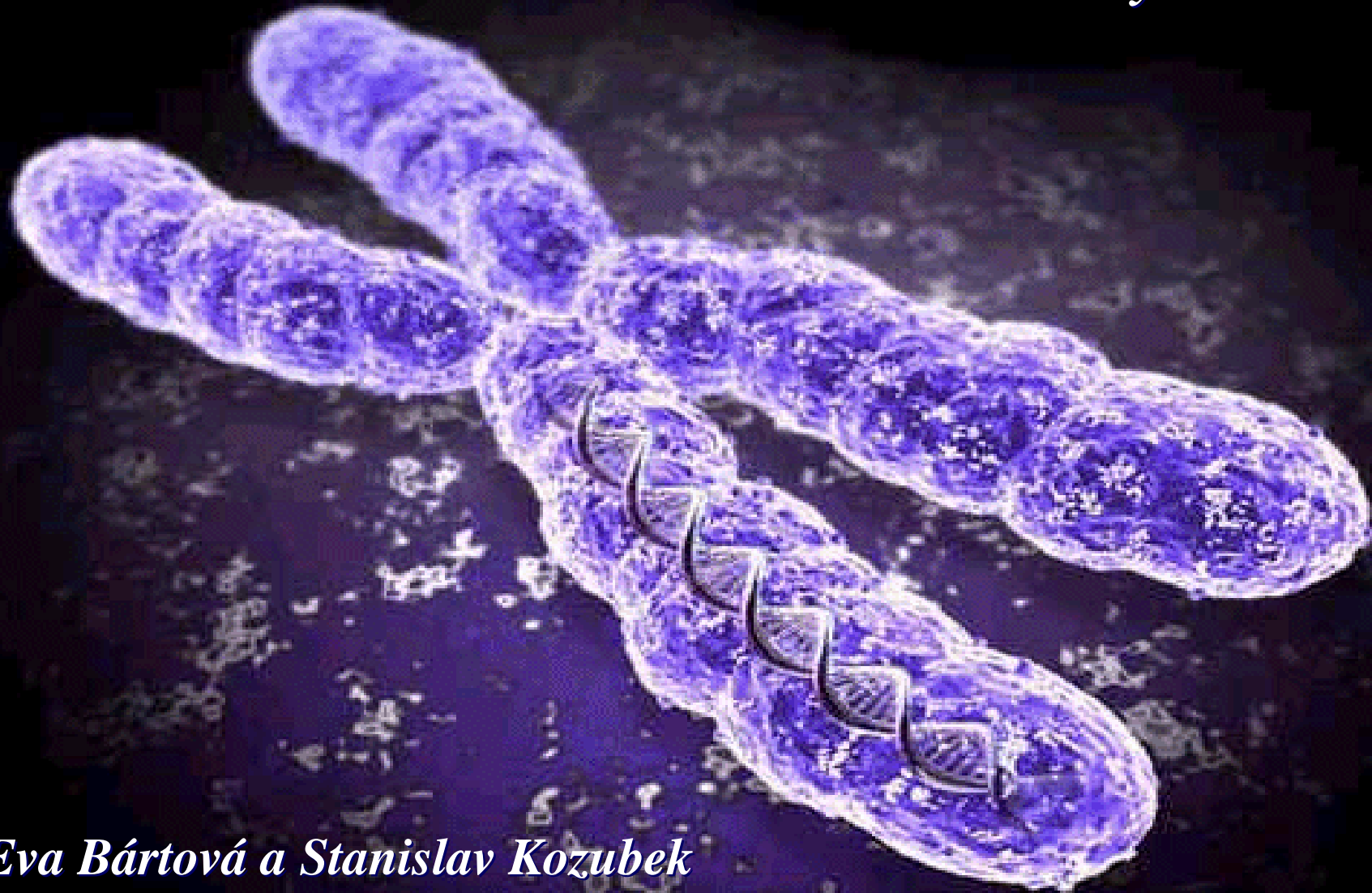


- **Kompartimentalizace interfazních jader**
- **Morfologické a jaderně-topografické aspekty buněčné diference.**
- **Apoptóza a struktura chromatinu.**
- **Cytoskelet a jaderná matrix - studium cytoskeletu pomocí mikroskopie s vysokým rozlišením.**
- **BAC/PAC knihovny a jejich využití pro FISH techniku**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

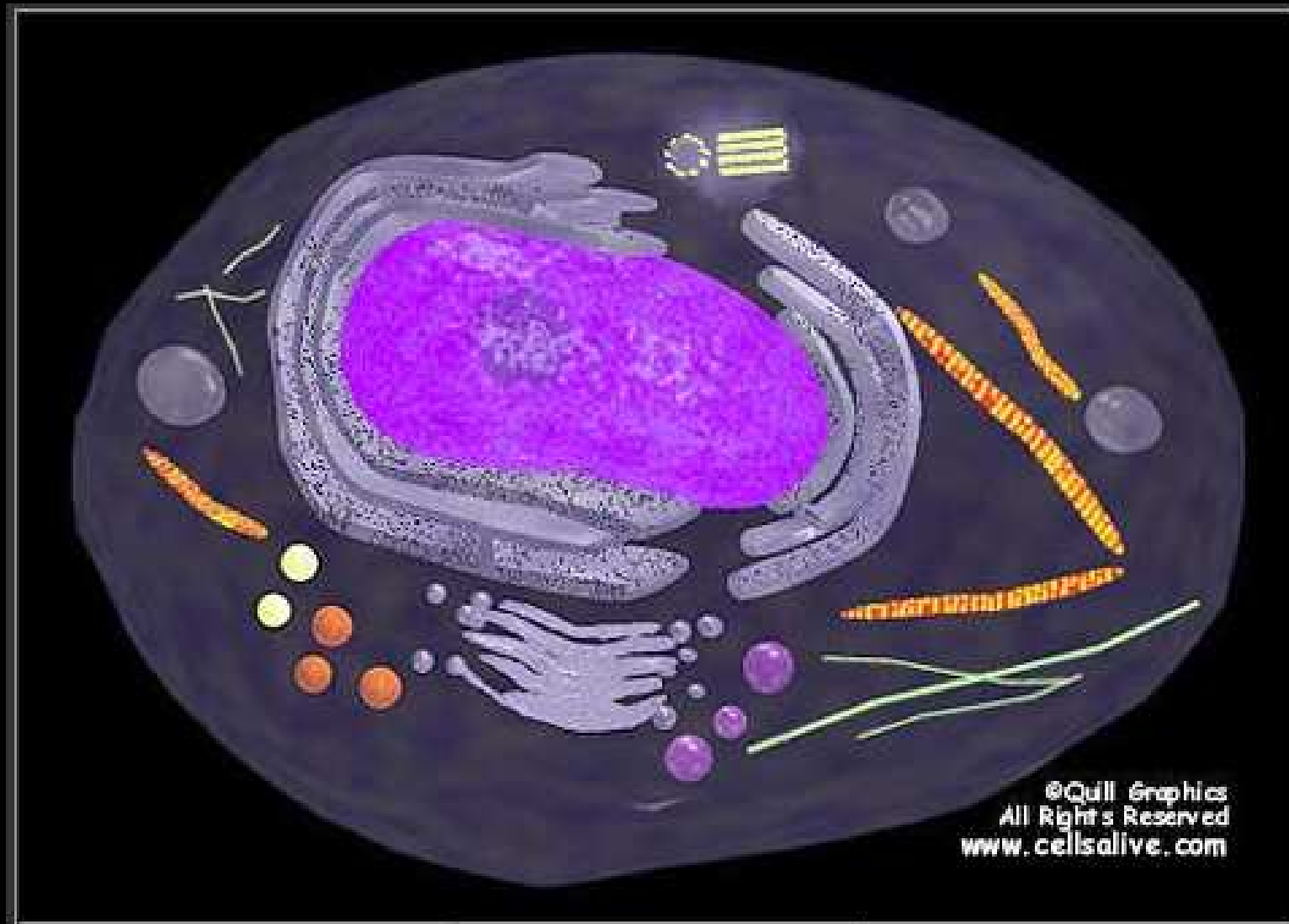
*Struktura chromatinu vyššího řádu*



*Eva Bártoová a Stanislav Kozubek*

[www.tqnyc.org](http://www.tqnyc.org)

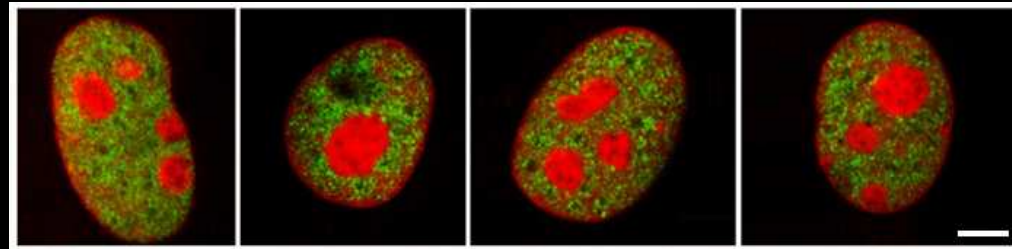
# Buněčné jádro



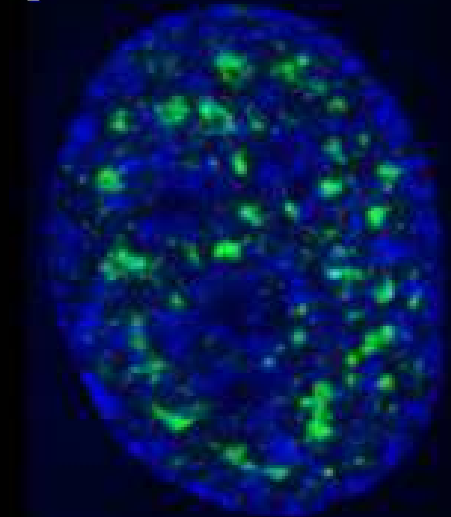
©Quill Graphics  
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[www.cellsalive.com](http://www.cellsalive.com)

## Nuclear compartments:

1. Nucleolus
2. Splicing speckles
3. Cajal bodies
4. PML bodies
5. snRNP
6. Clustery interchromatinový granulí (IGCs)



speckles



Cajal  
Bodies

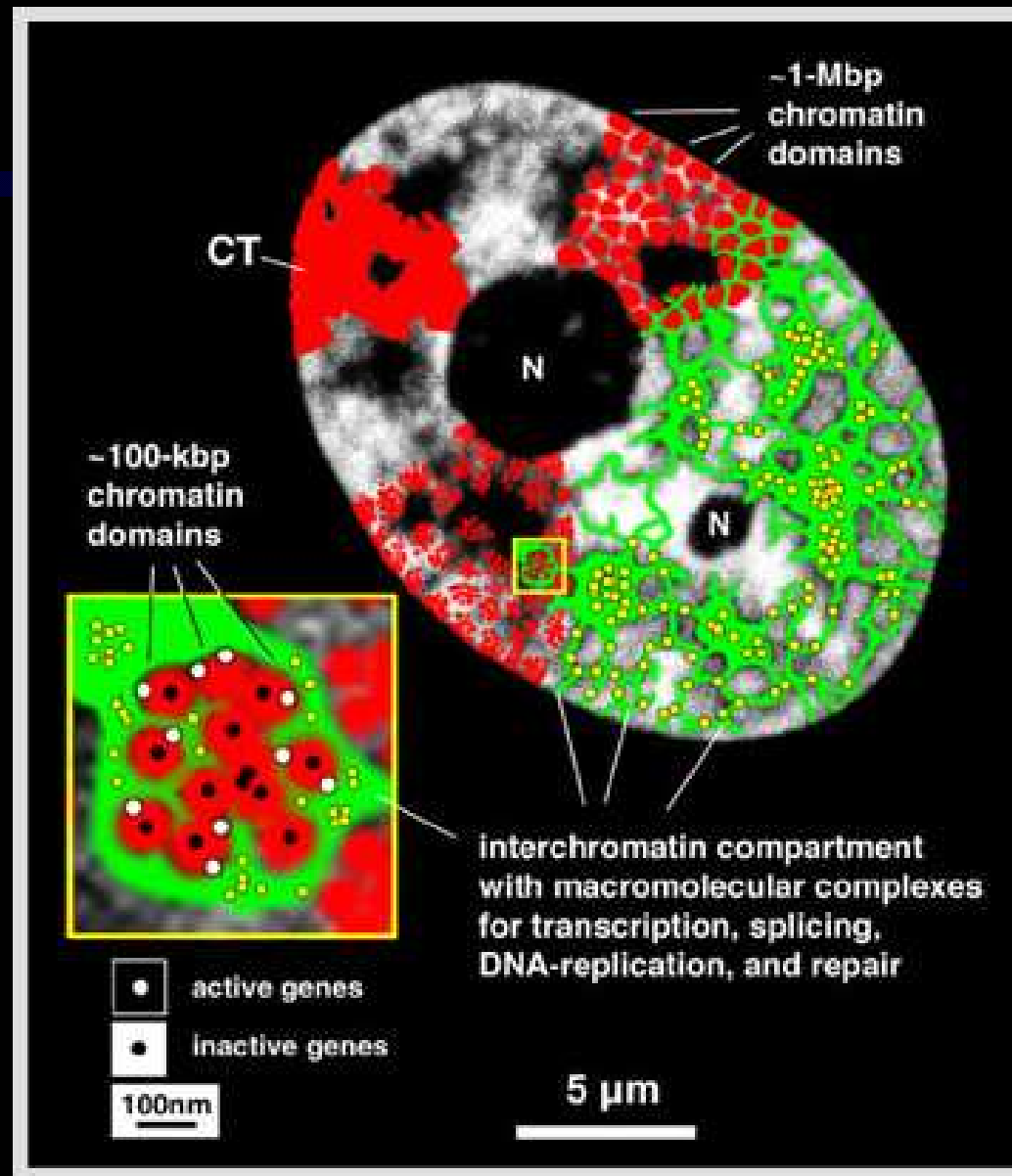


PML  
Nuclear  
Bodies

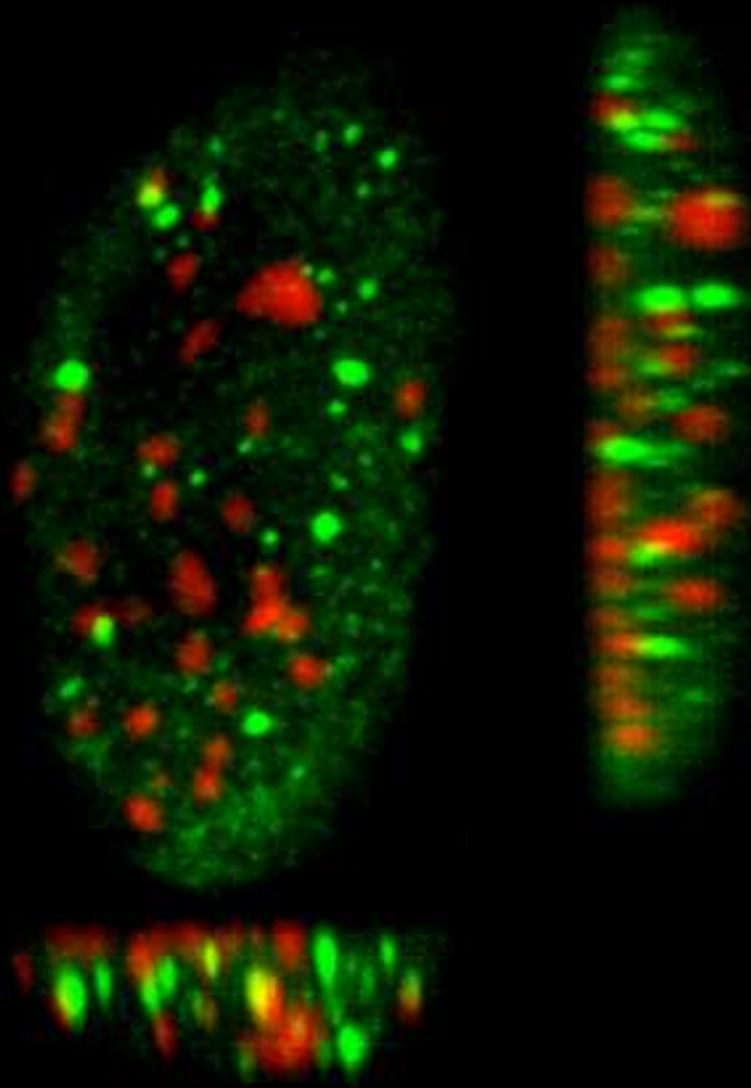
### Roles of snRNPs

- 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

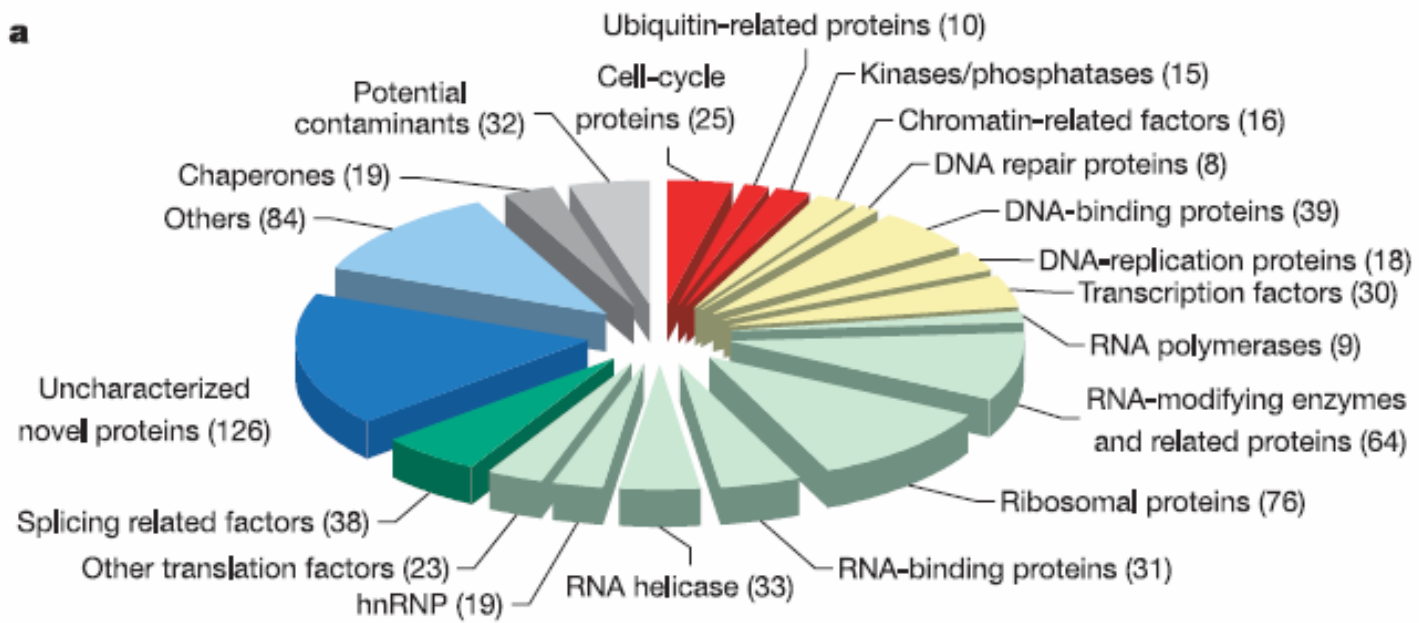
# CT-IC MODEL (T. Cremer)



