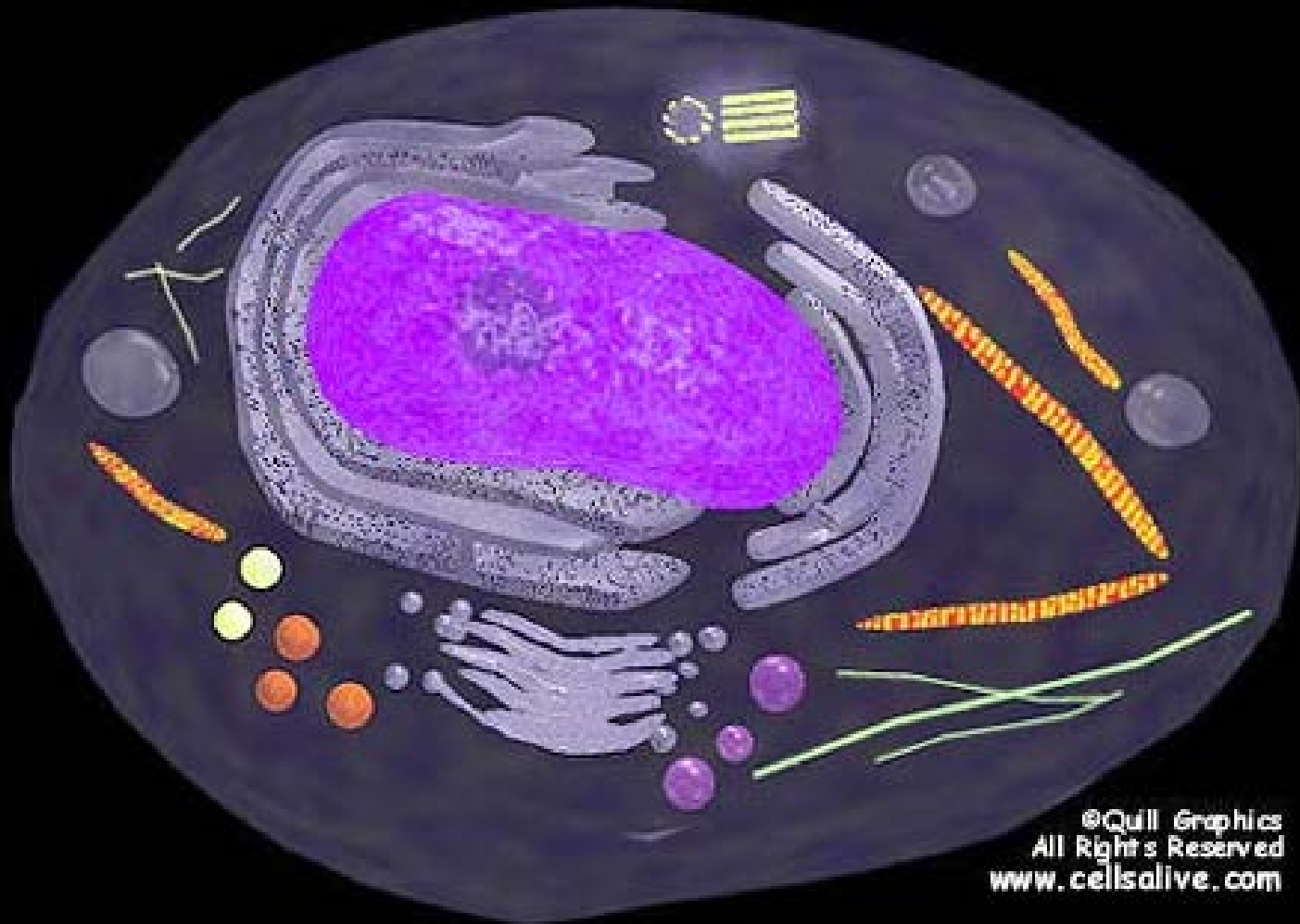


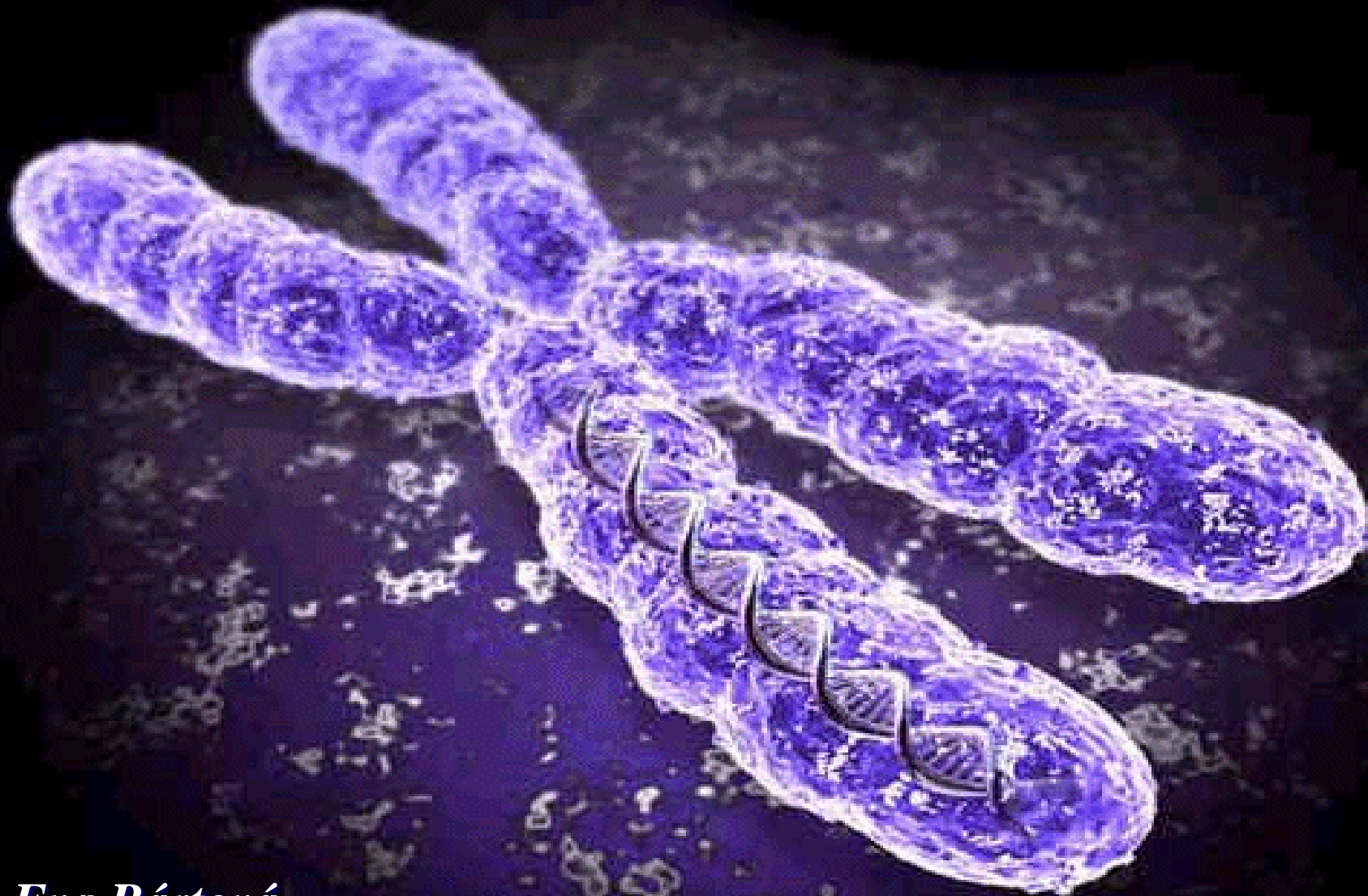
- **Struktura chromatinu - kompartmentalizace interfazních jader**
- **Morfologické a jaderně-topografické aspekty buněčné diferenciace.**
- **Apoptóza a struktura chromatinu.**
- **Cytoskelet a jaderná matrix - studium cytoskeletu pomocí mikroskopie s vysokým rozlišením.**
- **BAC/PAC knihovny a jejich využití pro FISH techniku**
- **Epigenetické procesy probíhající v buněčných jádrech.**
- **Cytogenetické a jaderně-topografické změny u nádorových buněk.**
- **Konfokální mikroskopie.**

Buněčné jádro



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www.cellsalive.com

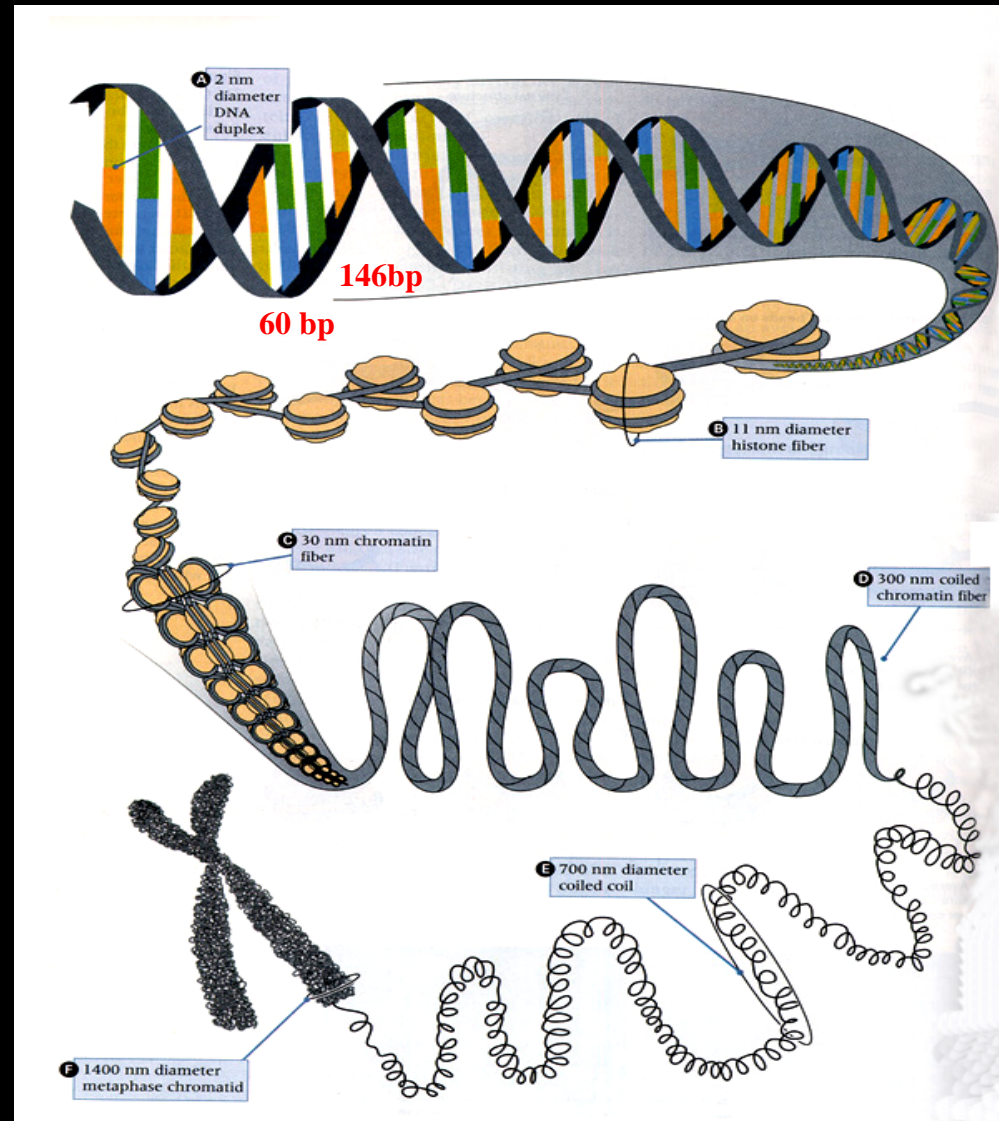
Struktura chromatinu vyššího řádu



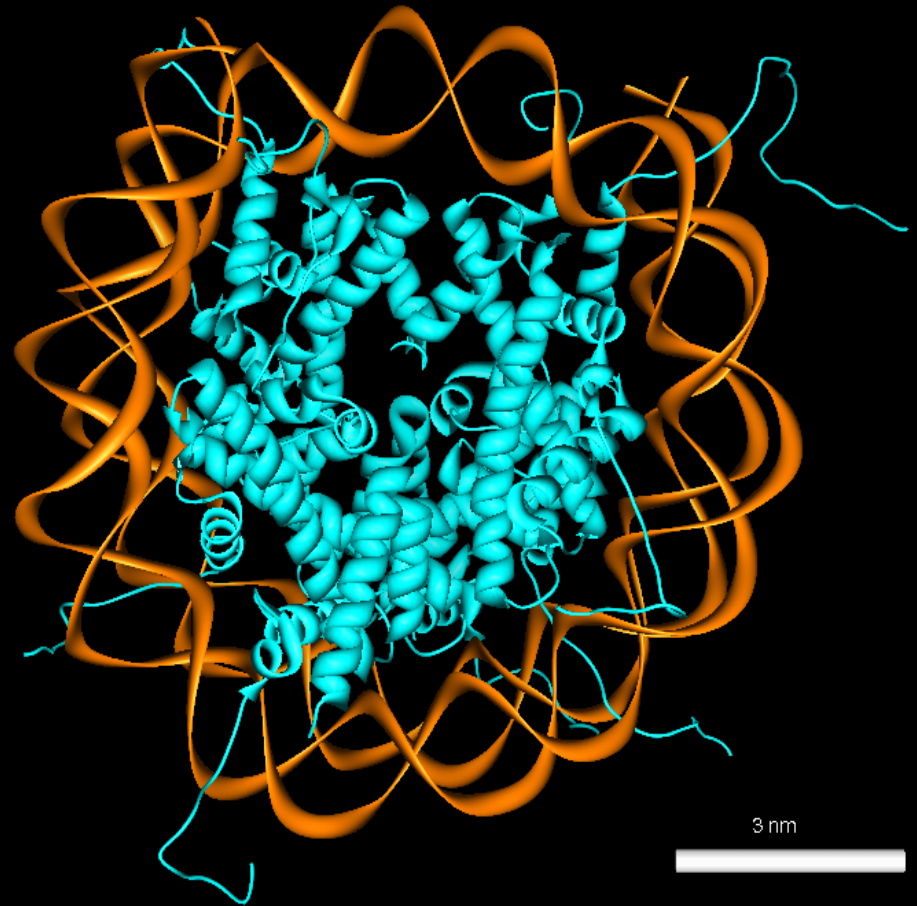
Eva Bártová

www.tqnyc.org

CHROMATIN: materiál jader eukaryotních buněk; nukleoproteinový komplex tvořený DNA vázanou na histony a další bílkoviny. V nedělicím se jádru lze rozlišit **euchromatin**, kde probíhá transkripce, a **heterochromatin**, který je transkripčně inaktivní.



HISTONY: skupina basických bílkovin v jádře eukaryotních buněk, kde vytvářejí reversibilní komplexy s DNA. Rozlišuje se pět typů histonů: H1, H2A, H2B, H3 a H4. Histony H2A, H2B, H3 a H4 tvoří vždy ve dvou kopiích oktamer, kolem nichž se obtáčí dvojšroubovicová DNA; tento útvar se nazývá nukleosom. Histon H1 je přítomen v menším množství než ostatní histony, a ačkoli je též vázán na DNA, není součástí nukleosomů. Histony se tak podílejí na uspořádání DNA v eukaryontním chromosomu do vlákna vyššího řádu.



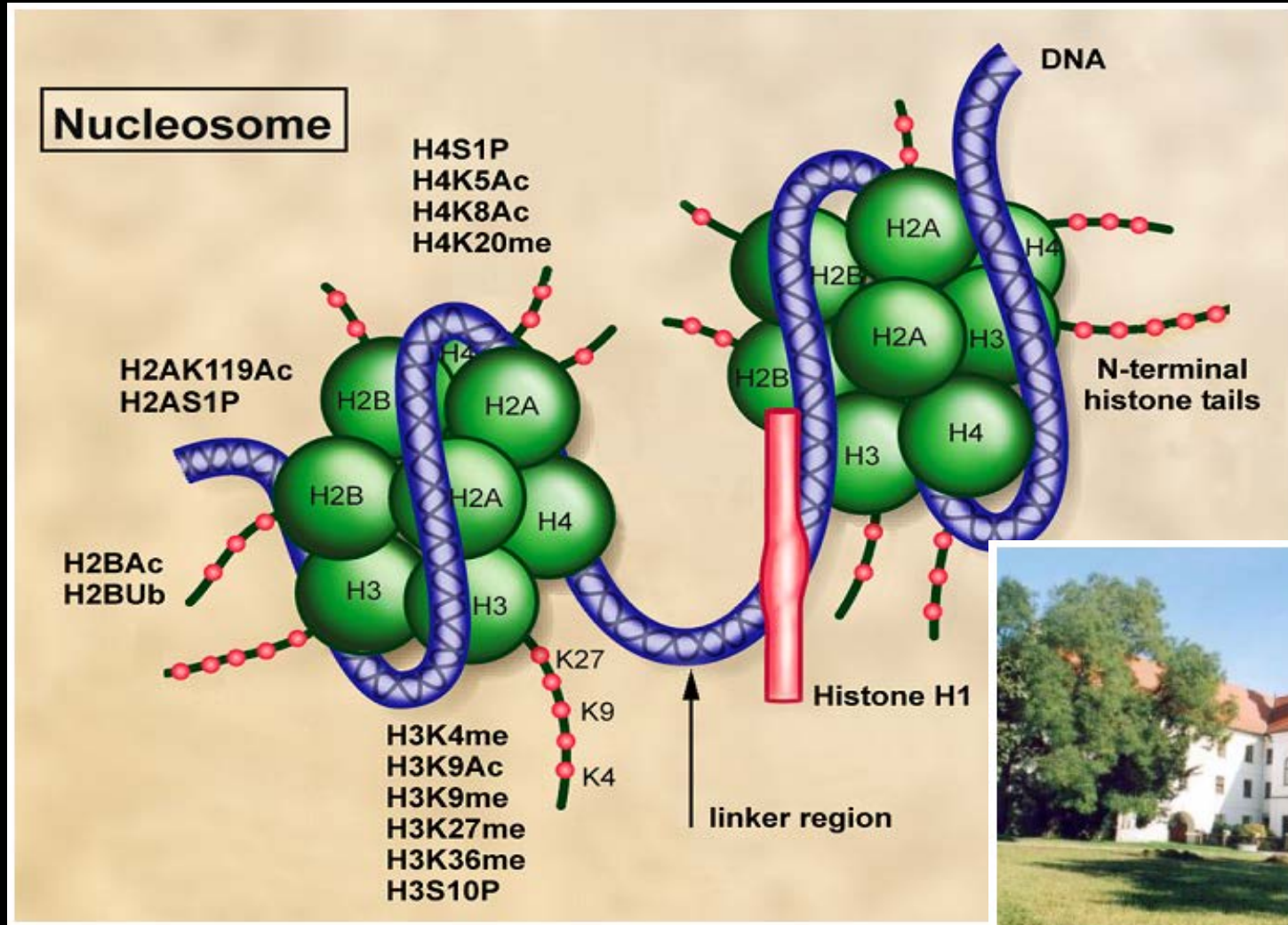
NUKLEOSOM



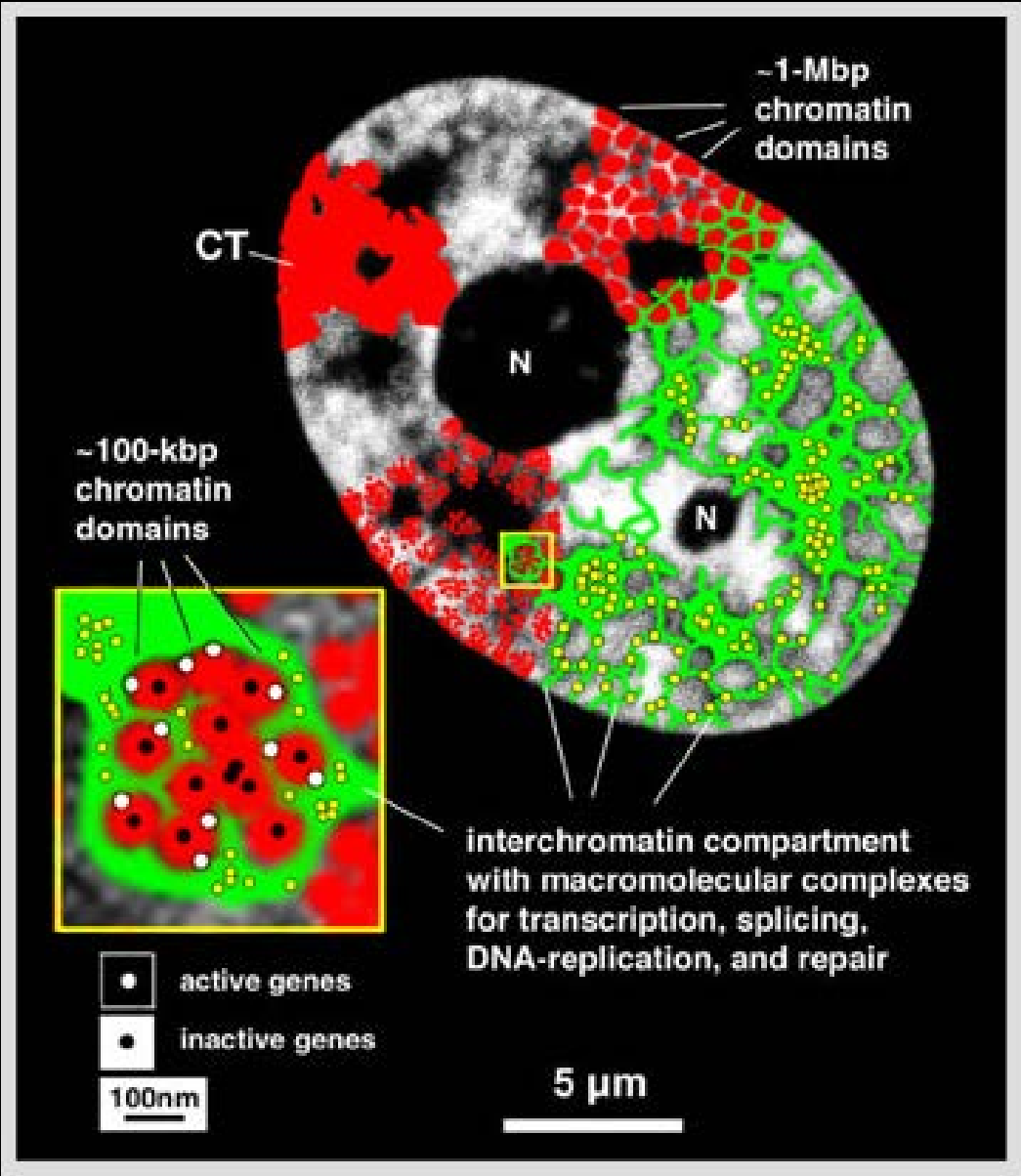
Histone signature

Brno nomenclature for histone modifications

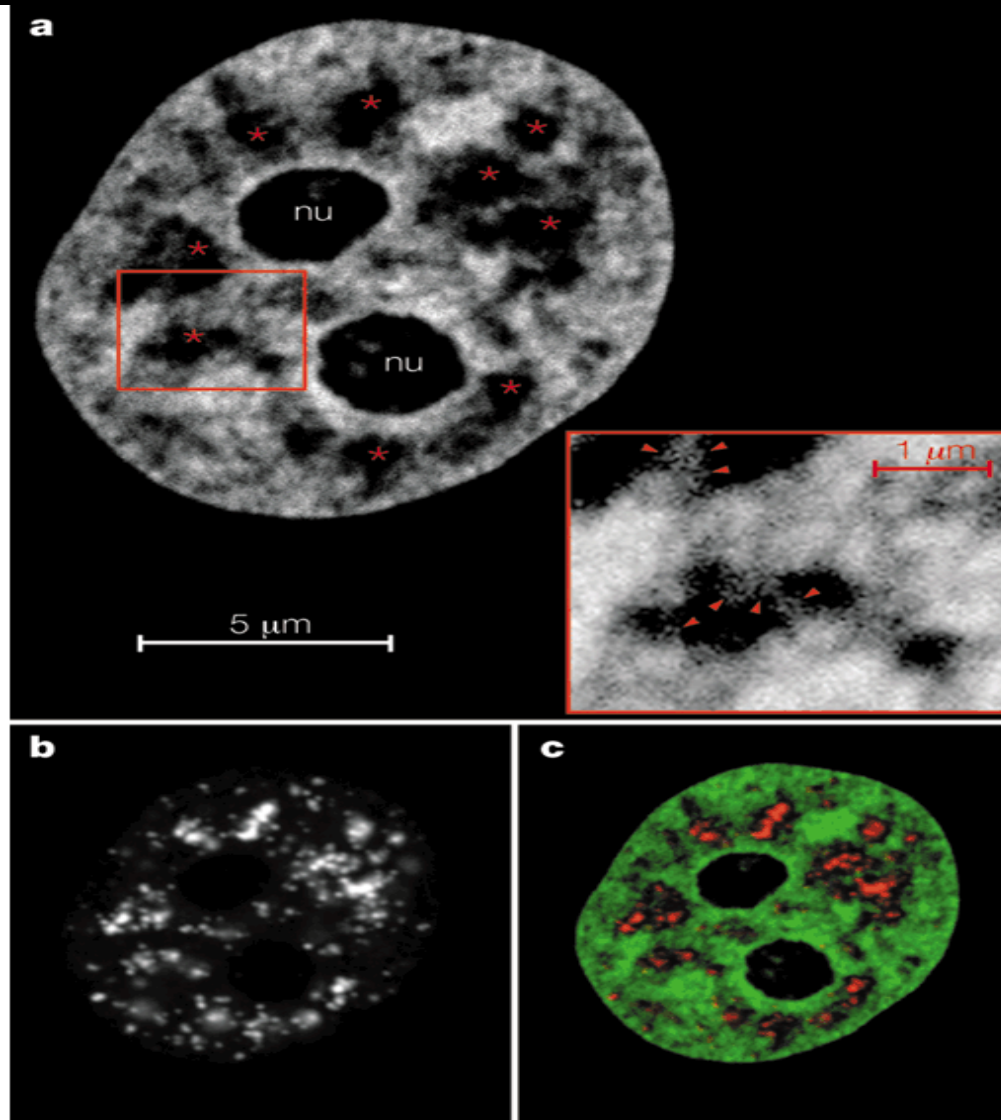
(Turner, 2005)



