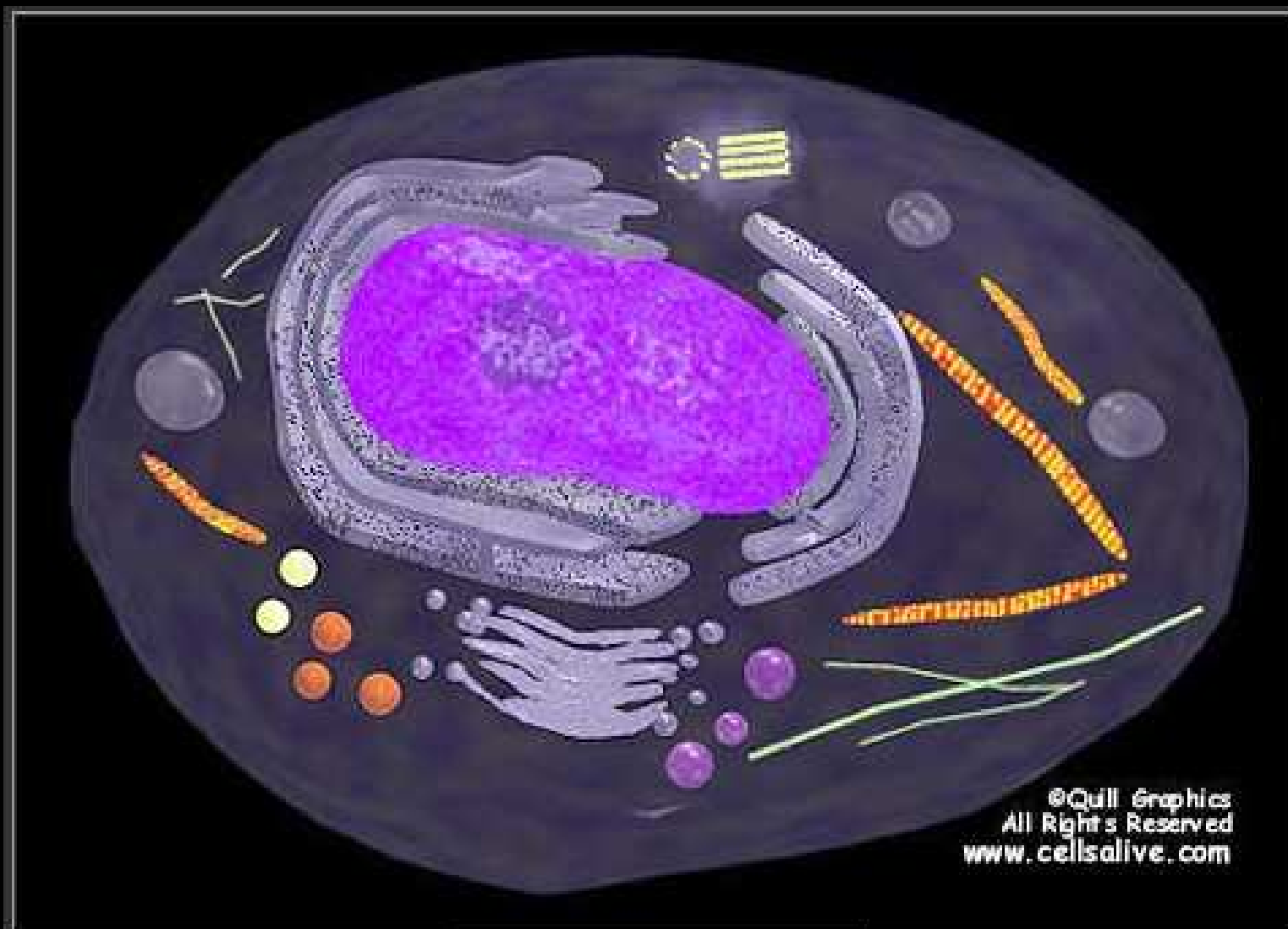
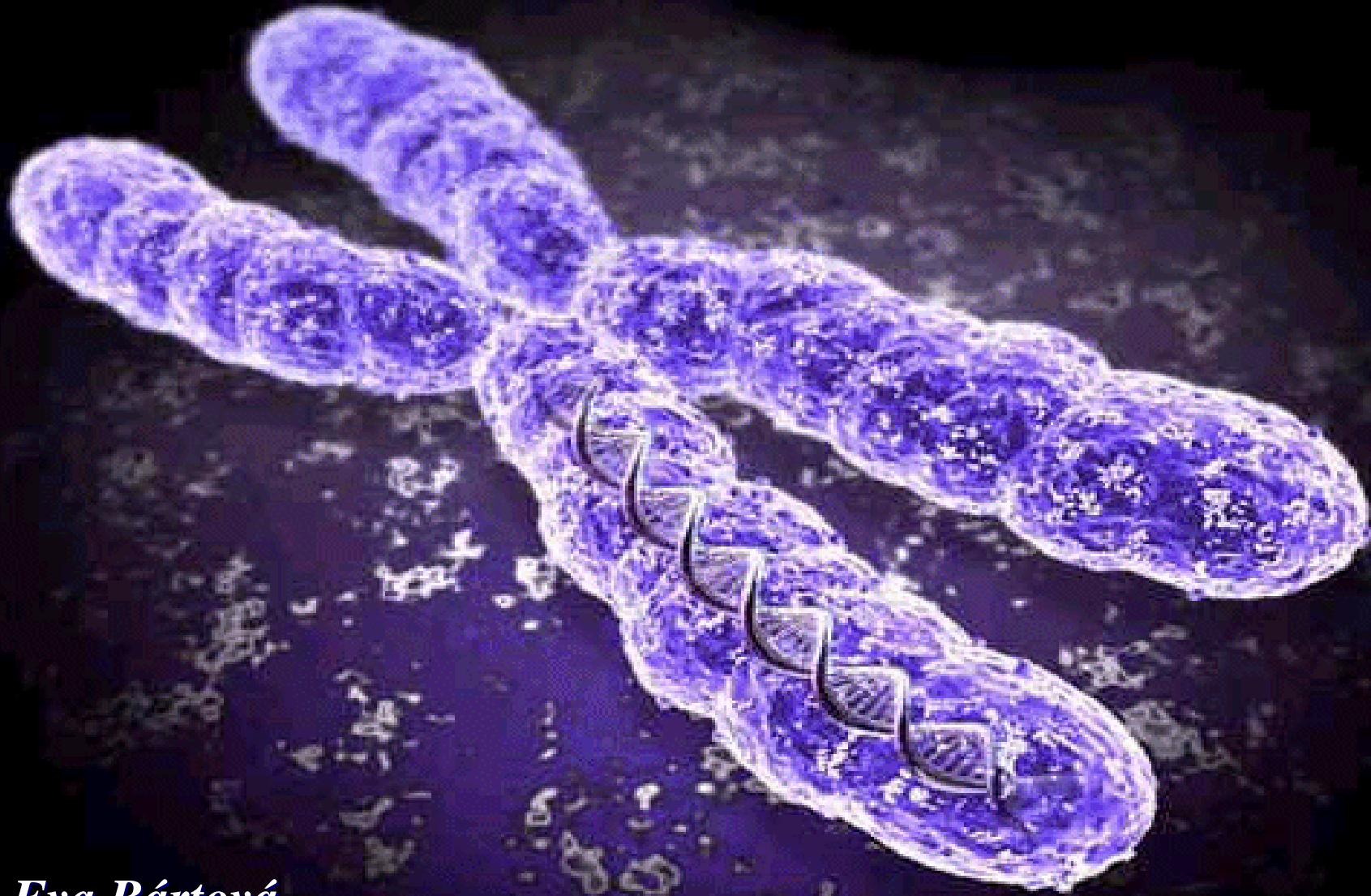


- **Kompartimentalizace interfazních jader**
- **Morfologické a jaderně-topografické aspekty buněčné diference.**
- **Apoptóza a struktura chromatinu.**
- **Cytoskelet a jaderná matrix - studium cytoskeletu pomocí mikroskopie s vysokým rozlišením.**
- **BAC/PAC knihovny a jejich využití pro FISH techniku**
- **Epigenetické procesy probíhající v buněčných jádrech.**
- **Cytogenetické a jaderně-topografické změny u vybraných typů nádorů.**
- **Patofyziologie, cytogenetika a jaderná topografie mnohočetného myelomu.**

Buněčné jádro



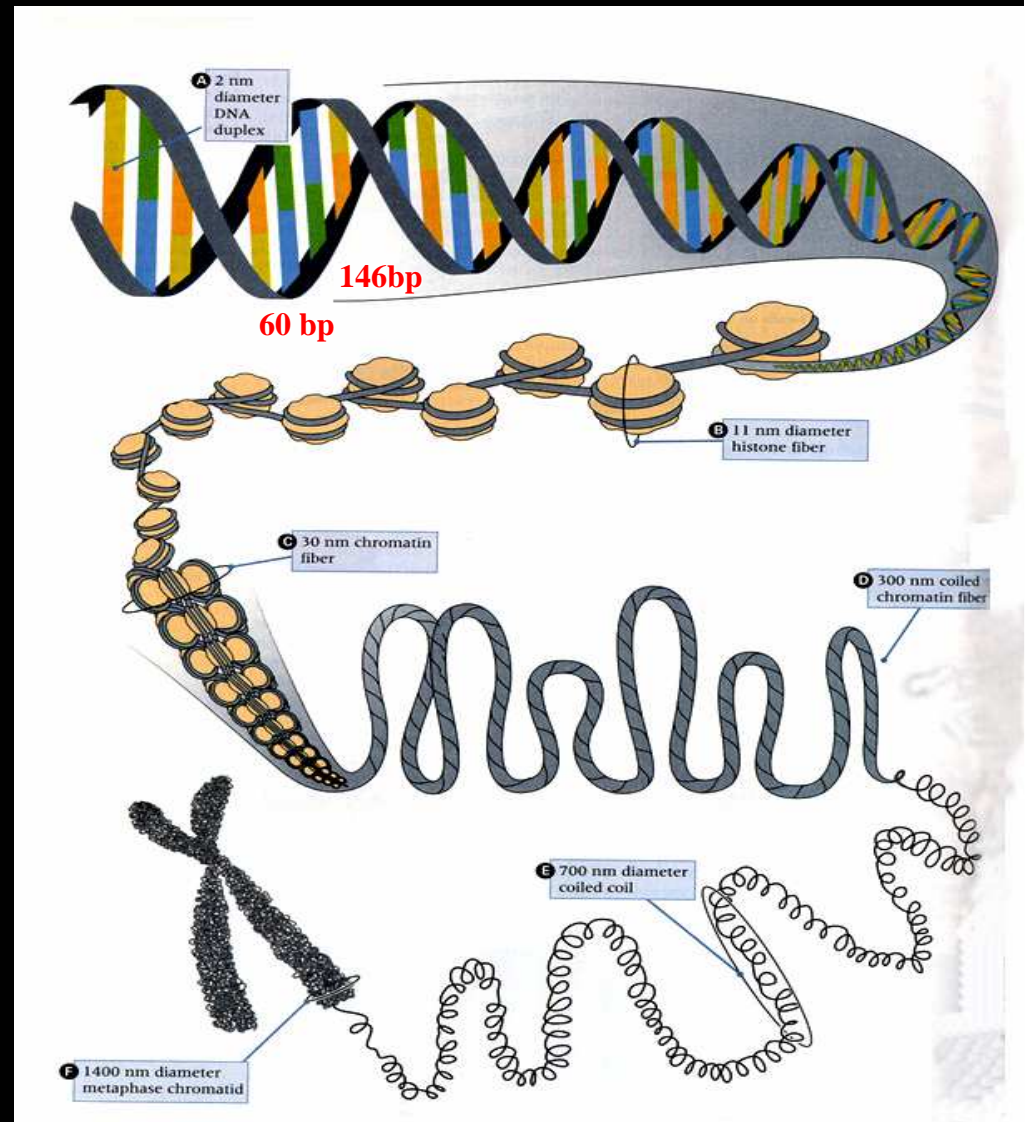
Struktura chromatinu vyššího řádu



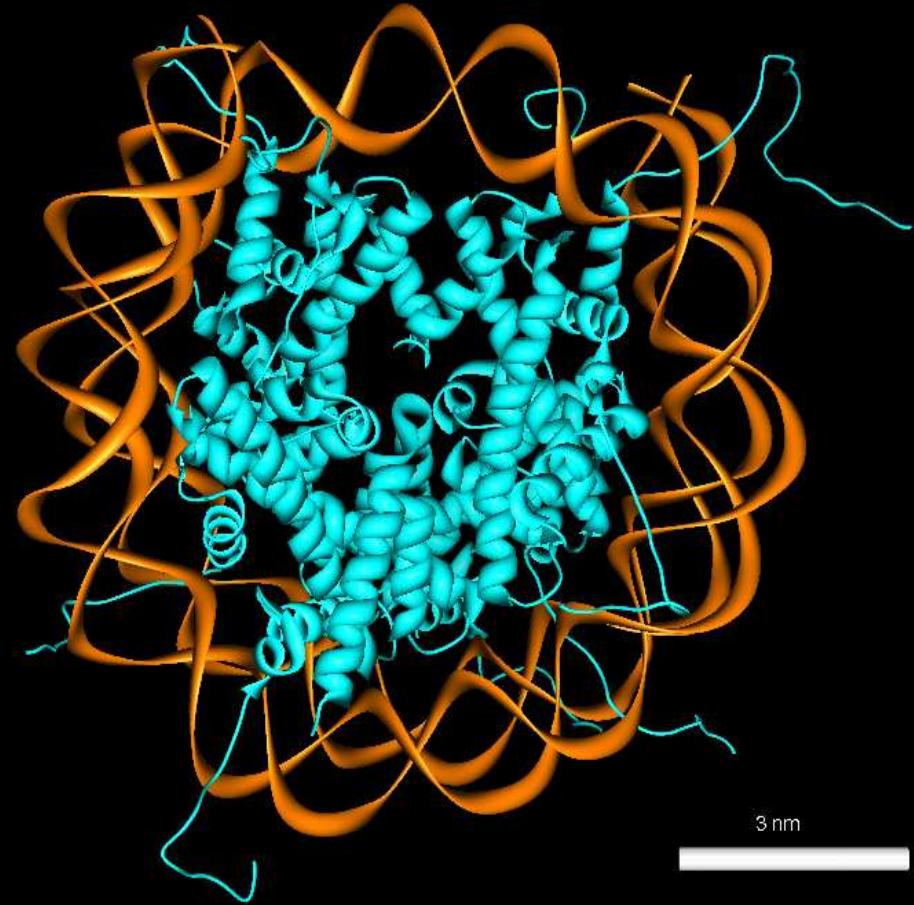
Eva Bártová

www.tqnyc.org

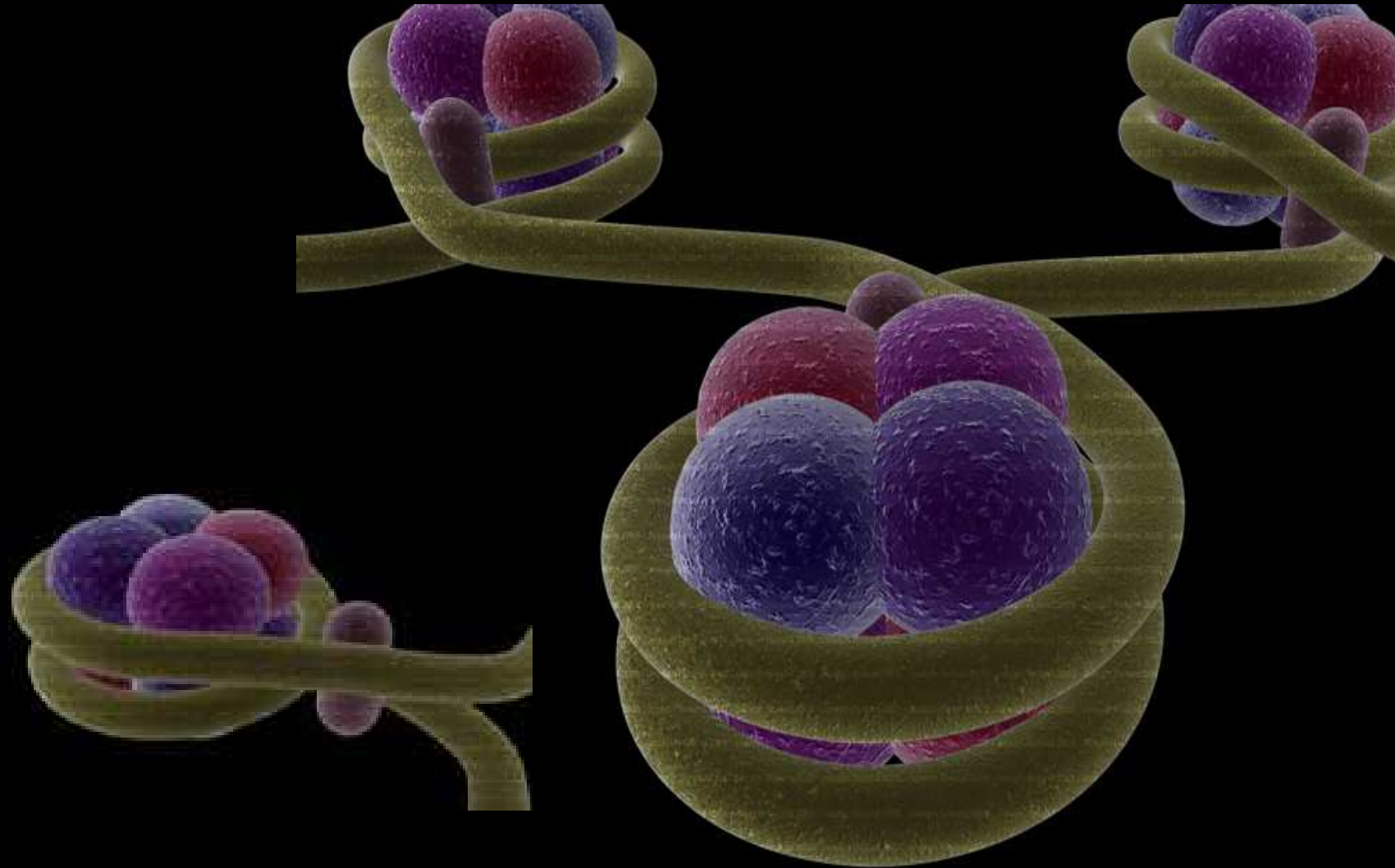
CHROMATIN: materiál jader eukaryotních buněk; nukleoproteinový komplex tvořený DNA vázanou na histony a další bílkoviny. V nedělicím se jádru lze rozlišit **euchromatin**, kde probíhá transkripce, a **heterochromatin**, který je transkripčně inaktivní.



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 oktamery, 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