# HP1 proteins



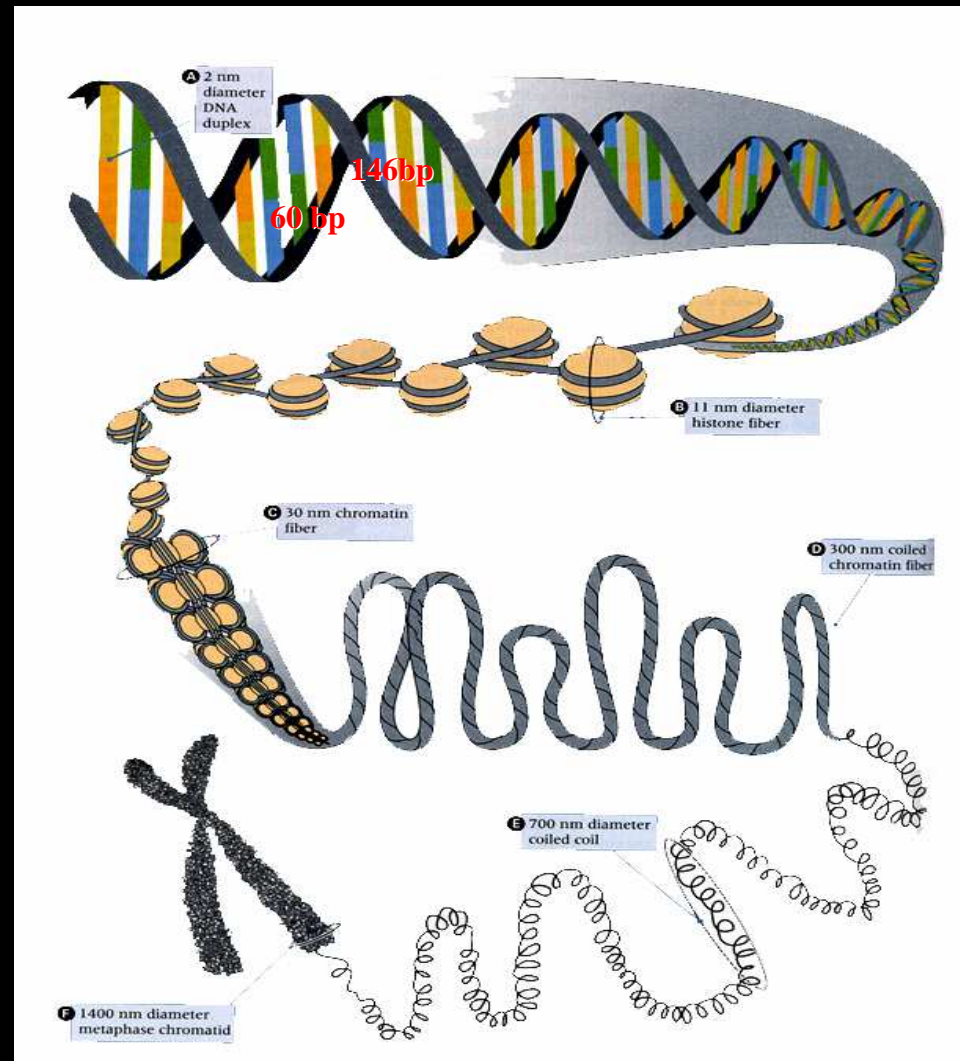
**a**



Andersen et al., Nature, 2005

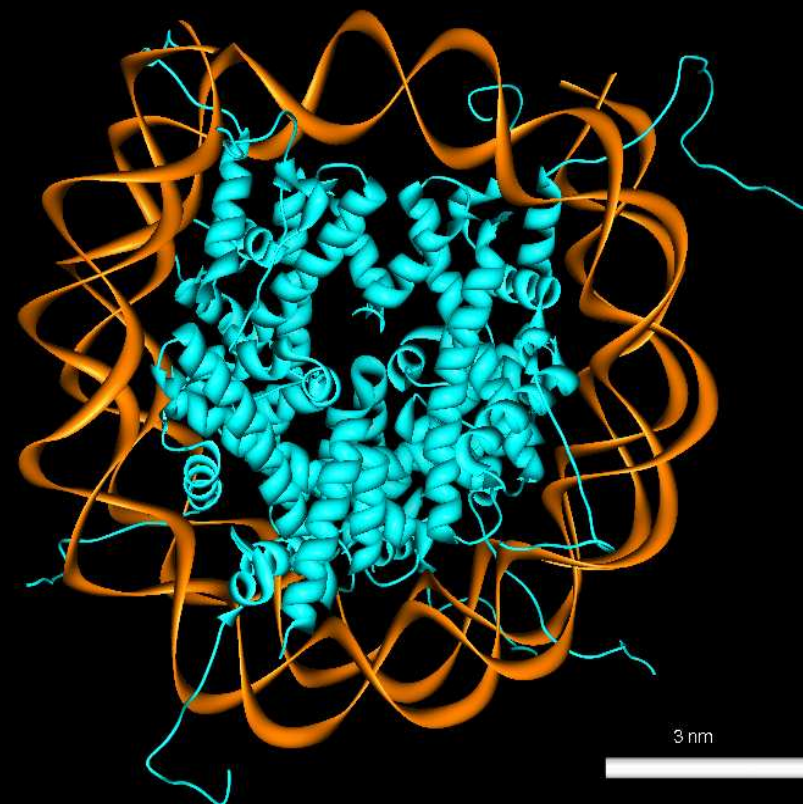


**CHROMATIN:** materiál jader eukaryotních buněk; nukleoproteinový komplex tvořený DNA vázanou na histony a další bílkoviny. V nedělicím se jádru lze rozlišit **euchromatin**, kde probíhá transkripce, a **heterochromatin**, který je transkripčně inaktivní.





**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.

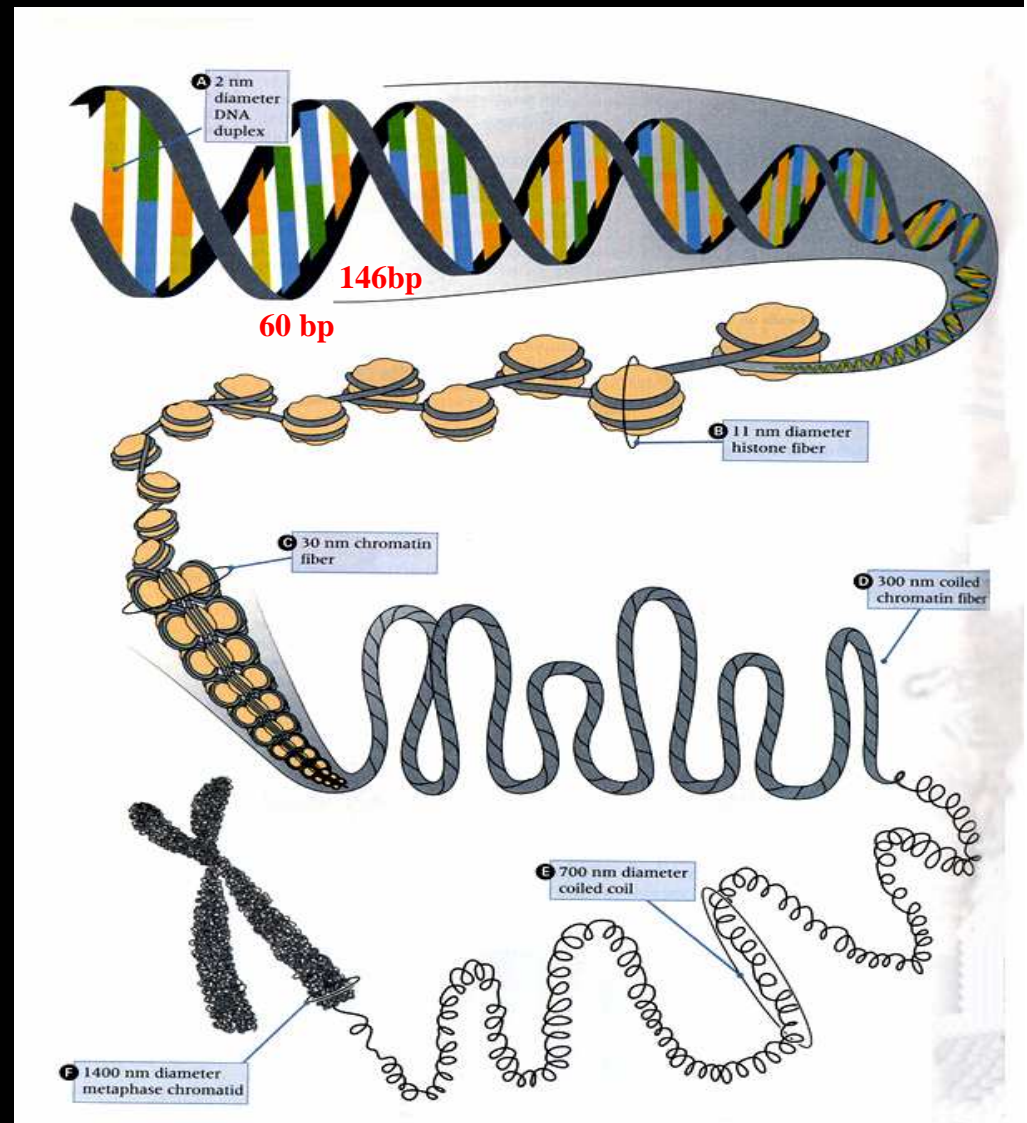


# NUKLEOSOM

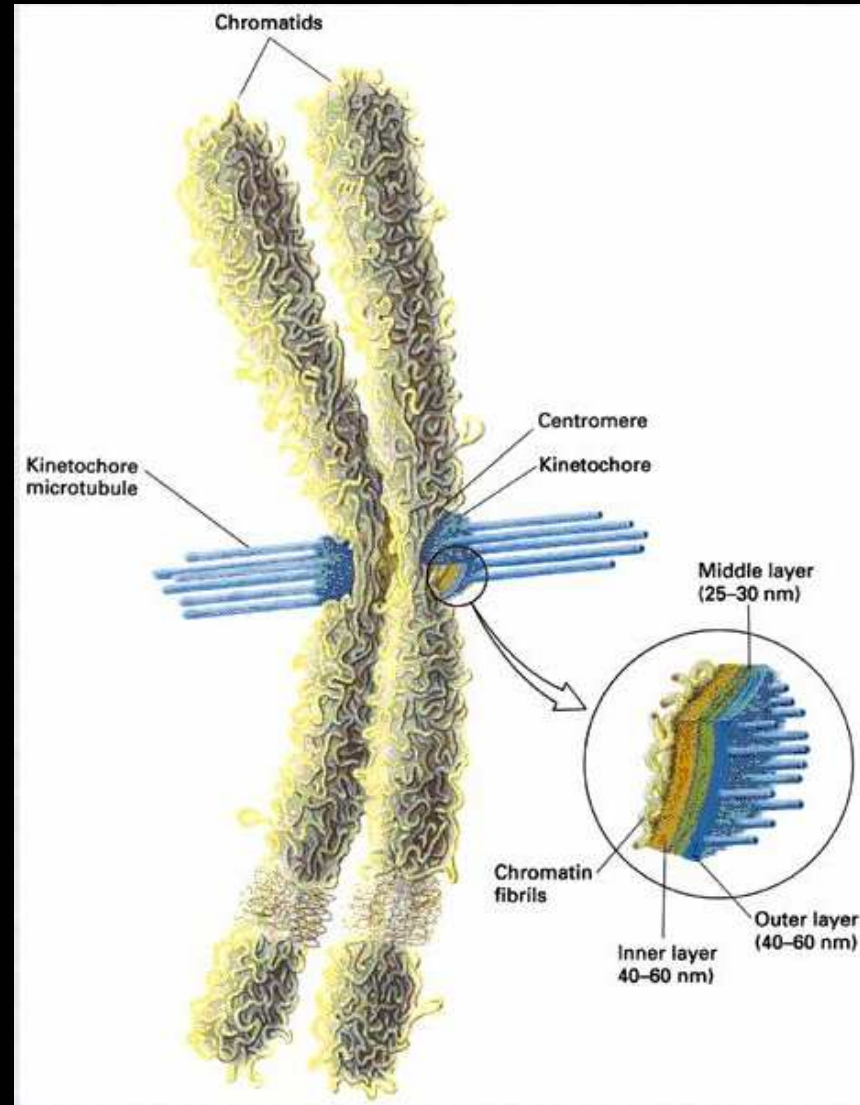
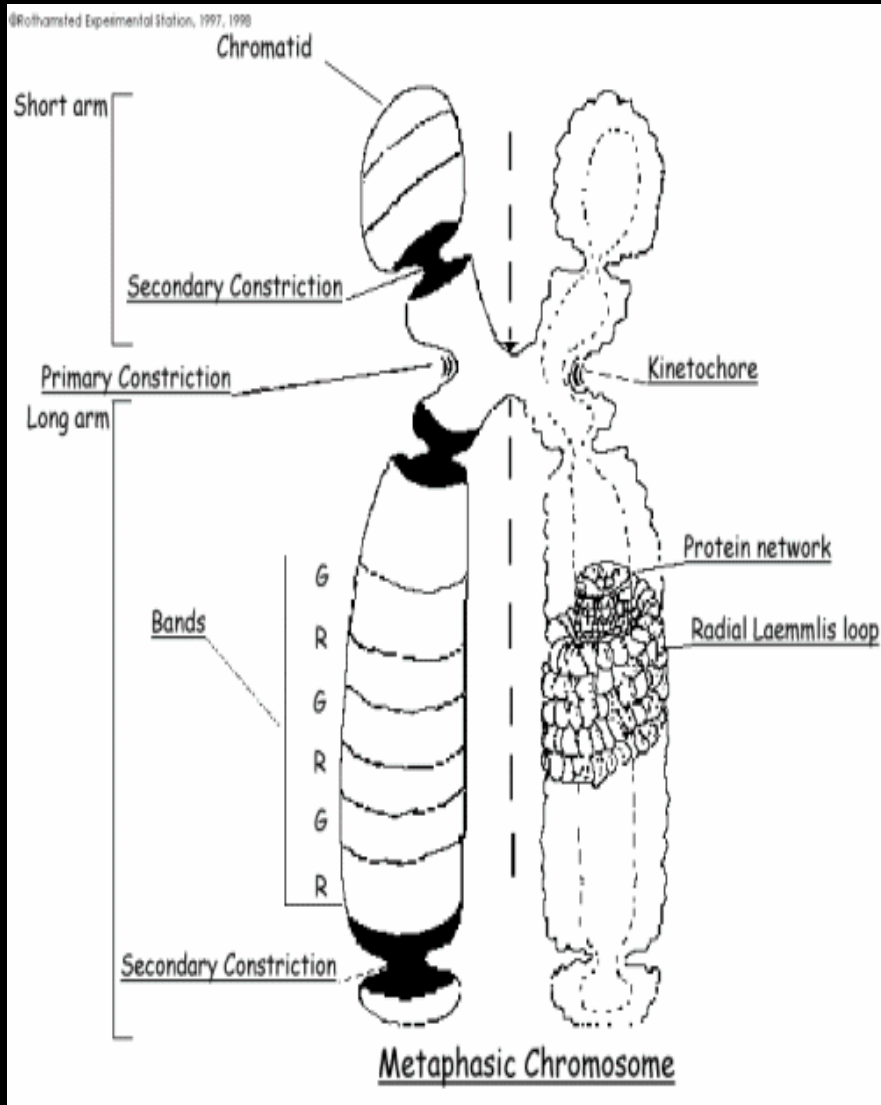


Joseph Roland 2003

**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.







Kondenzace chromatinu

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

The centromere is together with telomeres and origin of replications one of the essential parts of any eukaryotic chromosomes. The centromere is usually defined by specific DNA (AT-rich) sequences which are in higher eukaryotes typical tandem repetitive sequences, often called "satellite DNA". SAT1,2,3, alpha satellites, beta satellites.

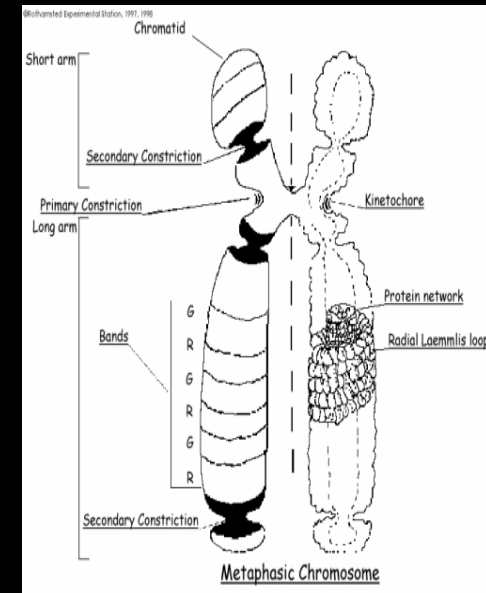
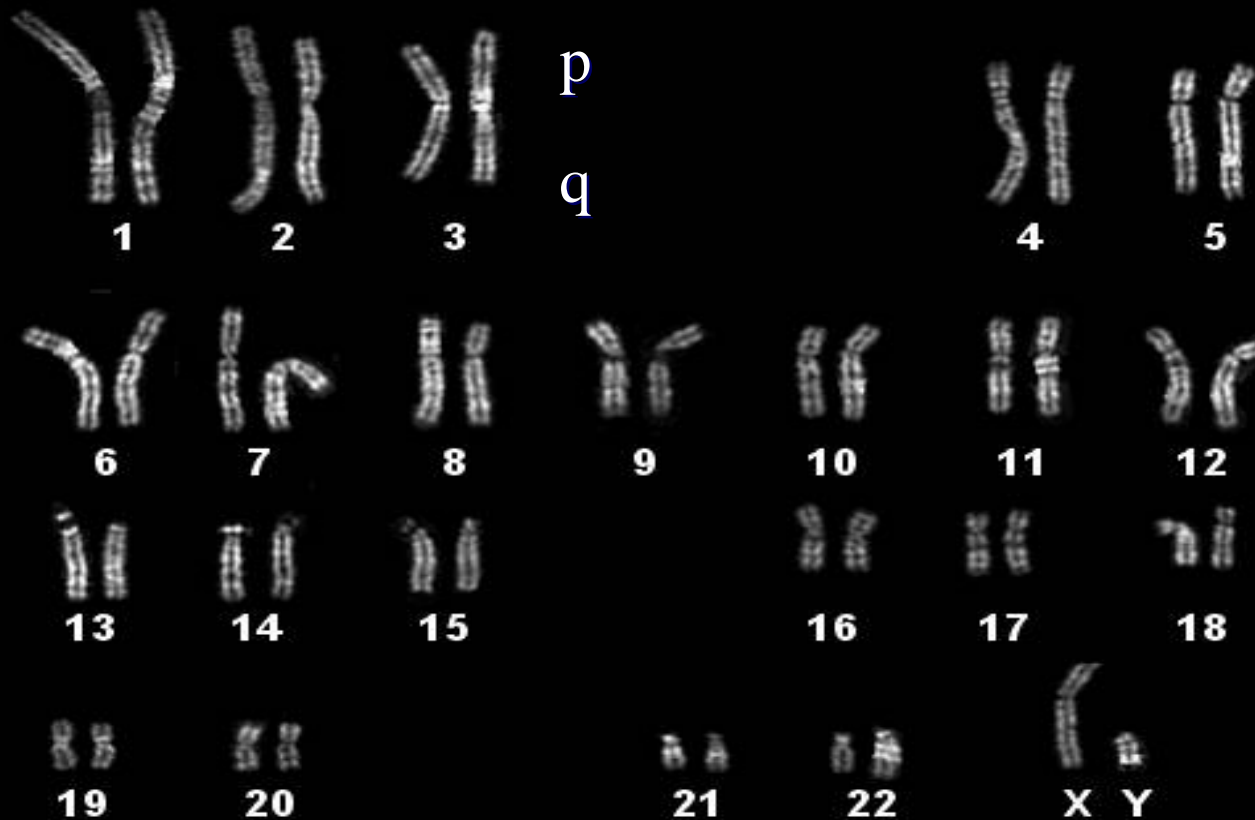
CENP A, B, C, G, H.

