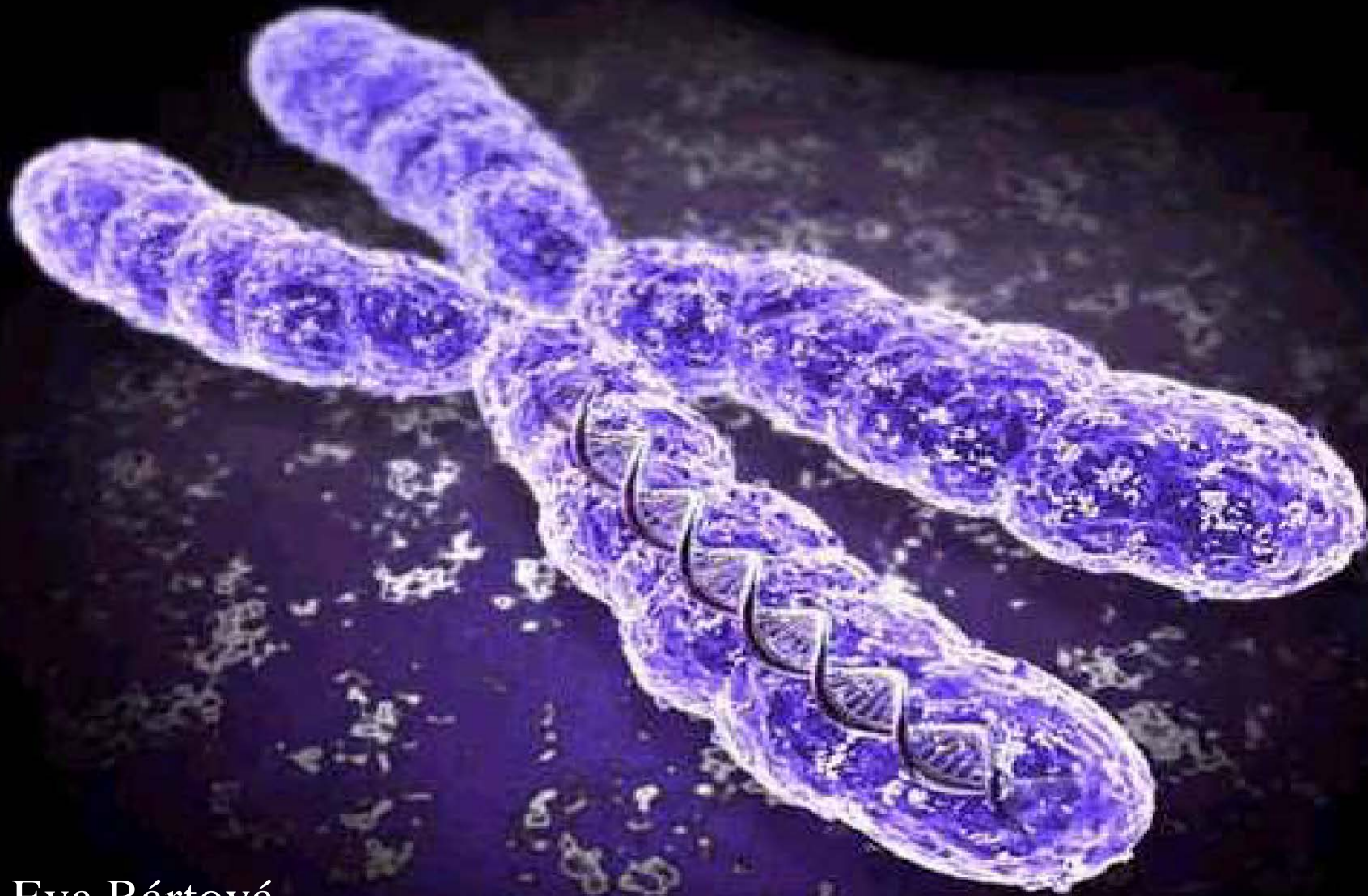


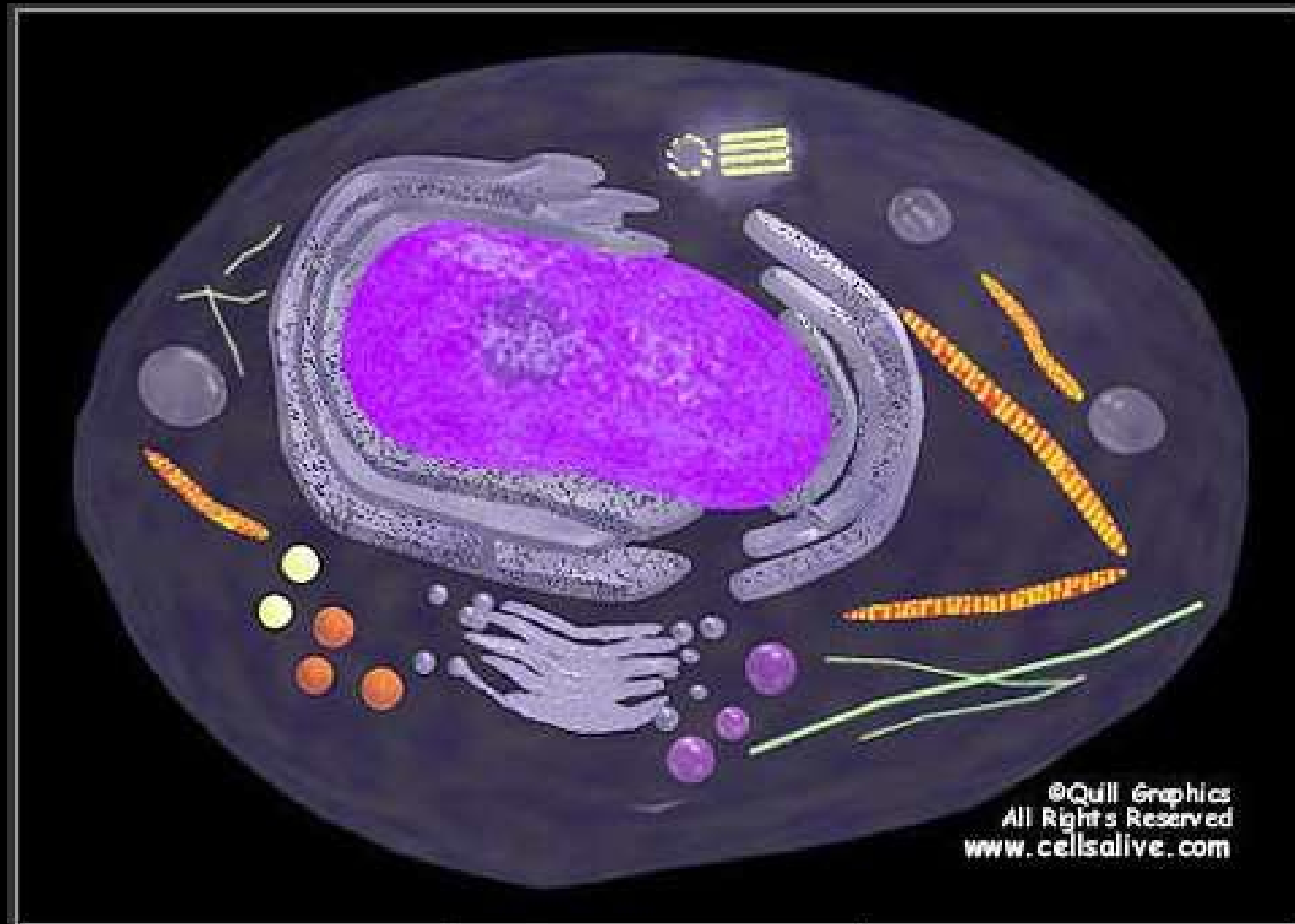
Struktura chromatinu vyššího řádu



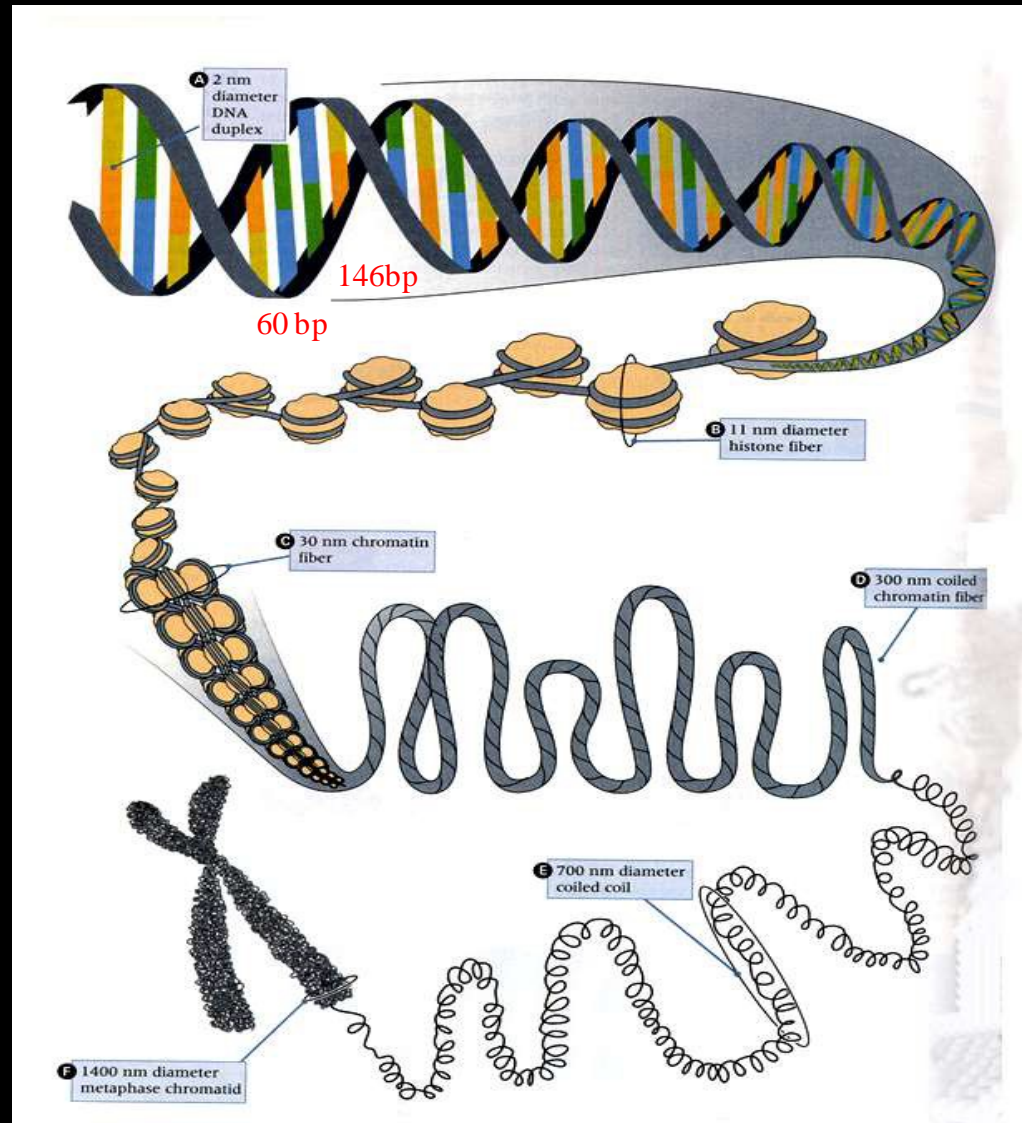
Eva Bártová

www.tqnyc.org

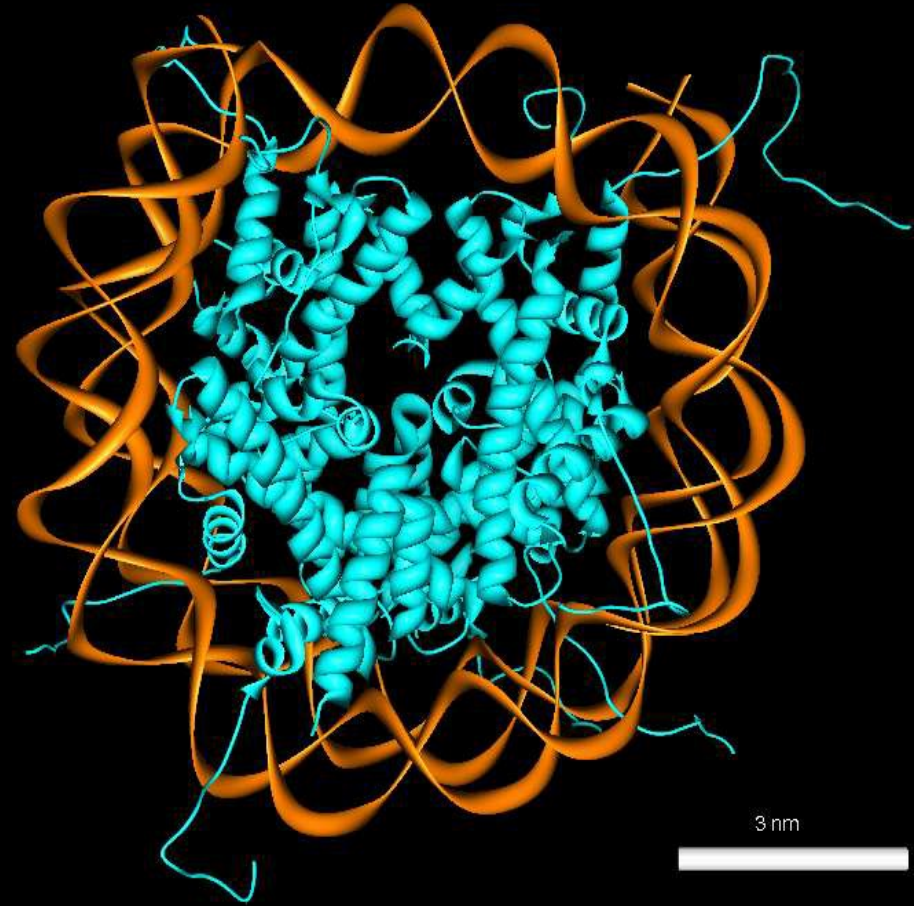
Struktura chromatinu



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.

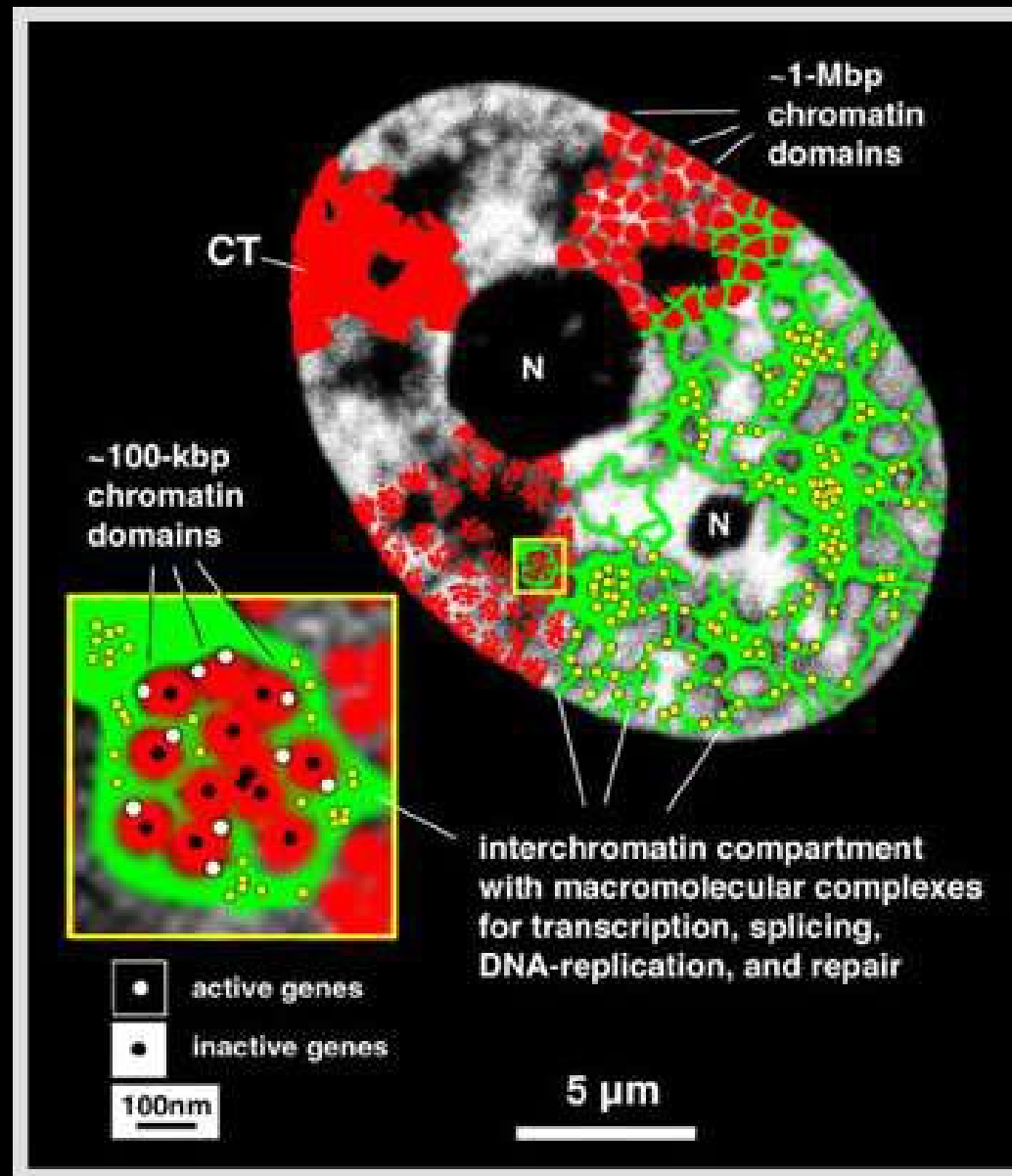


NUKLEOSOM

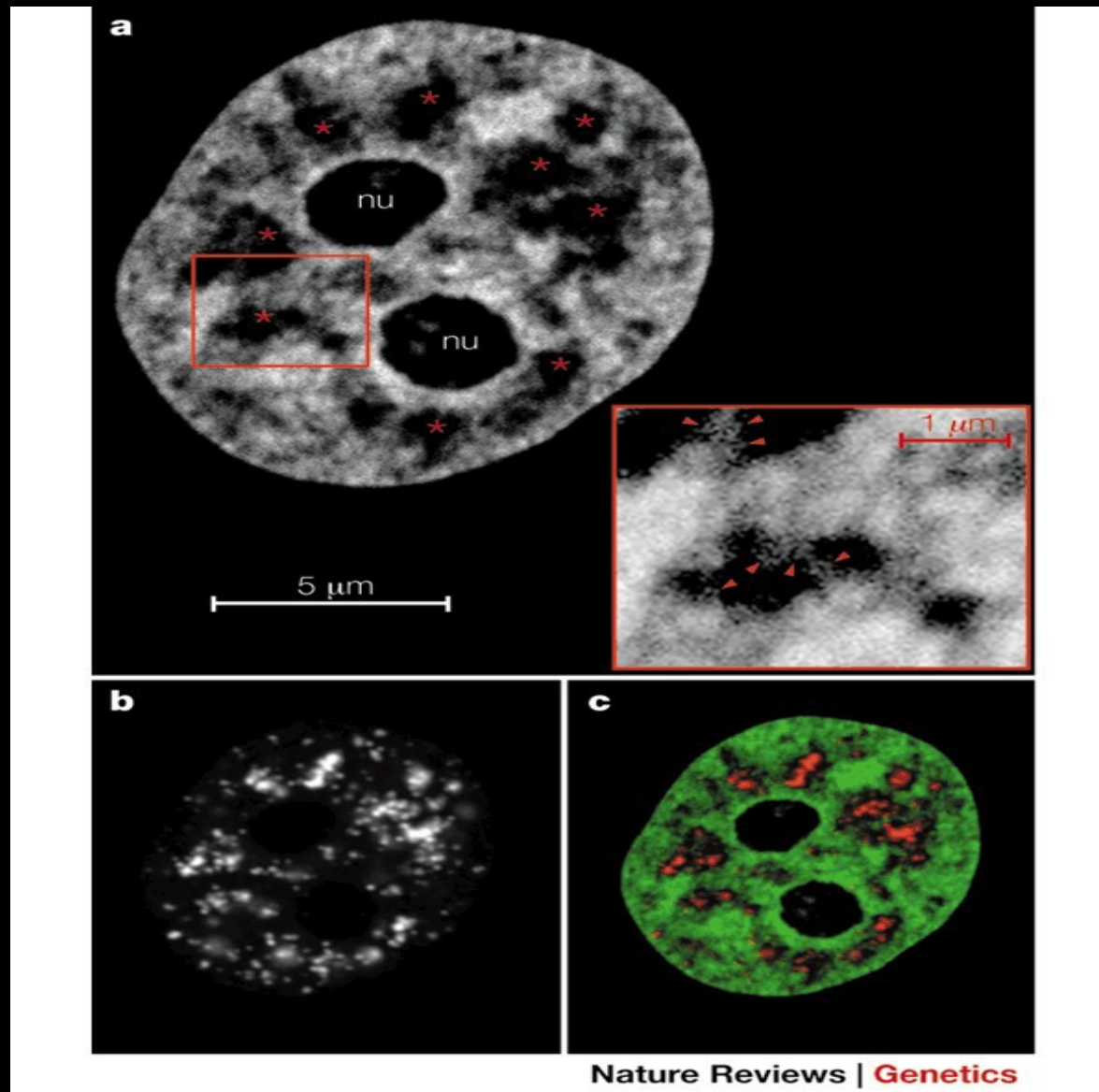


Joseph Roland 2003

CT-IC MODEL (T. Cremer)



