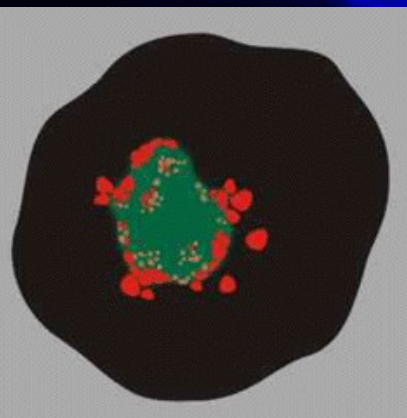


APOPTOSIS: ARCHITECTURE OF CHROMOSOMAL TERRITORIES IN APOPTOTIC CELLS

E. Bártová

*•Institute of Biophysics Academy of Sciences of
the Czech Republic*



Cellular death-by-suicide is part of normal development, and is termed apoptosis or programmed cell death (PCD). Cysteine Aspartate Specific ProteASEs – caspases – are active in apoptosis, as are p53, a tumor suppressor gene, and FAS gene, which is member 6 of the tumor necrosis factor receptor superfamily (TNF). In contrast to apoptosis, necrosis is cell death that results from cytotoxic, injurious stresses that are too severe for correction by the cellular stress response.

Apoptosis is a part of normal cell turnover and tissue homeostasis

„History“ of molecular biology of cell death



Kerr et al., 1972:

Identification of the cell death APOPTOSIS

Kerr, Wylie and Currie Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J.Cancer* 1972;26:239-257

1990

Horvitz (1992-3) identification of „cell death genes“ in *Caenorhabditis elegans* {*ced-3* (ICE), *ced-4* (0), *ced-9* (*bcl-2*)} (Cerretti 1992, Thornberry 1992) uncovering of the homology between *ced-3* gene product and ICE (interleukin-1 β converting enzyme)] protease



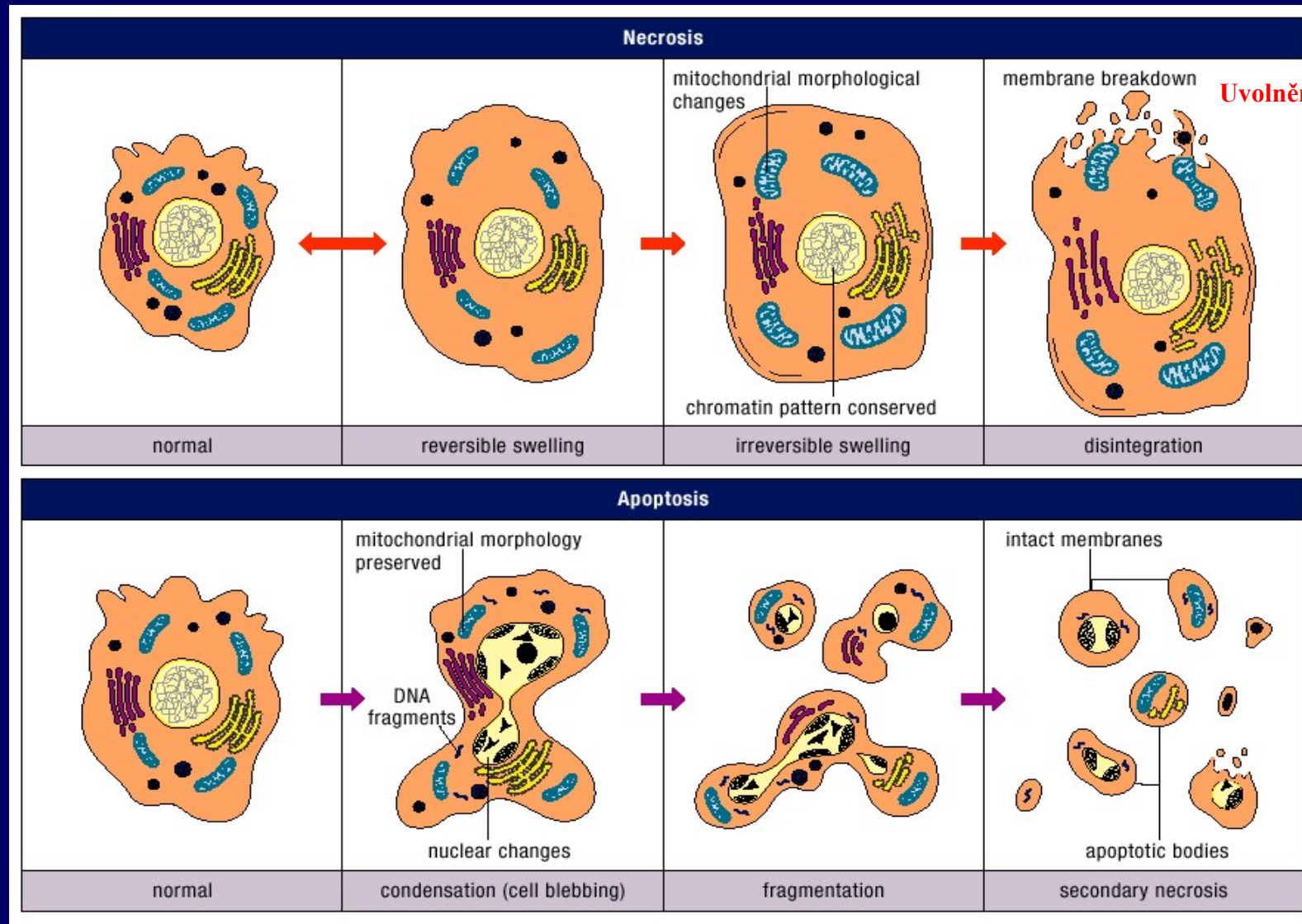
**Discovery of new family of mammalian cysteine proteases -
CASPASES**

Apoptosis is involved in a wide range of physiological and pathological processes.

- > Development (embryonic, neuronal development)**
- > Inflammation and involution of tissues**
- > In the immune system (Apoptosis is employed as a method of cytotoxic T-cell mediated killing of infected cells)**
- > In ageing**

Apoptosis plays a pivotal role in the pathophysiology of **ageing**'. The free radical theory of ageing links senescence to damage inflicted by **superoxide-derived radicals** and other oxidants generated primarily in mitochondrial respiration. The **mitochondrial theory of ageing**, proposes that ageing is the **result of accumulated free radical damage to mitochondrial DNA (mtDNA)**. The accumulation of errors in mtDNA leads to errors in the polypeptides encoded by mtDNA, i.e., the four mitochondrial enzymatic complexes. Defective complexes produce more free radicals leading to a vicious cycle of increasing mtDNA damage, radical generation, and possibly apoptosis

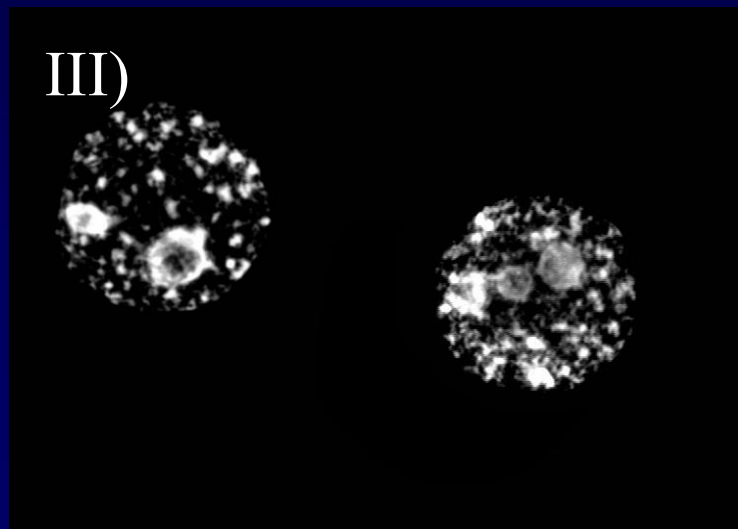
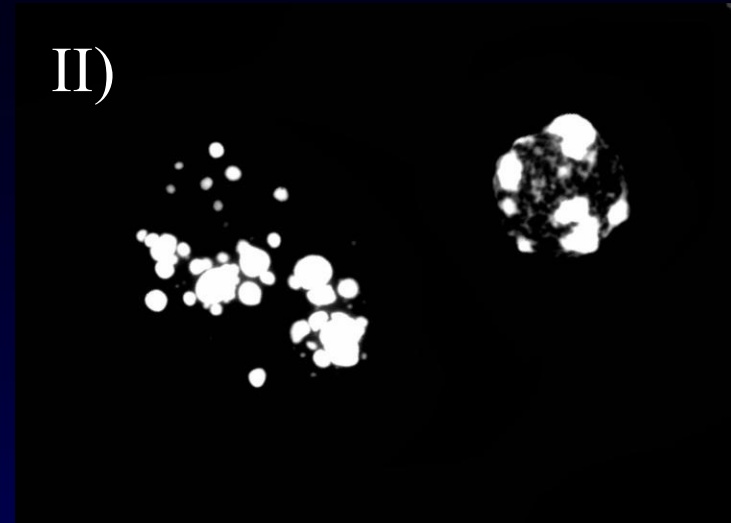
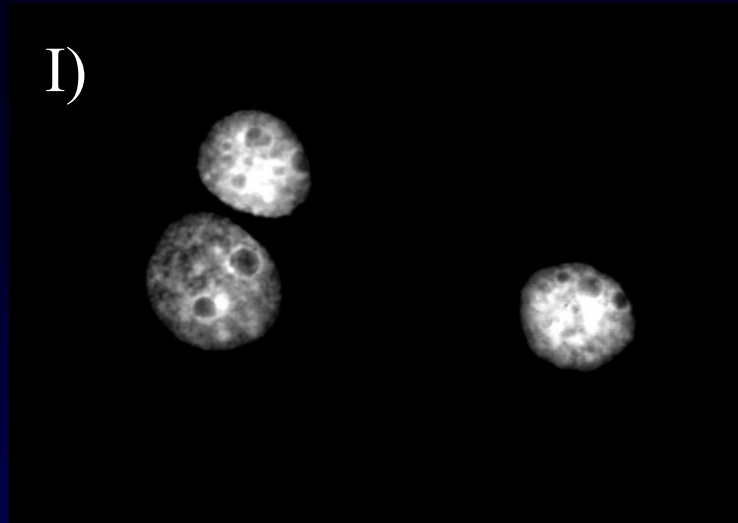
Apoptosis in contrast to necrotic cell death



Uvolnění lysosomálních enzymů

Nuclear morphology in HL-60 cells

(P. Mlejnek 2001)



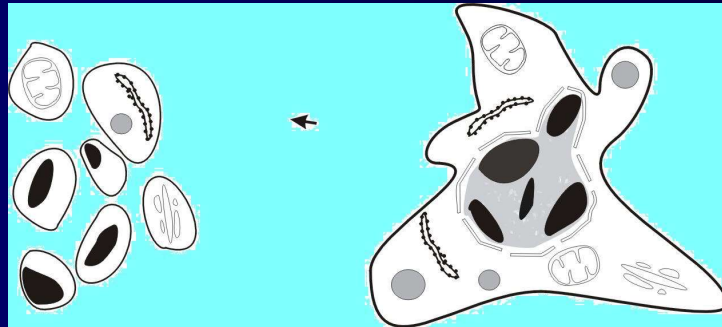
I) Control

II) Apoptosis

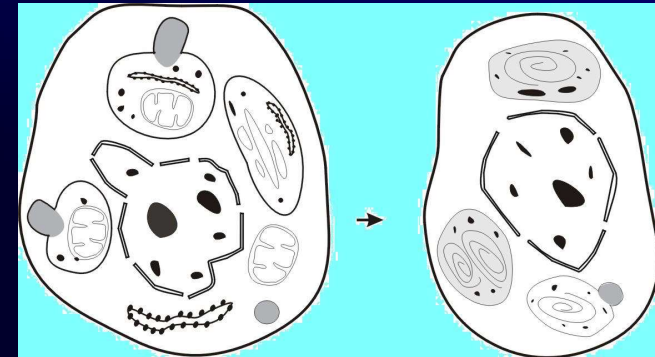
III) Necrosis

Cell death classification by Clarke

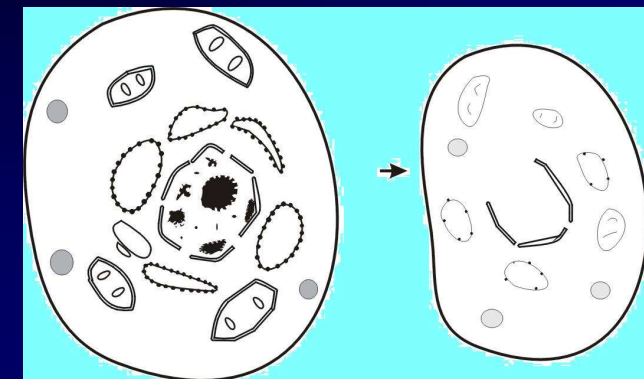
Apoptosis



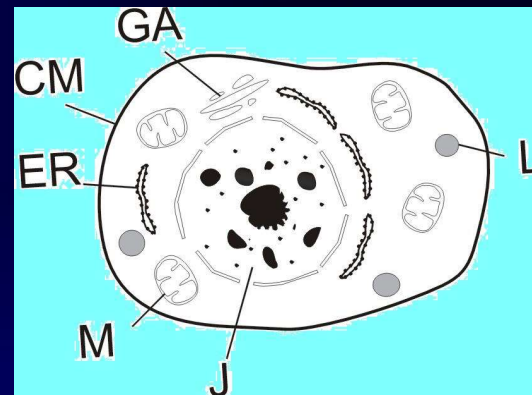
Autophagy



Nelysosomal disintegration



CM – cyt. membrane
J – nuclei
M – mitochondrion
ER – endopl. reticulum
GA – Golgy complex
L – lysosomes

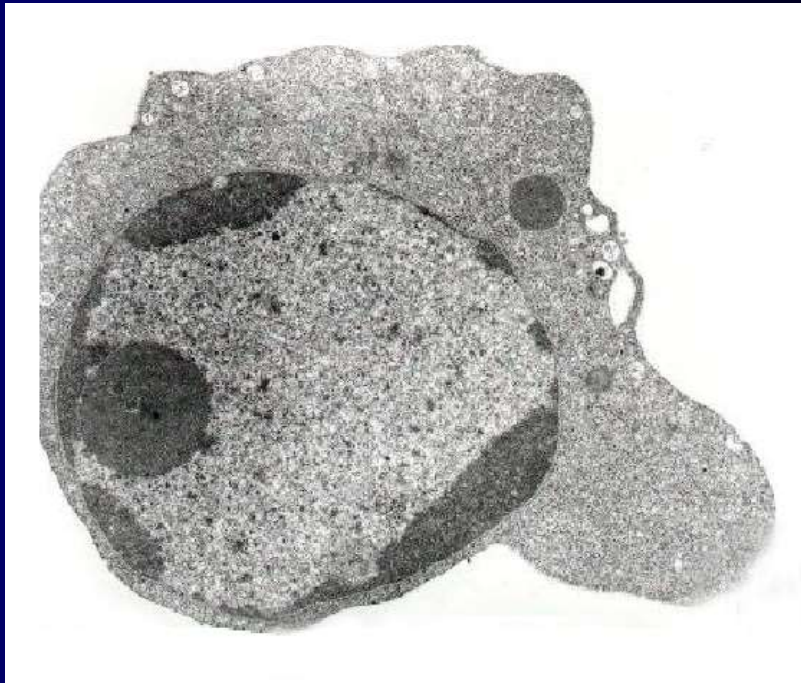
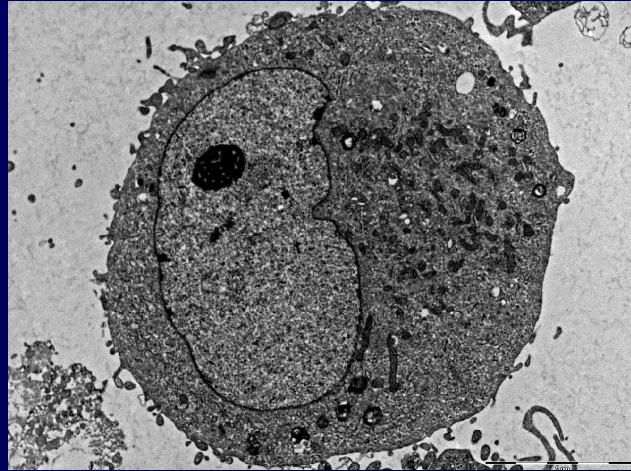


