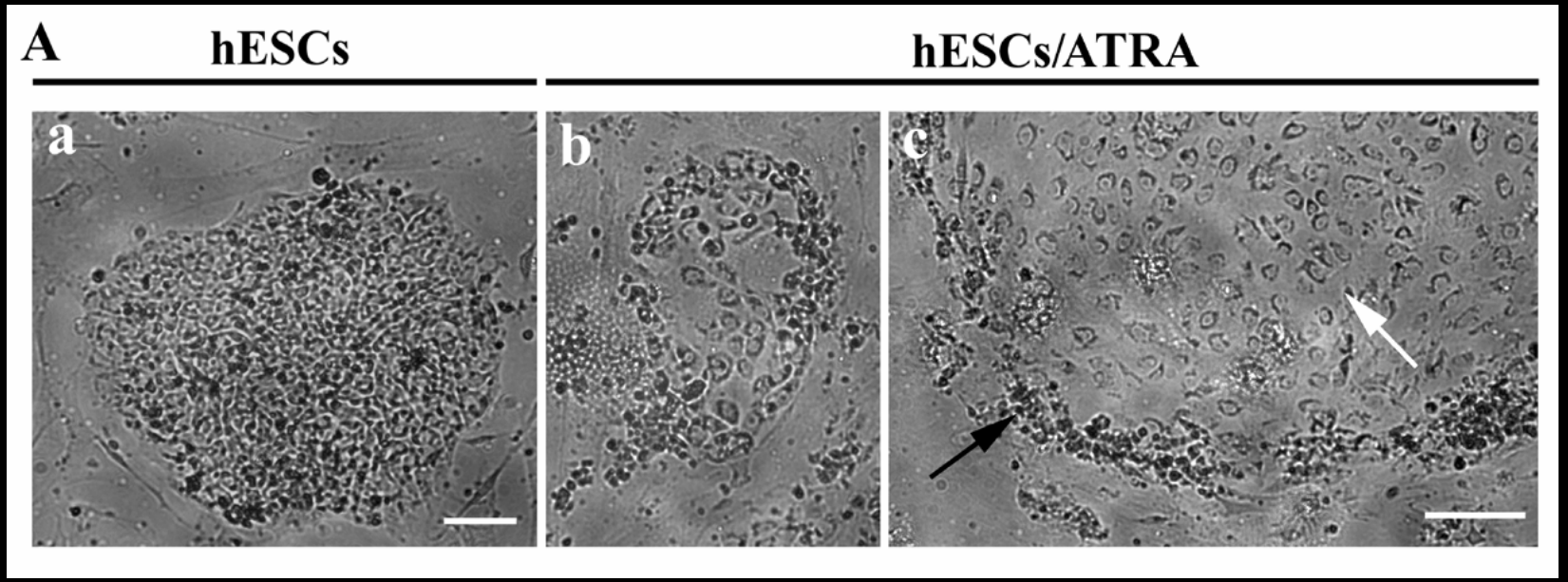
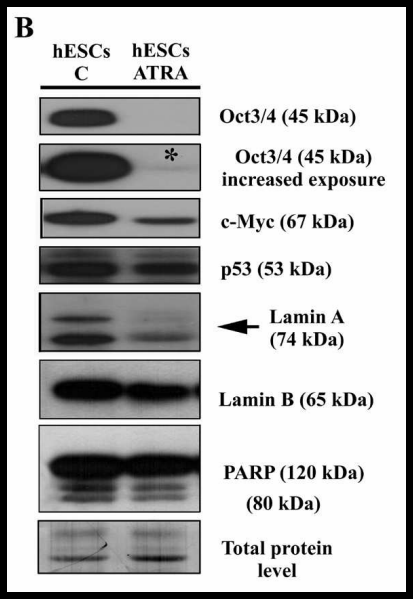
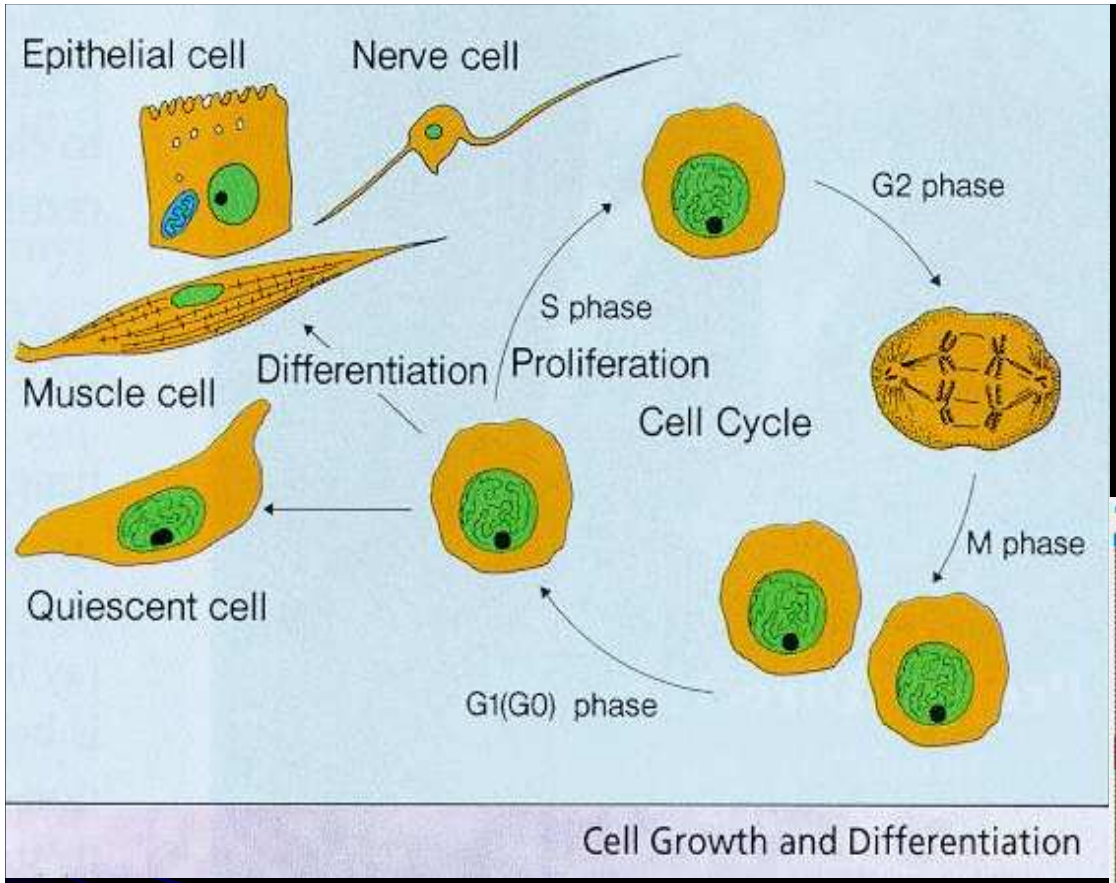
Diferenciace buněk a struktura chromatinu

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

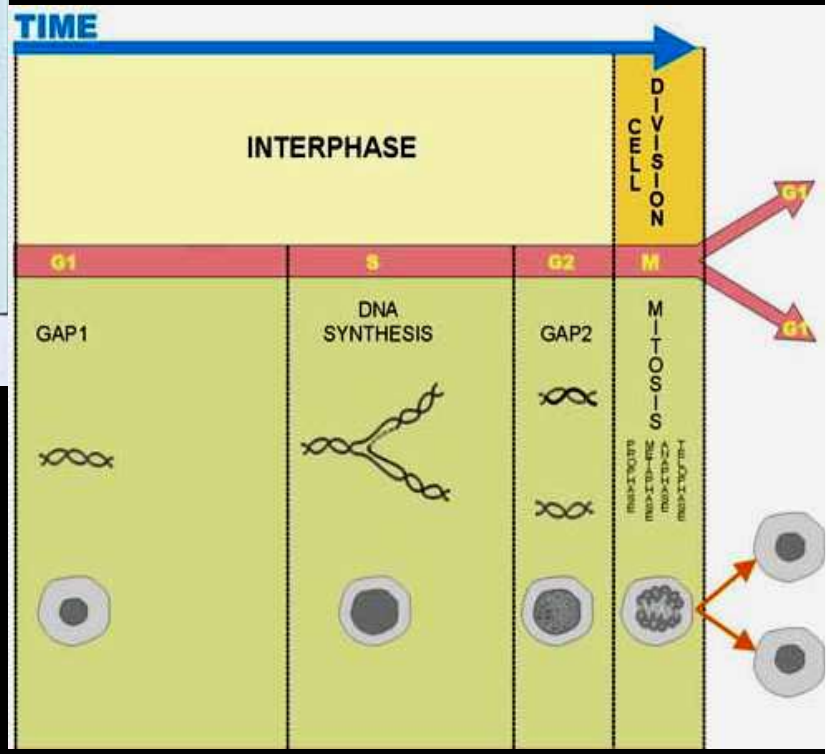
Morfologie lidských ES buněk





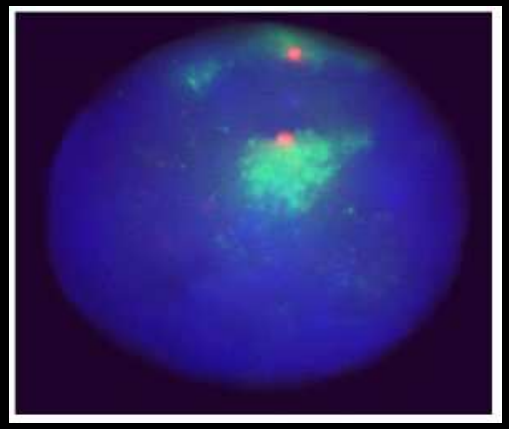


Cell Differentiation
Cell Growth



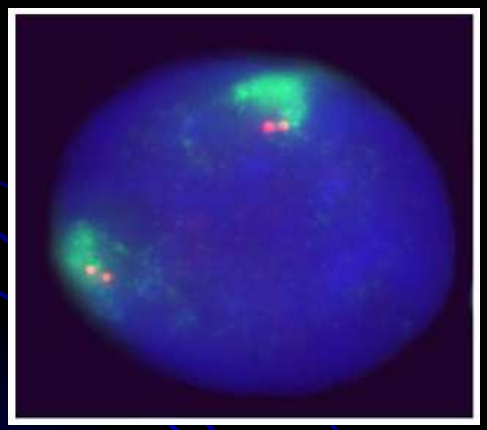
DNA/Histone synthesis

G1

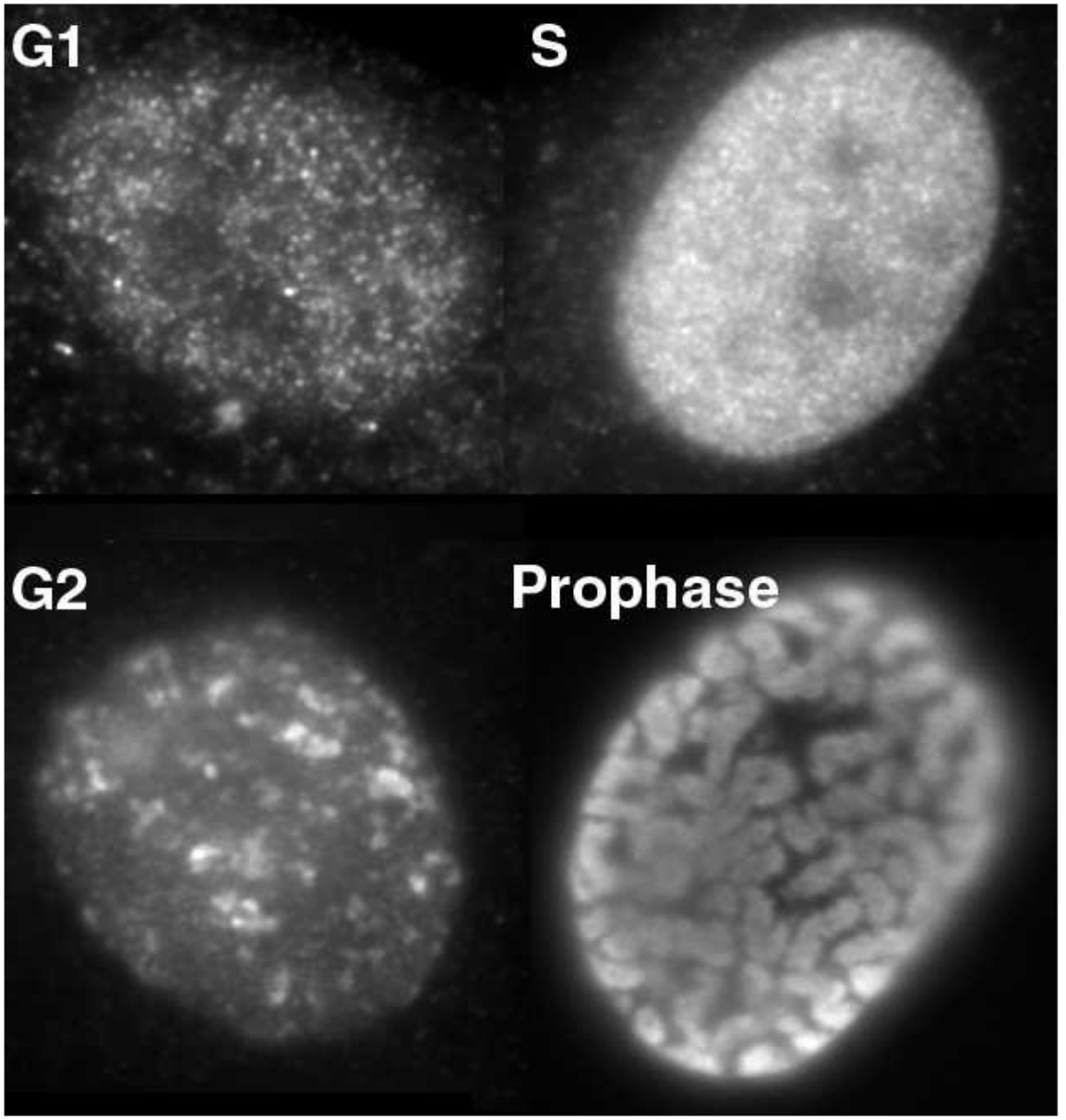


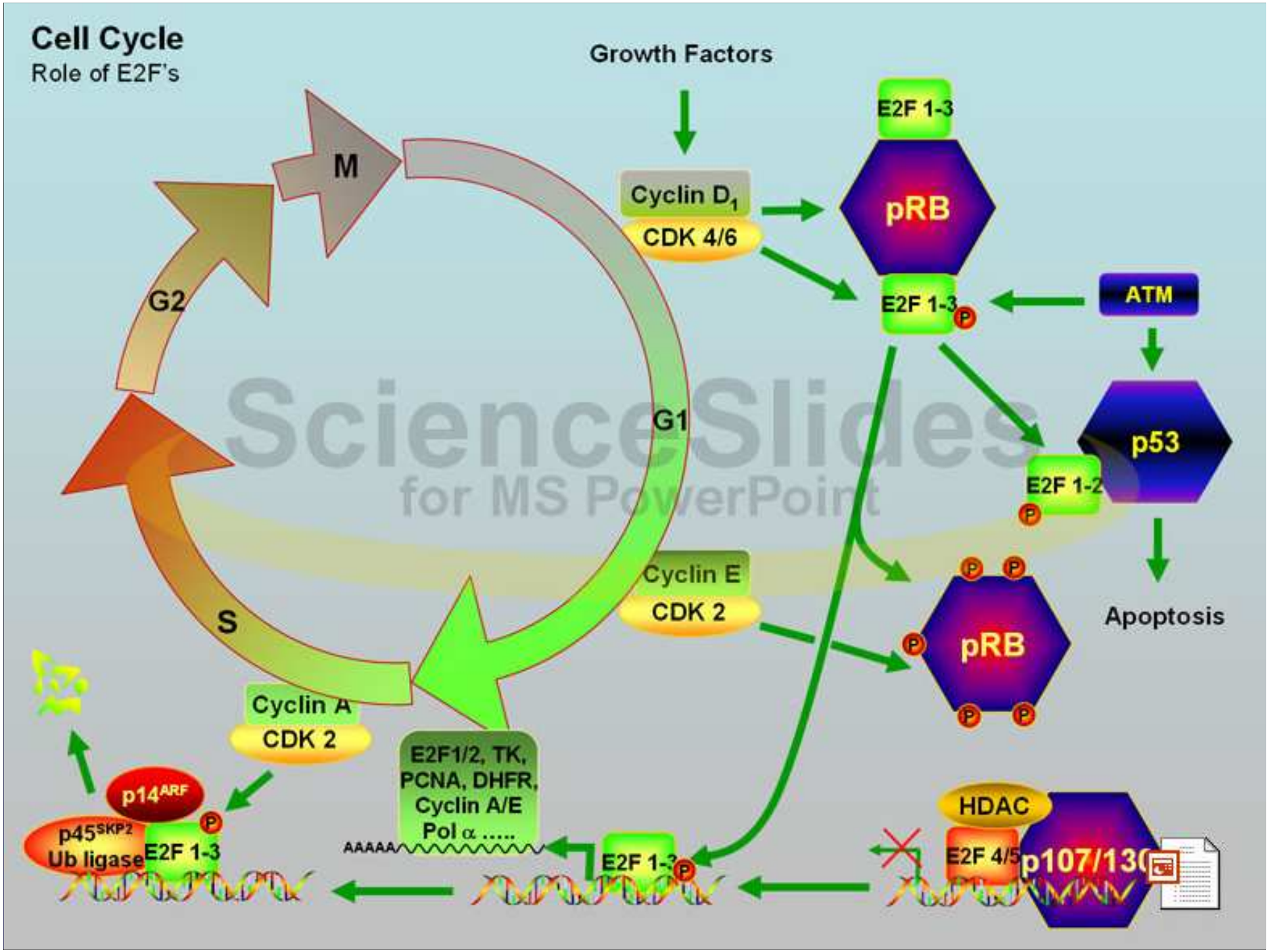
Rb1 gene

G2

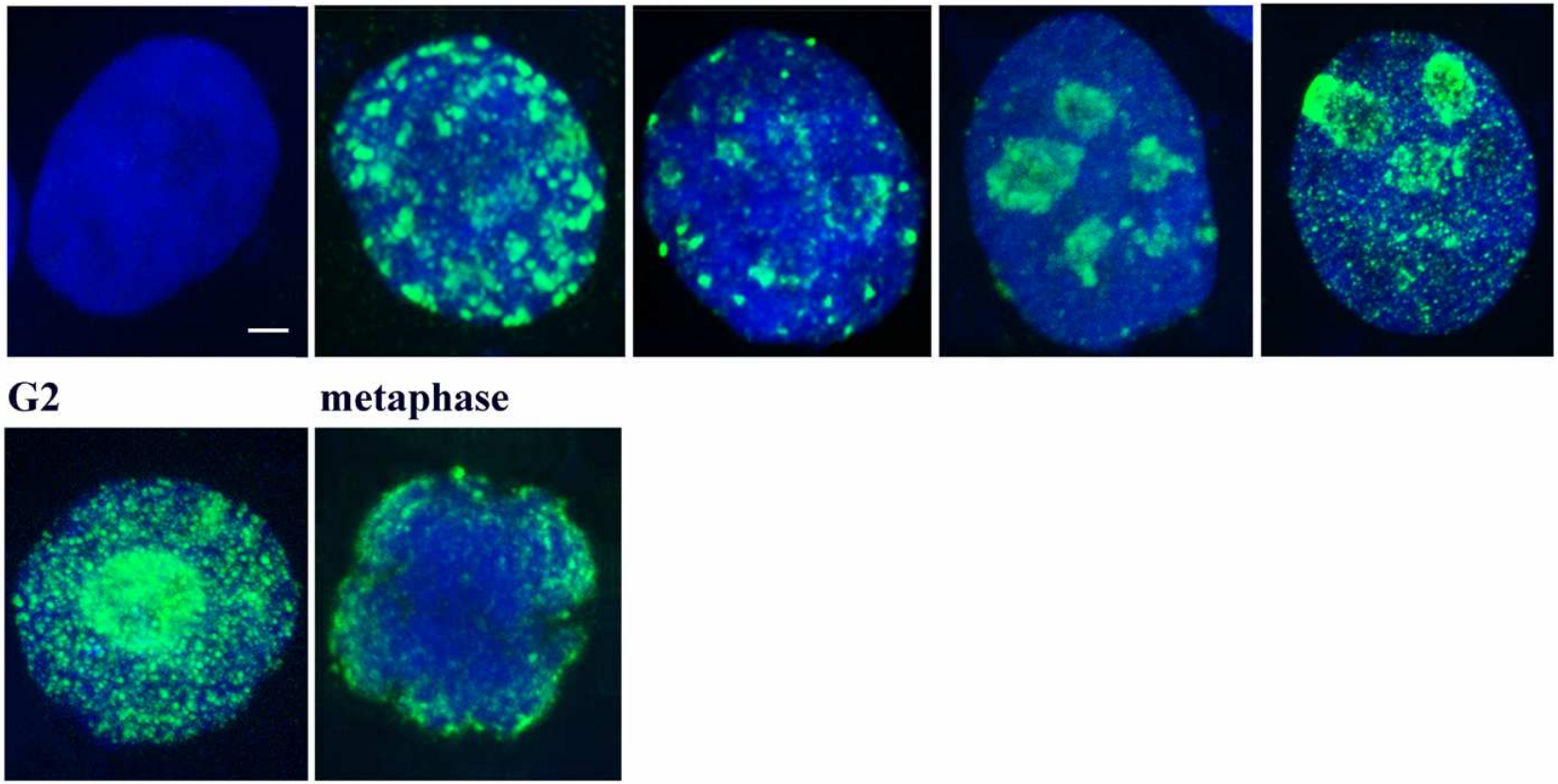


Anti-phospho H3

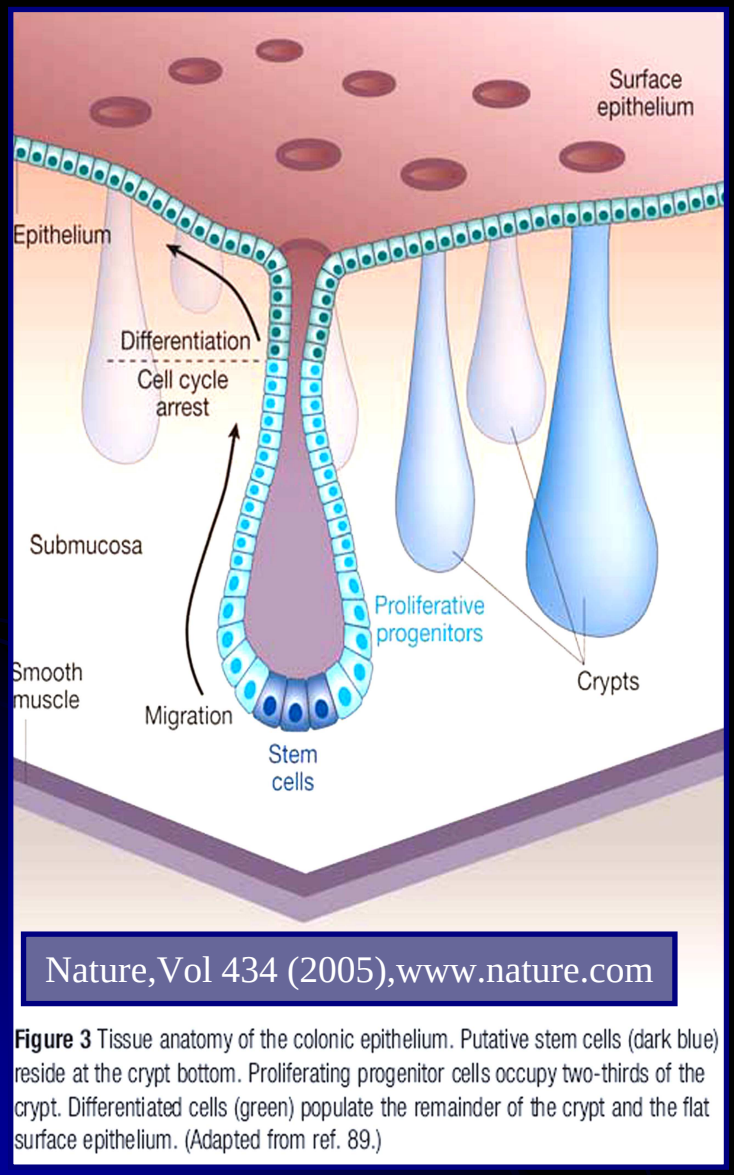



Cell Cycle
Role of E2F's
Growth Factors
M
G2
G1
S
Cyclin D1
CDK 4/6
E2F 1-3
pRB
E2F 1-3
P
ATM
p53
E2F 1-2
P
Apoptosis
Cyclin E
CDK 2
pRB
Cyclin A
CDK 2
E2F1/2, TK,
PCNA, DHFR,
Cyclin A/E
Pol α .....
AAAAA
p14ARF
p45SKP2
Ub ligase
E2F 1-3
E2F 1-3
HDAC
E2F 4/5
p107/130
ScienceSlides
for MS PowerPoint

pKi-67

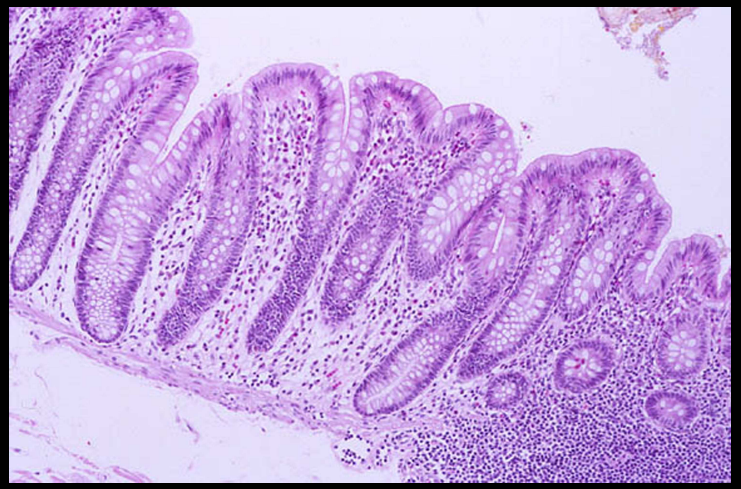


Enterocytic Cell Differentiation

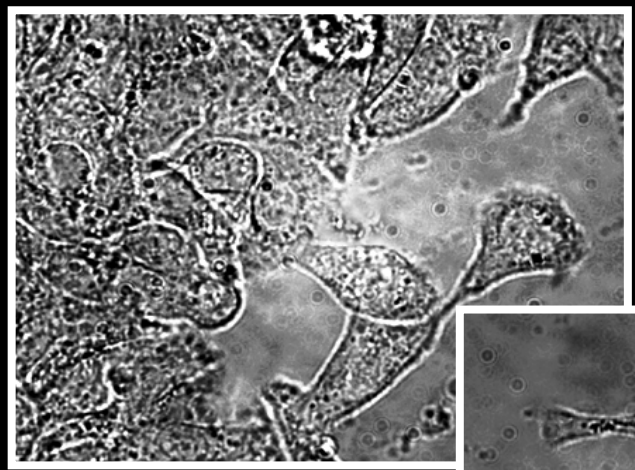


Nature,Vol 434 (2005),www.nature.com

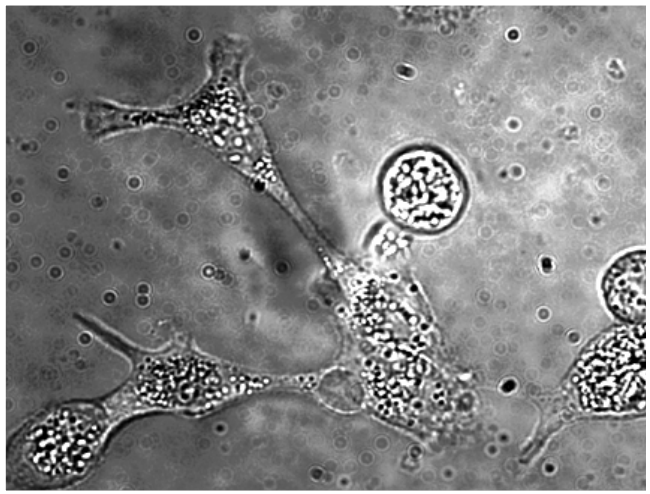
Figure 3 Tissue anatomy of the colonic epithelium. Putative stem cells (dark blue) reside at the crypt bottom. Proliferating progenitor cells occupy two-thirds of the crypt. Differentiated cells (green) populate the remainder of the crypt and the flat surface epithelium. (Adapted from ref. 89.)



