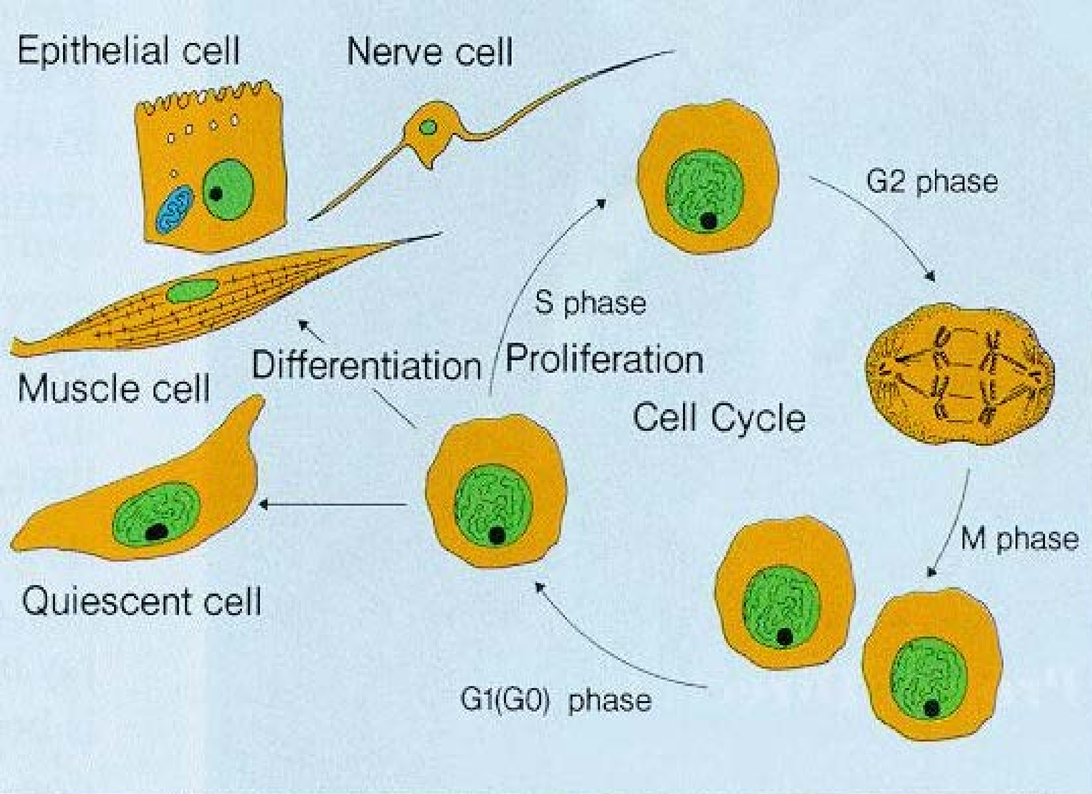
Buněčná diferenciace a struktura chromatinu

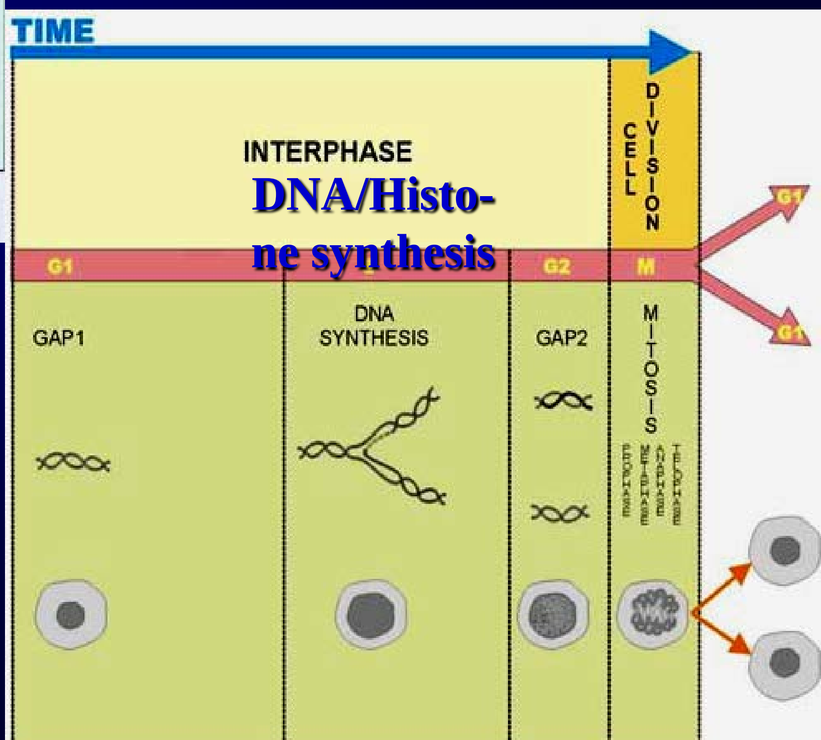
Buněčná diferenciace je proces při kterém buňky získávají nový fenotyp, který je spojen se specifickou buněčnou funkcí. Pro daný buněčný typ je charakteristická aktivace skupiny genů, které jsou zodpovědné za terminální diferenciaci.

http://www.youtube.com/watch?v=mUcE1Y_bOQE

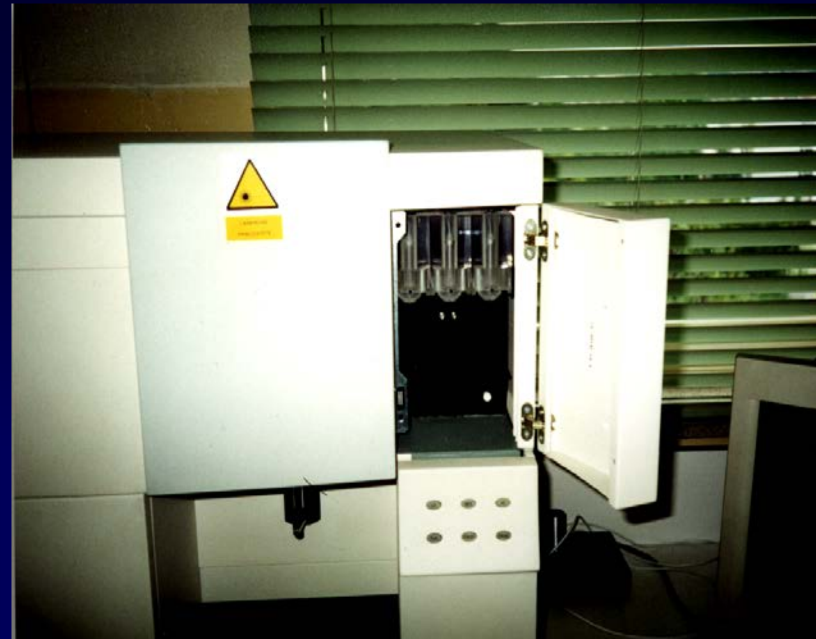


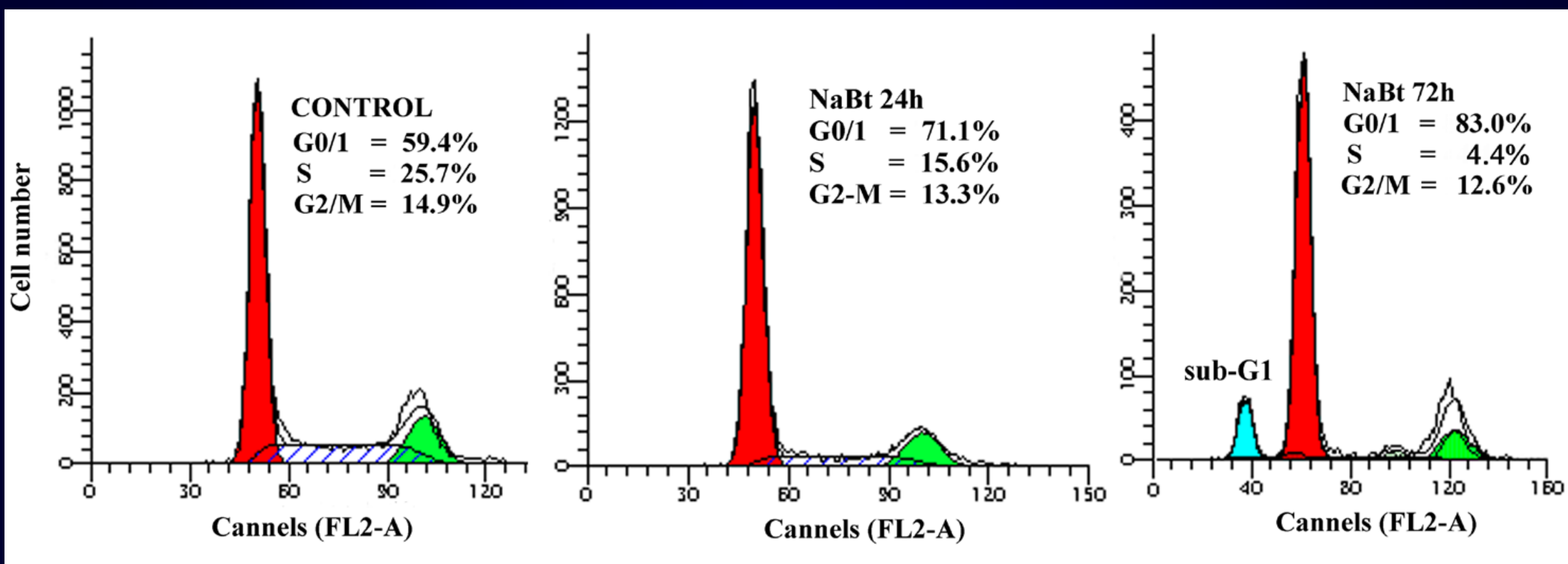
Cell Growth and Differentiation

Cell Differentiation
Cell Growth

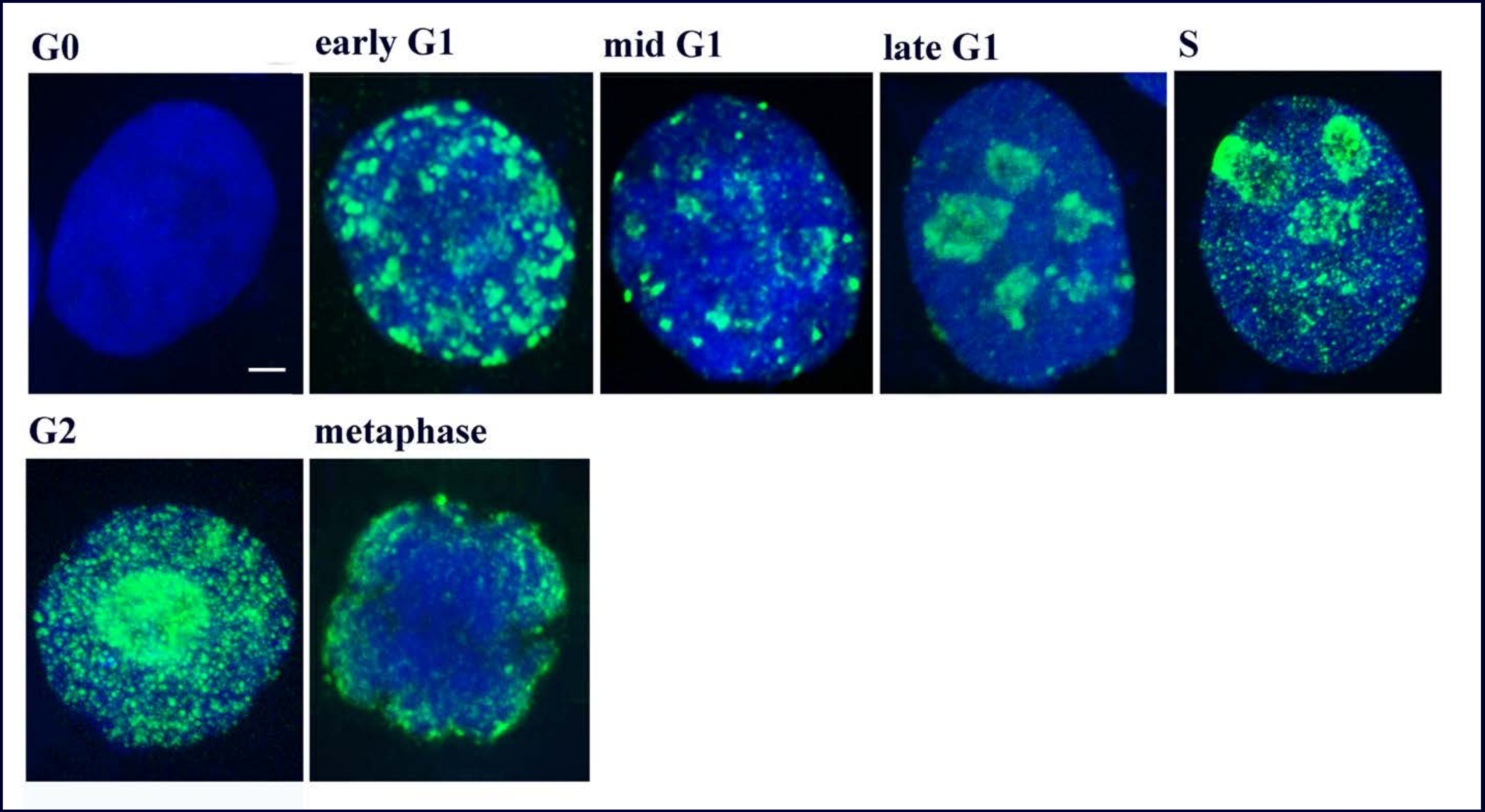


FCM




Cell number
CONTROL
G0/1 = 59.4%
S = 25.7%
G2/M = 14.9%
Cannels (FL2-A)
NaBt 24h
G0/1 = 71.1%
S = 15.6%
G2-M = 13.3%
Cannels (FL2-A)
NaBt 72h
G0/1 = 83.0%
S = 4.4%
G2/M = 12.6%
sub-G1
Cannels (FL2-A)

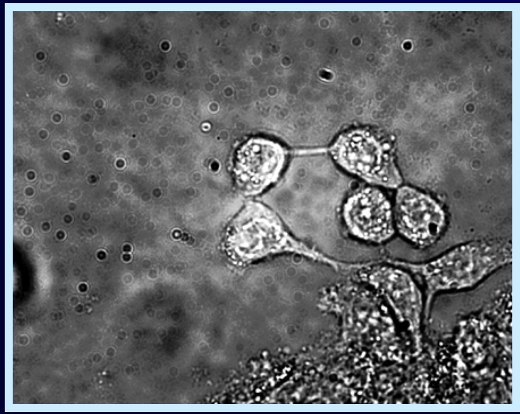
pKi-67



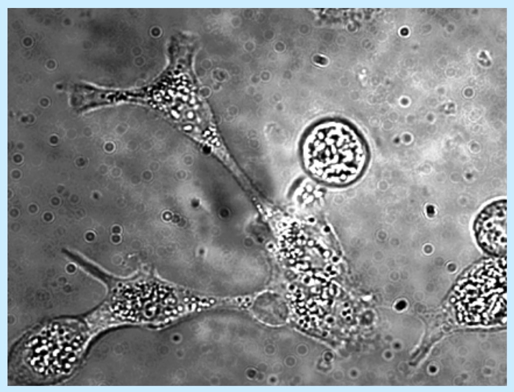
Andrea Harničarová et al. (2006)

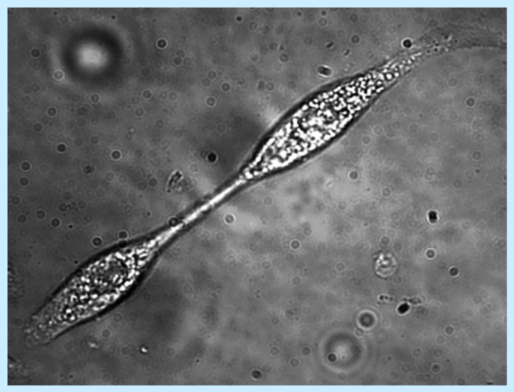
Enterocytic cell differentiation

Control

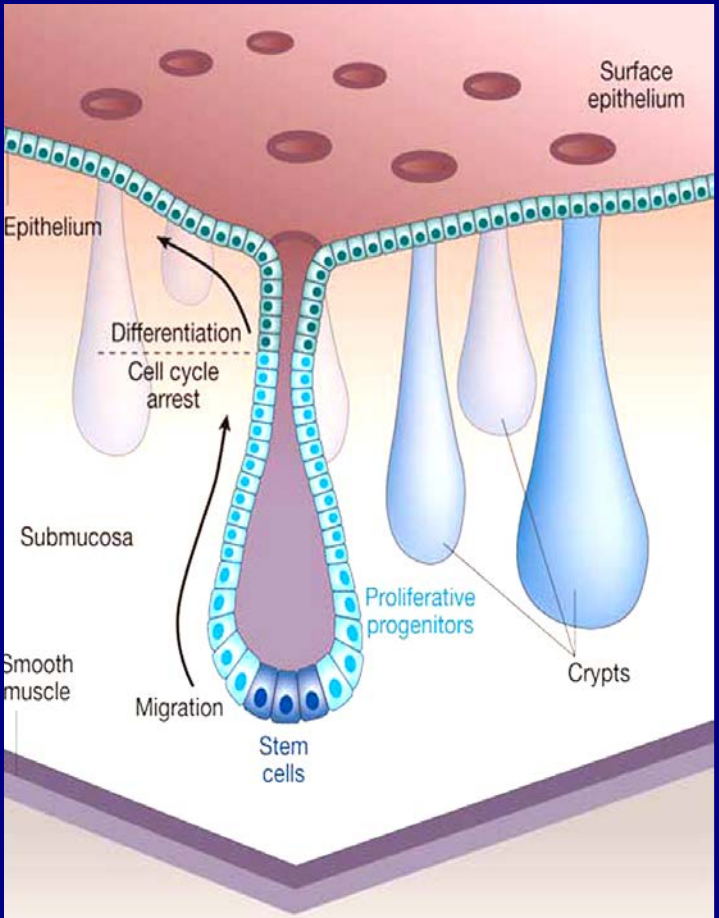


Sodium Butyrate



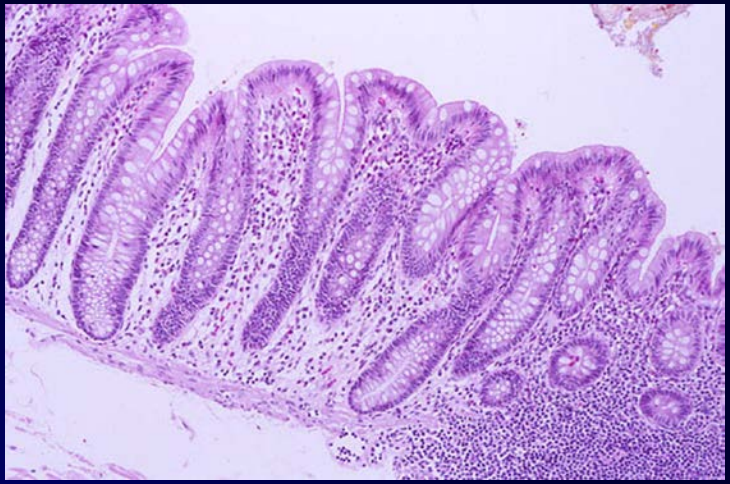


Enterocytic Cell Differentiation

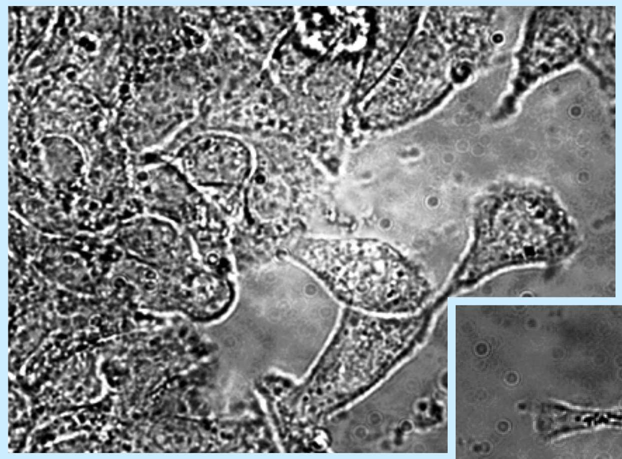


Nature,Vol 434 (2005),www.nature.com

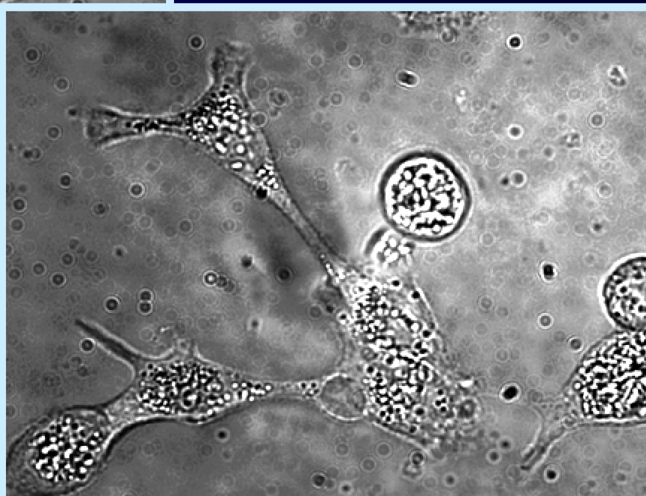
Figure 3 Tissue anatomy of the colonic epithelium. Putative stem cells (dark blue) reside at the crypt bottom. Proliferating progenitor cells occupy two-thirds of the crypt. Differentiated cells (green) populate the remainder of the crypt and the flat surface epithelium. (Adapted from ref. 89.)