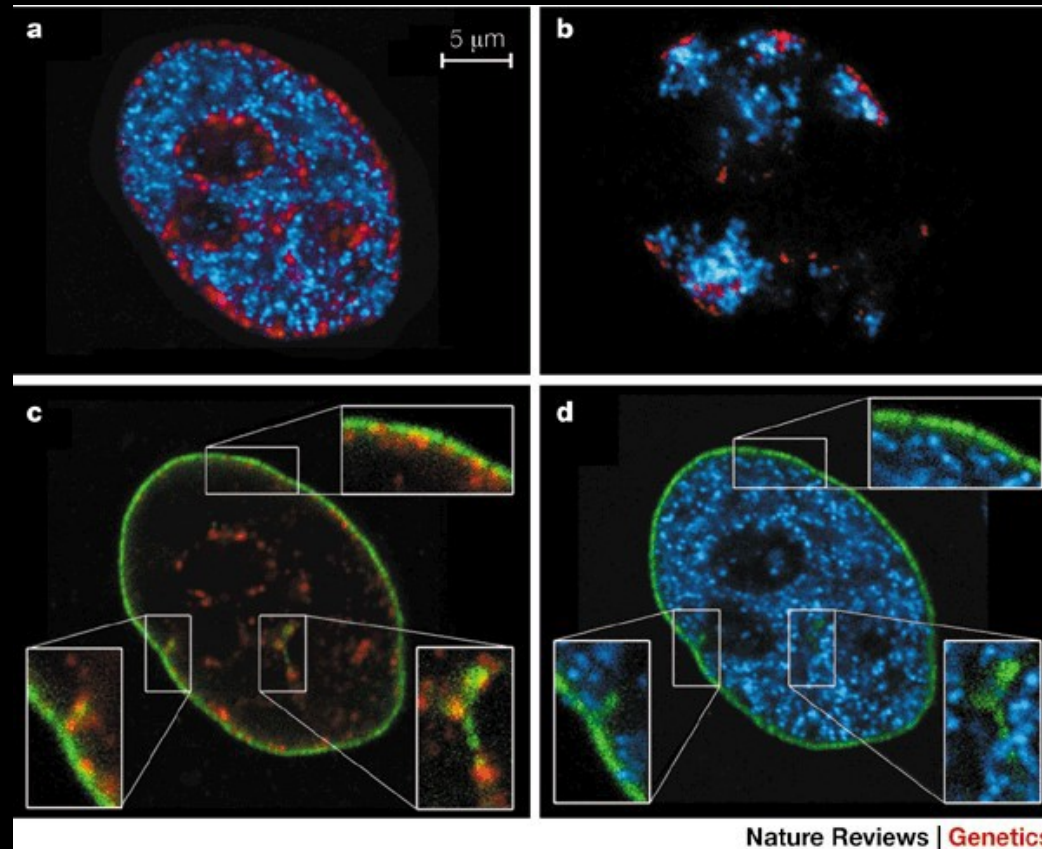
Chromatinové meziprostory



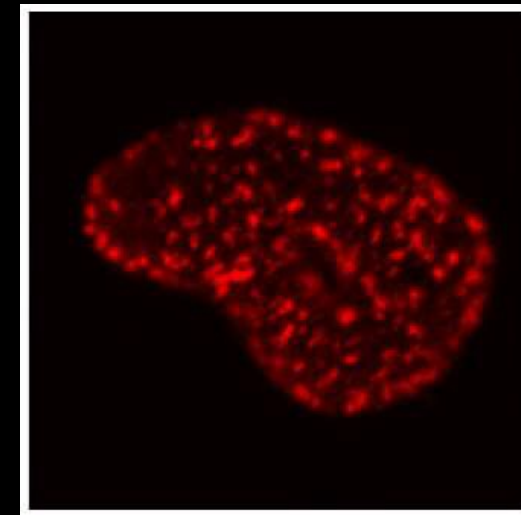
T. Cremer group, Munich

Struktura chromatinu a jederné procesy

Pozdně a časně se replikující chromatin



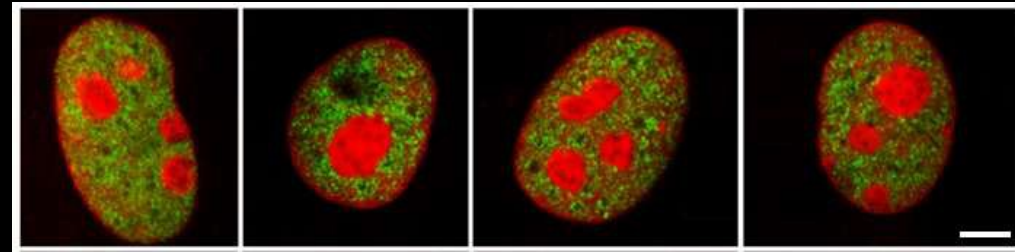
Replikační ohniska



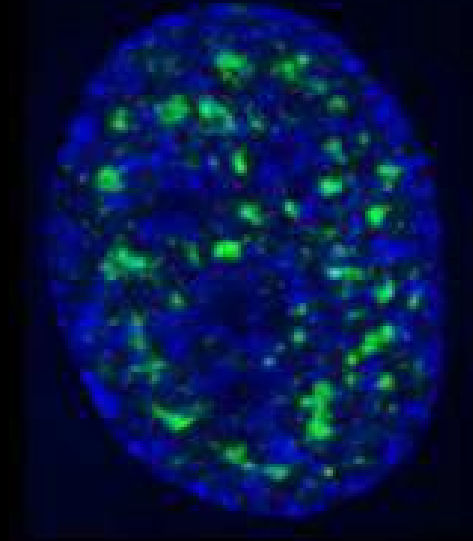
T. Cremer group, Munich

Nuclear compartments:

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



speckles



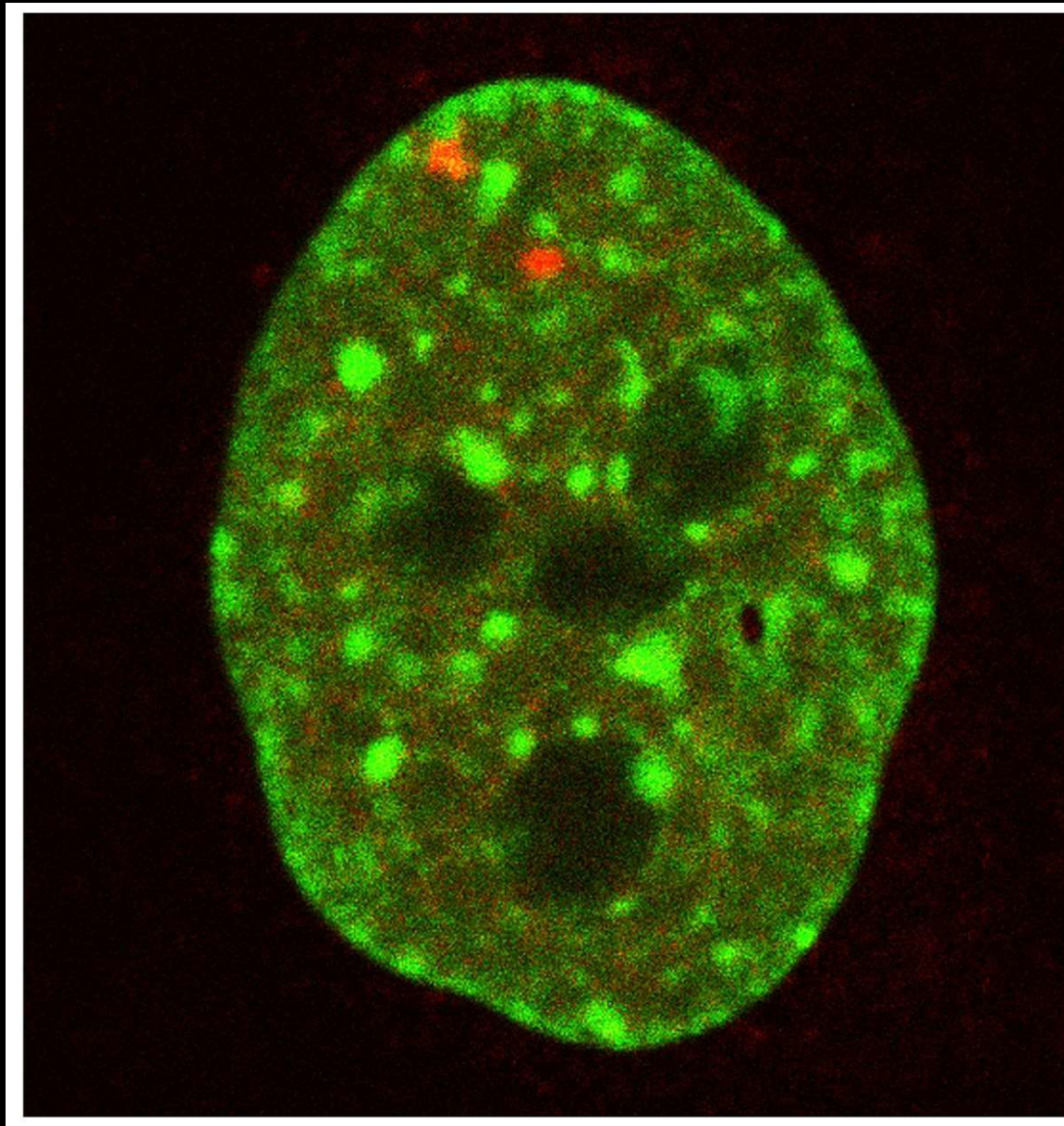
Cajal
Bodies

PML
Nuclear
Bodies

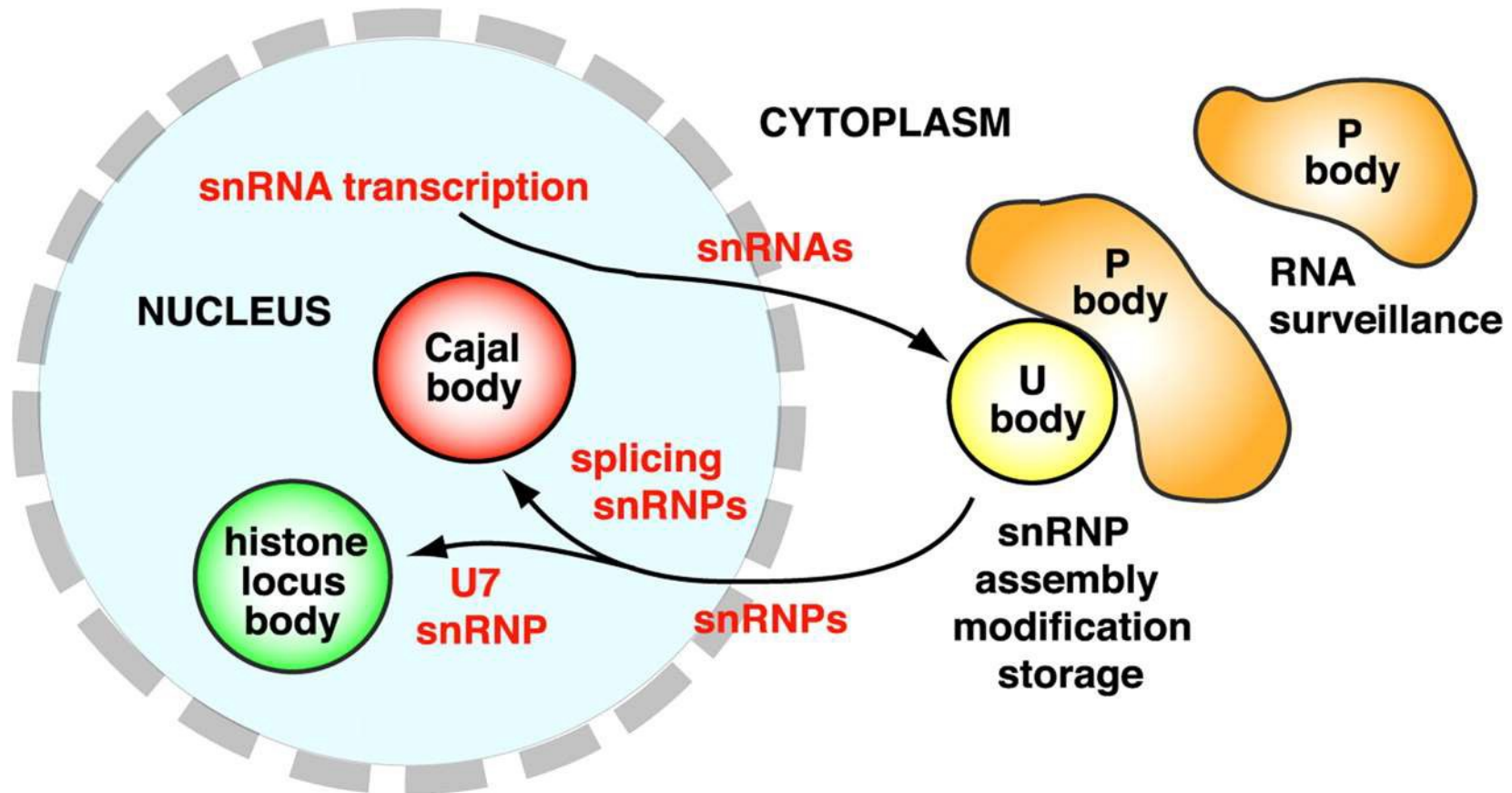
Roles of snRNPs

- **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

DNA repair foci



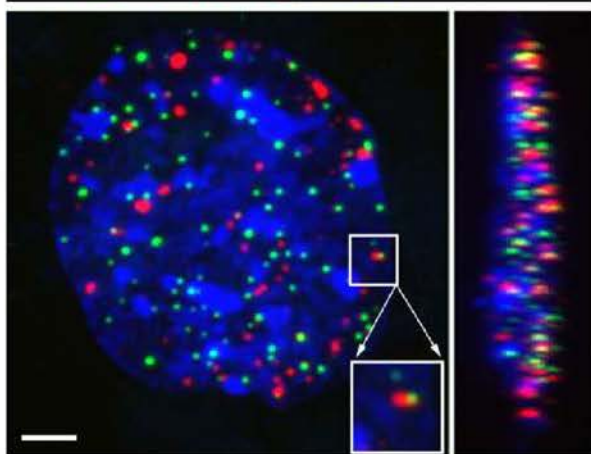
Nuclear and cytoplasmic bodies involved in snRNP assembly.



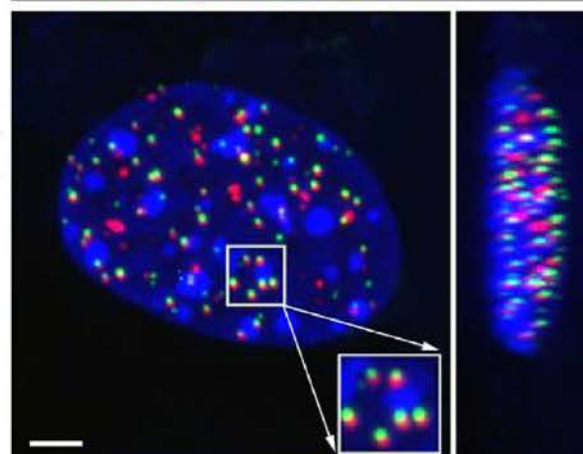
Liu J , Gall J G PNAS 2007;104:11655-11659

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

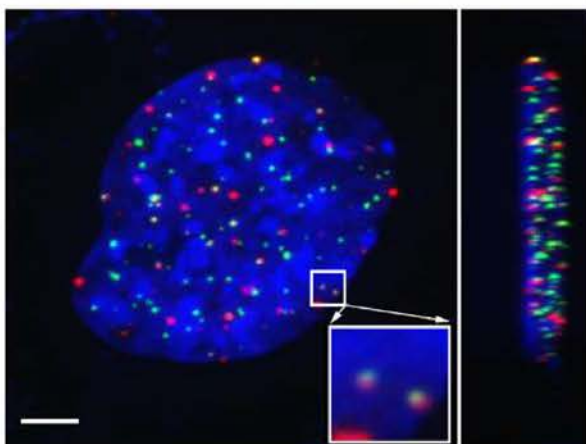
SUV39h (wt)

**GFP-TRF1 / PML / DNA**

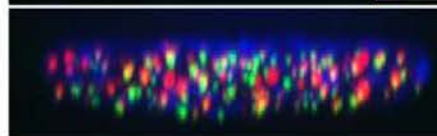
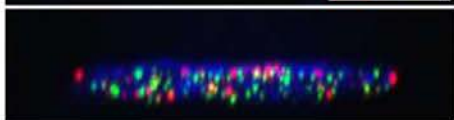
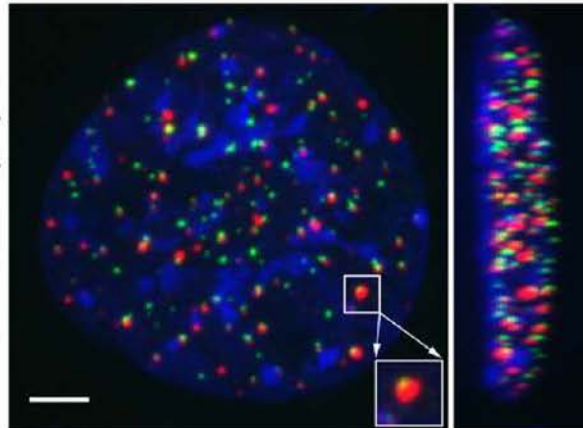
SUV39h (dn)

