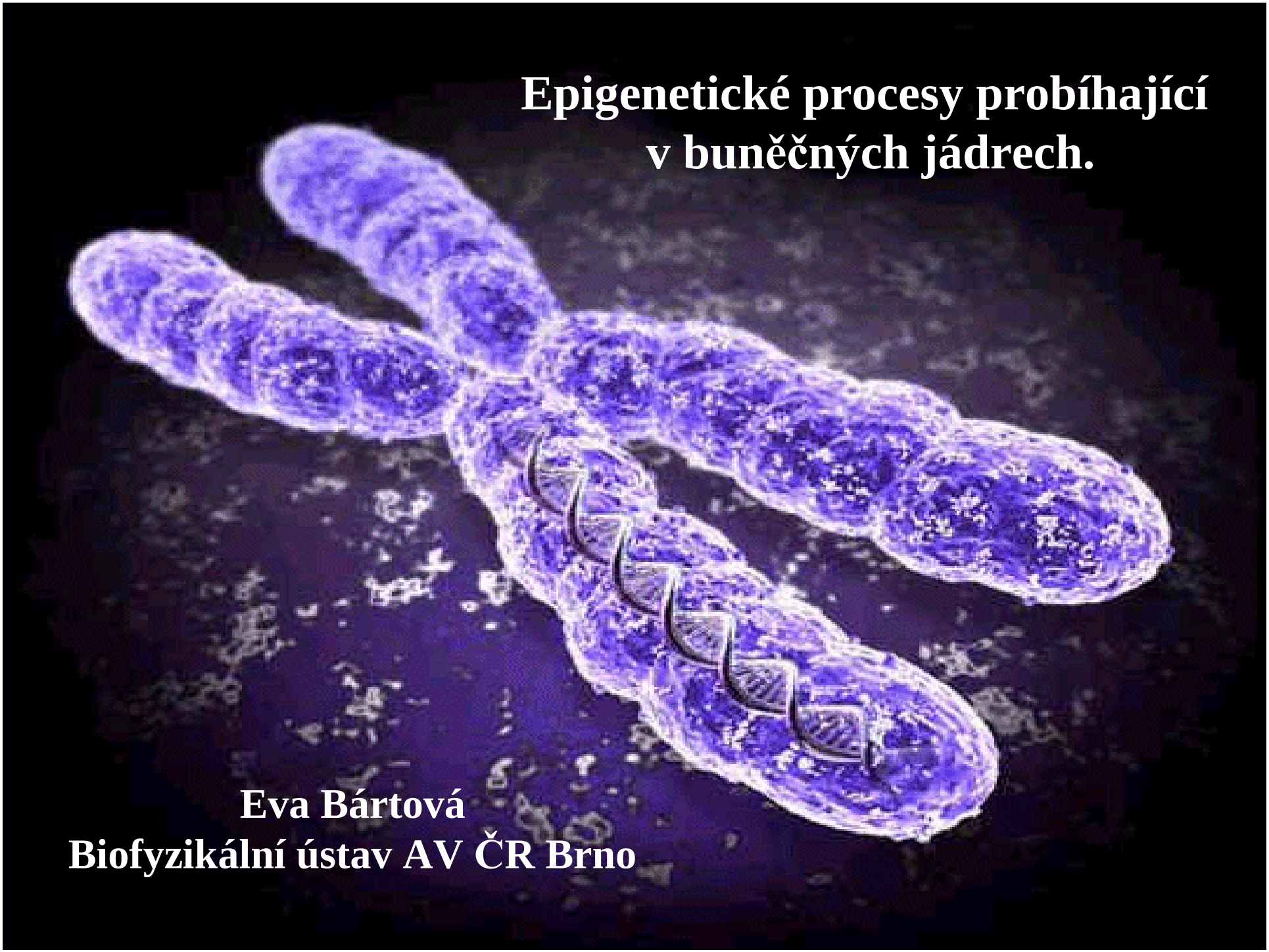
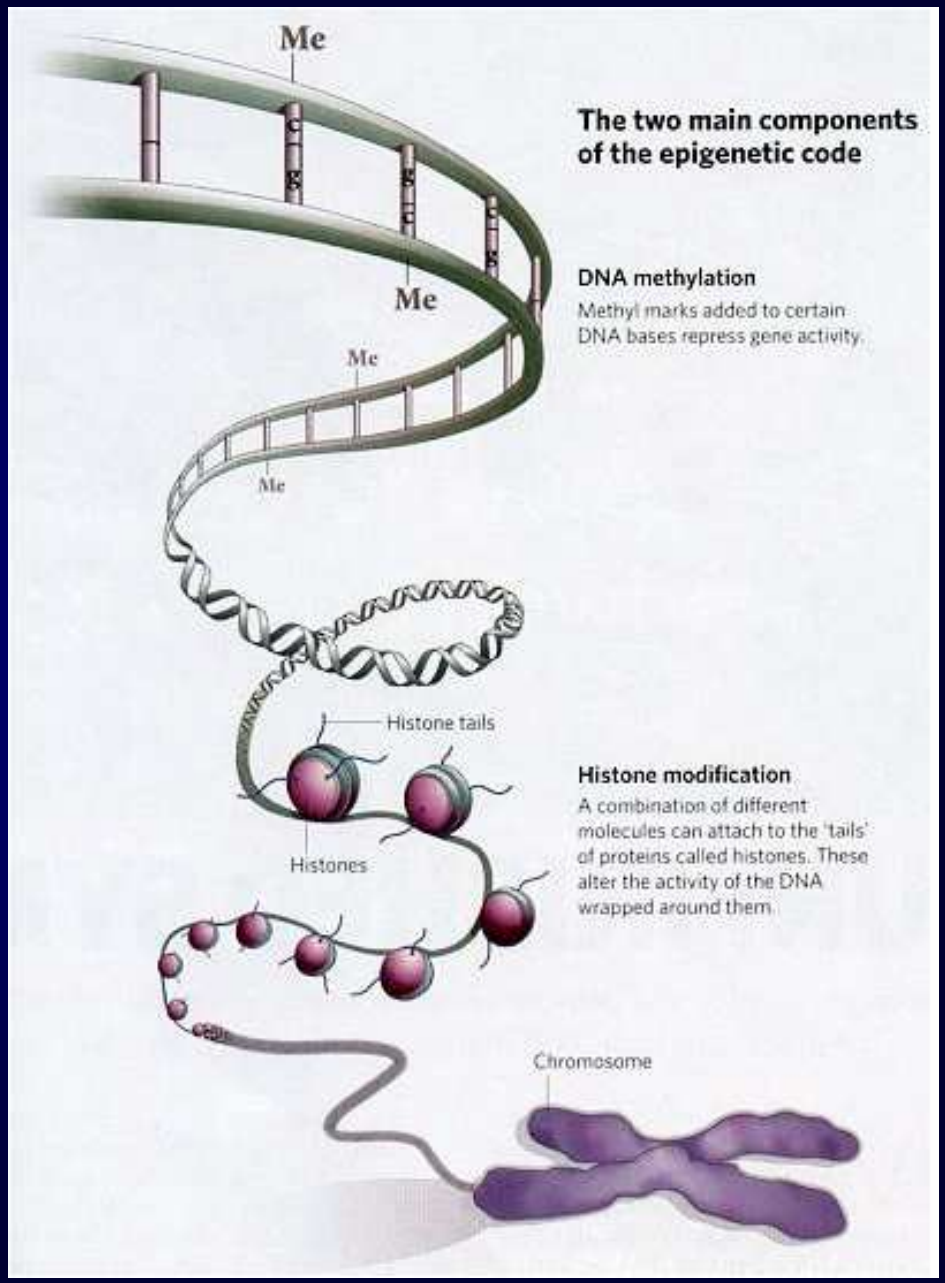
Epigenetické procesy probíhající v buněčných jádrech.

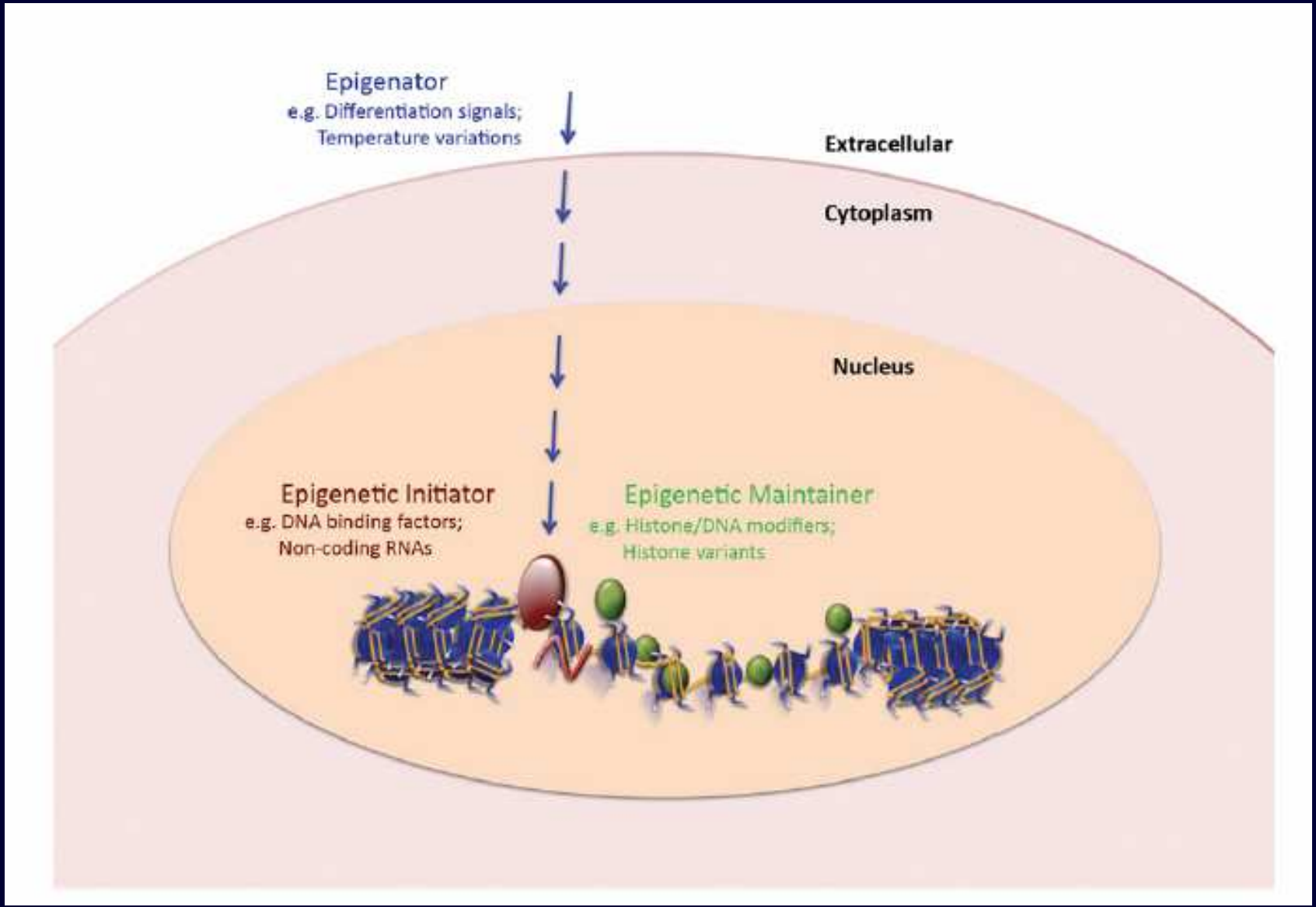


Eva Bártová
Biofyzikální ústav AV ČR Brno

What is epigenetics ?

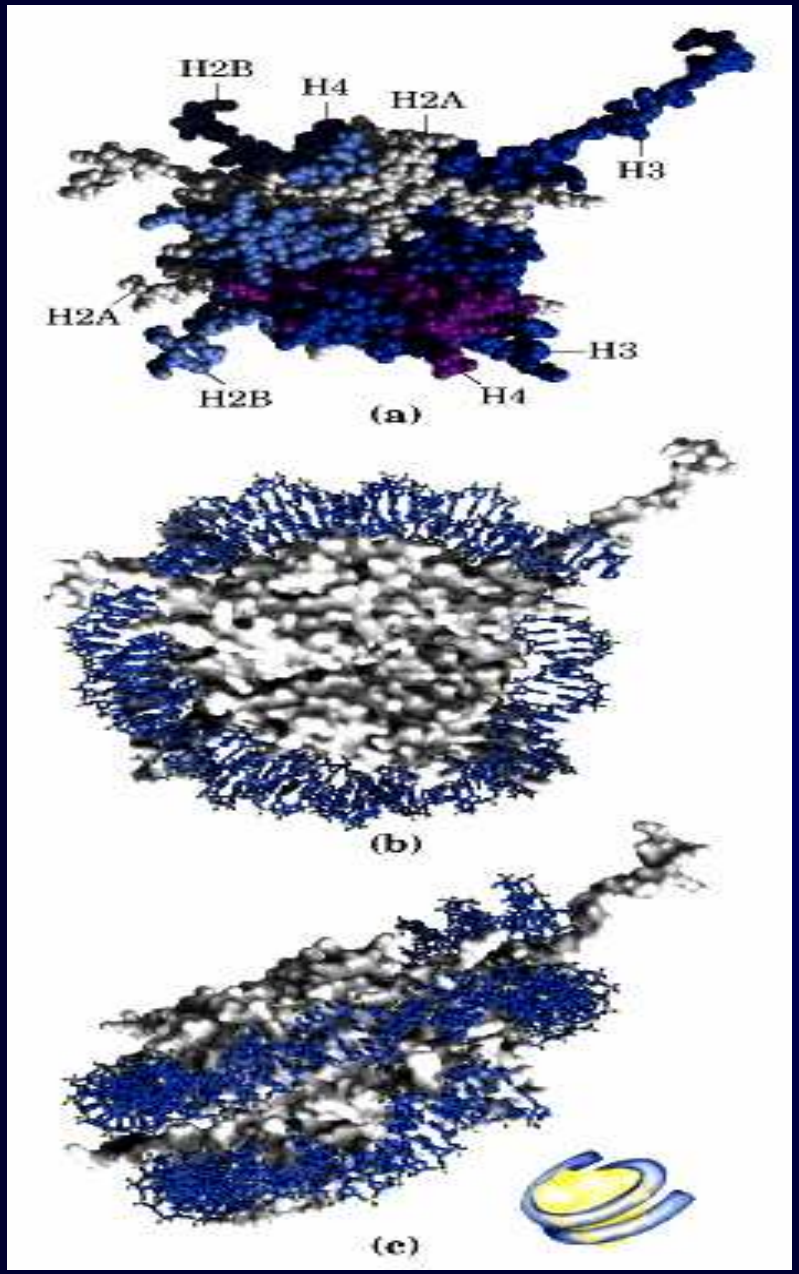
Epigenetics refers to heritable changes in the phenotype that occur irrespective of alterations in the DNA sequences.

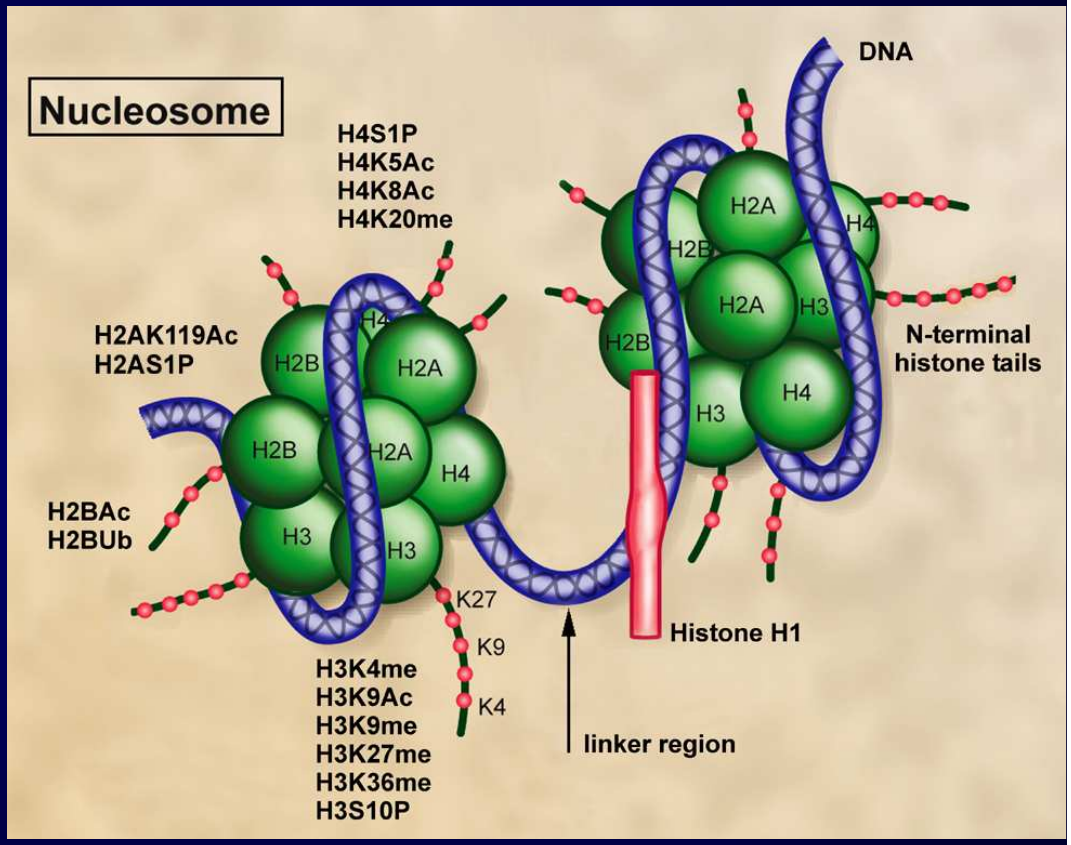


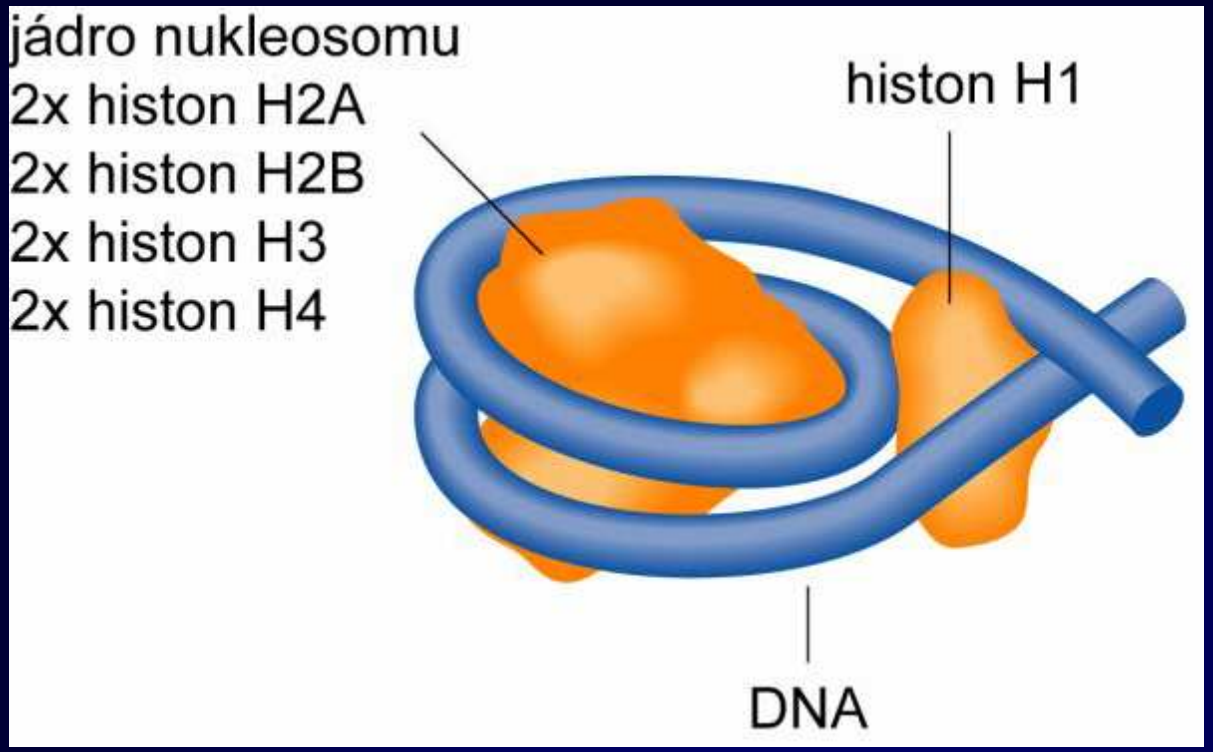


Berger et al., Genes Dev. (2009)

Nucleosome


H2B
H4
H2A
H3
H2A
H3
H2B
H4
(a)
(b)
(c)

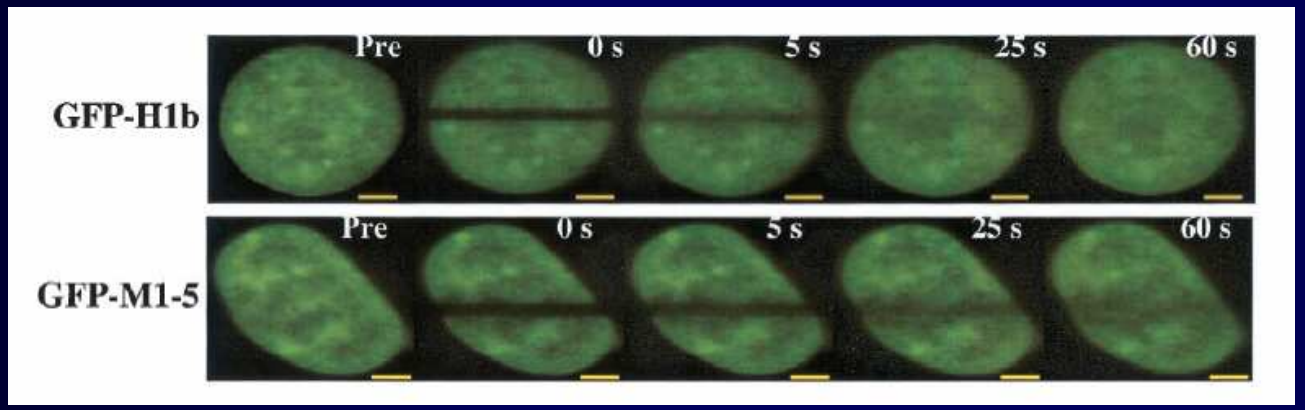

Nucleosome
DNA
H4S1P
H4K5Ac
H4K8Ac
H4K20me
H2AK119Ac
H2AS1P
H2BAc
H2BUb
H3K4me
H3K9Ac
H3K9me
H3K27me
H3K36me
H3S10P
K27
K9
K4
Histone H1
linker region
N-terminal
histone tails
H2A
H2B
H3
H4



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. Decreased mobility of GFP-H1.

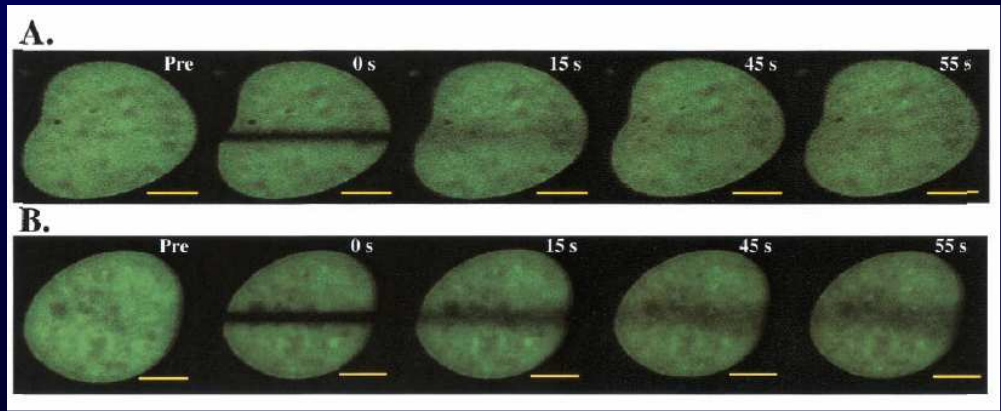


Experiments of E. Meshorer

Overexpression p21

GFP-H1b

GFP-M1-5



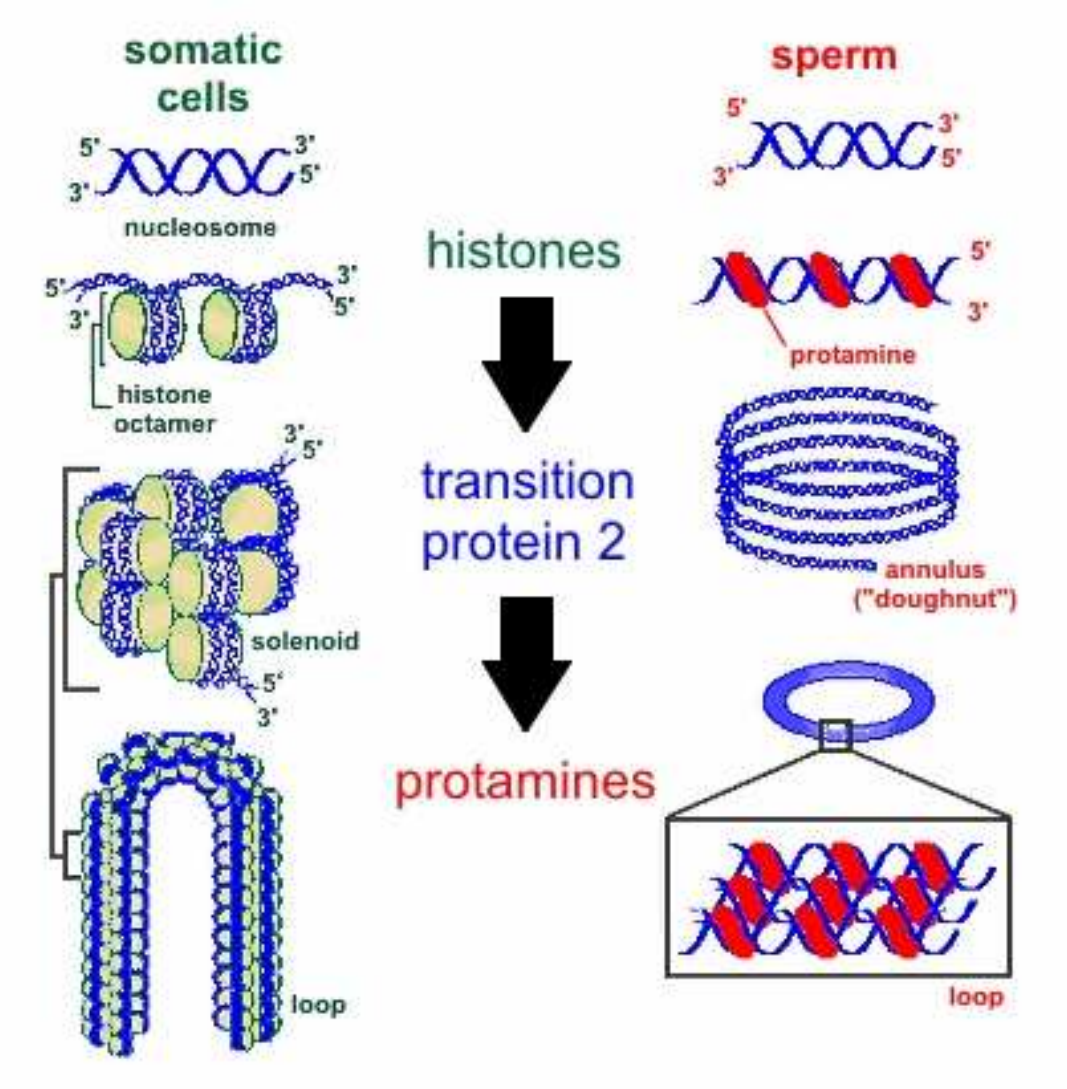
Varianty histonů

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 $H1^{o.}$

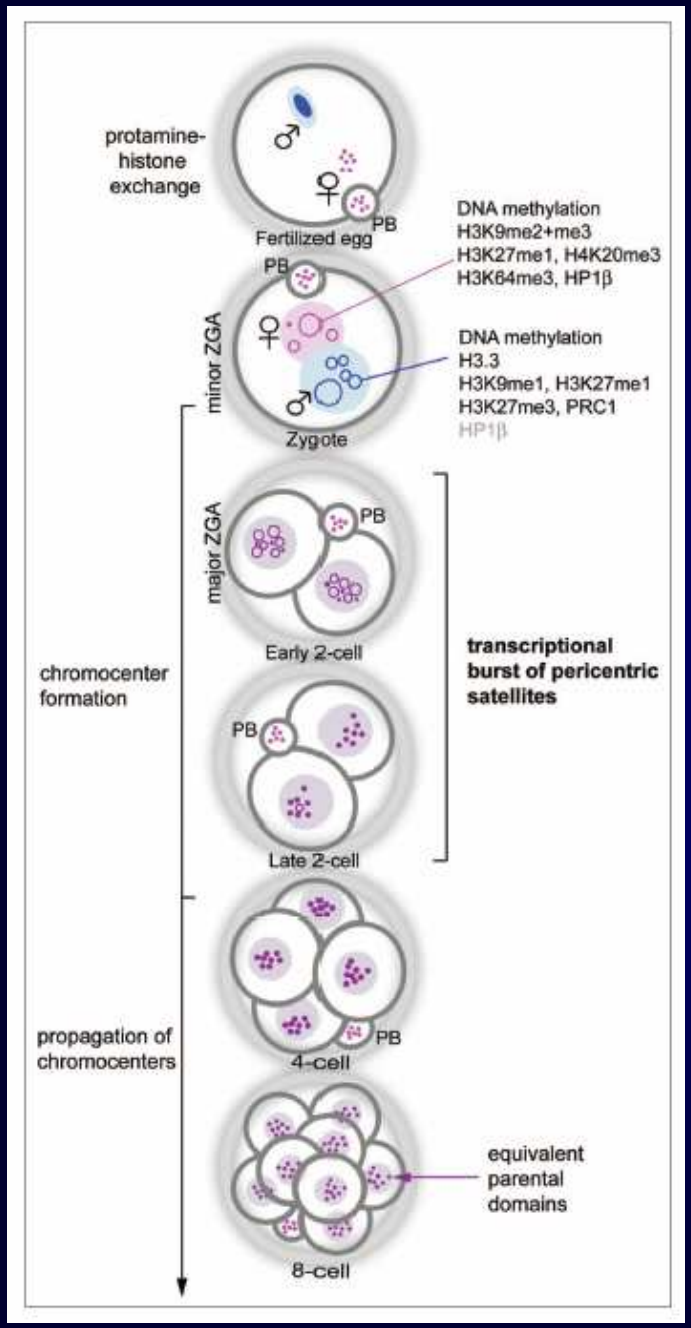
H2A: H2A.X, H2A.Z, MacroH2A, H2A-Bbd, H2AvD, H2A.X. varianta H2A.Z je konzervativní během evoluce. Macro H2A se vyskytuje u Xi, zatímco H2A-Bbd u Xa chromosomu a autosomů. H2A.Z se vyskytuje v intergenických oblastech.

