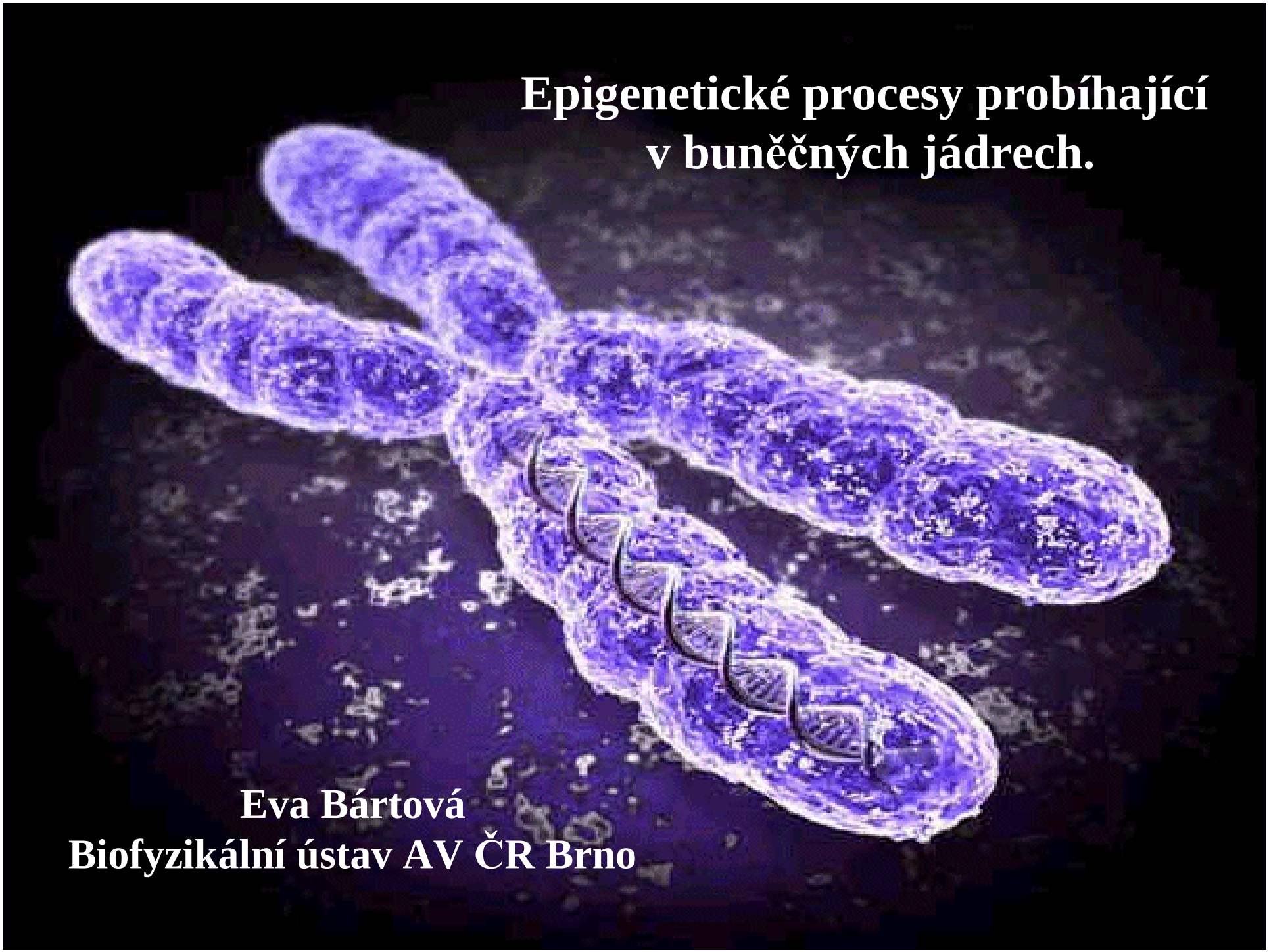
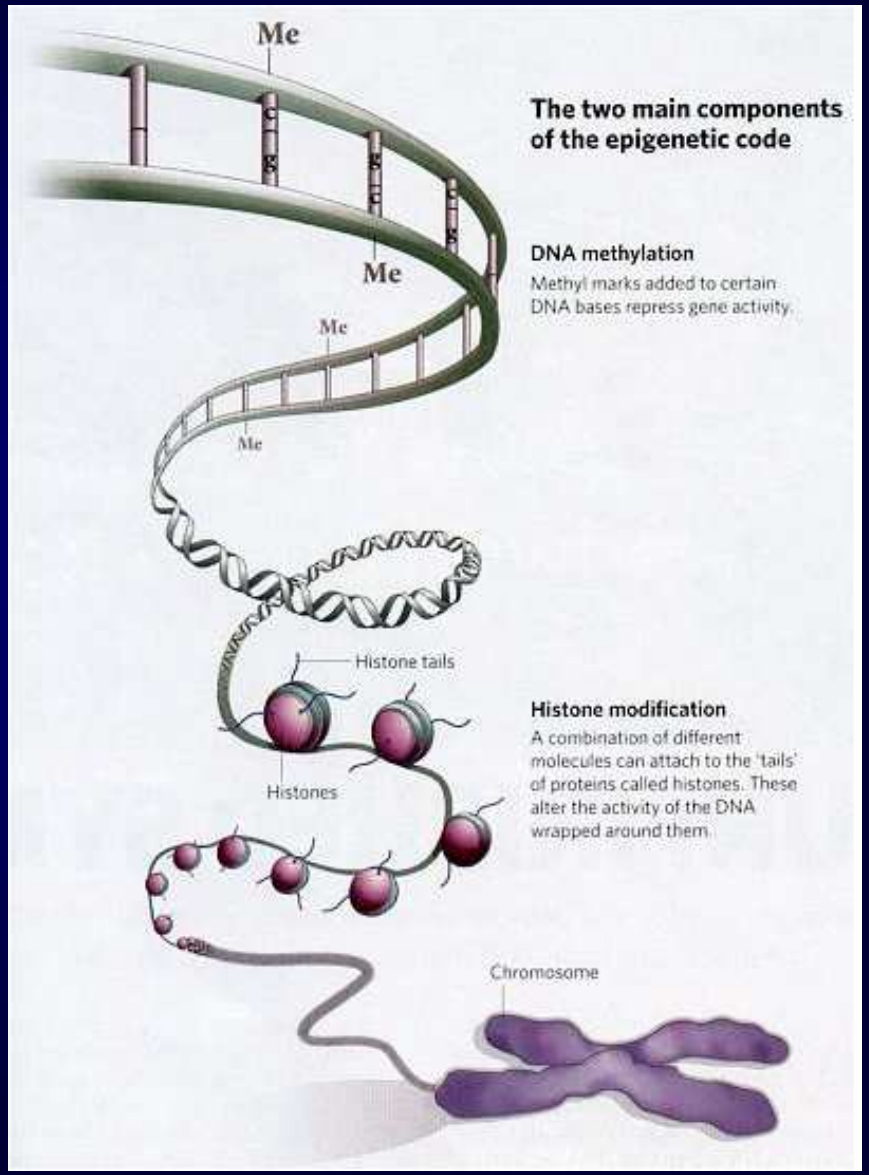
Epigenetické procesy probíhající v buněčných jádrech.



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.



Epigenator:

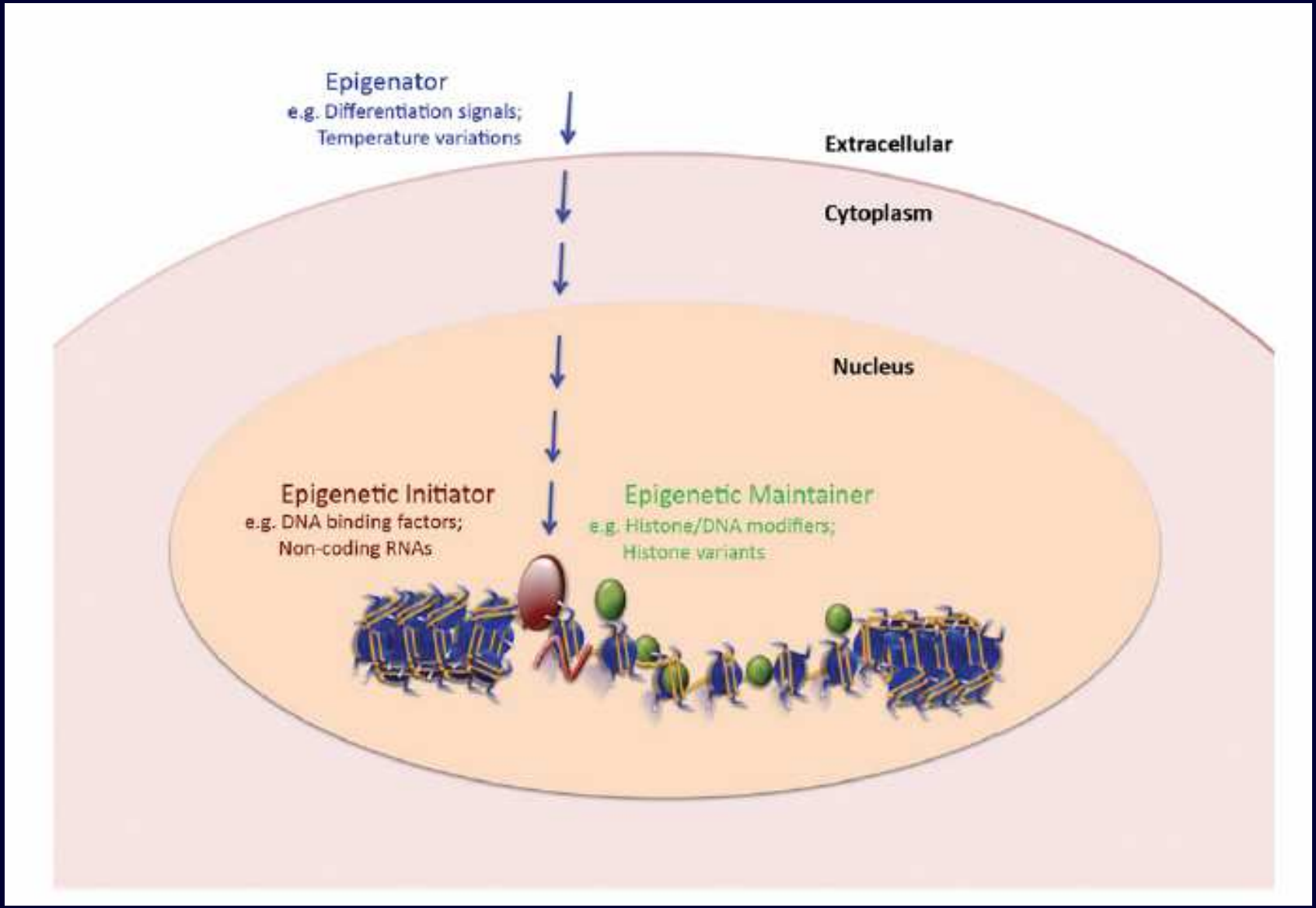
emanates from the environment and triggers and Intracellular pathway. Epigenetic signaling pathway could be a protein-protein interaction or modification-based events.

Epigenetic initiator:

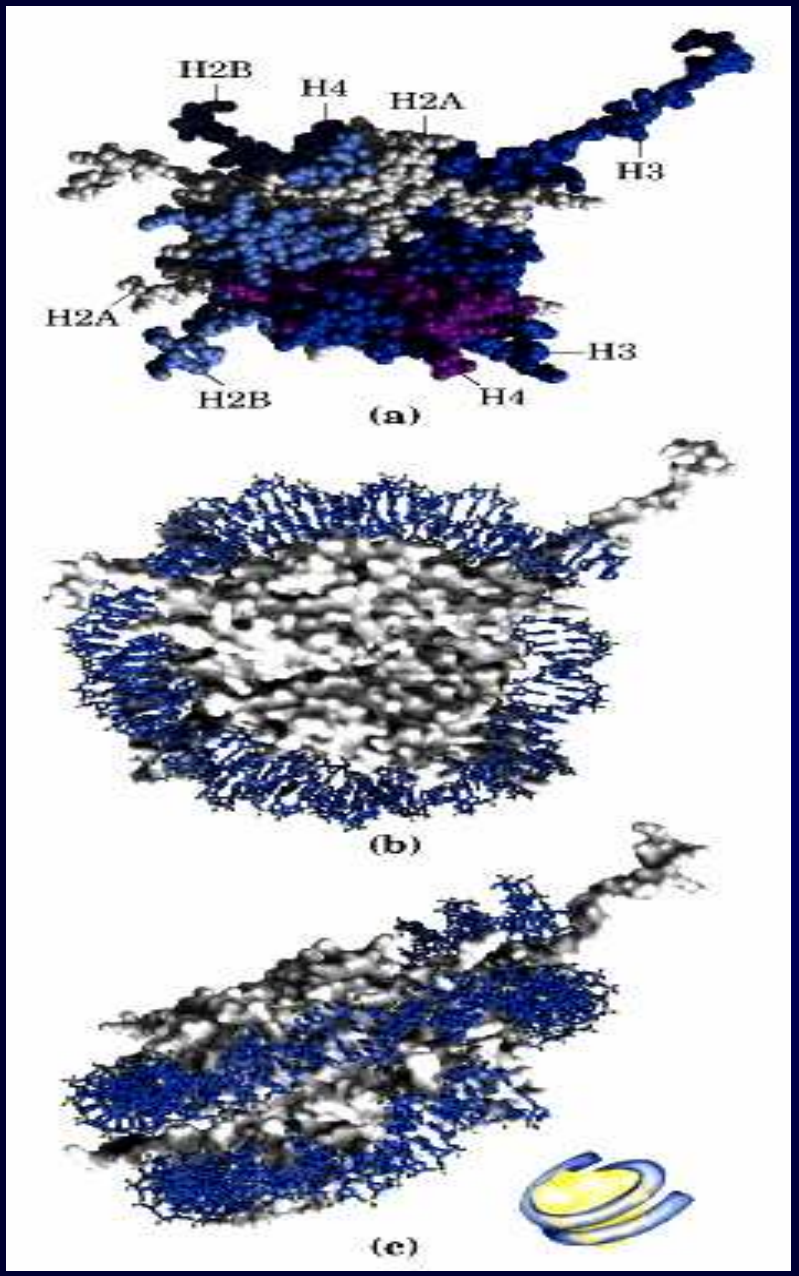
signal, which responds to the Epigenator and is necessary to define the precise location of epigenetic chromatin environment. Initiator could be DNA-binding protein, non-coding RNA, factor that coordinates chromatin structure.

Epigenetic Maintainer:

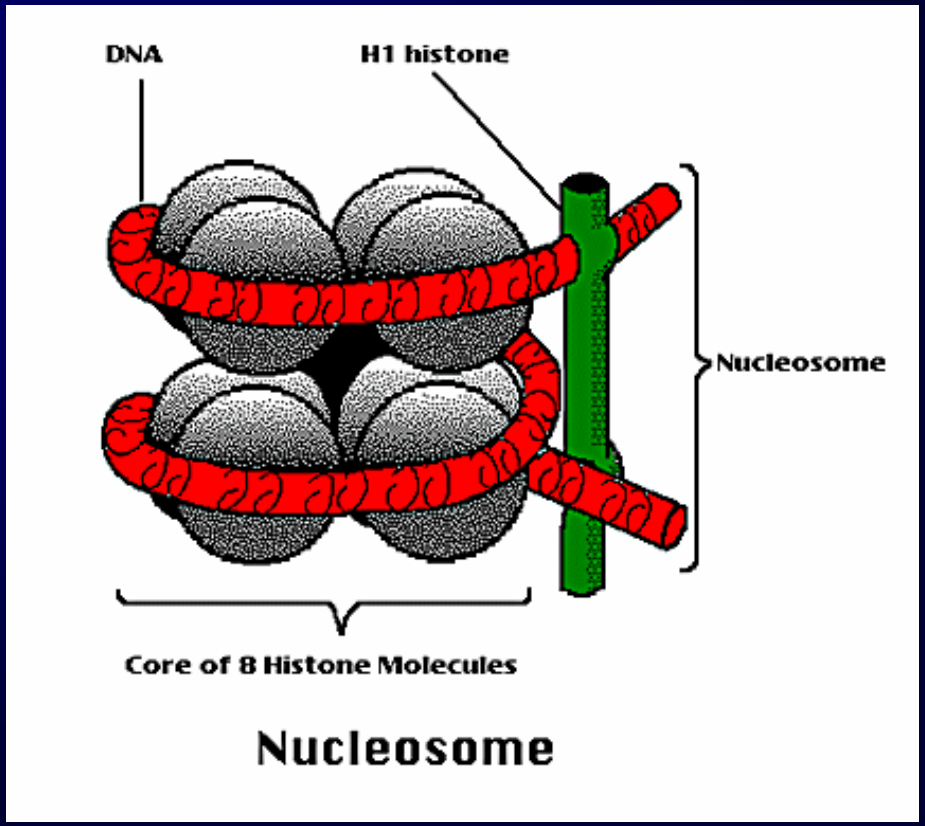
signal, which sustained the chromatin environment in the first and subsequent generation. It is DNA methylation, histone modification, histone variants, nucleosome positioning. (Berger S. et al., 2009)

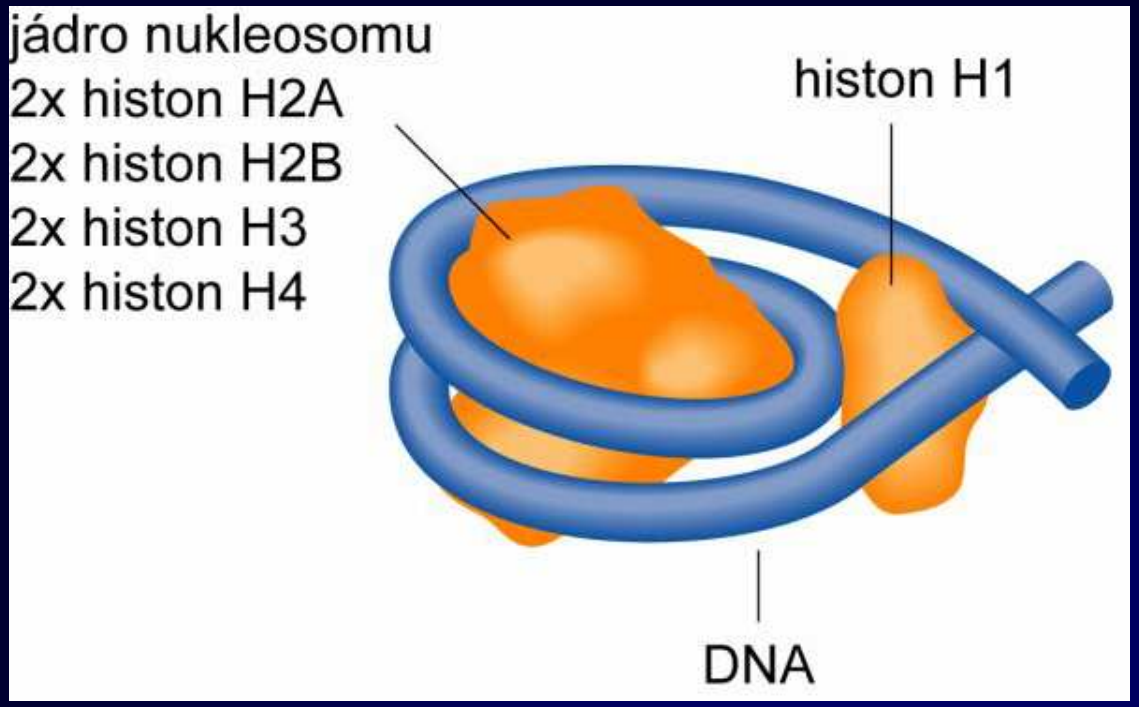


Berger et al., Genes Dev., 2009



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.

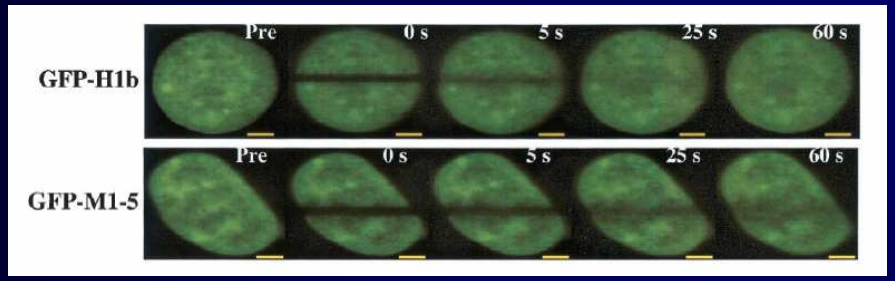




1. **Buňka může existovat i bez významně redukovaného množství H1.**
2. **H1 varianty nejsou hlavní determinanty buněčného fenotypu.**
3. **Funkce H1 variant je nejenom při utlumení transkripční aktivity ale také při její aktivaci (může snižovat nebo i zvyšovat expresi specifických genů.**
4. **H1 hraje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

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.

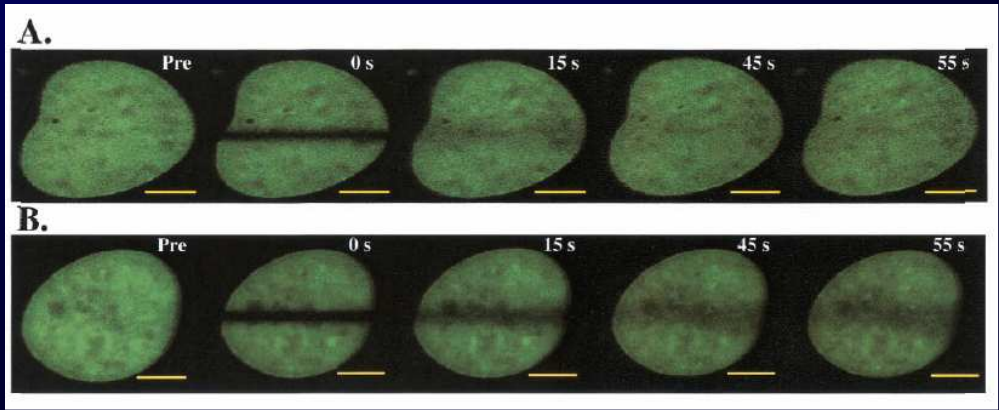


GFP-M1-5: five cyclin-dependent kinase phosphorylation consensus sites were mutated from serine or threonine residues into alanines

Overexpression p21

GFP-H1b

GFP-M1-5



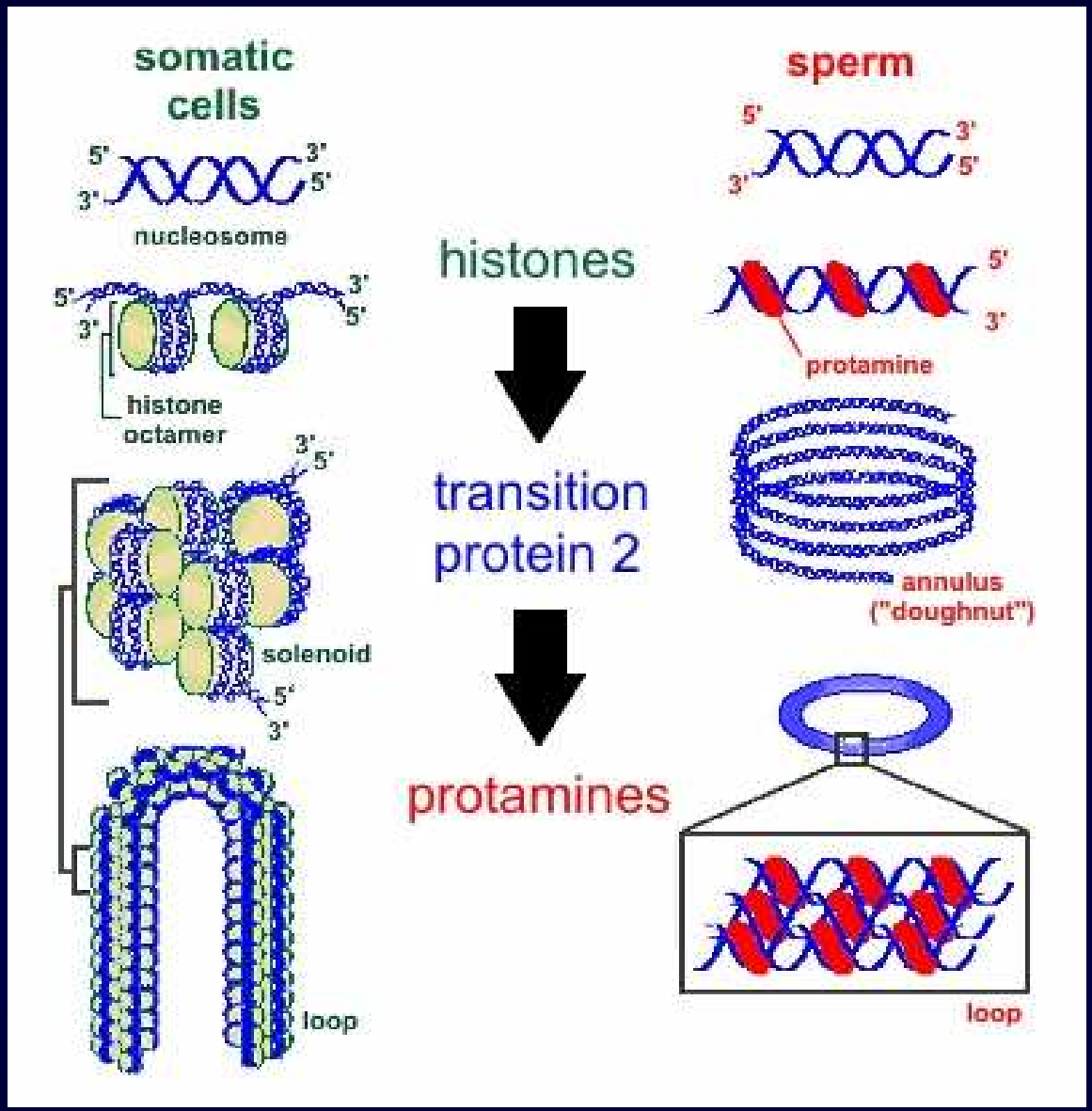
Varianty histonů

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 – nahrazení histonů protaniny

.

