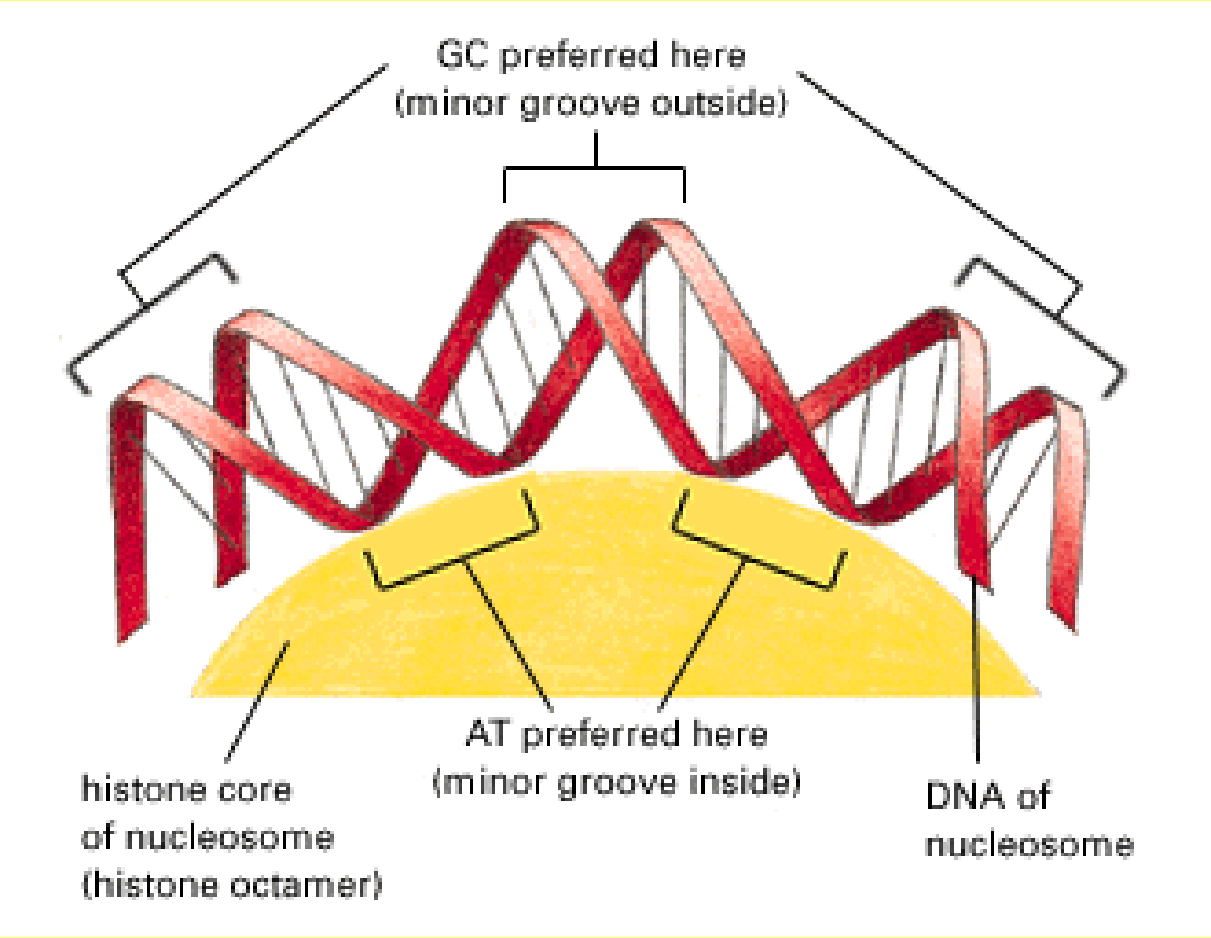
Nucleosomal particles are not identical

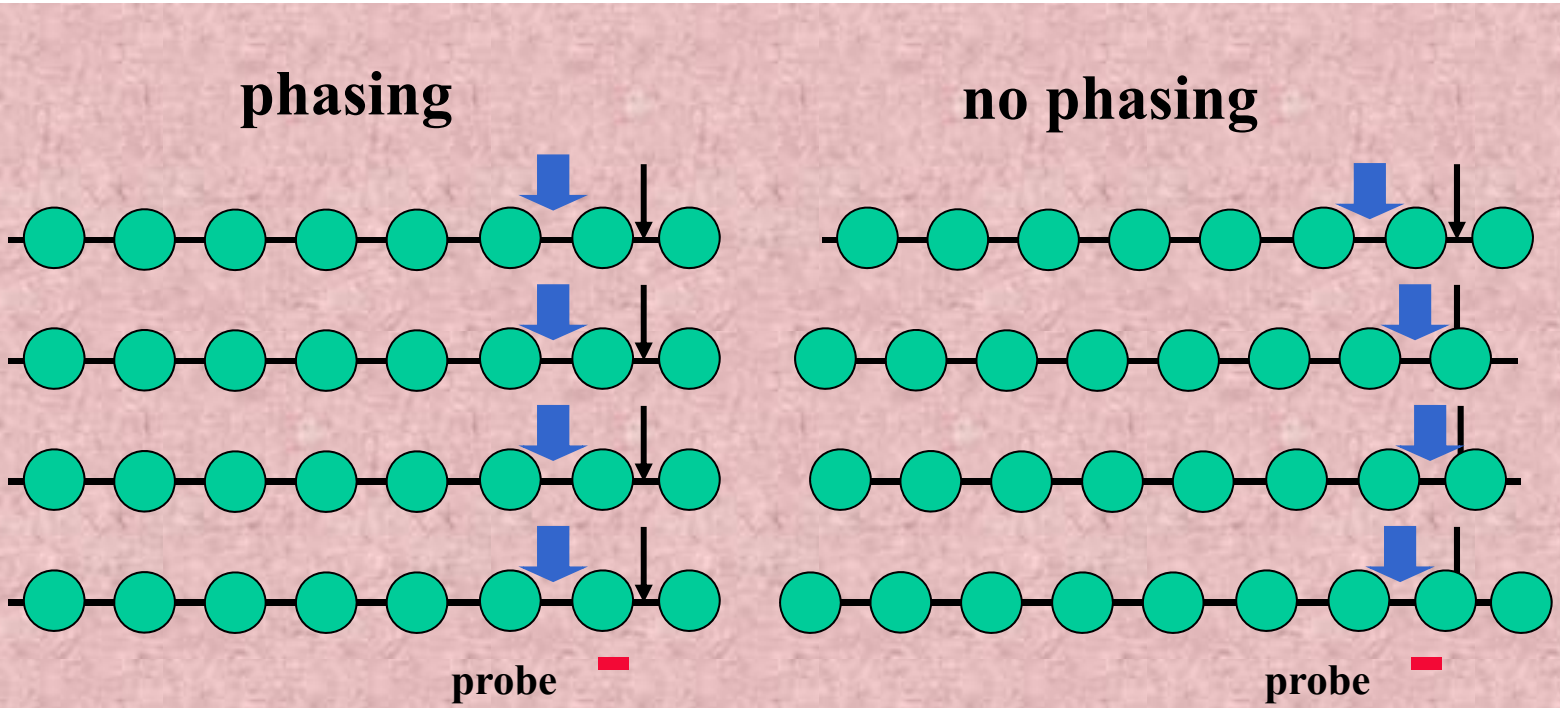

a
b
c
d
e
f
g
h
i
k
j

Nucleotide bases do not contact with DNA. Consequently, interaction of nucleosomal core
rand
from
core

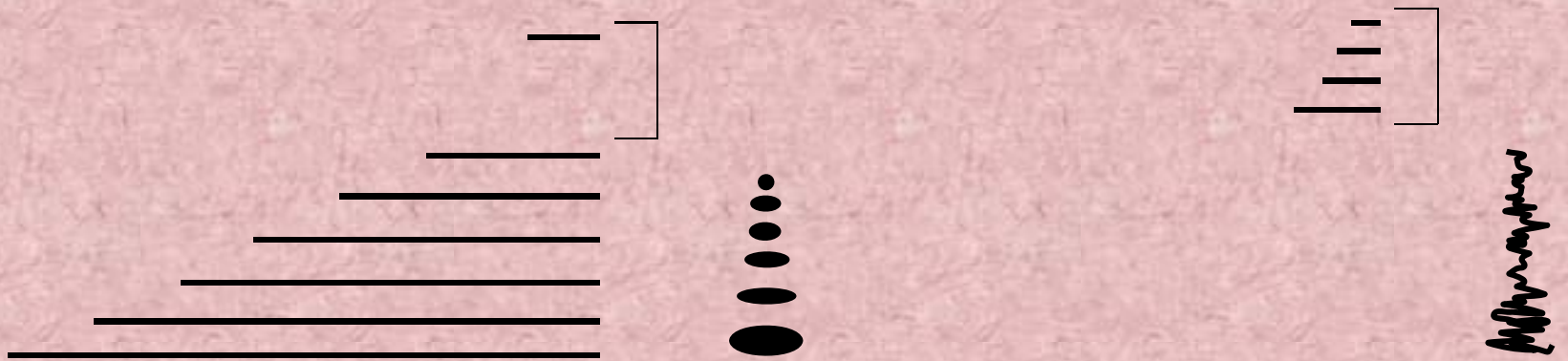
ma

Some DNA sequences are preferable for localization of nucleosomes due to enhanced ability of these sequences to wound around the nucleosomal core.





distribution of signals after treatment with micrococcal nuclease, isolation of DNA, treatment with restriction enzyme, electrophoresis, blotting and hybridization



Distribution of nucleosomes along the DNA reflects the ability of DNA to wound around the histone octamer.

Preferential positions of nucleosomes on DNA can be predicted by using special computer programs

In chromatin positions of nucleosomal cores may differ from the predicted because of interaction of sequence-specific DNA-binding proteins ex.: transcription factors) with their recognition sequences

FASING – fixed localisation of nucleosomes on the underlying DNA sequence

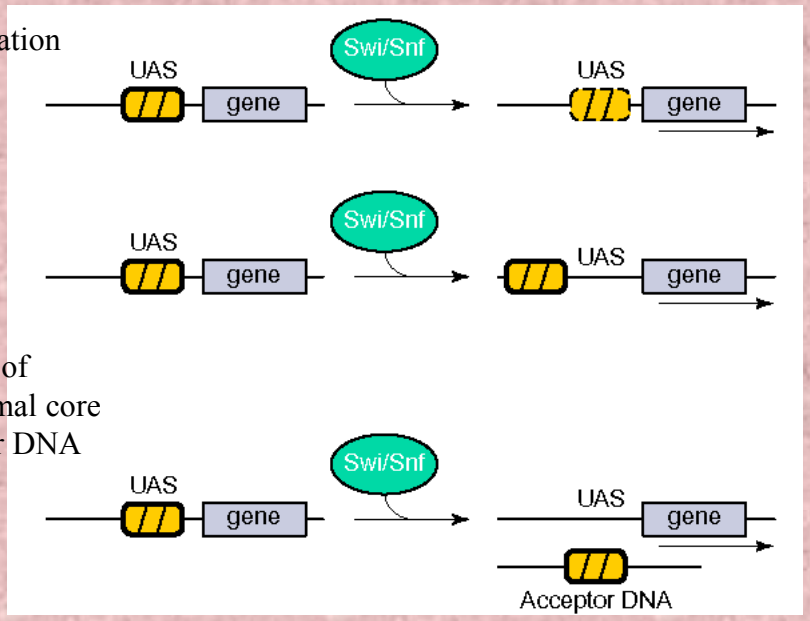
SPACING – average distance between nucleosomal cores

Localisation of nucleosomal cores on the regulatory DNA sequences may interphere with binding of transcription factors
That is why in cells there exist complexes of chromatin remodelling

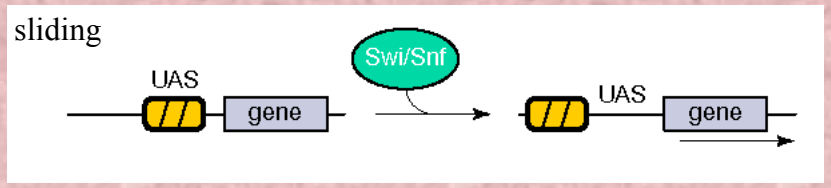
There are three major groups of chromatin remodelling complexes

GROP	PROTOTIPE	ATPase
SWI/SNF	swi2/snf2 (yeast)	swi2/snf2 (bromodomain)
ISWI	NURF (Drosophila)	ISWI
CHD	CHD-1 (yeast)	Chd3/Chd4 (Mi-2) (chromodomain)
	NURD (Drosophila)	

swi/snf activities



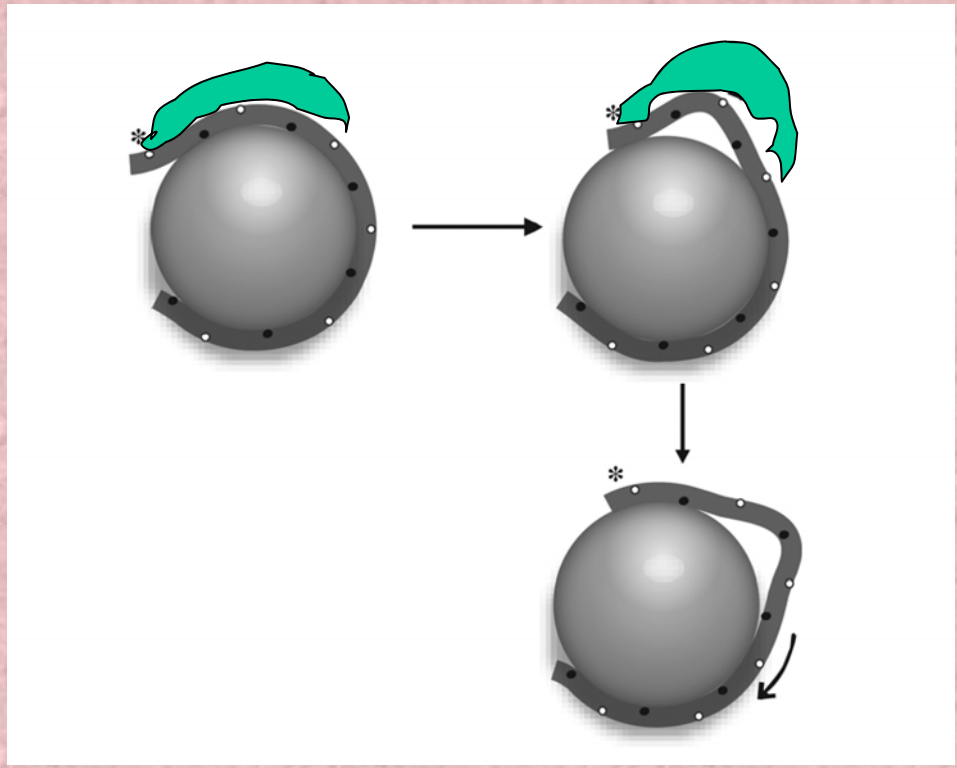
NURF activities



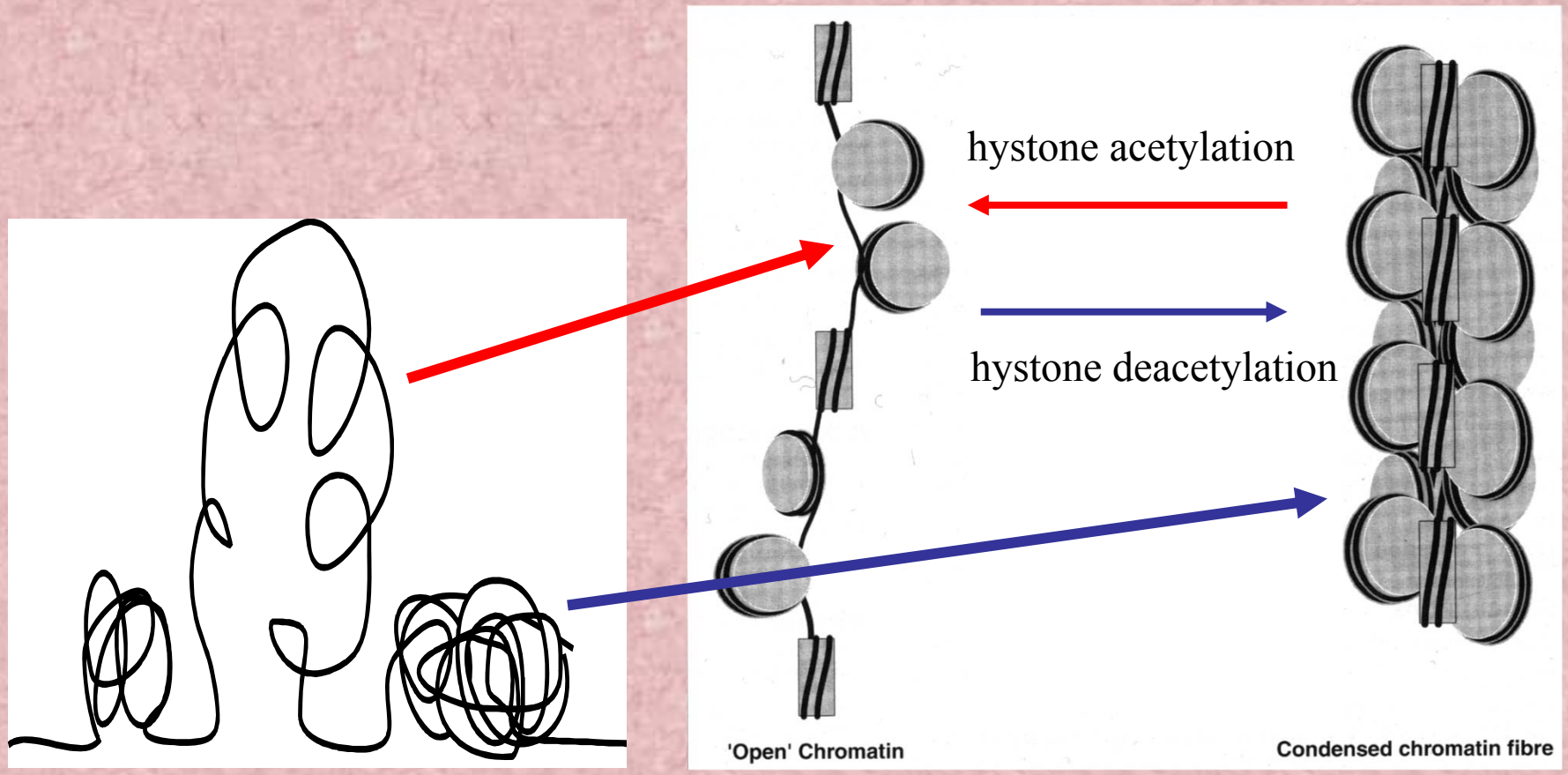
NURD activities

sliding + deacetylation des histones (HDAC1, HDAC2)

Nucleosomal cores are moved in a stepwise fashion
swi2/snf2 – 50 bp per 1 ATP
other remodelling complexes – 10 bp per 1 ATP



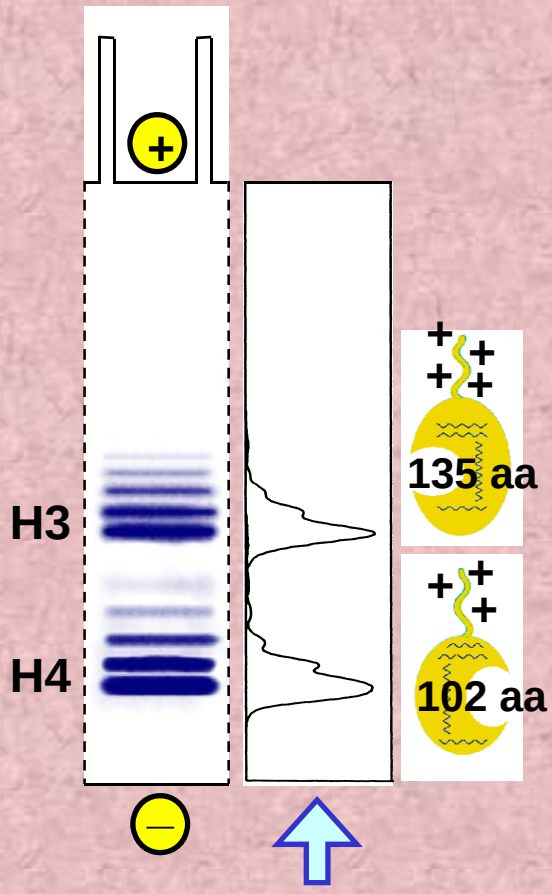
increased DNAse I sensitivity is due to the change in the mode of chromatin packaging (unfolding of 30 nm fiber).

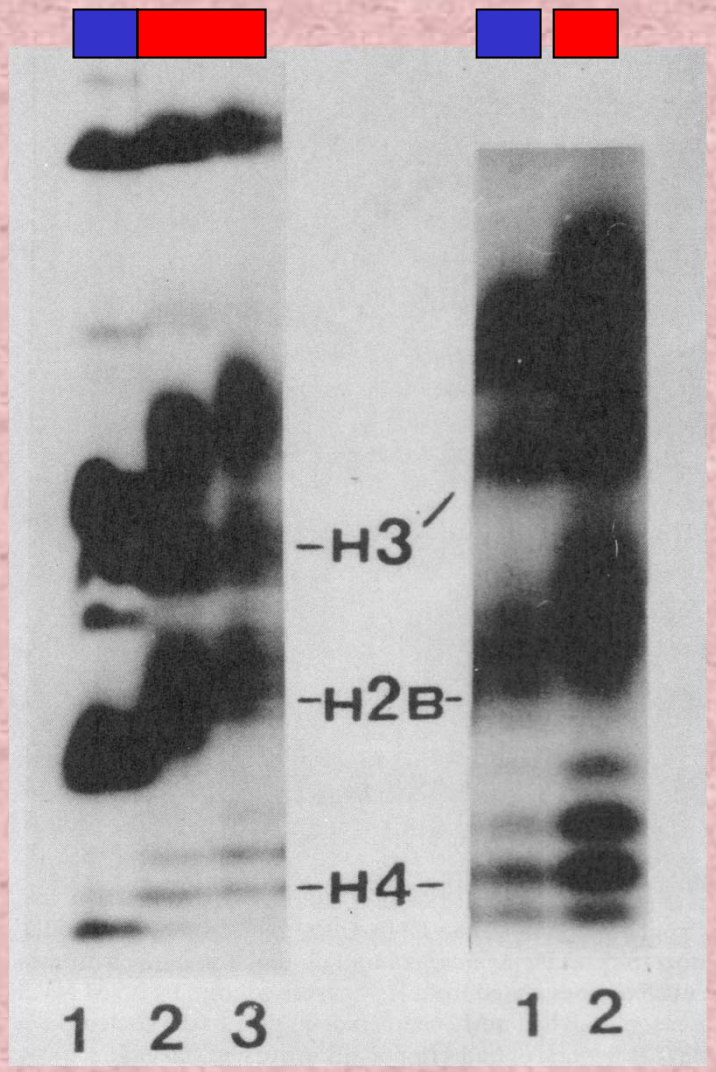


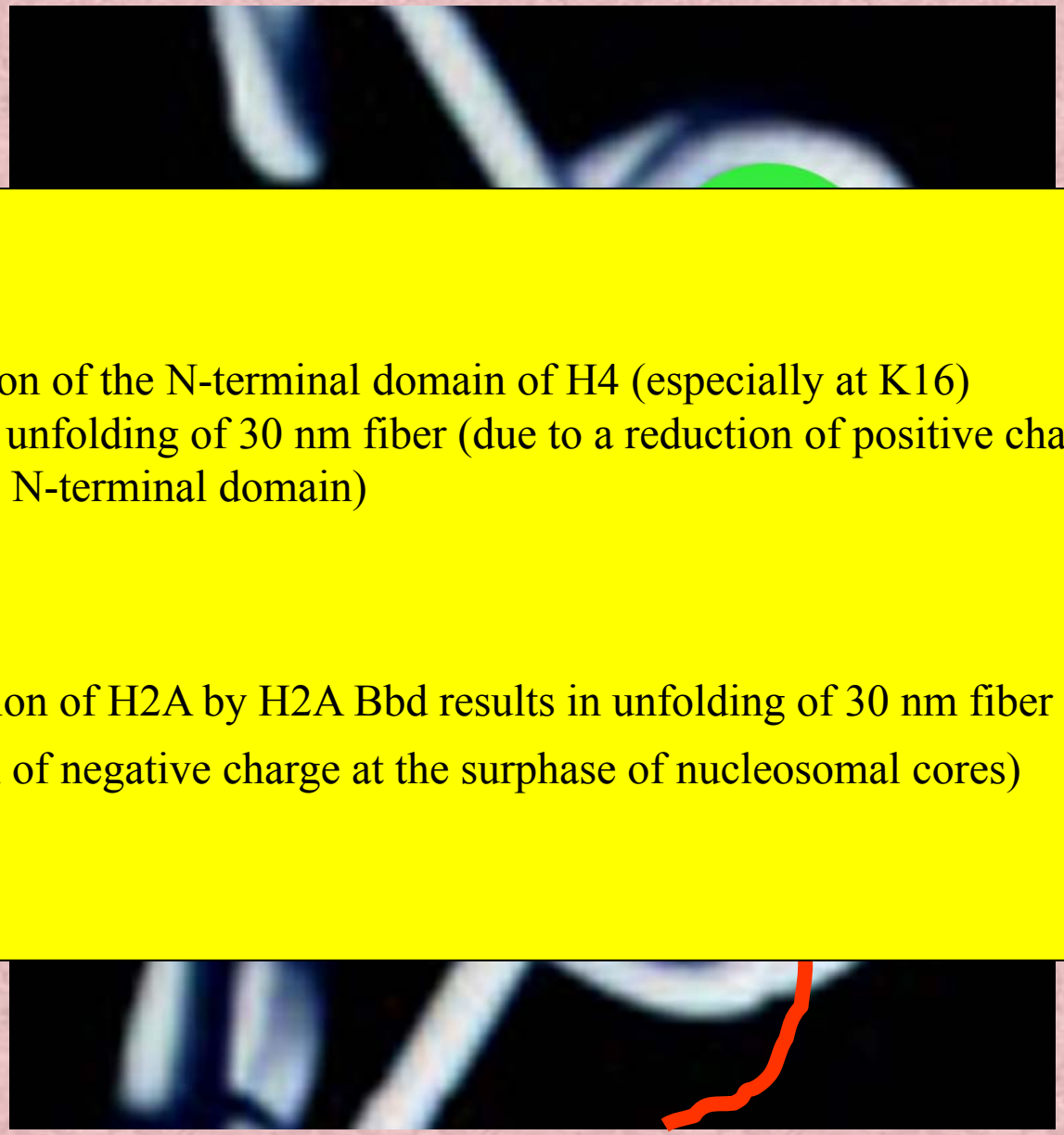
Active chromatin is characterized by increased level of hustone acetylation

(Acetic Acid – Urea)

1M acid, pH ~3 in 5-8 M urea



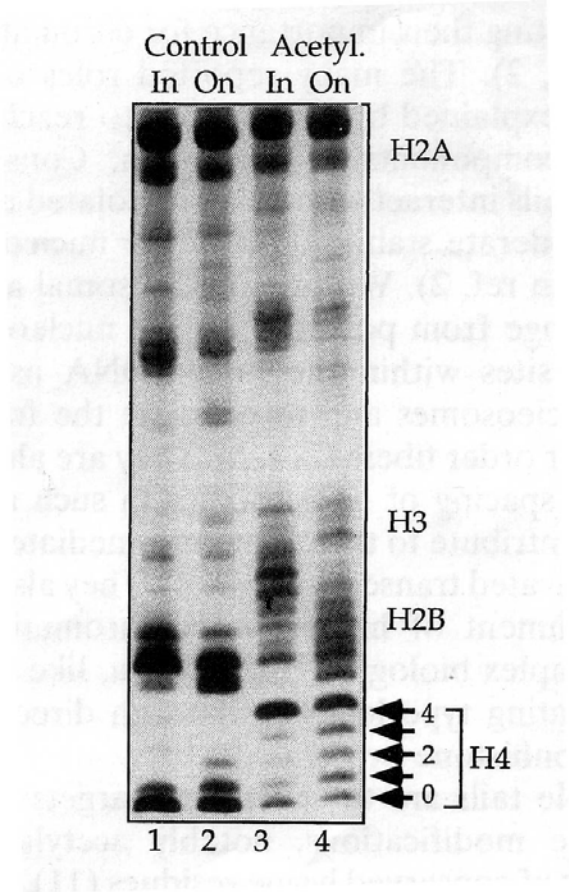


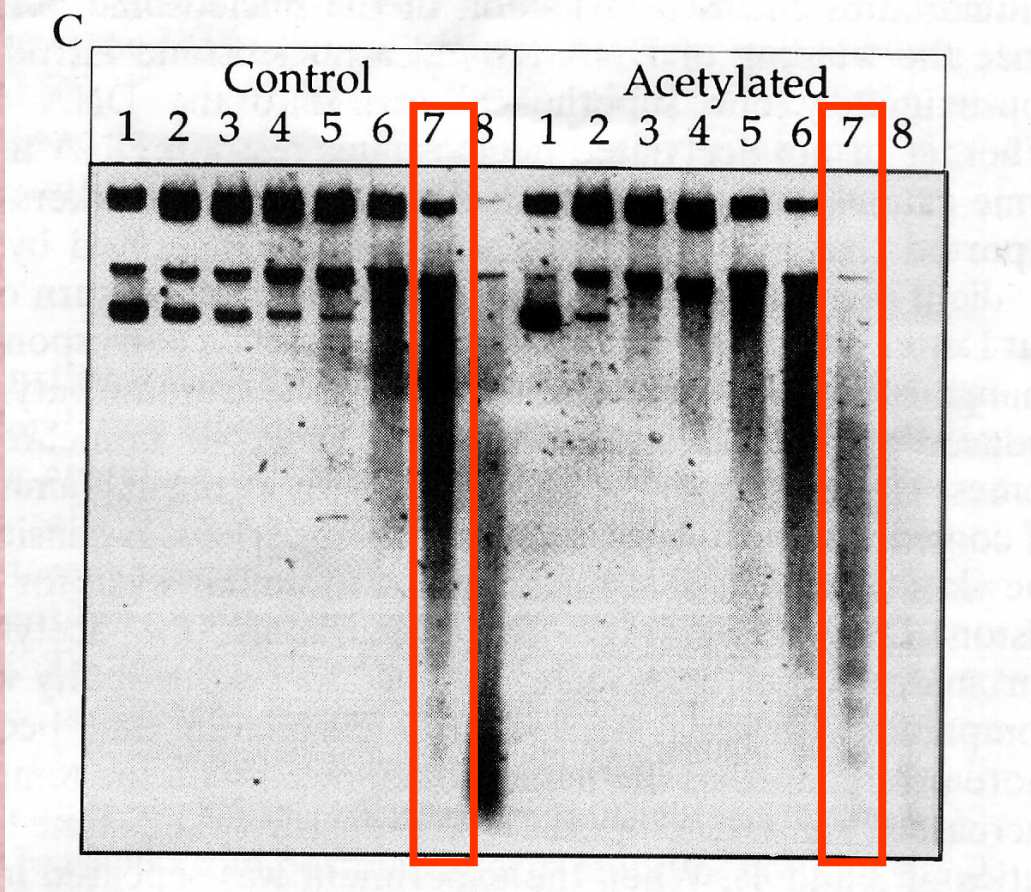


Acetylation of the N-terminal domain of H4 (especially at K16)
results in unfolding of 30 nm fiber (due to a reduction of positive charge
of the H4 N-terminal domain)

