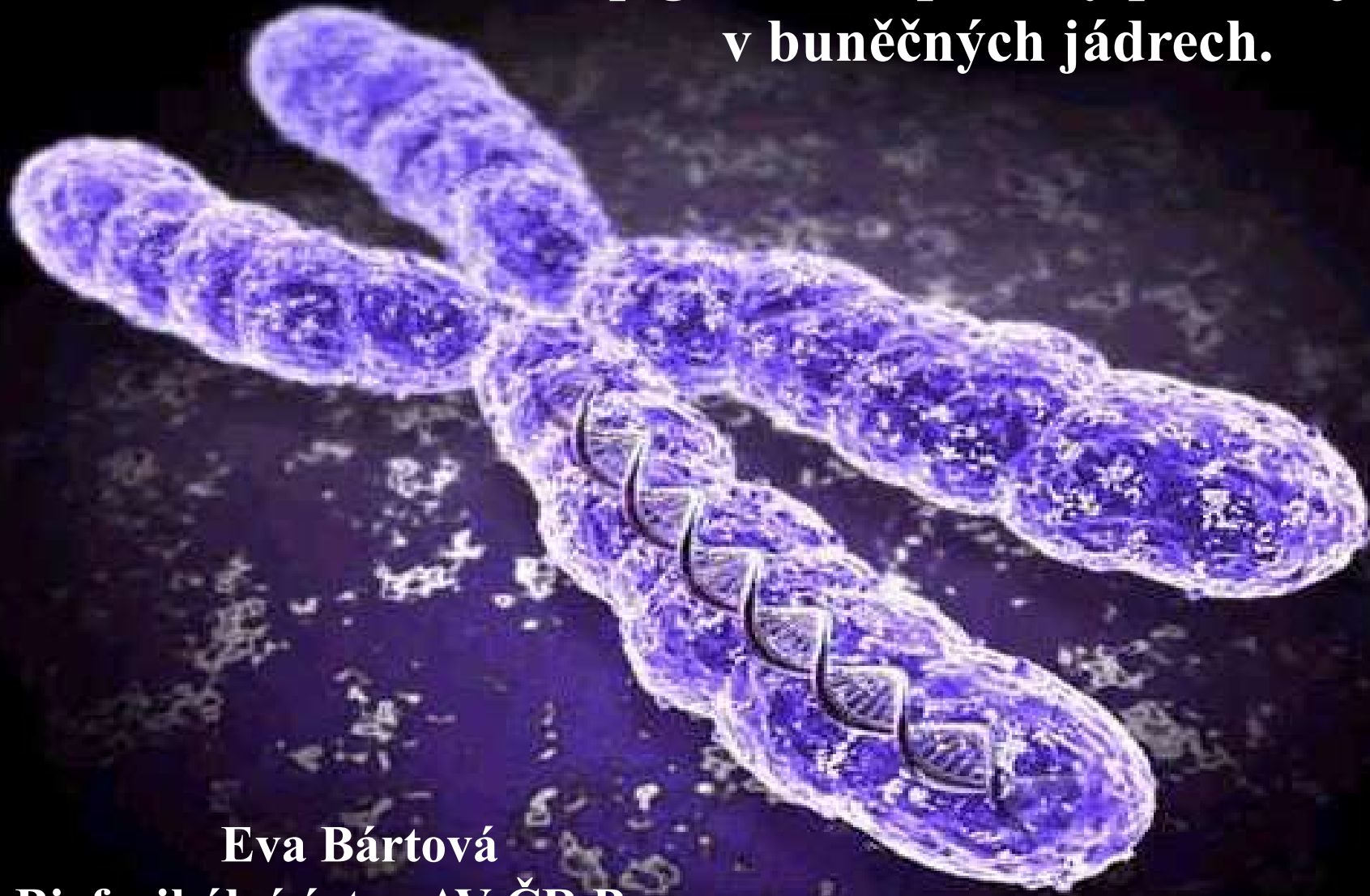


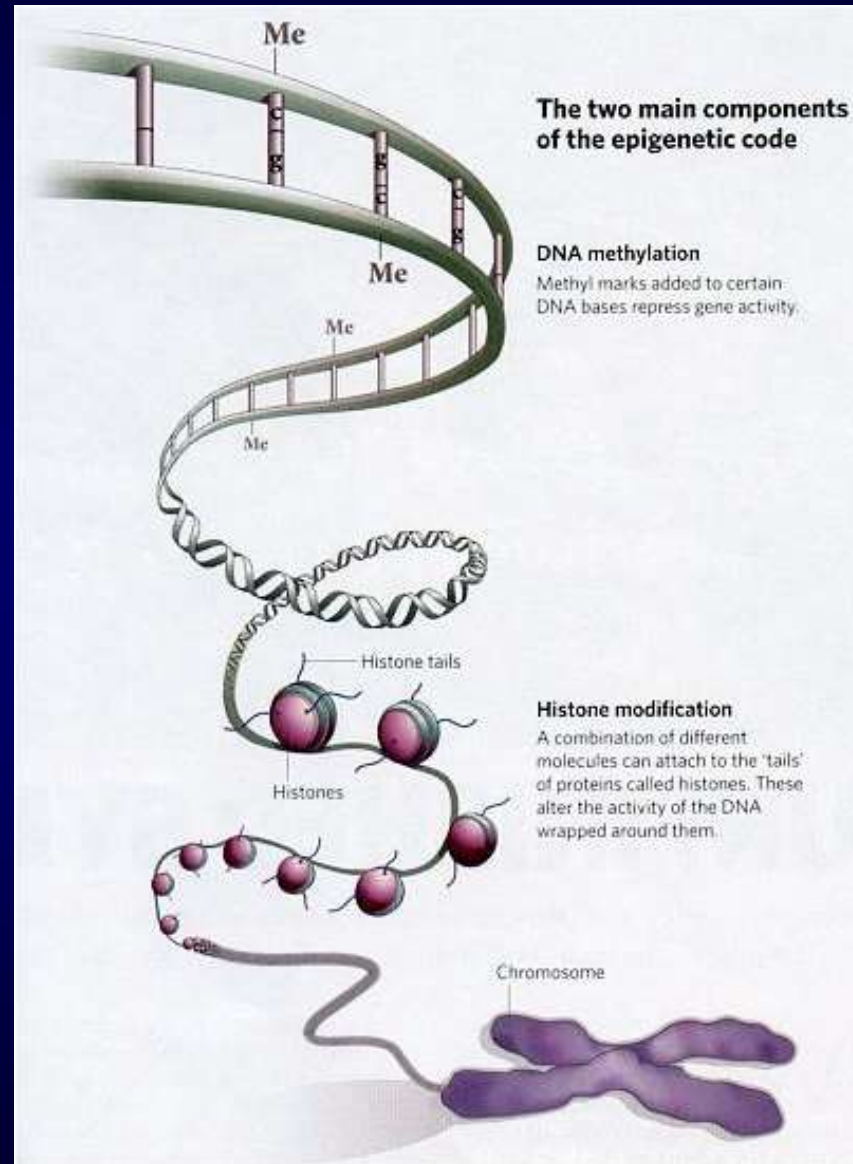
Epigenetické procesy probíhající v buněčných jádrech.

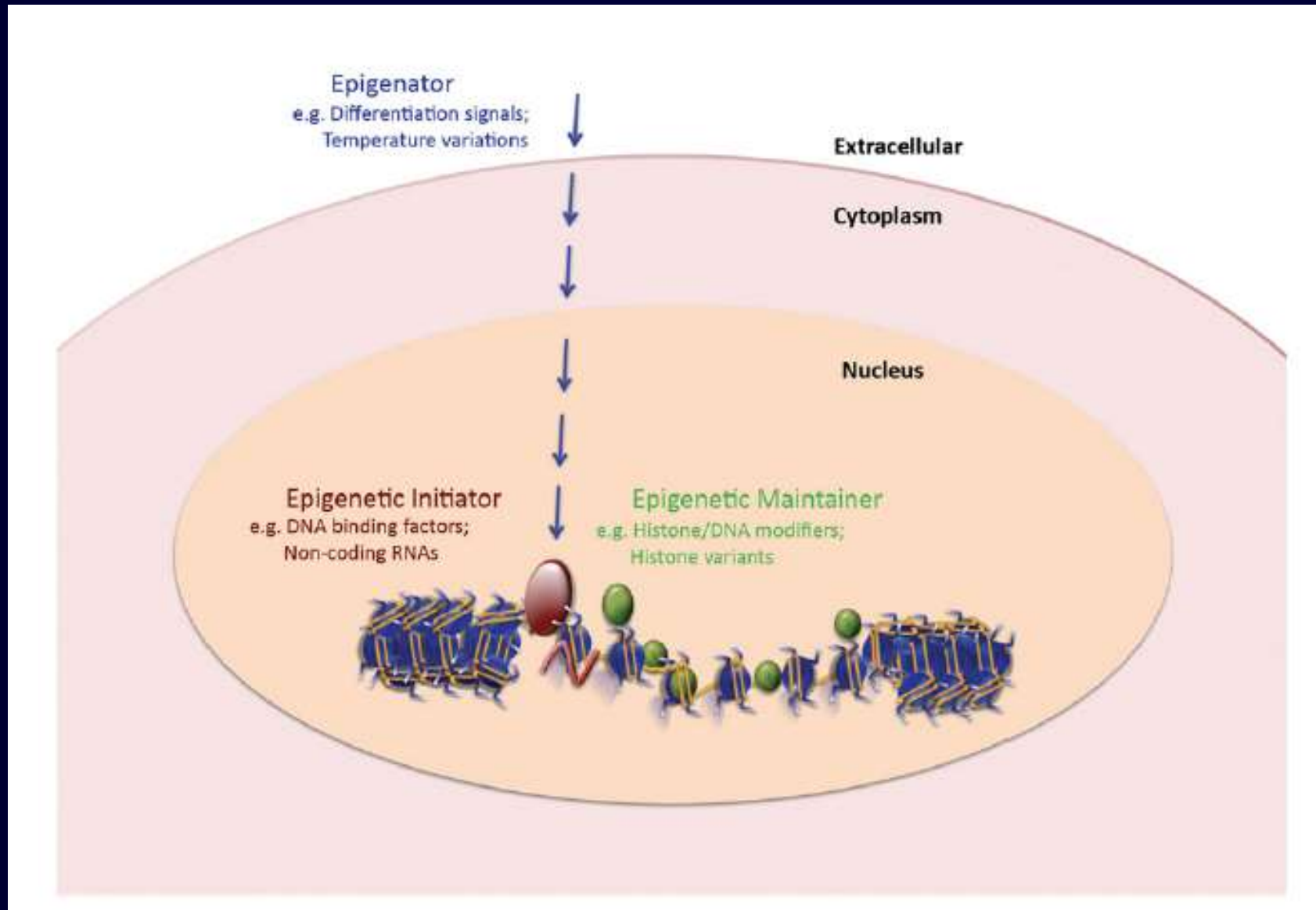


Eva Bártová
Biofyzikální ústav AV ČR Brno

What is epigenetics ?

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





Epigenator:

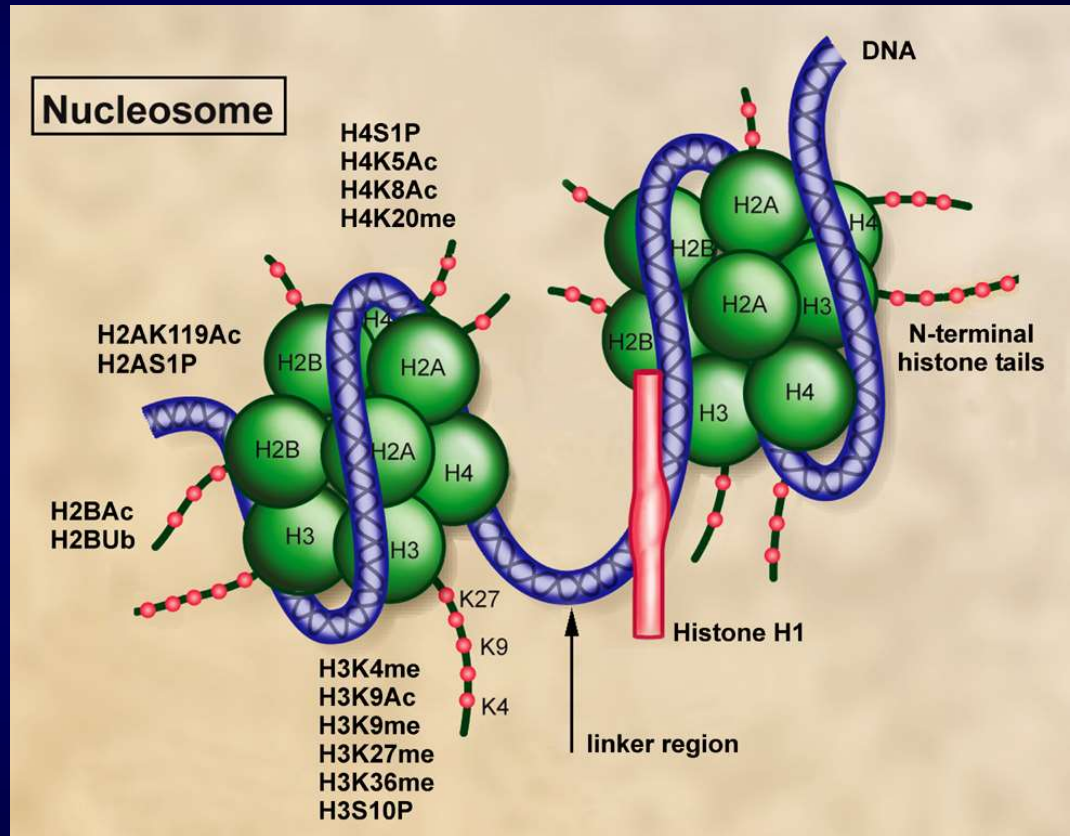
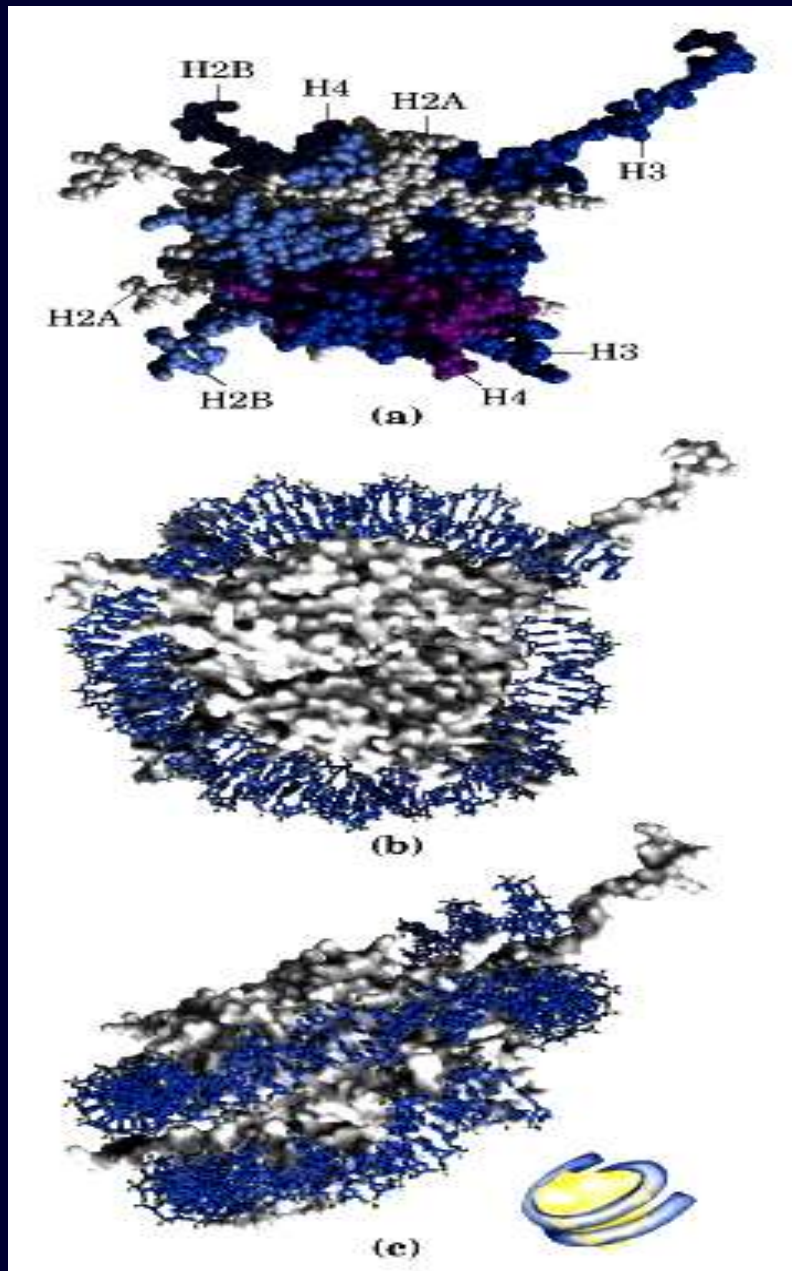
emanates from the environment and triggers and Intracellular pathway. Epigenetic signaling pathway could be a protein-protein interaction or modification-based events.

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)



jádro nukleosomu

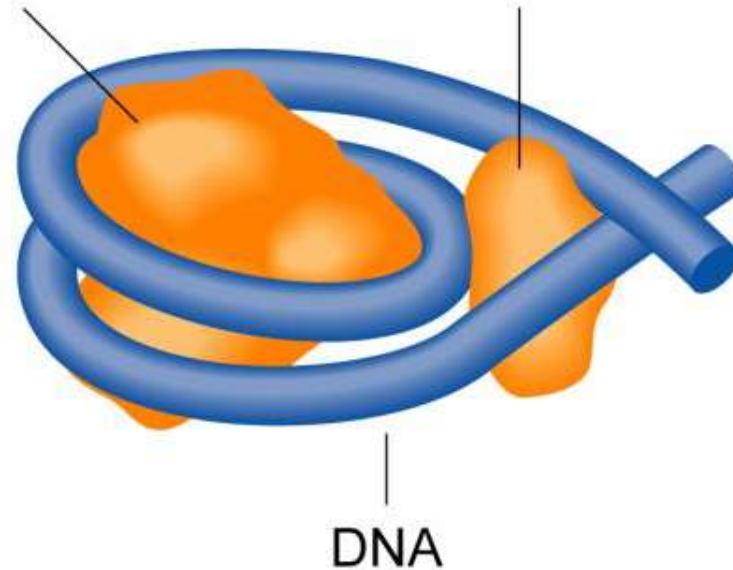
2x histon H2A

2x histon H2B

2x histon H3

2x histon H4

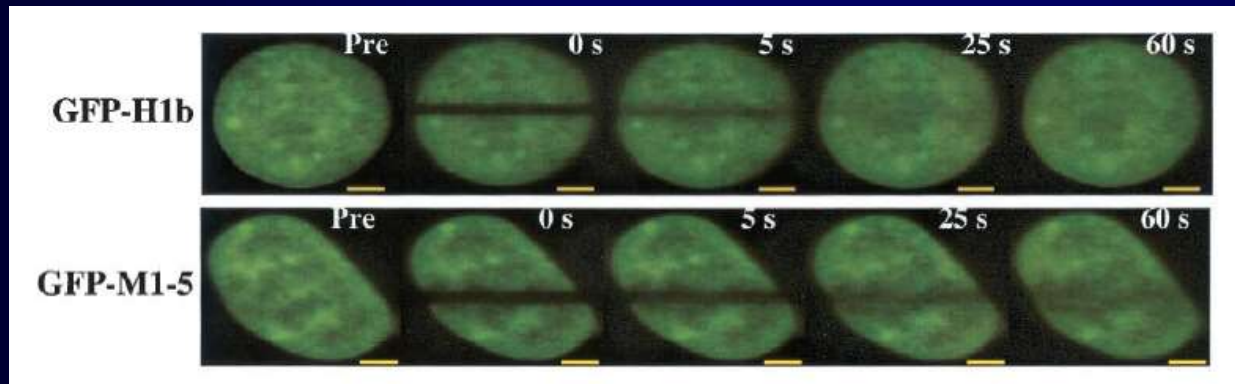
histon H1



1. **Buňka může existovat i bez významně redukováného 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**

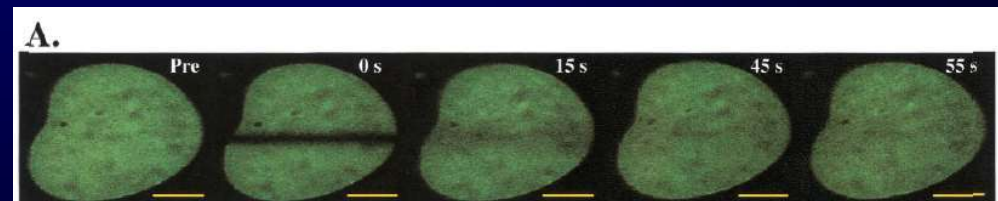
The linker histone H1 is involved in maintaining higher-order chromatin structures and displays dynamic nuclear mobility, which may be regulated by posttranslational modifications. H1 tail phosphorylation play in important role.