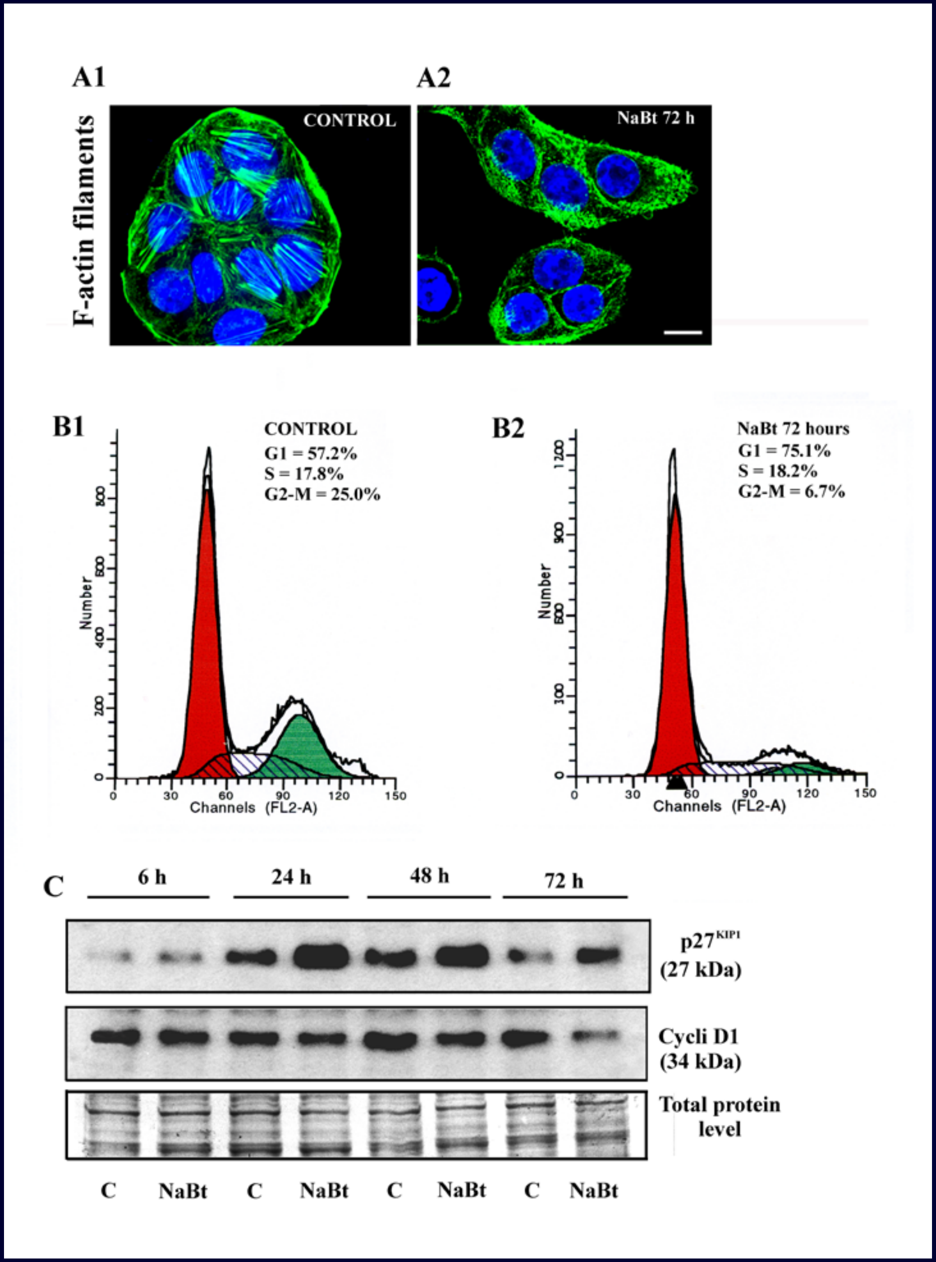
Control



NaBt



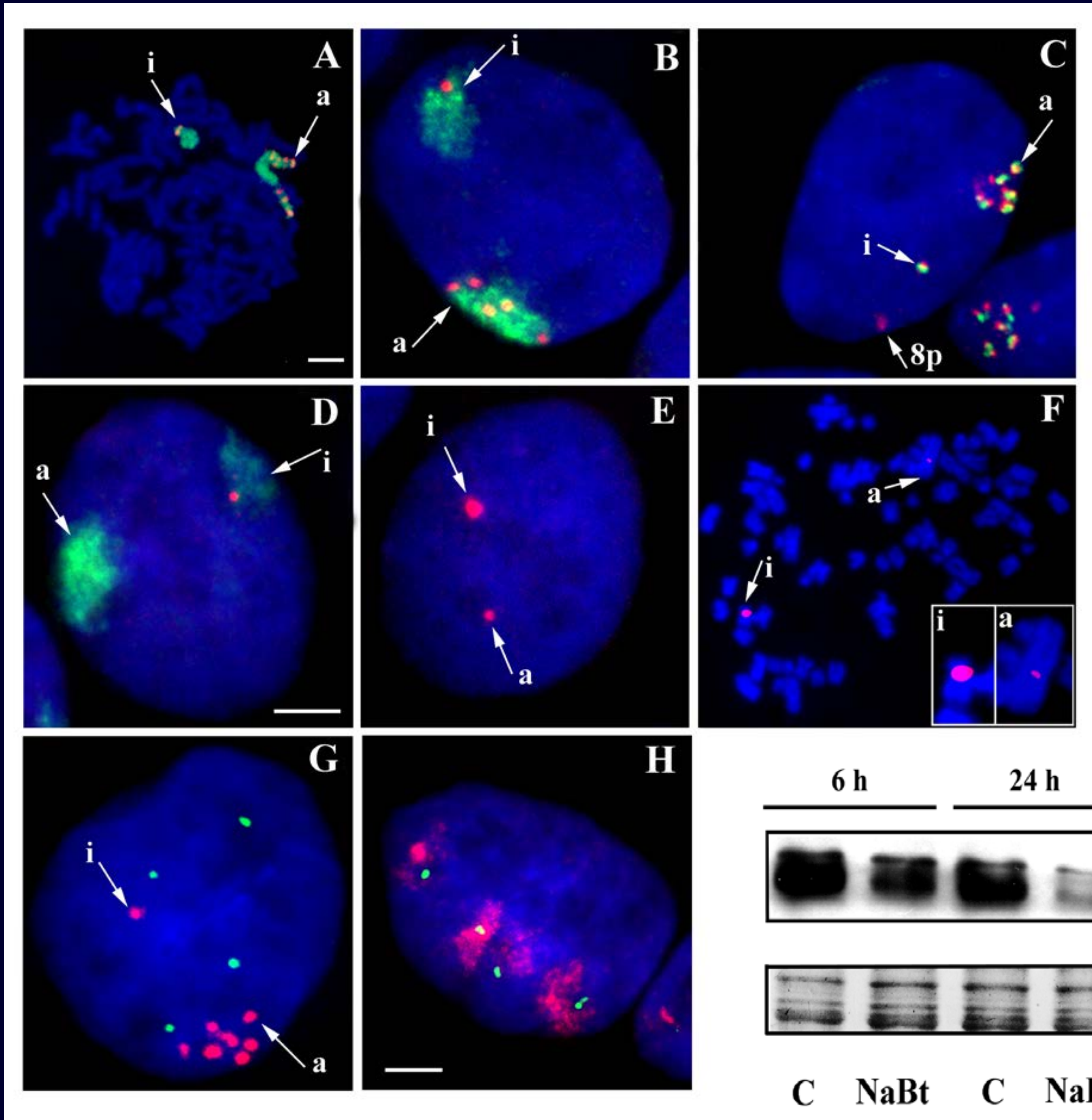
Enterocytic cell differentiation

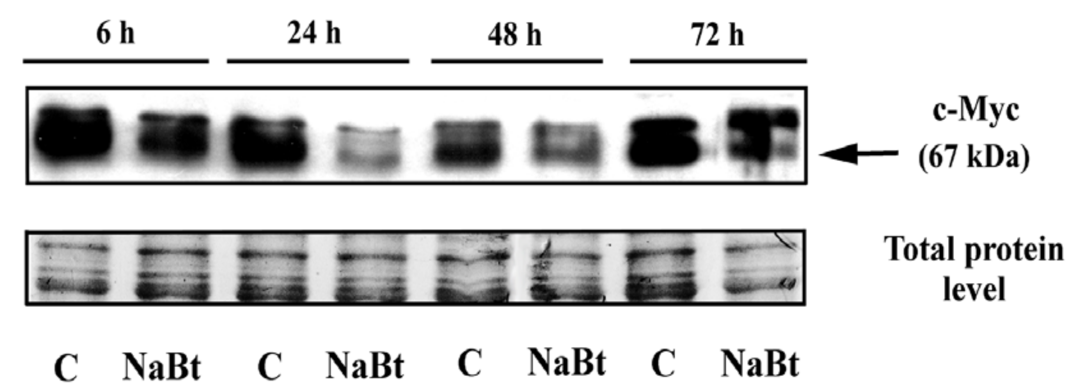


Harničarová et al., 2005

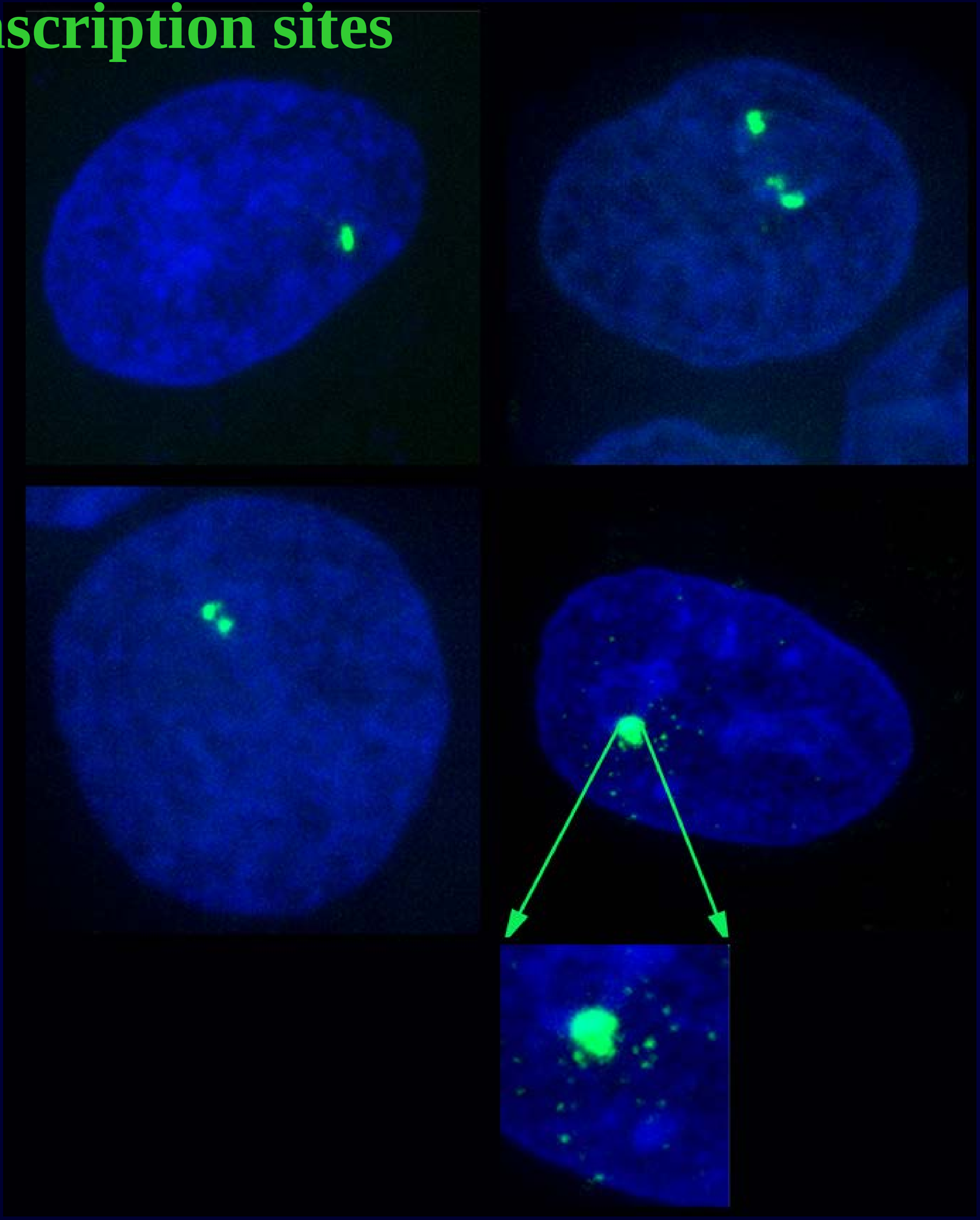
HSA 8 and related structures

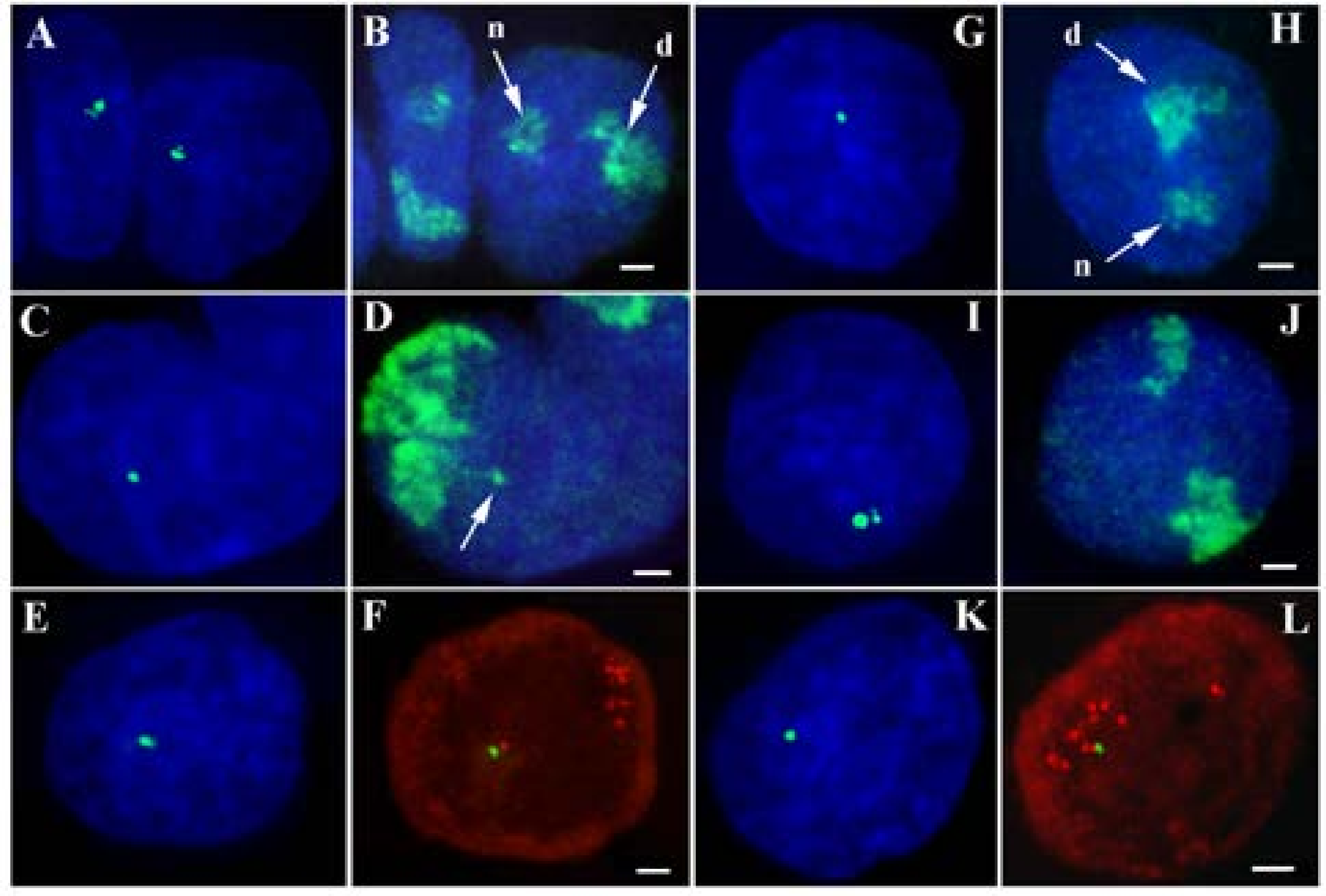
Harničarová et al., 2006



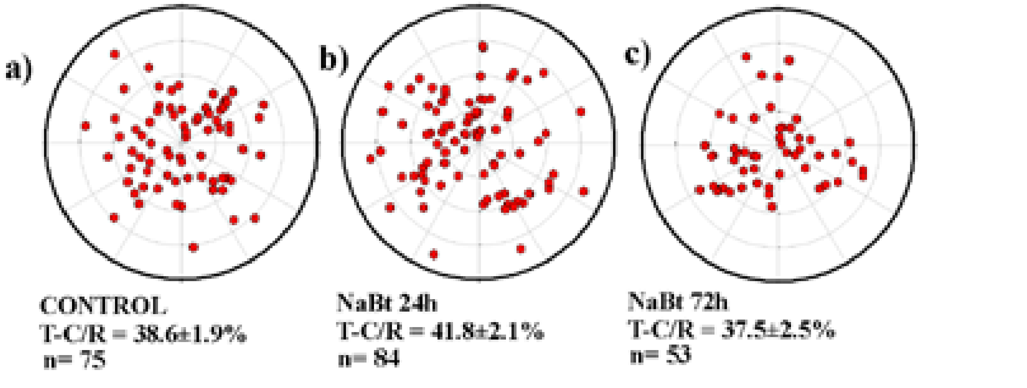


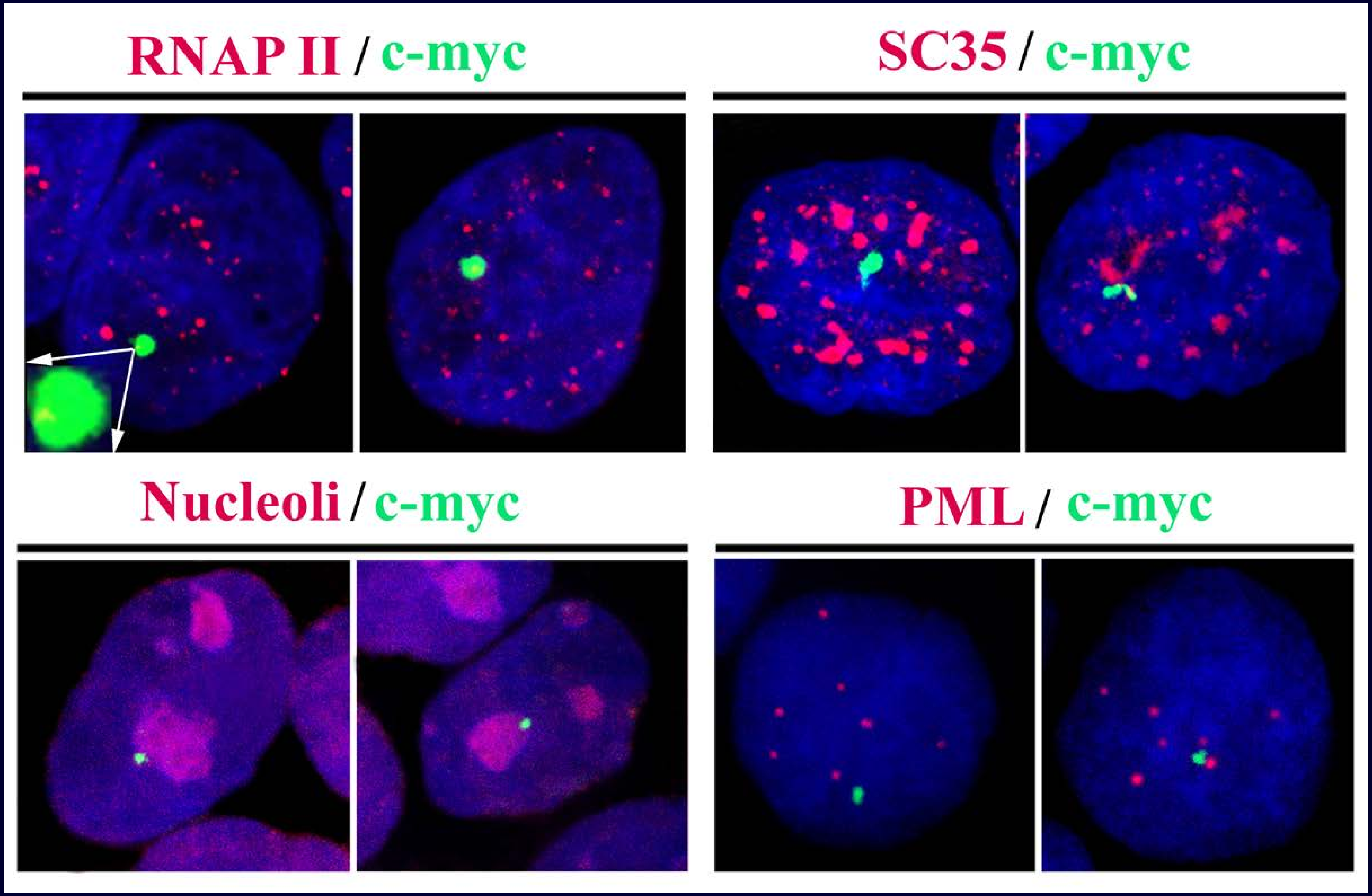
C-myc transcription sites



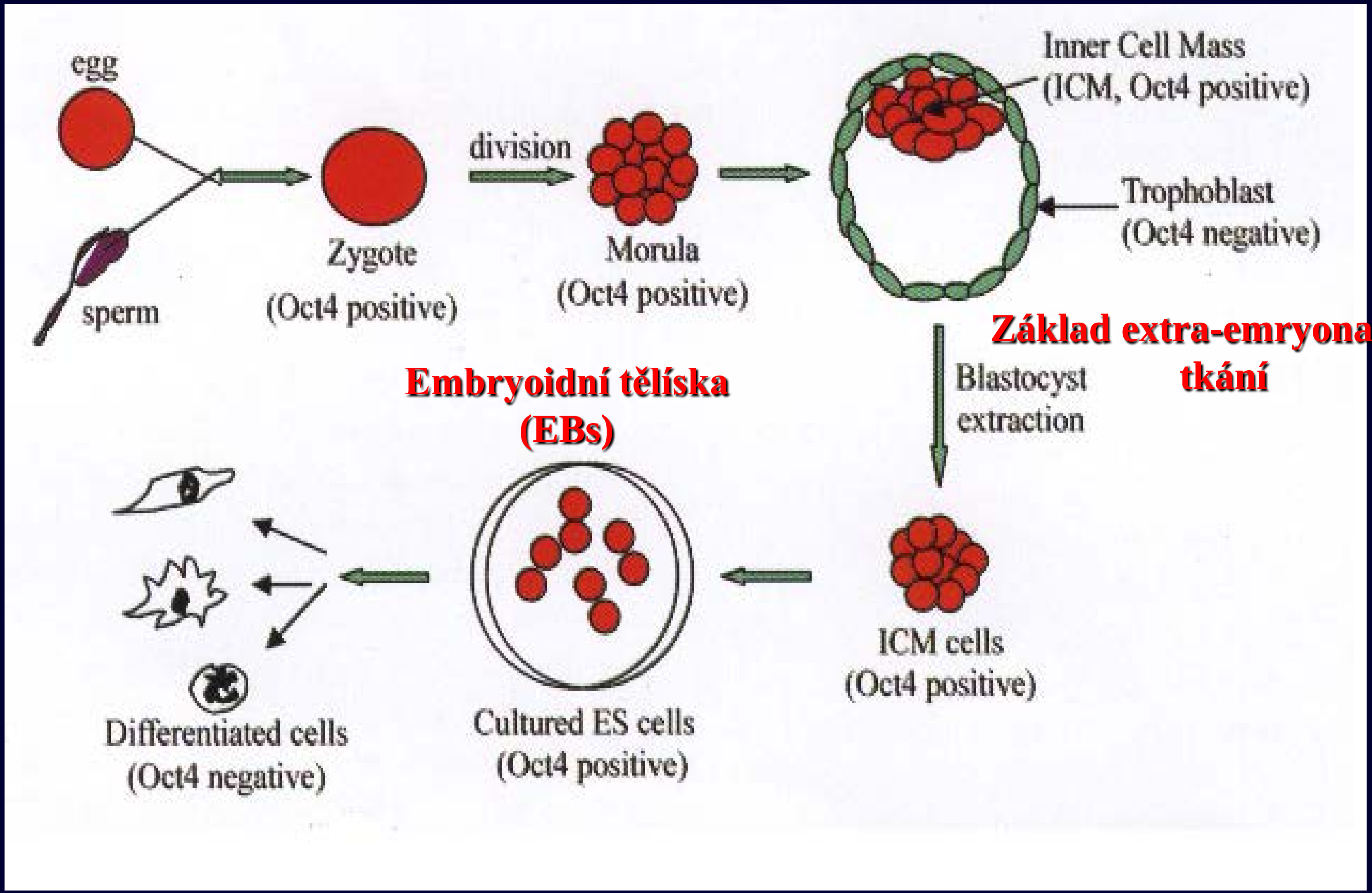


C-myc gene and c-myc transcription site in HT29 cells