Substitution of H2A by H2A Bbd results in unfolding of 30 nm fiber (because of reduction of negative charge at the surphase of nucleosomal cores)

Chromatin assembled in vitro from freeDNA and hyperacetylated hystones possesses the properties of active chromatin
Krajewski and Becker, 1998

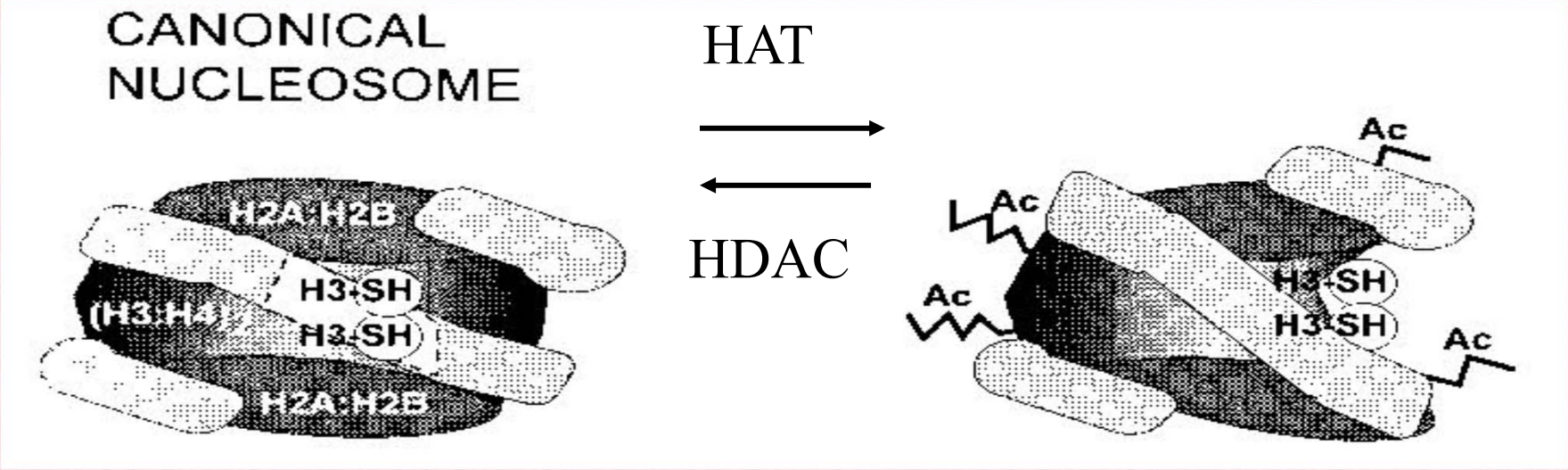




Hyperacetylated hystones were prepared from cells treated with trichostatine A, an inhibitor of histone deacetylases

Chromatin assembled from hyperacetylated hystones is characterized by elevated DNase I sensitivity

Acetylation of histone tails triggers spatial reorganization of the nucleosomal core. This reorganization is believed to facilitate transcription

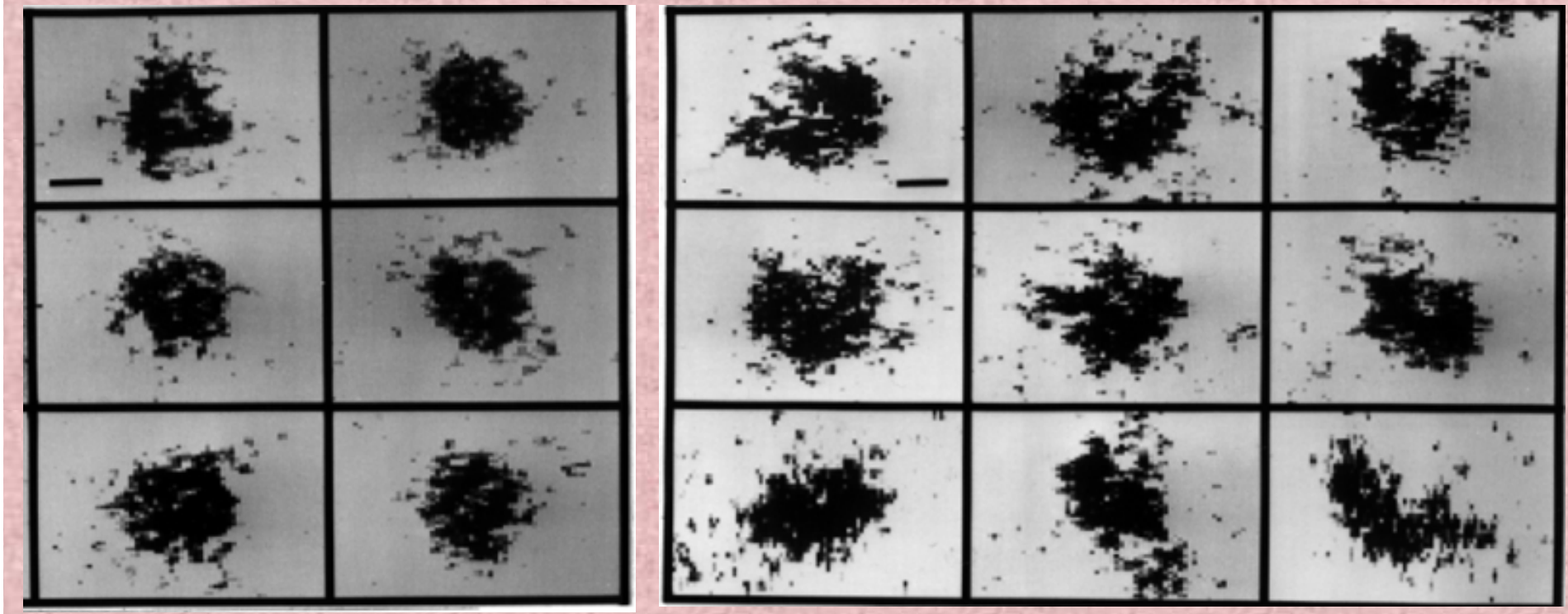


Bazett-Jones DP, Mendez E, Czarnota GJ, Ottensmeyer FP, Allfrey VG.

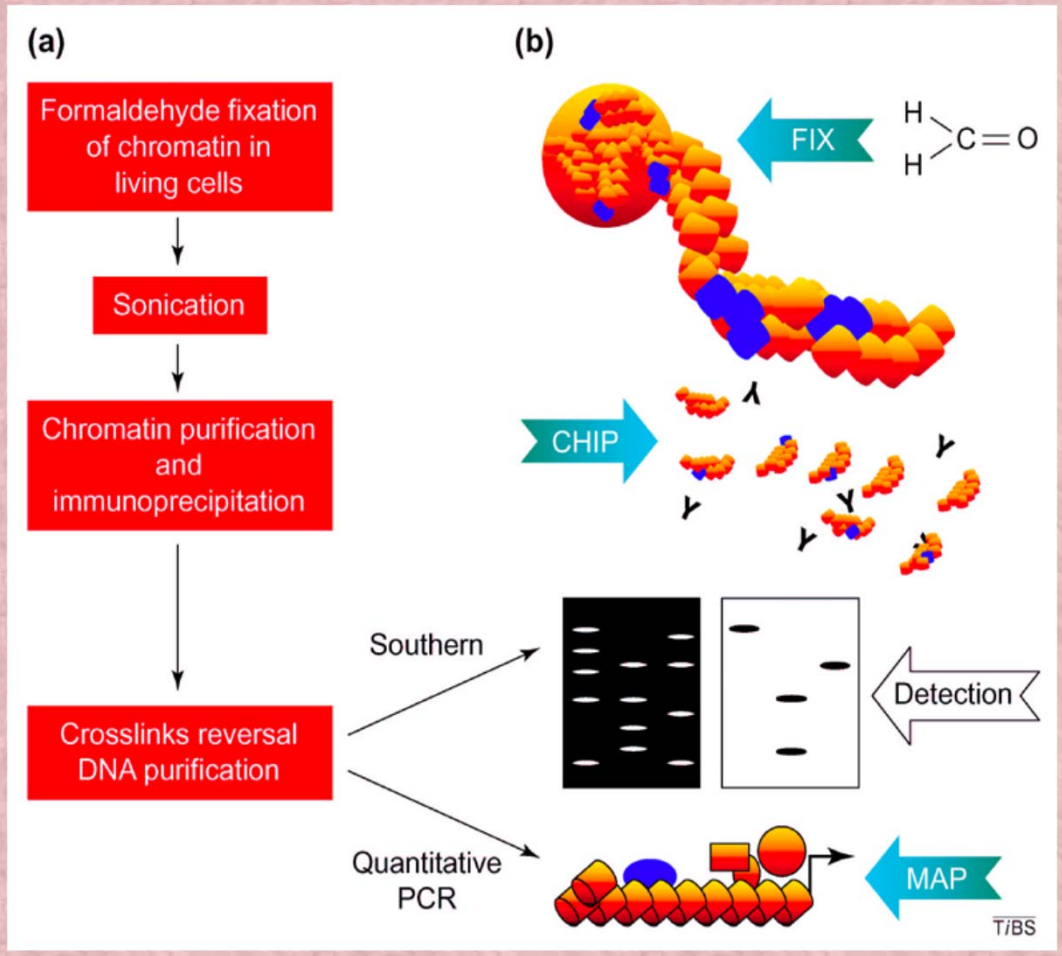
Nucleic Acids Res. 1996 Jan 15;24(2):321-9.

nucleosomes from non-active chromatin

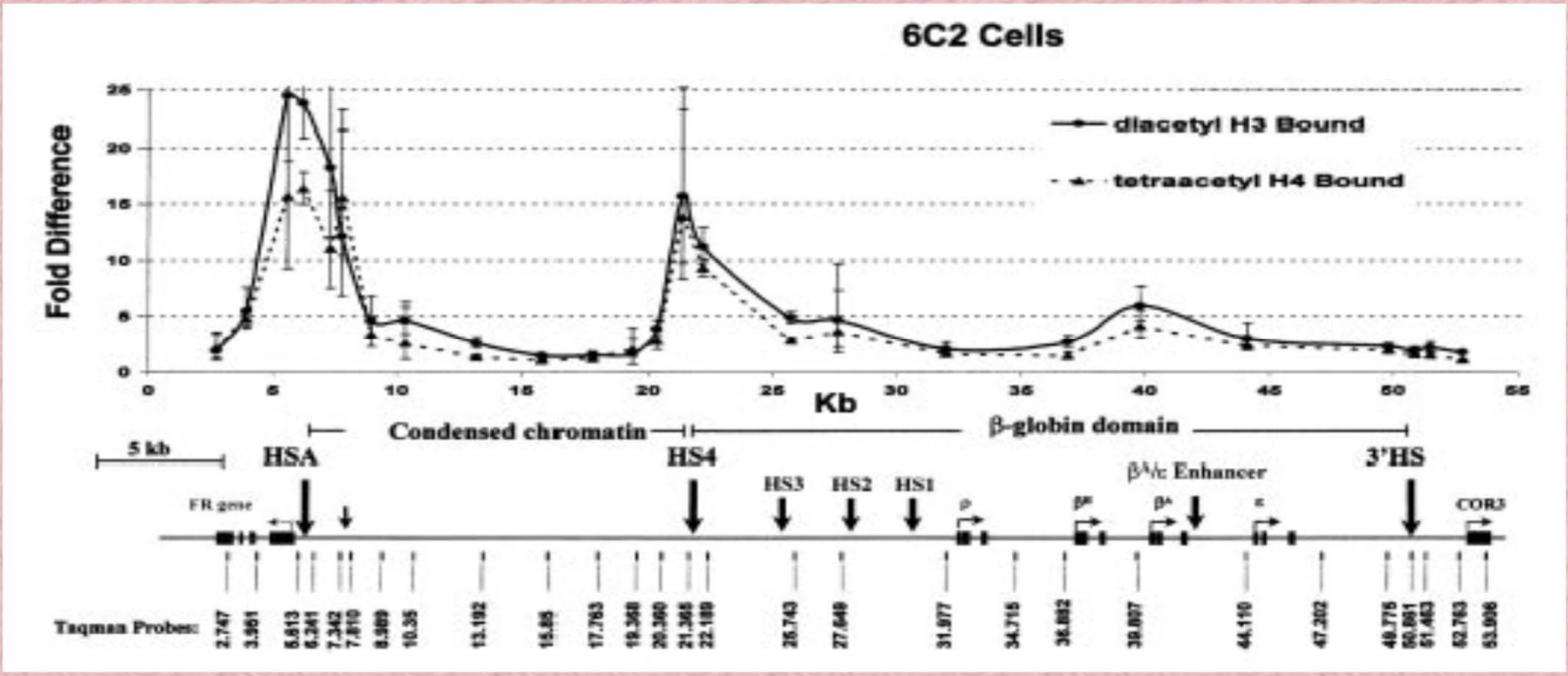
Nucleosomes from active chromatin



Distribution of modified histones within long genomic areas can be studied using chromatin immunoprecipitation (ChIP)



Genomic domains characterized by the presence of hyperacetylated histones H3 and H4 colocalise with DNase-sensitive domains



Active chromatin domains are characterized by elevated level of histone acetylation.

Distribution of acetylated histones along the genome can be studied by using Chromatin Immuno Presipitation (ChIP)

In domains of beta-globin genes and other domains of tissue-specific genes the regions containing hyperacetylated hystones roughly colocalise with DNase I-sensitive regions

Dynamics of chromatin domains is regulated by hystone acetylases (HAT) and histone desacetylases (HDAC)

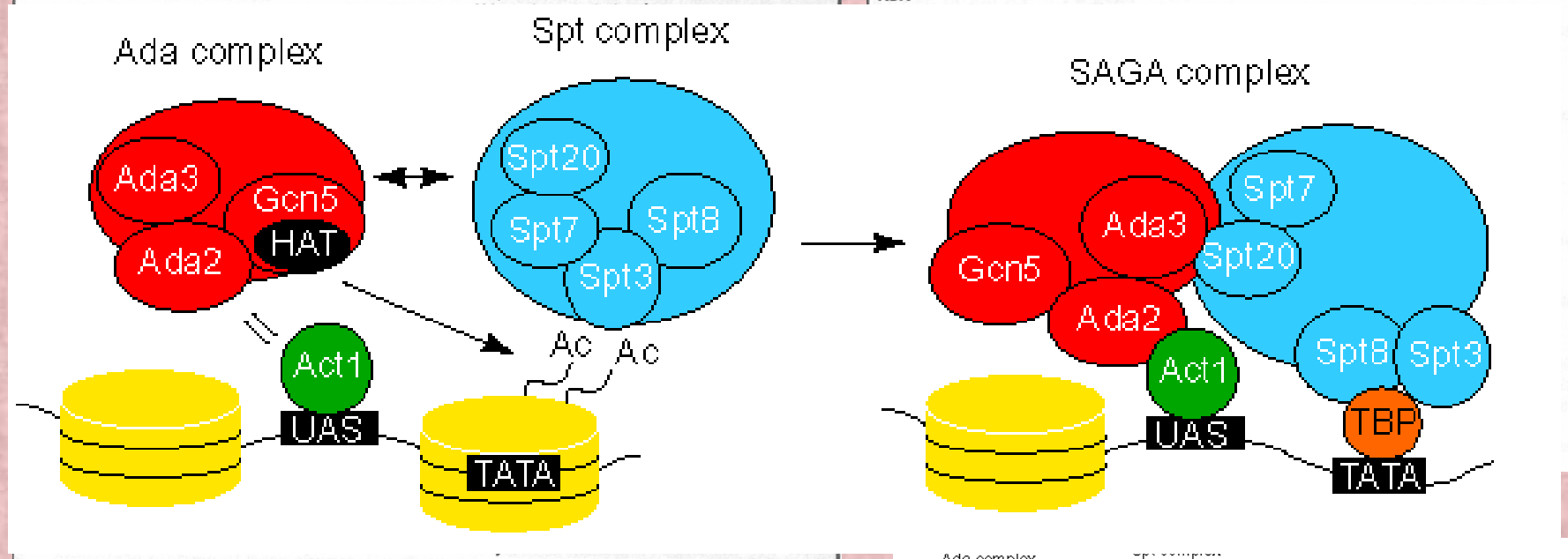
Both HAT and HDAC are present in large multu-subunit complexes

Table 2. Histone acetyltransferases and transcription

HAT complex	Catalytic subunit	Organism	HAT activity and roles in transcription
	p300/CBP	*H. sapiens*	Global transcriptional regulator HAT activity stimulates transcription *in vivo* Acetylates nucleosomal H2A, H2B, H3 and
ADA	GCN5	*S. cerevisiae*	Acetylates nucleosomal H3 and H2B
NuA3	unknown	*S. cerevisiae*	Acetylates nucleosomal H3
NuA4	ESA1	*S. cerevisiae*	Interacts with acidic activation domains HAT activity stimulates transcription *in vitro* Acetylates nucleosomal H4 and H2A
Elongator	ELP3	*S. cerevisiae*	Transcription elongation Acetylates histones H2A, H2B, H3 and H4

Table 1. Subunit composition of Gcn5p-containing HAT complexes[a]

Subunit function/ homology	Yeast ADA	Yeast SAGA	Human PCAF	Human TFTC[b]	Human STAGA[b]
Acetyltransferase	Gcn5	Gcn5	PCAF	Gcn5L	Gcn5L
ADA					
					Spt3
					TAF$_{II}$31



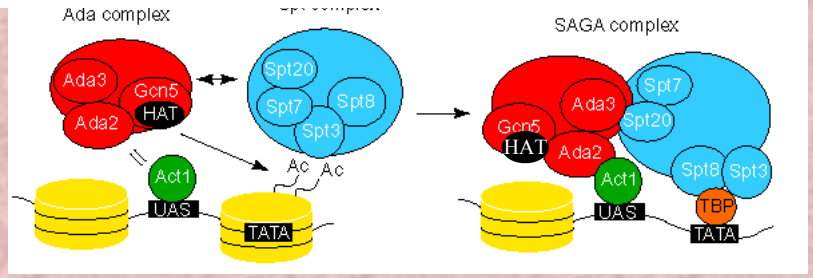


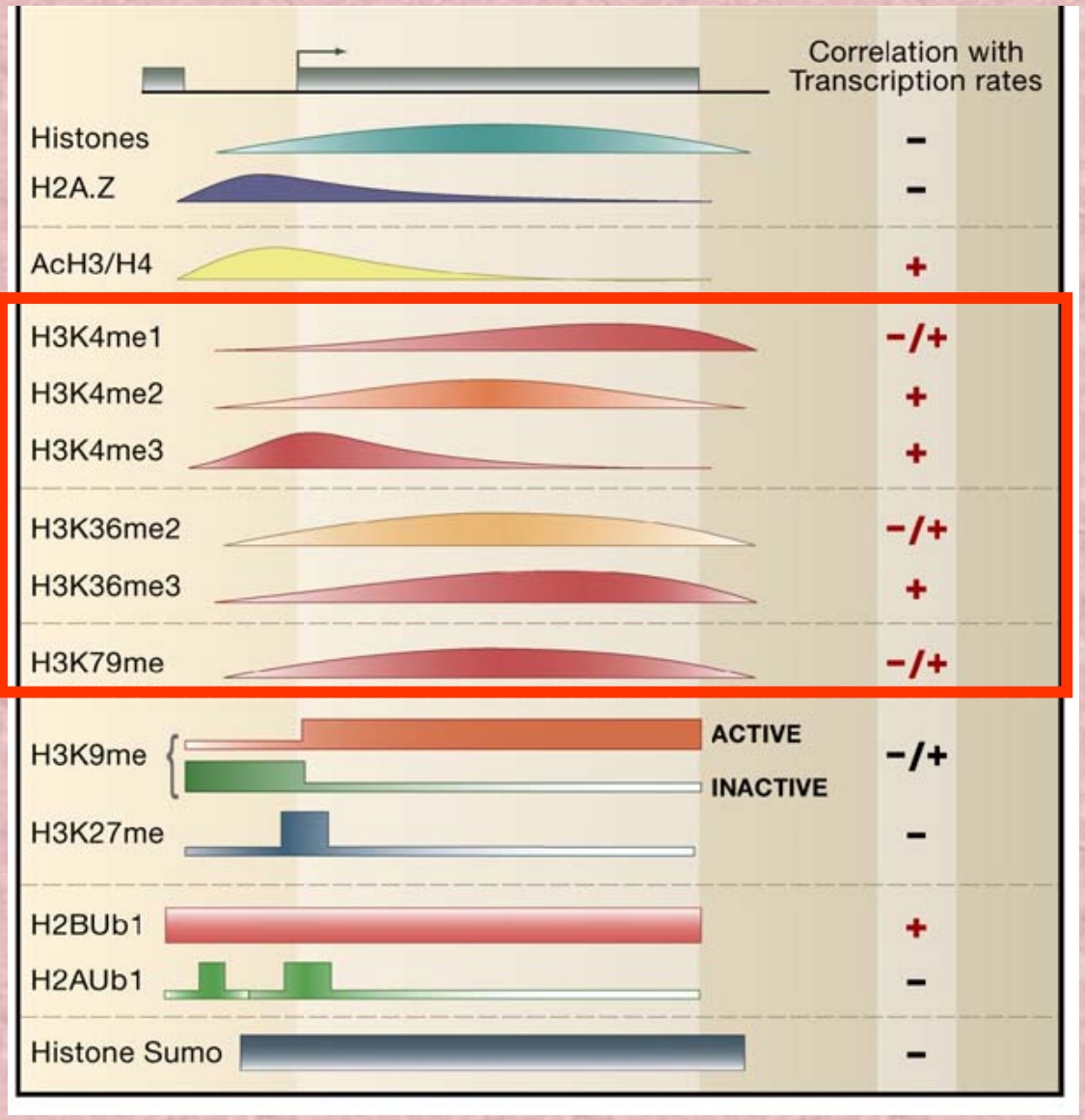
Table 1. Histone Modifications Associated with Transcription

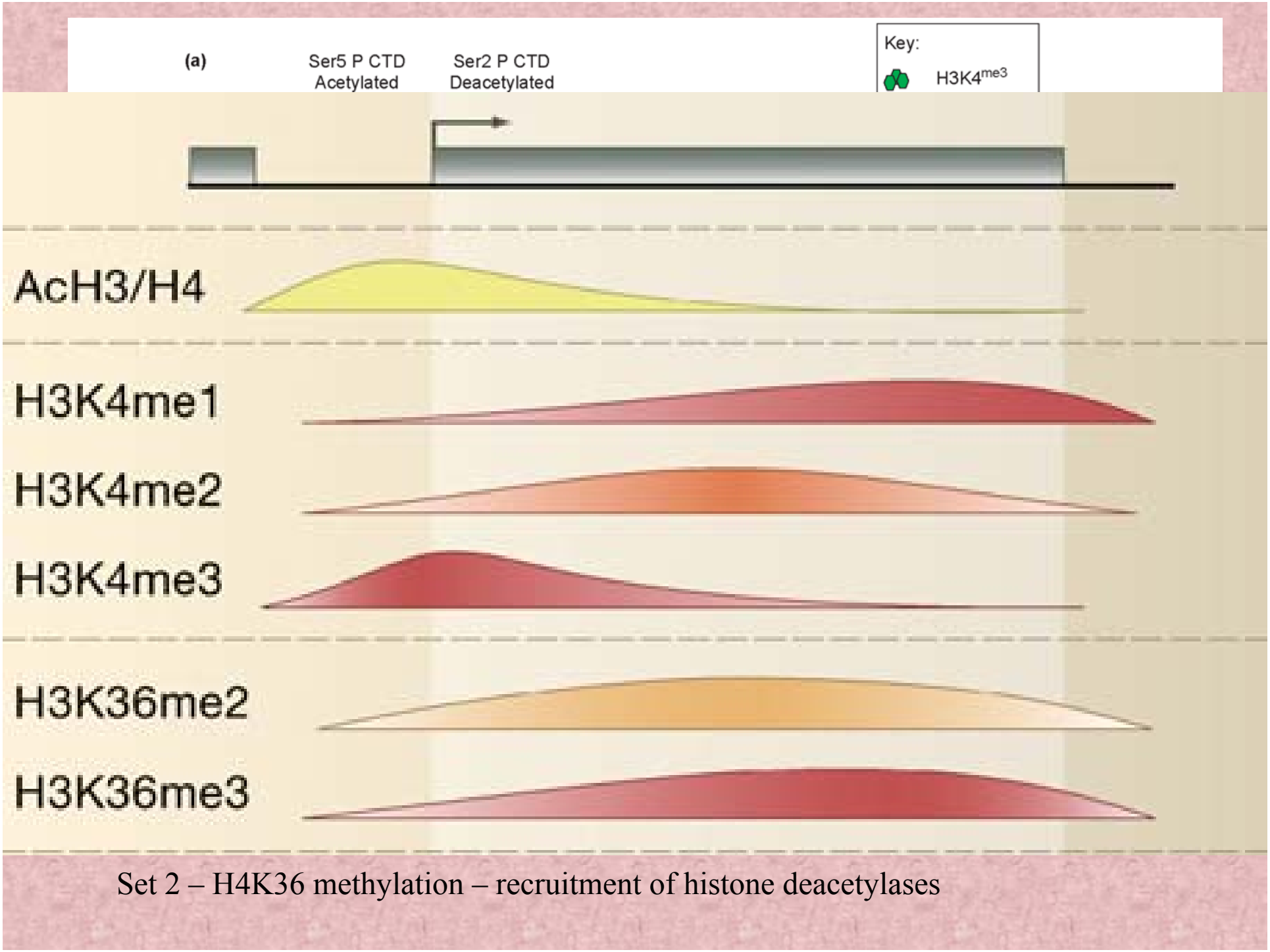
Modifications	Position		Enzymes				Recognition Module(s)[a]	Functions in Transcription
			S. cerevisiae	*S. pombe*	*Drosophila*	Mammals		
Methylation	H3	K4	Set1	Set1	Trx, Ash1	MLL, ALL-1, Set9/7, ALR-1/2, ALR, Set1	PHD, Chromo, WD-40	Activation
		K9	n/a	Clr4	Su(var)3-9, Ash1	Suv39h, G9a, Eu-HMTase I, ESET, SETBD1	Chromo (HP1)	Repression, activation
		K27				E(Z)	Ezh2, G9a	Repression
		K36	Set2			HYPB, Smyd2, NSD1	Chromo(Eaf3), JMJD	Recruiting the Rpd3S to repress internal initiation
		K79	Dot1			Dot1L	Tudor	Activation
	H4	K20		Set9	PR-Set7, Ash1	PR-Set7, SET8	Tudor	Silencing
Arg Methylation	H3	R2				CARM1		Activation
		R17				CARM1		Activation
		R26				CARM1		Activation
	H4	R3				PRMT1	(p300)	Activation
Phosphorylation	H3	S10	Snf1				(Gcn5)	Activation
Ubiquitination	H2B	K120/123	Rad6, Bre1	Rad6		UbcH6, RNF20/40	(COMPASS)	Activation
	H2A	K119				hPRC1L		Repression
Acetylation	H3	K56					(Swi/Snf)	Activation
	H4	K16	Sas2, NuA4		dMOF	hMOF	Bromodomain	Activation
	Htz1	K14	NuA4, SAGA					Activation

[a] The proteins that are indicated within the parentheses are shown to recognize the corresponding modifications but specific domains have yet to be determined.

Table 2. Histone Variants Involved in Transcription Regulation

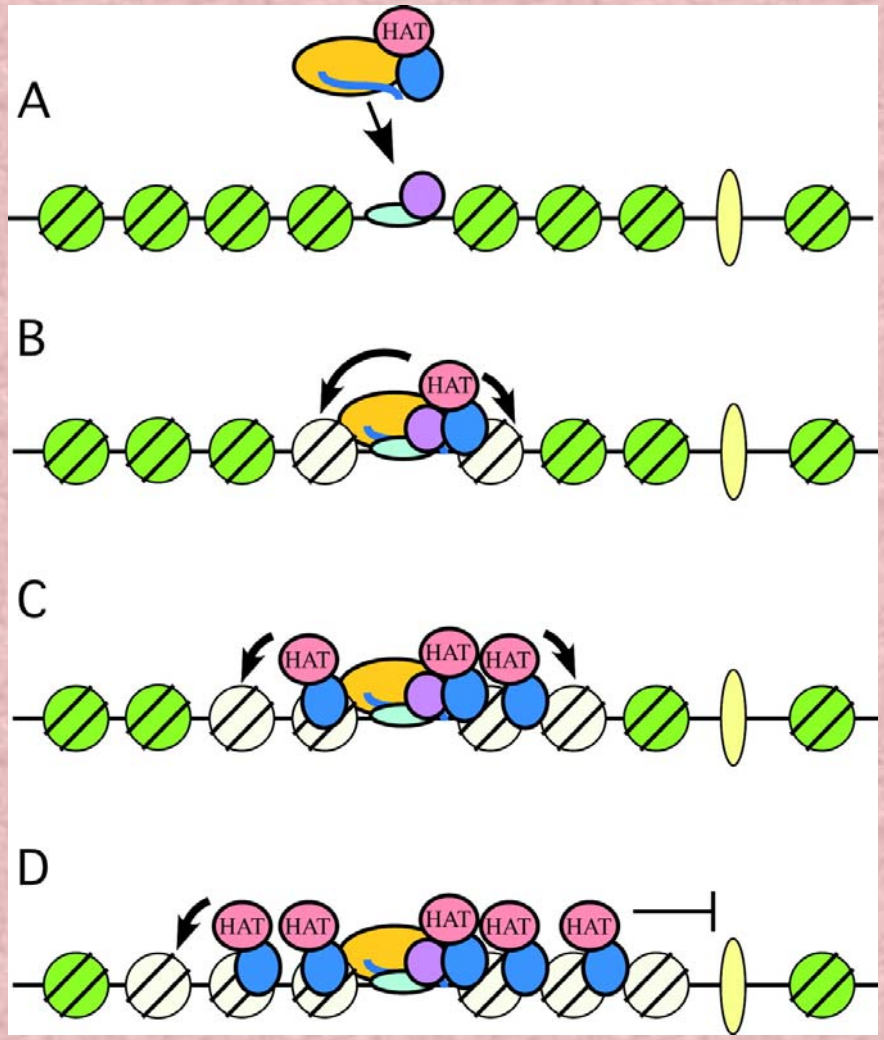
Histone	Variant Forms	Role(s) in Transcription	Localization	Structural Features	Functions
H3	H3.3	Transcription activation	Transcribing region	Different from canonical H3 in only four amino acids.	Active transcription triggers deposition and removal.
H2A	macroH2A	X chromosome inactivation	Inactive X chromosome	C-term nonhistone-like region is responsible for most of functions; histone-fold prevents sliding; prefers to form hybrid nucleosome.	Repressing initiation but not elongation; interfering histone acetylation by p300; it blocks sliding by ACF and remodeling by Swi/Snf; it inhibits transcription factor binding (NFkB).
	H2AZ	Transcription activation/ repression	Promoter, heterochromatin boundary	Loop1 differs from H2A, disfavors formation of hybrid nucleosome; C-term α helix is essential for recognition.	Facilitates TBP binding; is evicted upon activation; prevents elongation-associated modification and remodeling at promoter
	H2ABbd	Transcription activation	Active X chromosome and autosomes	Lack of C term; it only organizes 118–130 bp pf DNA and leaves each side 10 bp free DNA.	Swi and ACF fail to mobilize the H2ABbd nucleosome but can increase its accessibility. p300- and Gal4-VP16-activated transcription is more robust on H2ABbd nucleosomes; H2A.Bbd histone fold domain is responsible for the unusual properties of the H2A.Bbd nucleosome
	H2A.X	Repression	Canonical in yeast, generally distributed	A conserved C-term SQ(E/D) motif that becomes phosphorylated upon DNA damage.	


