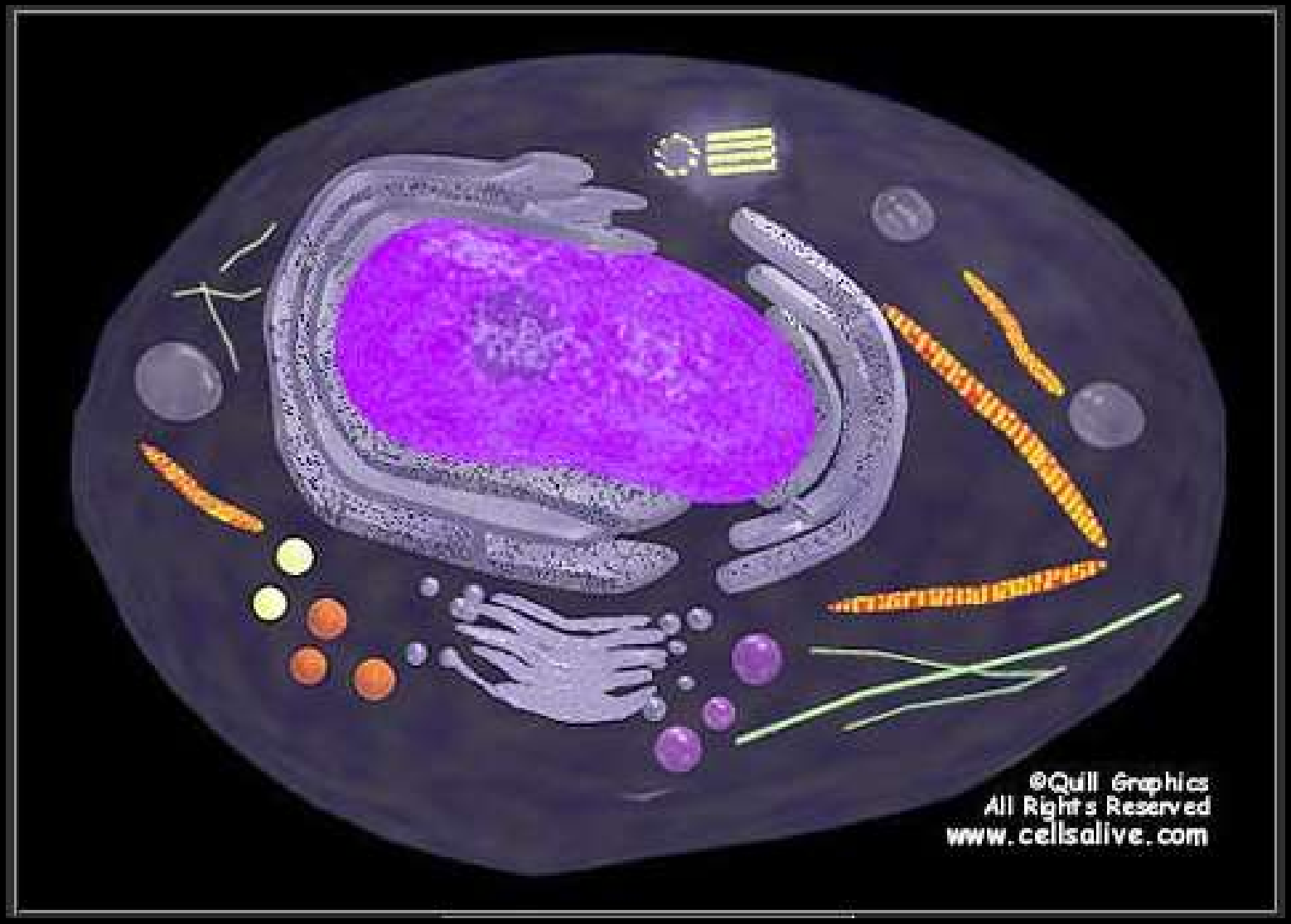
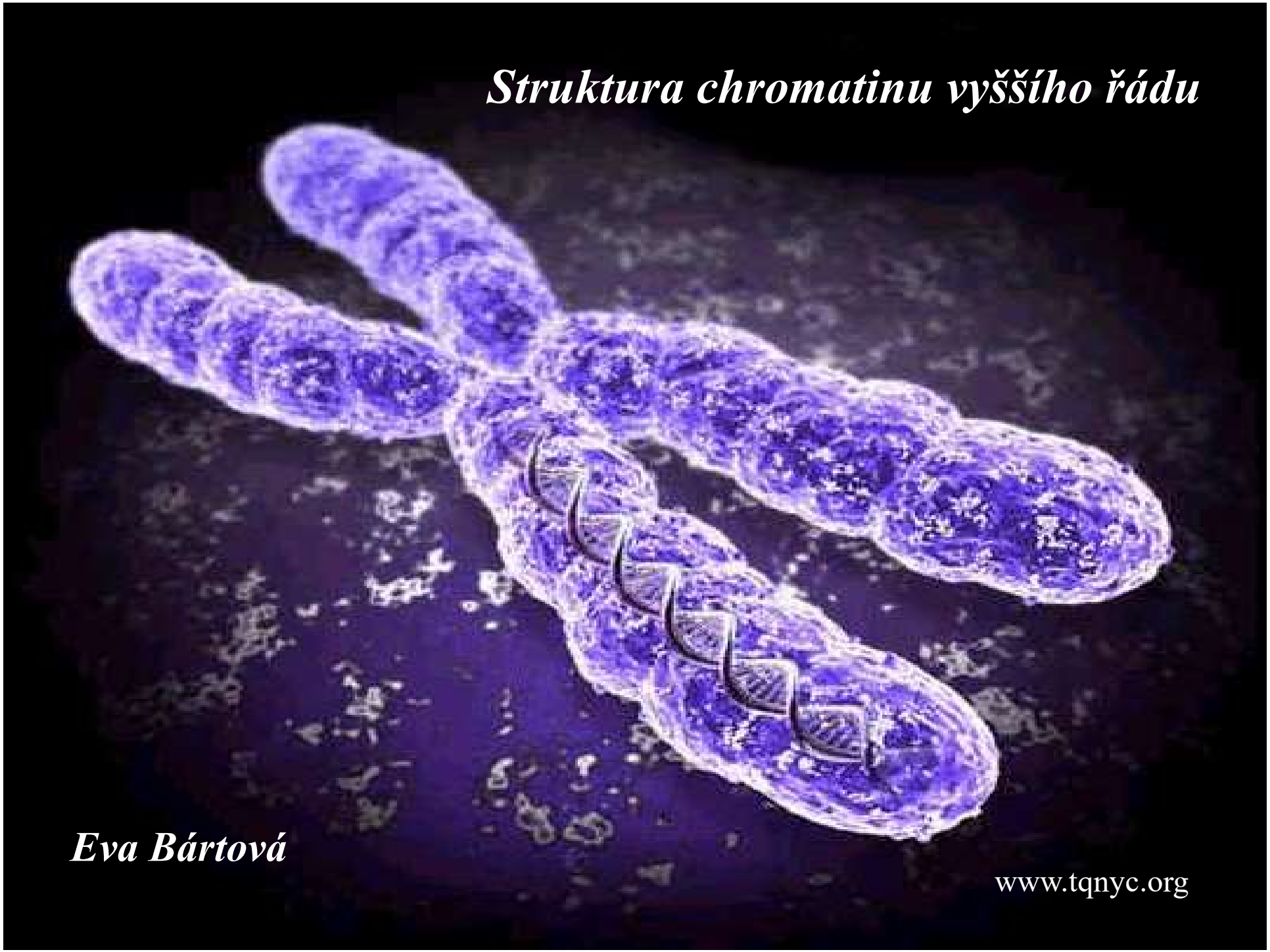
- Struktura chromatinu - kompartmentalizace interfazních jader
- Morfologické a jaderně-topografické aspekty buněčné diferenciace.
- Apoptóza a struktura chromatinu.
- Cytoskelet a jaderná matrix - studium cytoskeletu pomocí mikroskopie s vysokým rozlišením.
- BAC/PAC knihovny a jejich využití pro FISH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

Buněčné jádro



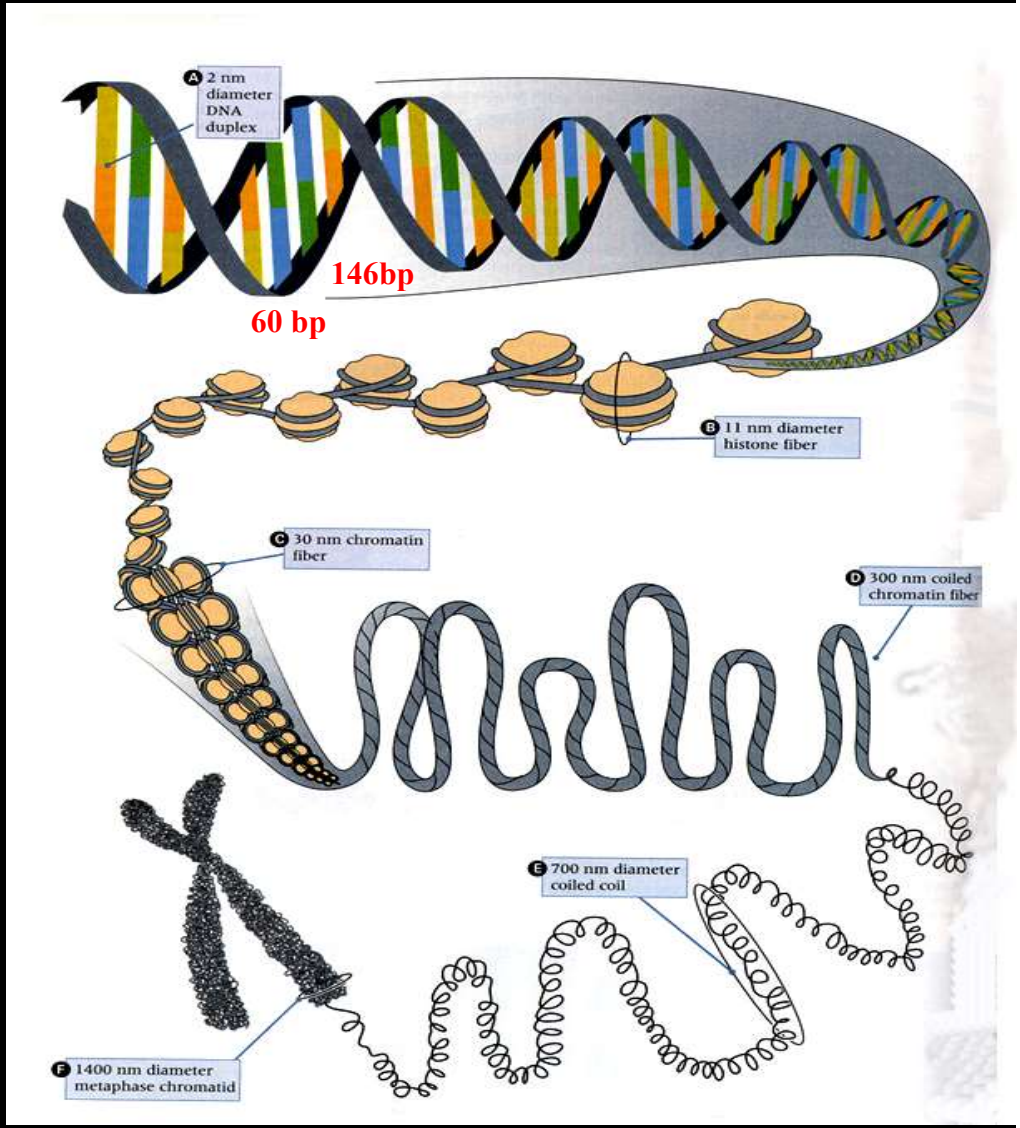
Struktura chromatinu vyššího řádu



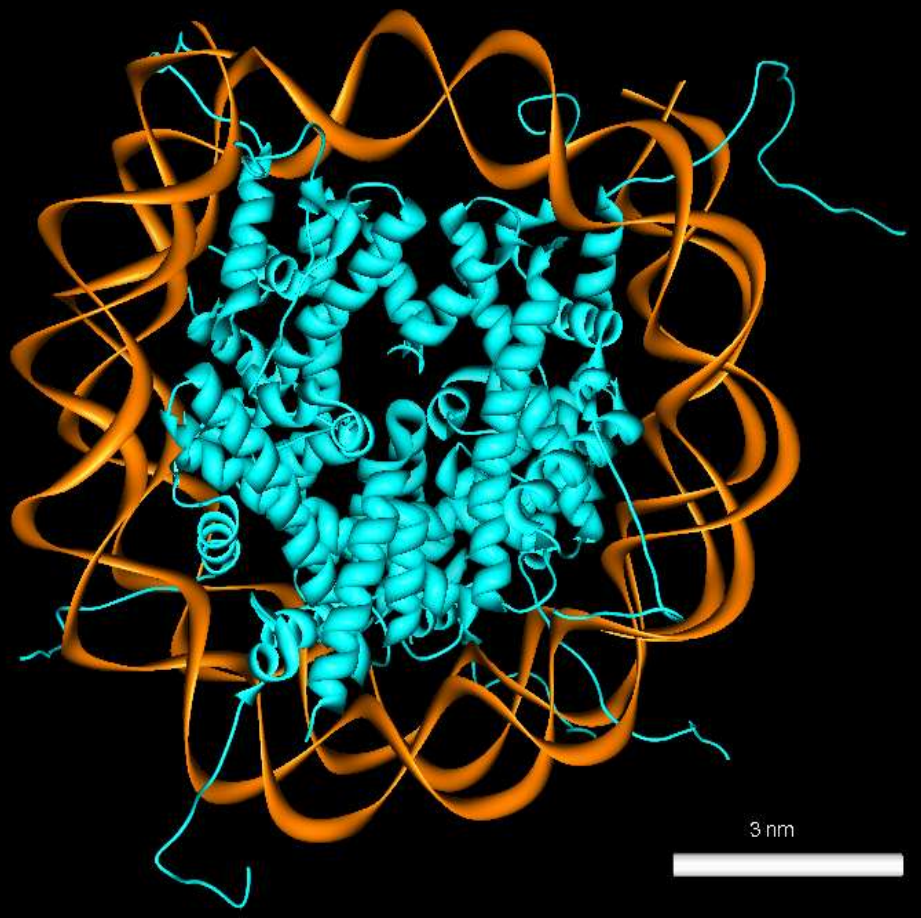
Eva Bártová

www.tqnyc.org

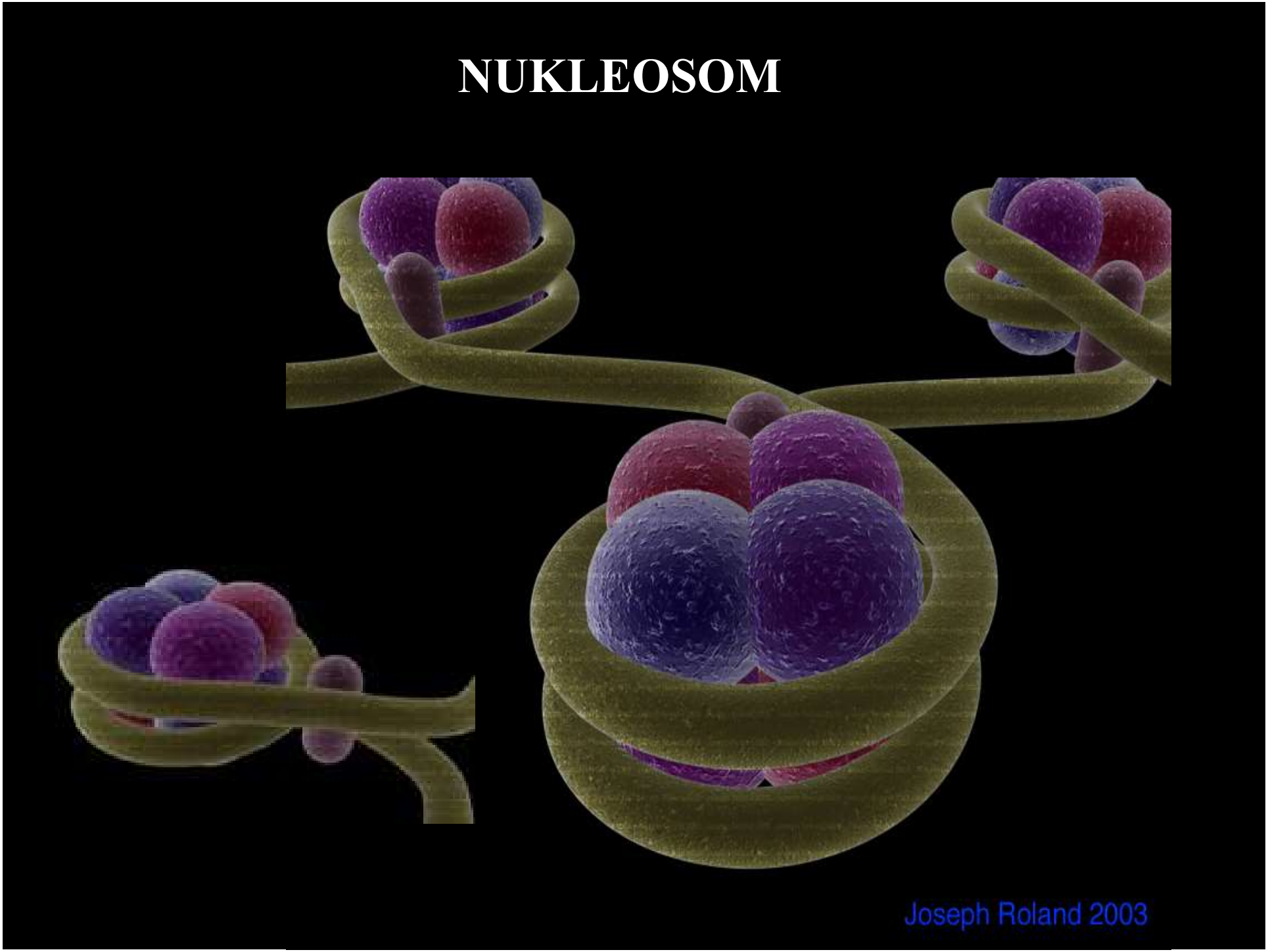
CHROMATIN: materiál jader eukaryotních buněk; nukleoproteinový komplex tvořený DNA vázanou na histony a další bílkoviny. V nedělícím se jádru lze rozlišit euchromatin, kde probíhá transkripce, a heterochromatin, který je transkripčně inaktivní.



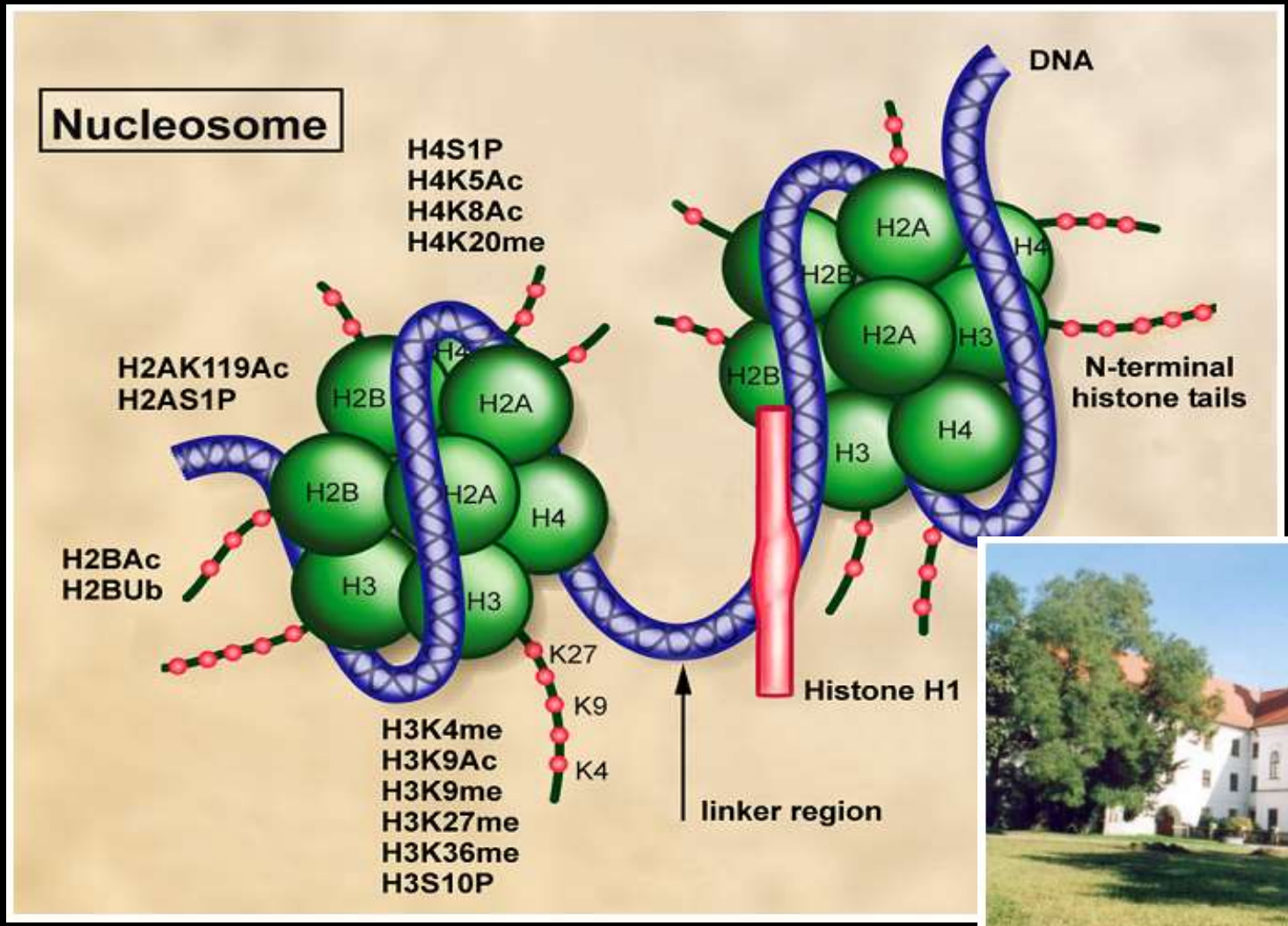
HISTONY: skupina basických bílkovin v jádře eukaryotních buněk, kde vytvářejí reversibilní komplexy s DNA. Rozlišuje se pět typů histonů: H1, H2A, H2B, H3 a H4. Histony H2A, H2B, H3 a H4 tvoří vždy ve dvou kopiích oktamery, kolem nichž se obtáčí dvojšroubovicová DNA; tento útvar se nazývá nukleosom. 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.



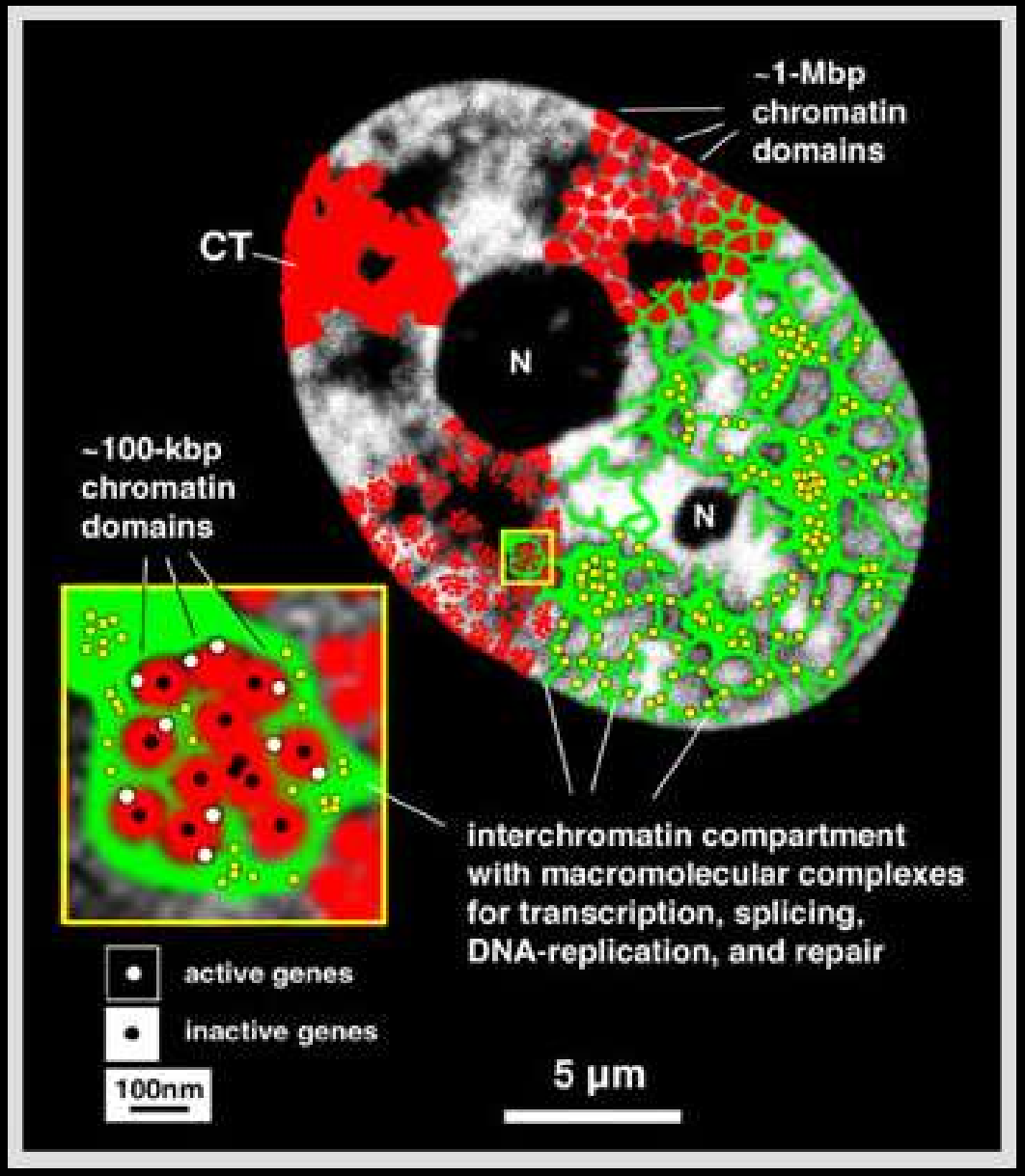
NUKLEOSOM



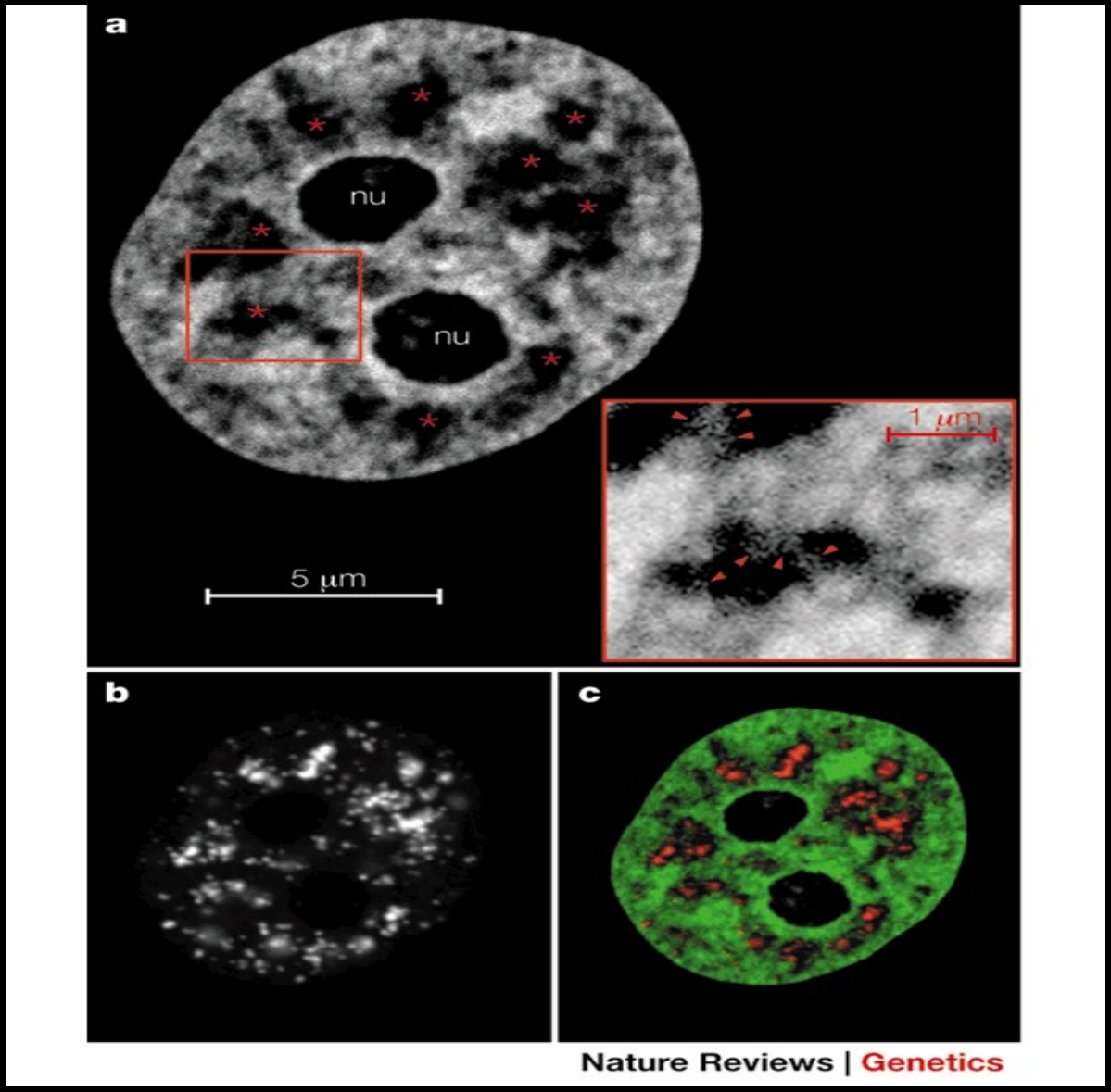
Histone signature
Brno nomenclature for histone modifications
(Turner, 2005)



