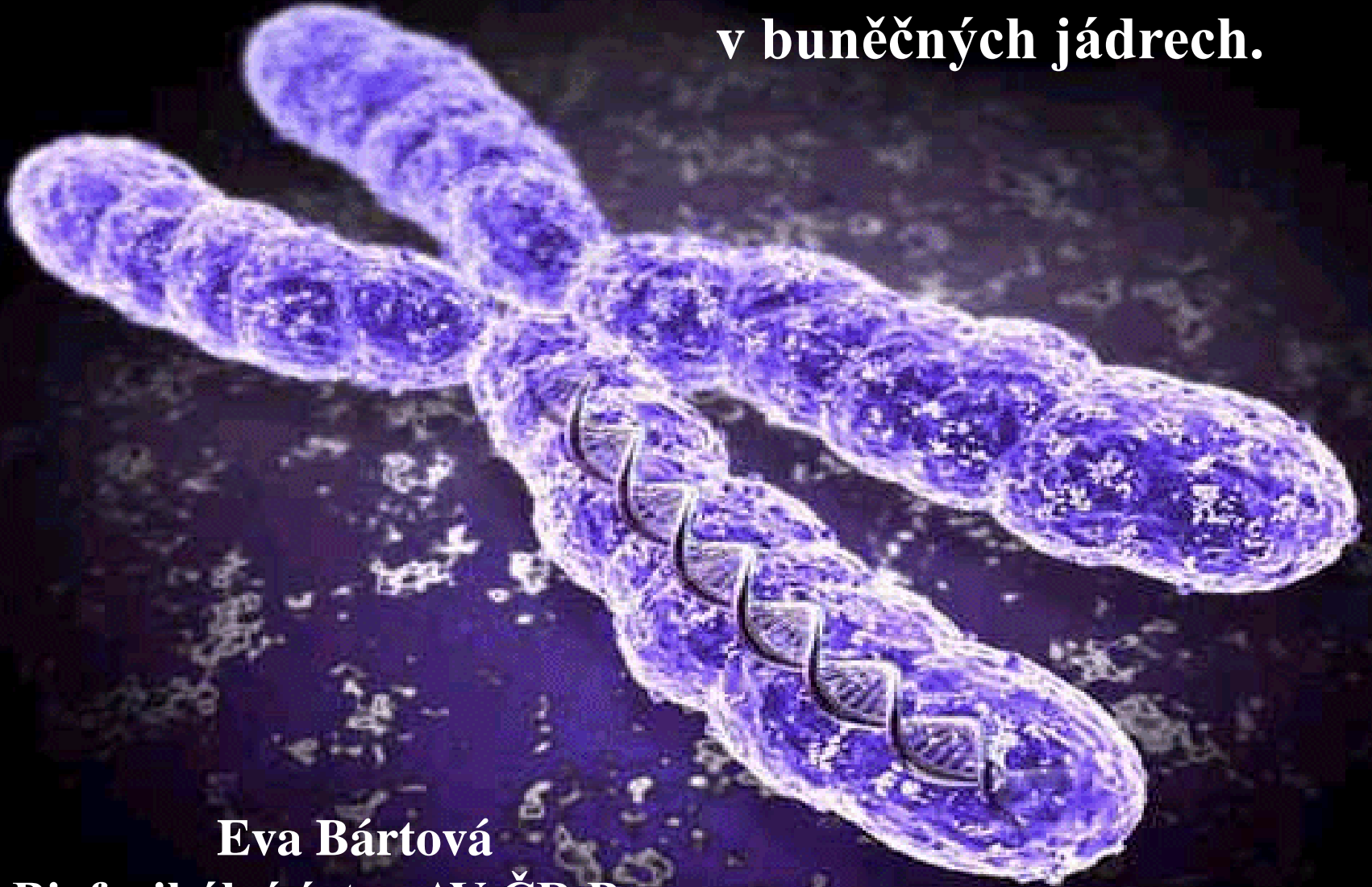


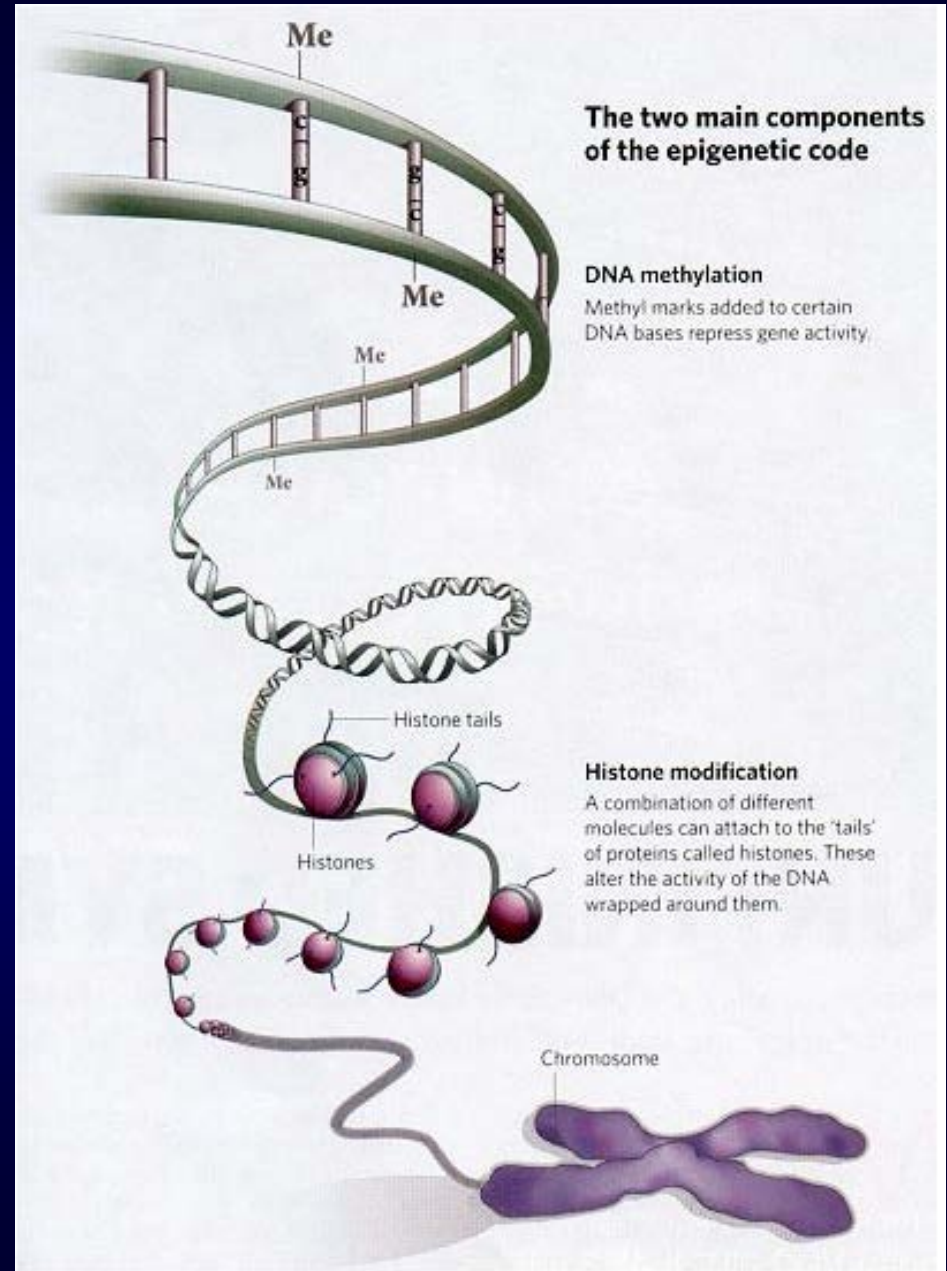
Epigenetické procesy probíhající v buněčných jádrech.

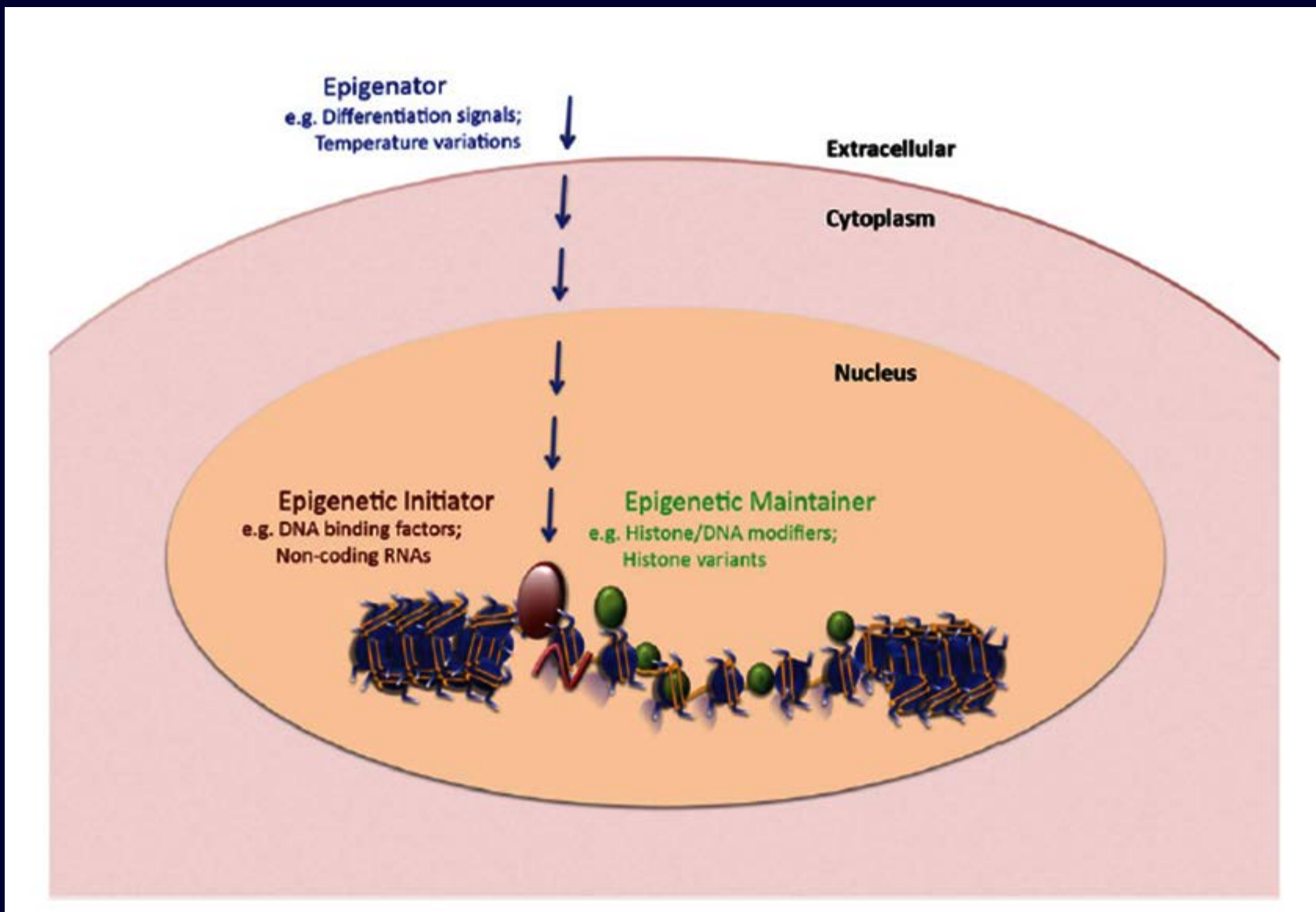


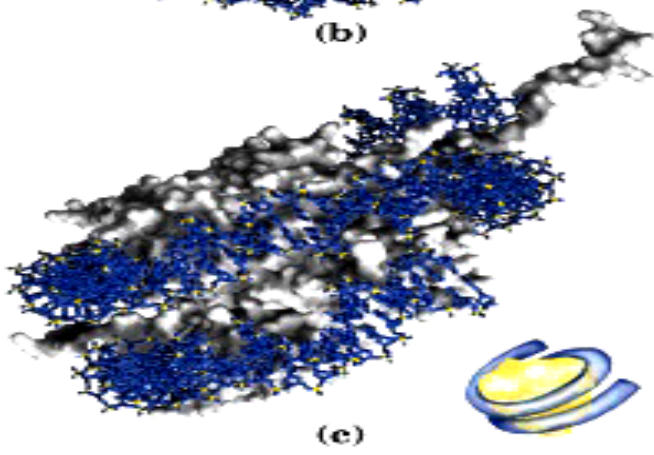
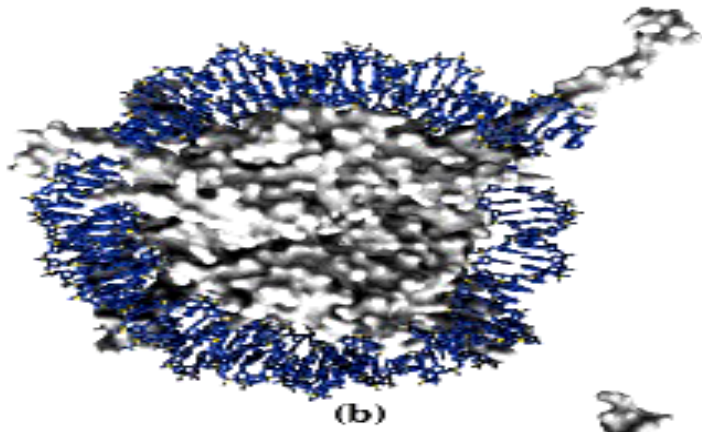
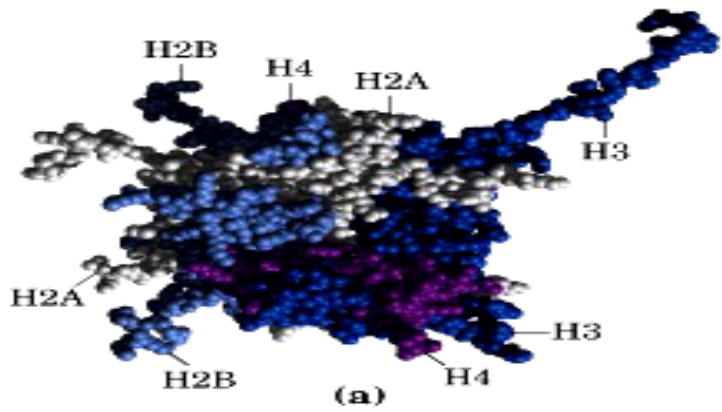
Eva Bártová
Biofyzikální ústav AV ČR Brno

What is epigenetics ?

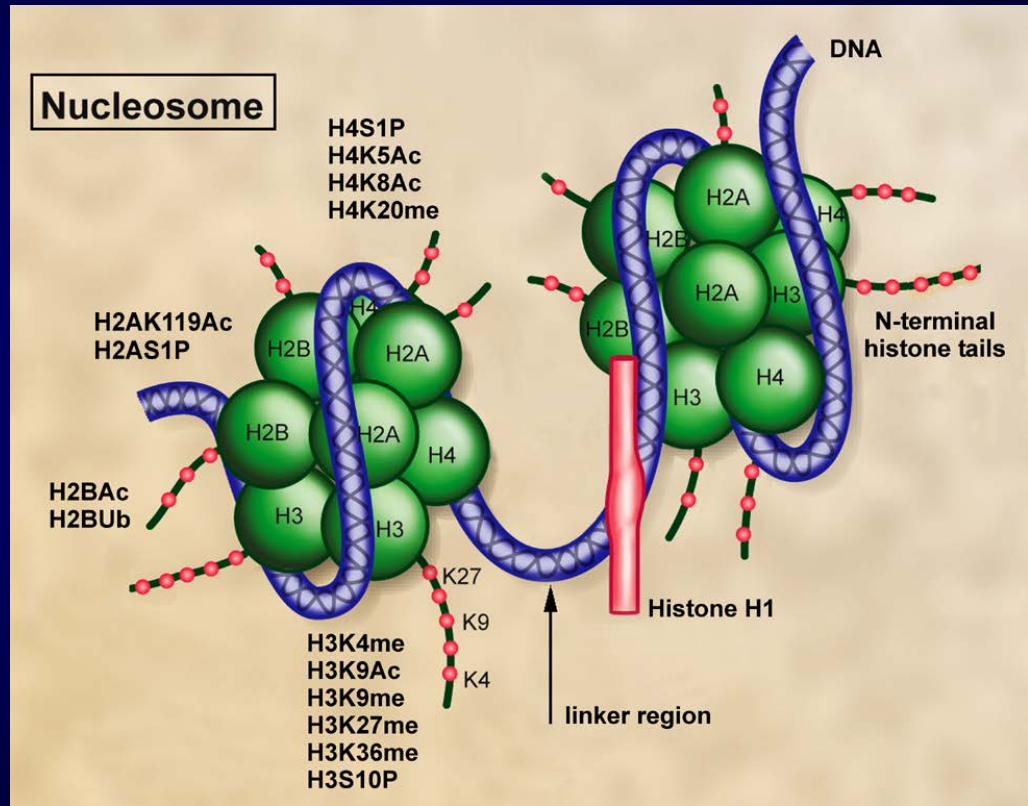
Epigenetics refers to heritable changes in the phenotype that occur irrespective of alterations in the DNA sequences.

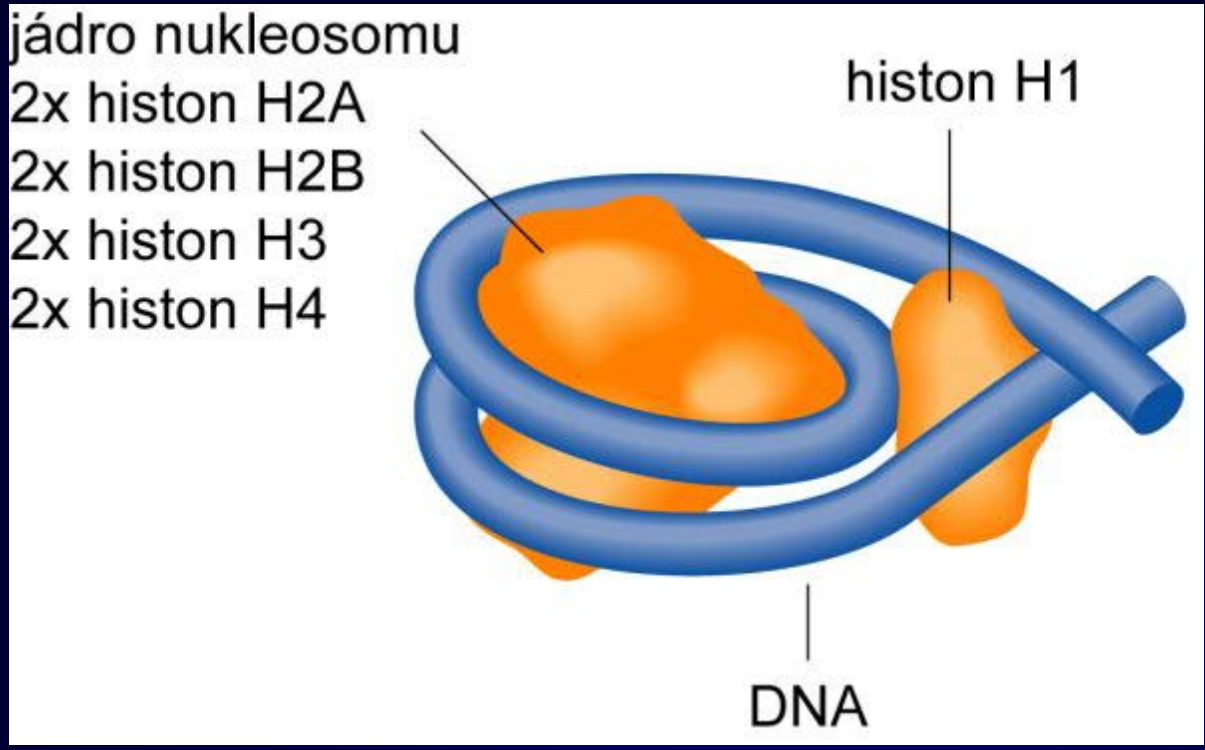






Nucleosome

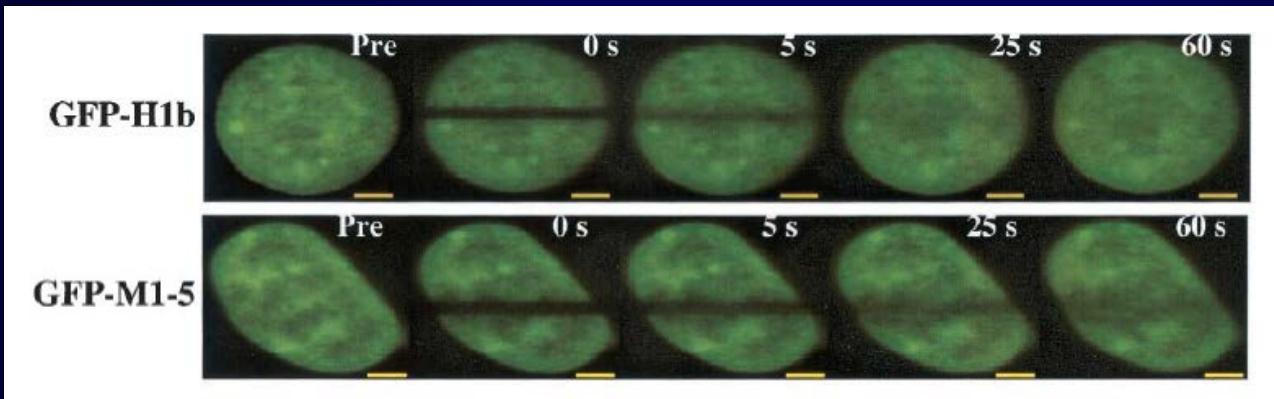




1. **Buňka může existovat i bez významně redukovaného množství H1.**
2. **H1 varianty nejsou hlavní determinanty buněčného fenotypu.**
3. **Funkce H1 variant je nejenom při utlumení transkripční aktivity ale také při její aktivaci (může snižovat nebo i zvyšovat expresi specifických genů).**
4. **H1 hraje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

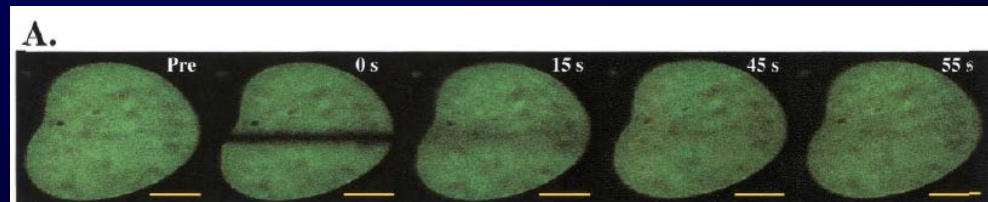
Using the technique of fluorescence recovery after photobleaching, Contreres et al., 2003 observed that the mobility of a GFP-wild-type H1 fusion protein is dependent on Cdk2 activity. GFP-H1 mobility was decreased in cells with low Cdk2 activity but not in the cells with bloked phophorylation of H1. **Decreased mobility of GFP-H1.**



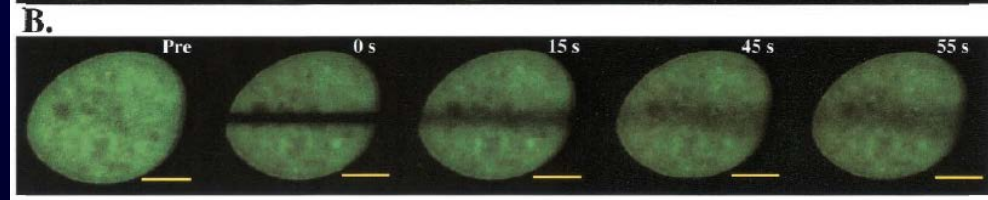
Experiments of E. Meshorer

Overexpression p21

GFP-H1b



GFP-M1-5

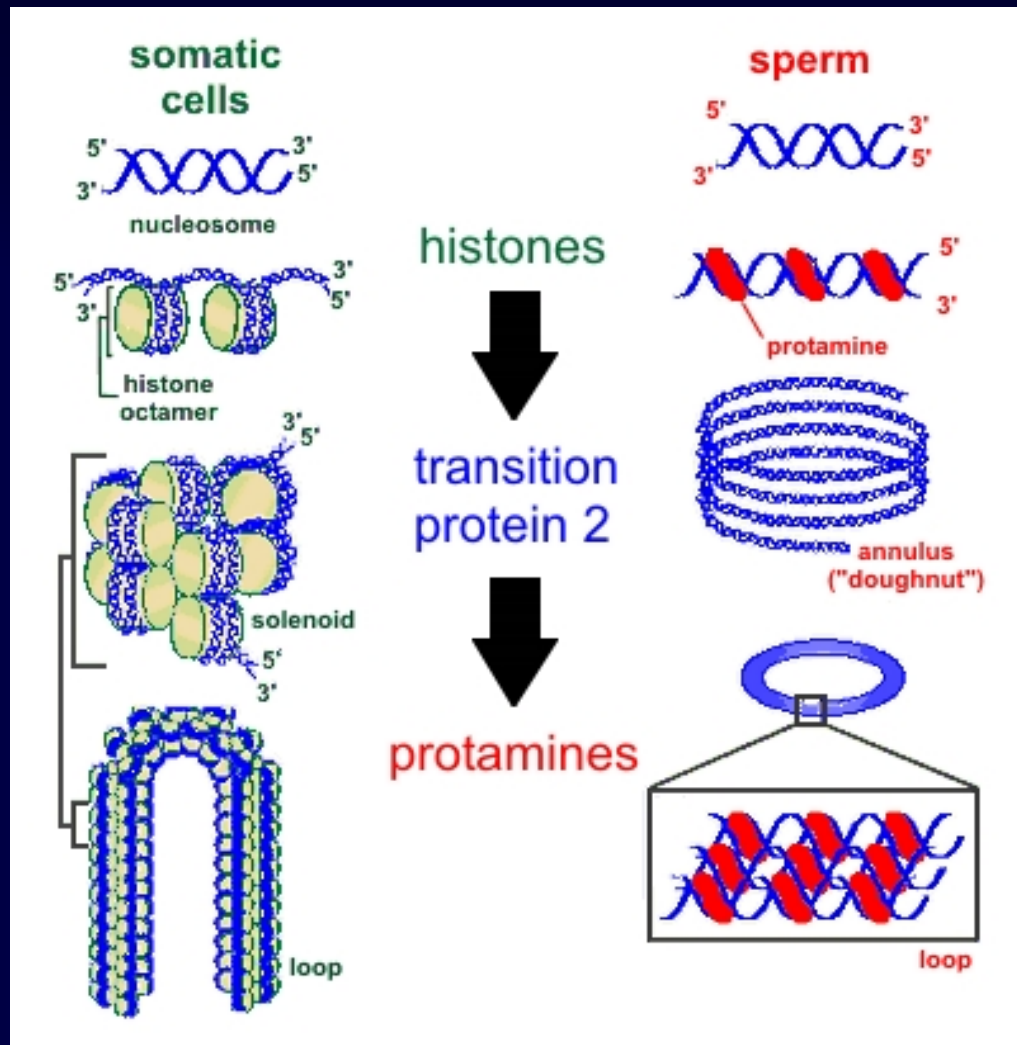


Varianty histonů

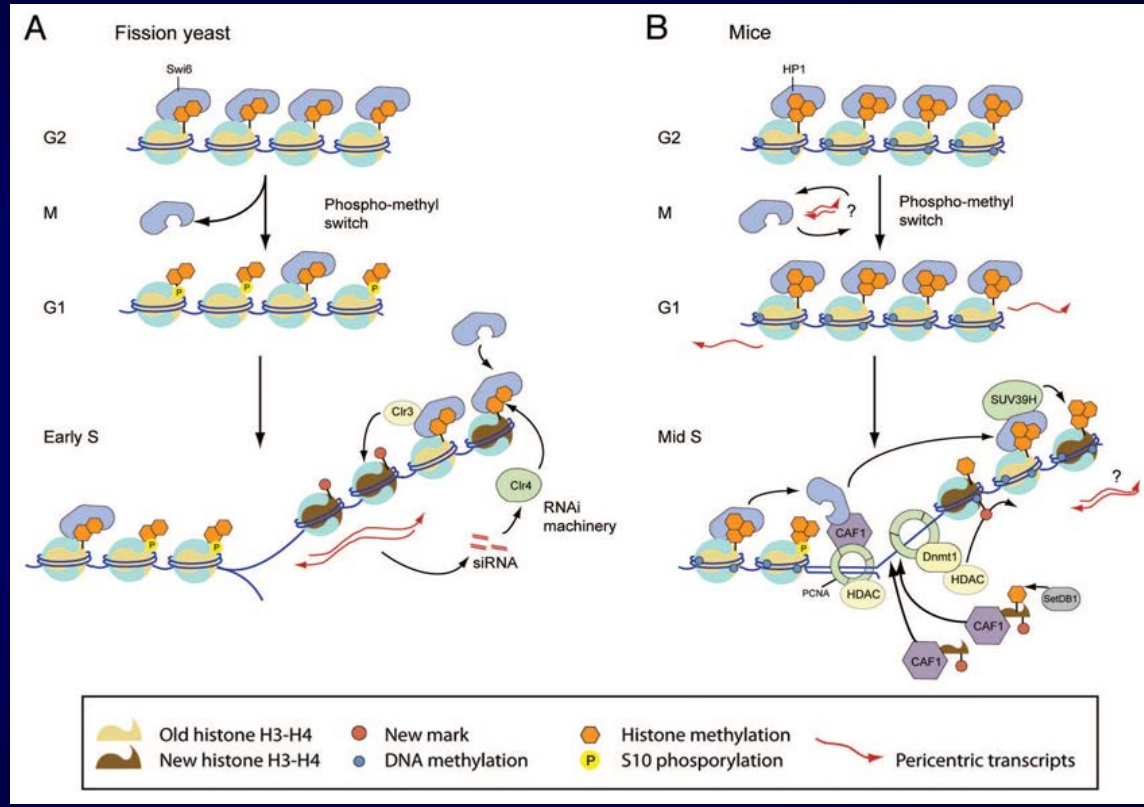
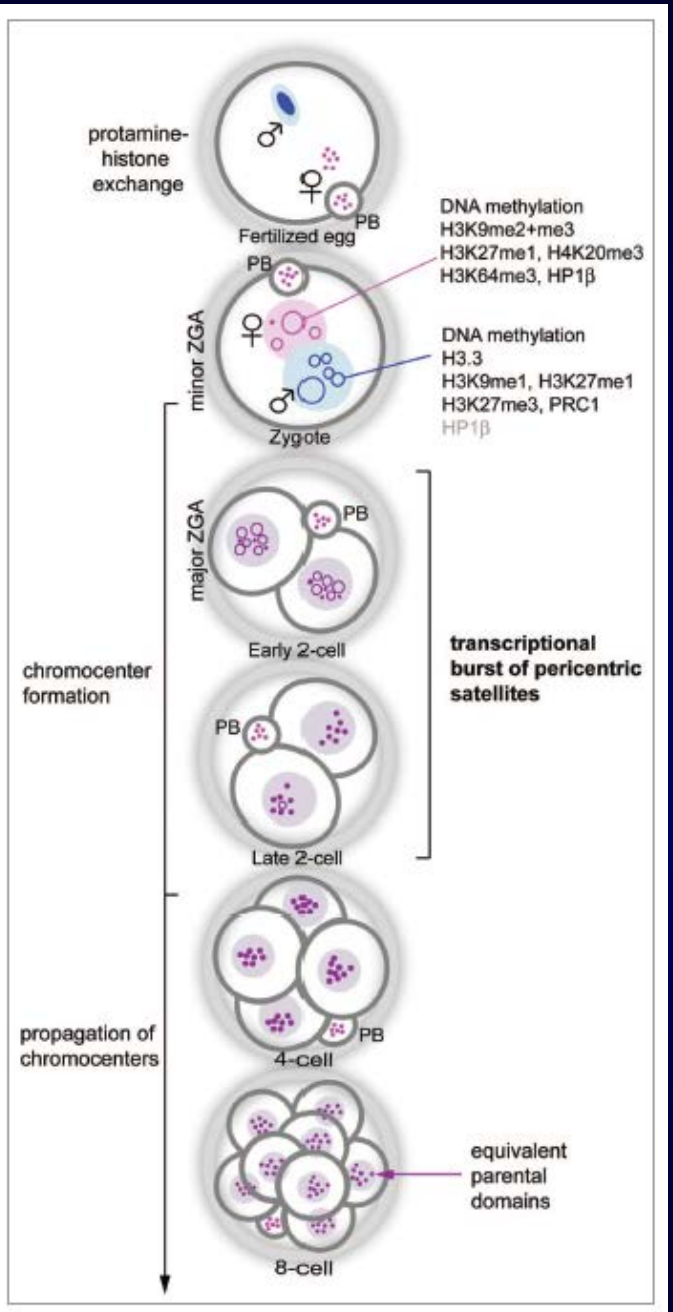
H1: varianty H1^o, H5 a testis-specific varianta H1. varianty H1 se různě uplatňují během buněčného cyklu, diferenciaci a vývoje. RA diferenciaci myších F9 je doprovázena zvýšenou transkripcí histonu H1^o.

