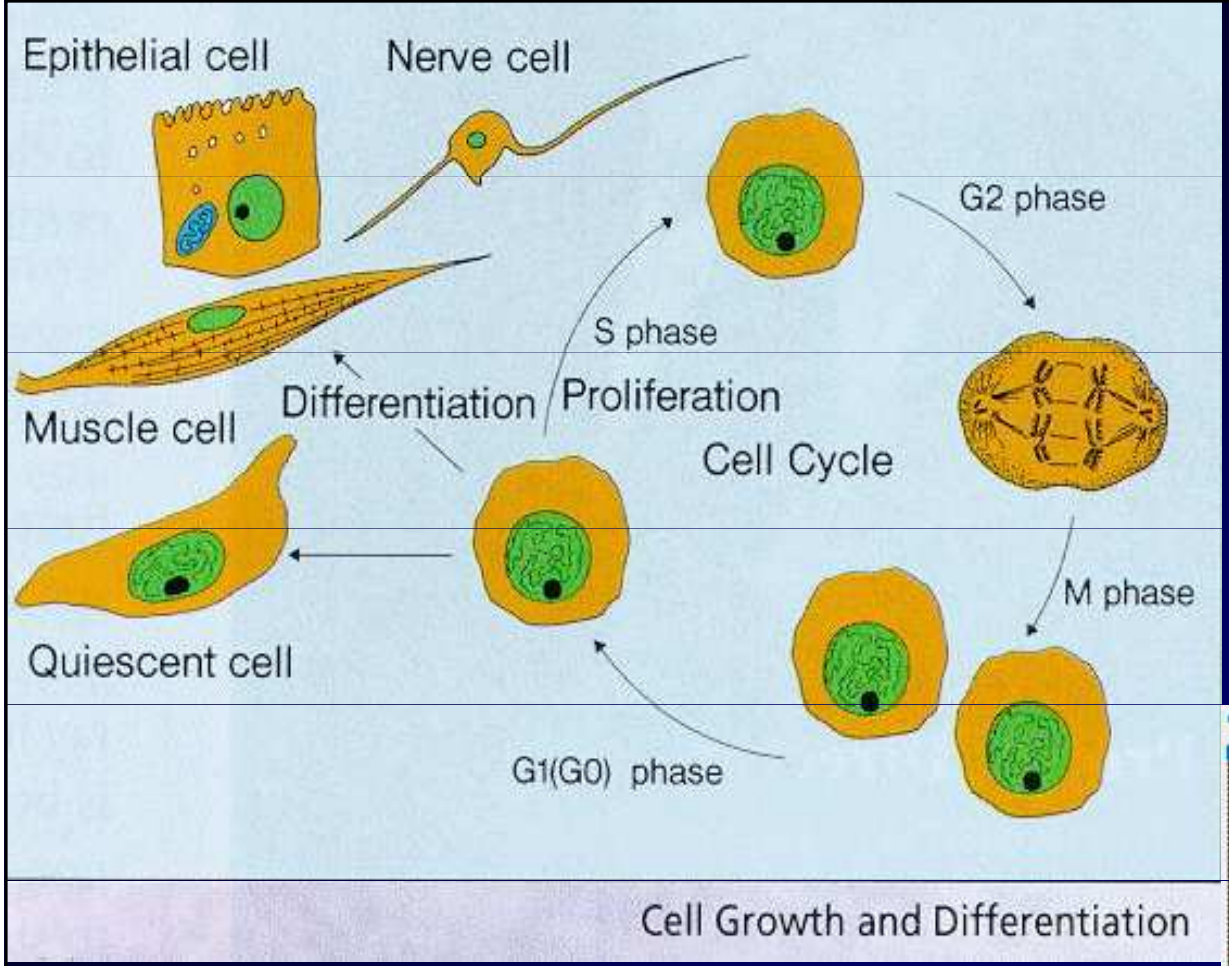
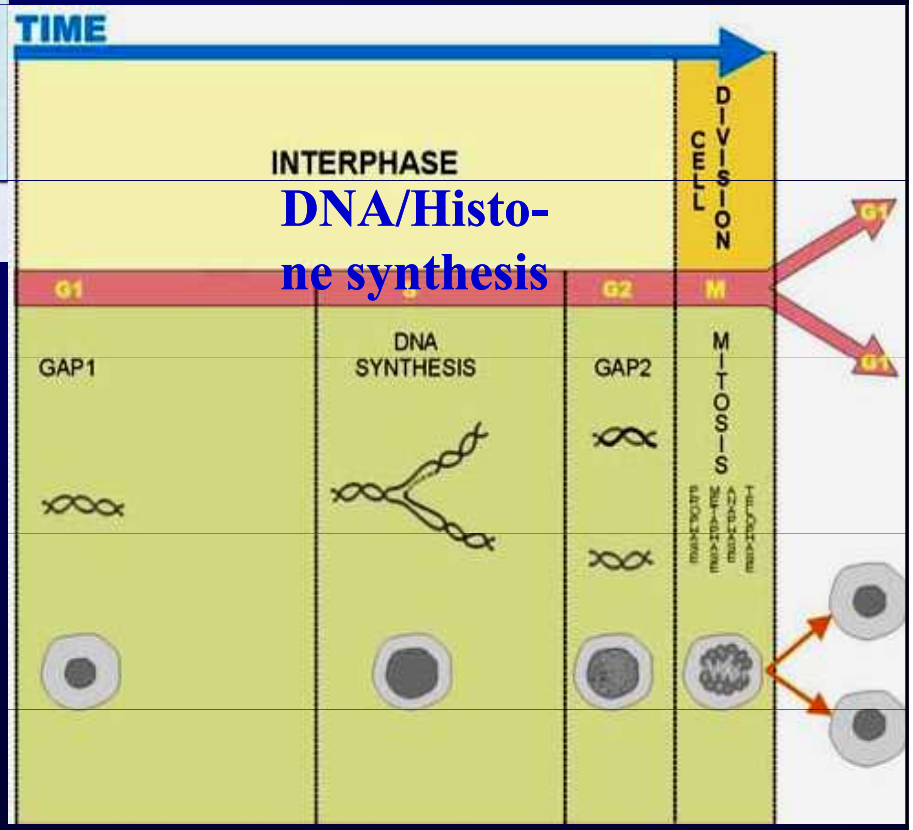
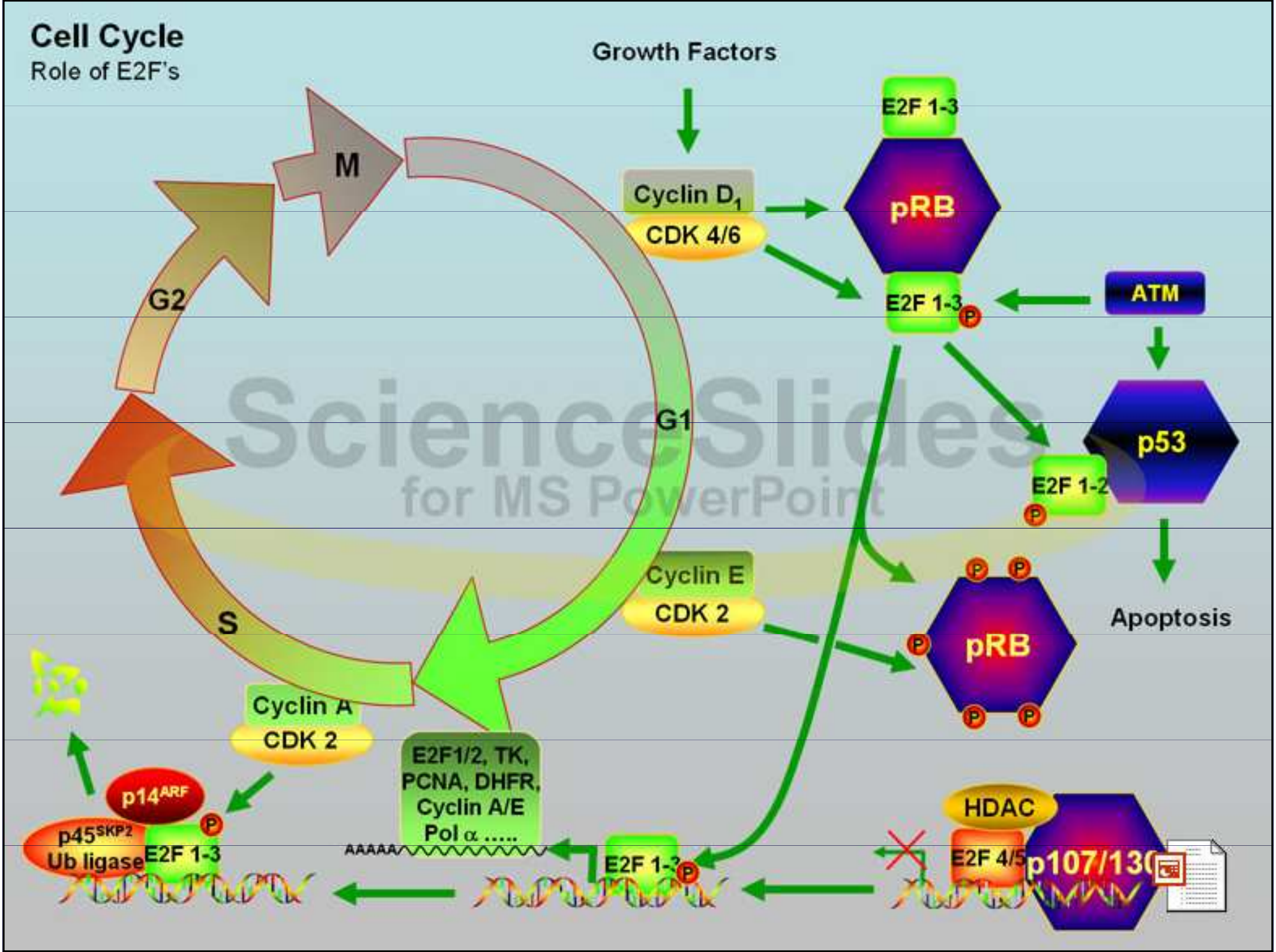
Buněčná diferenciace a struktura chromatinu

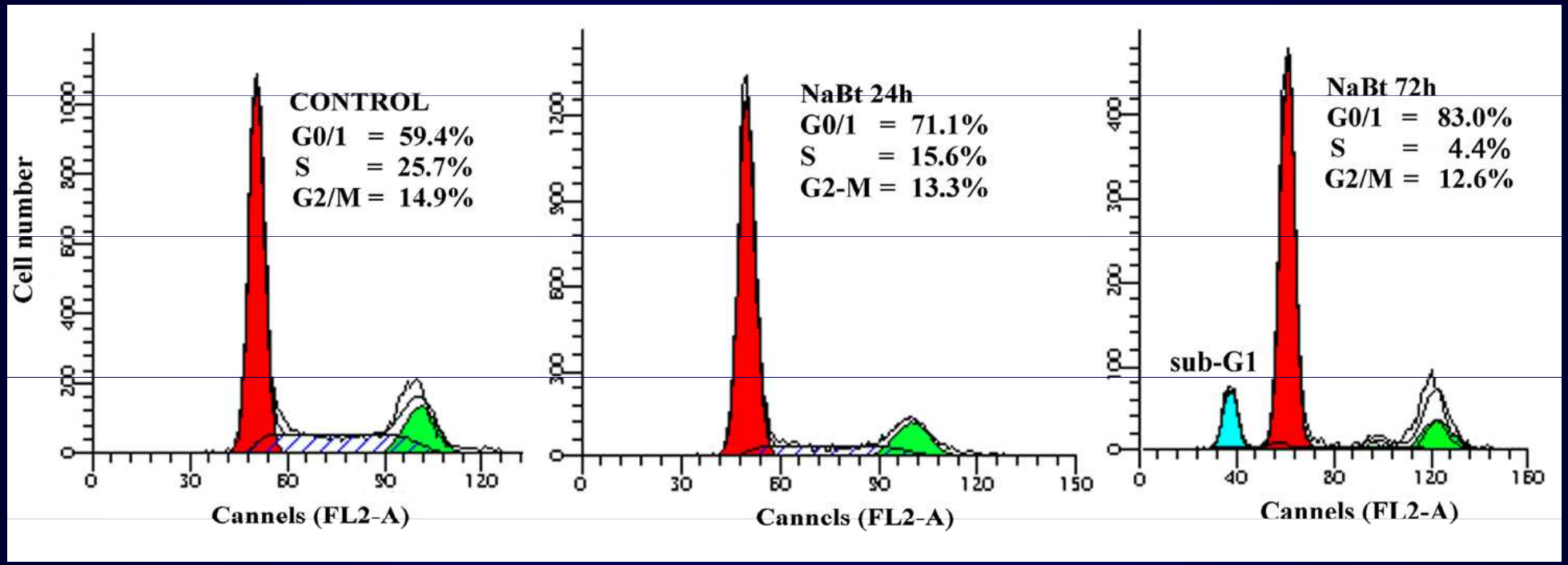
Buněčná diferenciace je proces při kterém buňky získávají nový fenotyp, který je spojen se specifickou buněčnou funkcí. Pro daný buněčný typ je charakteristická aktivace skupiny genů, které jsou zodpovědné za terminální diferenciaci.

Cell Differentiation
Cell Growth

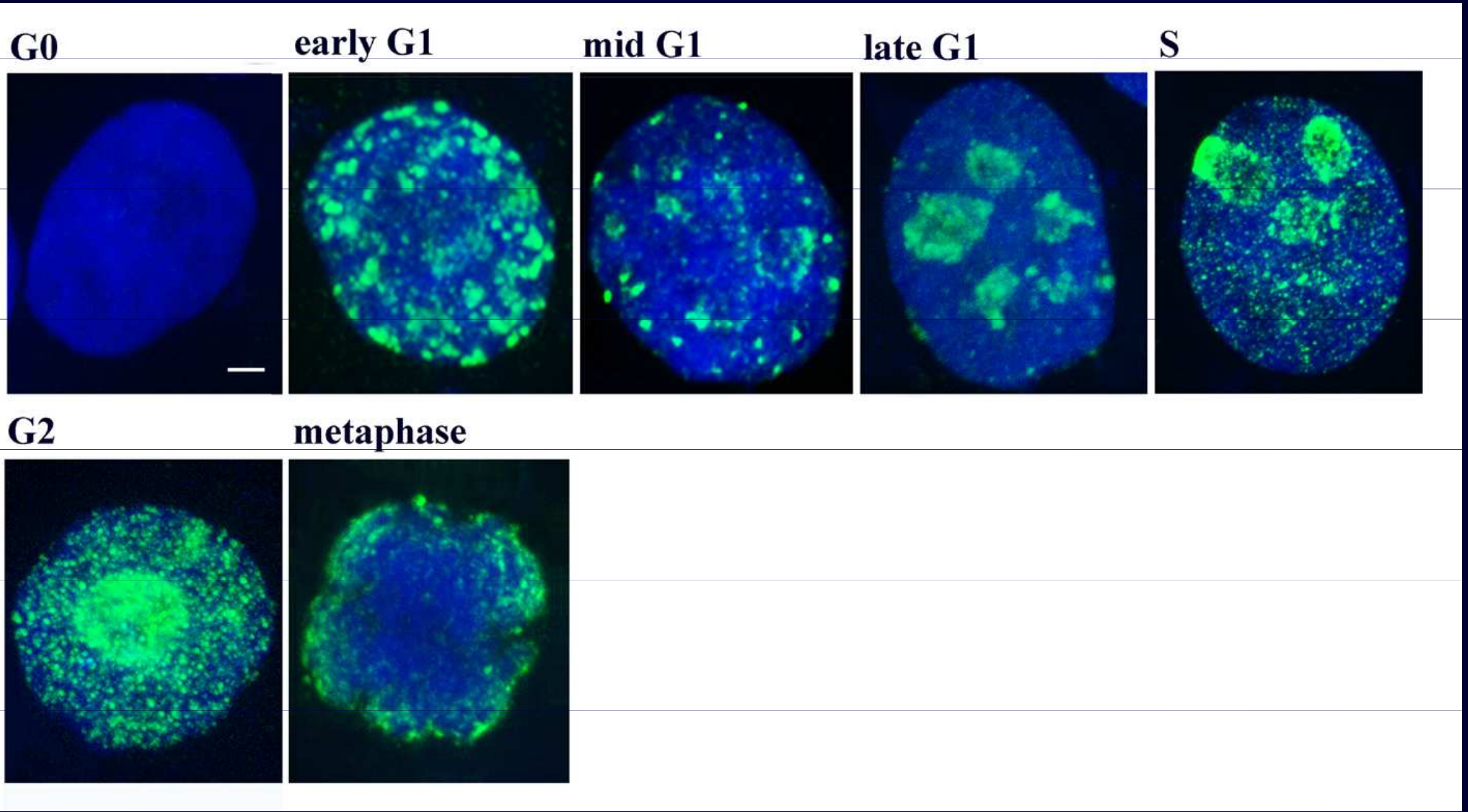





Cell Cycle
Role of E2F's
Growth Factors
M
G2
G1
S
Cyclin D1
CDK 4/6
E2F 1-3
pRB
E2F 1-3
P
ATM
p53
E2F 1-2
P
Apoptosis
Cyclin E
CDK 2
pRB
Cyclin A
CDK 2
E2F1/2, TK,
PCNA, DHFR,
Cyclin A/E
Pol α .....
AAAAA
p14ARF
p45SKP2
Ub ligase
E2F 1-3
E2F 1-3
HDAC
E2F 4/5
p107/130
ScienceSlides
for MS PowerPoint

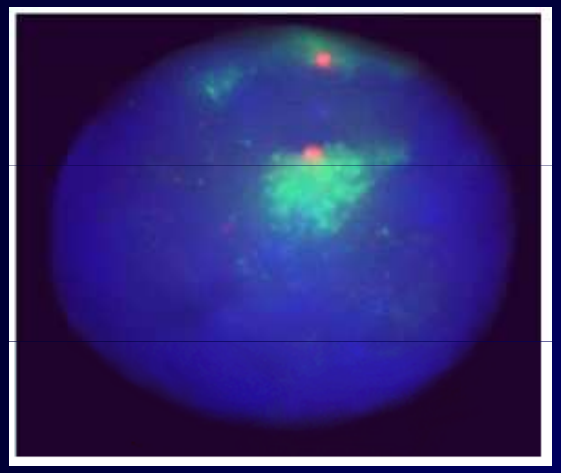

Cell number
CONTROL
G0/1 = 59.4%
S = 25.7%
G2/M = 14.9%
Cannels (FL2-A)
NaBt 24h
G0/1 = 71.1%
S = 15.6%
G2-M = 13.3%
Cannels (FL2-A)
NaBt 72h
G0/1 = 83.0%
S = 4.4%
G2/M = 12.6%
sub-G1
Cannels (FL2-A)