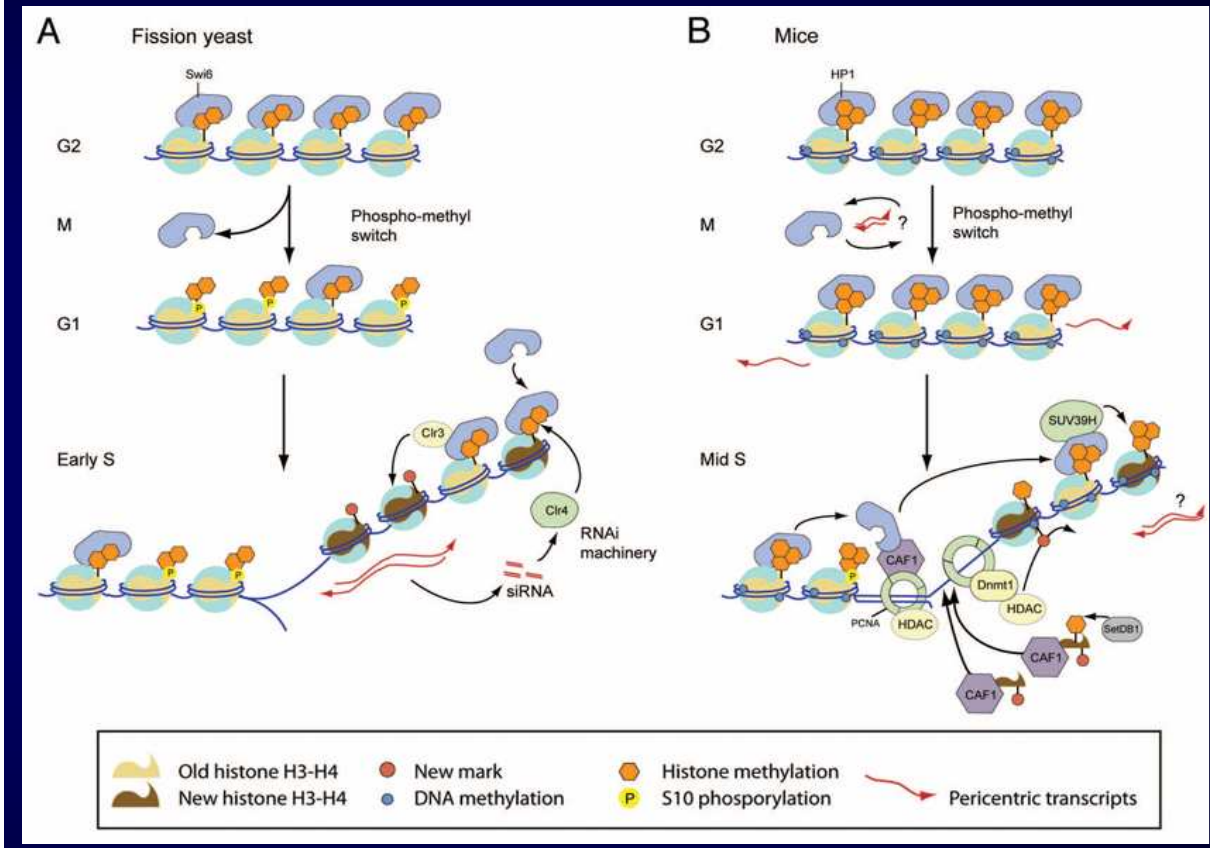
H2B: nemá varianty, uplatňuje se při regulaci kondenzace chromatinu, represi transkripce a během gametogeneze, H2B je zodpovědný za uspořádání chromatinu u spermií – nahrazení histonů protaniny

.

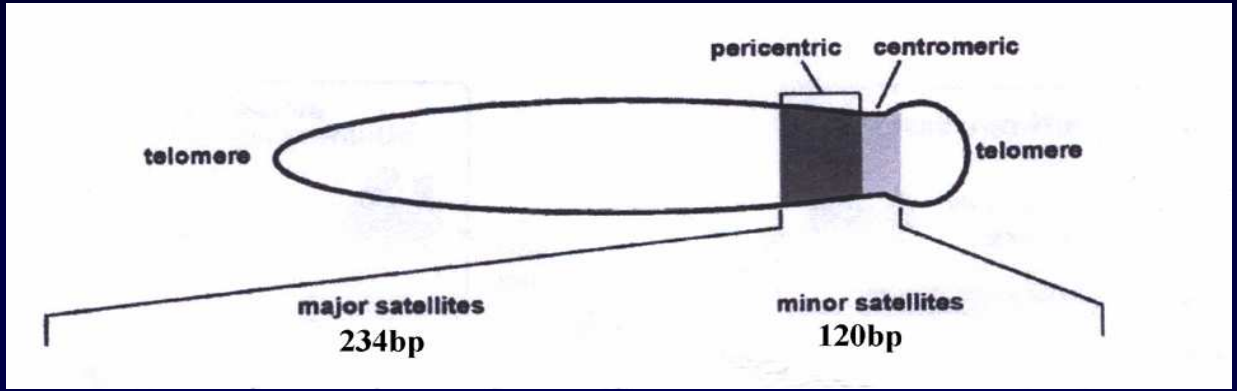


Protamines (P1/P2) are small, arginine-rich, nuclear proteins that replace histones late in the haploid phase of spermatogenesis and are believed essential for sperm head condensation and DNA stabilization. However, in humans and maybe other primates, 10-15% of the sperm's genome is packaged by histones thought to bind genes that are essential for early embryonic development.





Almouzni and Probst, Nucleus (2011)

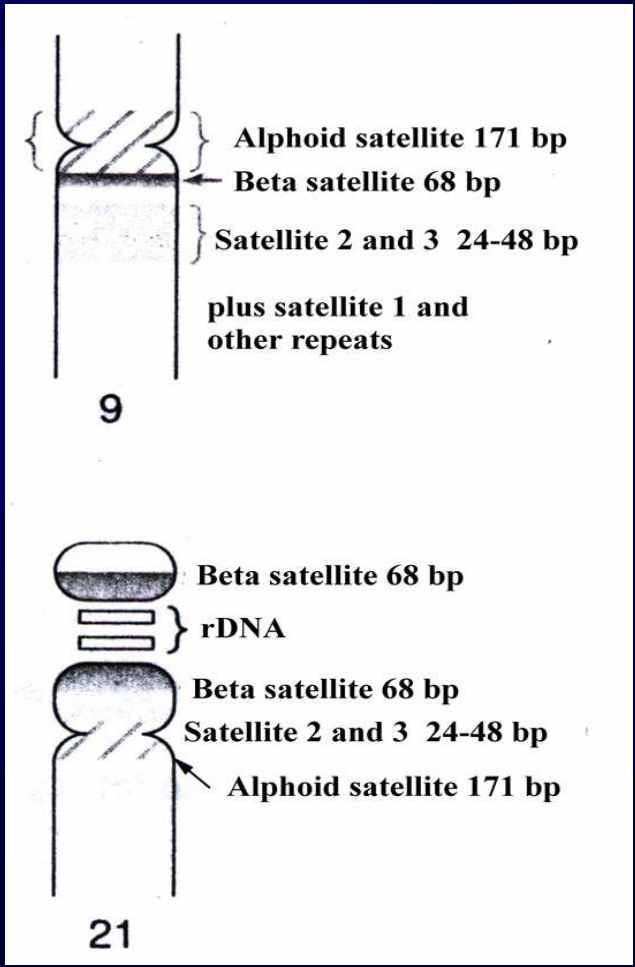


Varianty histonů

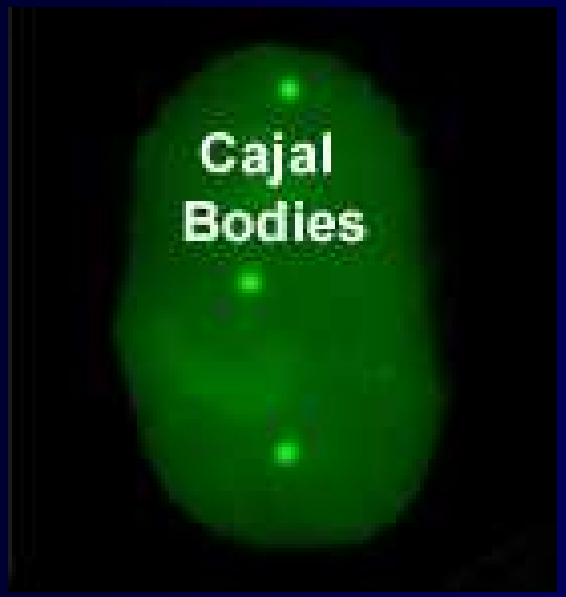
H3: existují dvě hlavní

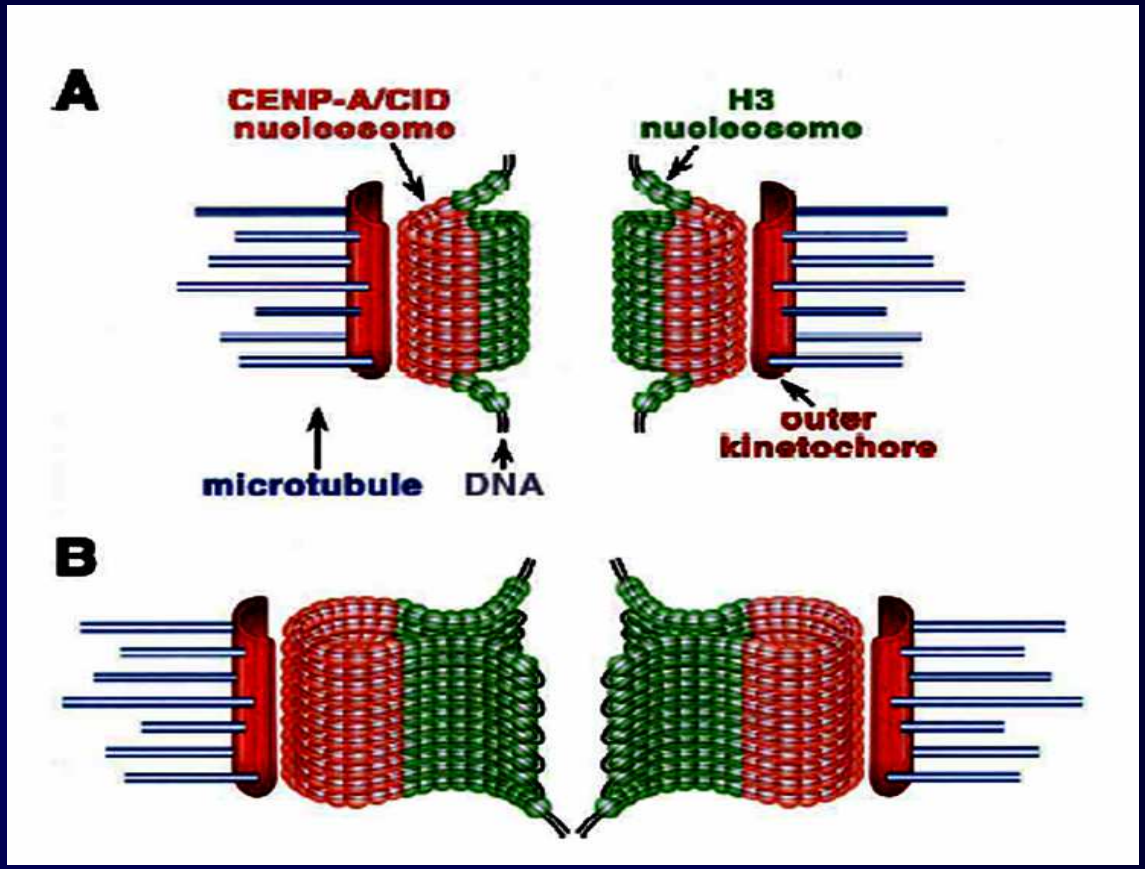
Varianty H3.3 a centromerické varianty

H3 (cenH3) = CENP-A-Z: jsou zodpovědné za vazbu kinetochoru a segregaci sesterských chromatid u eukaryot



Varianty histonů H3: **fosforylace CENP-A (Ser-7) je essenciální pro funkci kinetochoru. Overexprese CENP-A doprovází vznik aneuploidie u kolorektální karcinomů.**





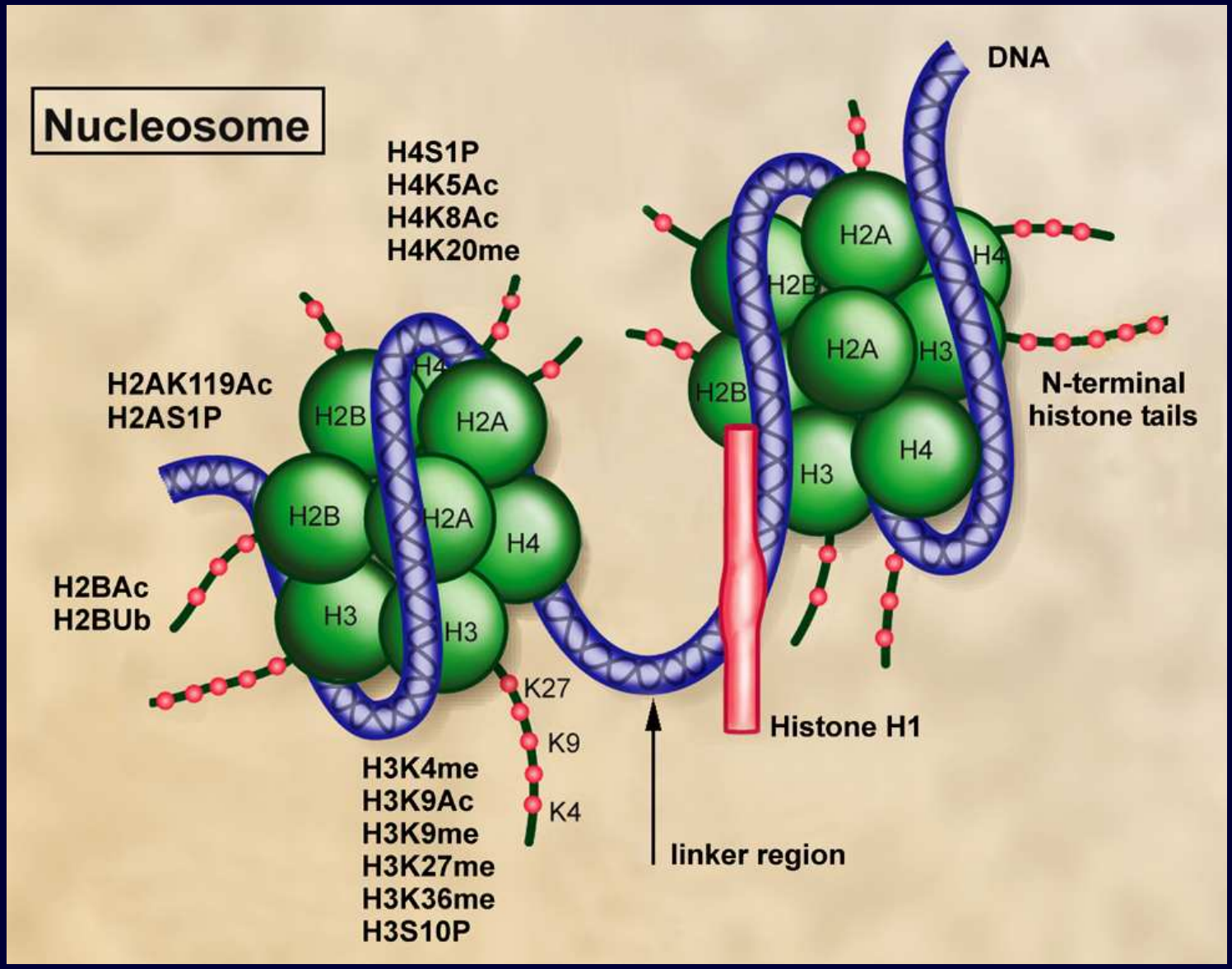
Varianty histonů H4: **většina genů kódujících hlavní histonové proteiny jsou exprimovány během S fáze buněčného cyklu. V případě H4, geny jsou konstitutivně exprimovány během buněčného cyklu. Pro H4 nejsou známy žádné varianty. Úpravy pre-mRNA histonů probíhají v Cajal bodies.**

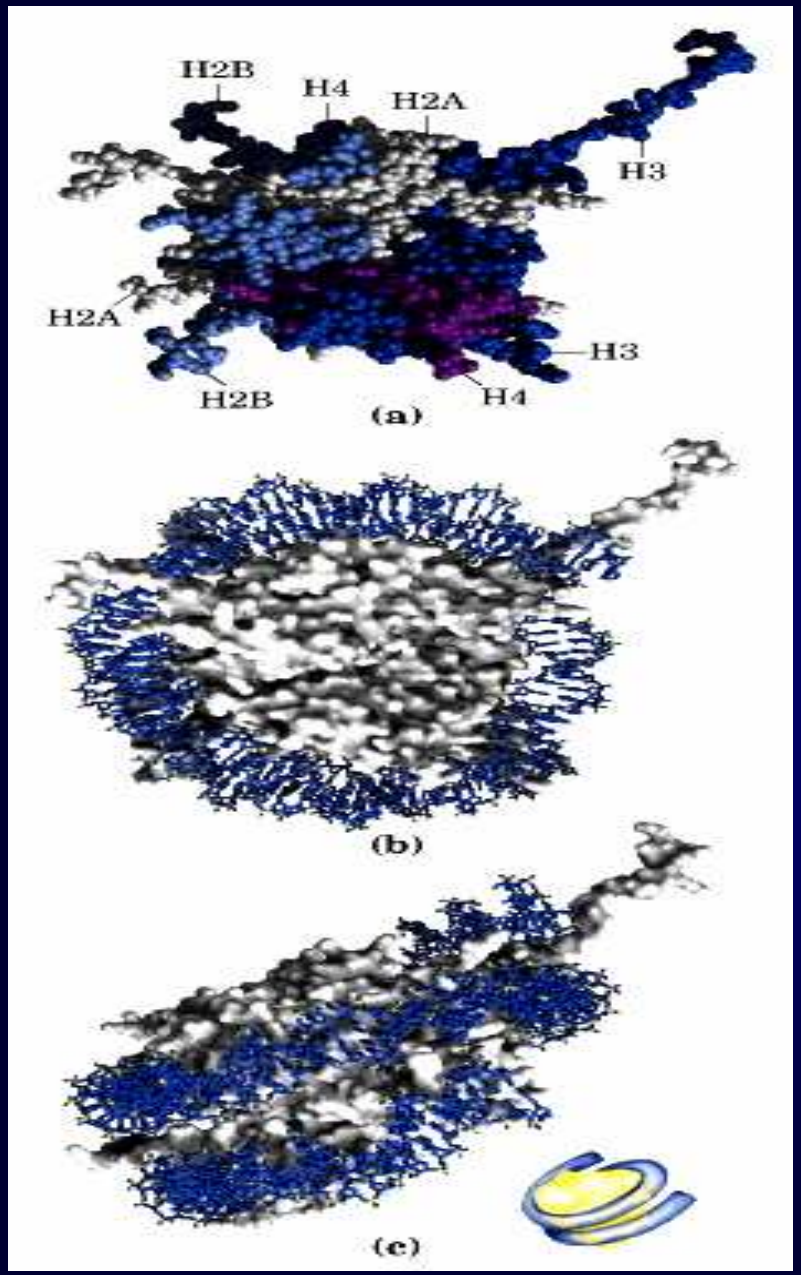
Biochemické modifikace histonů

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

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

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


Nucleosome
H4S1P
H4K5Ac
H4K8Ac
H4K20me
H2AK119Ac
H2AS1P
H2BAc
H2BUb
H3K4me
H3K9Ac
H3K9me
H3K27me
H3K36me
H3S10P
K27
K9
K4
H2B
H2A
H4
H3
DNA
N-terminal
histone tails
Histone H1
linker region



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

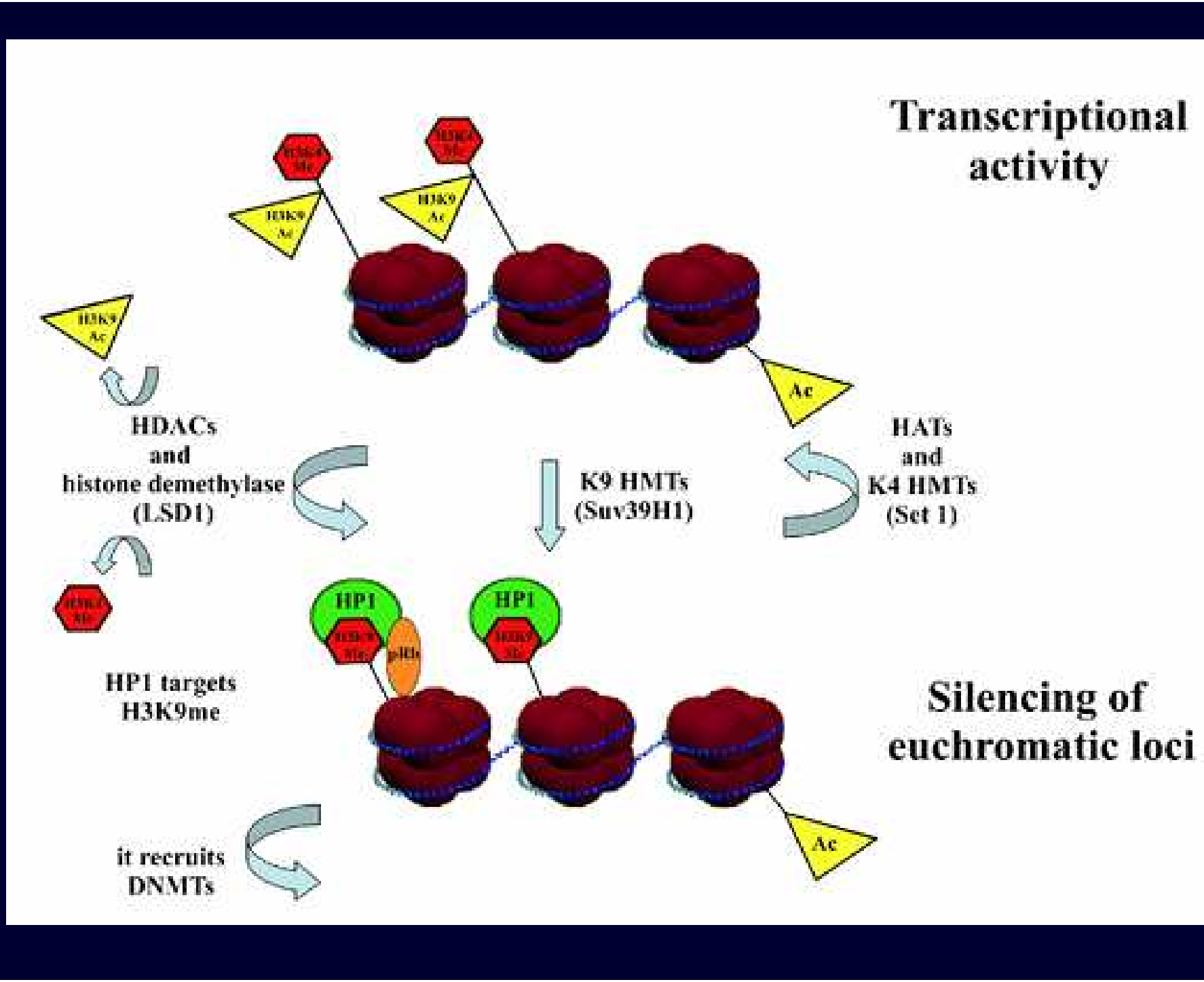
2004: Objev demethylace histonů za účasti aminové oxidasy LSD1 (KIAA0601) (Shi et al., Cell 2004). LSD1 specificky demethyluje H3 (K4), epigenetickou modifikaci zodpovědnou za transkripční aktivitu.

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

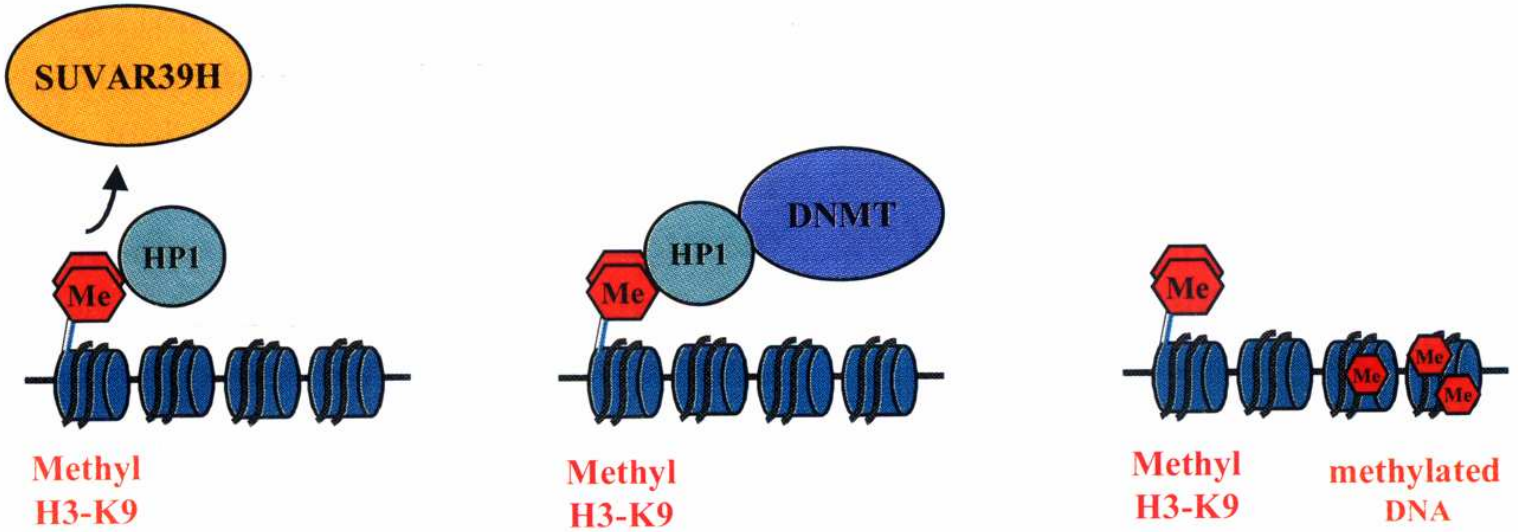
HDACs: Class I, II, III

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

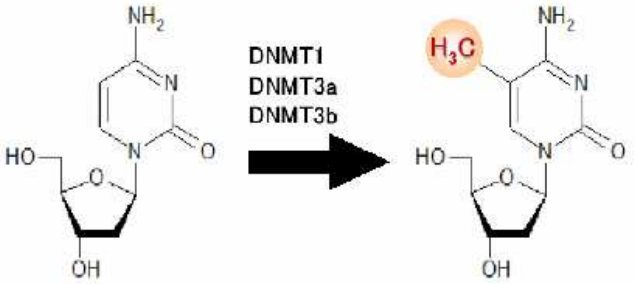
Demethylases: LSD1 (antagonizuje H3K4me-transcriptional activation), JHDM1b (H3K4me3), Jmjd2b (H3K9me3), JHDM2a, JMJ D2B (antagonizuje H3K9me2/me3 - heterochromatin formation)

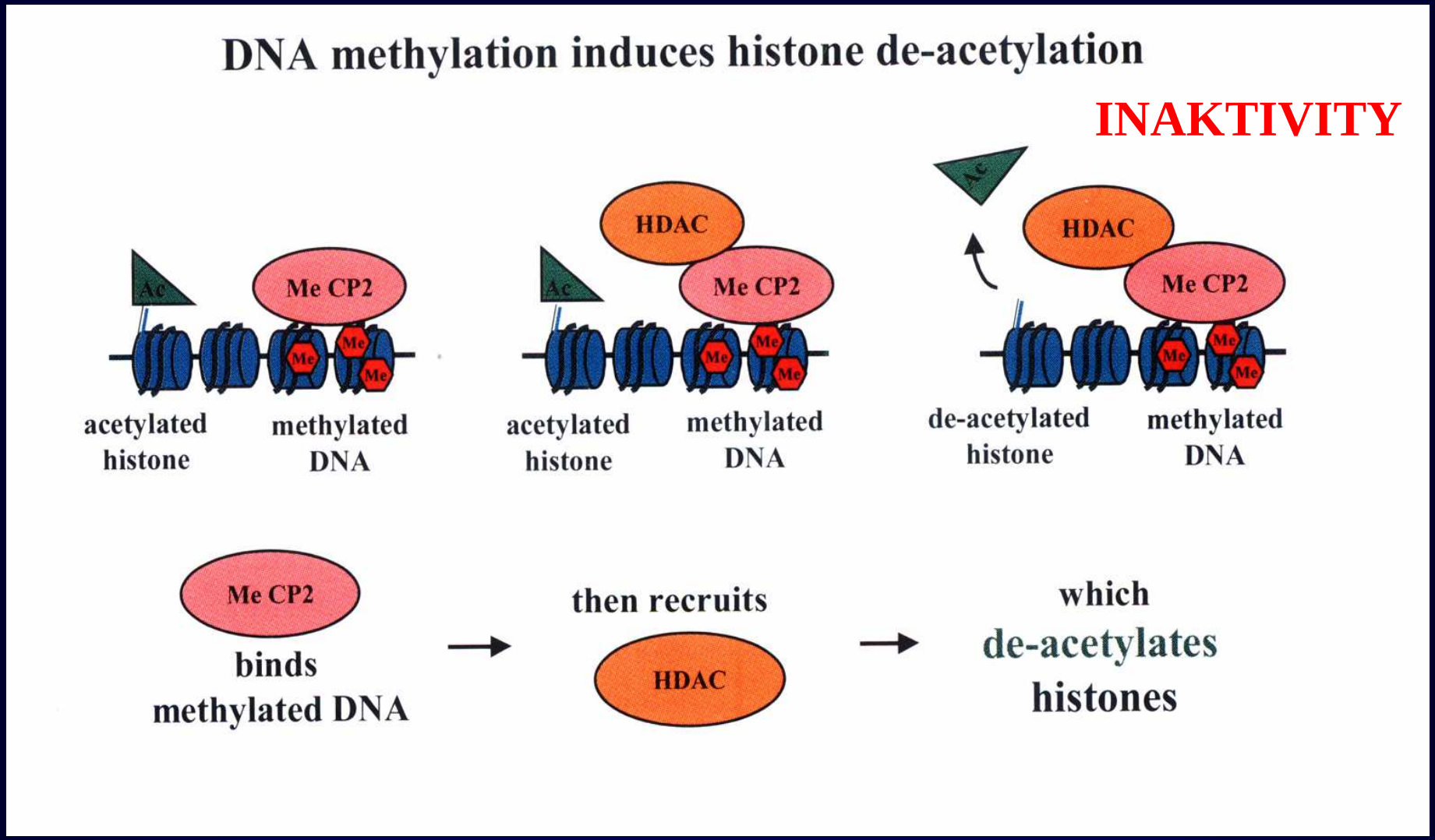

Transcriptional activity
H3K9 Ac
HDACs and histone demethylase (LSD1)
K9 HMTs (Suv39H1)
HATs and K4 HMTs (Set 1)
Ac
HP1
HP1
HP1 targets H3K9me
it recruits DNMTs
Silencing of euchromatic loci
Ac

Histone H3-K9 methylation induces DNA methylation



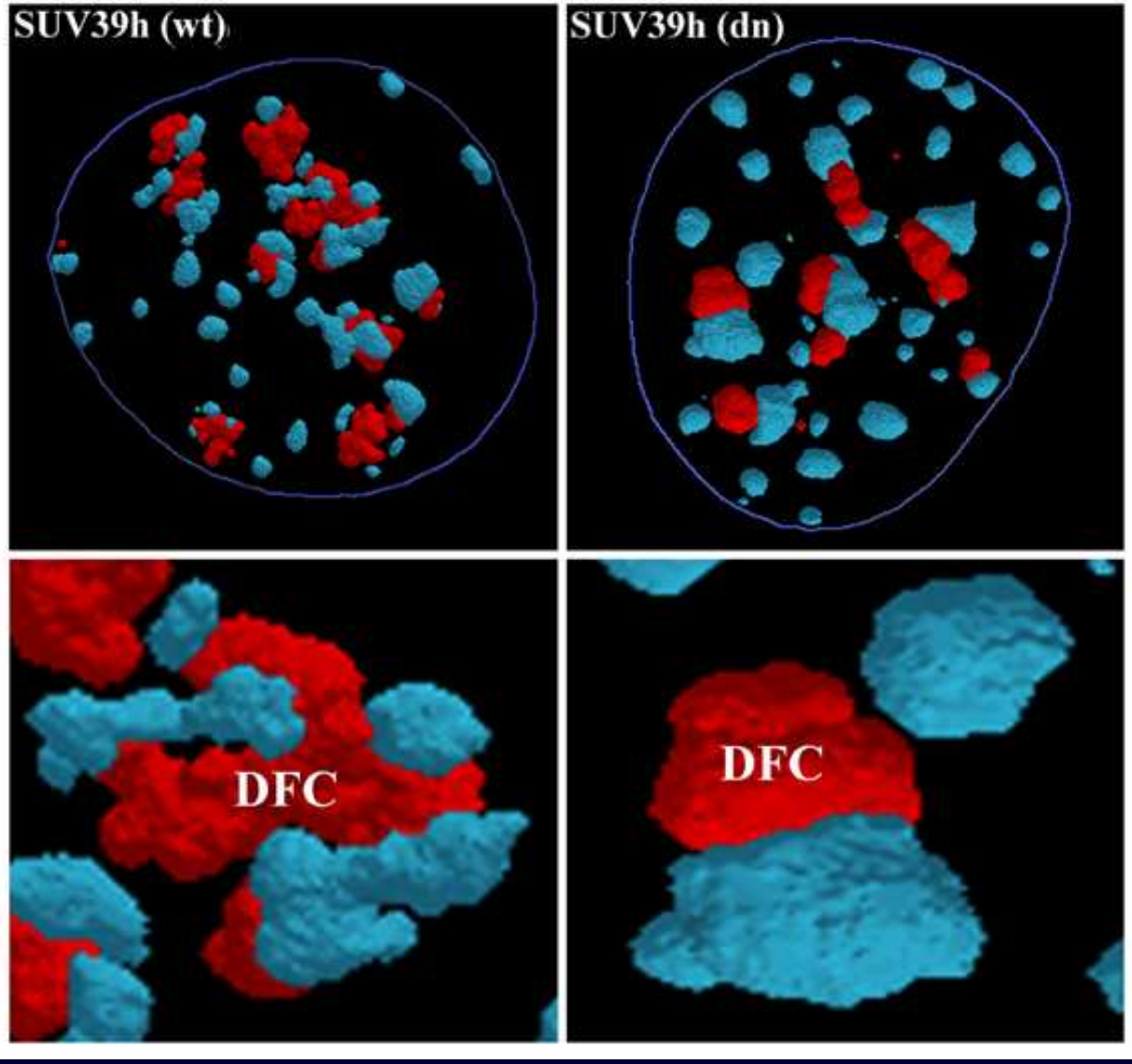
HP1 **targets** Methyl H3-K9 → **then recruits** DNMT → **which methylates DNA**

