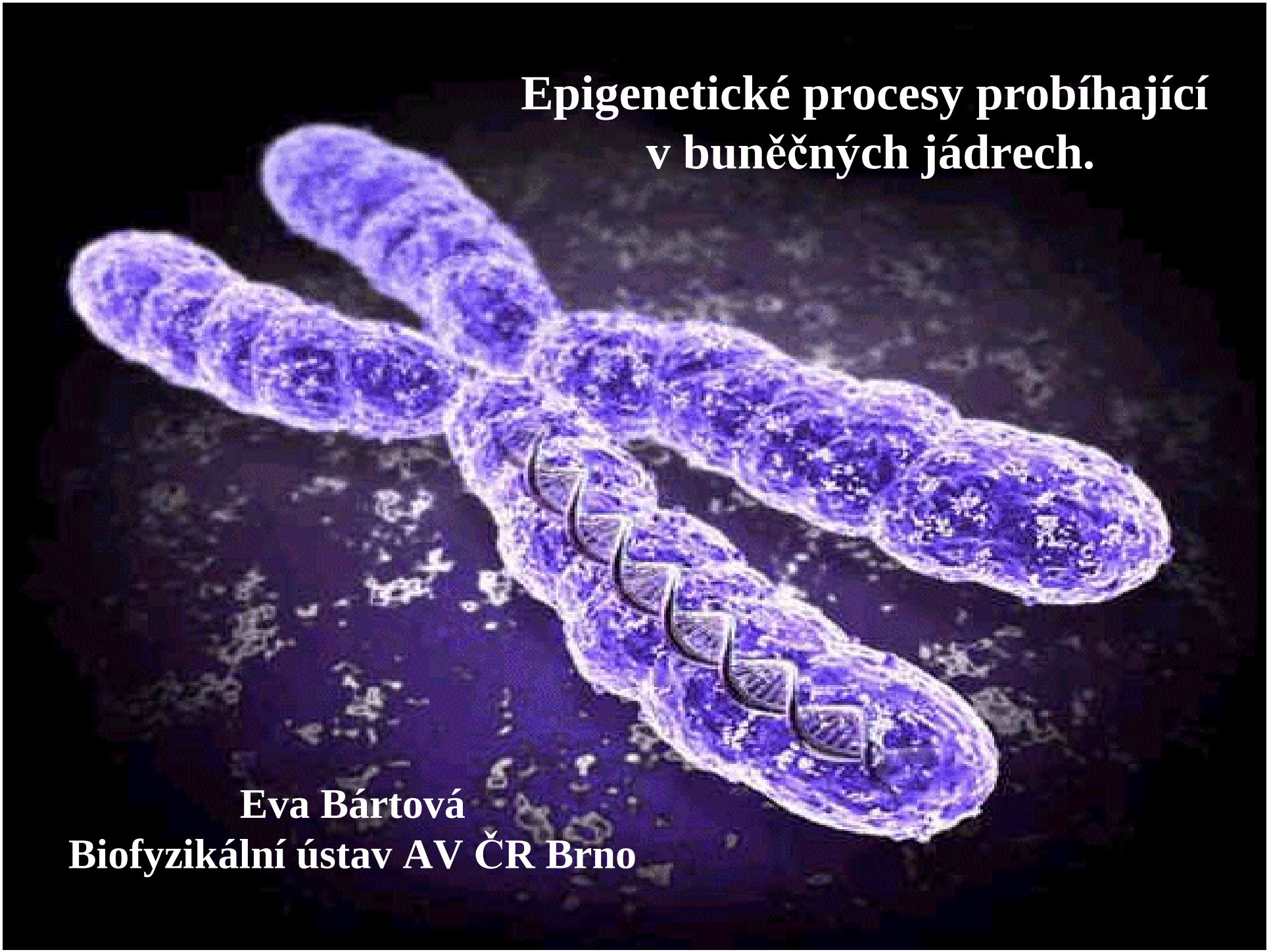
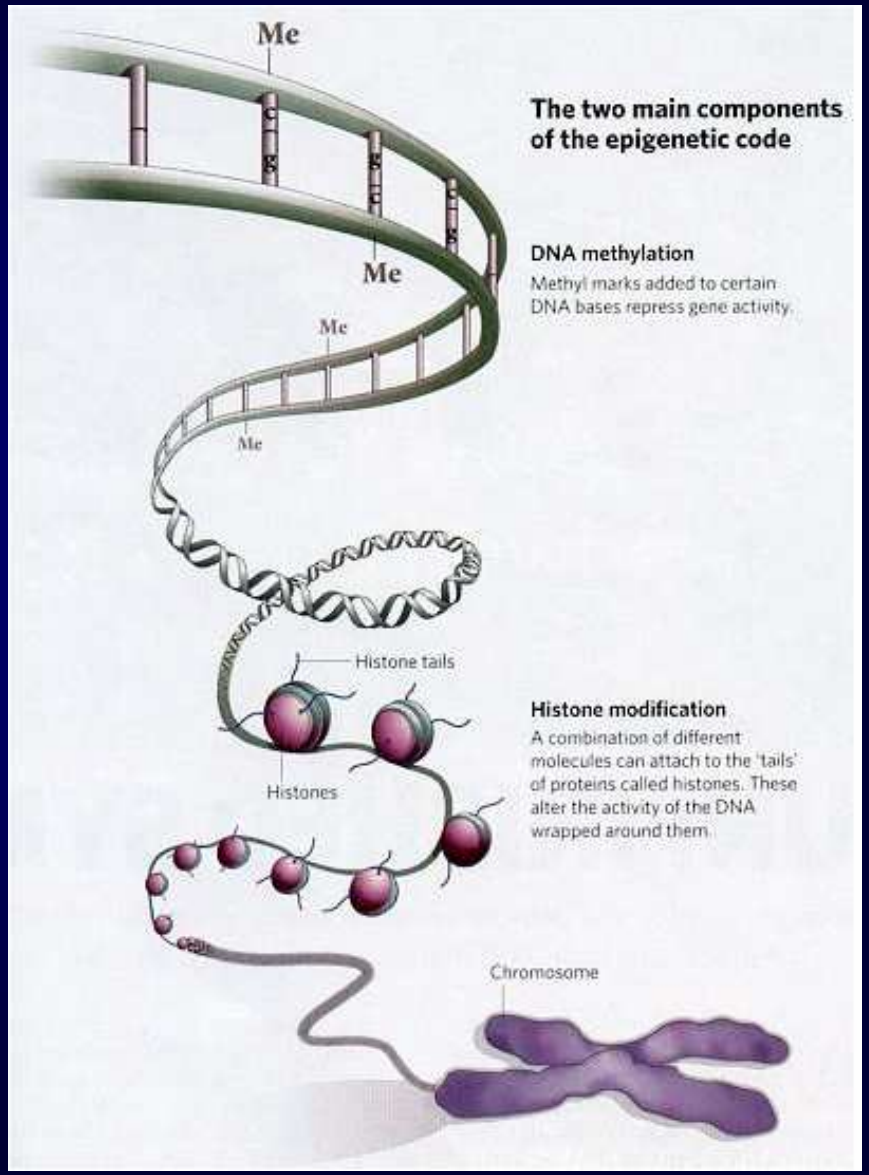
Epigenetické procesy probíhající v buněčných jádrech.

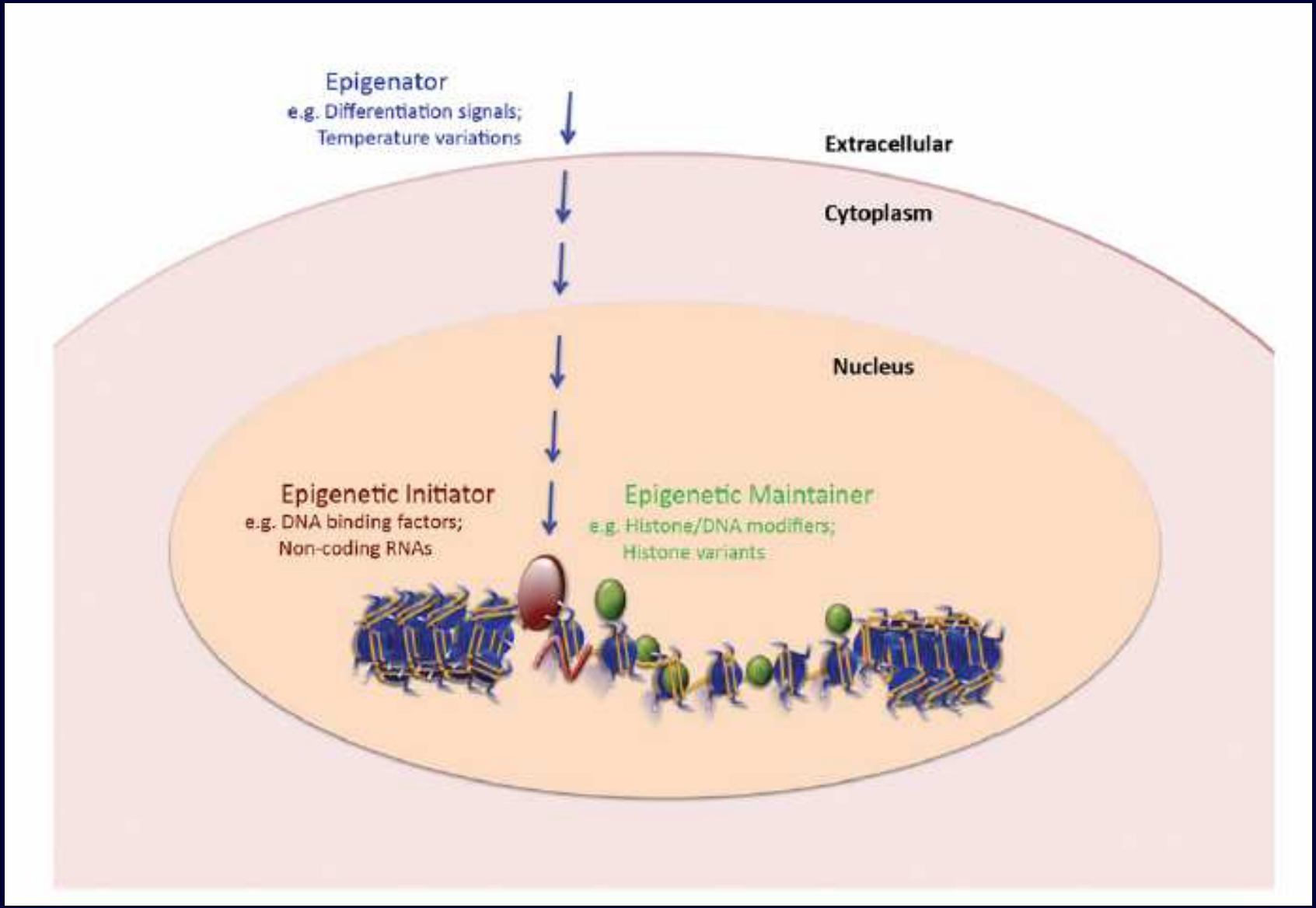


Eva Bártová
Biofyzikální ústav AV ČR Brno

What is epigenetics ?

Epigenetics refers to heritable changes in the phenotype that occur irrespective of alterations in the DNA sequences.





Berger et al., Genes Dev., 2009

Epigenator:

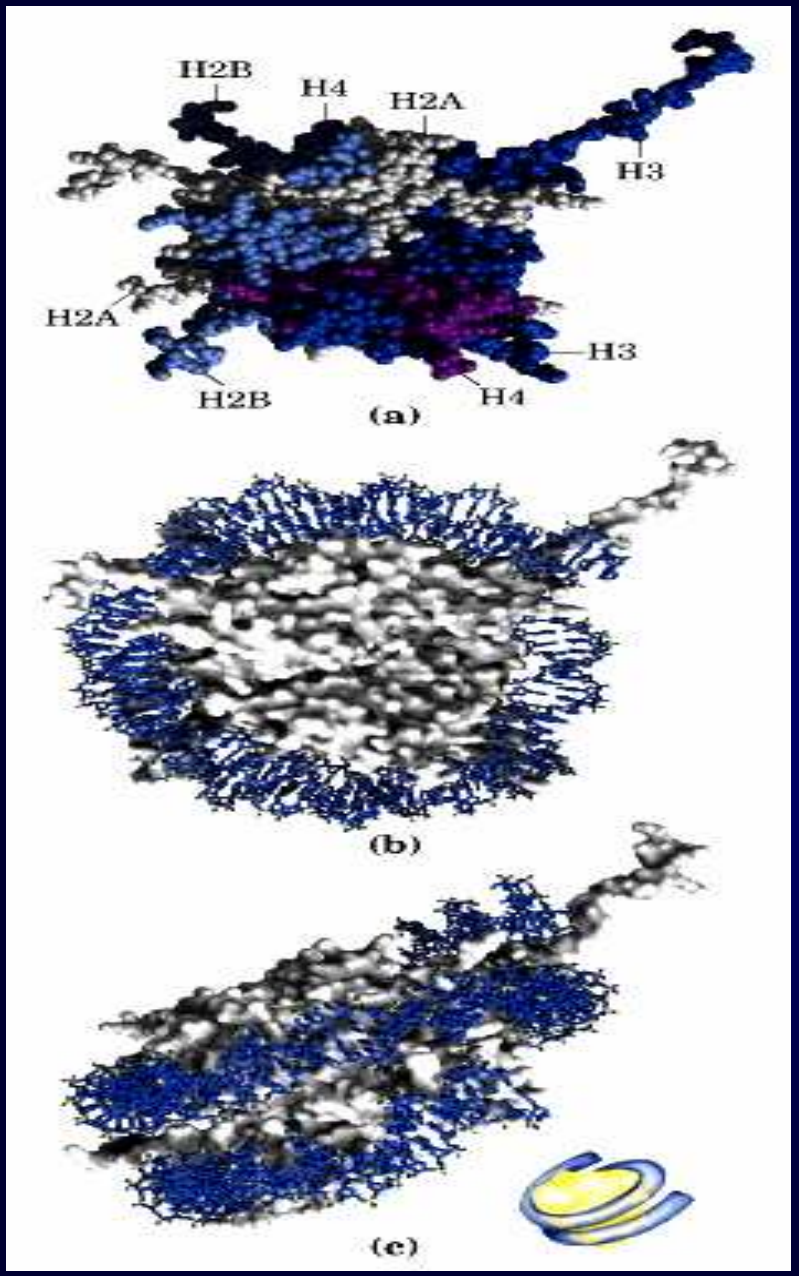
emanates from the environment and triggers and intracellular pathway. Epigenetic signaling pathway could be a protein-protein interaction or modification-based events.

Epigenetic initiator:

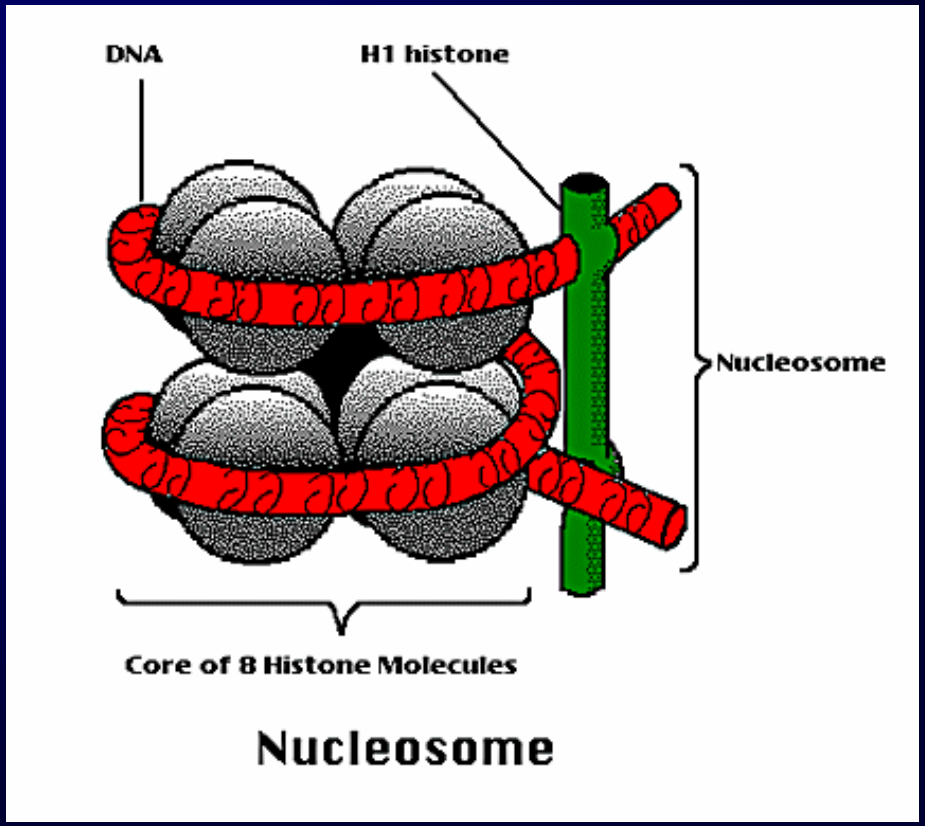
signal, which responds to the Epigenator and is necessary to define the precise location of epigenetic chromatin environment. Initiator could be DNA-binding protein, non-coding RNA, factor that coordinates chromatin structure.

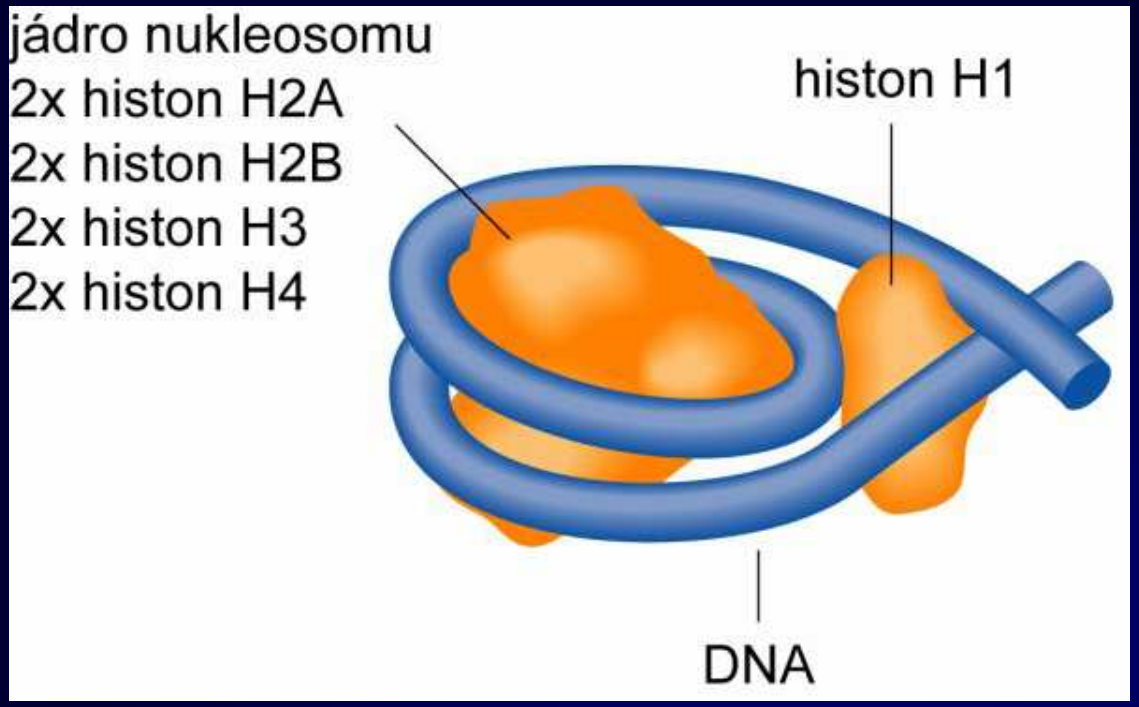
Epigenetic Maintainer:

signal, which sustained the chromatin environment in the first and subsequent generation. It is DNA methylation, histone modification, histone variants, nucleosome positioning. (Berger S. et al., 2009)



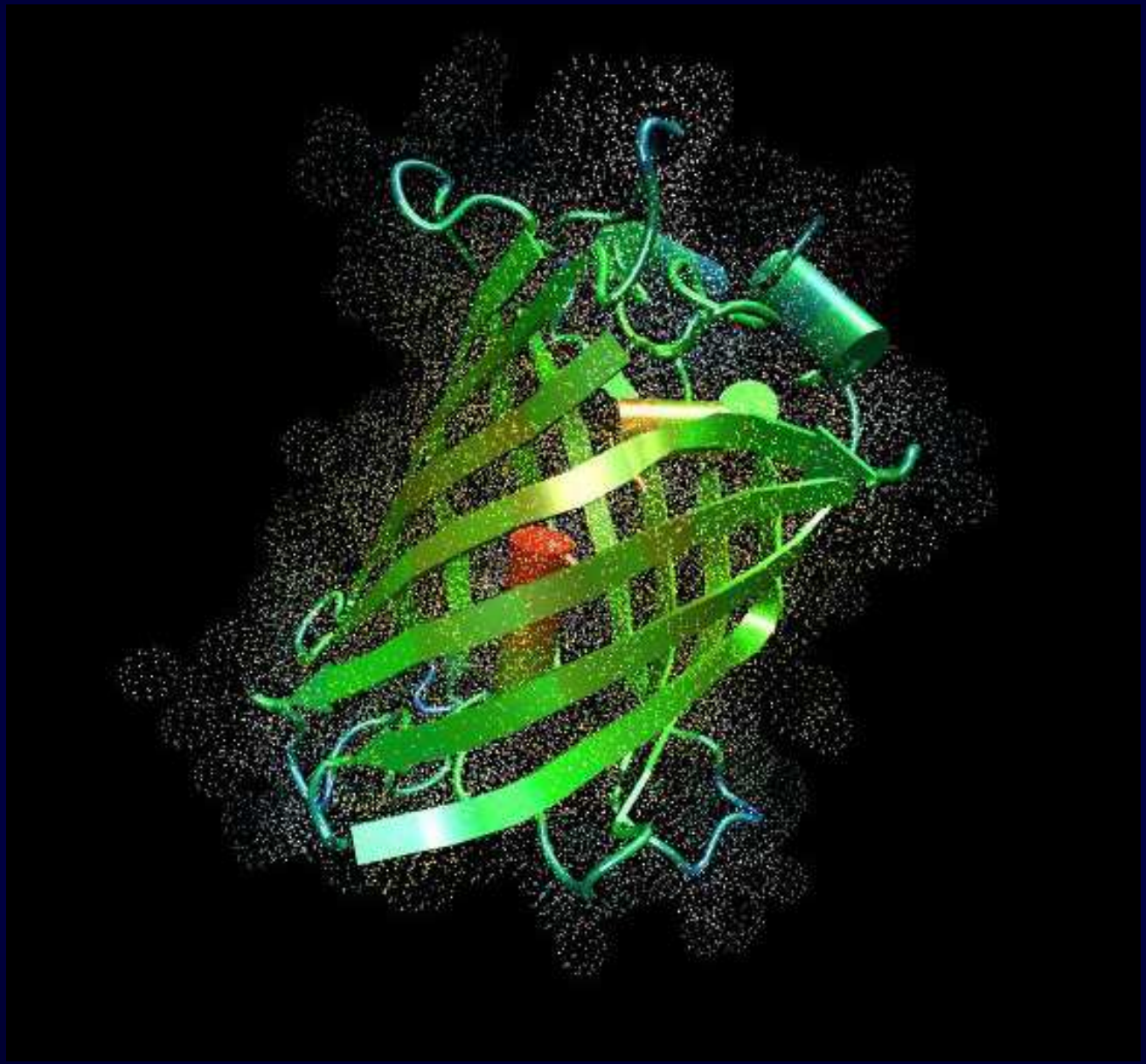
N-koncové oblasti histonů H2A, H2B H3a H4 (délka 16-44 aminokyselin nejsou součástí jádra nukleosomu, ale vybíhají do stran (volné konce). V linkerové oblasti – H1: funkce na kondenzaci chromatinu vyššího řádu.