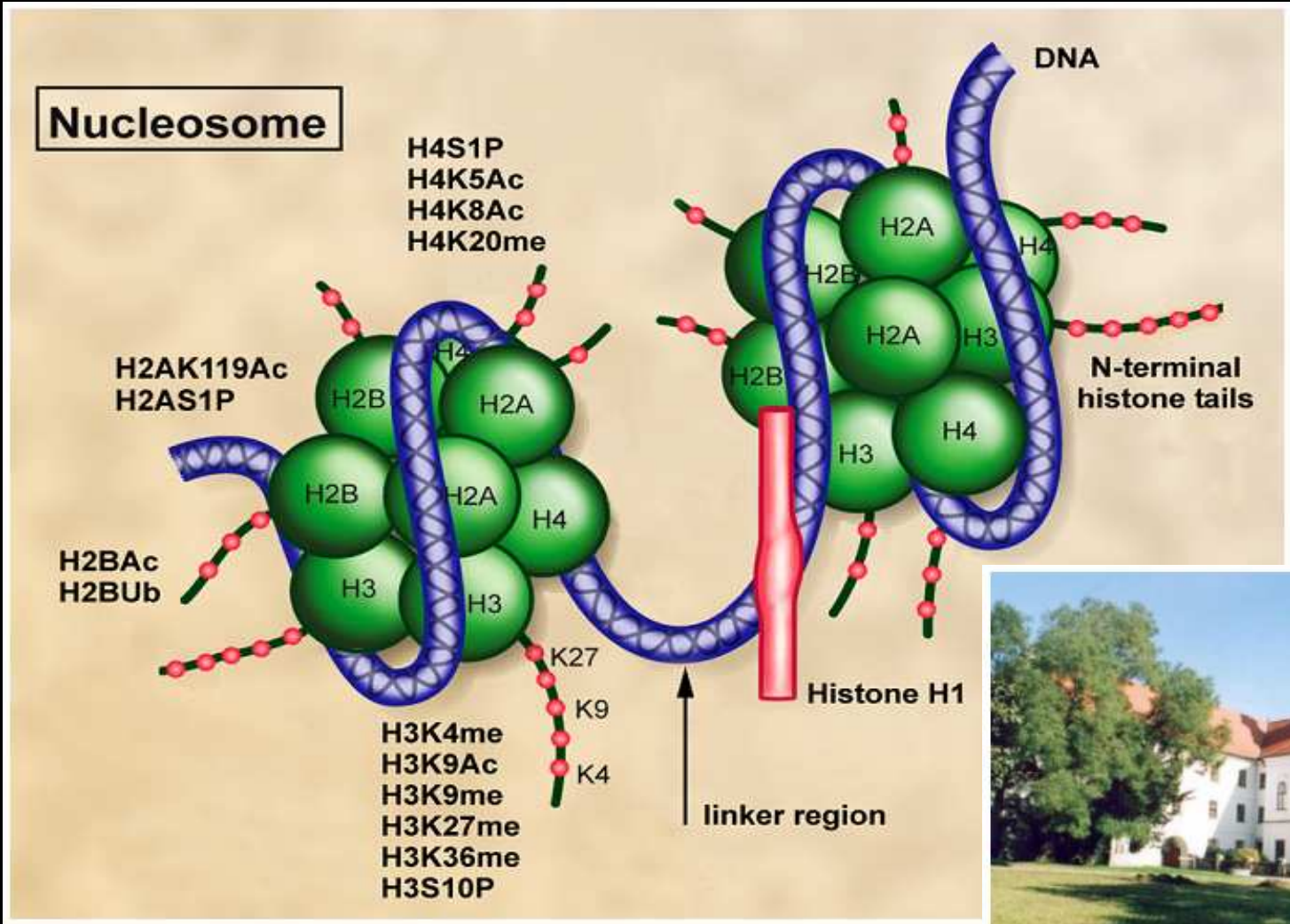


Joseph Roland 2003

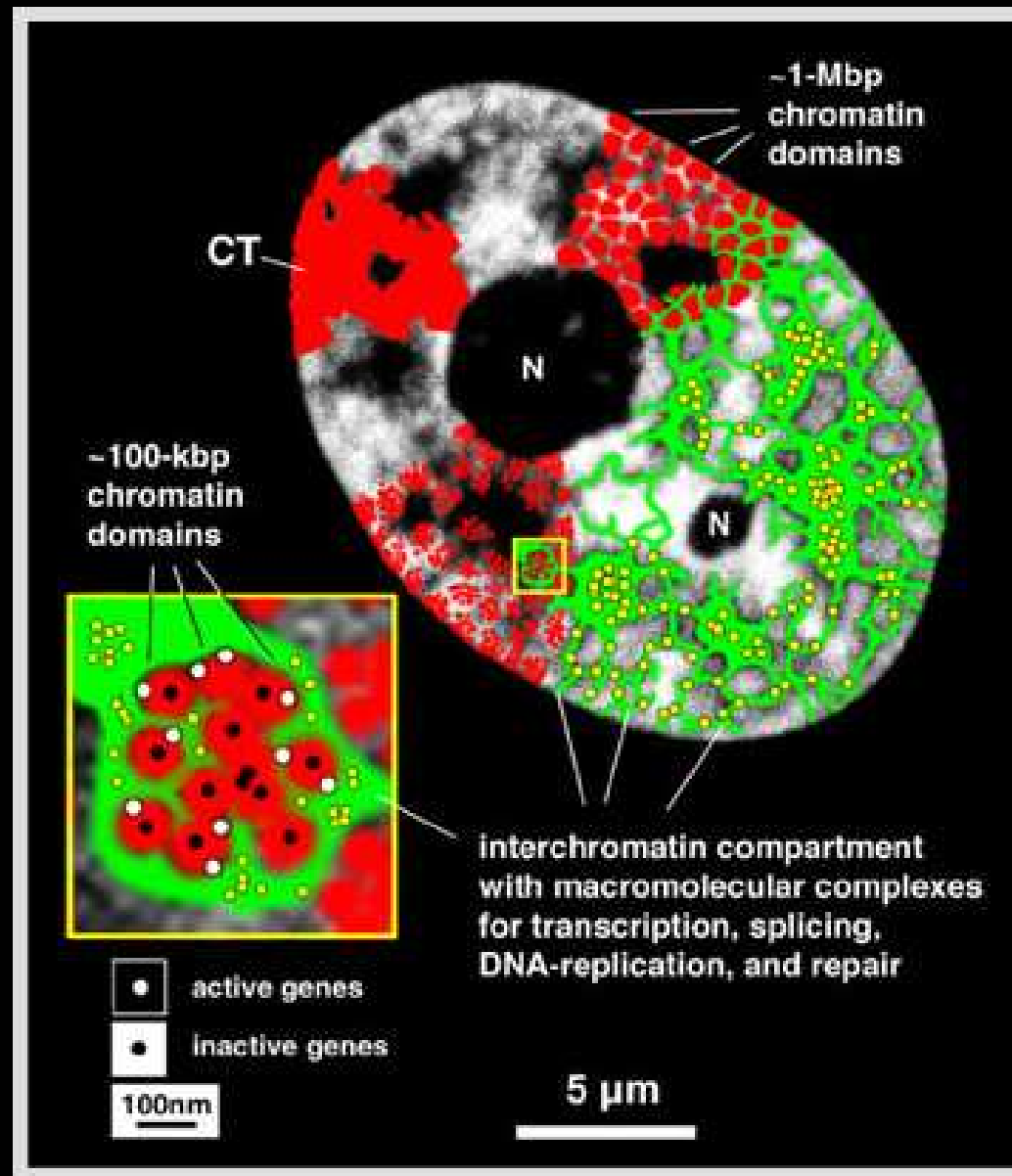
Histone signature

Brno nomenclature for histone modifications

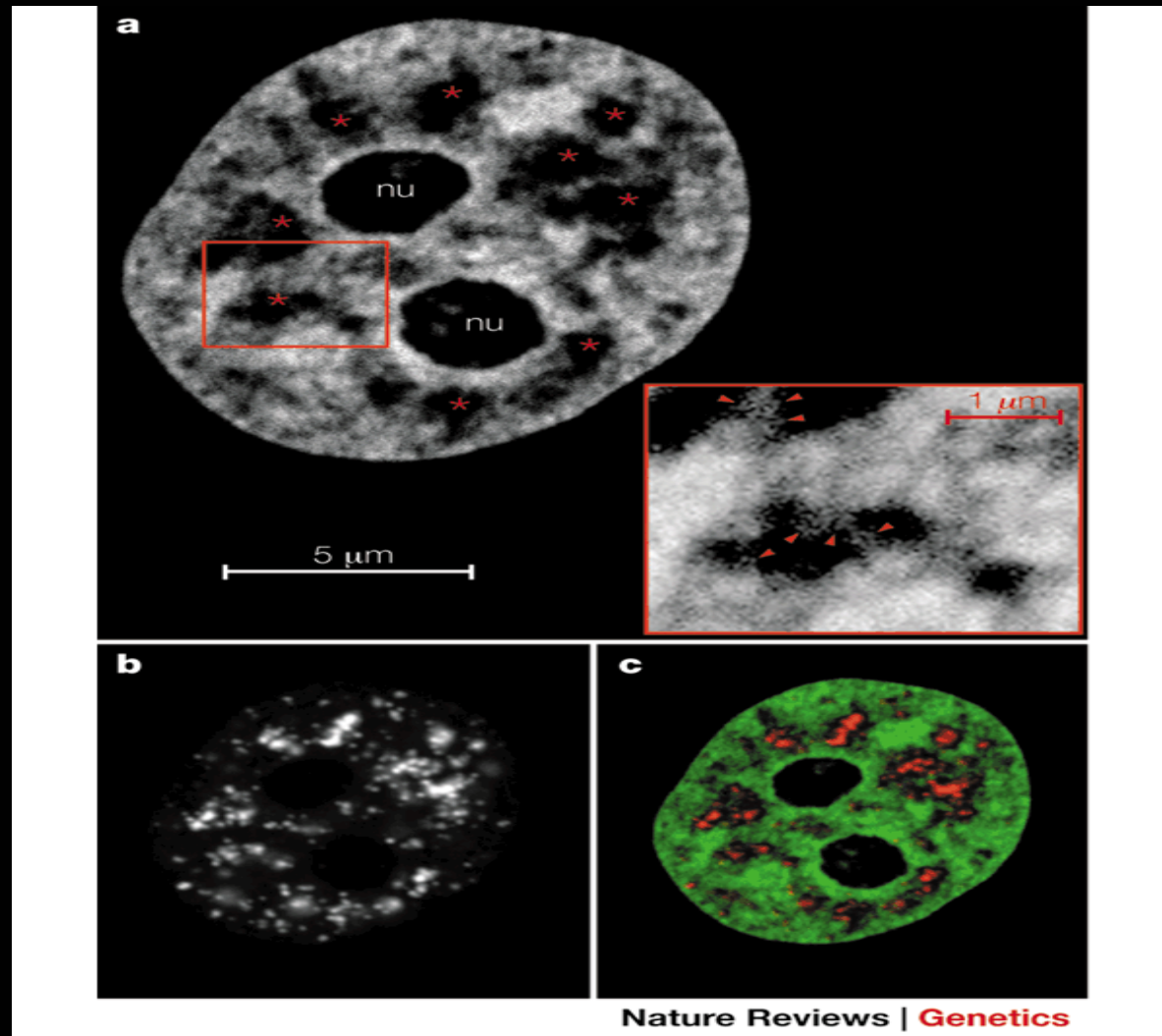
(Turner, 2005)



CT-IC MODEL (T. Cremer)



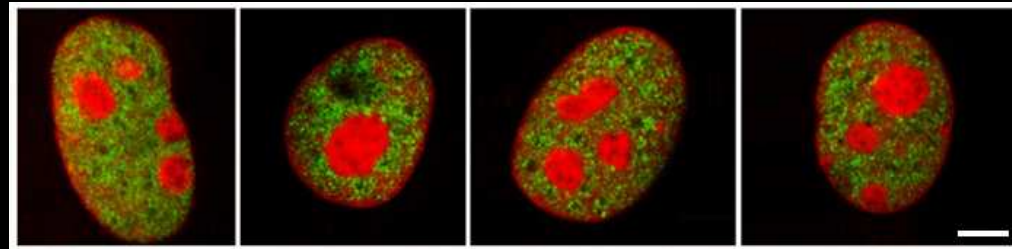
Chromatinové meziprostory



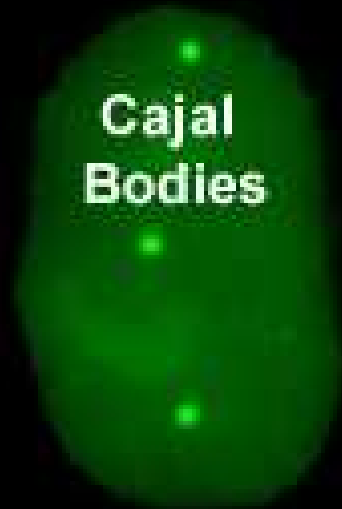
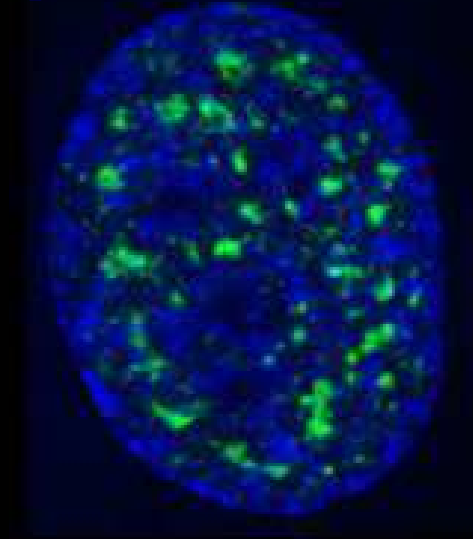
T. Cremer group, Munich

Nuclear compartments:

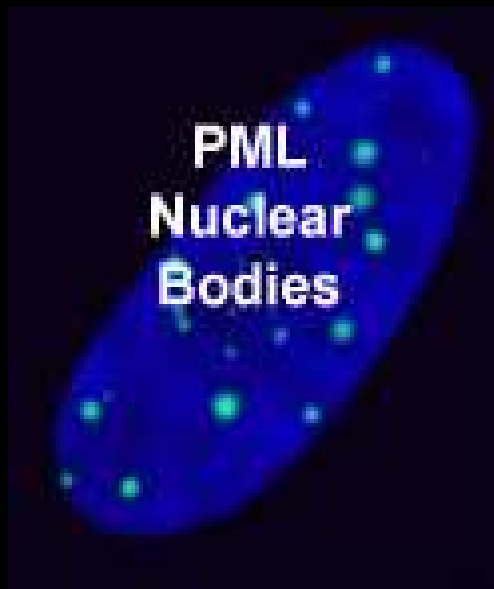
1. Nucleolus
2. Splicing speckles
3. Cajal bodies
4. PML bodies
5. snRNP



speckles



Cajal
Bodies

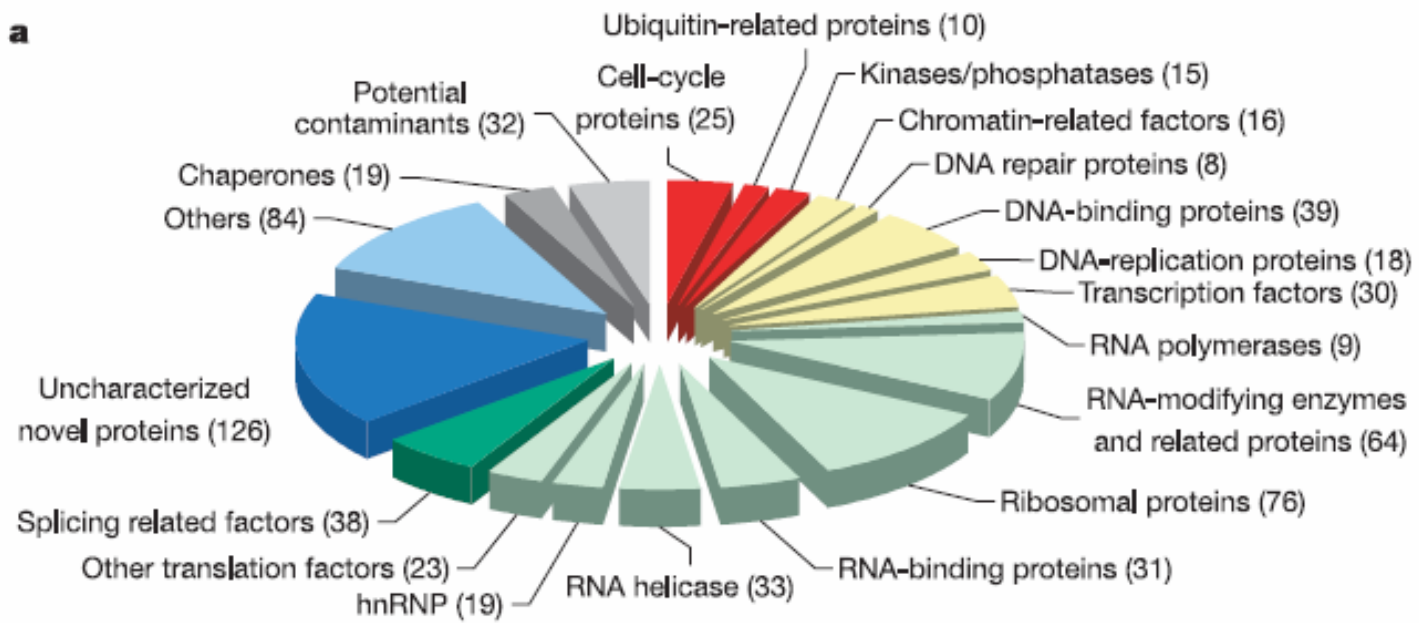


PML
Nuclear
Bodies

Roles of snRNPs

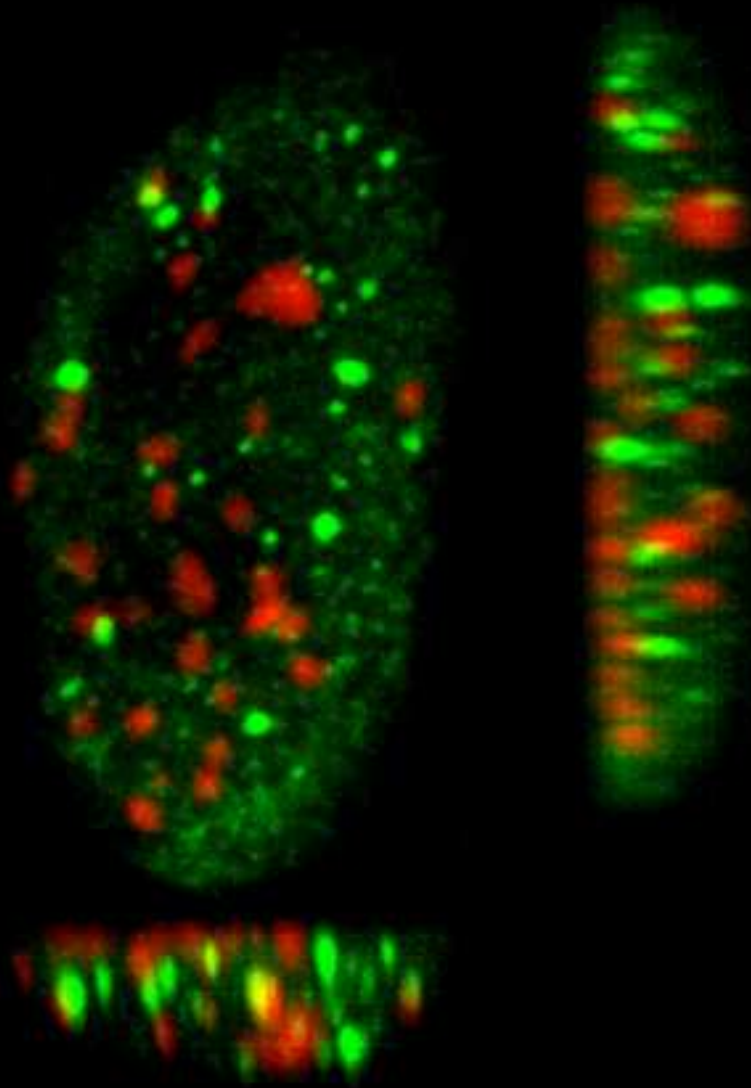
- U1 snRNP binds 5' splice site
- U2 snRNP binds to branch point
- U4/U6 snRNP. snRNAs are base paired. U6 is catalytic
- U5 snRNP contacts the 5' splice site
 - forms tri-snRNP complex with U4/U6

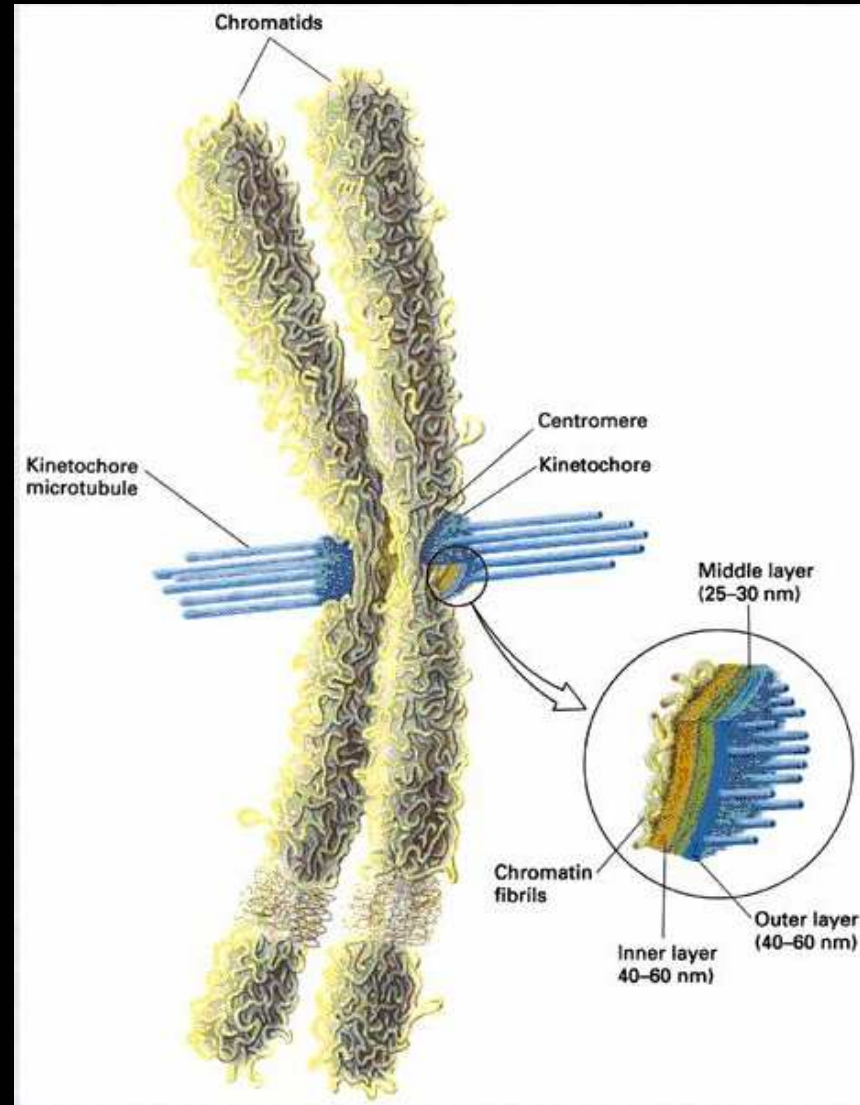
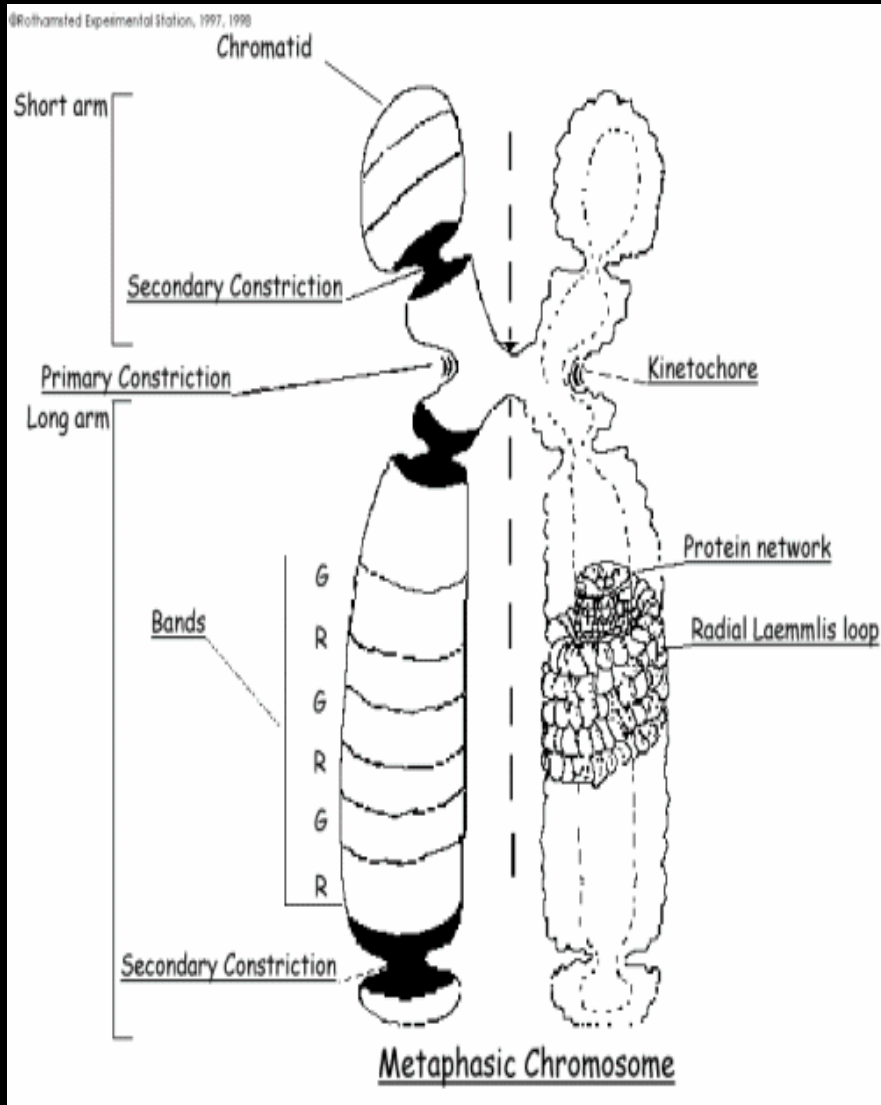
a



Andersen et al., Nature, 2005

HP1 proteins

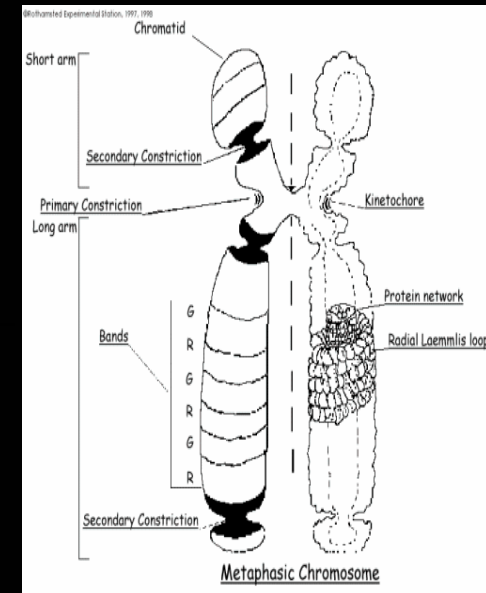
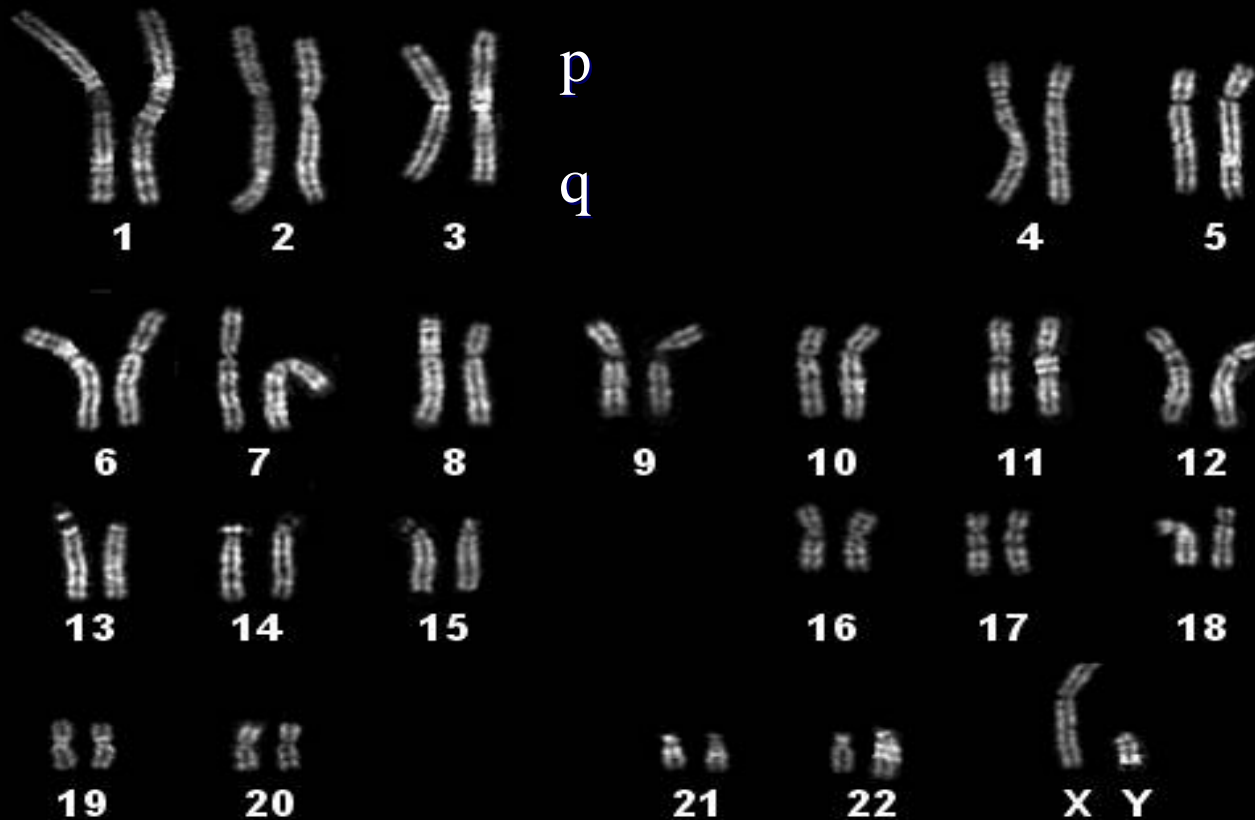