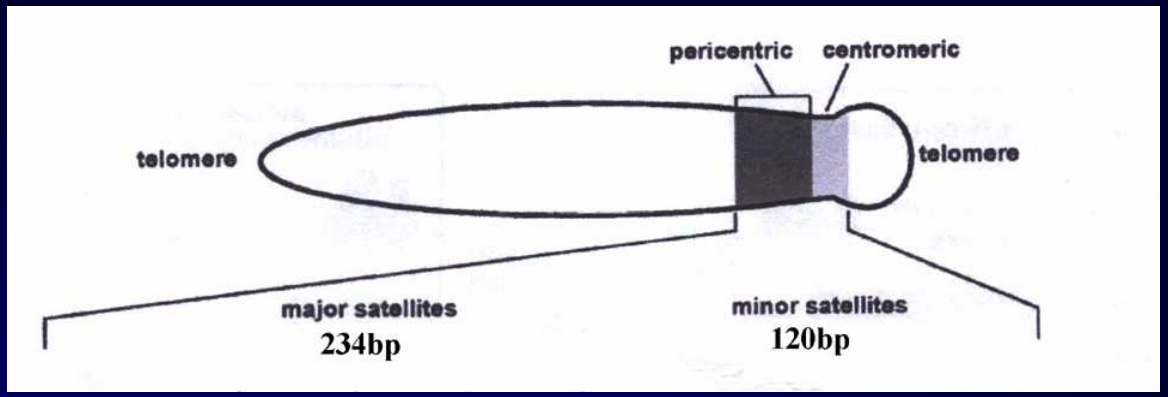

somatic cells
5'
3'
3'
5'
nucleosome
5'
3'
3'
5'
histone octamer
3'
5'
solenoid
5'
3'
loop
histones
transition protein 2
protamines
sperm
5'
3'
3'
5'
5'
3'
protamine
annulus ("doughnut")
loop

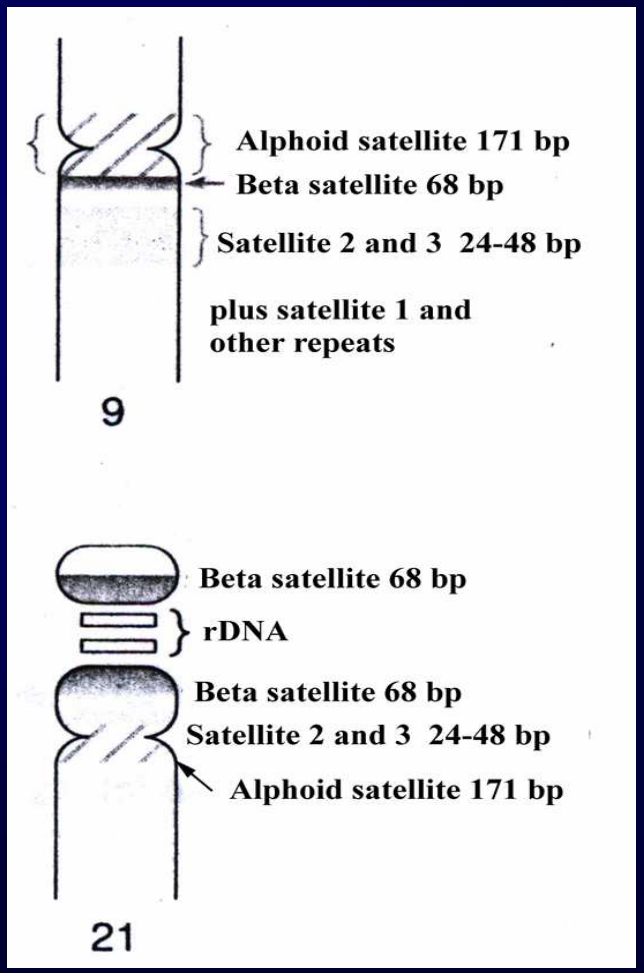
Varianty histonů

H3: existují dvě hlavní

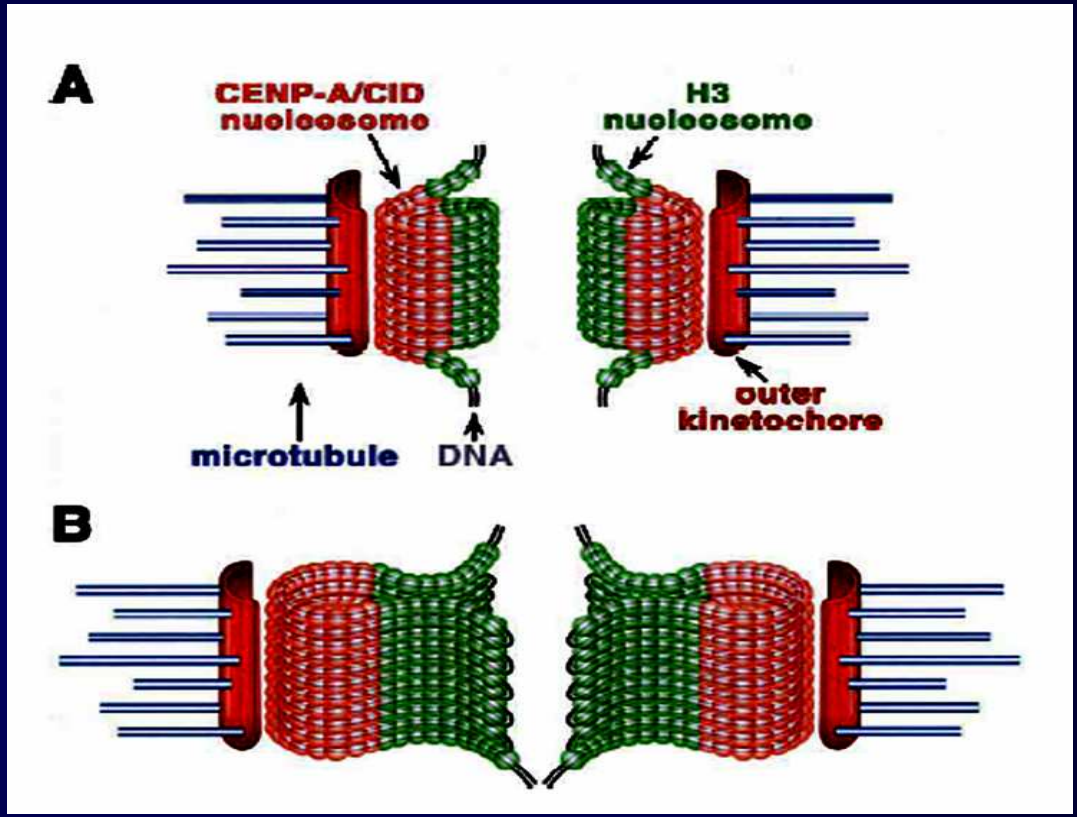
Varianty H3.3 a centromerické varianty

H3 (cenH3) = CENP-A-Z: 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 CENP-A plays an important role for aneuploidy in colorectal cancers.**



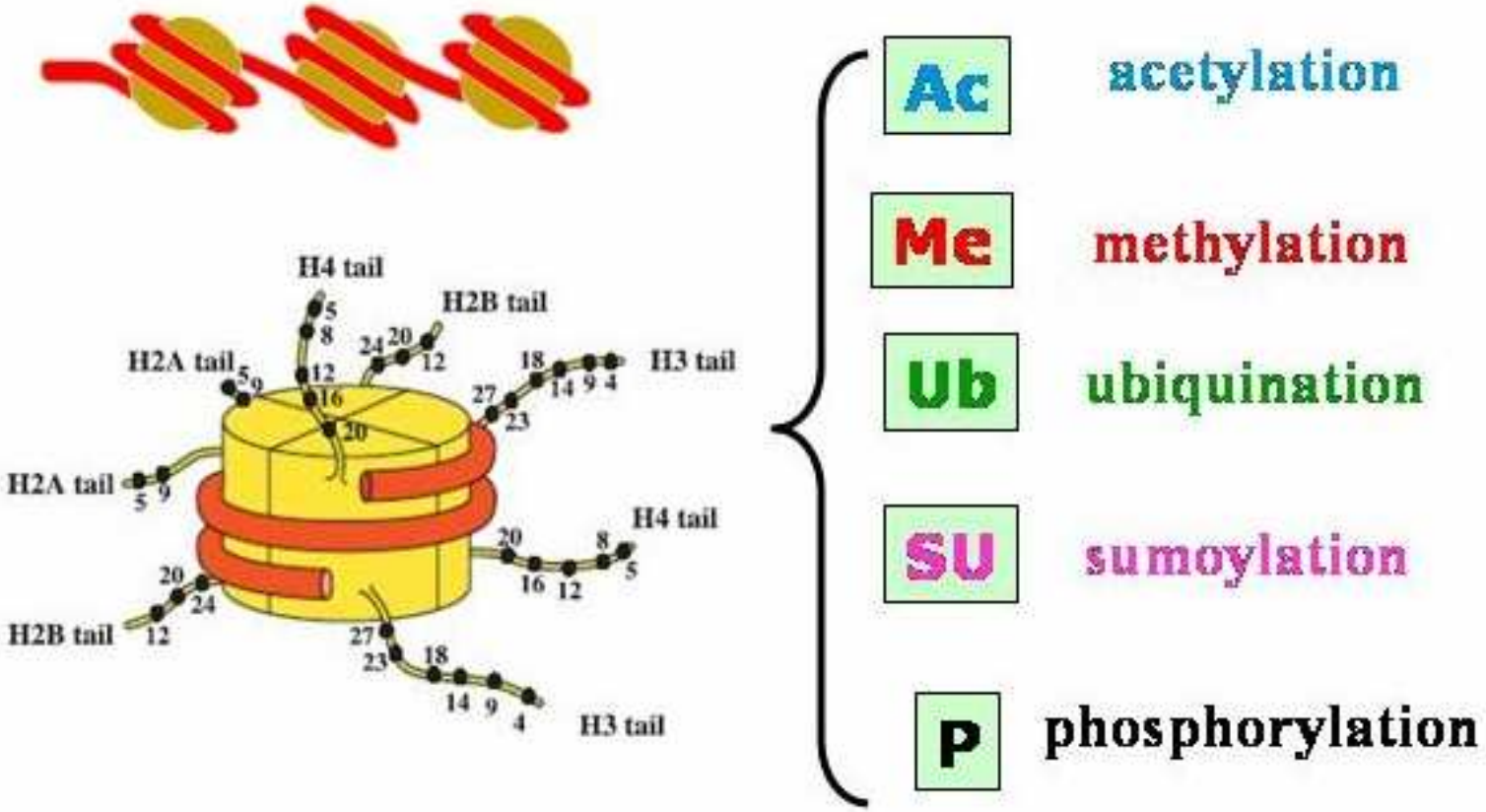
Varianty histonů H4: **většina genů kódujících hlavní histonové proteiny jsou exprimovány během S fáze buněčného cyklu. V případě H4, geny jsou konstitutivně exprimovány během buněčného cyklu. Pro H4 nejsou známy žádné varianty. Úpravy pre-mRNA histonů probíhají v Cajal bodies.**

Biochemické modifikace histonů

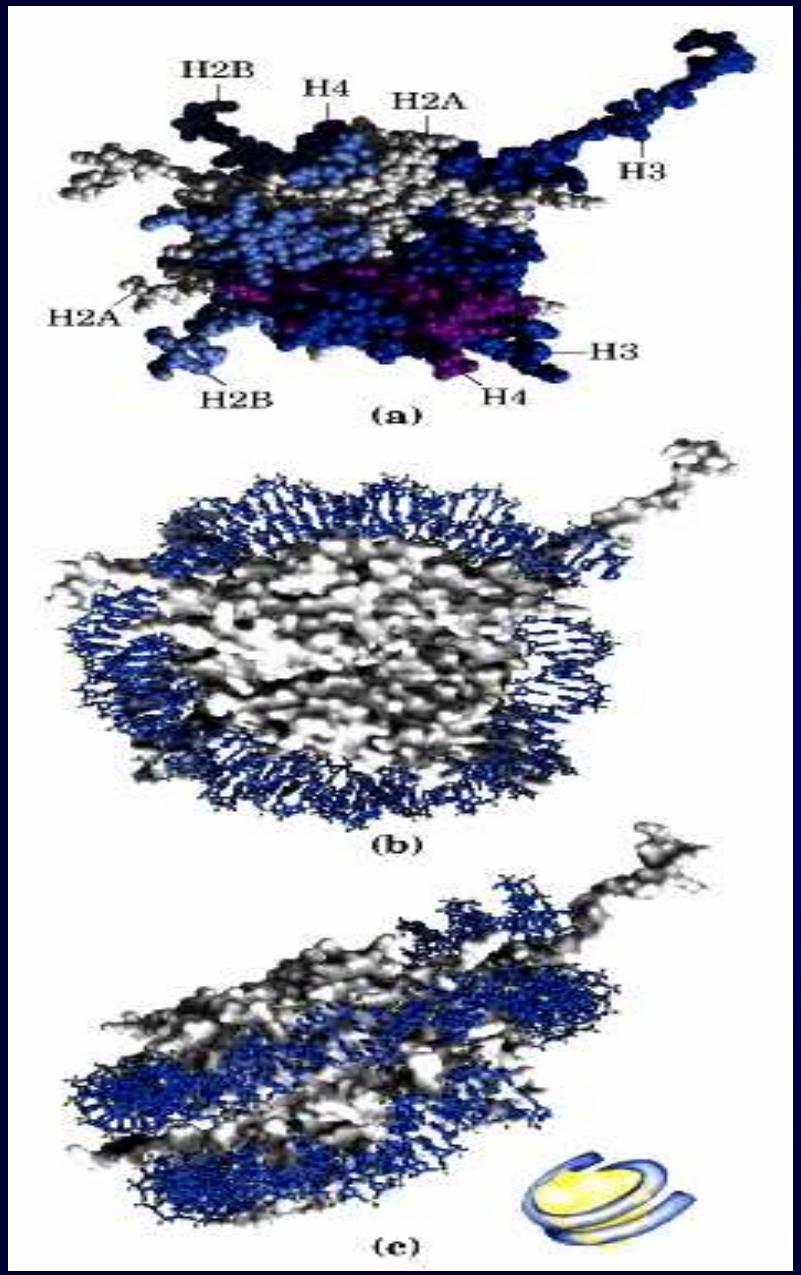
- **Dynamická struktura chromatinu je přímo ovlivněná post-translačními modifikacemi amino-konců histonů**

- **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)

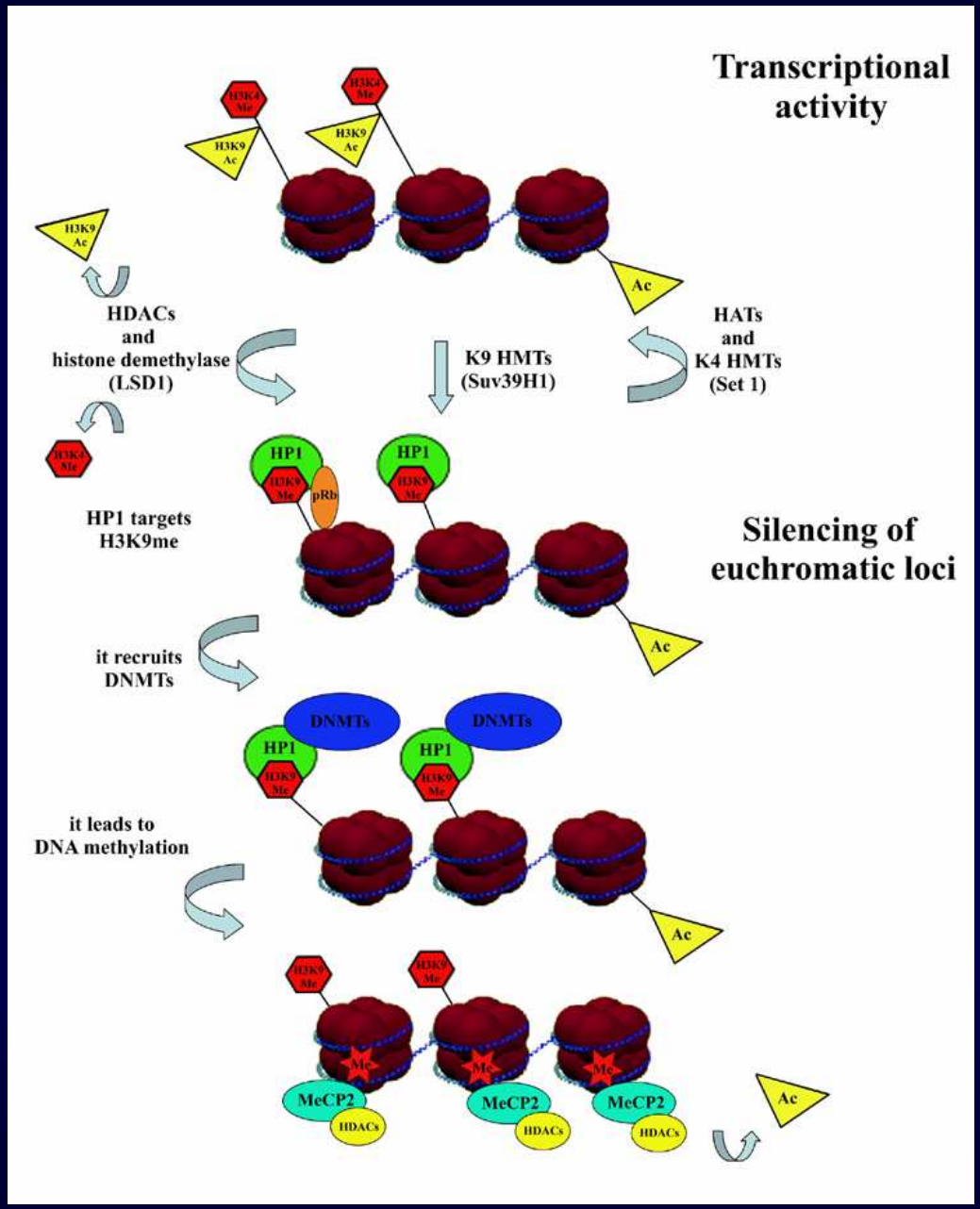
2004: Objev demethylace histonů za účasti aminové oxidasy LSD1 (KIAA0601) (Shi et al., Cell 2004). LSD1 specificky demethyluje H3 (K4), epigenetickou modifikaci zodpovědnou za transkripční aktivitu.

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

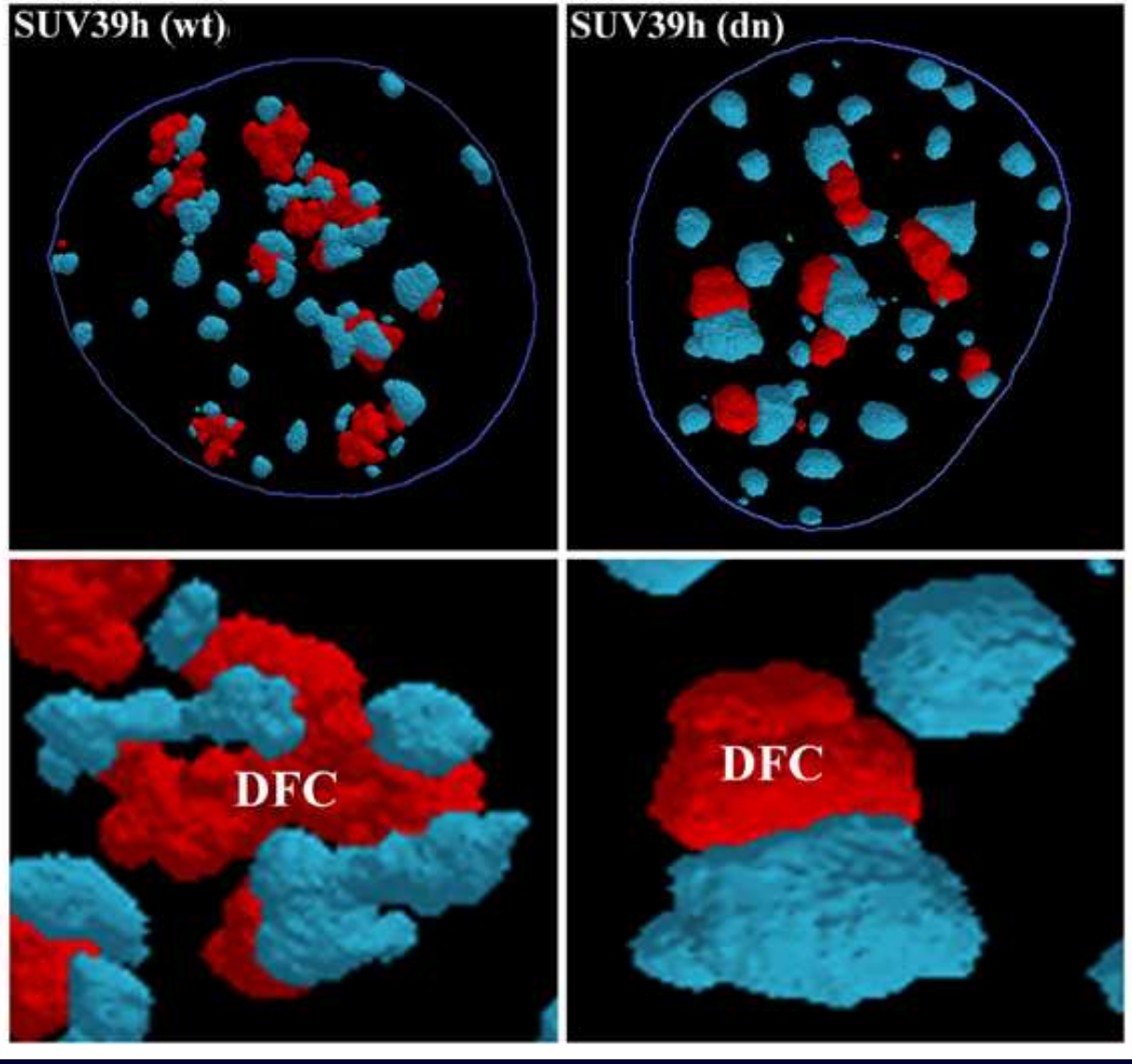
HDACs: Class I, II, III

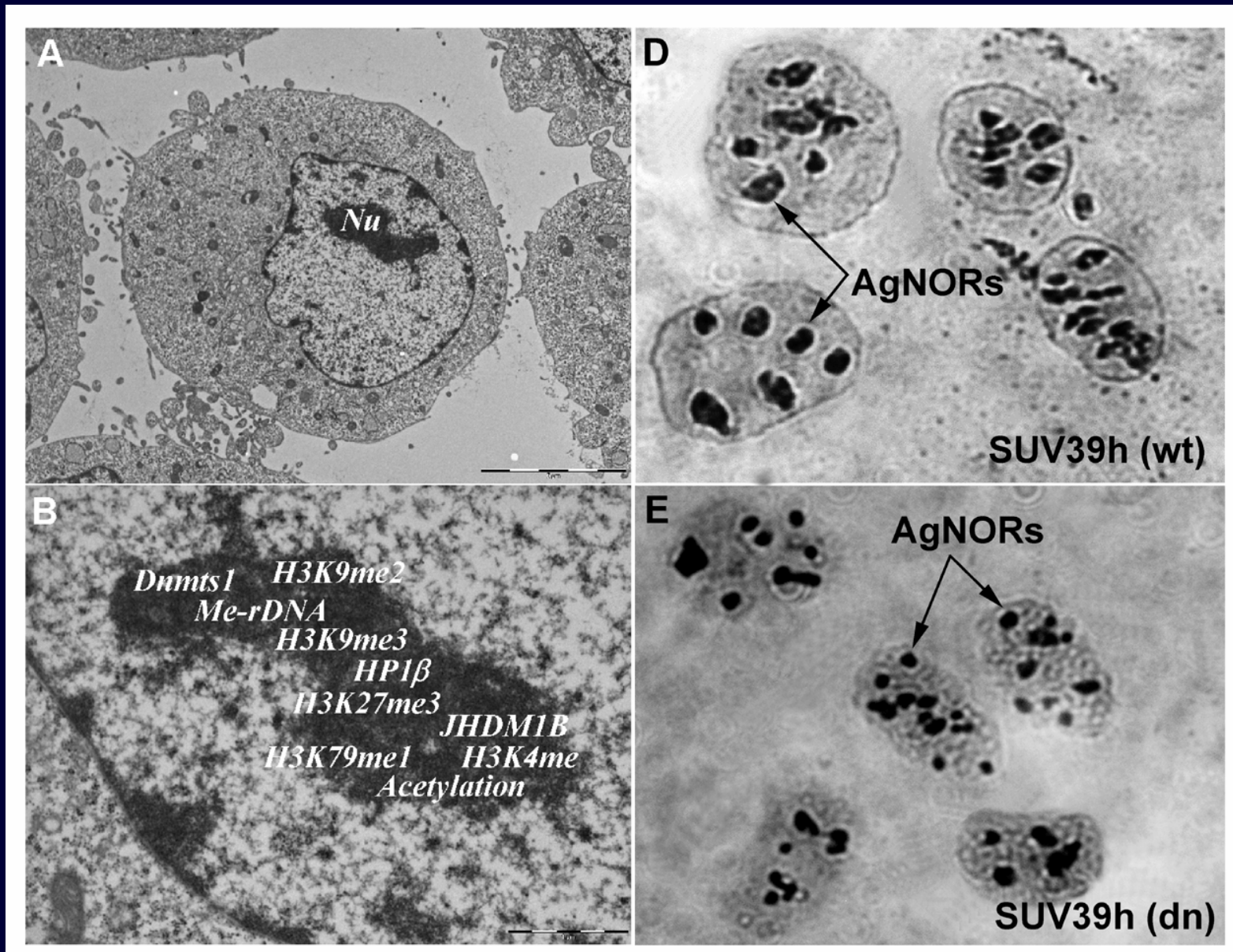
HMTs: SUV39H1, SUV39H2, G9a, MLL1, hSet 1, hSet 2, SUV4-20H1, SUV4-20H2, EZH2 (PcG silencing)

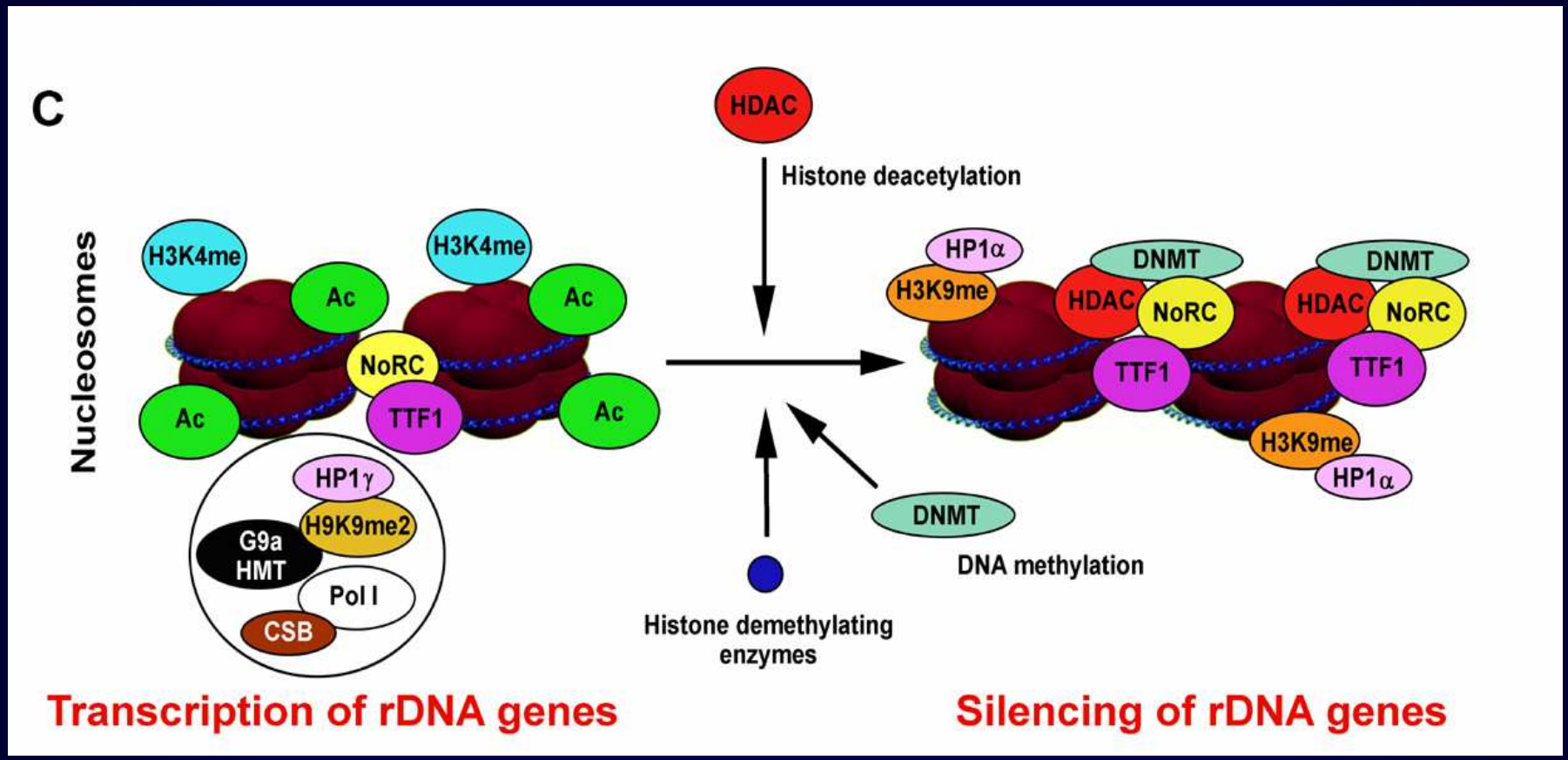
<u>Demethylases:</u> LSD1 (transcriptional activation), JHDM1b (H3K4me3), Jmjd2b (H3K9me3), JHDM2a, JMJ D2B (heterochromatin formation)