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 the mobility of GFP-H1.**

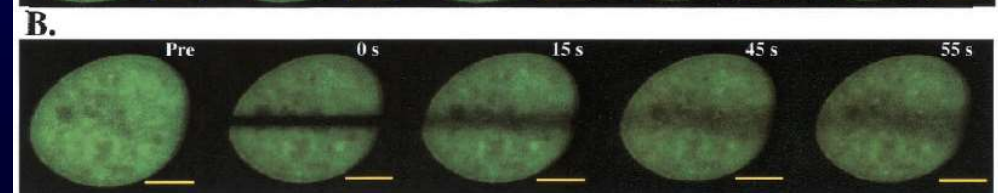


Overexpression p21

GFP-H1b



GFP-M1-5



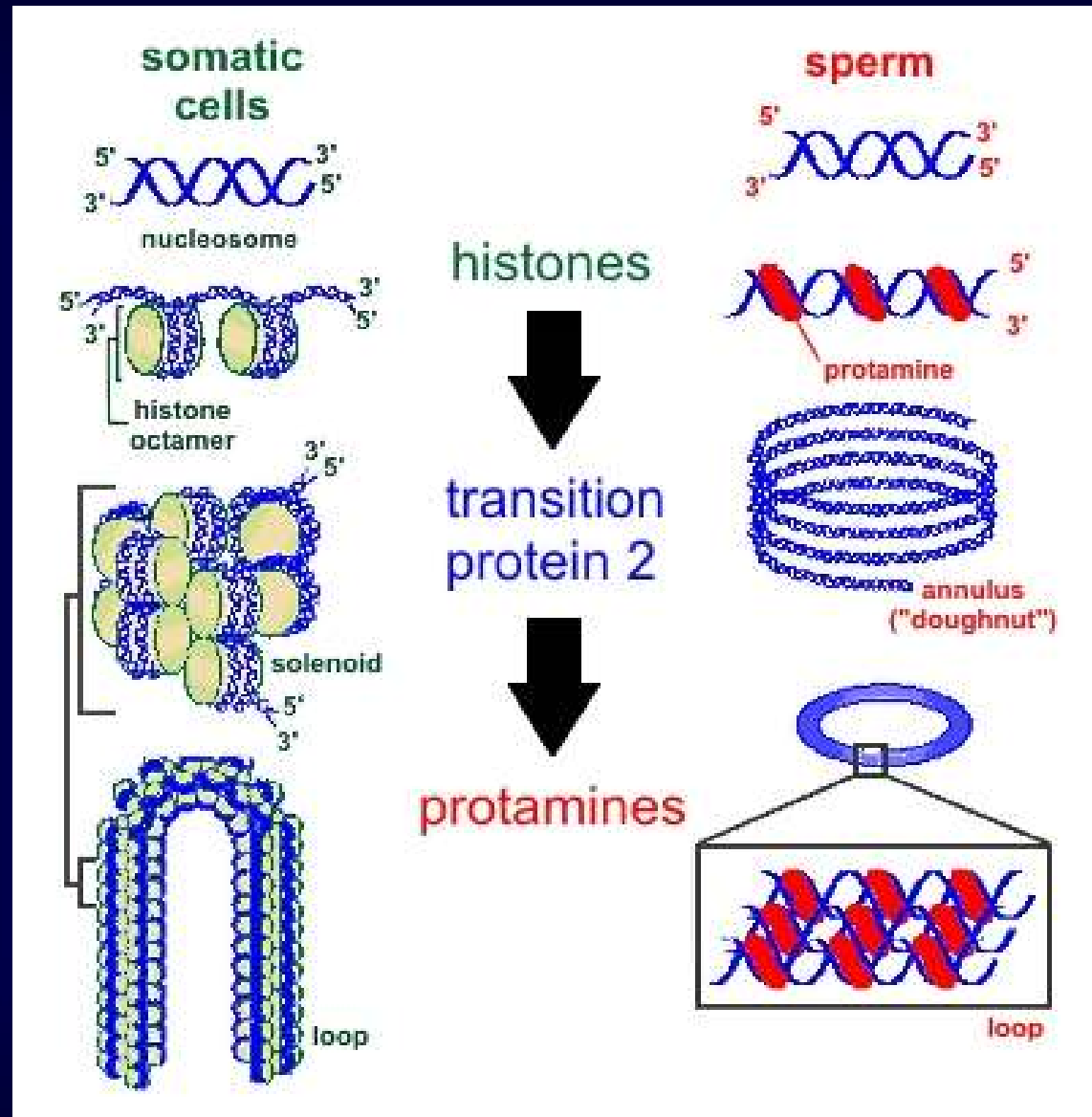
Varianty histonů

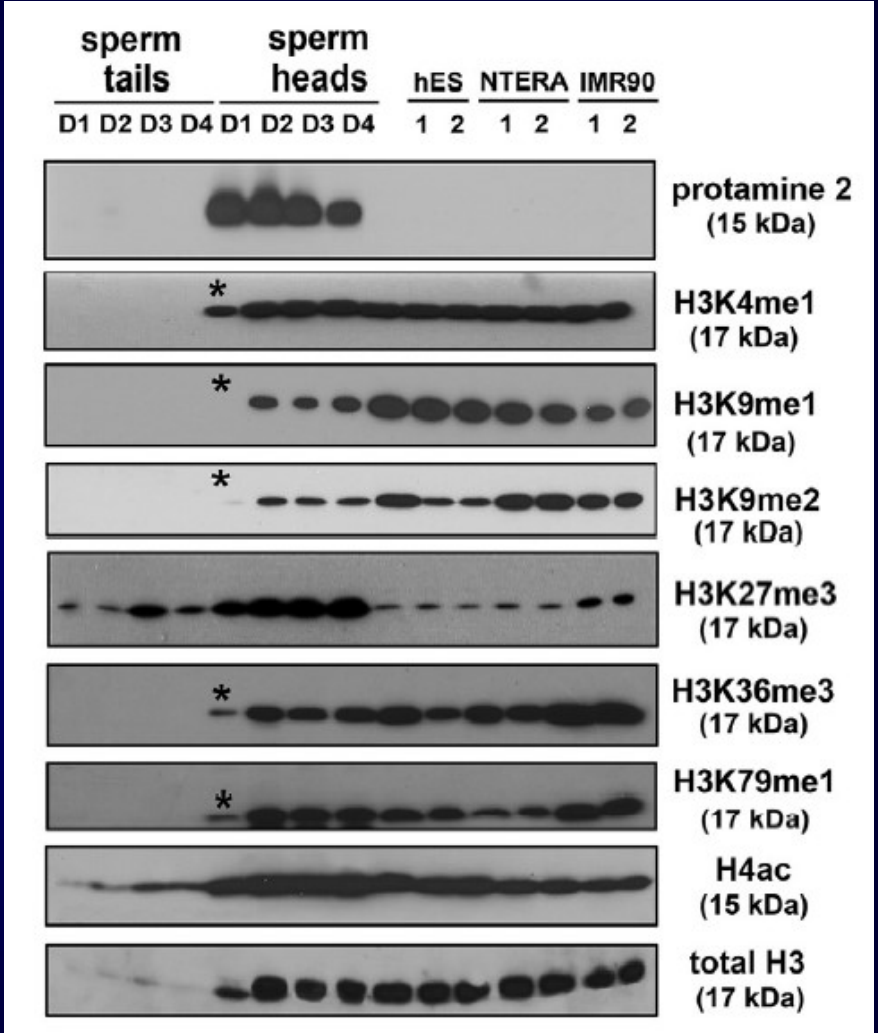
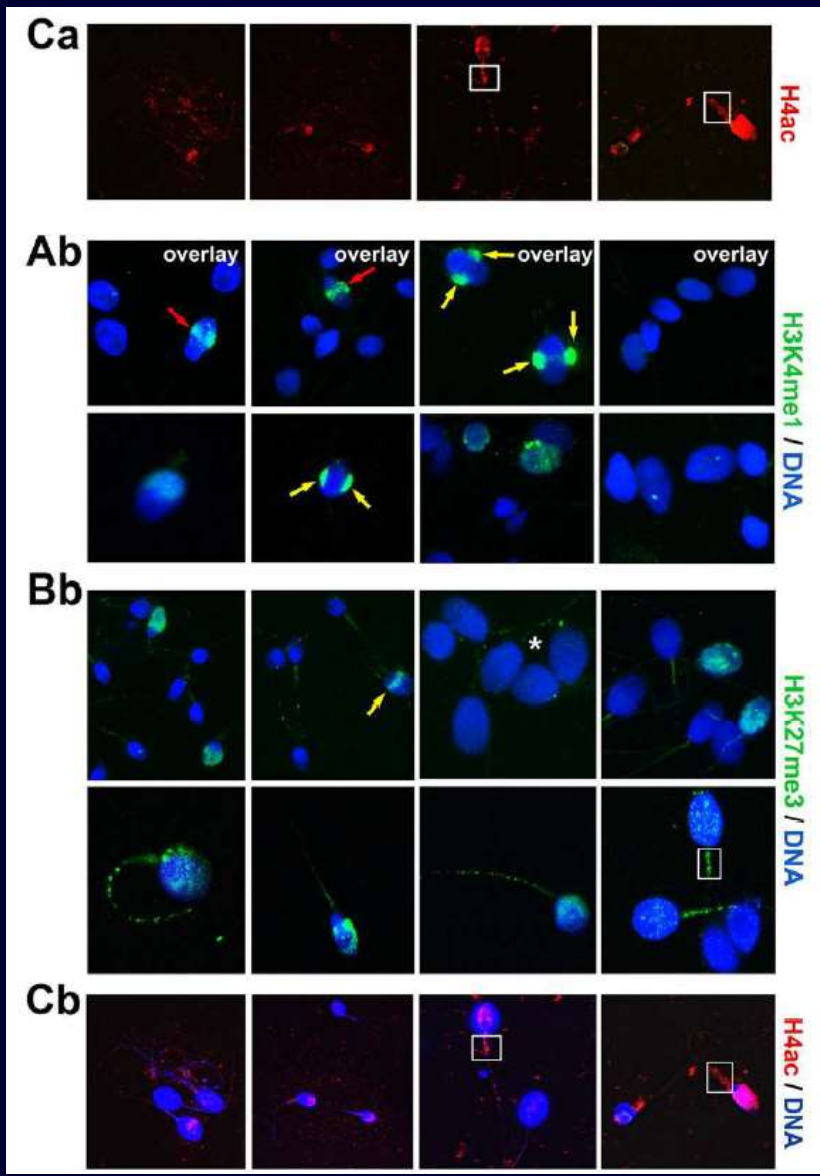
H1: varianty H1^o, H5 a testis-specific varianta H1. varianty H1 se různě uplatňují během buněčného cyklu, diferenciaci a vývoje. RA diferenciaci myších F9 je doprovázena zvýšenou transkripcí histonu H1^o.

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, represí transkripce a během gametogeneze, H2B je zodpovědný za uspořádání chromatinu u spermií – nahrazení histonů protaniny

.





Varianty histonů

H3: existují dvě hlavní

Varianty H3.3 a

centromerické varianty

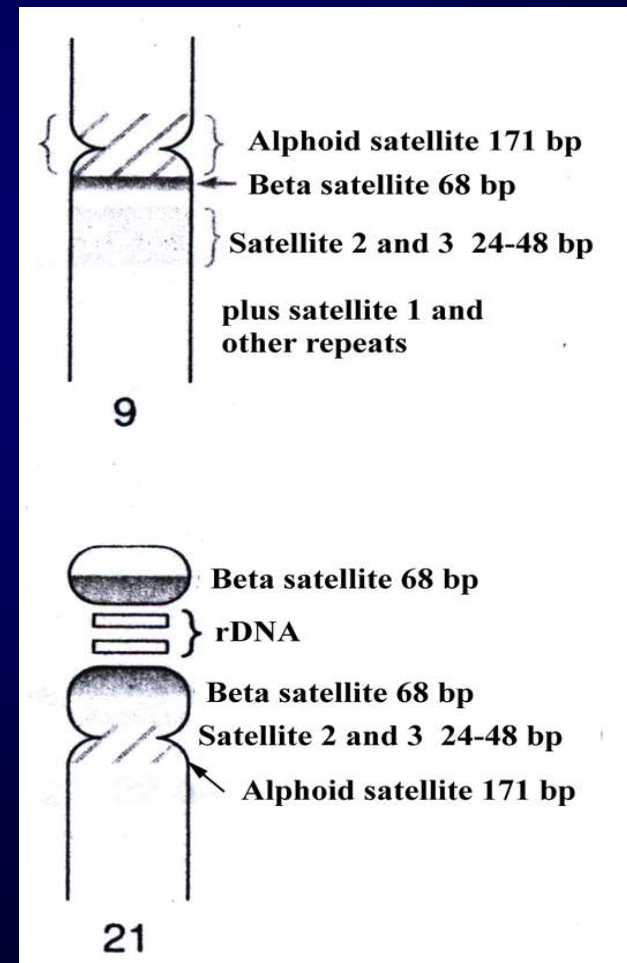
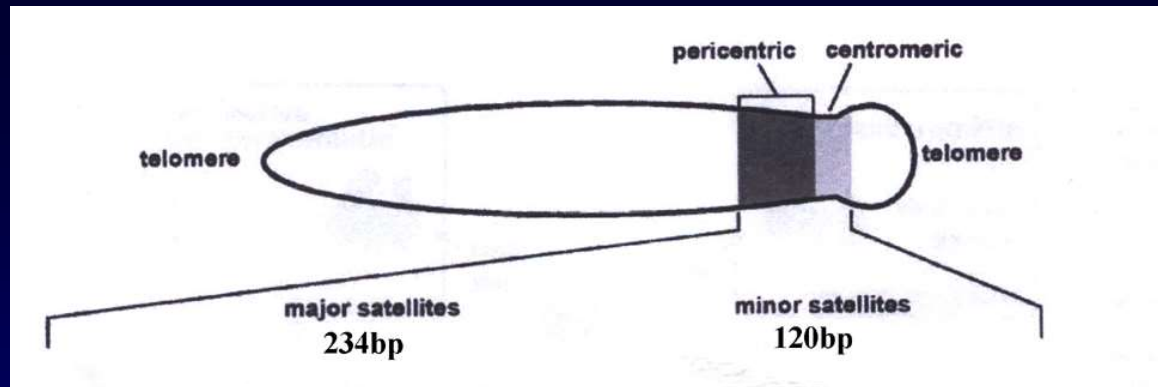
H3 (cenH3) = CENP-A-Z:

jsou zodpovědné za

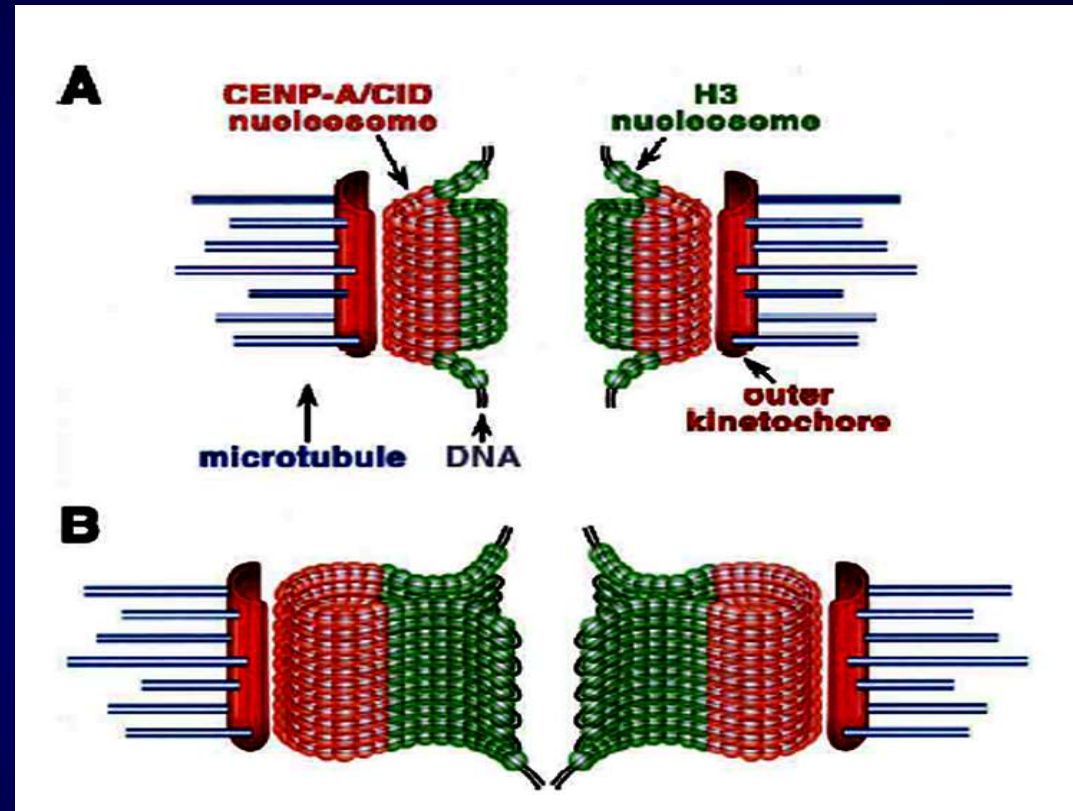
vazbu kinetochoru a

segregaci sesterských

chromatid u eukaryot

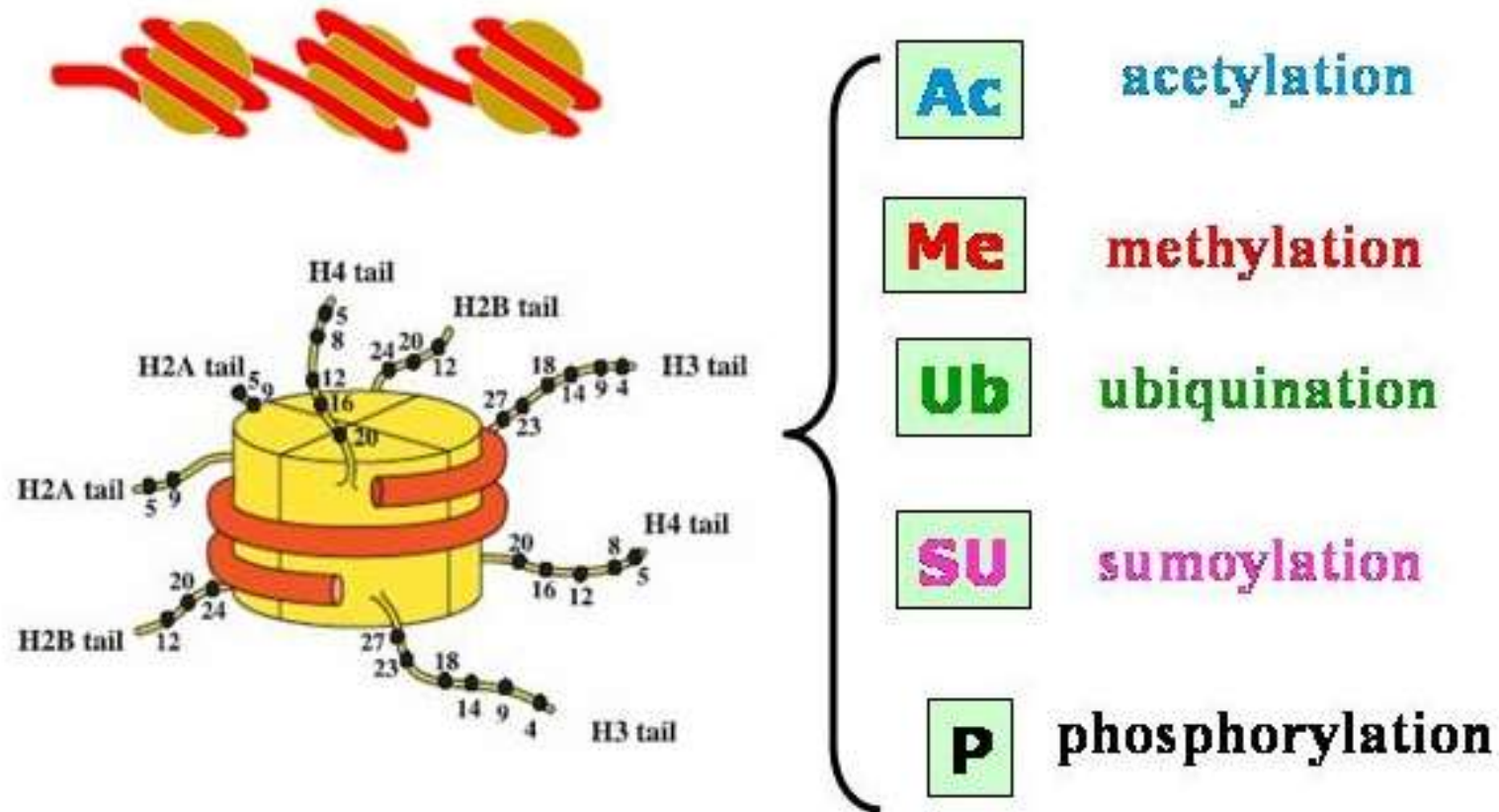


Varianty histonů H3: phosphorylation of CENP-A on Ser-7 is essential for kinetochore function. Overexpression of CENP-A plays an important role for aneuploidy in colorectal cancers.

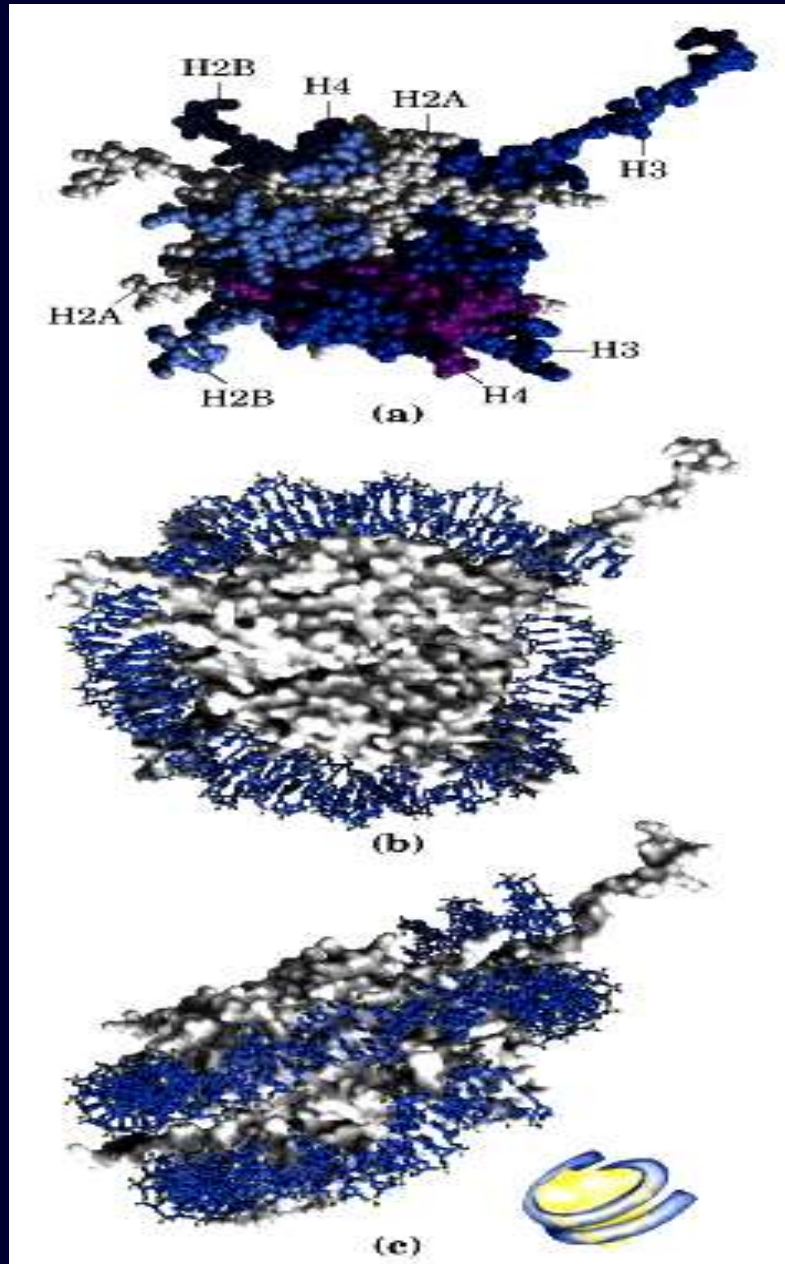


Varianty histonů H4: většina genů kódujících hlavní histonové proteiny jsou exprimovány během S fáze buněčného cyklu. V případě H4, geny jsou konstitutivně exprimovány během buněčného cyklu. Pro H4 nejsou známy žádné varianty. Úpravy pre-mRNA histonů probíhají v Cajal bodies.