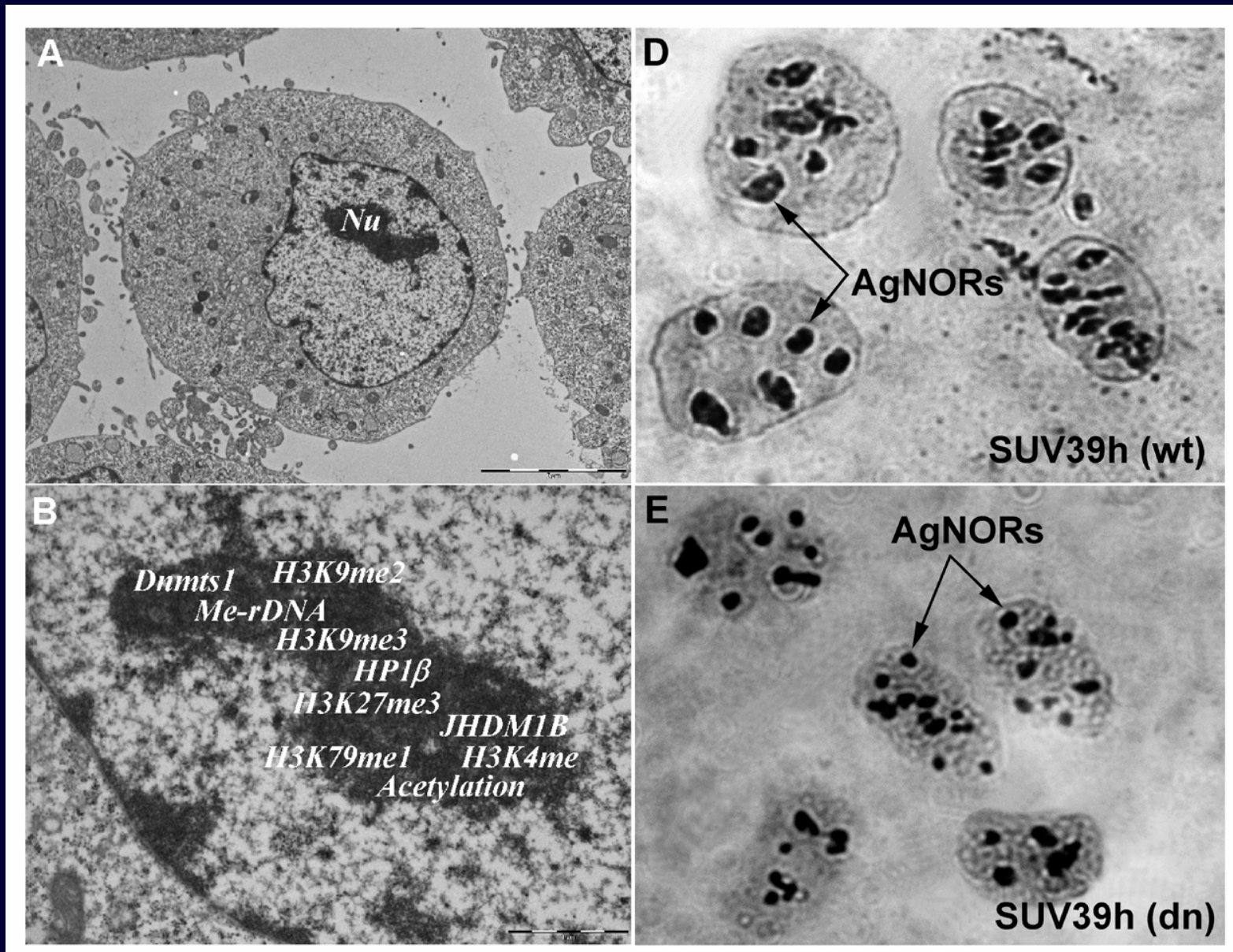


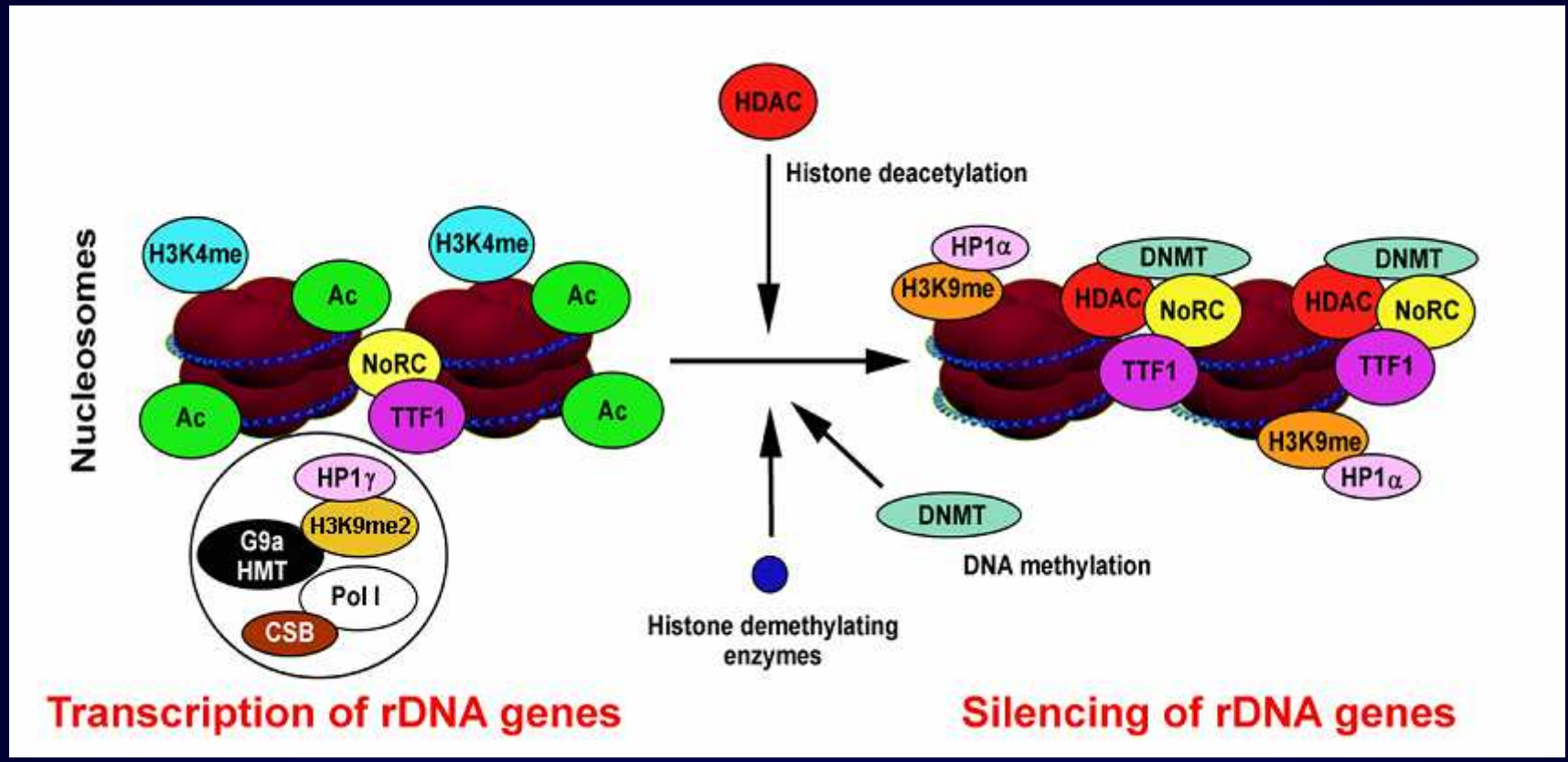


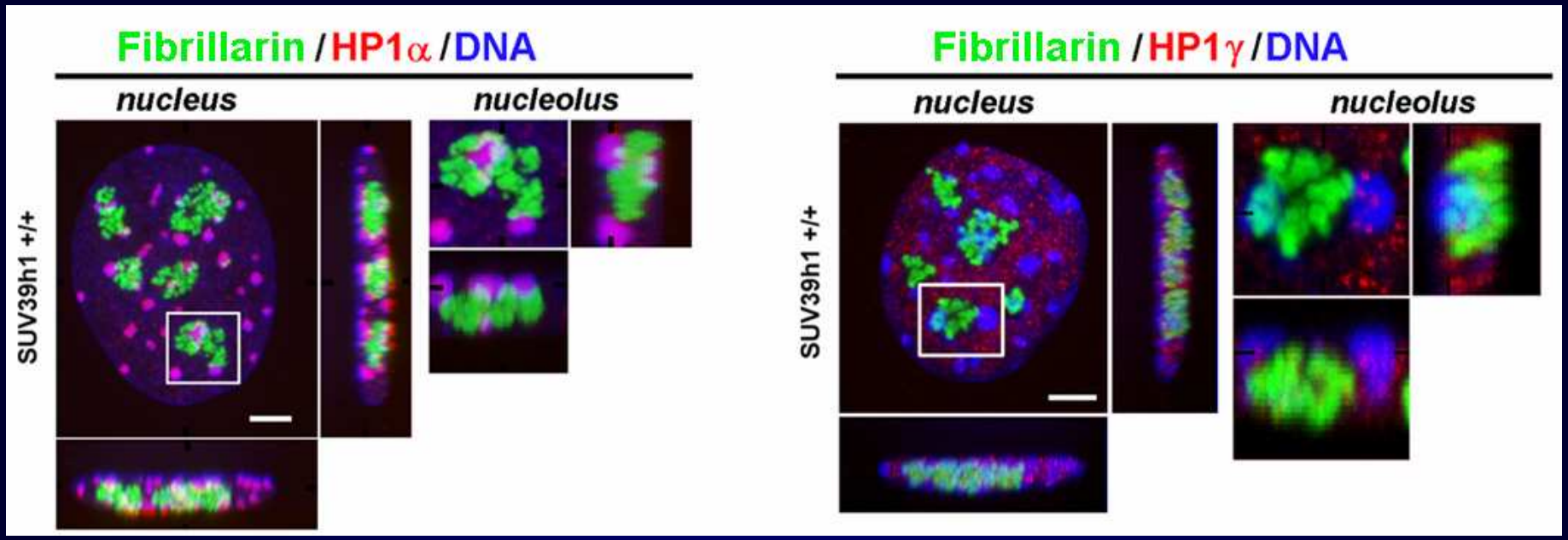
MeCP2: Methyl-CpG binding Protein, specifically binds to to methylated DNA

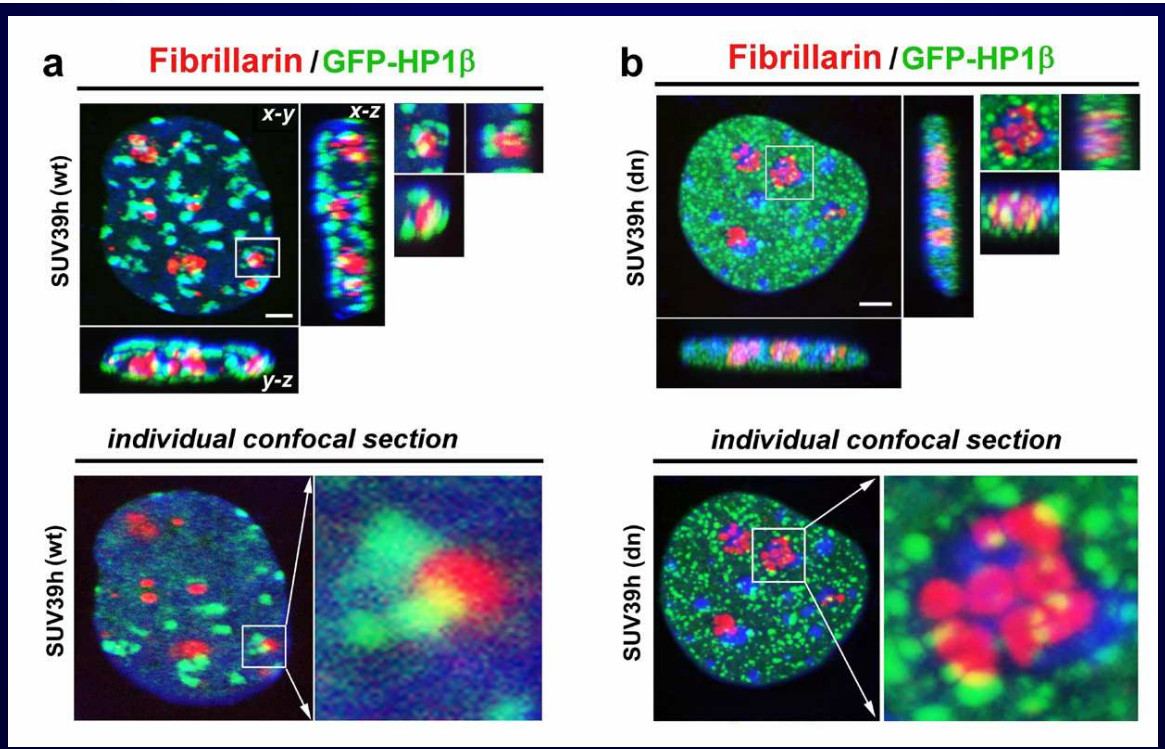
Fibrillarin / Chromocenters

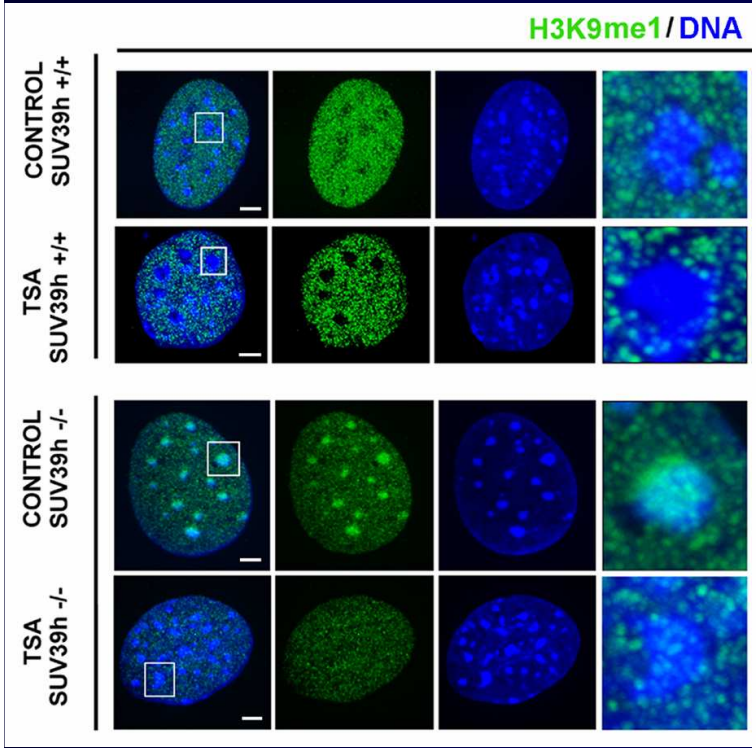


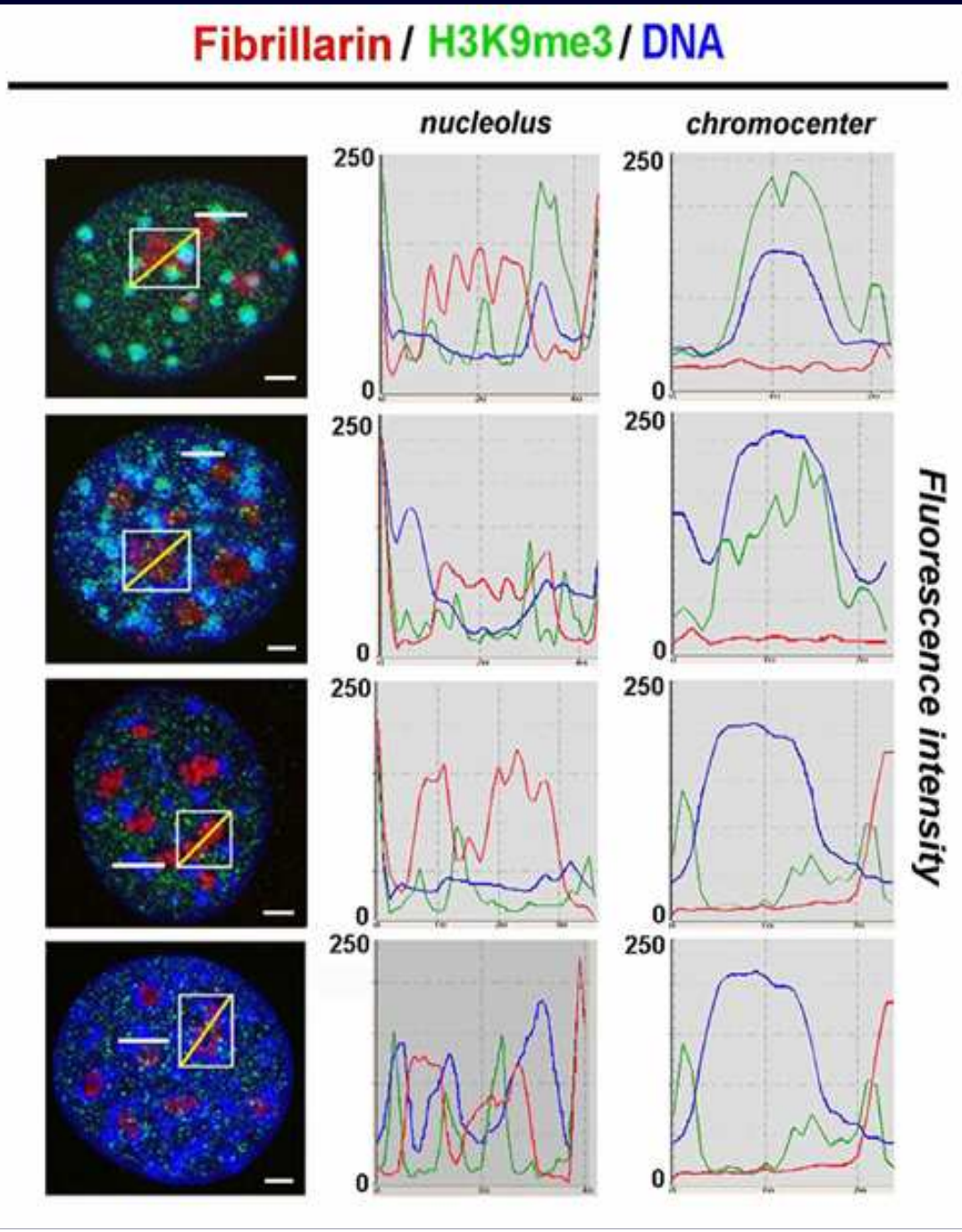

A
Nu
B
Dnmts1
H3K9me2
Me-rDNA
H3K9me3
HP1β
H3K27me3
JHDM1B
H3K79me1
H3K4me
Acetylation
D
AgNORs
SUV39h (wt)
E
AgNORs
SUV39h (dn)

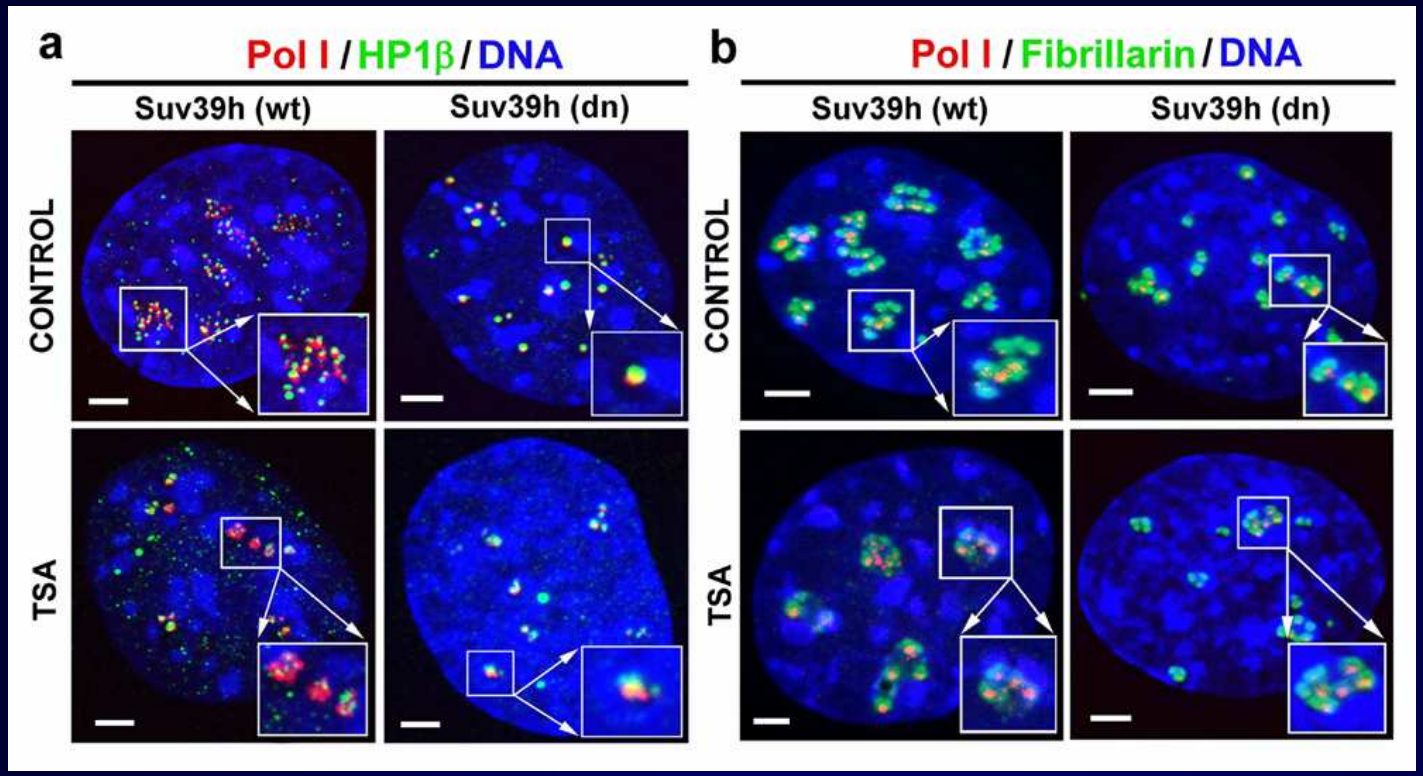

HDAC
Histone deacetylation
Nucleosomes
H3K4me
Ac
H3K4me
Ac
NoRC
Ac
TTF1
Ac
HP1γ
H3K9me2
G9a HMT
Pol I
CSB
HP1α
H3K9me
DNMT
HDAC
NoRC
TTF1
DNMT
HDAC
NoRC
TTF1
H3K9me
HP1α
DNMT
DNA methylation
Histone demethylating enzymes
Transcription of rDNA genes
Silencing of rDNA genes

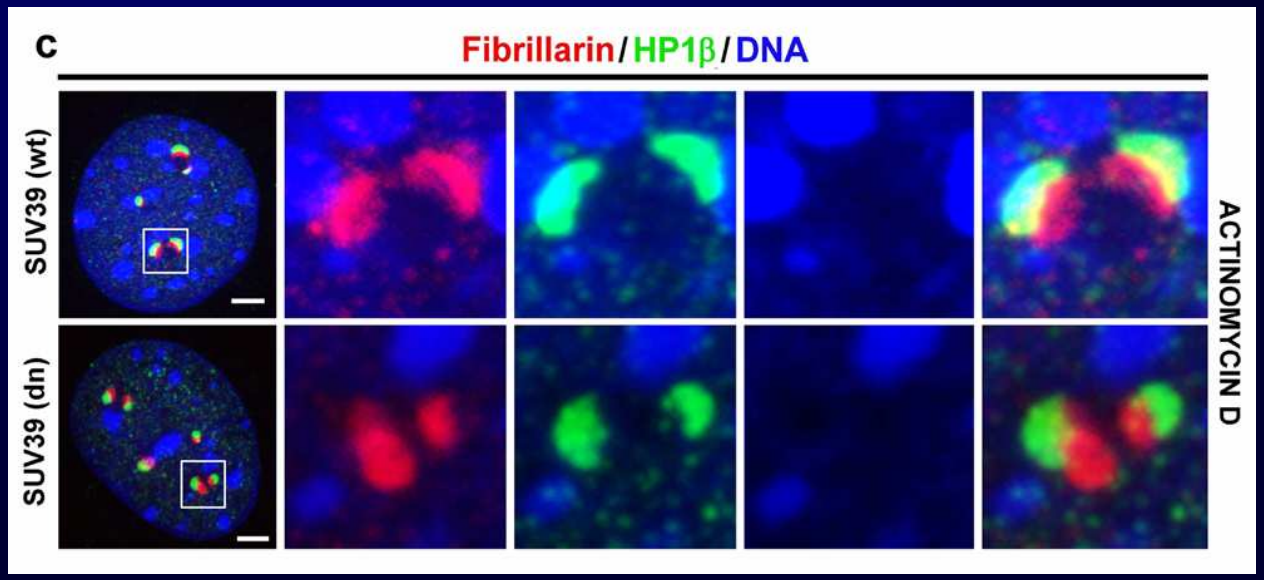

Fibrillarin / HP1α / DNA
nucleus
nucleolus
SUV39h1 +/+
Fibrillarin / HP1γ / DNA
nucleus
nucleolus
SUV39h1 +/+

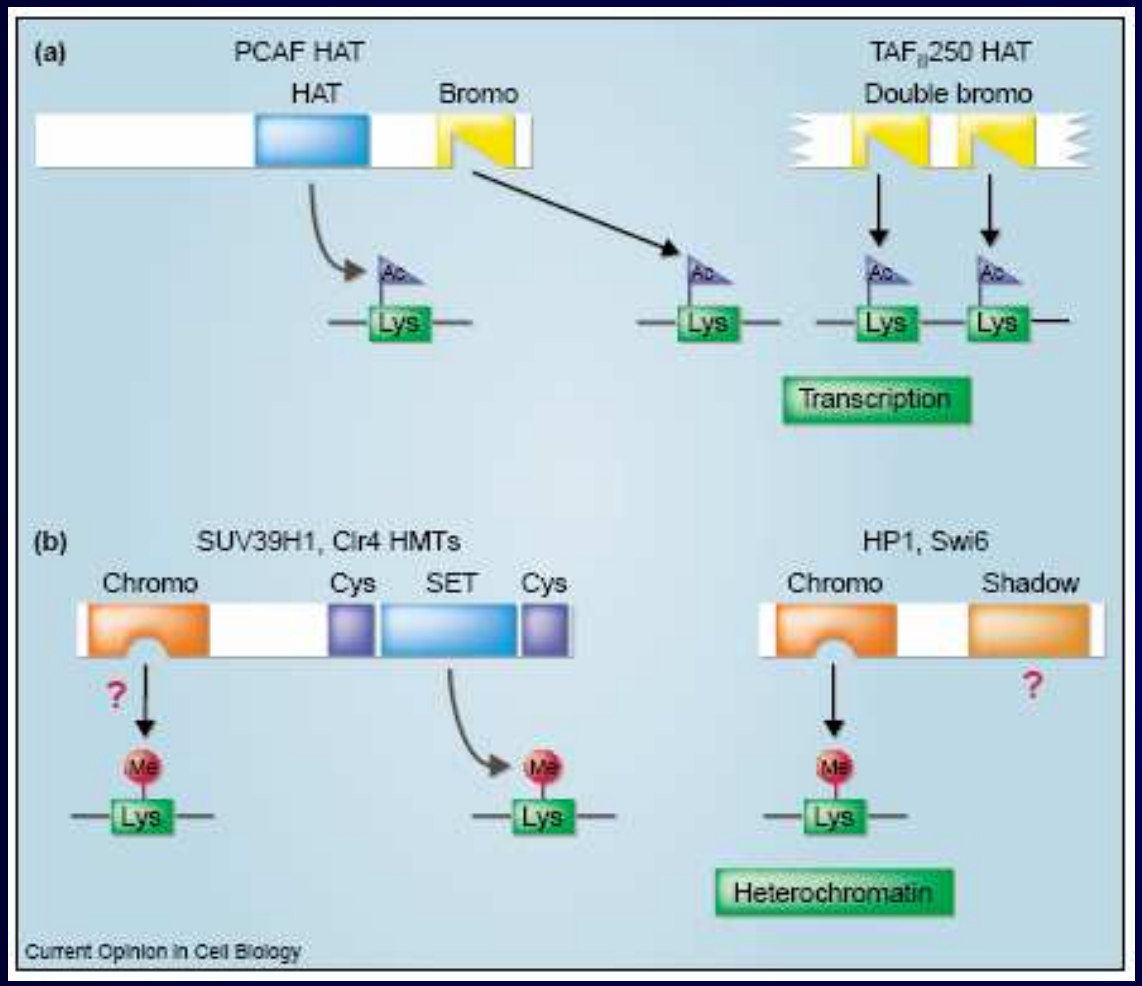

a
Fibrillarin / GFP-HP1β
x-y
x-z
y-z
SUV39h (wt)
b
Fibrillarin / GFP-HP1β
SUV39h (dn)
individual confocal section
SUV39h (wt)
individual confocal section
SUV39h (dn)