pKi-67



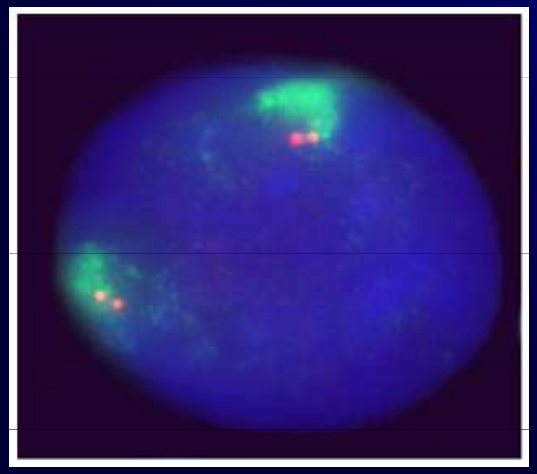
Andrea Harničarová

G1

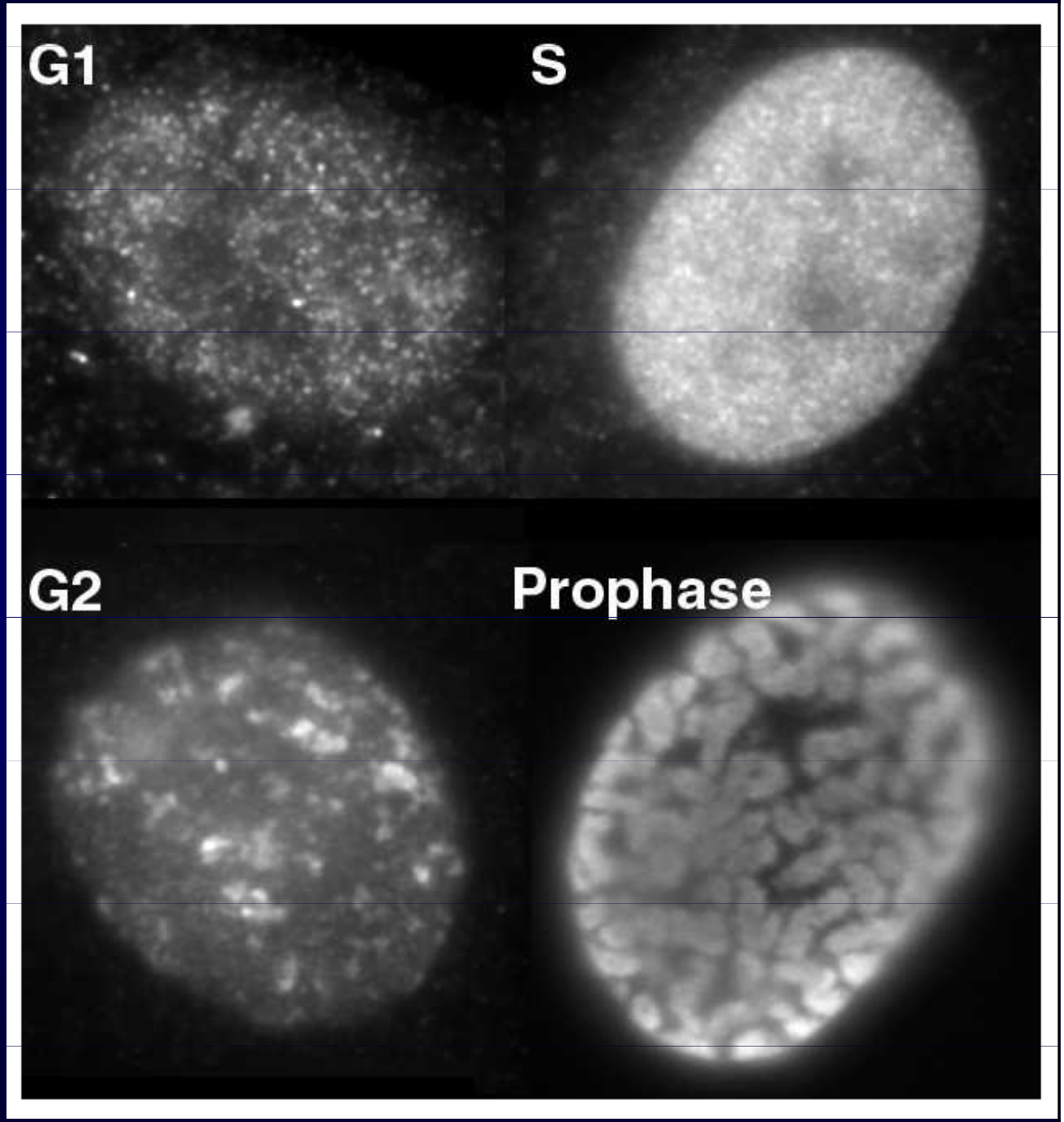


Rb1 gene

G2

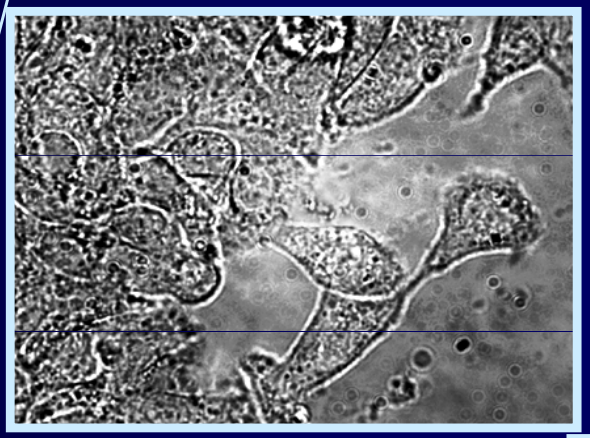


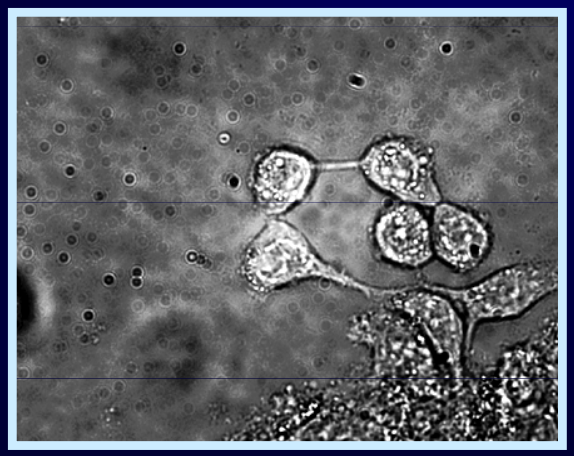
Anti-phospho H3



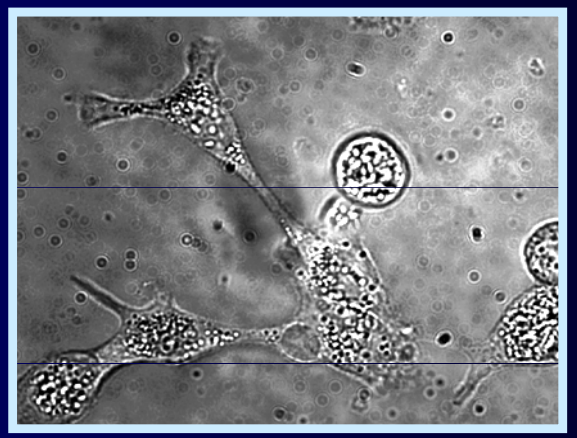
Enterocytic cell differentiation

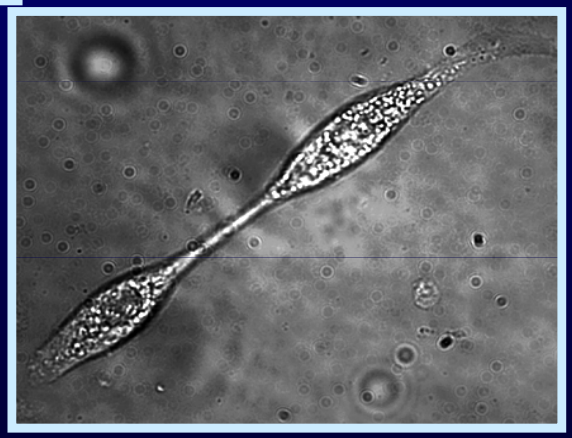
Control



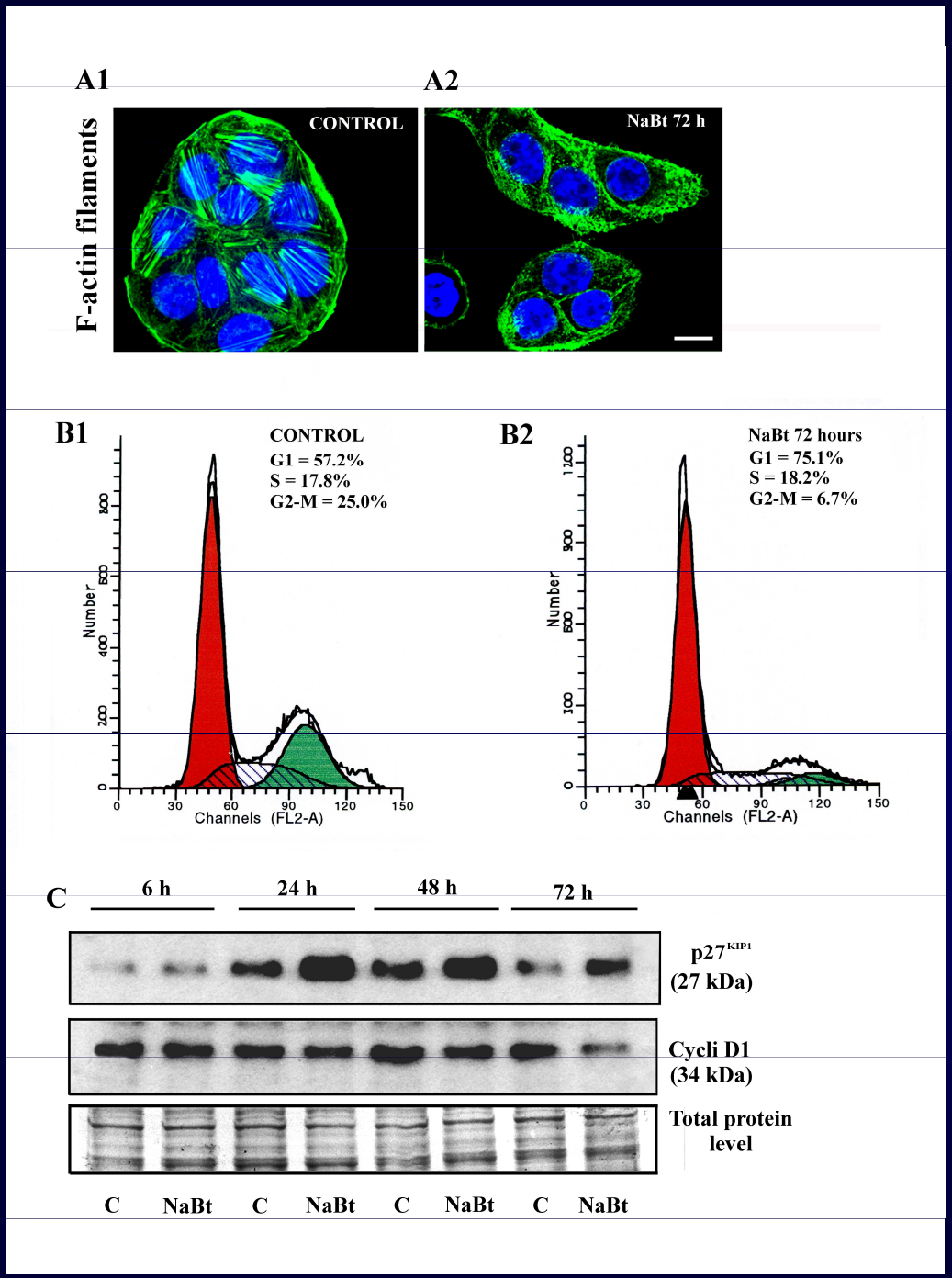


Sodium Butyrate



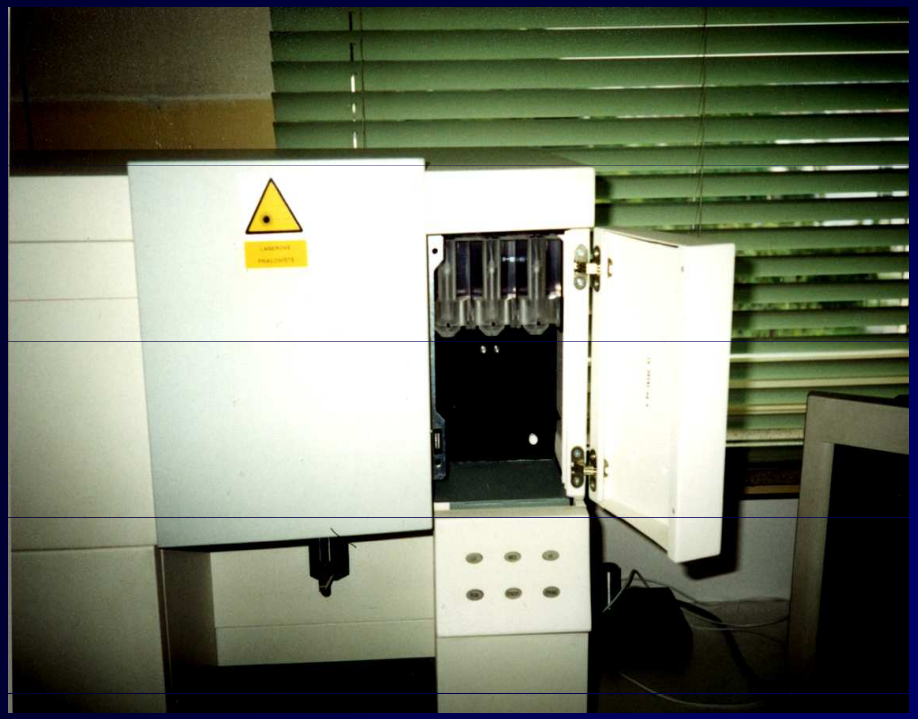


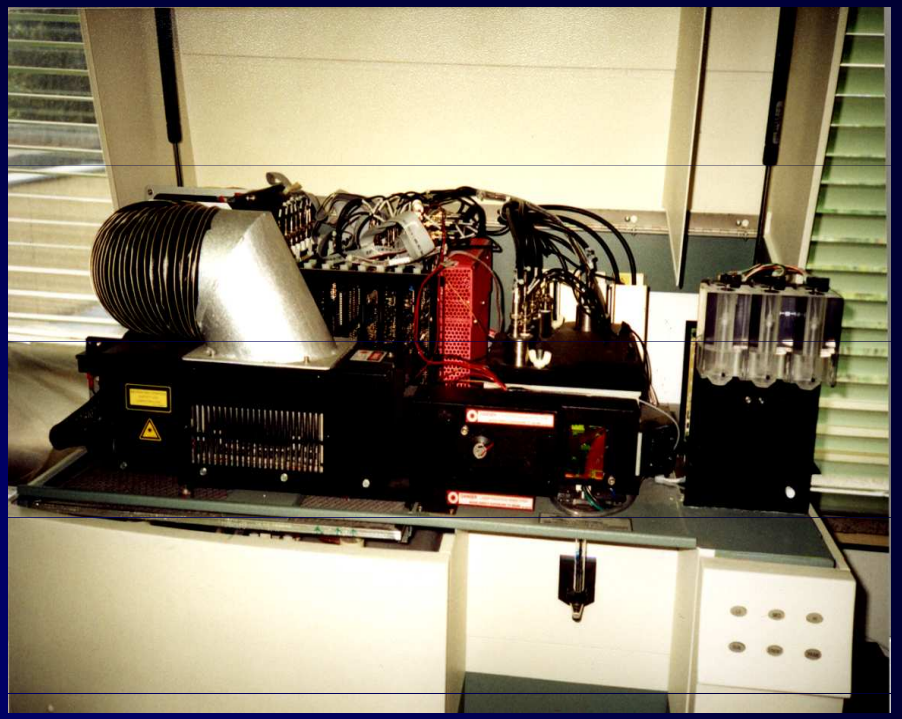
Enterocytic cell differentiation

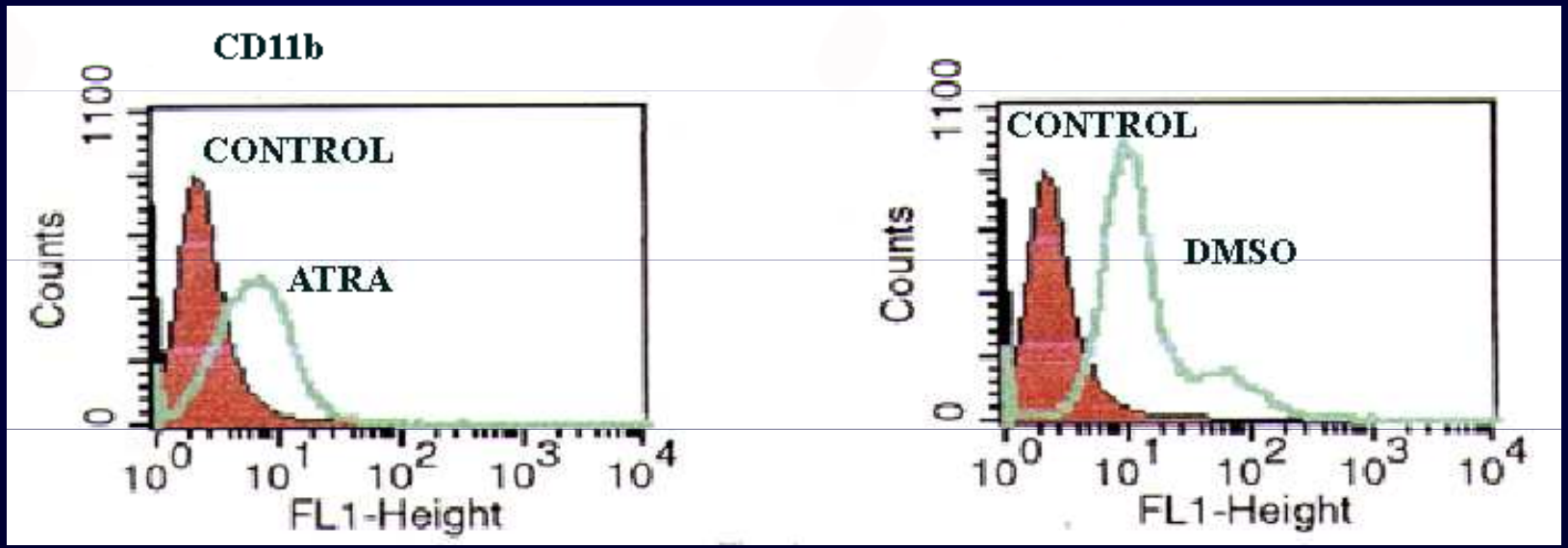


Harničarová et al., 2005

Flow Cytometry

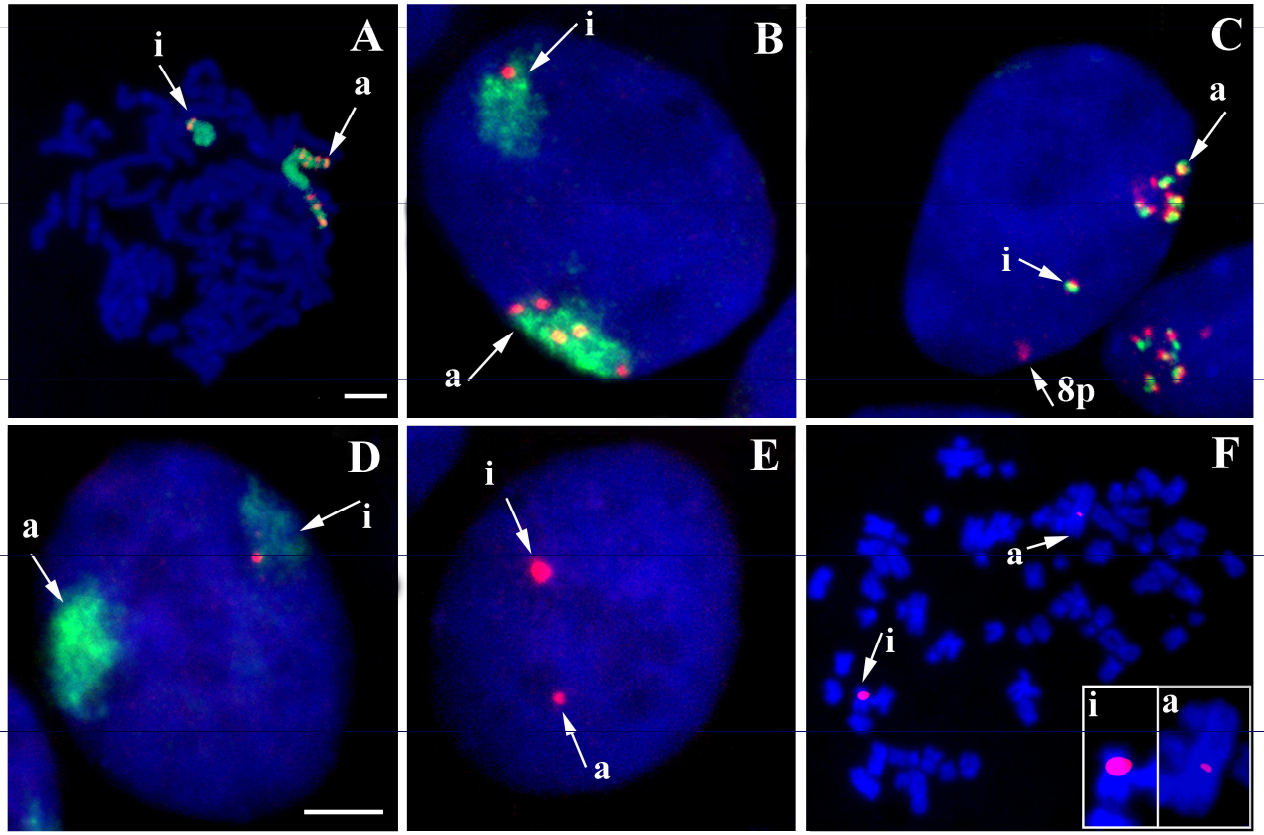


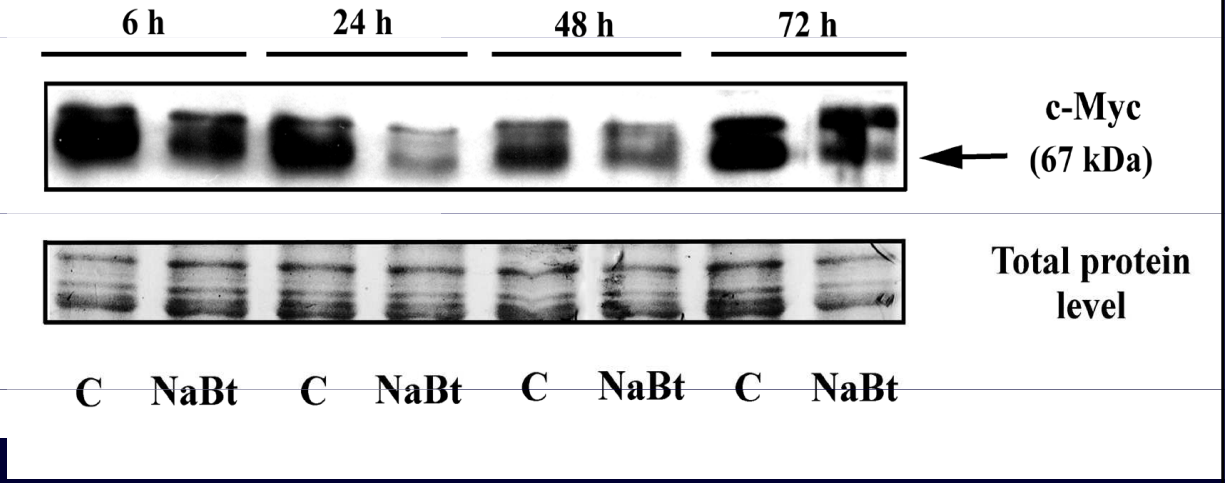




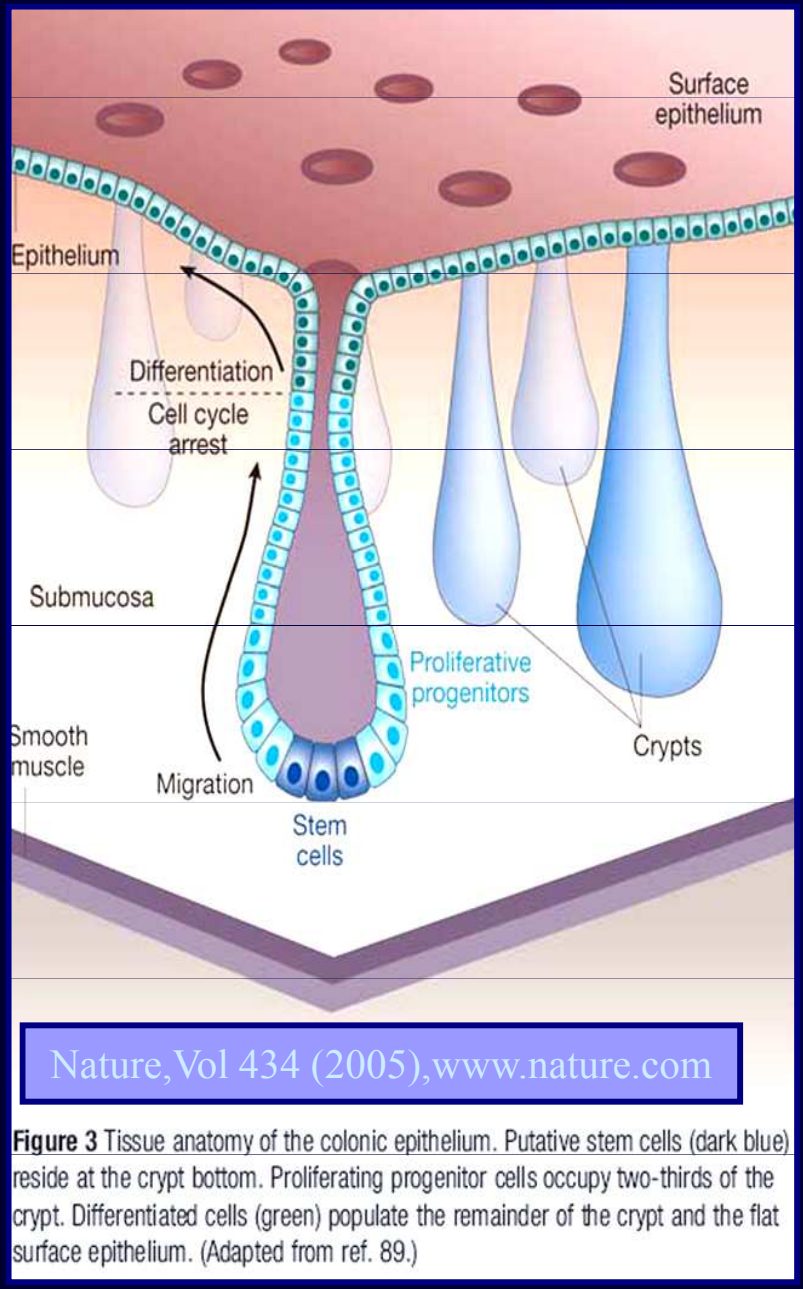
HSA 8 and related structures

Harničarová et al., 2006



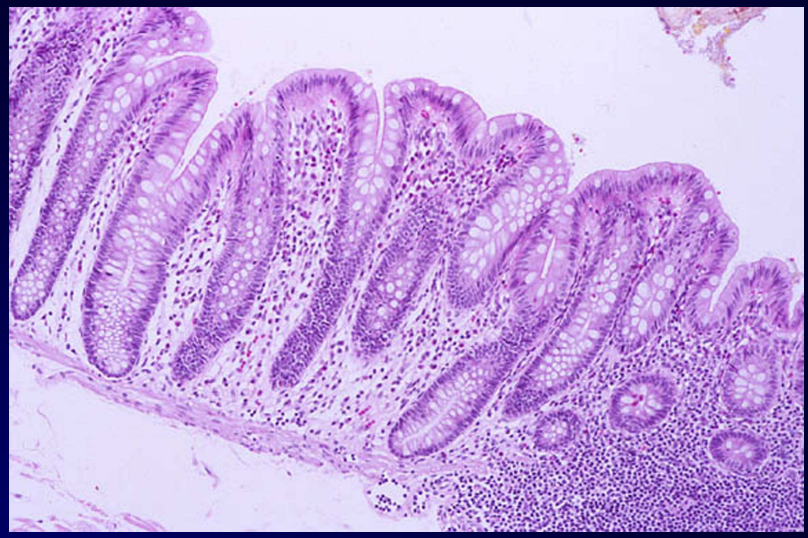


Enterocytic Cell Differentiation

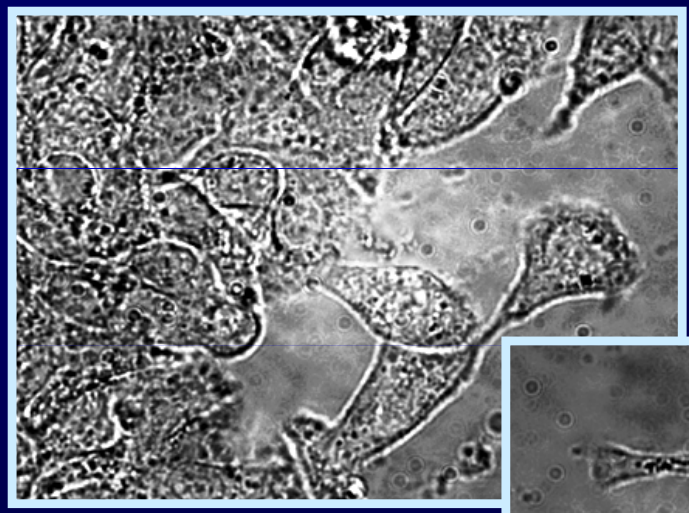


Nature, Vol 434 (2005), www.nature.com

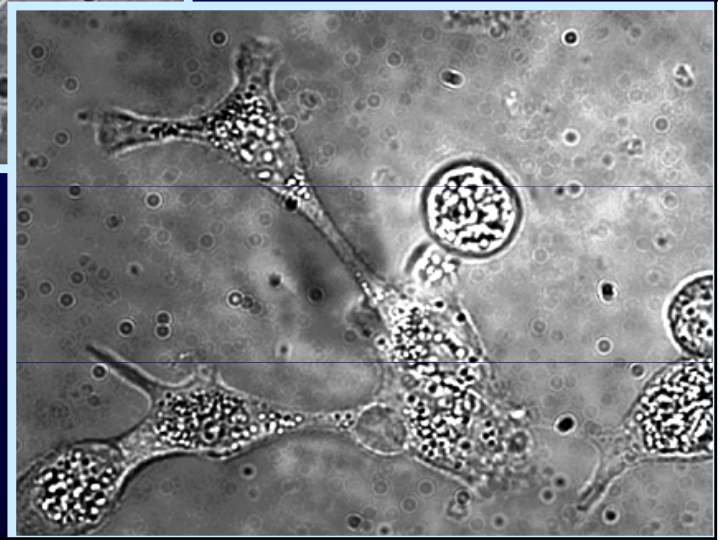
Figure 3 Tissue anatomy of the colonic epithelium. Putative stem cells (dark blue) reside at the crypt bottom. Proliferating progenitor cells occupy two-thirds of the crypt. Differentiated cells (green) populate the remainder of the crypt and the flat surface epithelium. (Adapted from ref. 89.)