CT-IC MODEL (T. Cremer)



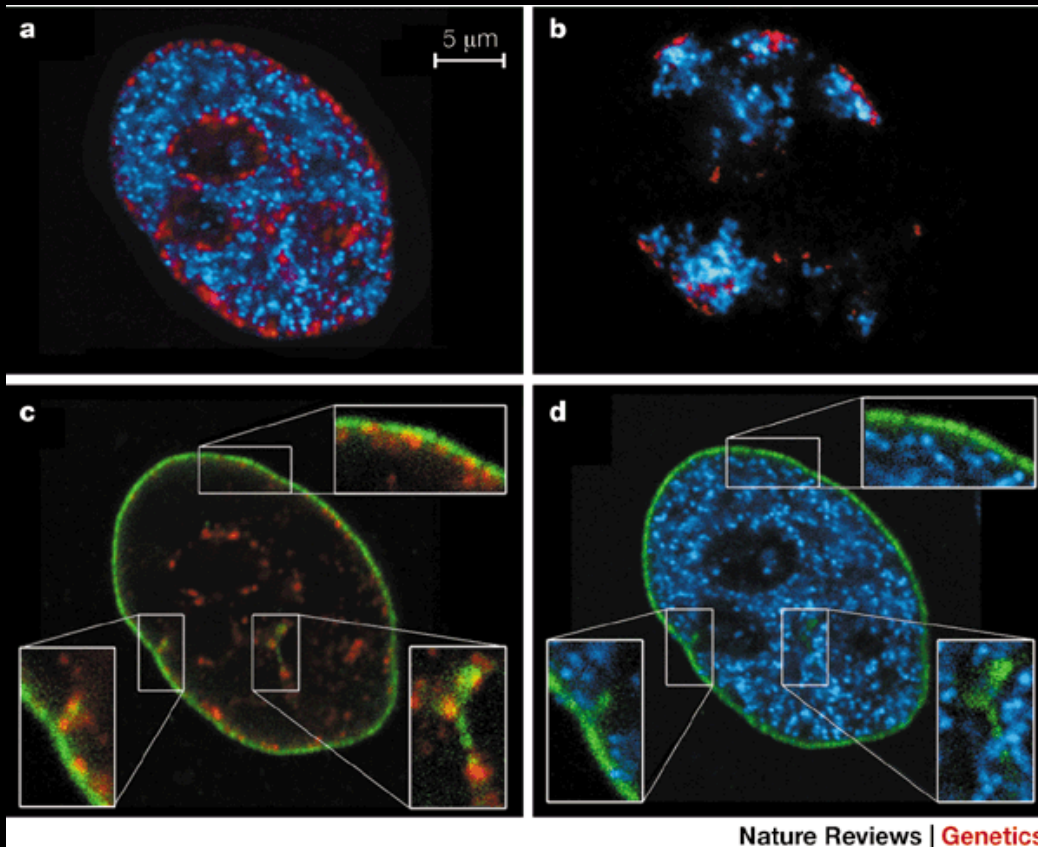
Chromatinové meziprostory



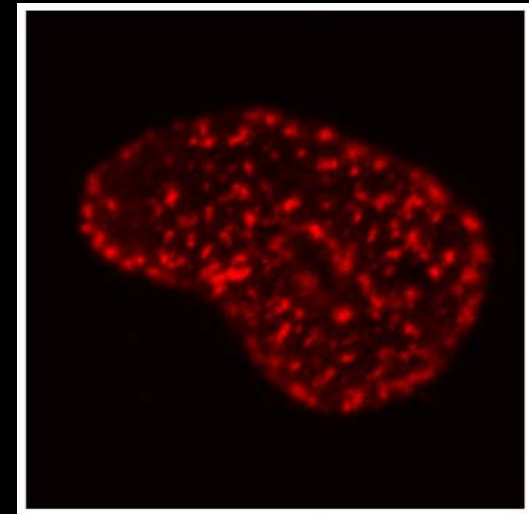
T. Cremer group, Munich

Struktura chromatinu a jederné procesy

Pozdně a časně se replikující chromatin

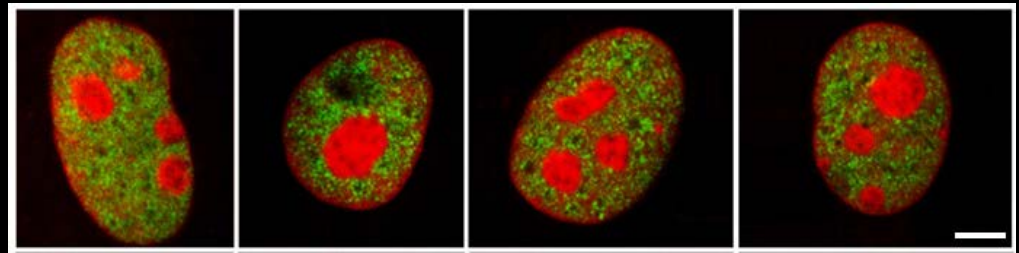


Replikační ohniska

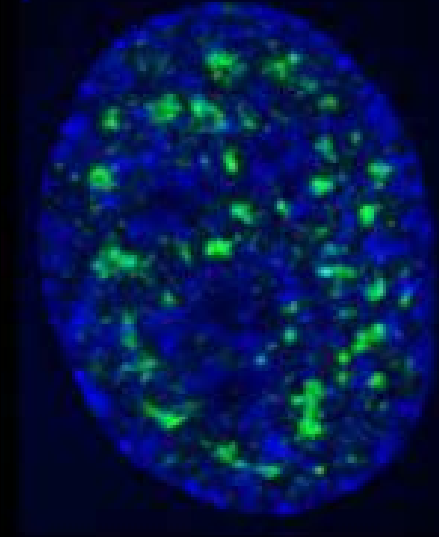


Nuclear compartments:

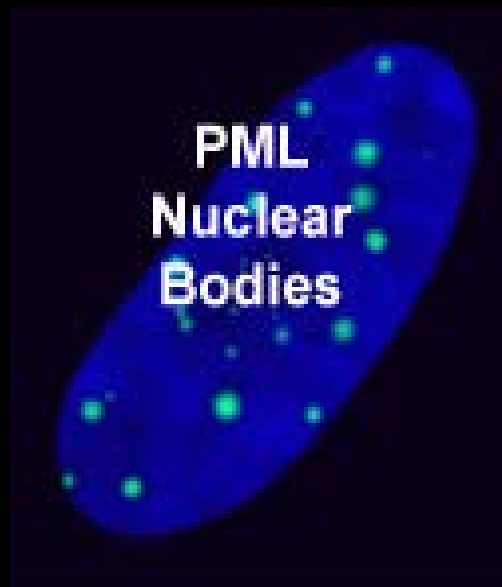
1. Nucleolus
2. Splicing speckles
3. Cajal bodies
4. PML bodies
5. snRNP



speckles



Cajal
Bodies

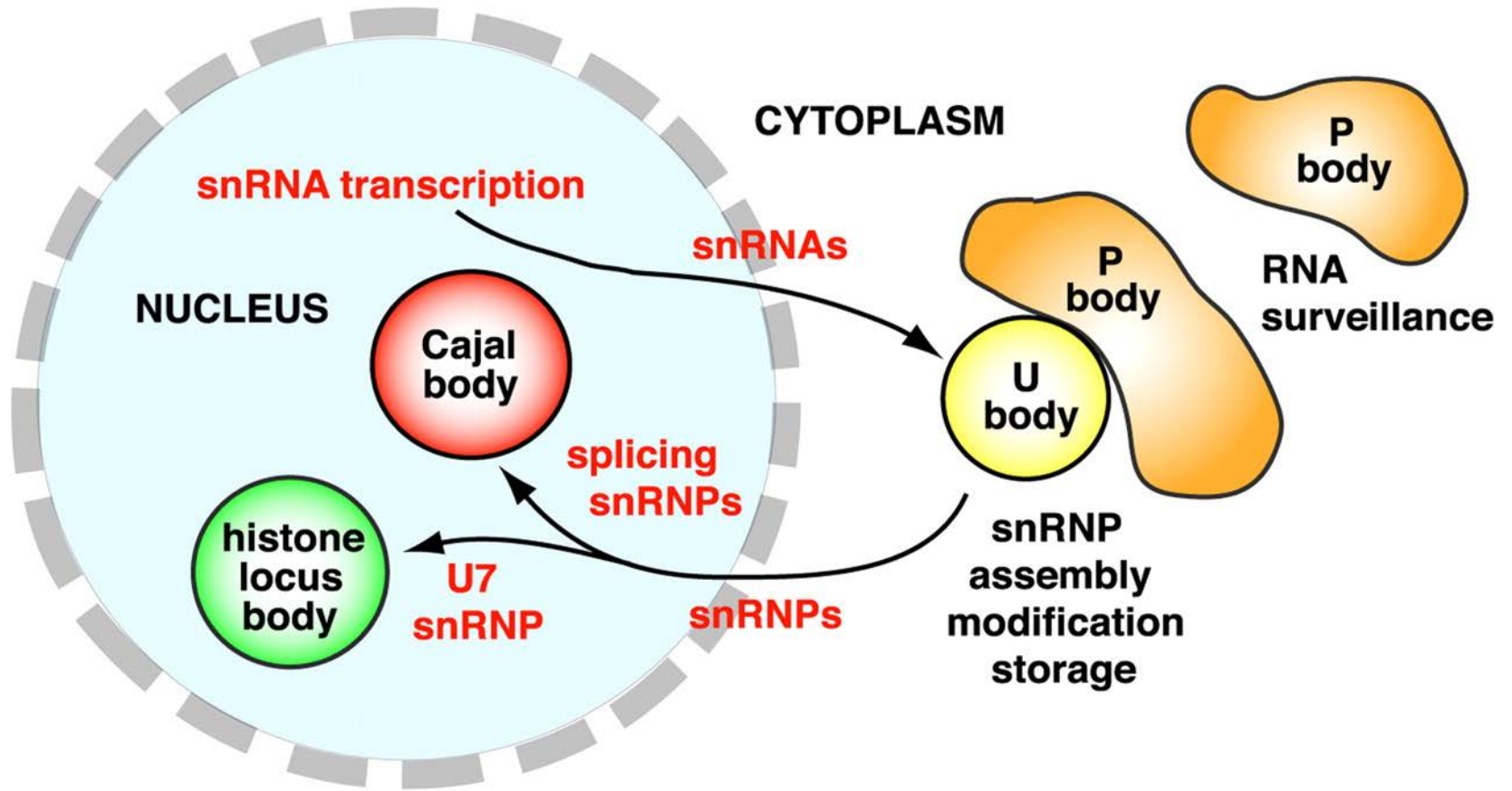


PML
Nuclear
Bodies

Roles of snRNPs

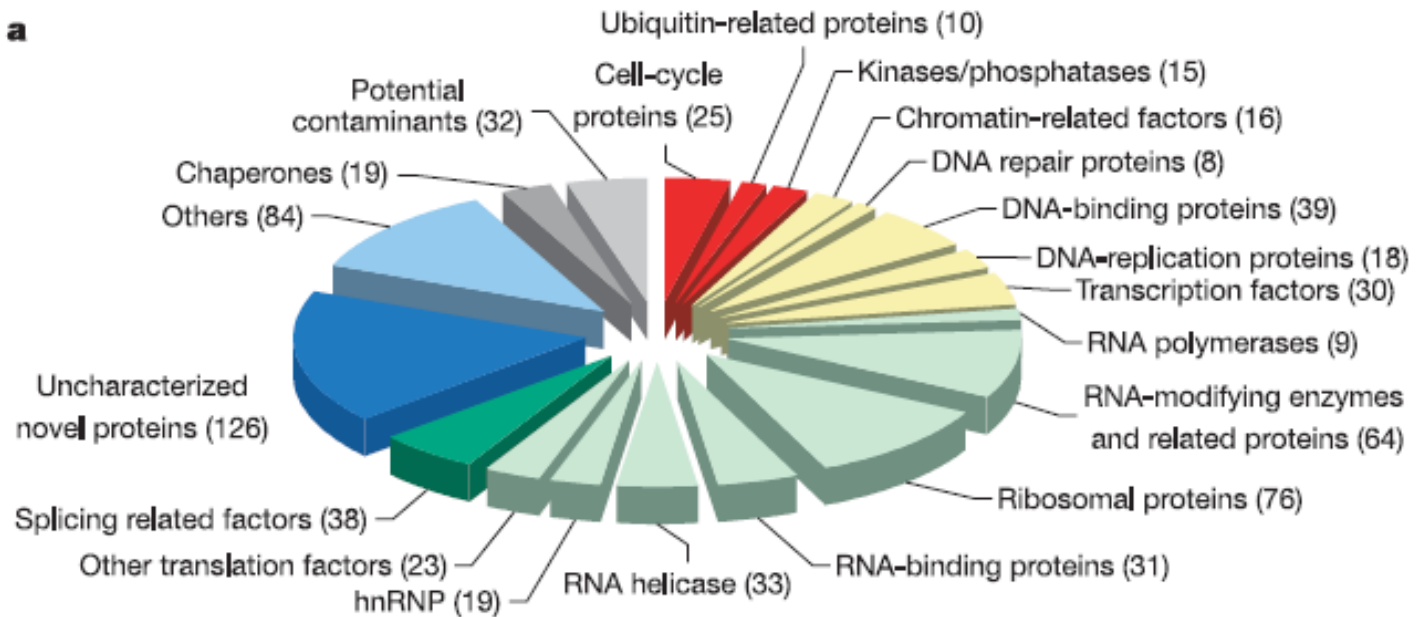
- **U1 snRNP** binds 5' splice site
- **U2 snRNP** binds to branch point
- **U4/U6 snRNP**. snRNAs are base paired. U6 is catalytic
- **U5 snRNP** contacts the 5' splice site
 - forms tri-snRNP complex with U4/U6

Nuclear and cytoplasmic bodies involved in snRNP assembly.



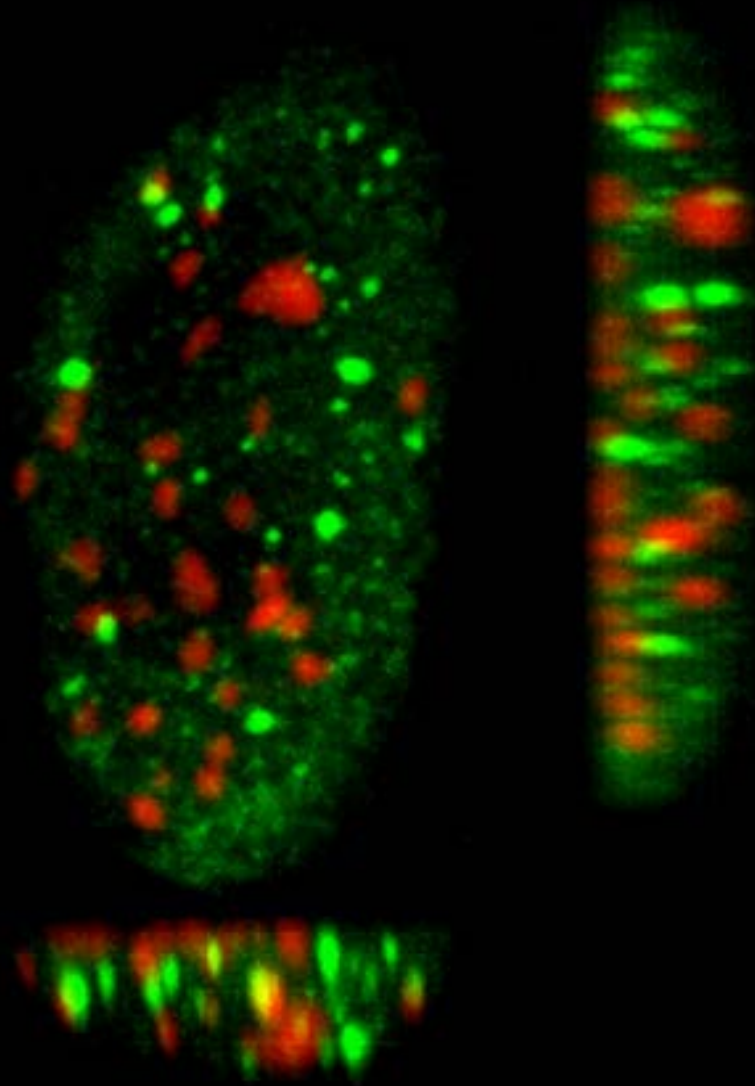
Liu J , Gall J G PNAS 2007;104:11655-11659

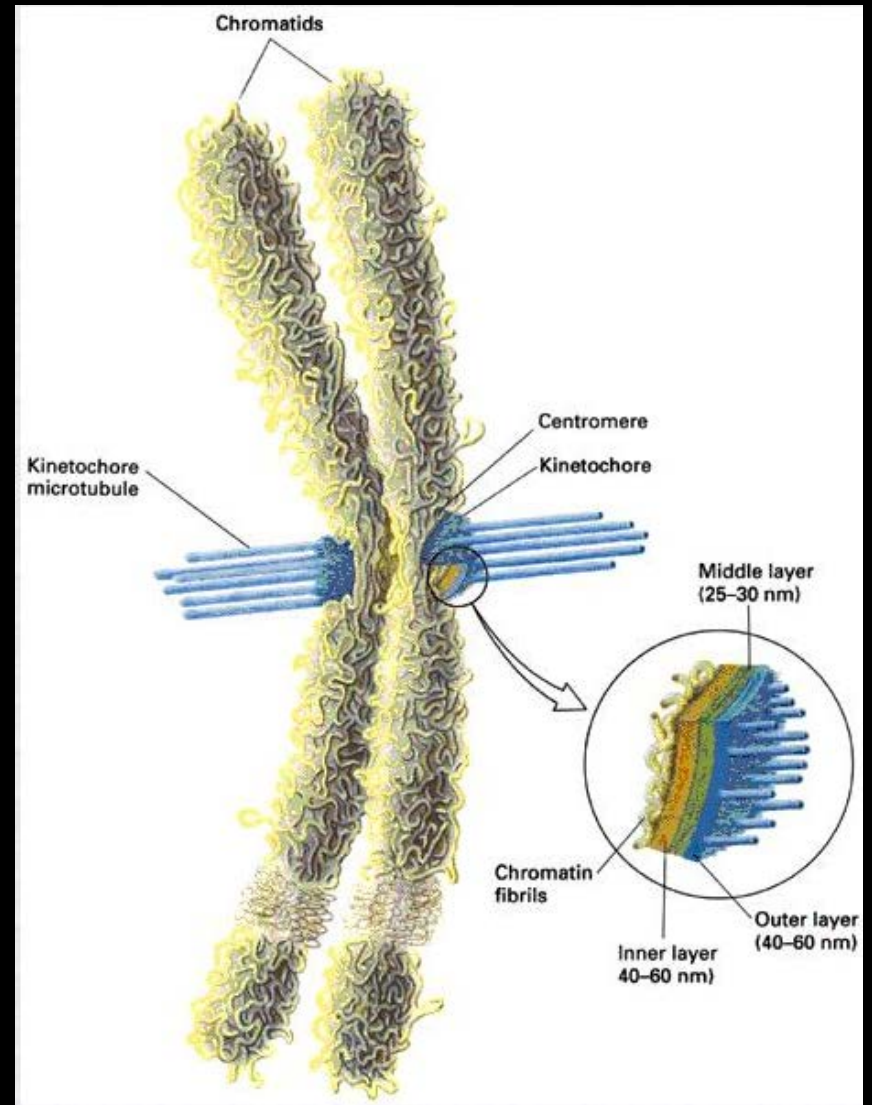
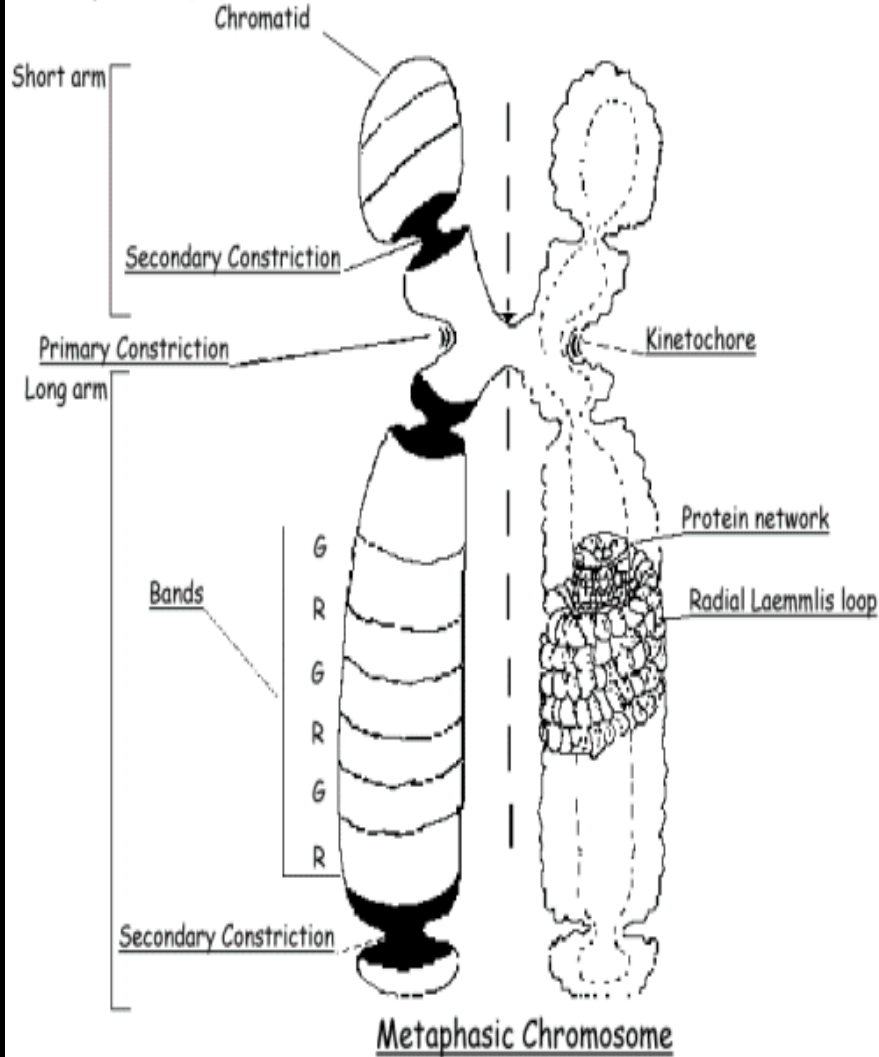
a



Andersen et al., Nature, 2005

HP1 proteins

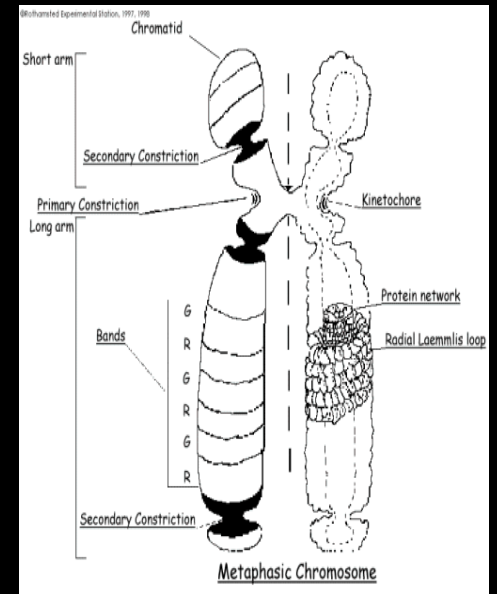
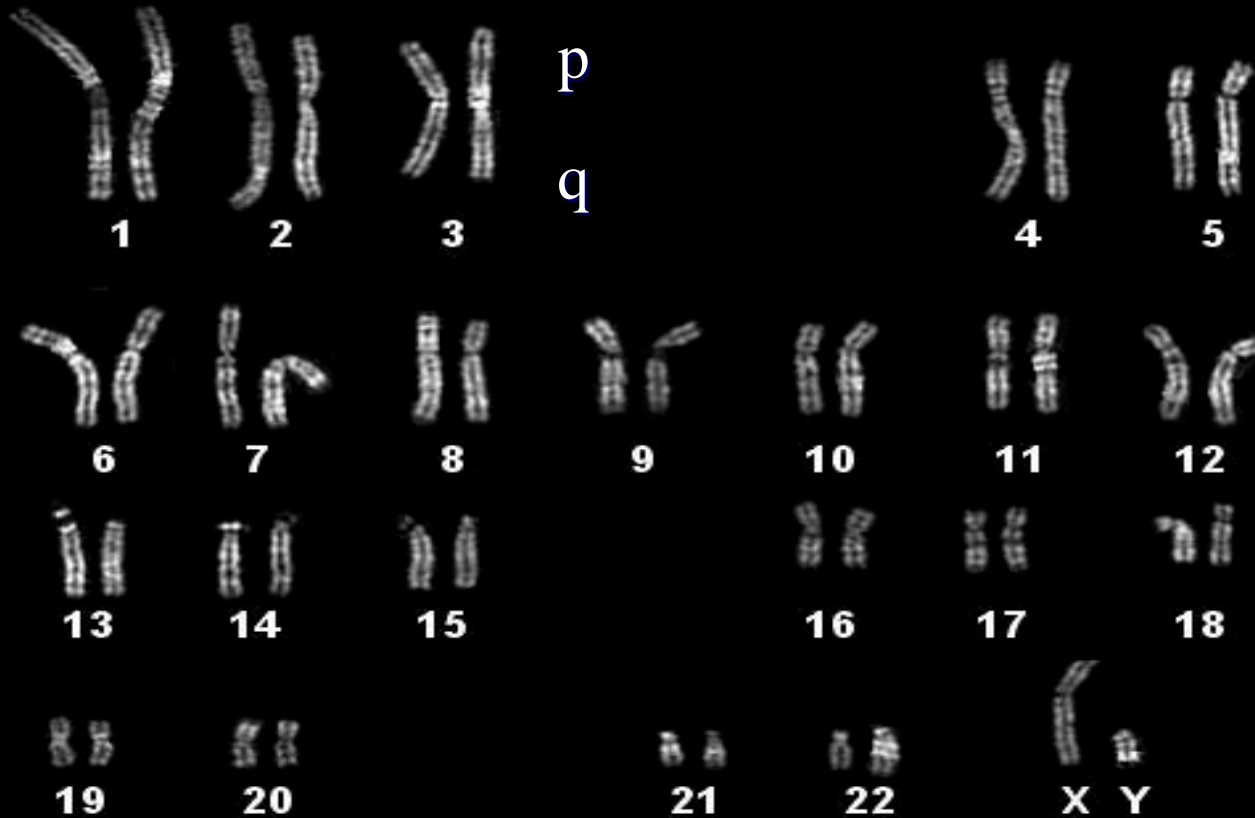




