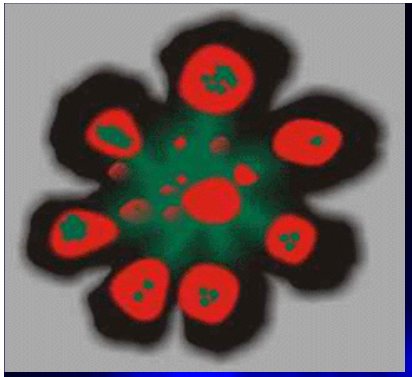
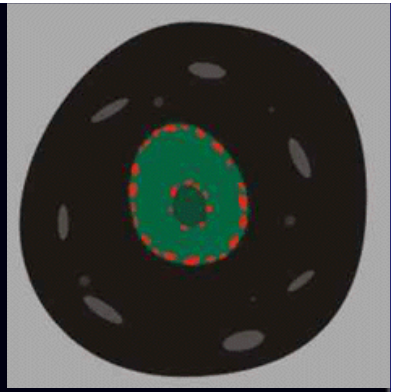
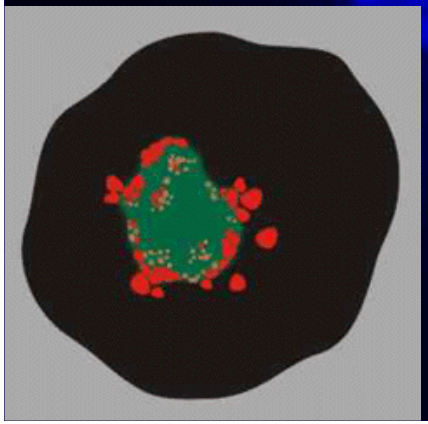


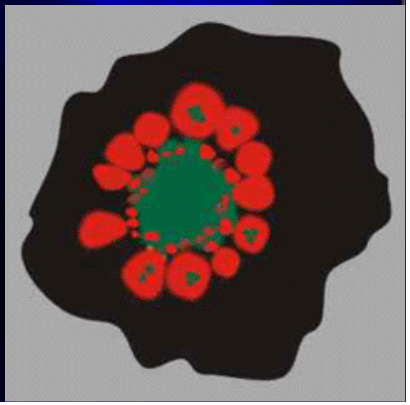


Apoptosis: Architecture of Chromosomal Territories in Apoptotic Cells

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 pehenomenon with wide-ranging implications in tissue kinestics. ***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-1β converting enzyme)] protease

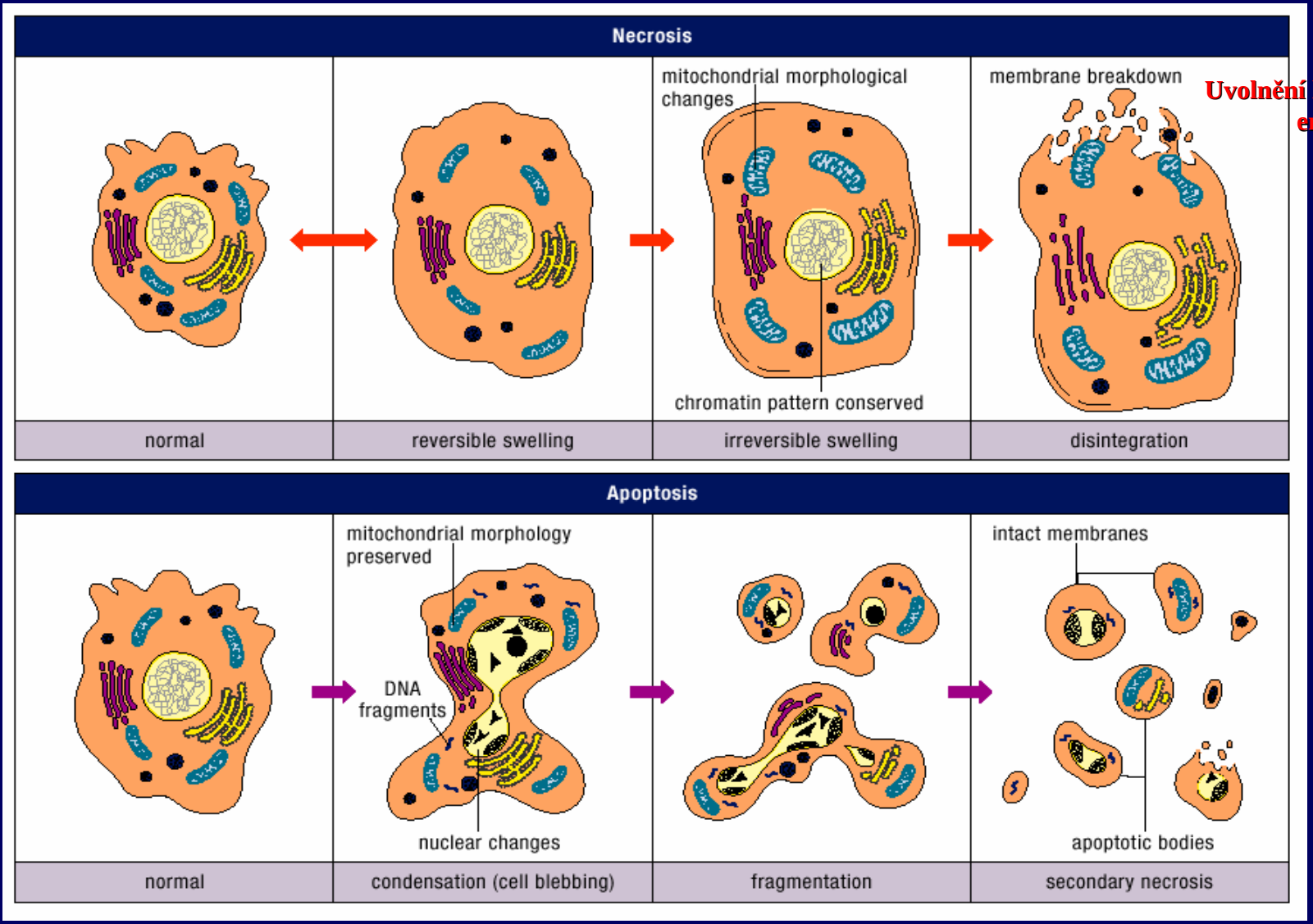
Discovery of new family of mammalian cysteine proteases - CASPASES

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

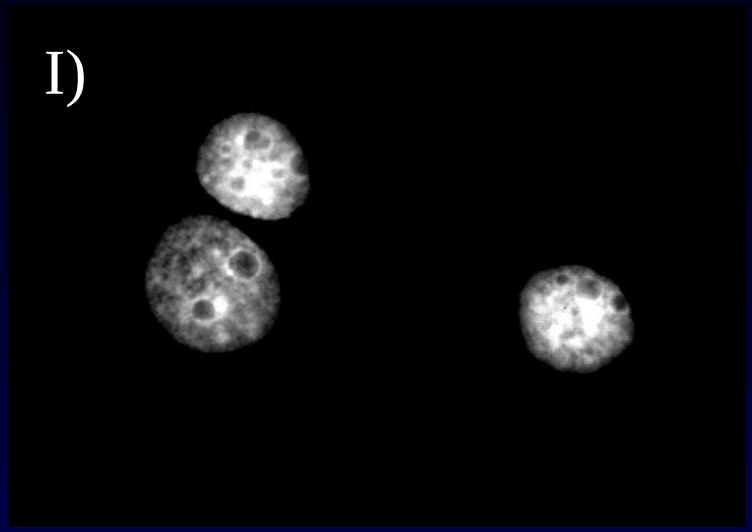


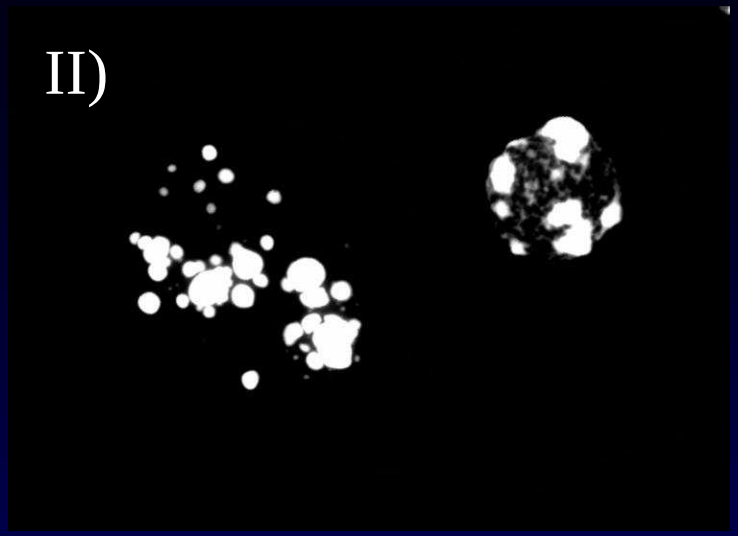
Uvolnění lysosomálních enzymů

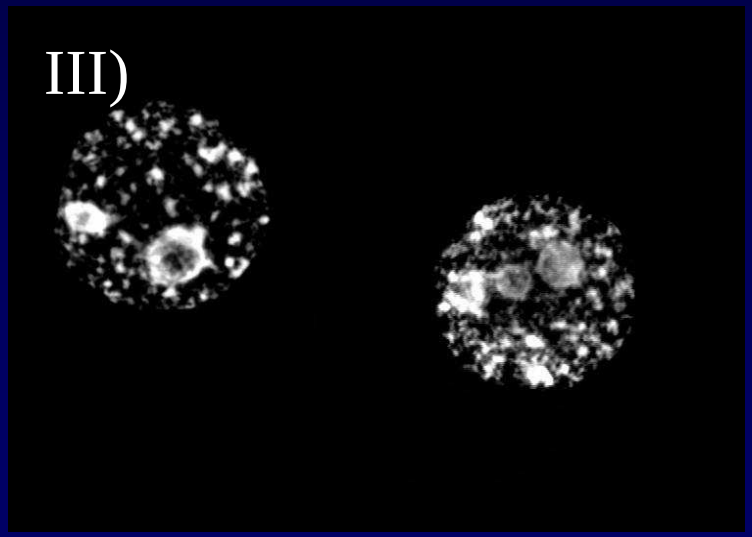
Roche: Cell Death - Apoptosis and Necrosis

Nuclear morfology in HL-60 cells

(P. Mlejnek 2001)