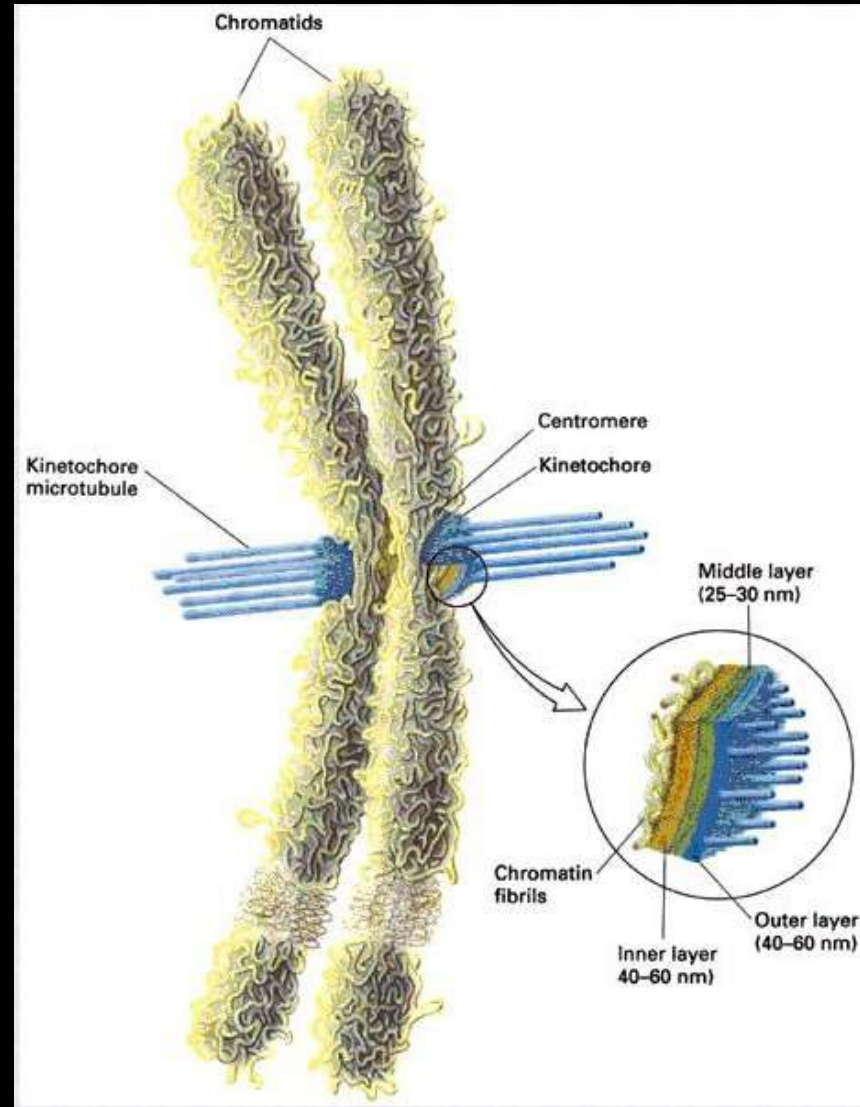
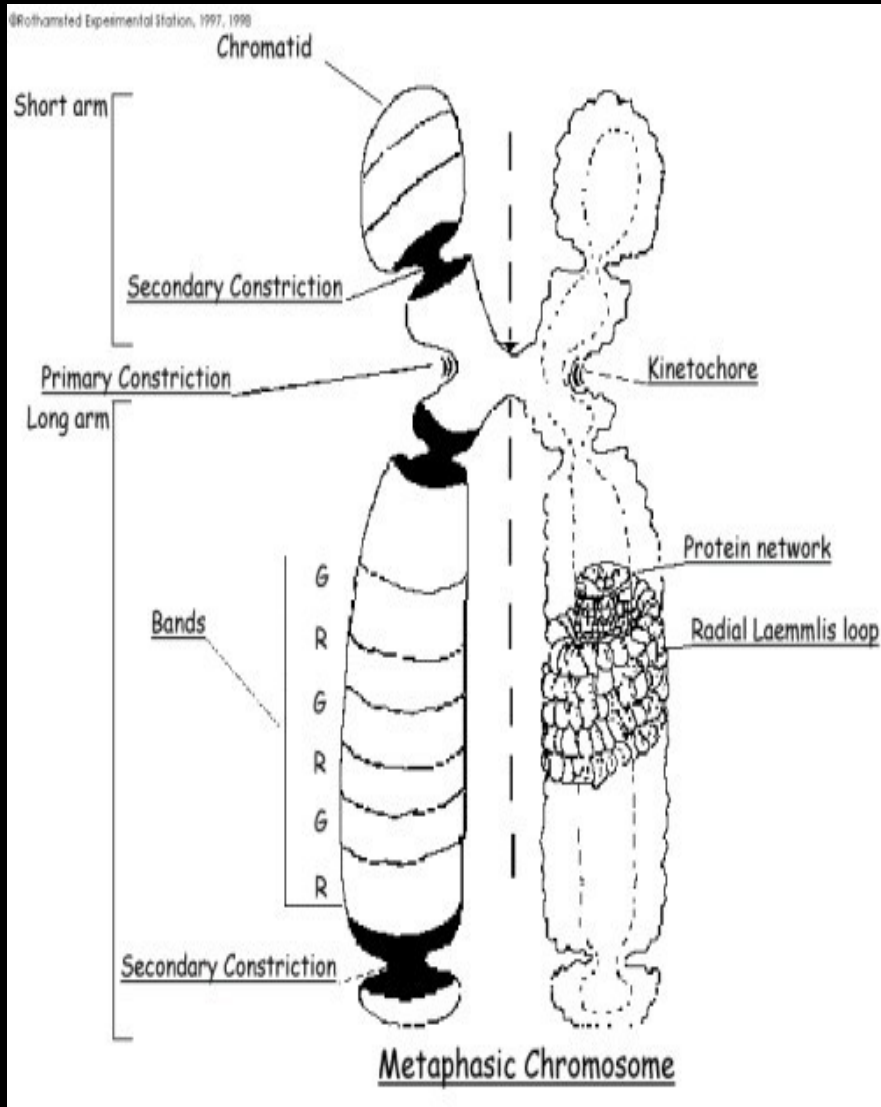
**GFP-TRF1 / PML / DNA**

LMNA (wt)

**GFP-TRF1 / PML / DNA**

LMNA (dn)

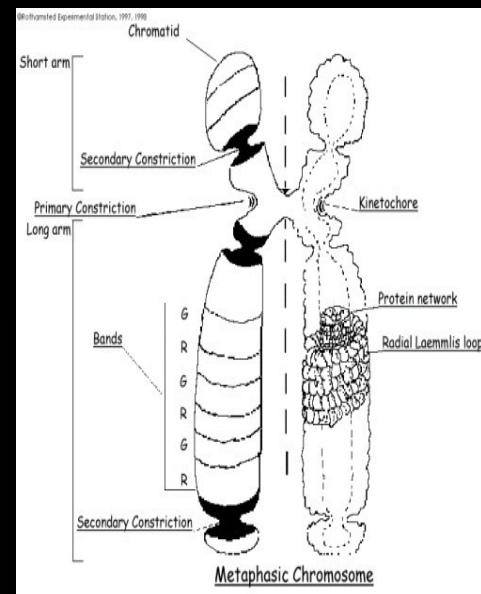
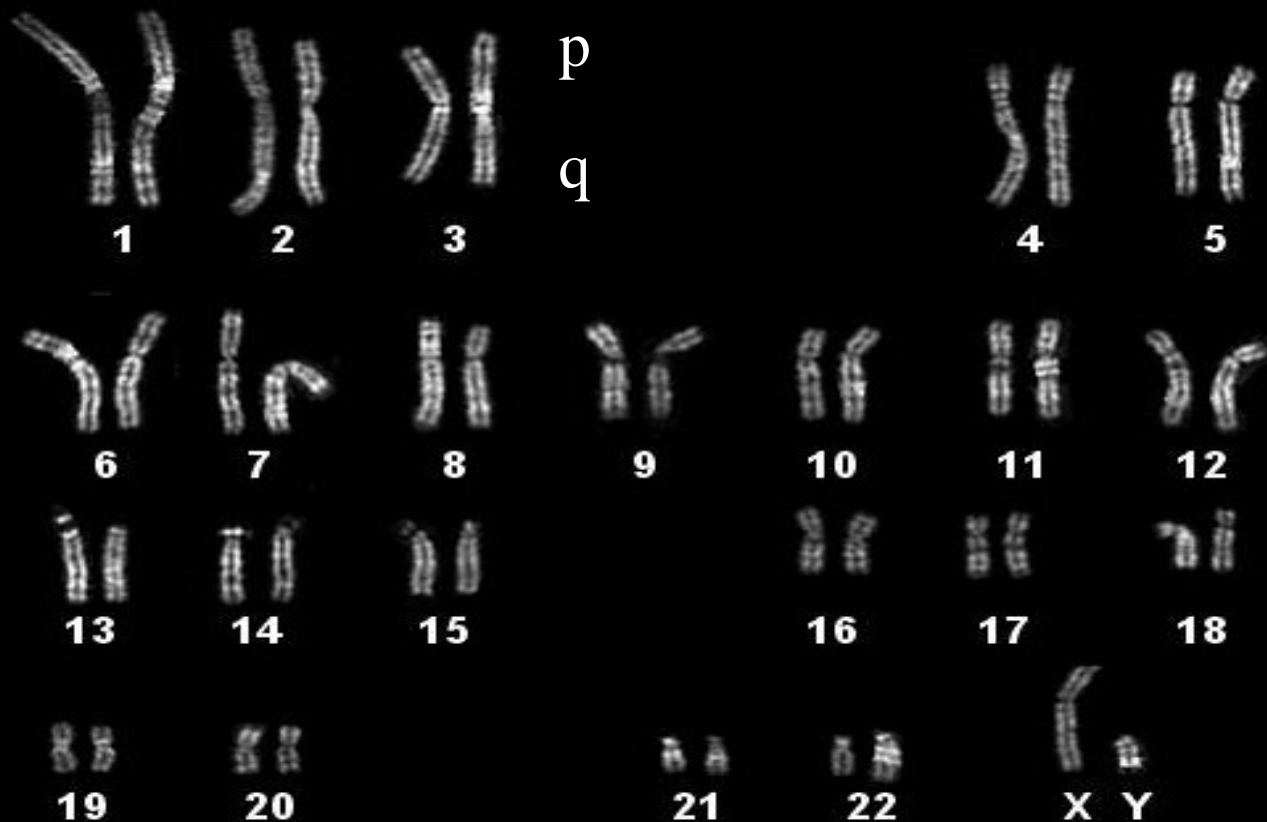




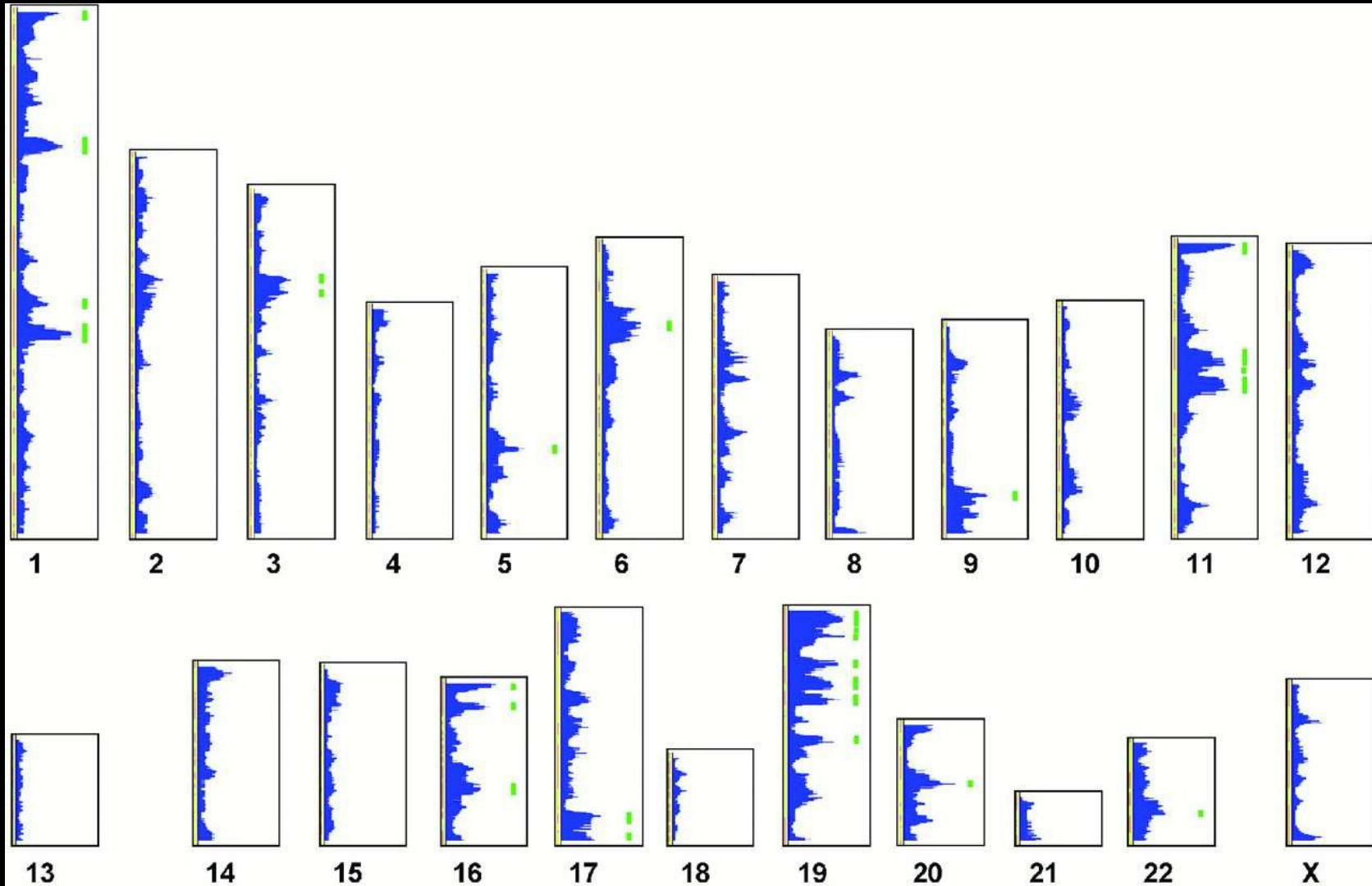
Kondenzace chromatinu

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

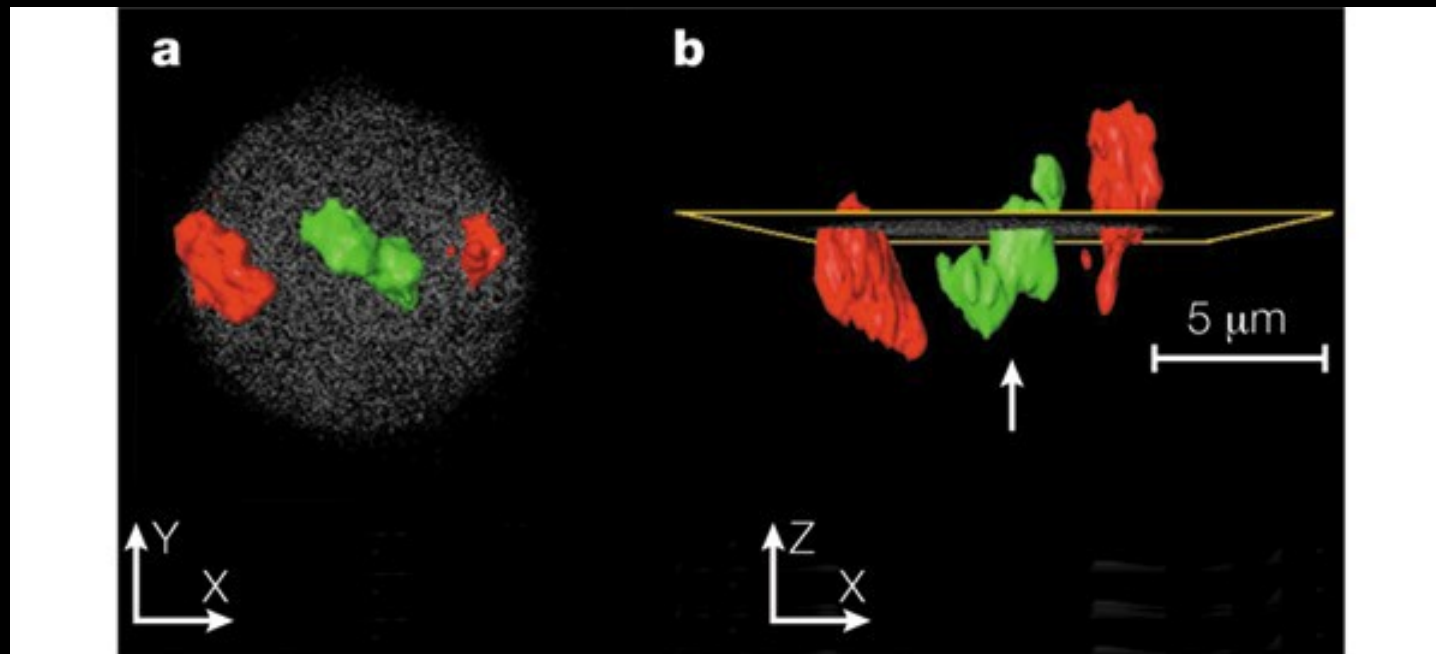
Typy chromozomů: meta- submeta-, akrocentrické



Transcriptome map (Caron et al., 2001)

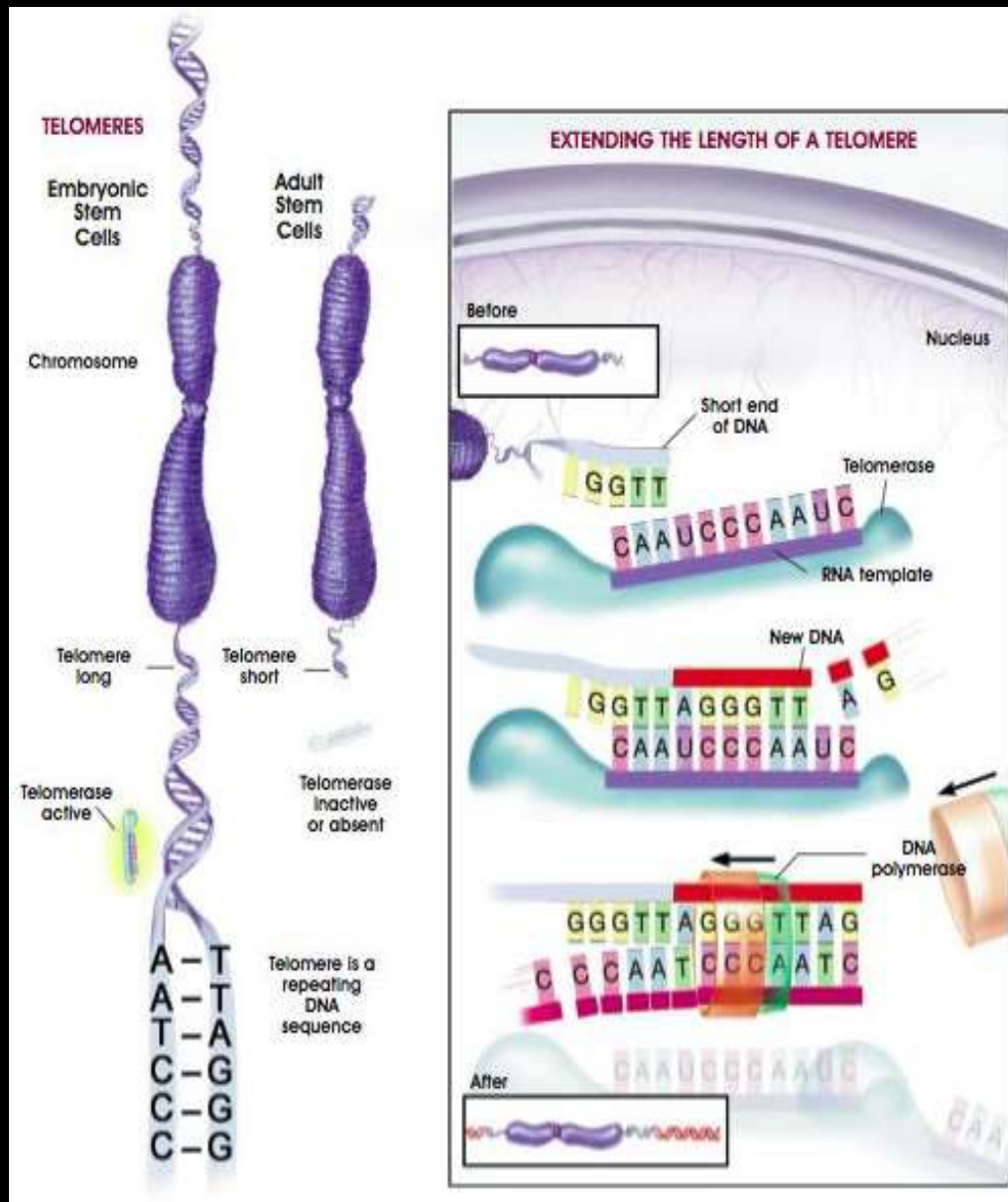


HSA 18 and 19 (positioning and gene density)