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



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)

H3K9me1 deposition in cytoplasm

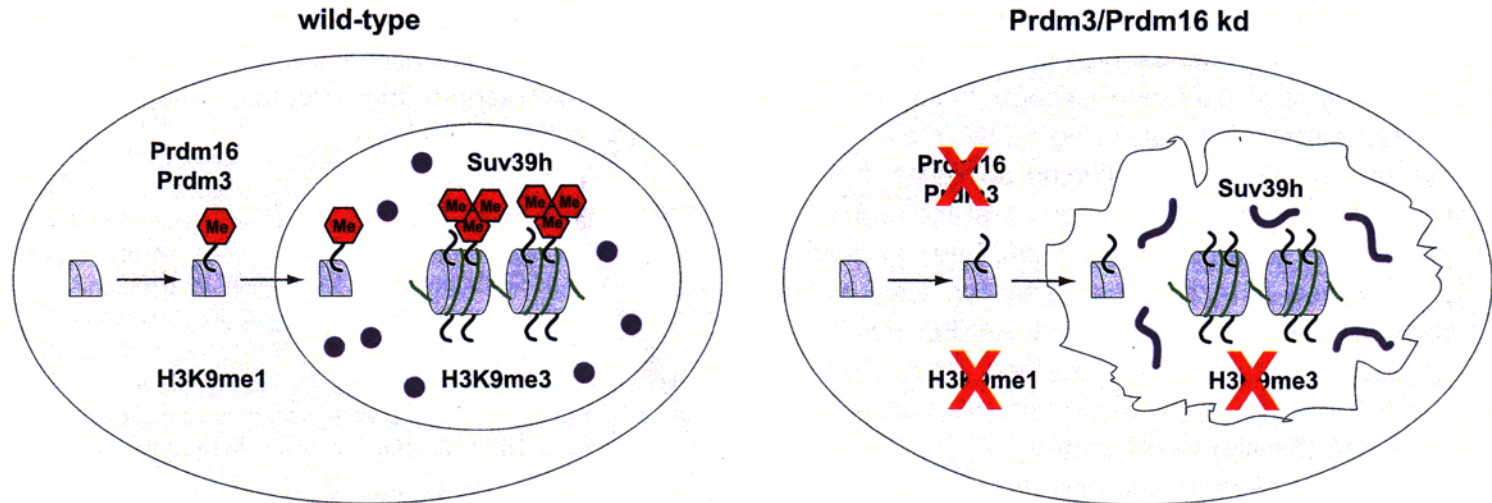
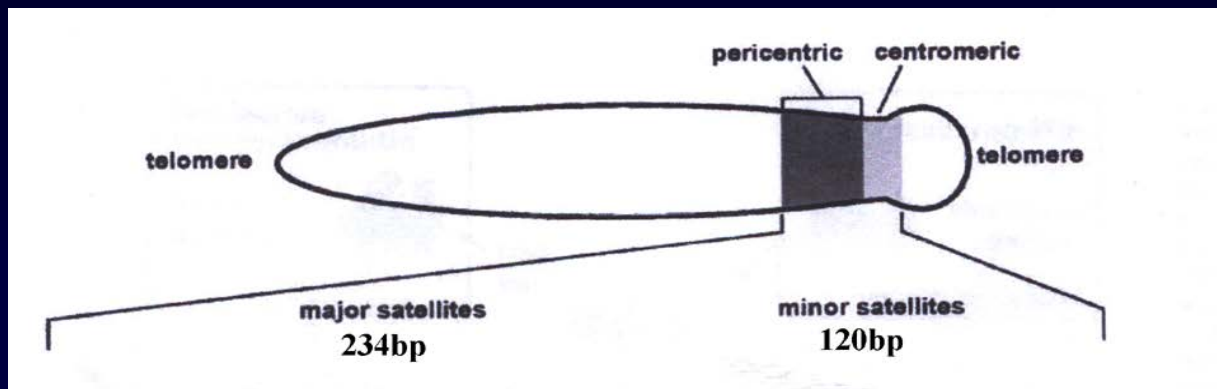


Figure 7. Deposition-Based Model for the Initiation and Maintenance of Mammalian Heterochromatin

In WT iMEFs, Prdm3 and Prdm16 direct H3K9me1 of cytoplasmic histone H3, which is then transported to the nucleus and incorporated into nucleosomes where it is converted by the Suv39h enzymes to H3K9me3 at heterochromatic foci (blue dots) that correspond to clusters of major satellite repeats. Upon Prdm3/Prdm16-depletion, cytoplasmic H3K9me1 is diminished, leading to insufficient generation of pericentric H3K9me3 by the Suv39h enzymes. In addition to the reduction of H3K9me1 (and H3K9me3) at pericentric heterochromatin, impaired Prdm3/Prdm16 function also results in the collapse of the major satellite repeat clusters (blue fibers) and breaks a proposed anchor in the nuclear lamina, which becomes unstructured (disorganized black outline). This model predicts the Prdm3 and Prdm16 KMTs as multi-functional enzymes with substrates that are not restricted to histone H3. See text for detailed explanation.



Varianty histonů

H3: existují dvě hlavní

Varianty H3.3 a

centromerické varianty

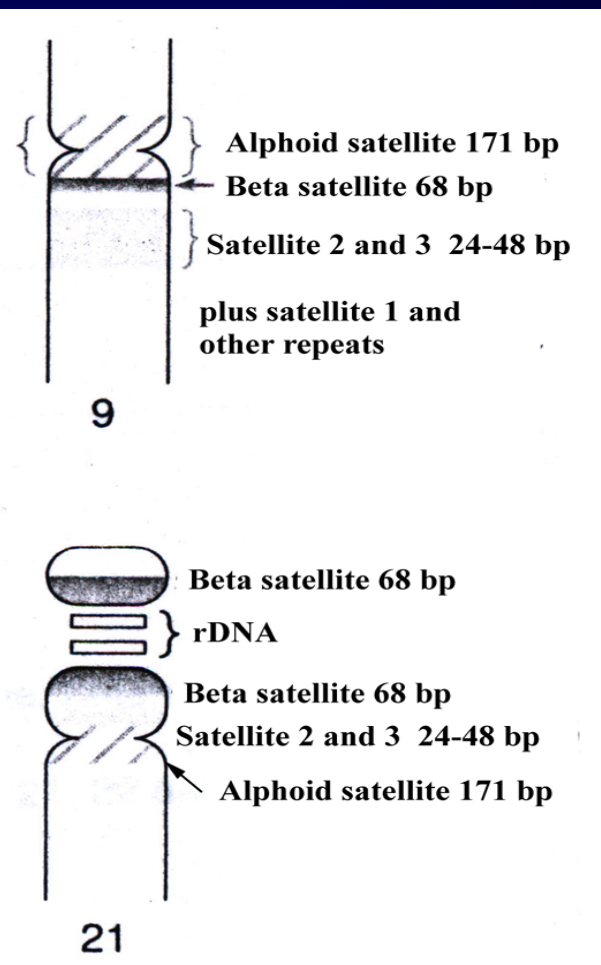
H3 (cenH3) = CENP-A-Z:

jsou zodpovědné za

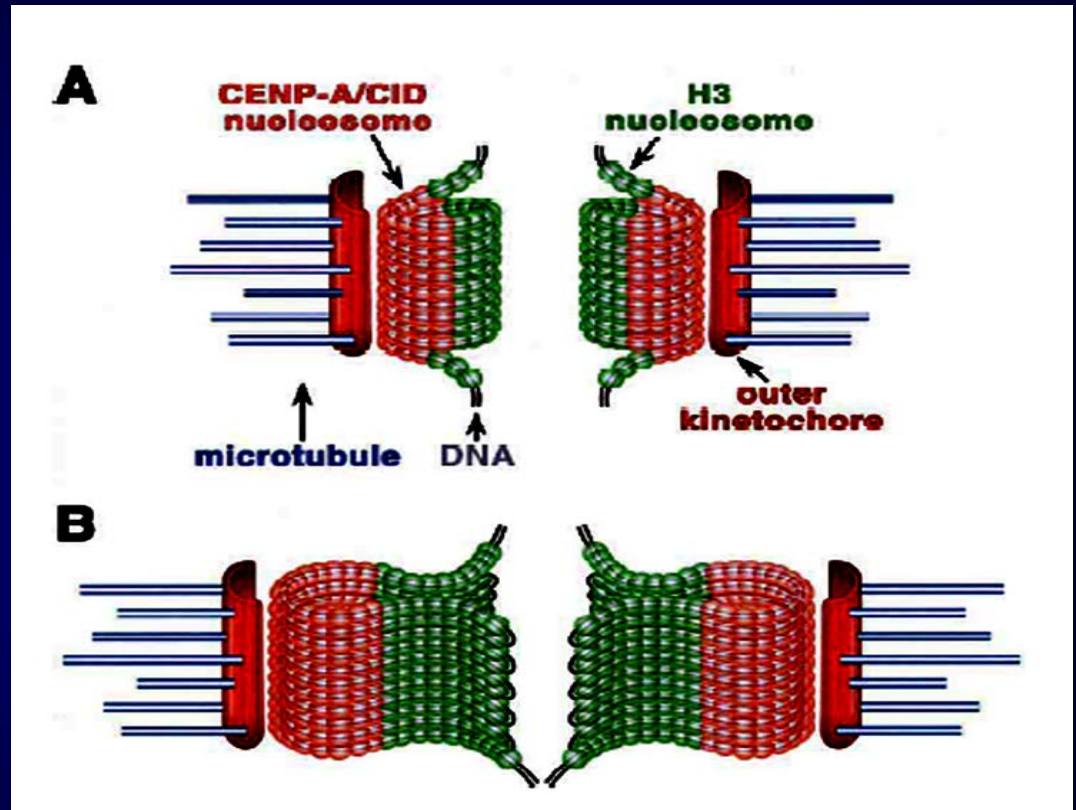
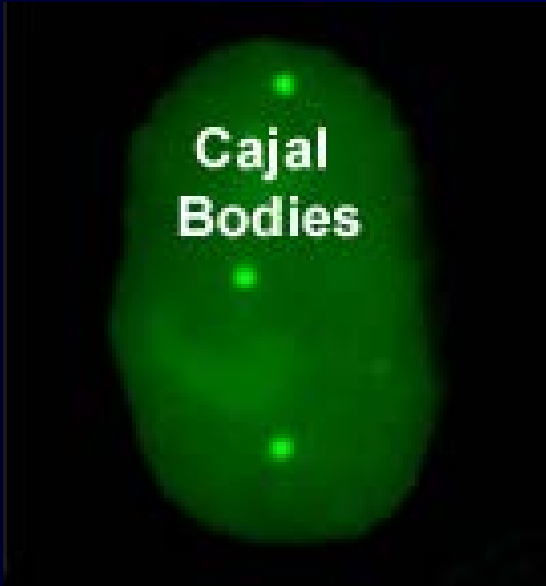
vazbu kinetochoru a

segregaci sesterských

chromatid u eukaryot



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

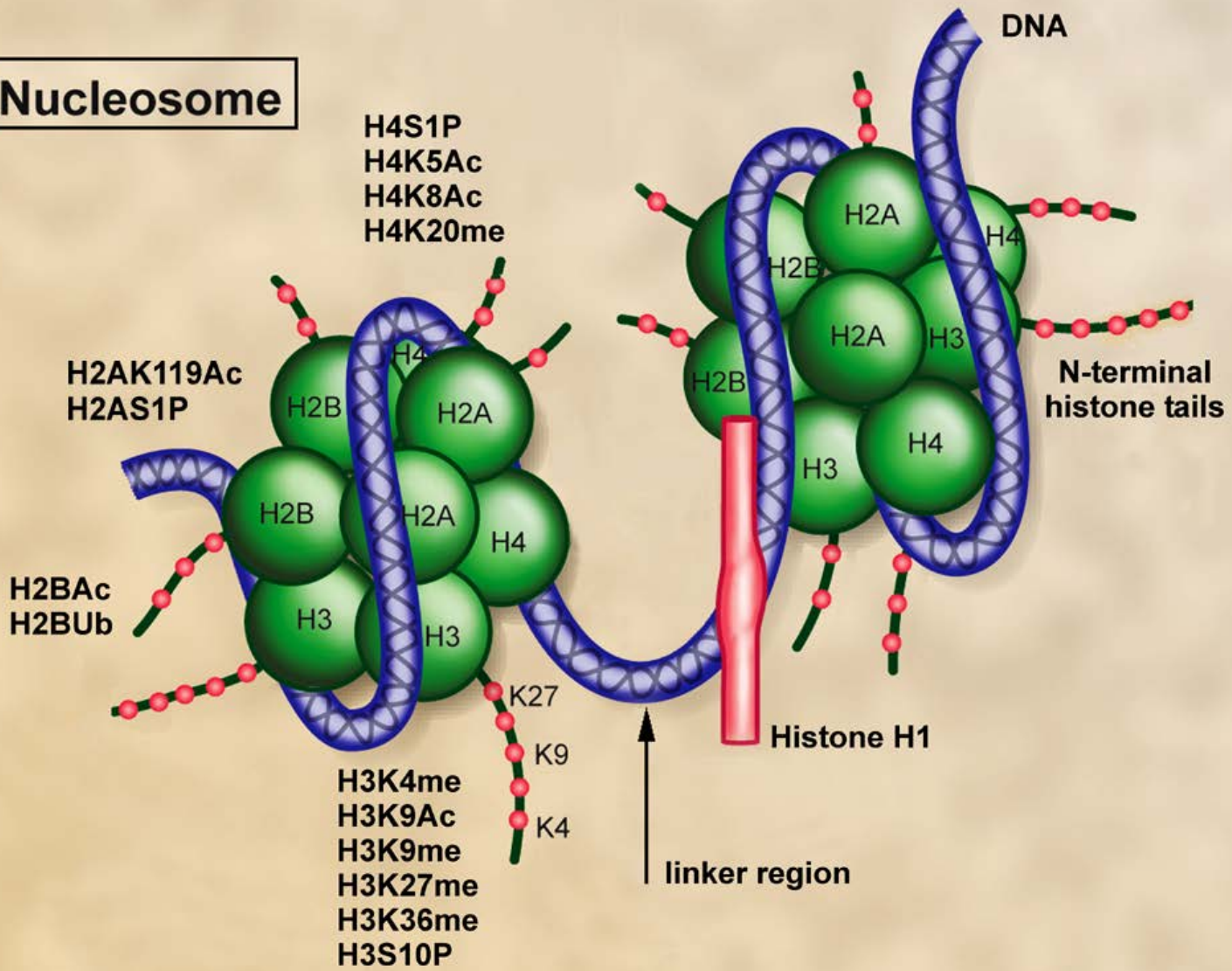


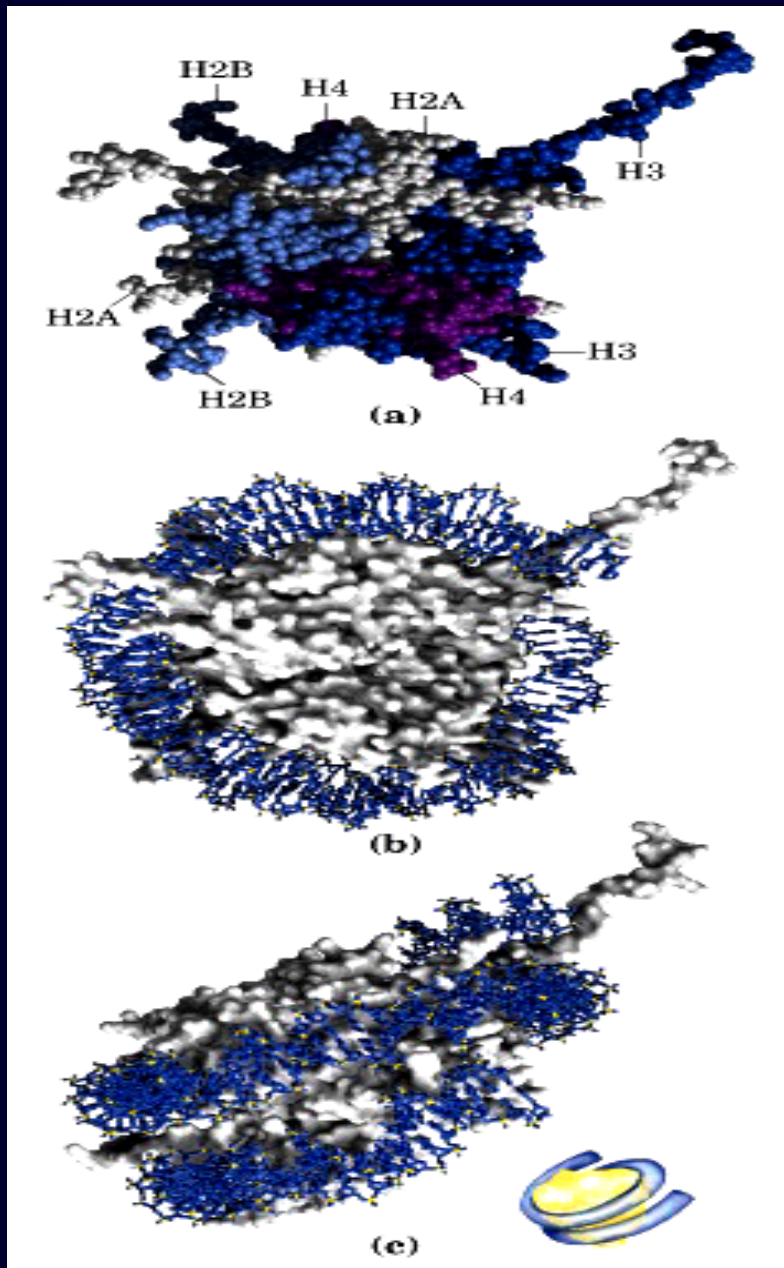
Varianty histonů H4: většina genů kódujících hlavní histonové proteiny jsou exprimovány během S fáze buněčného cyklu. V případě H4, geny jsou konstitutivně exprimovány během buněčného cyklu. Pro H4 nejsou známy žádné varianty. Úpravy pre-mRNA histonů probíhají v Cajal bodies.

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





Vztah mezi acetylací a metylací histonů: acetylace histonů je katalyzována histonovými acetyl-transferázami (HATs) a odstraňována 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.

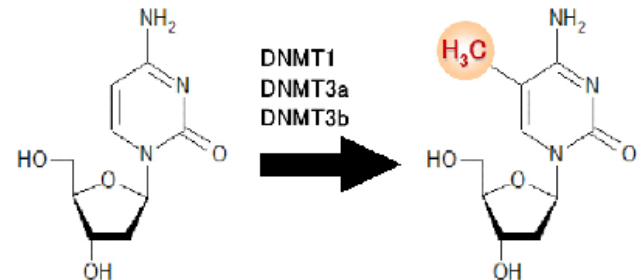
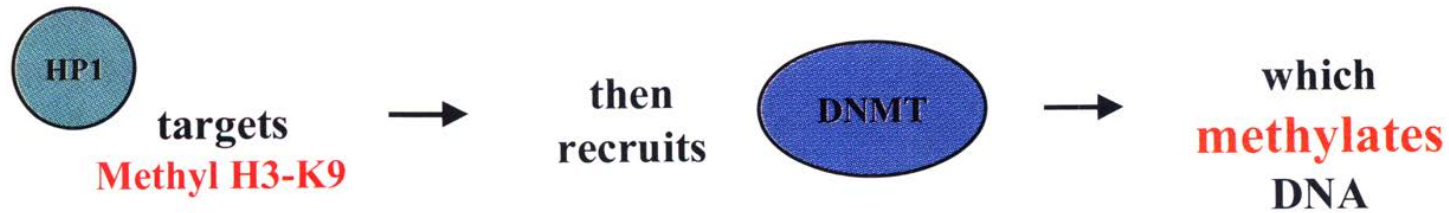
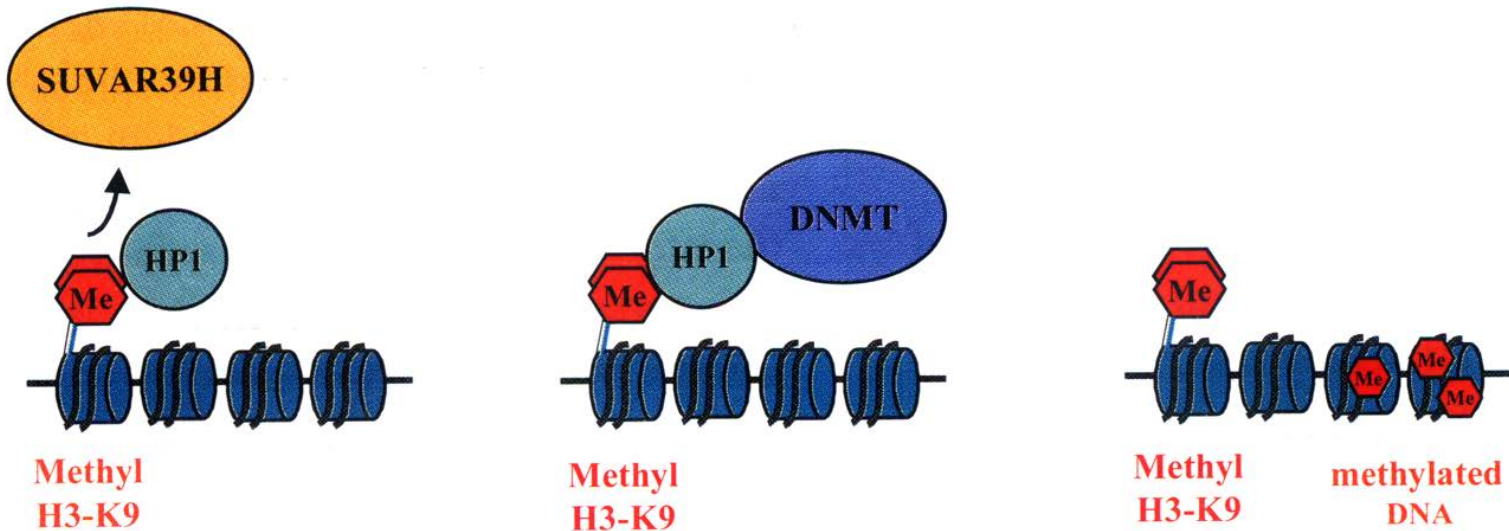
HATs: HAT1, PCAF, CBP/p300, TFIIC90, ELP3, SRC1, CLOCK
(see Allis et al., 2007).