Biochemické modifikace histonů



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: acylace histonů je katalyzována histon acetyltransferázami (HATs) a odstraňována histon deacetylázami (HDACs). HDACs odstraní acetyl skupinu, která je nahrazena methyl skupinou za účasti HMTs (Suv39H1- human, Clr4 – *S.pombe*)

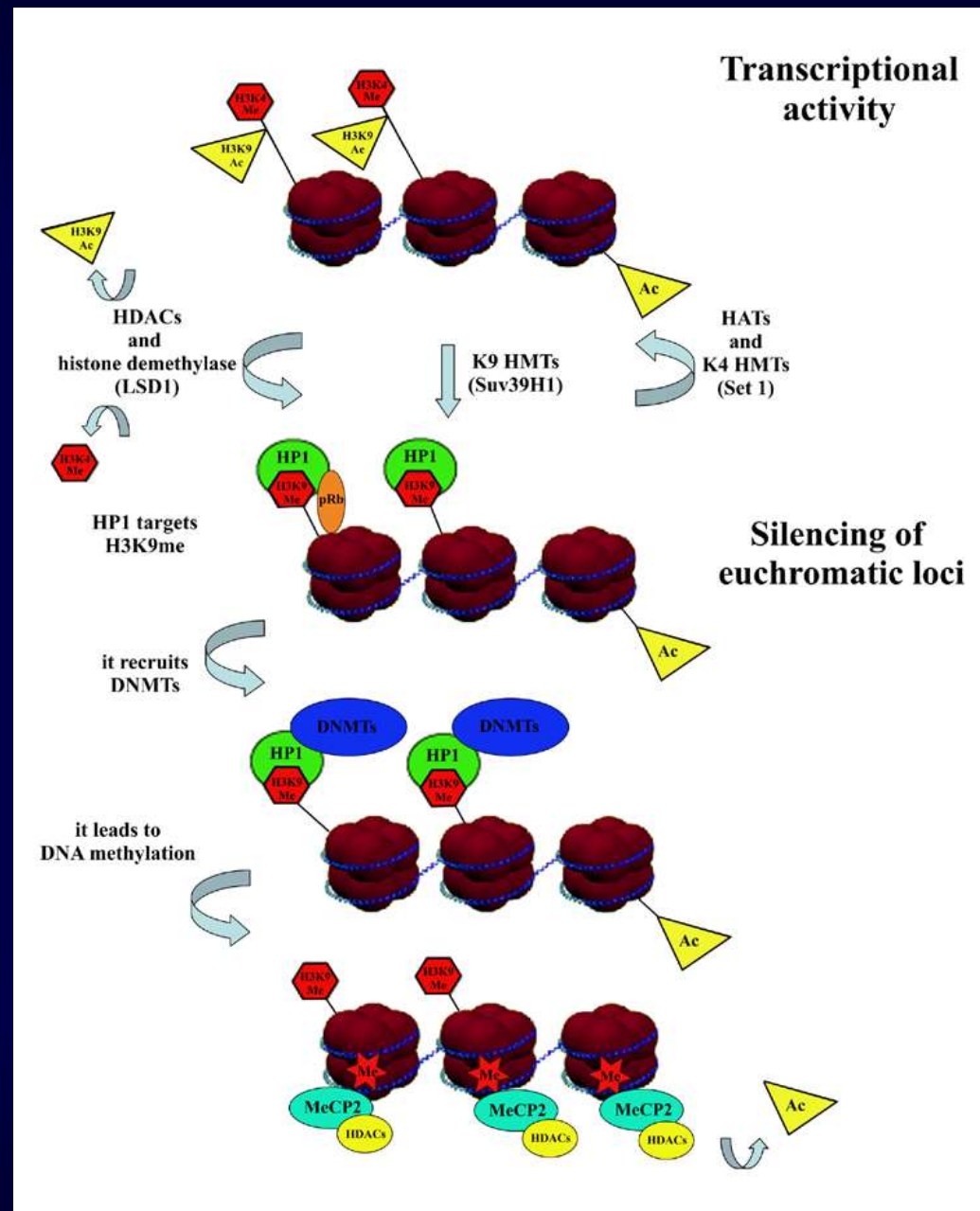
2004: Objev demethylace histonů za účasti aminové oxidasy **LSD1 (KIAA0601)** (Shi et al., Cell 2004). LSD1 specificky demethyluje H3 (K4), epigenetickou modifikaci zodpovědnou za transkripční aktivitu.

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

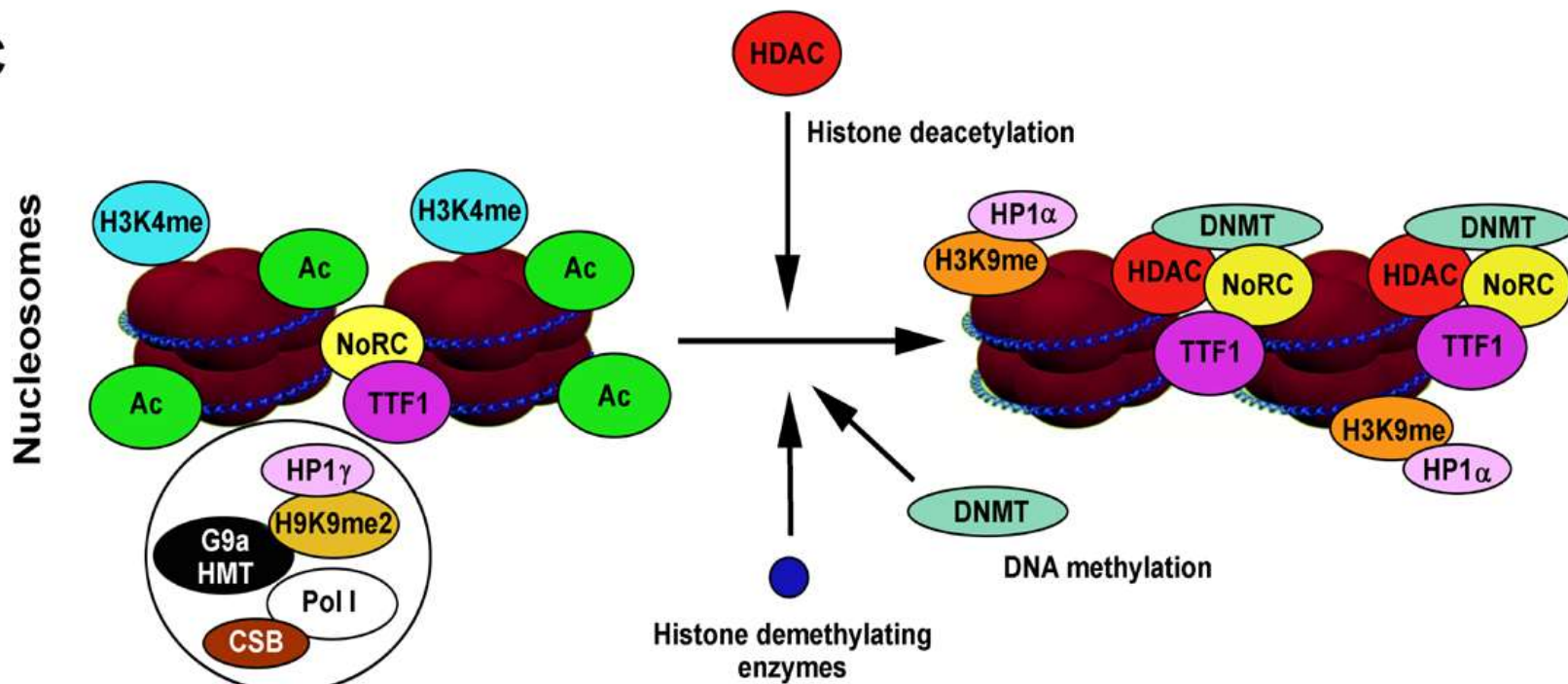
HDACs: Class I, II, III

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

**Demethylases: LSD1 (transcriptional activation),
JHD1b (H3K4me3), Jhd2b (H3K9me3), JHD2a, JMJ D2B
(heterochromatin formation)**



C

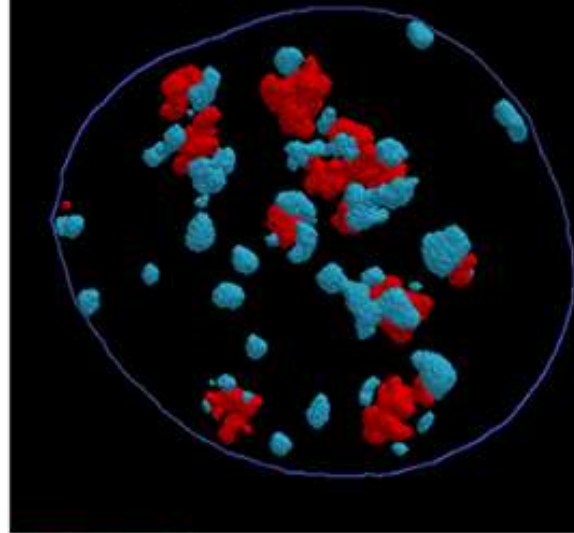


Transcription of rDNA genes

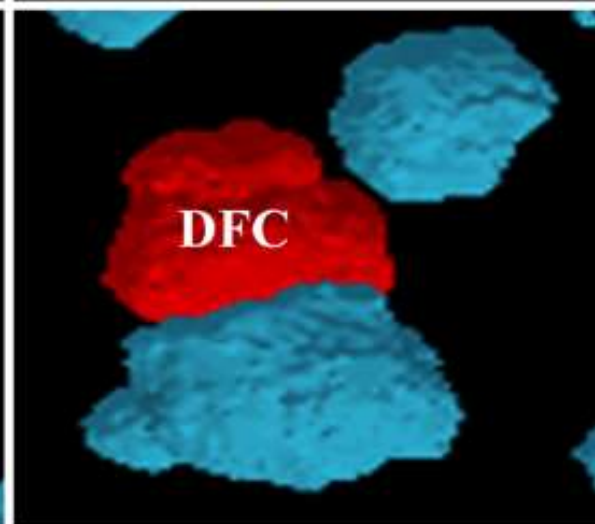
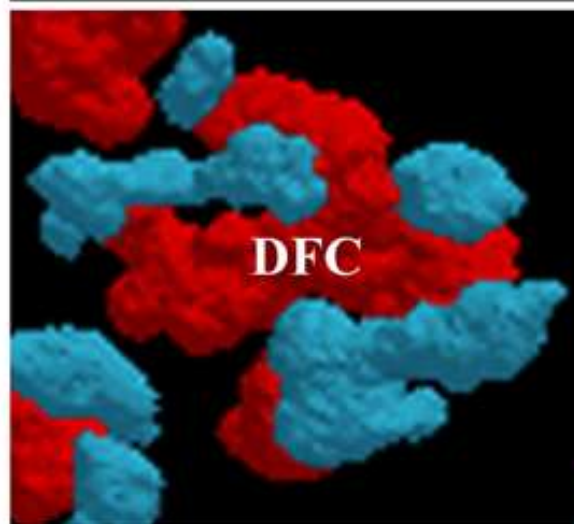
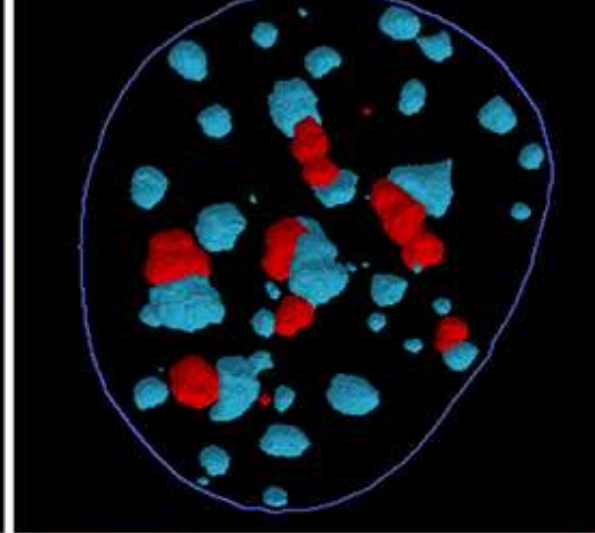
Silencing of rDNA genes

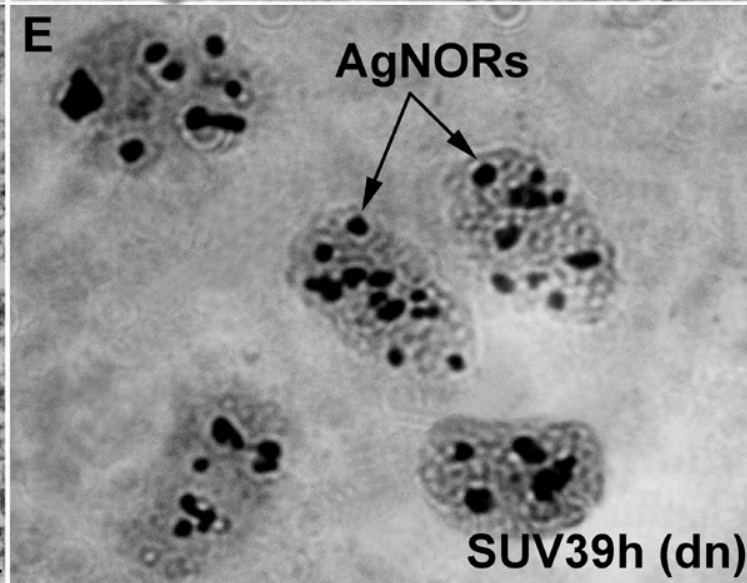
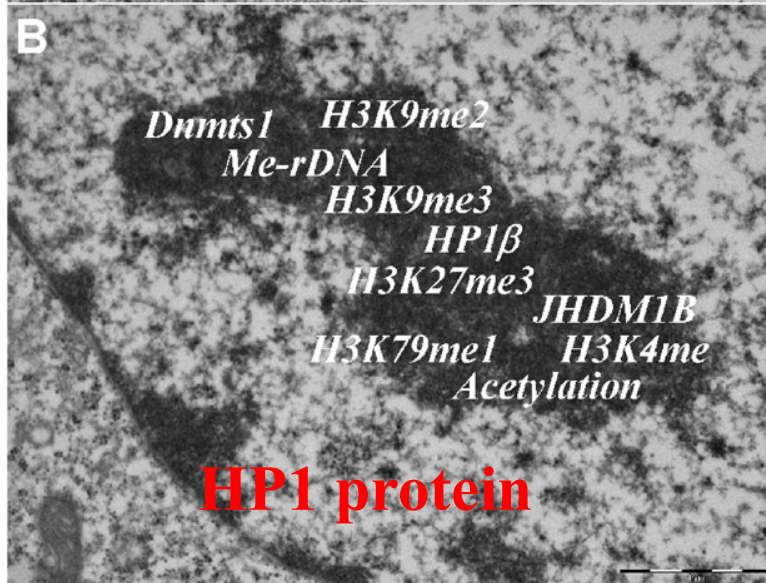
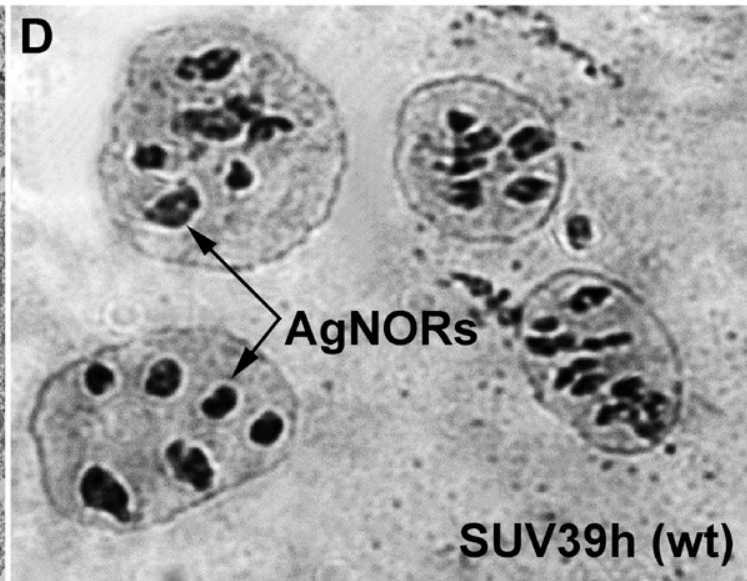
Fibrillarlin / Chromocenters

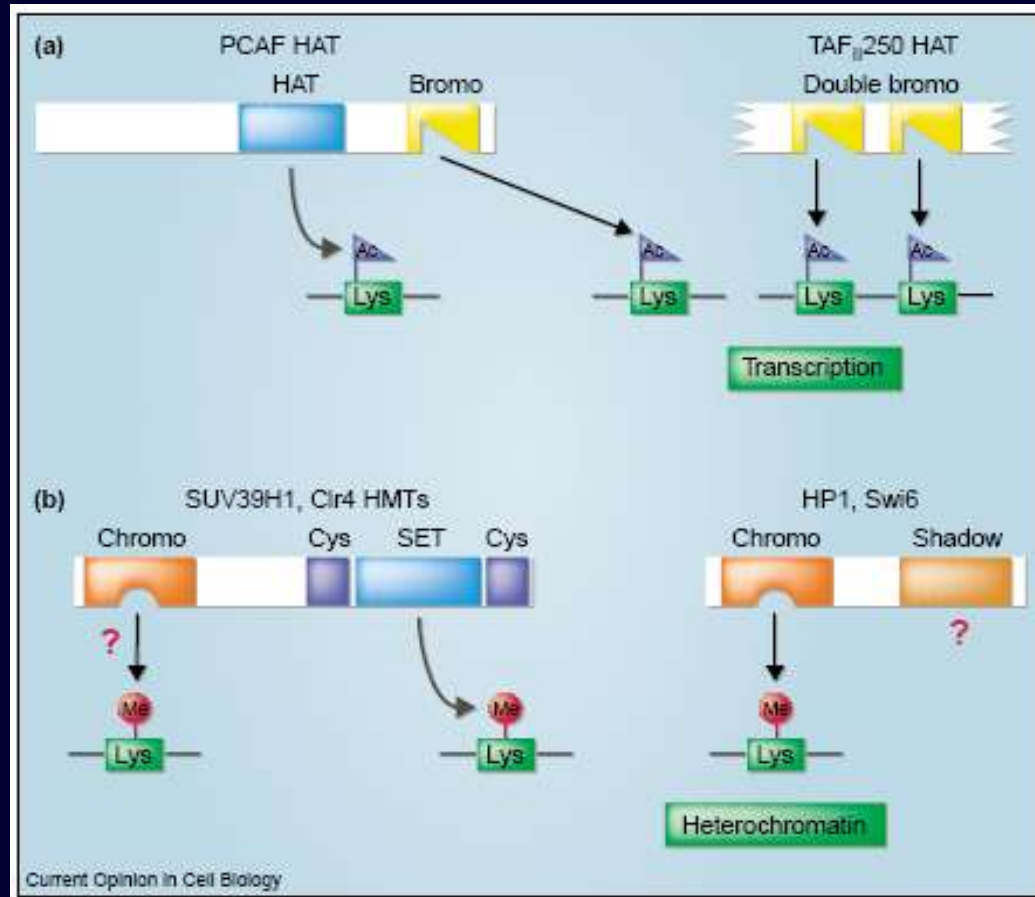
SUV39h (wt)



SUV39h (dn)







HP1 protein

CD: protein-chromatin

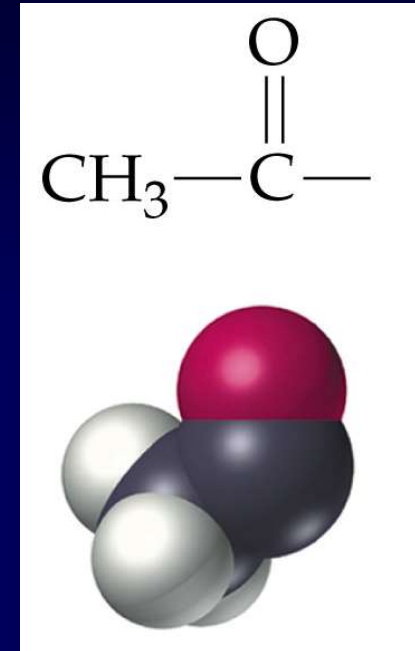
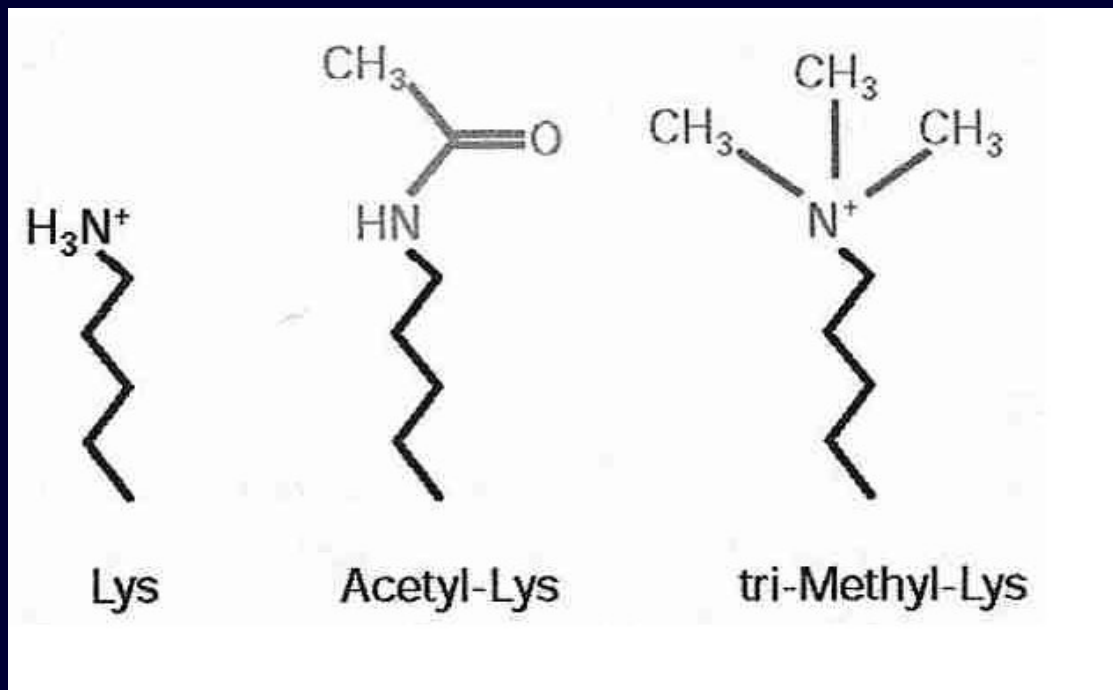
CSD: protein-protein