Correlation with Transcription rates
Histones
H2A.Z
AcH3/H4
H3K4me1
H3K4me2
H3K4me3
H3K36me2
H3K36me3
H3K79me
H3K9me
ACTIVE
INACTIVE
H3K27me
H2BUb1
H2AUb1
Histone Sumo
–
–
+
–/+
+
+
–/+
+
–/+
–/+
–
+
–
–



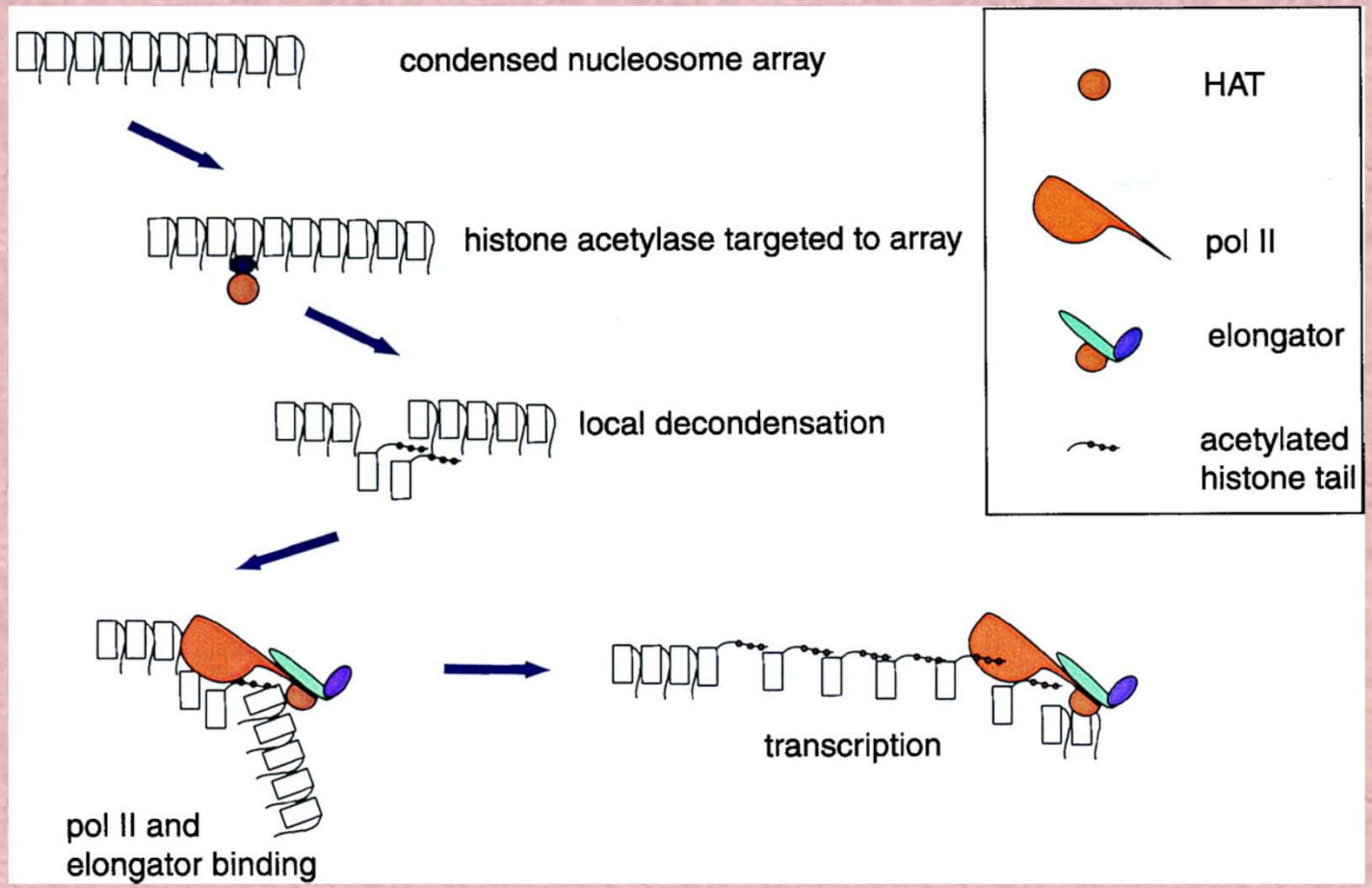
Set 2 – H4K36 methylation – recruitment of histone deacetylases

A model for hyperacetylated domain formation (processive spreading of H3K9 acetylation is mediated by HAT that contains bromodomain recognizing H3K9 acetylation

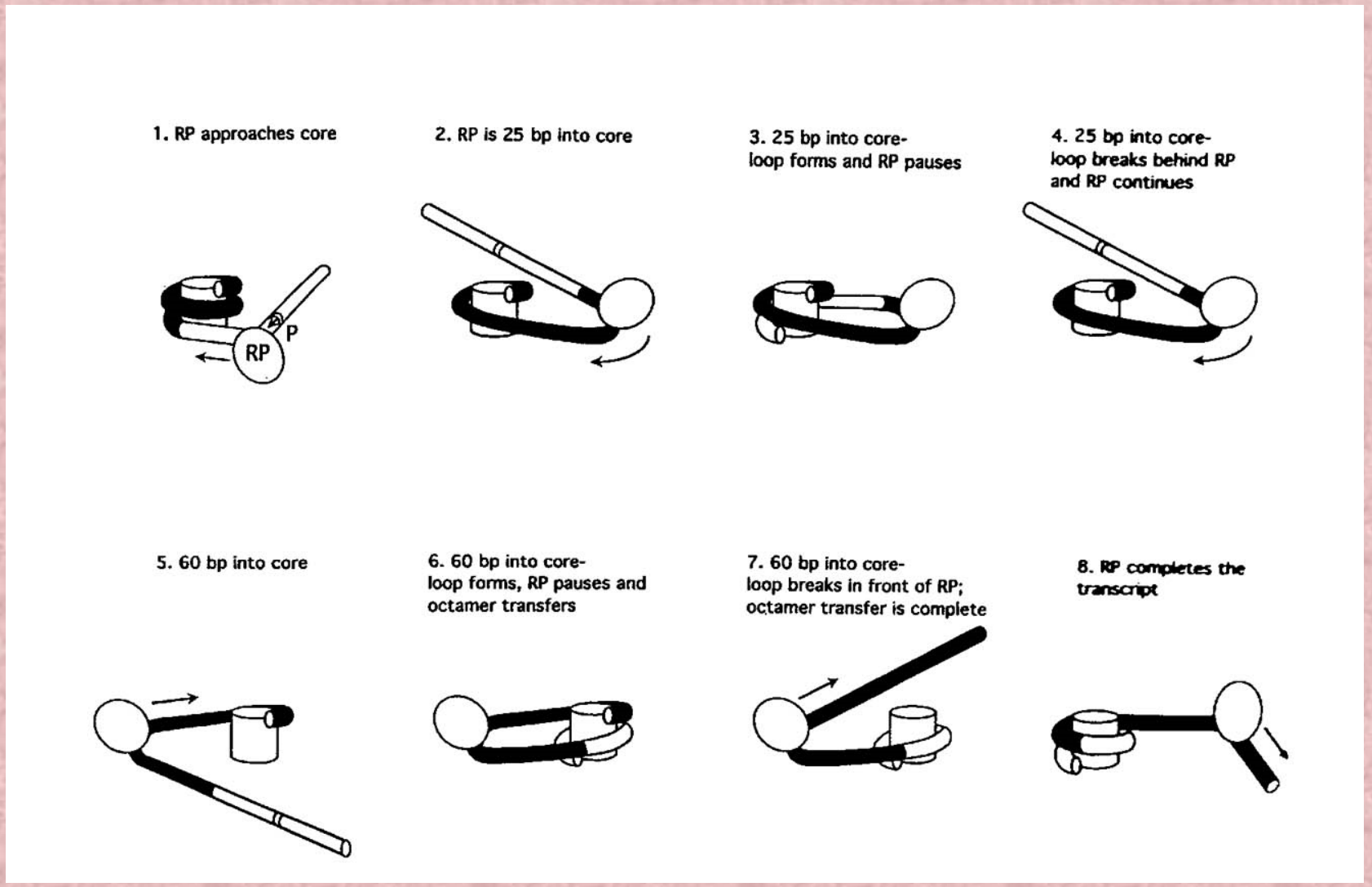


Bulger, M. J. Biol. Chem. 2005;280:21689-21692

Elongating Poi II can serve as a vehicle moving histone acetylases and chromatin remodeling complexes from LCR to Promoters
Travers, 1999



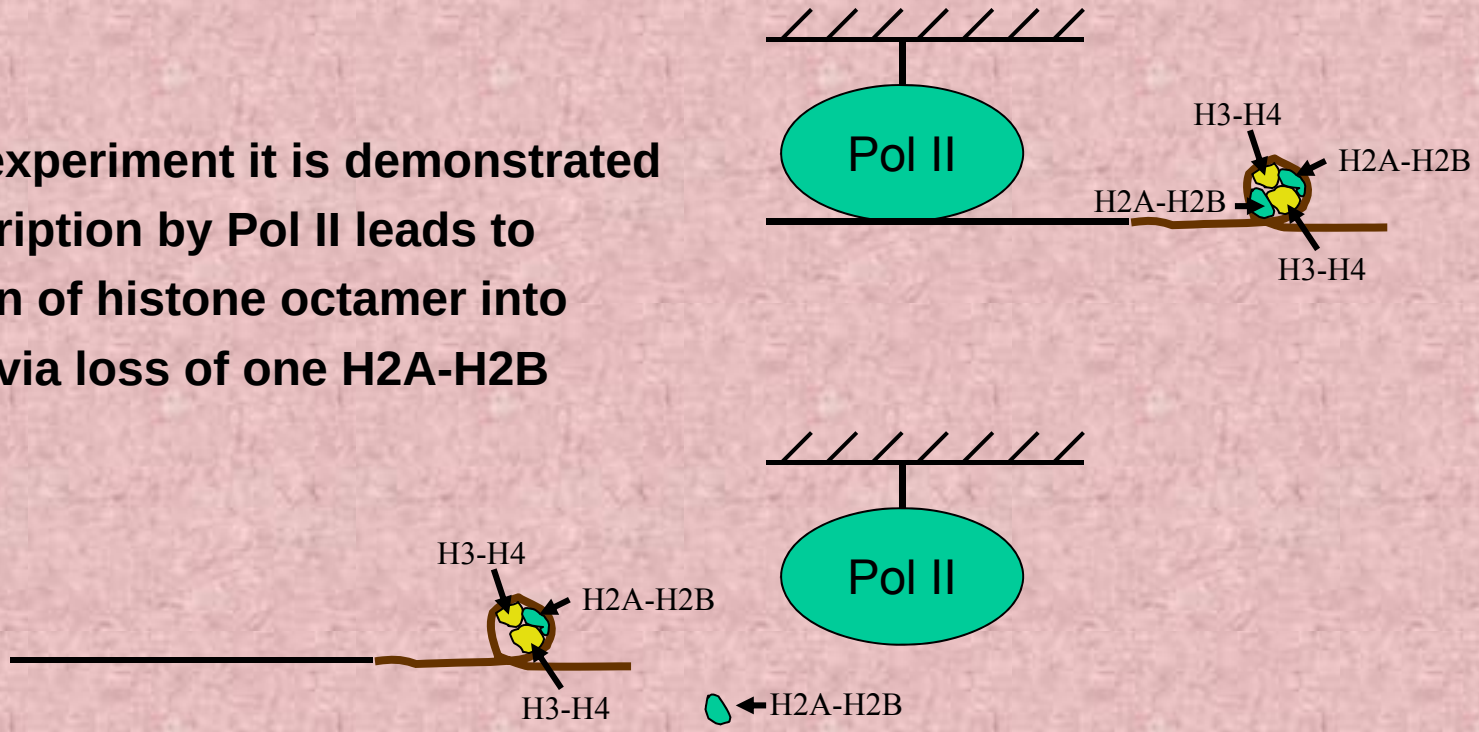
Transcription through nucleosomes: nucleosome core displacement ?



Partial dissociation of H2A-H2B dimmer (dimmers) from nucleosomal core is a key event permitting transcription by Pol II of chromatin templates

It is demonstrated experimentally that RNA pol II preferentially binds to chromatin in which nucleosomes lack one H2A-H2B dimmer.

In model experiment it is demonstrated that tanscription by Pol II leads to conversion of histone octamer into hexamer (via loss of one H2A-H2B dimmer)



The key role in displacement of H2A-H2B (H2A.Z-H2B) plays transcription elongation factor FACT

FACT

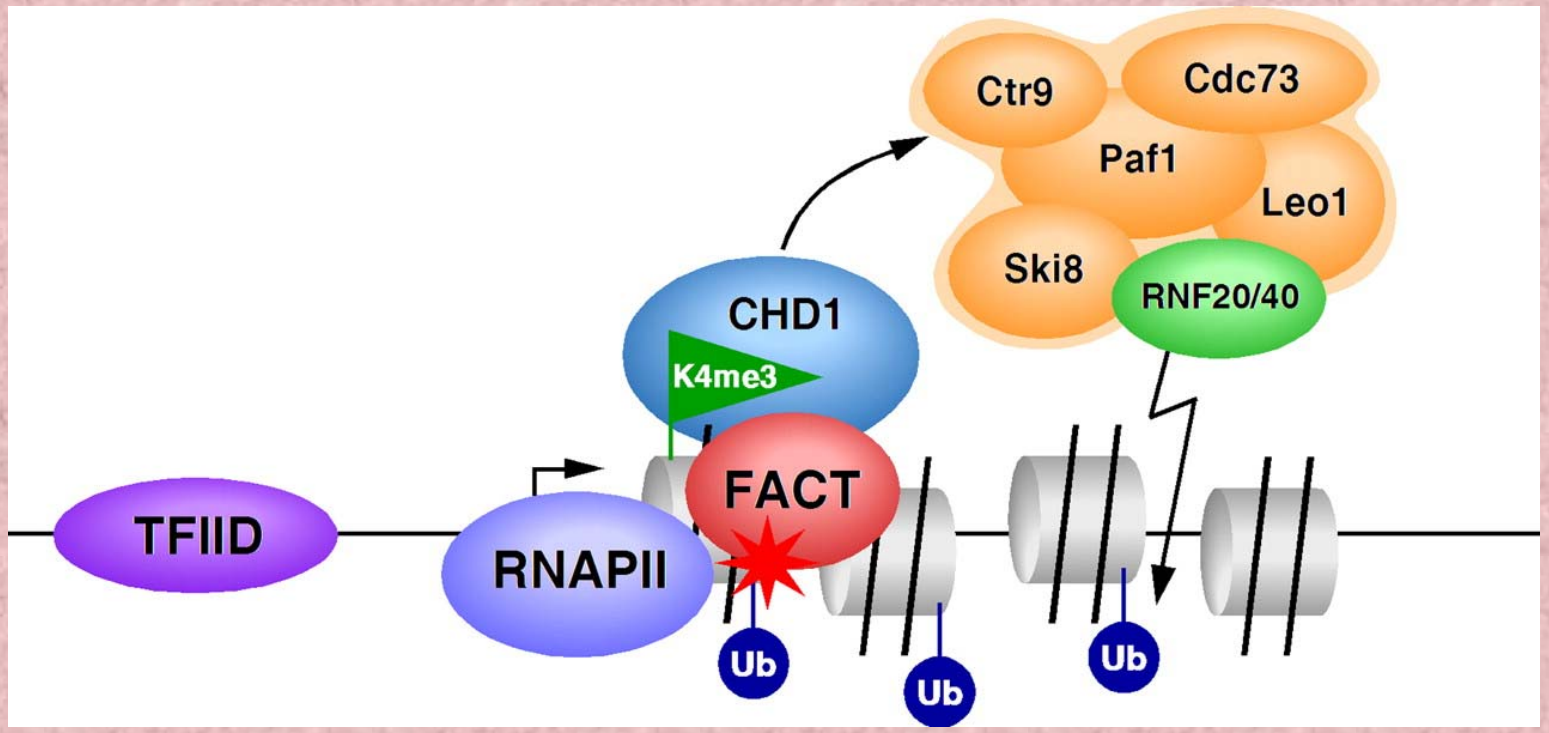
SSRP1 (HMG-box-containing protein)

p140/hSpt16 (human homolog of yeas Spt16./Cdc68)

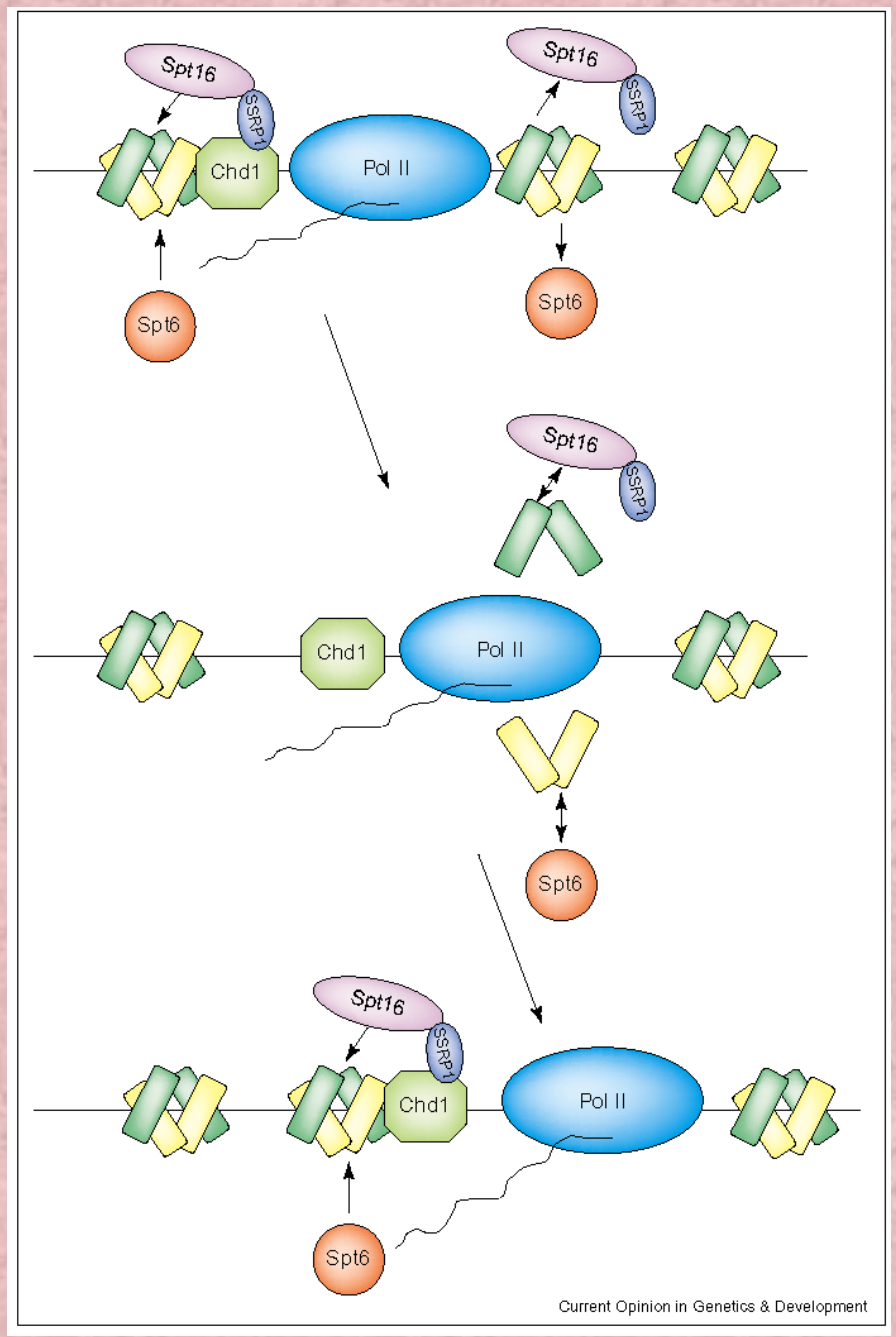
C-terminal domain of Spt16 is rich in negatively charged amino-acid residues, which is typical for all histone chaperons

FACT physically interacts with nucleosomes. In model system it should be present in equimolar amounts with nucleosomal cores in order to ensure the maximal transcription rate

Chromatin remodeling factor CHD1 recruits FACT to the regions reach in H3 trimethylated at K4 (promoters and 5'-regions of genes). Monoubiquitinilation of H2B at K120 facilates dissociation of H2A-H2B dimmers and thus transcription through nucleosomes.



Reinberg, D. et al. J. Biol. Chem. 2006;281:23297-23301



FACT participate both in displacement of H2A-H2B ahead of transcription complex and in reassembly of octamers behind the transcription complexes.

FACT interacts with dimmers H2A-H2B (green rectangles)

Another factor (Spt6) serves as H3-H4 shaperon

Chd1 participating in nucleosomes reassembly physically interacts with one of FACT subunits (SSRP1)

ACTIVE CHROMATIN - SUMMARY

1. Elevated level of histone acetylation

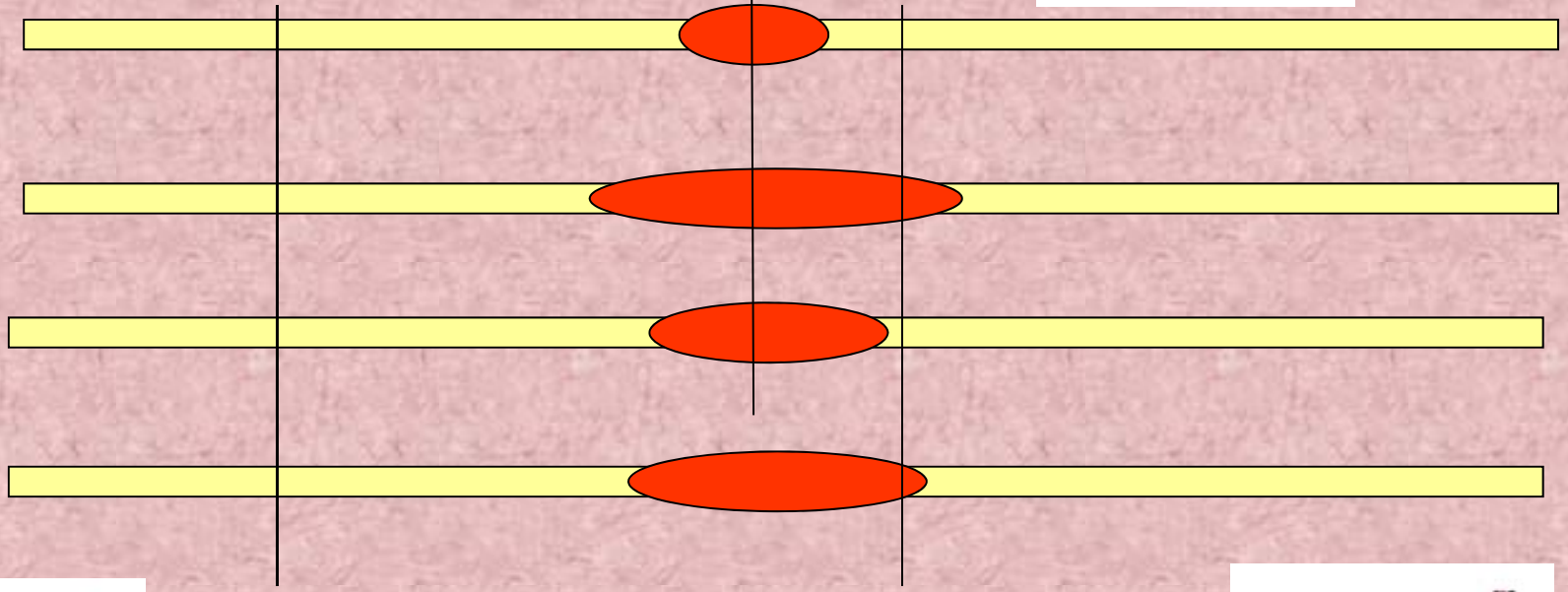
2. Partial loss of H2A-H2B (displacement mediated by FACT (Facilitated Chromatin Transcription)

3. Partial unfolding of nucleosomal cores (resulting in exposure of H3 disulphide groups)
4. Presence of specific variants of core histones (H3.3)
5. Methylation of H3 at several specific positions (K4, K36)
6. Partial loss of H1.

For a long time it was assumed that “defolt” state of chromatin is repressive while the active domains are organized under the control of special regulatory systems

Now it becomes increasingly evident that repressed chromatin domains are also organized under the control of specially regulatory systems and that “defolt” configuration of chromatin is in a way intermediate i.e. neither active nor repressive. This point of view was supported by studies of position effect variegation (“variegated expression”, PEV)

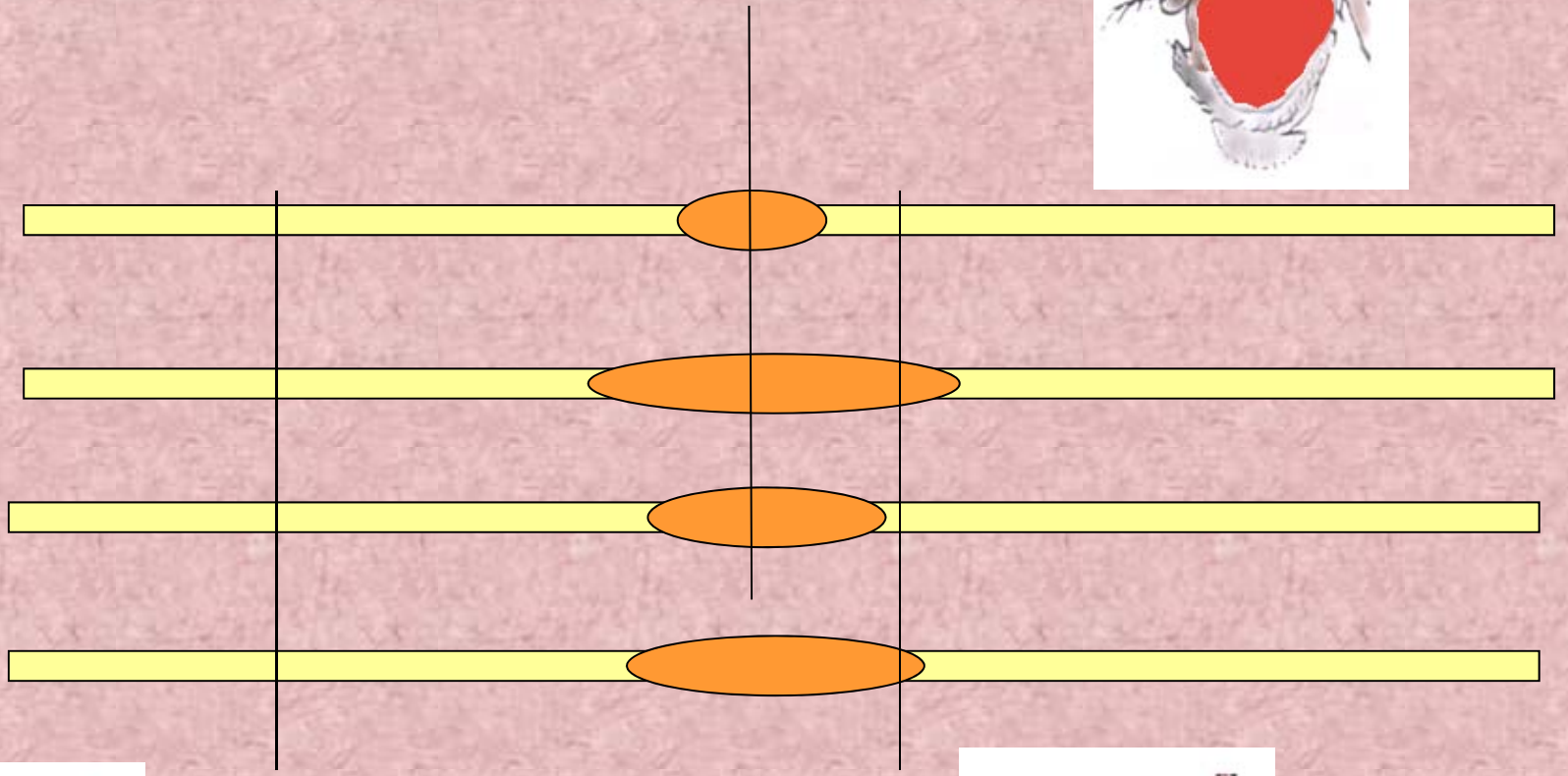
gene integrated here is repressed in all cells



gene integrated here is active in all cells

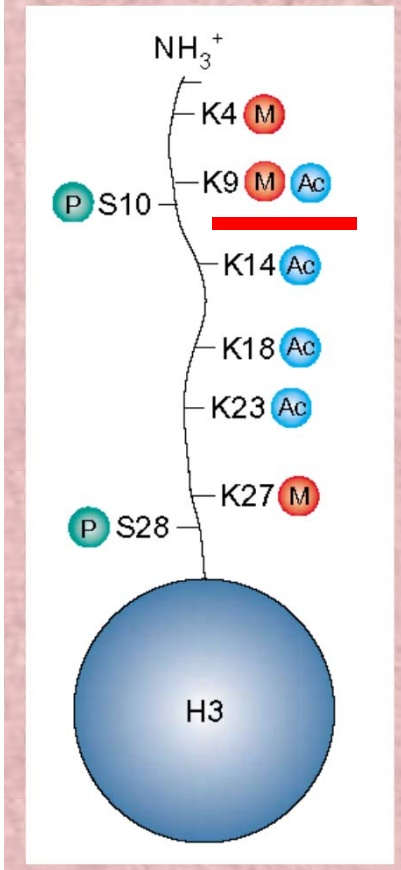
gene integrated here is active in some cells and is repressed in the others (mozaic expression)

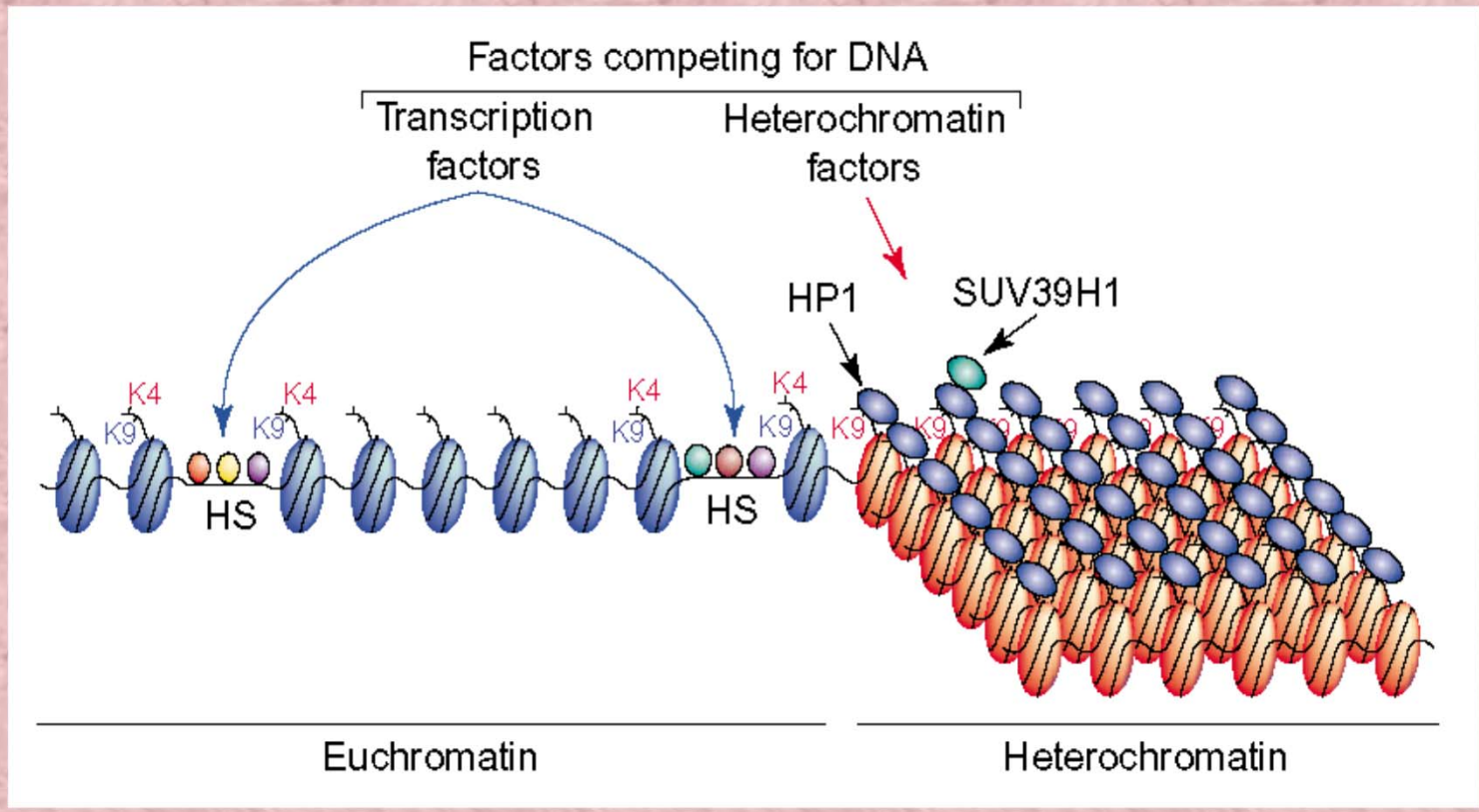
Mutations that suppress PEV make transgene active in any position



PEV is a concequence of the domain packaging into heterochromatin. There are mutations which suppress PEV (***Suvars***). Study of genes affected by these mutations made it possible to identify key protein products necessary for organization of heterochromatin.