HDACs: Class I, II, III

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

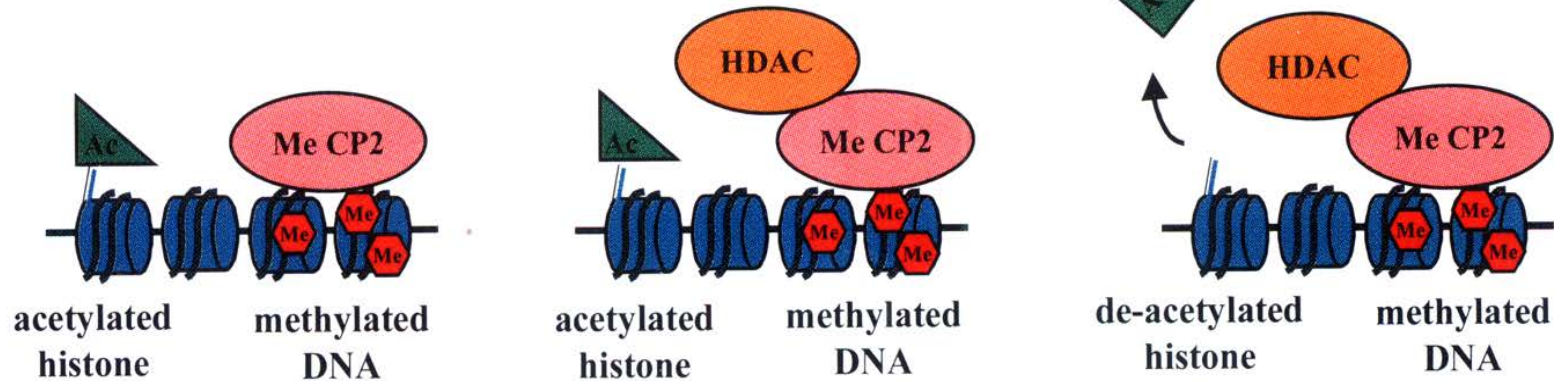
Demethylases: LSD1 (antagonizuje H3K4me-transcriptional activation), JHDM1b (H3K4me3), Jmjd2b (H3K9me3), JHDM2a, JMJD2B (antagonizuje H3K9me2/me3 - heterochromatin formation)

Histone H3-K9 methylation induces DNA methylation



DNA methylation induces histone de-acetylation

INAKTIVITY



Me CP2
binds
methylated DNA



then recruits

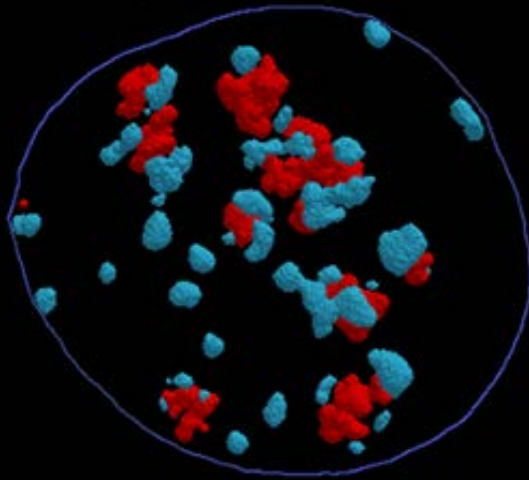


which
de-acetylates
histones

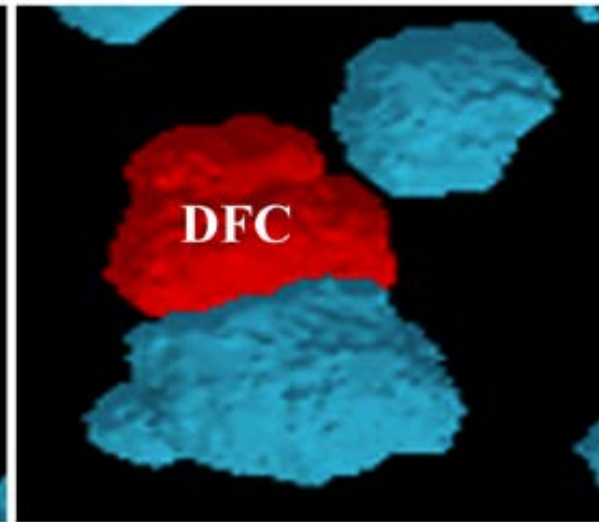
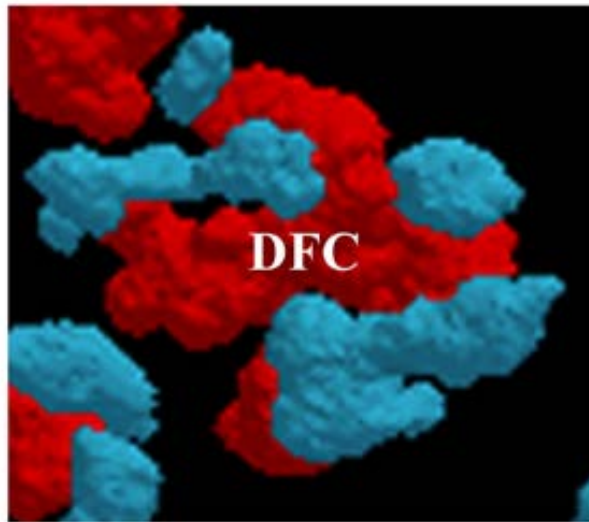
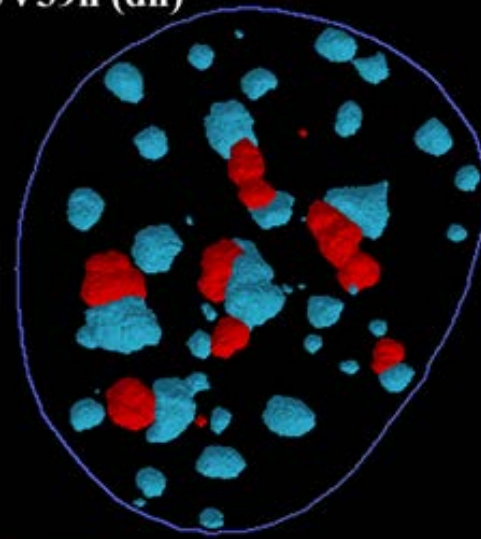
MeCP2: Methyl-CpG binding Protein, specifically binds to to methylated DNA

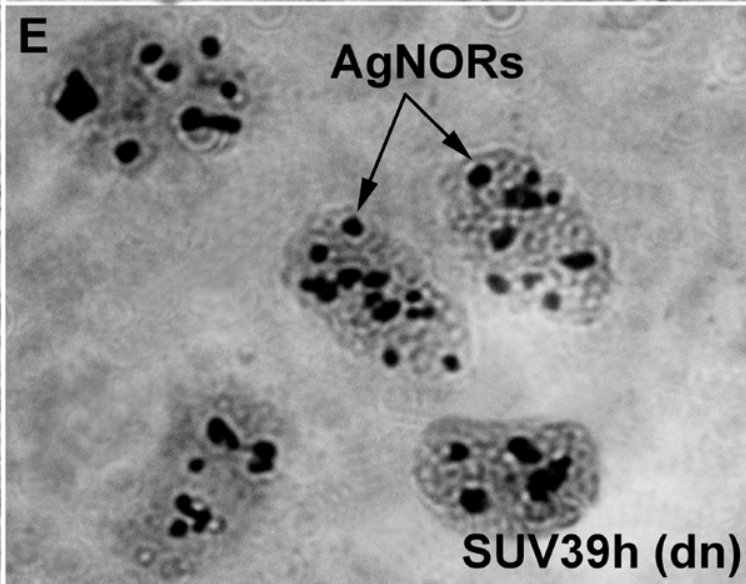
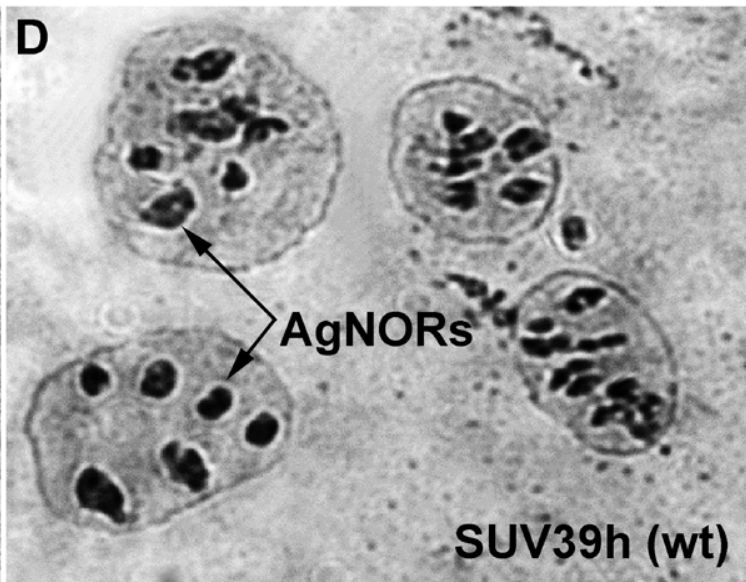
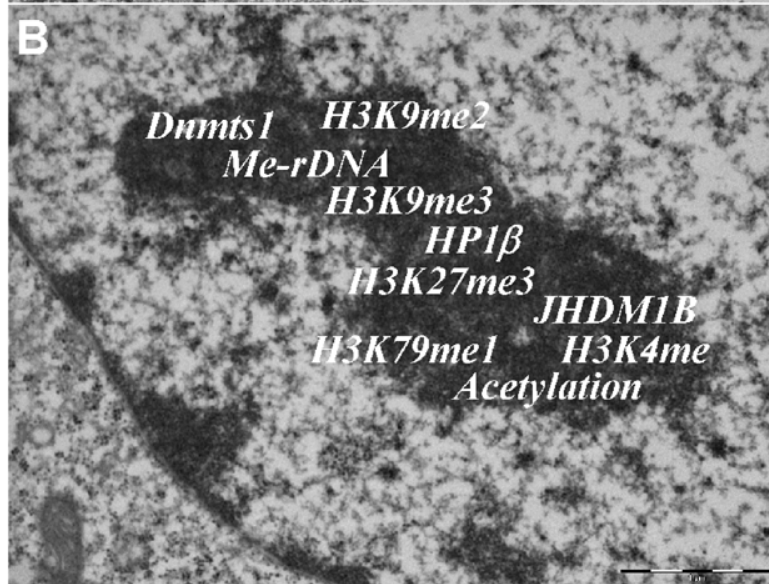
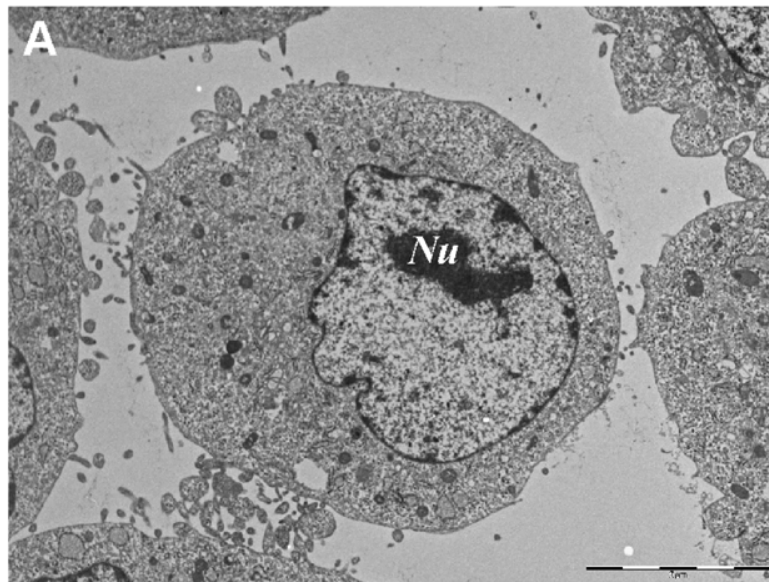
Fibrillarlin / Chromocenters

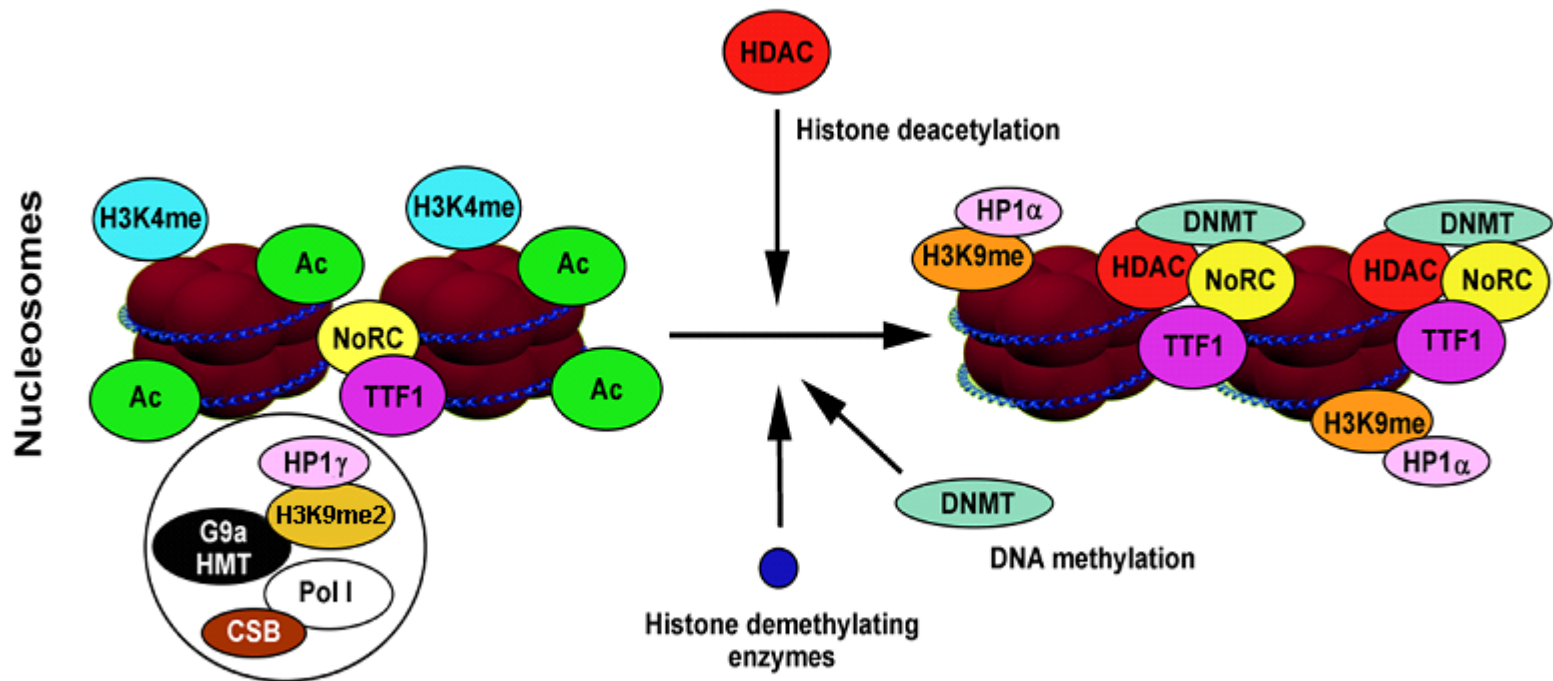
SUV39h (wt)



SUV39h (dn)



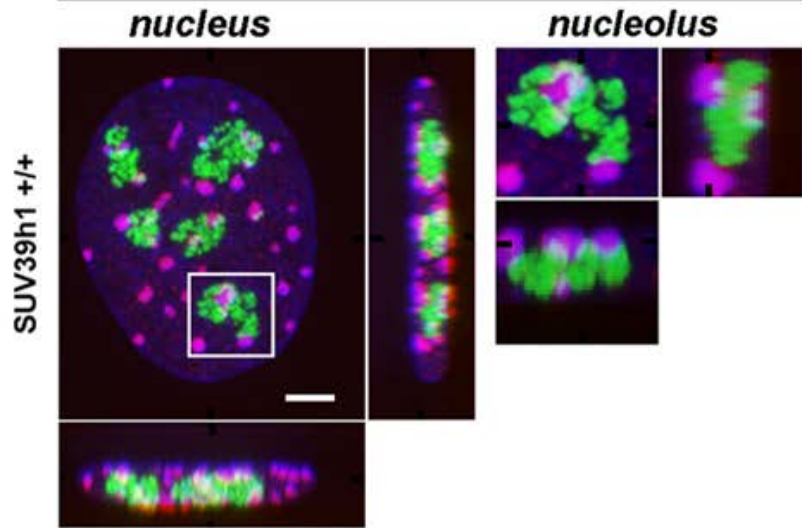




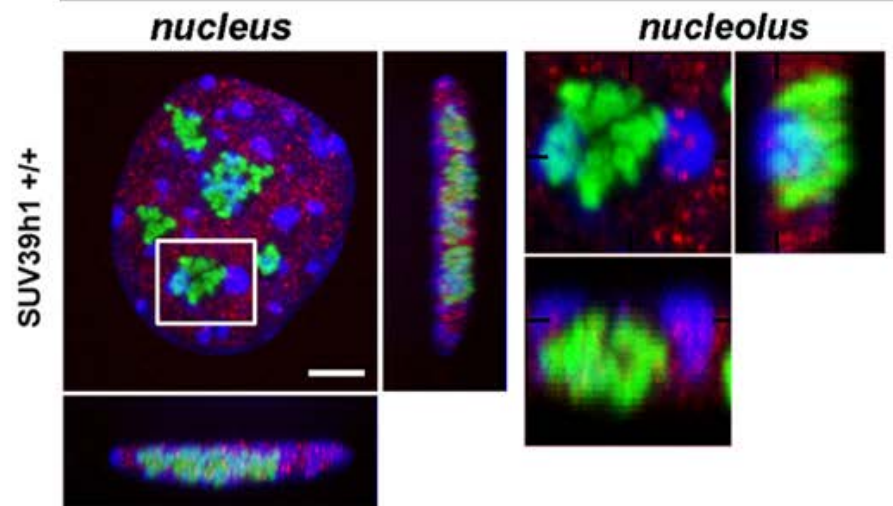
Transcription of rDNA genes

Silencing of rDNA genes

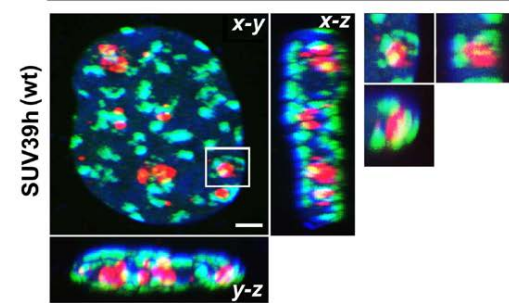
Fibrillarin / HP1 α / DNA



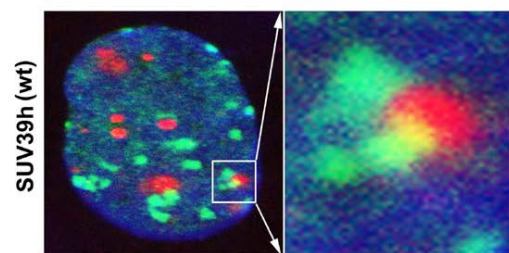
Fibrillarin / HP1 γ / DNA



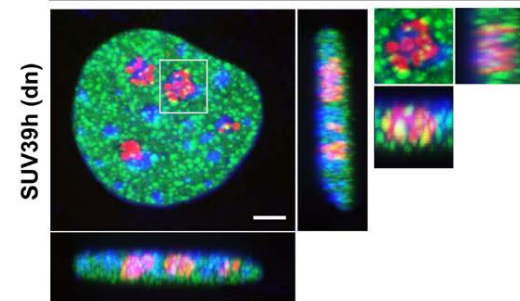
a Fibrillarin / GFP-HP1 β



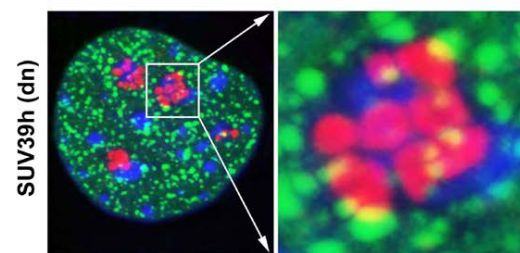
individual confocal section



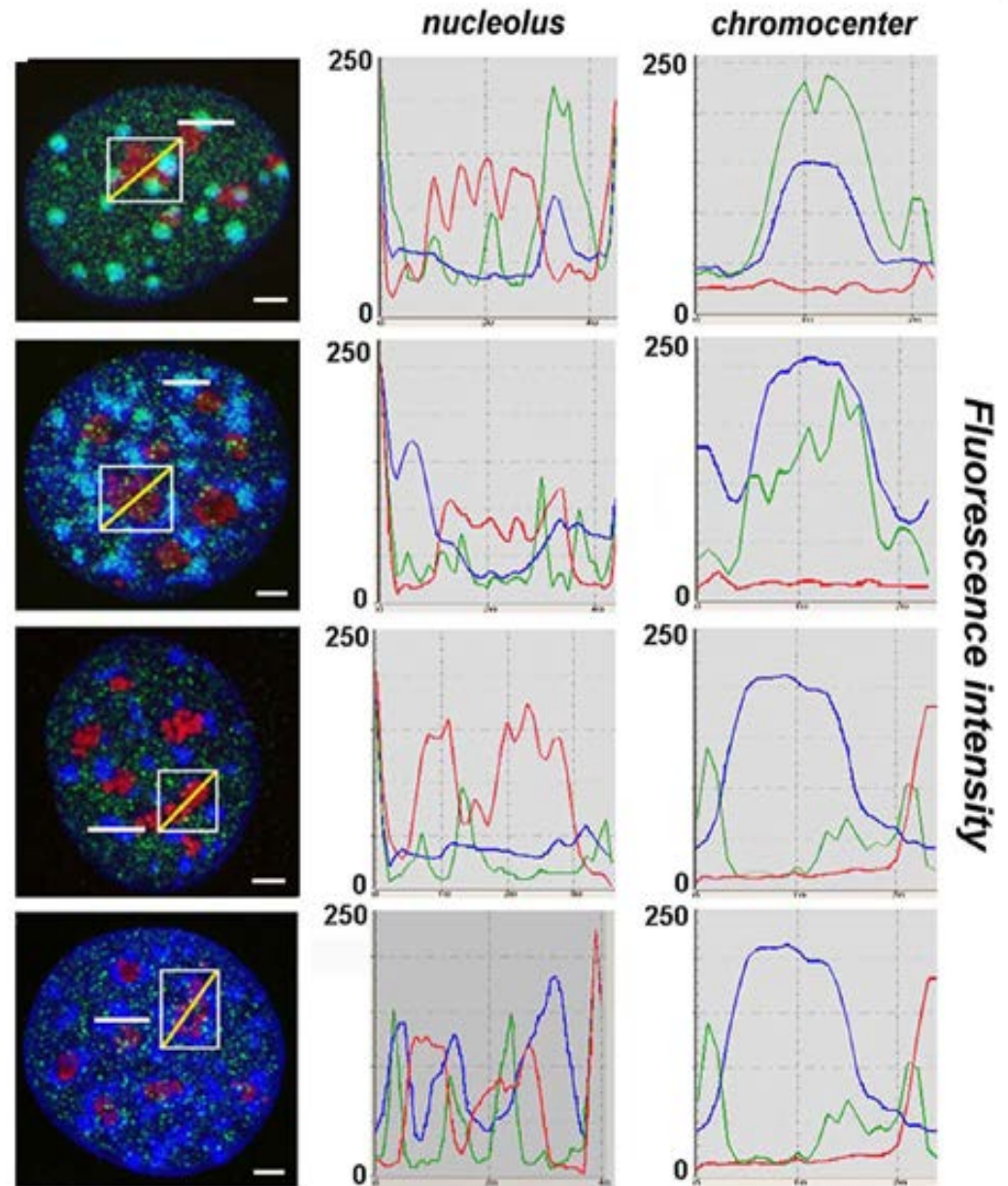
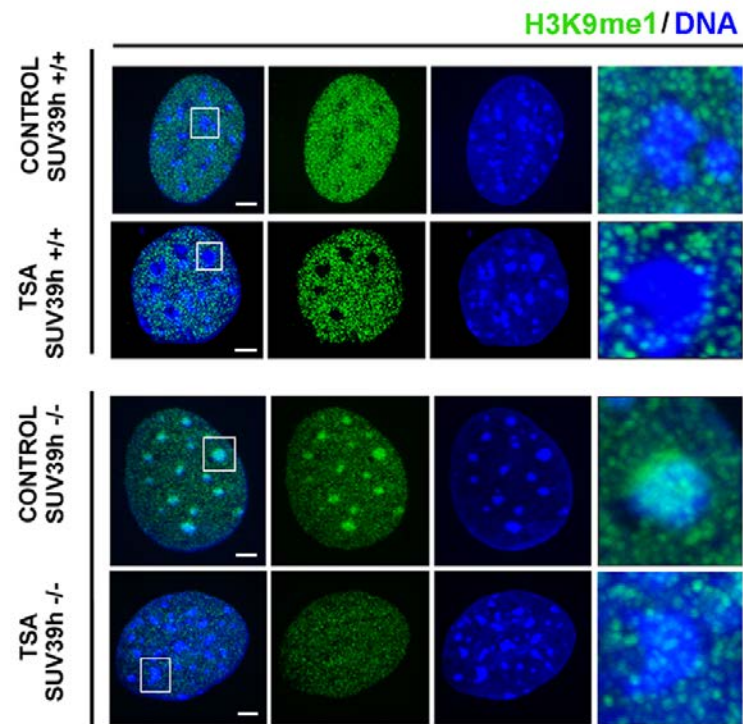
b Fibrillarin / GFP-HP1 β

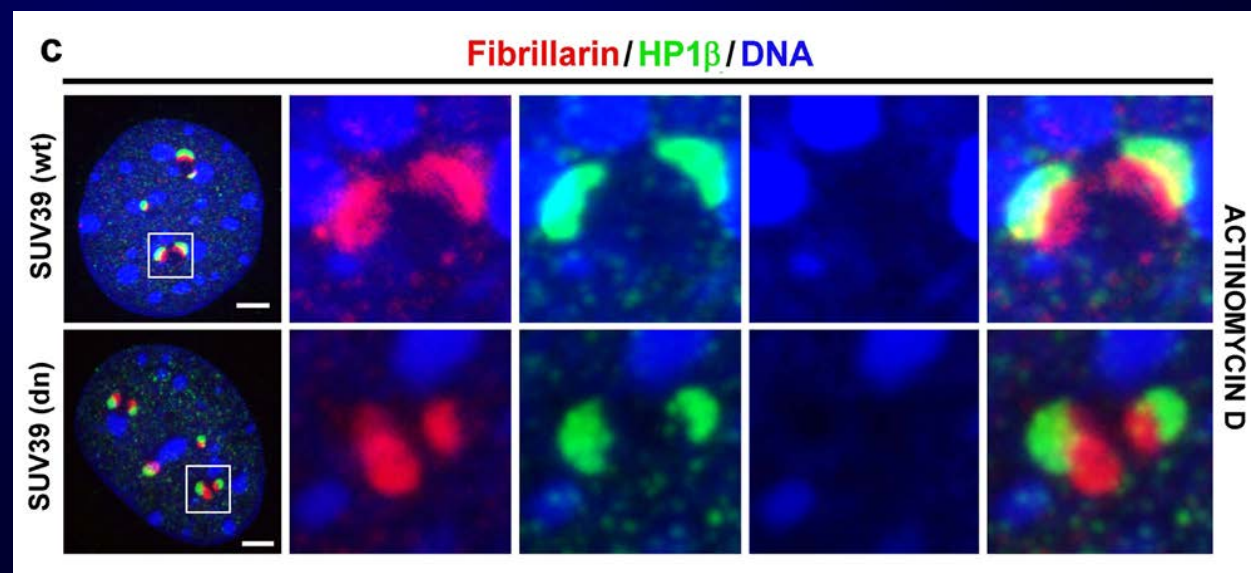
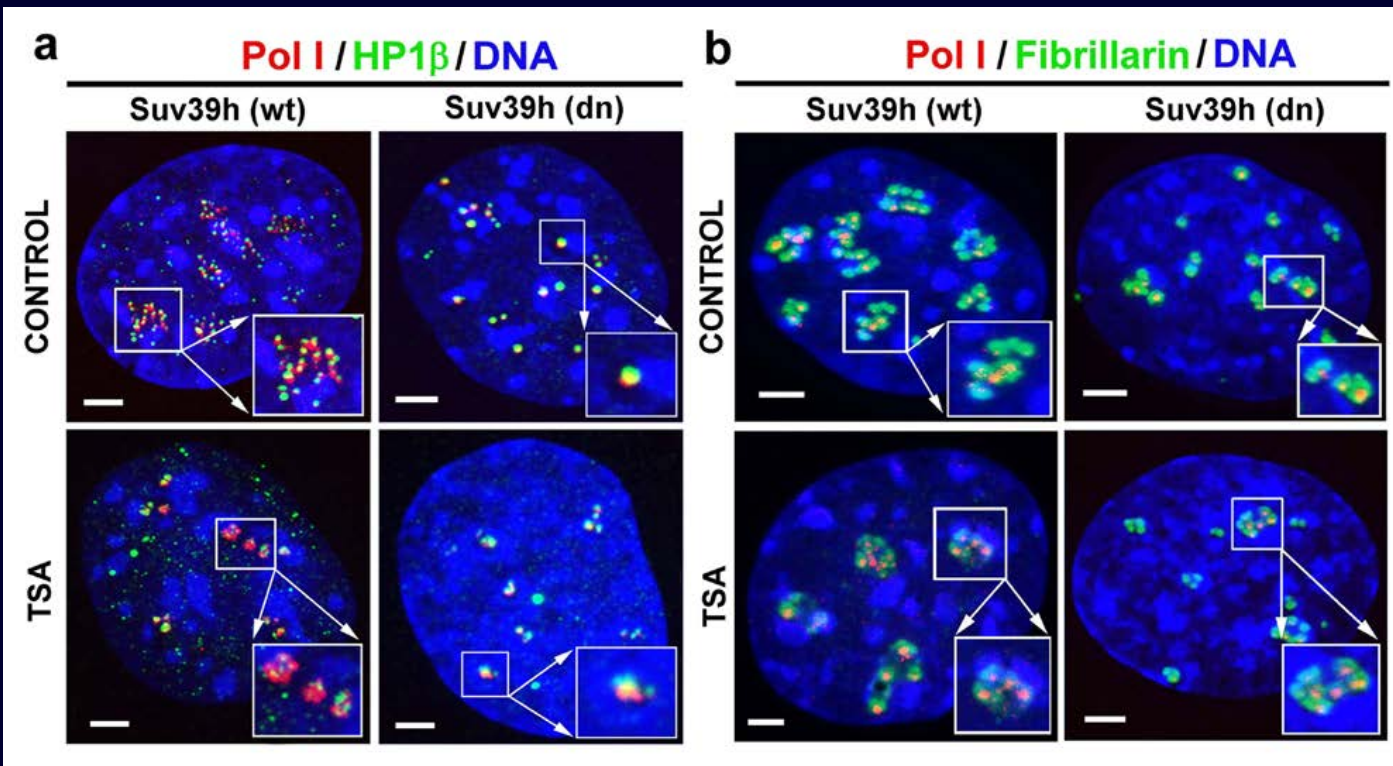