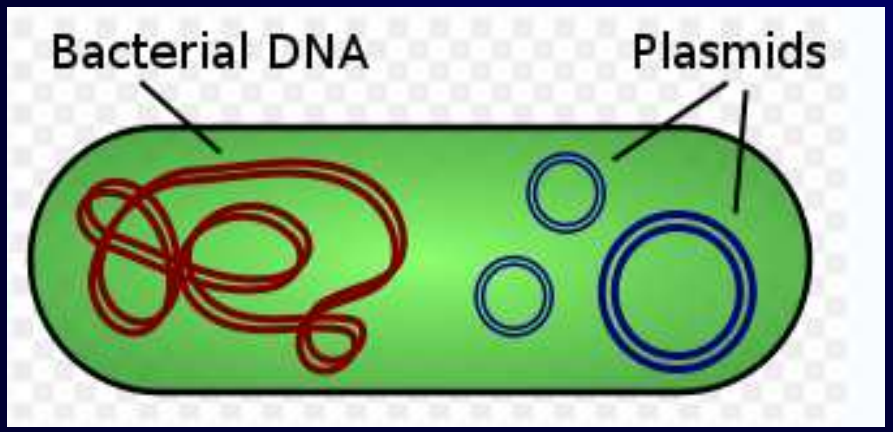


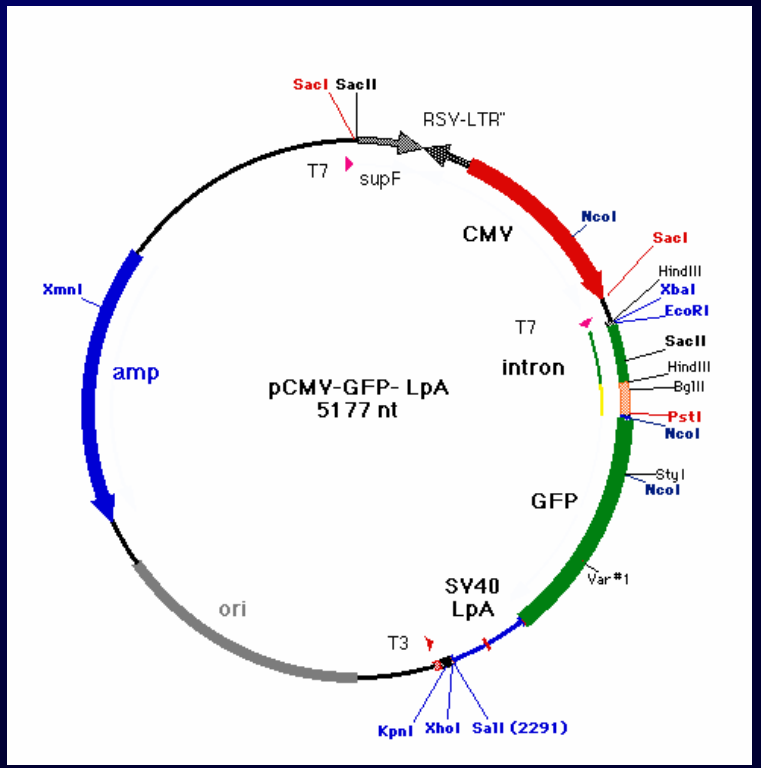
1. **Buňka může existovat i bez významně redukovaného množství H1.**
2. **H1 varianty nejsou hlavní determinanty buněčného fenotypu.**
3. **Funkce H1 variant je nejenom při utlumení transkripční aktivity ale také při její aktivaci (může snižovat nebo i zvyšovat expresi specifických genů.**
4. **H1 hraje 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**

GFP



Pacific jellyfish,
Aequorea victoria





A **plasmid** is an extra chromosomal DNA molecule separate from the chromosomal DNA which is capable of replicating independently from the chromosomal DNA.[1] In many cases, it is circular and double-stranded. Plasmids usually occur naturally in bacteria, but are sometimes found in eukaryotic organisms (e.g., the *2-micrometre-ring* in *Saccharomyces cerevisiae*).

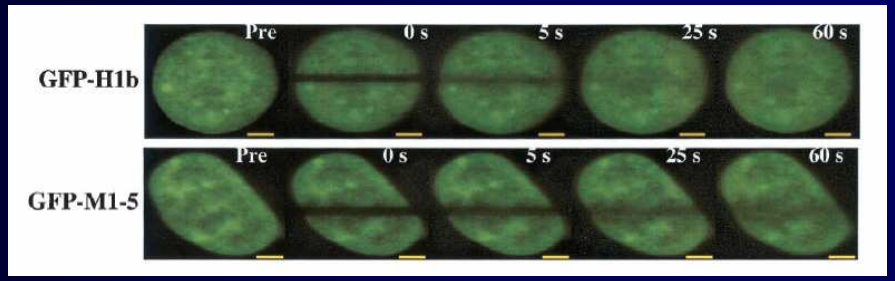
Plasmid size varies from 1 to over 1,000 kilobase pairs (kbp).[2][3][4] The number of identical plasmids within a single cell can range anywhere from one to even thousands under some circumstances. Plasmids can be considered to be part of the mobilome, since they are often associated with conjugation, a mechanism of horizontal gene transfer.

The term *plasmid* was first introduced by the American molecular biologist Joshua Lederberg in 1952.[5]

Plasmids are considered transferable genetic elements, or "replicons", capable of autonomous replication within a suitable host.

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.

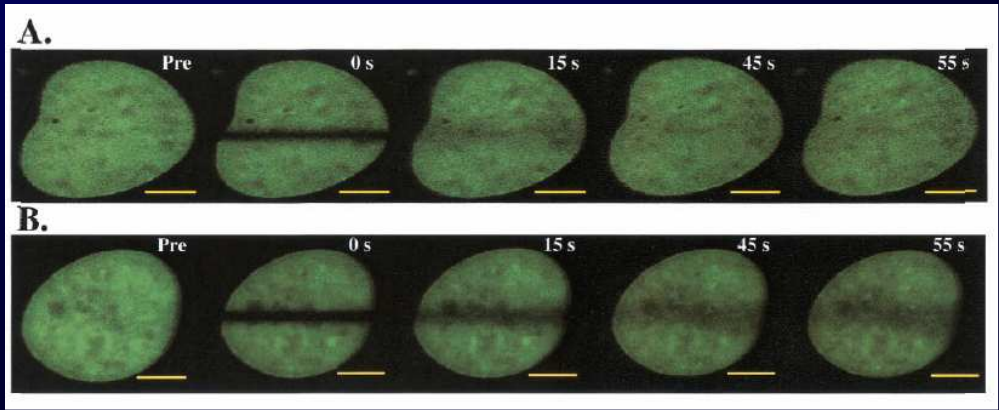


GFP-M1-5: five cyclin-dependent kinase phosphorylation consensus sites were mutated from serine or threonine residues into alanines

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.

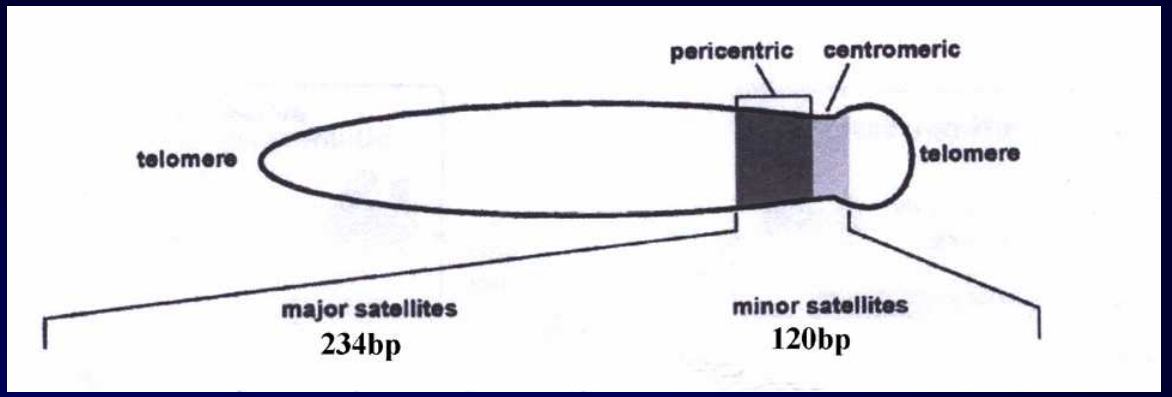
H2B: nemá varianty, uplatňuje se při regulaci kondenzace chromatinu, represi transkripce a během gametogeneze, H2B je zodpovědný za uspořádání chromatinu u spermií.

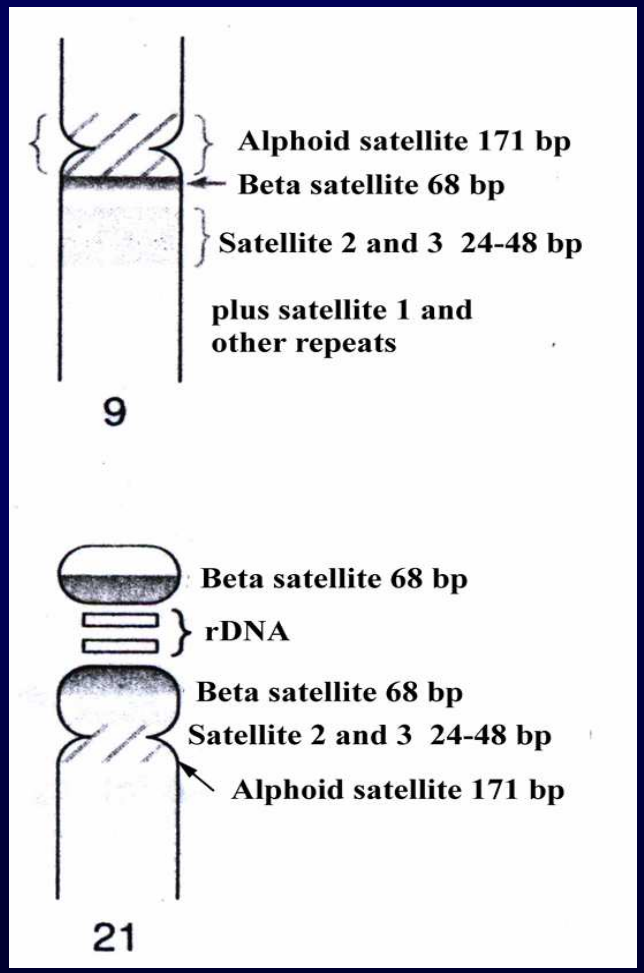
Varianty histonů

H3: existují dvě hlavní

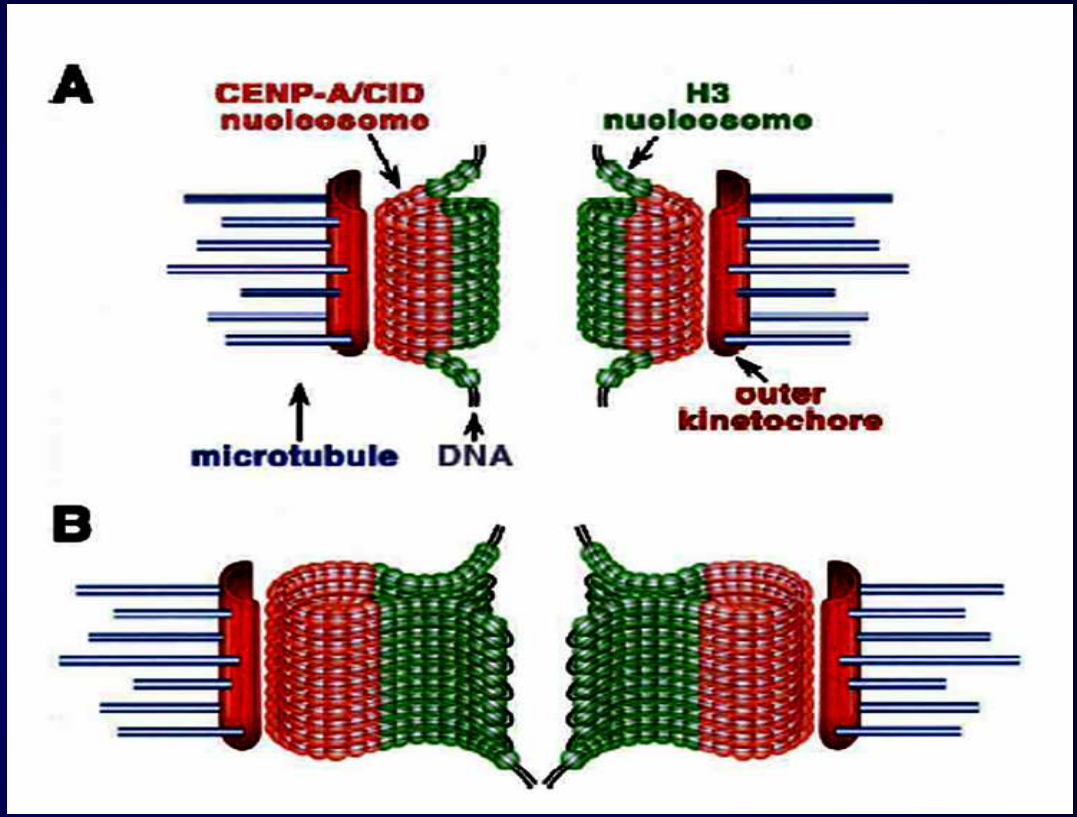
Varianty H3.3 a centromerické varianty

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.**



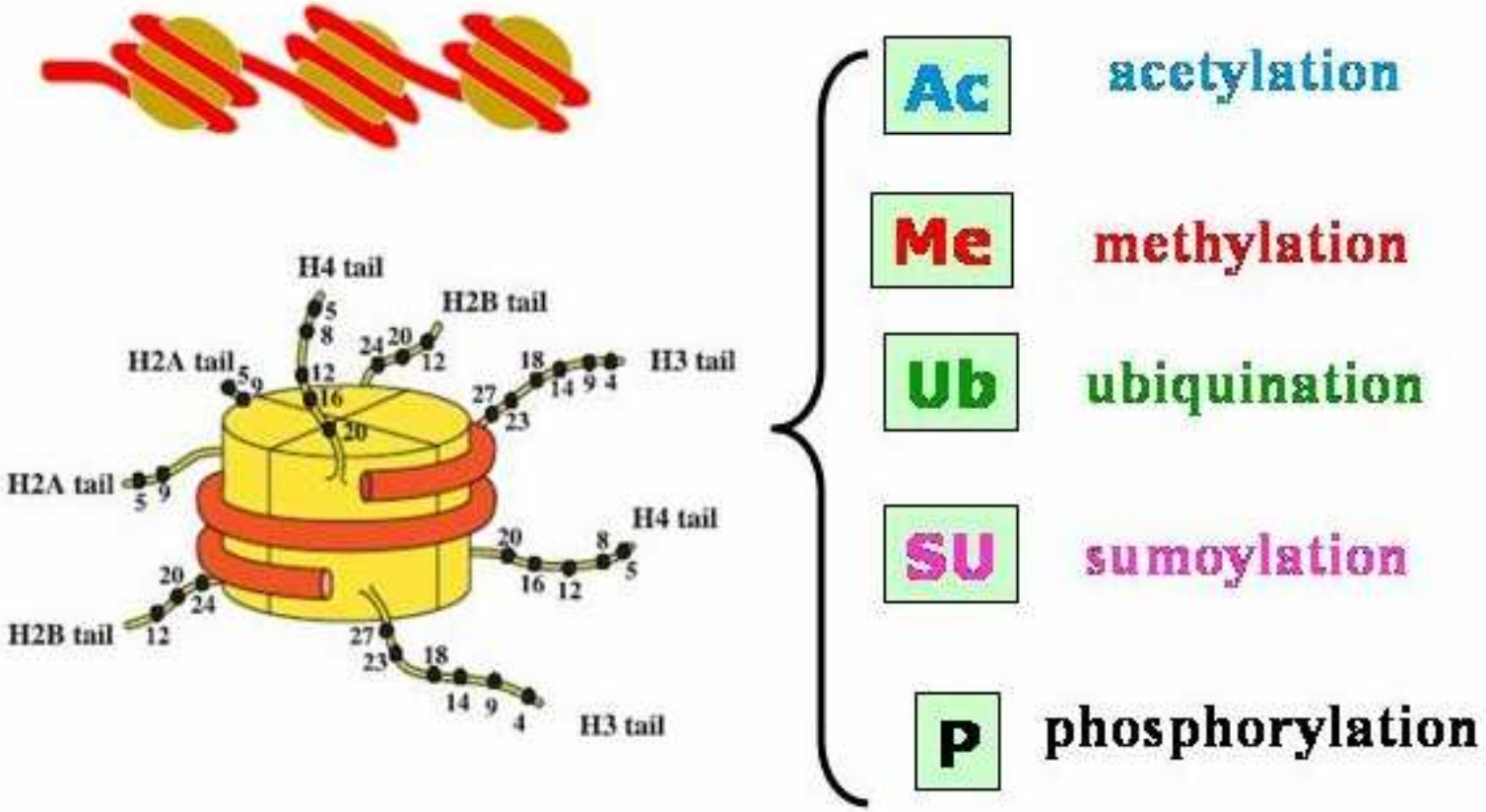
Varianty histonů H4: **většina genů kódujících hlavní histonové proteiny jsou exprimovány během S fáze buněčného cyklu. V případě H4, geny jsou konstitutivně exprimovány během buněčného cyklu. Pro H4 nejsou známy žádné varianty.**

Biochemické modifikace histonů

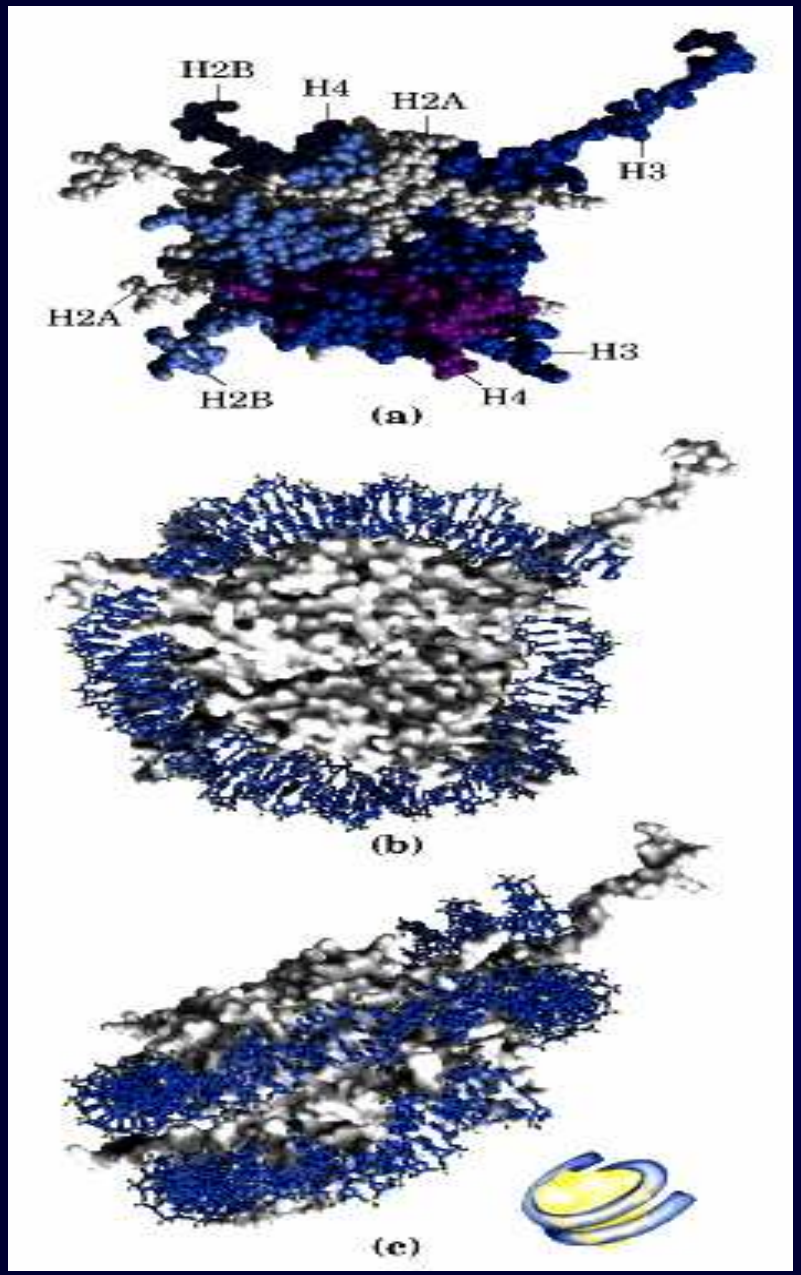
- **Dynamická struktura chromatinu je přímo ovlivněná post-translačními modifikacemi amino-konců histonů**

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

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



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



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)

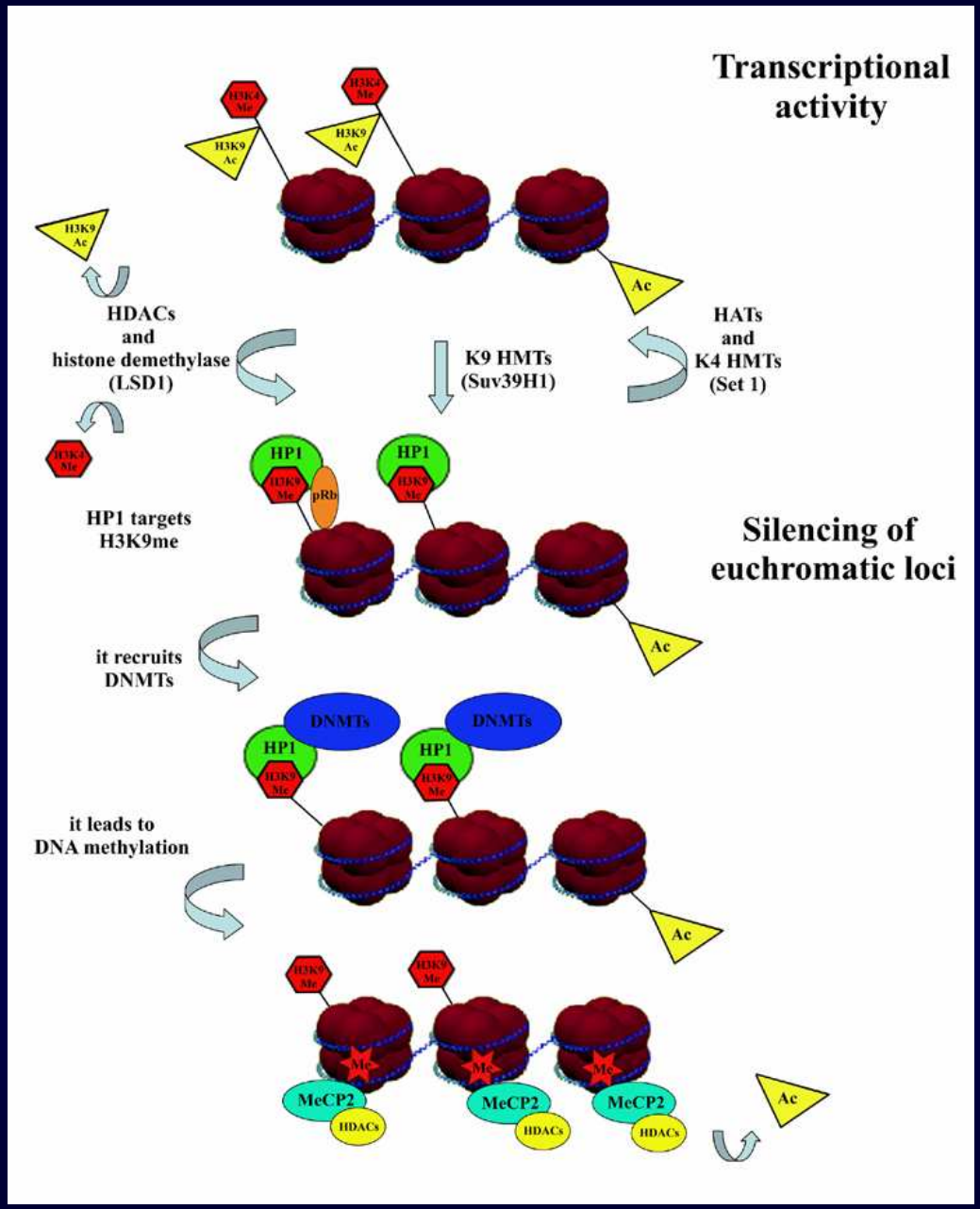
2004: Objev demethylace histonů za účasti aminové oxidasy LSD1 (KIAA0601) (Shi et al., Cell 2004). LSD1 specificky demethyluje H3 (K4), epigenetickou modifikaci zodpovědnou za transkripční aktivitu.

HATs: HAT1, PCAF, CBP/p300, TFIIC90, ELP3, SRC1, CLOCK
(see Allis et al., 2007).