HD: HP1-to-DNA and linker histones

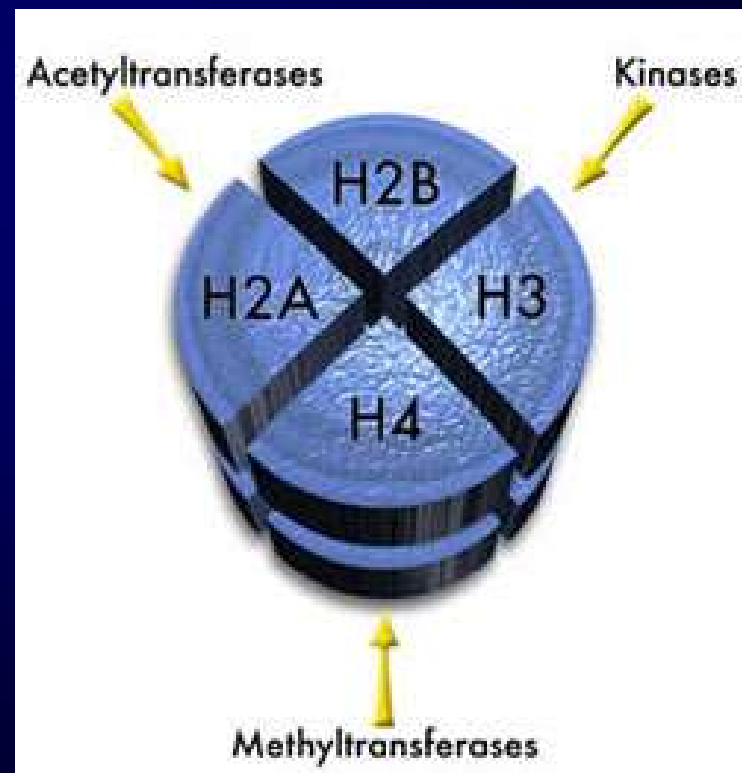
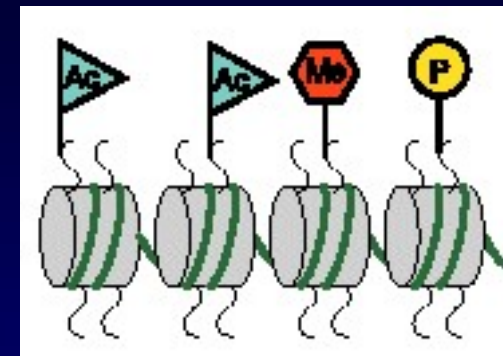
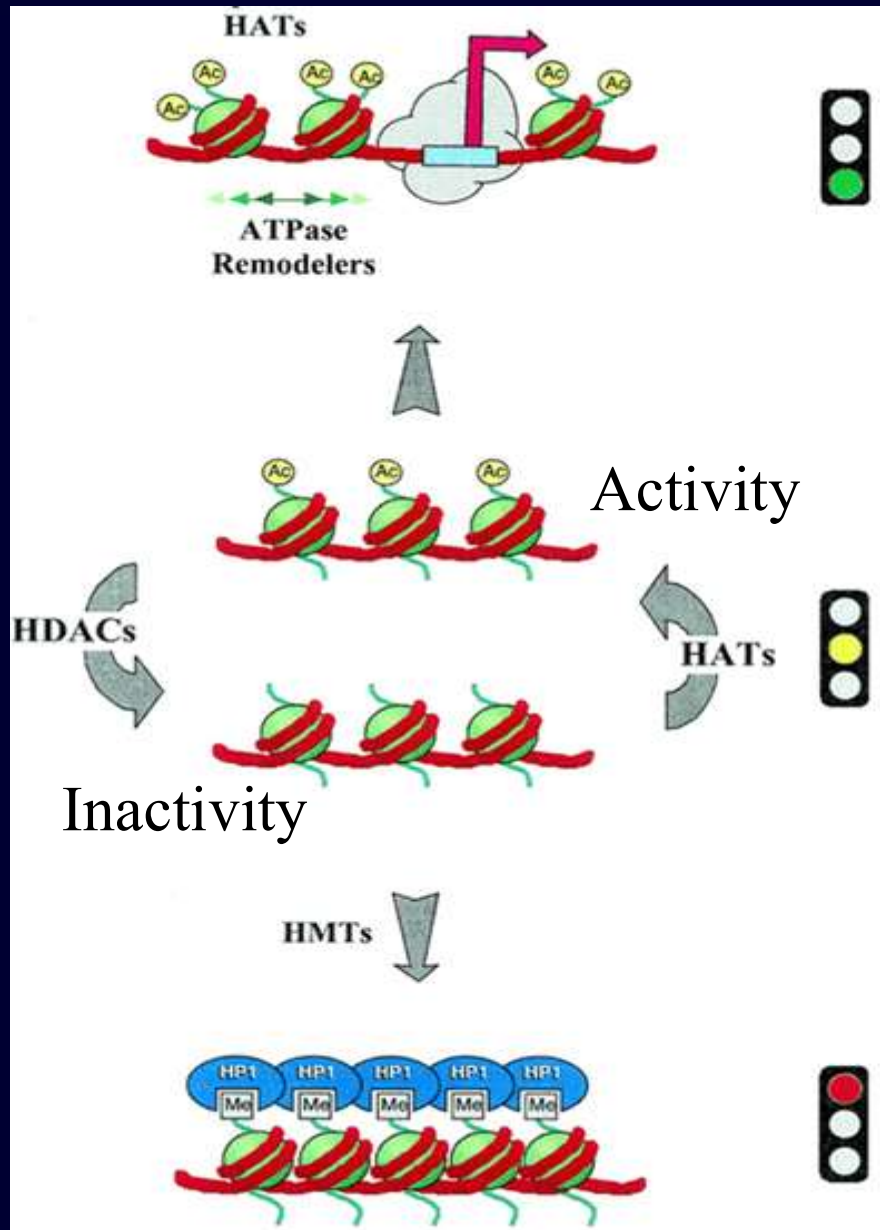
H3K9me1/me2/me3

HMTs:

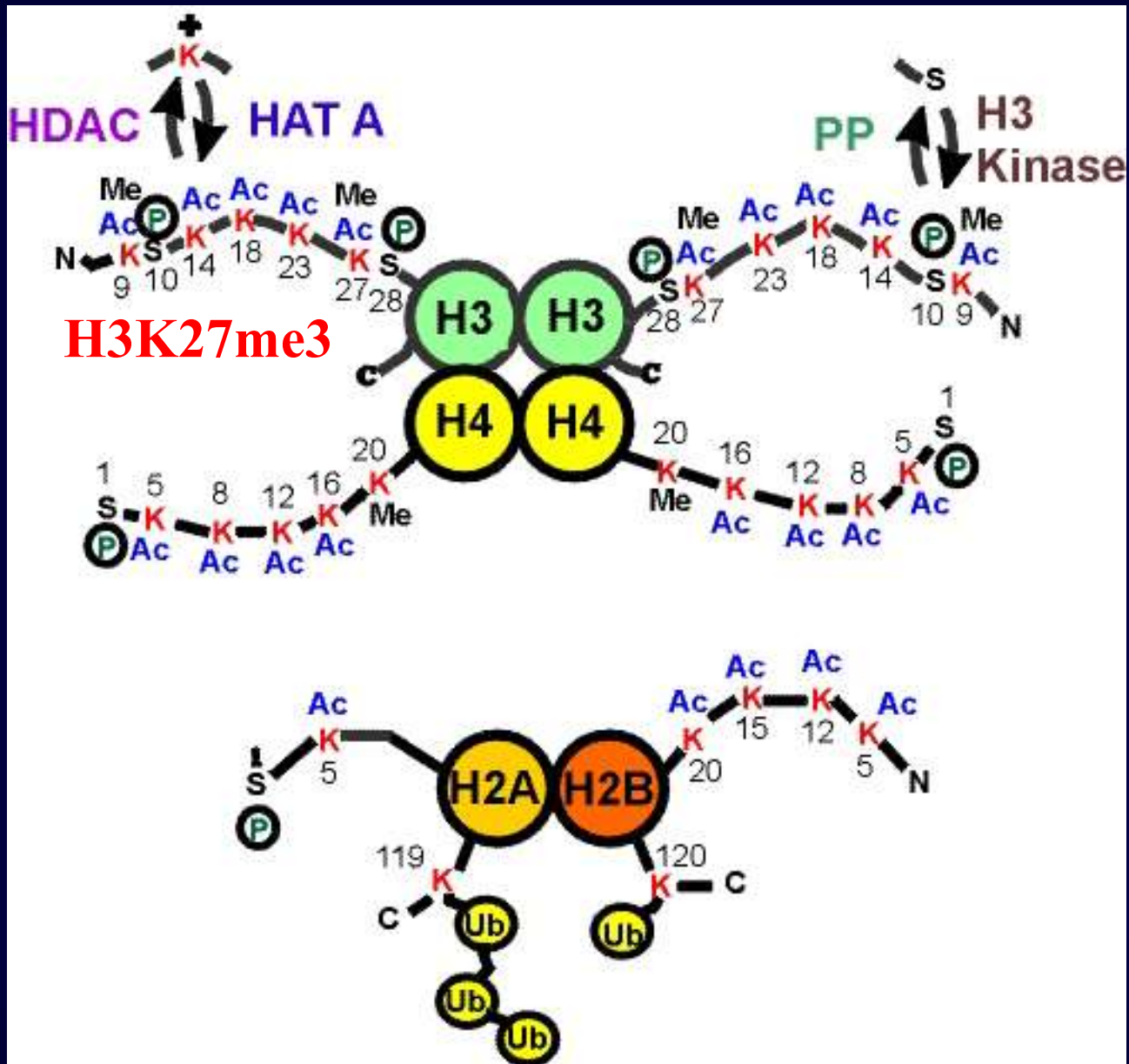
D. melanogaster: Su(var)3-9 je lokalizován v oblastech kondenzovaného chromatinu a je to klíčový regulátor v organizaci represivního chromatinu. Homolog u *S. pombe* je Clr4 umyší SUV39h1 a u lidských buněk SUV39H1. Tyto HMTs specificky methylojí H3(K9).



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



Ikaros, Helios



Inaktivace X chromosomu ve vztahu k epigenetickým modifikacím

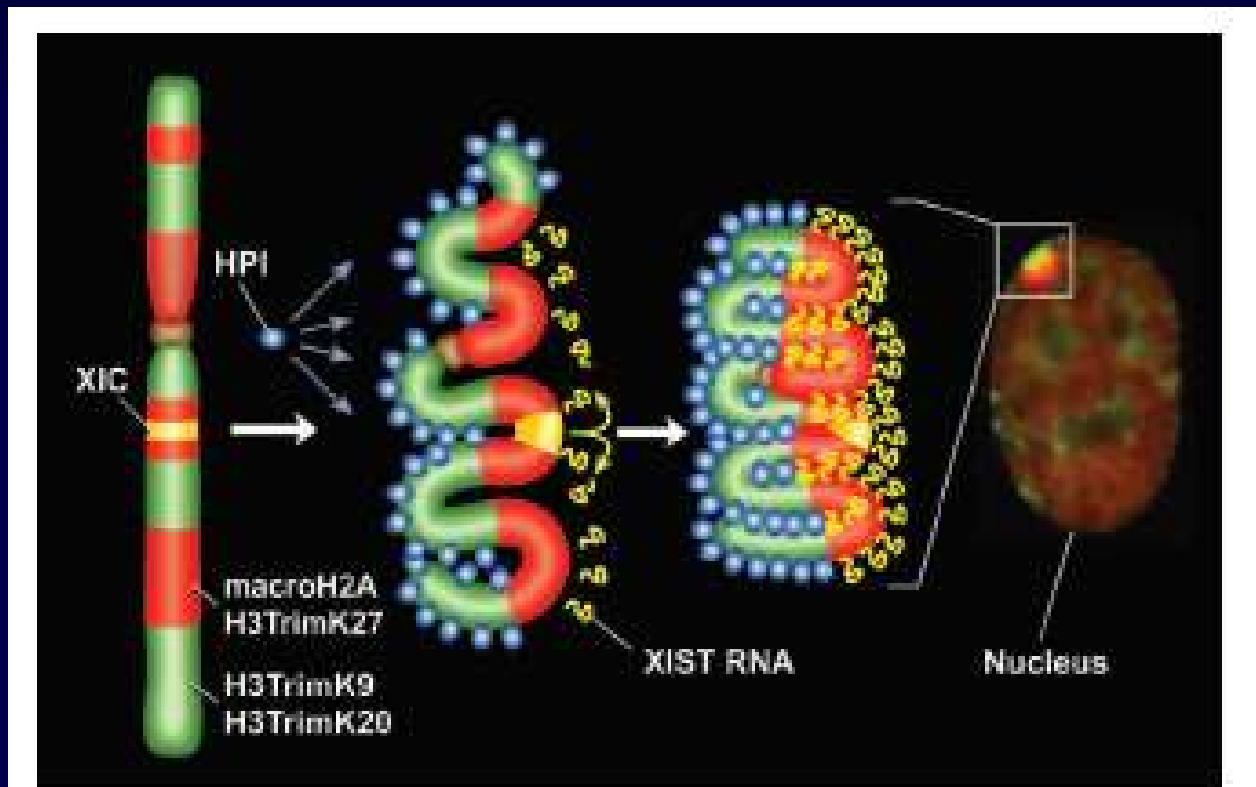
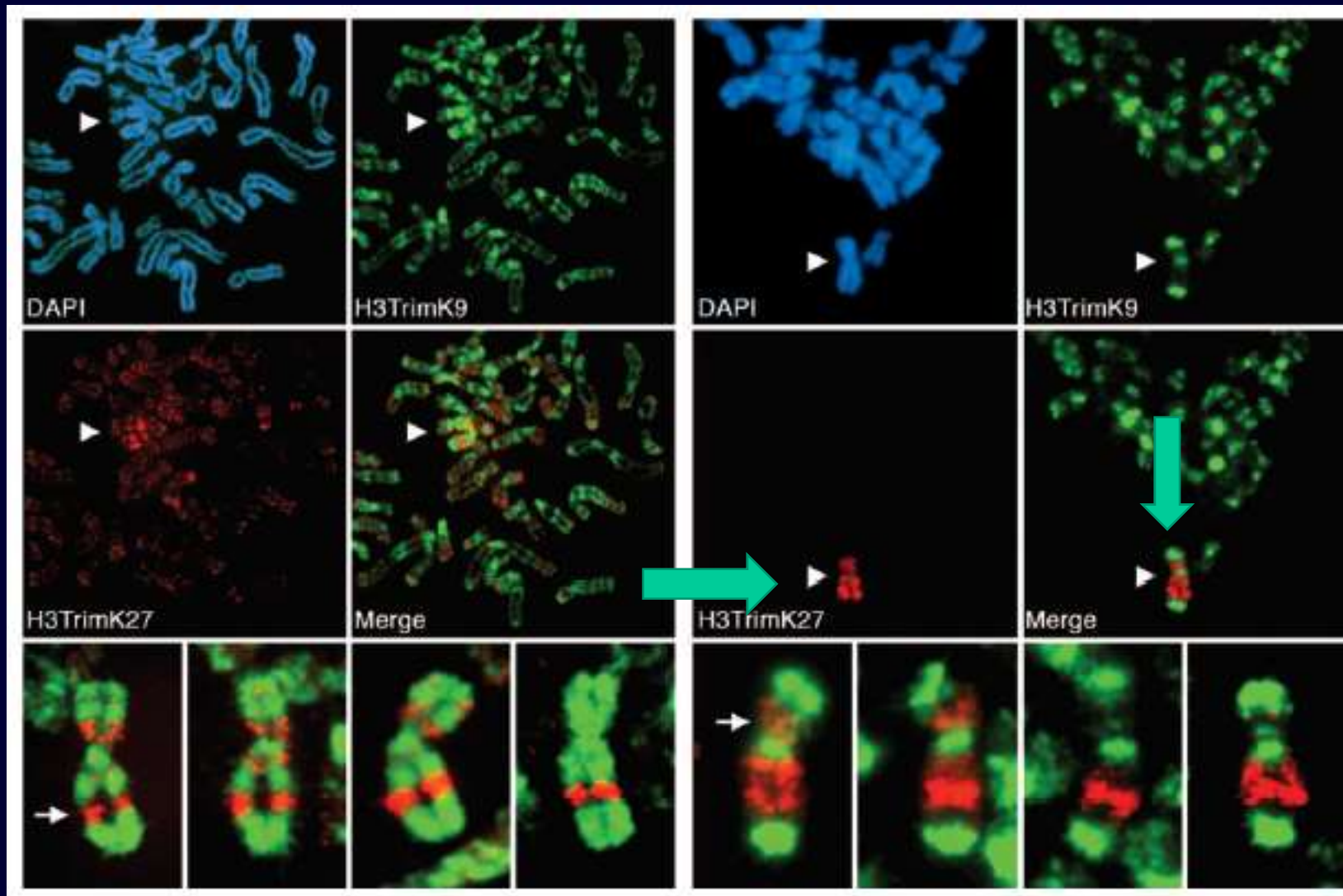
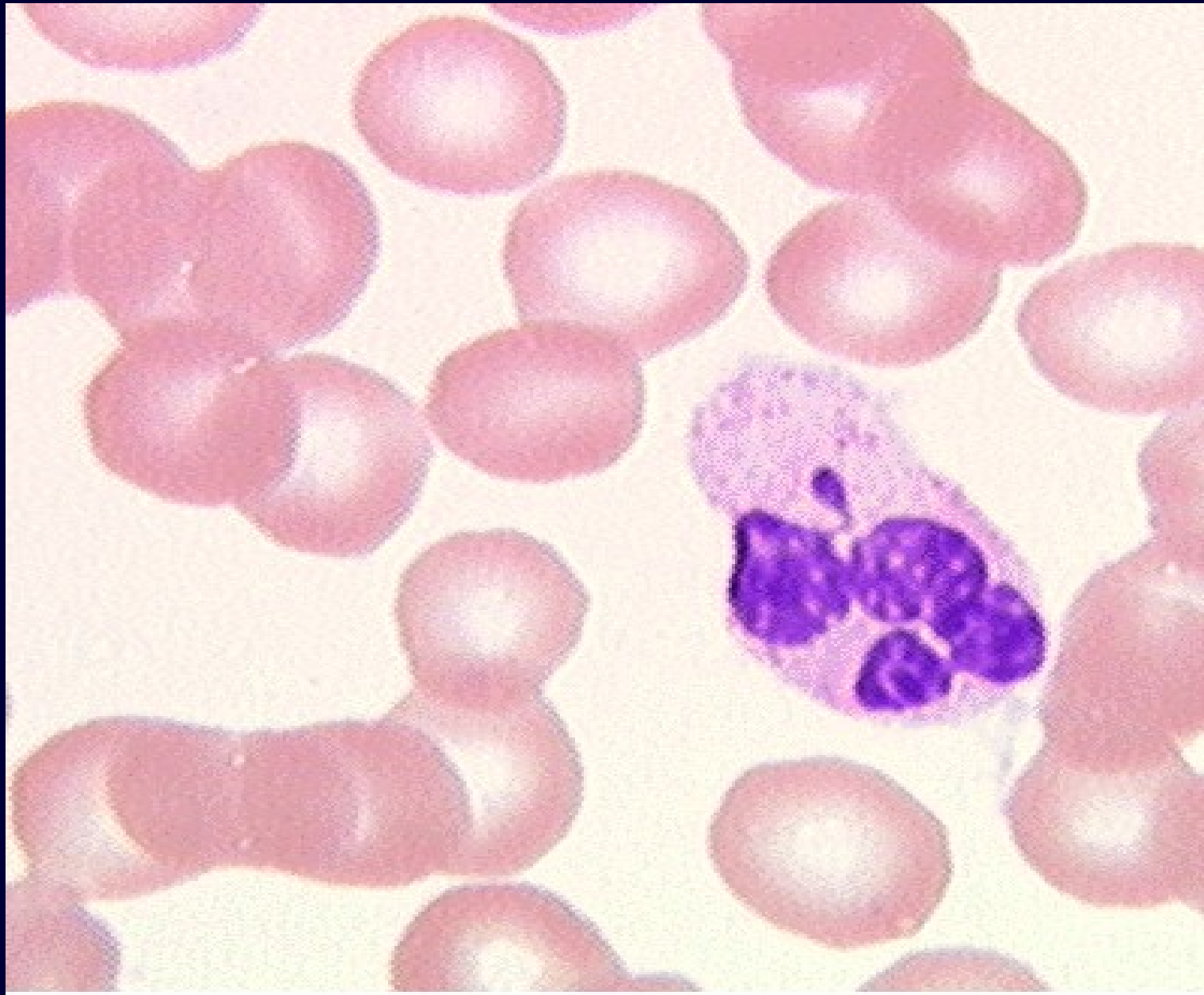


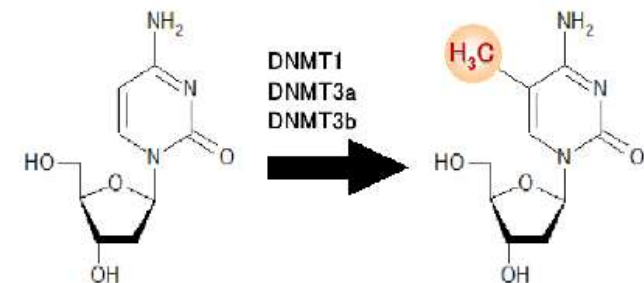
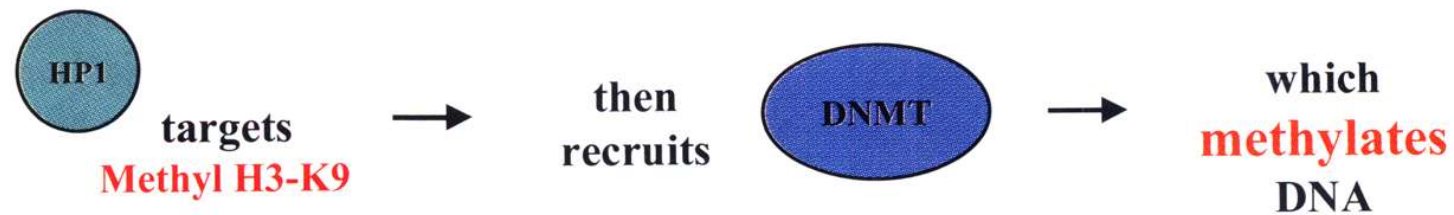
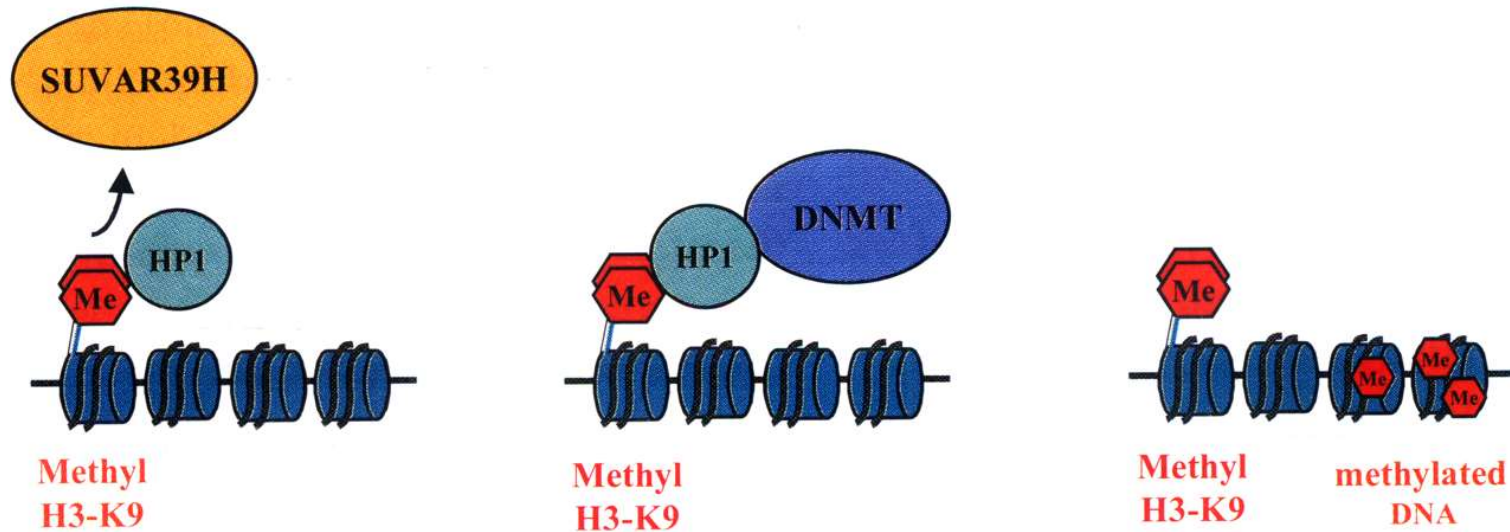
Fig. 4. Schematic model showing how heterochromatin of the Xi could transition between metaphase and interphase to be organized into the two nonoverlapping heterochromatin territories and to explain how XIST RNA could rapidly spread in cis outward from the X inactivation center (XIC) along only part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