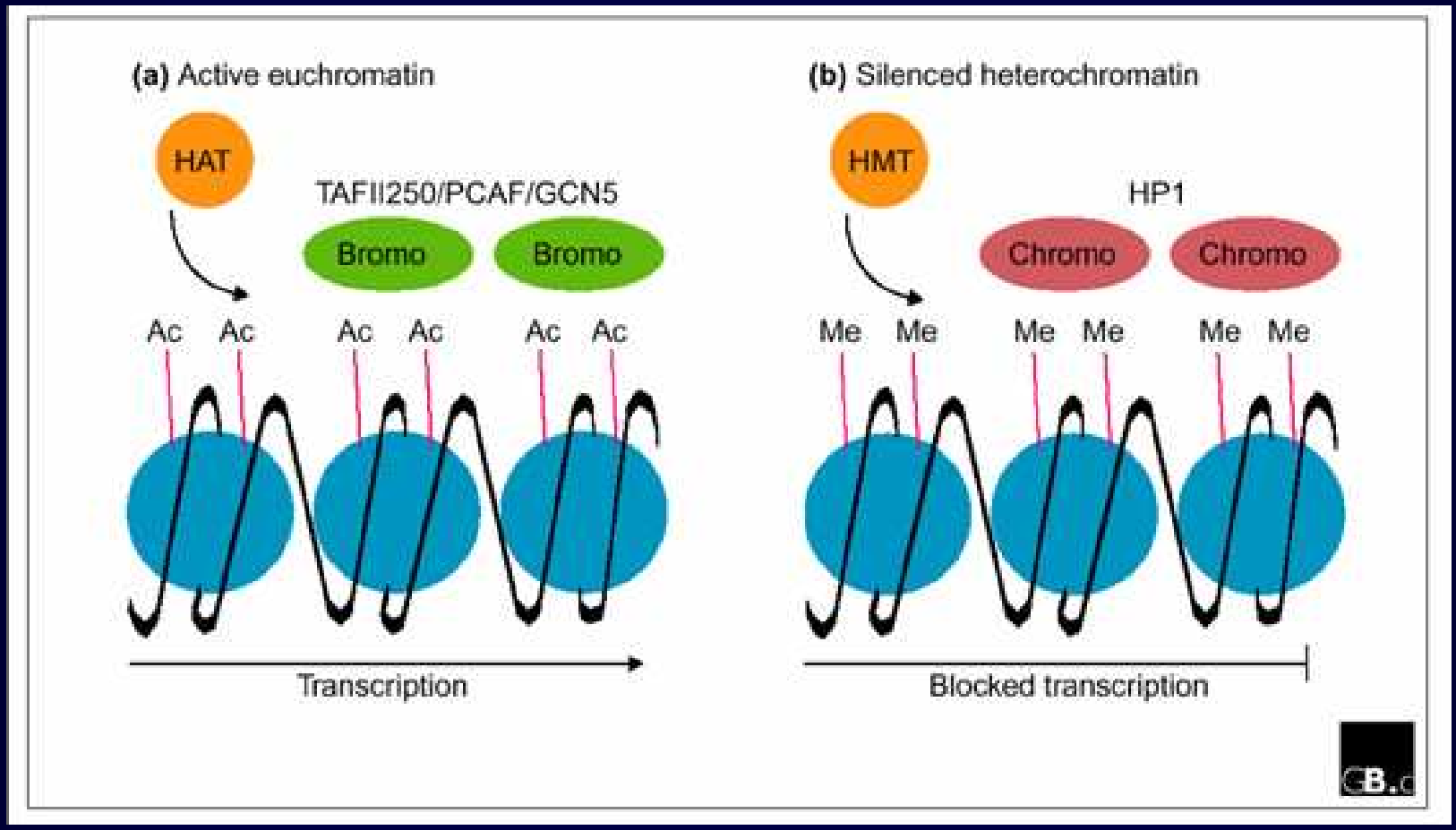

Transcriptional activity
H3K4 Me
H3K9 Ac
H3K4 Me
H3K9 Ac
H3K9 Ac
Ac
HDACs and histone demethylase (LSD1)
K9 HMTs (Suv39H1)
HATs and K4 HMTs (Set 1)
H3K4 Me
HP1
H3K9 Me
pRb
HP1
H3K9 Me
HP1 targets H3K9me
Silencing of euchromatic loci
Ac
it recruits DNMTs
DNMTs
HP1
H3K9 Me
DNMTs
HP1
H3K9 Me
it leads to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

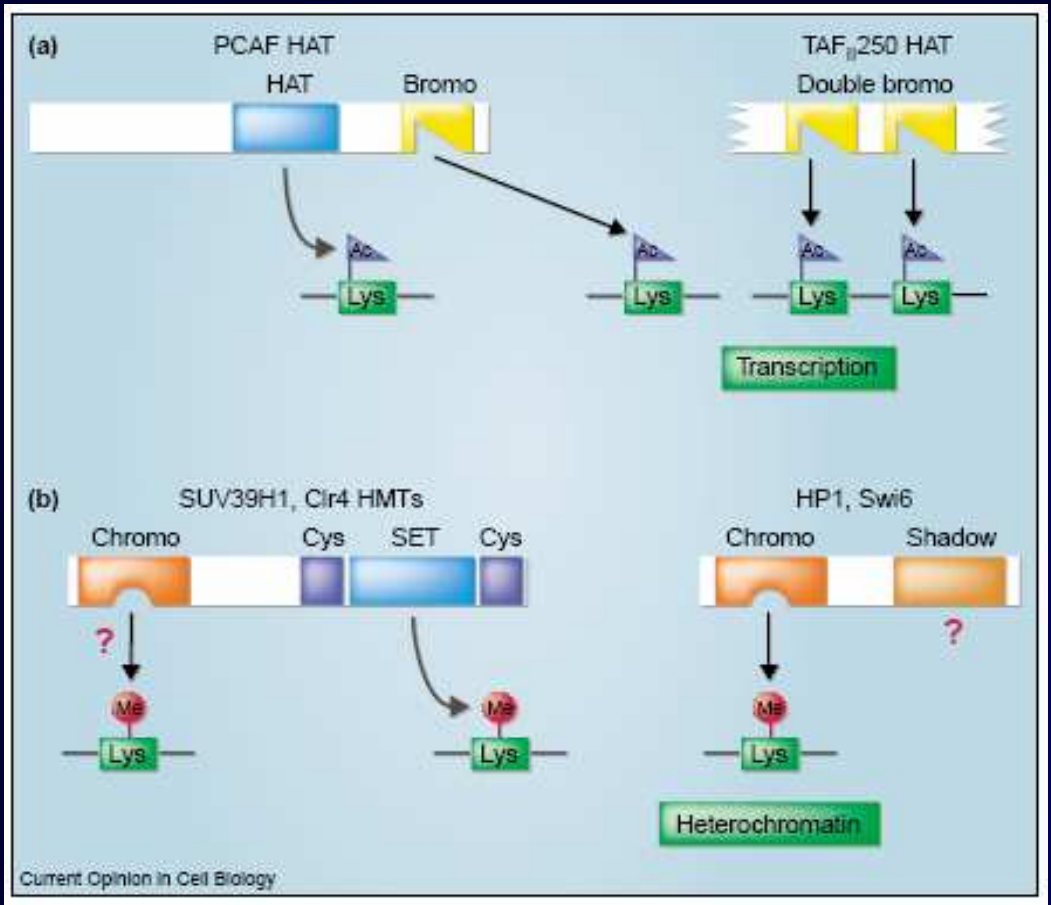
Fibrillarin / Chromocenters




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



HP1 protein

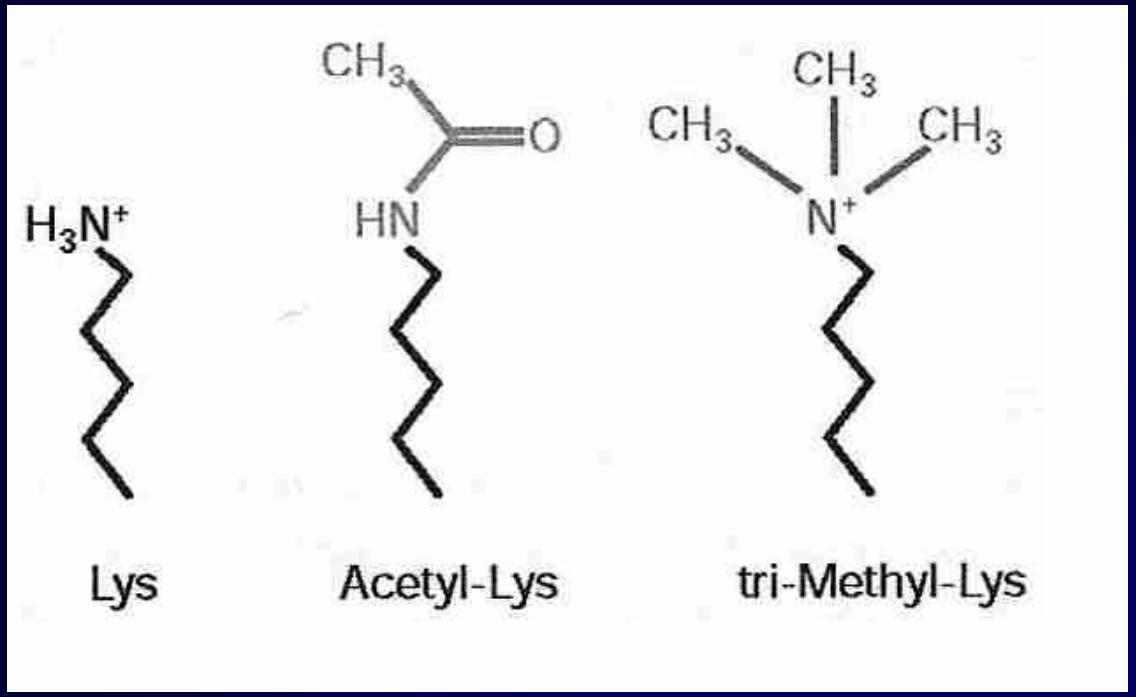
CD: protein-chromatin

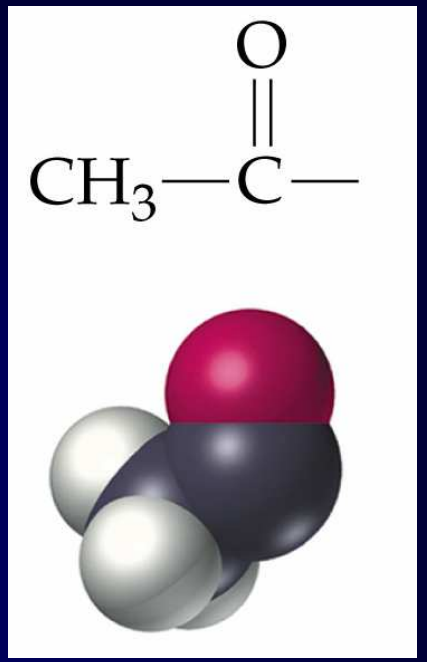
CSD: protein-protein

HD: HP1-to-DNA and linker histones

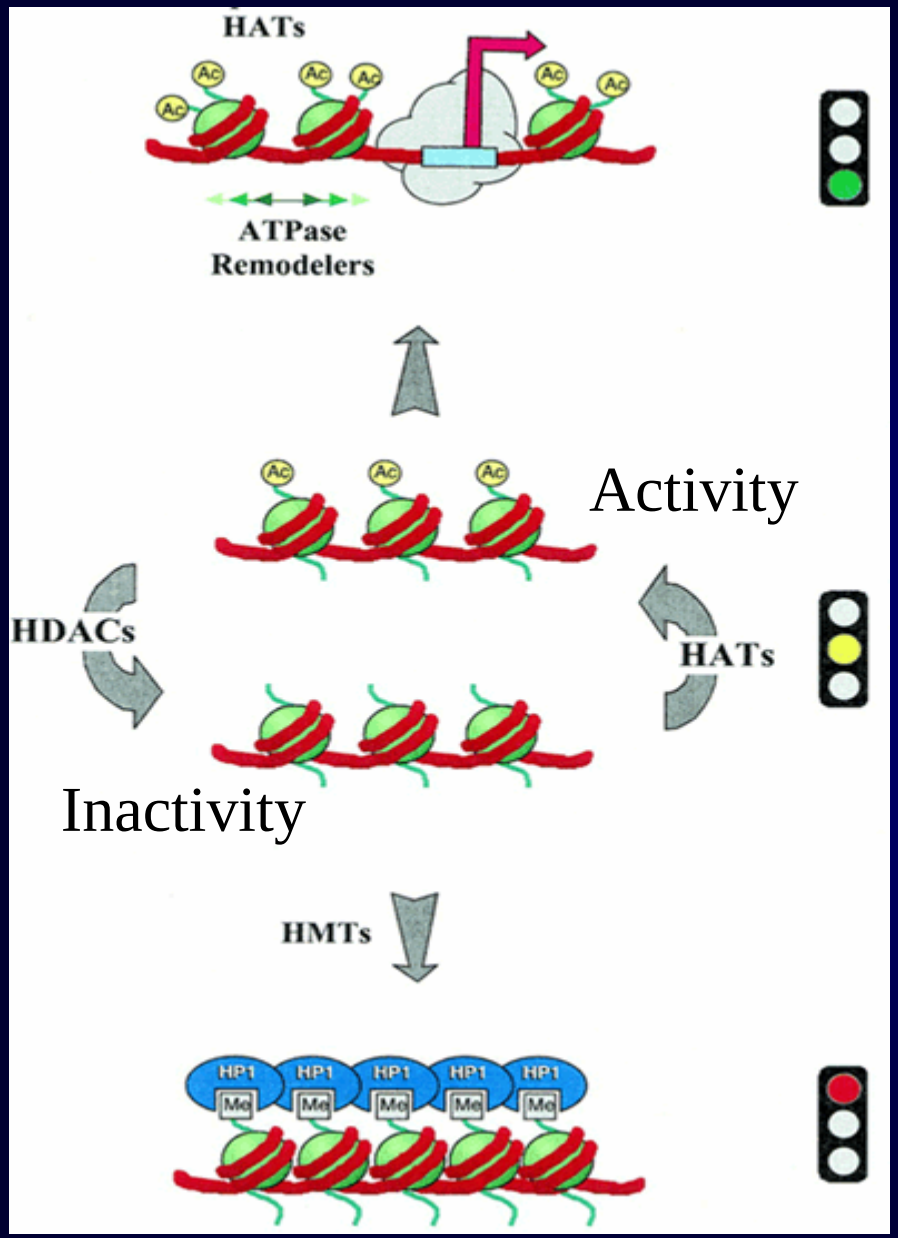
HMTs:

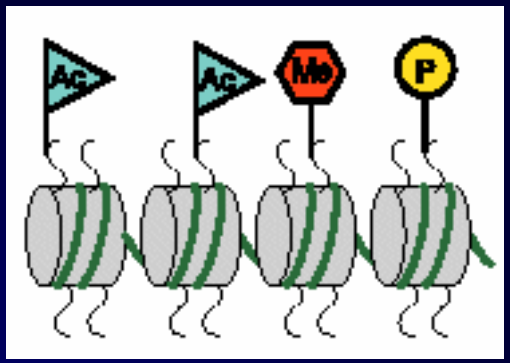
D. melanogaster: Su(var)3-9 je lokalizován v oblastech kondenzovaného chromatinu a je to klíčový regulátor v organizaci represivního chromatinu. Homolog u S.pombe je Clr4 umyší SUV39h1 a u lidských buněk SUV39H1. Tyto HMTs specificky methylují H3(K9).

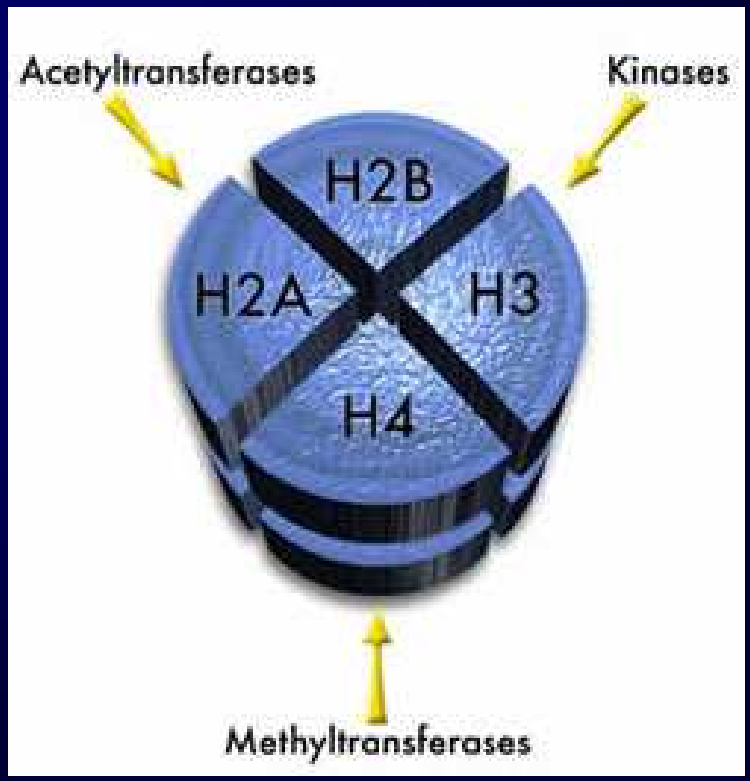




Primárním důsledkem histonových modifikací je snížení schopnosti histonových konců interagovat s dalšími složkami chromatinu, včetně DNA.

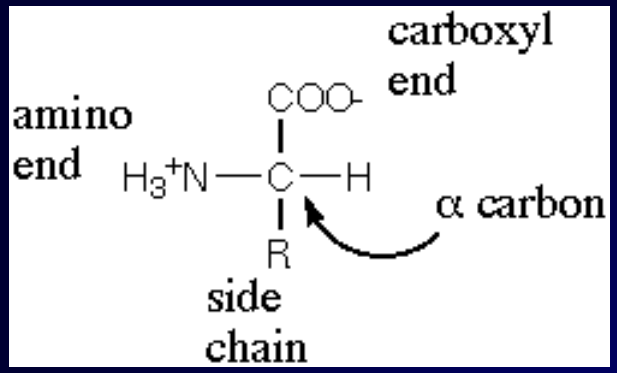


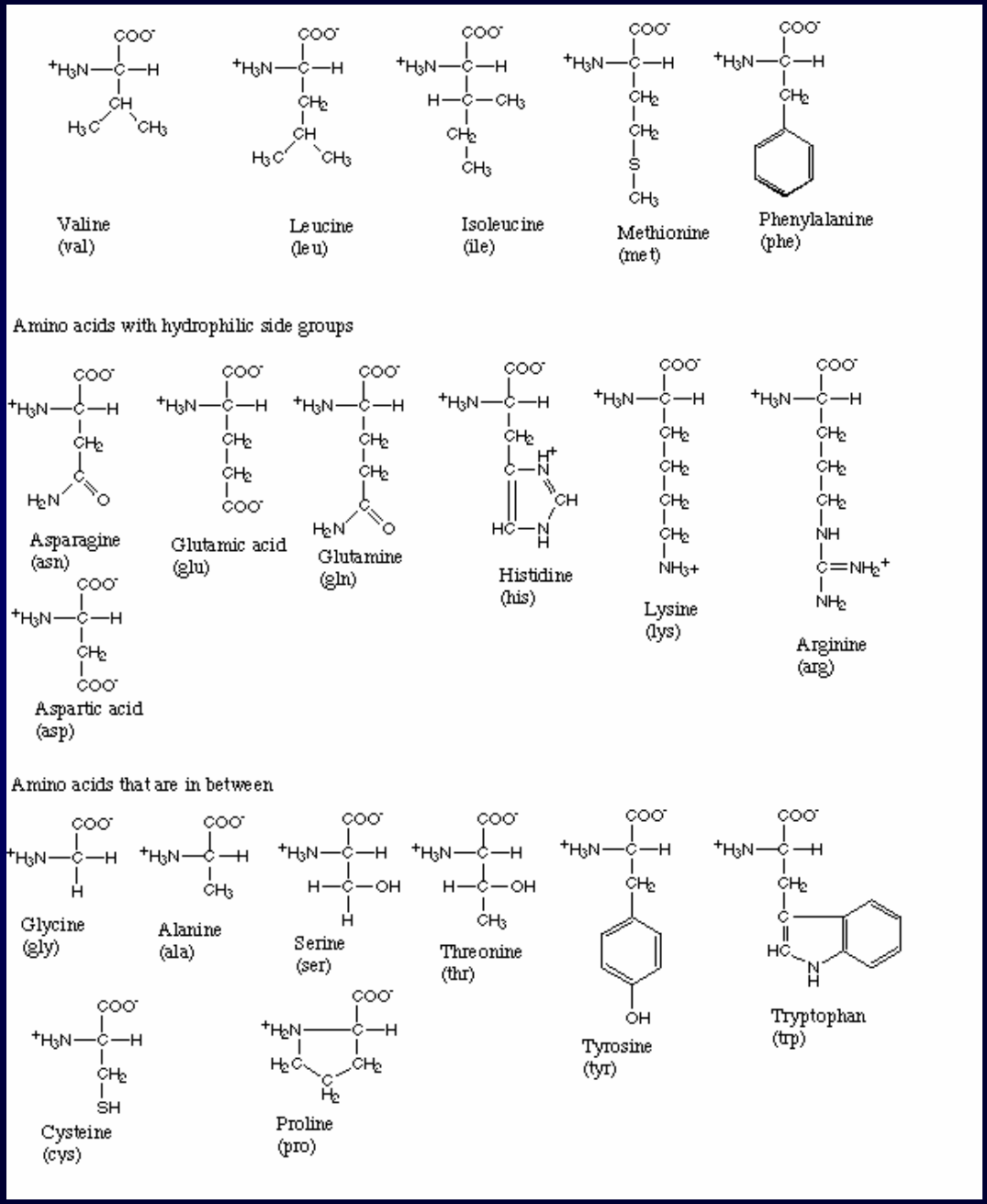




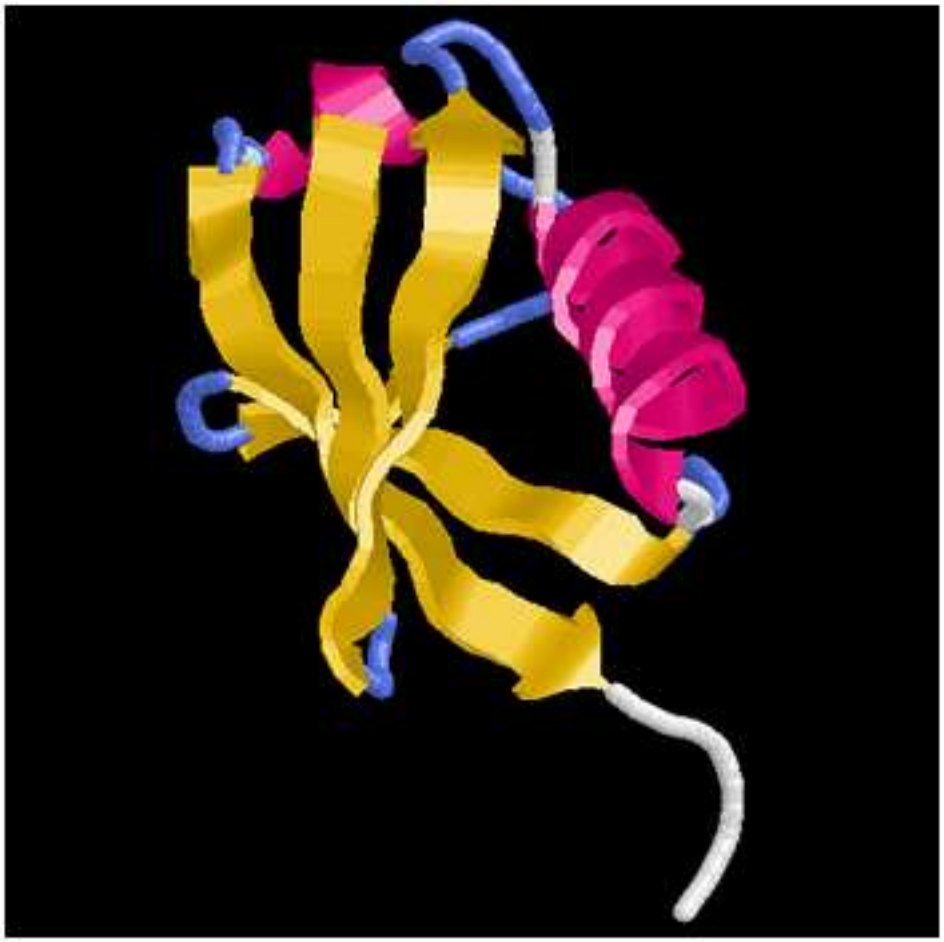
Ikaros, Helios

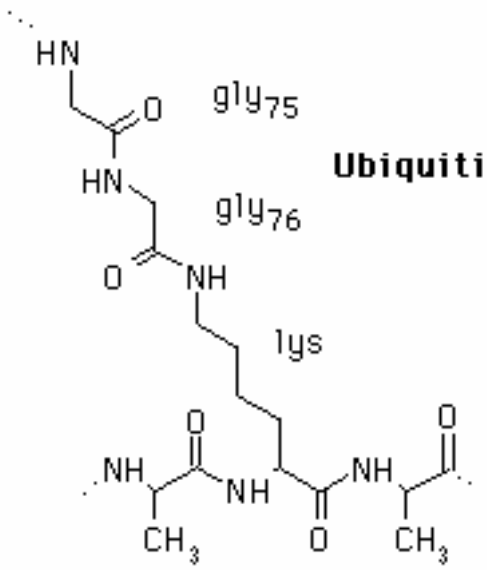
Amino acid


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

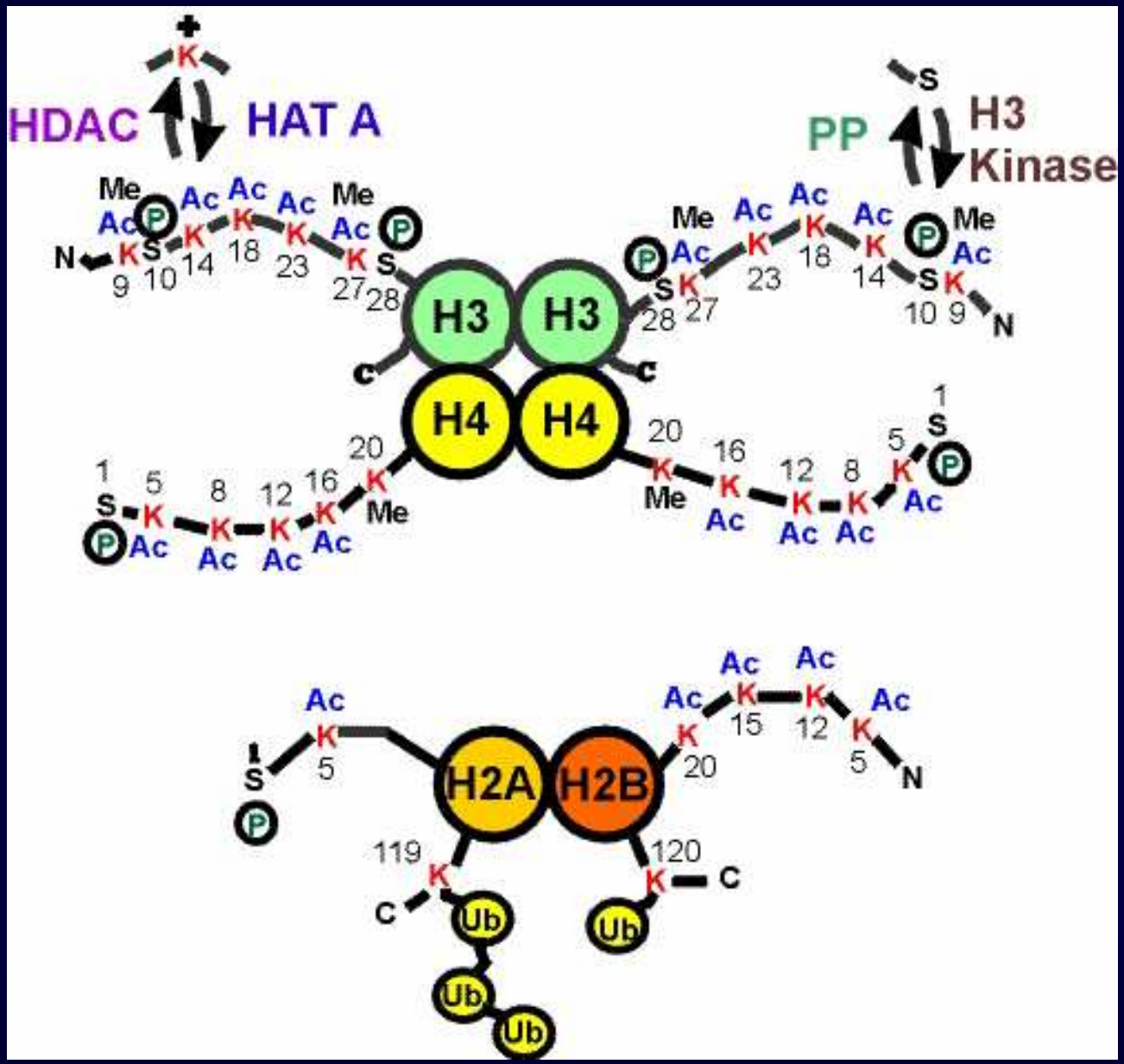

Valine
(val)
Leucine
(leu)
Isoleucine
(ile)
Methionine
(met)
Phenylalanine
(phe)
Amino acids with hydrophilic side groups
Asparagine
(asn)
Glutamic acid
(glu)
Glutamine
(gln)
Histidine
(his)
Lysine
(lys)
Arginine
(arg)
Aspartic acid
(asp)
Amino acids that are in between
Glycine
(gly)
Alanine
(ala)
Serine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