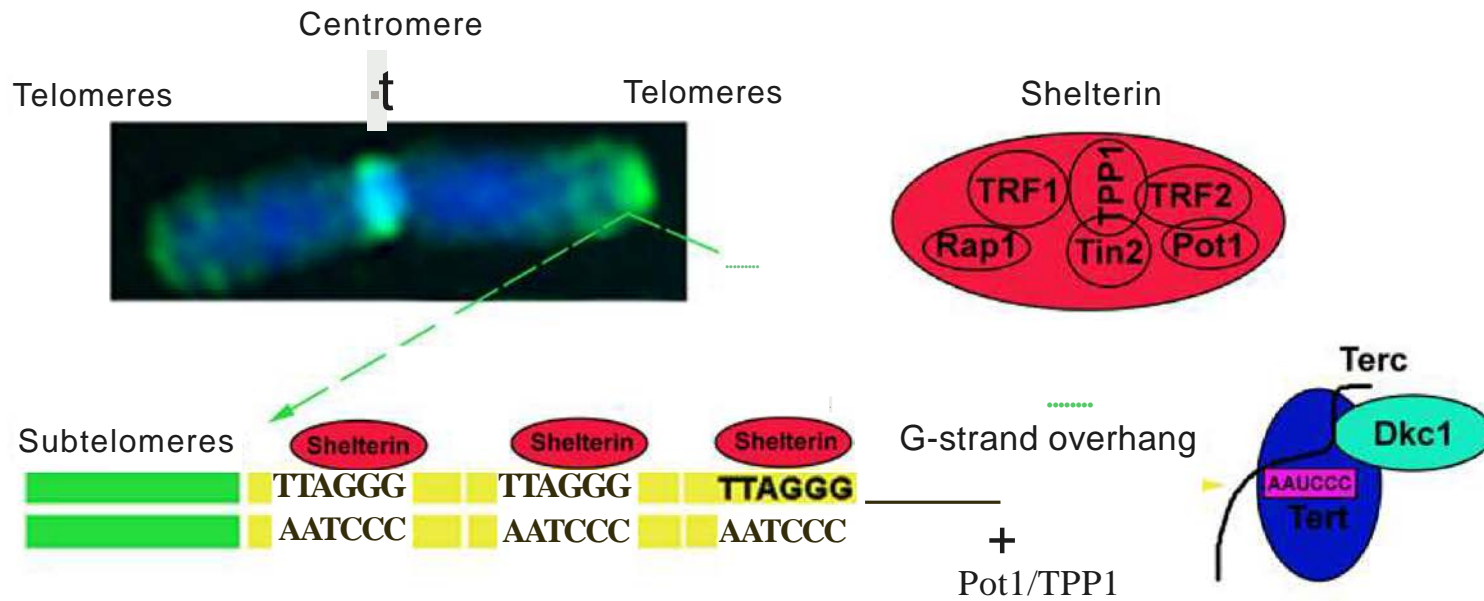


T. Cremer group, Munich

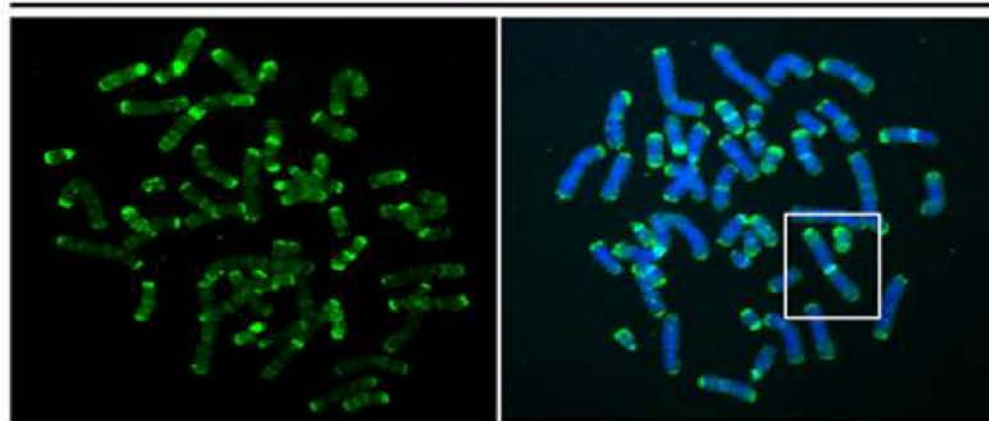
Telomeres (TTAGGG sequence and different proteins)



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



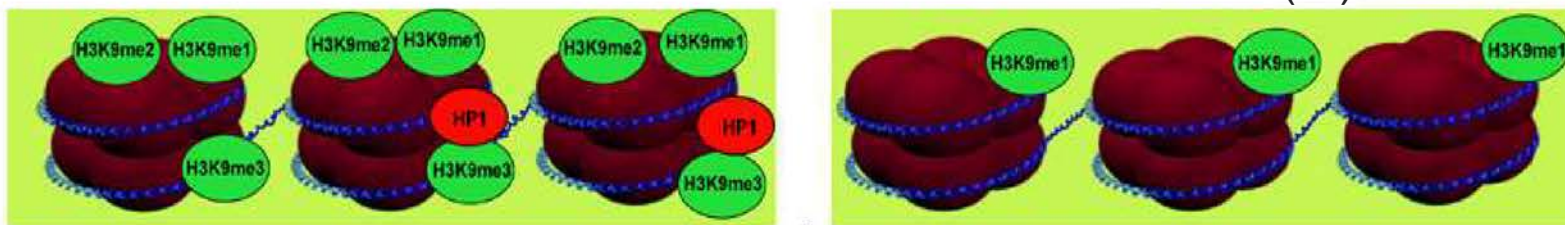
B

H3K9 methylation at telomeres

a)

SUV39h (wt)

SUV39h (dn)



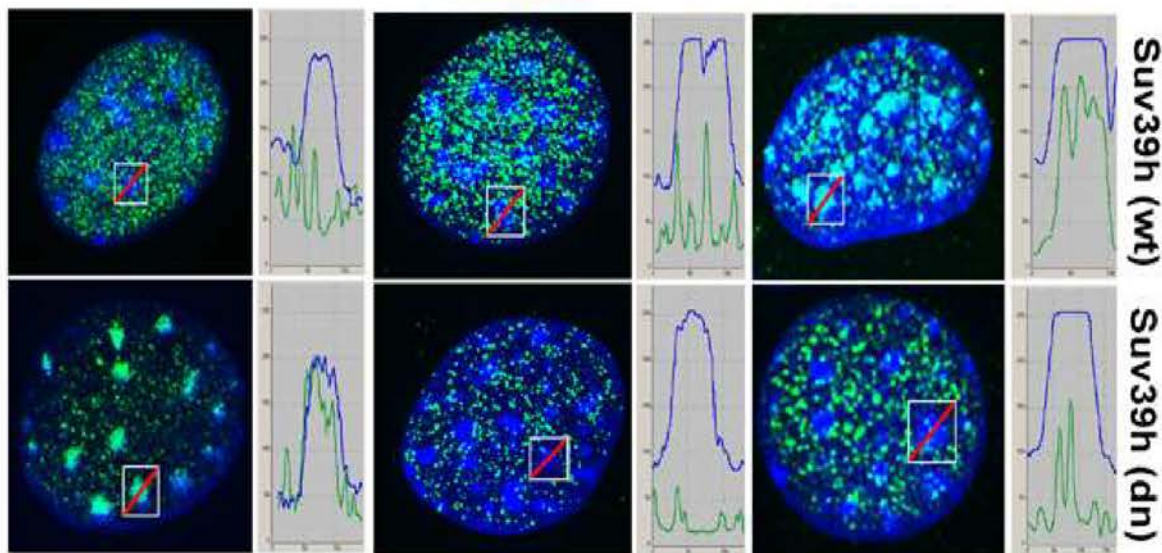
García-Cao et al. (2004)

b)

H3K9me1

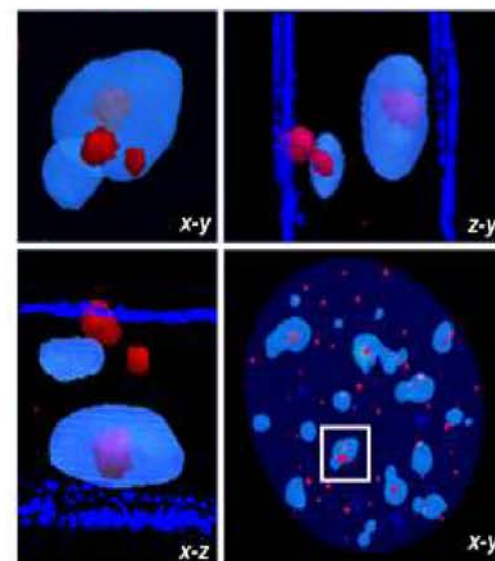
H3K9me2

H3K9me3



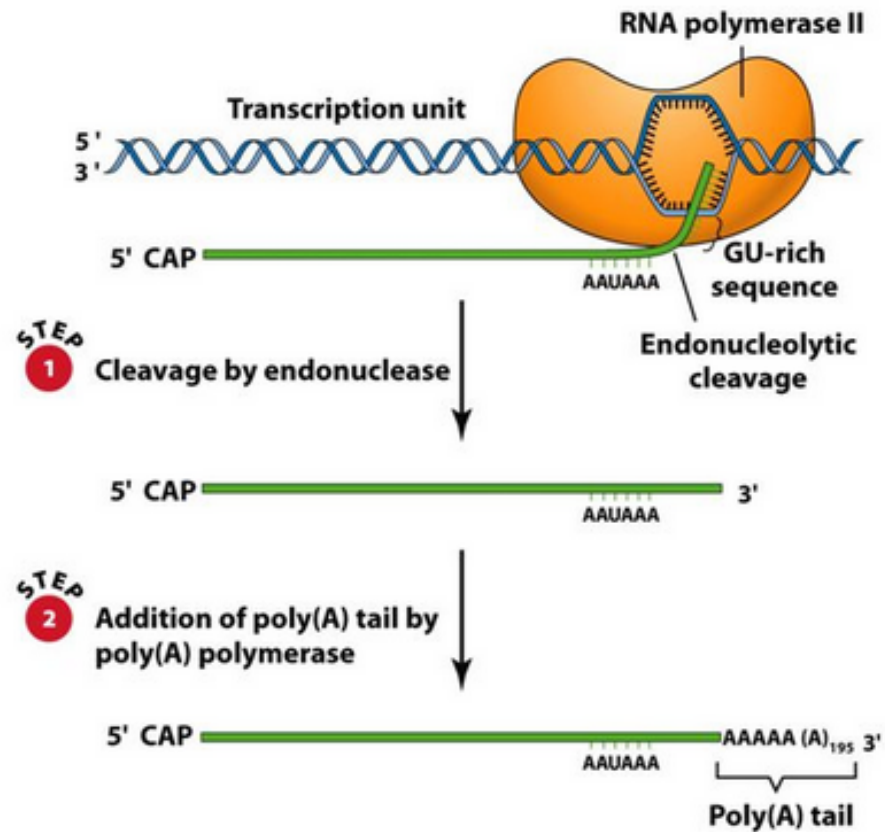
c)

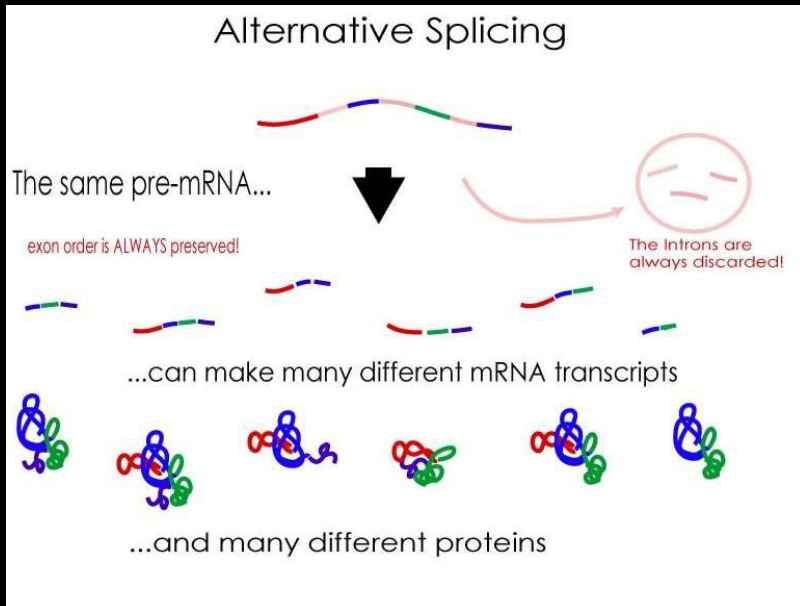
Telomeres/Chromocenters,



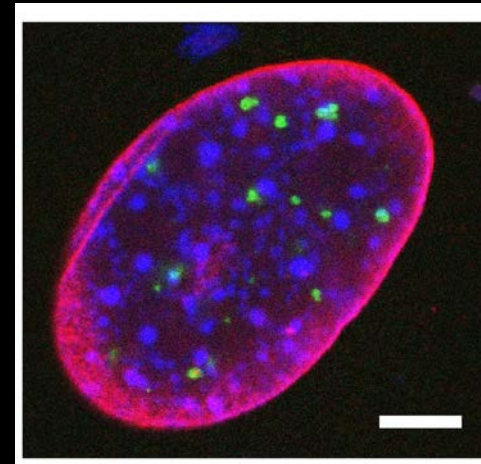
TRANSKRIPCE: přepis, biosynthesa řetězce RNA podle templátového řetězce DNA, přičemž jednotlivé nukleotidy jsou připojovány na základě komplementarity (viz base nukleových kyselin). Klíčovým enzymem této synthesy je RNA-polymerasa. Transkripce probíhá ve třech stupních: a) **iniciace** (zahájení), kdy 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.

Polyadenylate

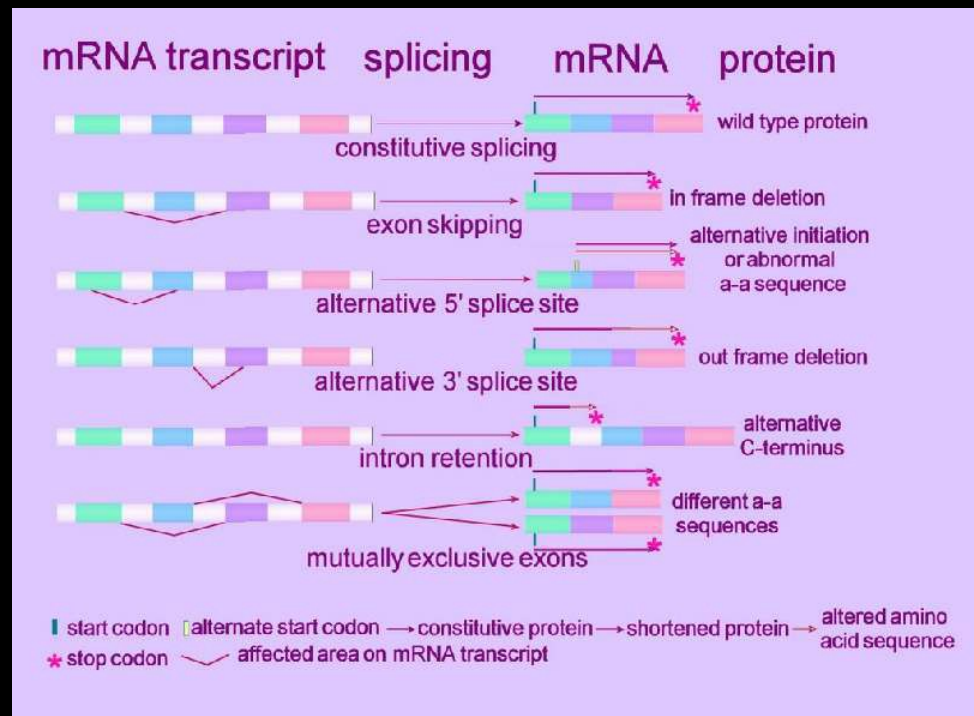


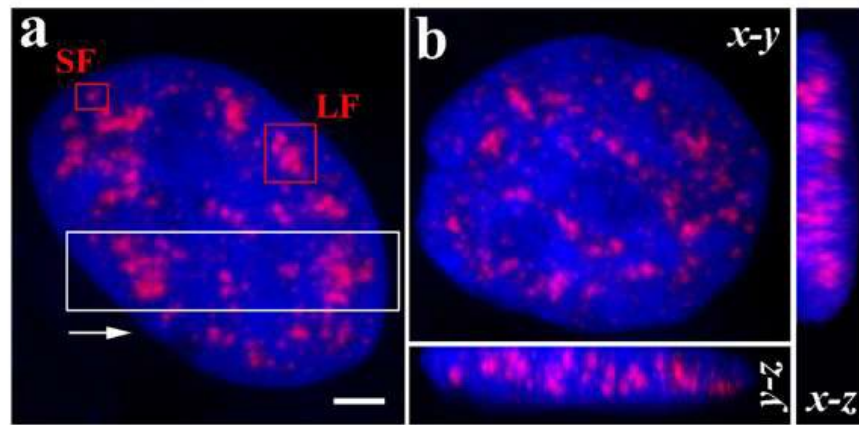


A-type lamins



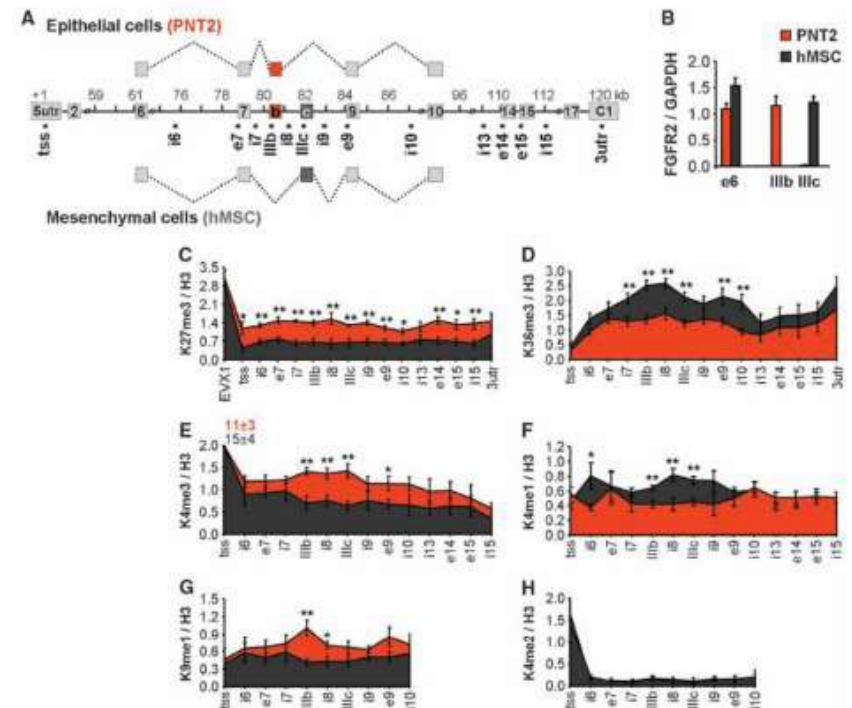
Alternative splicing is a form of epigenetic mechanism that enables a single gene to give rise to multiple, differentially spliced versions of a protein, increasing complexity without a change in the genome.



C**SC35 / DAPI**

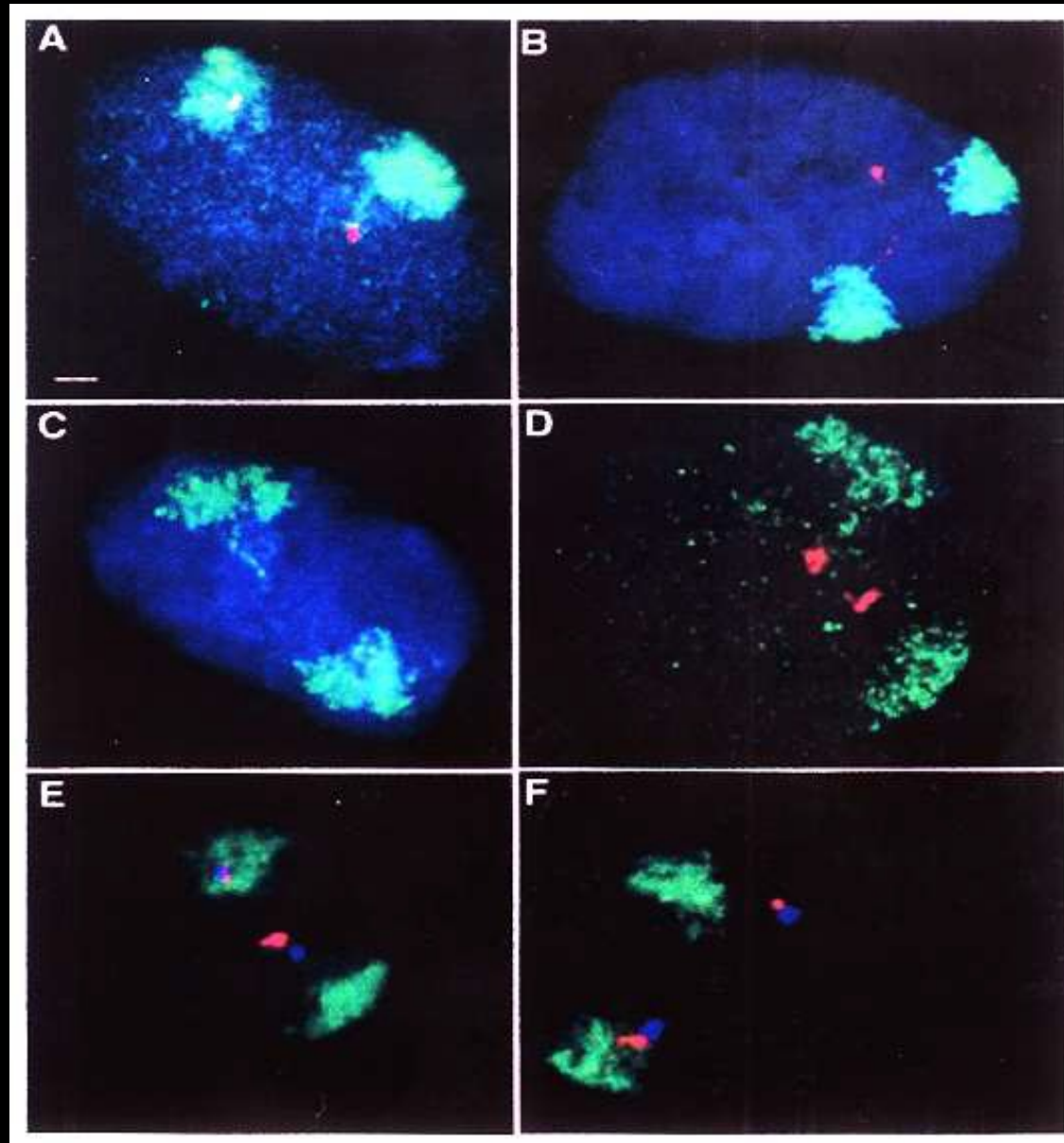
Faktory sestřihu jsou v SC-35 doménách, dále snRNP U1-U6 jsou součástí faktoru sestřihu SF2/ASF