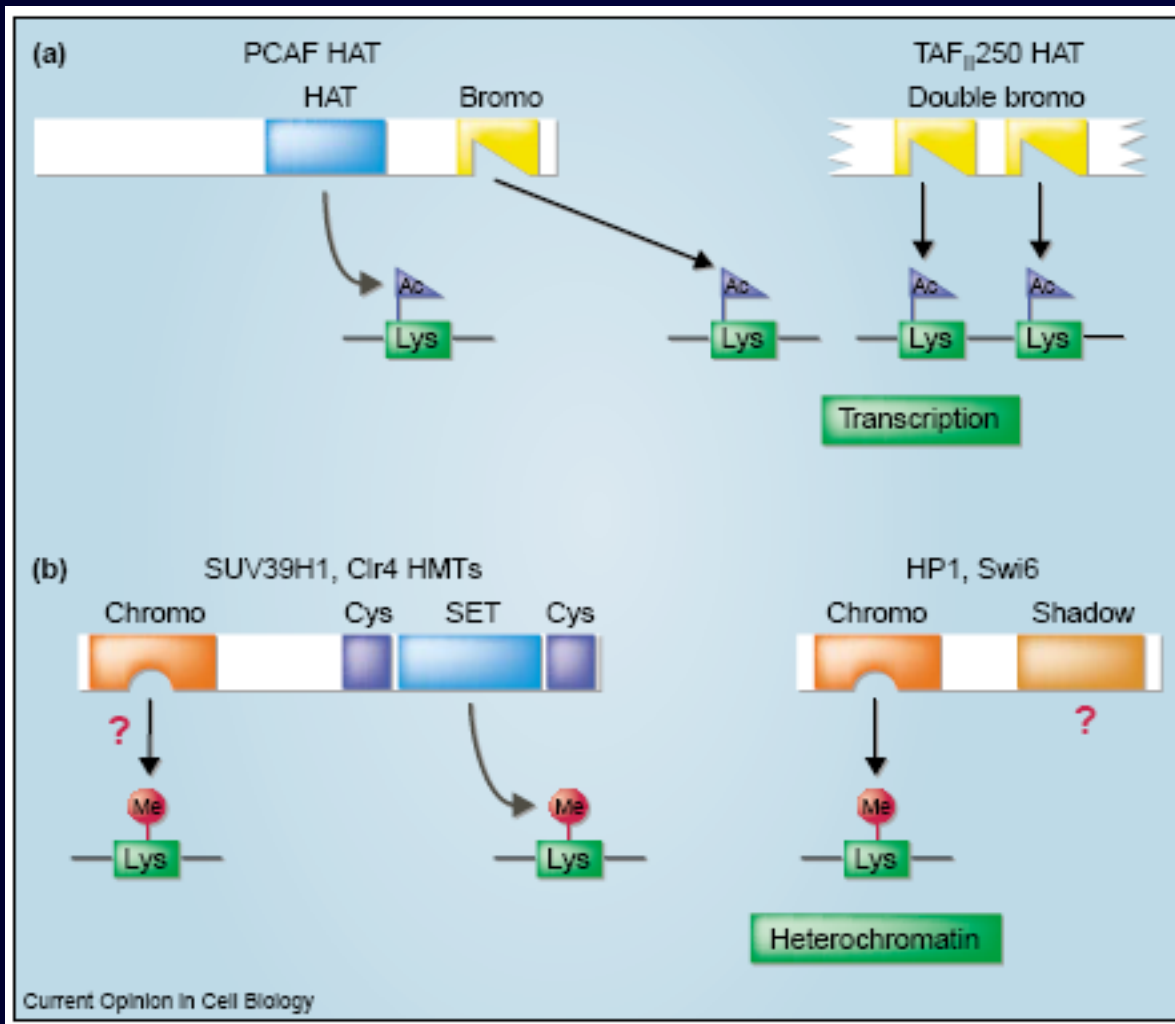
individual confocal section



Fibrillarin / H3K9me3 / DNA





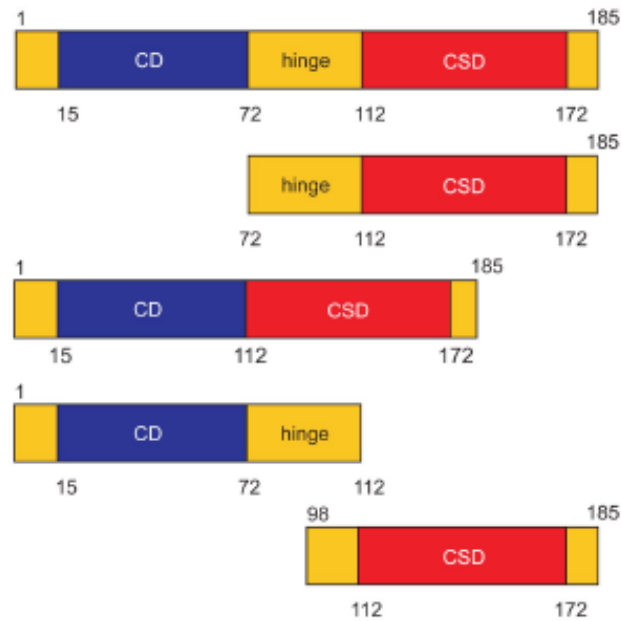


HP1 protein

CD: protein-chromatin

CSD: protein-protein

HD: HP1-to-DNA and linker histones



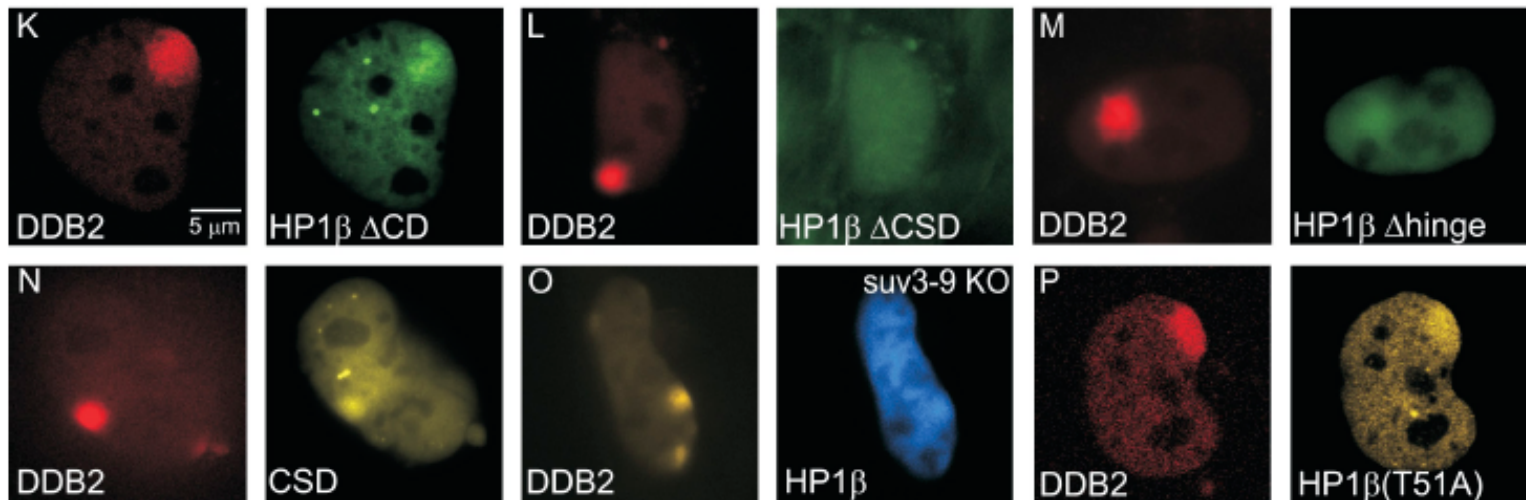
Full-length HP1β

HP1β ΔCD

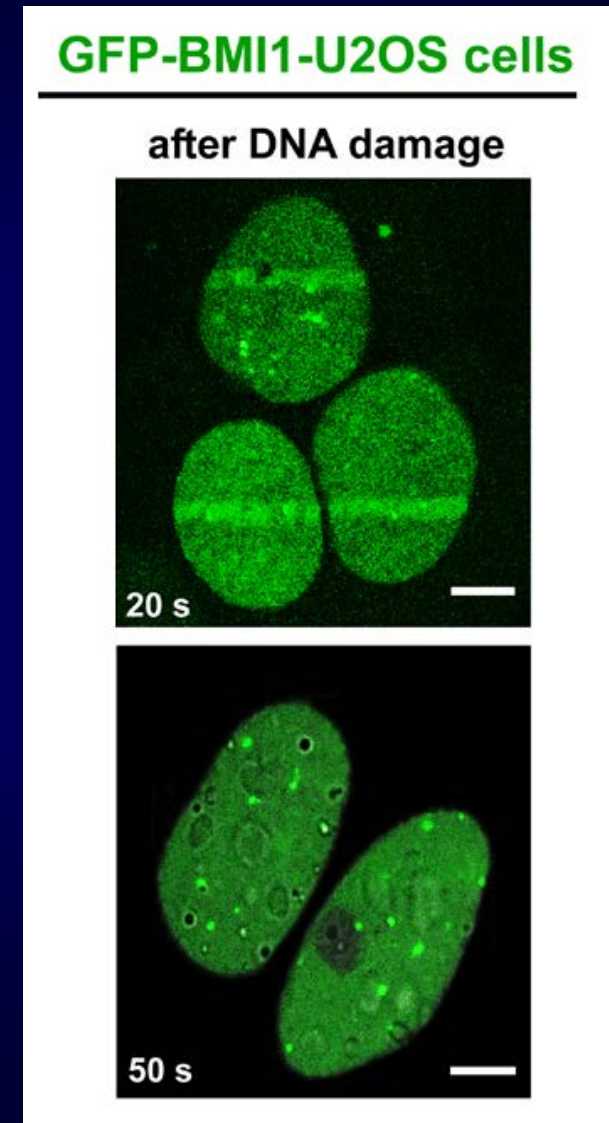
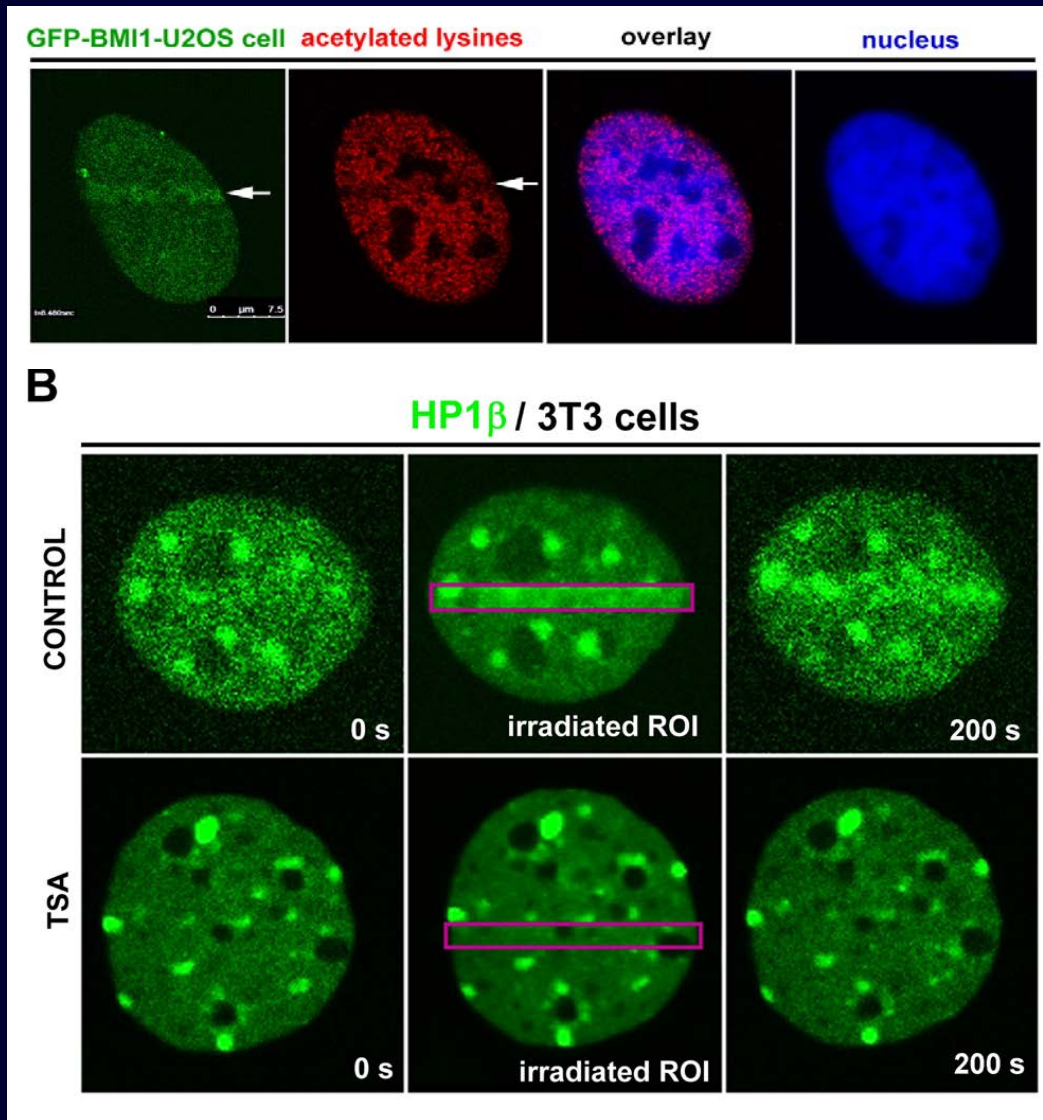
HP1β Δhinge

HP1β ΔCSD

CSD



HP1 β protein in DSBs



A

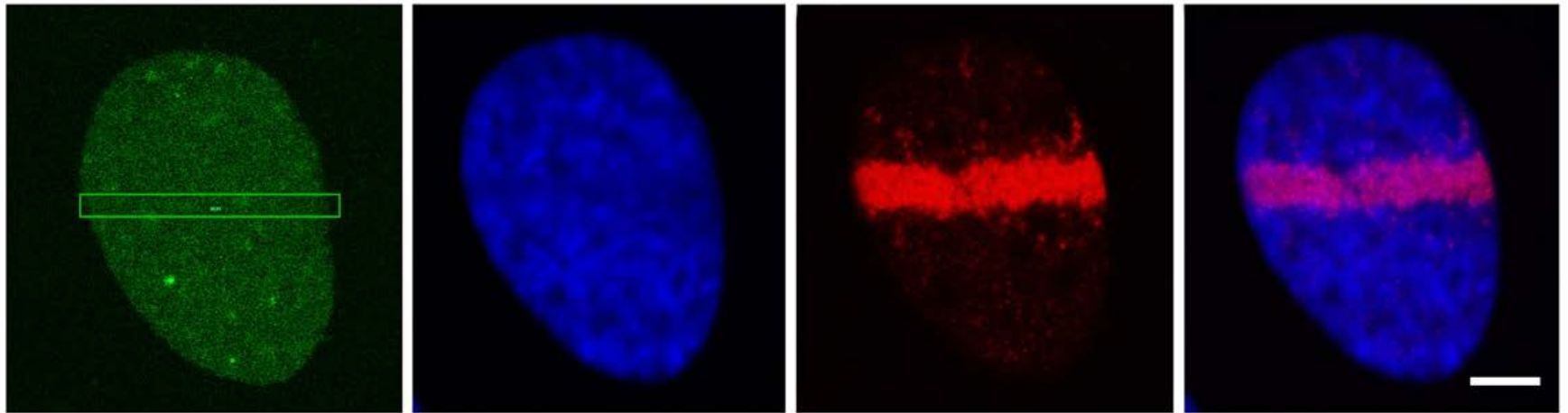
GFP-BMI1-U2OS cell
micro-irradiated

Nucleus

γ H2AX

γ H2AX / Nucleus

Non-treated cells



Experiments of Gabriela Šustáčková

DNA repair

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

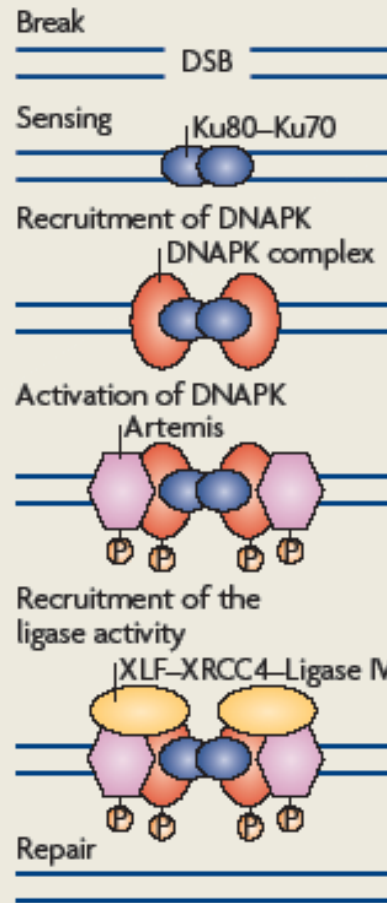
Non-homologous end joining

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.

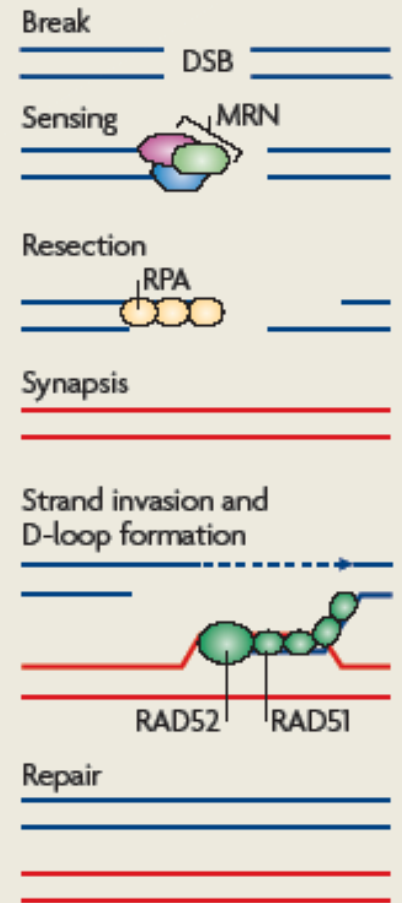
Homologous recombination repair

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.

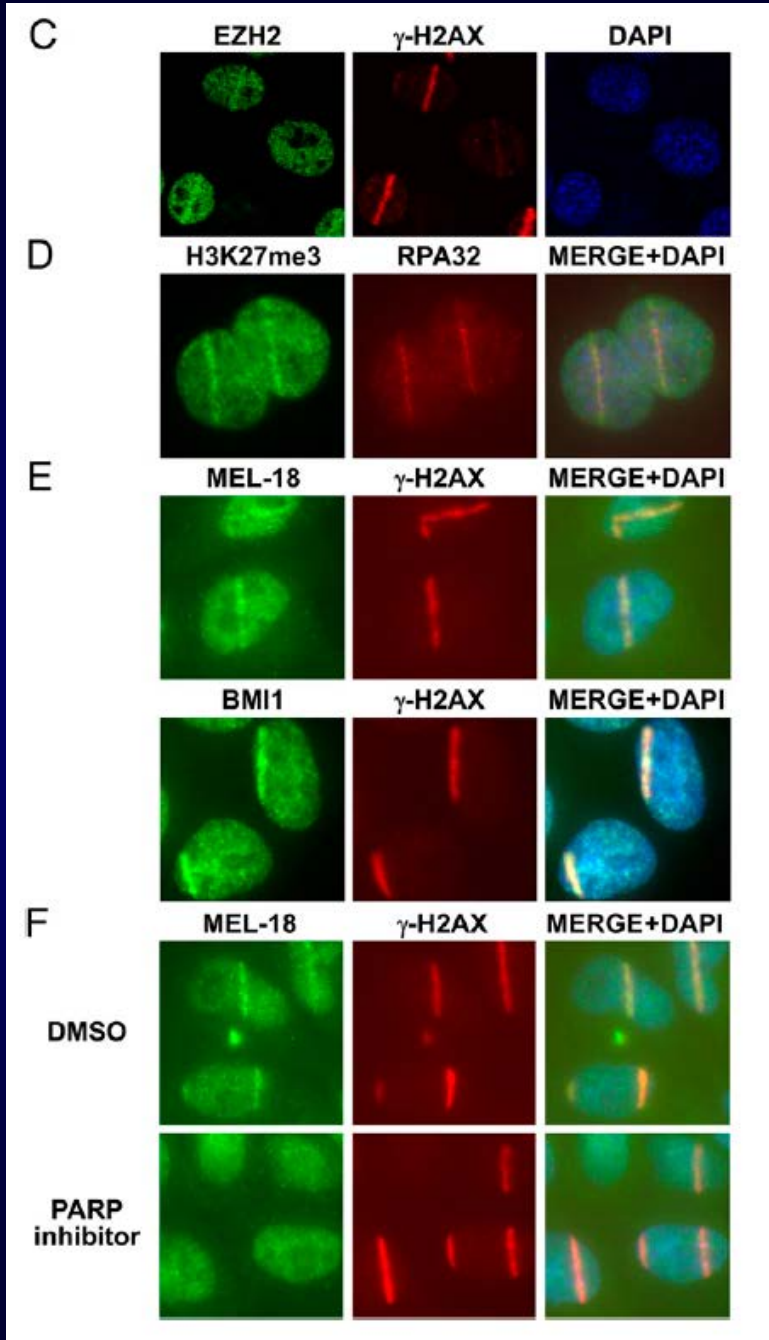
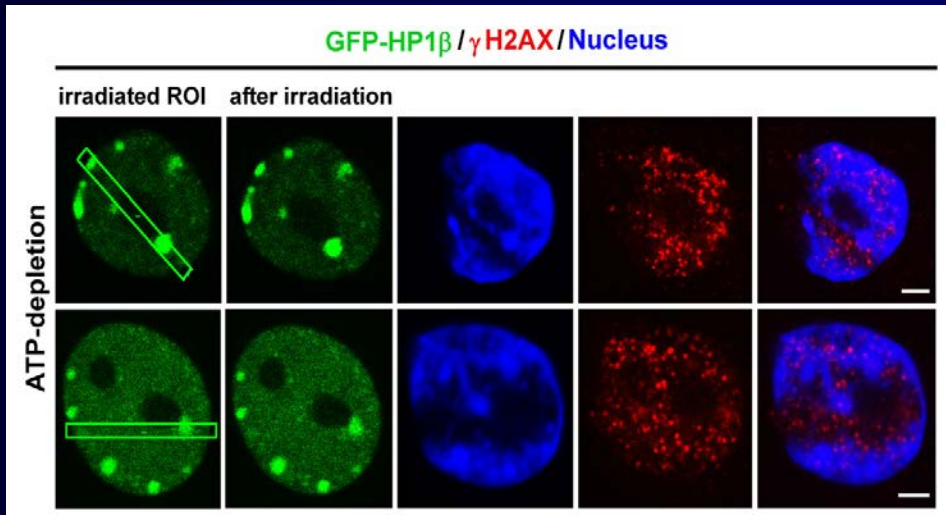
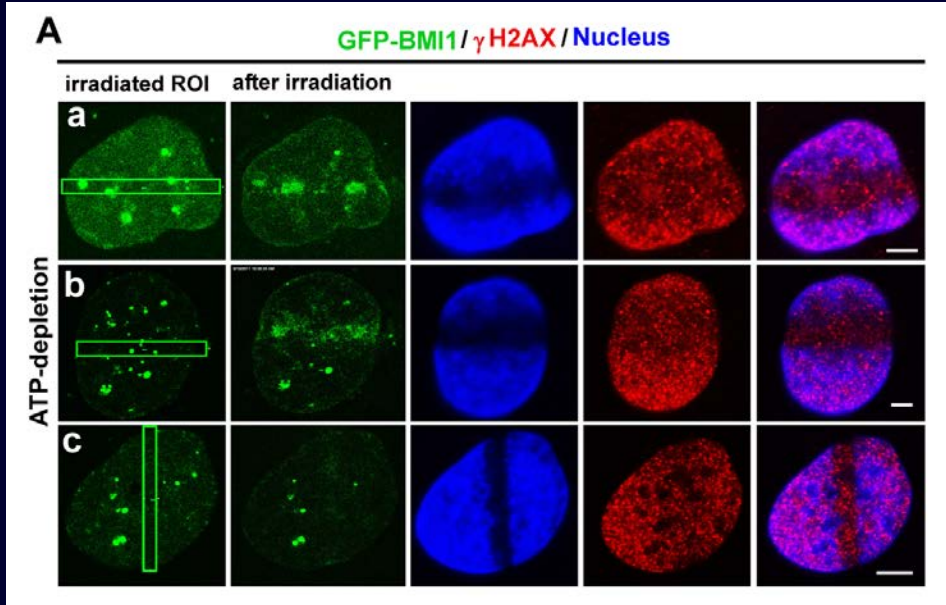
Non-homologous end joining



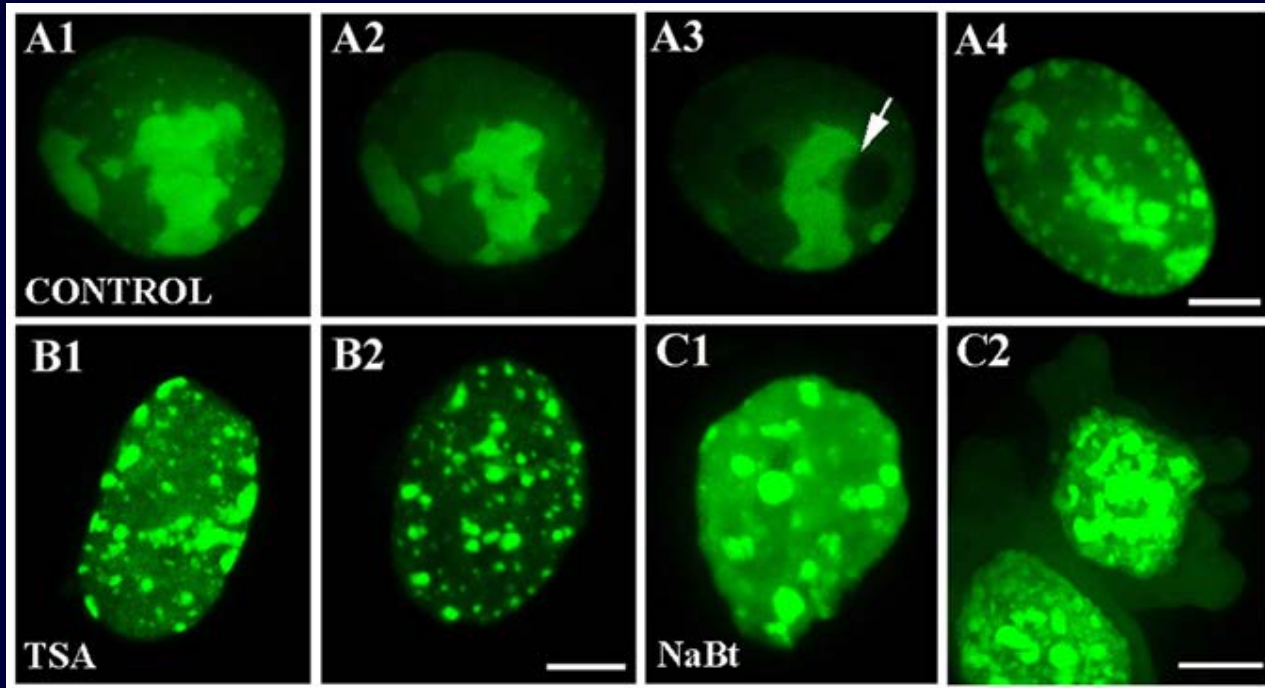
Homologous recombination



ATP depletion

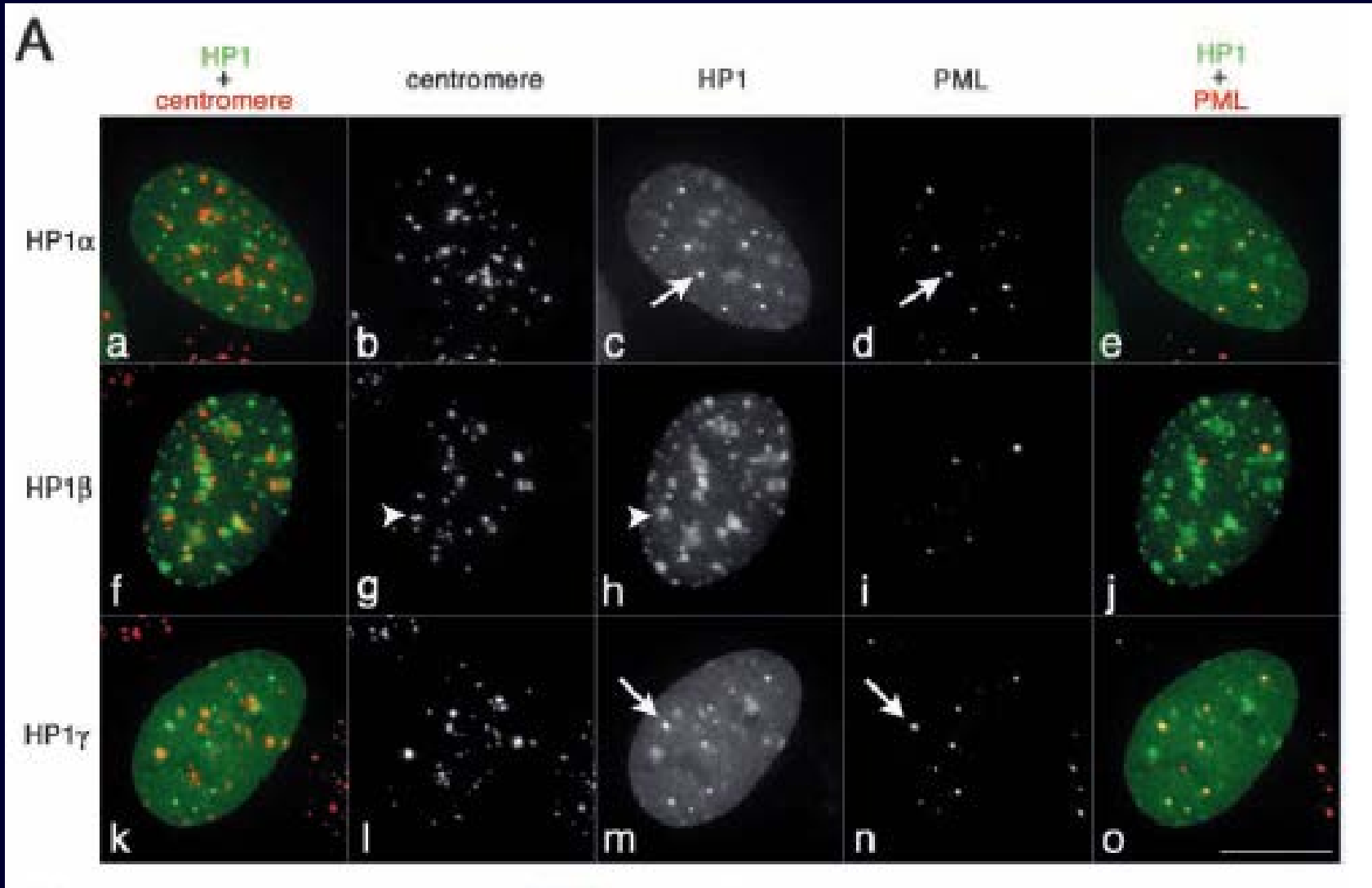


HP1 proteins



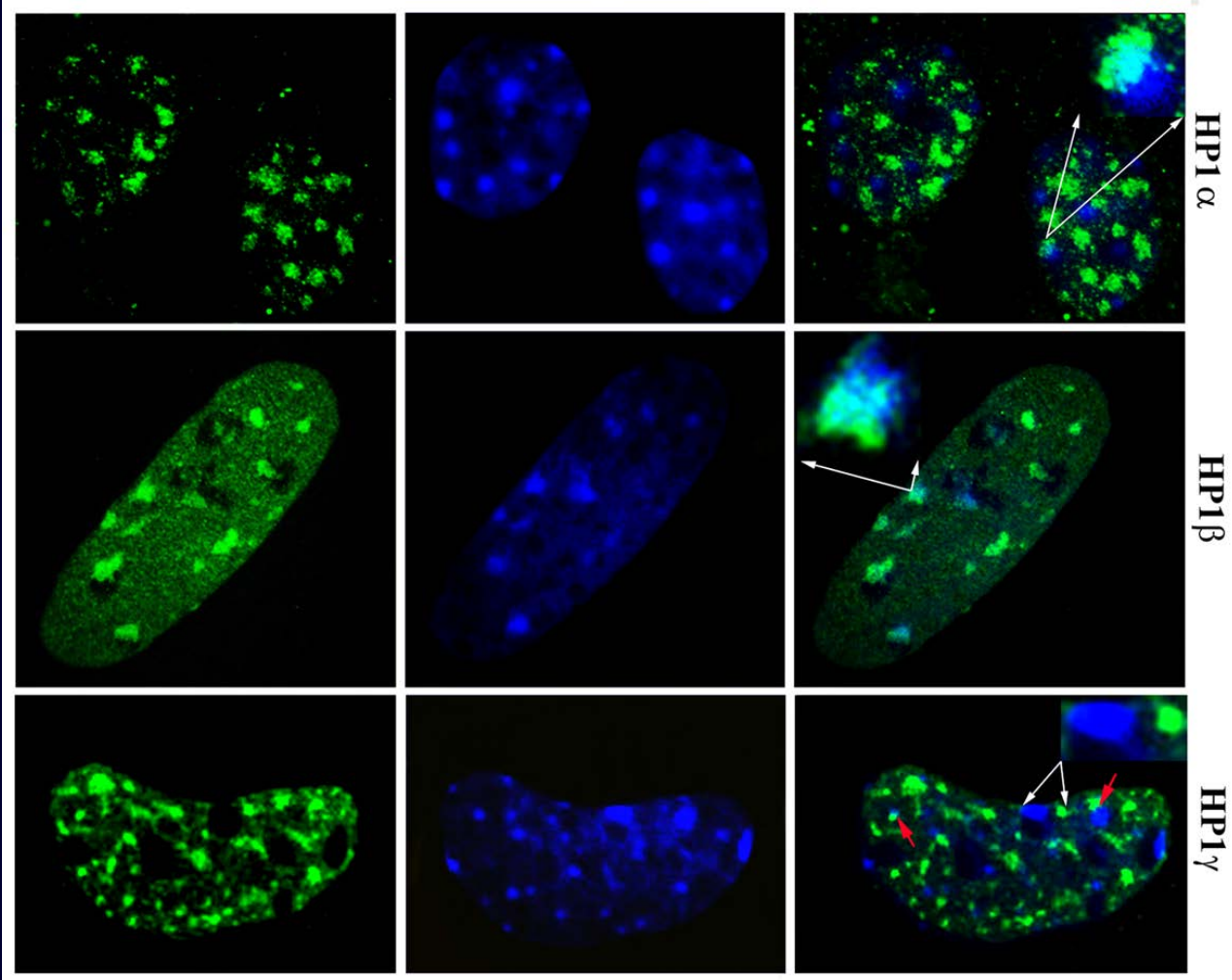
- HP1 proteiny jsou hlavní složkou heterochromatinu a hrají důležitou úlohu při jeho tvorbě. HPs mají vysokou afinitu k pericentromerickým a telometrickým oblastem chromosomů.
- HPs interagují s HMTs jako je SUV39h1 a SUV39h2, která jsou zodpovědné za metylaci H3(K9).