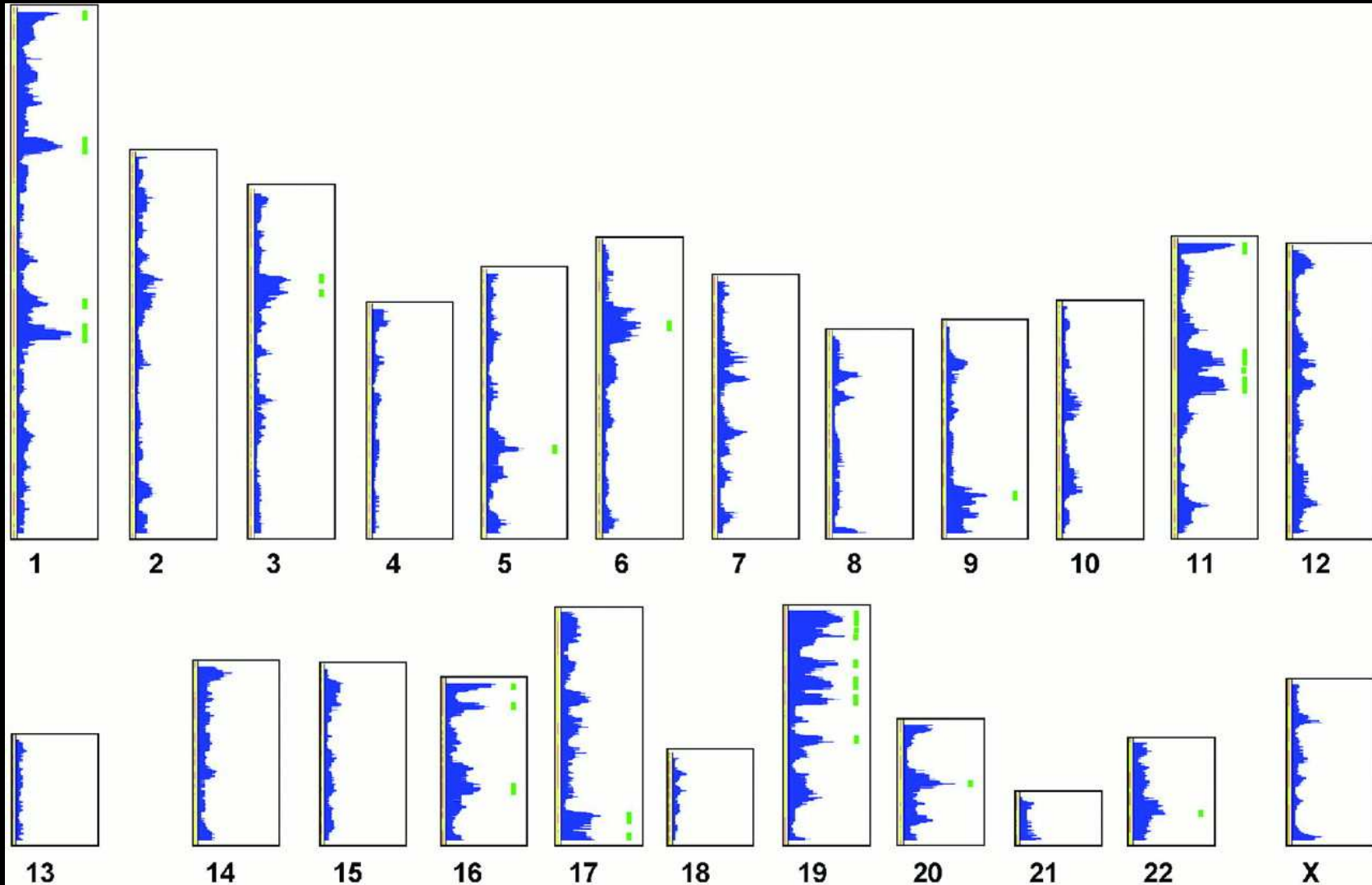
Kondenzace chromatinu

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

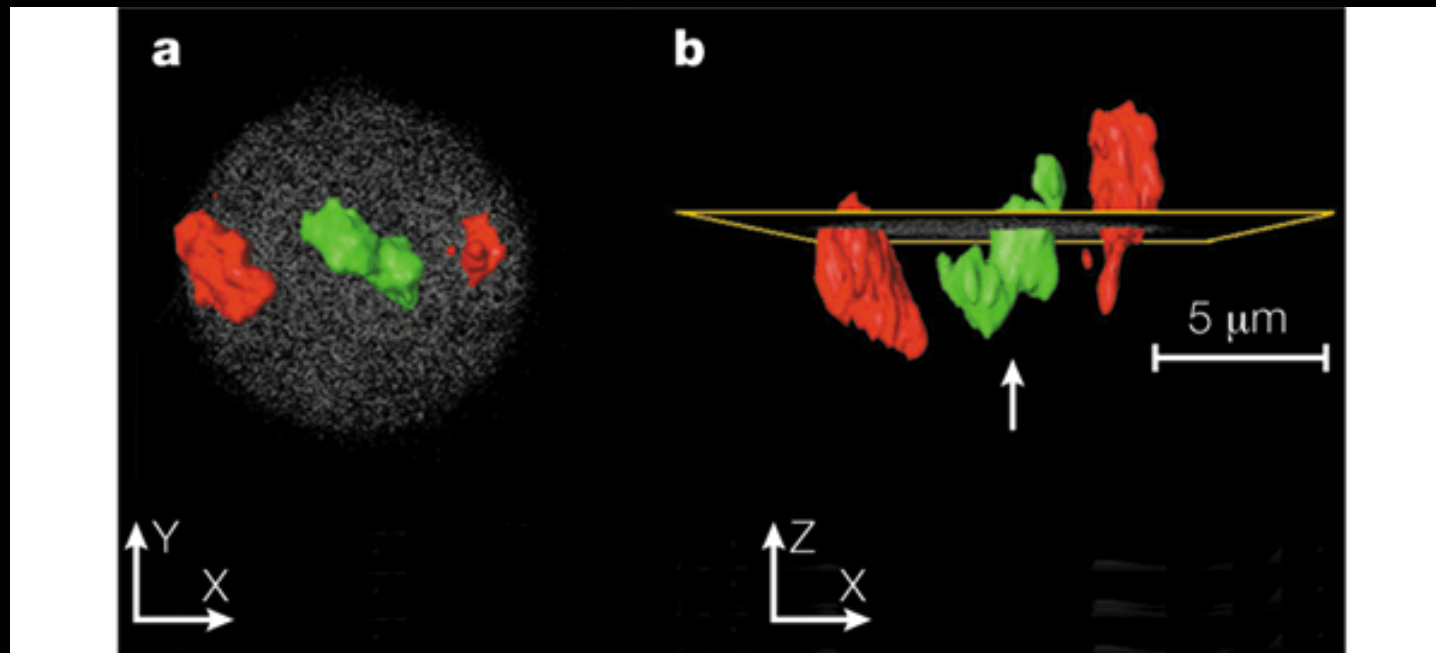
Typy chromozomů: meta- submeta-, akrocentrické



Transcriptome map (Caron et al., 2001)

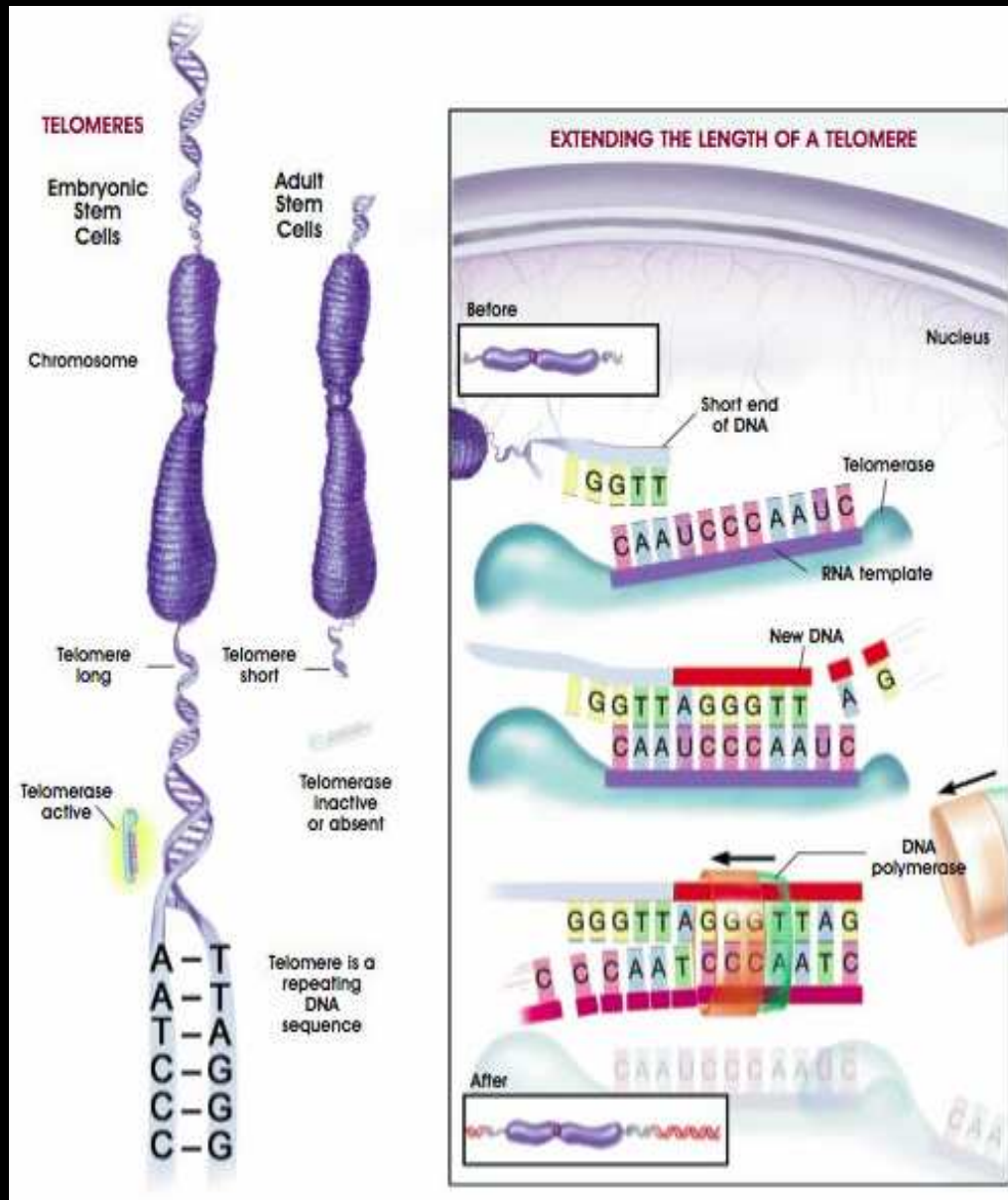


HSA 18 and 19 (positioning and gene density)

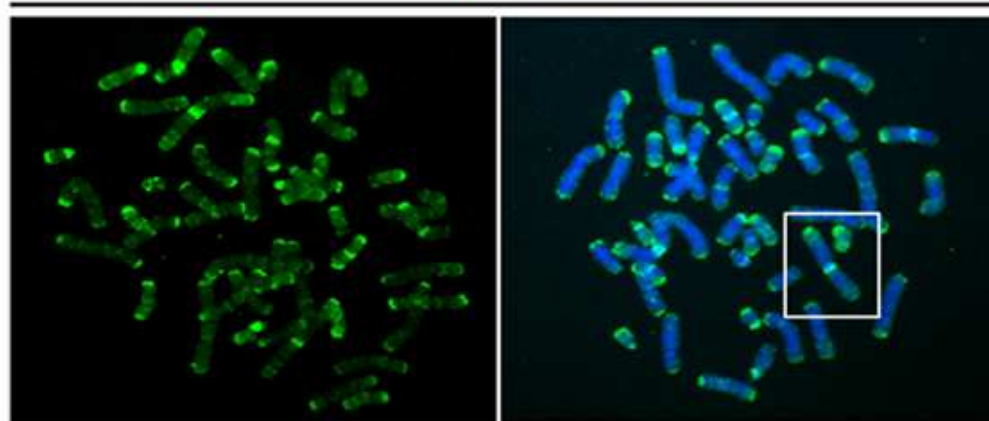
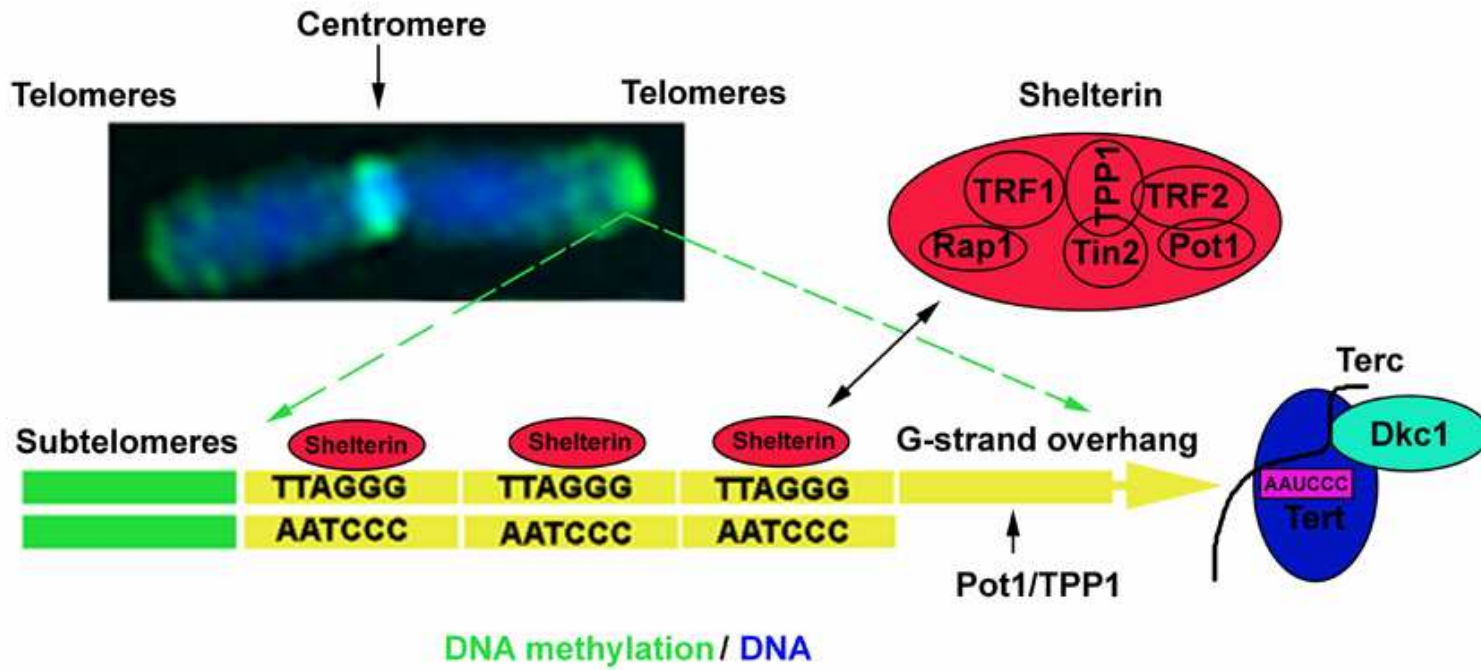


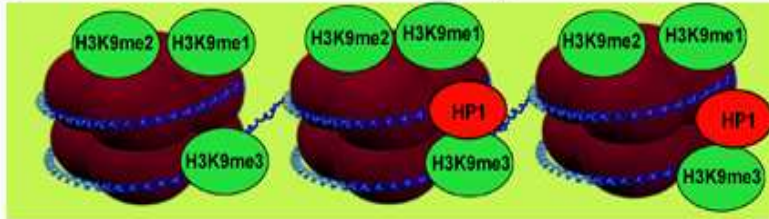
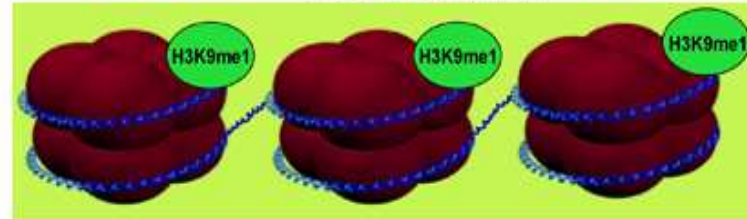
T. Cremer group, Munich

Telomeres (TTAGGG sequence and different proteins)

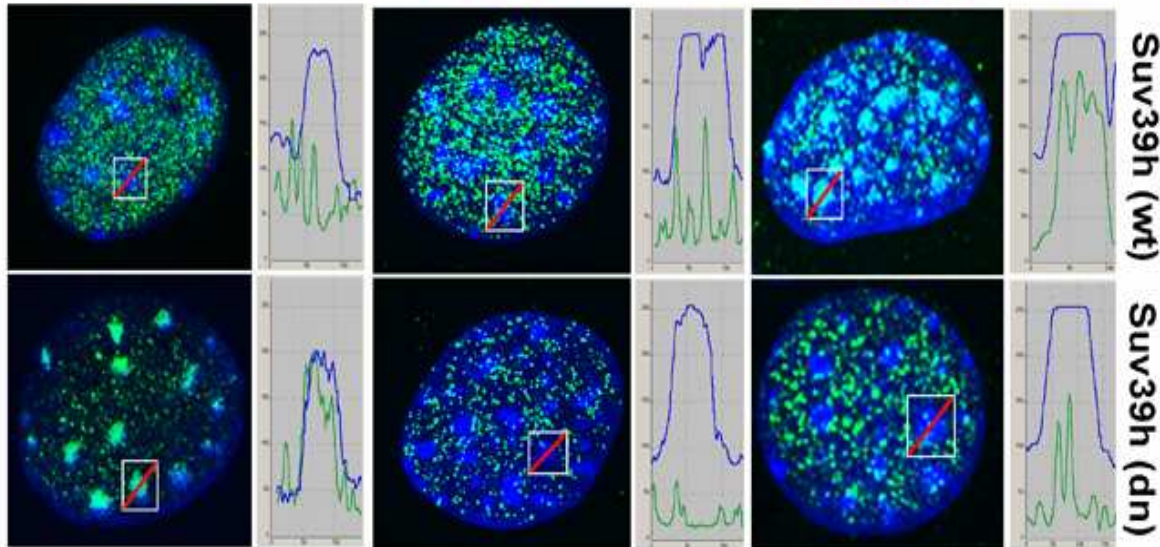
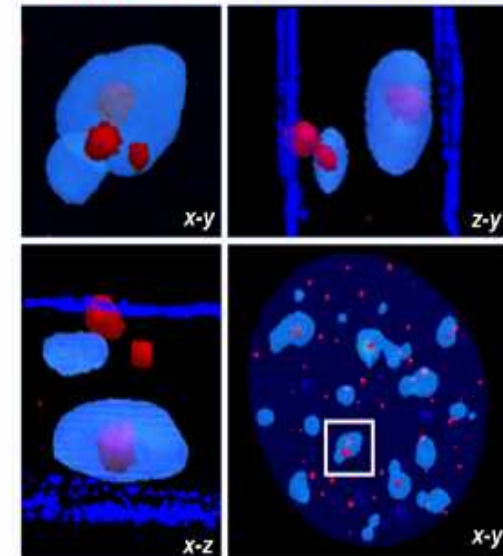


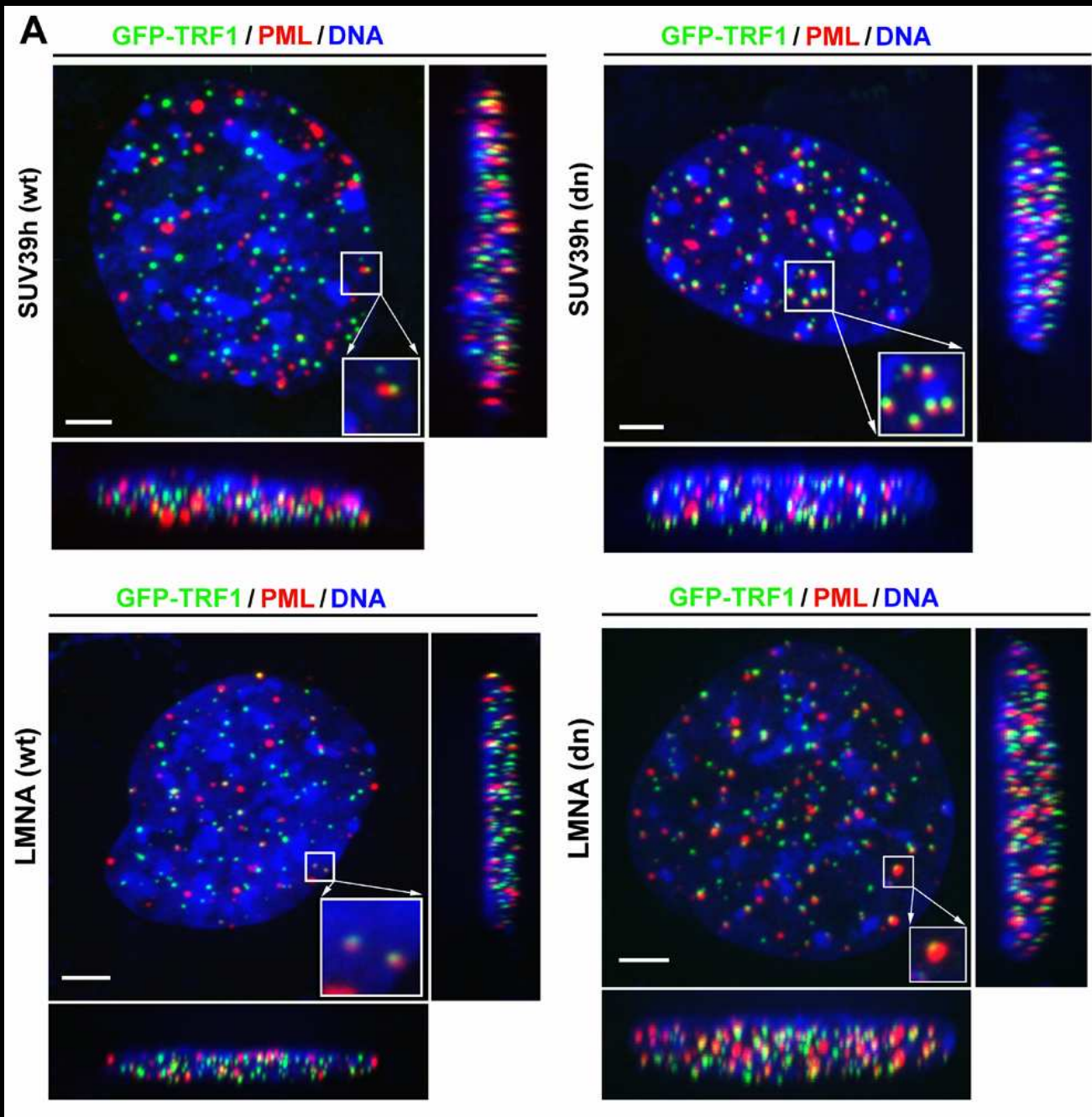
Telomerase is composed of two subunits, Telomerase Reverse Transcriptase (hTERT, the 'h' is for human) and hTR (Telomerase RNA). These two subunits are coded for by two different genes in the genome. Using hTR template, hTERT can add a six nucleotide repeating sequence, 5'-TTAGGG to the 3' strand of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

A

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

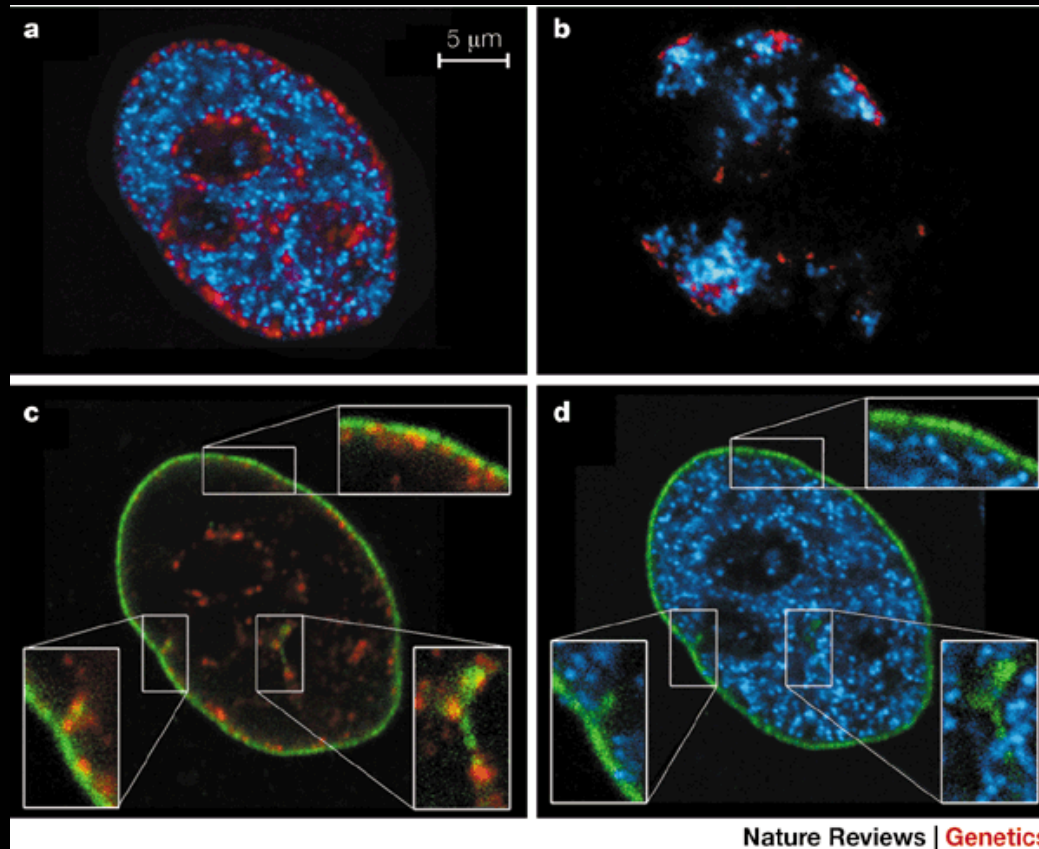
García-Cao et al. (2004)