H3K9me1 / DNA
CONTROL SUV39h +/+
TSA SUV39h +/+
CONTROL SUV39h -/-
TSA SUV39h -/-


Fibrillarin / H3K9me3 / DNA
nucleolus
chromocenter
250
0
Fluorescence intensity


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


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

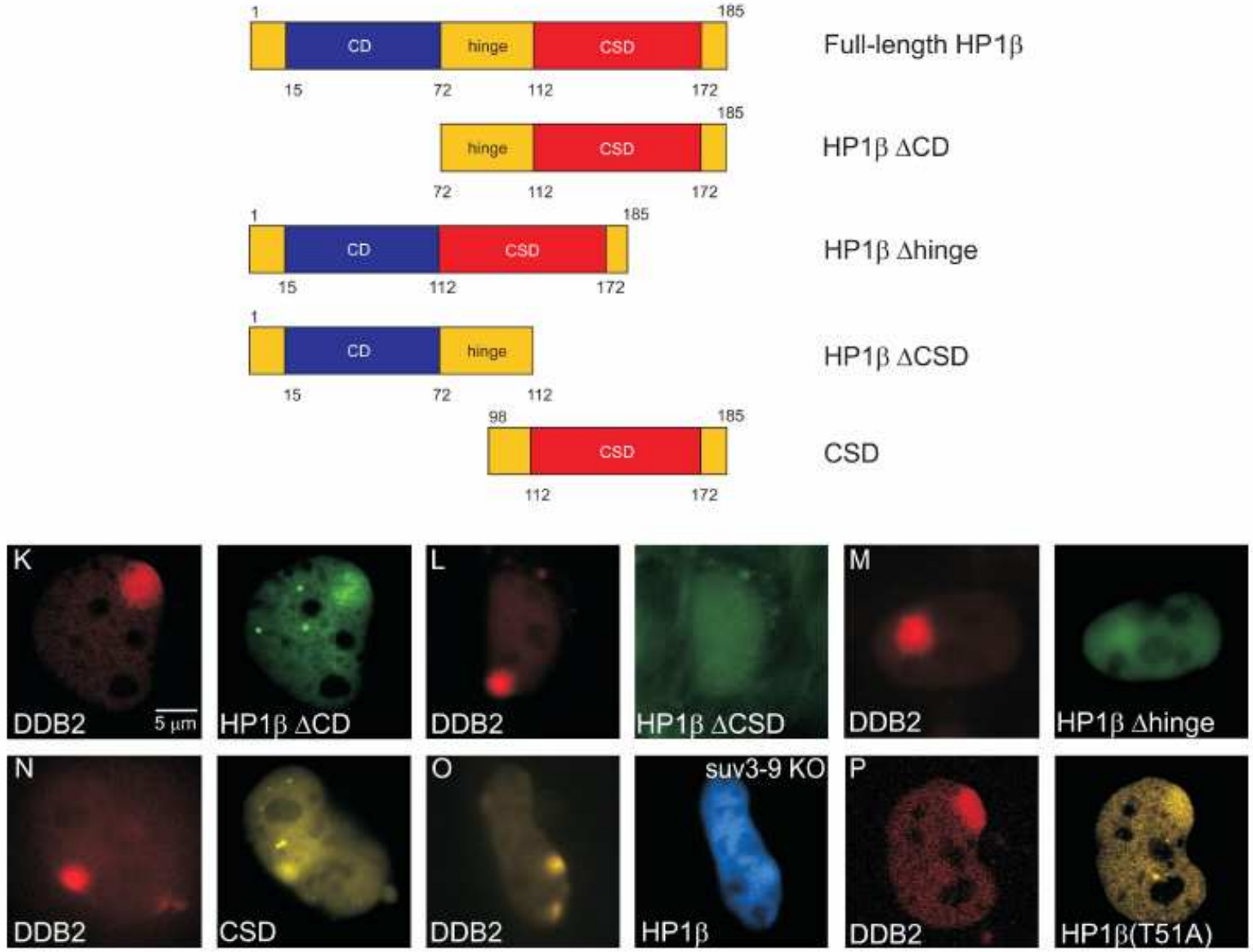


HP1 protein

CD: protein-chromatin

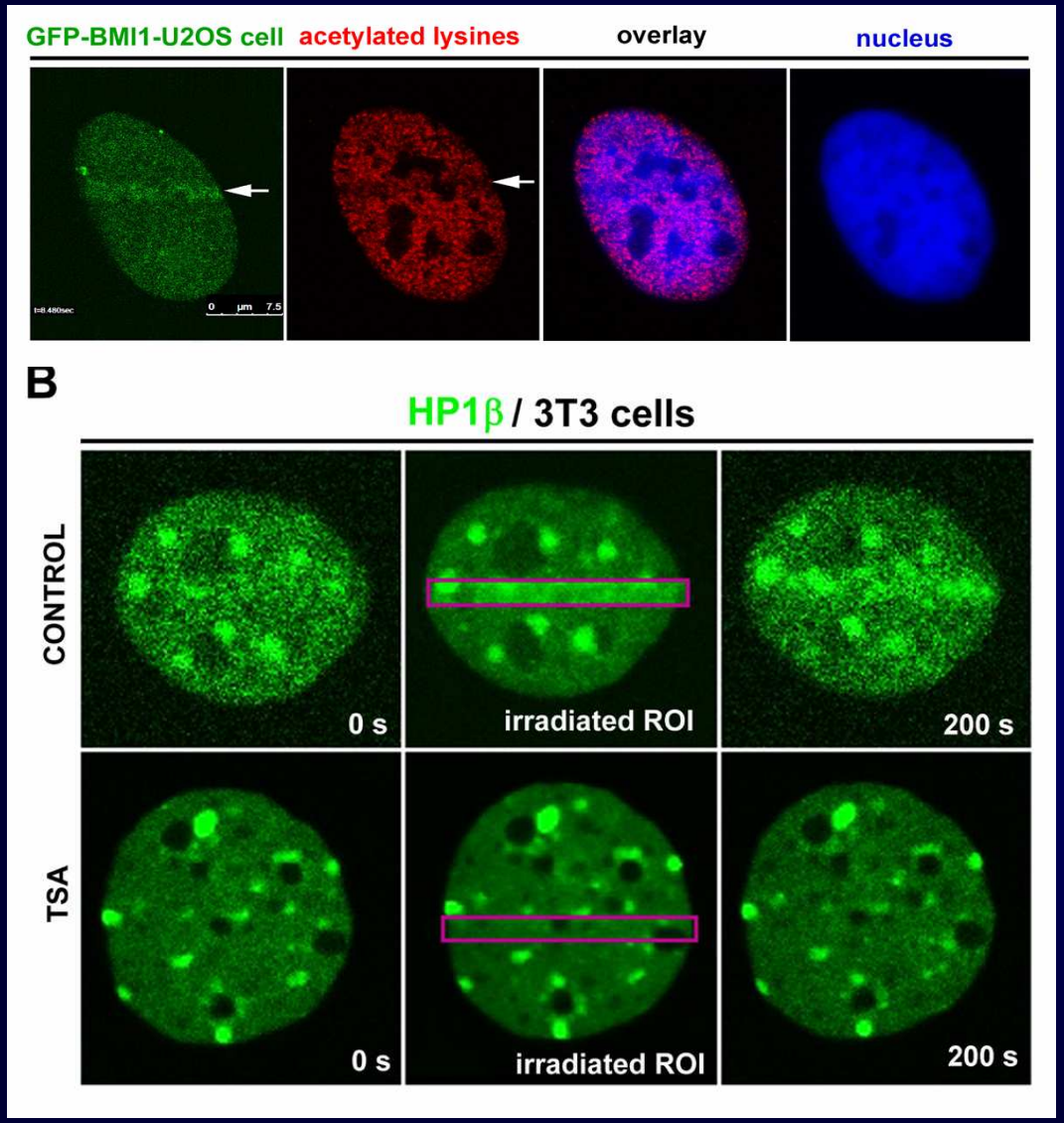
CSD: protein-protein

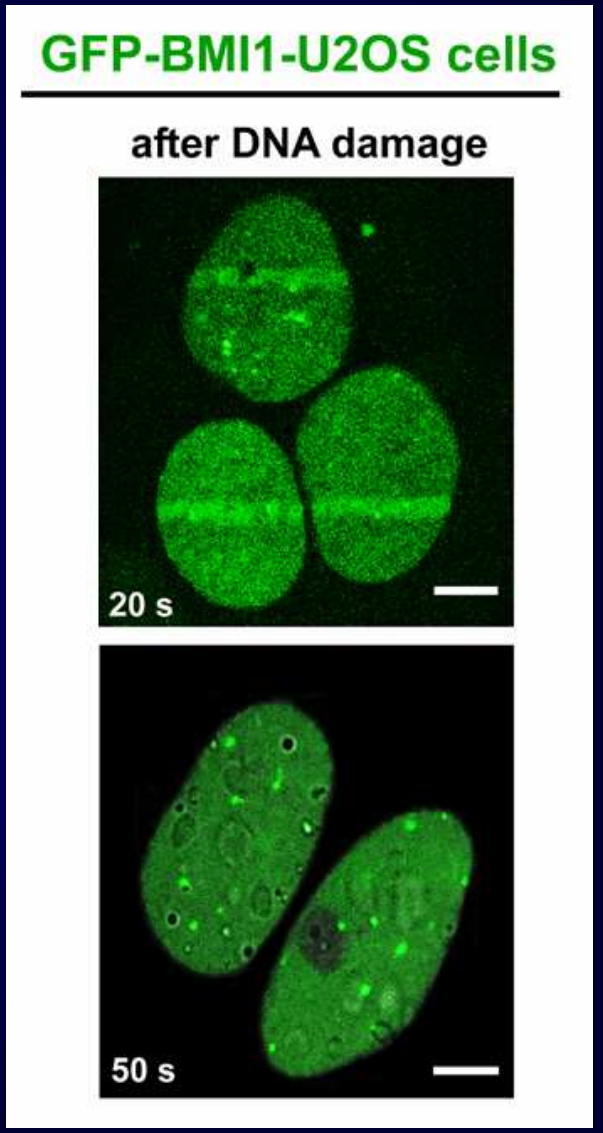
HD: HP1-to-DNA and linker histones



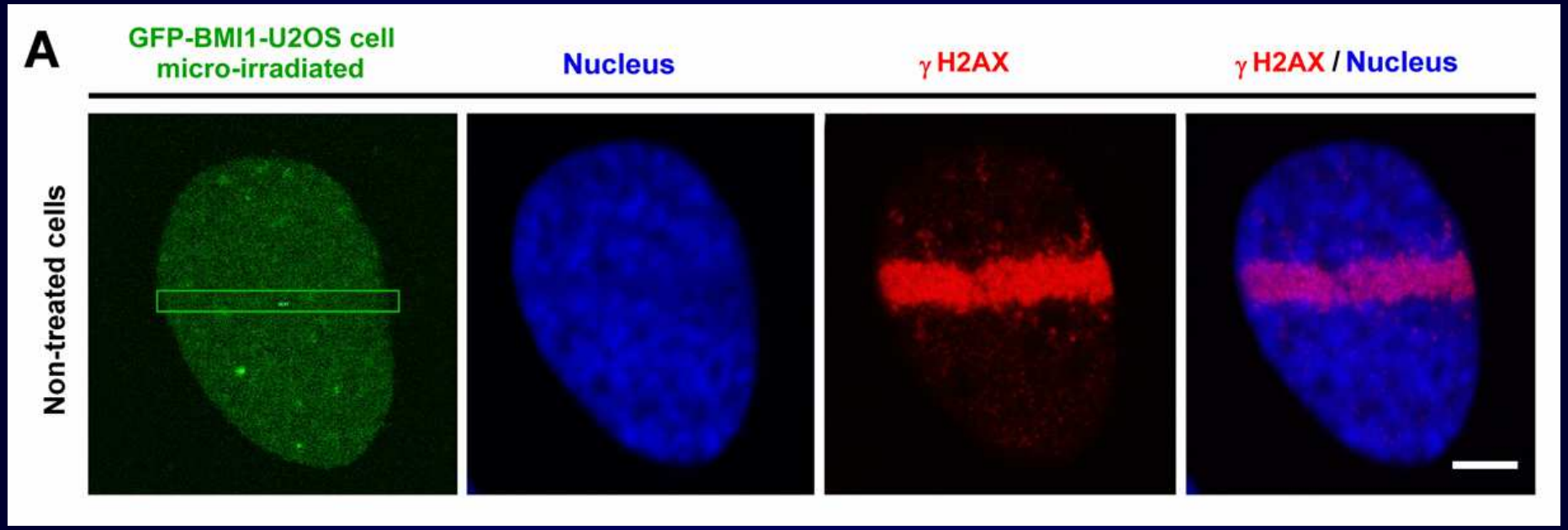
Lujsterburg et al. (2009)

HP1β protein in DSBs





Experiments of Gabriela Galiová and Lenka Stixová



Experiments of Gabriela Šustáčková

DNA repair

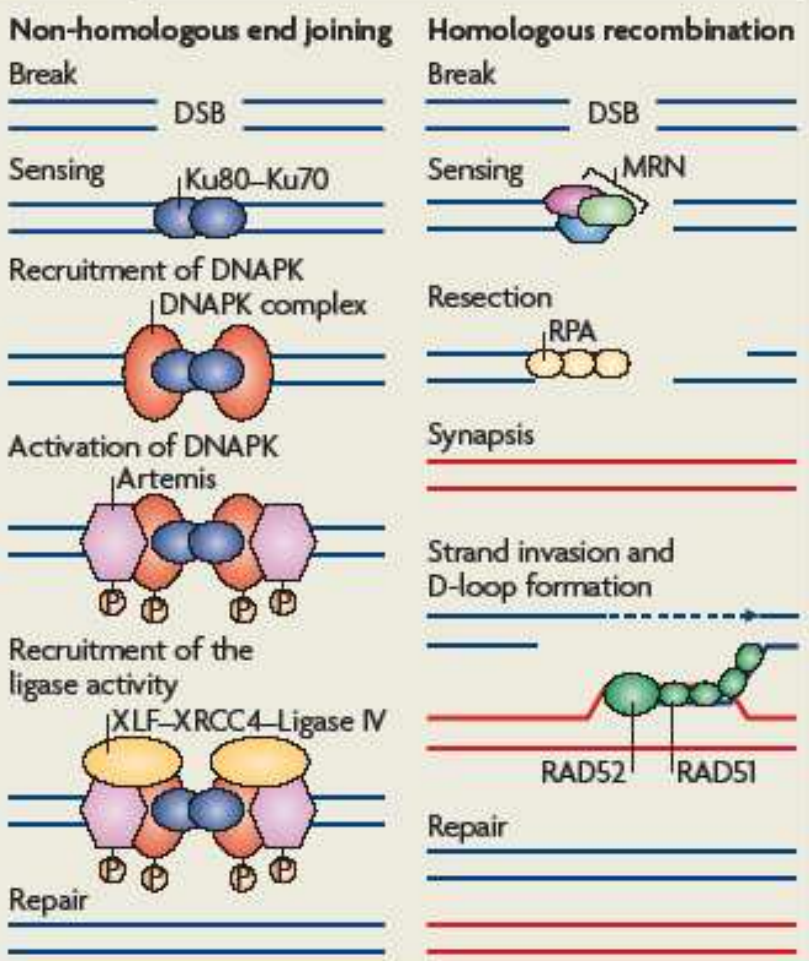
Box 1 | **The two main types of double-stranded DNA-break repair**

Non-homologous end joining

A DNA lesion (a double-stranded DNA break (DSB)) is sensed by the Ku80–Ku70 heterodimer, which in turn recruits the DNA-dependent protein kinase catalytic subunit DNAPKcs, resulting in assembly of the DNAPK complex and activation of its kinase activity (see the figure; left panel). Increasing evidence suggests that DNAPK functions as a regulatory component of non-homologous end joining (NHEJ), potentially facilitating and regulating the processing of DNA ends. DNAPK also increases the recruitment of XRCC4, DNA ligase IV, XLF and Artemis, which carry out the final rejoining reaction.

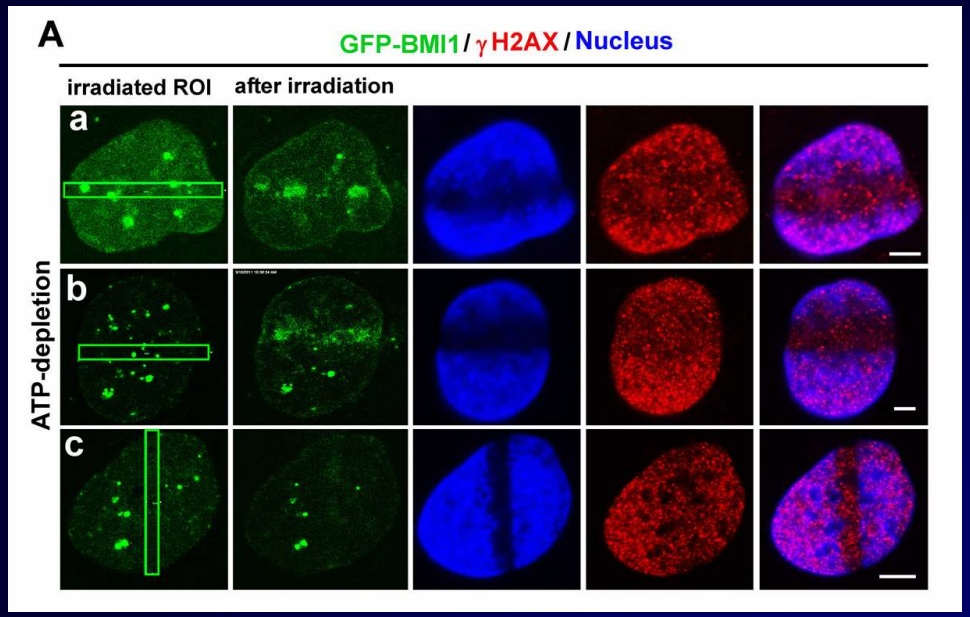
Homologous recombination repair

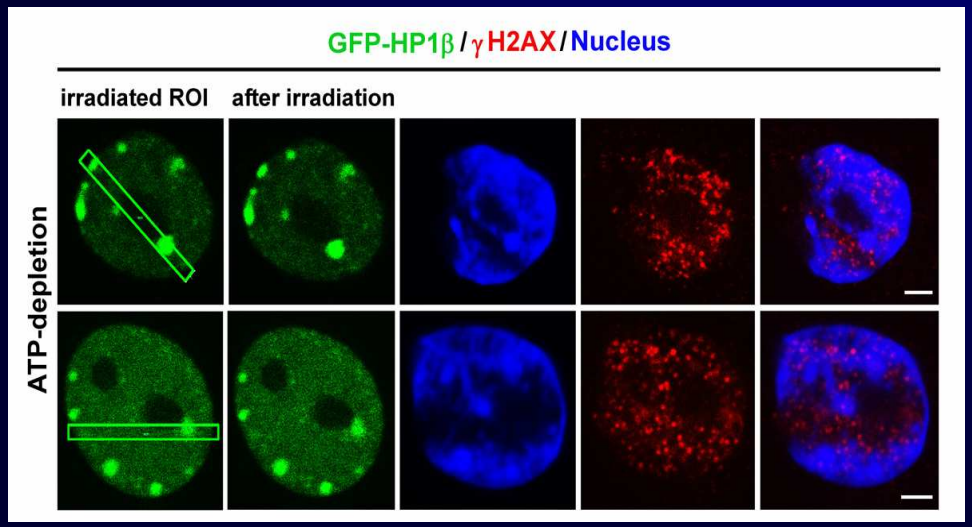
A DNA lesion is recognized by the MRN (MRE11–RAD50–NBS1) complex, which is recruited to the DSB to generate single-stranded DNA by resection (see the figure; right panel). The single-stranded ends are bound by replication protein A (RPA), RAD51 and RAD52 and can subsequently invade the homologous template, creating a D-loop and a Holliday junction, to prime DNA synthesis and to copy and ultimately restore genetic information that was disrupted by the DSB.

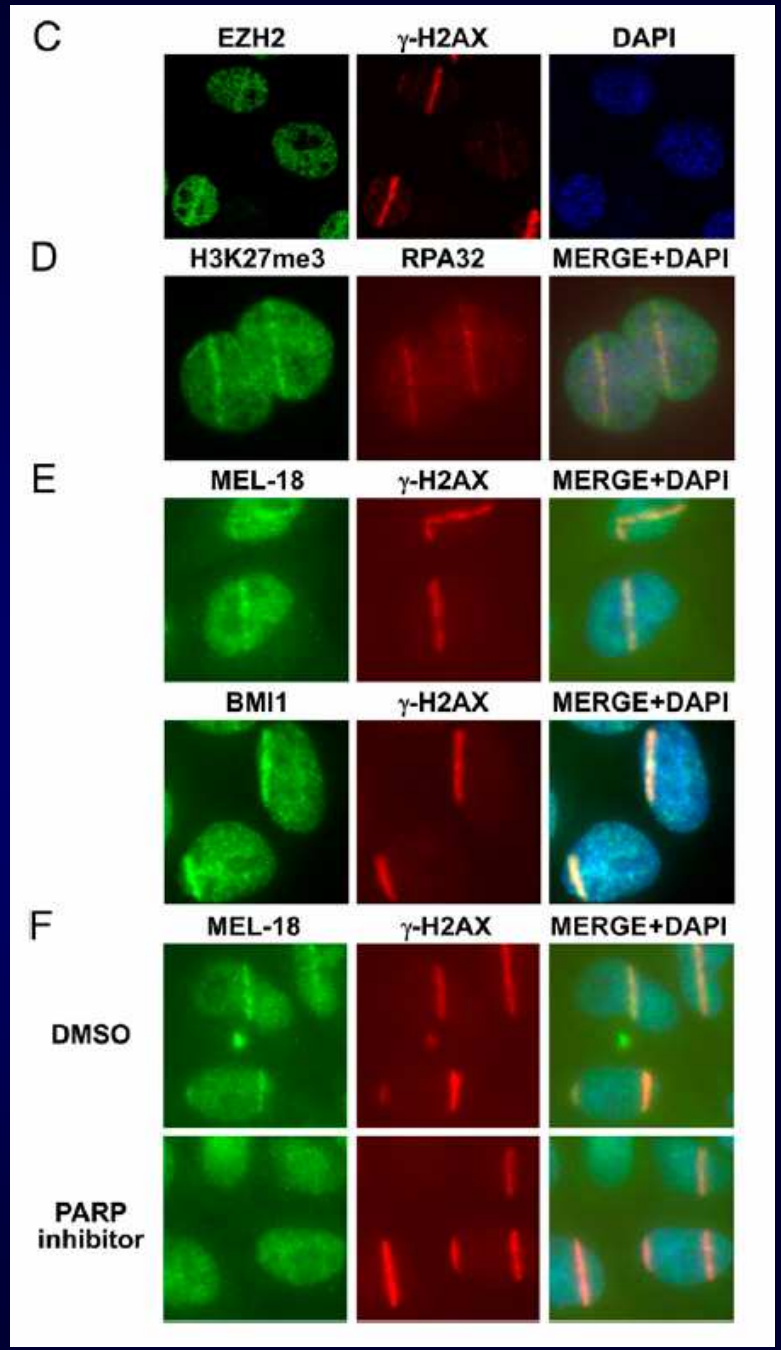


Misteli and Soutogou (2009)

ATP depletion



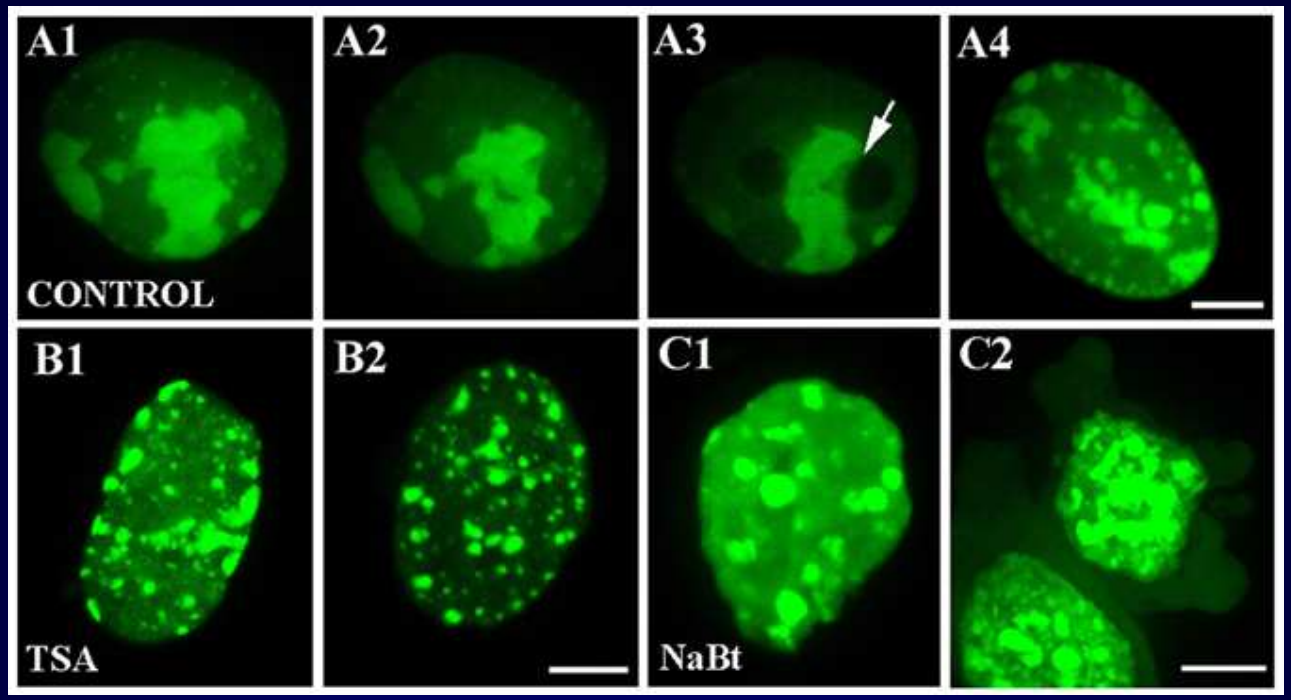




Šustáčková et al., JCP, 2011

Chou et al., PNAS (2010)

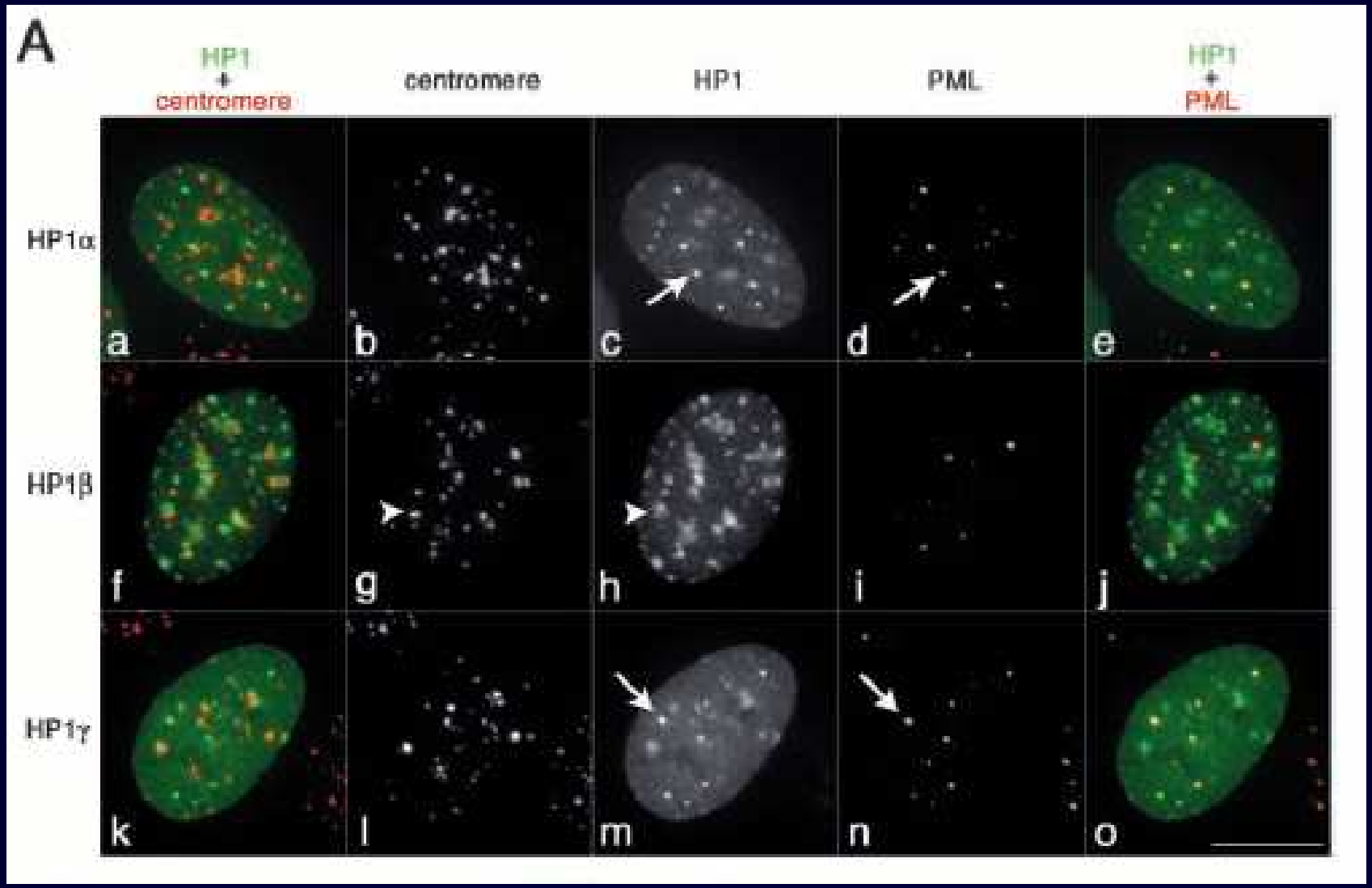
HP1 proteins



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

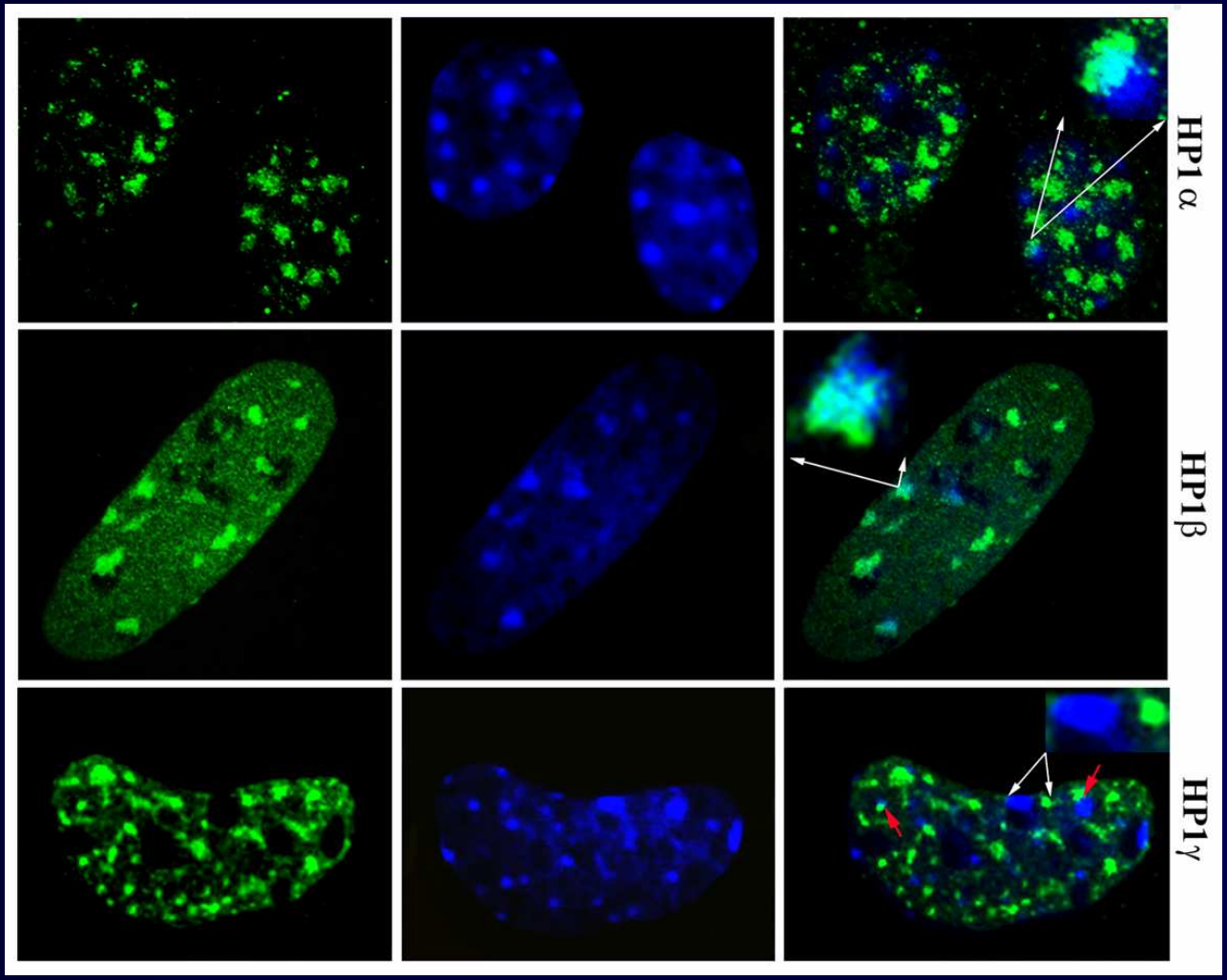
• HPs interagují s HMTs jako je SUV39h1 a SUV39h2, která jsou zodpovědné za methylaci H3(K9).