HP1 proteiny – v lidských buňkách jsou 3 sub-typy



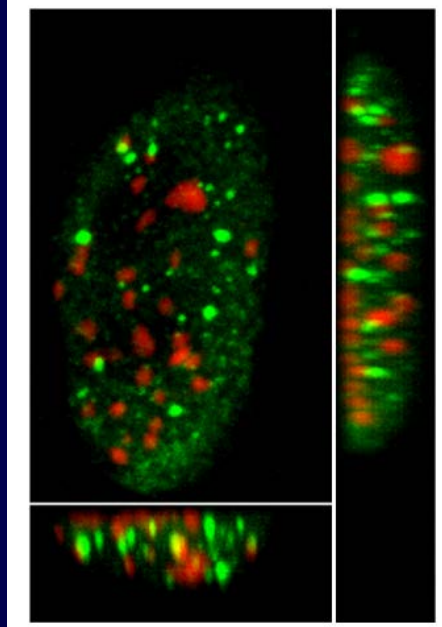
Hayakawa et al., 2003

HP1 proteiny u ECS



HP1 α HP1 β

CONTROL

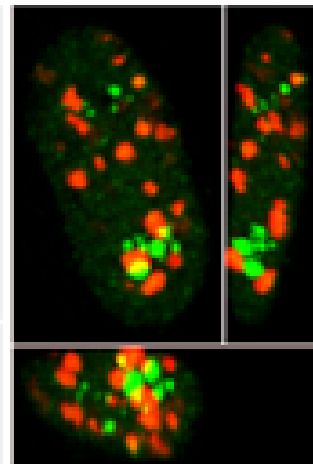
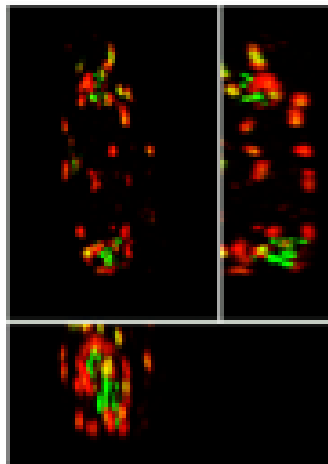
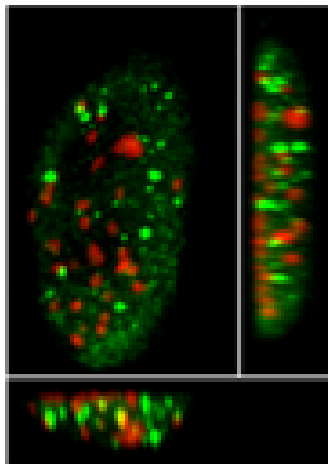


P19 / HP1 α / HP1 β

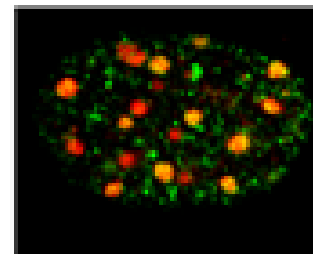
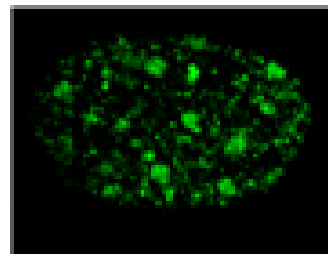
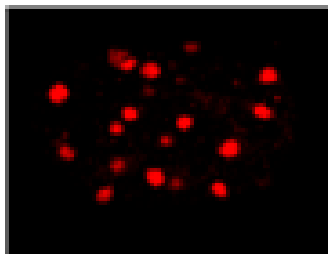
CONTROL

TSA

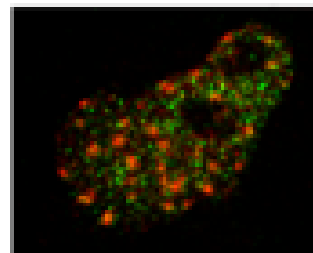
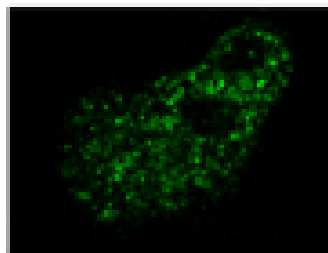
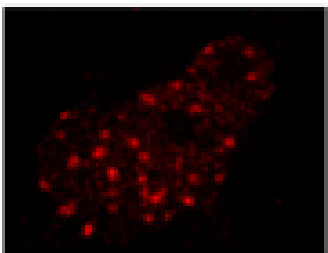
5-dAzaC

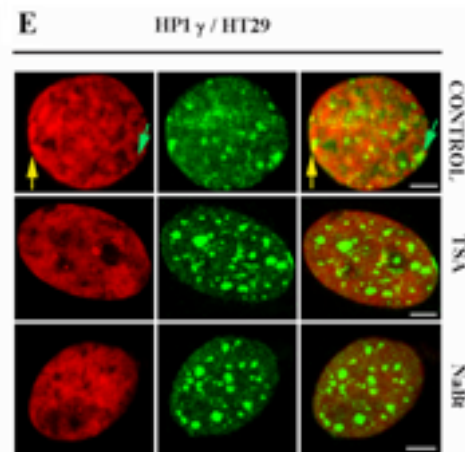
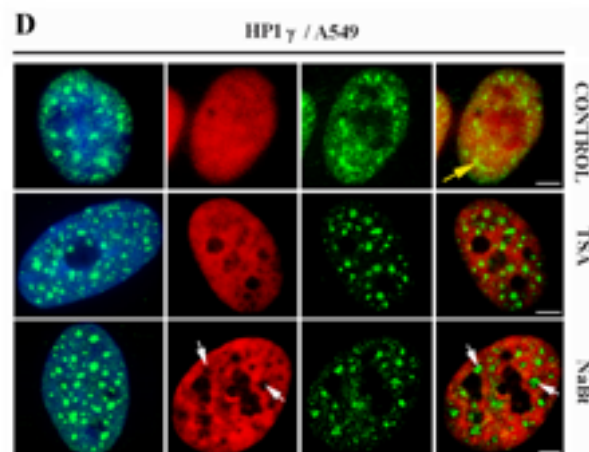
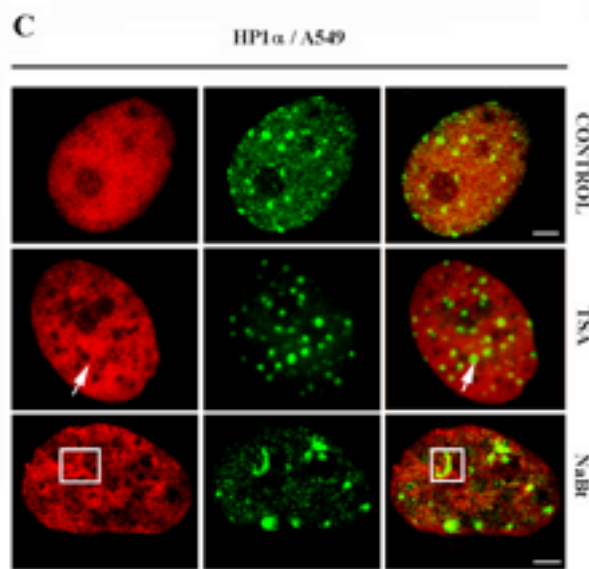
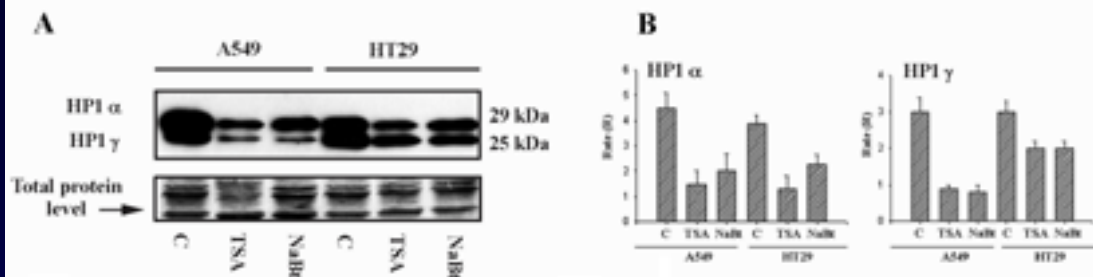


P19/IR



P19/SR



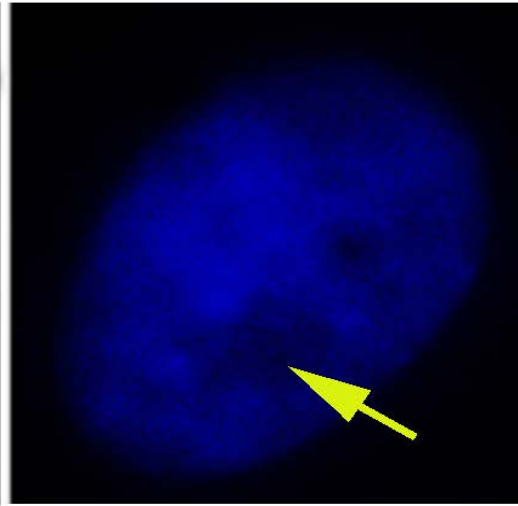
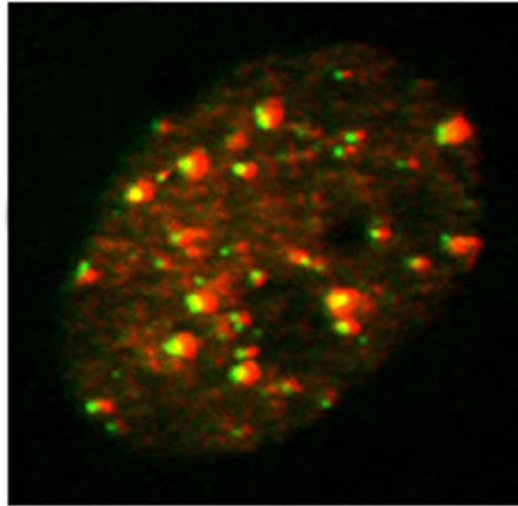


CENP-A / HP1 α

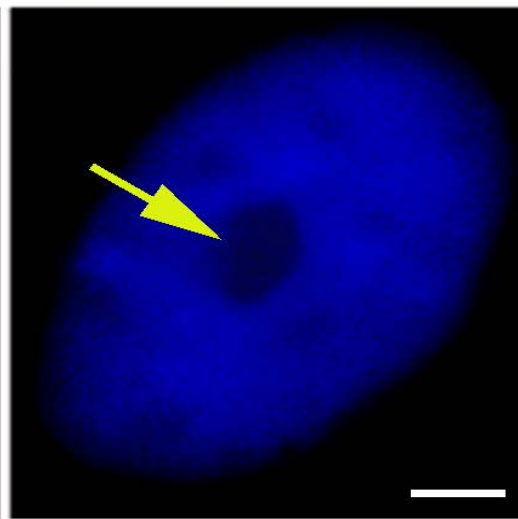
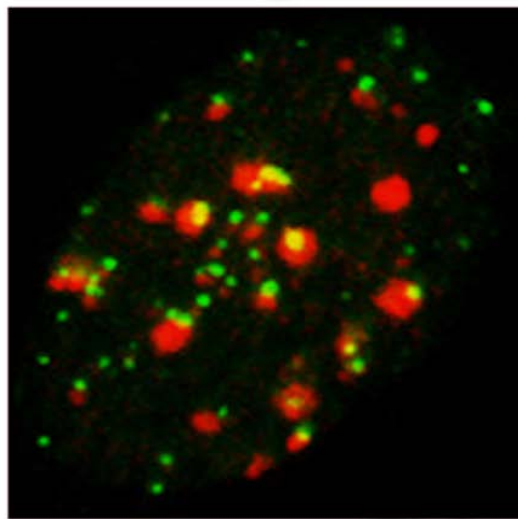
DAPI

Max. image

Mid. section



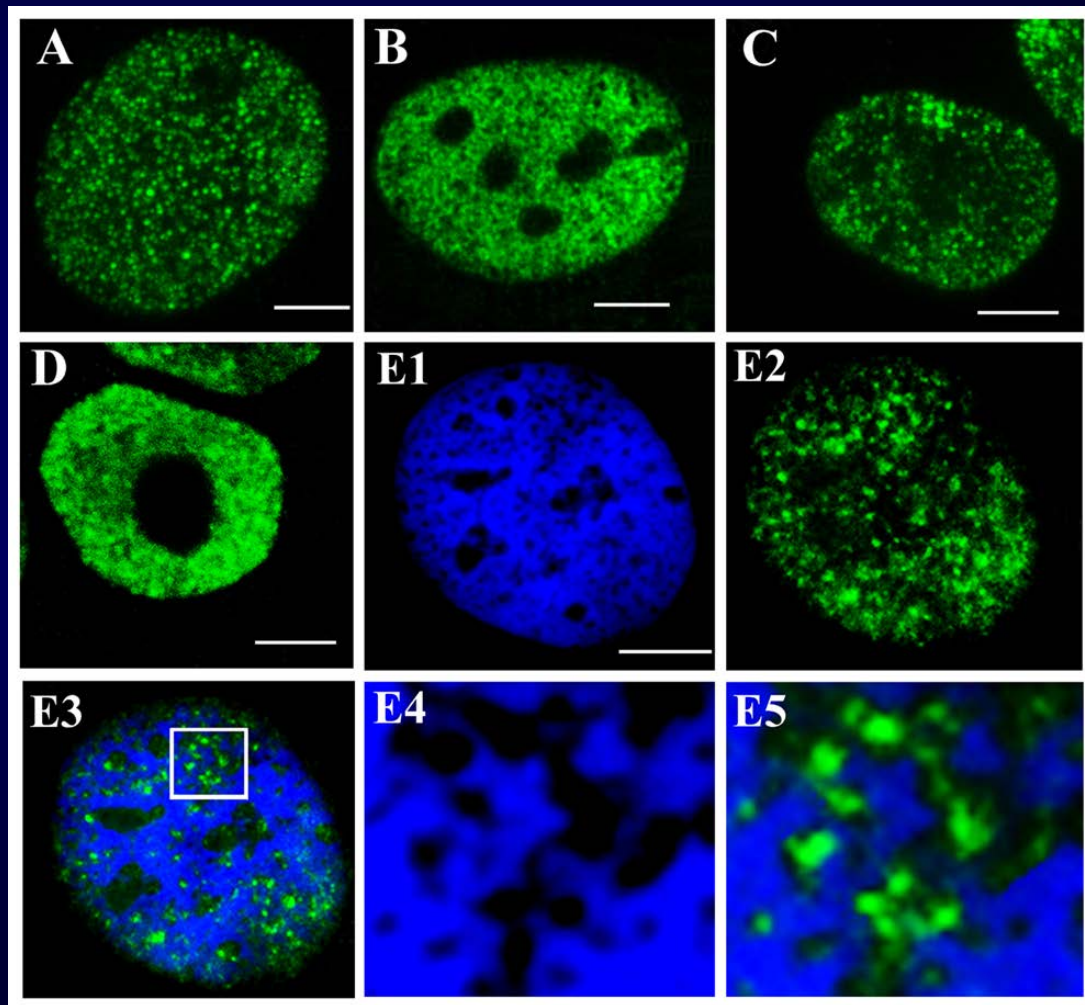
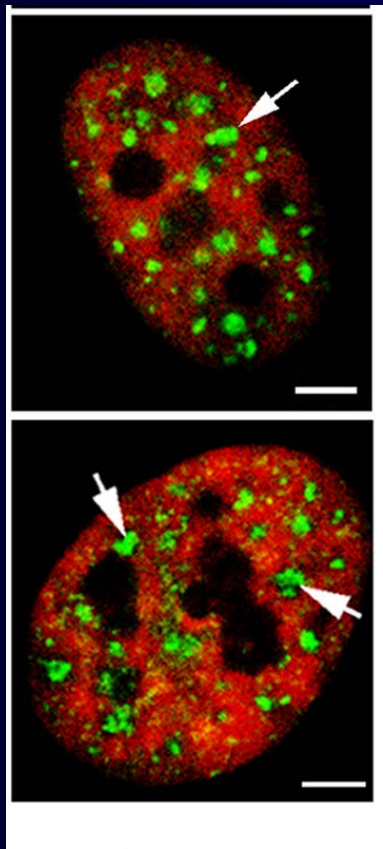
CONTROL



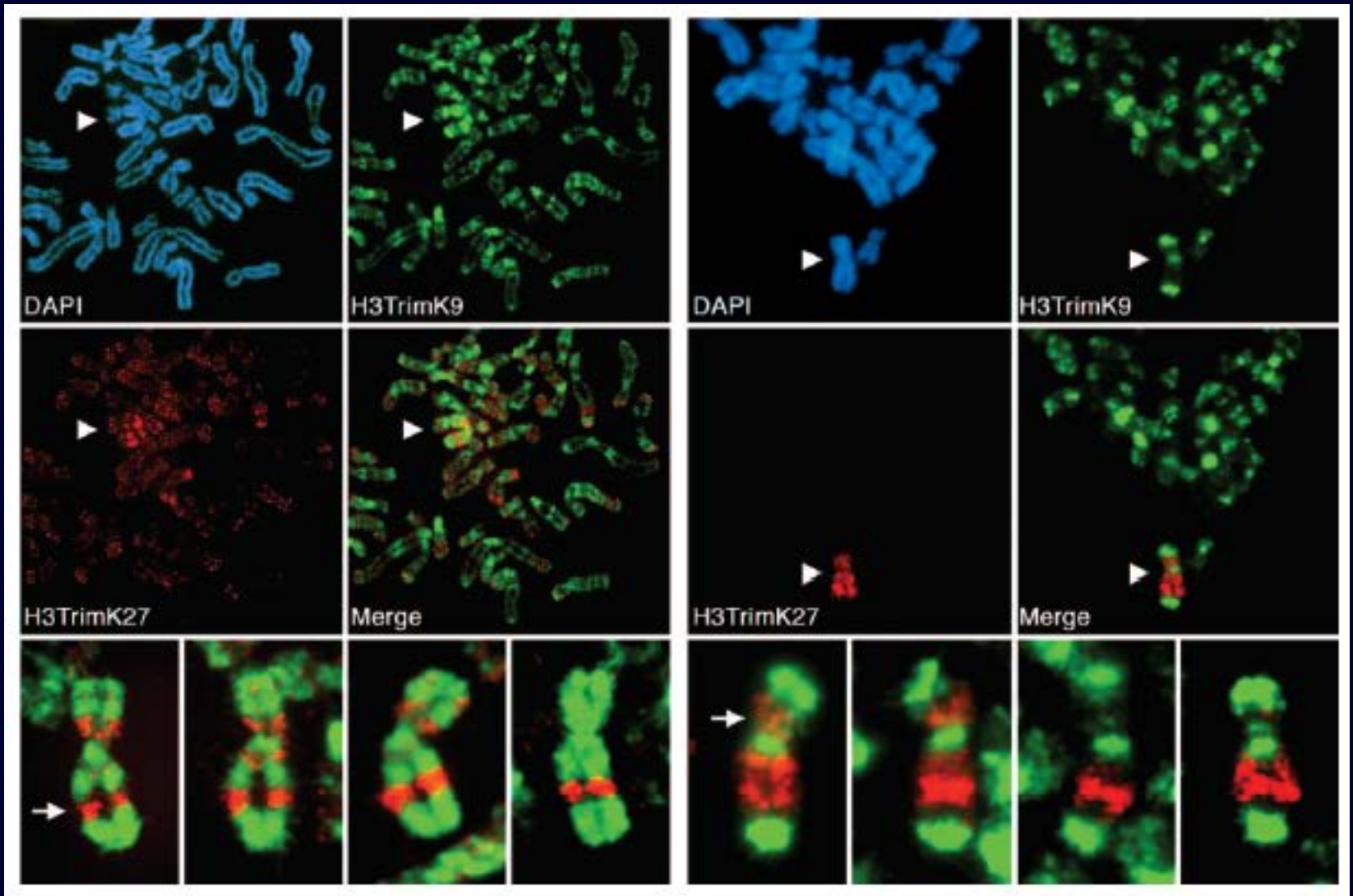
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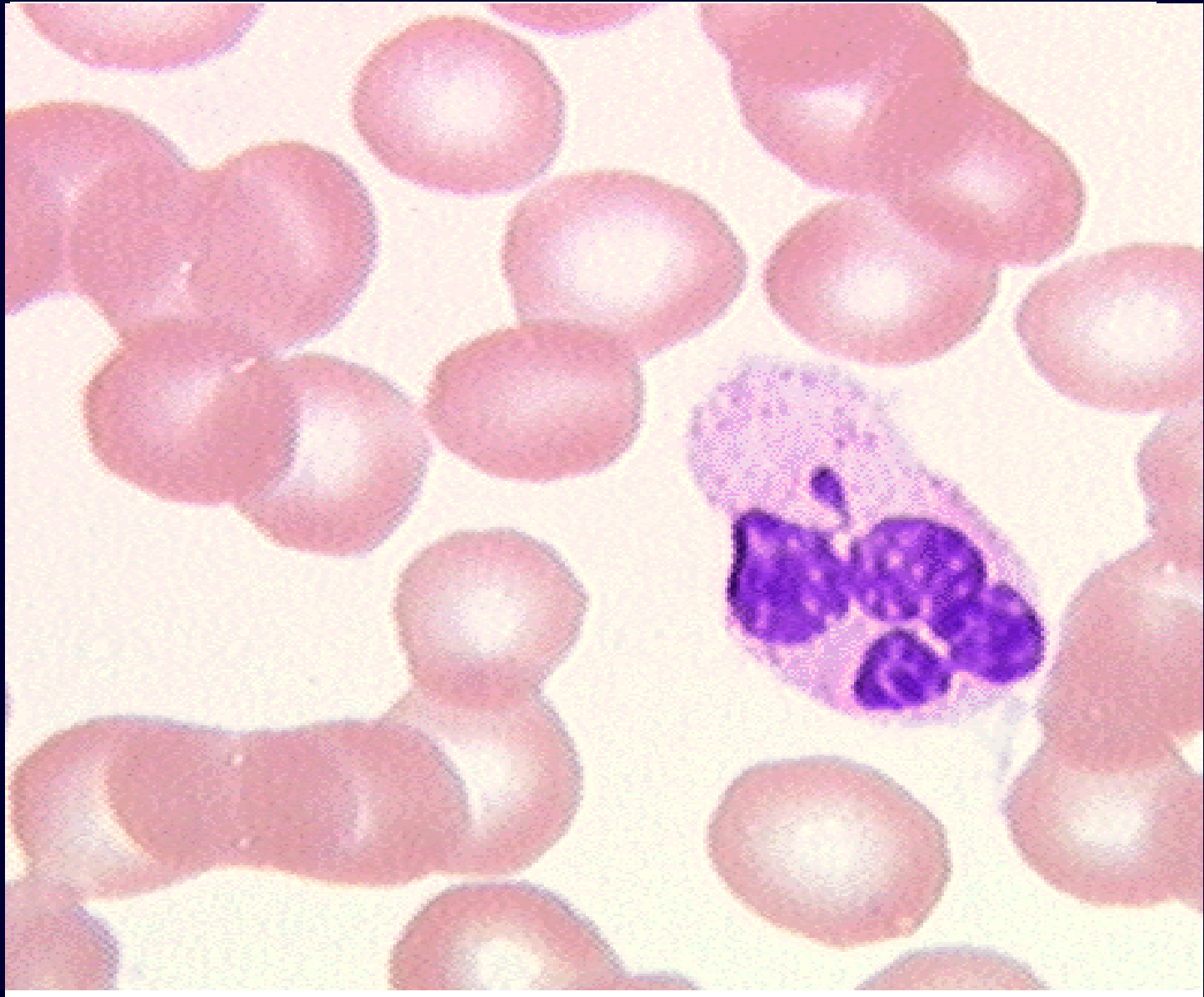
HP1 protein a H3(K4) di-methylation and IC spaces

HP1

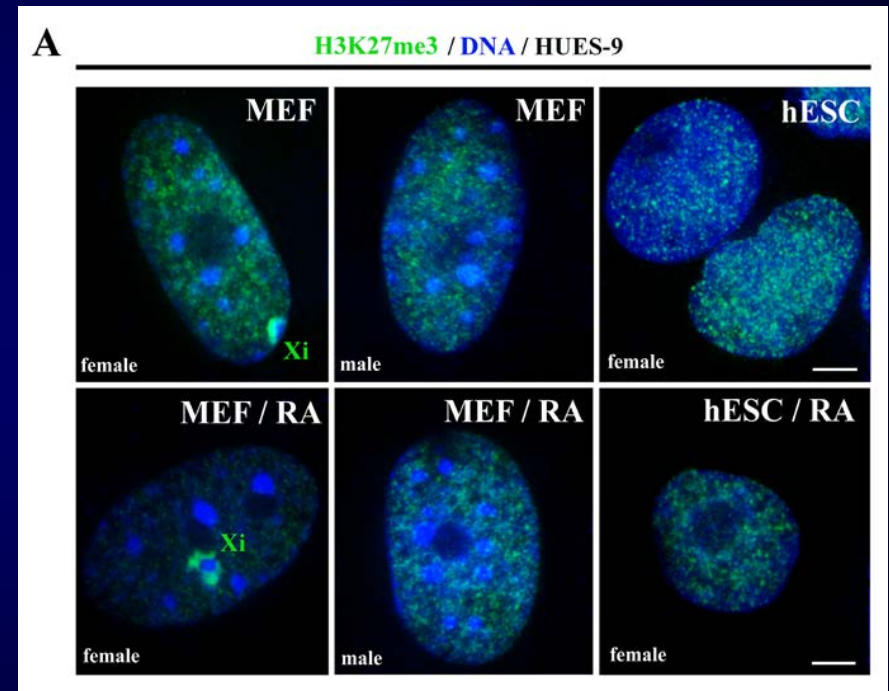
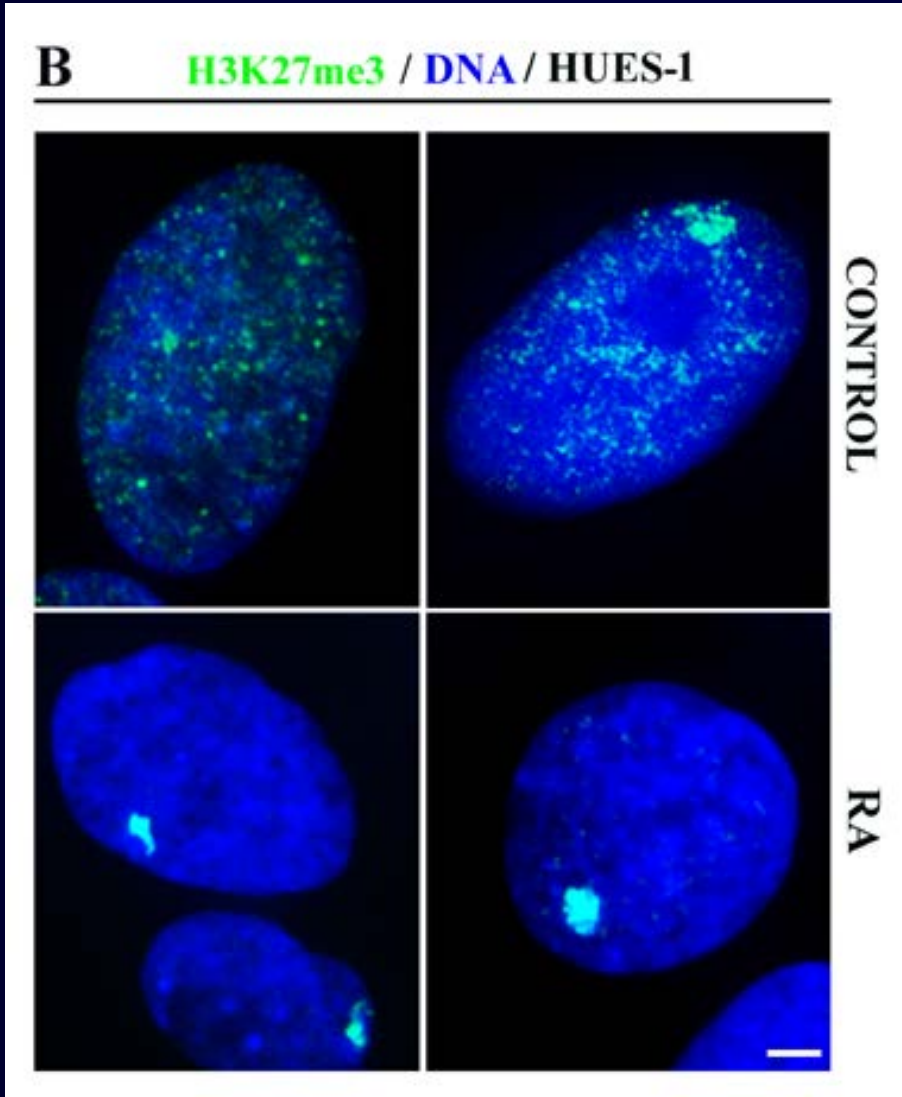