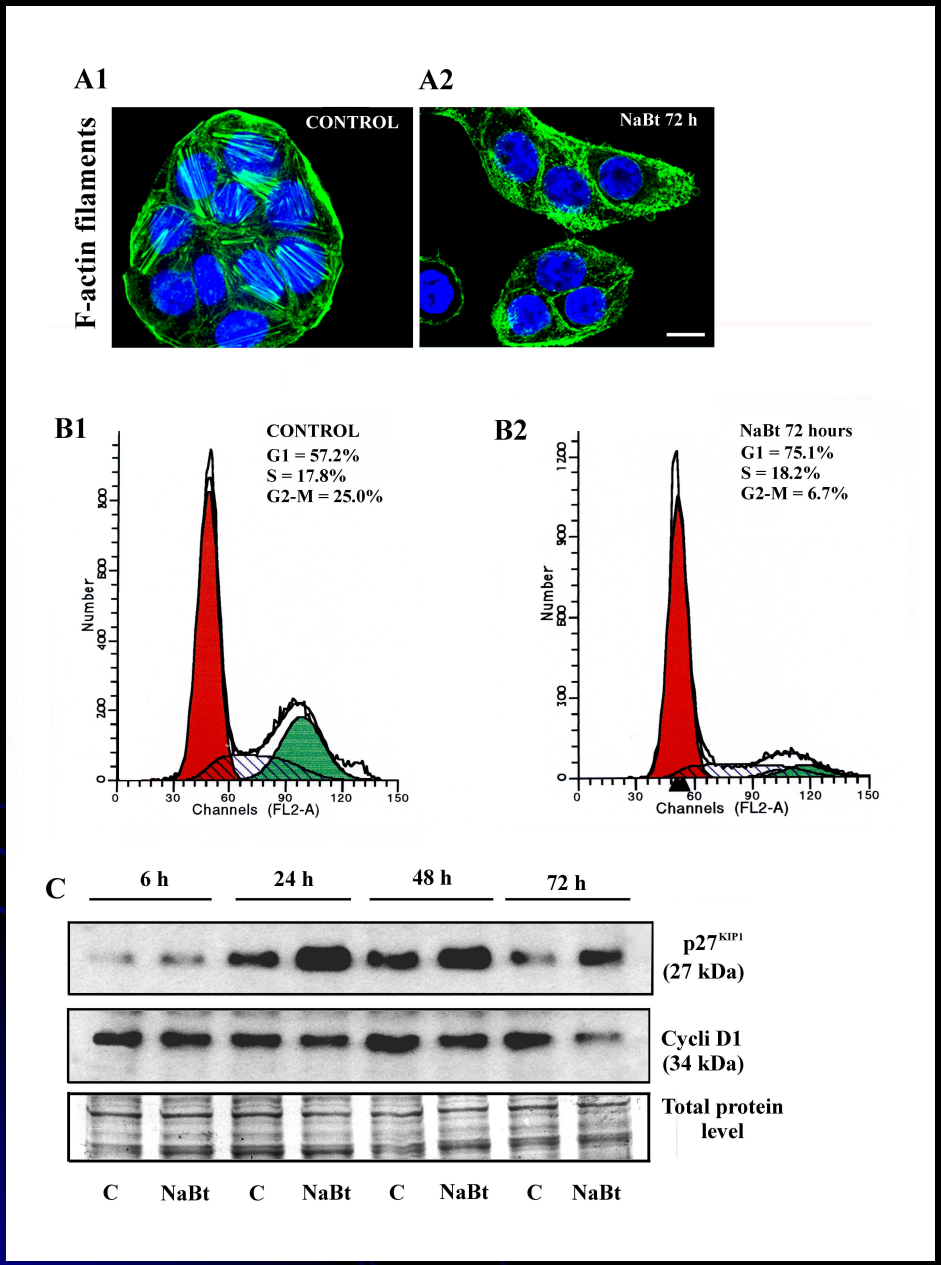
Control



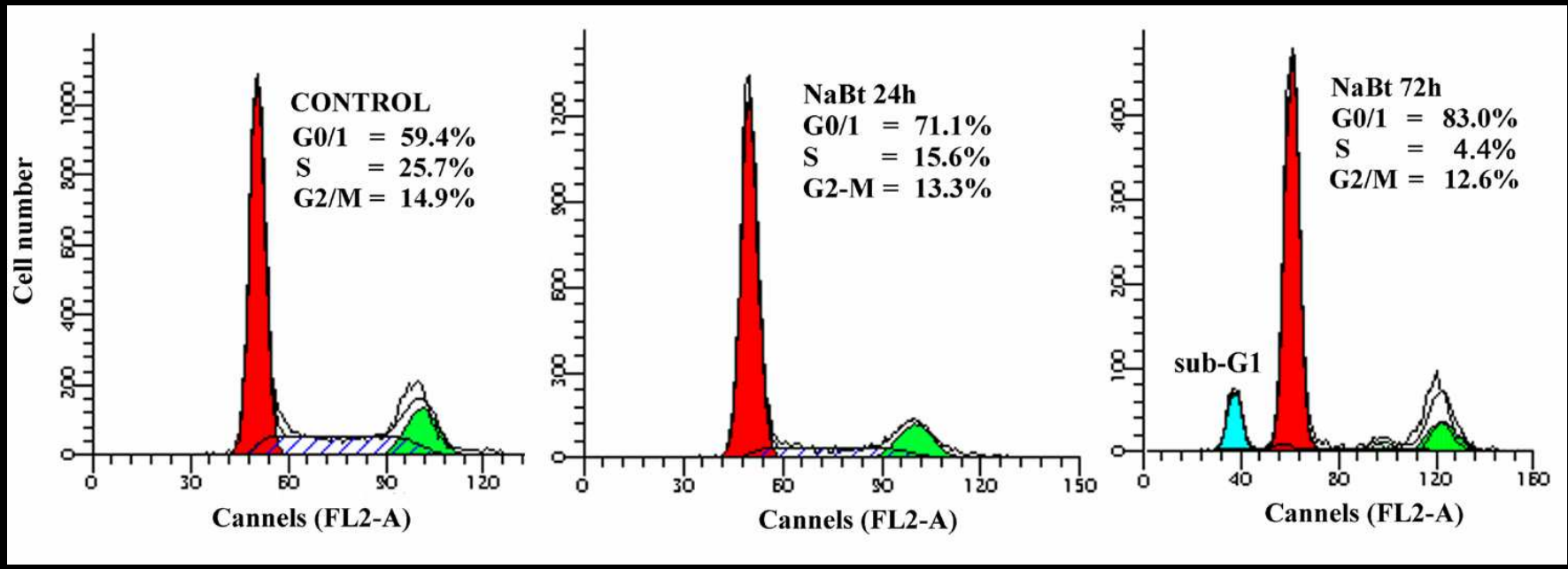
NaBt



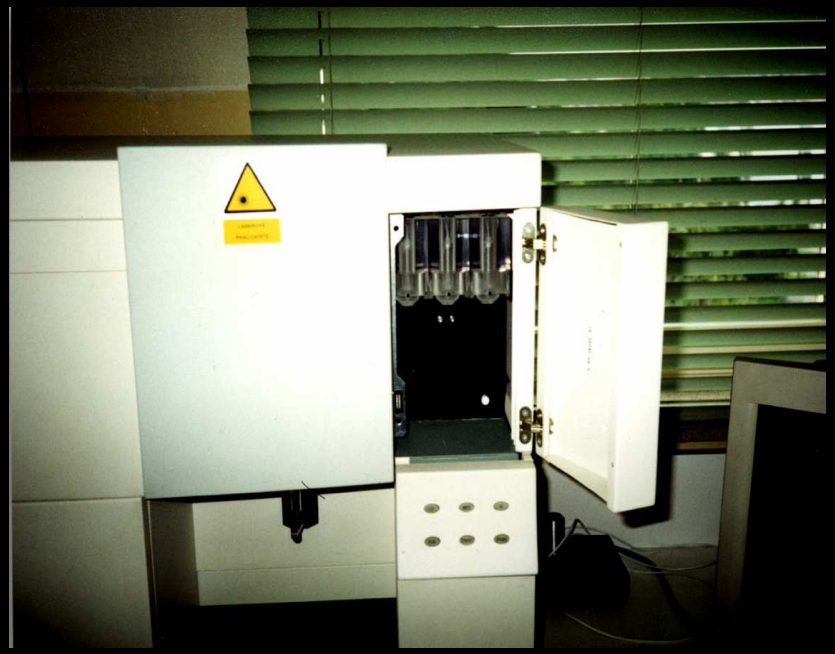
Enterocytic cell differentiation

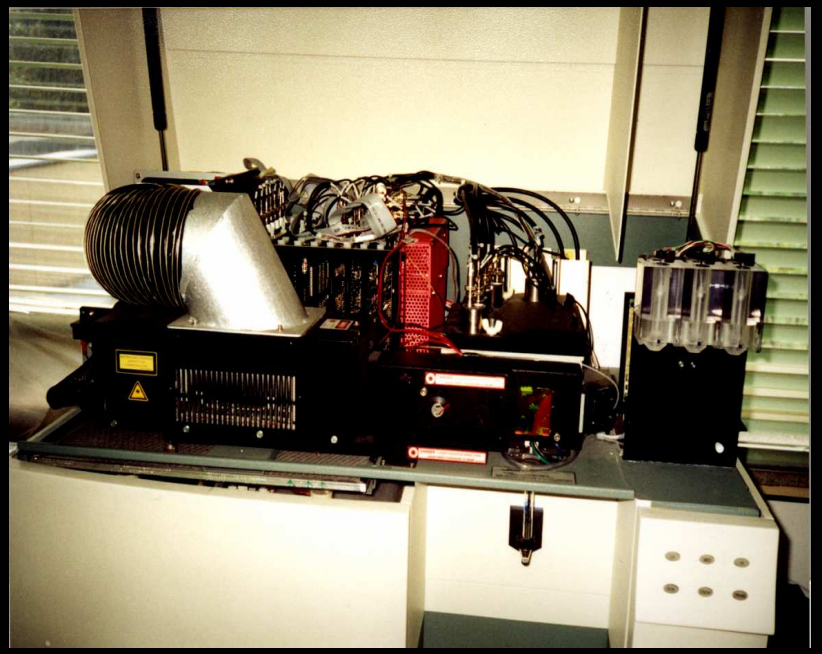


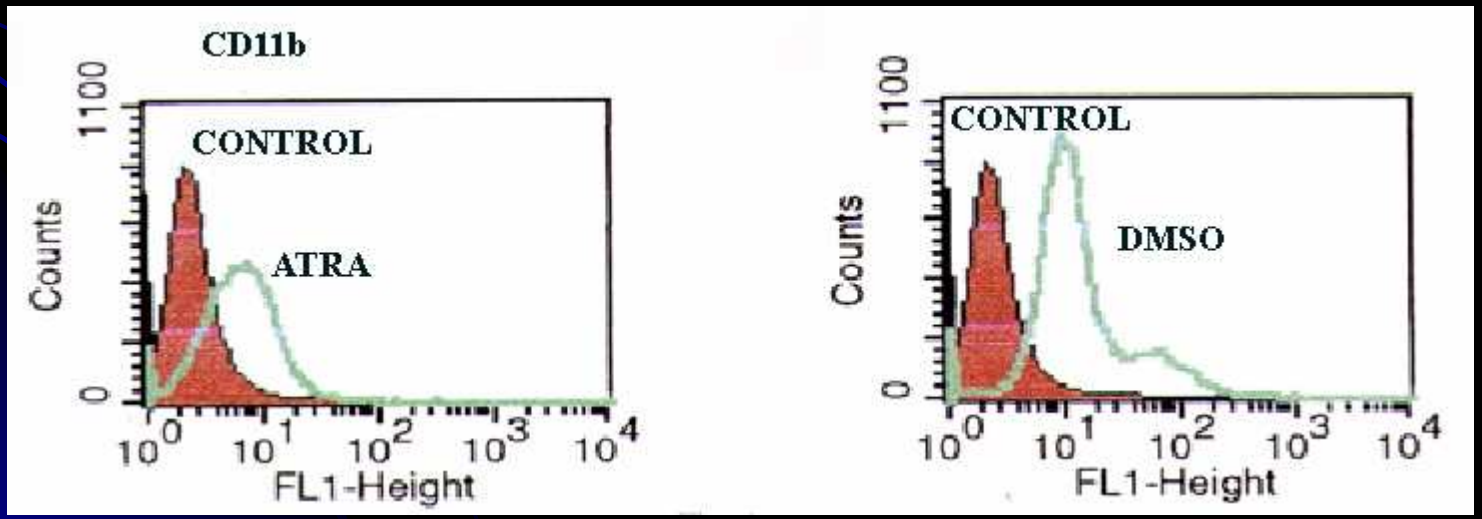
Harničarová et al., 2005

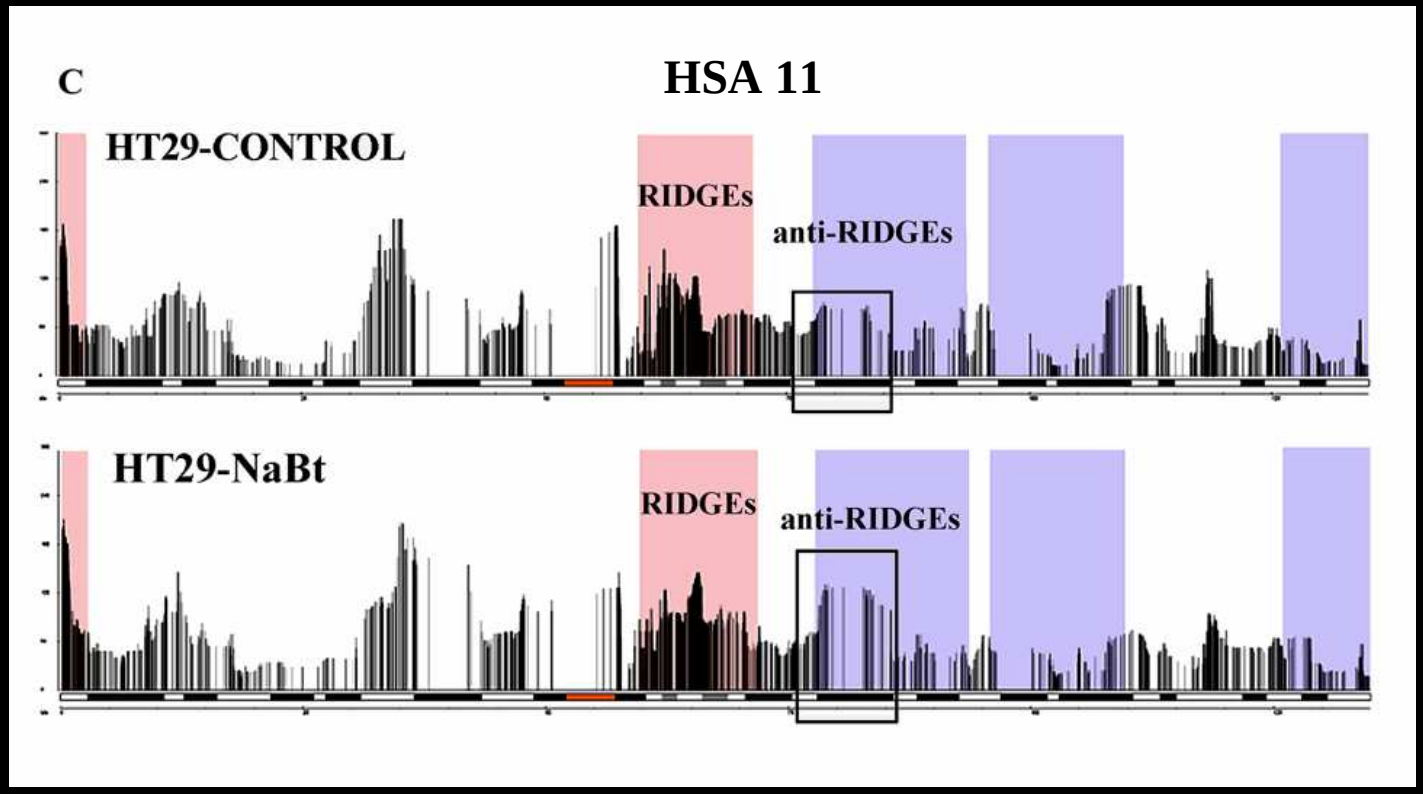

Cell number
CONTROL
G0/1 = 59.4%
S = 25.7%
G2/M = 14.9%
Cannels (FL2-A)
NaBt 24h
G0/1 = 71.1%
S = 15.6%
G2-M = 13.3%
Cannels (FL2-A)
NaBt 72h
G0/1 = 83.0%
S = 4.4%
G2/M = 12.6%
sub-G1
Cannels (FL2-A)

Flow Cytometry

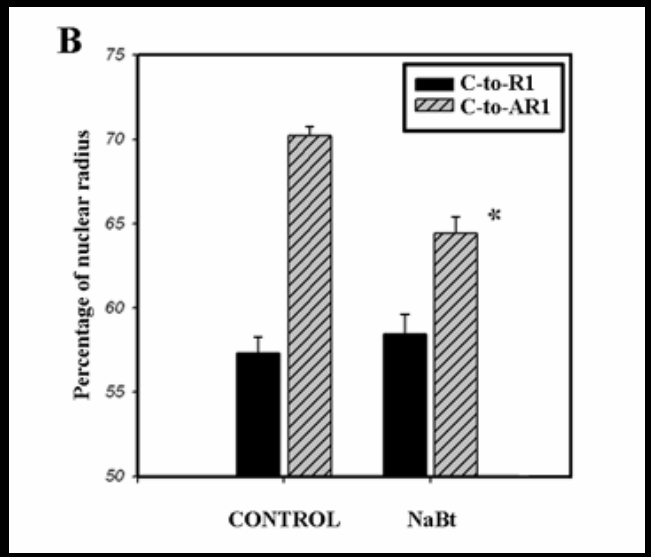






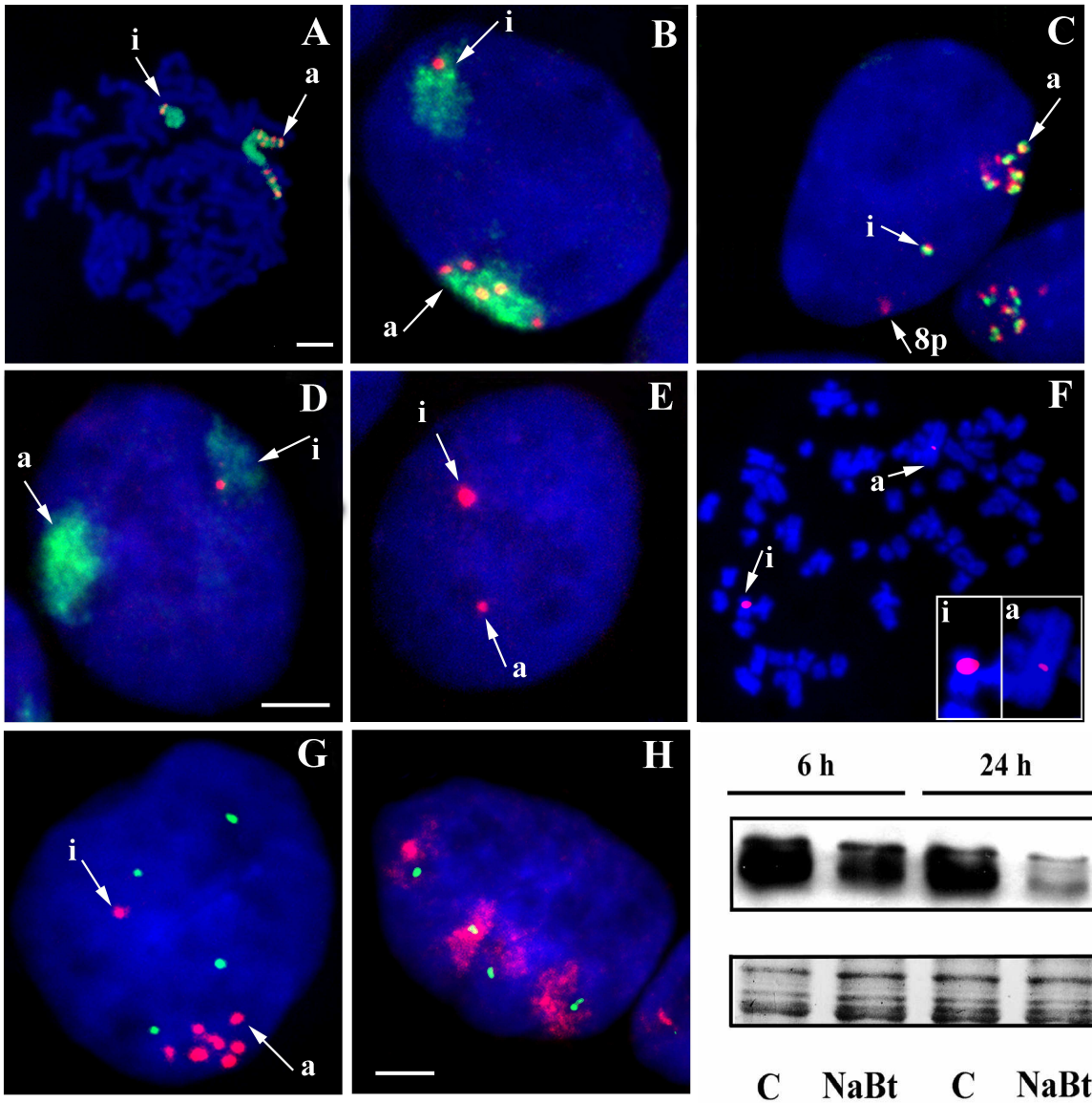


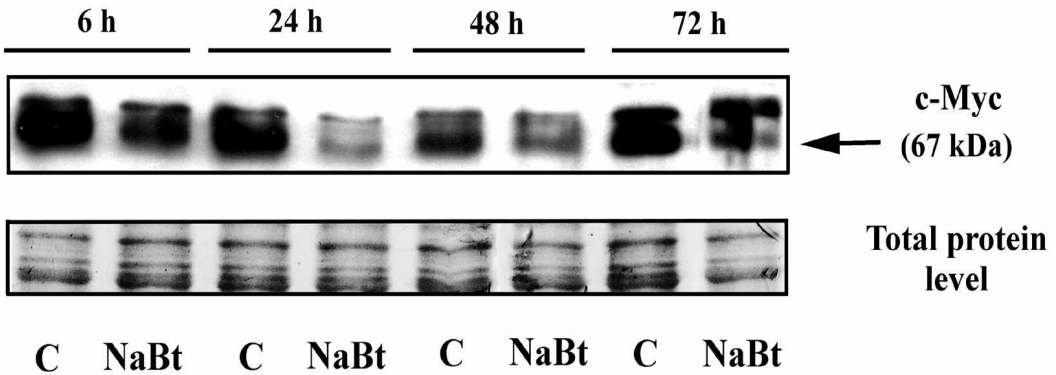
3D-genome project (LSHG-CT-2003-503441)

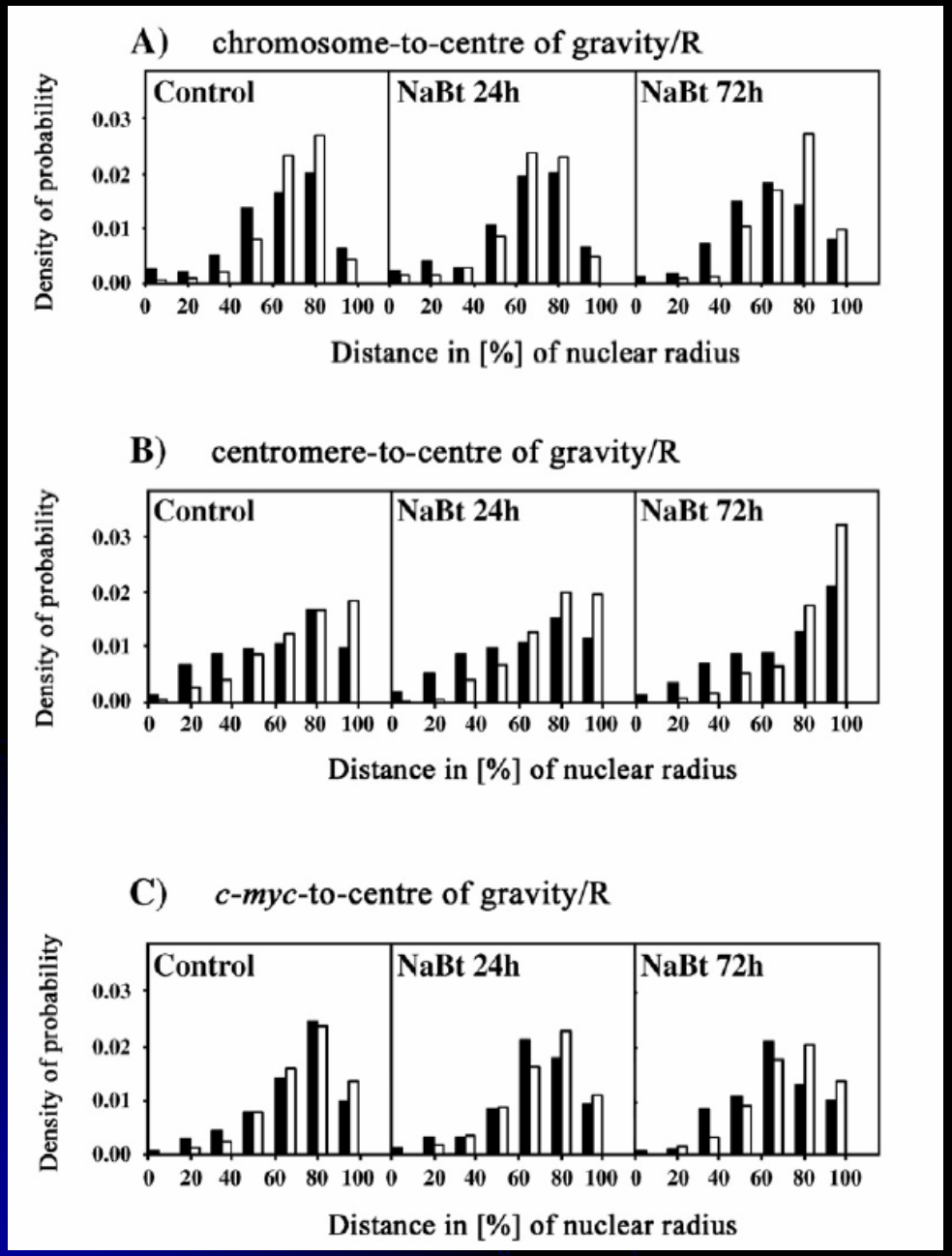


HSA 8 and related structures

Harničarová et al., 2006



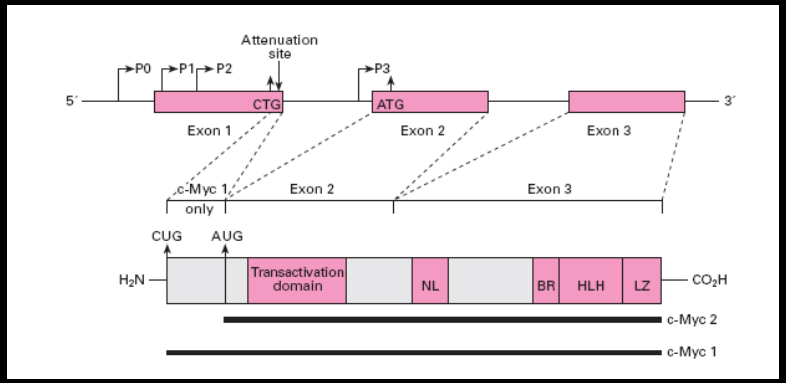




	Chromosome-to-center of gravity/R; mean ± SE in % of radius	
	Normal	Derivative
A		
Control (n=149)	67.7 ± 1.6	75.1 ± 1.6*
NaBt 24 h (n=132)	67.2 ± 1.5	71.9 ± 1.5
NaBt 72 h (n=153)	67.6 ± 1.6	73.6 ± 1.4*
	Centromere-to-center of gravity/R; mean ± SE in % of radius	
	Normal	Derivative
B		
Control (n=512)	63.4 ± 1.0	74.7 ± 0.9*
NaBt 24 h (n=536)	64.5 ± 1.1	76.4 ± 0.8*
NaBt 72 h (n=575)	70.5 ± 1.0#	83.1 ± 0.7*,#
	c-myc-to-center of gravity/R; mean ± SE in % of radius	
	Normal	Derivative
C		
Control (n=149)	71.5 ± 1.4	78.2 ± 0.7*
NaBt 24 h (n=132)	69.1 ± 1.2	76.9 ± 0.9
NaBt 72 h (n=153)	69.0 ± 1.7	76.0 ± 0.8*
	c-myc-to-center of gravity of chromosome/r; mean ± SE in % of radius of chromosome territory	
	Normal	Derivative
D		
Control (n=149)	63.9 ± 1.1	58.5 ± 0.7*
NaBt 24 h (n=132)	56.3 ± 1.0#	57.8 ± 0.7
NaBt 72 h (n=153)	57.2 ± 1.0#	59.9 ± 0.8