Inaktivace X chromosomu ve vztahu k epigenetickým modifikacím

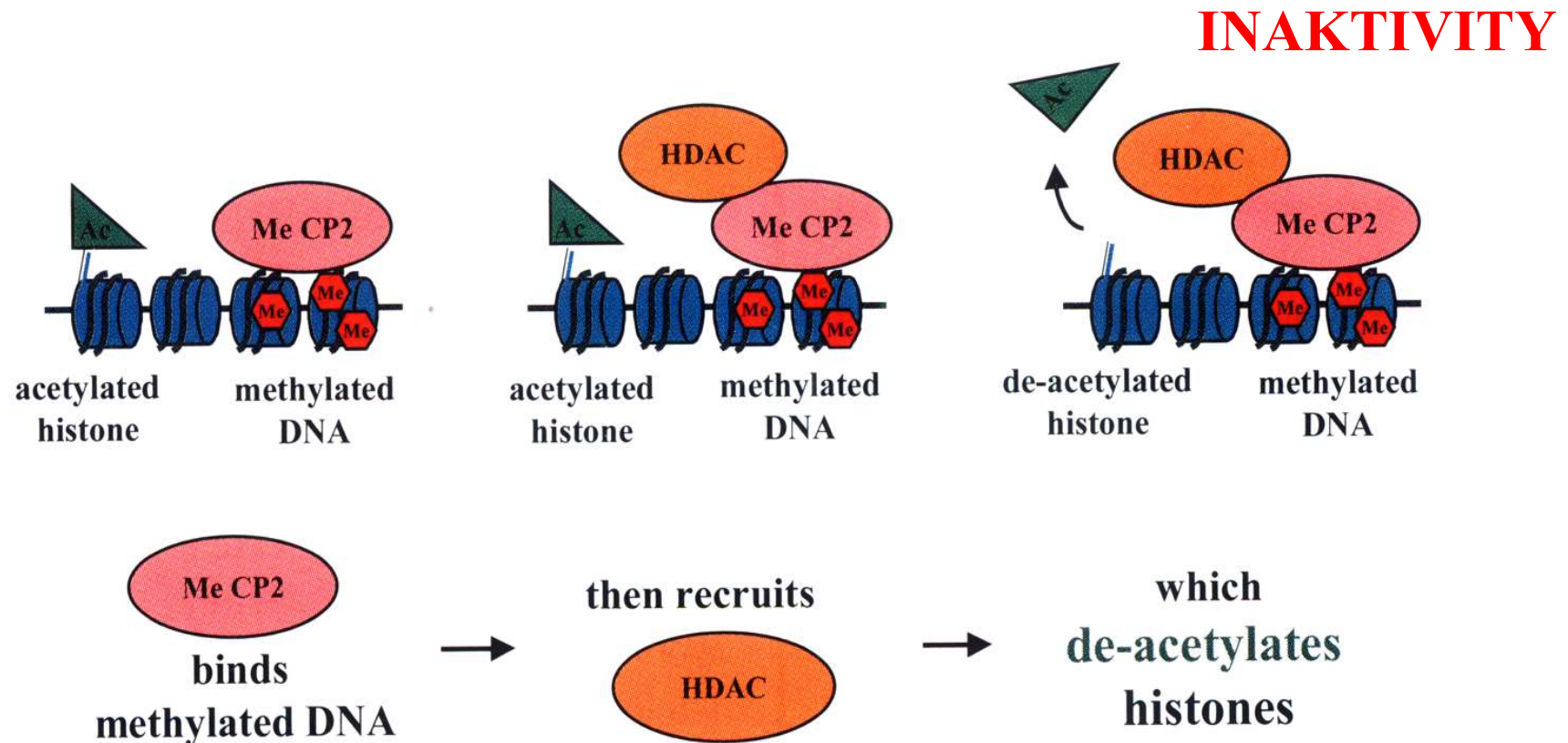




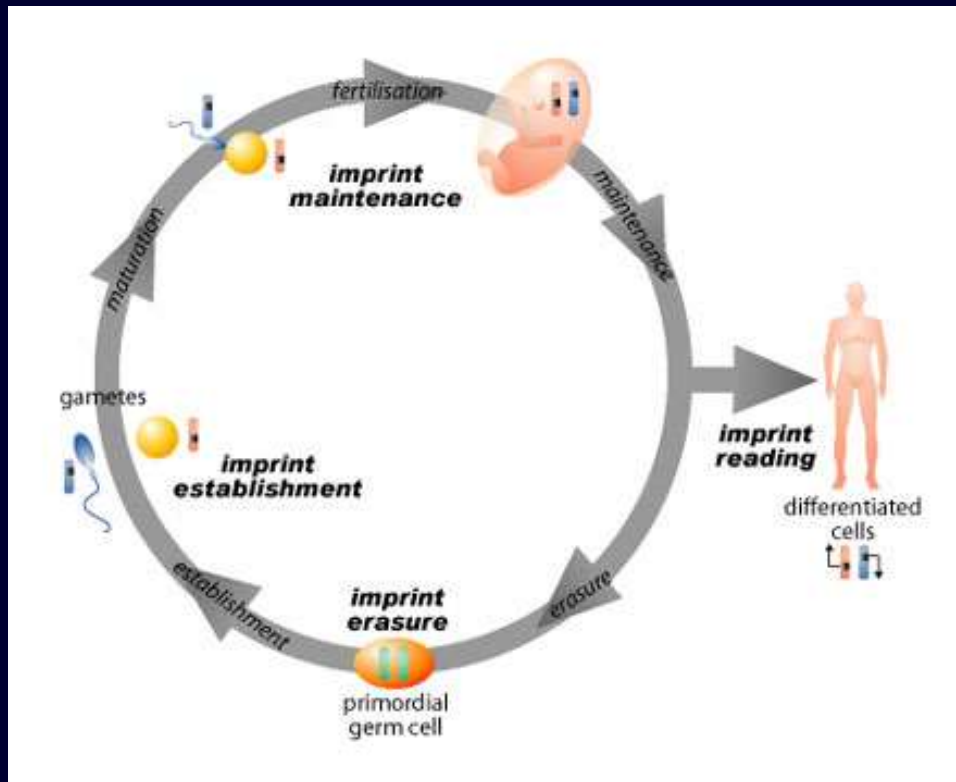
Histone H3-K9 methylation induces DNA methylation



DNA methylation induces histone de-acetylation



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

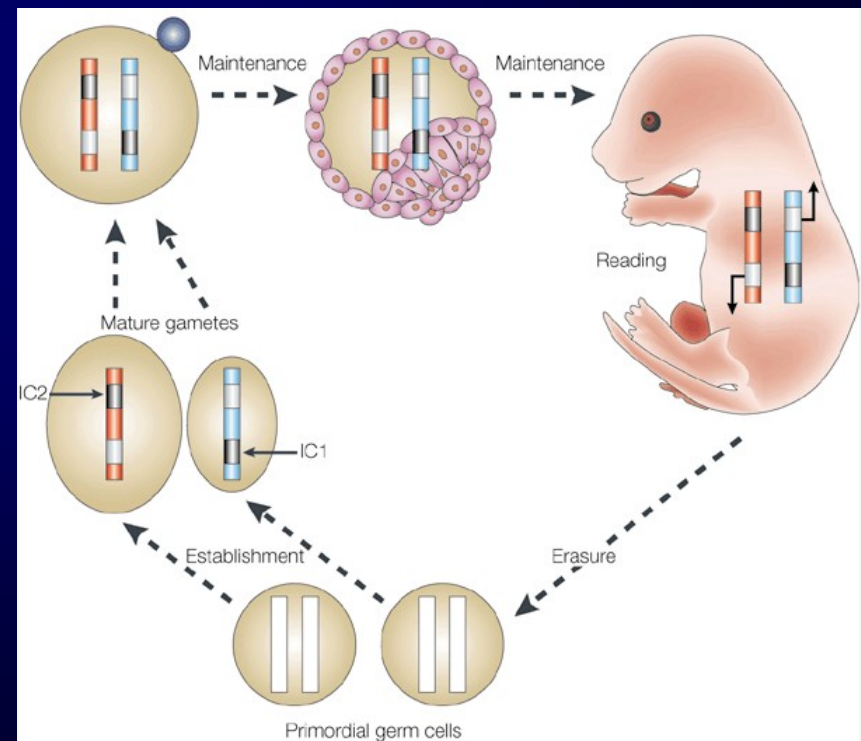


IMPRINTING

Myši embryo: samičí alela je zamethylována, nevyjadřuje se

Dospělý jedinec: obě alely jsou demethylovány

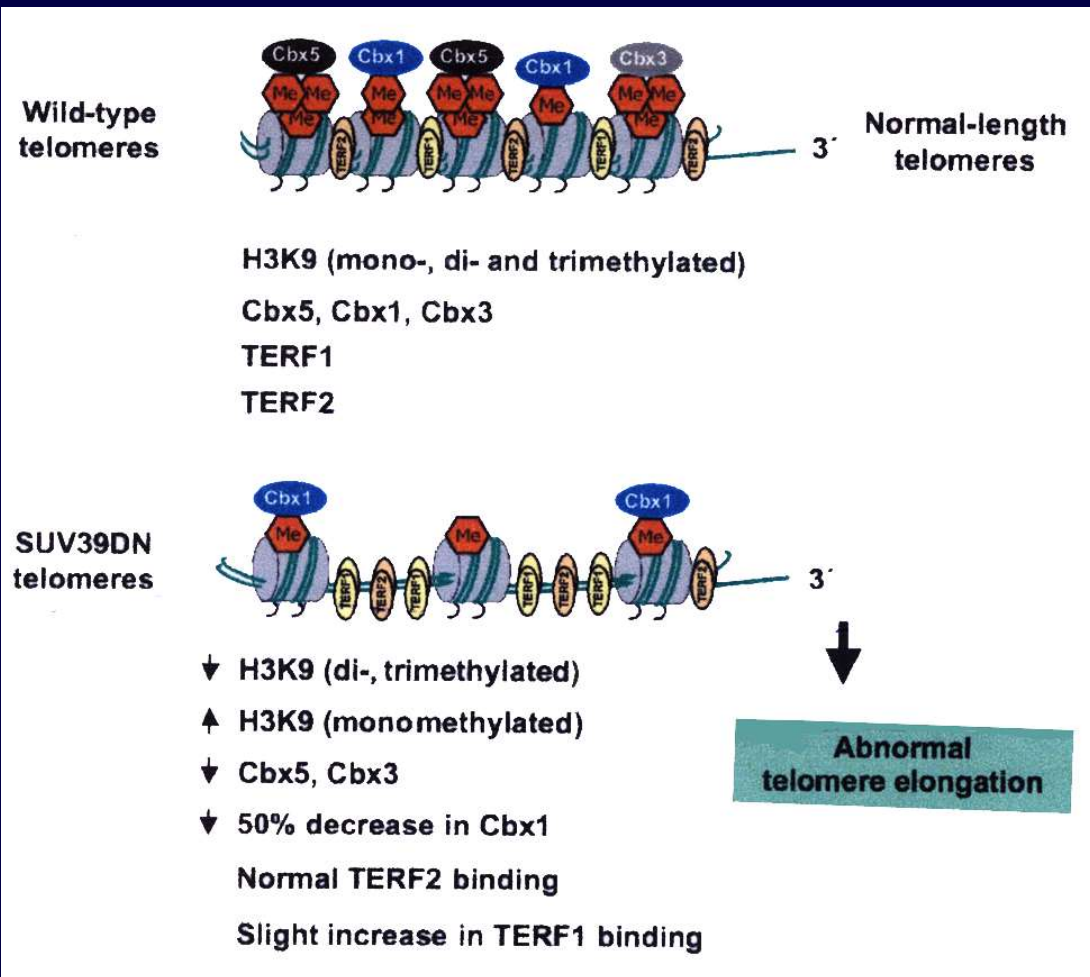
**Gametogeneze: se obnoví původní stav
Platí pro gen IGF II.**



ISSN1471-0056

Methylation state of telomeres

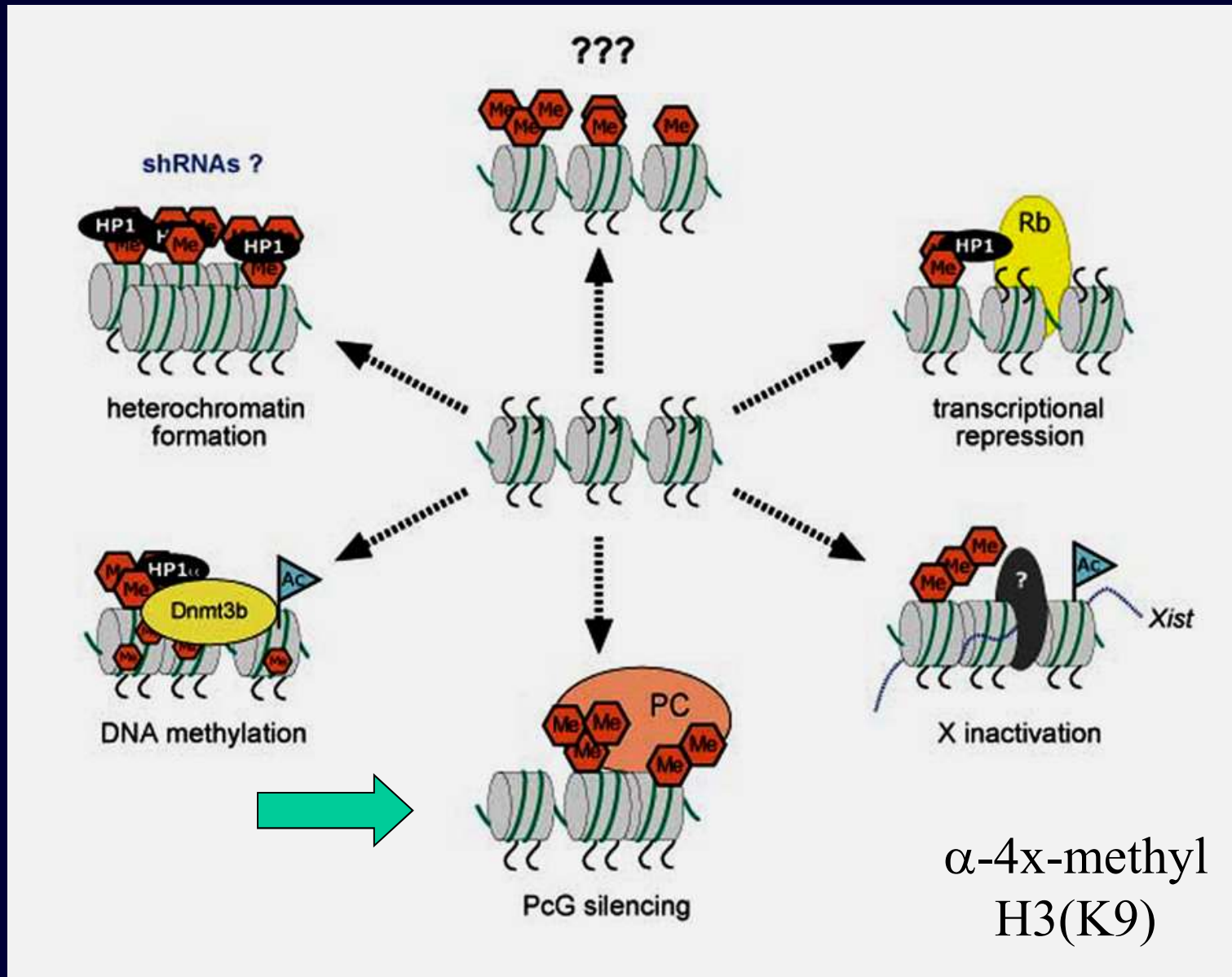
(Cbx1=HP1 β , Cbx3=HP1 γ , Cbx5 = HP1 α)



HP1 α (12q13)

HP1 β (17q21)

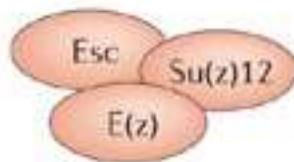
HP1 γ (7p15)



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.

a
Class II PcG complexes

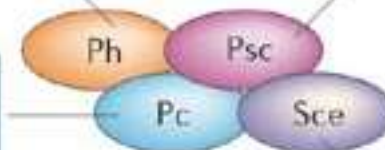


c
Class I PcG complexes

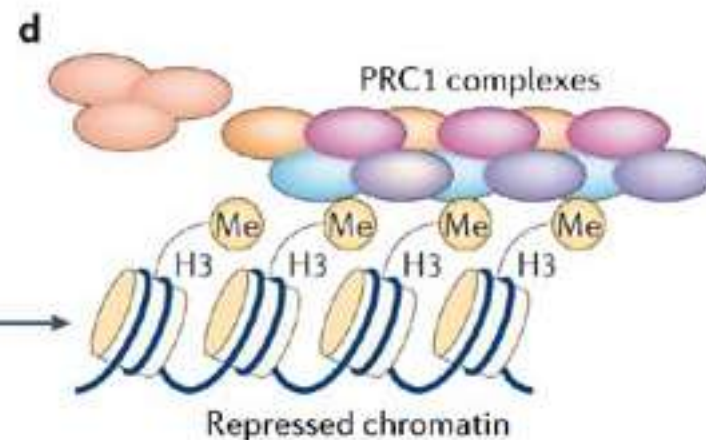
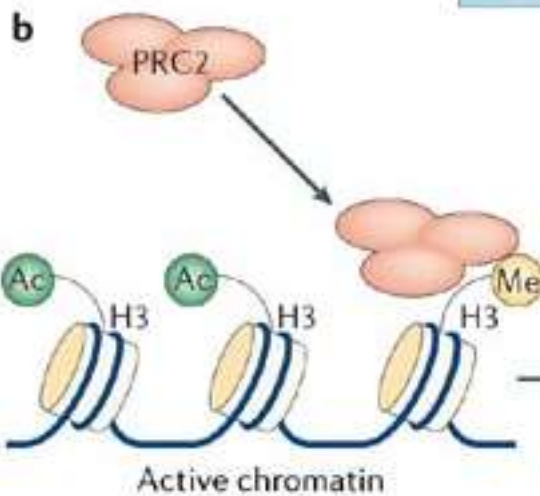
HPH1/EDR1/PHC1
HPH2/EDR2/PHC2
HPH3/EDR3/PHC3

PCGF1/RNF68/NSPC1
PCGF2/MEL18/RNF110
PCGF3/RNF3
PCGF4/BMI1/RNF51
PCGF5/RNF159
PCGF6/MBLR/RNF134

CBX2/HPC1
CBX4/HPC2
CBX6
CBX7
CBX8/HPC3



RING1a/RNF1
RING1b/RNF2



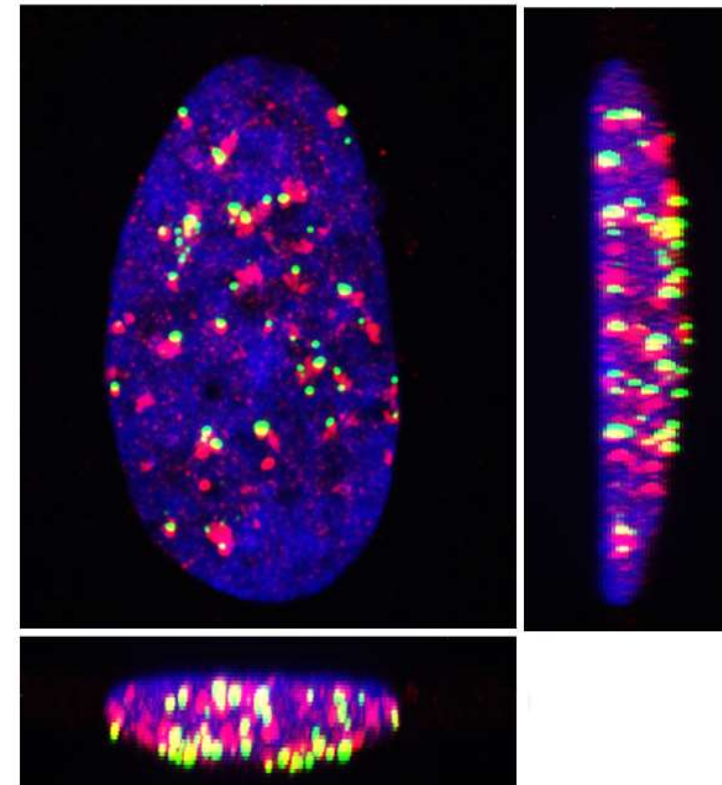
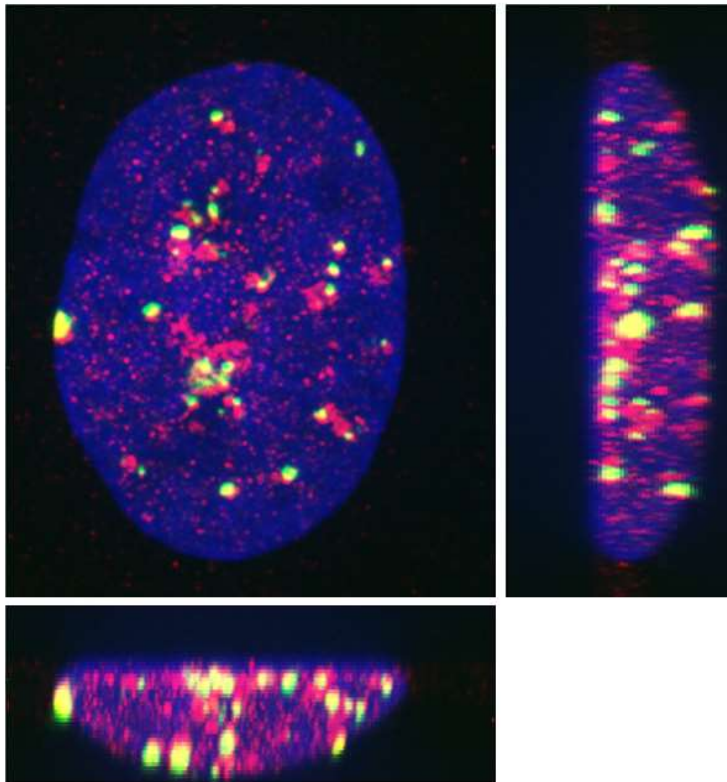
H3K27me3 / BMI1 / Nucleus