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

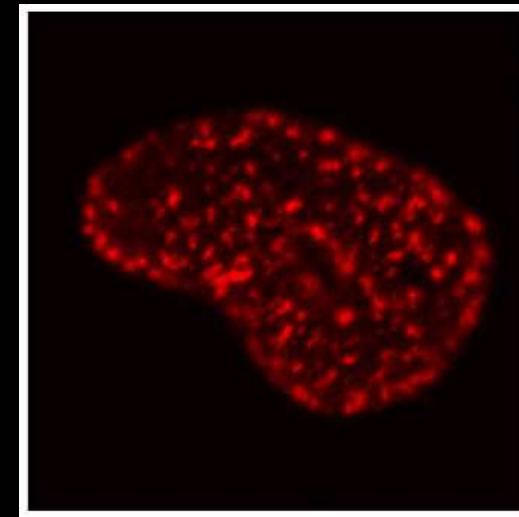


Struktura chromatinu a jederné procesy

Pozdně a časně se replikující chromatin

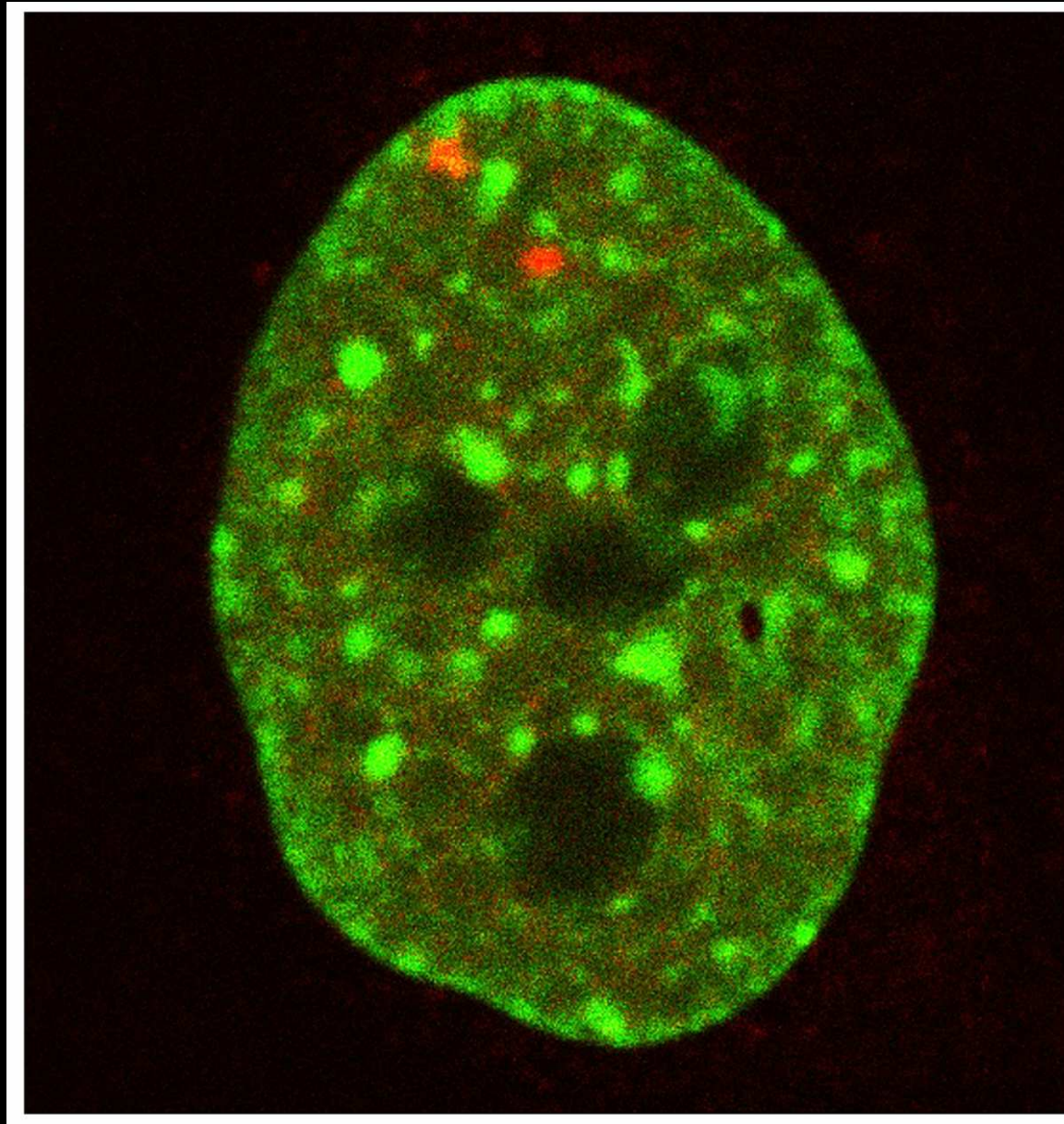


Replikační ohniska



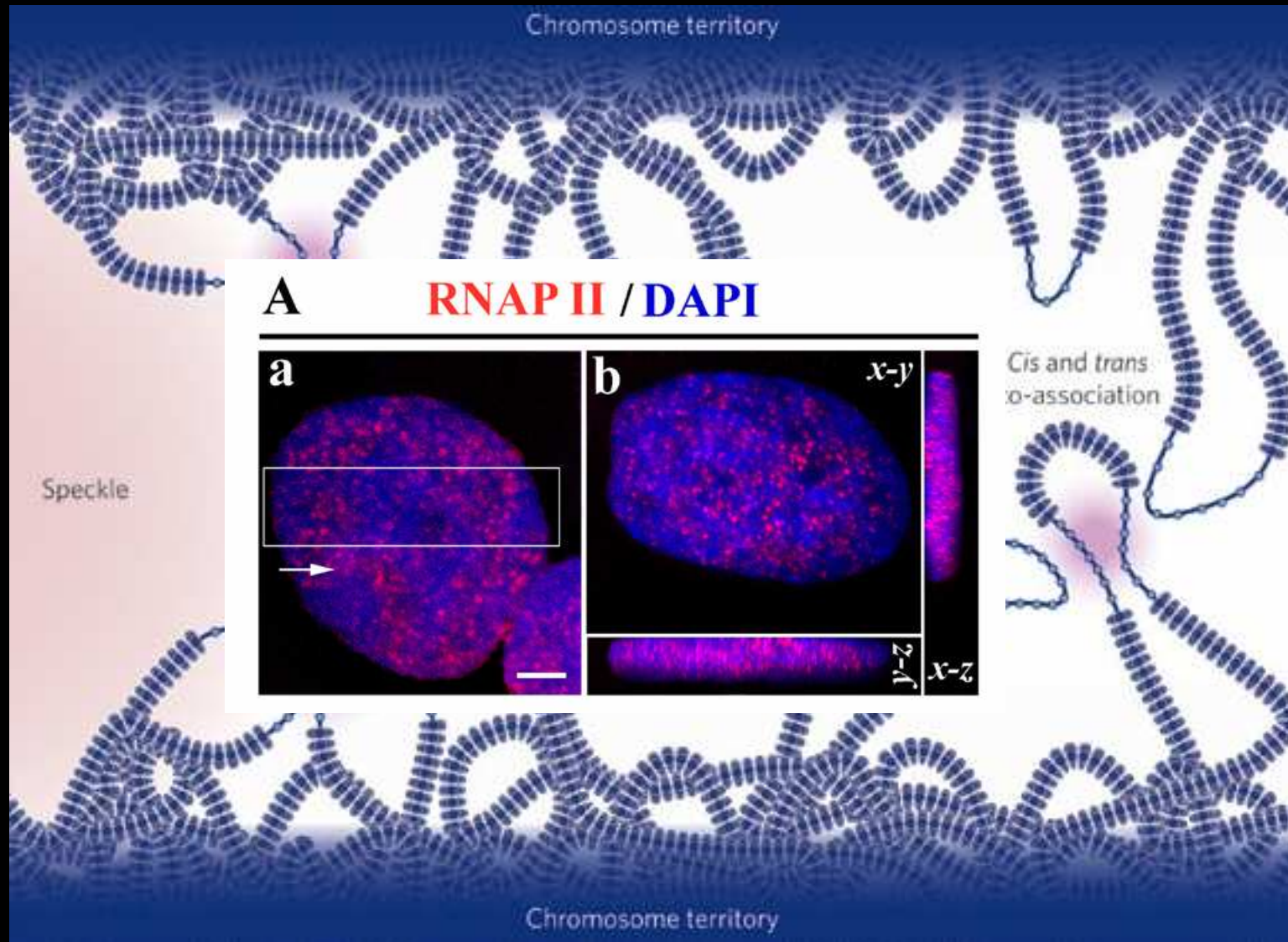
T. Cremer group, Munich

DNA repair foci



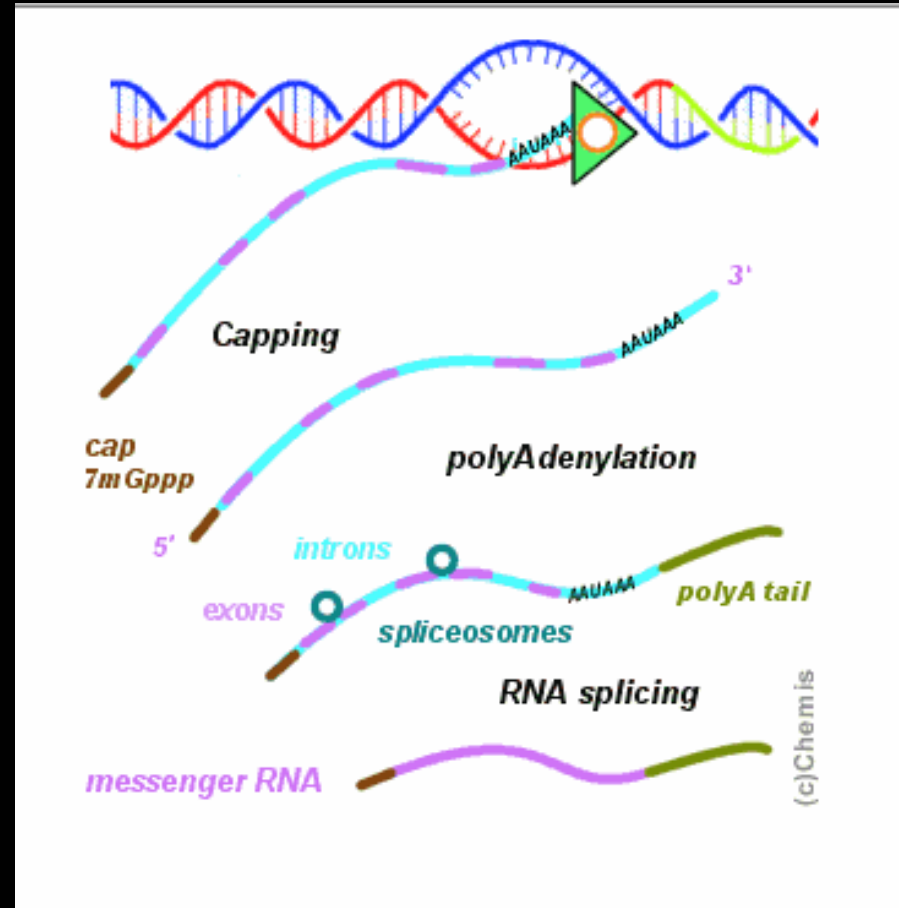
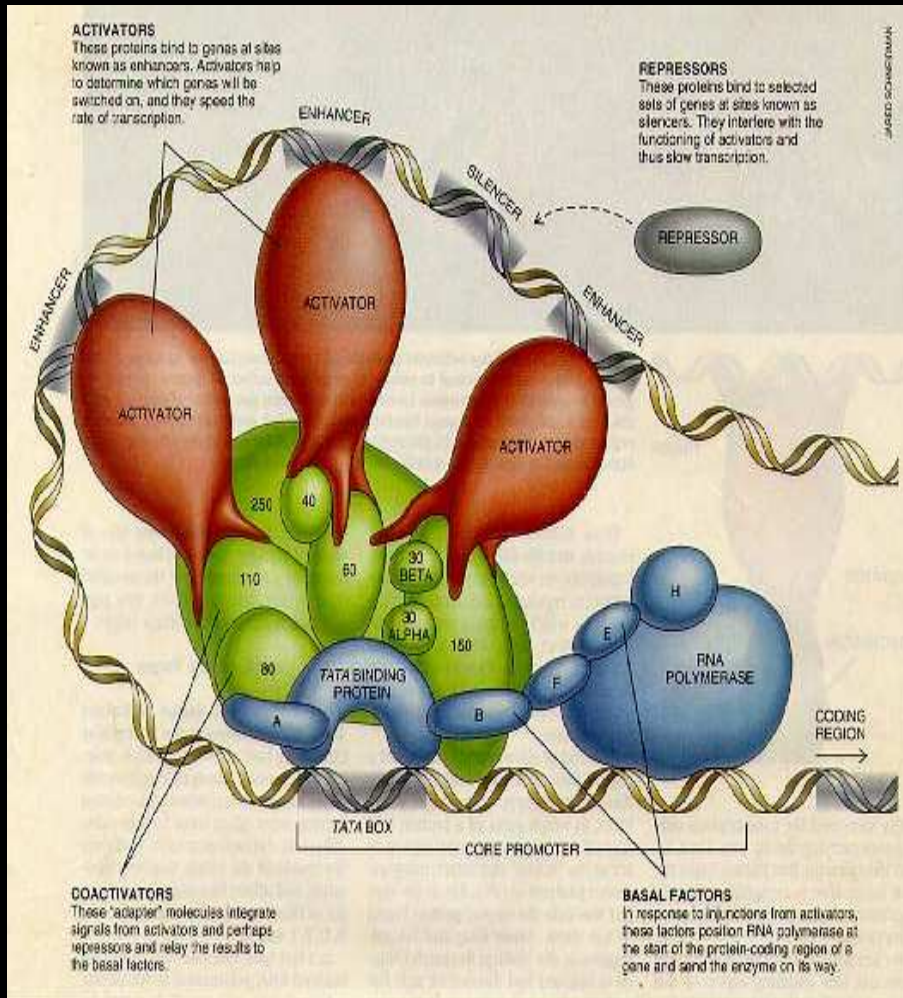
TRANSKRIPCE: přepis, biosynthesa řetězce RNA podle templátového řetězce DNA, přičemž jednotlivé nukleotidy jsou připojovány na základě komplementarity (viz base nukleových kyselin). Klíčovým enzymem této synthesy je RNA-polymerasa. Transkripce probíhá ve třech stupních: **a) iniciace** (zahájení), kdy 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 bílkoviny, tzv. terminačními neboli ρ (ro) faktory. Řízení transkripce jednotlivých genů patří k nejdůležitějším mechanismům regulace enzymové aktivity a diferenciace buněk.

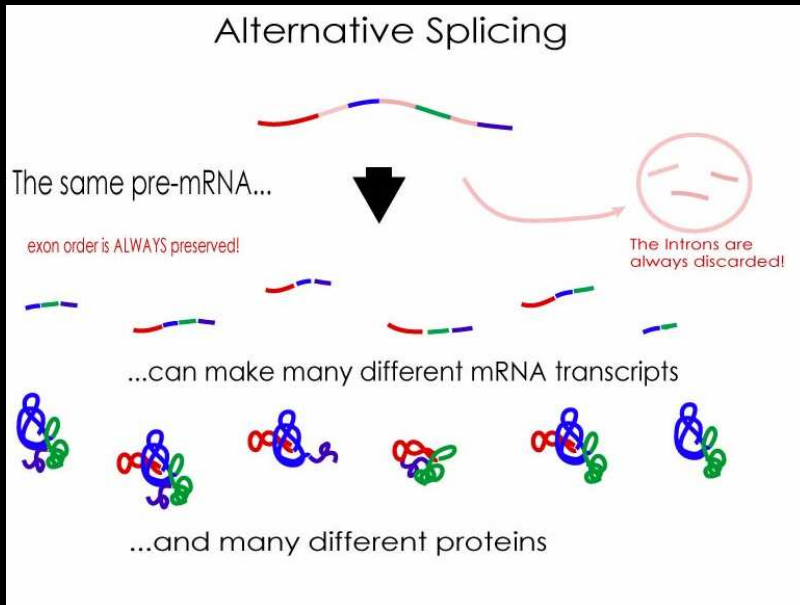
Transcription factories



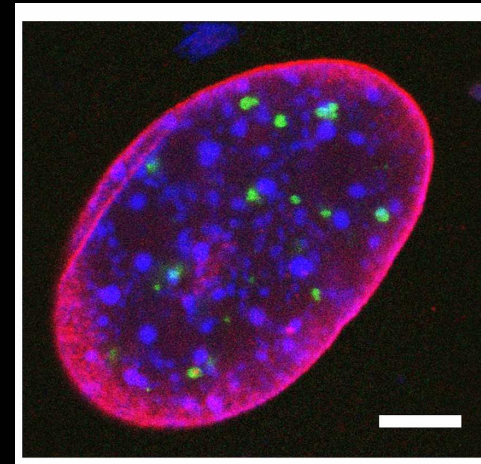
Cis acting elements: Enhancers, LCR

Trans acting elements: proteins, Ikoros, Helios, HP1 proteins

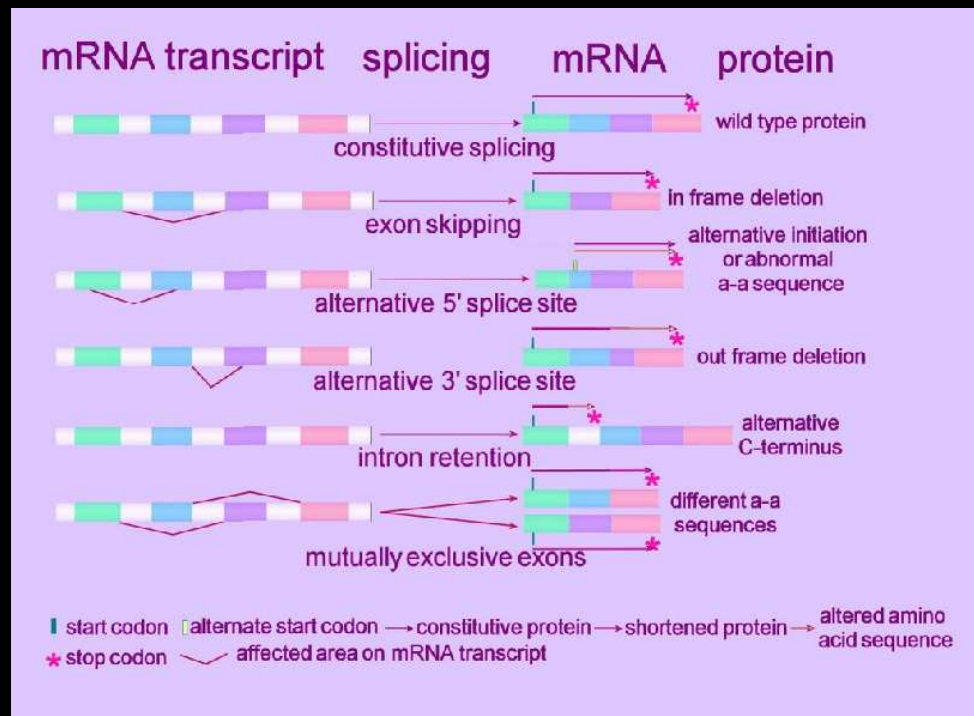


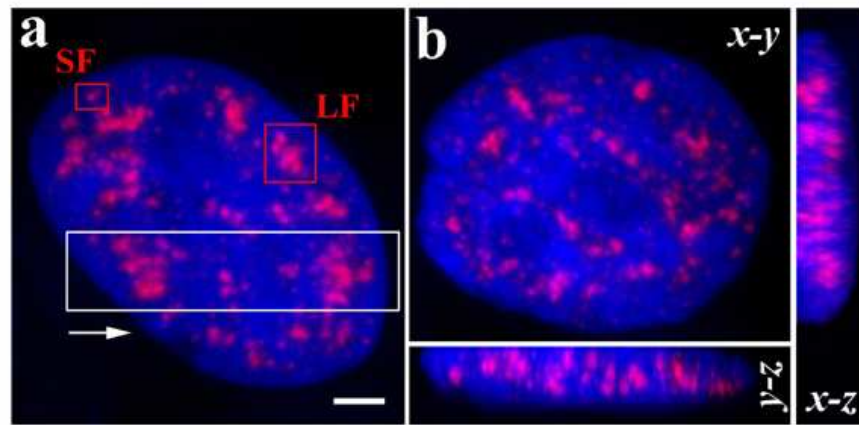


A-type lamins



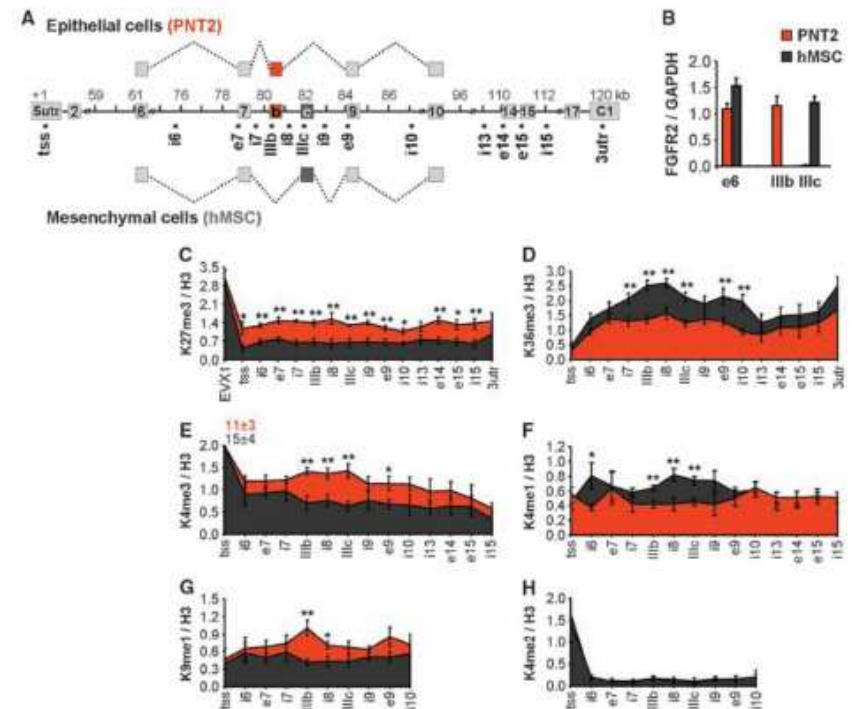
Alternative splicing is a form of epigenetic mechanism that enables a single gene to give rise to multiple, differentially spliced versions of a protein, increasing complexity without a change in the genome.