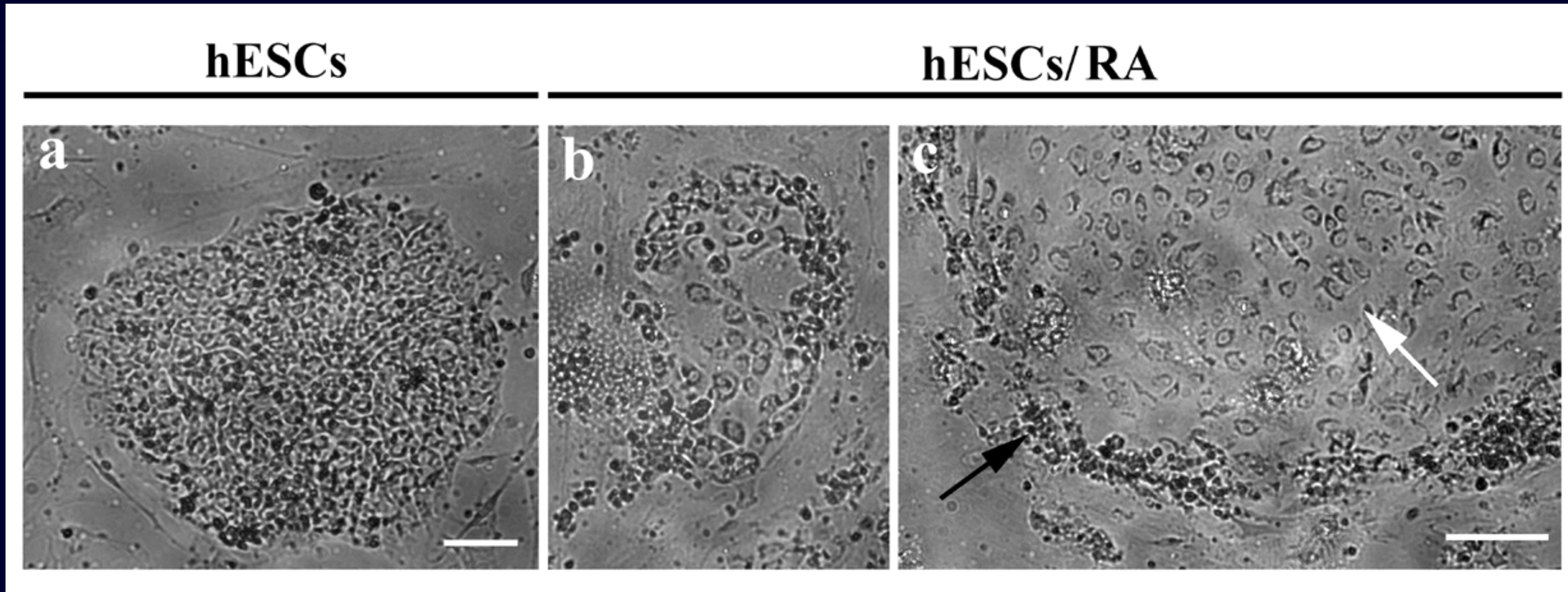



RNAP II / c-myc
SC35 / c-myc
Nucleoli / c-myc
PML / c-myc

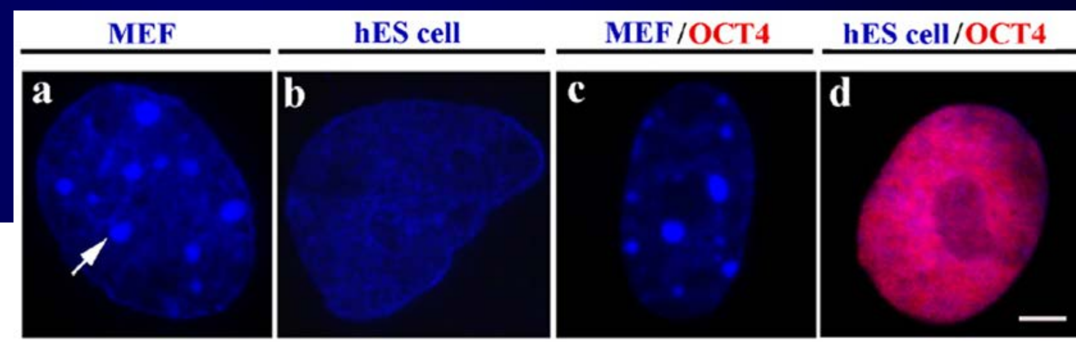
1. Differentiation of mouse emryonic cells (ES and EC)



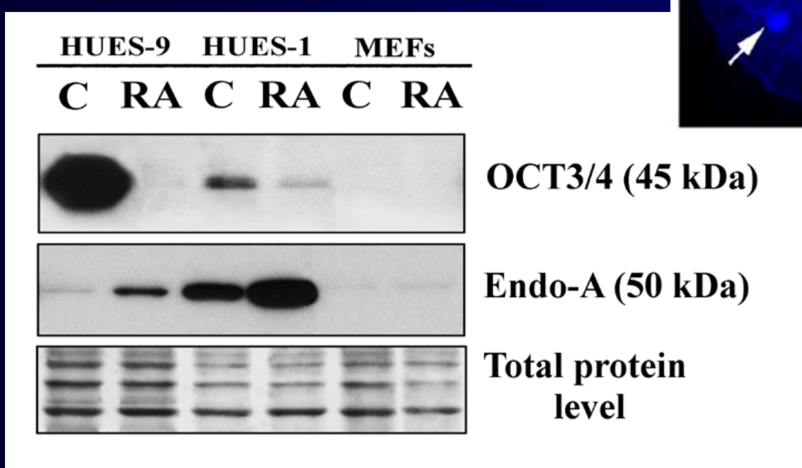
A



B

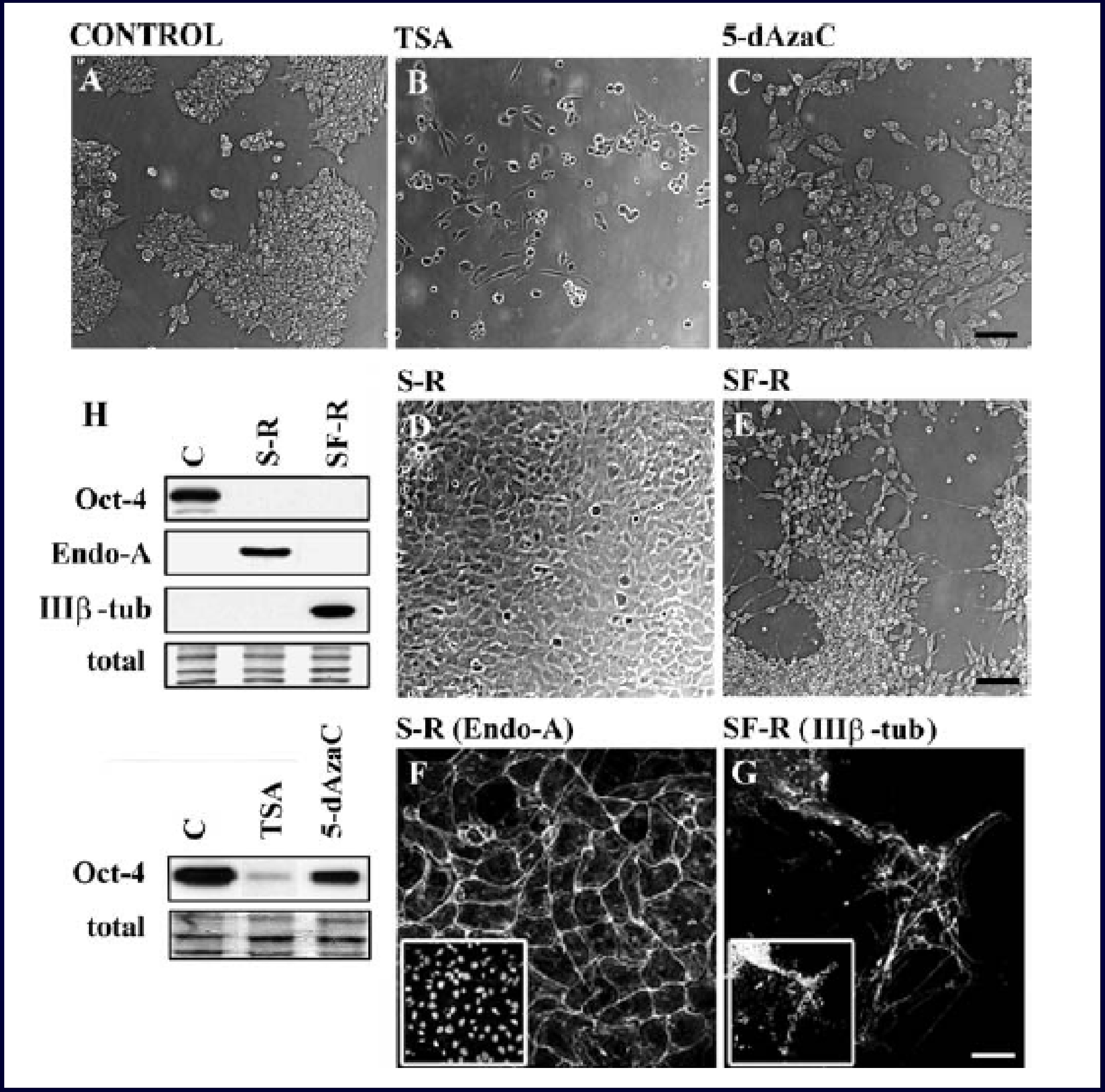


C

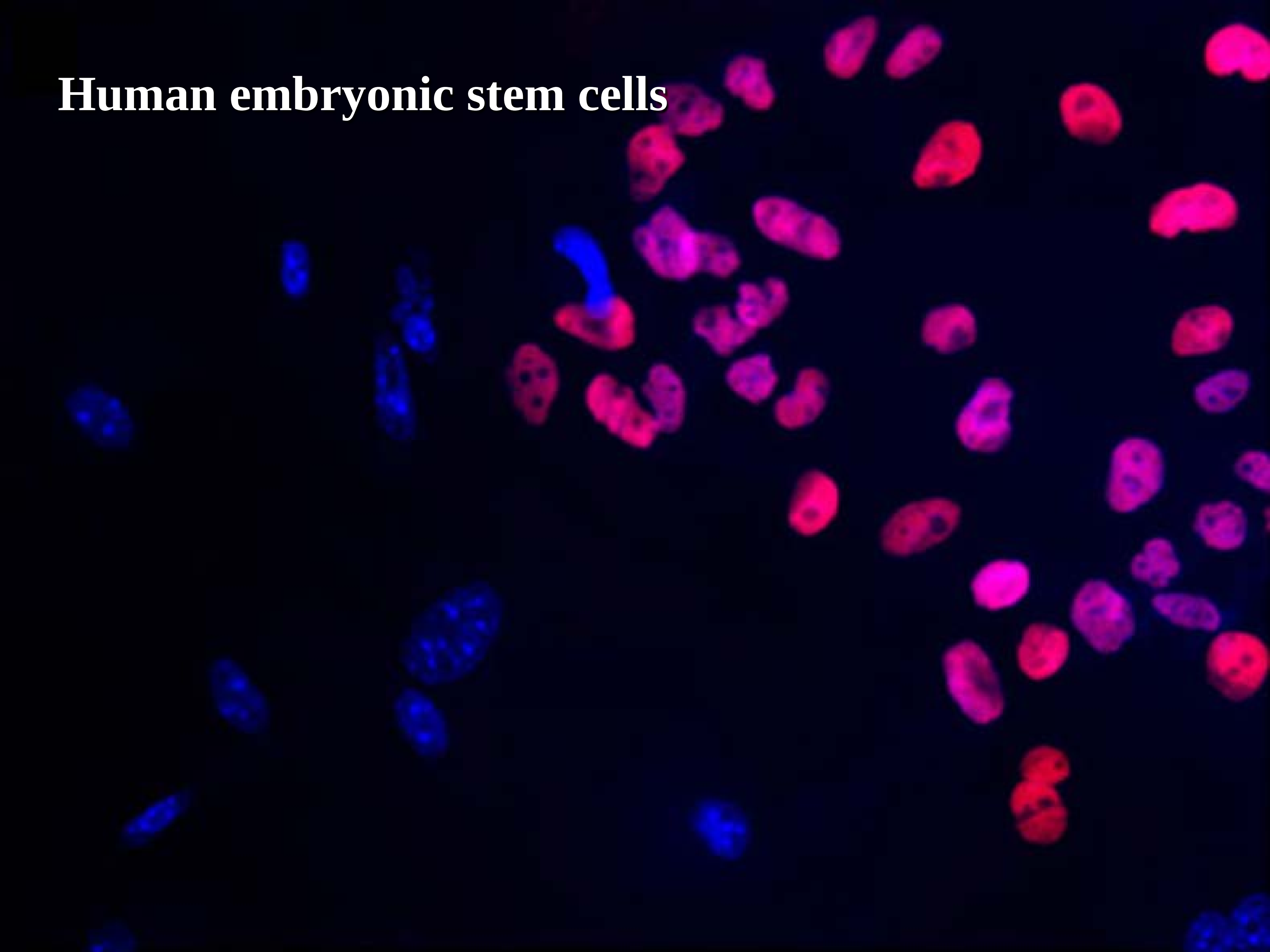


(Bártová et al., Differentiation, 2008)
(Bártová et al., Developmental Dynamics, 2008)

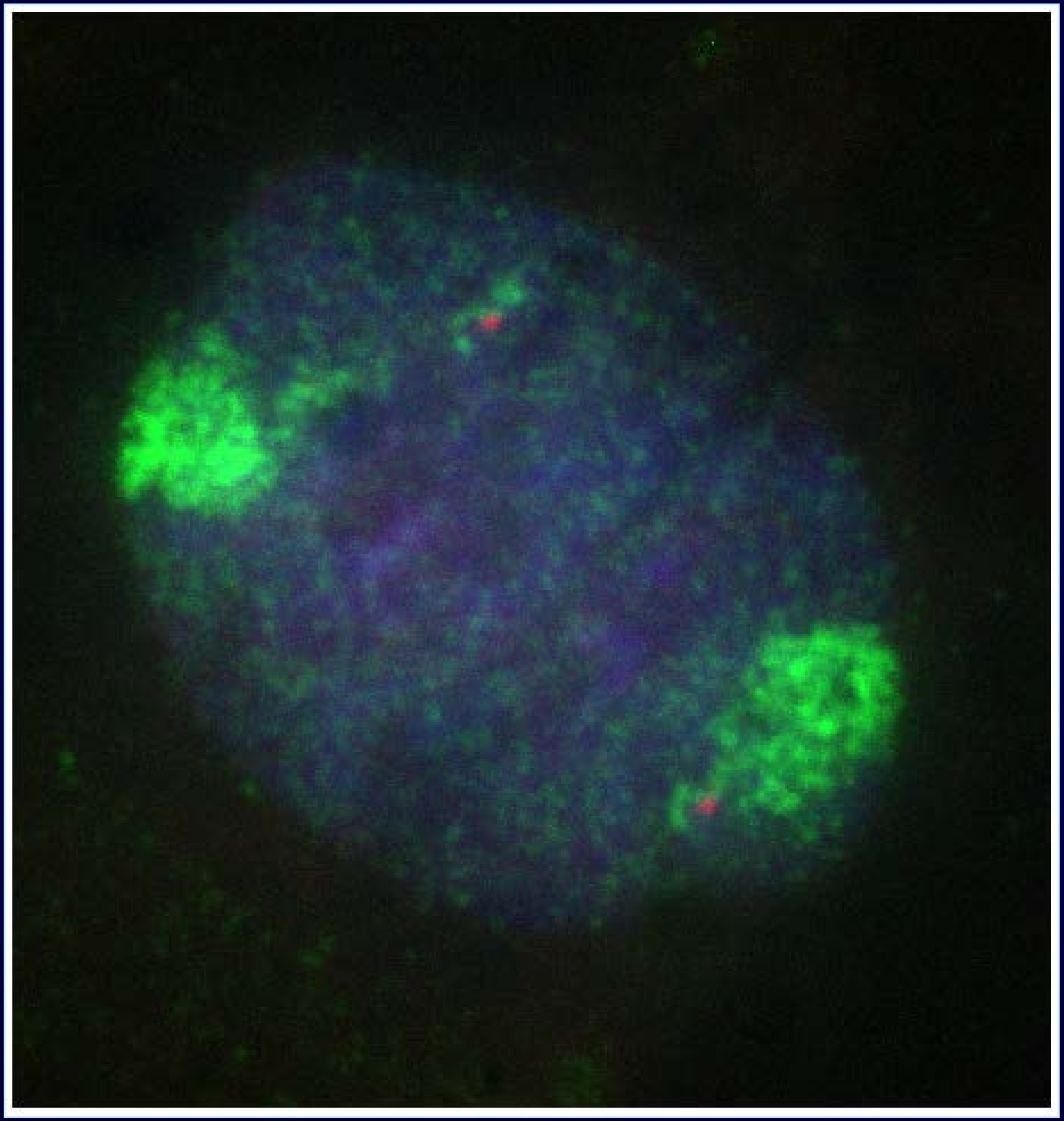
Mouse embyonal carcinoma cells P19

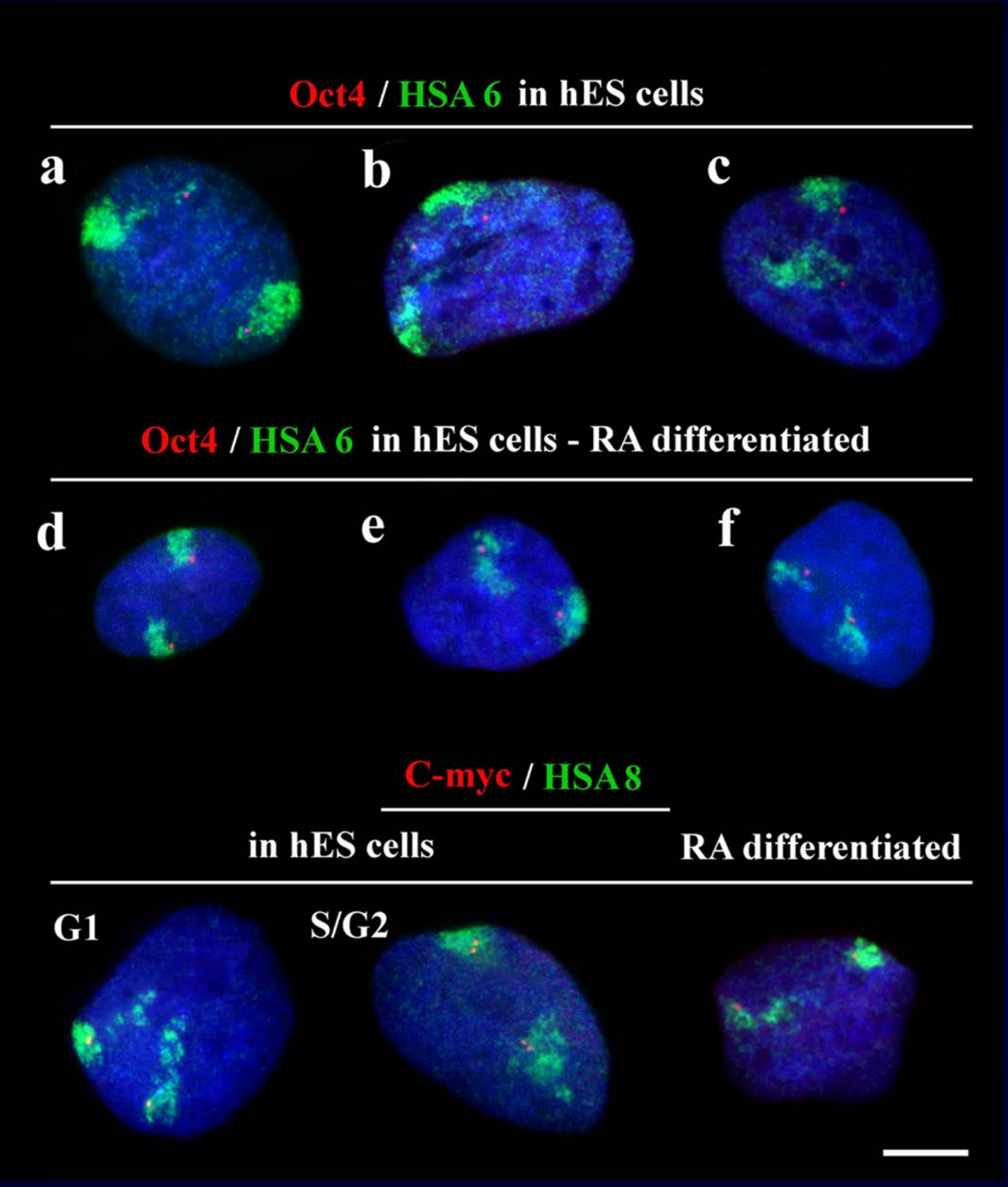


(Bártová et al., Histochem. Cell Biol., 2007)