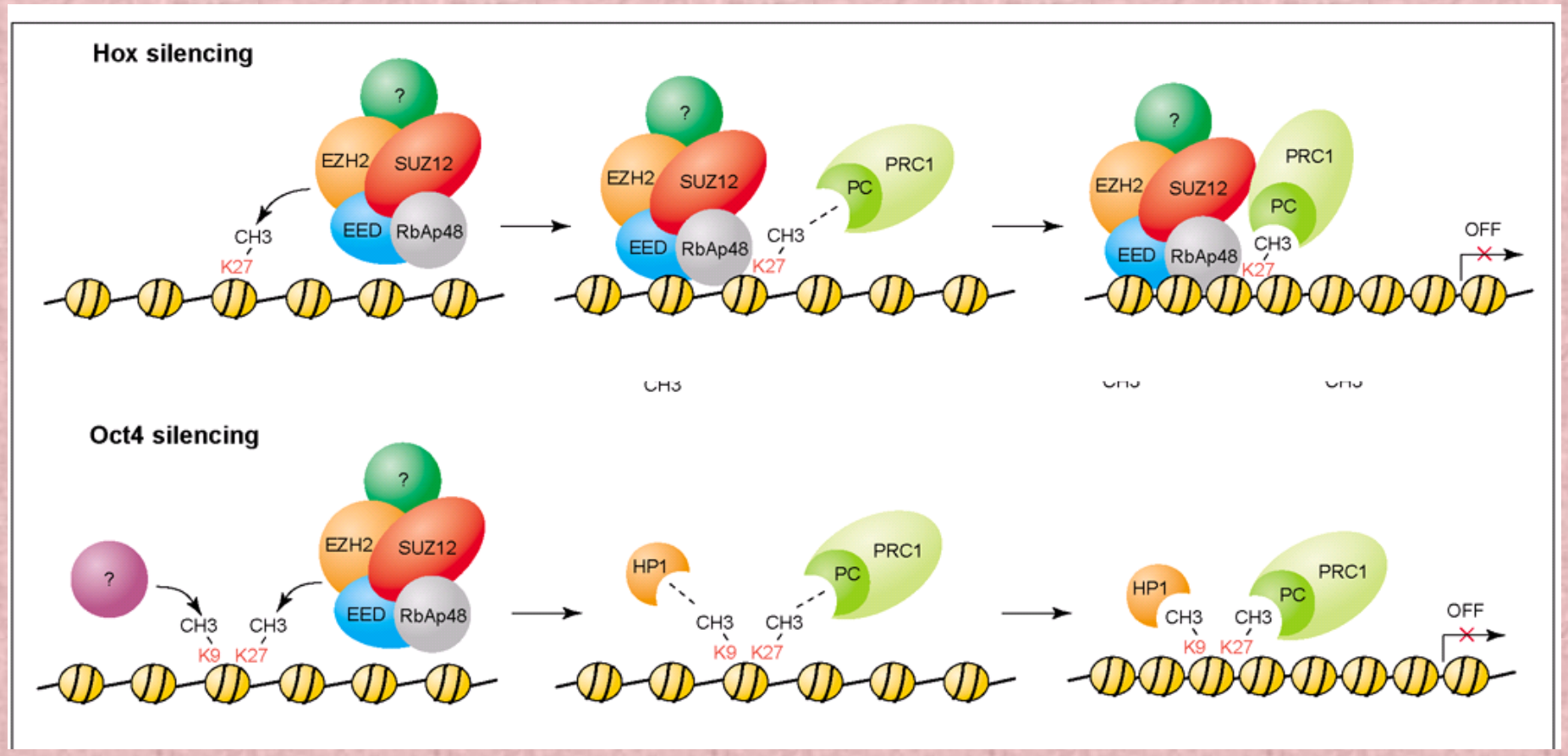
The most important among them are **histonemethylases** and **structural proteins of heterochromatin** (**HP1** in Drosophila and other higher eukaryoytes, **Sir** in yeast)



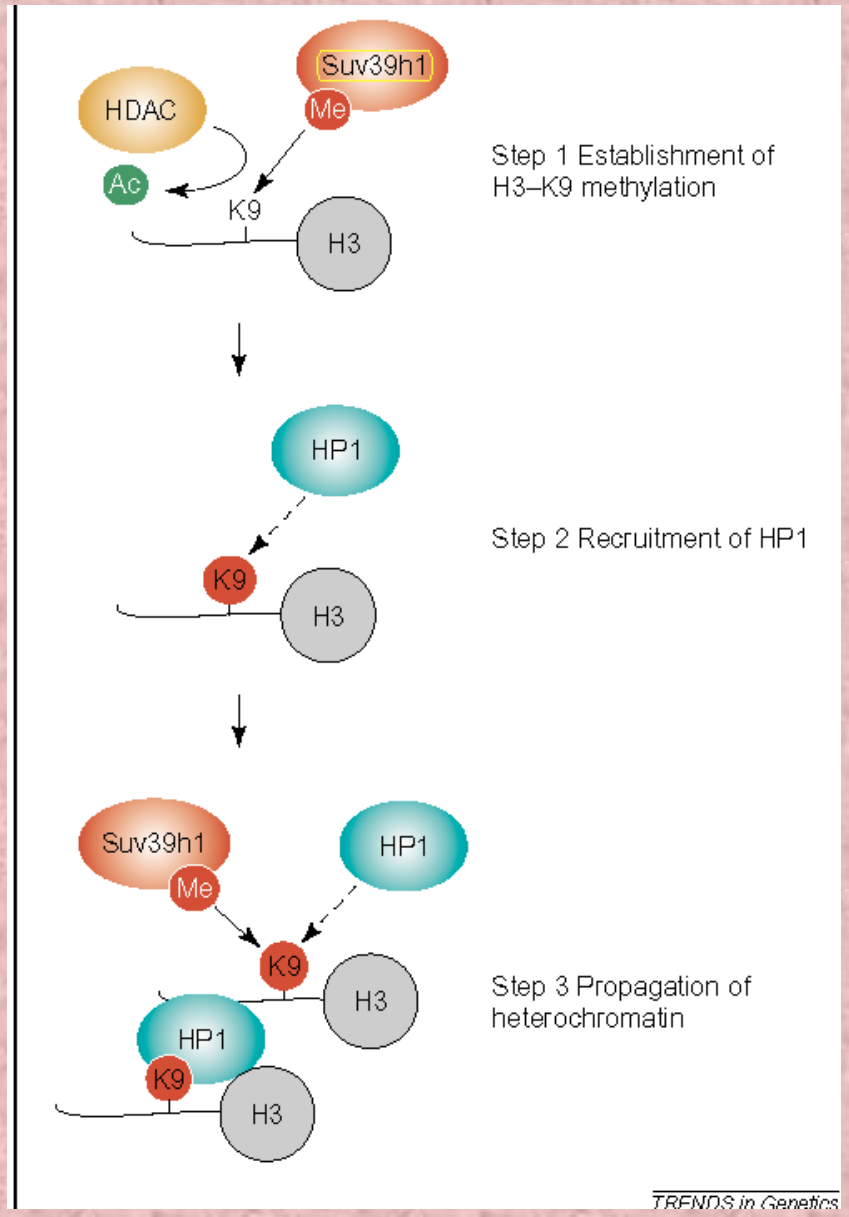


Methylation of H3 at positions K9 and K27 is especially important for heterochromatin formation

Constitutive heterochromatin (centromeres)	H3K9 dimethylation H3K9 trimethylation par SuVar39 recruitment of HP1 (chromodomain)
Facultative heterochromatine (inactive X-chromosome) Hox gene inactivation	H3K27 di- et tri-methylation par EZ (enhancer of Zesta) recruitment of Polycomb (Pc) and other repressors


Hox silencing
?
EZH2
SUZ12
EED
RbAp48
CH3
K27
PRC1
PC
OFF
Oct4 silencing
HP1
K9

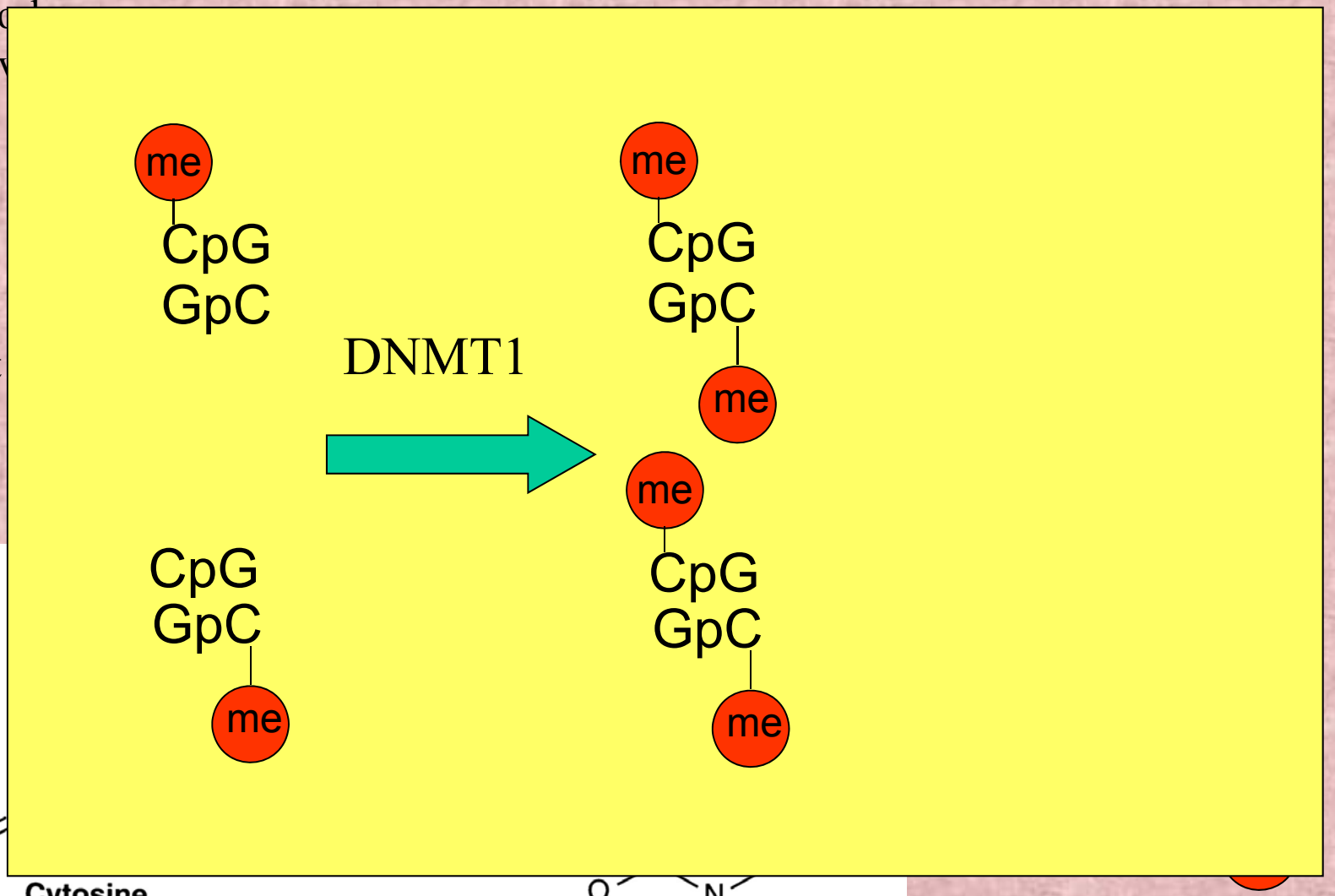
Spreading of heterochromatin



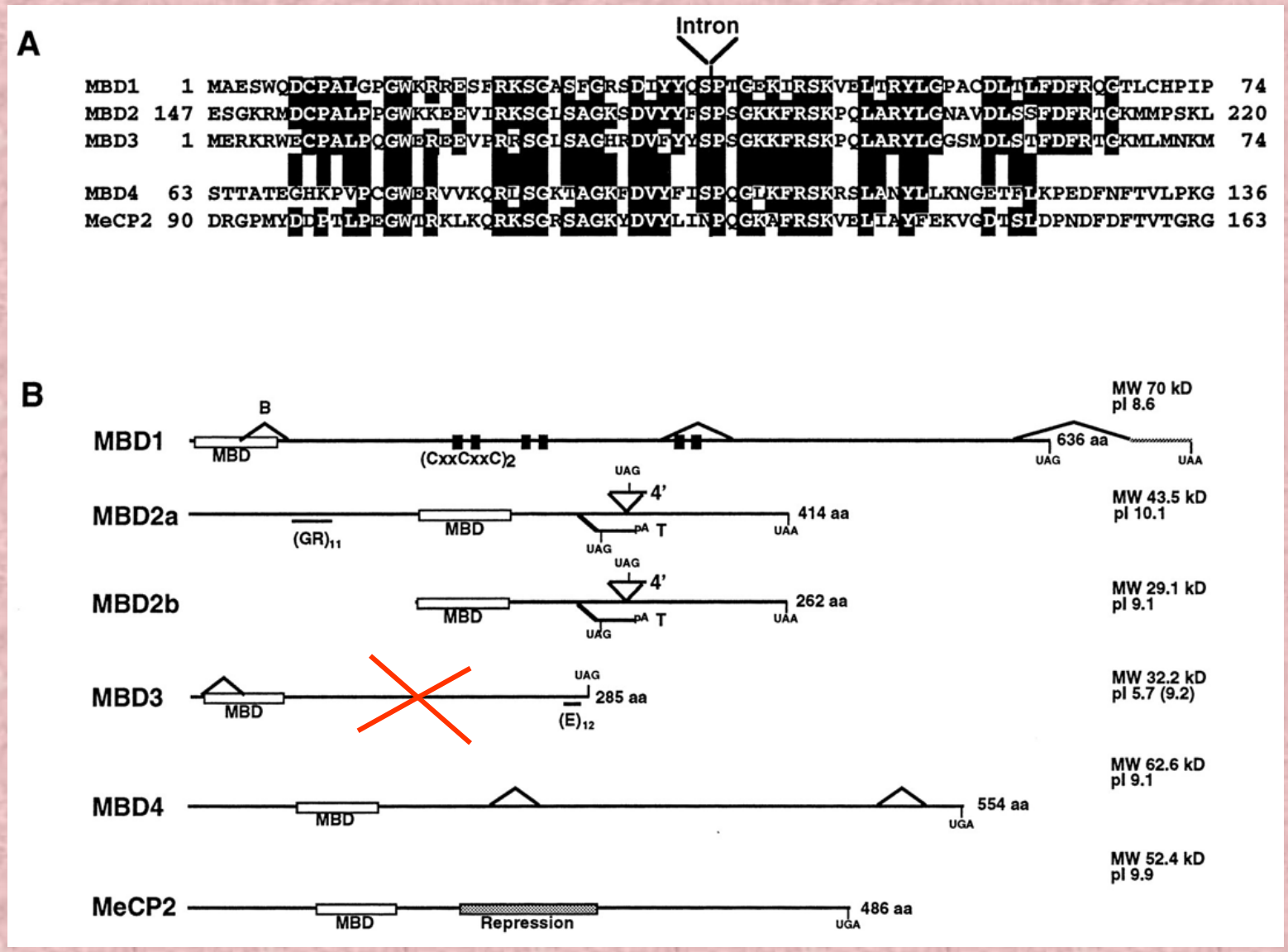
H3 methylated in position K9 attracts Hp1. The latter in turn attracts histonmethylase that methylate neighboring H3 on position K9
In this way the spreading of heterochromatin occurs

Coding DNA sequences constitute 10% or even less of the whole length of the genome of higher eukaryotes. In differentiated cells of multicellular organisms most of the genes do not wo
Inactiv

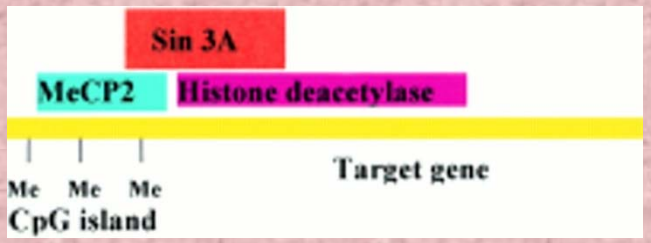
Most

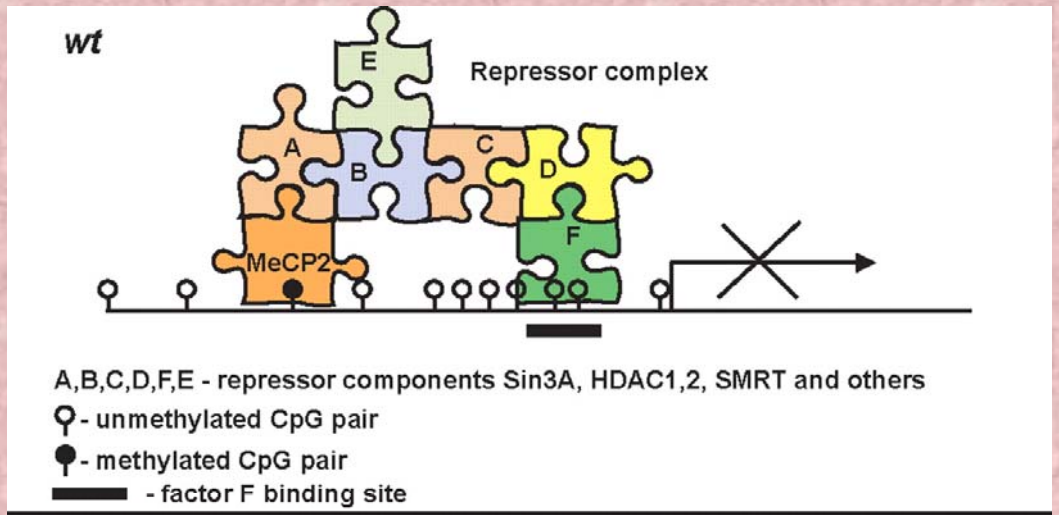


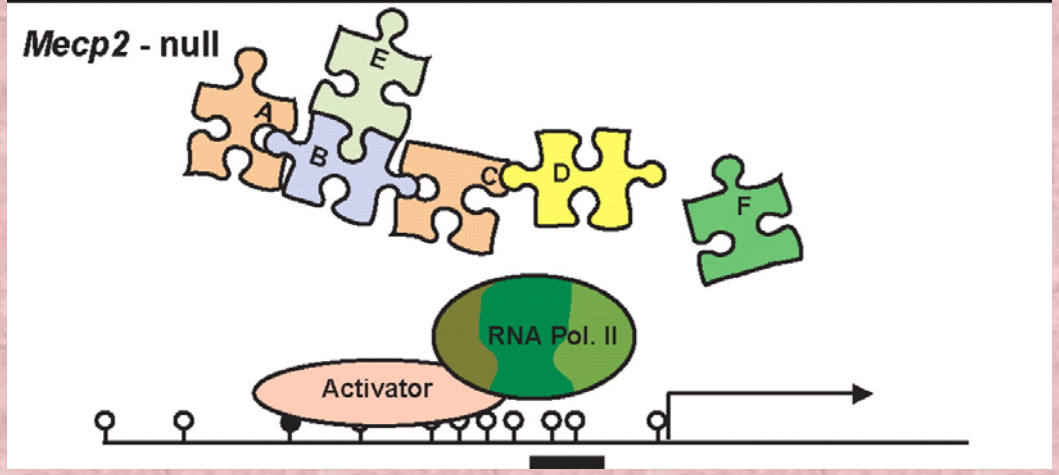
Methylated DNA is recognized by proteins possessing the MBD domain
The most known Me-CpG binding protein is MeCP2



MeCP2 possesses the MBD domain and a transcription repression domain). In addition it can attract Sin 3 complex containing histondeacetylases HDAC1 и HDAC2.



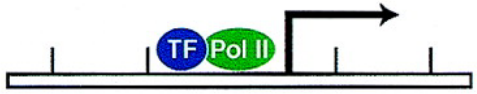




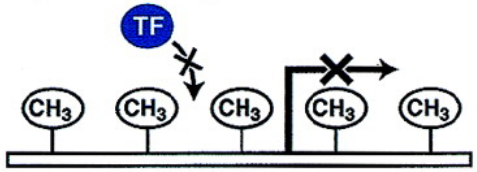
DNA Methylation and Transcriptional Repression

1. Direct interference with transcription activator factor binding

a. Active transcription



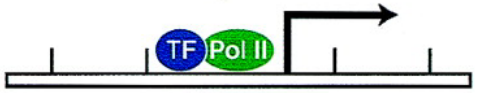
b. Repression by inhibition of TF binding



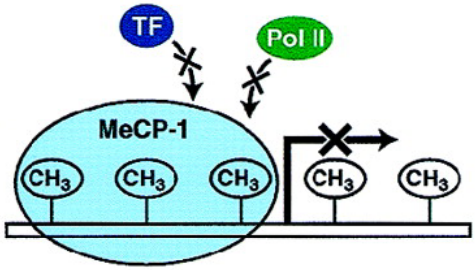
Examples: Methylation sensitive TF: *AP-2, E2F, NFkB*
Methylation insensitive TF: *Sp1*

2. Specific transcriptional repressors

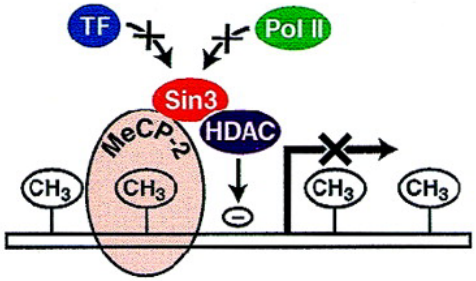
a. Active transcription



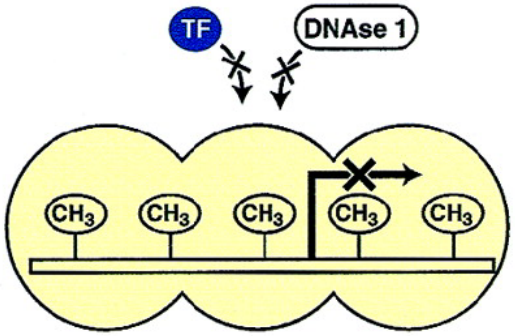
b. Repression by MeCP-1



c. Repression by MeCP-2



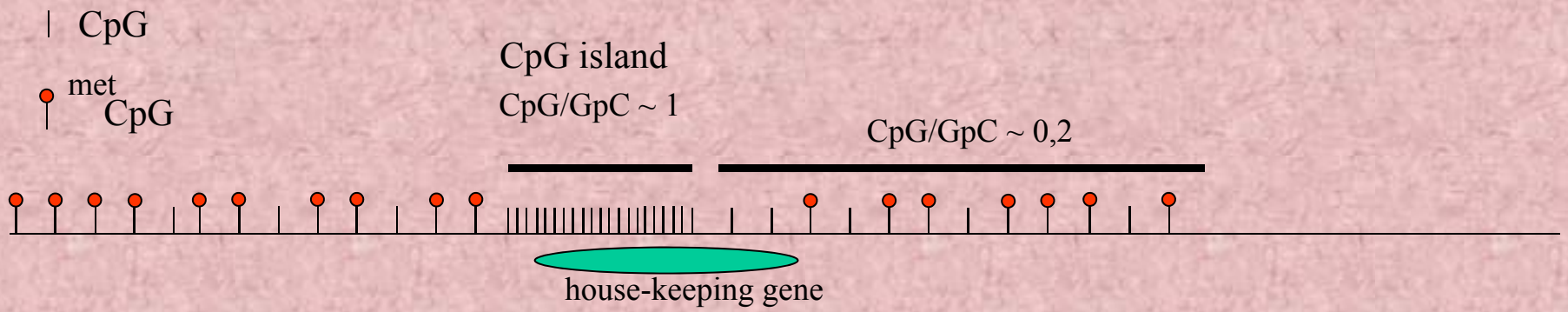
3. Inactive chromatin structure formation



methylated CpG: 

unmethylated CpG: 

CpG islands



CpG islands usually contain recognition sites for transcription factor Sp1 CCGCCC

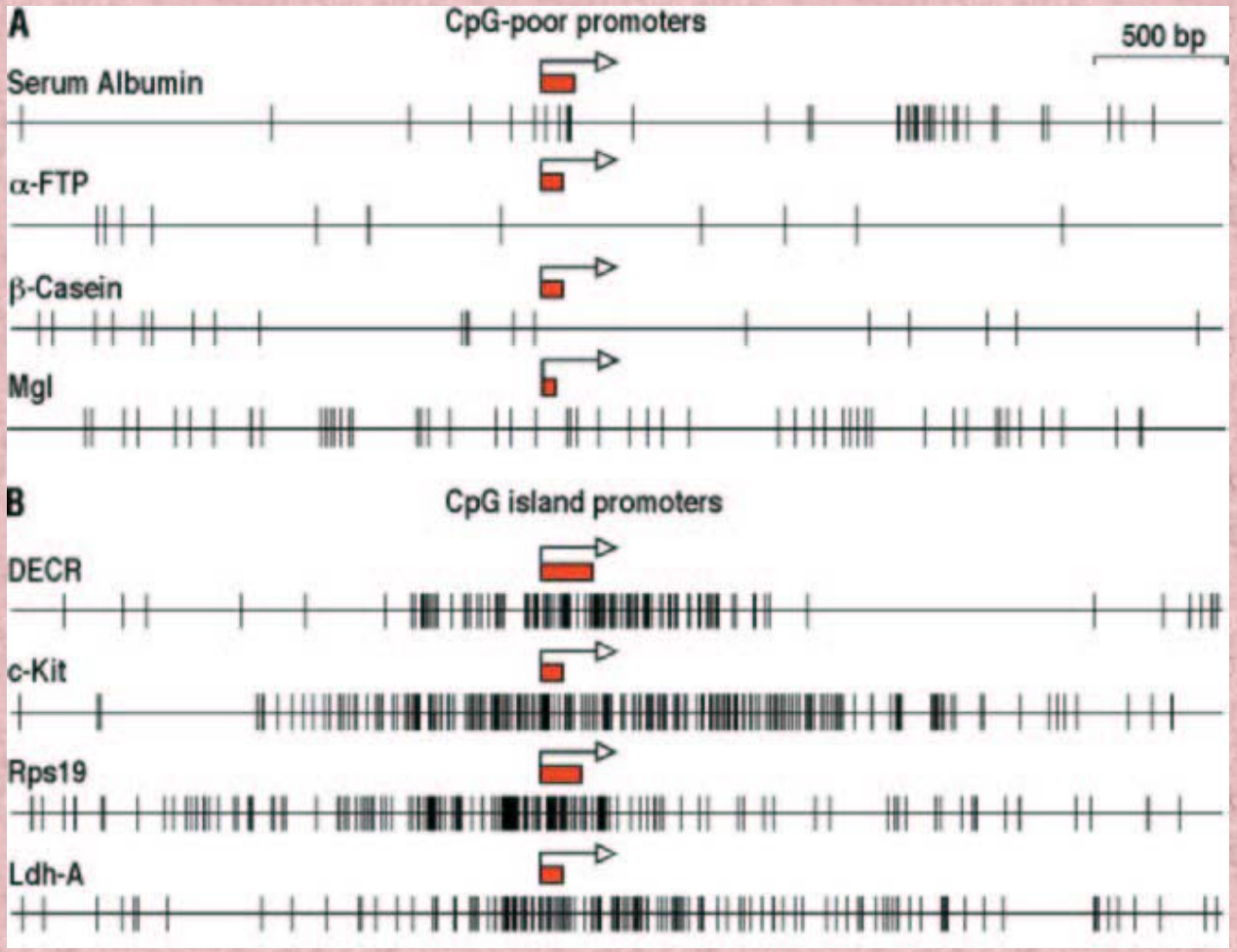
met

C ⟶ T

E.Coli CpG/GpC = 1

Human CpG/GpC = 0.2

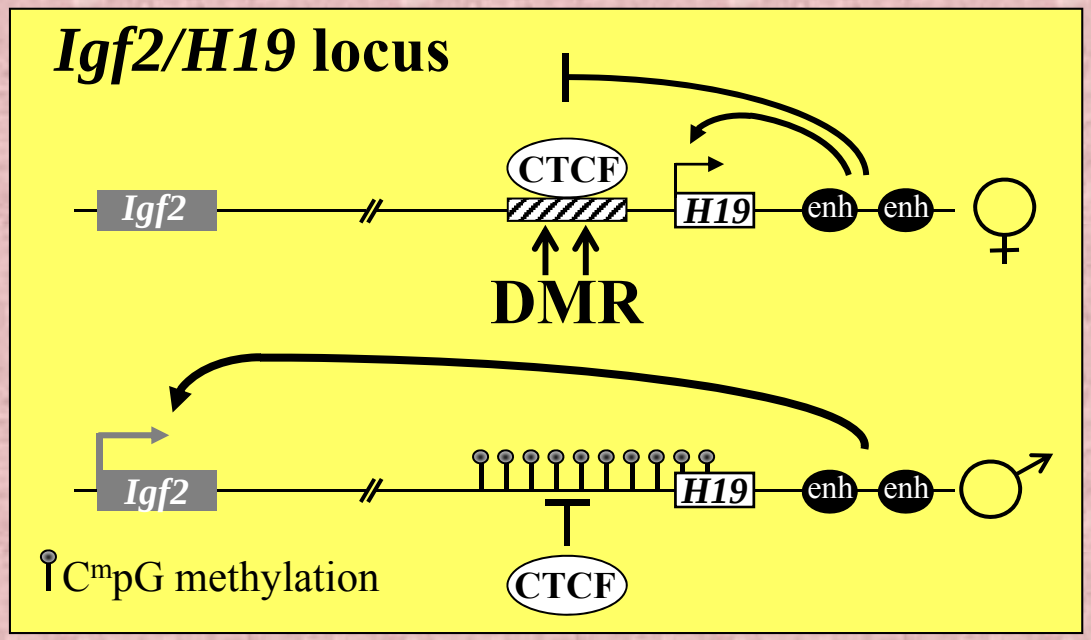
Most of the promoters of house-keeping genes are located within CpG islands