CONTROL

TSA

maximum image

maximum image

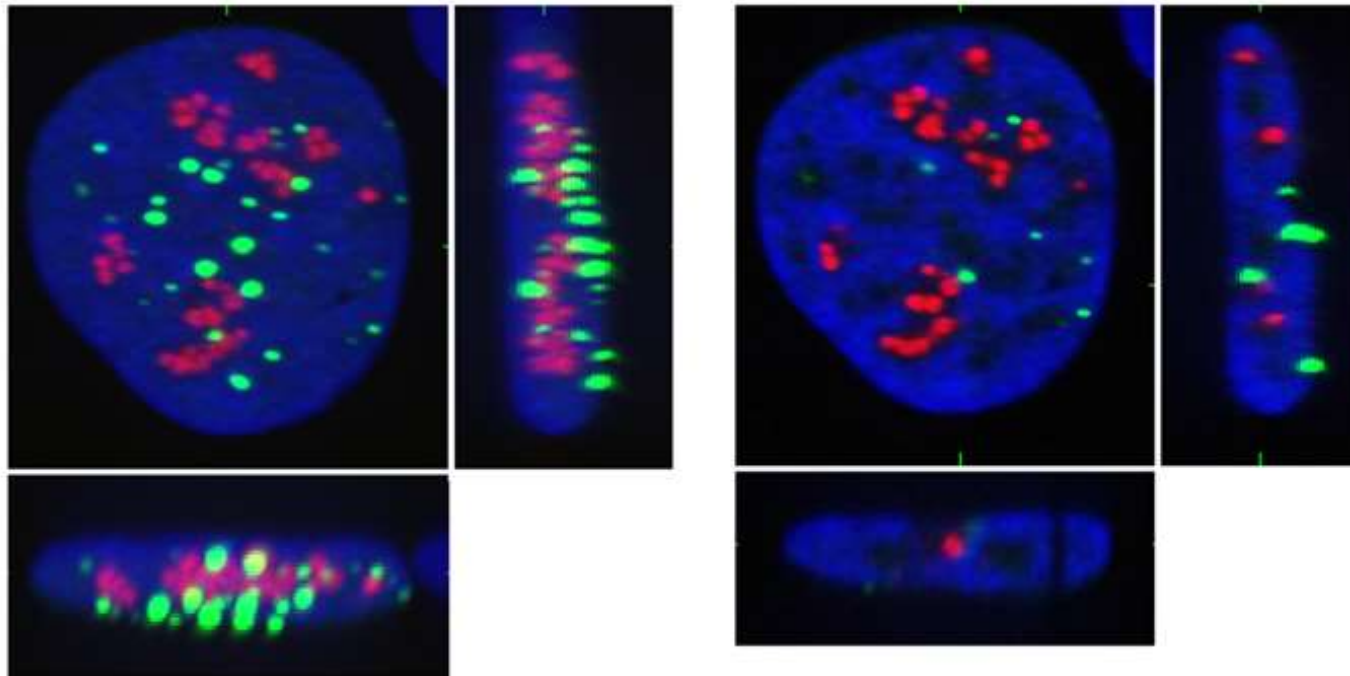


Fibrillarin / BMI1 / Nucleus

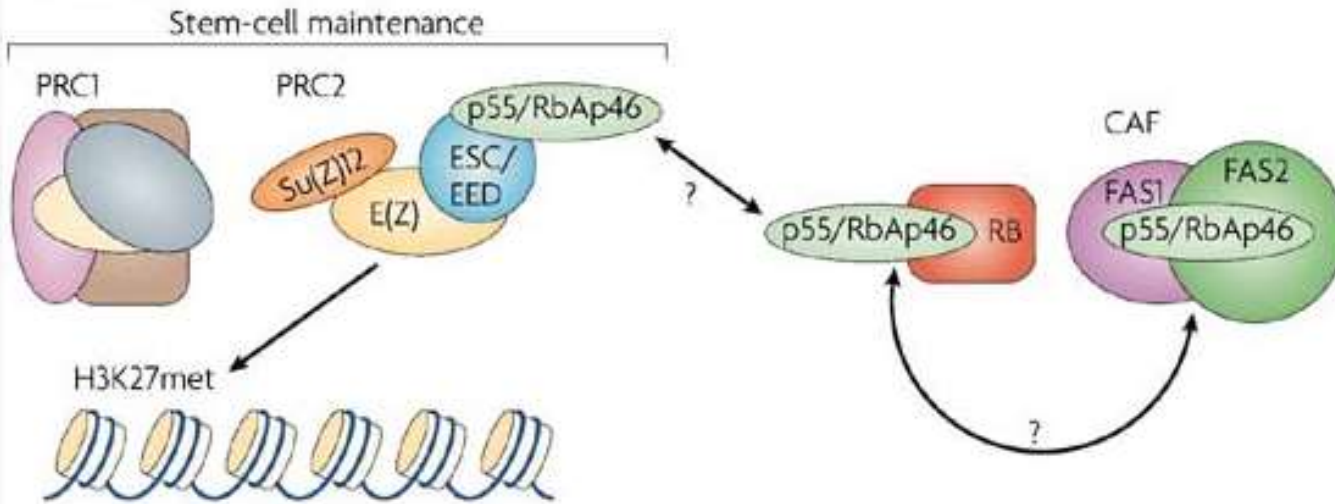
CONTROL

maximum image

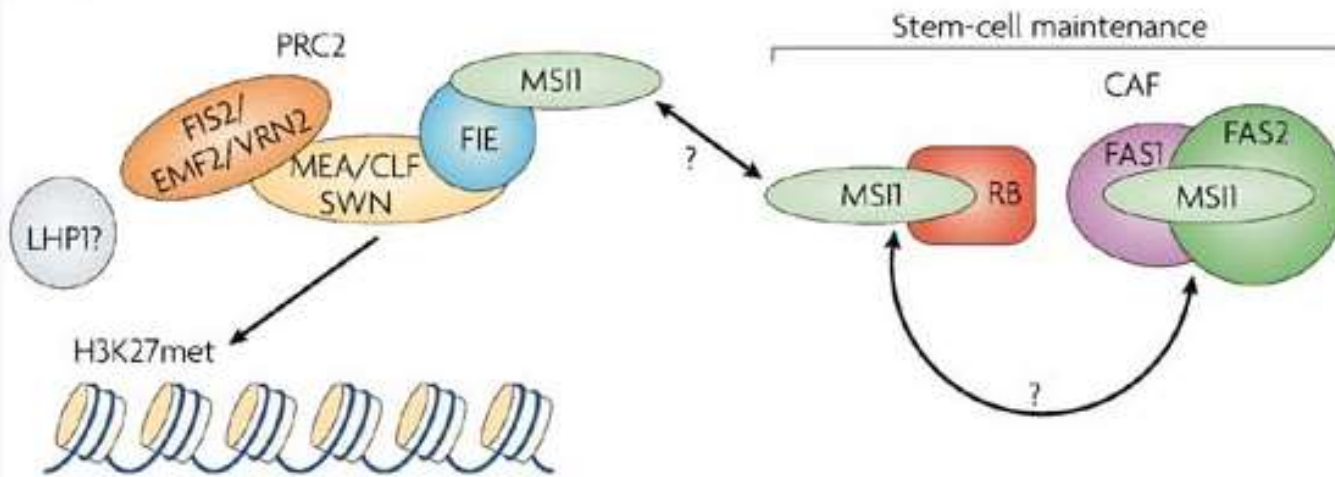
individual section

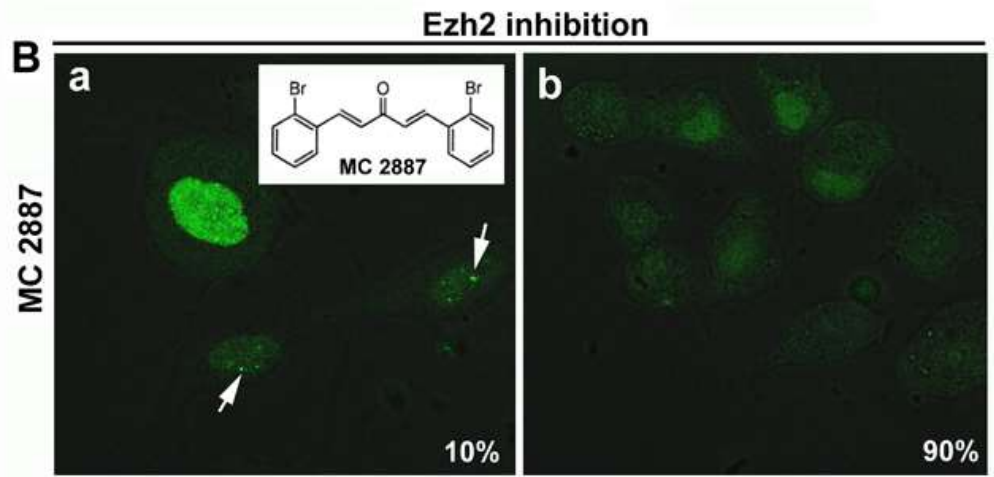
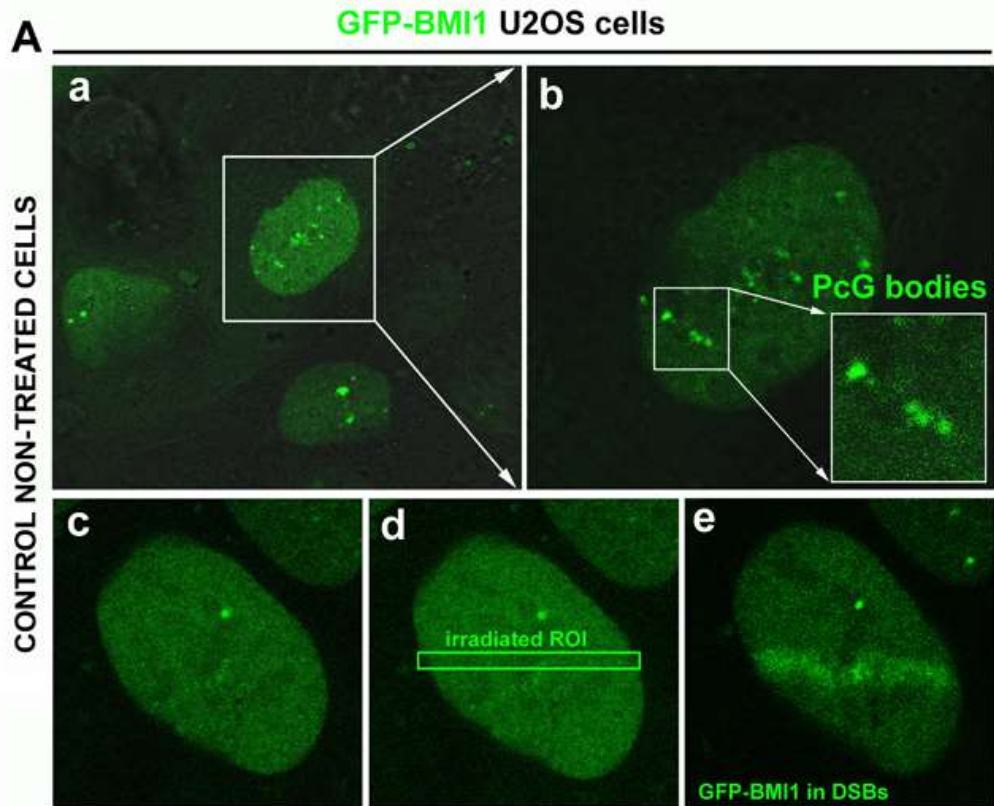


a Animals

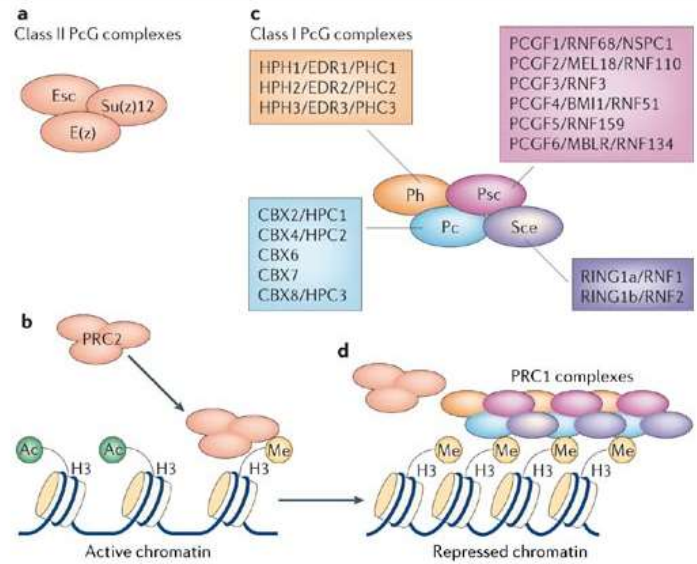


b Plants





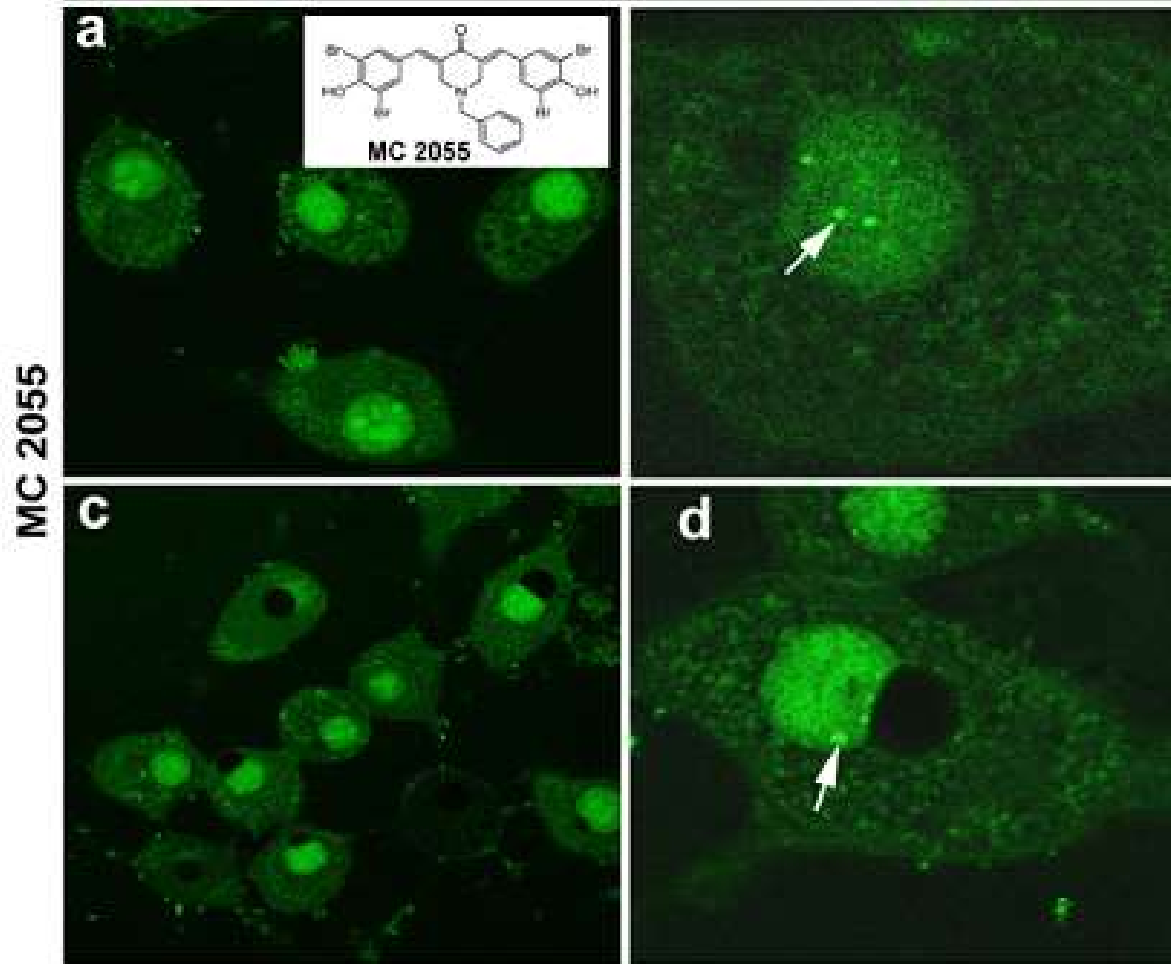
PcG – related proteins



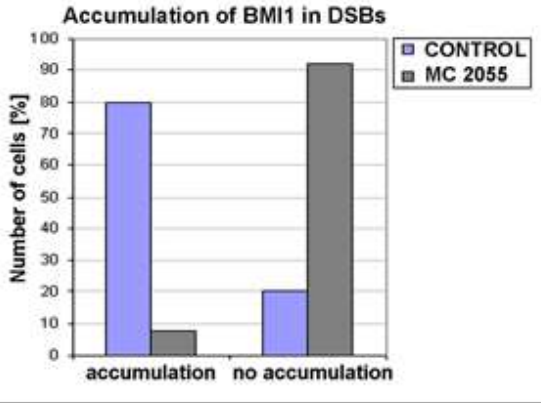
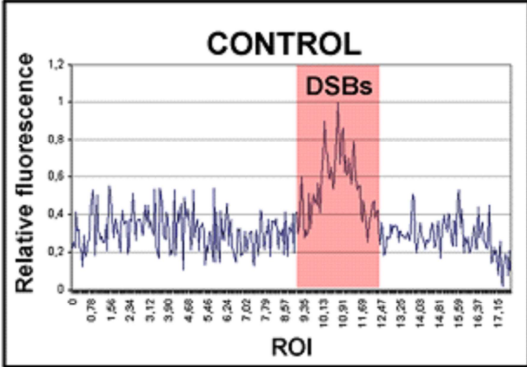
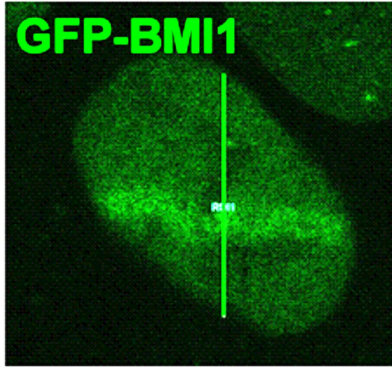
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Nature Reviews | Molecular Cell Biology

GFP-BMI1

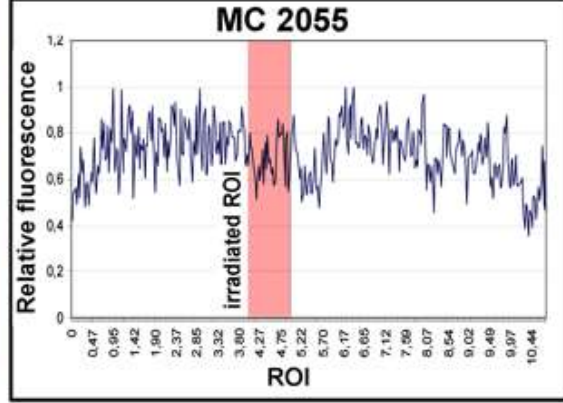
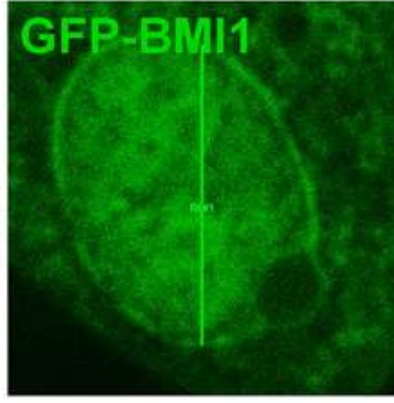
Ezh2 inhibition



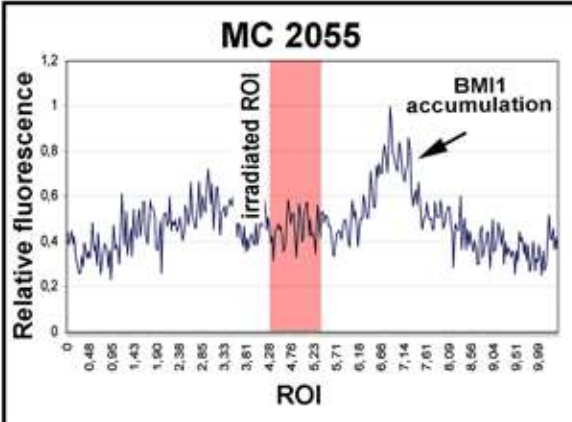
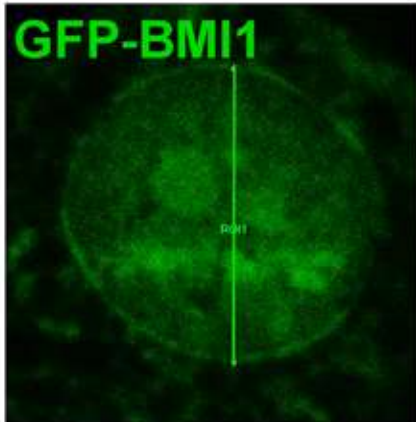
CONTROL



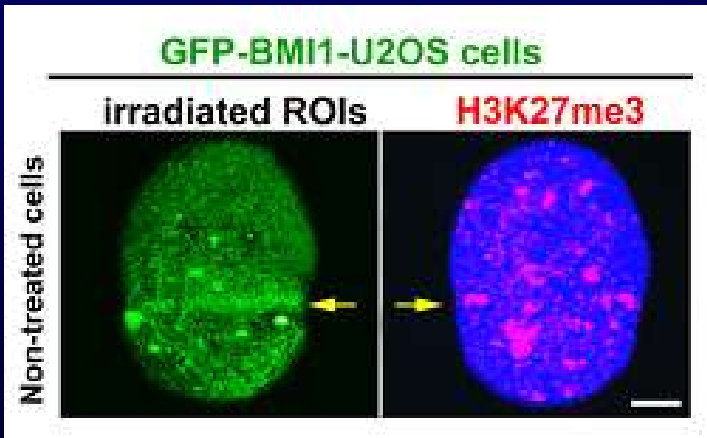
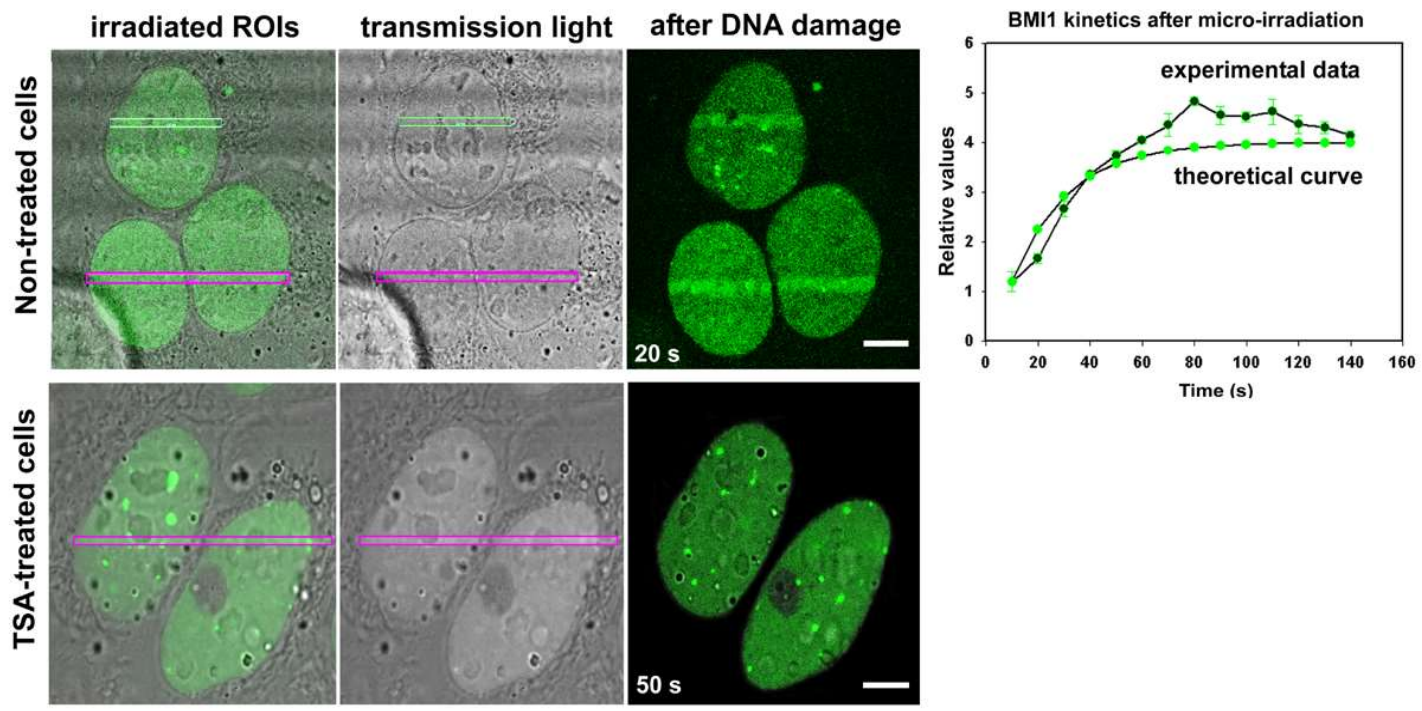
MC 2055