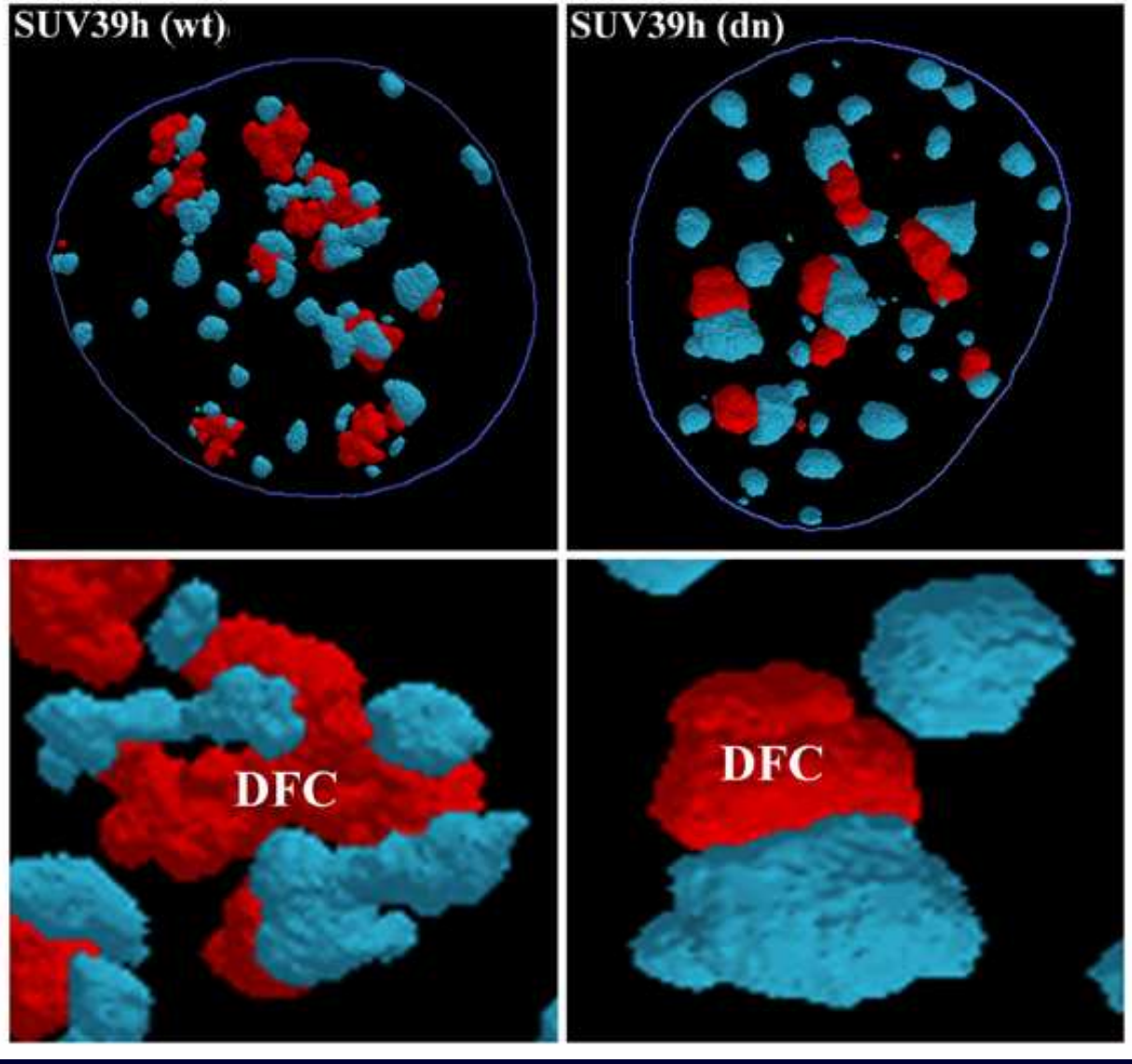
HDACs: Class I, II, III

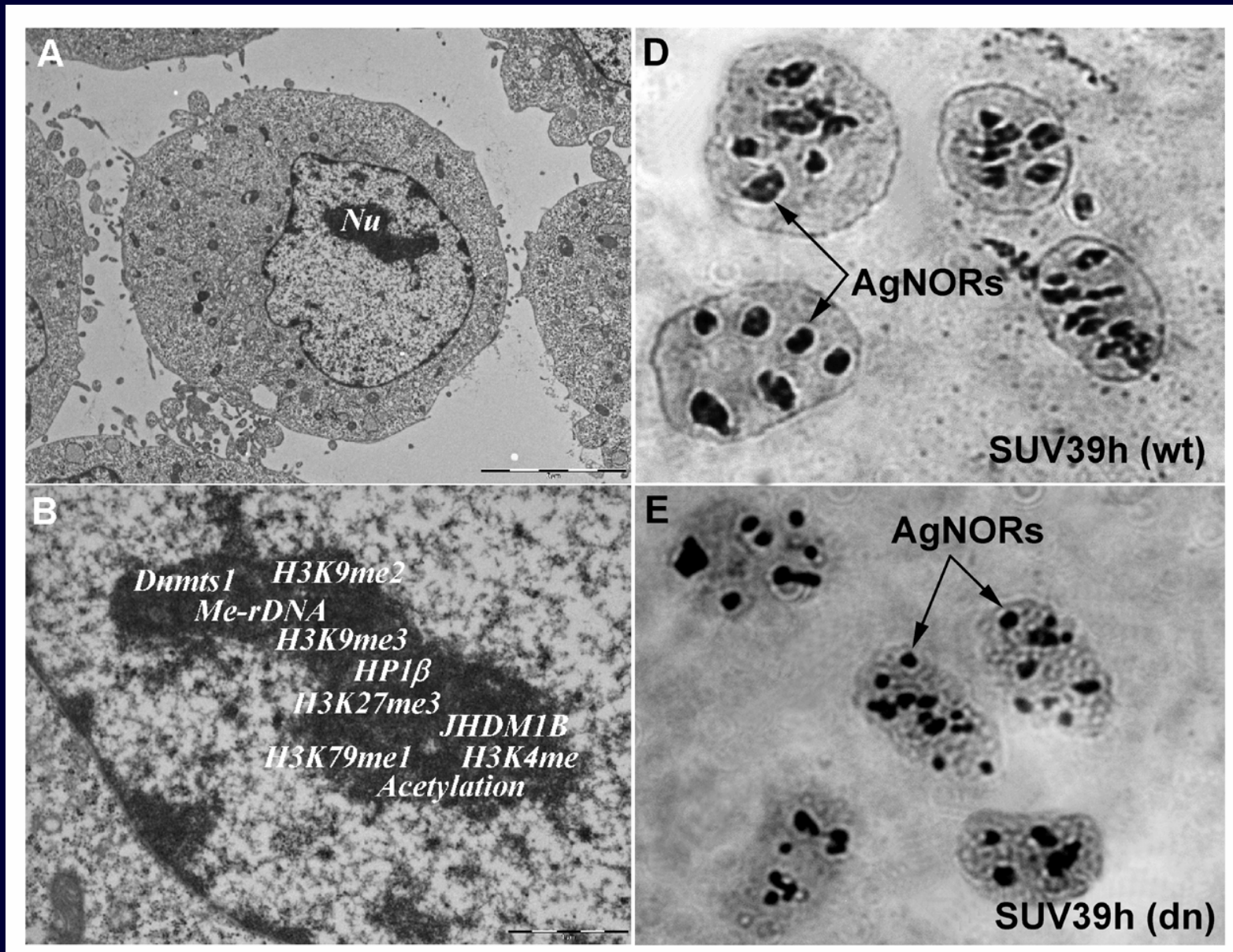
HMTs: SUV39H1, SUV39H2, G9a, MLL1, hSet 1, hSet 2, SUV4-20H1, SUV4-20H2, EZH2 (PcG silencing)

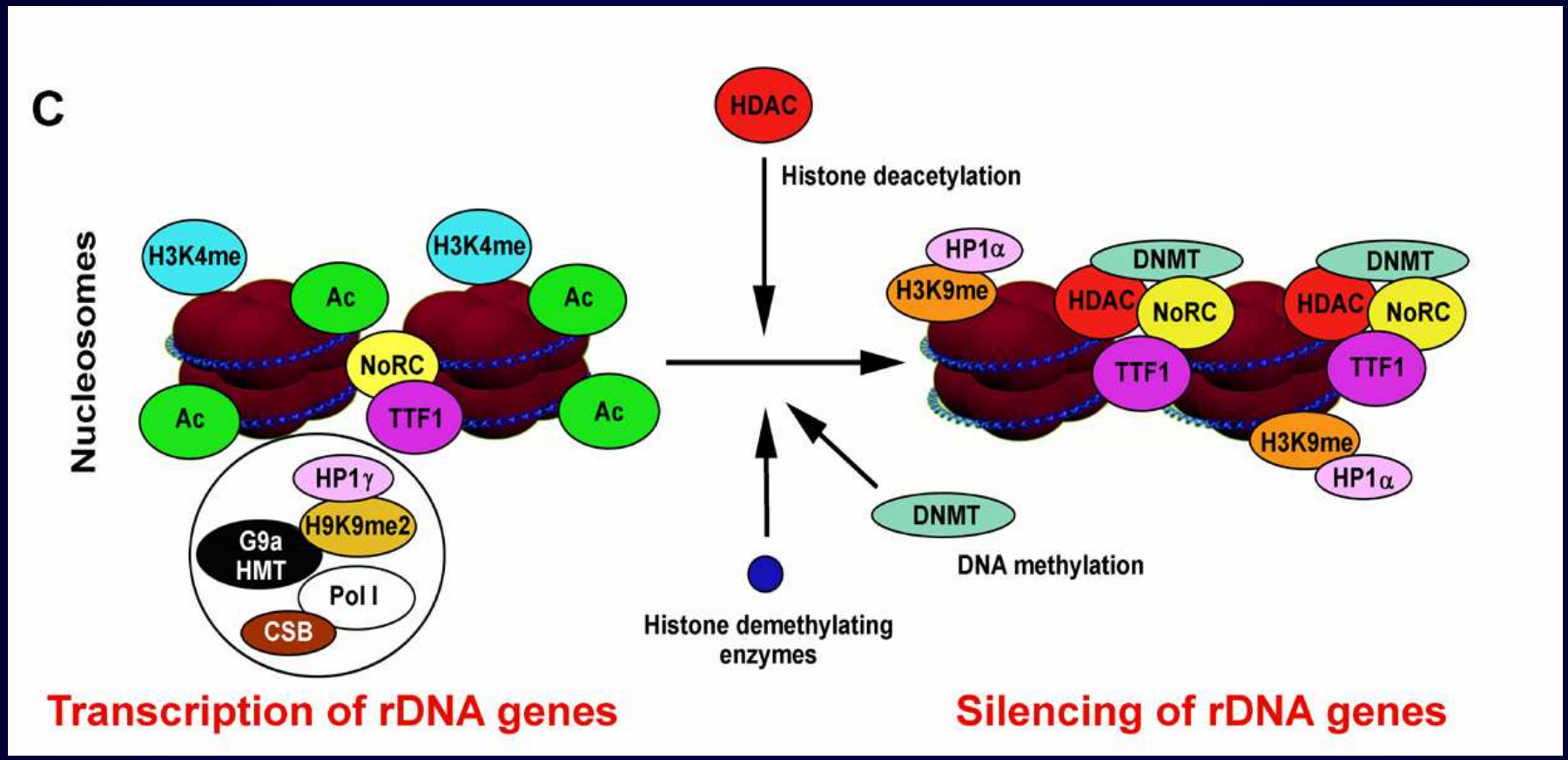
<u>Demethylases:</u> LSD1 (transcriptional activation), JHDM1b (H3K4me3), Jmjd2b (H3K9me3), JHDM2a, JMJ D2B (heterochromatin formation)

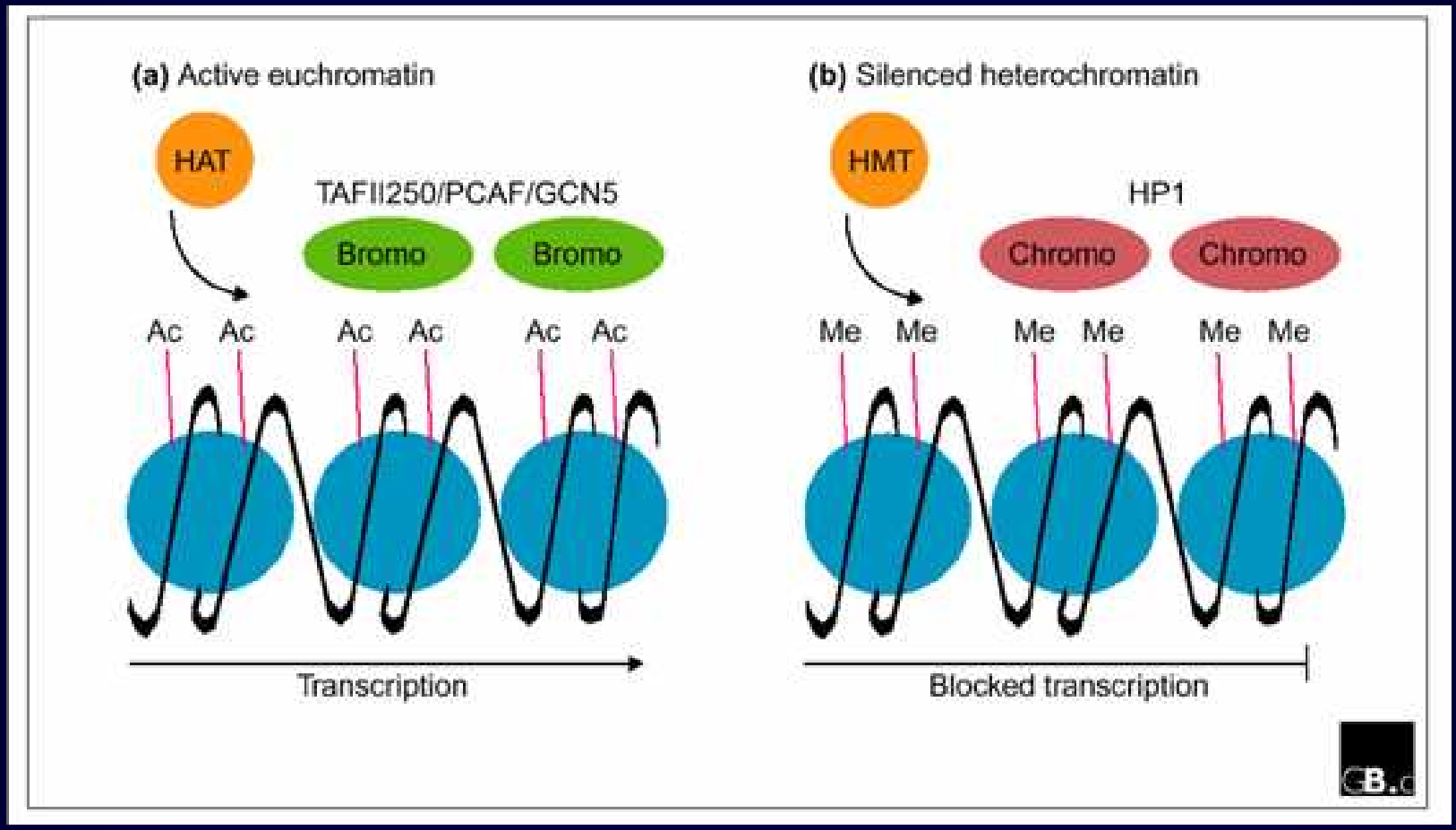

Transcriptional activity
H3K4 Me
H3K9 Ac
H3K4 Me
H3K9 Ac
H3K9 Ac
Ac
HDACs and histone demethylase (LSD1)
K9 HMTs (Suv39H1)
HATs and K4 HMTs (Set 1)
H3K4 Me
HP1
H3K9 Me
pRb
HP1
H3K9 Me
HP1 targets H3K9me
Silencing of euchromatic loci
Ac
it recruits DNMTs
DNMTs
HP1
H3K9 Me
DNMTs
HP1
H3K9 Me
it leads to DNA methylation
Ac
H3K9 Me
H3K9 Me
Me
Me
Me
MeCP2
HDACs
MeCP2
HDACs
MeCP2
HDACs
Ac

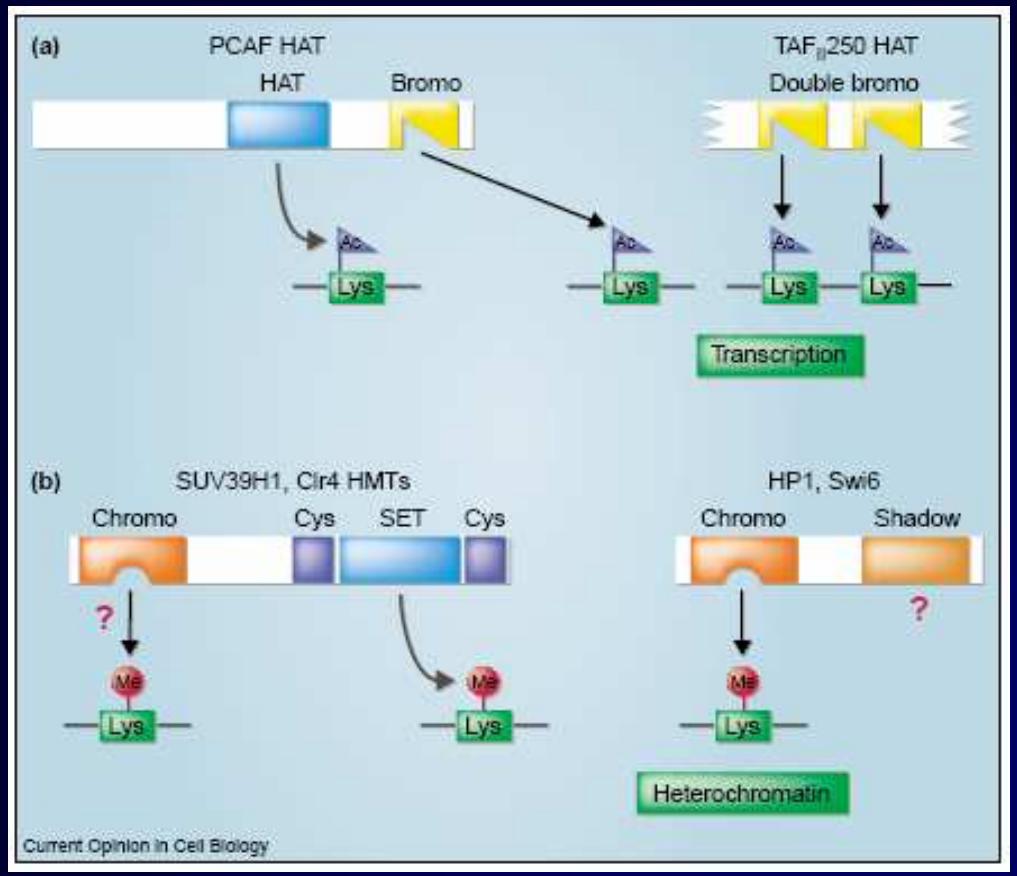
Fibrillarin / Chromocenters




A
Nu
B
Dnmts1
H3K9me2
Me-rDNA
H3K9me3
HP1β
H3K27me3
JHDM1B
H3K79me1
H3K4me
Acetylation
D
AgNORs
SUV39h (wt)
E
AgNORs
SUV39h (dn)


C
HDAC
Histone deacetylation
Nucleosomes
H3K4me
Ac
NoRC
TTF1
HP1γ
H9K9me2
G9a HMT
Pol I
CSB
HP1α
H3K9me
DNMT
HDAC
NoRC
TTF1
DNMT
DNA methylation
Histone demethylating enzymes
Transcription of rDNA genes
Silencing of rDNA genes


(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

HP1α (CBX5)
MGKKTKRT_{phos}AD$S_{phos}S_{phos}S_{phos}S_{phos}$EDEEEYVVEKVLDRRVV$K_{fml}$GQVEYLL$K_{fml/me1/ac}$W
K_{ac}GFS_{fml}EEHNTWEPEK_{ac}NLDCPELIS_{fml}EFMKKYK KMKEGENNKPR EK SESNKR$K_{ac/fml/me1}$
S_{fml}NFS_{fml}N$S_{phos/fml}$ADDI$K_{ac}S_{fml}$KKK_{ac}REQSNDIARGFERGLEPEKIIGATDSCGDLMFLMKW
$K_{fml/ac/me1}$DTDEADLVLAKEANVK_{fml}CPQIVIAFYEERL TWHAYPEDAENK_{fml}EKETAKS

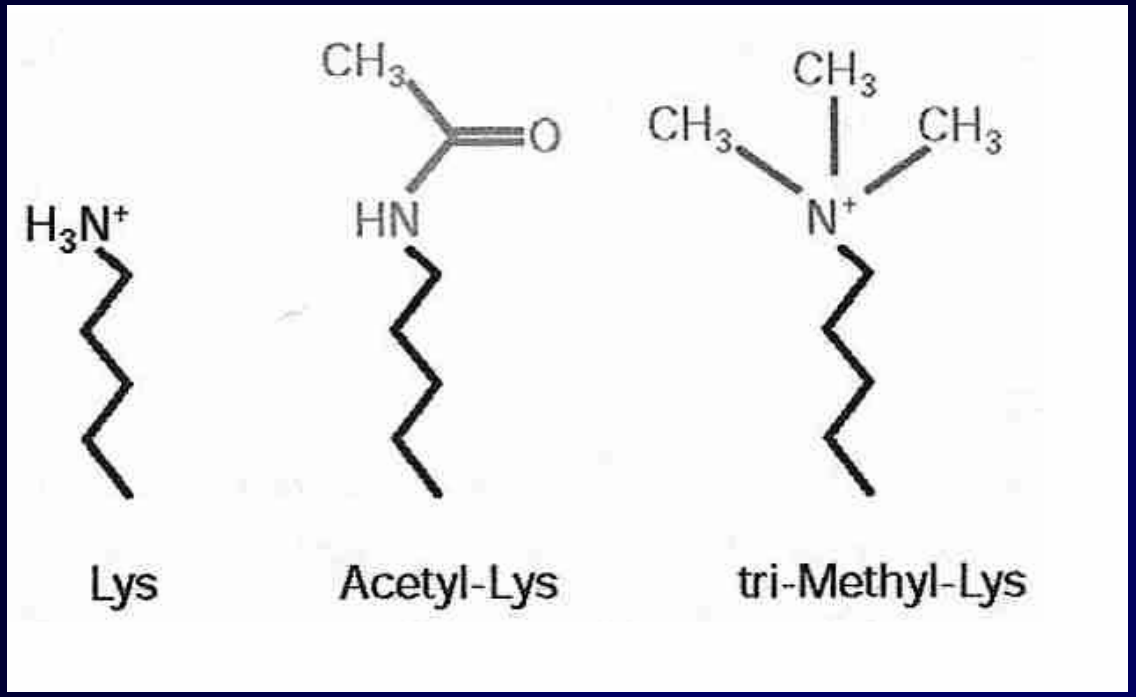
HP1β (CBX1)
MGKKQNKK_{me1}VEEVLEEEEEEYVVEKVLDRRVV$K_{ac/fml}$G$K_{ac/me1/fml}$VEYLL$K_{ac/fml}$WKGF
SDEDNTWEPEENLDCPDLIAEFLQSQKTAHETDK SEGGKRKADSDSEDKGE ESKPK KKK
EESEKPRGFARGLEPERIIGATDSSGELMFLMKW $K_{fml/ac}$NSDEADLVPAK_{fml}EANVKCPQV
VISFYEERLTWHSYPSEDDDKKDDKN

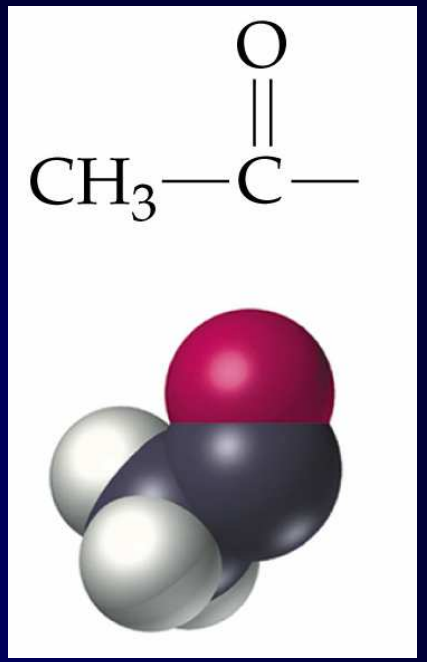
HP1γ (CBX3)
MASNK TTLQKMGKK QNGKSK$K_{fml/ac/me1}$VEEAEPEEFVVE$K_{fml/ac}$VLDRRVVNG$K_{fml/me1/ac}$V
EYFLK_{fml}WKGFTDADNTWEPEENLDCPELIEAFLNSQKAGKEKD GTKRKS_{phos}LS_{phos}DSES
DDSKSK KKRDAADKPR GFAR GLDPERIIGATDSSGELMFLMKW $K_{fml/ac/me1}$DS_{fml}DEADLV
LAKEANMKCPQIVIAFYEERLTWH $S_{phos/fml}$CPEDEAQ

FIG. 2. **PTM maps of HP1 modifications.** Over 90% sequence coverage was obtained on all isoforms (shown in *red*). Residues with PTMs are shown in *black bold type*. PTMs identified include phosphorylation (*phos*), acetylation (*ac*), monomethylation (*me1*), and formylation (*fml*).

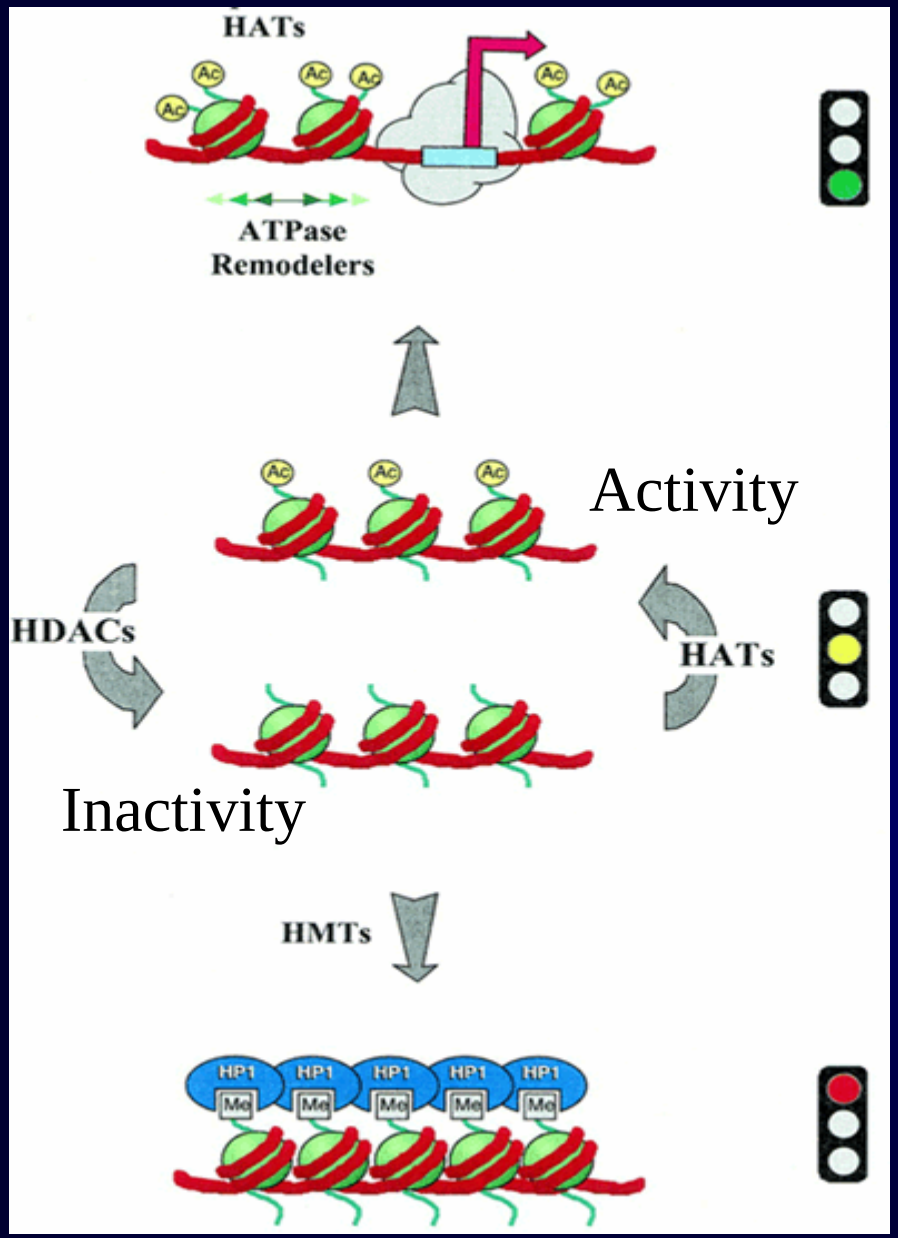
HMTs:

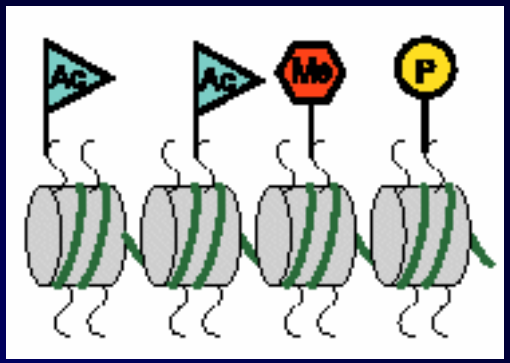
D. melanogaster: Su(var)3-9 je lokalizován v oblastech kondenzovaného chromatinu a je to klíčový regulátor v organizaci represivního chromatinu. Homolog u S.pombe je Clr4 umyší SUV39h1 a u lidských buněk SUV39H1. Tyto HMTs specificky methylují H3(K9).