CT-IC MODEL (T. Cremer)

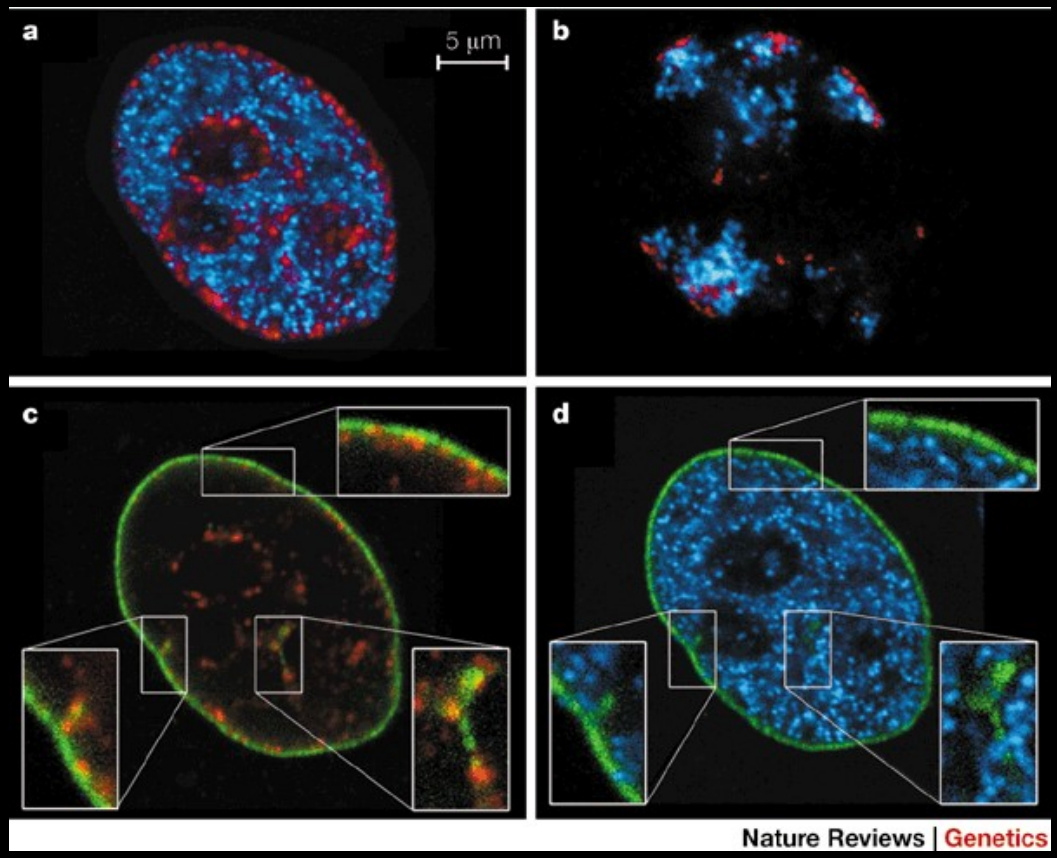


Chromatinové meziprostory

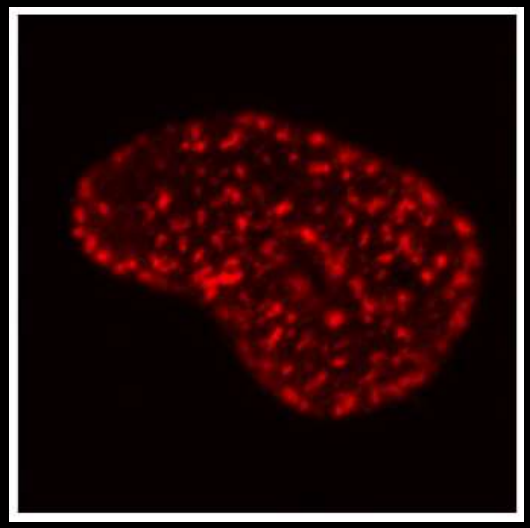


T. Cremer group, Munich

Struktura chromatinu a jederné procesy
Pozdně a časně se replikující chromatin



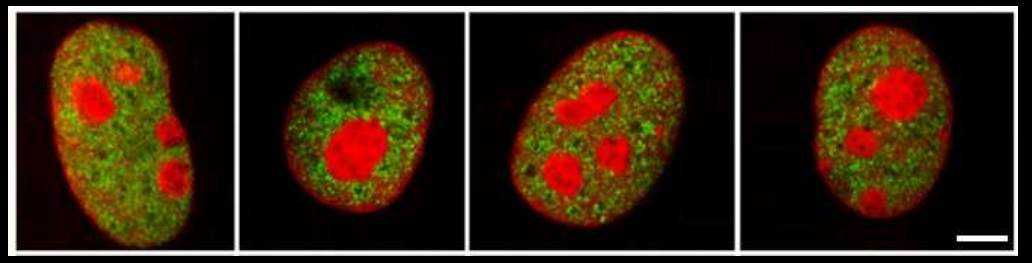
Replikační ohniska



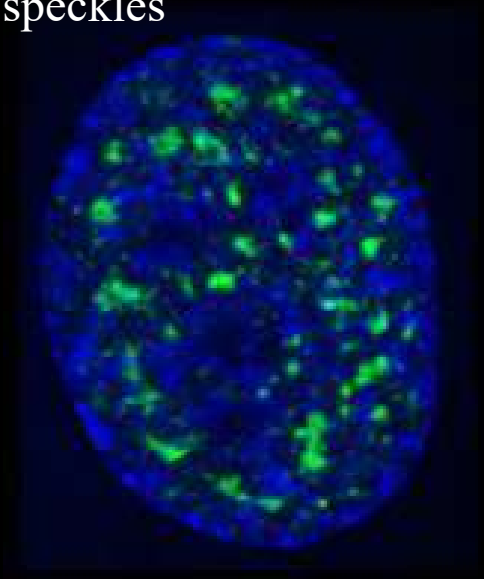
T. Cremer group, Munich

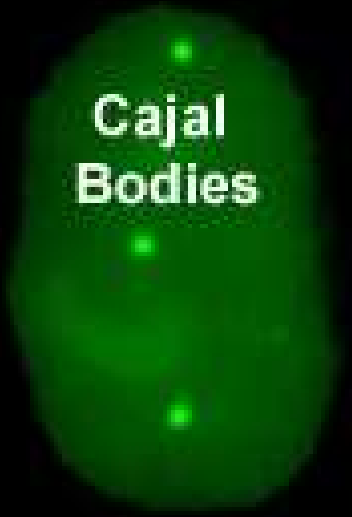
Nuclear compartments:

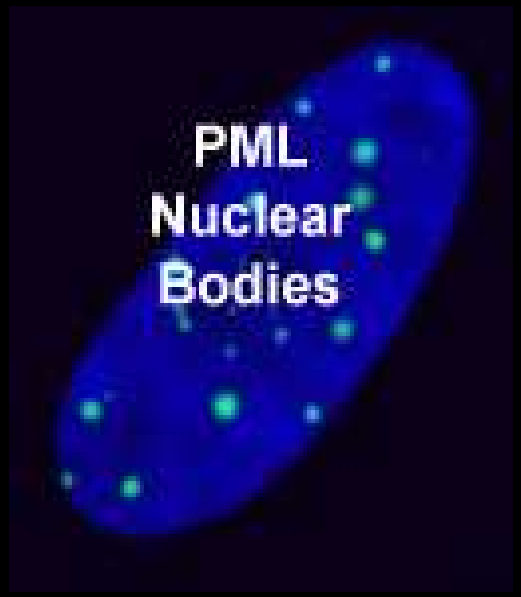
1. Nucleolus
2. Splicing speckles
3. Cajal bodies
4. PML bodies
5. snRNP

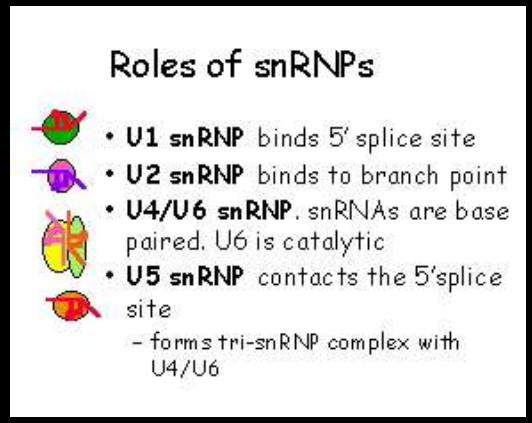


speckles

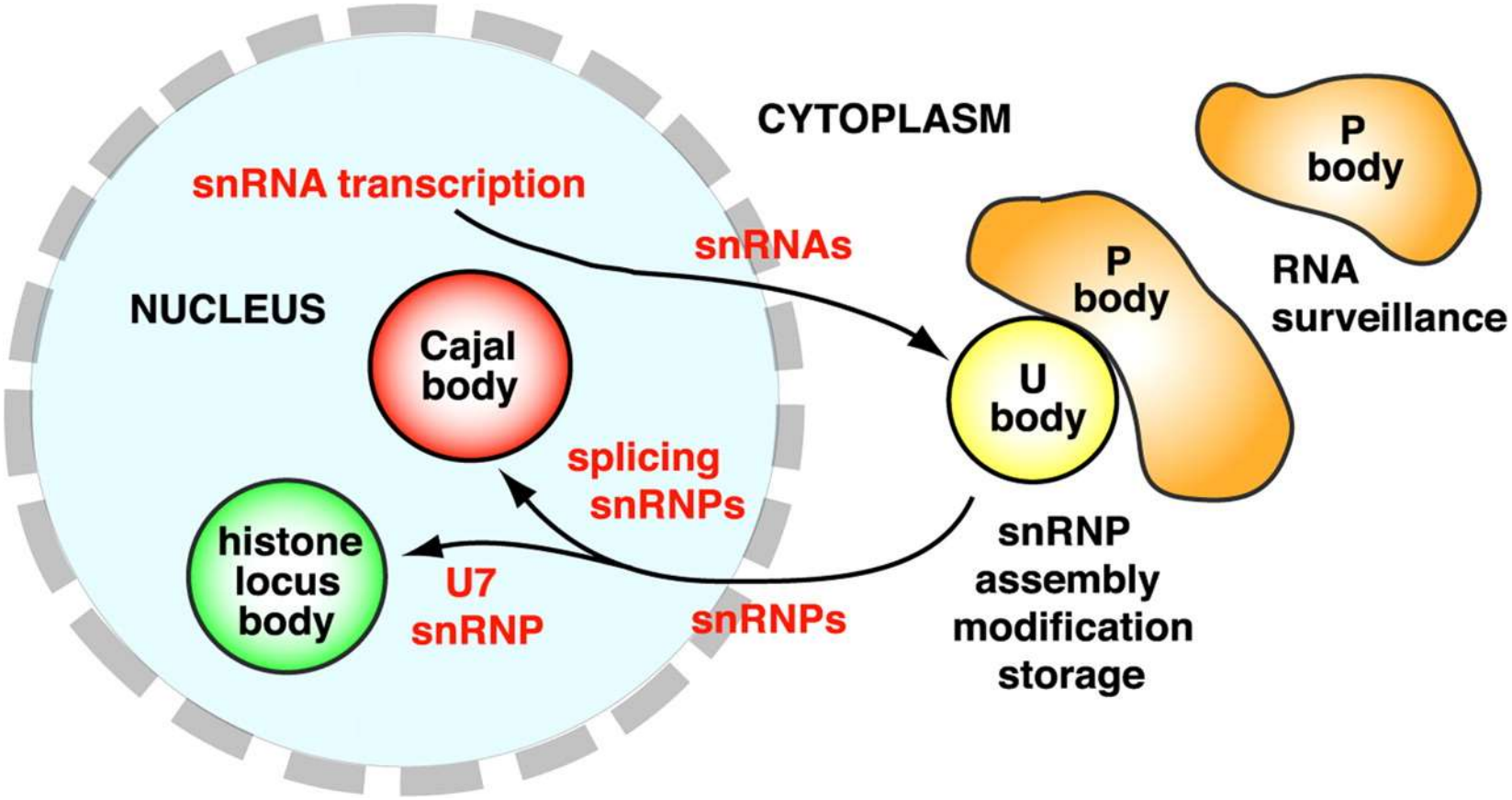






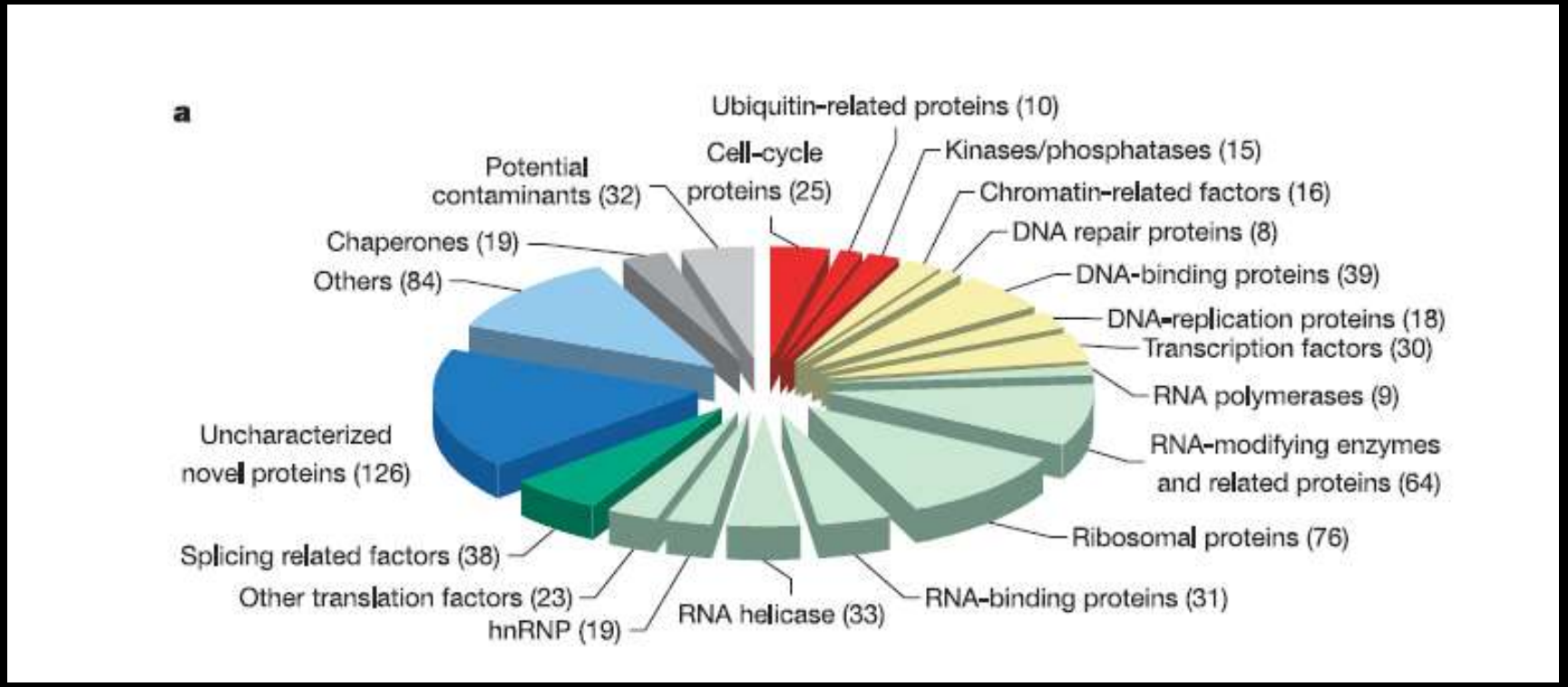


Nuclear and cytoplasmic bodies involved in snRNP assembly.



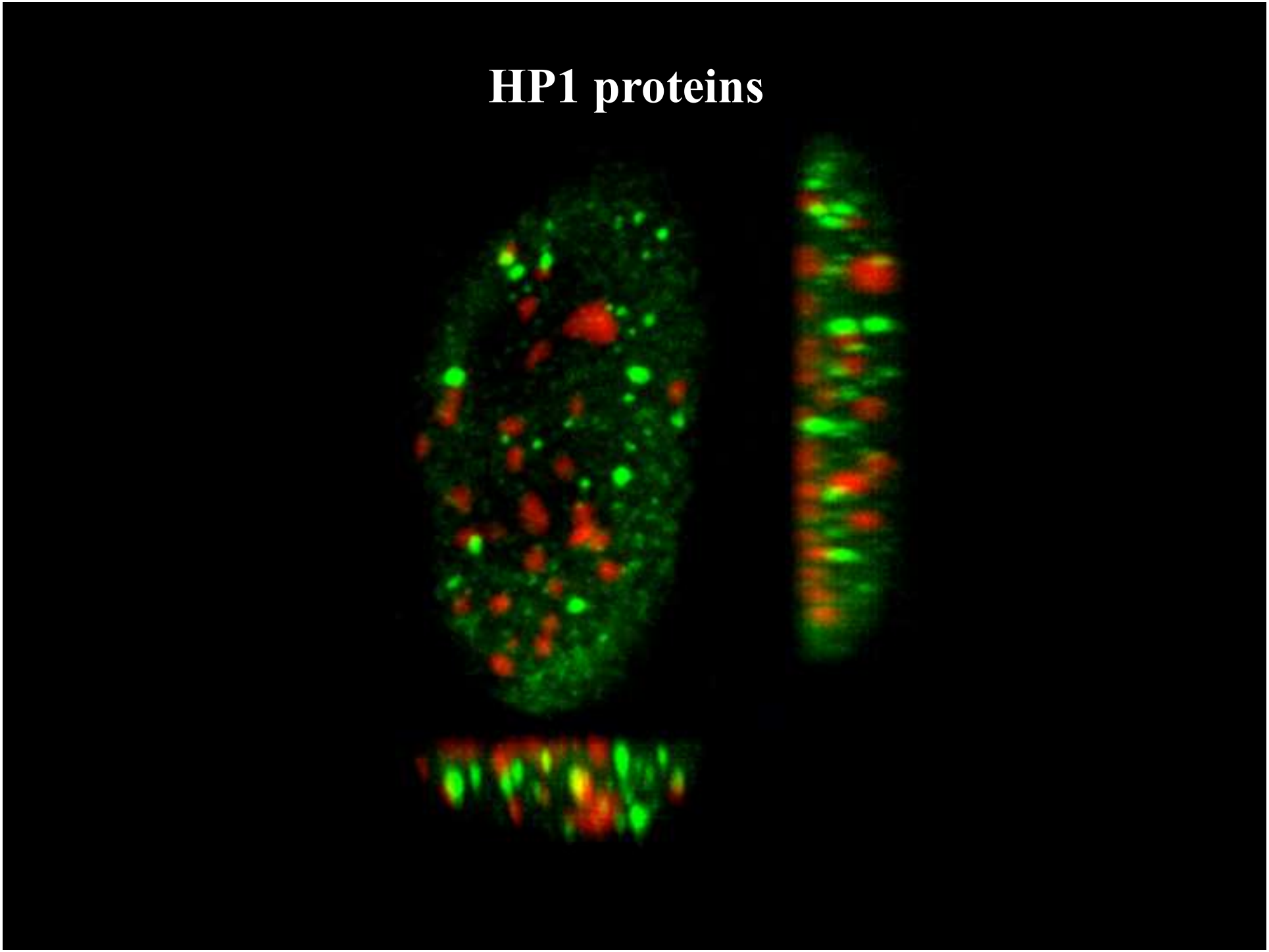
Liu J , Gall J G PNAS 2007;104:11655-11659

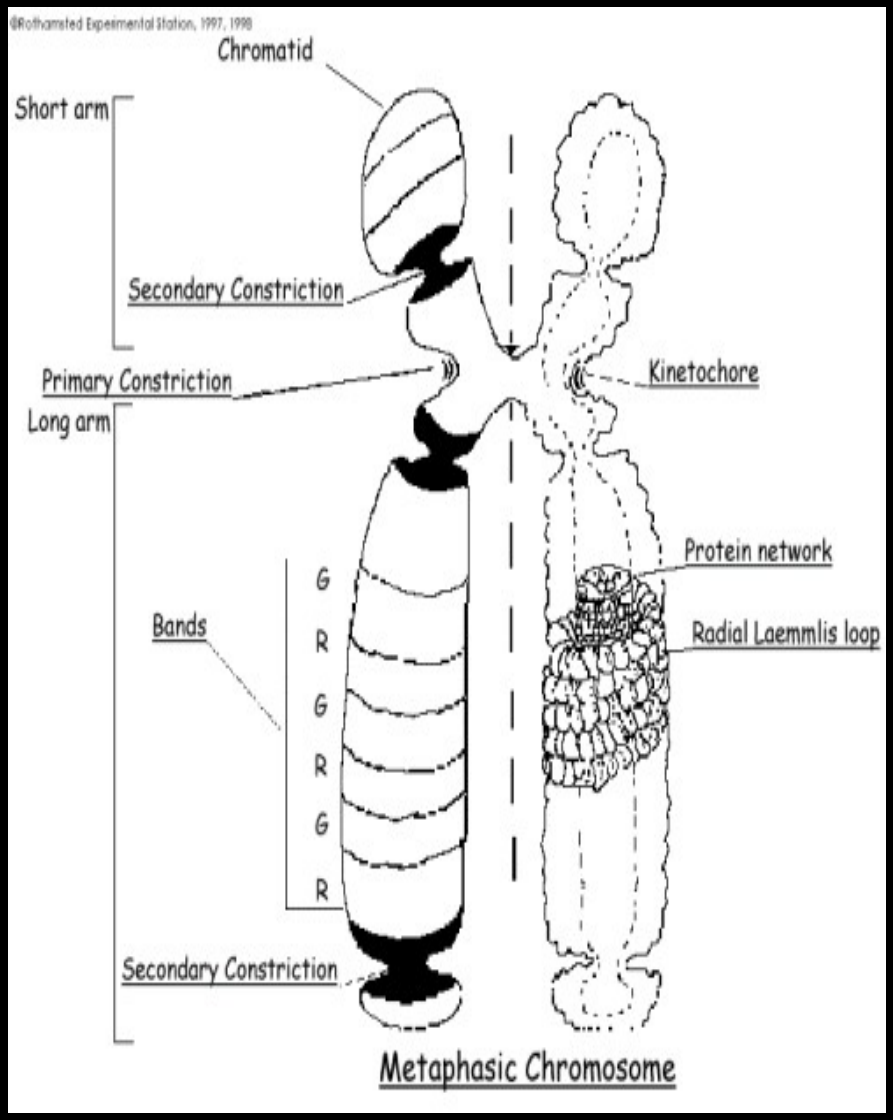
©2007 by National Academy of Sciences



Andersen et al., Nature, 2005

HP1 proteins





Kondenzace chromatinu

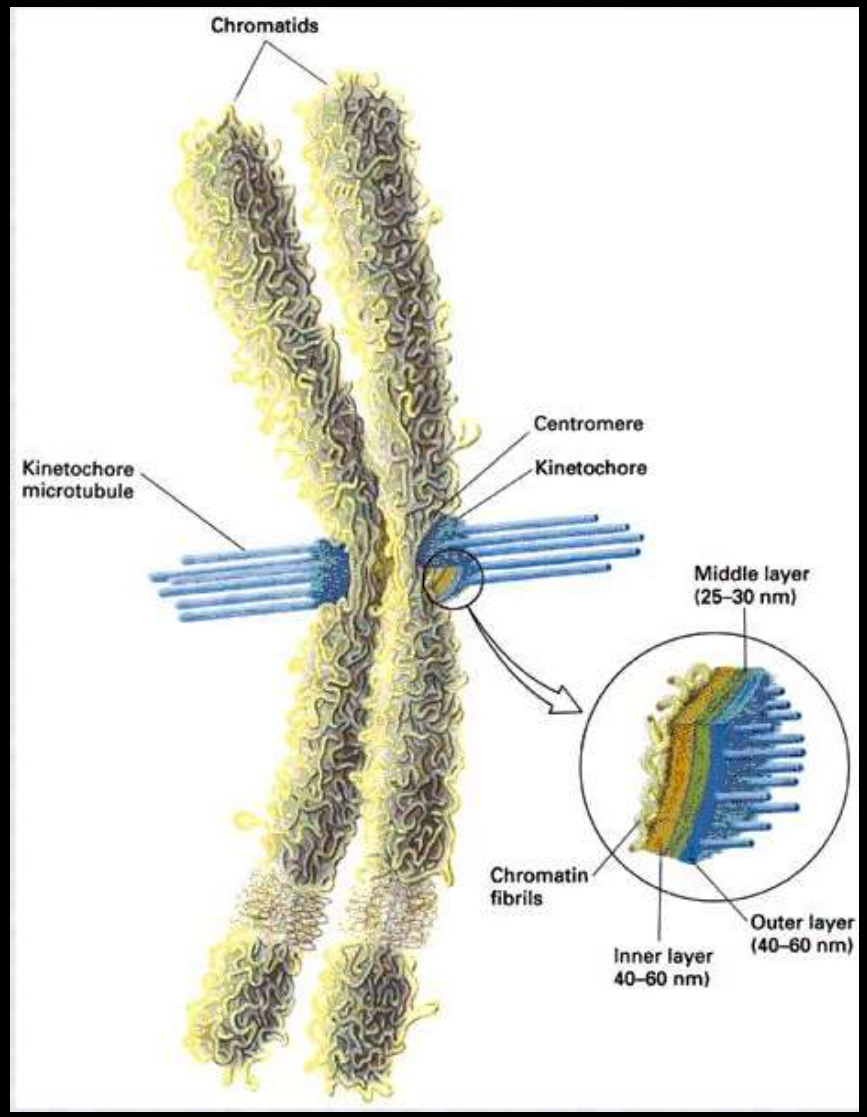
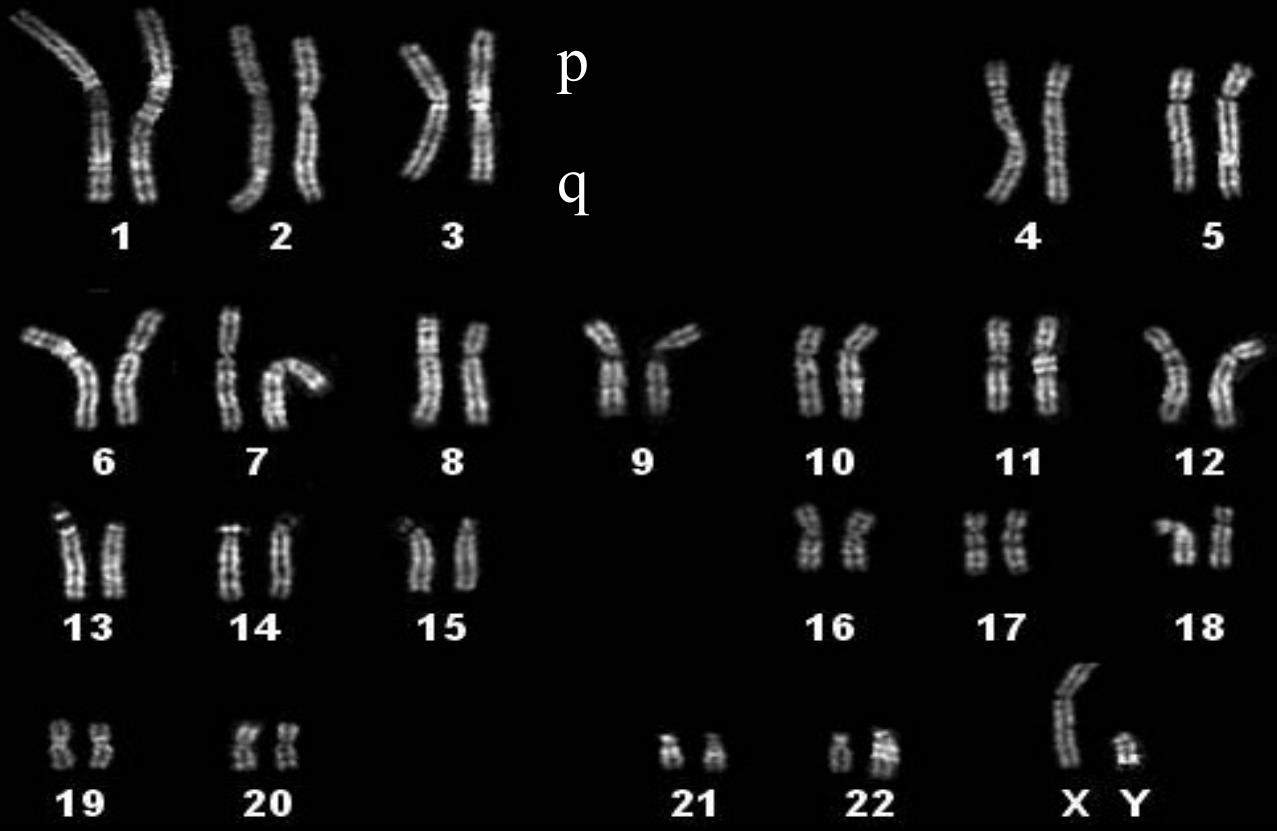
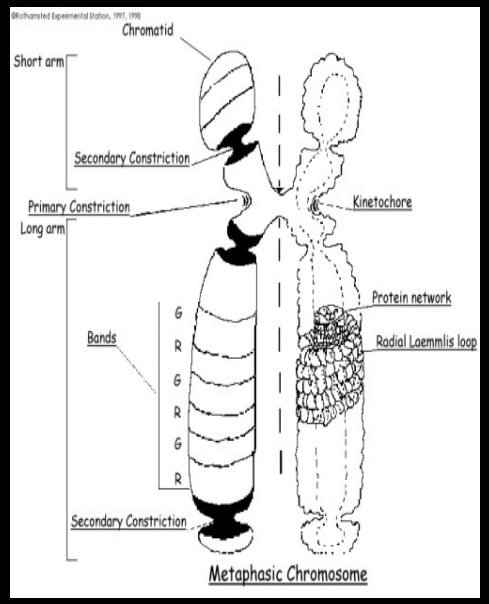