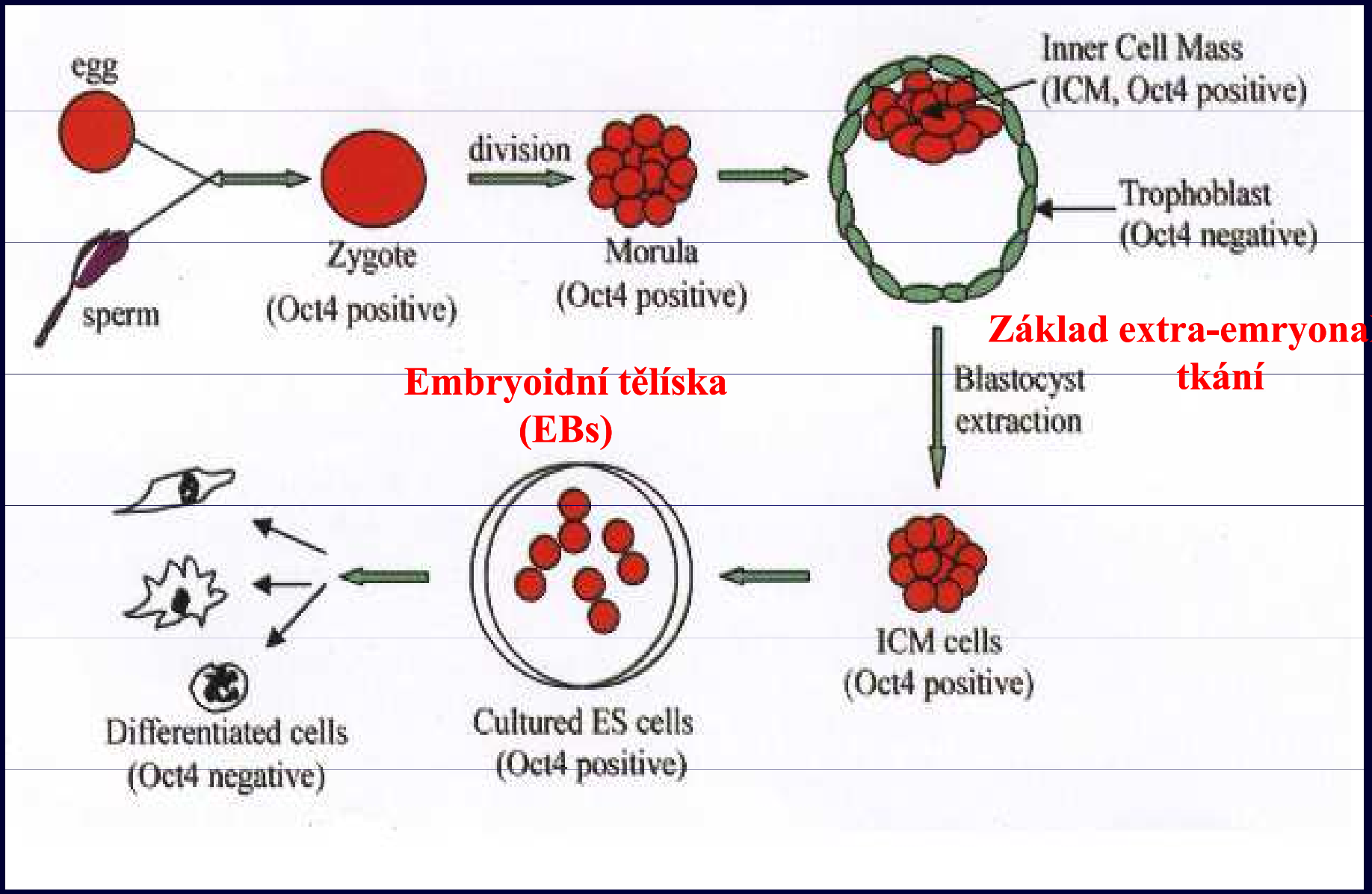
Control



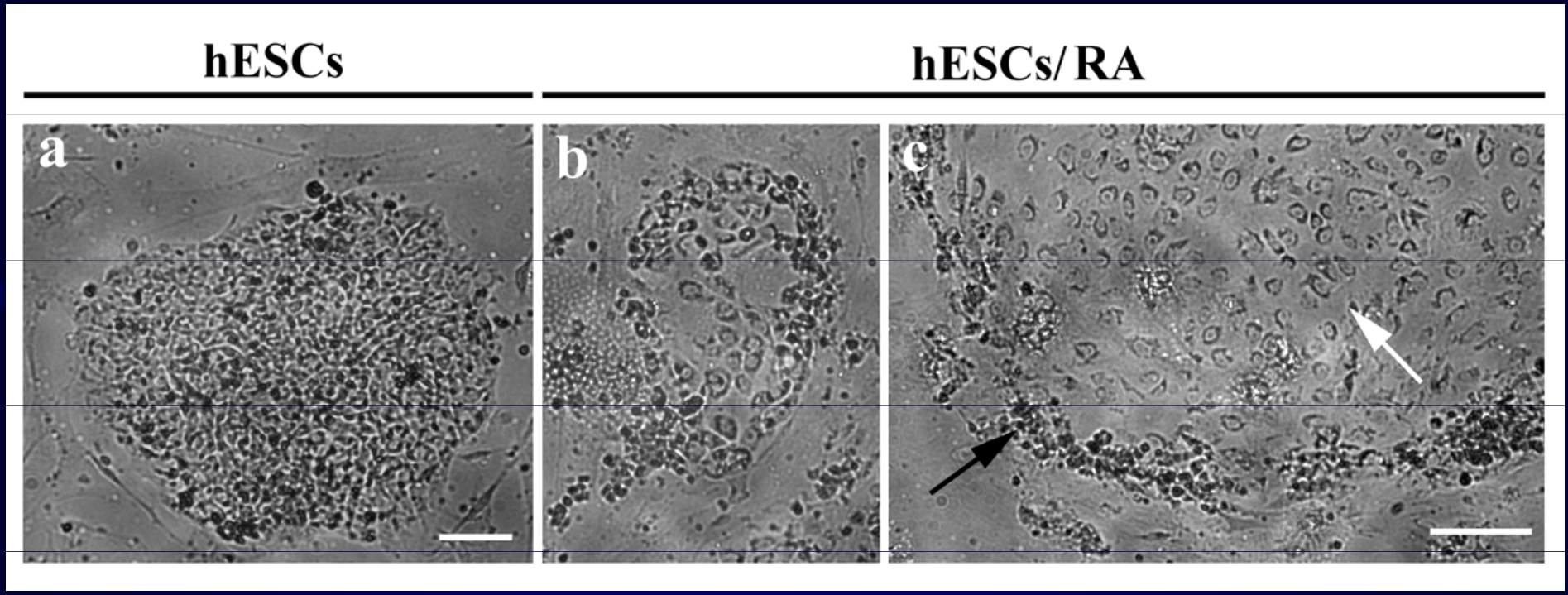
NaBt



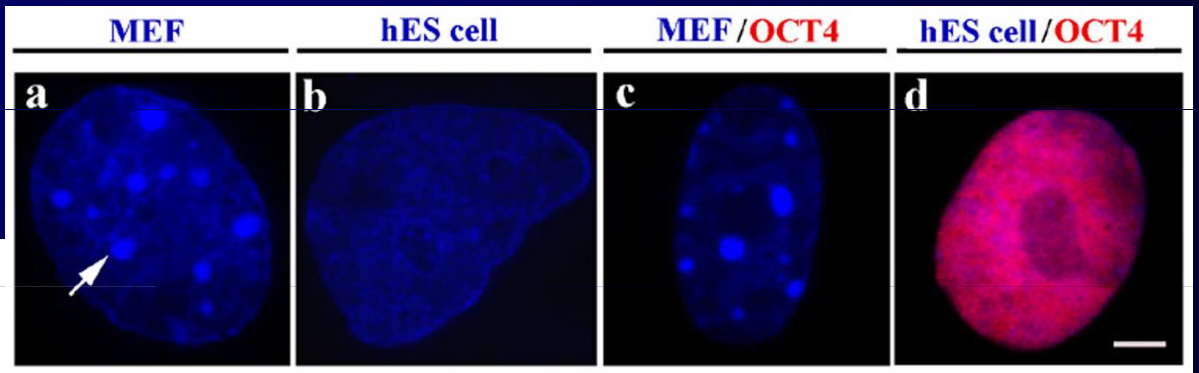
1. Differentiation of mouse emryonic cells (ES and EC)



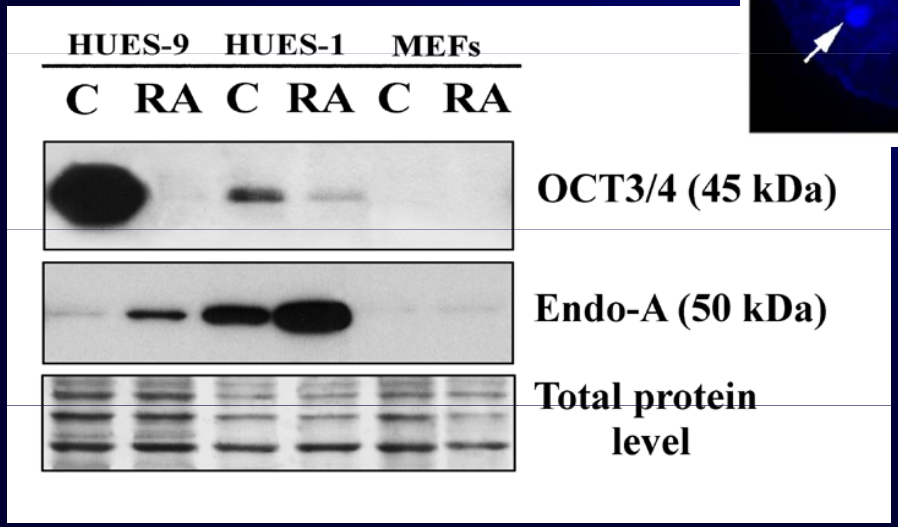
A



B

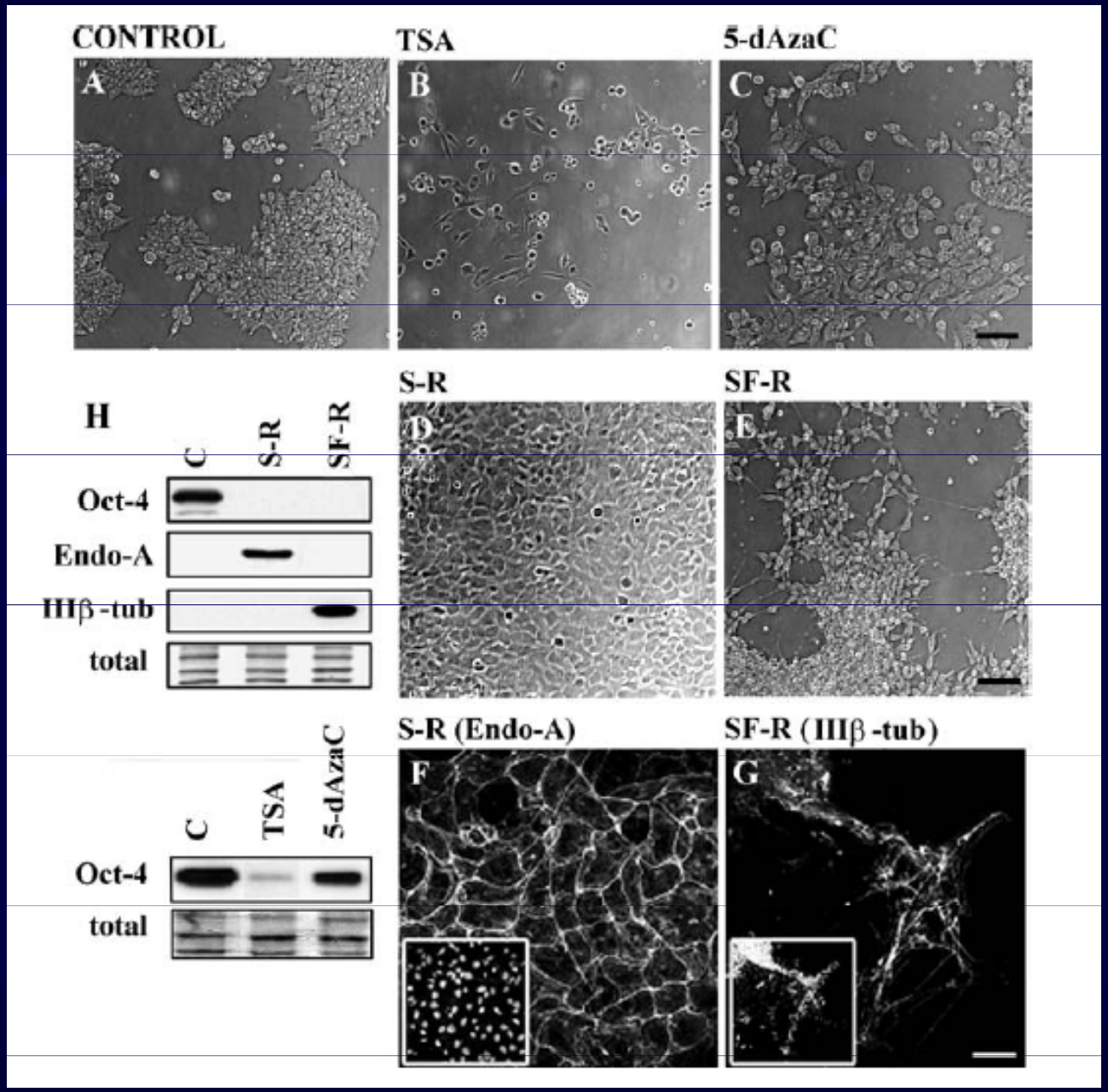


C

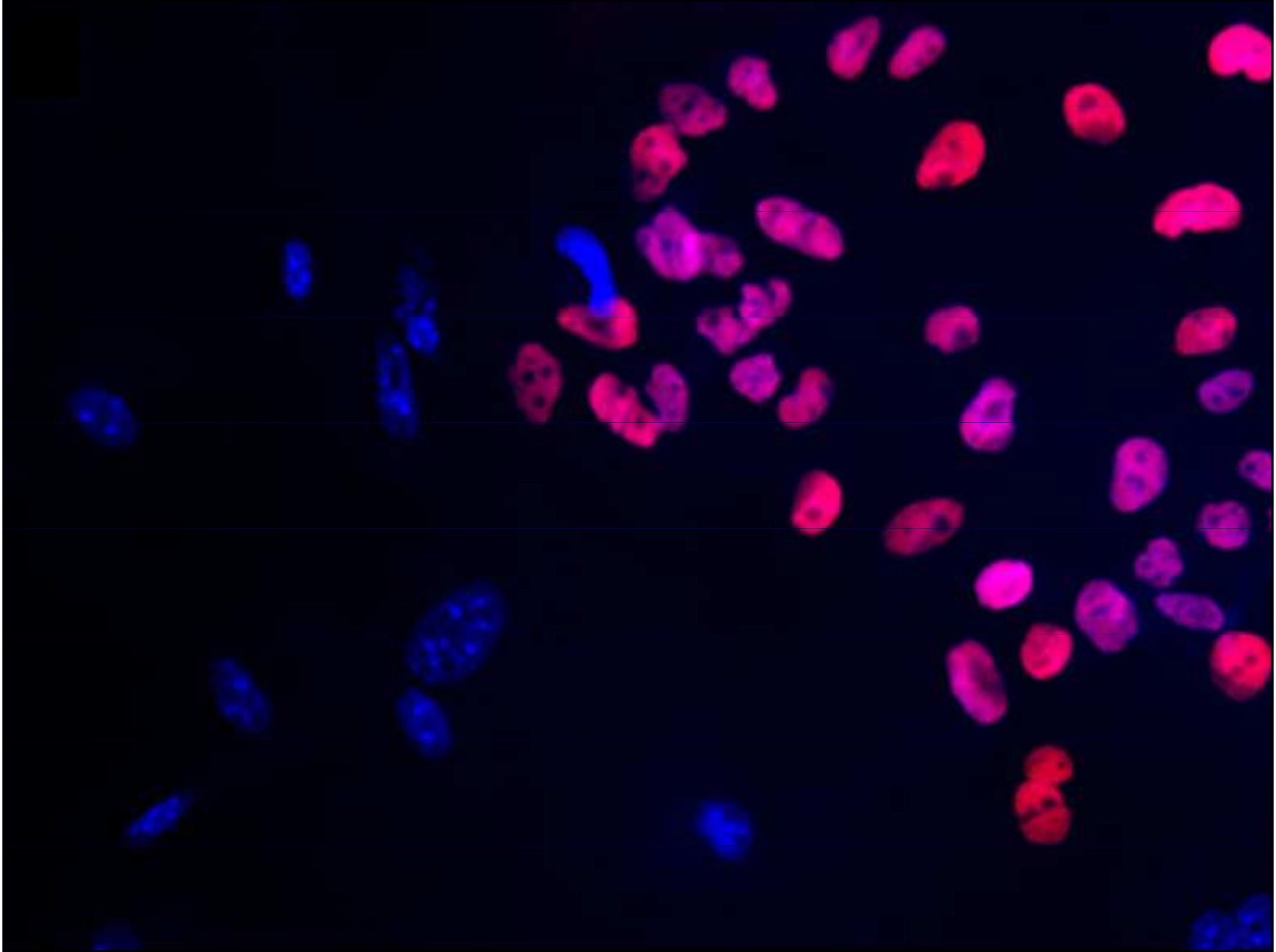


(Bártová et al., Differentiation, 2008)
(Bártová et al., Developmental Dynamics, 2008)

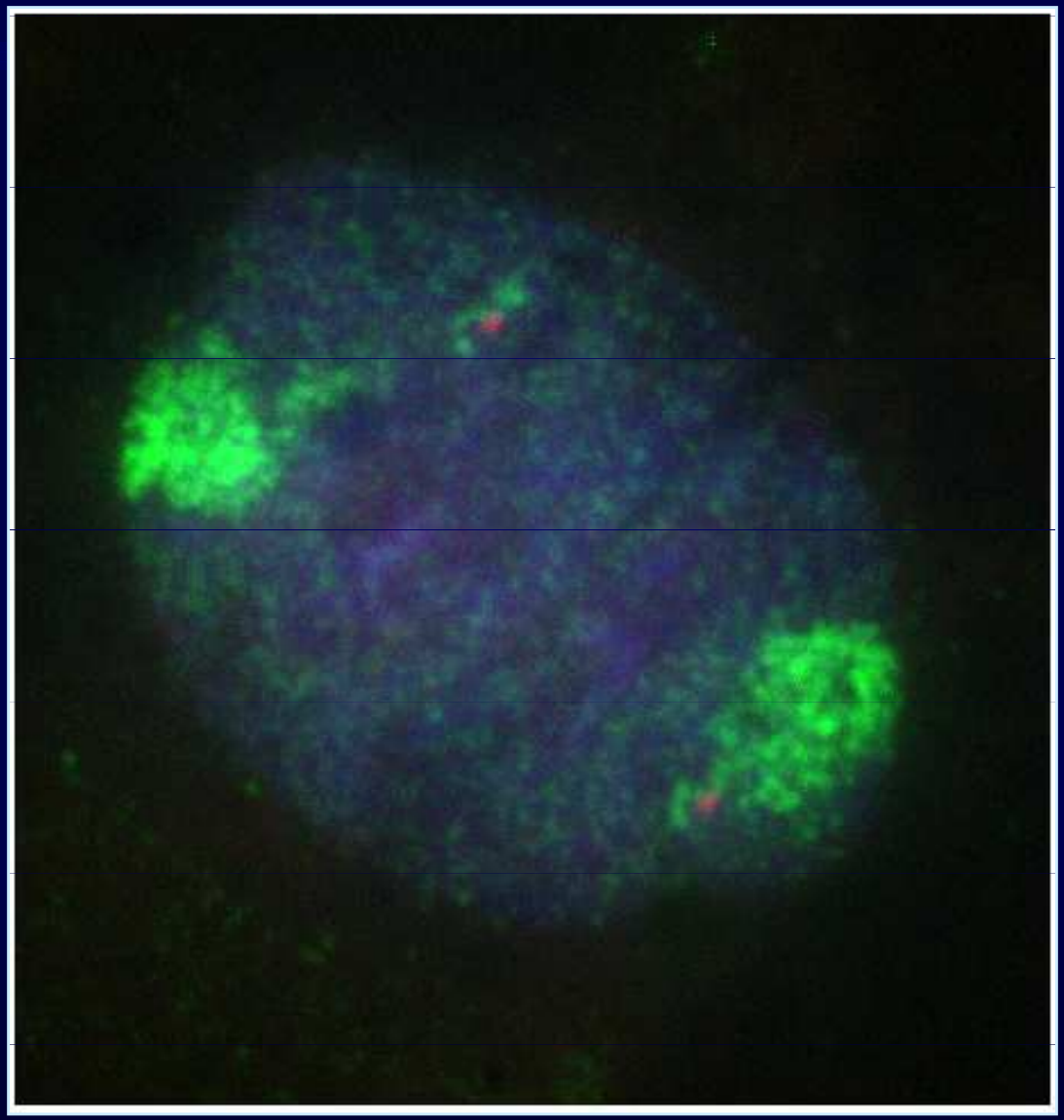
Mouse embyonal carcinoma cells P19

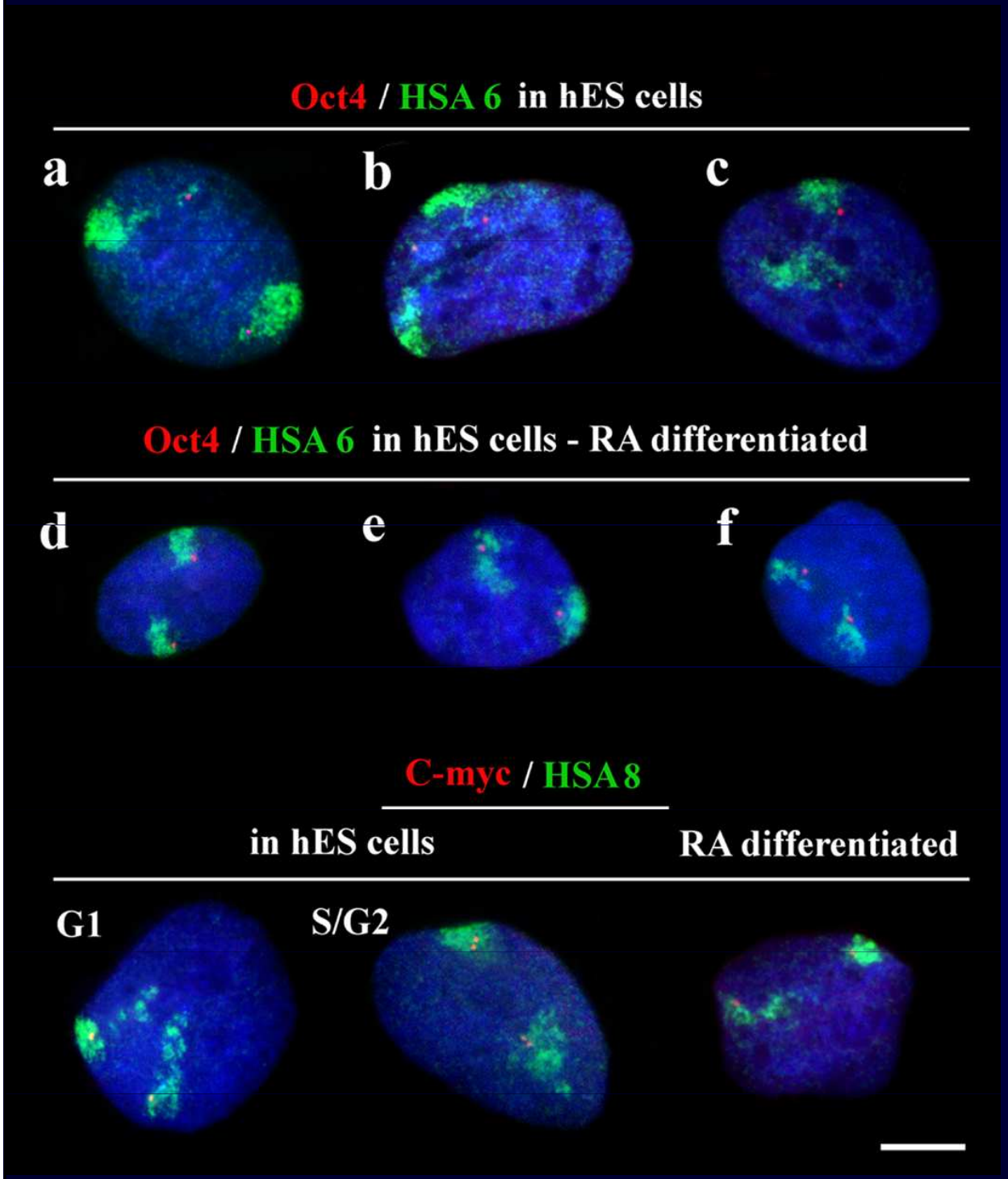


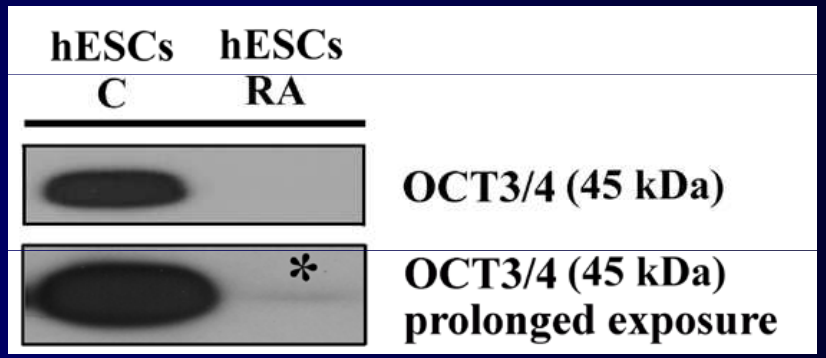
(Bártová et al., Histochem. Cell Biol., 2007)

