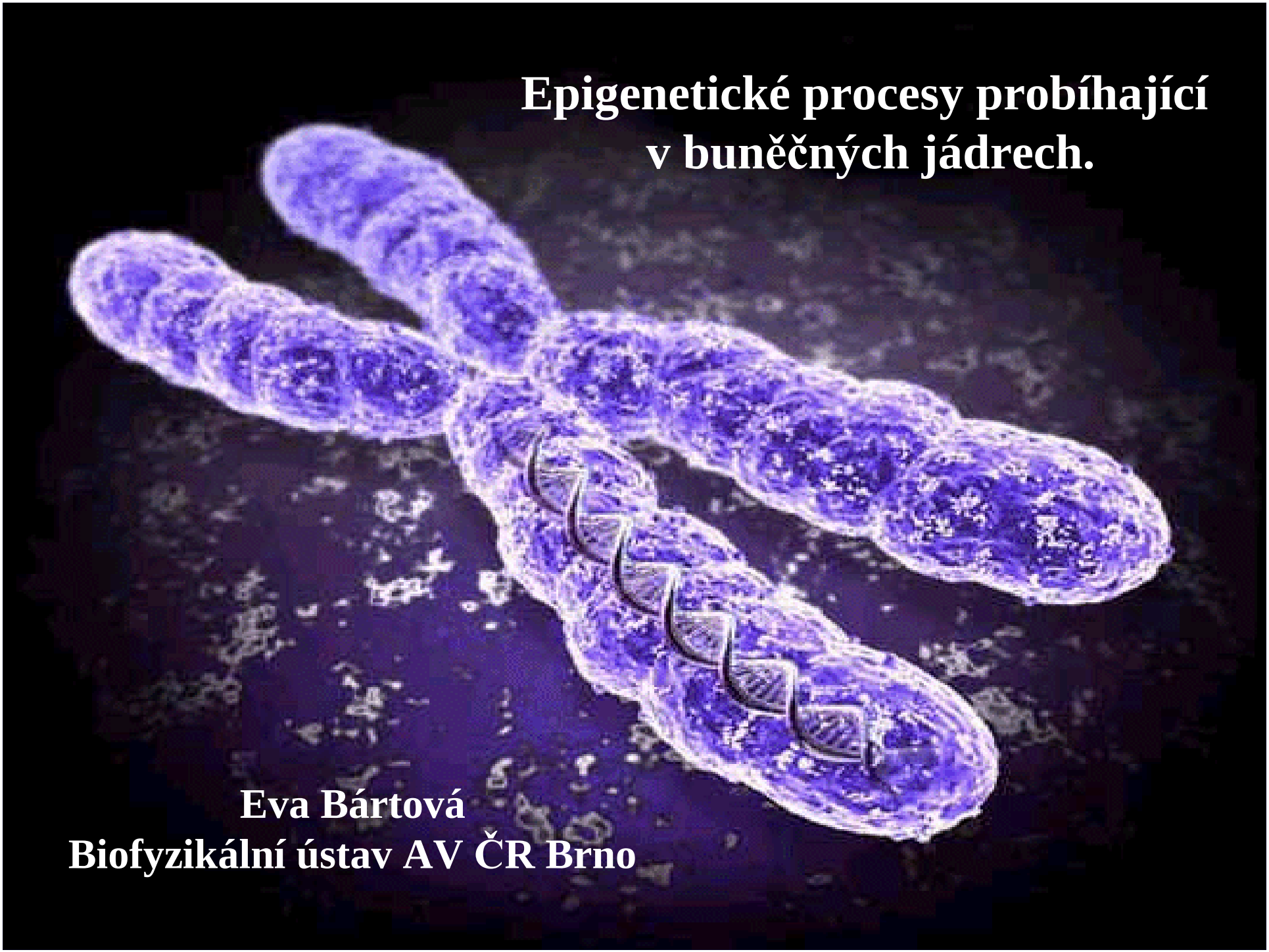
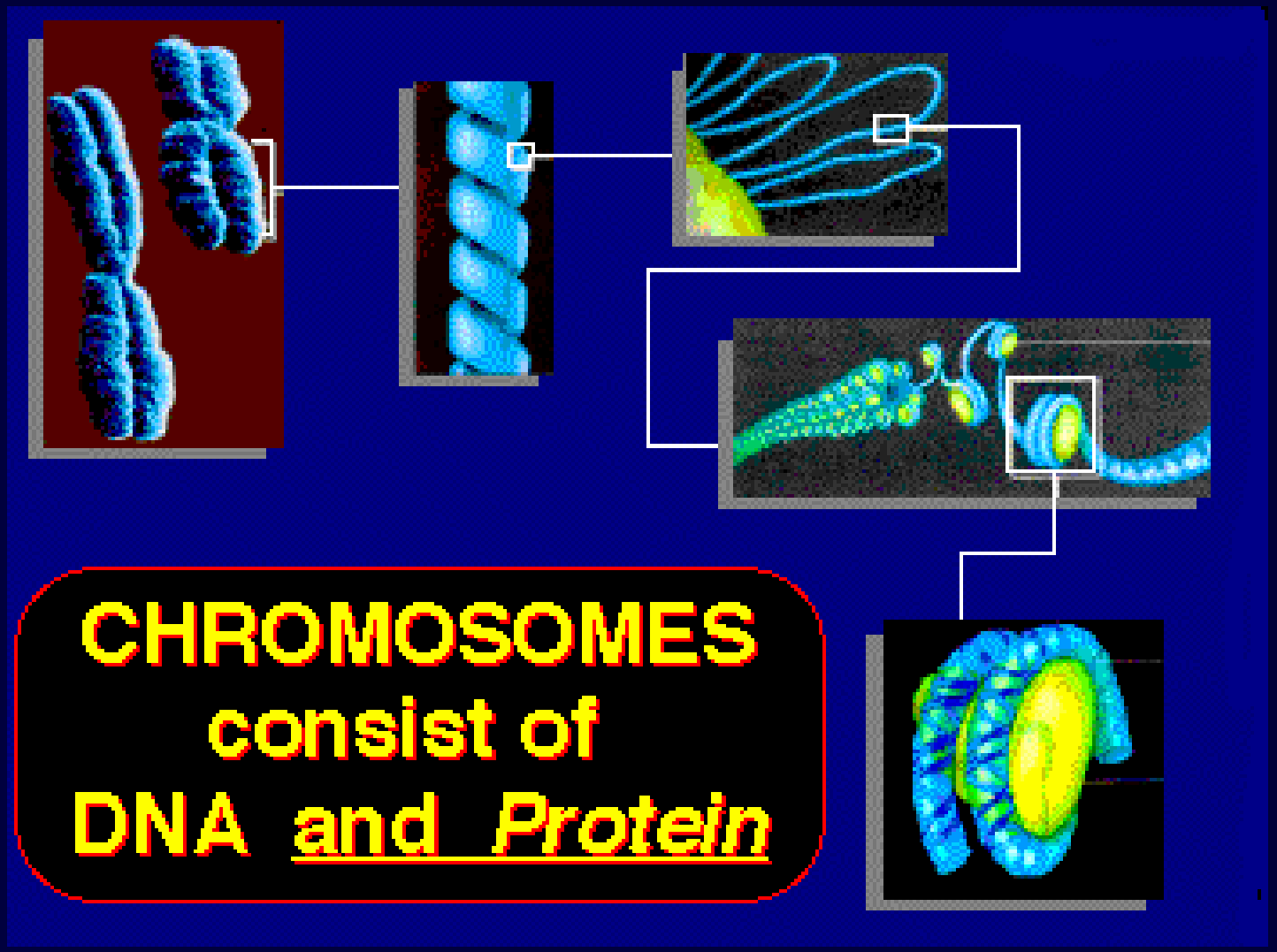
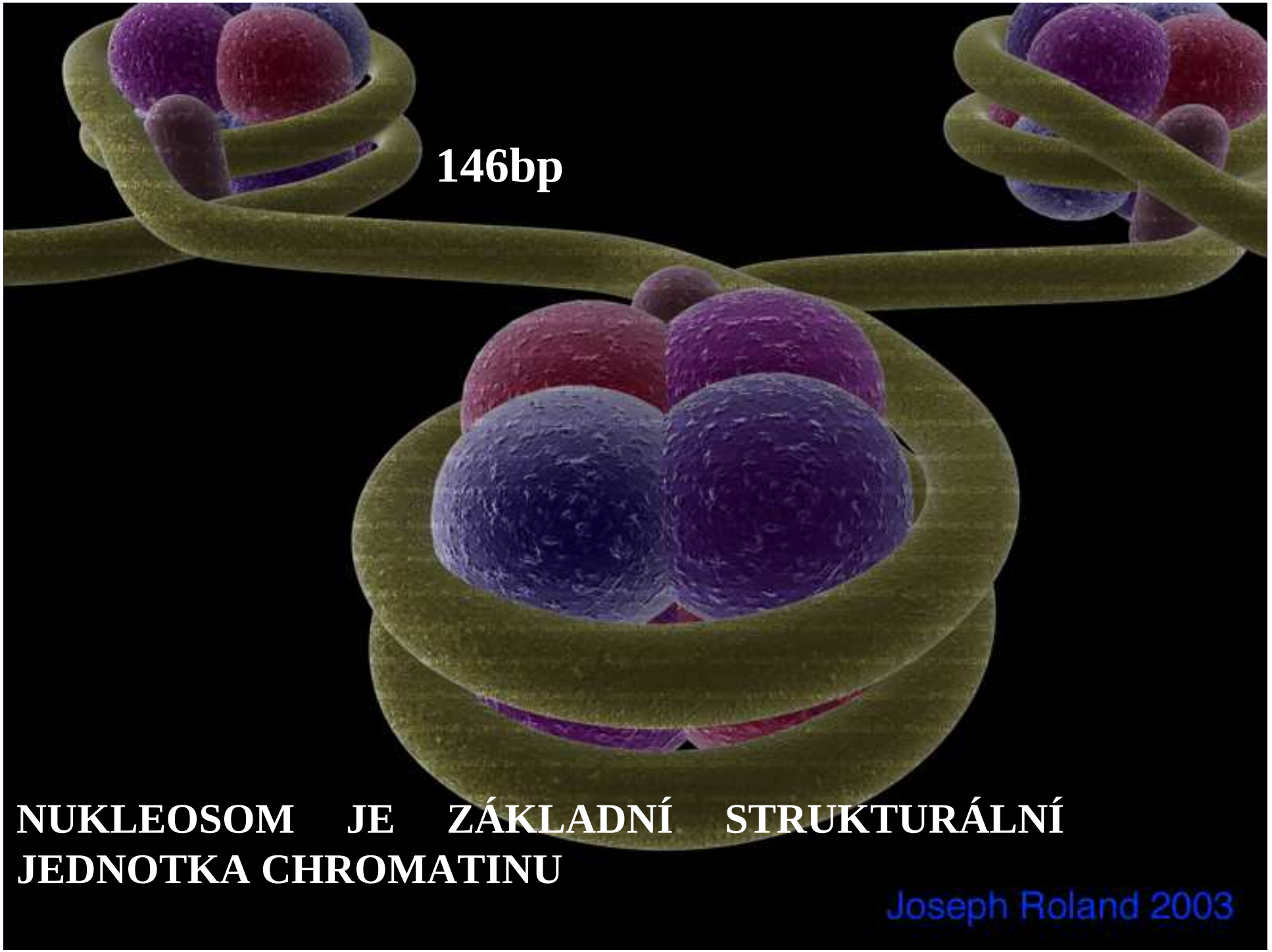
Epigenetické procesy probíhající v buněčných jádrech.

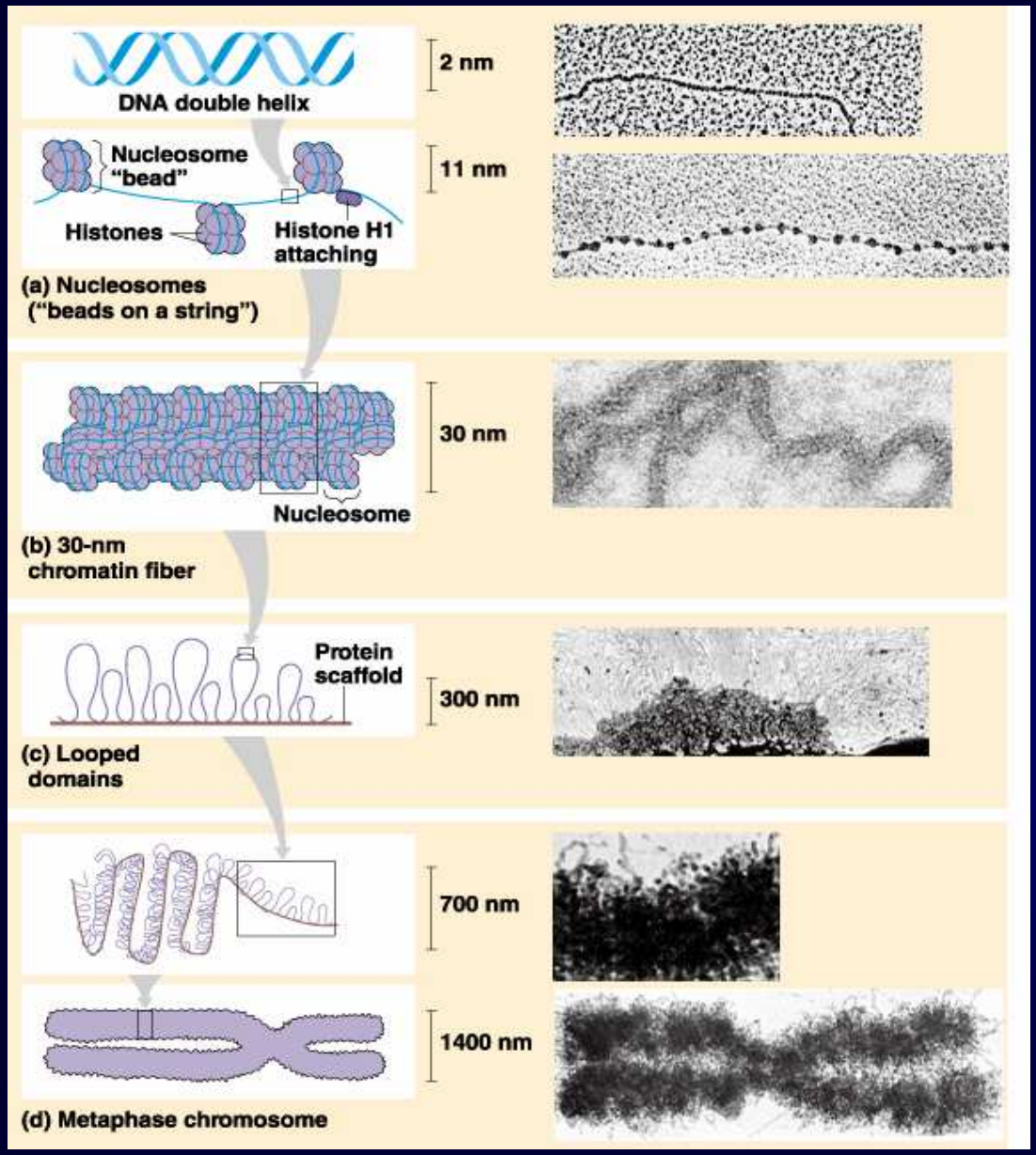


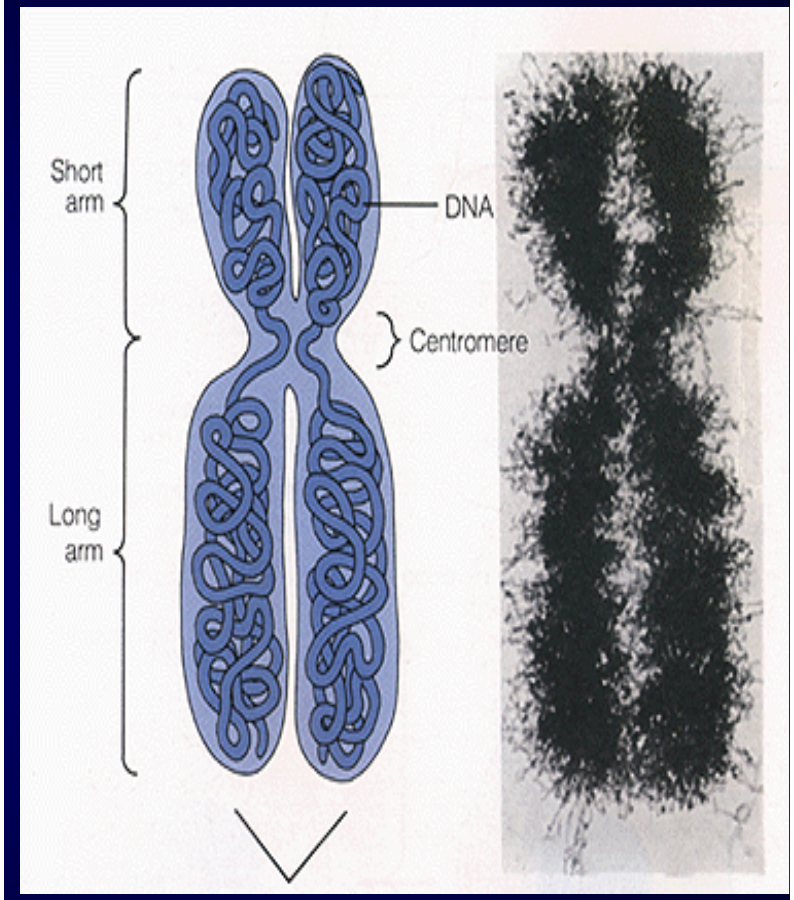
Eva Bártová
Biofyzikální ústav AV ČR Brno

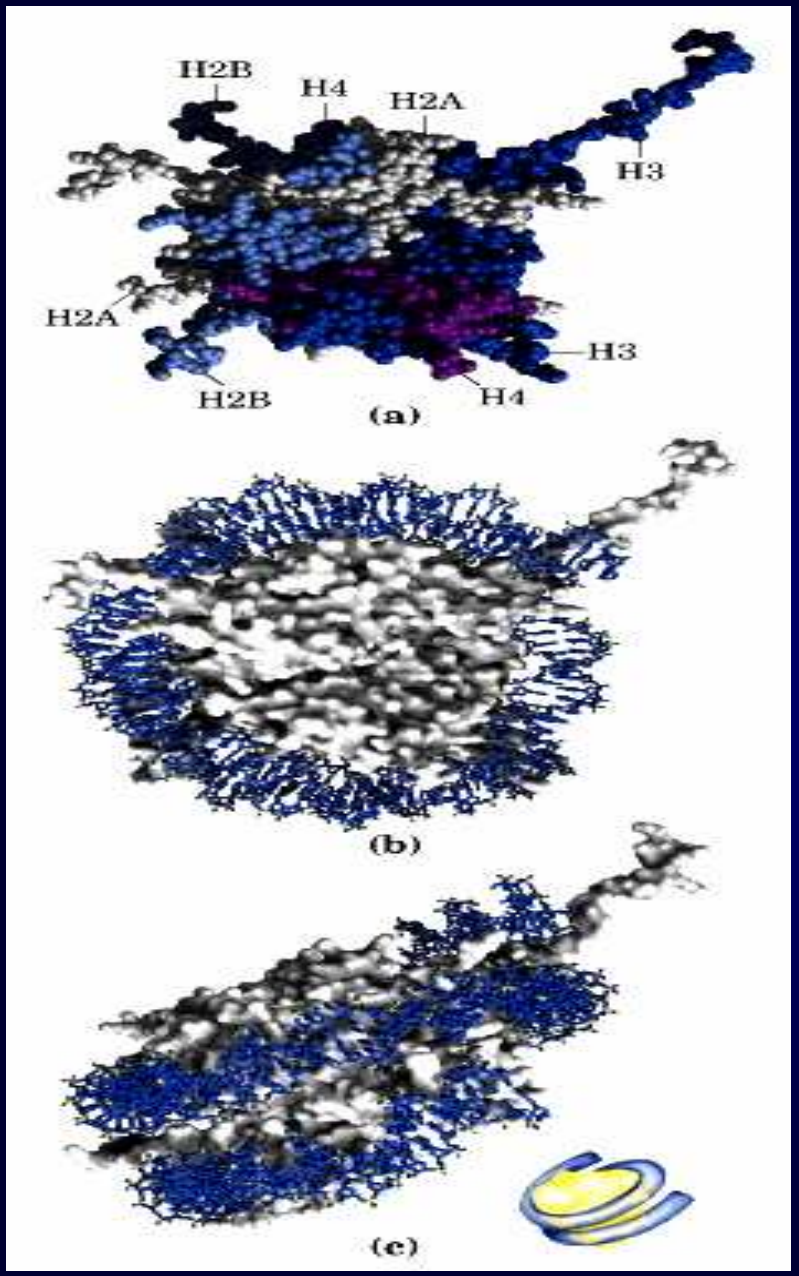

CHROMOSOMES
consist of
DNA and Protein



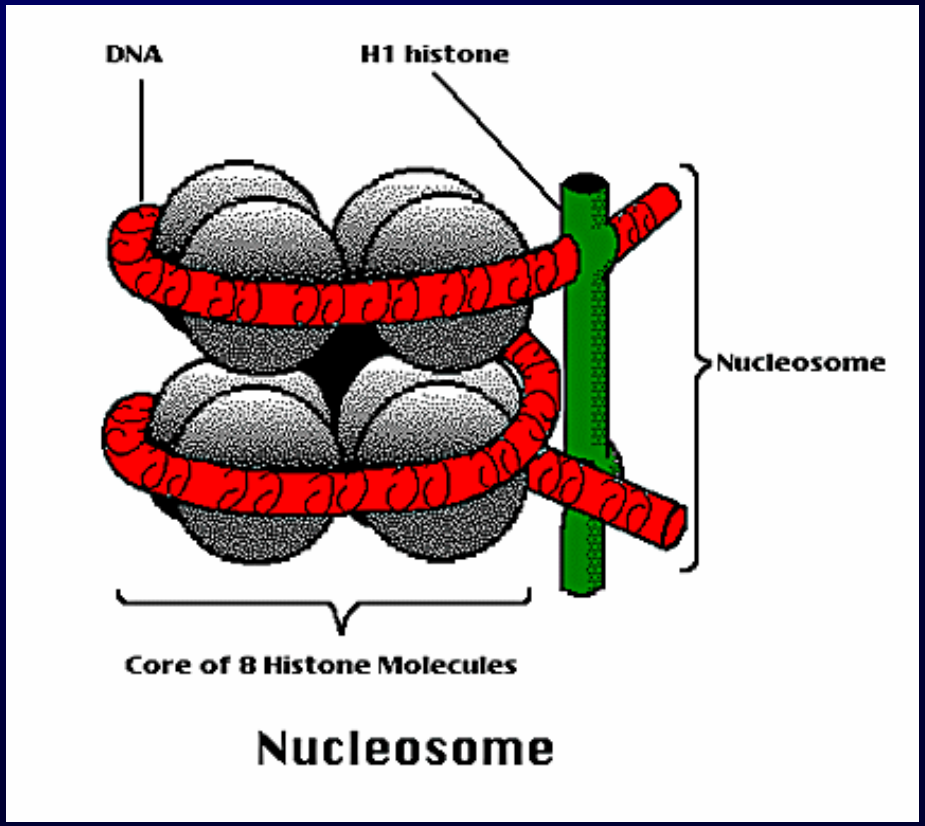
NUKLEOSOM JE ZÁKLADNÍ STRUKTURÁLNÍ JEDNOTKA CHROMATINU

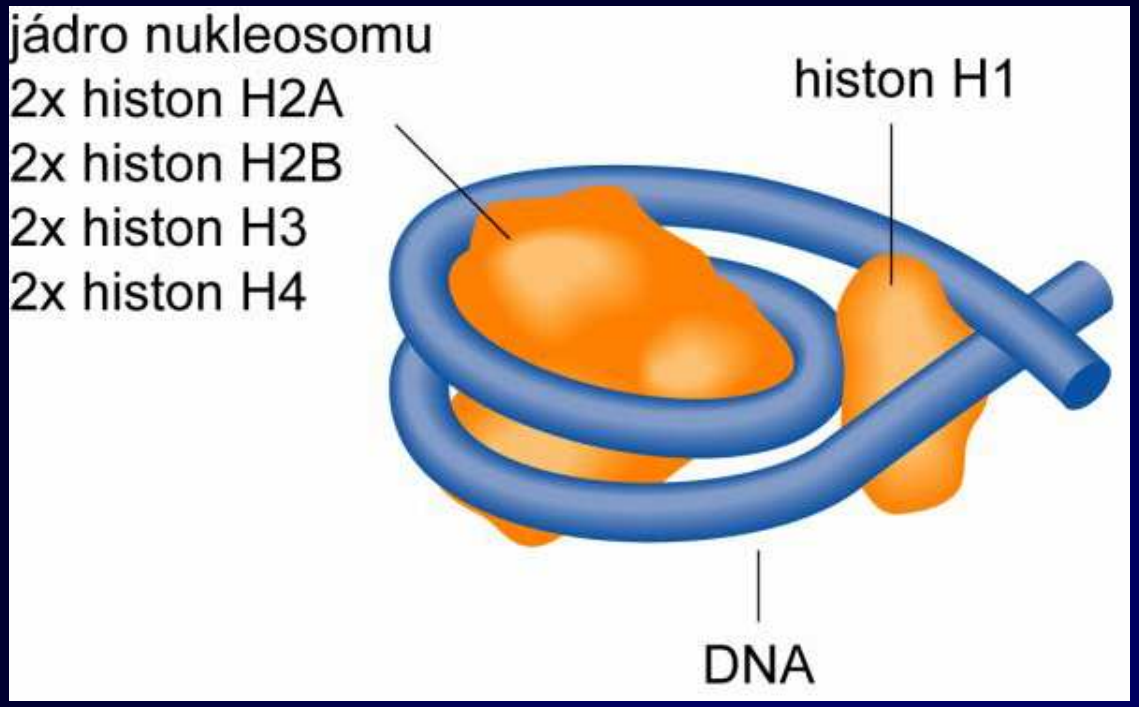

DNA double helix
2 nm
Nucleosome "bead"
Histones
Histone H1 attaching
11 nm
(a) Nucleosomes ("beads on a string")
30 nm
Nucleosome
(b) 30-nm chromatin fiber
Protein scaffold
300 nm
(c) Looped domains
700 nm
1400 nm
(d) Metaphase chromosome


Short arm
DNA
Centromere
Long arm



N-koncové oblasti histonů H2A, H2B H3a H4 (délka 16-44 aminokyselin nejsou součástí jádra nukleosomu, ale vybíhají do stran (volné konce). V linkerové oblasti – H1: funkce na kondenzaci chromatinu vyššího řádu.