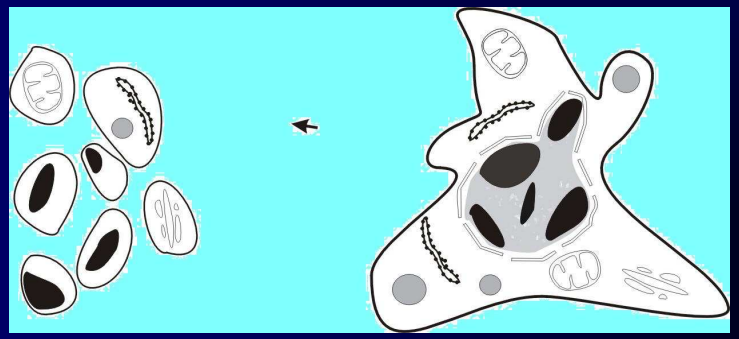
I) Control

II) Apoptosis

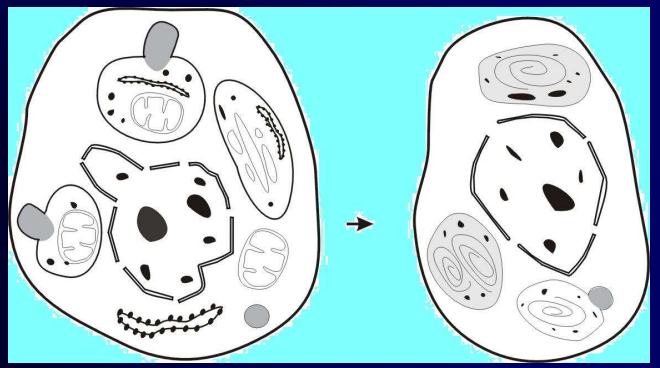
III) Necrosis

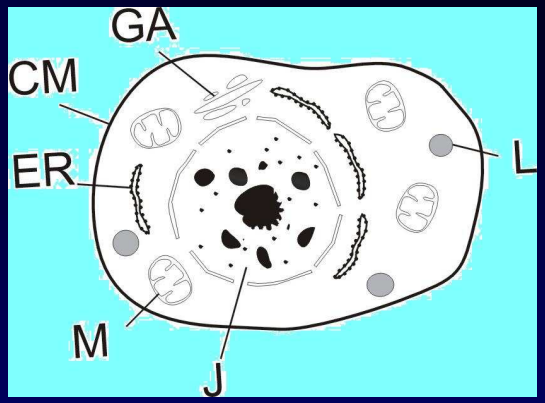
Cell death classification by Clarke

Apoptosis

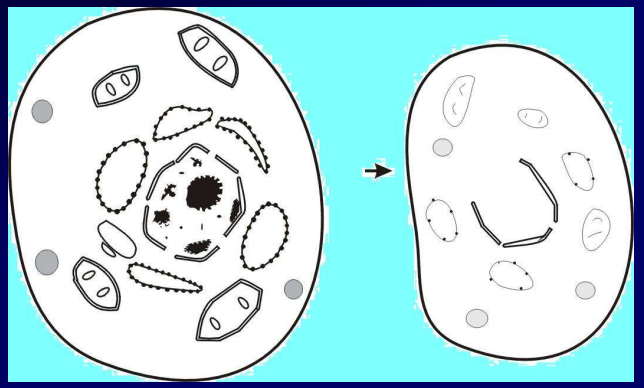


Autophagy





Nelysosomal disintegration



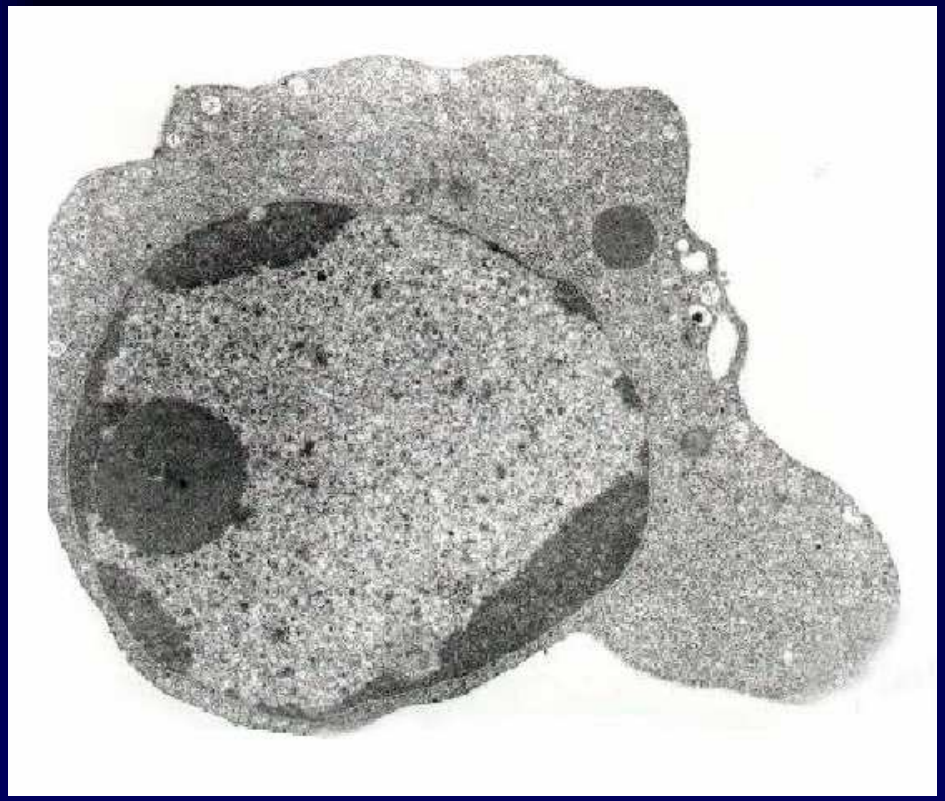
CM – cyt. membrane
J – nuclei
M – mitochondrion
ER – endopl. reticulum
GA – Golgy complex
L – lysosomes

Cell death classification by Clarke

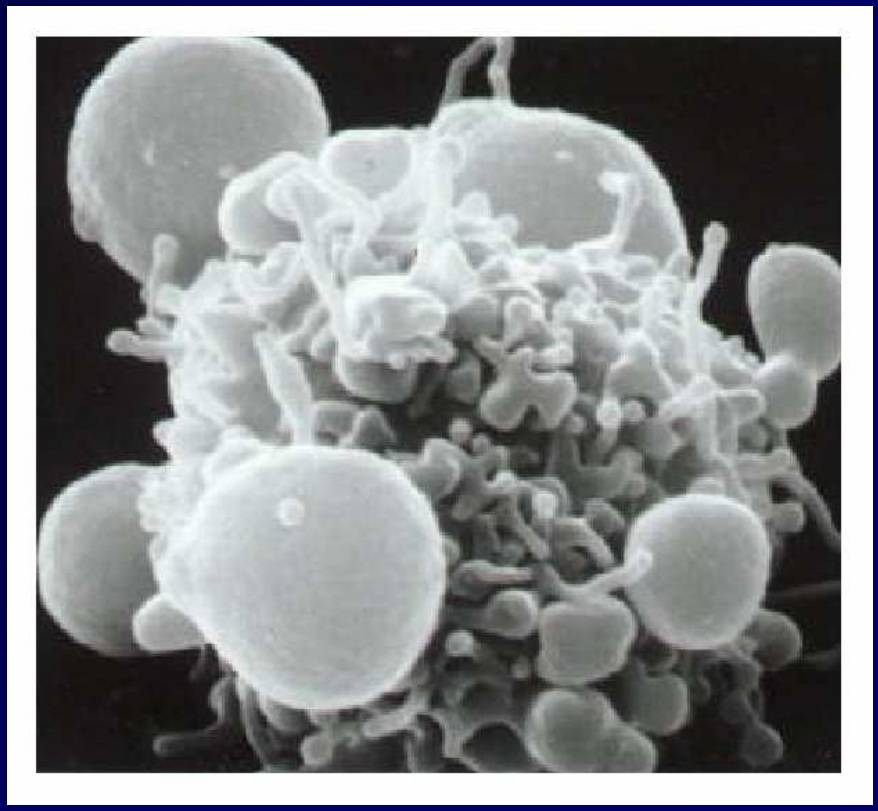
- Apoptosis - heterophagy, final cell destruction is done by lysosomes of other cells
- Autophagy - final cell destruction is done by its own lysosomes
- Nonlysosomal disintegration - cell destruction is mediated by unknown nonlysosomal proteases

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



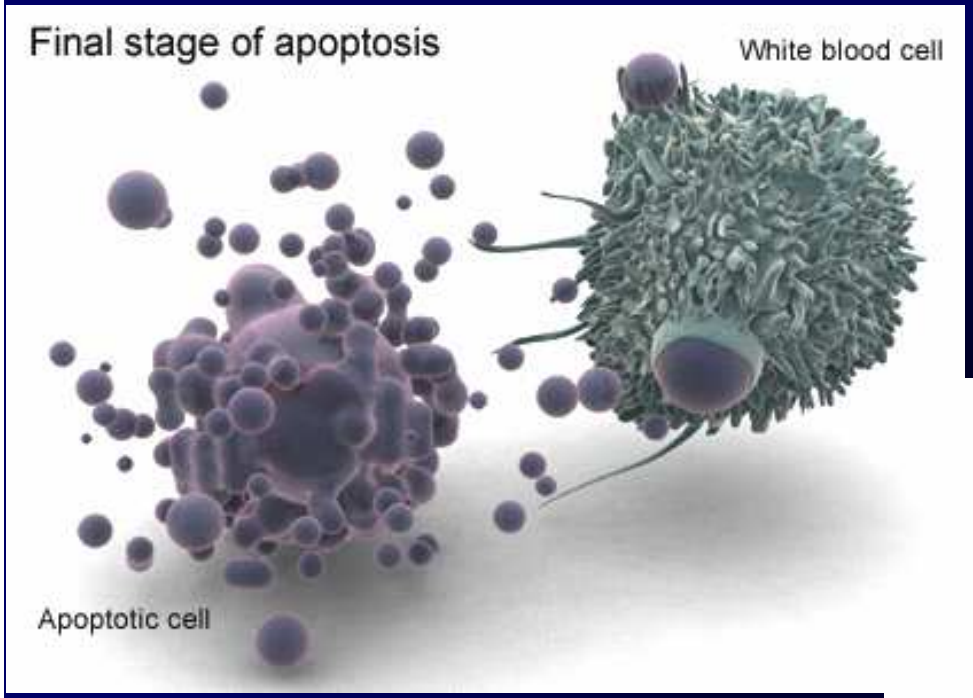
Transmission electron micrograph



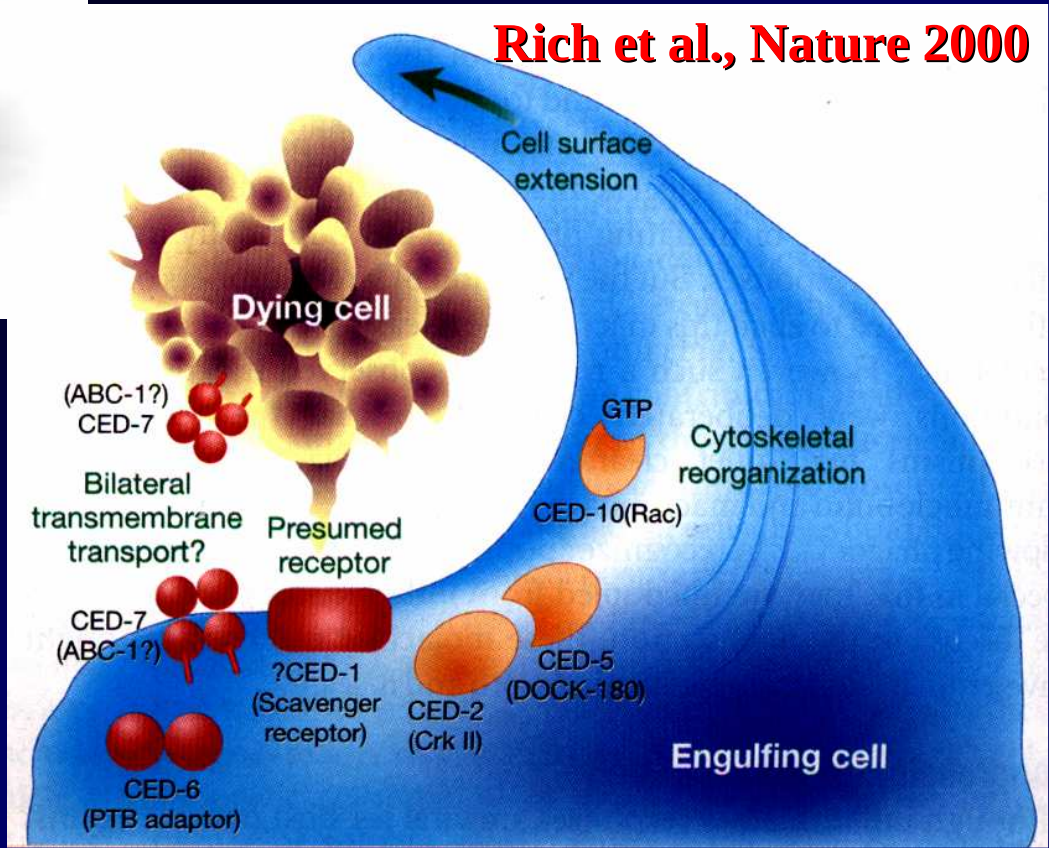
Scanning electron micrograph

C-Knudson@uniowa.edu

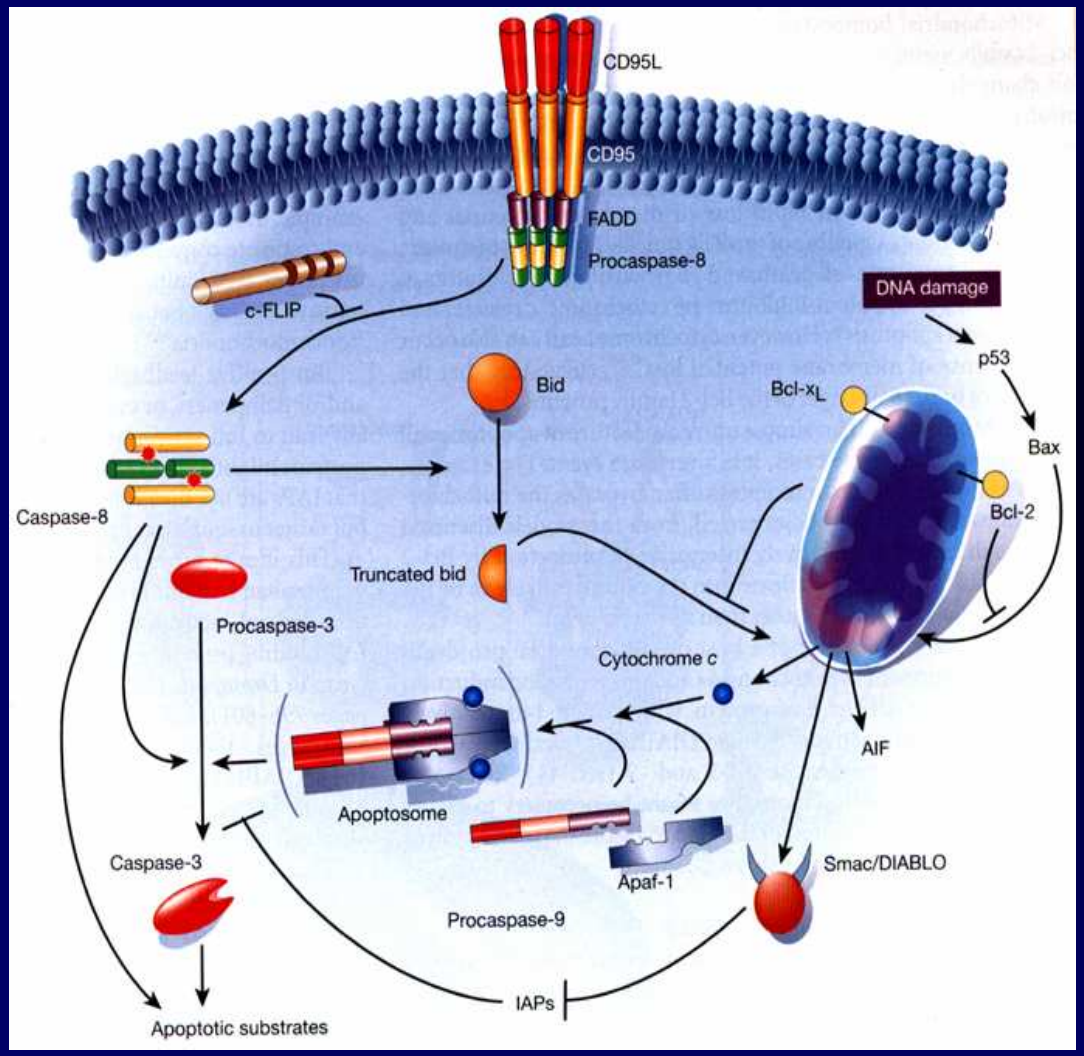
Apoptotic DNA degradation is followed by phogocytosis of apoptotic bodies



