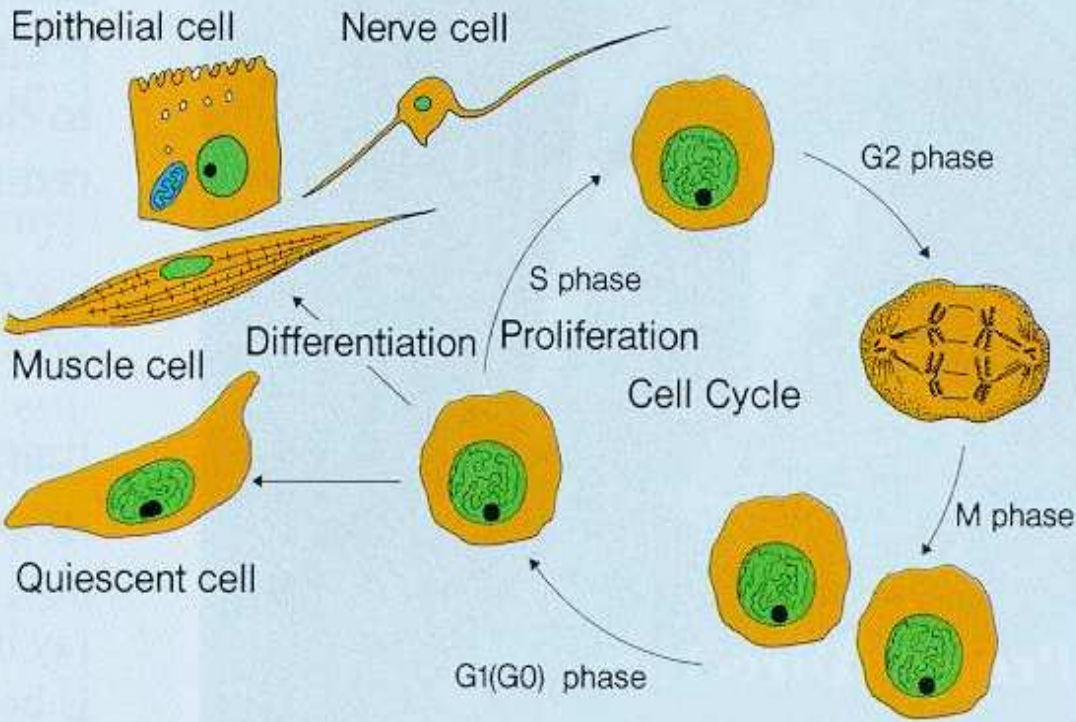


Buněčná diferenciaci a struktura chromatinu

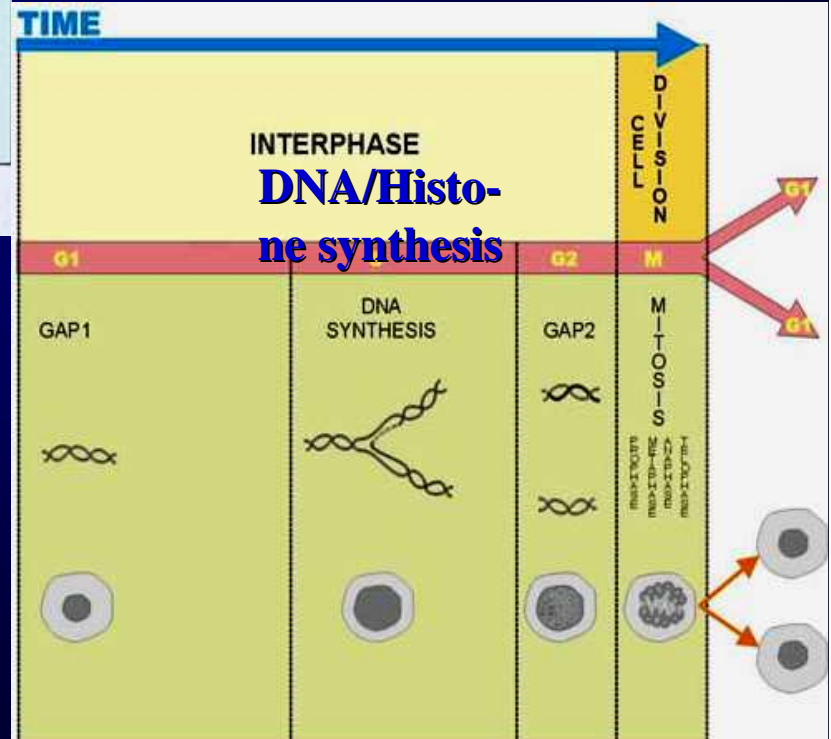
Buněčná diferenciaci je proces při kterém buňky získávají nový fenotyp, který je spojen se specifickou buněčnou funkcí. Pro daný buněčný typ je charakteristická aktivace skupiny genů, které jsou zodpovědné za terminální diferenciaci.



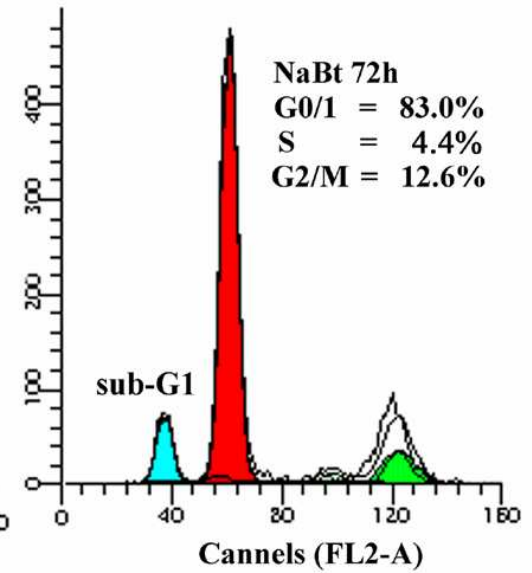
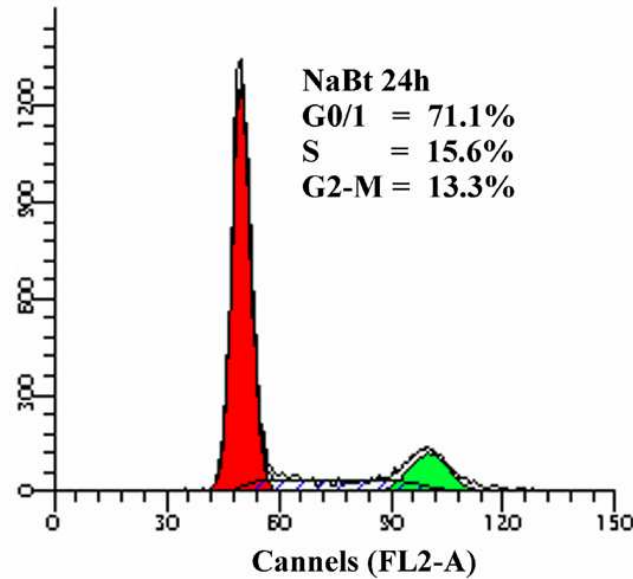
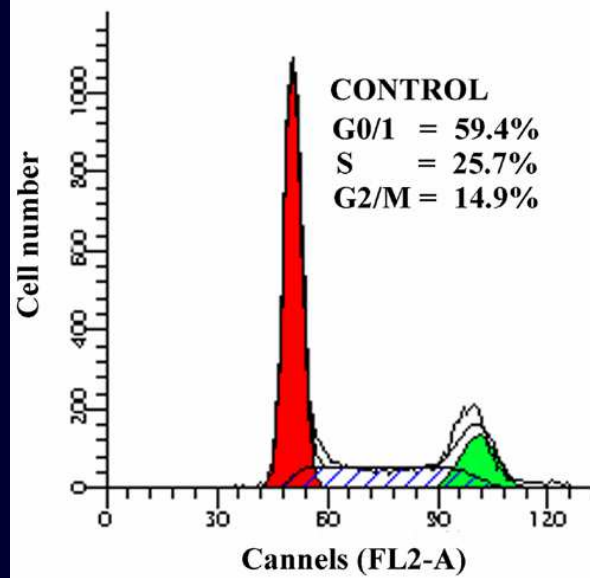
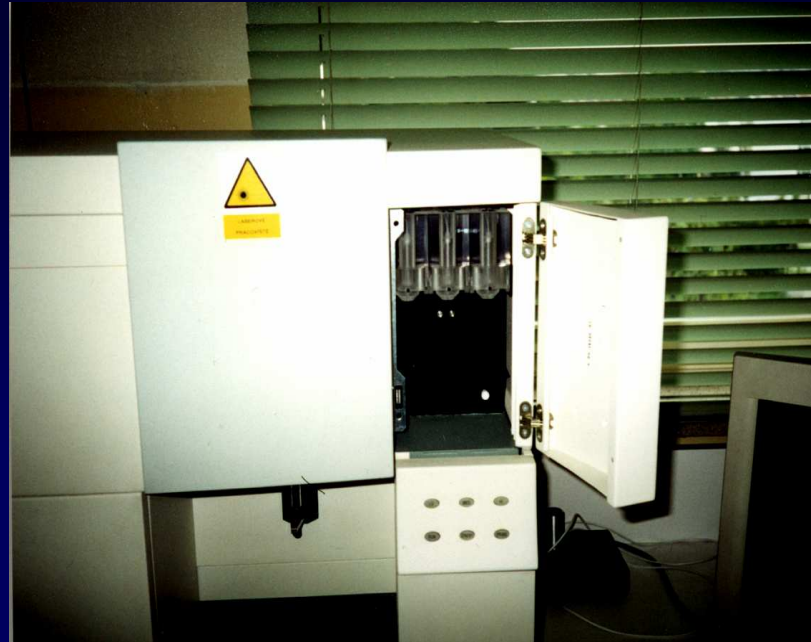
Cell Growth and Differentiation

Cell Differentiation

Cell Growth

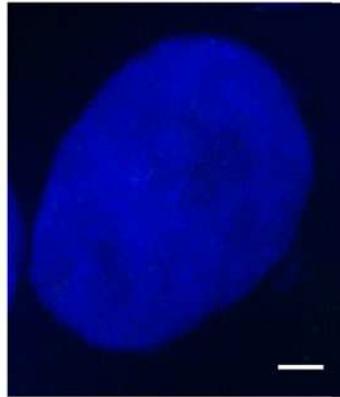


FCM

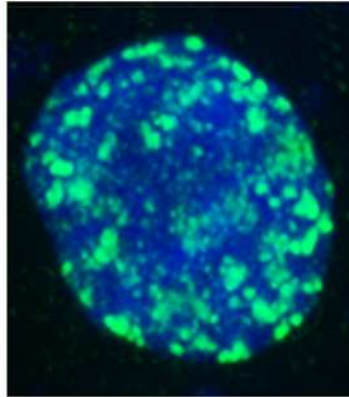


pKi-67

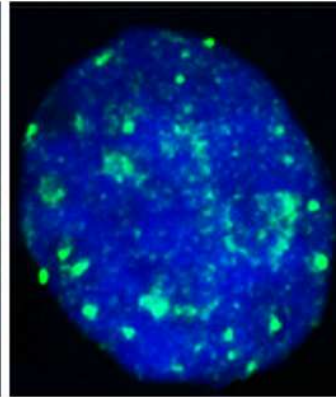
G0



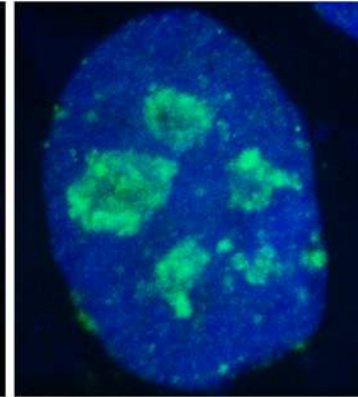
early G1



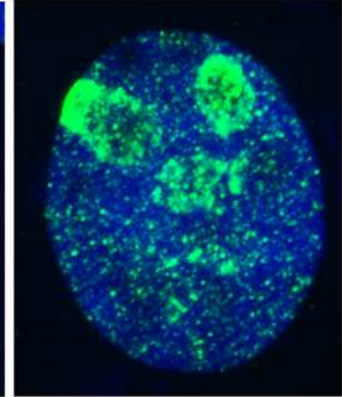
mid G1



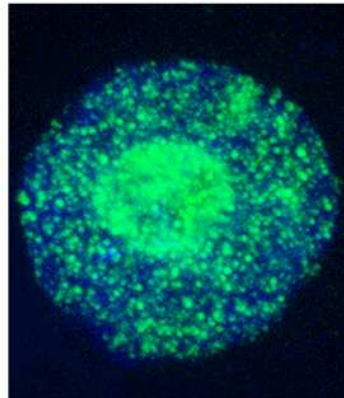
late G1



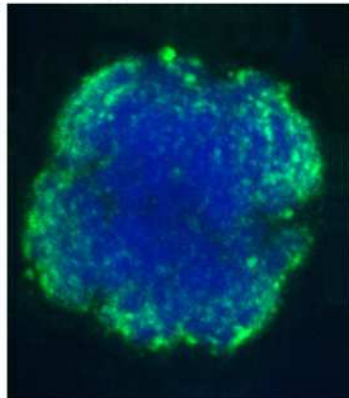
S



G2

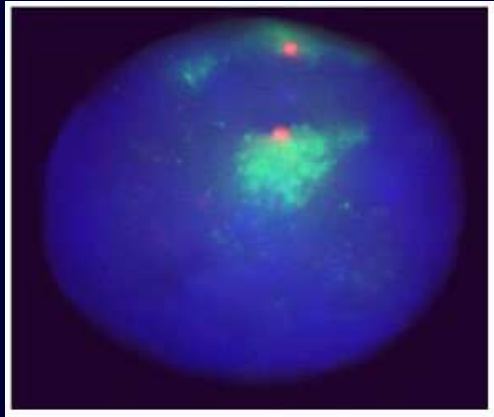


metaphase



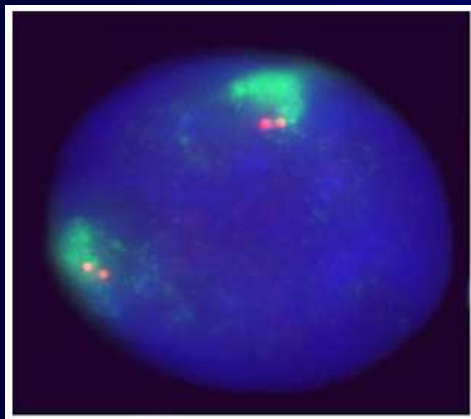
Andrea Harničarová et al. (2006)

G1



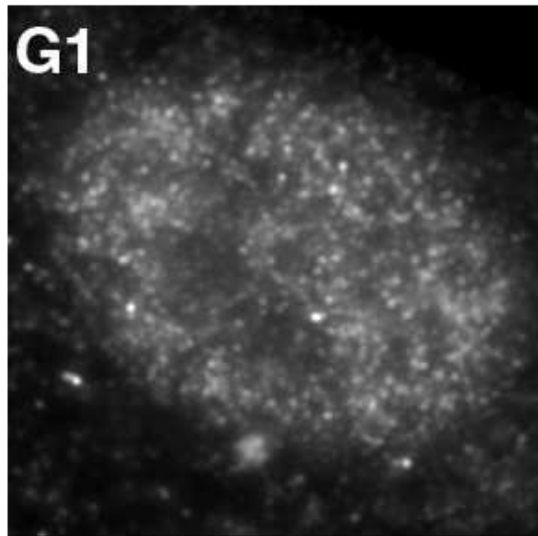
Rb1 gene

G2

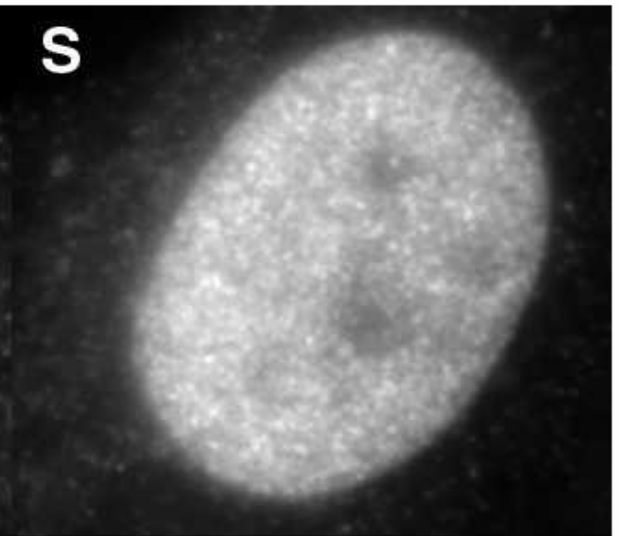


Anti-phospho H3

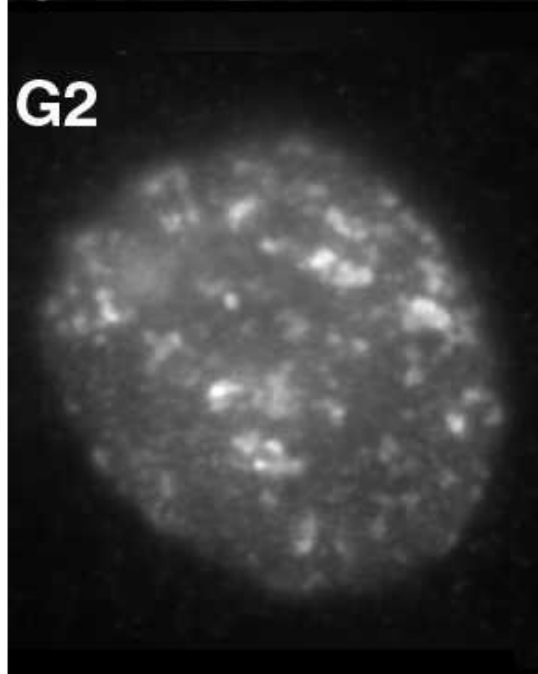
G1



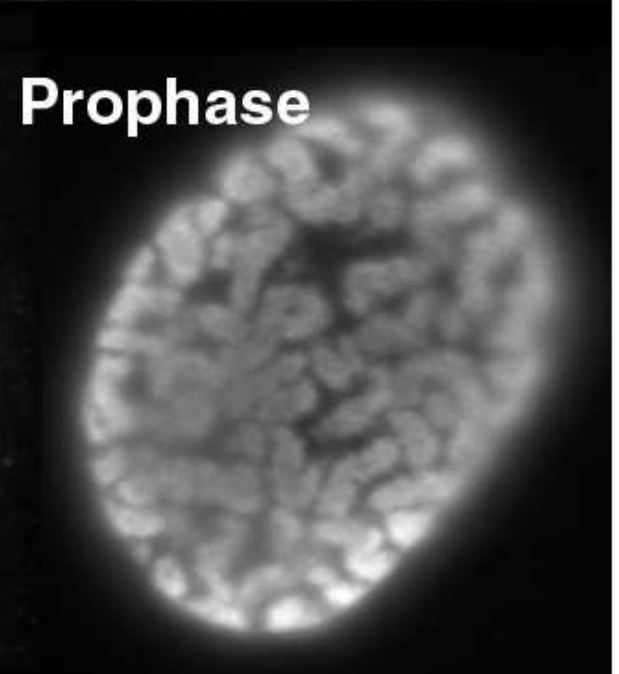
S



G2



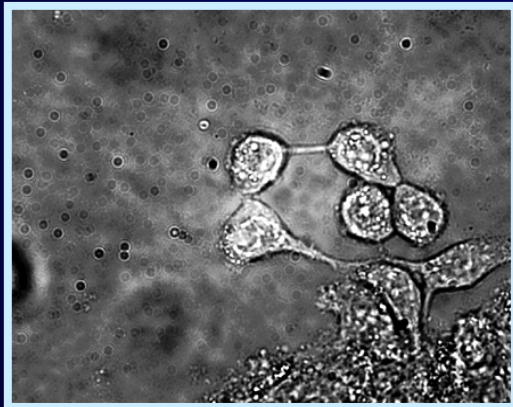
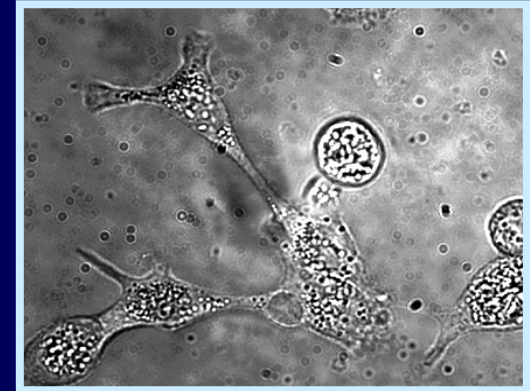
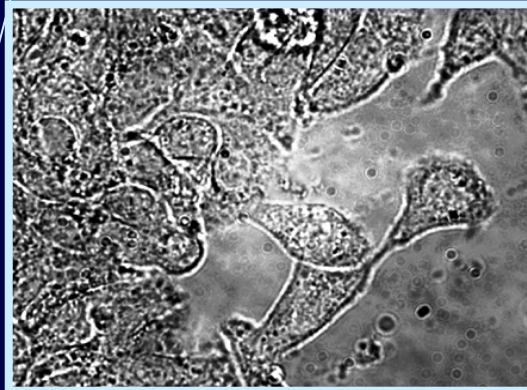
Prophase



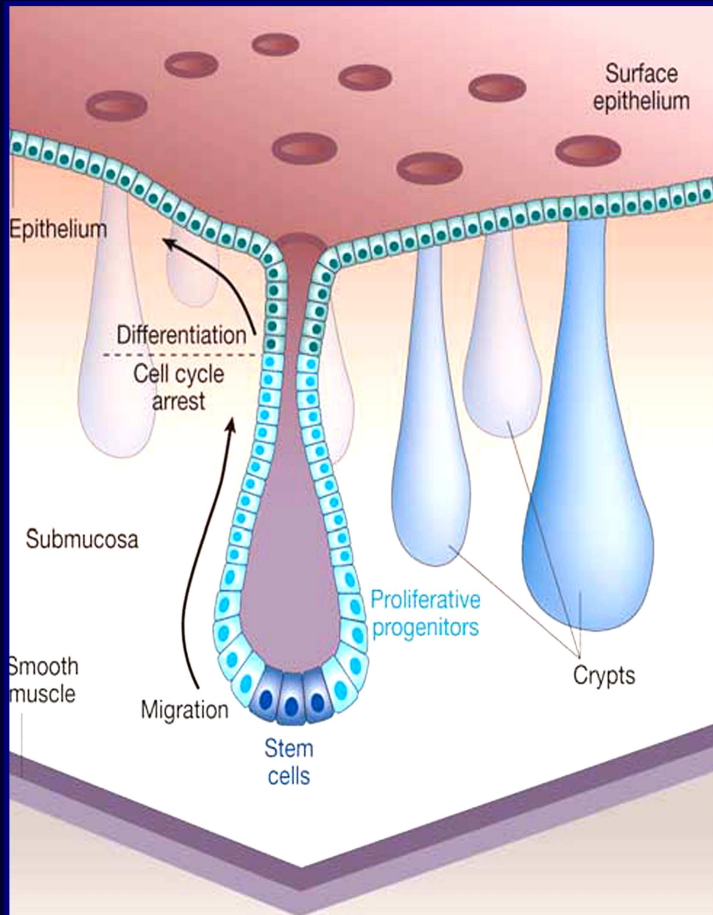
Enterocytic cell differentiation

Control

Sodium Butyrate



Enterocytic Cell Differentiation

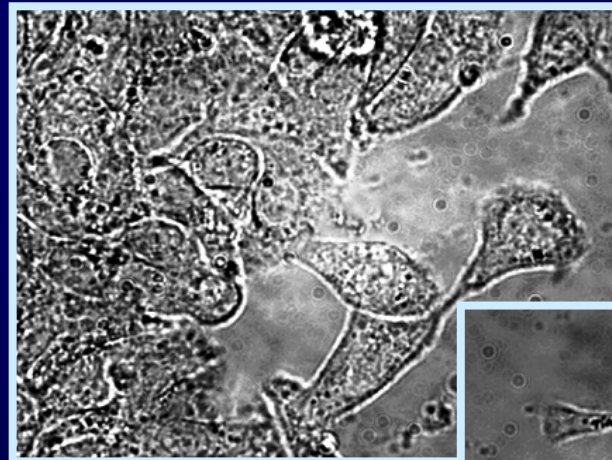


Nature, Vol 434 (2005), www.nature.com

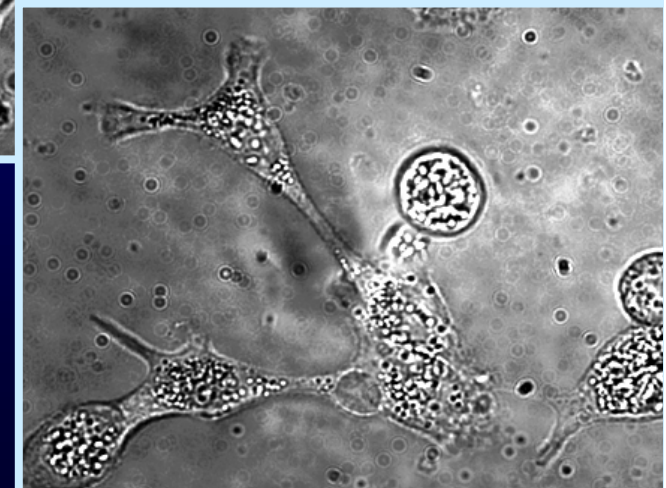
Figure 3 Tissue anatomy of the colonic epithelium. Putative stem cells (dark blue) reside at the crypt bottom. Proliferating progenitor cells occupy two-thirds of the crypt. Differentiated cells (green) populate the remainder of the crypt and the flat surface epithelium. (Adapted from ref. 89.)