Harničarová et al., 2006

Human *c-myc* gene and two resultant protein products



Ryan and Birnie, 1996

Sequences of oligoprobes were used according to Singer group and are follows

(1) 5′-TCG T*AG TCG AGG T*CA TAG TTC CT*G TTG GTG AAG CT*A ACG TT*G AGG GGC AT-3′
(2) 5′-CCA CAT* ACA GTC CTG GAT* GAT GAT TTT T*TG ATG AAG GT*C TCG TCG T*CC G-3′
(3) 5′-TGA CCT* TTT GCC AGG AGC CT*G CCT CTT TT*C CAC AGA AAC AAC AT*C GAT* TT-3′
(4) 5′-CTG GT*G CAT TTT CGG T*TG TTG CTG AT*C TGT CTC AGG ACT* CTG ACA CT*G TC-3′
(5) 5′-GGC CTT* TTC ATT* GTT TT*C CAA CTC CGG GAT* CTG GT*C ACG CAG GGC AAA AA-3′

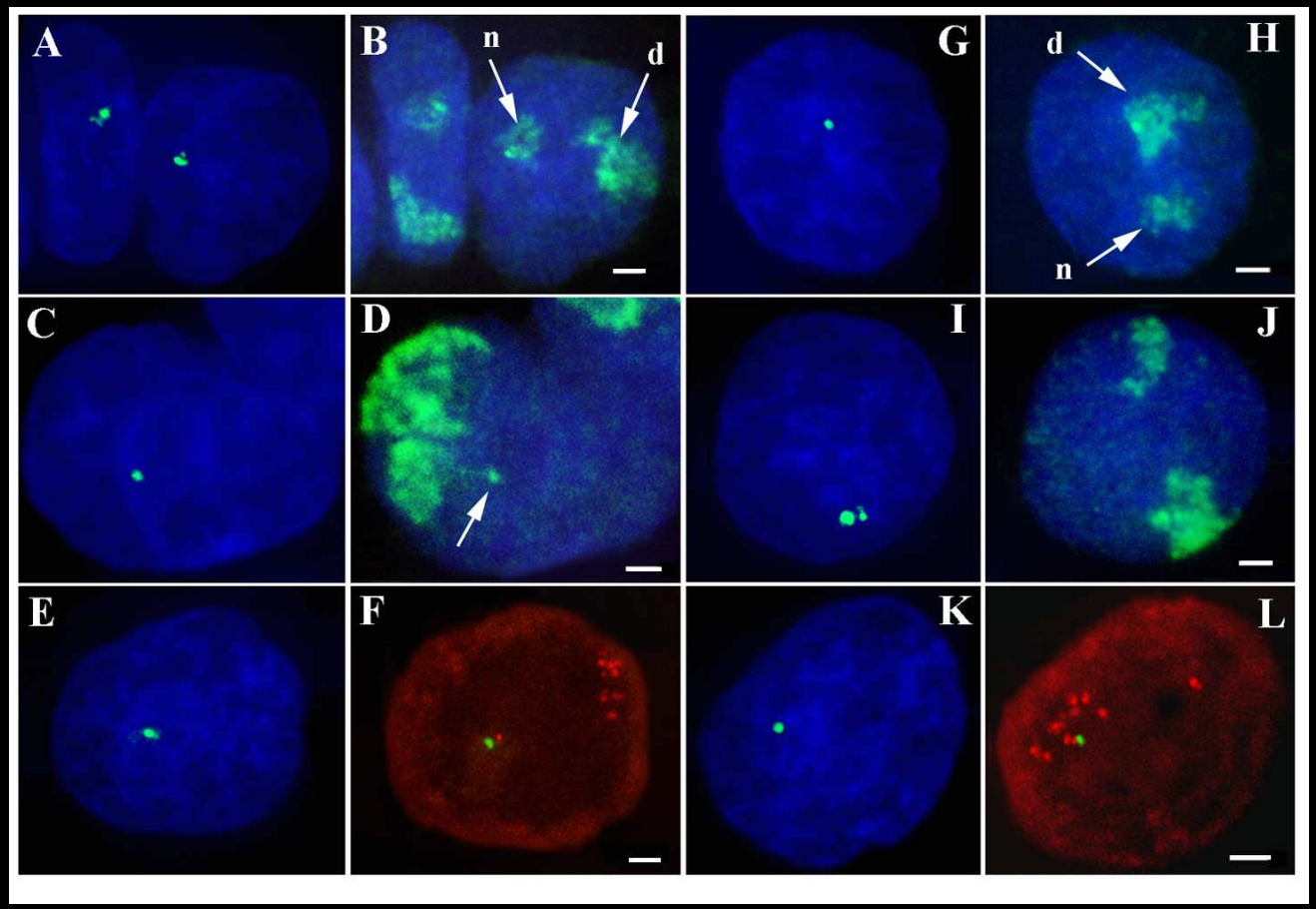
EXON 1
5'CCCCCGAGCTGTGCTGCTCGCGGCCGCCAC
CGCCGGGCCCCGGCCGTCCCTGGCTCCCCTCC
TGCCTCGAGAAGGGCAGGGCTTCTCAGAGGC
TTGGCGGGAAAAAGAACGGAGGGAGGGATC
GCGCTGAGTATAAAAGCCGGTTTTCGGGGCT
TTATCTAACTCGCTGTAGTAATTCCAGCGAGA
GGCAGAGGGAGCGAGCGGGCGGCCGGCTAG
GGTGGAAGAGCCGGGCGAGCAGAGCTGCGC
TGCGGGCGTCCTGGGAAGGGAGATCCGGAGC
GAATAGGGGGCTTCGCCTCTGGCCCAGCCCT
CCCGCTGATCCCCCAGCCAGCGGTCCGCAAC
CCTTGCCGCATCCACGAAACTTTGCCCATAGC
AGCGGGCGGGCACTTTGCACTGGAACTTACA
ACACCCGAGCAAGGACGCGACTCTCCCGACG
CGGGGAGGCTATTCTGCCCATTTGGGGACAC
TTCCCCGCCGCTGCCAGGACCCGCTTCTCTGA
AAGGCTCTCCTTGCAGCTGCTTAGACGCTGG
ATTTTTTTCGGGTAGTGGAAAACCAG 3'

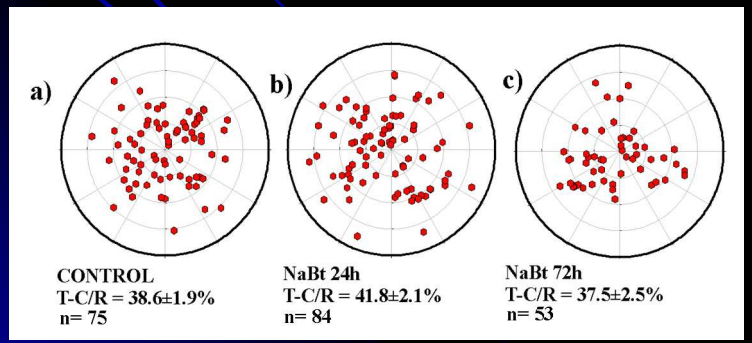
INTRON 1-2

EXON 2
5' CAGCCTCCCGCGACG
(1)ATGCCCCTCAACGTTAGCTTCACCAACAGGAACTATGACCTCG A
CTACGACTCGGTGCAGCCGTATTTCTACTGCGACGAGGAGGAGAACT
TCTACCAGCAGCAGCAGAGCGAGCTGCAGCCCCCGGCGCCCAG
CGAGGATATCTGGAAGAAATTCGAGCTGCTGCCCACCCCGCCCCTGT
CCCCTAGCCGCCGCTCCGGGCTCTGCTCGCCCTCCTACGTTGCGGTC
ACACCCTTCTCCCTTCGGGGAGACAACGACGGCGGTGGCGGGAGCTT
CTCCACGGCCGACCAGCTGGAGATGGTGACCGAGCTGCTGGGAGGA
GACATGGTGAACCAGAGTTTCATCTGCGACC
(2)CGGACGACGAGACCTTCATCAAAAACATCATCATCCAGGACT GTATGTGG
AGCGGCTTCTCGGCCGCCGCCAAGCTCGTCTCAGAGAAGCTGGCCTC
CTACCAGGCTGCGCGCAAAGACAGCGGCAGCCCGAACCCCGCCCGC
GGCCACAGCGTCTGCTCCACCTCCAGCTTGTACCTGCAGGATCTGAG
CGCCGCCGCCTCAGAGTGCATCGACCCCTCGGTGGTCTTCCCCTACC
CTCTCAACGACAGCAGCTCGCCCAAGTCCTGCGCCTCGCAAGACTCC
AGCGCCTTCTCTCCGTCCTCGGATTCTCTGCTCTCCTCGACGGAGTCC
TCCCCGCAGGGCAGCCCCGAGCCCCTGGTGCTCCATGAGGAGACAC
CGCCCACCACCAGCAGCGACTCTG 3'

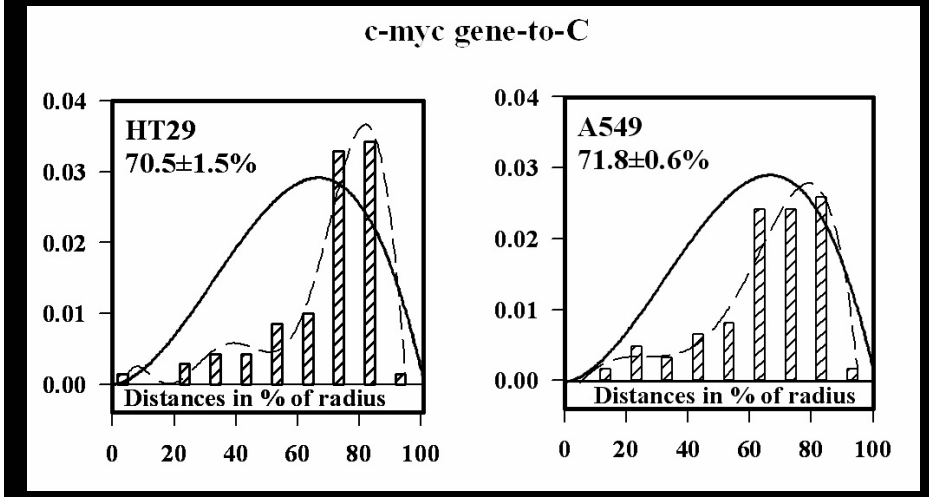
INTRON 2-3

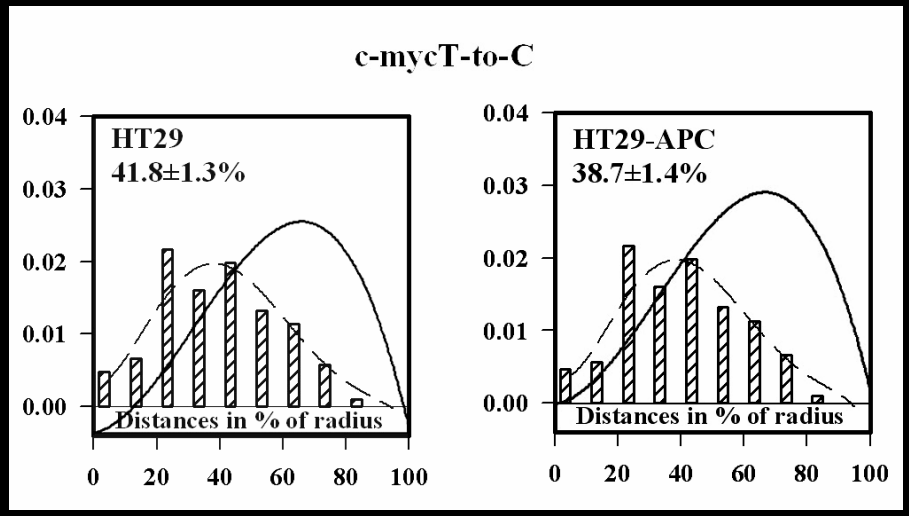
EXON 3
5' AGGAGGAACAAGAAGATGAGGAAG
(3)AAATCGATGTTGTTTCTGTGGAAAAGAGGCAGGCTCCTGGCAAAAGGT CA
GAGTCTGGATCACCTTCTGCTGGAGGCCACAGCAAACCTCCTCACAGCCCACT
GGTCCTCAAGAGGTGCCACGTCTCCACACATCAGCACAACTACGCAGCGCCT
CCCTCCACTCGGAAGGACTATCCTGCTGCCAAGAGGGTCAAGTTG
(4)GACAGTGTCAGAGTCCTGAGACAGATCAGCAACAACCGAAAATGCACC AG
CCCCAGGTCCTCGGACACCGAGGAGAATGTCAAGAGGCGAACACACAACGTC TTGGAGCGCCAGAGAGGAACGAGCTAAAACGGAGCT
(5)TTTTTGCCCTGCGTGACCAGATCCCGGAGTTGGAAAACAATGAAAAGG CC
CCCAAGGTAGTTATCCTTAAAAAAGCCACAGCATACATCCTGTCCGTCCAAGC
AGAGGAGCAAAAGCTCATTTCTGAAGAGGACTTGTTGCGGAAACGACGAGA
ACAGTTGAAACACAAACTTGAACAGCTACGGAACTCTTGTGCGTAAGGAAAA
GTAAGGAAAACGATTCCTTCTAACAGAAATGTCCTGAGCAATCACCTATGAA
CTTGTTTCAAATGCATGATCAAATGCAACCTCACAACCTTGGCTGAGTCTTGA
GACTGAAAGATTTAGCCATAATGTAAACTGCCTCAAATTGGACTTTGGGCATA
AAAGAACTTTTTTATGCTTACCATCTTTTTTTTTTCTTTAACAGATTTGTATTTA
AGAATTGTTTTTAAAAAATTTTAAGATTTACACAATGTTTCTCTGTAAATATTG
CCATTAAATGTAAATAACTTTAATAAAACGTTTATAGCAGTTACACAGAATTT
CAATCCTAGTATATAGTACCTAGTATTATAGGTACTATAAACCCTAATTTTTTT
TATTTAAGTACATTTTGCTTTTTAAAGTTGATTTTTTTCTATTGTTTTTAGAAAA
AATAAAATAACTGGCAAATATATCATTGAGCC 3'

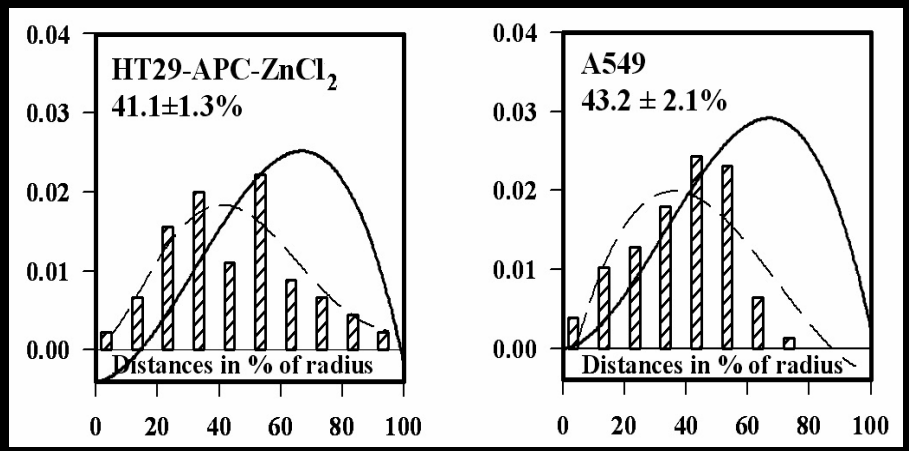


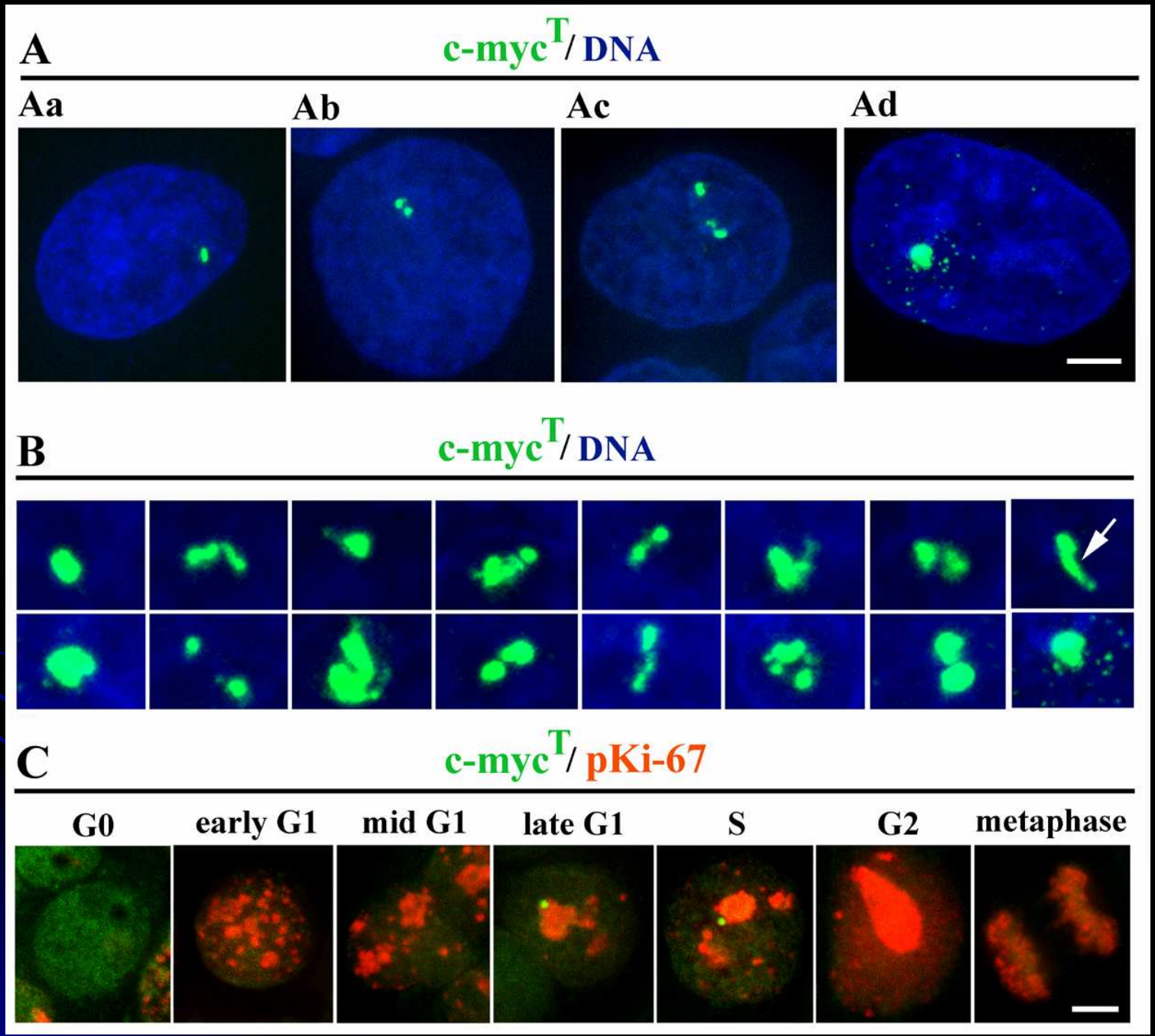


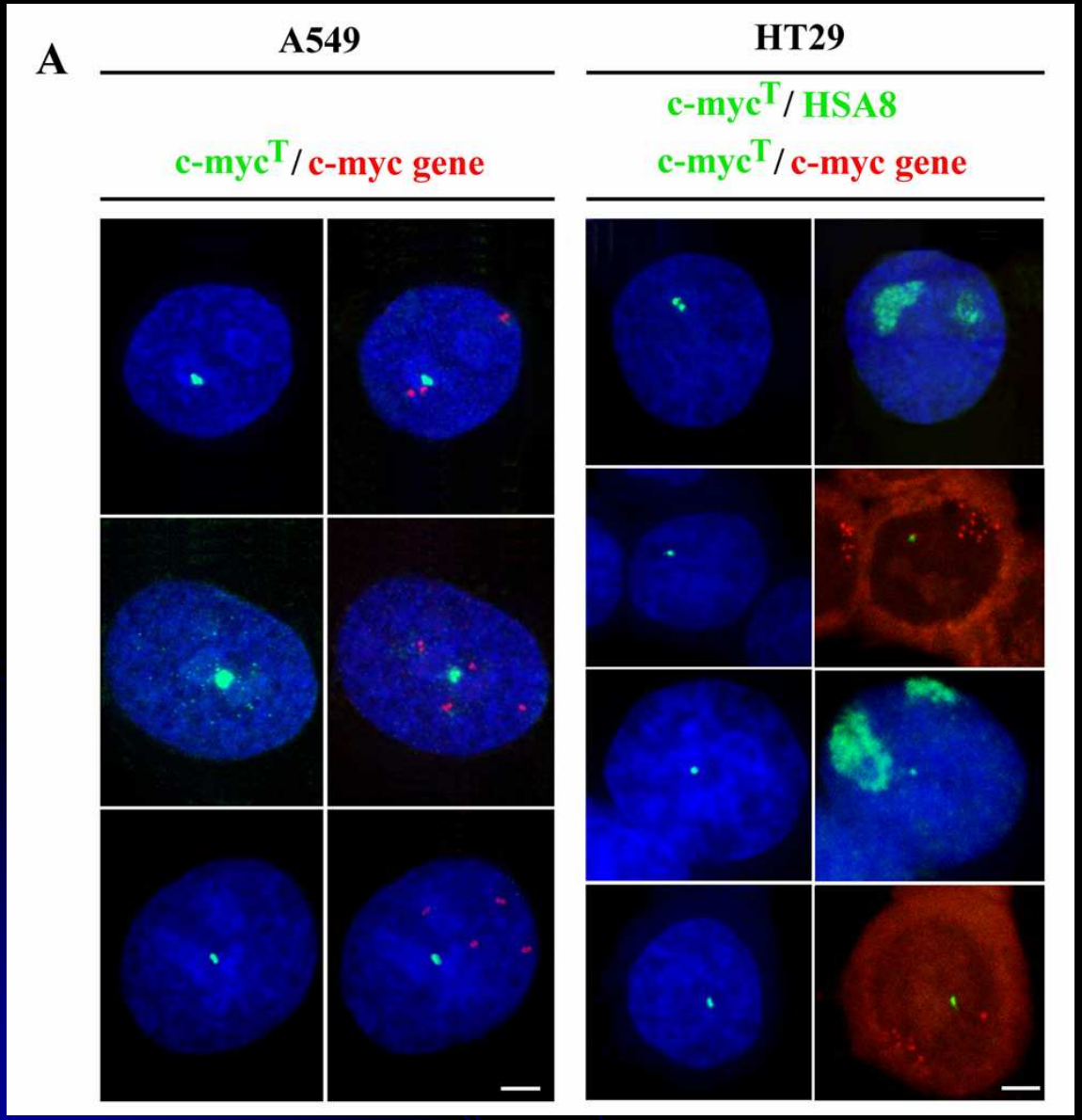
	Distance of inactive (active) *c-myc* gene-to-center of chromosome territory mean±SE in % of radius	
	Normal chromosome territory	Derivative chromosome territory
Control	61.0±3.8 (77.9±5.0)*	60.3±2.1 (77.9±5.0)*
NaBt 24 h	54.9±3.7 (80.3±5.3)*	60.4±2.1 (72.4±5.7)*
NaBt 72 h	53.8±3.0 (79.6±5.7)*	59.0±2.2 (80.0±5.1)*

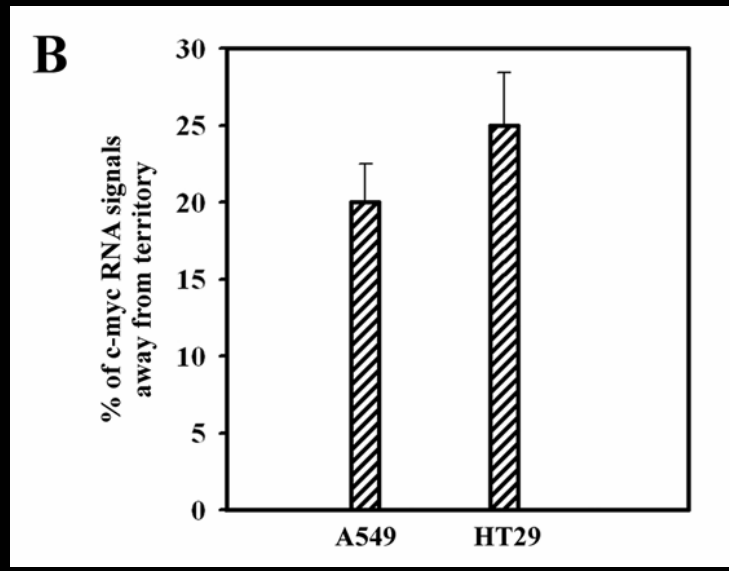

c-myc gene-to-C
HT29
70.5±1.5%
A549
71.8±0.6%
Distances in % of radius
0.04
0.03
0.02
0.01
0.00
0
20
40
60
80
100