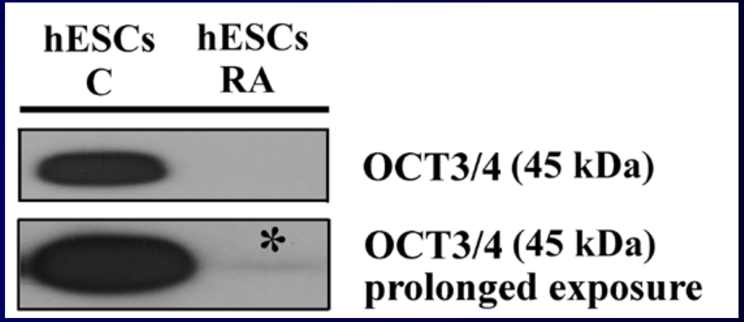

Human embryonic stem cells

Oct3/4 and HSA6 in human ESCs

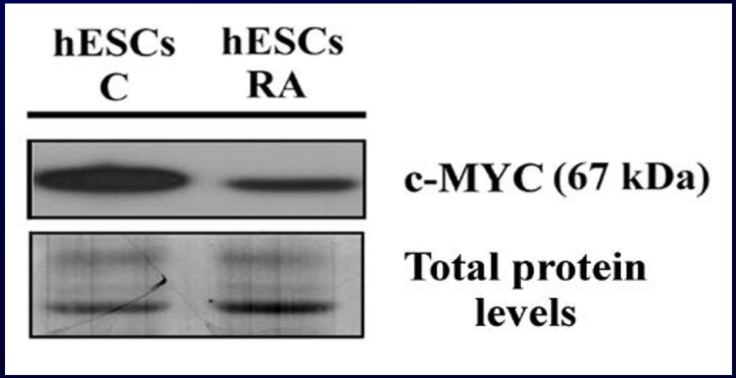




Oct4 / HSA 6 in hES cells
a
b
c
Oct4 / HSA 6 in hES cells - RA differentiated
d
e
f
C-myc / HSA 8
in hES cells
RA differentiated
G1
S/G2

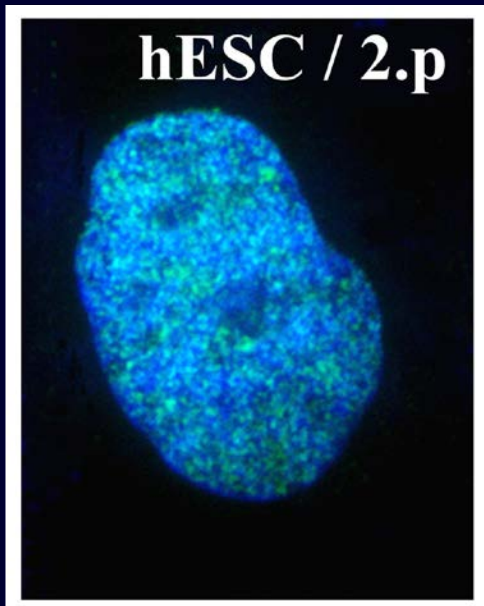


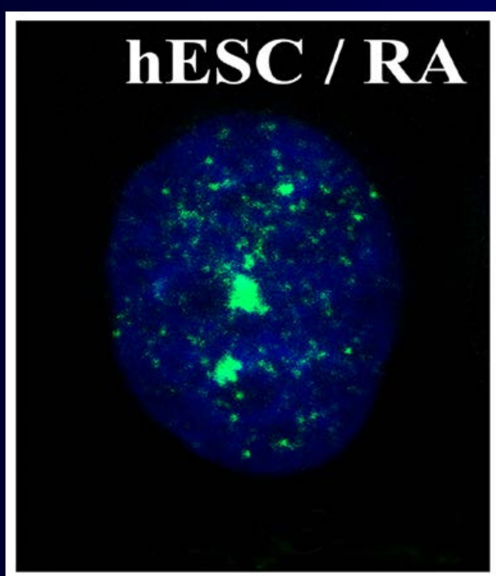
hESCs C
hESCs RA
OCT3/4 (45 kDa)
*
OCT3/4 (45 kDa) prolonged exposure

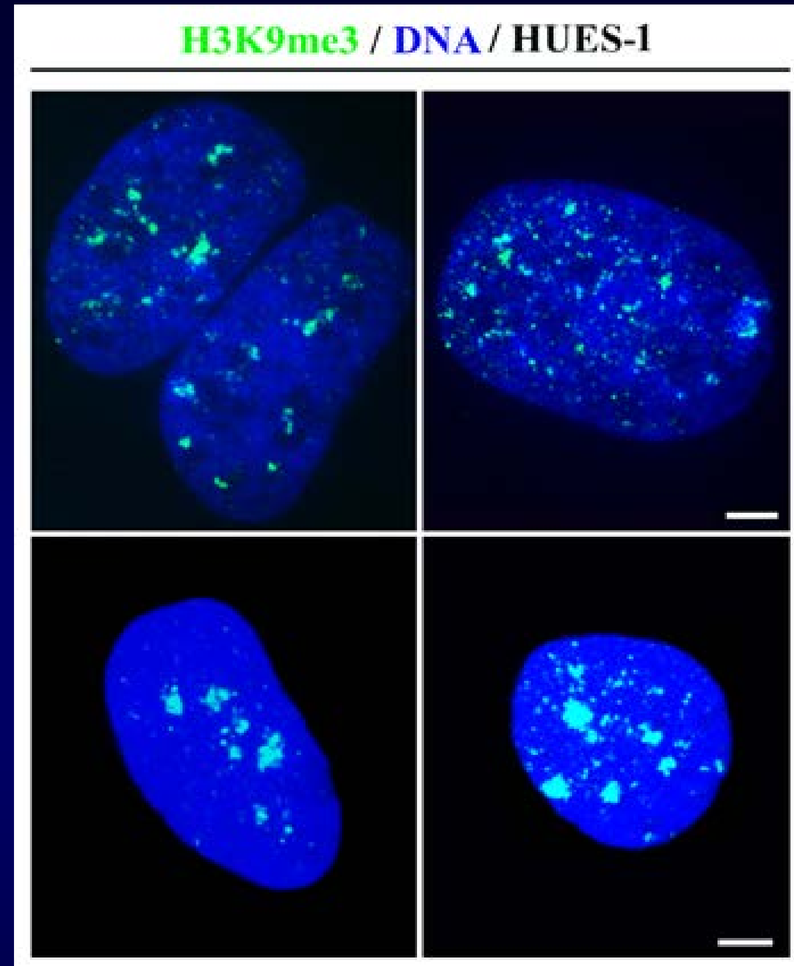


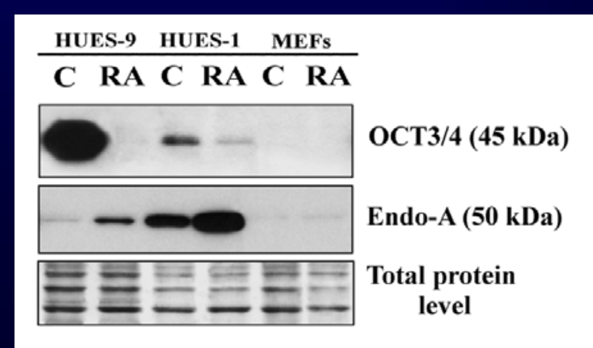
hESCs C
hESCs RA
c-MYC (67 kDa)
Total protein levels