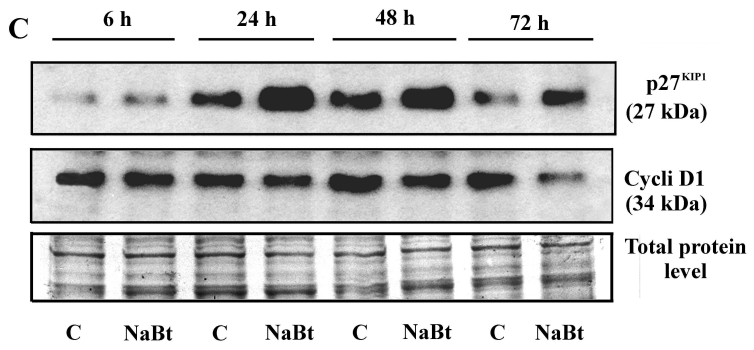
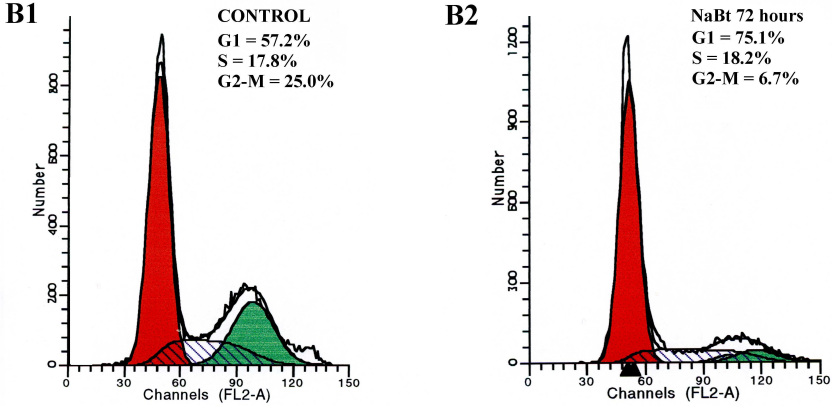
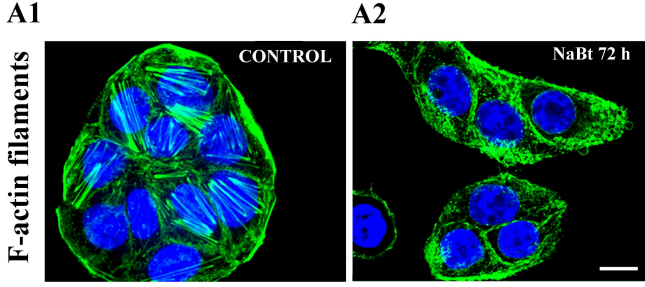
Control



NaBt



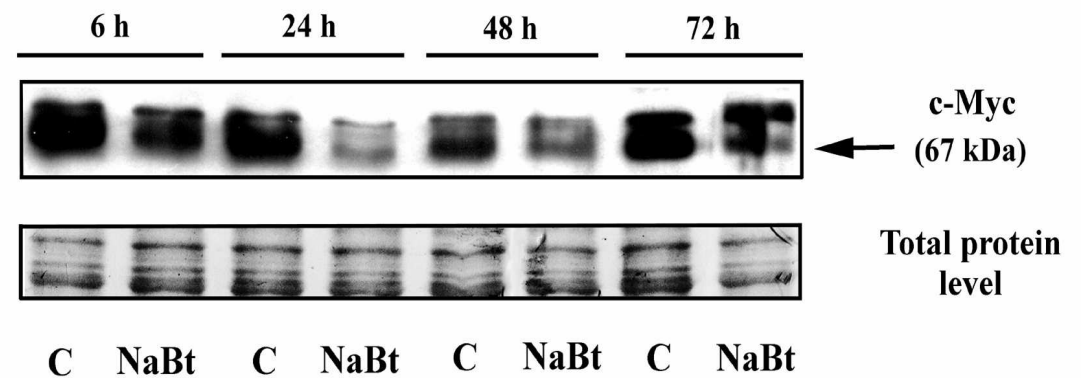
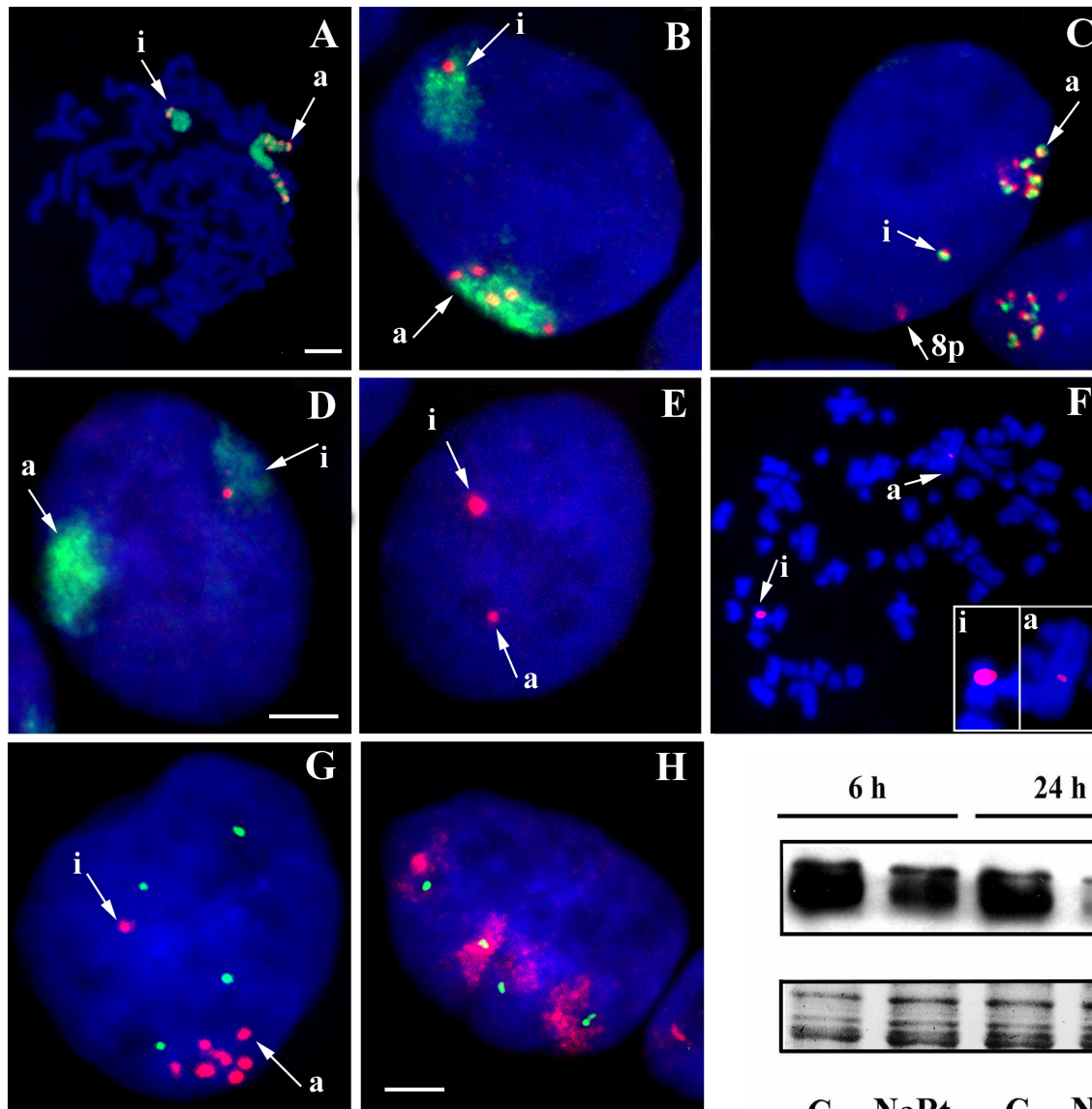
Enterocytic cell differentiation



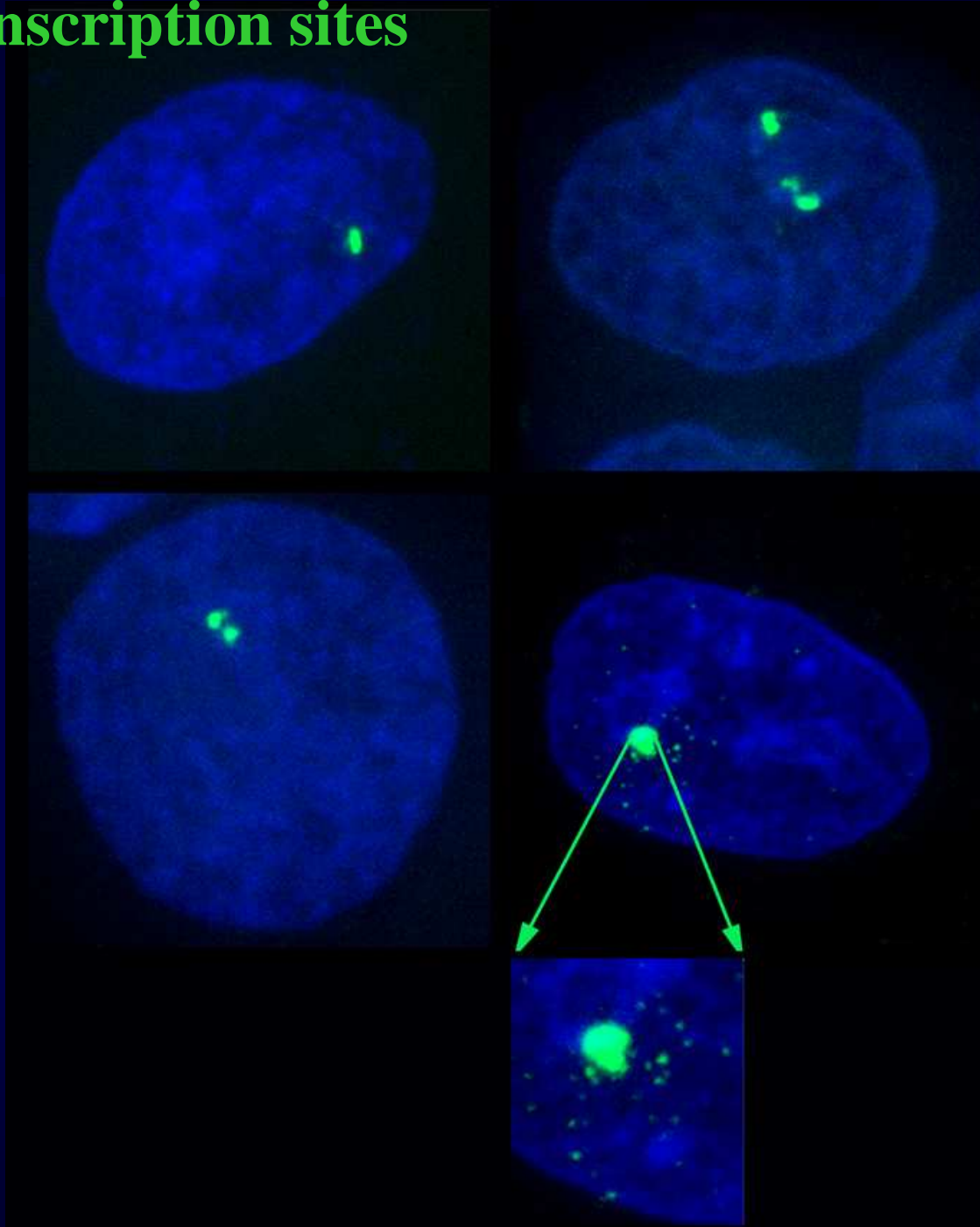
Harničarová et al., 2005

HSA 8 and related structures

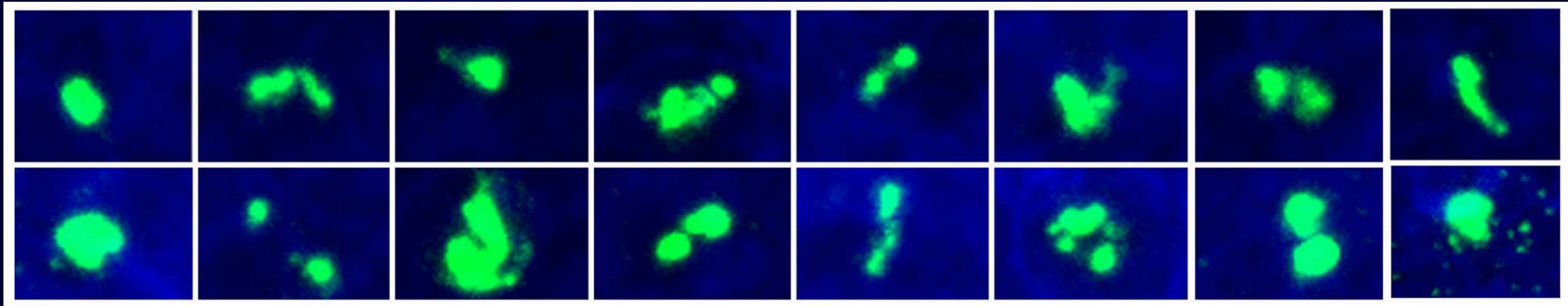
Harničarová et al., 2006

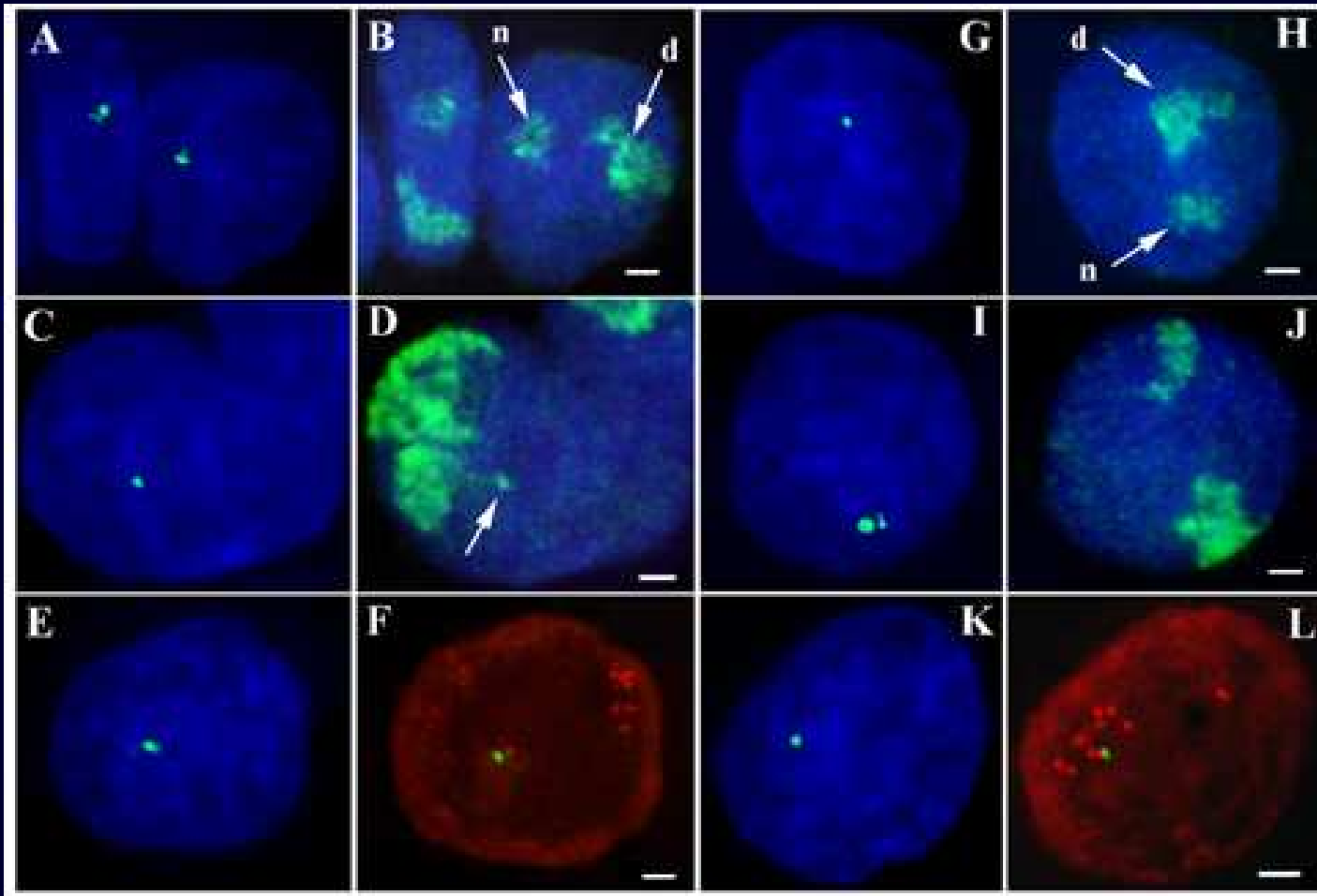


C-myc transcription sites

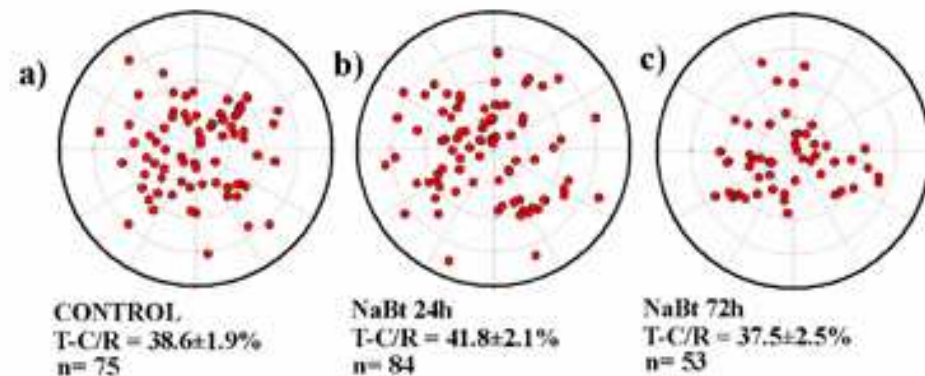


Cell-to-cell variability





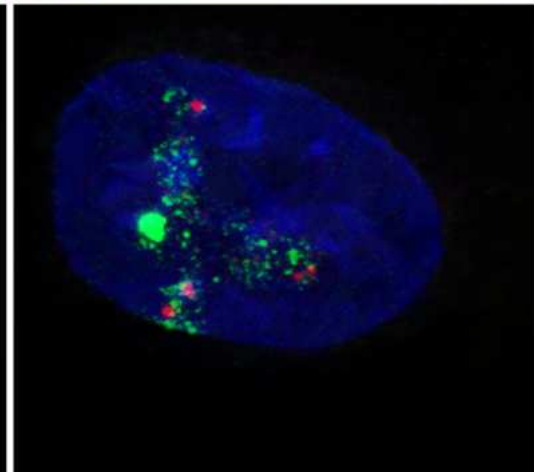
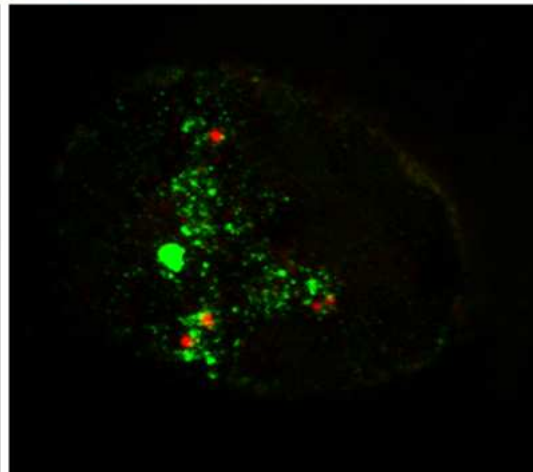
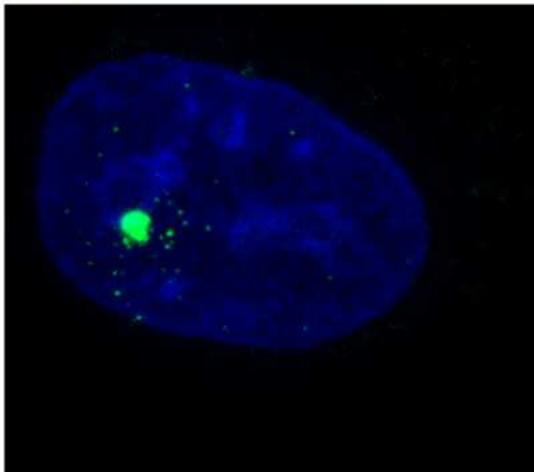
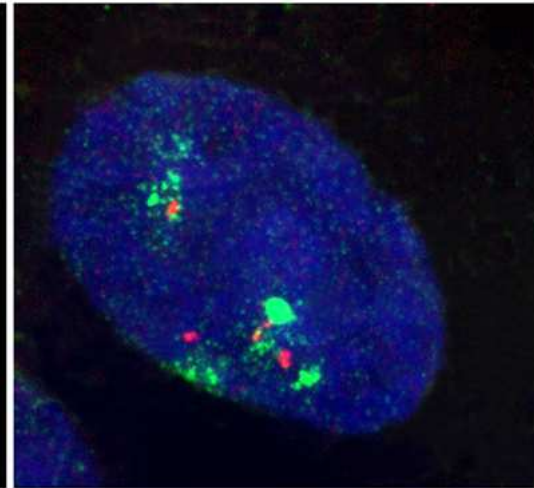
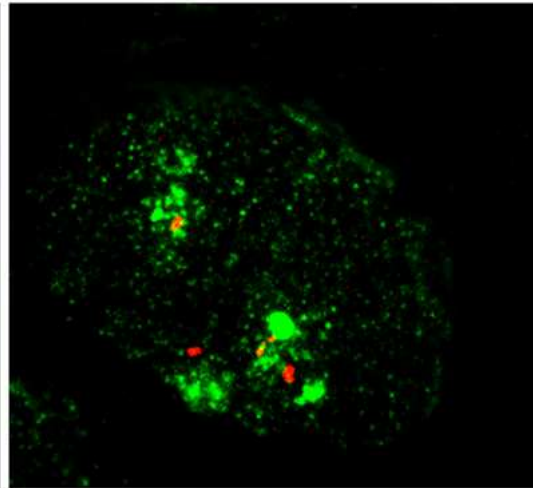
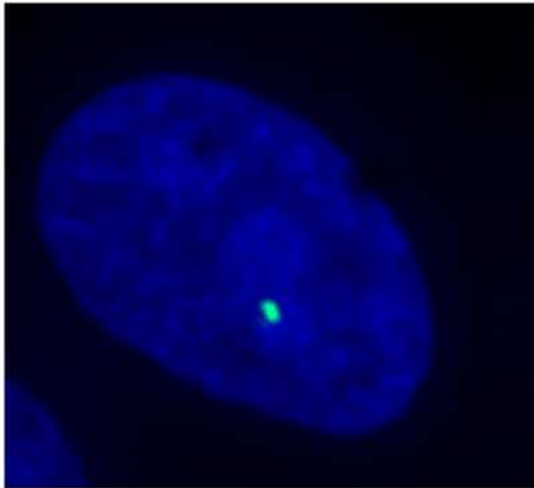
C-myc gene and c-myc transcription site in HT29 cells