HP1 proteiny – v lidských buňkách jsou 3 sub-typy

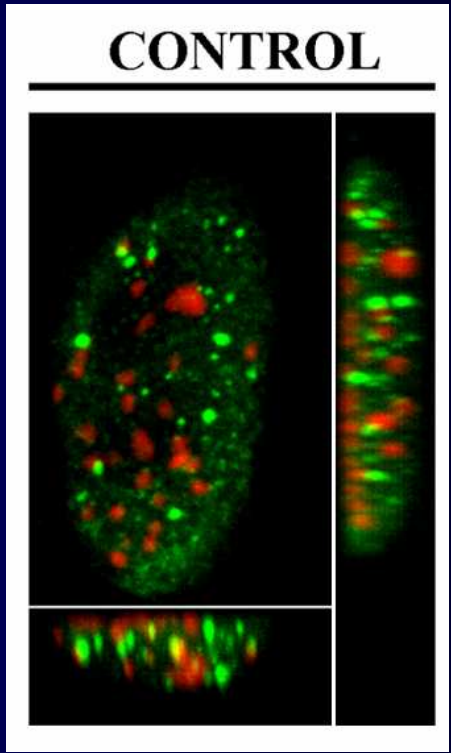


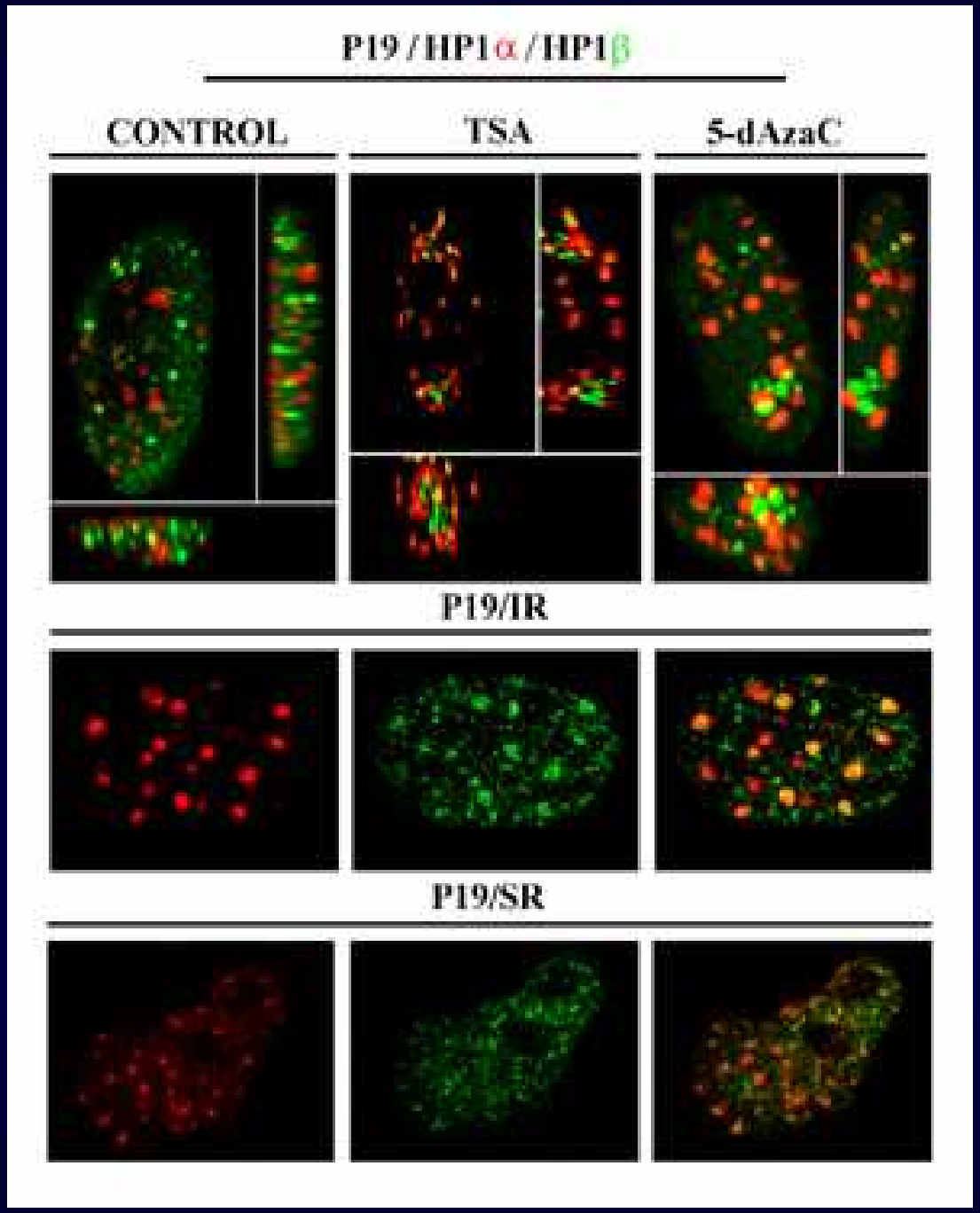
Hayakawa et al., 2003

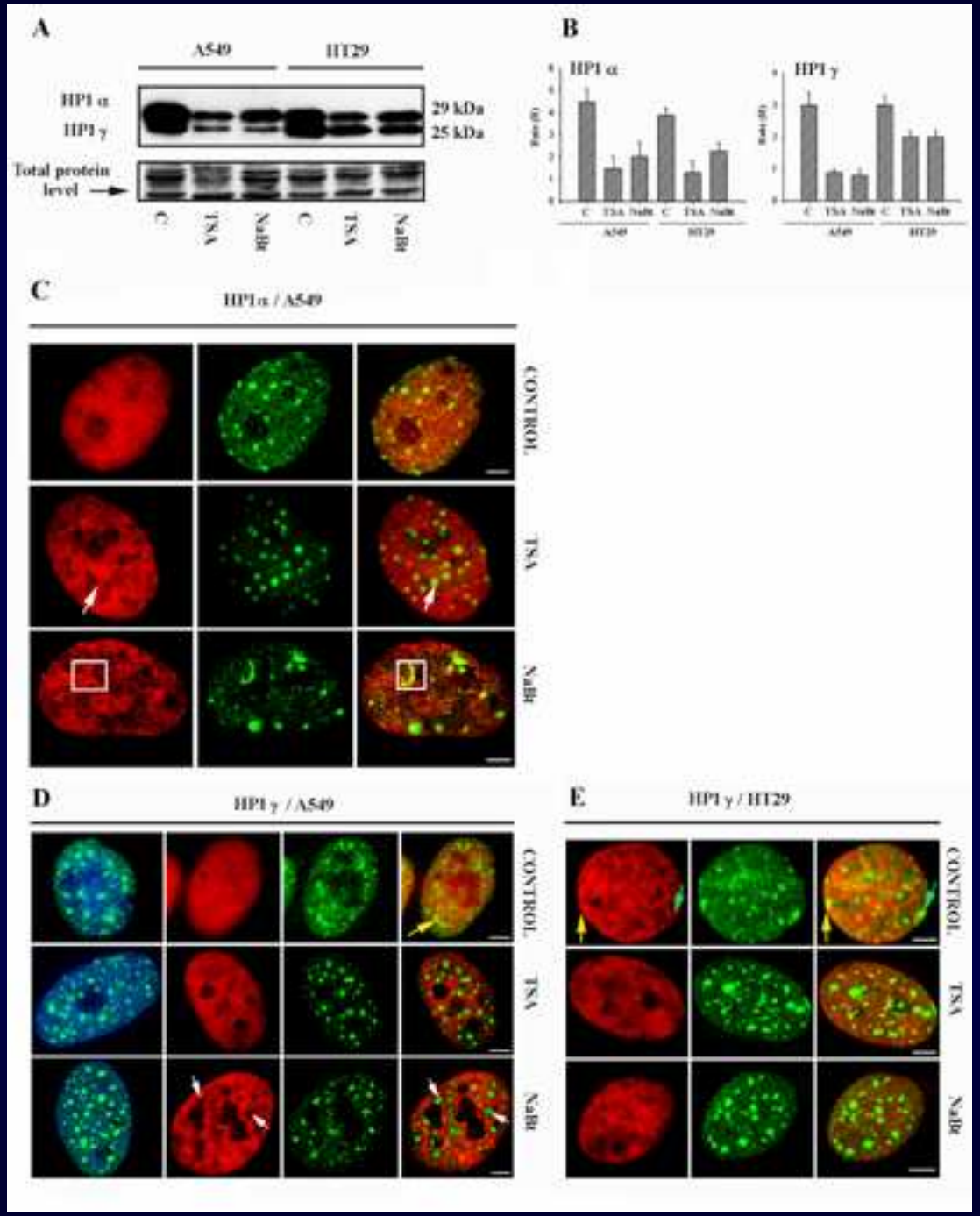
HP1 proteiny u ECS

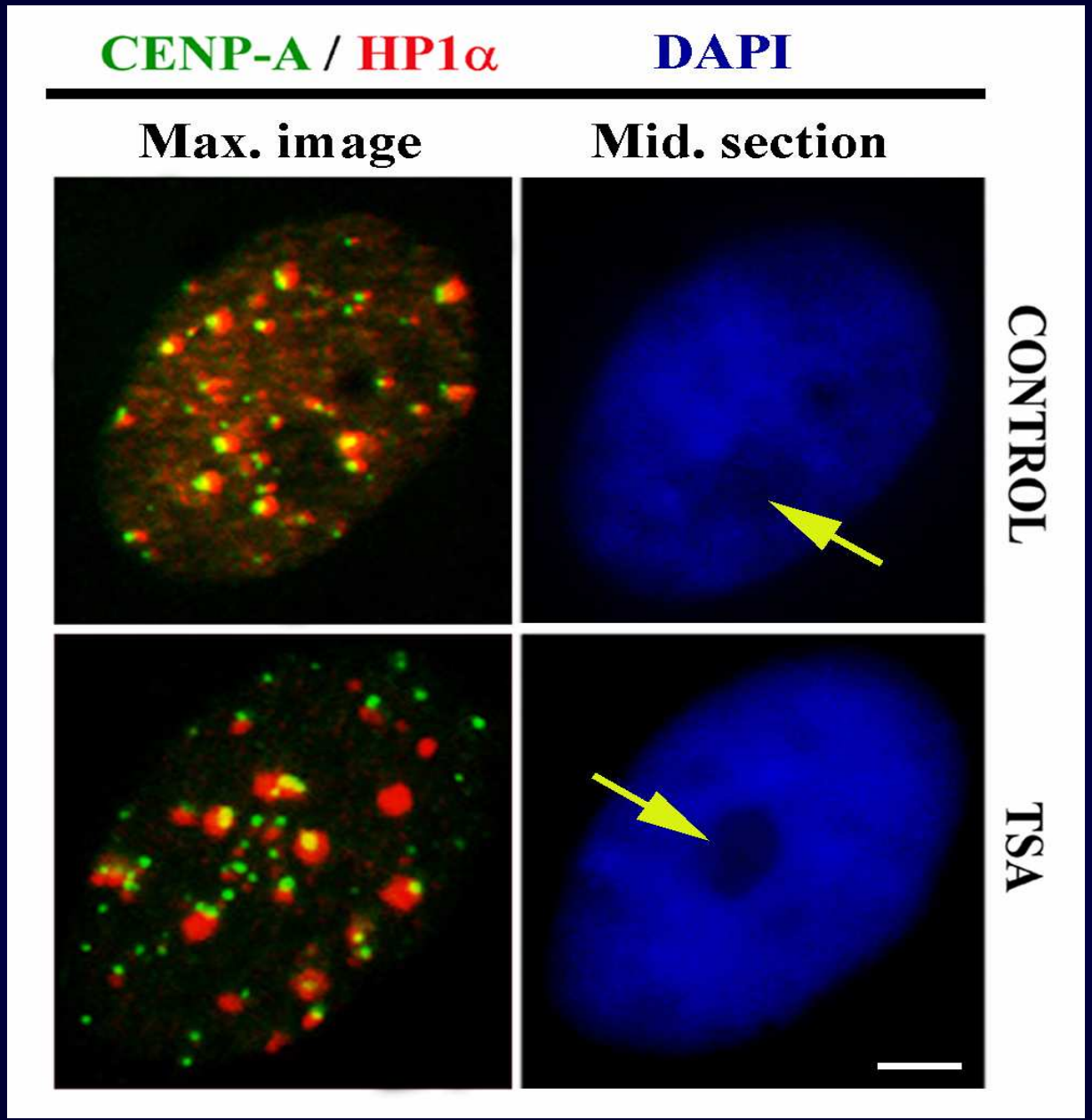


HP1α HP1β



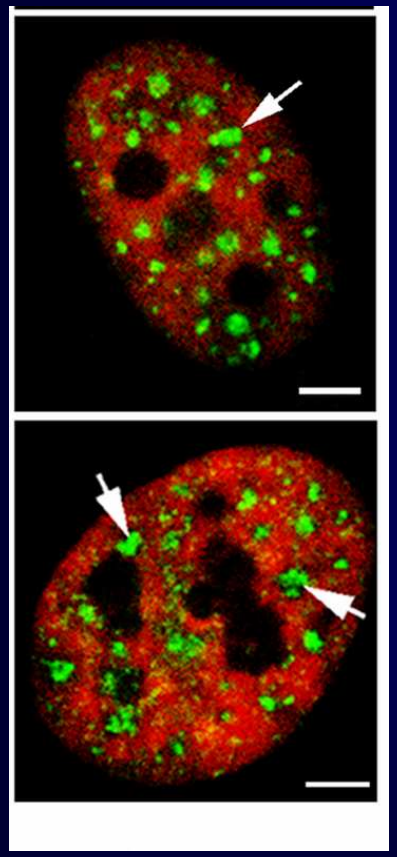

P19 / HP1α / HP1β
CONTROL
TSA
5-dAzaC
P19/IR
P19/SR

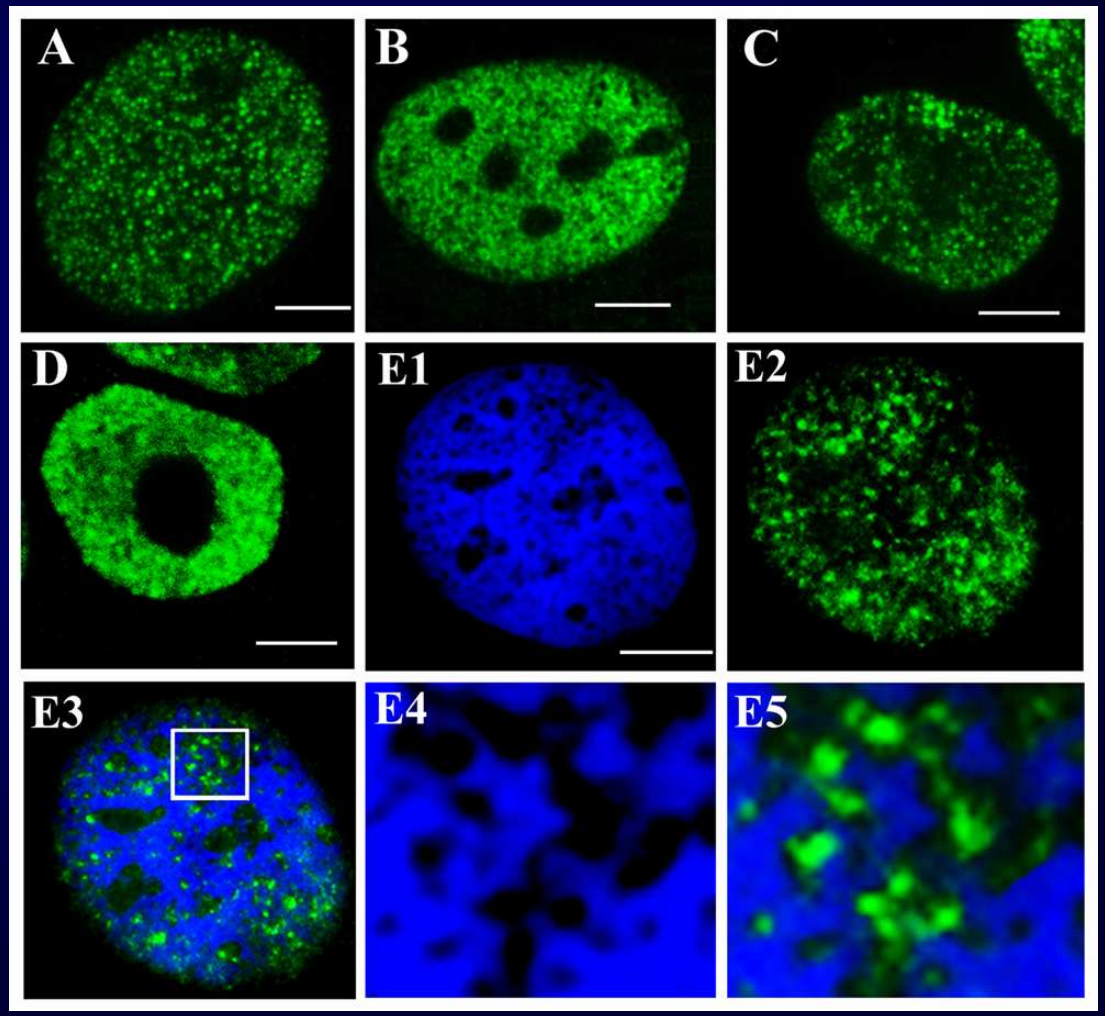

A
A549
HT29
HP1 α
HP1 γ
29 kDa
25 kDa
Total protein level
C
TSA
NaBt
B
HP1 α
HP1 γ
C
TSA
NaBt
A549
HT29
C
HP1α / A549
CONTROL
TSA
NaBt
D
HP1 γ / A549
CONTROL
TSA
NaBt
E
HP1 γ / HT29
CONTROL
TSA
NaBt

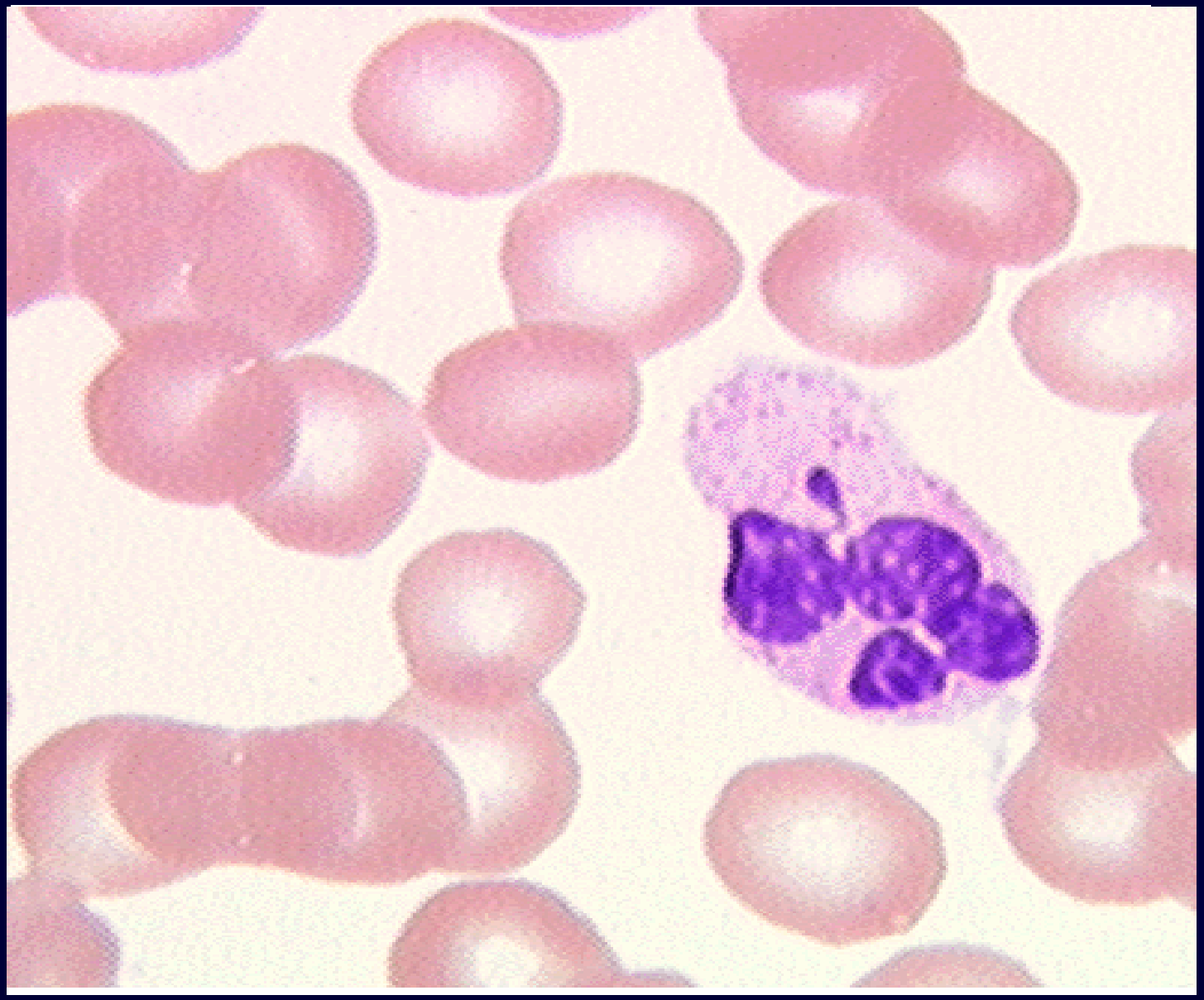

CENP-A / HP1α
DAPI
Max. image
Mid. section
CONTROL
TSA

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

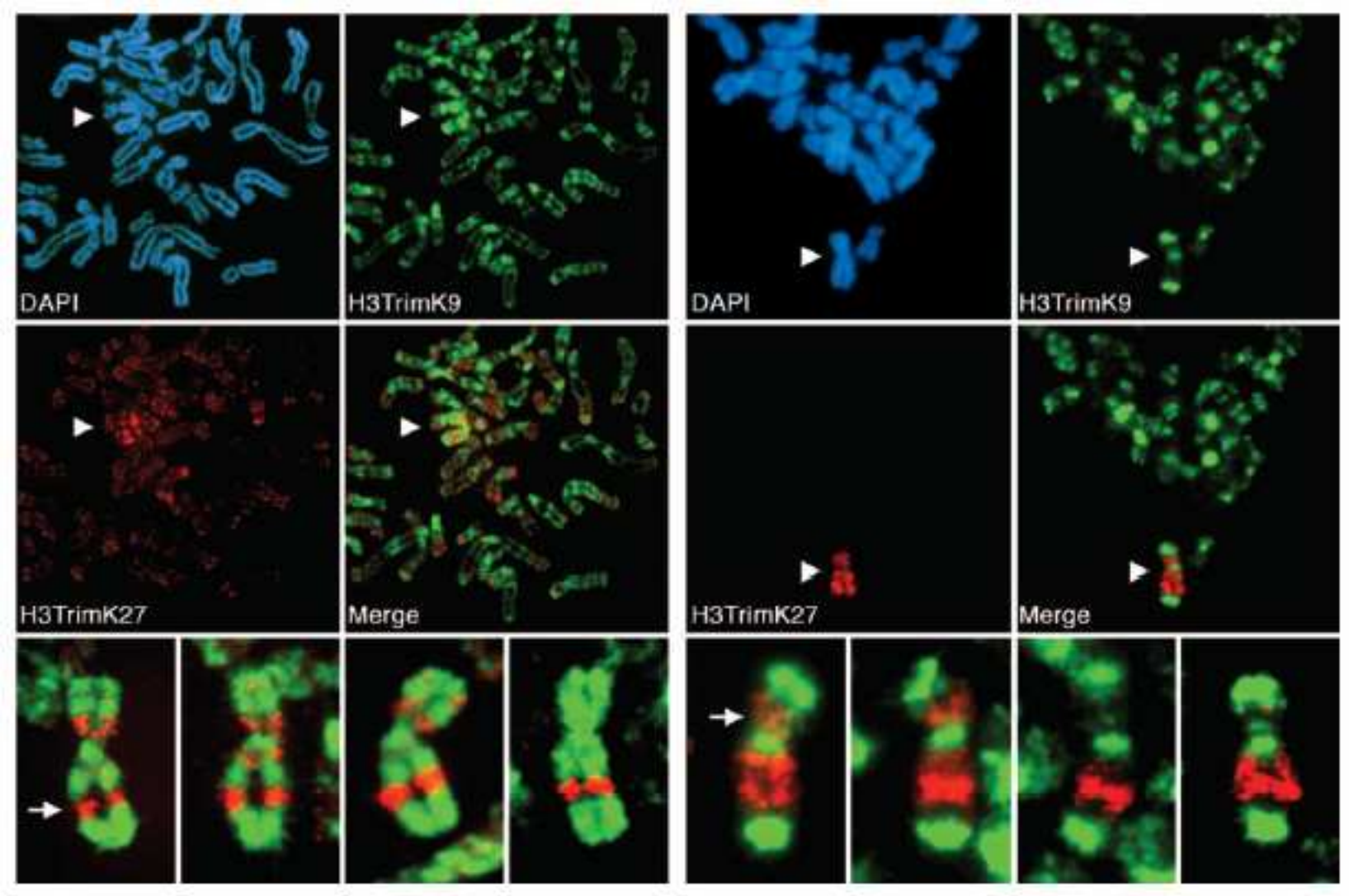
HP1





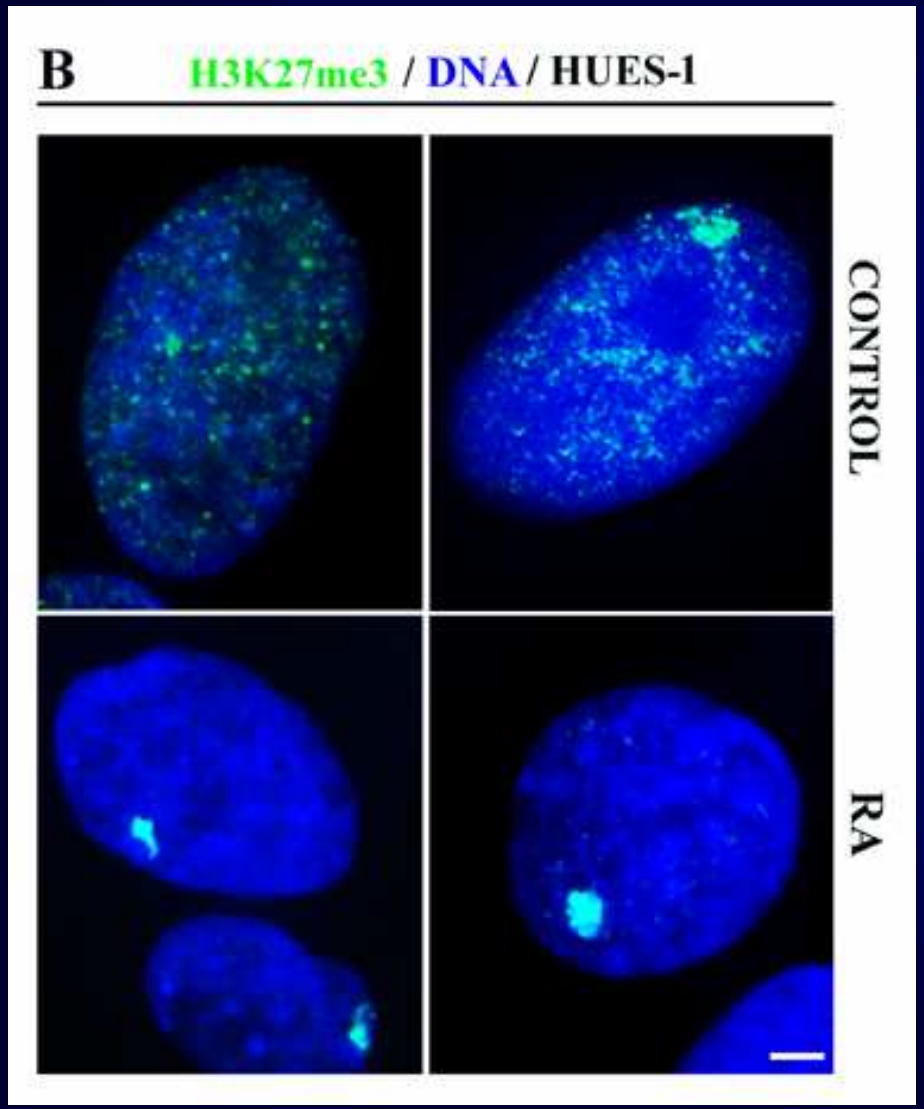


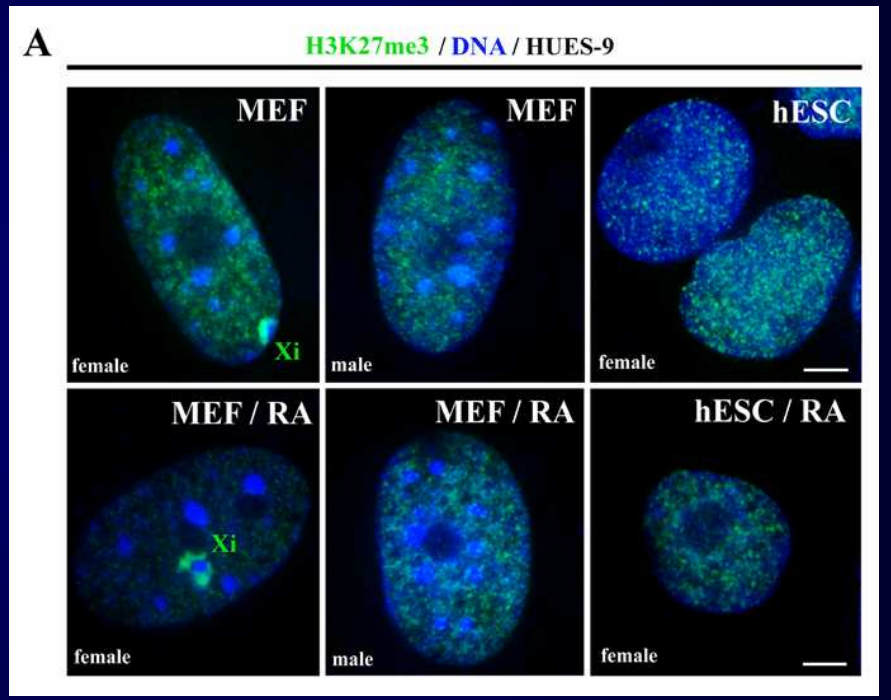
Inaktivace X chromosomu ve vztahu k epigenetickým modifikacím