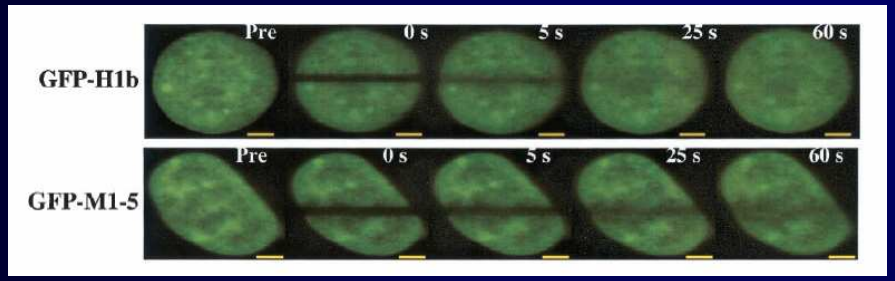




1. **Organismus může žít s i bez významně redukovaného množství H1.**
2. **H1 varianty nejsou hlavní determinanty buněčného fenotypu.**
3. **Funkce H1 variant je nejenom při utlumení transkripční aktivity ale také při její aktivaci (může snižovat nebo i zvyšovat expresi specifických genů.**
4. **H1 hraje důležitou úlohu v kondensaci chromatinu. Spíše je důležitý pro stabilizaci nukleosomů než pro vlastní řízení kondenzace chromatinu.**
5. **Experimentálně navozená redukce H1 vede ke zkrácení linkerové DNA**

The linker histone H1 is involved in maintaining higher-order chromatin structures and displays dynamic nuclear mobility, which may be regulated by posttranslational modifications. H1 tail phosphorylation play in important role.

Using the technique of fluorescence recovery after photobleaching, Contreres et al., 2003 observed that the mobility of a GFP-wild-type H1 fusion protein is dependent on Cdk2 activity. GFP-H1 mobility was decreased in cells with low Cdk2 activity but not in the cells with bloked phophorylation of H1. Blocking the activity of Cdk2 by p21 expression decreased the mobility of GFP-H1. These data suggest that CDK2 phosphorylates histone H1 in vivo, resulting in a more open chromatin structure by destabilizing of nucleosomes.

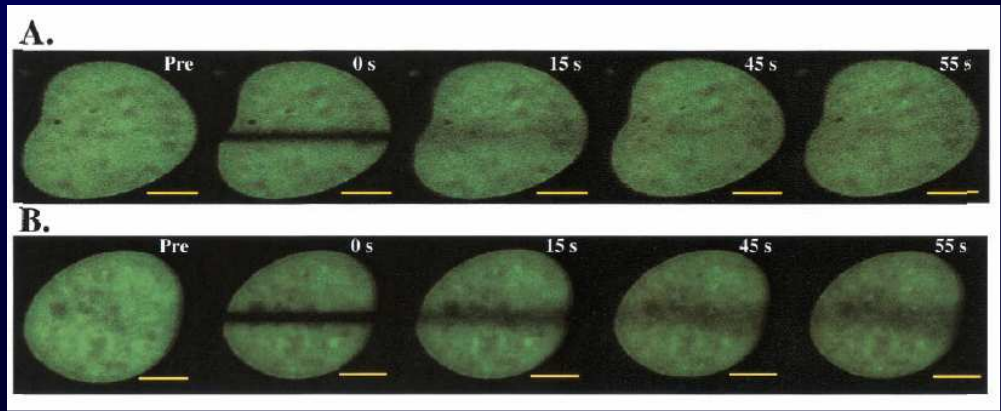


GFP-M1-5: five cyclin-dependent kinase phosphorylation consensus sites were mutated from serine or threonine residues into alanines

Overexpression p21

GFP-H1b

GFP-M1-5



Varianty histonů

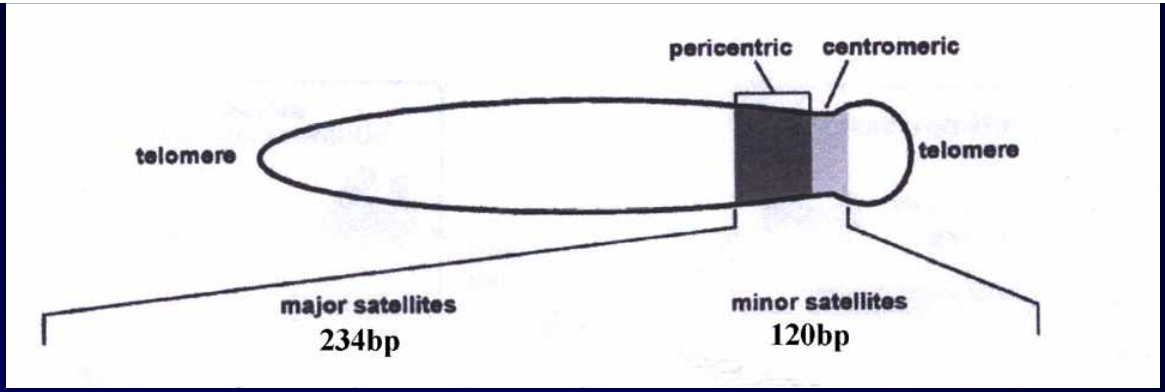
H1: varianty $H1^o$, H5 a testis-specific varianta H1. varianty H1 se různě uplatňují během buněčného cyklu, diferenciace a vývoje. RA diferenciace myších F9 je doprovázena zvýšenou transkripci histonu $H1^o$.

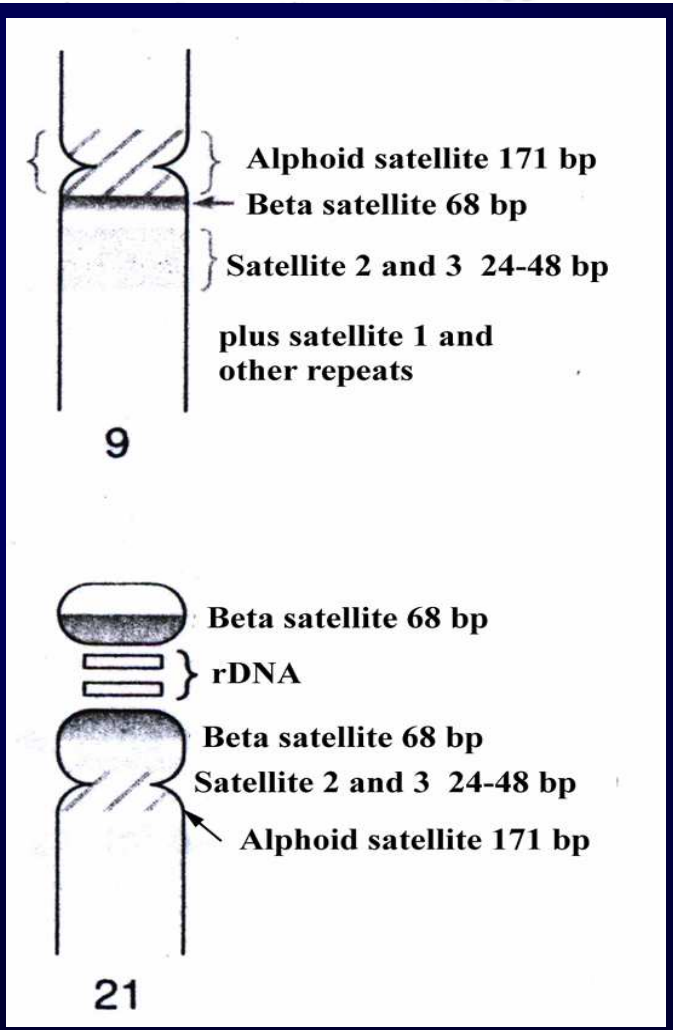
H2A: H2A.X, H2A.Z, MacroH2A, H2A-Bbd, H2AvD, H2A.X. varianta H2A.Z je konzervativní během evoluce. Macro H2A se vyskytuje u Xi, zatímco H2A-Bbd u Xa chromosomu a autosomů. H2A.Z se vyskytuje v intergenických oblastech.

H2B: nemá varianty, uplatňuje se při regulaci kondenzace chromatinu, represi transkripce a během gametogeneze, H2B je zodpovědný za uspořádání chromatinu u spermií.

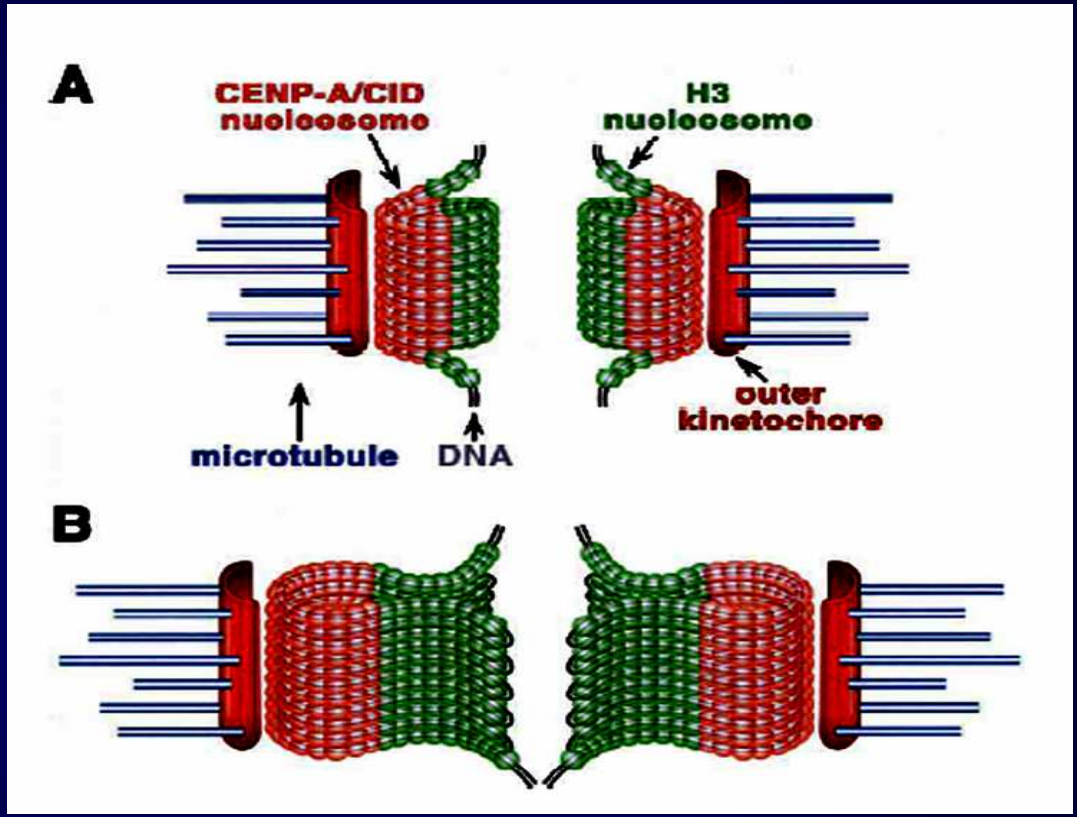
Varianty histonů

H3: existují dvě hlavní Varianty H3.3 a centromerické varianty H3 (cenH3) = CENP A: jsou zodpovědné za vazbu kinetochoru a segregaci sesterských chromatid u eukaryot





Varianty histonů H3: **phosphorylation of CENP-A on Ser-7 is essential for kinetochore function. Overexpression of CENPA plays an important role for aneuploidy in colorectal cancers.**



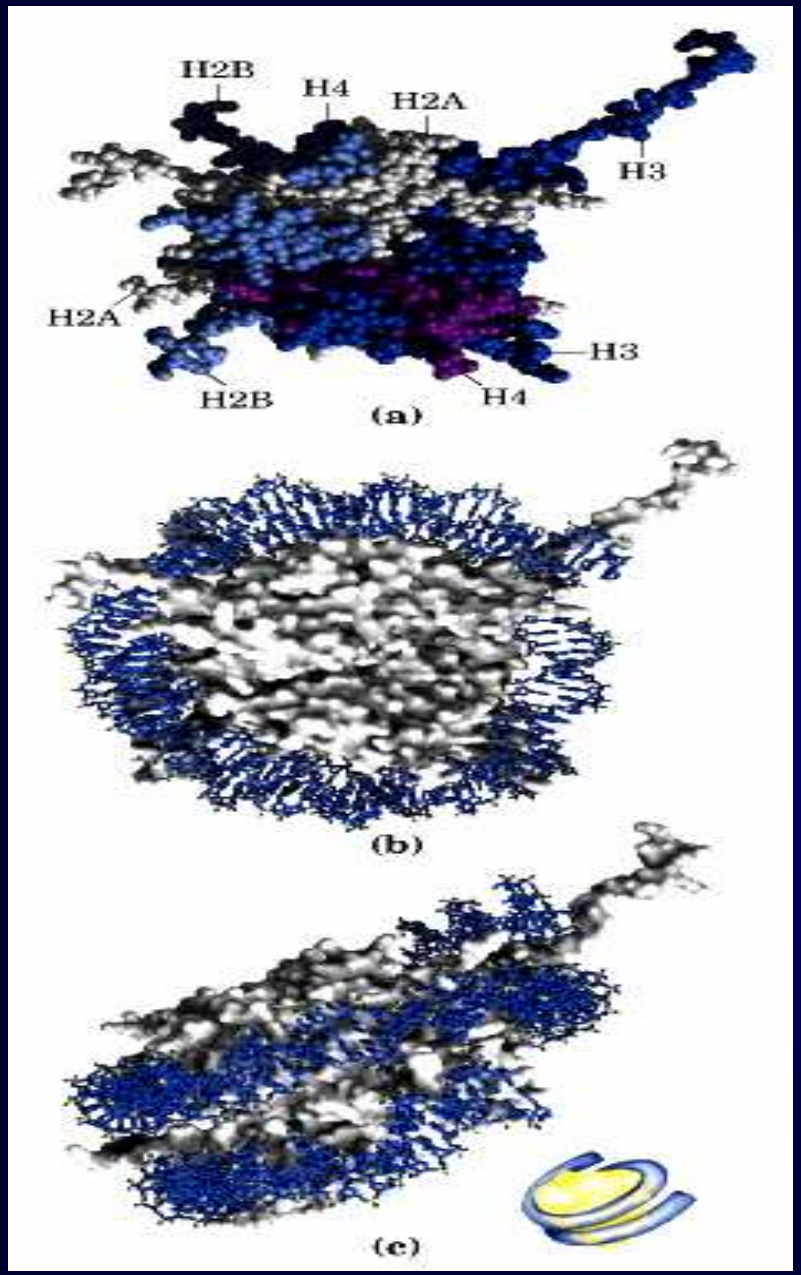
Varianty histonů H4: **většina genů kódujících hlavní histonové proteiny jsou exprimovány během S fáze buněčného cyklu. V případě H4, geny jsou konstitutivně exprimovány během buněčného cyklu. Pro H4 nejsou známy žádné varianty.**

Chemické modifikace histonů

- **Dynamická struktura chromatinu je přímo ovlivněná postranslačními modifikacemi amino-konců histonů**

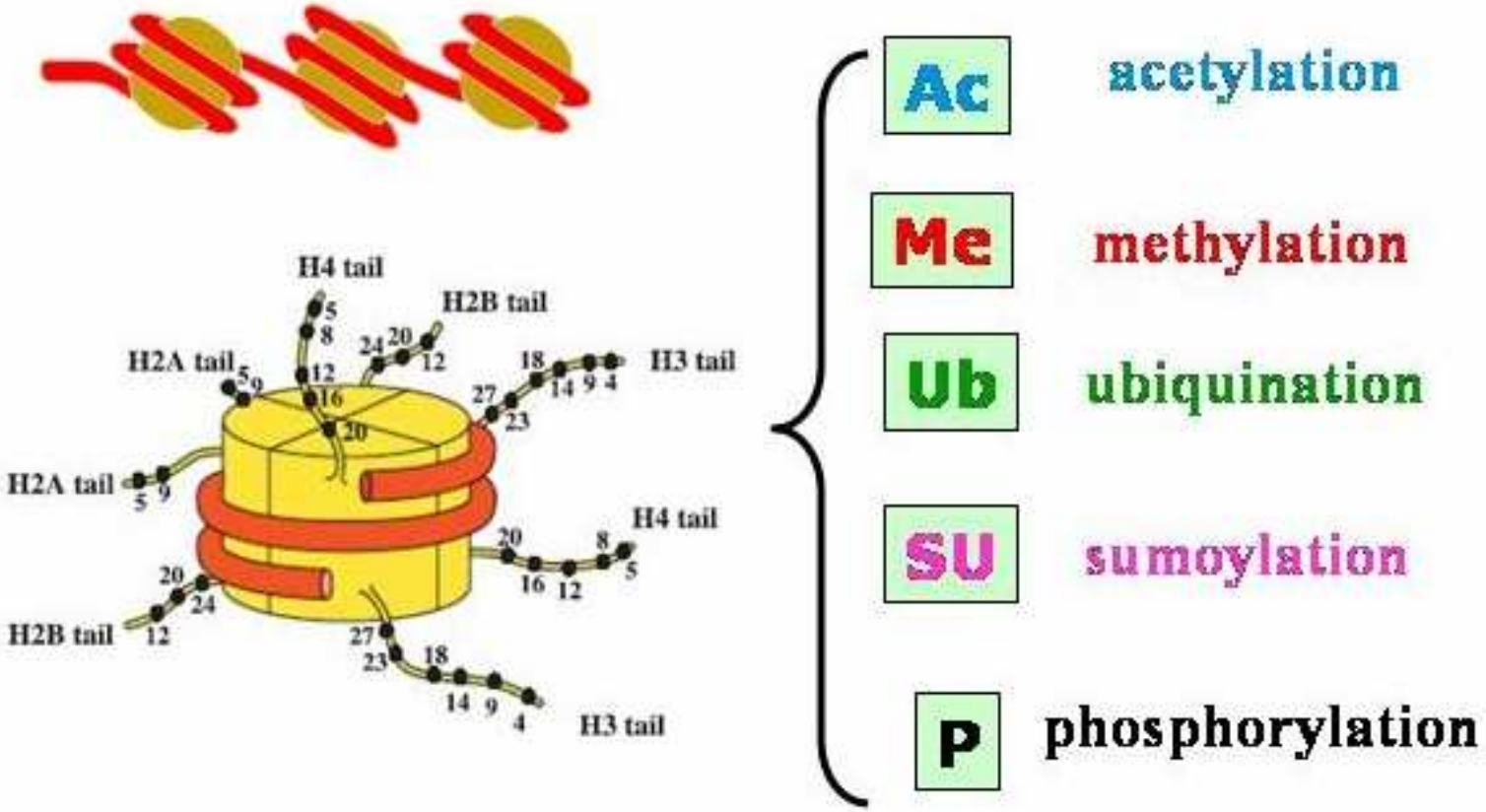
- **Typy histonových modifikací:**
  a) acetylace,
  b) methylace,
  c) fosforylace,
  d) polyadenylace,
  e) ubiquitinace

- **Methylace histonů byla objevena již před 30 lety.**

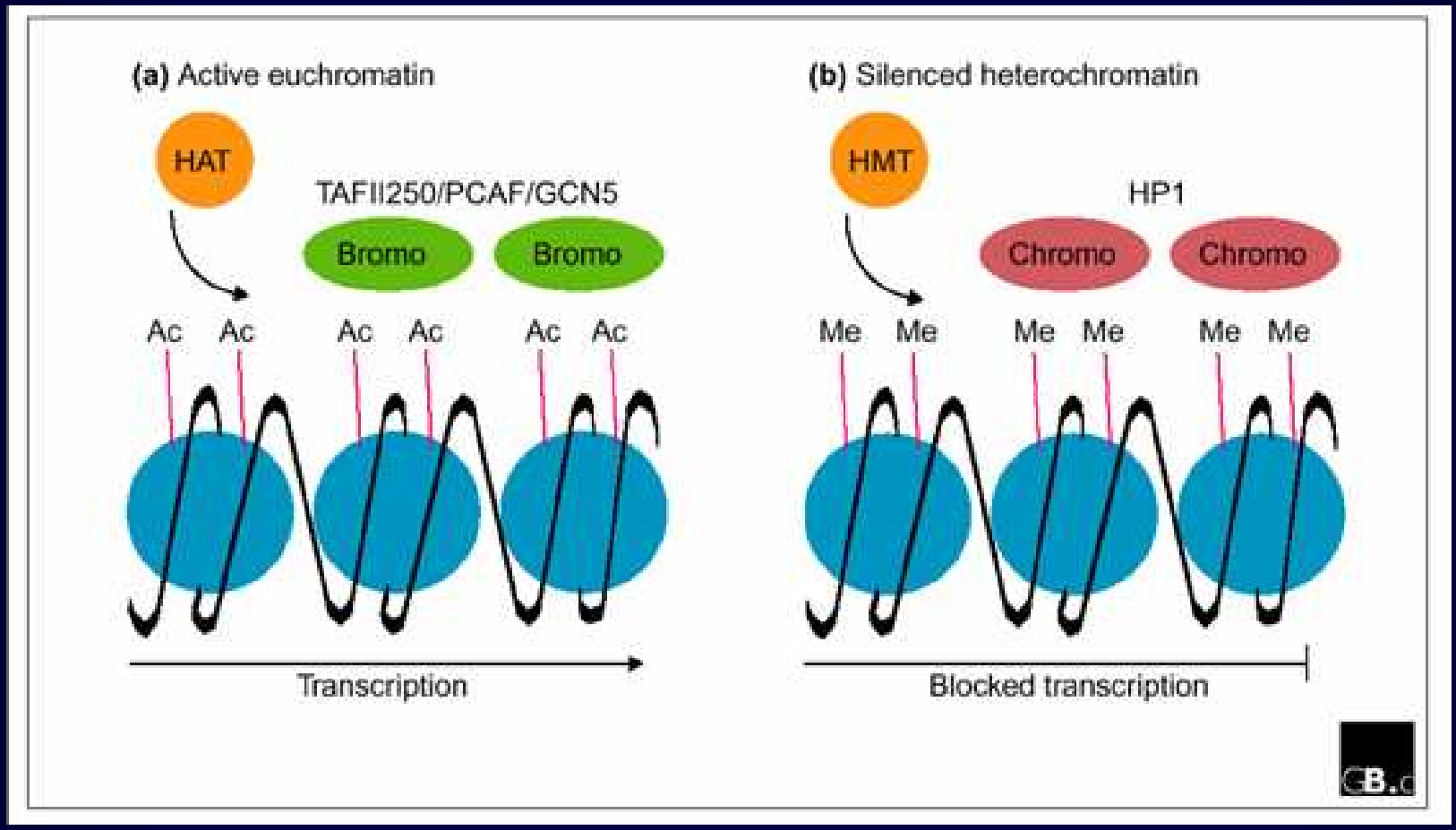


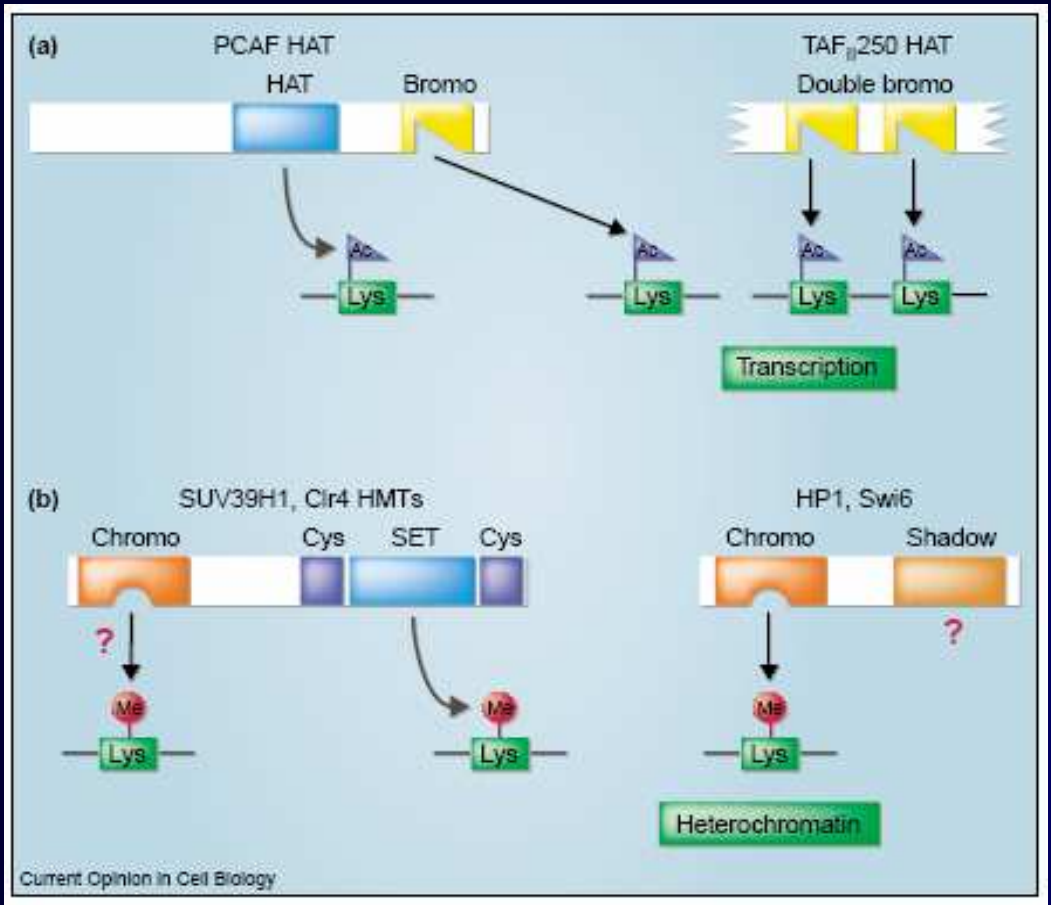
Vztah mezi acetylací a metylací histonů: acetylace histonů je katalyzována histon acetyl-transferázami (HATs) a odstraňo-vána histon deacetylázami (HDACs). HDACs odstraní acetyl-skupinu, která je nahrazena methyl skupinou za účasti HMTs (Suv39H1- human, Clr4 – S.pombe)

2004: Objev demethylace histonů za účasti amin oxidasy LSD1 (KIAA0601) (Shi et al., Cell 2004). LSD1 specificky demethyluje H3 (K4), epigenetickou modifikaci zodpovědnou za transkripční aktivitu.