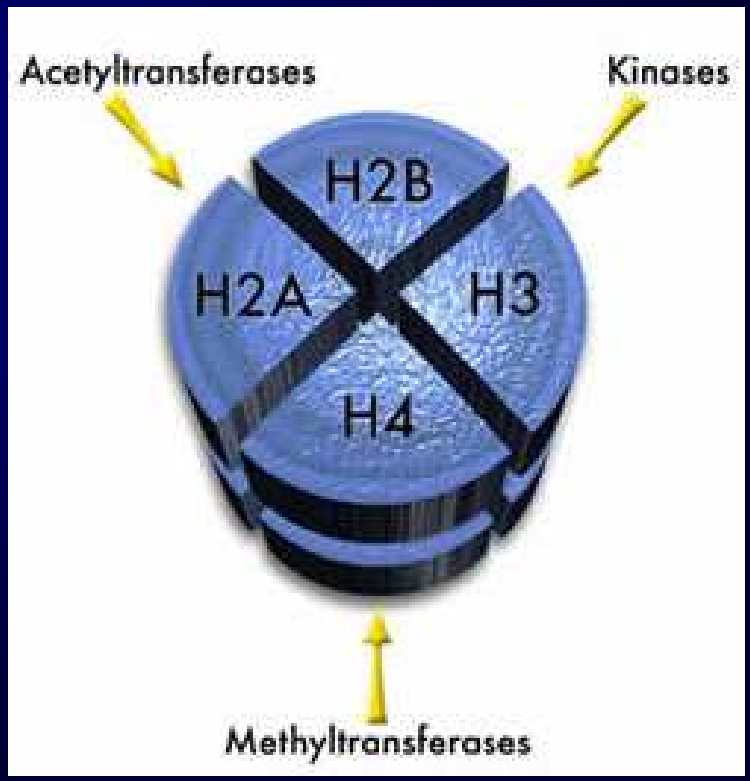




Primárním důsledkem histonových modifikací je snížení schopnosti histonových konců interagovat s dalšími složkami chromatinu, včetně DNA.

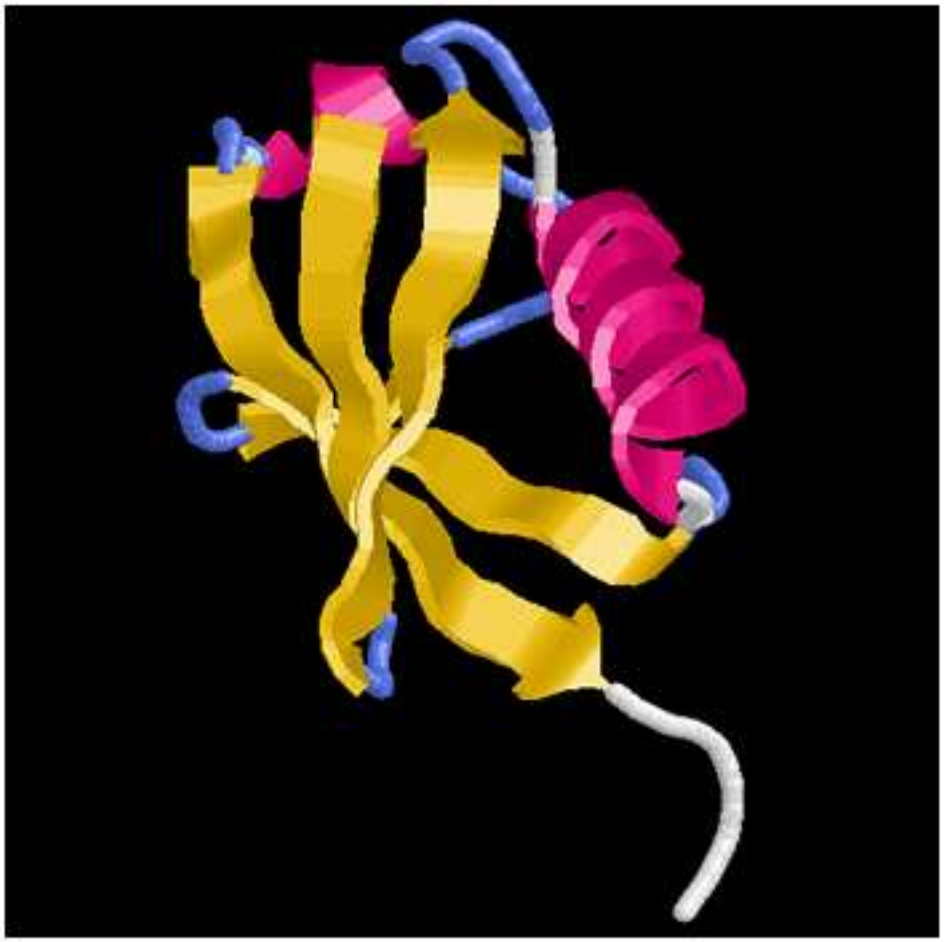


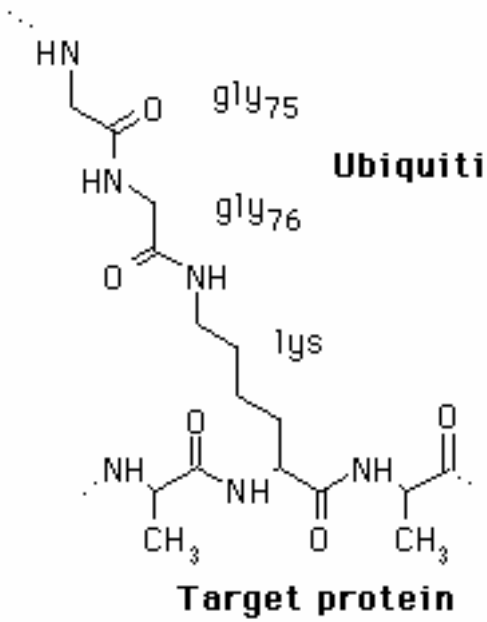




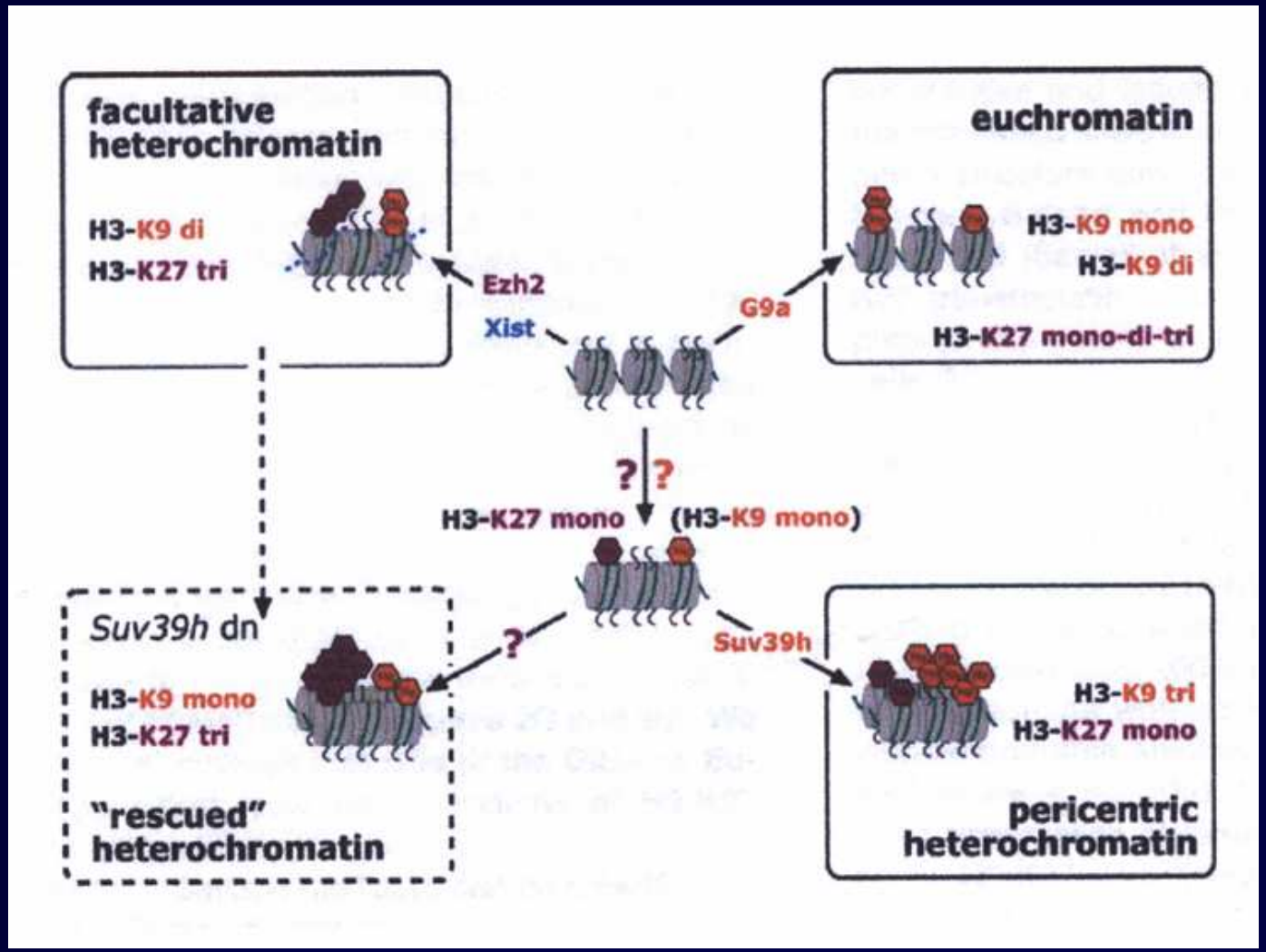
Ikaros, Helios

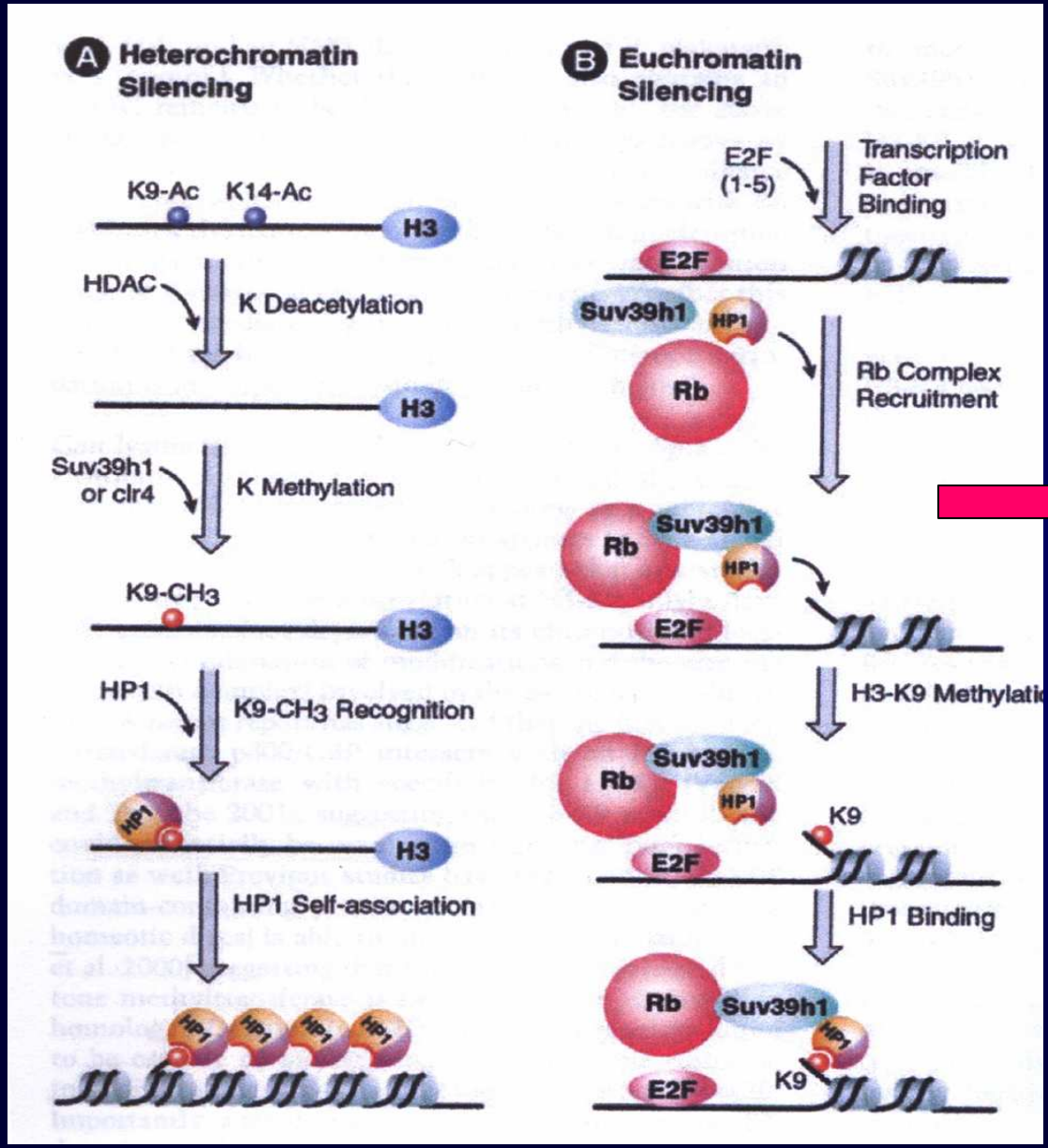
Ubiquitin structure





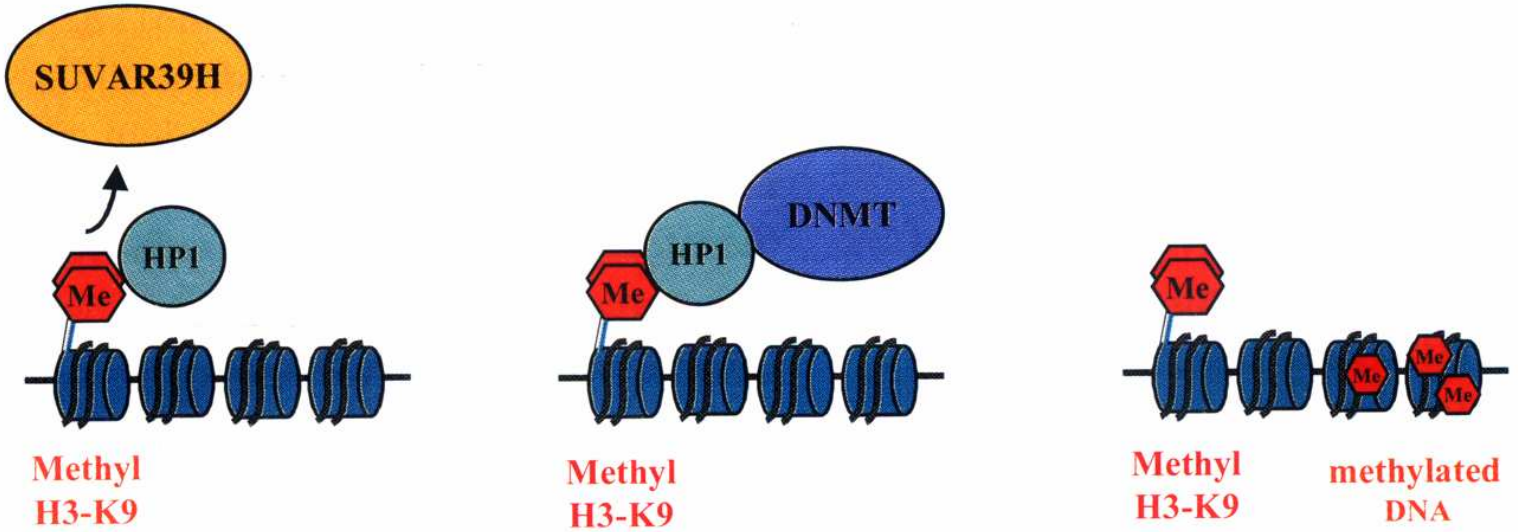
Ubiquitination of histones has been reported *in vivo* although the most prevalent ubiqutination occurs in H2A and H2B. One of the widely studied proteins that undergoes ubiquitination for its activity is p53.