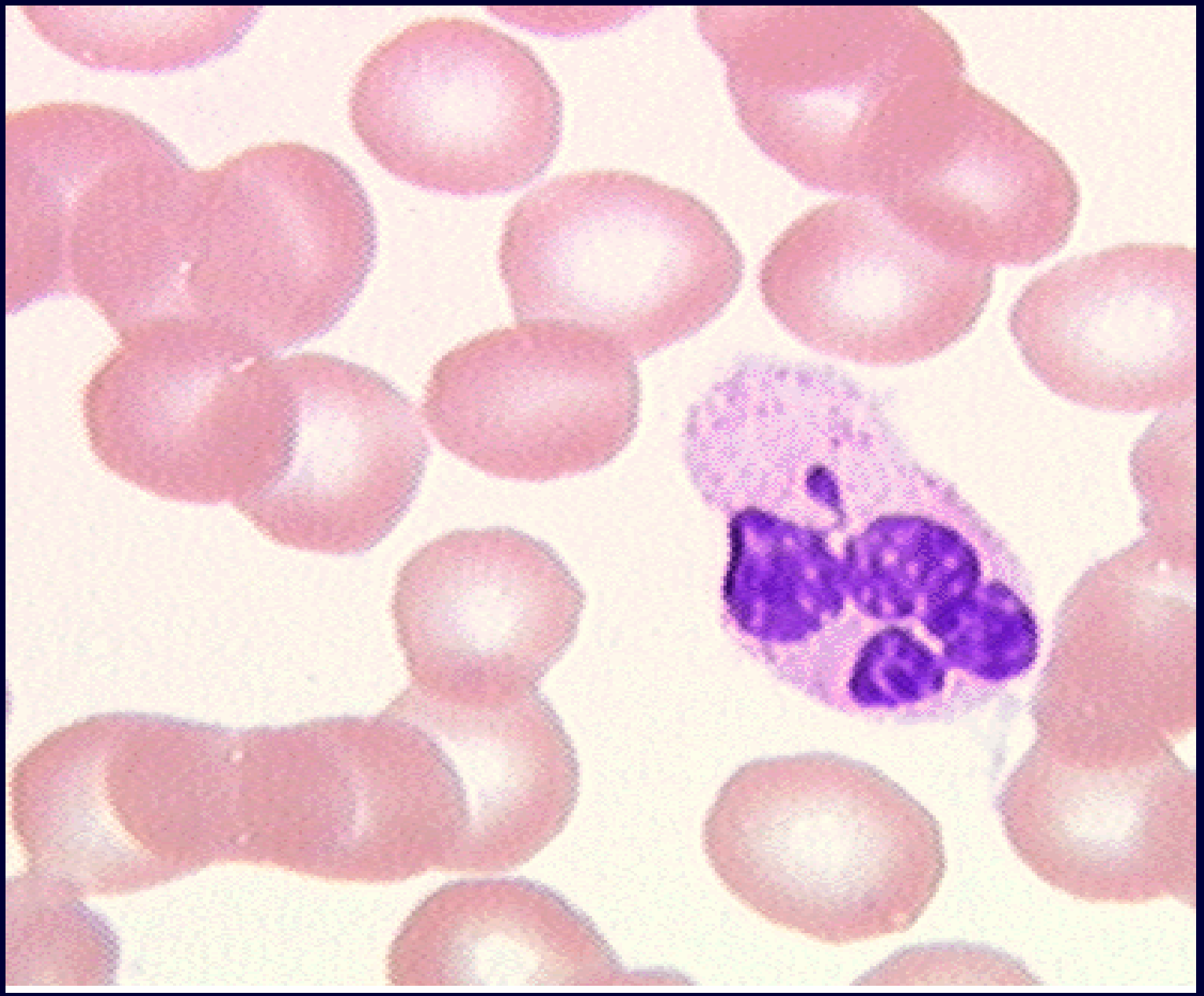
Ubiquitin structure



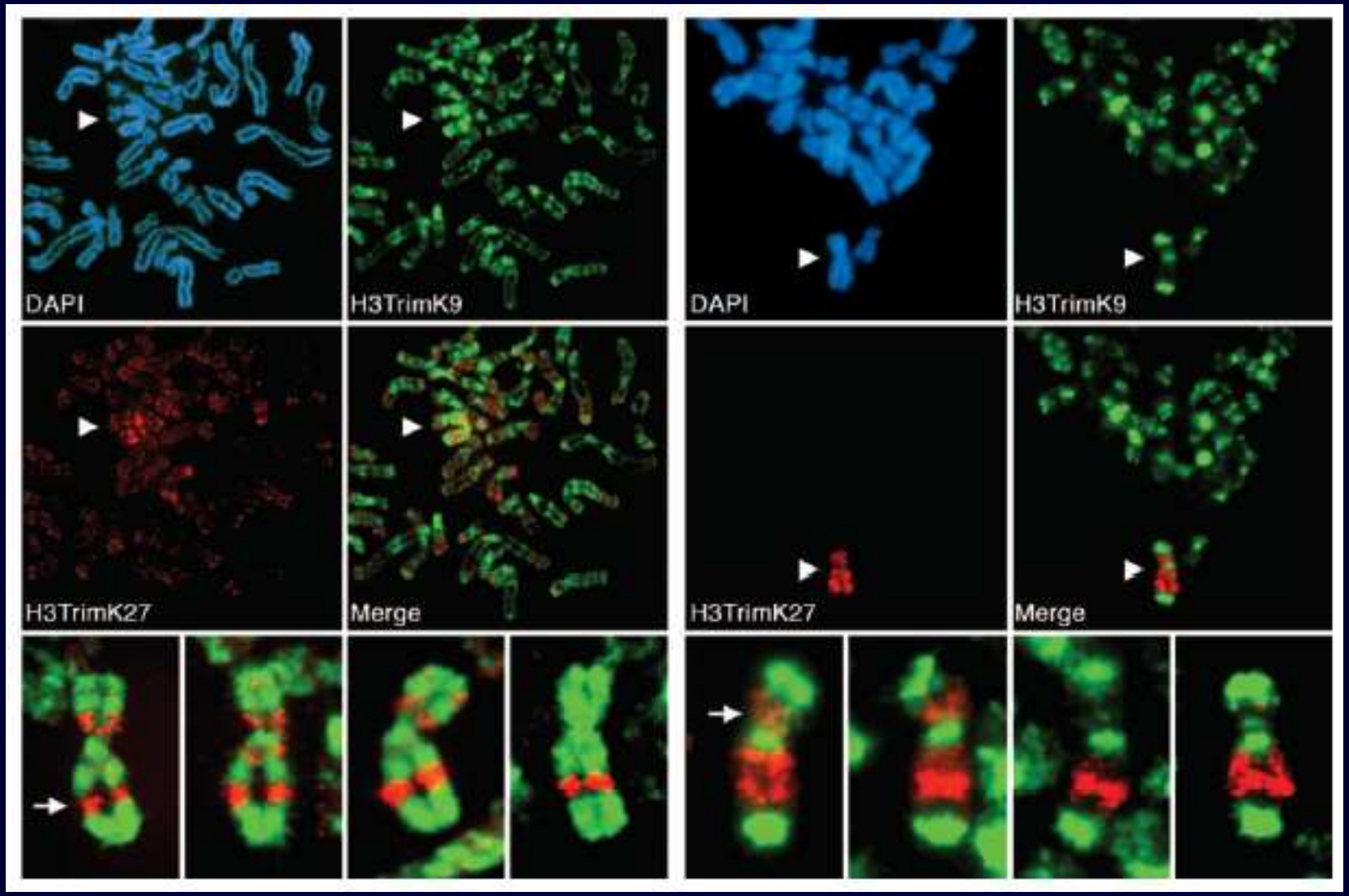


Ubiquitination of histones has been reported *in vivo* although the most prevalent ubiqutination occurs in H2A and H2B. One of the widely studied proteins that undergoes ubiquitination for its activity is p53.


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



Inaktivace X chromosomu ve vztahu k epigenetickým modifikacím



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

Inaktivace X chromosomu ve vztahu k epigenetickým modifikacím

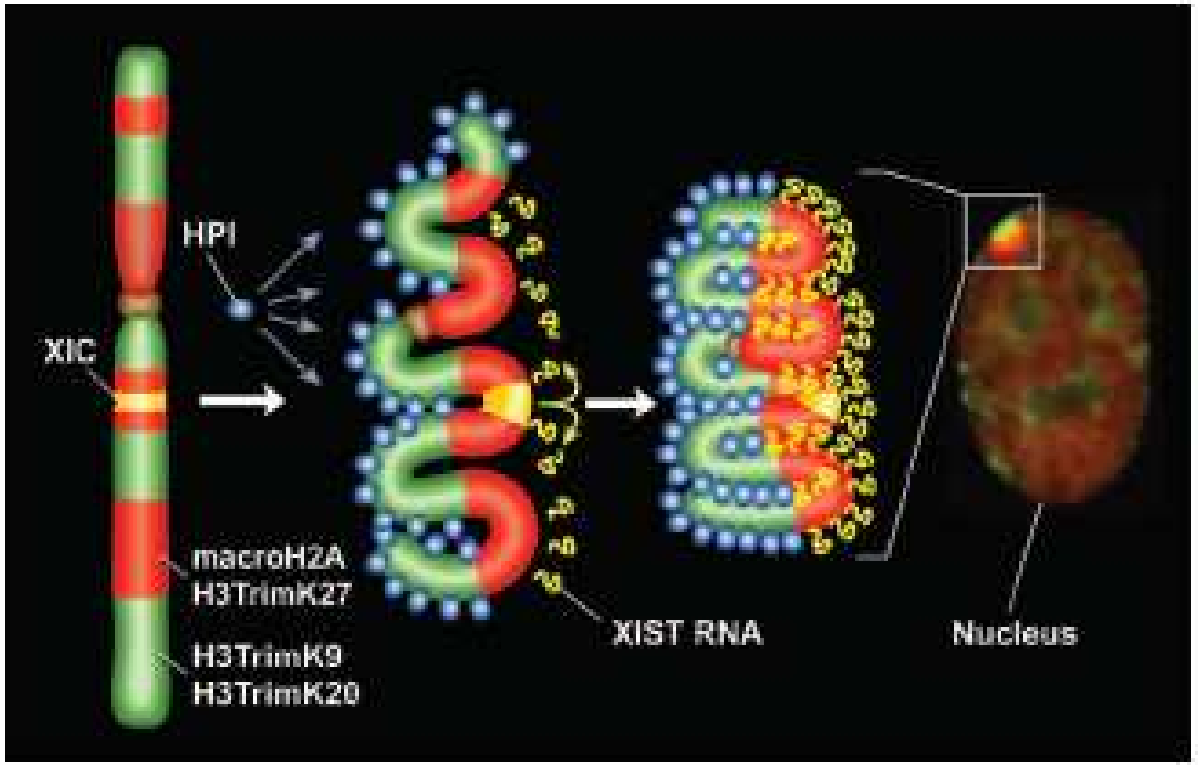
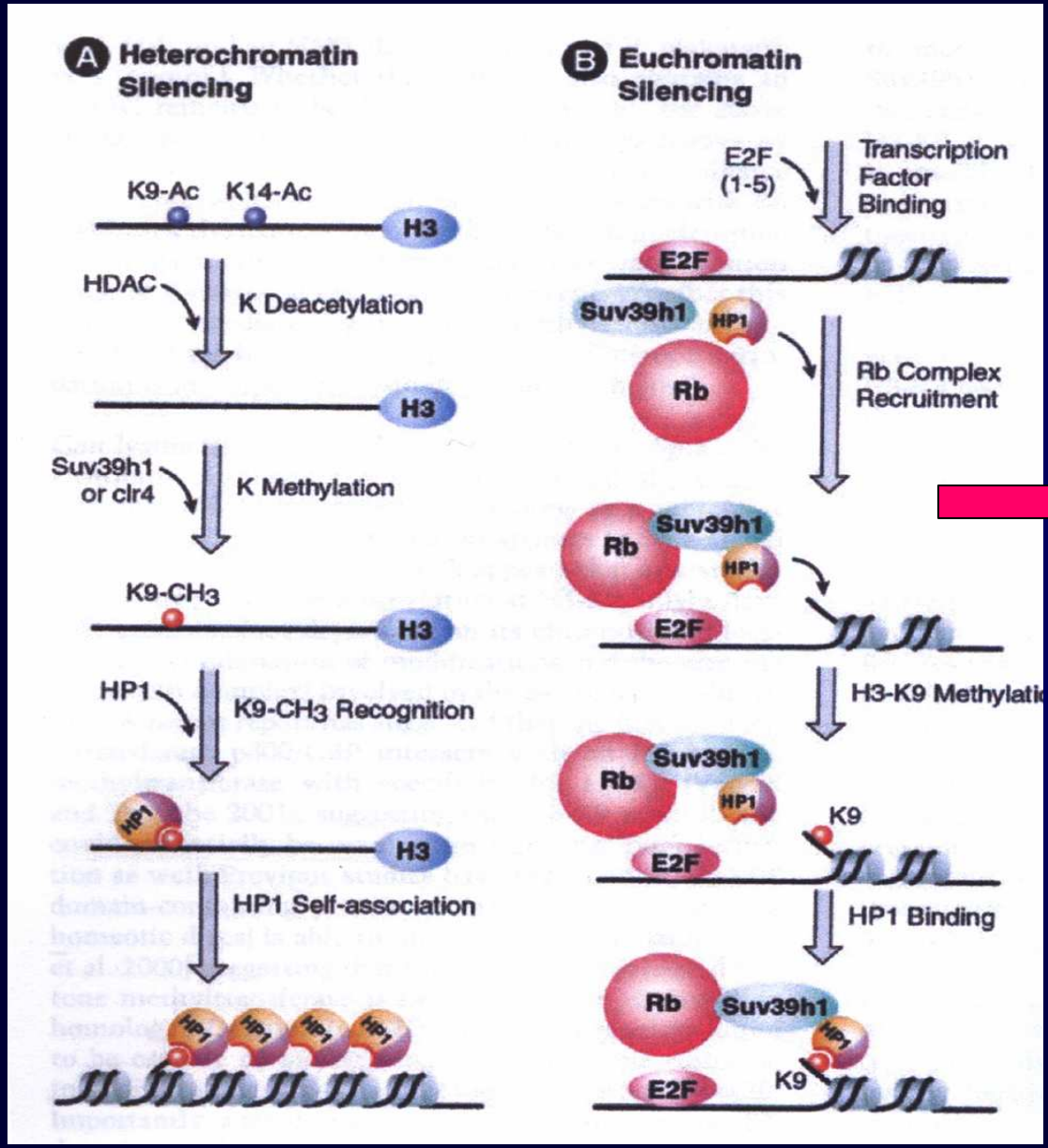
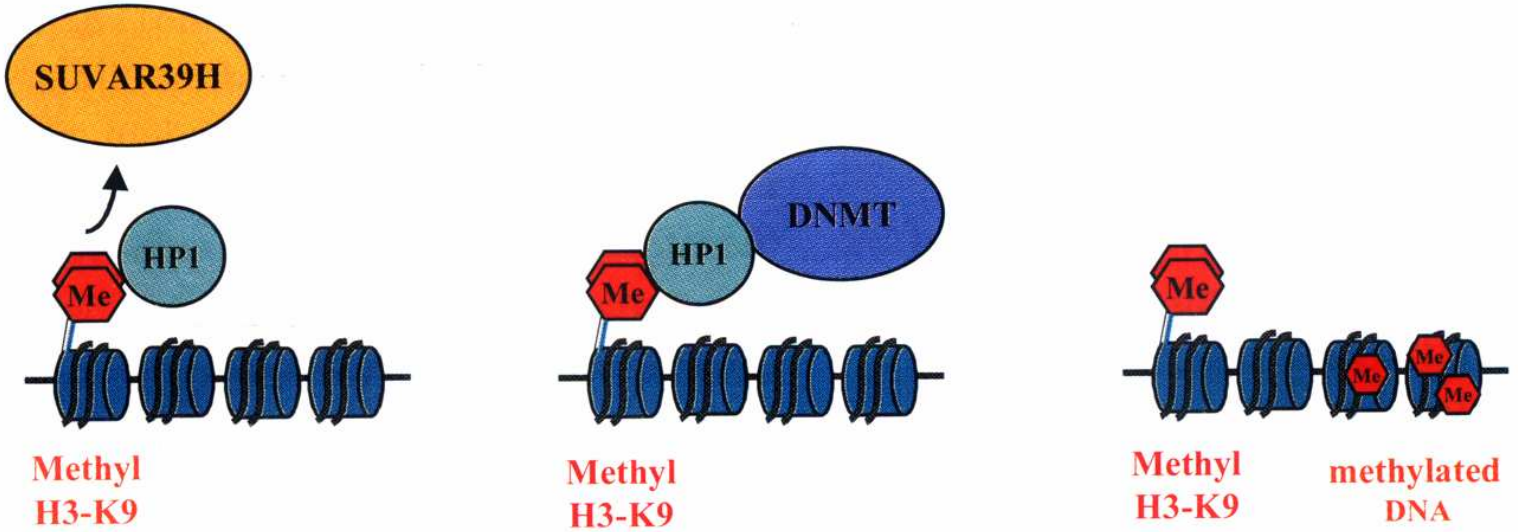


Fig. 4. Schematic model showing how heterochromatin of the Xi could transition between metaphase and interphase to be organized into the two nonoverlapping heterochromatin territories and to explain how XIST RNA could rapidly spread in cis outward from the X inactivation center (XIC) along only part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