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

Inactivation of X chromosome in hESC





Inaktivace X chromosomu ve vztahu k epigenetickým modifikacím

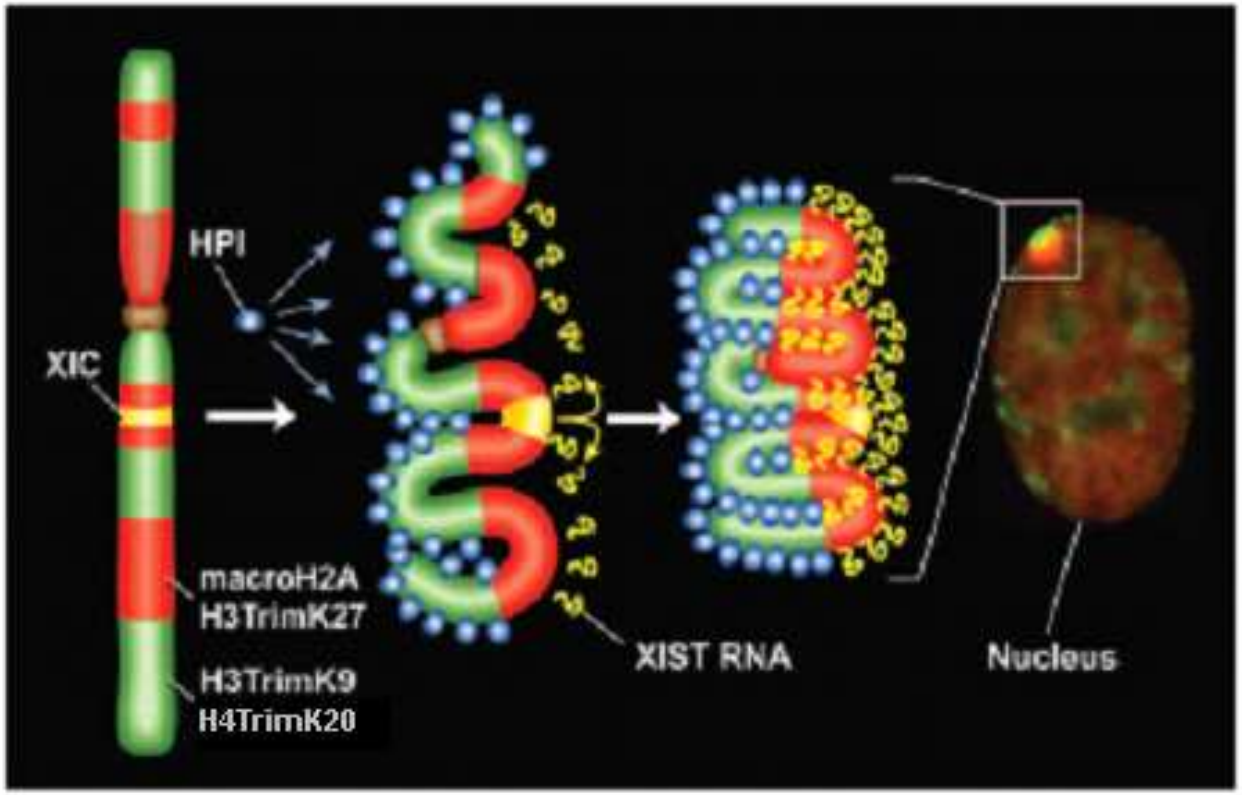
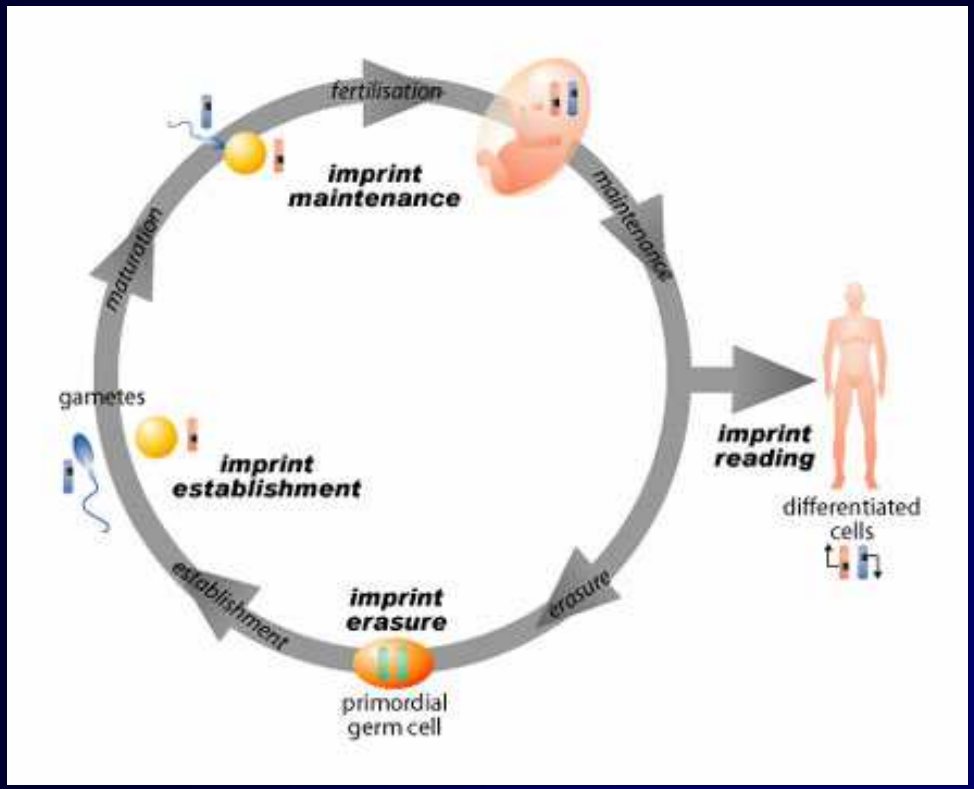


Fig. 4. Schematic model showing how heterochromatin of the Xi could transition between metaphase and interphase to be organized into the two nonoverlapping heterochromatin territories and to explain how XIST RNA could rapidly spread in cis outward from the X inactivation center (XIC) along only part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

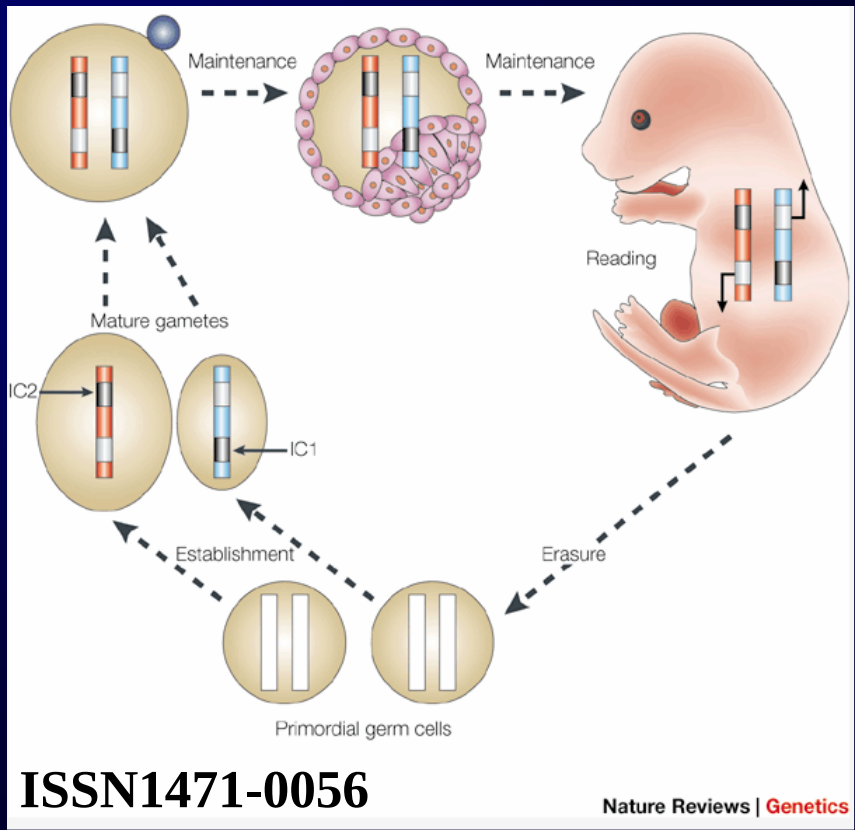


IMPRINTING

Myší embryo: samičí alela je zamethylována, nevyjadřuje se

Dospělý jedinec: obě alely jsou demethylovány

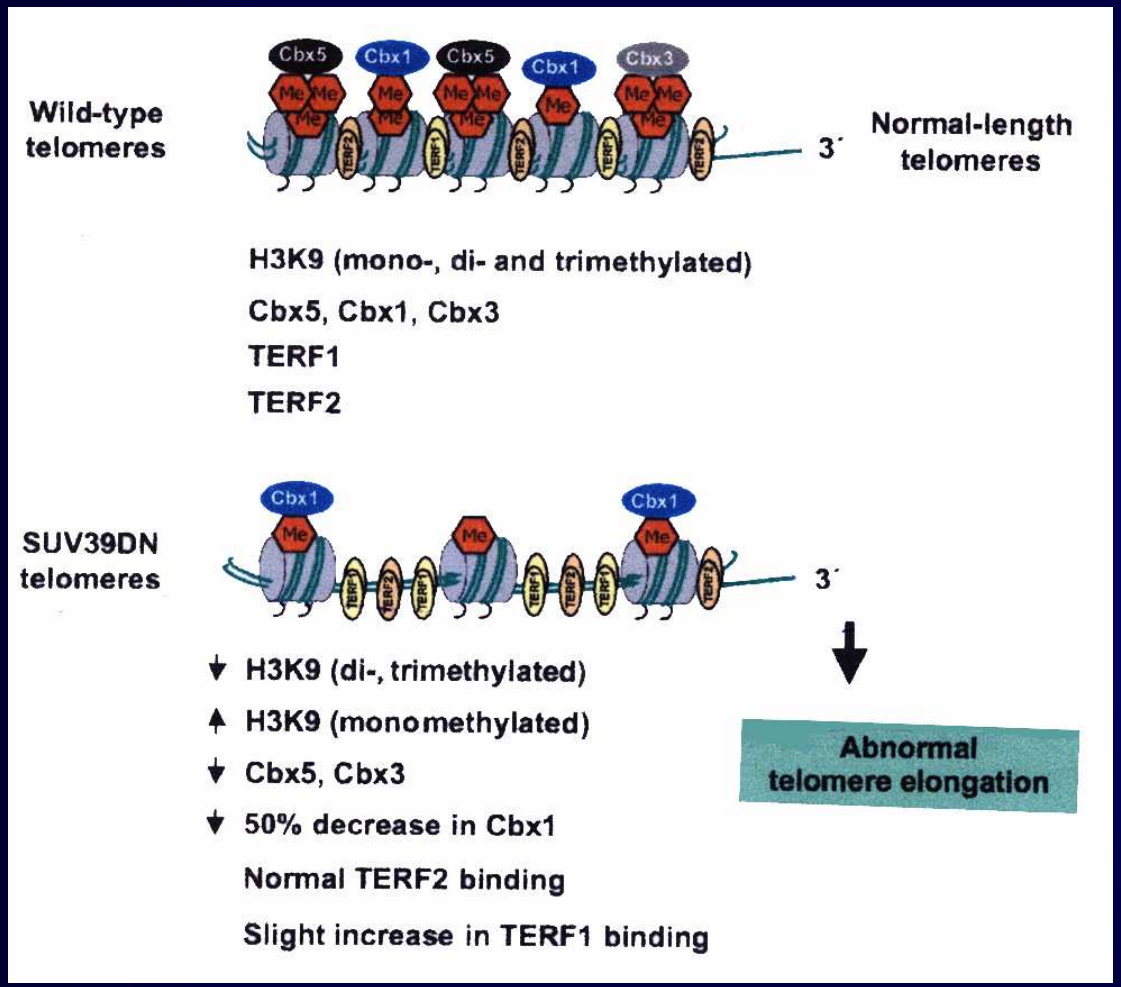
Gametogeneze: se obnoví původní stav
Platí pro gen IGF II.

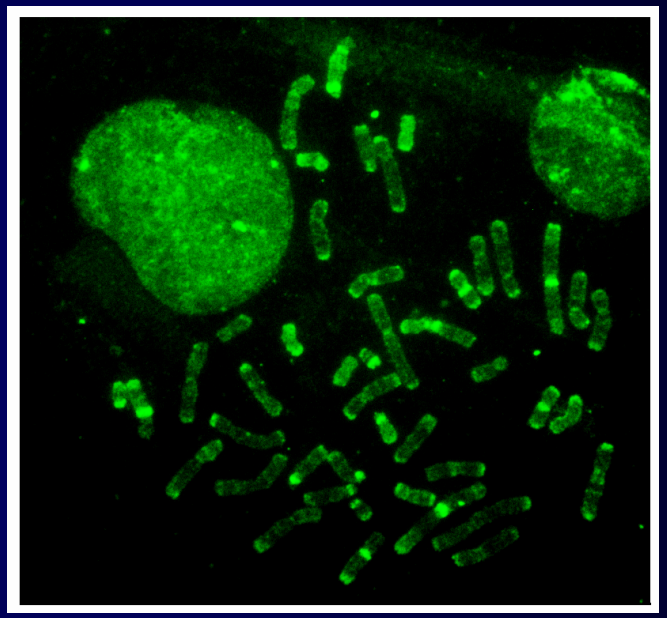


1. Independent of the classical Mendelian inheritance.
2. Methylation and histone modifications in order to achieve monoallelic gene expression.

Methylation state of telomeres

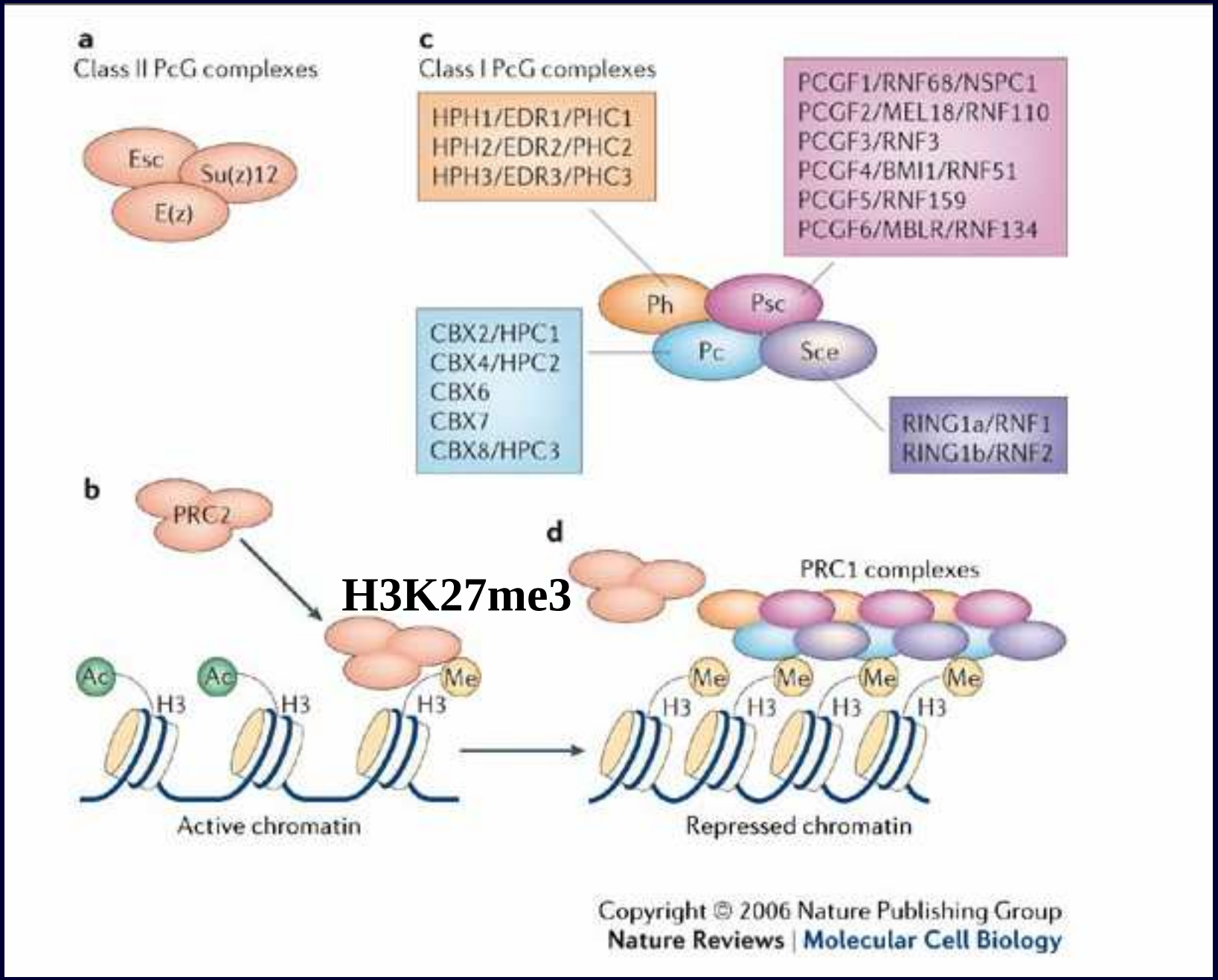
(Cbx1=HP1β, Cbx3=HP1γ, Cbx5 = HP1α)





Experiments of E. Bártová

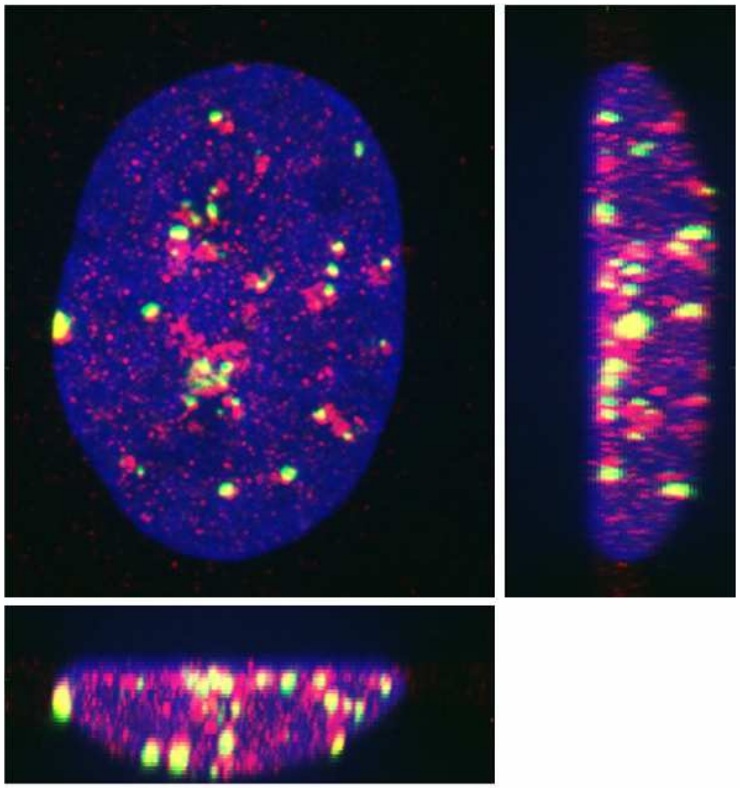
***HP1α* (12q13)** ***HP1β* (17q21)** ***HP1γ* (7p15)**


a
Class II PcG complexes
Esc
Su(z)12
E(z)
c
Class I PcG complexes
HPH1/EDR1/PHC1
HPH2/EDR2/PHC2
HPH3/EDR3/PHC3
PCGF1/RNF68/NSPC1
PCGF2/MEL18/RNF110
PCGF3/RNF3
PCGF4/BMI1/RNF51
PCGF5/RNF159
PCGF6/MBLR/RNF134
Ph
Psc
Pc
Sce
CBX2/HPC1
CBX4/HPC2
CBX6
CBX7
CBX8/HPC3
RING1a/RNF1
RING1b/RNF2
b
PRC2
H3K27me3
Ac
Ac
Me
H3
H3
H3
Active chromatin
d
PRC1 complexes
Me
Me
Me
Me
H3
H3
H3
H3
Repressed chromatin
Copyright © 2006 Nature Publishing Group
Nature Reviews | Molecular Cell Biology

H3K27me3 / BMI1 / Nucleus

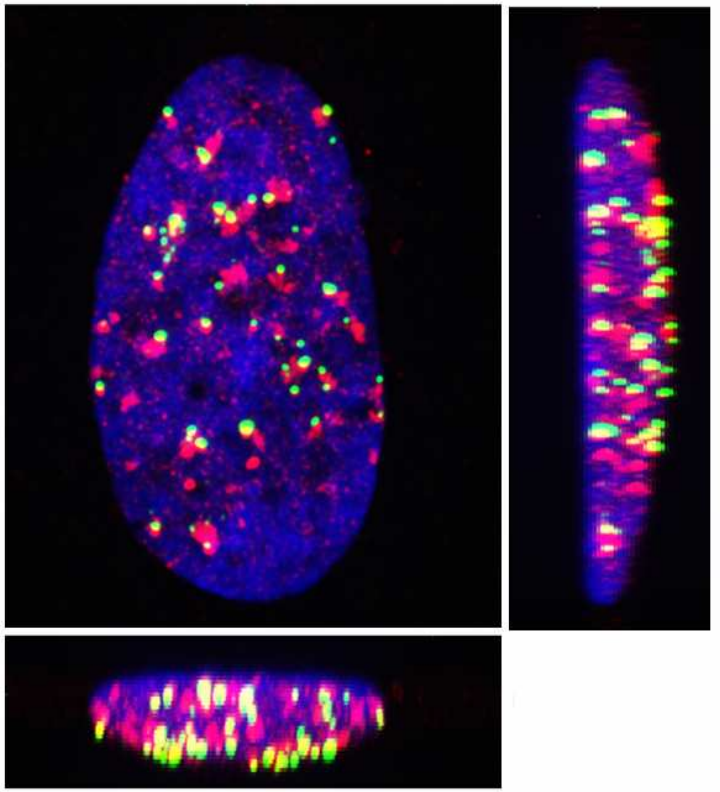
CONTROL

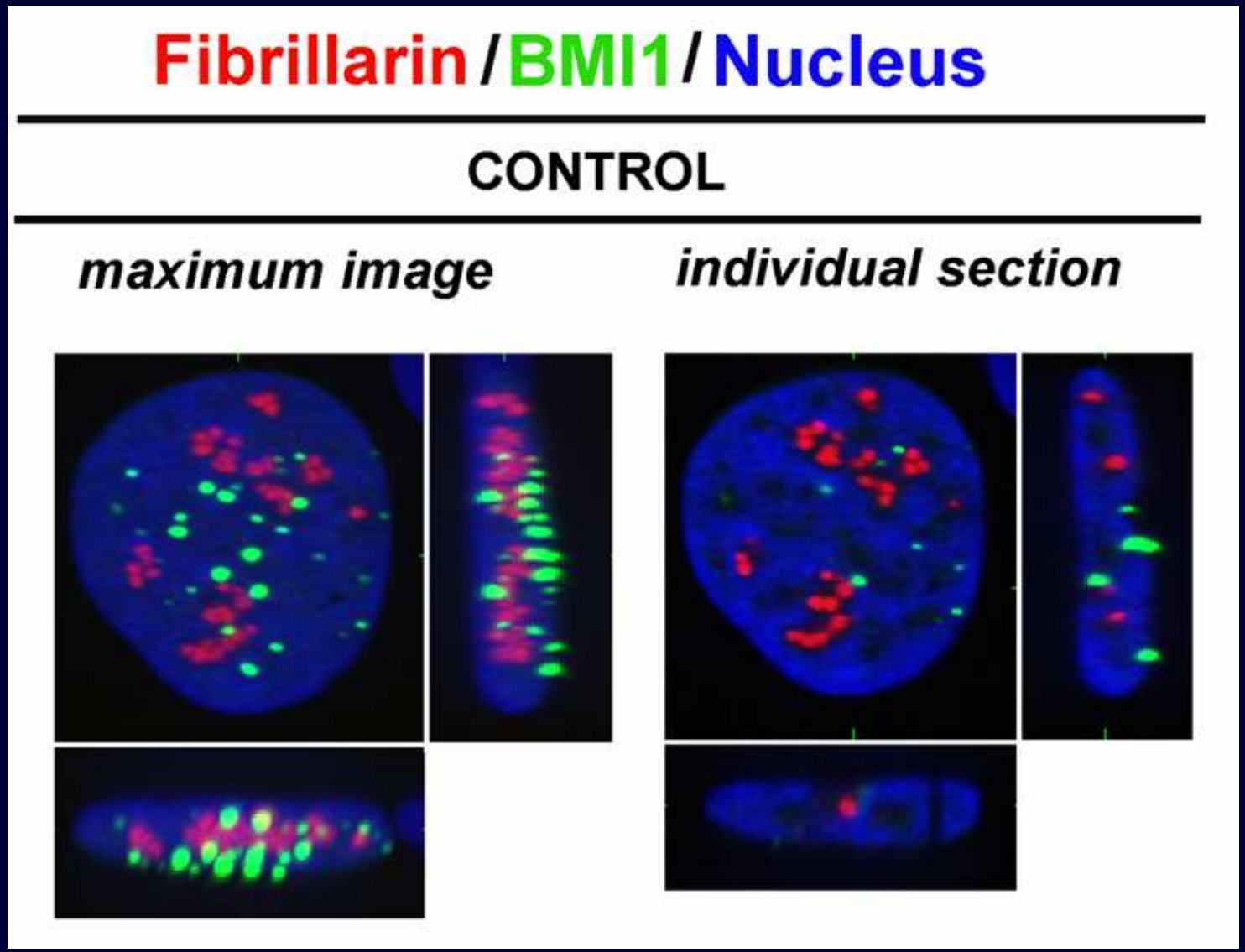
maximum image



TSA

maximum image

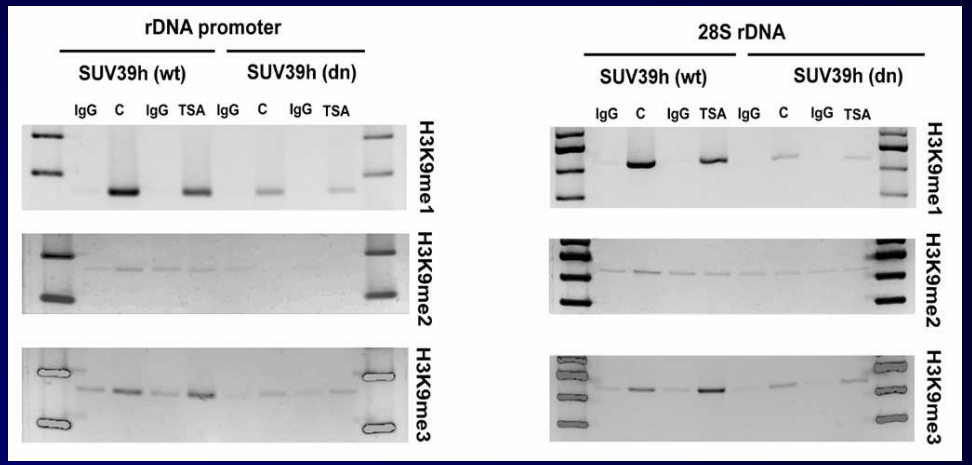


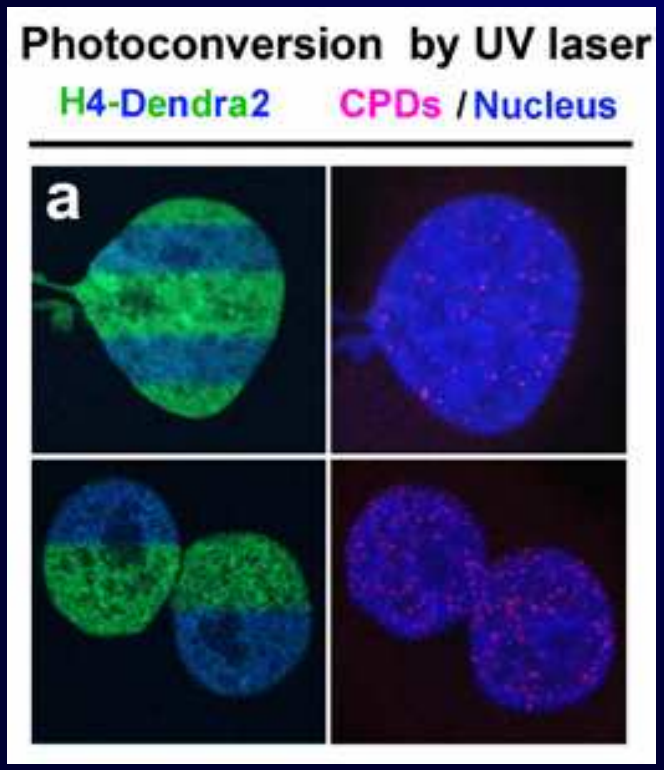

Fibrillarin / BMI1 / Nucleus
CONTROL
maximum image
individual section

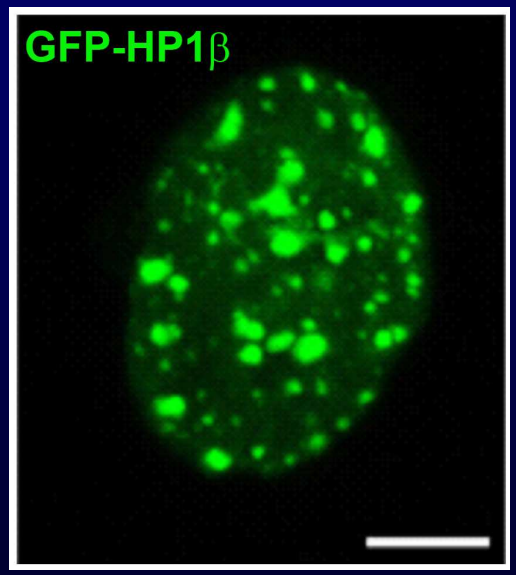
Metody

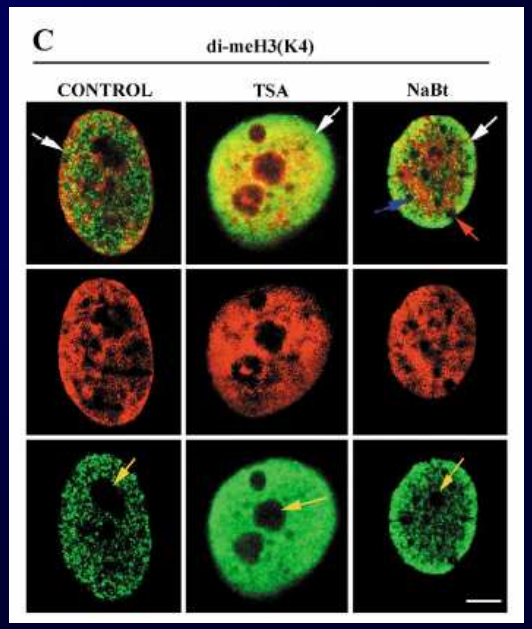
Metody vhodné ke studiu histonového kódu

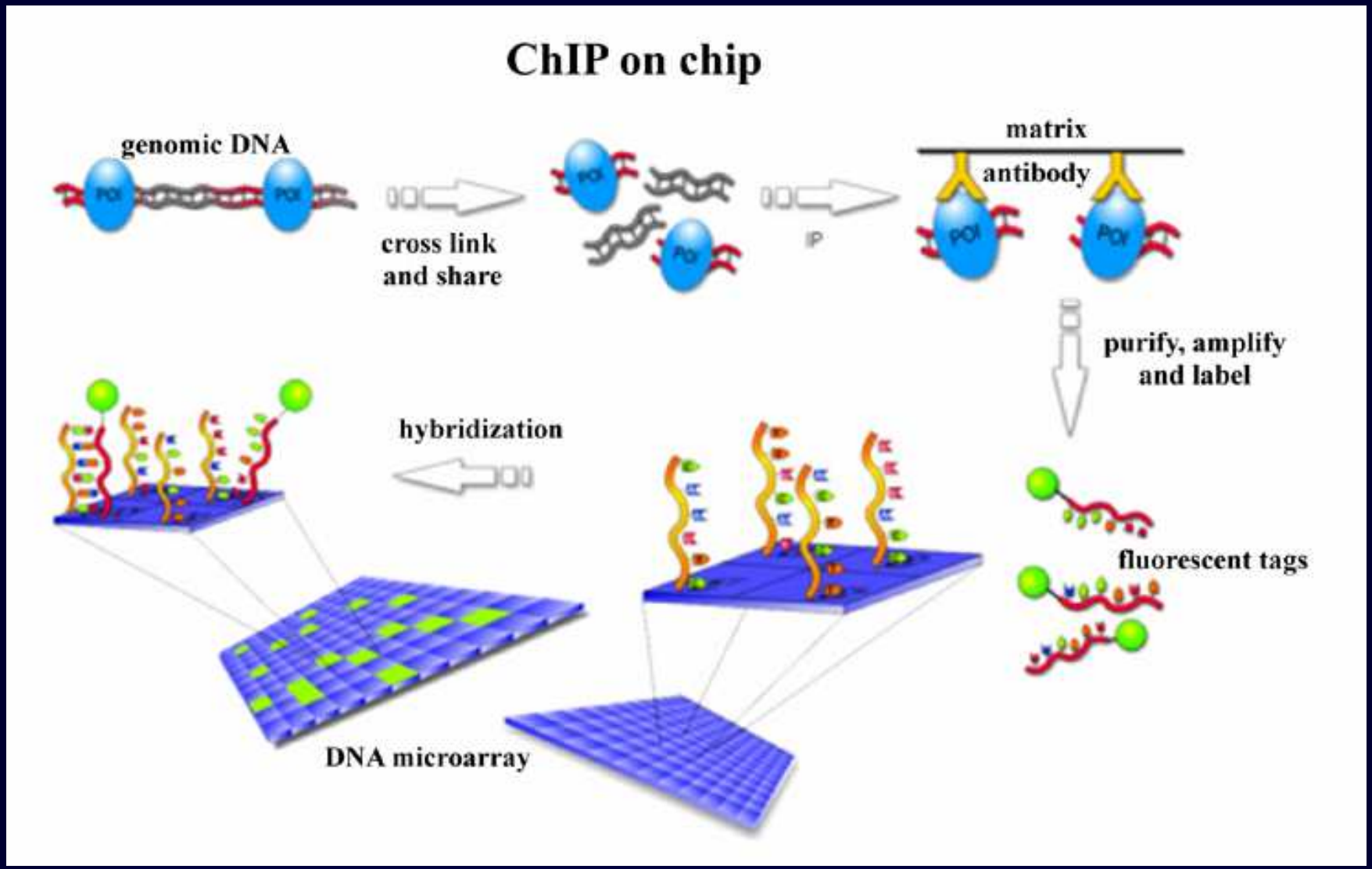
1. imunofluorescence-konfokální mikroskopie
2. GFP-technologie
3. ChIP-PCR
4. ChIP-on-chip