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

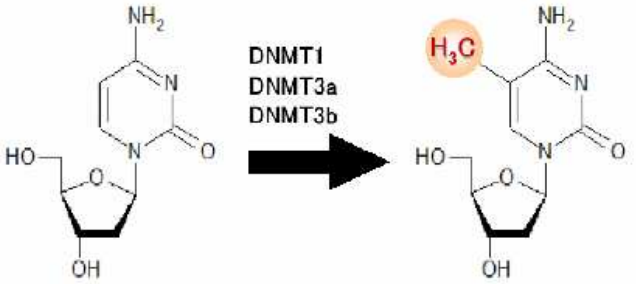


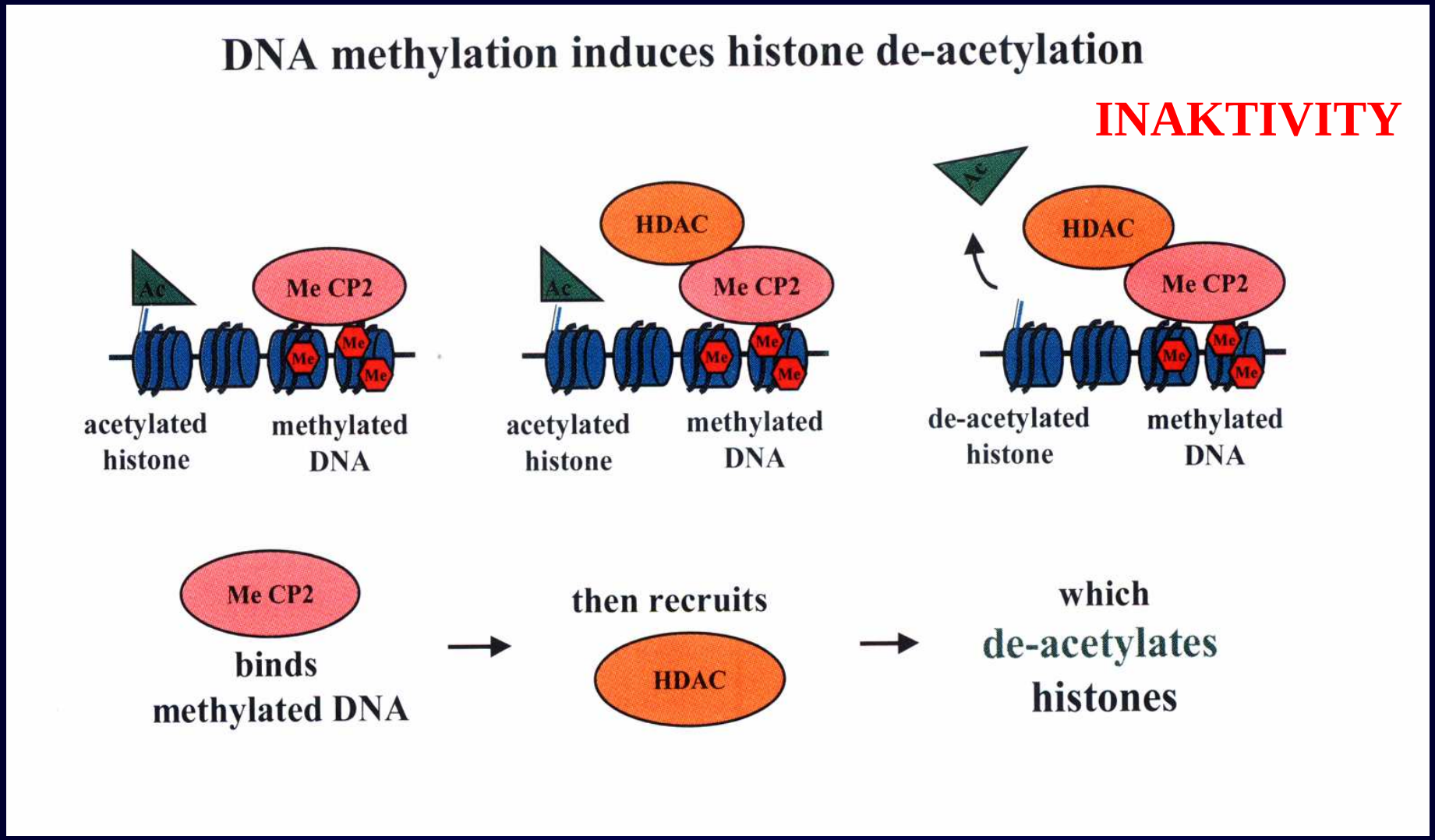
Repression of cyclin E promoter

Histone H3-K9 methylation induces DNA methylation

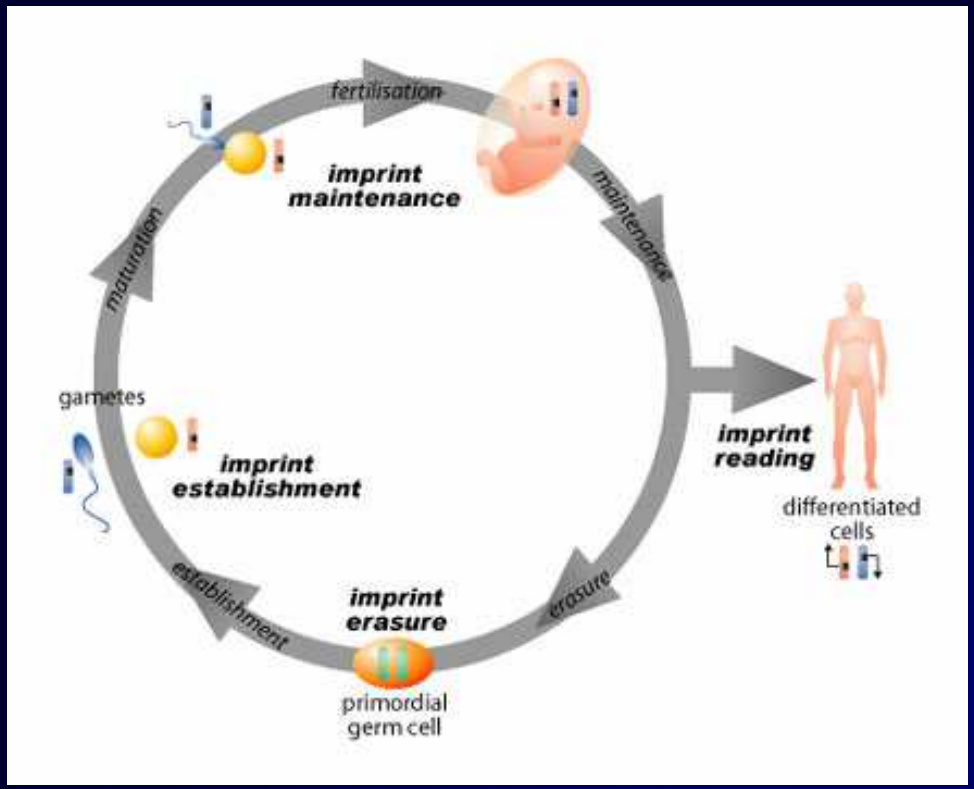


HP1 **targets** Methyl H3-K9 → **then recruits** DNMT → **which methylates DNA**





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

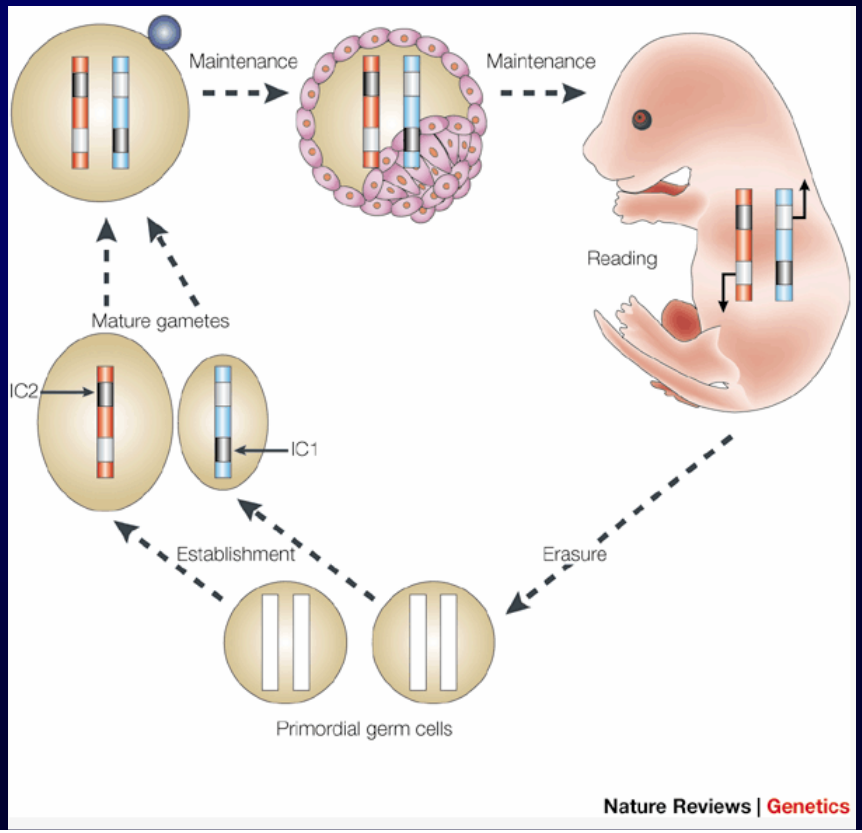


IMPRINTING

Myší embryo: samičí alela je zamethylována, nevyjadřuje se

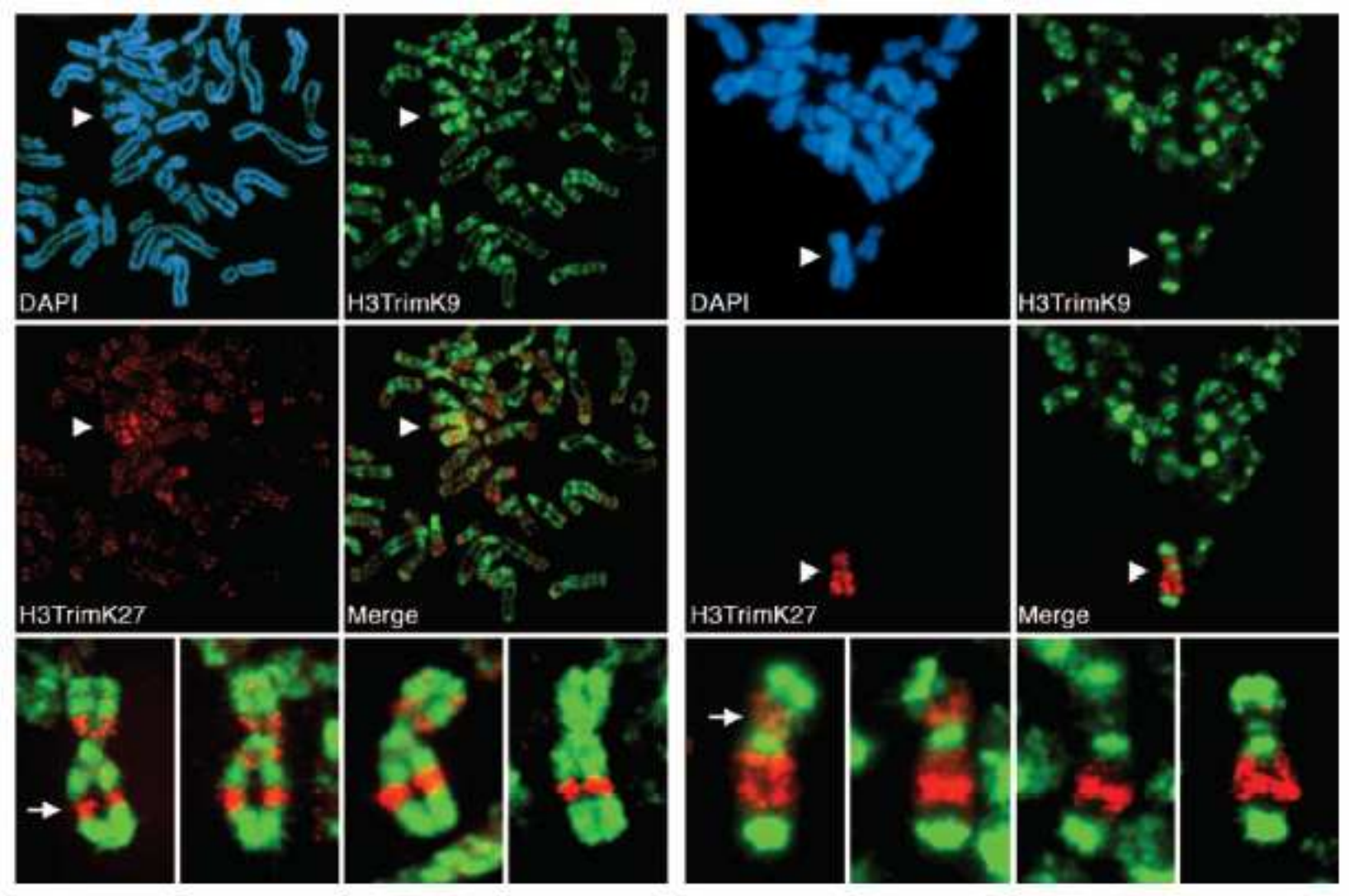
Dospělý jedinec: obě alely jsou demethylovány

Gametogeneze: se obnoví původní stav
Platí pro gen IGF II.



ISSN1471-0056

Inaktivace X chromosomu ve vztahu k epigenetickým modifikacím



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

Inaktivace X chromosomu ve vztahu k epigenetickým modifikacím

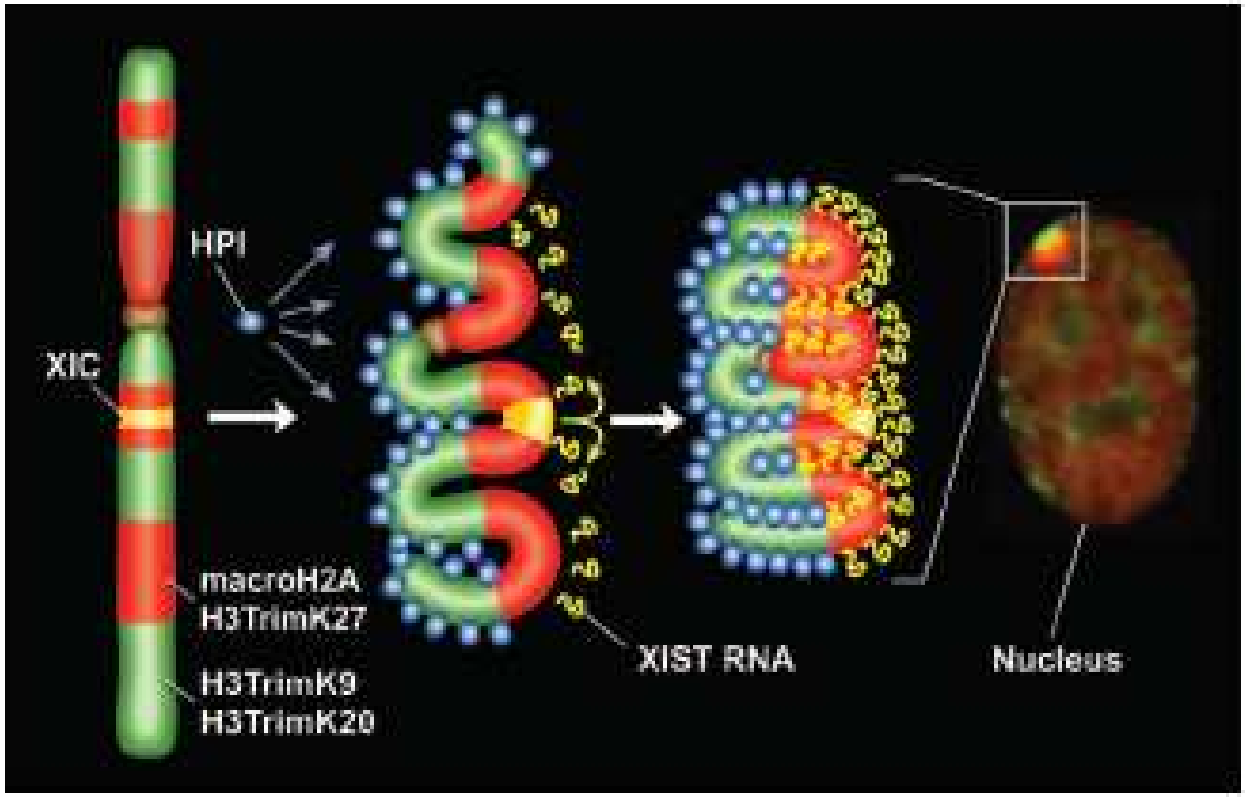
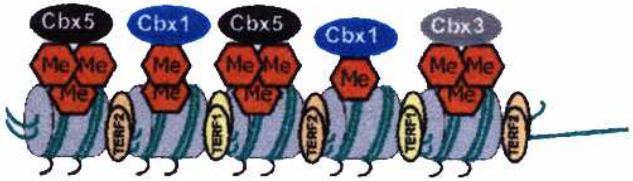


Fig. 4. Schematic model showing how heterochromatin of the Xi could transition between metaphase and interphase to be organized into the two nonoverlapping heterochromatin territories and to explain how XIST RNA could rapidly spread in cis outward from the X inactivation center (XIC) along only part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

Brian Chadwich, Florida University

Methylation state of telomeres

Wild-type telomeres



3´

Normal-length telomeres

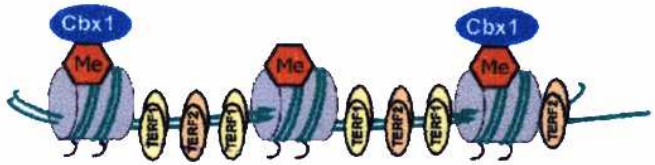
H3K9 (mono-, di- and trimethylated)

Cbx5, Cbx1, Cbx3

TERF1

TERF2

SUV39DN telomeres



3´

↓ H3K9 (di-, trimethylated)

↑ H3K9 (monomethylated)

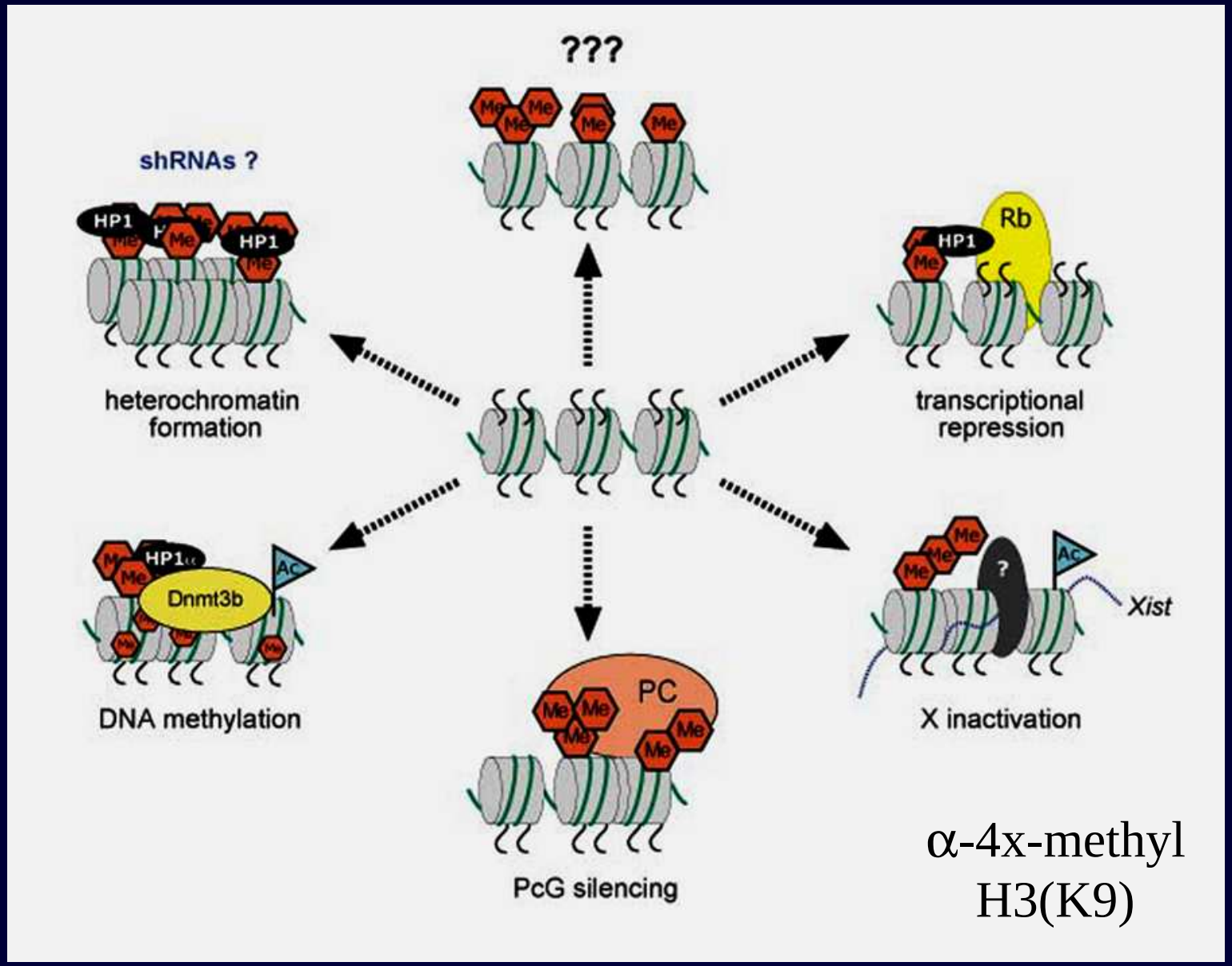
↓ Cbx5, Cbx3

↓ 50% decrease in Cbx1

Normal TERF2 binding

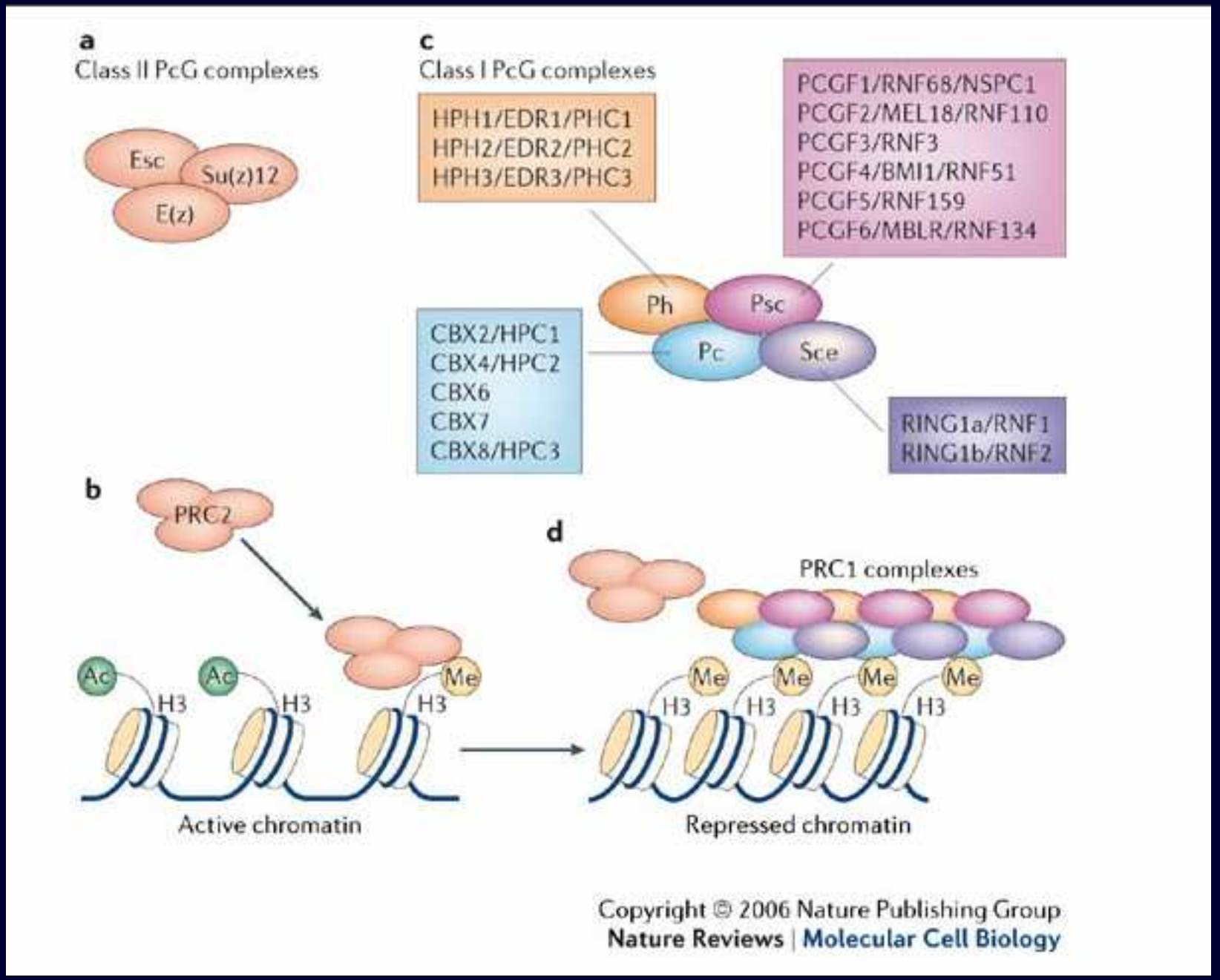
Slight increase in TERF1 binding

Abnormal telomere elongation



FAKULTATIVNÍ HETEROCHROMATIN

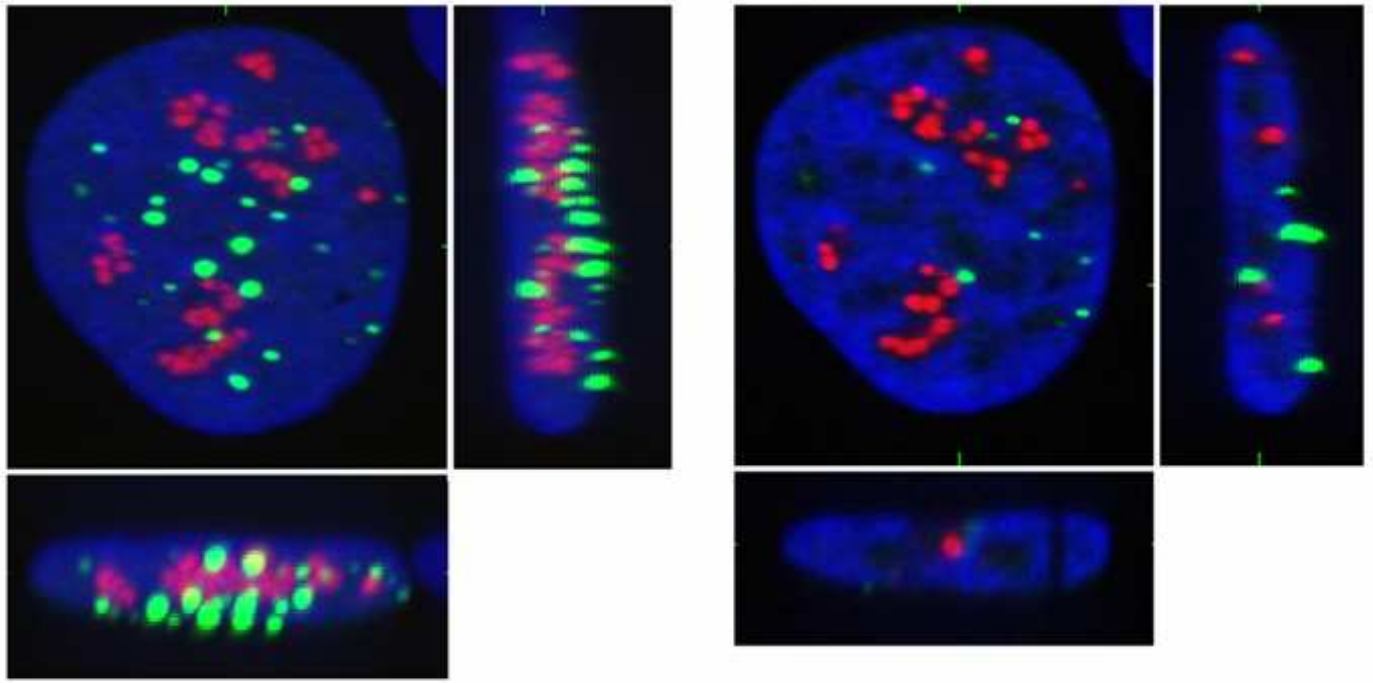
Polycomb group (PcG) proteins are highly conserved regulatory factors that were initially discovered in Drosophila. PcG genes are best known for their role in maintaining silent expression states of Hox genes during development, while trithorax group (trxG) proteins maintain Hox gene expression patterns in the appropriate spatial domains. PcG and trxG proteins are also involved in the regulation of normal cell proliferation, and their mutation has been linked to defects in stem cell fates and to cancer. They act by regulating chromatin structure and chromosome architecture at their target loci.