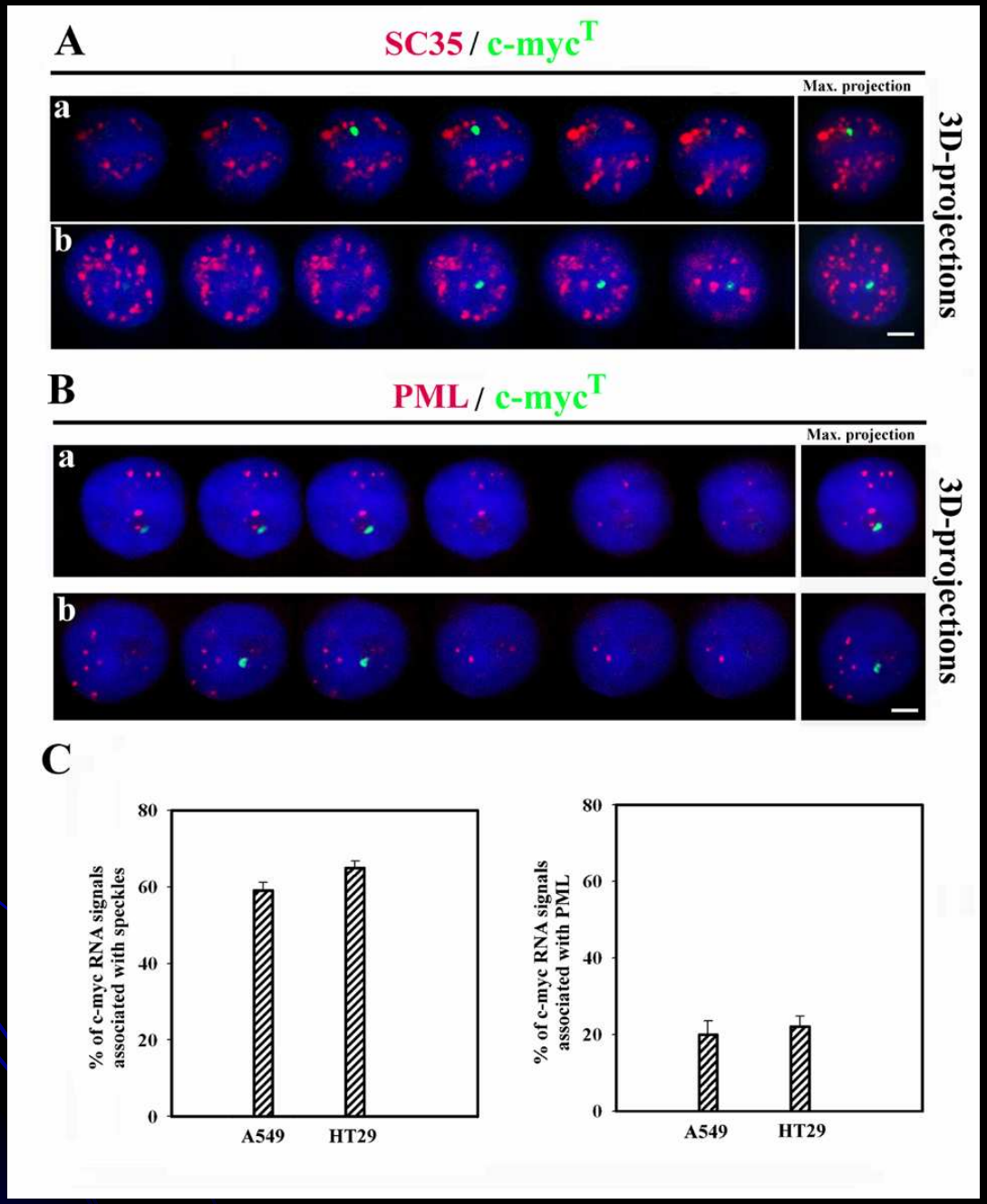

c-mycT-to-C
HT29
41.8±1.3%
HT29-APC
38.7±1.4%
Distances in % of radius
0.04
0.03
0.02
0.01
0.00
0
20
40
60
80
100

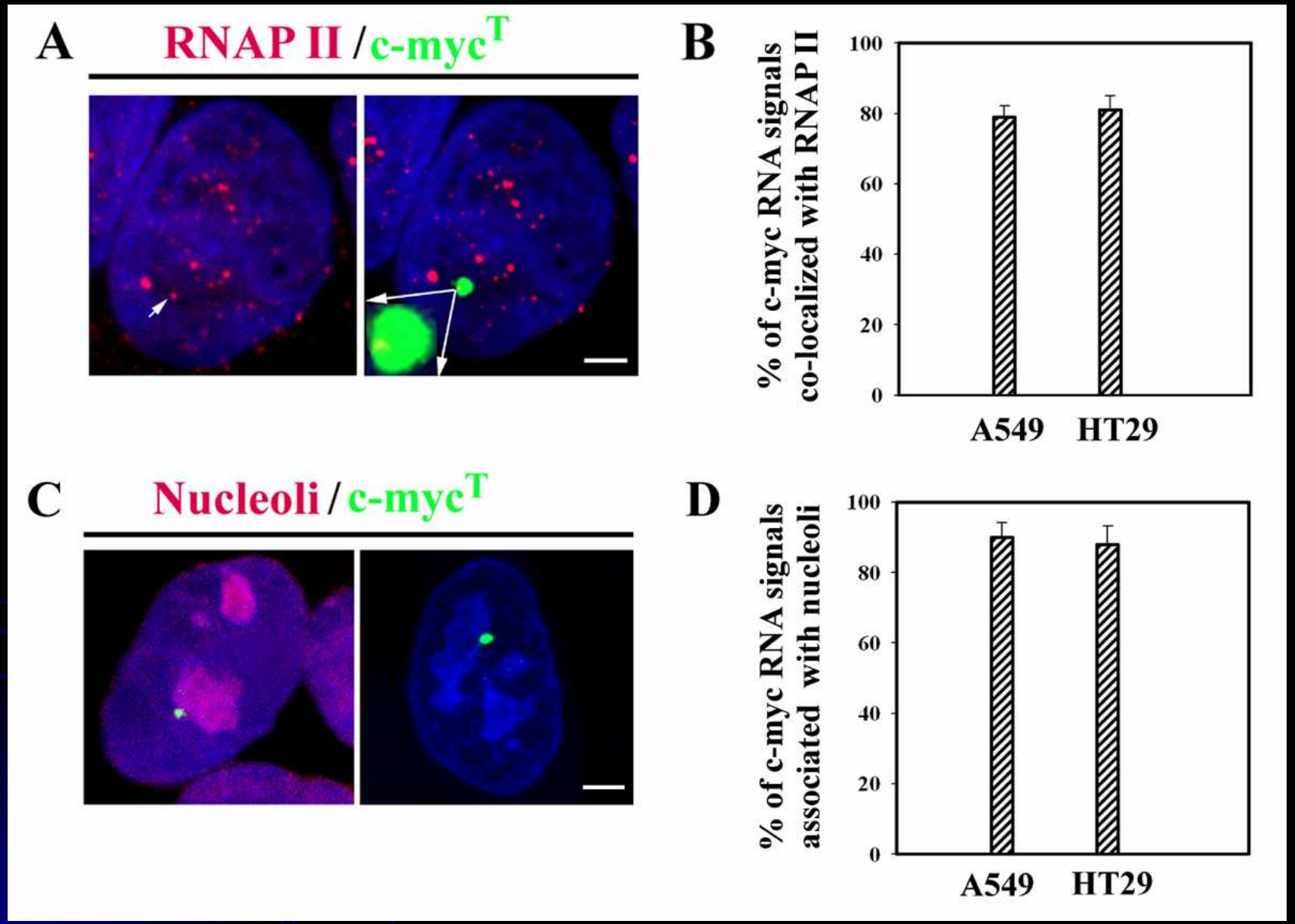

HT29-APC-ZnCl2
41.1±1.3%
A549
43.2 ± 2.1%
Distances in % of radius
0.04
0.03
0.02
0.01
0.00
0
20
40
60
80
100


A
c-myc^T / DNA
Aa
Ab
Ac
Ad
B
c-myc^T / DNA
C
c-myc^T / pKi-67
G0
early G1
mid G1
late G1
S
G2
metaphase

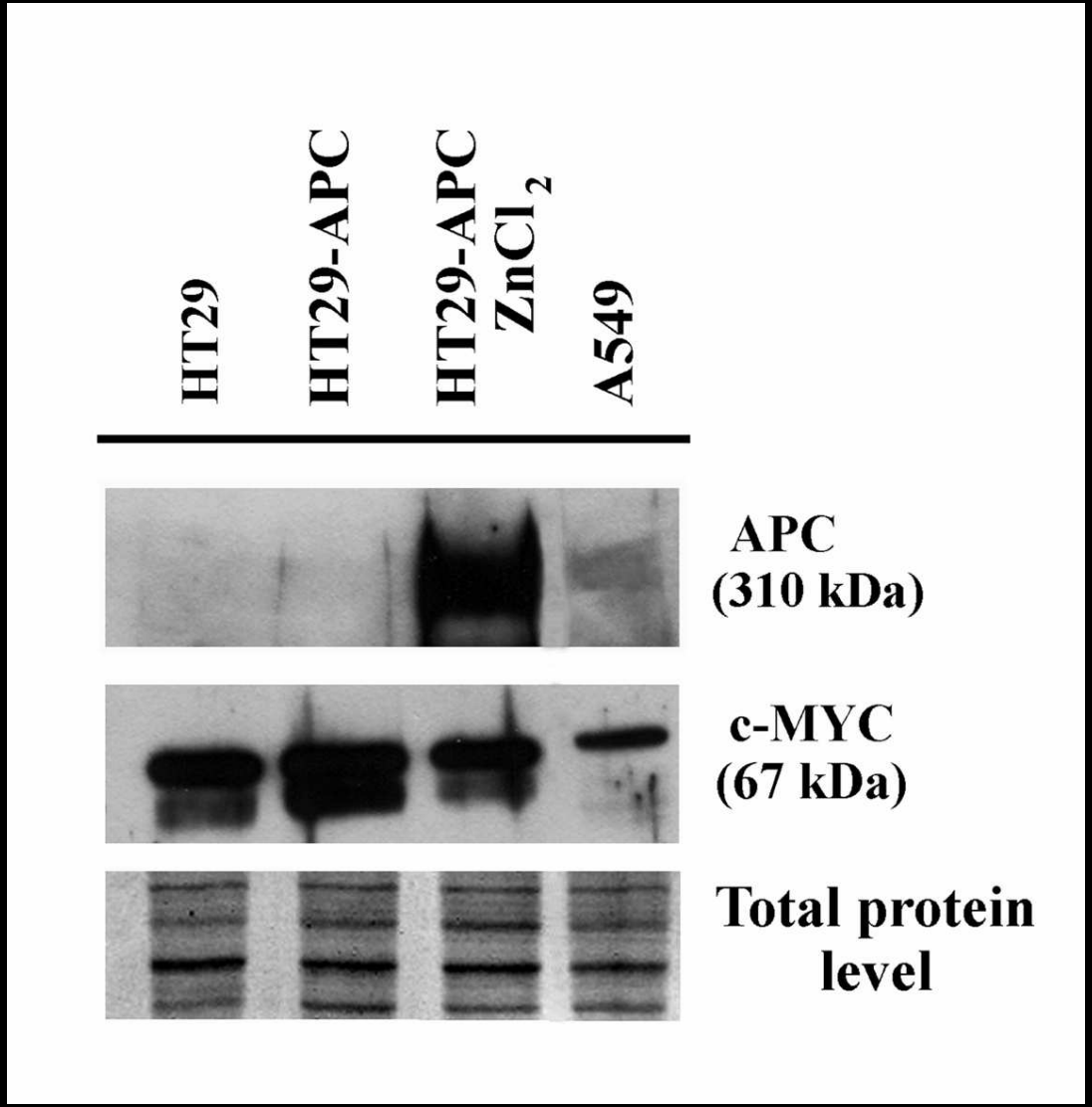

A
A549
HT29
c-myc^T / c-myc gene
c-myc^T / HSA8
c-myc^T / c-myc gene


B
% of c-myc RNA signals away from territory
30
25
20
15
10
5
0
A549
HT29

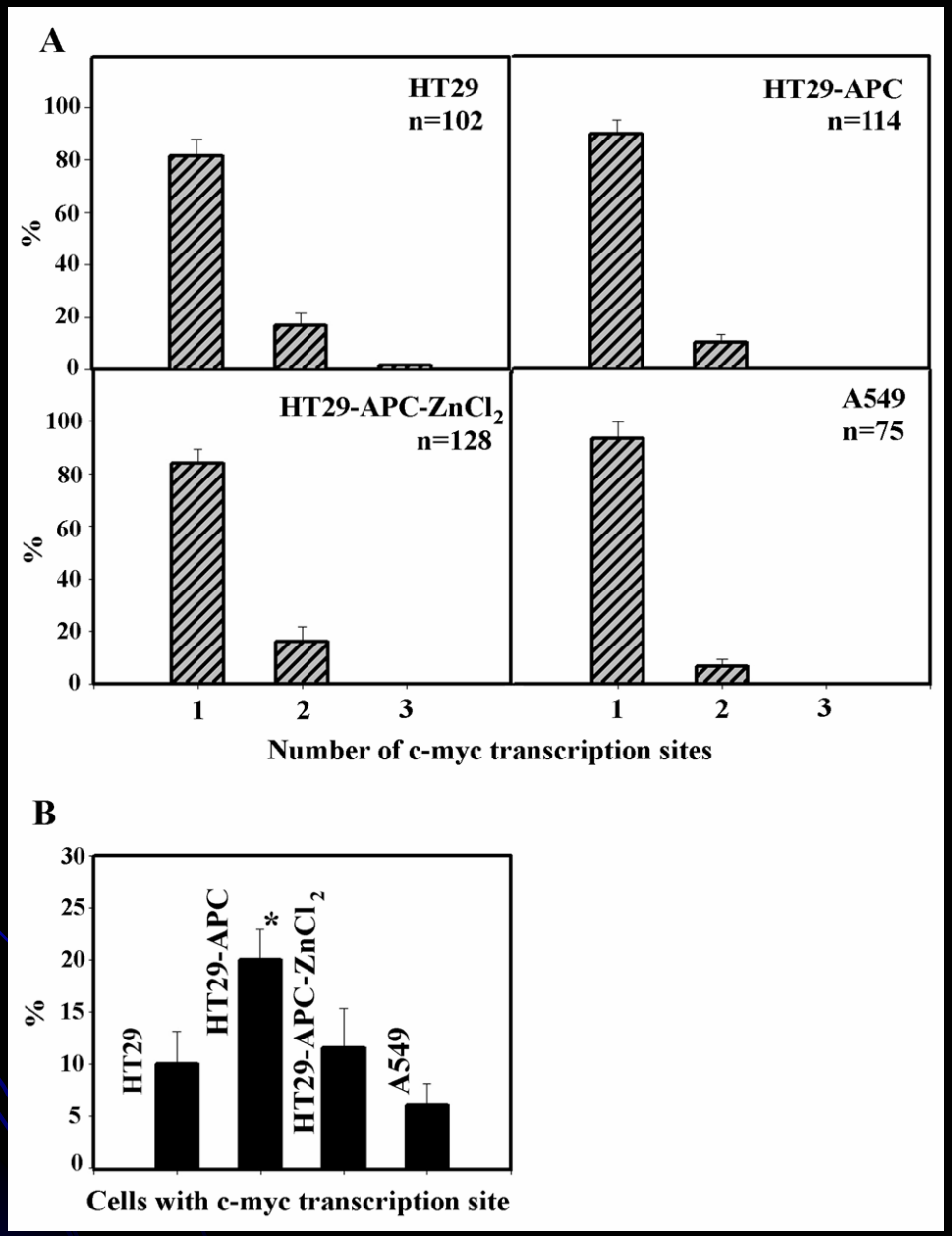

A
SC35 / c-myc^T
Max. projection
a
b
3D-projections
B
PML / c-myc^T
Max. projection
a
b
3D-projections
C
% of c-myc RNA signals associated with speckles
80
60
40
20
0
A549
HT29
% of c-myc RNA signals associated with PML
80
60
40
20
0
A549
HT29

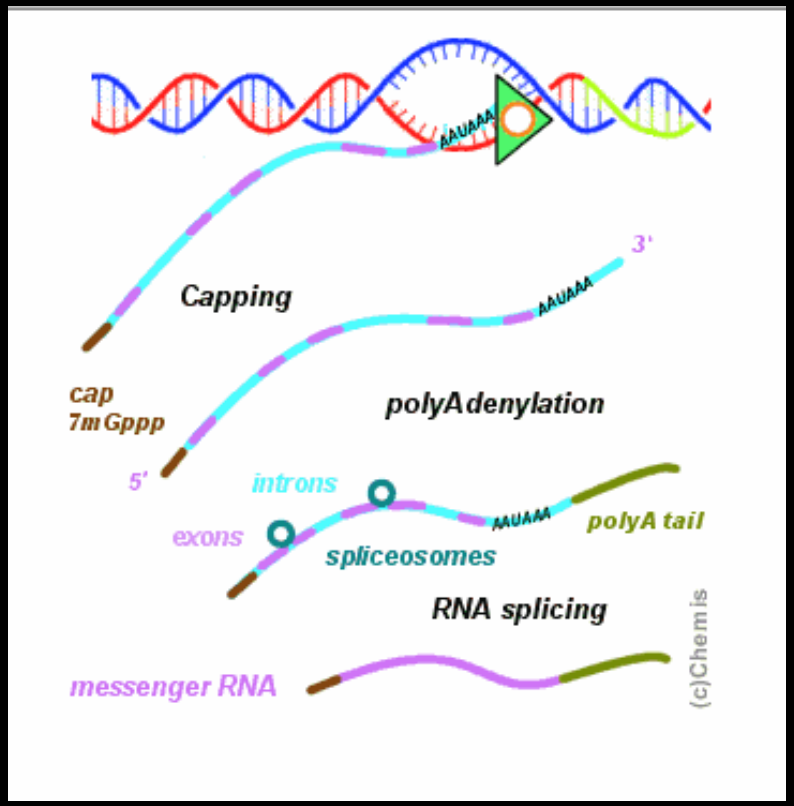

A
RNAP II / c-myc^T
B
% of c-myc RNA signals co-localized with RNAP II
100
80
60
40
20
0
A549
HT29
C
Nucleoli / c-myc^T
D
% of c-myc RNA signals associated with nucleoli
100
80
60
40
20
0
A549
HT29

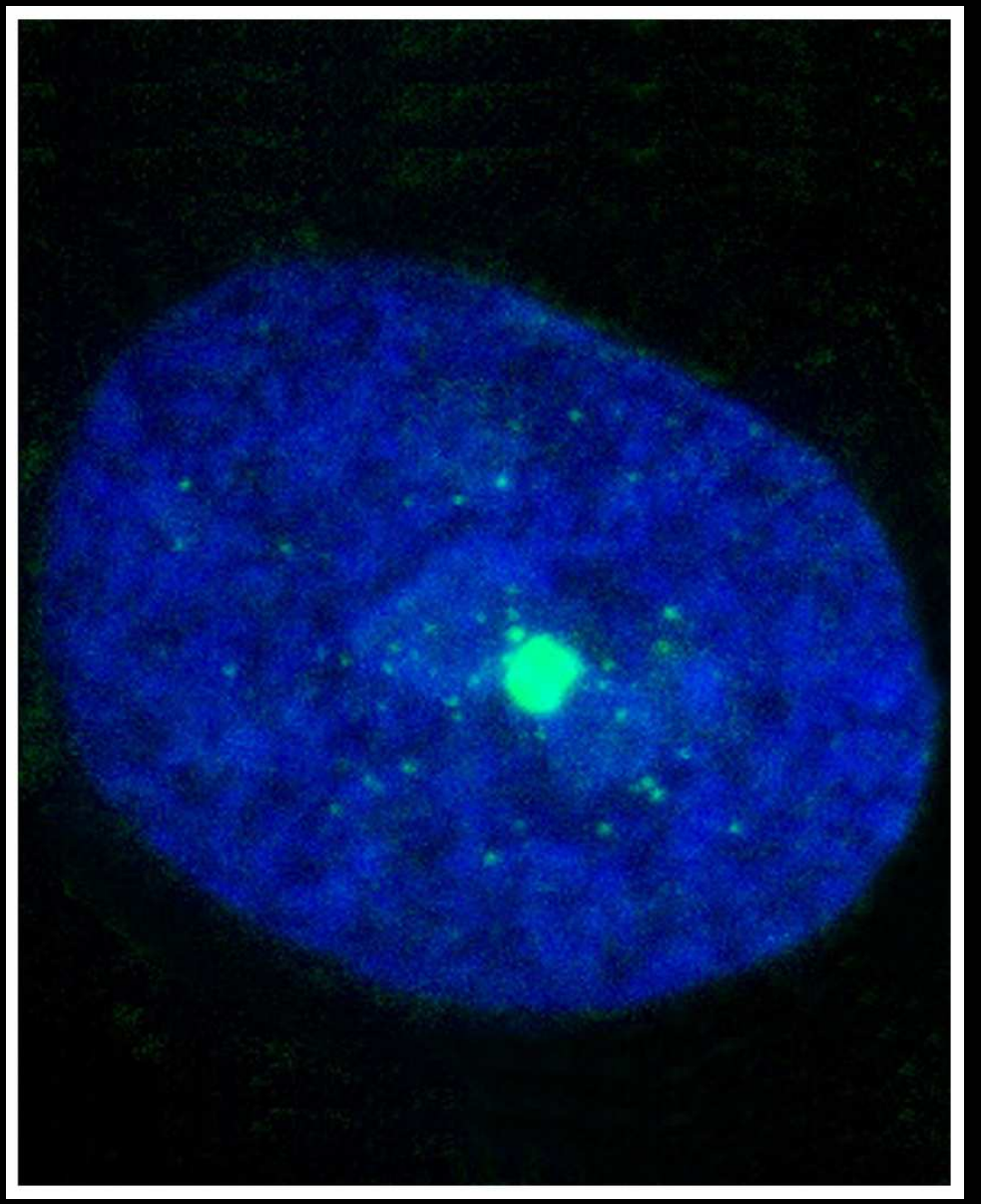
Western blot analysis



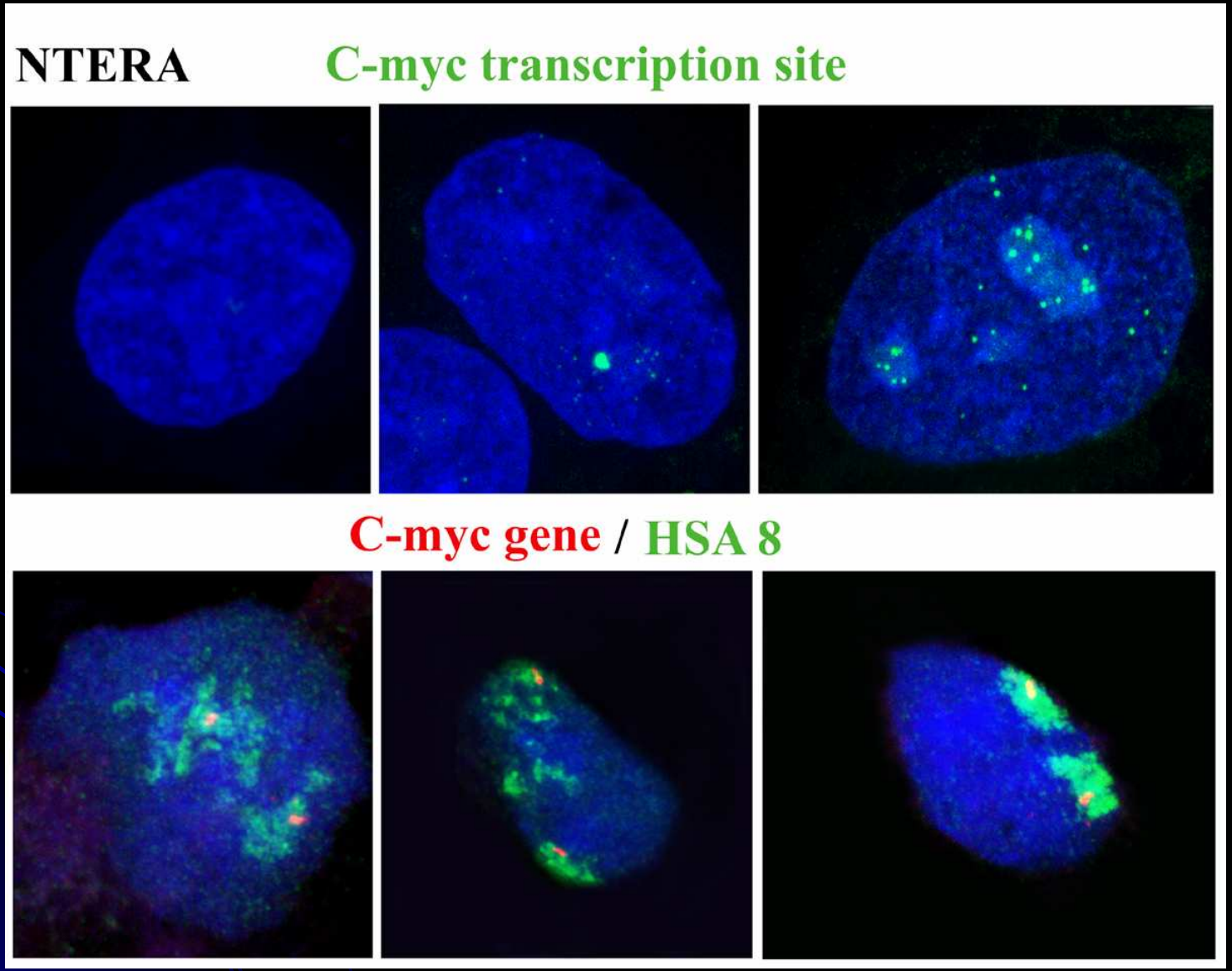
Number of c-myc RNA signals



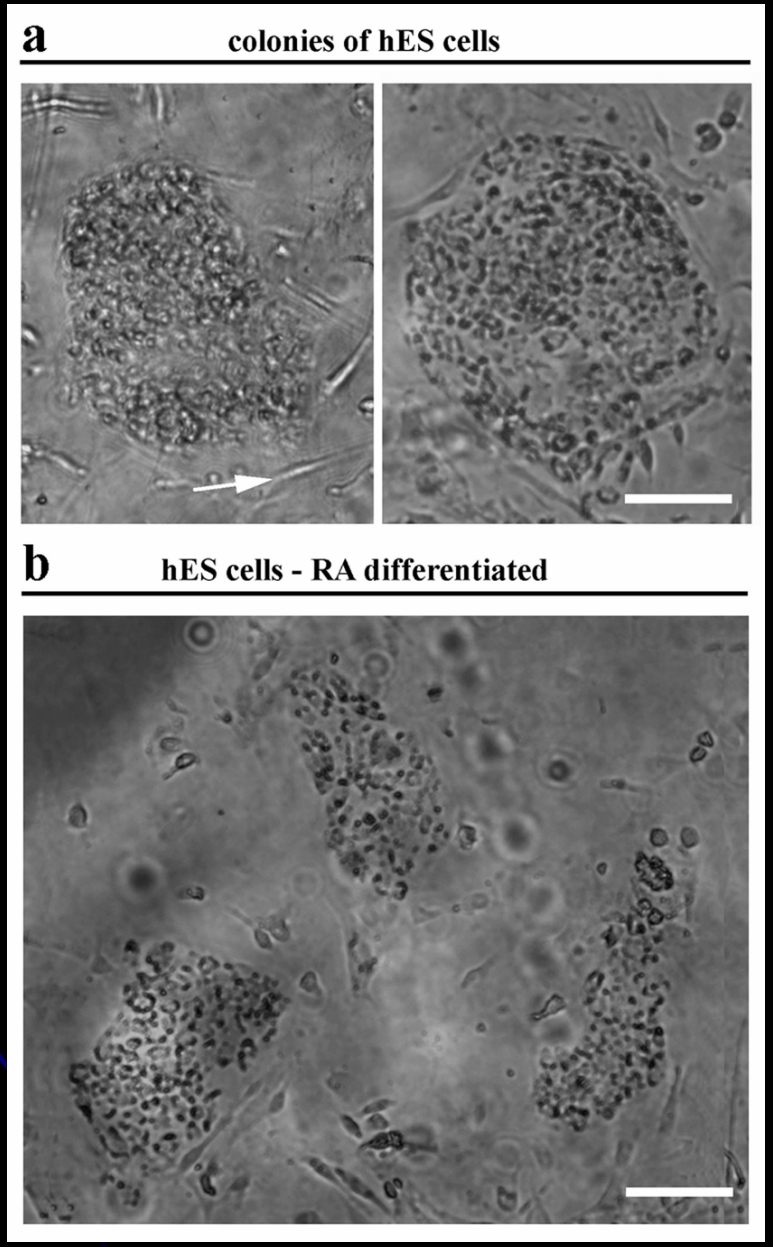

AAUAAA
Capping
3'
AAUAAA
cap
7mGppp
polyAdenylation
5'
introns
exons
AAUAAA
polyA tail
spliceosomes
RNA splicing
messenger RNA
(c)Chemis