Cell death classification by Clarke

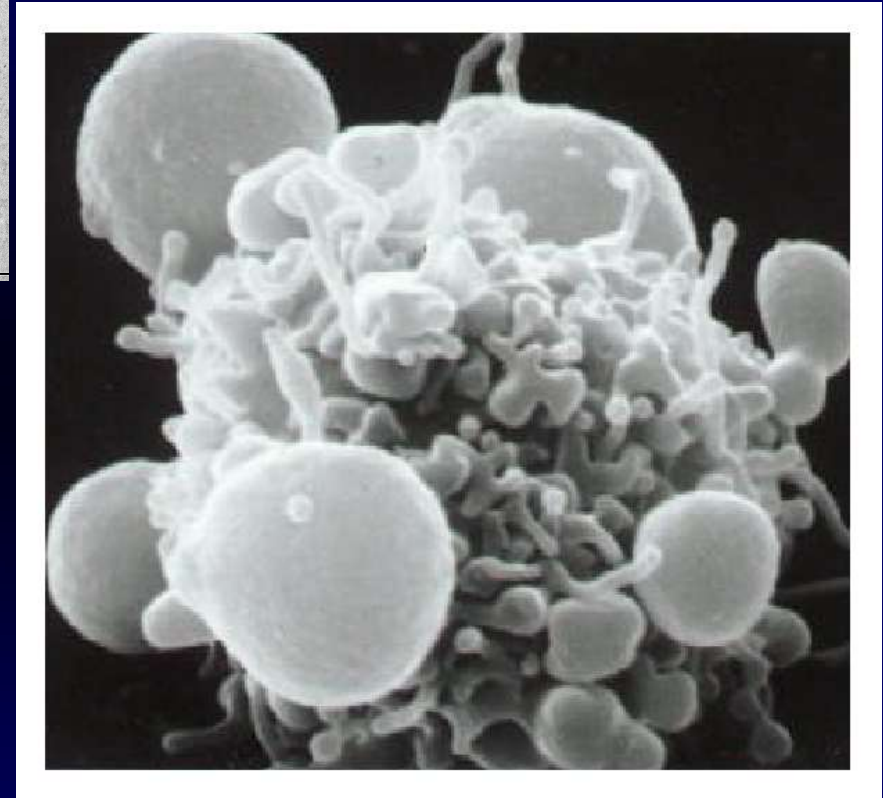
- **Apoptosis** - heterophagy, final cell destruction is done by lysosomes of other cells
- **Autophagy** - final cell destruction is done by its own lysosomes
- **Nonlysosomal disintegration** - cell destruction is mediated by unknown nonlysosomal proteases

Anoikis is a form of programmed cell death which is induced by anchorage-dependent cells detaching from the surrounding extracellular matrix (ECM)[1]. Usually cells stay close to the tissue to which they belong since the communication between proximal cells as well as between cells and ECM provide essential signals for growth or survival. When cells are detached from the ECM, i.e. there is a loss of normal cell-matrix interactions, they may undergo anoikis. However, metastatic tumor cells may escape from anoikis and invade other organs.

Morphological features of apoptosis



Transmission electron micrograph



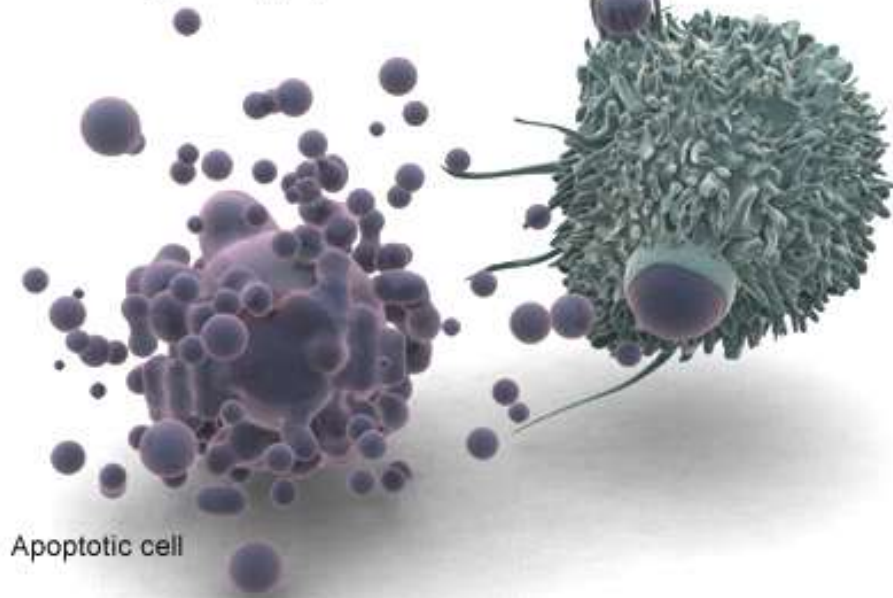
Scanning electron micrograph

C-Knudson@uniowa.edu

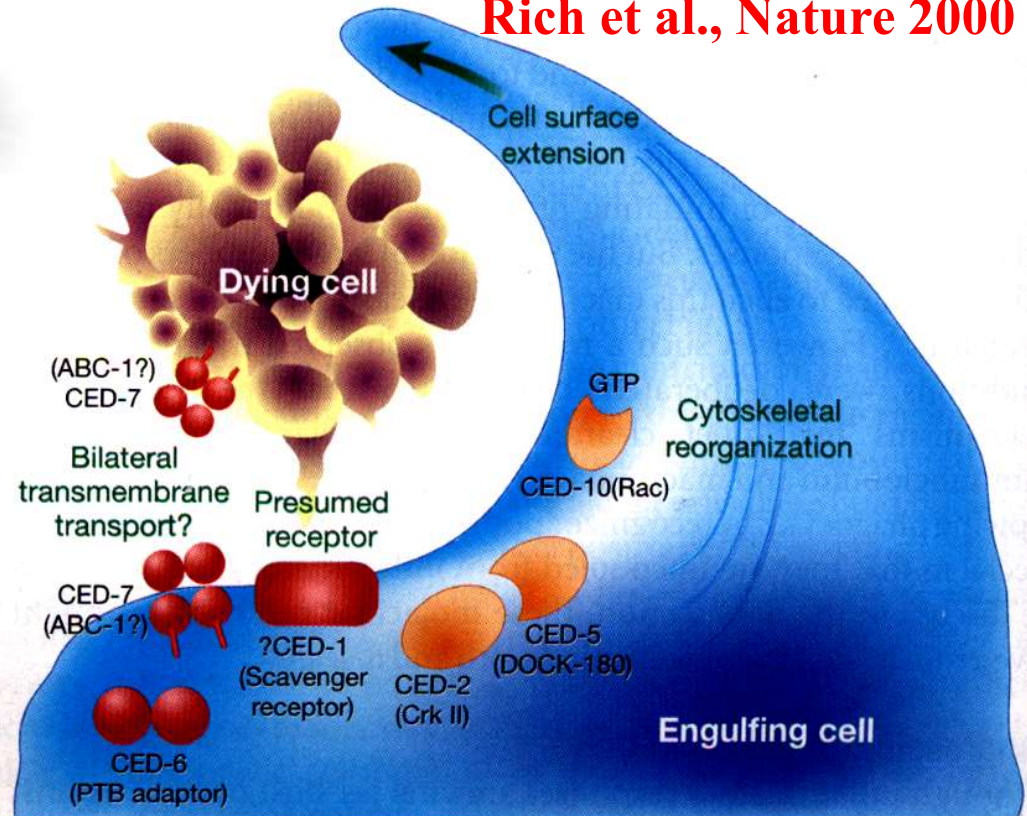
Apoptotic DNA degradation is followed by phagocytosis of apoptotic bodies

Final stage of apoptosis

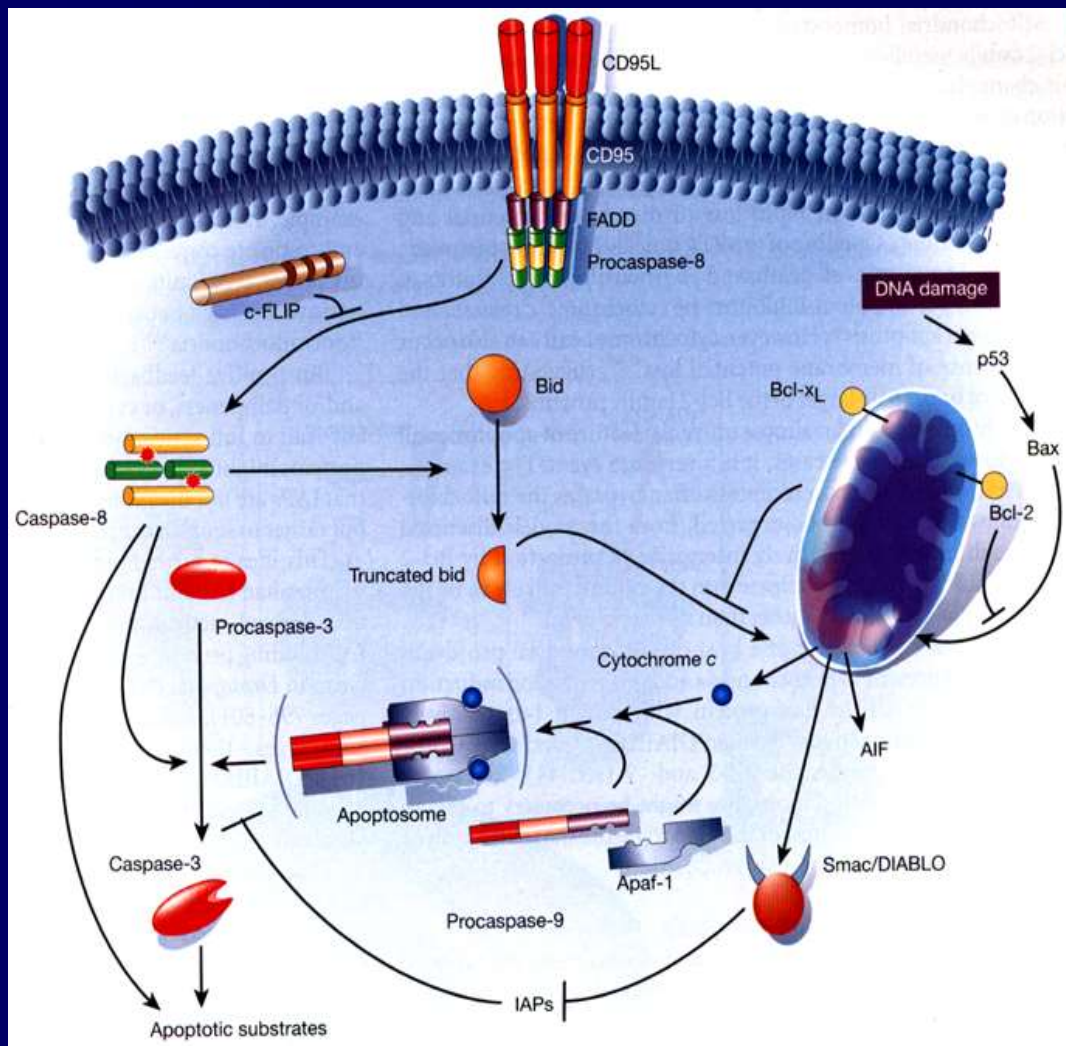
White blood cell



Rich et al., Nature 2000



Two major apoptotic pathways in mammalian cells

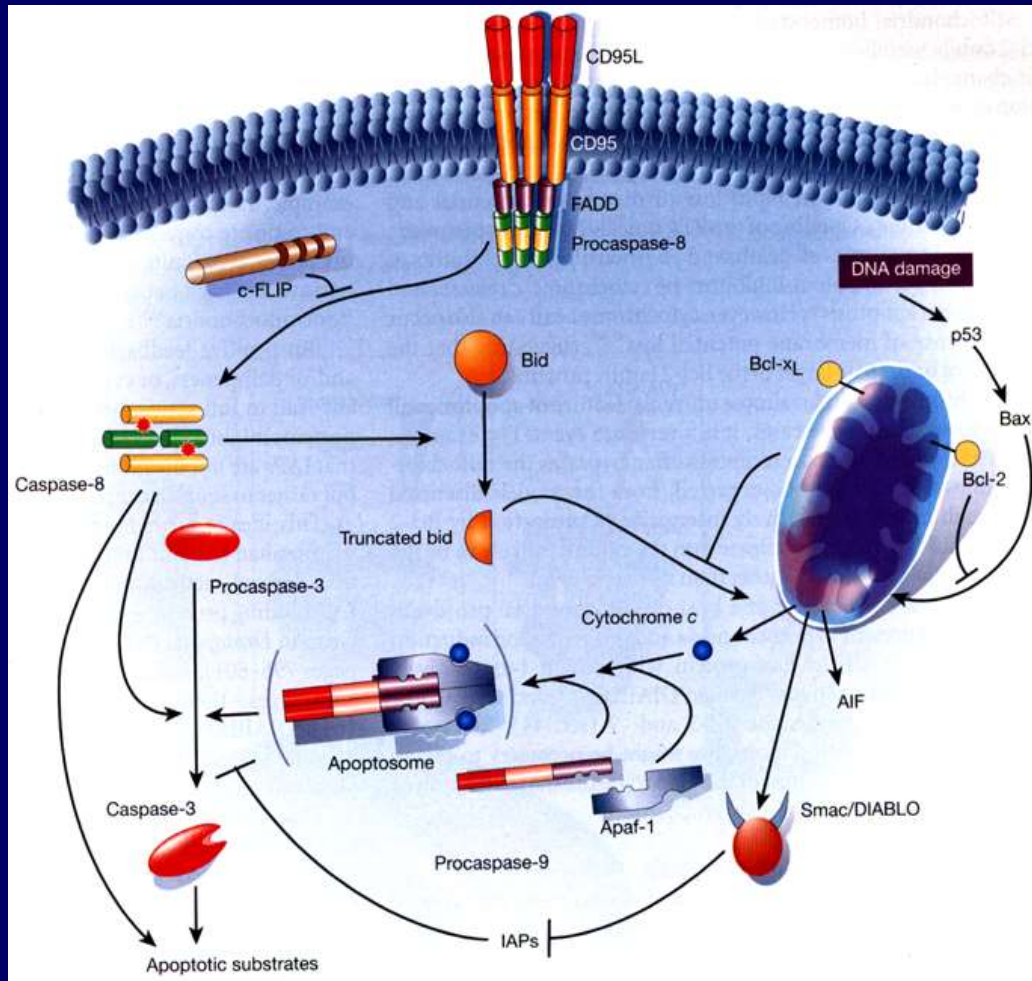


Death-receptor pathway:

Death receptor superfamily:
CD95 receptor and tumour necrosis factor receptor. CD95 ligand binds to CD95 receptor - to form death inducing signaling complex. This complex recruits via the adaptor molecule FADD (Fas-associated death domain protein). Procaspase 8 binds to this complex in order to activate Caspase-8 and subsequently activation of Caspase-3 is induced. Activation of procaspase-8 can be blocked through degenerate caspase homologue c-FLIP.