Rich et al., Nature 2000



Two major apoptotic pathways in mammalian cells

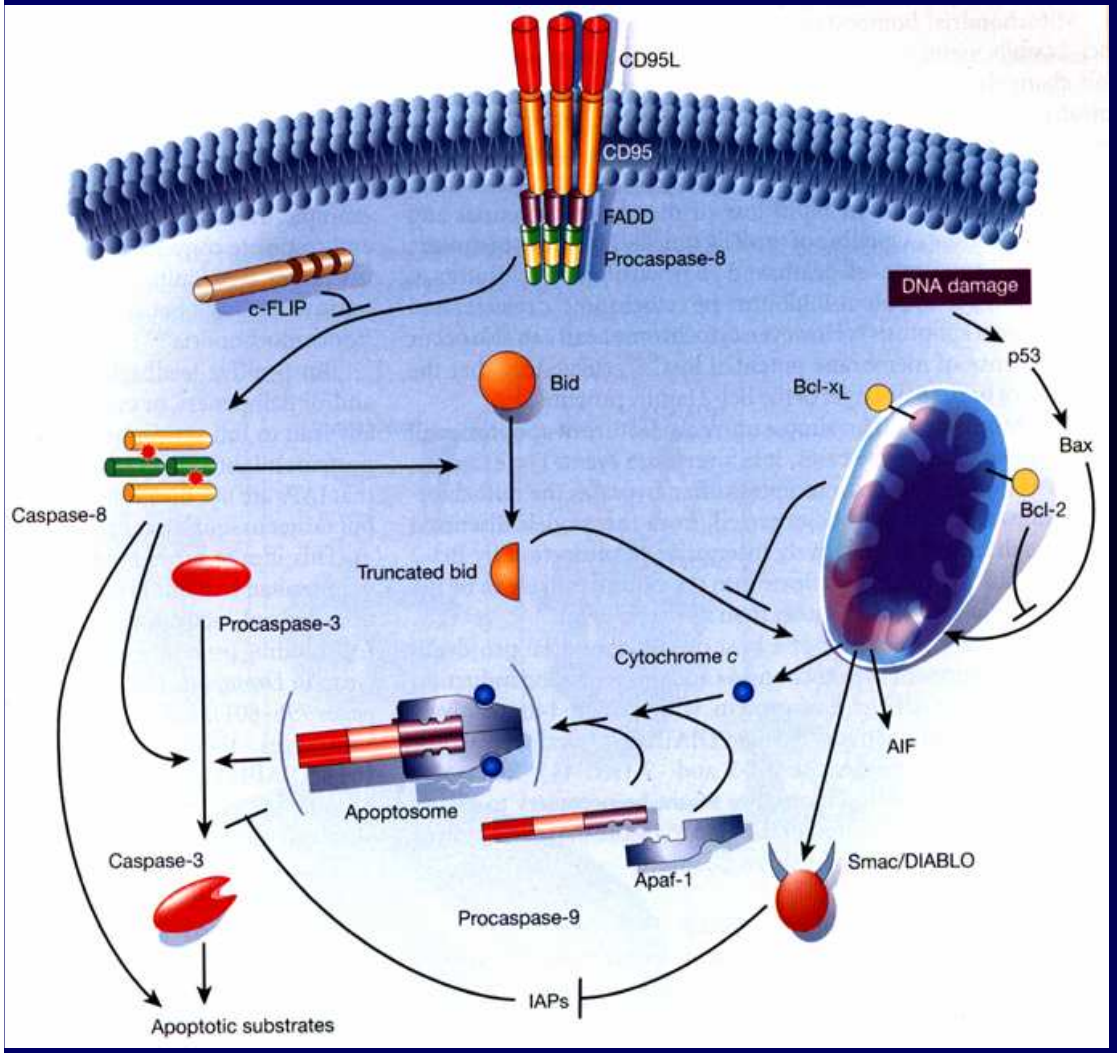


Death-receptor pathway:

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 subsequenty activation of Caspase-3 is induced. Activation of procaspase-8 can be blocked through degenerate caspase homoloque c-FLIP.

Hengartner M.O., Nature 2000

Two major apoptotic pathways in mammalian cells



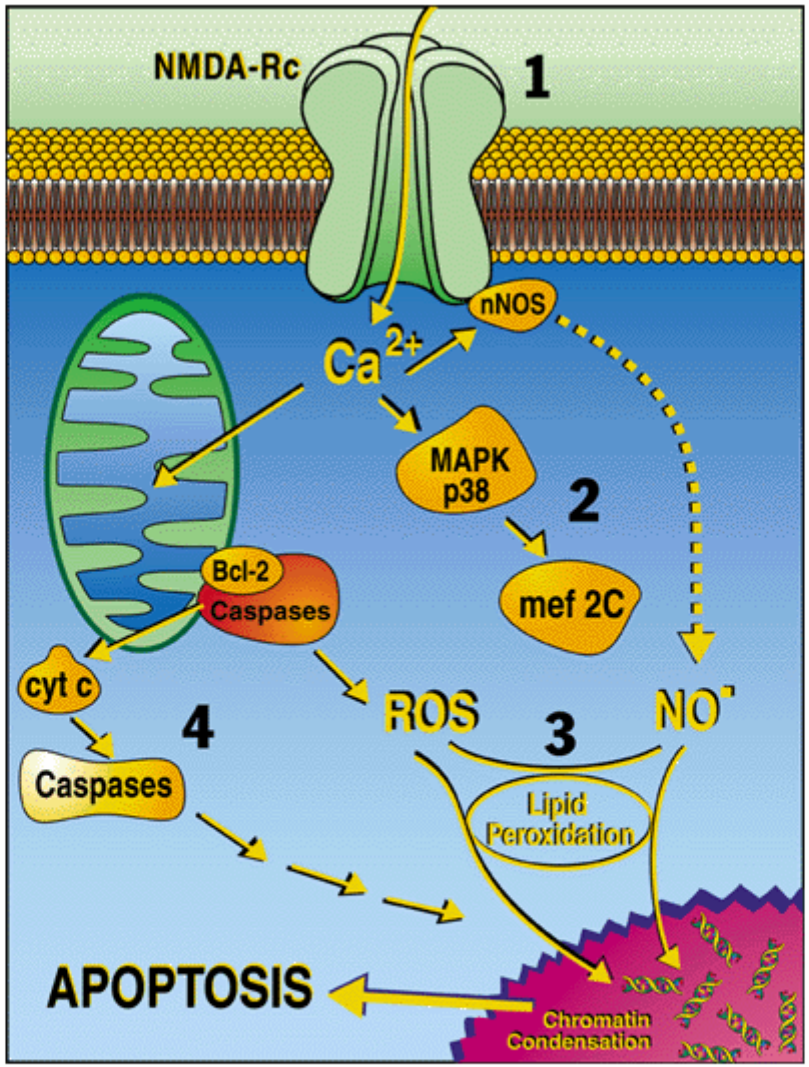
Hengartner M.O., Nature 2000

The mitochondrial pathway

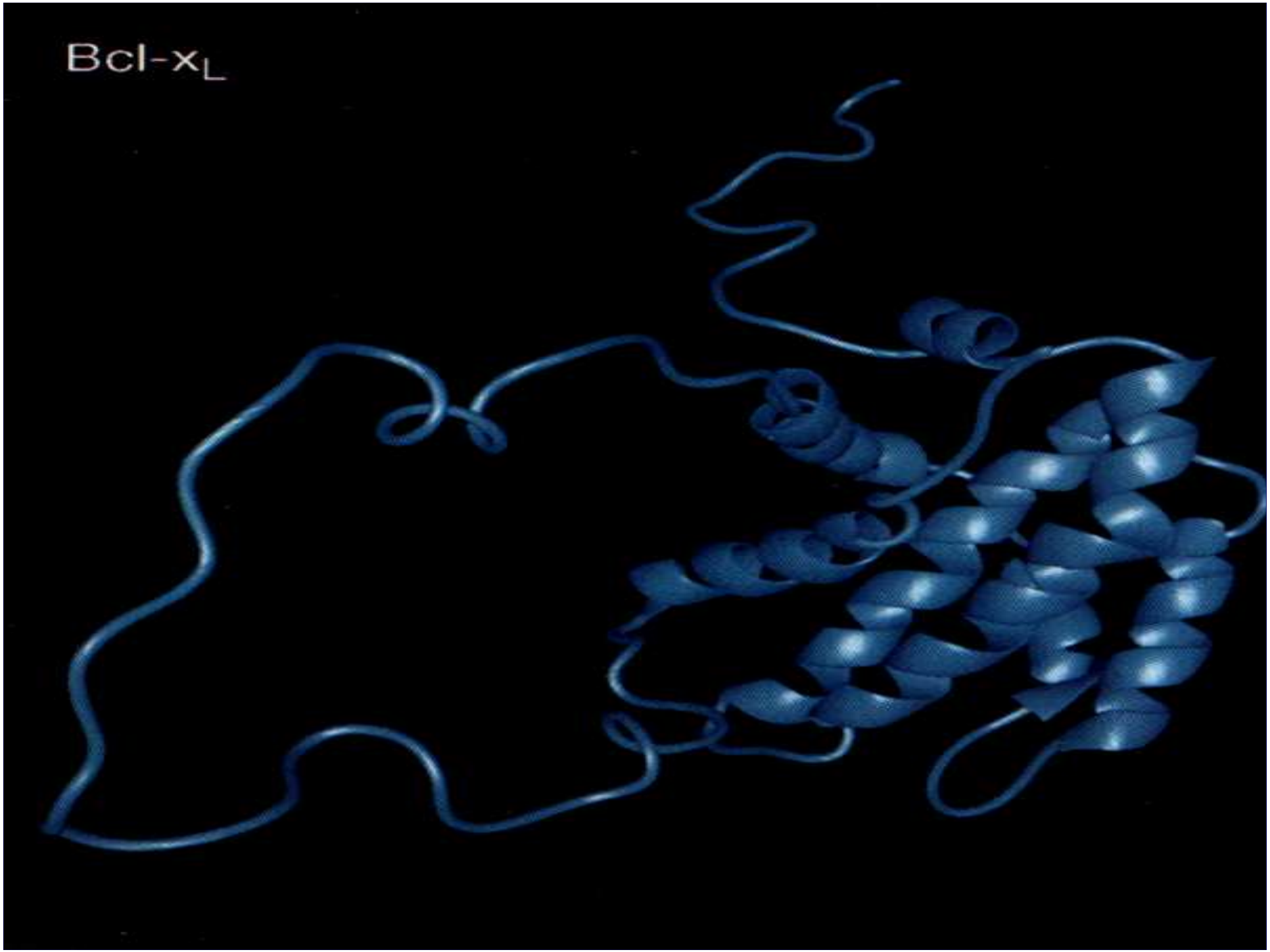
- activated after DNA damage
- proapoptotic members of Bcl-2 family,located on the surface of mitochondria, are activated
- Cytochrome c is released from mitochondria and forms complex with Apaf-1 and Procaspase 9.
- The complex is called APOPTOSOME.