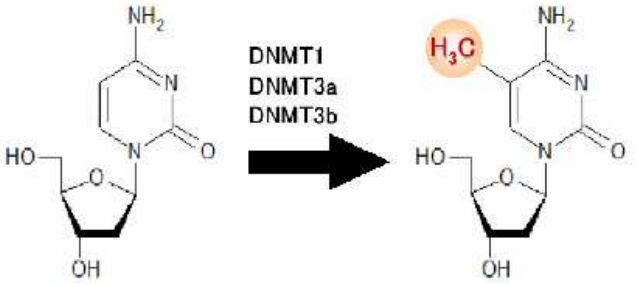


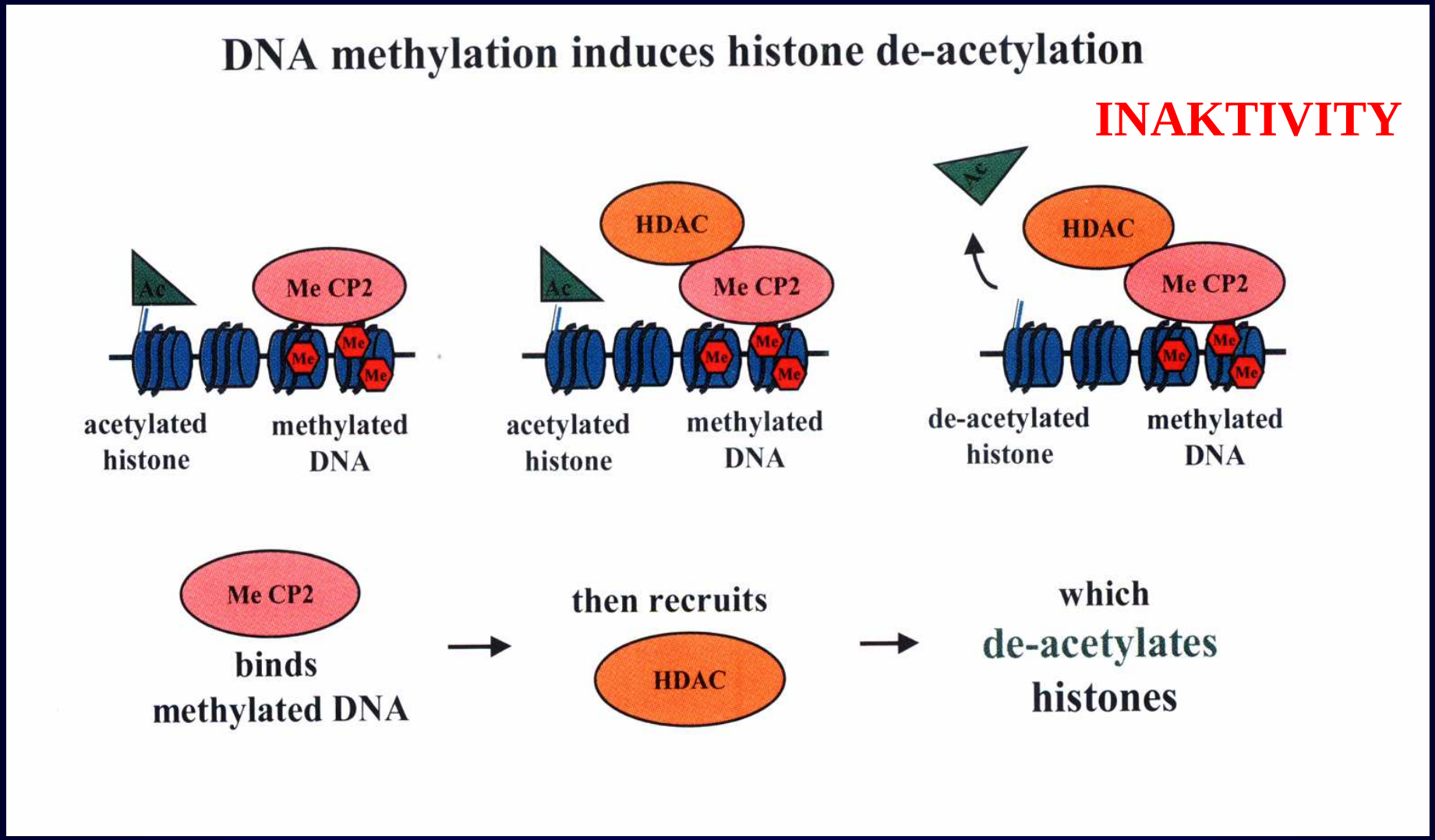
Repression of cyclin E promoter

Histone H3-K9 methylation induces DNA methylation

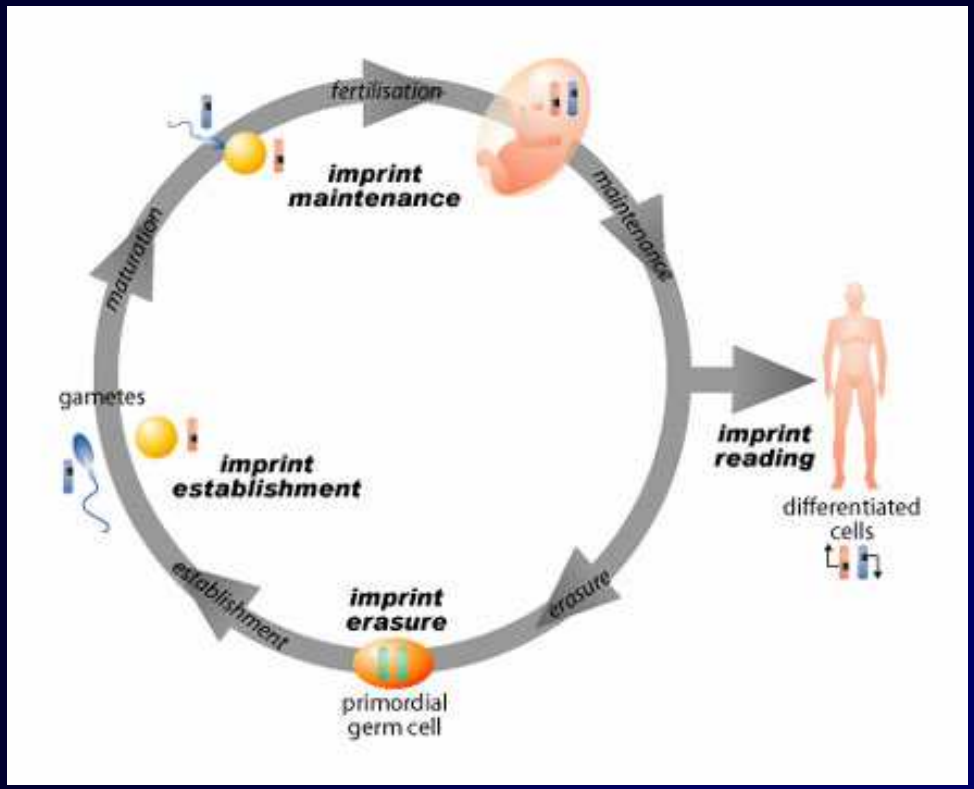


HP1 **targets** Methyl H3-K9 → **then recruits** DNMT → **which methylates DNA**





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

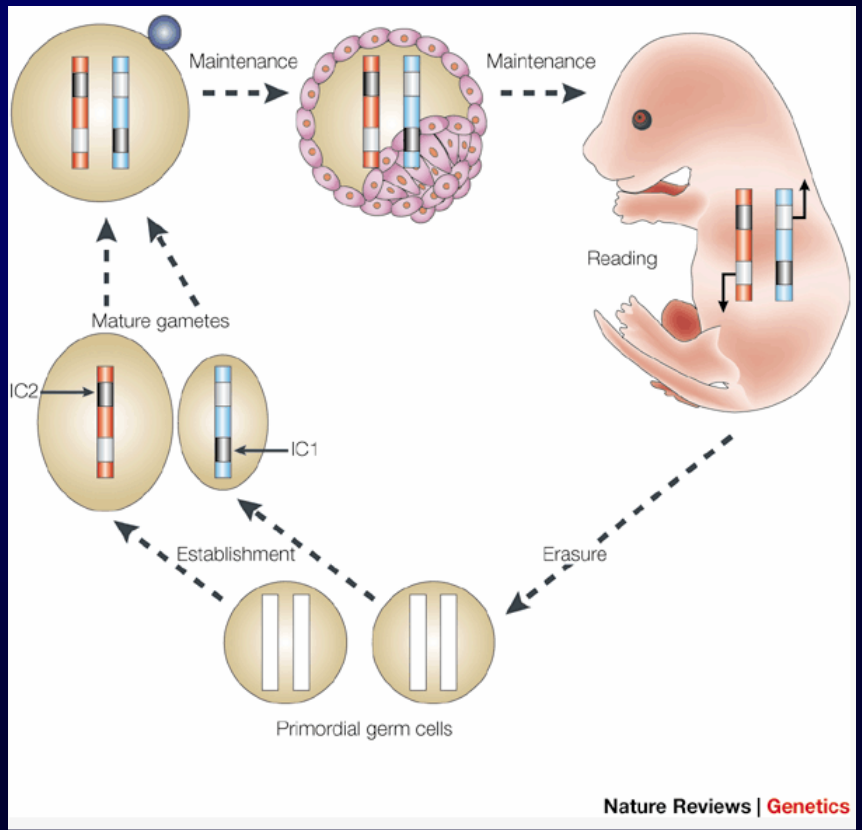


IMPRINTING

Myší embryo: samičí alela je zamethylována, nevyjadřuje se

Dospělý jedinec: obě alely jsou demethylovány

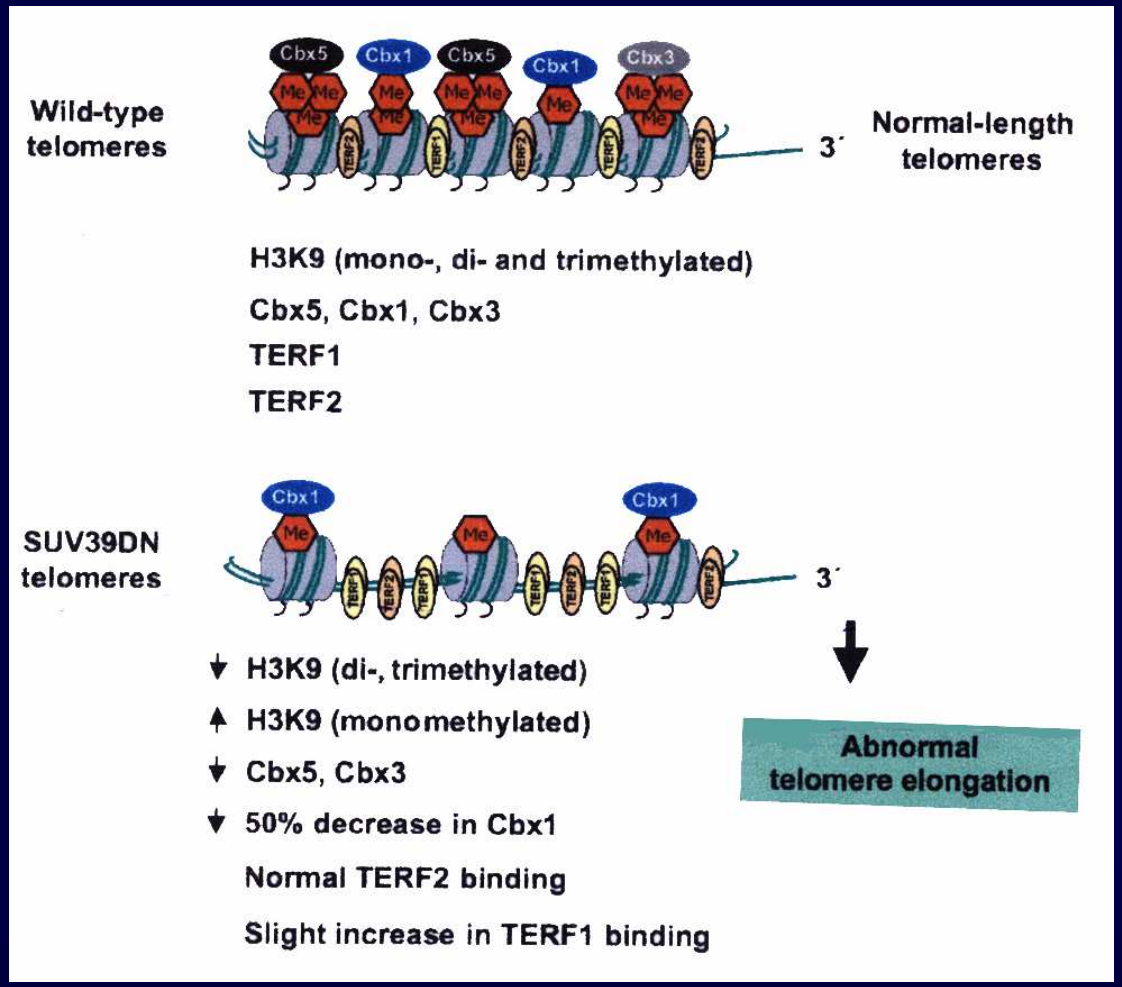
Gametogeneze: se obnoví původní stav
Platí pro gen IGF II.



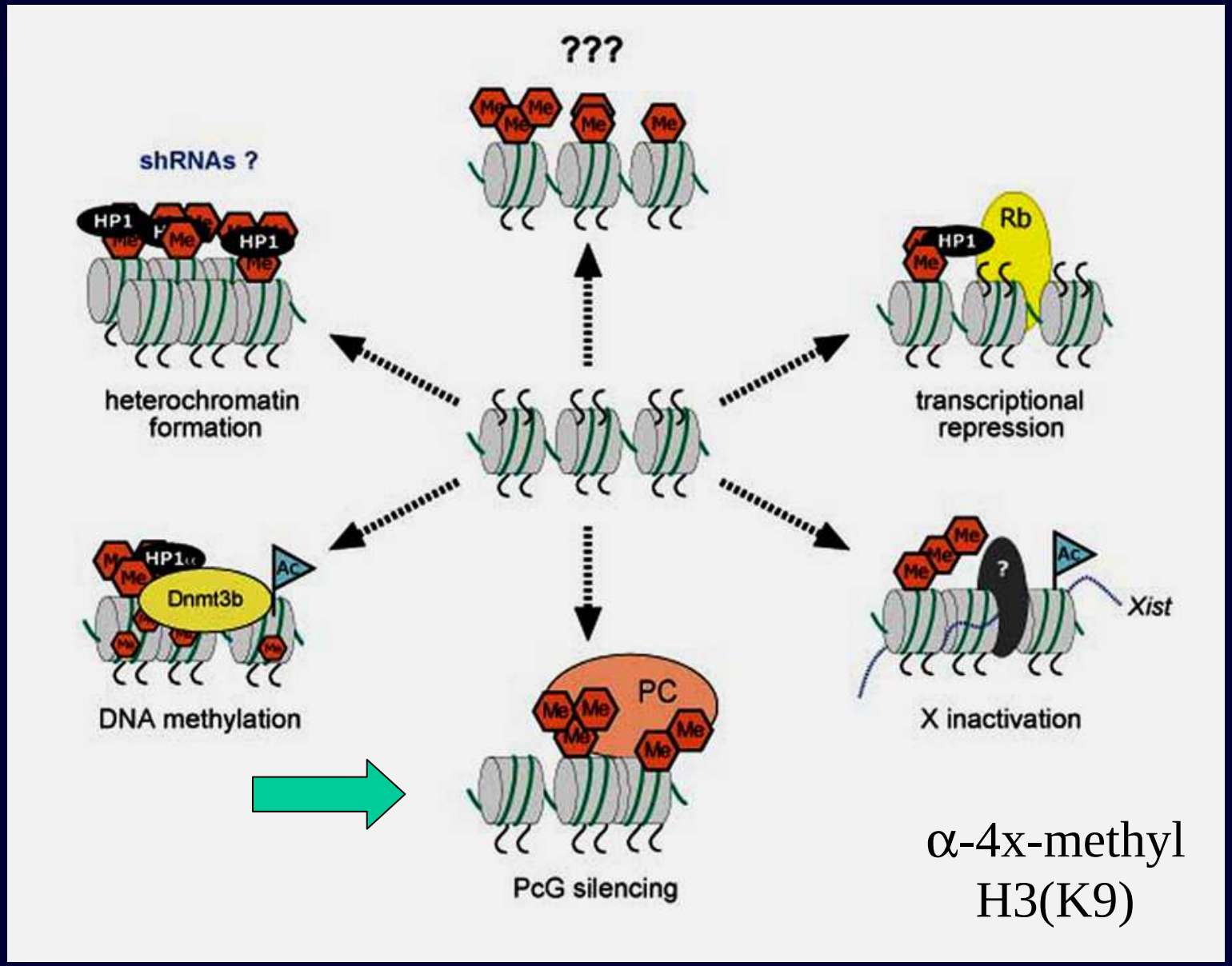
ISSN1471-0056

Methylation state of telomeres

(Cbx1=HP1β, Cbx3=HP1γ, Cbx5 = HP1α)

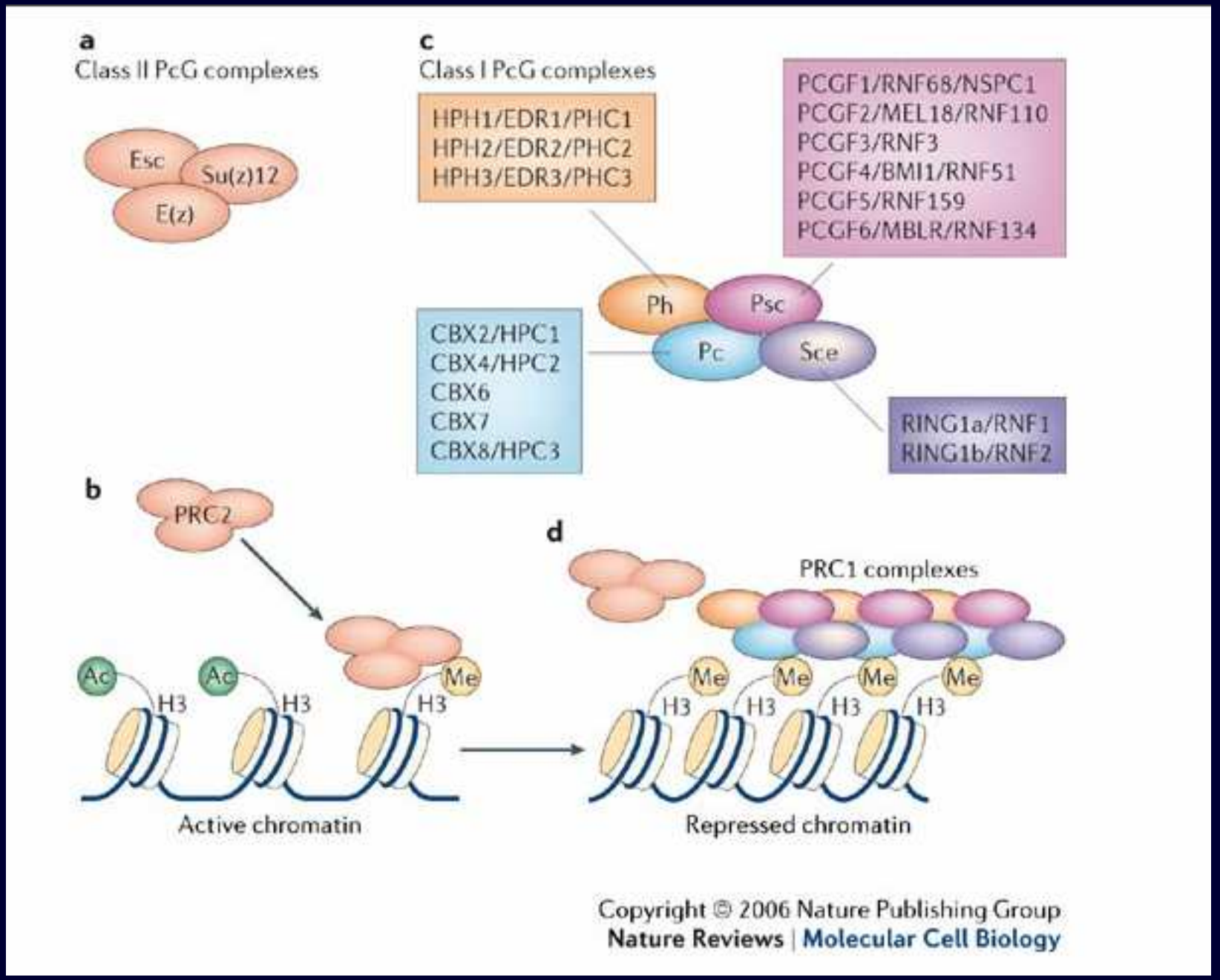


HP1α (12q13) *HP1β* (17q21) *HP1γ* (7p15)



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.



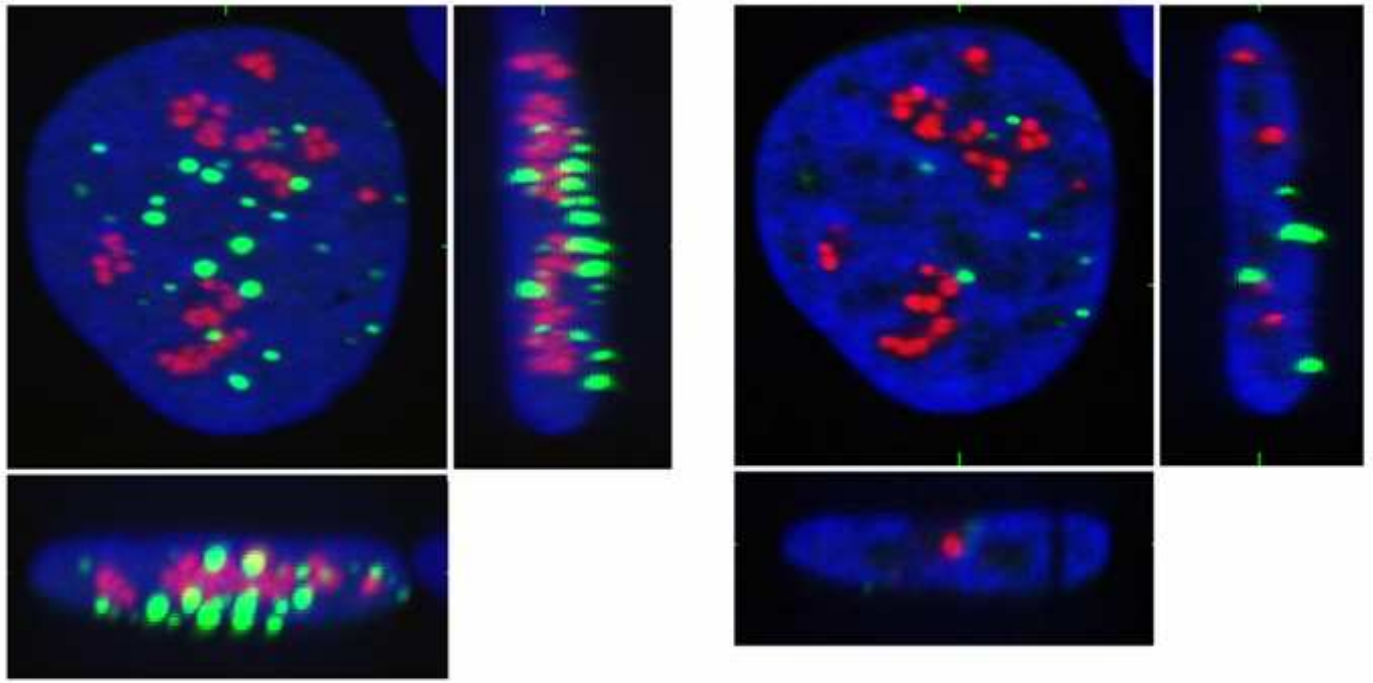
Year	Brief description of the main findings	Pubmed link
1978	Ed Lewis's founding Polycomb paper identifying a role for the *Pc* gene in the regulation of homeotic genes	go!
1985	Characterization of the *trithorax* gene as a regulator of homeotic gene expression Role of PcG proteins in the maintenance of homeotic gene expression, i.e. in the process of "cellular memory"	go! go!
1988	Antagonism between *Polycomb* and *trithorax* genes	go!
1989	Polytene chromosome binding pattern of Pc	go!
1991	Identification of Bmi-1, the first mammalian PcG gene Role of Bmi-1 in Cancer	go! go: a! b!
1992	Involvement of Trithorax in leukemia	go!
1993	Characterization of PREs in Drosophila Chromatin IP of Polycomb	go: a! b! c! go!
1994	Bmi-1 action as a bona fide mammalian PcG protein	go!
1997	Analysis of PcG proteins in plants PcG proteins and epigenetic regulation of gene expression by "cosuppression"	go! go!
1999	Purification of the PRC1 complex Role of PcG in cell proliferation	go! go!
2000	trxG proteins and histone acetylation	go: a! b!
2001	Link between PcG proteins and the basal transcriptional machinery PcG proteins and genomic imprinting in mammals	go: a! b! go!
2002	Characterization of the E(z)-Esc / PRC2 complex - Histone methyltransferase activity trxG proteins and histone methylation	go: a! b! c! d! go: a! b!
2003	Binding of the PC chromo domain to histone H3 methylated at Lysine 27 PcG proteins and X-inactivation Polycomb as a Sumo E3 protein	go: a! b! go: a! b! go!
2004	PRC1 proteins mediate histone ubiquitination Identification of a PRC3 complex related to PRC2 and identification of histone H1 methylation activity	go! go!
2005	Identification of a link between PcG proteins and DNA methylation Role for PcG proteins in the phenomenon of transdetermination in *Drosophila*	go: a! b! go: a! b!

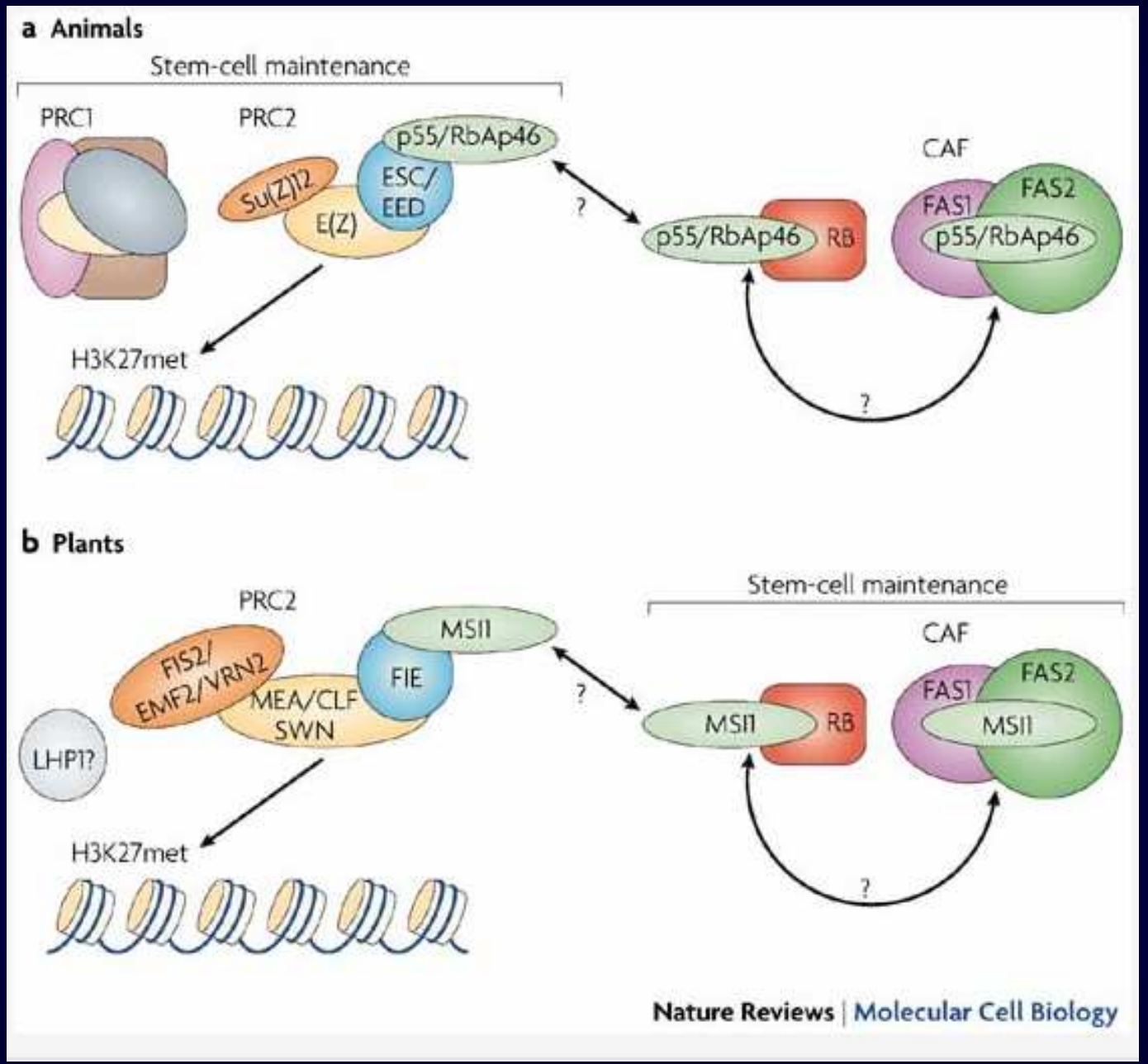
2006: Genome-wide mapping of the down-stream target sites for PcG proteins

Fibrillarin / BMI1 / Nucleus

CONTROL

maximum image ***individual section***

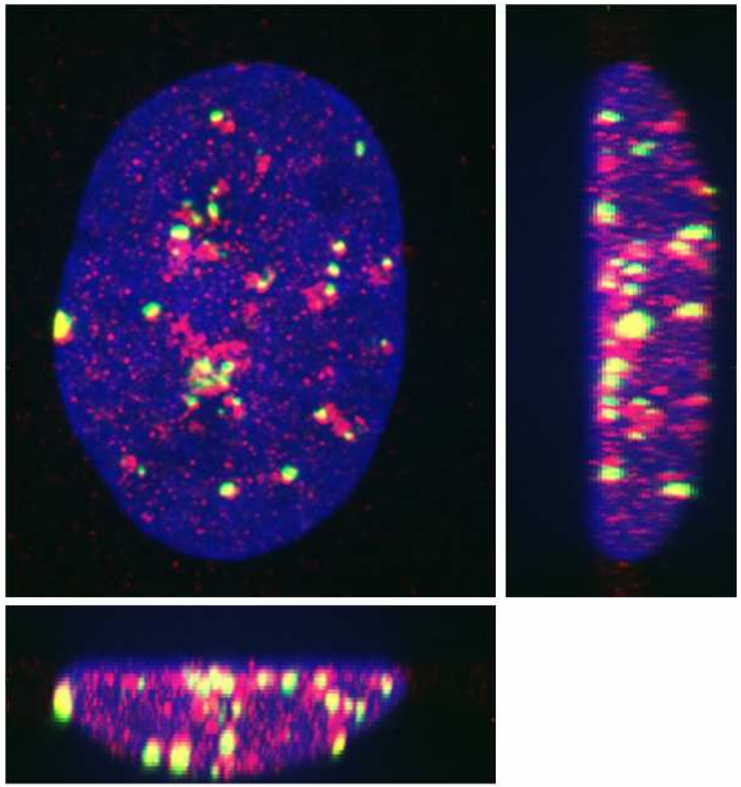



a Animals
Stem-cell maintenance
PRC1
PRC2
p55/RbAp46
Su(Z)12
ESC/
EED
E(Z)
?
CAF
FAS1
FAS2
p55/RbAp46
RB
p55/RbAp46
H3K27met
?
b Plants
PRC2
Stem-cell maintenance
MSI1
FIS2/
EMF2/VRN2
FIE
MEA/CLF
SWN
?
CAF
FAS1
FAS2
MSI1
RB
MSI1
LHP1?
H3K27met
?
Nature Reviews | Molecular Cell Biology