Hengartner M.O., Nature 2000

Two major apoptotic pathways in mammalian cells



Hengartner M.O., Nature 2000

- **The mitochondrial pathway**
- **activated after DNA damage**
- **proapoptotic members of Bcl-2 family, located on the surface of mitochondria, are activated**
- **Cytochrome c is released from mitochondria and forms complex with Apaf-1 and Procaspase 9.**
- **The complex is called APOPTOSOME.**

Both apoptotic pathways converge on the level of Caspase-3 activation

Caspase-3 activation is antagonized by IAP released from mitochondria

The Bcl-2 Family

Anti-Apoptotic



Pro -Apoptotic



BH4



BH1



Transmembrane Domain

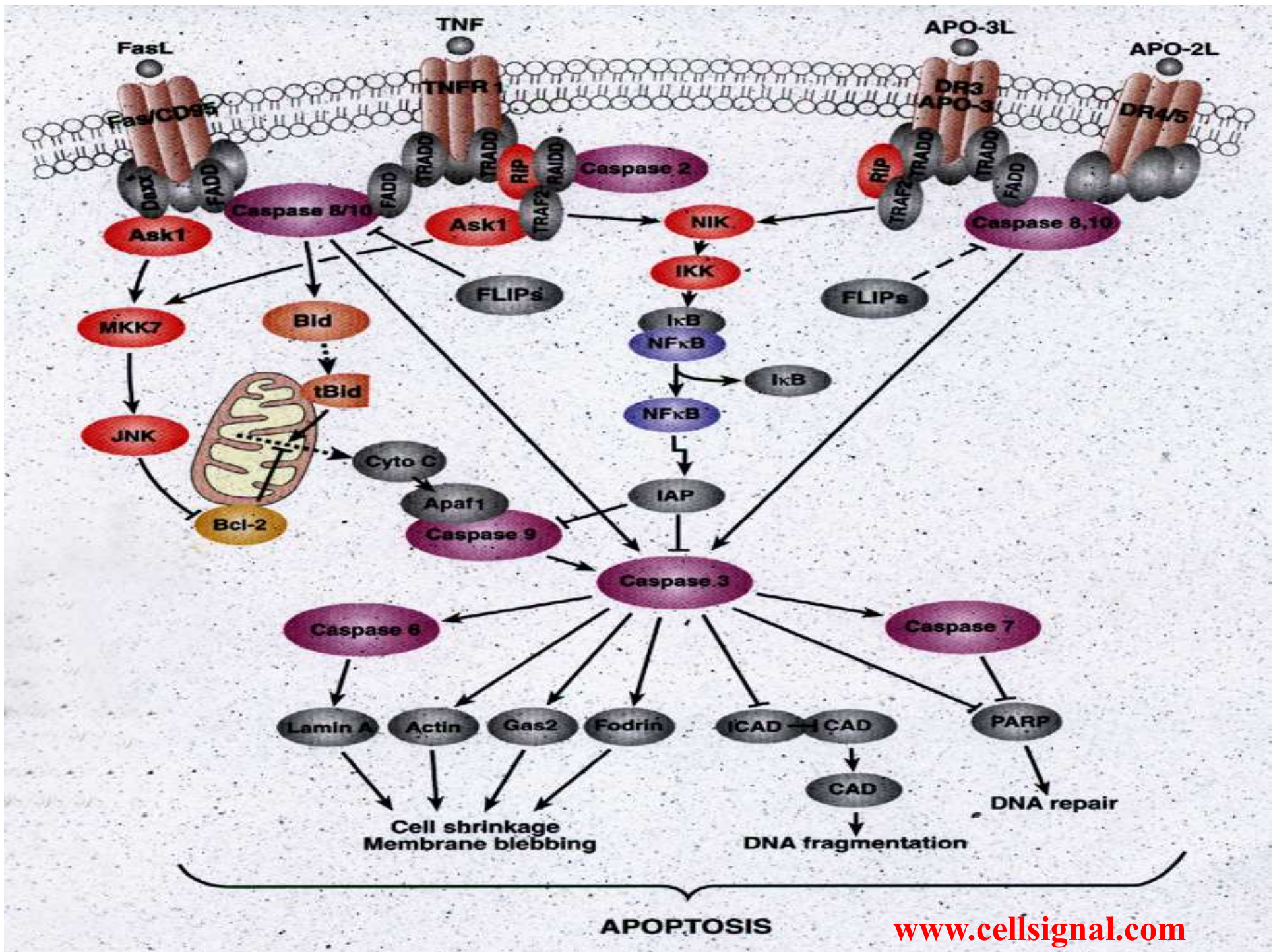


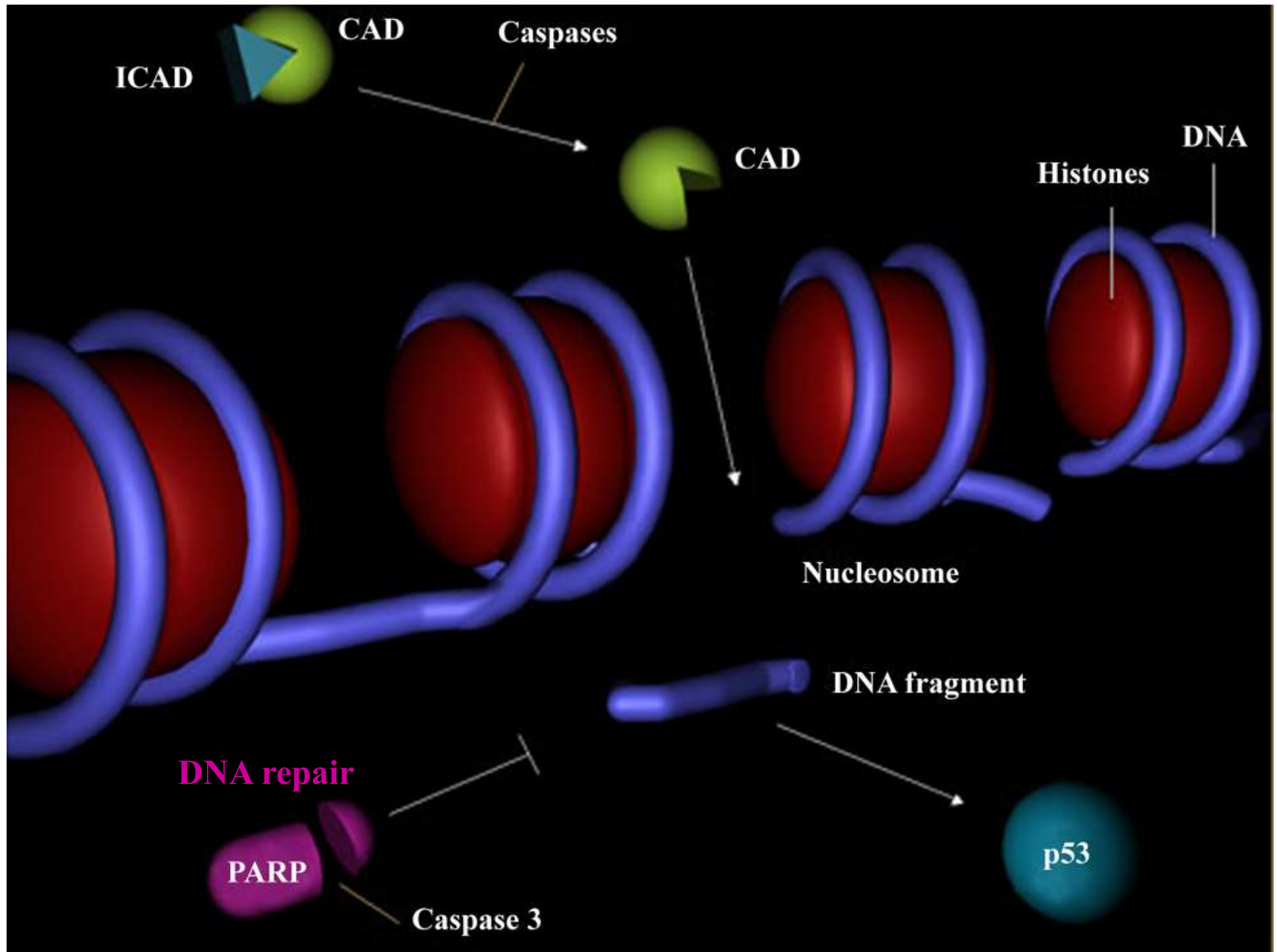
BH3

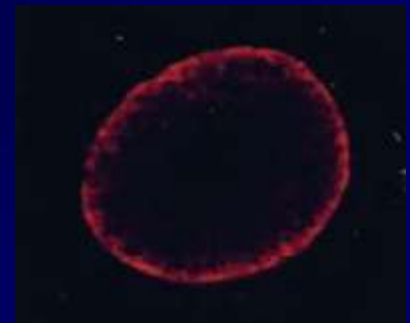
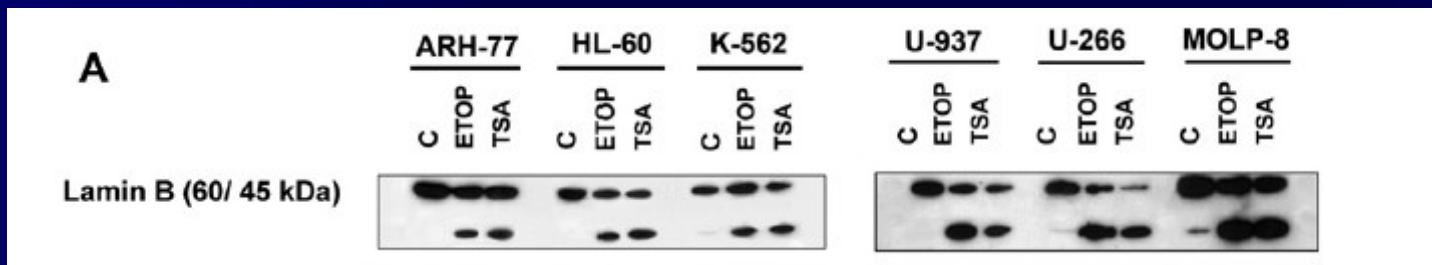
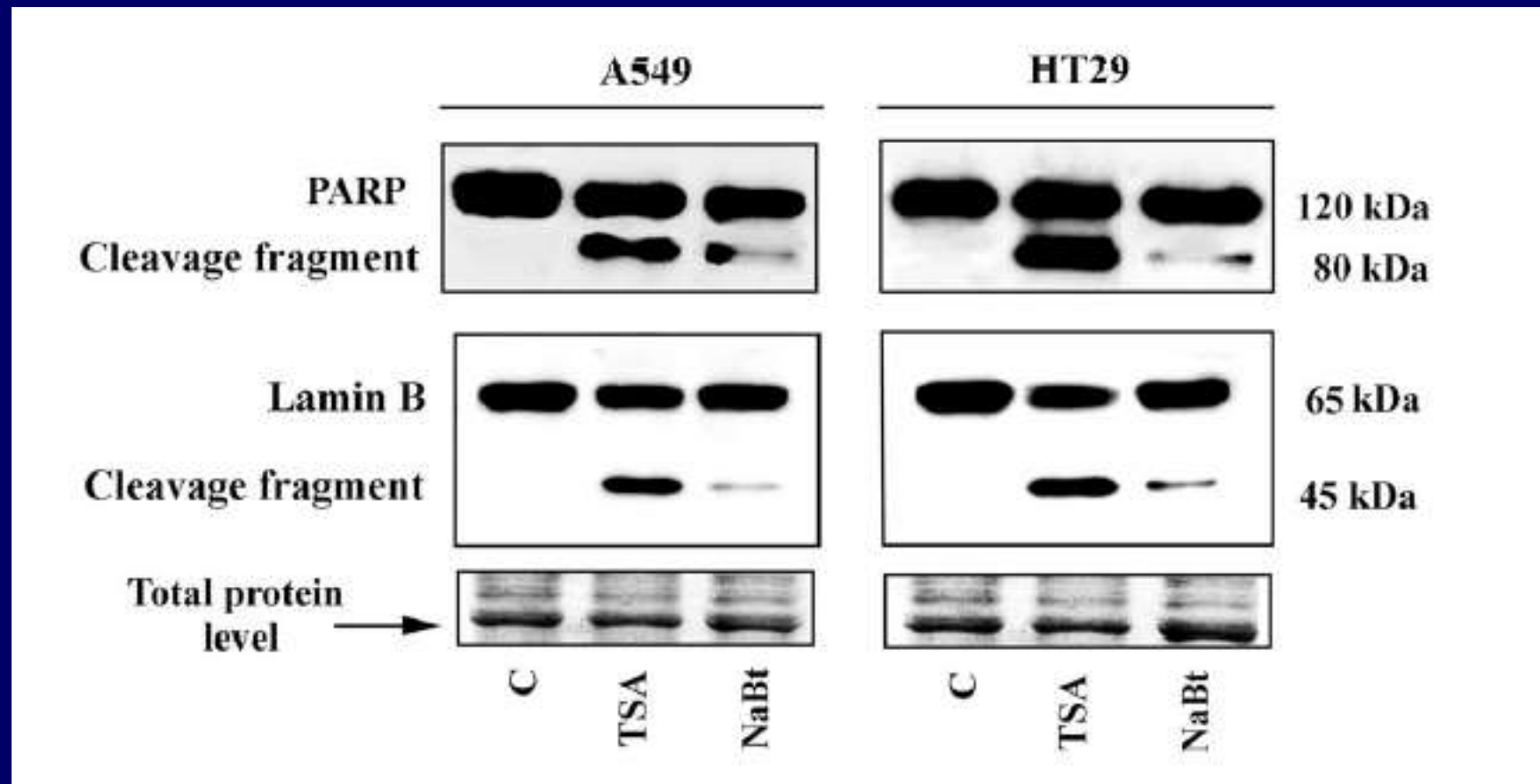