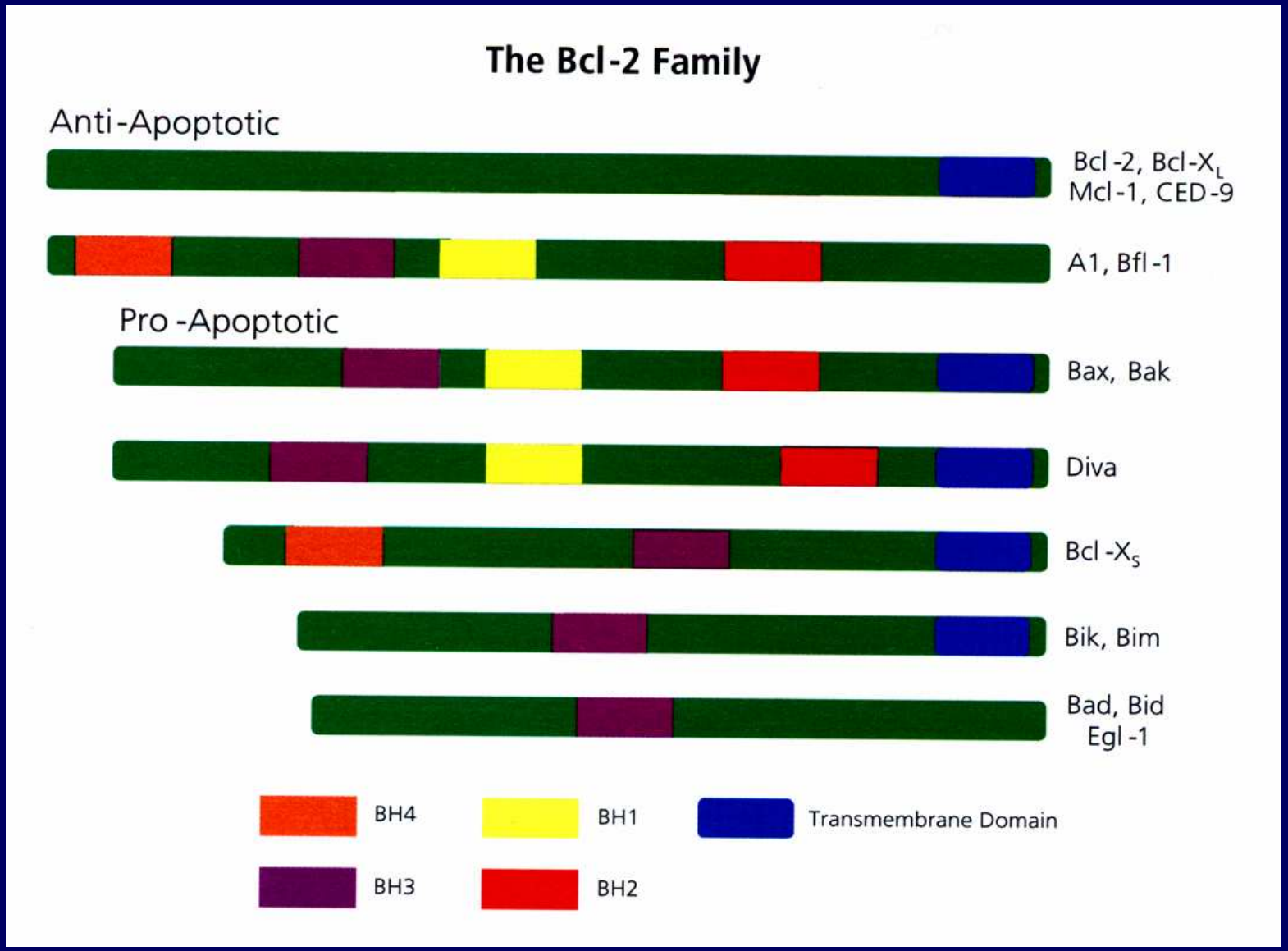
Both apoptotic pathways converge on the level of Caspase-3 activation

Caspase-3 activation is antagonized by IAP released from mitochondria

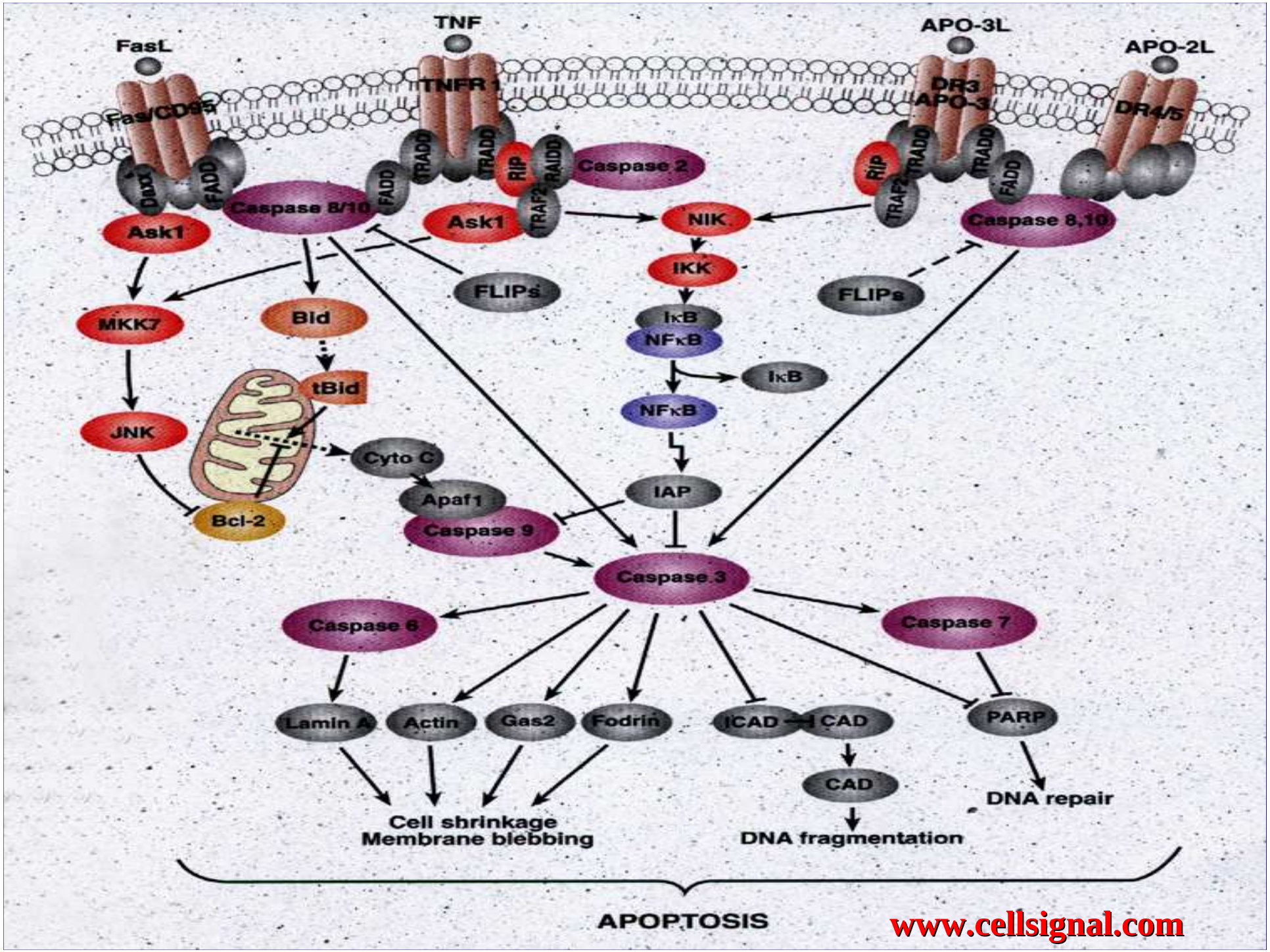


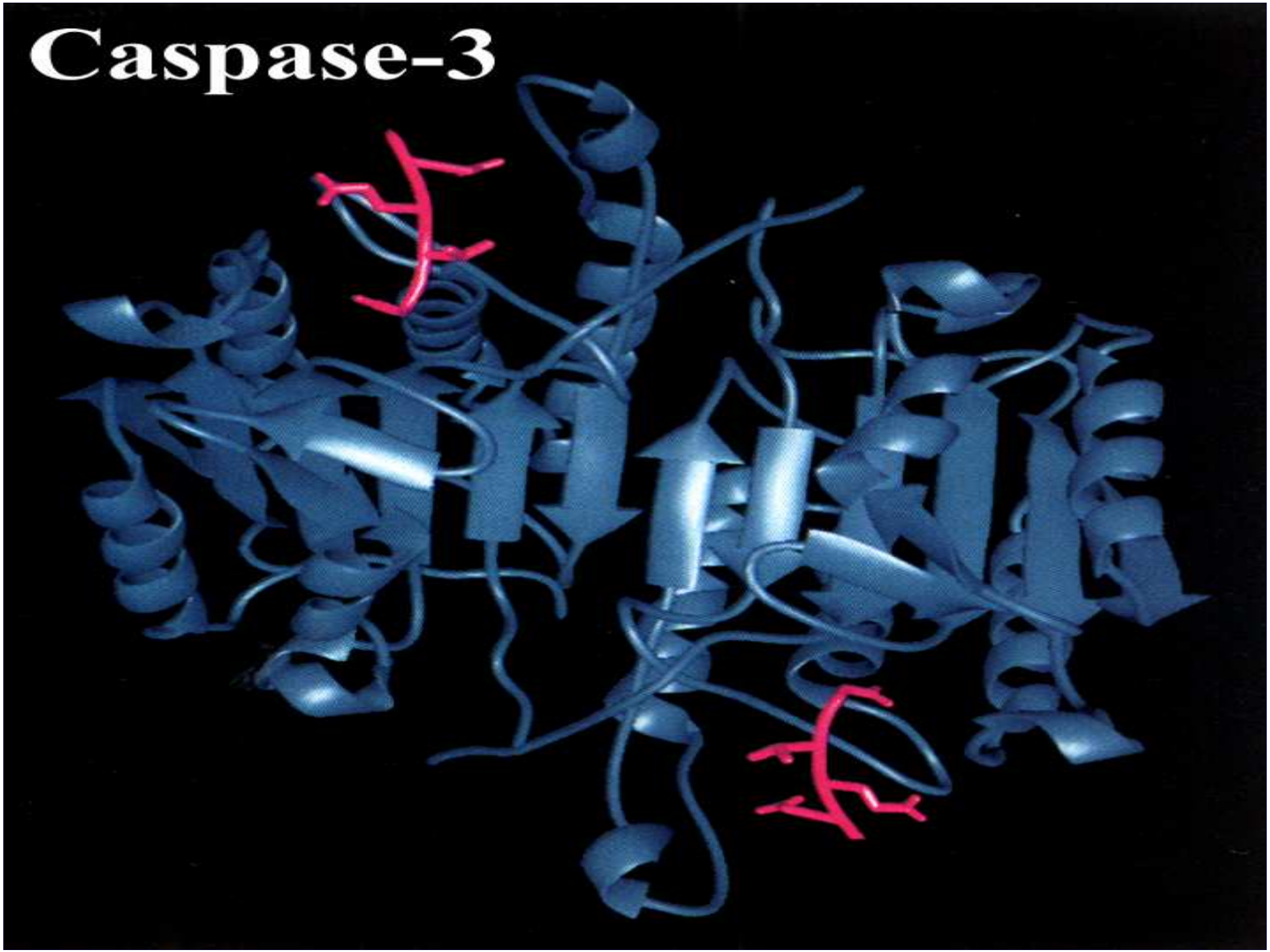
Schematic illustration of the signaling pathways discovered or characterized in the Neurodegenerative Disease Program that can be targeted to prevent neuronal apoptosis and thus treat various neurologic diseases. Drug or molecular therapies are being developed to (1) antagonize NMDA receptors (NMDA-Rc), (2) modulate activation of the p38 mitogen activated kinase (MAPK) - MEF2C (transcription factor) pathway, (3) prevent toxic reactions of free radicals such as nitric oxide (NO) and reactive oxygen species (ROS), and (4) inhibit apoptosis-inducing enzymes including caspases.

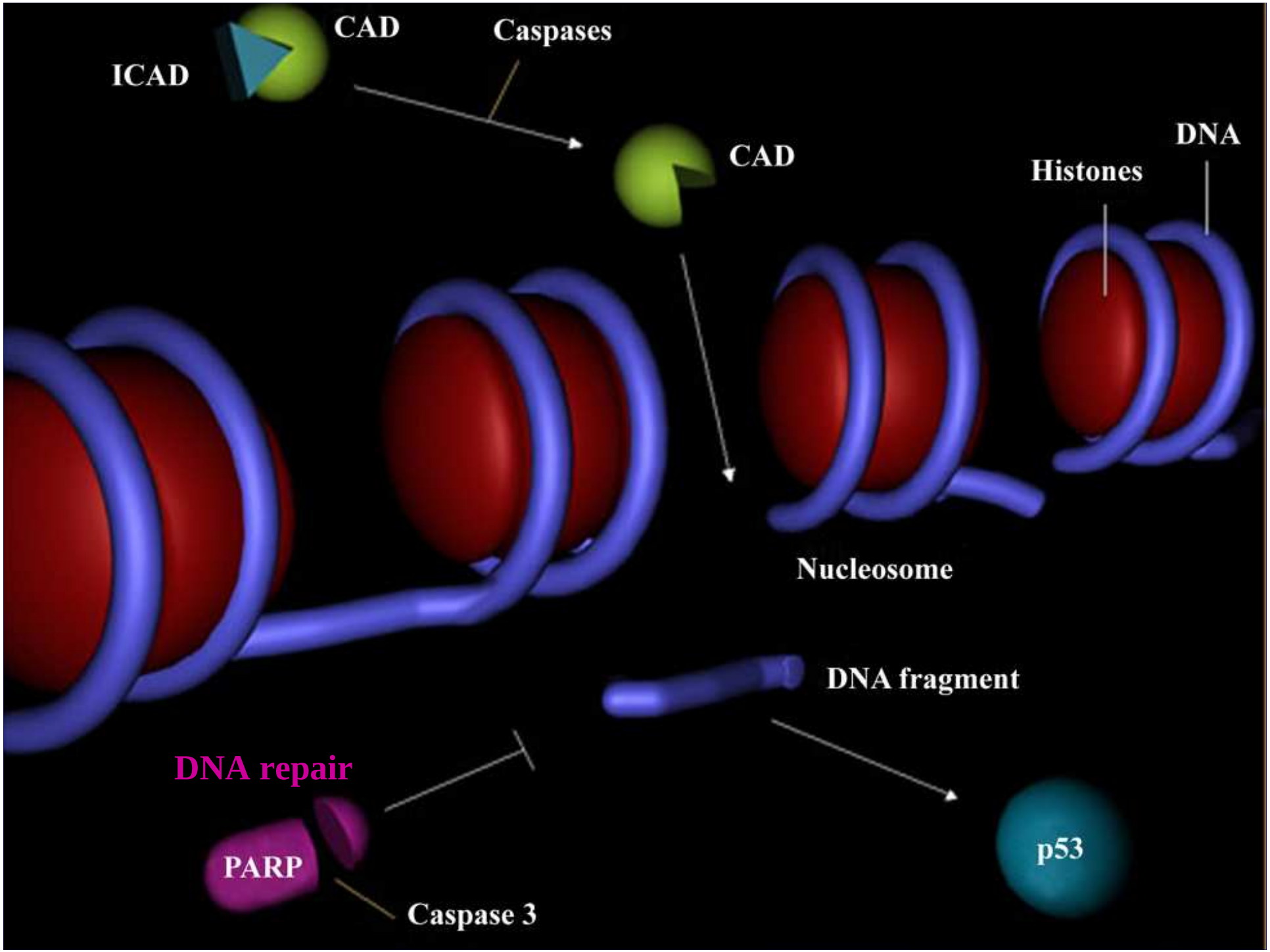

Bcl-x_L



Sigma (Apoptosis and Life Science)