Inaktivace X chromosomu ve vztahu k epigenetickým modifikacím





Inactivation of X chromosome in hESC



Inaktivace X chromosomu ve vztahu k epigenetickým modifikacím

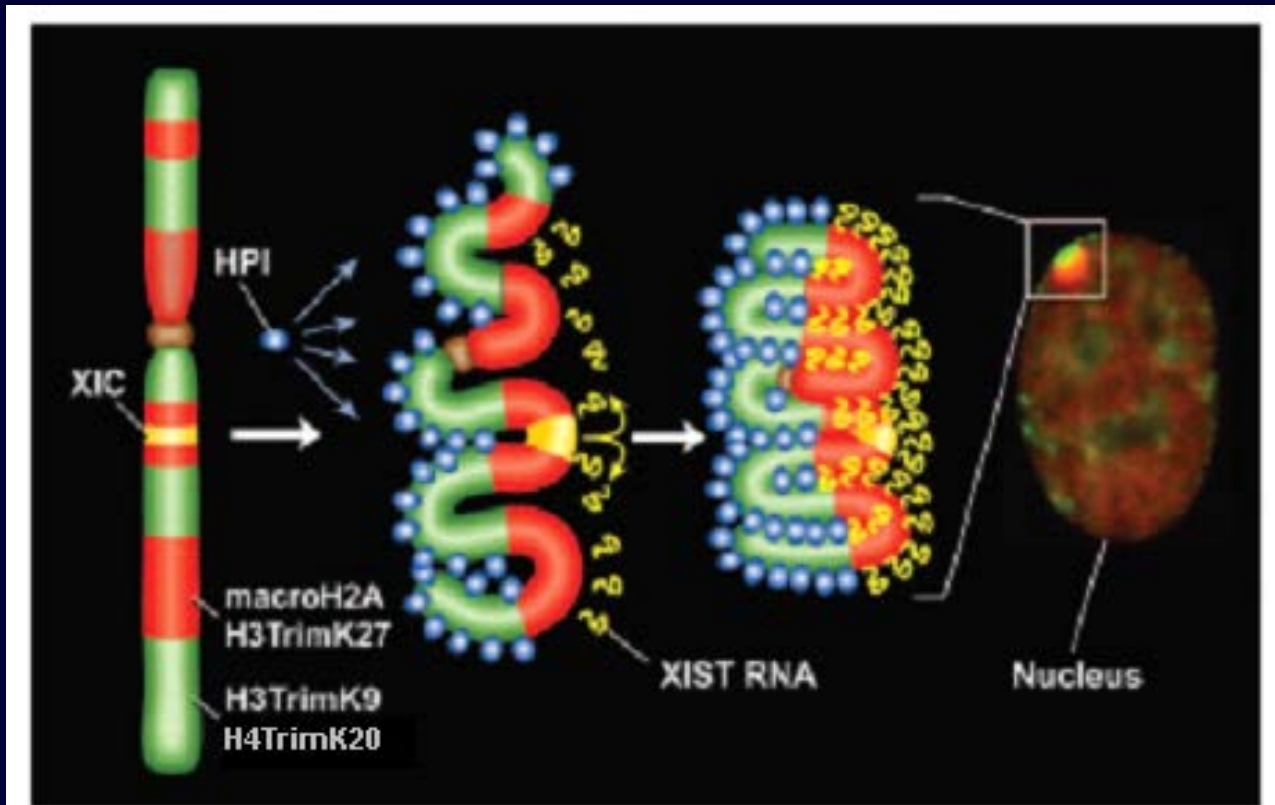
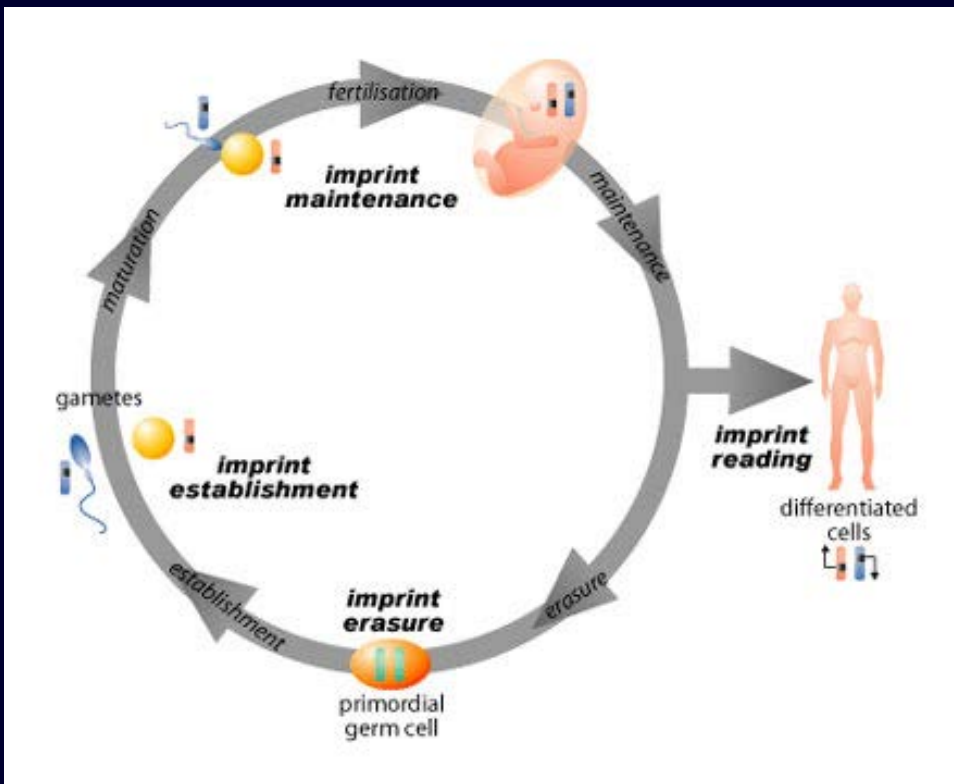


Fig. 4. Schematic model showing how heterochromatin of the Xi could transition between metaphase and interphase to be organized into the two nonoverlapping heterochromatin territories and to explain how XIST RNA could rapidly spread in cis outward from the X inactivation center (XIC) along only part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

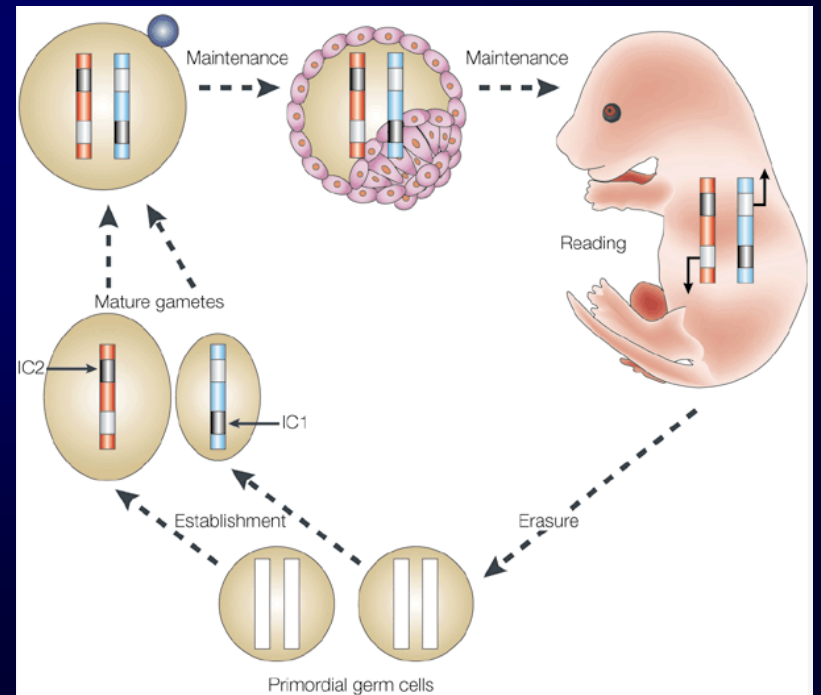


IMPRINTING

Myší embryo: samičí alela je zamethylována, nevyjadřuje se

Dospělý jedinec: obě alely jsou demethylovány

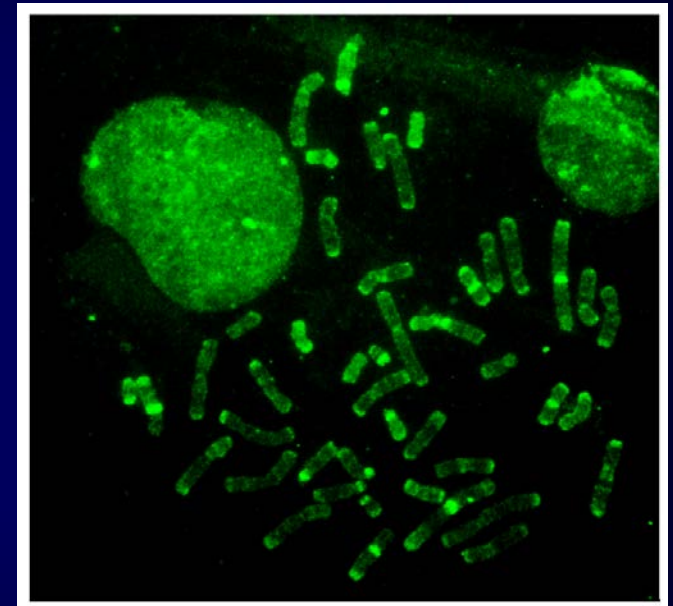
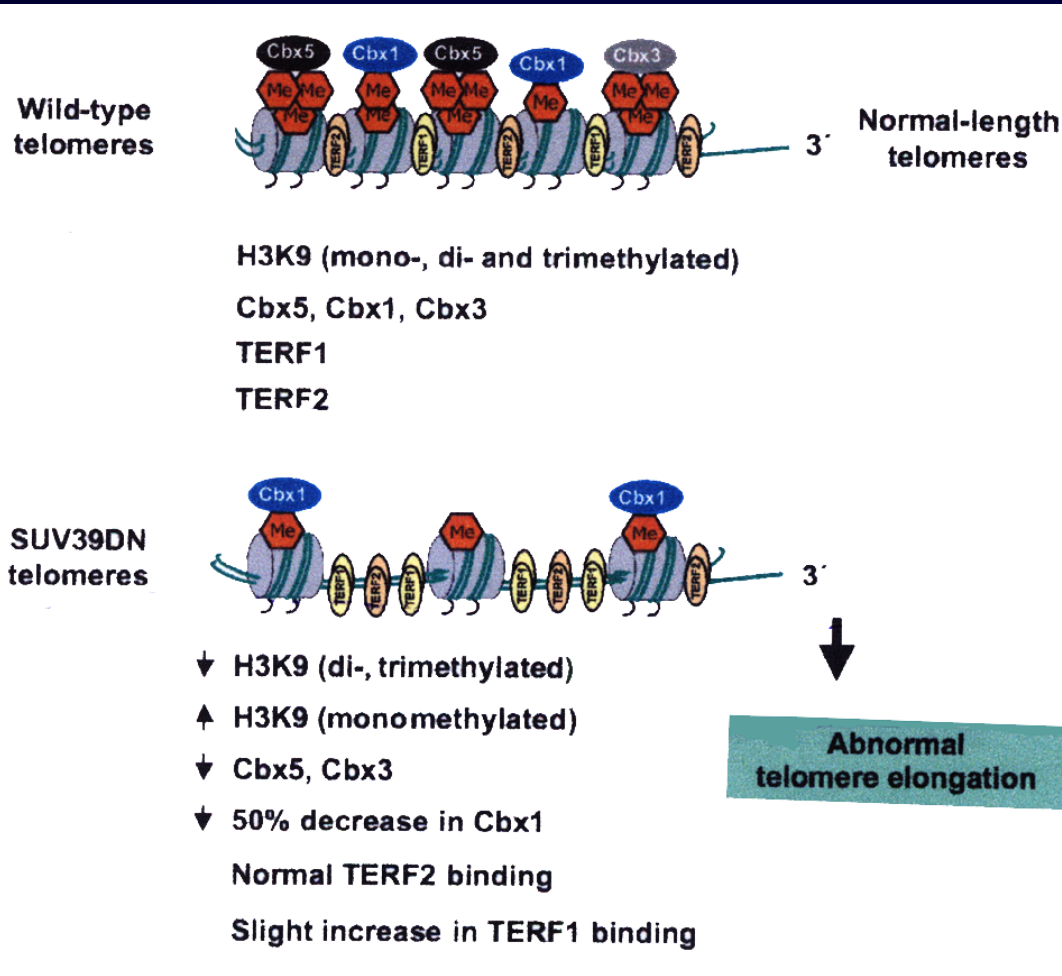
**Gametogeneze: se obnoví původní stav
Platí pro gen IGF II.**



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

Methylation state of telomeres

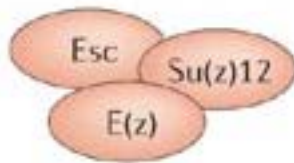
(Cbx1=HP1 β , Cbx3=HP1 γ , Cbx5 = HP1 α)



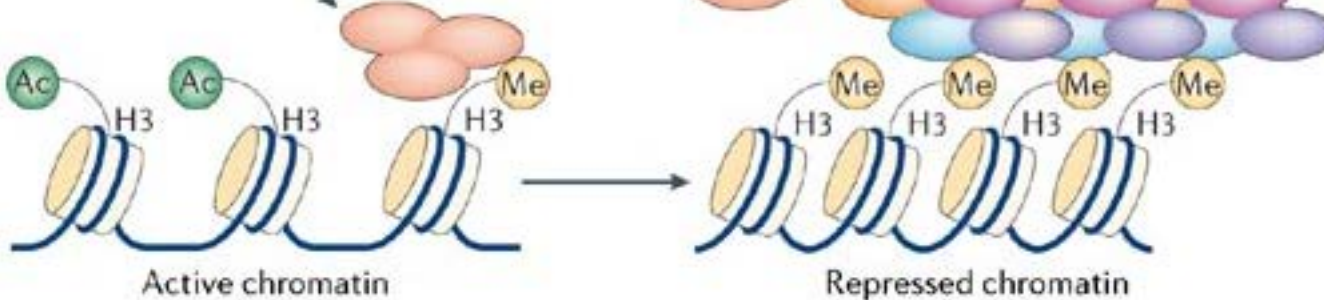
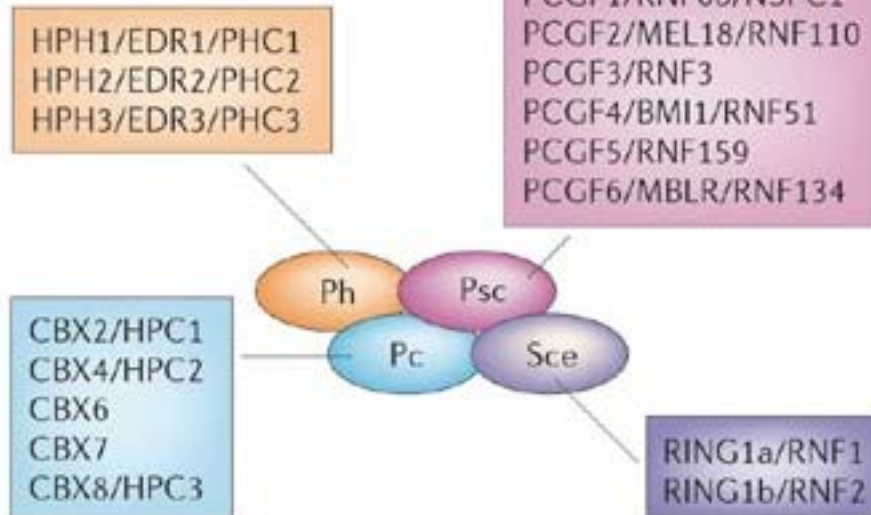
Experiments of E. Bártová

HP1 α (12q13) HP1 β (17q21) HP1 γ (7p15)

a
Class II PcG complexes



c
Class I PcG complexes



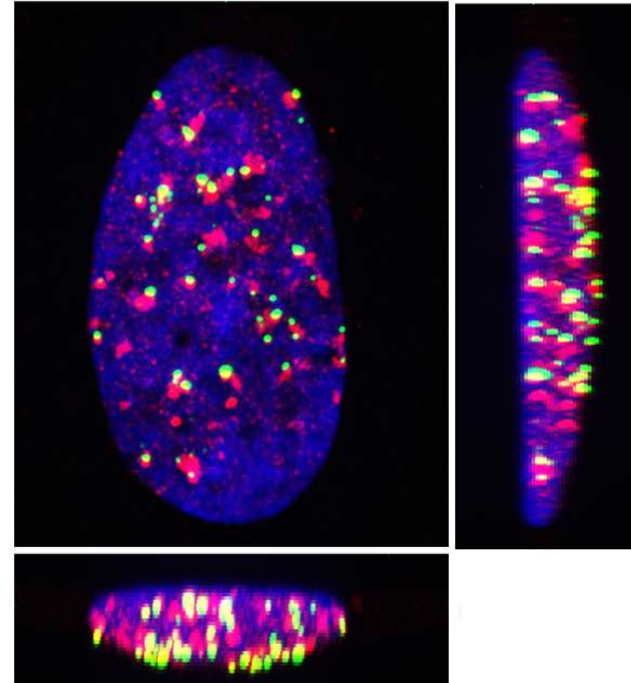
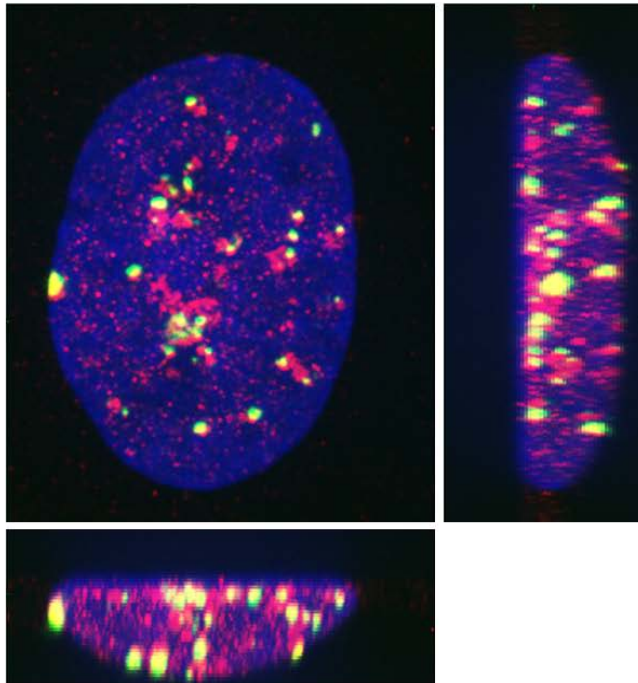
H3K27me3 / BMI1 / Nucleus

CONTROL

TSA

maximum image

maximum image

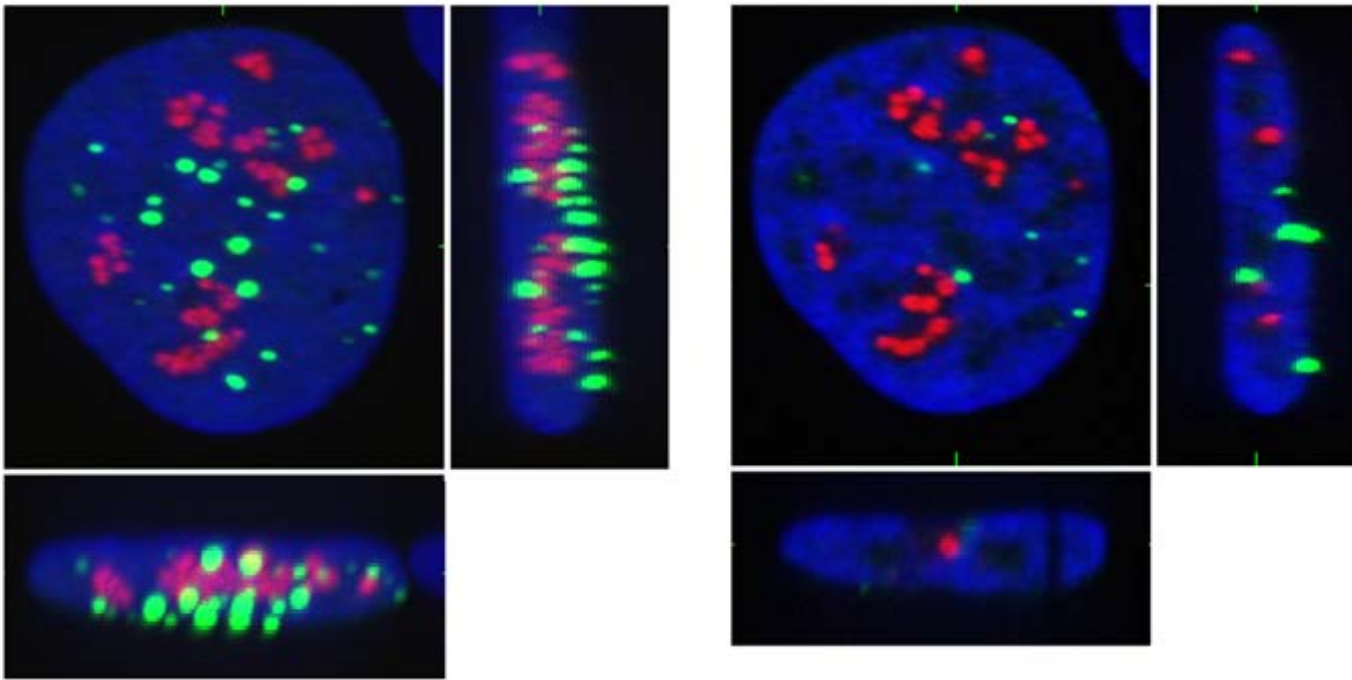


Fibrillarin / BMI1 / Nucleus

CONTROL

maximum image

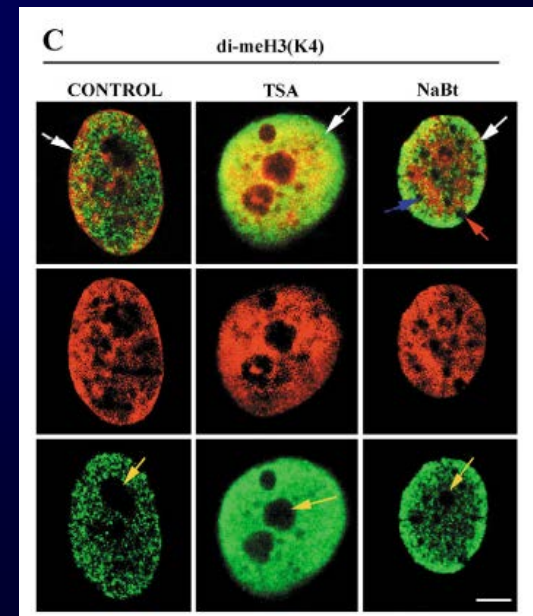
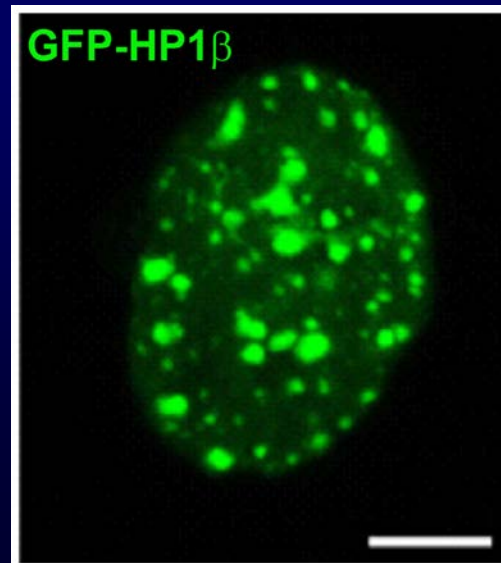
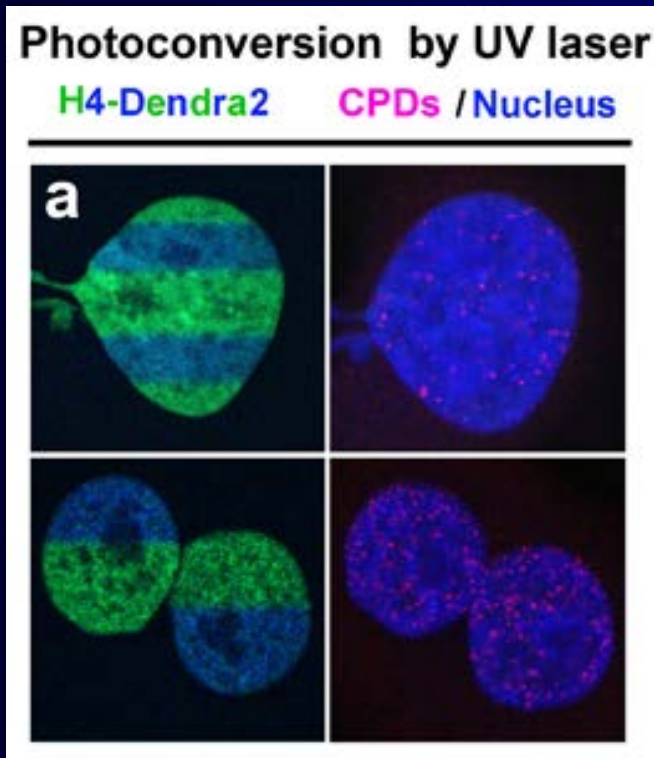
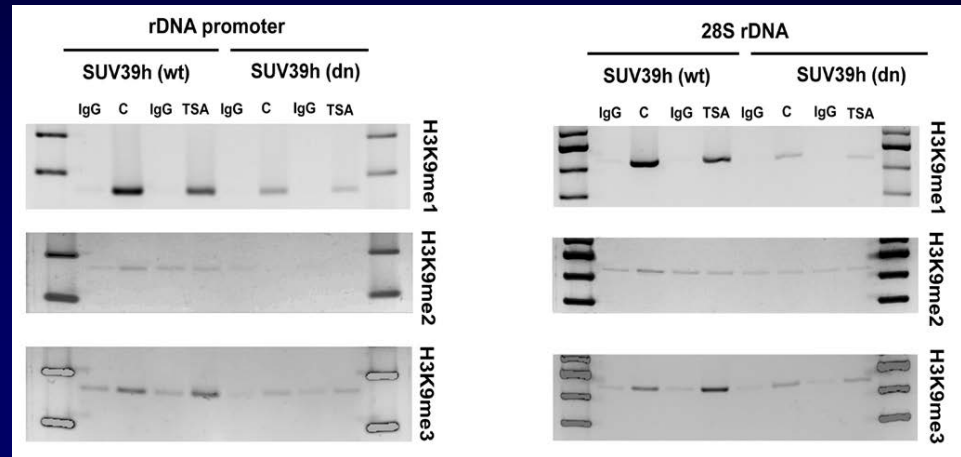
individual section