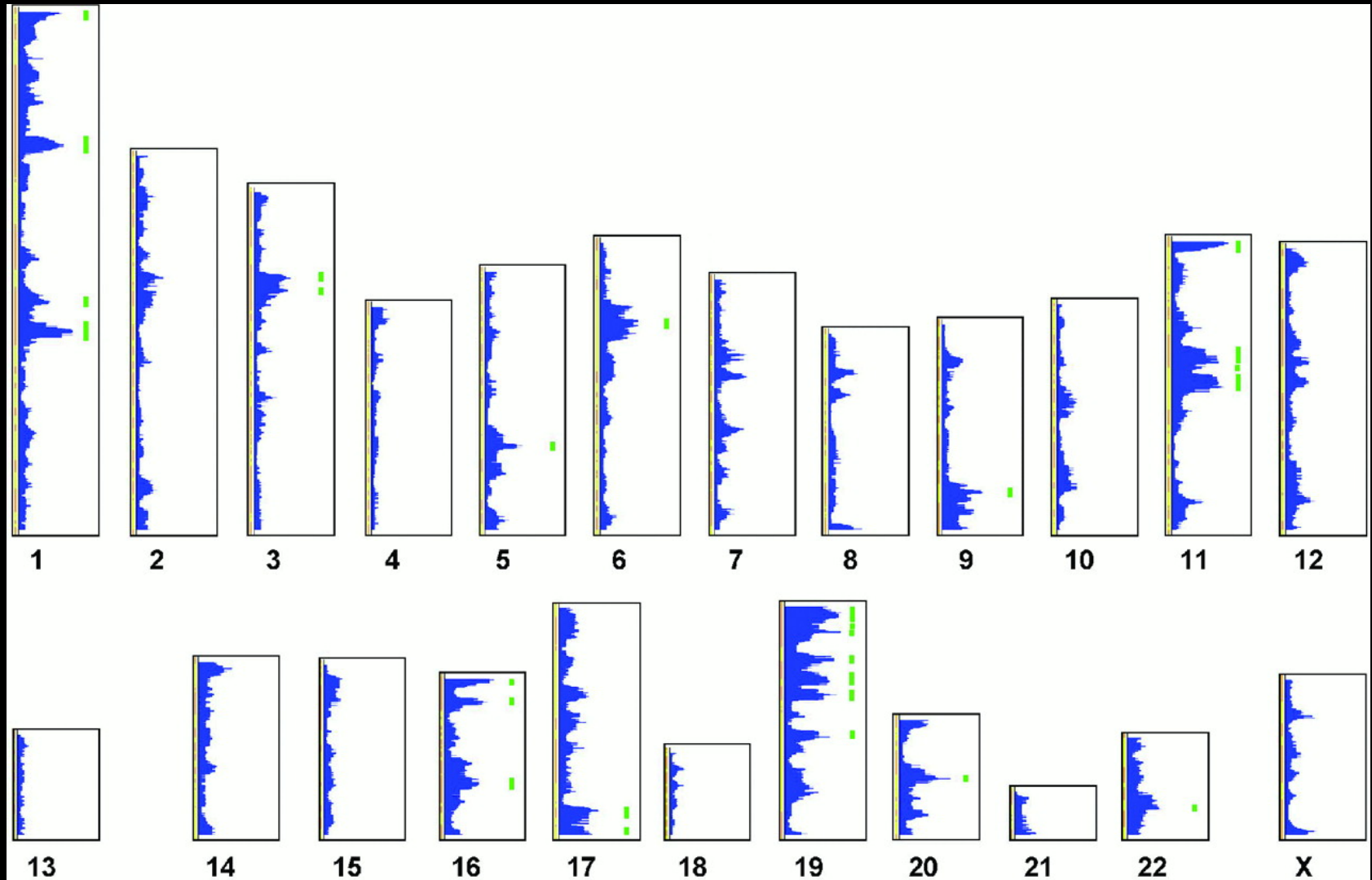
Kondenzace chromatinu

Figure 23-38, p. 1094, Molecular Cell Biology, 3rd ed., Lodish, et al., copyright 1995,

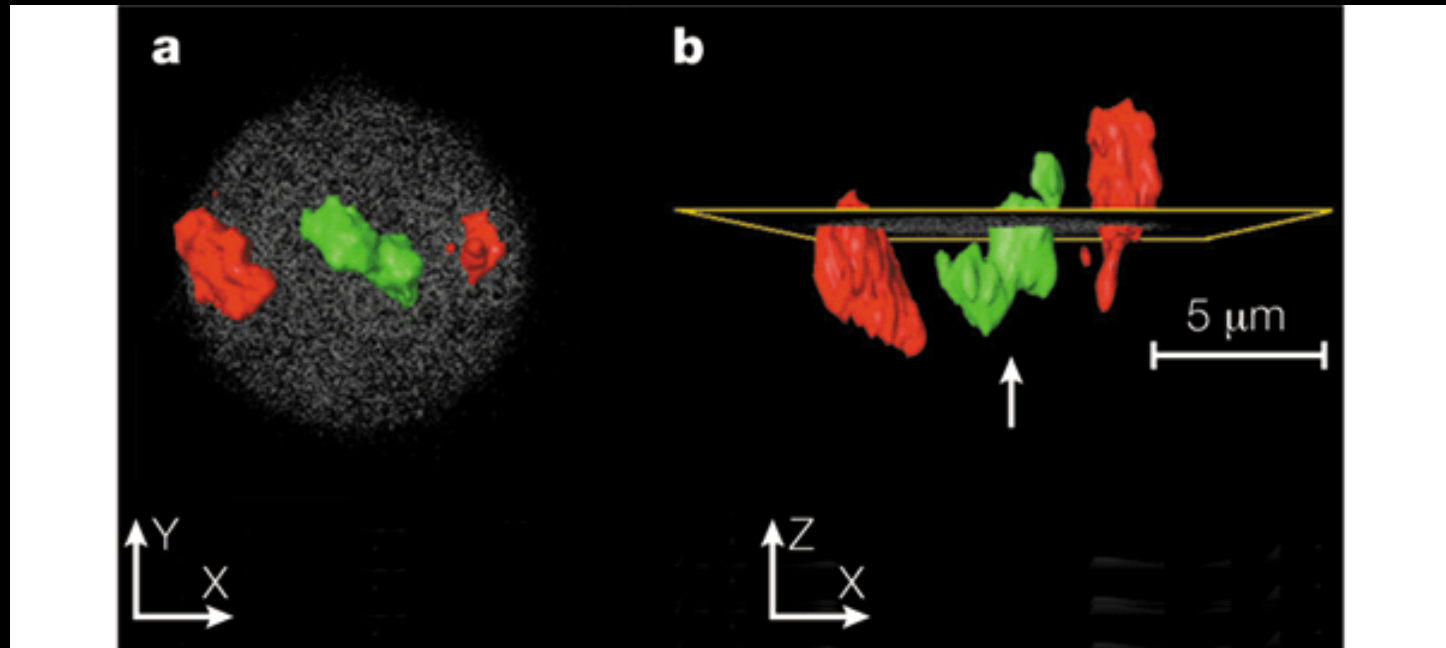
Typy chromozomů: meta- submeta-, akrocentrické



Transcriptome map (Caron et al., 2001)

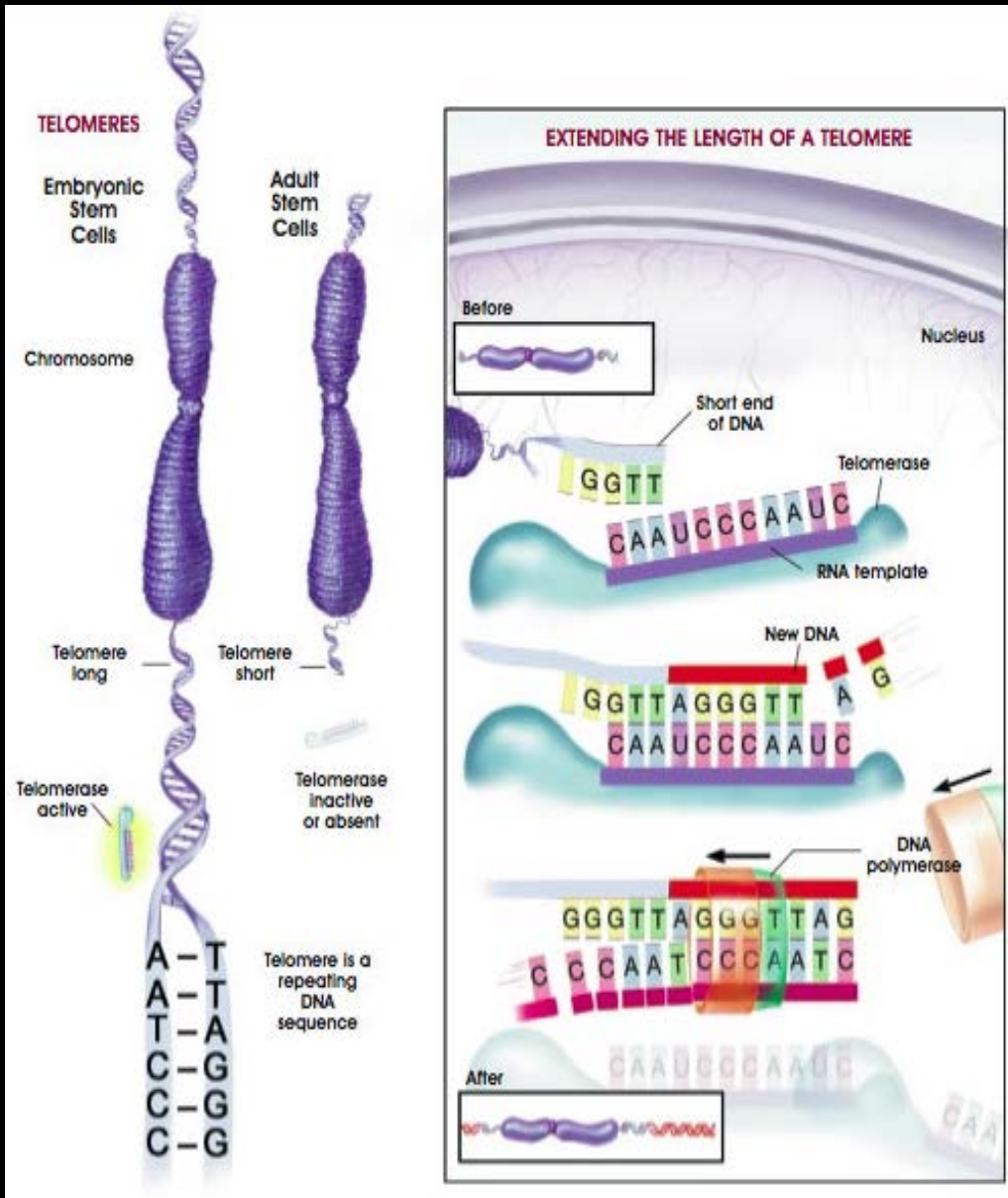


HSA 18 and 19 (positioning and gene density)

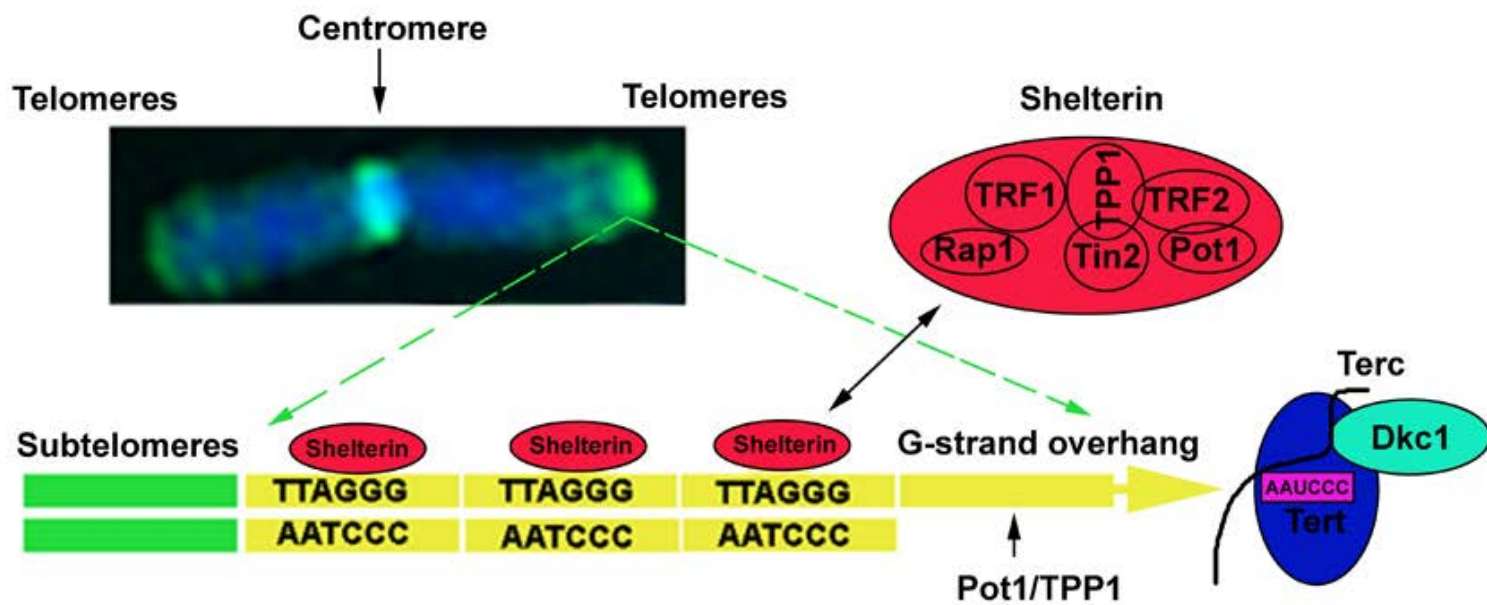


T. Cremer group, Munich

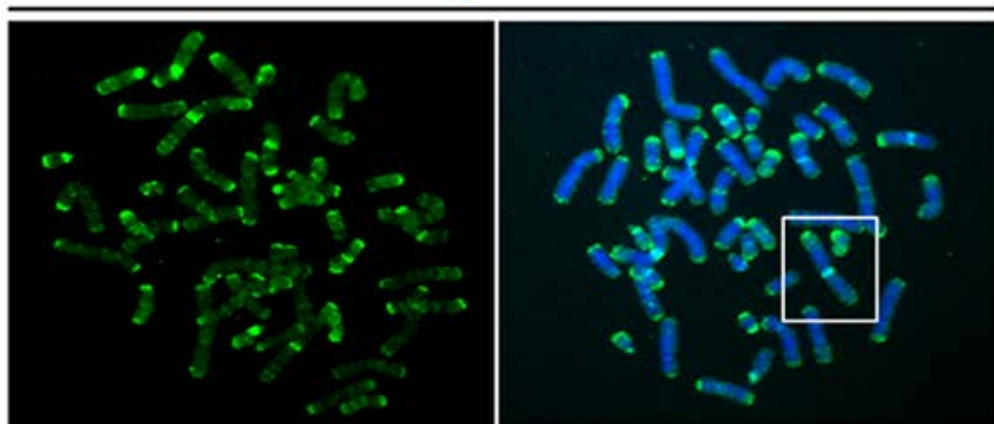
Telomeres (TTAGGG sequence and different proteins)

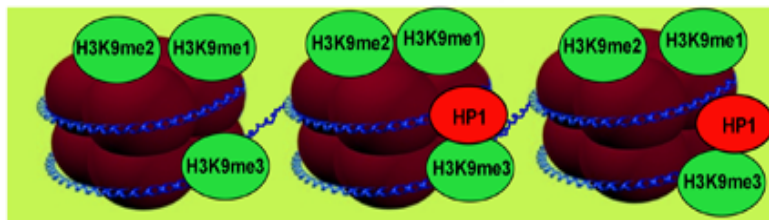
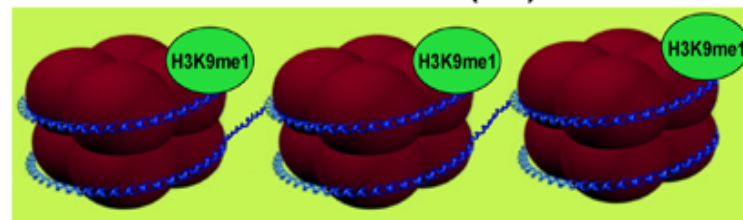


Telomerase is composed of two subunits, Telomerase Reverse Transcriptase (hTERT, the 'h' is for human) and hTR (Telomerase RNA). These two subunits are coded for by two different genes in the genome. Using hTR template, hTERT can add a six nucleotide repeating sequence, 5'-TTAGGG to the 3' strand of chromosomes. This repeating TTAGGG sequence is called the telomere. The template region of hTR is 3'-CCCAAUCCC - 5'.

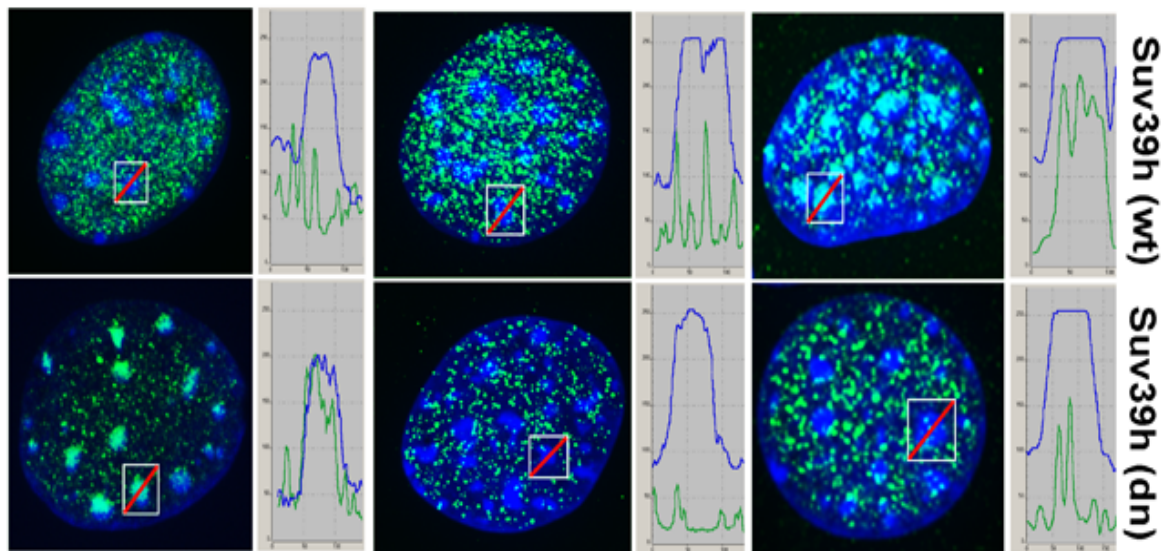
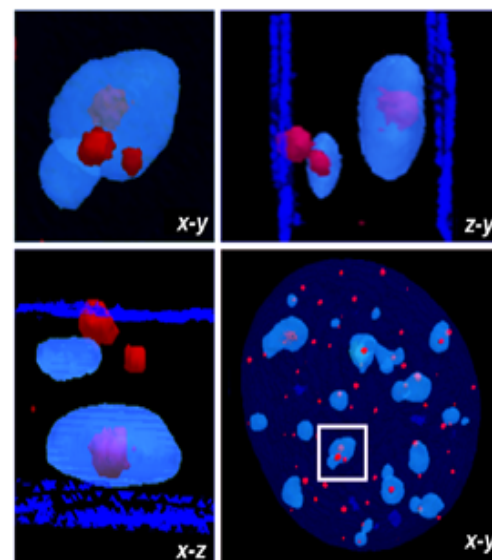
A

DNA methylation / DNA



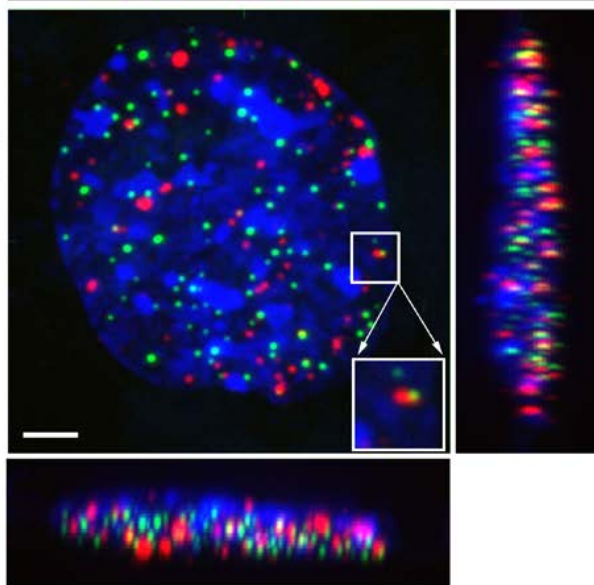
B**H3K9 methylation at telomeres****a)****SUV39h (wt)****SUV39h (dn)**

García-Cao et al. (2004)

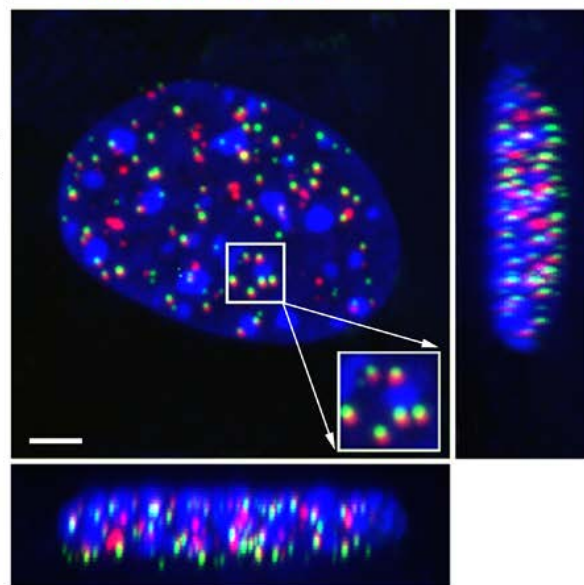
b)**H3K9me1****H3K9me2****H3K9me3****c)****Telomeres / Chromocenters**

A**GFP-TRF1 / PML / DNA**

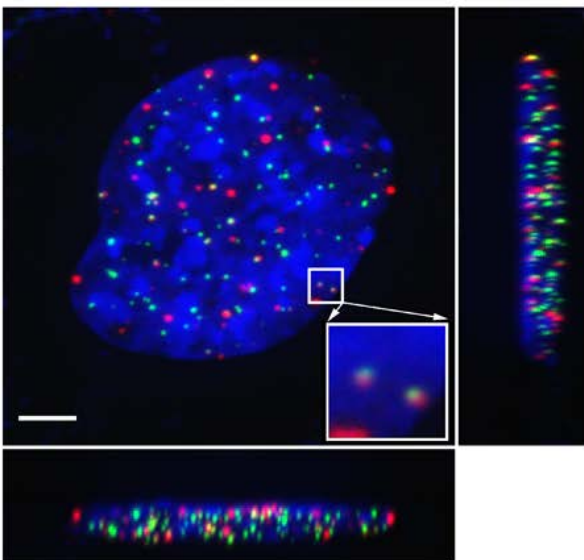
SUV39h (wt)

**GFP-TRF1 / PML / DNA**

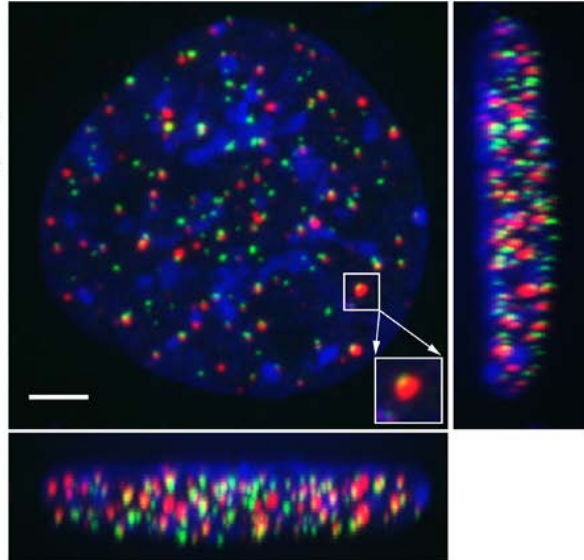
SUV39h (dn)