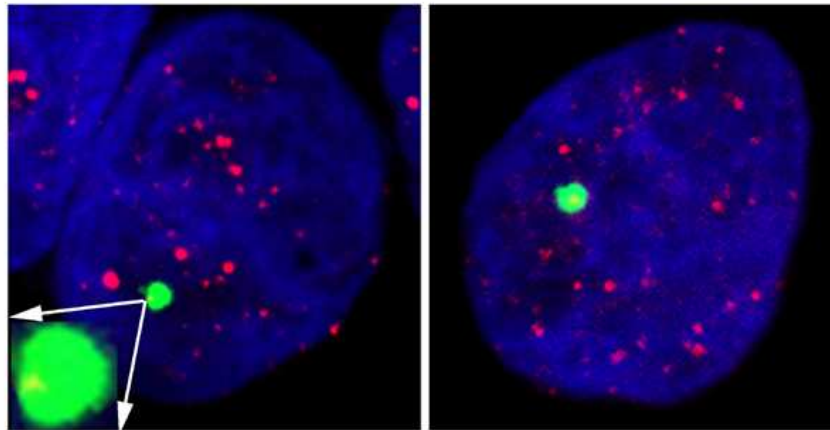
C-MYC transcript

HSA8/C-MYC gene

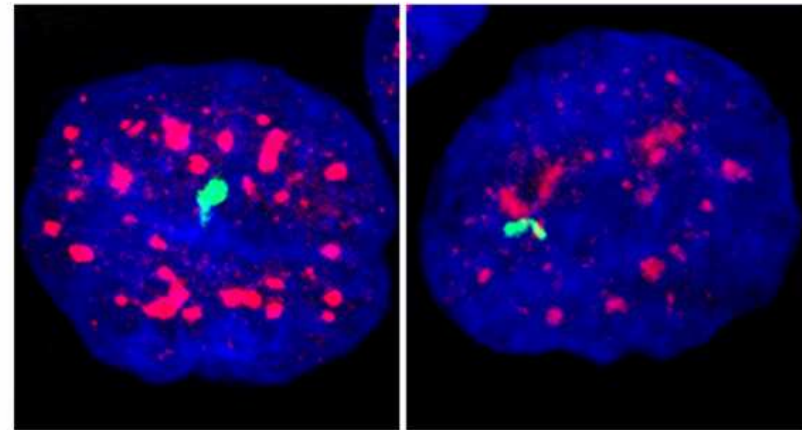
OVERLAY



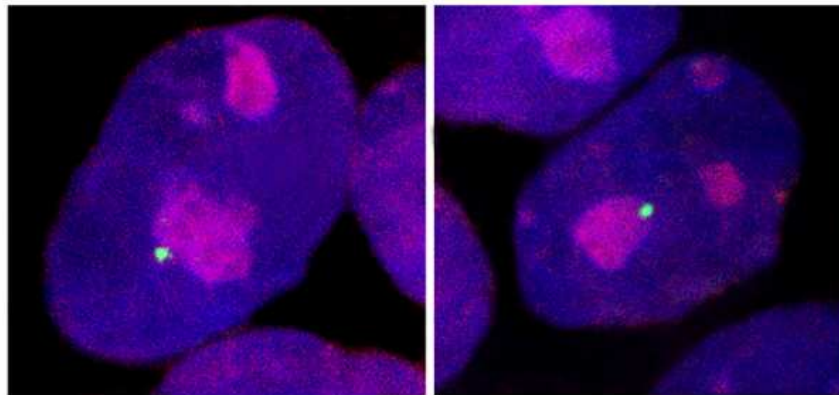
RNAP II / c-myc



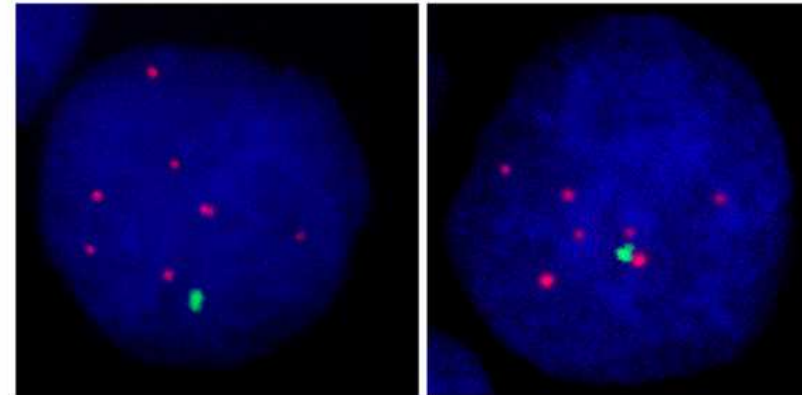
SC35 / c-myc

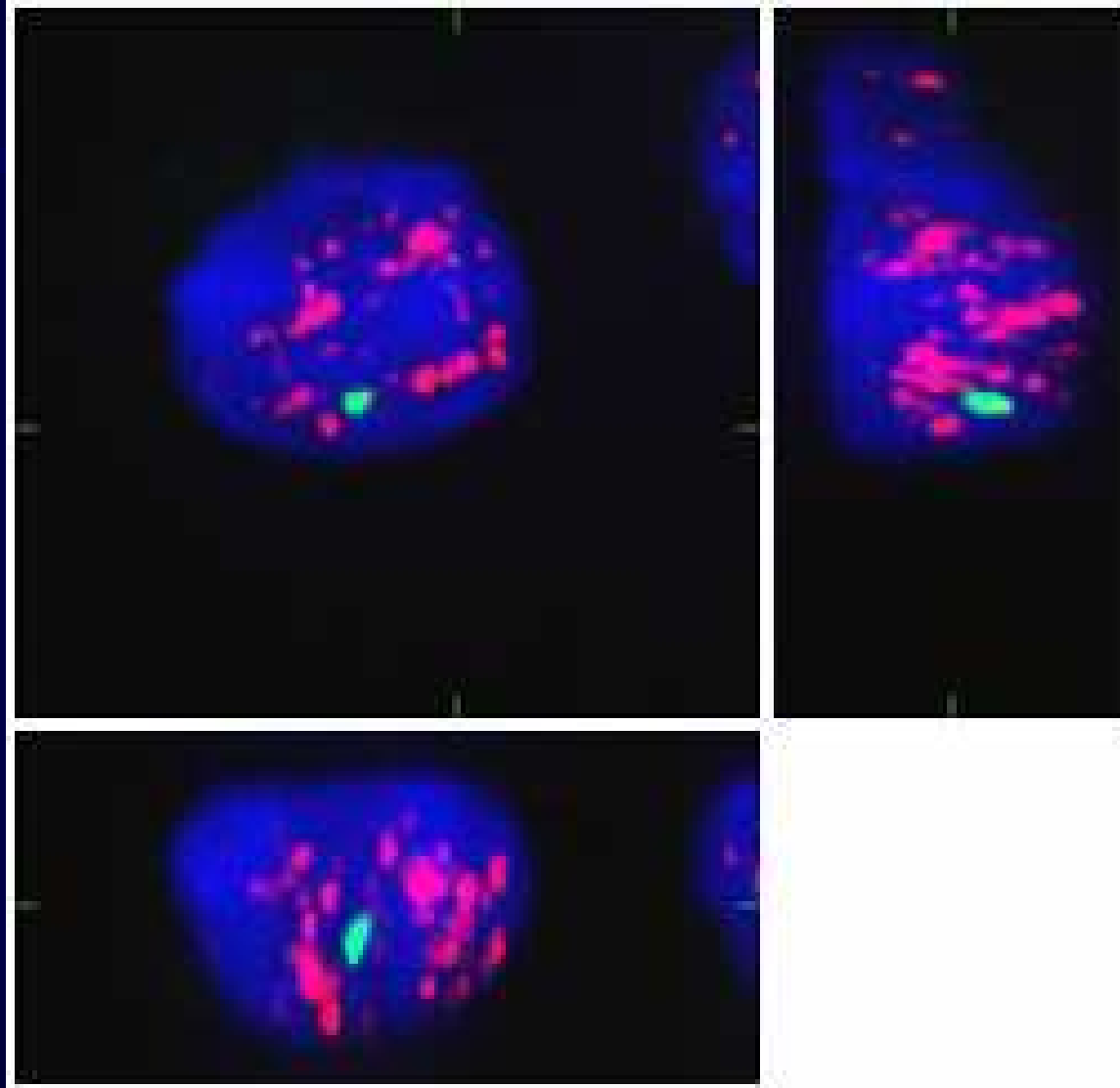


Nucleoli / c-myc

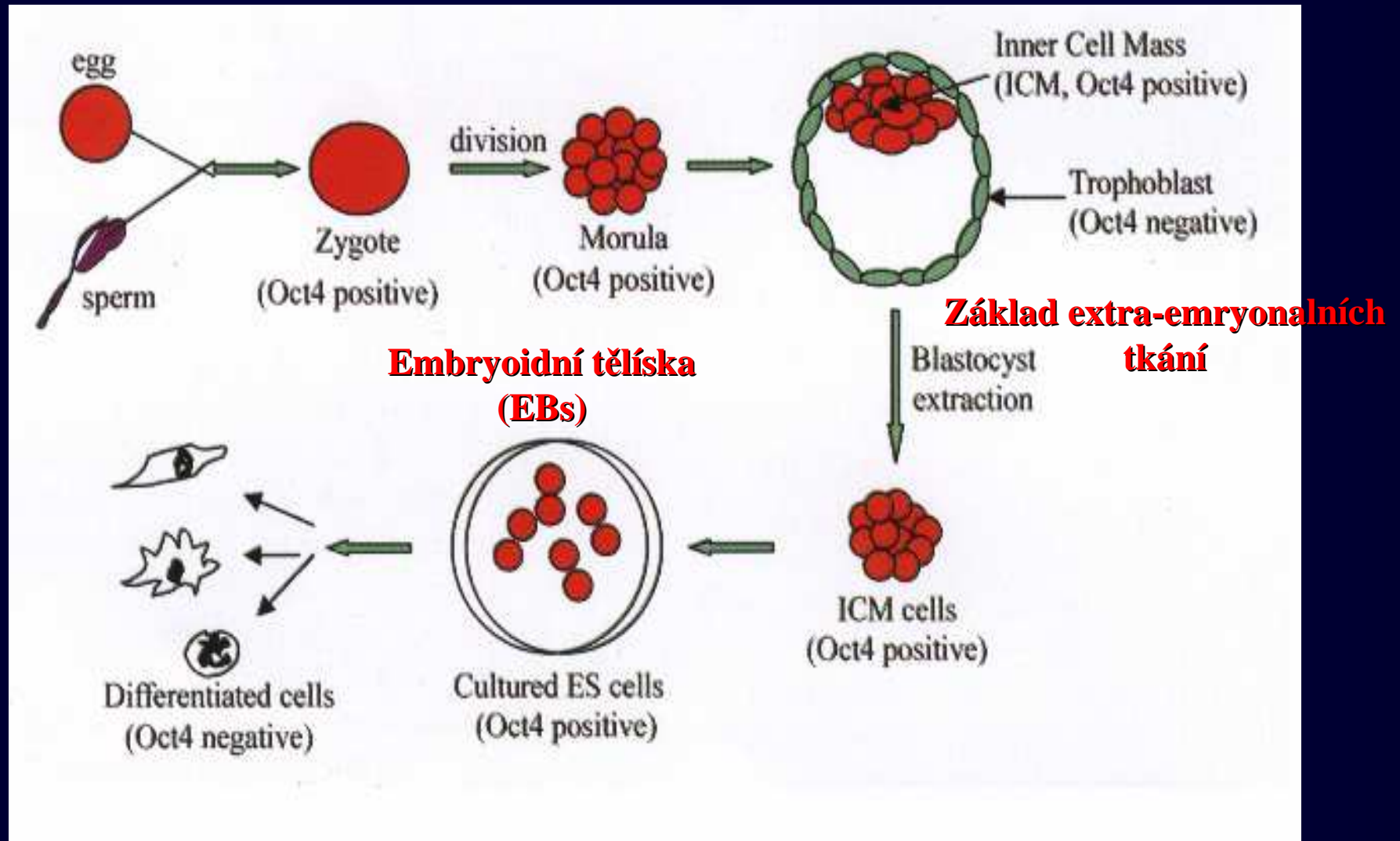


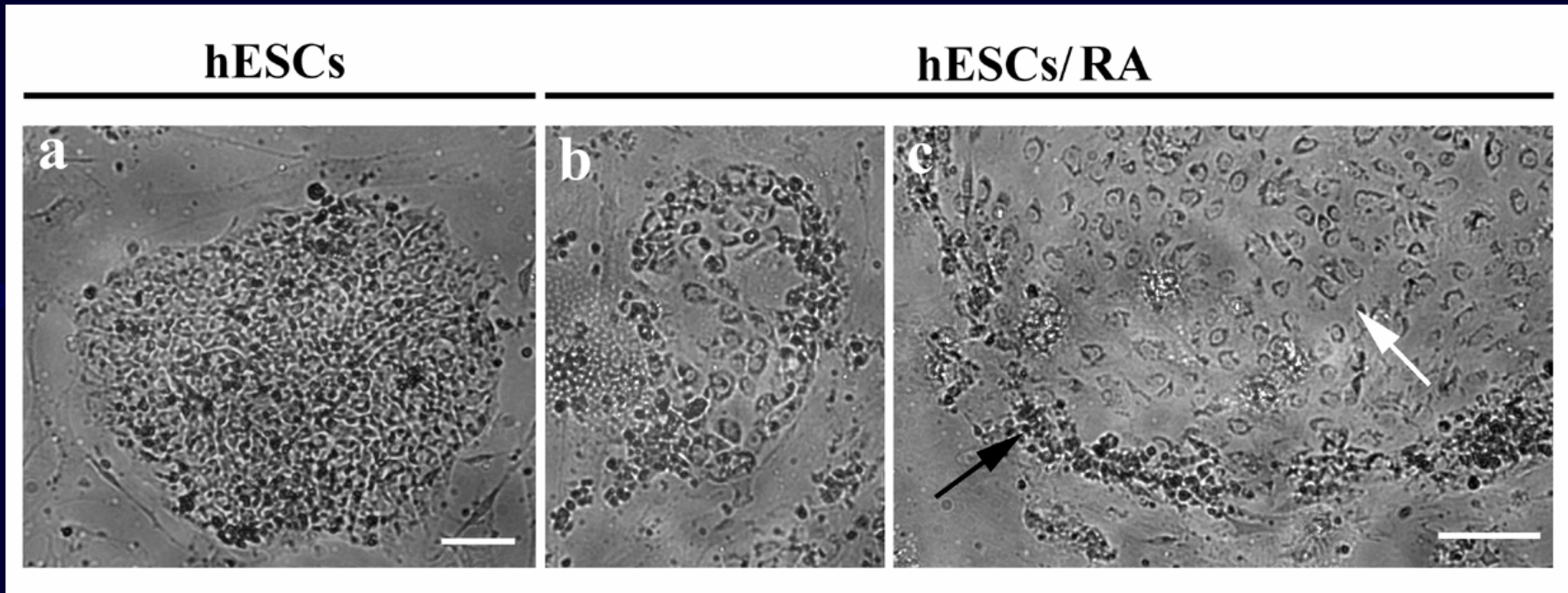
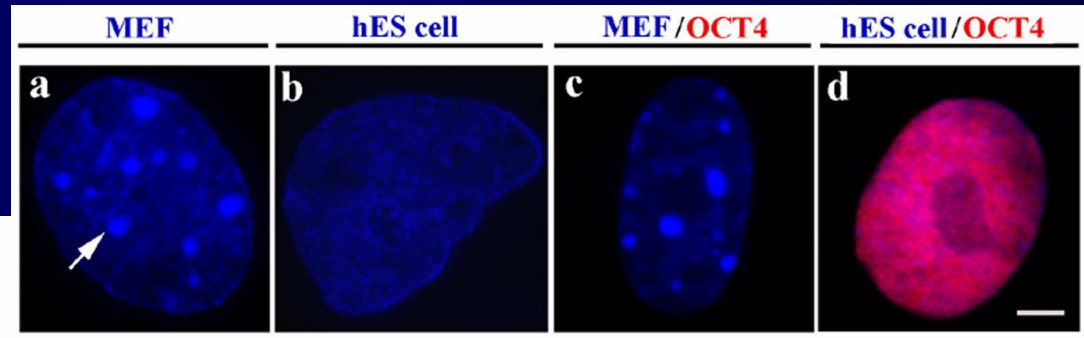
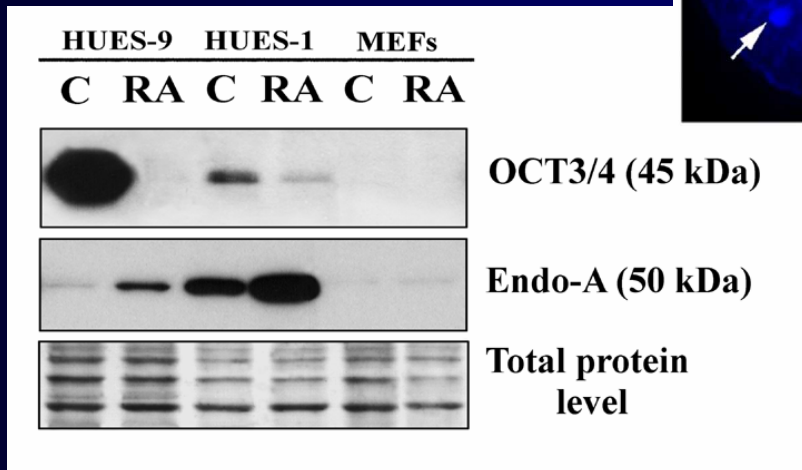
PML / c-myc





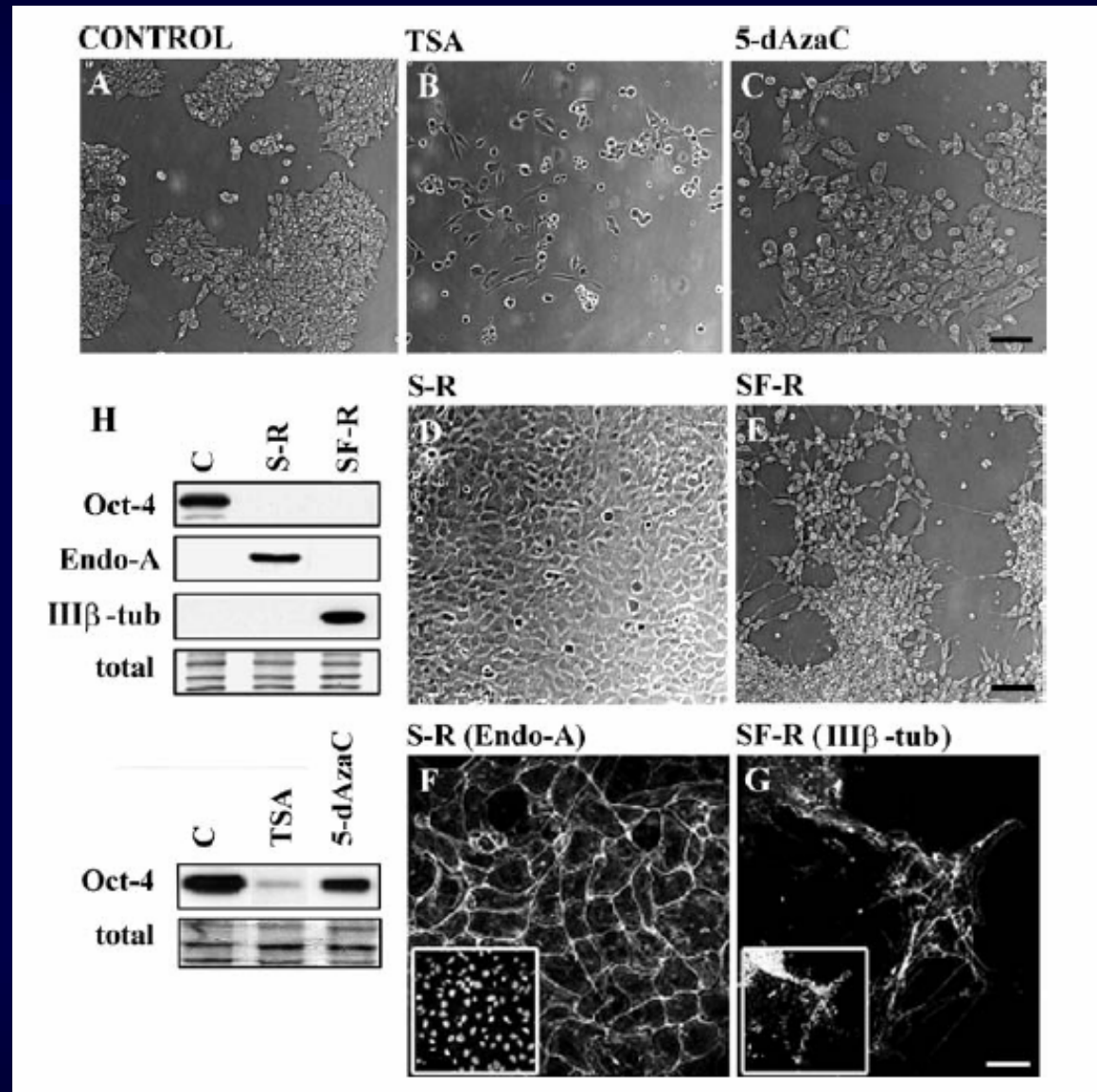
1. Differentiation of mouse embryonic cells (ES and EC)



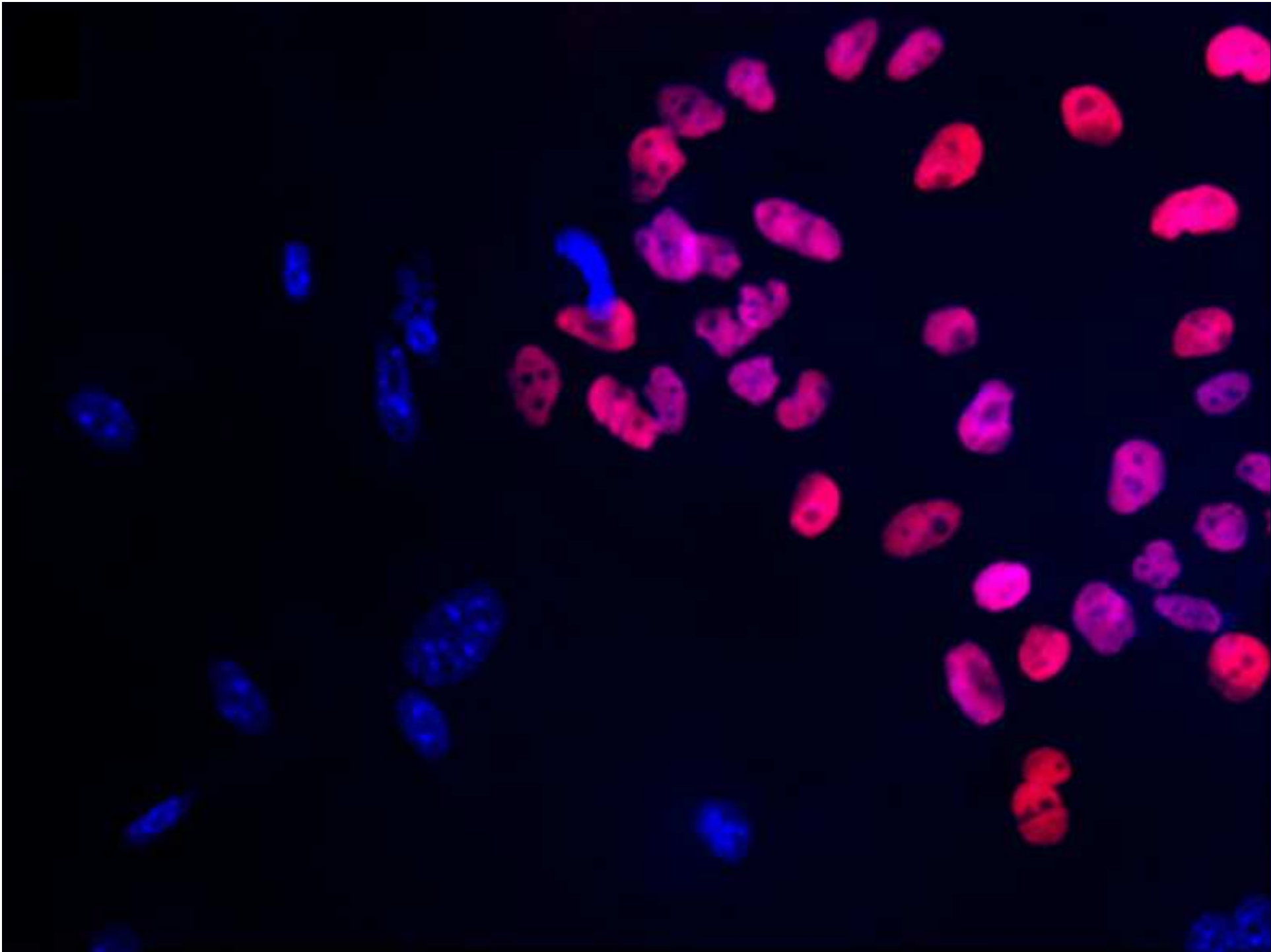
A**B****C**

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

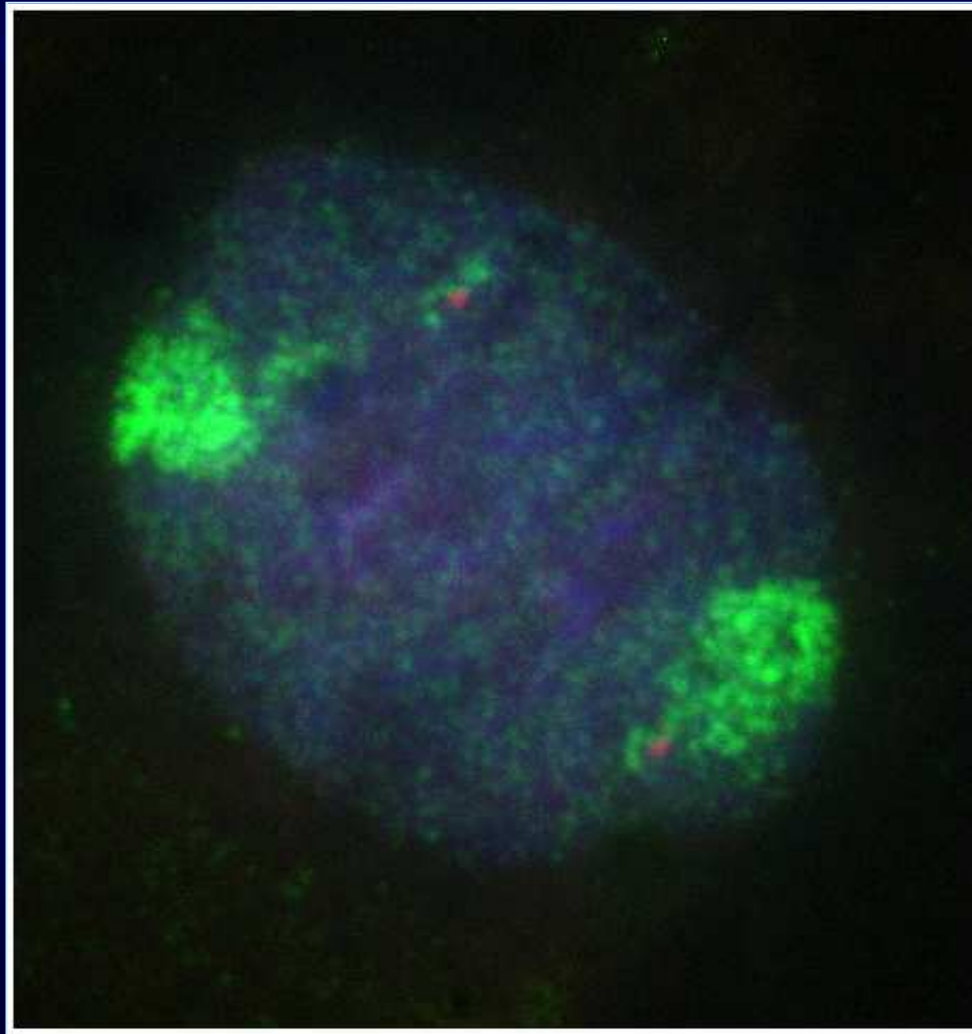
Mouse embryonal carcinoma cells P19



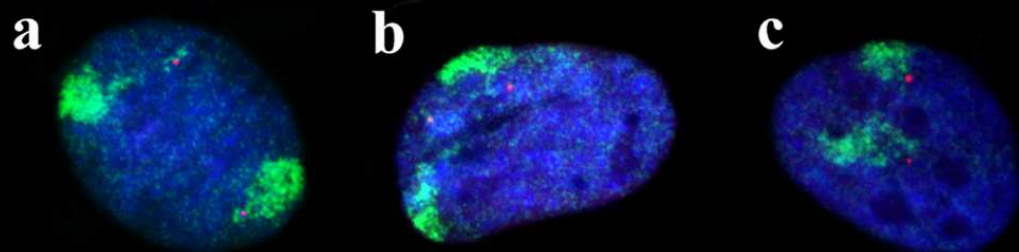
(Bártová et al., Histochem. Cell Biol., 2007)