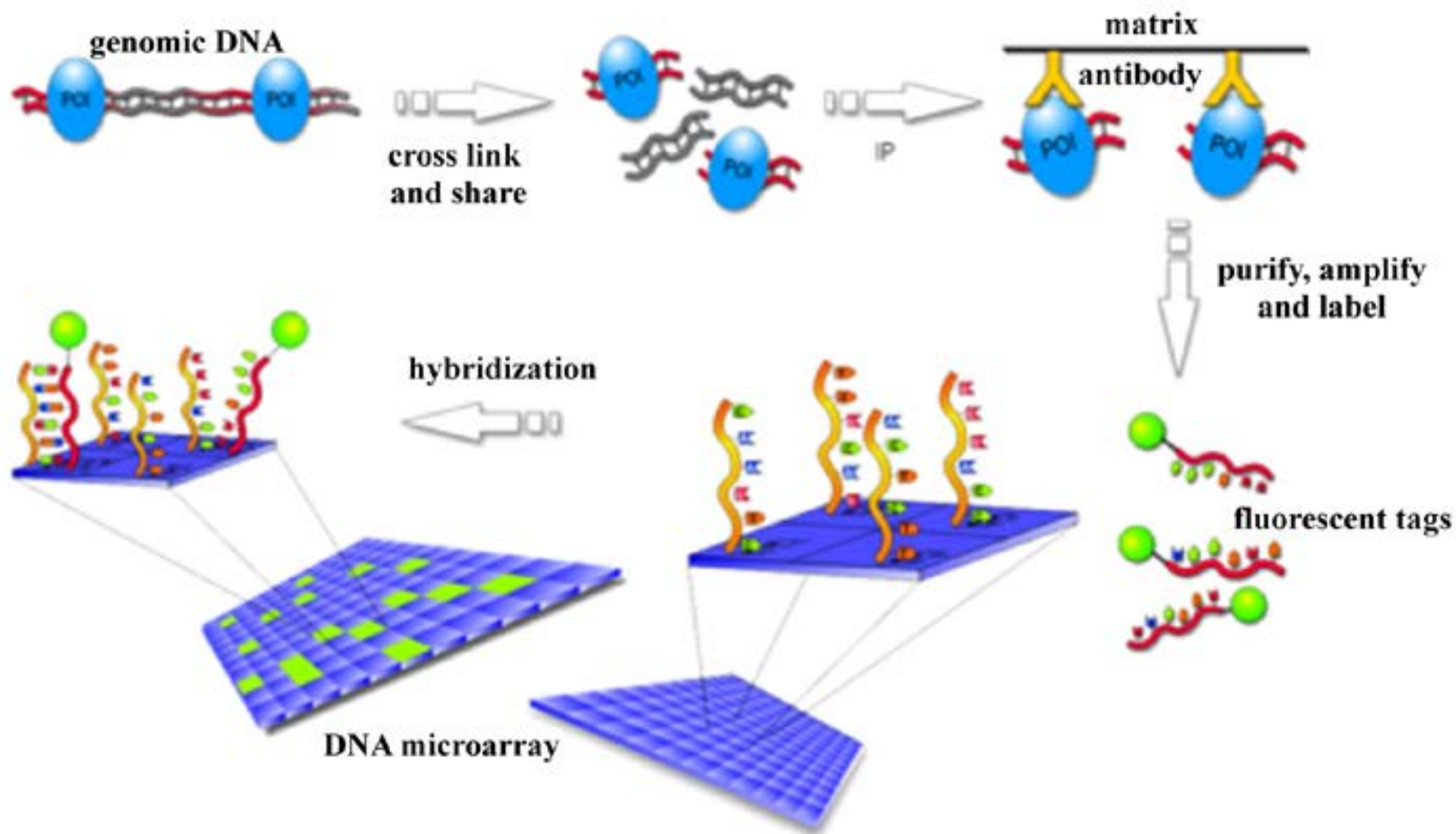
Metody

Metody vhodné ke studiu histonového kódu

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

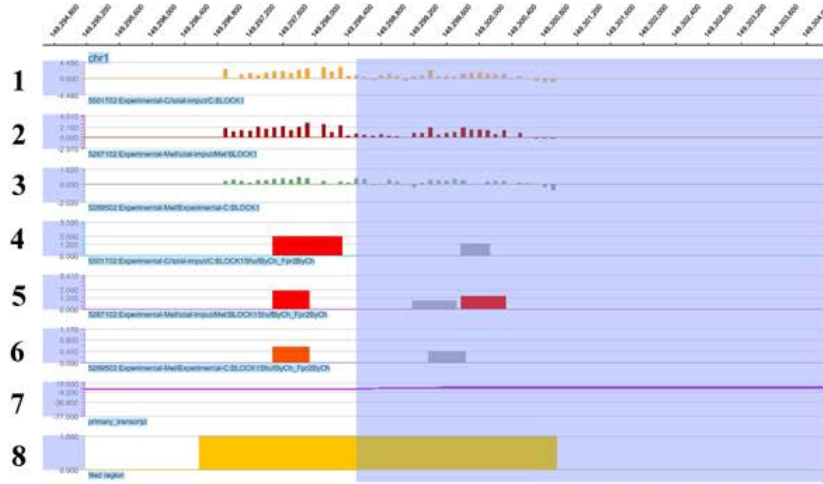


ChIP on chip



H3K9 acetylation in ARH77 cells

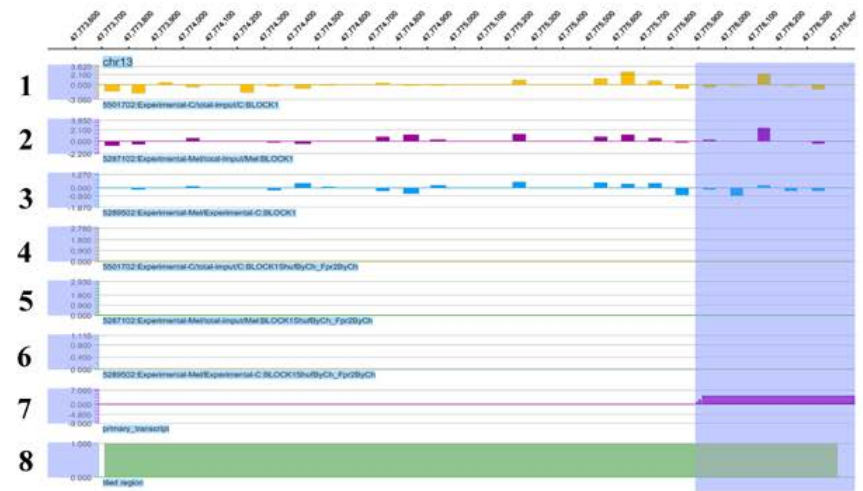
AF1Q



TP53



RB1

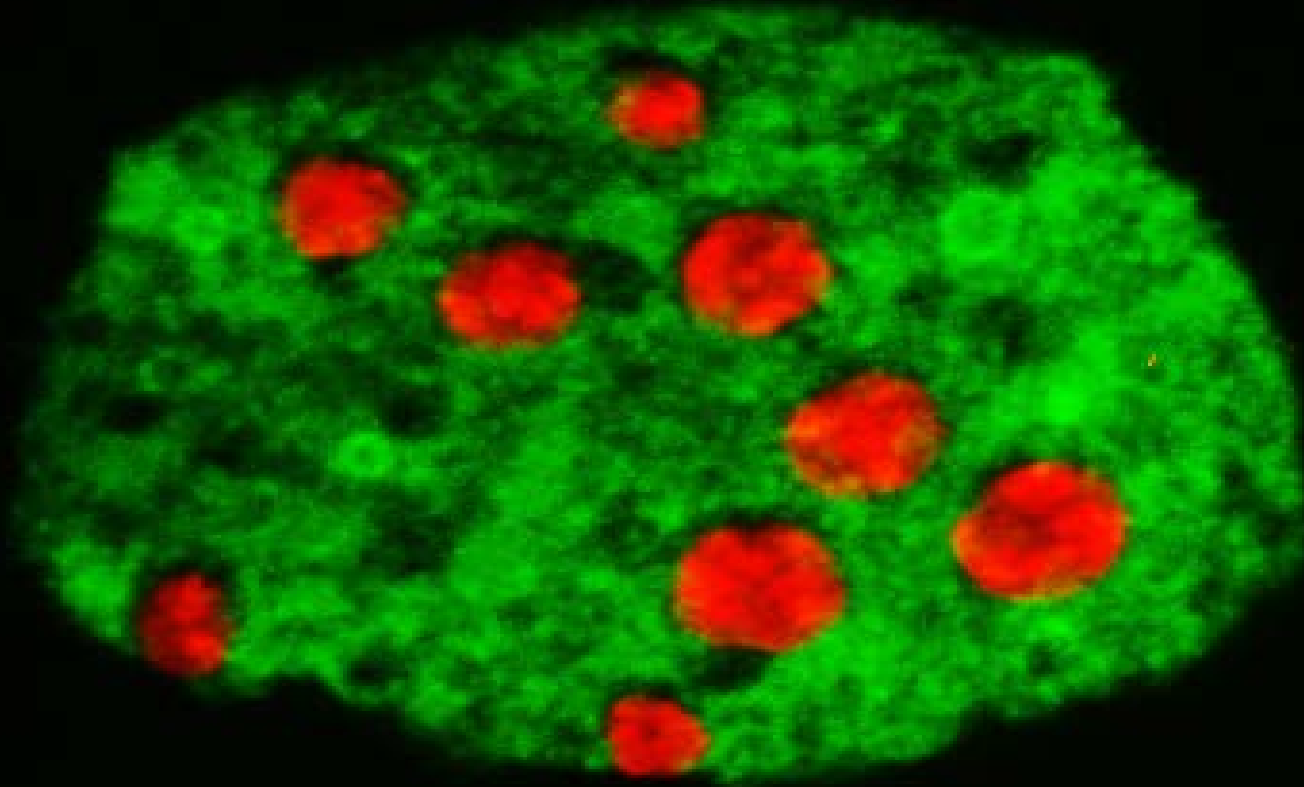


ASTN2-TRIM32



Ukázkové experimenty

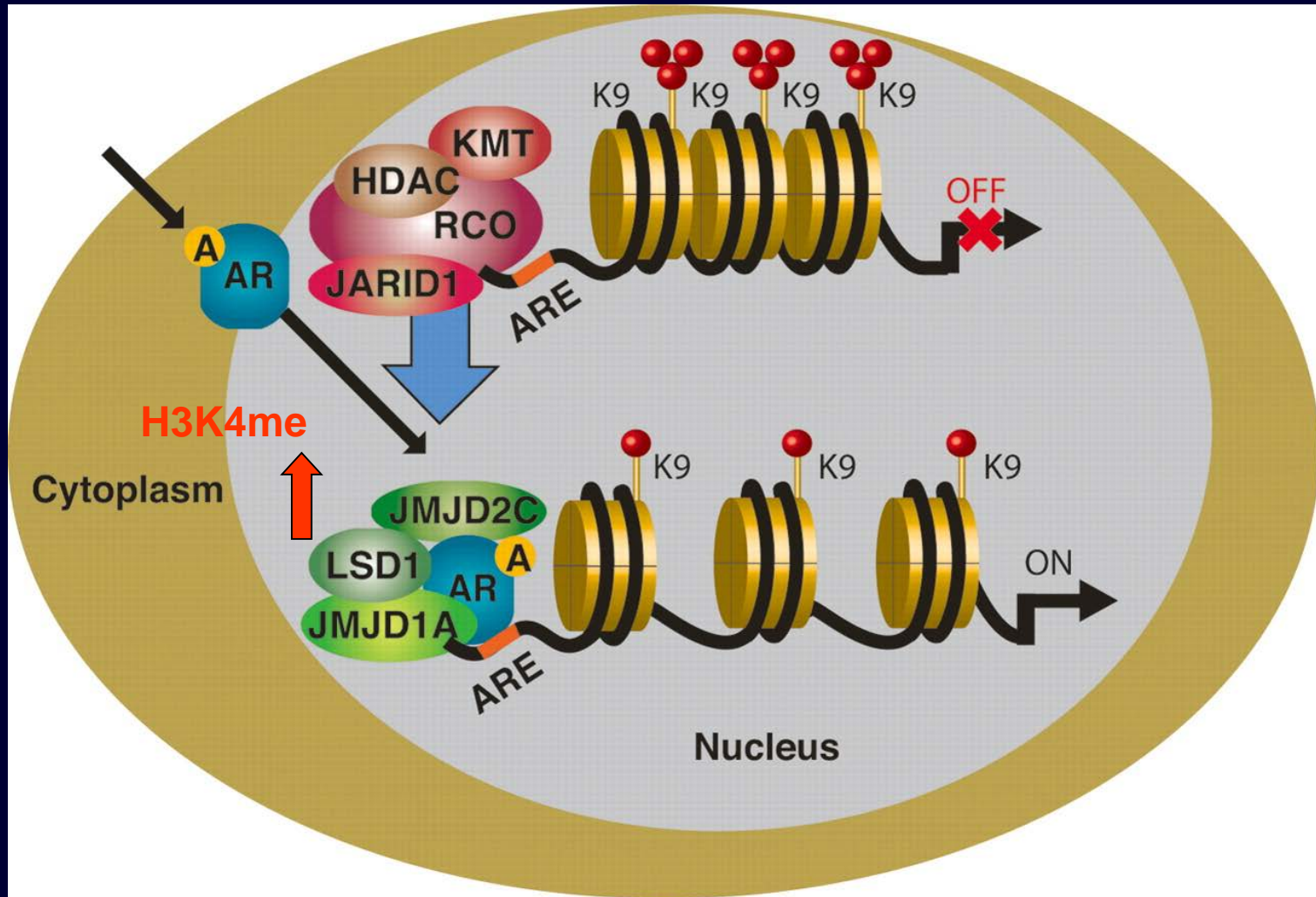
NUCLEAR ARRANGEMENT AND KINETIC PROPERTIES OF JMJD2B HISTONE DEMETHYLASE



**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**

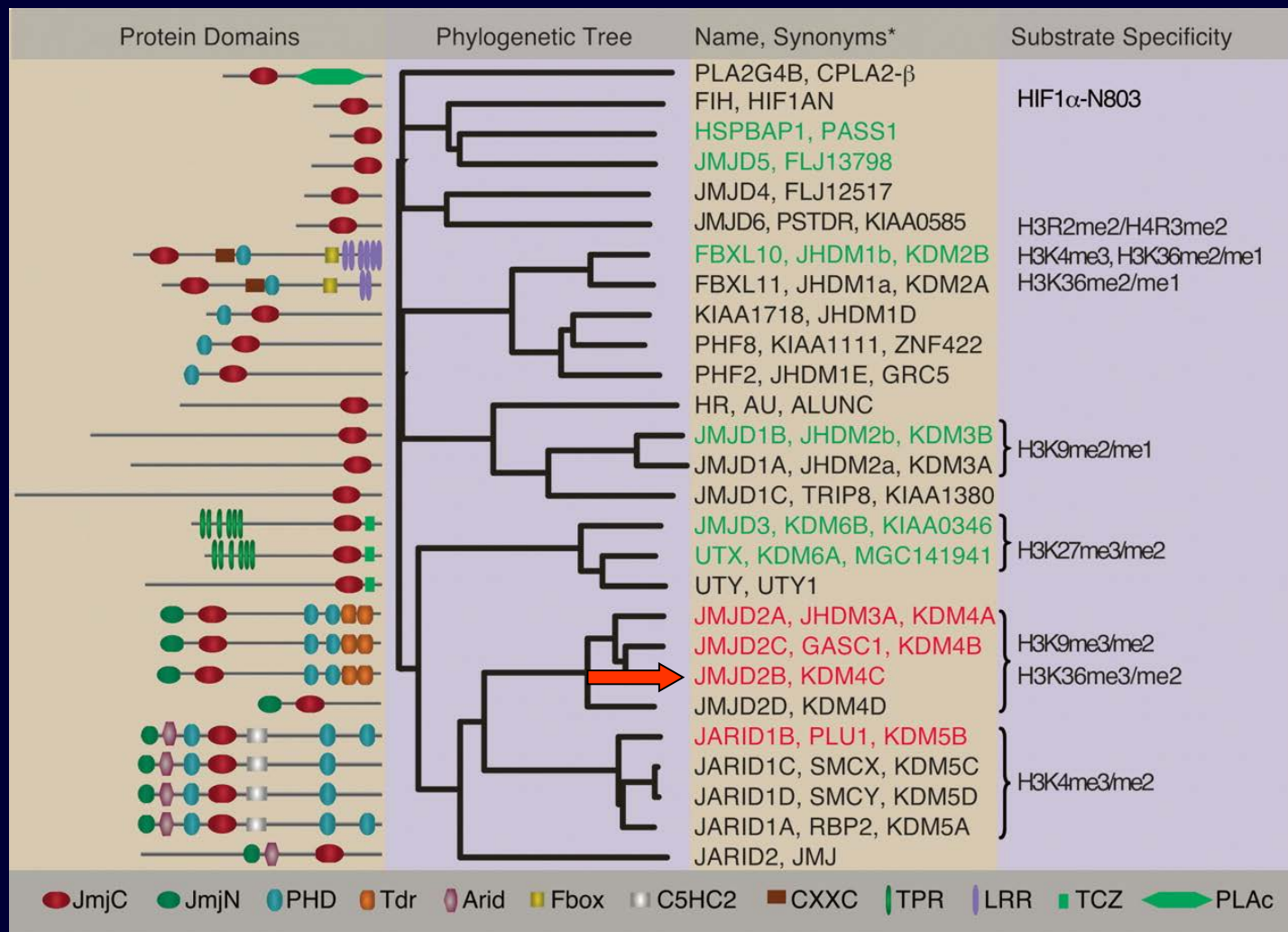
The involvement of demethylases in AR-mediated transcription.



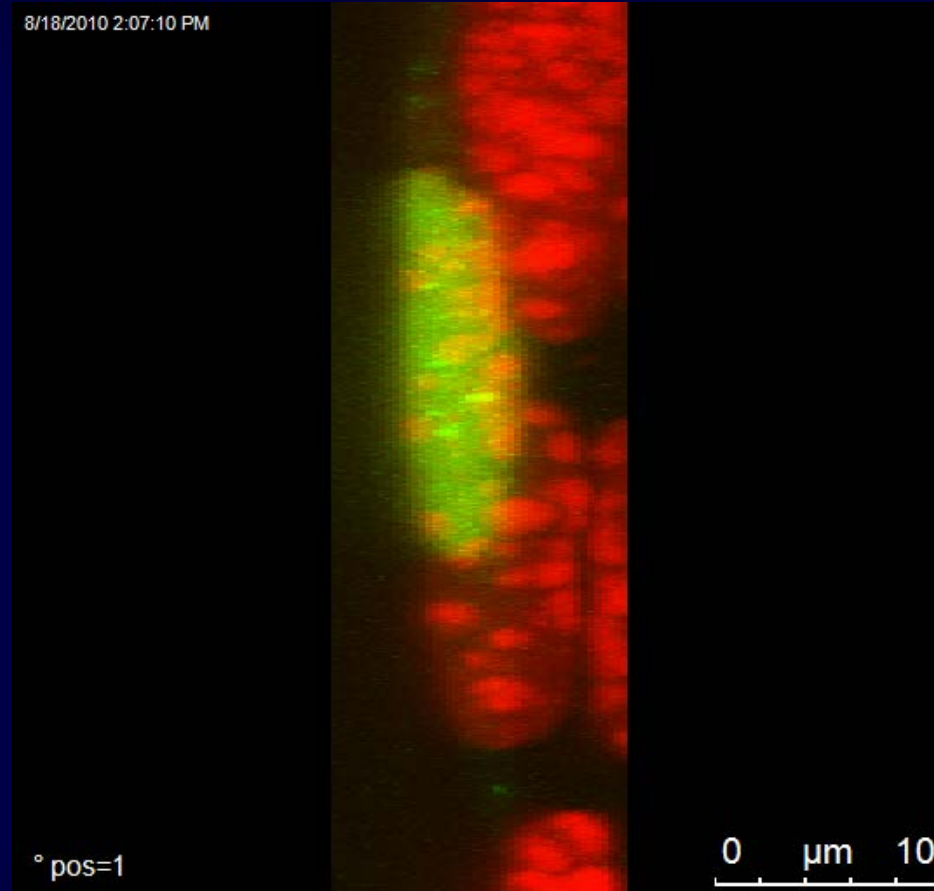
Cloos PA et al. *Genes Dev.* 2008;22:1115-1140



Phylogenetic tree of the JmjC family of demethylases.

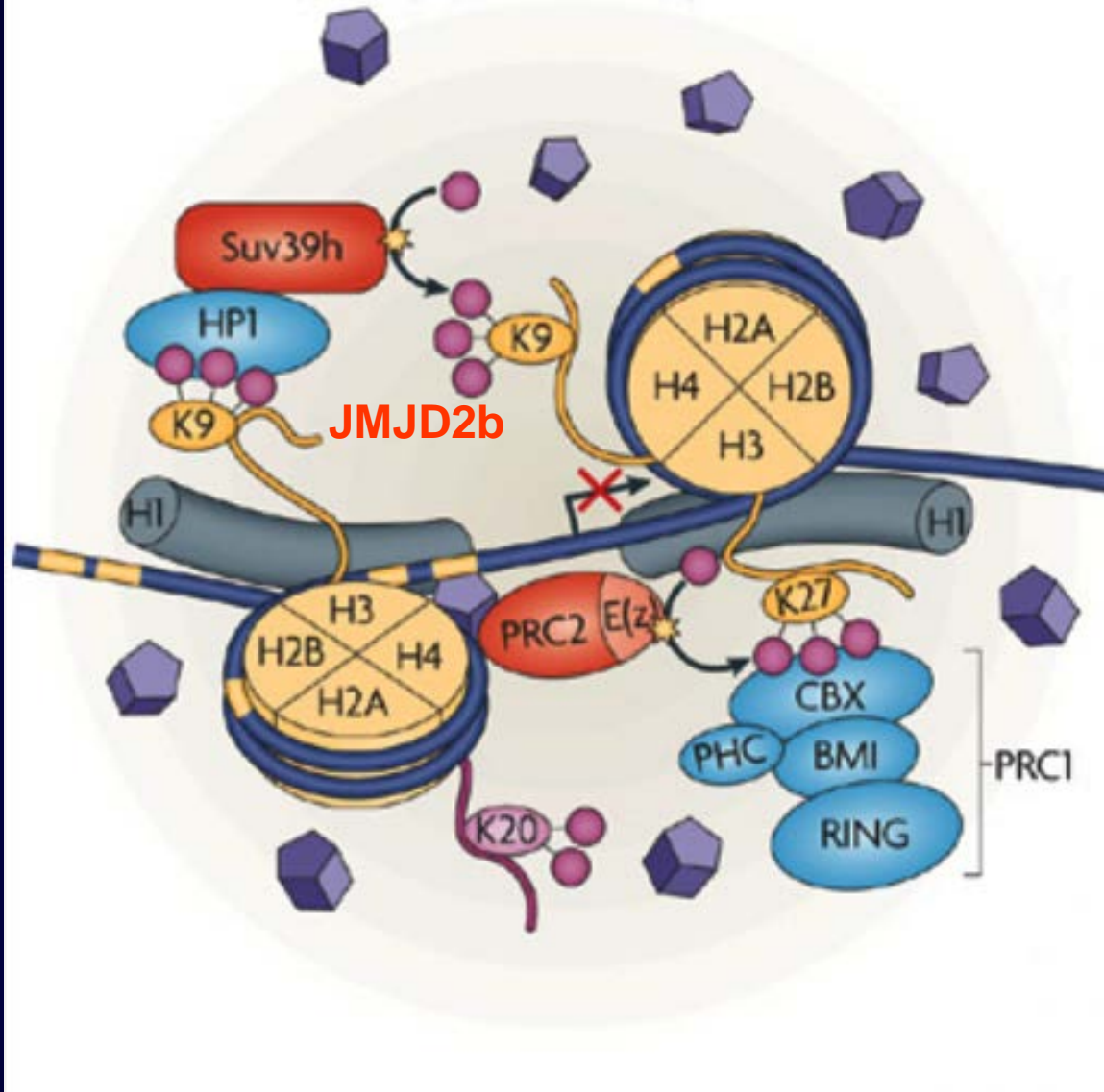


JMJD2b histone lysine demethylase



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

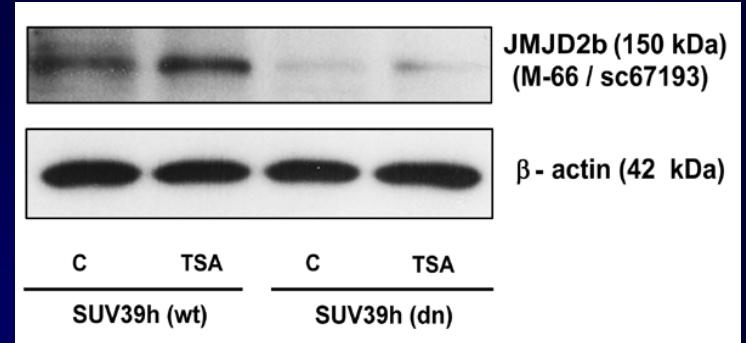
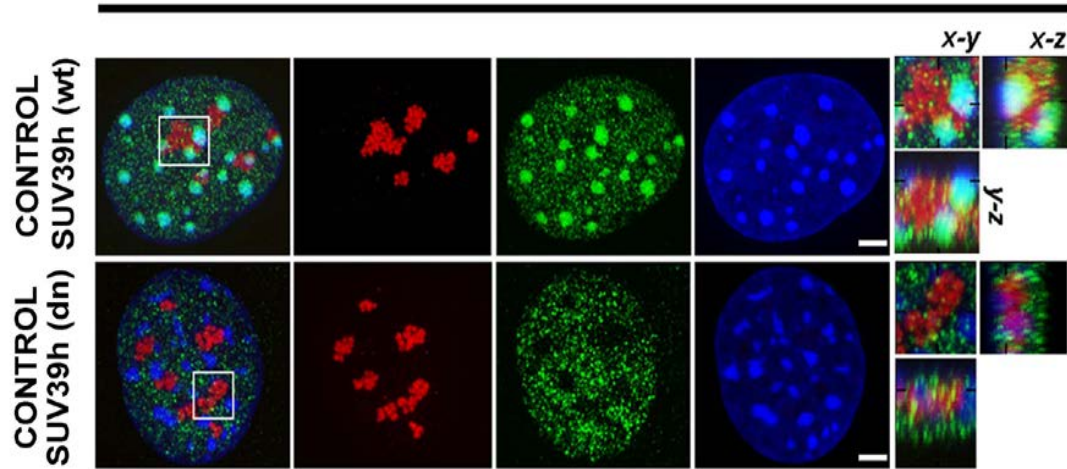
a Somatic interphase (repressed locus)



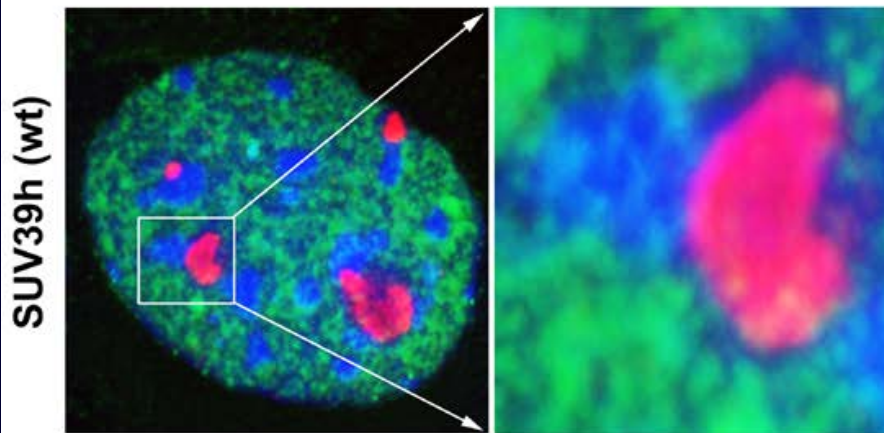
Dieter Egli, Garrett Birkhoff & Kevin Eggan (2008)
Nature Reviews Molecular Cell Biology 9, 505-516

JMJD2b histone lysine demethylase

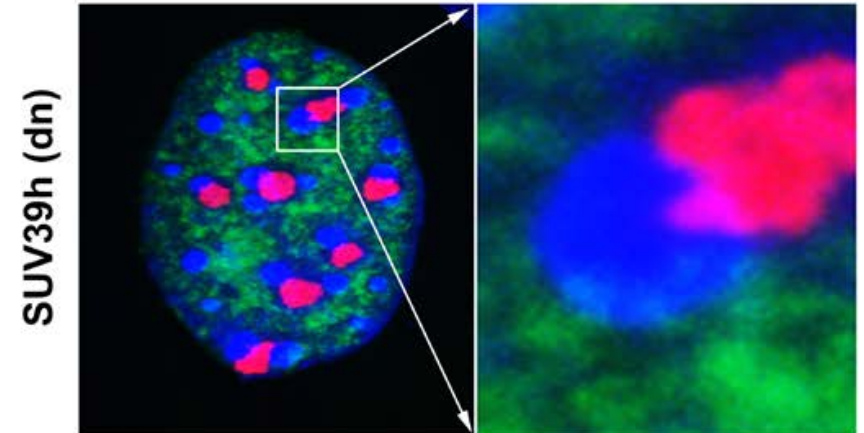
Fibrilarin / H3K9me3 / DNA



Jmjd2b / Fibrillar / chromocenters

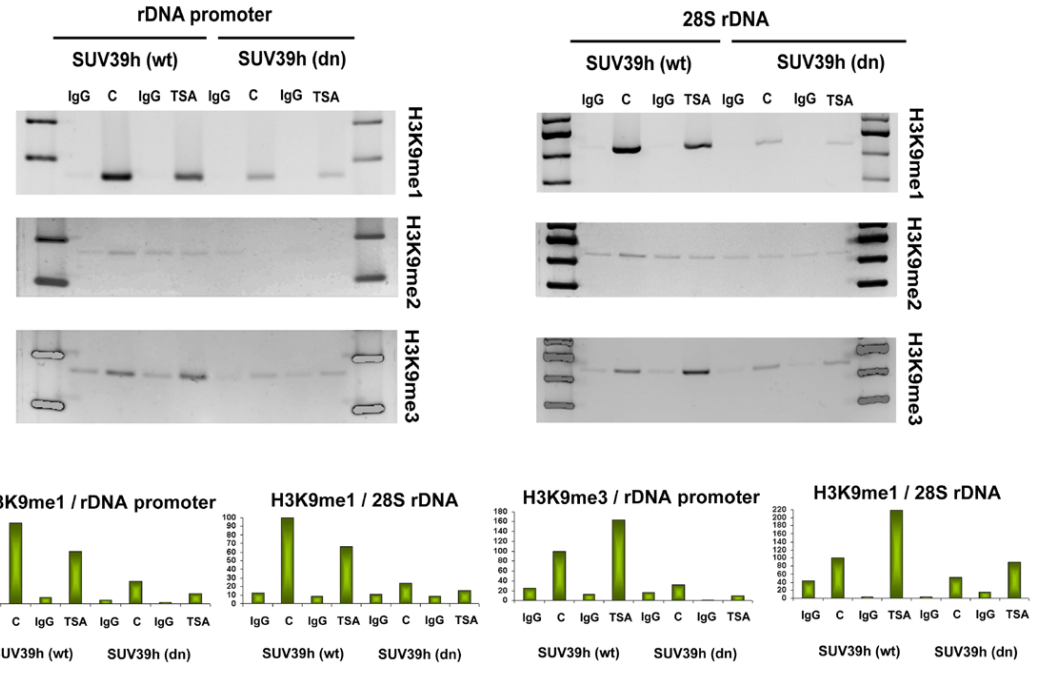
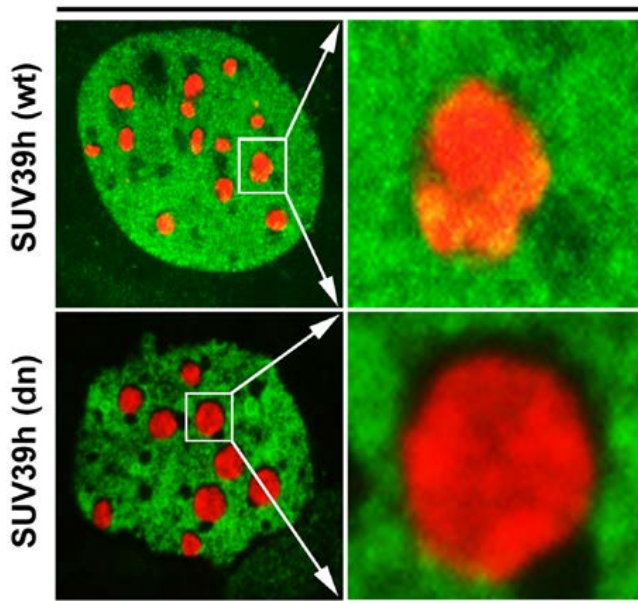