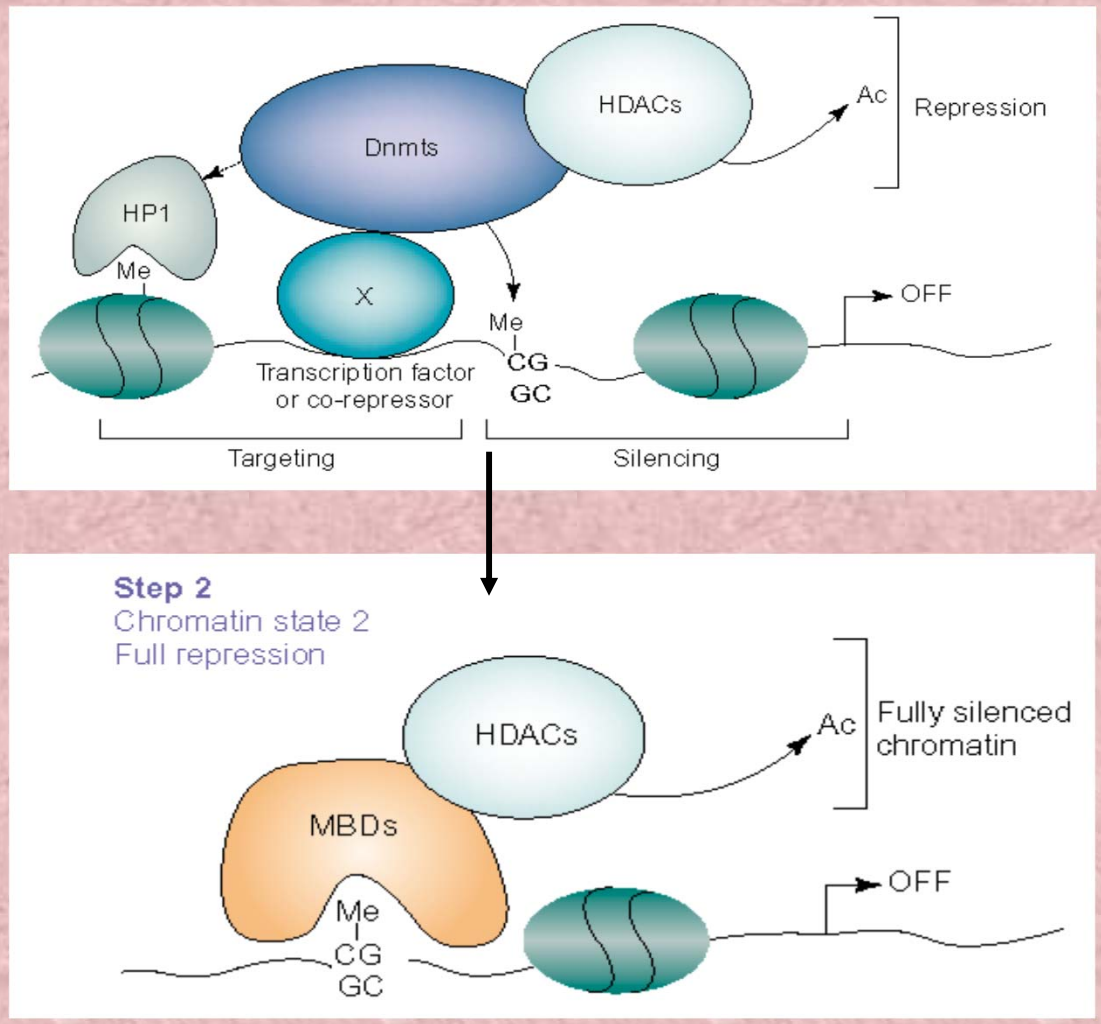
Imprinting is an allele-specific modification that leads to differential expression between the parental alleles in somatic cells. DNA methylation play a key role in establishment of imprinted regions

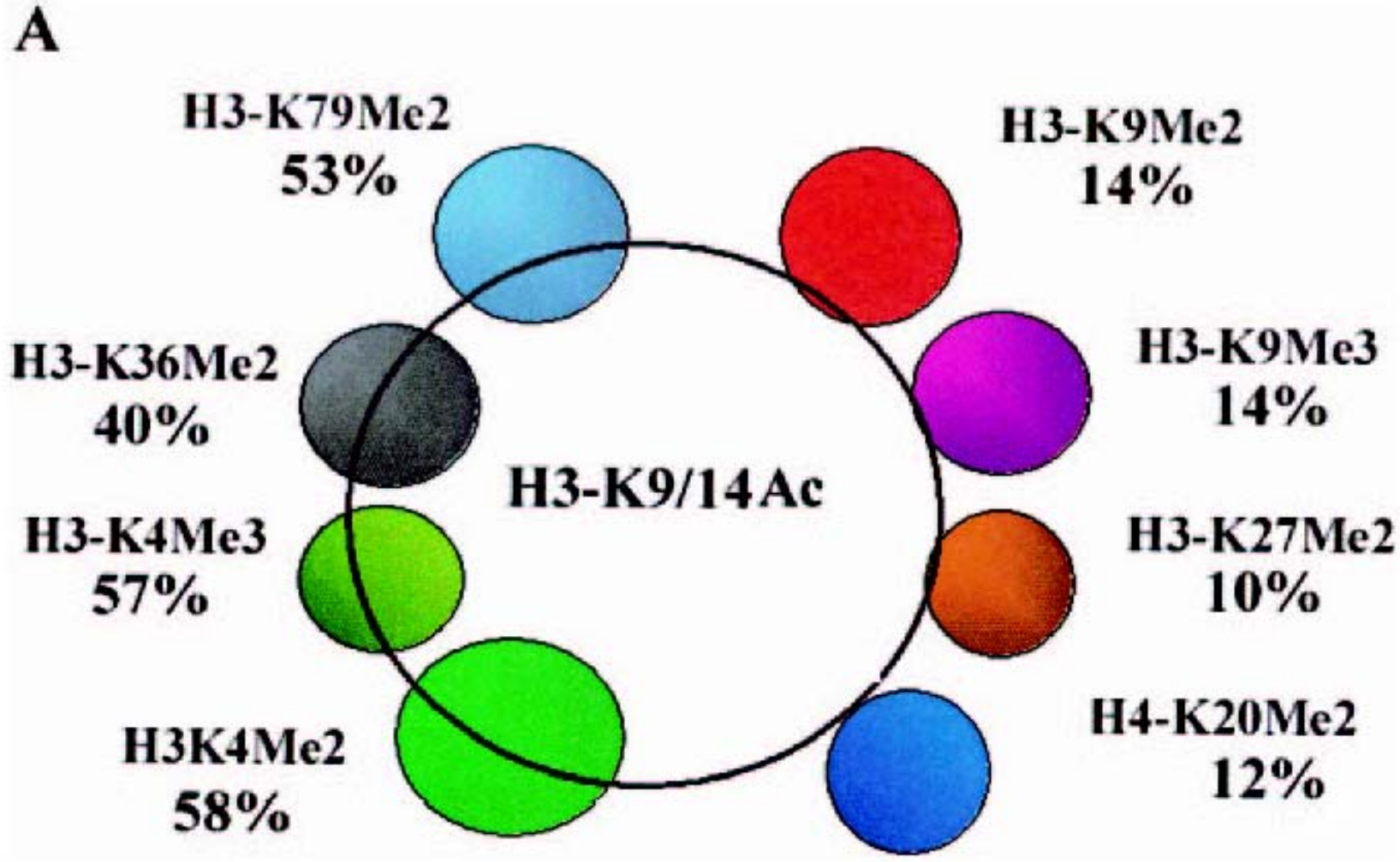
One can consider imprinting as one of examples of organization of repressed genomic domain

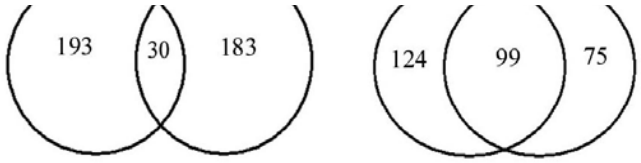
There are special regulatory elements that control establishment of imprinted domains (**ICR, Imprinting Choice Regions**). Conditional (methylation-sensitive) insulators play a key role in the work of ICRs.



For quite a long time it was thought the DNA methylation is a primary modification and that histon-methylases are attracted by methylated DNA. Now it is proven that the real scenario is the opposite one. Mutation affecting function of H3 lys9 methyltransferase interferes with DNA methylation. Thus, histone methylation is a primary event in chromatin domain silencing. DNA methylation is necessary to make the silencing irreversible.




A
H3-K79Me2
53%
H3-K9Me2
14%
H3-K36Me2
40%
H3-K9Me3
14%
H3-K9/14Ac
H3-K4Me3
57%
H3-K27Me2
10%
H3K4Me2
58%
H4-K20Me2
12%


193
30
183

124
99
75

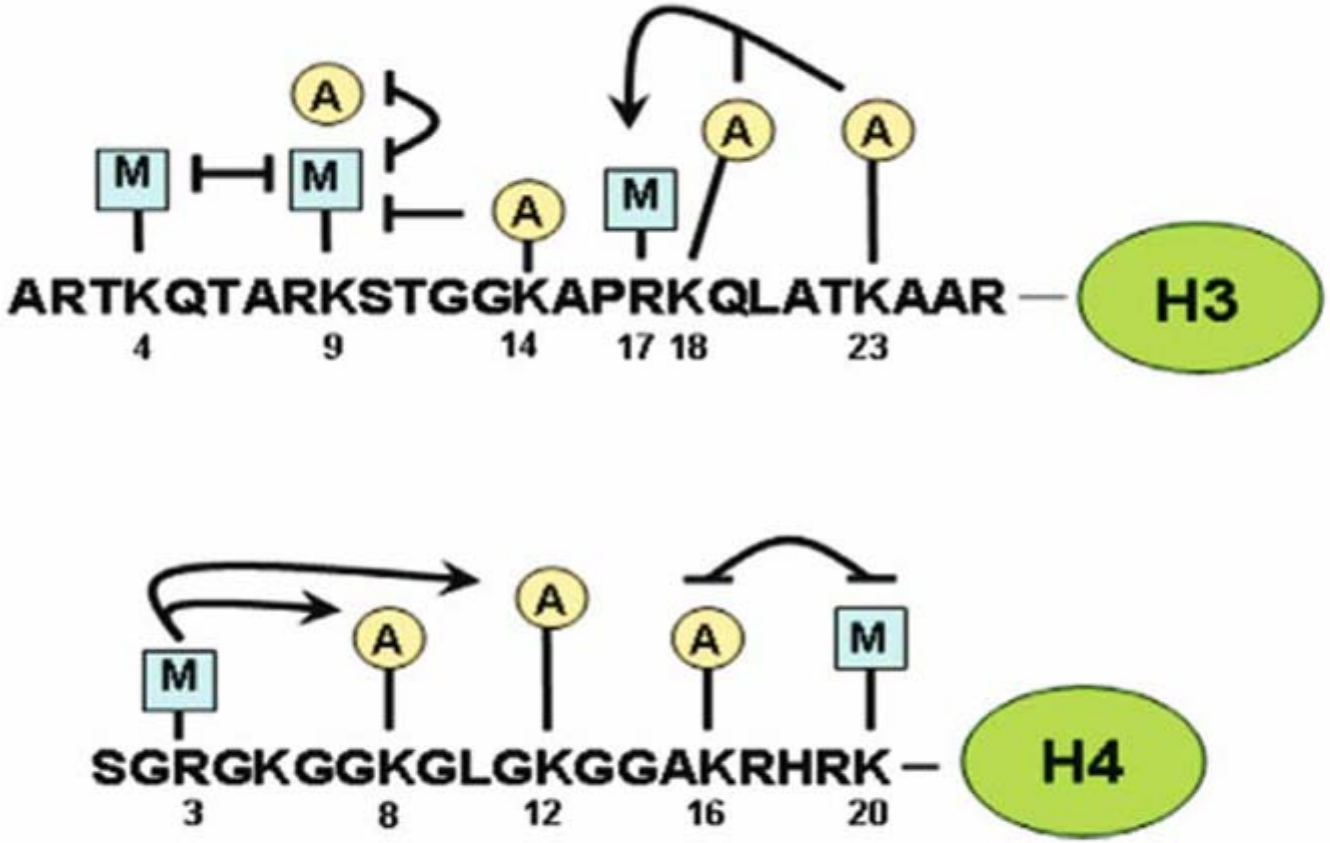
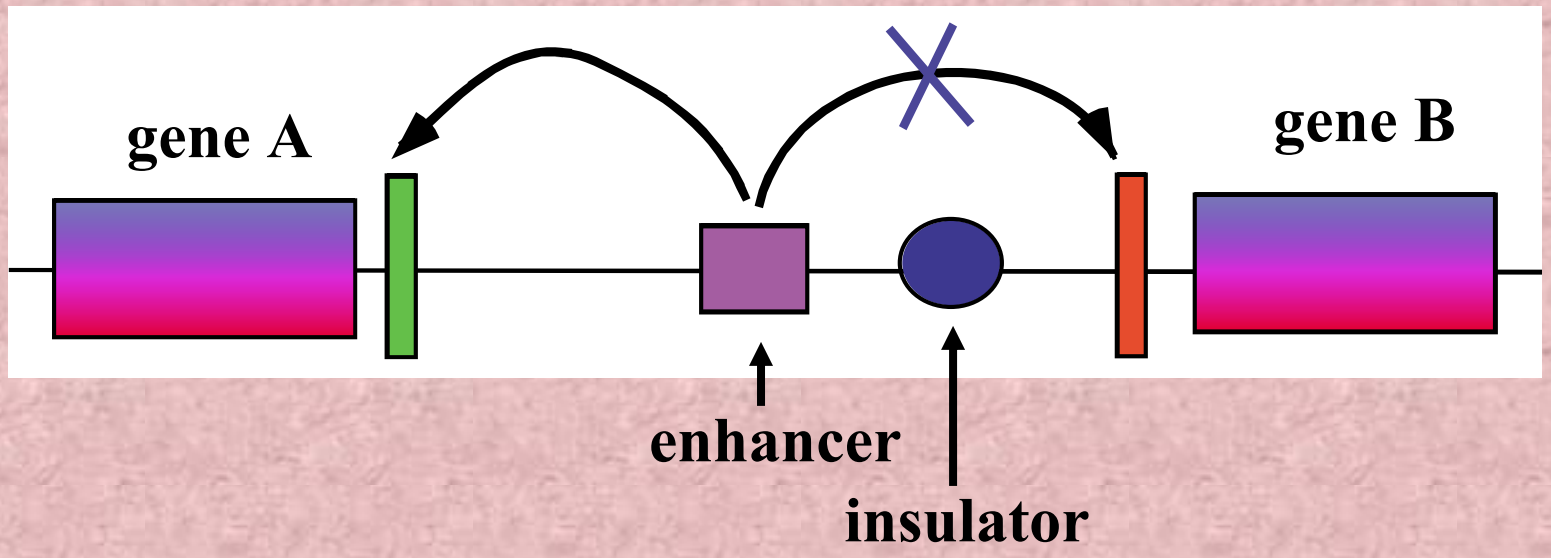


Plate 22. Interplay between histone acetylation and methylation. Acetylated (A) and methylated (M) residues are indicated. Positive effects are represented by normal arrows; negative effects are represented by tee arrow. (See Chapter 16 Figure **??**, p. 353.)

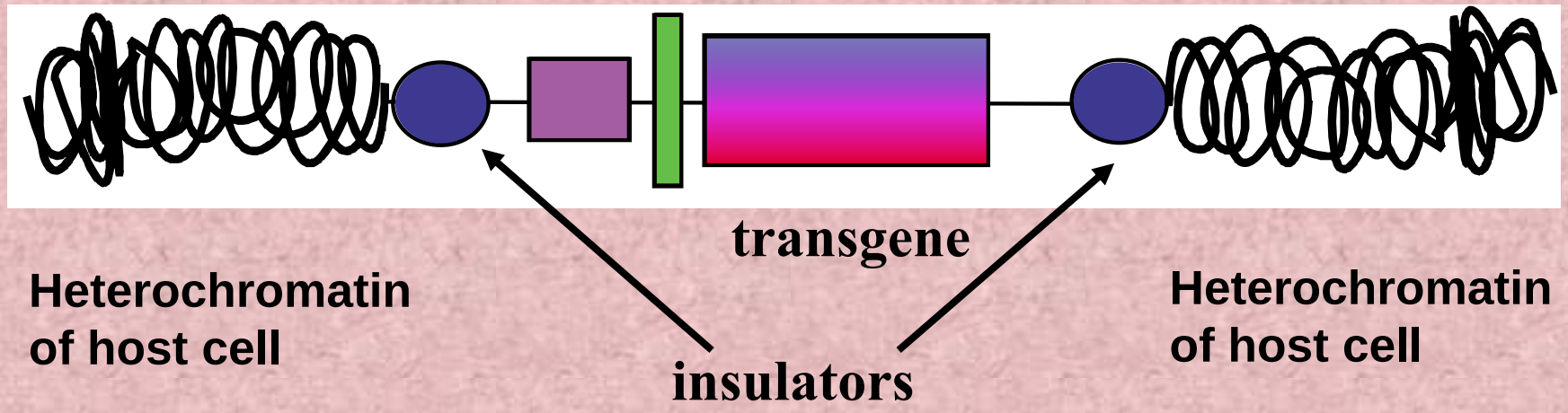
Insulators – regulatory elements of the genome that may separate functionally genomic domains

Being placed between an enhancer and a promoter insulator blocs action of the enhancer on this promoter. At the same time insulator does not suppress the activity of the enhancer in general. This is a principal difference between enhancers and silencers



Insulators – functional elements of the genome that can protect a transgene from position effects (bordering activity)

Insulators block spreading of negative signals from heterochromatin of host cells

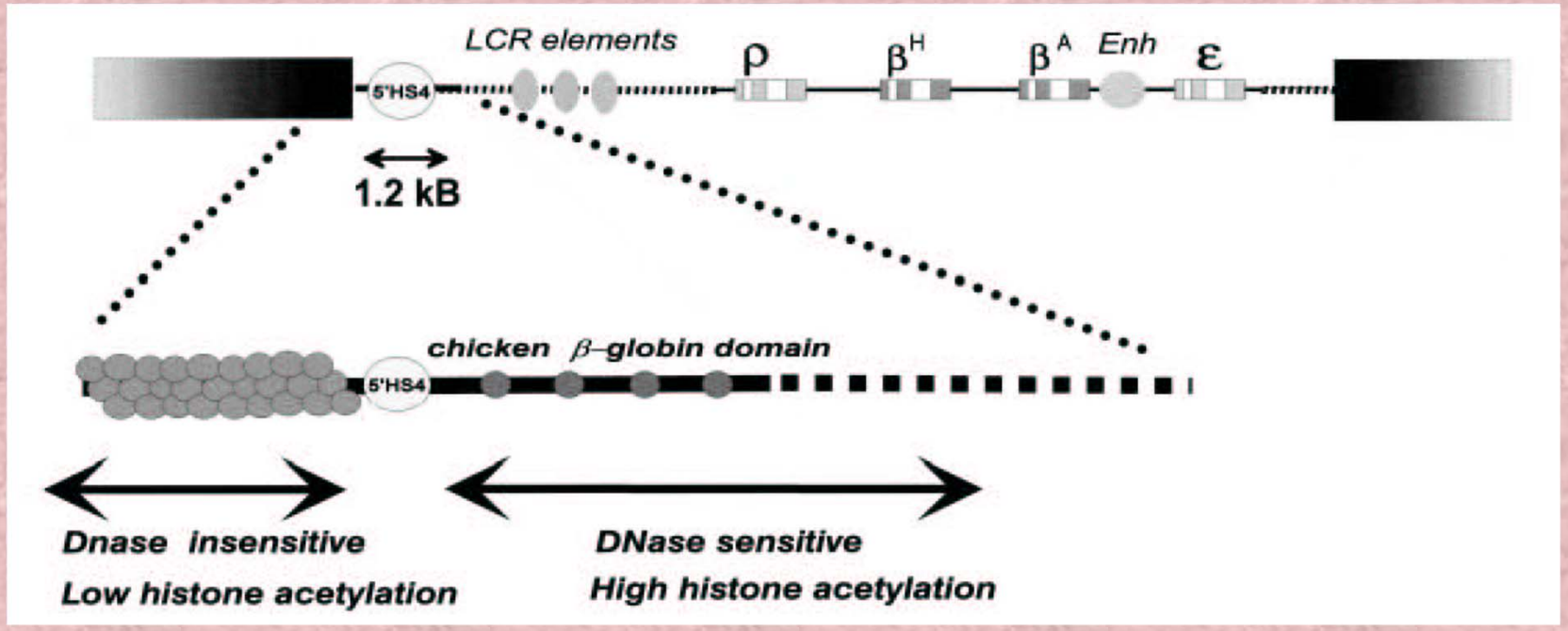


Some insulators possess both enhancer-blocking and domain bordering activities

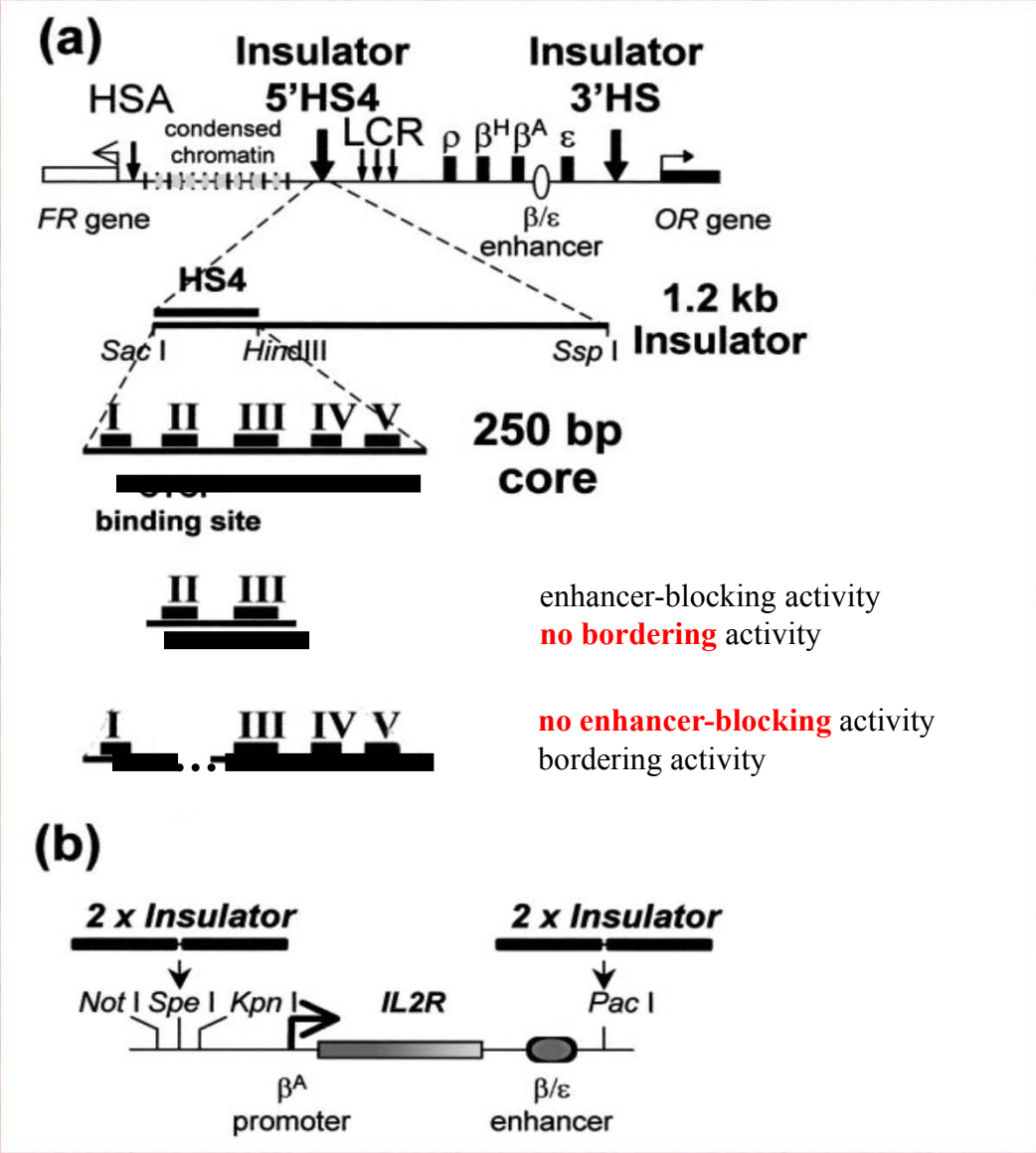
Other insulators possess only one of these activities

In complex insulators possessing both enhancer-blocking and domain bordering activities distinct functional domains are responsible for these activities

One of the best-studied insulator possessing both bordering and enhancer-blocking activities is located at the 5' end of the chicken β-globin gene domain