**GFP-TRF1 / PML / DNA**

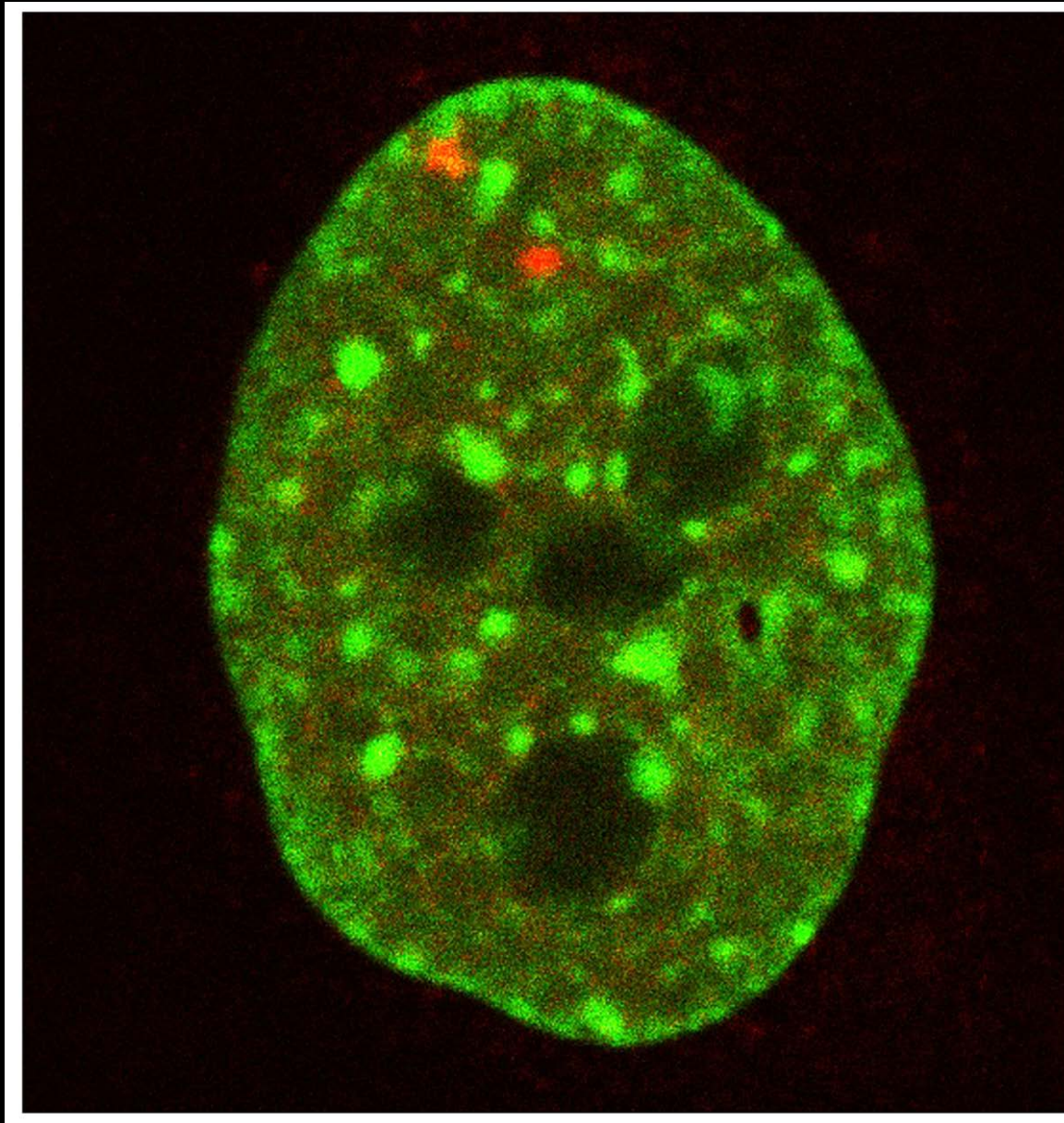
LMNA (wt)

**GFP-TRF1 / PML / DNA**

LMNA (dn)



DNA repair foci



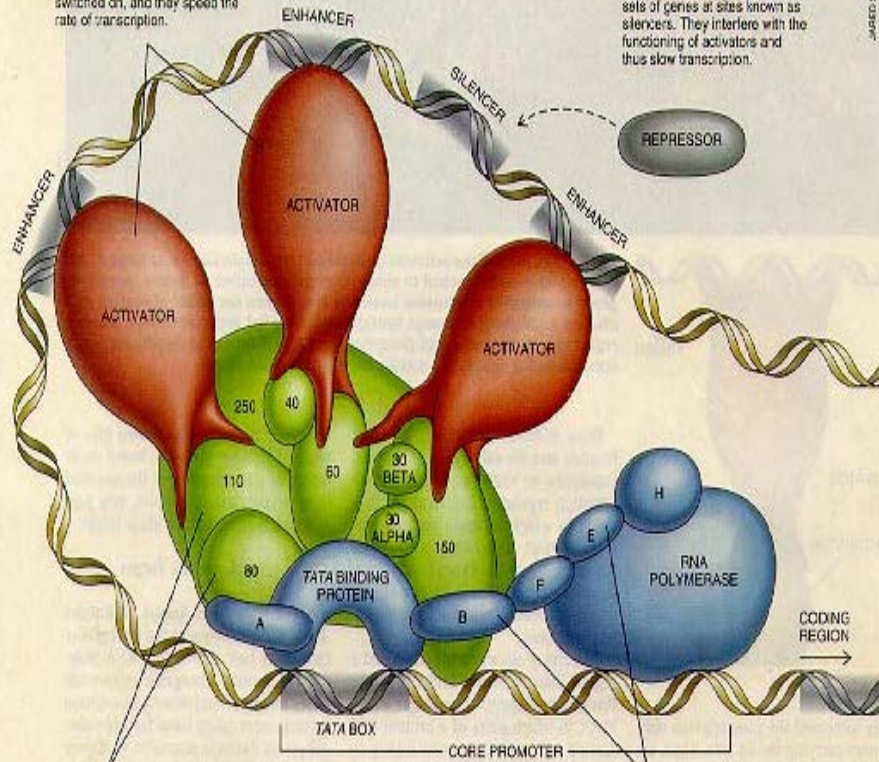
TRANSKRIPCE: přepis, biosynthesa řetězce RNA podle templátového řetězce DNA, přičemž jednotlivé nukleotidy jsou připojovány na základě komplementarity (viz base nukleových kyselin). Klíčovým enzymem této synthesy je RNA-polymerasa. Transkripce probíhá ve třech stupních: **a) iniciace** (zahájení), kdy se RNA-polymerasa váže na specifickou sekvenci DNA (viz promotor) a přesunuje se k místu, kde začíná vlastní synthesa; **b) elongace**, kdy se RNA-polymerasa posunuje podél řetězce DNA, uvolňuje kódující řetězec a podle templátového řetězce postupně syntetisuje novou RNA tím, že na volnou 3'-OH skupinu ribosy připojuje komplementární nukleotidy, jejichž donorem jsou nukleosidtrifosfáty; vznikající RNA se postupně uvolňuje od komplexu s DNA a dvojitý helix DNA se samovolně obnovuje; **c) terminace** (ukončení synthesy a úplné uvolnění RNA) je signalisováno zvláštními sekvencemi ve struktuře DNA, které jsou rozpoznávány bílkovinami, tzv. terminačními neboli ρ (ro) faktory. Řízení transkripce jednotlivých genů patří k nejdůležitějším mechanismům regulace enzymové aktivity a diferenciaci buněk.

ACTIVATORS

These proteins bind to genes at sites known as enhancers. Activators help to determine which genes will be switched on, and they speed the rate of transcription.

REPRESSORS

These proteins bind to selected sets of genes at sites known as silencers. They interfere with the functioning of activators and thus slow transcription.



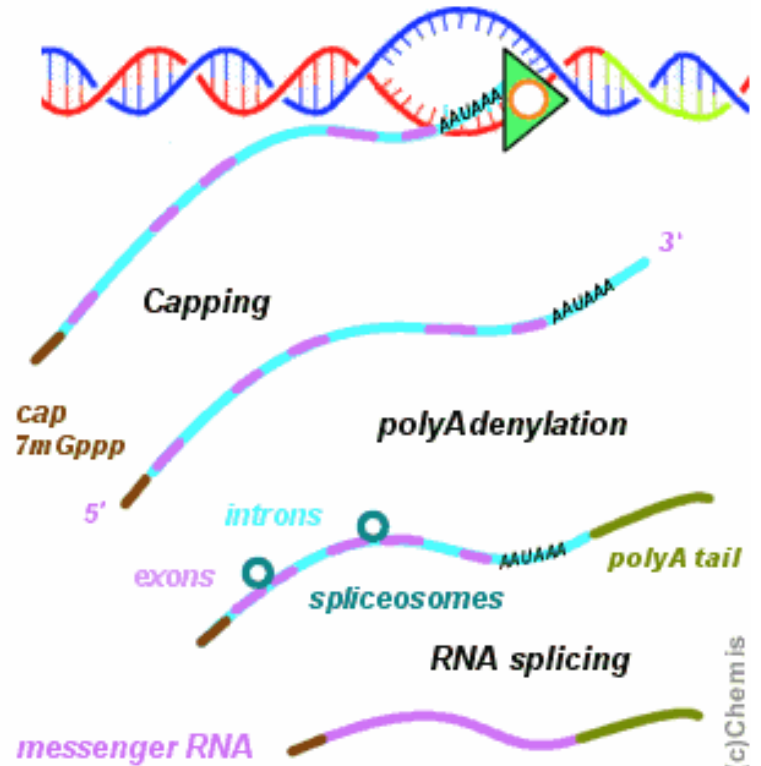
COACTIVATORS

These "adapter" molecules integrate signals from activators and perhaps repressors and relay the results to the basal factors.

BASAL FACTORS

In response to injunctions from activators, these factors position RNA polymerase at the start of the protein-coding region of a gene and send the enzyme on its way.

JARED SCHNEIDMAN



(c)Chemis

Alternative Splicing

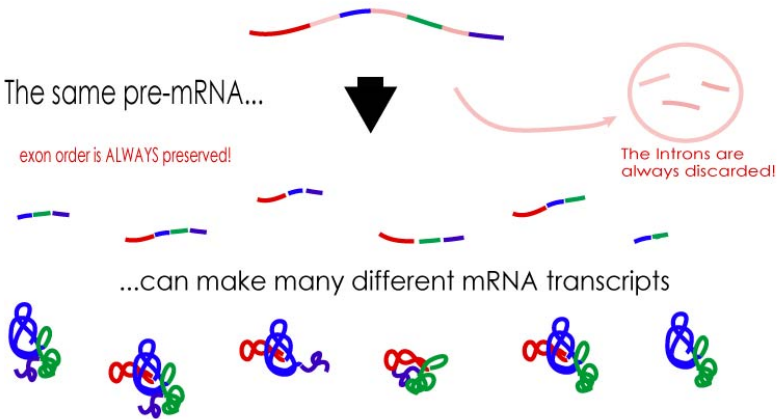
The same pre-mRNA...

exon order is ALWAYS preserved!

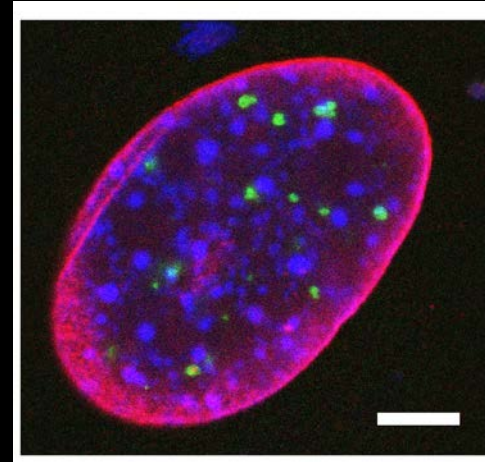
The introns are always discarded!

...can make many different mRNA transcripts

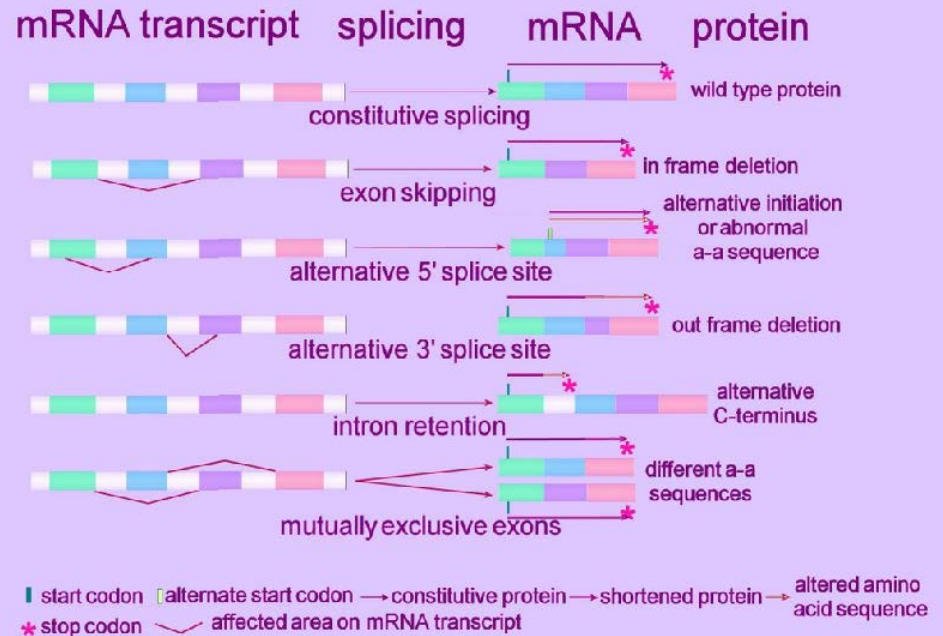
...and many different proteins



A-type lamins

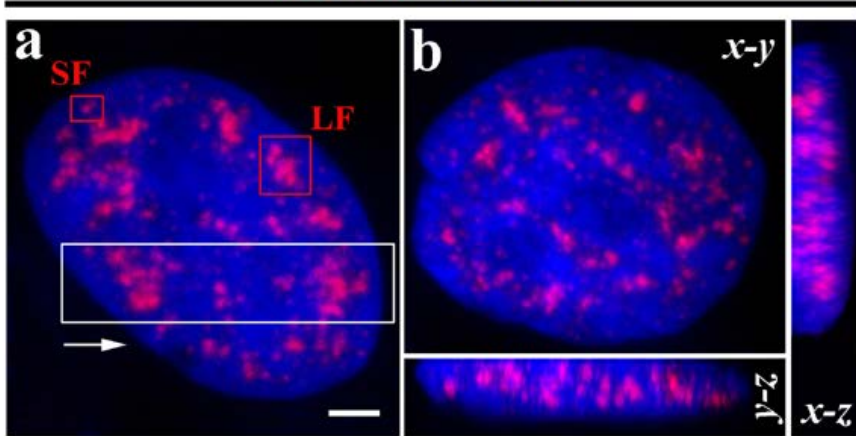


Alternative splicing is a form of epigenetic mechanism that enables a single gene to give rise to multiple, differentially spliced versions of a protein, increasing complexity without a change in the genome.



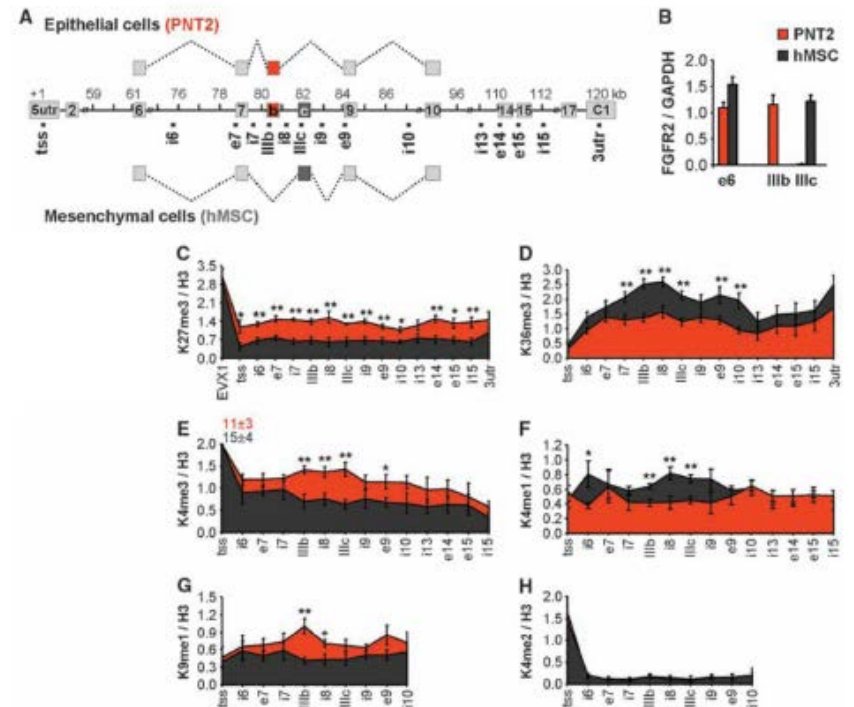
C

SC35/DAPI



Faktory sestřihu jsou v SC-35 doménách, dále snRNP U1-U6 jsou součástí faktoru sestřihu SF2/ASF

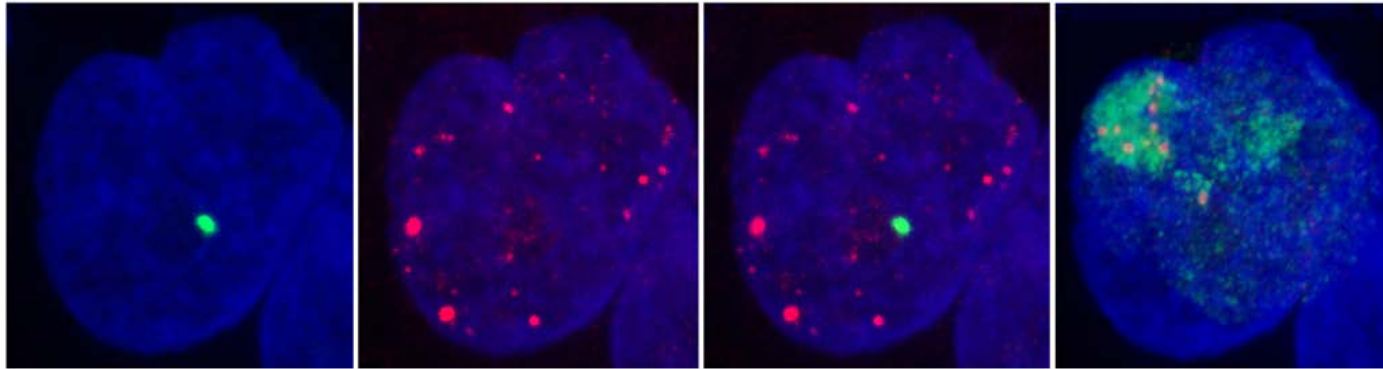
Bartova group



Luco et al., Science (2010)