FasL
TNF
APO-3L
APO-2L
Fas/CD95
TNFR1
DR3
APO-3
DR4/5
DAXX
FADD
TRADD
RIP
RAIDD
TRAF2
Caspase 2
Caspase 8/10
Caspase 8,10
Ask1
NIK
IKK
FLIPs
MKK7
Bid
tBid
JNK
Cyto C
Apaf1
Caspase 9
Bcl-2
IAP
IκB
NFκB
Caspase 3
Caspase 6
Caspase 7
Lamin A
Actin
Gas2
Fodrin
ICAD
CAD
PARP
DNA repair
Cell shrinkage
Membrane blebbing
DNA fragmentation
APOPTOSIS
www.cellsignal.com

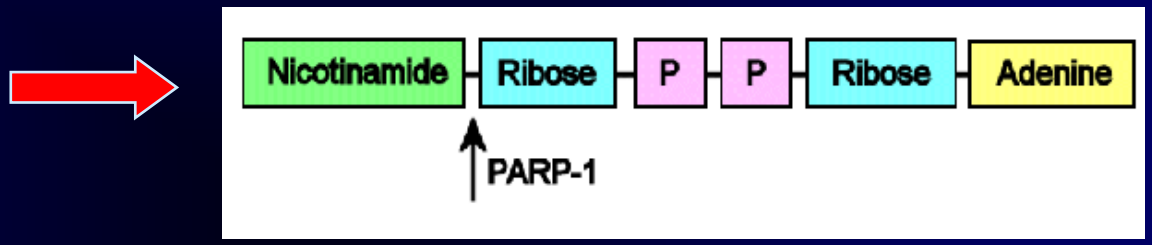

Caspase-3


CAD
Caspases
ICAD
CAD
DNA
Histones
Nucleosome
DNA fragment
DNA repair
PARP
Caspase 3
p53

DNA damage stimulates apoptosis. For example **p53** is a tumour suppressor gene. MDM2 inhibits the activity of p53 participating in the ubiquitination of p53. p53 is activated when MDM2 is inhibited by signalling from factors such as DNA damage. p53 is a transcription factor. Active p53 induces the transcription of many genes, including Bax, which promotes apoptosis by stimulating the release of cytochrome c and the formation of **apoptosomes.**

PARP-1 is a nuclear enzyme involved in DNA repair. When overactive, it can cause apoptosis or necrosis. PARP-1 is activated by single stranded DNA. Active PARP-1 cleaves NAD+ as shown in figure.

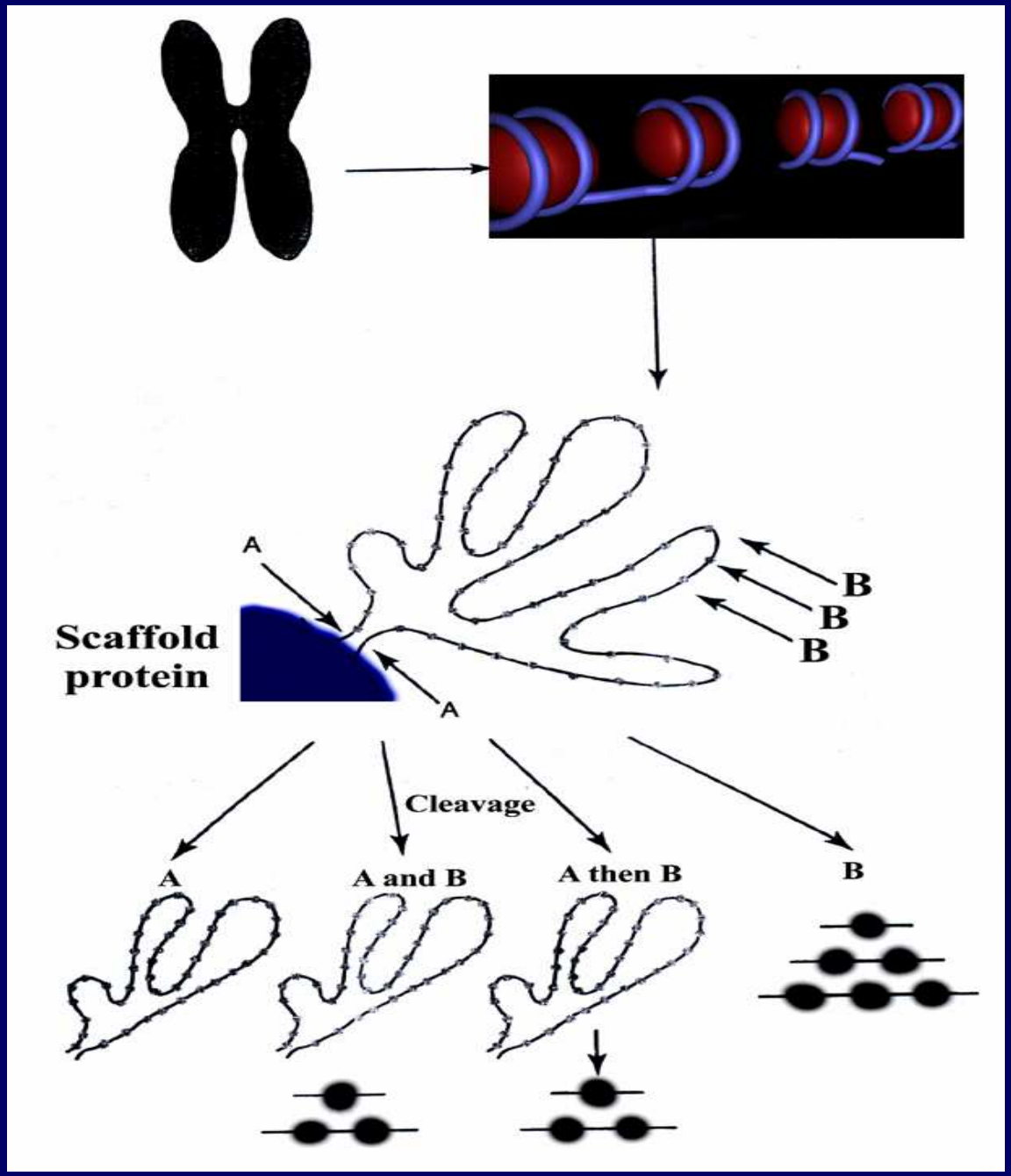
Cleavage of NAD+ by PARP-1.



PARP-1 catalyses the addition of an ADP-ribose polymer of 50-200 residues to nuclear proteins such as histones, which stimulates DNA repair enzymes. However, overactive PARP-1 causes depletion of NAD+**,** and consequently the depletion of ATP**.**

- ATP depletion leads to ion pump failure. The cell swells and bursts due to osmotic pressure. This is **necrosis.**
- Alternatively, the depletion of NAD+ from mitochondria appears to induce **AIF translocation** from the mitochondria to the cytoplasm**.** This leads to **apoptosis.**
- **There may be a PARP-1 activity threshold, which determines whether the cell engages in DNA repair, apoptosis or necrosis.**

Apoptosis is ATP dependent. Apoptosis involves chromatin fragmentation, which would be predicted to cause PARP-1 overactivity and drive the cell into necrosis.

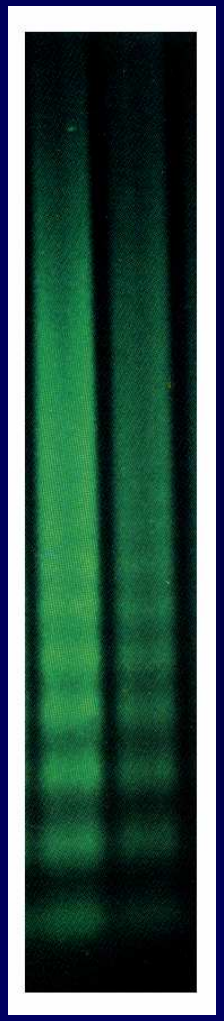


DNA fragmentation during apoptosis

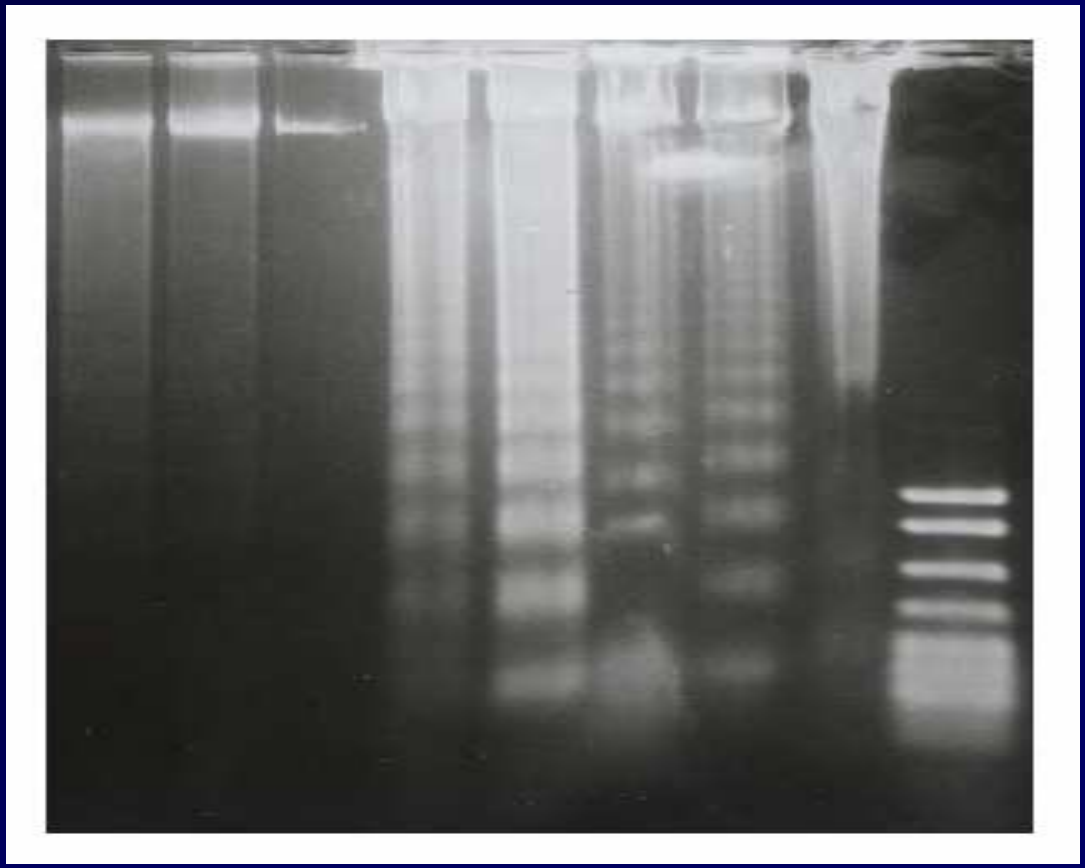
1. High molecular weight DNA fragmentation (50-300 kbp)
2. Oligonucleosomal DNA fragmentation (180-200 bp)
3. Single- strand cleavage