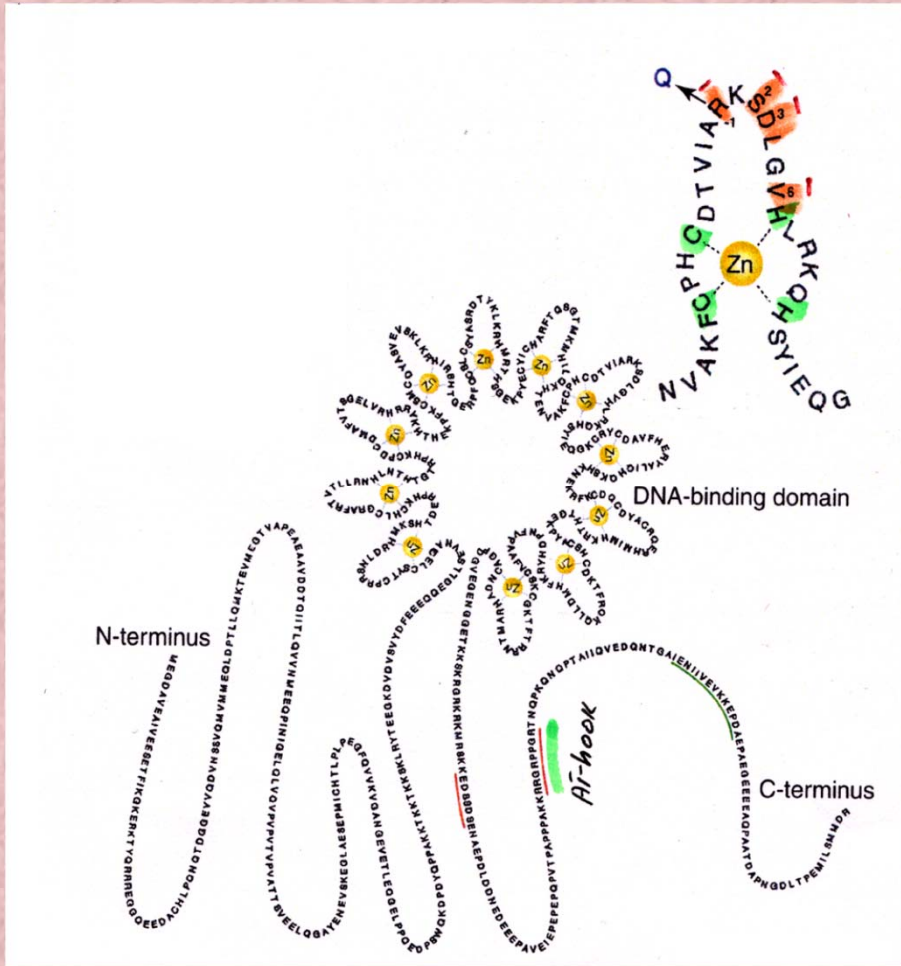


Deletional analysis of an insulator

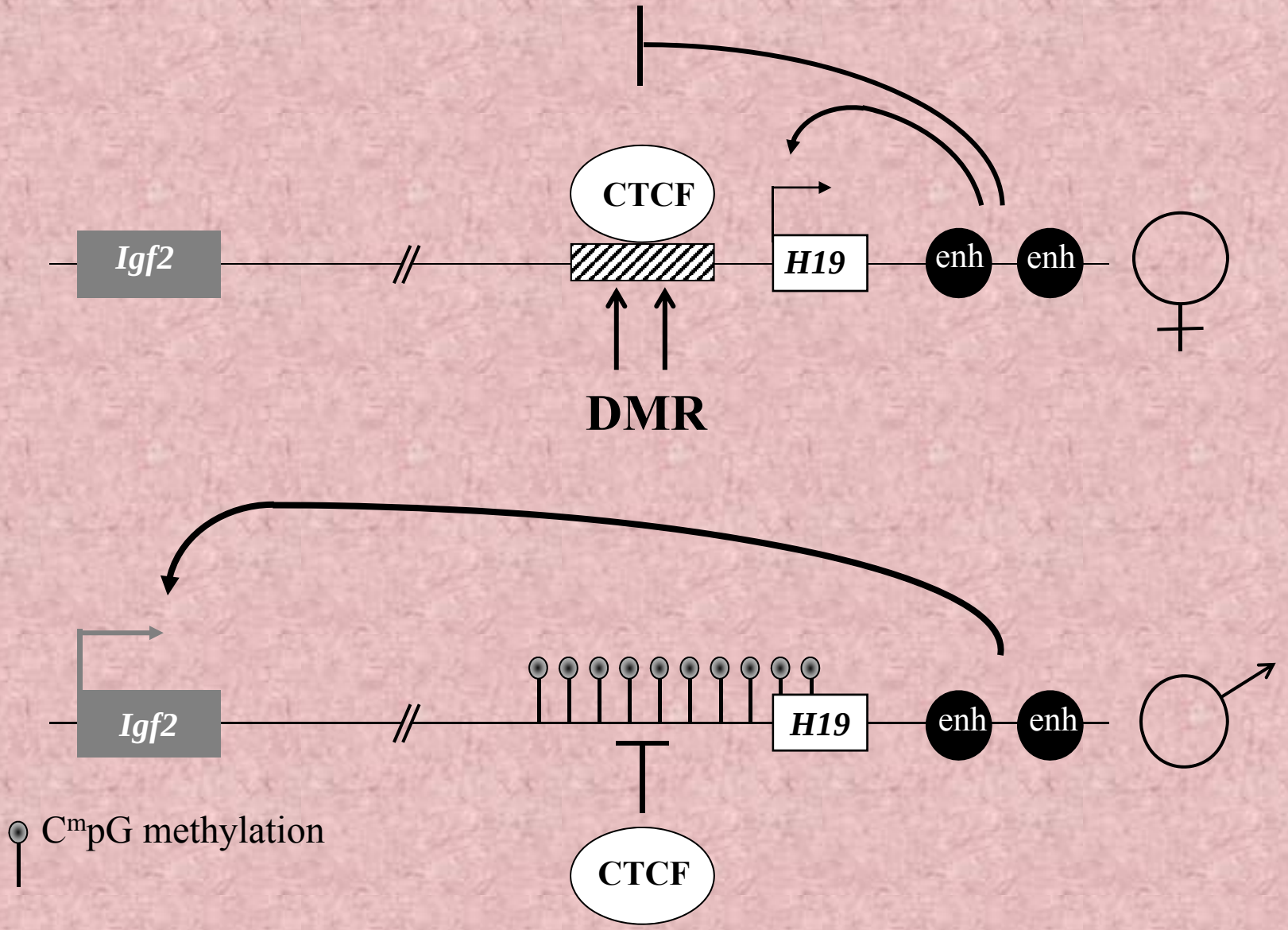


CTCF bind to all known vertebrate "enhancer-blocking" elements and this binding is

necessary and sufficient for enhancer-blocking activity



Igf2/H19 locus



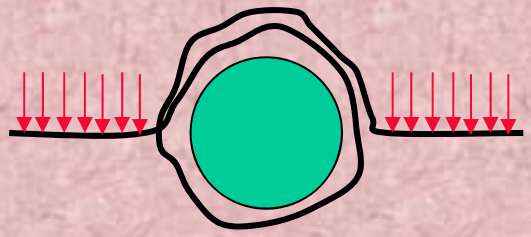
Replication of chromatin

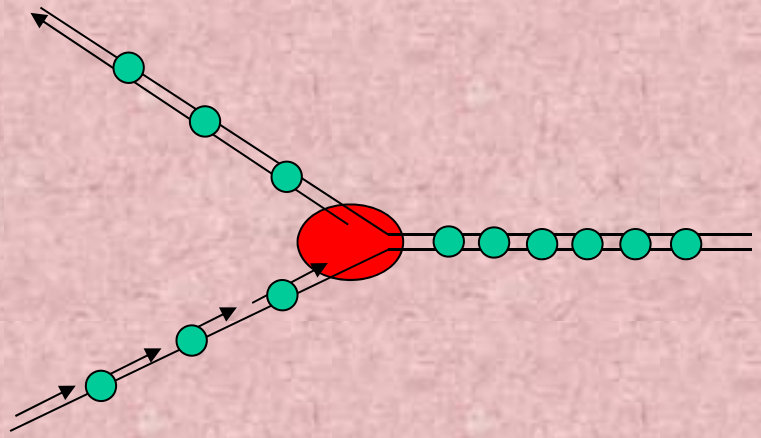
What happens with nucleosomes during replication

How epigenetic profiles are reproduced (How variant histones are incorporated and how histone modifications are reproduced)

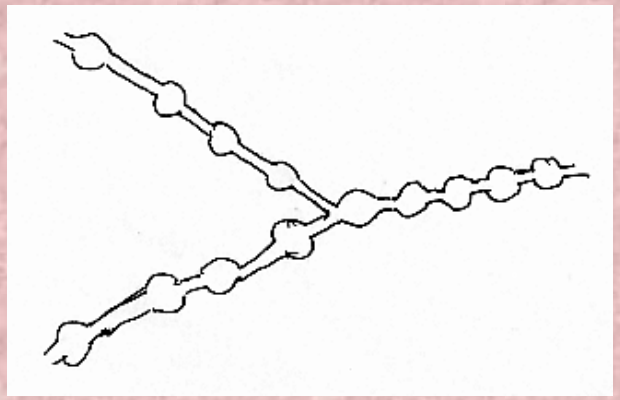
Using trioxalen crosslinking it is possible to demonstrate that ald nucleosomes are transferred to both new DNA chains. As a result, the density of nucleosomes is diminished by half

Trioxalen introduces links between DNA chains only in spacer regions



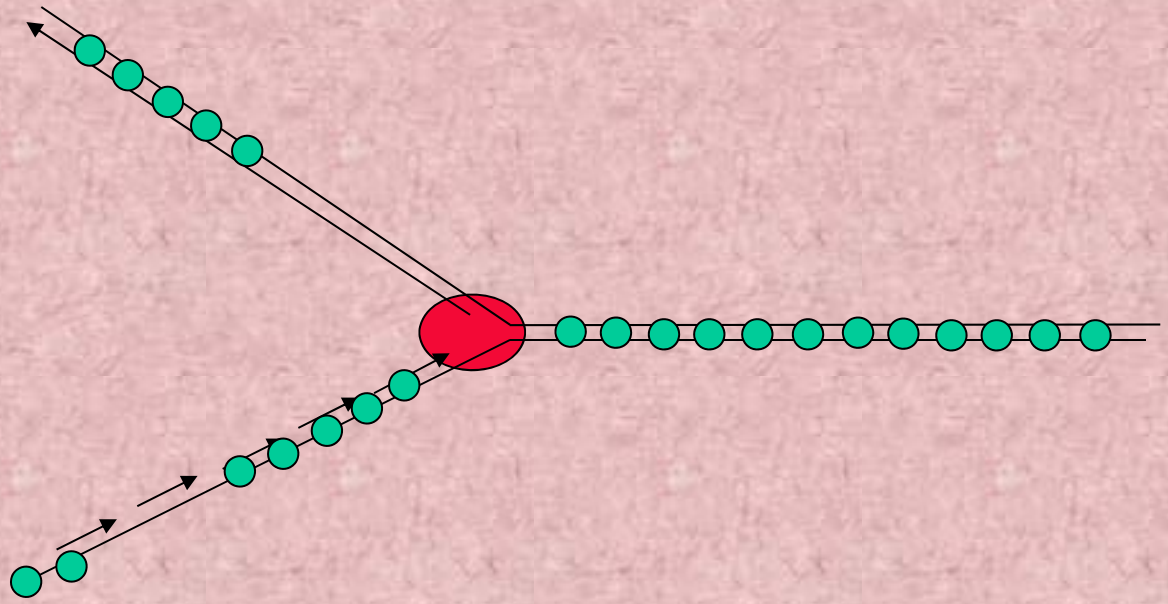


Uner conditions of suppression of histone synthesis one can observe the following structures after trioxalen cross-linking, protein digestion and DNA denaturation



More detailed studies have demonstrated that after digestion of nascent chromatin with micrococcal nuclease one can observe the normal nucleosomal pattern. Thus the transferred on new DNA chain old nucleosomes are likely to be organized in short blocks

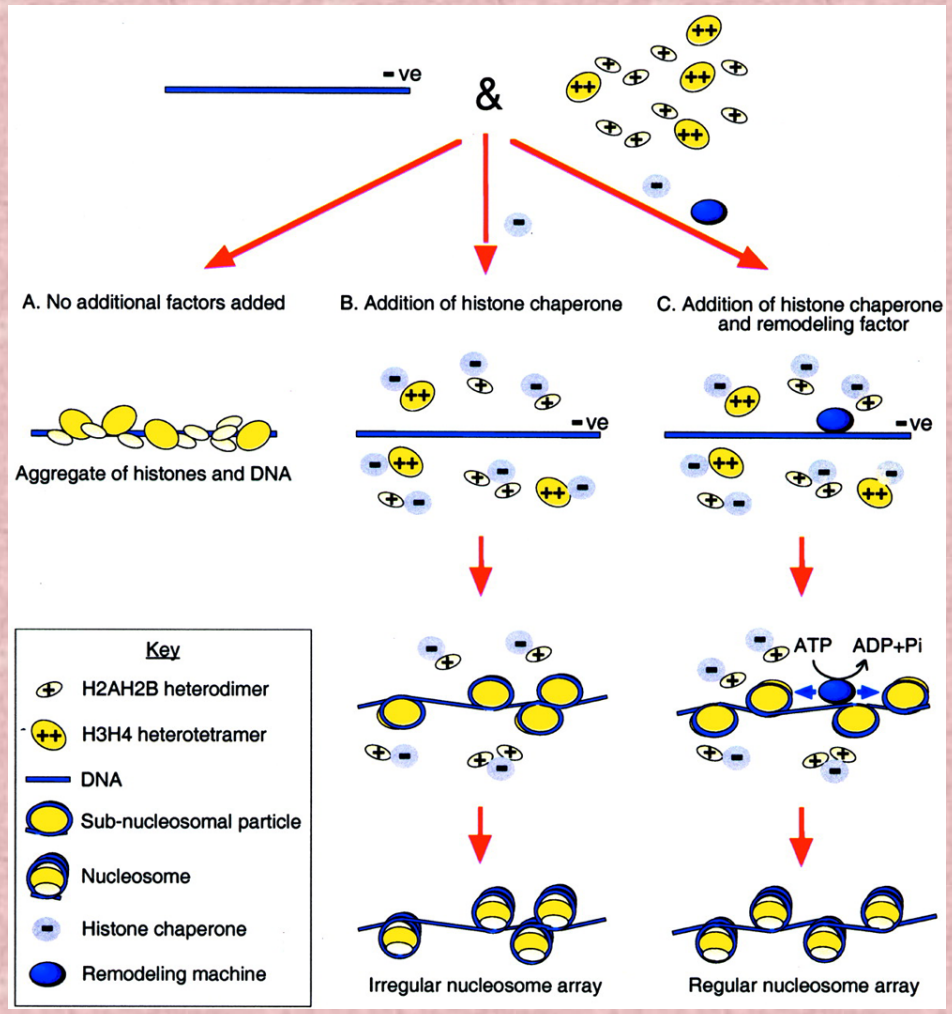
This is likely to be due to the limited processivity of the chromatin remodeling factor ASF1

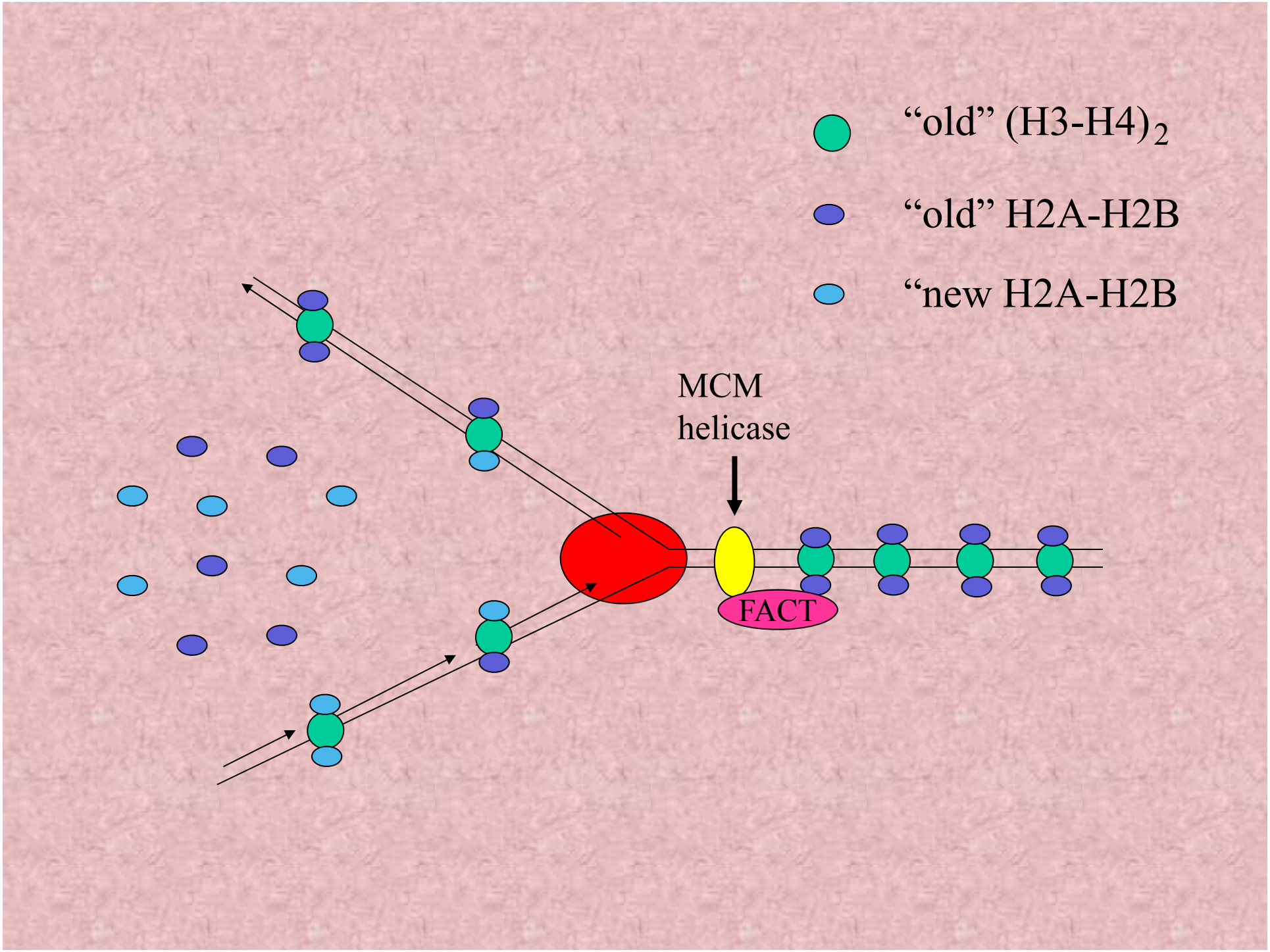


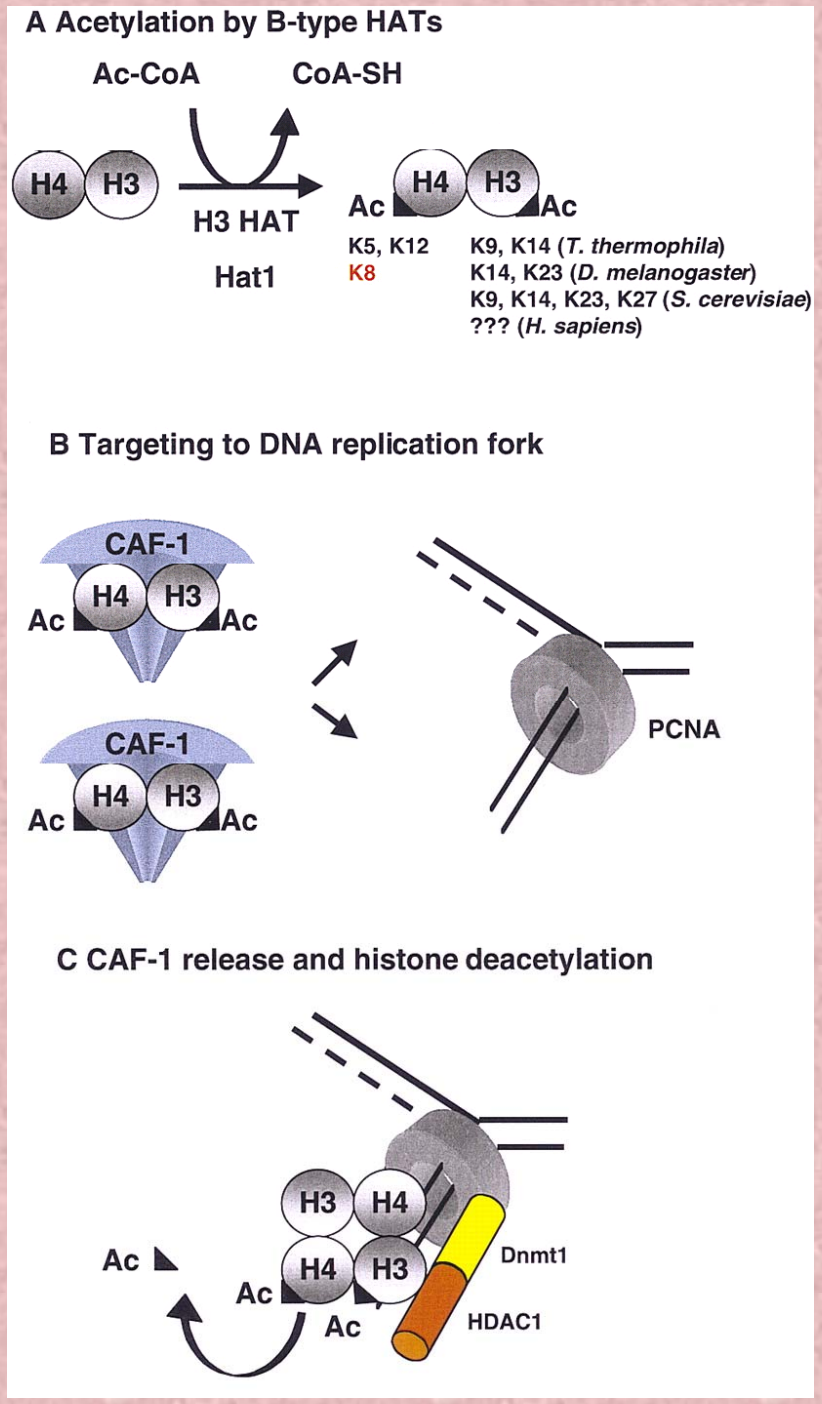
After mixing in a test-tube DNA and histoones one can obtain large irregylat DNA-protein agregates.

Nurmal nucleosomes are assembled in vitro after reducing the charges (in the presence of a shaperon or under conditions of elevated salt concentration). These nucleosomes are not regularly distributed along DNA (spacing is not normal)

To achieve normal spacing one should add chromatin remodelling complex (ACF or CHRAC)

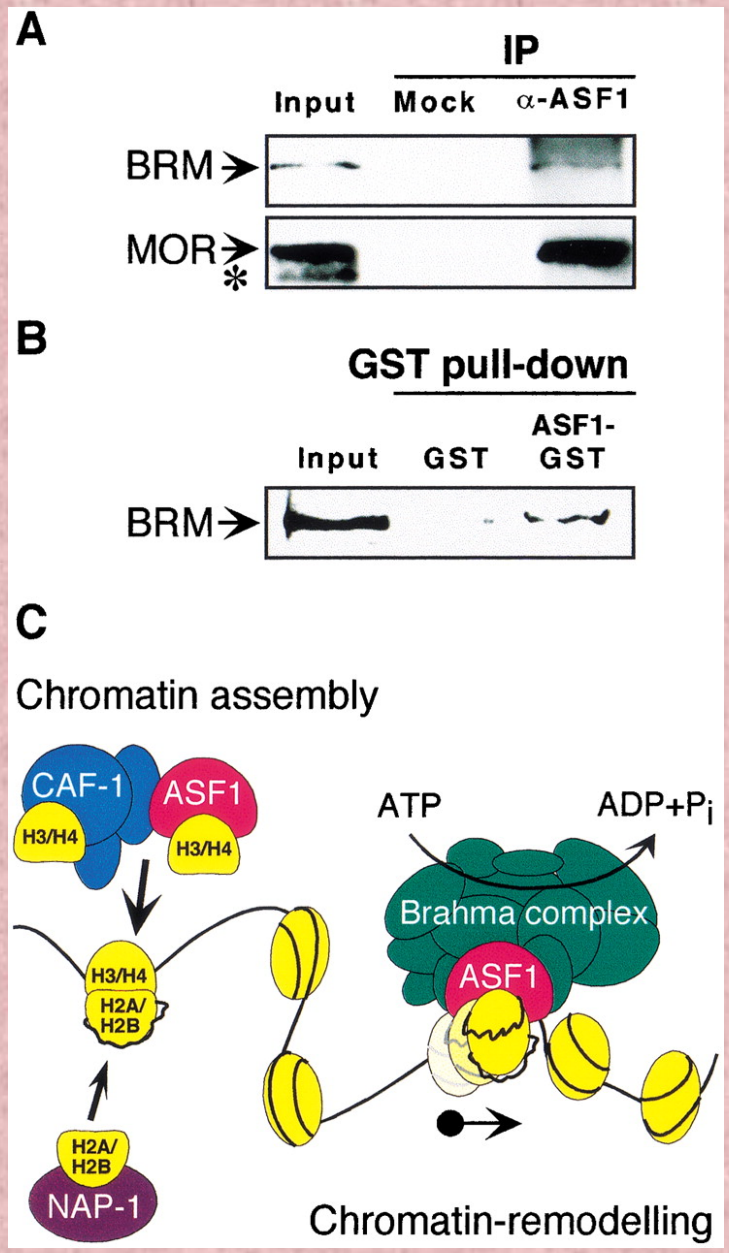



"old" (H3-H4)2
"old" H2A-H2B
"new H2A-H2B
MCM
helicase
FACT



Replication-dependent assembly of nucleosomes is mediated in two steps.
First, tetramer (H3-H4)2 is assembled from two heterodimeres H3-H4. The heterodimeres are self-assembled in cytoplasm from newly-synthesised histones. These histones are specially acethylated by Hat1 (which do not participate modification of histones assembled in nucleosomes). Heterodimers H3-H4 are transferred to the nucleus by chaperons CAF-1 (Chromatin Assembly Factor 1) and ASF-1 (Ati Silencing Function protein1). ASF1 complexed to histones is also called RCAF).
CAF1 is recruited to, replication forks by PCNA.
ASF1 is recruited by CAF1.

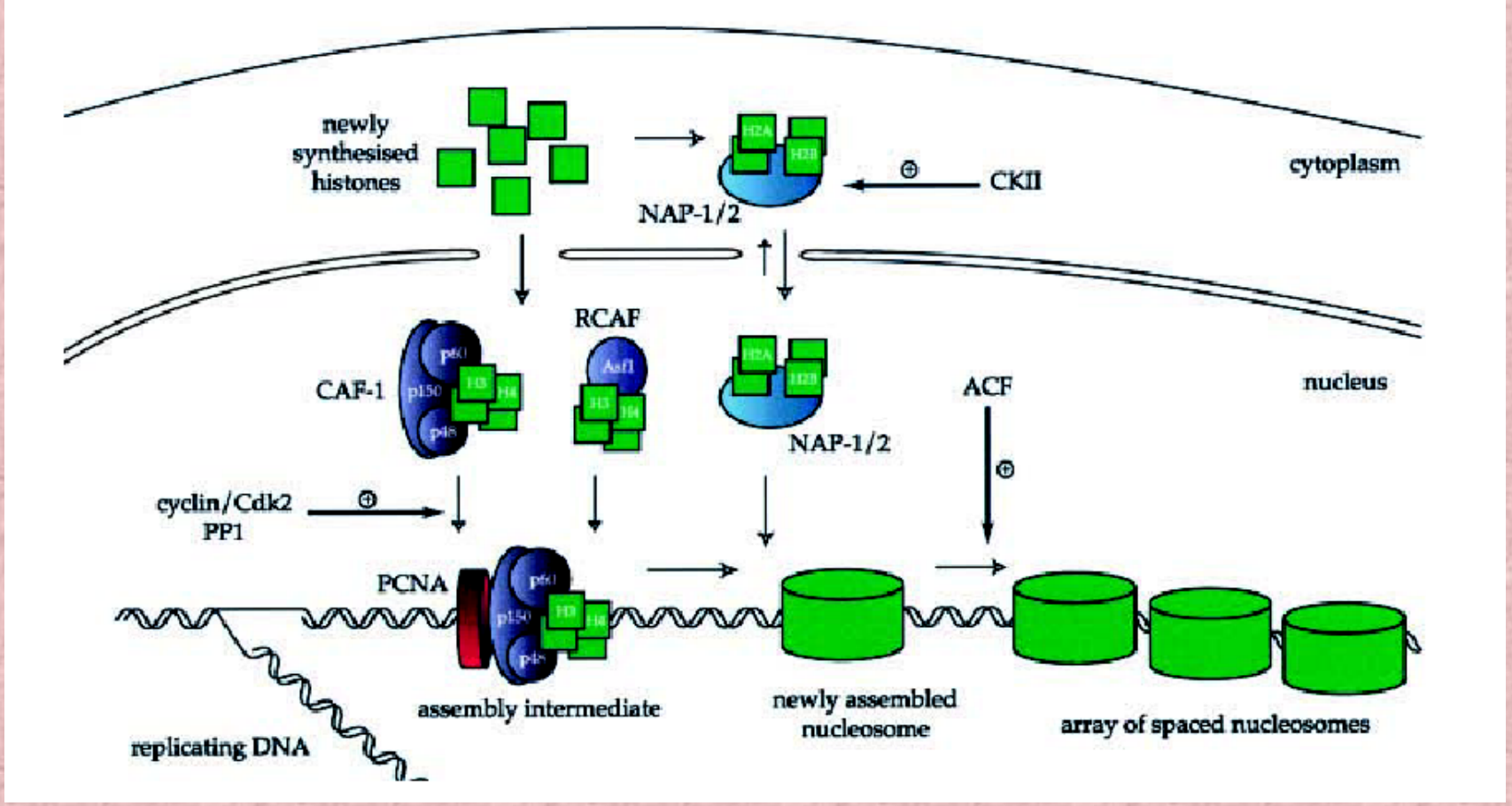
Typical for newly-synthesised histones acetilation is removed by HDAC1 after some lag period
During this lag perion nucleosomal particles are unstable and substitution of main histone forms by variant forms is possible.