Jmjd2b / Fibrillar / chromocenters

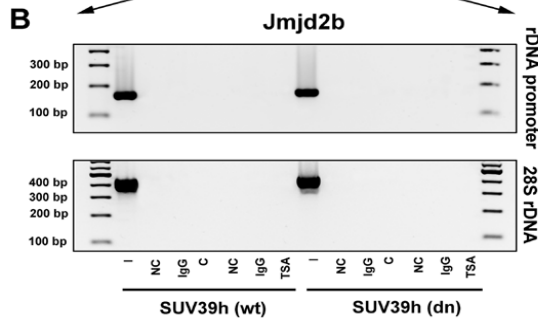
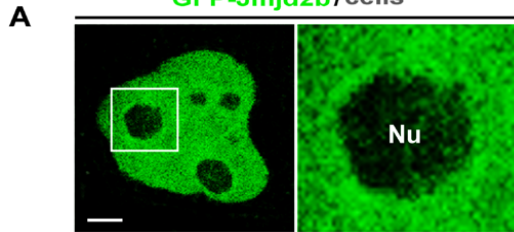


Jmjd2b histone lysine demethylase

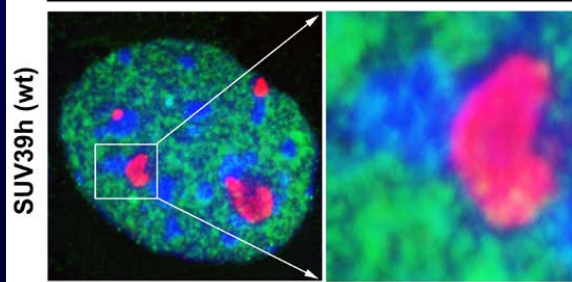
GFP-Jmjd2b / Fibrillarin



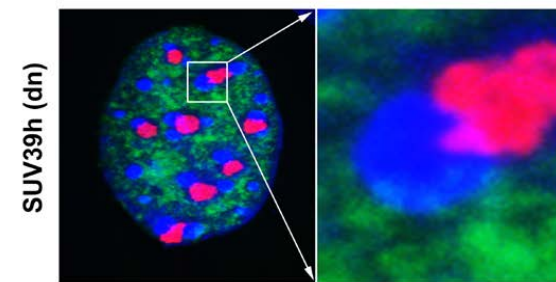
GFP-Jmjd2b / cells

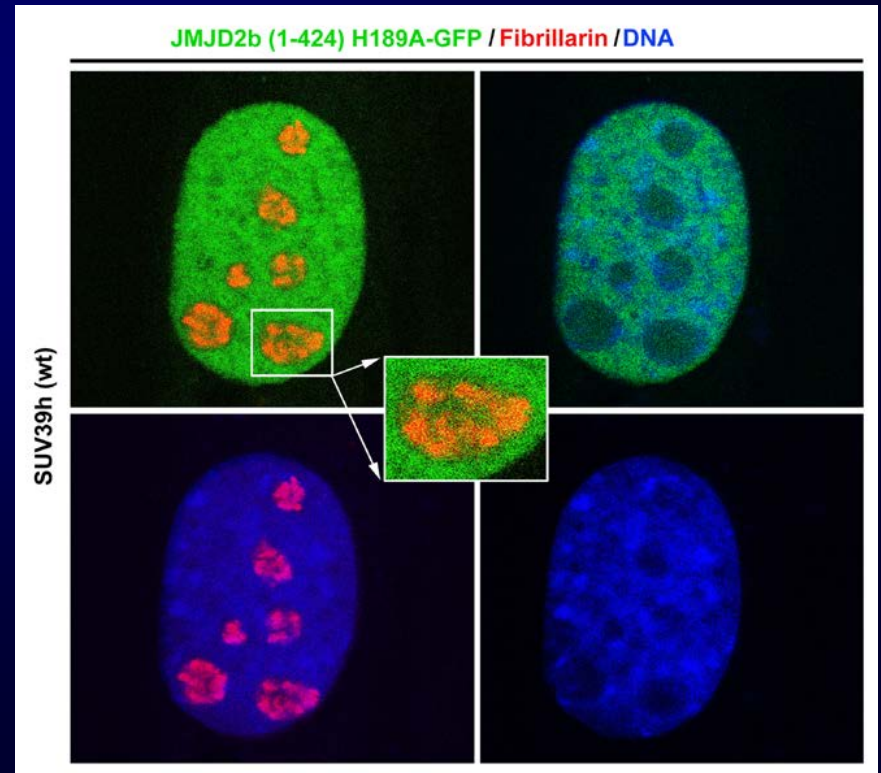
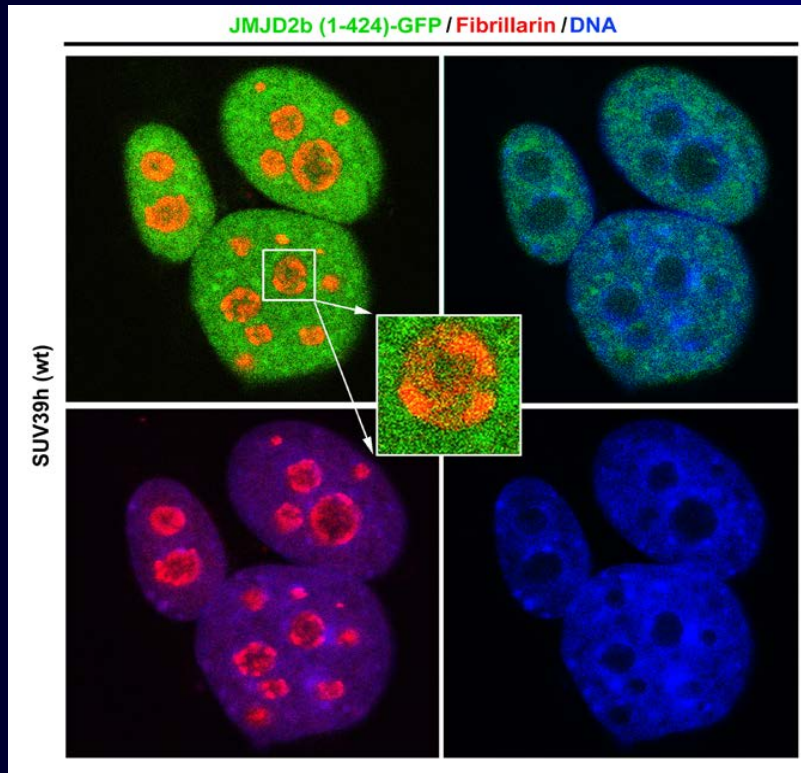
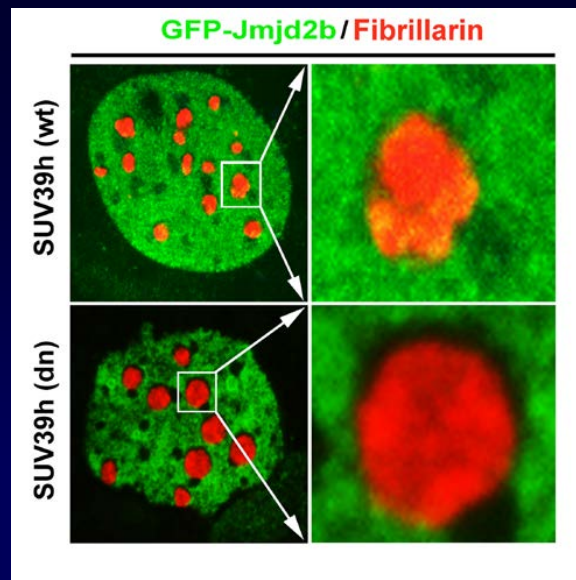


Jmjd2b / Fibrillarin / chromocenters

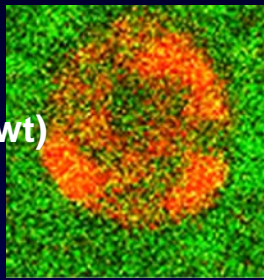


Jmjd2b / Fibrillarin / chromocenters

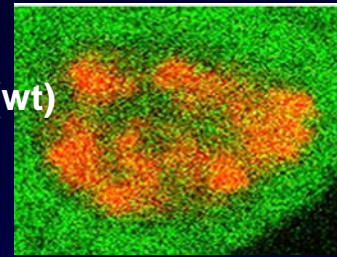




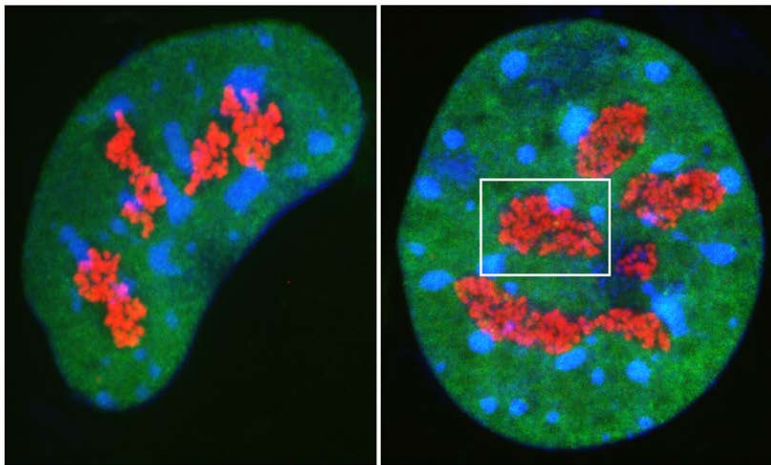
SUV39h (wt)



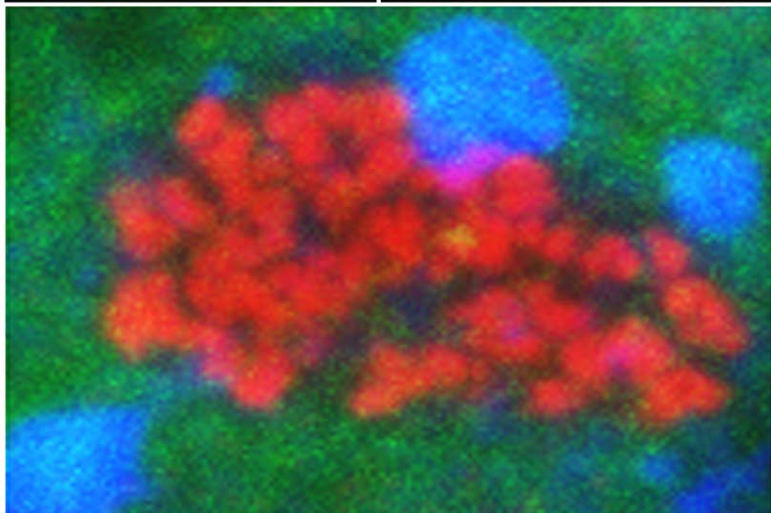
SUV39h (wt)



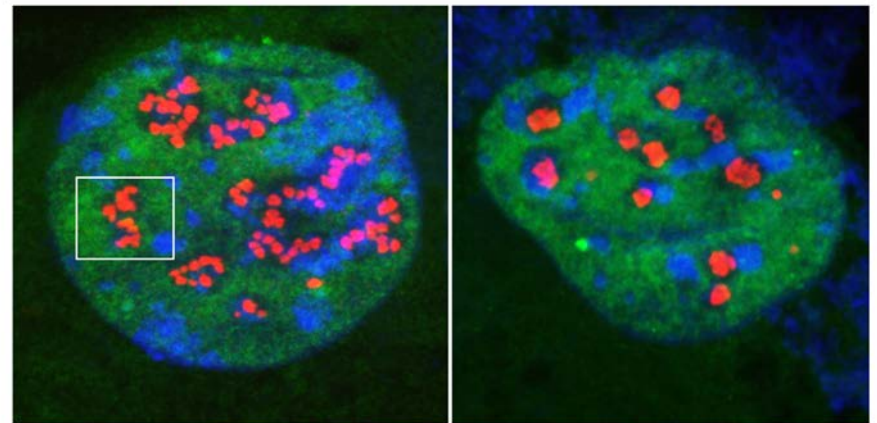
JMJD2b (1-424)-GFP / Fibrillarin / Chromocenters



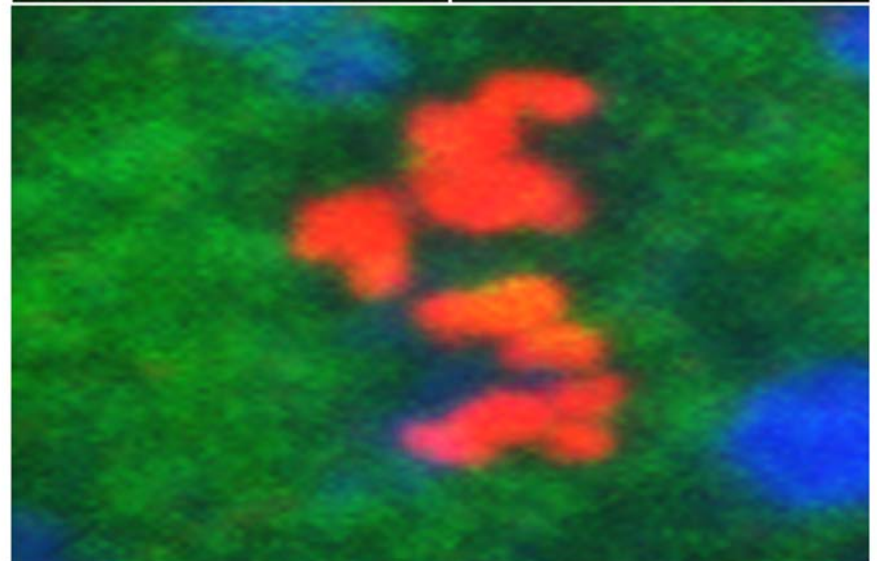
SUV39h (dn)

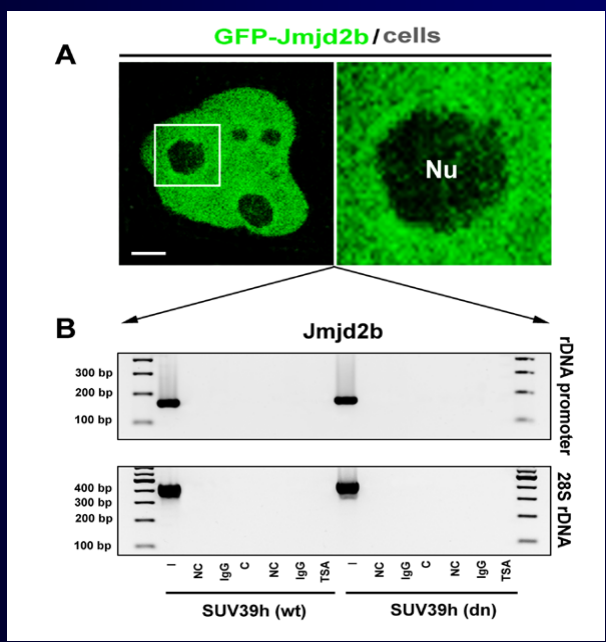
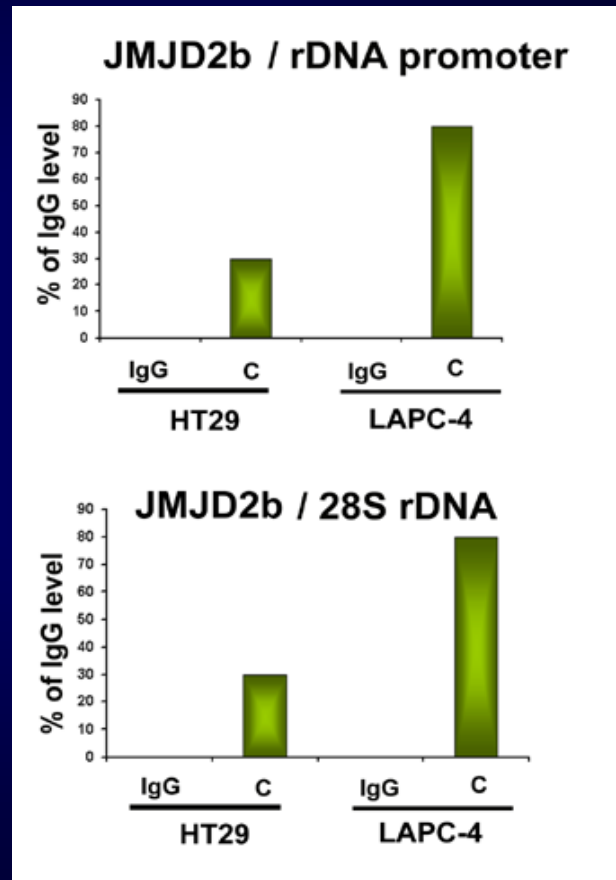
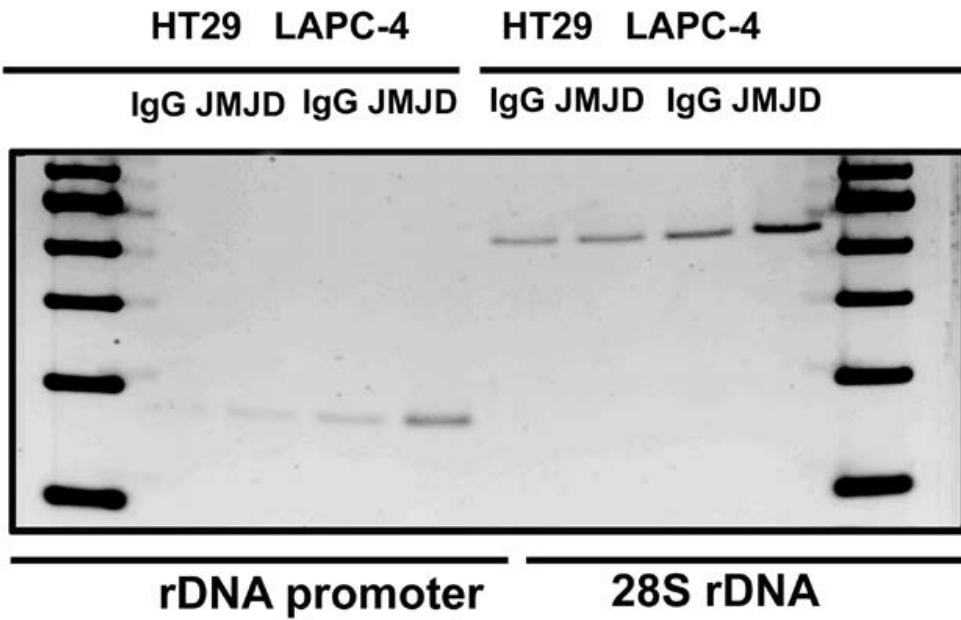


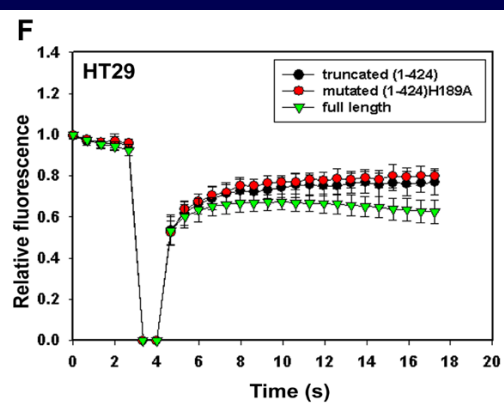
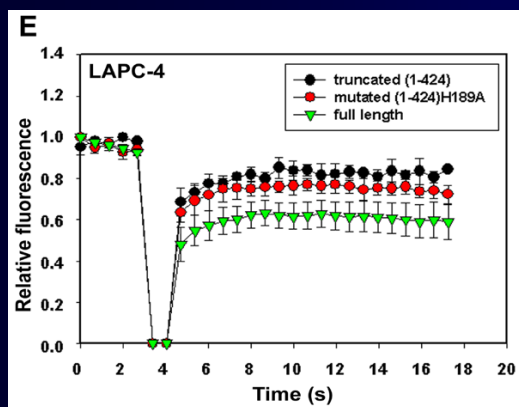
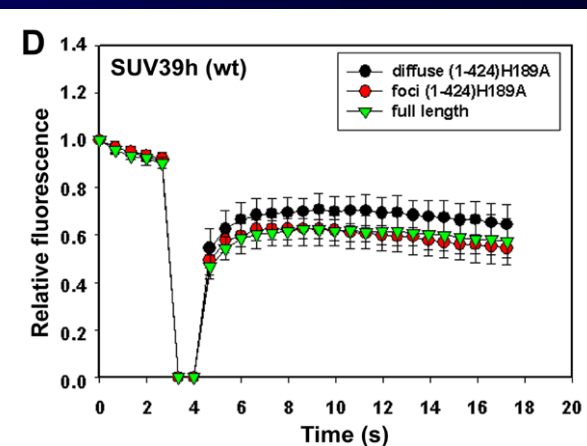
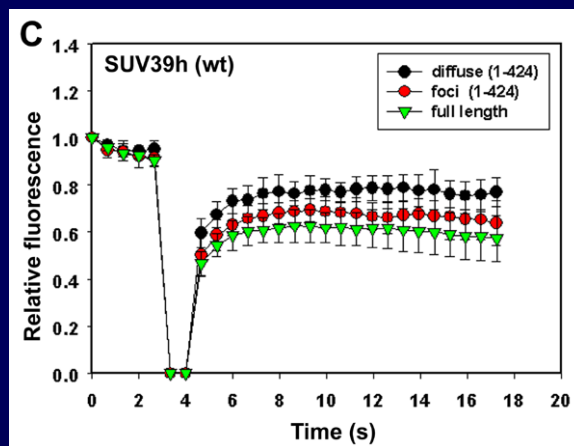
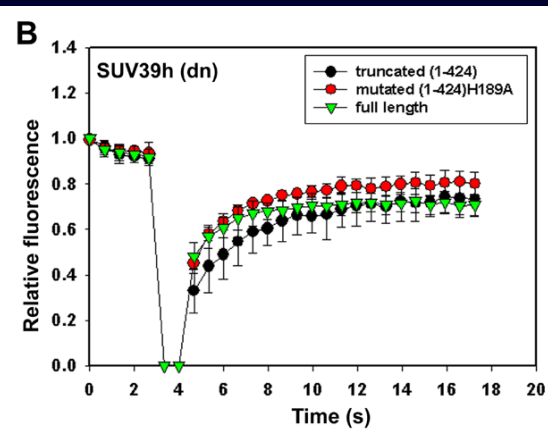
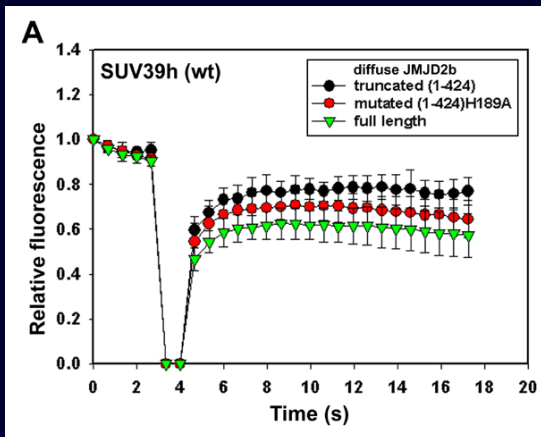
JMJD2b (1-424)H189-GFP / Fibrillarin / Chromocenters

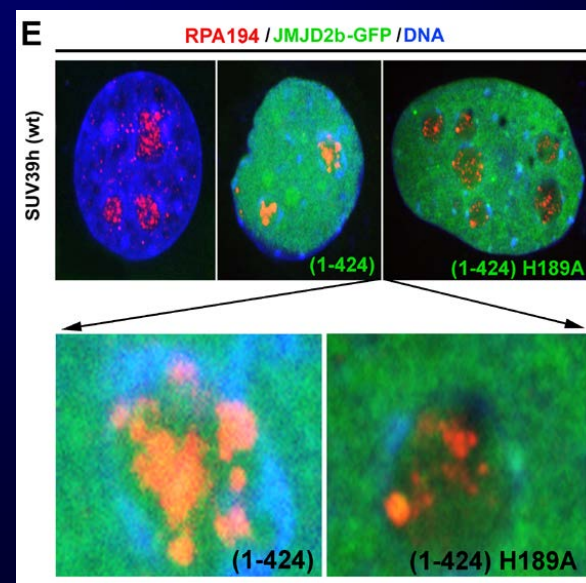
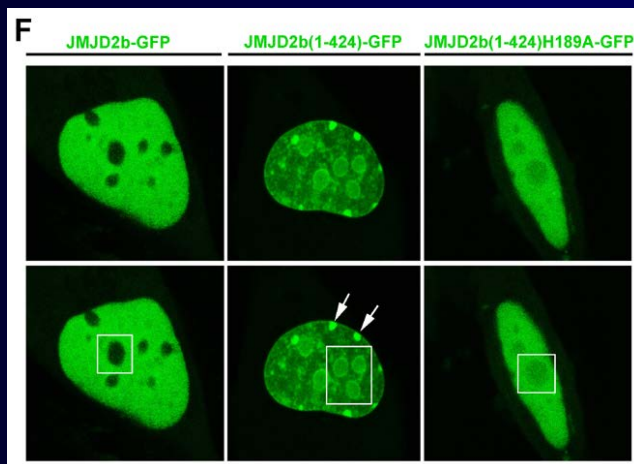
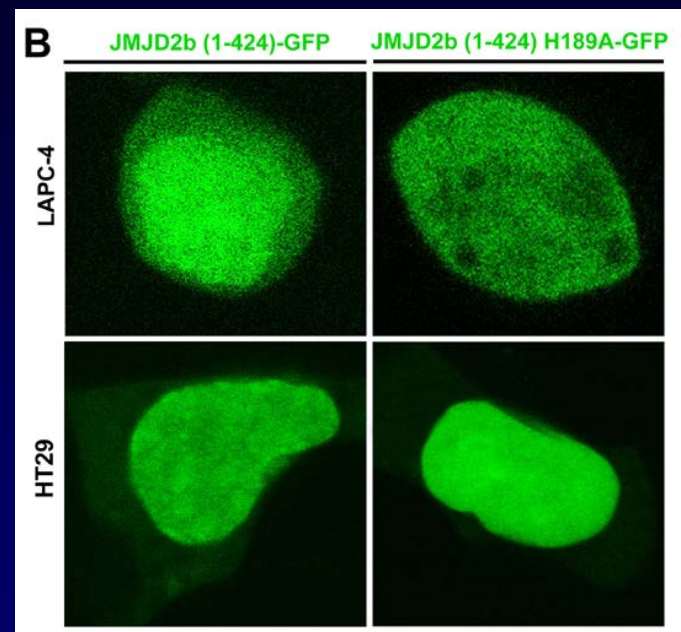
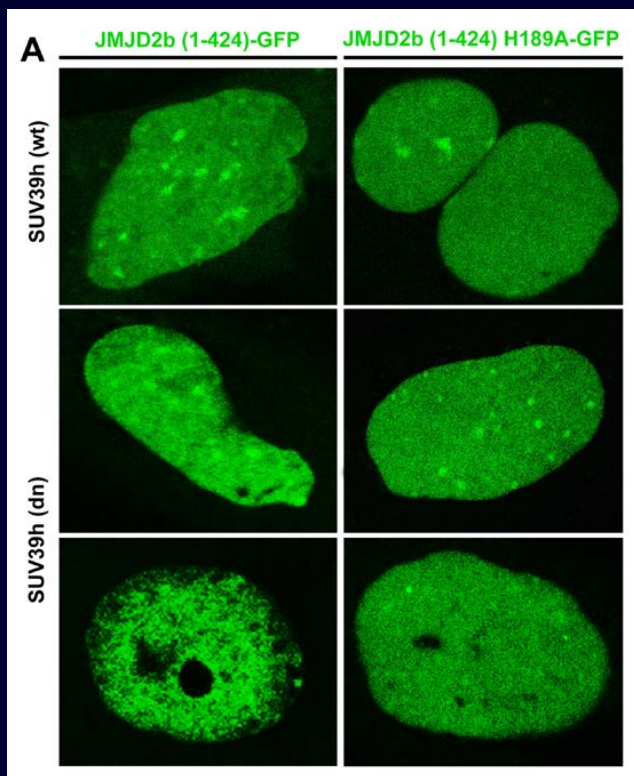


SUV39h (dn)











**Eva Bártová, Gabriela Šustáčková,
Lenka Stixová, Soňa Legartová, Darya Orlova, Veronika Foltánková**
Institute of Biophysics, the Academy of Sciences of the Czech Republic, v.v.i., Brno

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.