Bartova group



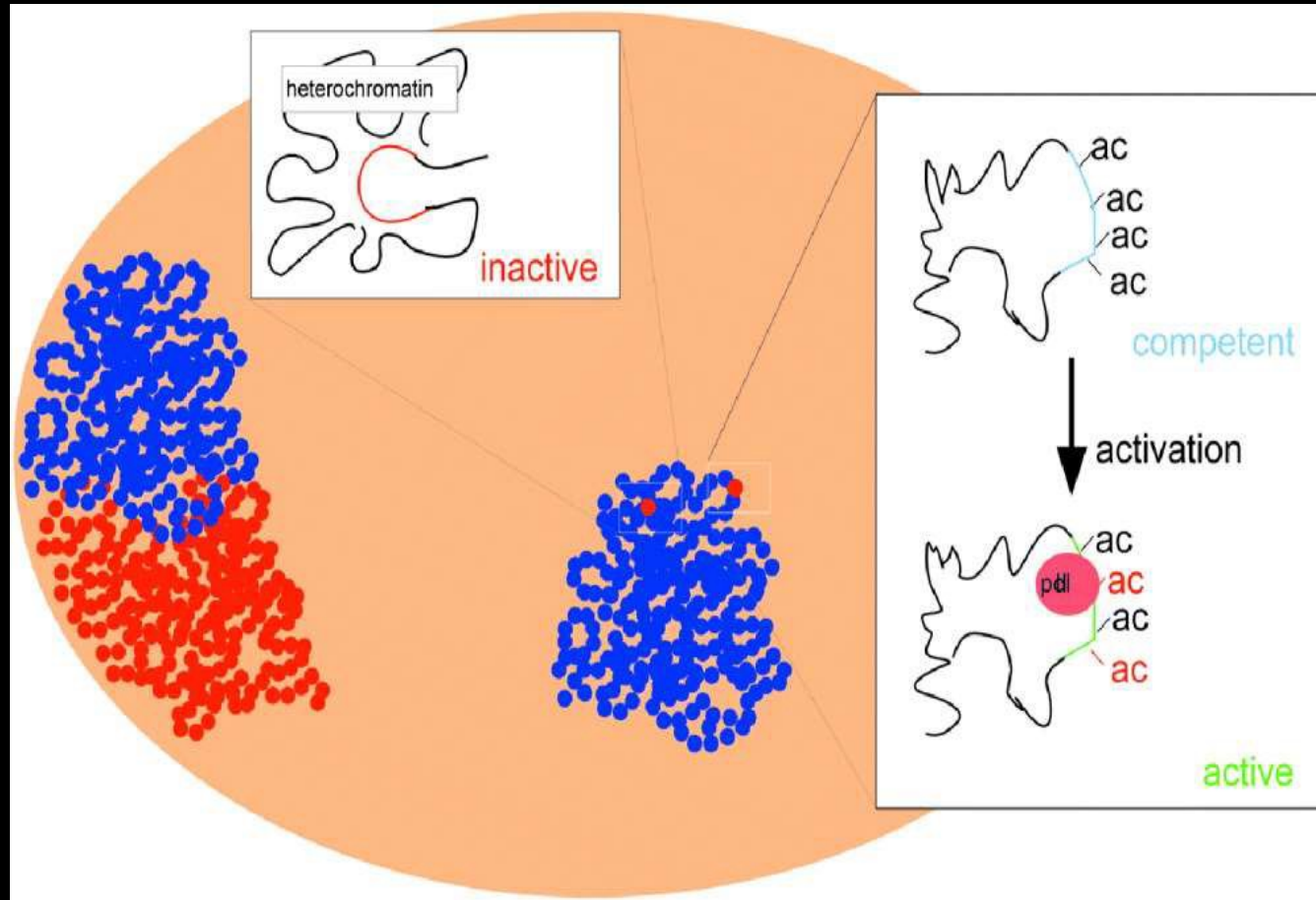
Luco et al., Science (2010)

MHC on HSA6



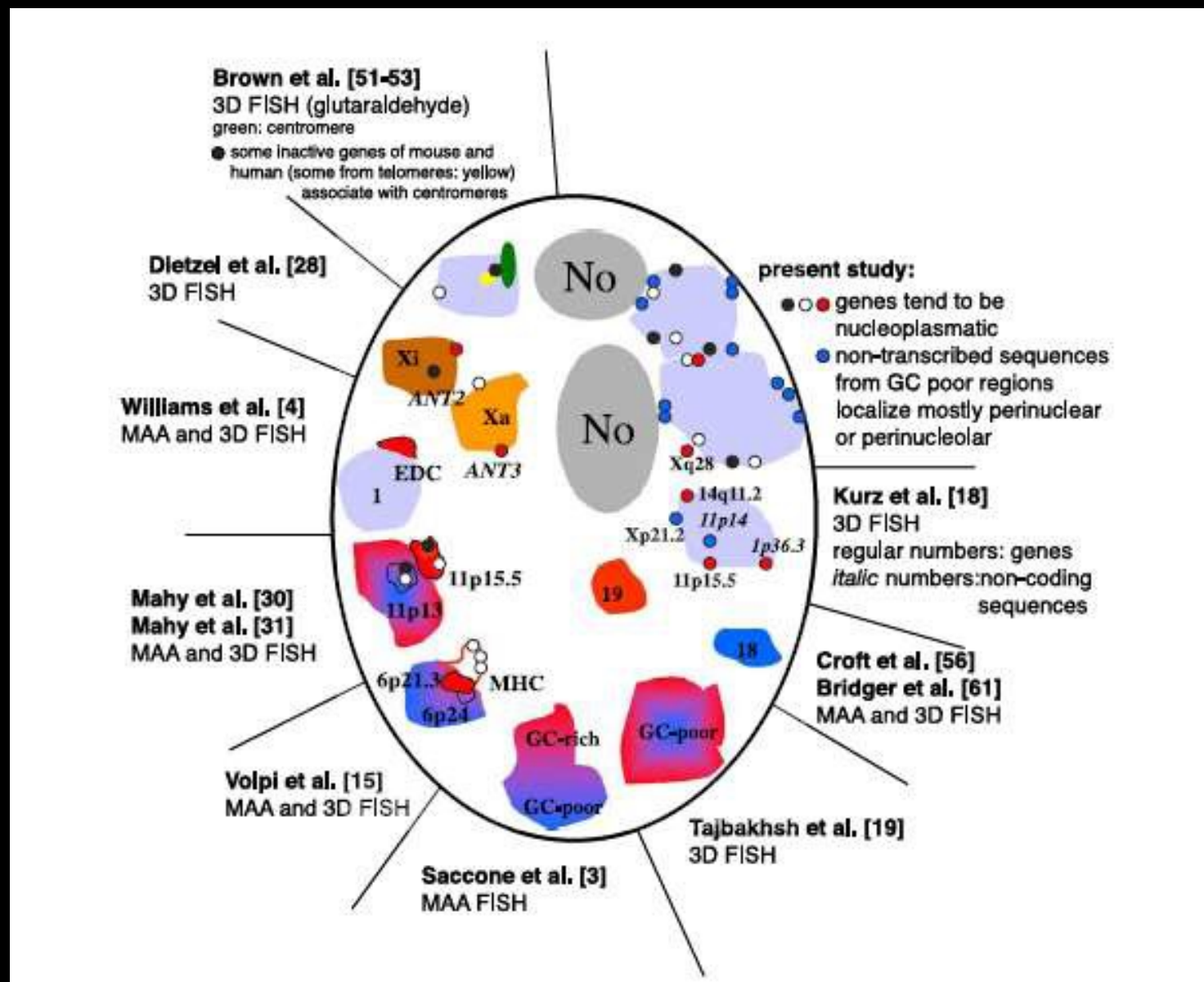
Volpi et al., 2000

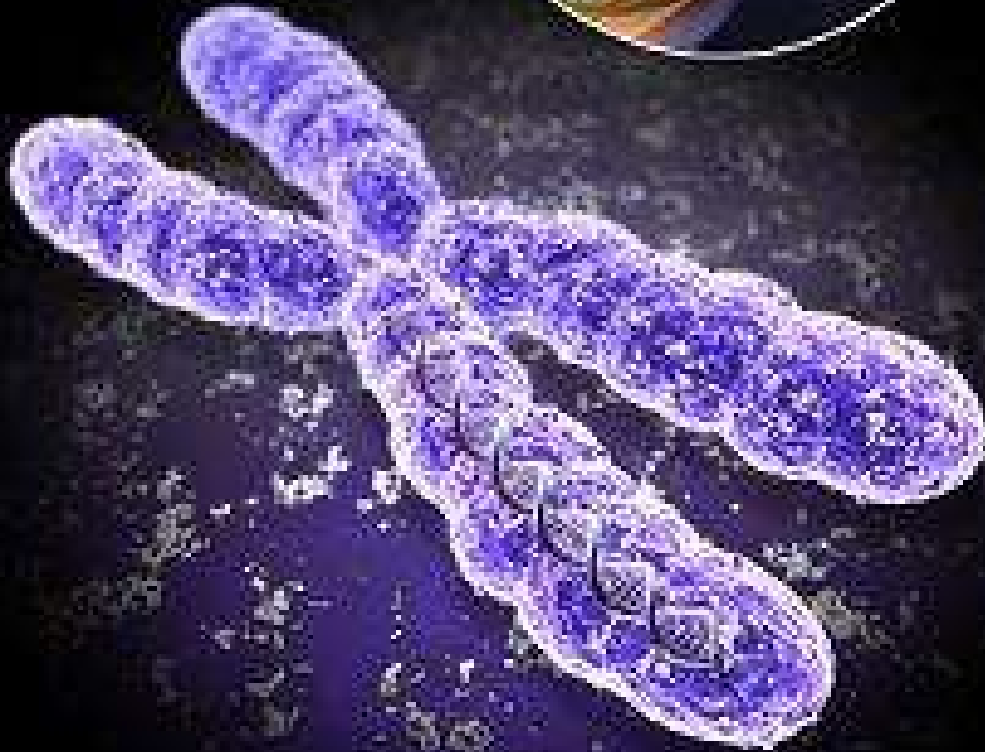
Active / inactive chromatin



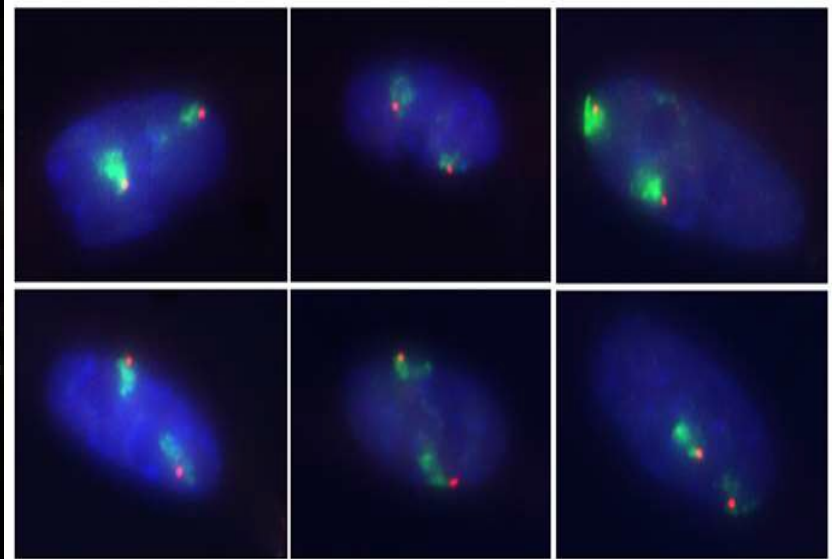
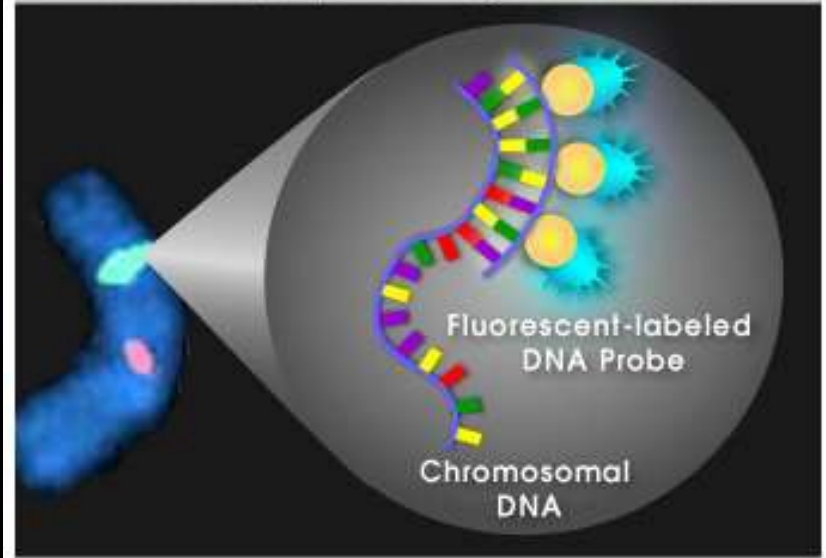
T. Cremer group, Munich

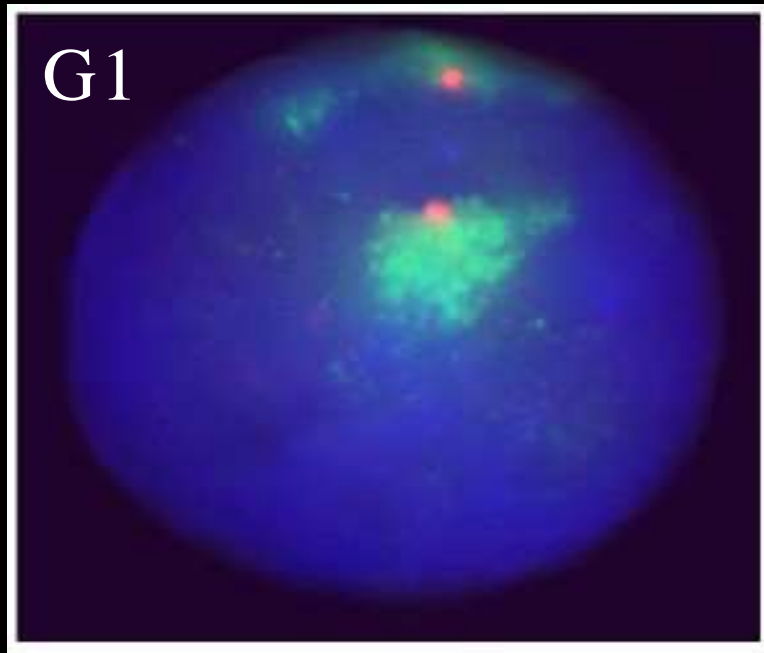
INTERMINGLING



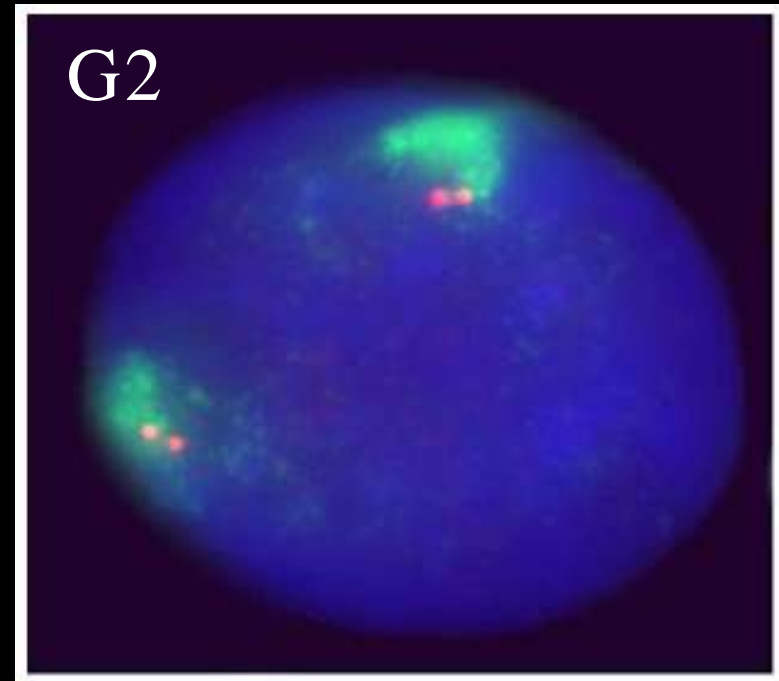


Chromosome prepared using FISH technique



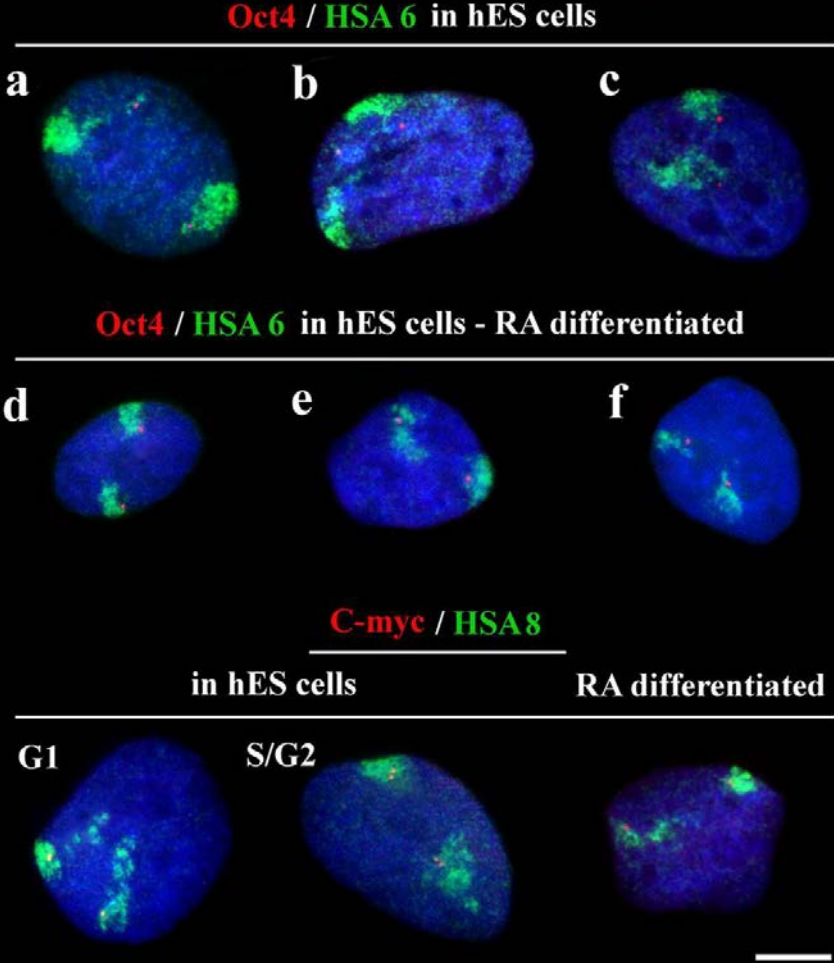
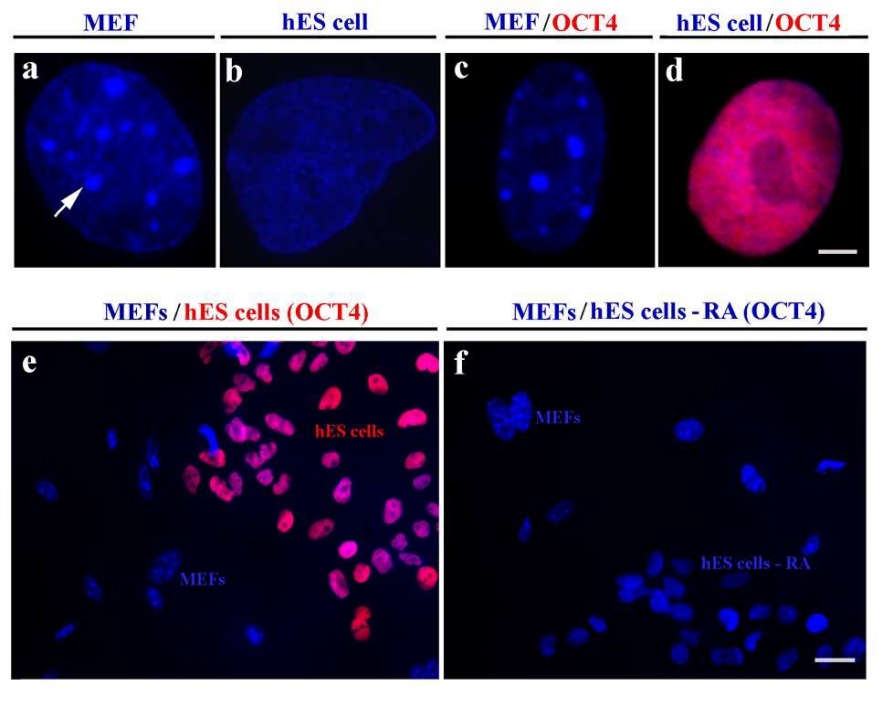