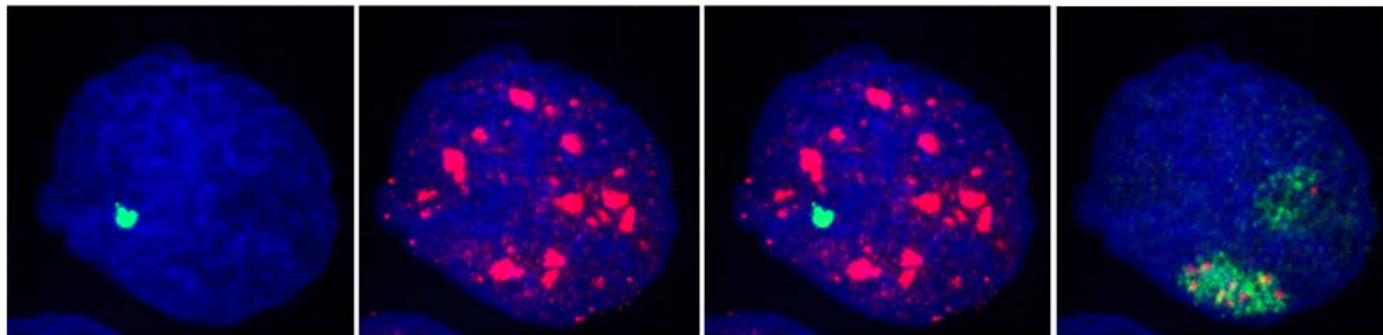
RNAP II / c-myc^T

c-myc gene
HSA8

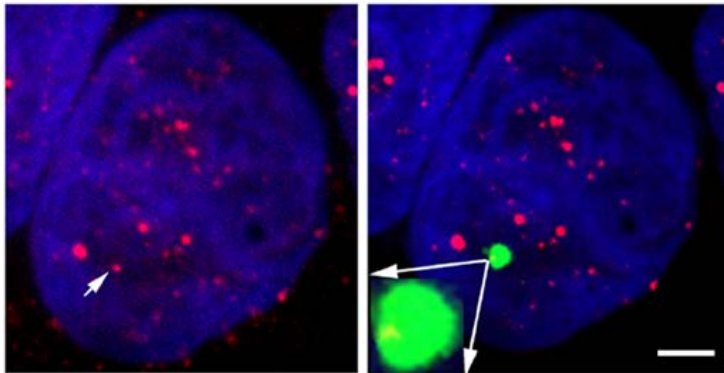


SC35 / c-myc^T

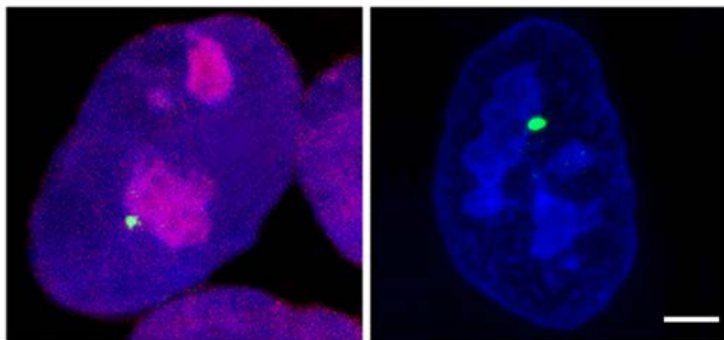
c-myc gene
HSA8



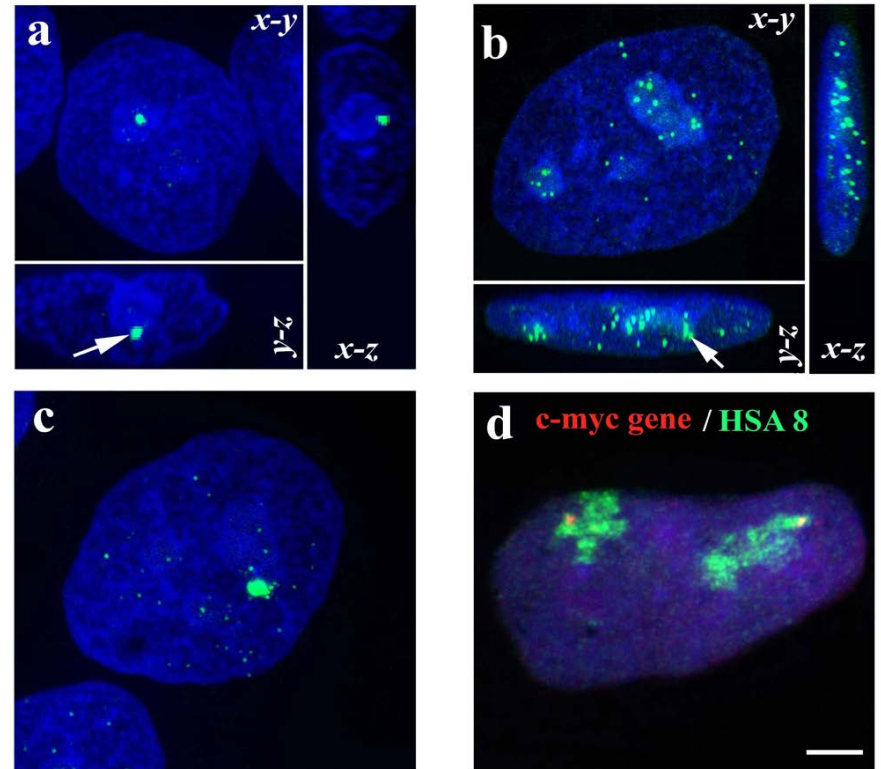
A RNAP II / c-myc^T



C Nucleoli / c-myc^T

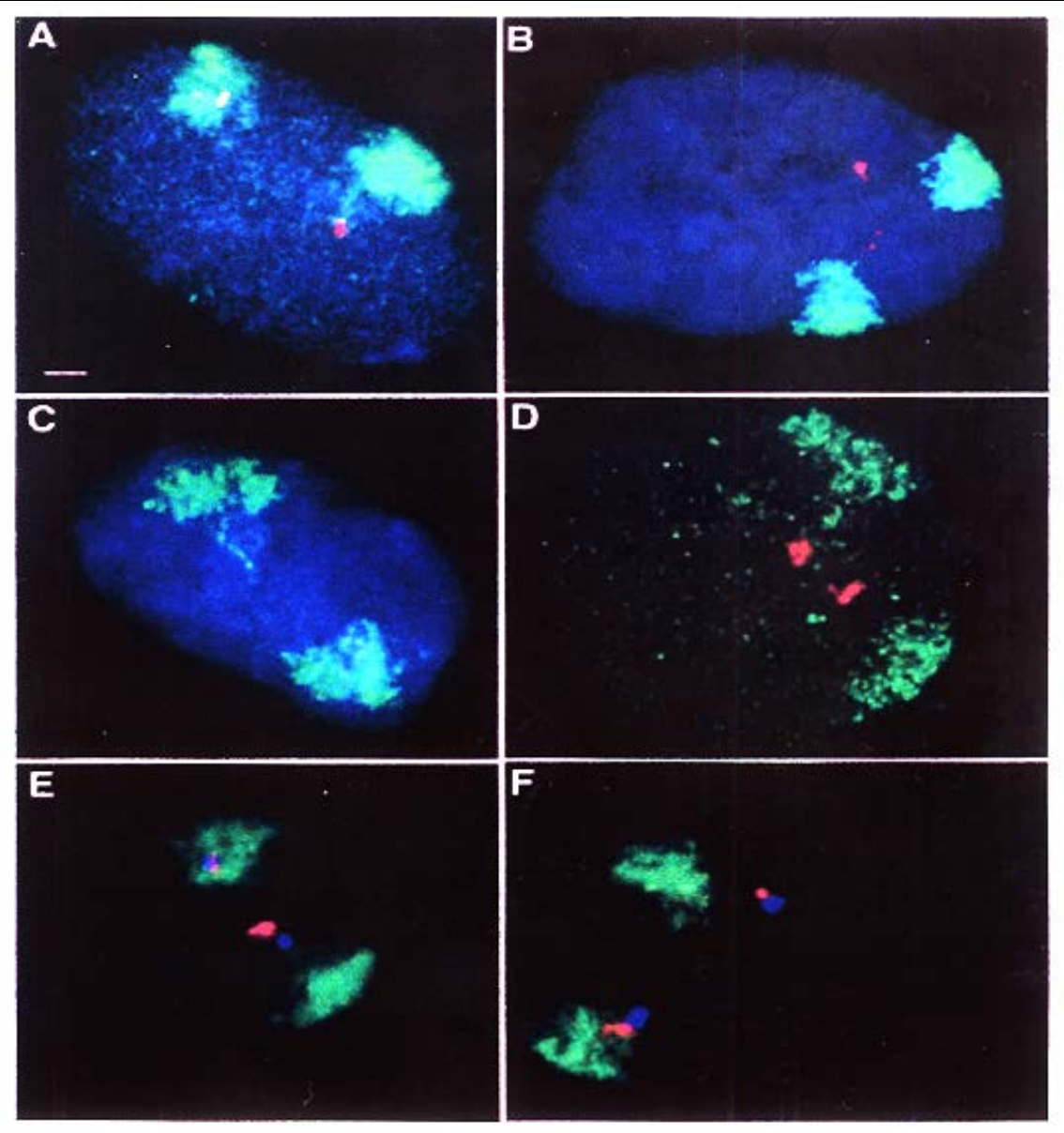


A c-myc^T / DNA

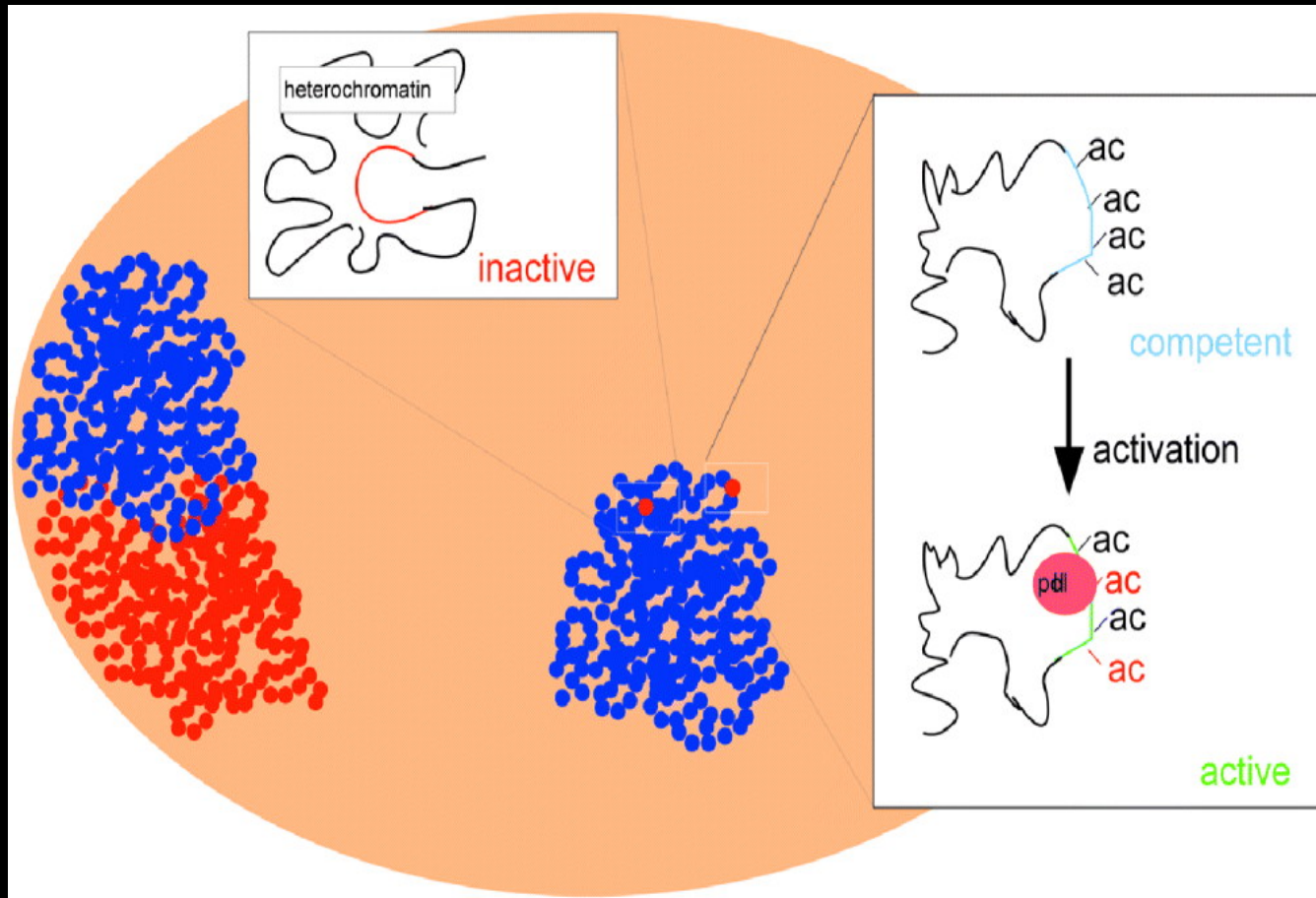


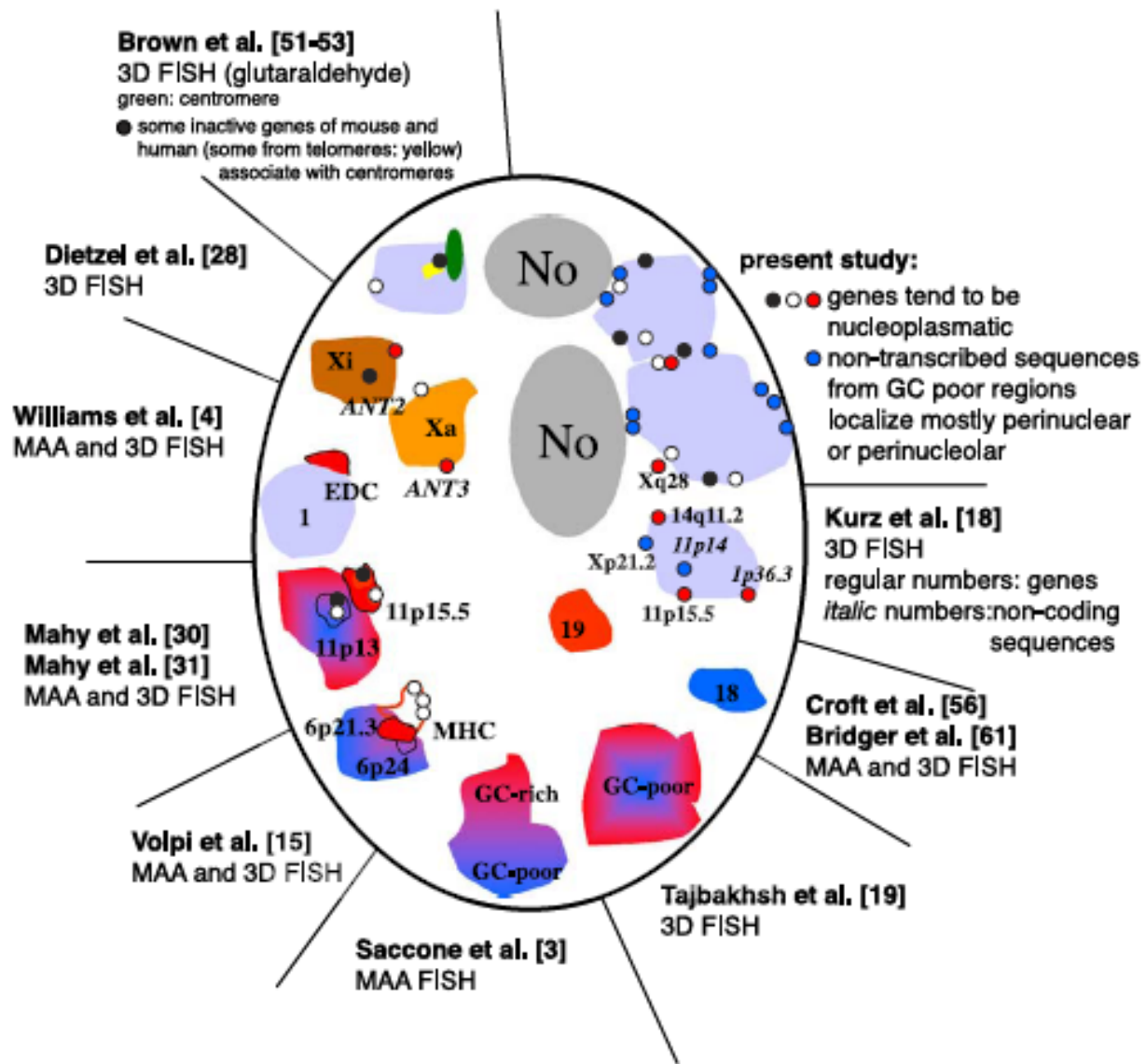
Note: Interleukin-1beta pre-mRNA splicing proceeds in cytoplasm of enucleated platelets (Denis M. et al., 2005).

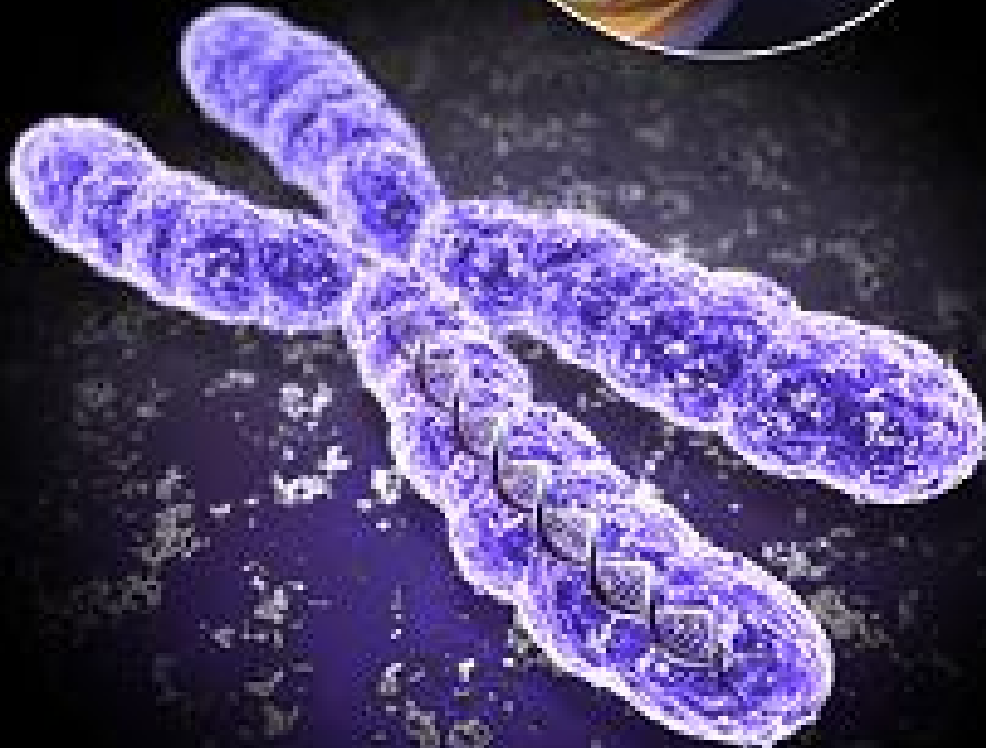
MHC on HSA 6



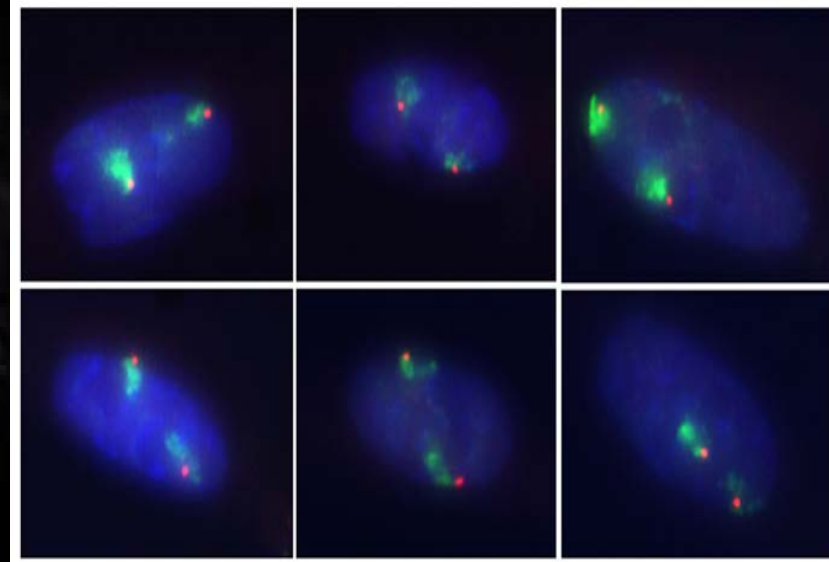
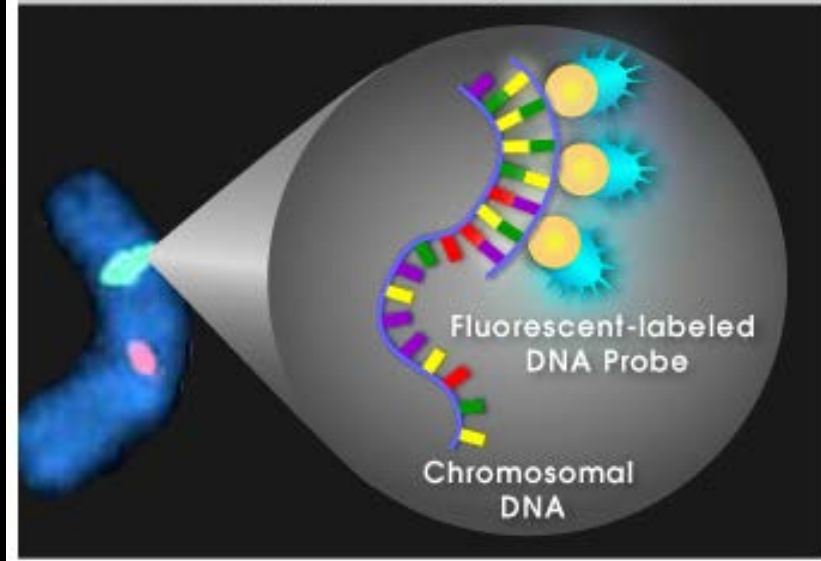
Active / inactive chromatin

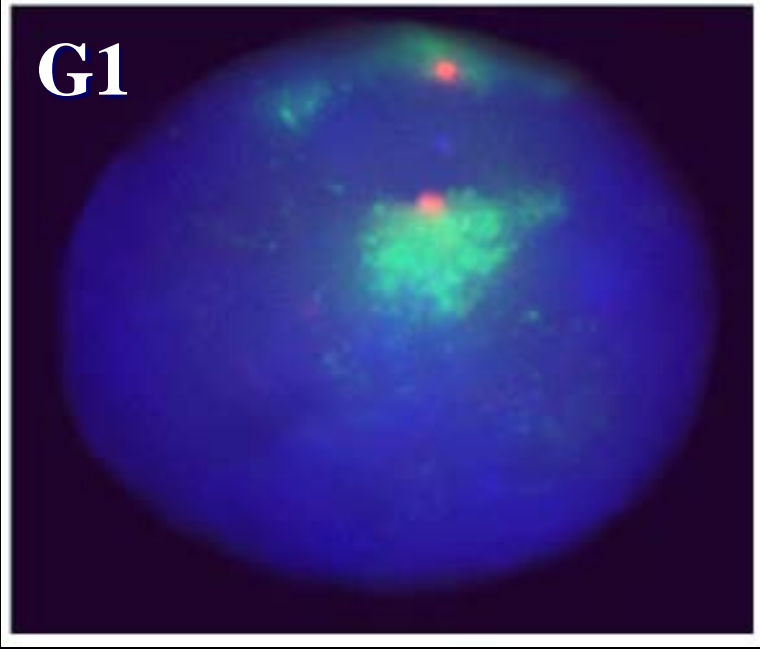




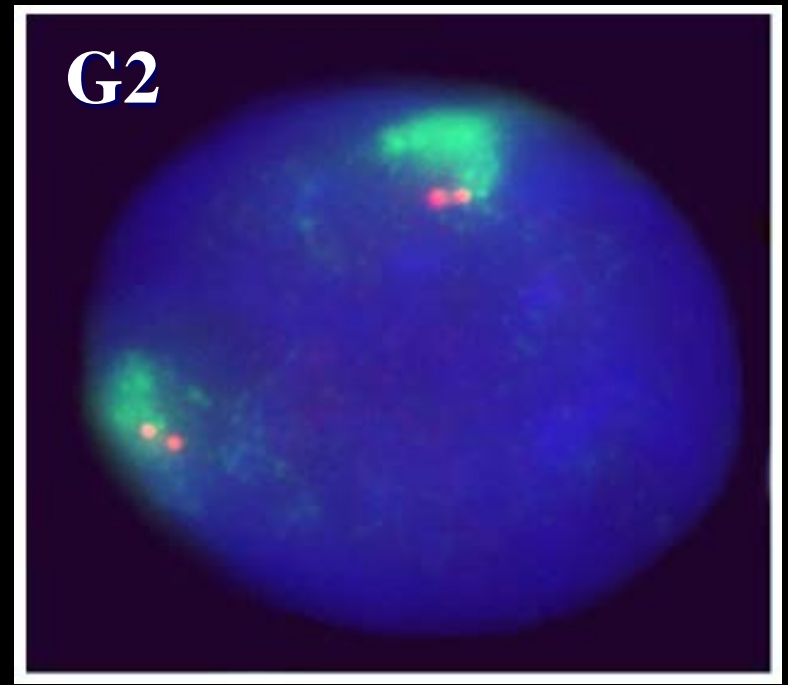


Chromosome prepared using FISH technique

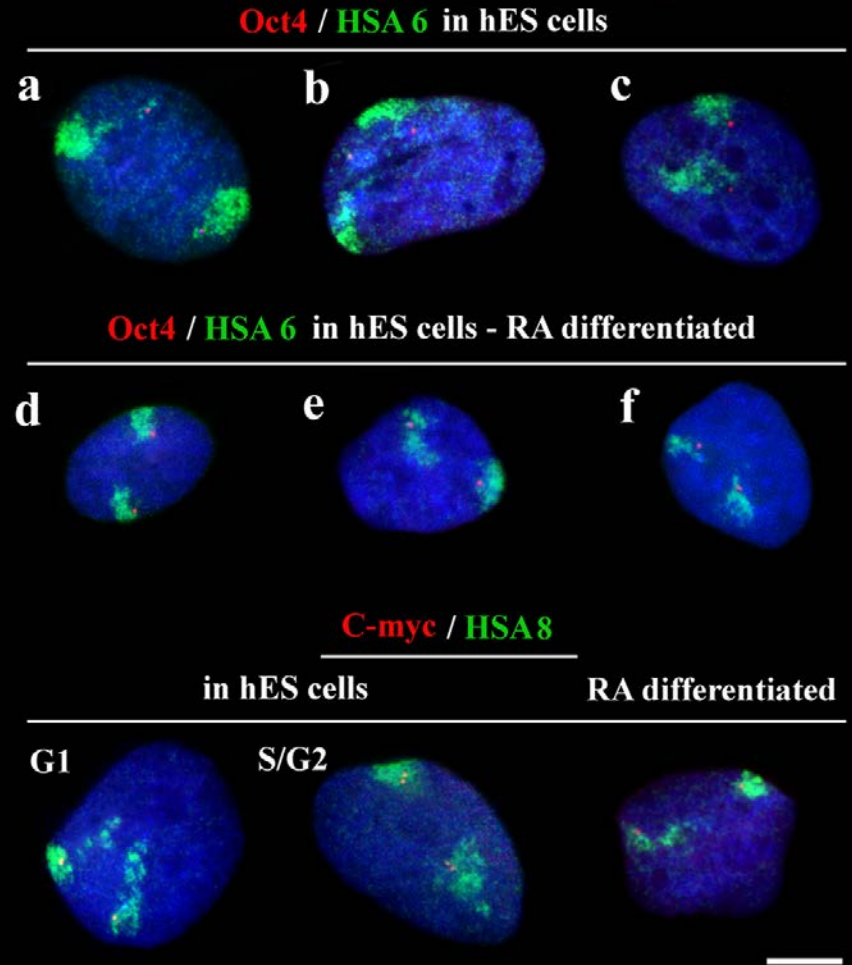
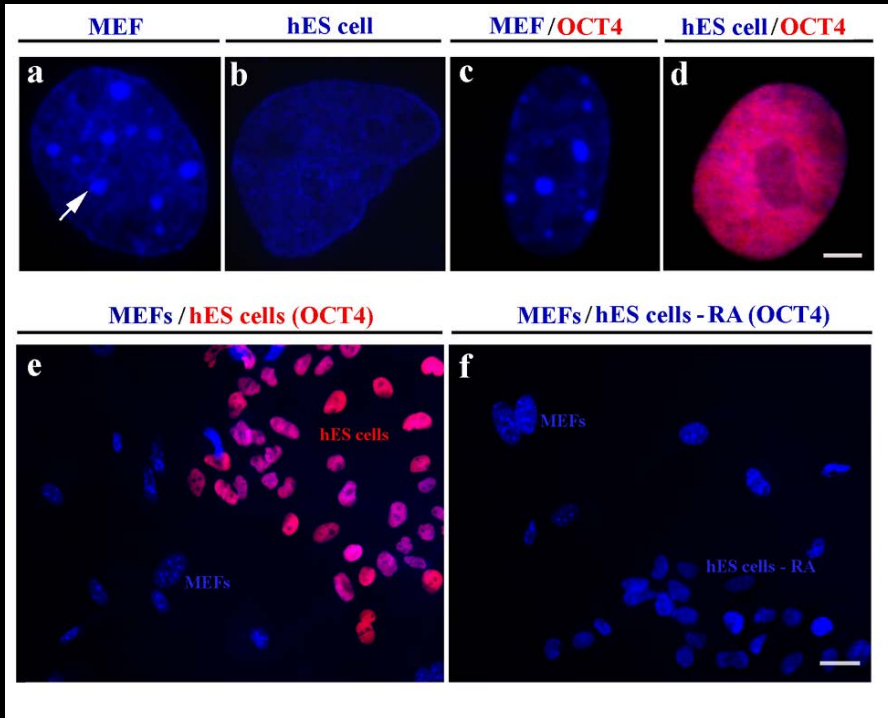




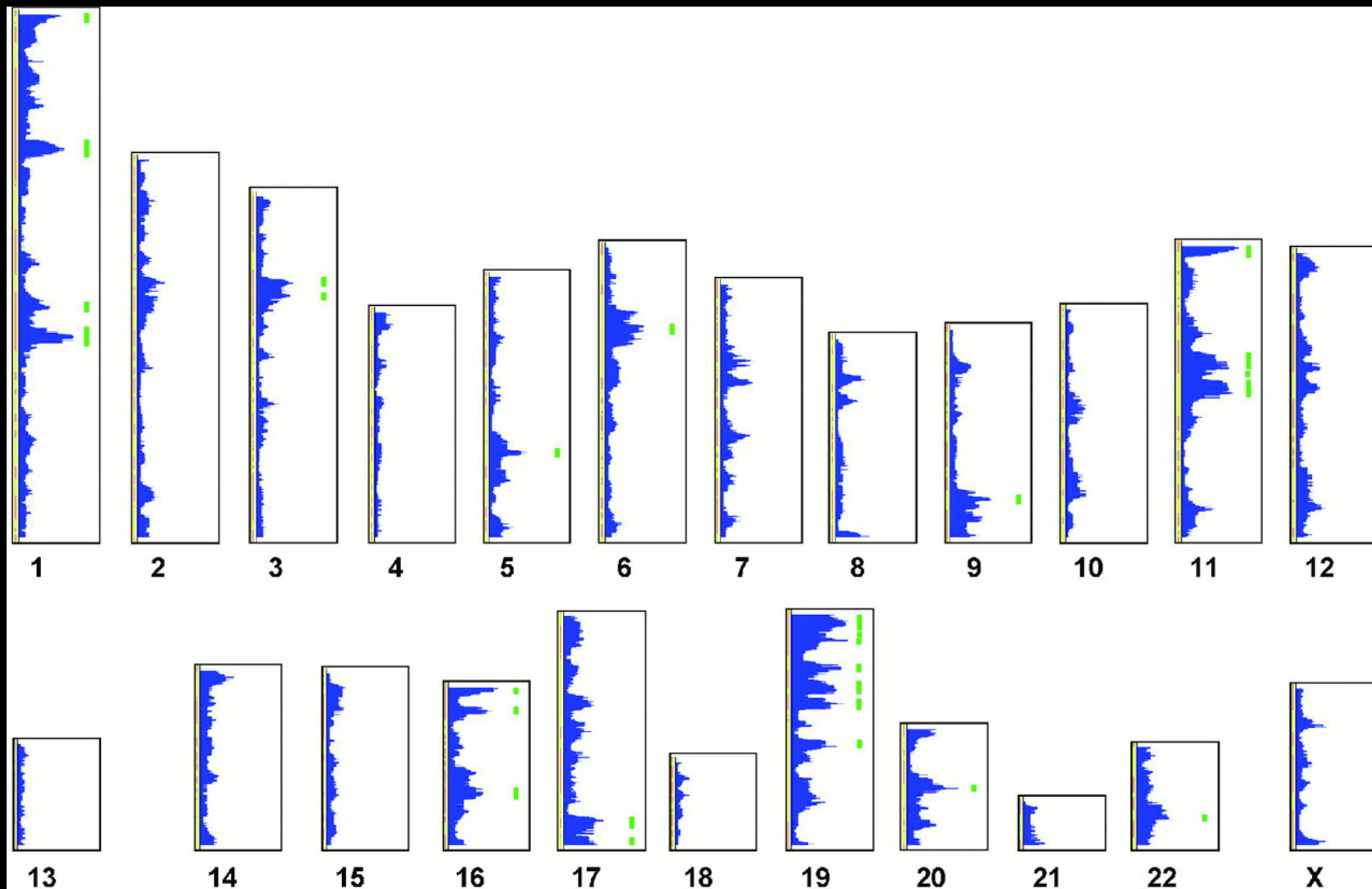
Rb1 gene

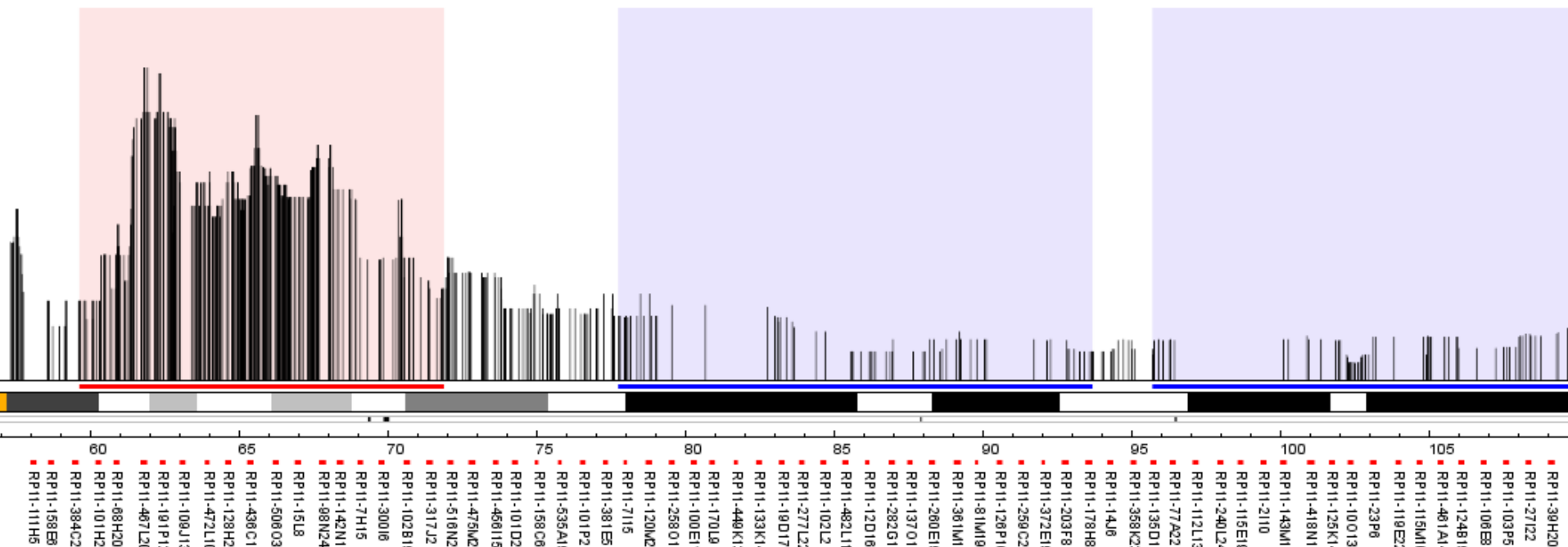
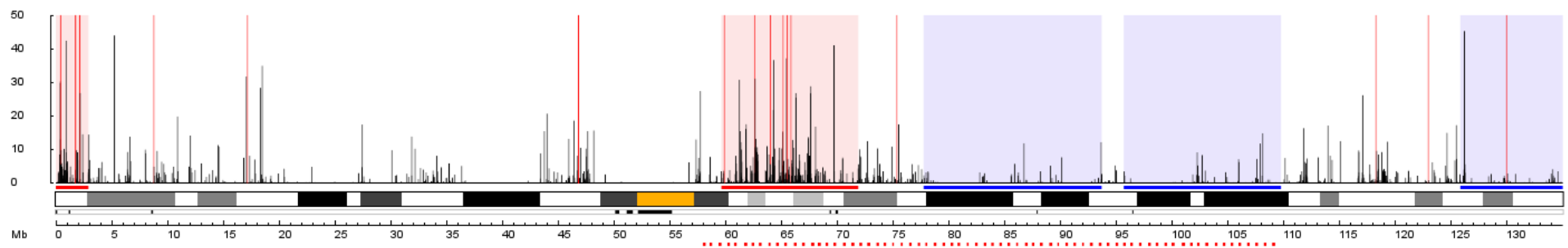
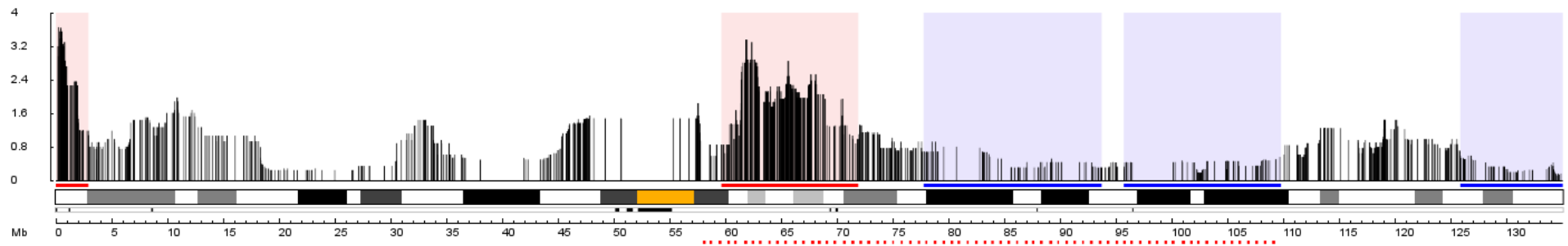