BH2

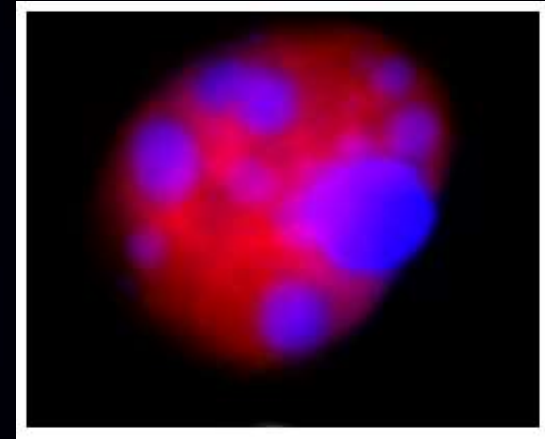
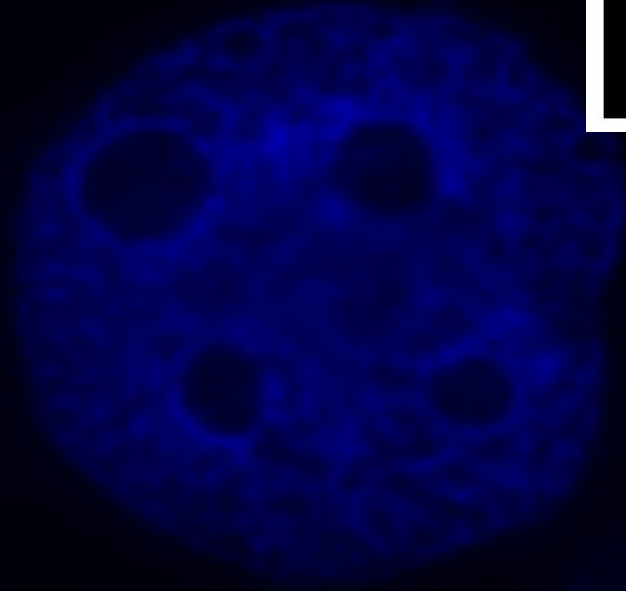
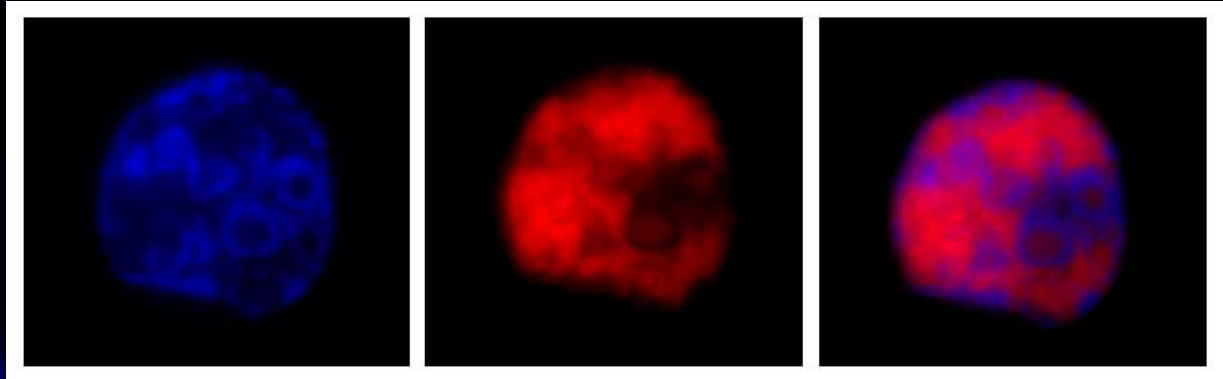
**Sigma (Apoptosis
and Life Science)**



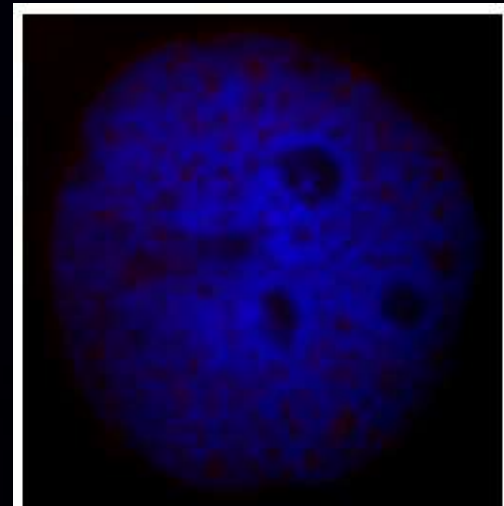
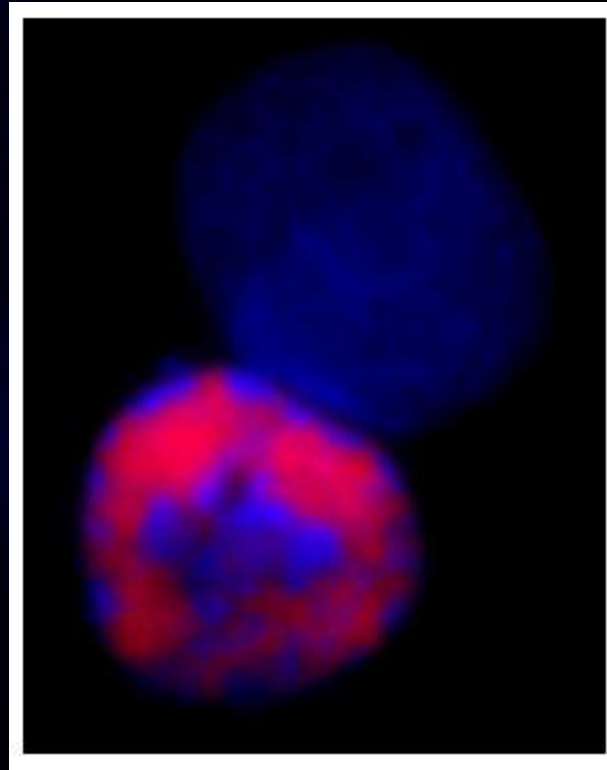


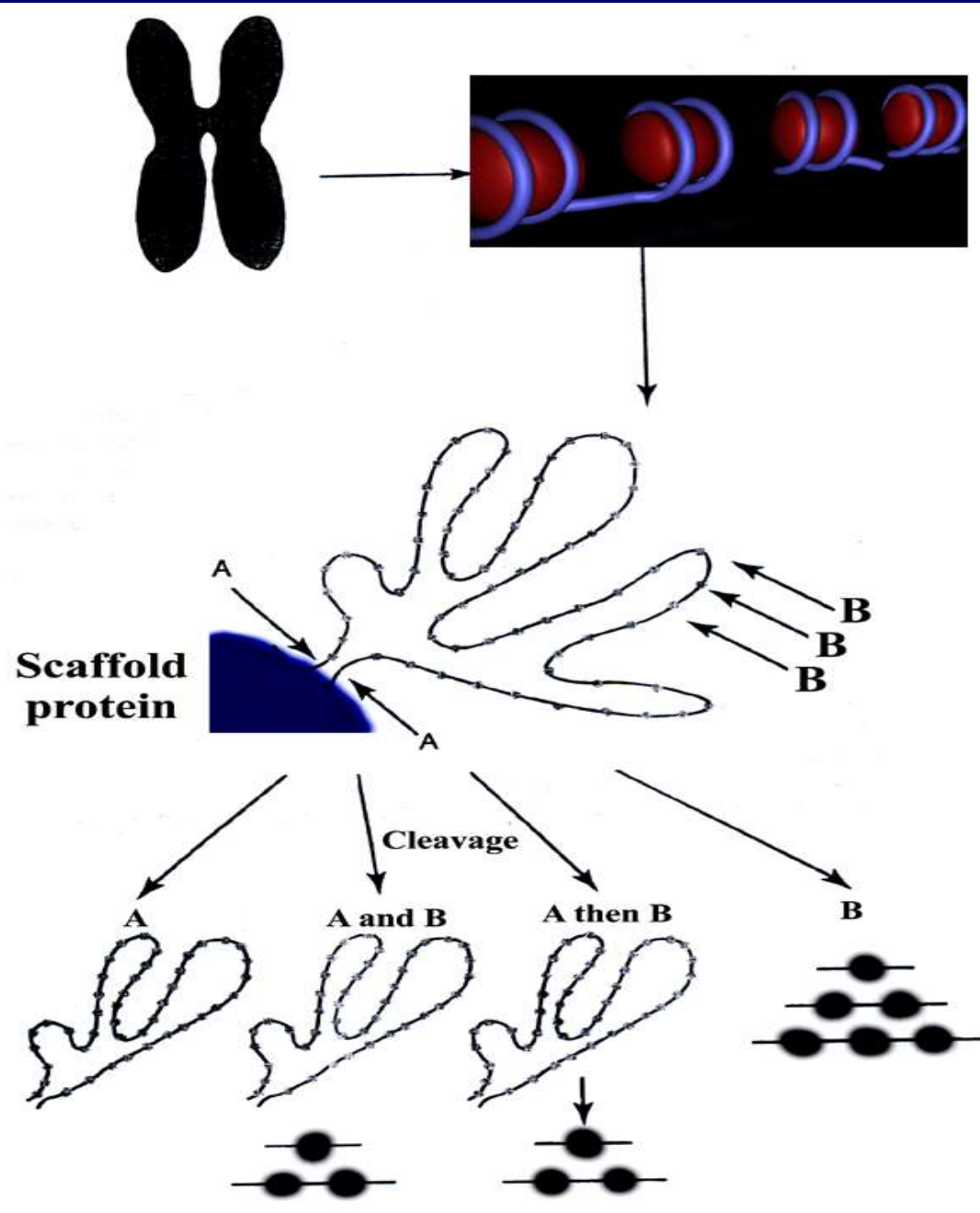


**Anti-PARP p85
fragment pAb**



**Poly(ADP-ribosylation)
and apoptosis**





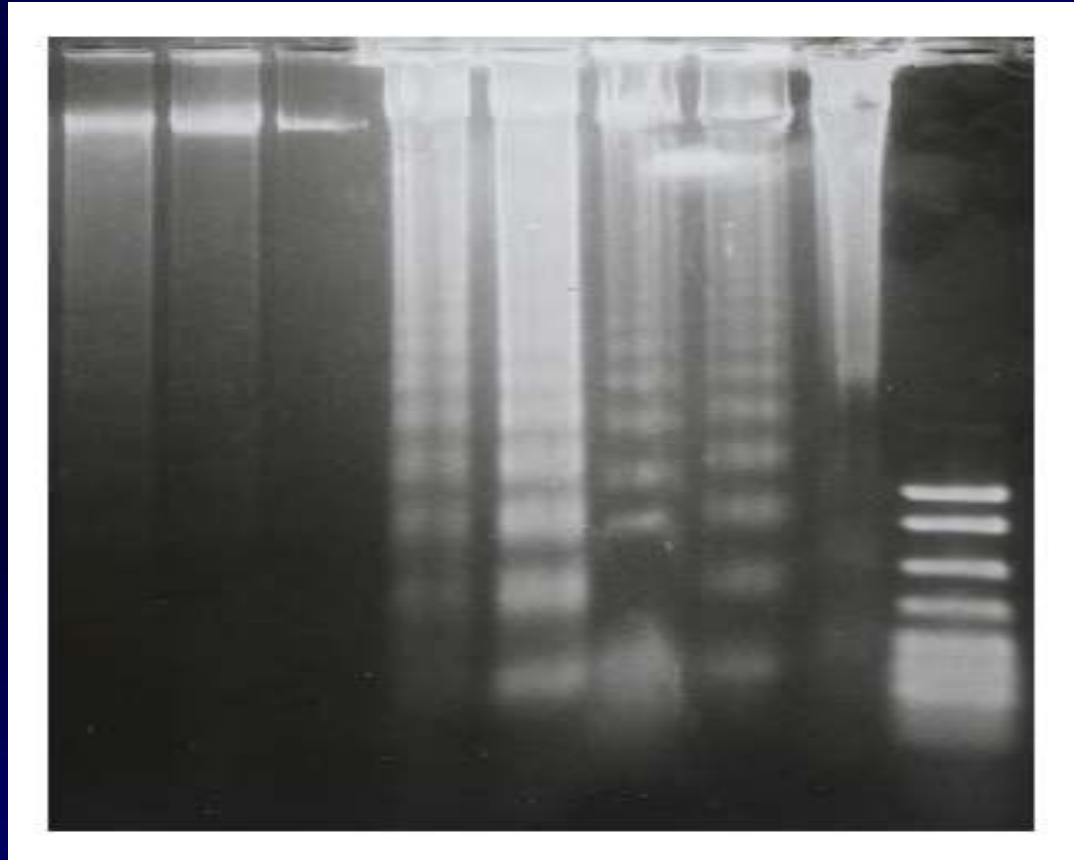
DNA fragmentation during apoptosis

1. High molecular weight DNA fragmentation (50-300 kbp)
2. Oligonucleosomal DNA fragmentation (180-200 bp)
3. Single- strand cleavage

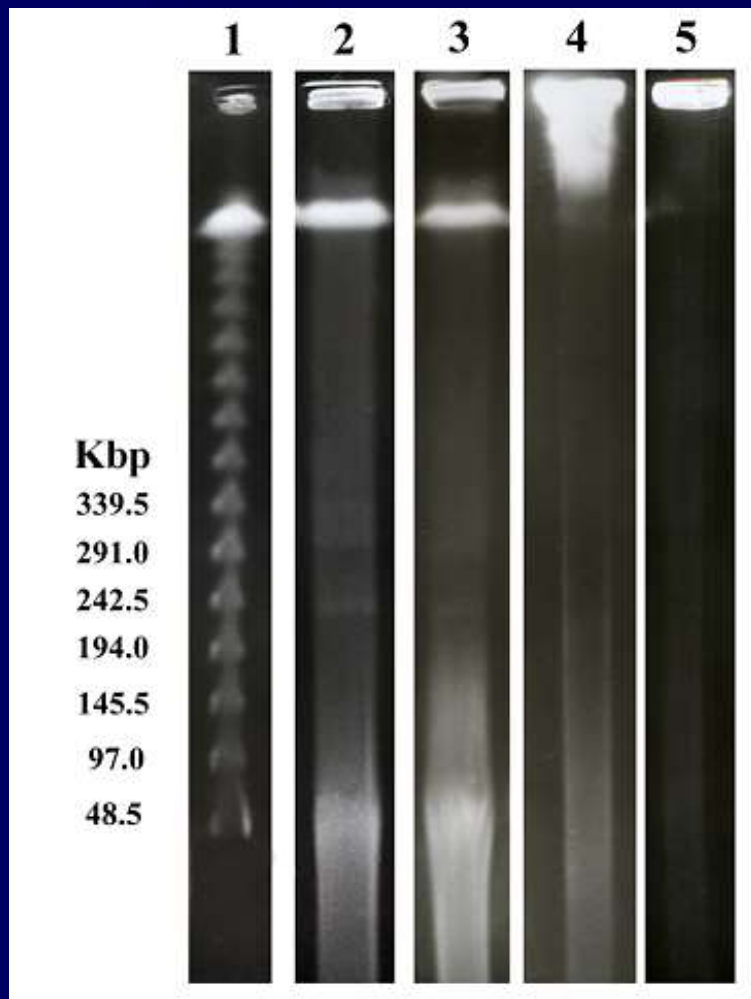
Bortner C.D. et al., 1995

APOPTOSIS DETECTION

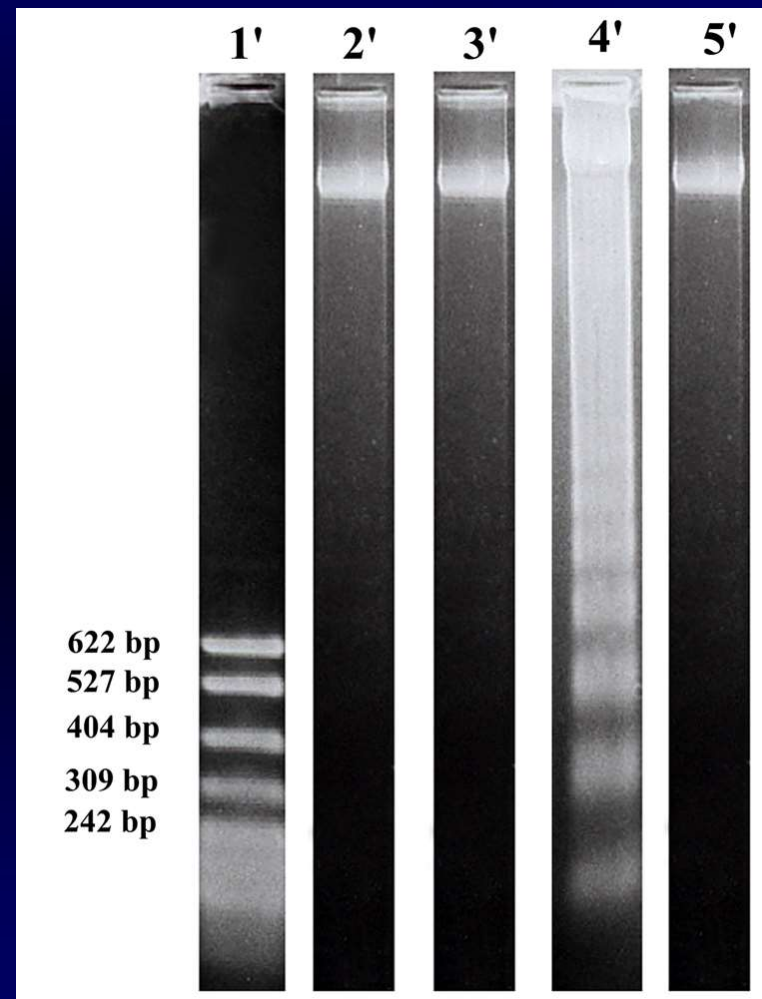
DNA fragmentation test



Large and oligonucleosomal DNA fragmentation in apoptotic cells (M. Fojtová, BFÚ Brno)