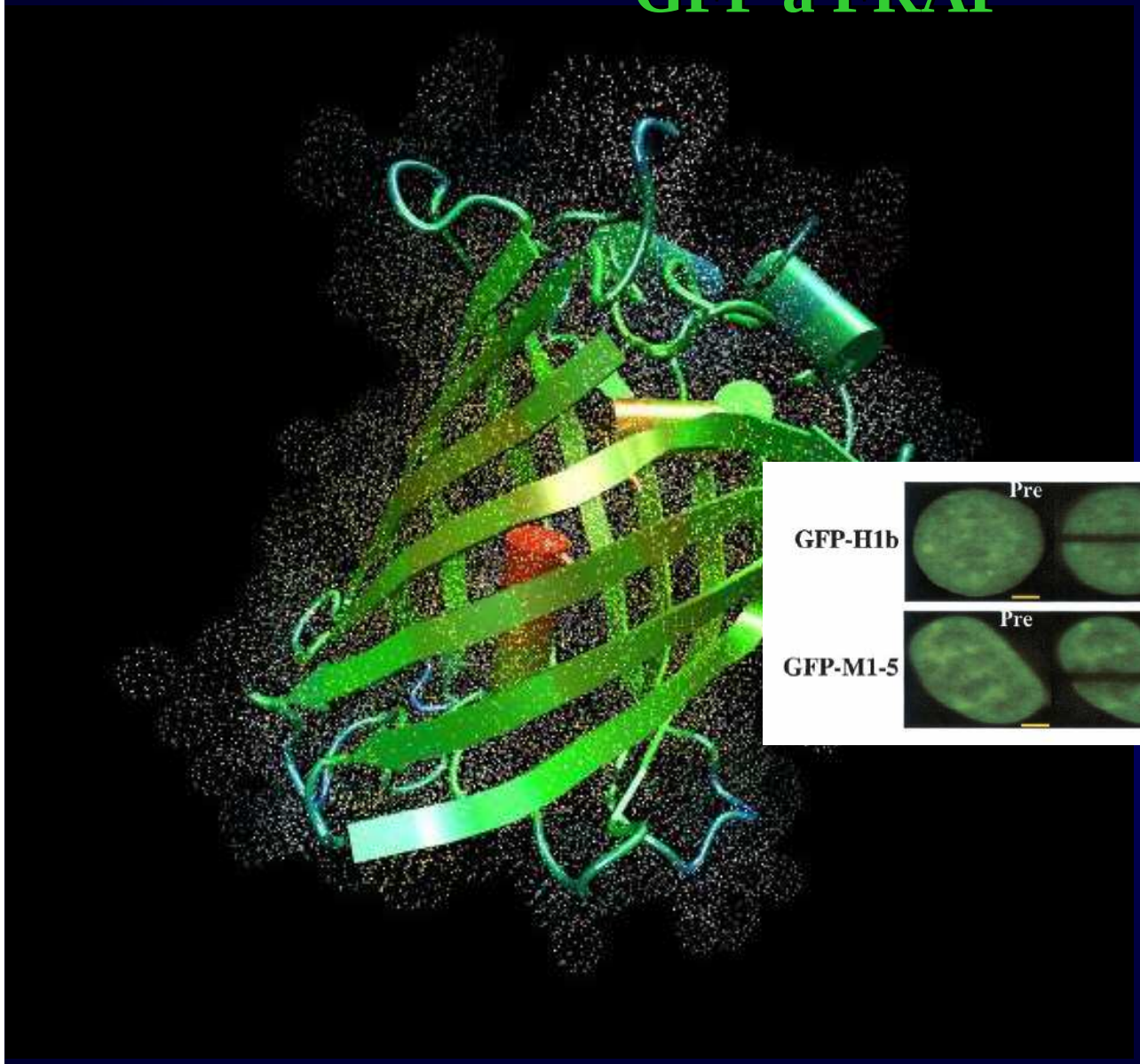
Fibrillarin / BMI1 / Nucleus

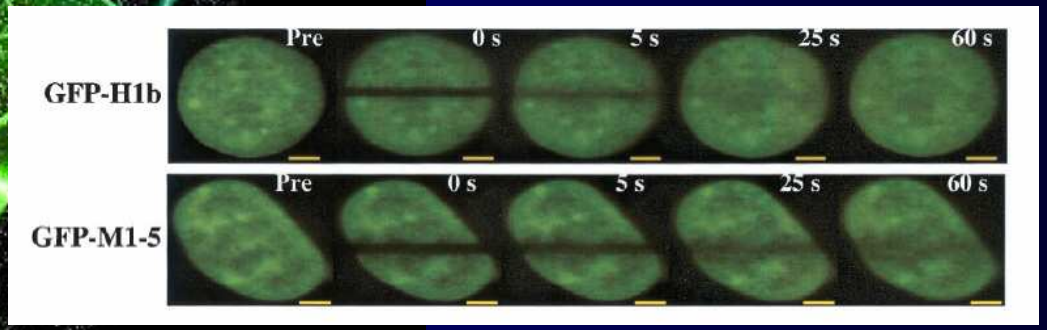
CONTROL

maximum image ***individual section***

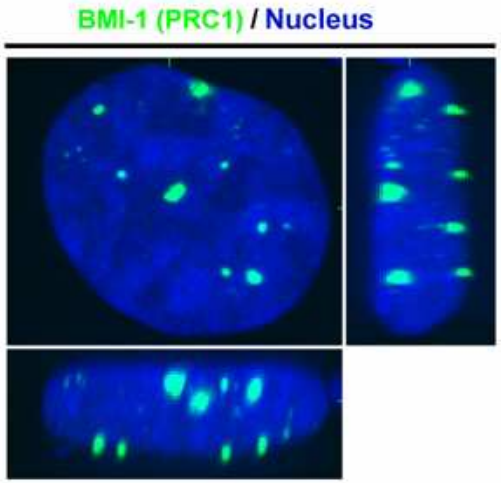


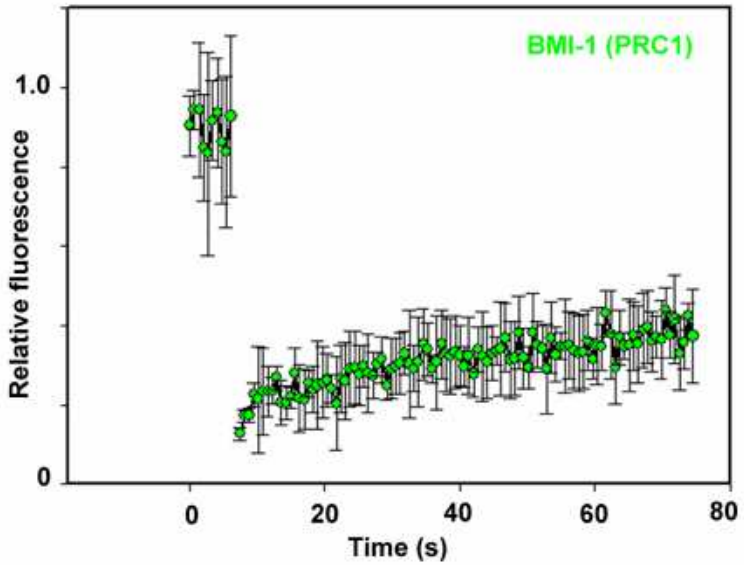
GFP a FRAP

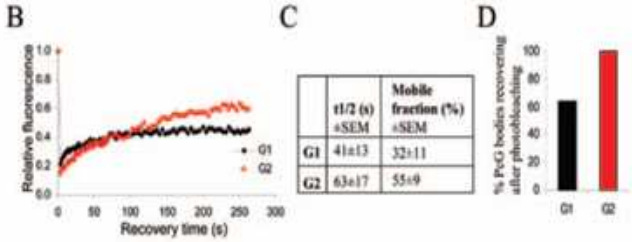


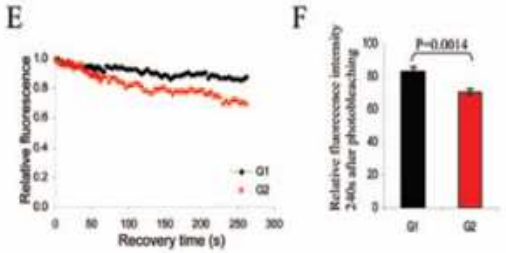


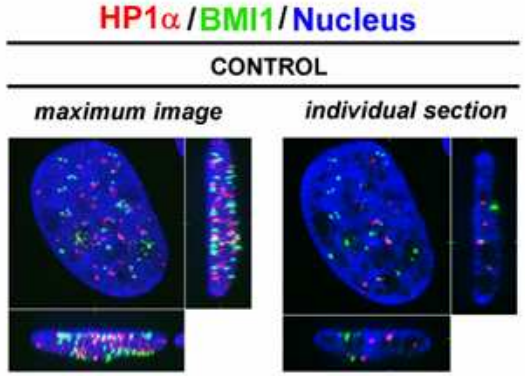
FRAP / GFP-BMI





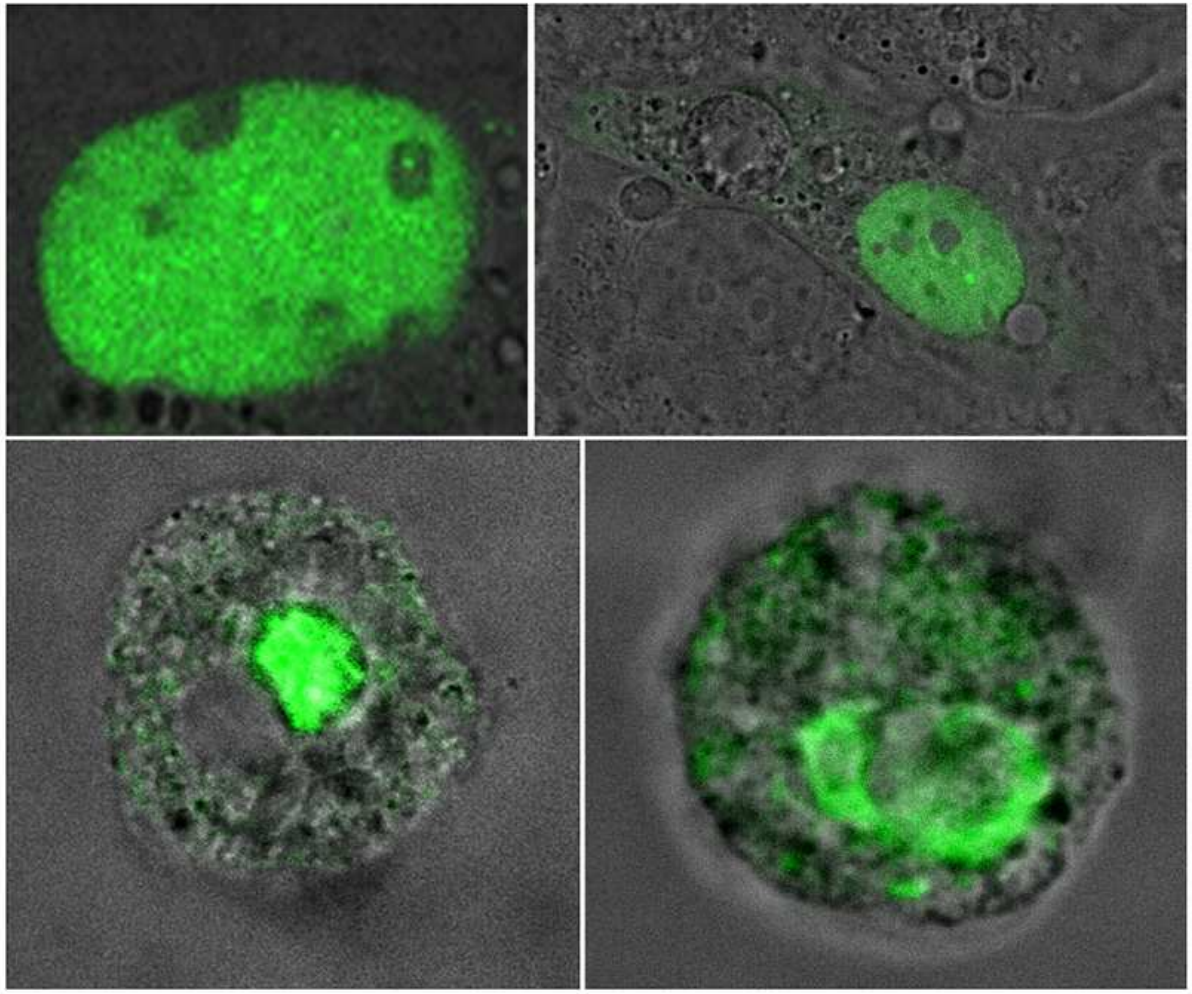


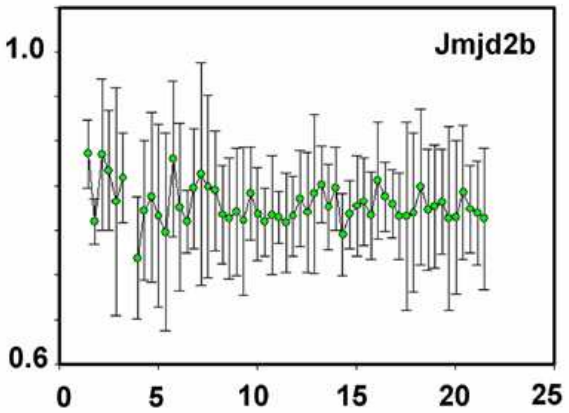


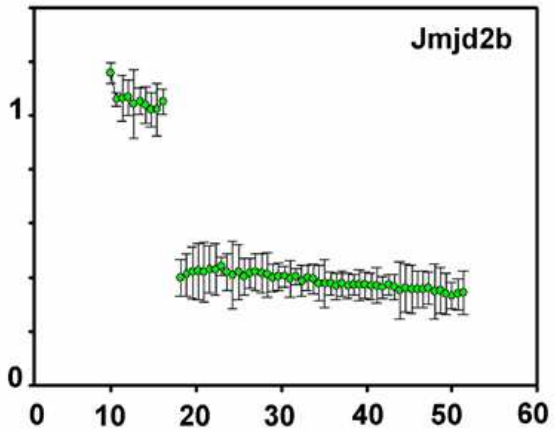


Hernandéz-Munoz et al. (2005)

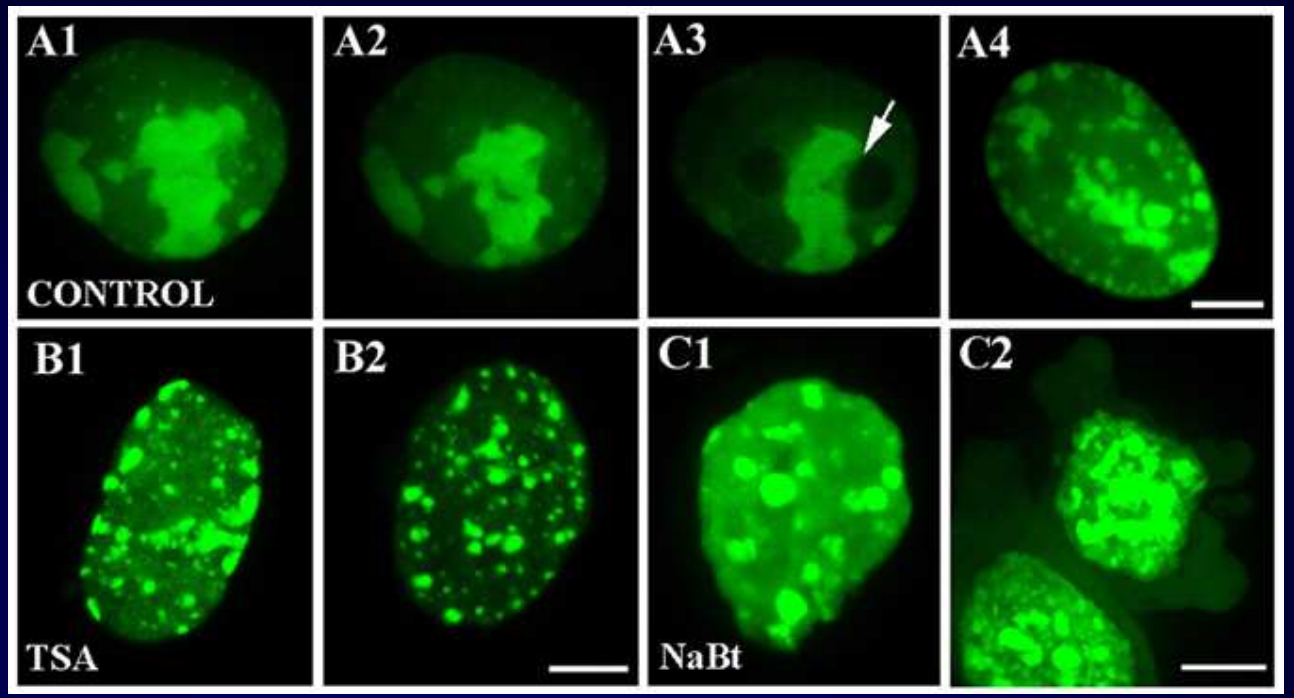
GFP-Jmjd2b / cells



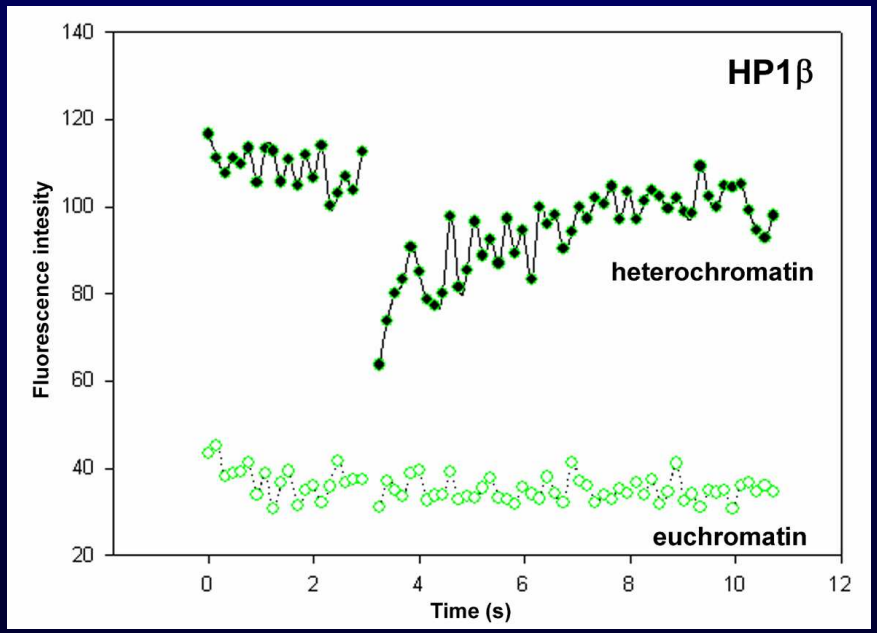




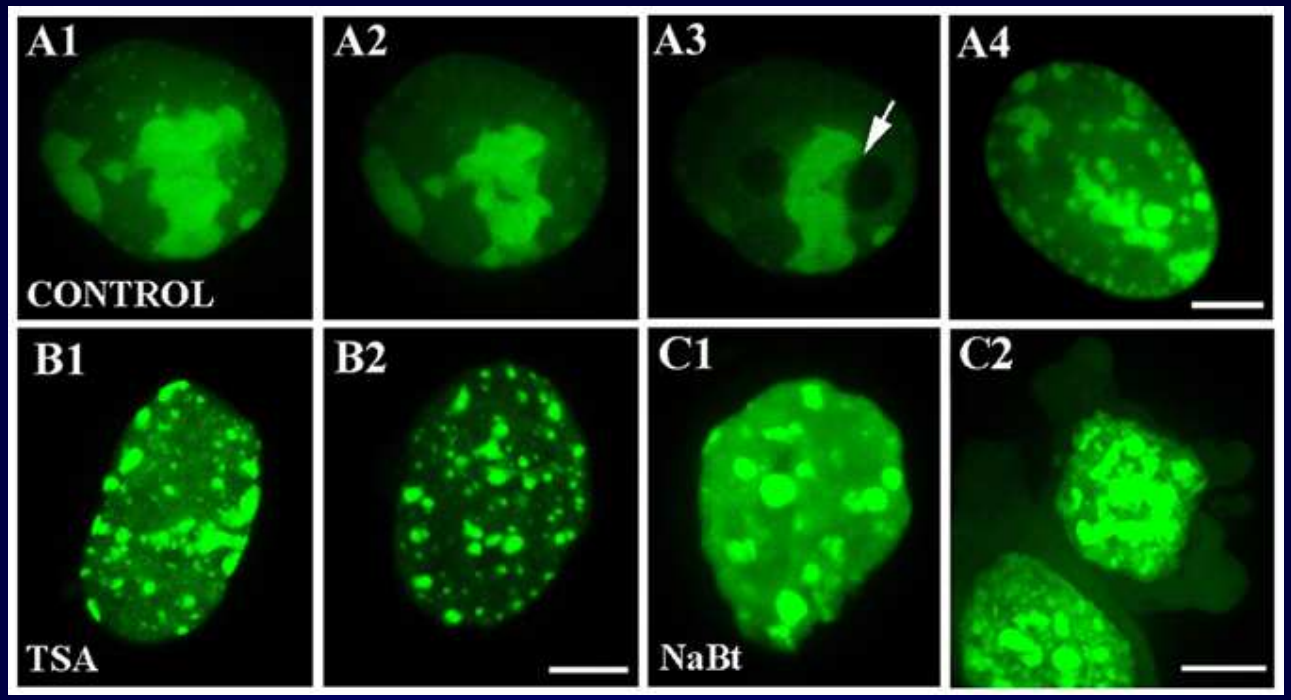
HP1β protein



FRAP – HP1β



HP1 proteins

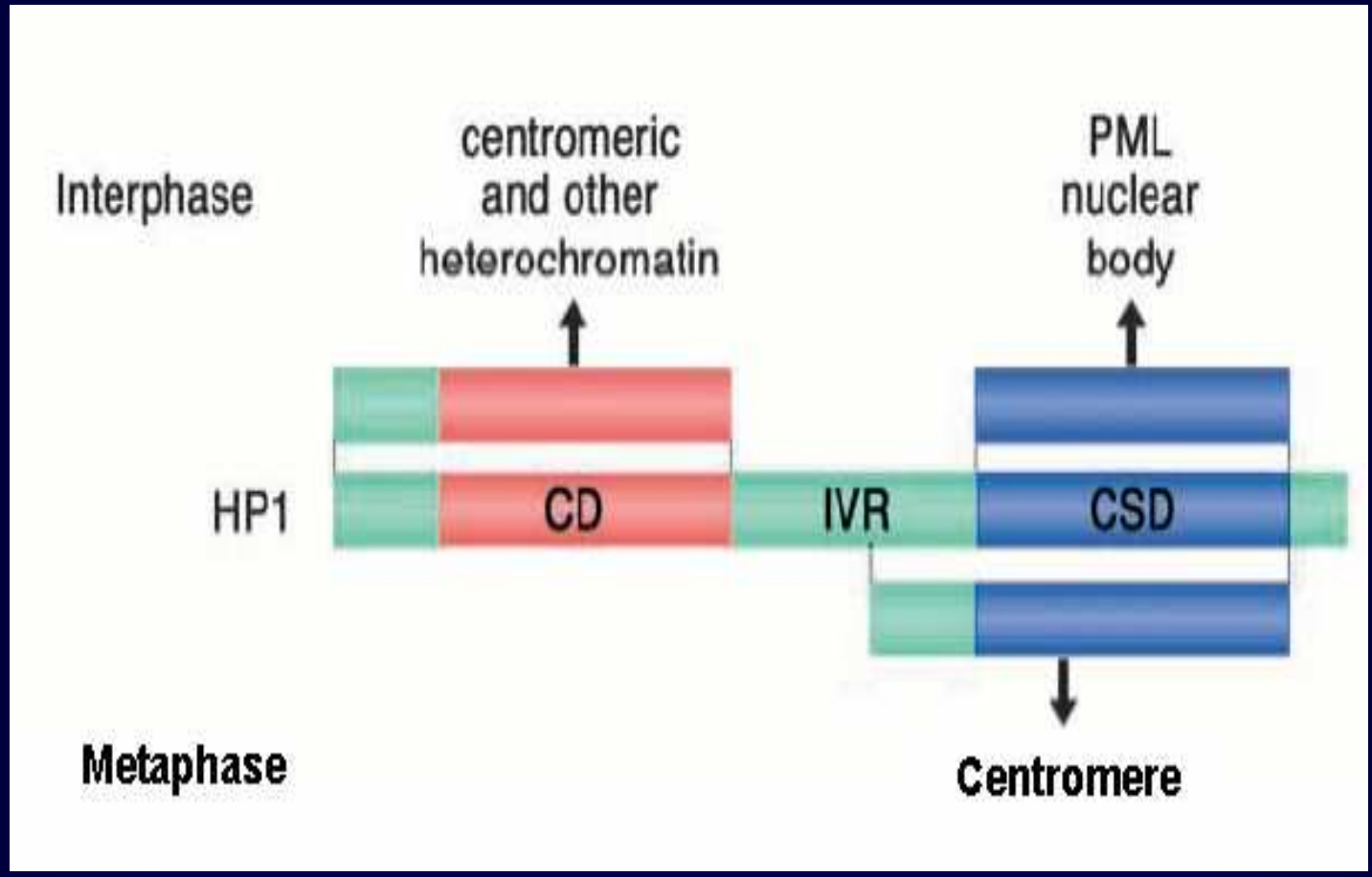


•**HP1 proteiny jsou hlavní složkou heterochromatinu a hrají důležitou úlohu při jeho tvorbě. HPs jsou mají vysokou afinitu k pericentromerickým a telometrickým oblastem chromosomů.**

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

HP1 proteins:

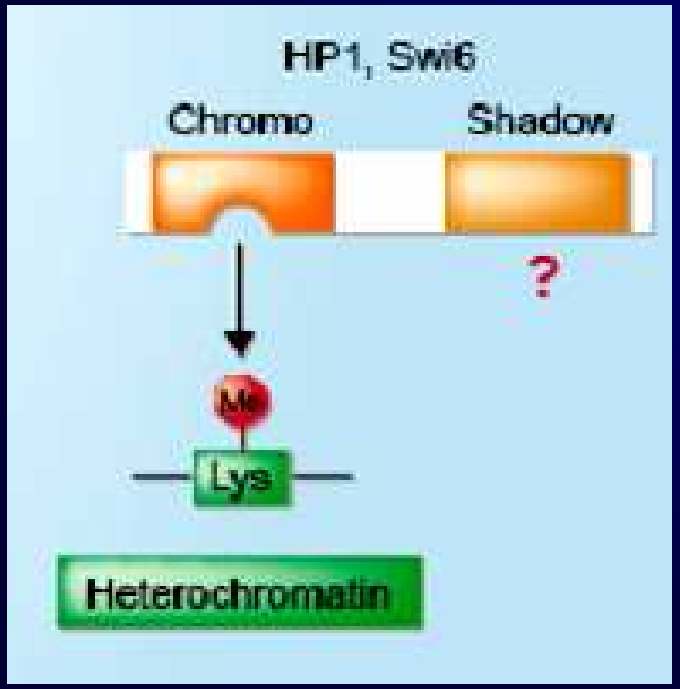
1. Heterochromatin protein (dHP1) was first identified in *Drosophila* and shown to localise to heterochromatin by antibody staining.
2. Mutation of HP1 gene decrease the effect of **PEV** (position effect variegation) on gene expression.
3. Null mutations of HP1 are lethal due to chromosome loss during 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 α and γ 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)