Genes in human embryonic stem cells

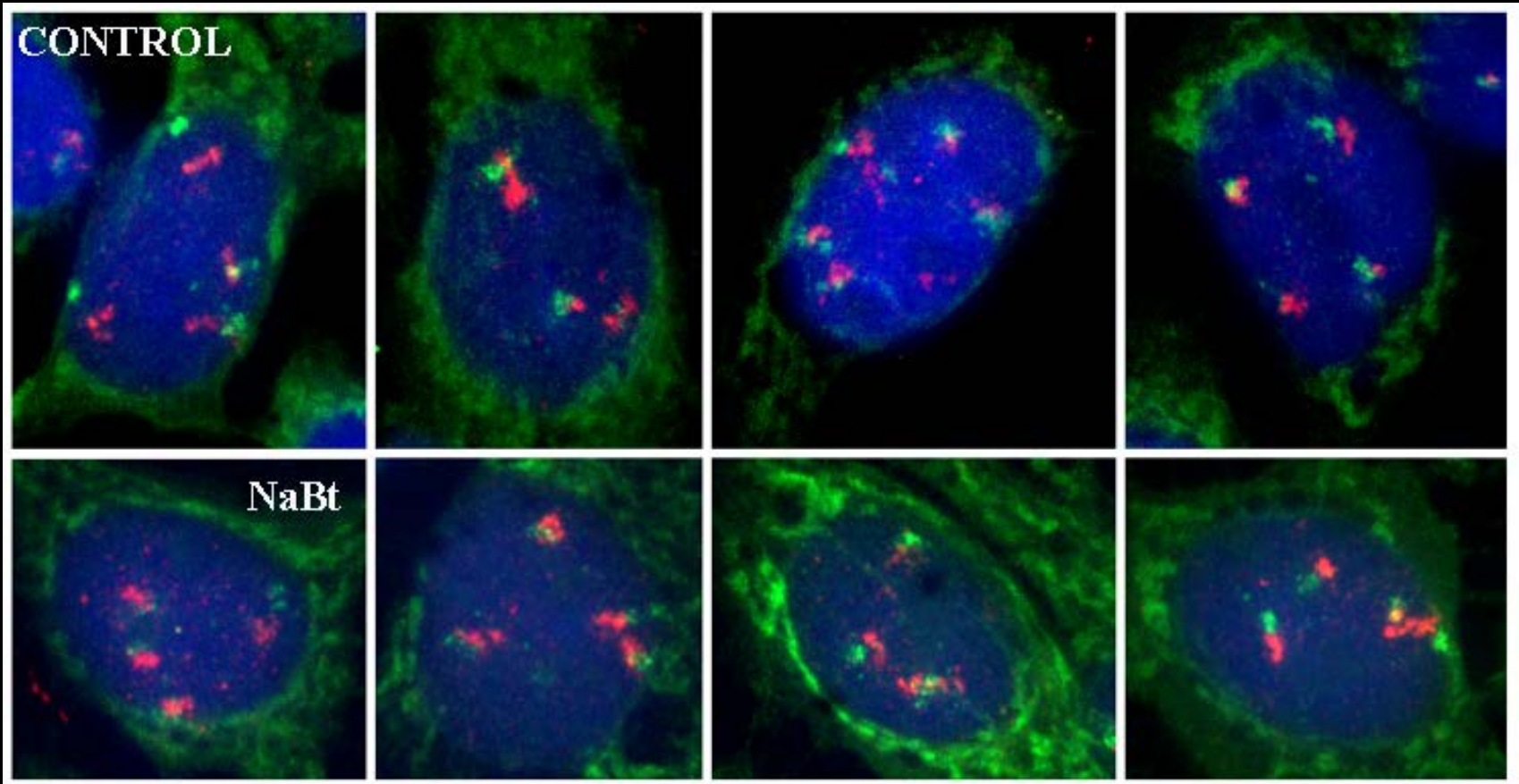


Transcriptome map (Caron et al., 2001)





Enterocytic cell differentiation and **RIDGE/ANTI-RIDGE**



Center of nucleus- to-gene distances

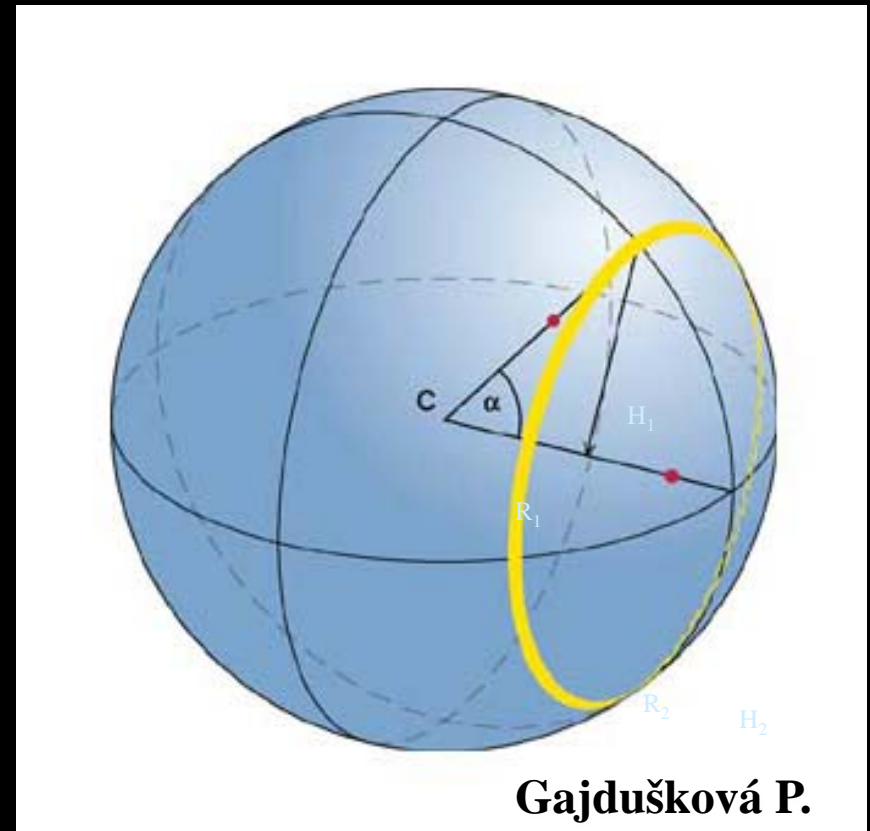
$$CS = \frac{\overline{CR_i}}{\overline{CH_i}} * 100$$

Gene-to-gene distances

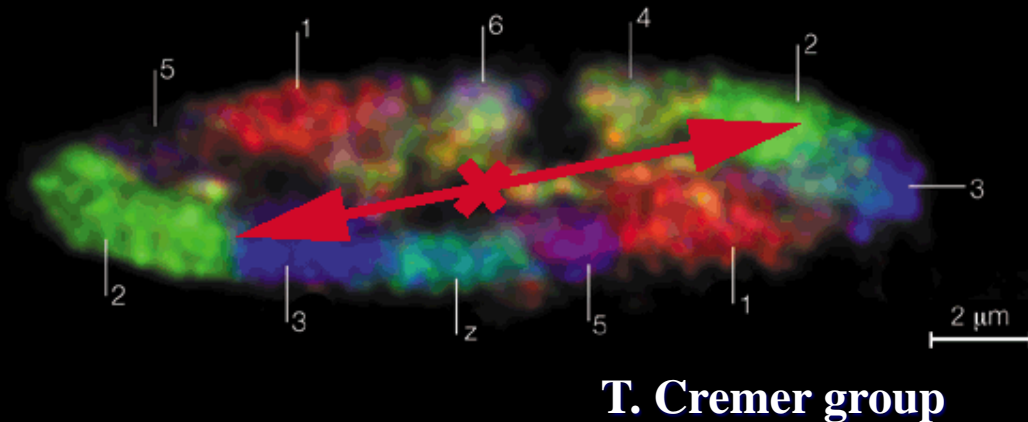
$$SS = \frac{\overline{R_1 R_2}}{(\overline{CH_1} + \overline{CH_2})/2} * 100$$

Gene-center of nucleus-gene angles

$$\cos(\alpha) = \frac{\overline{CR_1} * \overline{CR_2}}{|\overline{CR_1}| * |\overline{CR_2}|}$$

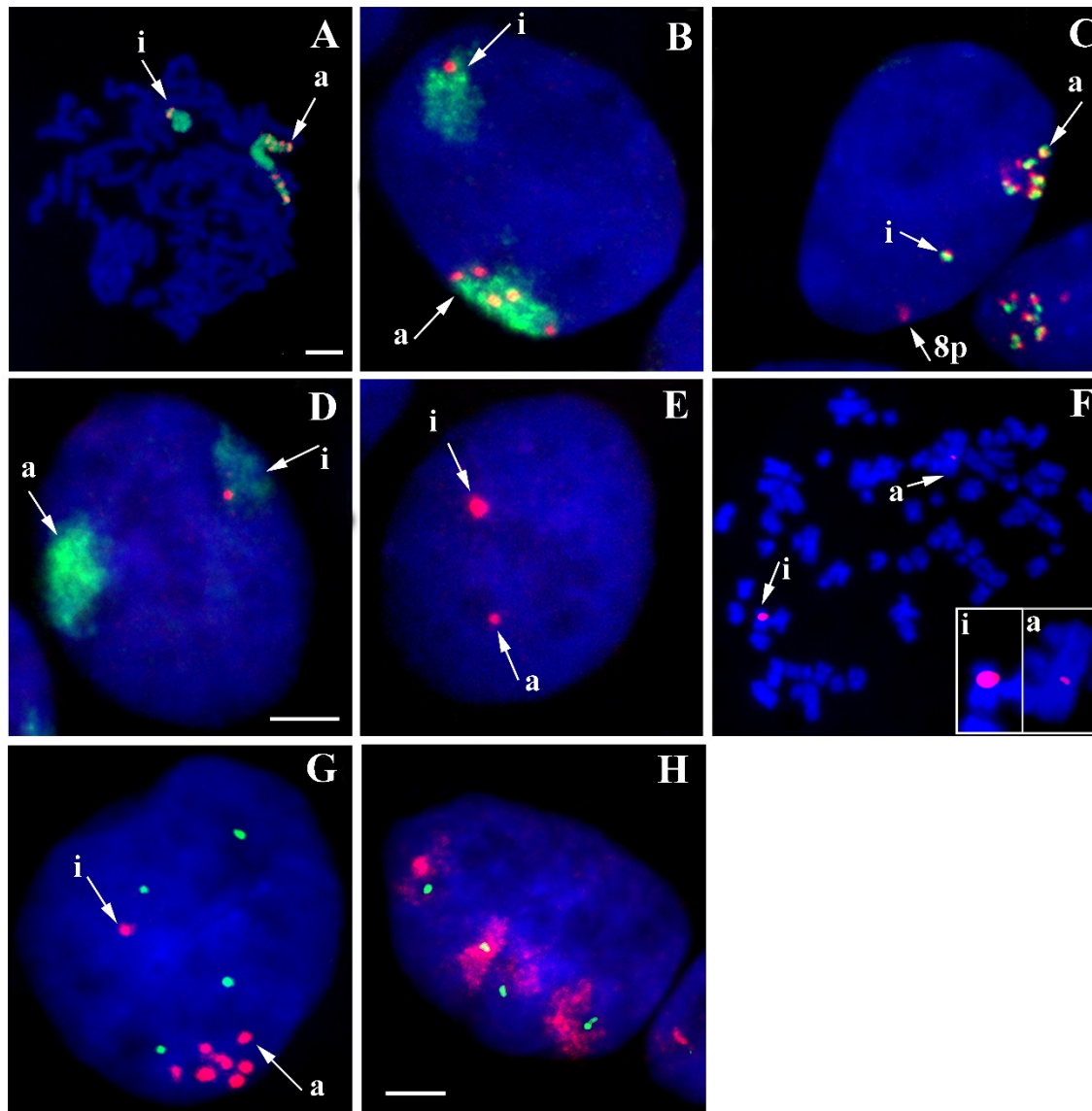


d



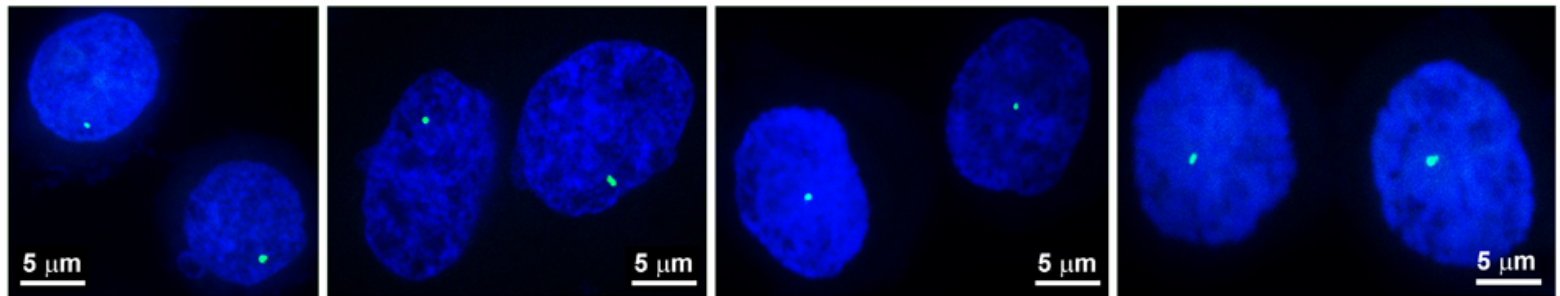
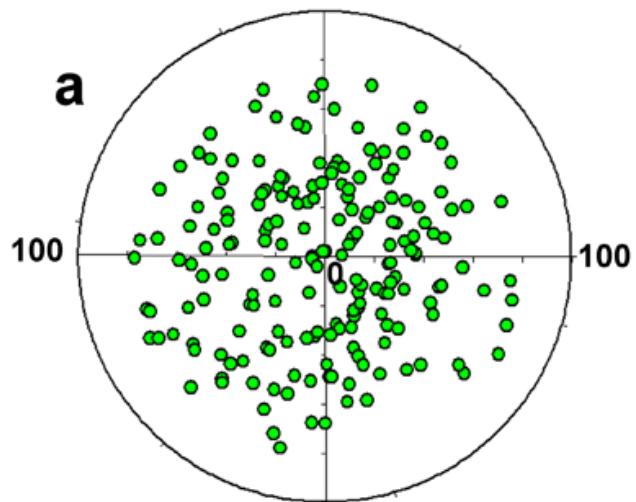
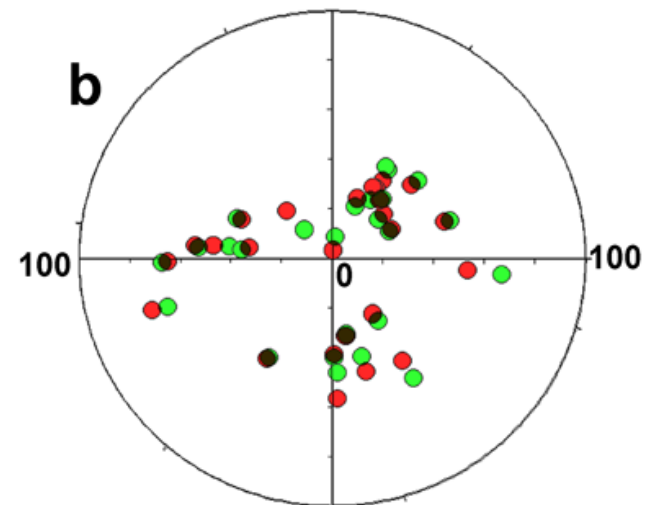
HSA 8 and related structures

Harničarová et al., 2005



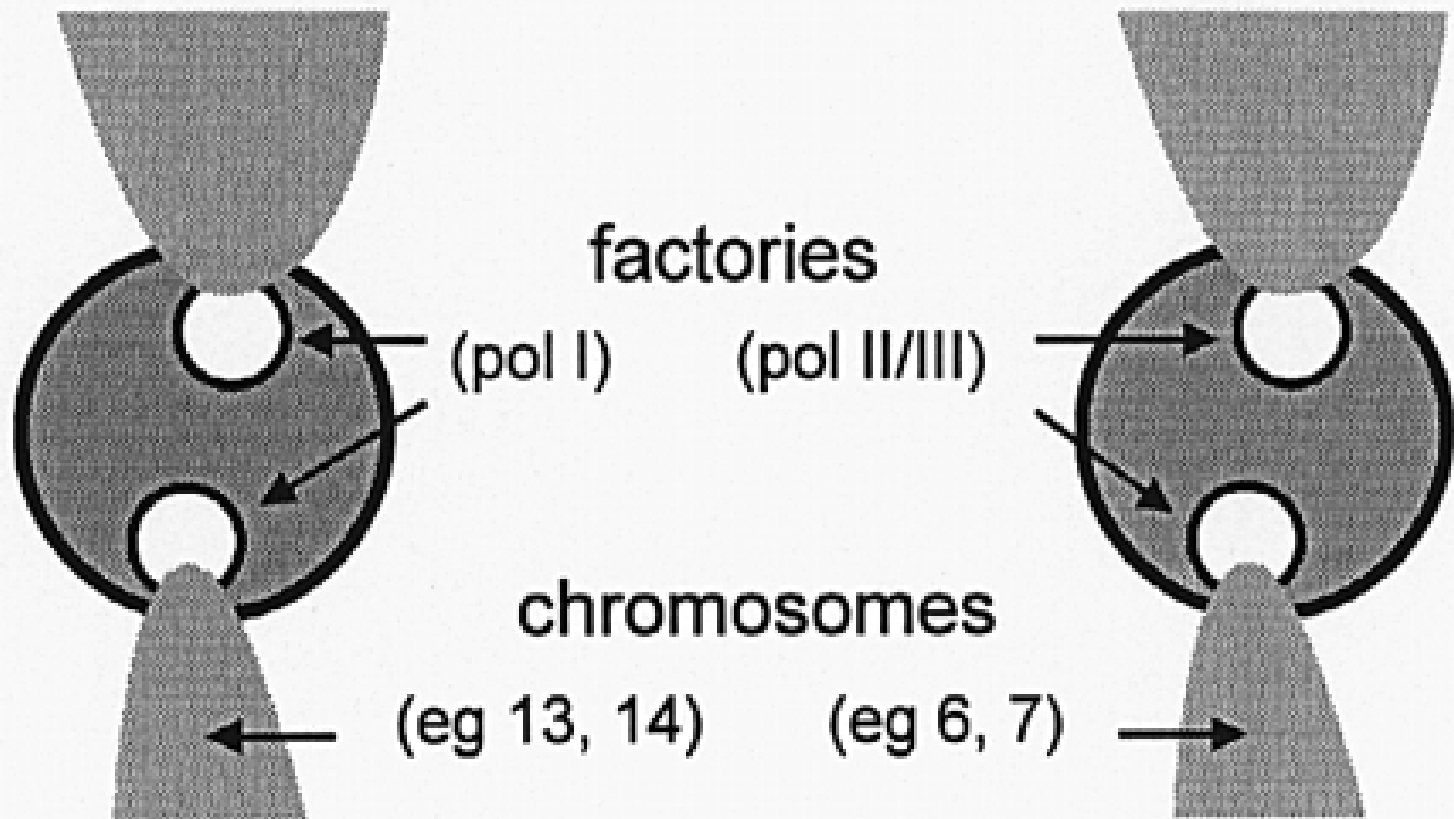
A**c-myc transcripts / Nucleoli**

sister cells

**B****c-myc transcripts in whole cell population****c-myc transcripts in sister cells**

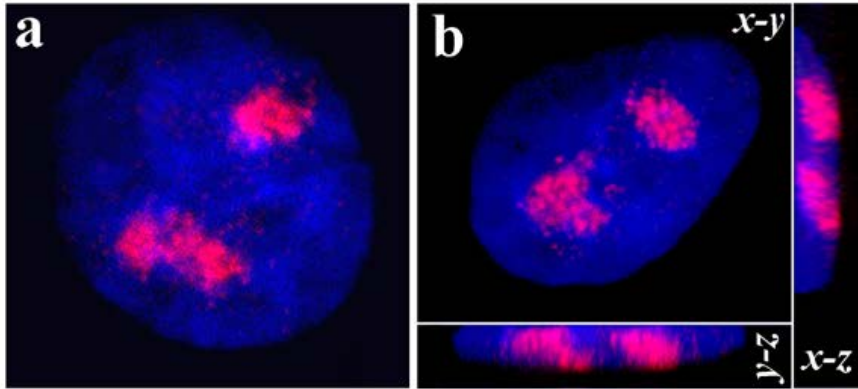
Nucleolus

OPT domain

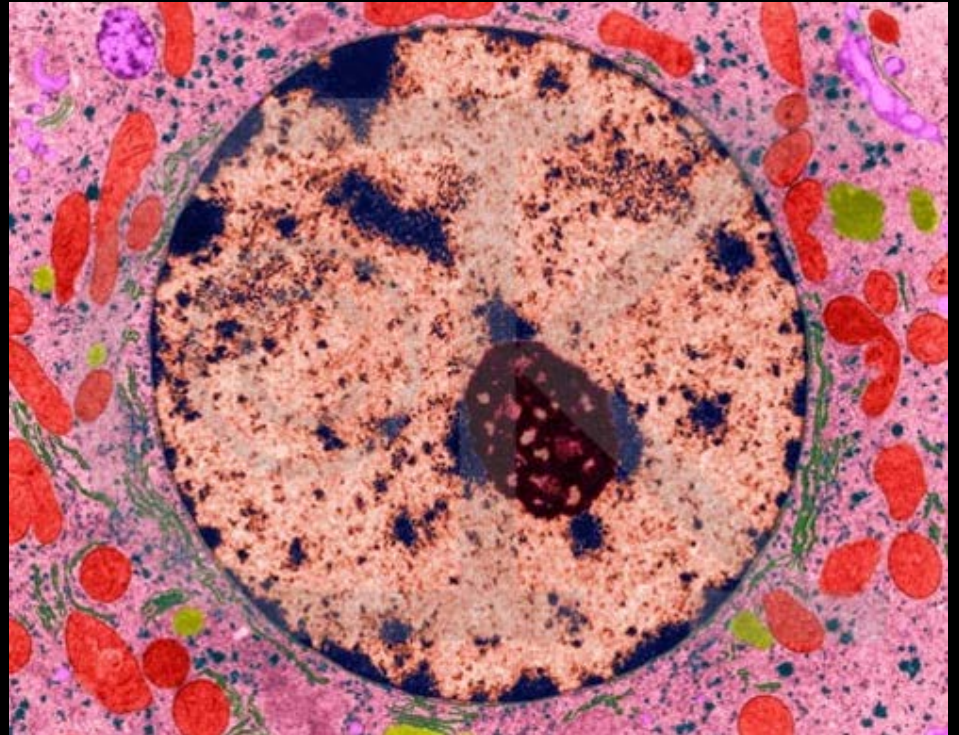


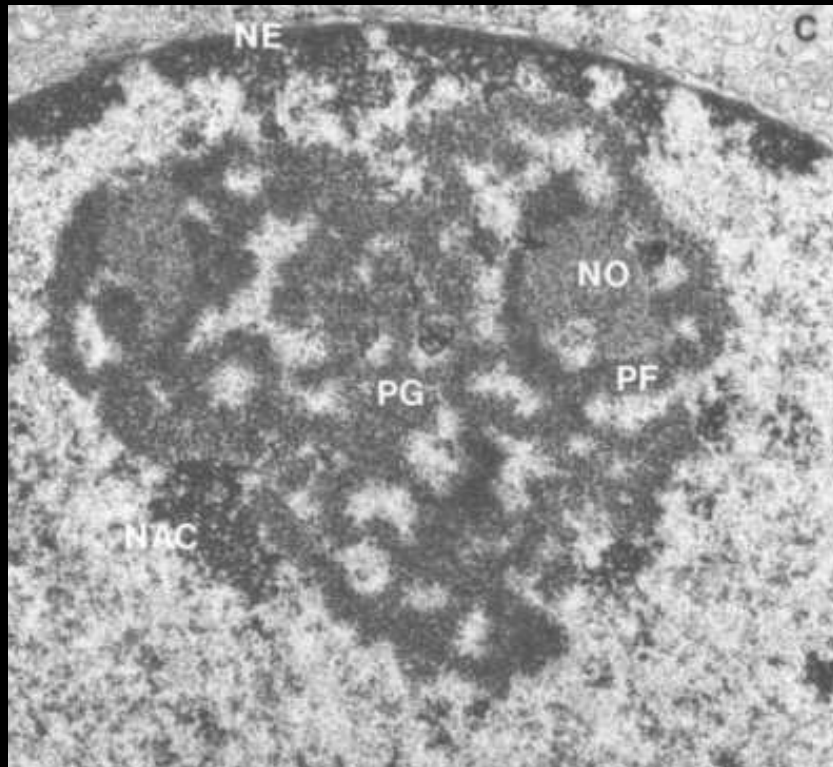
B

Nucleoli / DAPI



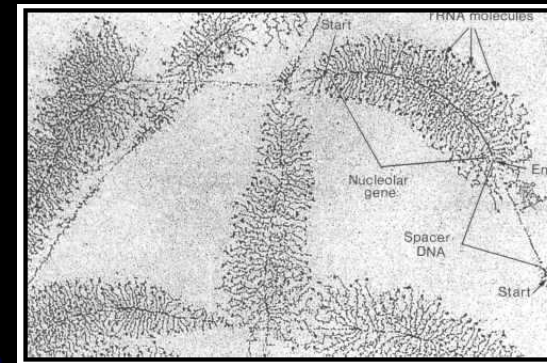
Nucleolus





NOR

400 (540) rDNA genů



FIBRILÁRNÍ CENTRUM (FC):