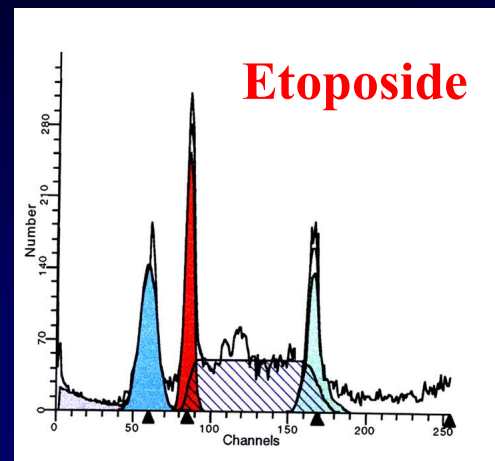
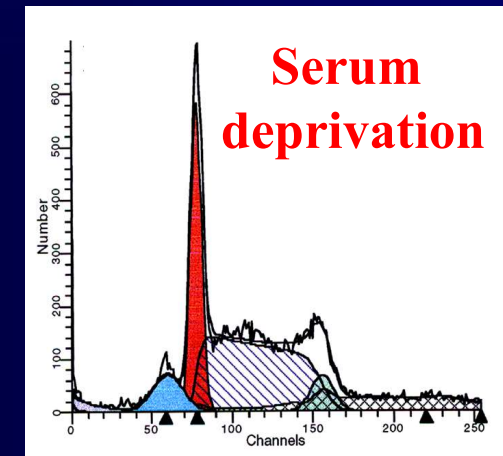
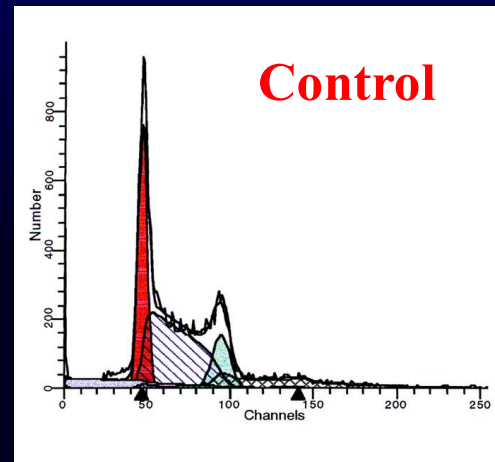
Field inversion electrophoresis (FIGE)



DNA fragmentation test

Apoptosis was detected in human erythroleukemia cell line K-562 and human retinoblastoma cell line Y79

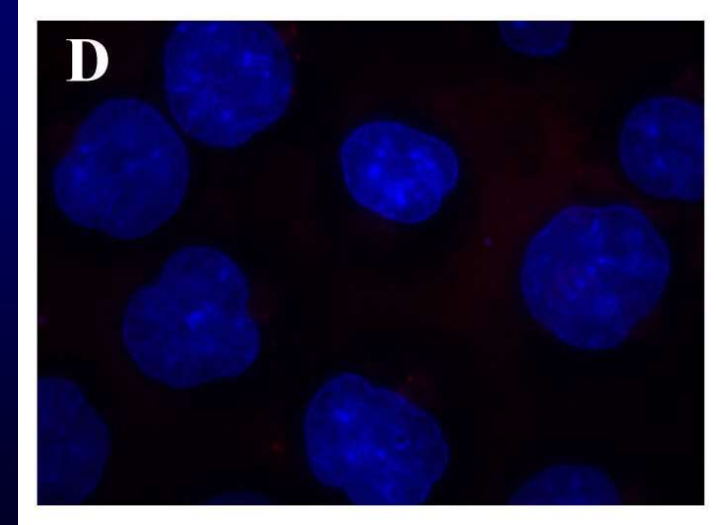
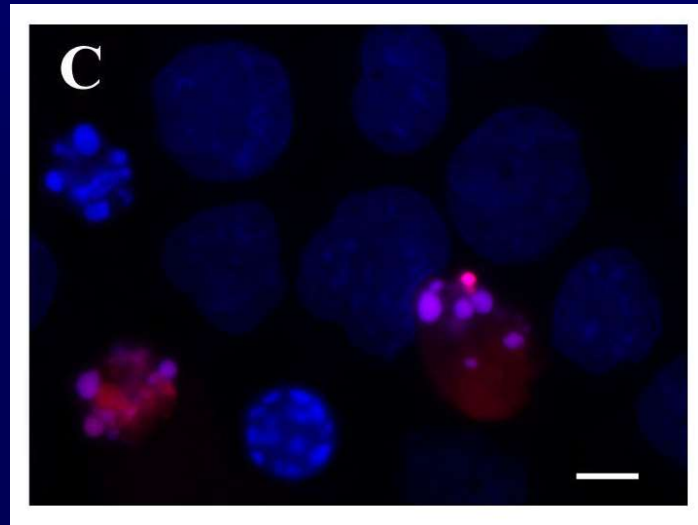
1. Etoposide
2. Cis-platin
3. Vincristine
4. Gamma-irradiation
5. Serum deprivation



Hoechst33342 / PI

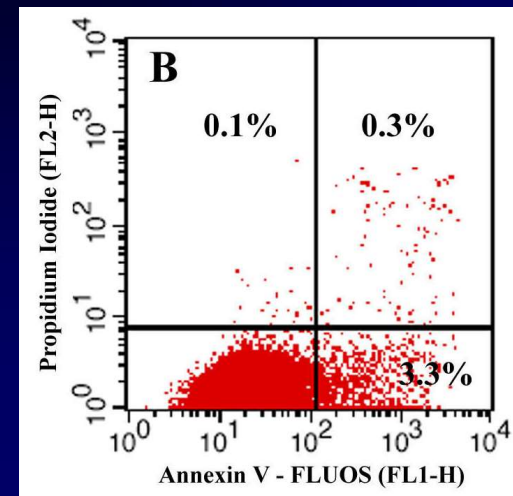
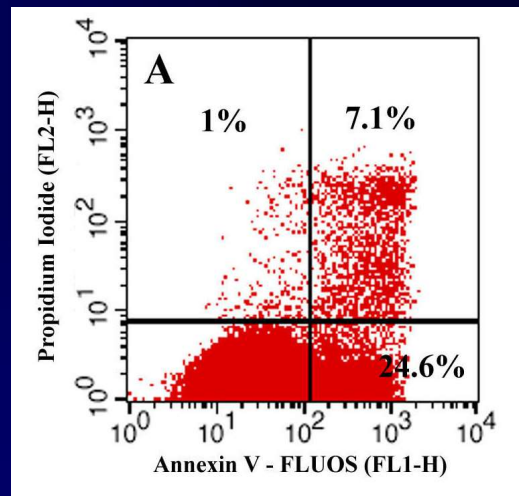
Etoposide

Control

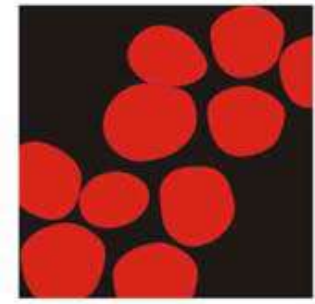
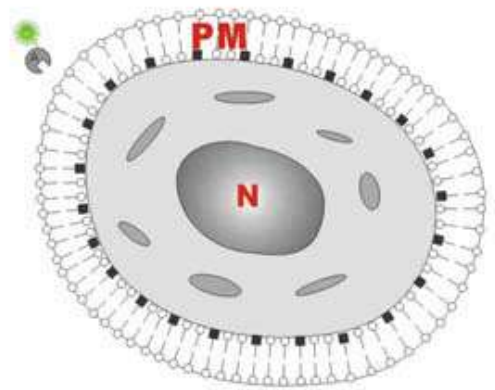


Anexin V binds to phosphatidylserines that are traslocated from the inner side od the plasma membrane to the cell surface soon after the induction of apoptosis

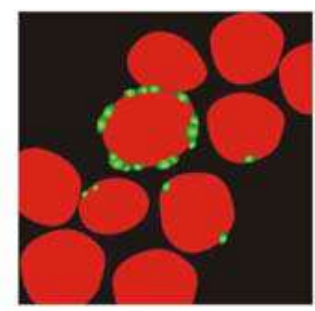
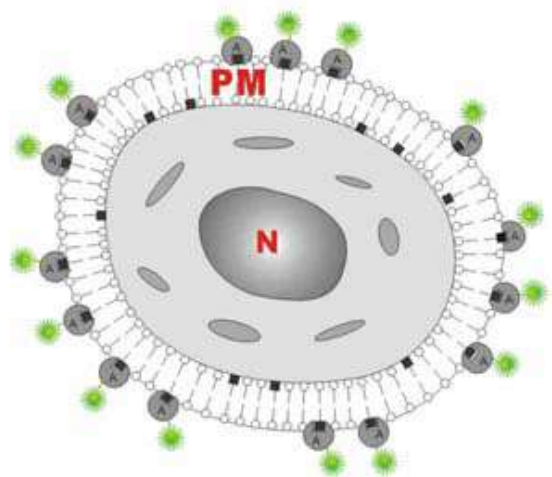
Annexin V / PI



- Phospholipid
- Phosphatidylserin
- Annexin with a green fluorescent label



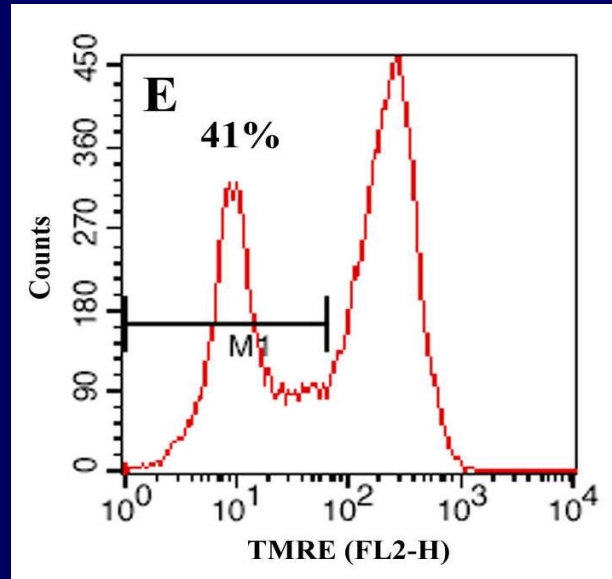
NORMAL, HEALTHY CELL



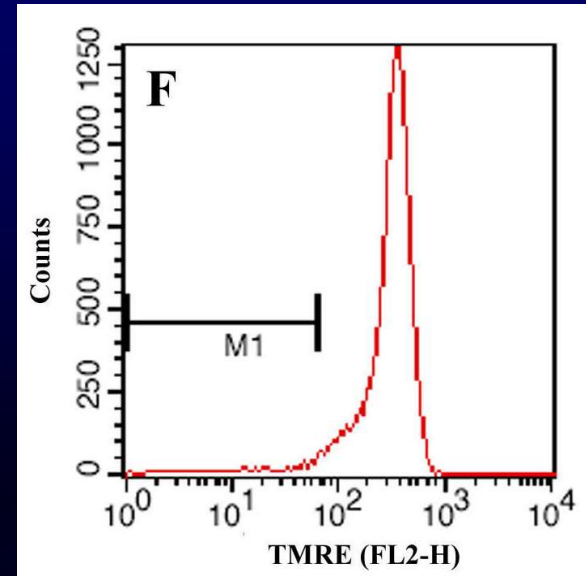
APOPTOTIC CELL

TMRE

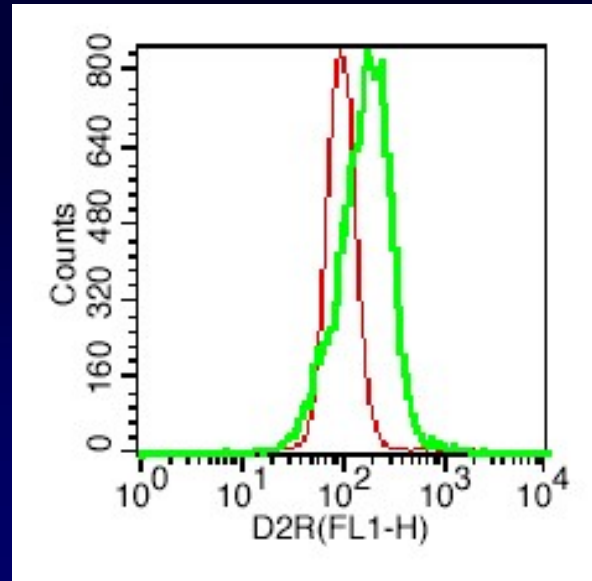
Etoposide

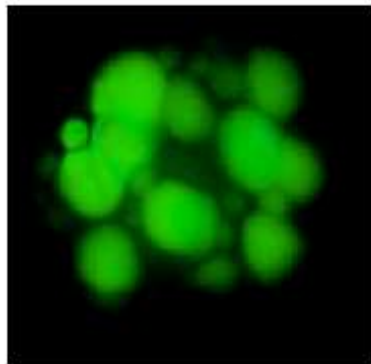
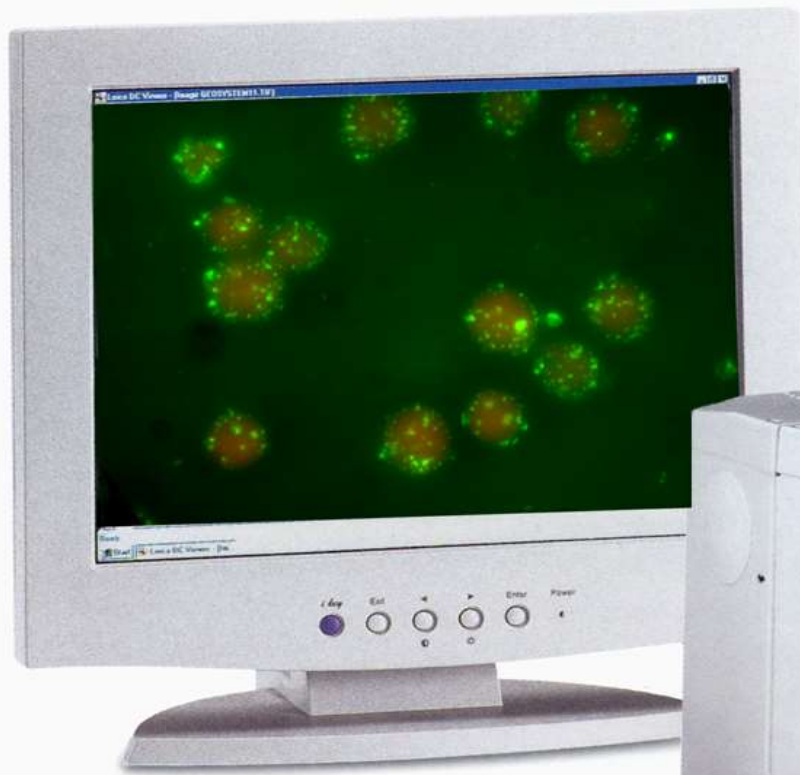