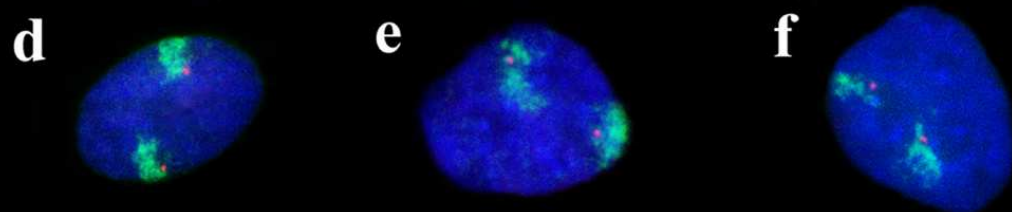
Oct3/4 and HSA6 in human ESCs



Oct4 / HSA 6 in hES cells



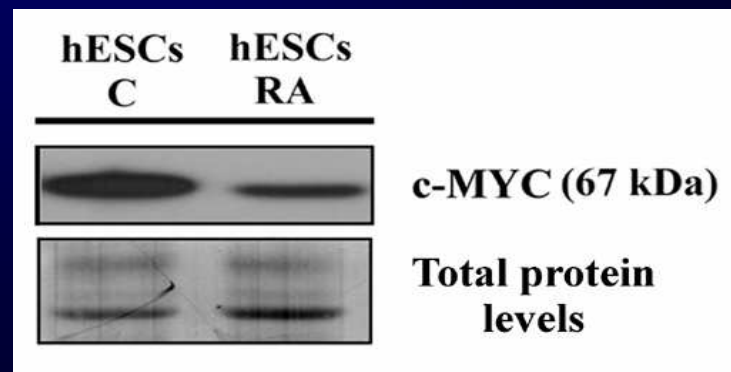
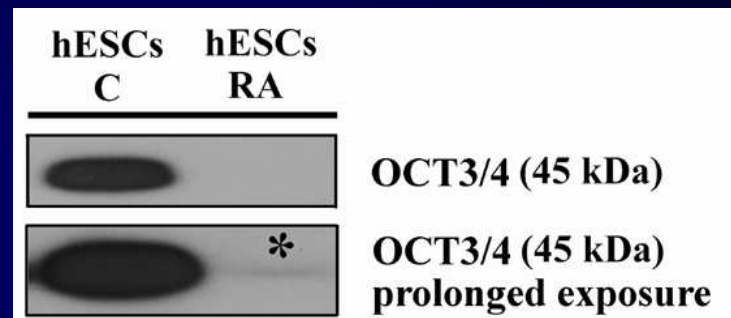
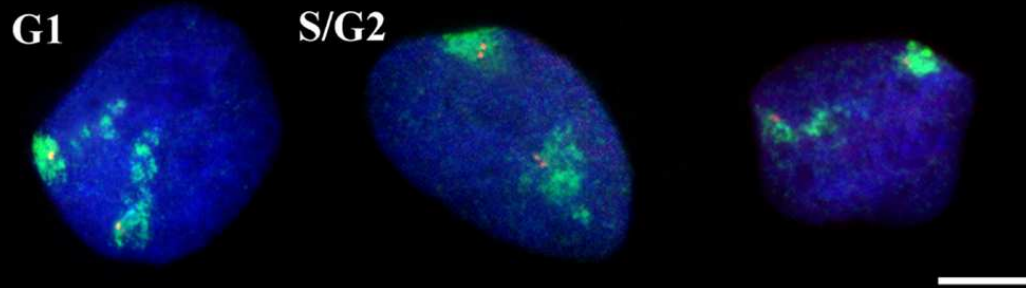
Oct4 / HSA 6 in hES cells - RA differentiated



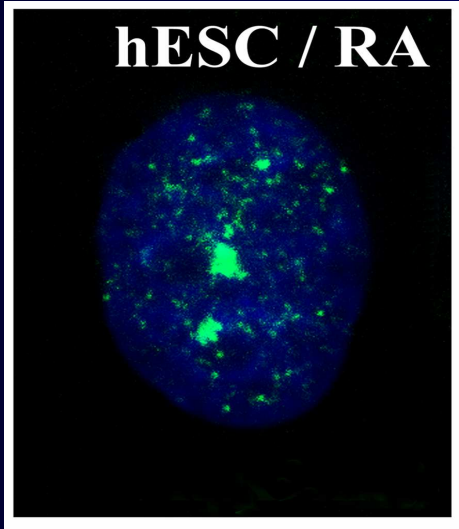
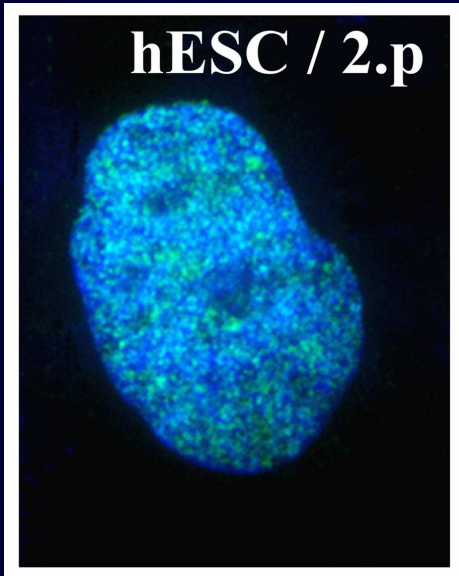
C-myc / HSA 8

in hES cells

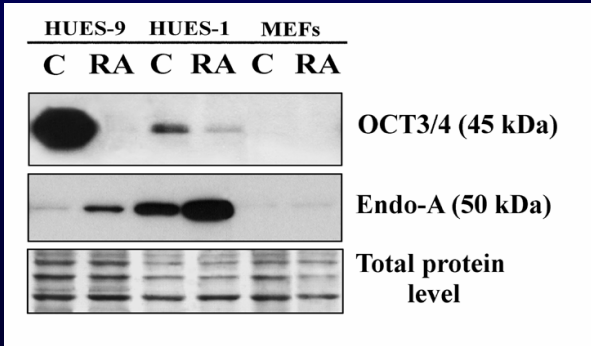
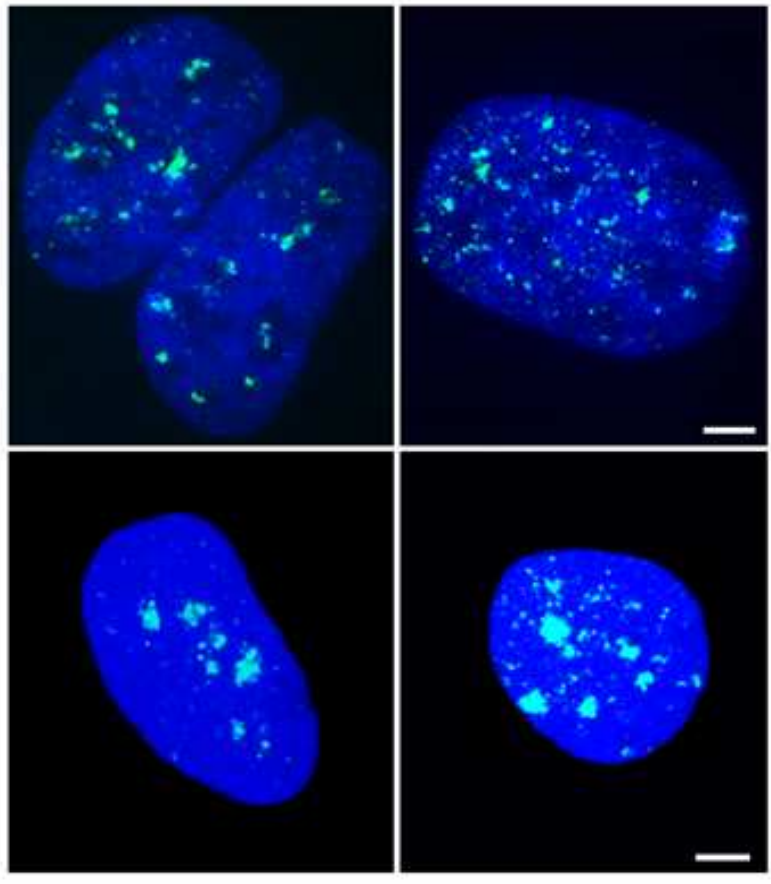
RA differentiated



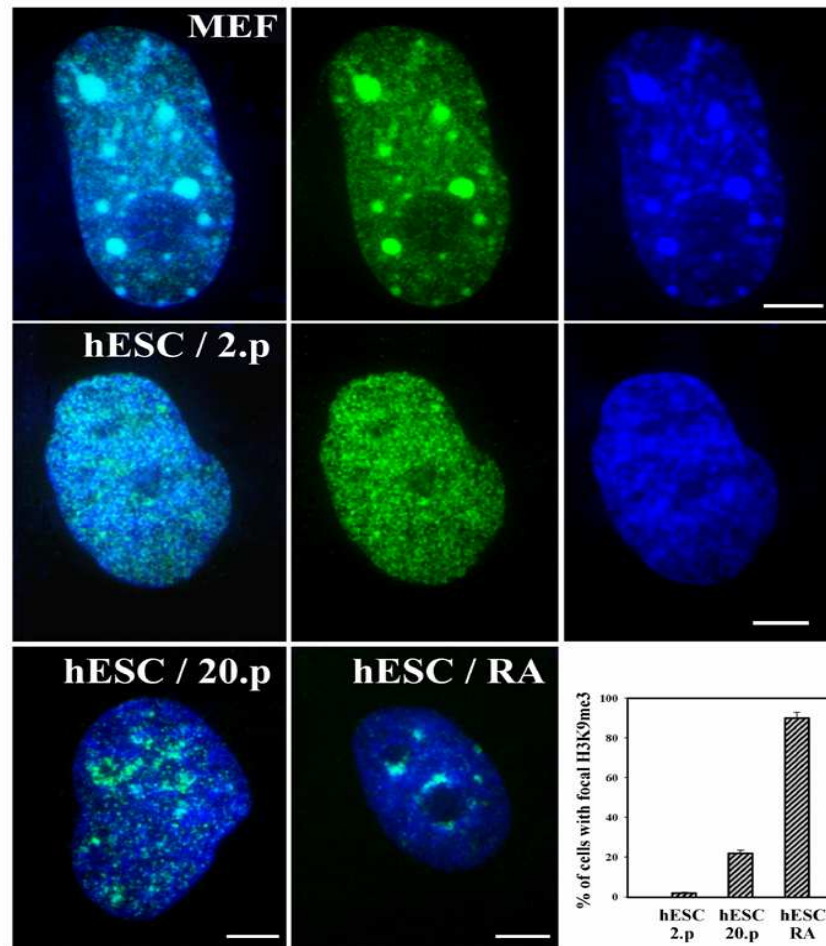
H3K9me3 / HUES-9



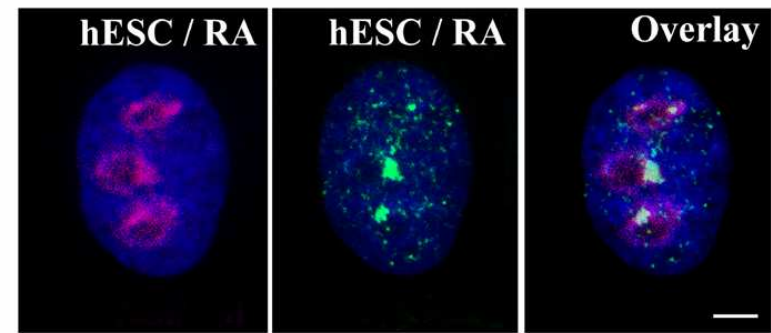
H3K9me3 / DNA / HUES-1



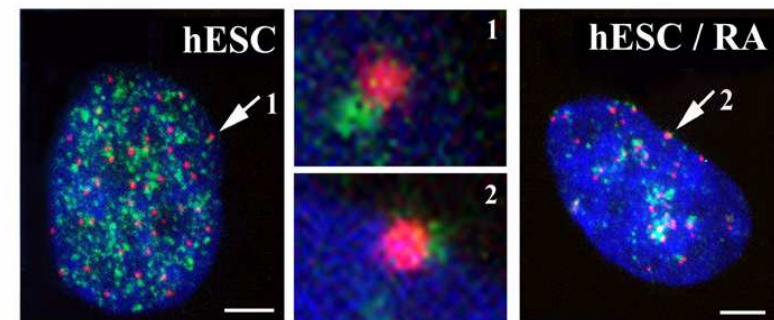
A H3K9me3 / DNA / HUES-9



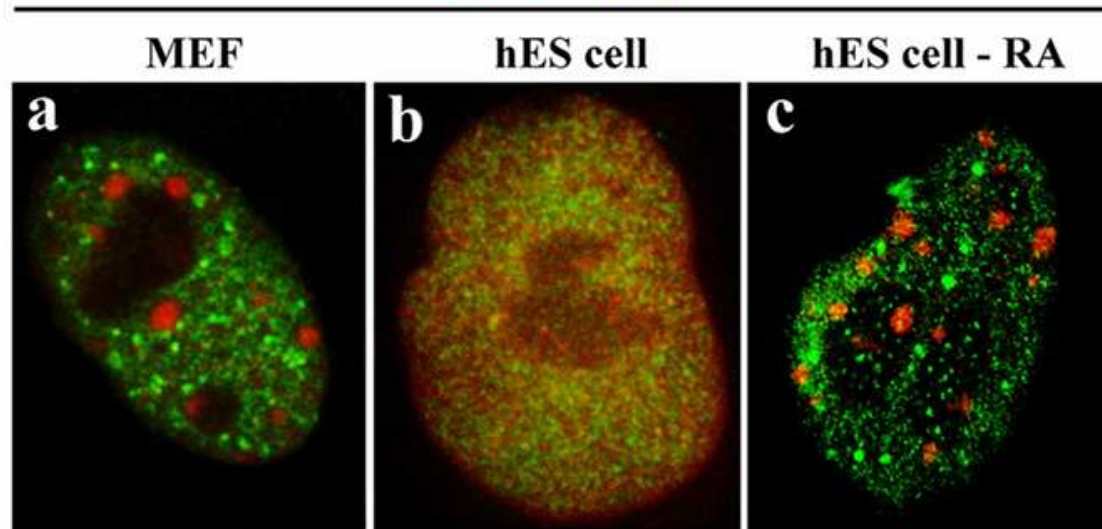
B H3K9me3 / Nucleoli / DNA



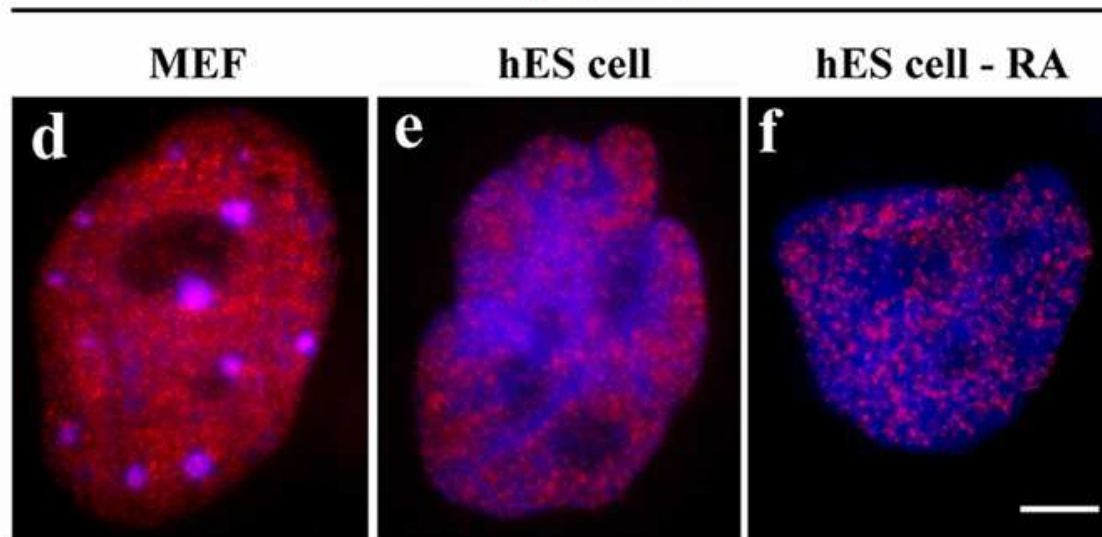
C H3K9me3 / CENP-A / DNA

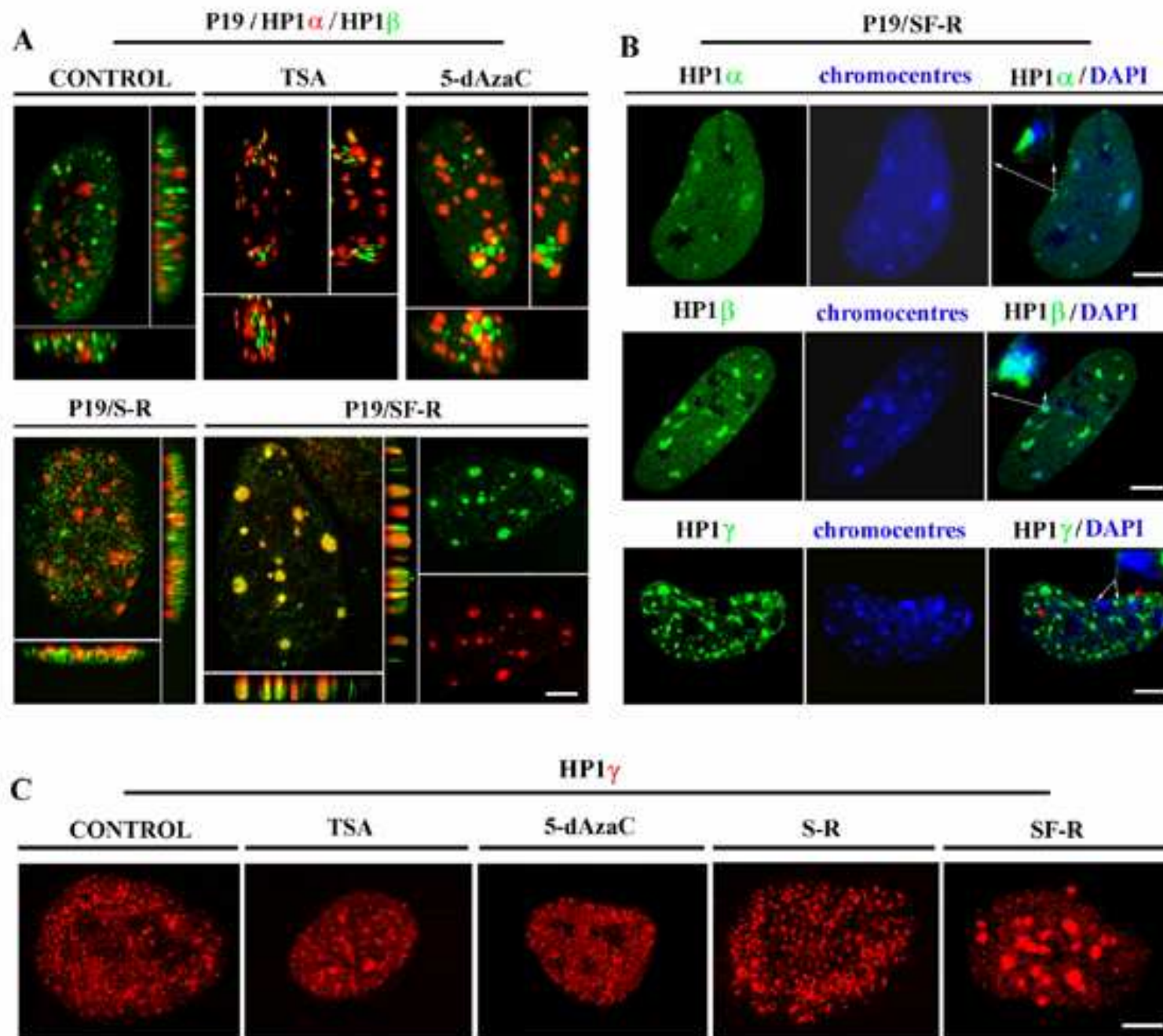


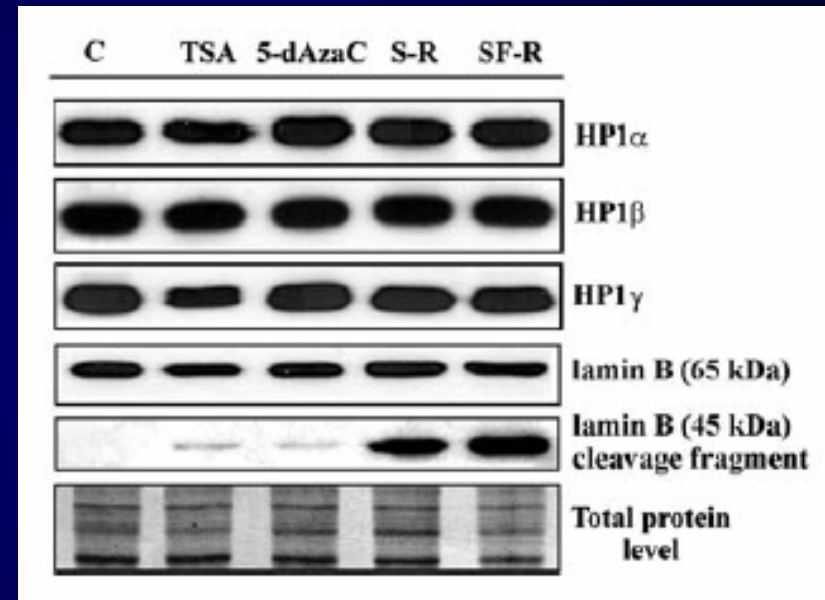
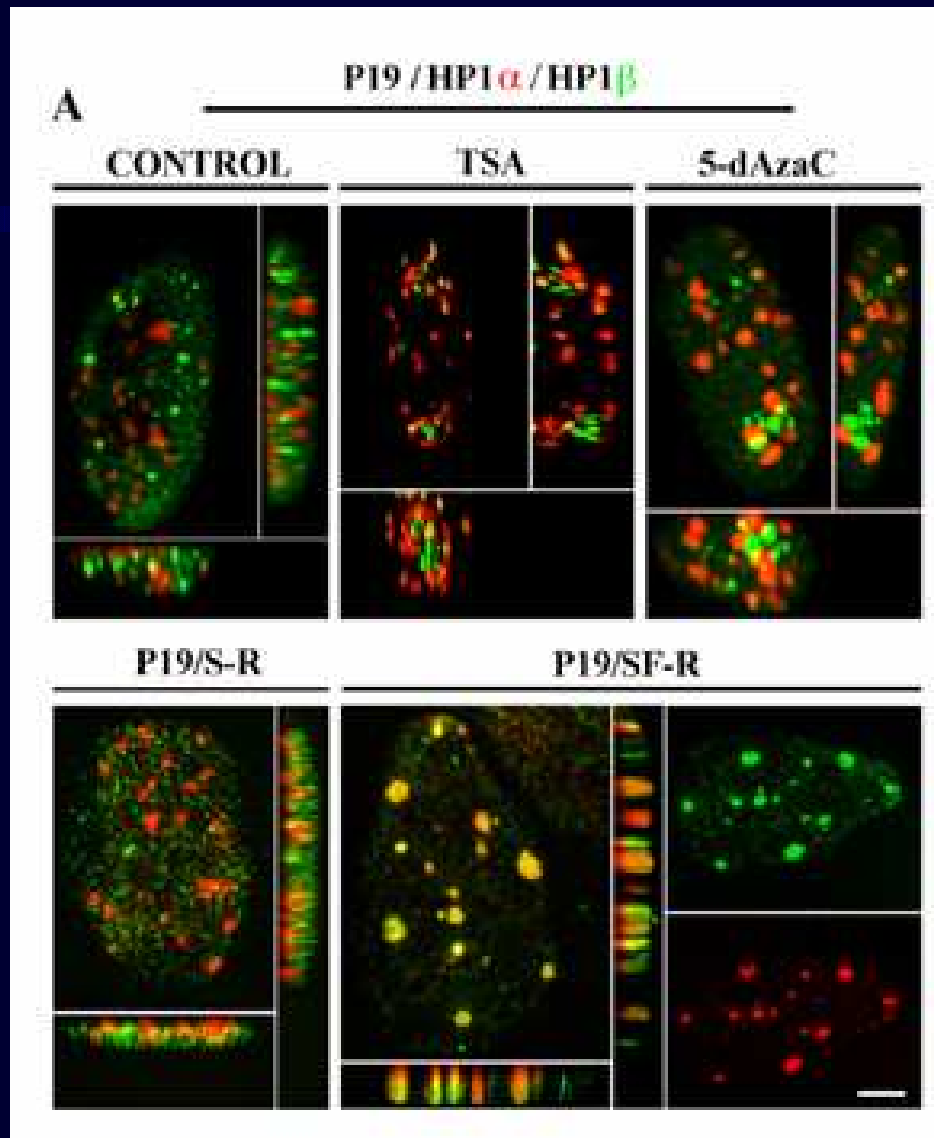
HP1 α / HP1 β