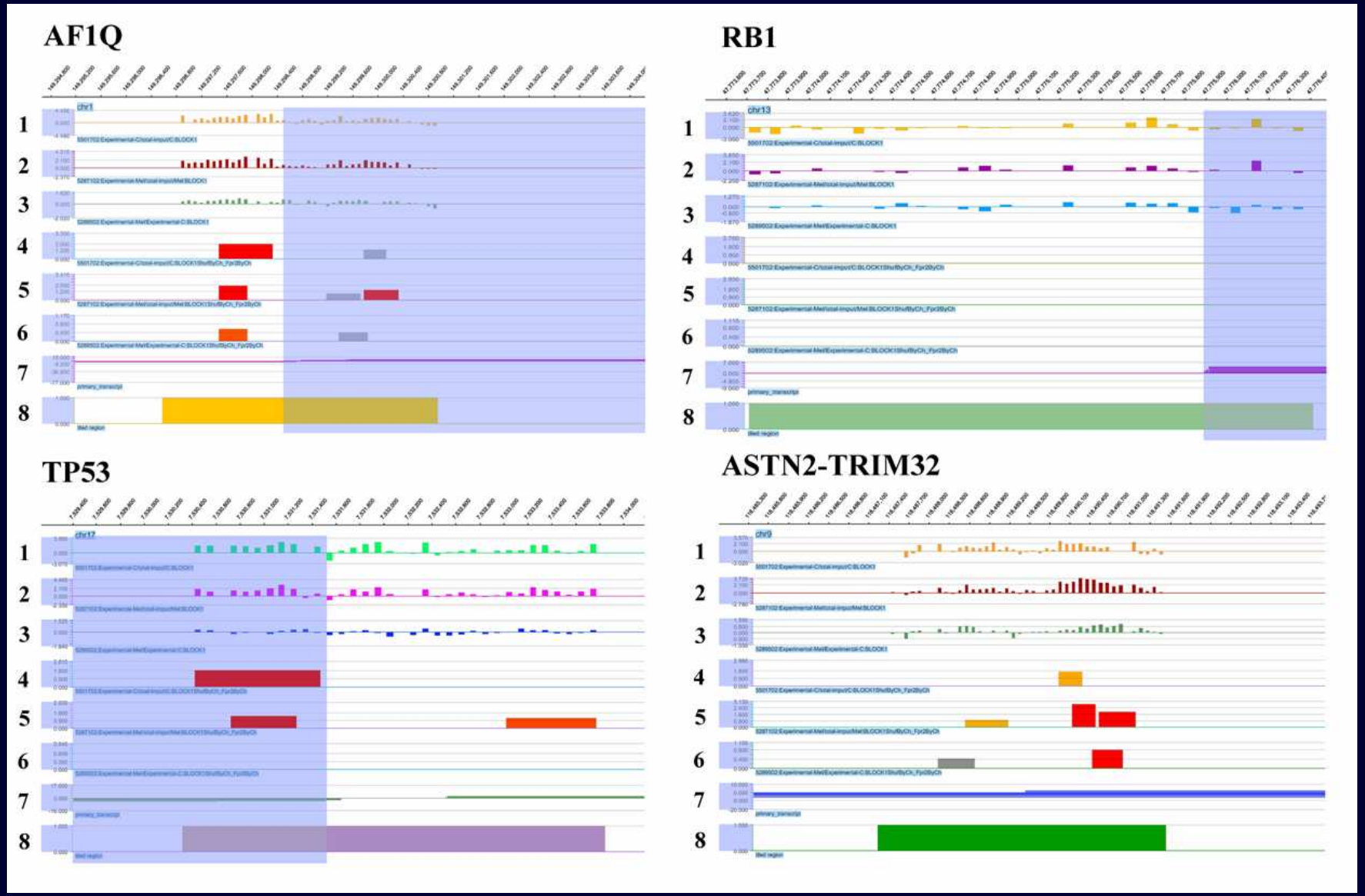






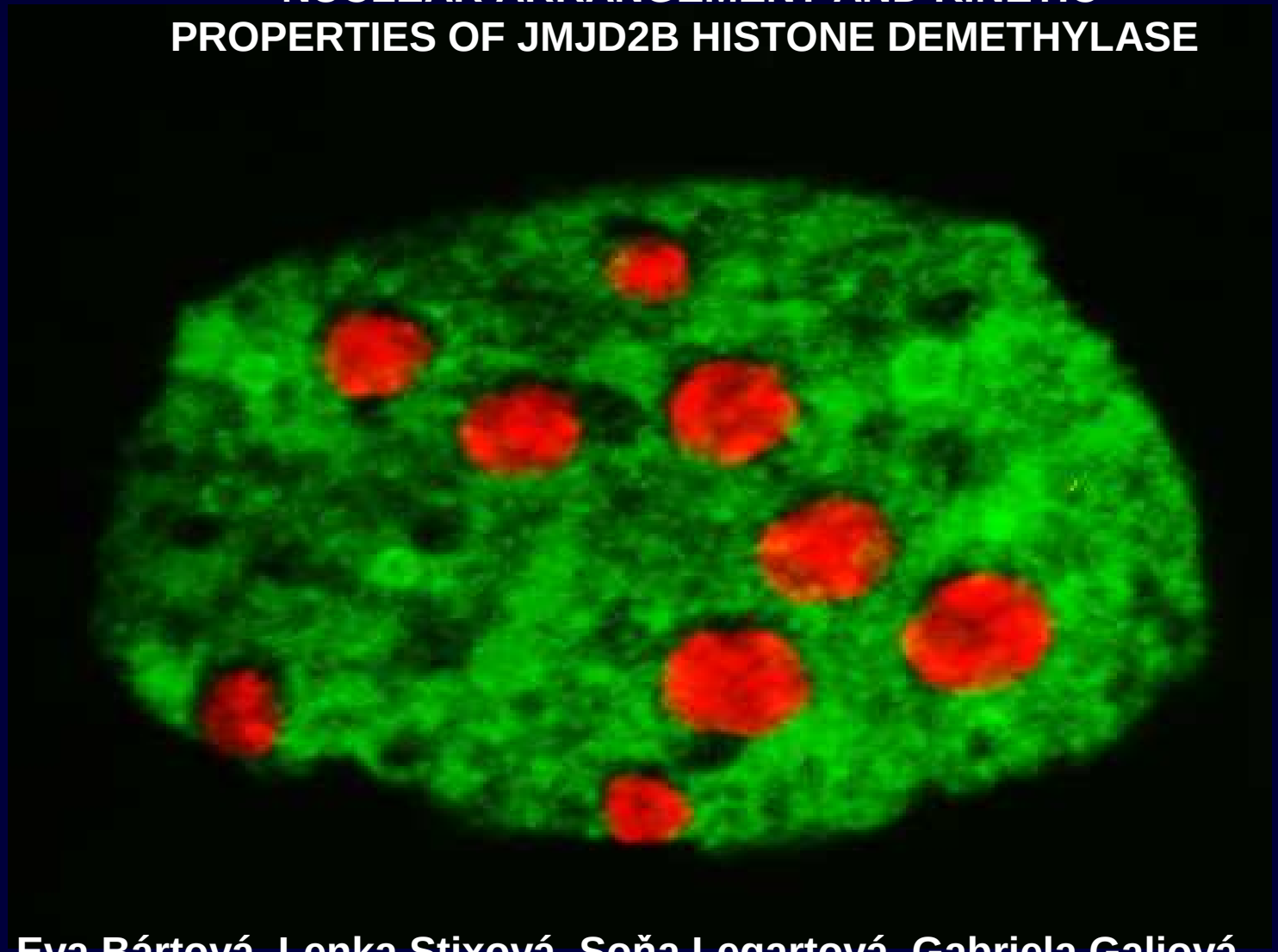

ChIP on chip
genomic DNA
POI
POI
cross link
and share
POI
POI
IP
matrix
antibody
POI
POI
purify, amplify
and label
fluorescent tags
hybridization
DNA microarray

H3K9 acetylation in ARH77 cells



Ukázkové experimenty

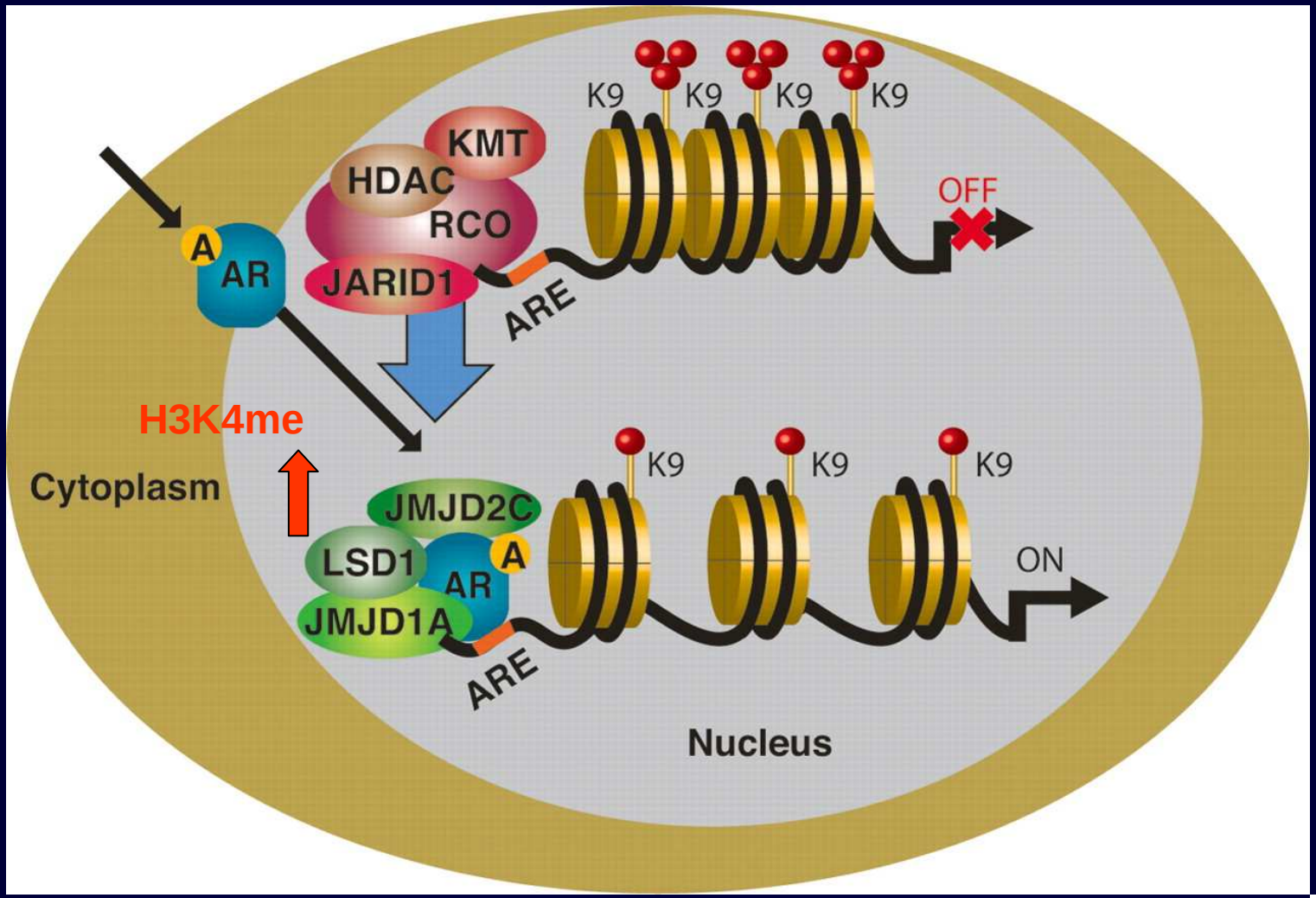
NUCLEAR ARRANGEMENT AND KINETIC PROPERTIES OF JMJD2B HISTONE DEMETHYLASE



Eva Bártová, Lenka Stixová, Soňa Legartová, Gabriela Galiová and Stanislav Kozubek

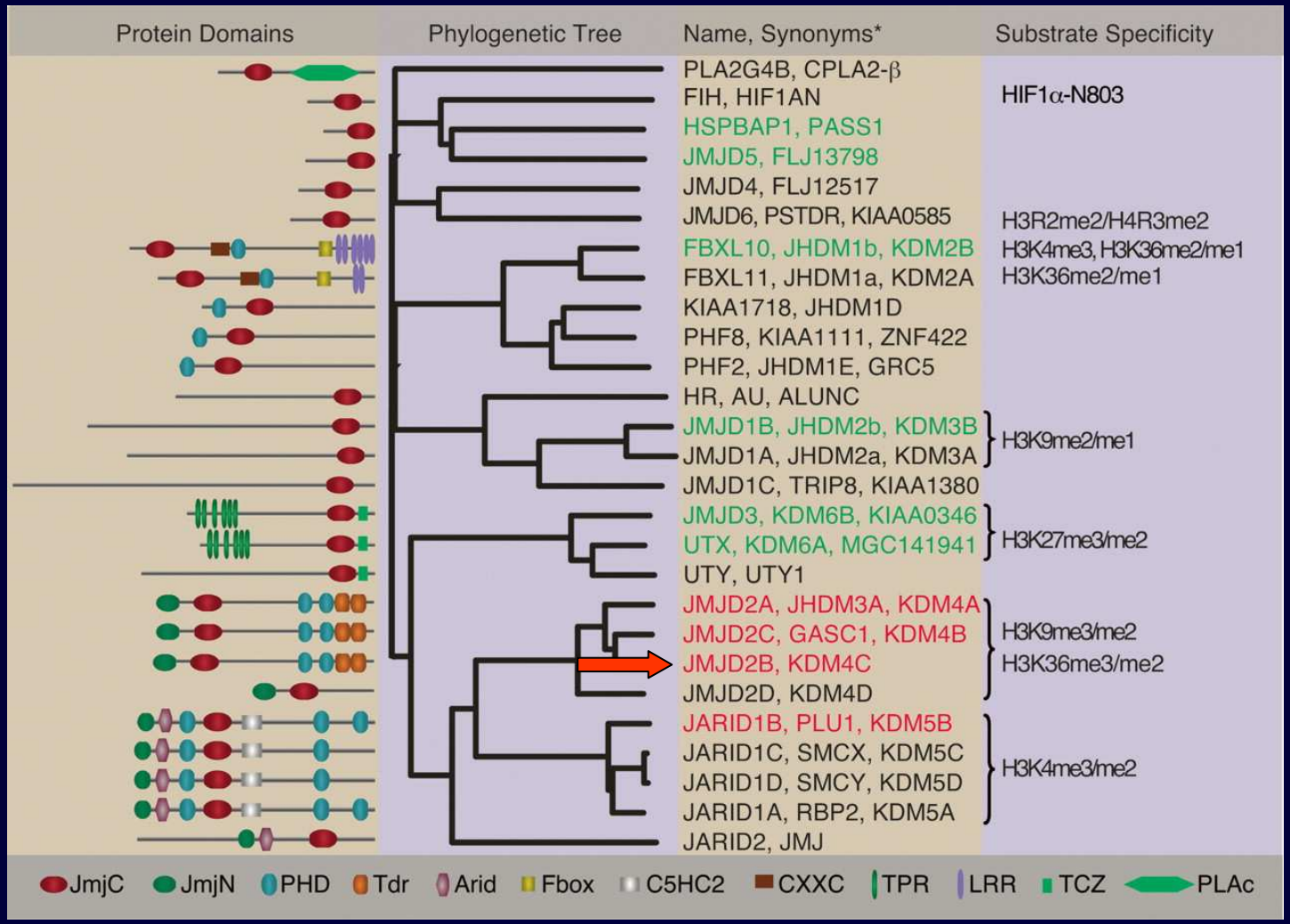
Institute of Biophysics, the Academy of Sciences of the Czech Republic, v.v.i., Brno

The involvement of demethylases in AR-mediated transcription.



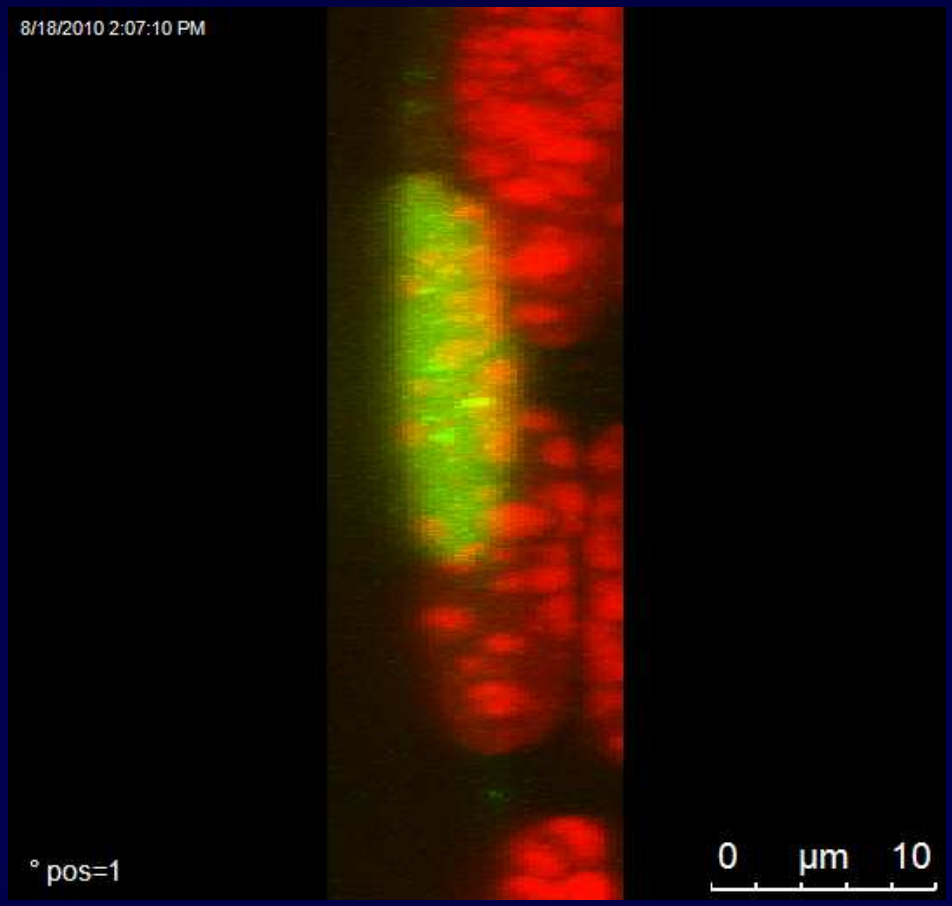
Cloos P A et al. Genes Dev. 2008;22:1115-1140

Phylogenetic tree of the JmjC family of demethylases.

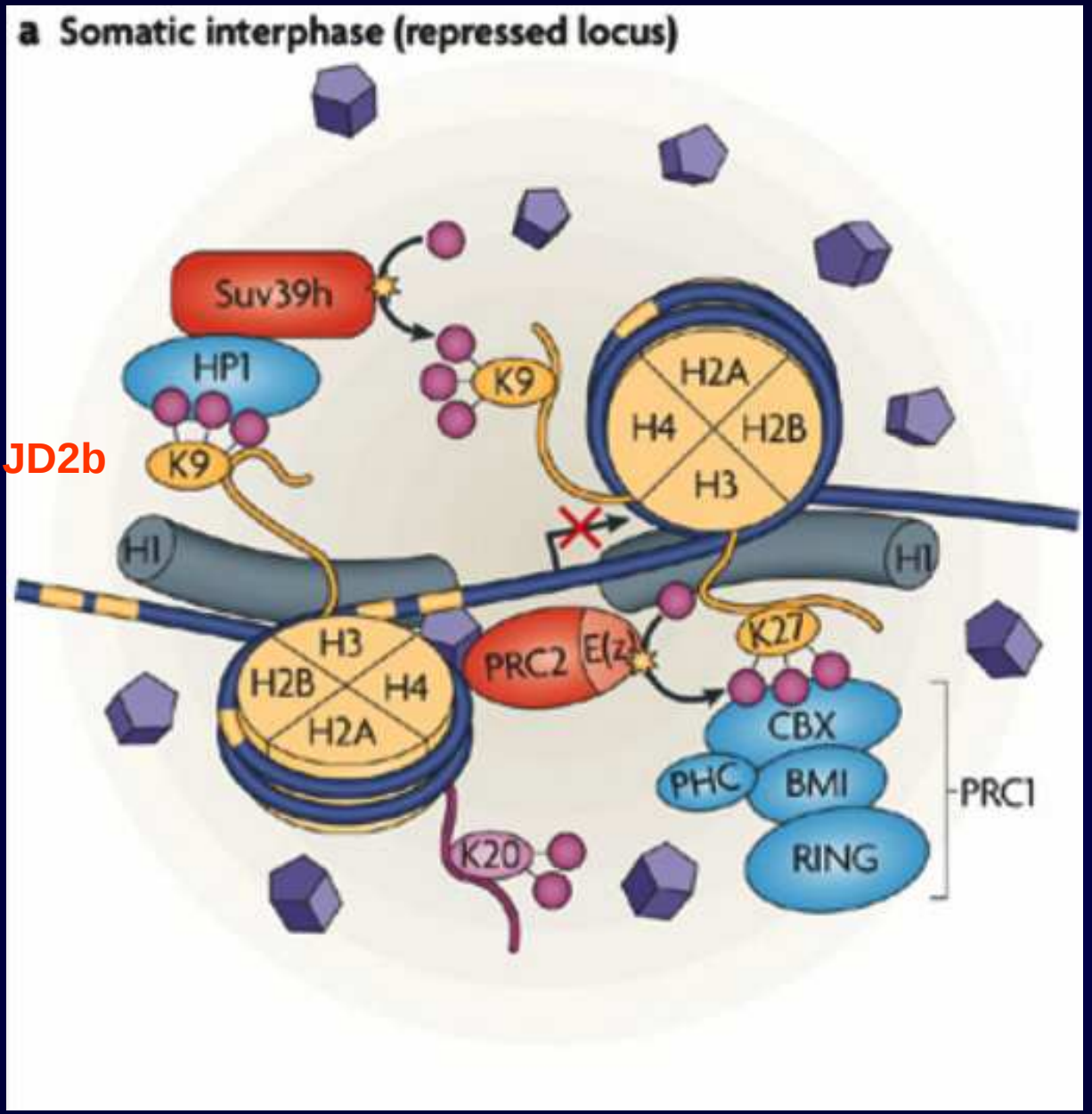


Cloos P A et al. Genes Dev. 2008;22:1115-1140

JMJD2b histone lysine demethylase



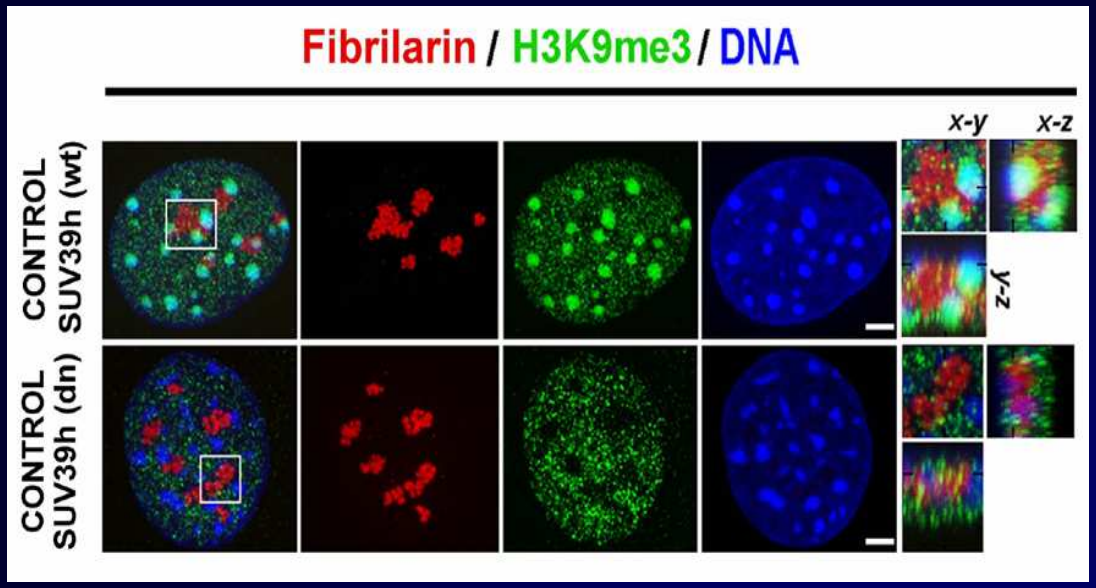
Mutant genetic background affects functional re-arrangement and kinetic properties of JMJD2b histone demethylase

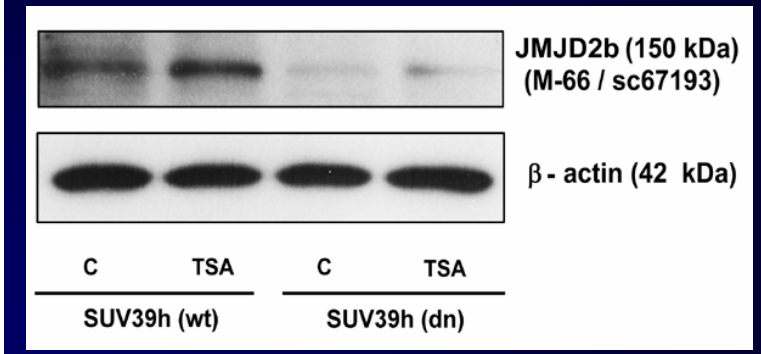


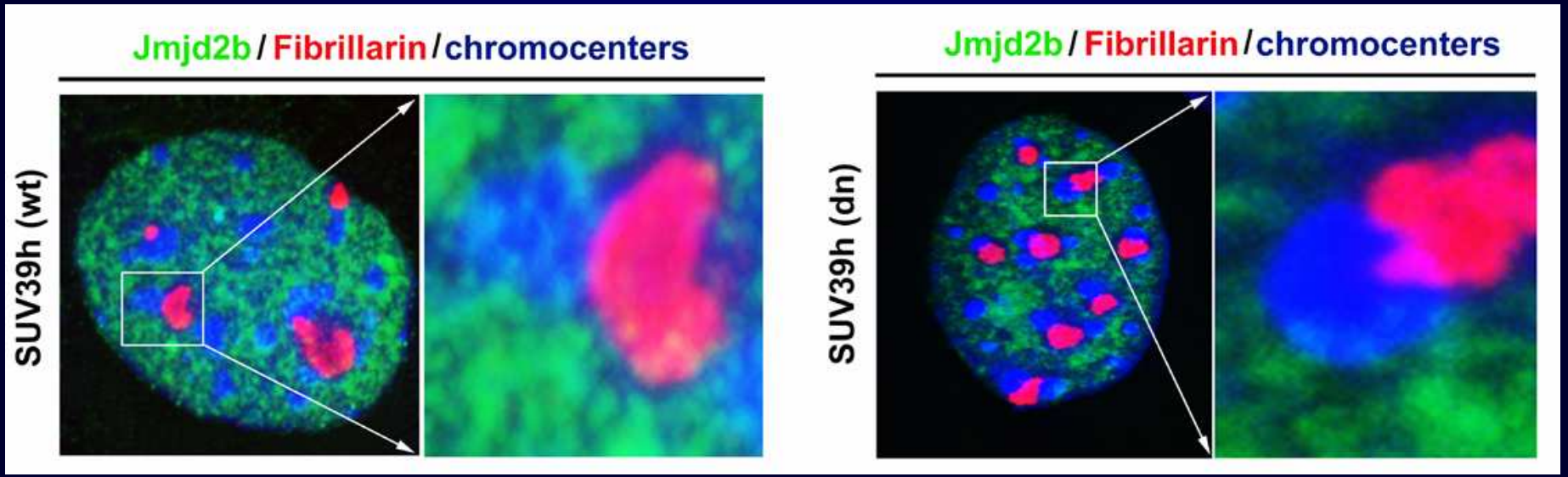
JMJD2b

Dieter Egli, Garrett Birkhoff & Kevin Eggan (2008)
Nature Reviews Molecular Cell Biology 9, 505-516