The figure illustrates nucleosome models and major posttranslational modifications which play essential roles in gene expression regulation and disease processes

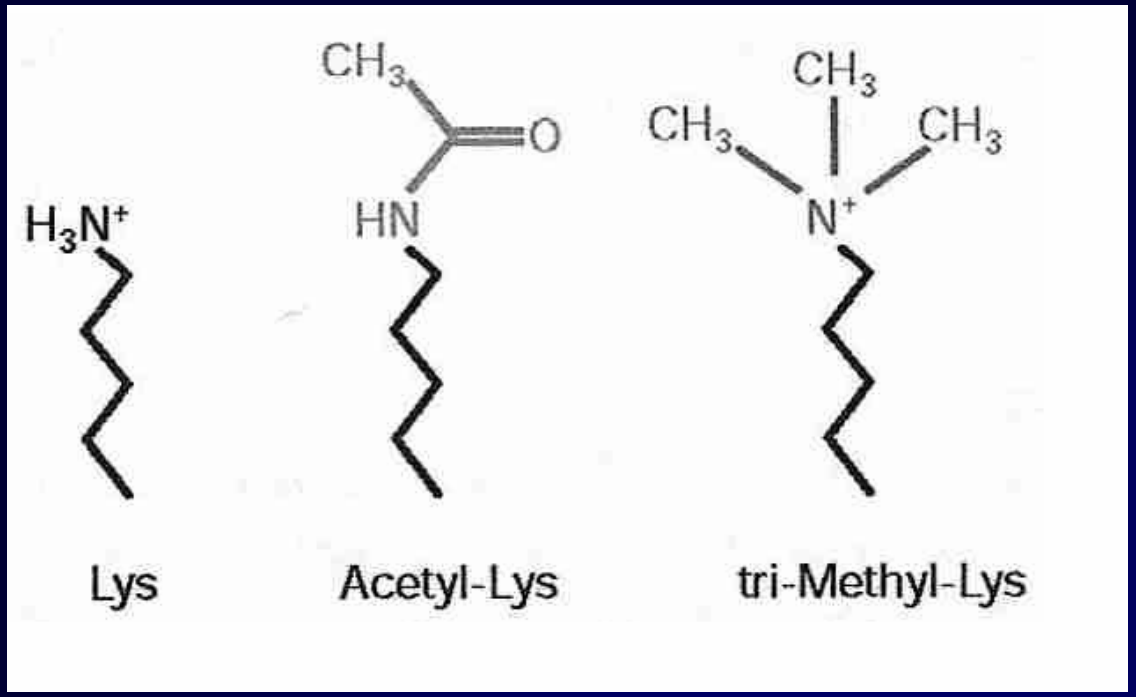

(a) Active euchromatin
HAT
TAFII250/PCAF/GCN5
Bromo
Bromo
Ac Ac Ac Ac Ac Ac
Transcription
(b) Silenced heterochromatin
HMT
HP1
Chromo
Chromo
Me Me Me Me Me Me
Blocked transcription

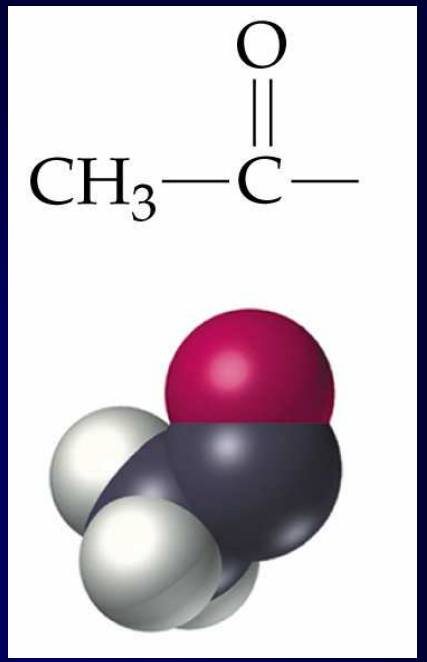


CD: protein-chromatin
CSD: protein-protein
HD: HP1-to-DNA and linker histones

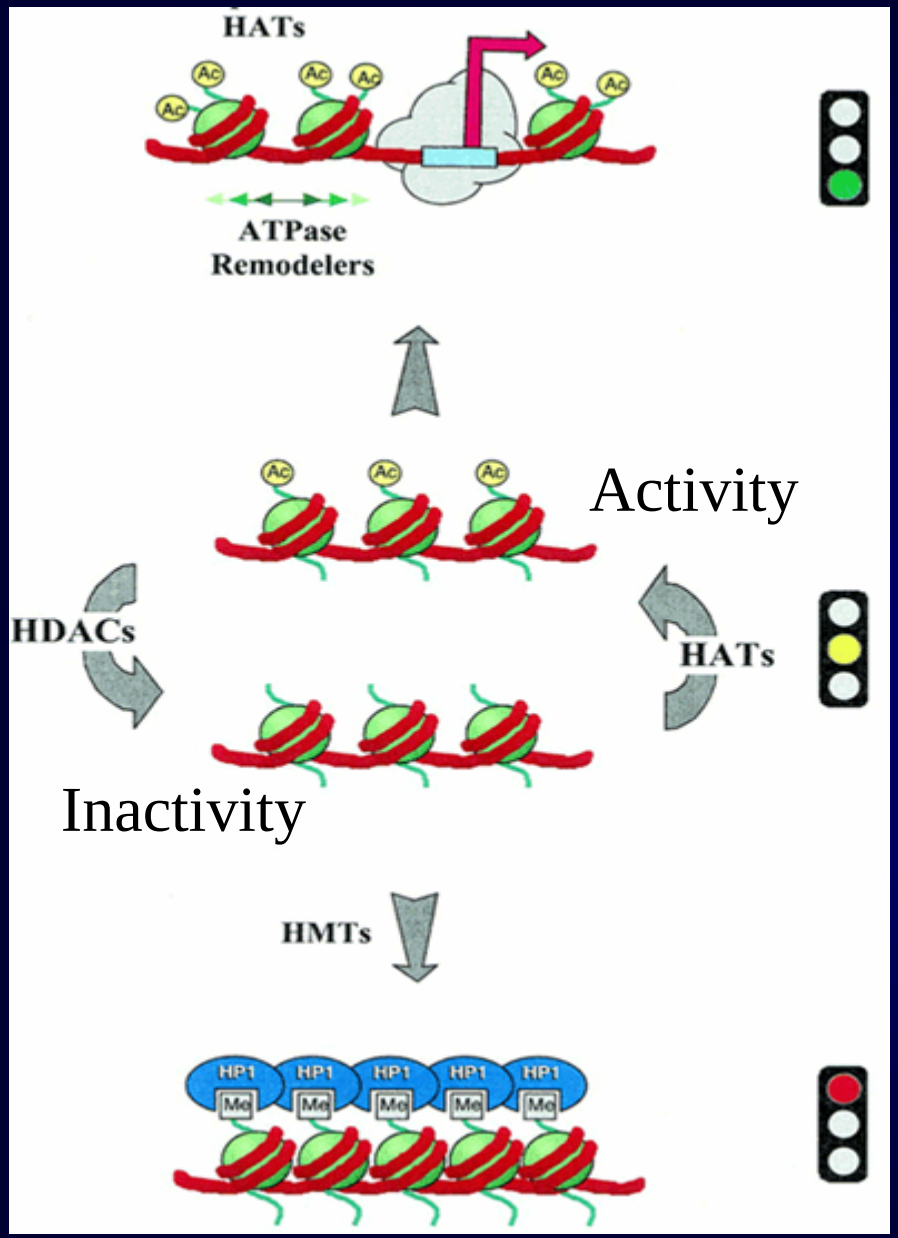
HMTs:

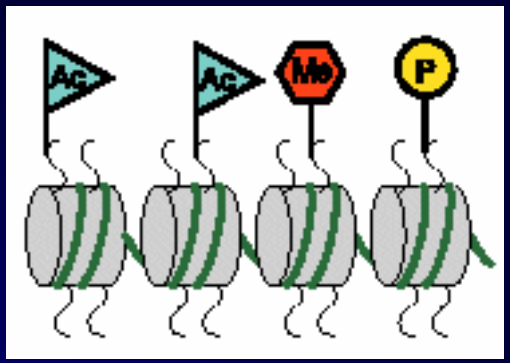
D. melanogaster: Su(var)3-9 je lokalizován v oblastech kondenzovaného chromatinu a je to klíčový regulátor v organizaci represivního chromatinu. homolog u S.pombe je Clr4 umyší SUV39h1 a u lidských buněk SUV39H1. Tyto HMTs specificky methylují H3(K9).

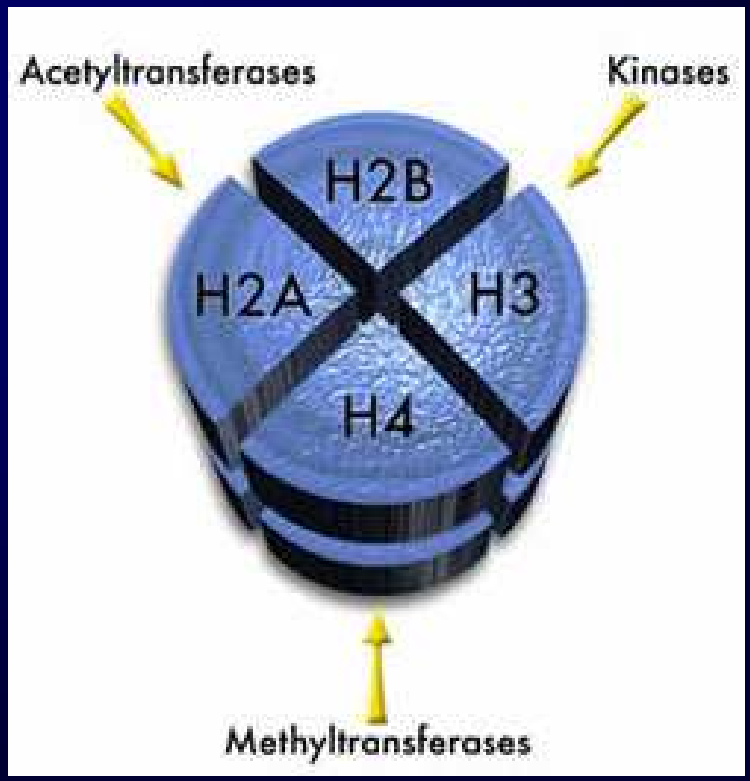




Primárním důsledkem histonových modifikací je snížení schopnosti histonových konců interagovat s dalšími složkami chromatinu, včetně DNA.

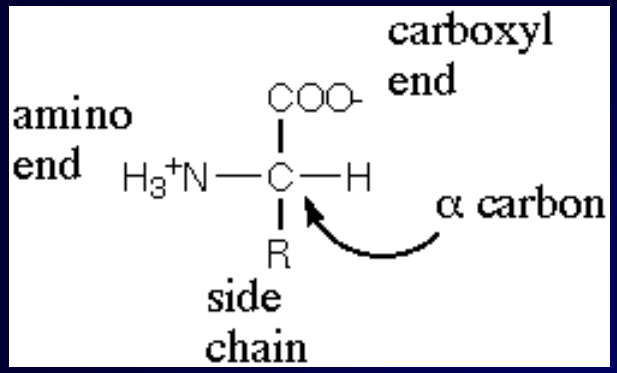


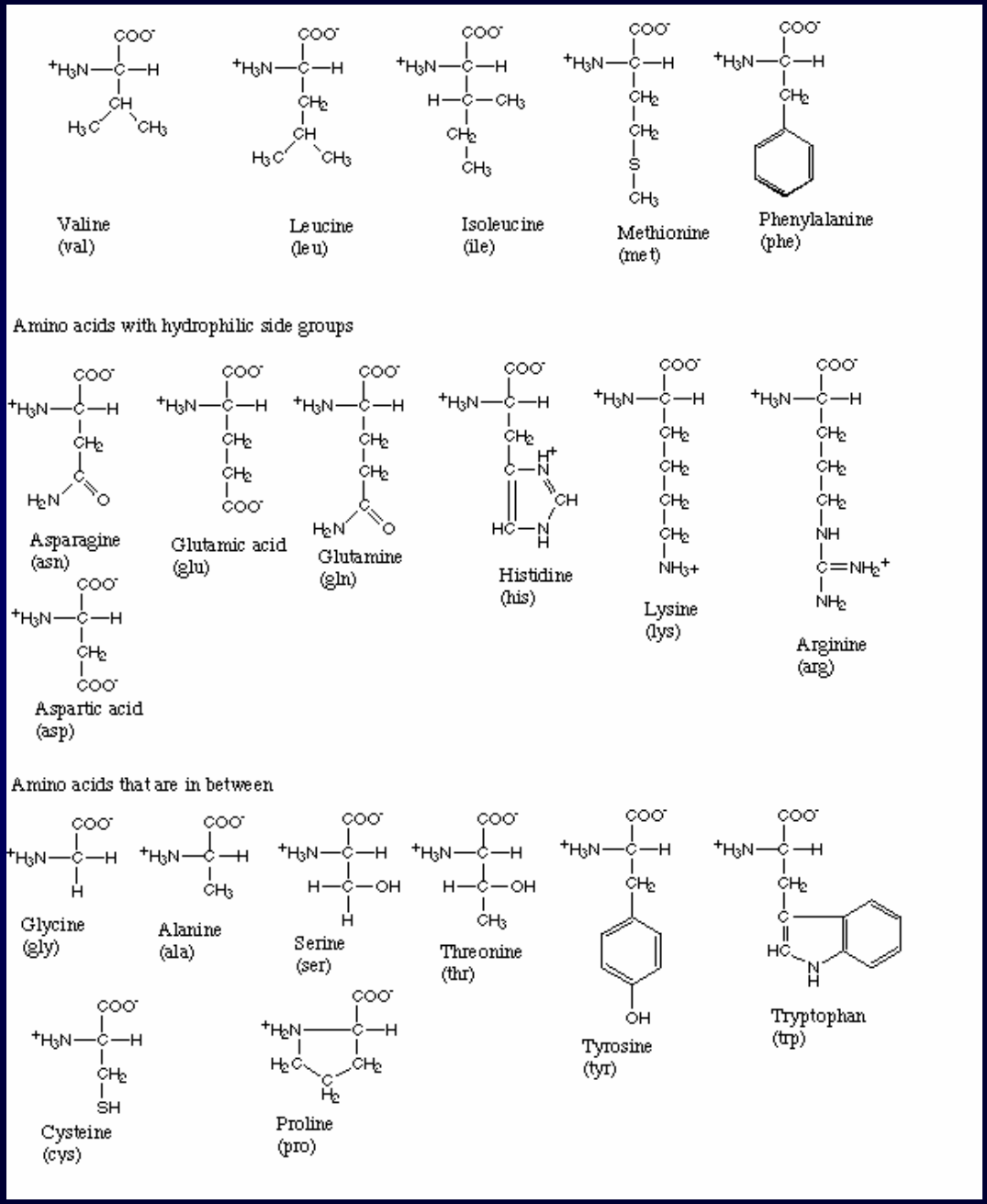


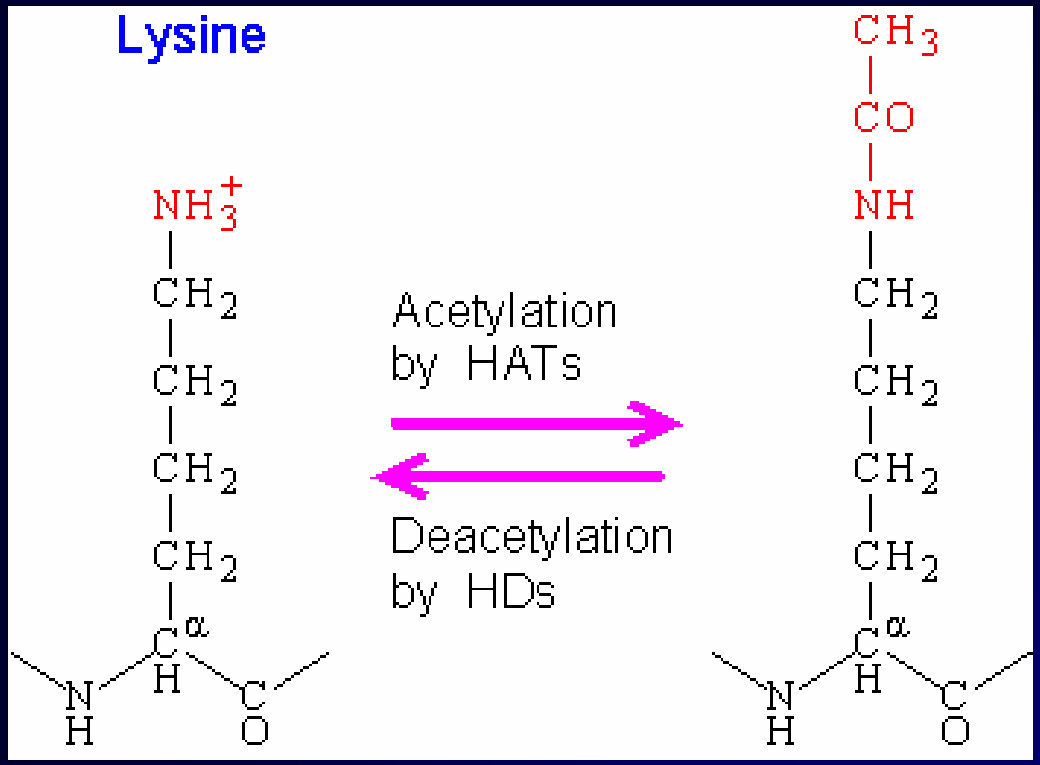


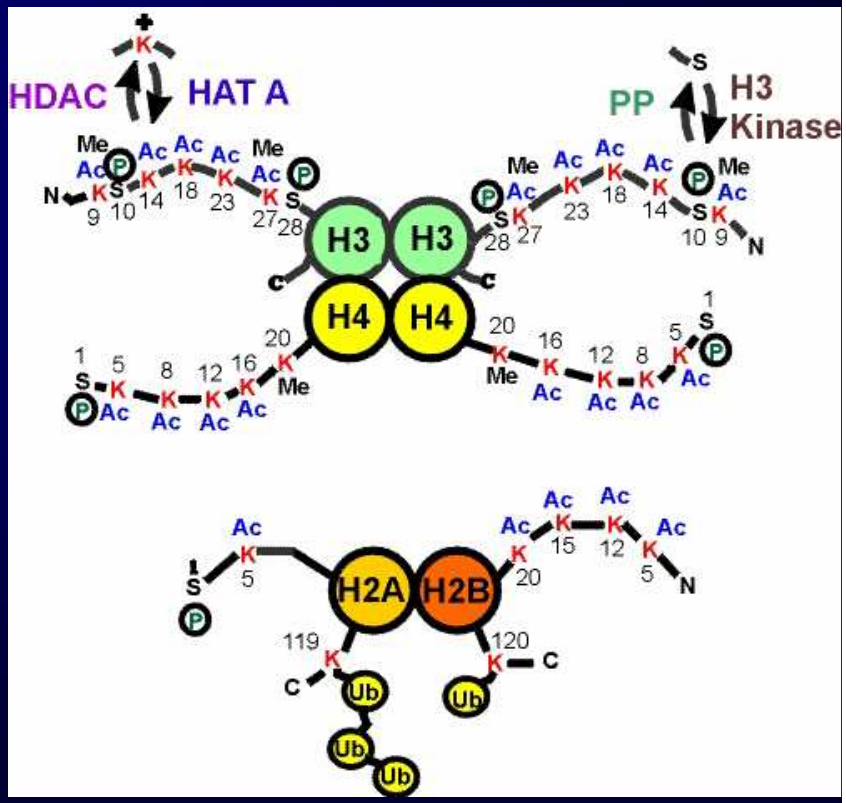
Ikaros, Helios

Amino acid

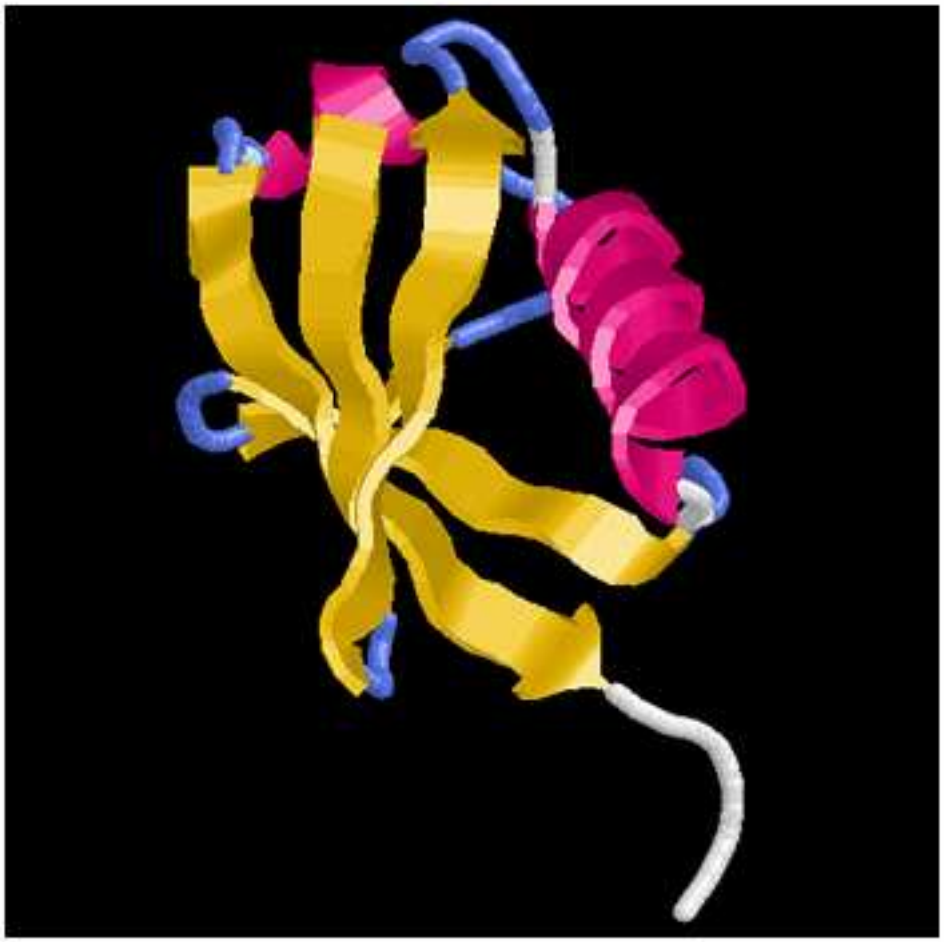

carboxyl
end
amino
end
COO-
H3+N
C
H
α carbon
R
side
chain

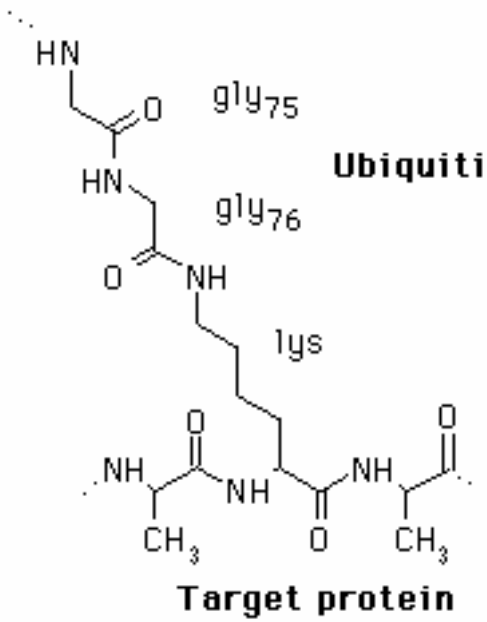

Valine
(val)
Leucine
(leu)
Isoleucine
(ile)
Methionine
(met)
Phenylalanine
(phe)
Amino acids with hydrophilic side groups
Asparagine
(asn)
Glutamic acid
(glu)
Glutamine
(gln)
Histidine
(his)
Lysine
(lys)
Arginine
(arg)
Aspartic acid
(asp)
Amino acids that are in between
Glycine
(gly)
Alanine
(ala)
Serine
(ser)
Threonine
(thr)
Tyrosine
(tyr)
Tryptophan
(trp)
Cysteine
(cys)
Proline
(pro)


Lysine
Acetylation
by HATs
Deacetylation
by HDs


HDAC
HAT A
PP
H3
Kinase
H3
H3
H4
H4
H2A
H2B
Ub
Ub
Ub
Ub

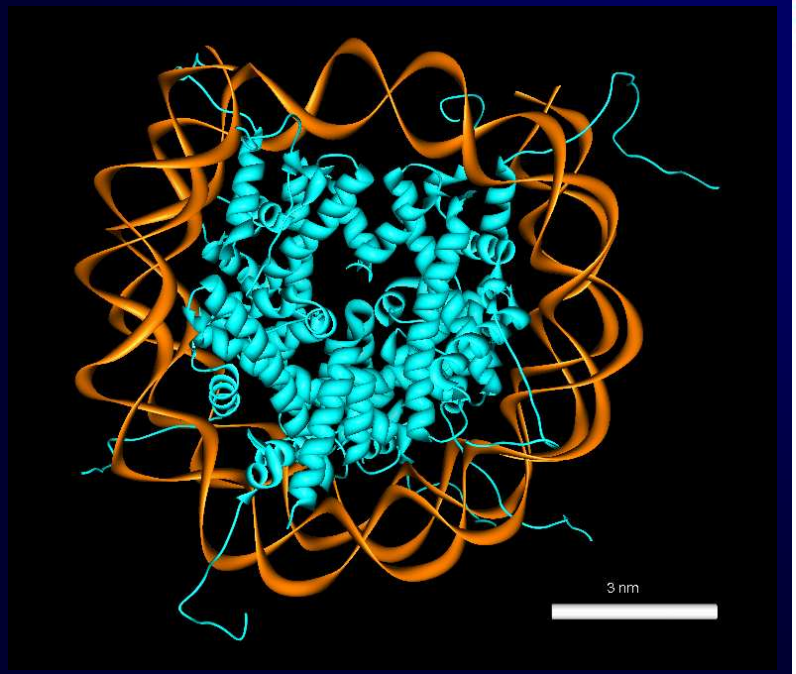
Ubiquitin structure





Ubiquitination of histones has been reported *in vivo* although the most prevalent ubiqutination occurs in H2A and H2B. One of the widely studied proteins that undergoes ubiquitination for its activity is p53.