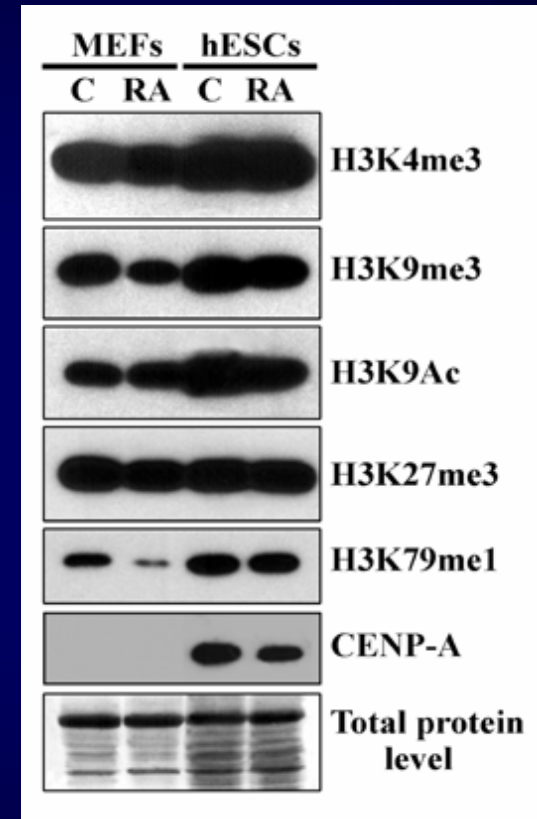
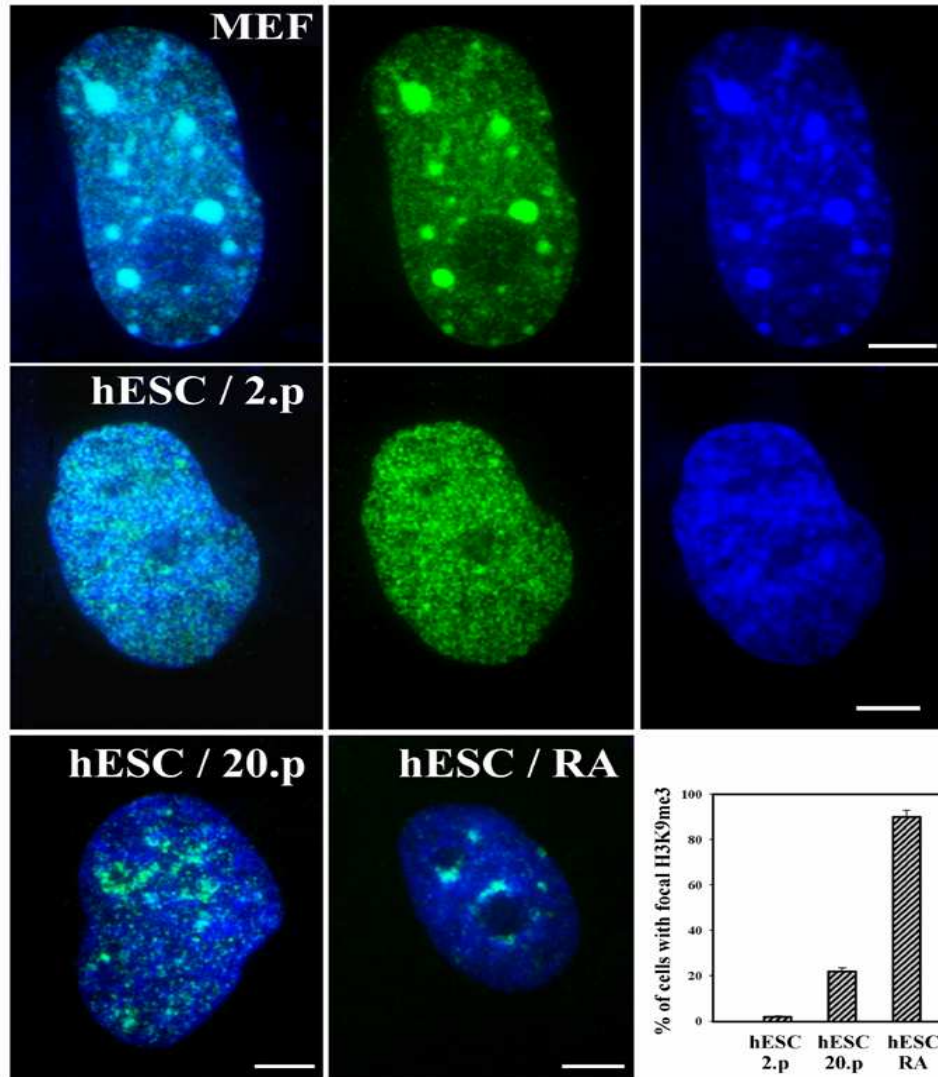
HP1 γ / nucleus



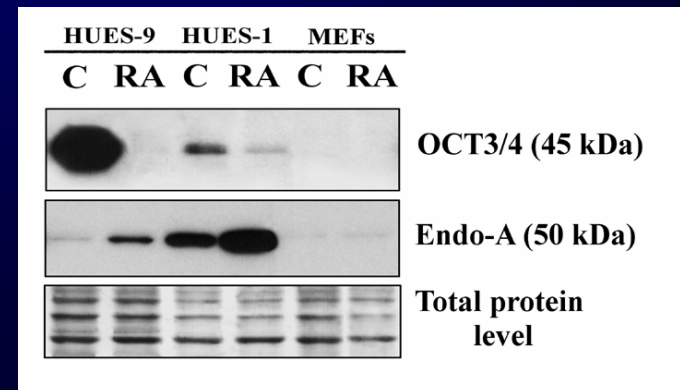
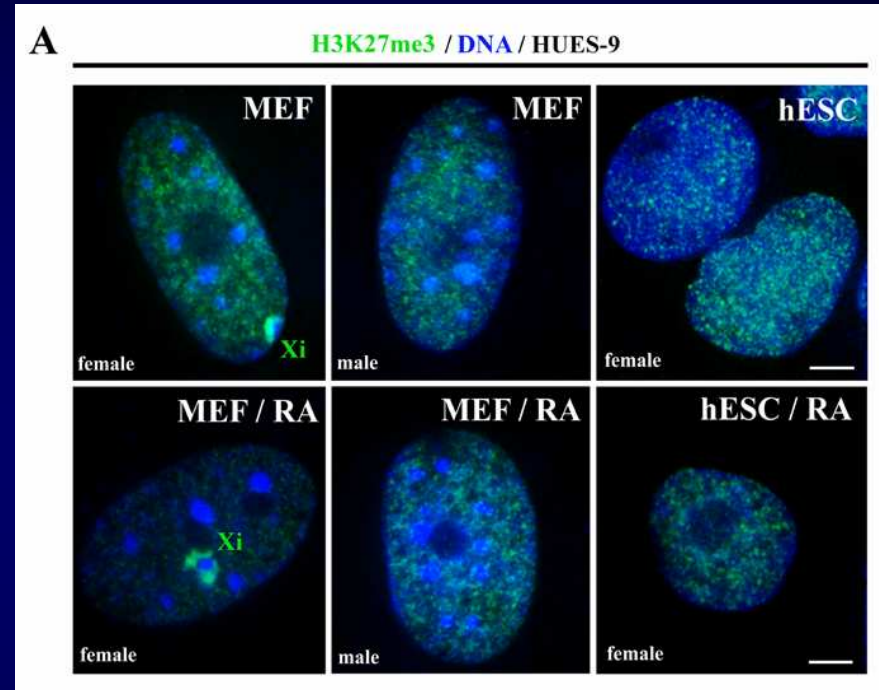
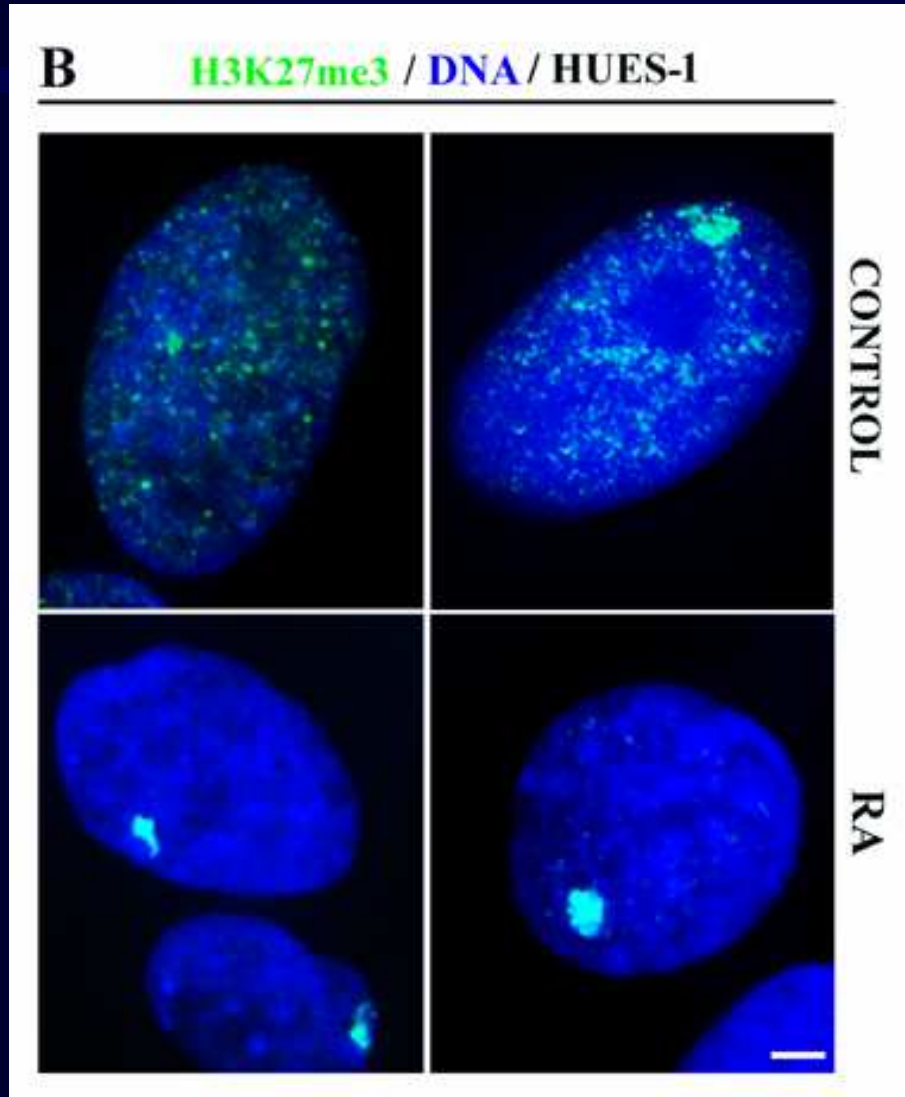


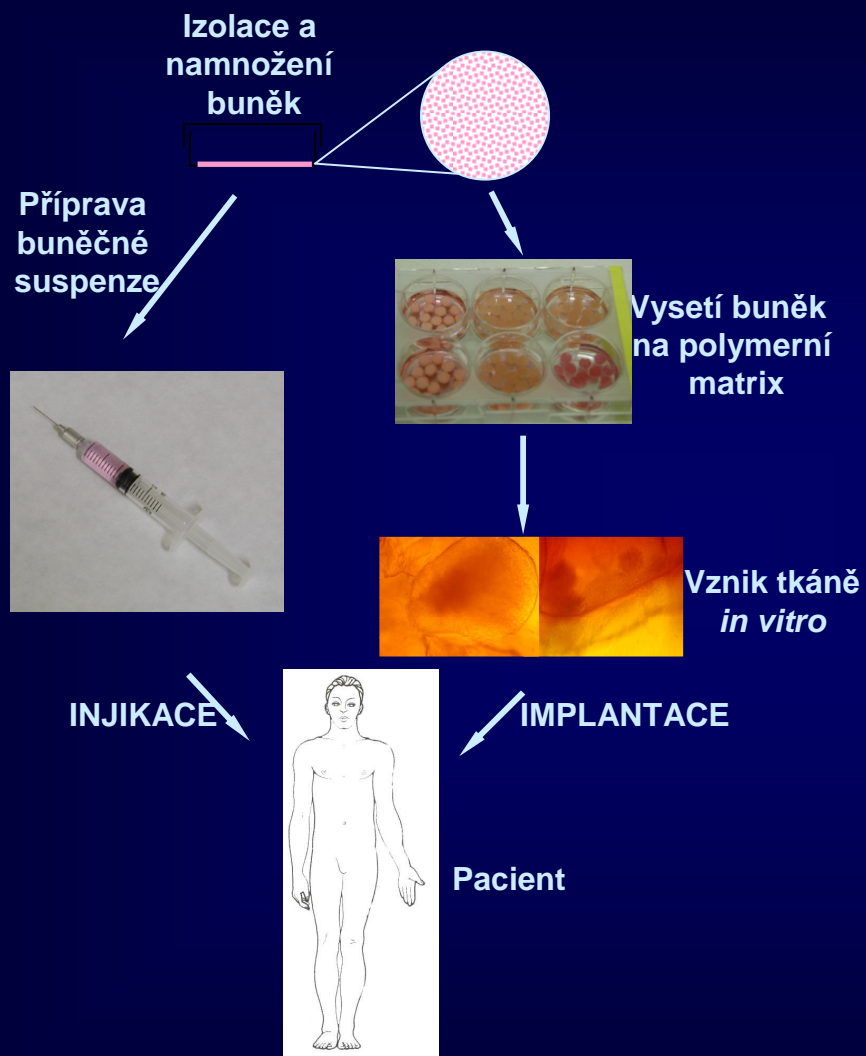


A **H3K9me3 / DNA / HUES-9**



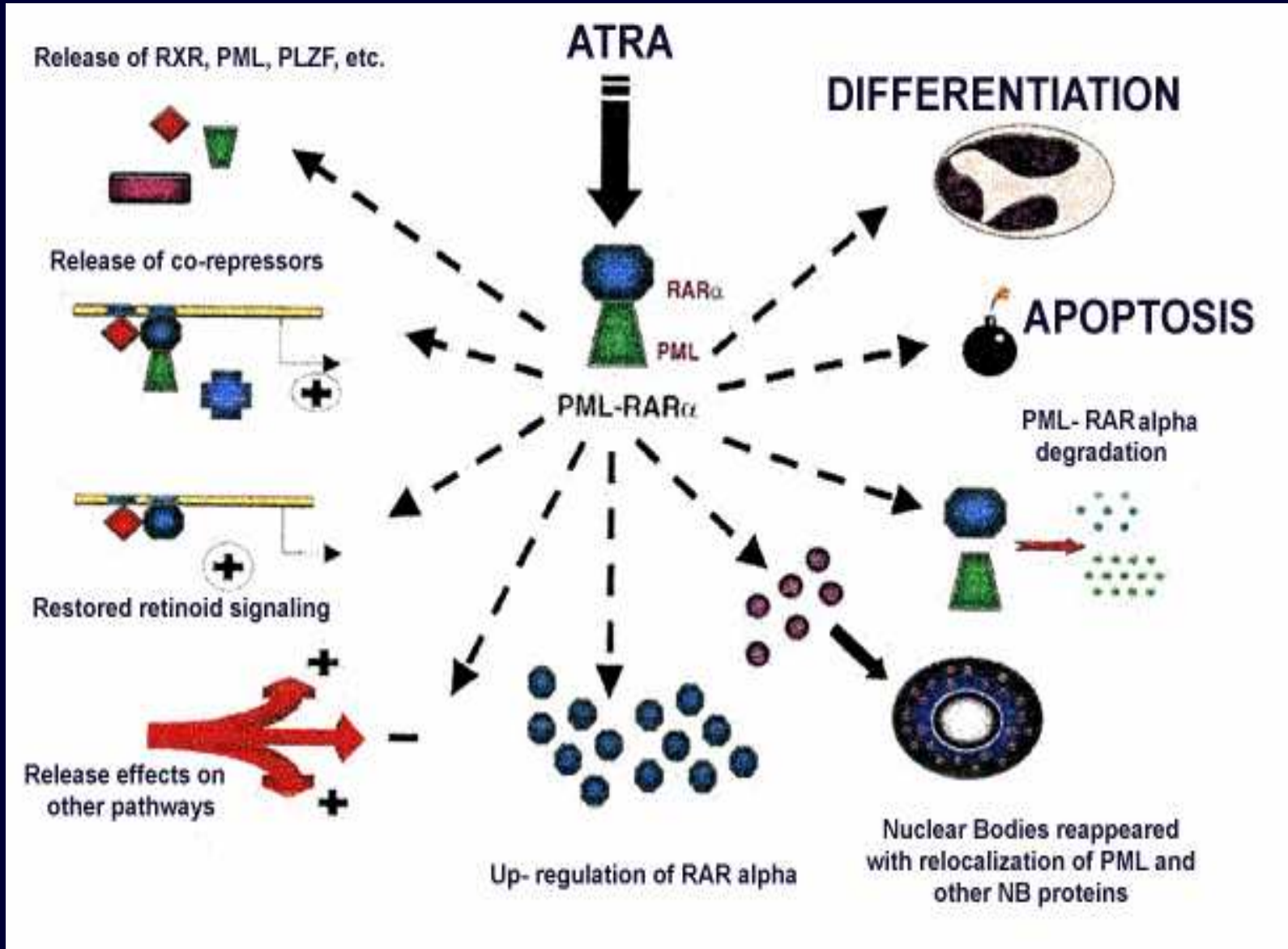
Inactivation of X chromosome in hESC





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 injekce suspenze buněk nebo implantací nové trojrozměrné tkáně na místo již odstraněné nefunkční části orgánu.