C**SC35 / DAPI**

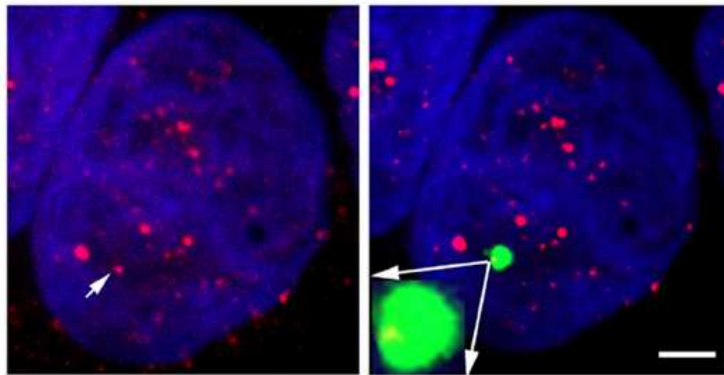
Faktory sestřihu jsou v SC-35 doménách, dále snRNP U1-U6 jsou součástí faktoru sestřihu SF2/ASF

Bartova group

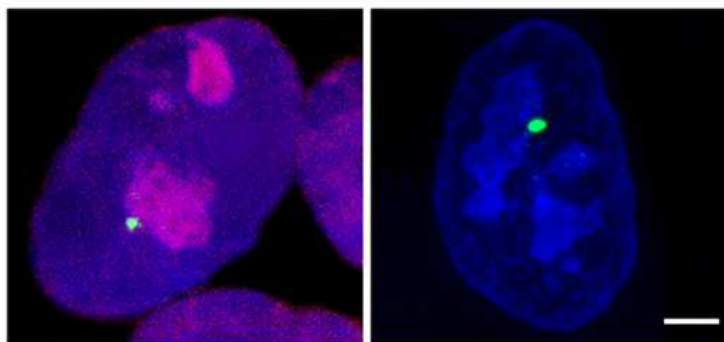


Luco et al., Science (2010)

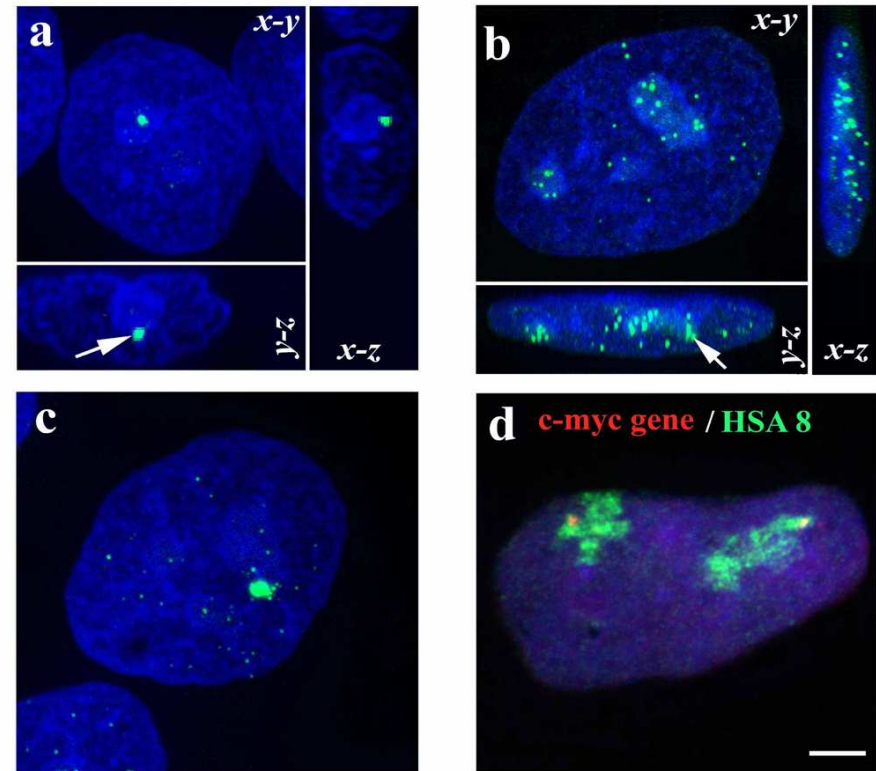
A RNAP II / c-myc^T



C Nucleoli / c-myc^T



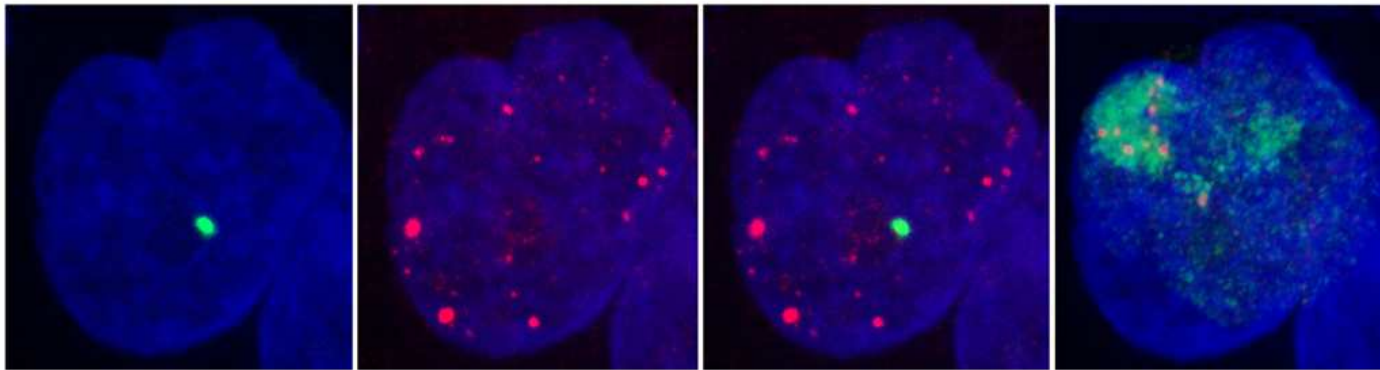
A c-myc^T / DNA



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

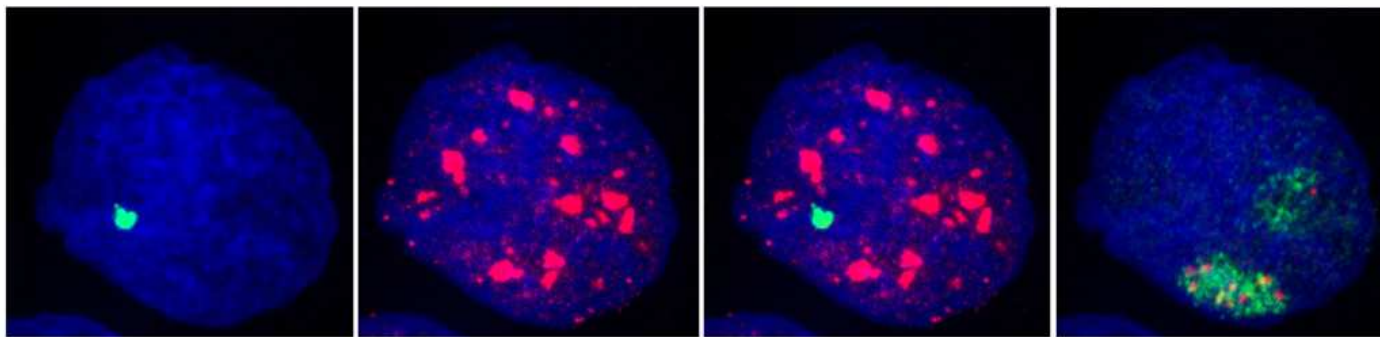
RNAP II / c-myc^T

c-myc gene
HSA8

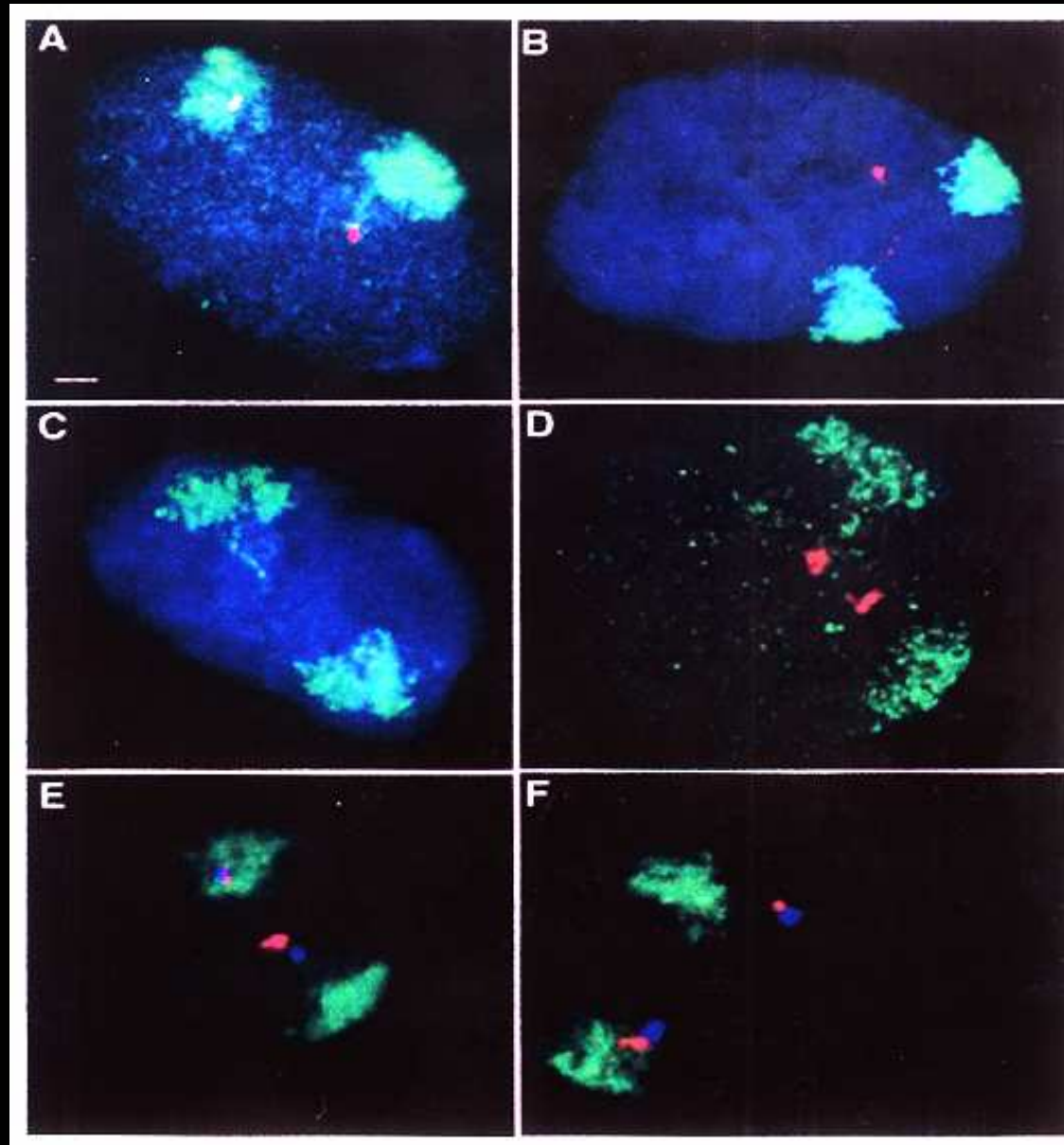


SC35 / c-myc^T

c-myc gene
HSA8

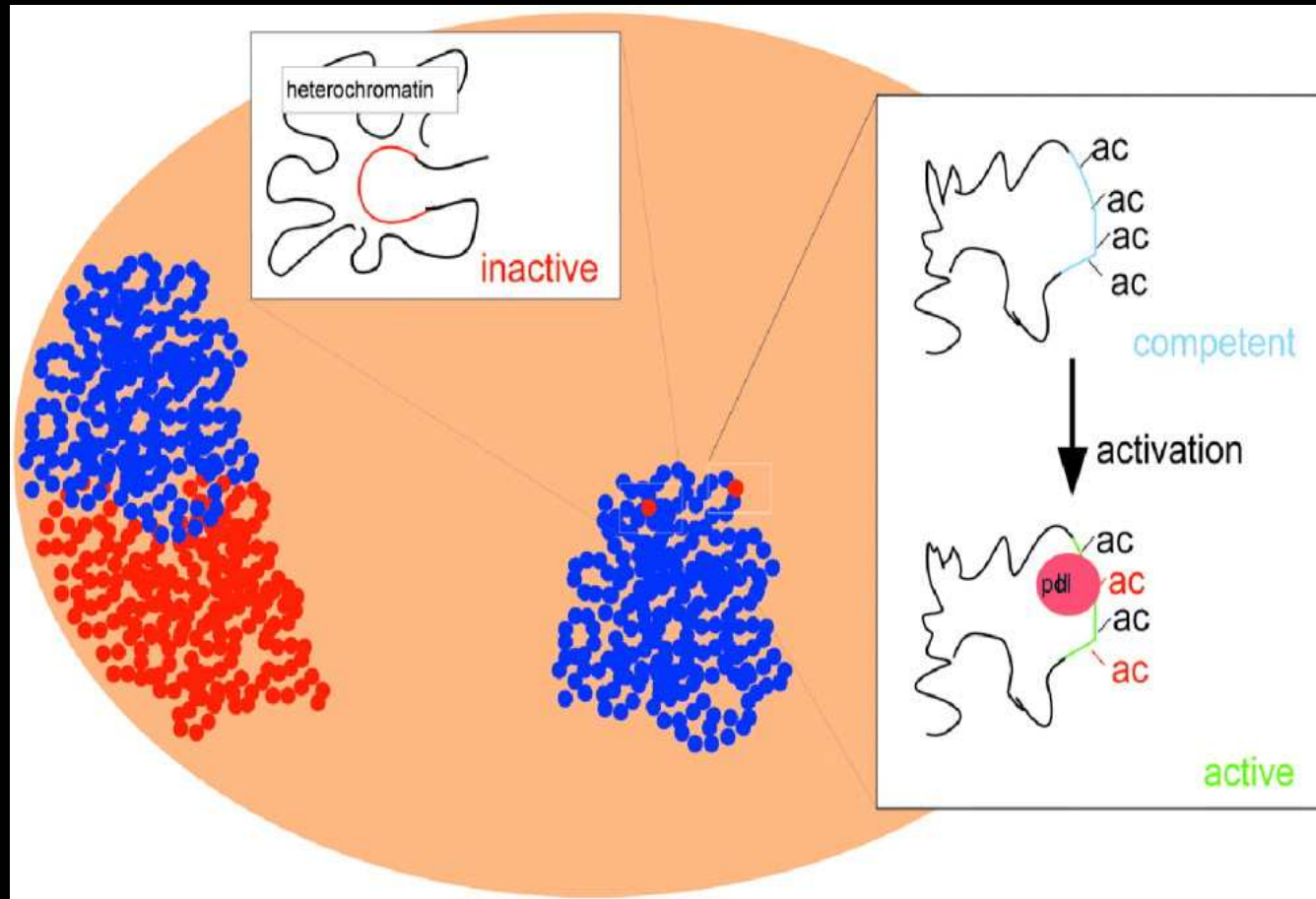


MHC on HSA 6



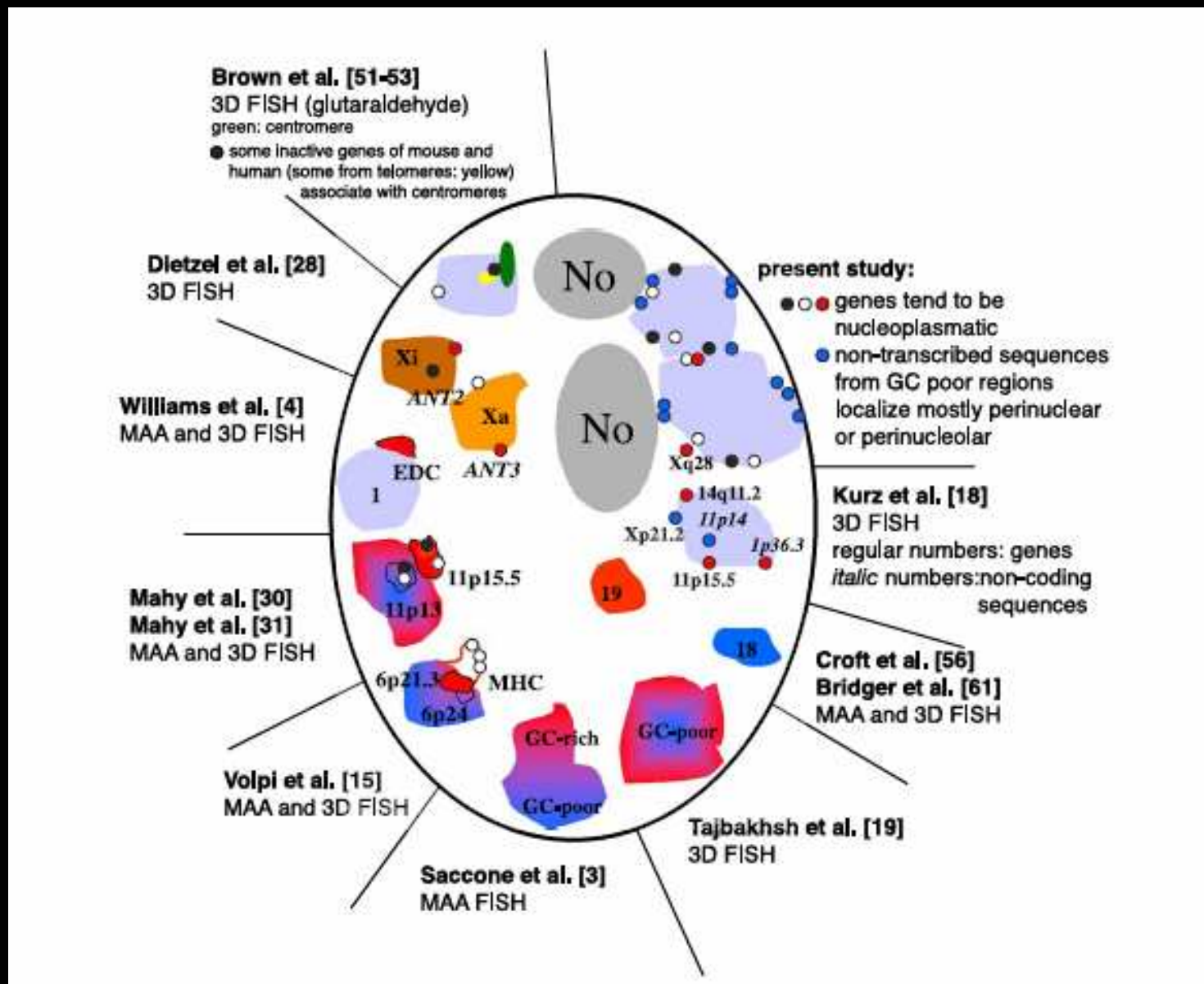
Volpi et al., 2000

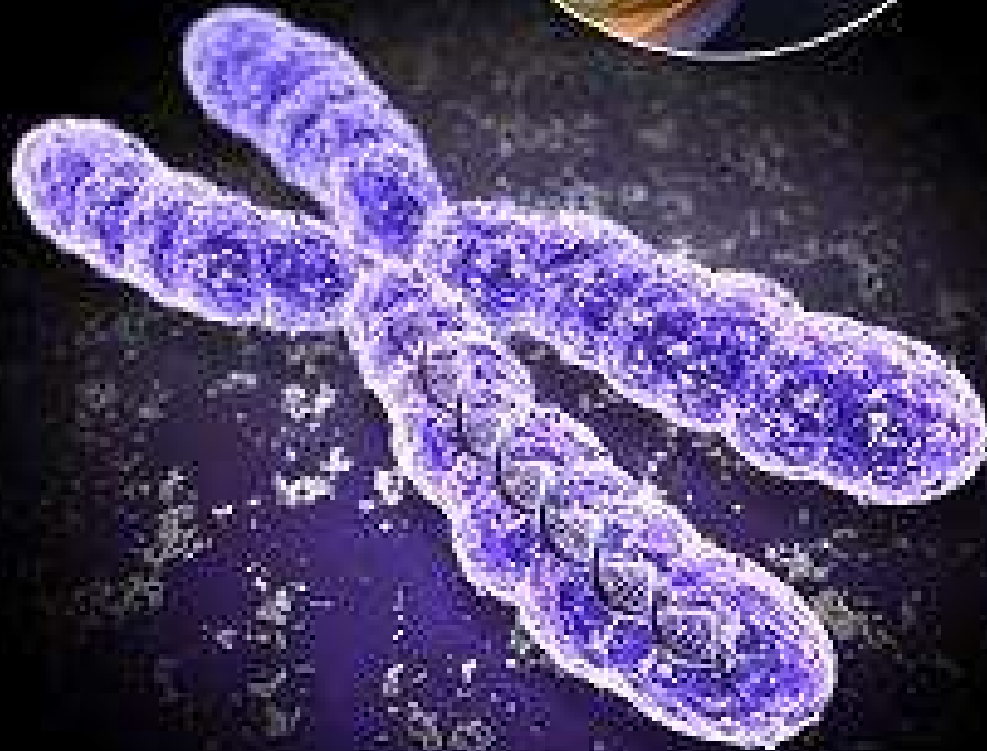
Active / inactive chromatin



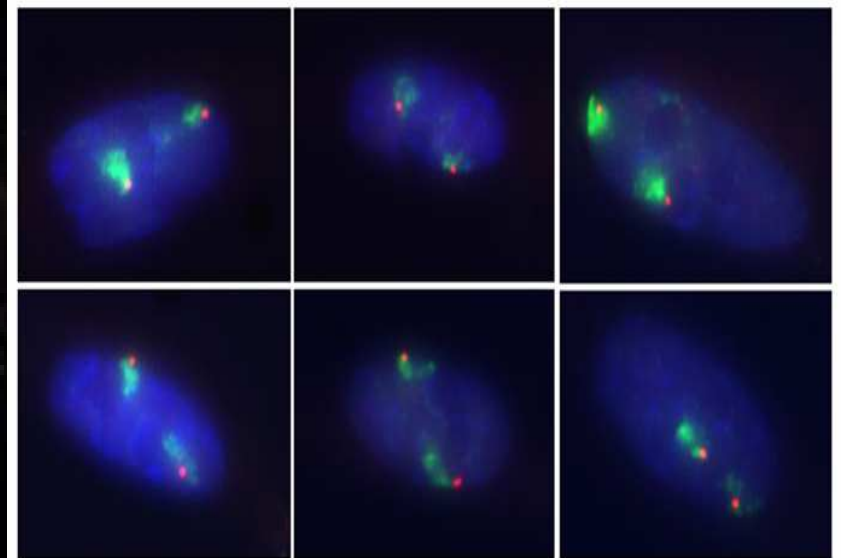
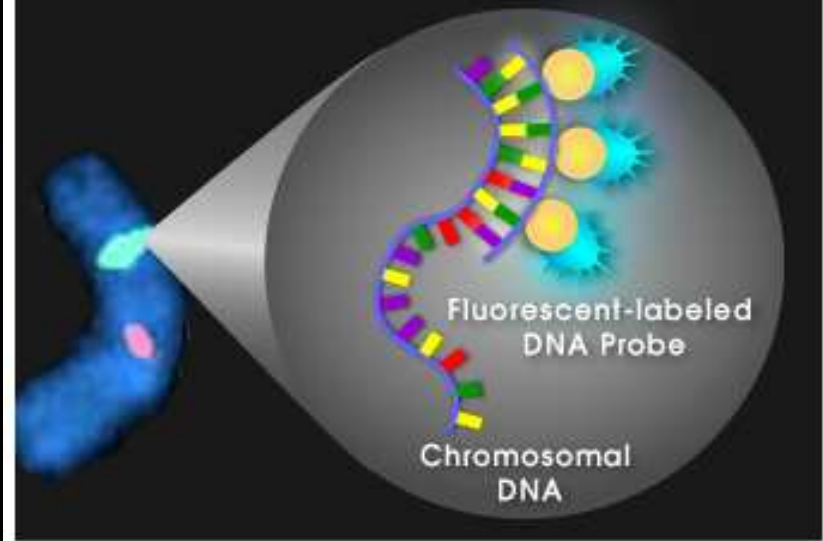
T. Cremer group, Munich