Figure 9. Interplay between different post-translational modifications occurring on histone H3 and H4 amino-terminal tails. Residues that are known to be acetylated (Ac), methylated (Me), and phosphorylated (P) are indicated. Positive and negative affects are indicated.



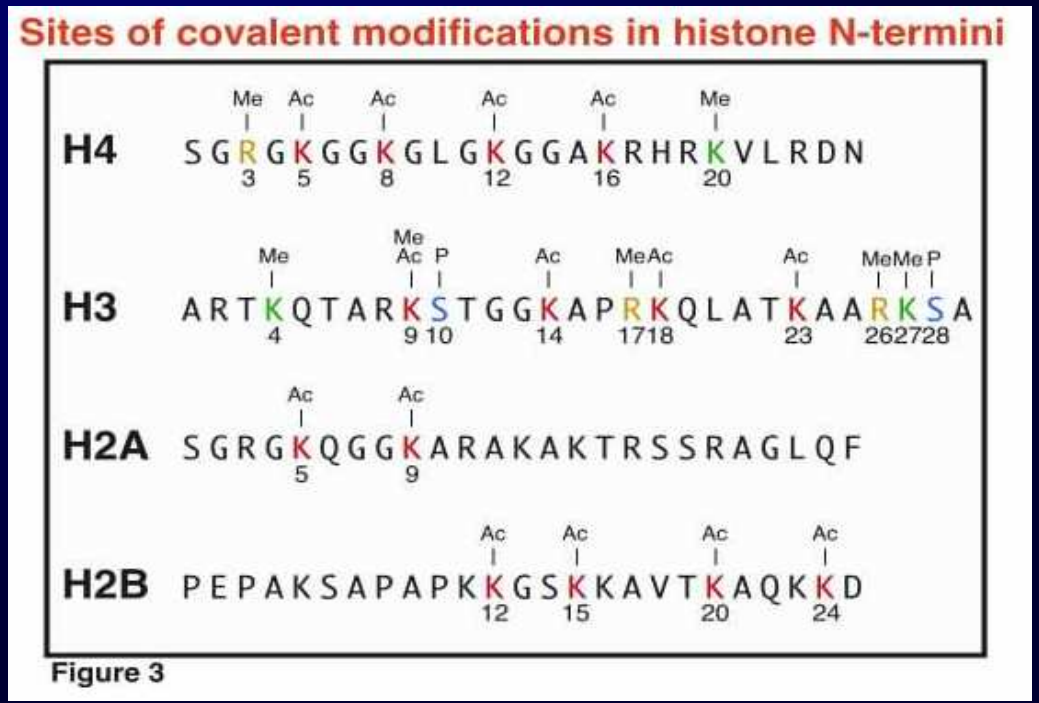
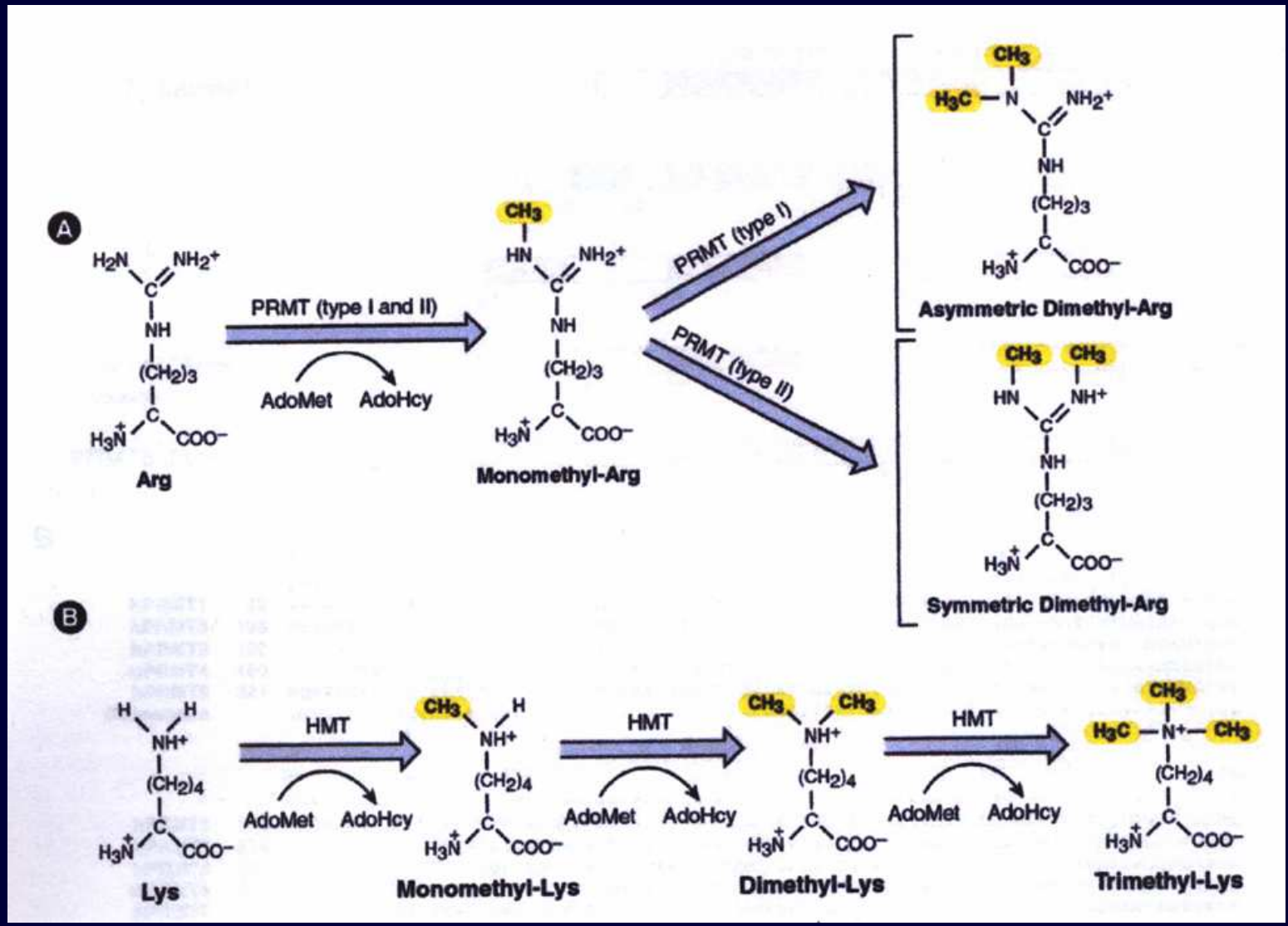
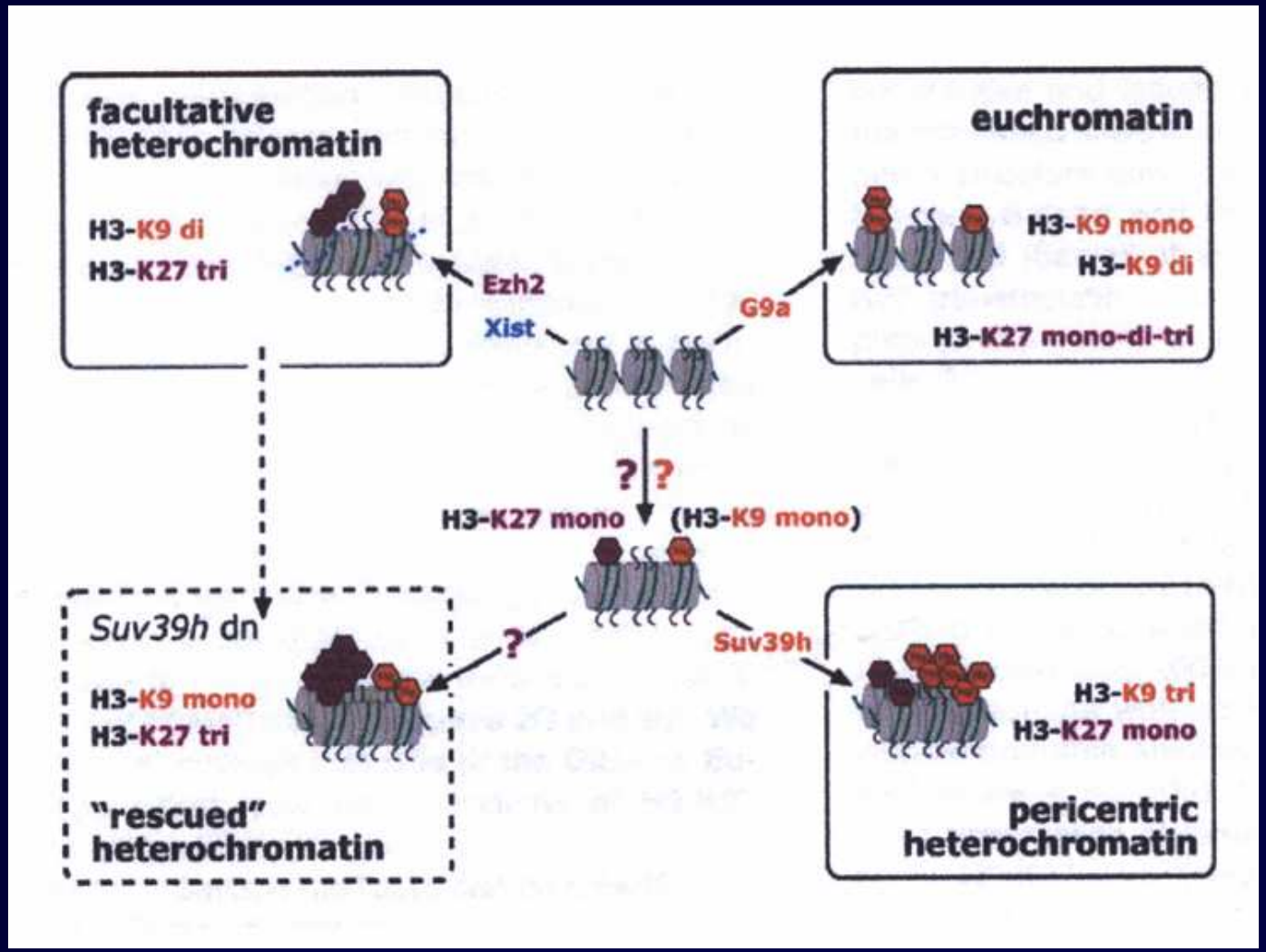


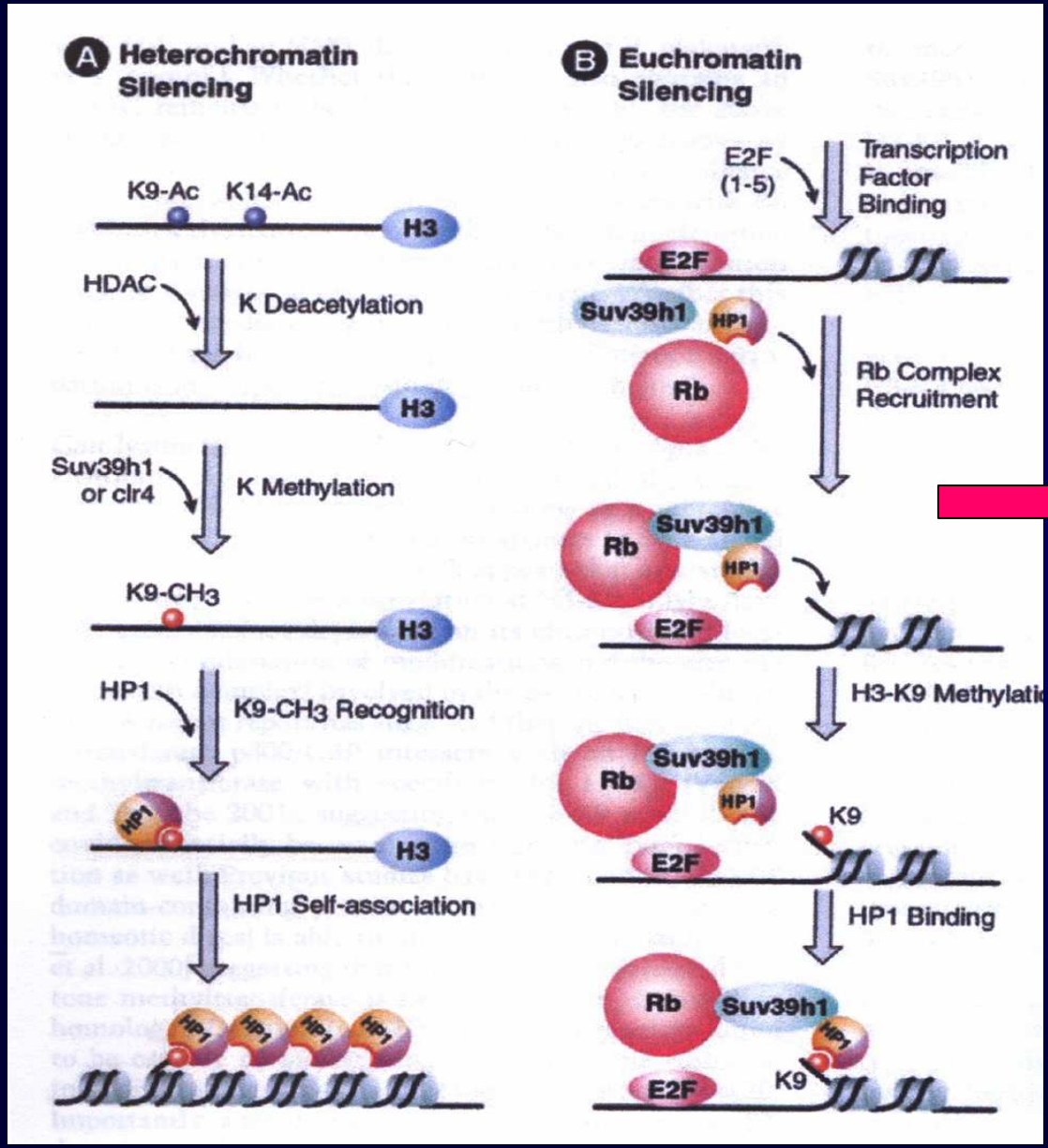
Figure 3

TRANSKRIPČNÍ AKTIVACE: H3-R2-Me, H3- Ac-K9, H3- K27-Me (Xi), H3-K36-Me, H3- K79-Me (telomeric silencing), H4-K20-Me (mitotic condensation)

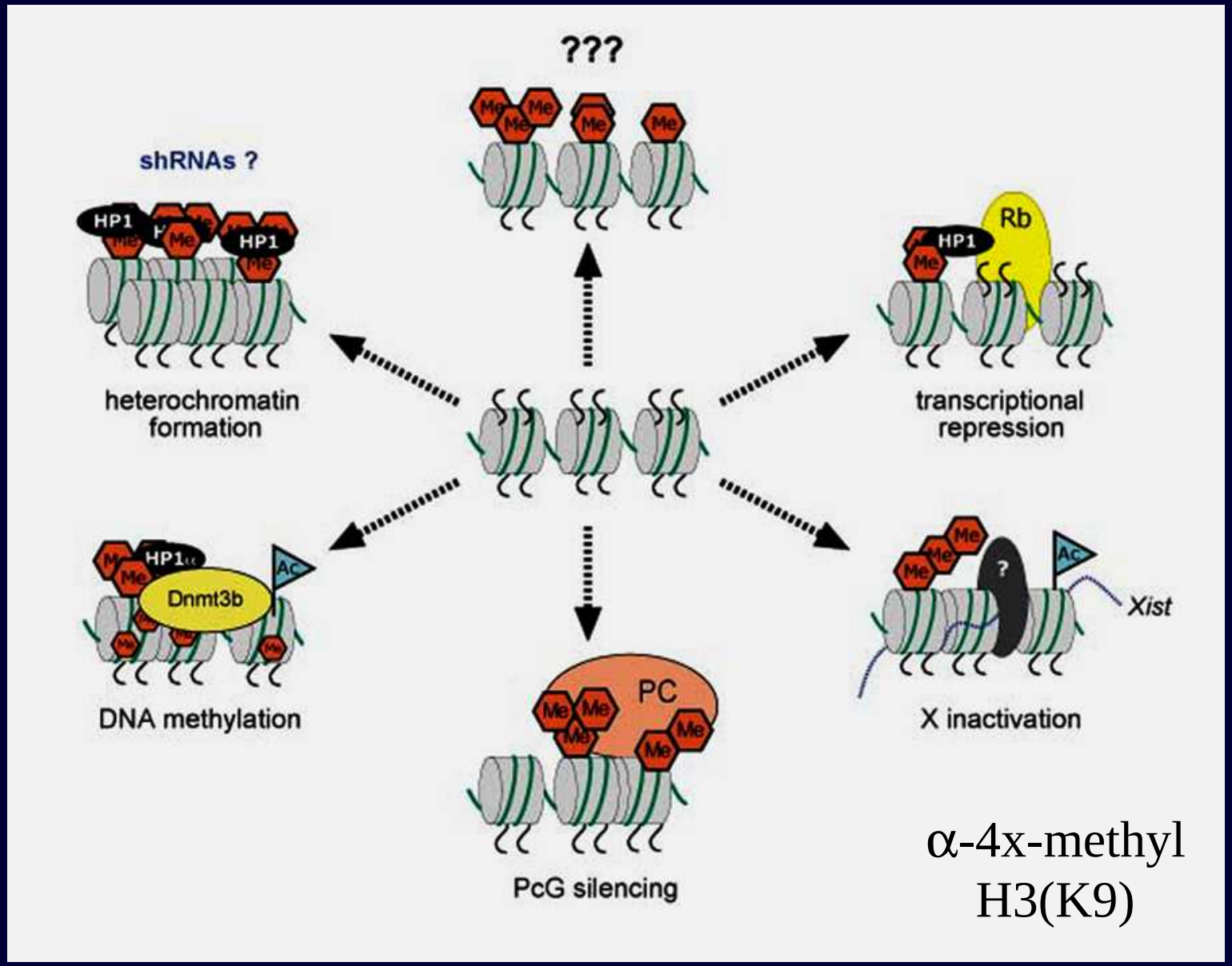
TRANSKRIPČNÍ INAKTIVACE: H3-Me-K9, H3-S10-P, H3-K17-Ac, H3- R-17-Me, H3-K18-Ac, H3-K23-Ac, H4-R3-Me, H4-K8-Ac,


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

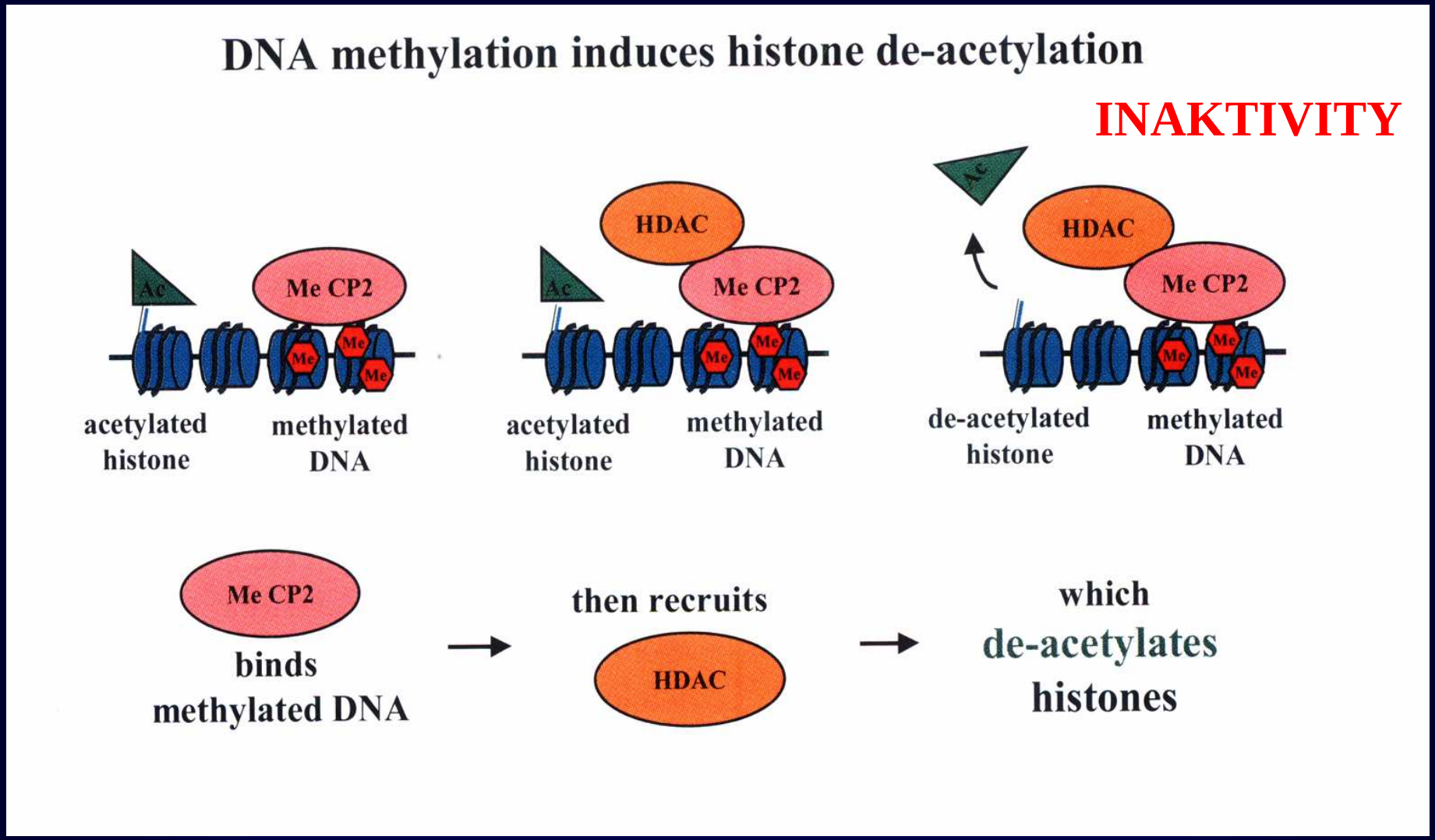

facultative heterochromatin
H3-K9 di
H3-K27 tri
Ezh2
Xist
G9a
euchromatin
H3-K9 mono
H3-K9 di
H3-K27 mono-di-tri
? ?
H3-K27 mono
(H3-K9 mono)
Suv39h dn
H3-K9 mono
H3-K27 tri
"rescued" heterochromatin
?
Suv39h
H3-K9 tri
H3-K27 mono
pericentric heterochromatin