The kinetochores are the sites where the spindle fibers attach. Kinetochores and the spindle apparatus are responsible for the movement of the two sister chromatids to opposite poles of dividing cell nucleus during anaphase.

A centromere functions in sister chromatid adhesion, kinetochore formation, and pairing of homologous chromosomes.

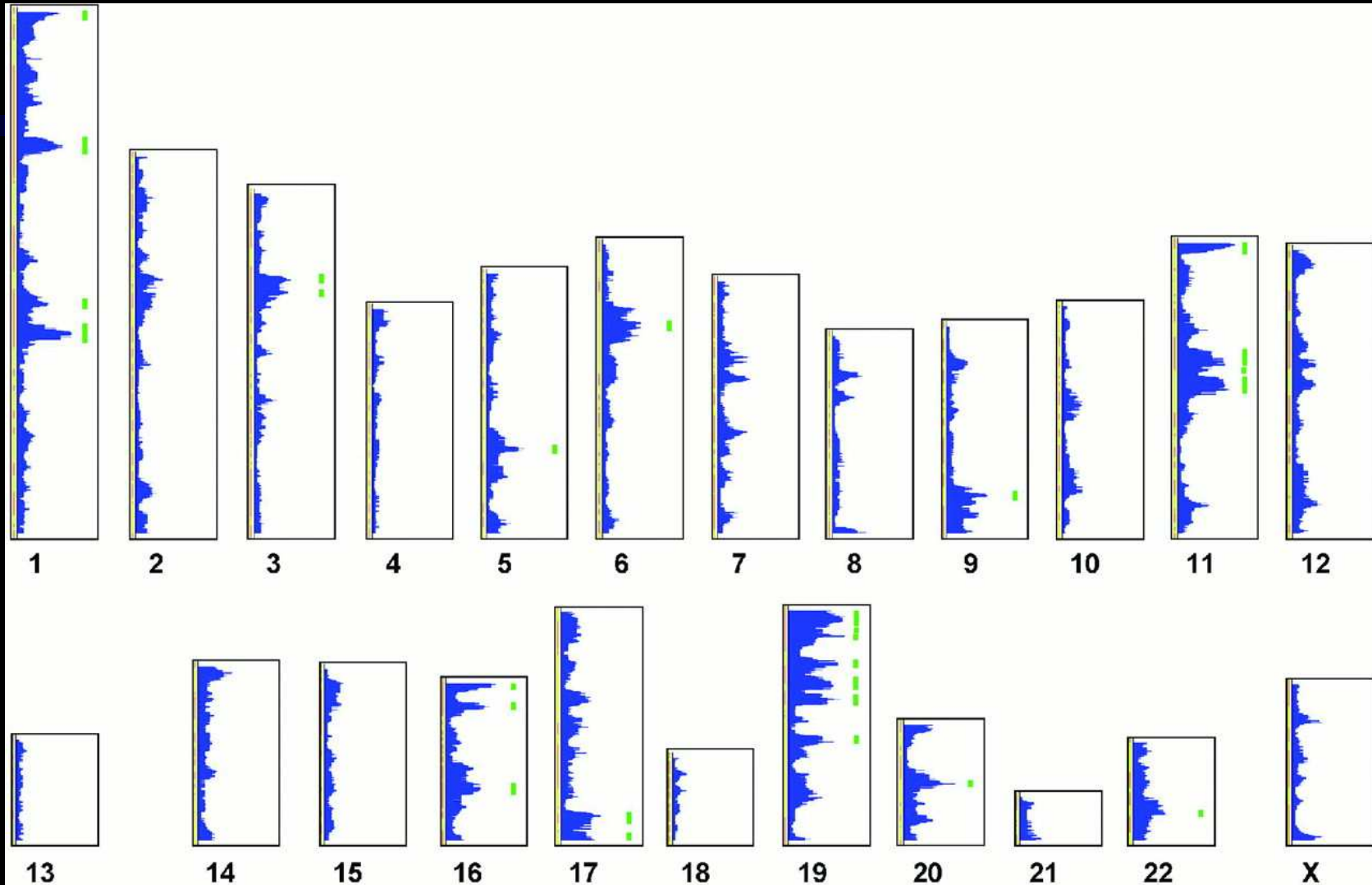
When the centromere doesn't function properly, the chromosomes don't align and separate properly, resulting in the wrong number of chromosomes in the daughter cells (aneuploidy), and conditions such as Down syndrome, if the cells survive at all.

# Typy chromozomů: meta- submeta-, akrocentrické

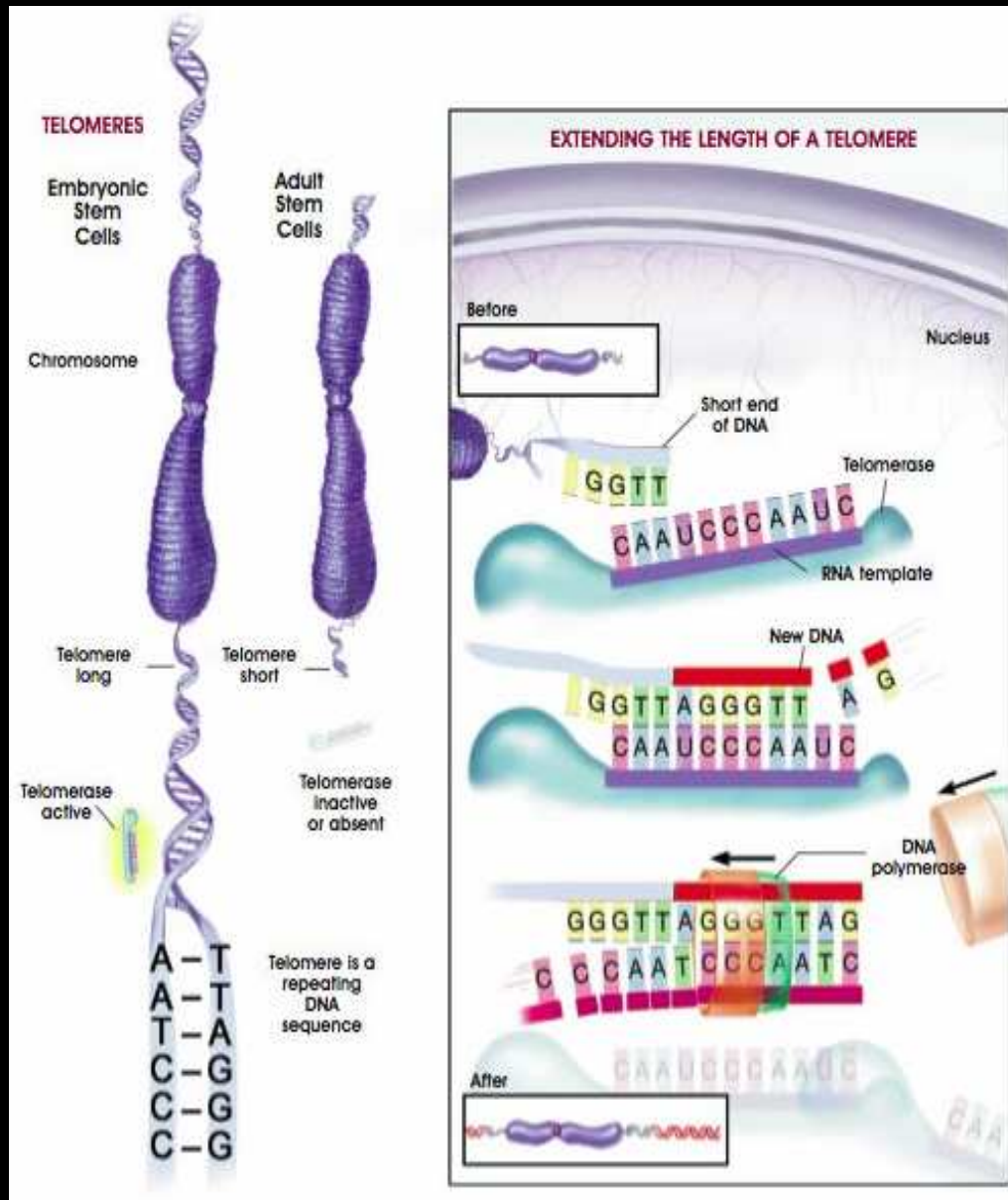




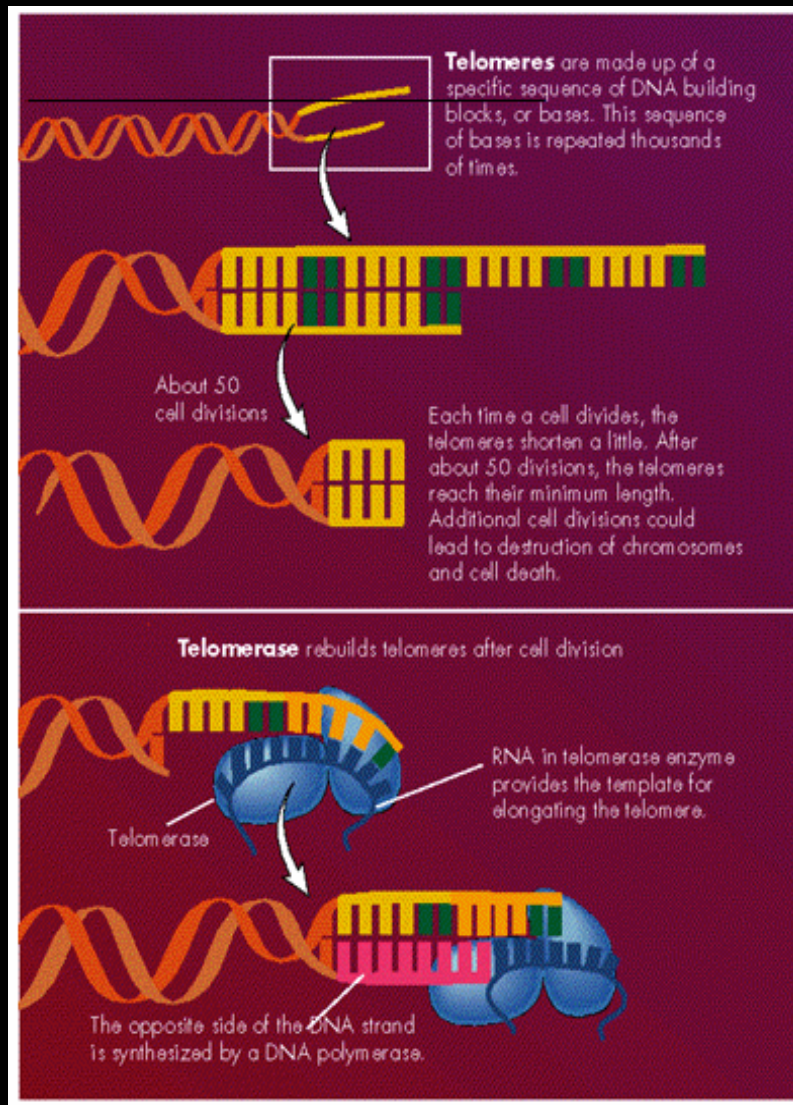
# Transcriptome map (Caron et al., 2001)



# Telomeres (TTAGGG sequence and different proteins)

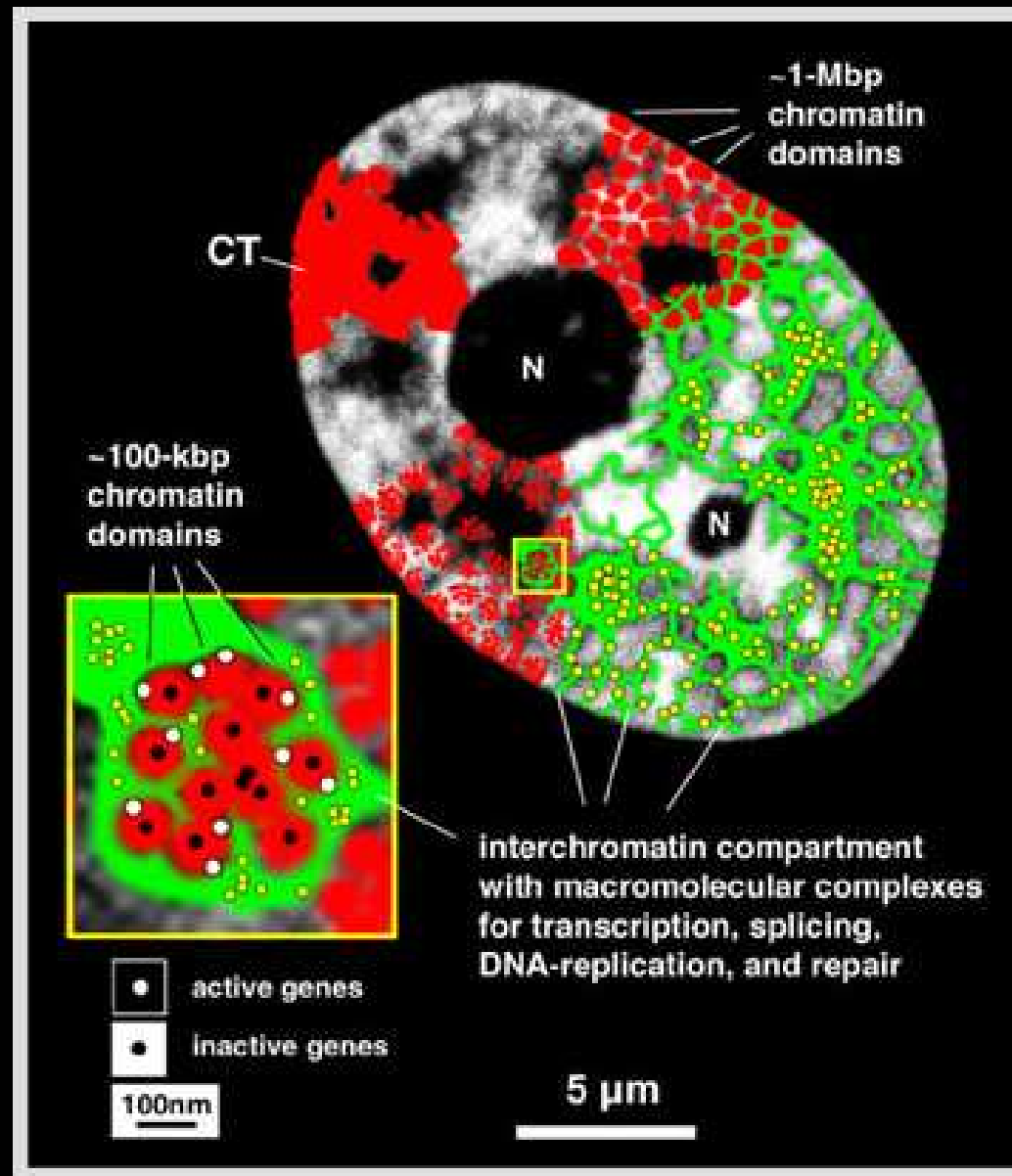


**Telomerase** is composed of two subunits, Telomerase Reverse Transcriptase (hTERT, the 'h' is for human) and hTR (Telomerase RNA). These two subunits are coded for by two different genes in the genome. Using hTR template, hTERT can add a six nucleotide repeating sequence, 5'-TTAGGG to the 3' strand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



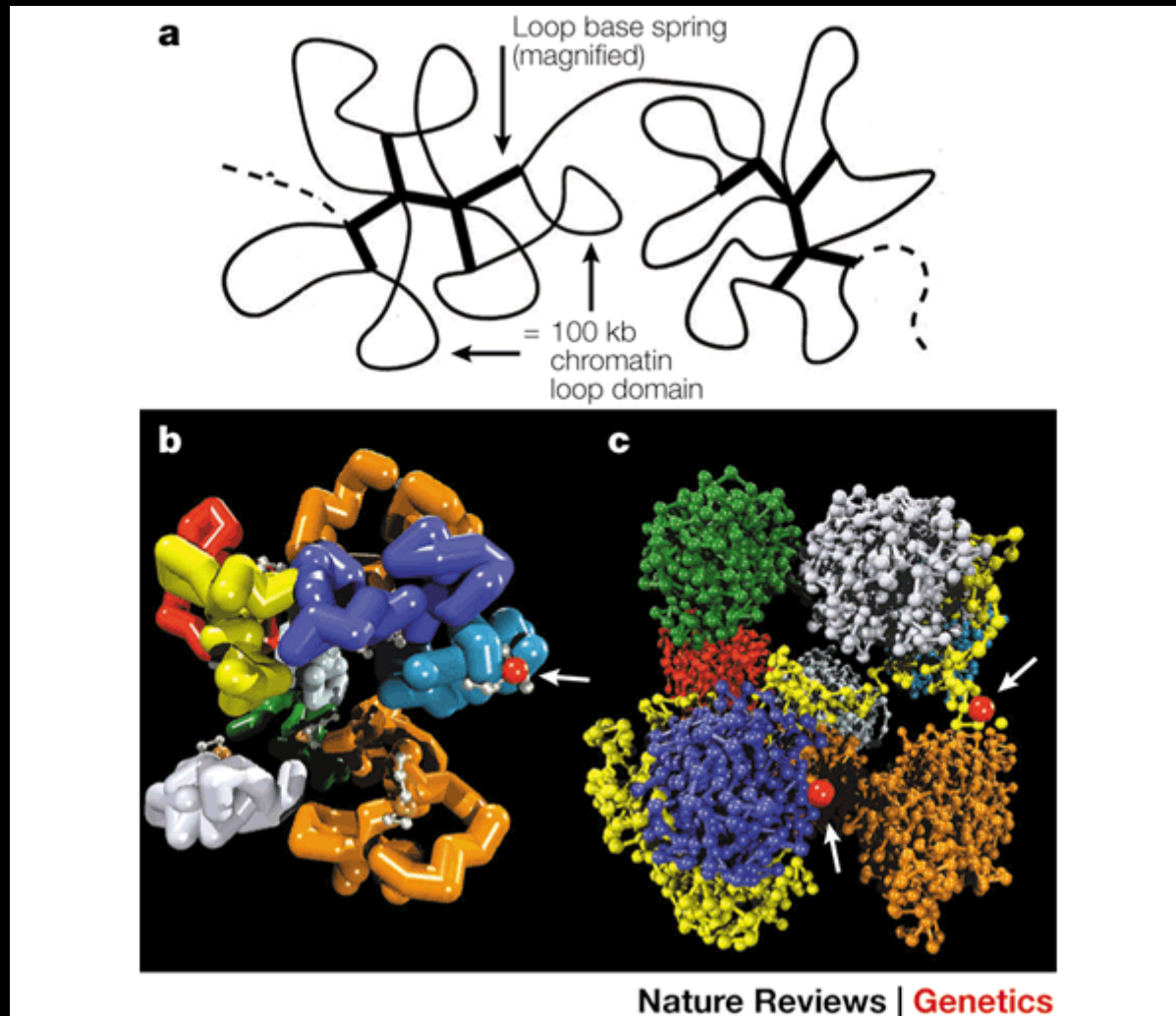
Telomerase is an enzyme that lengthens telomeres by adding on repeating sequences of DNA. Telomerase binds to the ends of the telomere via an RNA template that is used for the attachment of a new strand of DNA. Telomerase adds several repeated DNA sequences then releases and a second enzyme, DNA Polymerase, attaches the opposite or complementary strand of DNA completing the double stranded extension of the chromosome ends. High levels of telomerase activity are detected in embryonic stem cells and cancer cells, whereas little or no telomerase activity is present in most mature, differentiated cell types.