Control



**CaspSCREEN (tm)
BioVision kit**

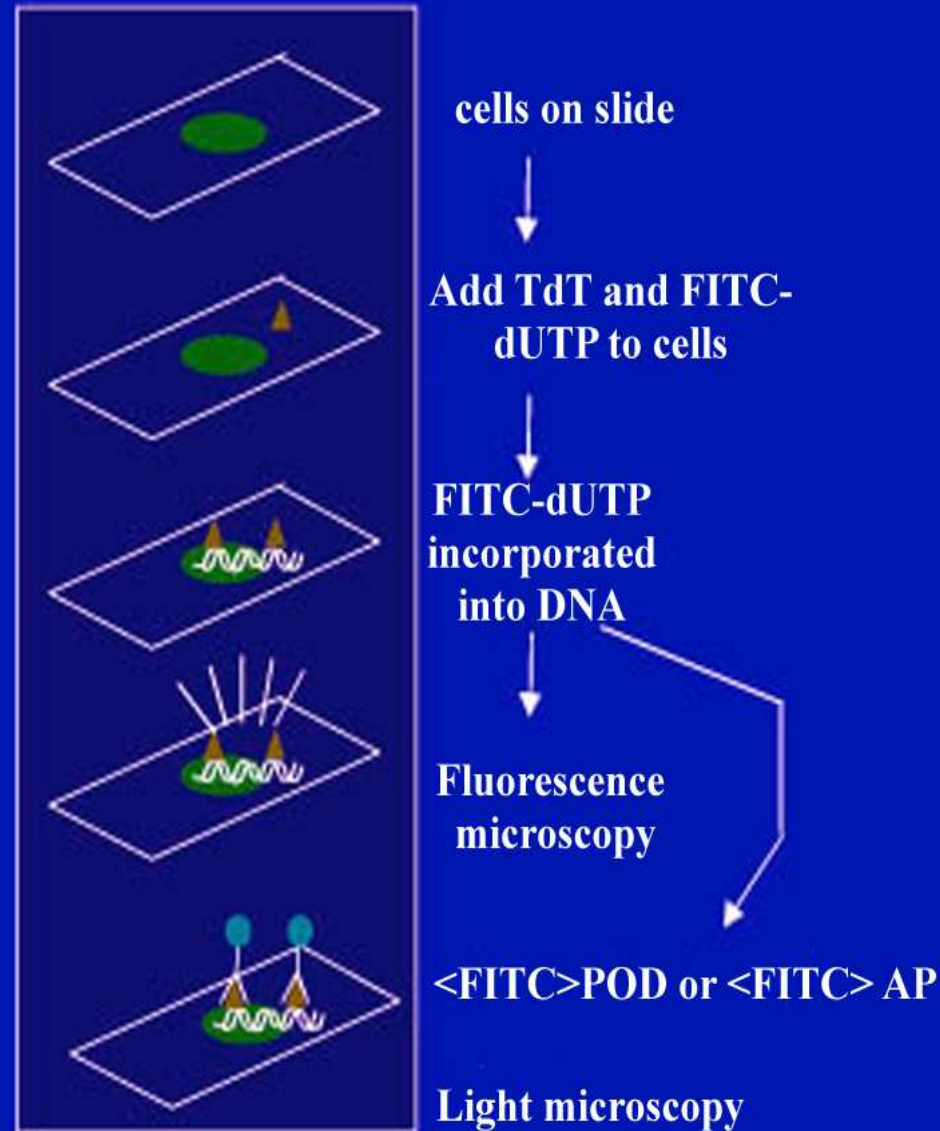


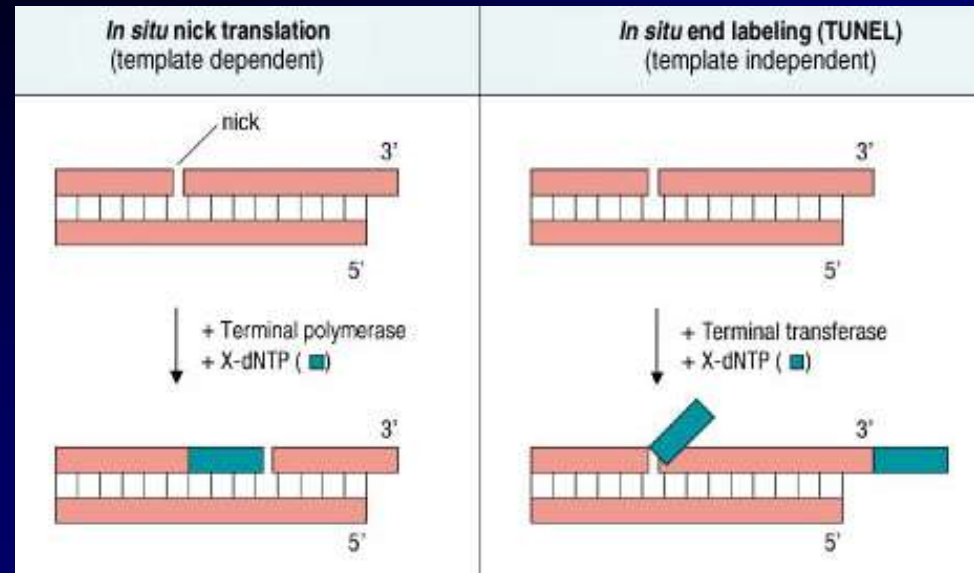
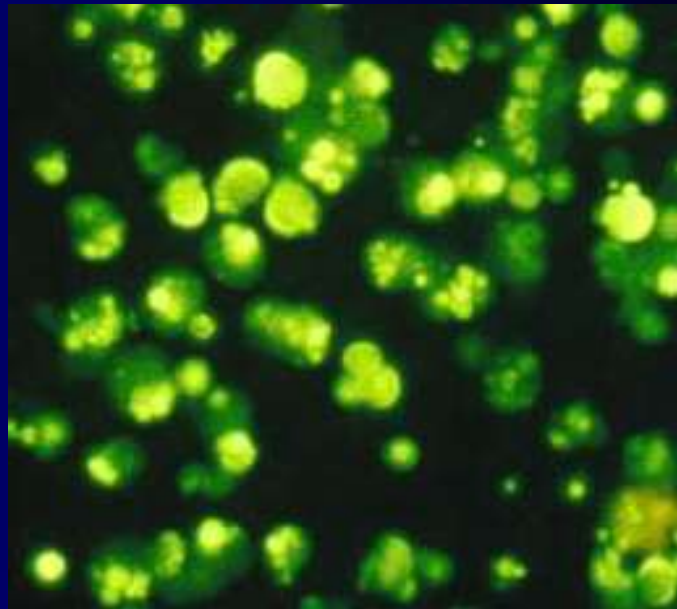
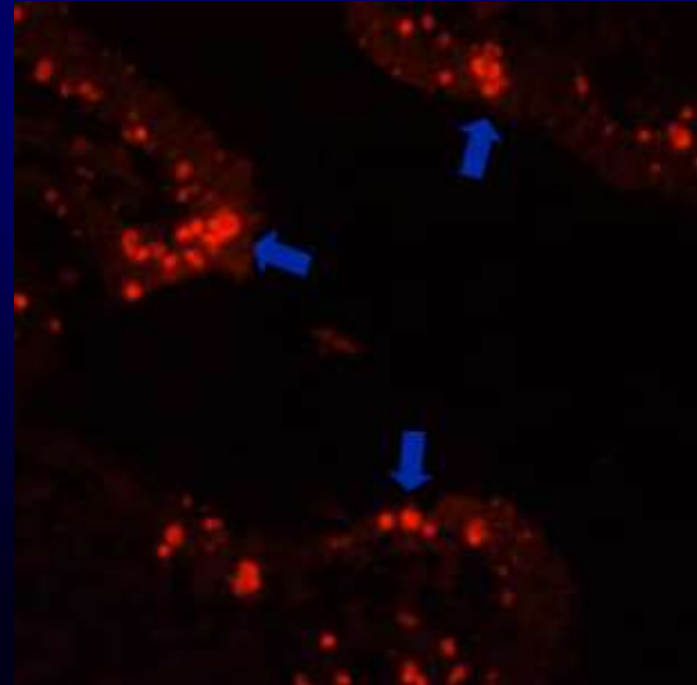
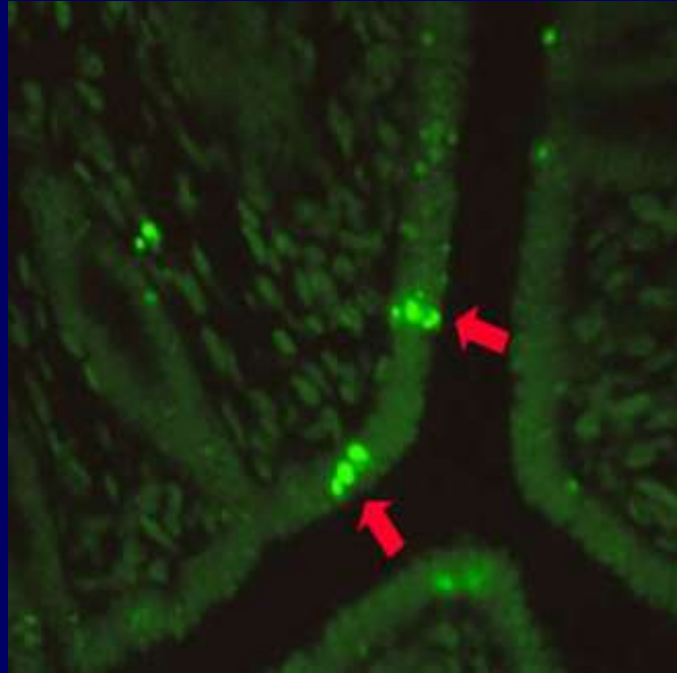


DNA Fragmentation - TUNEL

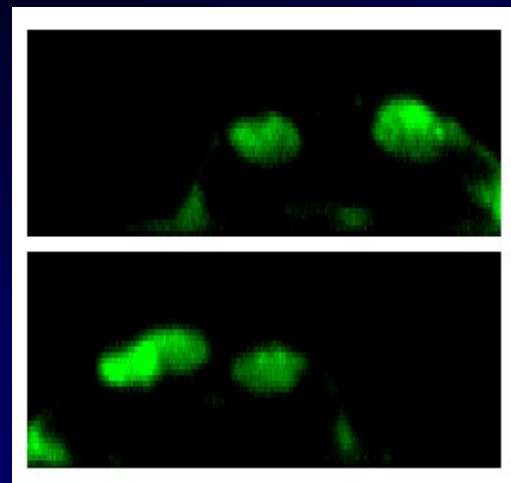
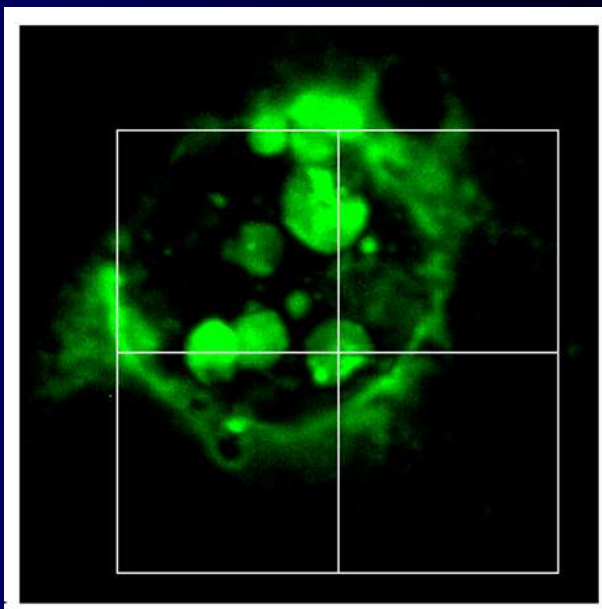
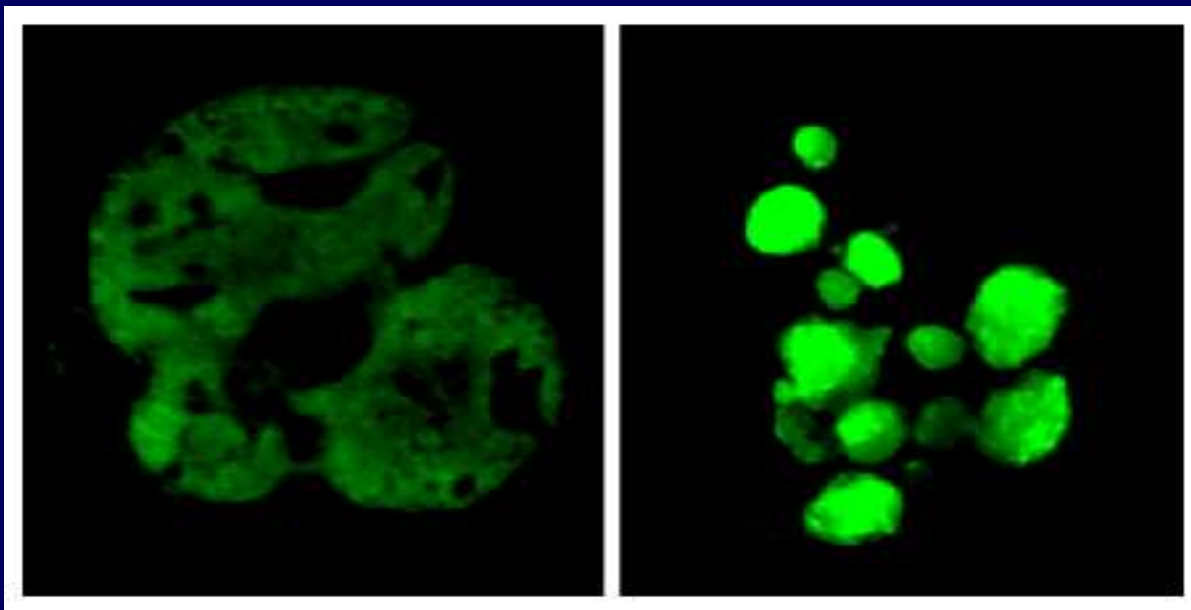


In Situ Cell Death Detection Kit - Test Principle

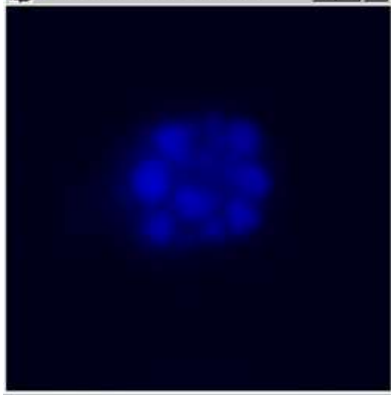




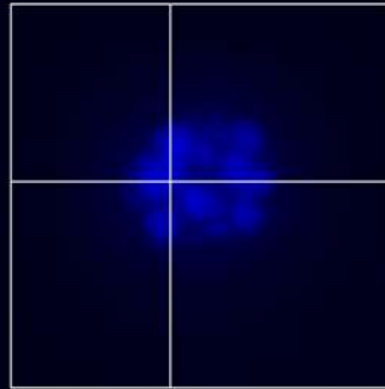
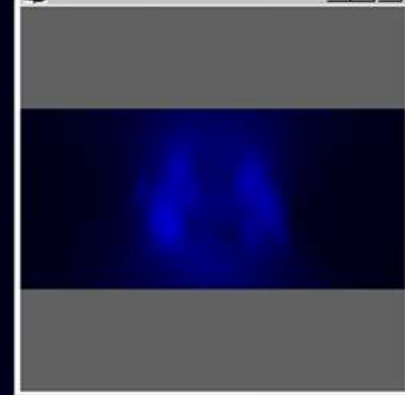
The results of TUNEL test