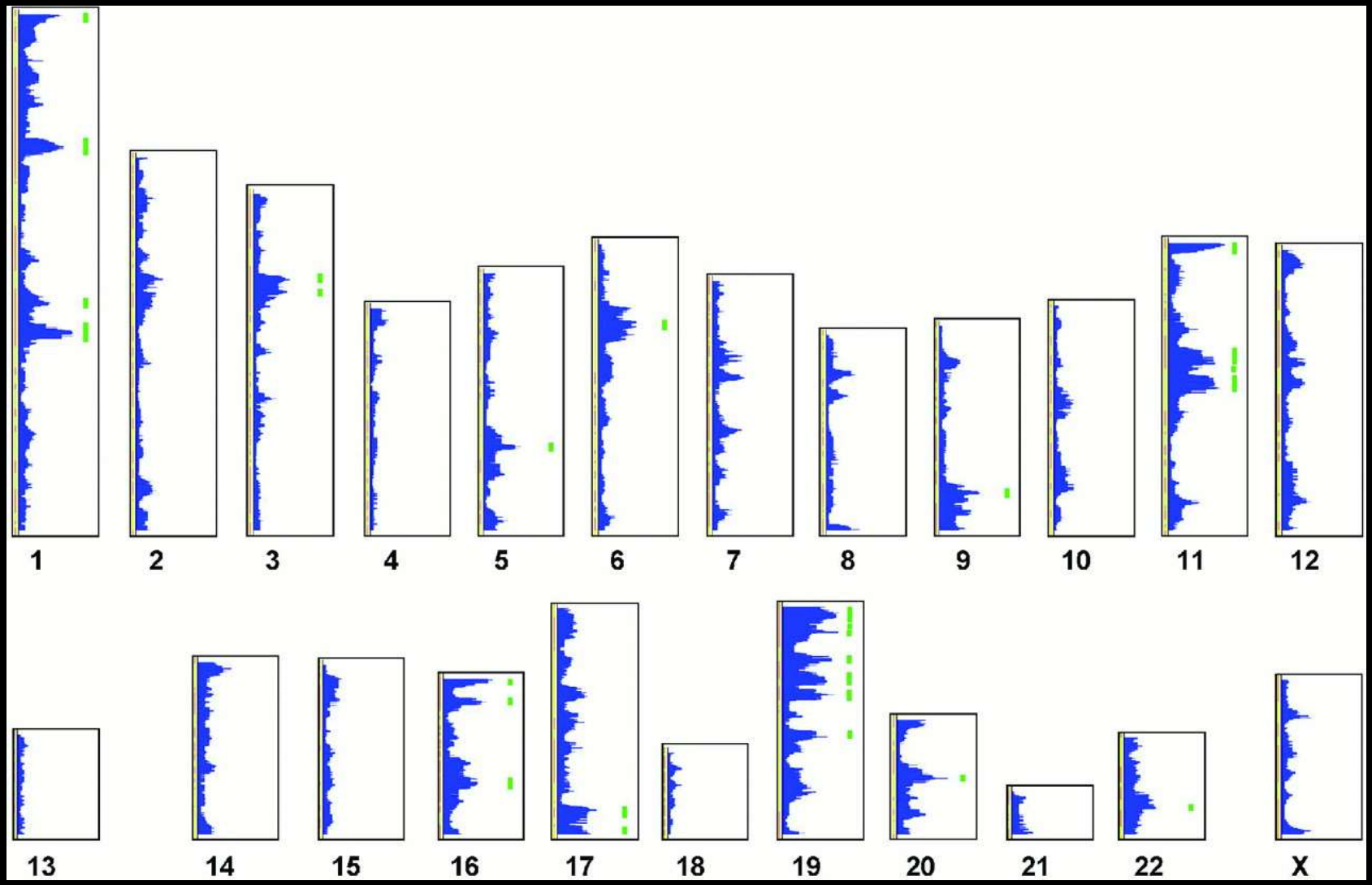
Figure 23-38, p. 1094, Molecular Cell Biology, 3rd ed., Lodish, et al., copyright 1995,

Typy chromozomů: meta- submeta-, akrocentrické

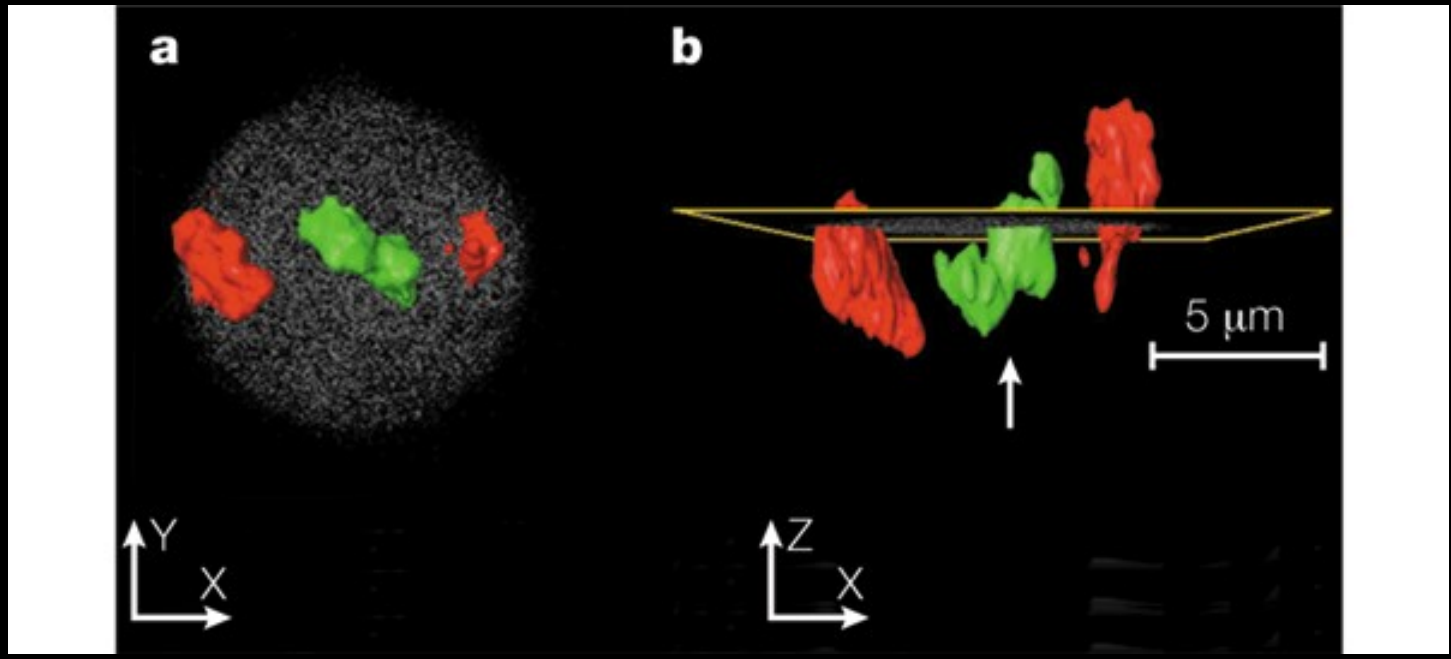




Transcriptome map (Caron et al., 2001)

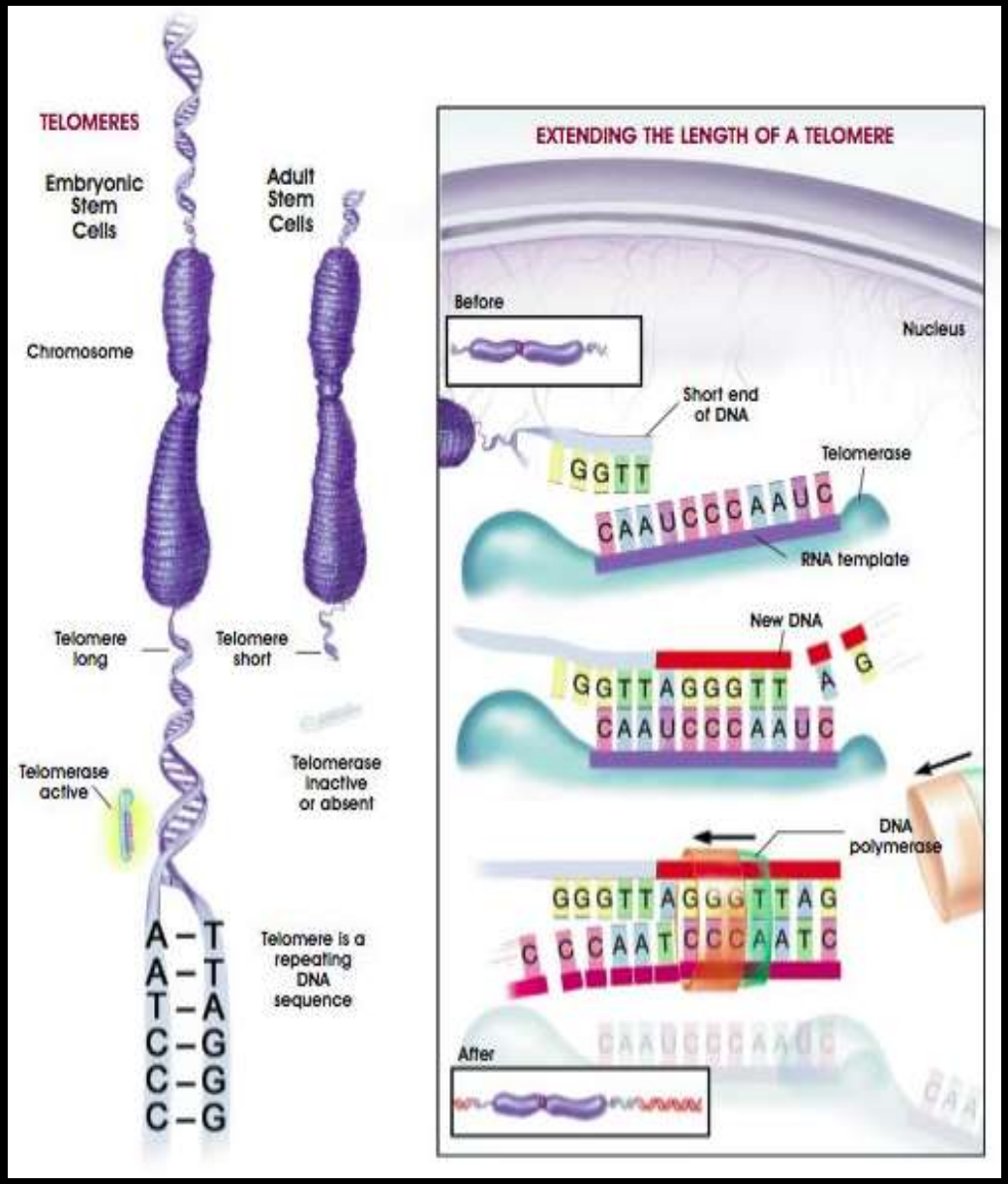


HSA 18 and 19 (positioning and gene density)

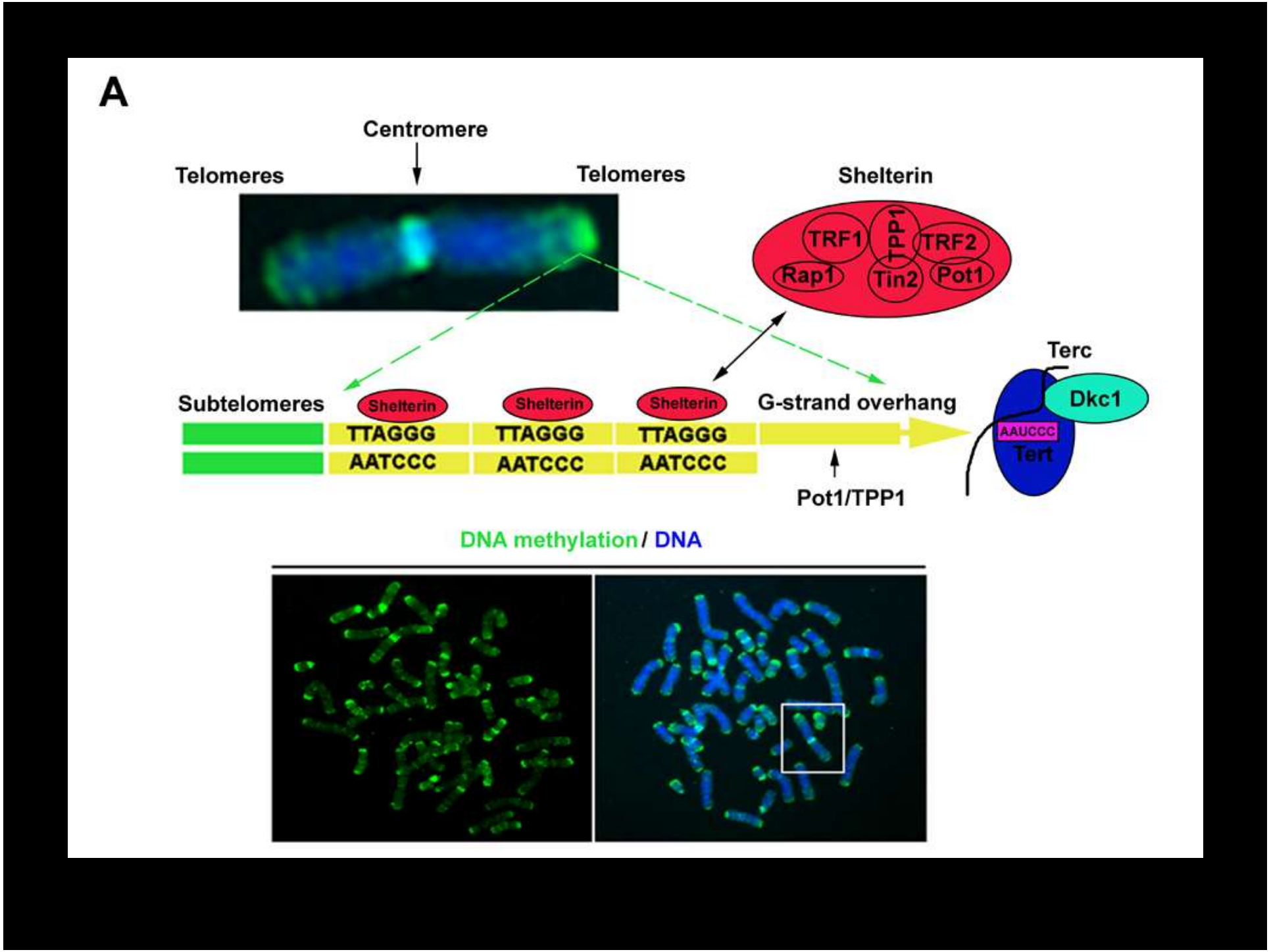


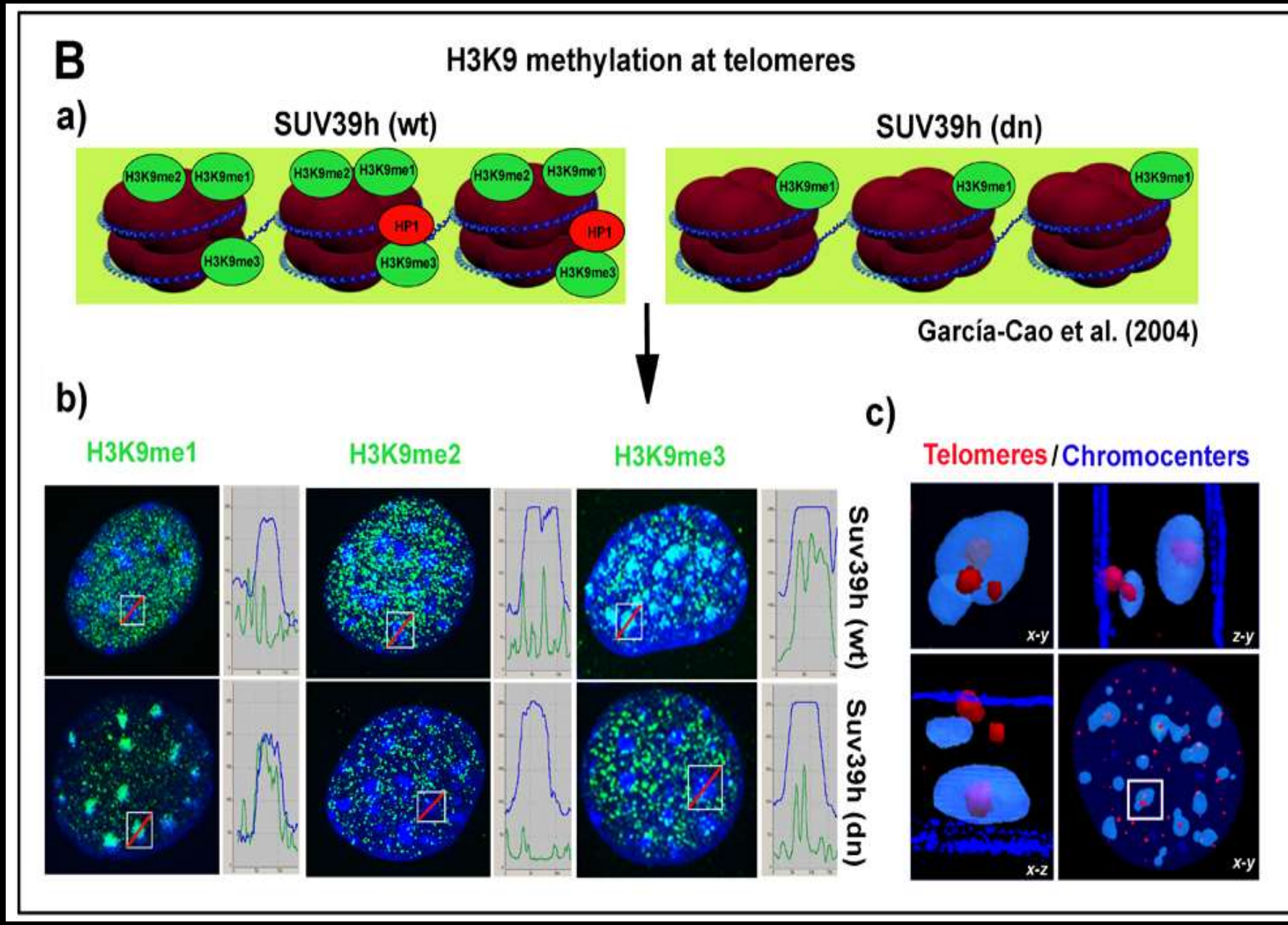
T. Cremer group, Munich

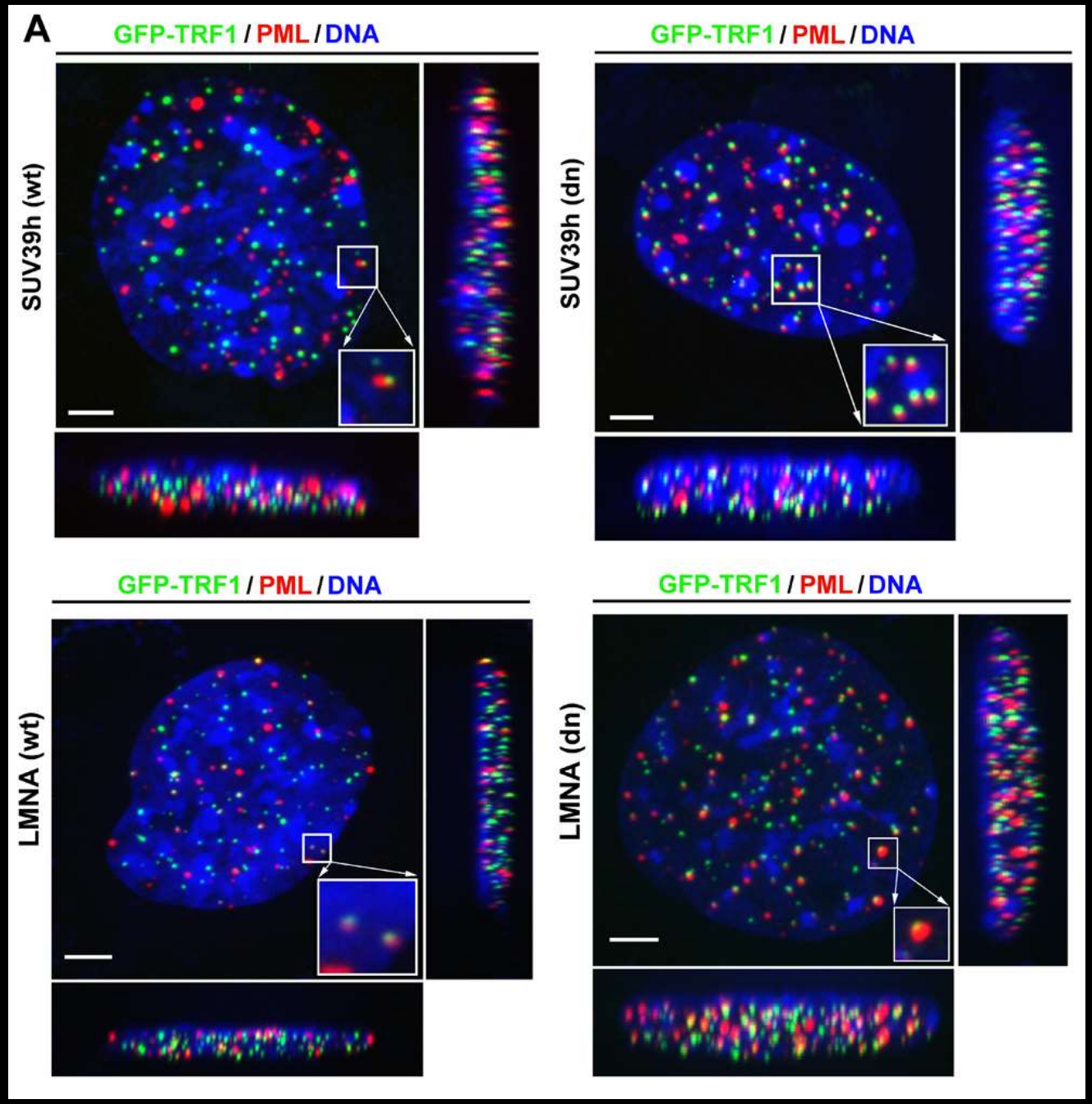
Telomeres (TTAGGG sequence and different proteins)