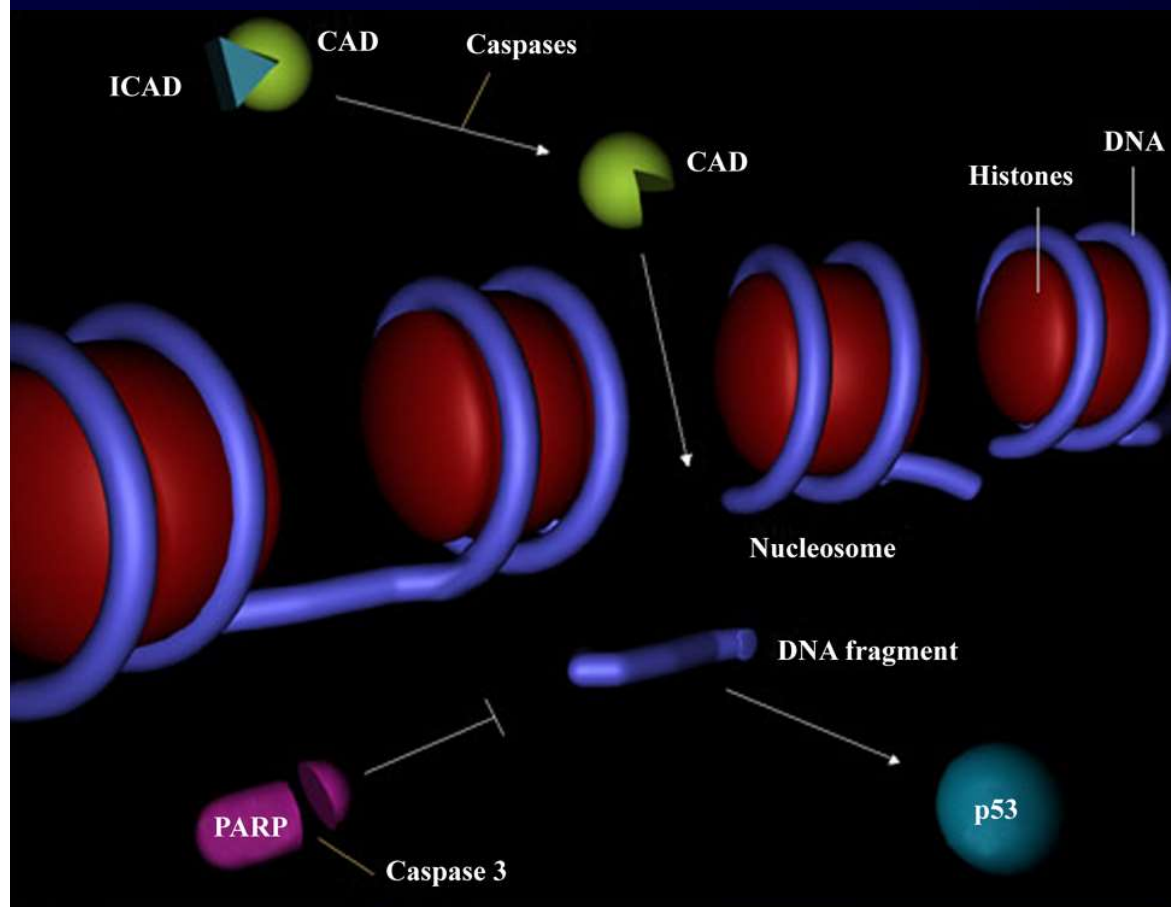
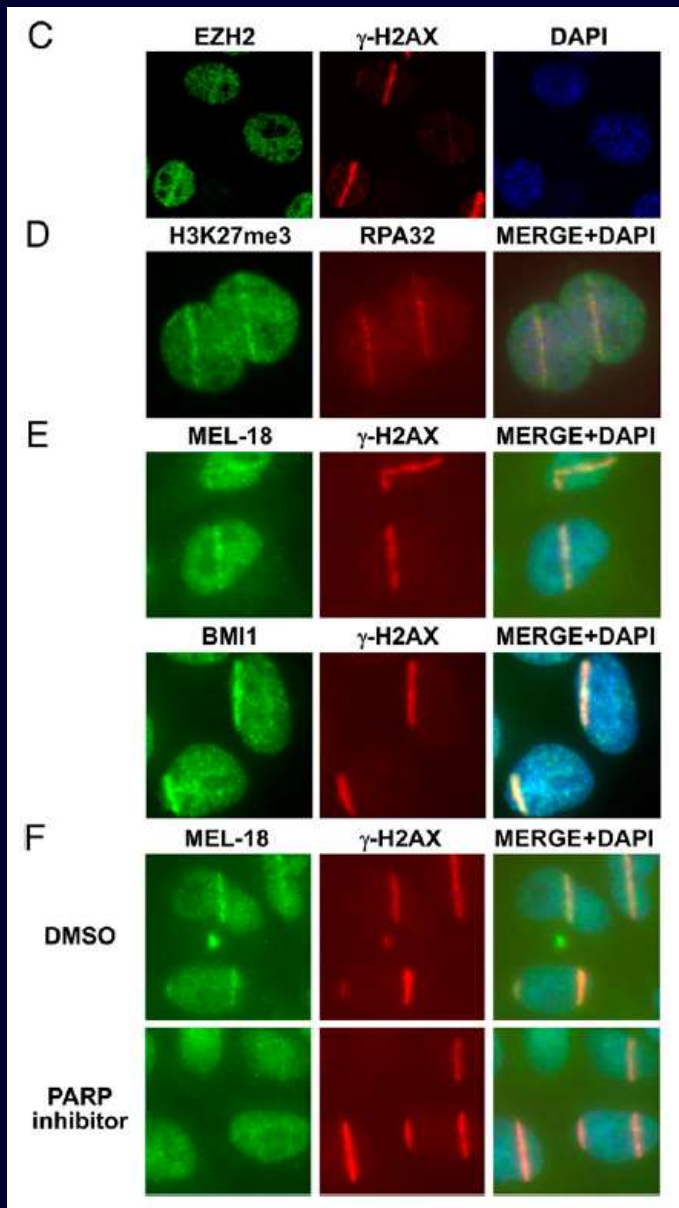


MC 2055



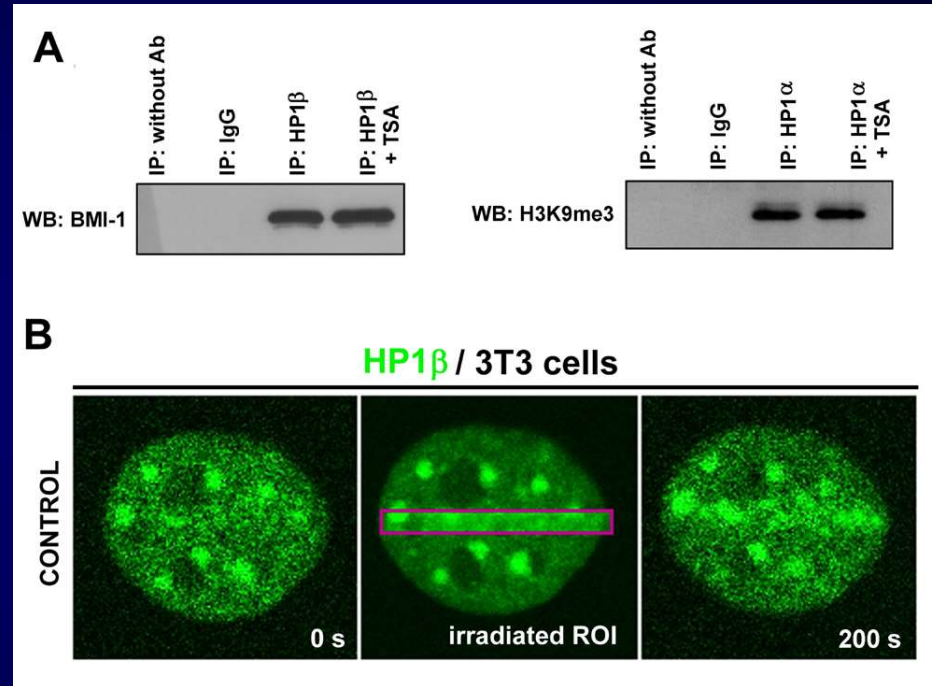
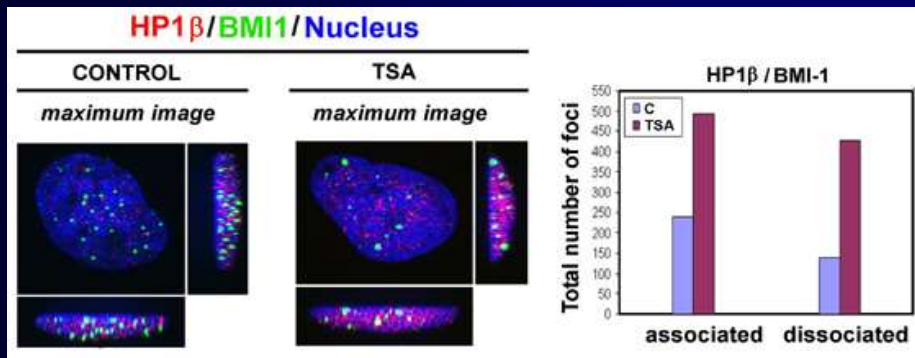
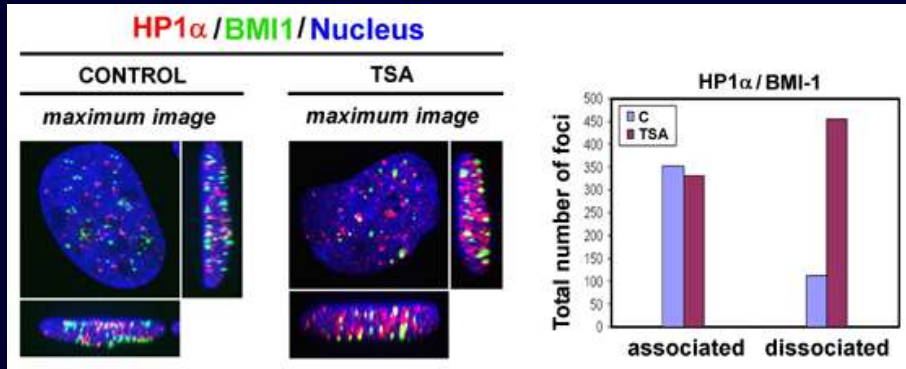
GFP-BMI1-U2OS cells



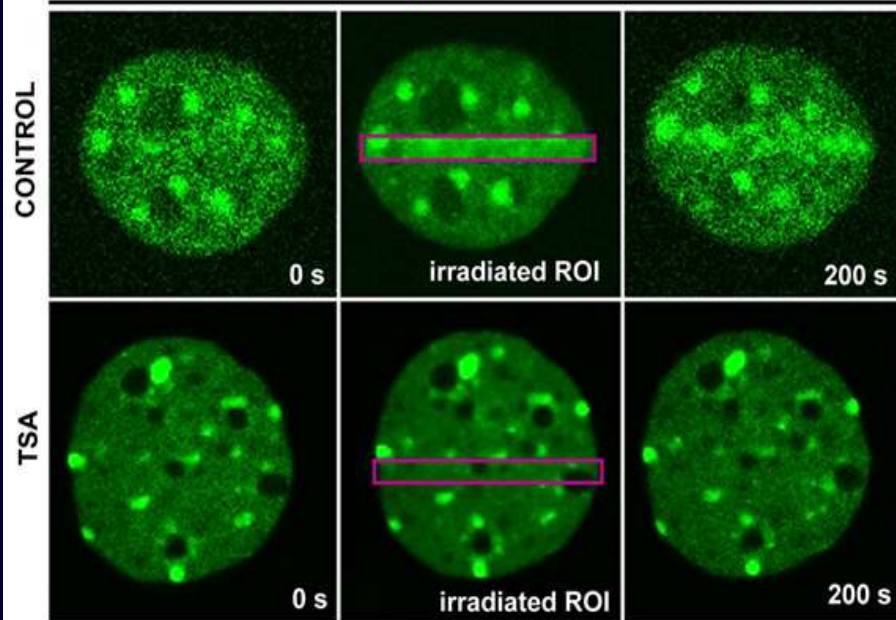


DNA repair

Chou et al., PNAS (2010)

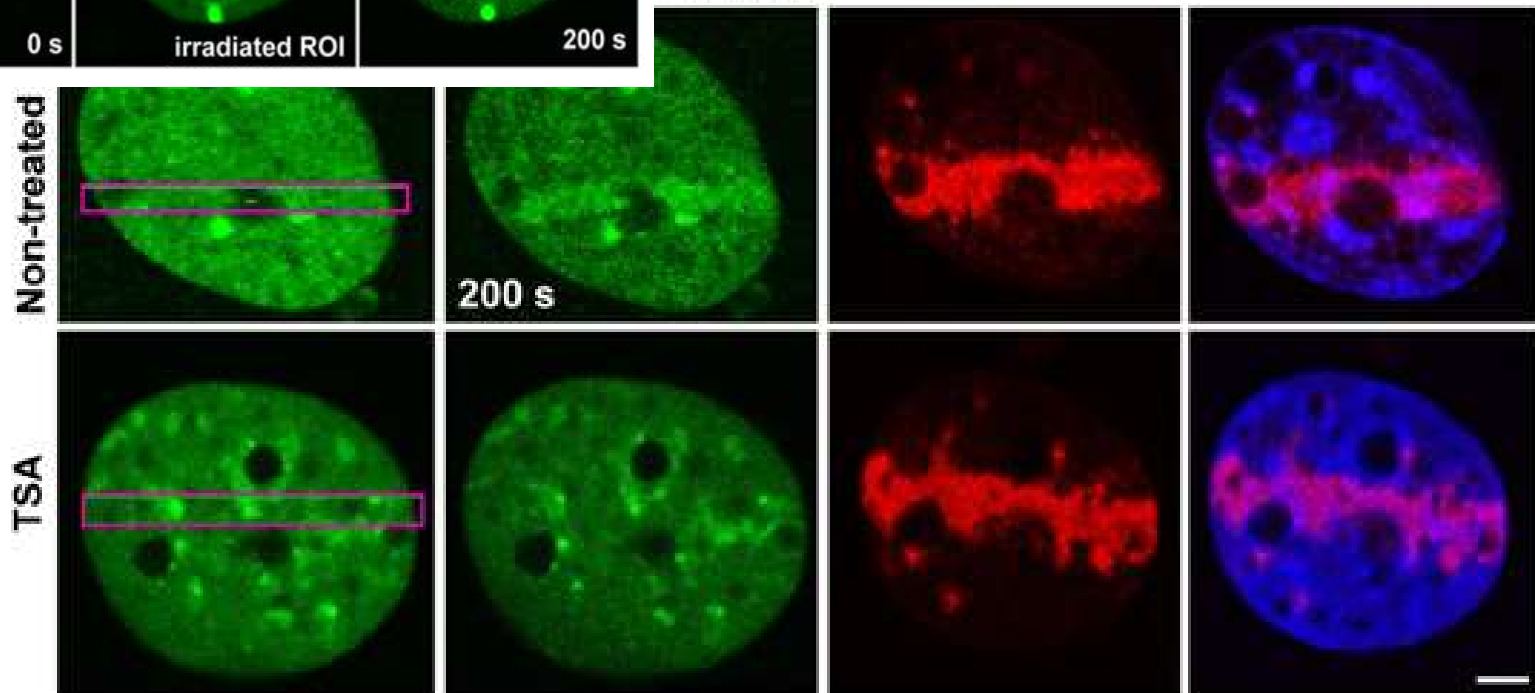


HP1 β / 3T3 cells

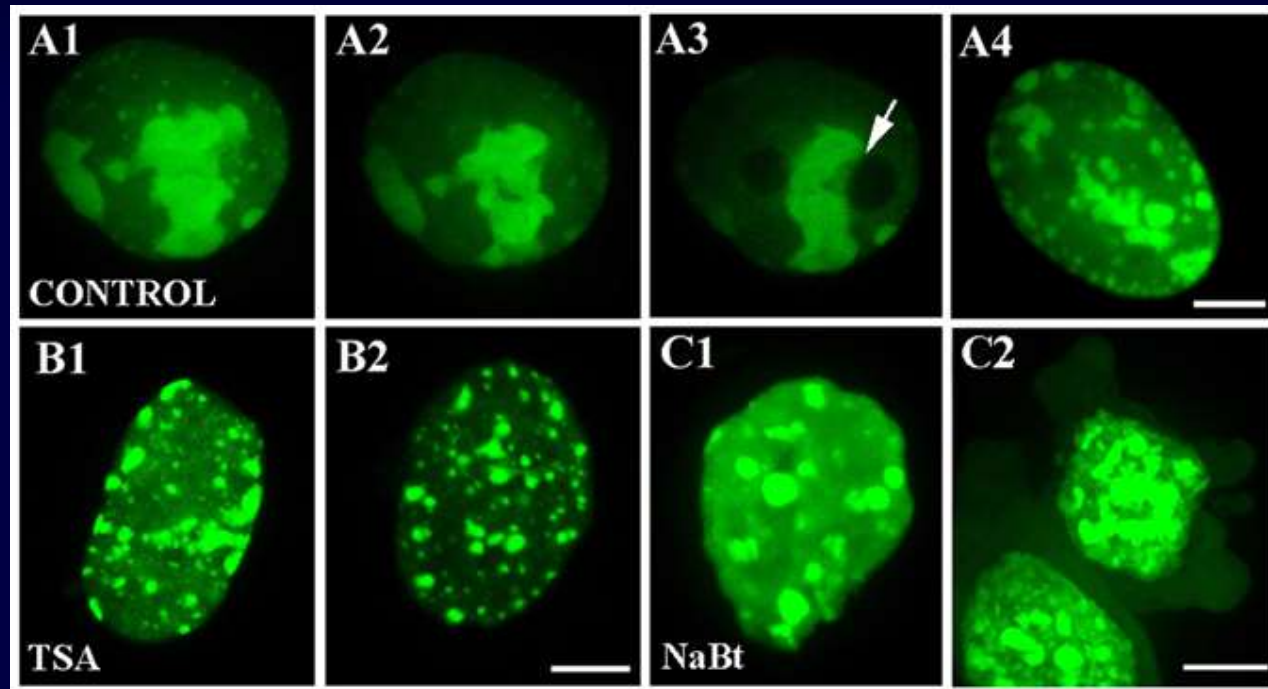


γ H2AX / 3T3 cell nuclei

damage



HP1 proteins

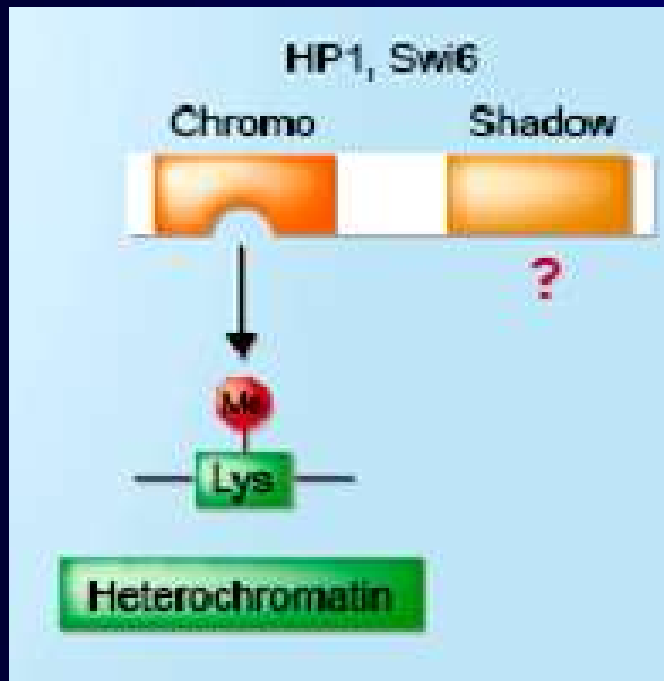


- HP1 proteiny jsou hlavní složkou heterochromatinu a hrají důležitou úlohu při jeho tvorbě. HPs mají vysokou afinitu k pericentromerickým a telometrickým oblastem chromosomů.
- HPs interagují s HMTs jako je SUV39h1 a SUV39h2, která jsou zodpovědné za metylaci H3(K9).