Repression of cyclin E promoter

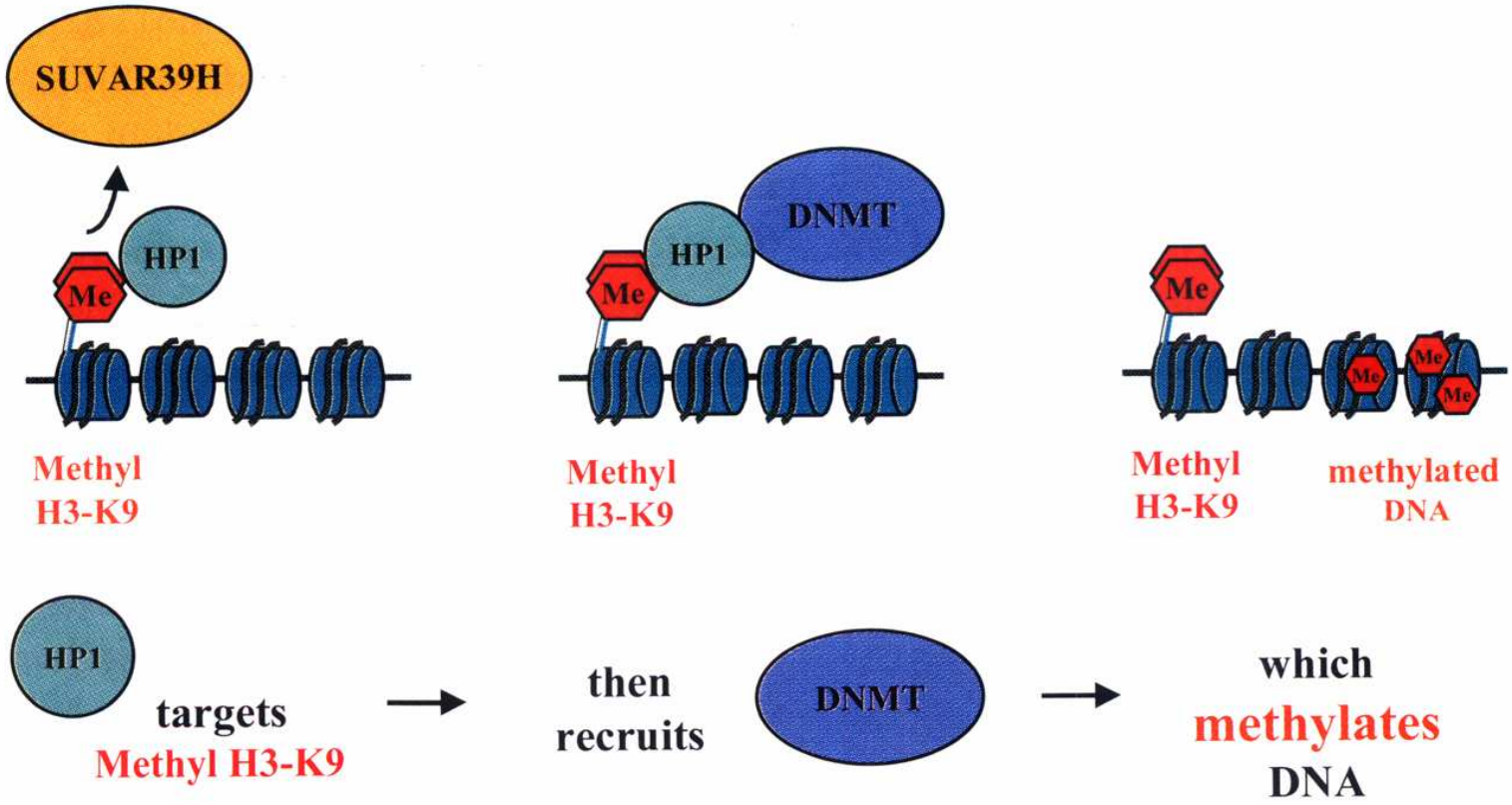


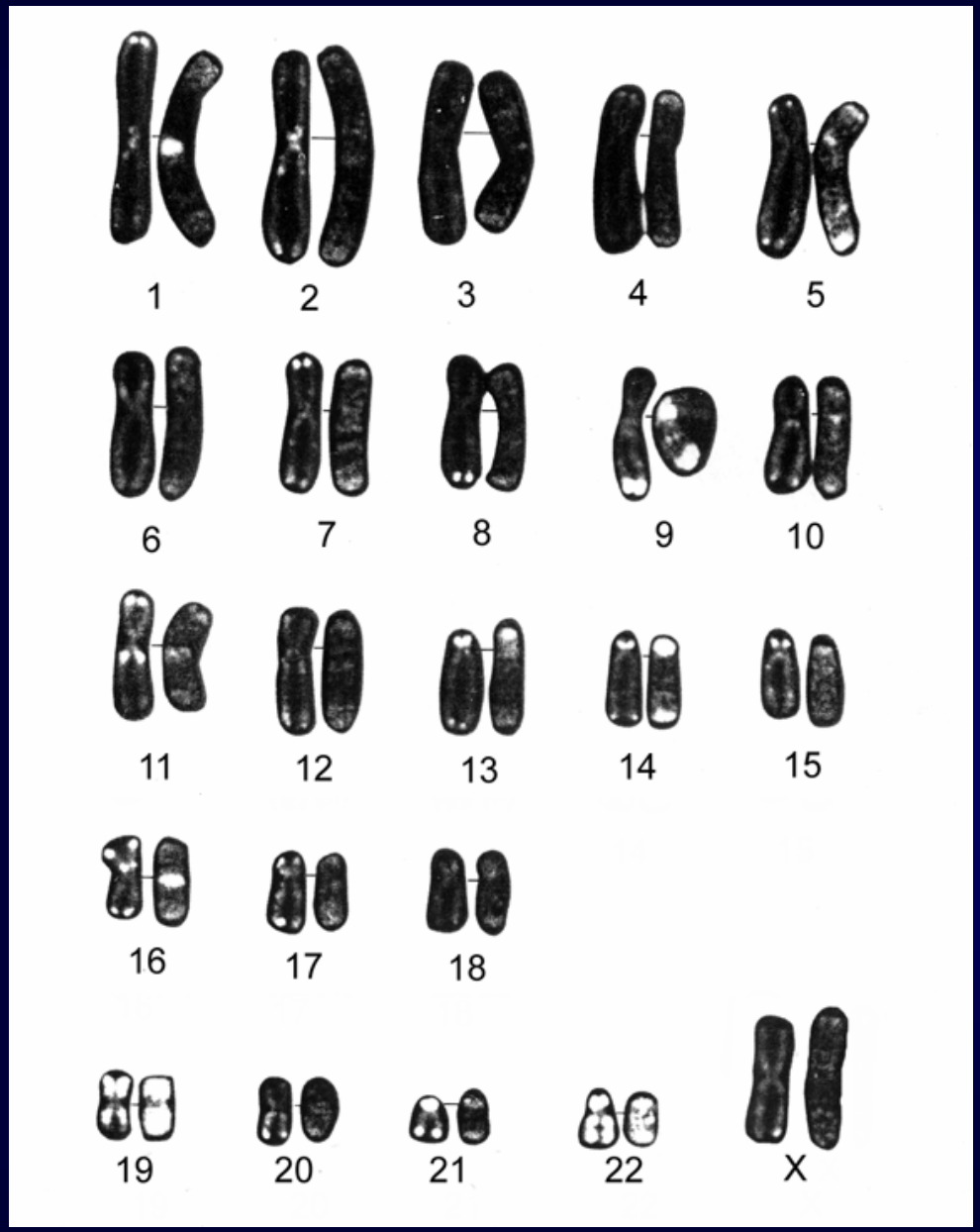
FAKULTATIVNÍ HETEROCHROMATIN



MeCP2: Methyl-CpG binding Protein, specifically binds to to methylated DNA

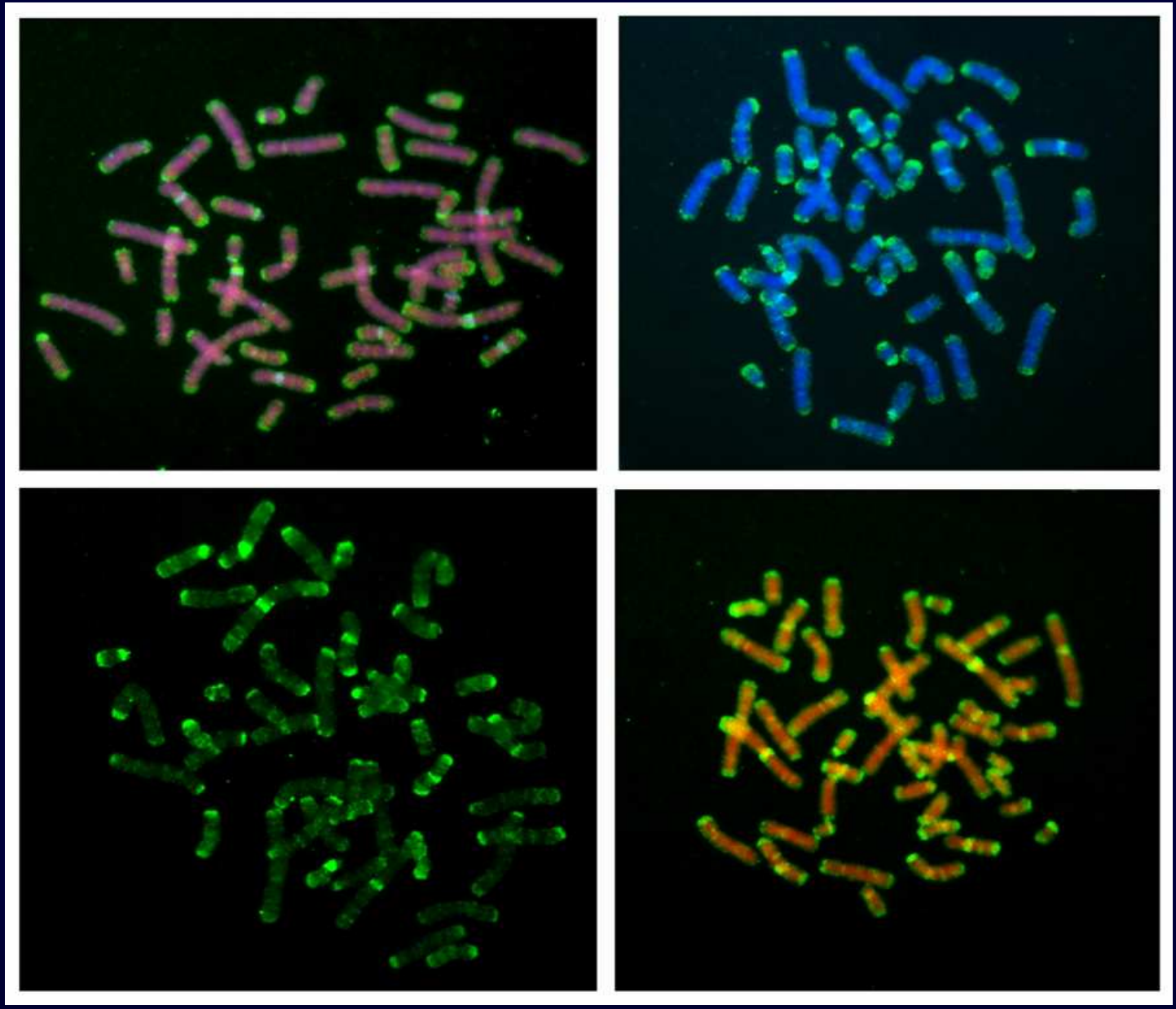
Histone H3-K9 methylation induces DNA methylation





Barbin et al., 1994

DNA methylated regions on human chromosomes



Methylation state of centromeres

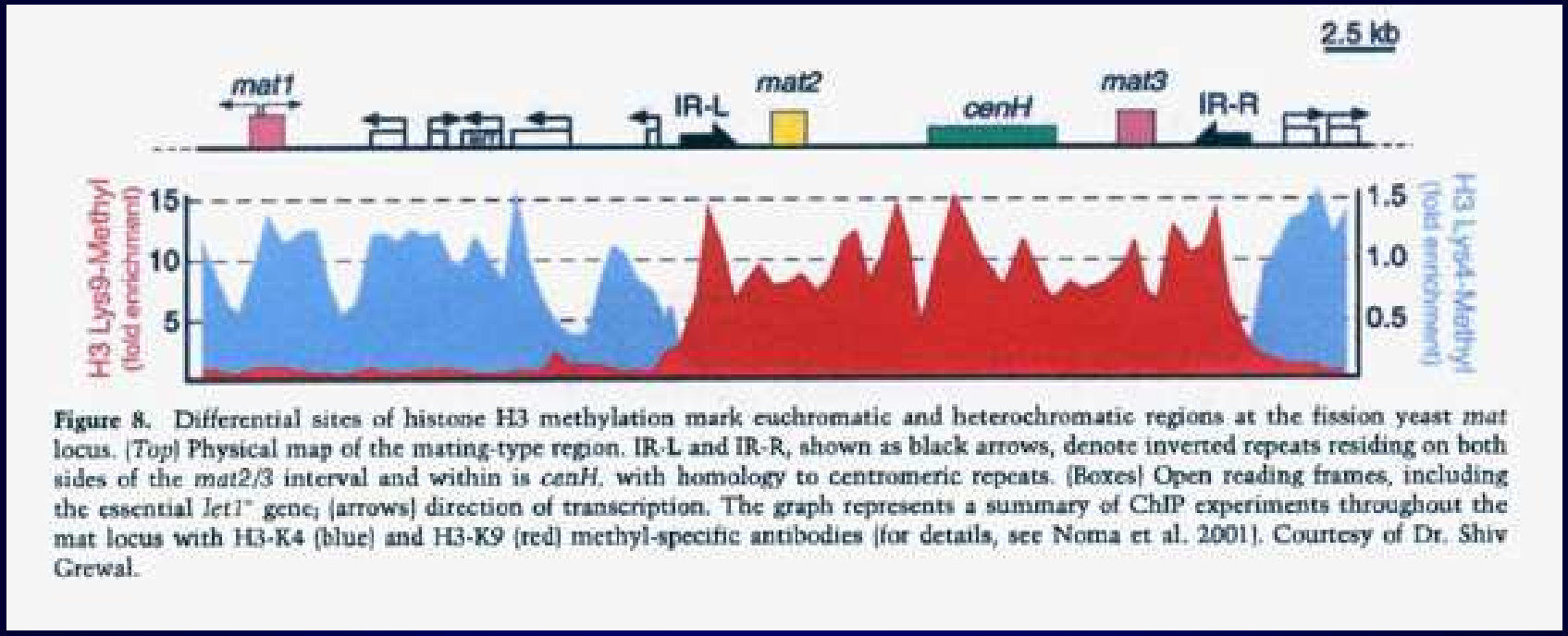
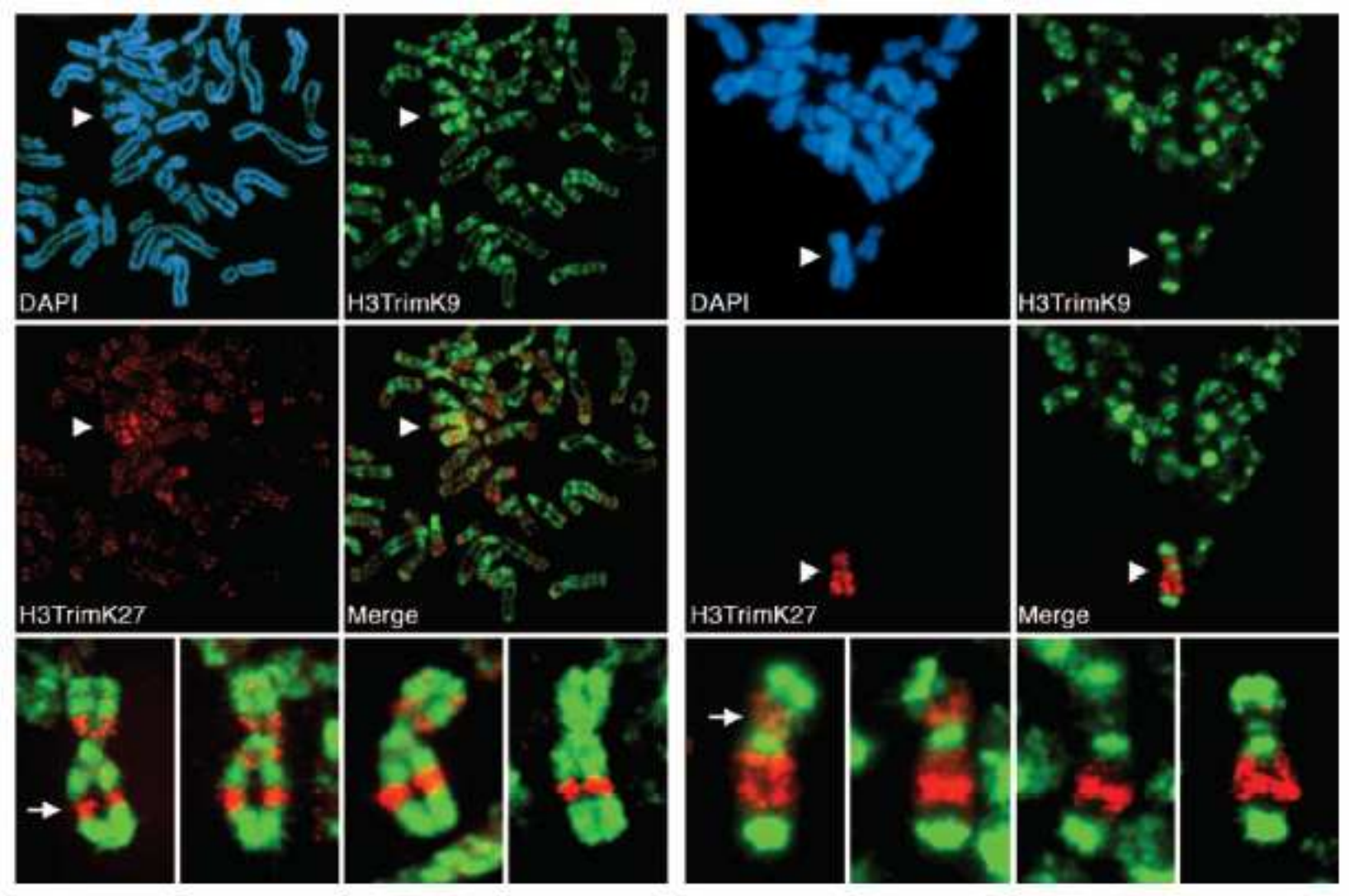


Figure 8. Differential sites of histone H3 methylation mark euchromatic and heterochromatic regions at the fission yeast *mat* locus. (*Top*) Physical map of the mating-type region. IR-L and IR-R, shown as black arrows, denote inverted repeats residing on both sides of the *mat2/3* interval and within is *cenH*, with homology to centromeric repeats. (Boxes) Open reading frames, including the essential *let1*$^+$ gene; (arrows) direction of transcription. The graph represents a summary of ChIP experiments throughout the mat locus with H3-K4 (blue) and H3-K9 (red) methyl-specific antibodies (for details, see Noma et al. 2001). Courtesy of Dr. Shiv Grewal.

•Methylation of H3(K9), H3(K27) and H3(K20) are associated with the repressive chromatin state whereas H3(K4), H3(K36) and H3(K79) methylations and/or histone acetylation have been correlated with active chromatin (summary Fischle et al., 2003; Lachner et al., 2003).

- Centromeric heterochromatin: mono- or di-meH3-K9
- Pericentric heterochromatin: tri-meH3K9
- Euchromatin: di-meH3-K4 and Acetylated
- Xi is α-4x-methyl H3(K9)

Inaktivace X chromosomu ve vztahu k epigenetickým modifikacím



Chadwick nad Willard, PNAS, 101, p.17450-17455

Inaktivace X chromosomu ve vztahu k epigenetickým modifikacím

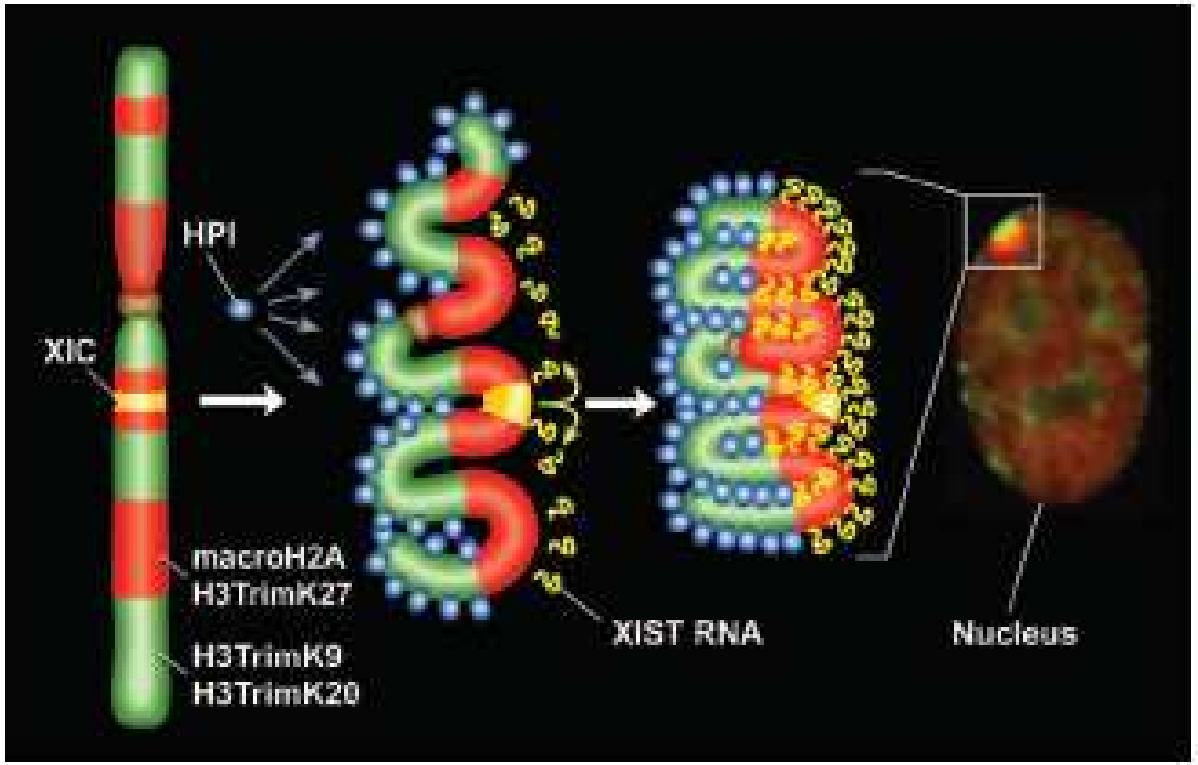
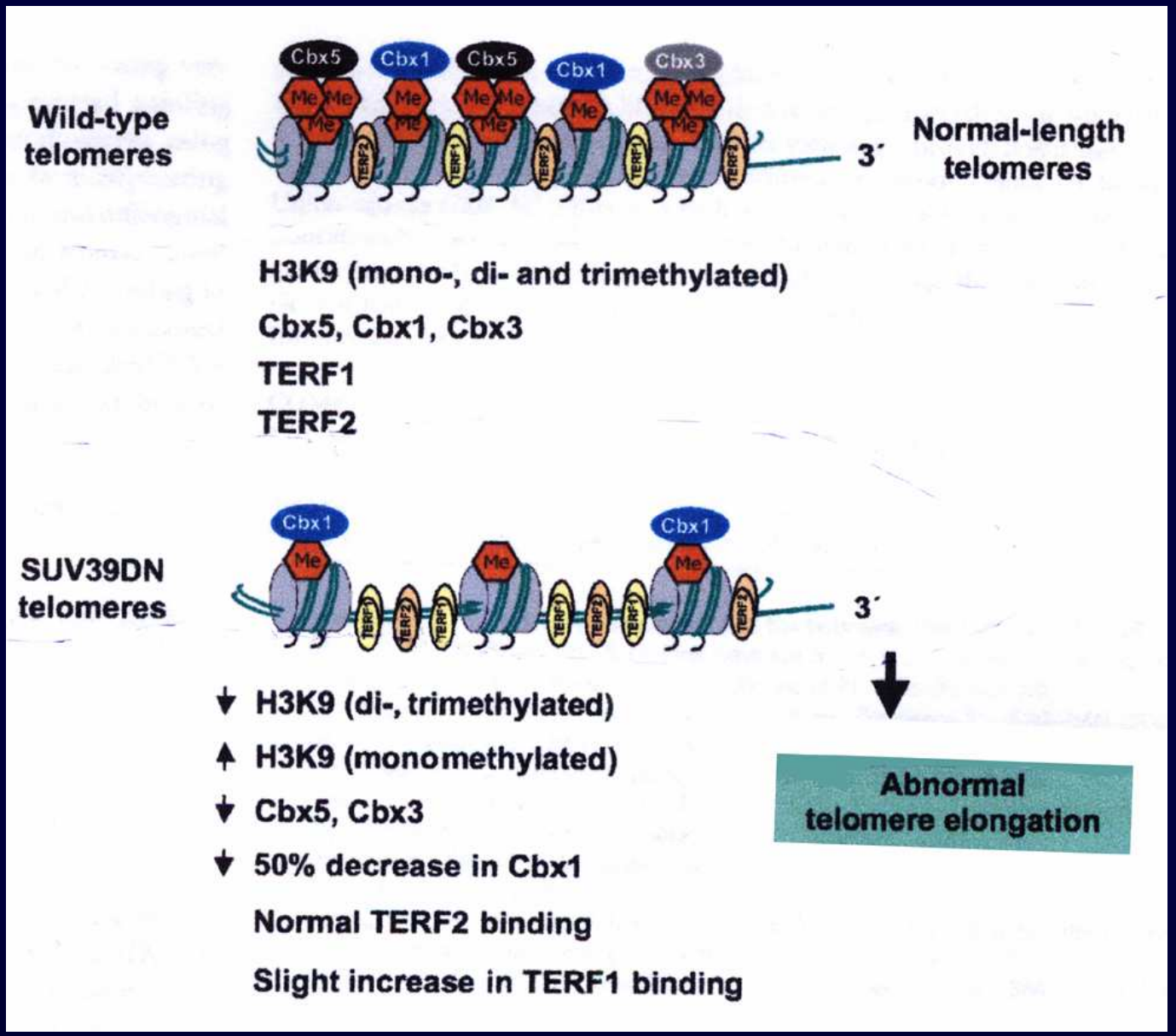
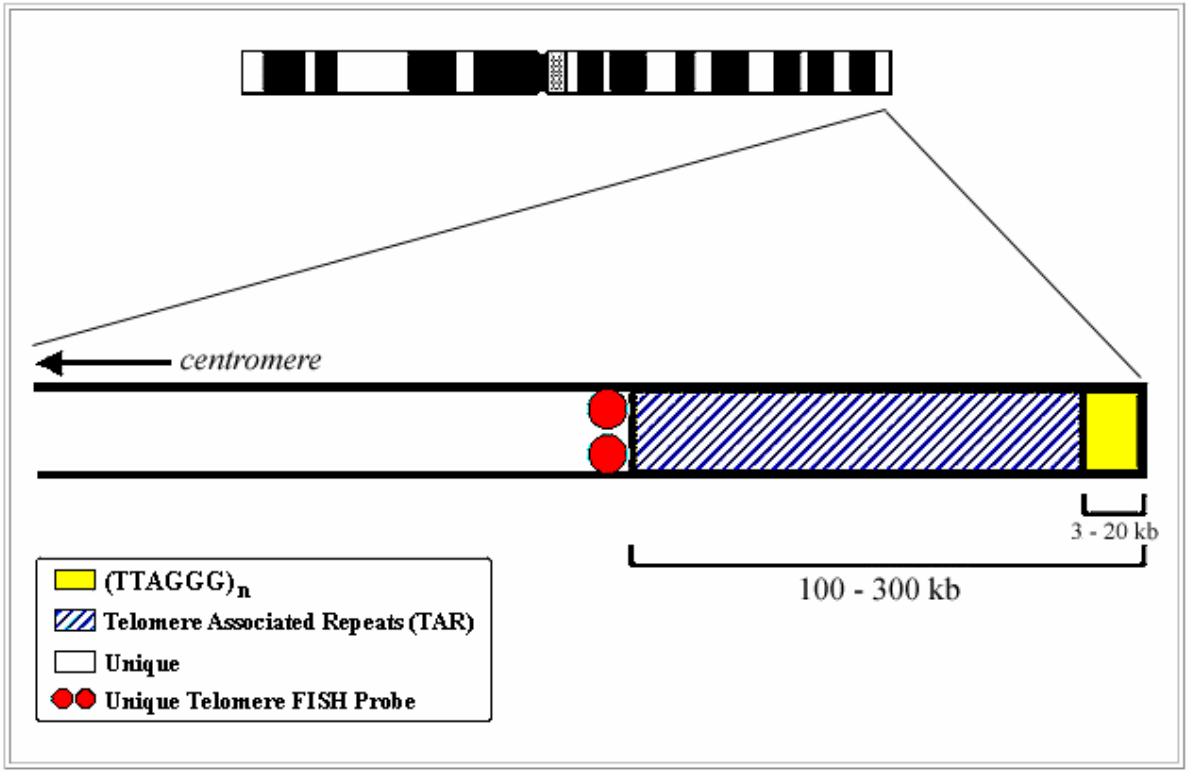


Fig. 4. Schematic model showing how heterochromatin of the Xi could transition between metaphase and interphase to be organized into the two nonoverlapping heterochromatin territories and to explain how XIST RNA could rapidly spread in cis outward from the X inactivation center (XIC) along only part of the Xi. See main text for details.