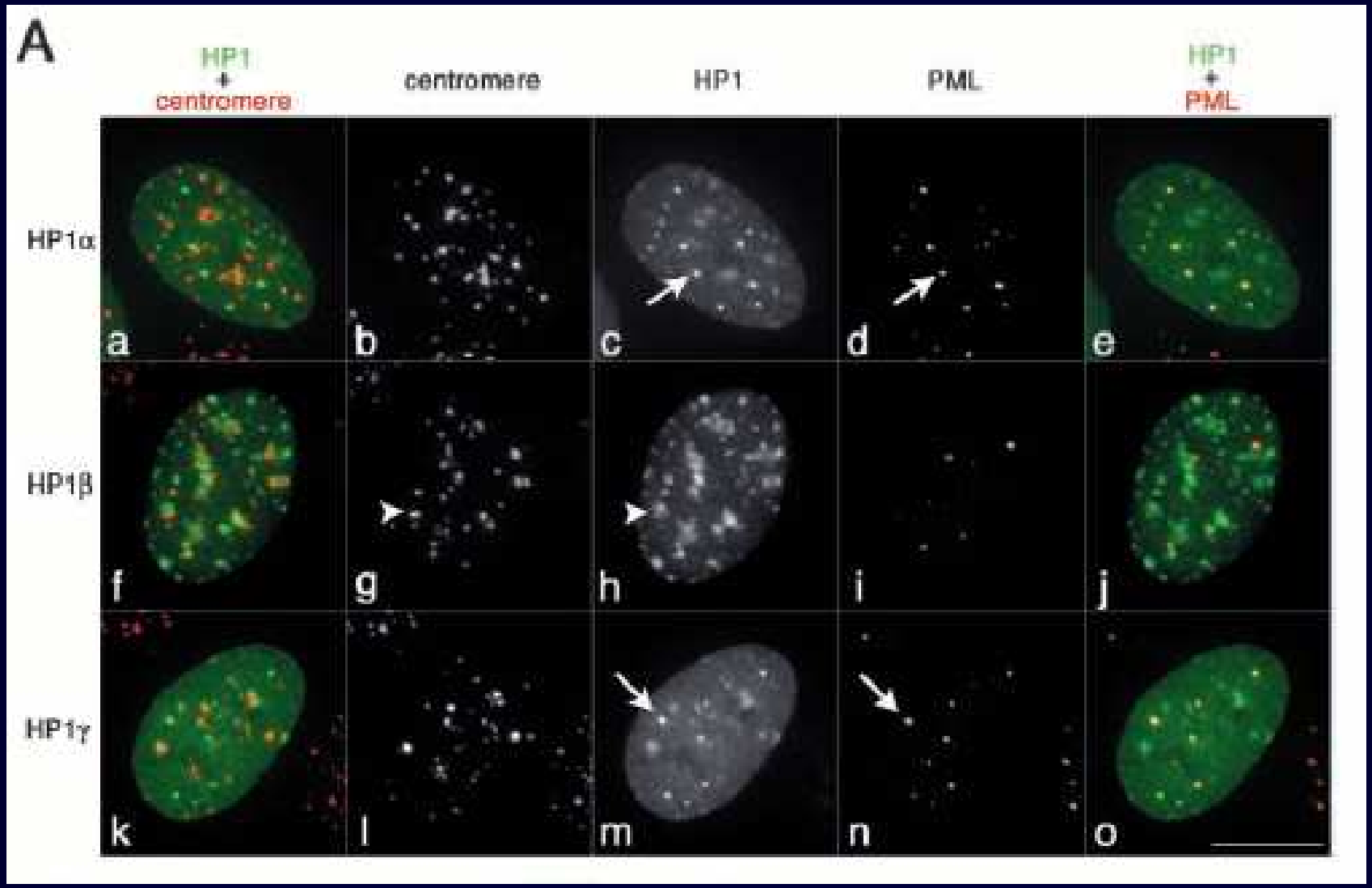
FUNKCE HPs

a) **Uspořádání chromatinu**

b) **Regulace transkripce**

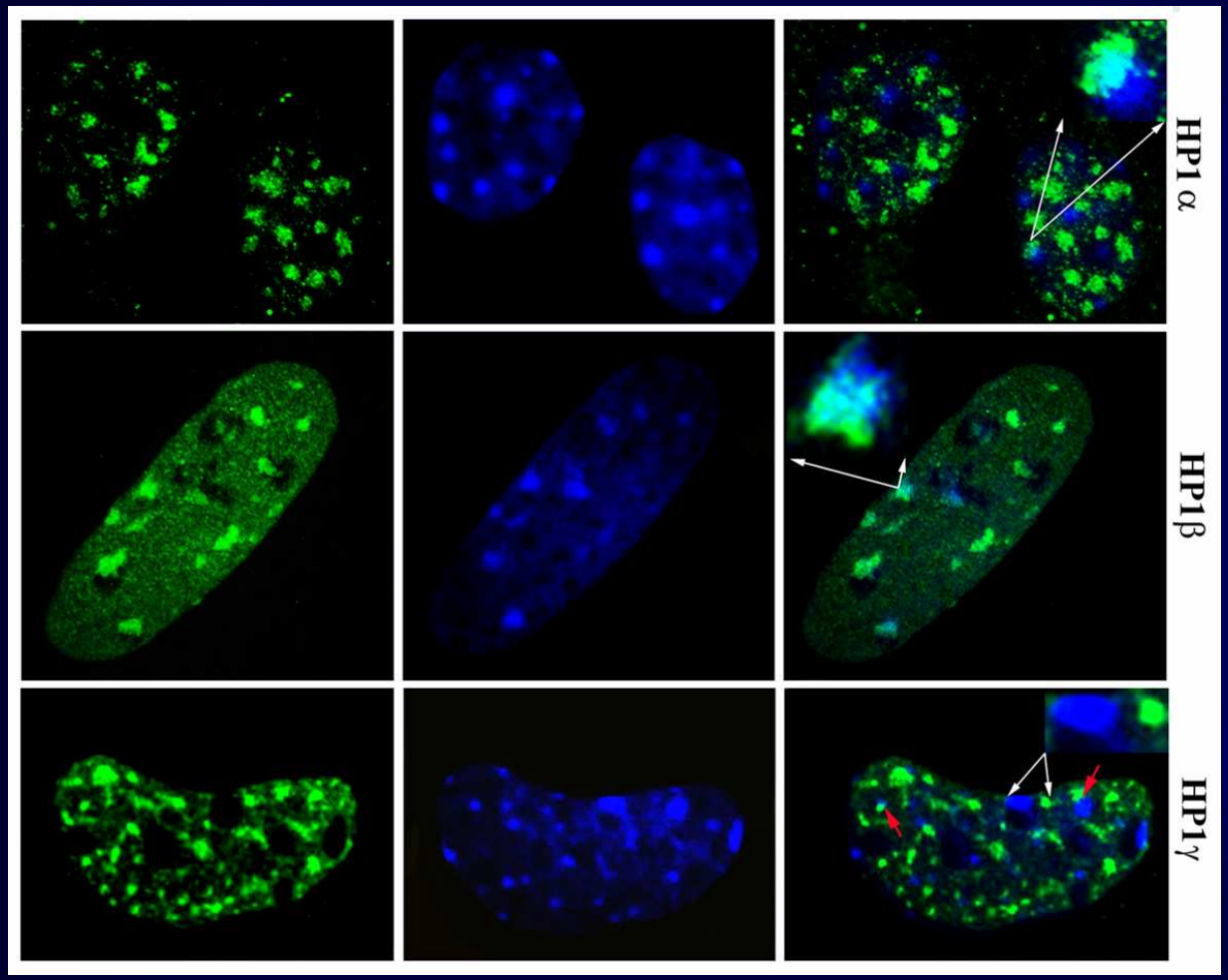
c) **Optimální regulace délky telomer a zprostředkování procesu telomeric silencing**

HP1 proteiny – v lidských buňkách jsou 3 sub-typy

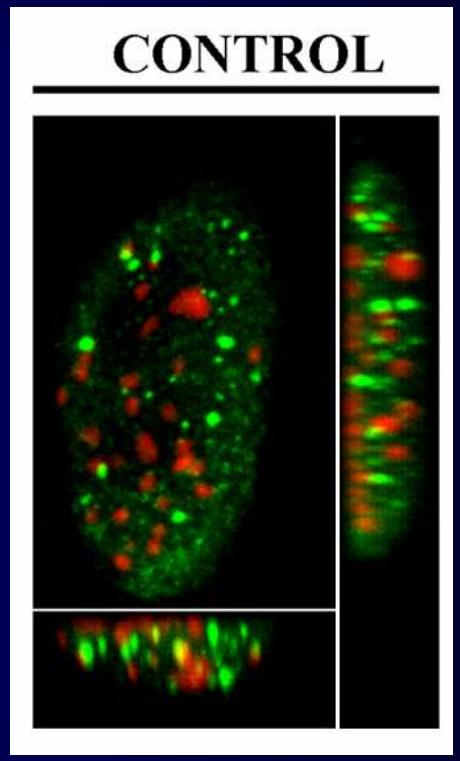


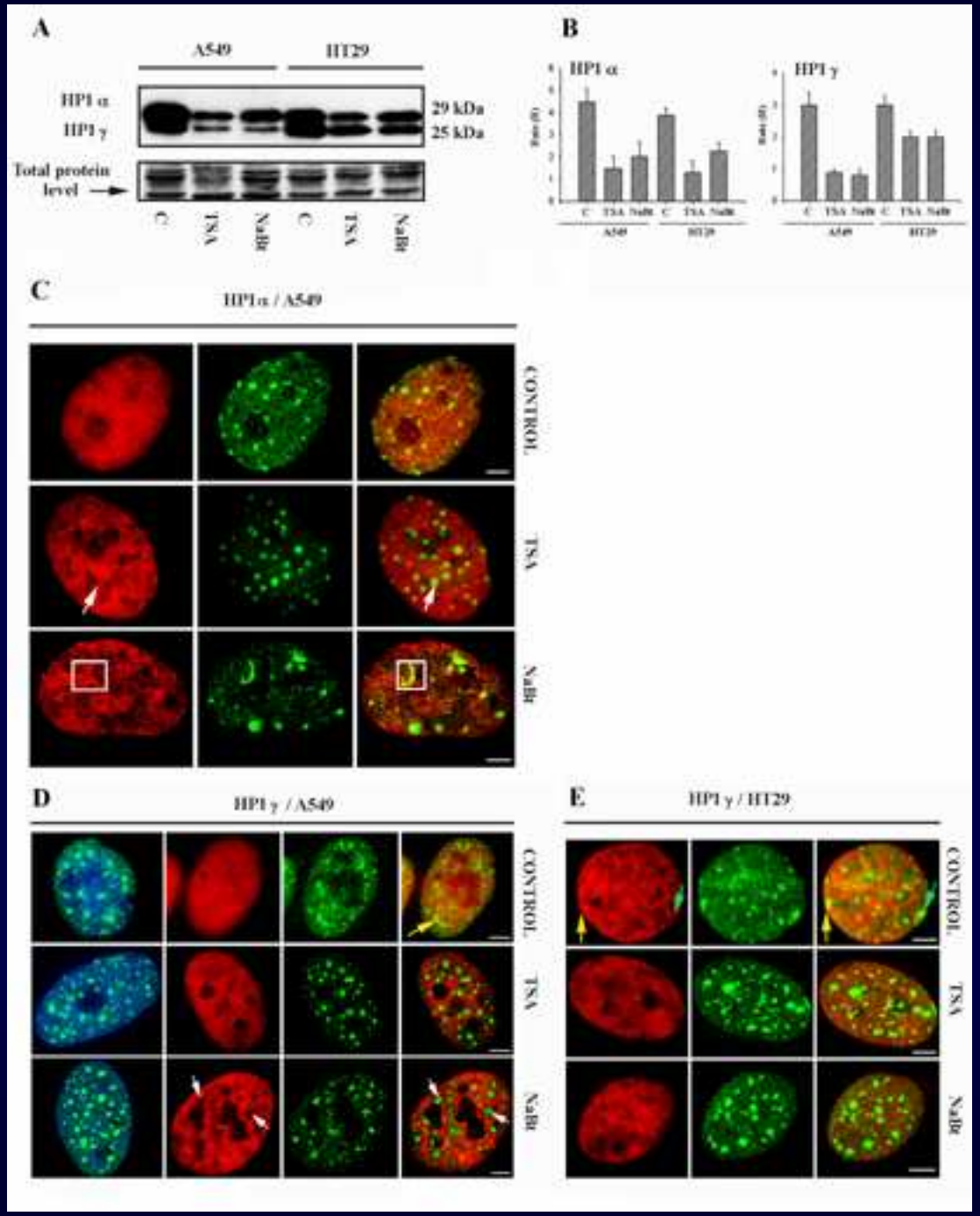
Hayakawa et al., 2003

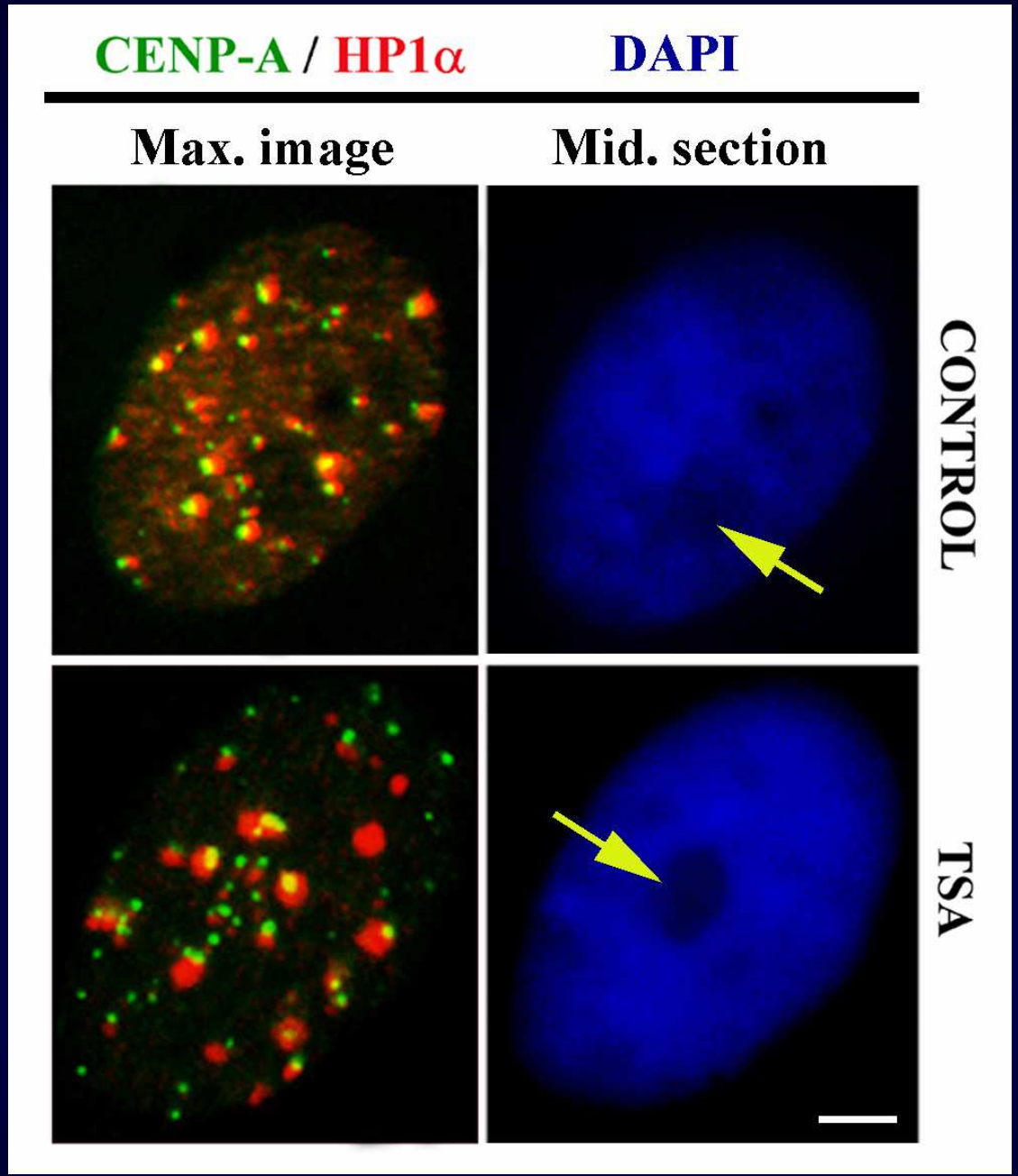
Neuronal cell differentiation of EC cells - HP1 proteins