H3K27me3 / BMI1 / Nucleus

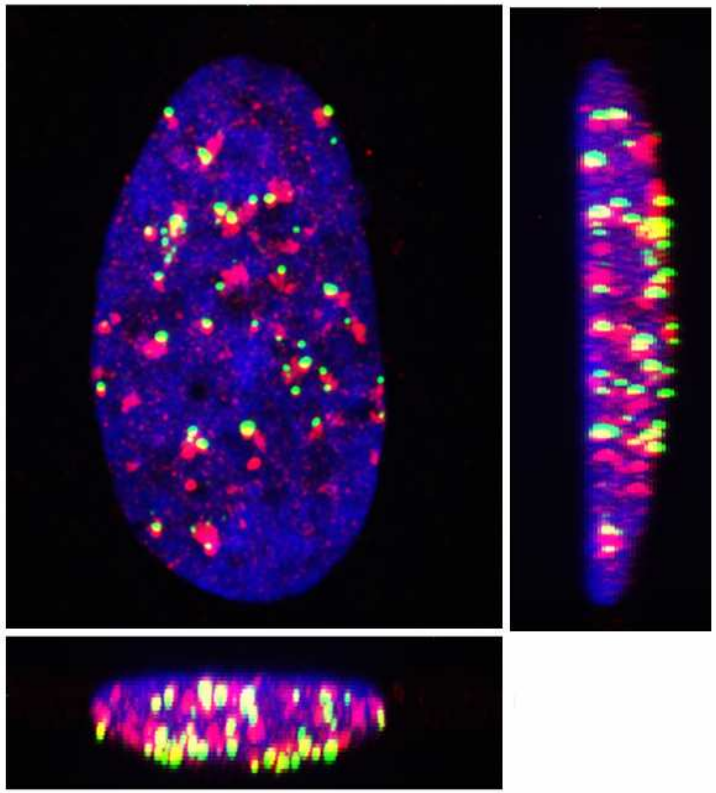
CONTROL

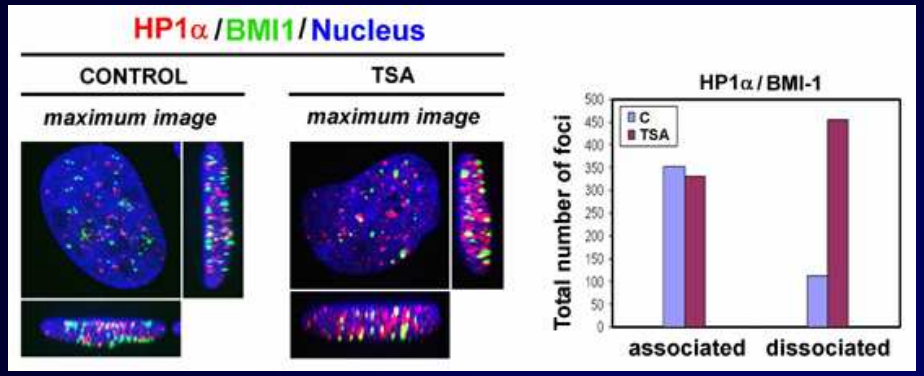
maximum image

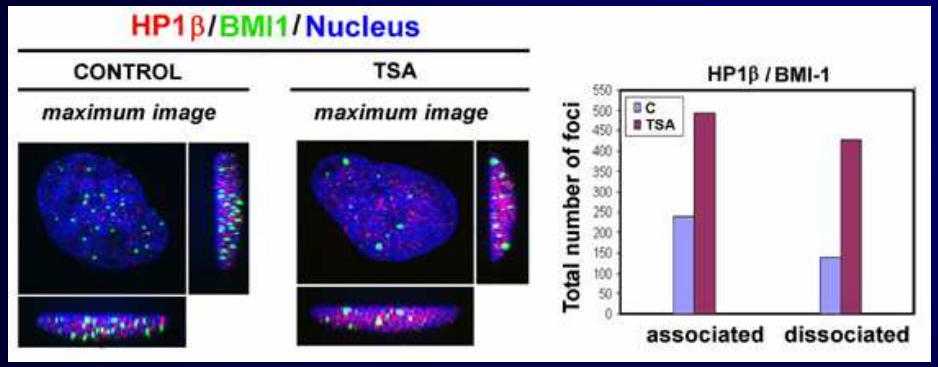


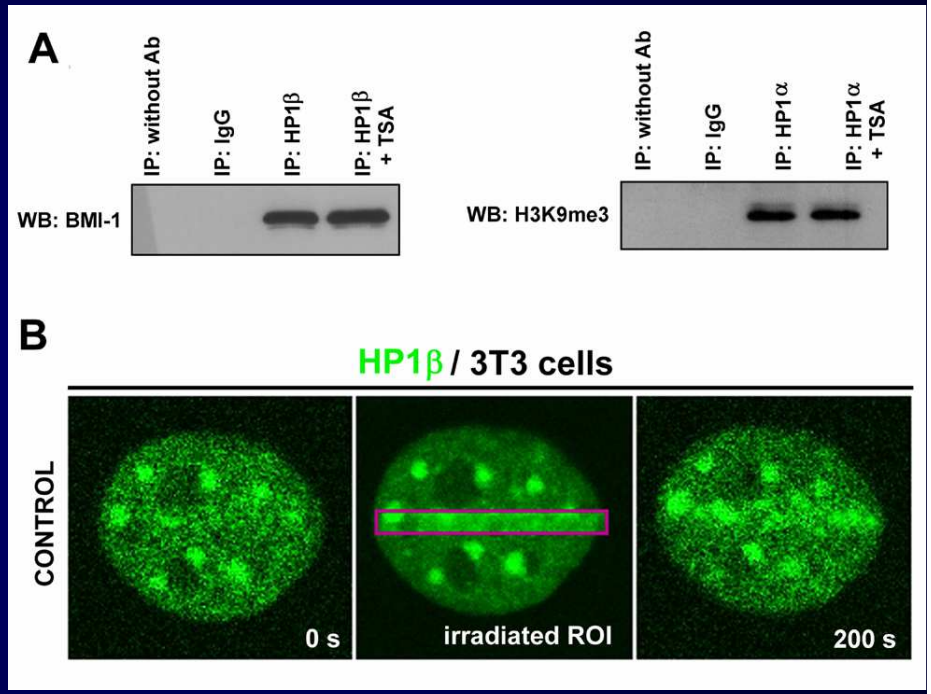
TSA

maximum image



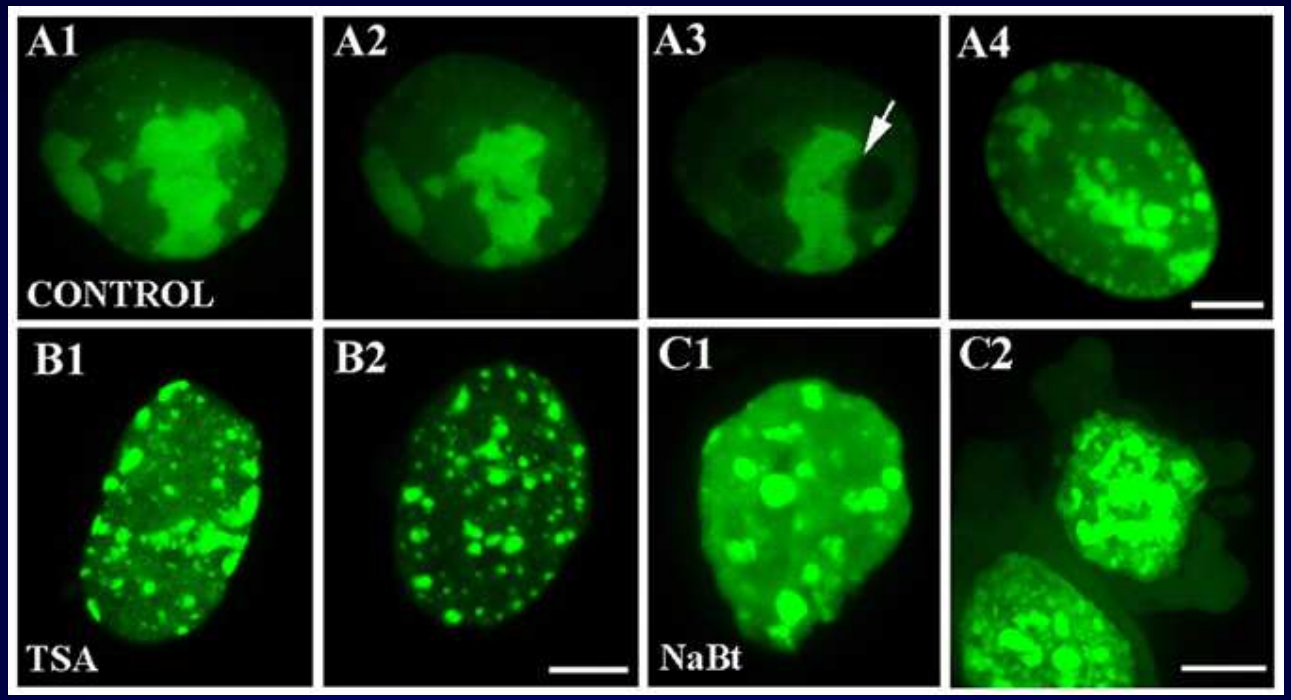






Experiments of Gabriela Galiová and Lenka Stixová

HP1 proteins

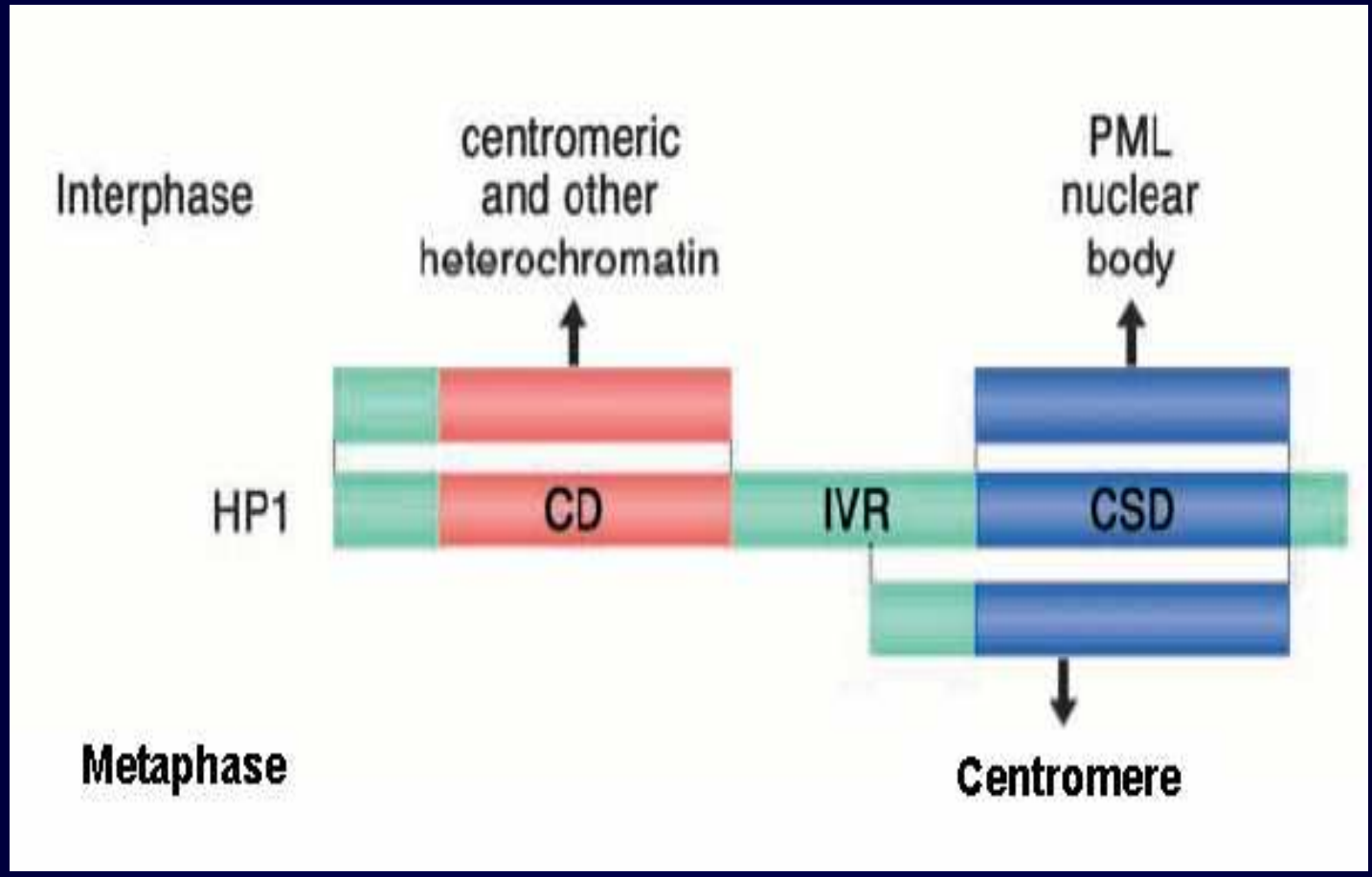


•HP1 proteiny jsou hlavní složkou heterochromatinu a hrají důležitou úlohu při jeho tvorbě. HPs jsou mají vysokou afinitu k pericentromerickým a telometrickým oblastem chromosomů.

• HPs interagují s HMTs jako je SUV39h1 a SUV39h2, která jsou zodpovědné za methylaci H3(K9).

HP1 proteins:

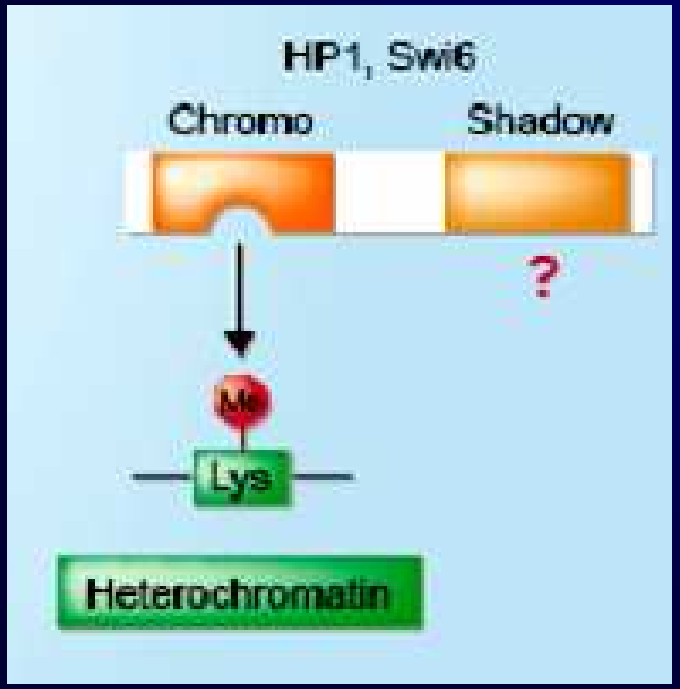
1. Heterochromatin protein (dHP1) was first identified in *Drosophila* and shown to localise to heterochromatin by antibody staining.
2. Mutation of HP1 gene decrease the effect of **PEV** (position effect variegation) on gene expression.
3. Null mutations of HP1 are lethal due to chromosome loss during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

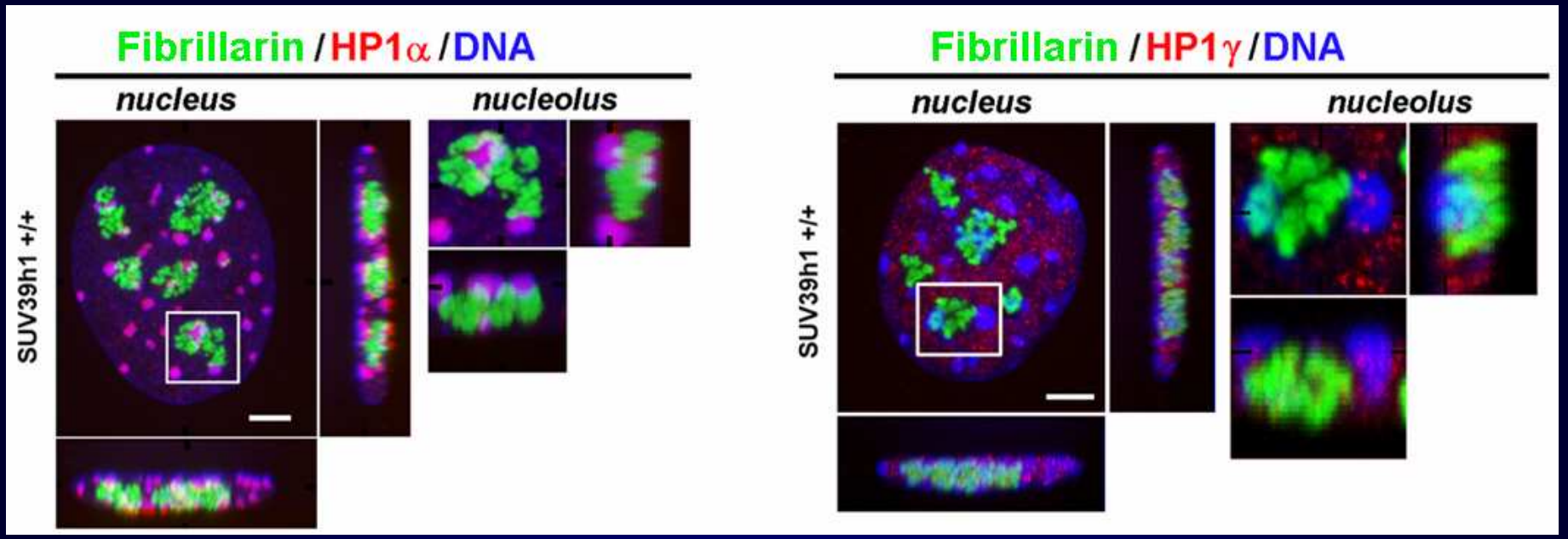


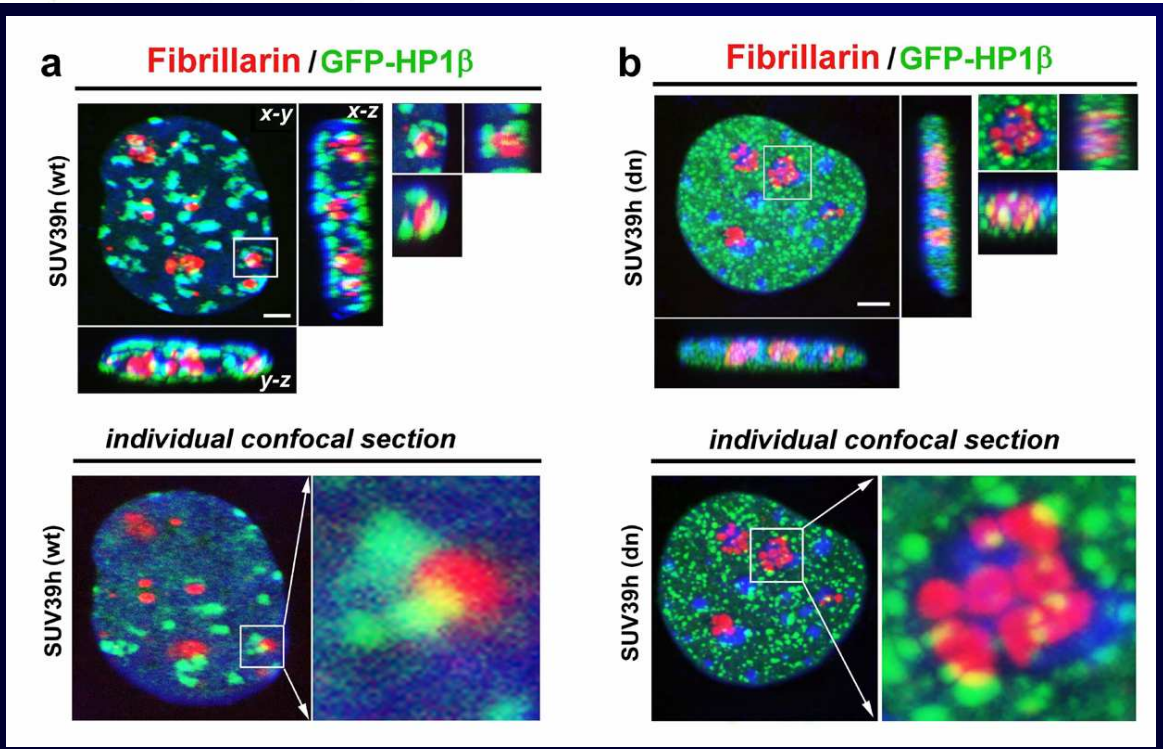
FUNKCE HP1s

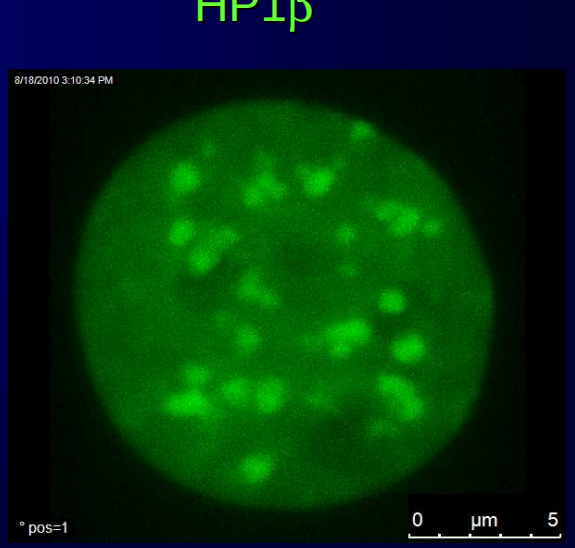
a) **Uspořádání chromatinu**

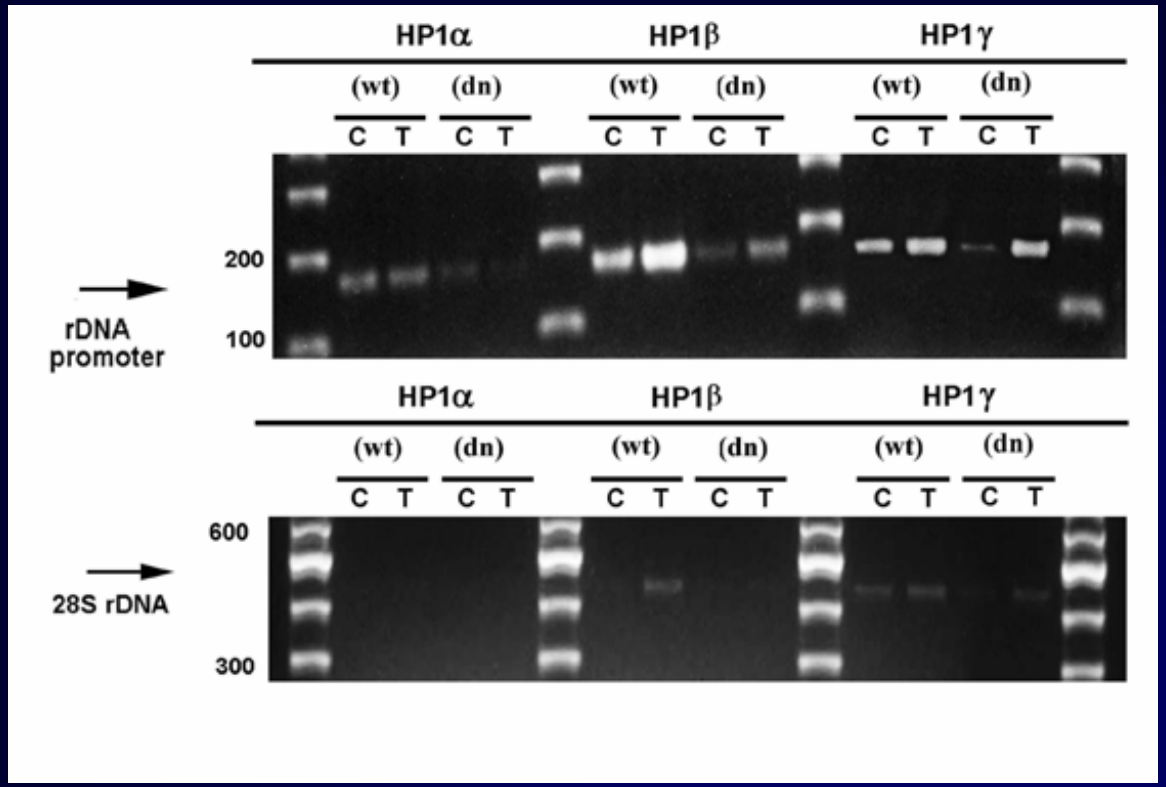
b) **Regulace transkripce**

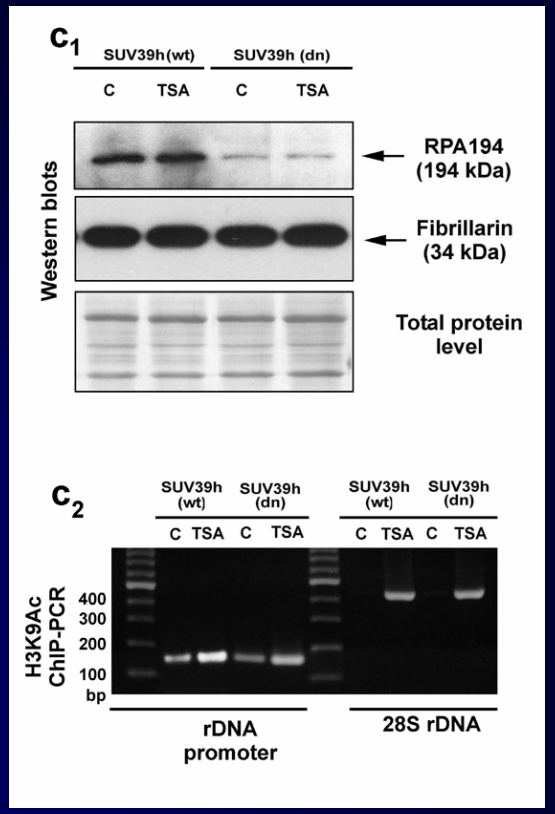
c) **Optimální regulace délky telomer a zprostředkování procesu telomeric silencing**

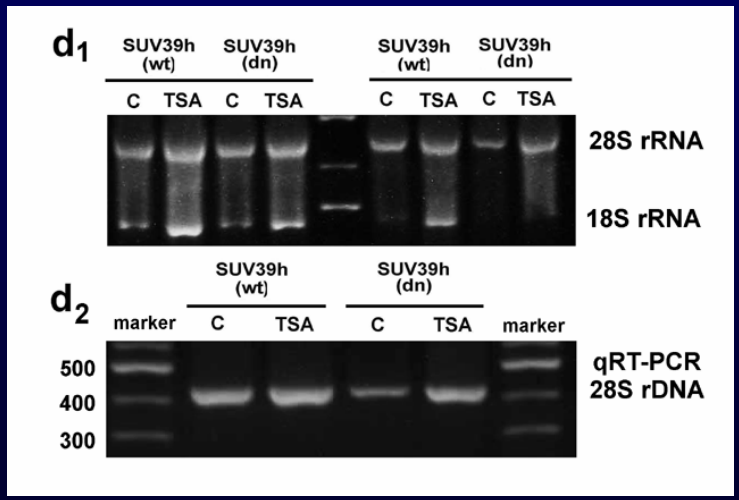

Fibrillarin / HP1α / DNA
nucleus
nucleolus
SUV39h1 +/+
Fibrillarin / HP1γ / DNA
nucleus
nucleolus
SUV39h1 +/+