# CT-IC MODEL (T. Cremer)



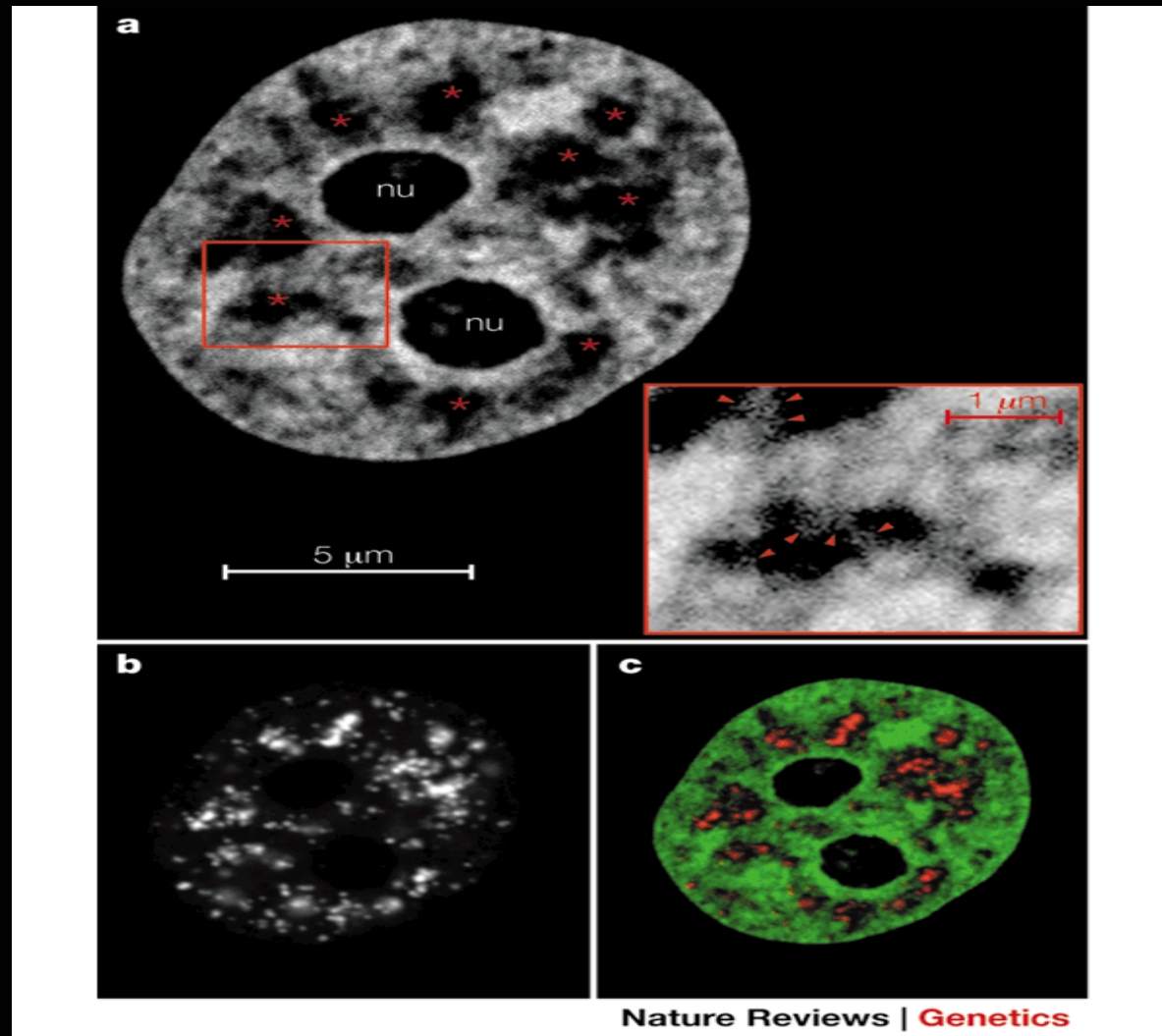


# Chromatin loops



T. Cremer group, Munich

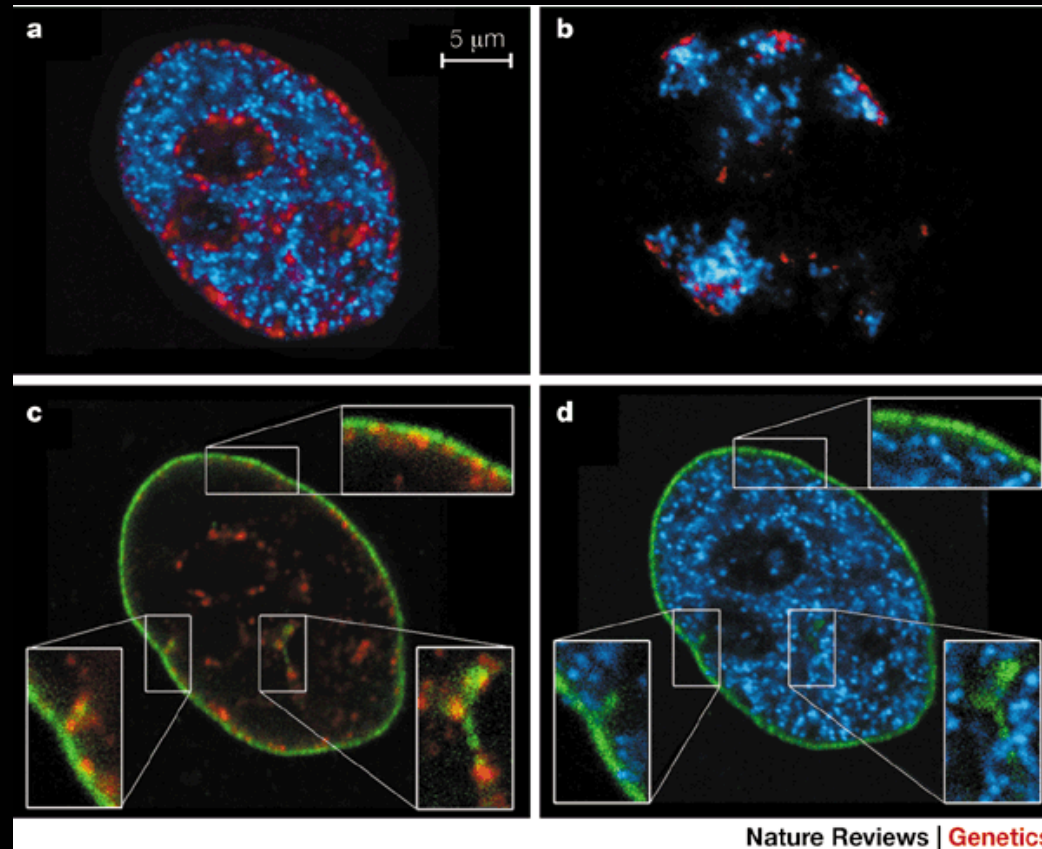
# Chromatinové meziprostory



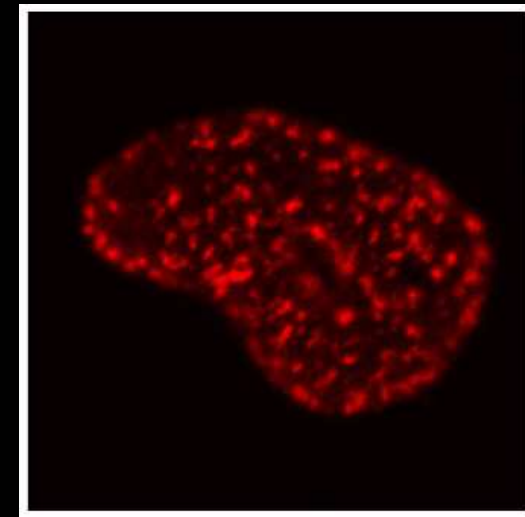
T. Cremer group, Munich



# Pozdně a časně se replikující chromatin

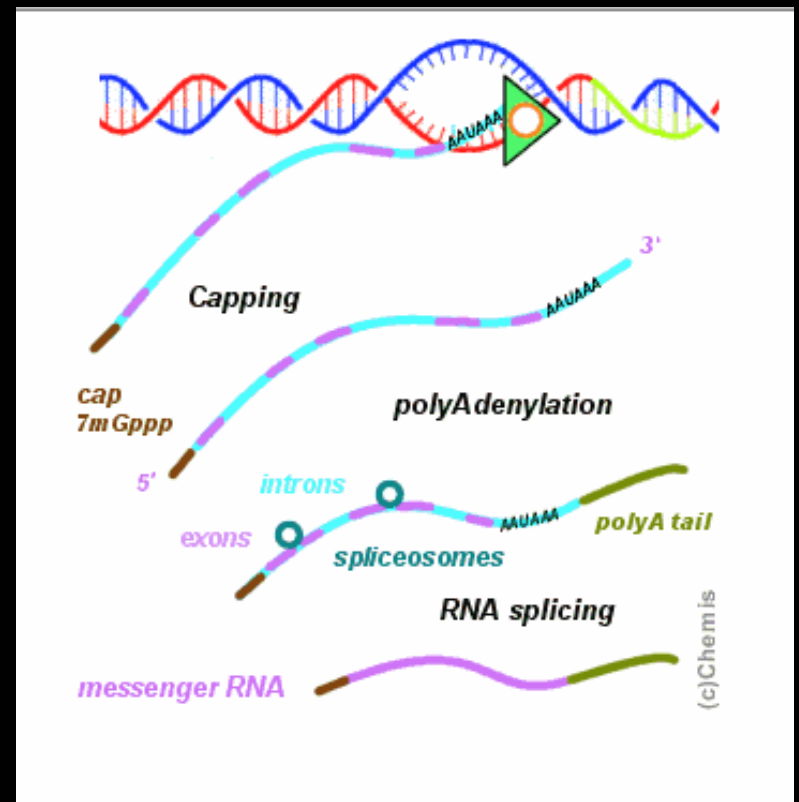
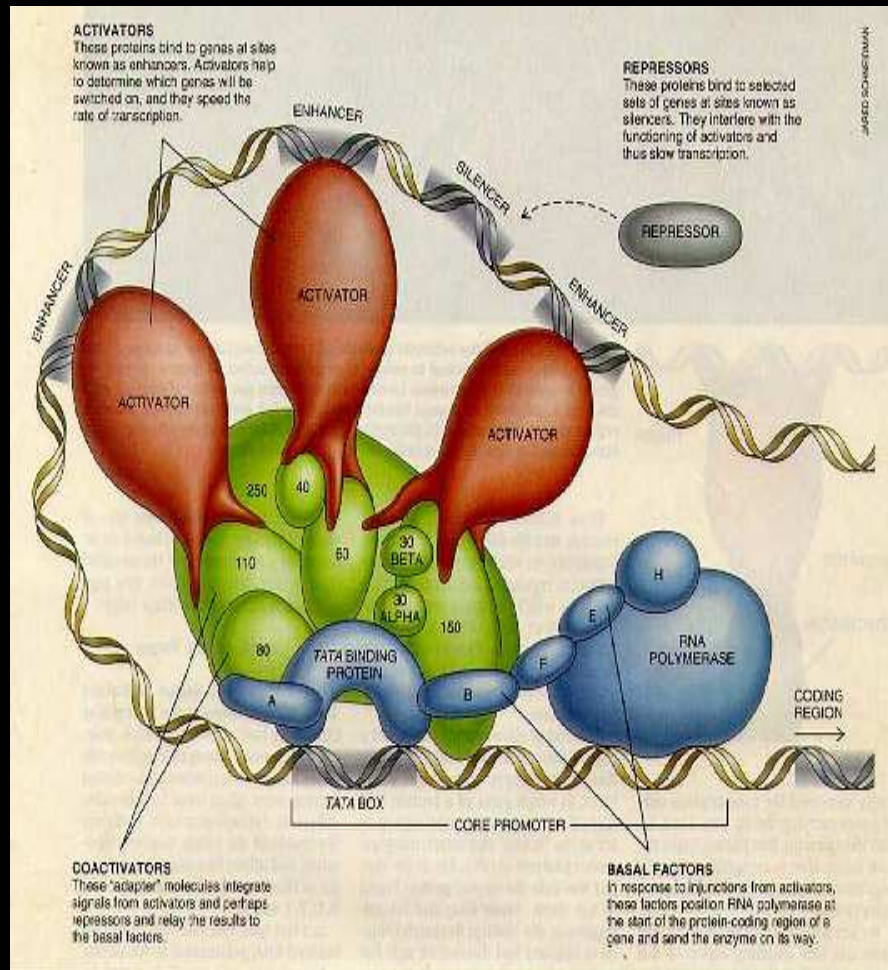


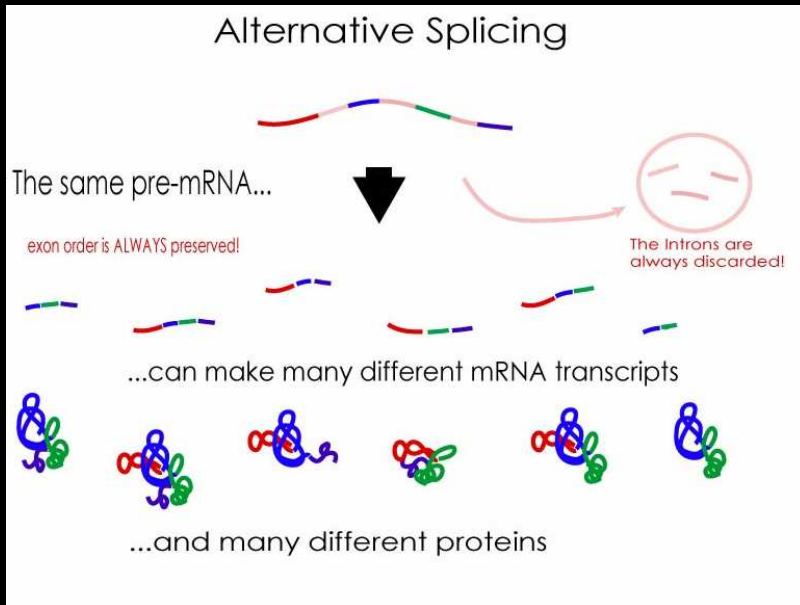
Replikační ohniska



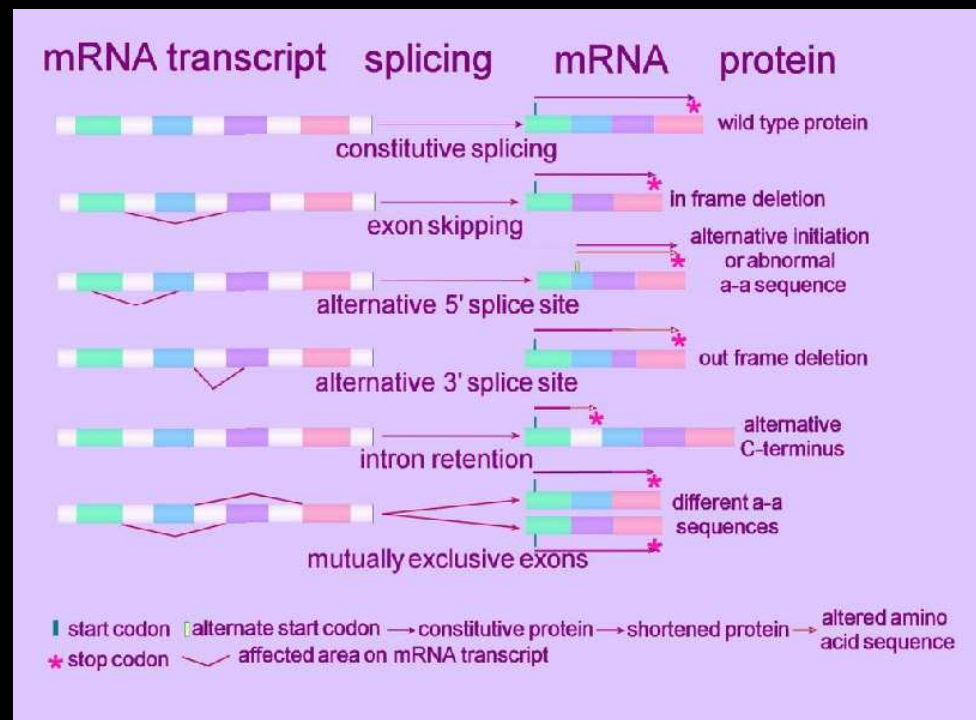
T. Cremer group, Munich

**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 dvojité 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 diferenciaci buněk.



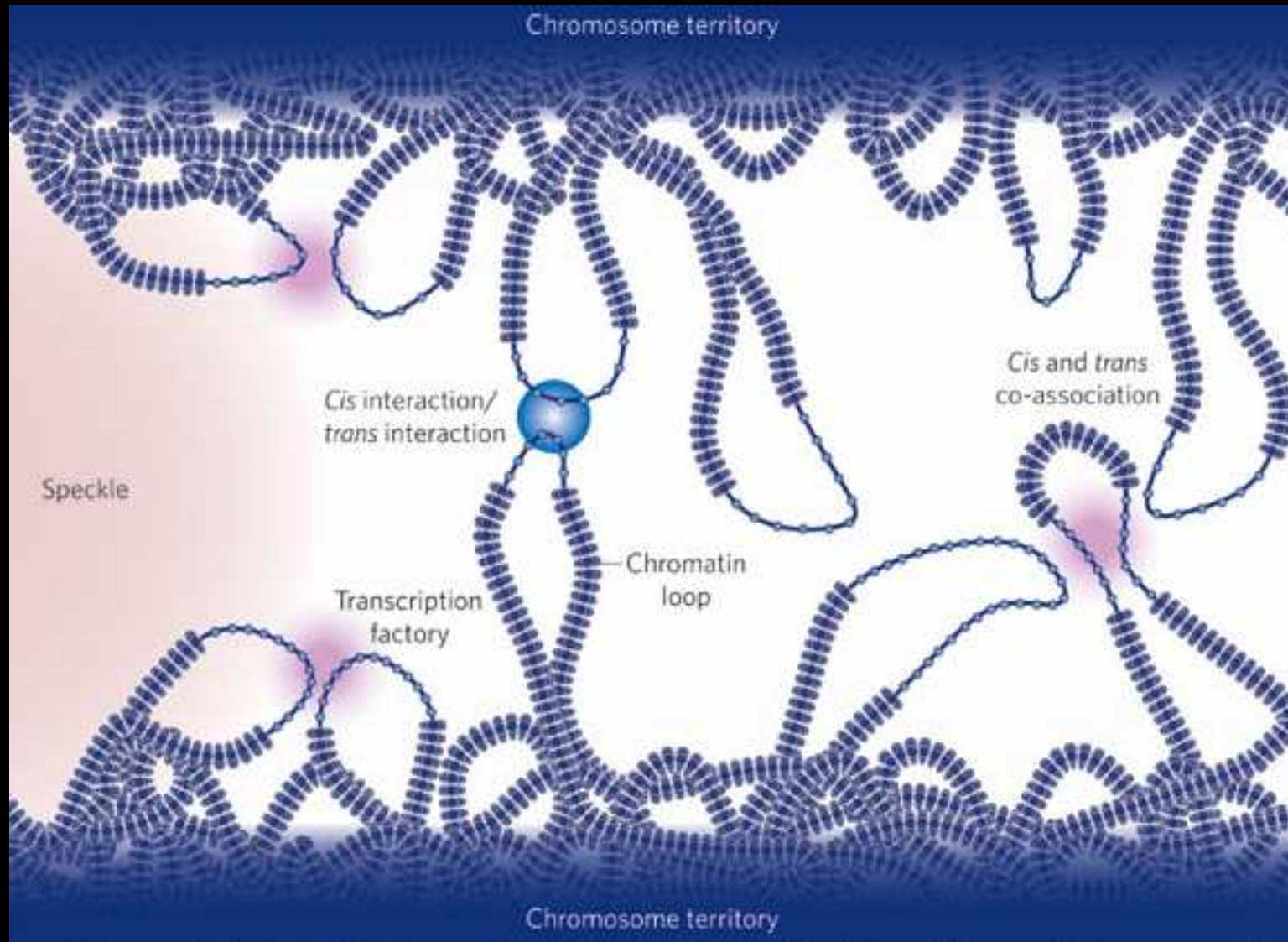


Alternative splicing is a form of epigenetic mechanism that enables a single gene to give rise to multiple, differentially spliced versions of a protein, increasing complexity without a change in the genome.