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

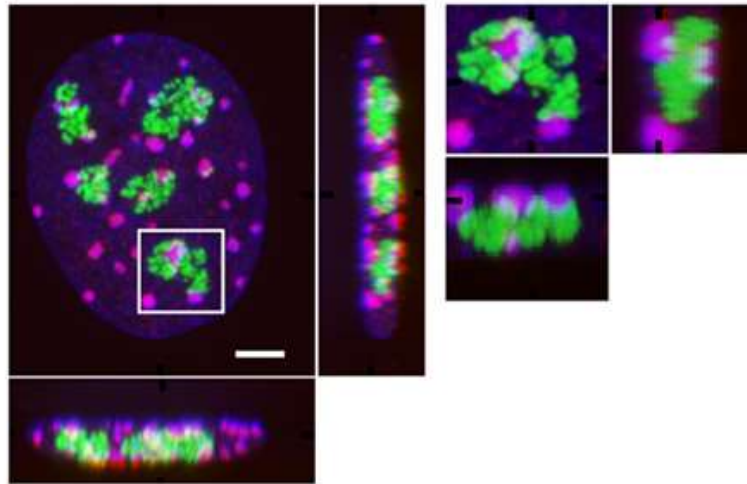
c) Optimální regulace délky telomer a zprostředkování procesu telomeric silencing

Fibrillarin / HP1 α / DNA

nucleus

nucleolus

SUV39h1 +/+

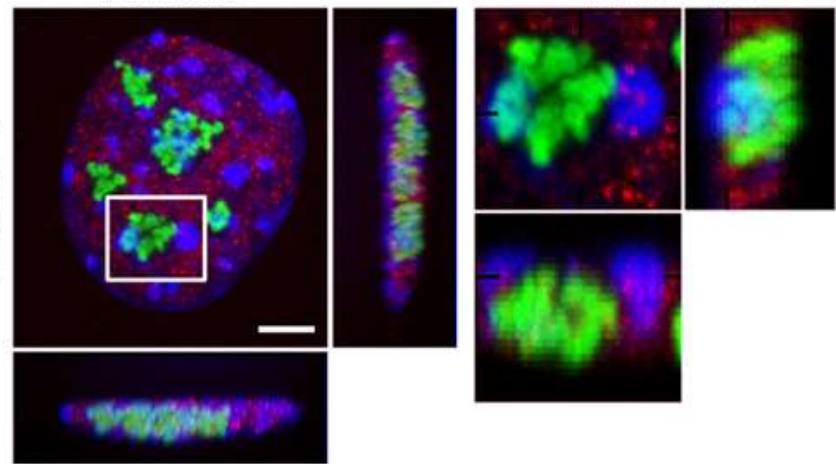


Fibrillarin / HP1 γ / DNA

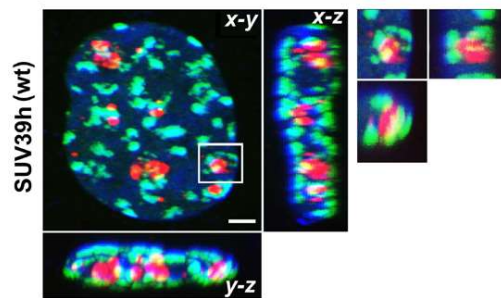
nucleus

nucleolus

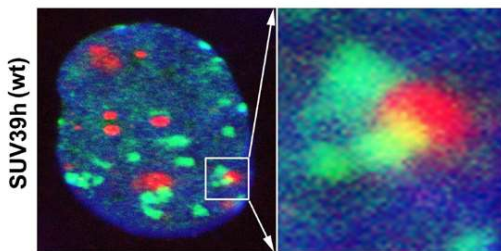
SUV39h1 +/+



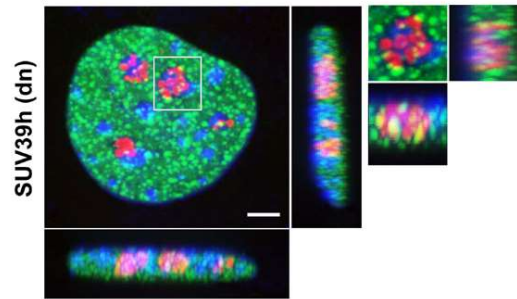
a Fibrillarin / GFP-HP1 β



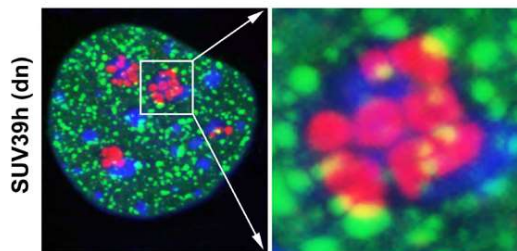
individual confocal section



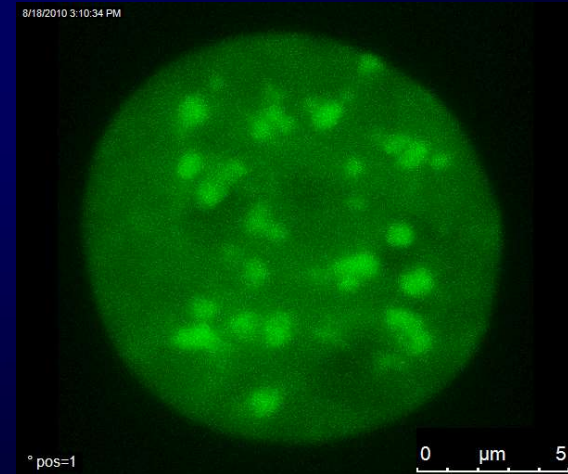
b Fibrillarin / GFP-HP1 β



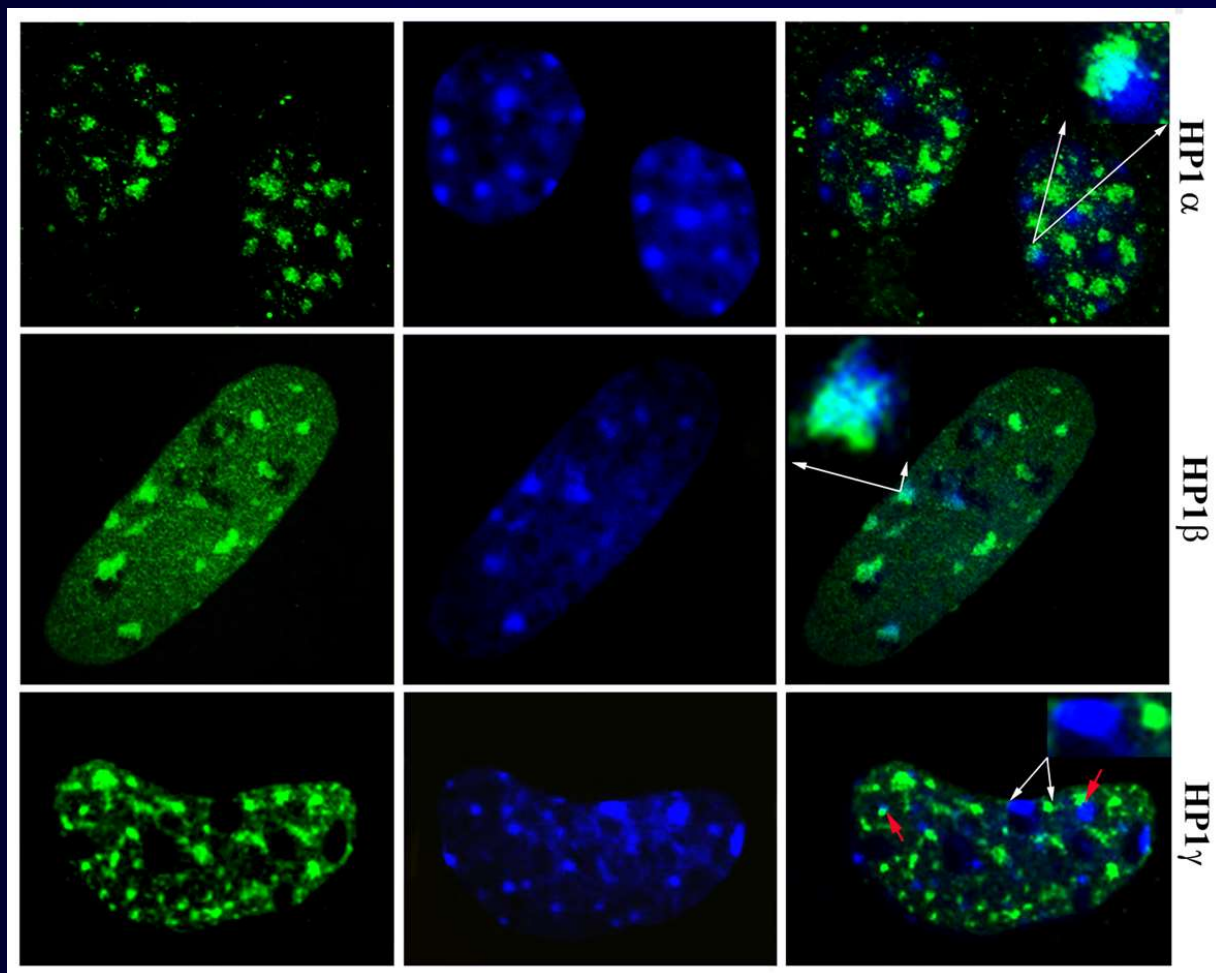
individual confocal section



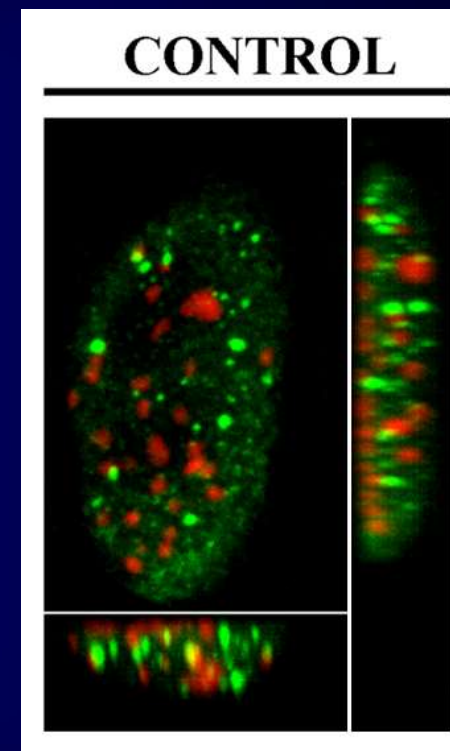
HP1 β



HP1 proteiny u ECS



HP1 α HP1 β

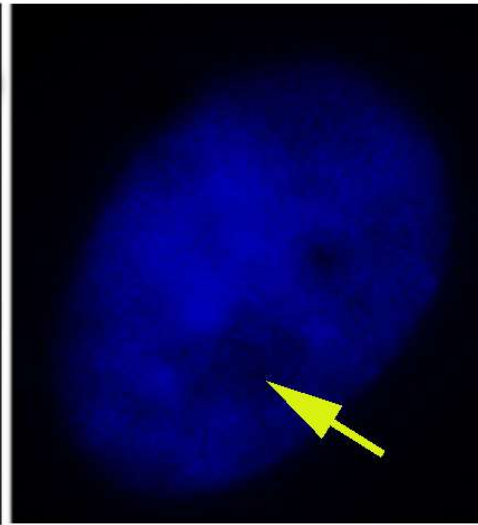
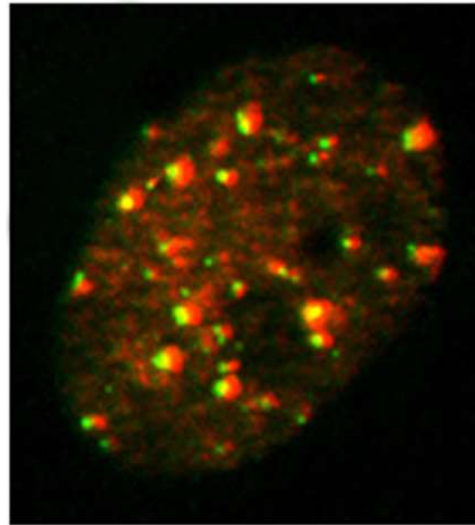


CENP-A / HP1 α

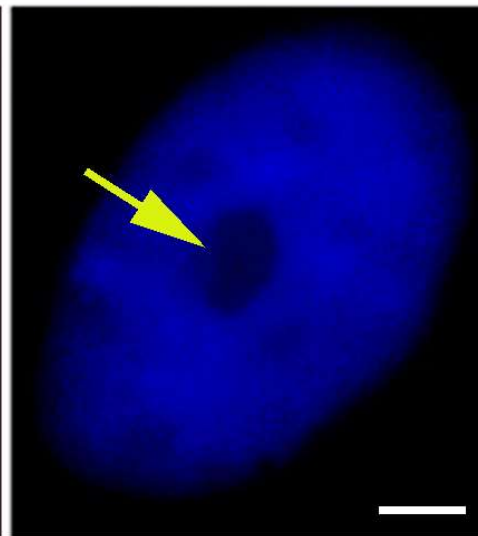
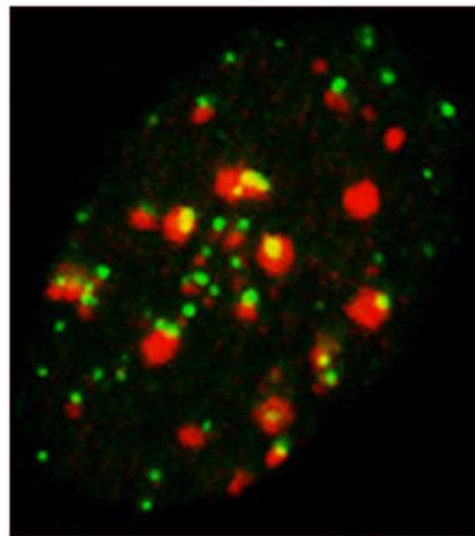
DAPI

Max. image

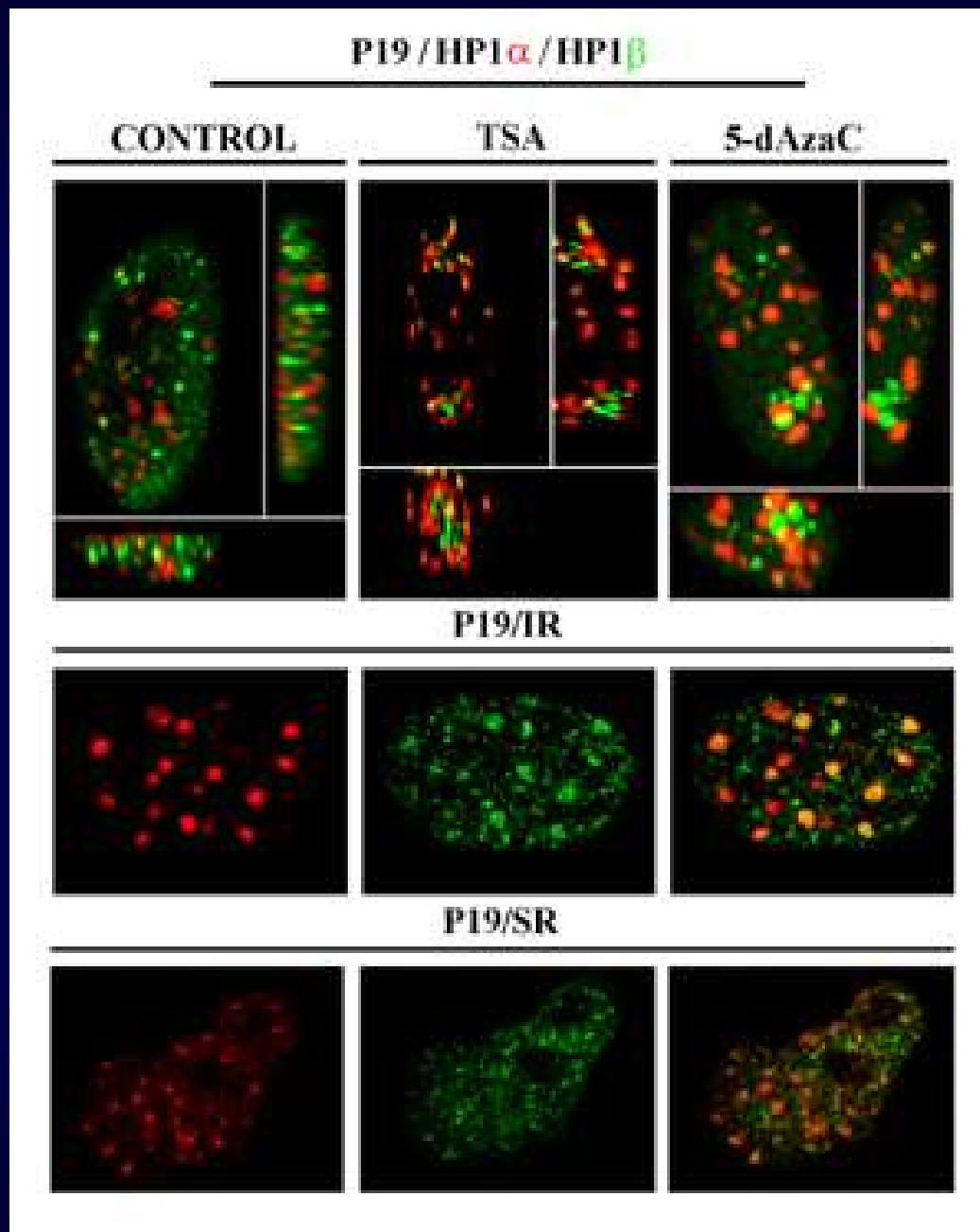
Mid. section



CONTROL

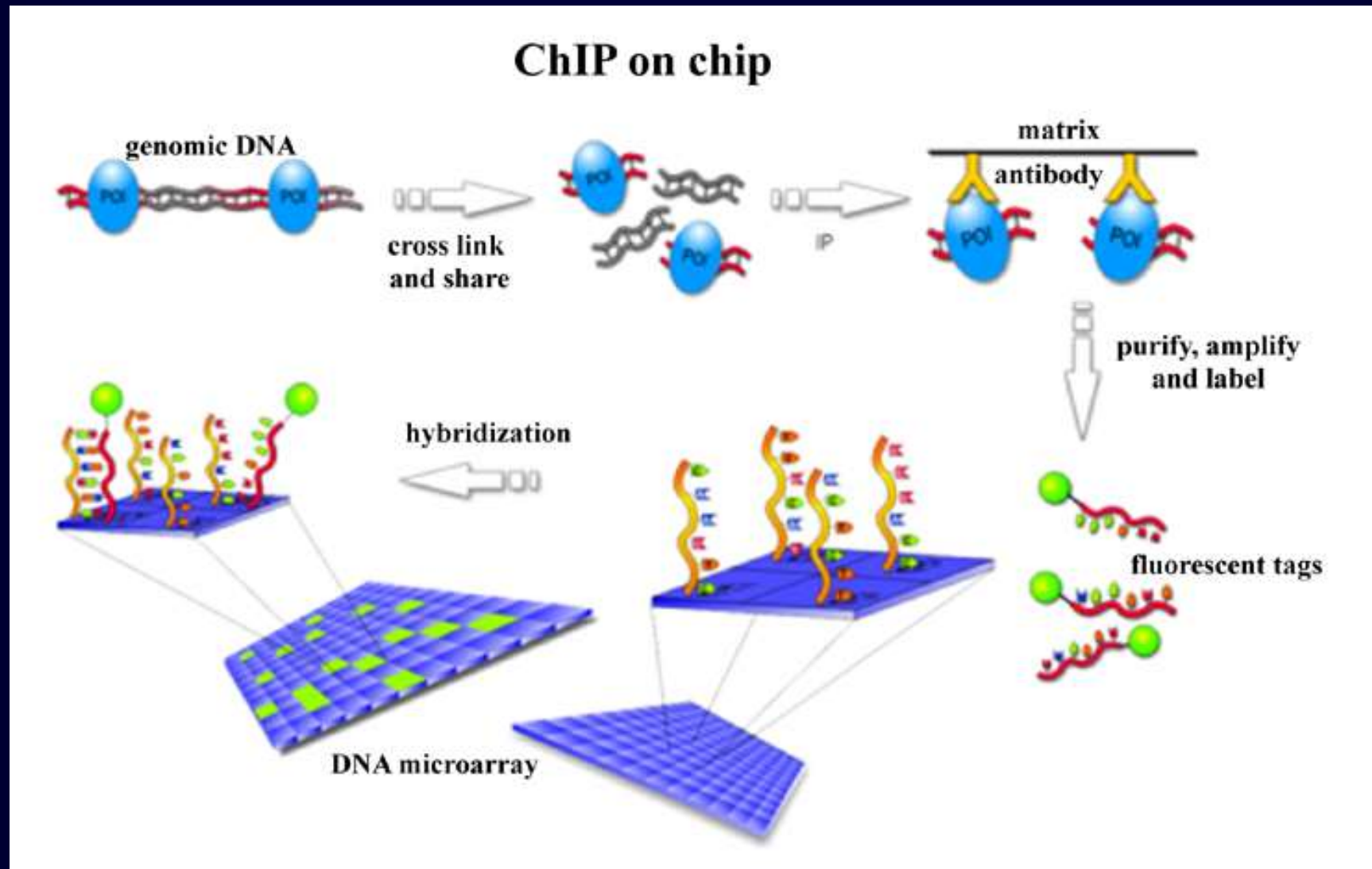


TSA

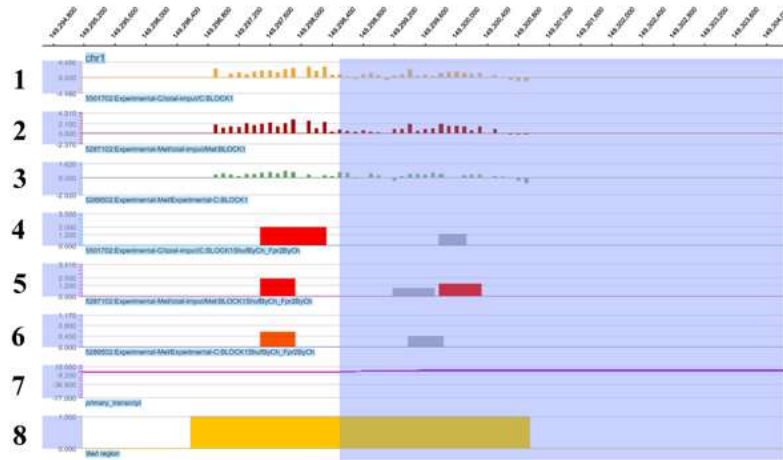


**Metoda
IHC**

Ligation mediated PCR



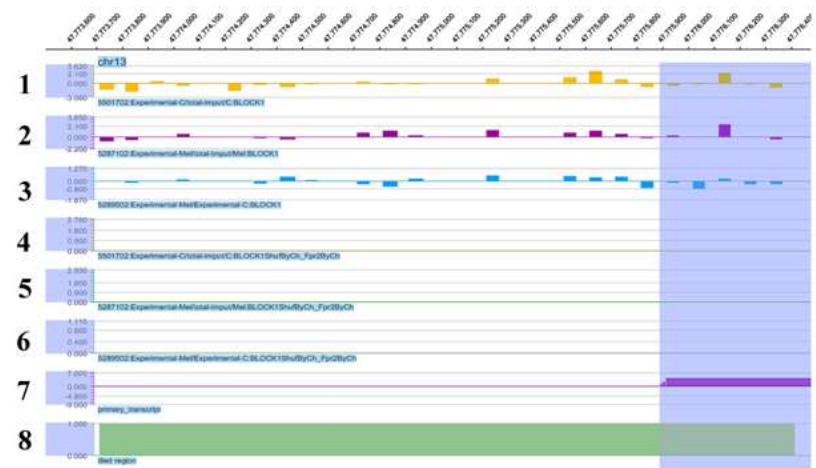
AF1Q



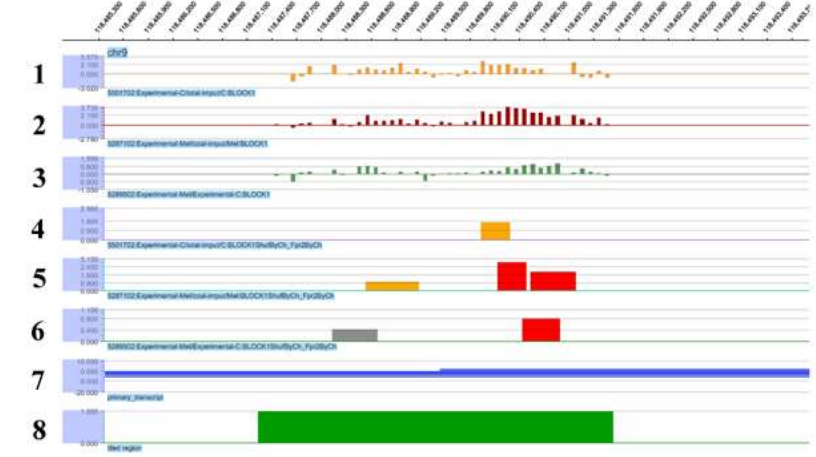
TP53



RB1



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**