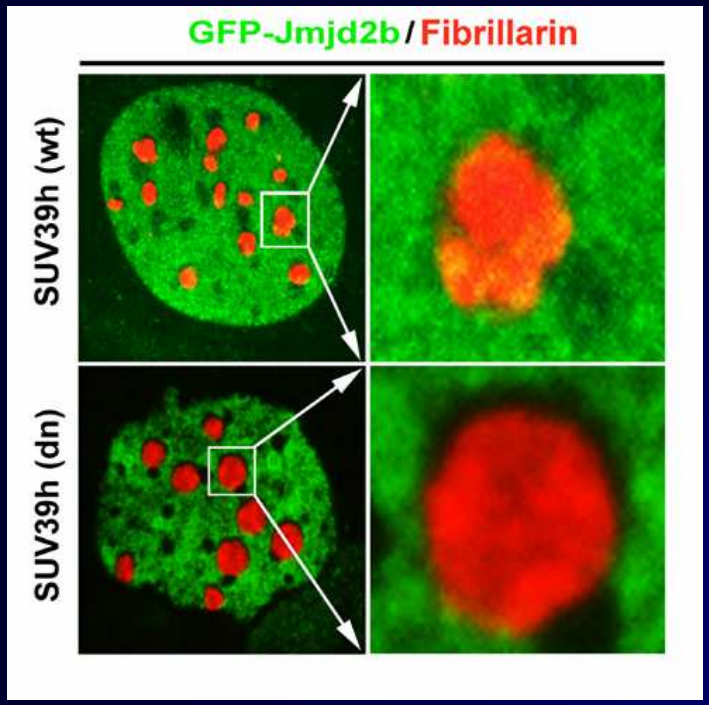
JMJD2b histone lysine demethylase

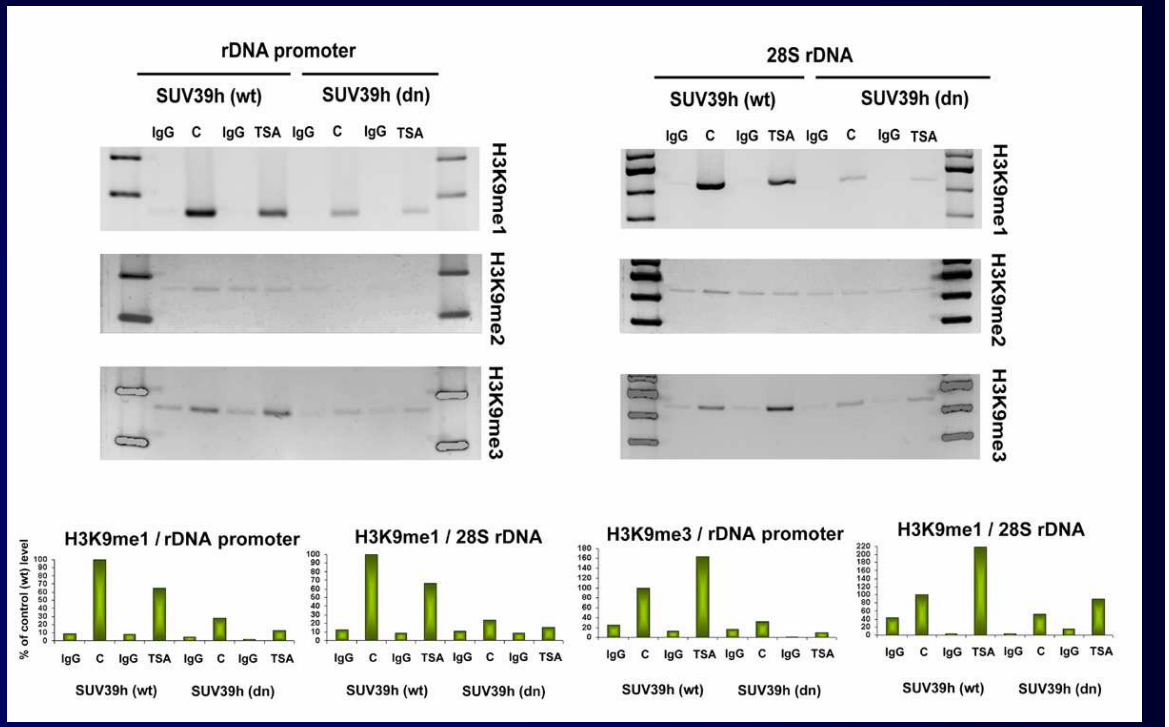


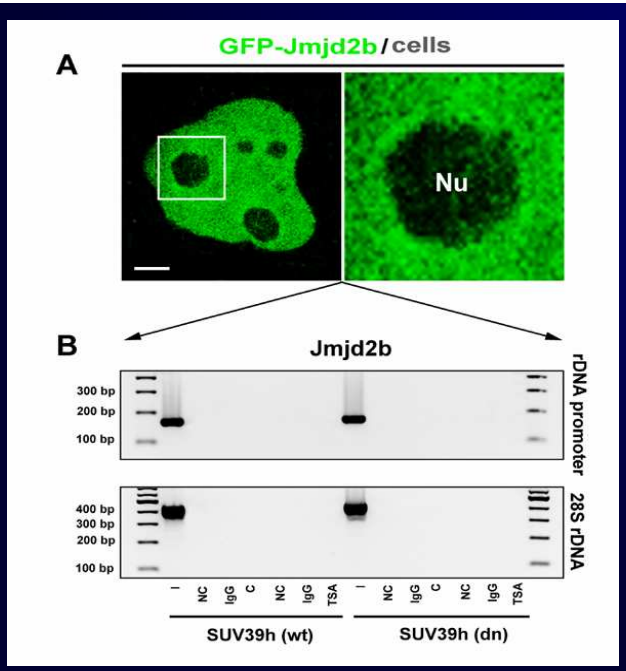


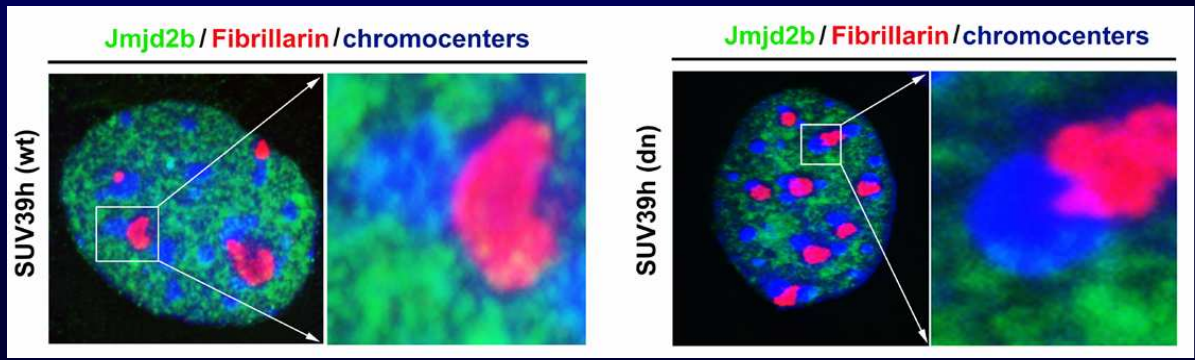


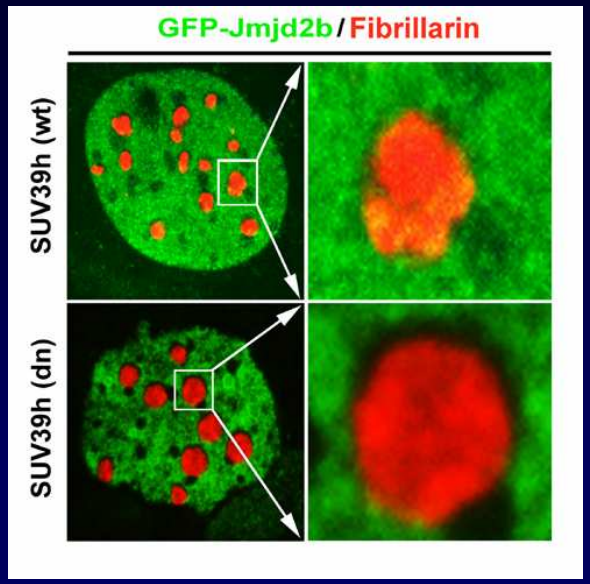
Jmjd2b histone lysine demethylase

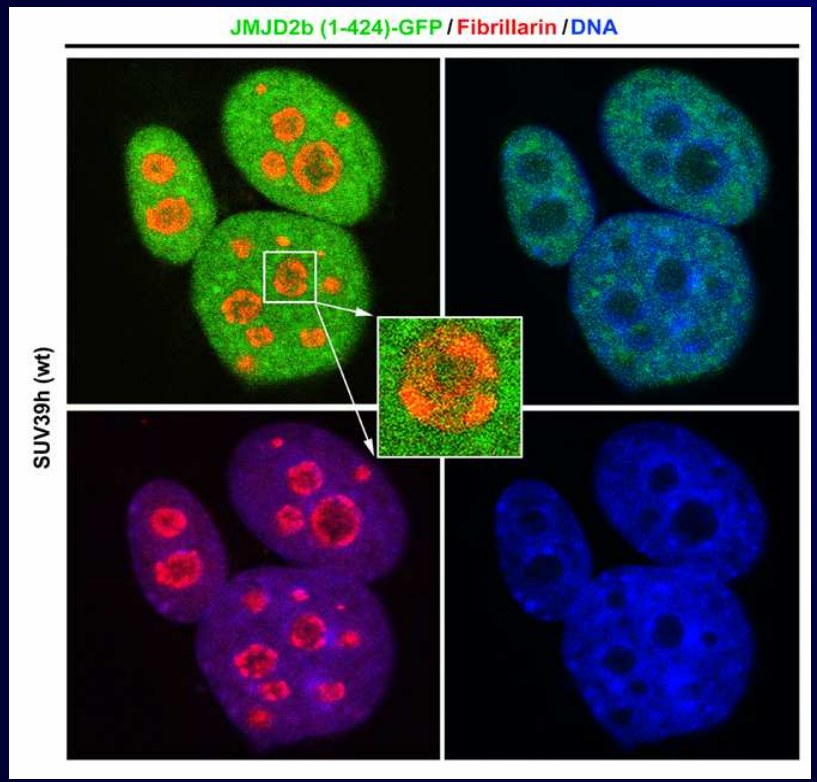


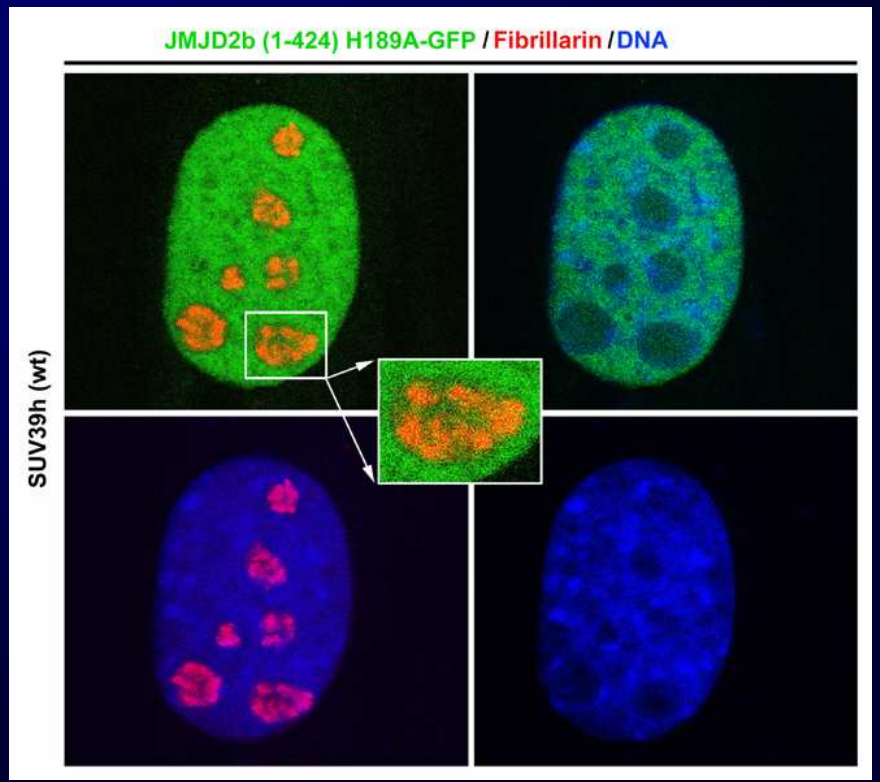




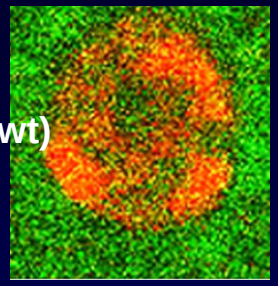



GFP-Jmjd2b / Fibrillarin
SUV39h (wt)
SUV39h (dn)

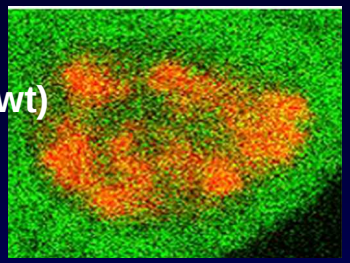

JMJD2b (1-424)-GFP / Fibrillarin / DNA
SUV39h (wt)


JMJD2b (1-424) H189A-GFP / Fibrillarin / DNA
SUV39h (wt)

SUV39h (wt)

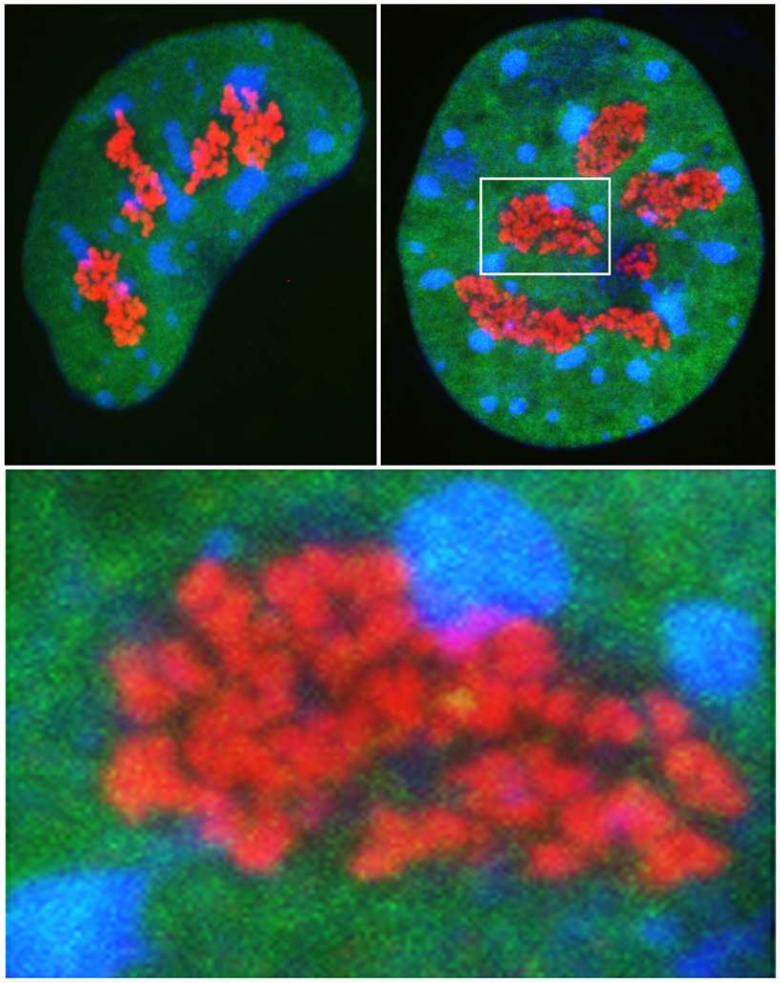


SUV39h (wt)



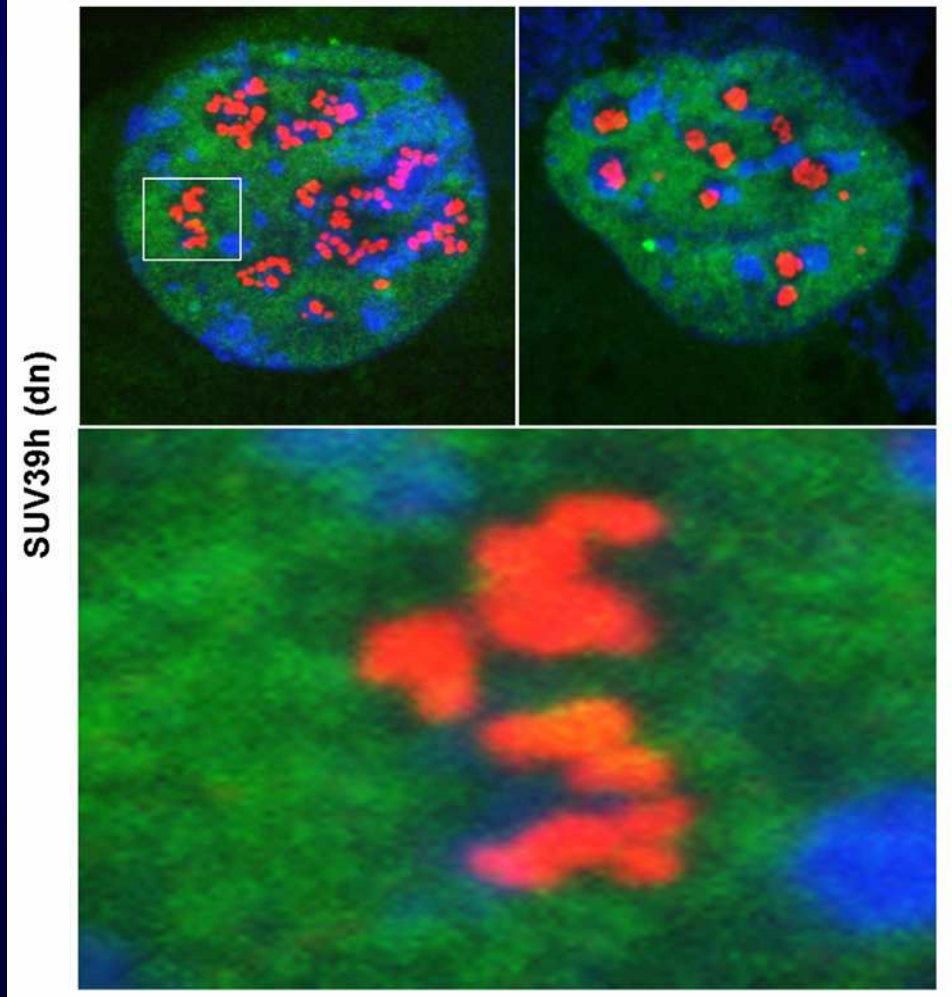
JMJD2b (1-424)-GFP / Fibrillarin / Chromocenters

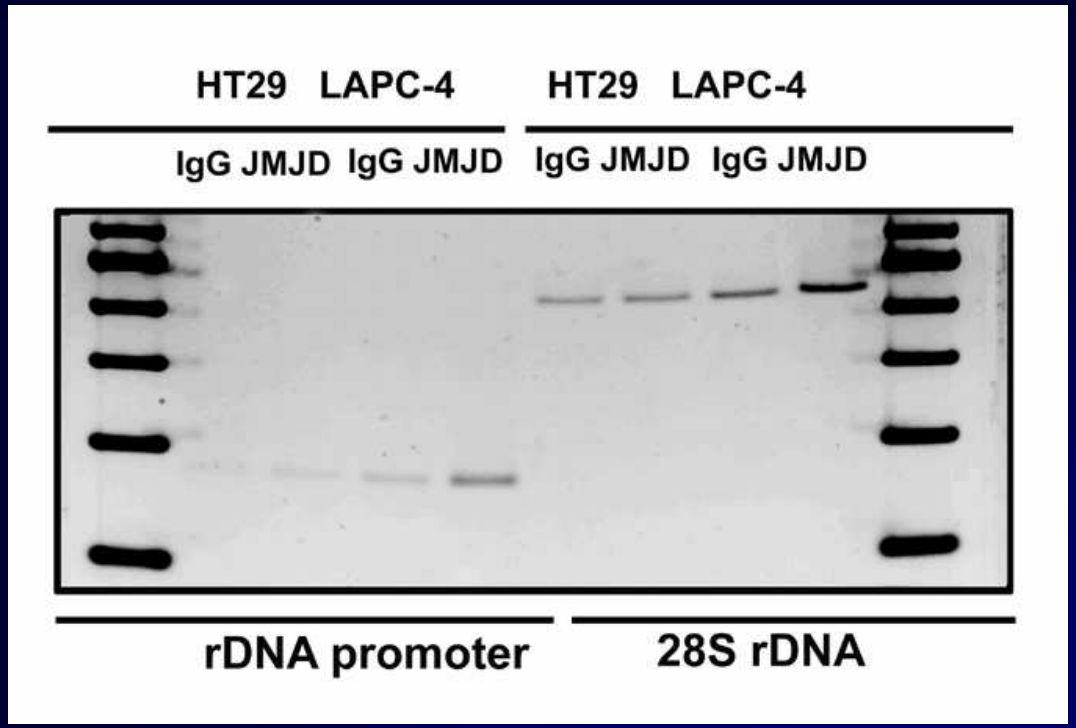
SUV39h (dn)

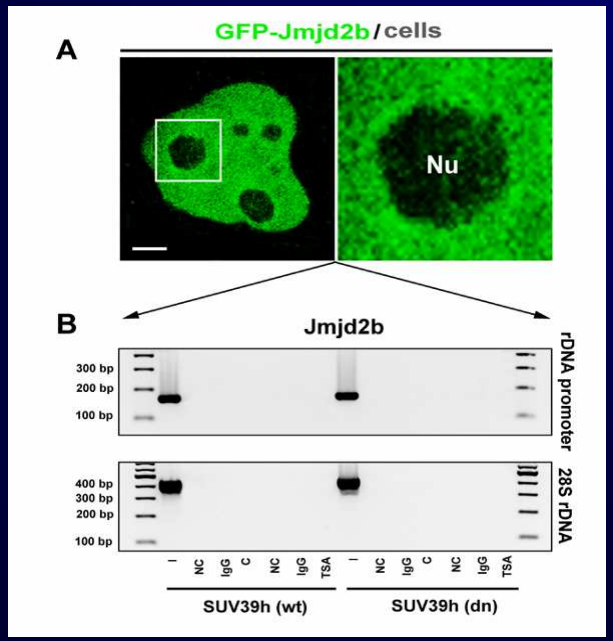


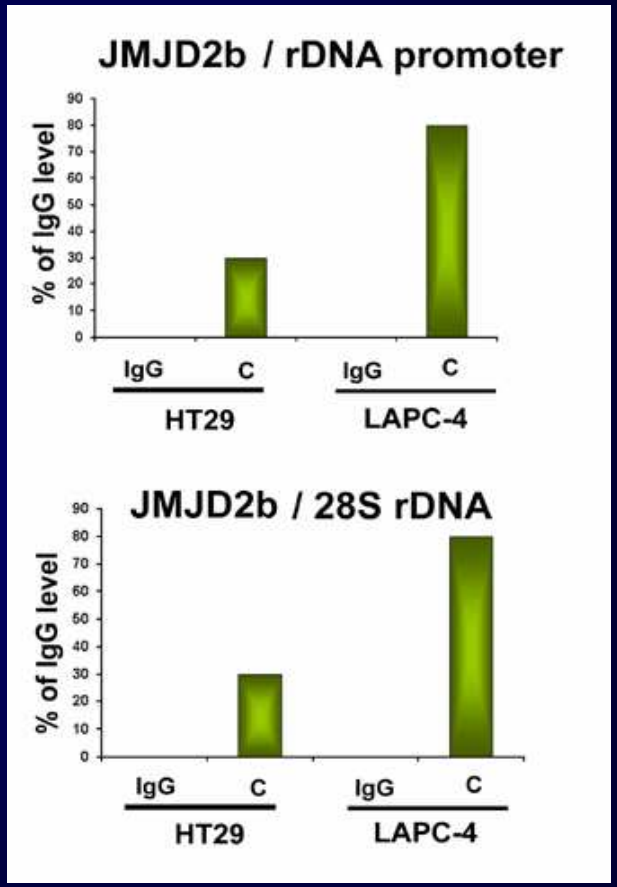
JMJD2b (1-424)H189-GFP / Fibrillarin / Chromocenters

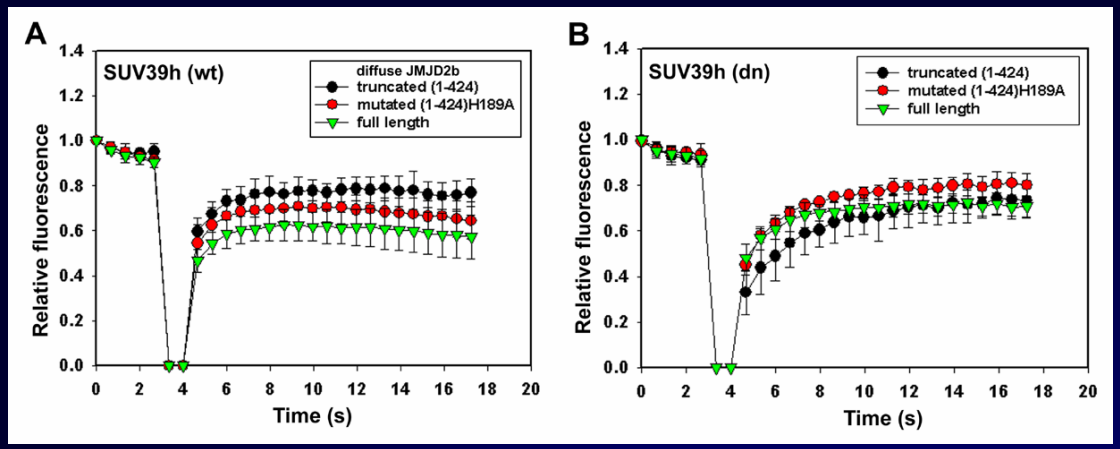
SUV39h (dn)

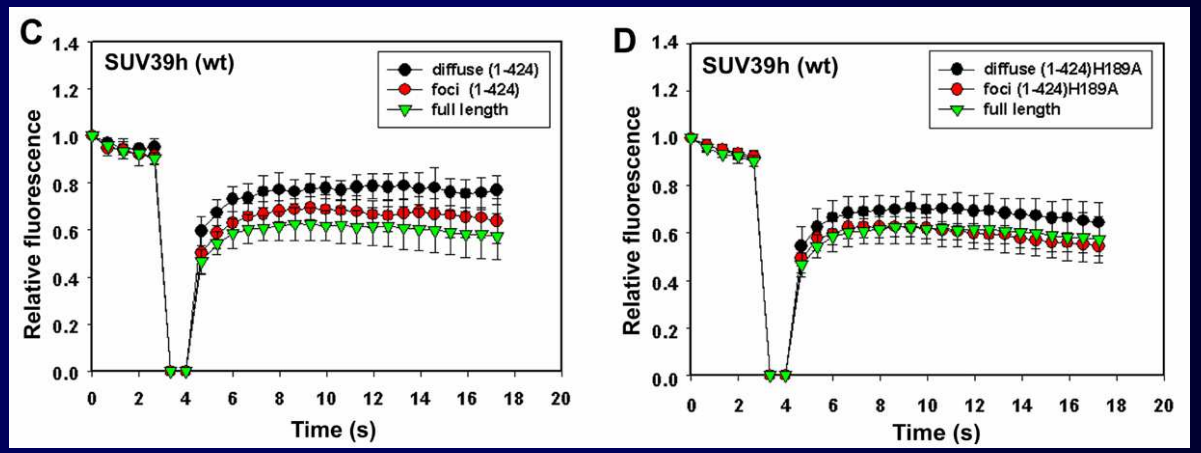


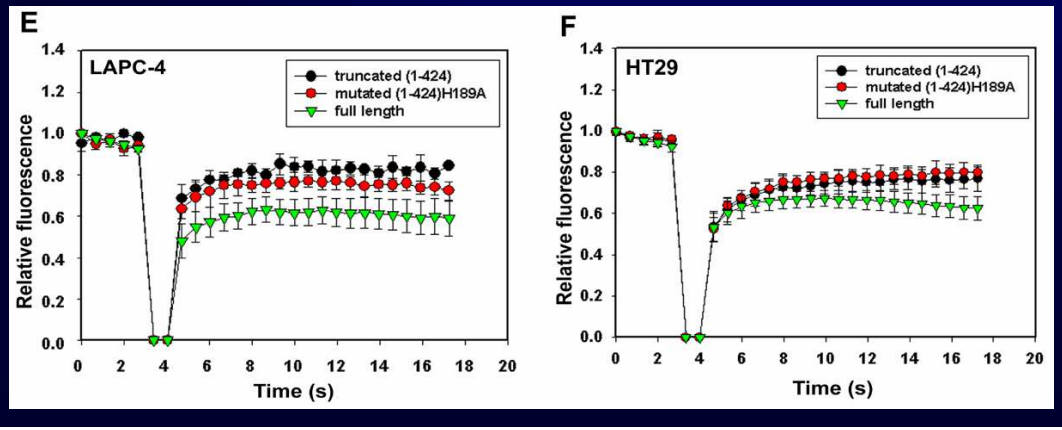

HT29 LAPC-4 HT29 LAPC-4
IgG JMJD IgG JMJD IgG JMJD IgG JMJD
rDNA promoter
28S rDNA

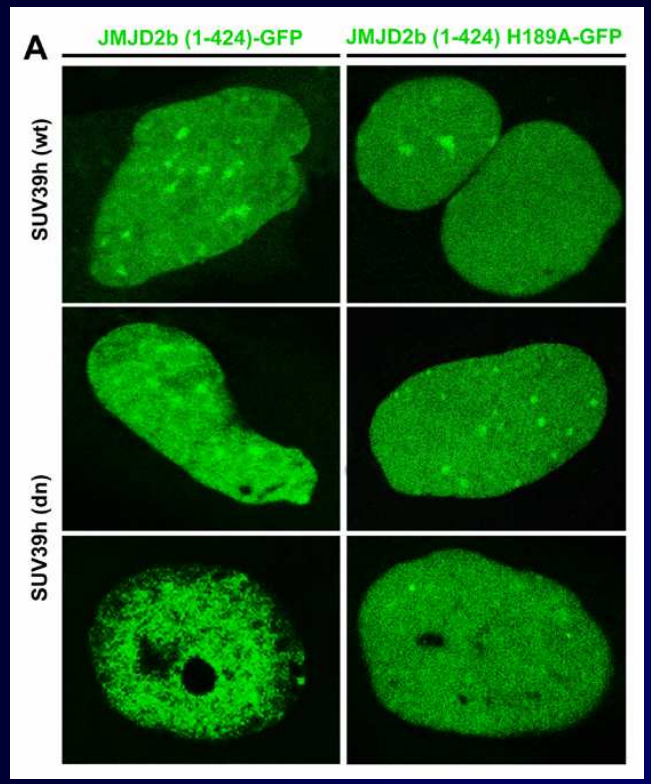

A
GFP-Jmjd2b/cells
Nu
B
Jmjd2b
300 bp
200 bp
100 bp
rDNA promoter
400 bp
300 bp
200 bp
100 bp
28S rDNA
I NC IgG C NC IgG TSA I NC IgG C NC IgG TSA
SUV39h (wt)
SUV39h (dn)

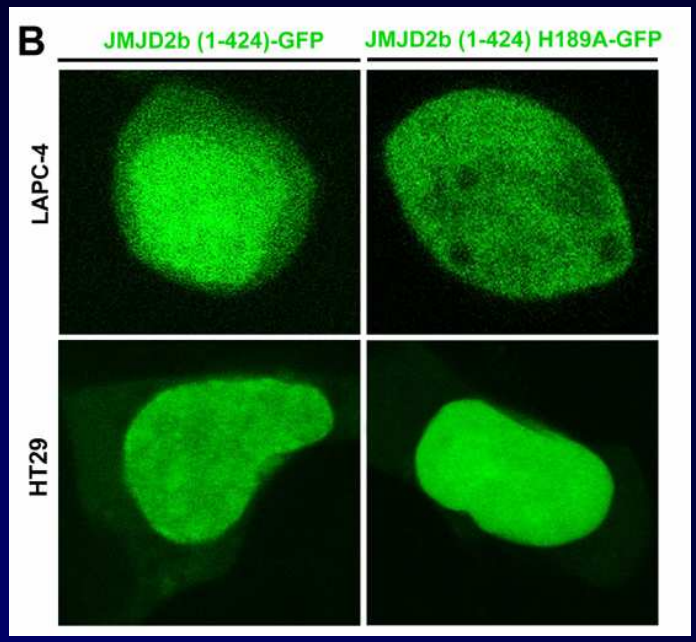

JMJD2b / rDNA promoter
% of IgG level
IgG C IgG C
HT29 LAPC-4
JMJD2b / 28S rDNA
% of IgG level
IgG C IgG C
HT29 LAPC-4

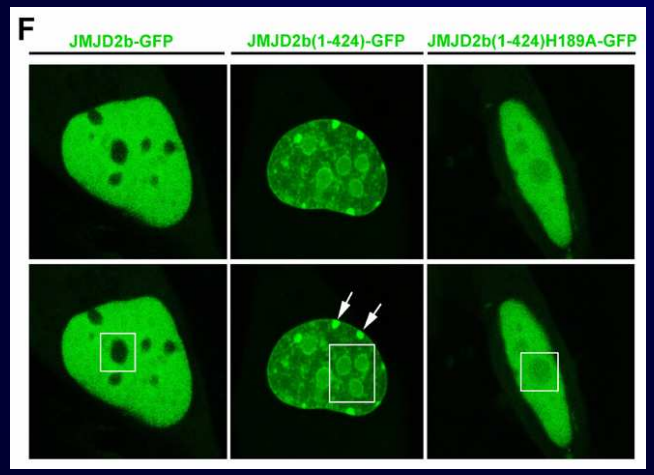

A
SUV39h (wt)
diffuse JMJD2b
truncated (1-424)
mutated (1-424)H189A
full length
Relative fluorescence
Time (s)
B
SUV39h (dn)
truncated (1-424)
mutated (1-424)H189A
full length
Relative fluorescence
Time (s)

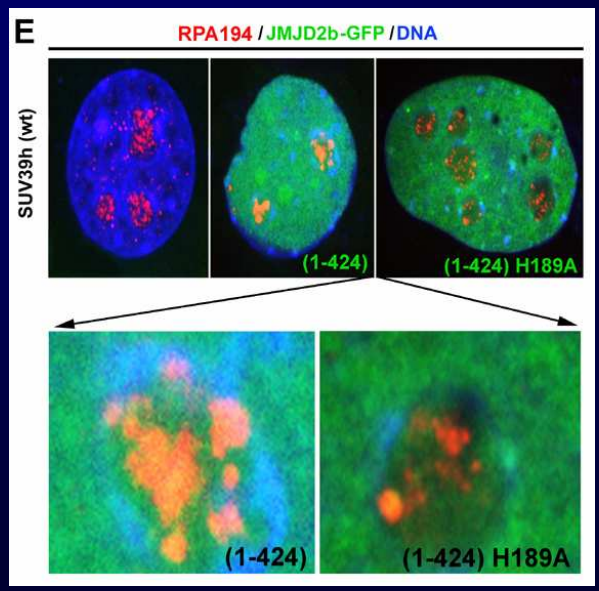

C
SUV39h (wt)
diffuse (1-424)
foci (1-424)
full length
Relative fluorescence
Time (s)
D
SUV39h (wt)
diffuse (1-424)H189A
foci (1-424)H189A
full length
Relative fluorescence
Time (s)


E
LAPC-4
truncated (1-424)
mutated (1-424)H189A
full length
Relative fluorescence
Time (s)
F
HT29
truncated (1-424)
mutated (1-424)H189A
full length
Relative fluorescence
Time (s)


A
JMJD2b (1-424)-GFP
JMJD2b (1-424) H189A-GFP
SUV39h (wt)
SUV39h (dn)


B
JMJD2b (1-424)-GFP
JMJD2b (1-424) H189A-GFP
LAPC-4
HT29


F
JMJD2b-GFP
JMJD2b(1-424)-GFP
JMJD2b(1-424)H189A-GFP


E
RPA194 / JMJD2b-GFP / DNA
SUV39h (wt)
(1-424)
(1-424) H189A
(1-424)
(1-424) H189A

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Projects: Ministry of Education Youth and Sports of the Czech Republic; the research projects LC535, LC06027, and ME 919. The Academy of Sciences of the Czech Republic: AVOZ50040702 and AVOZ50040507 and the Grant Agency of the Czech Republic by grant no. P302/10/1022. European Union project COST TD09/05 and corresponding national COST-CZ project LC11020. Marie Curie project PIRSES-GA-2010-269156.