Bone marrow

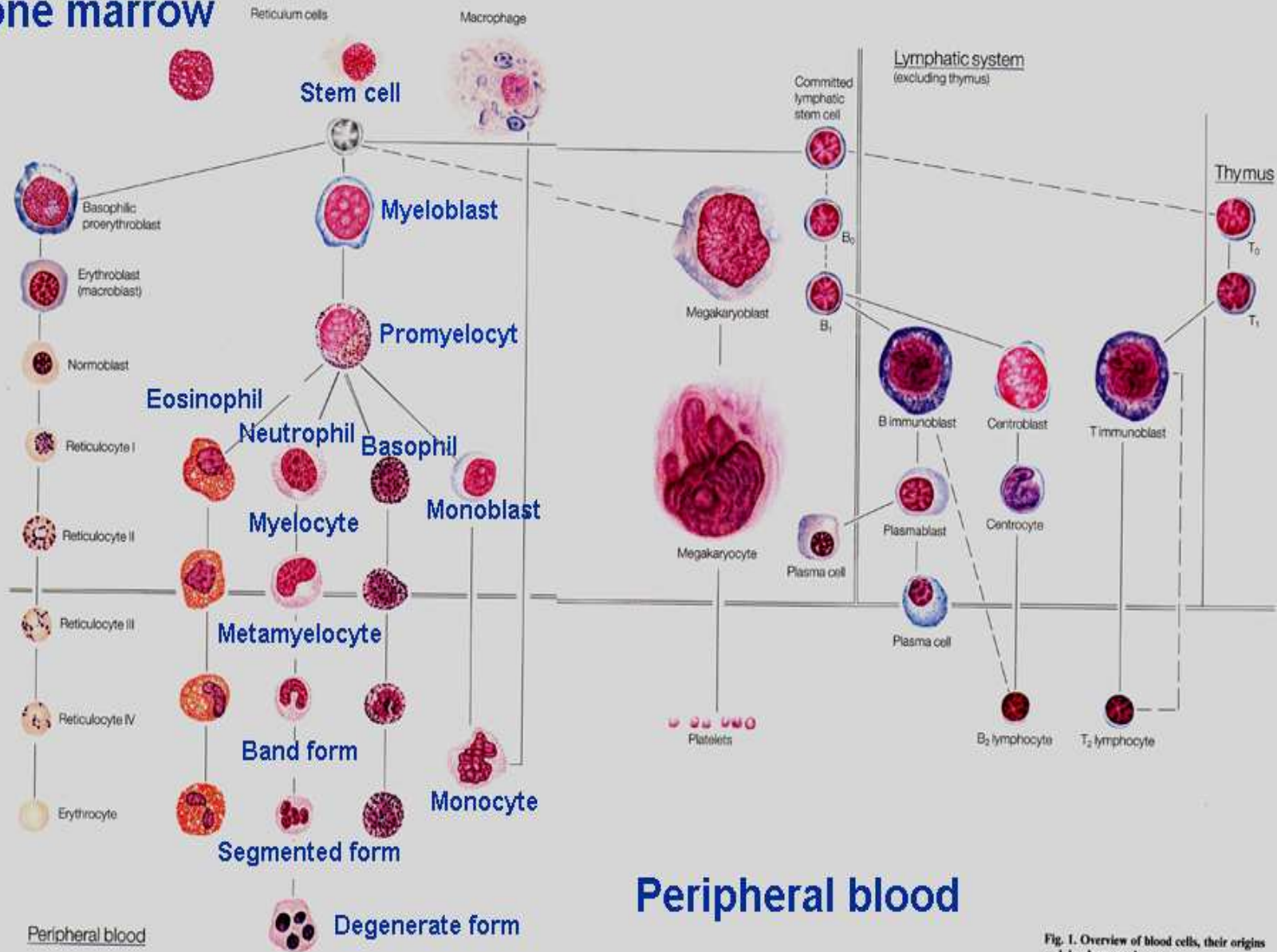
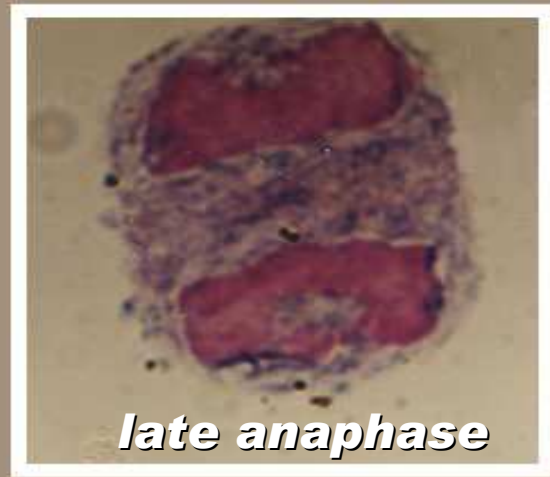
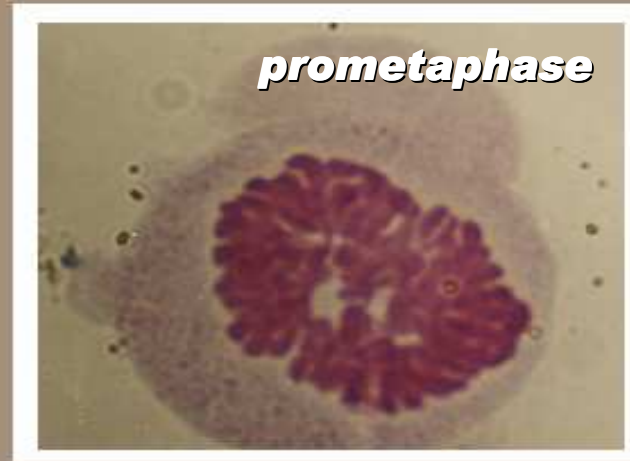
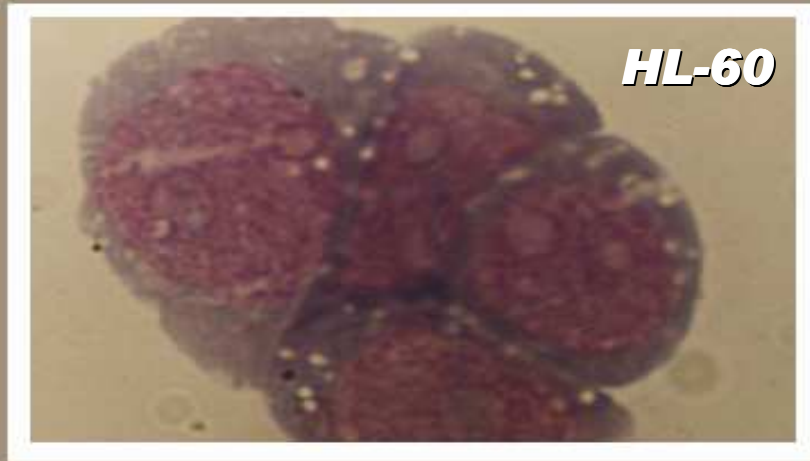
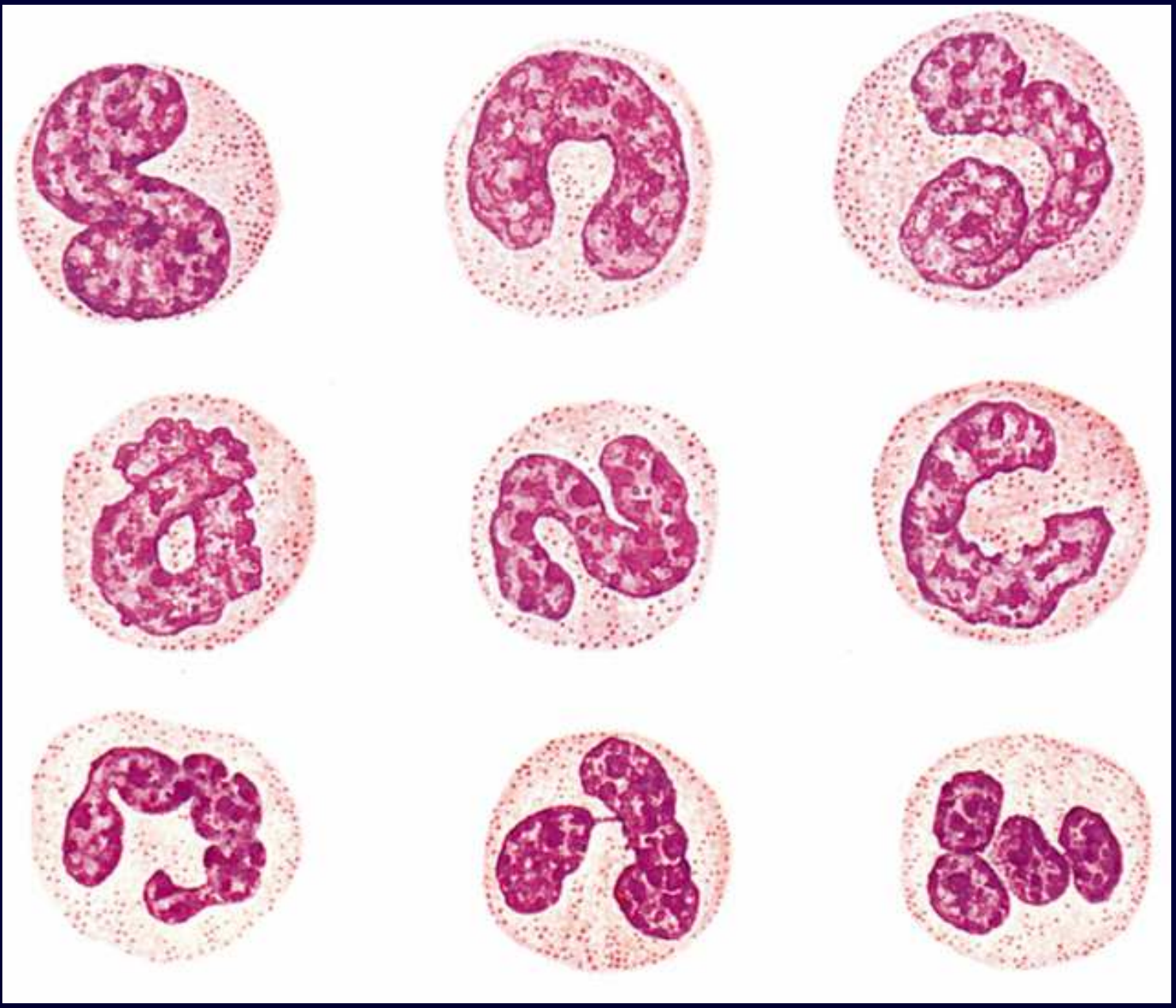
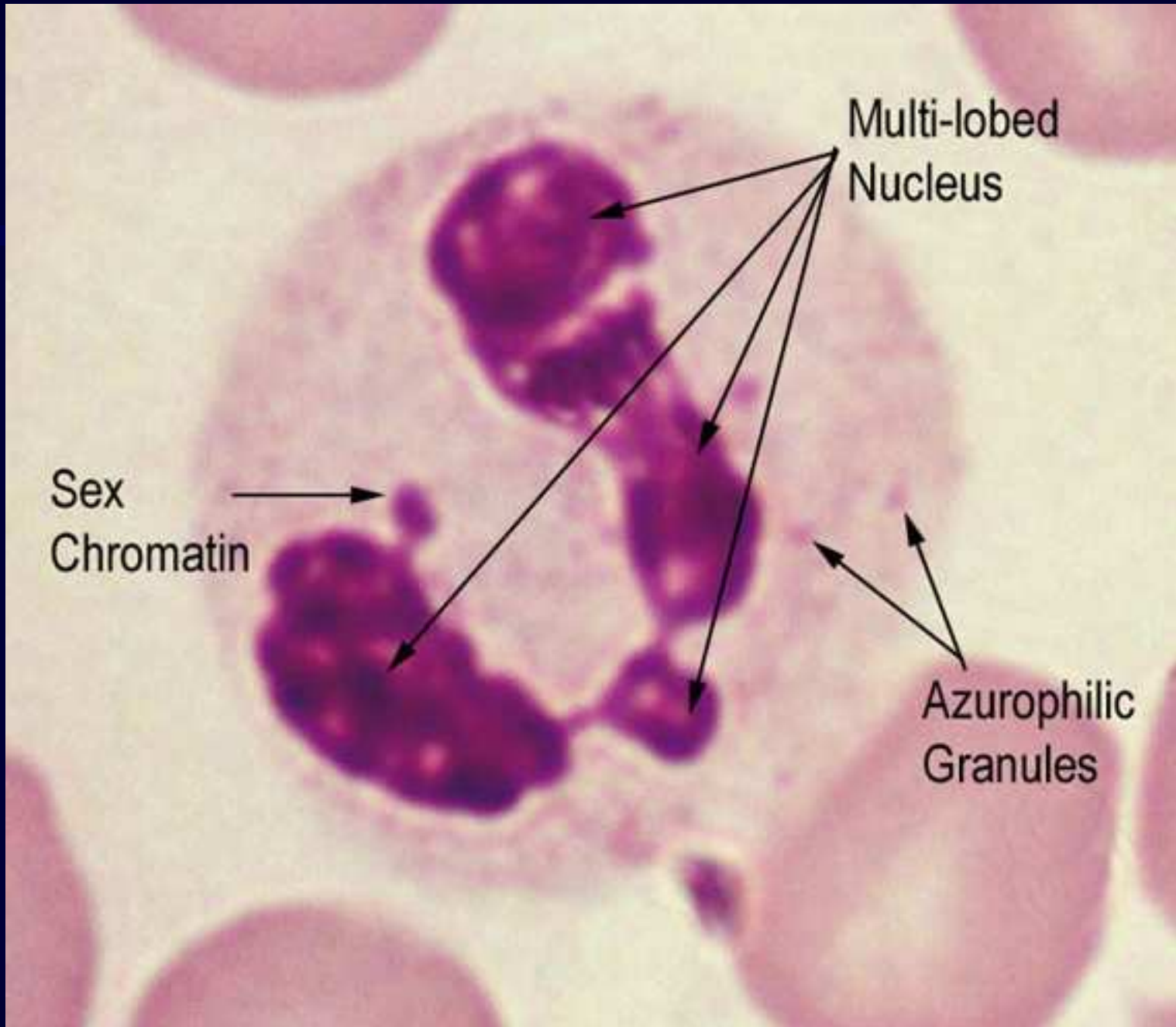


Fig. 1. Overview of blood cells, their origins and developmental stages

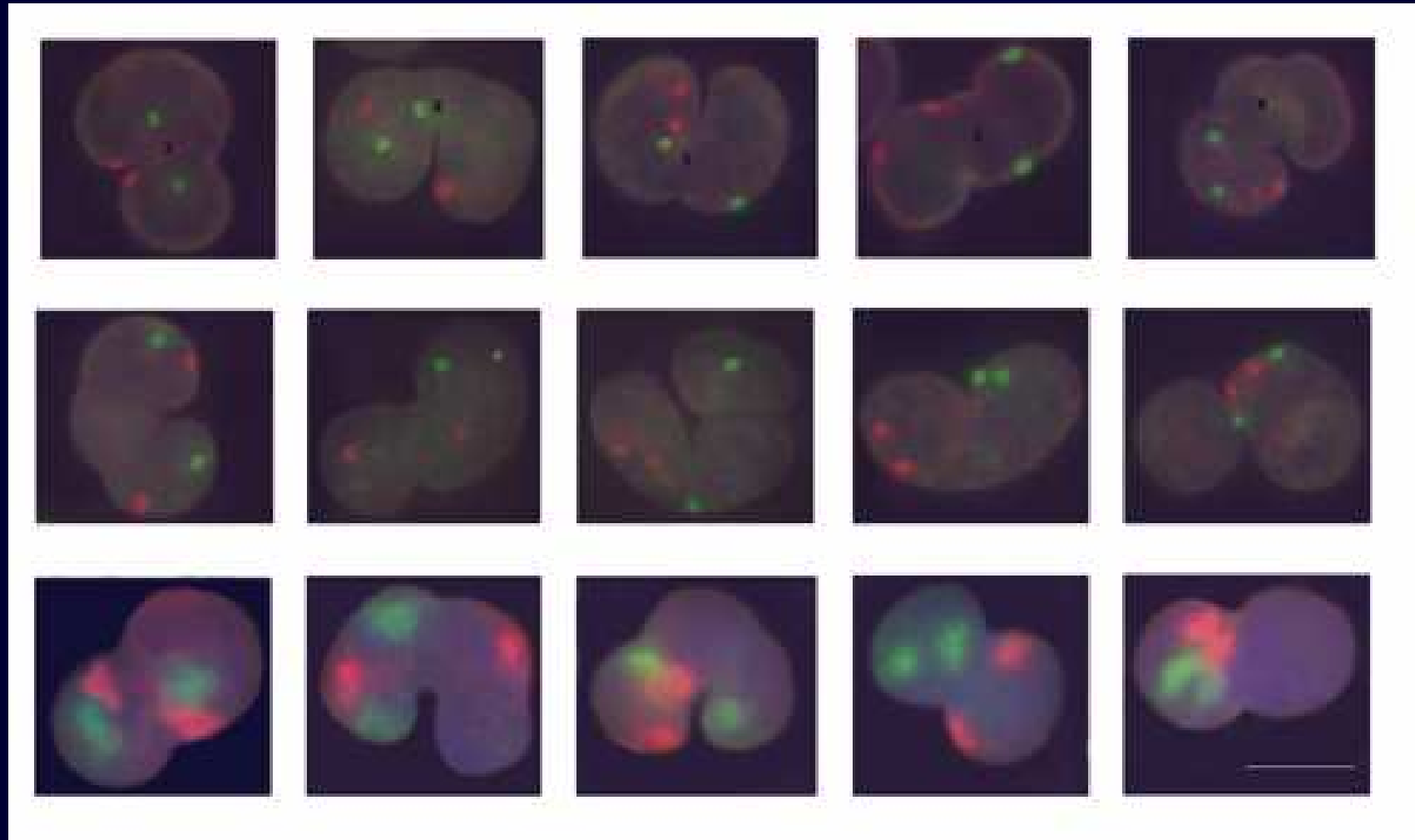
Morphology of human leukemic promyelocytic cell line HL60 and neutrophilic granulocyte

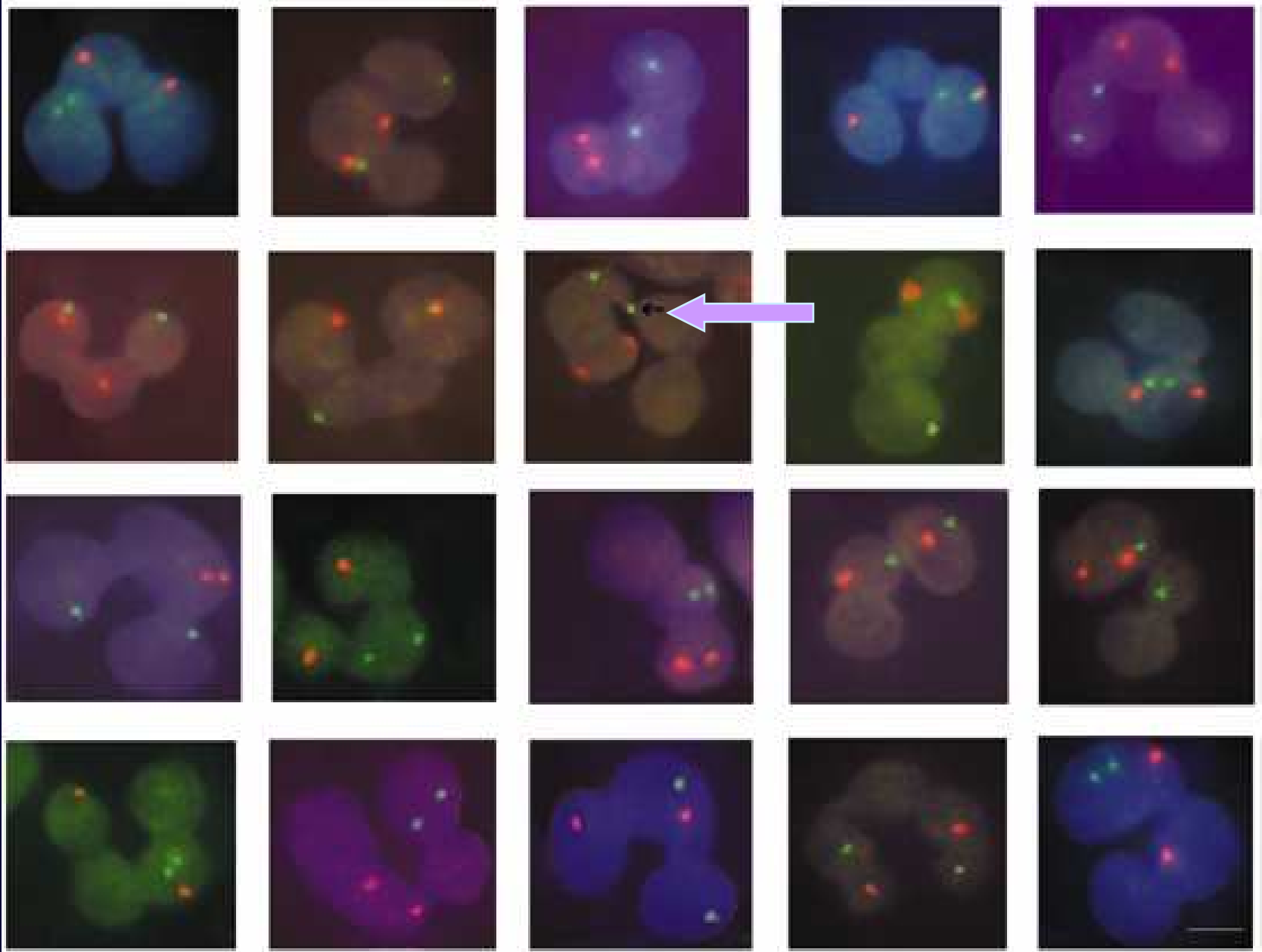




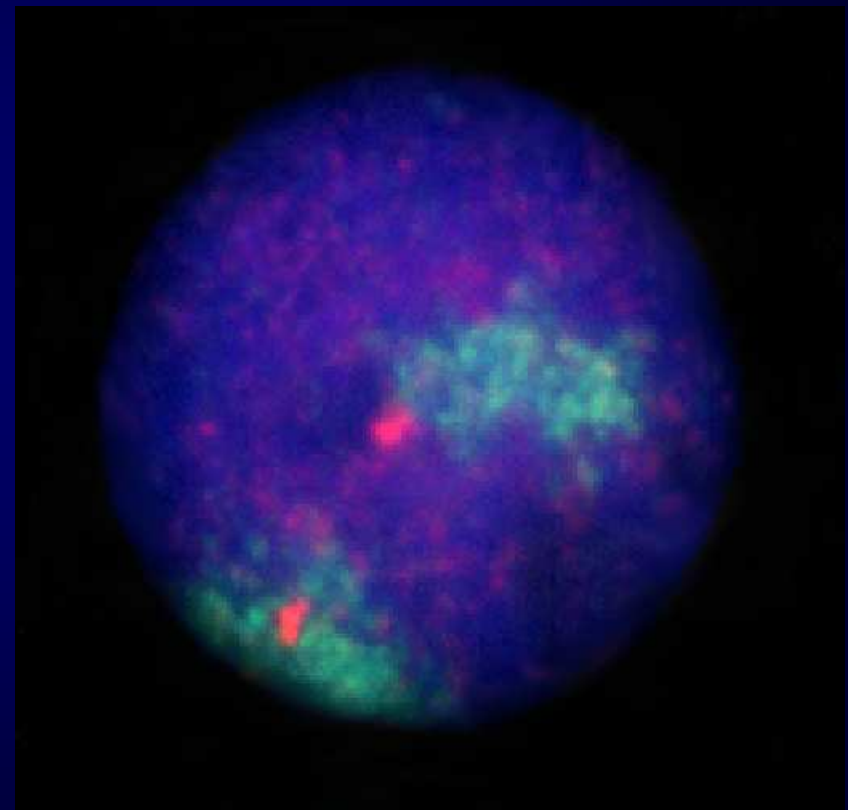
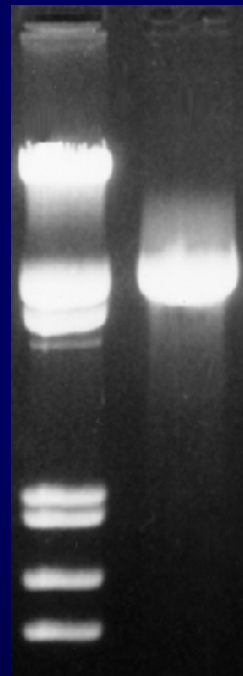
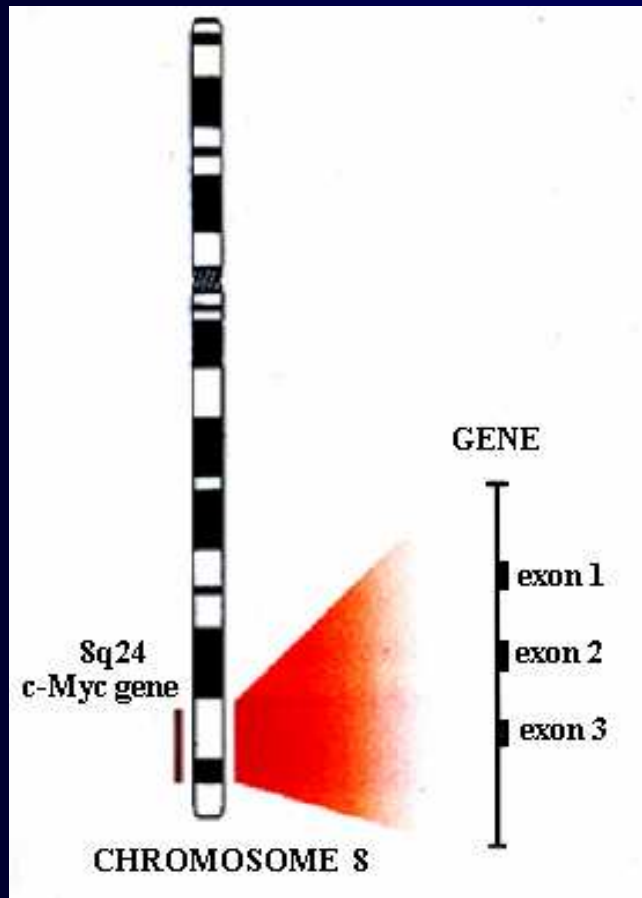