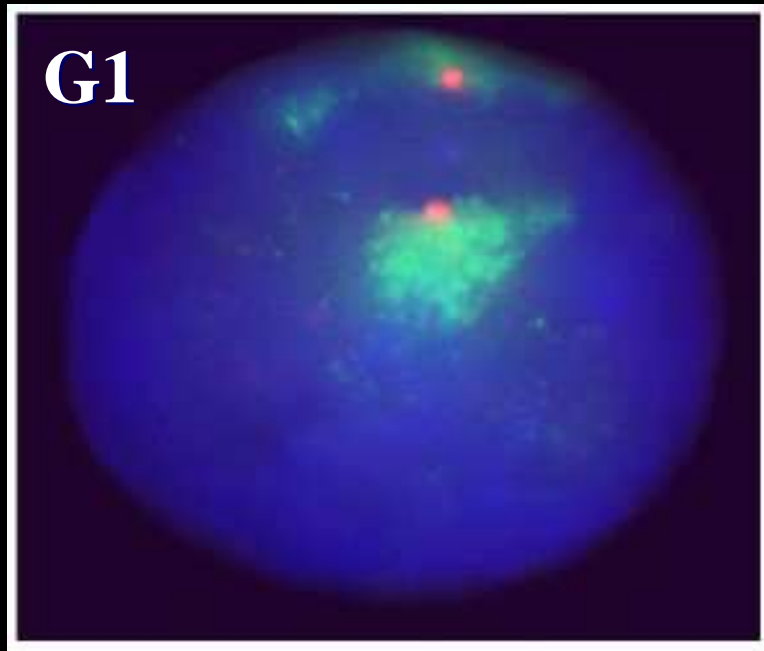
INTERMINGLING



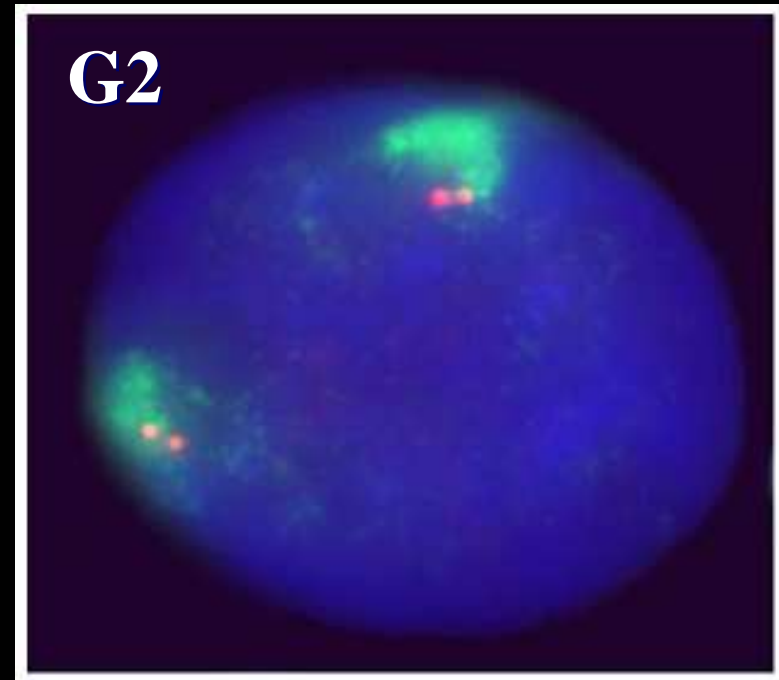


Chromosome prepared using FISH technique



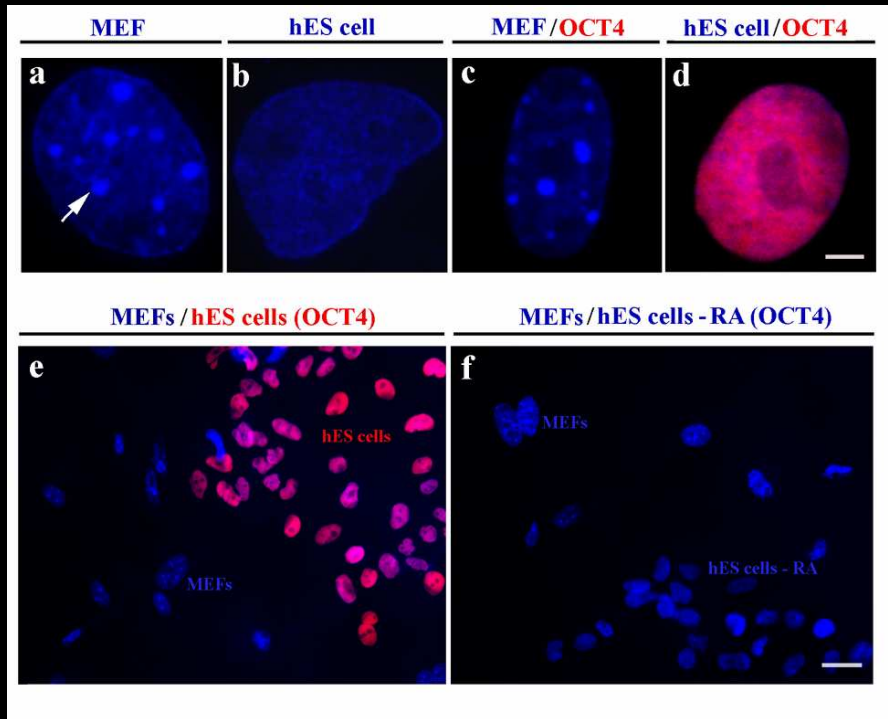


Rb1 gene

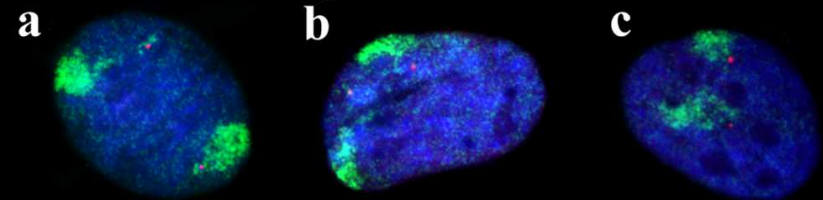


Bártová et al., 2002

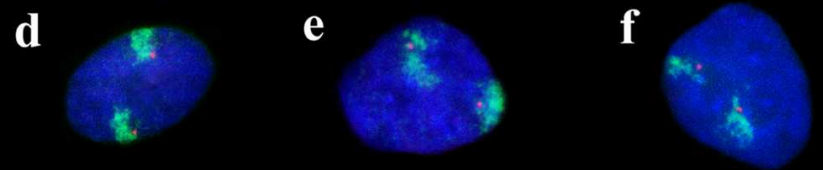
Genes in human embryonic stem cells



Oct4 / HSA 6 in hES cells



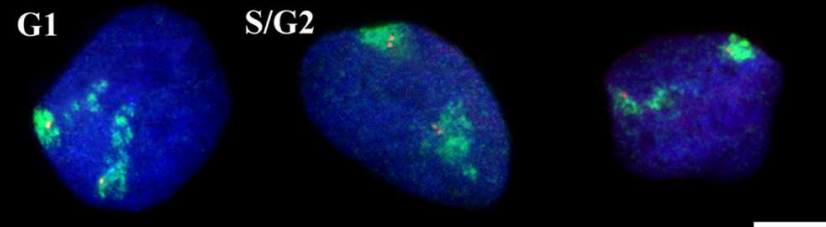
Oct4 / HSA 6 in hES cells - RA differentiated



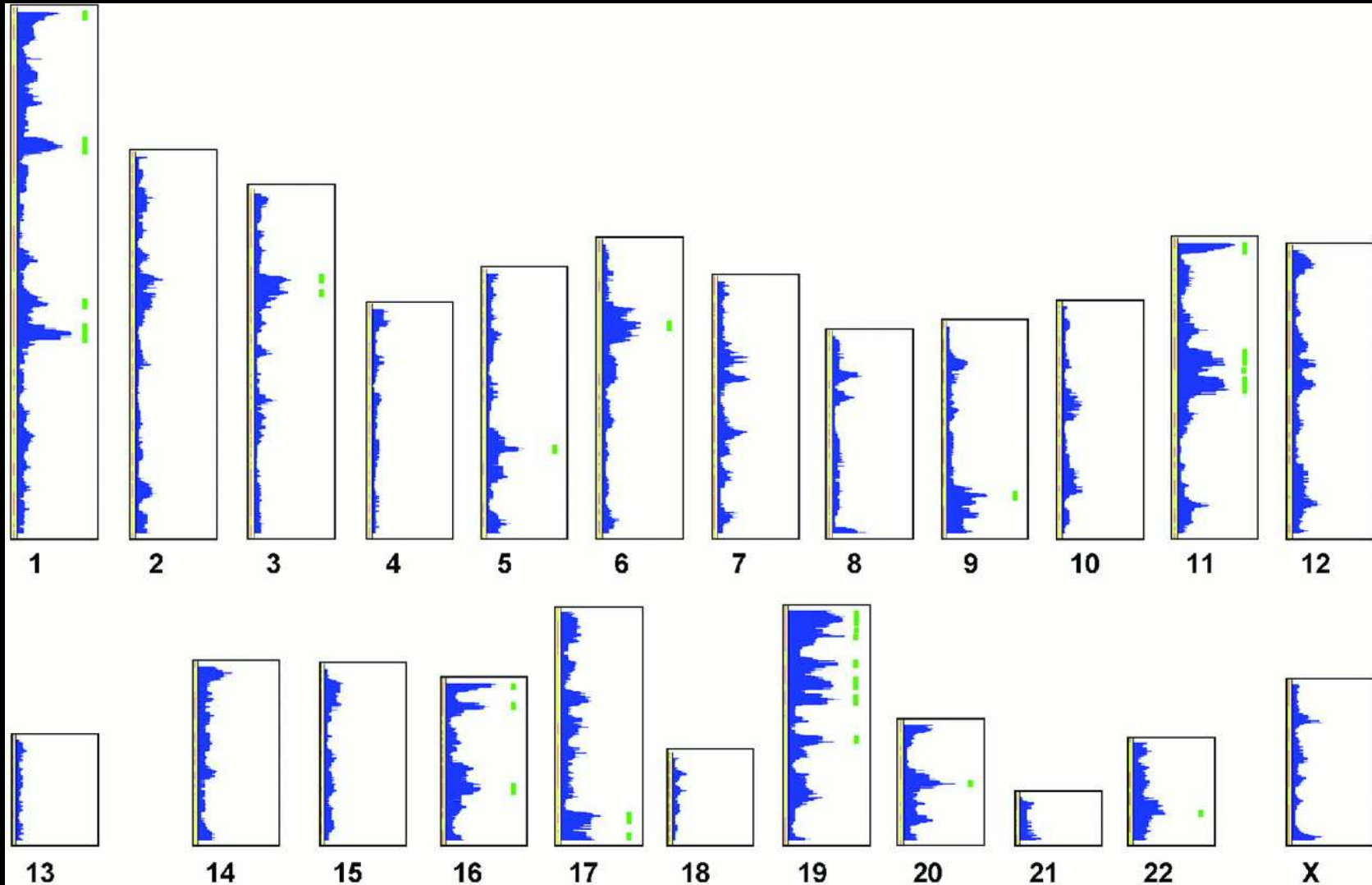
C-myc / HSA 8

in hES cells

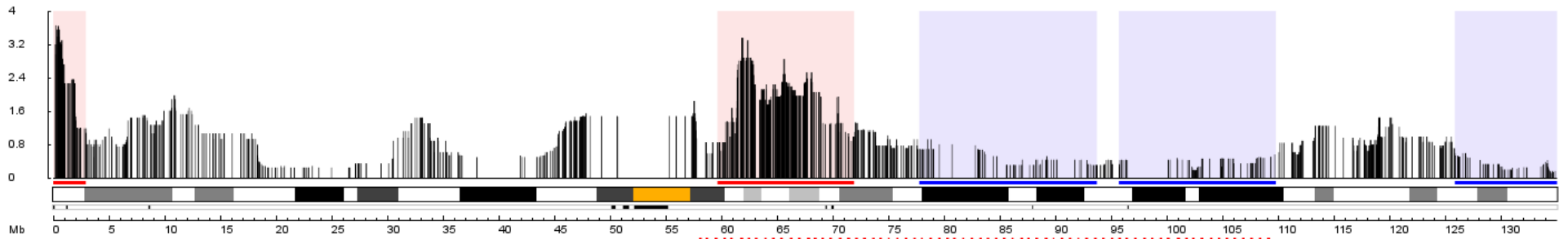
RA differentiated



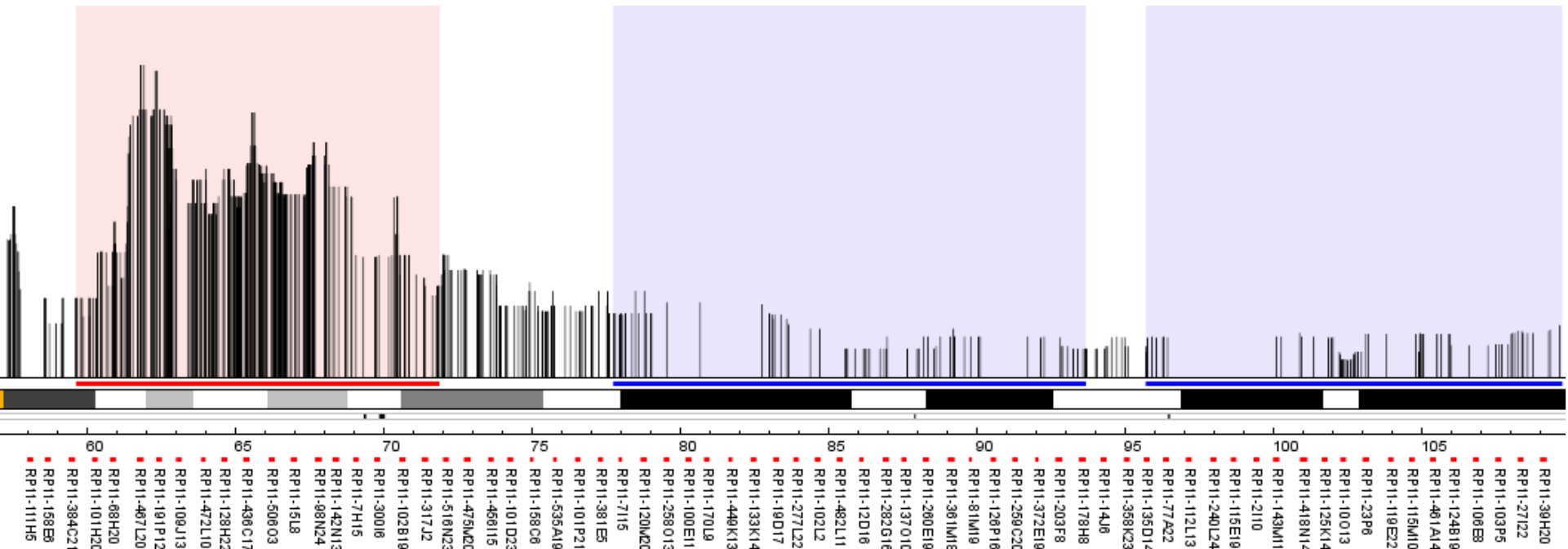
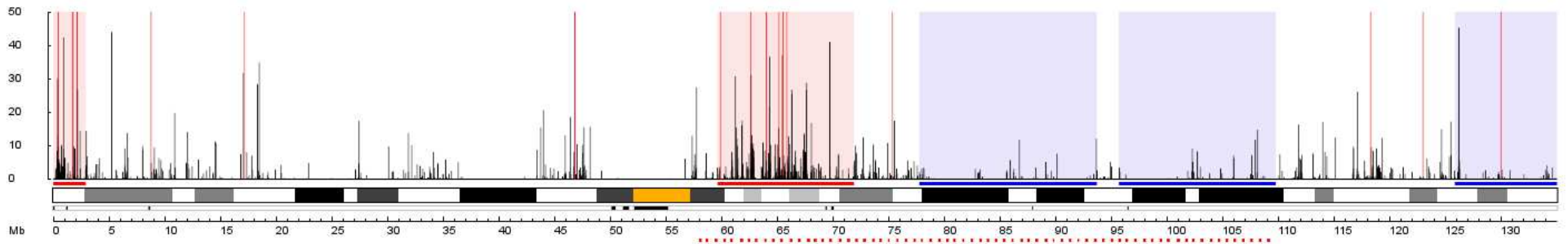
Transcriptome map (Caron et al., 2001)



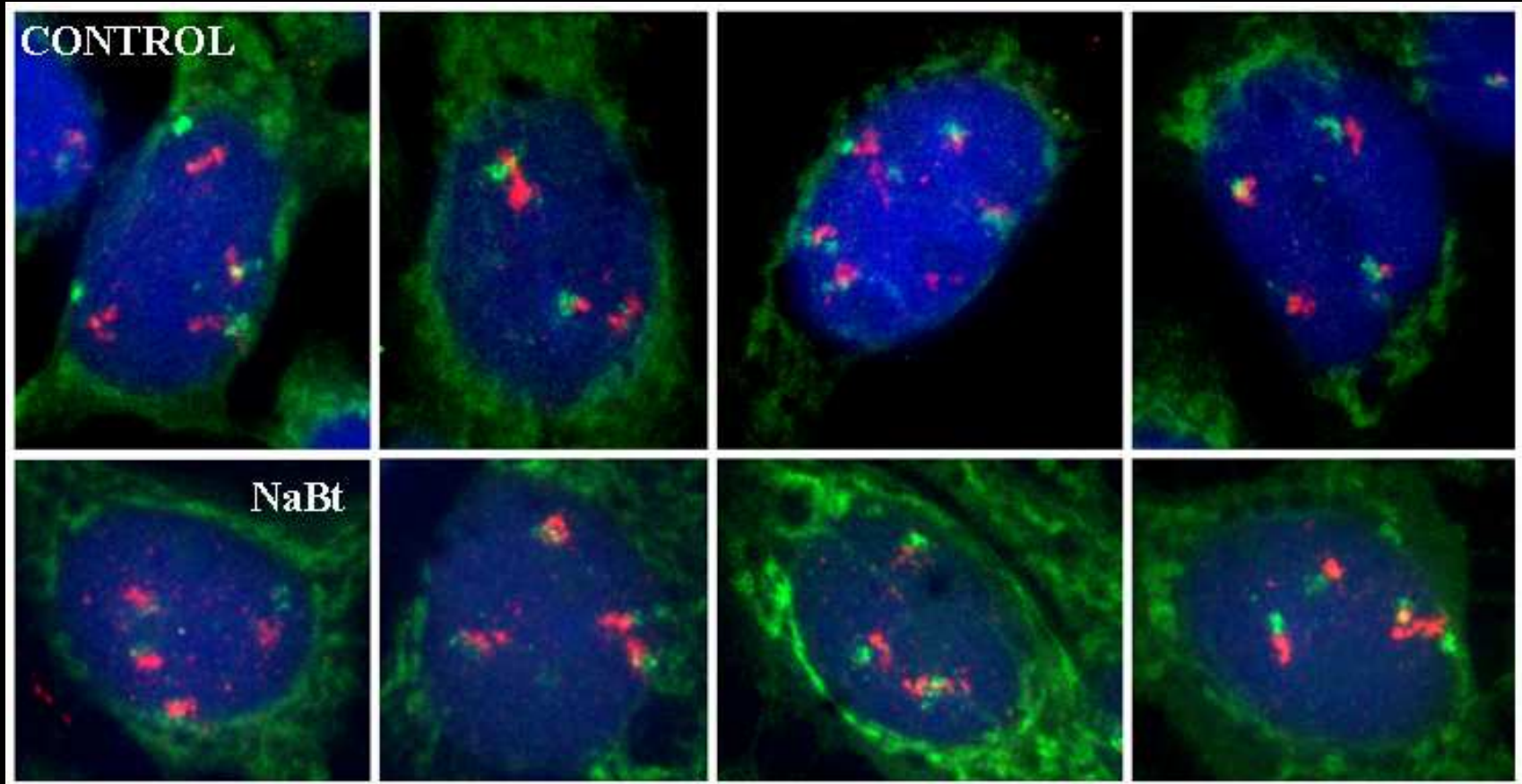
hg15 chr11-norm_htm-hs202libs-mm-49-100000-4-ver0.88



hg15 chr11-norm_htm-hs202libs-100000-50-ver0.88



Enterocytic cell differentiation and RIDGE/ANTI-RIDGE



Center of nucleus- to-gene distances

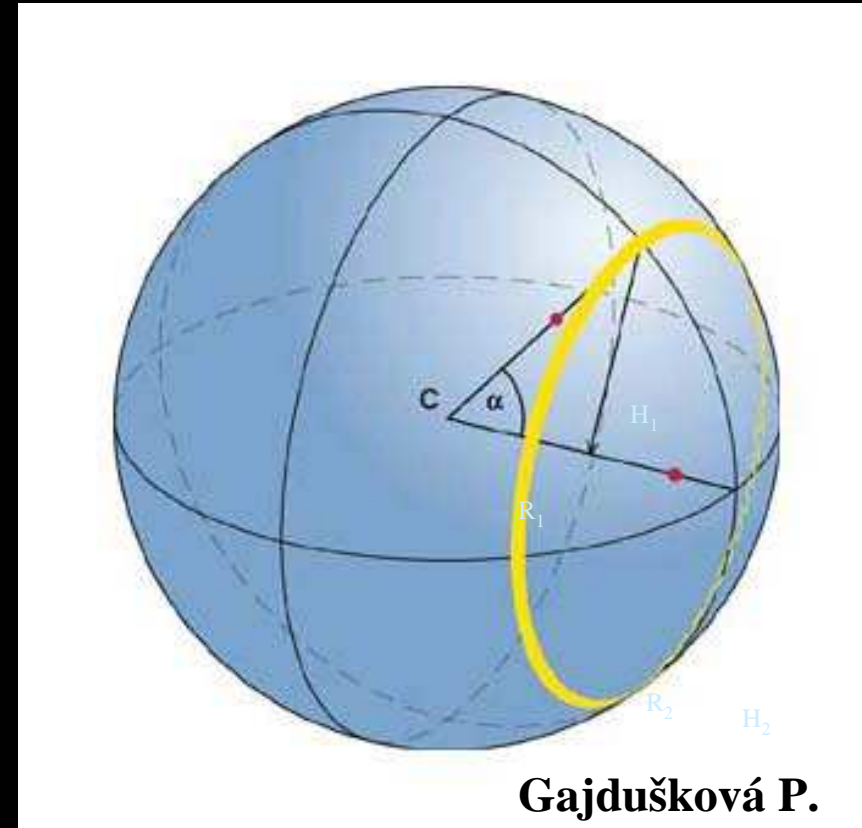
$$CS = \frac{\overline{CR_i}}{\overline{CH_i}} * 100$$

Gene-to-gene distances

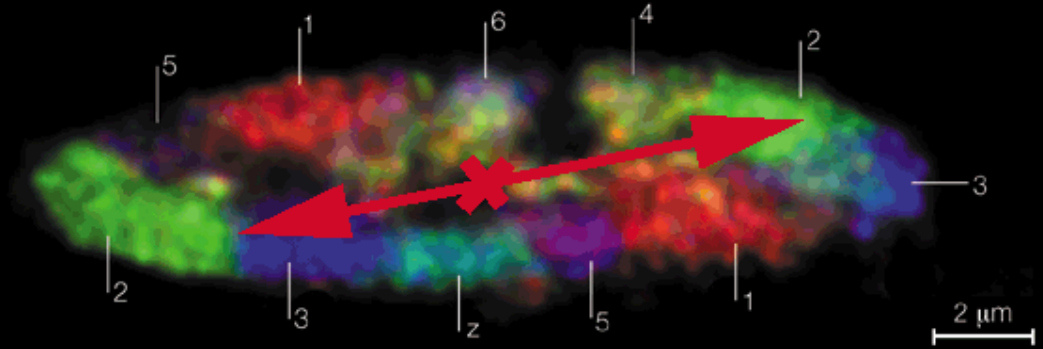
$$SS = \frac{\overline{R_1 R_2}}{(\overline{CH_1} + \overline{CH_2})/2} * 100$$

Gene-center of nucleus-gene angles

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



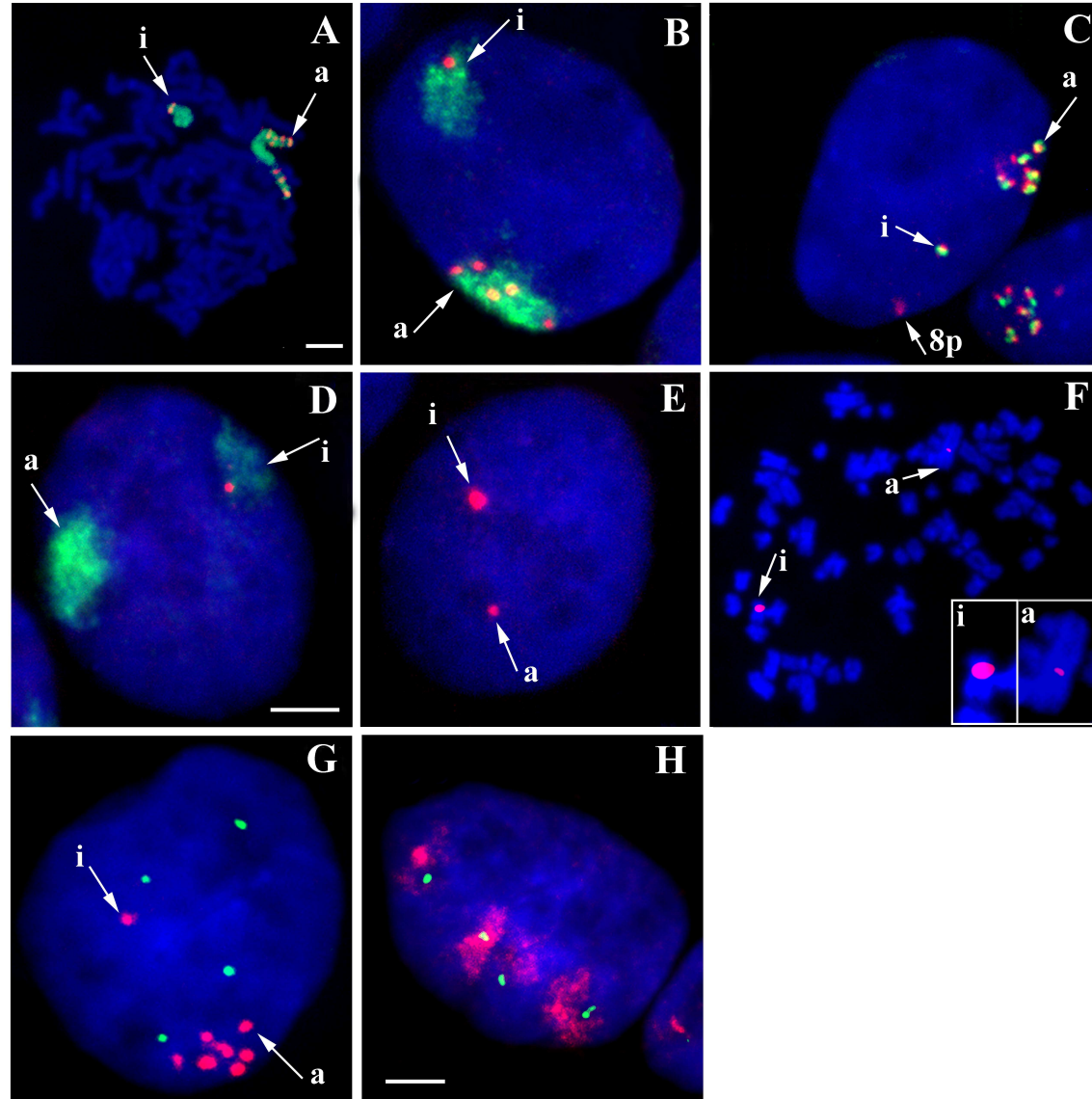
d

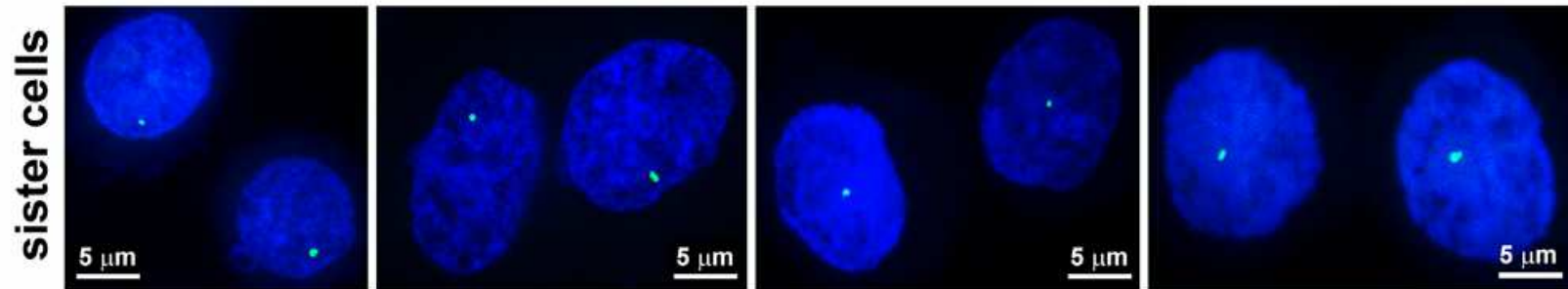
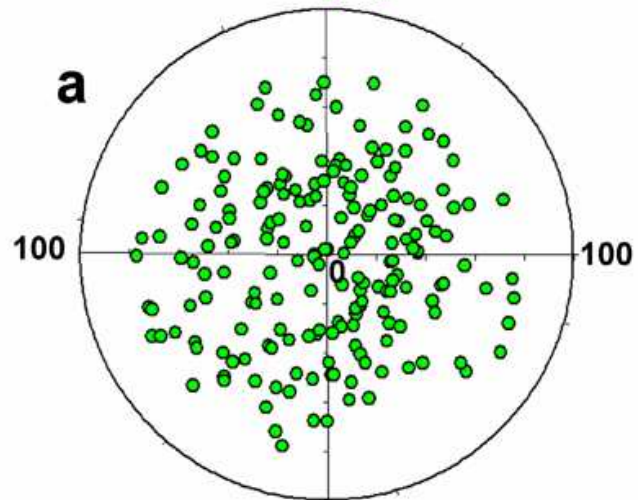
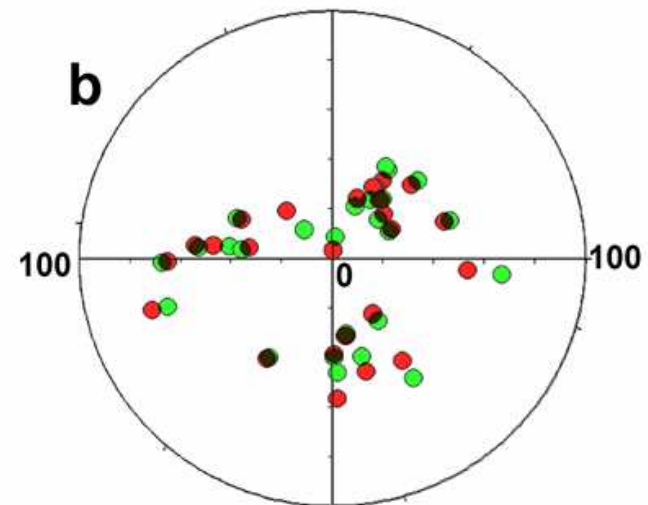