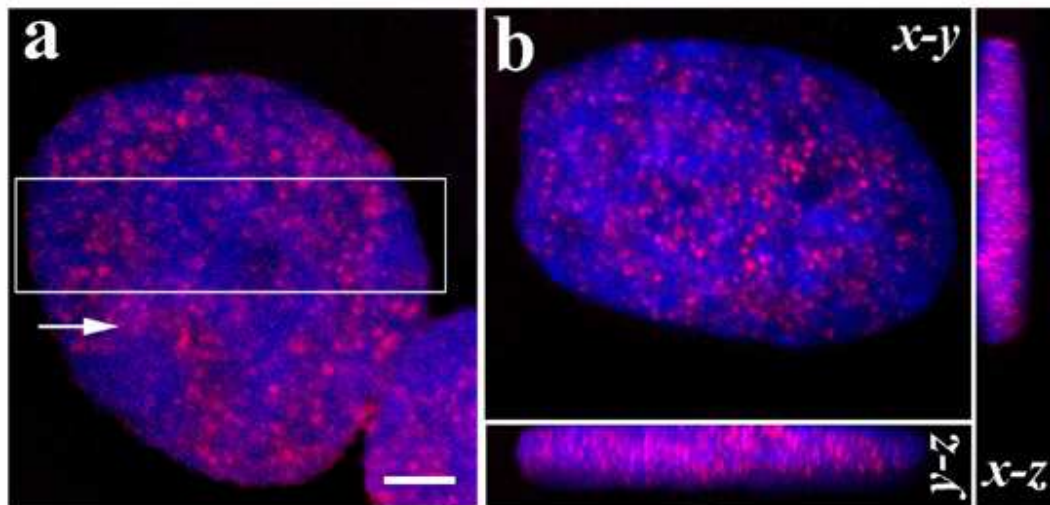
# Transcription factories



Cis acting elements: Enhancers, LCR

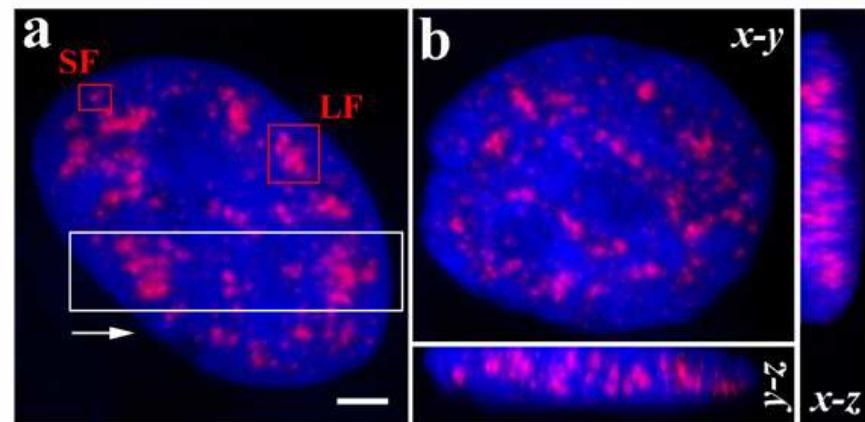
Trans acting elements: proteins, Ikoros, Helios, HP1 proteins

## A RNAP II / DAPI



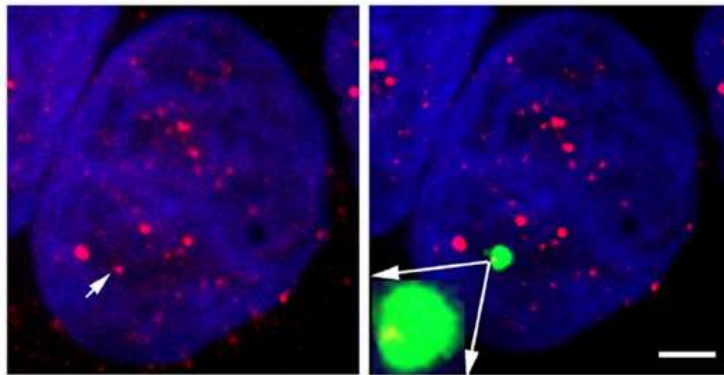
Faktory sestřihu jsou v SC-35 doménách, dále snRNP U1-U6 jsou součástí faktoru sestřihu SF2/ASF

## C SC35 / DAPI

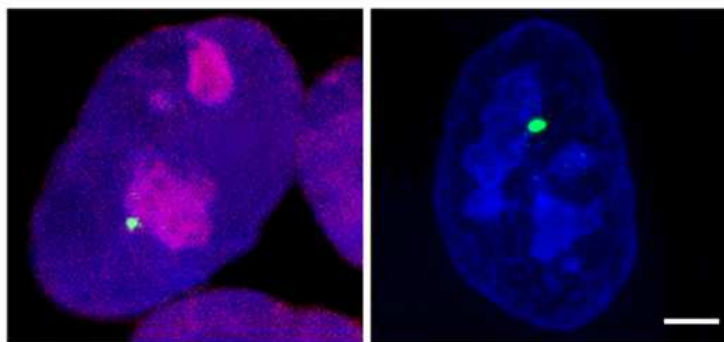




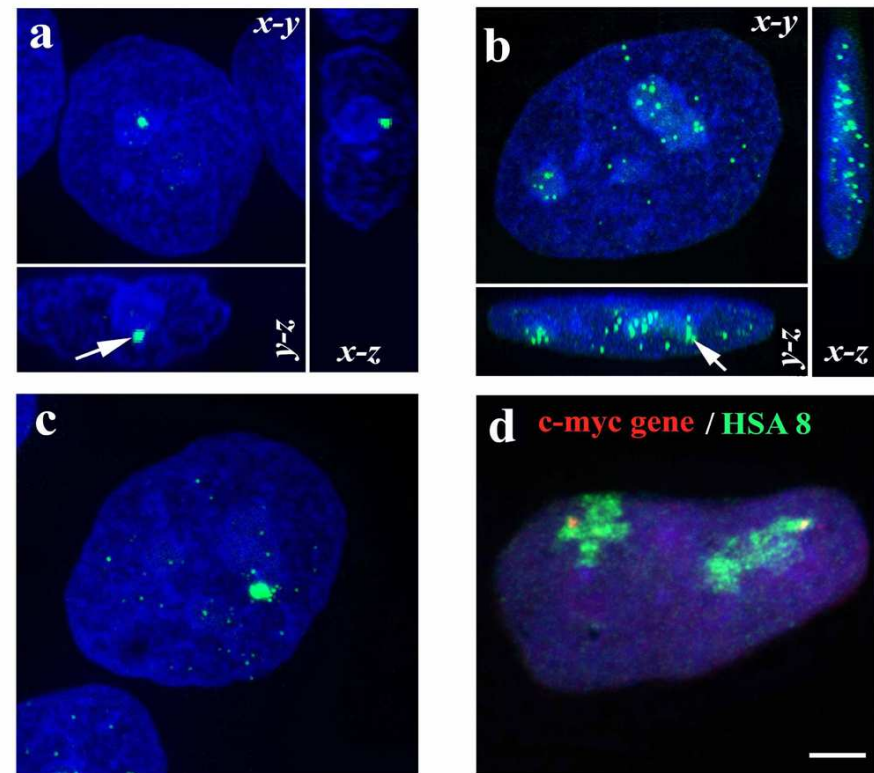
**A** RNAP II / c-myc<sup>T</sup>



**C** Nucleoli / c-myc<sup>T</sup>



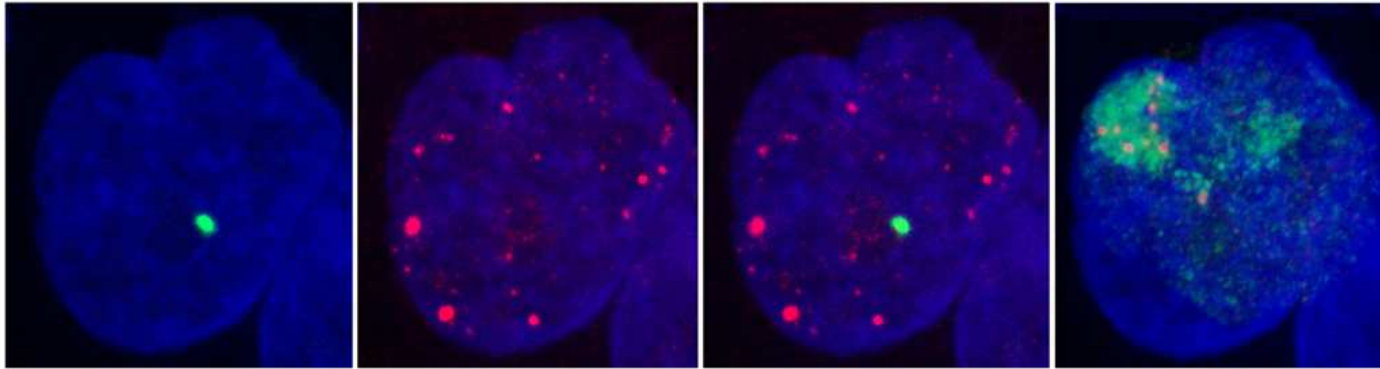
**A** c-myc<sup>T</sup> / DNA



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

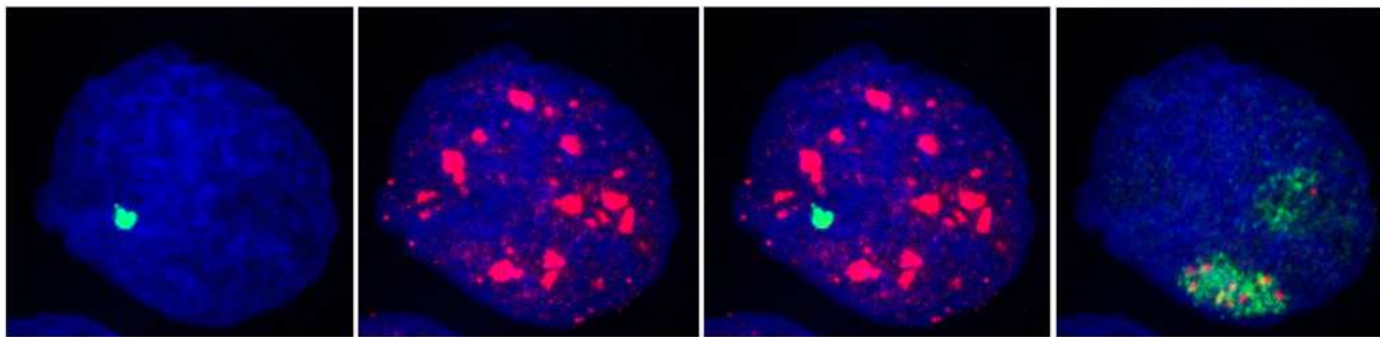
RNAP II / c-myc<sup>T</sup>

c-myc gene  
HSA8

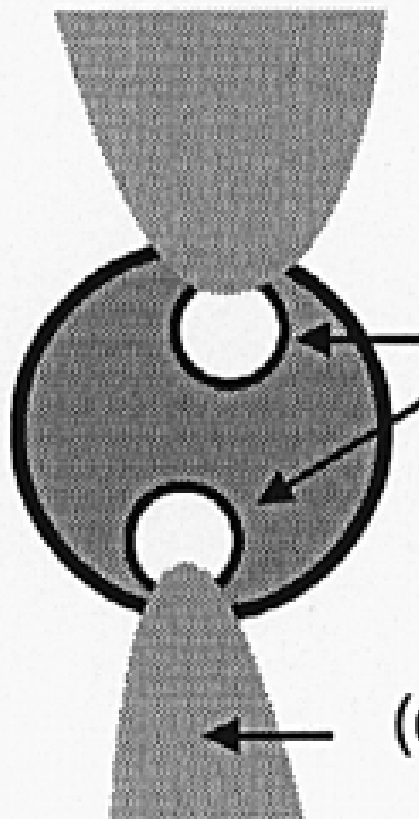


SC35 / c-myc<sup>T</sup>

c-myc gene  
HSA8



## Nucleolus



(pol I)

(eg 13, 14)

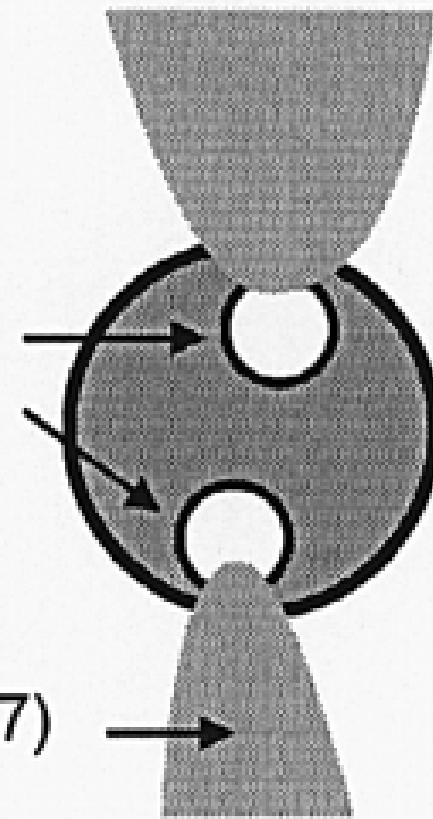
## factories

(pol II/III)

## chromosomes

(eg 6, 7)

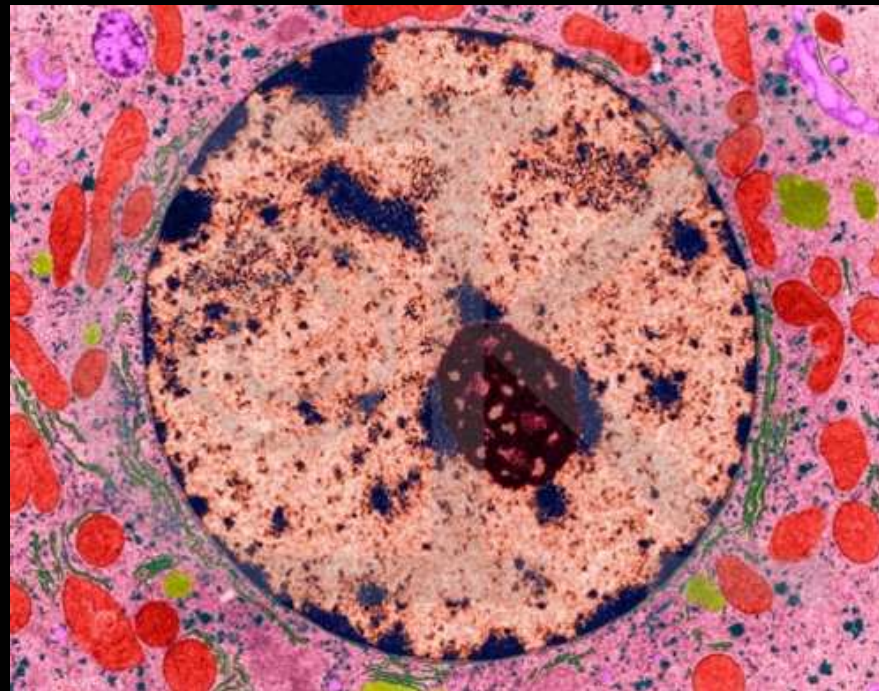
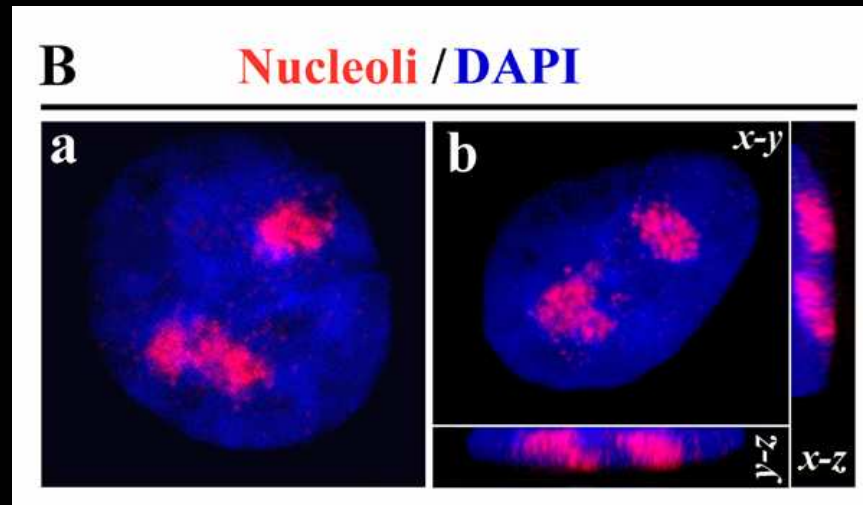
## OPT domain



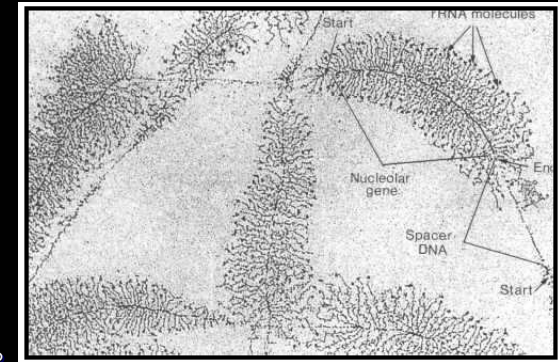
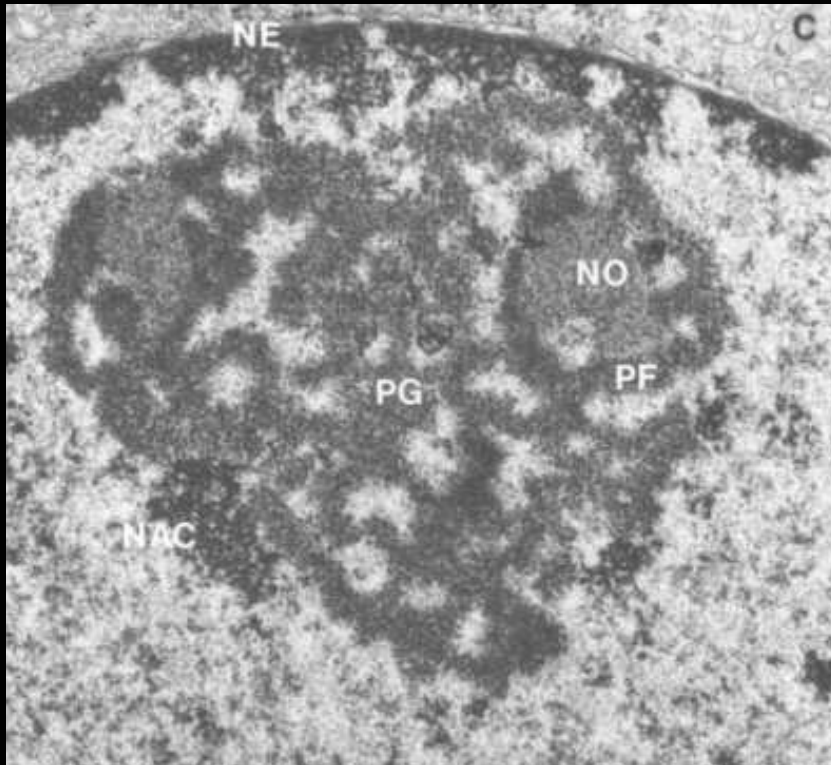
(pol II/III)