Methylation state of telomeres



Human Telomere Structure

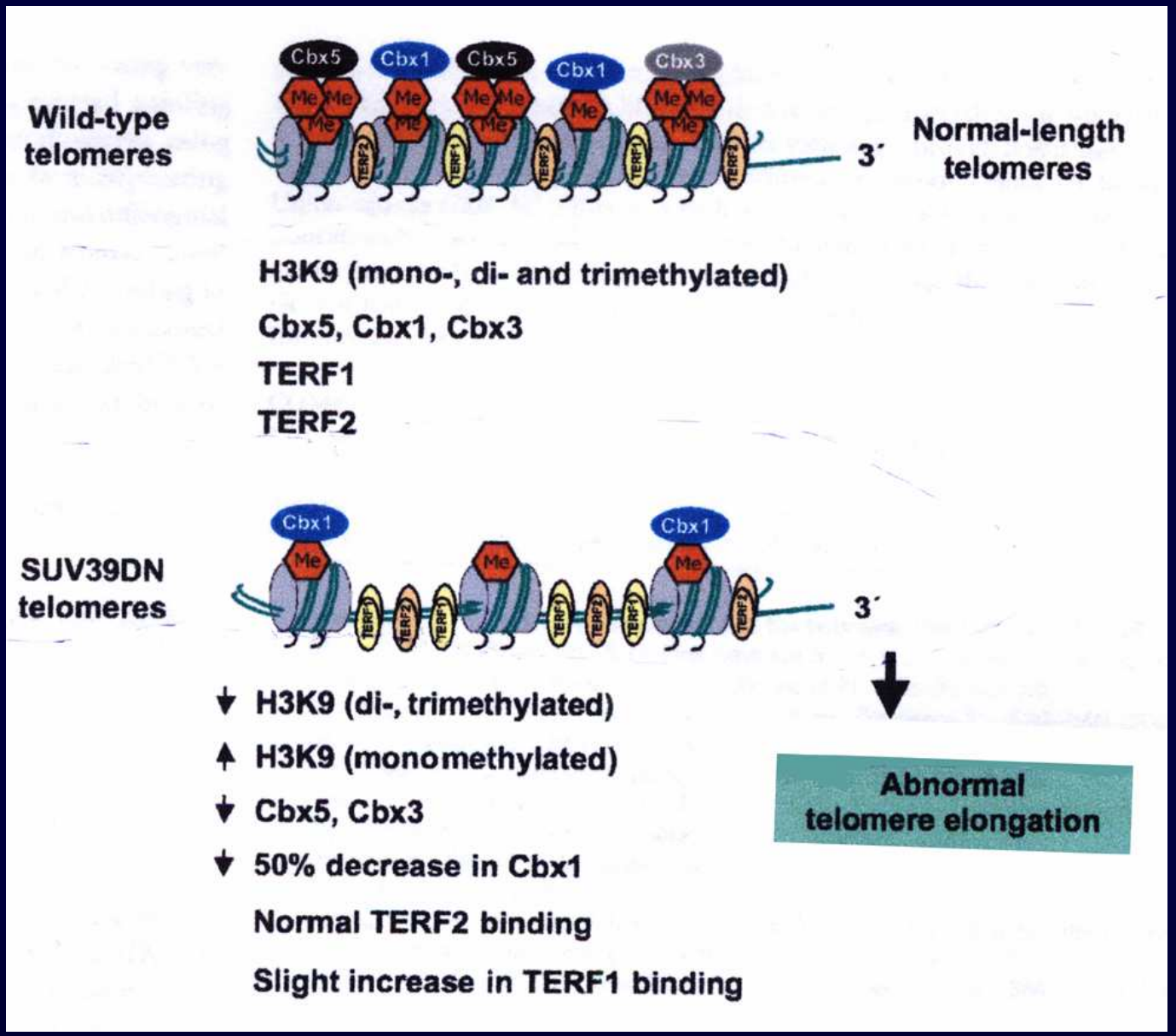


A telomere is a repeating DNA sequence (TTAGGG) at the end of the body's chromosomes. The telomere can reach a length of 15,000 base pairs. Telomeres function by preventing chromosomes from losing base pair sequences at their ends. They also stop chromosomes from fusing to each other. However, each time a cell divides, some of the telomere is lost (usually 25-200 base pairs per division). When the telomere becomes too short, the chromosome reaches a "critical length" and can no longer replicate. This means that a cell becomes "old" and dies by a process called *apoptosis*. Telomere activity is controlled by two mechanisms: erosion and addition. Erosion, as mentioned, occurs each time a cell divides. Addition is determined by the activity of telomerase. (view animation)

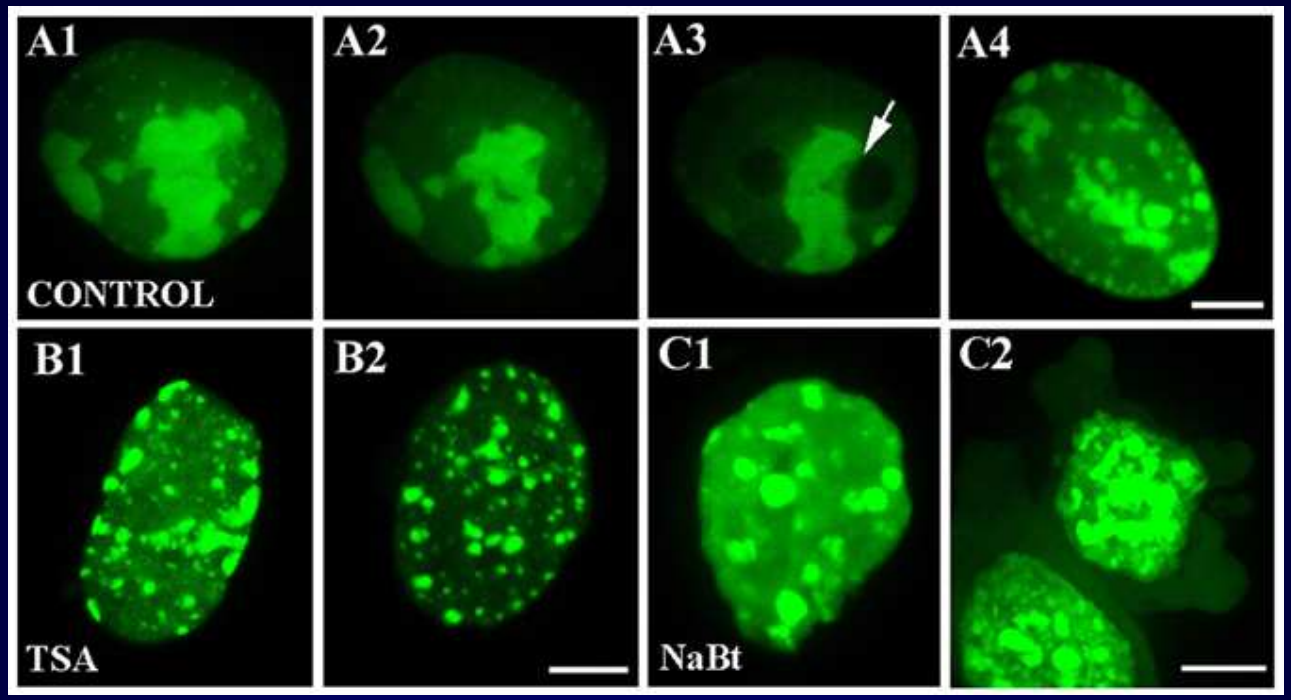
Telomerase, also called telomere terminal transferase, is an enzyme made of protein and *RNA* subunits that elongates chromosomes by adding TTAGGG sequences to the end of existing chromosomes. Telomerase is found in fetal tissues, adult *germ cells*, and also tumor cells. Telomerase activity is regulated during development and has a very low, almost undetectable activity in *somatic* (body) cells. Because these somatic cells do not regularly use telomerase, they age. The result of aging cells is an aging body. If telomerase is activated in a cell, the cell will continue to grow and divide. This "immortal cell" theory is important in two areas of research: aging and cancer.

Catalytic subunit of telomerase, hTERT

Methylation state of telomeres



HP1 proteins

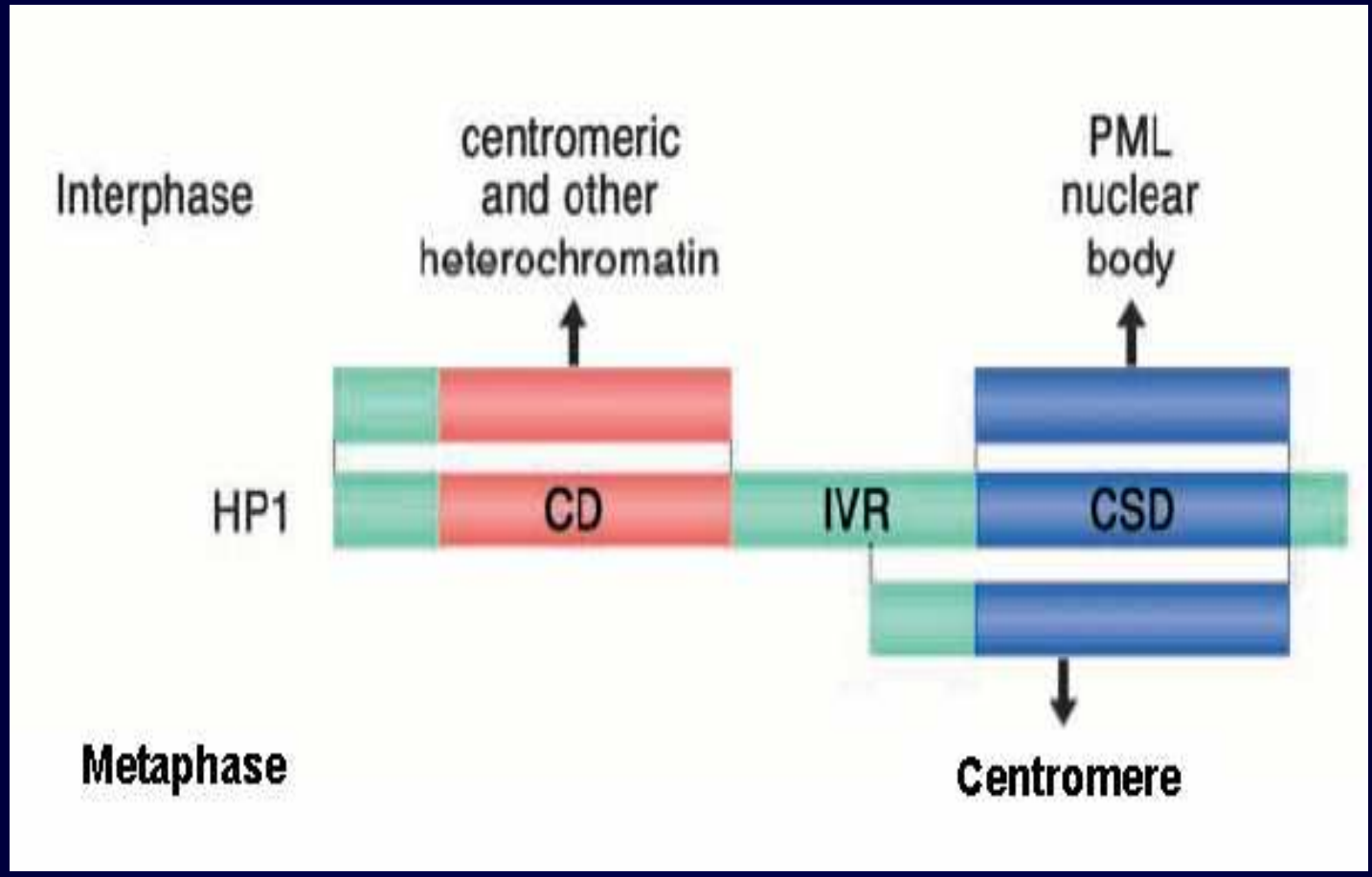


•**HP1 proteiny jsou hlavní složkou heterochromatinu a hrají důležitou úlohu při jeho tvorbě. HPs jsou mají vysokou afinitu k pericentromerickým a telometrickým oblastem chromosomů.**

• **HPs interagují s HMTs jako je SUV39h, která je zodpovědná za methylaci H3(K9).**

HP1 proteins:

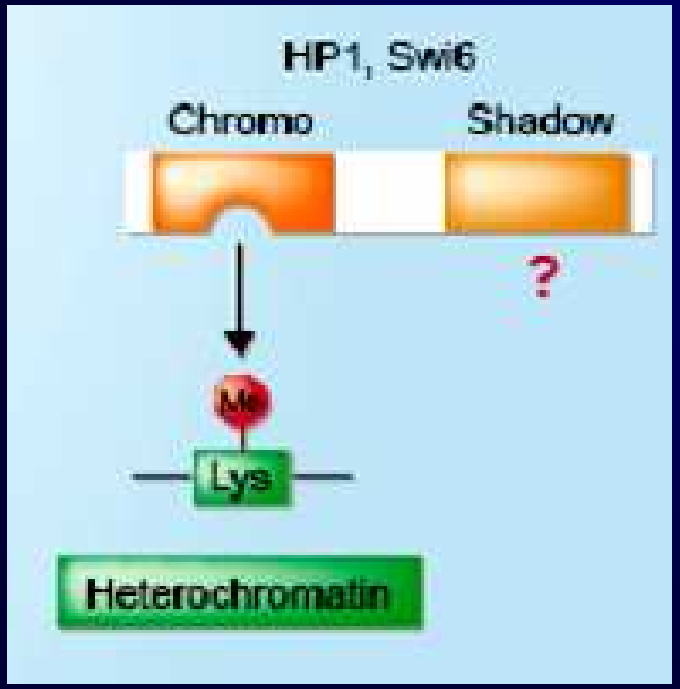
1. Heterochromatin protein (dHP1) was first identified in *Drosophila* and shown to localise to heterochromatin by antibody staining.
2. Mutation of HP1 gene decrease the effect of **PEV** (position effect variegation) on gene expression.
3. Null mutations of HP1 are lethal due to chromosome loss during cell division.
4. Homologous protein to HP1 are these of Polycomb group (Pc). Both Pc and HP1 share a common amino acid sequence of the chromodomain (chromatin modification) which is thought to mediate protein/protein interactions. This domain is highly conserved from yeast to man.
5. Three genes for mammalian HP1 have been identified: α, β, and γ.
6. To date only a and g HP1 proteins have been identified in *Xenopus laevis*. We want to determine the role of HP1 proteins in *Xenopus* development.


Interphase
centromeric
and other
heterochromatin
PML
nuclear
body
HP1
CD
IVR
CSD
Metaphase
Centromere

HPs se skládají z vysoce konzervativních oblastí:

a) N-terminální chromodomény (CD)

b) strukturálně odvozené C-terminální chromo-shadow domény (CSD)



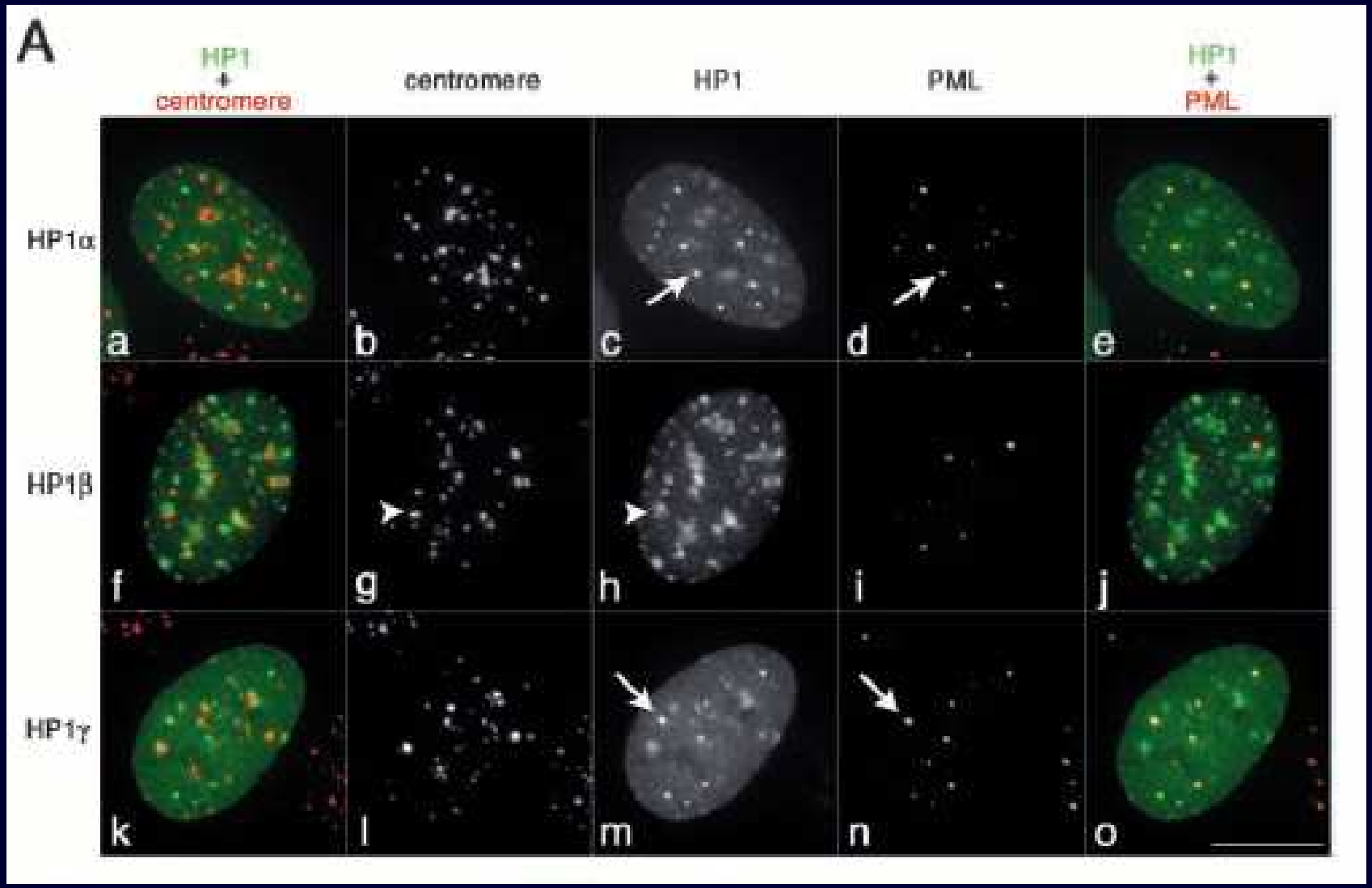
FUNKCE HPs

a) **Uspořádání chromatinu**

b) **Regulace transkripce**

c) **Optimální regulace délky telomer a zprostředkování procesu telomeric silencing**

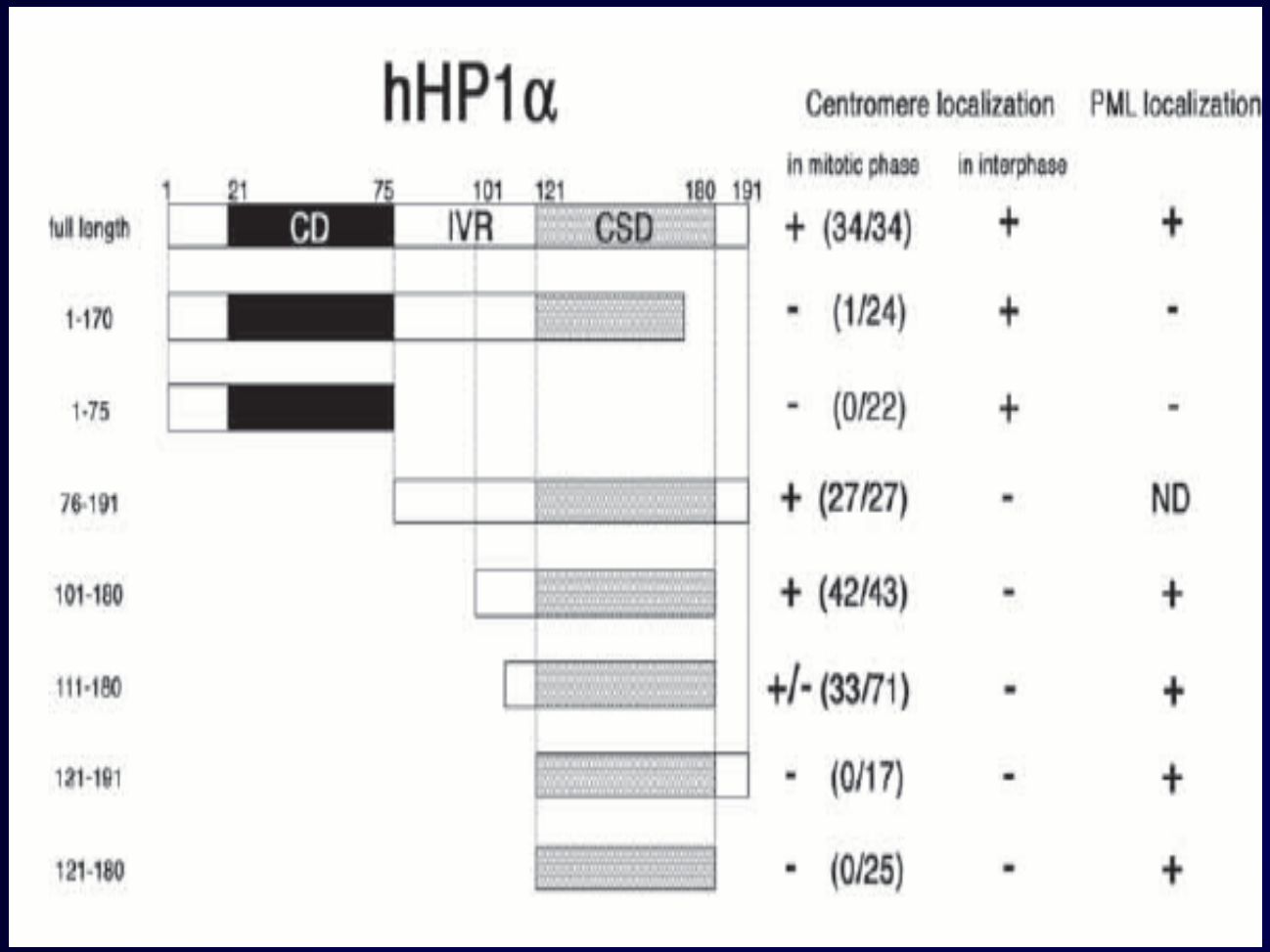
HP1 proteiny – v lidských buňkách jsou 3 sub-typy

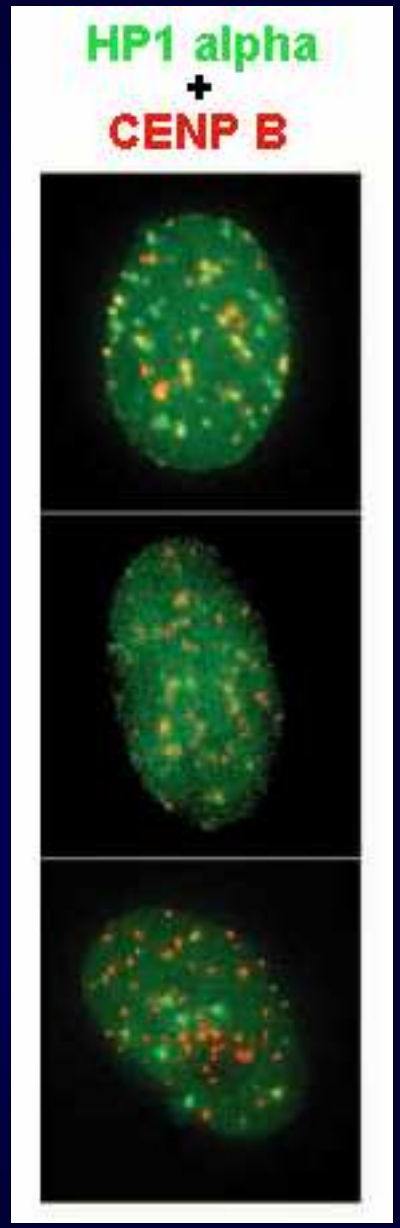


Hayakawa et al., 2003

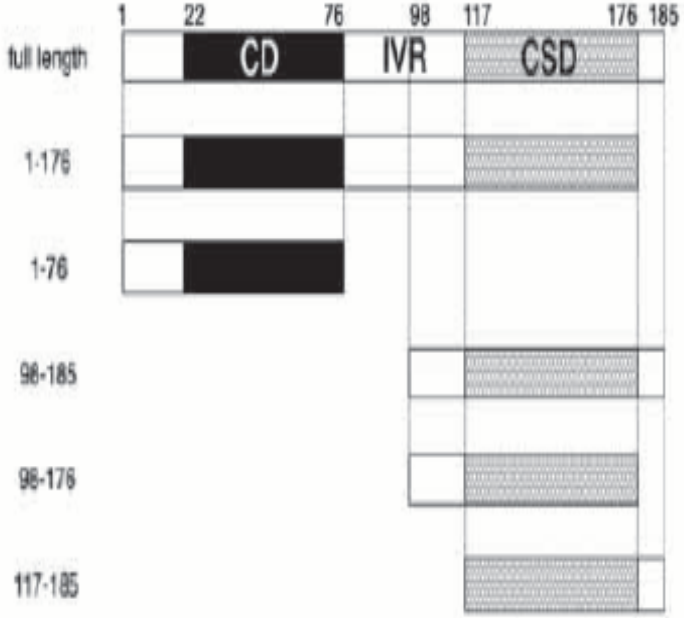
Savčí jádra obsahují 10-30 sférických struktur zvaných **PML bodies** (PODs, ND10 nebo Kremer bodies). Gen kódující PML je fúzován s genem kódujícím receptor pro kyselinu retinouvou a to u akutní promyelocytické leukemie (APL), Jde o translokaci t(15;17). PML bodies jsou cílem mnoha virů při časné infekci, jsou místem iniciace transkripce u virů.