T. Cremer group

HSA 8 and related structures

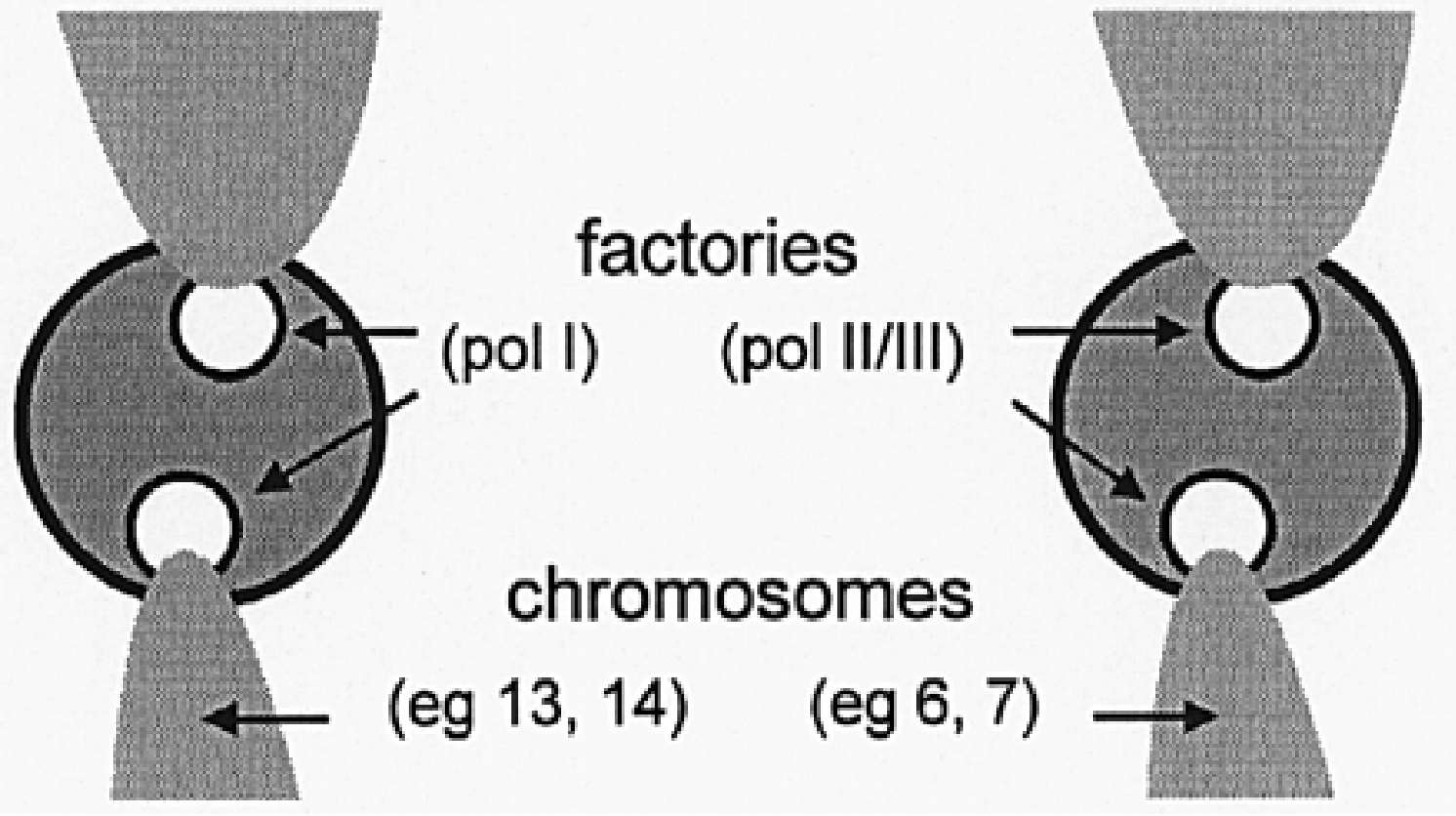
Harničarová et al., 2005



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

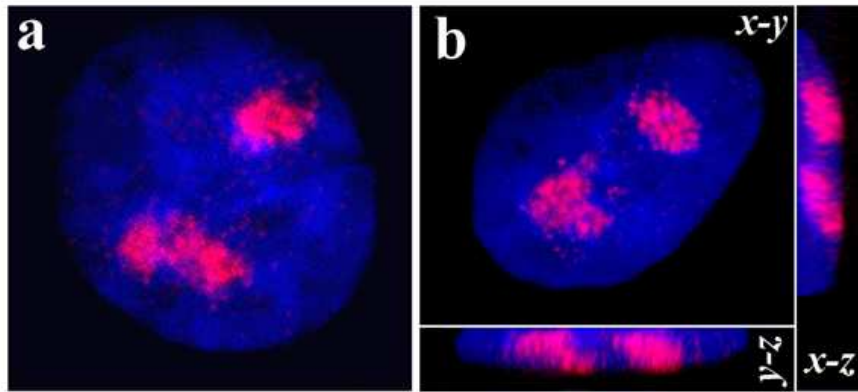
Nucleolus

OPT domain

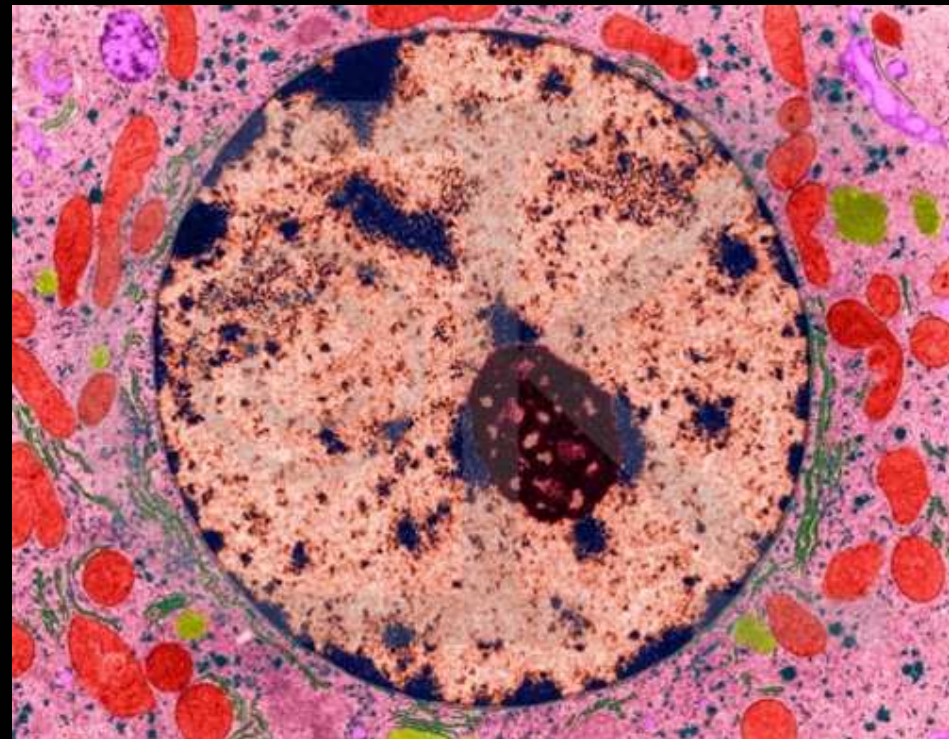


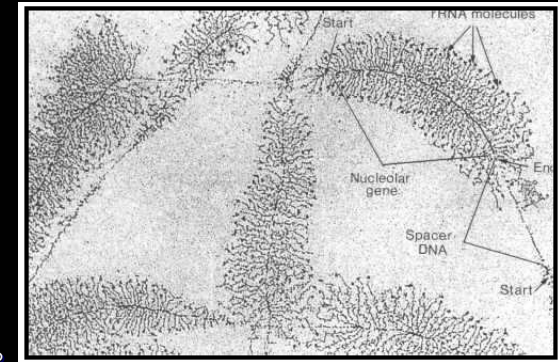
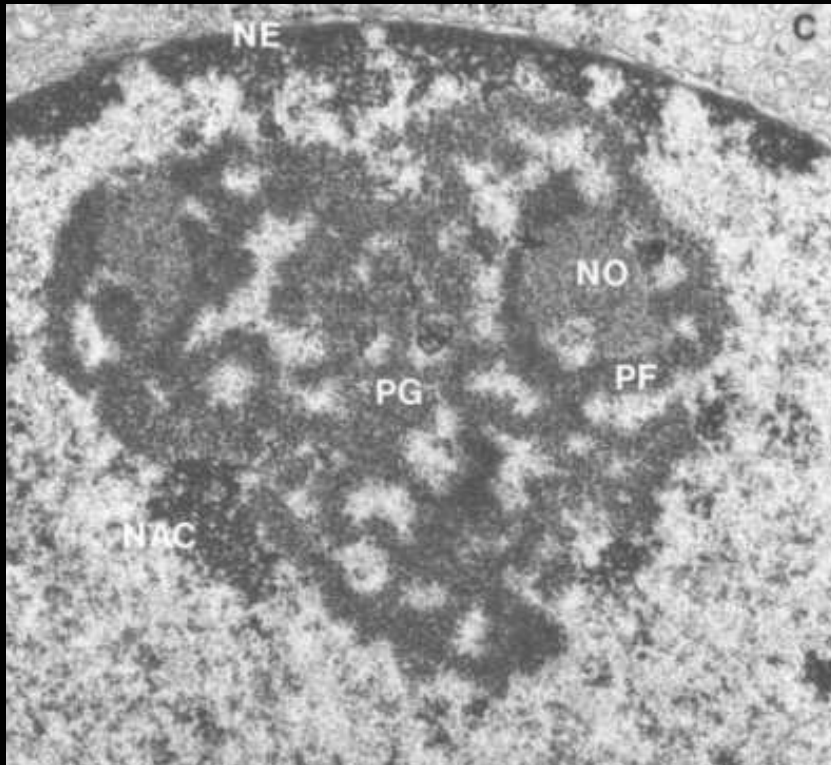
B

Nucleoli / DAPI



Nucleolus





NOR

400 (540) rDNA genů

FIBRILÁRNÍ CENTRUM (FC):

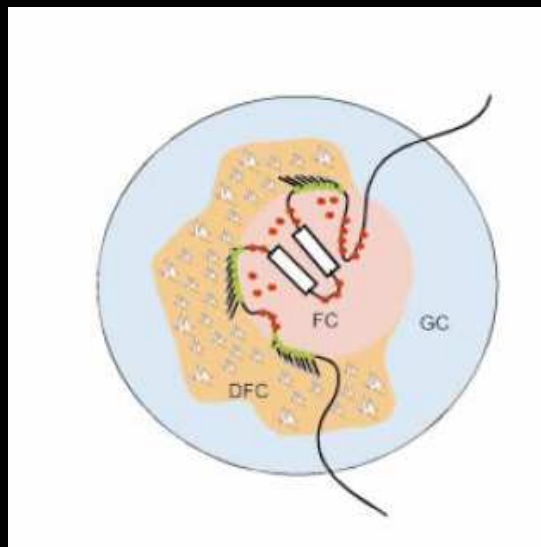
zodpovídá za změny v buněčné aktivitě

DENSE FIBRILAR COMPONENT (DFC):

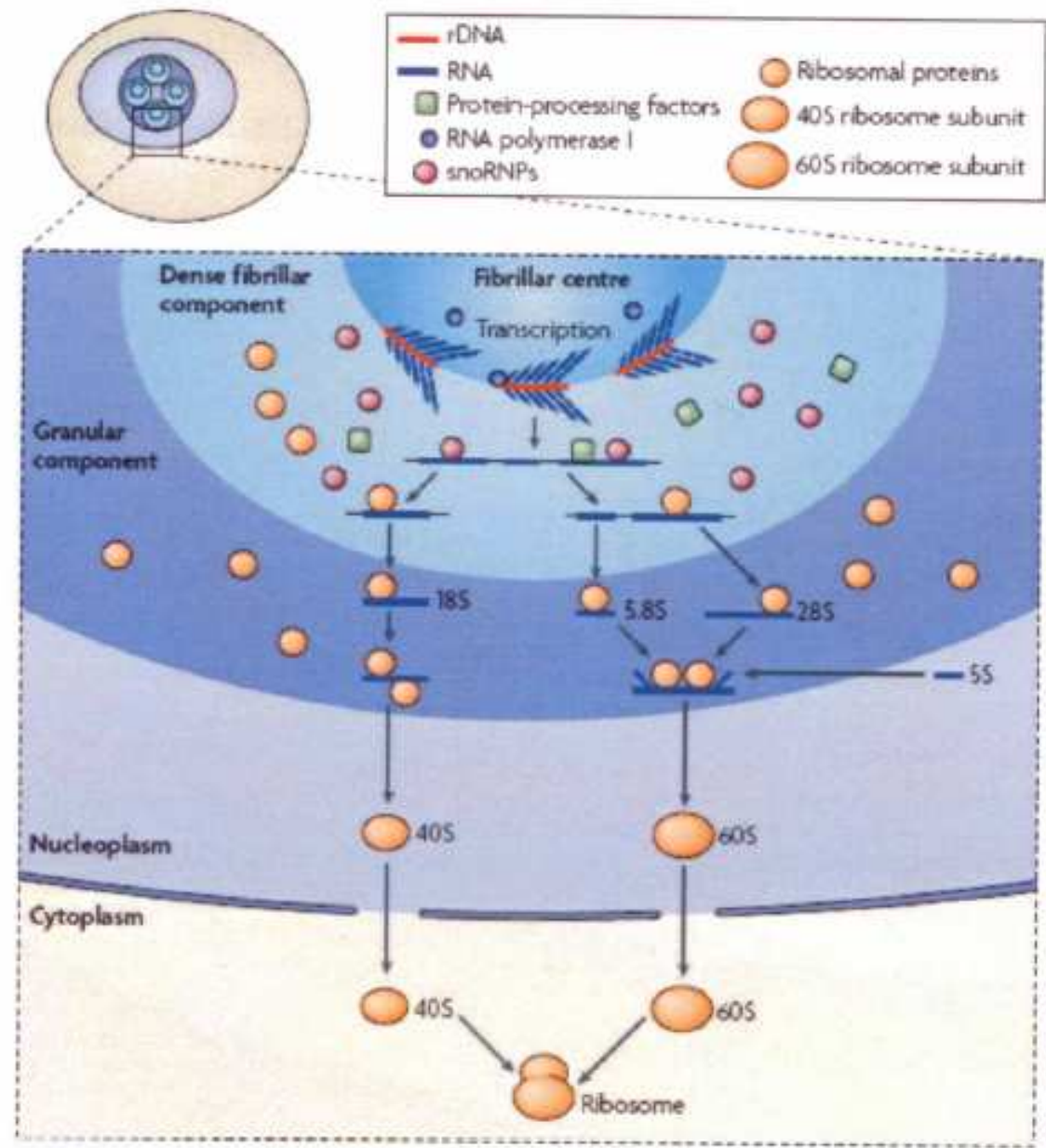
místo syntézy ribozomálních podjednotek

GRANULAR COMPONENTS:

ukotvuje DFC a FC



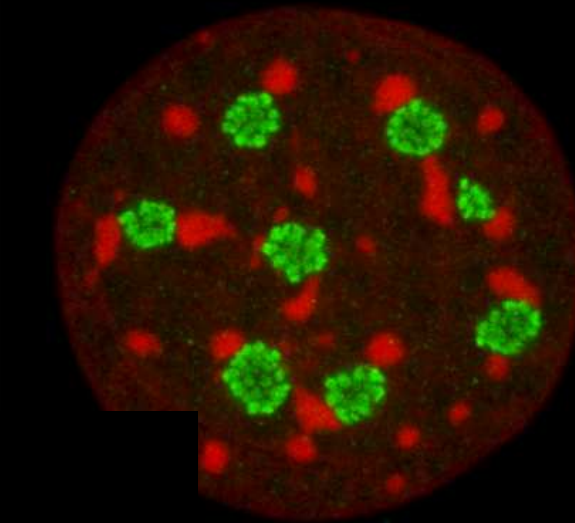
Tvorba ribozomů je komplexní proces zahrnující transkripci 45S prekurzorické rRNA, její vyžrávání, modifikaci a asociaci s ribozomálními proteiny a 5S rRNA, která se syntetizuje mimo jádérko. Vyžrávání rRNA probíhá v procesomu, který obsahuje mnoho komplexů a snRNA



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



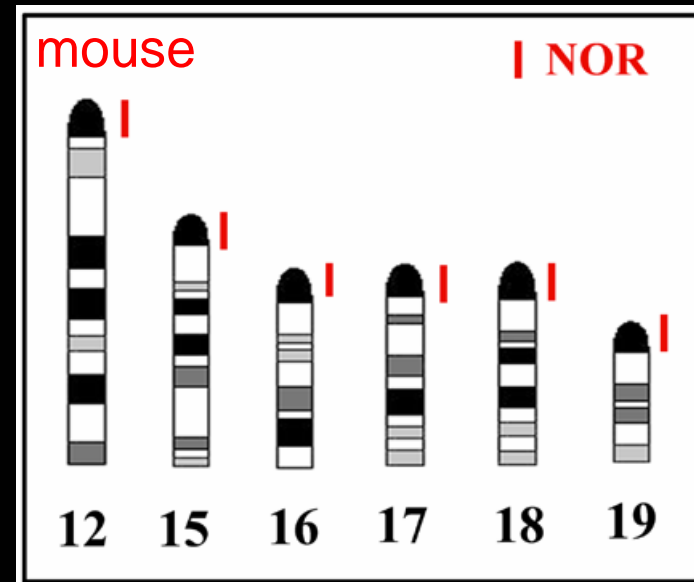
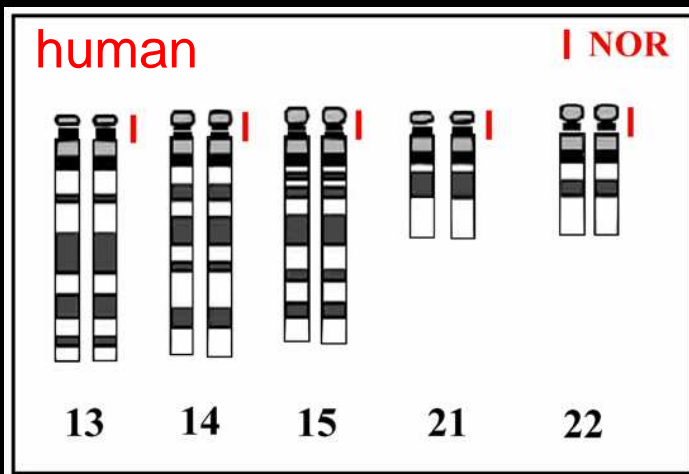
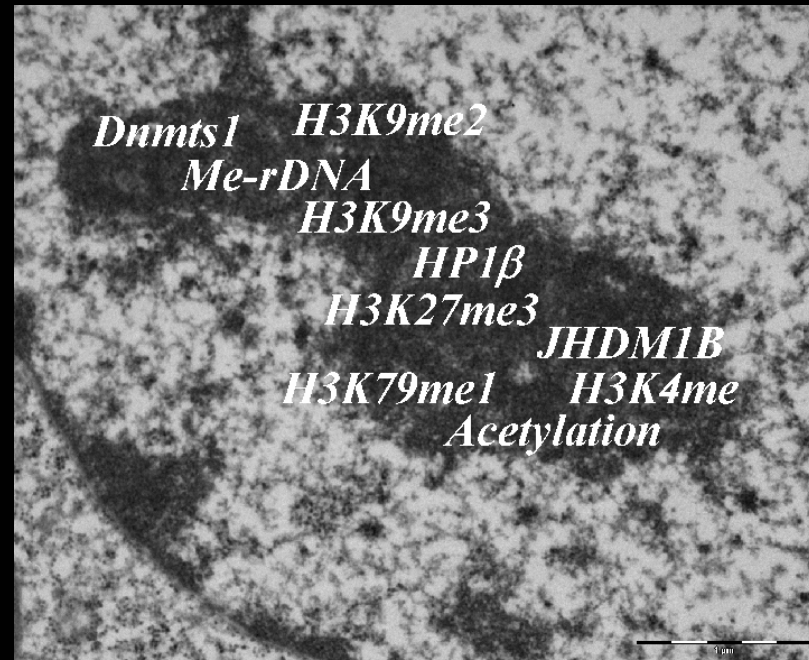
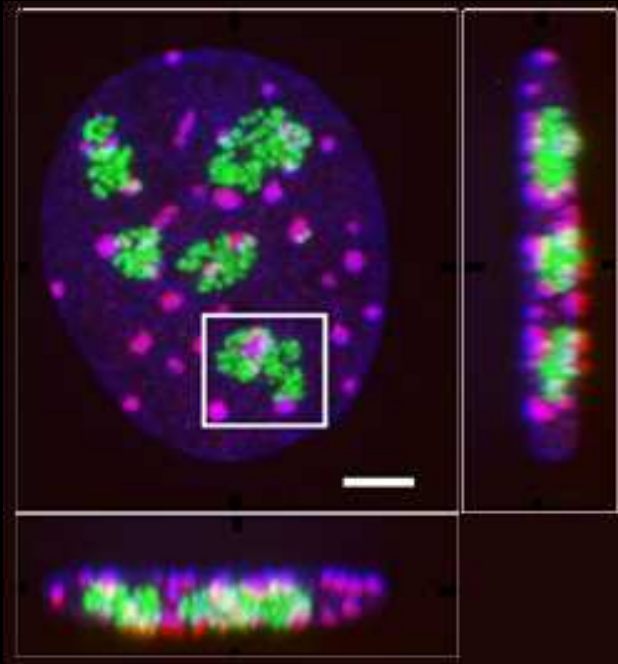
12/8/2010 3:40:48 PM