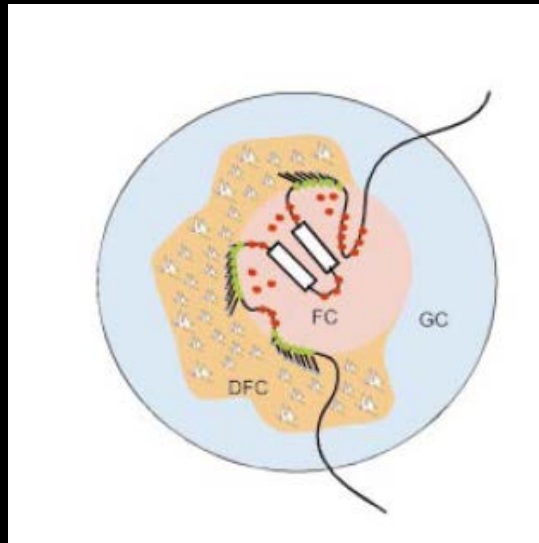
zodpovídá za změny v buněčné aktivitě

DENSE FIBRILAR COMPONENT (DFC):

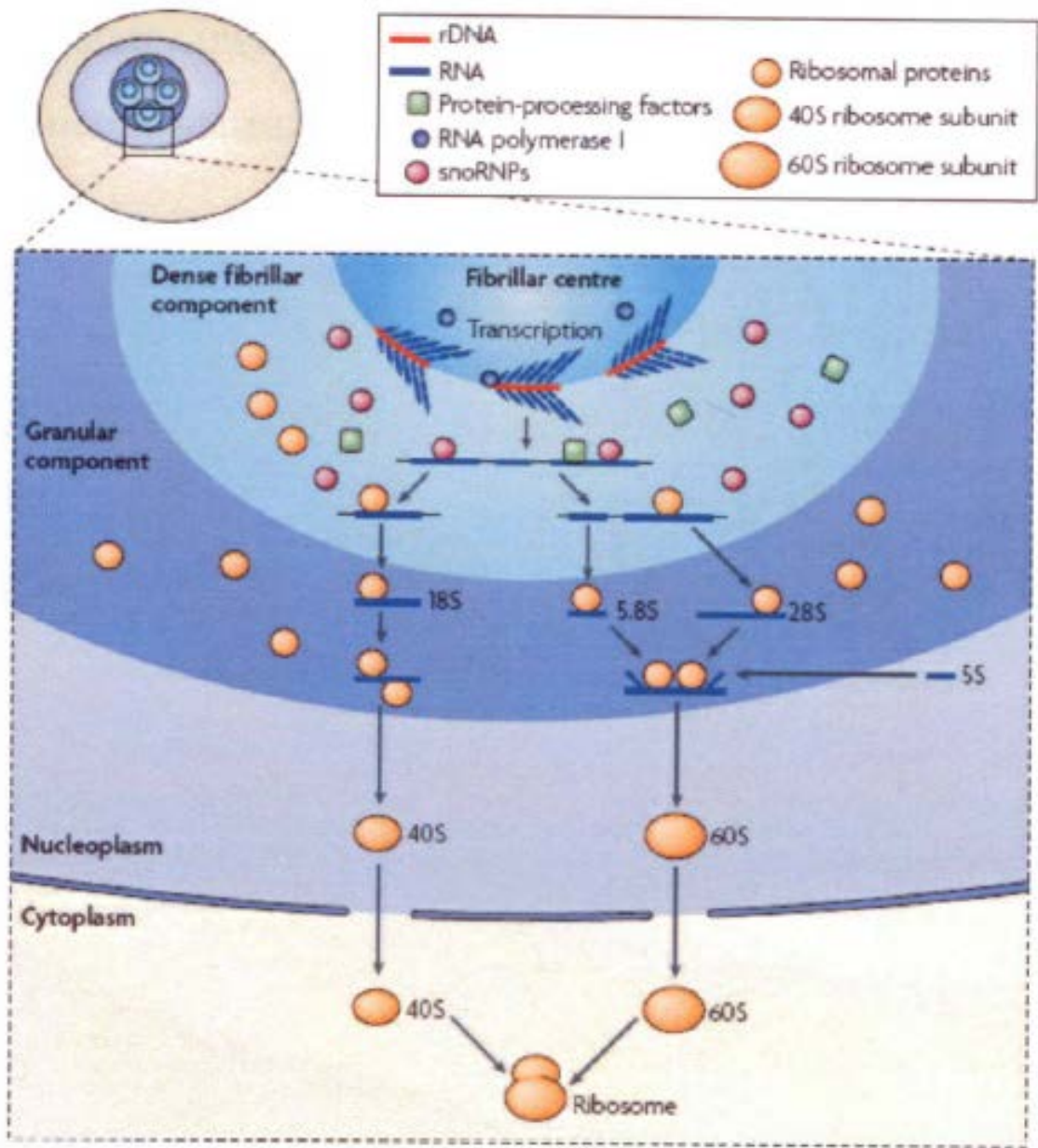
místo syntézy ribozomálních podjednotek

GRANULAR COMPONENTS:

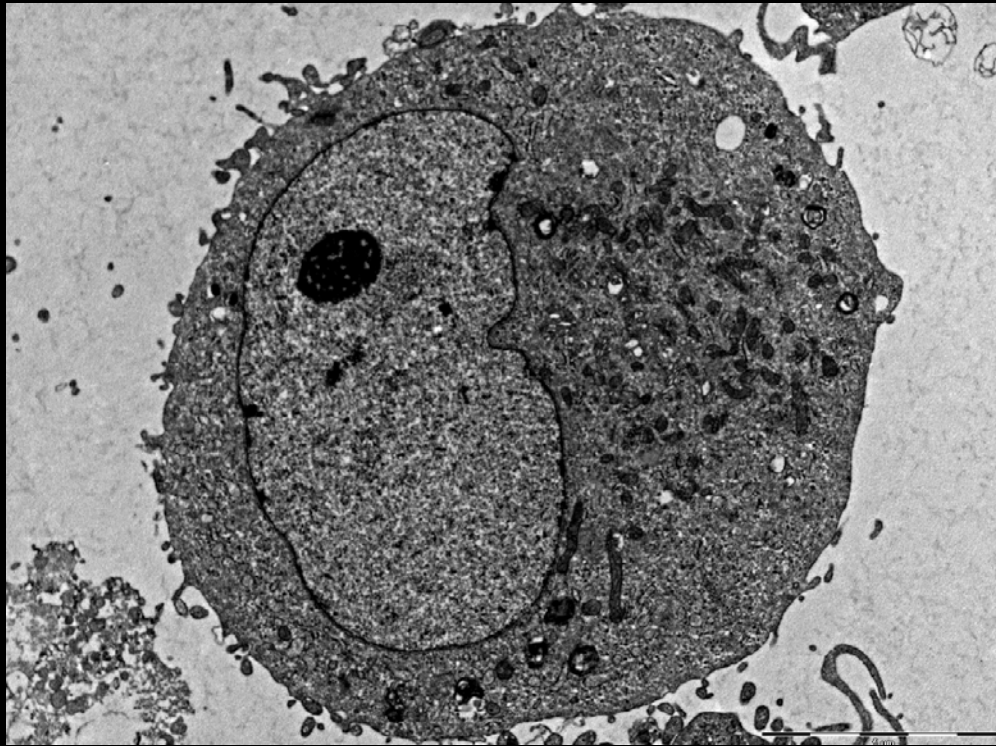
ukotvuje DFC a FC



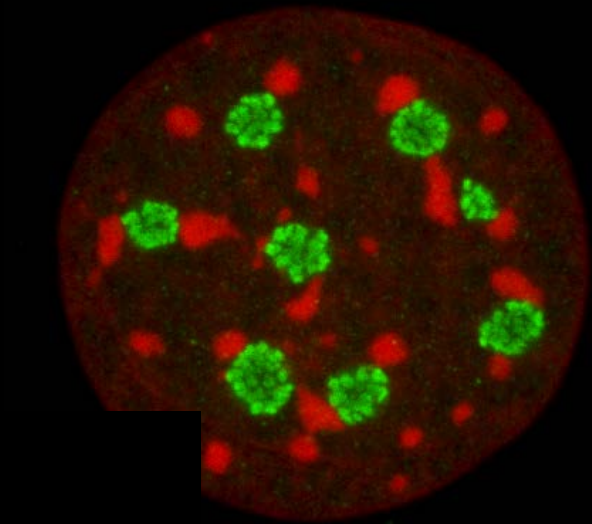
Tvorba ribozomů je komplexní proces zahrnující transkripci 45S prekurzorické rRNA, její vyžívání, modifikaci a asociaci s ribozomálními proteiny a 5S rRNA, která se syntetizuje mimo jádérko. Vyžívání rRNA probíhá v procesomu, který obsahuje mnoho komplexů a snRNA




SUV39h-independent association of HP1 β with fibrillarin-positive nucleolar regions



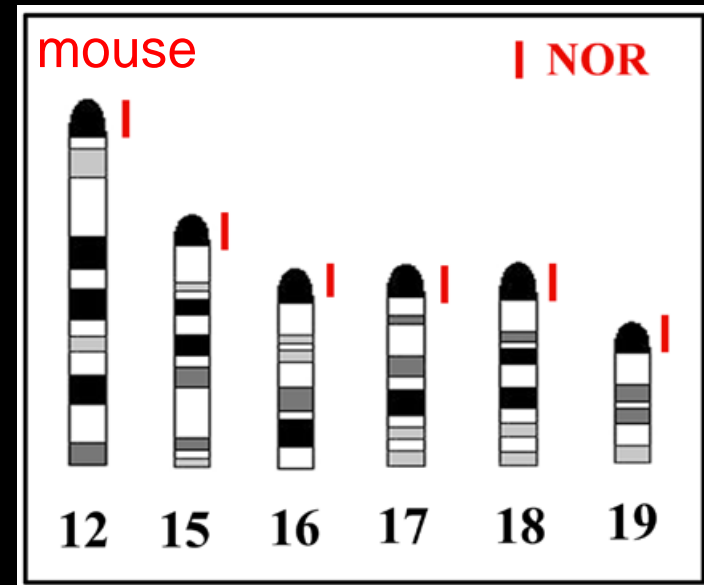
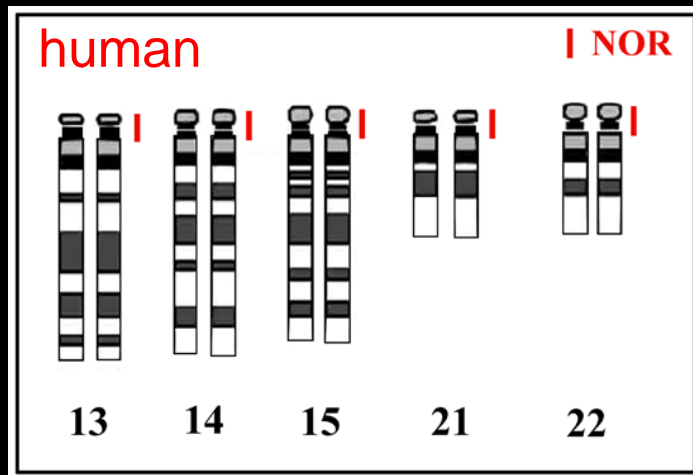
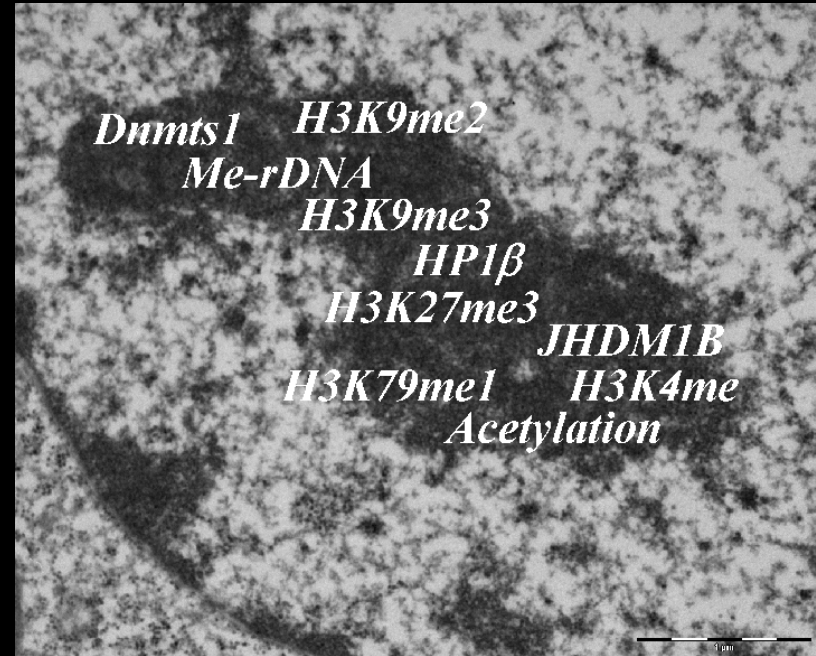
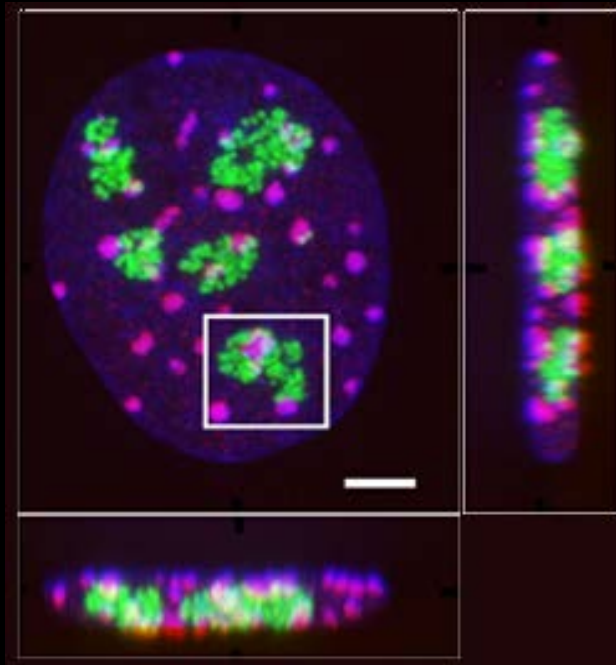
12/8/2010 3:40:48 PM



° pos=1 

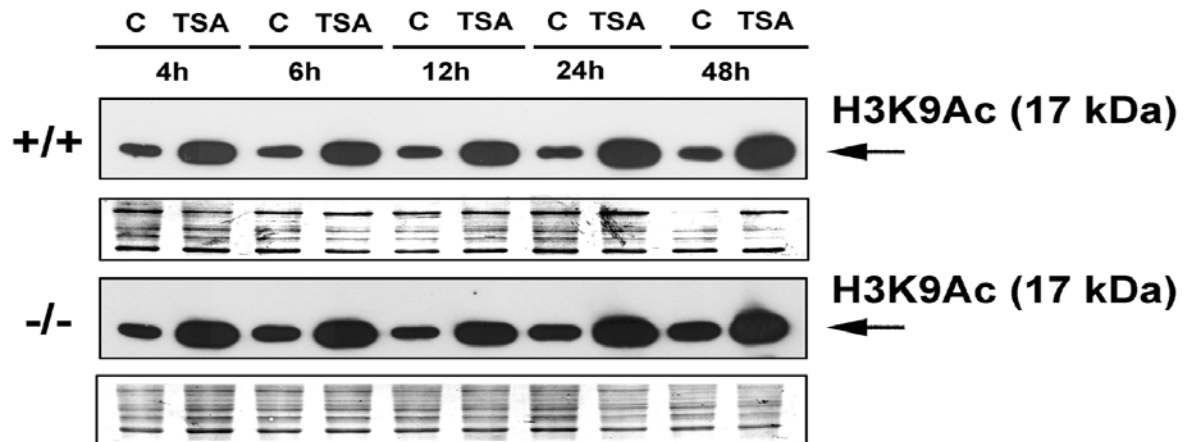
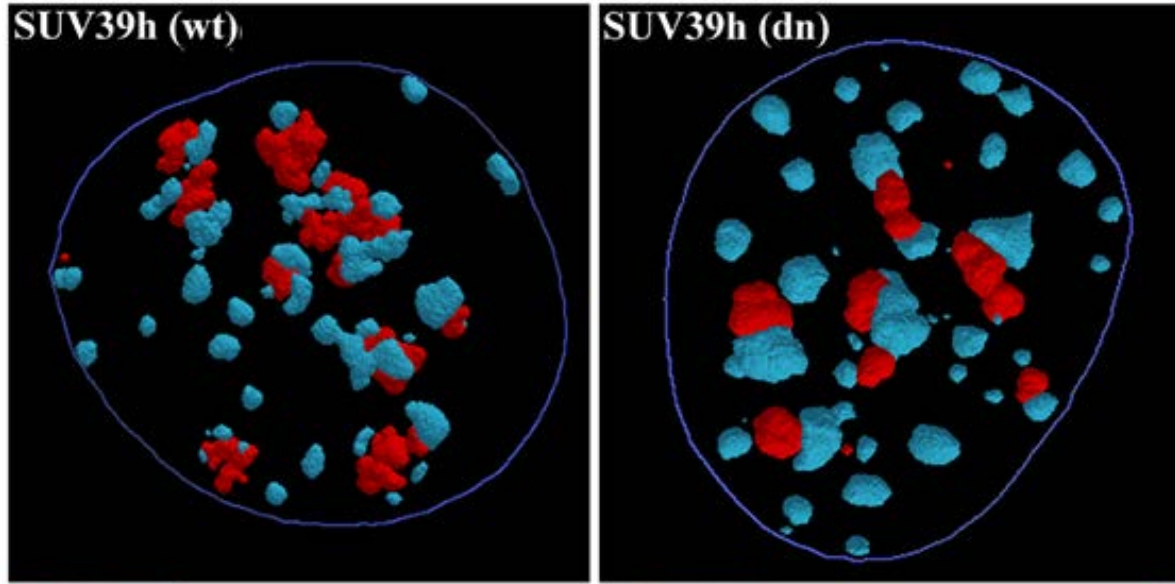
Epigenetics of Nucleoli