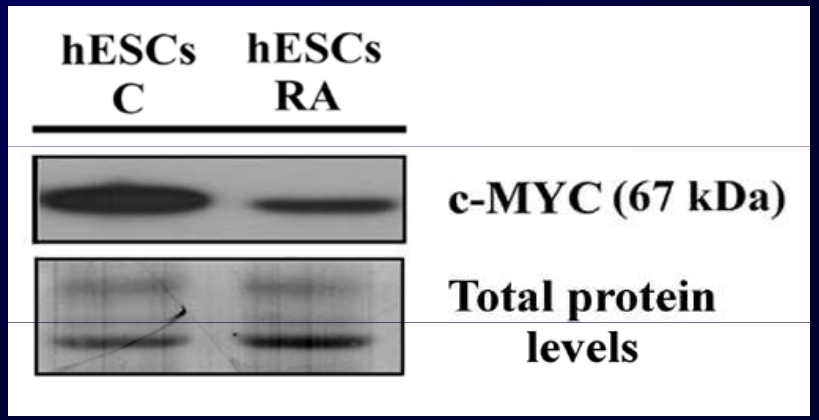


Oct3/4 and HSA6 in human ESCs

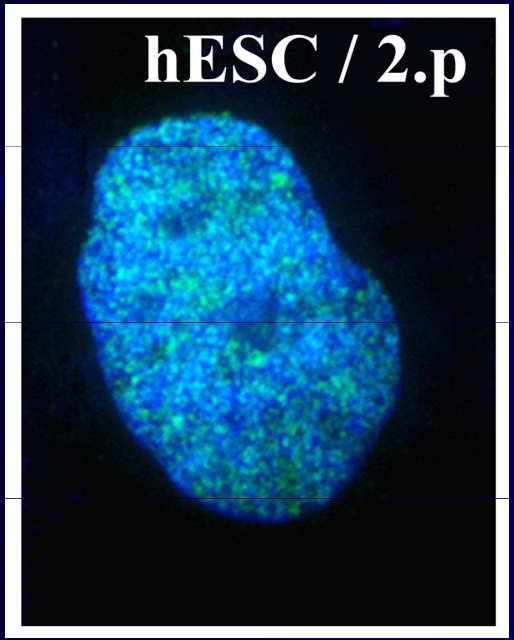


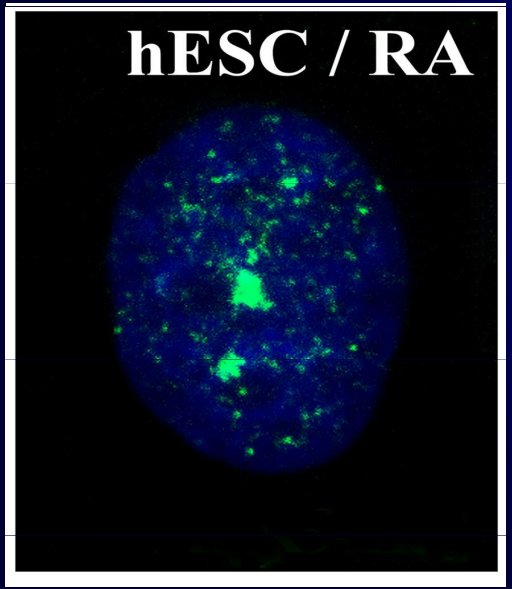

Oct4 / HSA 6 in hES cells
a
b
c
Oct4 / HSA 6 in hES cells - RA differentiated
d
e
f
C-myc / HSA 8
in hES cells
RA differentiated
G1
S/G2

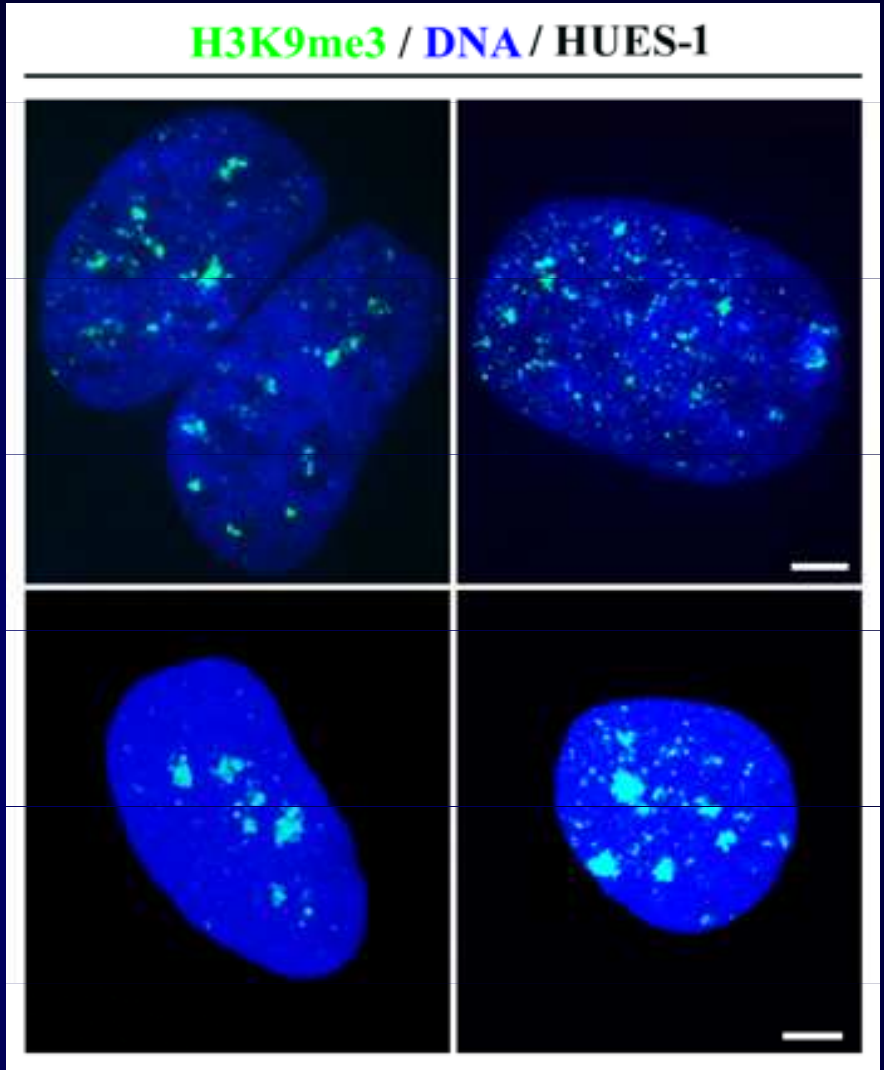

hESCs C
hESCs RA
OCT3/4 (45 kDa)
*
OCT3/4 (45 kDa) prolonged exposure

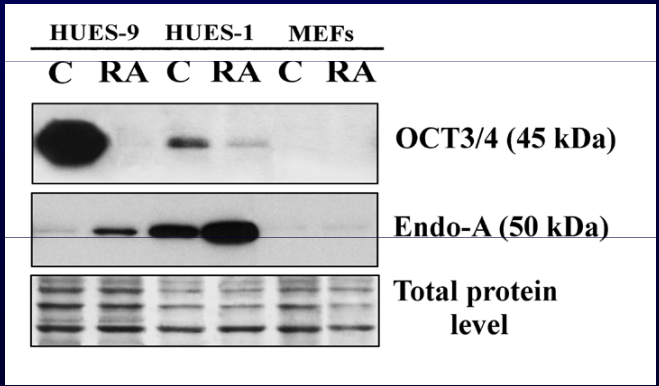

hESCs C
hESCs RA
c-MYC (67 kDa)
Total protein levels

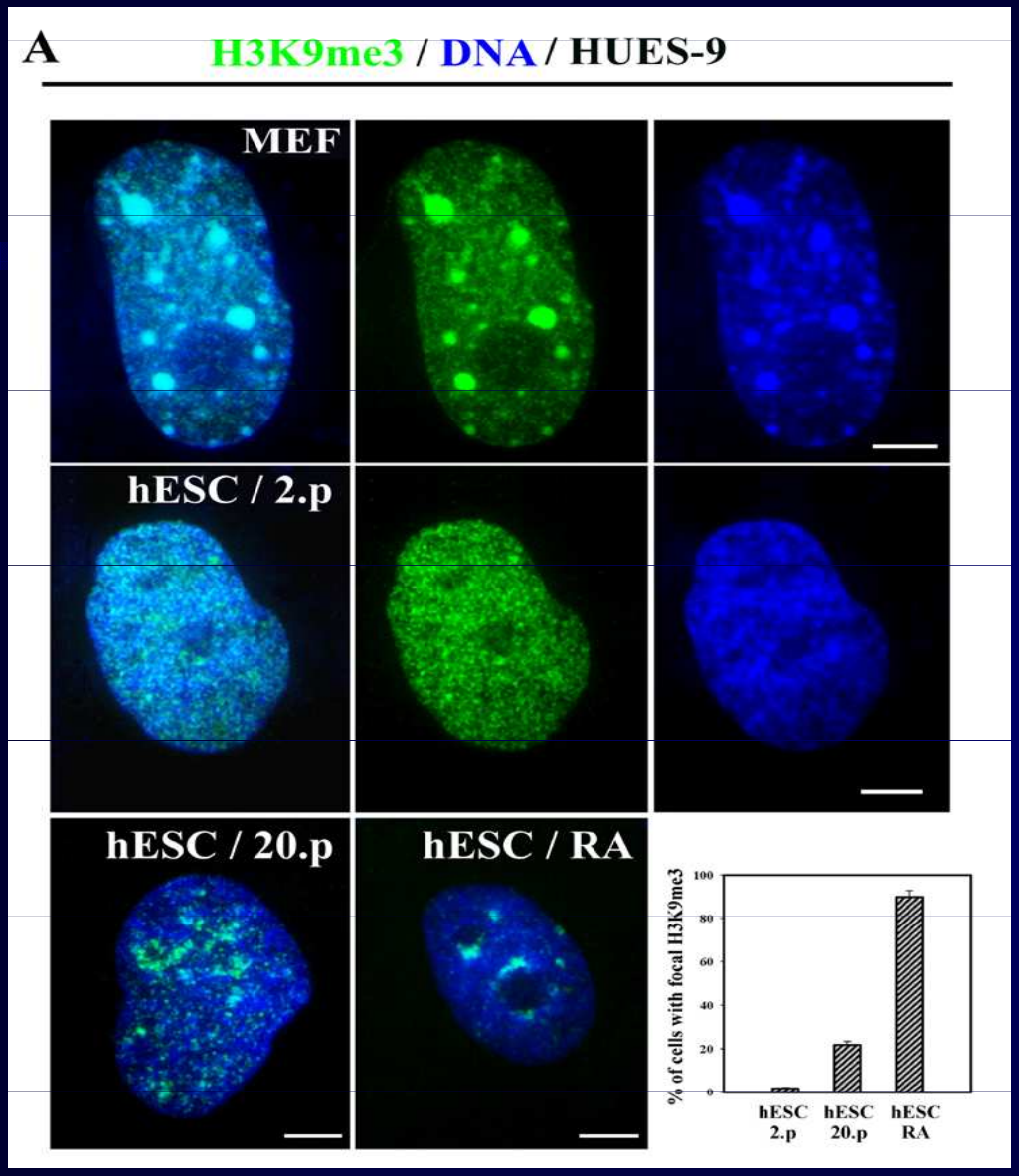
H3K9me3 / HUES-9

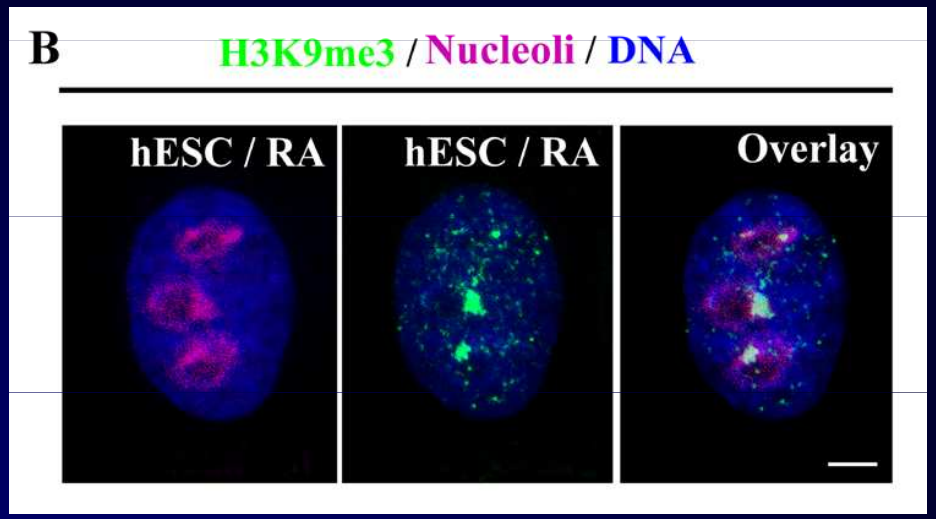


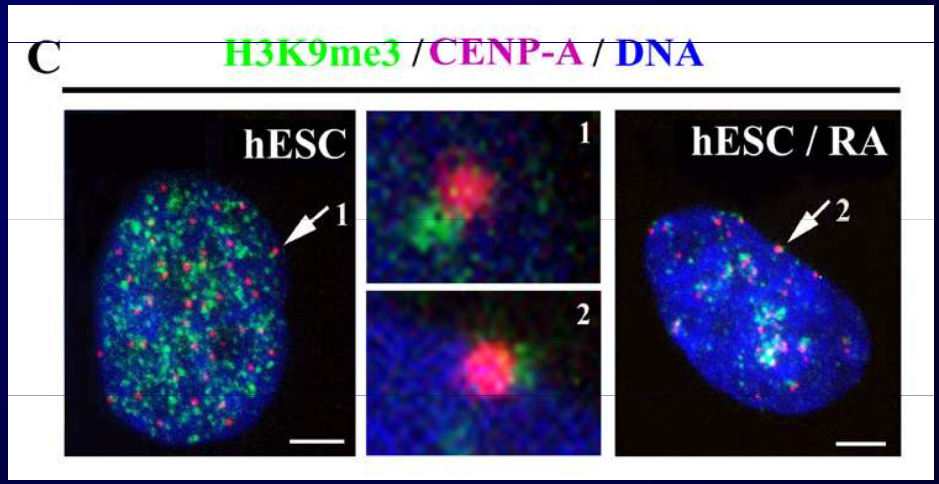


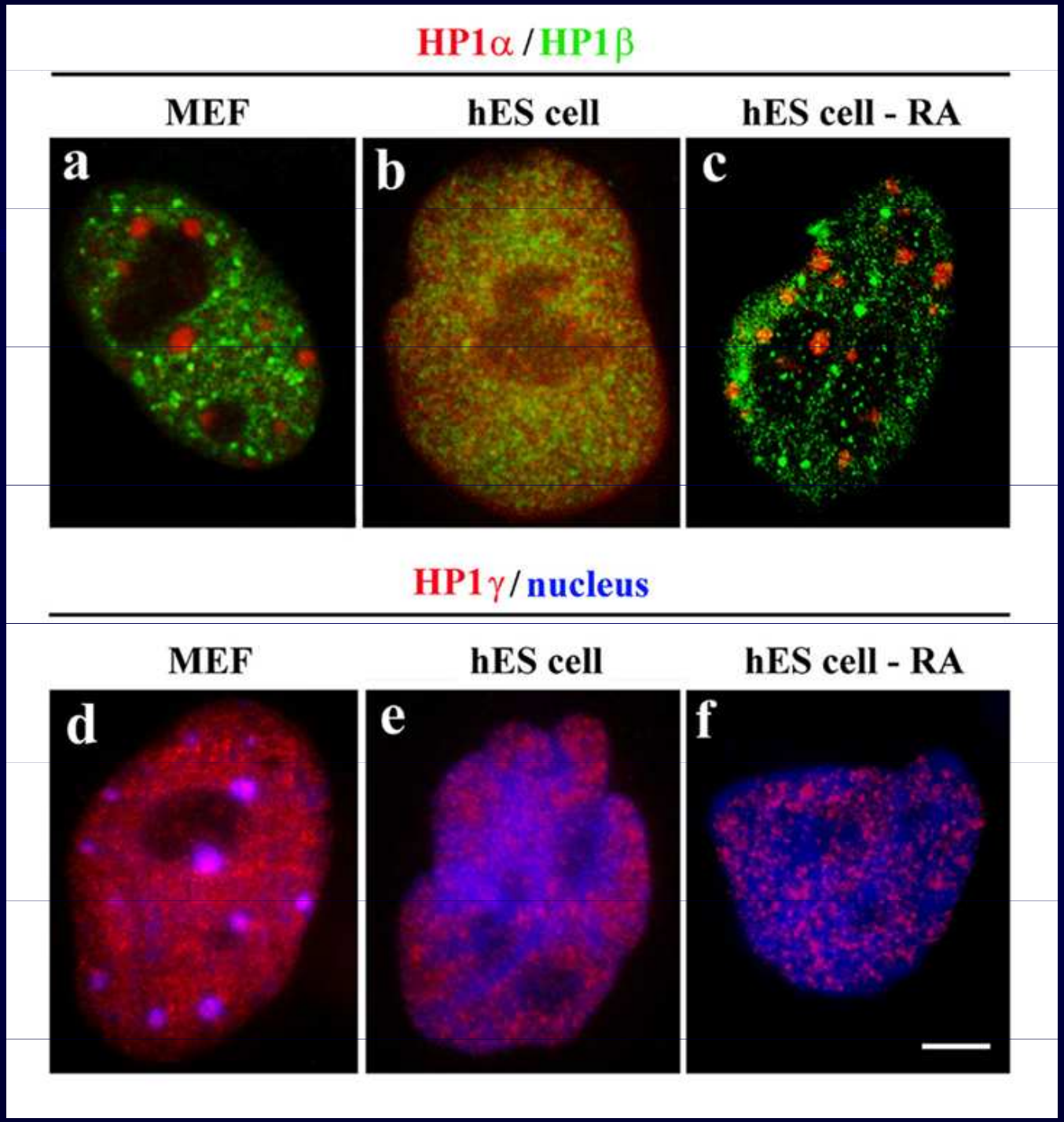


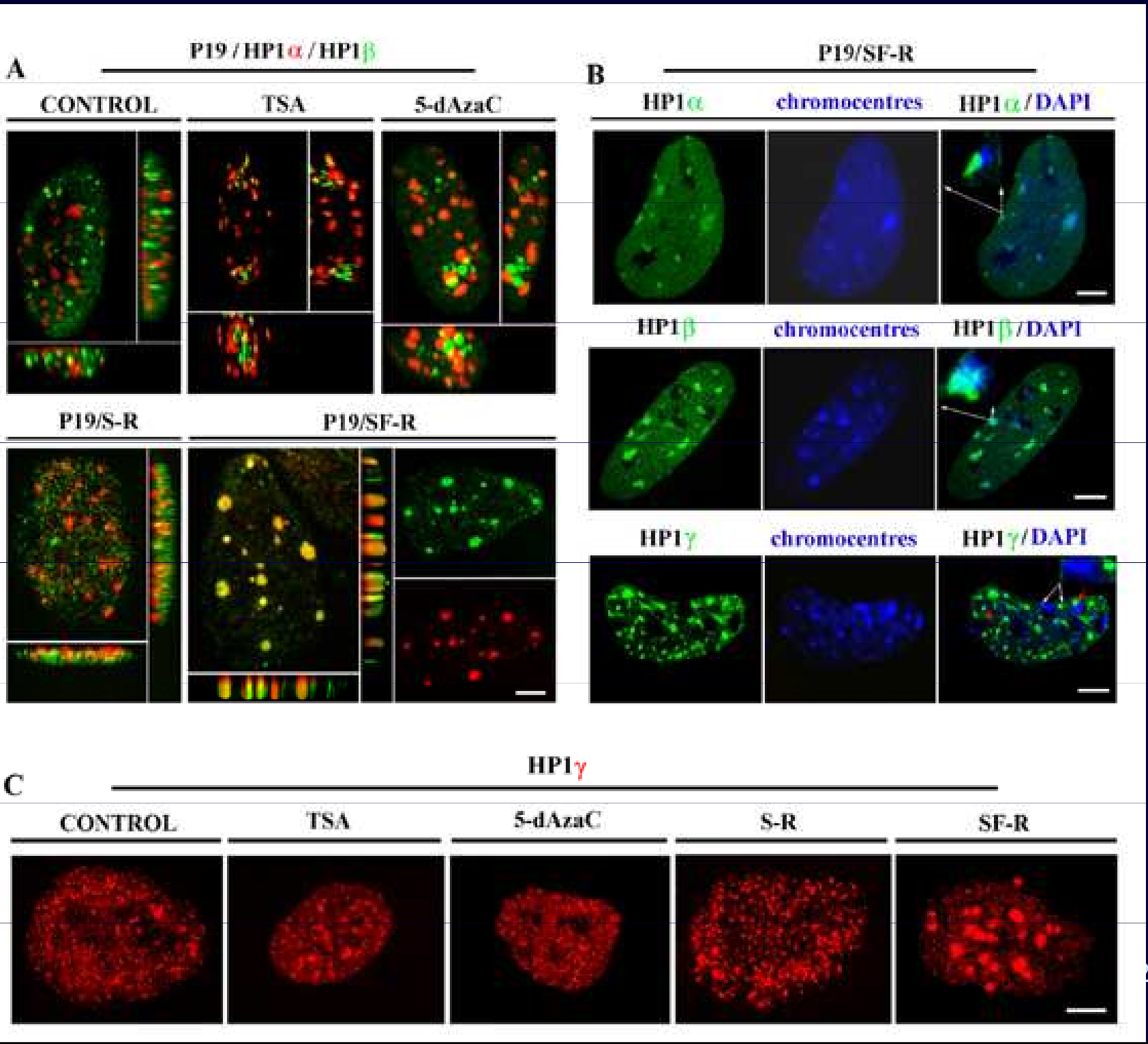


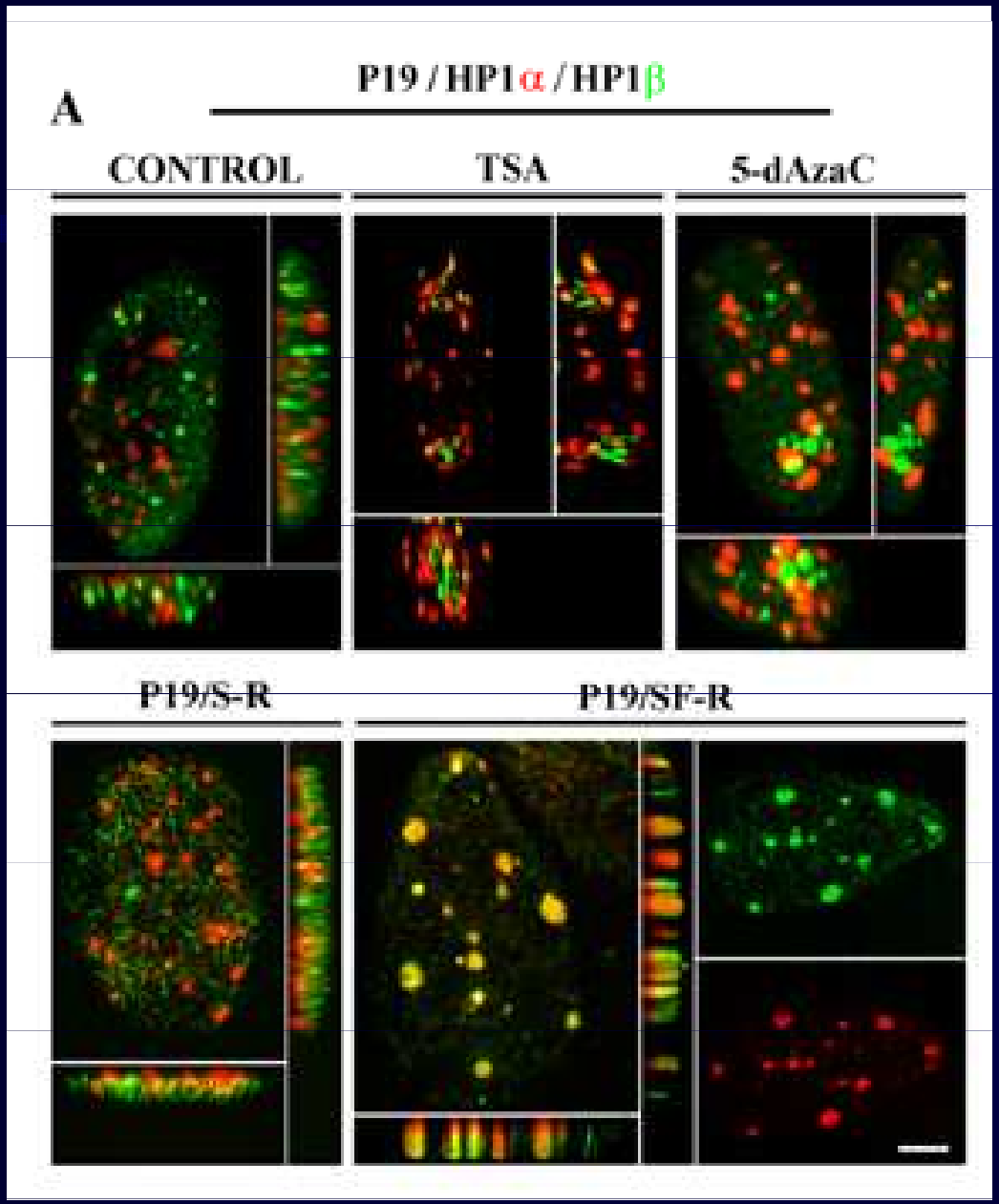

A
H3K9me3 / DNA / HUES-9
MEF
hESC / 2.p
hESC / 20.p
hESC / RA
% of cells with focal H3K9me3
100
80
60
40
20
0
hESC 2.p
hESC 20.p
hESC RA