(eg 6, 7)

# Nucleolus







## NOR

400 (540) rDNA genů

## FIBRILÁRNÍ CENTRUM (FC):

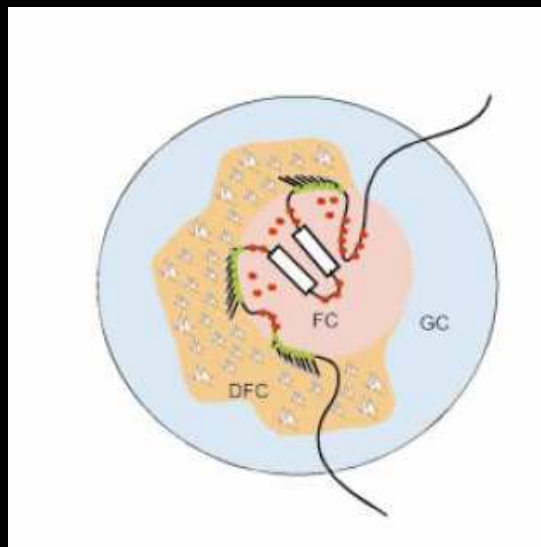
zodpovídá za změny v buněčné aktivitě

## DENSE FIBRILAR COMPONENT (DFC):

místo syntézy ribozomálních podjednotek

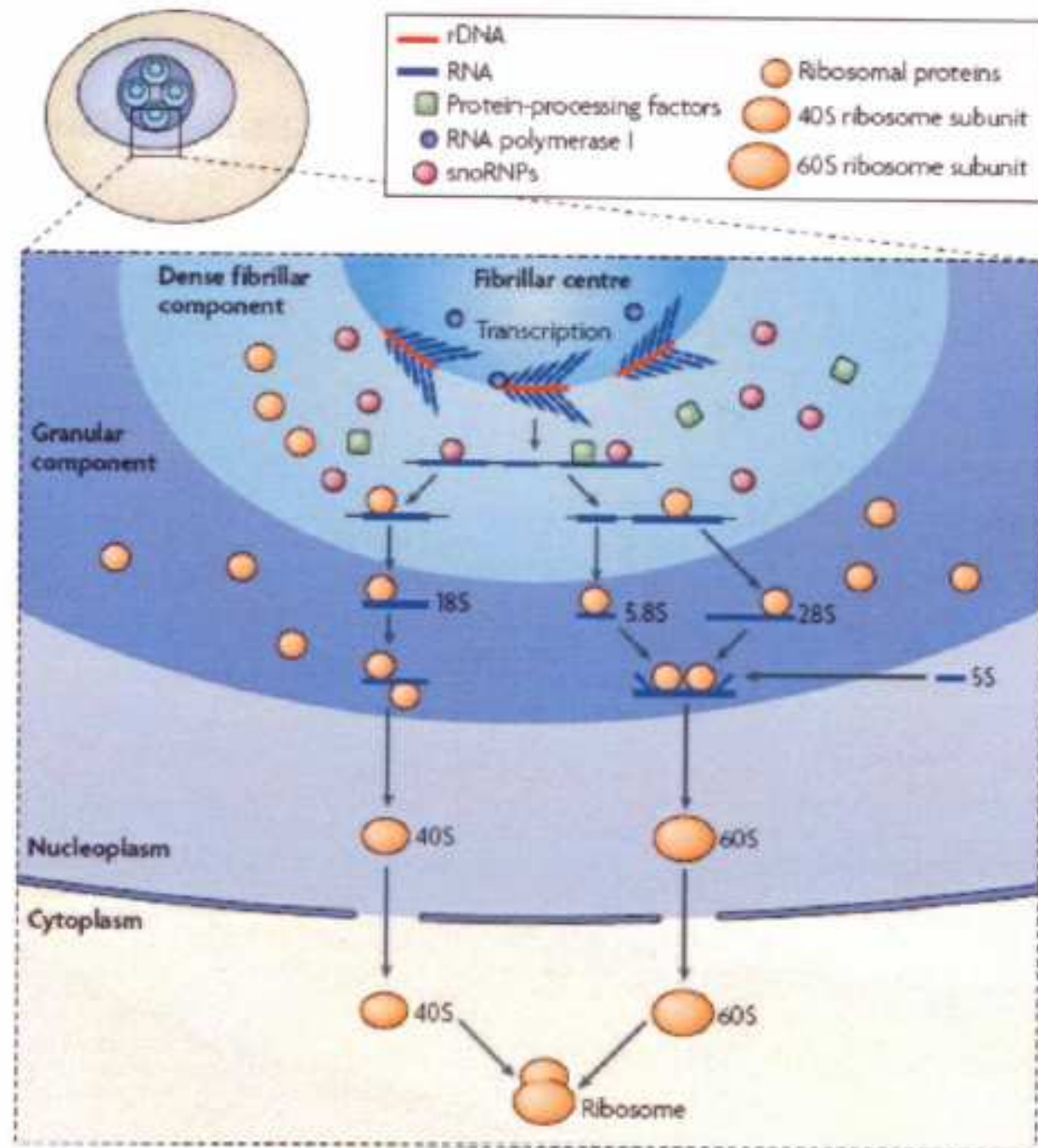
## GRANULAR COMPONENTS:

ukotvuje DFC a FC

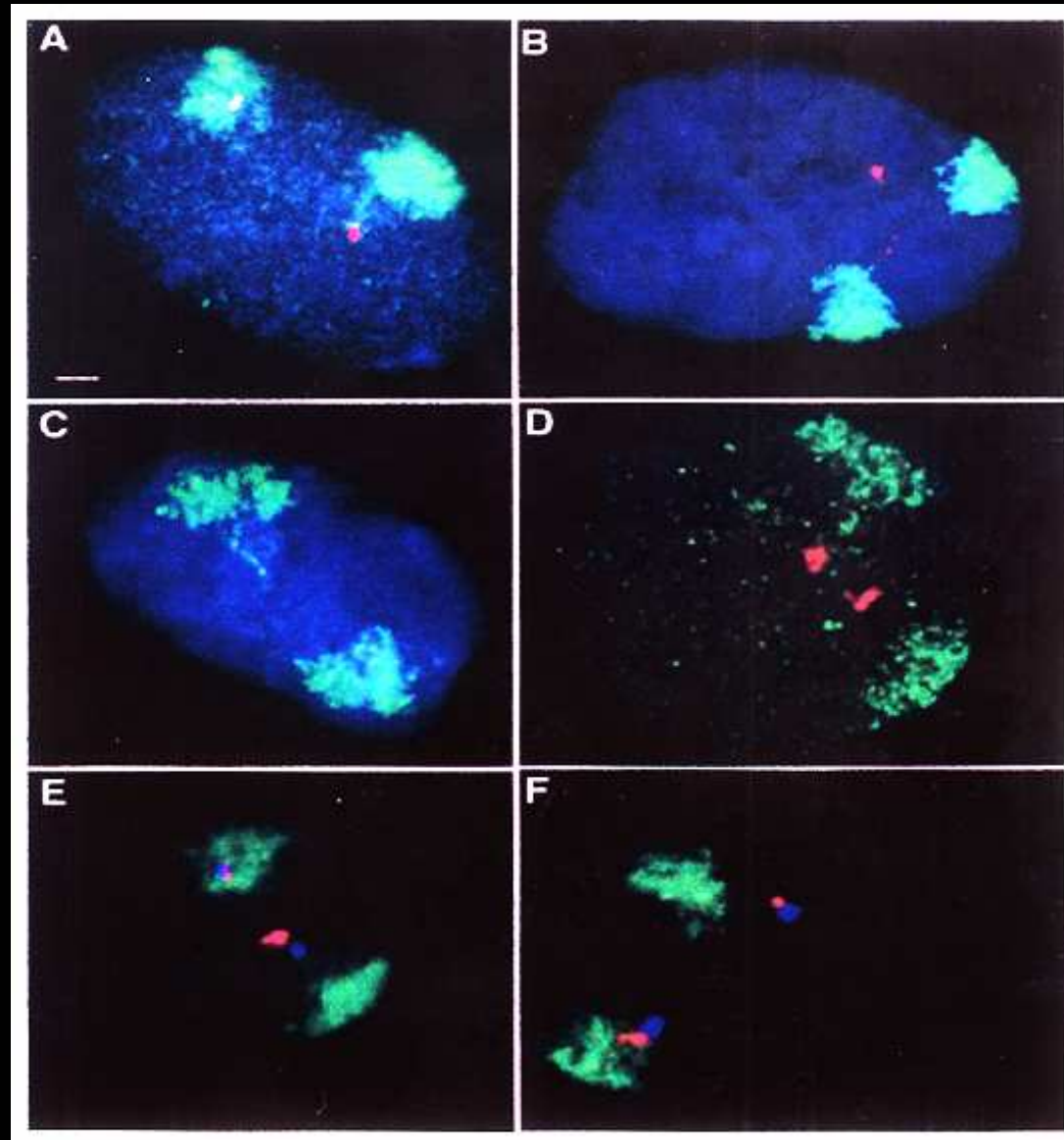


Tvorba ribozomů je komplexní proces zahrnující transkripci 45S prekurzorické rRNA, její vyžrávání, modifikaci a asociaci s ribozomálními proteiny a 5S rRNA, která se syntetizuje mimo jádérko. Vyžrávání rRNA probíhá v procesomu, který obsahuje mnoho komplexů a snRNA