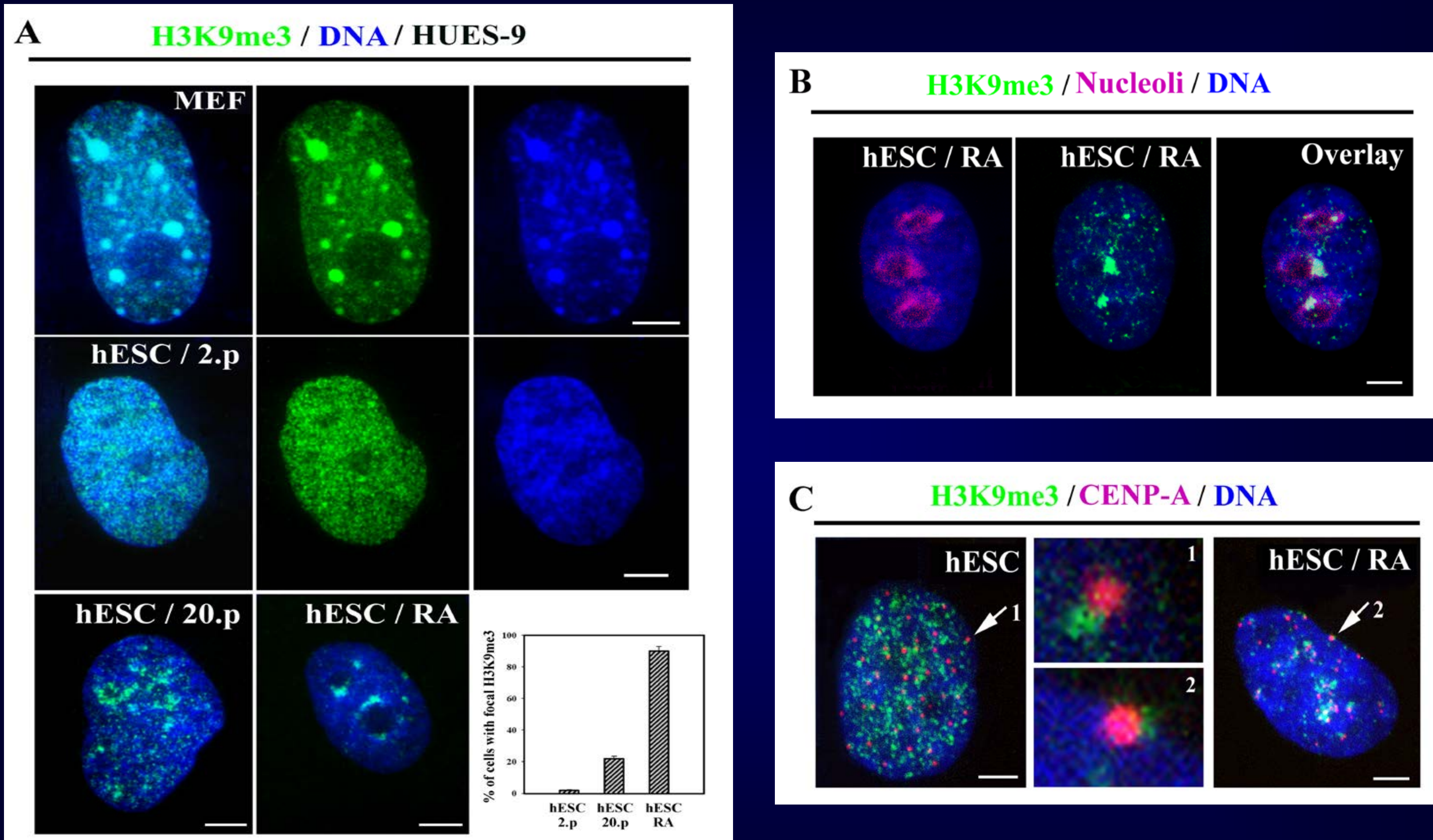
H3K9me3 / HUES-9

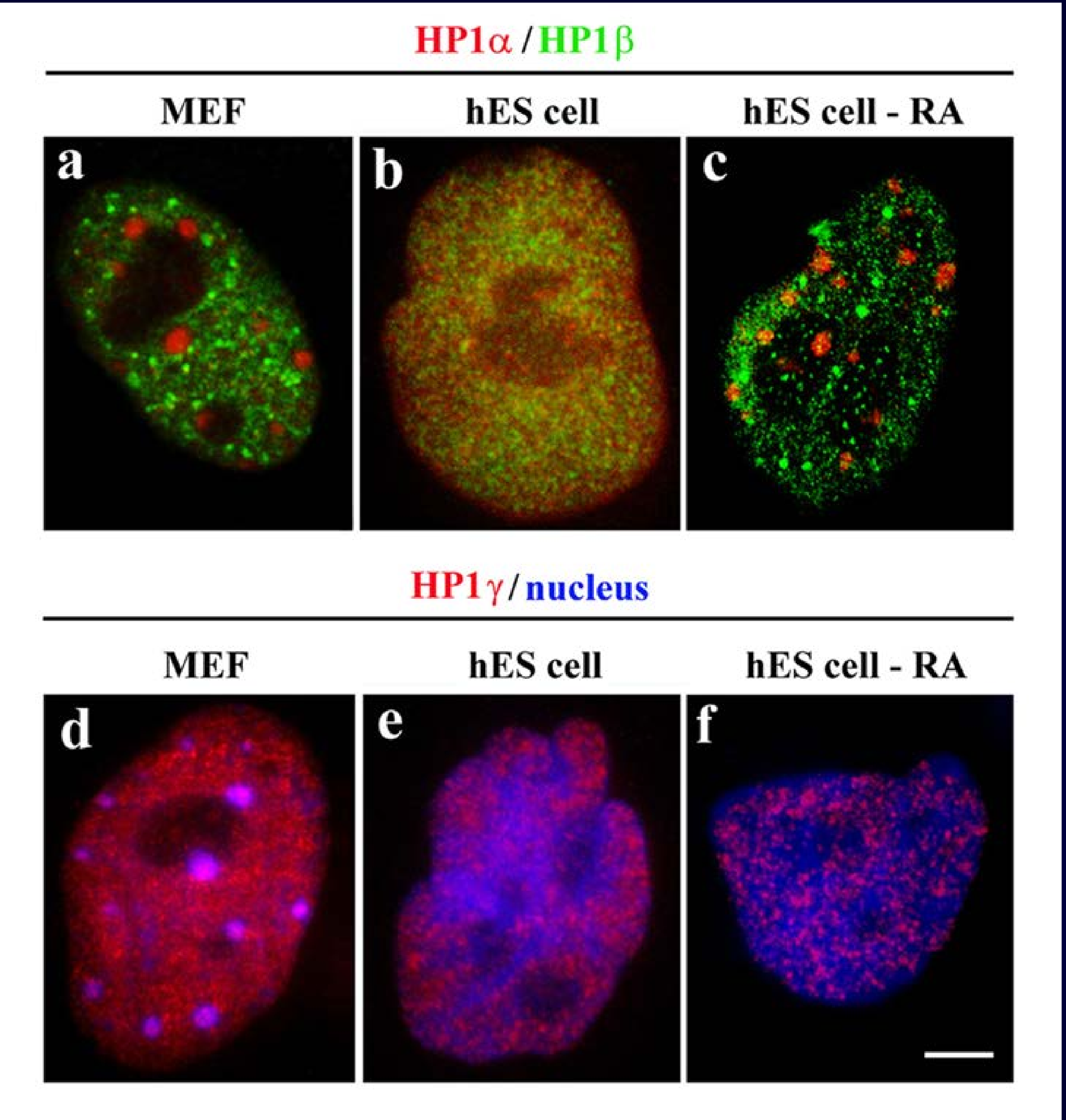


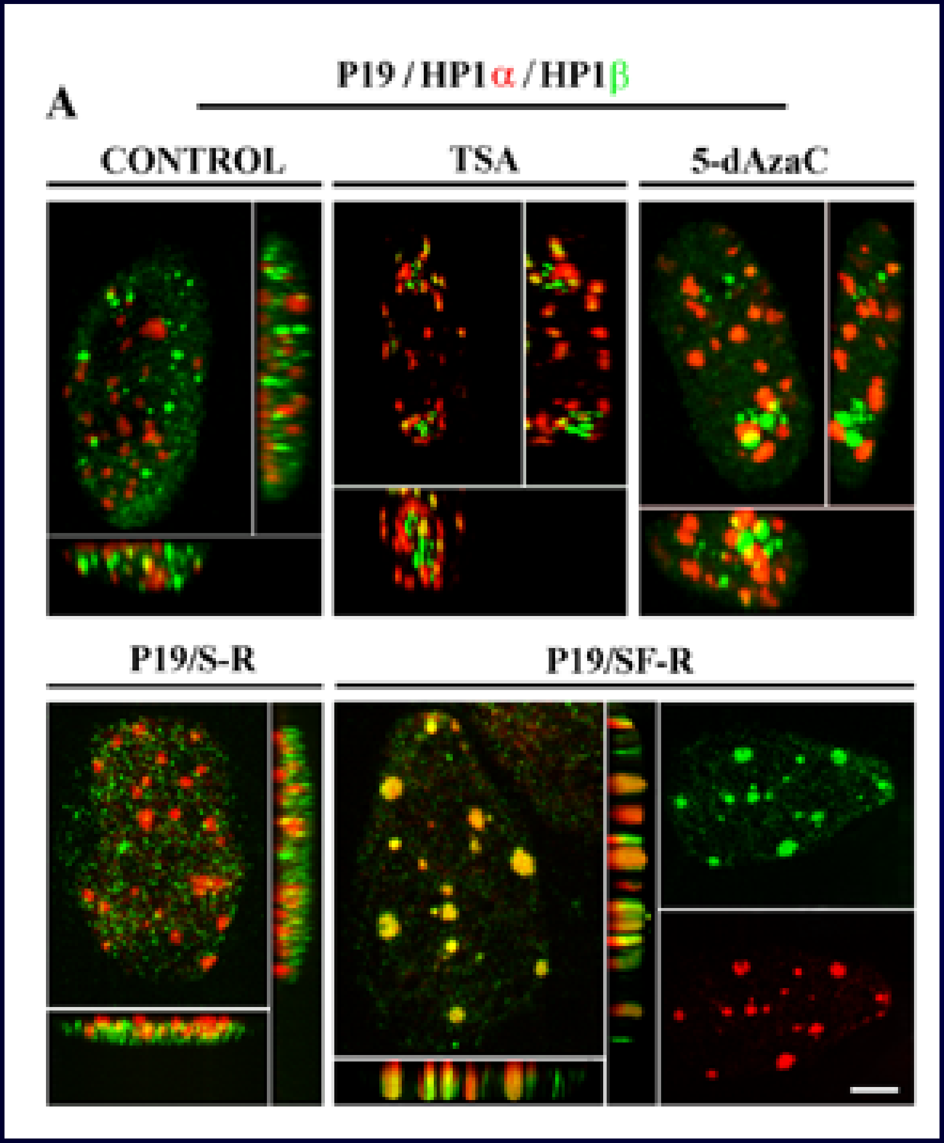


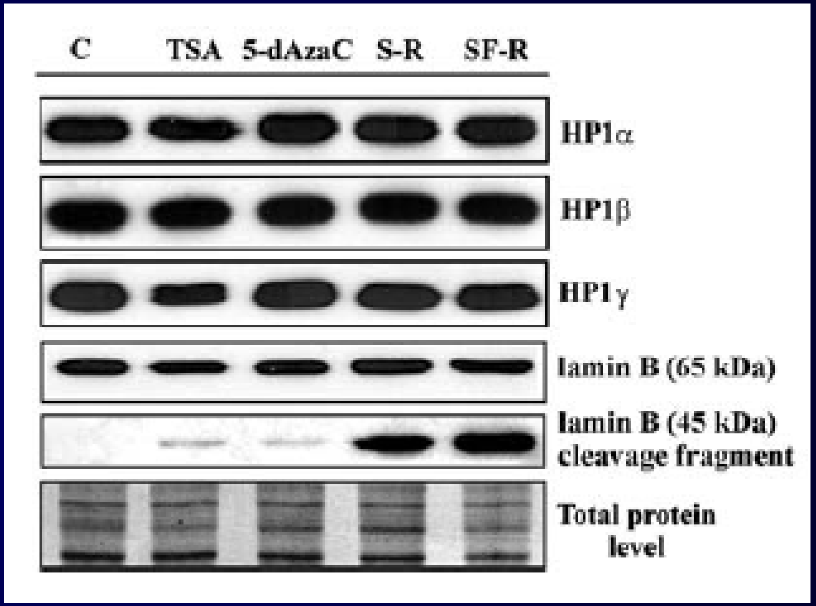


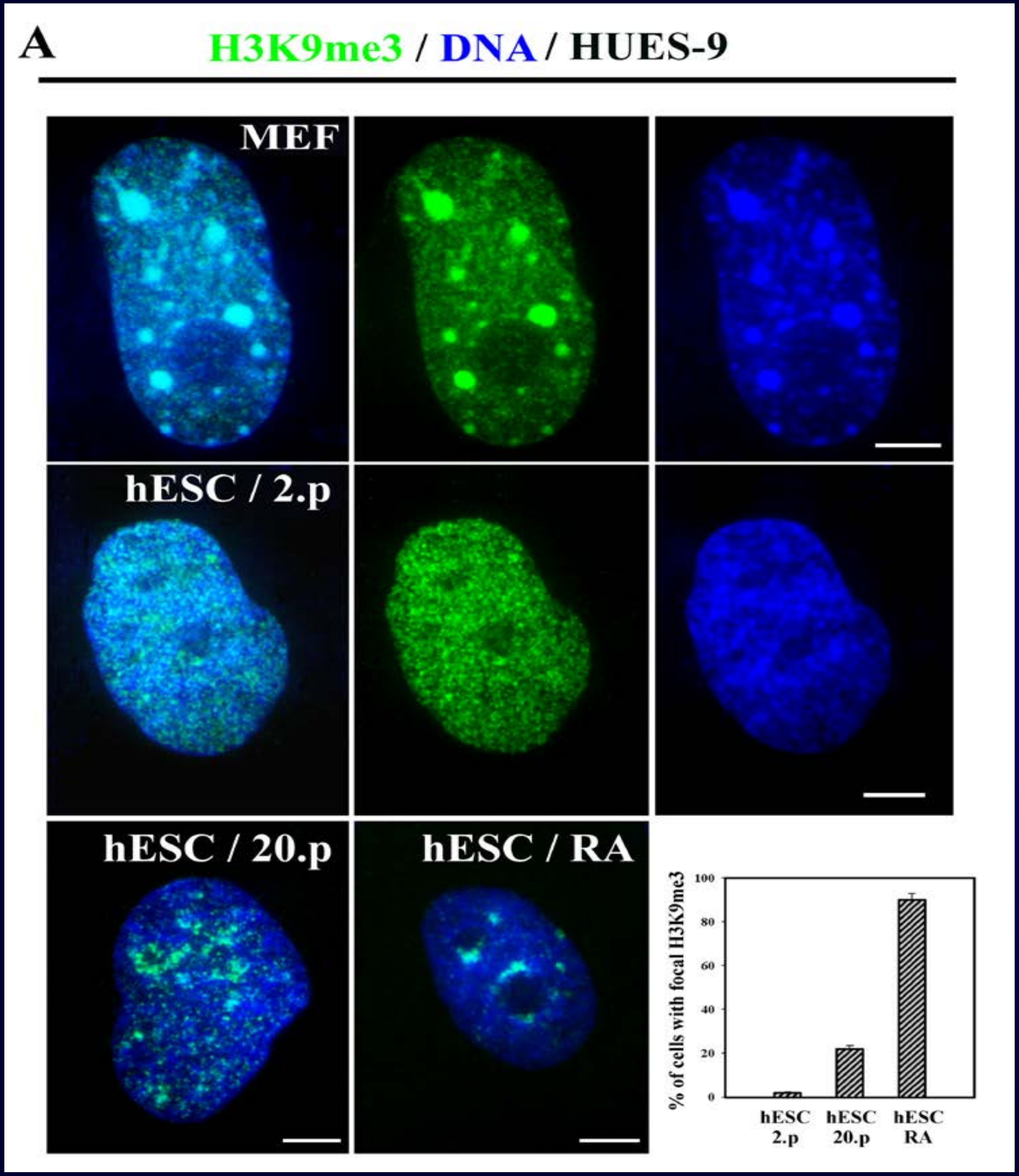


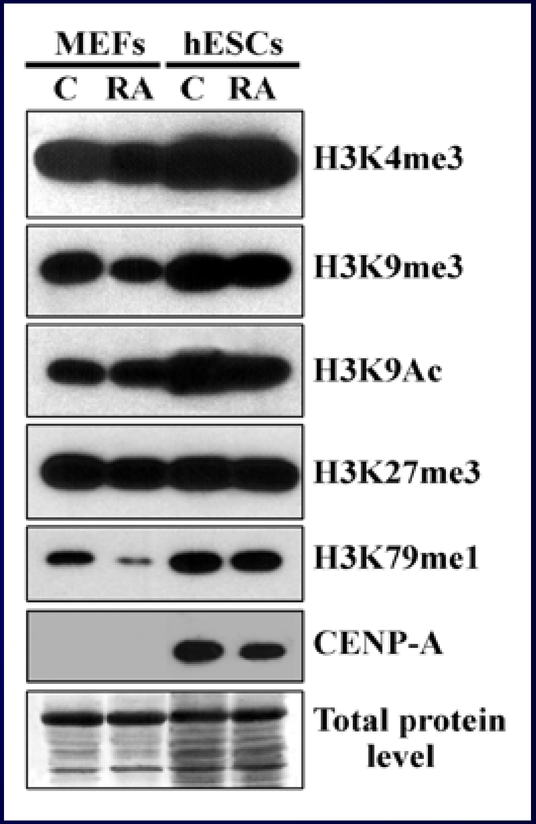

A
H3K9me3 / DNA / HUES-9
MEF
hESC / 2.p
hESC / 20.p
hESC / RA
% of cells with focal H3K9me3
100
80
60
40
20
0
hESC 2.p
hESC 20.p
hESC RA
B
H3K9me3 / Nucleoli / DNA
hESC / RA
hESC / RA
Overlay
C
H3K9me3 / CENP-A / DNA
hESC
1
1
2
hESC / RA
2


HP1α / HP1β
MEF
hES cell
hES cell - RA
a
b
c
HP1γ / nucleus
MEF
hES cell
hES cell - RA
d
e
f

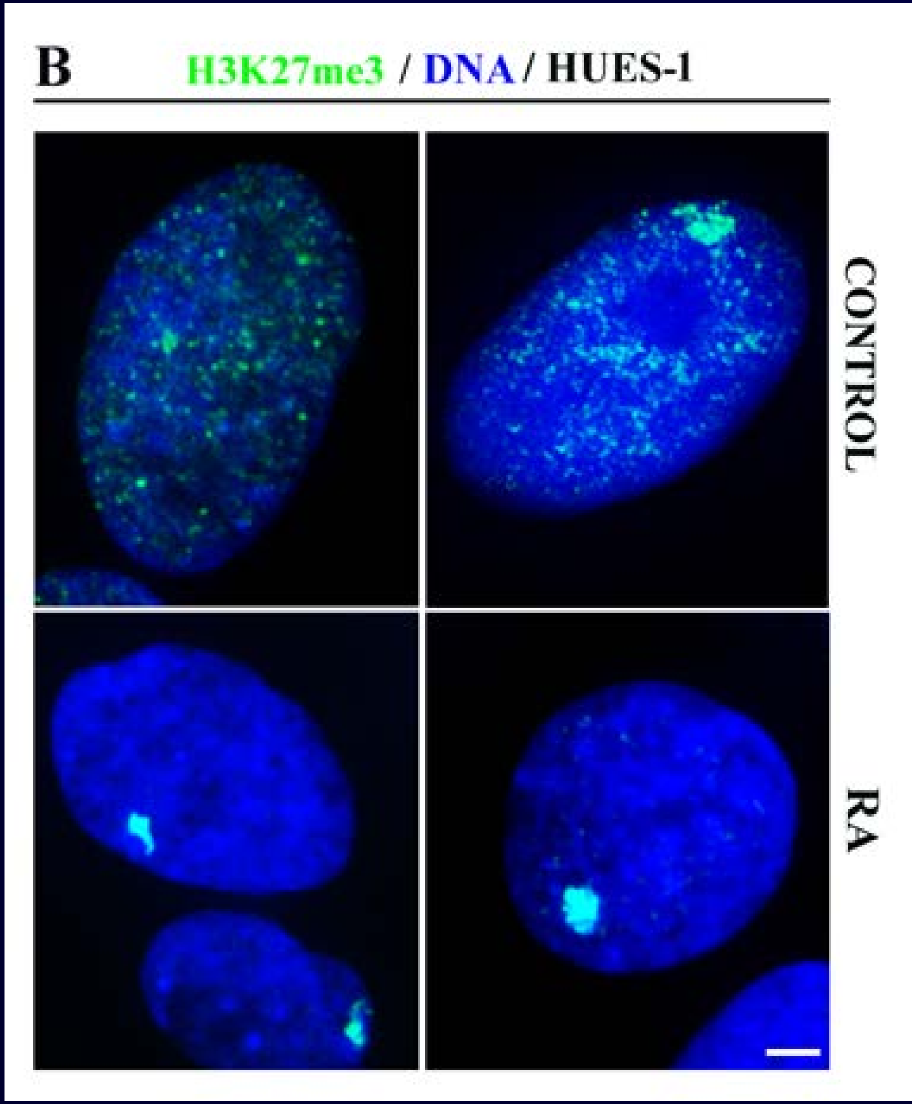

A
P19 / HP1α / HP1β
CONTROL
TSA
5-dAzaC
P19/S-R
P19/SF-R

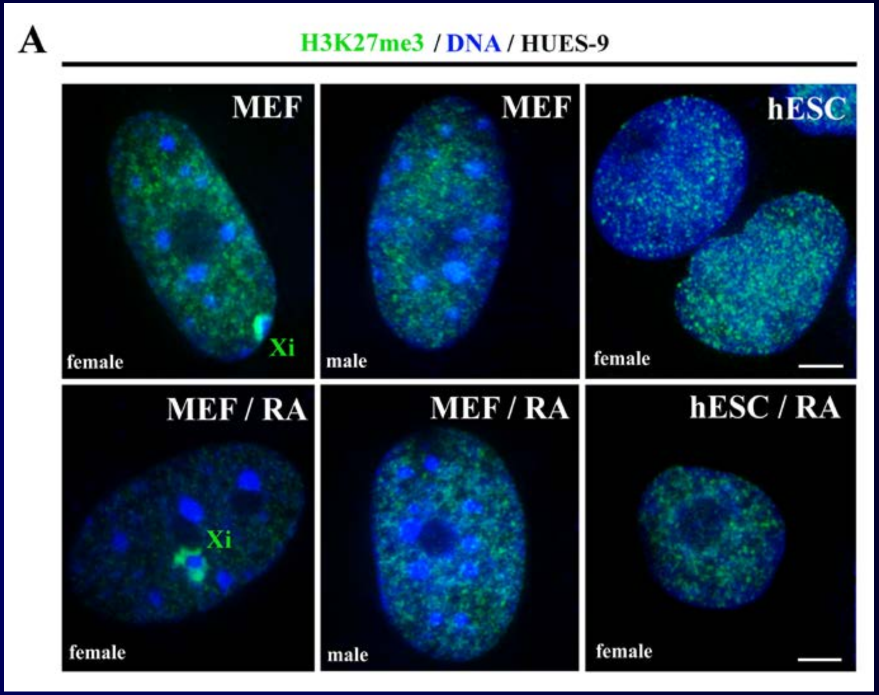

C
TSA
5-dAzaC
S-R
SF-R
HP1α
HP1β
HP1γ
lamin B (65 kDa)
lamin B (45 kDa) cleavage fragment
Total protein level

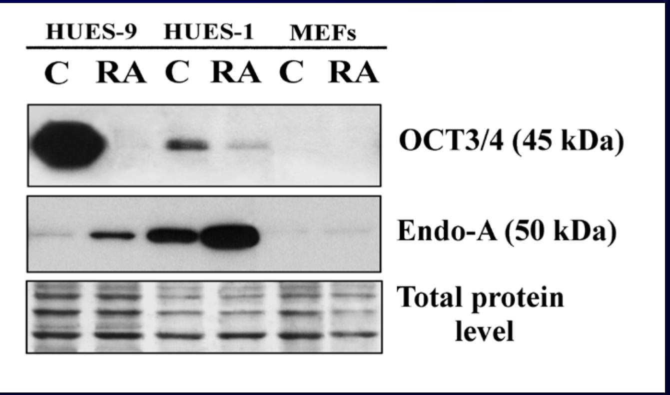

A
H3K9me3 / DNA / HUES-9
MEF
hESC / 2.p
hESC / 20.p
hESC / RA
% of cells with focal H3K9me3
100
80
60
40
20
0
hESC 2.p
hESC 20.p
hESC RA


MEFs
hESCs
C
RA
C
RA
H3K4me3
H3K9me3
H3K9Ac
H3K27me3
H3K79me1
CENP-A
Total protein level

Inactivation of X chromosome in hESC







Xi and ESC differentiation Masui et al., Cell (2011)

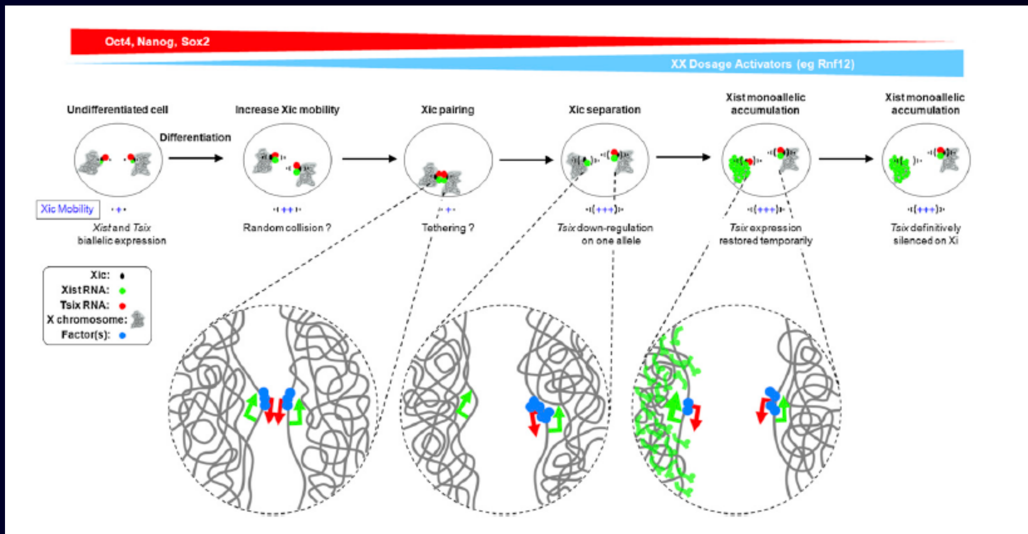
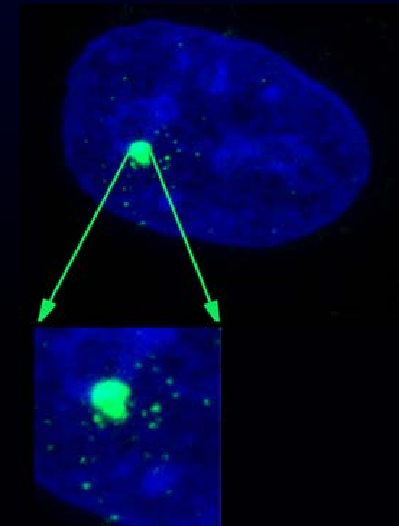
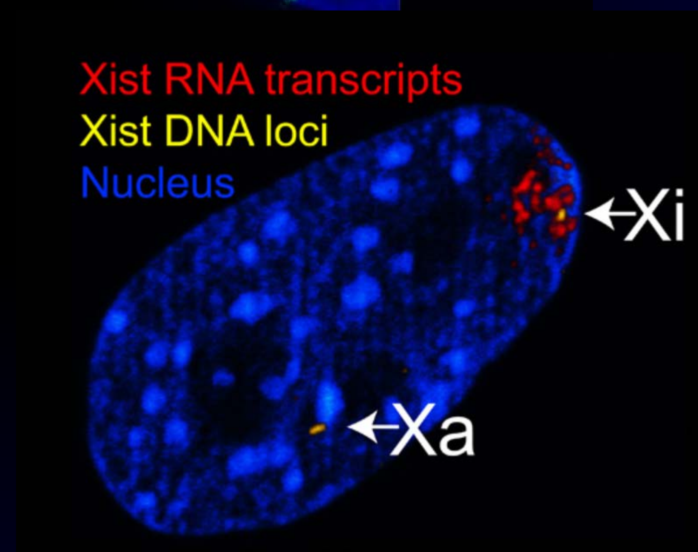


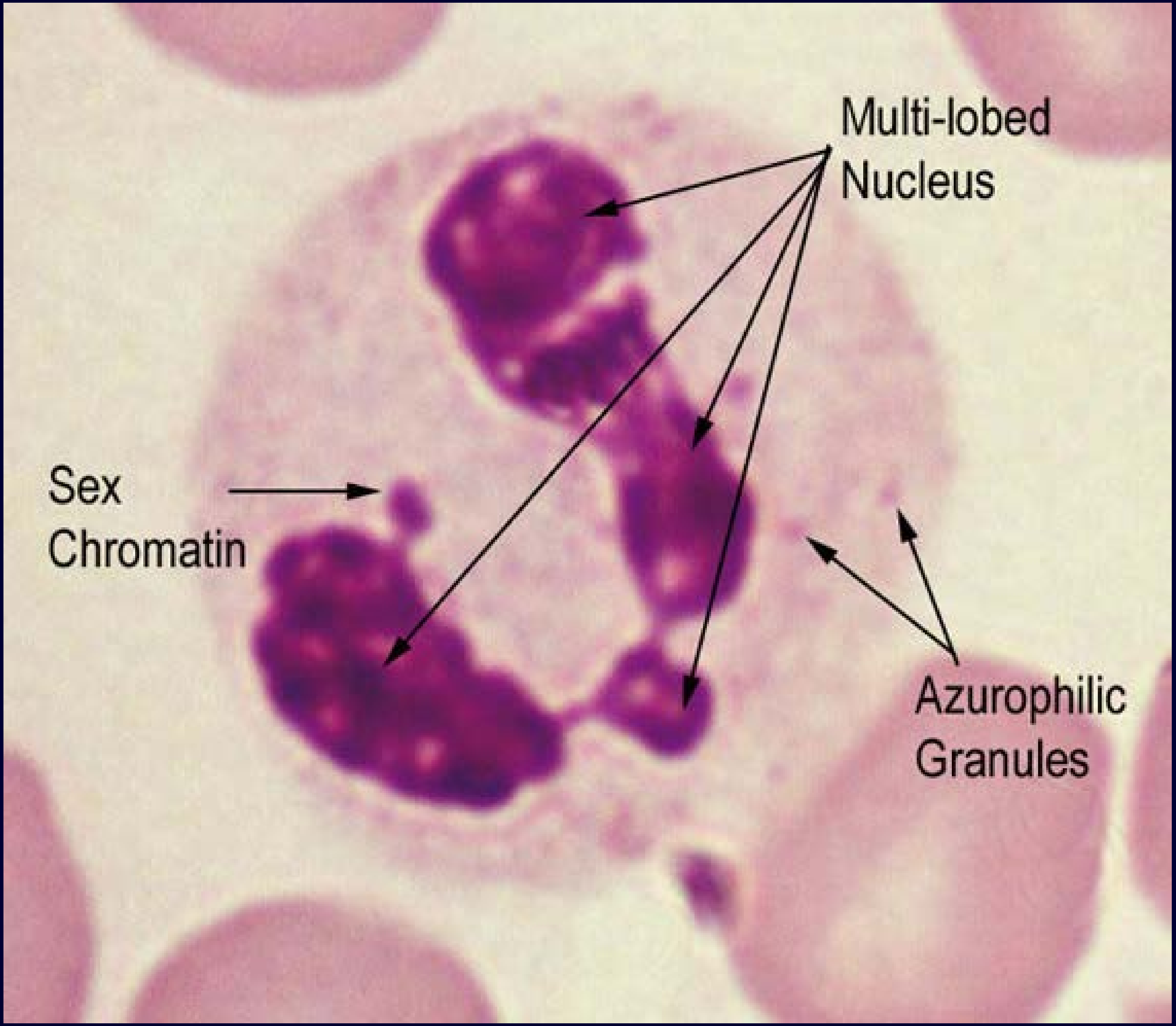
Figure 6. Model for Monoallelic *Tsix* and *Xist* Regulation downstream of Pairing at *Tsix/Xite*

From left, nucleus of an undifferentiated ES cell, where both the X chromosomes are actively transcribed and *Tsix* is biallelically expressed, *Xist* being more weakly expressed. Upon differentiation, the mobility of chromosomes increases; this may result in increased frequency of random collisions between the two Xic loci. Once the two loci come together, they stay in close proximity (d $\leq$ 2 μm) for ~45 min and display reduced mobility, indicative of possible tethering to each other, either directly or via some common nuclear compartment. Immediately after pairing, *Tsix* expression becomes monoallelic transiently, through asymmetric distribution of certain factor(s) at the locus (schematically shown below). This transient downregulation of *Tsix* can provide an opportunity for upregulation of *Xist* transcription in *cis* and thus initiate XCI. *Tsix* monoallelic expression may return to a biallelic pattern at later time points, but this will no longer be able to prevent *Xist* RNA accumulation, and ultimately *Tsix* will be silenced by *Xist* in *cis*, ensuring that asymmetric *Xist* expression is locked in.