Bortner C.D. et al., 1995

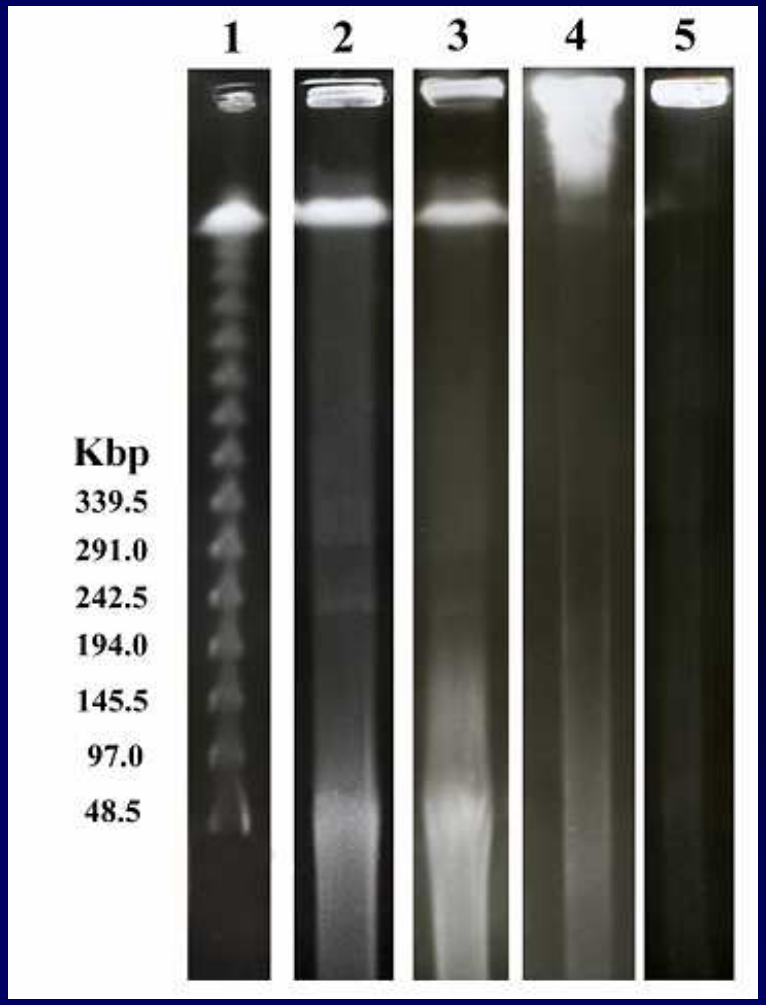
APOPTOSIS DETECTION



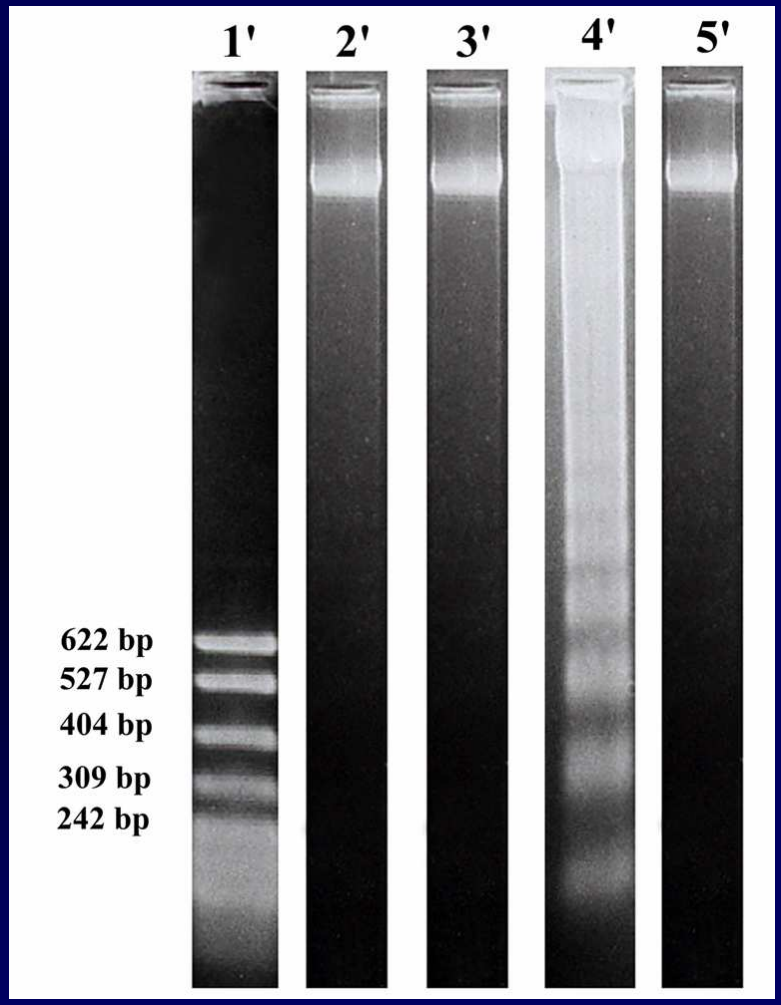
DNA fragmentation test



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

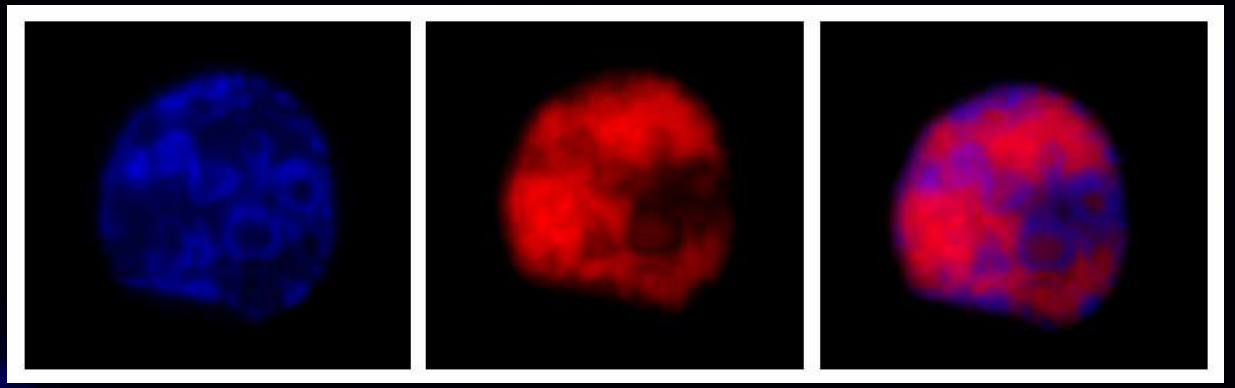


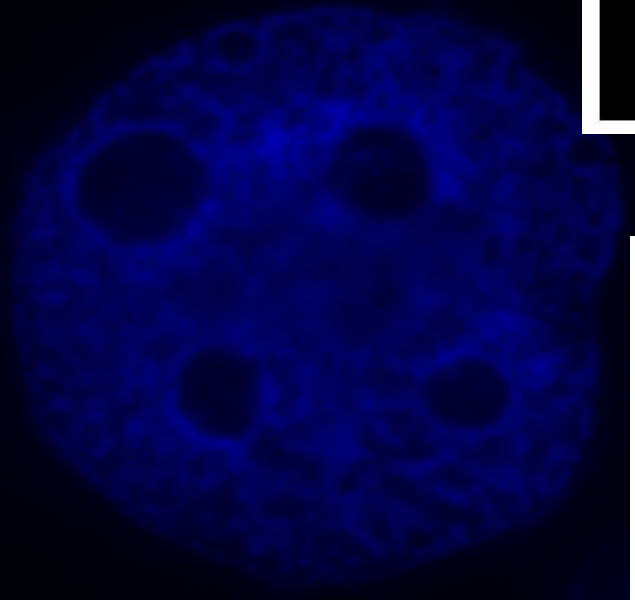
Field inversion electrophoresis (FIGE)