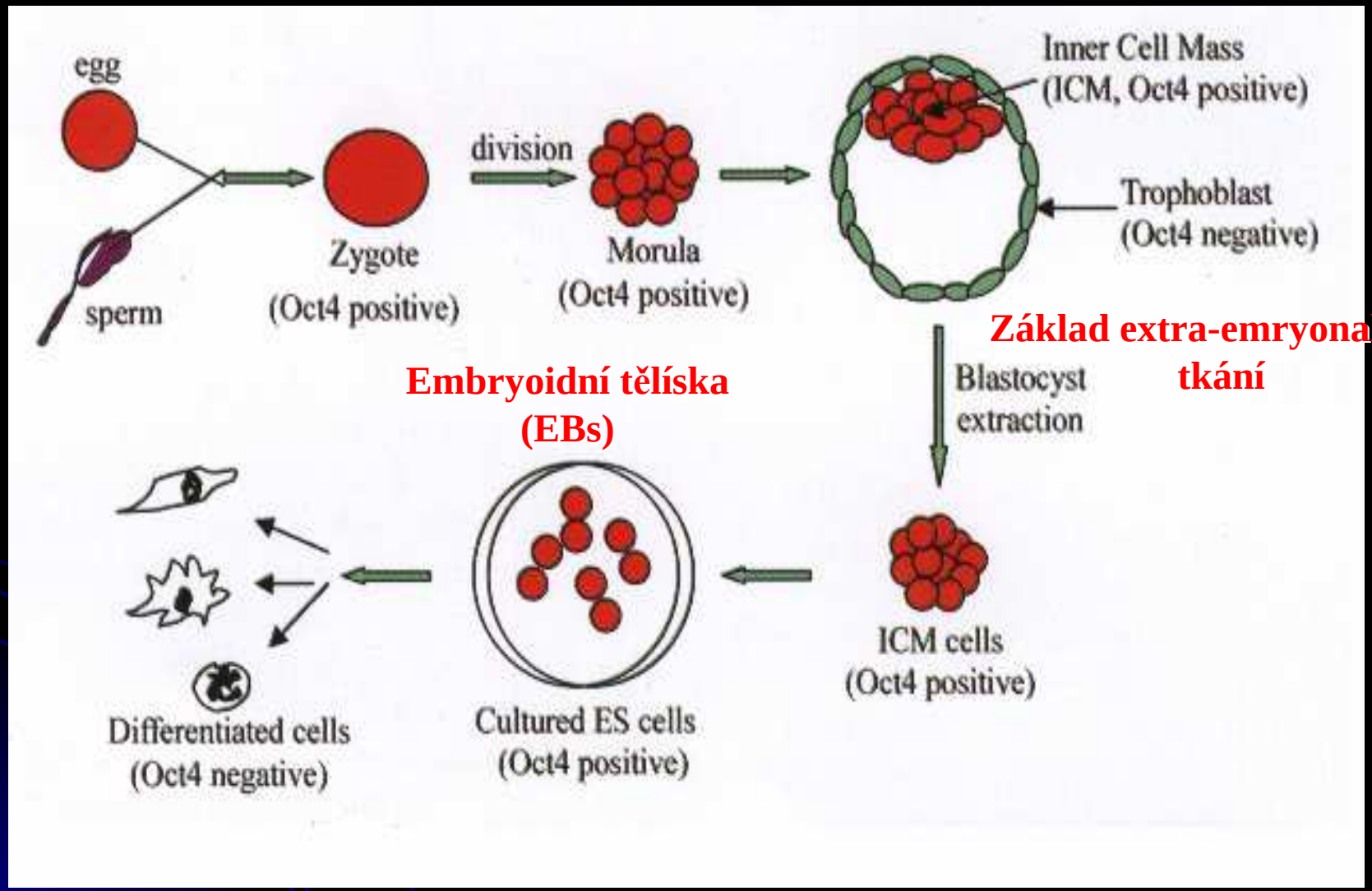
Human embryonal (teratocarcinoma) cells NTERA

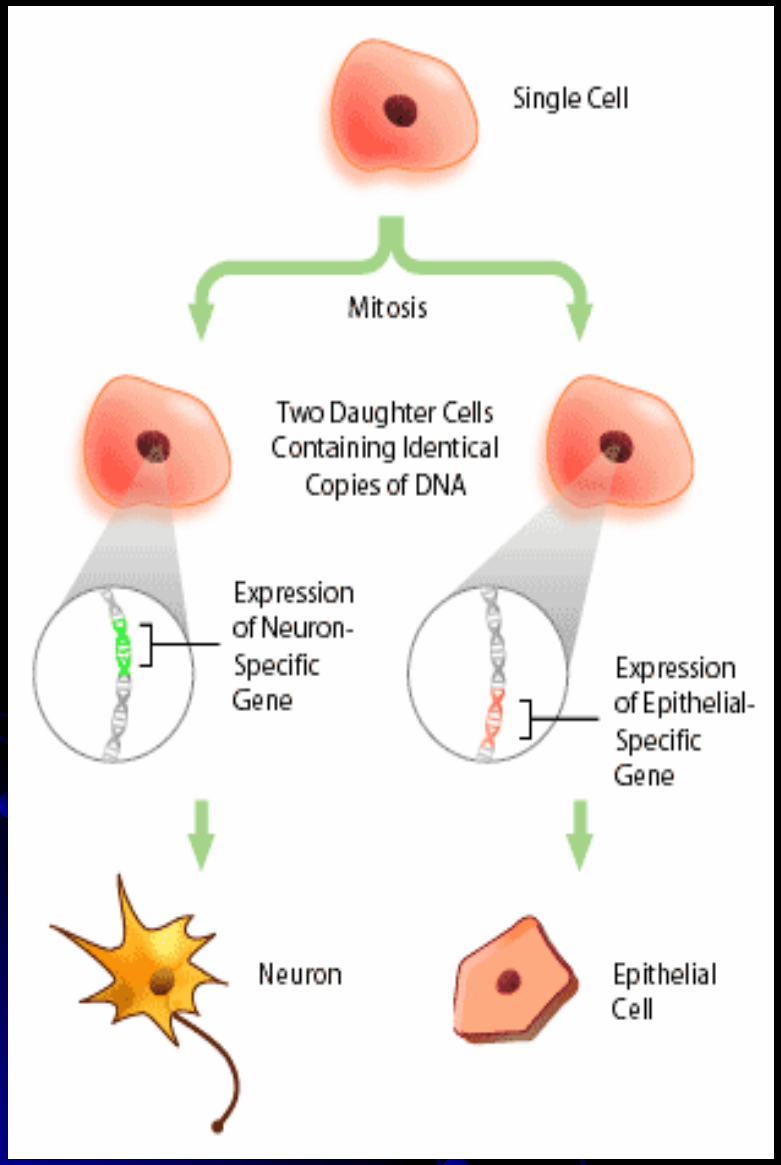


Gene expression and structure in human embryonic stem cells



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





ES: Embryonální kmenové buňky (ES) jsou imortalizované buněčné linie derivované z vnitřní buněčné masy 3.5 denní blastocysty. Tyto buňky se mohou rozmnožovat v nevydiferencovaném stavu za přítomnosti LIF faktoru. ES buňky mají schopnost diferencovat in vitro v progenitorová stádia. ES buňky tvoří tak zvaná emryoidní tělíska (EBs), což jsou tří-dimenzionální struktury se schopností diferencovat do různých buněčných typů, například: hematopoietických, myogenických, neurálních a jiných. Ovlivnění těchto buněk například RA vede k indukci neurální diferenciace.

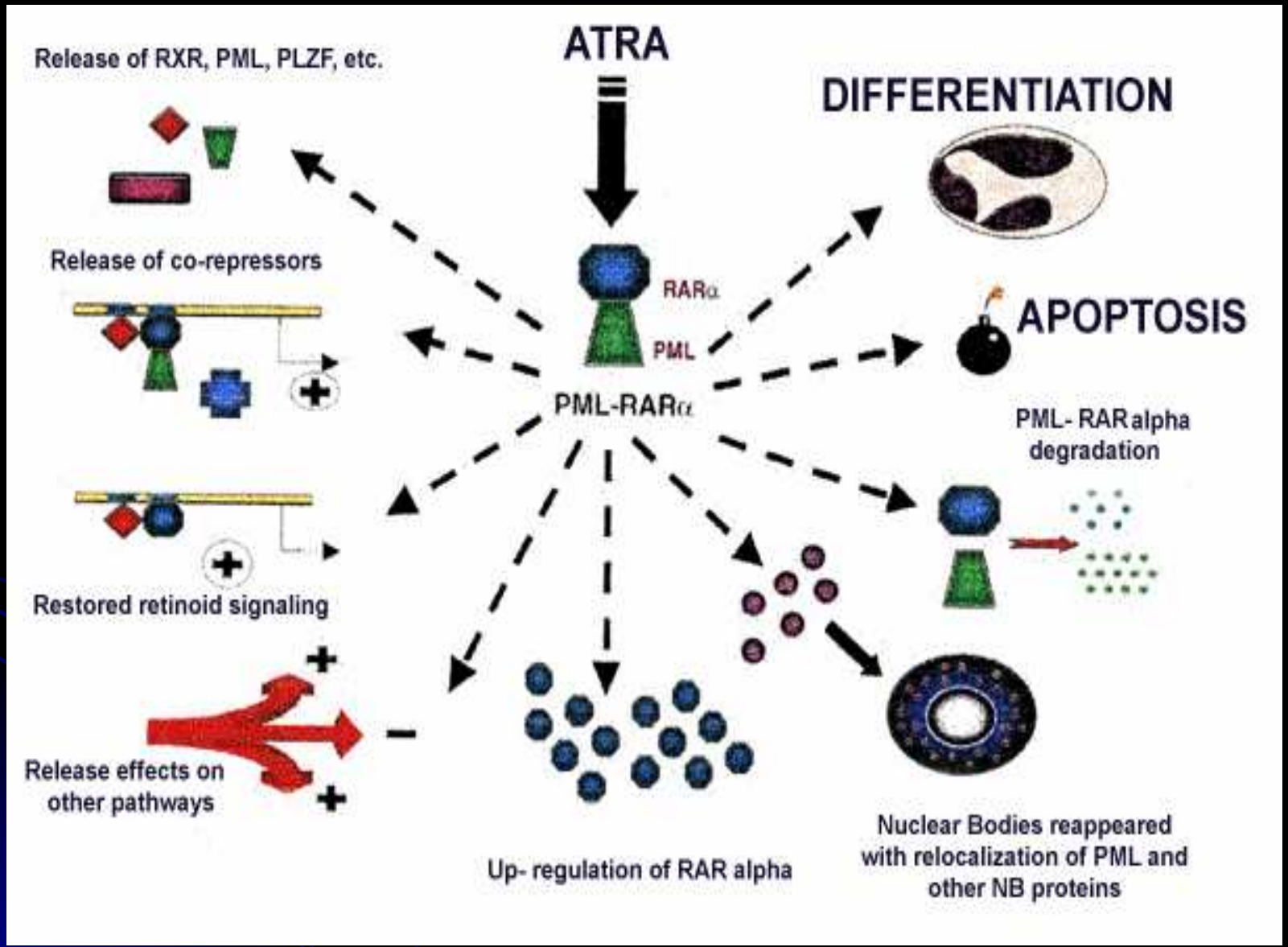
Neurální diferenciace ES / EC buněk

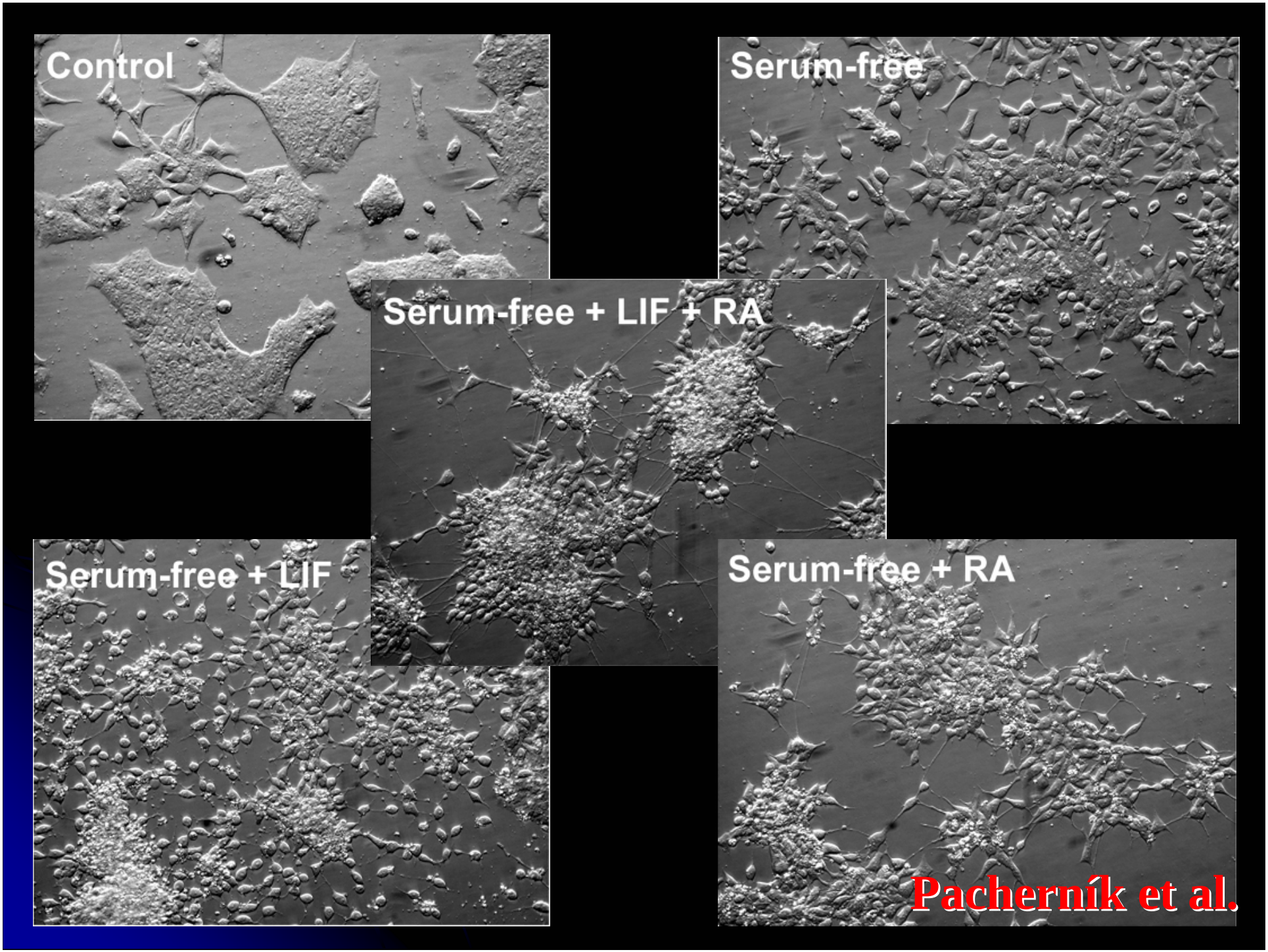
Indukce

- spontánně v EB
- spontánně v monovrstvě
- v EB v přítomnosti kyseliny retinové (RA)
- v monovrstvě bez přítomnosti séra
- v monovrstvě bez přítomnosti séra + RA ?

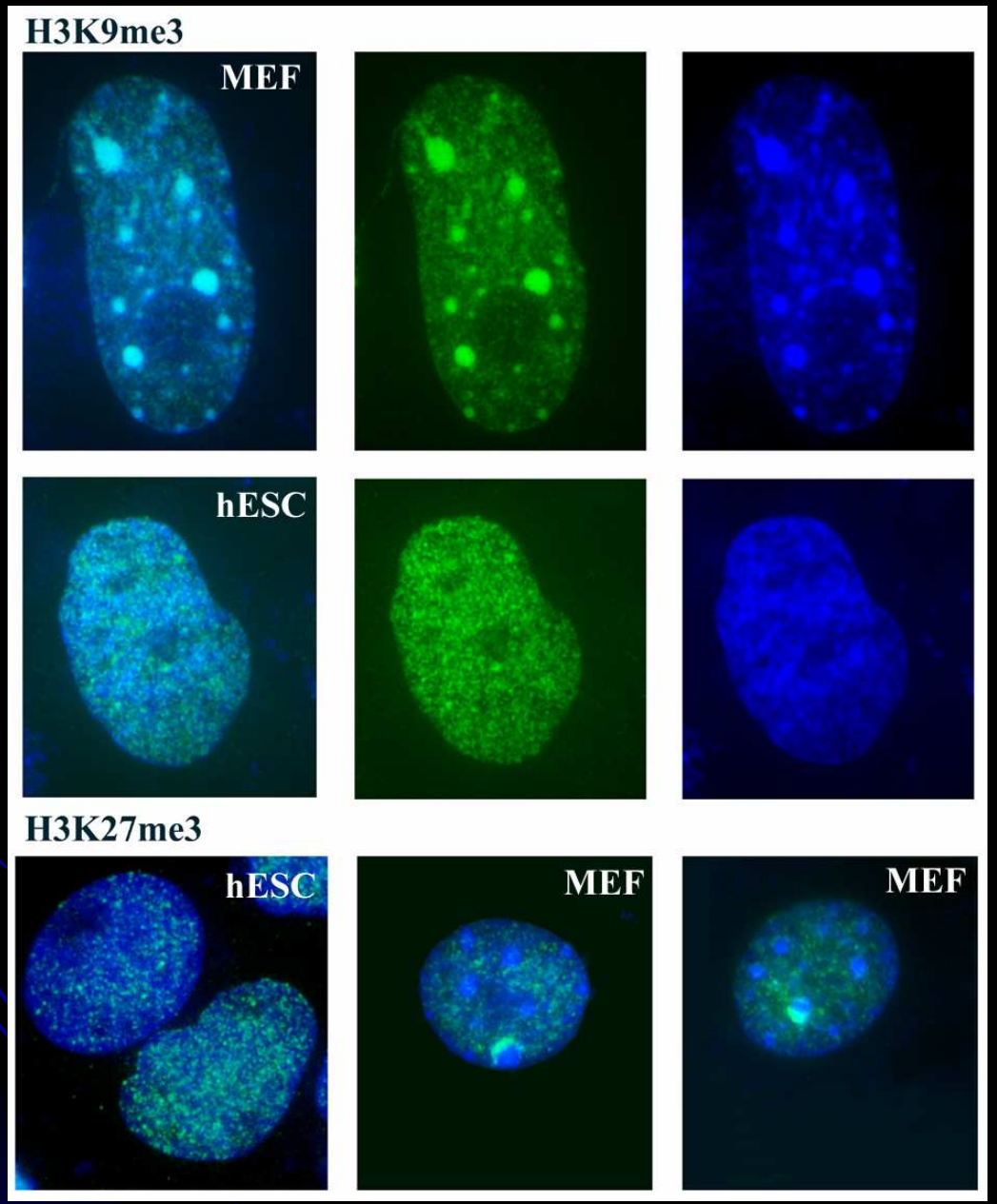
Selekce

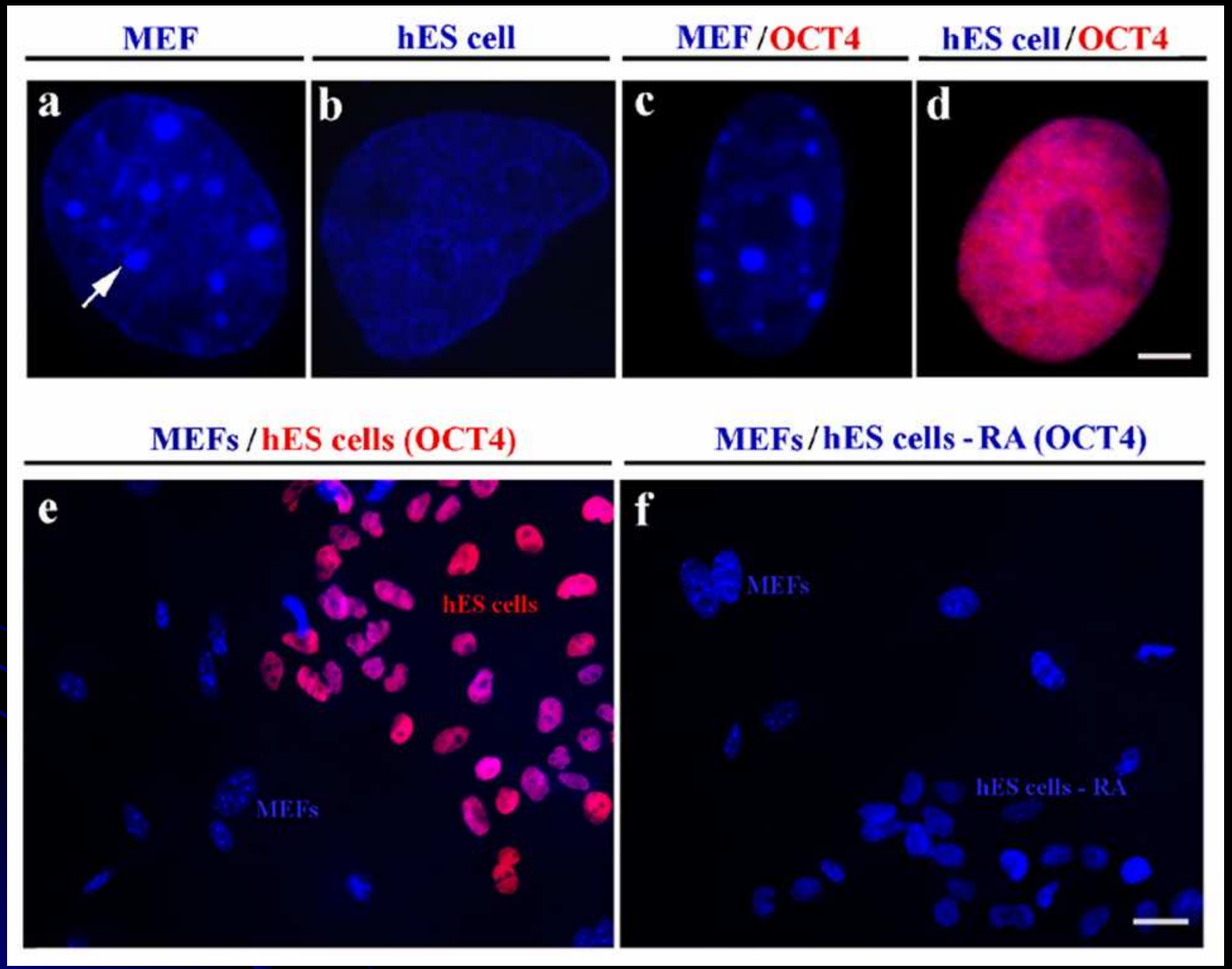
- bezsérové médium
- doplňky média: ITS, N2, B27
- inhibitory jiných diferenciací: Noggin, Chordin, Follistatin


Release of RXR, PML, PLZF, etc.
ATRA
DIFFERENTIATION
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

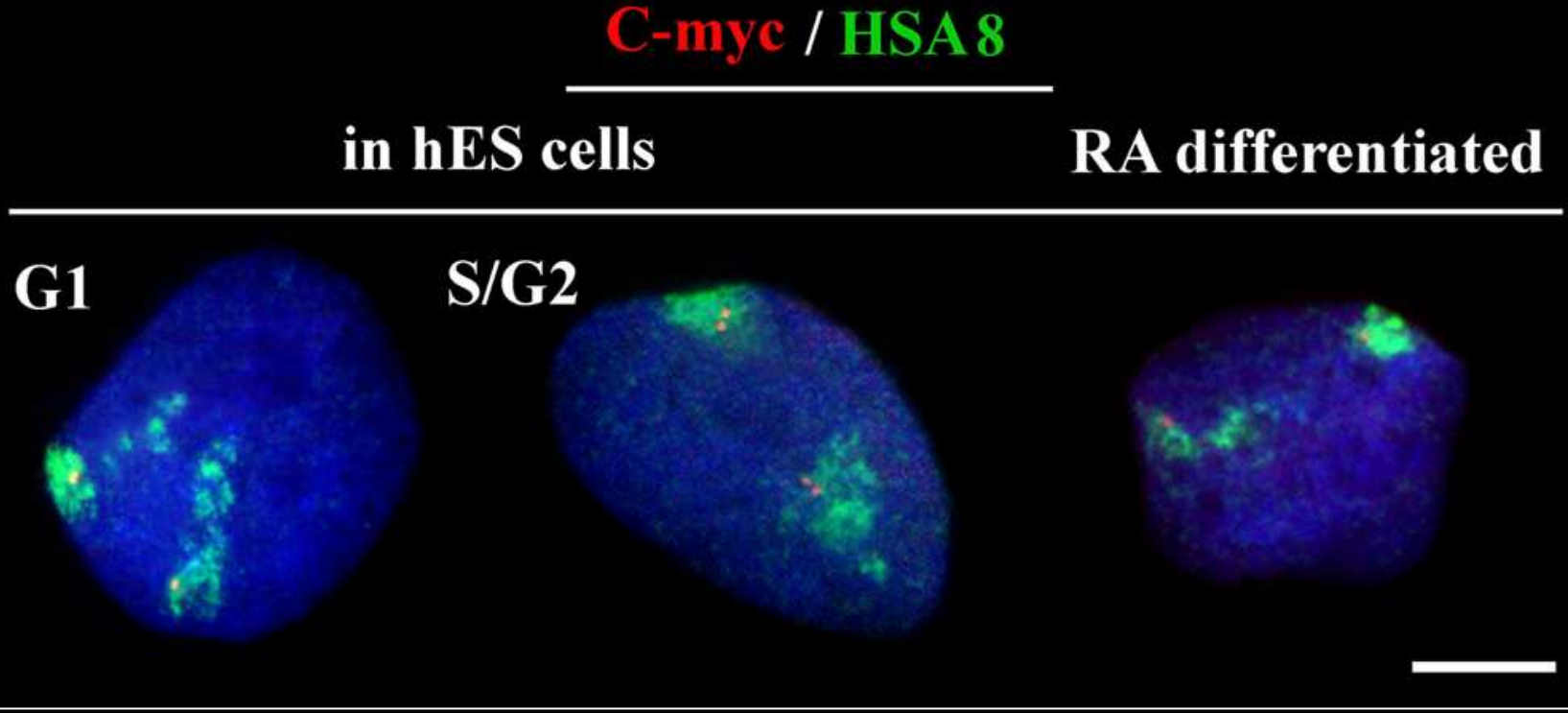

Control
Serum-free
Serum-free + LIF + RA
Serum-free + LIF
Serum-free + RA
Pacherník et al.