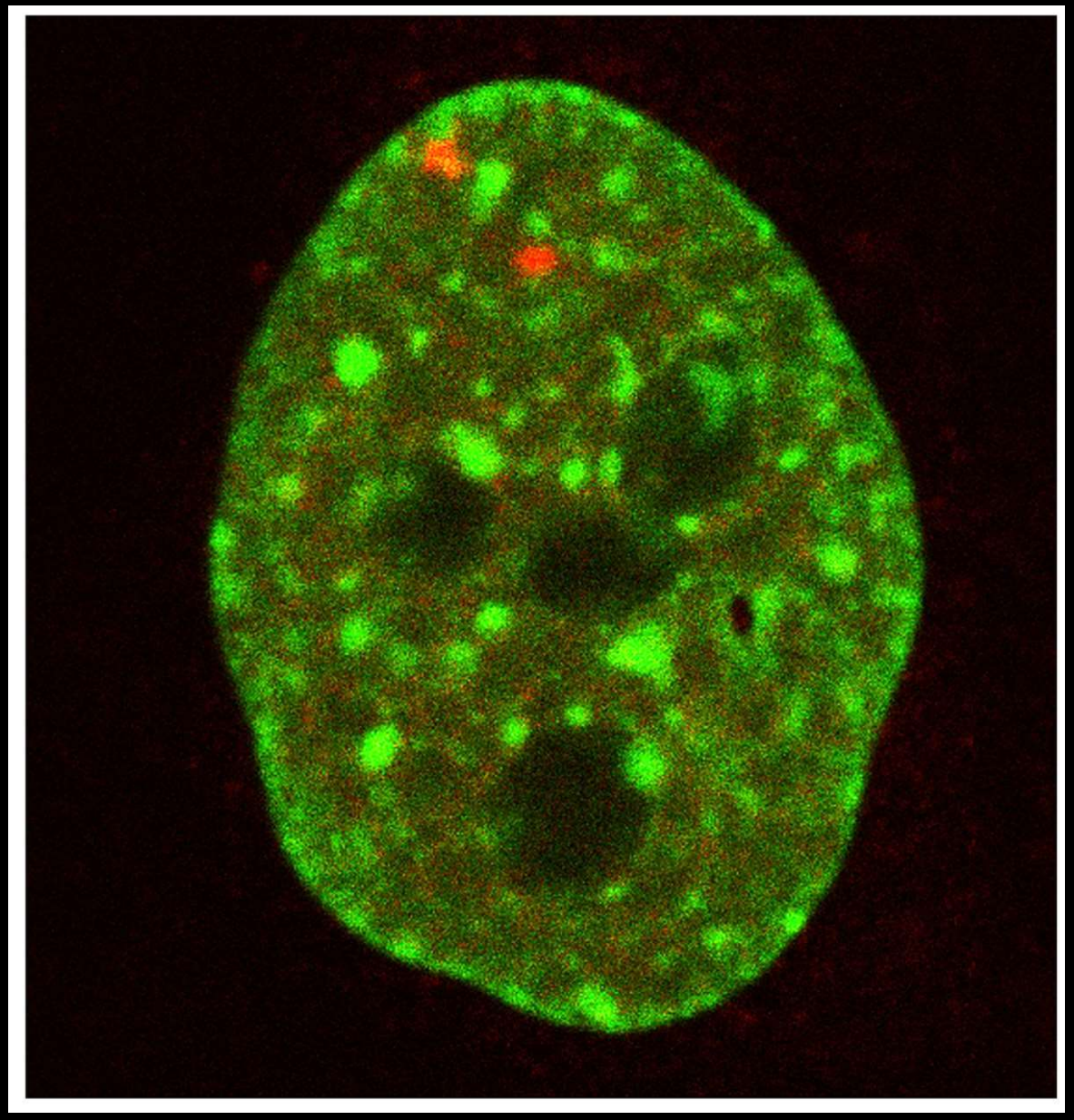
Telomerase is composed of two subunits, Telomerase Reverse Transcriptase (hTERT, the 'h' is for human) and hTR (Telomerase RNA). These two subunits are coded for by two different genes in the genome. Using hTR template, hTERT can add a six nucleotide repeating sequence, 5'-TTAGGG to the 3' strand of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


A
Centromere
Telomeres
Telomeres
Shelterin
TRF1
TPP1
TRF2
Rap1
Tin2
Pot1
Terc
Dkc1
AAUCCC
Tert
Subtelomeres
Shelterin
Shelterin
Shelterin
G-strand overhang
TTAGGG
TTAGGG
TTAGGG
AATCCC
AATCCC
AATCCC
Pot1/TPP1
DNA methylation / DNA


B
H3K9 methylation at telomeres
a)
SUV39h (wt)
SUV39h (dn)
H3K9me2
H3K9me1
H3K9me3
HP1
García-Cao et al. (2004)
b)
H3K9me1
H3K9me2
H3K9me3
Suv39h (wt)
Suv39h (dn)
c)
Telomeres / Chromocenters
x-y
z-y
x-z
x-y

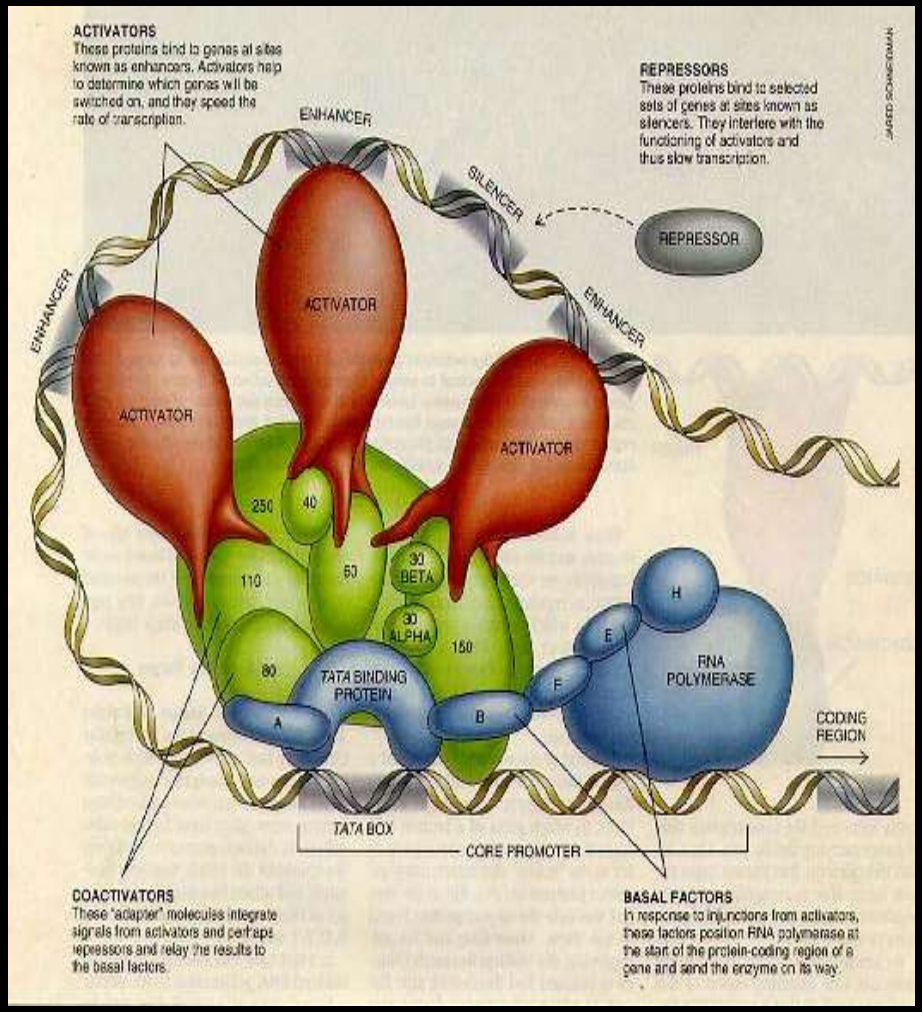

A
GFP-TRF1 / PML / DNA
SUV39h (wt)
GFP-TRF1 / PML / DNA
SUV39h (dn)
GFP-TRF1 / PML / DNA
LMNA (wt)
GFP-TRF1 / PML / DNA
LMNA (dn)

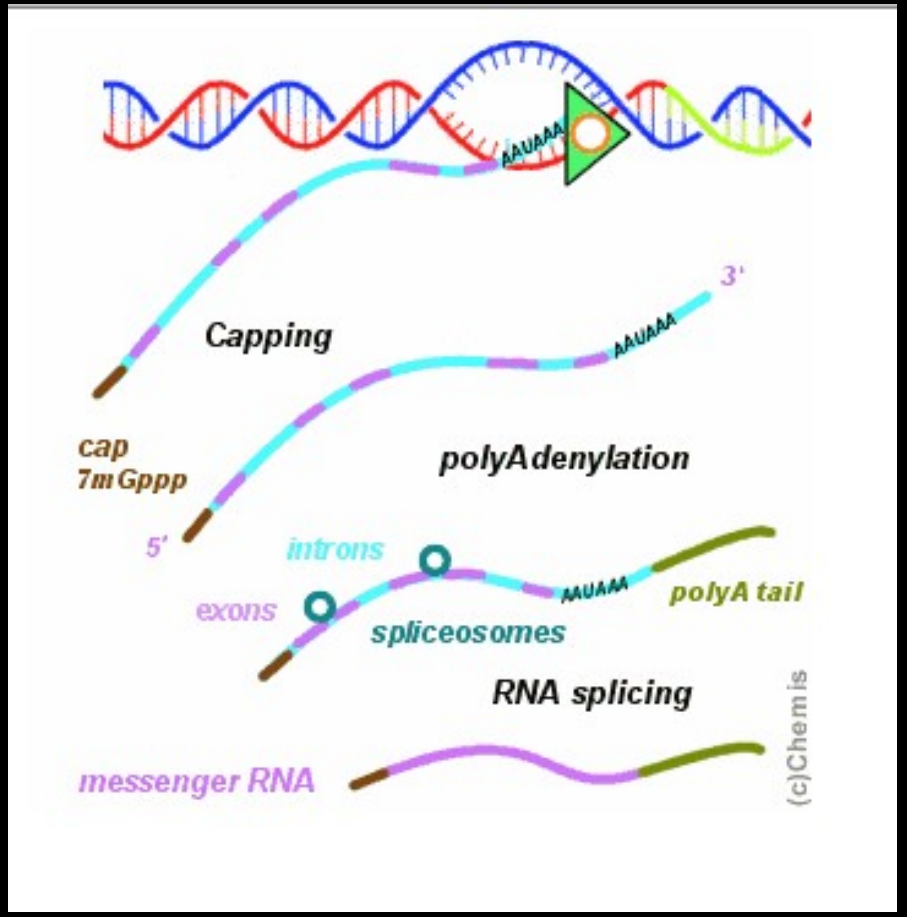
DNA repair foci

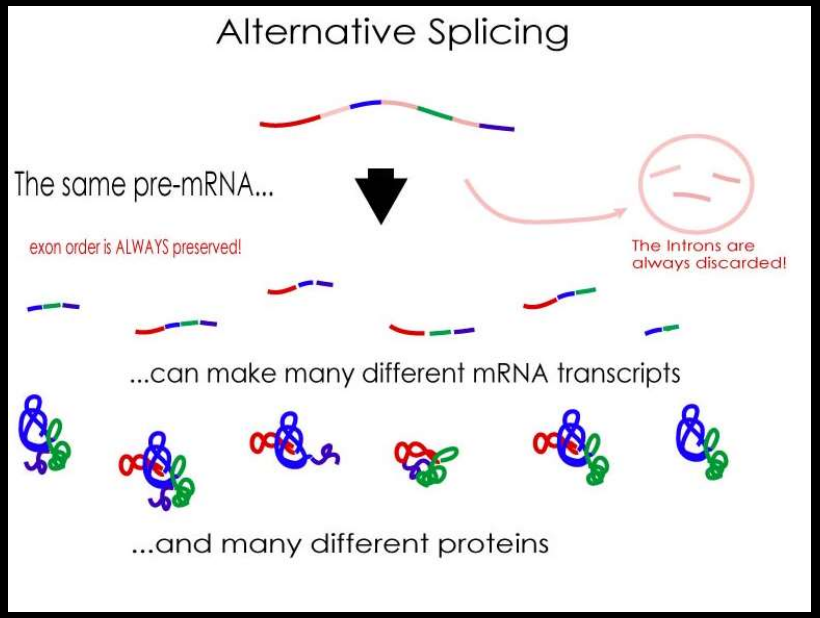


TRANSKRIPCE: přepis, biosynthesa řetězce RNA podle templátového řetězce DNA, přičemž jednotlivé nukleotidy jsou připojovány na základě komplementarity (viz base nukleových kyselin). Klíčovým enzymem této synthesy je RNA-polymerasa. Transkripce probíhá ve třech stupních: **a)** iniciace (zahájení), kdy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

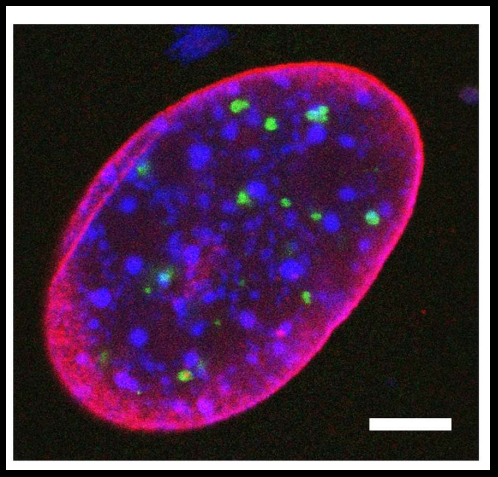
diferenciace buněk.


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.
JARED SCHNEIDMAN
ENHANCER
SILENCER
REPRESSOR
ENHANCER
ENHANCER
ACTIVATOR
ACTIVATOR
ACTIVATOR
250
40
110
60
30 BETA
30 ALPHA
150
80
TATA BINDING PROTEIN
A
B
E
F
H
RNA POLYMERASE
CODING REGION
TATA BOX
CORE PROMOTER
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.

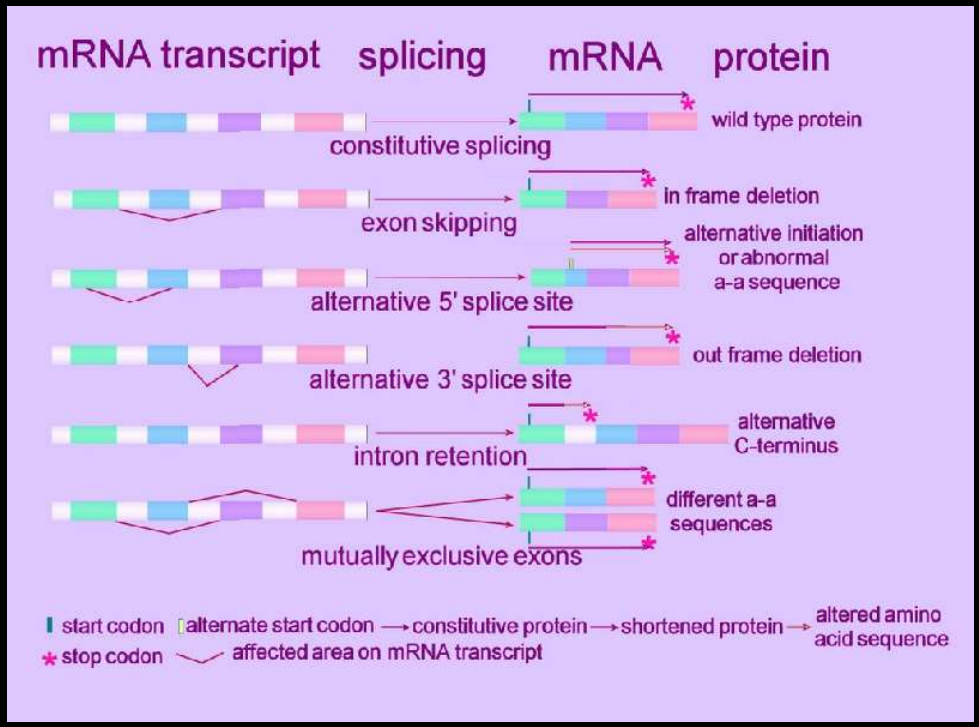

AAUAAA
Capping
3'
AAUAAA
cap
7mGppp
polyAdenylation
5'
introns
exons
AAUAAA
polyA tail
spliceosomes
RNA splicing
messenger RNA
(c)Chemis

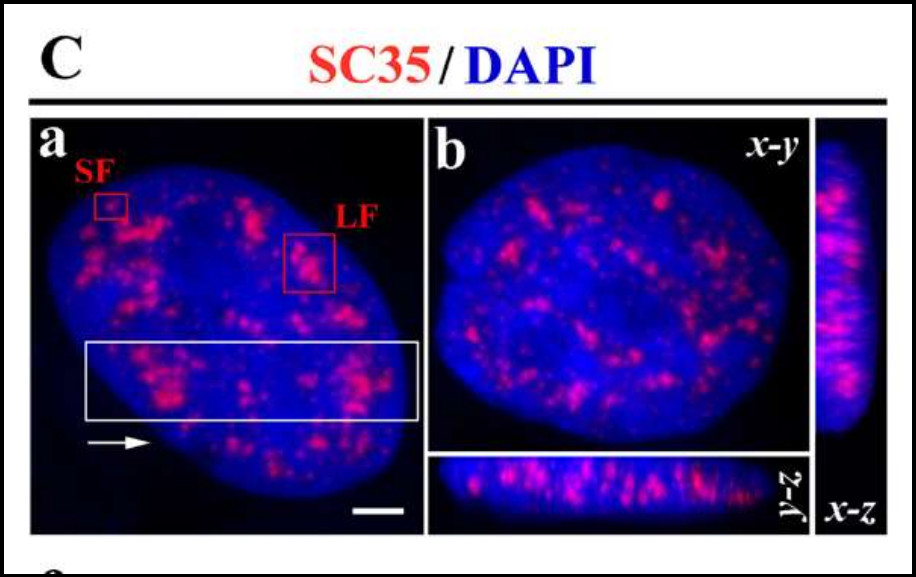


A-type lamins



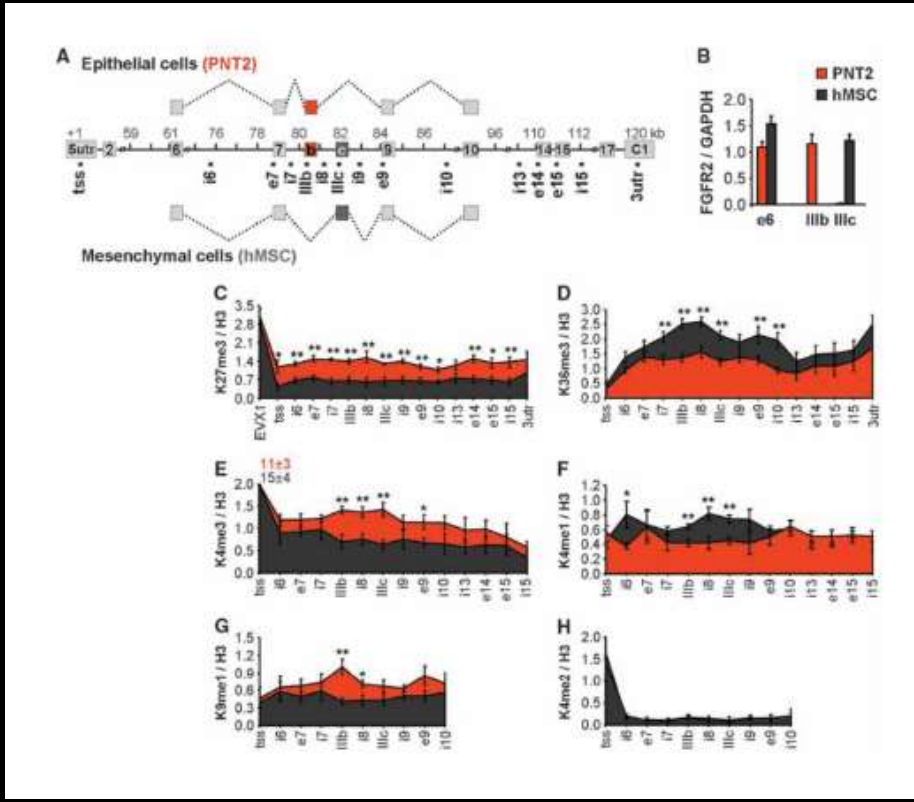
Alternative splicing is a form of epigenetic mechanism that enables a single gene to give rise to multiple, differentially spliced versions of a protein, increasing complexity without a change in the genome.



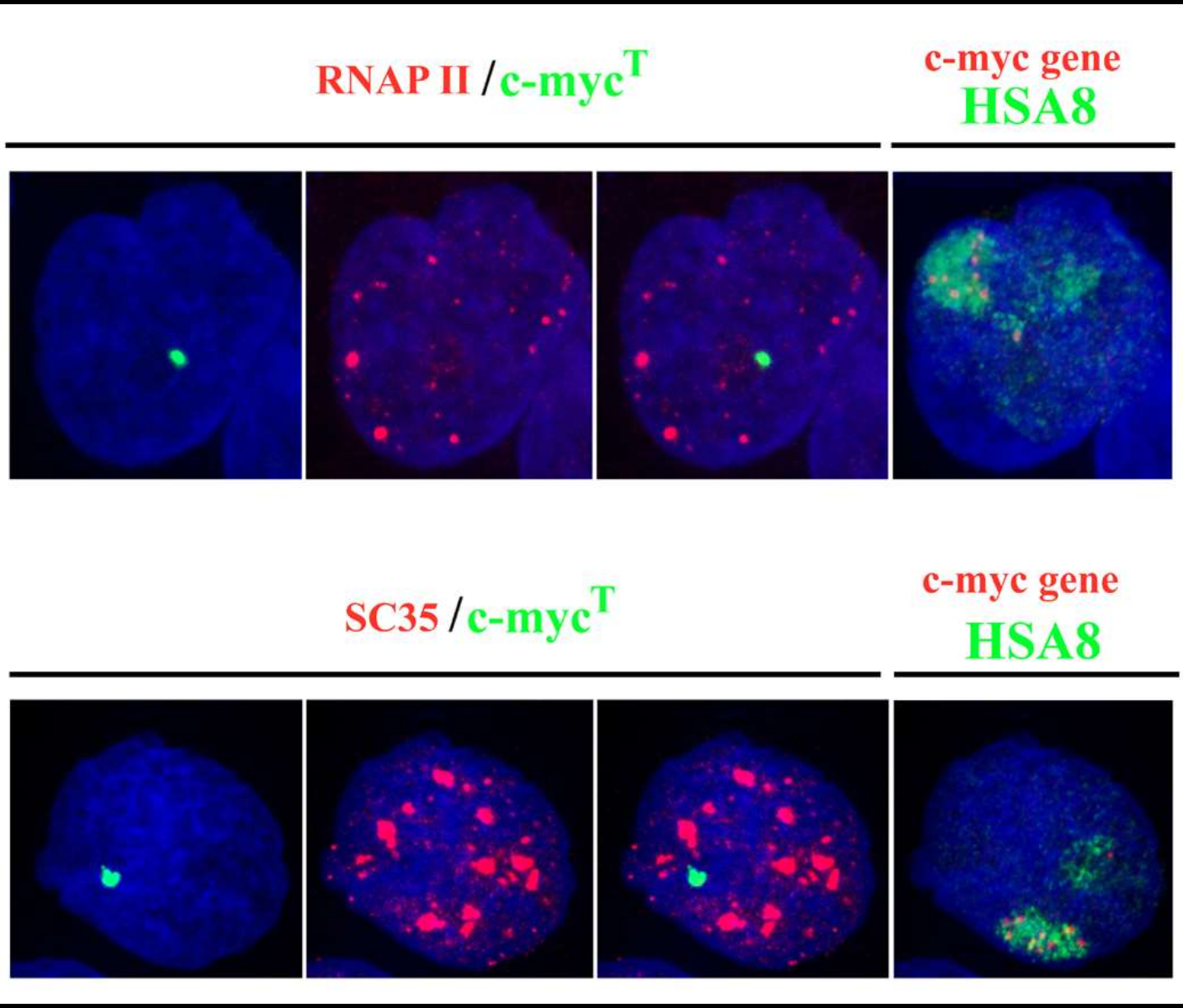


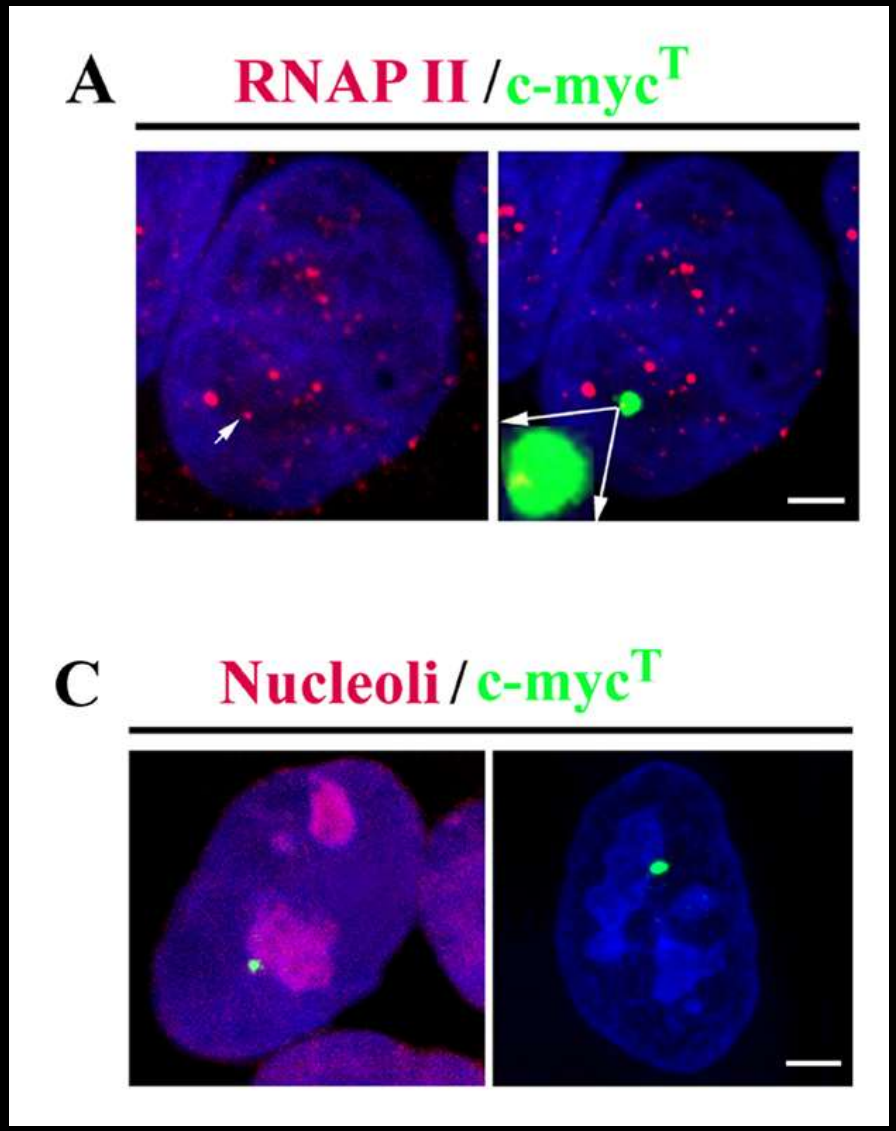
Bartova group

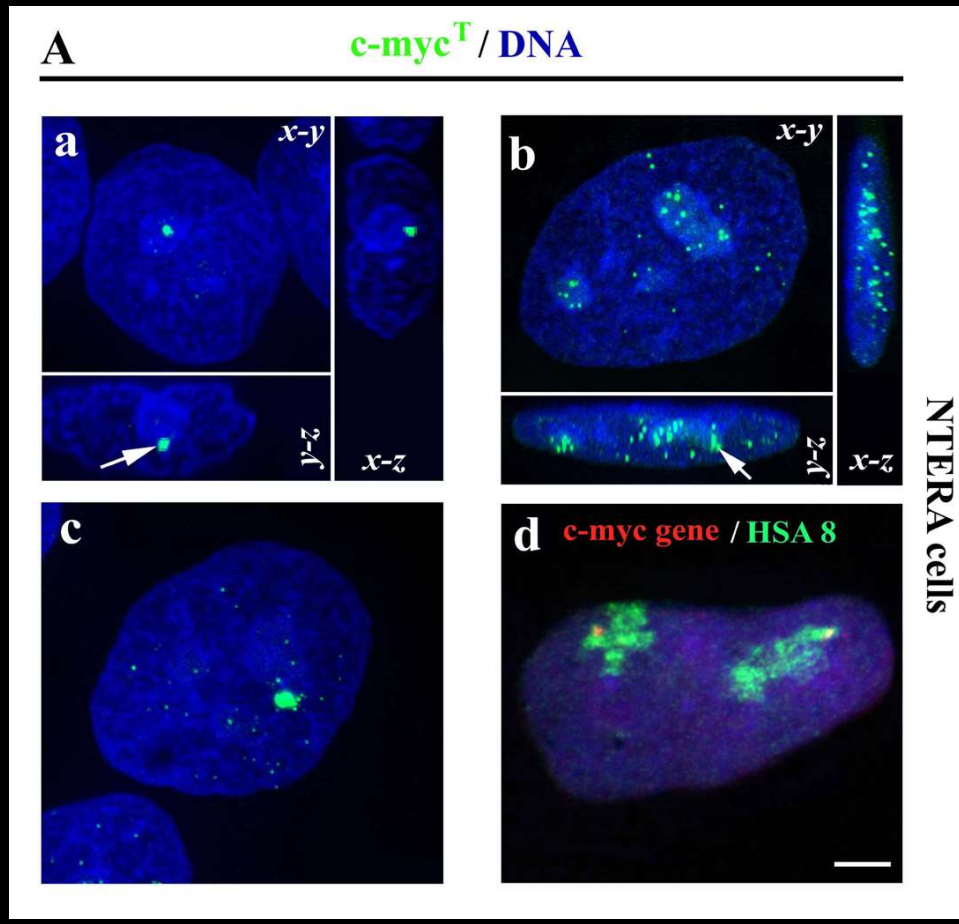
Faktory sestřihu jsou v SC-35 doménách, dále snRNP U1-U6 jsou součástí faktoru sestřihu SF2/ASF