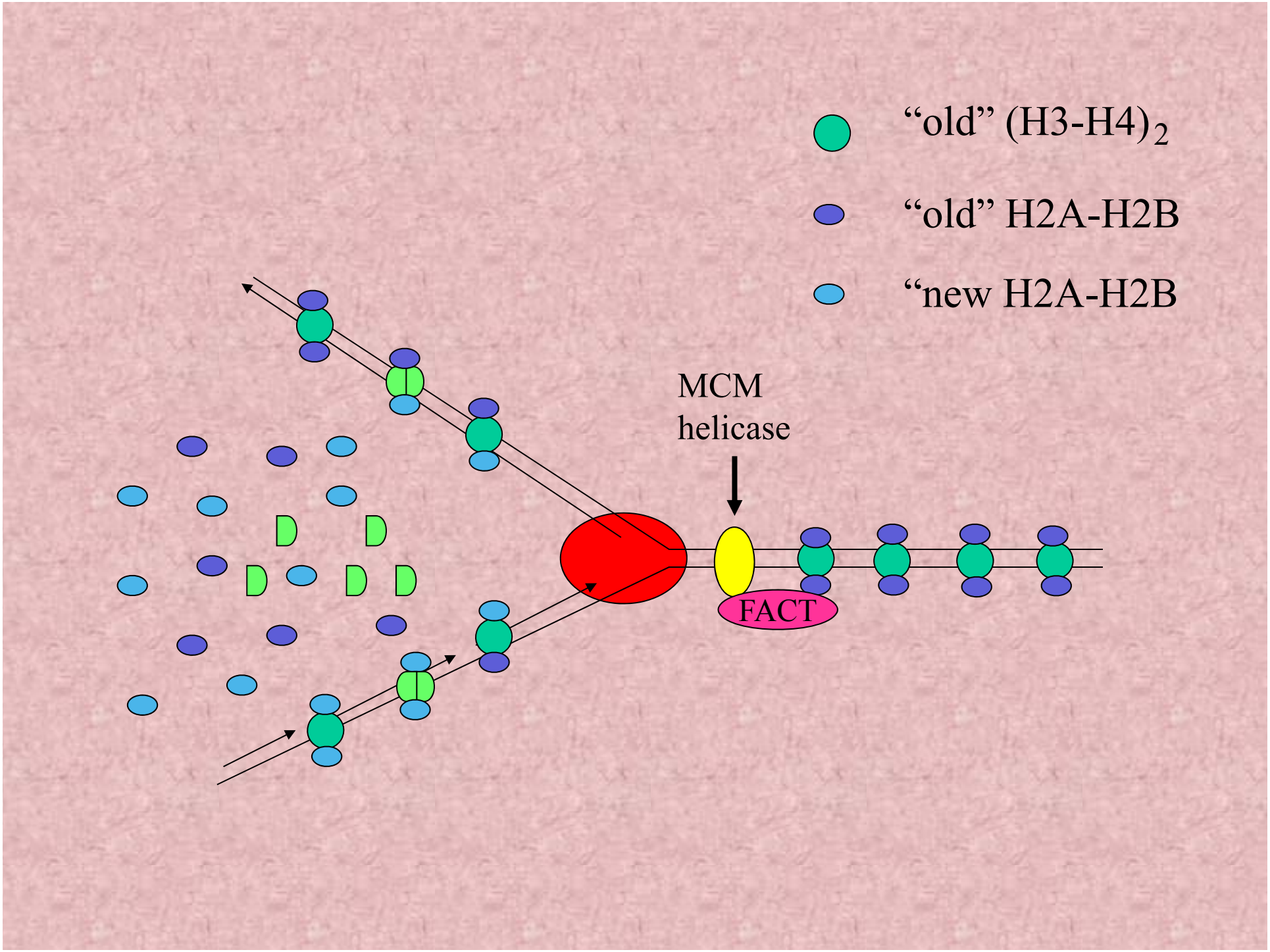


Using chromatin immunoprecipitation assay (ChIP) it was demonstrated that ASF interacts with a chromatinremodelling complex Brahma. In this way the latter is recruited to replication forks

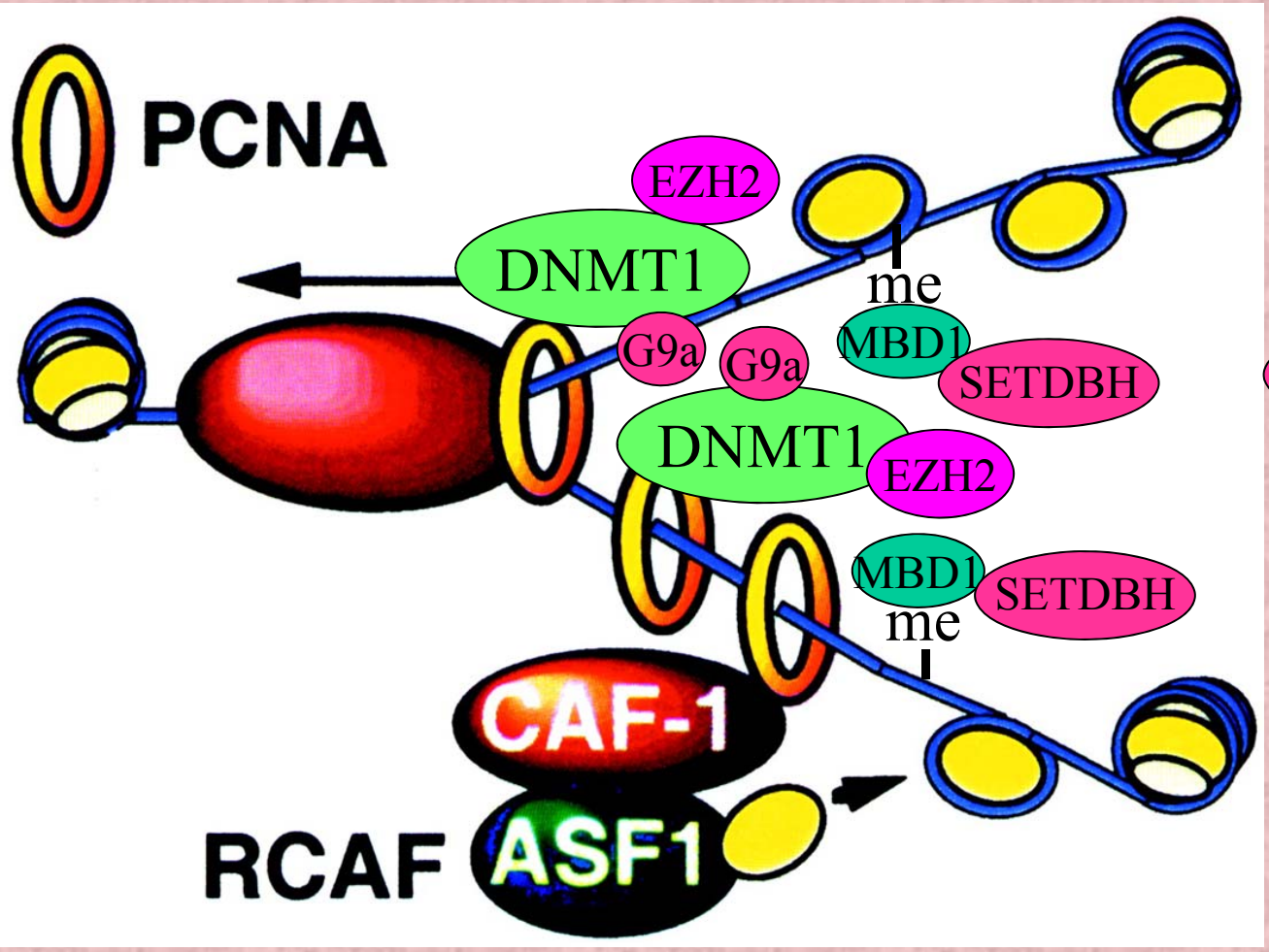
Heterodimers H2A-H2B are self-assembled in cytoplasm and are transferred to the nucleus by chaperon NAP1 (Nucleosome Assembly Protein 1)

Cromatin remodelling complexes RCF and ACF take care of correct distribution of nucleosomal particles




"old" (H3-H4)2
"old" H2A-H2B
"new H2A-H2B
MCM
helicase
FACT

Reproduction of modifications profilsof histones after replication constitutes an important task. In case of repressed chromatin domains methylation of DNA shows the regions where repressive modifications should be made. PCNA recruits DNMT1. DNMT1 reestablish simmetric pattern of CpG methylation. It also recruits H3K9 and H3K27 histone methylases. Methylated CpG recruits MBD1-SETDBH complex



Replication independent nucleosome assembly

Incorporation of some variant forms is strictly restricted to specific phases of the cell cycle (CenpA – G1)

Incorporation of some variant formes (H3.3) is coupled to transcription

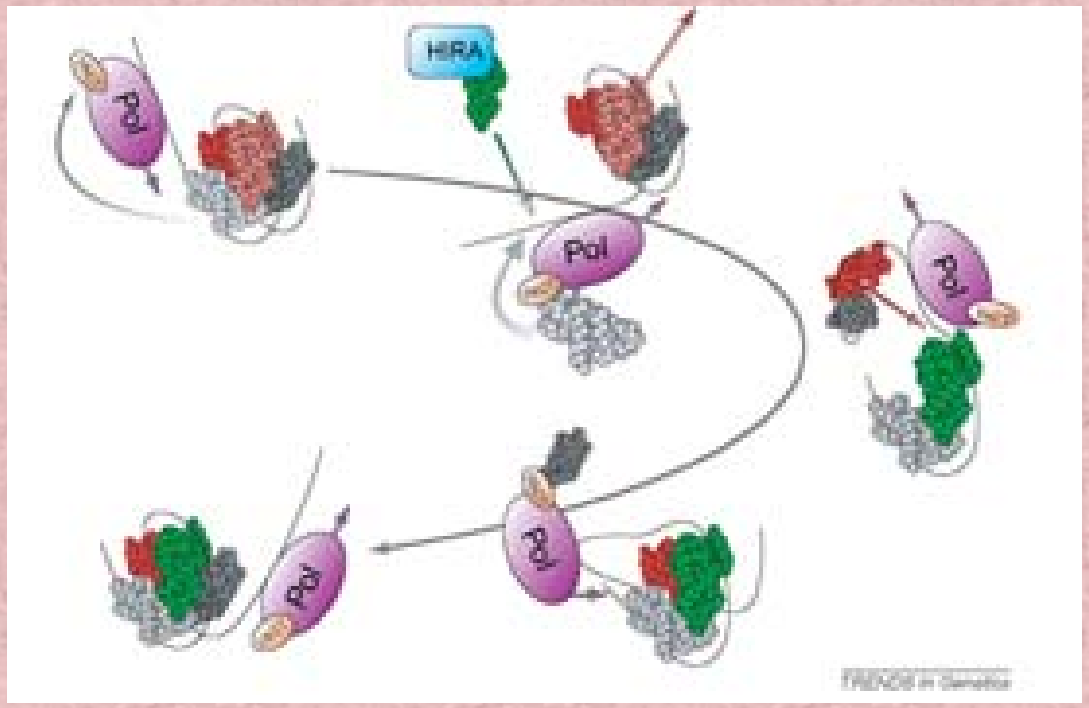
Special shaperons are used for substitution of main formes of histones by different variant formes

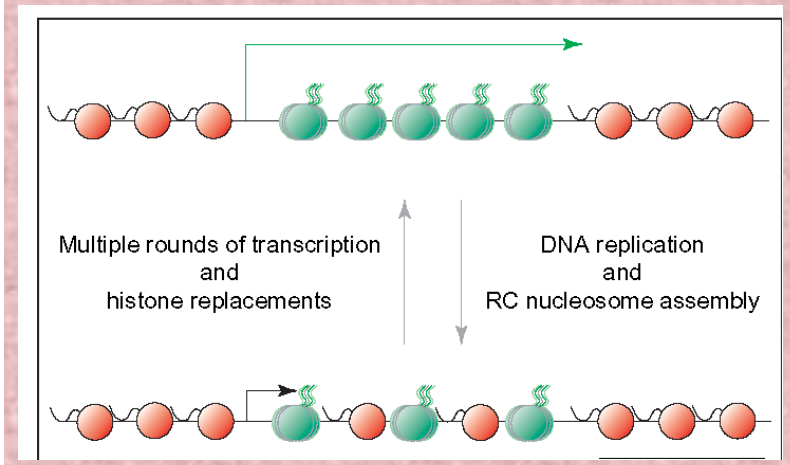
HIRA (histone regulator A) substitution of H3 (K9acet) by H3.3

Swr1- substitution of H2A-H2B by H2A.Z-H2B (in fact H2A-H2B to H2A-Htz as Swr1 is found in yeast cells)

Swr1 contains Swi2 ATPase similar to Snf2. Thus this chaperon possess the properties of a chromatin remodelling complex

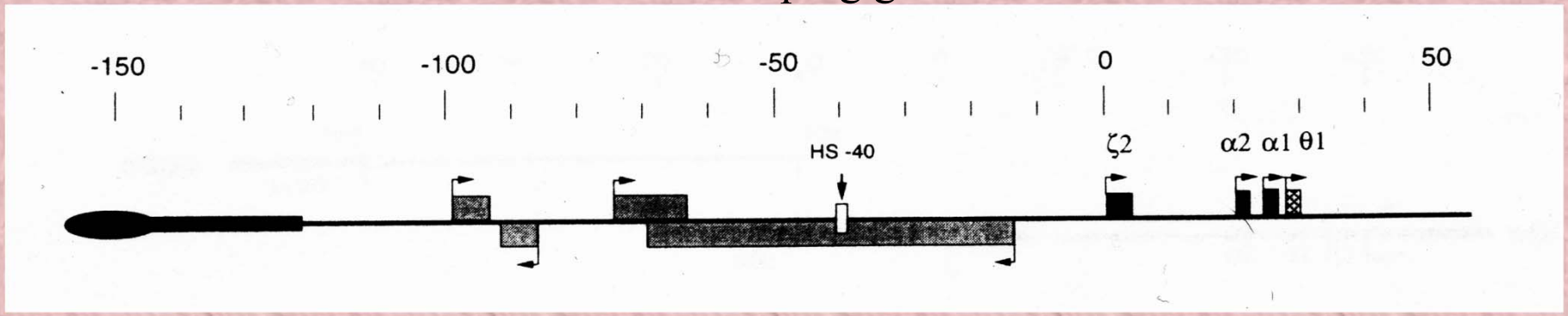
Destabilisation of nucleosomes caused by transcription may facilitate substitution of major histone forms by variant forms



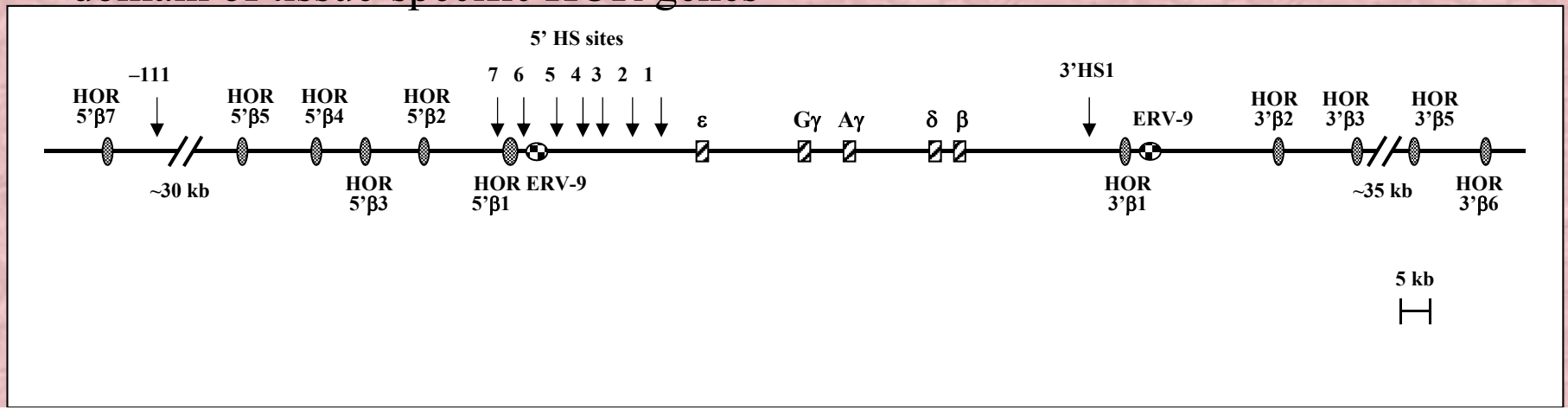


Along with classical domains of tissue specific genes that are characterized by cell lineage-dependent sensitivity to DNase I, there are “mixed” domains which contains both tissue-specific and hose-keeping genes or non-related tissue-specific genes.

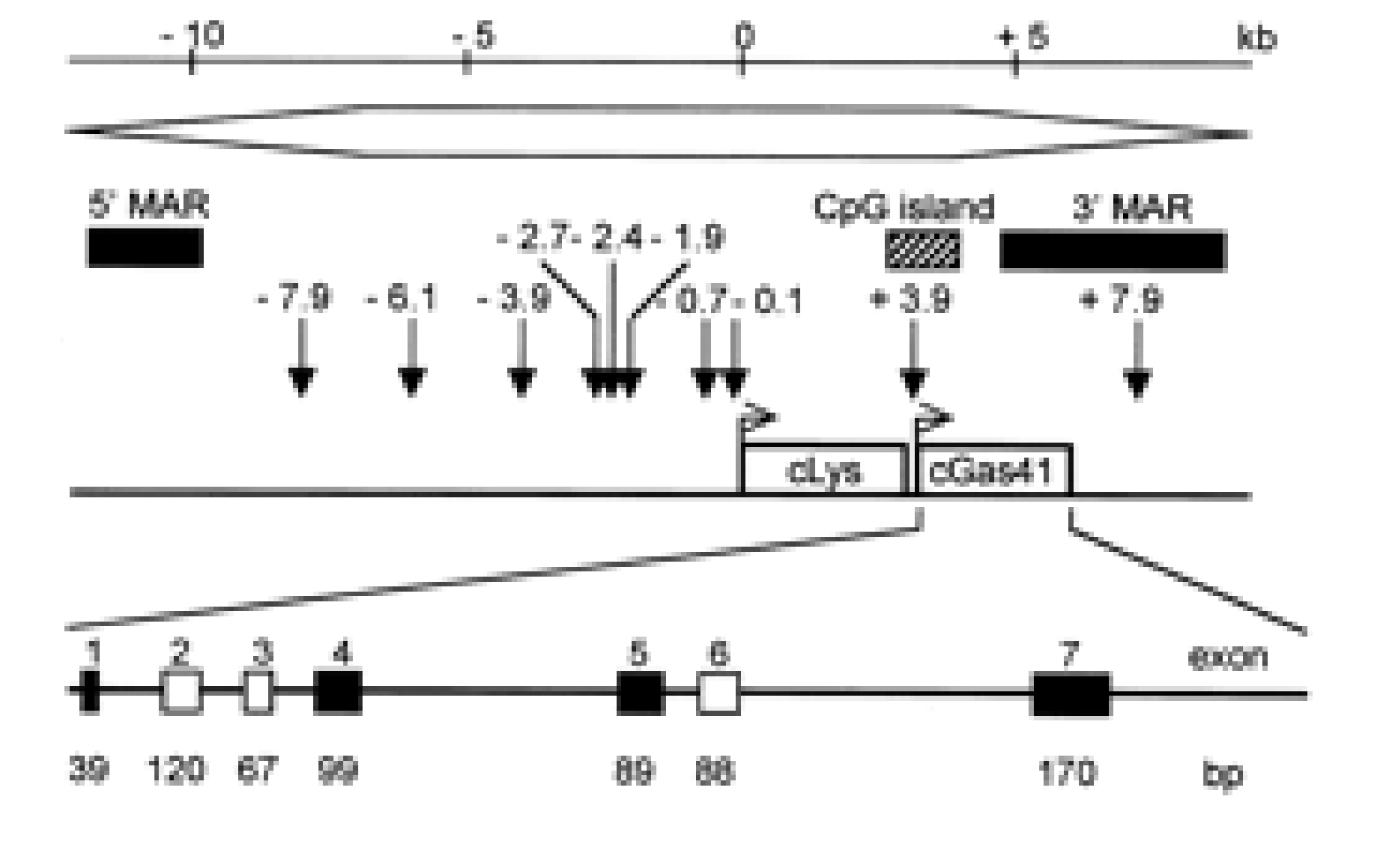
Major upstream regulatory element of human alpha-globin gene domain is locaten in the introne of a house-keeping gene



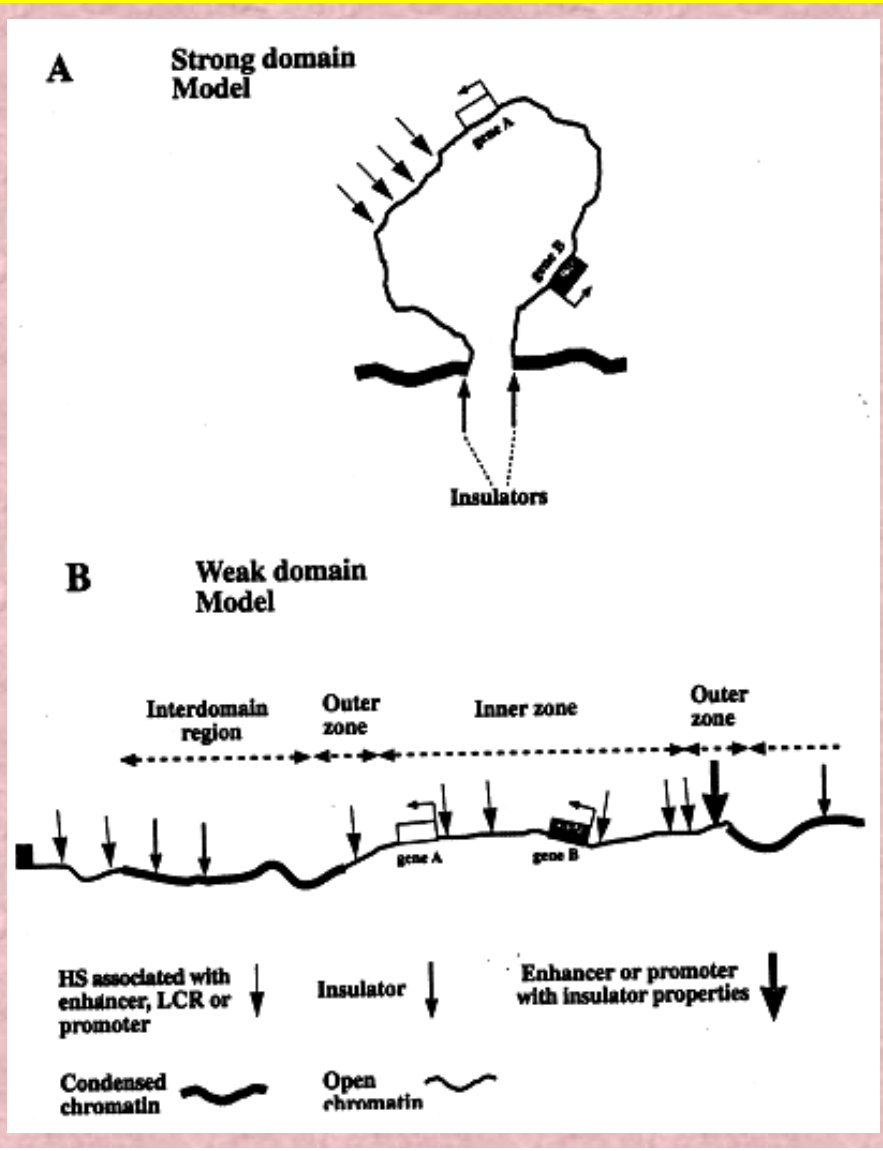
“classical” tissue-specific domain of beta-globin genes is located within a larger domain of tissue-specific HOR genes



In chicken lysosyme gene domain a housekeeping gene is present

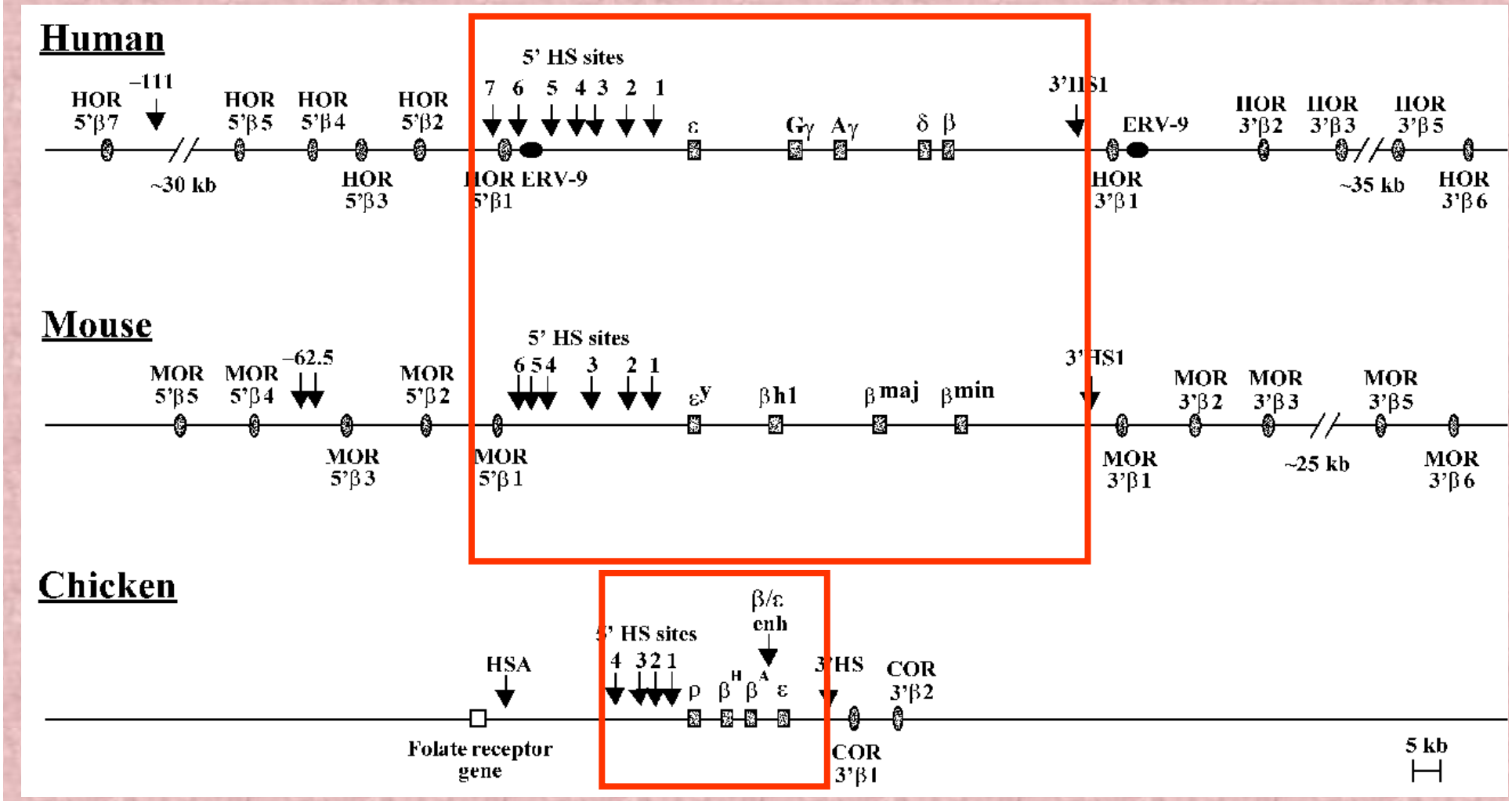


Domains with defined and "soft" (or dispersed) borders



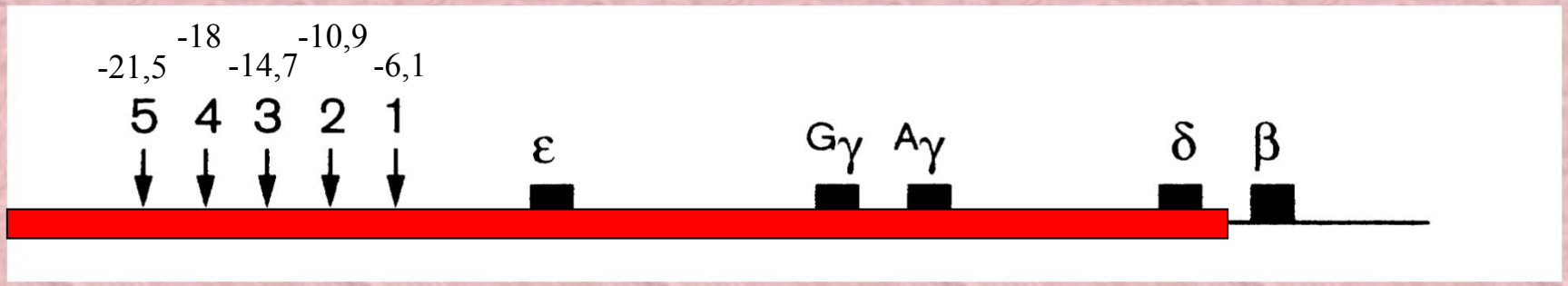
Vertebrate domains of beta-globin genes are typical examples of « strong » domains or domains with defined borders

DNase-sensitive in erythroid cells
DNase resistent in cells of other lineages

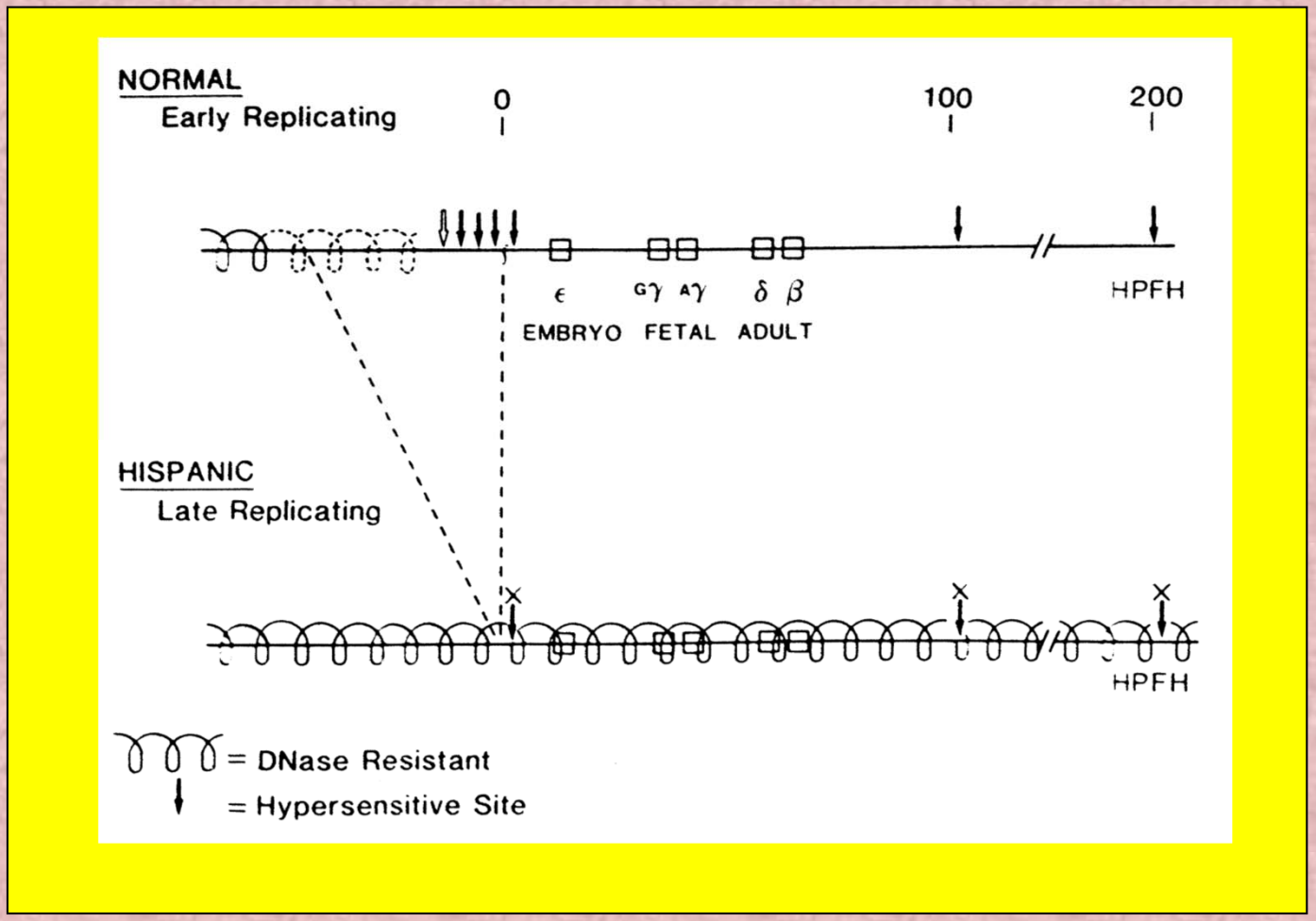


Human beta-globin gene domain

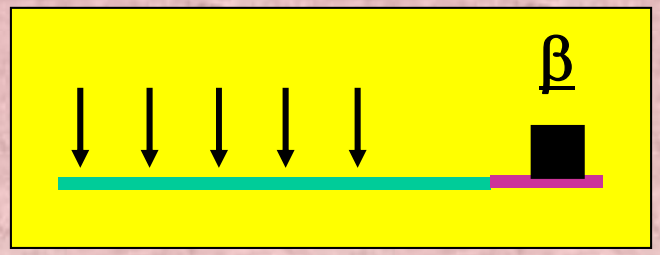
Deletions resulting in development of thalassemias

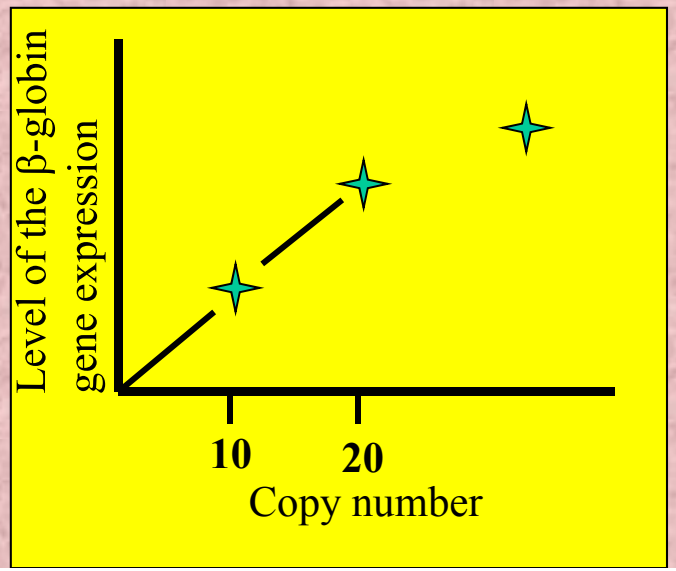


In erythroid cells bearing “Spanish” deletion the domain of β-globin genes becomes DNase-resistant and late-replicating



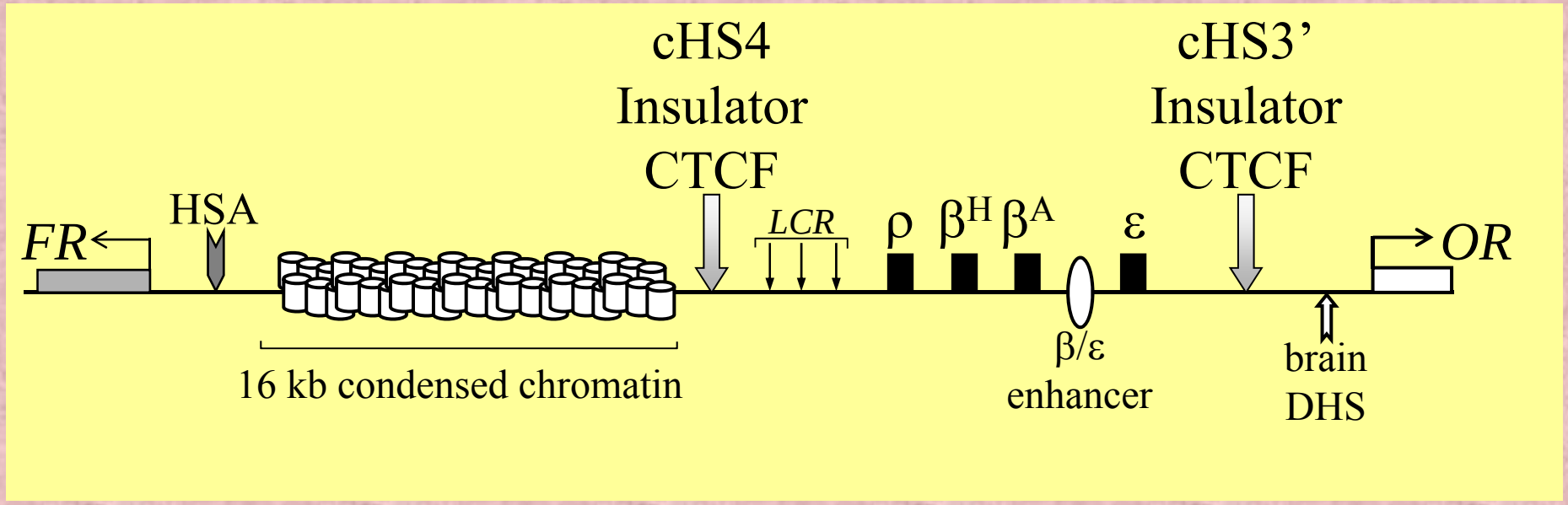
Regulatory elements located in the 5' area of the human domain of β-globin gene domain ensure high level of expression of linked transgenes and dampen the position effect variegation (Grosveld et al. 1987).