Epigenetics of hES Cells

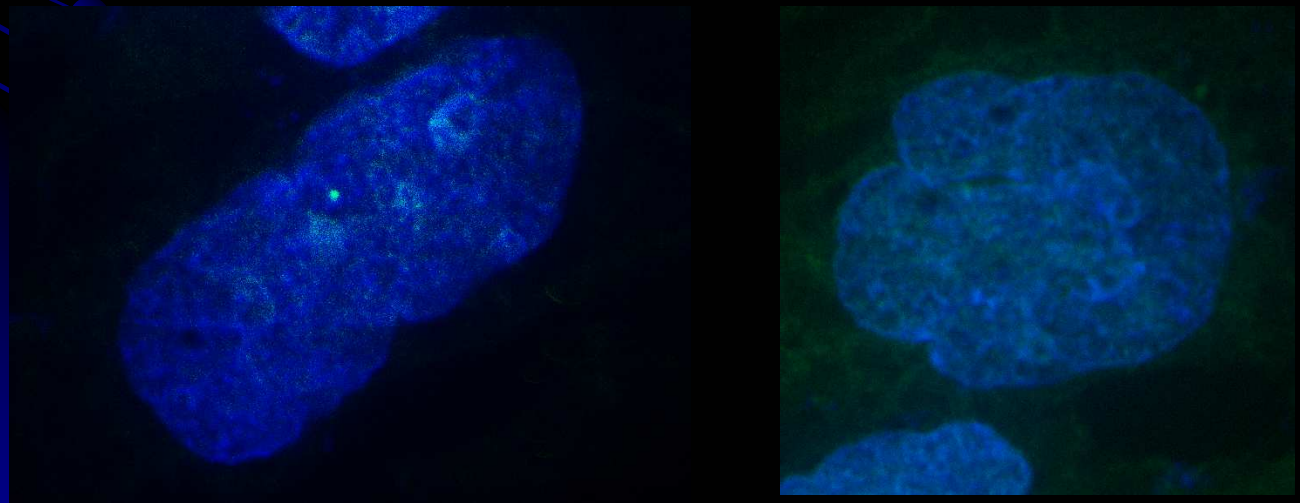



MEF
hES cell
MEF/OCT4
hES cell/OCT4
a
b
c
d
MEFs/hES cells (OCT4)
MEFs/hES cells - RA (OCT4)
e
hES cells
MEFs
f
MEFs
hES cells - RA

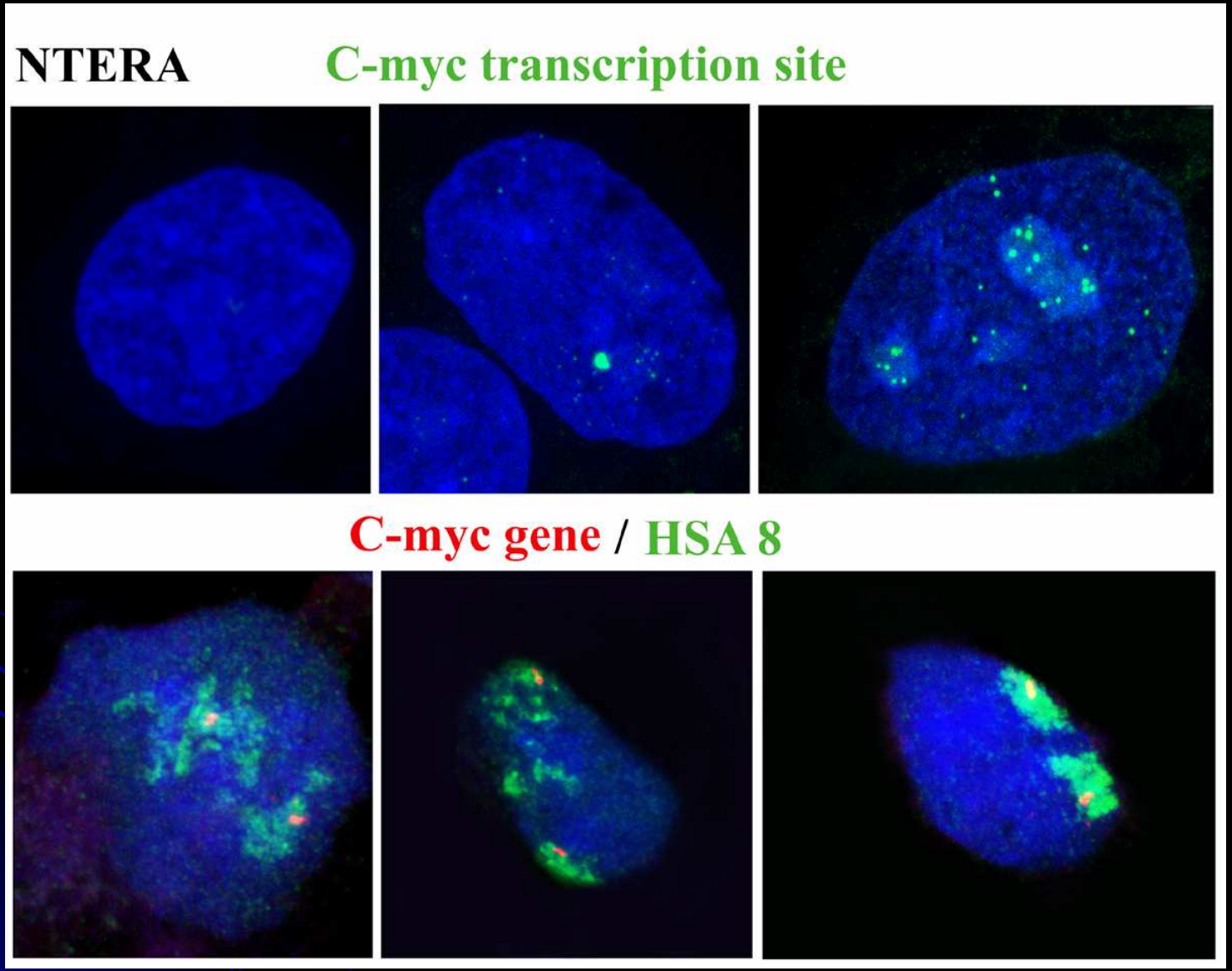
The c-myc gene



The c-myc transcription site

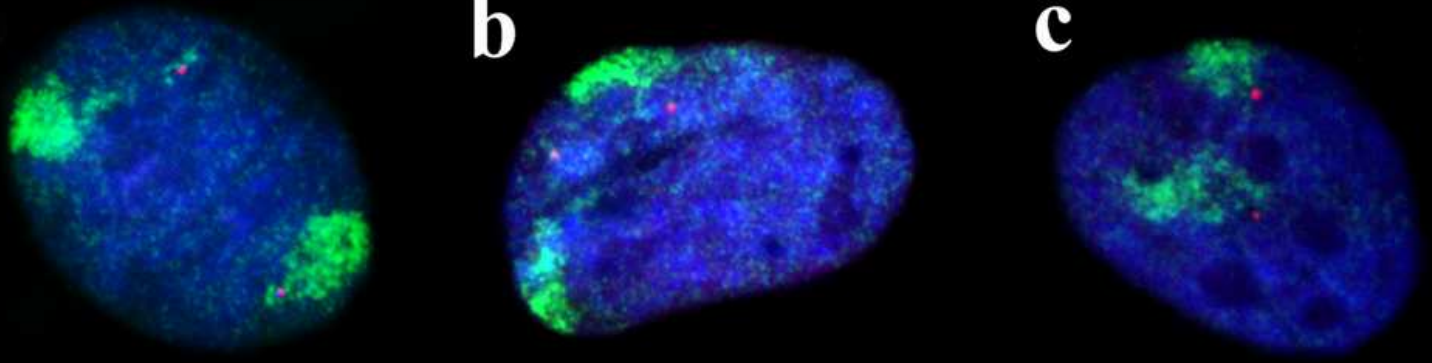


Human embryonal (teratocarcinoma) cells NTERA



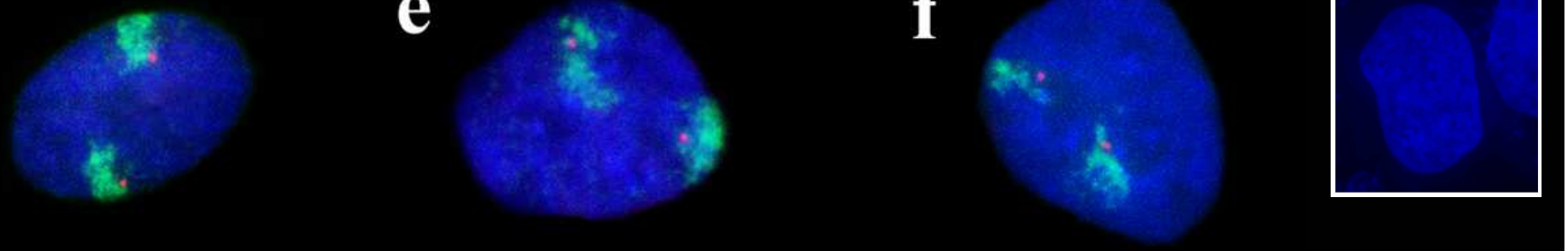
Oct4 / HSA 6 in hES cells

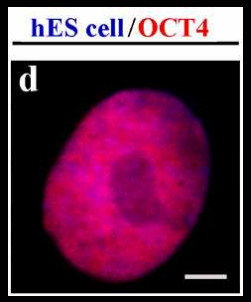
a b c



Oct4 / HSA 6 in hES cells - RA differentiated

d e f





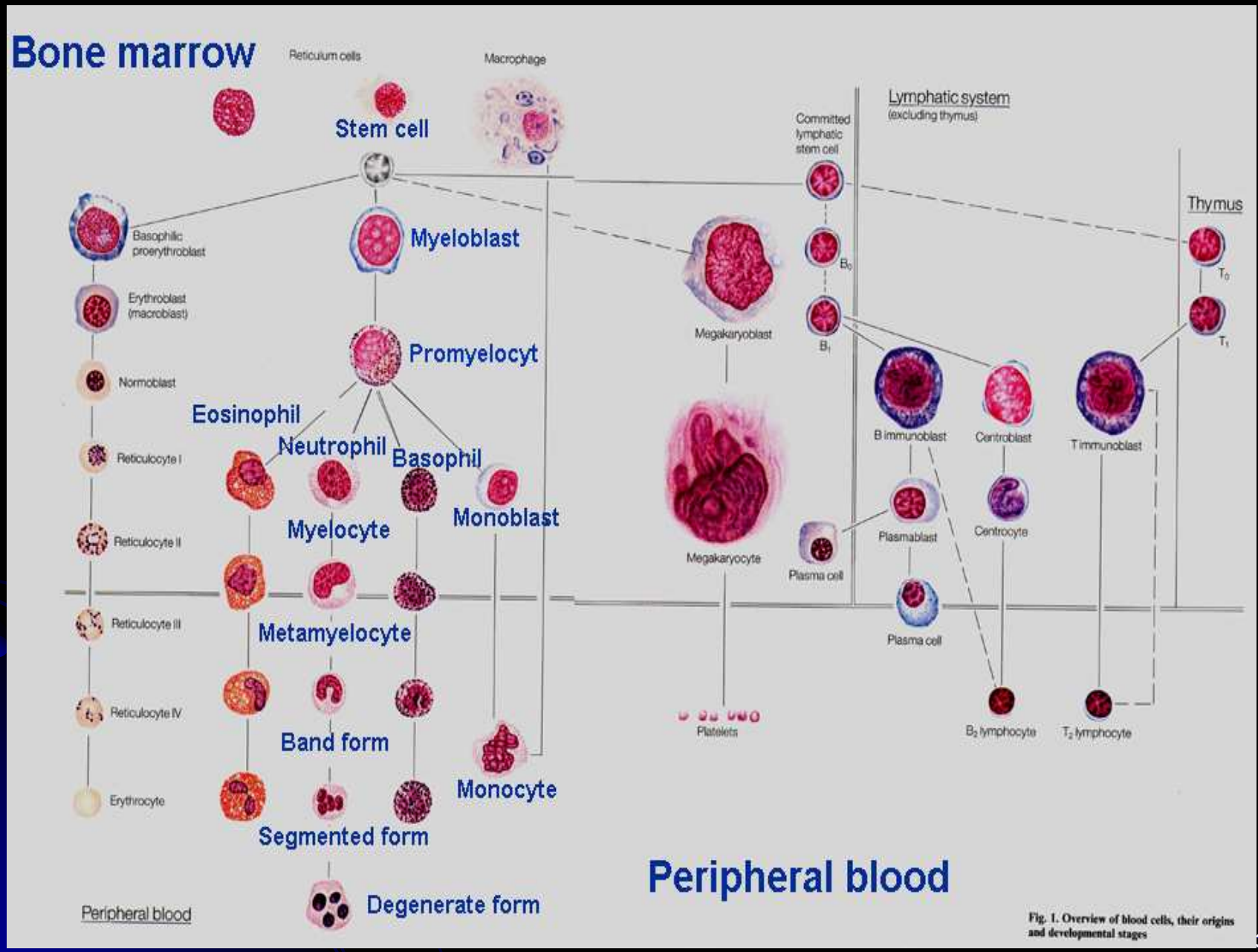
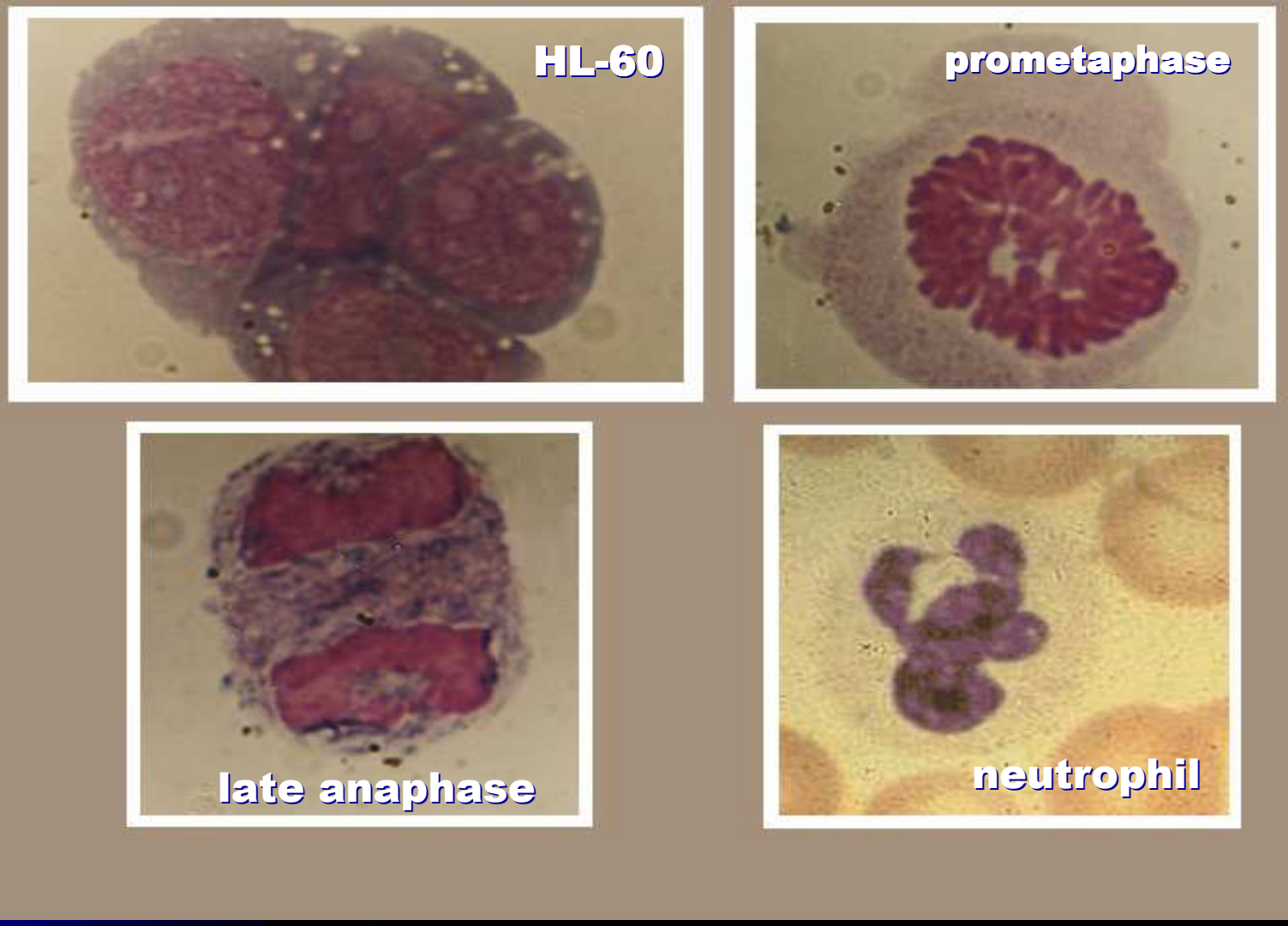
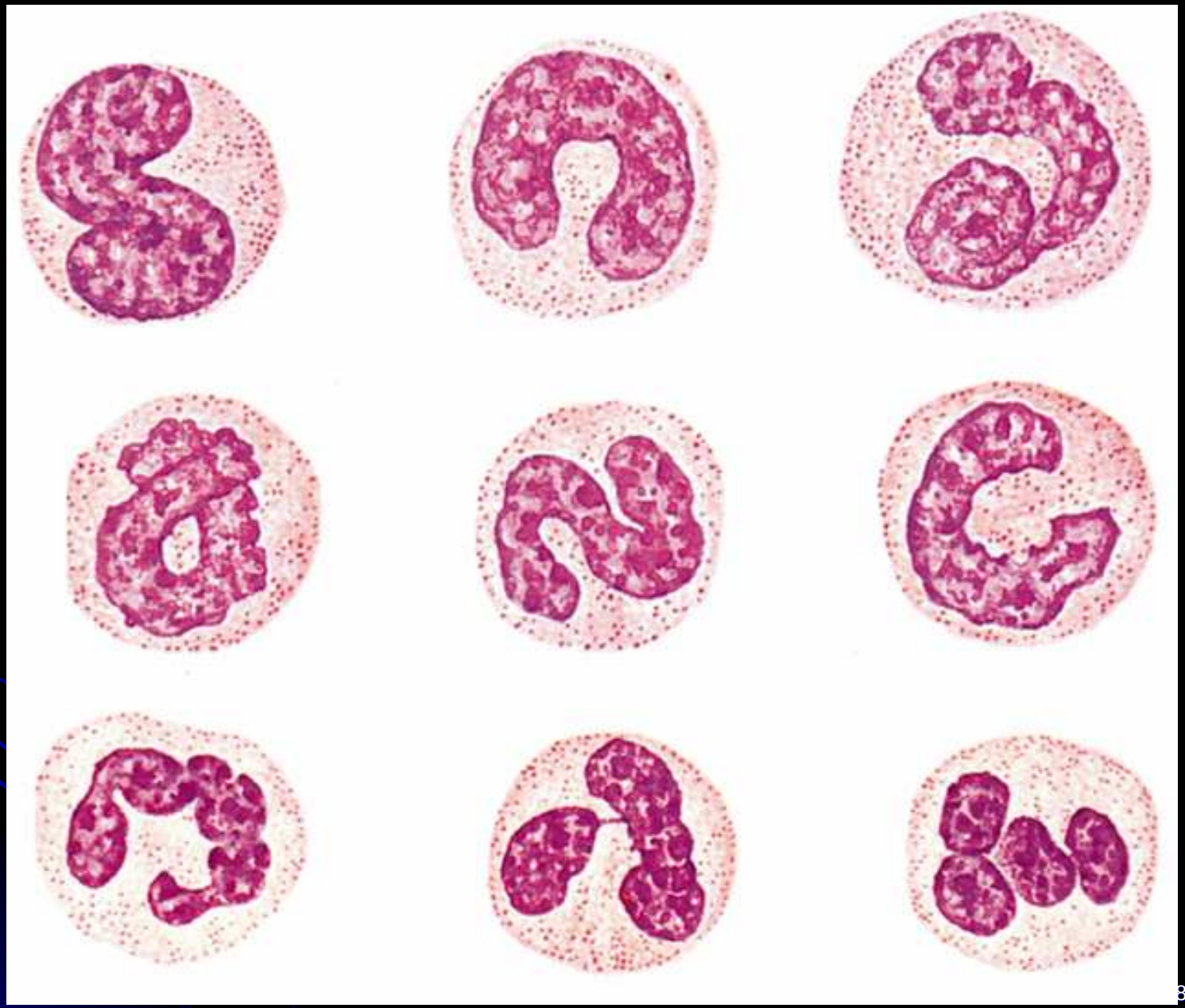


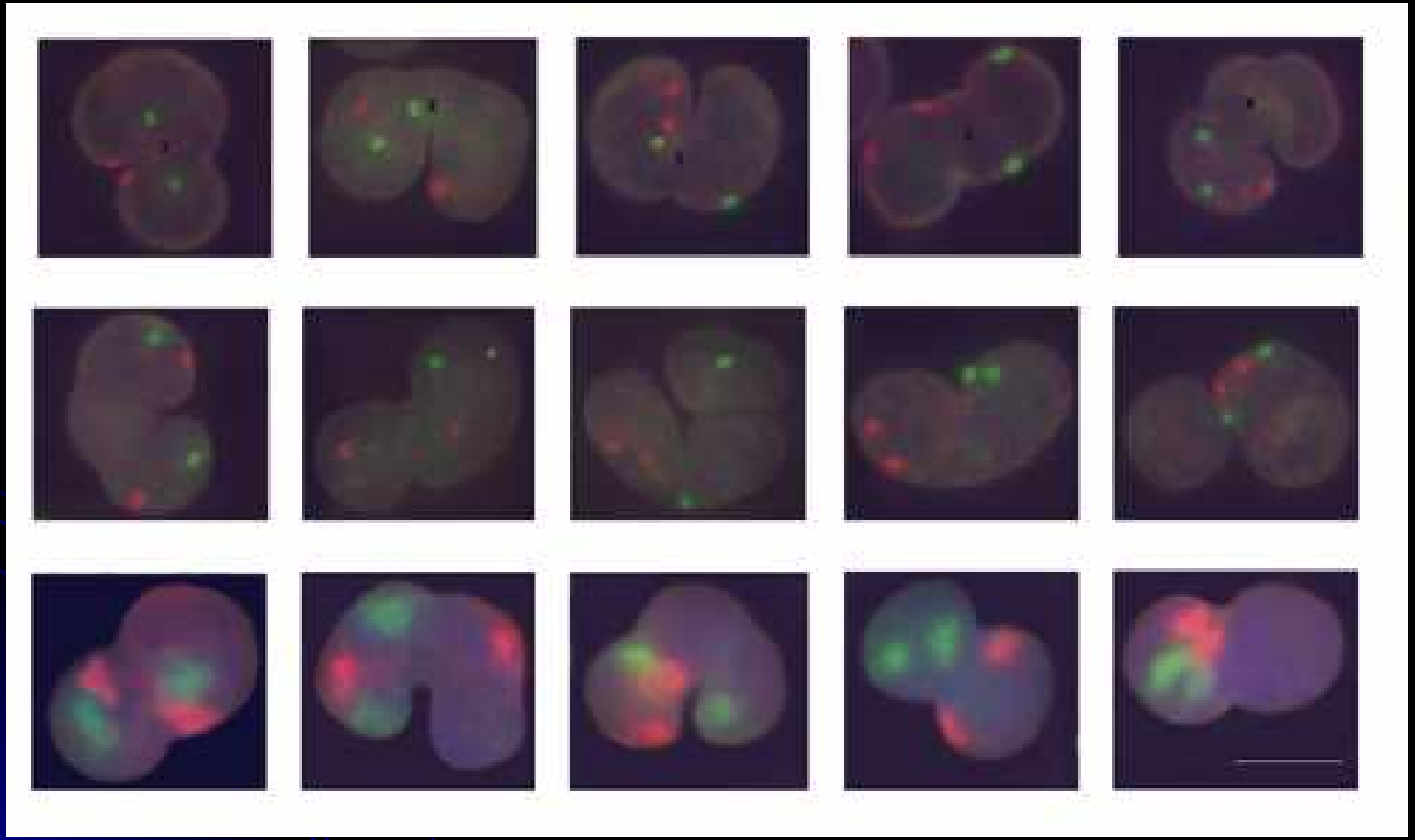
Fig. 1. Overview of blood cells, their origins and developmental stages

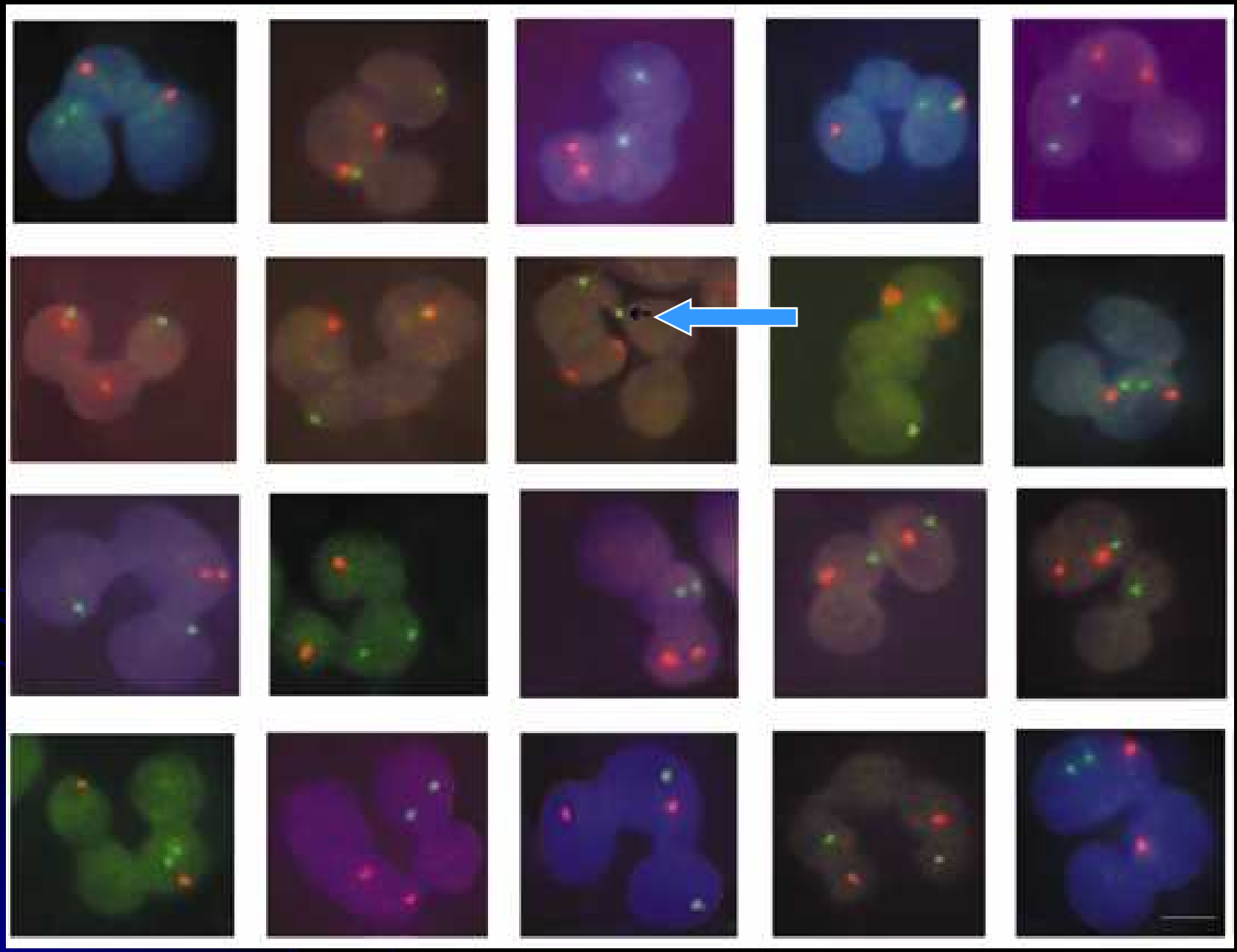
Morphology of human leukemic promyelocytic cell line HL60 and neutrophilic granulocyte