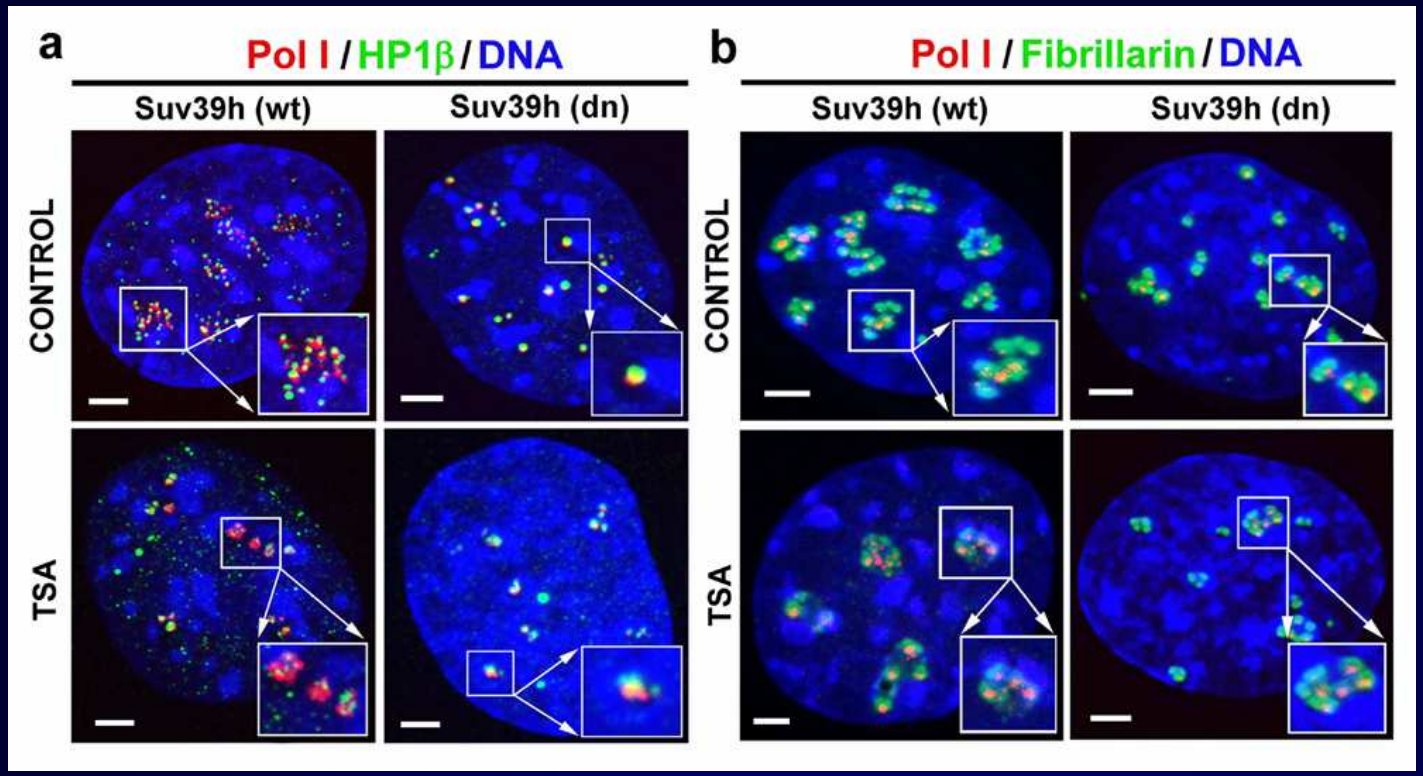

a
Fibrillarin / GFP-HP1β
x-y
x-z
y-z
SUV39h (wt)
b
Fibrillarin / GFP-HP1β
SUV39h (dn)
individual confocal section
SUV39h (wt)
individual confocal section
SUV39h (dn)

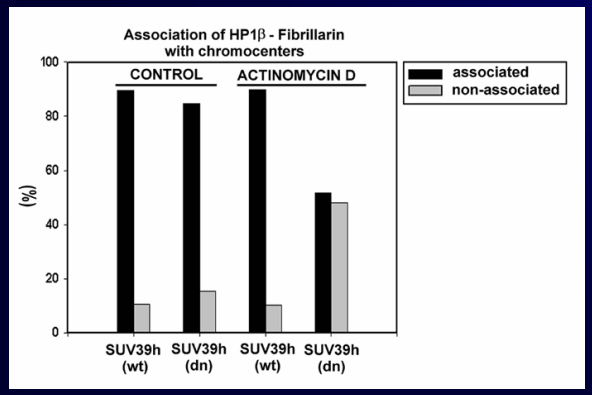

HP1β
8/18/2010 3:10:34 PM
° pos=1
0
µm
5

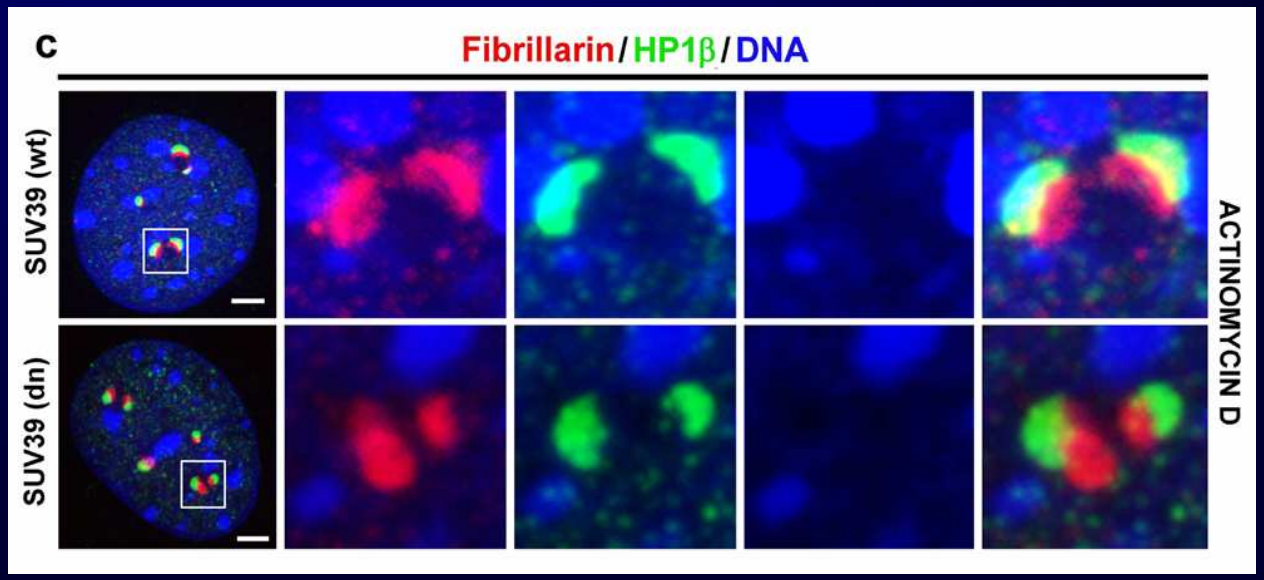

HP1α
HP1β
HP1γ
(wt)
(dn)
C T C T C T C T C T C T
200
100
rDNA promoter
600
300
28S rDNA


c1
SUV39h(wt)
SUV39h (dn)
C TSA C TSA
Western blots
RPA194 (194 kDa)
Fibrillarin (34 kDa)
Total protein level
c2
SUV39h (wt)
SUV39h (dn)
H3K9Ac ChIP-PCR
400
300
200
100
bp
rDNA promoter
28S rDNA

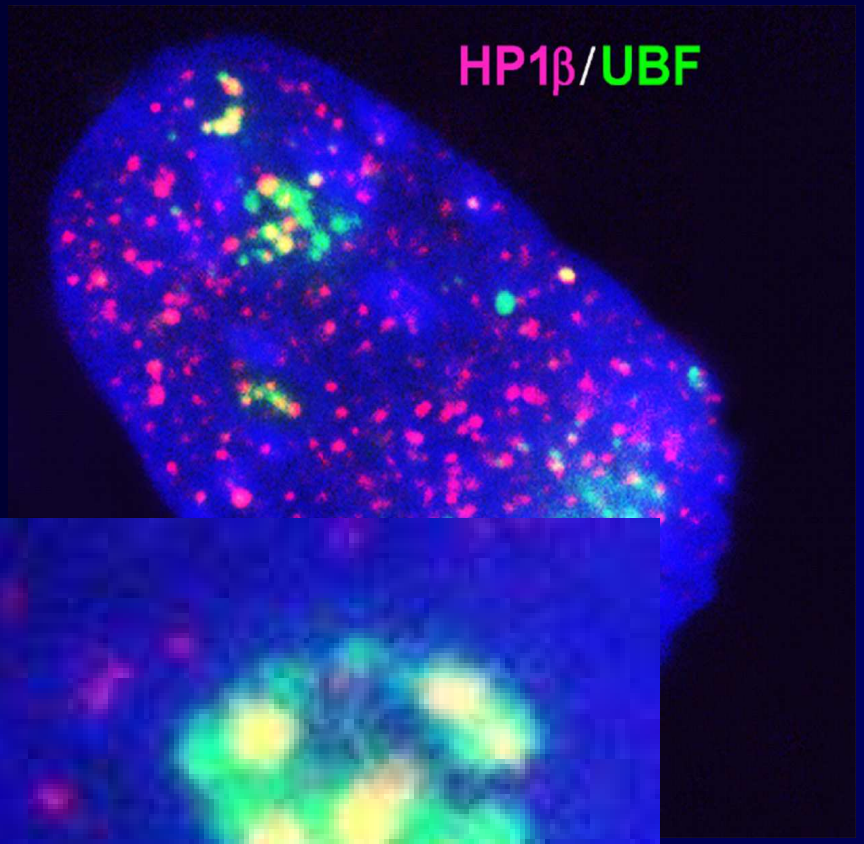

d1
SUV39h (wt)
SUV39h (dn)
C TSA C TSA C TSA C TSA
28S rRNA
18S rRNA
d2
marker
500
400
300
qRT-PCR 28S rDNA

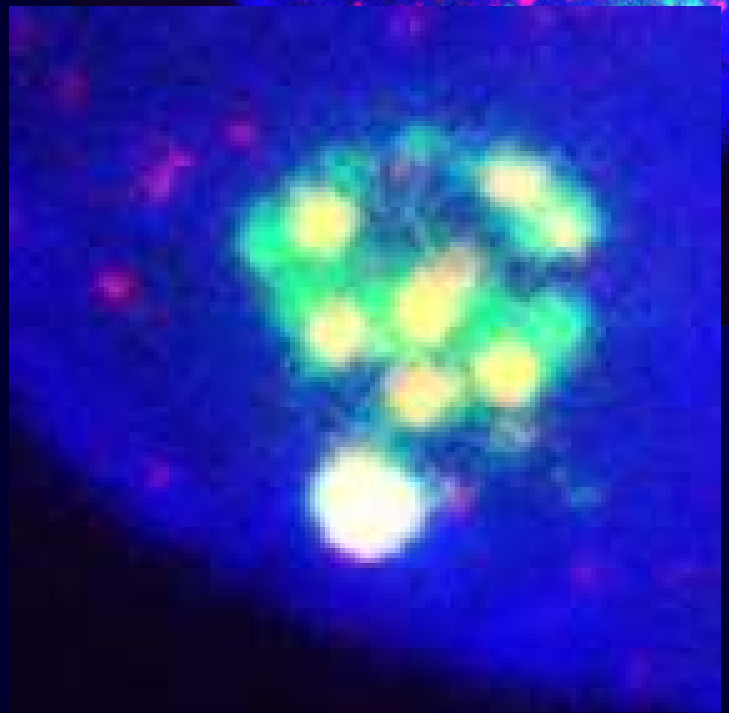

a
Pol I / HP1β / DNA
Suv39h (wt)
Suv39h (dn)
CONTROL
TSA
b
Pol I / Fibrillarin / DNA
Suv39h (wt)
Suv39h (dn)
CONTROL
TSA

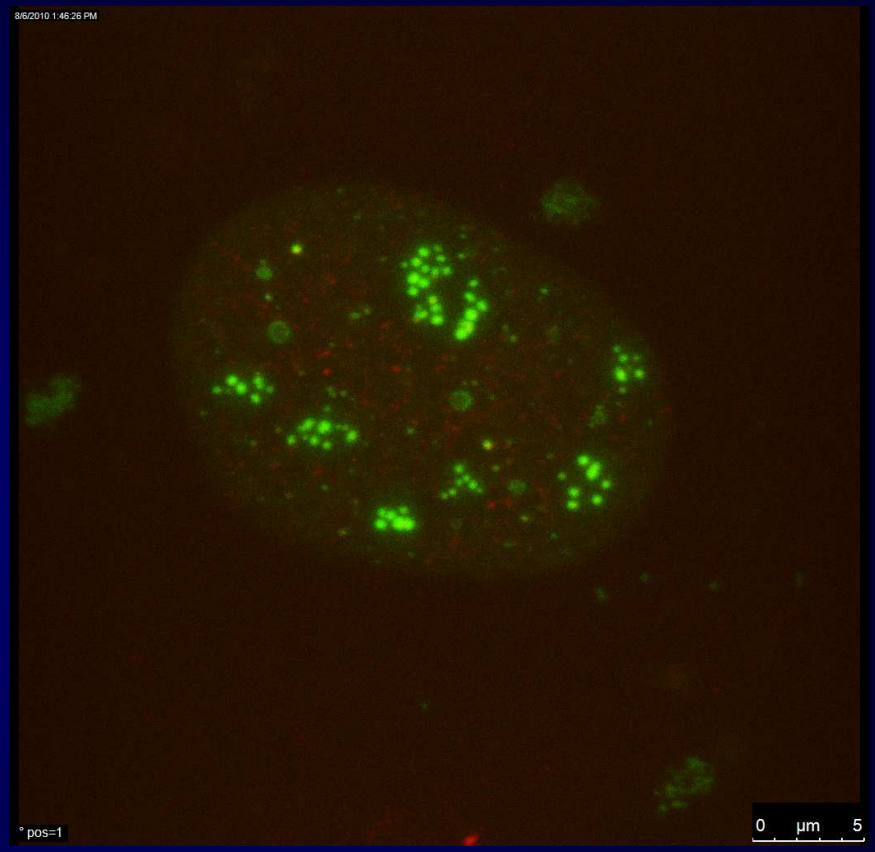

Association of HP1β - Fibrillarin
with chromocenters
CONTROL
ACTINOMYCIN D
associated
non-associated
(%)
100
80
60
40
20
0
SUV39h (wt)
SUV39h (dn)
SUV39h (wt)
SUV39h (dn)


c
Fibrillarin / HP1β / DNA
SUV39 (wt)
SUV39 (dn)
ACTINOMYCIN D

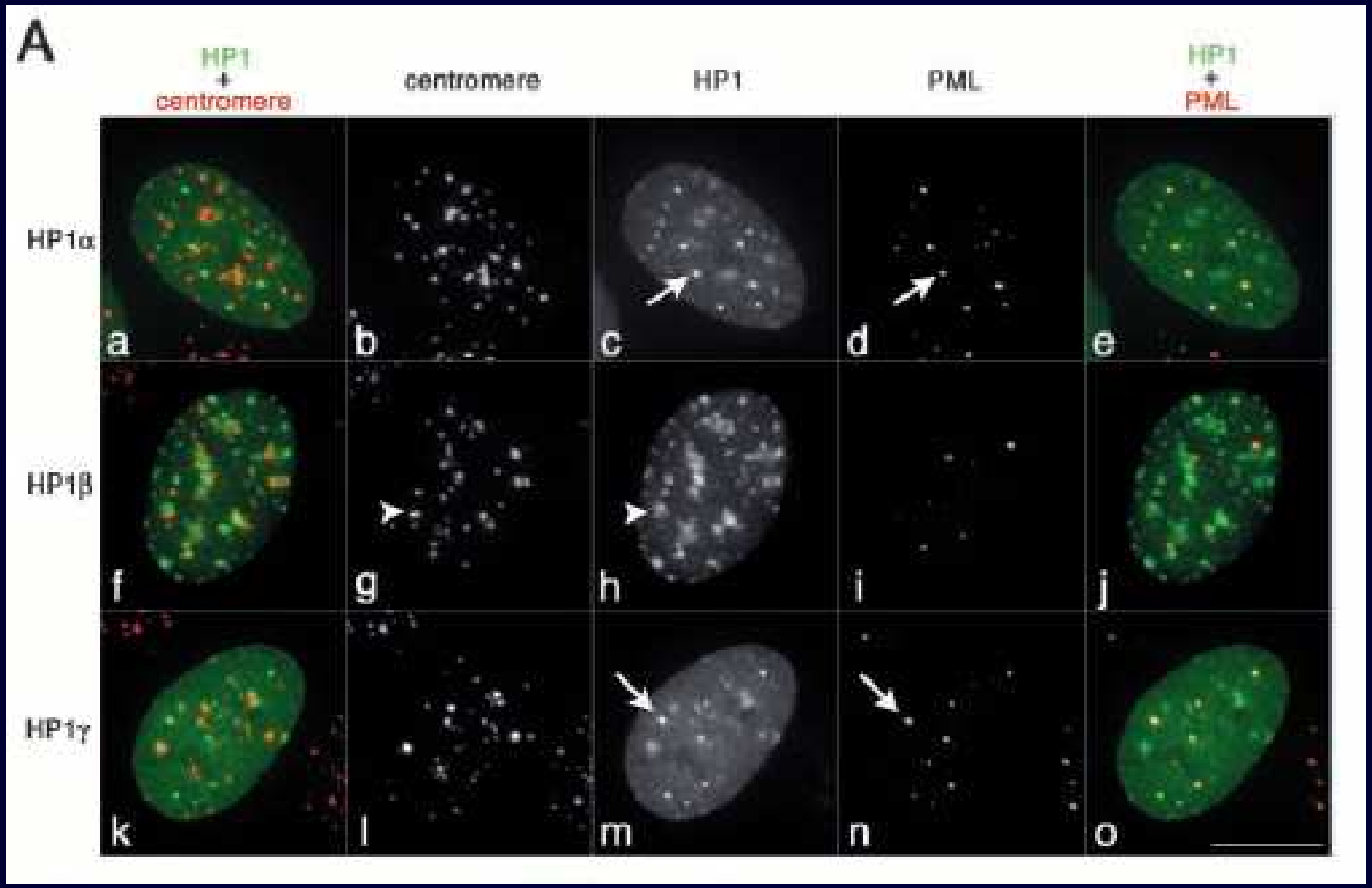
MEF cells







HP1 proteiny – v lidských buňkách jsou 3 sub-typy

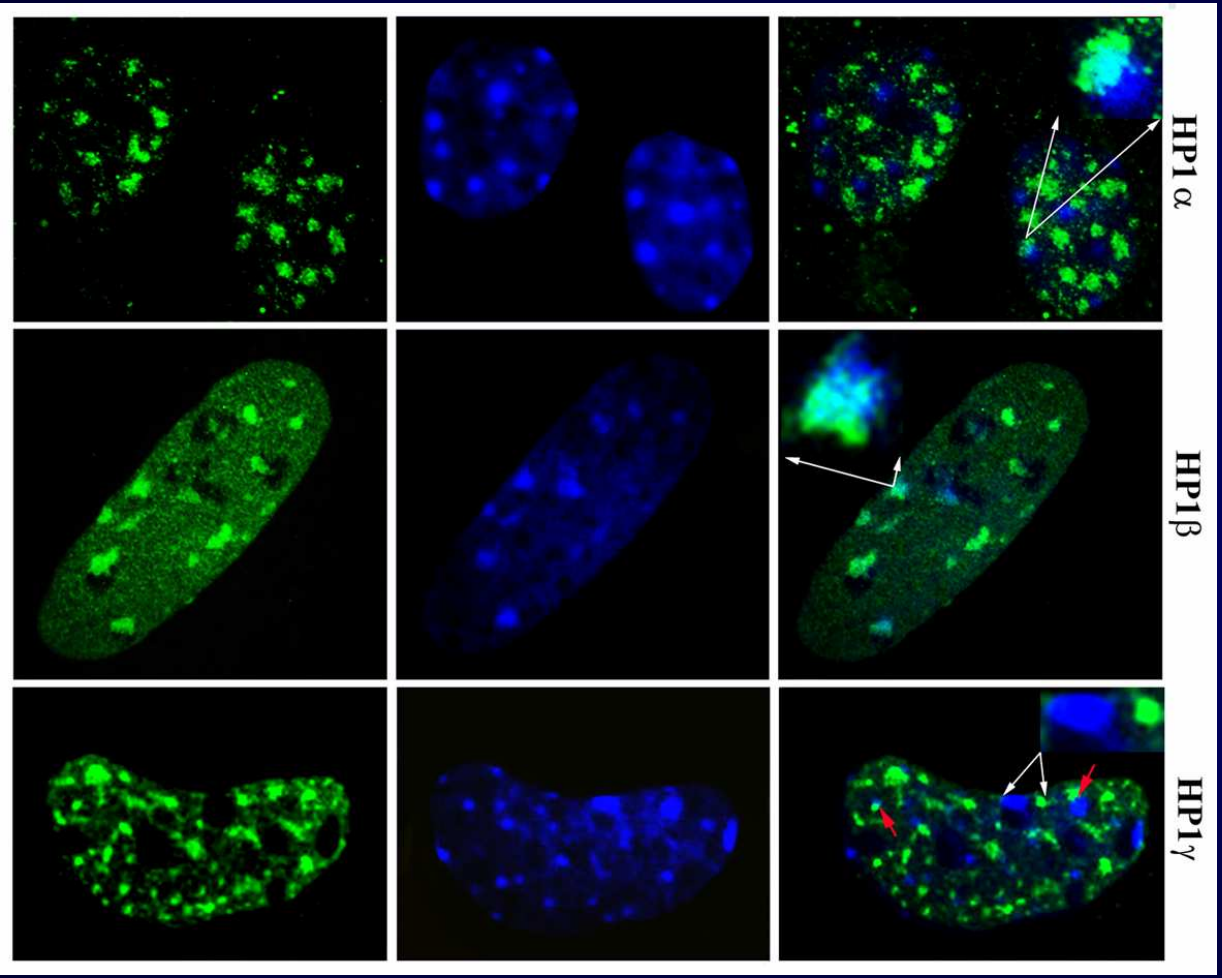


Hayakawa et al., 2003

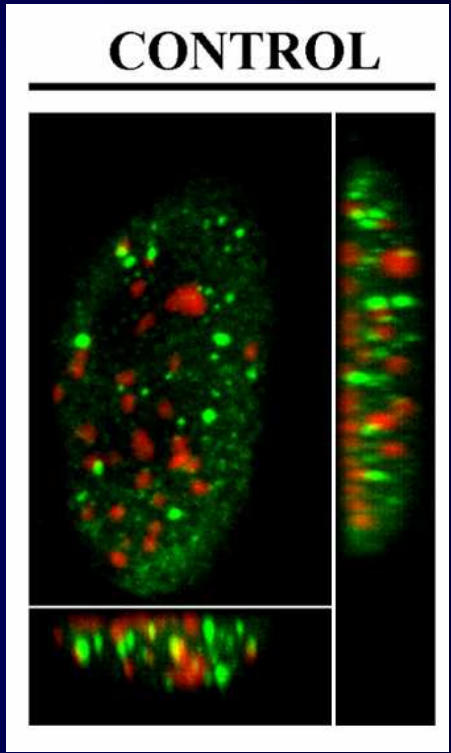
Savčí jádra obsahují 10-30 sférických struktur zvaných **PML bodies** (PODs, ND10 nebo Kremer bodies). Gen kódující PML je fúzován s genem kódujícím receptor pro kyselinu retinouvou a to u akutní promyelocytické leukemie (APL), Jde o translokaci t(15;17). PML bodies jsou cílem mnoha virů při časné infekci,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

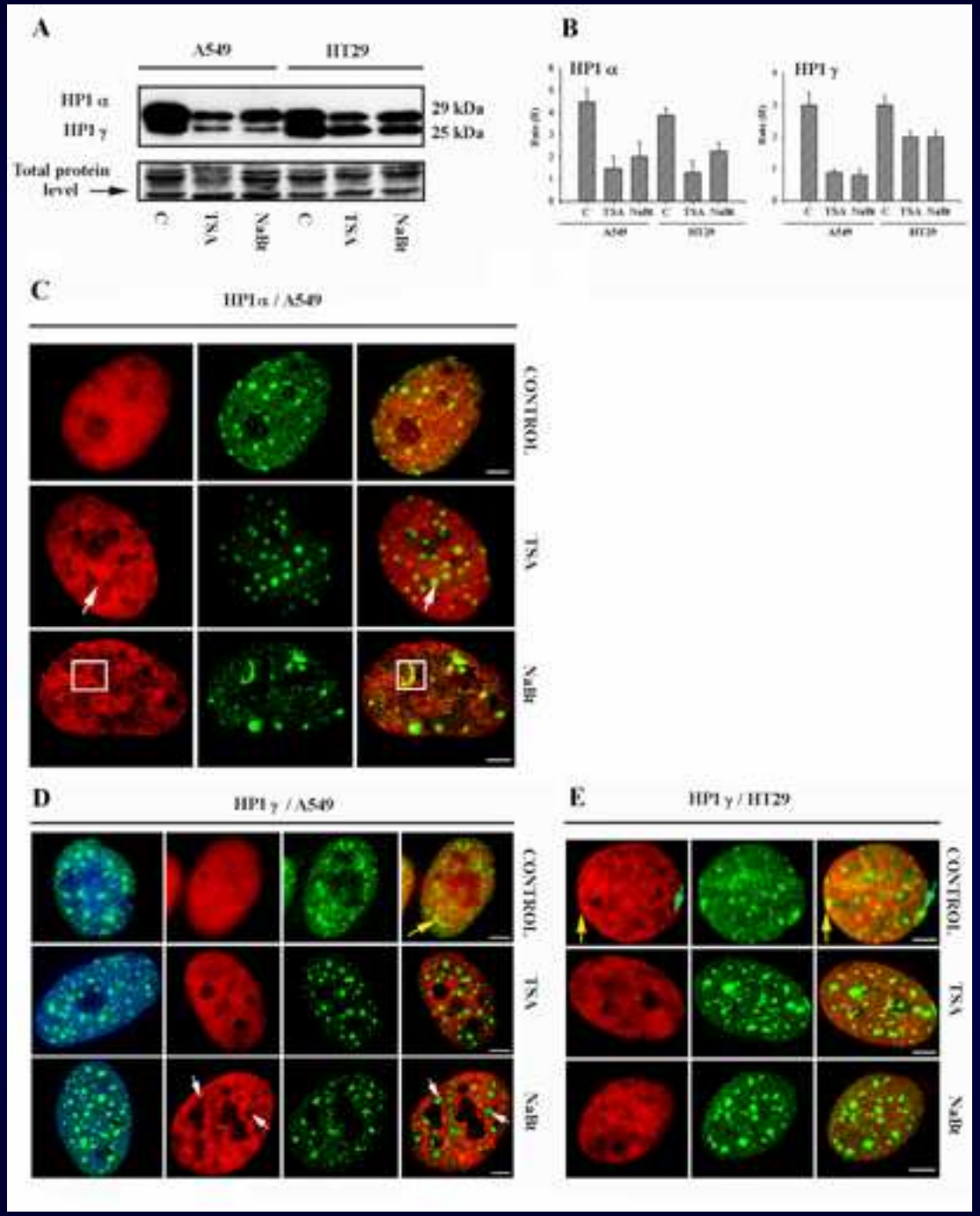
PML bodies interagují s mnoha proteiny podobnými HDAC, které se však neshromažďují v PML bodies. PML jsou zahrnuty v řadě procesů jako je buněčný růst, apoptóza, imunitní odpovědi a regulace transkripce. PML jsou také místem degradace některých proteinů, asociují nejen s HP1, ale i se specifickými geny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