Rb1 gene

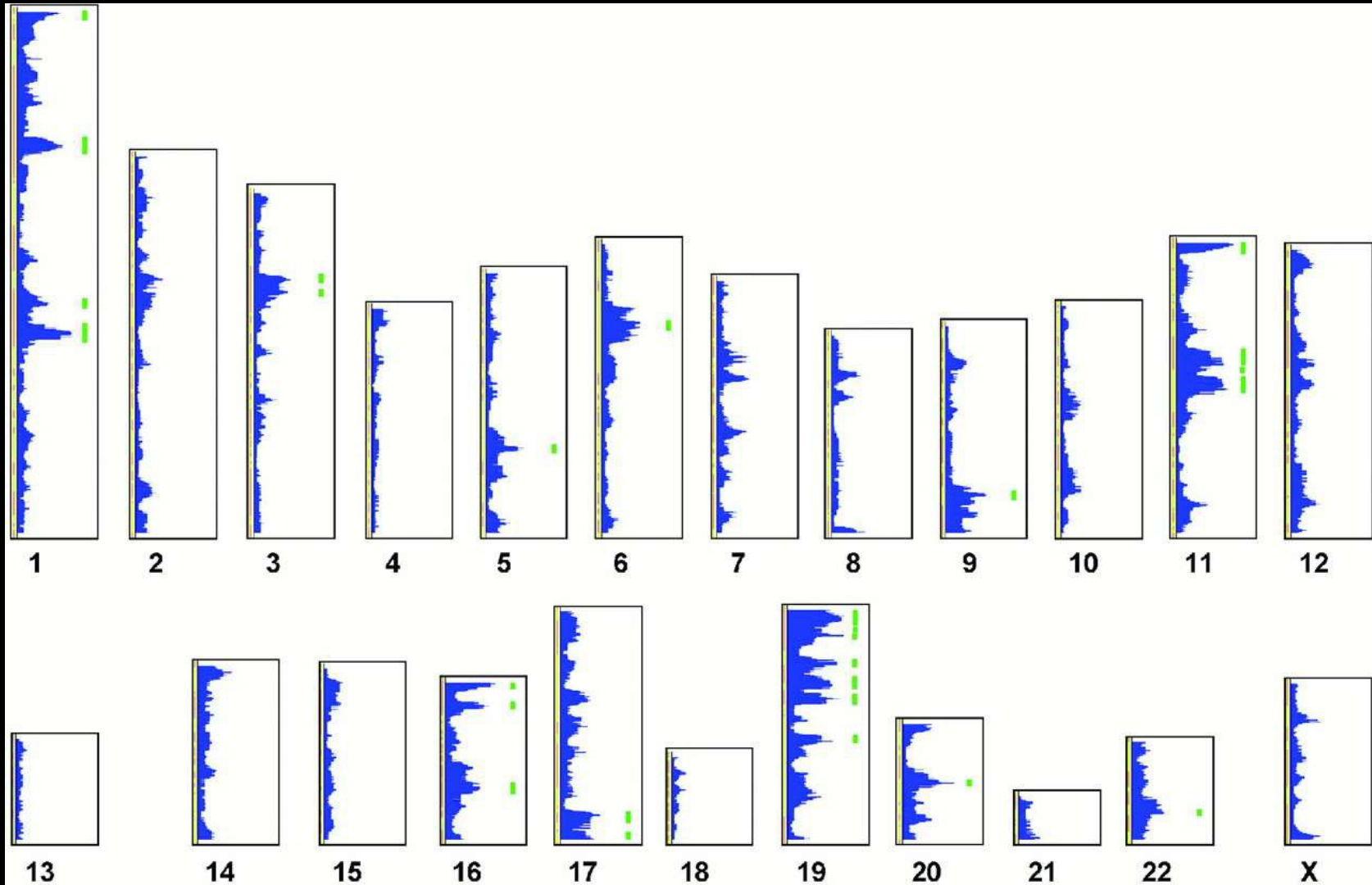


Bártová et al., 2002

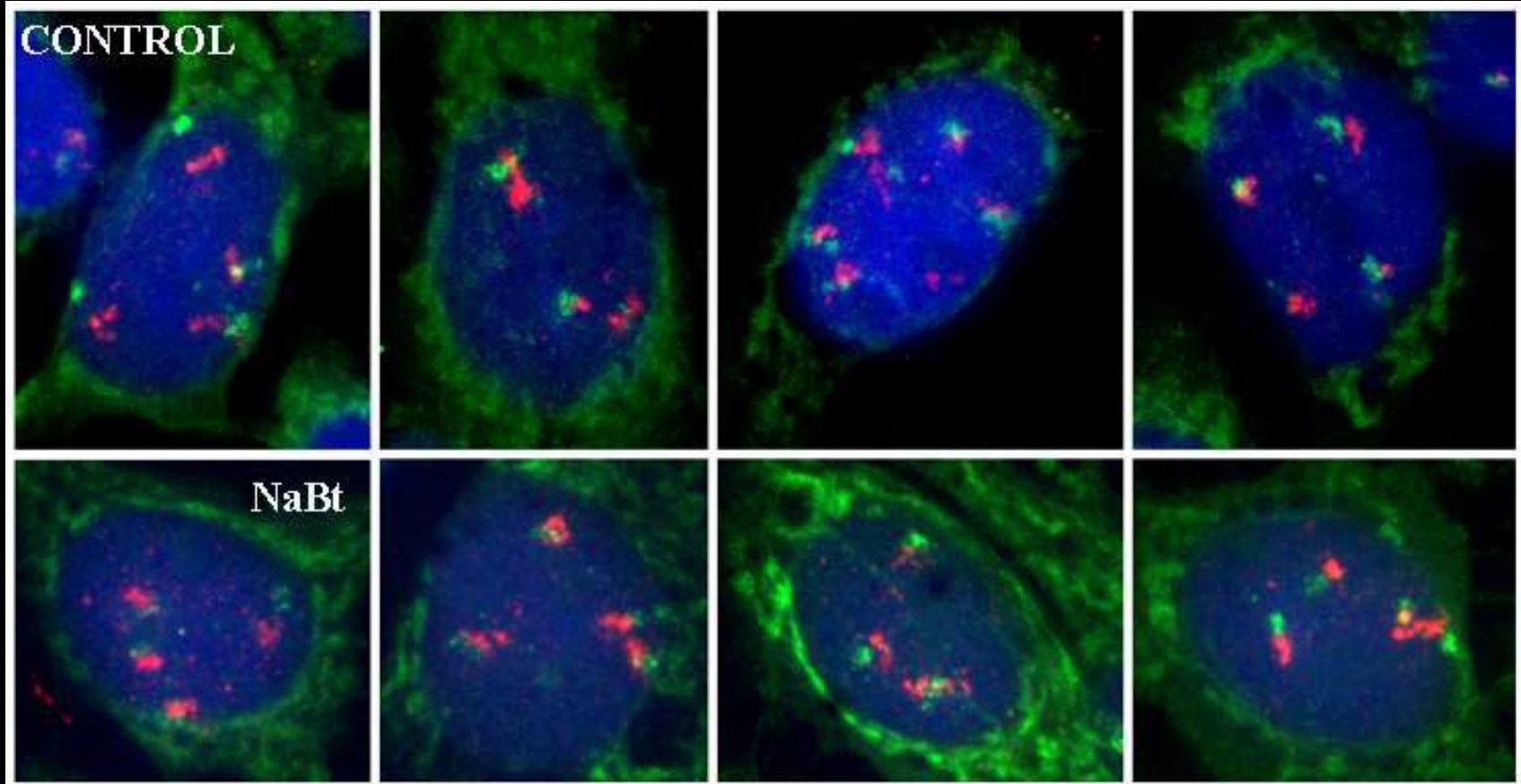
Genes in human embryonic stem cells



Transcriptome map (Caron et al., 2001)



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



Center of nucleus-
to-gene distances

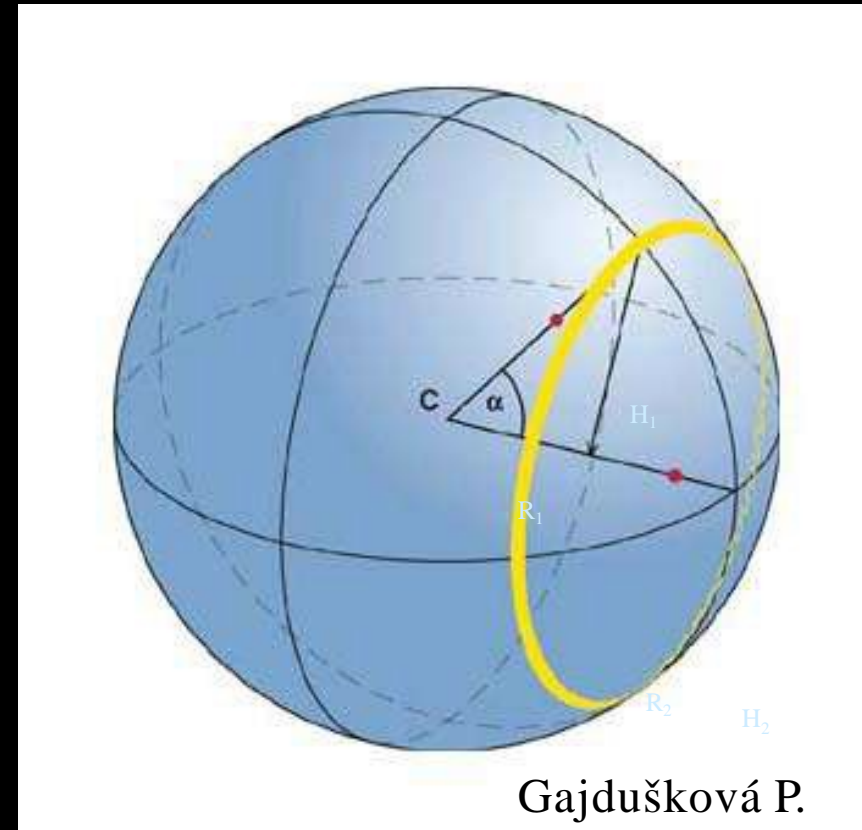
$$CS \square \frac{\overline{CR_i}}{\overline{CH_i}} * 100$$

Gene-to-gene
distances

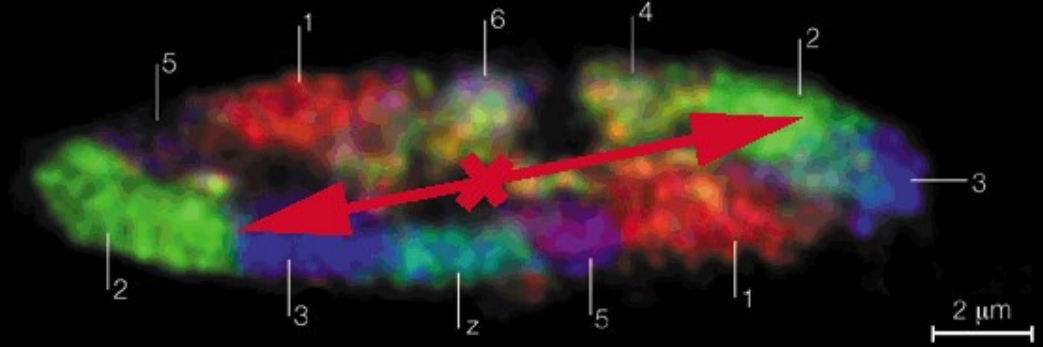
$$SS \square \frac{\overline{R_1 R_2}}{(\overline{CH_1} \square \overline{CH_2})/2} * 100$$

Gene-center of
nucleus-gene
angles

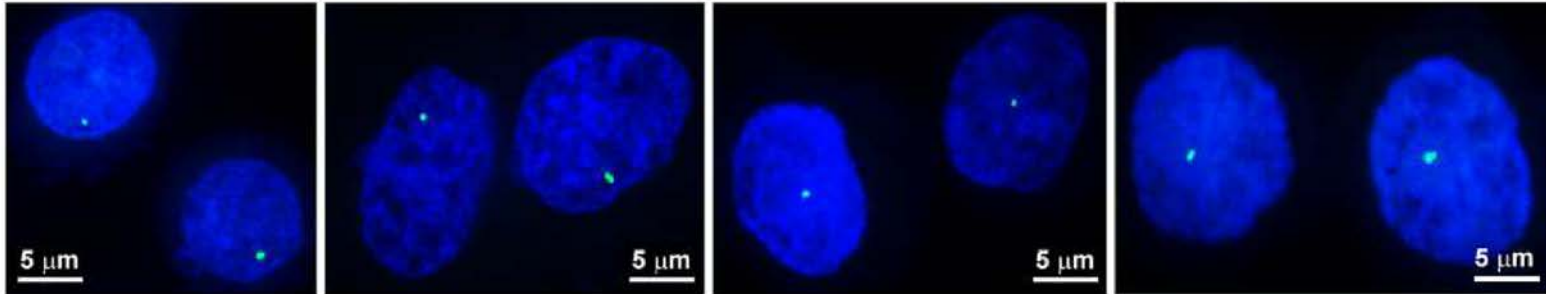
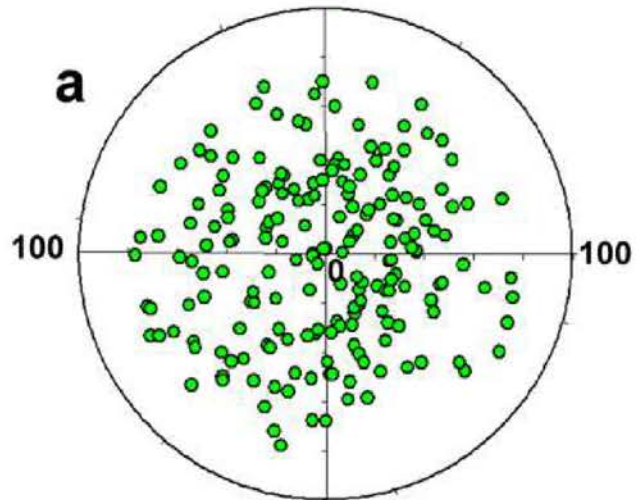
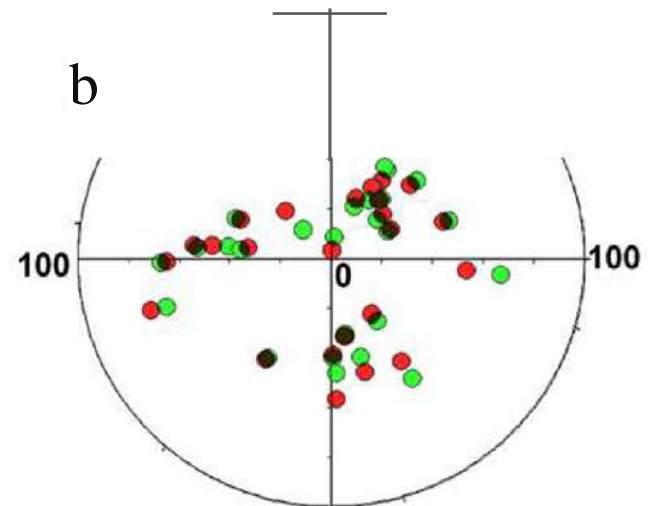
$$\cos(\square) \square \frac{\overline{CR_1} * \overline{CR_2}}{|\overline{CR_1}| * |\overline{CR_2}|}$$



d

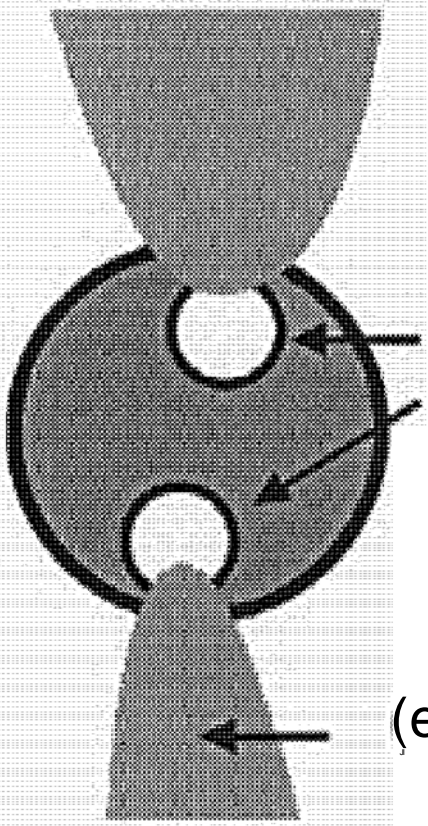


T. Cremer group

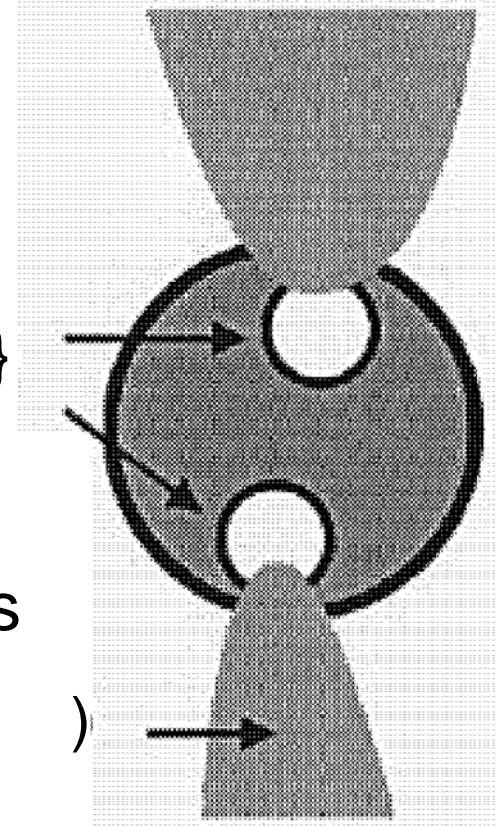
A**c-myc transcripts / Nucleoli****sister cells****B****c-myc transcripts in whole cell population****c-myc transcripts in sister cells**

Nucleolus

OPT domain

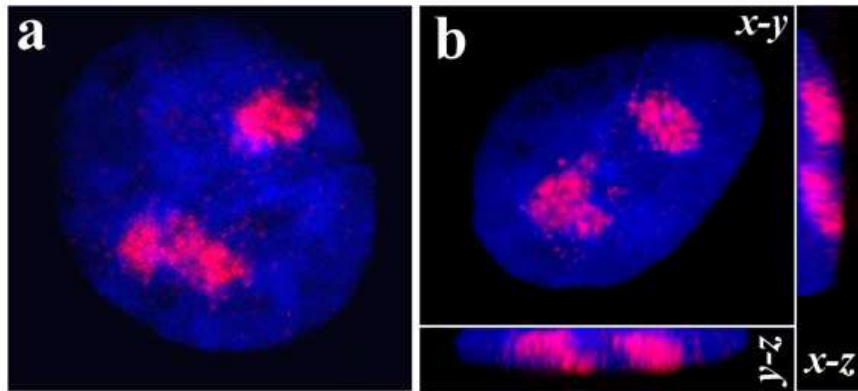


factorr s
(pol I) (pol Ir/III)
ct1ron1os n1 s
(eq ,14) (eg)