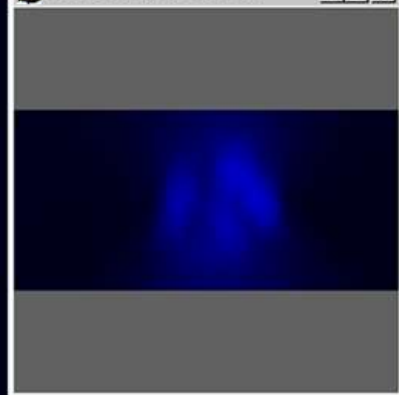
R:000 G:000 B:018 X:161 Y:320



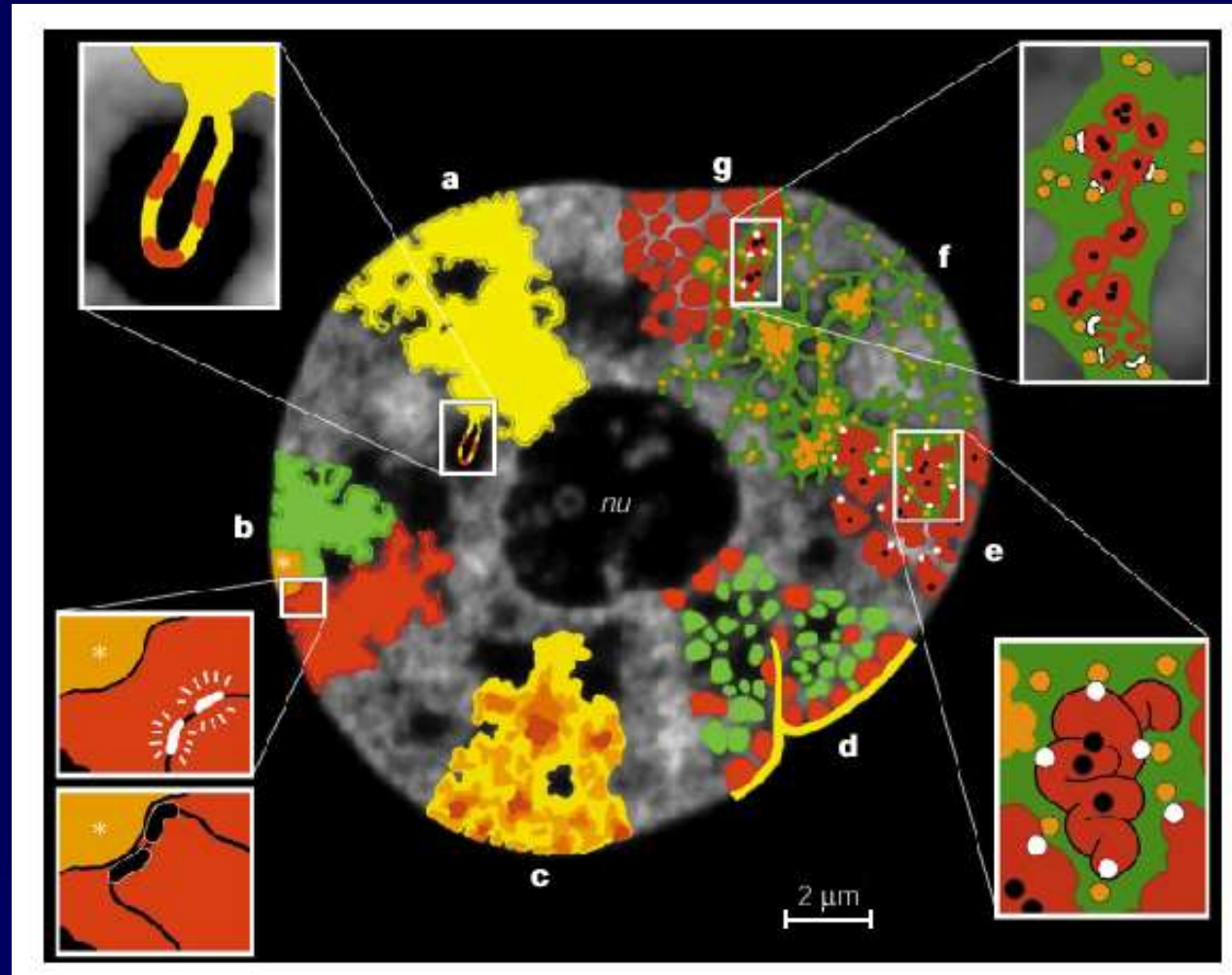
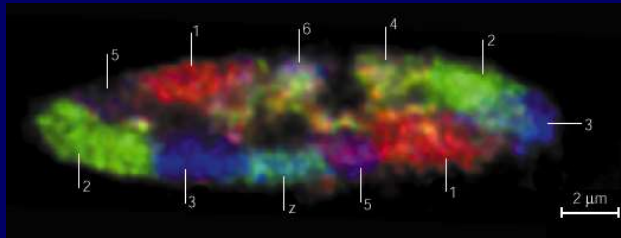
R:000 G:000 B:017 X:138 Y:10



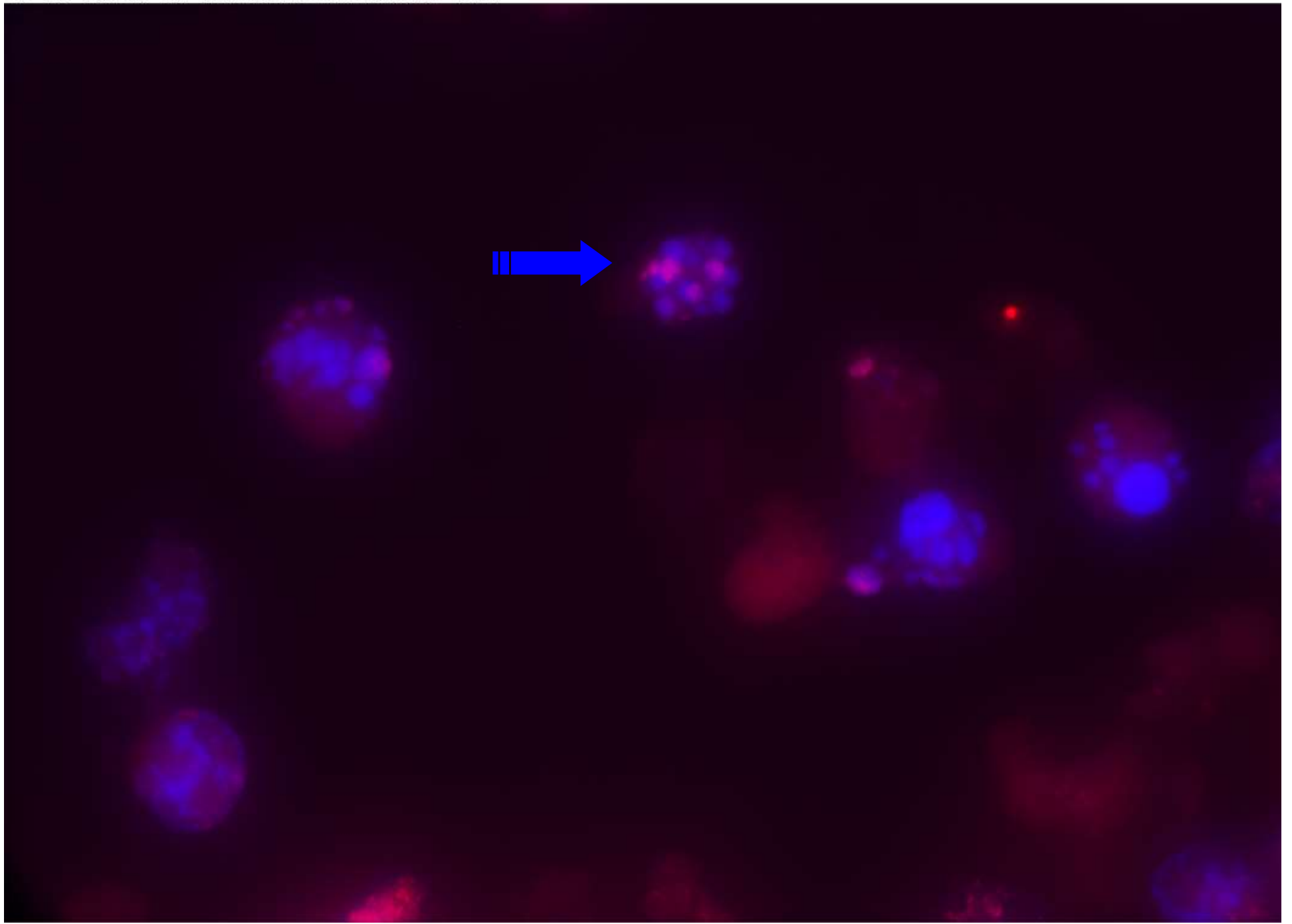
R:000 G:000 B:018 X:369 Y:19

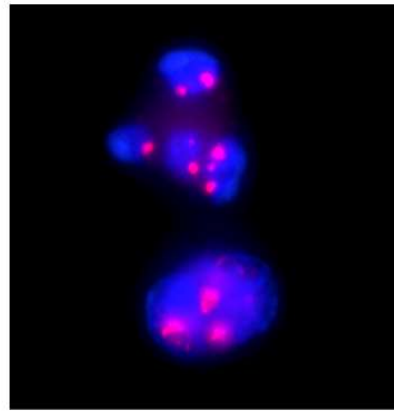
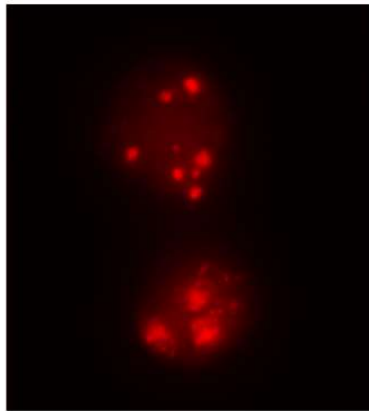
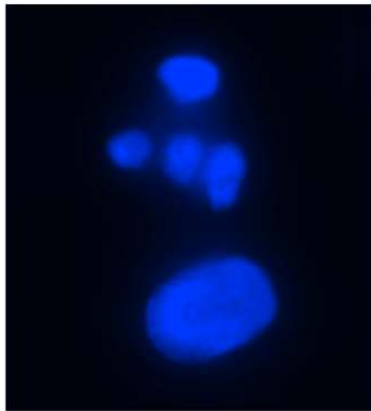


Nuclear organisation of chromosomal territories

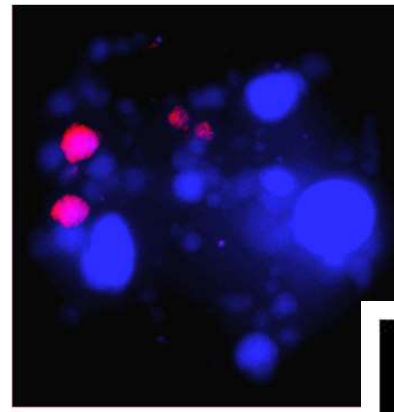
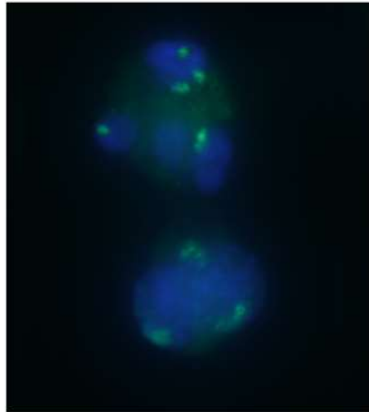
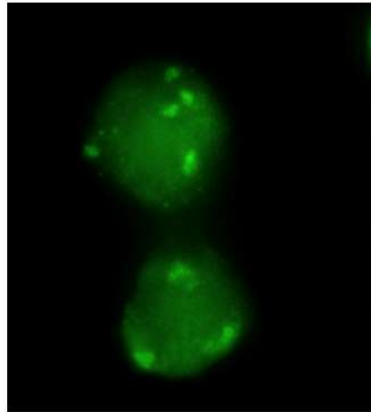


(Cremer T. and Cremer C., 2001)