Topographic Types of Human Granulocytes

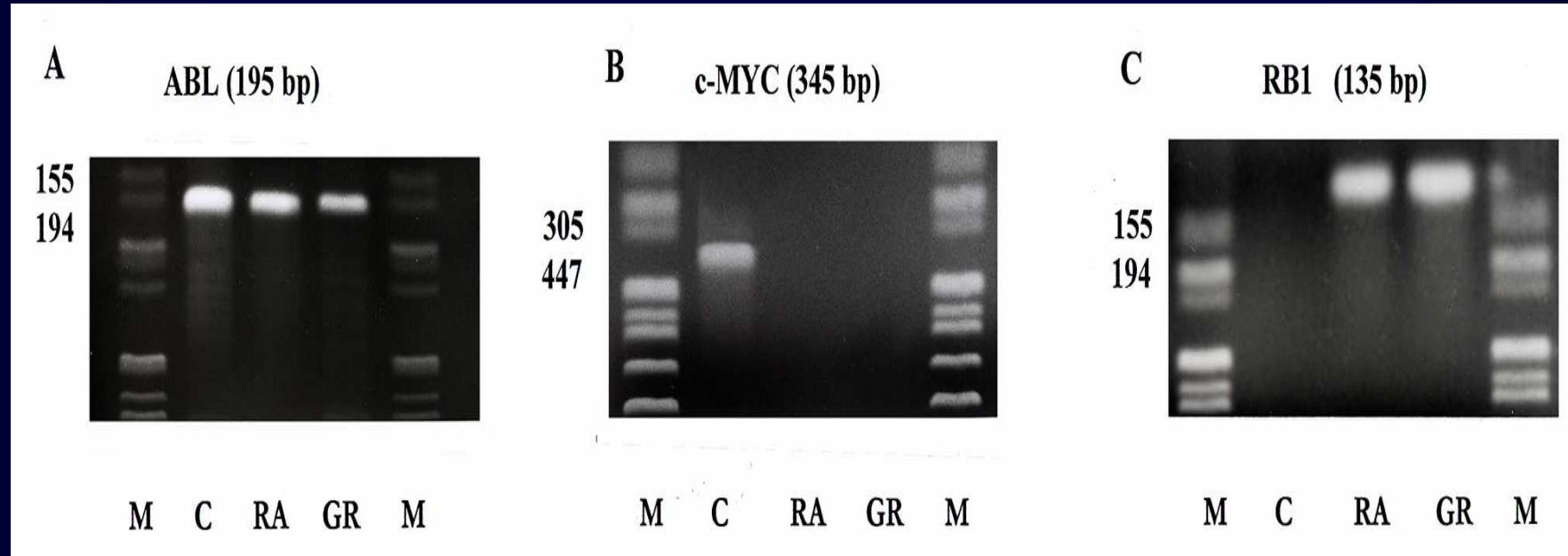




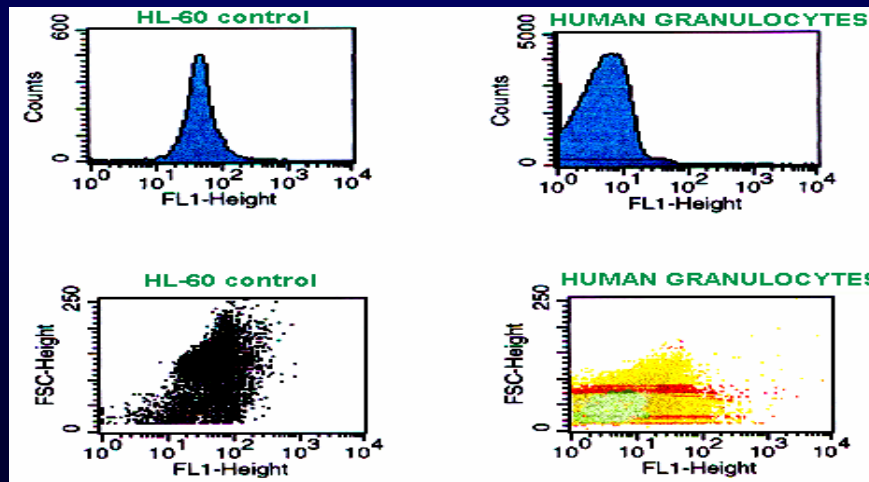
The C-myc Gene Nuclear Location



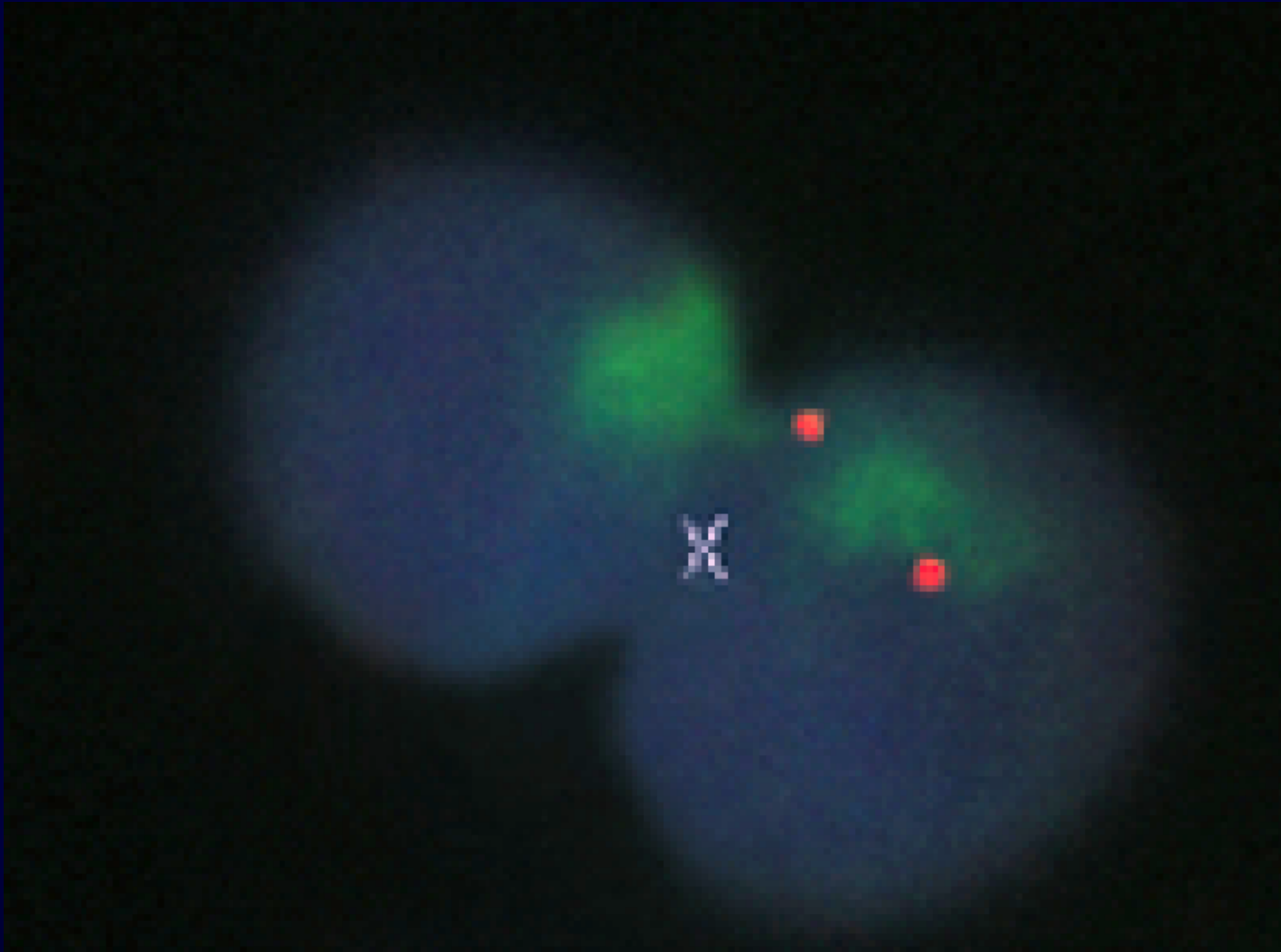
Changes in the expression of selected genes



FCM
c-myc

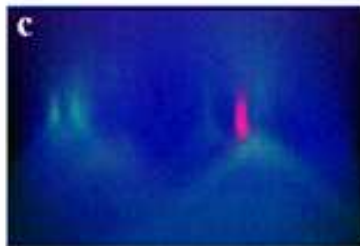
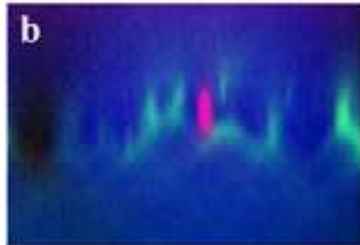
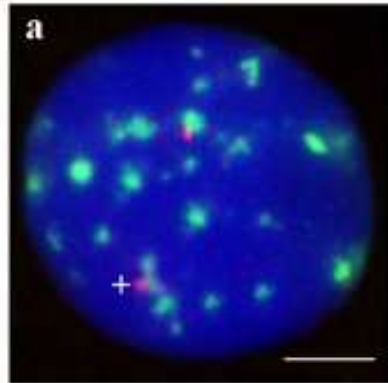


The C-myc gene nuclear topography in granulocytic nuclei

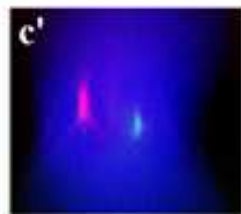
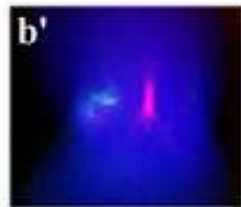
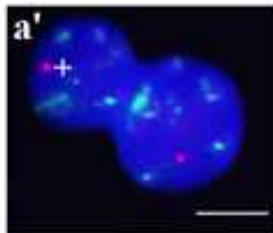


Centromeric silencing

A

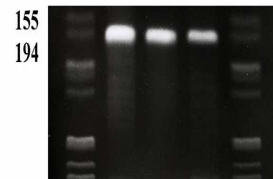


B



A

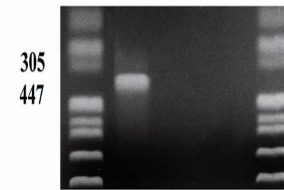
ABL (195 bp)



M C RA GR M

B

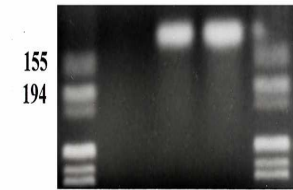
c-MYC (345 bp)



M C RA GR M

C

RB1 (135 bp)



M C RA GR M

Bone marrow

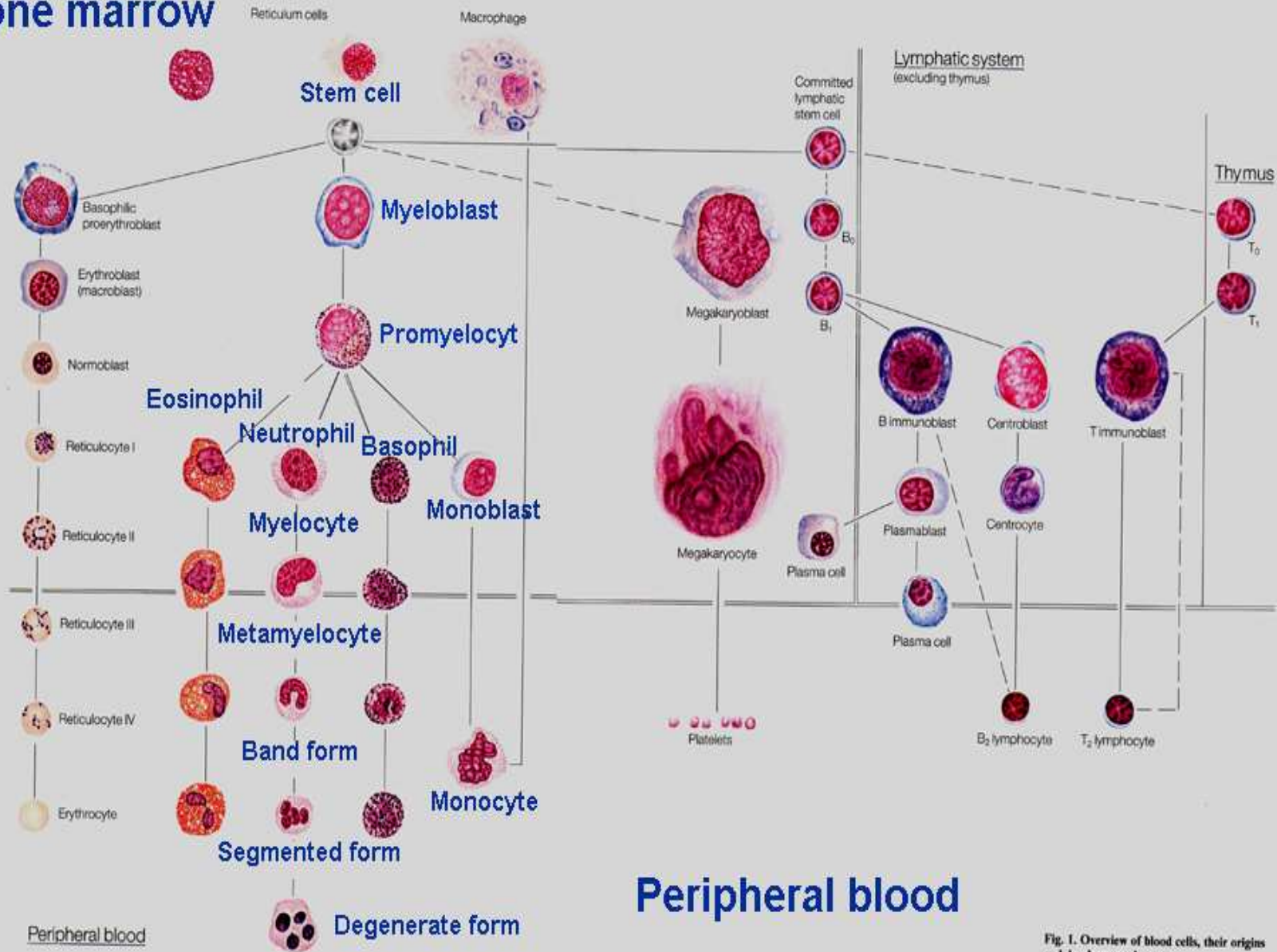
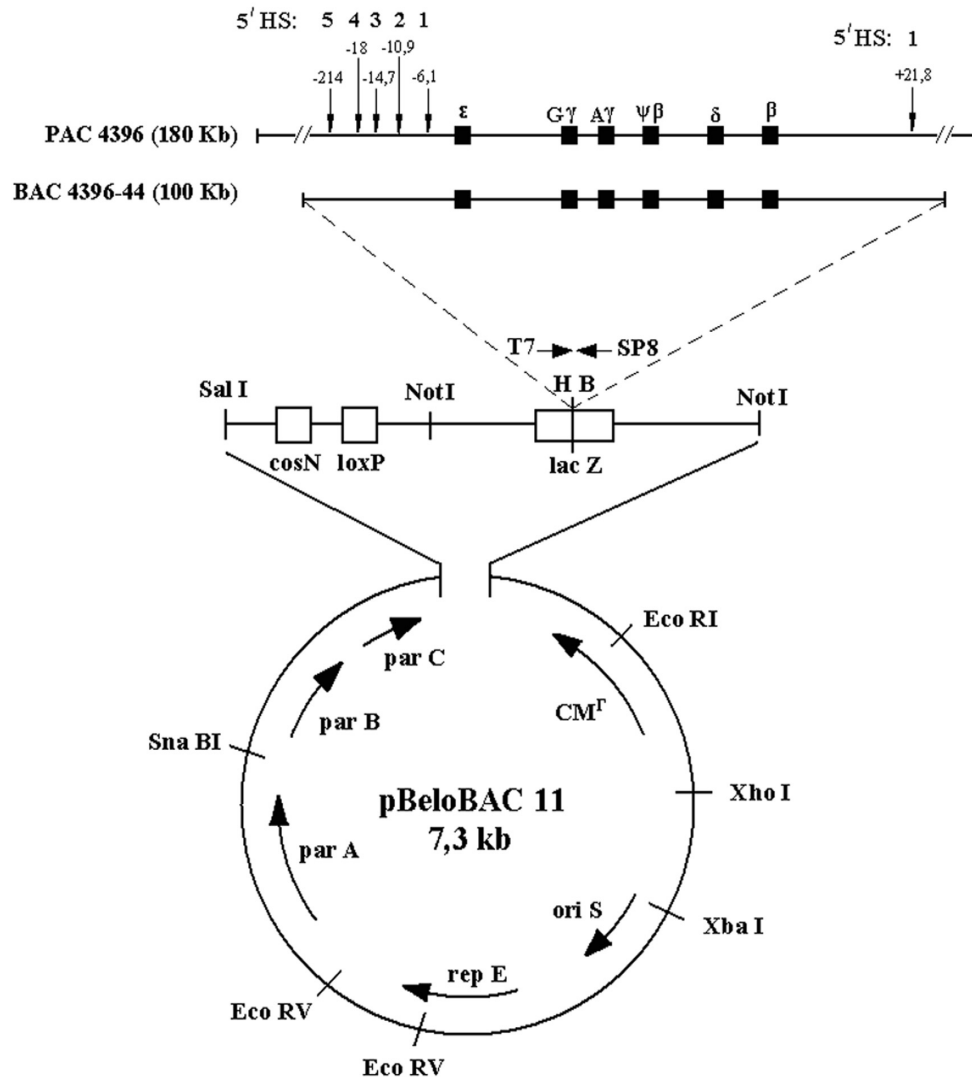


Fig. 1. Overview of blood cells, their origins and developmental stages

Beta-like globin gene cluster

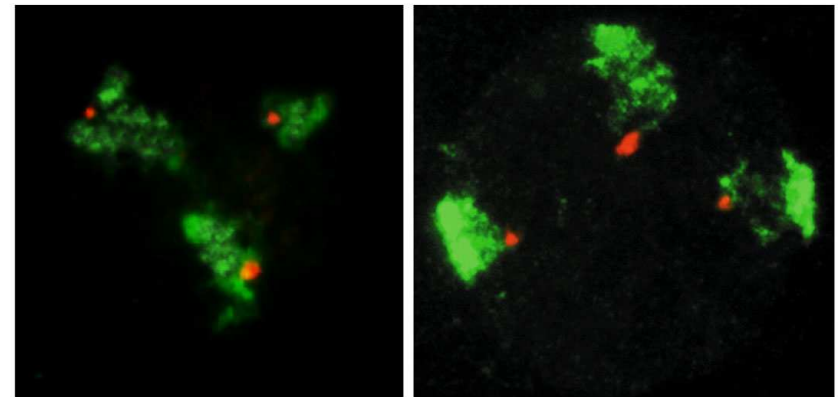
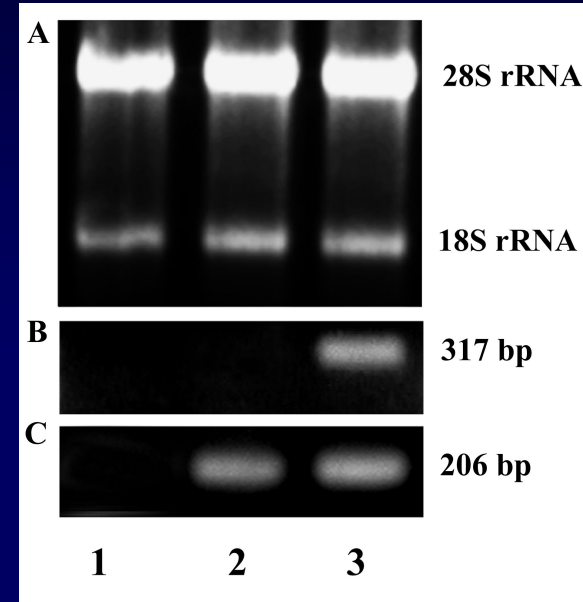
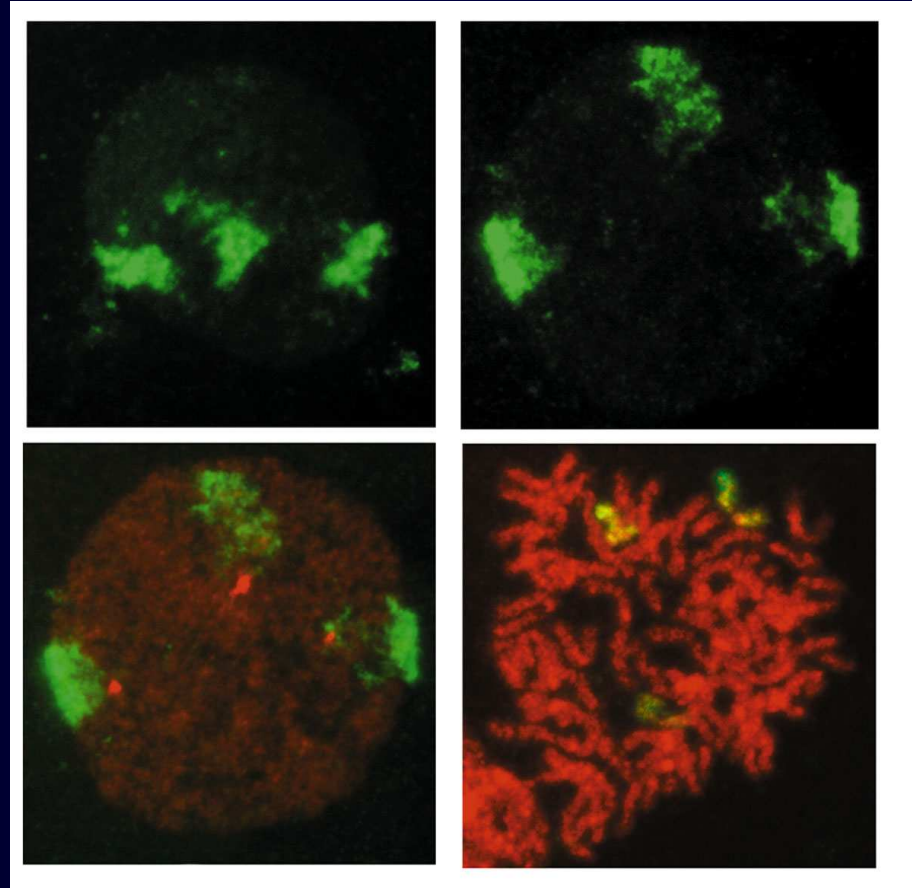


Arrayed on chromosome 11, encodes one embryonic (ϵ) and two fetal ($G\gamma$, $A\gamma$) and two adult (δ , β) globin chains. Expression of β -like genes undergoes a developmental related switching mechanism:

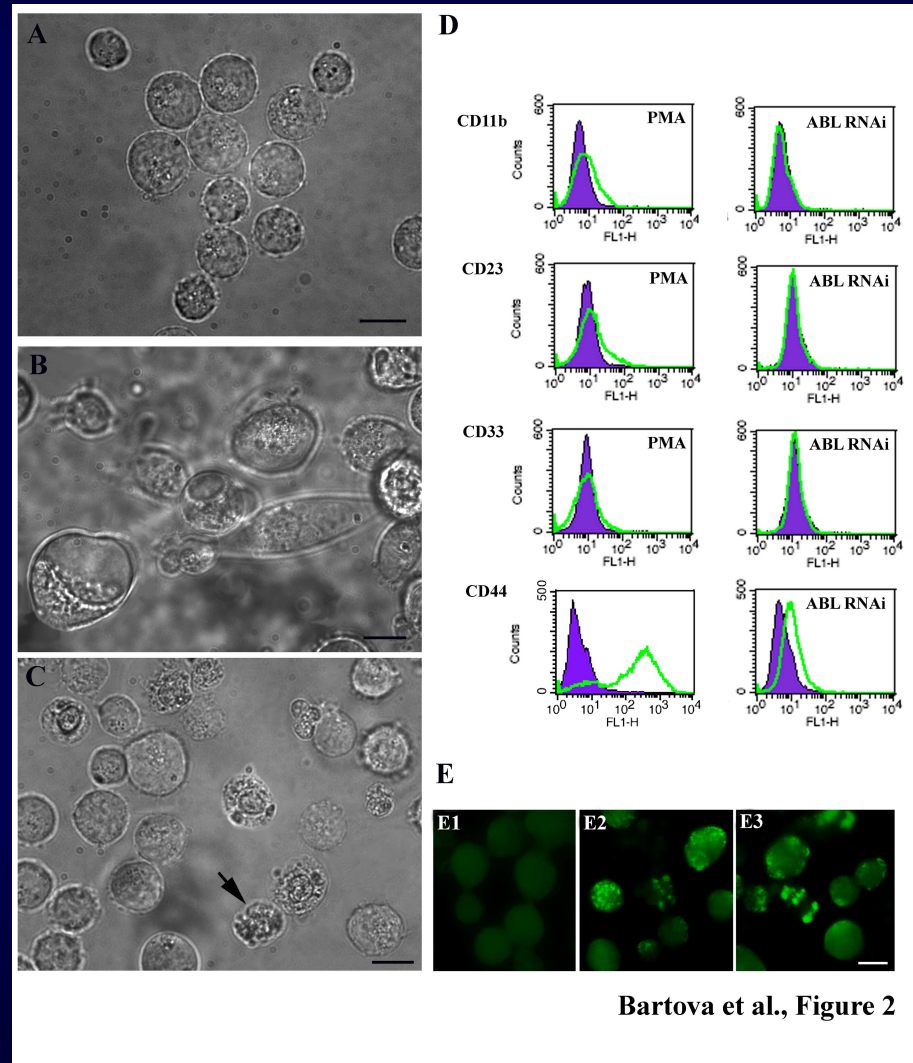
- ϵ : expressed in early embryo
- fetal γ : fetal life.
- δ , β : adulthood.