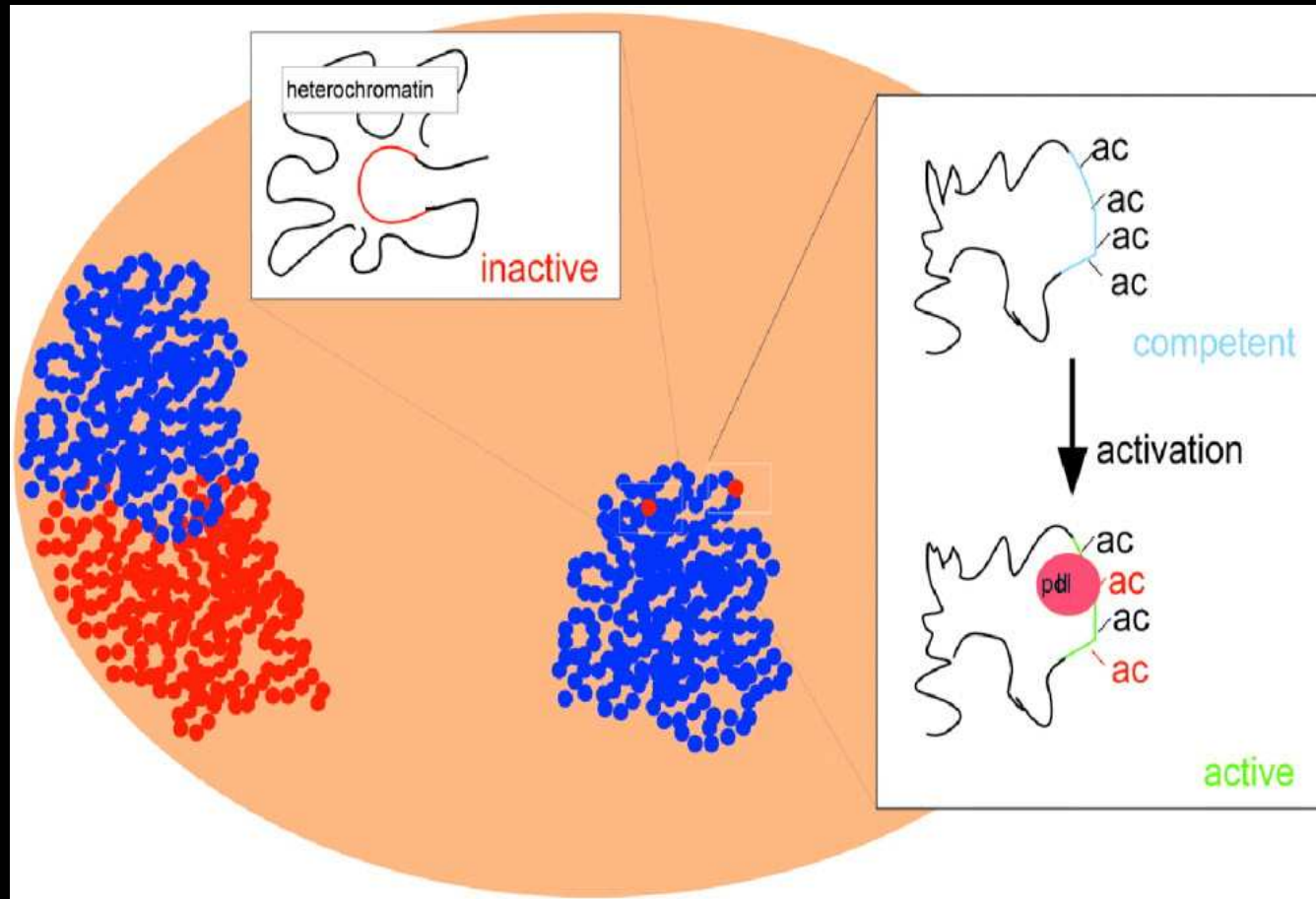


# MHC on HSA 6



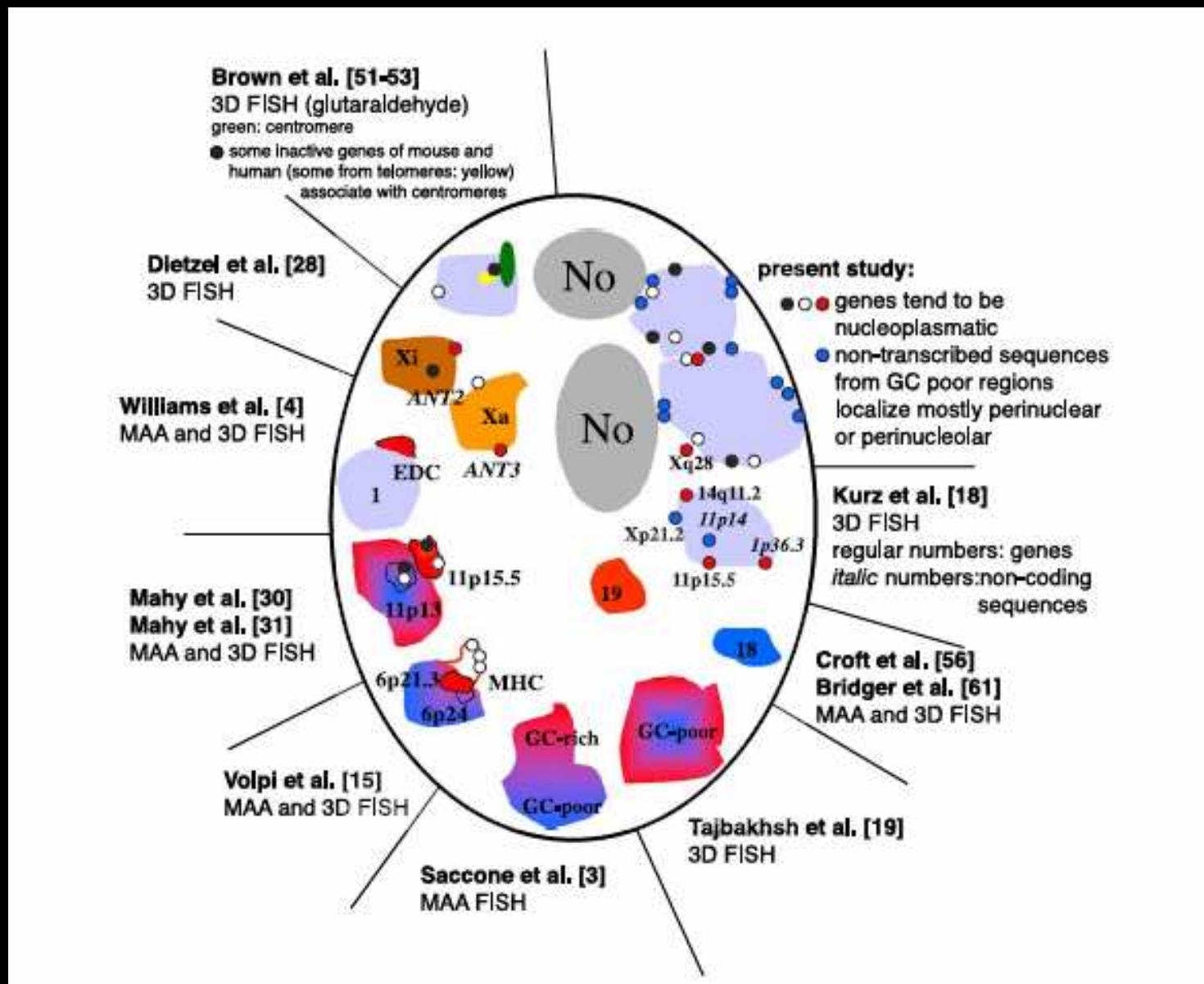
Volpi et al., 2000

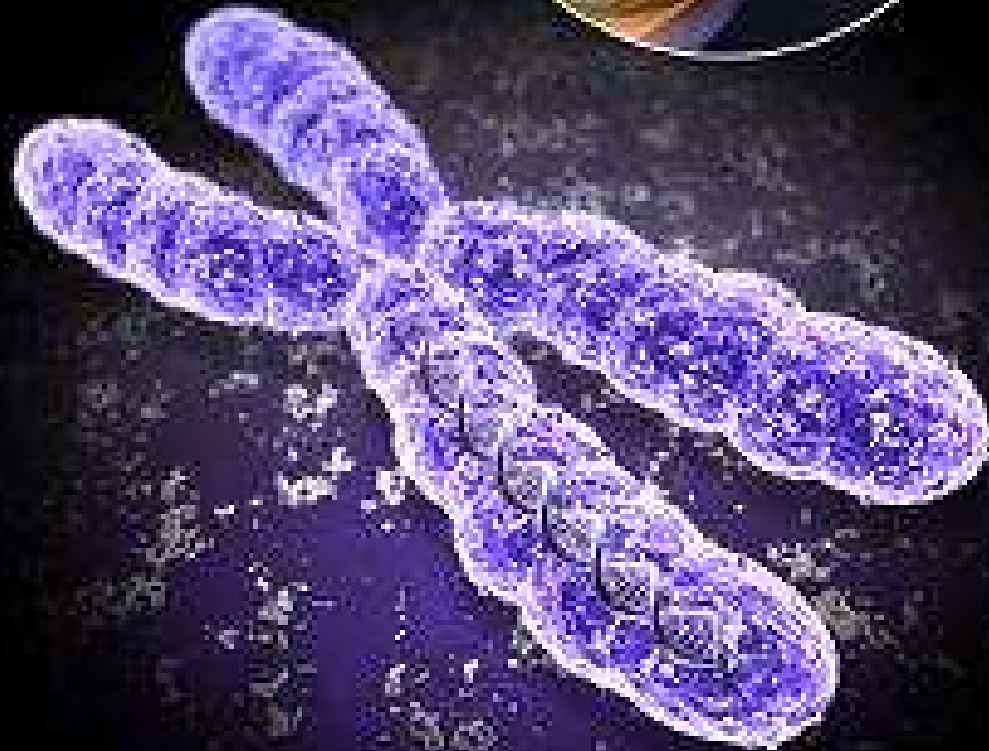
# Active / inactive chromatin



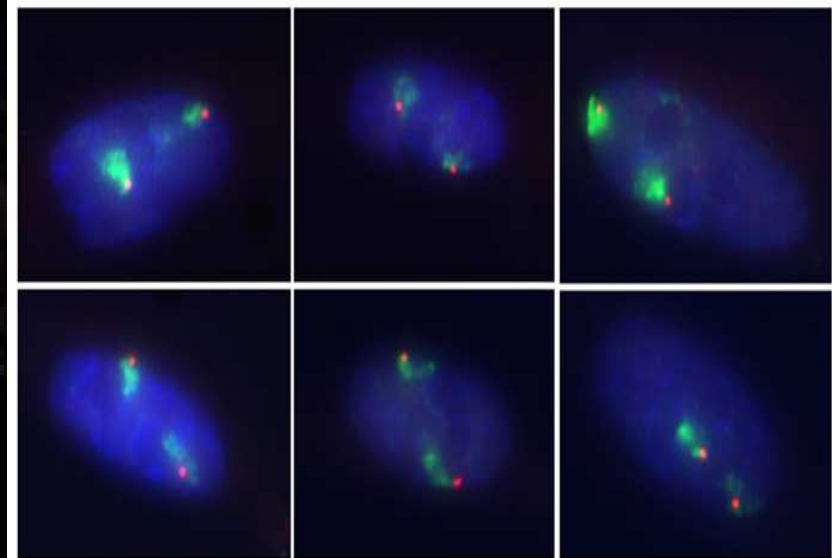
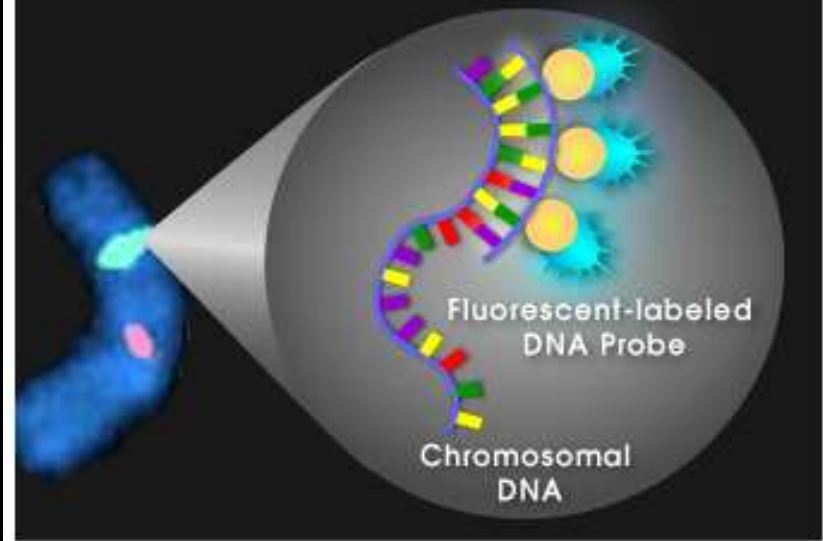
T. Cremer group, Munich

**INTERMINGLING**

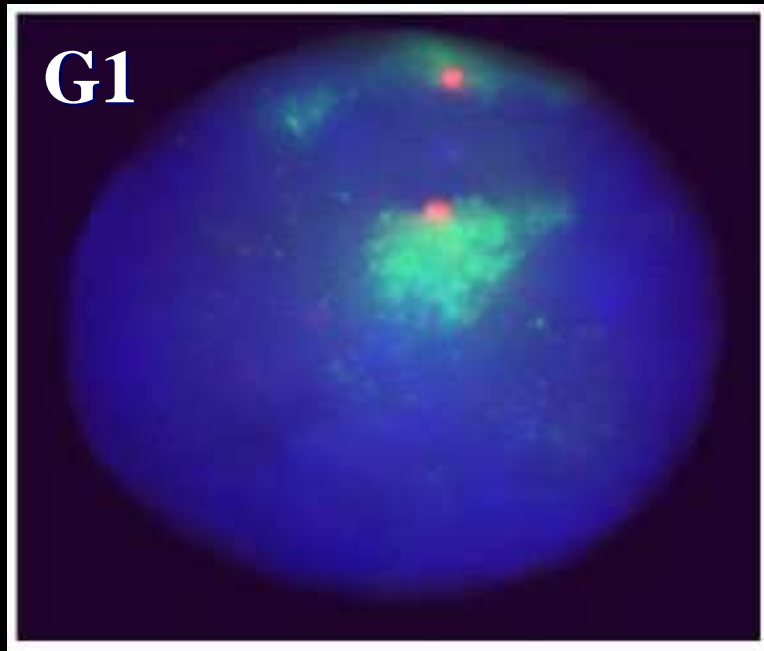




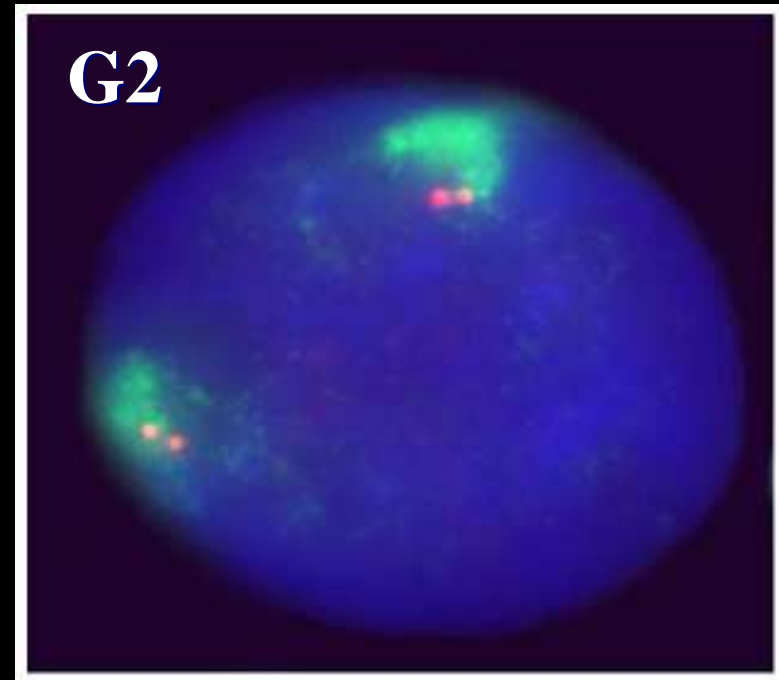
Chromosome prepared using FISH technique





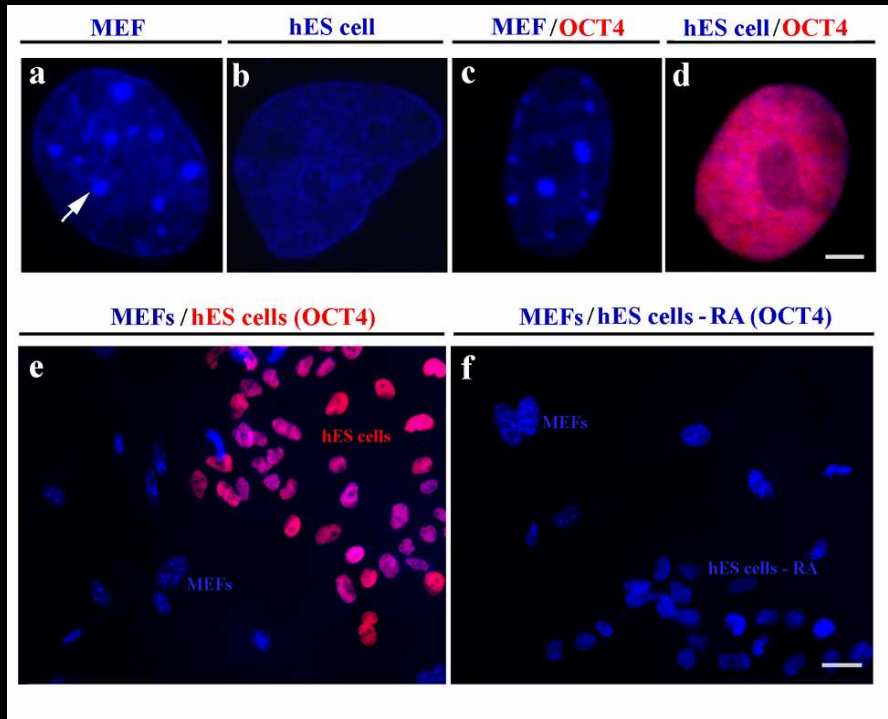


**Rb1 gene**

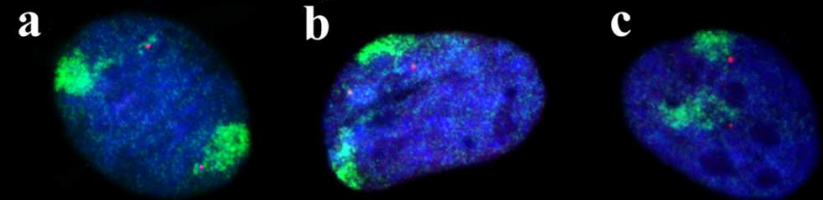


Bártová et al., 2002

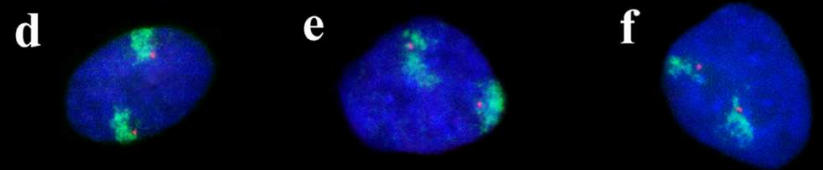
# Genes in human embryonic stem cells



Oct4 / HSA 6 in hES cells



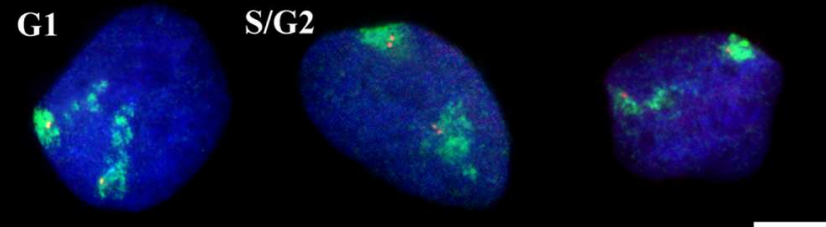
Oct4 / HSA 6 in hES cells - RA differentiated



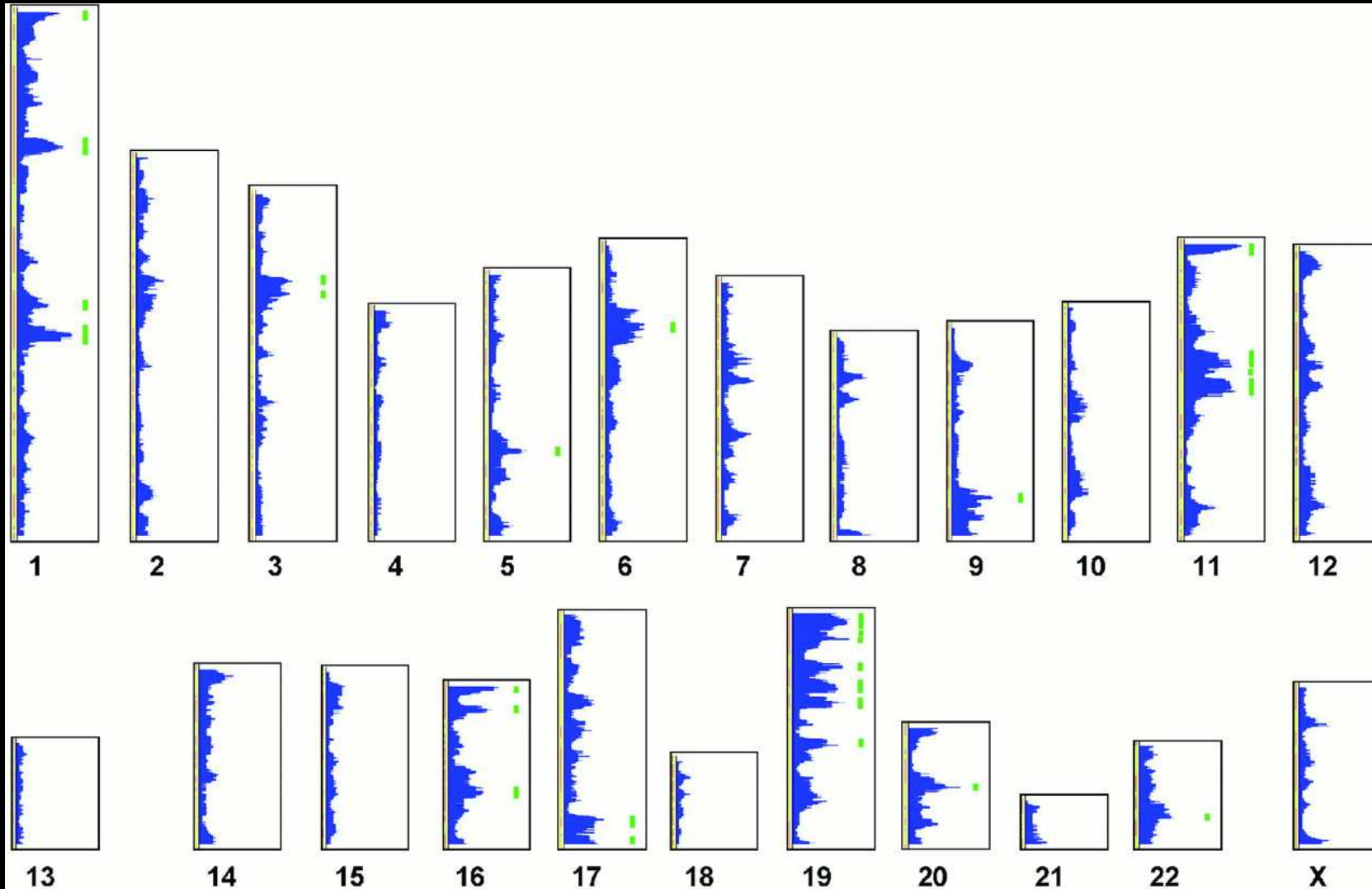
C-myc / HSA 8

in hES cells

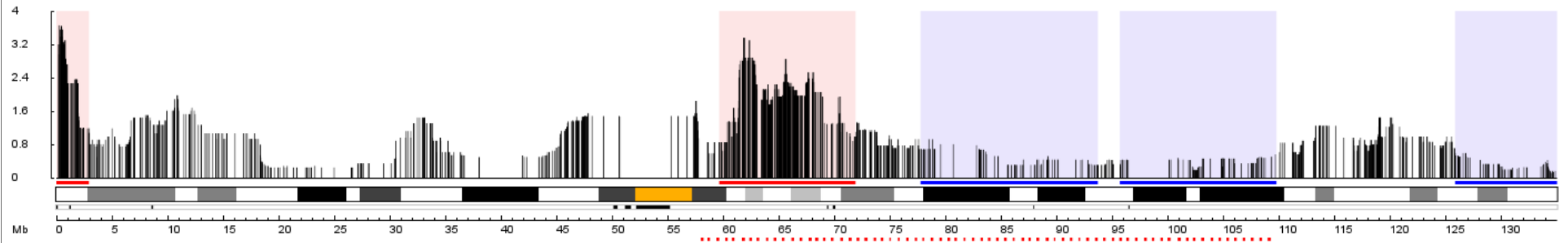
RA differentiated



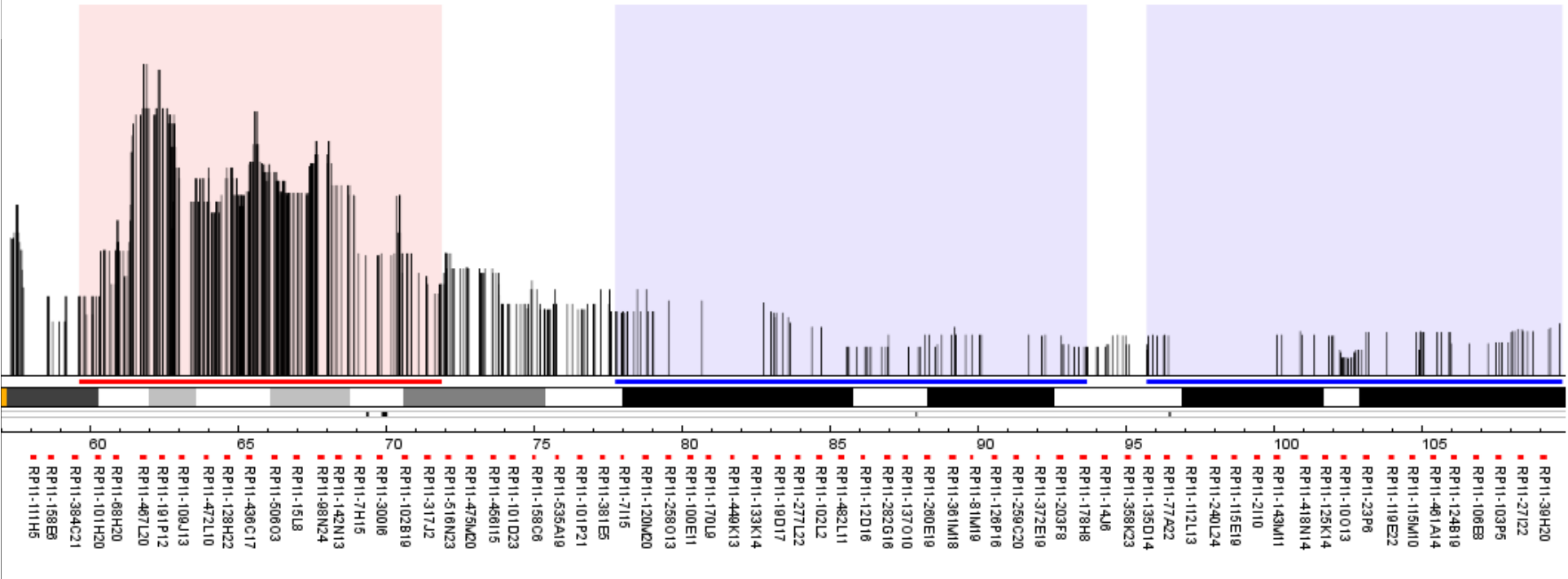
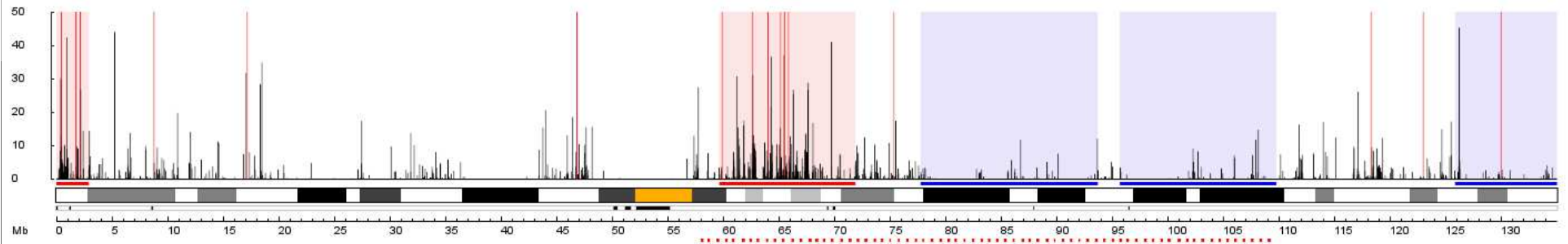
# Transcriptome map (Caron et al., 2001)