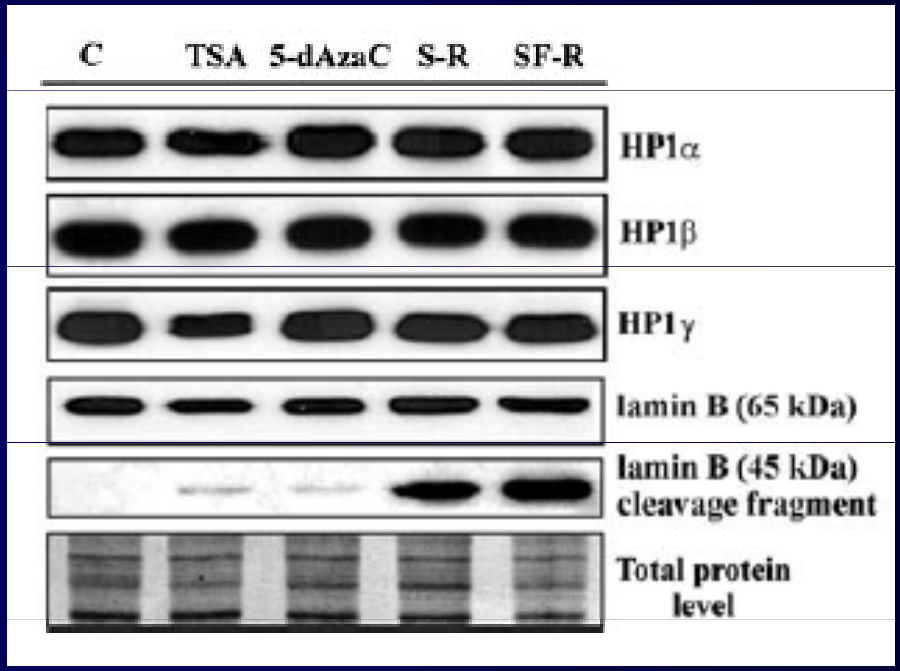

B
H3K9me3 / Nucleoli / DNA
hESC / RA
hESC / RA
Overlay

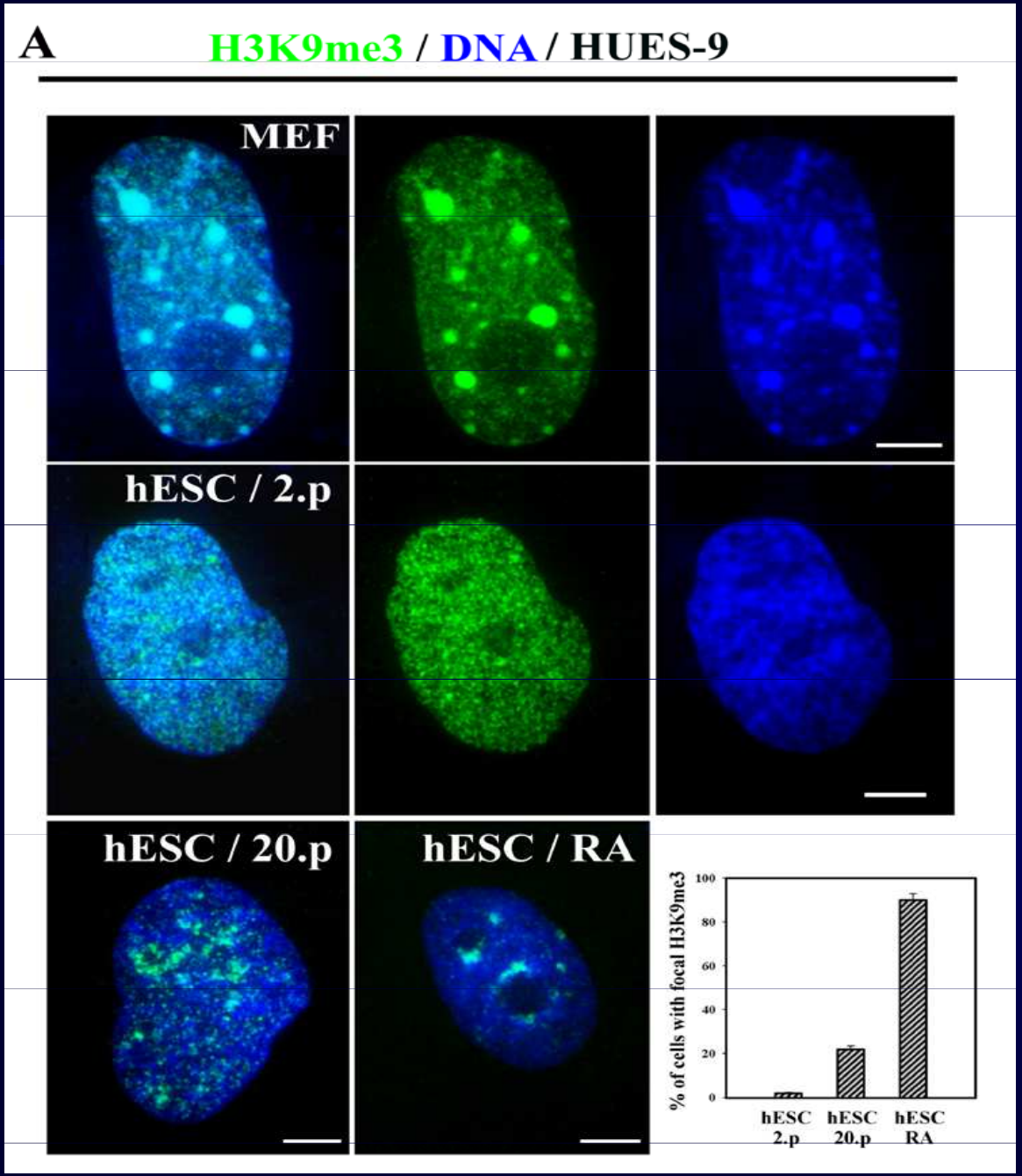

C
H3K9me3 / CENP-A / DNA
hESC
1
1
2
hESC / RA
2

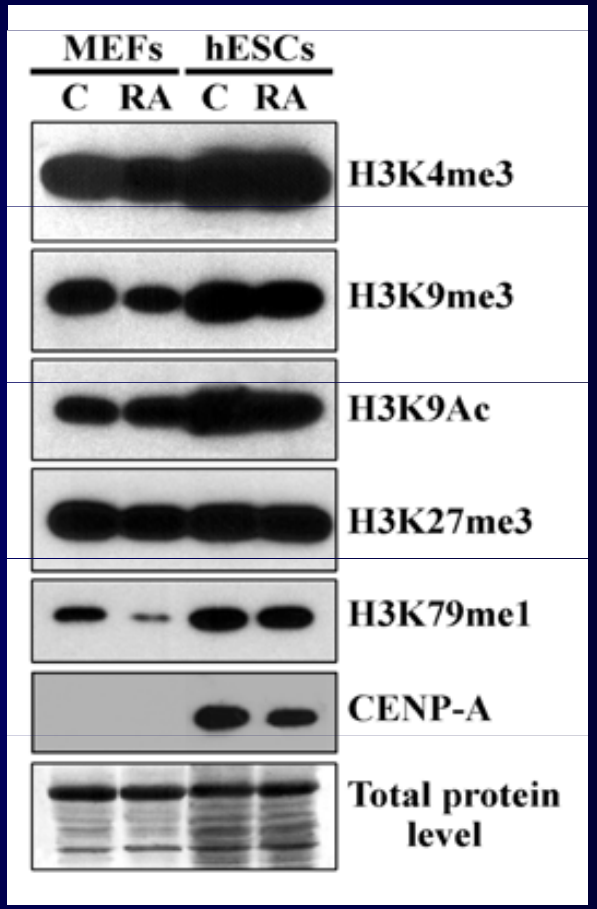

HP1α / HP1β
MEF
hES cell
hES cell - RA
a
b
c
HP1γ / nucleus
MEF
hES cell
hES cell - RA
d
e
f


A
P19 / HP1α / HP1β
CONTROL
TSA
5-dAzaC
P19/S-R
P19/SF-R
B
P19/SF-R
HP1α
chromocentres
HP1α/DAPI
HP1β
chromocentres
HP1β/DAPI
HP1γ
chromocentres
HP1γ/DAPI
C
HP1γ
CONTROL
TSA
5-dAzaC
S-R
SF-R

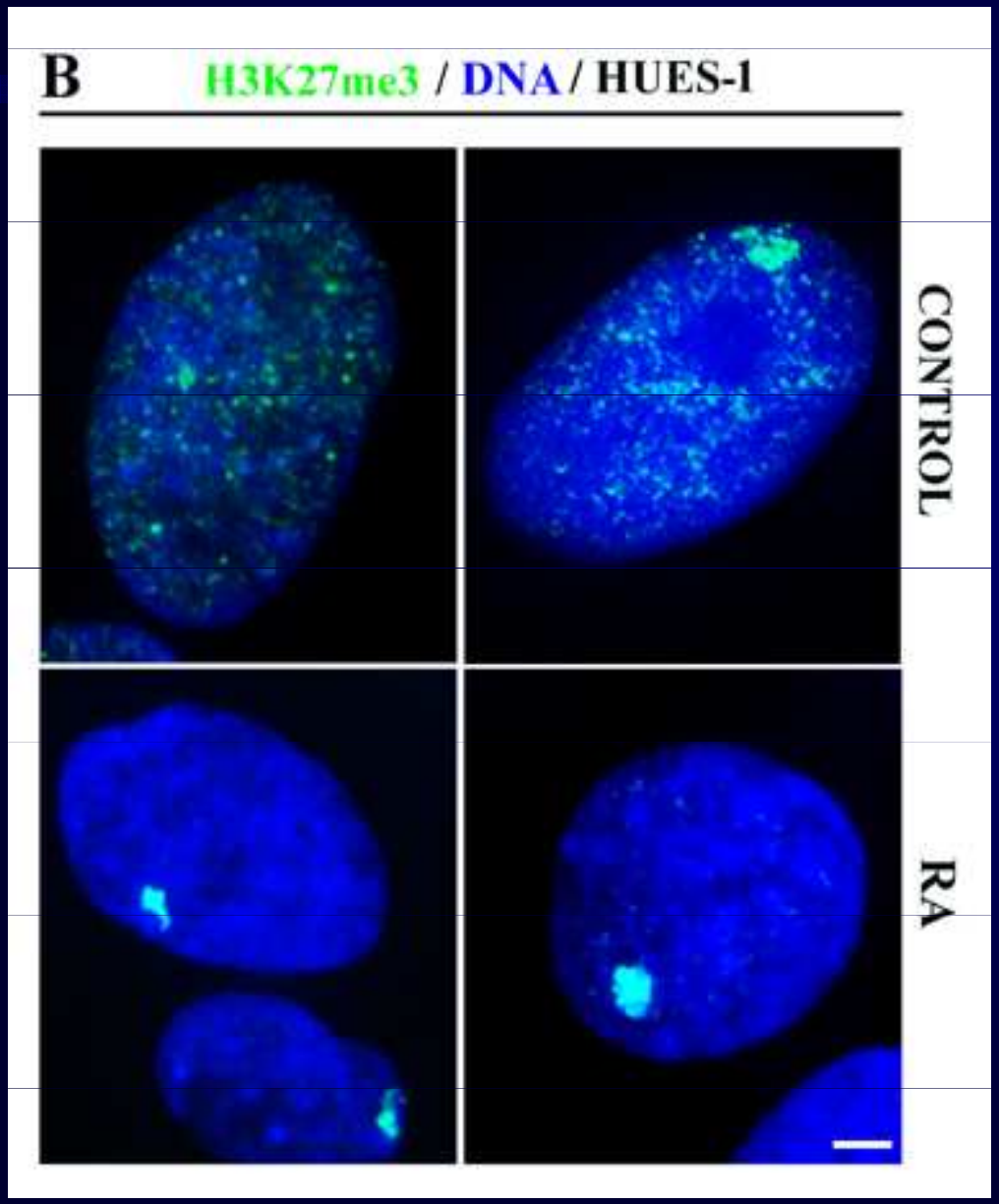

A
P19 / HP1α / HP1β
CONTROL
TSA
5-dAzaC
P19/S-R
P19/SF-R

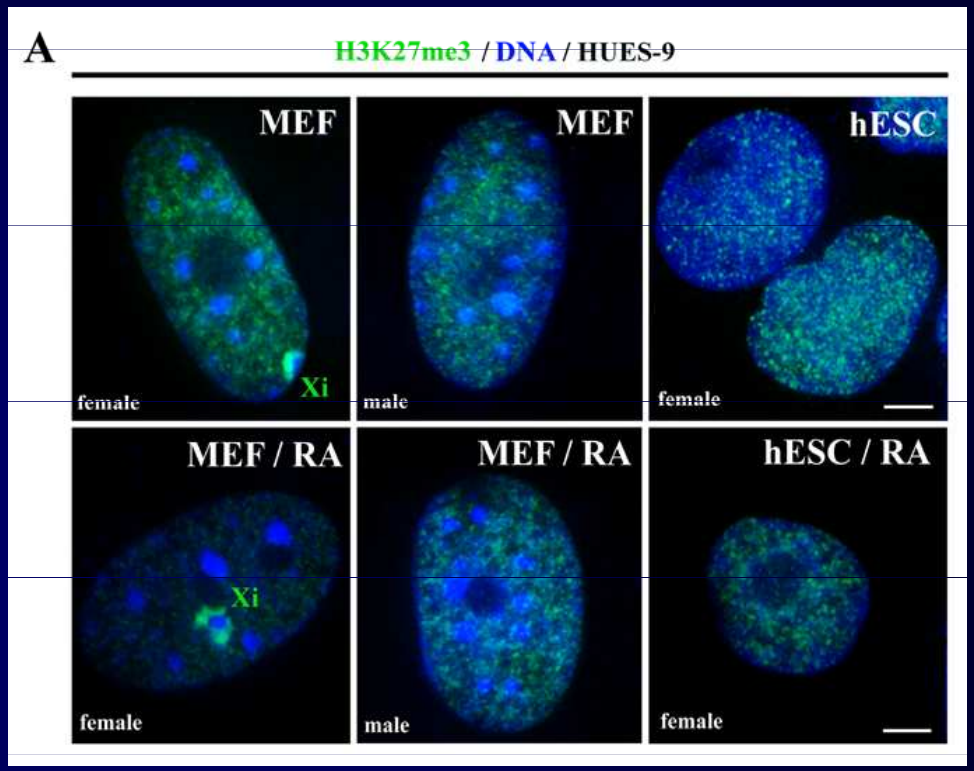

C
TSA
5-dAzaC
S-R
SF-R
HP1α
HP1β
HP1γ
lamin B (65 kDa)
lamin B (45 kDa) cleavage fragment
Total protein level

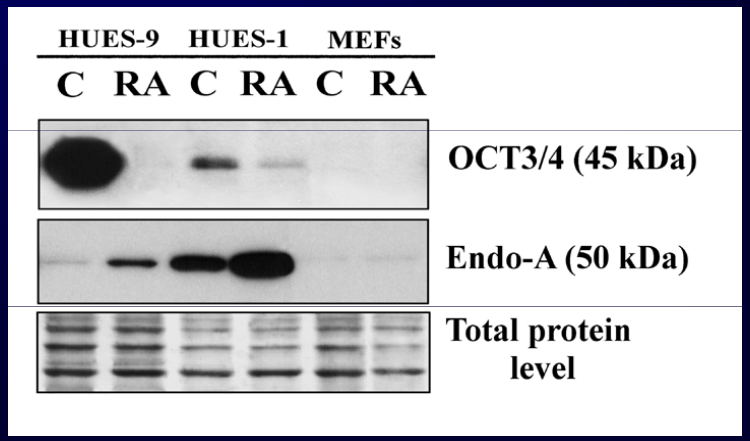

A
H3K9me3 / DNA / HUES-9
MEF
hESC / 2.p
hESC / 20.p
hESC / RA
% of cells with focal H3K9me3
100
80
60
40
20
0
hESC 2.p
hESC 20.p
hESC RA

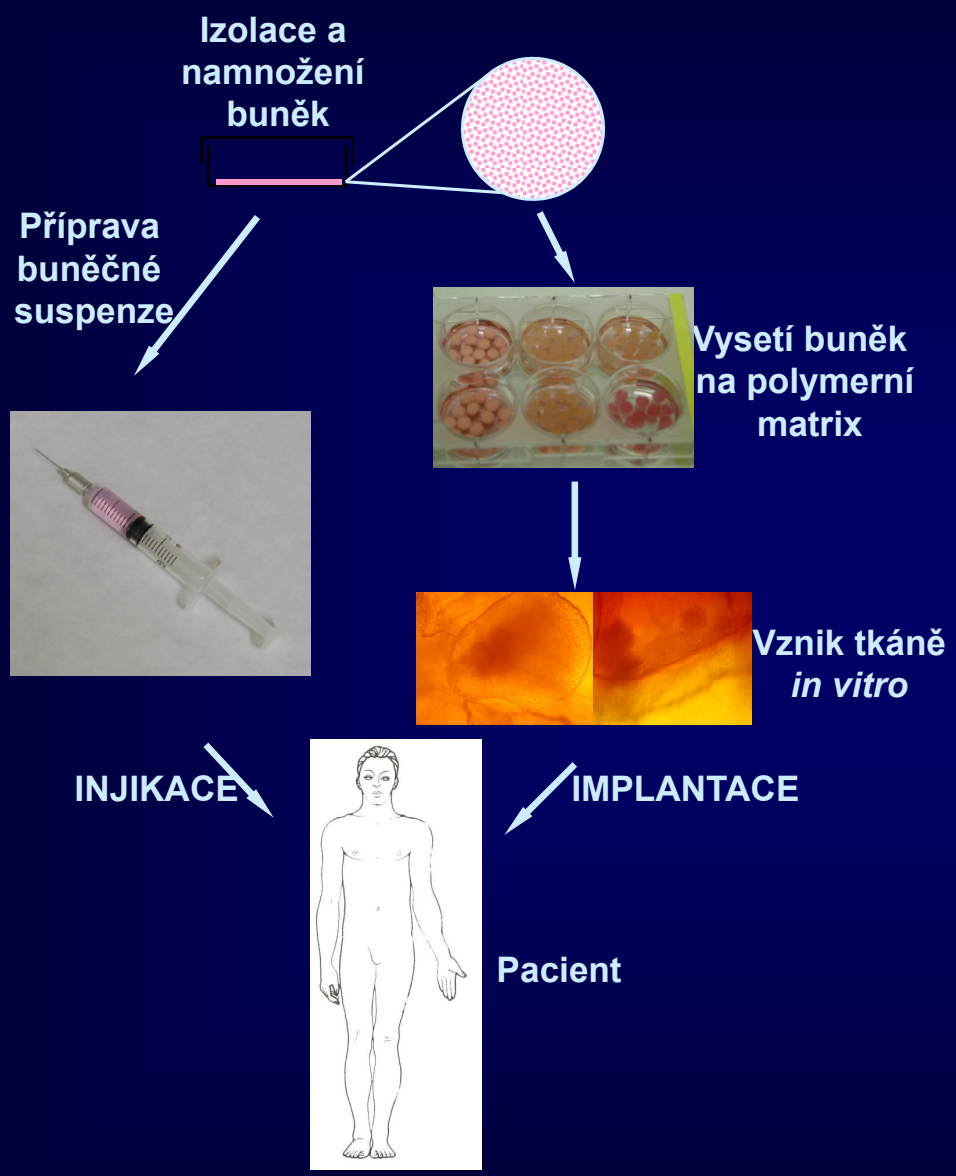

MEFs
hESCs
C
RA
C
RA
H3K4me3
H3K9me3
H3K9Ac
H3K27me3
H3K79me1
CENP-A
Total protein level

Inactivation of X chromosome in hESC



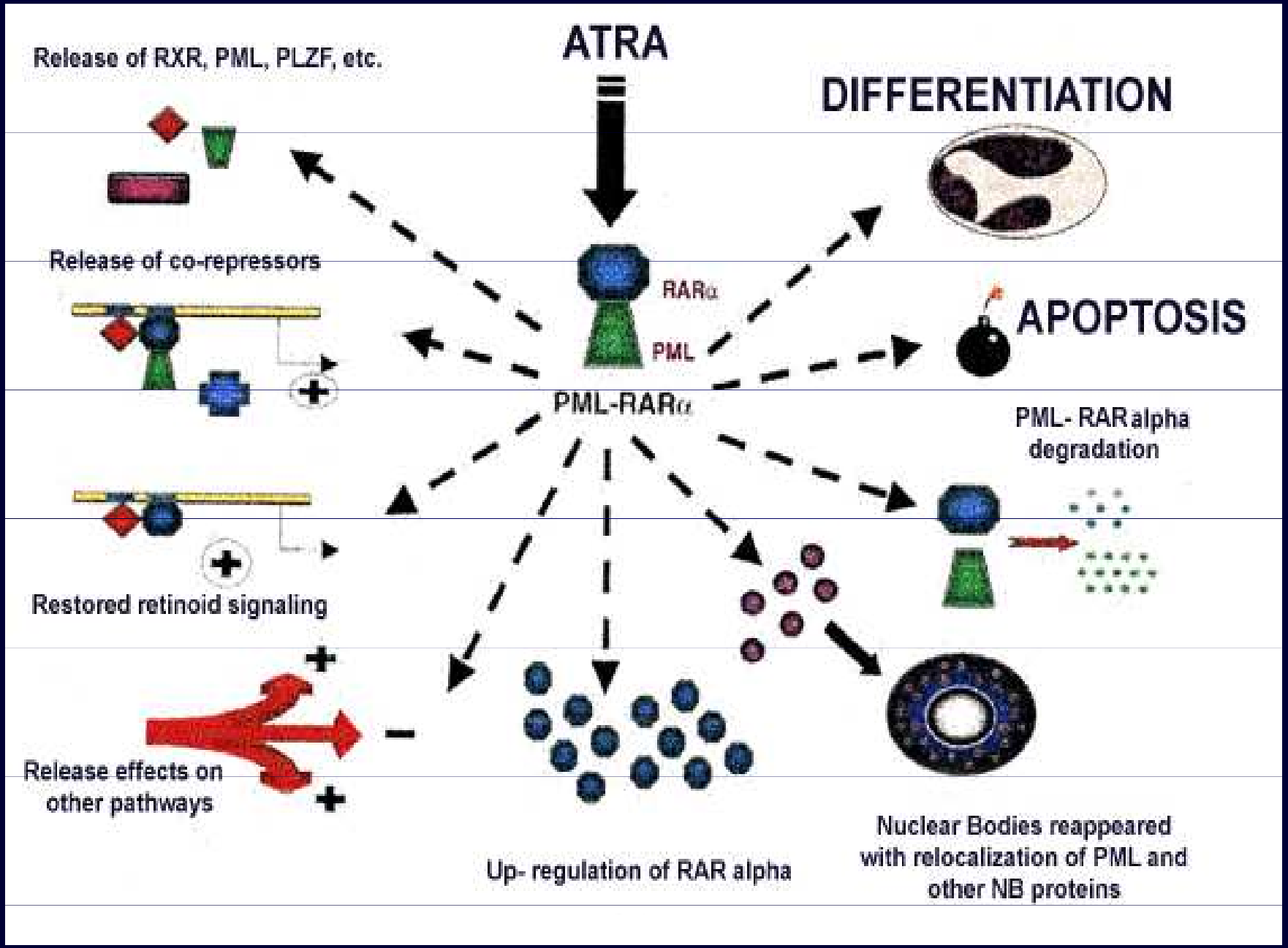


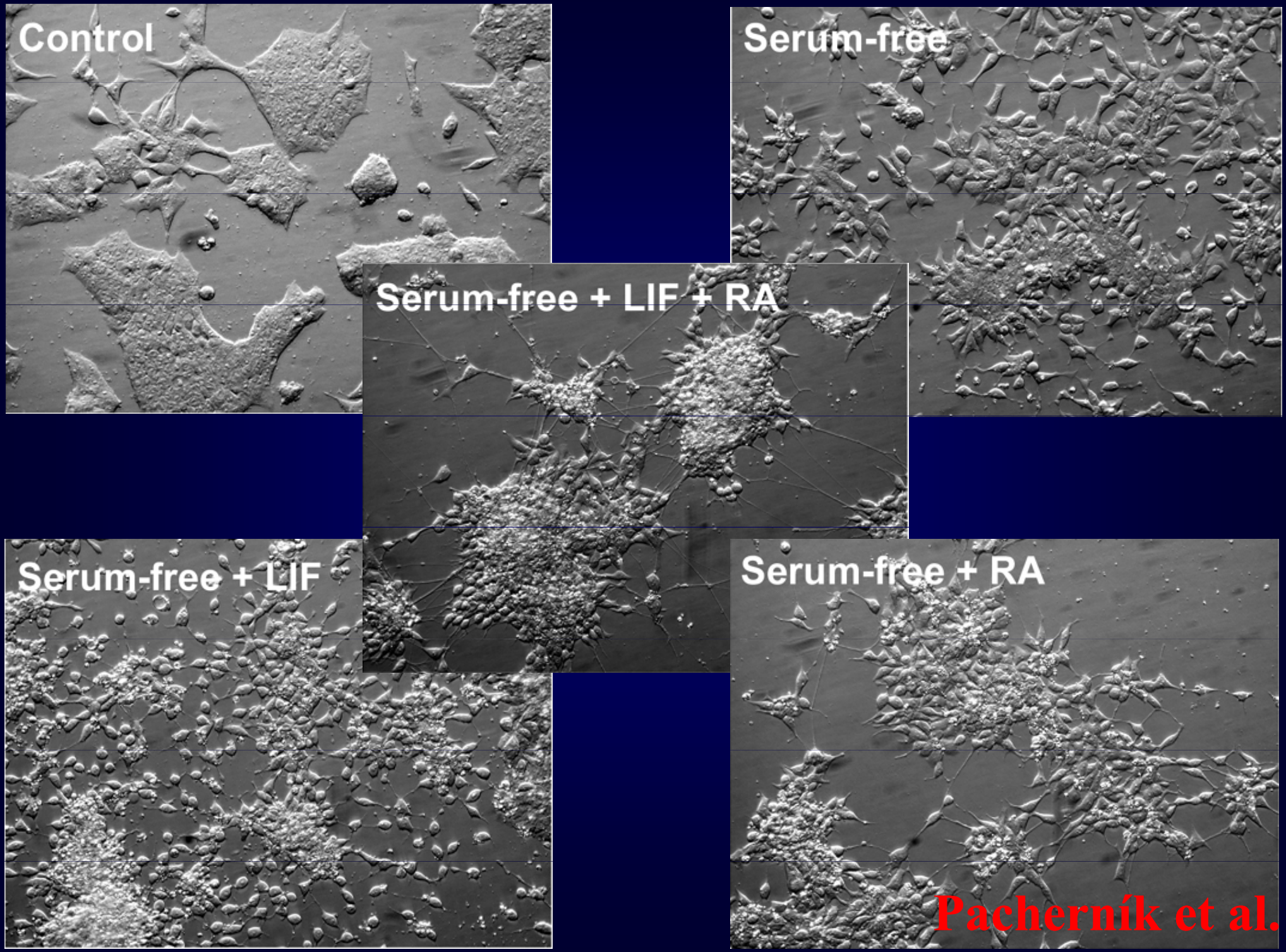




Schématické znázornění přístupů k buněčné terapii.

Buňky získané biopsií, diferenciací z ES nebo jiným způsobem se nechají narůst *in vitro*. Transplantace pak může být provedena pomocí injikace suspenze buněk nebo implantací nové trojrozměrné tkáně na místo již odstraněné nefunkční části orgánu.


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


Control
Serum-free
Serum-free + LIF + RA
Serum-free + LIF
Serum-free + RA
Pacherník et al.