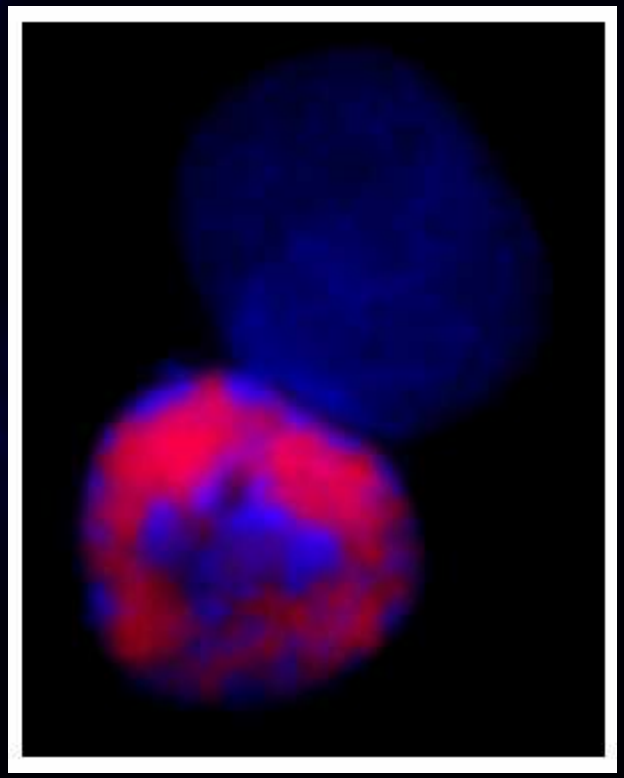


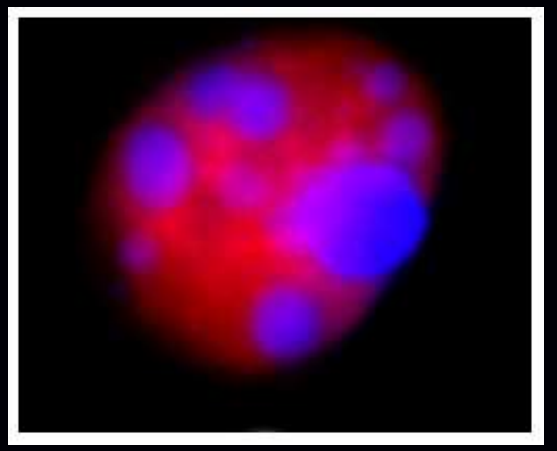
DNA fragmentation test

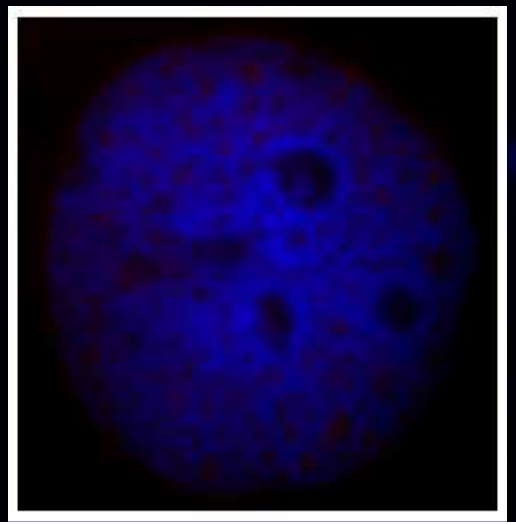
Anti-PARP p85 fragment pAb





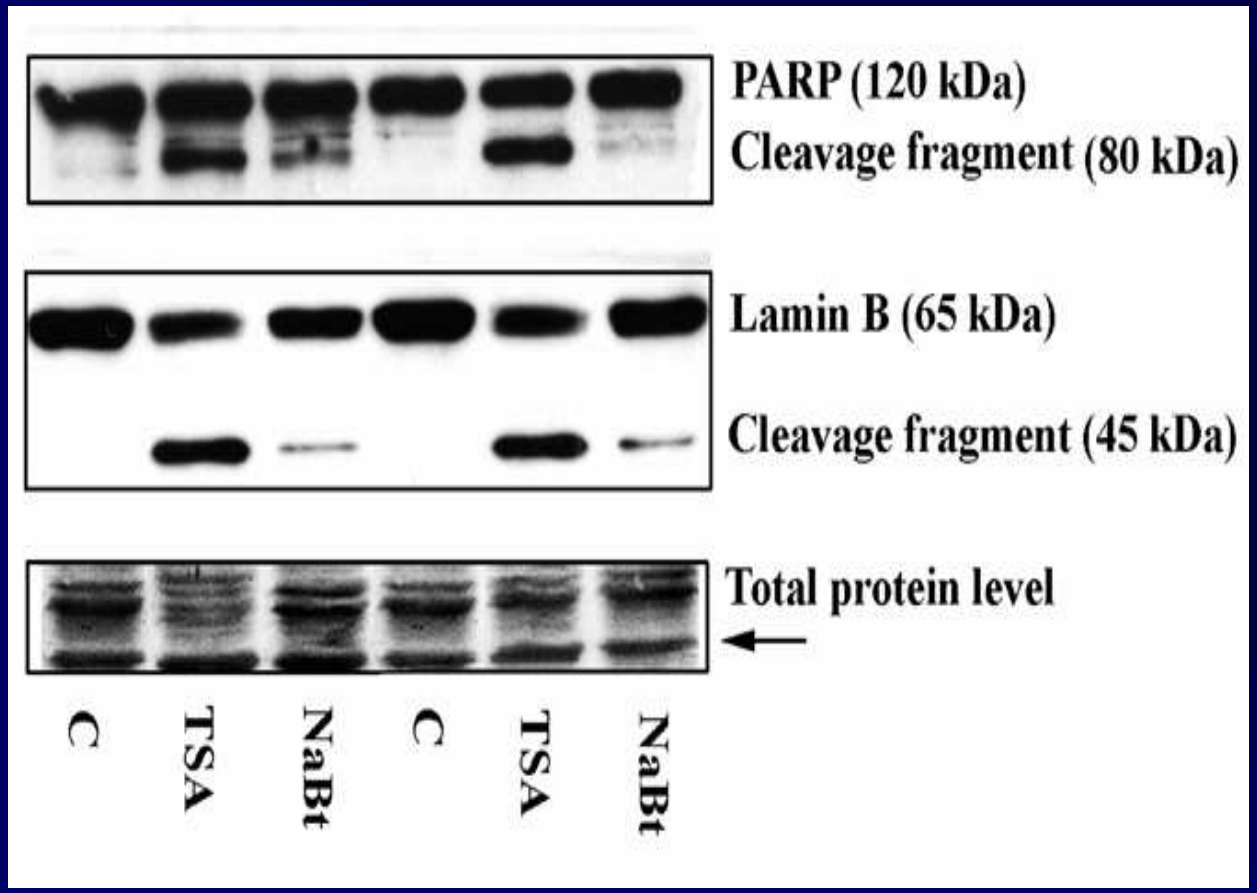


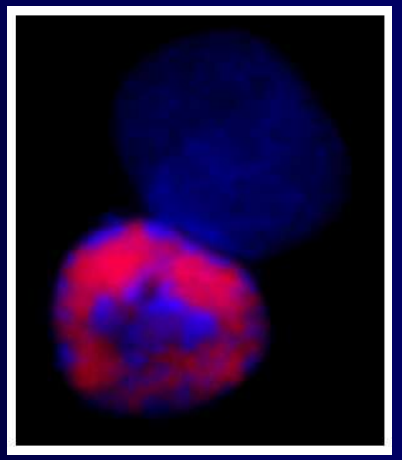




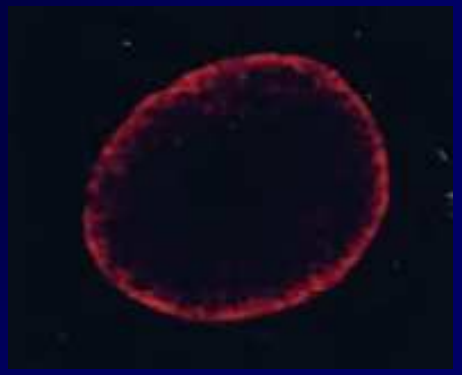
Poly(ADP-ribosyl)ation and apoptosis

Western blots and detection of apoptosis





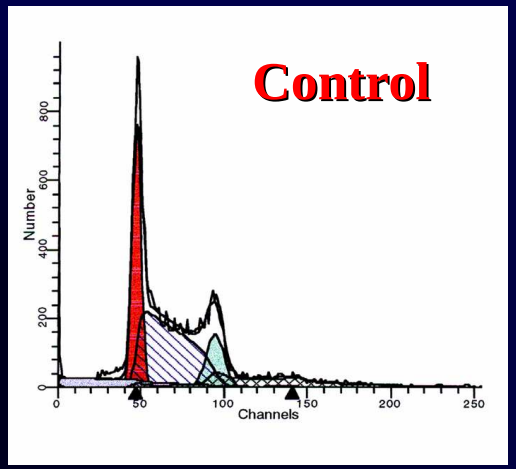
PARP cleavage

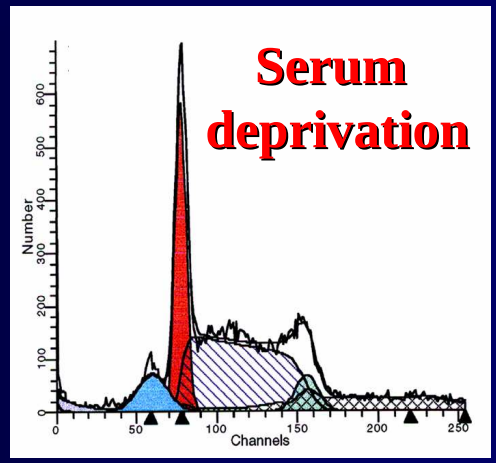


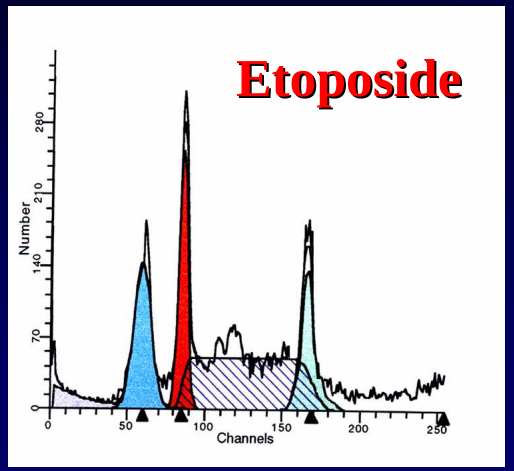
Lamin B

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



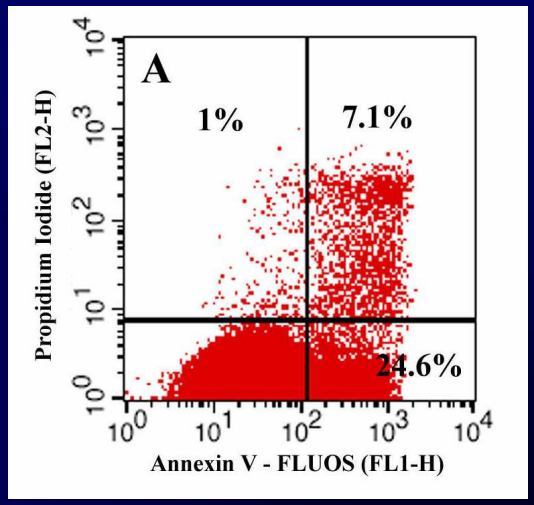


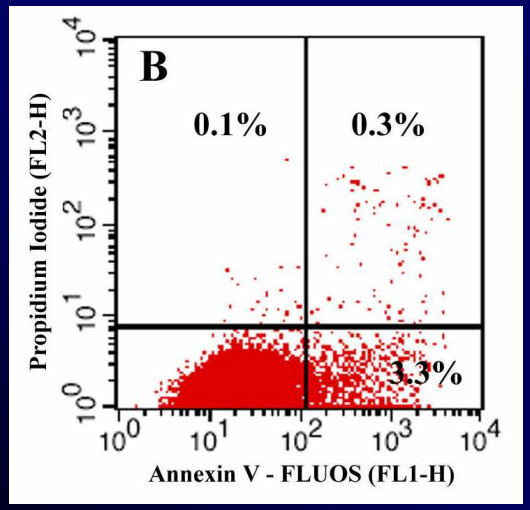


Etoposide

Control

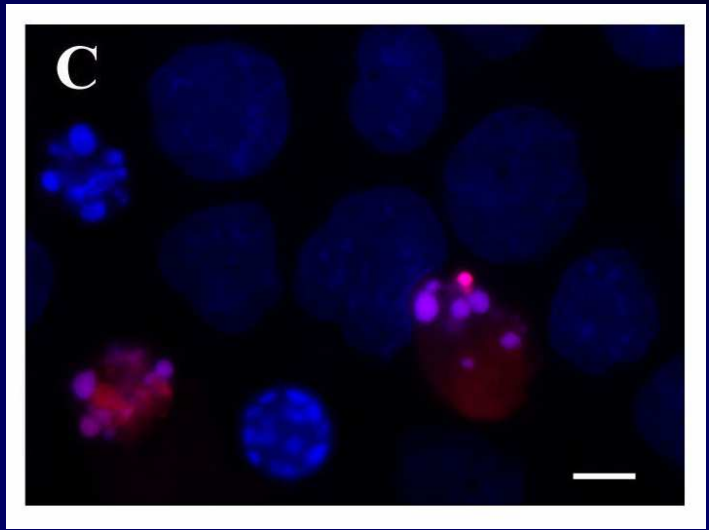
Annexin V / PI

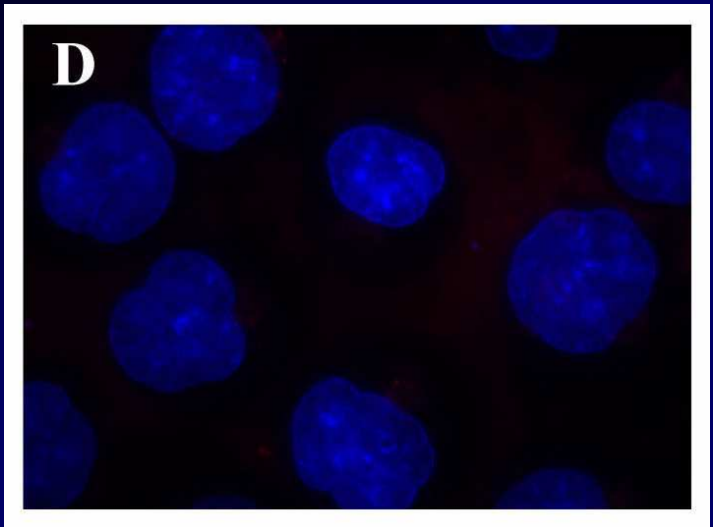


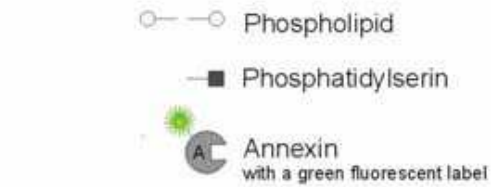


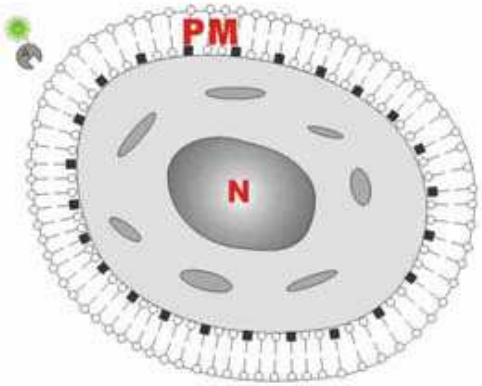
Anexin V binds to phosphatidylserines that are traslocated from the inner side od the plasma membrane to the cell surface soon after the induction of apoptosis