Luco et al., Science (2010)

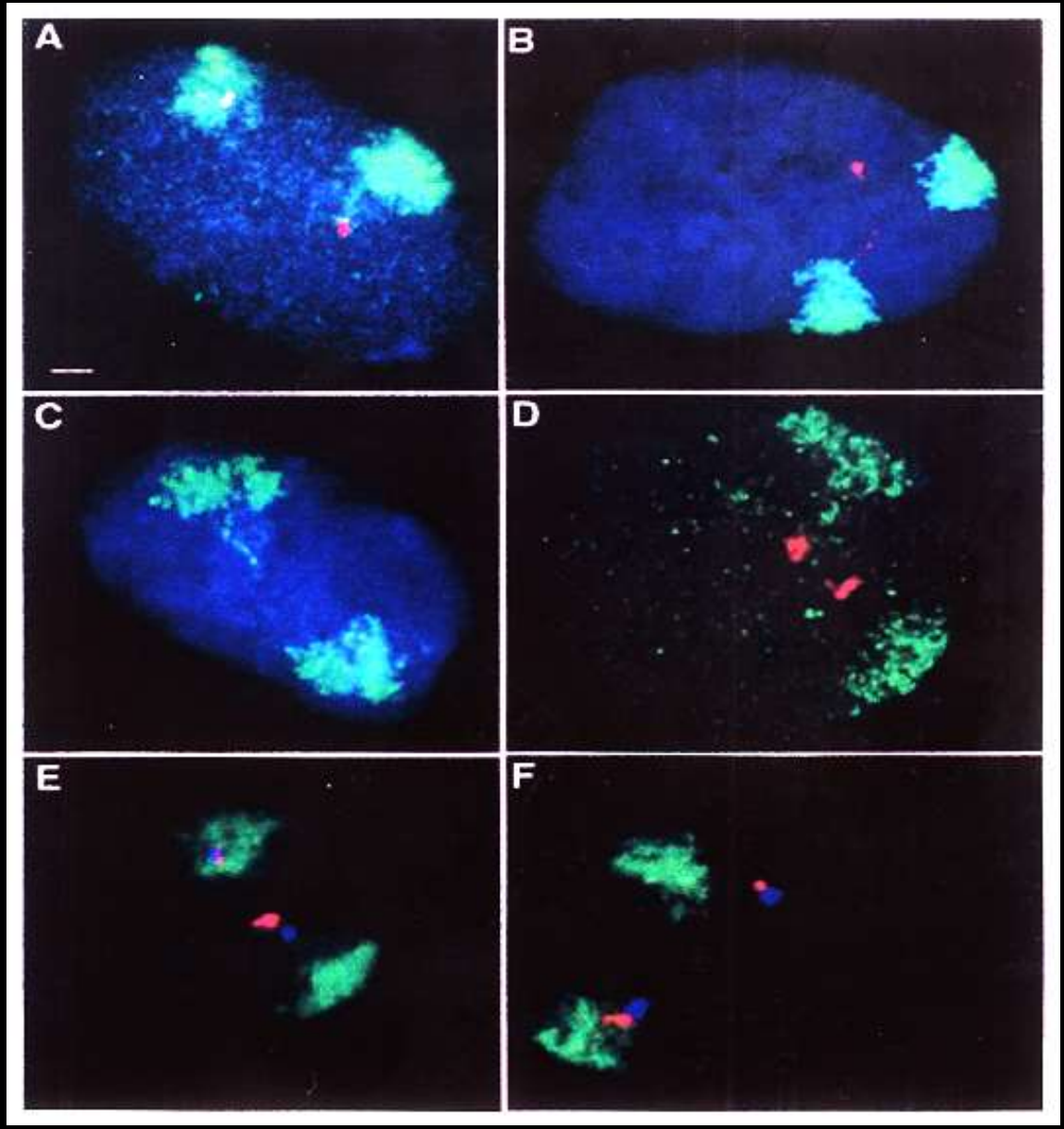

RNAP II / c-myc^T
c-myc gene
HSA8
SC35 / c-myc^T
c-myc gene
HSA8





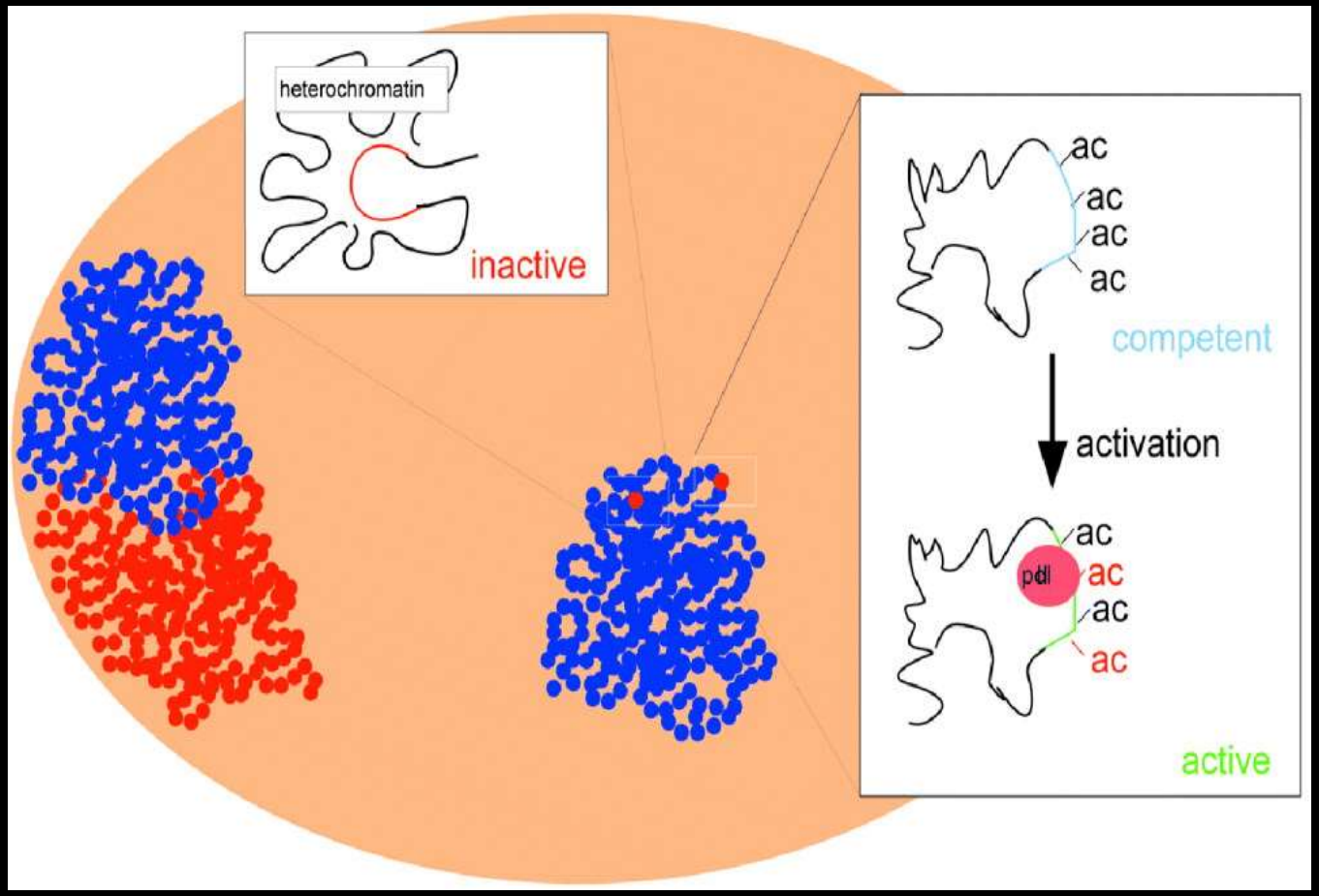
Note: Interleukin-1beta pre-mRNA splicing proceeds in cytoplasm of enucleated platelets (Denis M. et al., 2005).

MHC on HSA 6



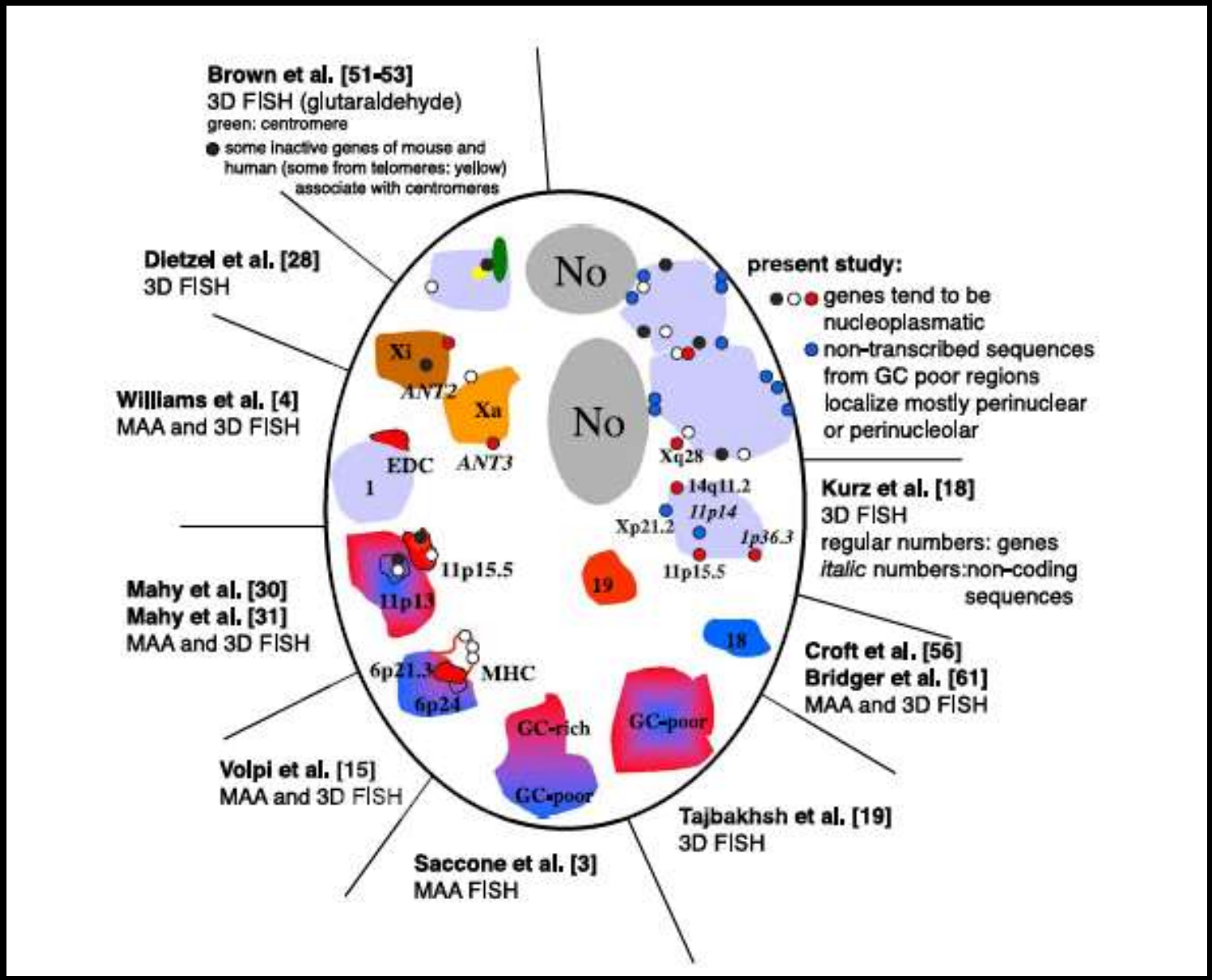
Volpi et al., 2000

Active / inactive chromatin

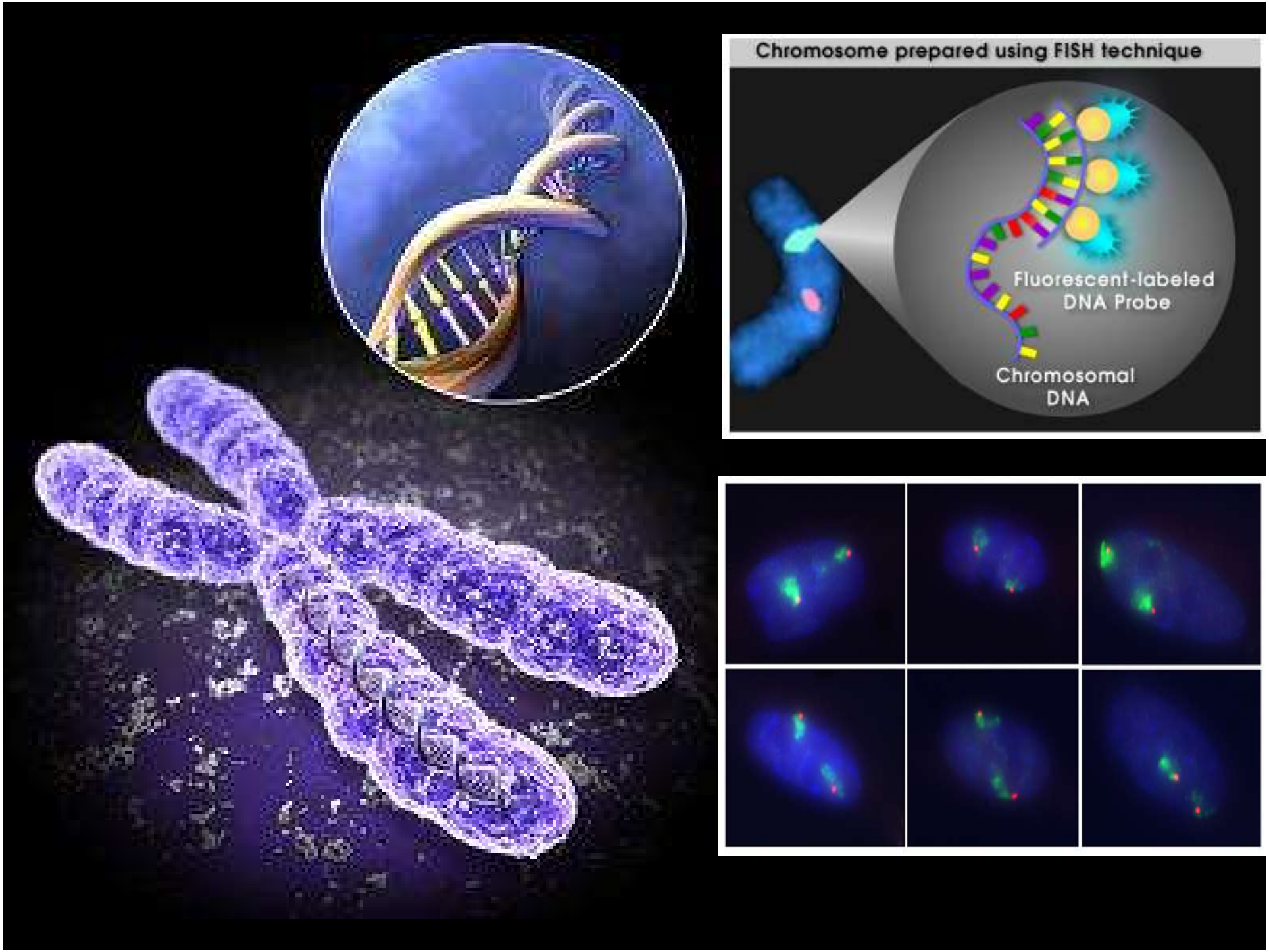


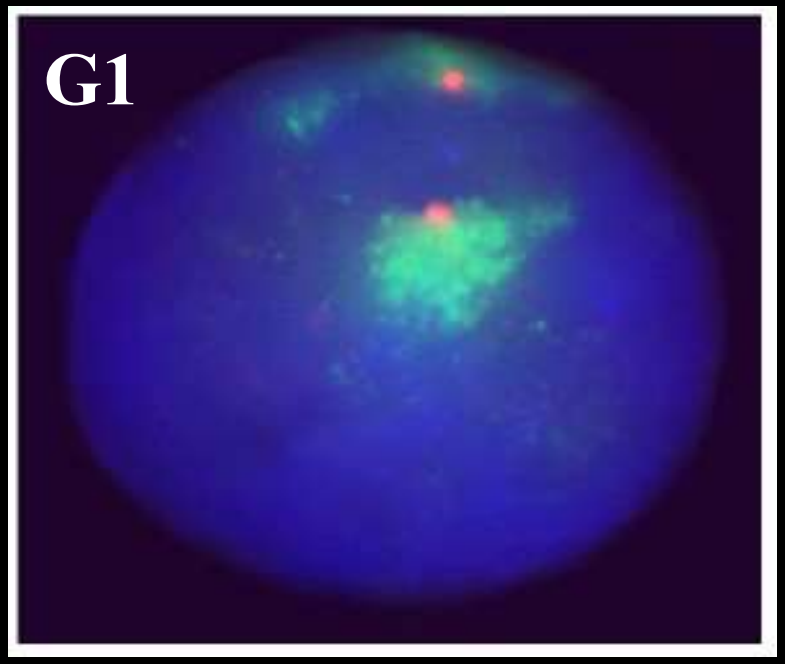
T. Cremer group, Munich

IMTERMINGLING

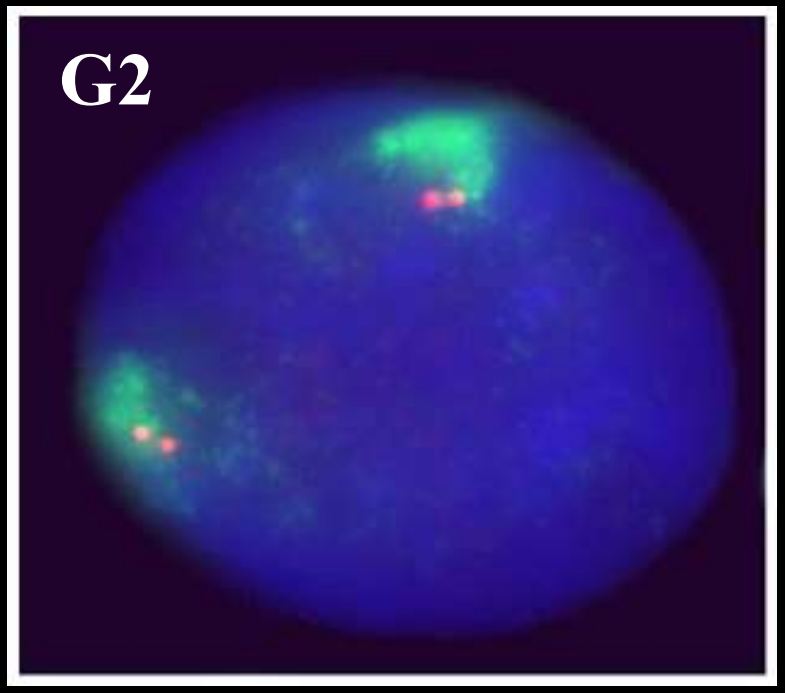


Scheuermann et al., Exp. Cell Res. (2004)


Chromosome prepared using FISH technique
Fluorescent-labeled DNA Probe
Chromosomal DNA