PML bodies interagují s mnoha proteiny podobnými HDAC, které se však neshromažďují v PML bodies. PML jsou zahrnuty v řadě procesů jako je buněčný růst, apoptóza, imunitní odpovědi a regulace transkripce. PML jsou také místem degradace některých proteinů, asociují nejen s HP1, ale i se specifickými geny jako je p53 a jeho protein TP53. PML NBs obsahují nově syntetizovanou RNA, výsledkem je významná úloha PML bodies v regulaci genové exprese.

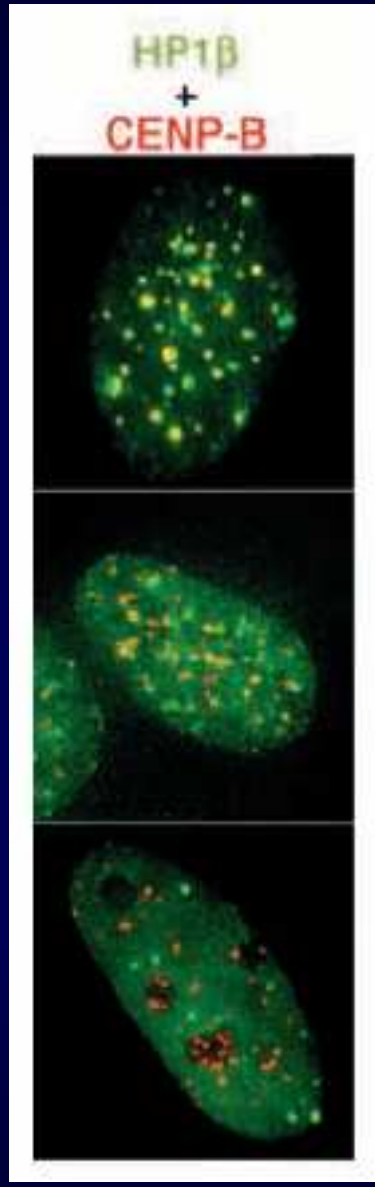

hHP1α
Centromere localization
PML localization
in mitotic phase
in interphase
1
21
75
101
121
180
191
CD
IVR
CSD
full length
+ (34/34)
+
+
1-170
- (1/24)
+
-
1-75
- (0/22)
+
-
76-191
+ (27/27)
-
ND
101-180
+ (42/43)
-
+
111-180
+/- (33/71)
-
+
121-191
- (0/17)
-
+
121-180
- (0/25)
-
+

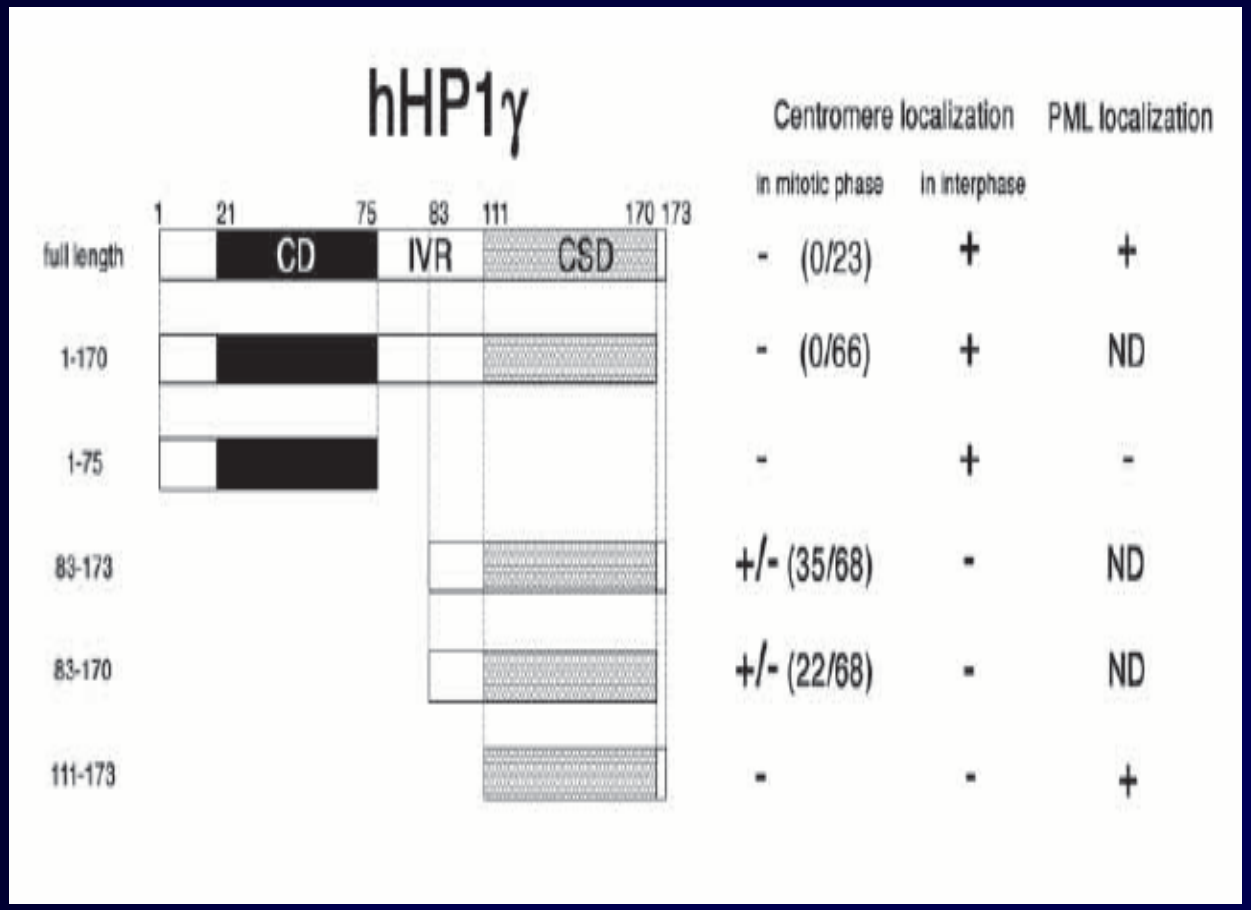

HP1 alpha
+
CENP B

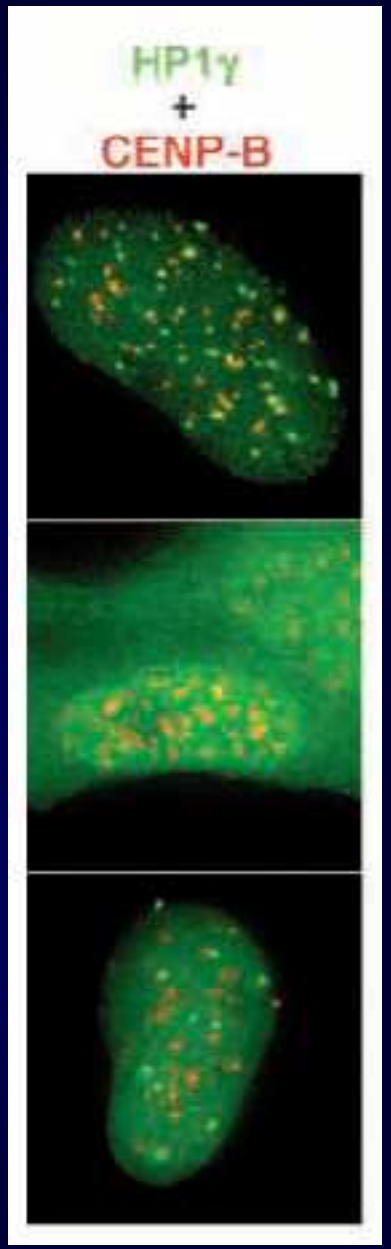
hHP1β

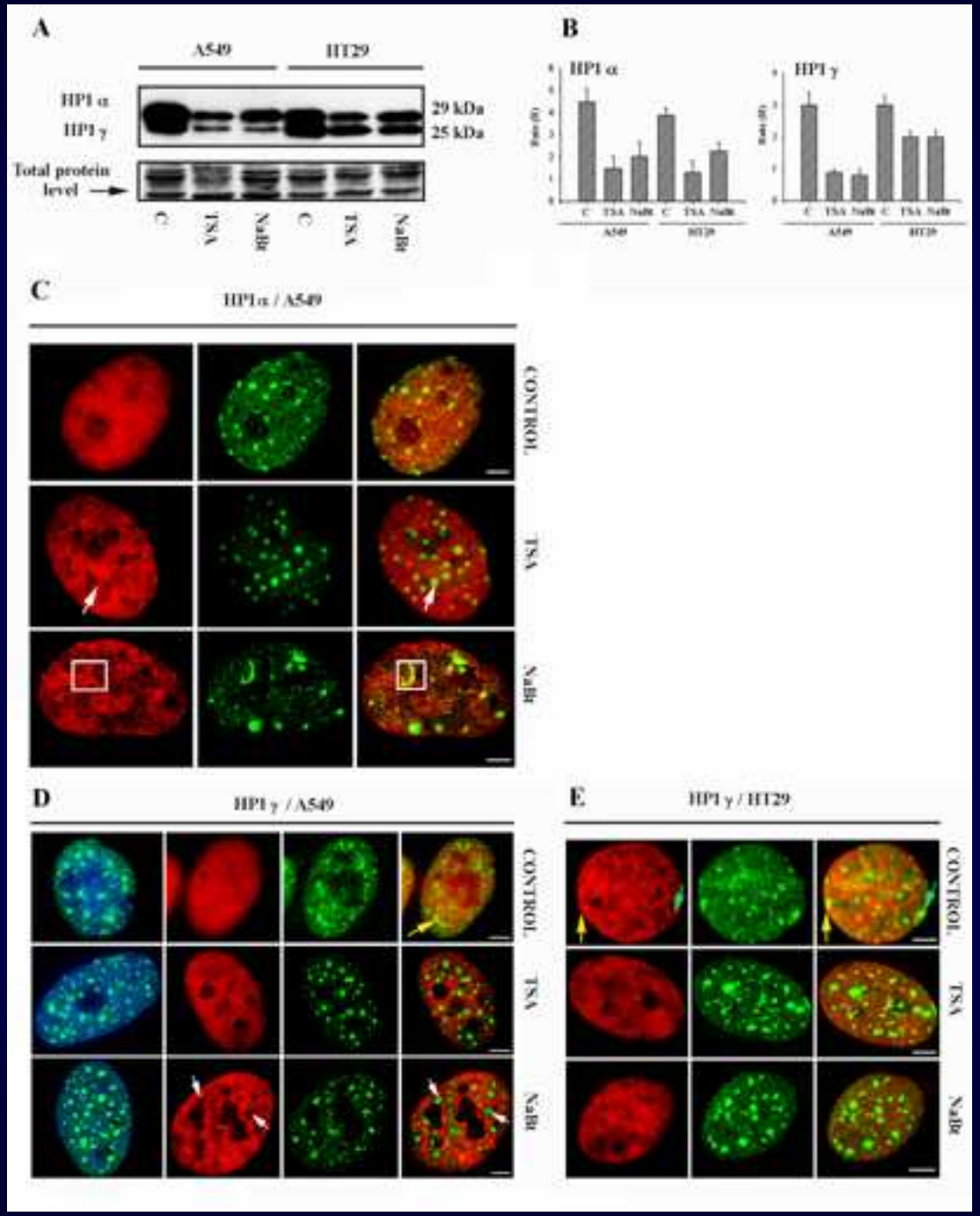


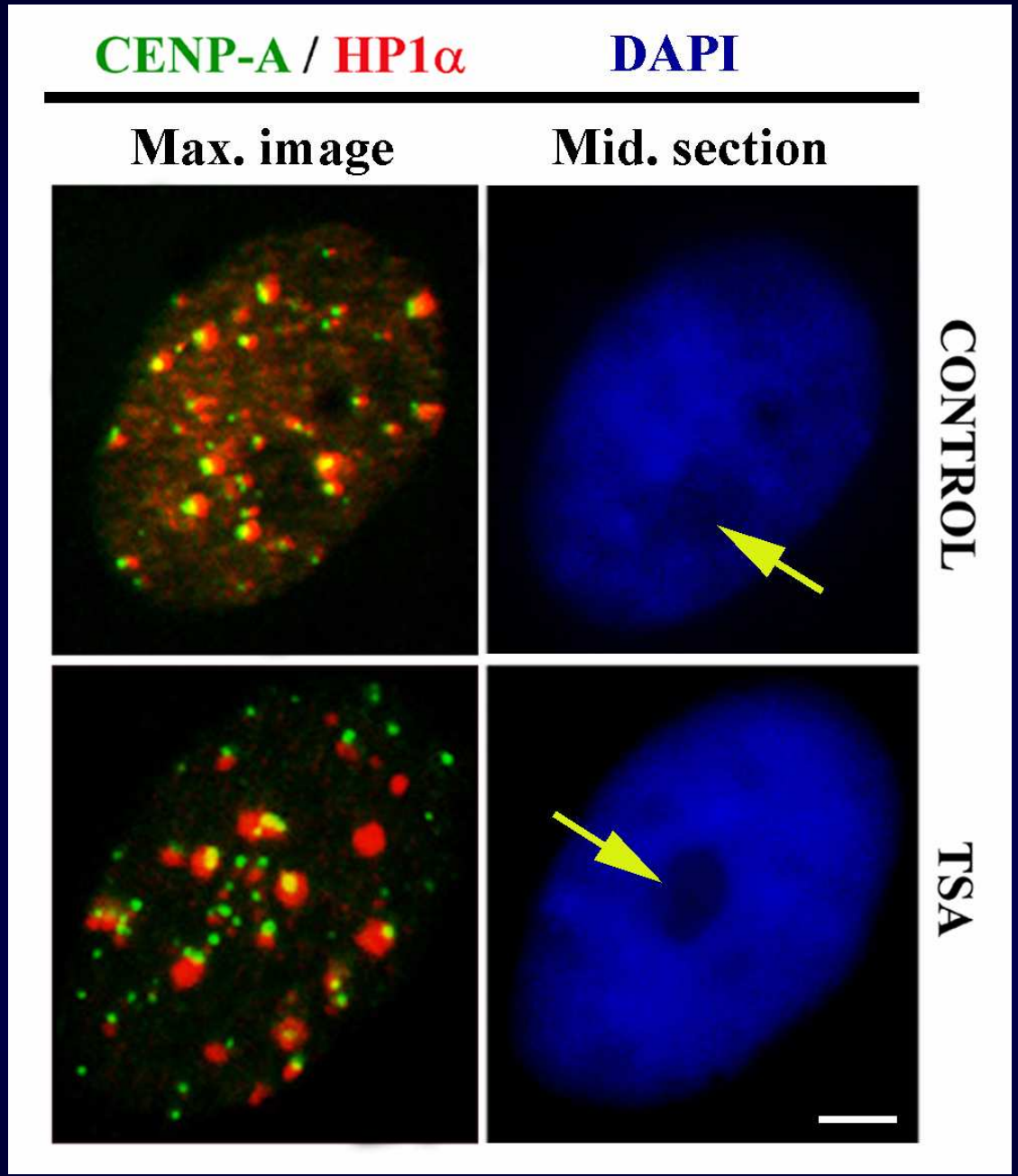
	Centromere localization		PML localization
	in mitotic phase	in interphase	
full length	- (0/23)	+	+
1-176	- (9/64)	+	ND
1-76	-	+	-
98-185	+/- (32/67)	-	ND
98-176	+/- (42/69)	-	ND
117-185	-	-	+

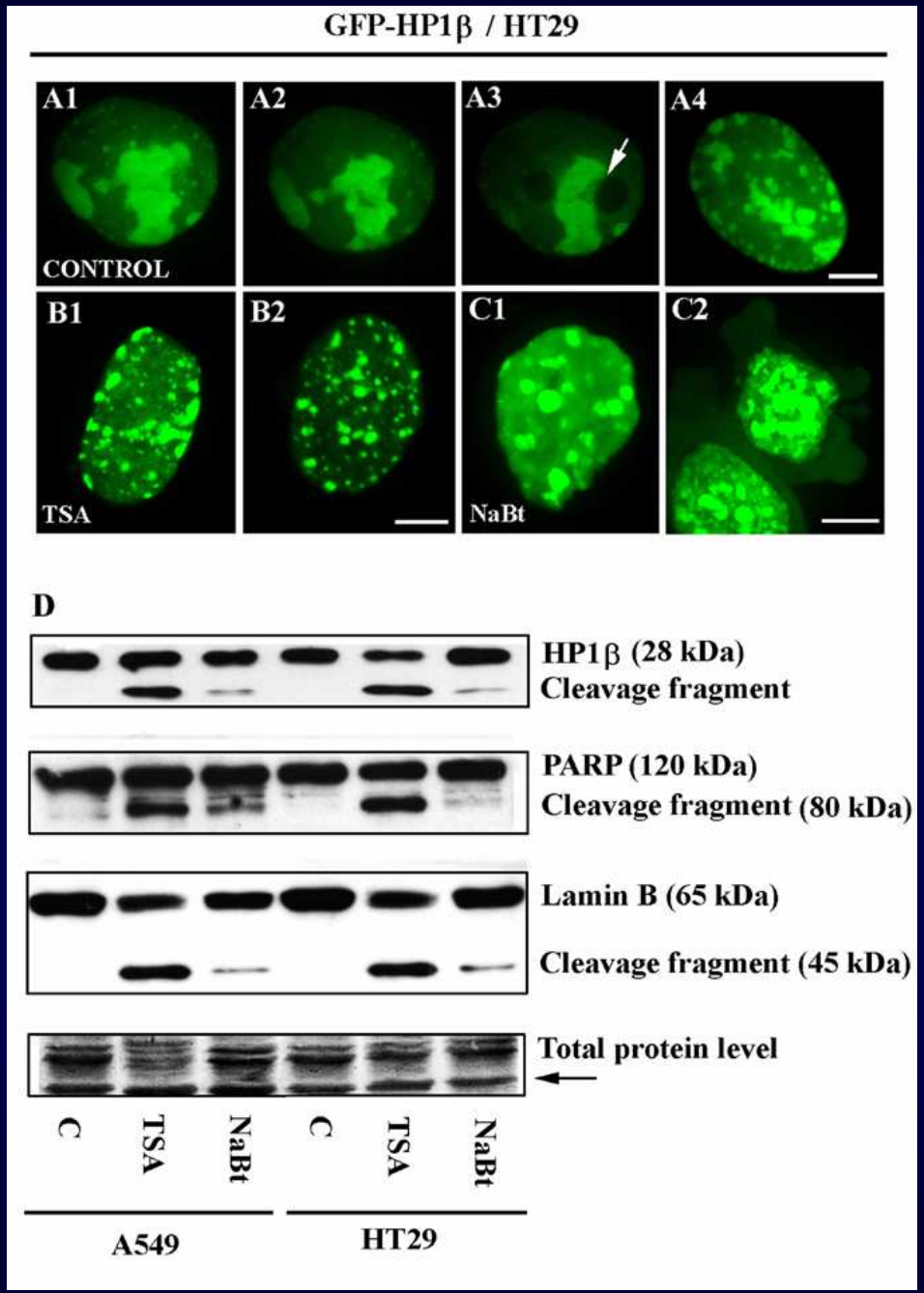


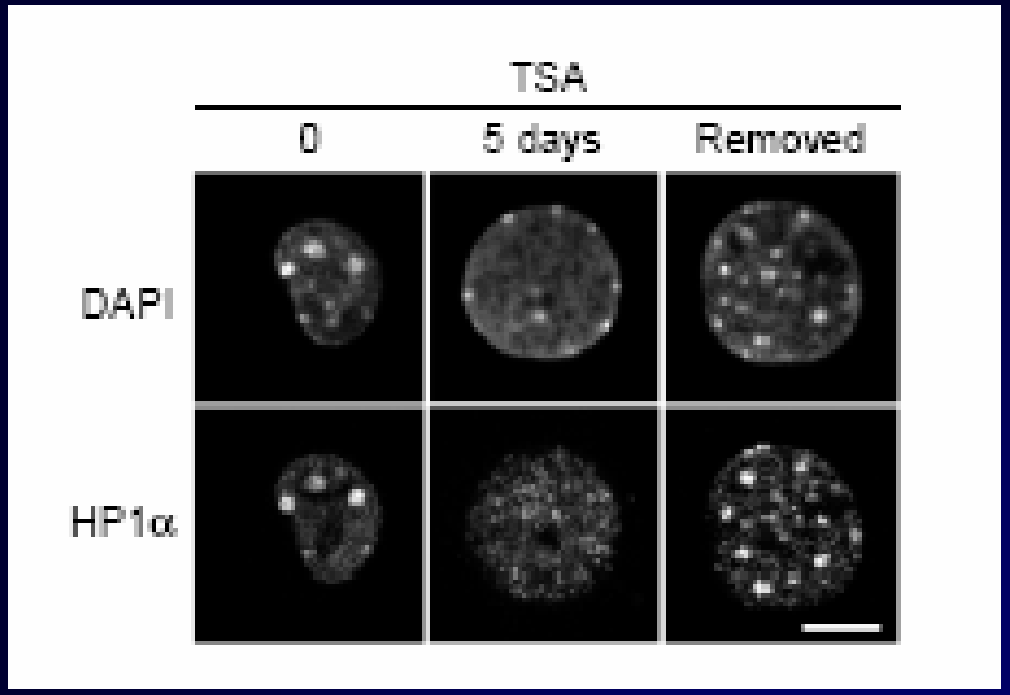

hHP1γ
1
21
75
83
111
170 173
CD
IVR
CSD
full length
1-170
1-75
83-173
83-170
111-173
Centromere localization
PML localization
in mitotic phase
in interphase
- (0/23)
+
+
- (0/66)
+
ND
-
+
-
+/- (35/68)
-
ND
+/- (22/68)
-
ND
-
-
+


HP1γ
+
CENP-B

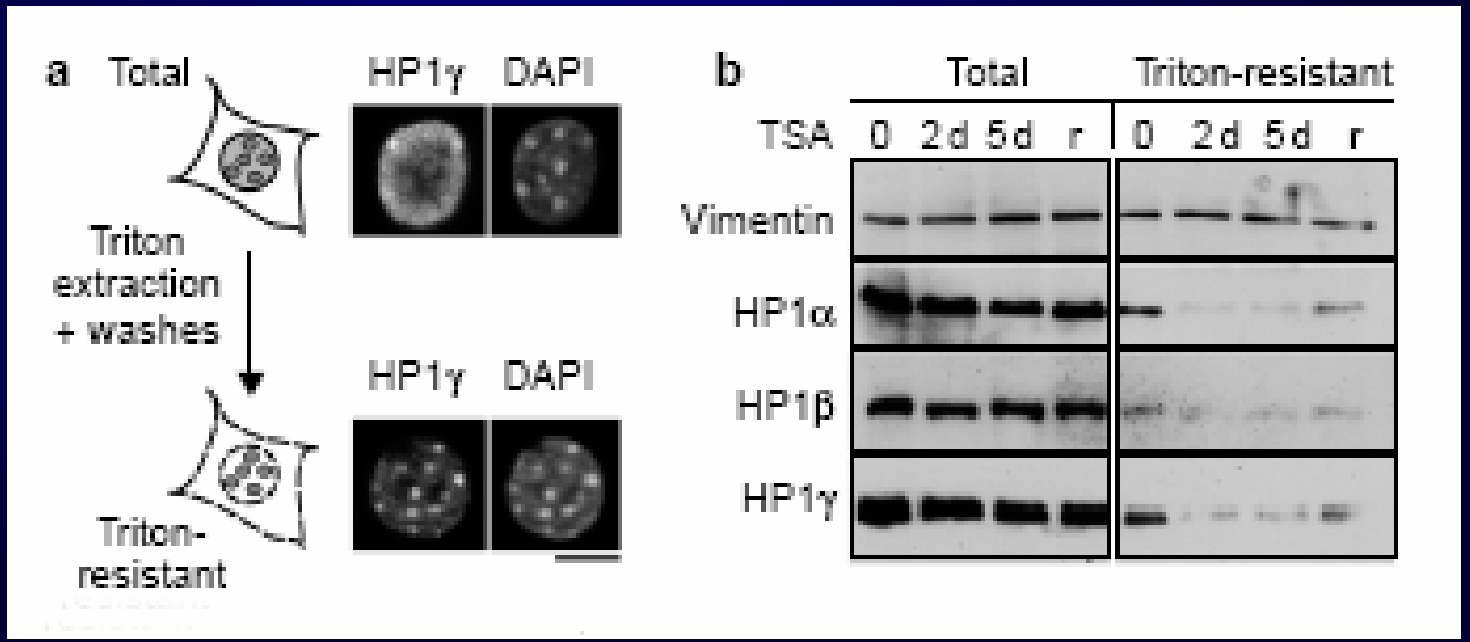

A
A549
HT29
HP1 α
HP1 γ
29 kDa
25 kDa
Total protein level
C
TSA
NaBt
B
HP1 α
HP1 γ
C
TSA
NaBt
A549
HT29
C
HP1α / A549
CONTROL
TSA
NaBt
D
HP1 γ / A549
CONTROL
TSA
NaBt
E
HP1 γ / HT29
CONTROL
TSA
NaBt