Fibrillar/chromocenters

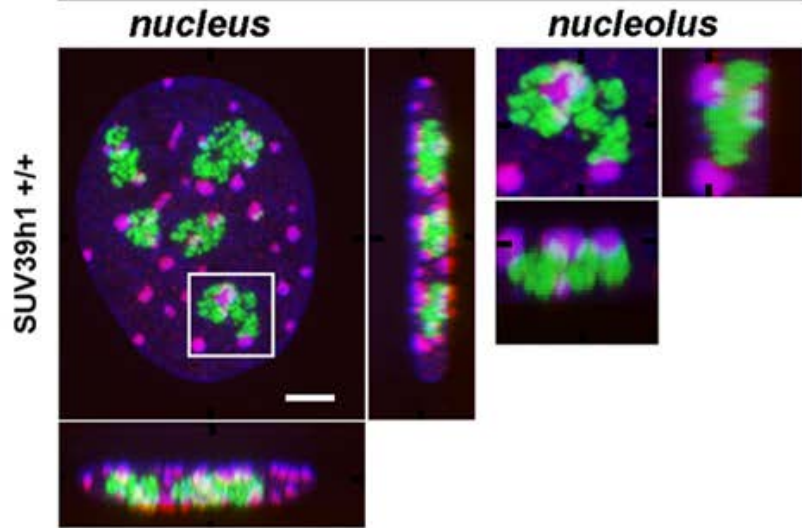


Differences between epigenetics of nucleoli and surrounding chromatin

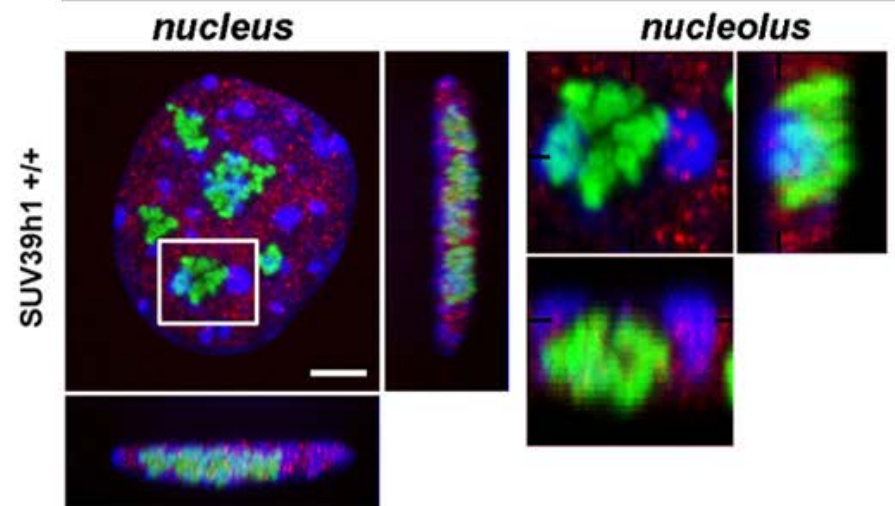
Fibrillar / Chromocenters



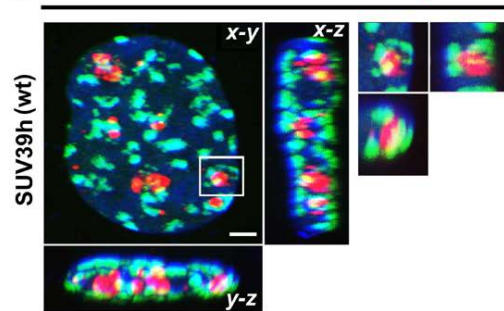
Fibrillarin / HP1 α / DNA



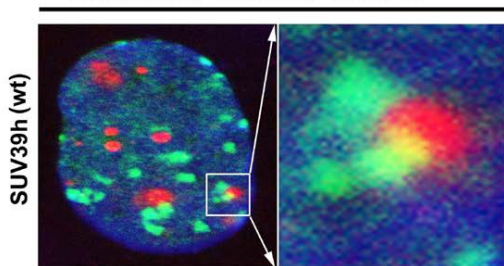
Fibrillarin / HP1 γ / DNA



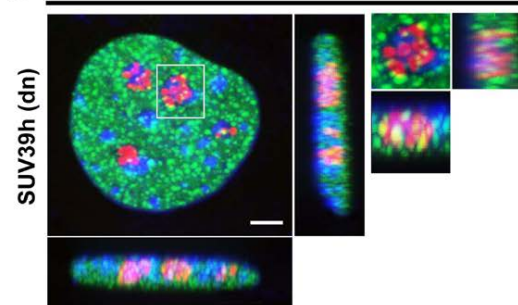
a Fibrillarin / GFP-HP1 β



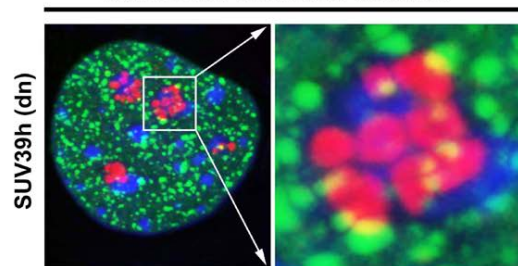
individual confocal section



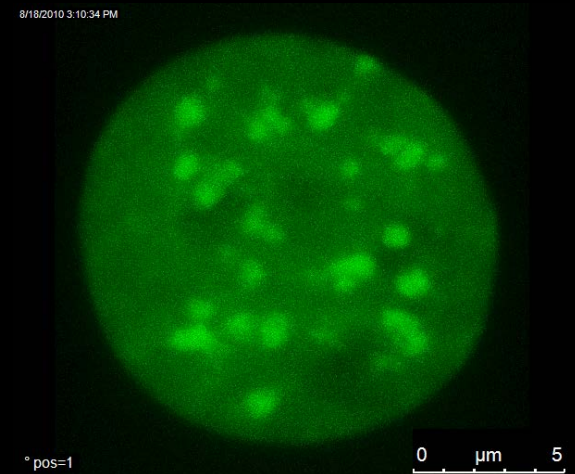
b Fibrillarin / GFP-HP1 β

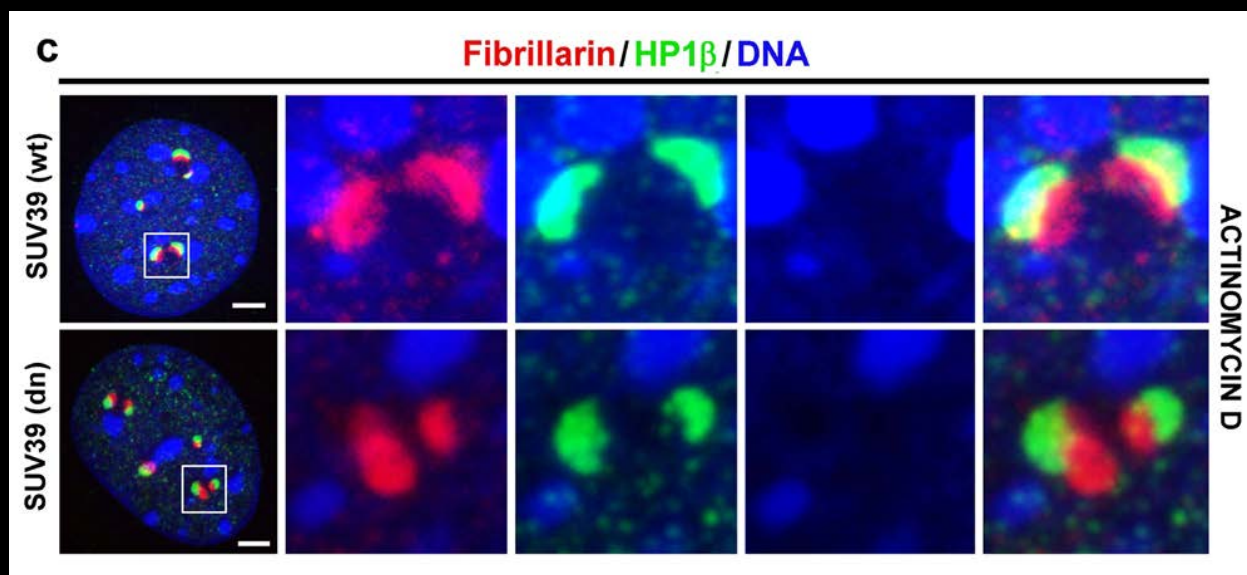
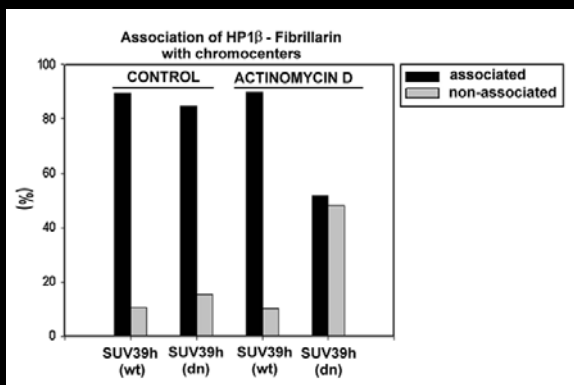
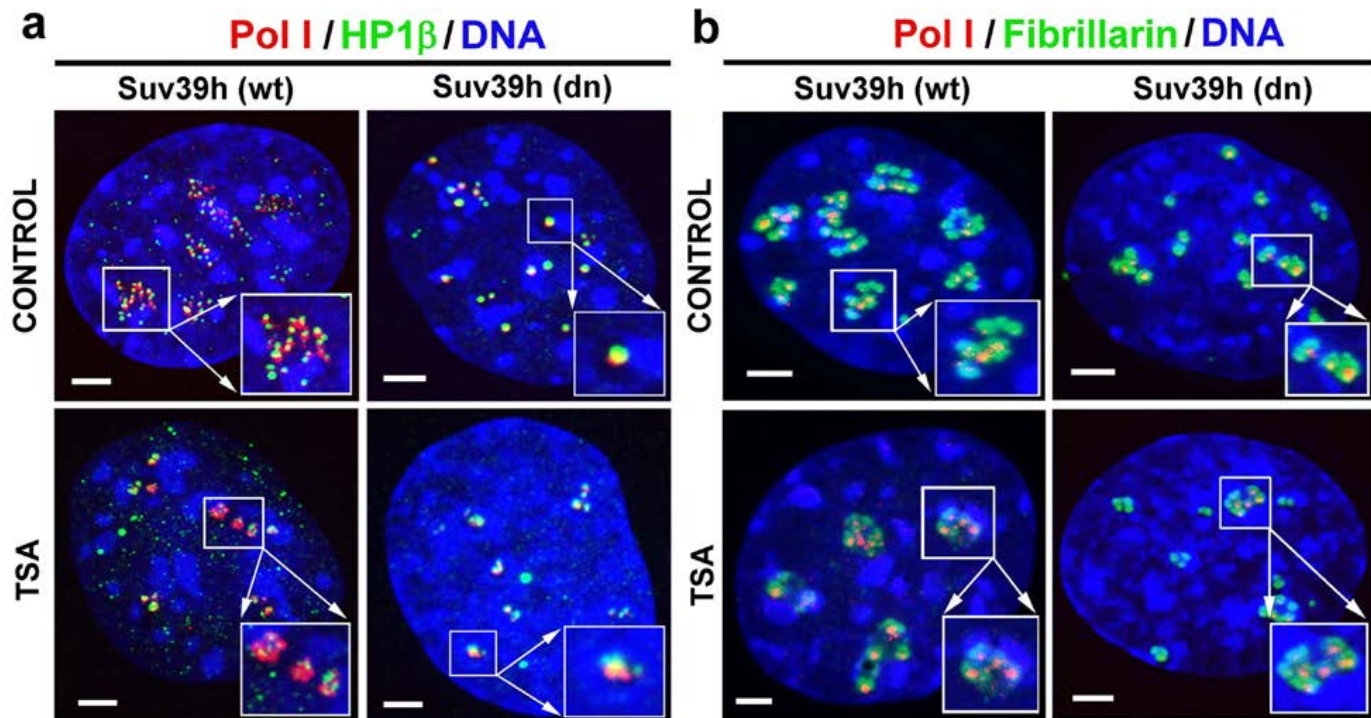


individual confocal section

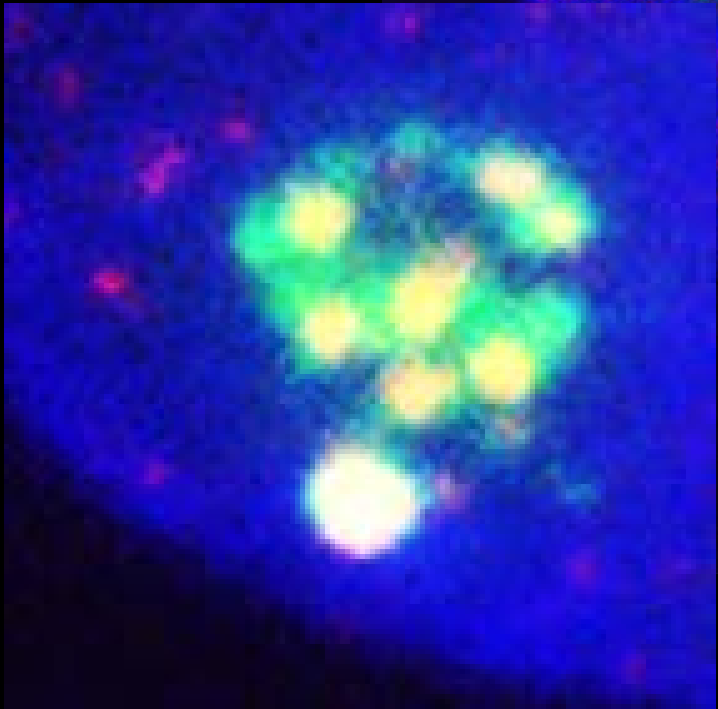
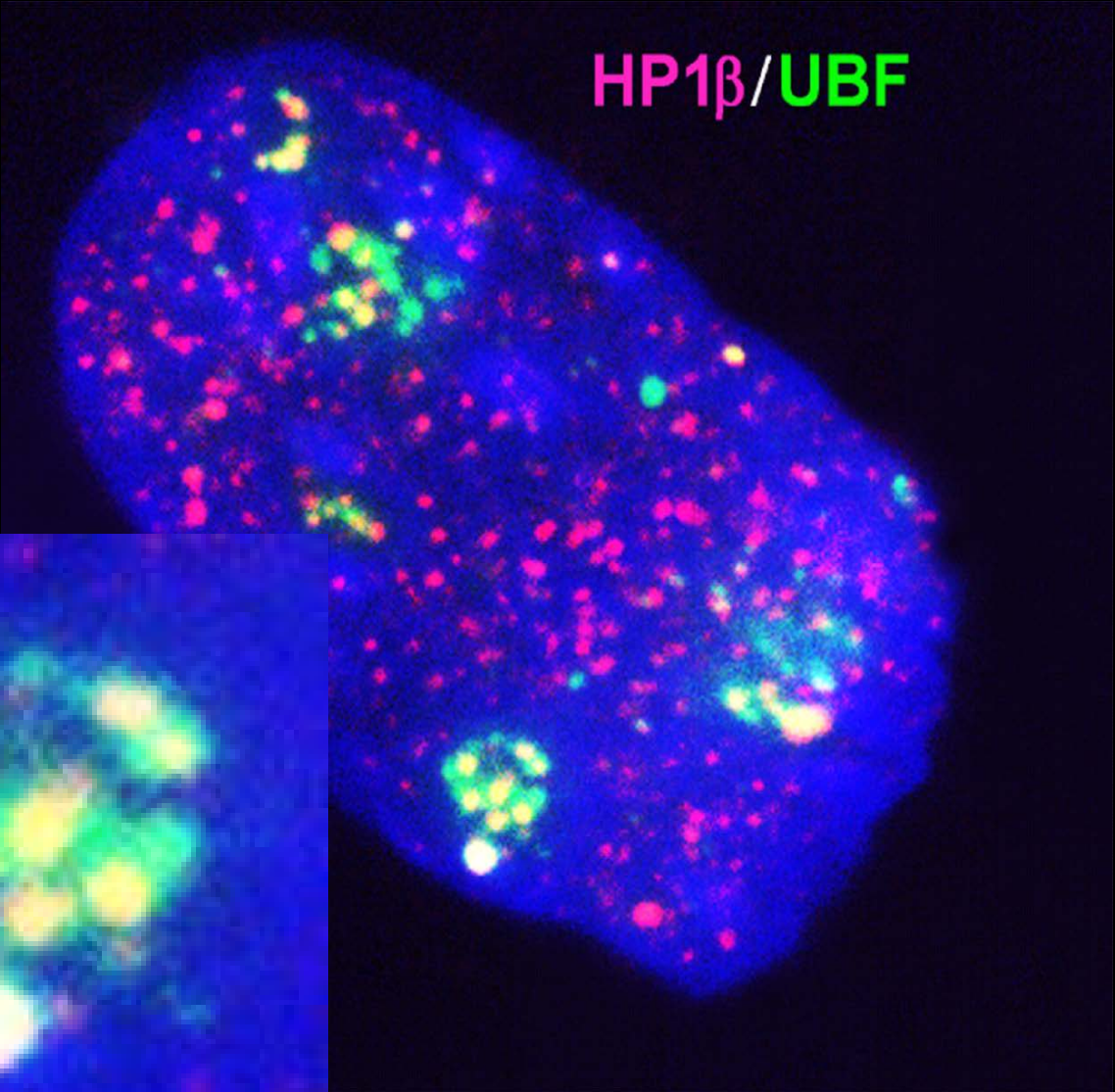


HP1 β



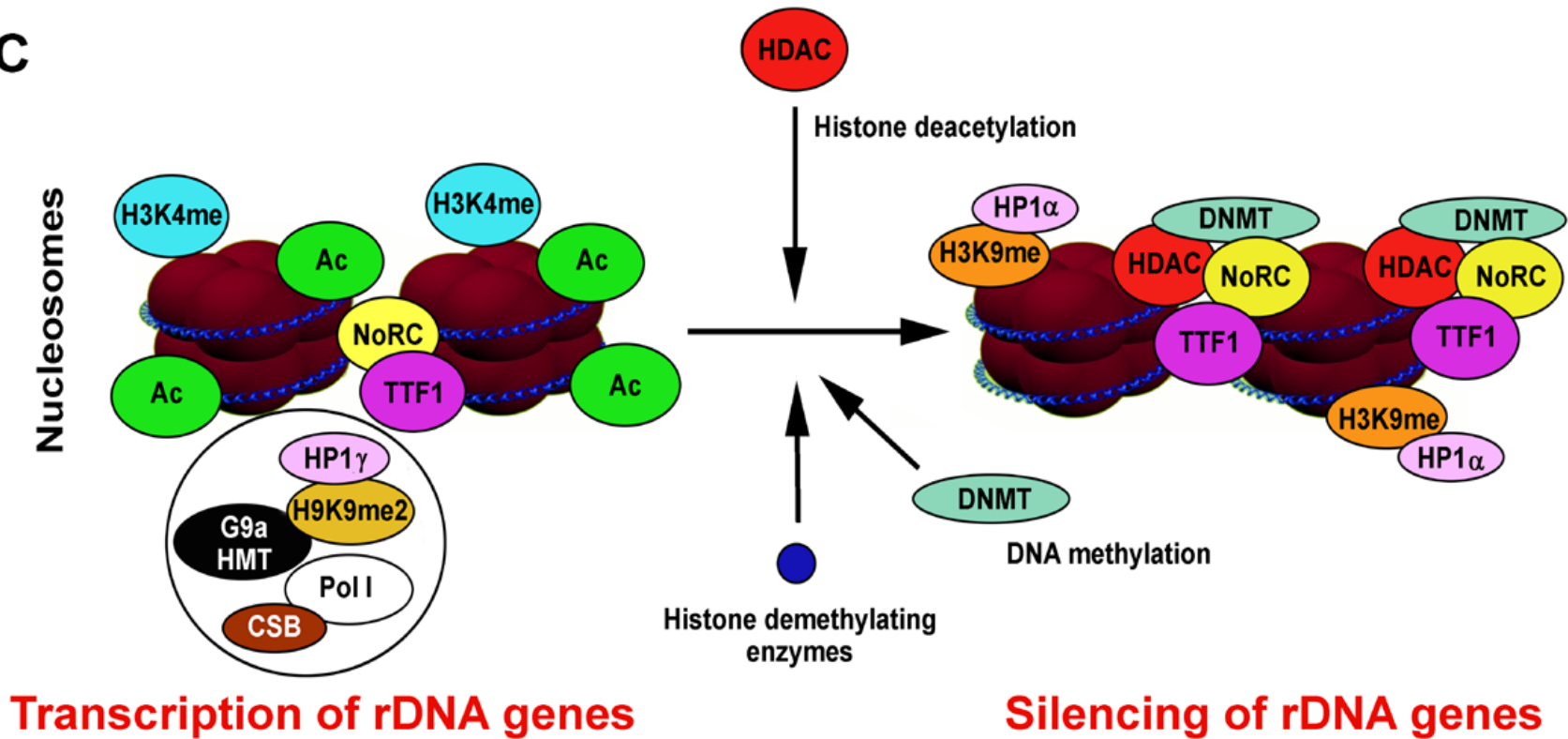


MEF cells



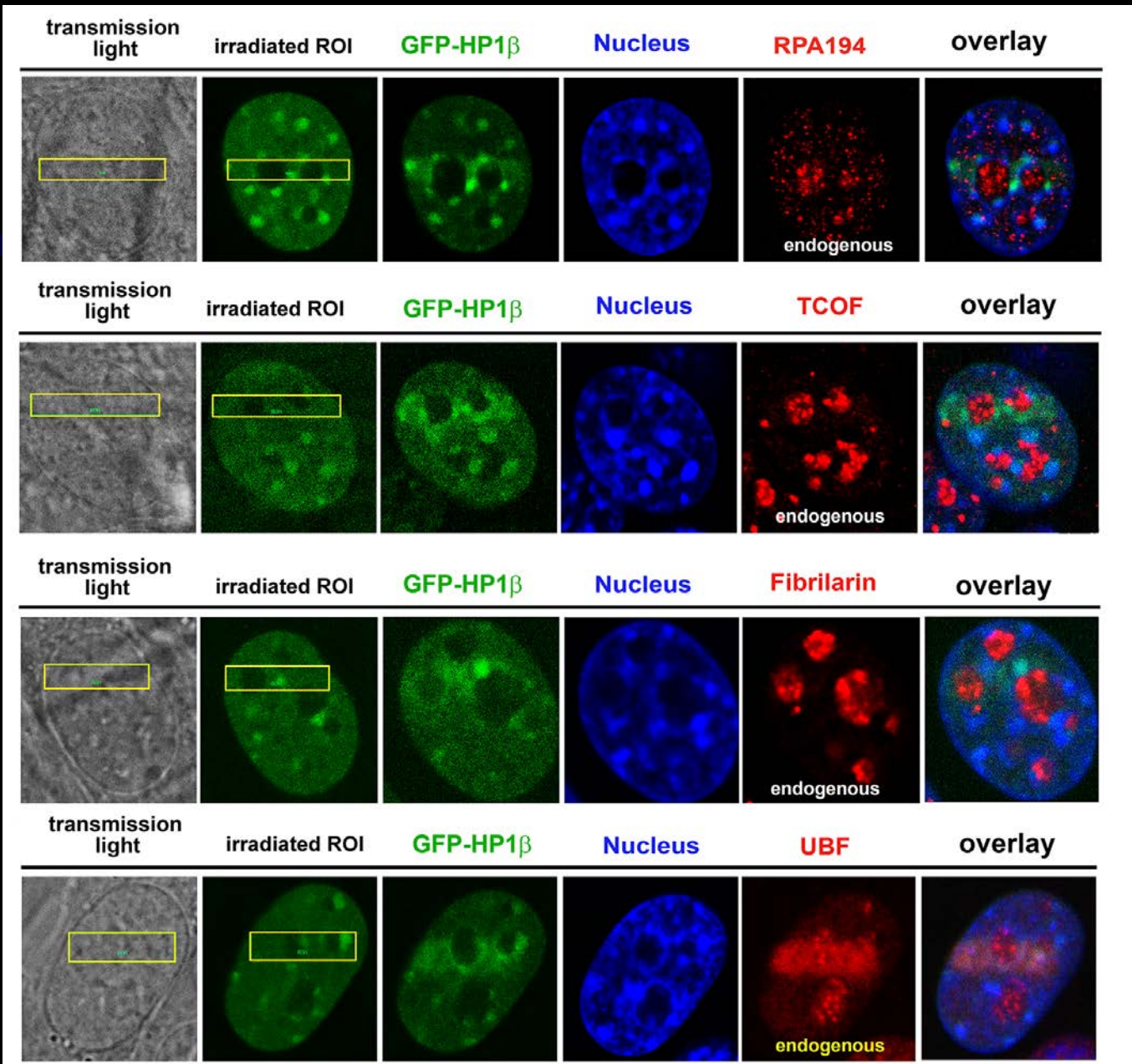
Epigenetics of nucleoli

C



Yuan et al. (2007)

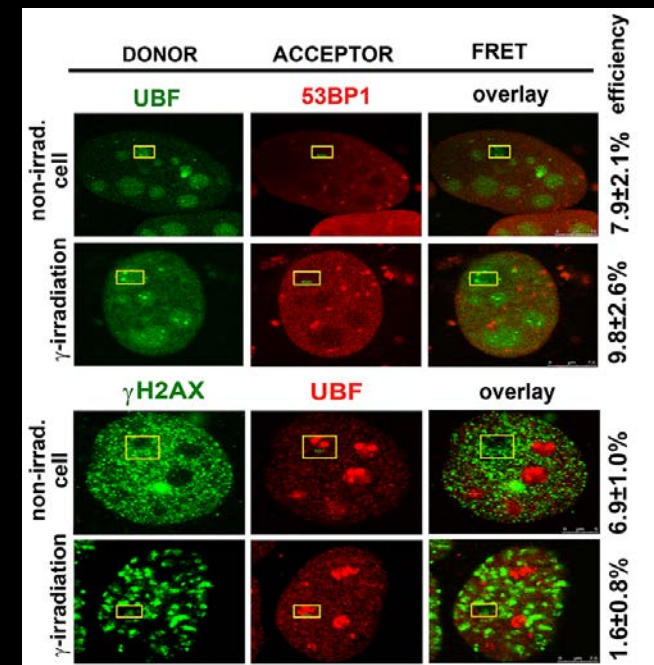
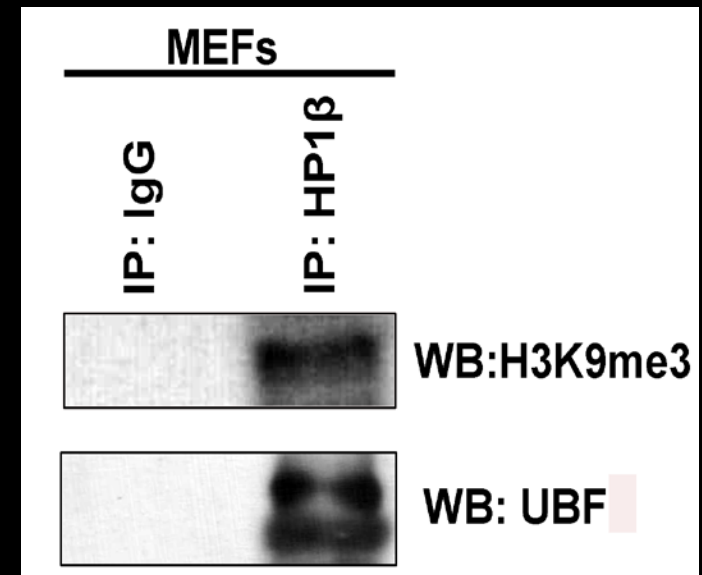
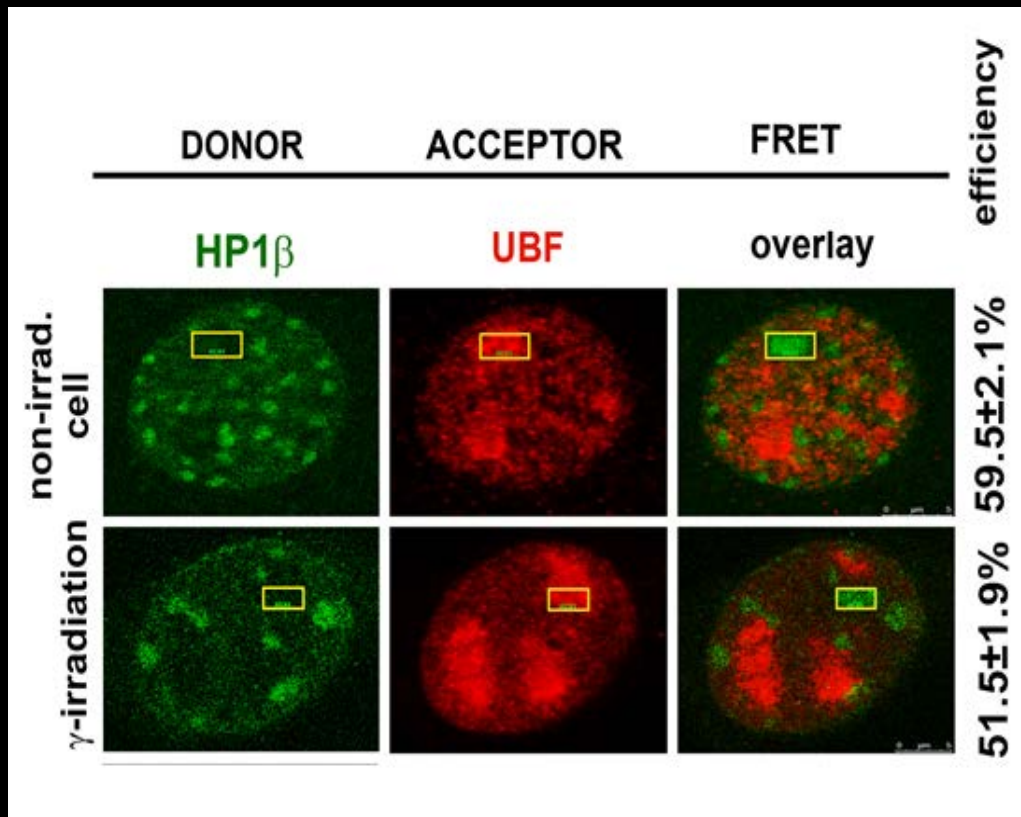
Bártová et al., JHC (2009)



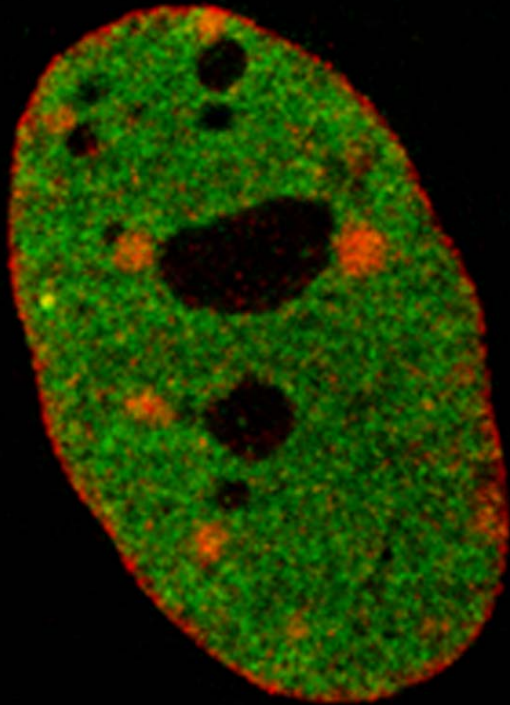
Experiments: Petra Sehnalová

HP1 β interacts with UBF FRET analysis

HP1 β – UBF
FRET efficiency > 50%



Experiments: Petra Sehnalová



ZÁVĚR

Struktura chromatinu hraje důležitou úlohu v regulaci jaderných procesů jako je replikace, transkripce, sestřih a DNA reparace.