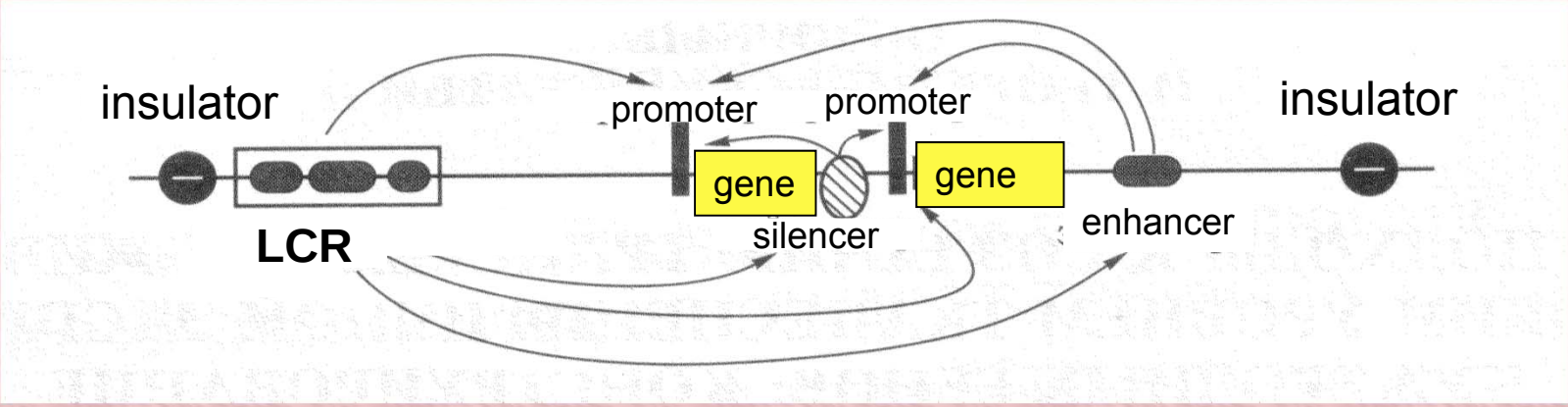


Regulatory elements similar to beta-globin gene domain LCR were found in other vertebrate beta-globin gene domains and also in several other genomic domains.

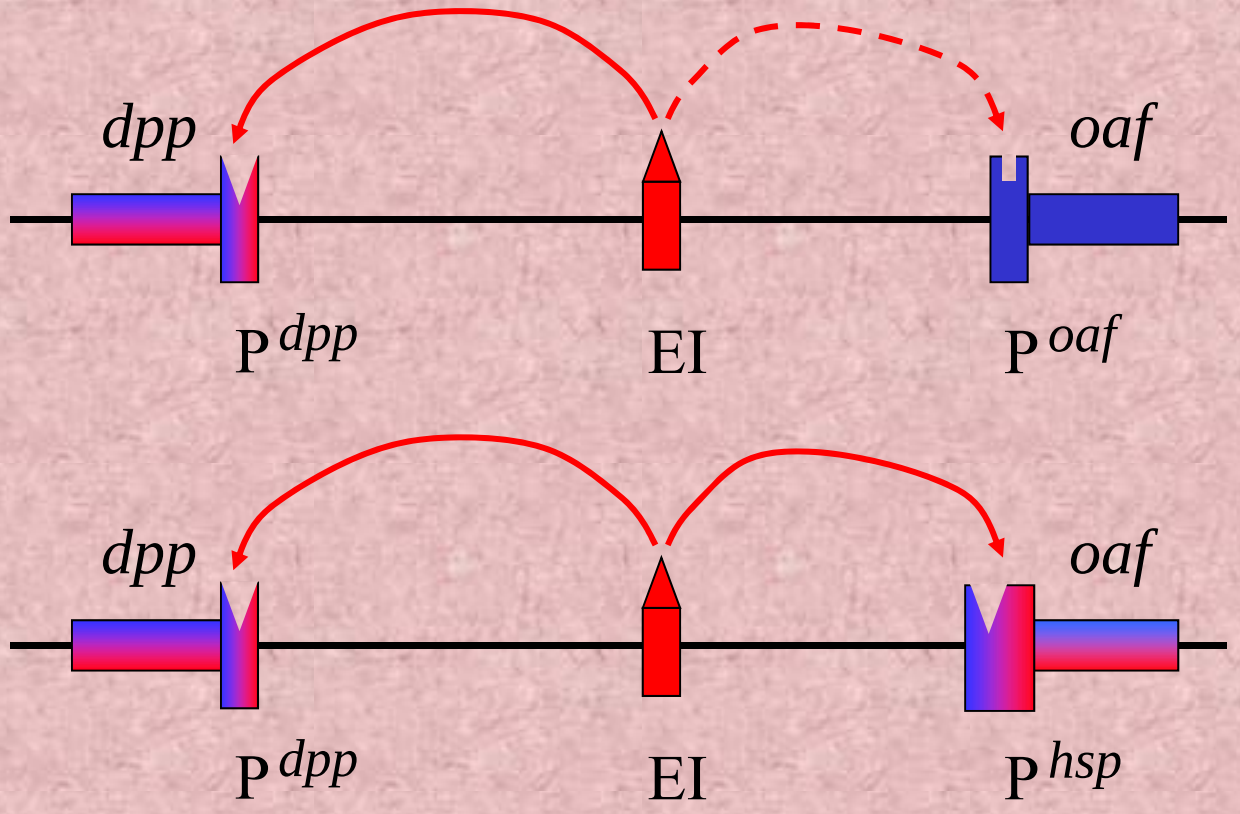
Along with LCR « strong » genomic domains may contain other enhancer elements



Regulatory systems of genomic domains with defined borders

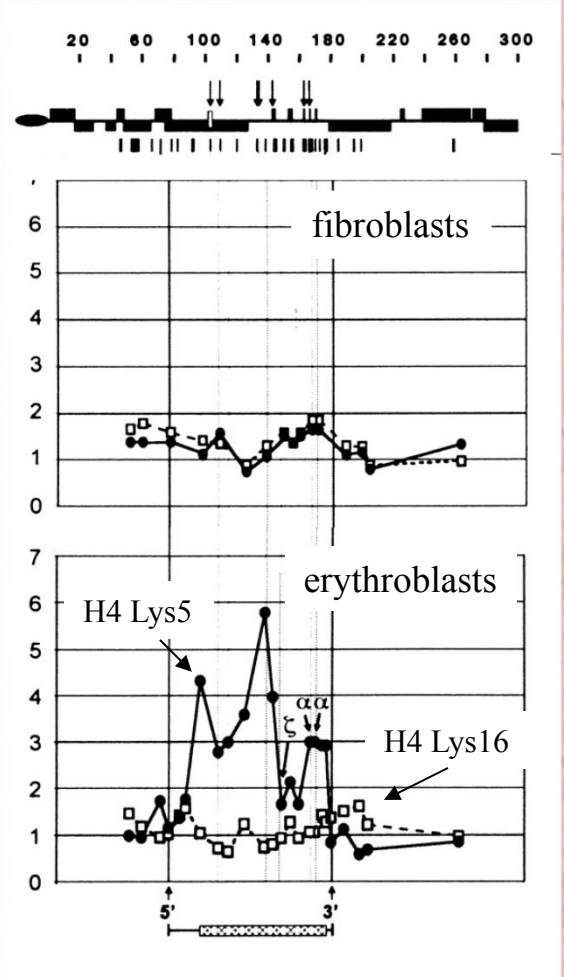


In overlapping genomic domains the functional isolation of regulatory systems is important. This functional isolation is achieved in different ways including utilization of tissue-specific or enhancers that recognize only one promoter or several promoters of functionally related genes

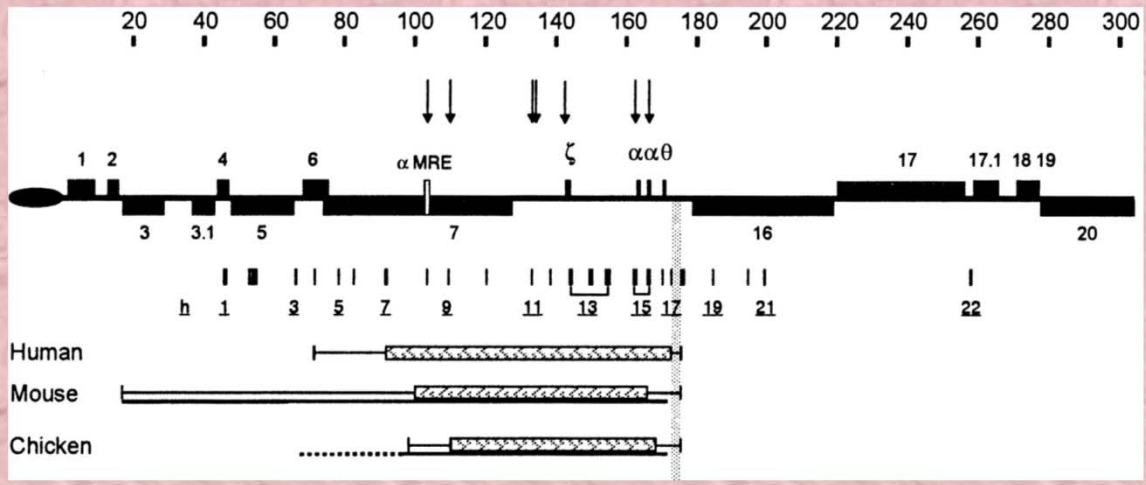


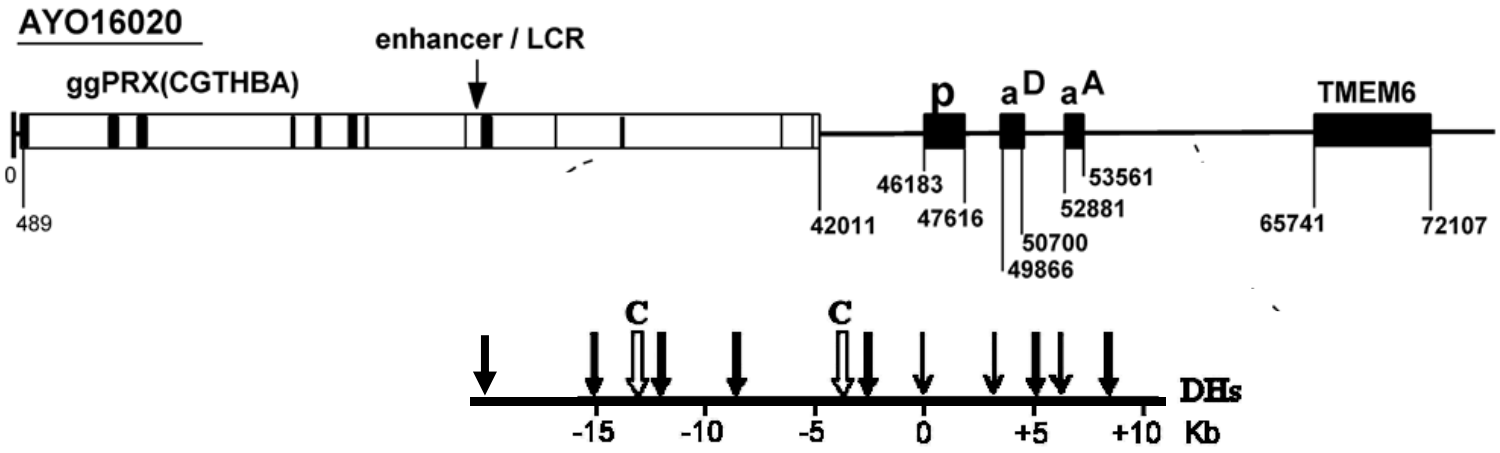
Some tissue-specific gene domains are located in permanently open chromatin areas. Even in this case they may be distinguished by

targeted histone acetylation



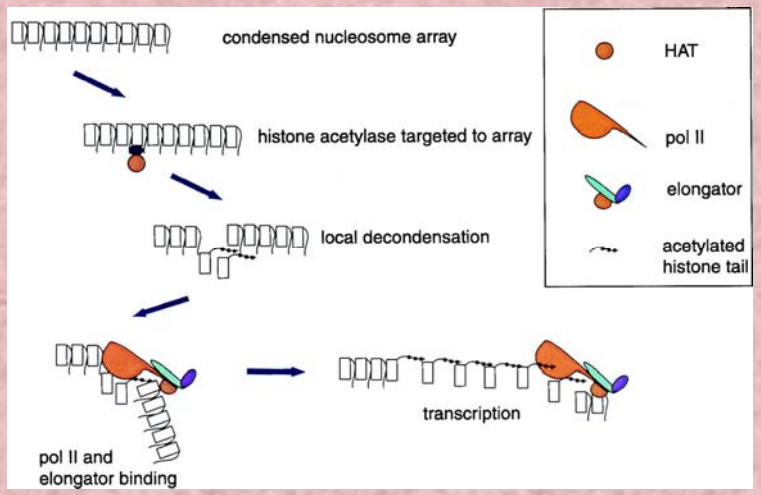
Anguita et al., 2001, PNAS 98, 12114-12119



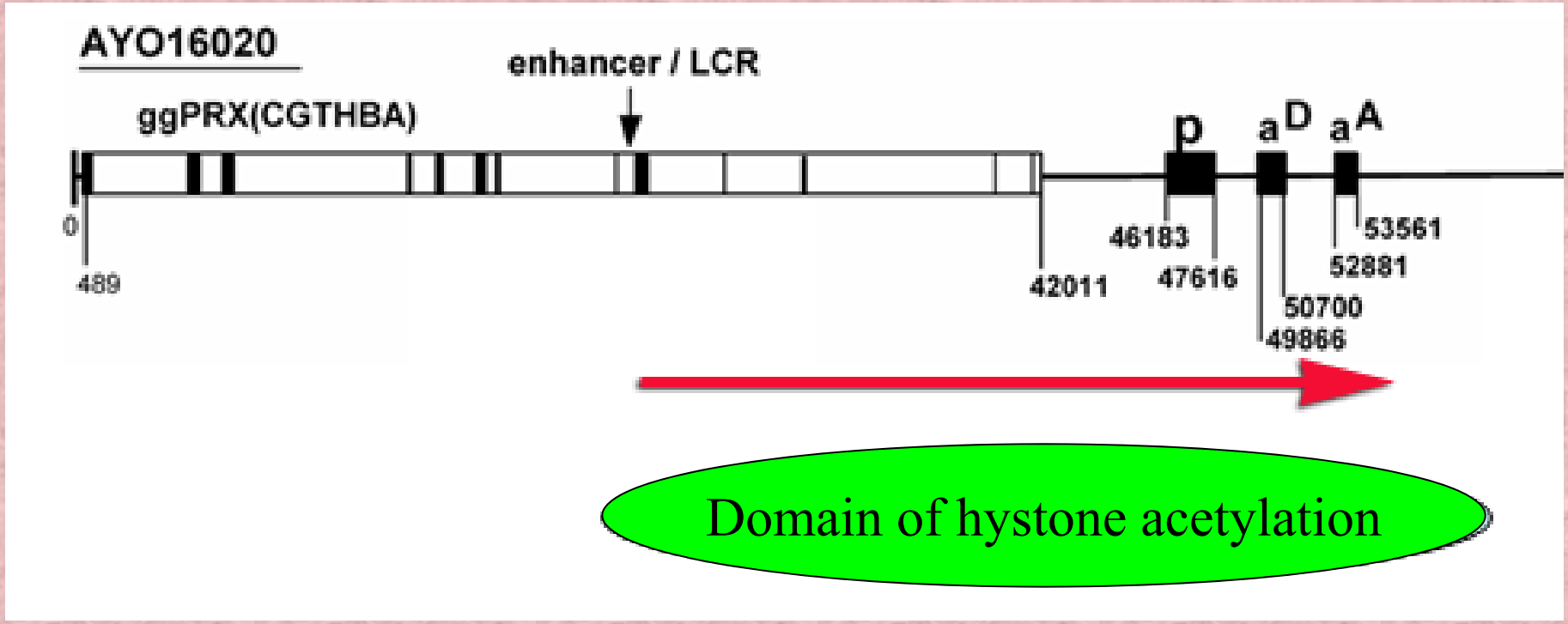


Erythroid-specific histone acetylation

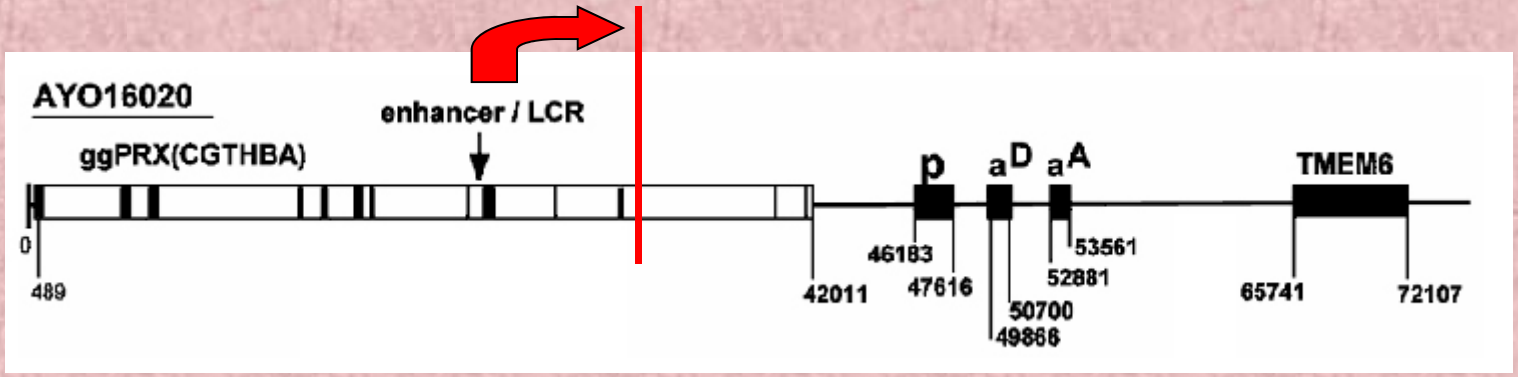
Chicken alpha-globin gene domain is a typical example of domains with non-defined borders. Still it is possible to recognize this domain by analysis of distribution of erythroid-specific DHs or by analysis of distribution of acetylated histones



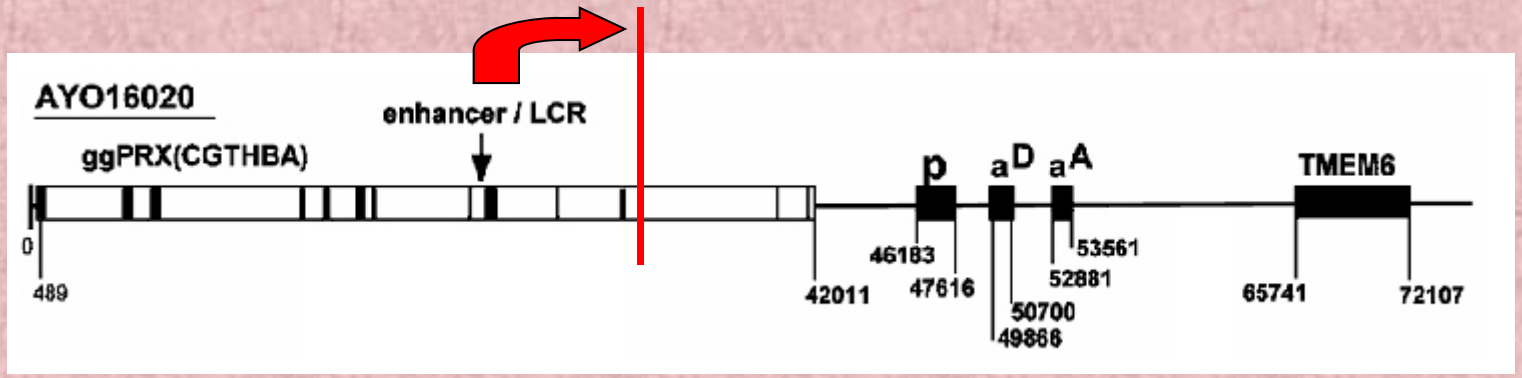
Activation of alpha-globin gene domain in pre-erythroblasts is likely to proceed via transcription-dependent spreading of hystone acetylation (Travers hypothesis).



conditional insulator

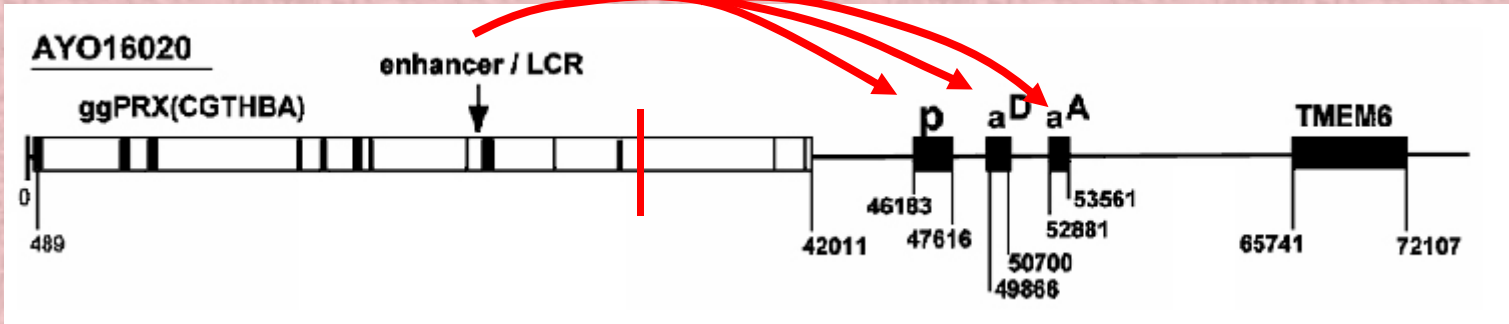


non-erythroid cells



site-specific
histone hyperacetylation

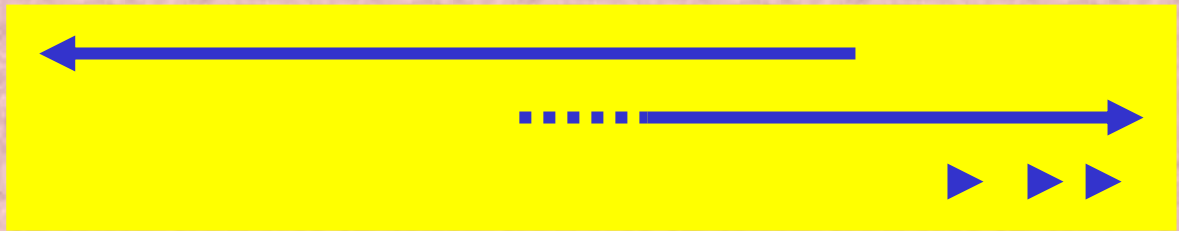
pre-erythroblasts



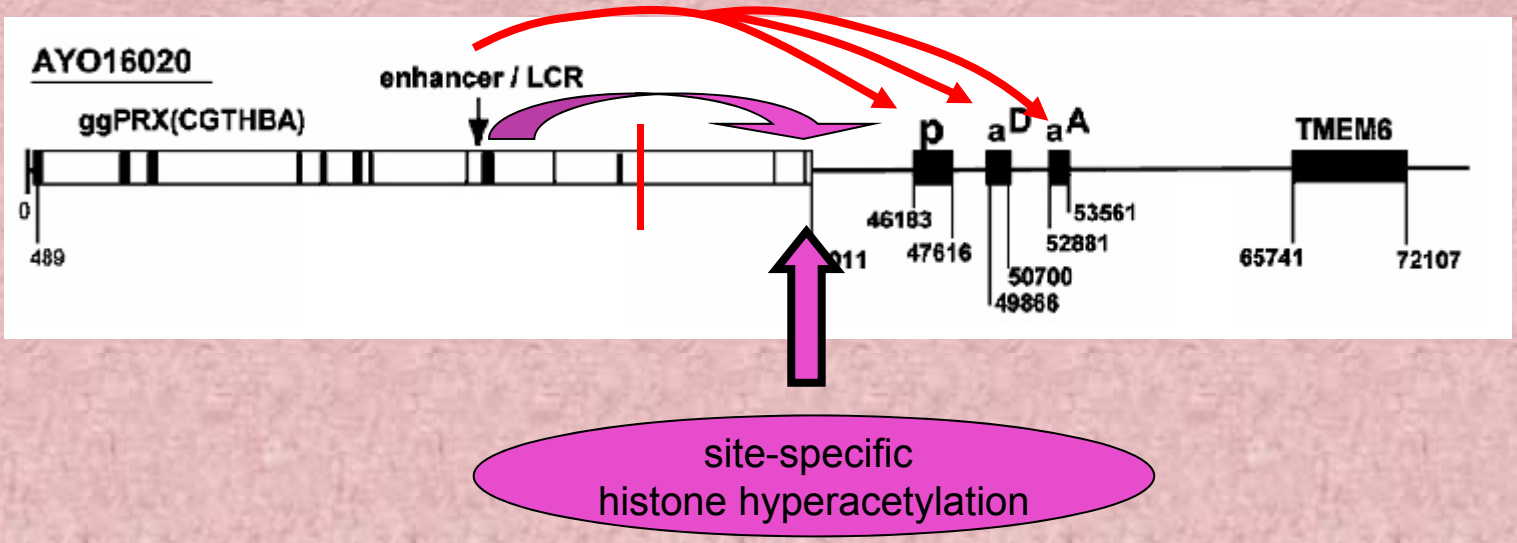
site-specific
histone hyperacetylation

erythroblasts

(active expression of globin genes)

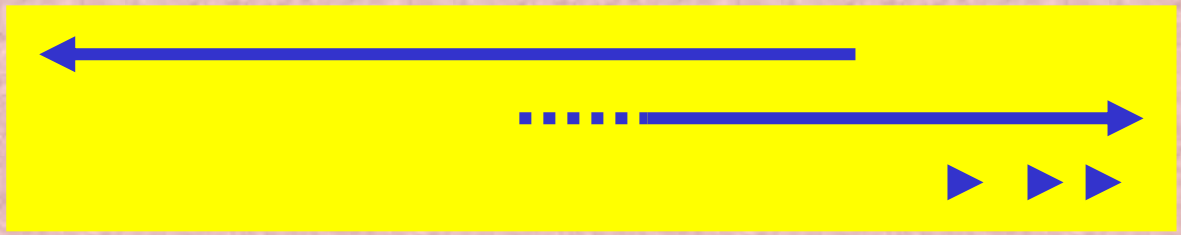


Regulatory systems activating expression of globin genes may also stimulate expression of a house-keeping gene ggPRX. This is prevented by activation of conditional CTCF-dependent silencer

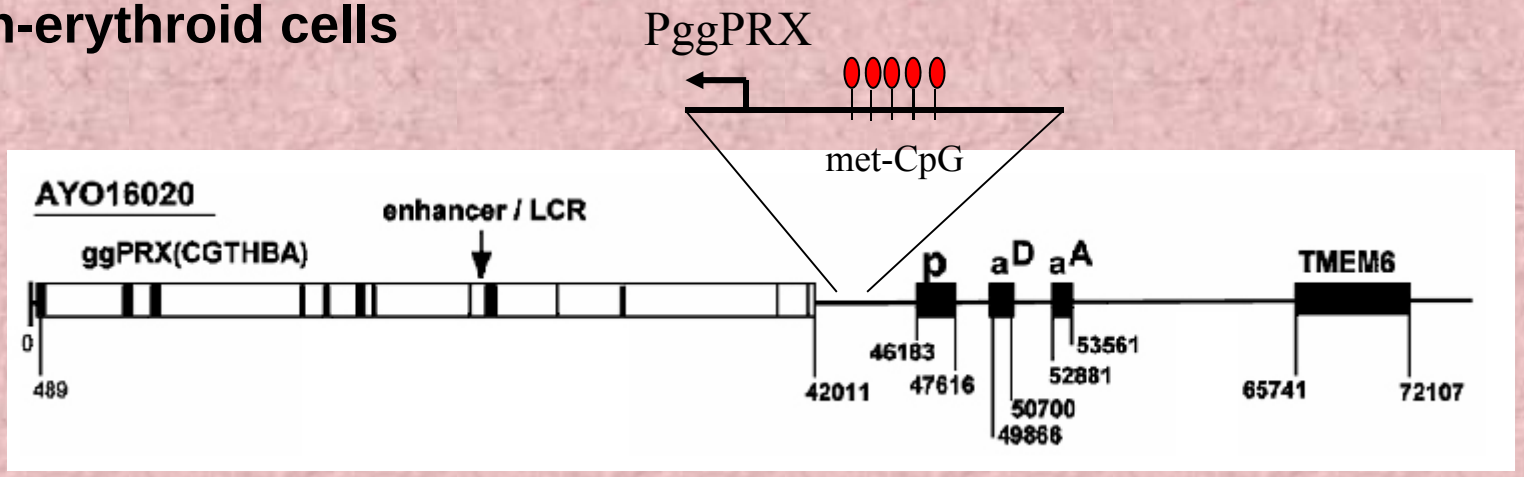


erythroblasts

(active expression of globin genes)



non-erythroid cells



erythroid cells