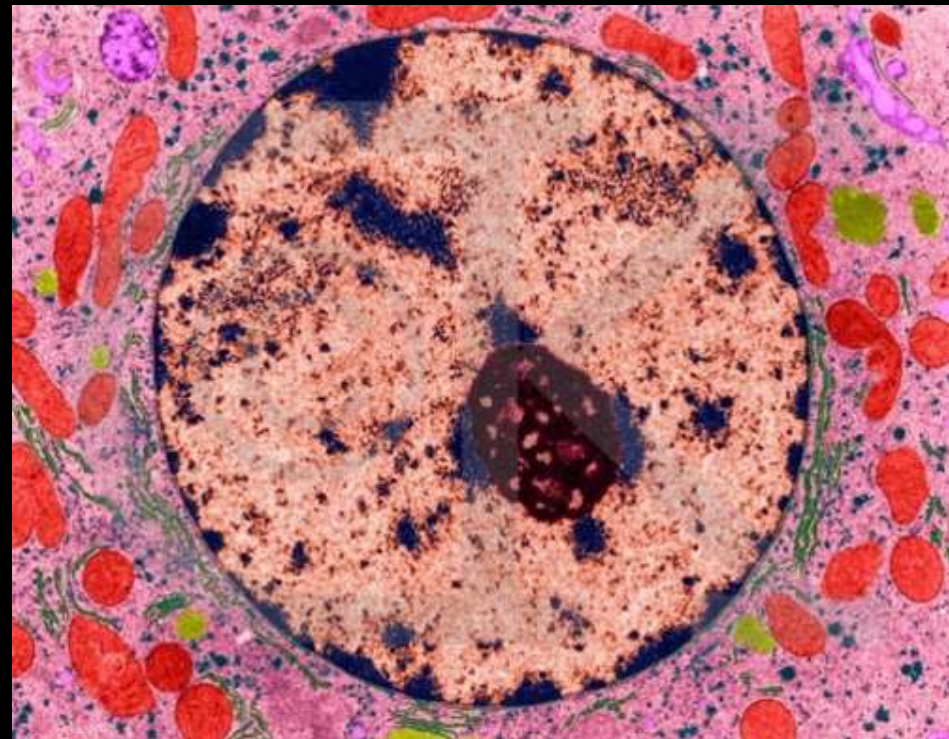
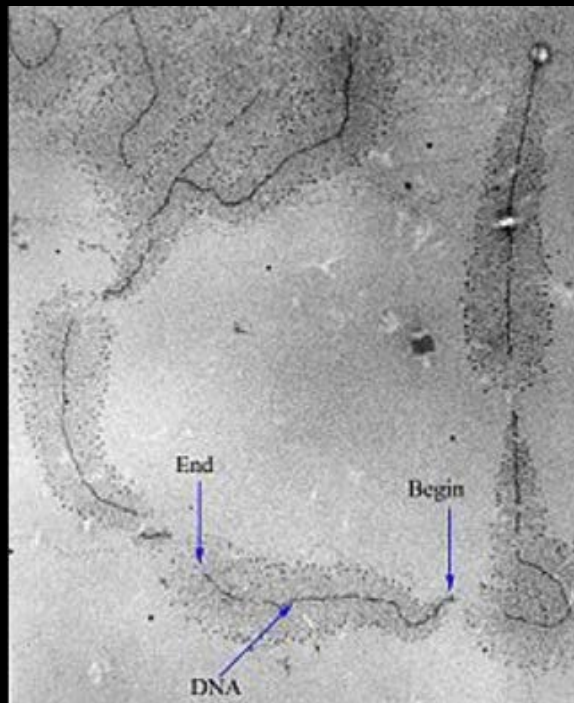


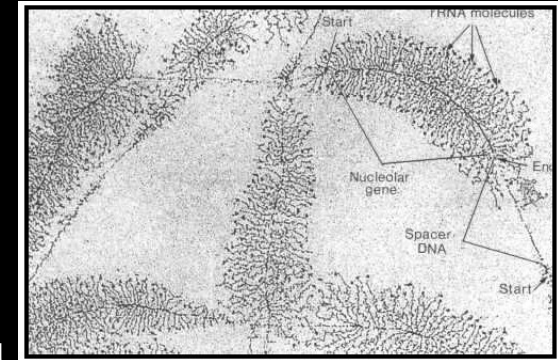
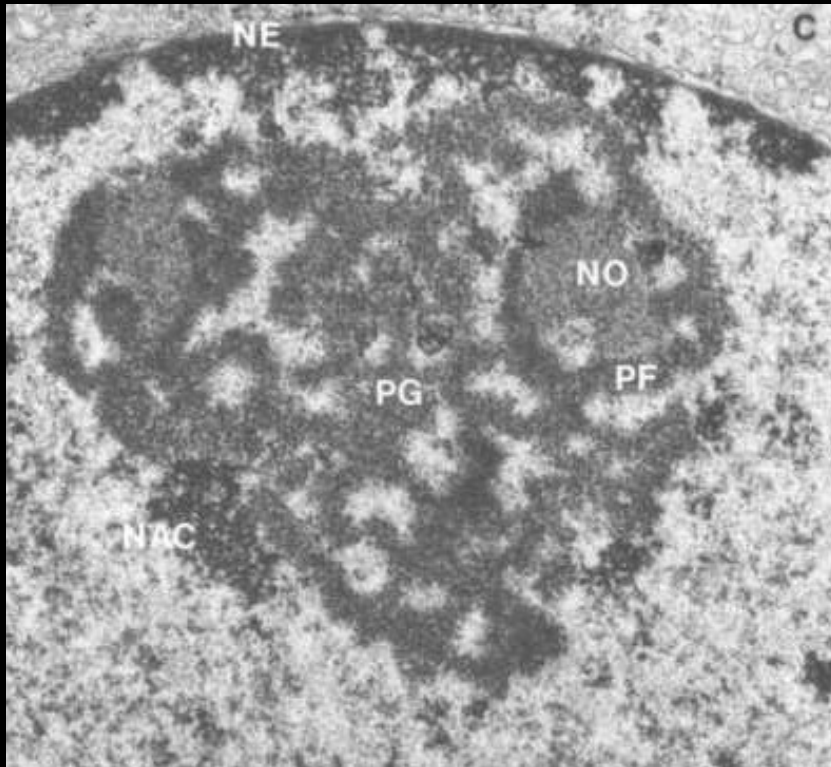
B

Nucleoli / DAPI



Nucleolus





NOR

400 (540) rDNA genů

FIBRILÁRNÍ CENTRUM (FC):

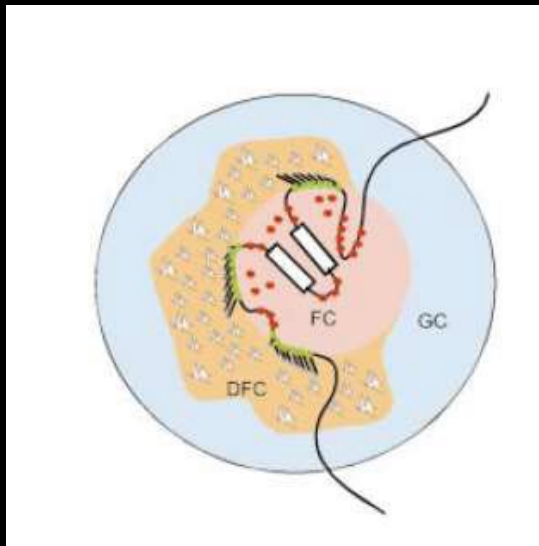
zodpovídá za změny v buněčné aktivitě

DENSE FIBRILAR COMPONENT (DFC):

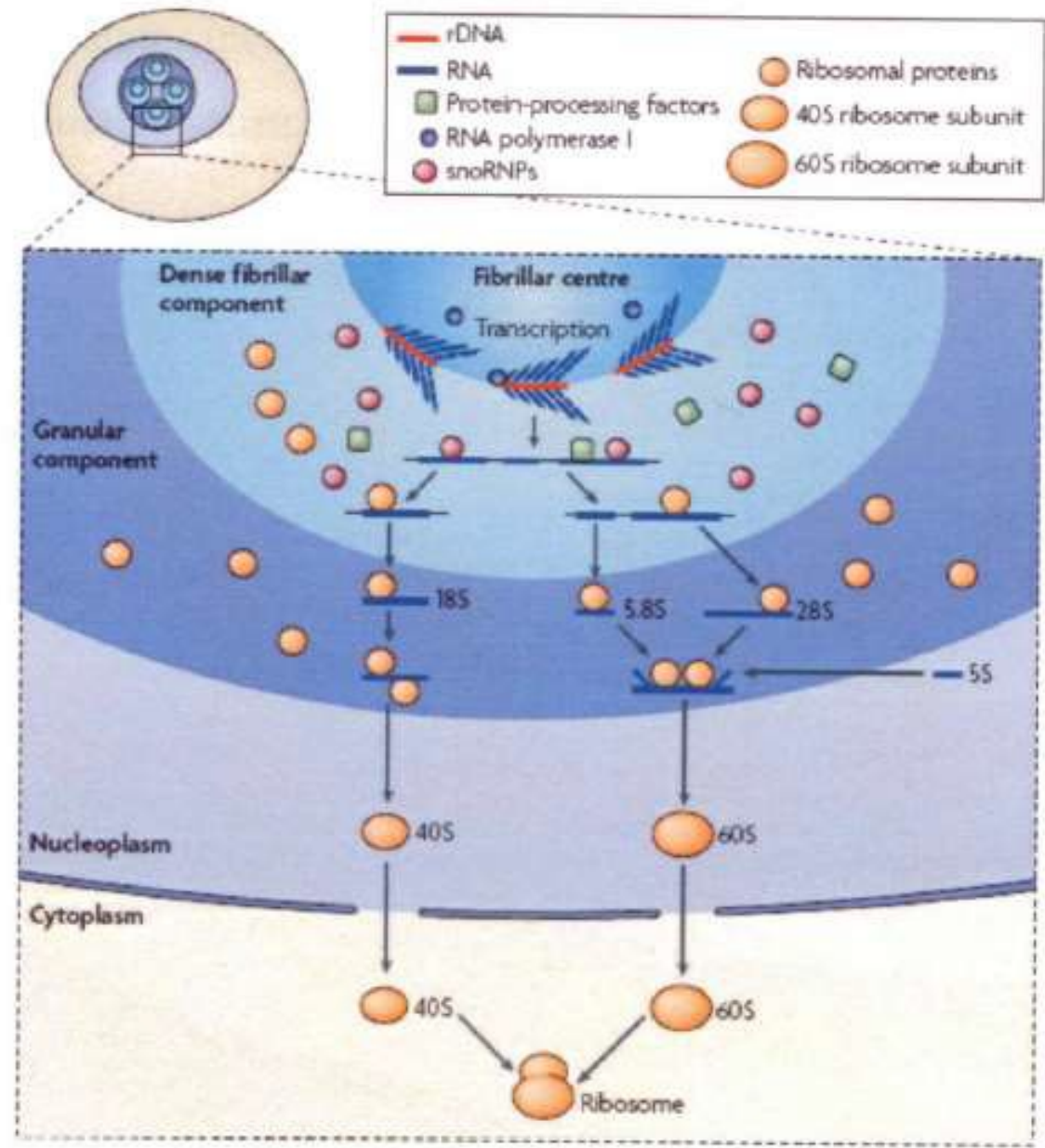
místo syntézy ribozomálních podjednotek

GRANULAR COMPONENTS:

ukotvuje DFC a FC



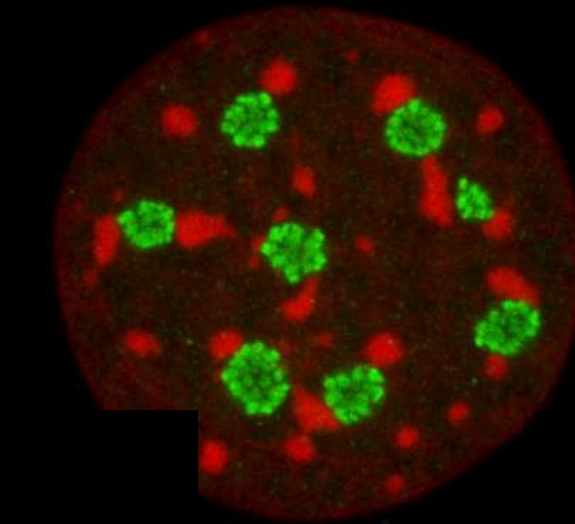
Tvorba ribozomů je komplexní proces zahrnující transkripci 45S prekurzorické rRNA, její vyhrávání, modifikaci a asociaci s ribozomálními proteiny a 5S rRNA, která se syntetizuje mimo jádérko. Vyhrávání rRNA probíhá v procesomu, který obsahuje mnoho komplexů a snRNA



SUV39h-independent association of HP1b with fibrillar- positive nucleolar regions



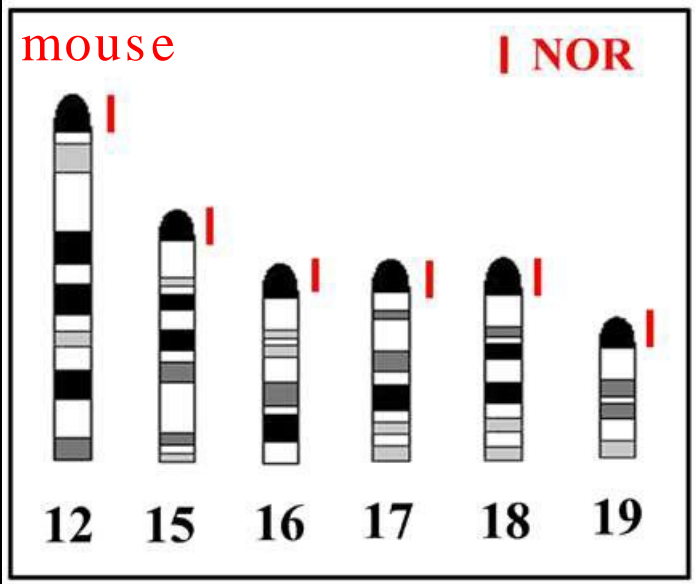
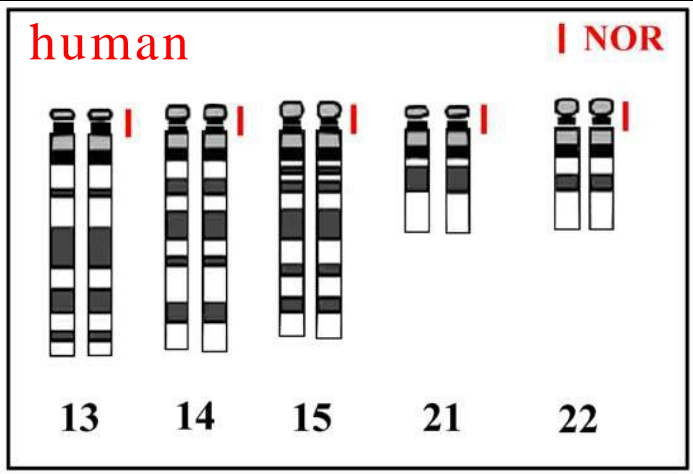
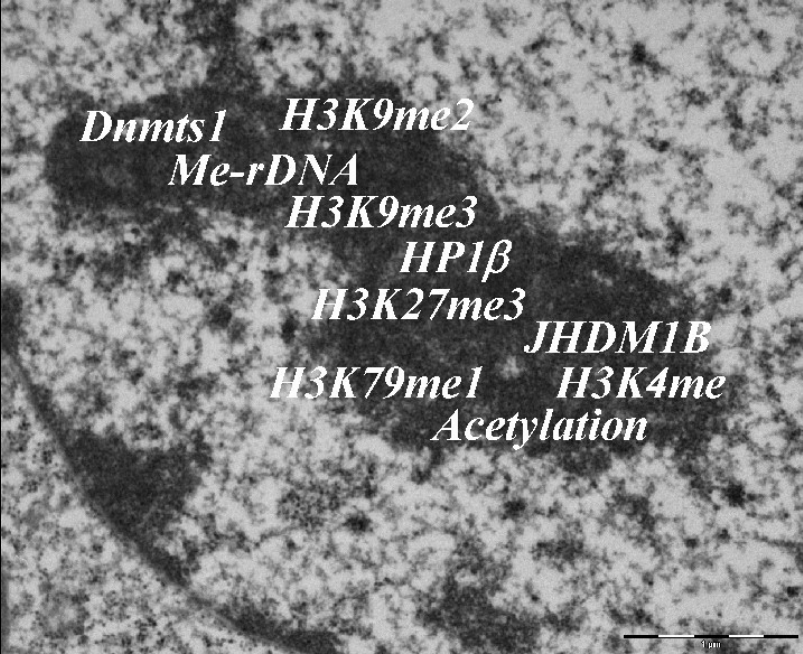
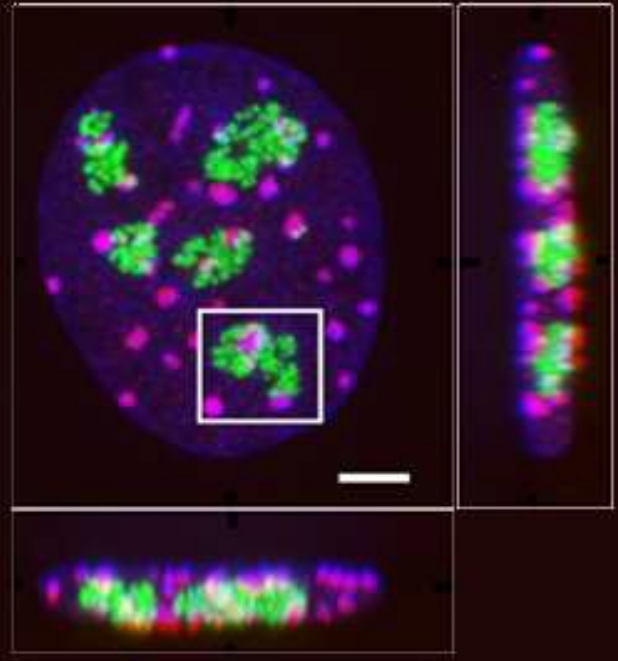
12/8/2010 3:40:48 PM



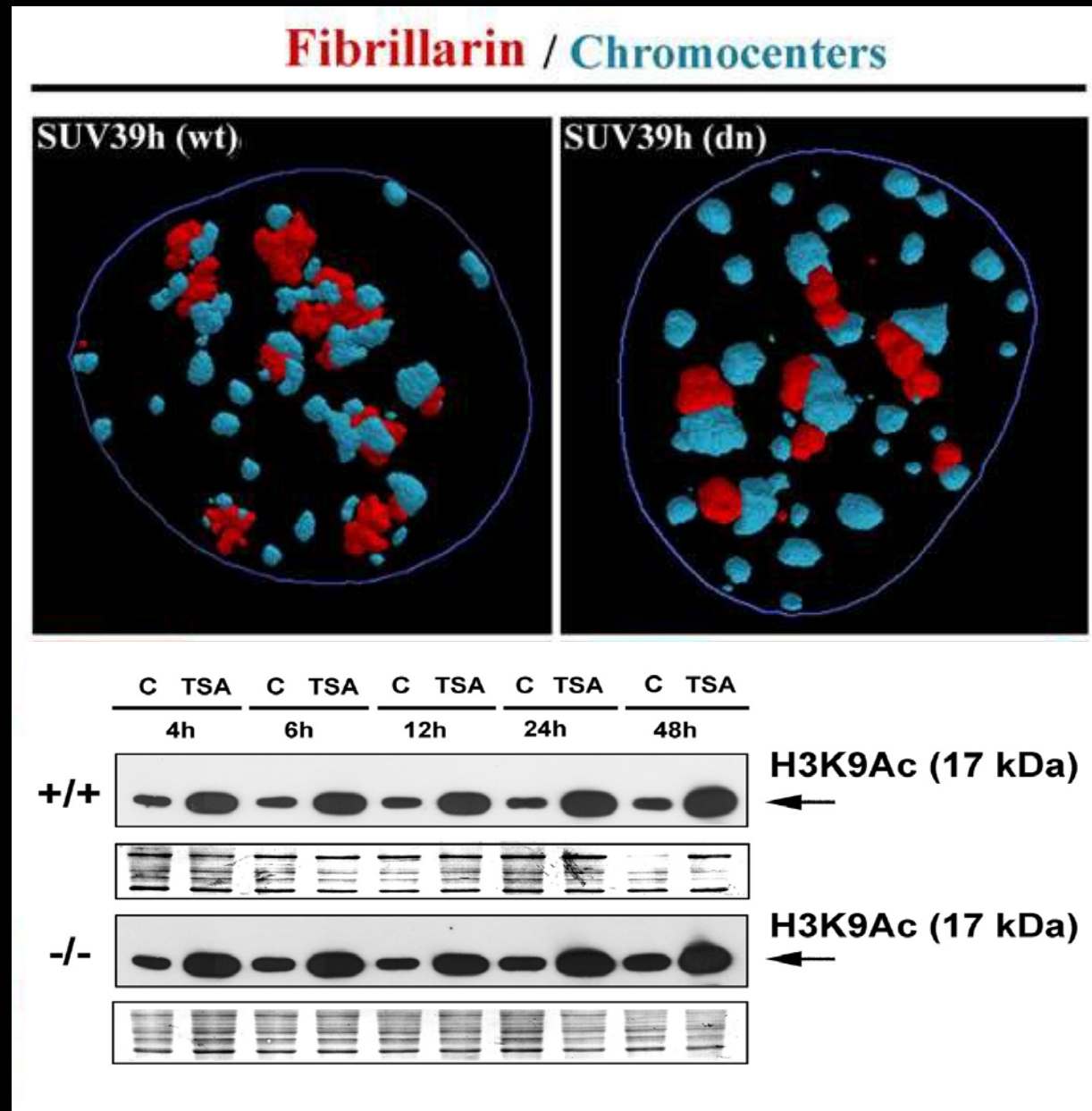
° pos=1 1 10

Epigenetics of Nucleoli

Fibrillarin/chromocenters



Differences between epigenetics of nucleoli and surrounding chromatin

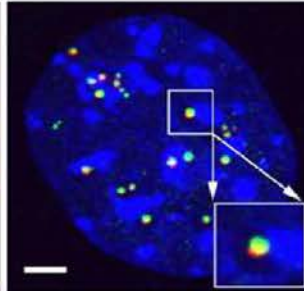
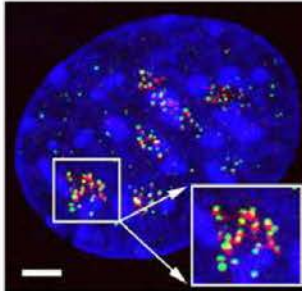


a**Pol I / HP1 / DNA**

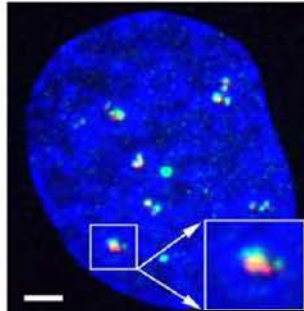
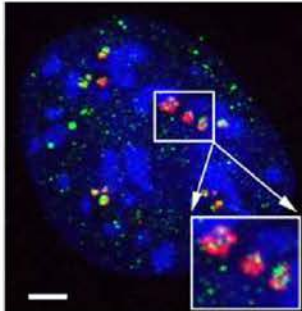
Suv39h (wt)