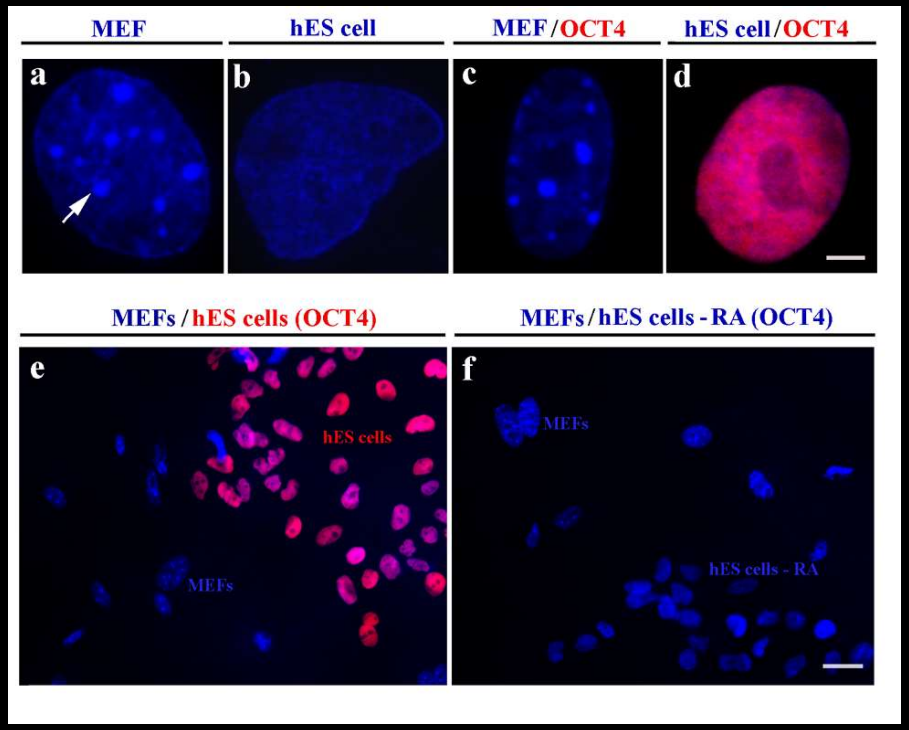


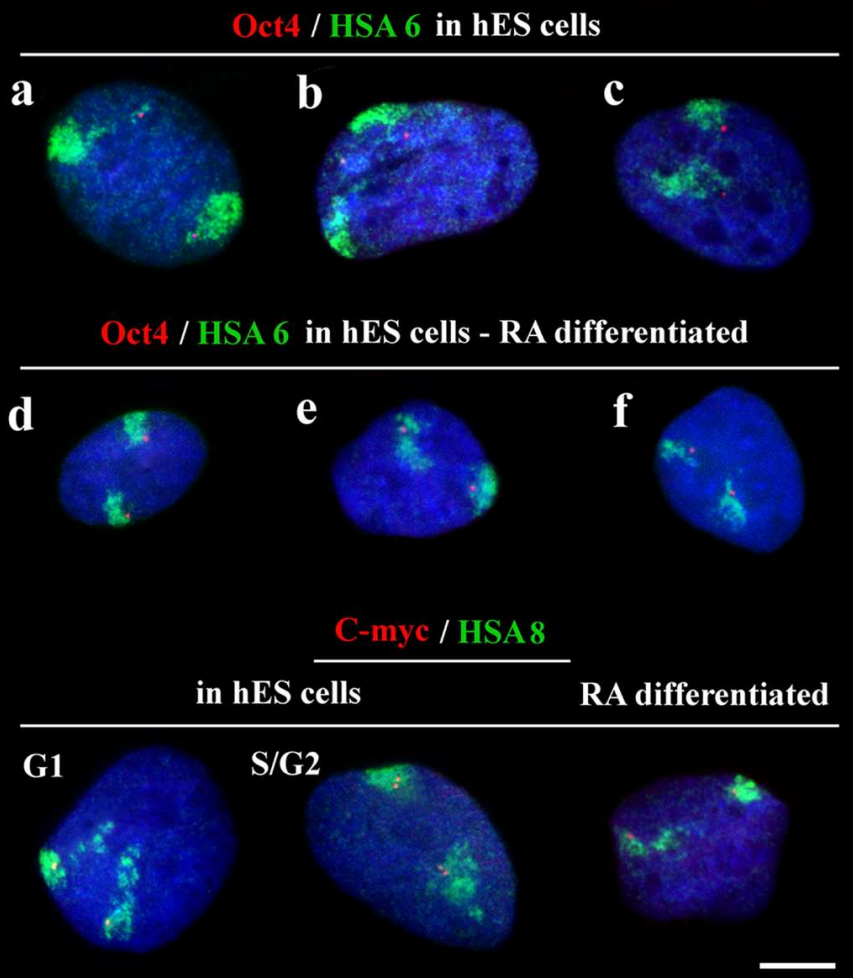
Rb1 gene



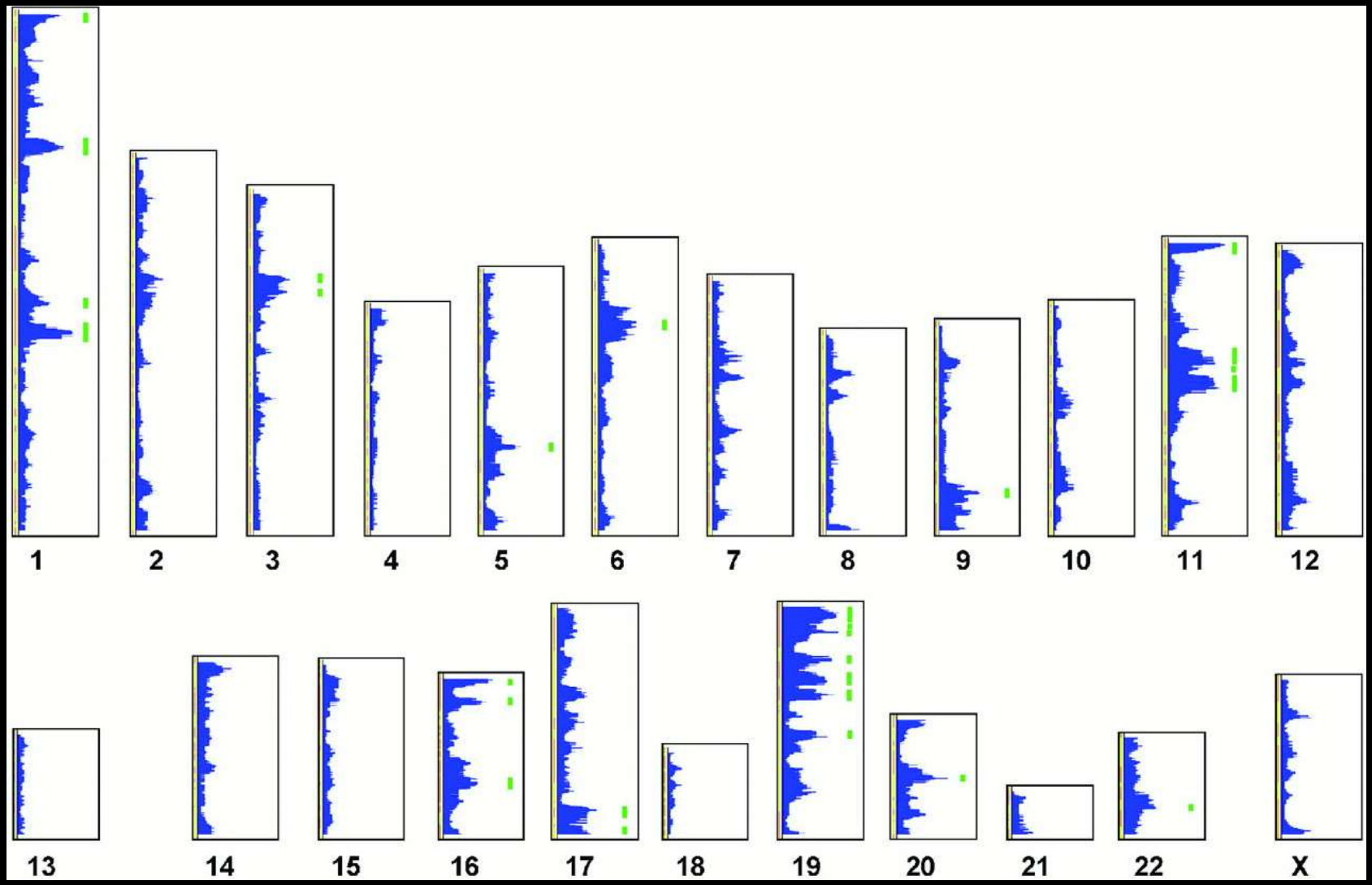
Bártová et al., 2002

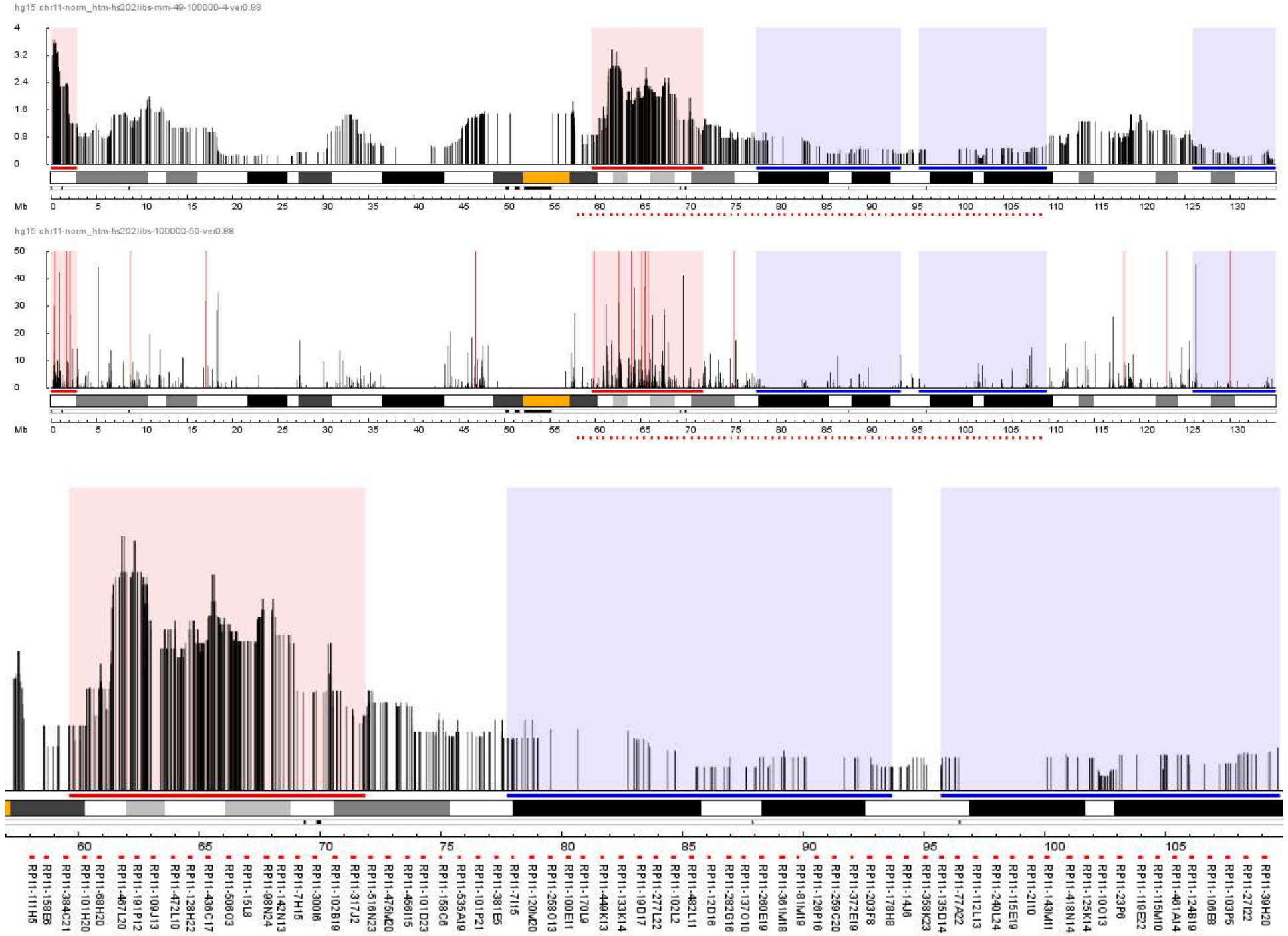
Genes in human embryonic stem cells



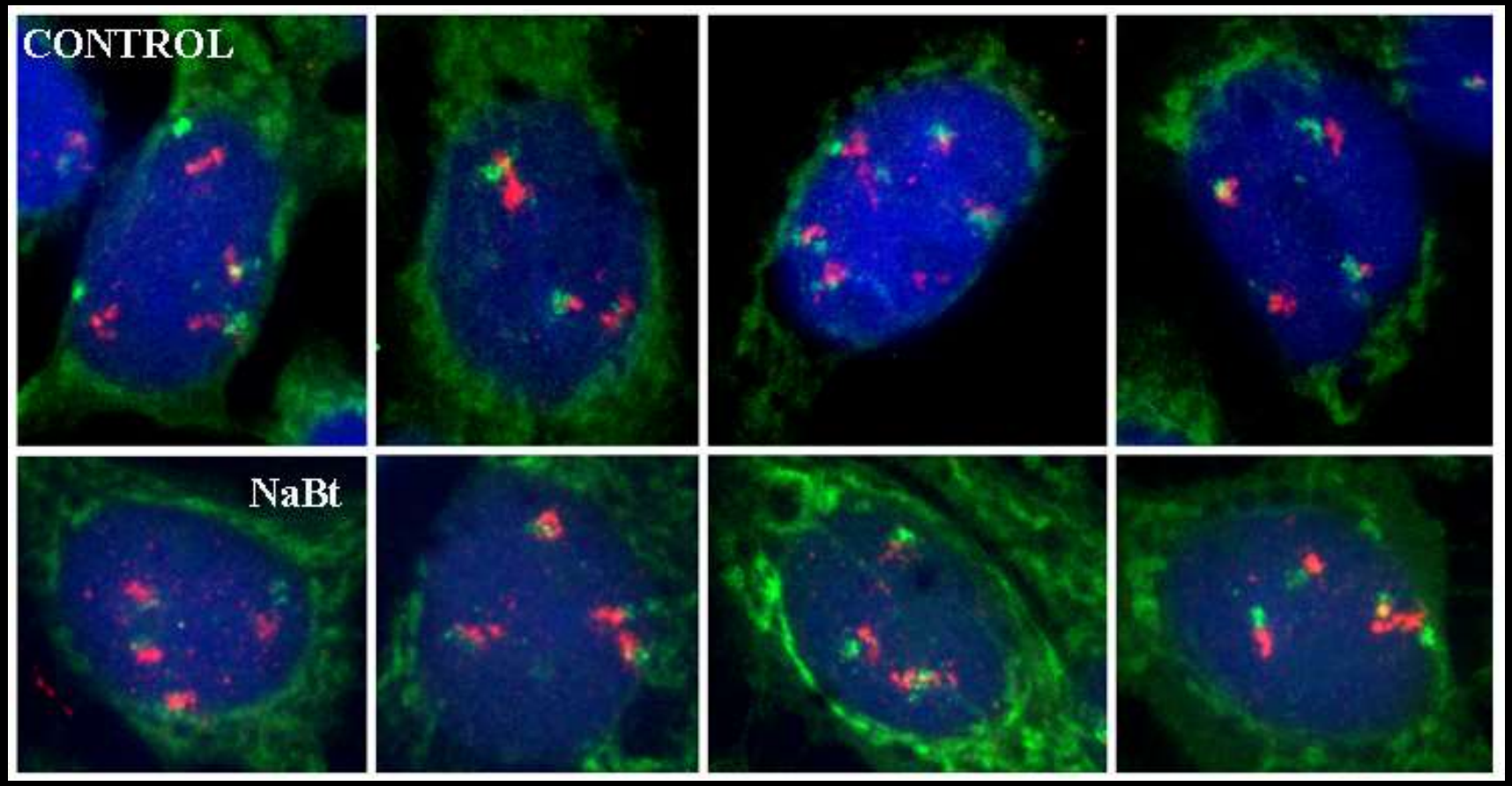


Transcriptome map (Caron et al., 2001)




hg15 chr11-norm_htm-hs202libs-mm-49-100000-4-ver0.88
hg15 chr11-norm_htm-hs202libs-100000-50-ver0.88
Mb
RP11-111H5
RP11-158E8
RP11-384C21
RP11-101H20
RP11-68H20
RP11-467L20
RP11-191P12
RP11-109J13
RP11-472L10
RP11-128H22
RP11-436C17
RP11-506O3
RP11-15L8
RP11-98N24
RP11-142N13
RP11-7H15
RP11-300I6
RP11-102B19
RP11-317J2
RP11-516N23
RP11-475M20
RP11-466I15
RP11-101D23
RP11-158C6
RP11-535A19
RP11-101P21
RP11-381E5
RP11-7I15
RP11-120M20
RP11-258O13
RP11-100E11
RP11-170L9
RP11-449K13
RP11-133K14
RP11-19D17
RP11-277L22
RP11-102L2
RP11-482L11
RP11-12D16
RP11-282G16
RP11-137O10
RP11-260E19
RP11-381M18
RP11-81M19
RP11-126P16
RP11-259C20
RP11-372E19
RP11-203F8
RP11-178H8
RP11-14J6
RP11-358K23
RP11-135D14
RP11-77A22
RP11-112L13
RP11-240L24
RP11-115E19
RP11-2I10
RP11-143M11
RP11-418N14
RP11-125K14
RP11-10O13
RP11-23P6
RP11-119E22
RP11-115M10
RP11-481A14
RP11-124B19
RP11-106E8
RP11-103P5
RP11-27I22
RP11-39H20

Enterocytic cell differentiation and RIDGE/ANTI-RIDGE



Center of nucleus-to-gene distances

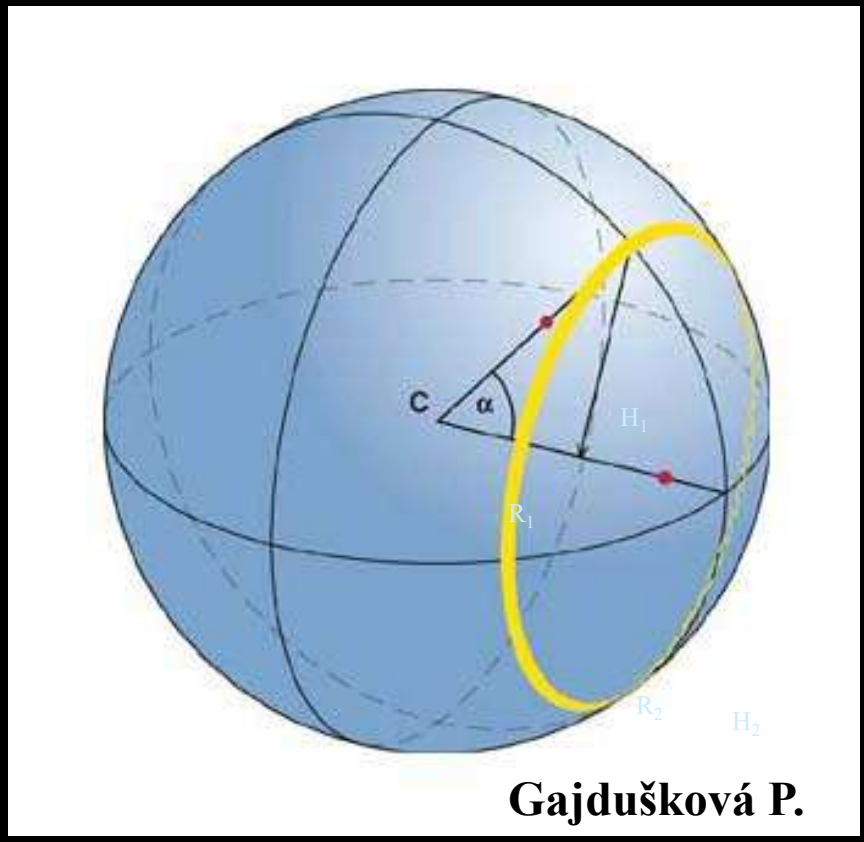
$$CS = \frac{\overline{CR_i}}{\overline{CH_i}} * 100$$

Gene-to-gene distances

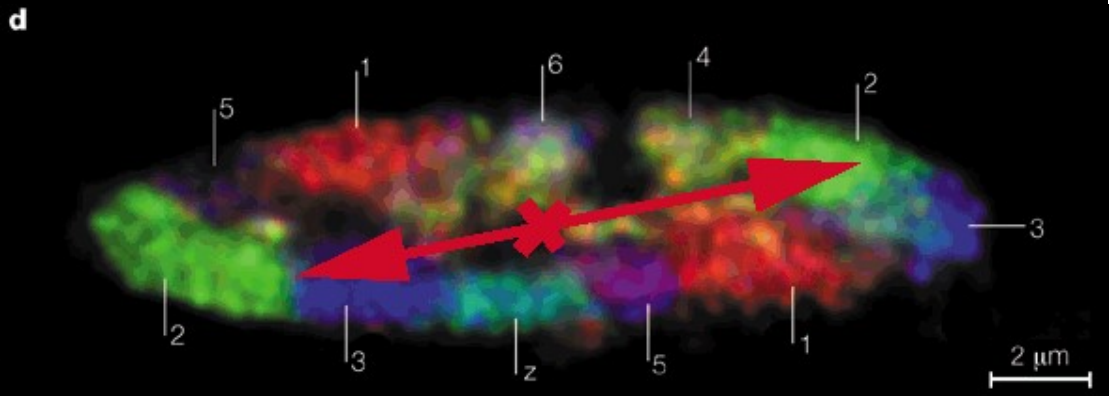
$$SS = \frac{\overline{R_1R_2}}{(\overline{CH_1} + \overline{CH_2})/2} * 100$$

Gene-center of nucleus-gene angles

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



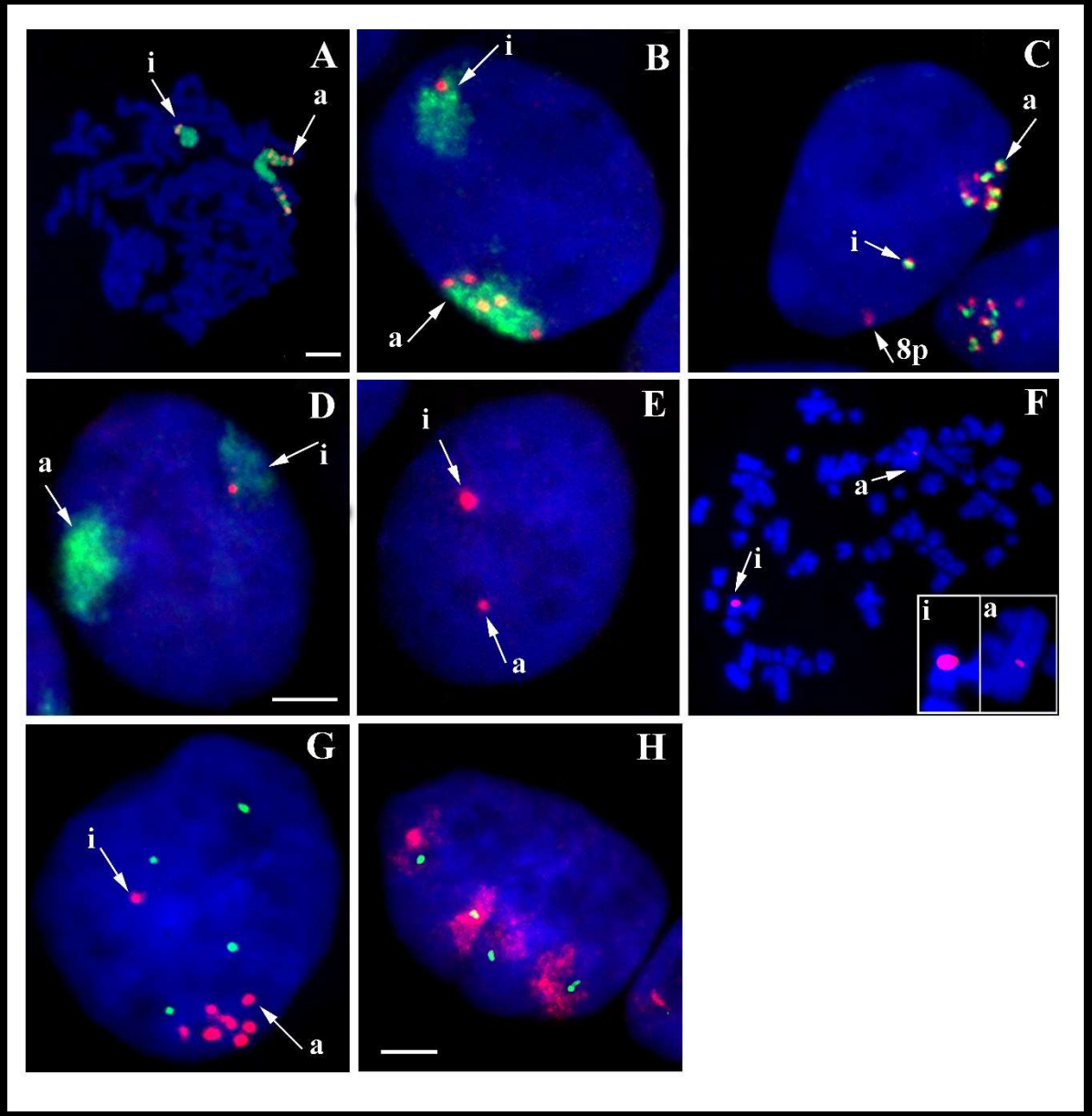
Gajdušková P.

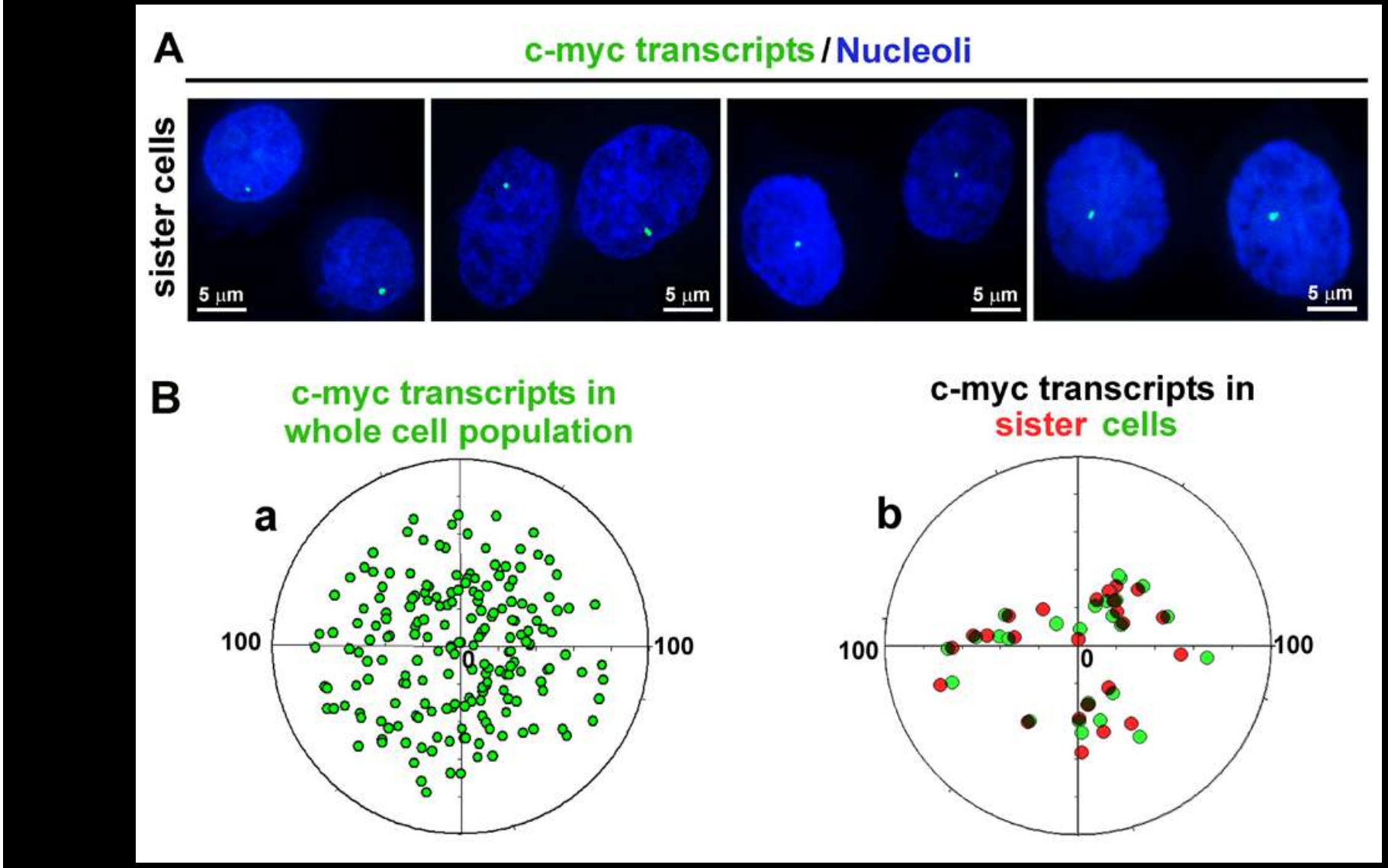


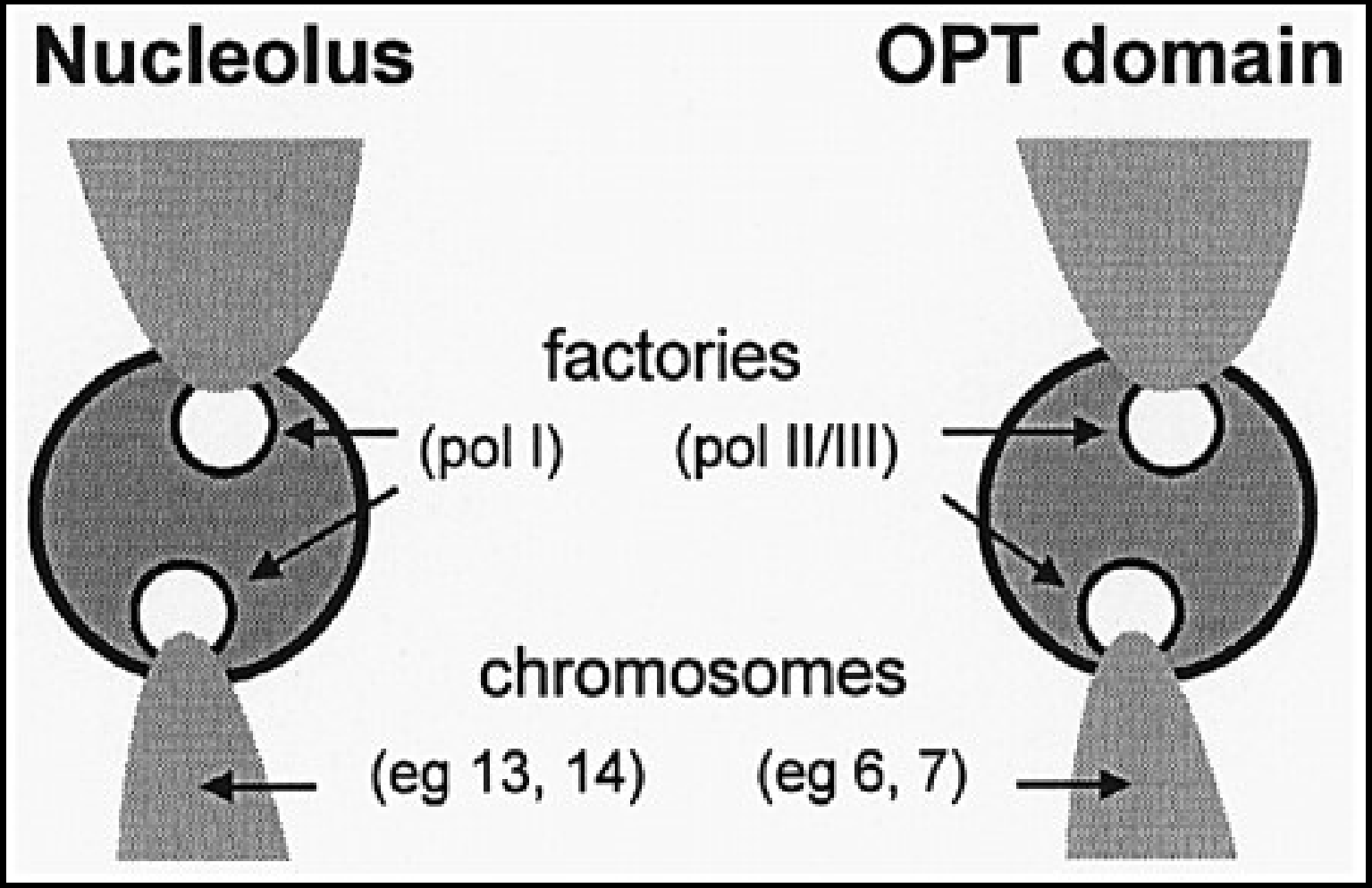
T. Cremer group

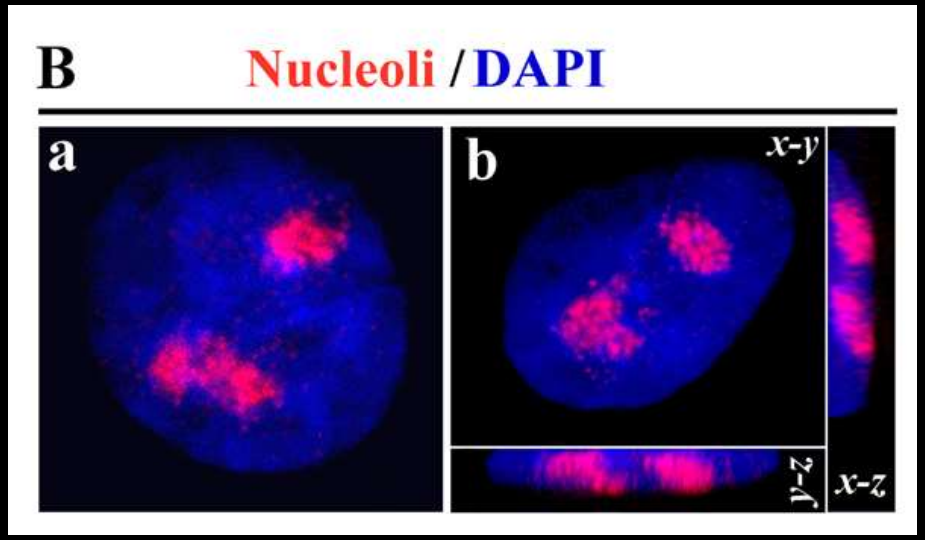
HSA 8 and related structures

Harničarová et al., 2005

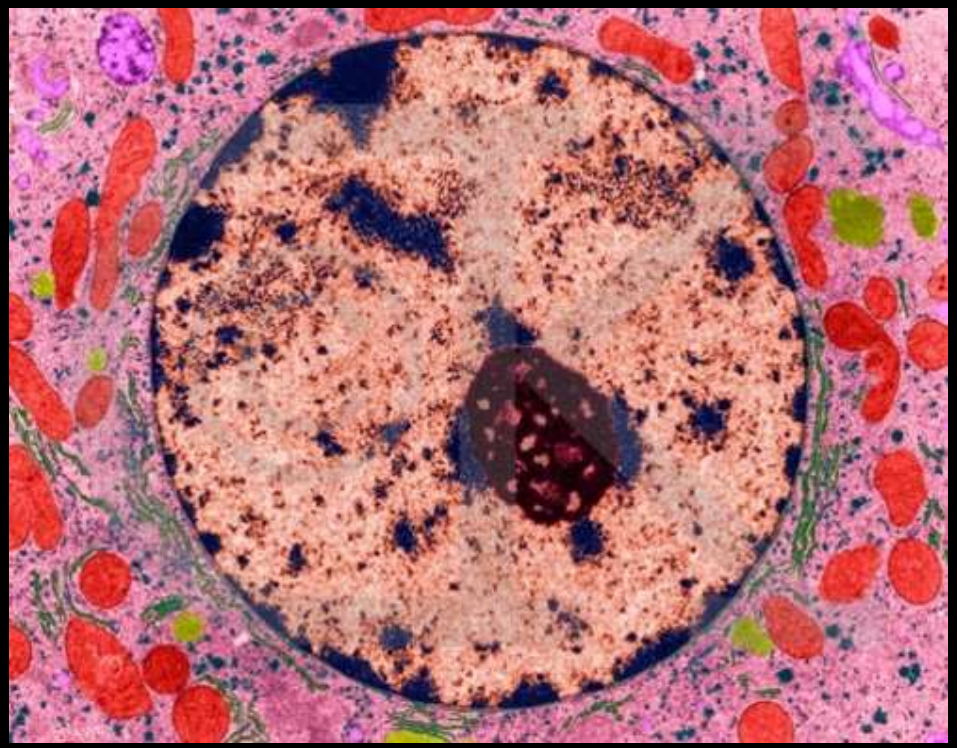


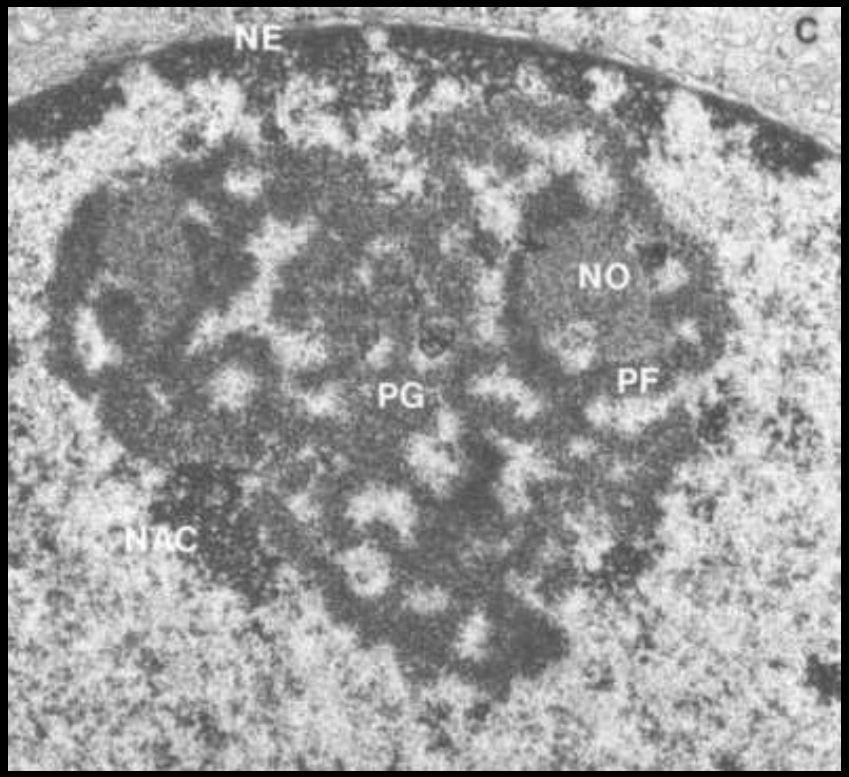

A
c-myc transcripts / Nucleoli
sister cells
5 μm
5 μm
5 μm
5 μm
B
c-myc transcripts in
whole cell population
a
100
0
100
c-myc transcripts in
sister cells
b
100
0
100