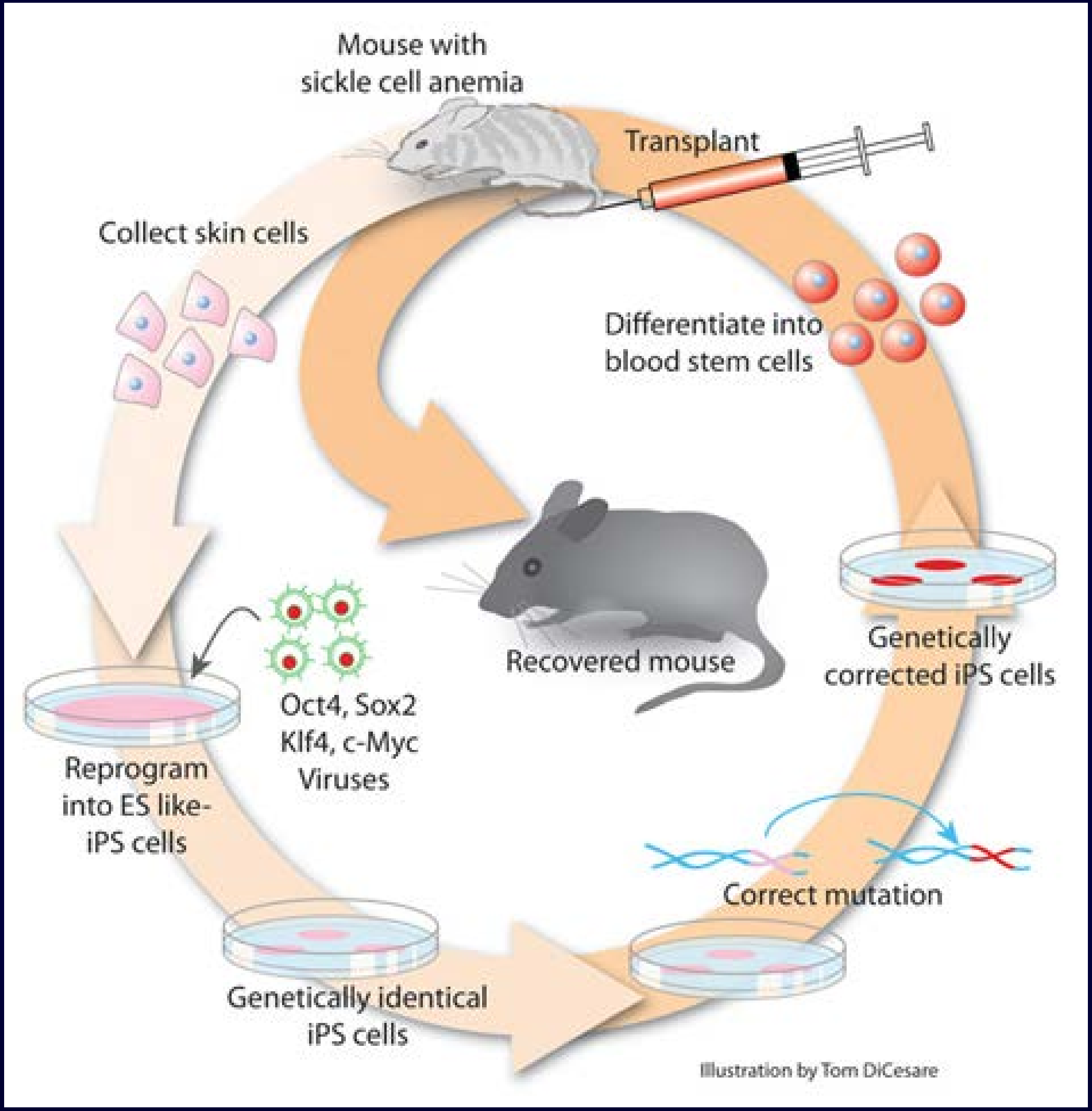




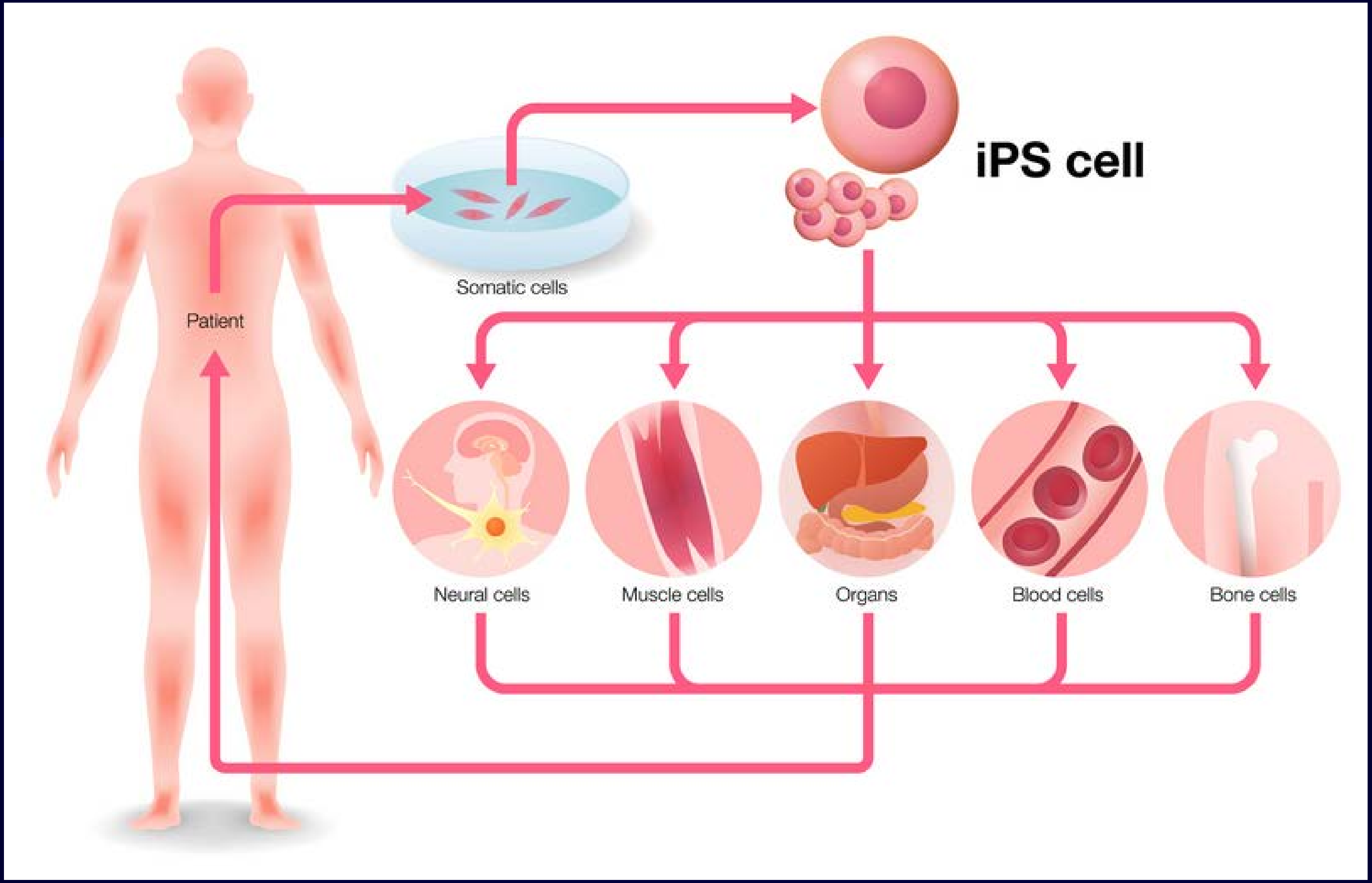
https://www.google.com/search?q=inactive+chromosome+X&client=firefox-b-d&source=lnms&tbm=isch&sa=X&ved=0ahUKEwiC6-KJ95zhAhVBQxUIHWljDk0Q_AUIDigB&biw=1268&bih=578&dpr=1.5#imgrc=bqJA0DbtY8F6XM:


Multi-lobed
Nucleus
Sex
Chromatin
Azurophilic
Granules

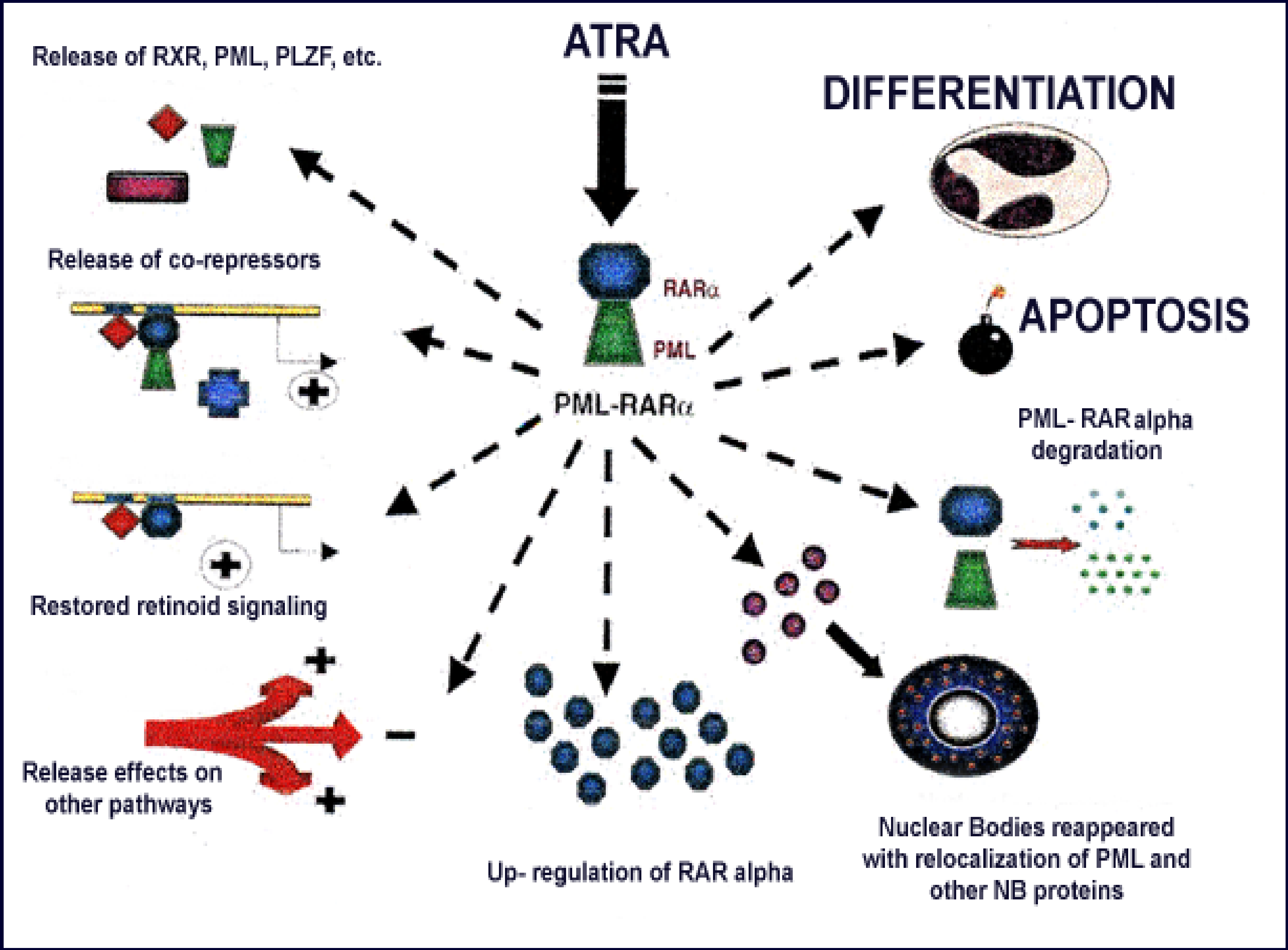
iPSC



Mouse with
sickle cell anemia
Transplant
Collect skin cells
Differentiate into
blood stem cells
Recovered mouse
Genetically
corrected iPS cells
Oct4, Sox2
Klf4, c-Myc
Viruses
Reprogram
into ES like-
iPS cells
Correct mutation
Genetically identical
iPS cells
Illustration by Tom DiCesare



iPS cell
Somatic cells
Patient
Neural cells
Muscle cells
Organs
Blood cells
Bone cells


ATRA
DIFFERENTIATION
Release of RXR, PML, PLZF, etc.
Release of co-repressors
RARα
PML
APOPTOSIS
PML-RARα
PML- RAR alpha
degradation
Restored retinoid signaling
Release effects on
other pathways
Up- regulation of RAR alpha
Nuclear Bodies reappeared
with relocalization of PML and
other NB proteins

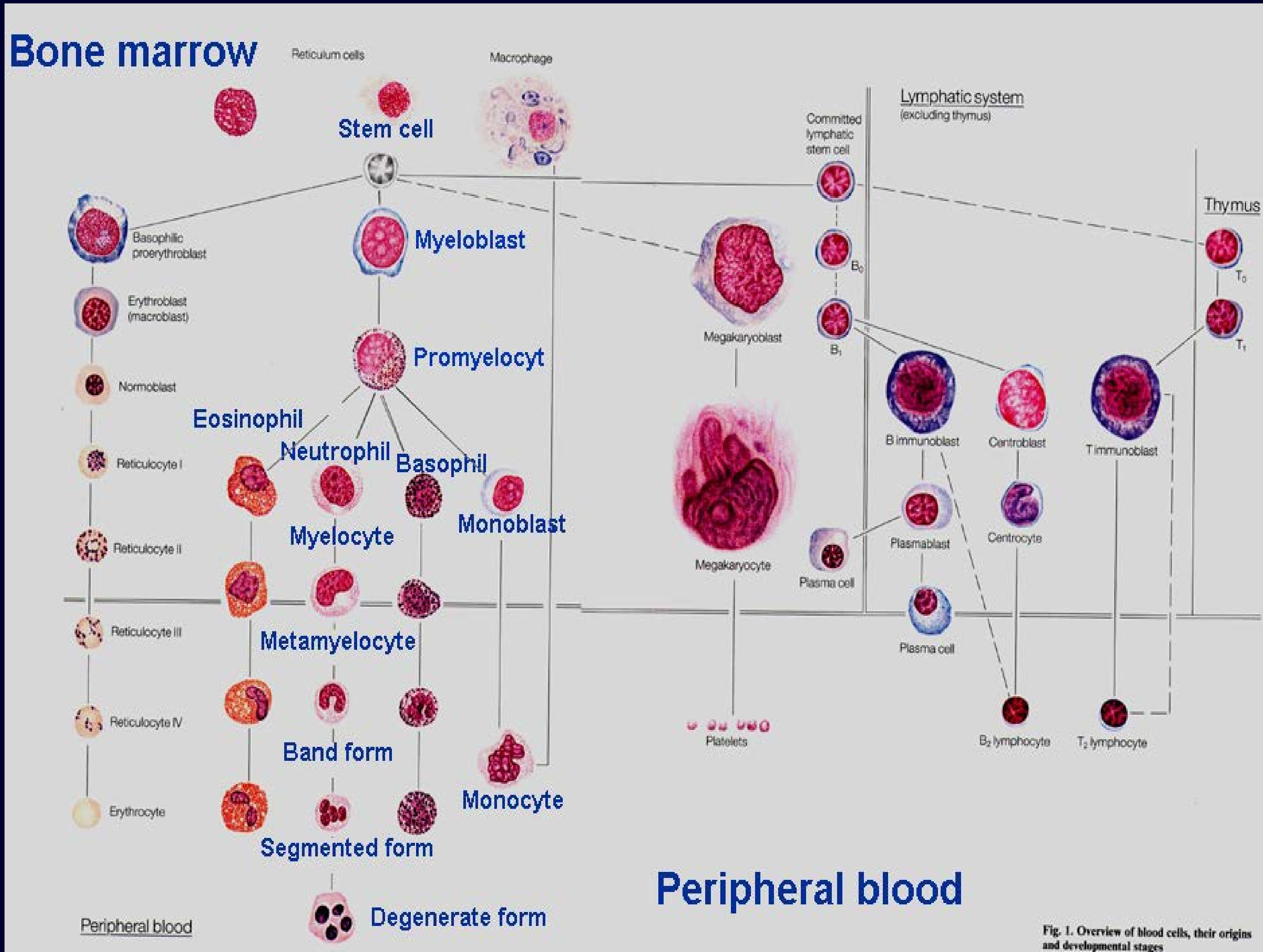
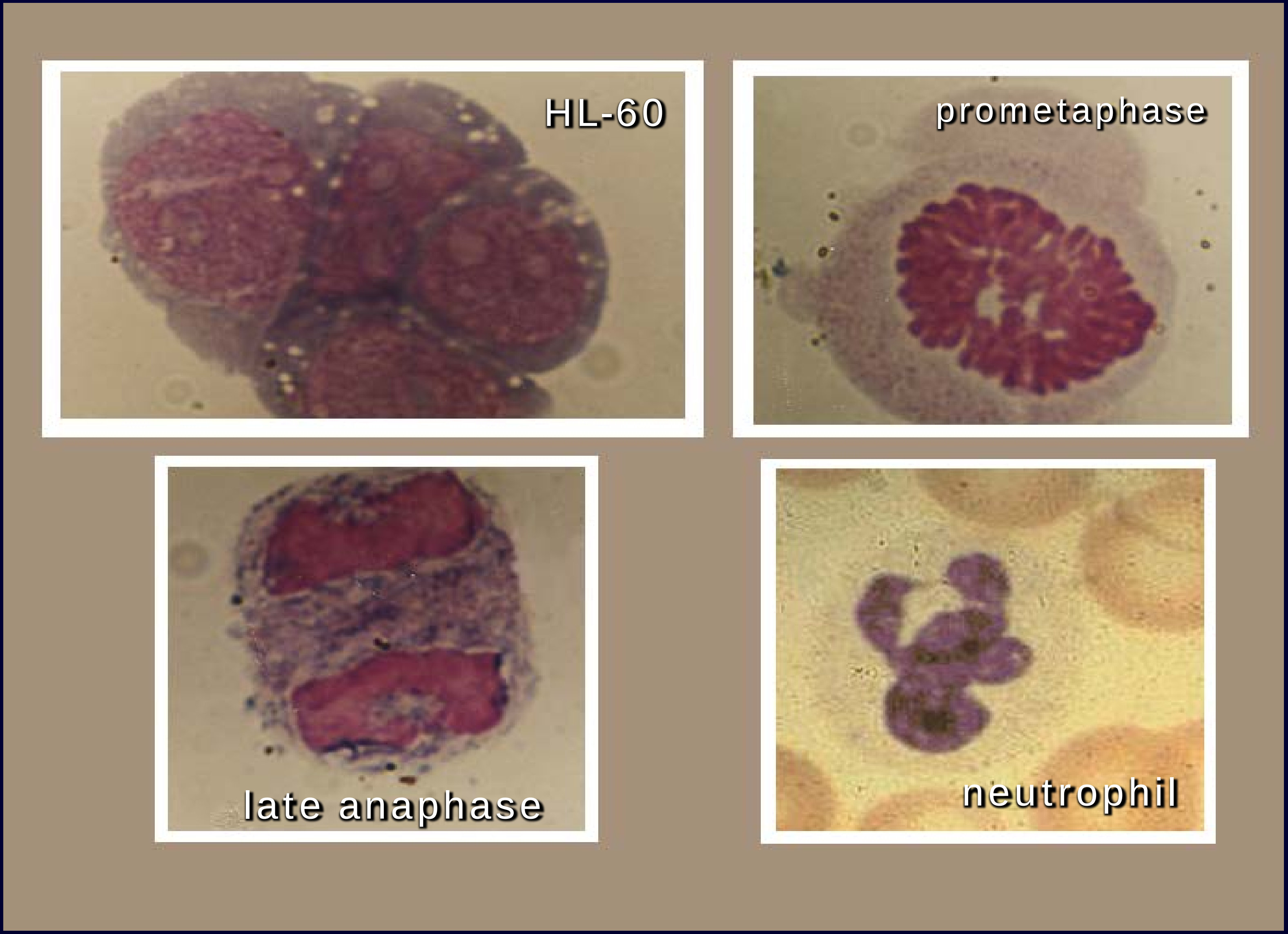
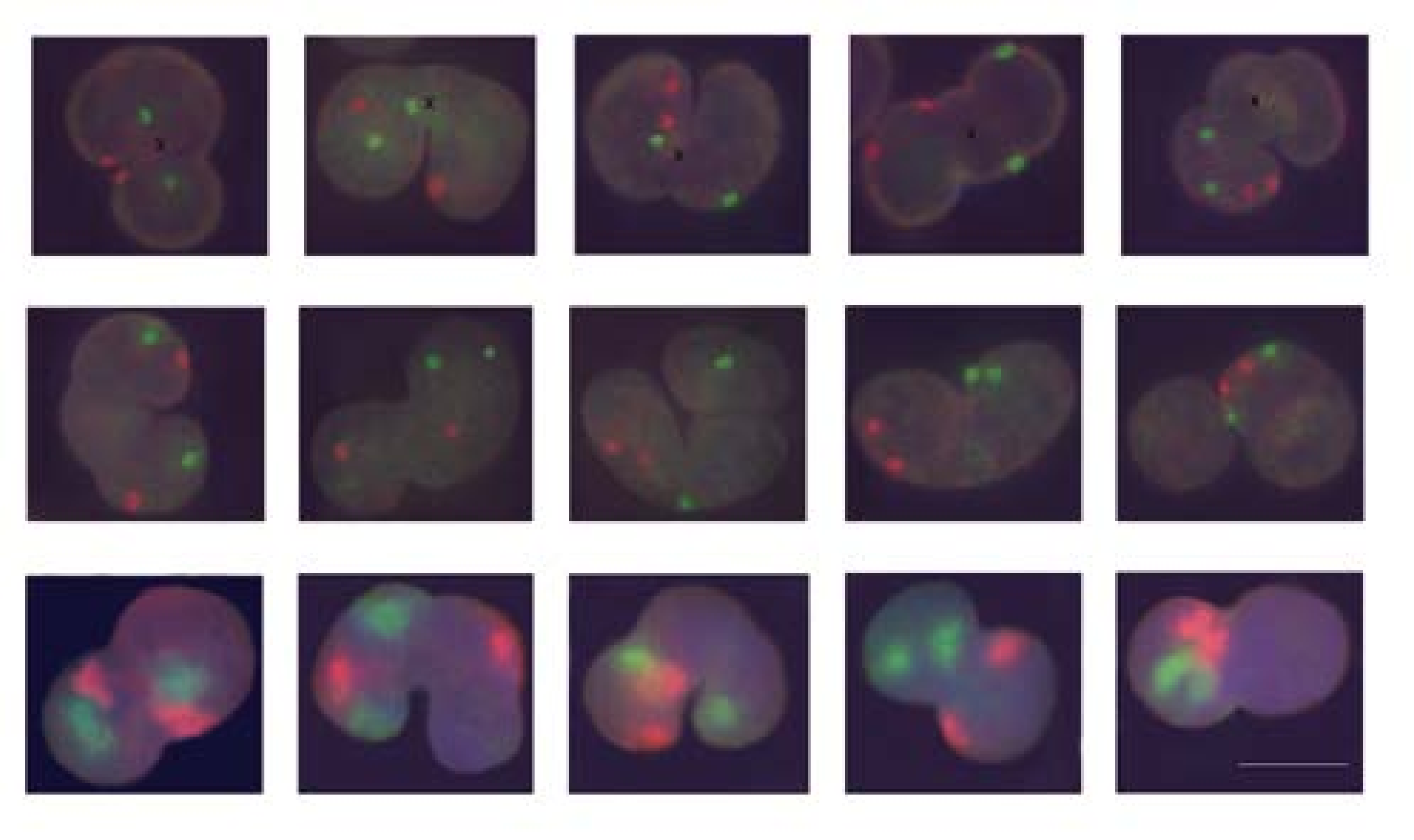