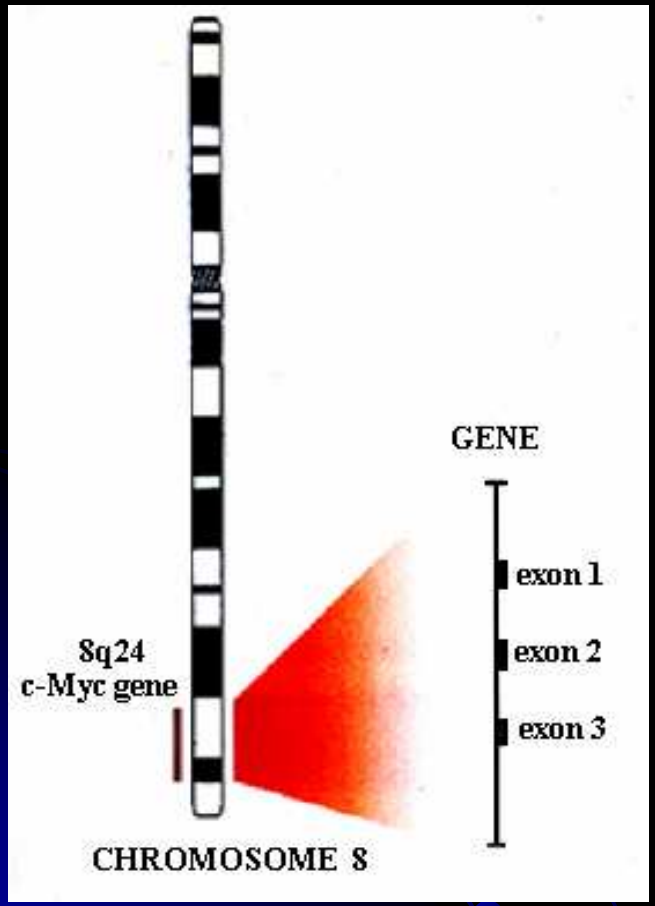


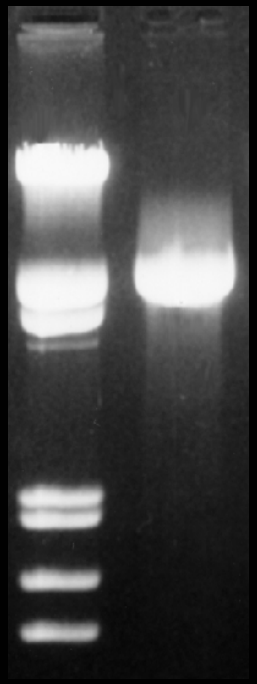
Topographic Types of Human Granulocytes

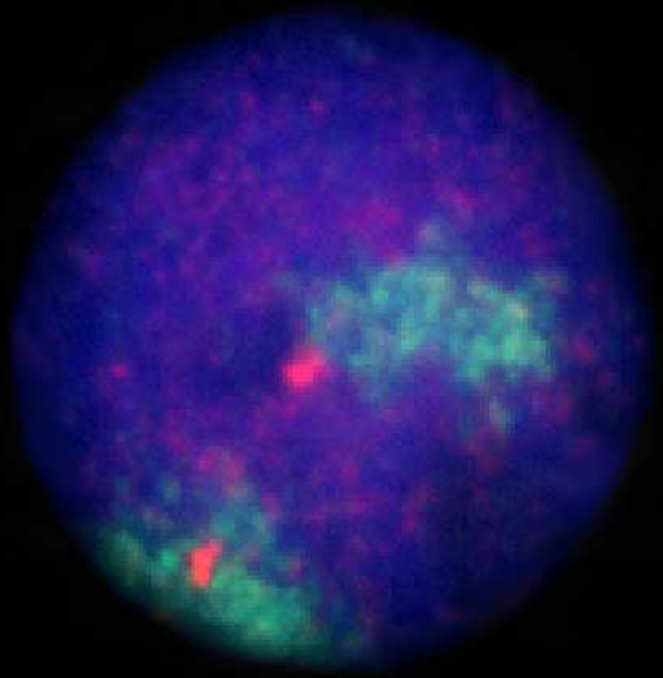




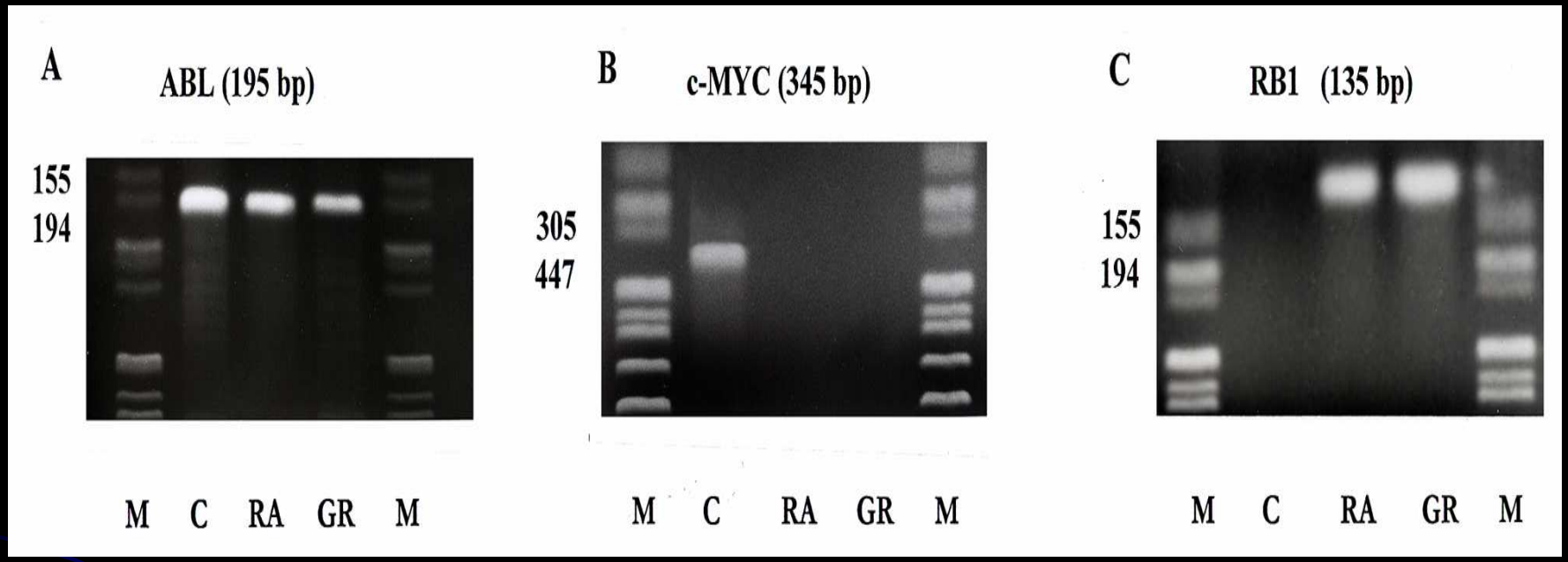
The C-myc Gene Nuclear Location



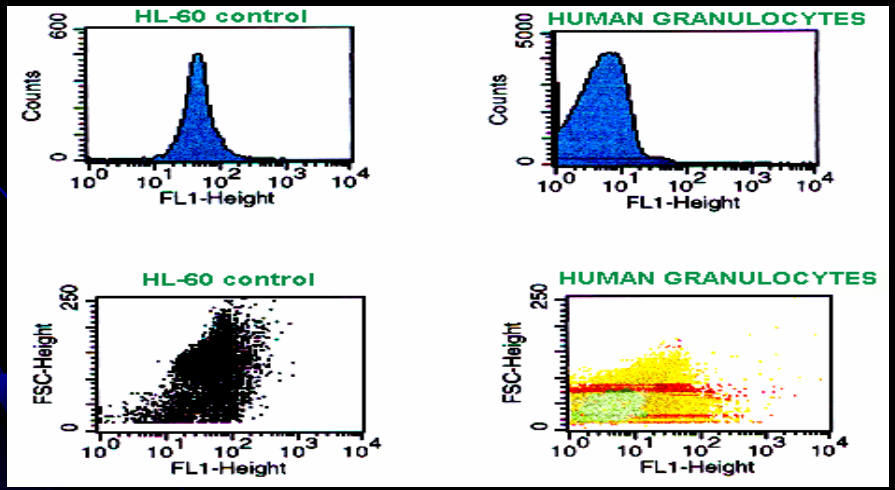




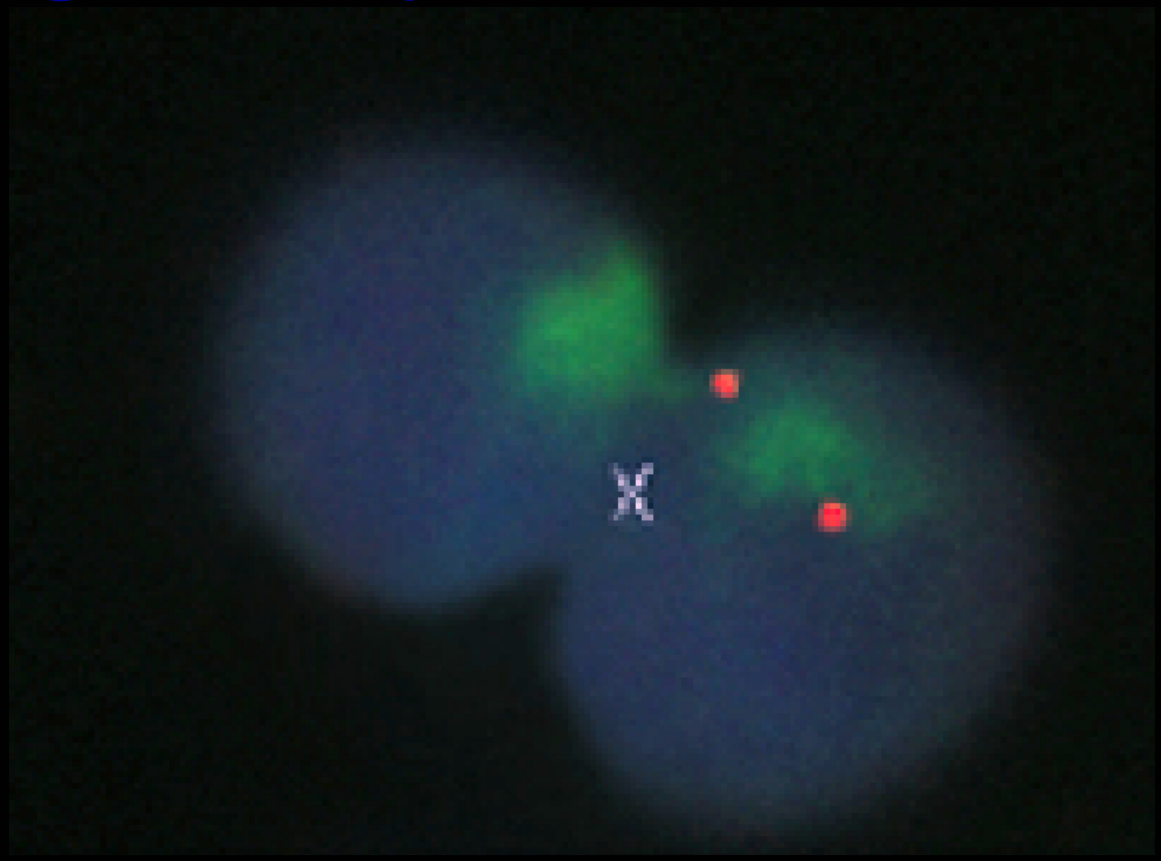
Changes in the expression of selected genes

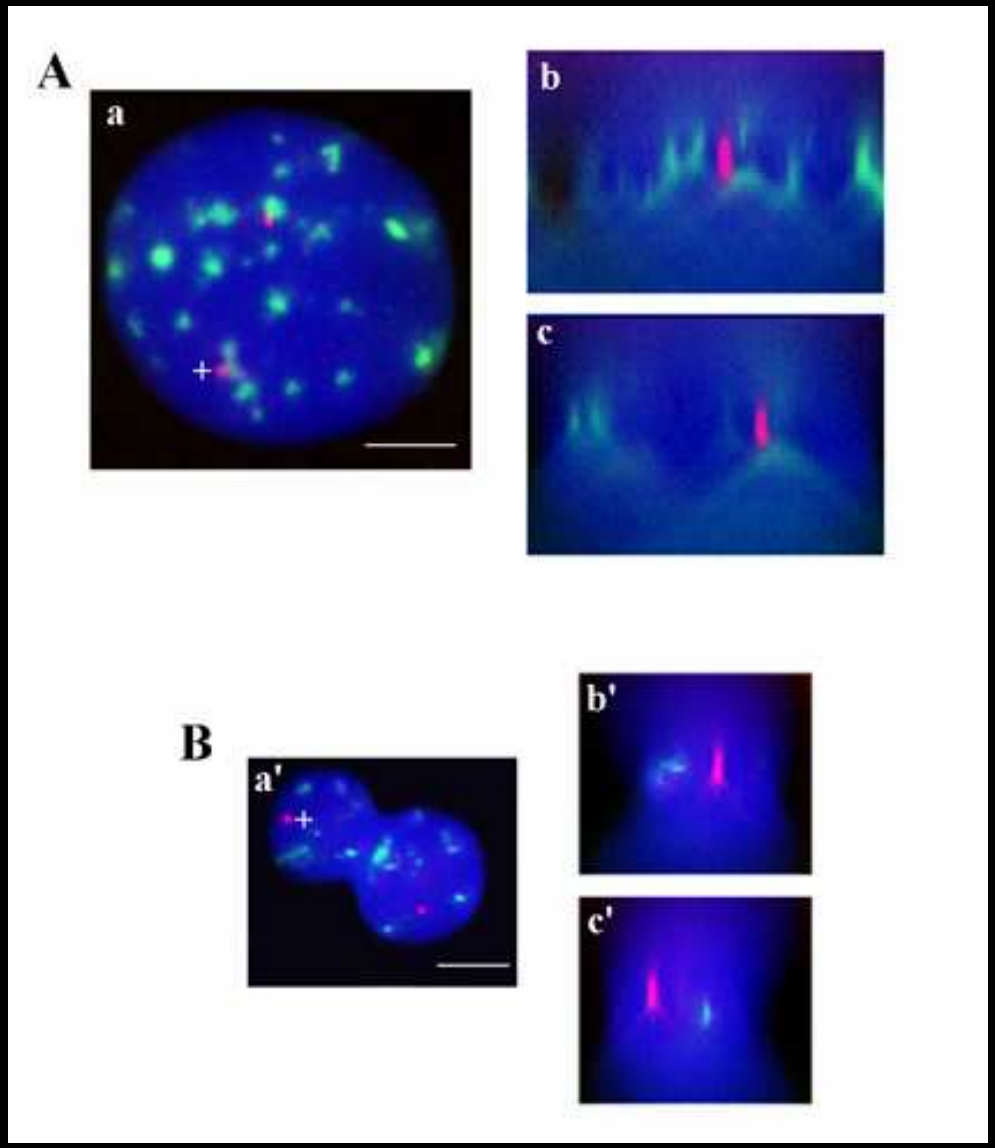


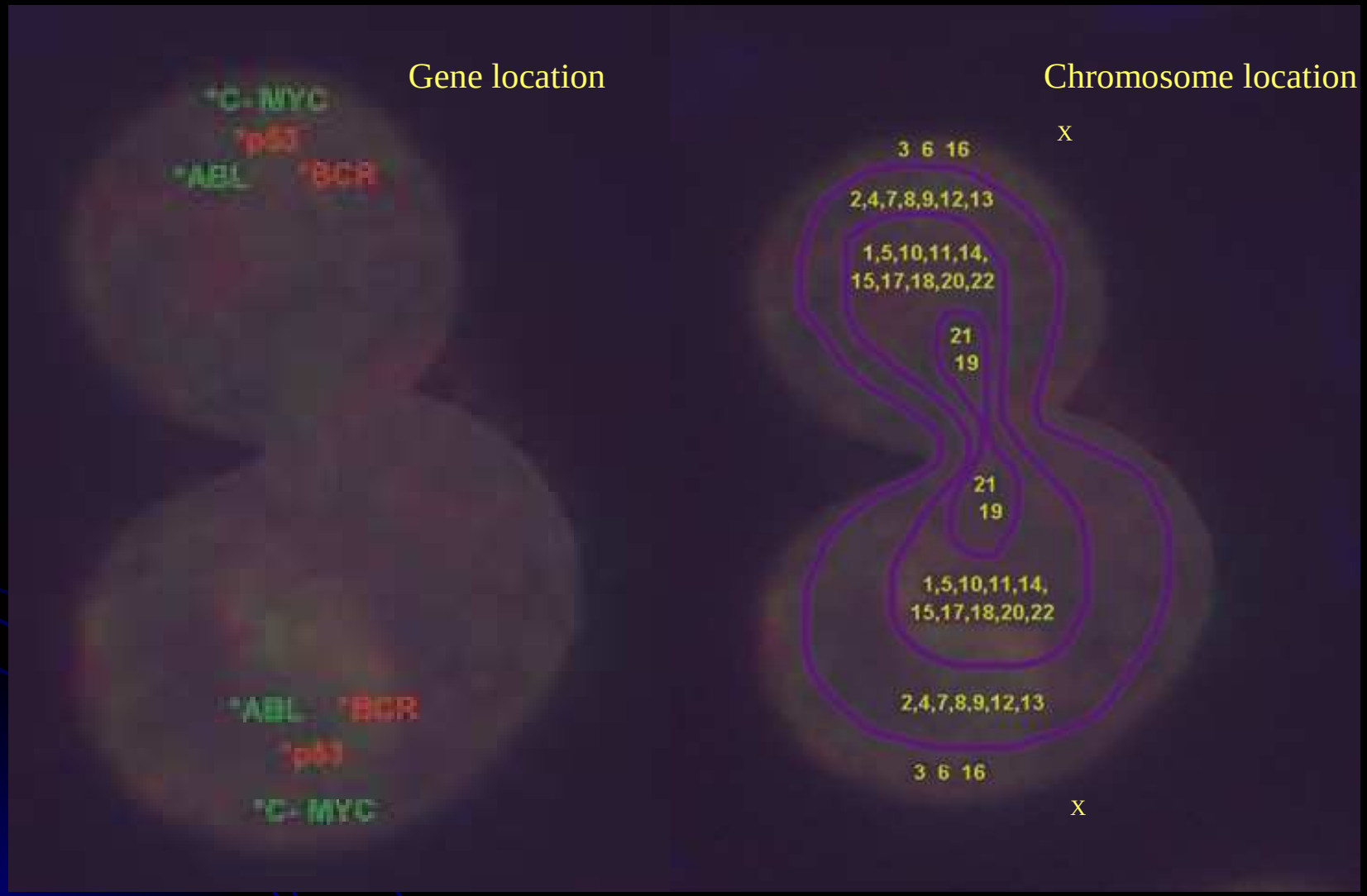
FCM c-myc



The C-myc gene nuclear topography in granulocytic nuclei




A
a
b
c
B
a'
b'
c'


Gene location
Chromosome location
*C-MYC
*p53
*ABL
*BCR
*ABL
*BCR
*p53
*C-MYC
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
X

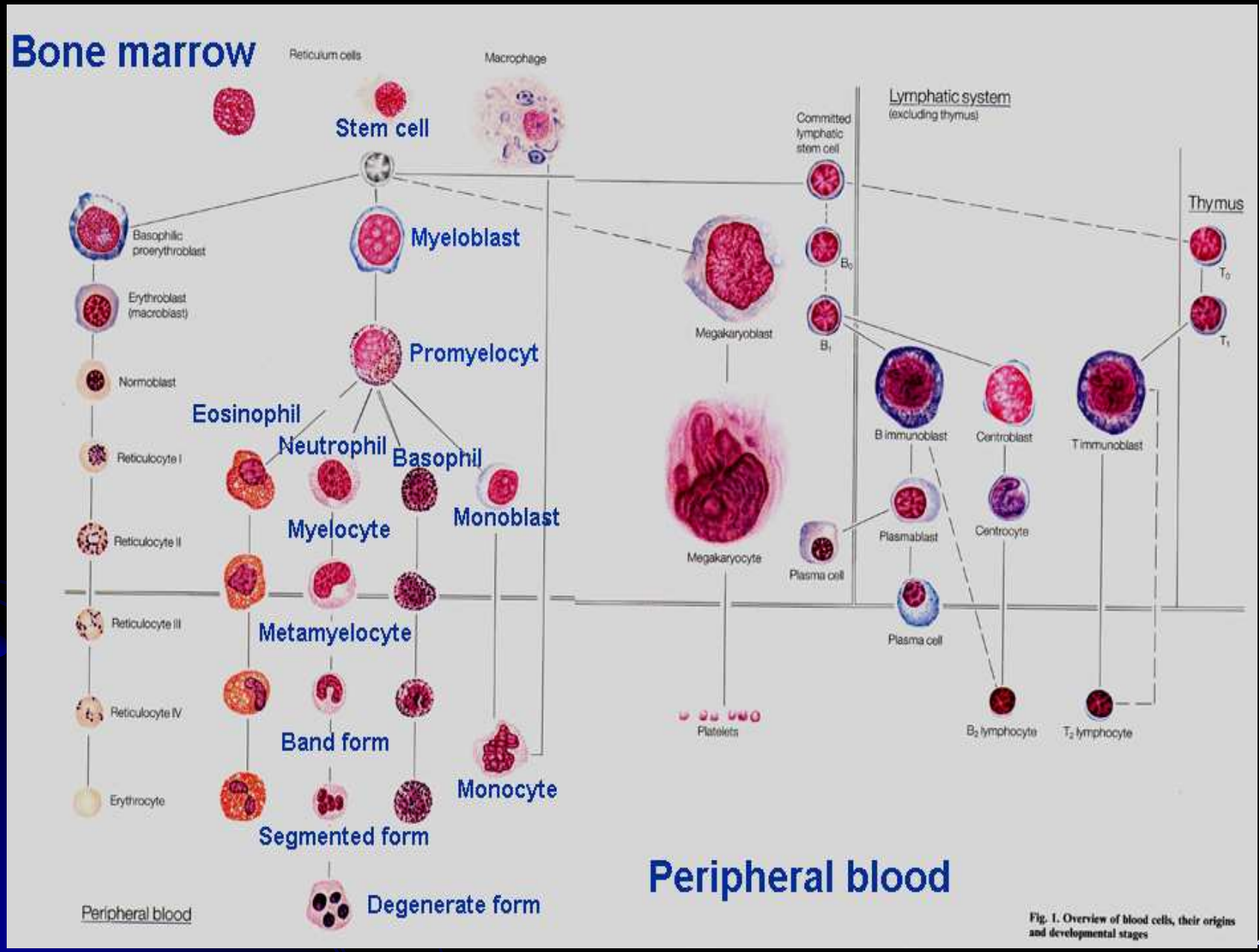
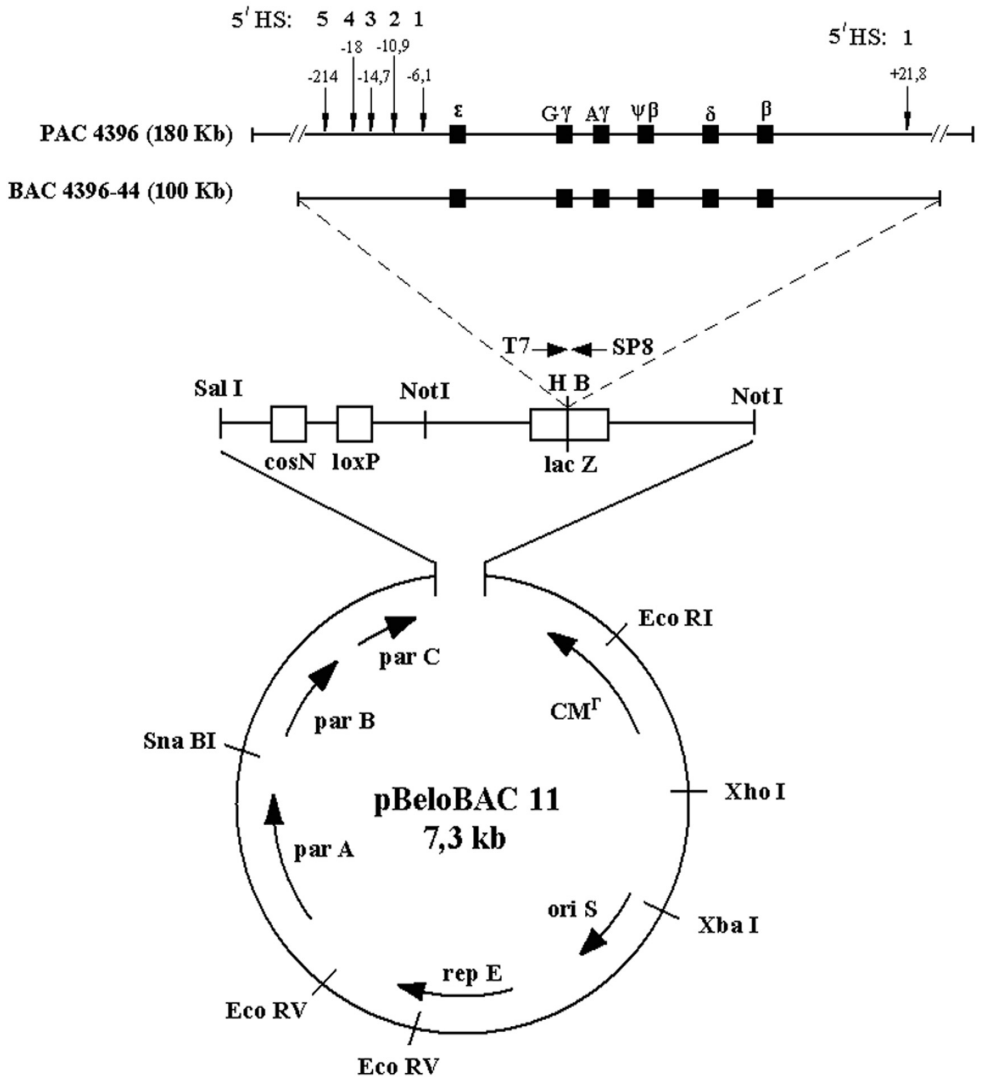


Fig. 1. Overview of blood cells, their origins and developmental stages

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:

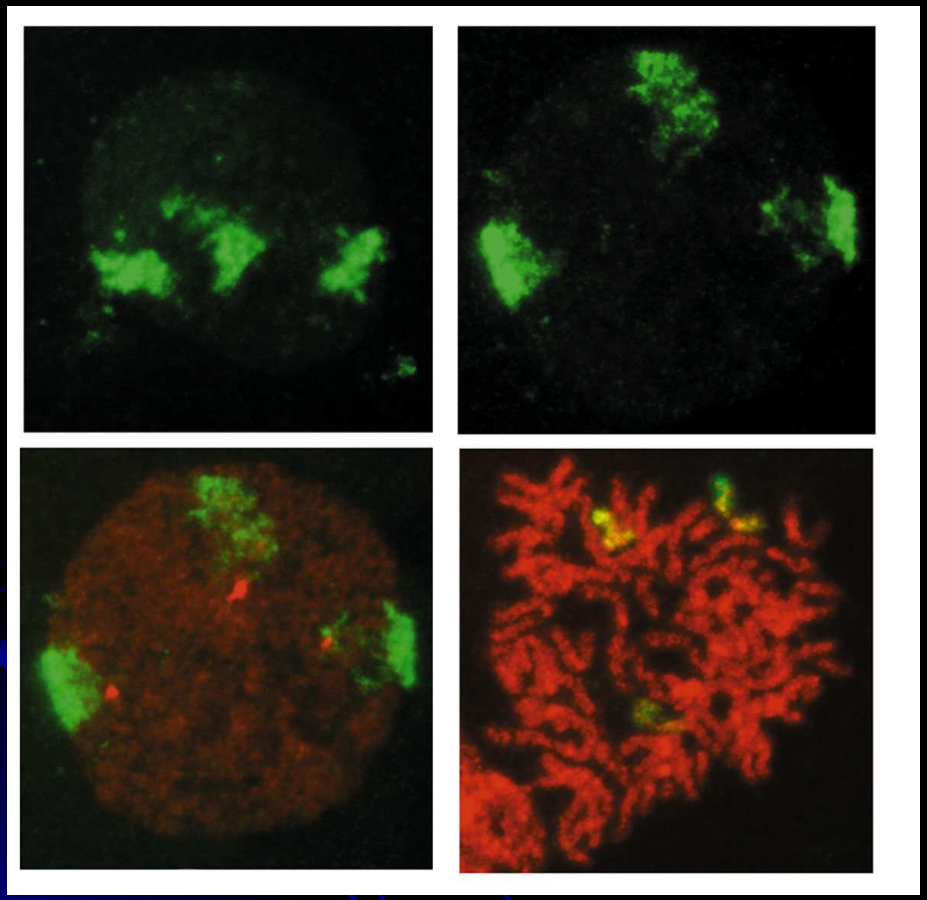
ε: expressed in early embryo

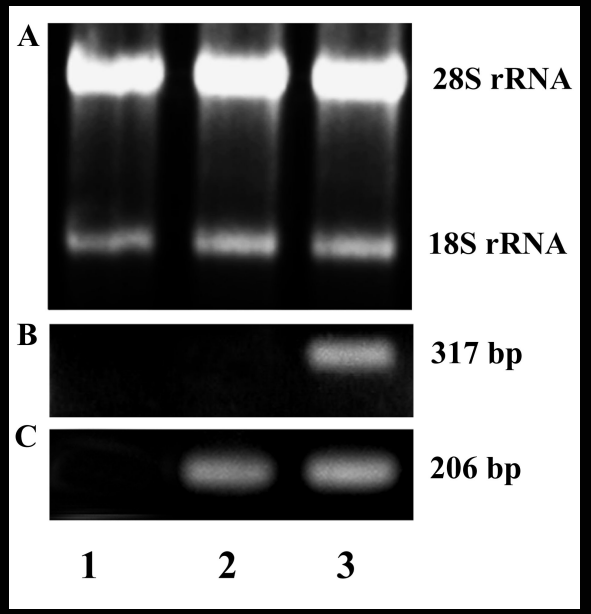
fetal γ: fetal life.