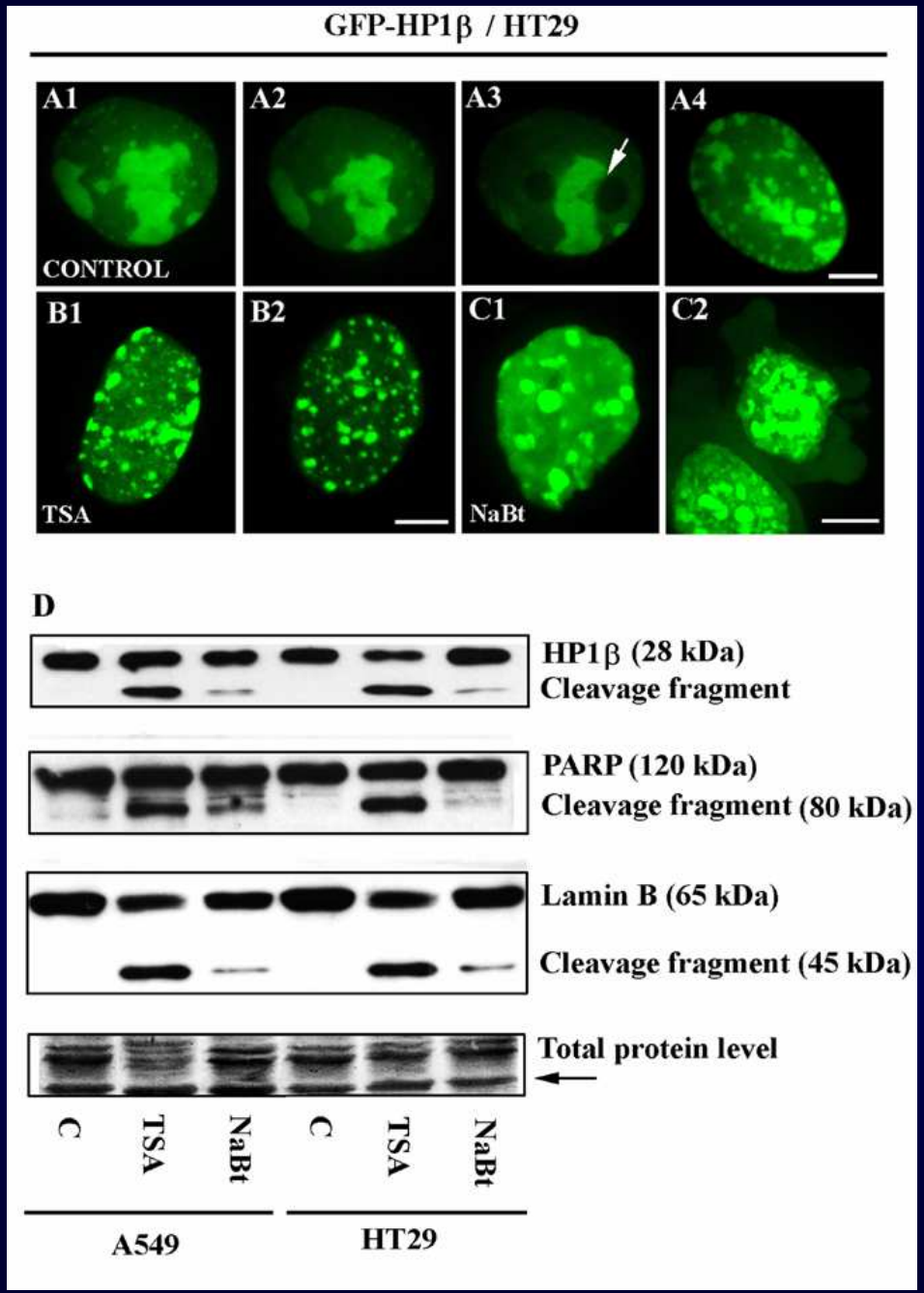


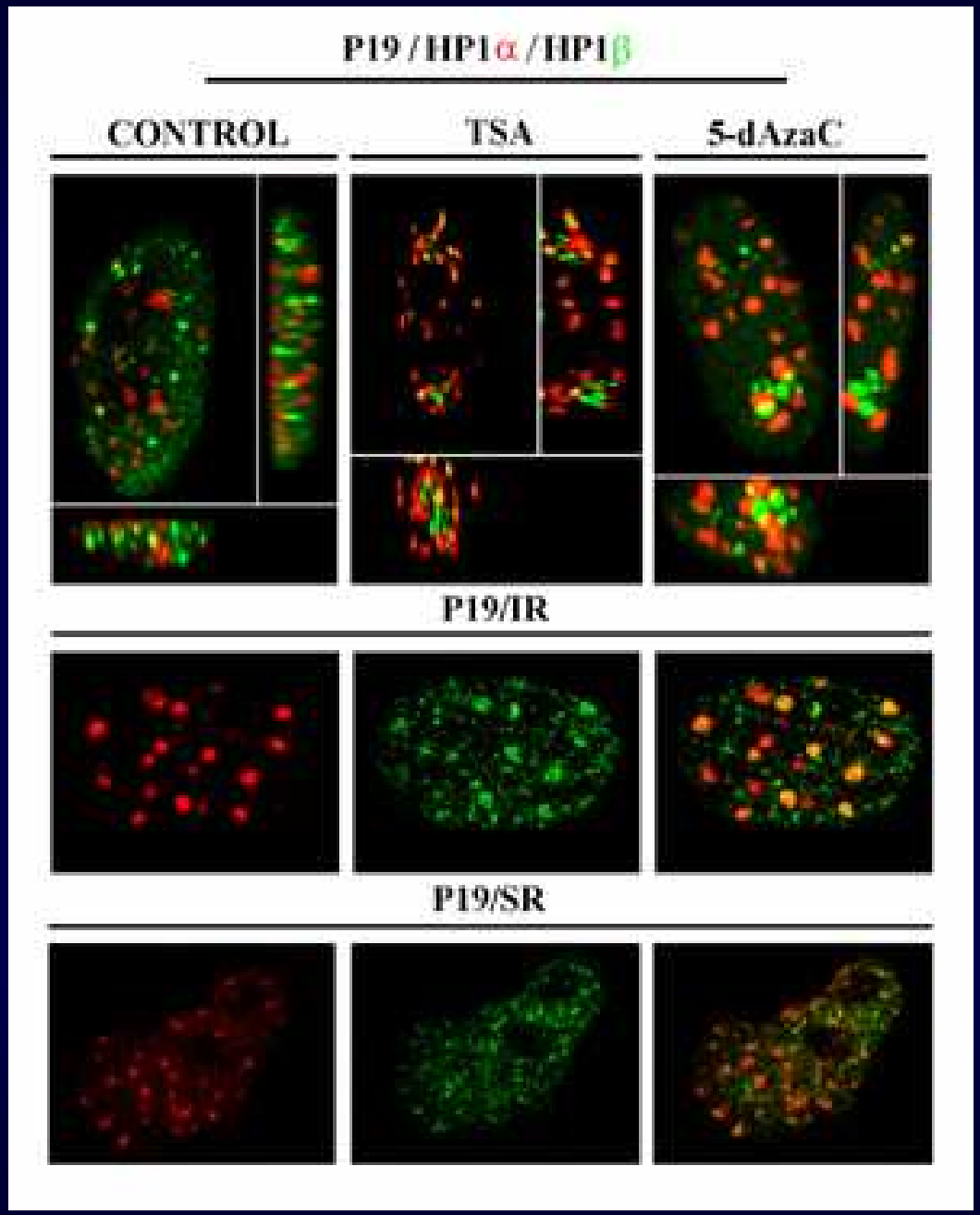
HP1α HP1β

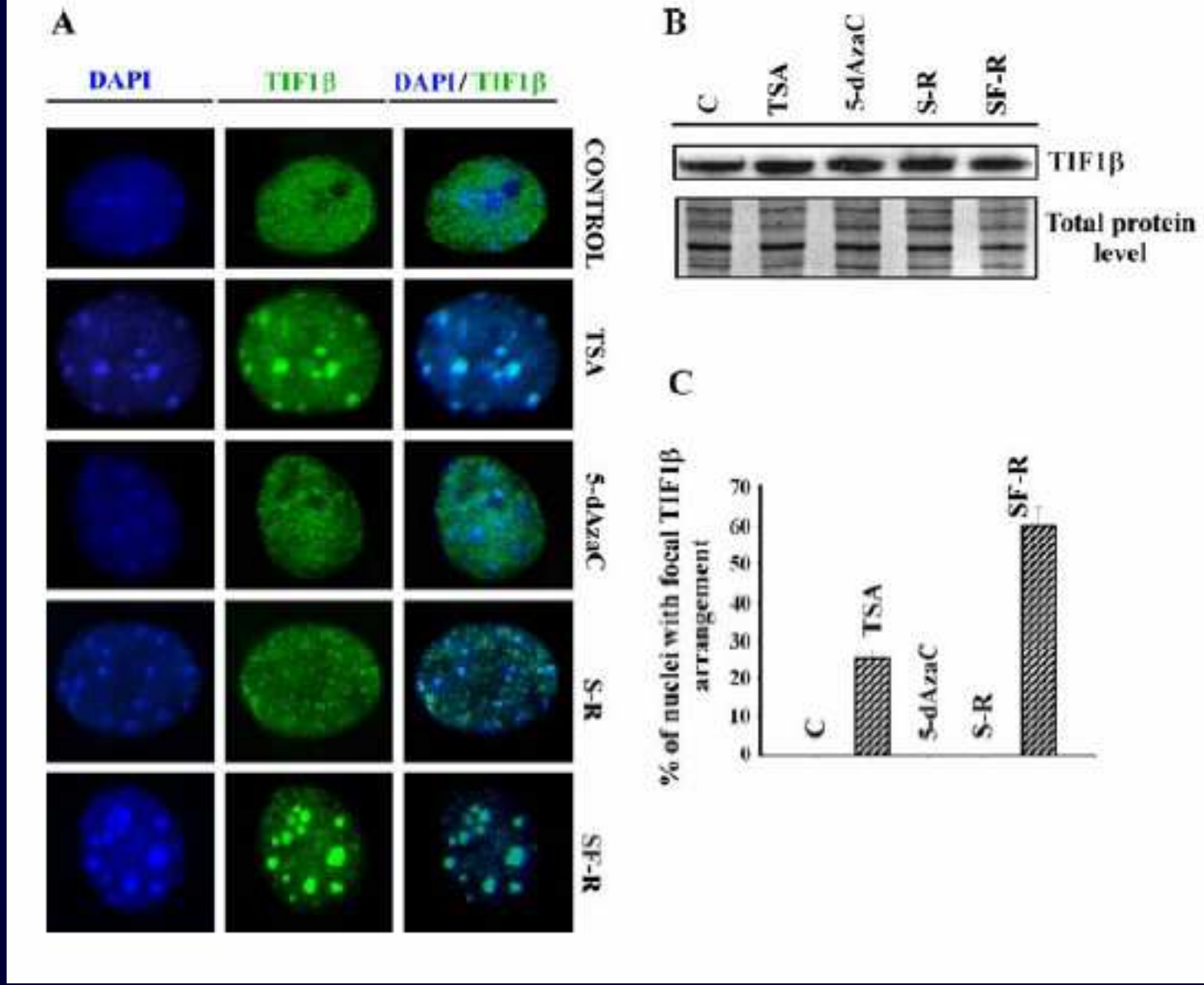


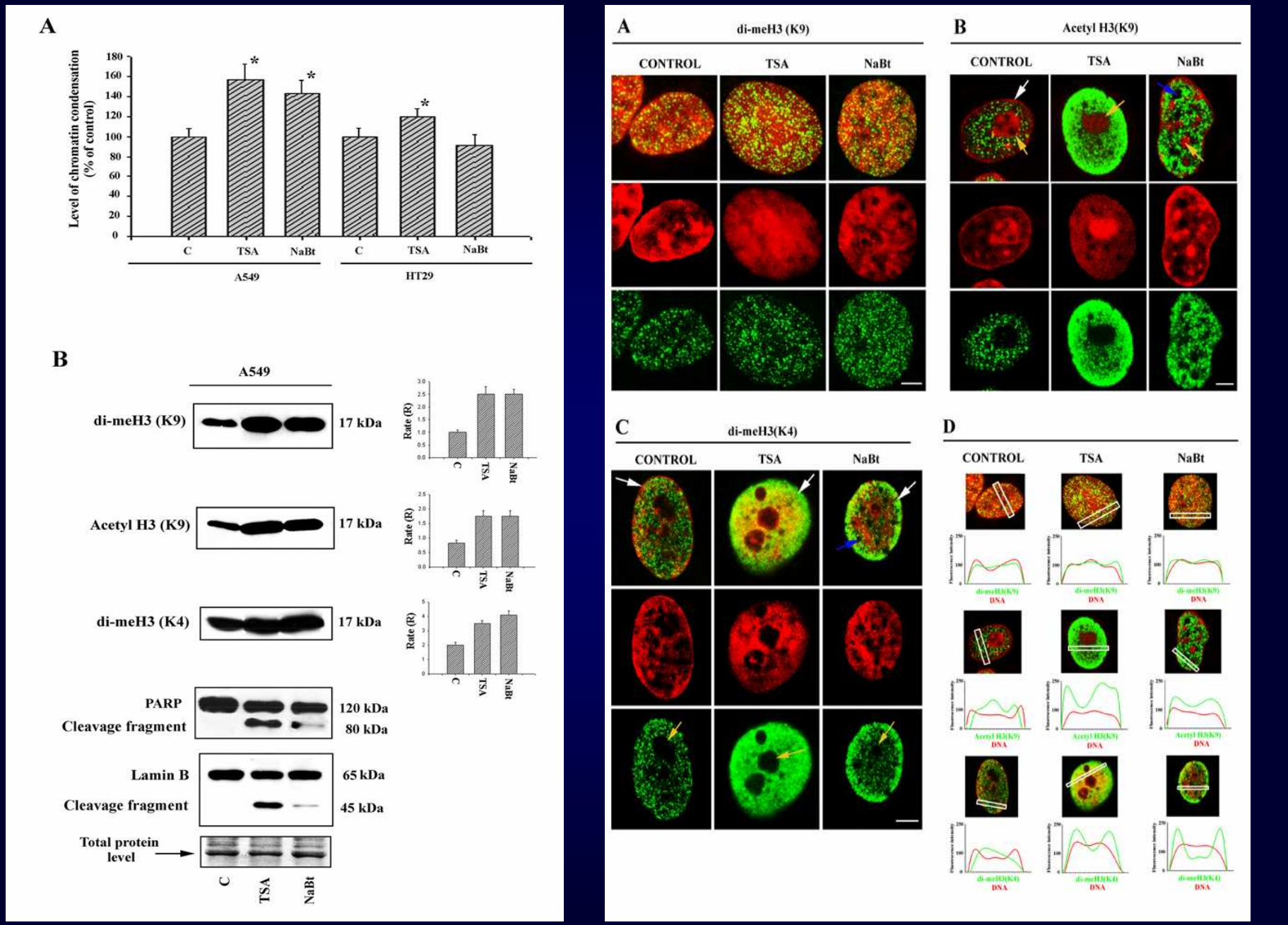

A
A549
HT29
HP1 α
HP1 γ
29 kDa
25 kDa
Total protein level
C
TSA
NaBt
B
HP1 α
HP1 γ
C
TSA
NaBt
A549
HT29
C
HP1α / A549
CONTROL
TSA
NaBt
D
HP1 γ / A549
CONTROL
TSA
NaBt
E
HP1 γ / HT29
CONTROL
TSA
NaBt


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

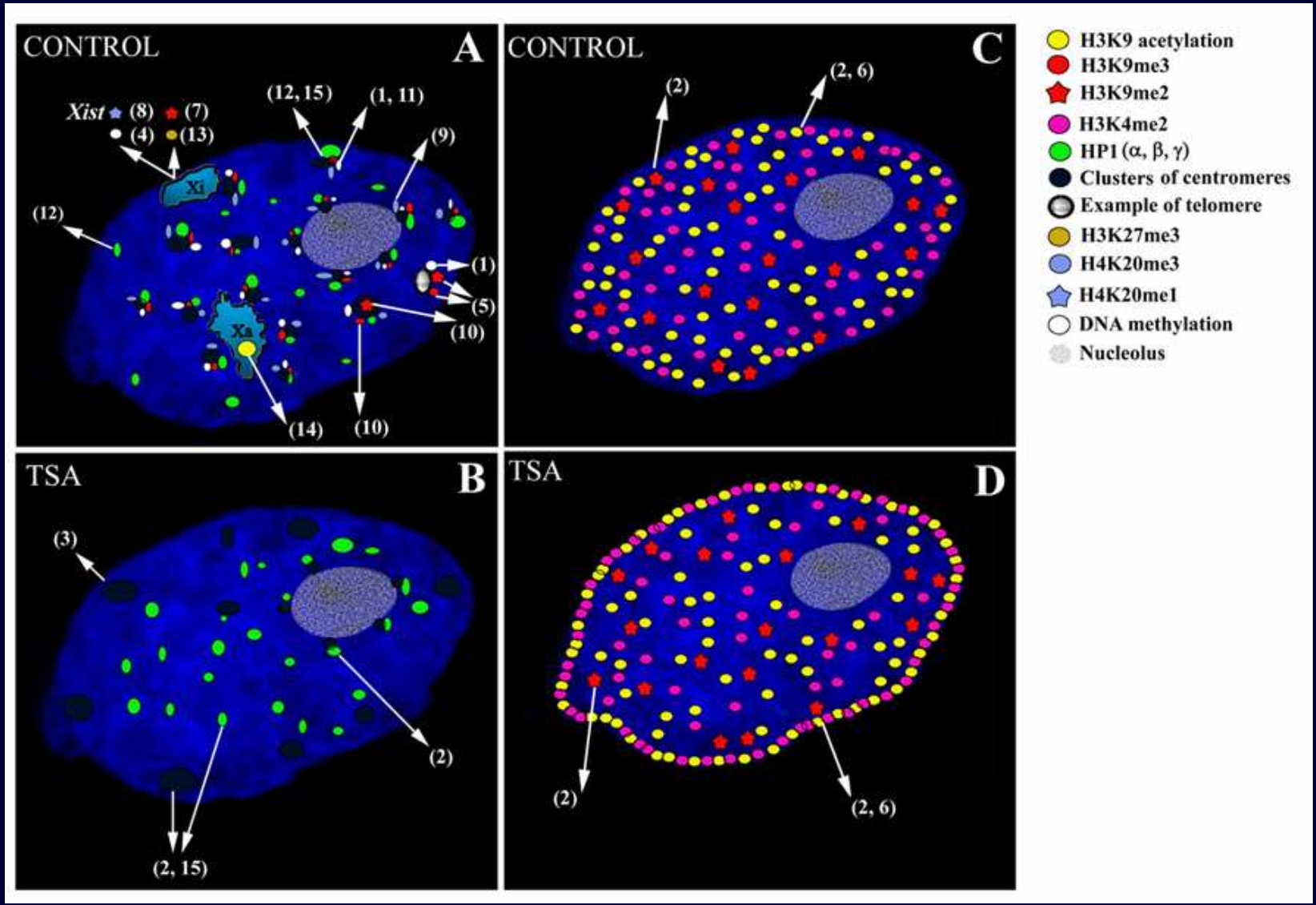

GFP-HP1β / HT29
A1
A2
A3
A4
CONTROL
B1
B2
C1
C2
TSA
NaBt
D
HP1β (28 kDa)
Cleavage fragment
PARP (120 kDa)
Cleavage fragment (80 kDa)
Lamin B (65 kDa)
Cleavage fragment (45 kDa)
Total protein level
C
TSA
NaBt
C
TSA
NaBt
A549
HT29


P19 / HP1α / HP1β
CONTROL
TSA
5-dAzaC
P19/IR
P19/SR

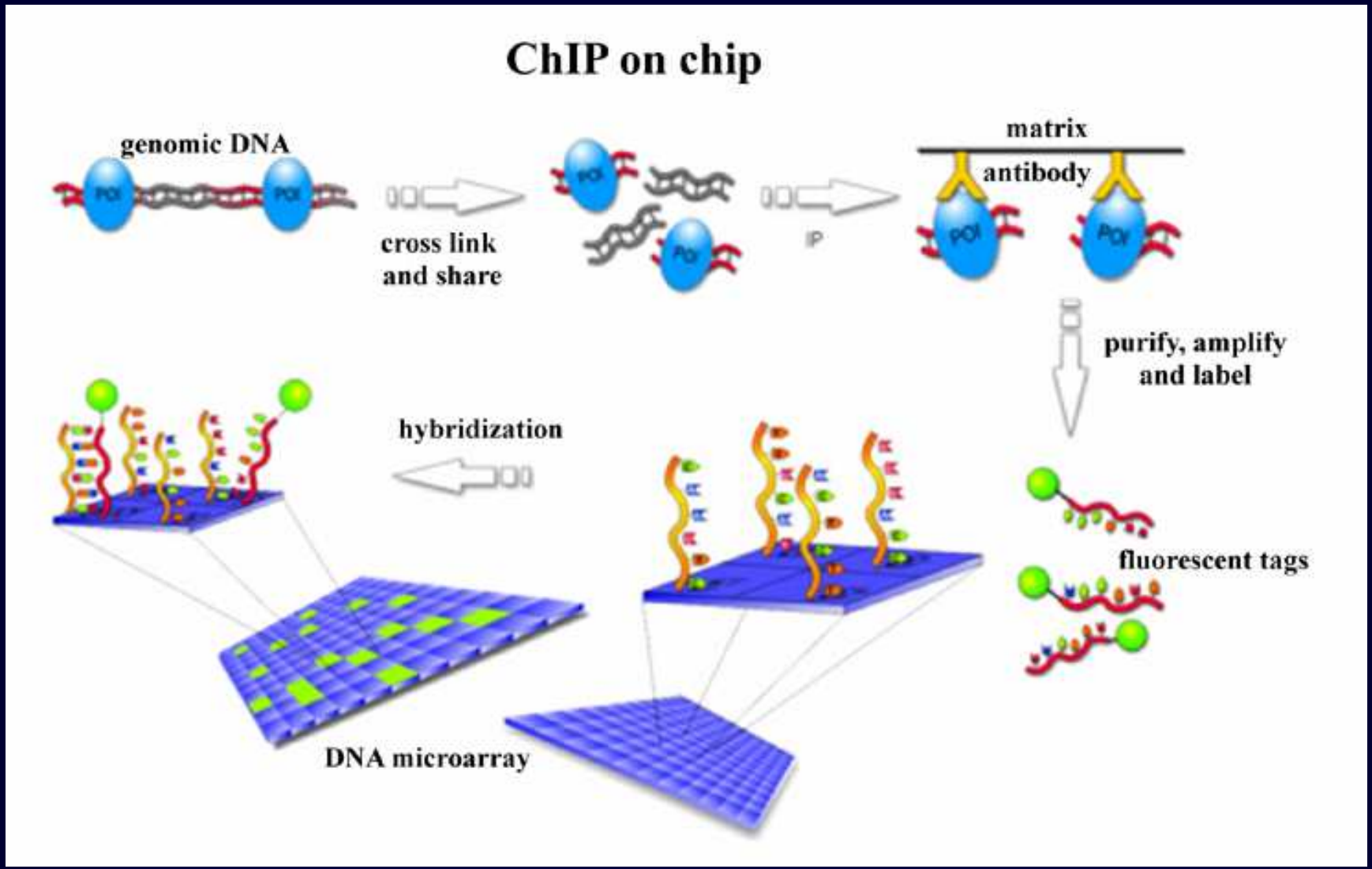

A
DAPI
TIF1β
DAPI/ TIF1β
CONTROL
TSA
5-dAzaC
S-R
SF-R
B
C
TSA
5-dAzaC
S-R
SF-R
TIF1β
Total protein level
C
% of nuclei with focal TIF1β arrangement
0
10
20
30
40
50
60
70
C
TSA
5-dAzaC
S-R
SF-R

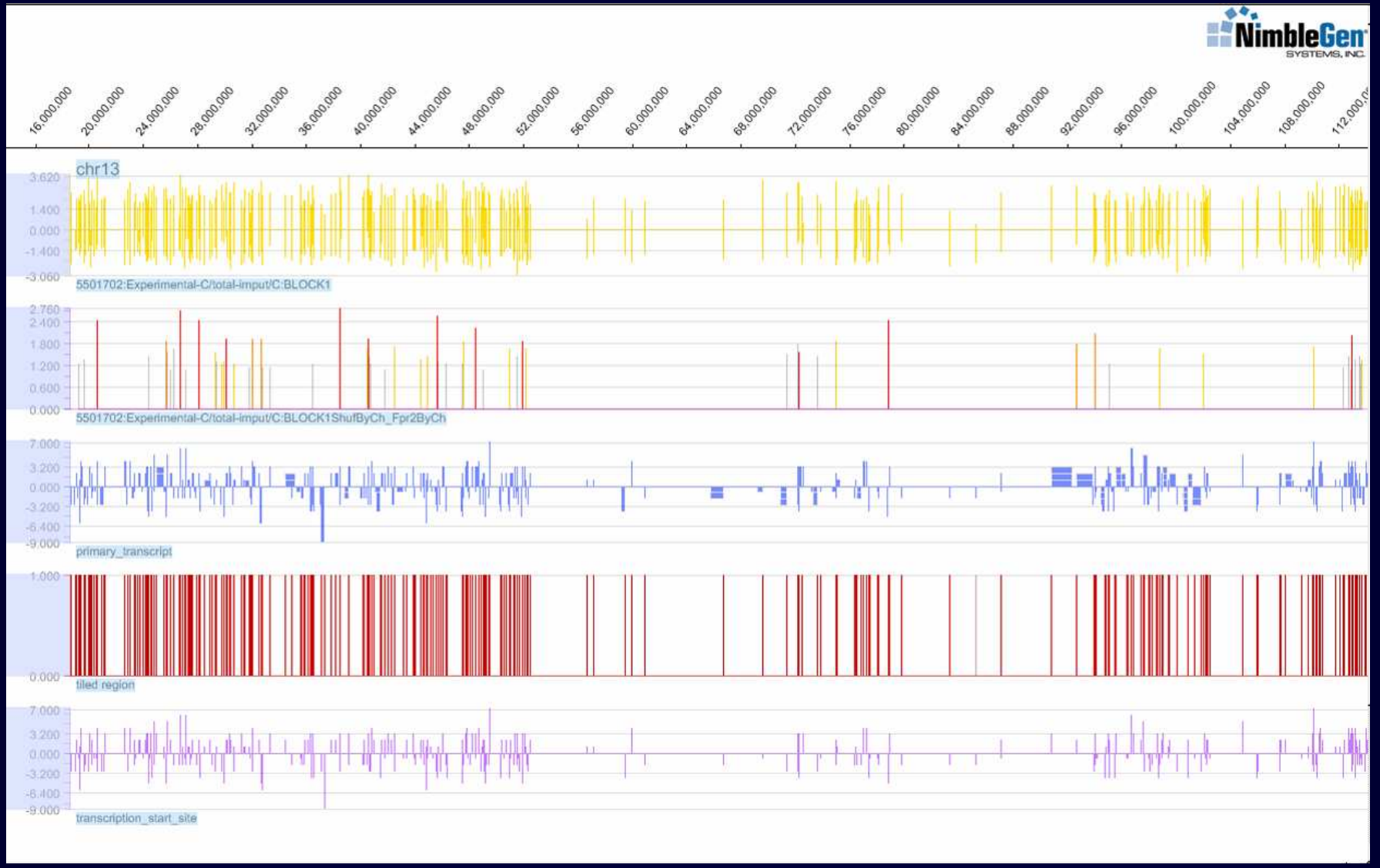

A
Level of chromatin condensation (% of control)
180
160
140
120
100
80
60
40
20
0
C
TSA
NaBt
C
TSA
NaBt
A549
HT29
B
A549
di-meH3 (K9)
17 kDa
Acetyl H3 (K9)
17 kDa
di-meH3 (K4)
17 kDa
PARP
Cleavage fragment
120 kDa
80 kDa
Lamin B
Cleavage fragment
65 kDa
45 kDa
Total protein level
Rate (R)
C
TSA
NaBt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

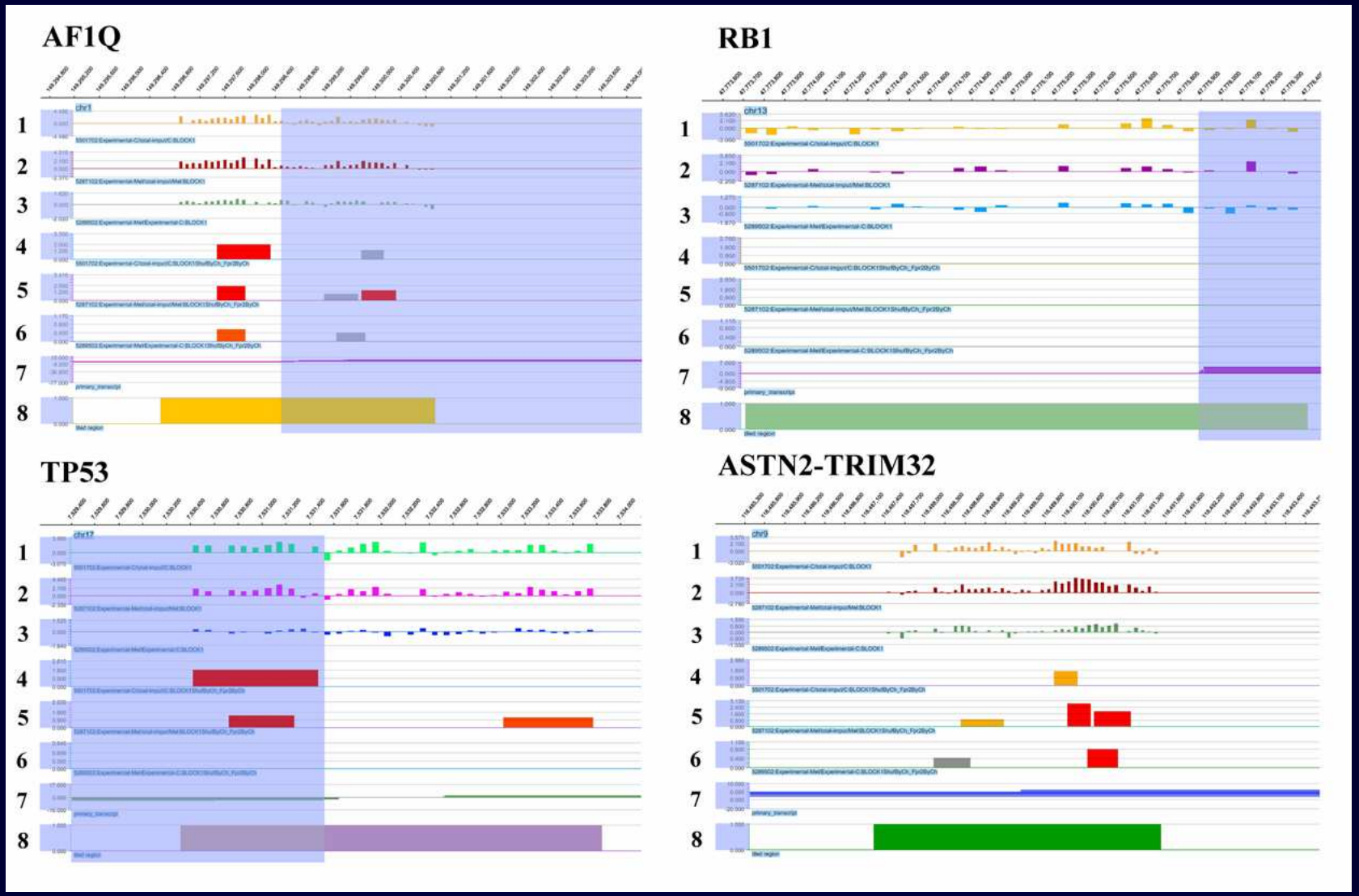
SHRNUTÍ


CONTROL
A
Xist (8) (7)
(4) (13)
(12,15)
(1, 11)
(9)
Xi
(12)
(1)
(5)
(10)
Xa
(14)
(10)
CONTROL
C
(2)
(2, 6)
TSA
B
(3)
(2)
(2, 15)
TSA
D
(2)
(2, 6)
H3K9 acetylation
H3K9me3
H3K9me2
H3K4me2
HP1 (α, β, γ)
Clusters of centromeres
Example of telomere
H3K27me3
H4K20me3
H4K20me1
DNA methylation
Nucleolus

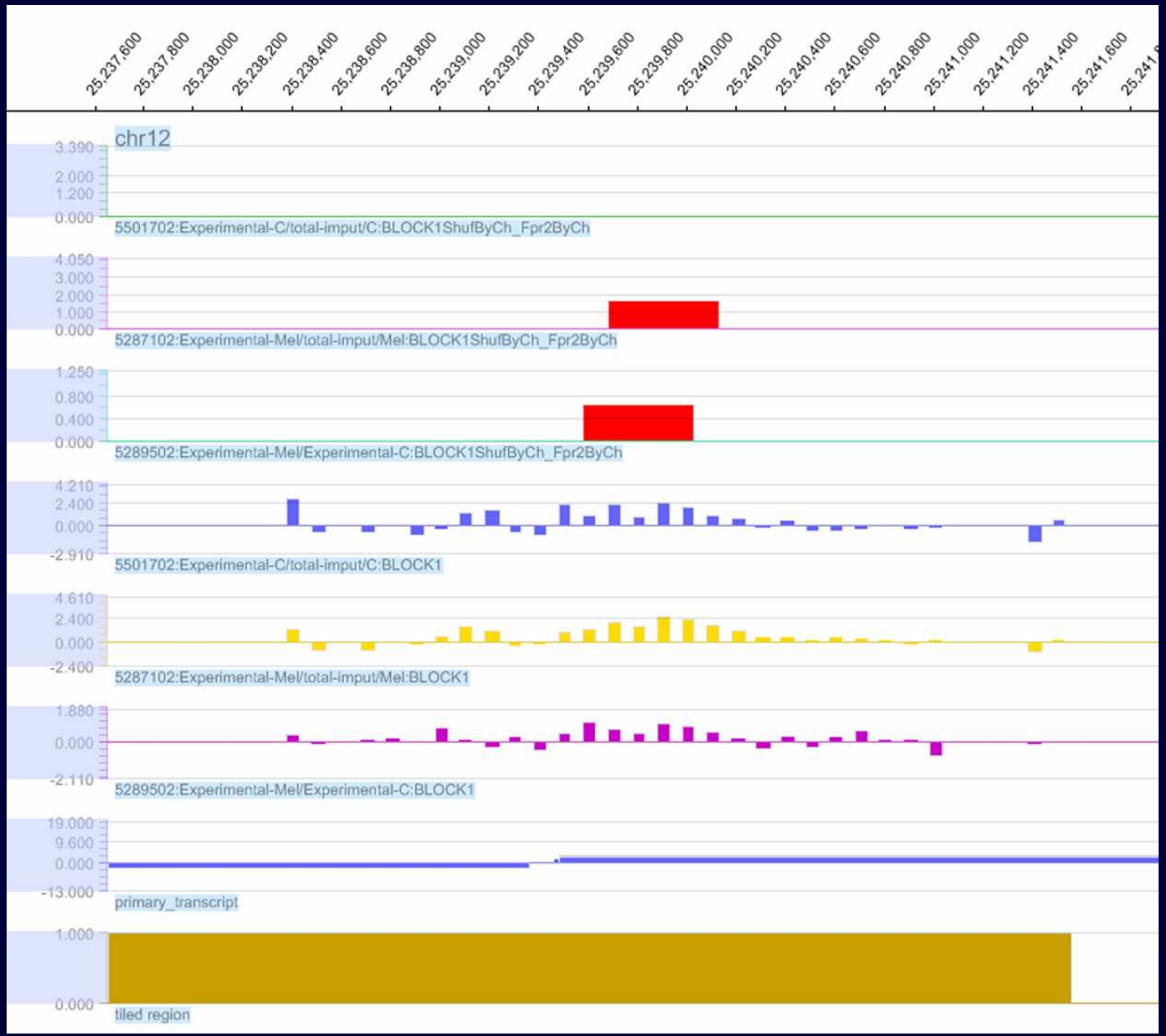
Ligation mediated PCR




NimbleGen
SYSTEMS, INC.
16,000,000
20,000,000
24,000,000
28,000,000
32,000,000
36,000,000
40,000,000
44,000,000
48,000,000
52,000,000
56,000,000
60,000,000
64,000,000
68,000,000
72,000,000
76,000,000
80,000,000
84,000,000
88,000,000
92,000,000
96,000,000
100,000,000
104,000,000
108,000,000
chr13
3.620
1.400
0.000
-1.400
-3.060
5501702:Experimental-C/total-imput/C:BLOCK1
2.760
2.400
1.800
1.200
0.600
0.000
5501702:Experimental-C/total-imput/C:BLOCK1ShufByCh_Fpr2ByCh
7.000
3.200
0.000
-3.200
-6.400
-9.000
primary_transcript
1.000
0.000
tiled region
7.000
3.200
0.000
-3.200
-6.400
-9.000
transcription_start_site


AF1Q
RB1
TP53
ASTN2-TRIM32

Cancer susceptibility gene 1



Shrnutí problematiky

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