CENP-A / HP1α
DAPI
Max. image
Mid. section
CONTROL
TSA

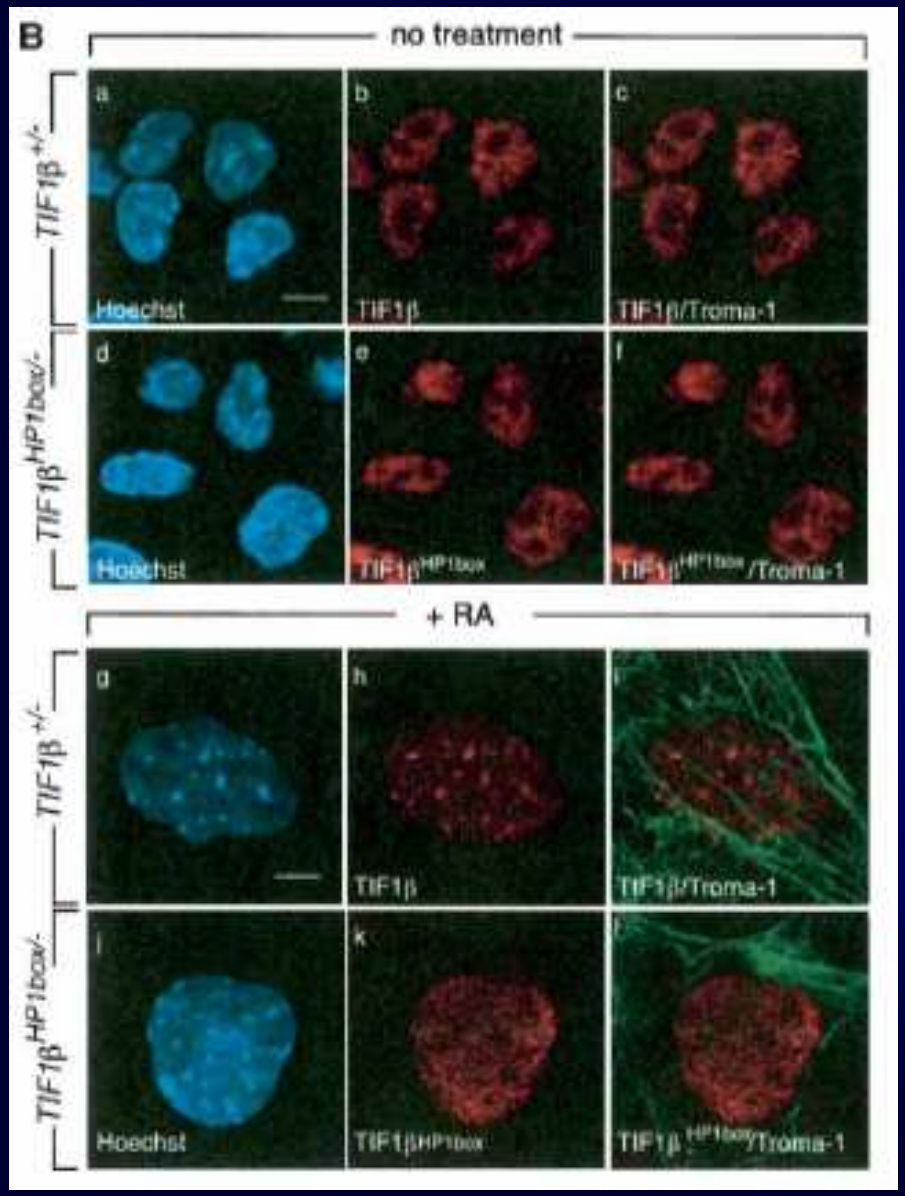

GFP-HP1β / HT29
A1
A2
A3
A4
CONTROL
B1
B2
C1
C2
TSA
NaBt
D
HP1β (28 kDa)
Cleavage fragment
PARP (120 kDa)
Cleavage fragment (80 kDa)
Lamin B (65 kDa)
Cleavage fragment (45 kDa)
Total protein level
C
TSA
NaBt
C
TSA
NaBt
A549
HT29

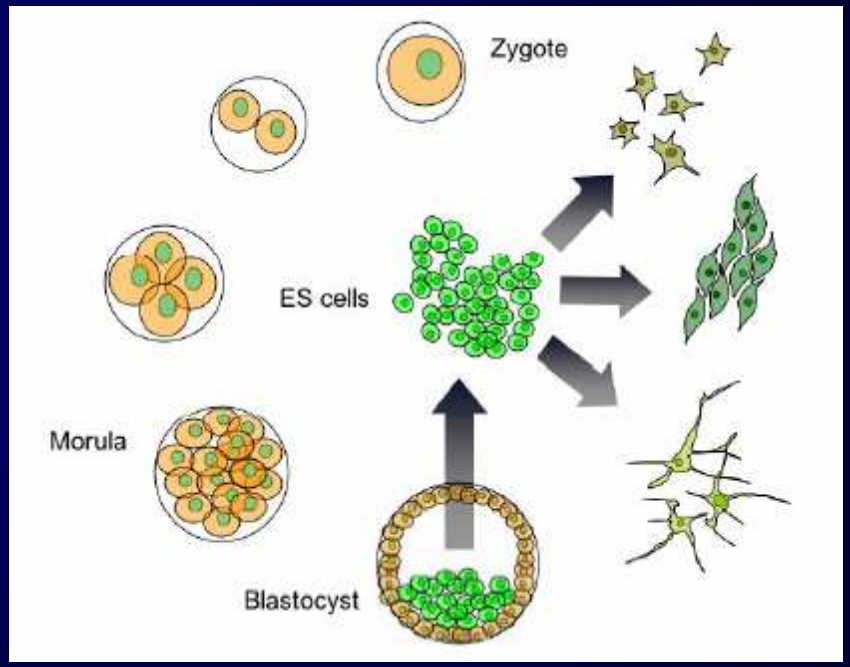


Taddei et al., 2001

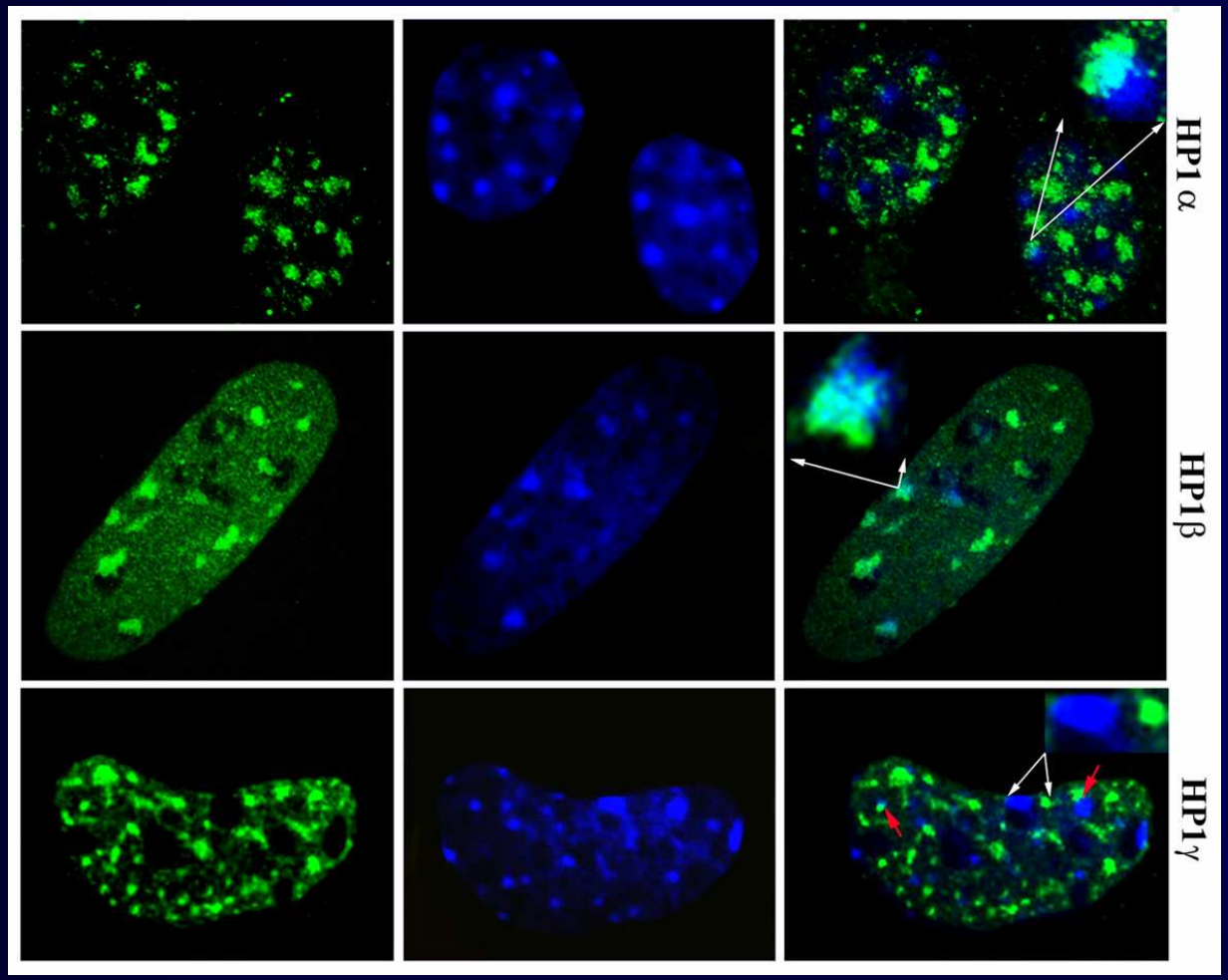


TIF1 beta and chromocentres and HP1 protein

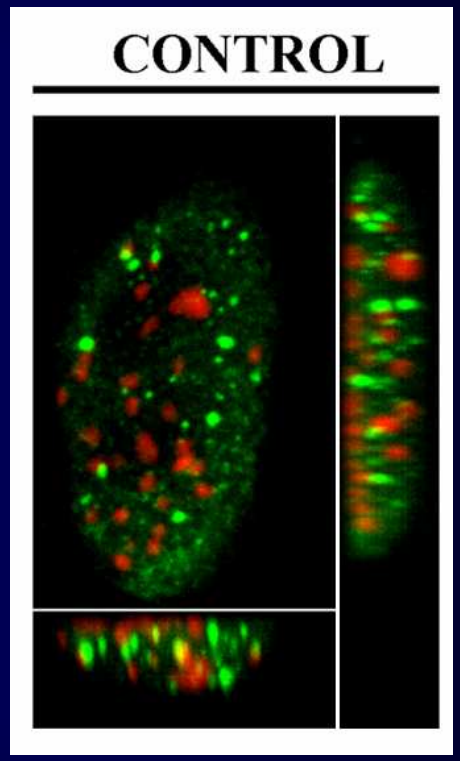


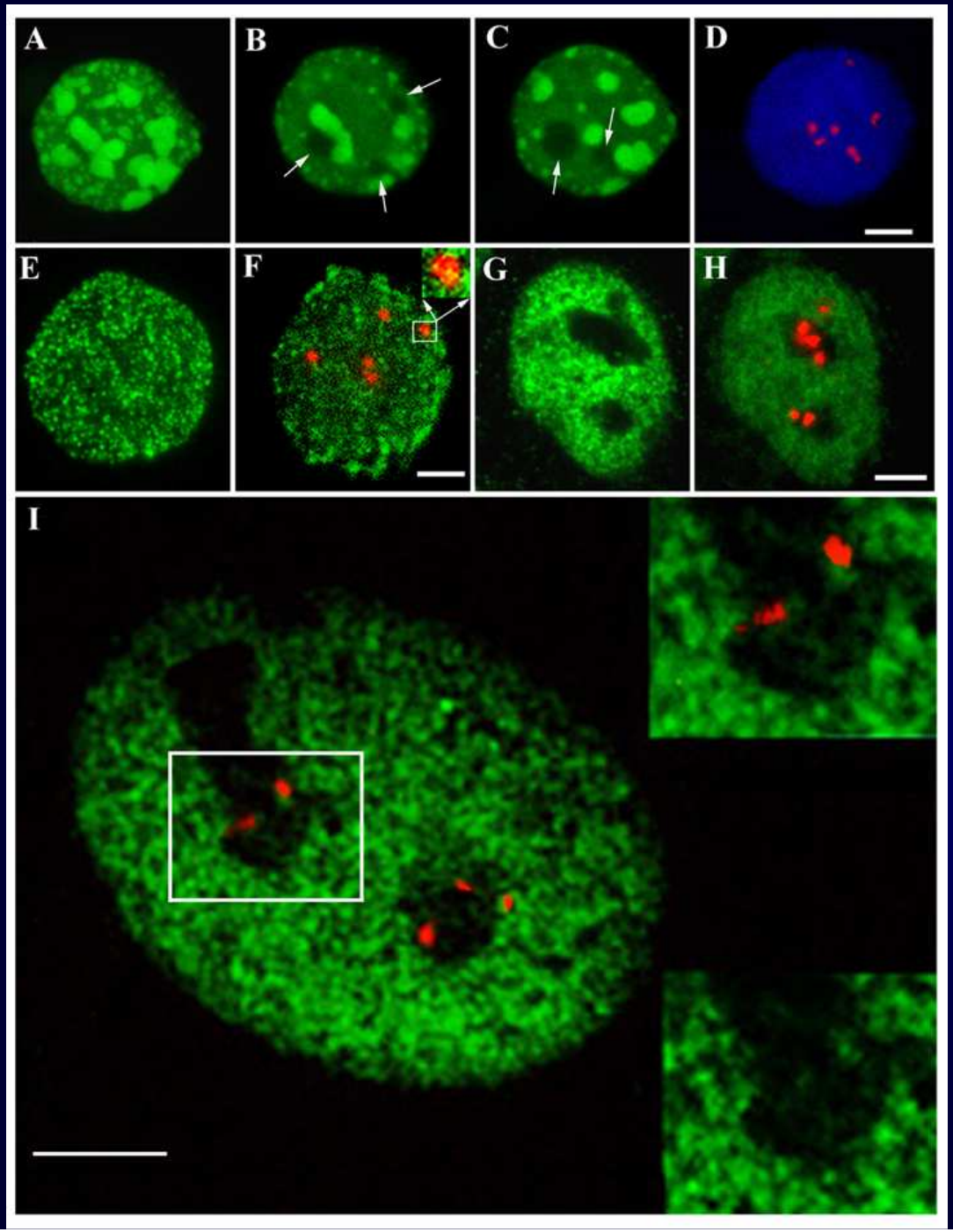


Neuronal cell differentiation of EC cells - HP1 proteins

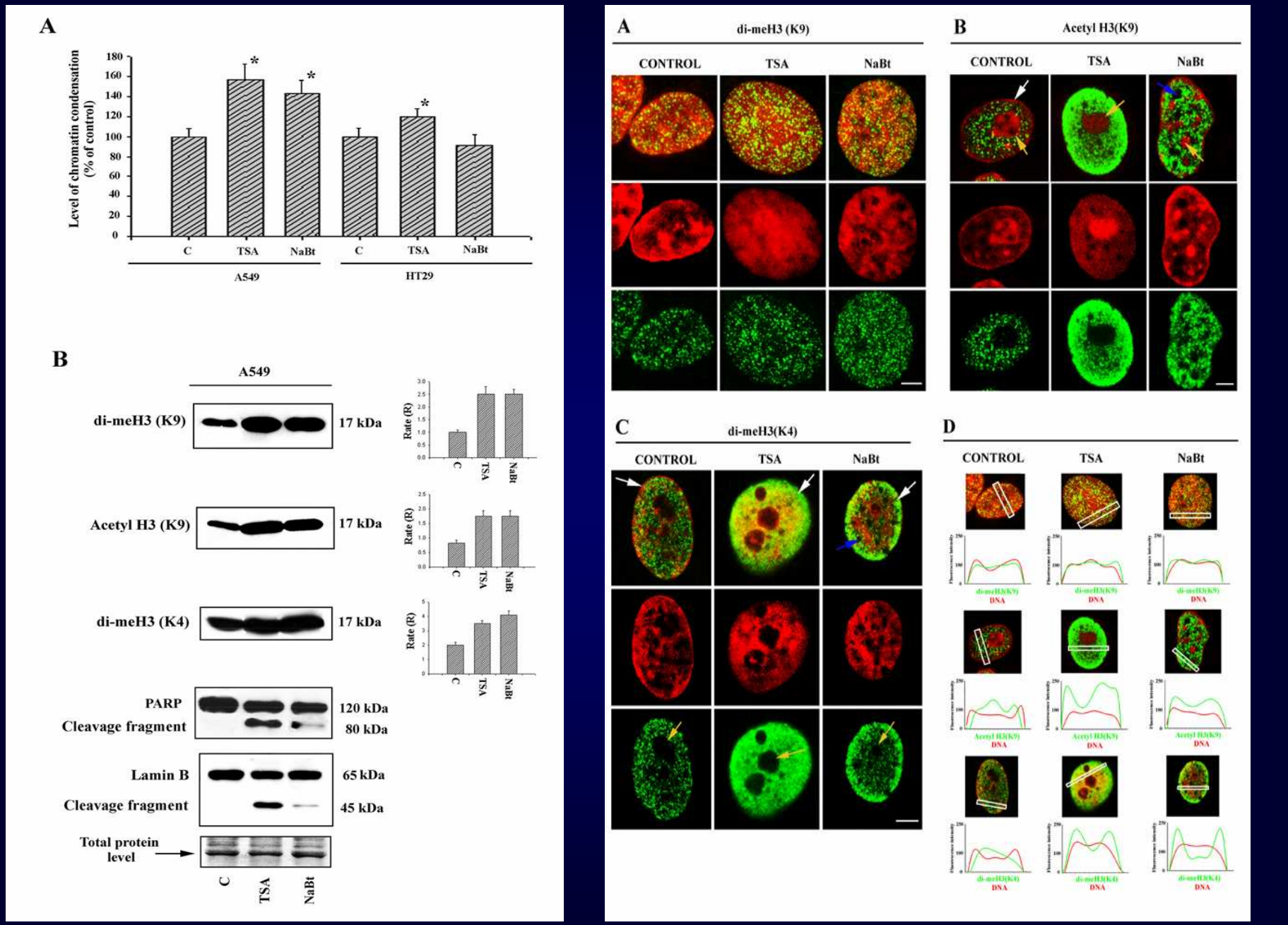


HP1α HP1β

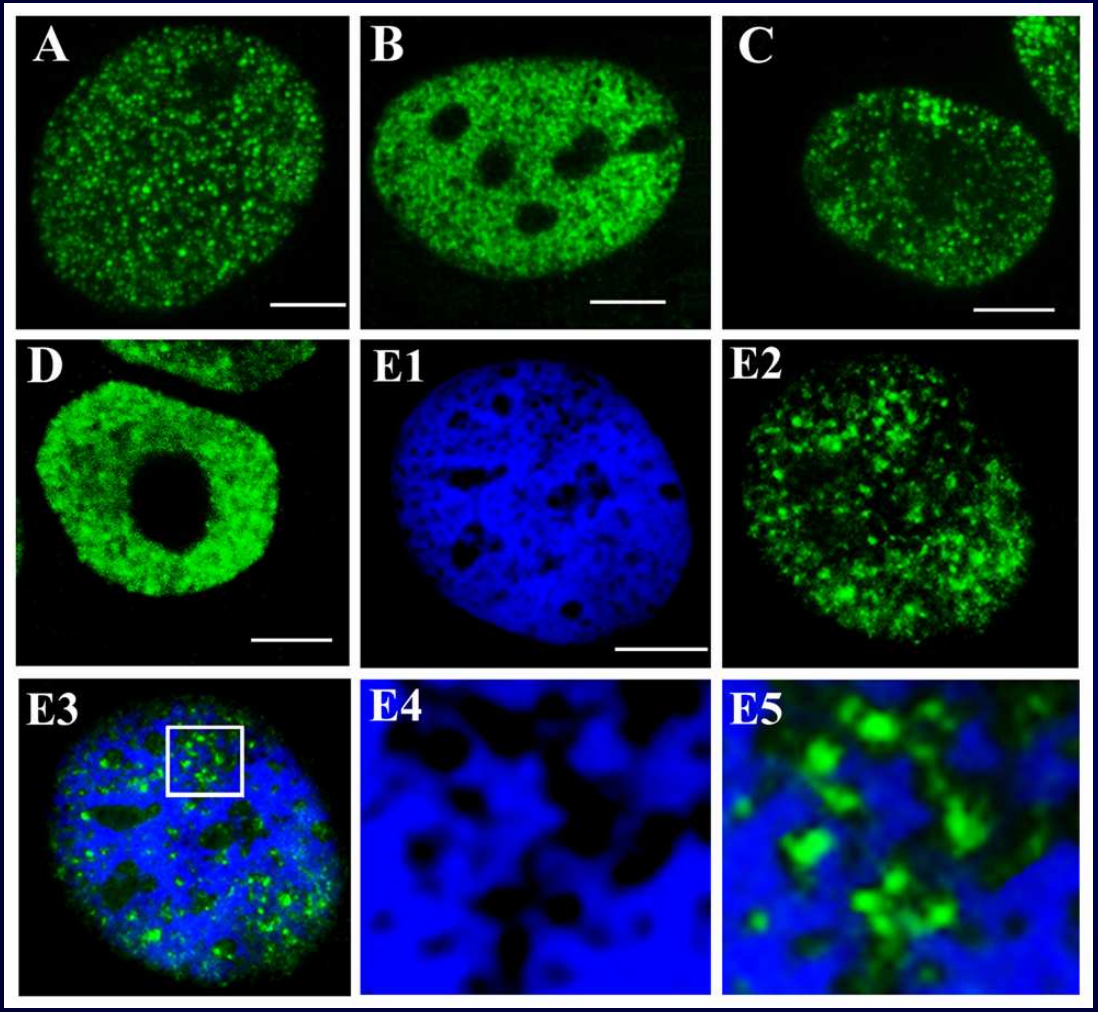




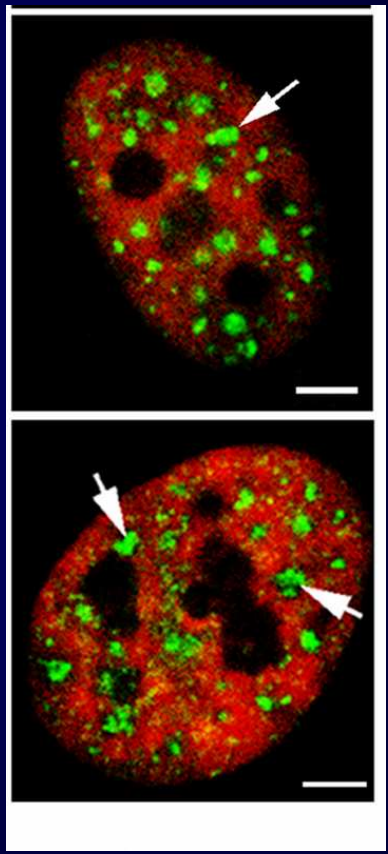
Cen 14/22


A
Level of chromatin condensation (% of control)
180
160
140
120
100
80
60
40
20
0
C
TSA
NaBt
C
TSA
NaBt
A549
HT29
B
A549
di-meH3 (K9)
17 kDa
Acetyl H3 (K9)
17 kDa
di-meH3 (K4)
17 kDa
PARP
Cleavage fragment
120 kDa
80 kDa
Lamin B
Cleavage fragment
65 kDa
45 kDa
Total protein level
Rate (R)
C
TSA
NaBt
A
di-meH3 (K9)
CONTROL
TSA
NaBt
B
Acetyl H3(K9)
CONTROL
TSA
NaBt
C
di-meH3(K4)
CONTROL
TSA
NaBt
D
CONTROL
TSA
NaBt
di-meH3(K9)
DNA
Acetyl H3(K9)
DNA
di-meH3(K4)
DNA

H3(K4) di-methylation and IC spaces



HP1



Shrnutí problematiky

1. Organizace chromatinu, struktura nukleosomů
2. Varianty histonů
3. Epigenetické modifikace histonů a jejich funkce (obecně)
4. Epigenetické modifikace centromer, Xi a telomer
5. HP1 proteiny – struktura a funkce
6. Účinky HDACi
7. Methylace DNA versus methylace histonů