δ, β: adulthood.

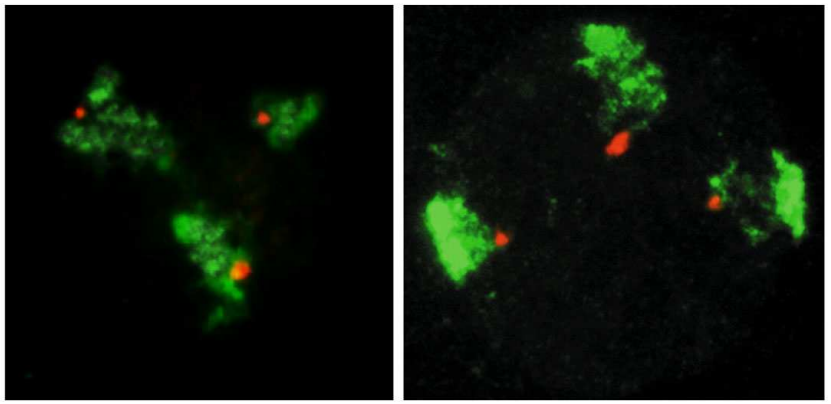
Changes in β-like gene expression acompany erythoid cell differentiation

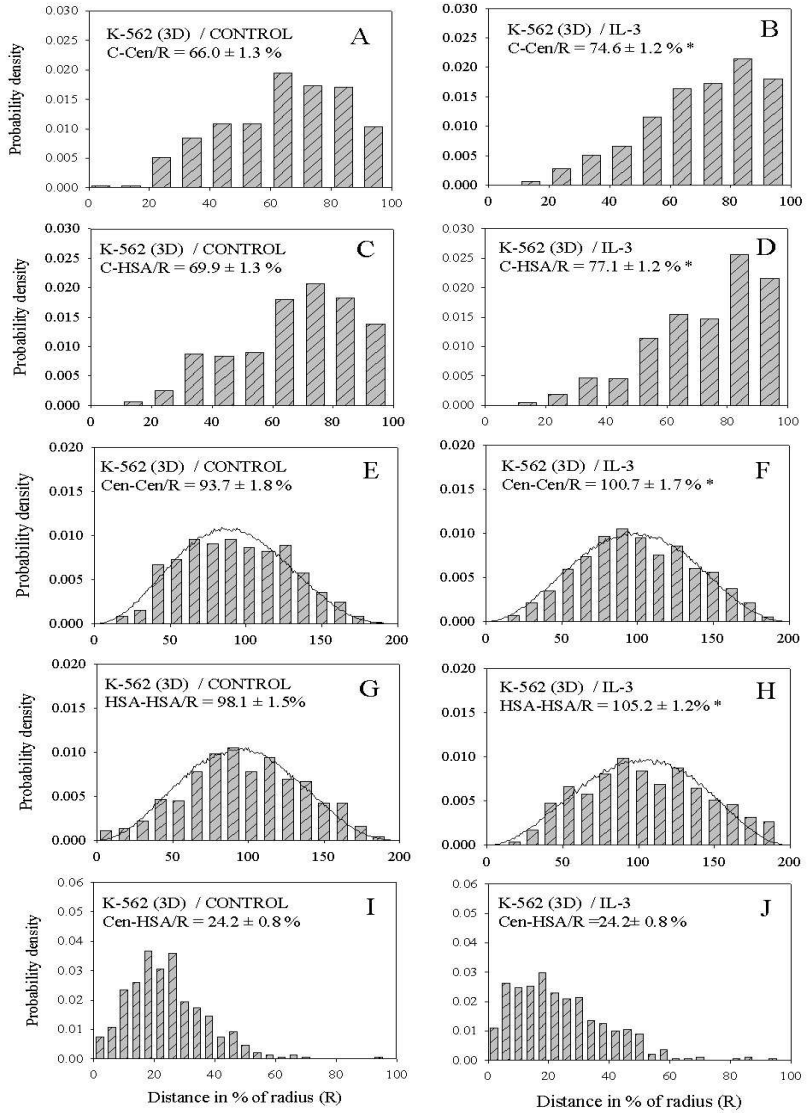
Differentiation of human hemopoietic cells into erythroid pathway



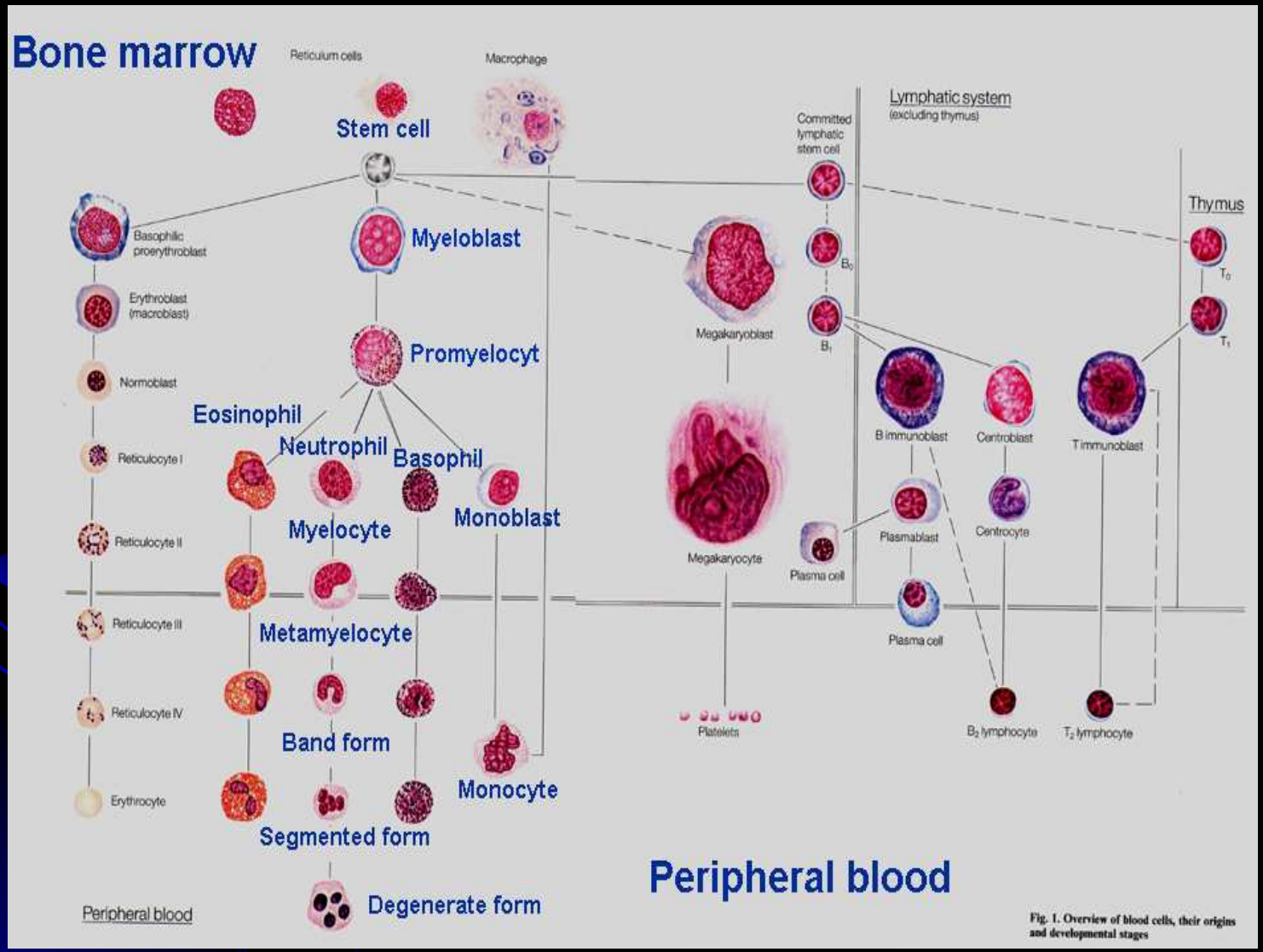


K IL-3

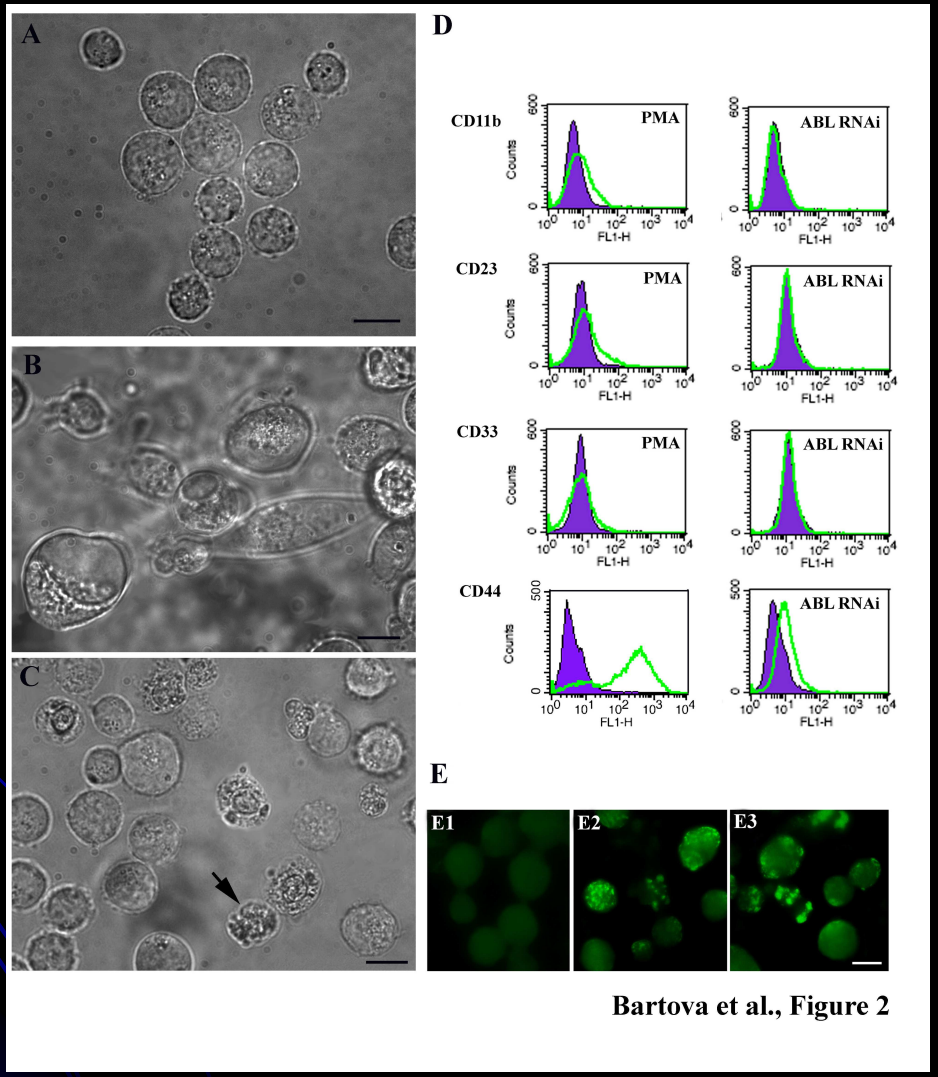



K-562 (3D) / CONTROL
C-Cen/R = 66.0 ± 1.3 %
A
K-562 (3D) / IL-3
C-Cen/R = 74.6 ± 1.2 % *
B
K-562 (3D) / CONTROL
C-HSA/R = 69.9 ± 1.3 %
C
K-562 (3D) / IL-3
C-HSA/R = 77.1 ± 1.2 % *
D
K-562 (3D) / CONTROL
Cen-Cen/R = 93.7 ± 1.8 %
E
K-562 (3D) / IL-3
Cen-Cen/R = 100.7 ± 1.7 % *
F
K-562 (3D) / CONTROL
HSA-HSA/R = 98.1 ± 1.5%
G
K-562 (3D) / IL-3
HSA-HSA/R = 105.2 ± 1.2% *
H
K-562 (3D) / CONTROL
Cen-HSA/R = 24.2 ± 0.8 %
I
K-562 (3D) / IL-3
Cen-HSA/R =24.2± 0.8 %
J
Probability density
Distance in % of radius (R)

Differentiation of human hemopoietic cells into megakaryocytes

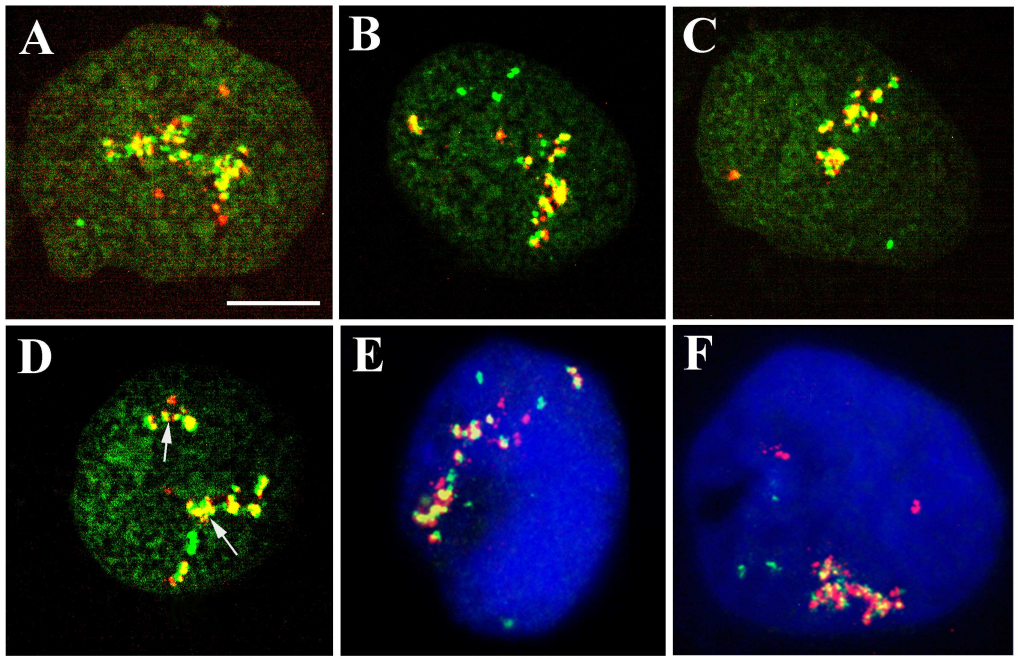


Differentiation of human hemopoietic cells into megakaryocytes

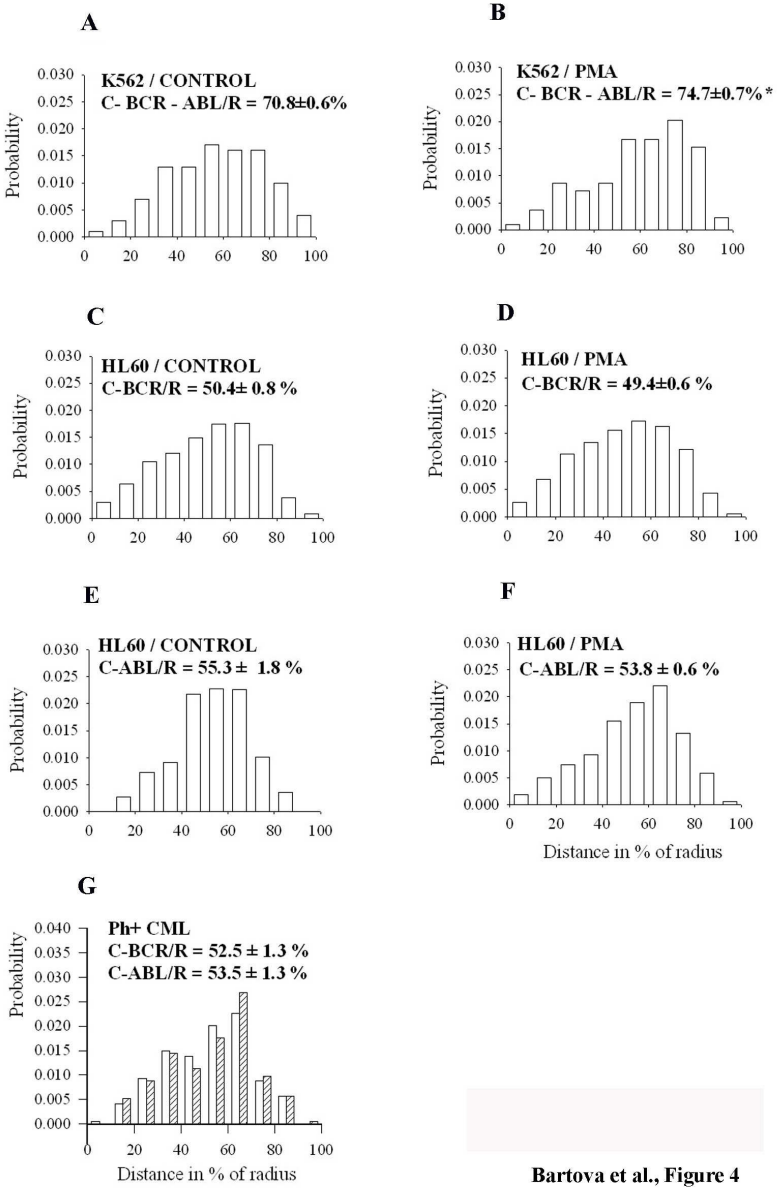


Bartova et al., Figure 2

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

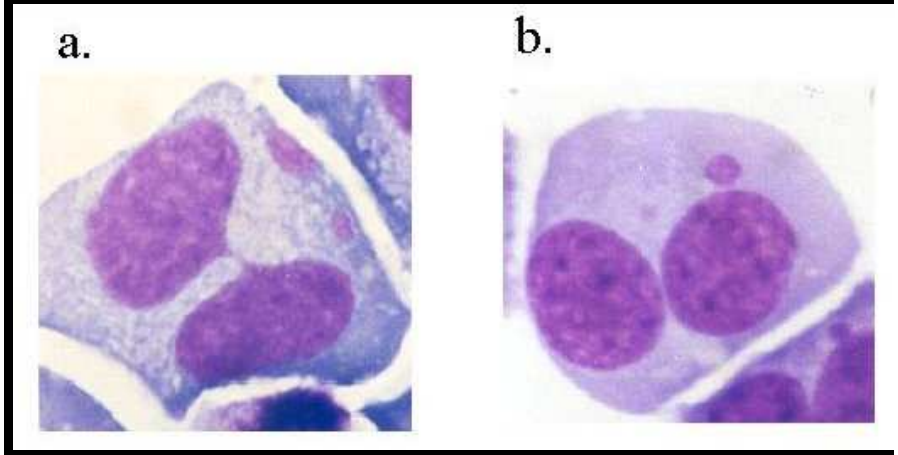


Bartova et al., Figure 3

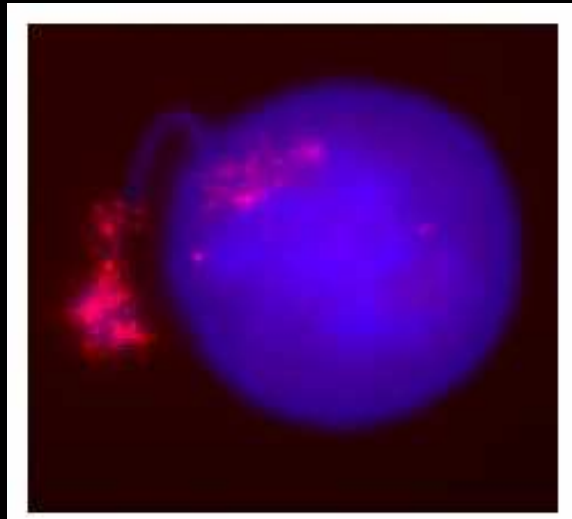


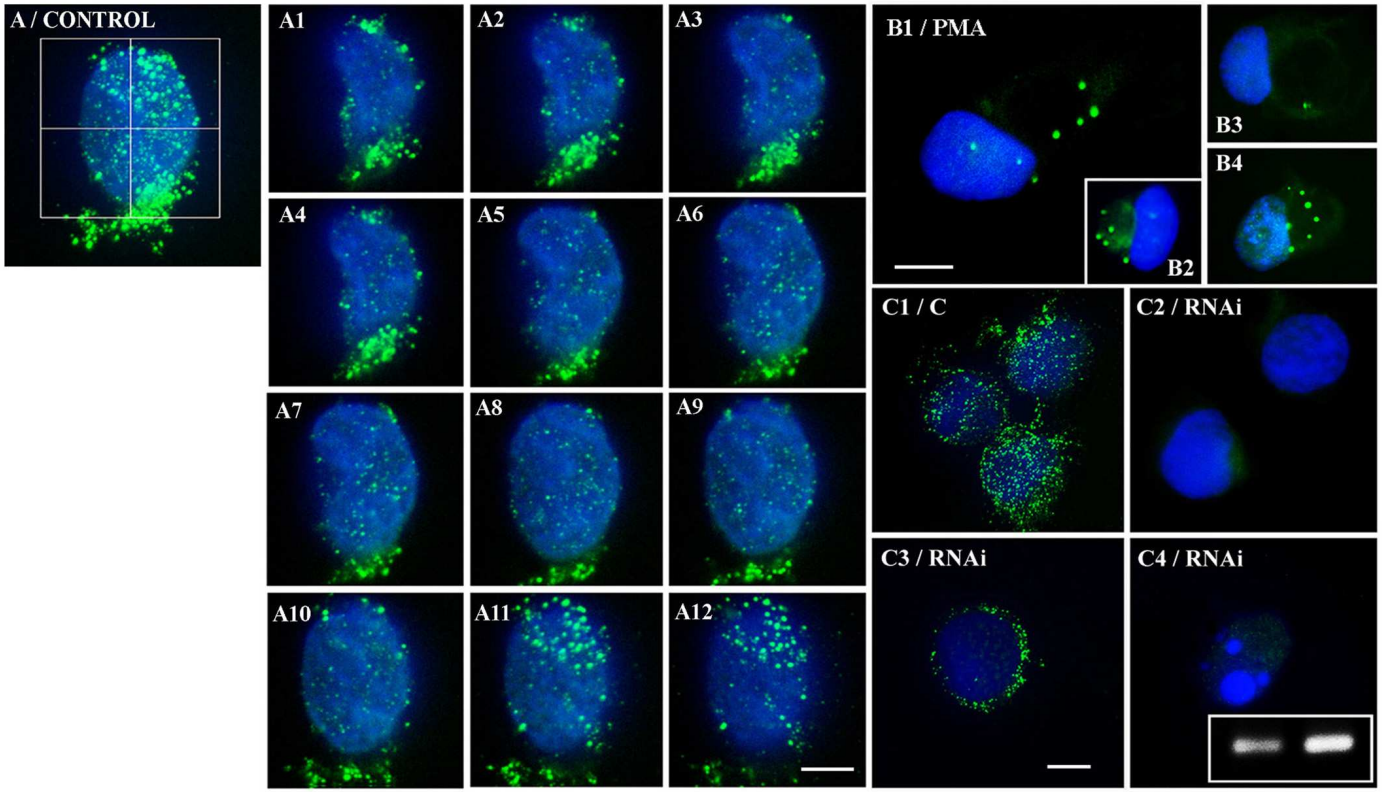
Bartova et al., Figure 4

MICRONUCLEI

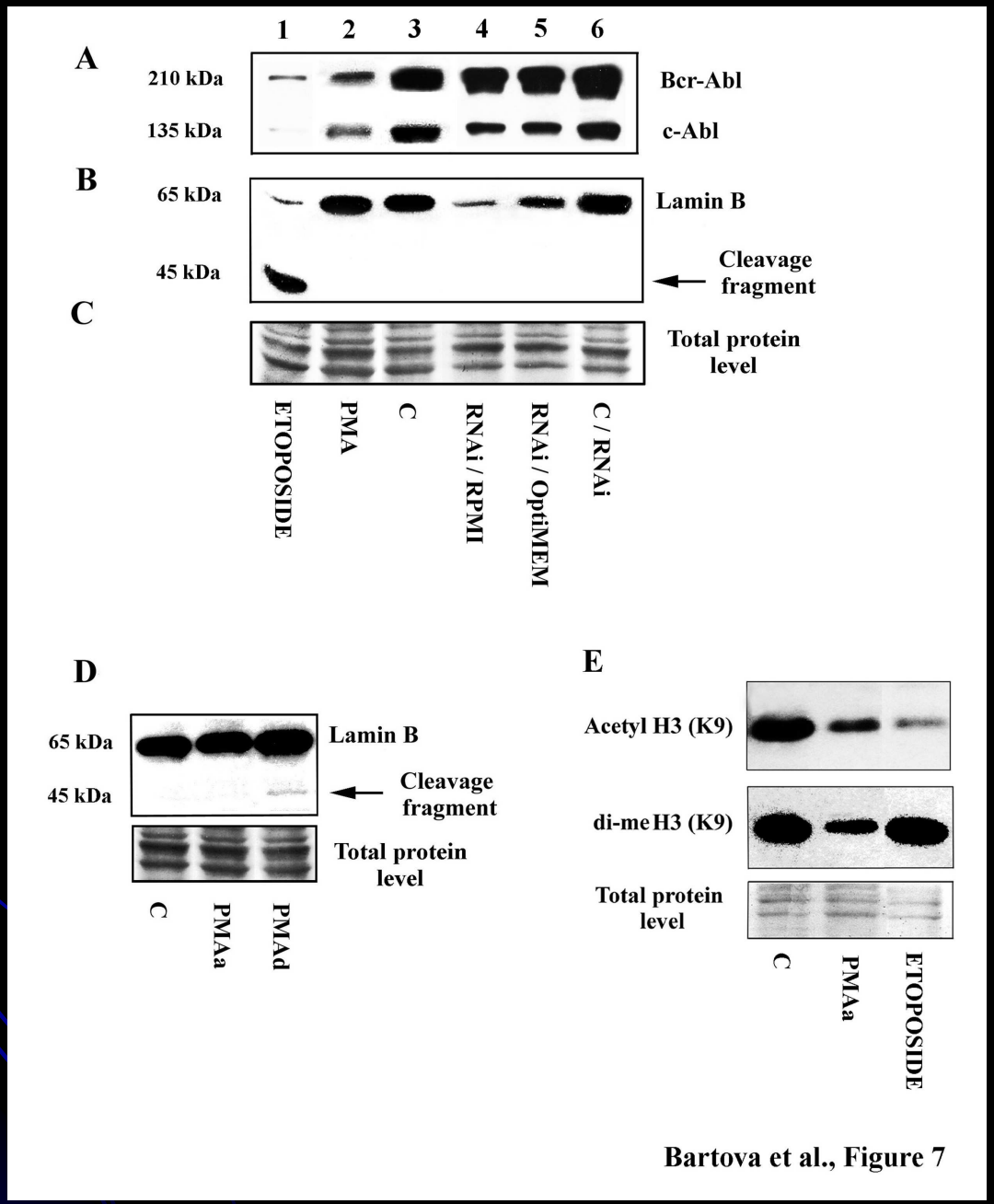


N-myc





Bartova et al., Figure 6



Bartova et al., Figure 7

ZÁVĚR

Diferenciace je charakteristická nejenom specifickými změnami na úrovni morfologie buněk, ale významně se mění i struktura chromatinu. Tyto strukturální změny v genomu mají velký význam z hlediska transkripční aktivity genů.