Changes in β -like gene expression accompany erythroid cell differentiation

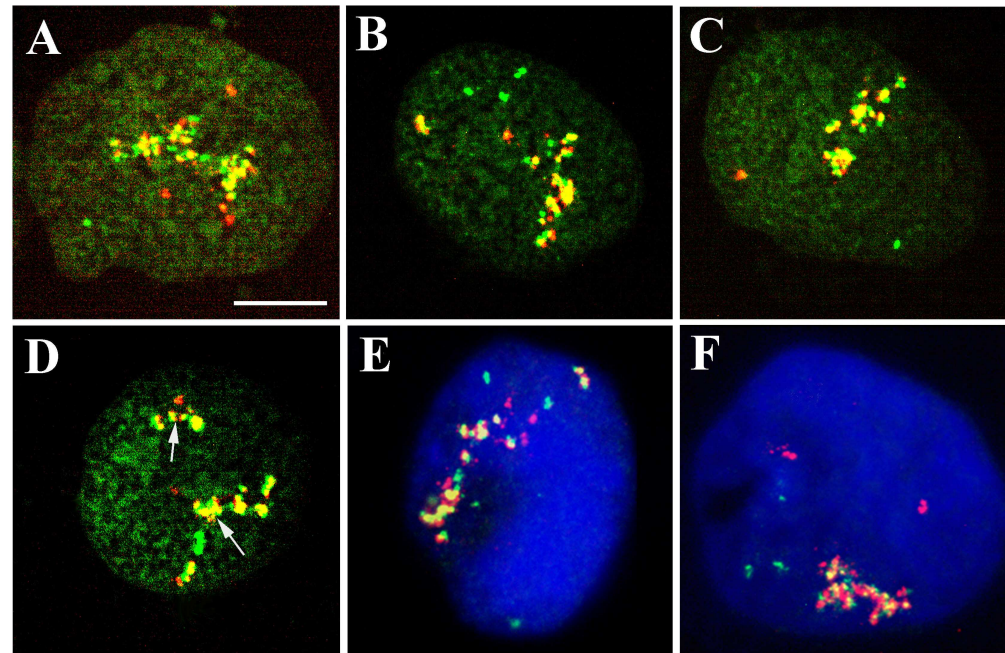
Differentiation of human hemopoietic cells into erythroid pathway



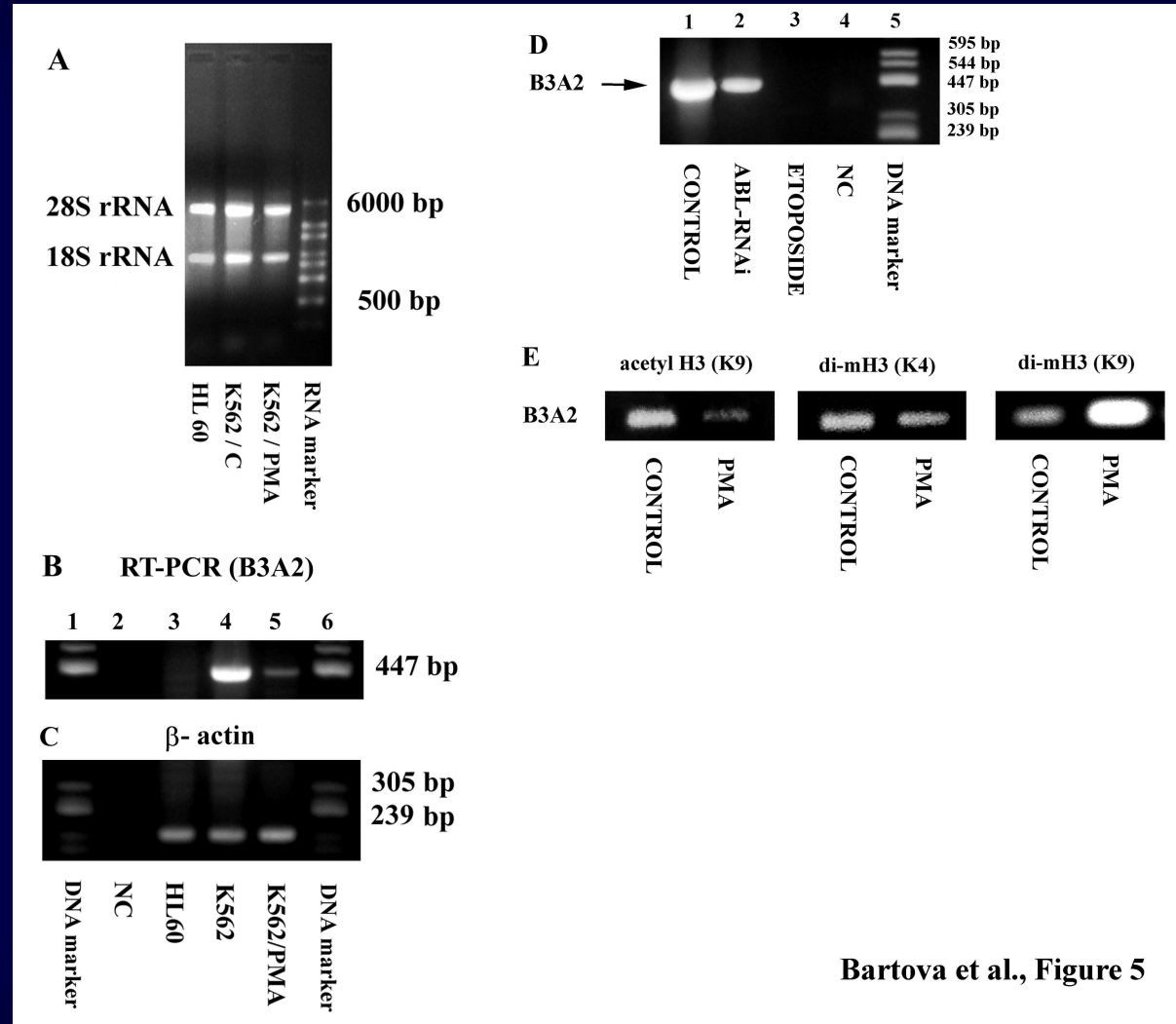
Differentiation of human hemopoietic cells into megakaryocytes



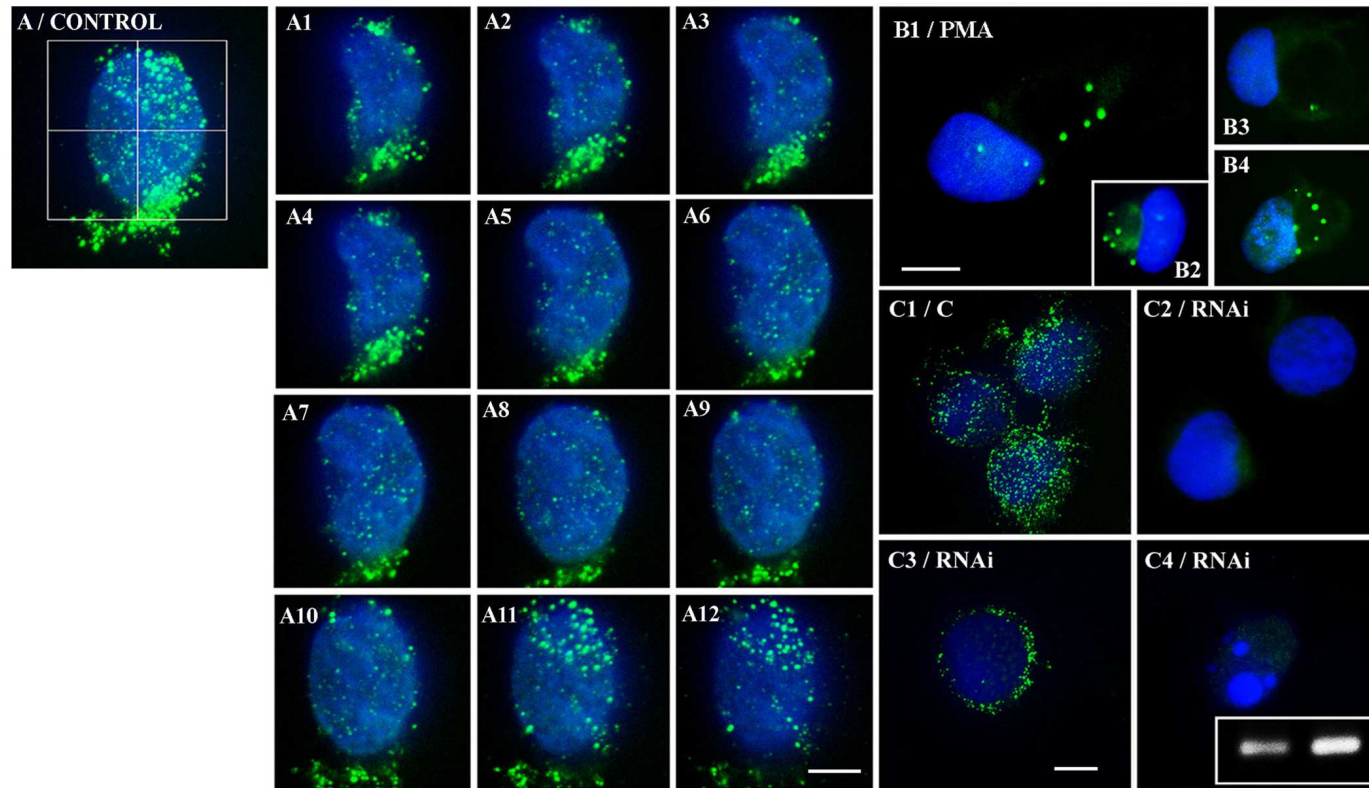
BCR (red signals) and ABL genes (green signals)



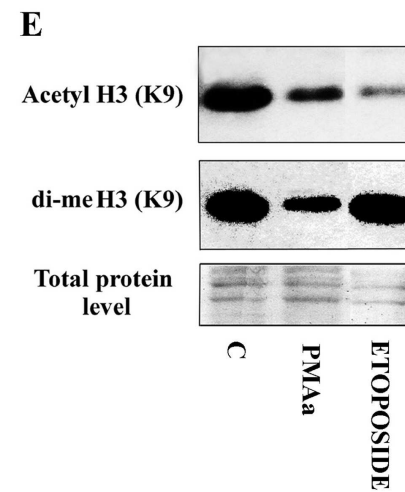
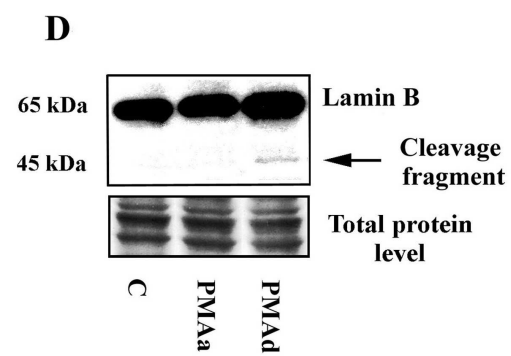
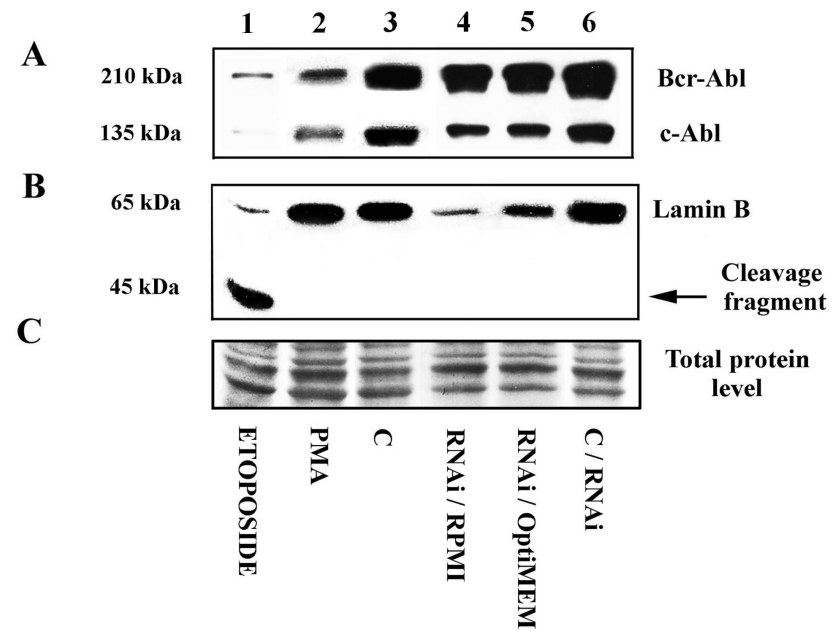
Bartova et al., Figure 3



Bartova et al., Figure 5



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 změny v genomu mají velký význam z hlediska aktivity genů a množství jejich proteinů. Tyto všechny uvedené buněčné faktory určují vznik daného buněčného typu.

RNA interference = RNA interferencia (RNAi)

Princíp: biologický proces, v ktorom molekuly ssRNA o veľkosti 20-25 nukleotidov sa zúčastňujú pri post - transkripčných génových reguláciách a v procese formovania štruktúry chromatinovej

História: 1990 - skupina **Richarda Jorgensena**

experiment za účelom zvýraznenia purpurovej farby petúnie – po vnesení extra kópií zodpovedného génu pozorovali opačný jav, ktorý nevedeli vysvetliť

1998 - **Andrew Z. Fire** a **Craig C. Mello**

dsRNA injektovaná do *Caenorhabditis elegans* spôsobovala „umlčanie“ súhlasných génov

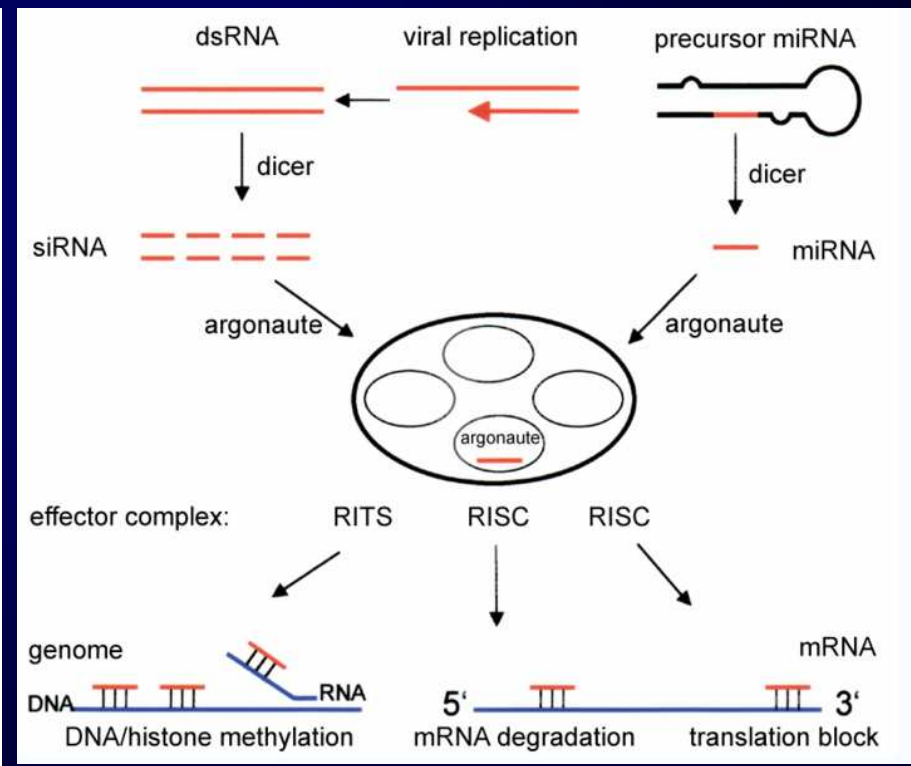
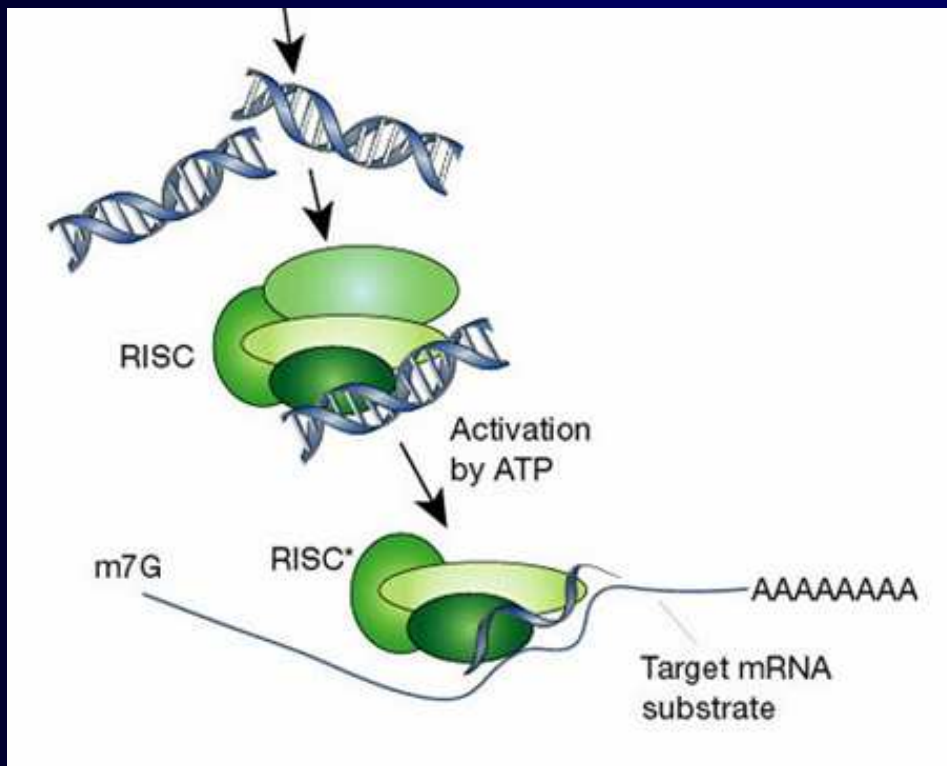
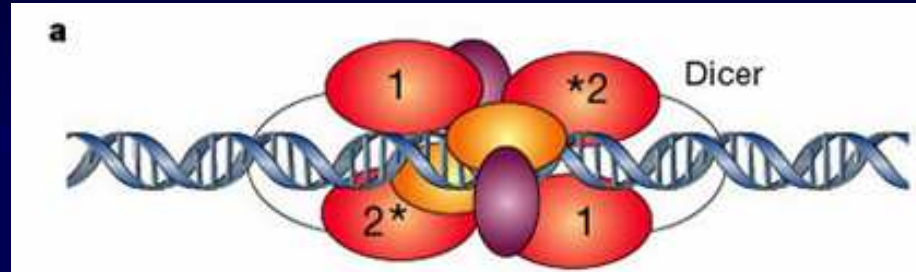
1999 - **Benitecs Graham**

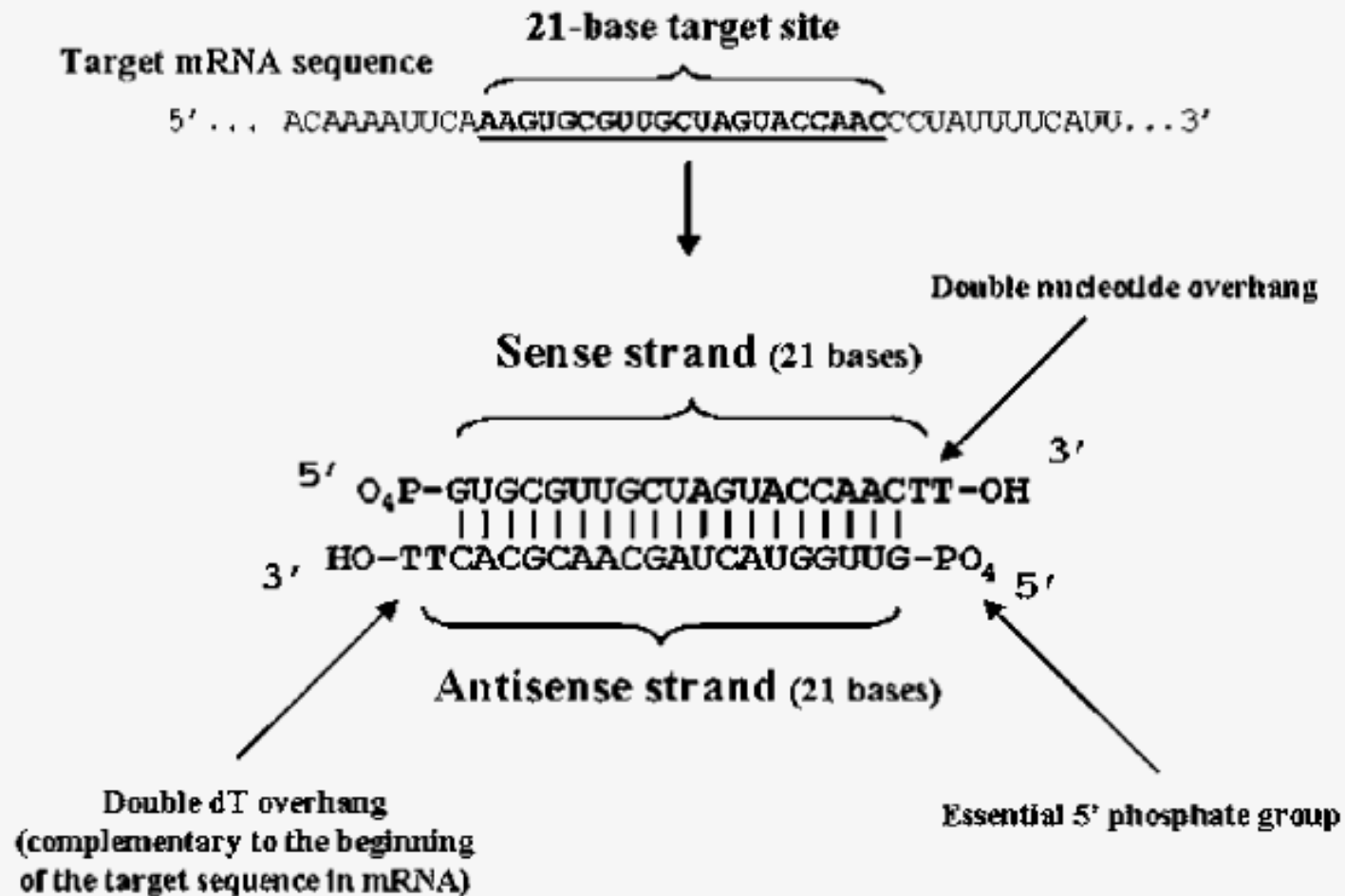
ukázala všeobecnosť javu RNAi a vytvorila DNA konštrukty, ktoré spúšťajú proces RNAi v ľudských a živočíšnych bunkách

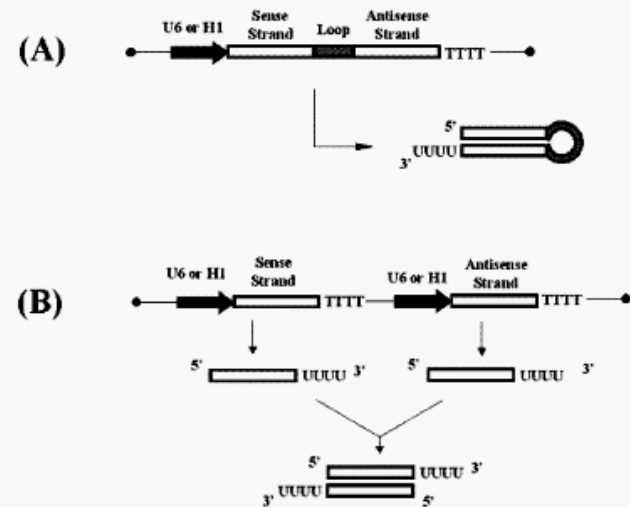
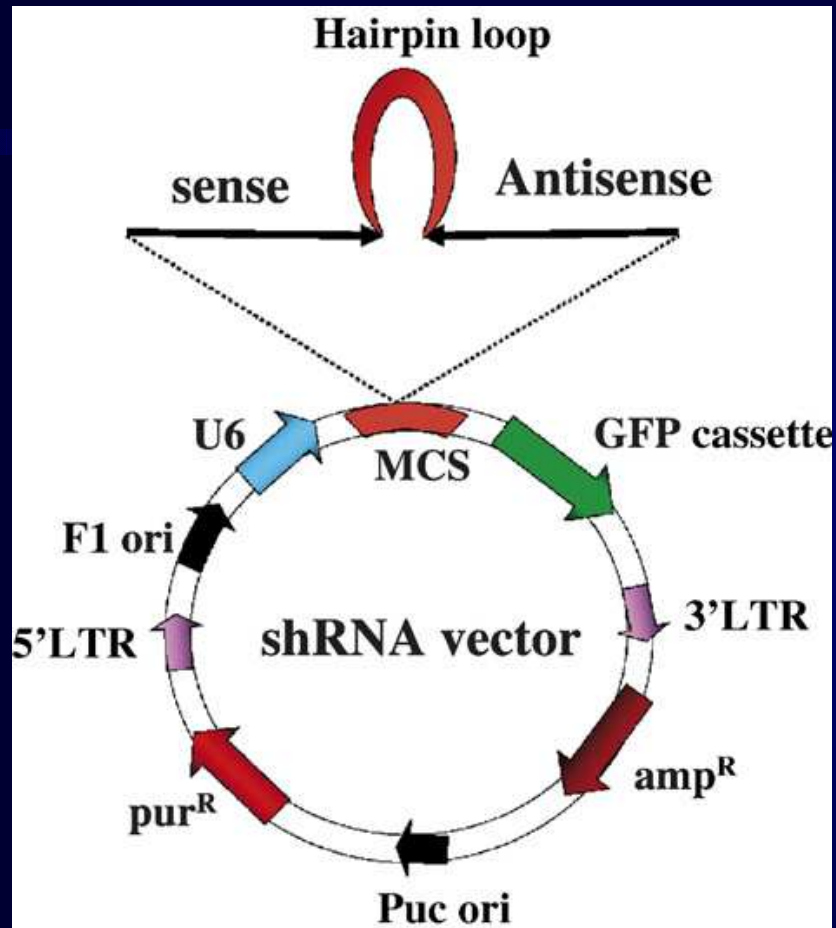
2006 - **Andrew Z. Fire** a **Craig C. Mello** získali Nobelovu cenu

objavenie, že dsRNA vyvoláva supresiu genovej aktivity, spôsobom homologickej závislosti v procese RNA interferencie

Mechanizmus procesu RNAi







Grafická predstava