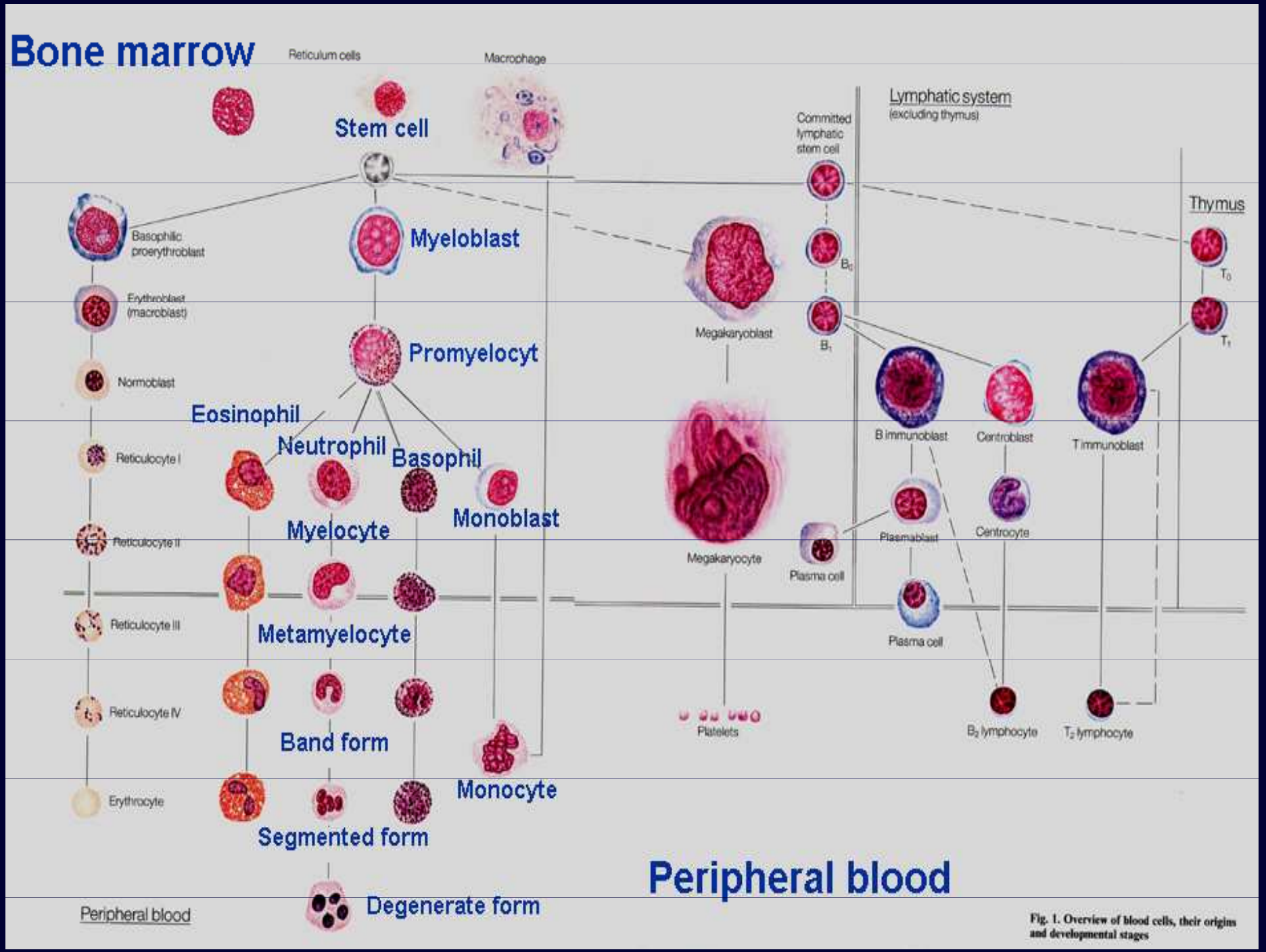
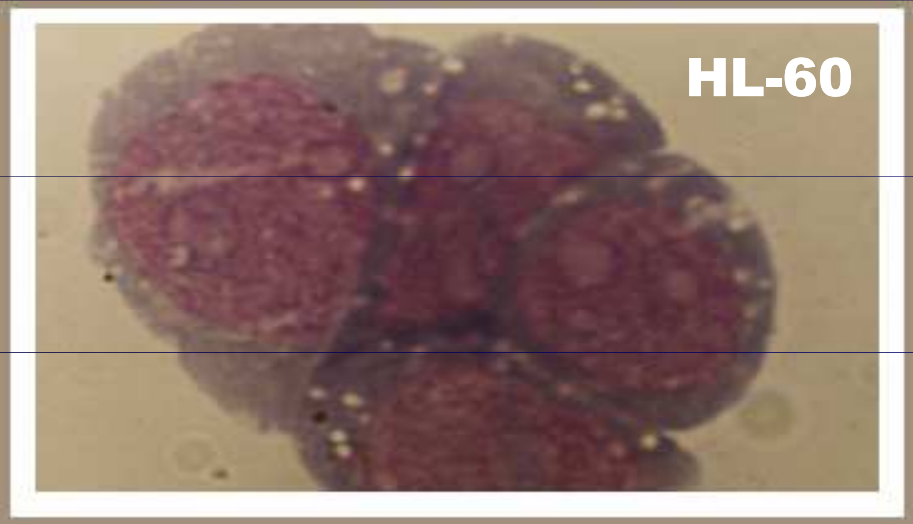
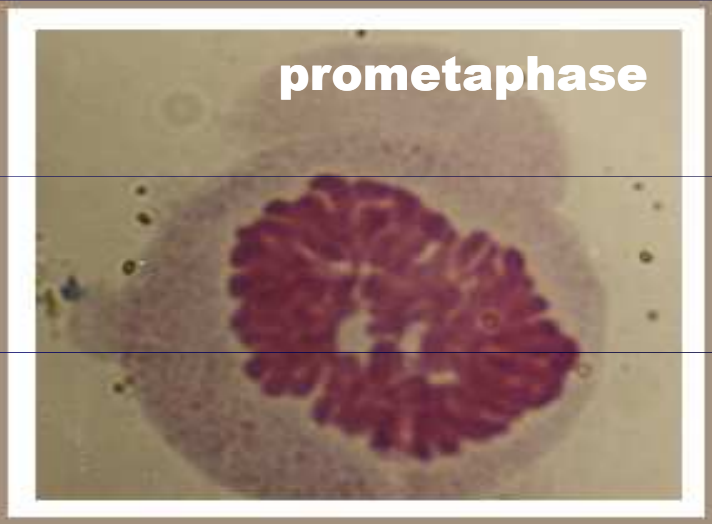
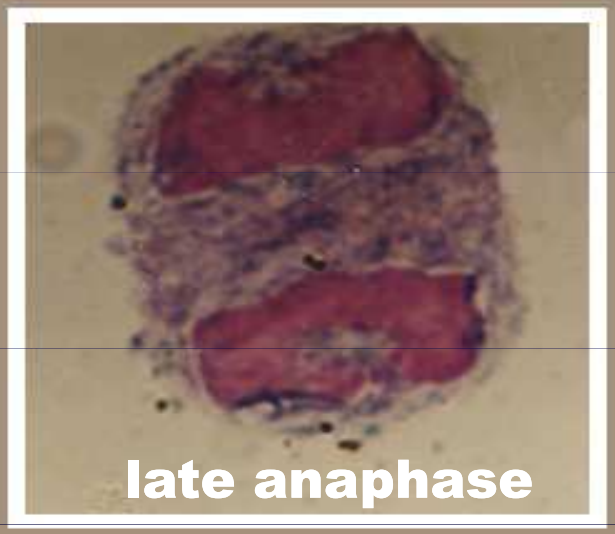


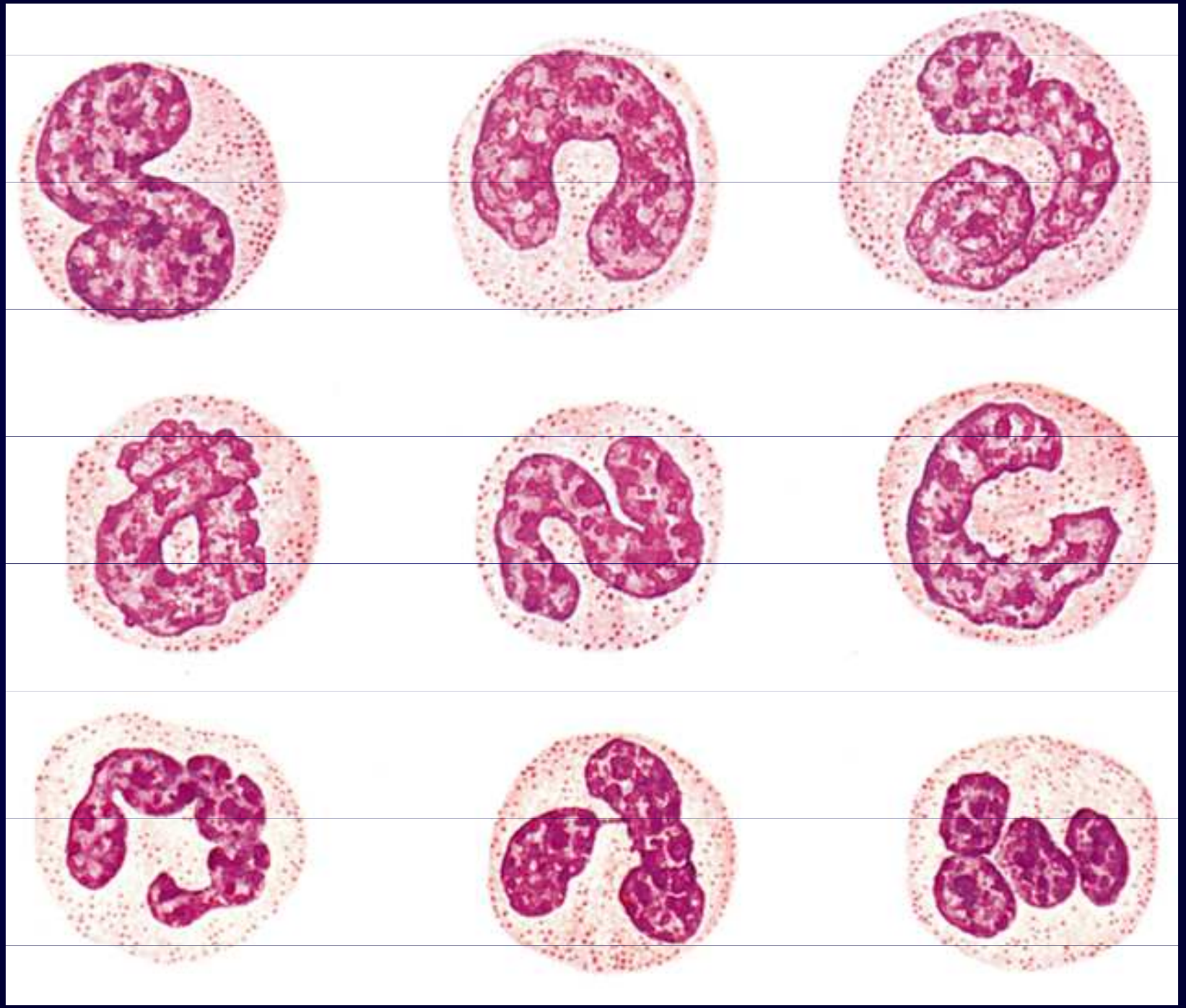
Fig. 1. Overview of blood cells, their origins and developmental stages

Morphology of human leukemic promyelocytic cell line HL60 and neutrophilic granulocyte