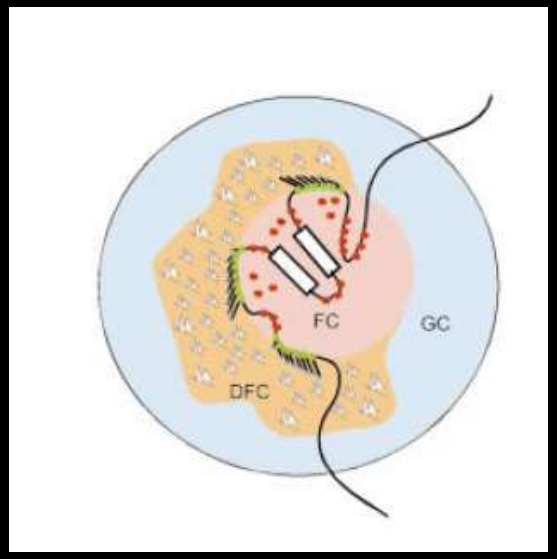

Nucleolus
OPT domain
factories
(pol I)
(pol II/III)
chromosomes
(eg 13, 14)
(eg 6, 7)

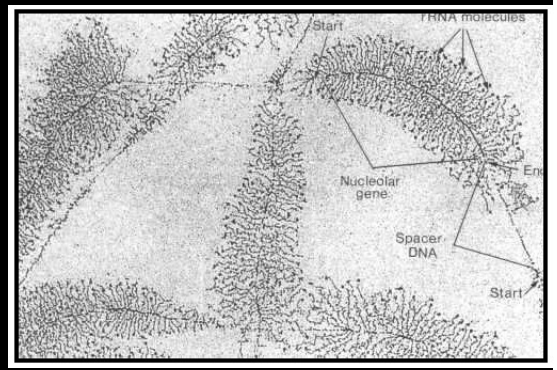


Nucleolus









NOR

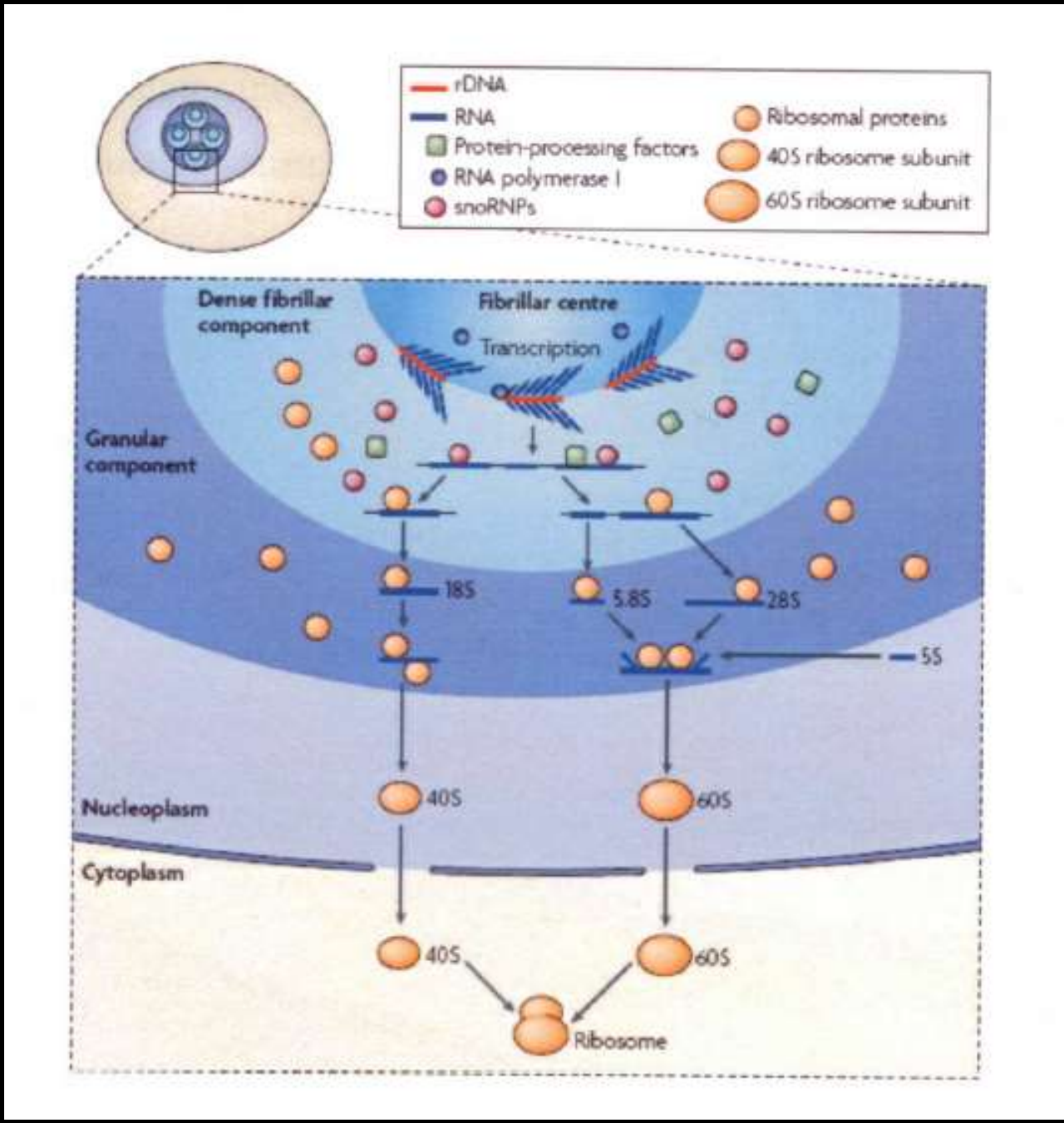
400 (540) rDNA genů

FIBRILÁRNÍ CENTRUM (FC):
zodpovídá za změny v buněčné aktivitě

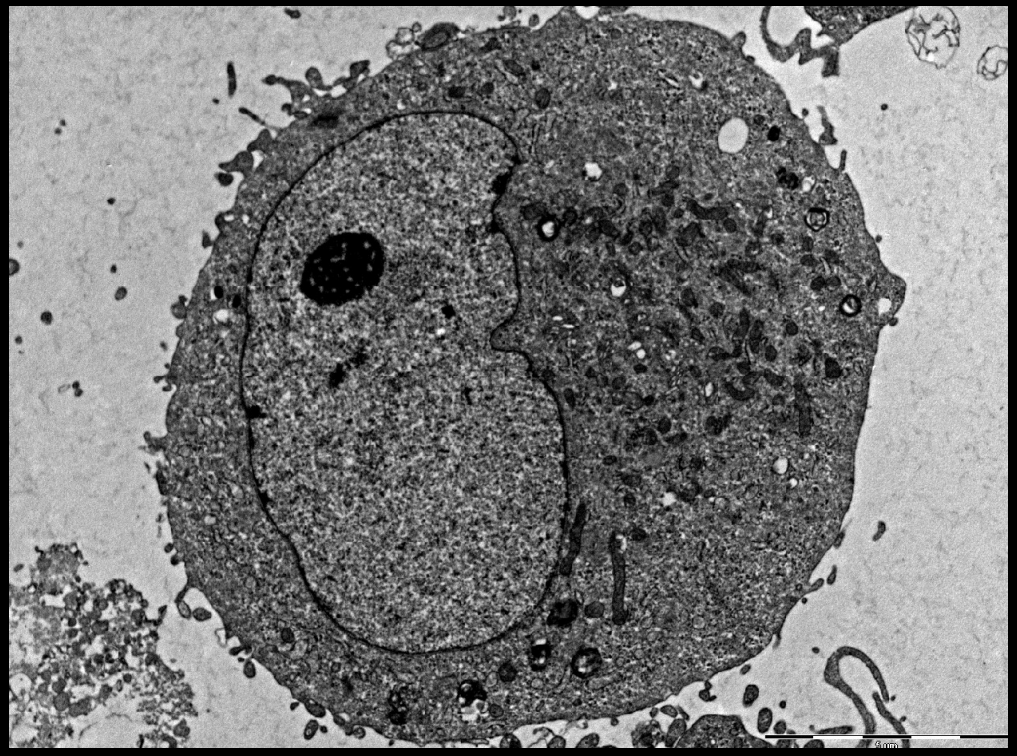
DENSE FIBRILAR COMPONENT (DFC):
místo syntézy ribozomálních podjednotek

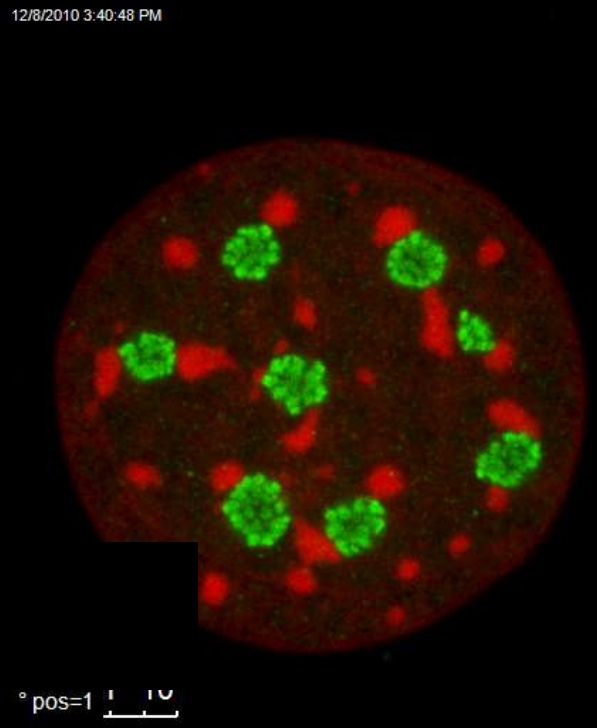
GRANULAR COMPONENTS:
ukotvuje DFC a FC

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


rDNA
RNA
Protein-processing factors
RNA polymerase I
snoRNPs
Ribosomal proteins
40S ribosome subunit
60S ribosome subunit
Dense fibrillar component
Fibrillar centre
Transcription
Granular component
18S
5.8S
28S
5S
Nucleoplasm
40S
60S
Cytoplasm
40S
60S
Ribosome

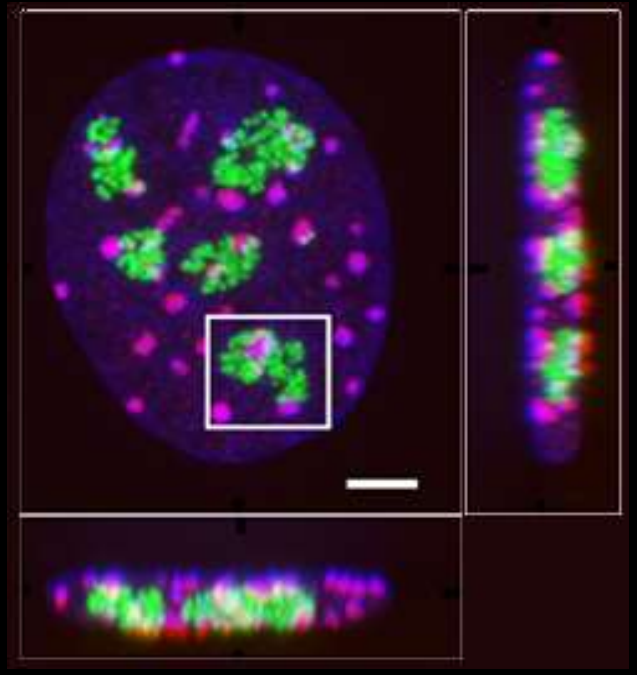
SUV39h-independent association of HP1β with fibrillarin-positive nucleolar regions