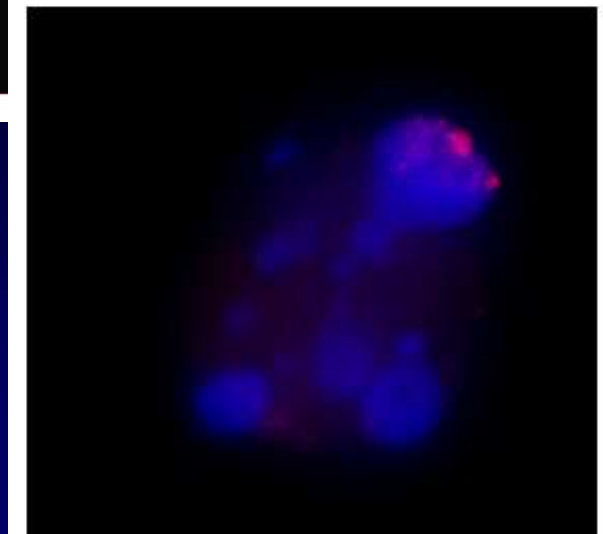




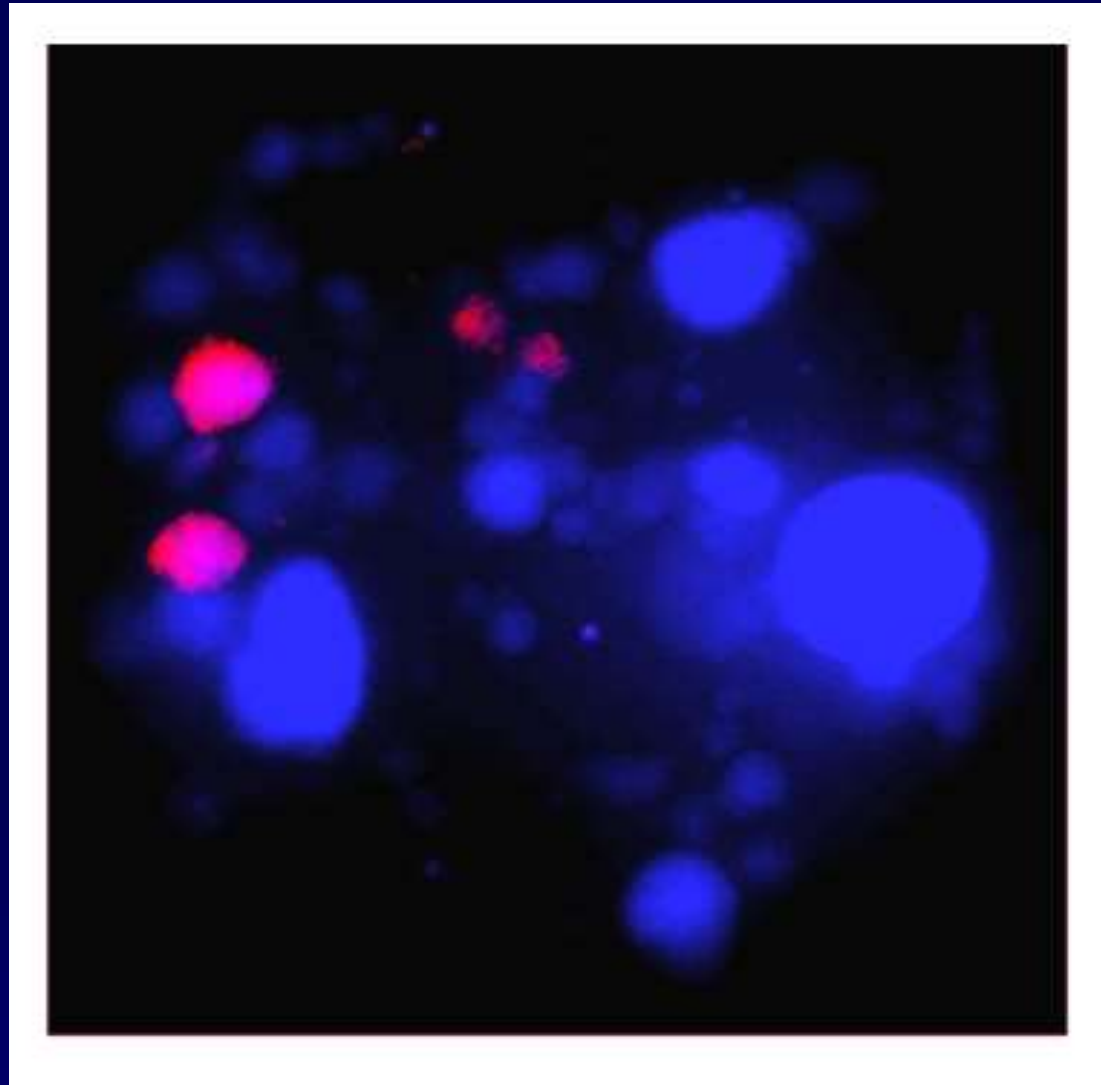
Territory of chromosome
11 and **17**



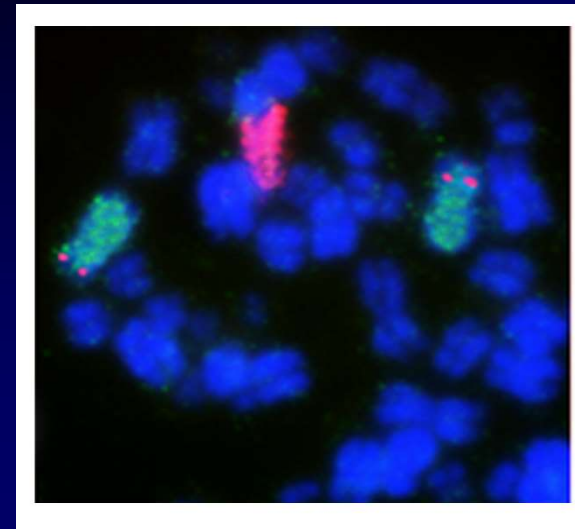
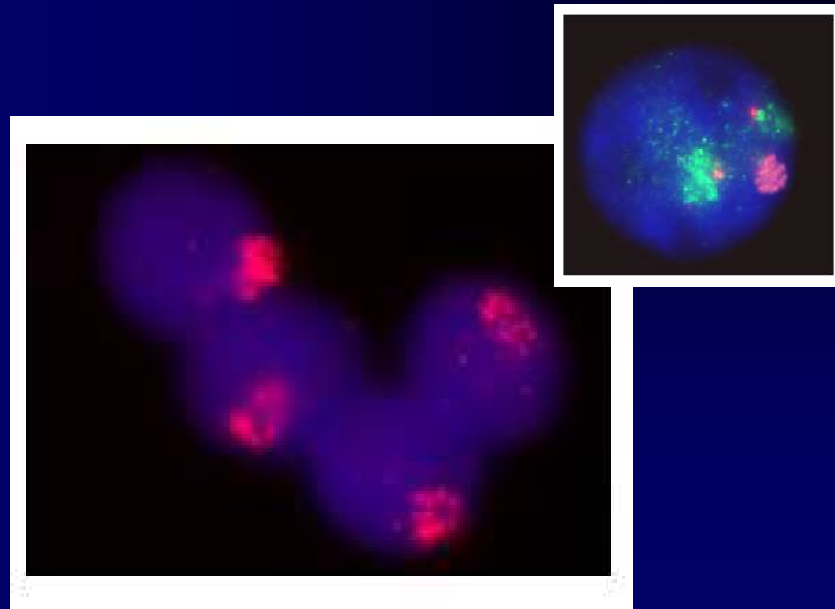
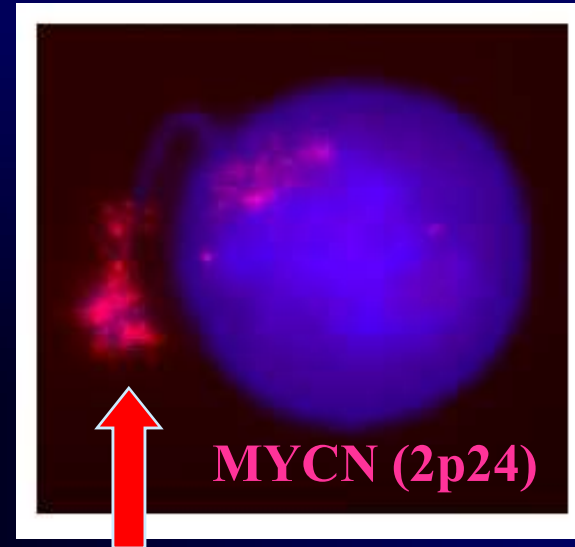
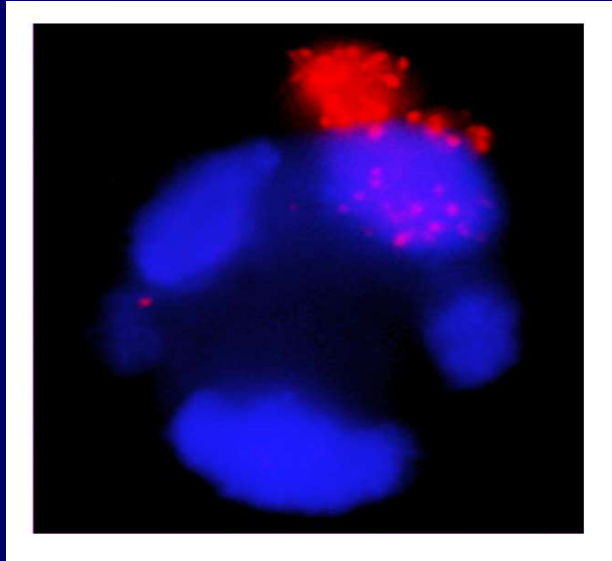
Territory of chromosome **3**



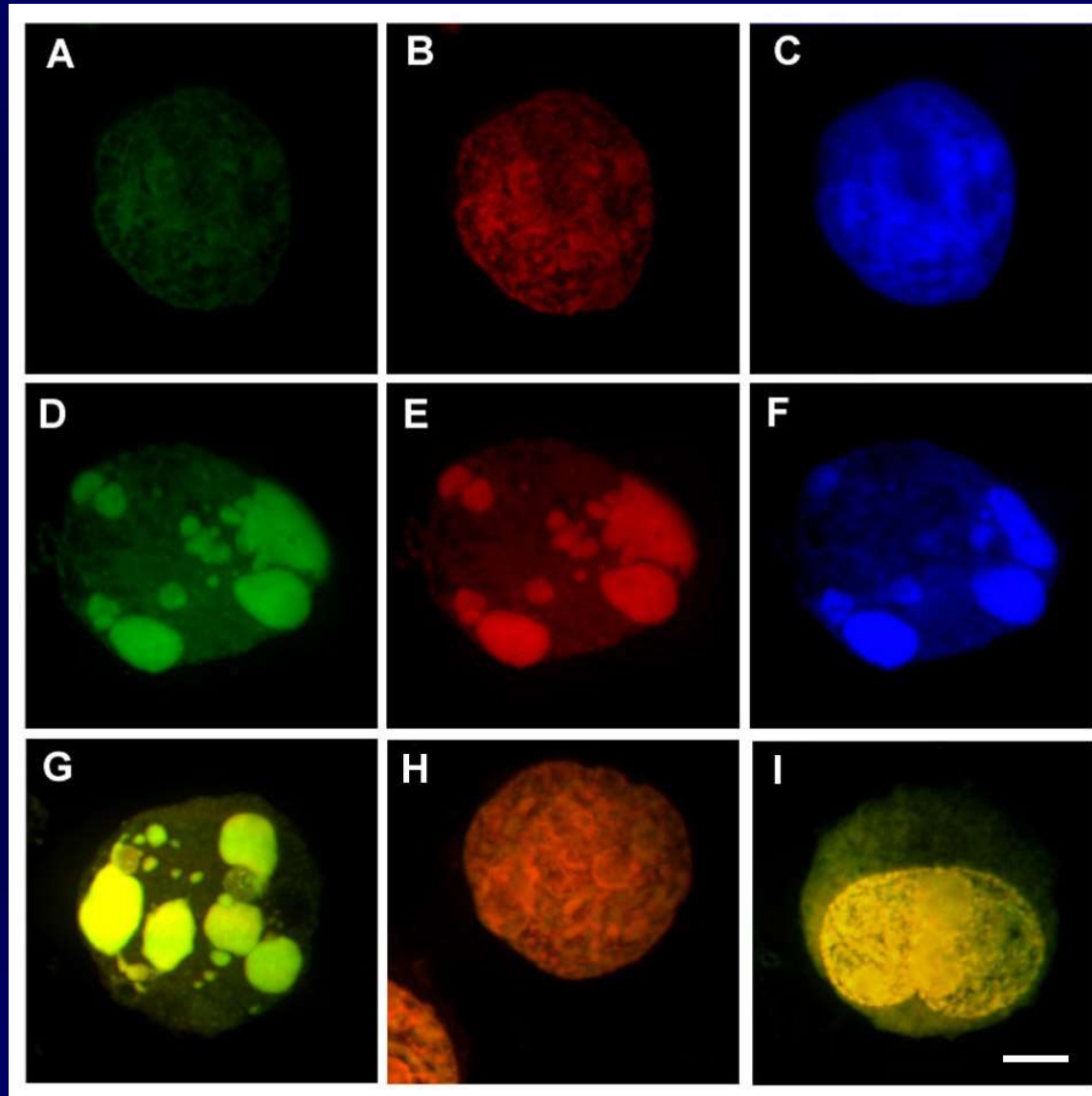
Arcitecture of chromosomal territories during apoptosis



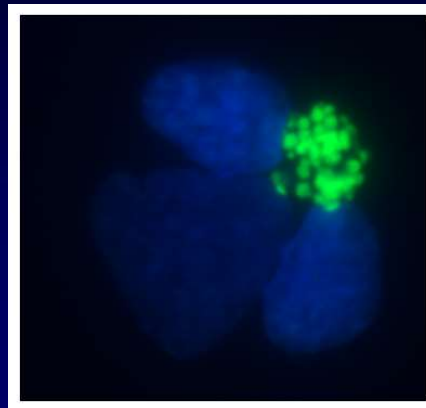
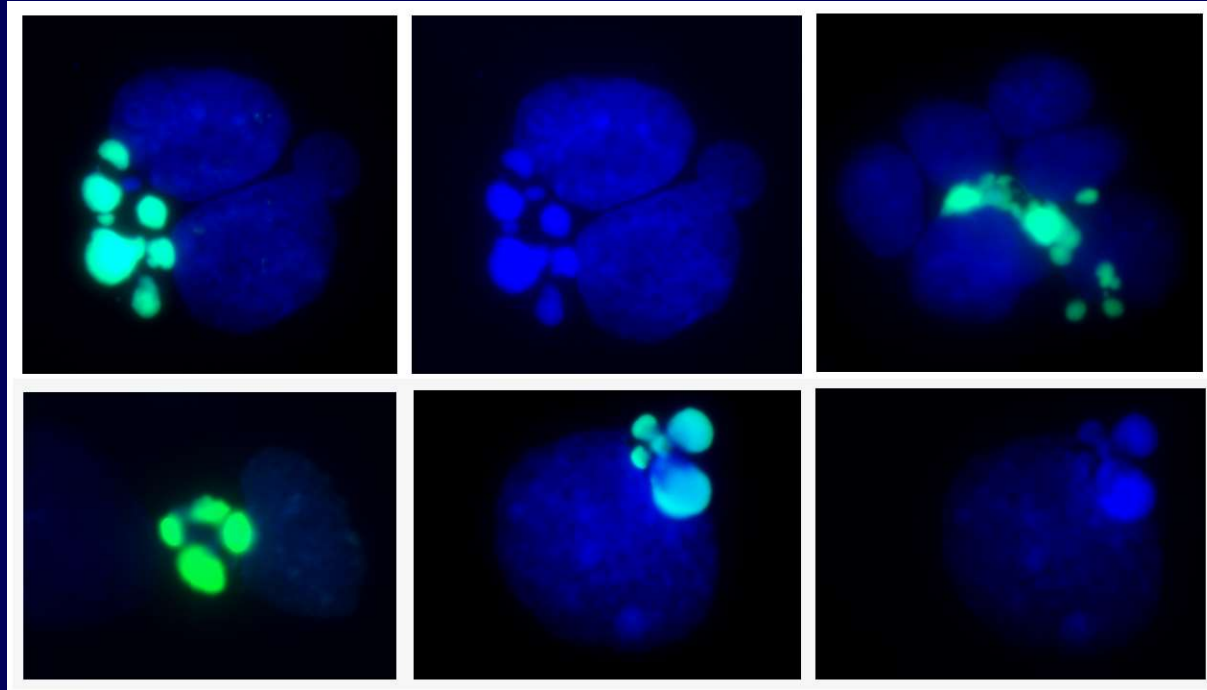
Retinoblastoma Y79 cells and HSR



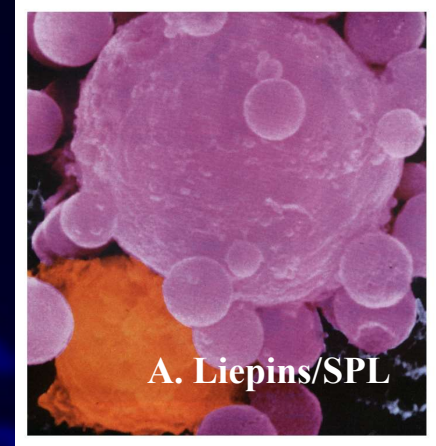
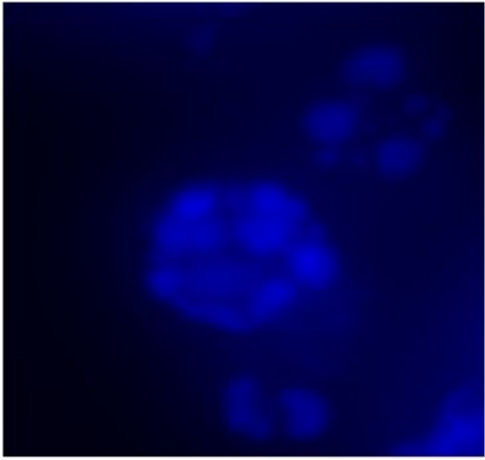
TUNEL and PI staining of fixed cells



Apoptosis in patient suffering from retinoblastoma TUNEL and DAPI staining



Conclusions



A. Liepins/SPL

- * Differences in DNA fragmentation
- * Differences in the number of nuclear apoptotic bodies
- * Chromosomal territories cleaved into high molecular DNA fragments were variably disassembled into apoptotic bodies whose induction is the main effort of anticancer therapy.
- * Apoptotic nuclear segmentation can be observed at centromeric regions.
- * Disassembly of chromosomal territories was also found in pre-apoptotic (TUNEL positive) nuclei.
- * Apoptosis can be observed not only after experimental and/or clinical treatment but also spontaneously.