Fig. 1. Overview of blood cells, their origins and developmental stages

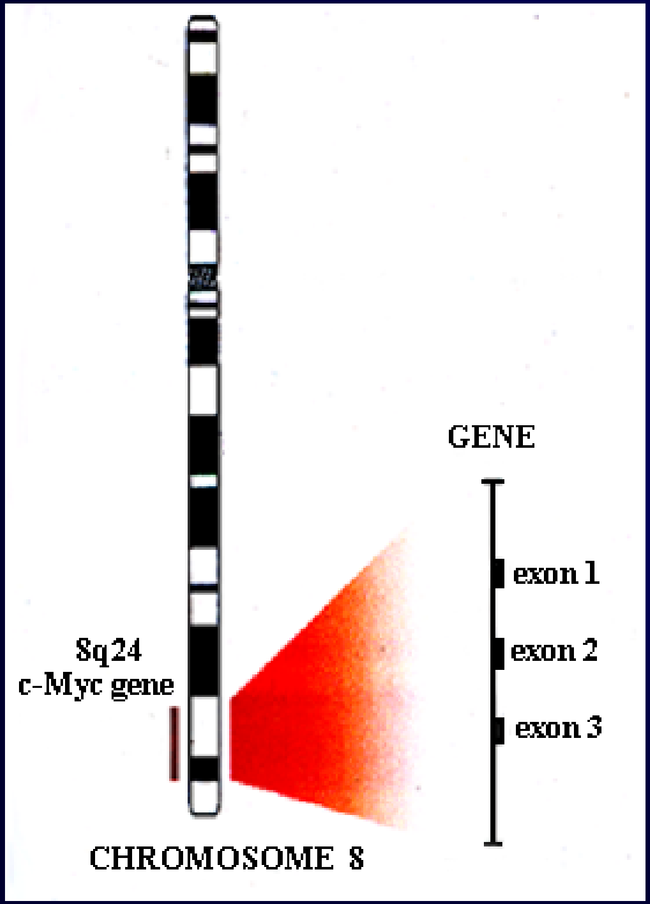
Morphology of human leukemic promyelocytic cell line HL60 and neutrophilic granulocyte

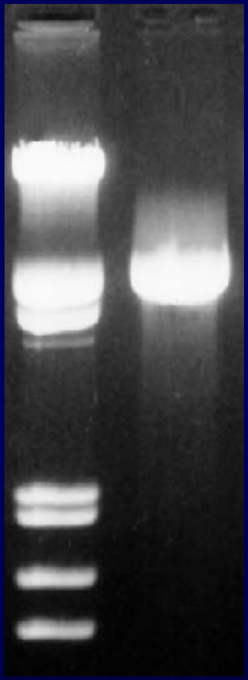


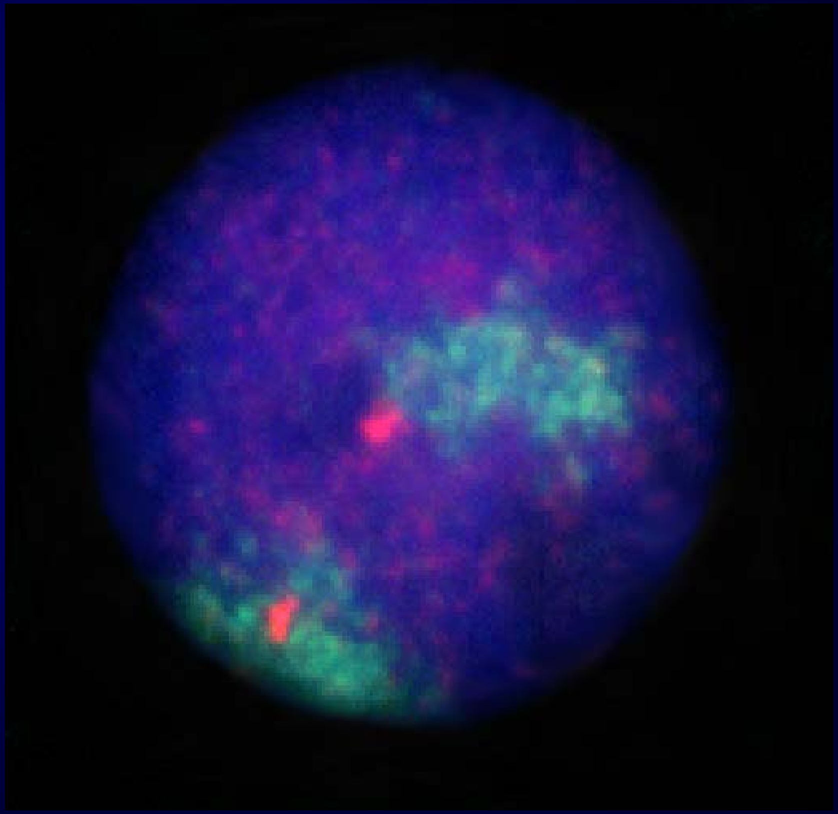
Topographic Types of Human Granulocytes



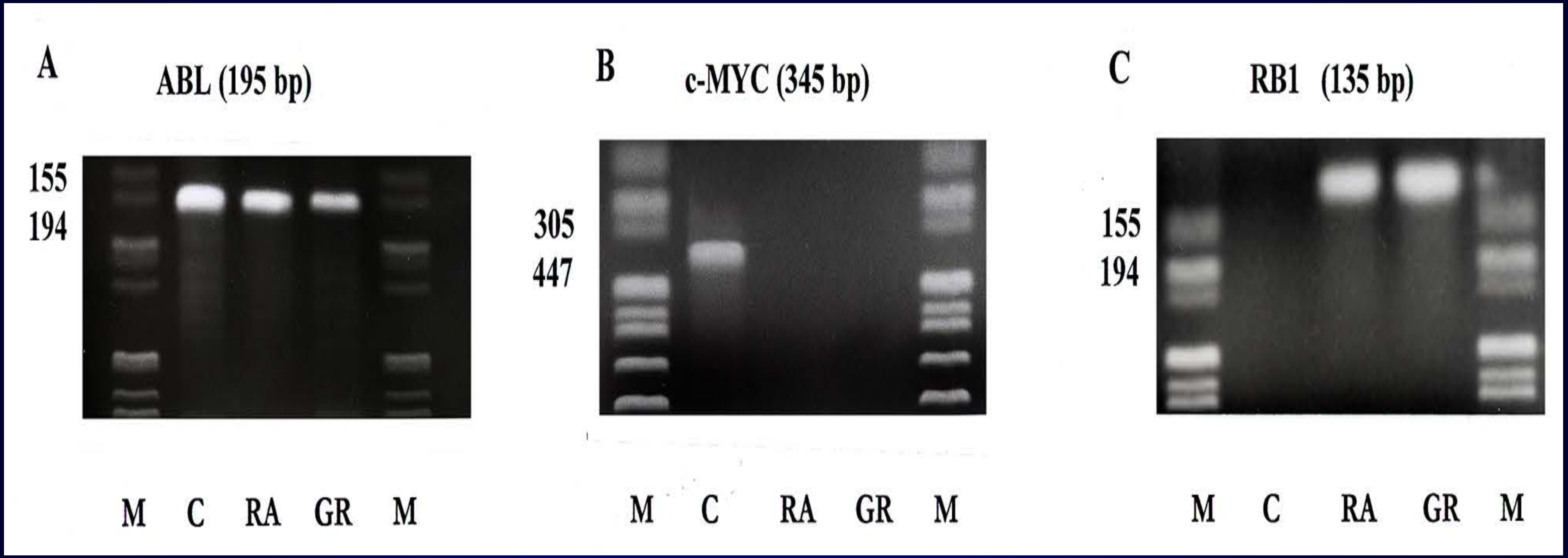
The C-myc Gene Nuclear Location



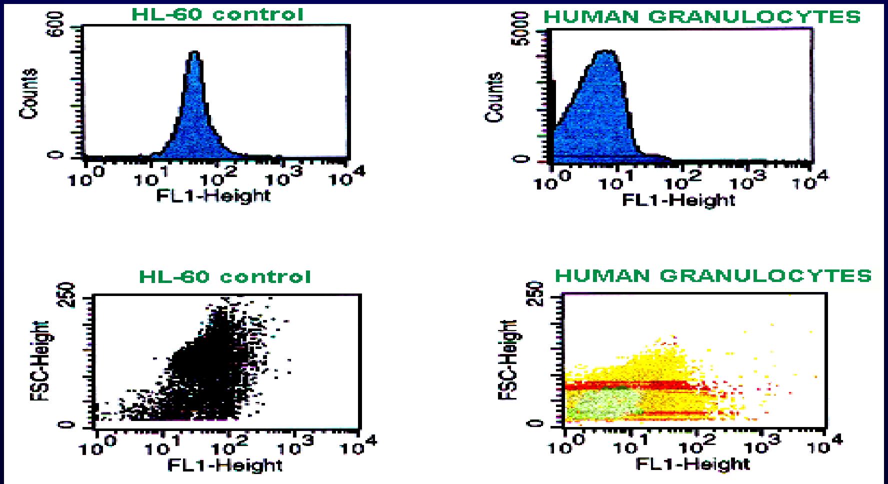




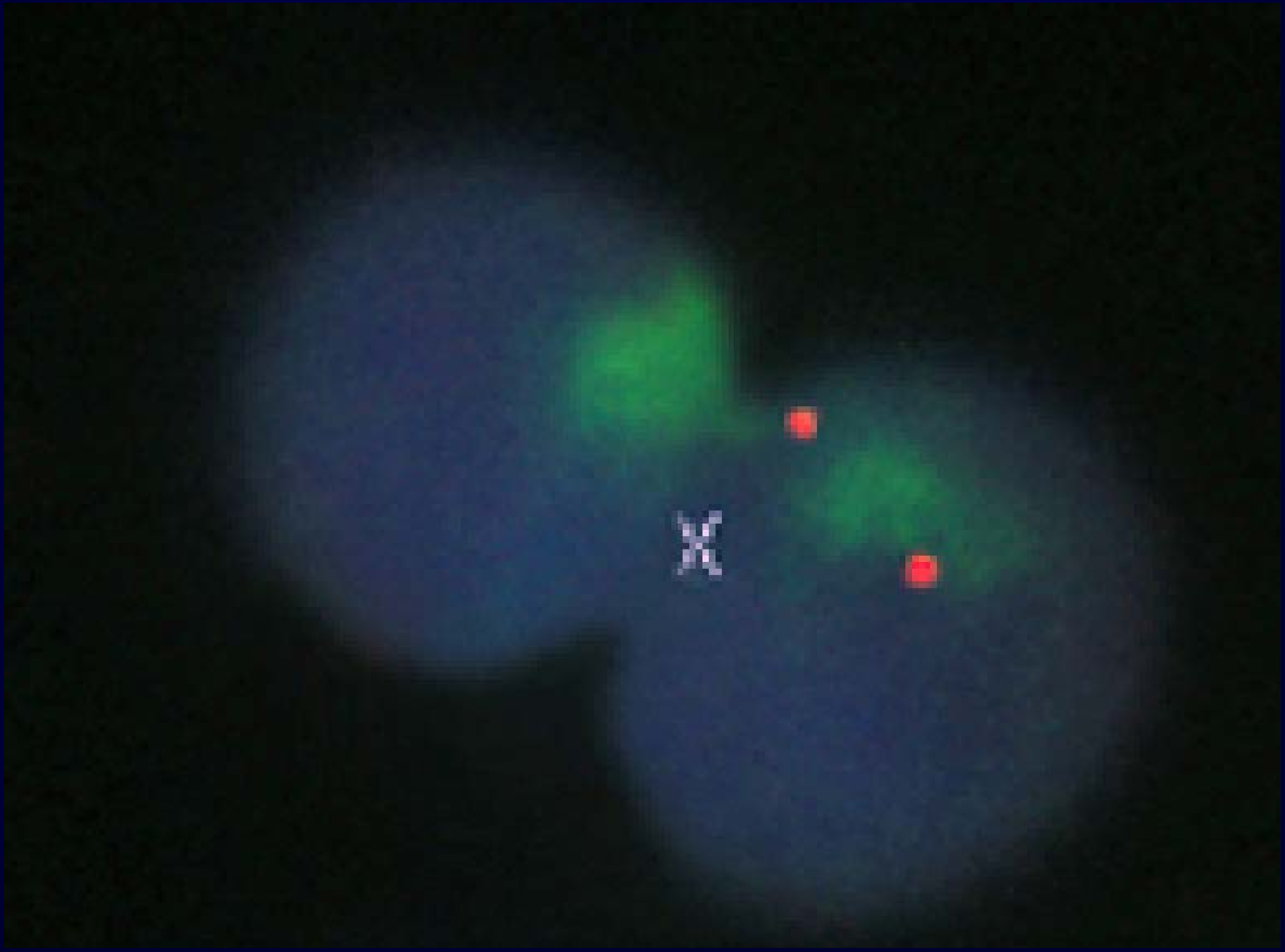
Changes in the expression of selected genes



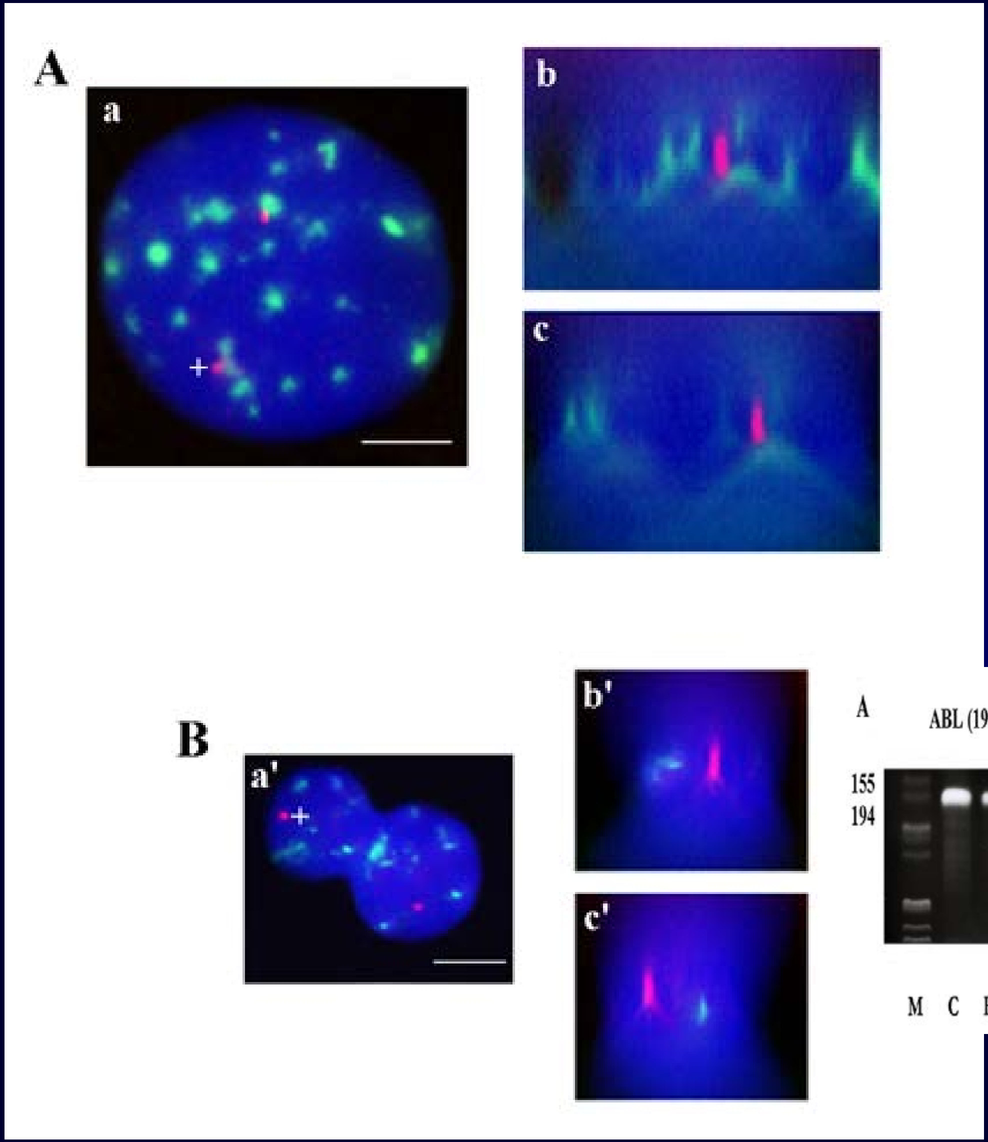
FCM
c-myc

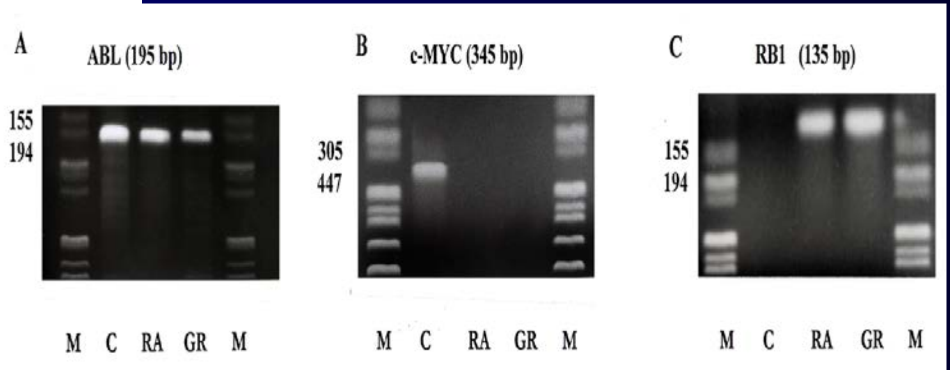


The C-myc gene nuclear topography in granulocytic nuclei



Centromeric silencing and Rb1 gene





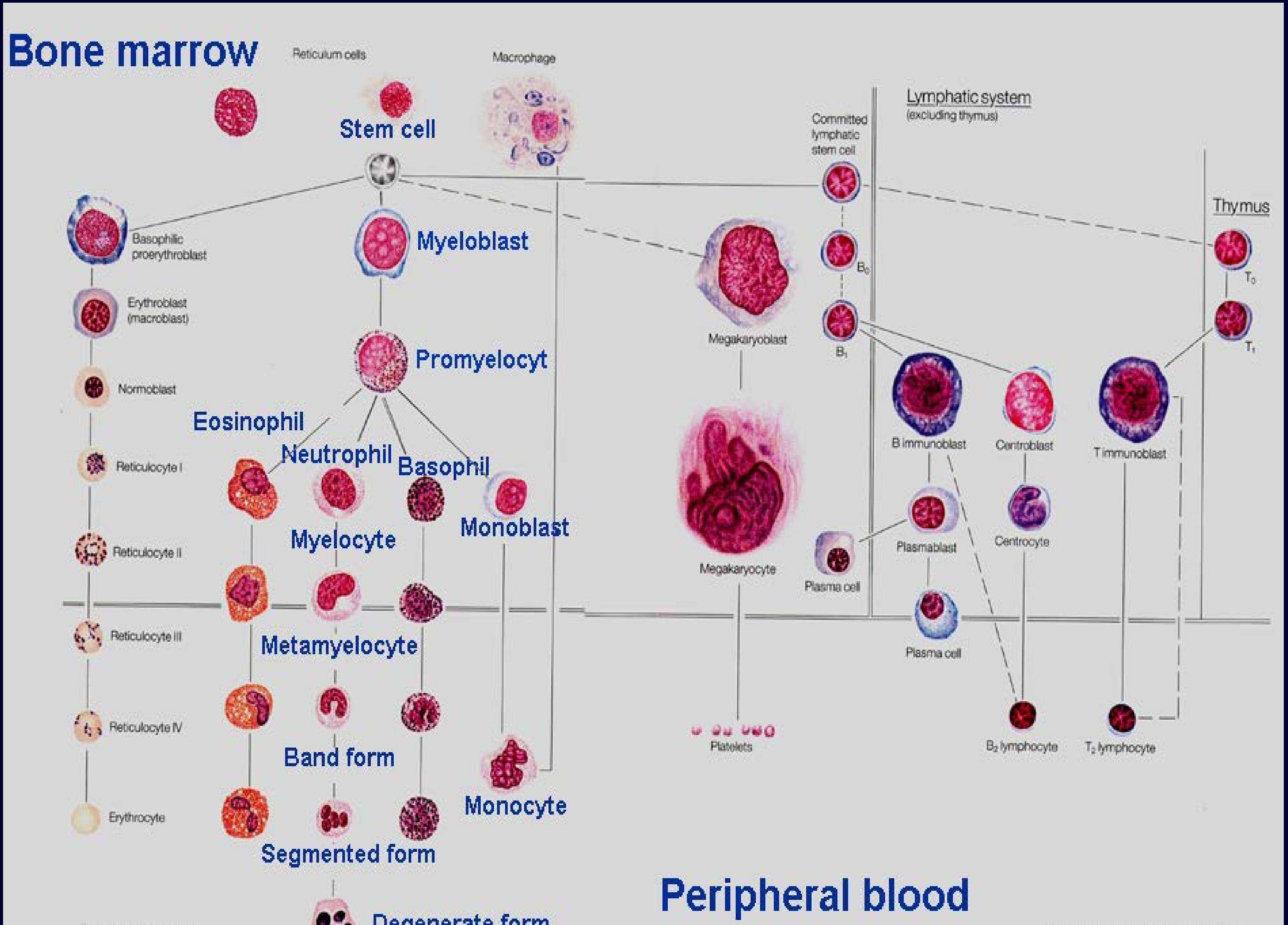
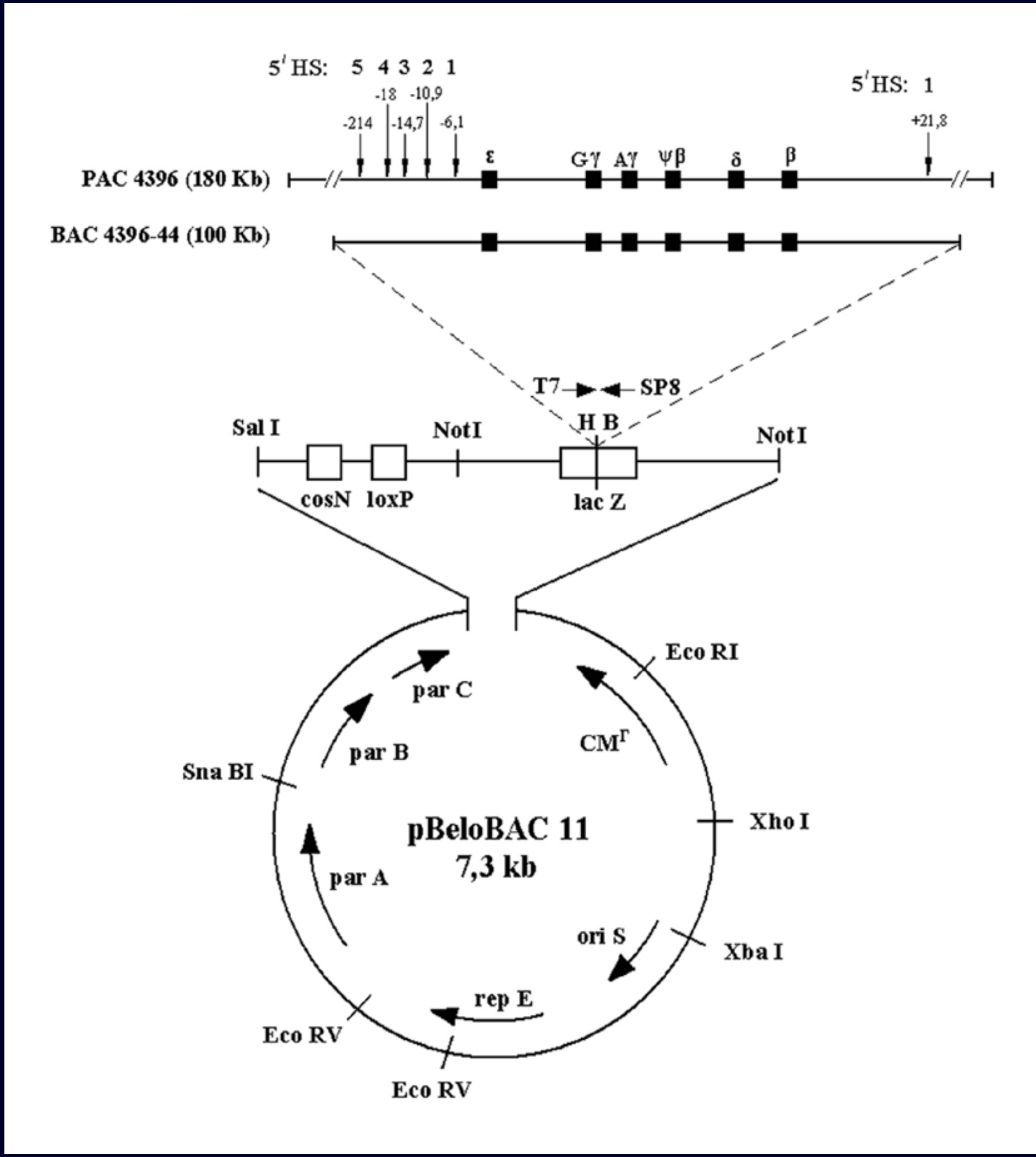