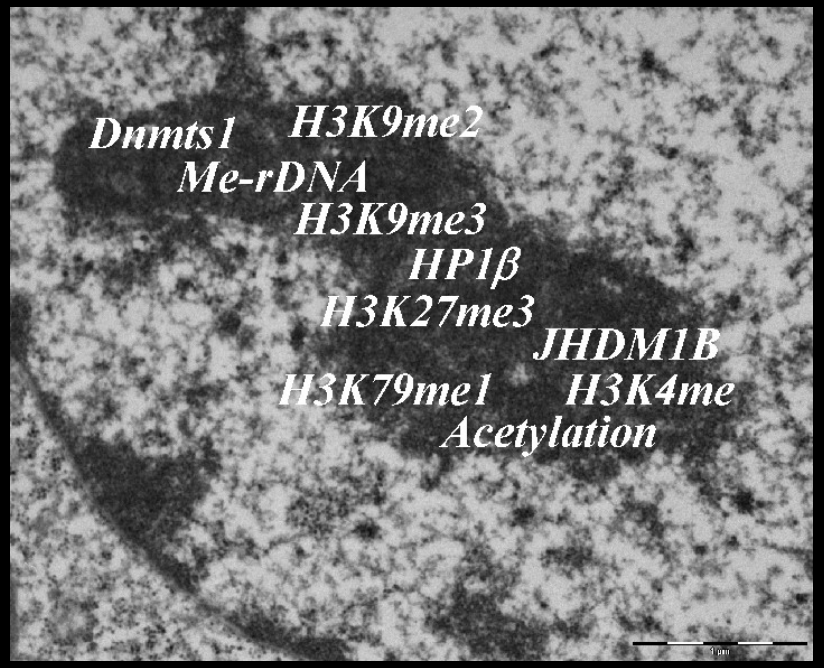


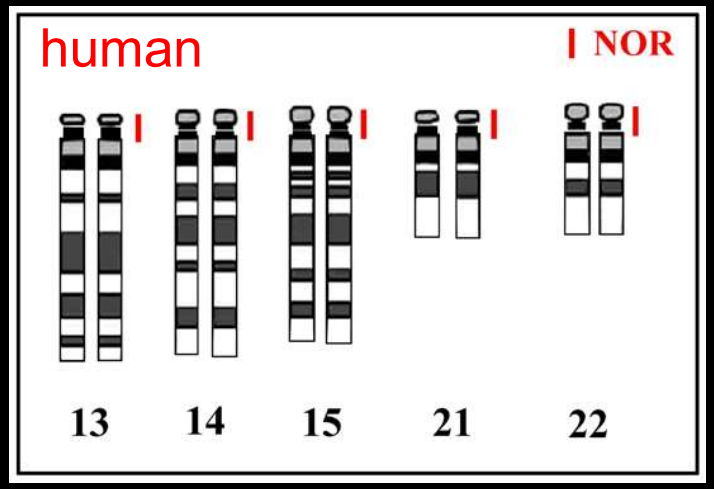


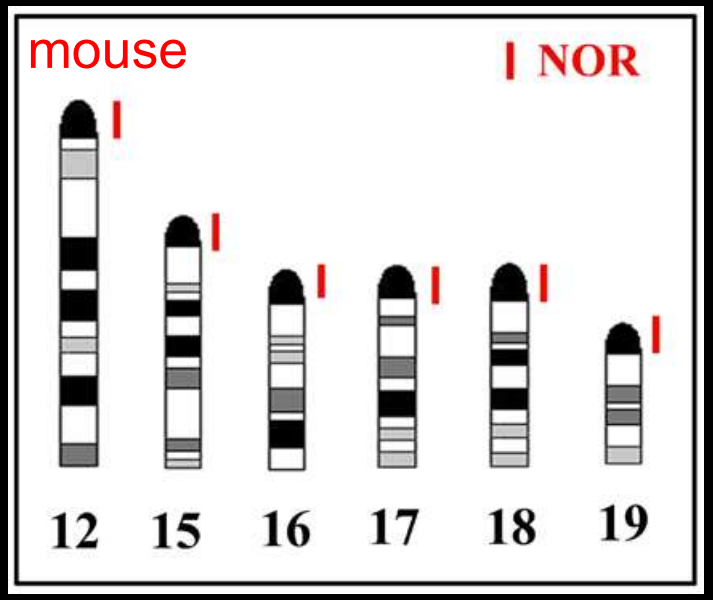
Epigenetics of Nucleoli

Fibrillarin/chromocenters

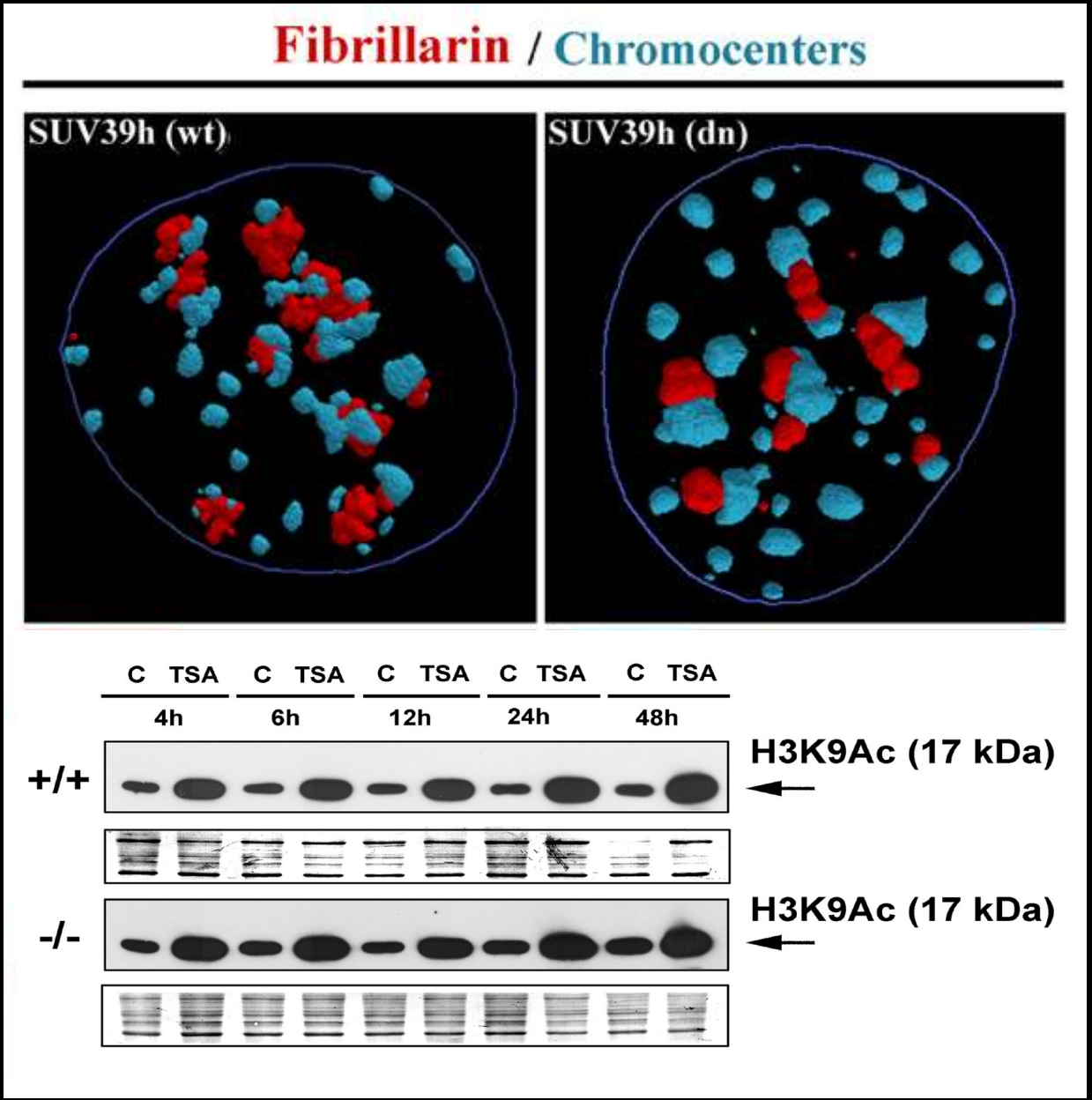


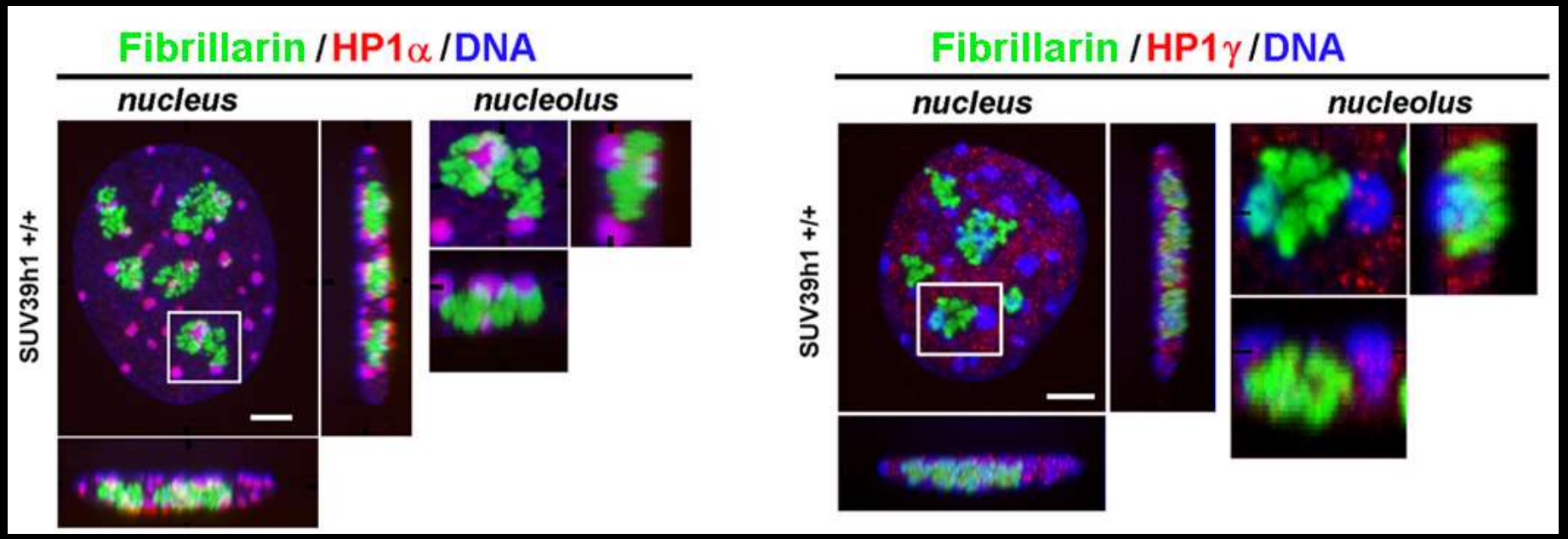


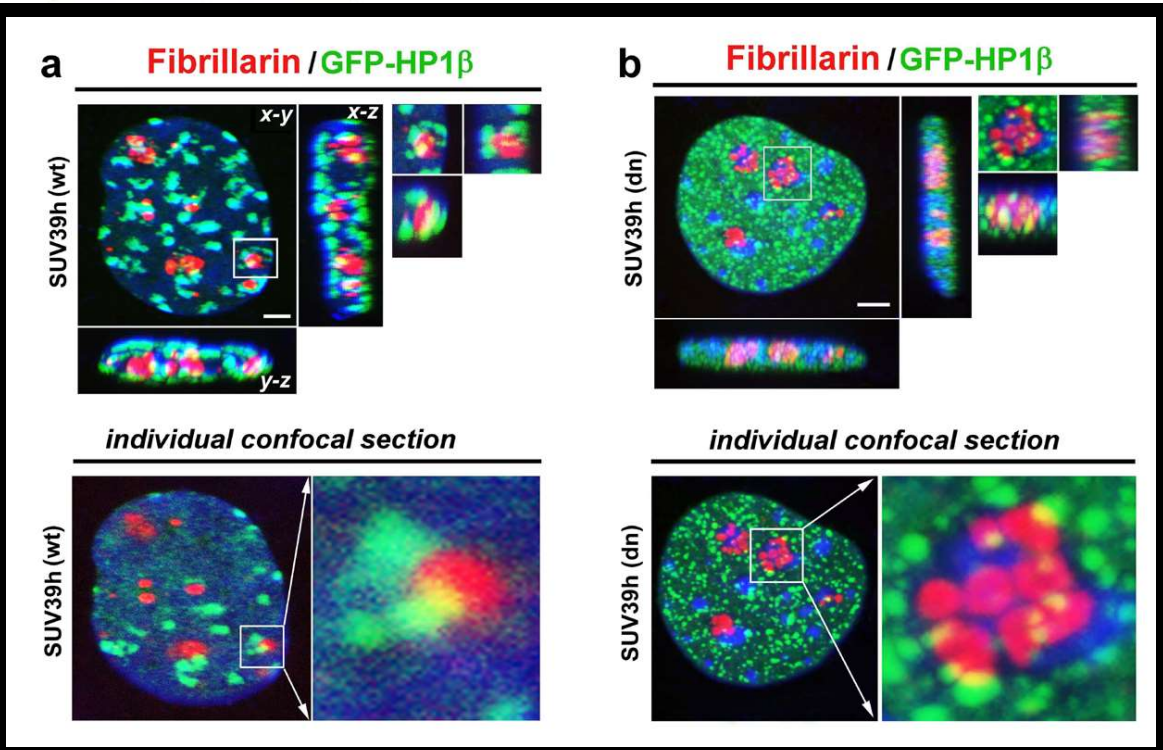


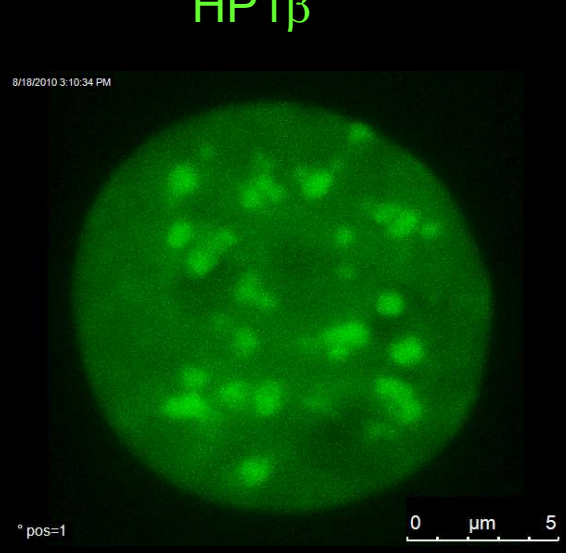


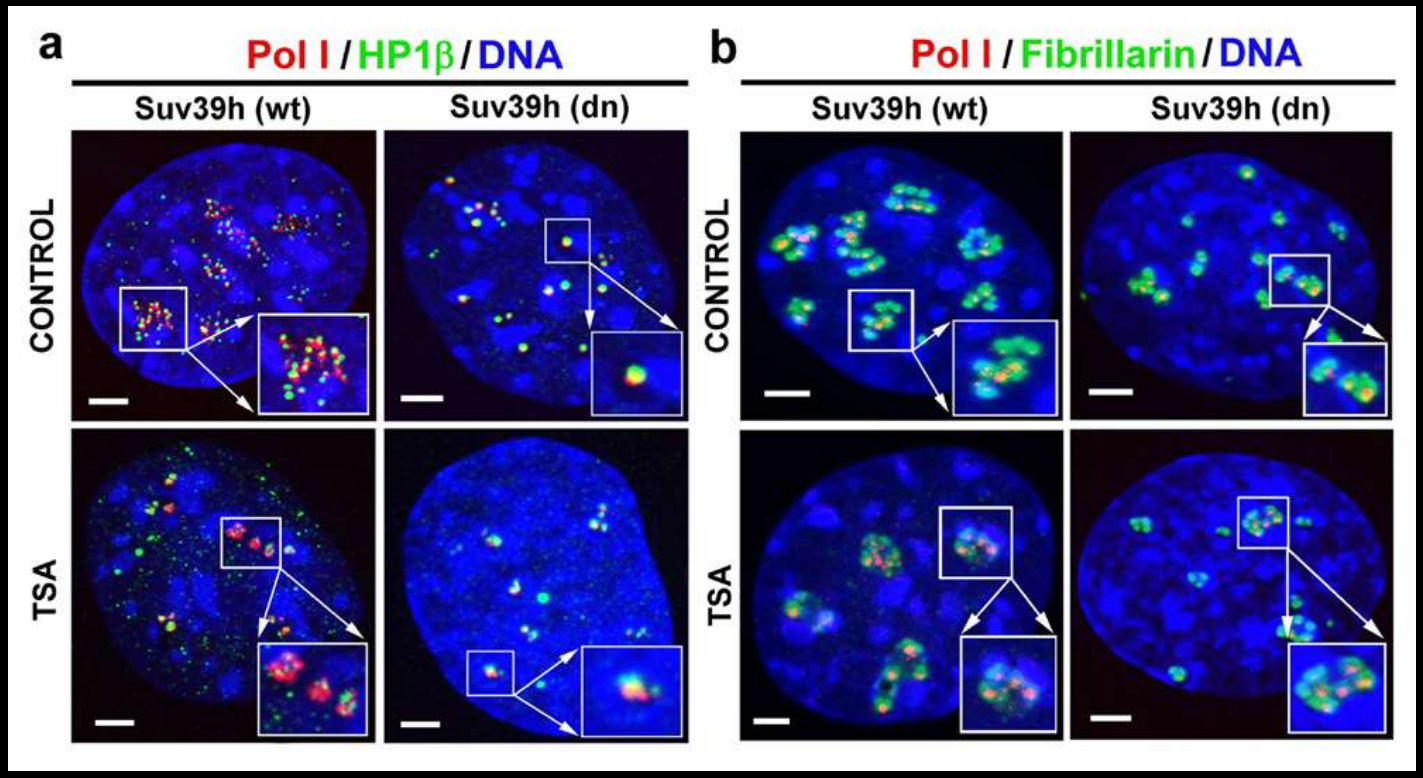
Differencies between epigenetics of nucleoli and surrounding chromatin

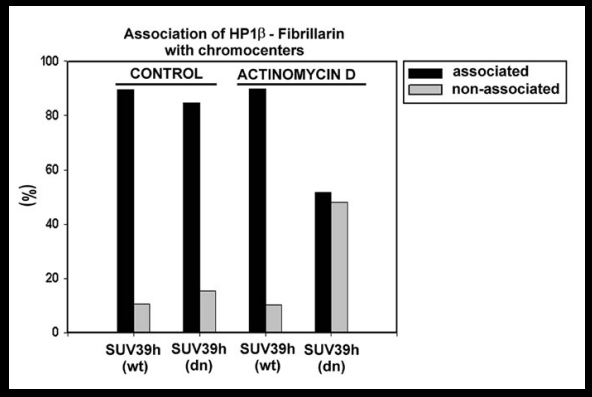


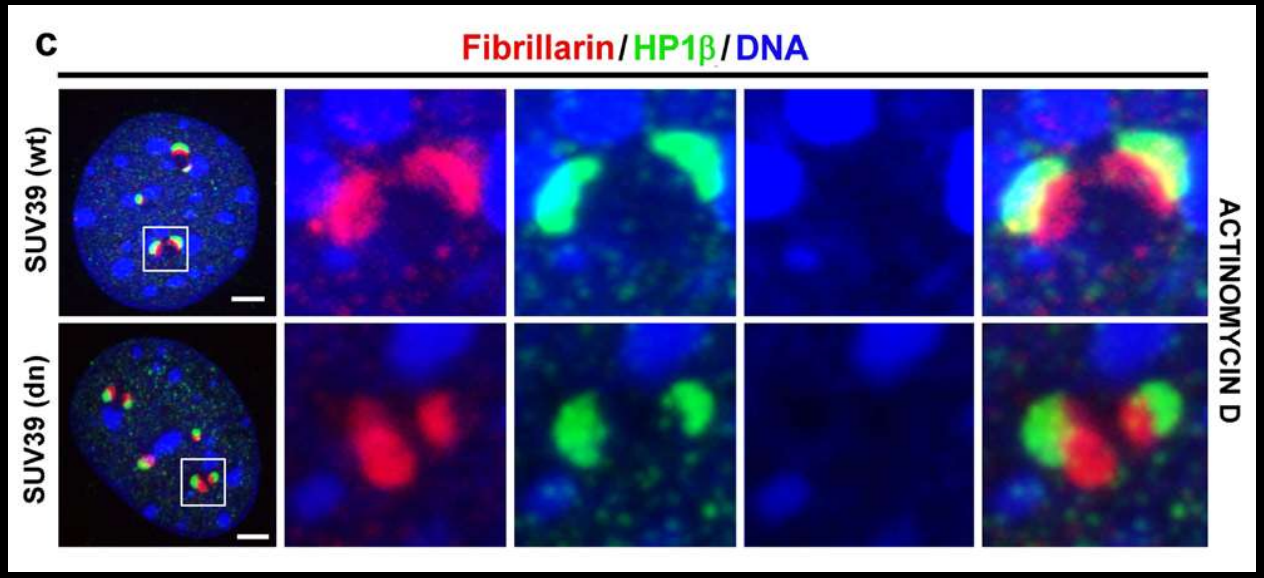

Fibrillarin / HP1α / DNA
nucleus
nucleolus
SUV39h1 +/+
Fibrillarin / HP1γ / DNA
nucleus
nucleolus
SUV39h1 +/+


a
Fibrillarin / GFP-HP1β
x-y
x-z
y-z
SUV39h (wt)
b
Fibrillarin / GFP-HP1β
SUV39h (dn)
individual confocal section
SUV39h (wt)
individual confocal section
SUV39h (dn)

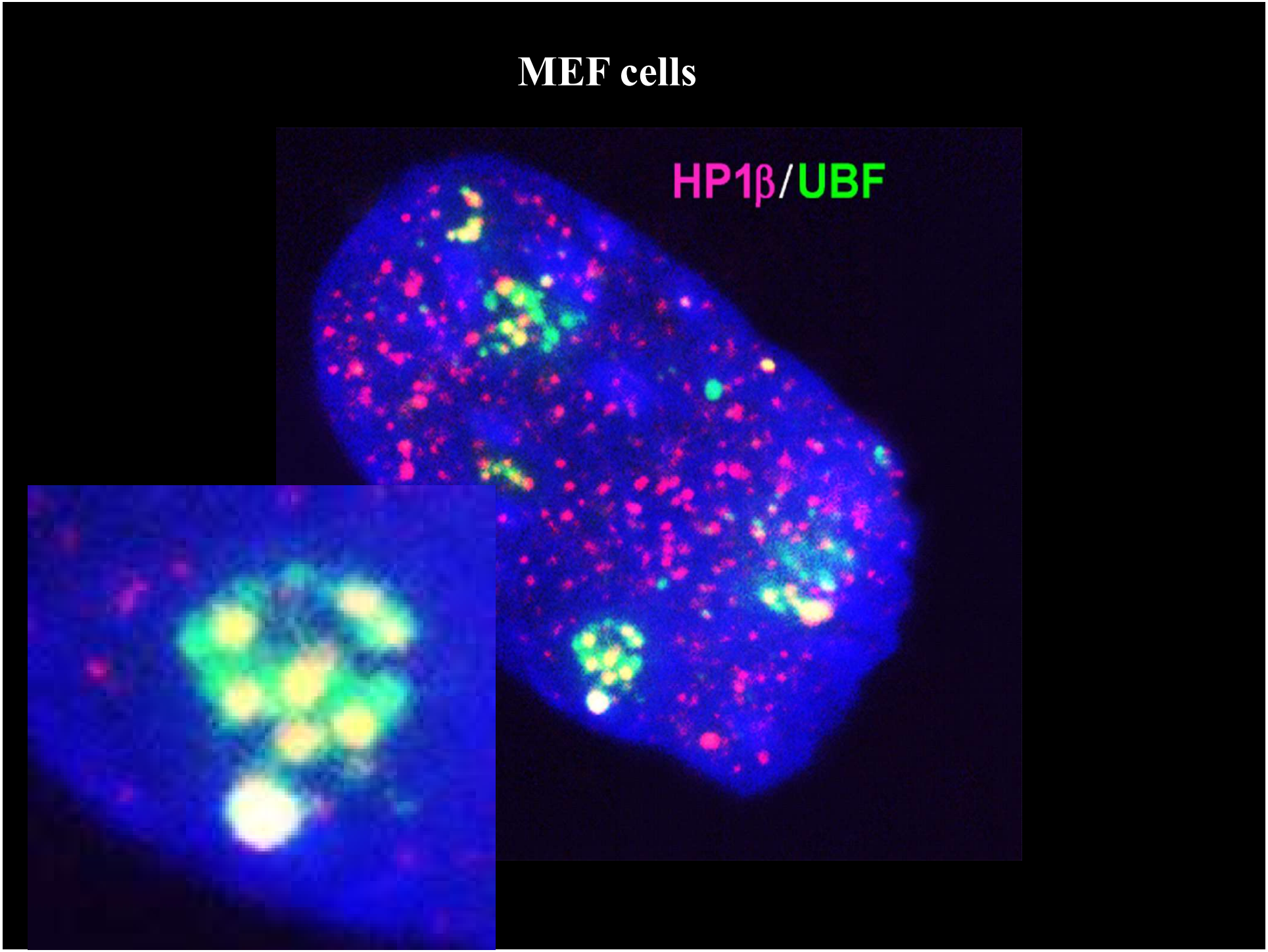

HP1β
8/18/2010 3:10:34 PM
° pos=1
0
µm
5


a
Pol I / HP1β / DNA
Suv39h (wt)
Suv39h (dn)
CONTROL
TSA
b
Pol I / Fibrillarin / DNA
Suv39h (wt)
Suv39h (dn)
CONTROL
TSA

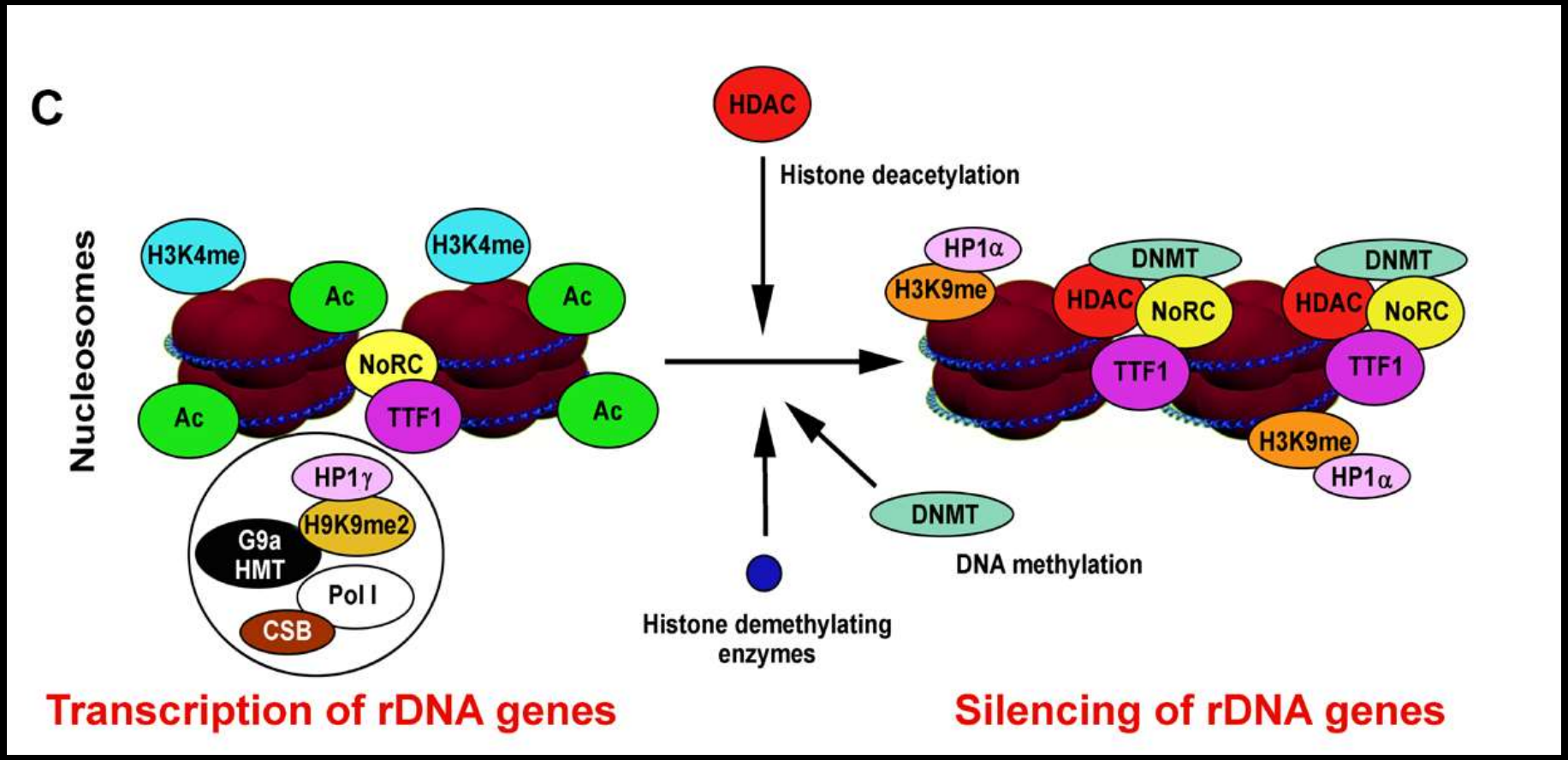

Association of HP1β - Fibrillarin
with chromocenters
CONTROL
ACTINOMYCIN D
associated
non-associated
(%)
100
80
60
40
20
0
SUV39h (wt)
SUV39h (dn)
SUV39h (wt)
SUV39h (dn)


c
Fibrillarin / HP1β / DNA
SUV39 (wt)
SUV39 (dn)
ACTINOMYCIN D

MEF cells

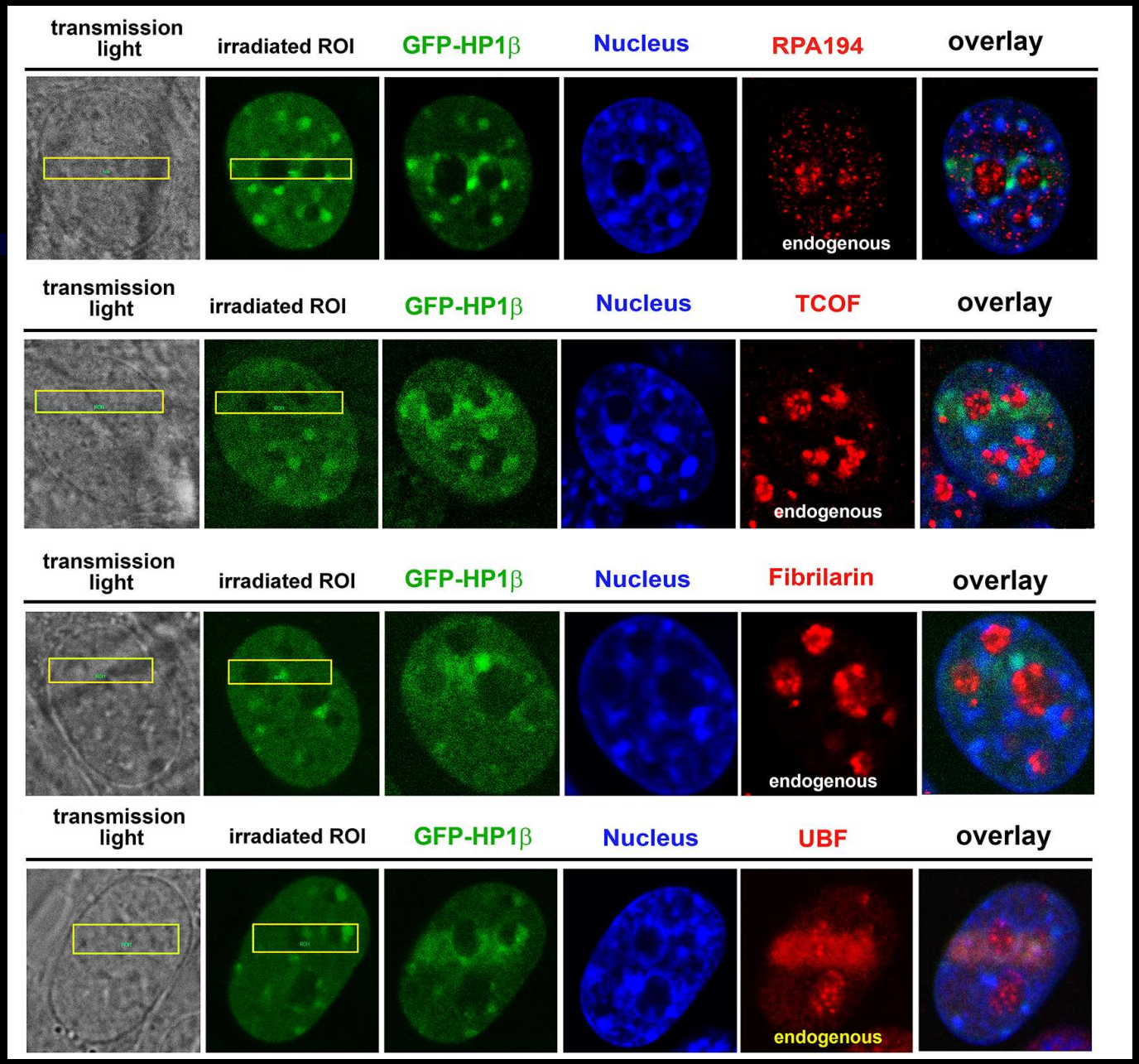


Epigenetics of nucleoli



Yuan et al. (2007)

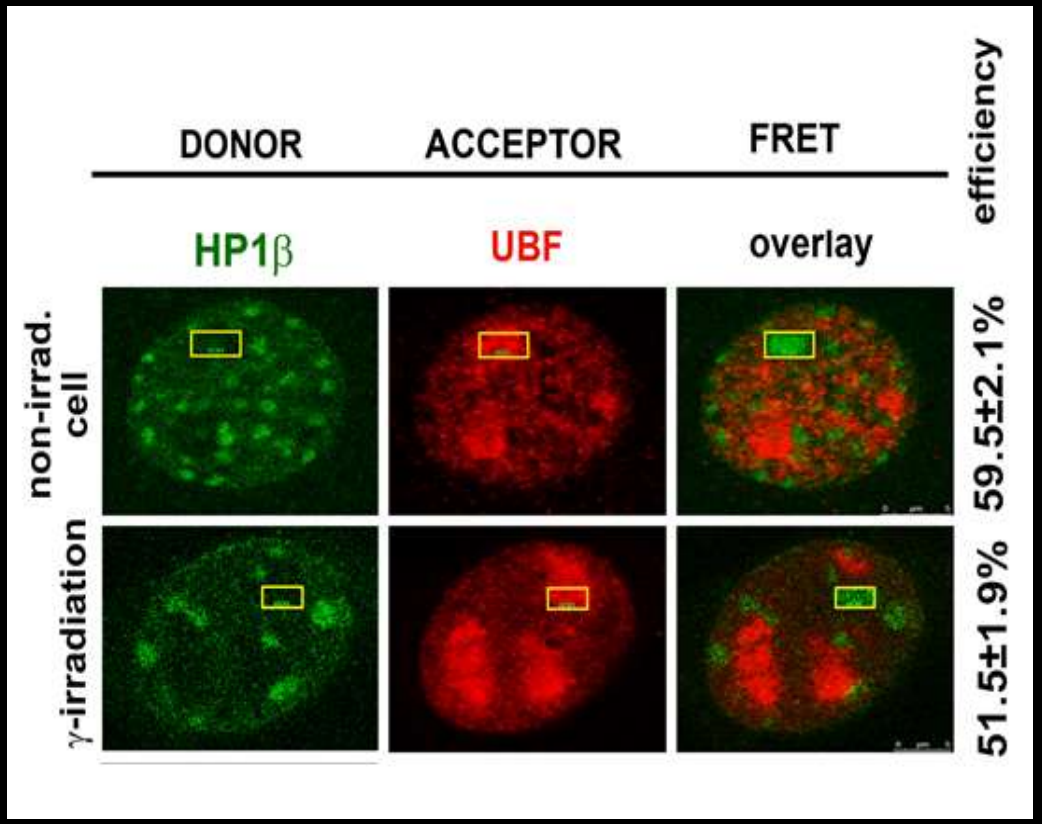
Bártová et al., JHC (2009)

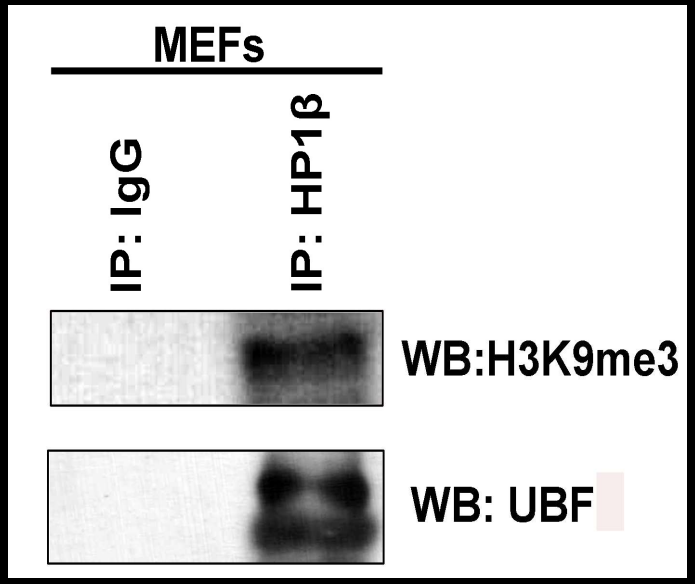


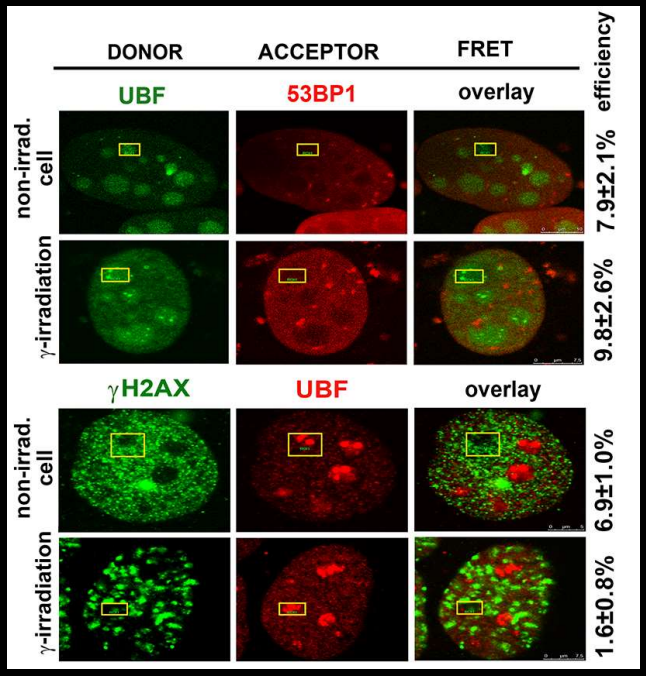
Experiments: Petra Sehnalová

HP1β interacts with UBF
FRET analysis

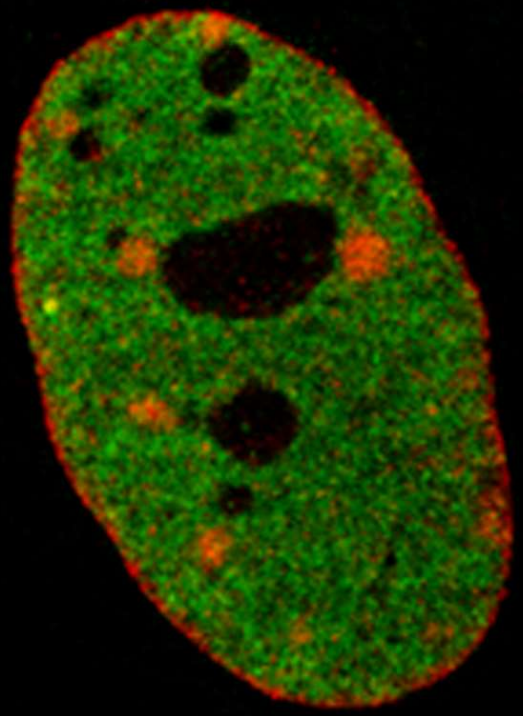
HP1β – UBF
FRET efficiency > 50%







Experiments: Petra Sehnalová



ZÁVĚR

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