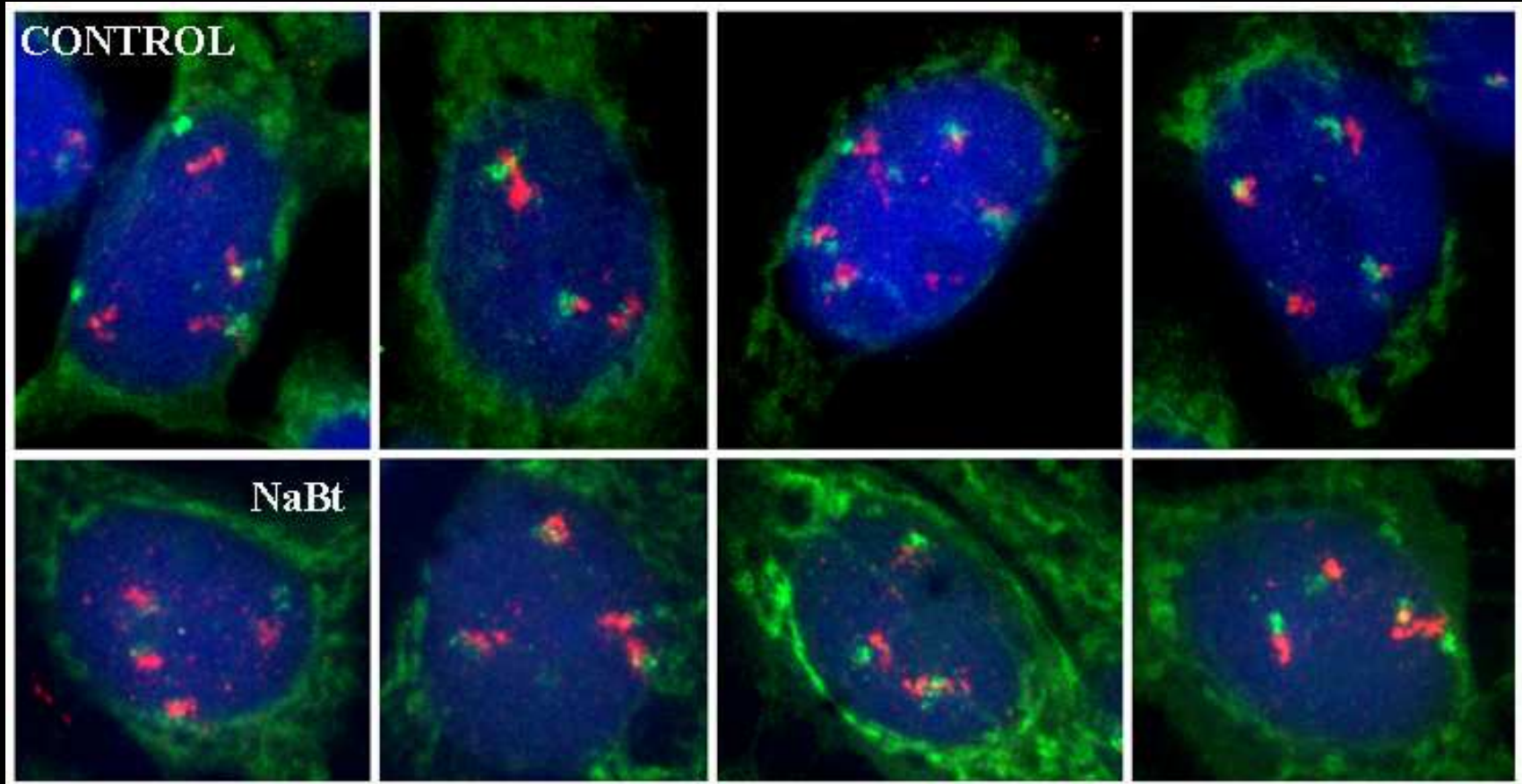
hg15 chr11-norm\_htm-hs202libs-mm-49-100000-4-ver0.88



hg15 chr11-norm\_htm-hs202libs-100000-50-ver0.88

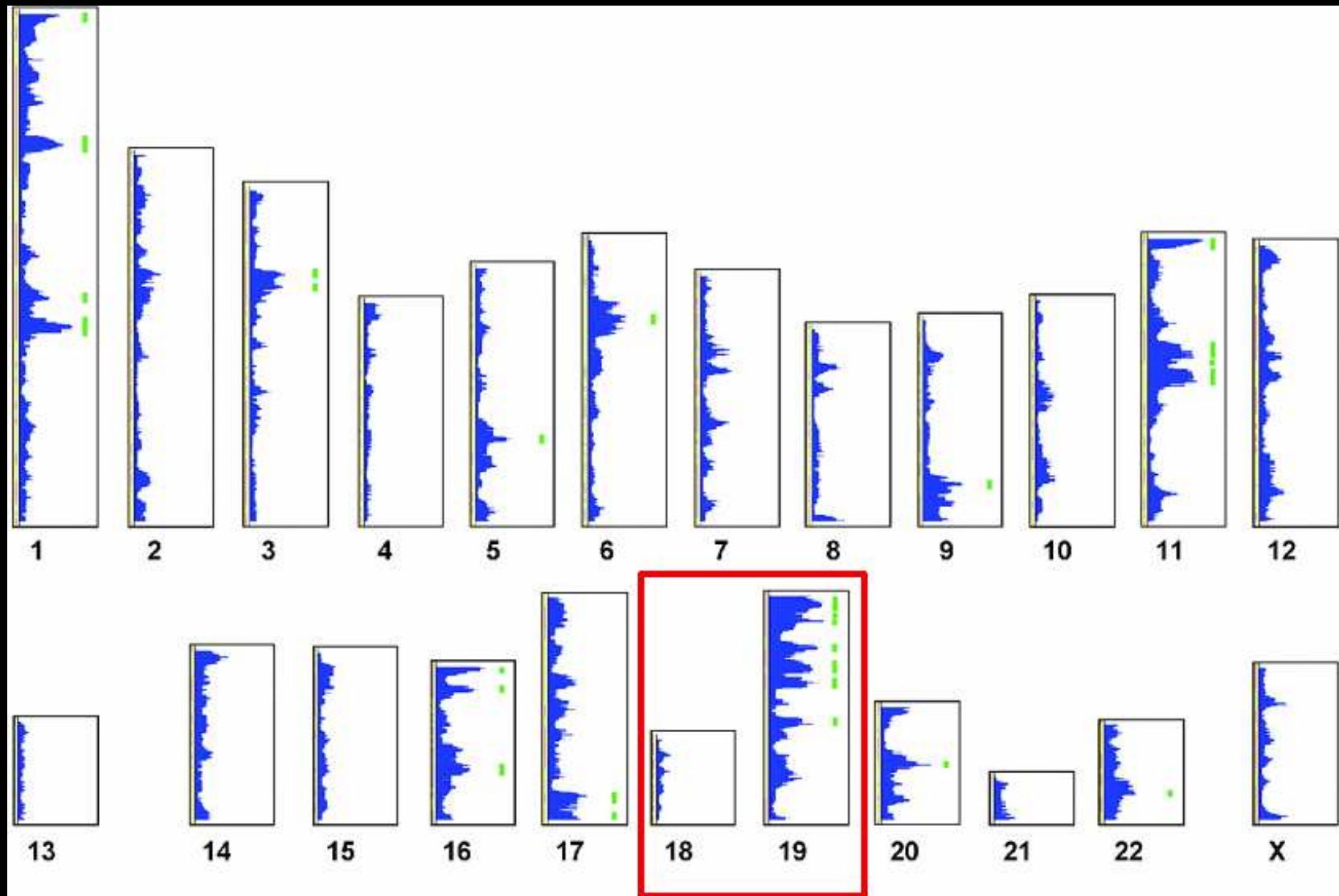


# Enterocytic cell differentiation and RIDGE/ANTI-RIDGE

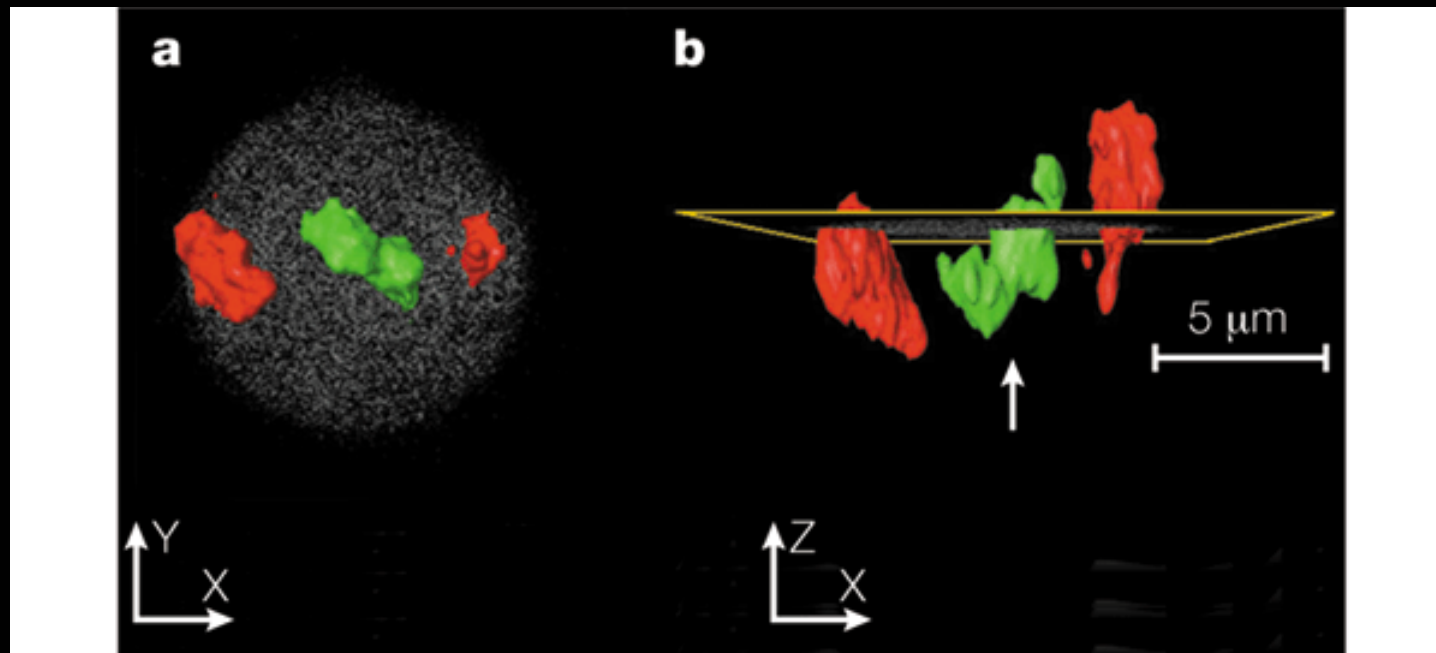




# Transcriptome map (Caron et al., 2001)



## HSA 18 and 19 (positioning x gene density)



T. Cremer group, Munich

## Center of nucleus- to-gene distances

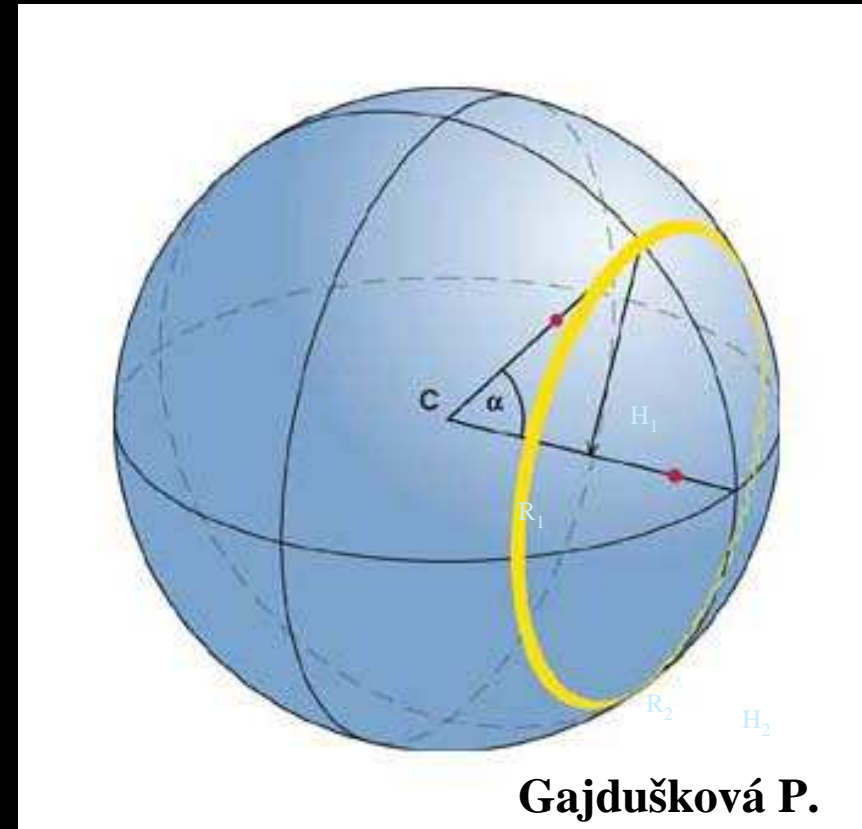
$$CS = \frac{\overline{CR_i}}{\overline{CH_i}} * 100$$

## Gene-to-gene distances

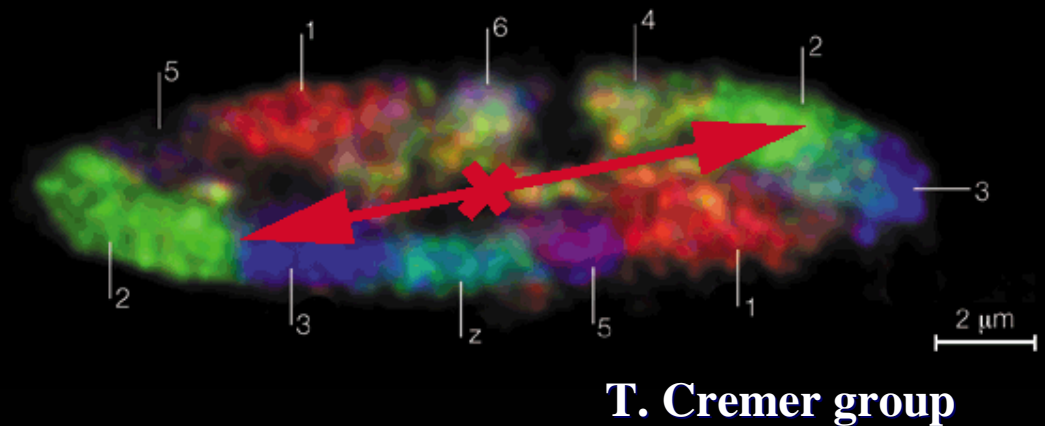
$$SS = \frac{\overline{R_1 R_2}}{(\overline{CH_1} + \overline{CH_2})/2} * 100$$

## Gene-center of nucleus-gene angles

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

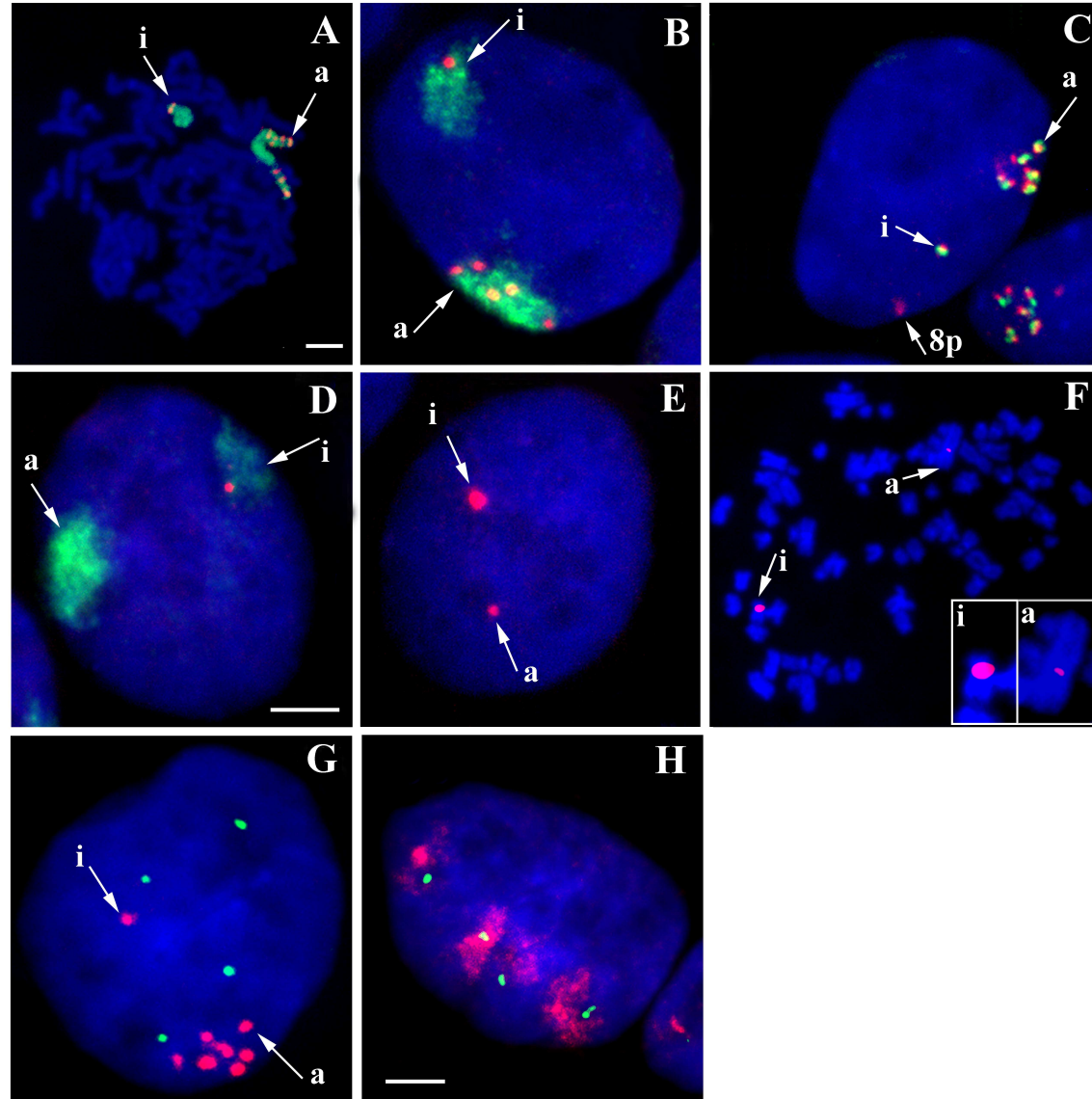


d



# HSA 8 and related structures

Harničarová et al., 2005





## ZÁVĚR

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