Suv39h (dn)

CONTROL



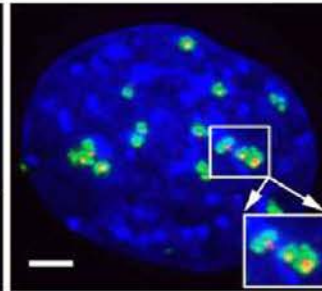
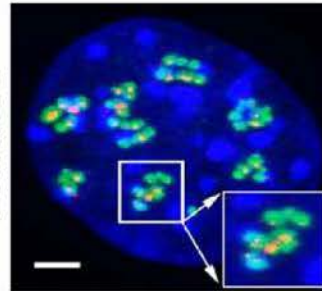
TSA

**b****Pol I / Fibrillarin / DNA**

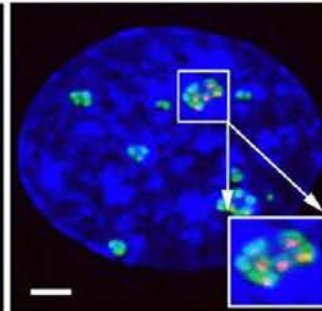
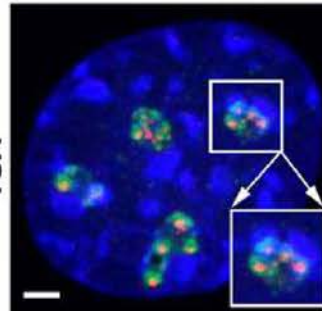
Suv39h (wt)

Suv39h (dn)

CONTROL

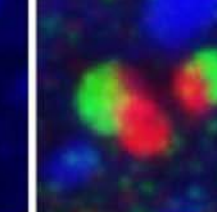
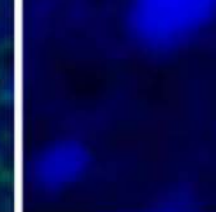
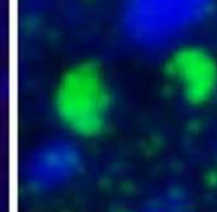
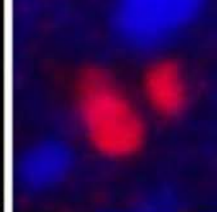
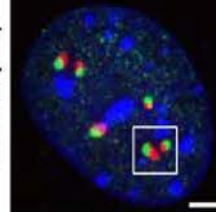
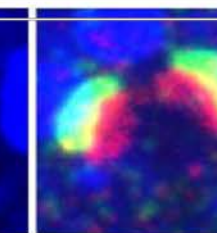
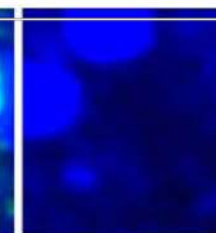
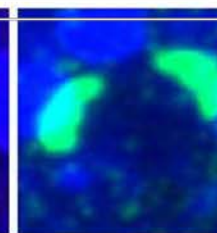
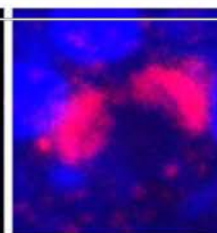
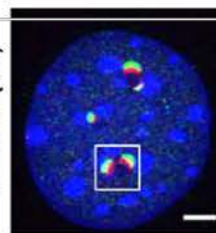


TSA

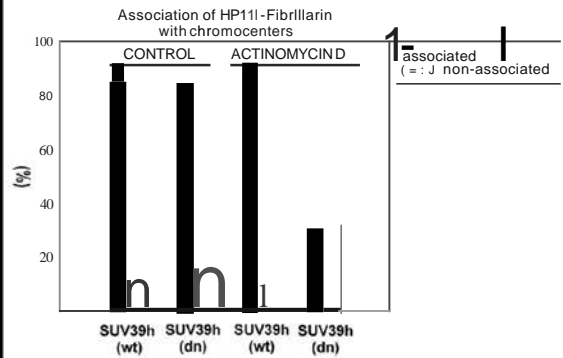
**c****Fibrillarin / HP1 / DNA**

SUV39 (wt)

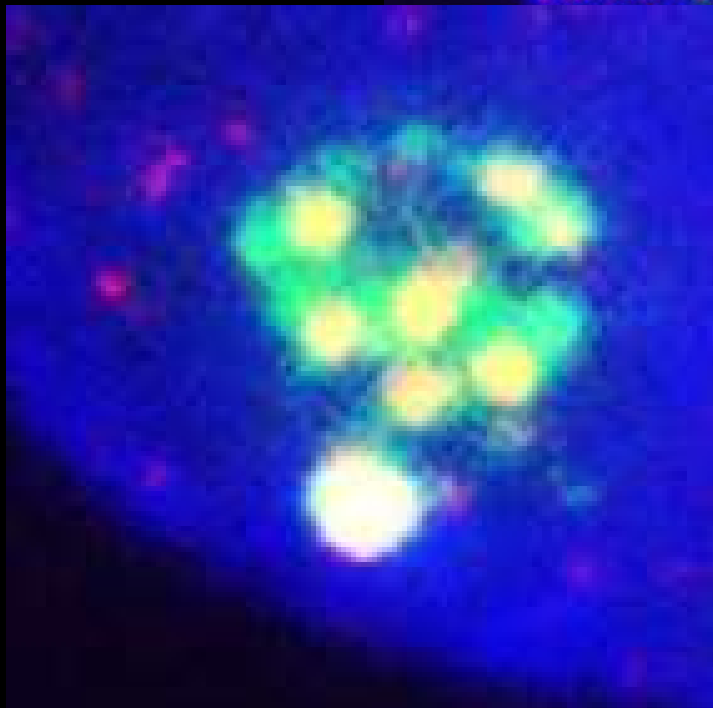
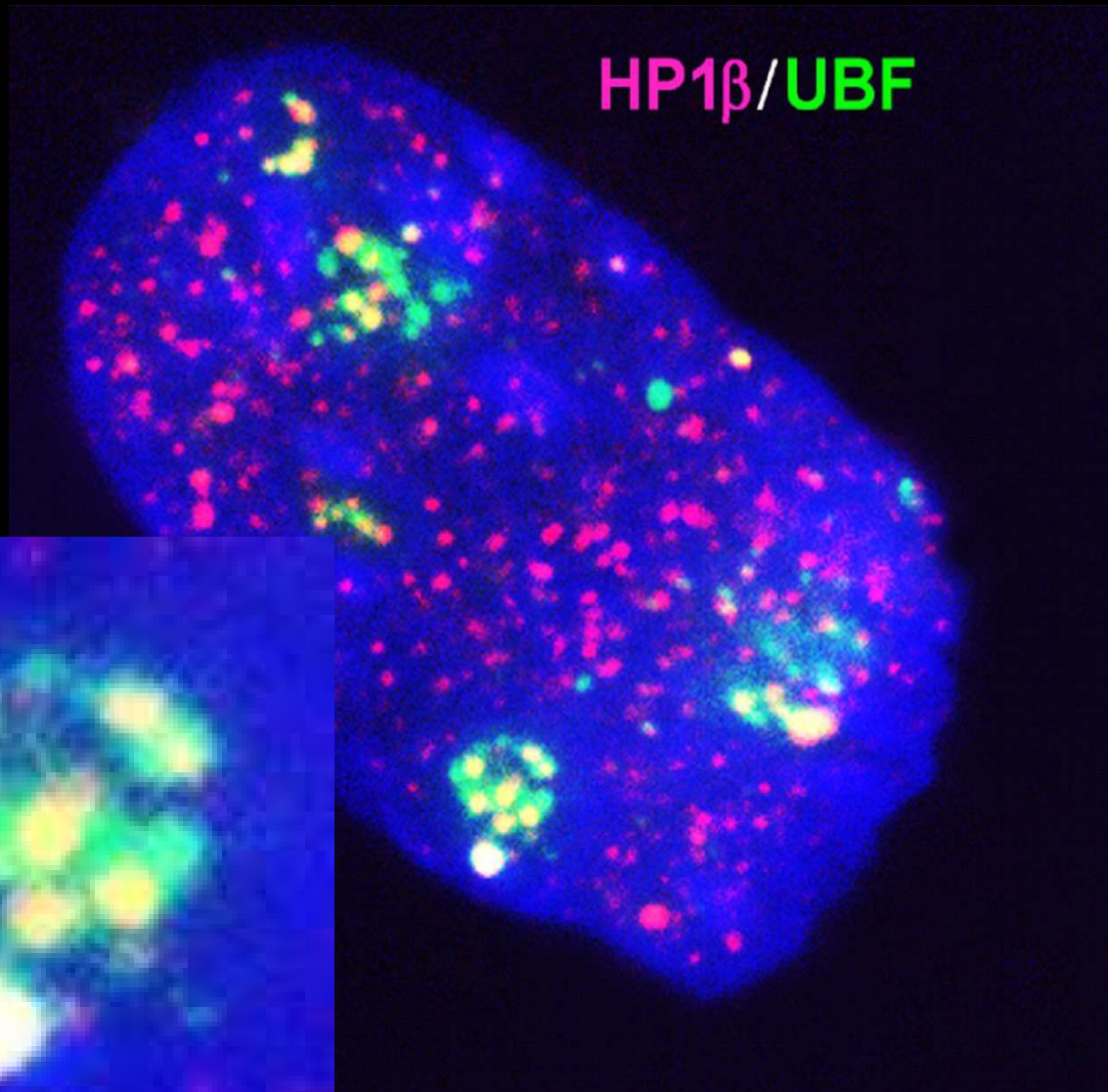
SUV39 (dn)

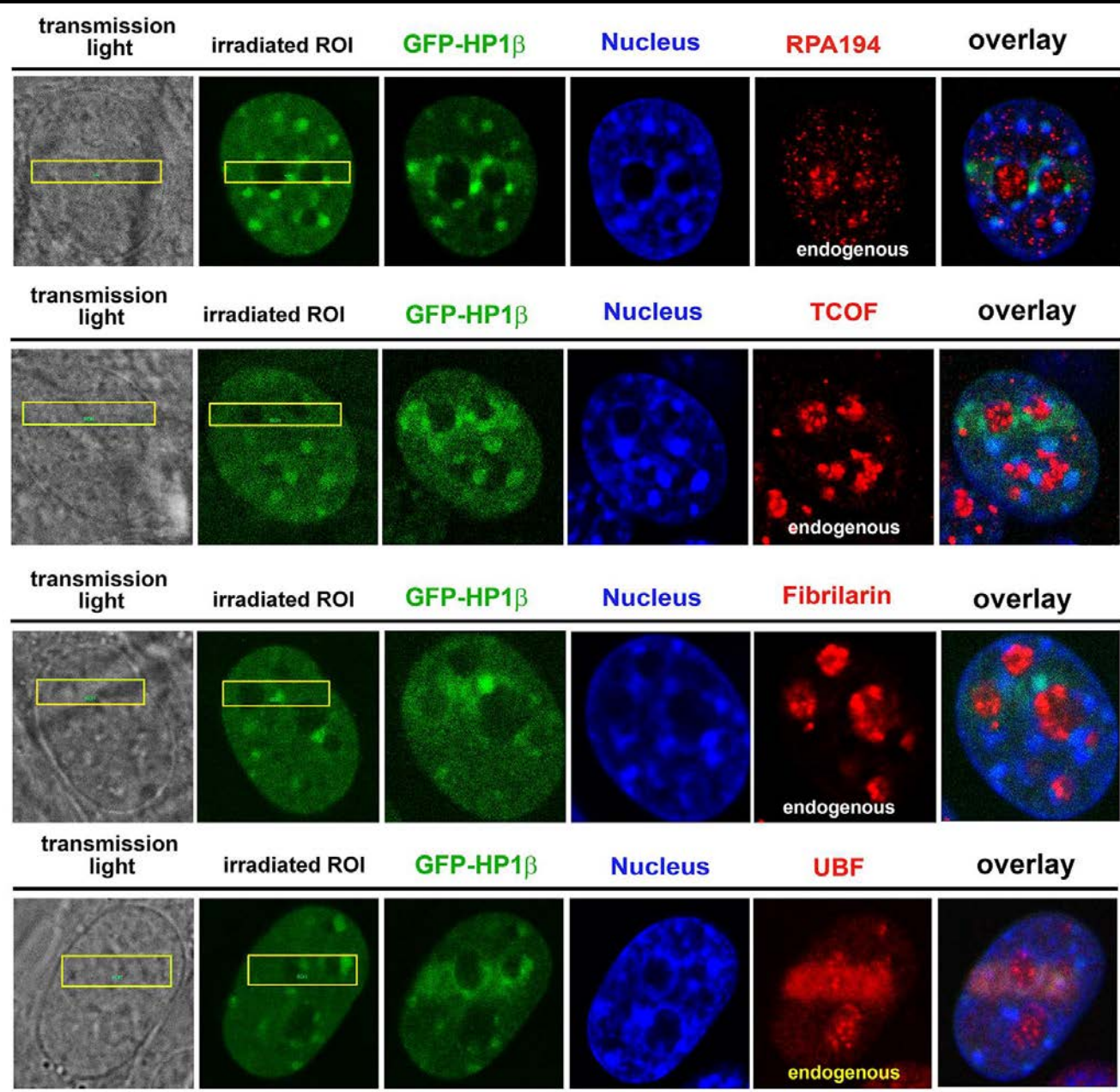


ACTINOMYCIN D



MEF cells



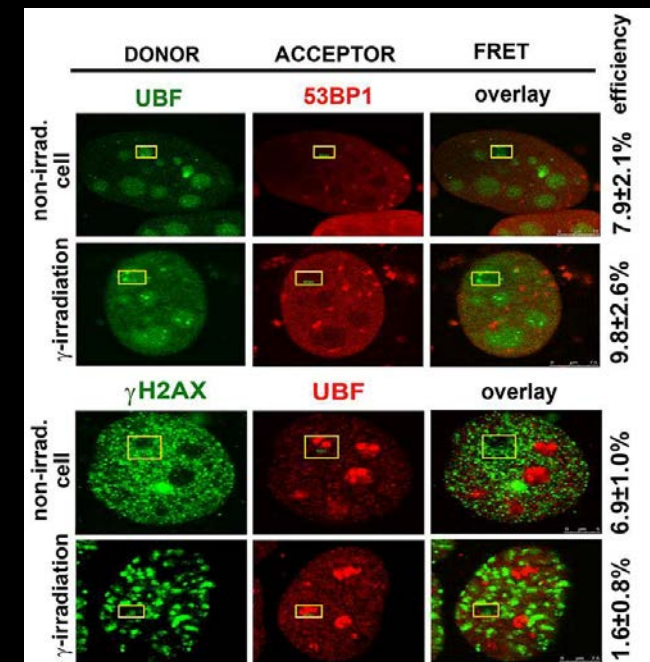
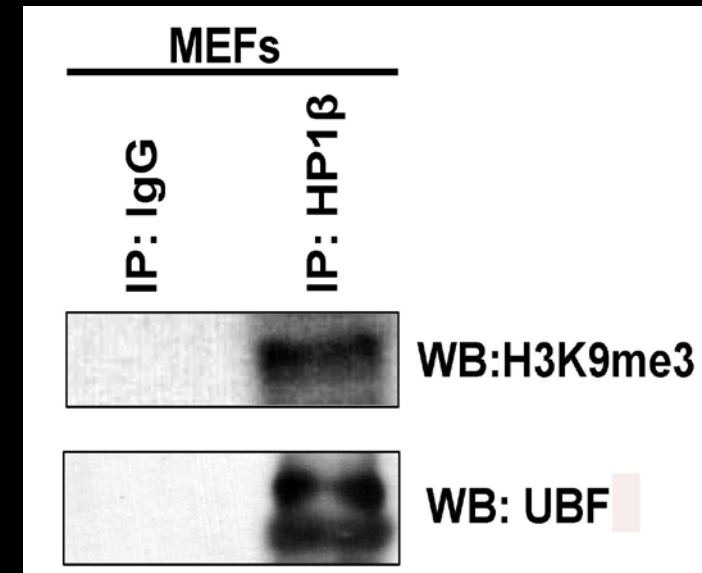
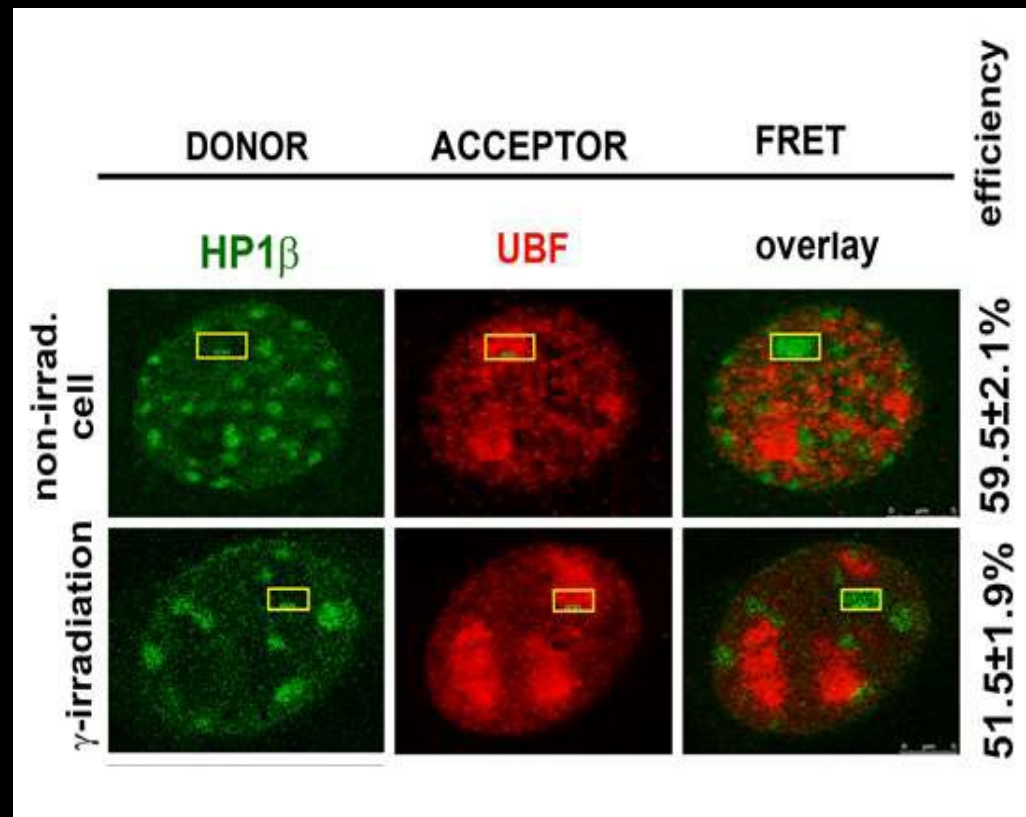


Experiments: Petra Sehnalová

HP1b interacts with UBF

FRET analysis

HP1 β – UBF
FRET efficiency > 50%



Experiments: Petra Sehnalová