Fig. 1. Overview of blood cells, their origins and developmental stages

Beta-like globin gene cluster



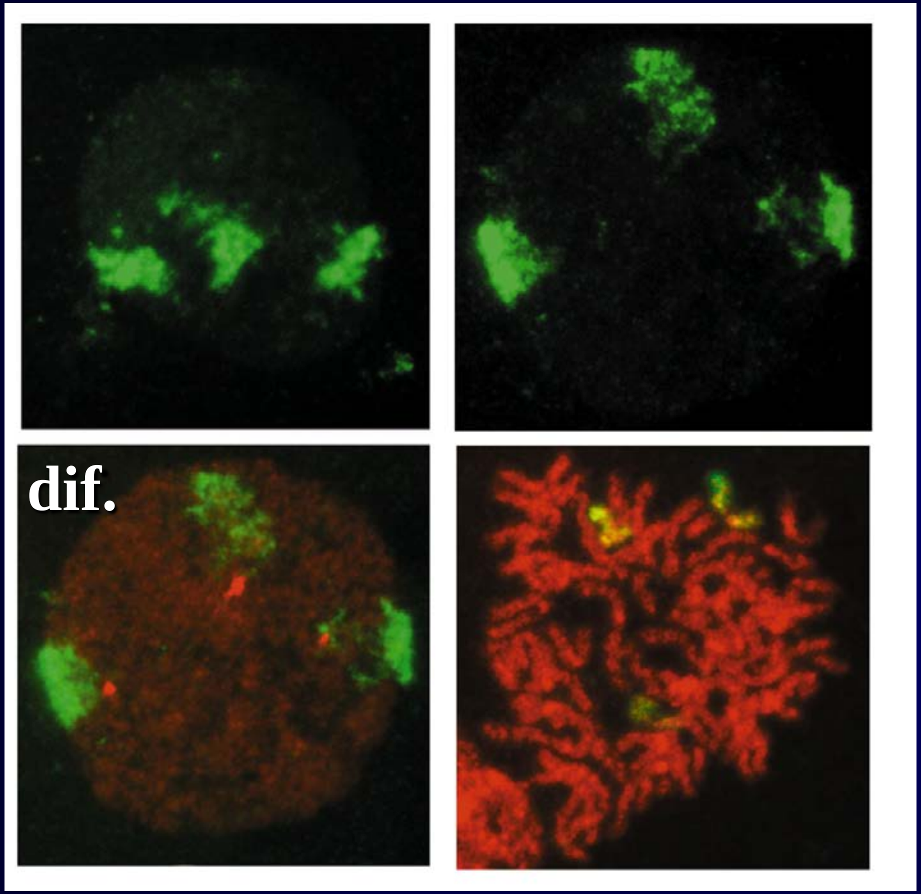
Arrayed on chromosome 11, encodes one embryonic (ε) and two fetal (Gγ, Aγ) and two adult (δ, β) globin chains. Expression of β-like genes undergoes a developmental related switching mechanism:

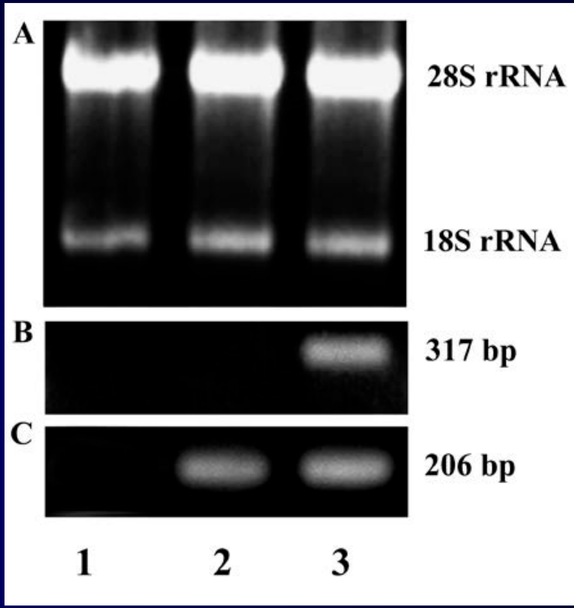
ε: expressed in early embryo fetal γ: fetal life.

δ, β: adulthood.

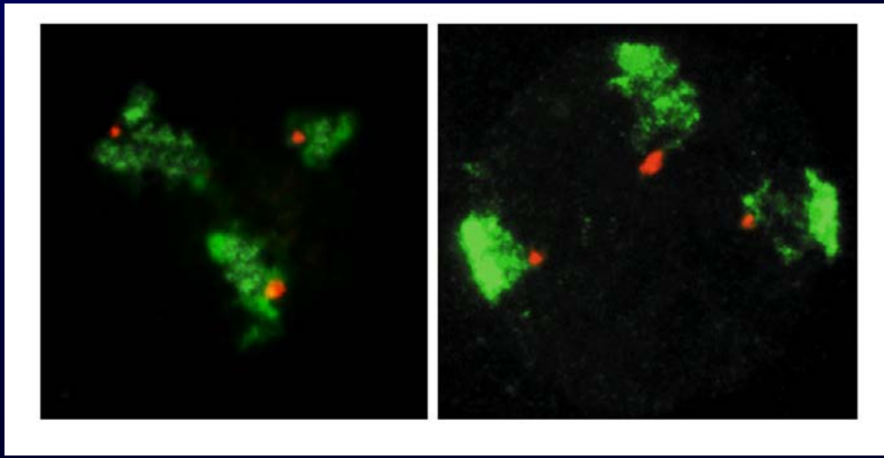
Changes in β-like gene expression acompany erythoid cell differentiation

Differentiation of human hemopoietic cells into erythroid pathway

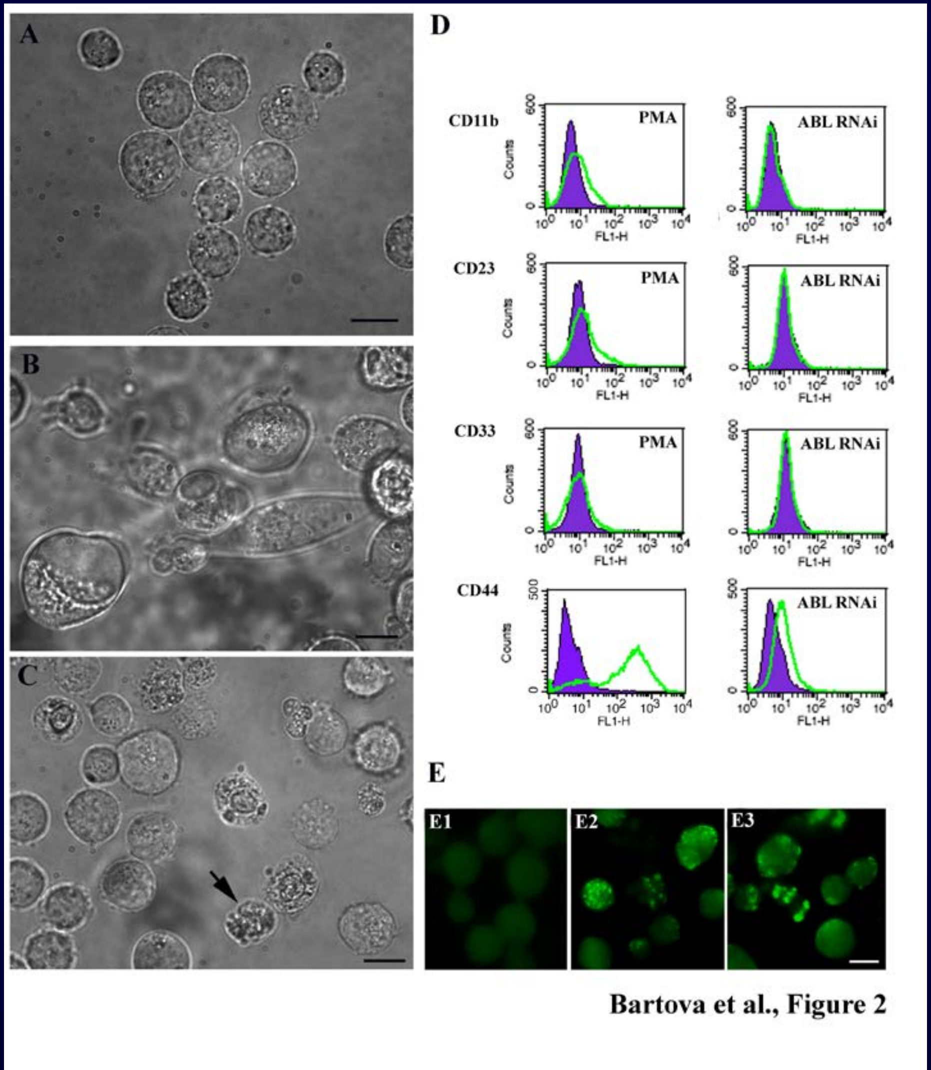




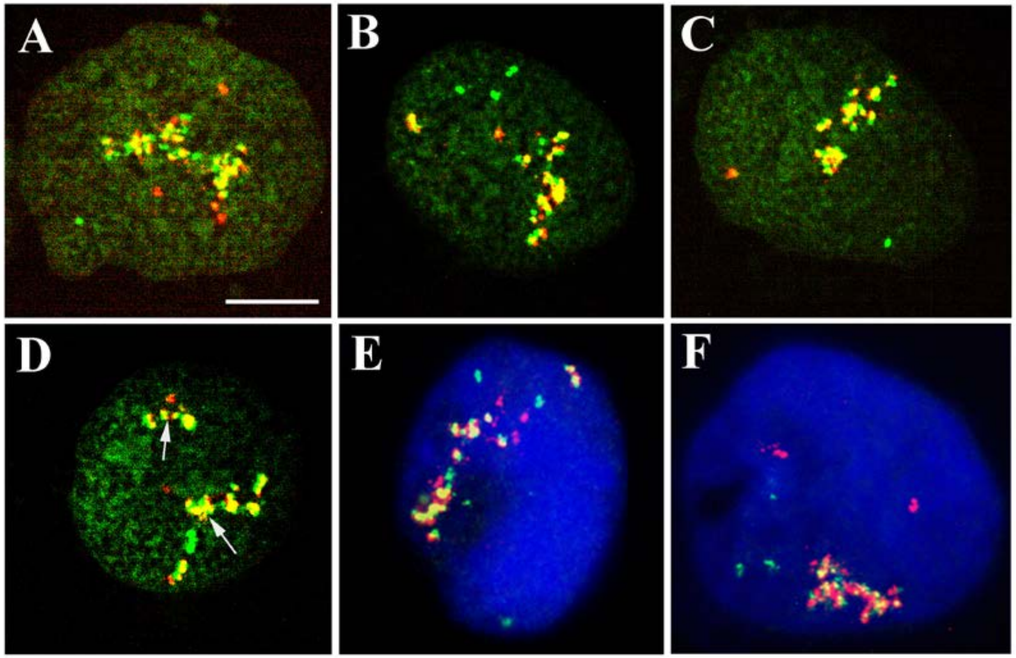
Non-dif. Dif.



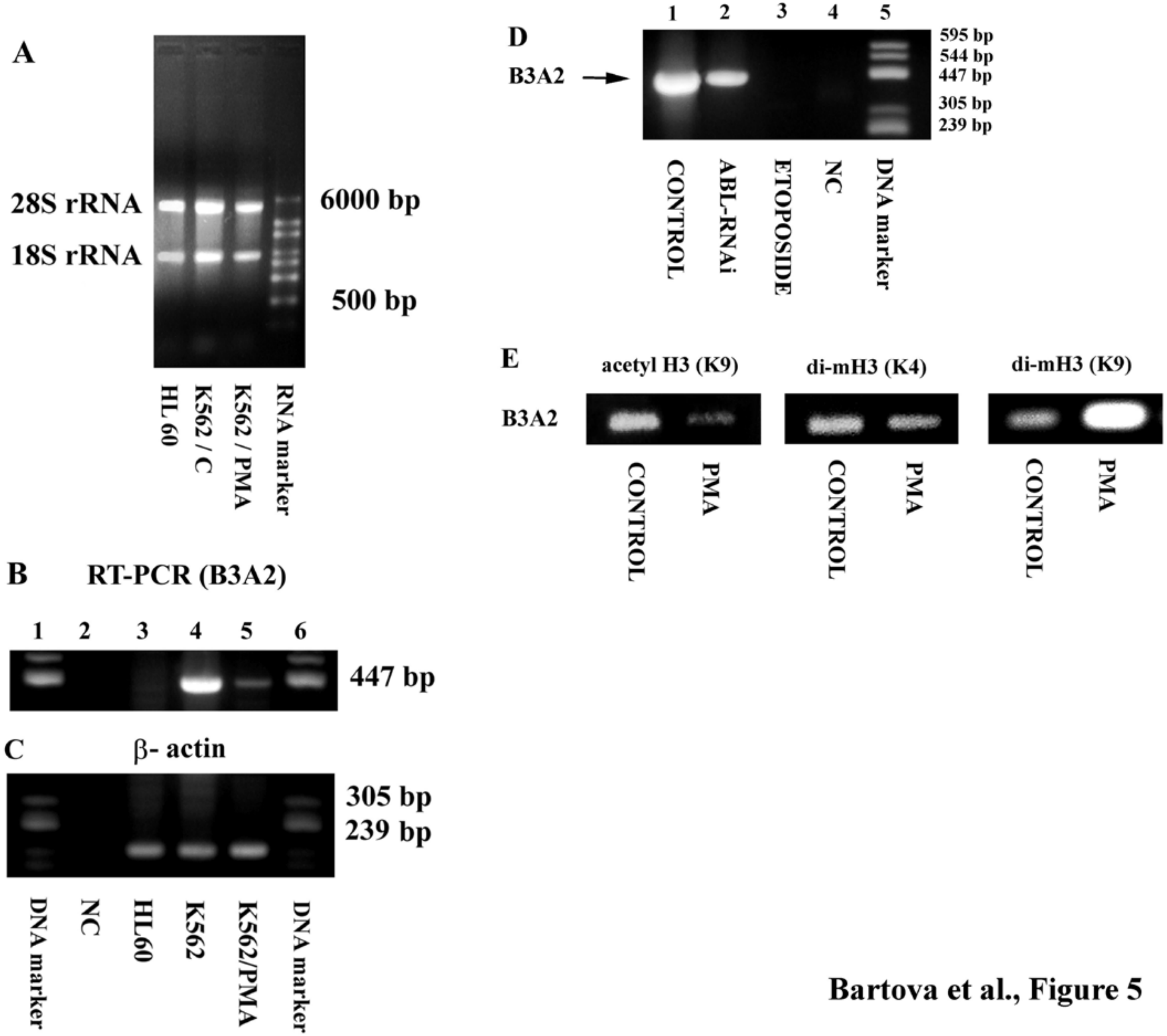
Differentiation of human hemopoietic cells into megakaryocytes



BCR (red signals) and ABL genes (green signals)

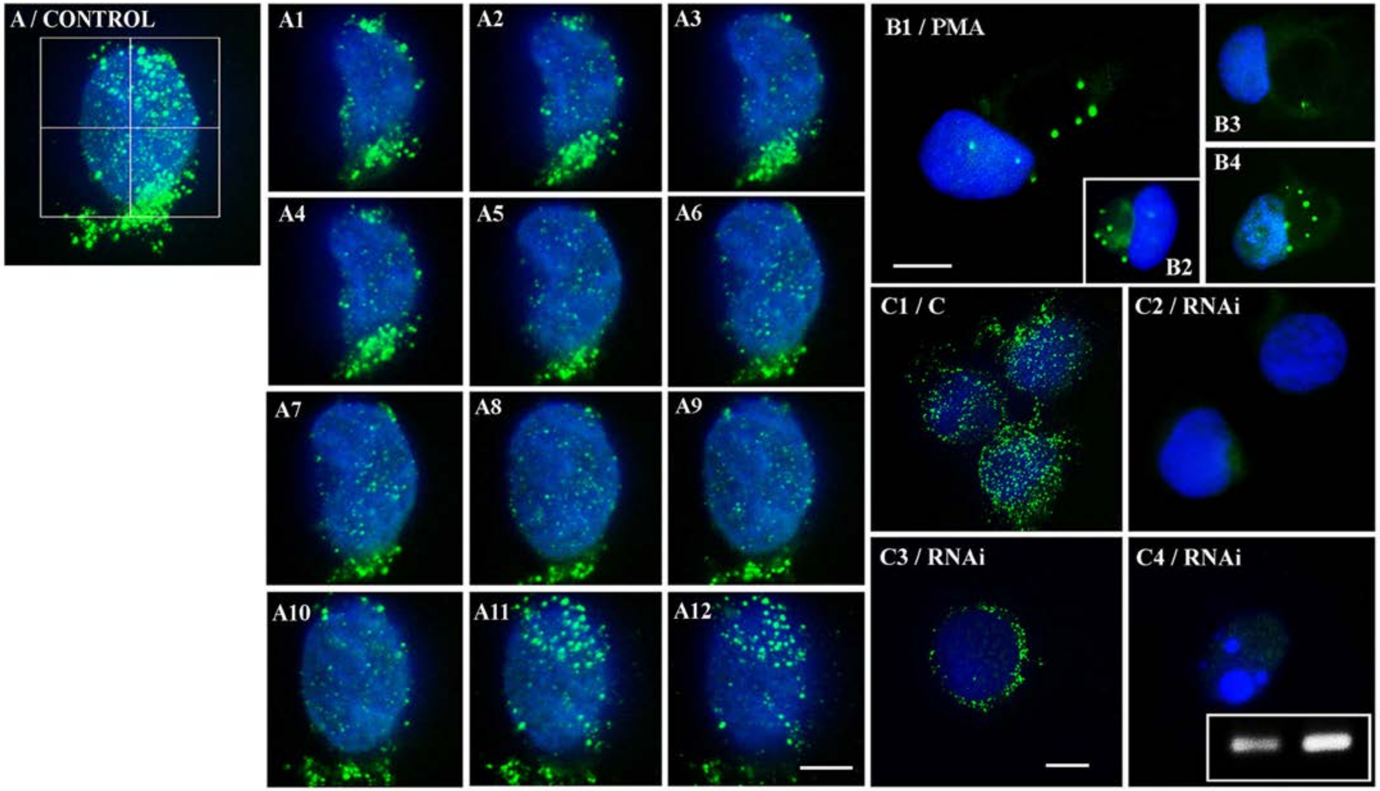


Bartova et al., Figure 3



Bartova et al., Figure 5

Abl protein



Bartova et al., Figure 6

ZÁVĚR

Diferenciace je charakteristická nejenom specifickými změnami na úrovni morfologie buněk, ale významně se mění i struktura chromatinu. Tyto změny v genomu mají velký význam z hlediska aktivity genů. Všechny uvedené faktory určují vznik specifického buněčného typu.

http://www.youtube.com/watch?v=fGNchPdlaGU