° pos=1 1 10

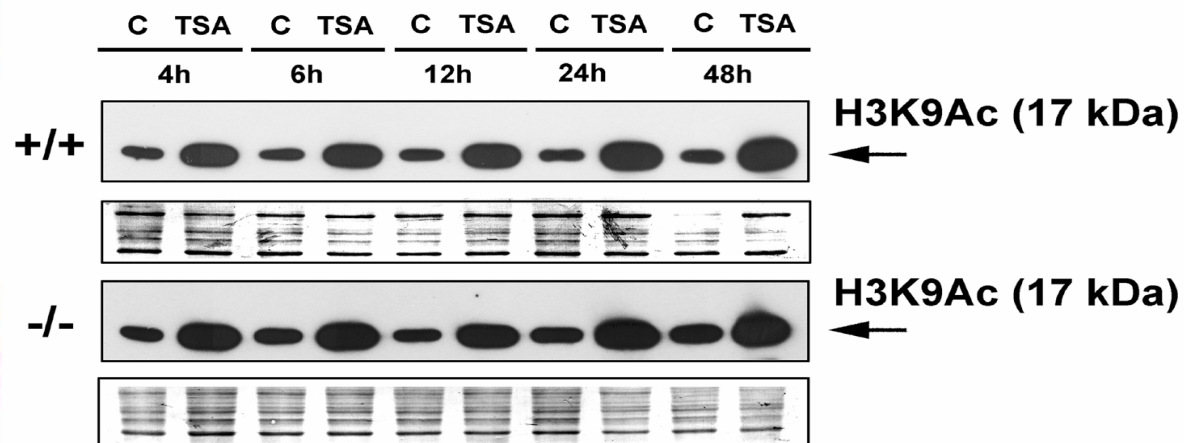
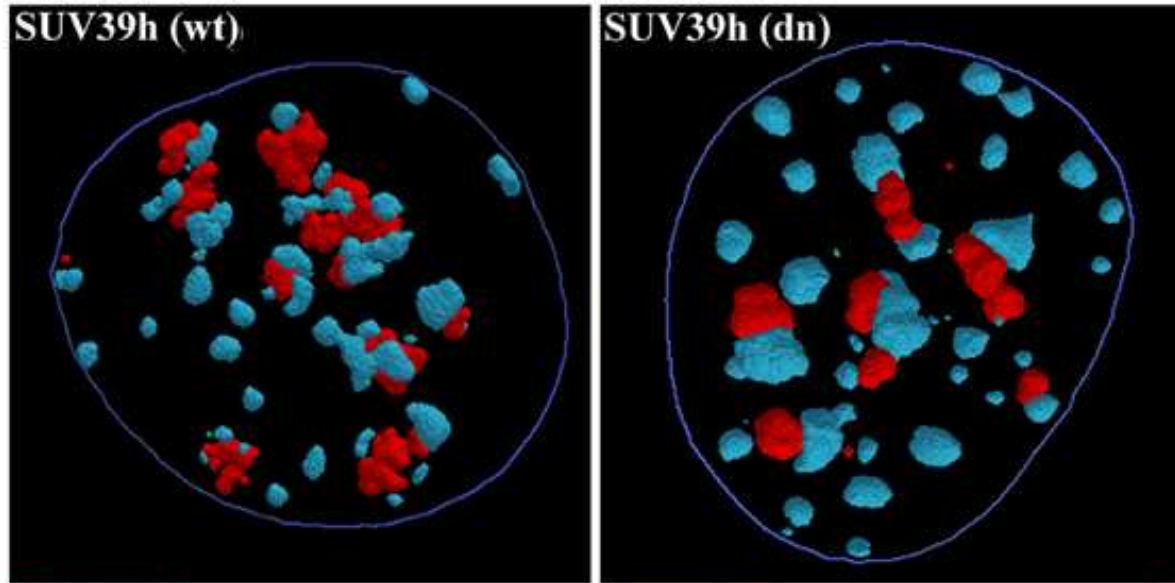
Epigenetics of Nucleoli

Fibrillarin/chromocenters

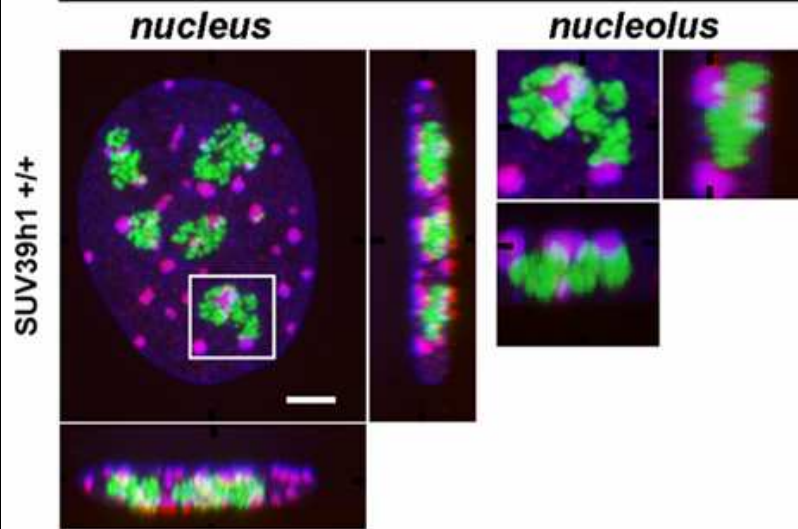


Differences between epigenetics of nucleoli and surrounding chromatin

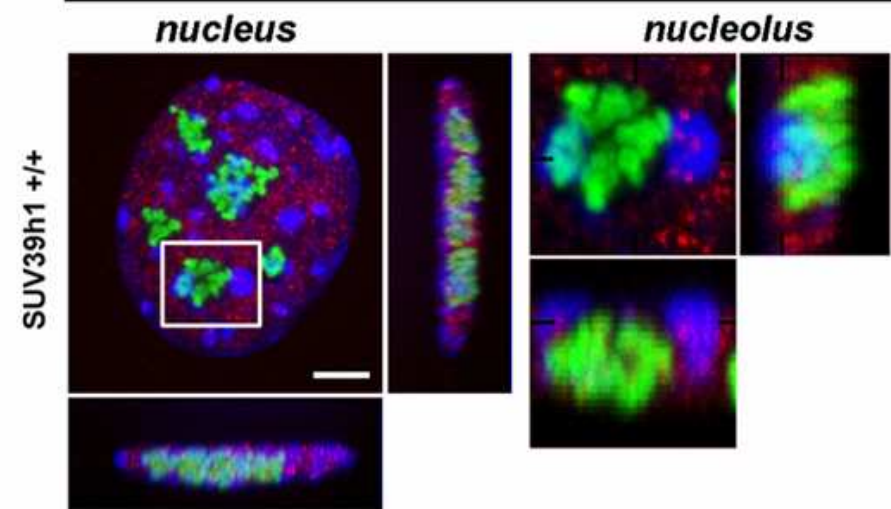
Fibrillarin / Chromocenters



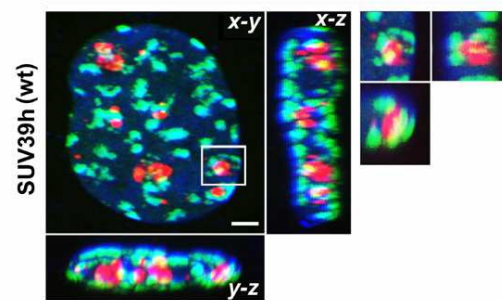
Fibrillarin / HP1 α / DNA



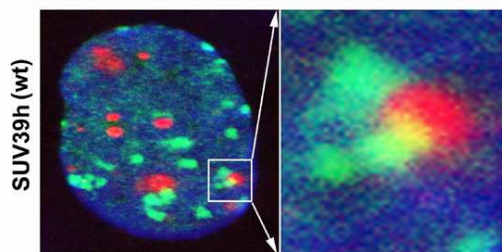
Fibrillarin / HP1 γ / DNA



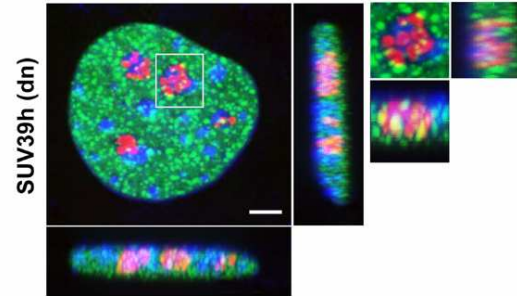
a Fibrillarin / GFP-HP1 β



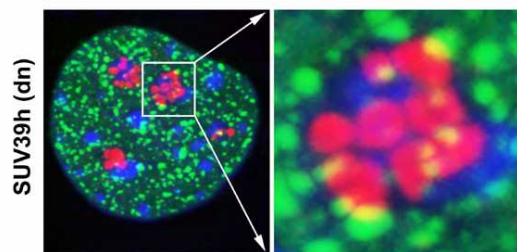
individual confocal section



b Fibrillarin / GFP-HP1 β

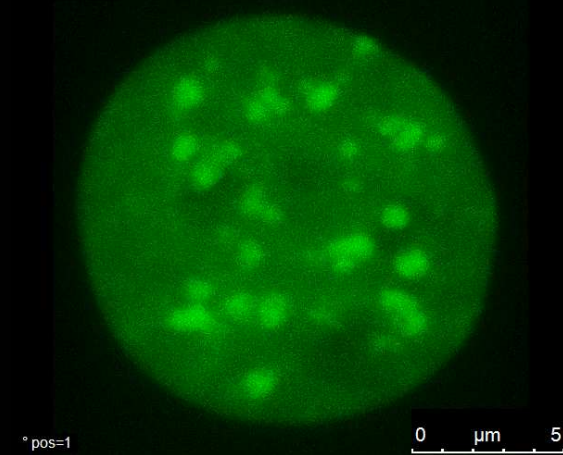


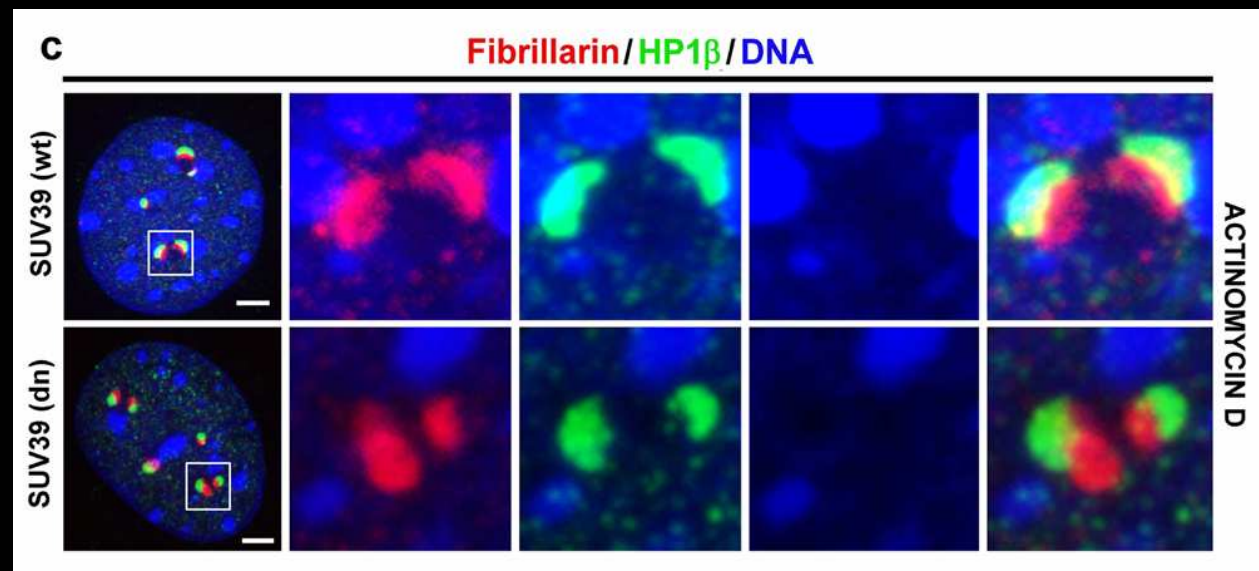
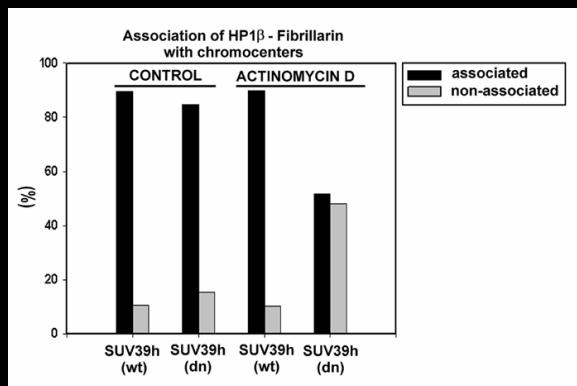
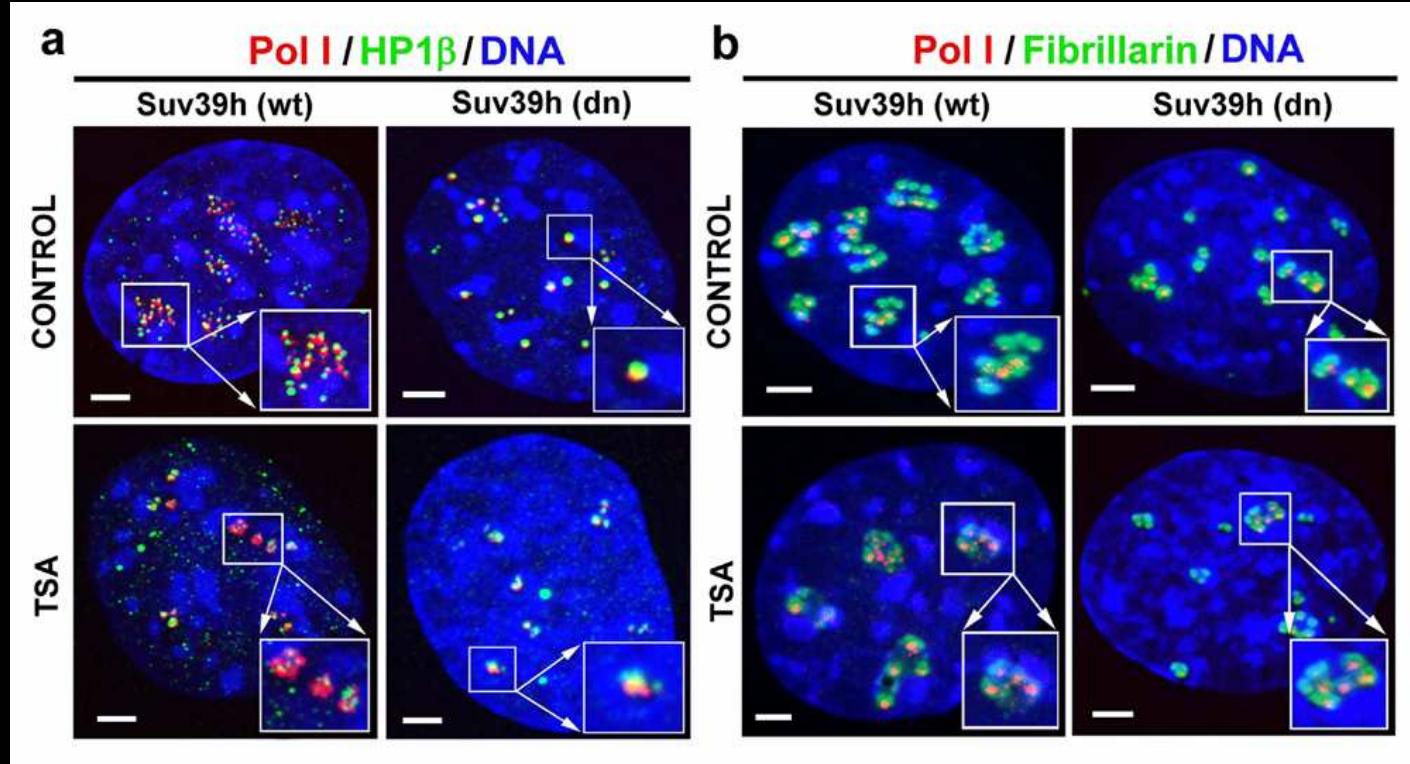
individual confocal section



HP1 β

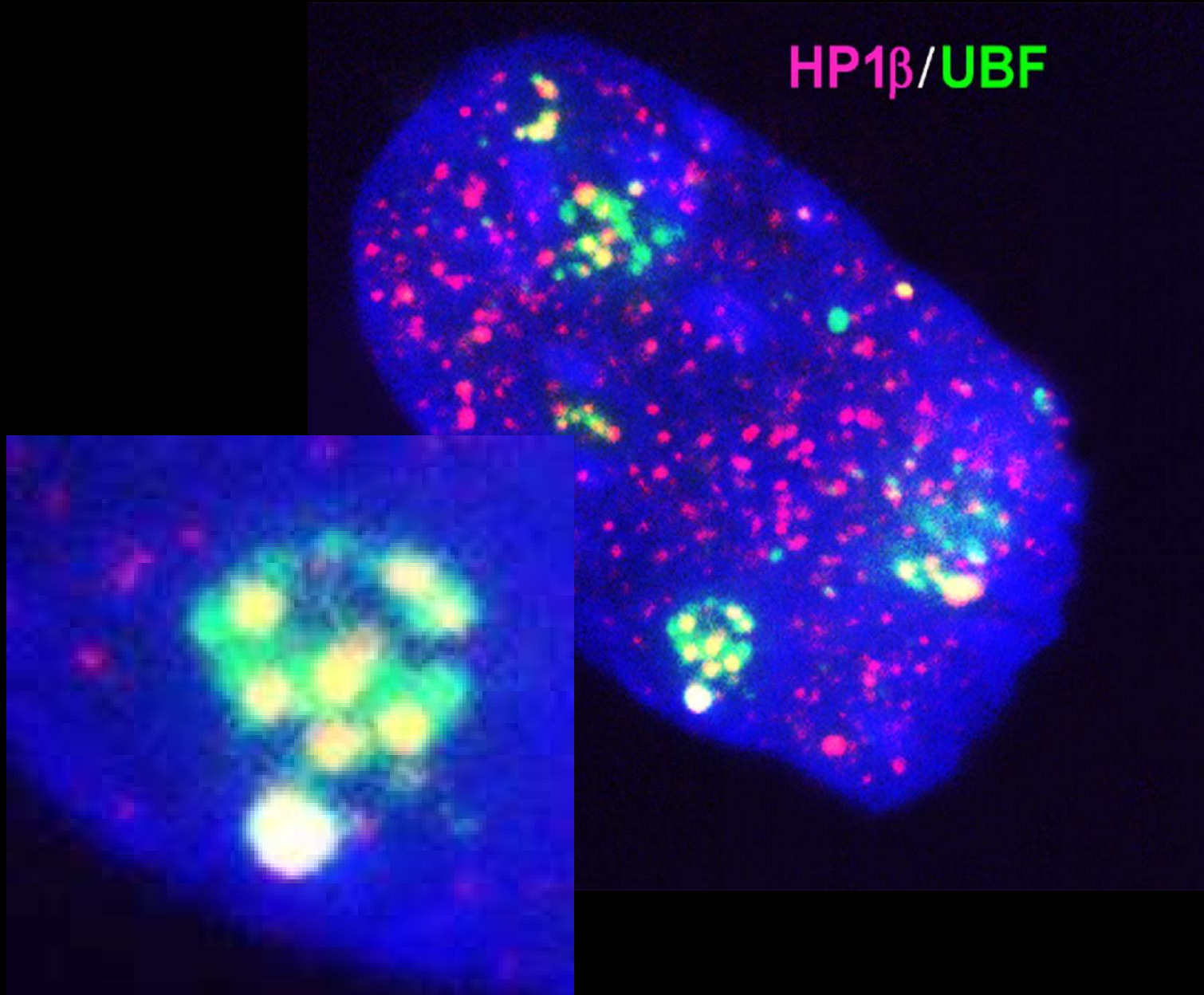
8/18/2010 3:10:34 PM





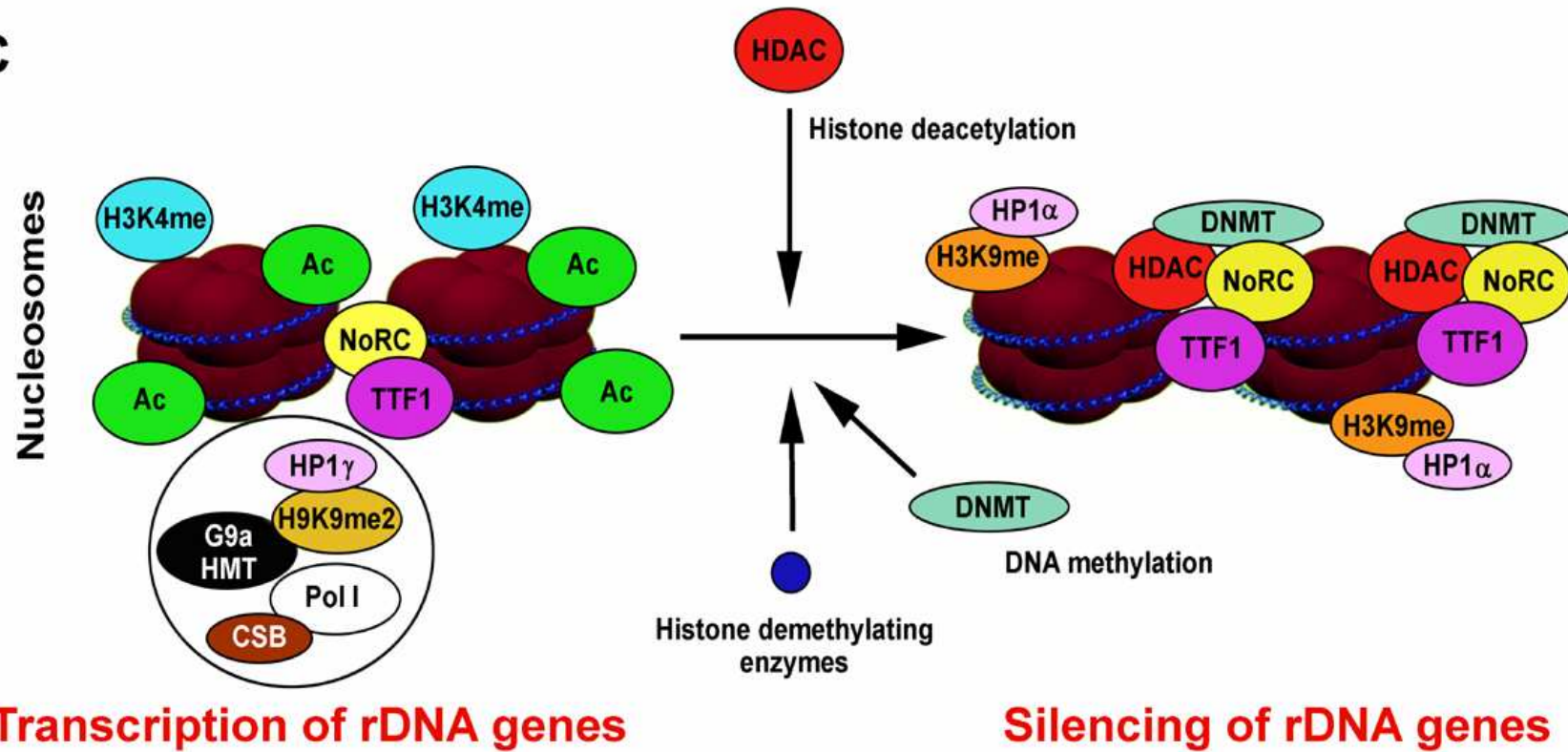
MEF cells

HP1 β /UBF



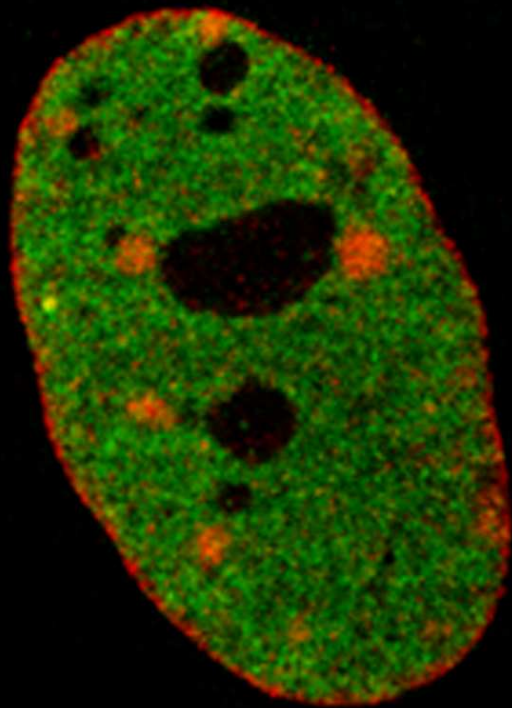
Epigenetics of nucleoli

C



Yuan et al. (2007)

Bártová et al., JHC (2009)



ZÁVĚR

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