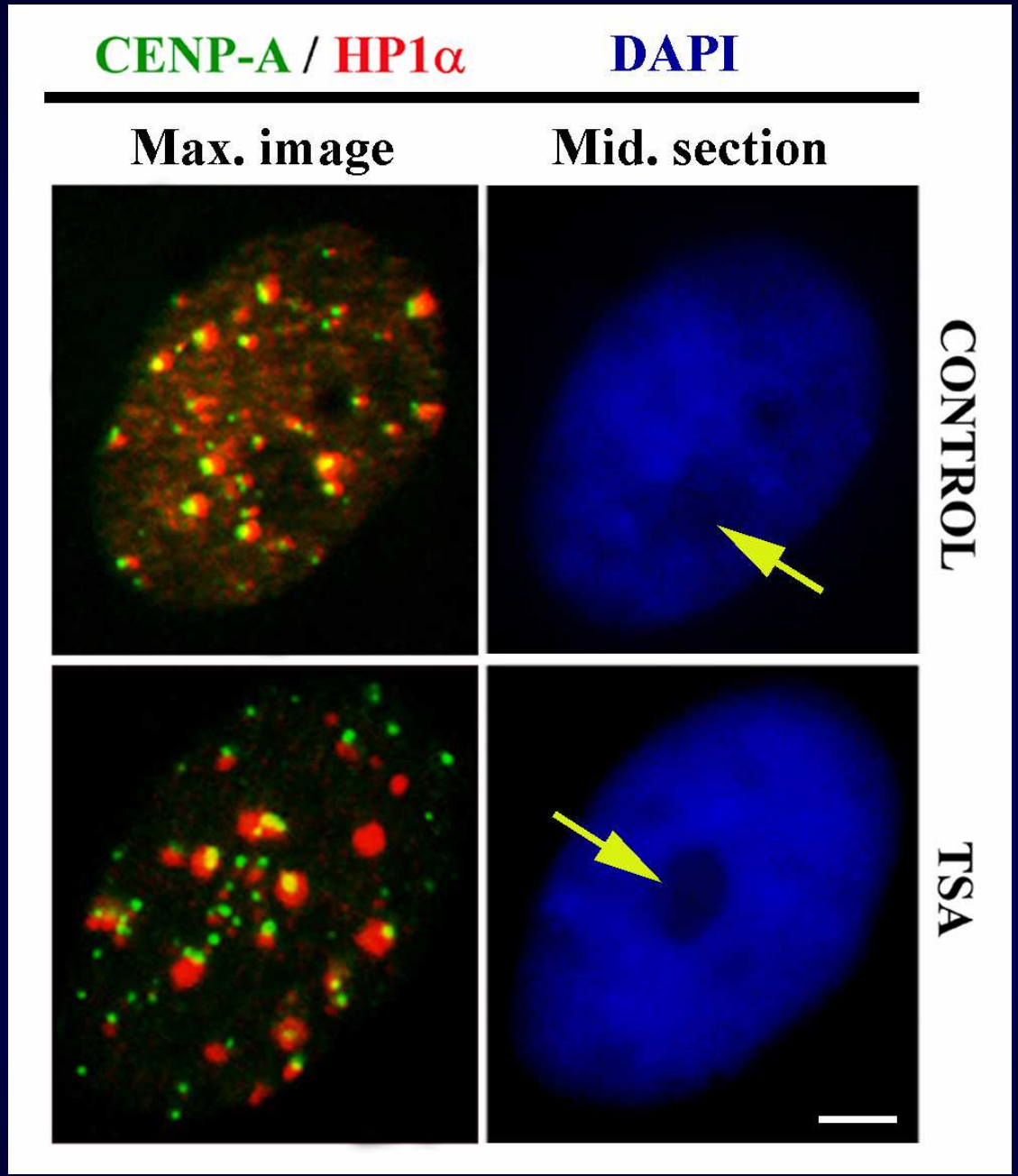
HP1 proteiny u ECS

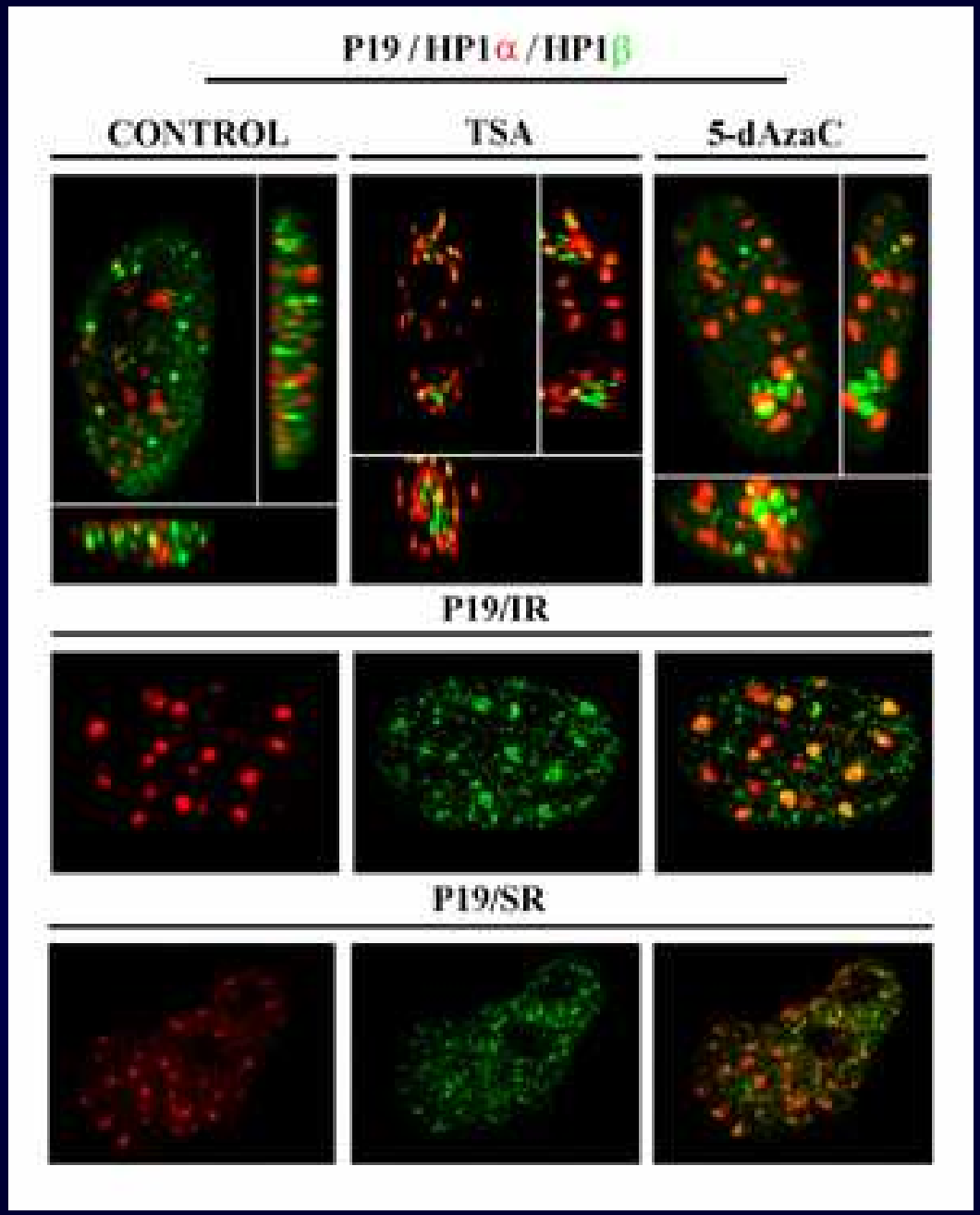


HP1α HP1β



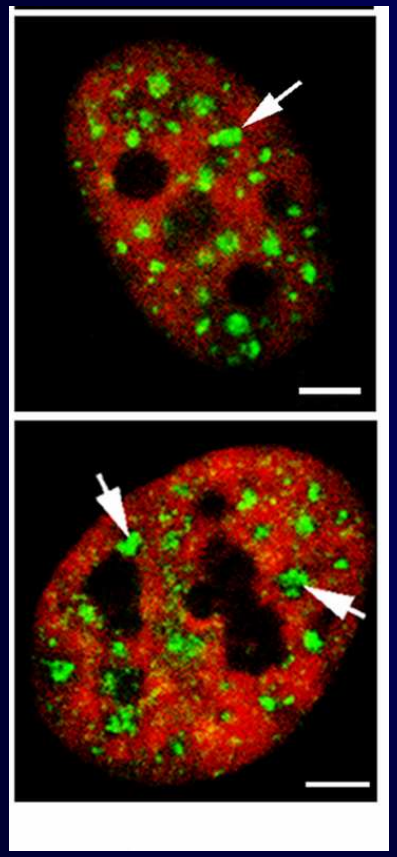

A
A549
HT29
HP1 α
HP1 γ
29 kDa
25 kDa
Total protein level
C
TSA
NaBt
B
HP1 α
HP1 γ
C
TSA
NaBt
A549
HT29
C
HP1α / A549
CONTROL
TSA
NaBt
D
HP1 γ / A549
CONTROL
TSA
NaBt
E
HP1 γ / HT29
CONTROL
TSA
NaBt

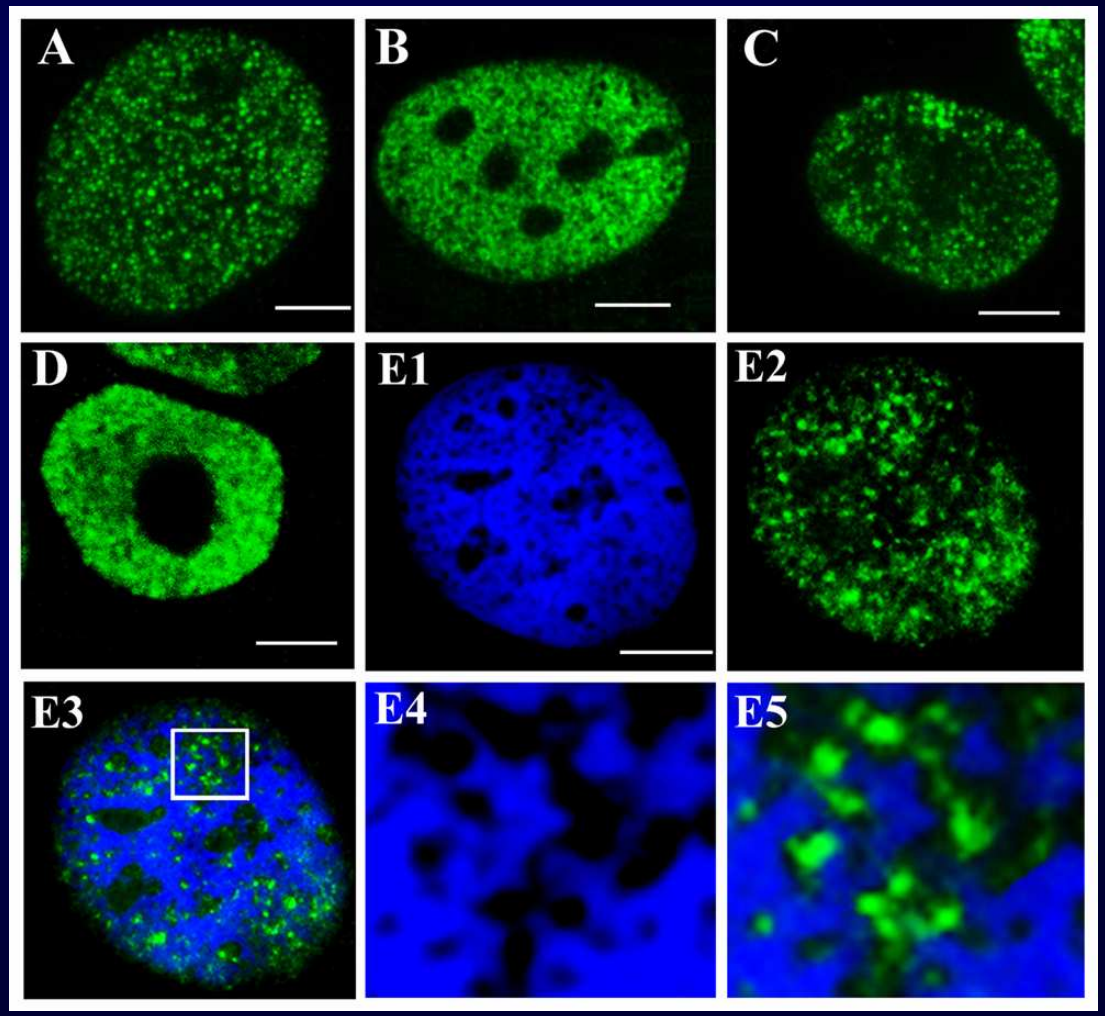

CENP-A / HP1α
DAPI
Max. image
Mid. section
CONTROL
TSA


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

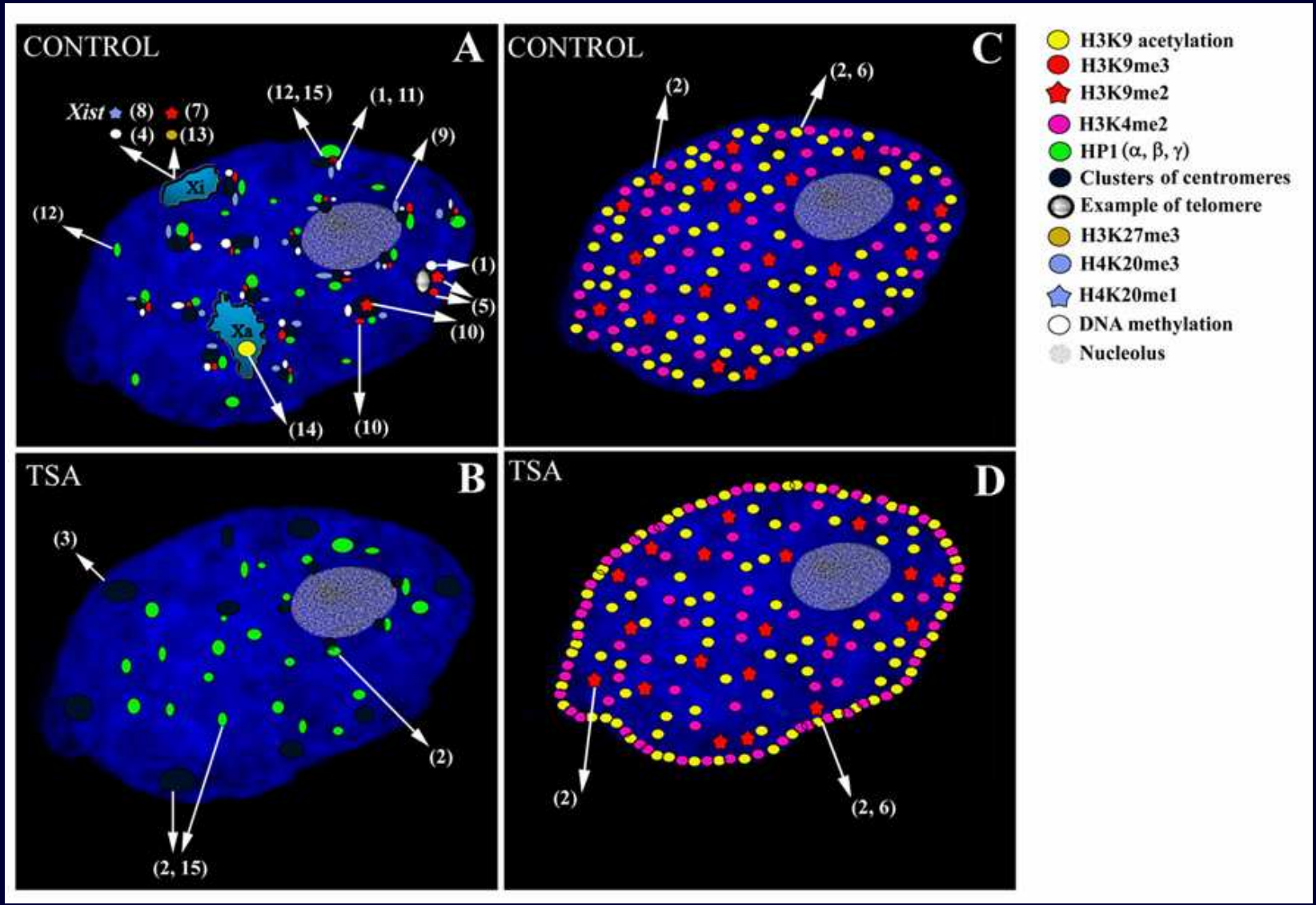
HP1 protein a H3(K4) di-methylation and IC spaces

HP1

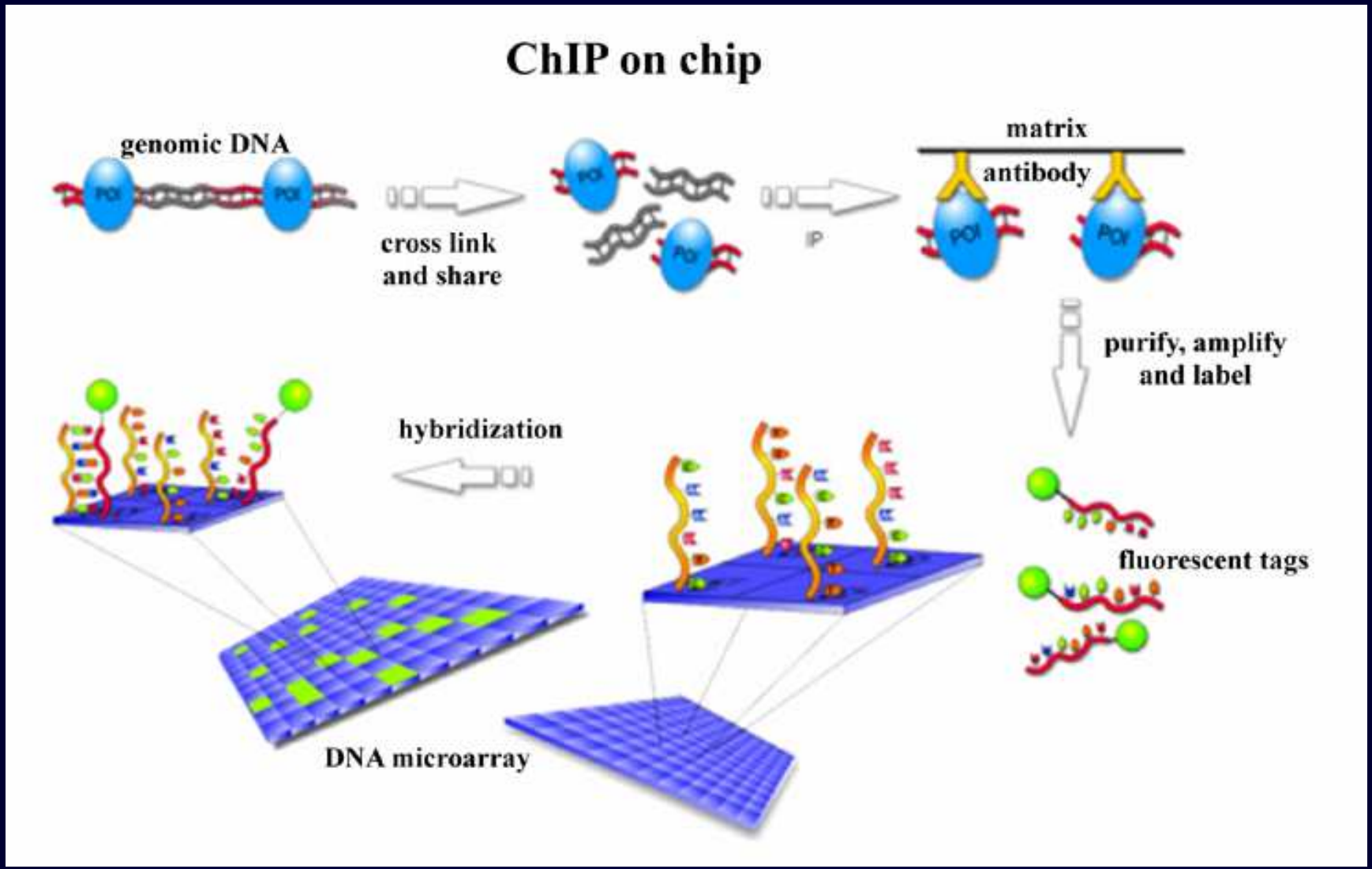


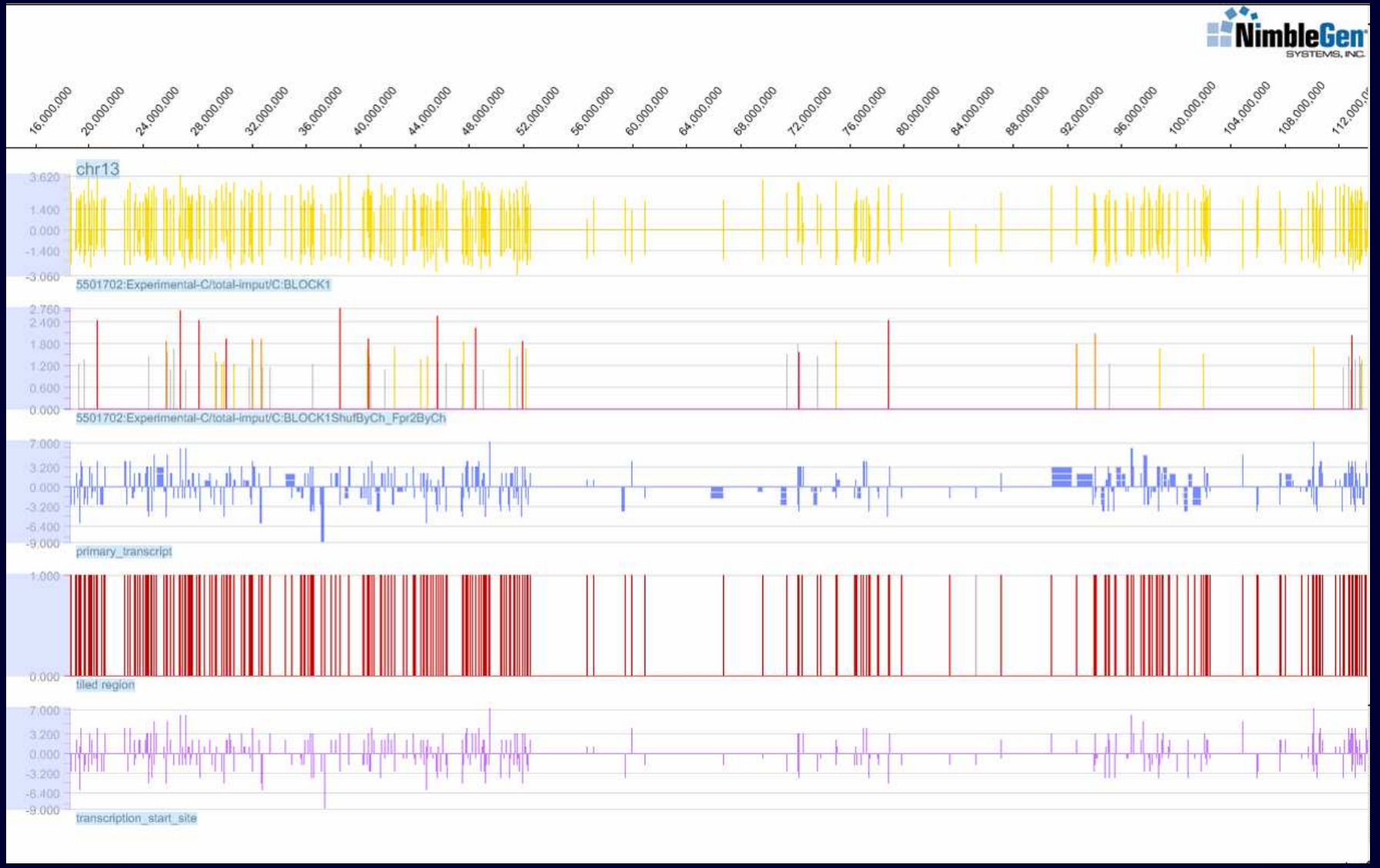


SHRNUTÍ


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.
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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
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0.000
-3.200
-6.400
-9.000
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3.200
0.000
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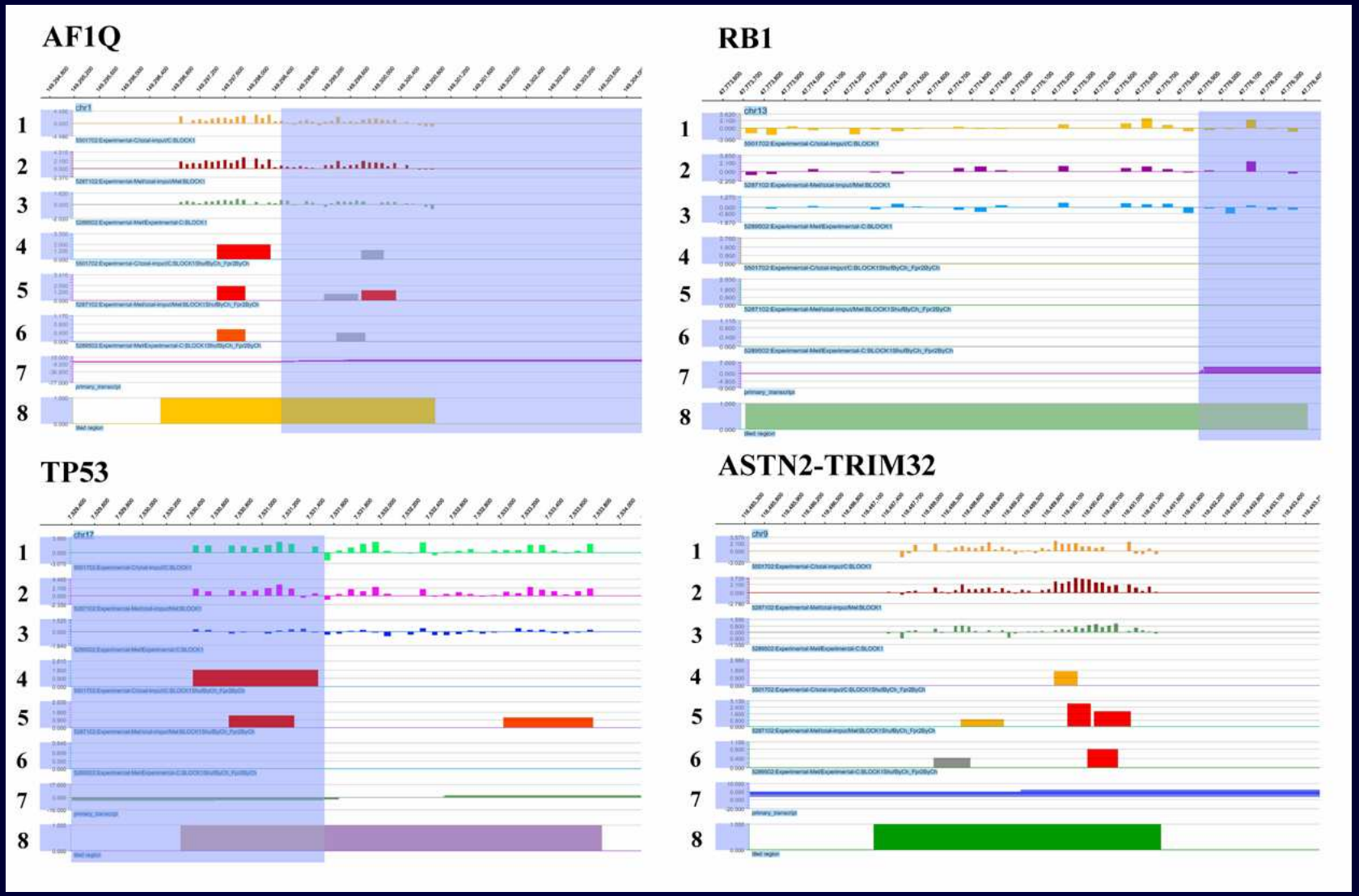
AF1Q
RB1
TP53
ASTN2-TRIM32

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 protein a jeho sub-typy – struktura a funkce
6. Účinky HDACi
7. Methylace DNA versus methylace histonů