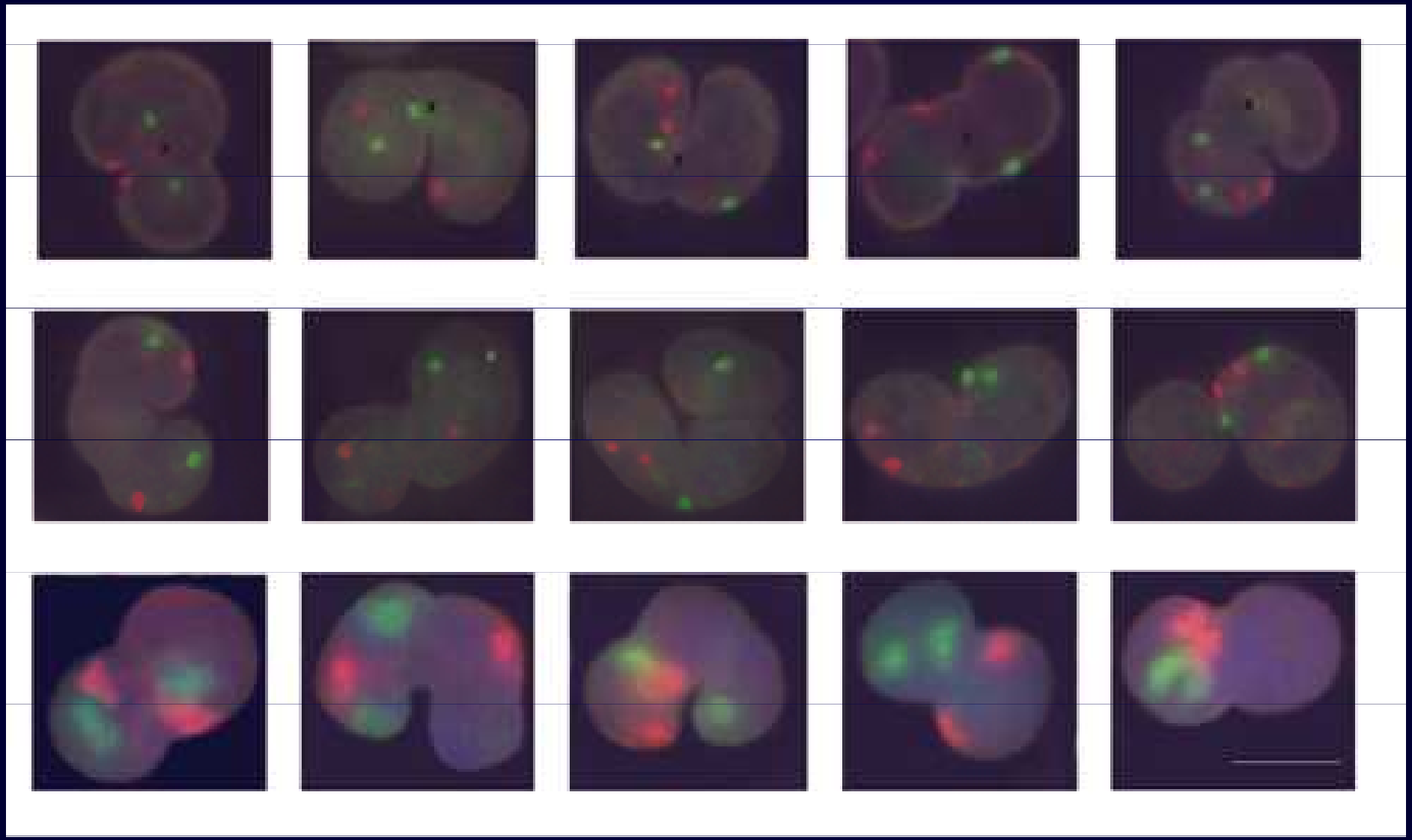


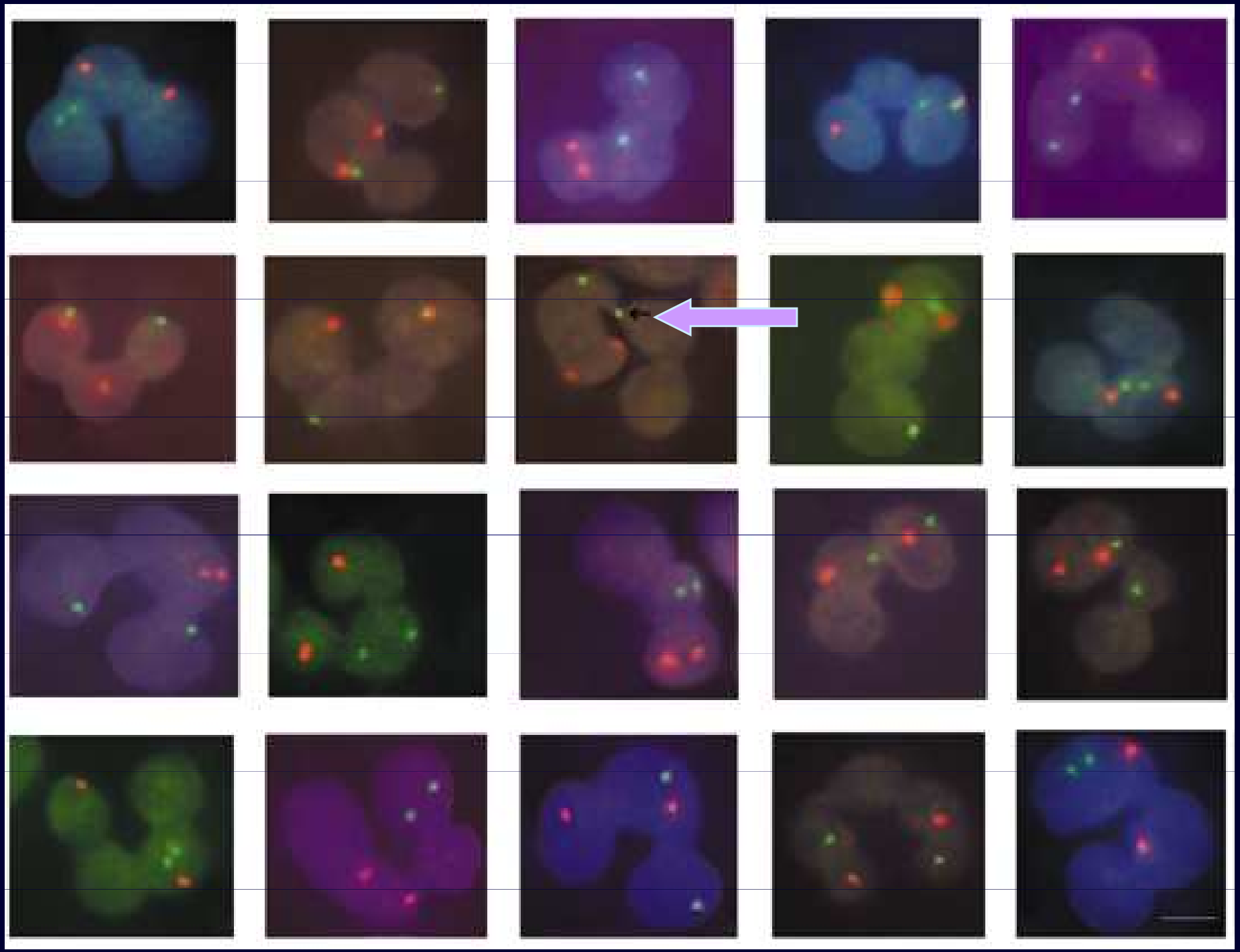




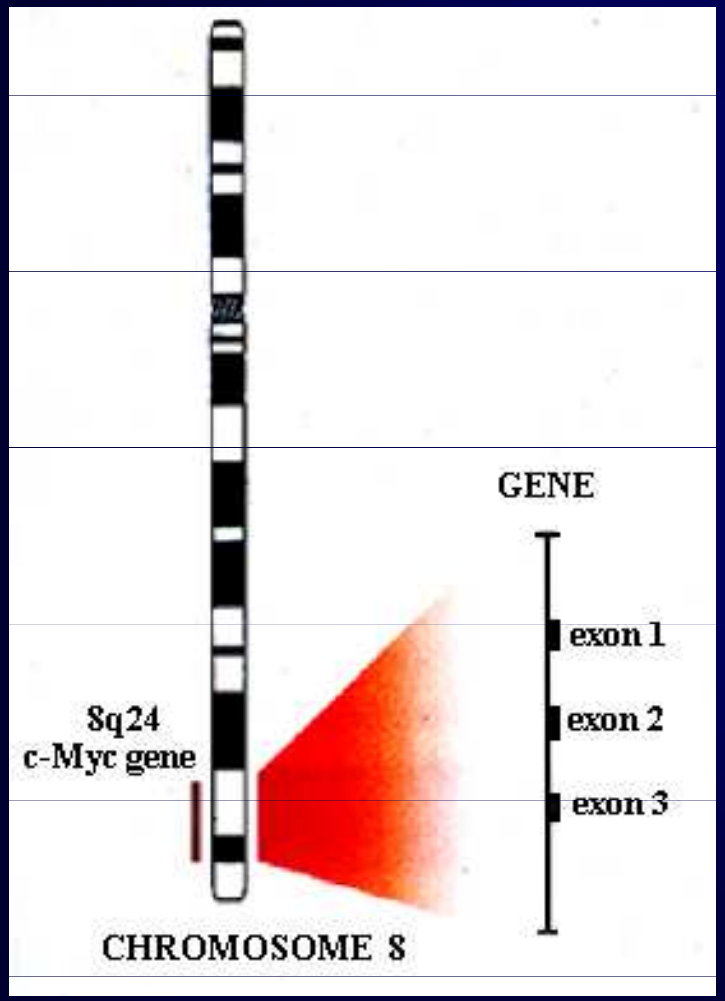


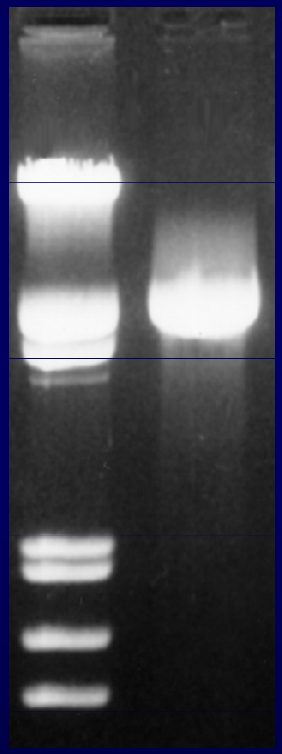
Topographic Types of Human Granulocytes

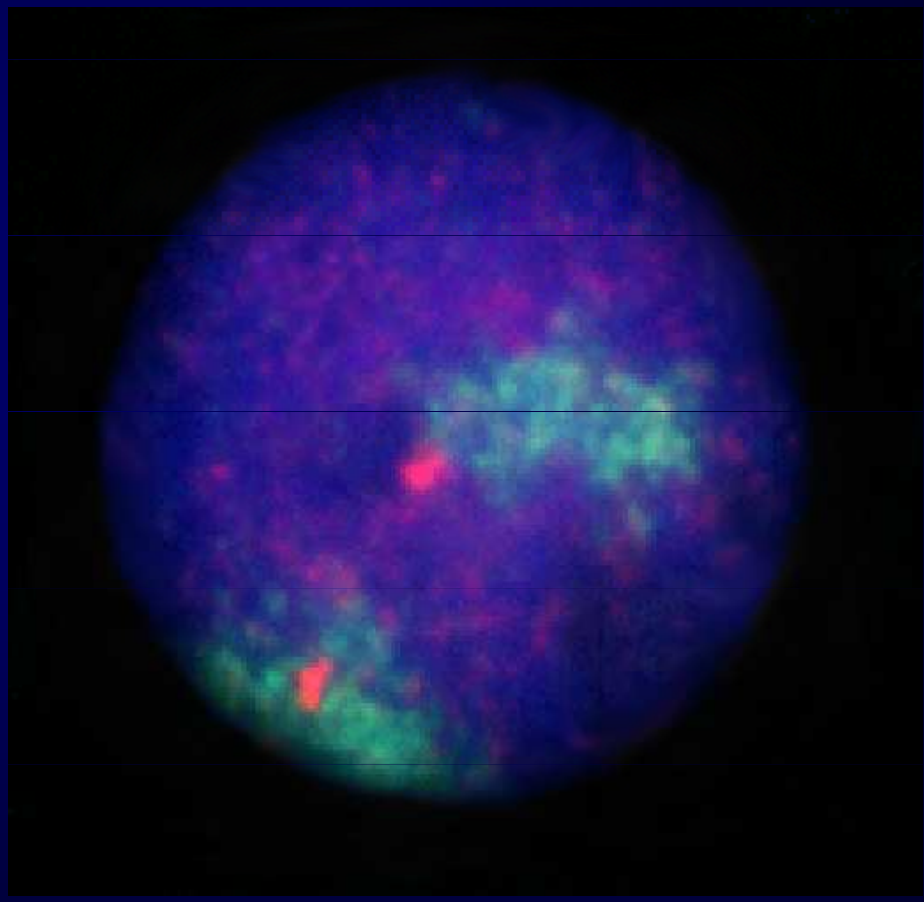




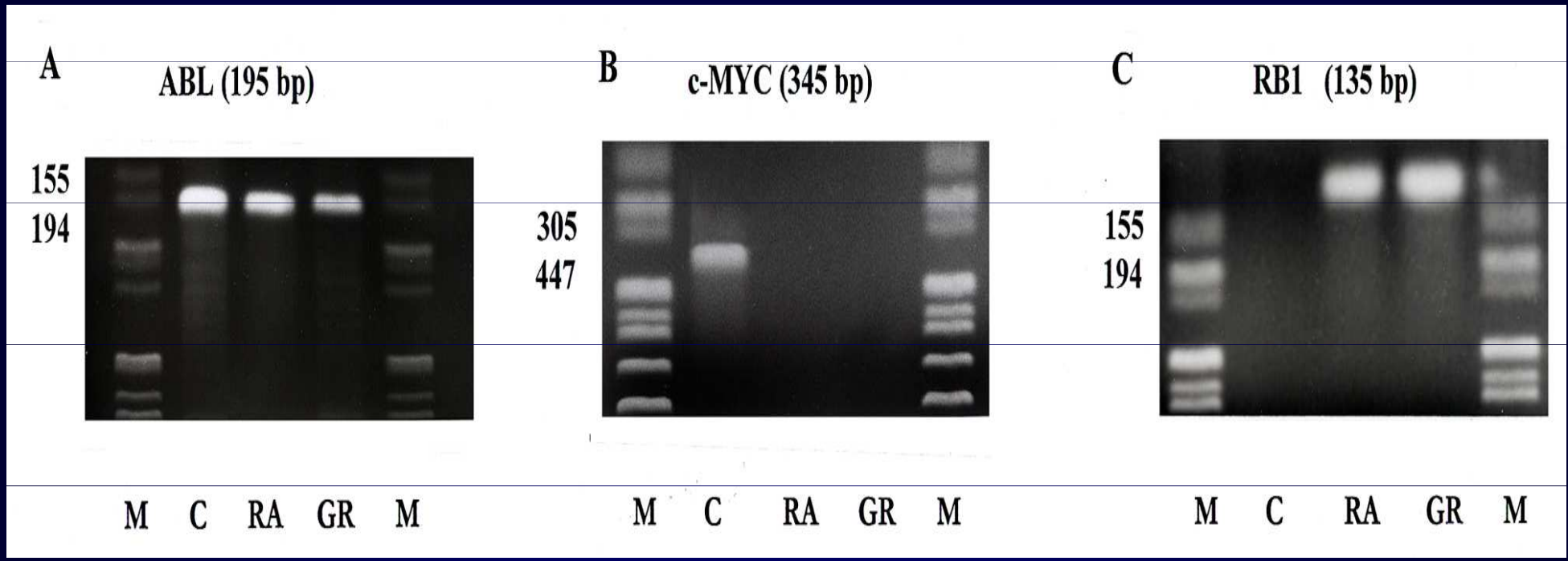
The C-myc Gene Nuclear Location



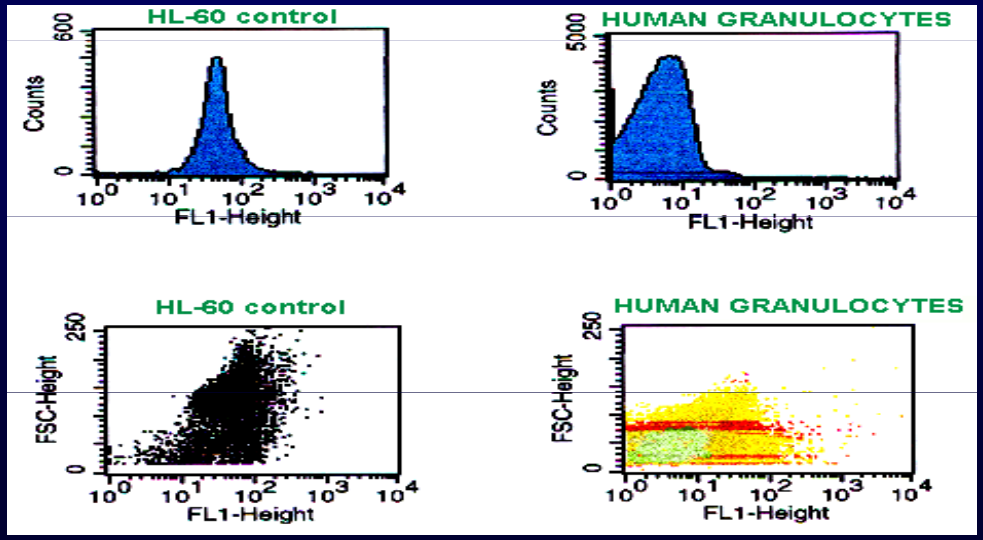




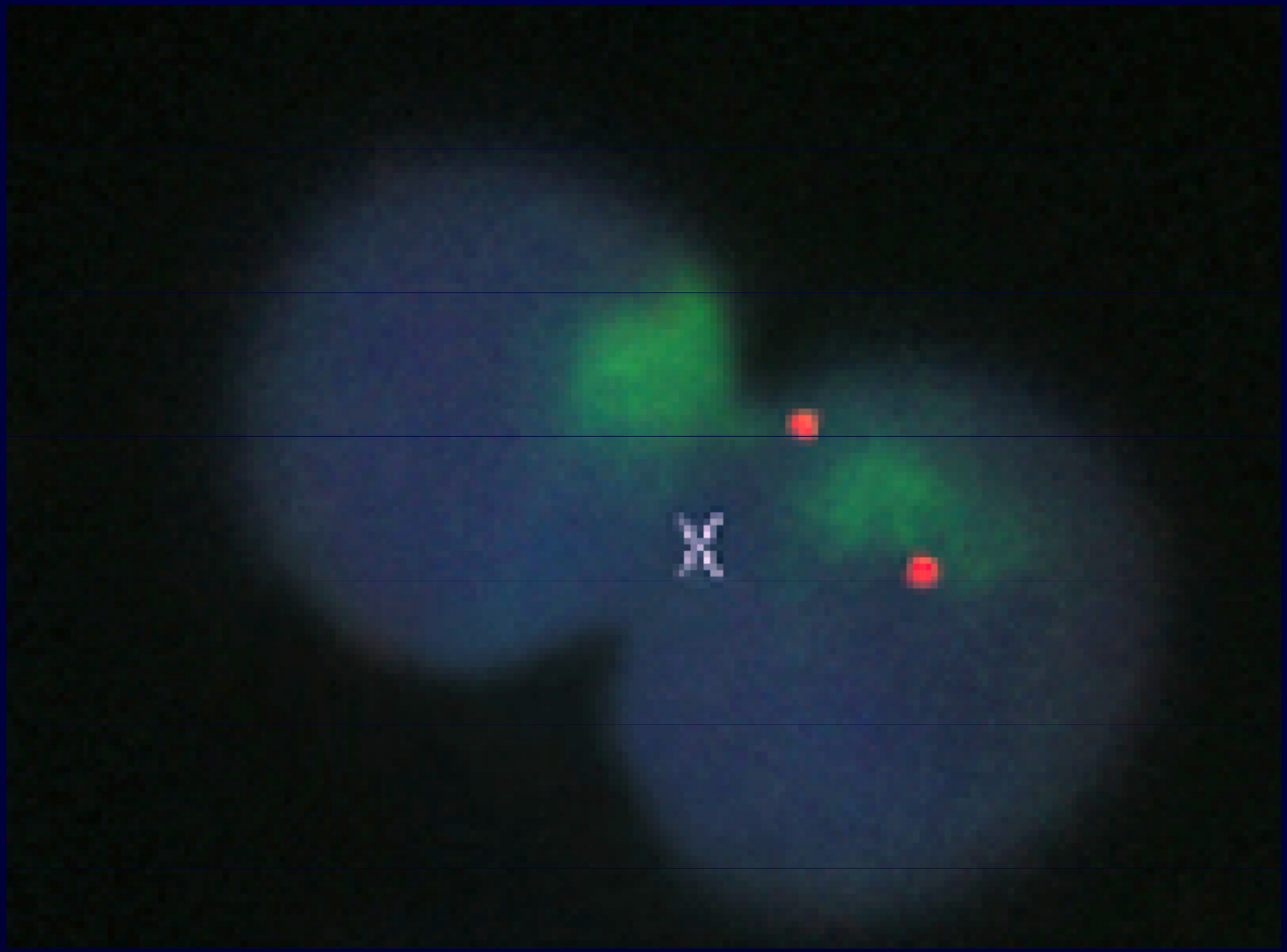
Changes in the expression of selected genes



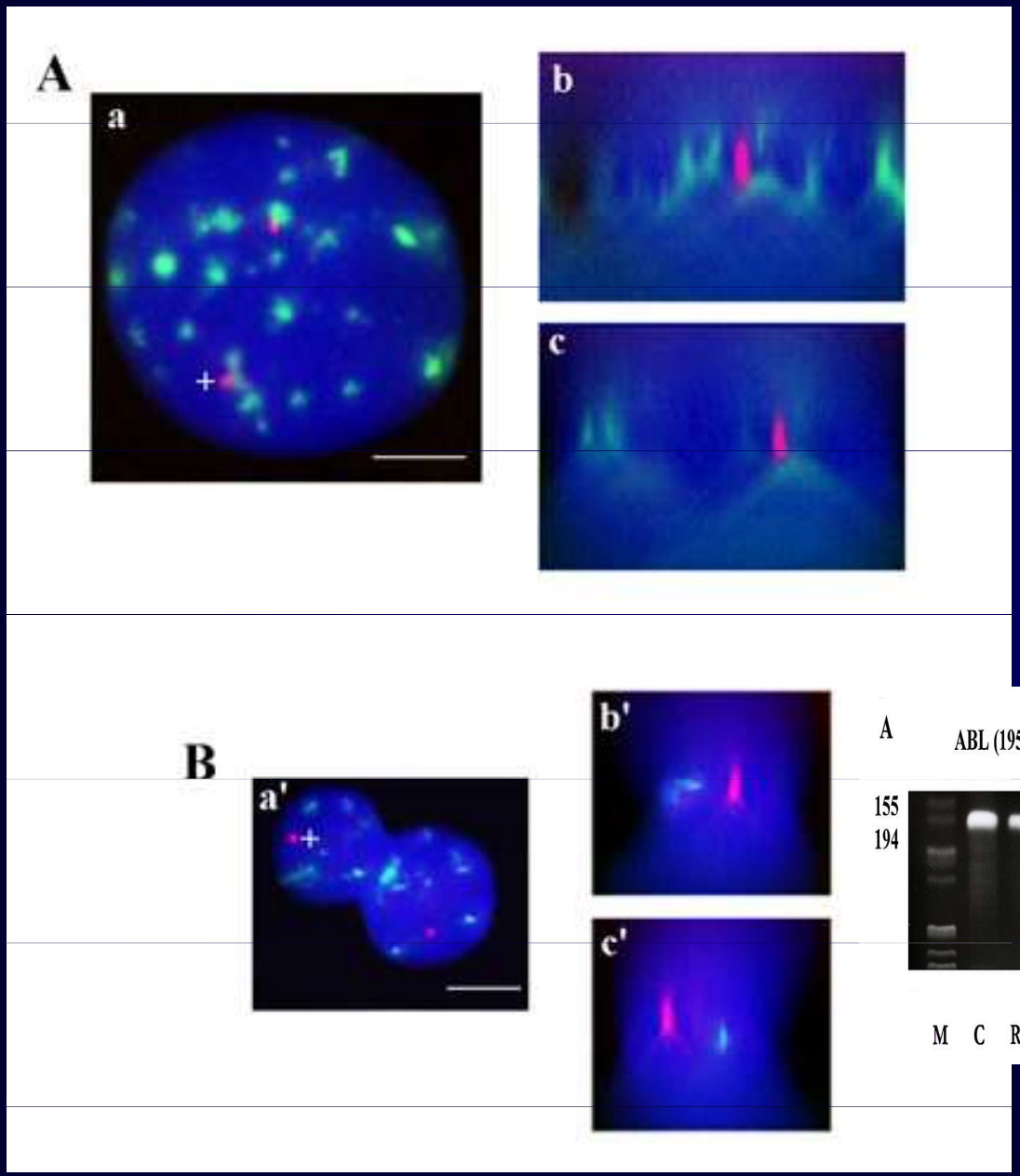
FCM c-myc

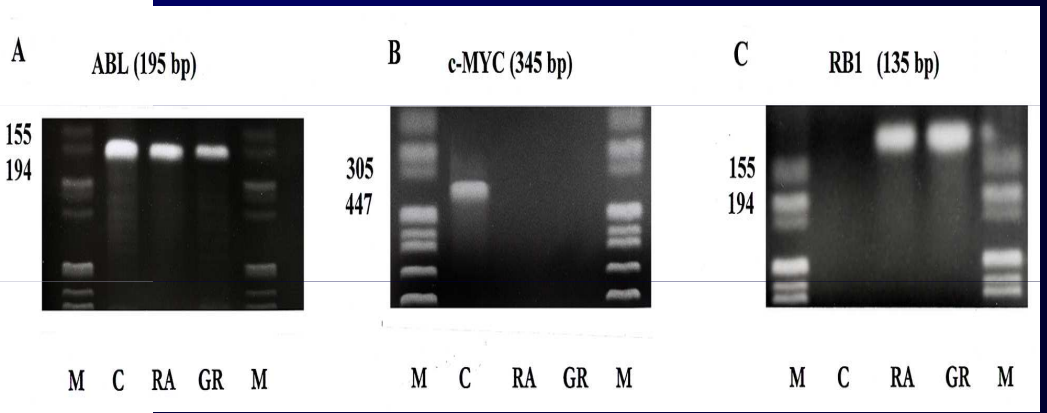


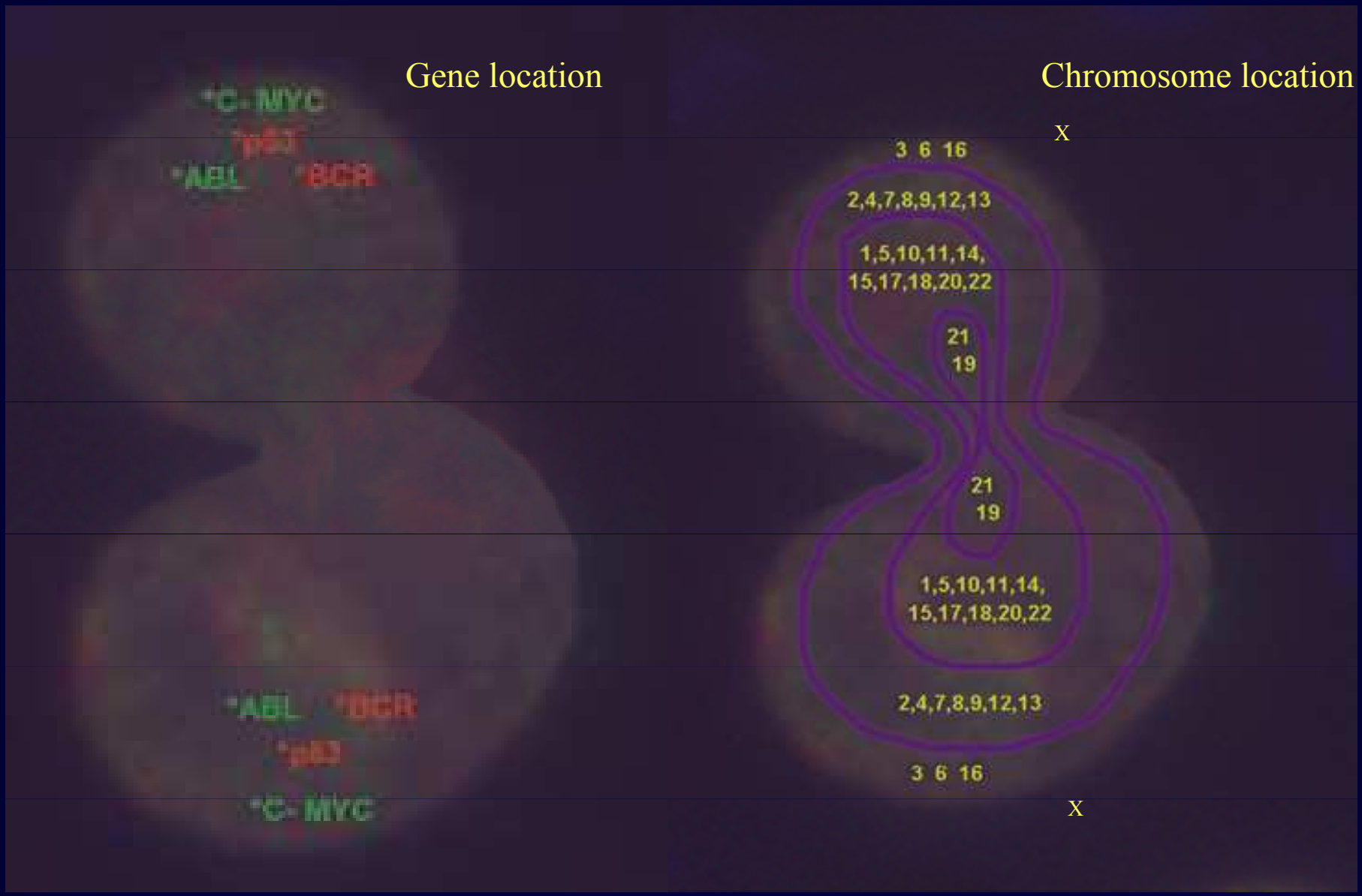
The C-myc gene nuclear topography in granulocytic nuclei



Centromeric silencing






Gene location
Chromosome location
*C-MYC
*P53
*ABL
*BCR
*ABL
*BCR
*P53
*C-MYC
X
3 6 16
2,4,7,8,9,12,13
1,5,10,11,14,
15,17,18,20,22
21
19
21
19
1,5,10,11,14,
15,17,18,20,22
2,4,7,8,9,12,13
3 6 16
X

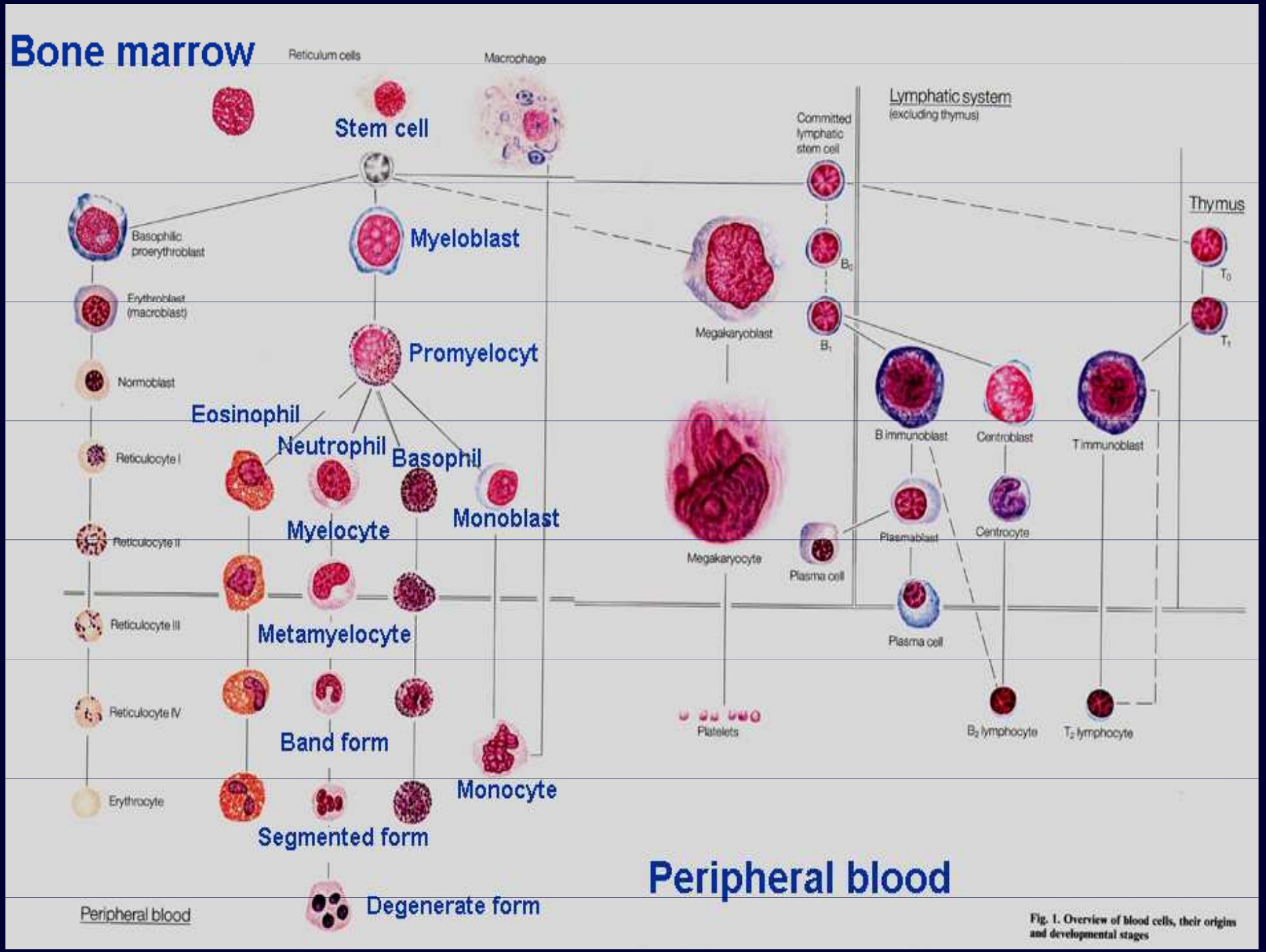
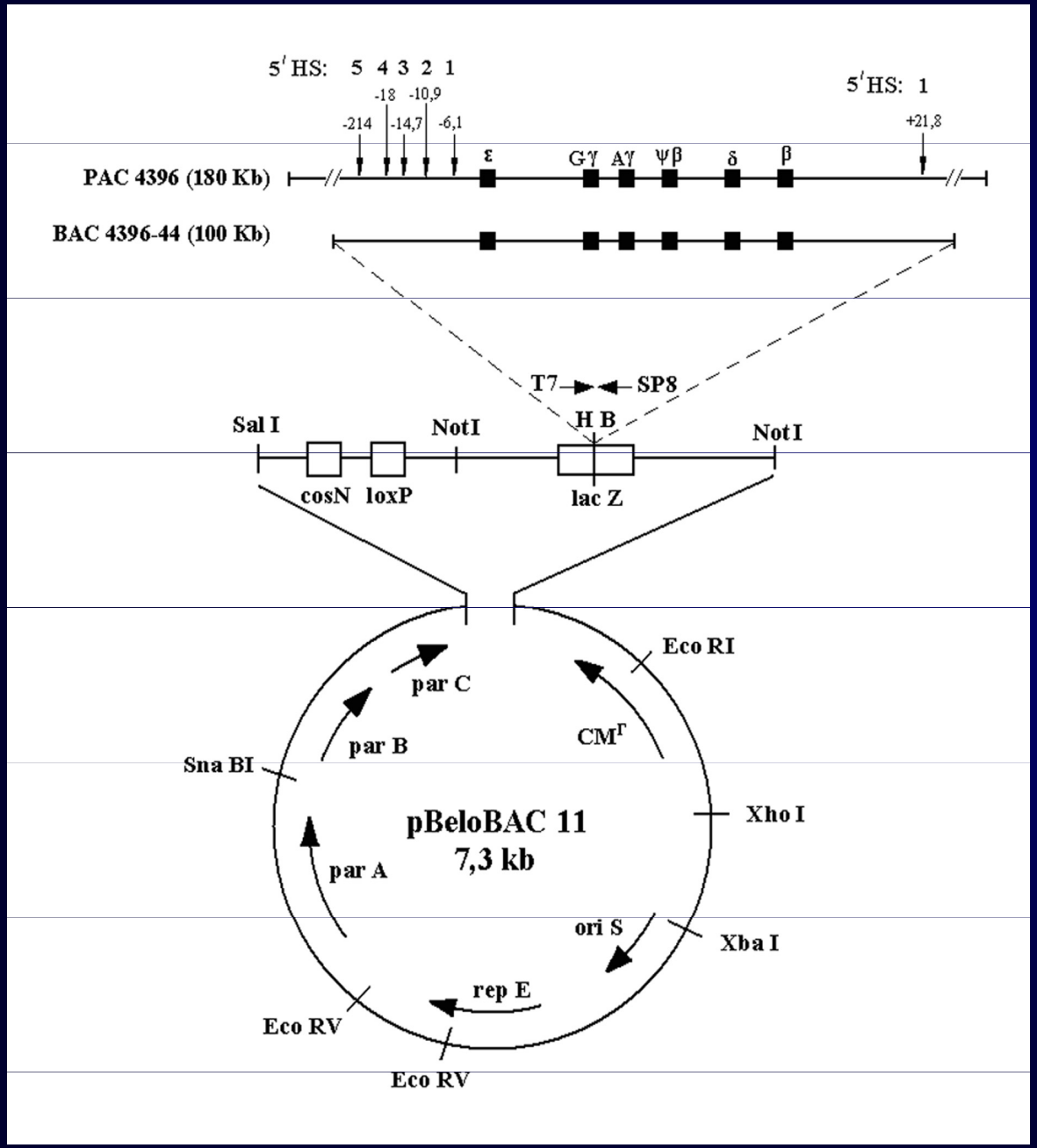


Fig. 1. Overview of blood cells, their origins and developmental stages

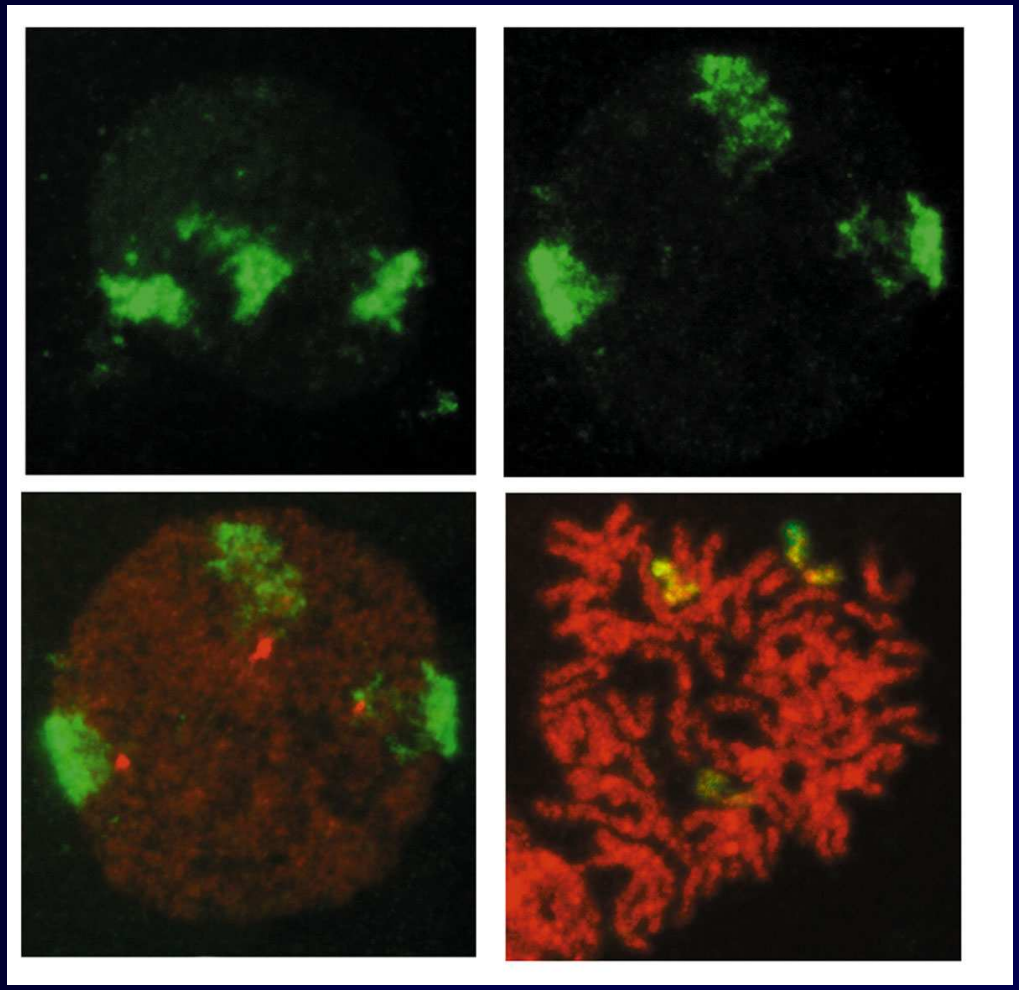
Beta-like globin gene cluster

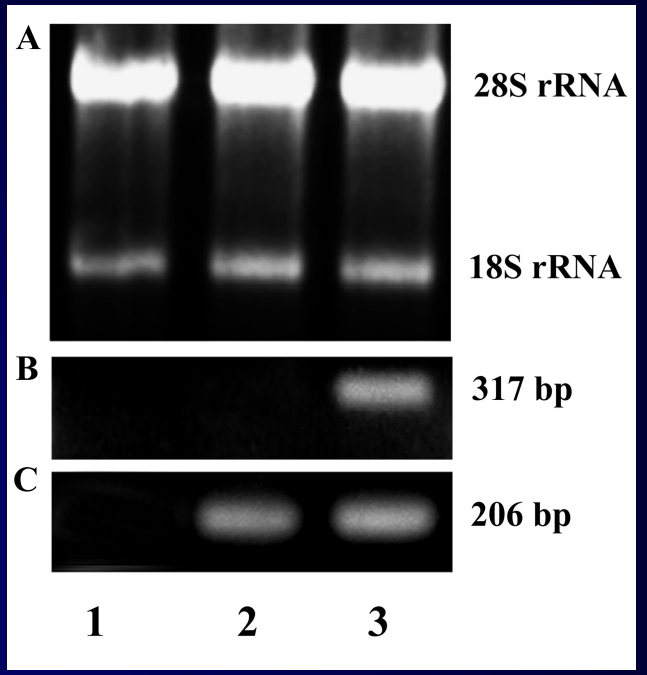


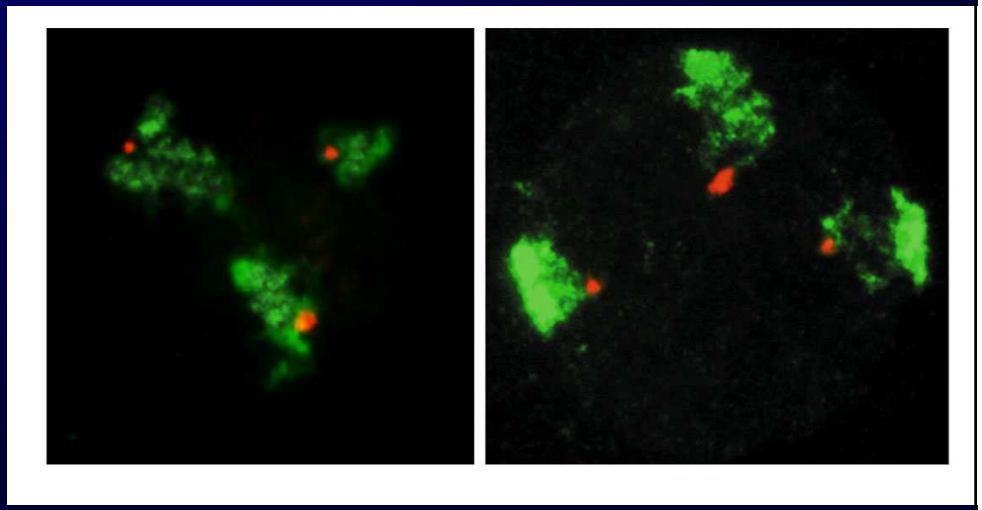
Arrayed on chromosome 11, encodes one embryonic (ε) and two fetal (Gγ, Aγ) and two adult (δ, β) globin chains. Expression of β-like genes undergoes a developmental related switching mechanism:
ε: expressed in early embryo
fetal γ: fetal life.
δ, β: adulthood.

Changes in β-like gene expression acompany erythoid cell differentiation

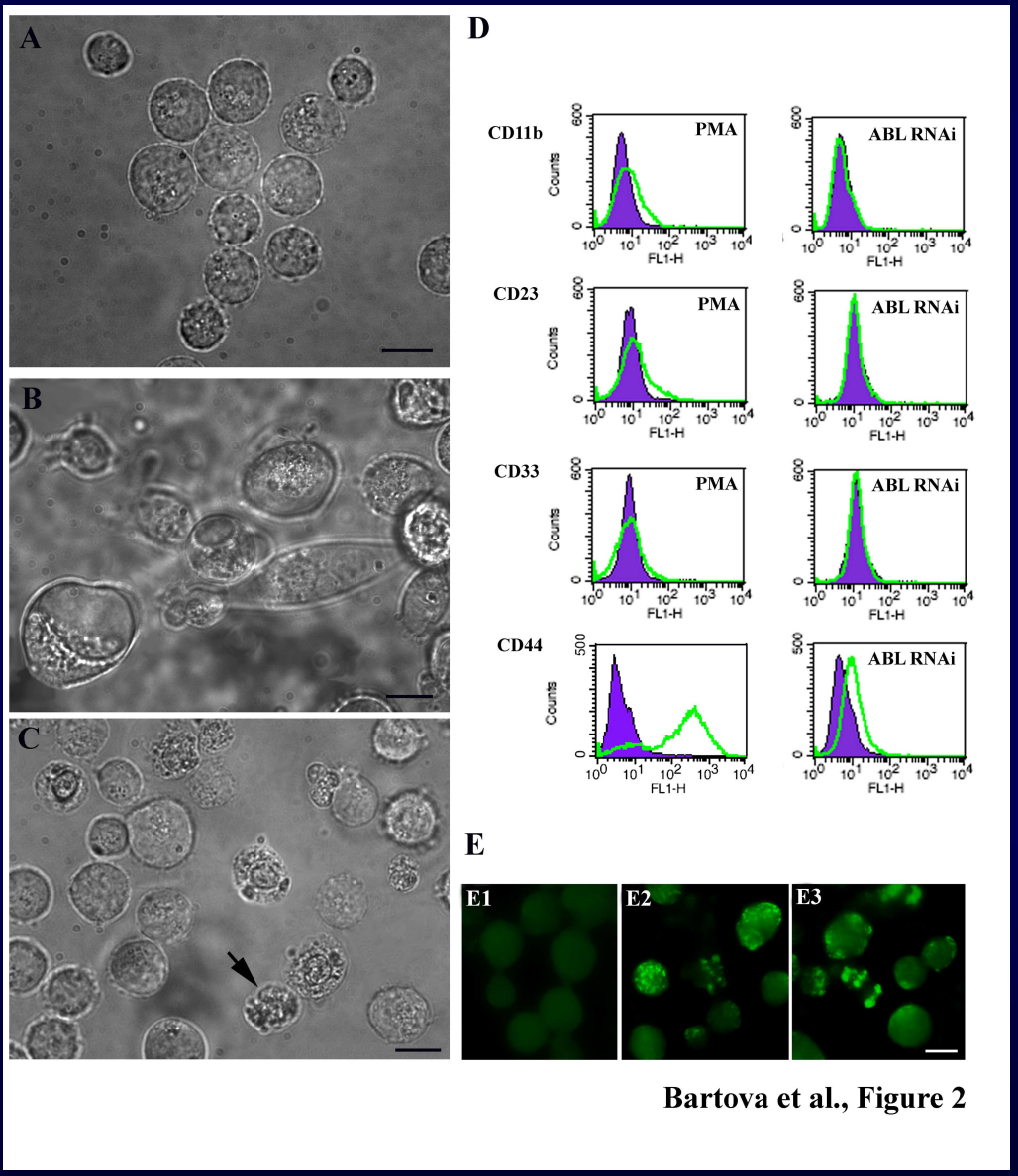
Differentiation of human hemopoietic cells into erythroid pathway





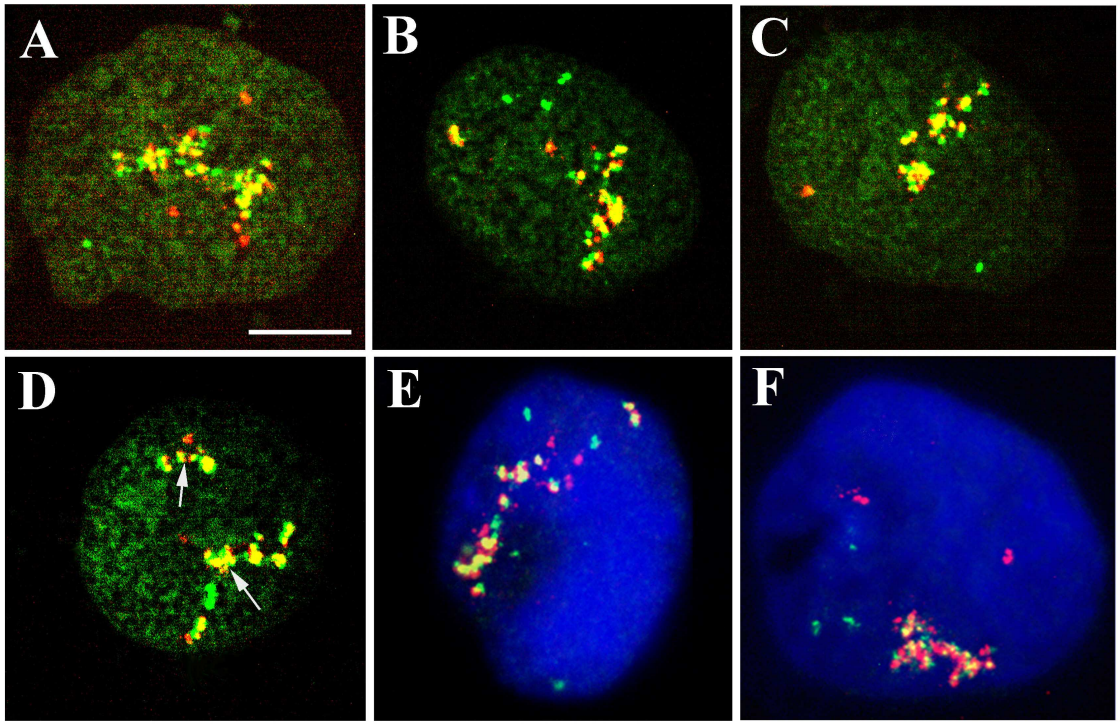


Differentiation of human hemopoietic cells into megakaryocytes

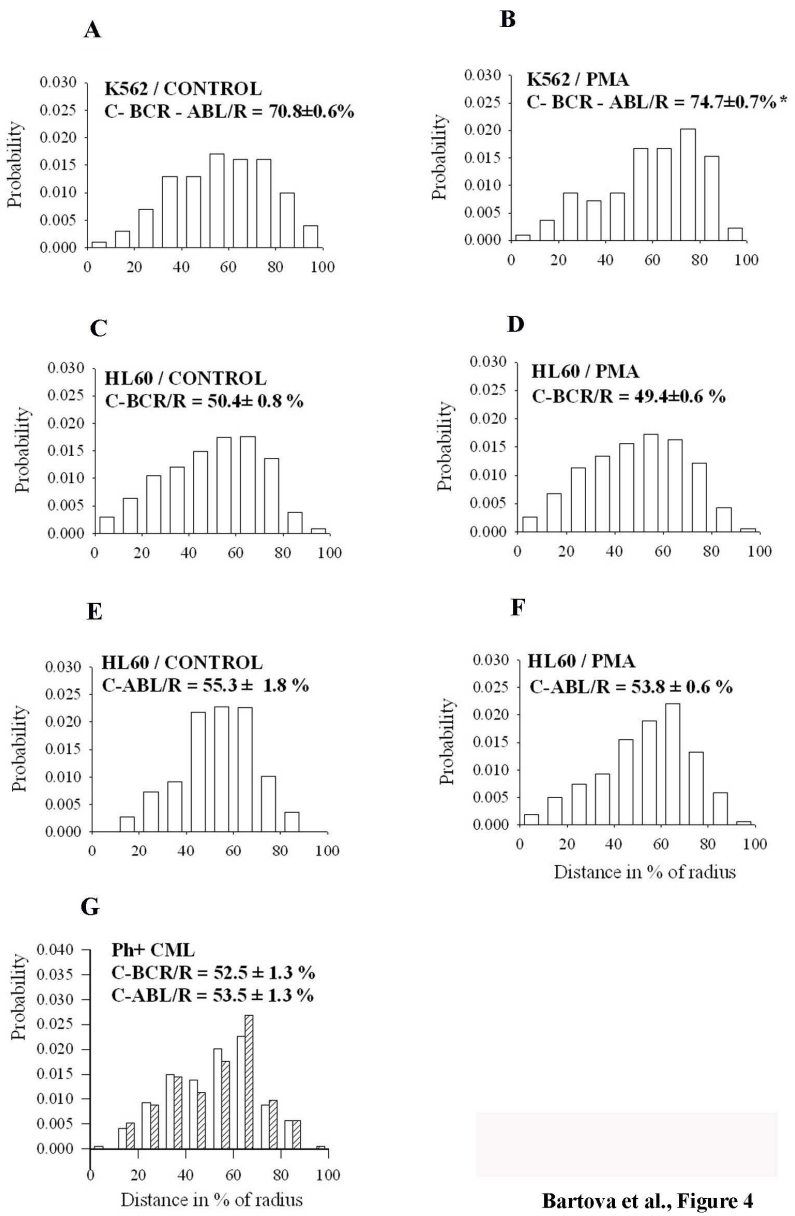


Bartova et al., Figure 2

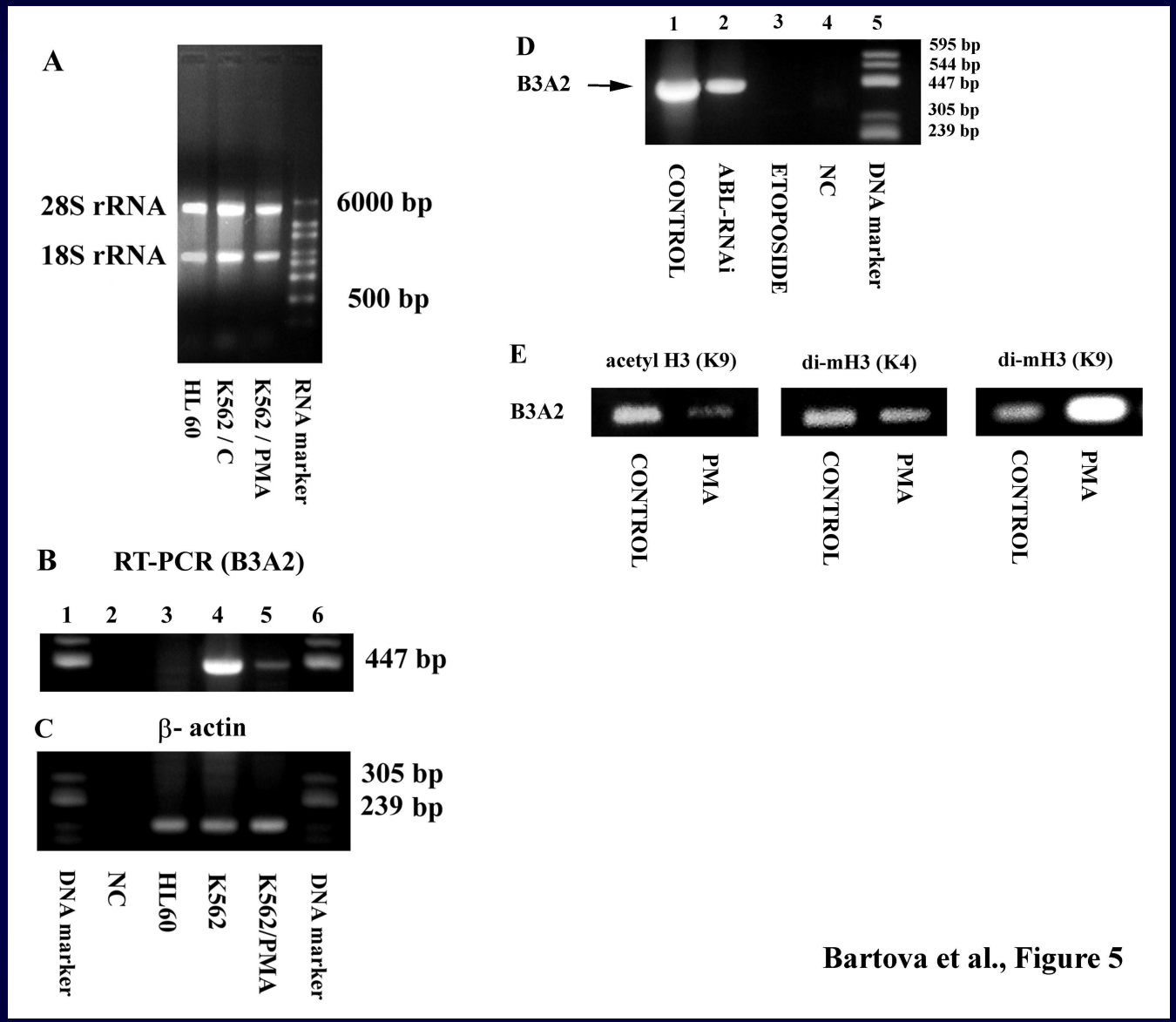
BCR (red signals) and ABL genes (green signals)



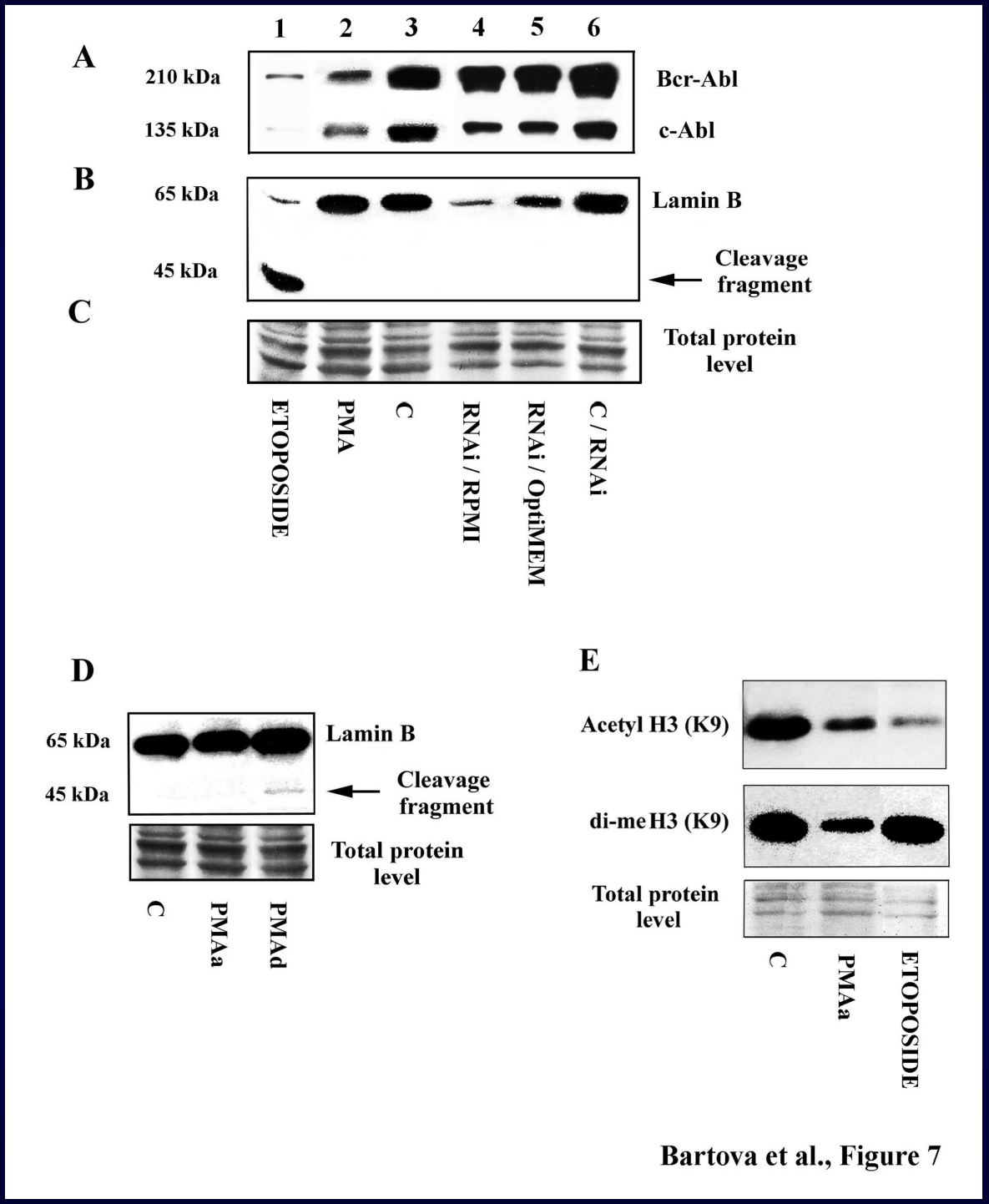
Bartova et al., Figure 3



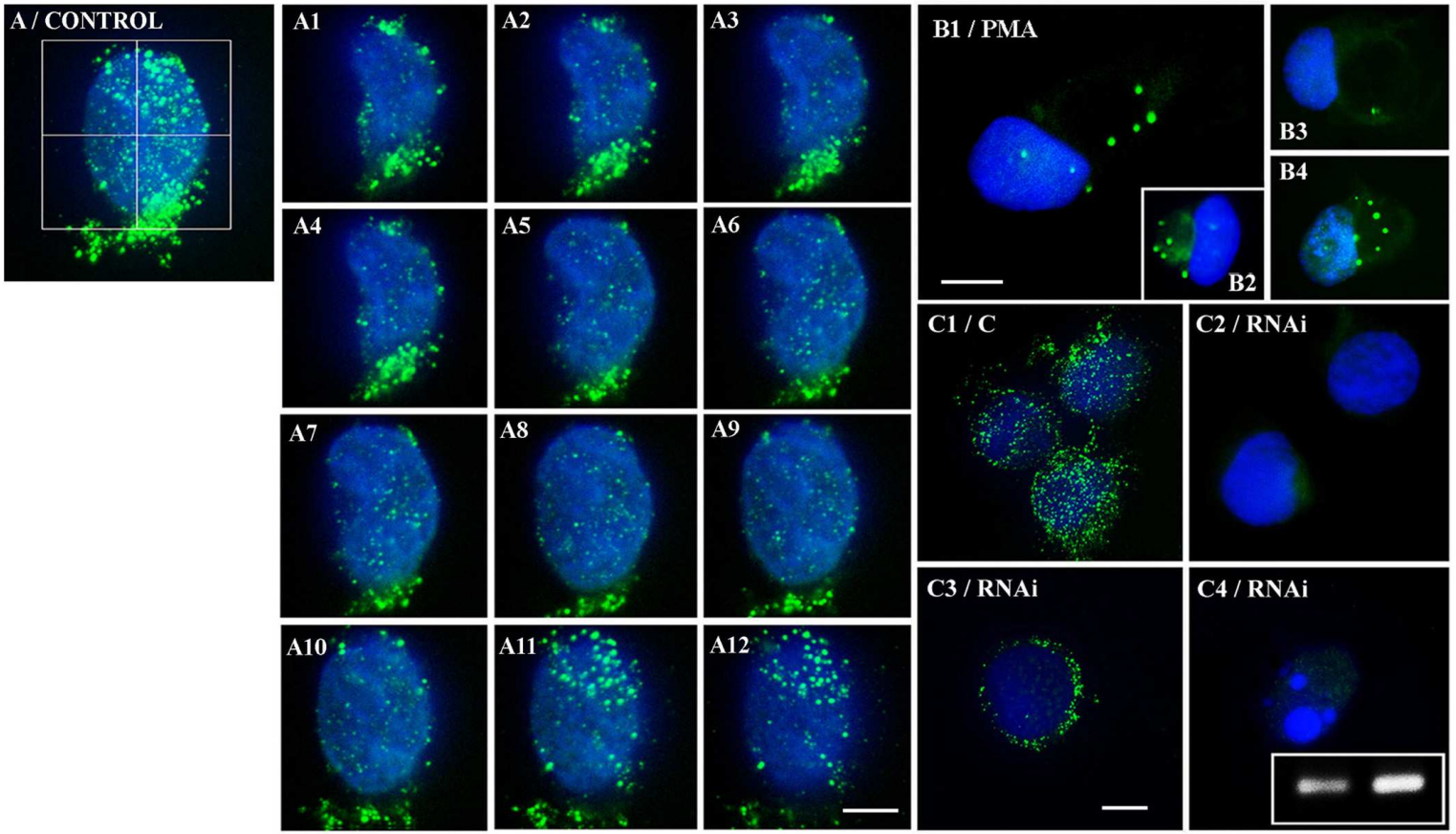
Bartova et al., Figure 4



Bartova et al., Figure 5



Bartova et al., Figure 7



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 a množství jejich proteinů. Tyto všechny uvedené buněčné faktory určují vznik daného buněčného typu.