Hoechst33342 / PI

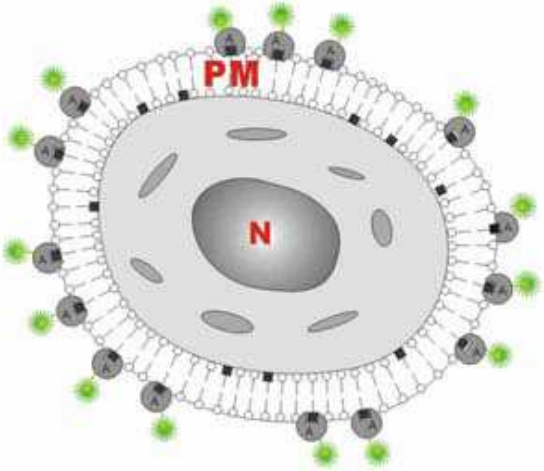




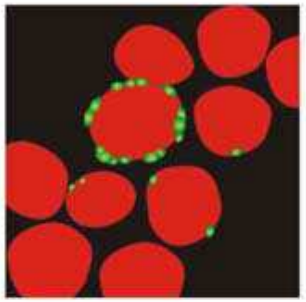




NORMAL, HEALTLHY CELL

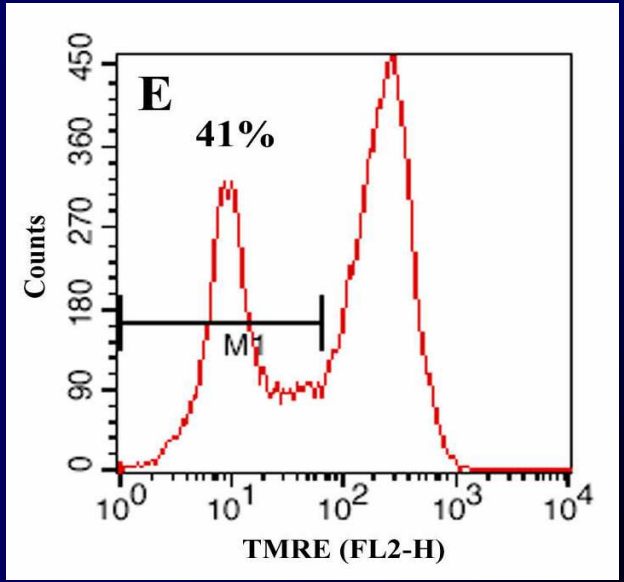


APOPTOTIC CELL

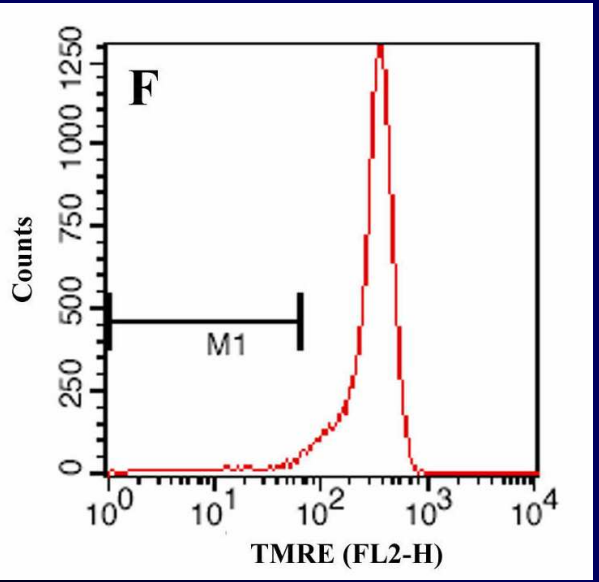


TMRE

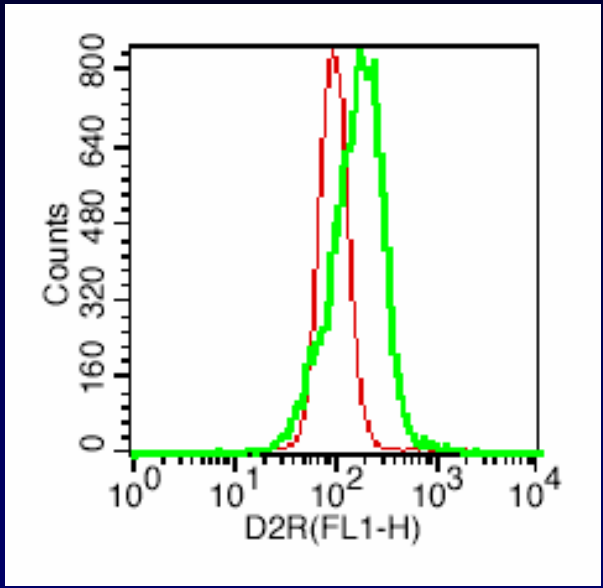
Etoposide



Control



CaspSCREEN (tm)
BioVision kit

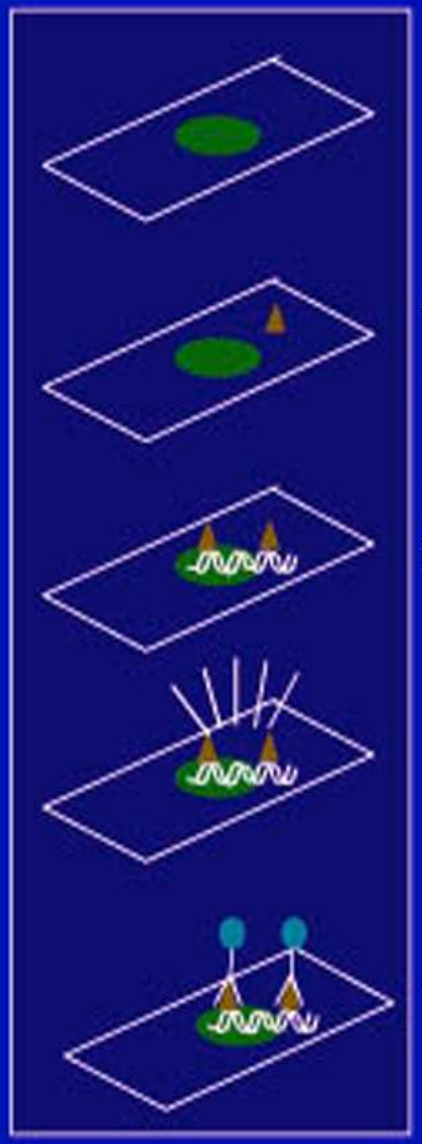


DNA Fragmentation - TUNEL

In Situ Cell Death Detection Kit - Test Principle

Roche

Diagnostics



cells on slide

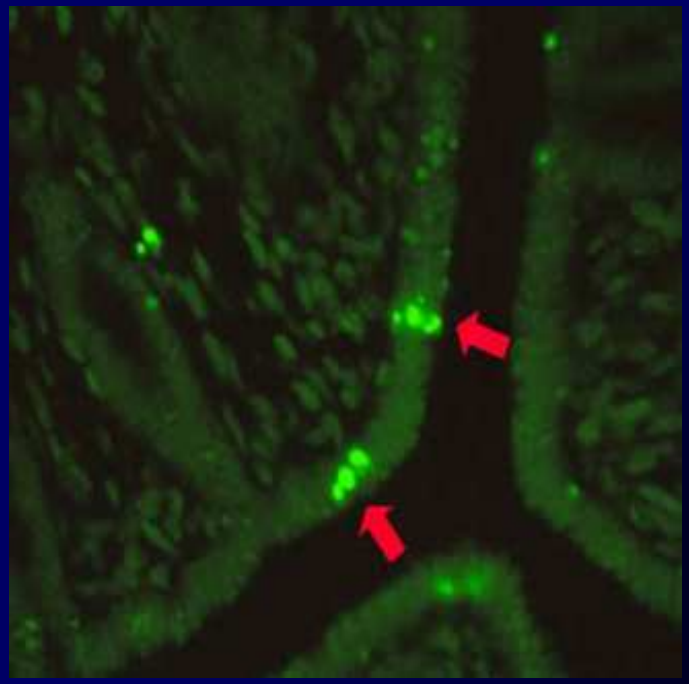
Add TdT and FITC-dUTP to cells

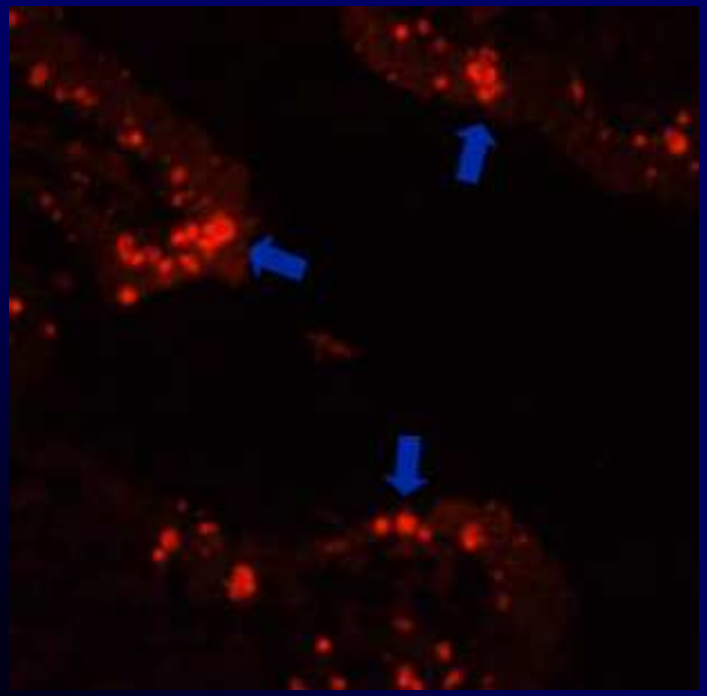
FITC-dUTP incorporated into DNA

Fluorescence microscopy

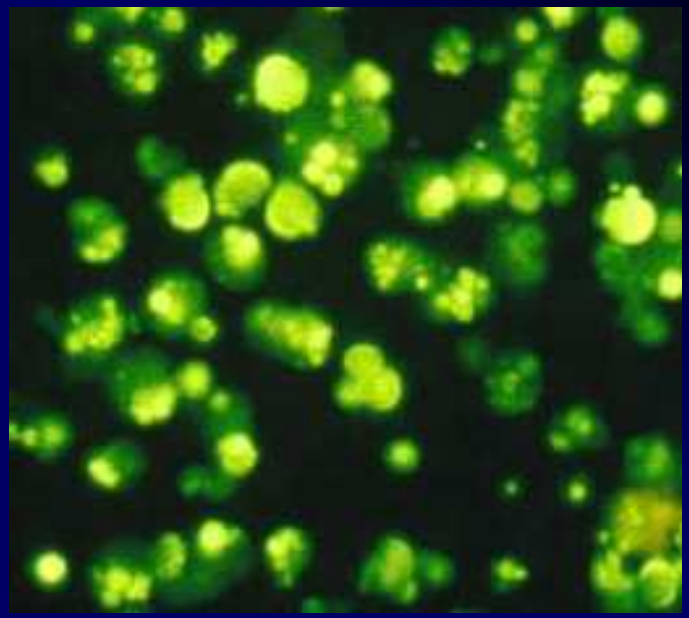
<FITC>POD or <FITC> AP

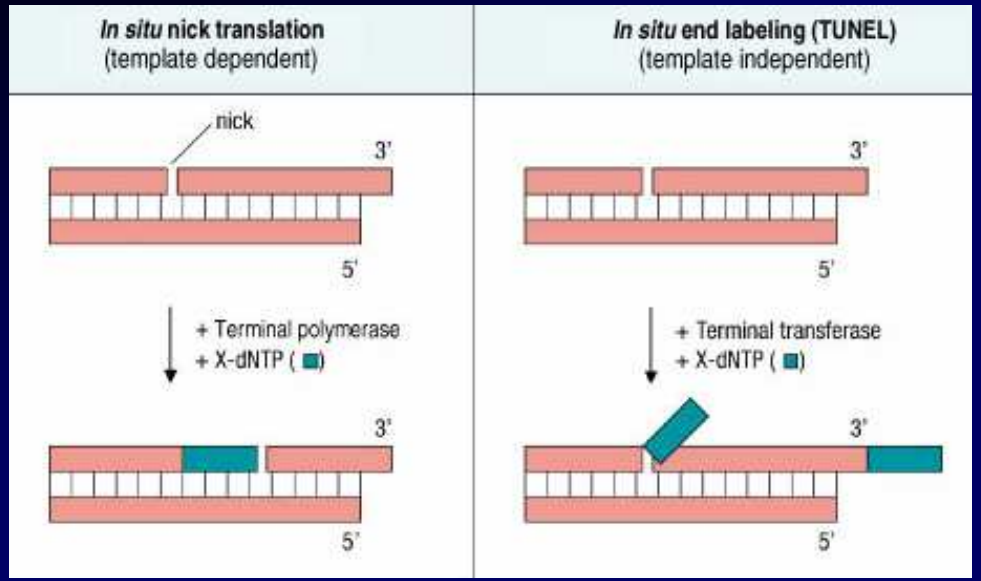
Light microscopy



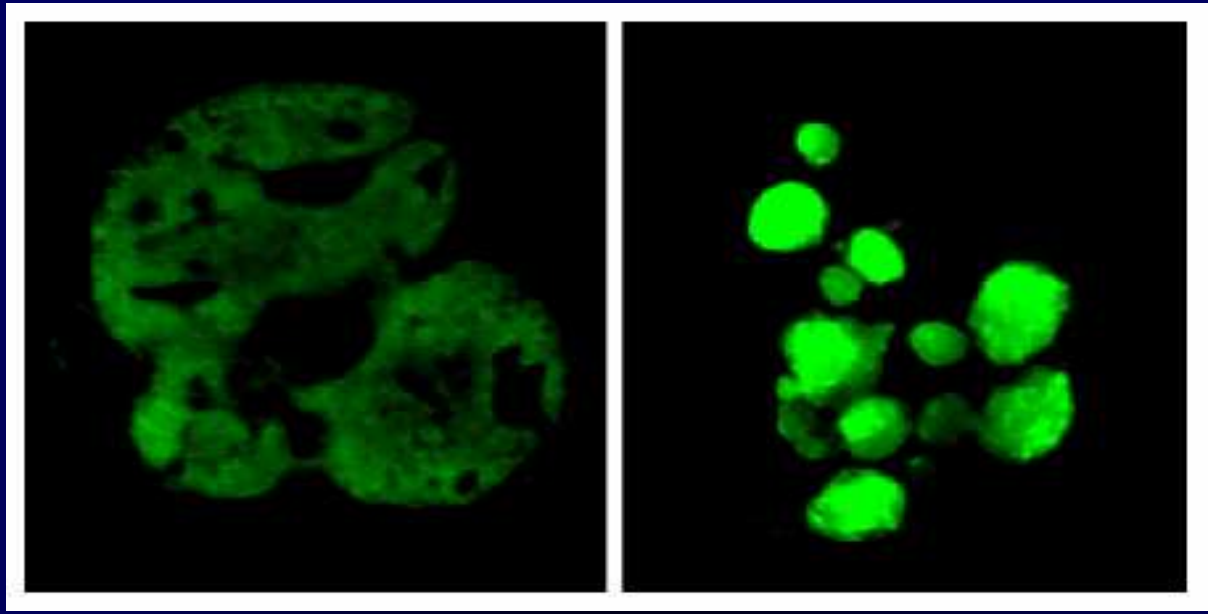


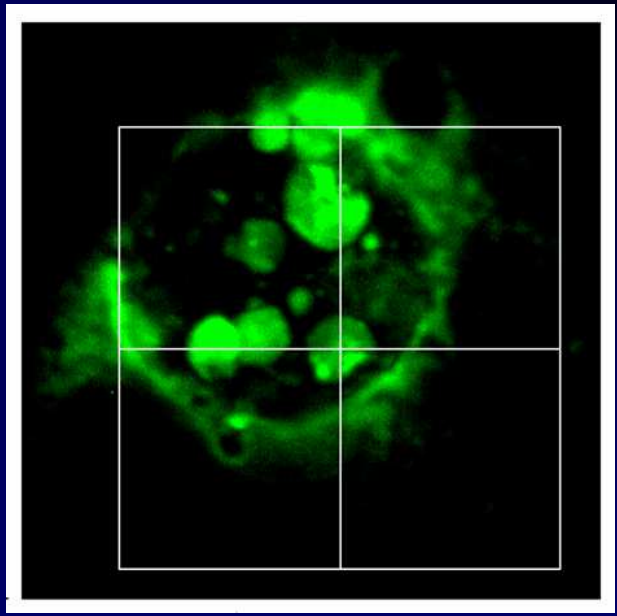
Roche

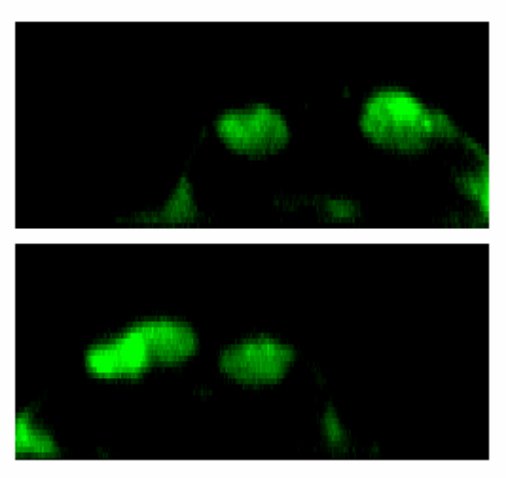


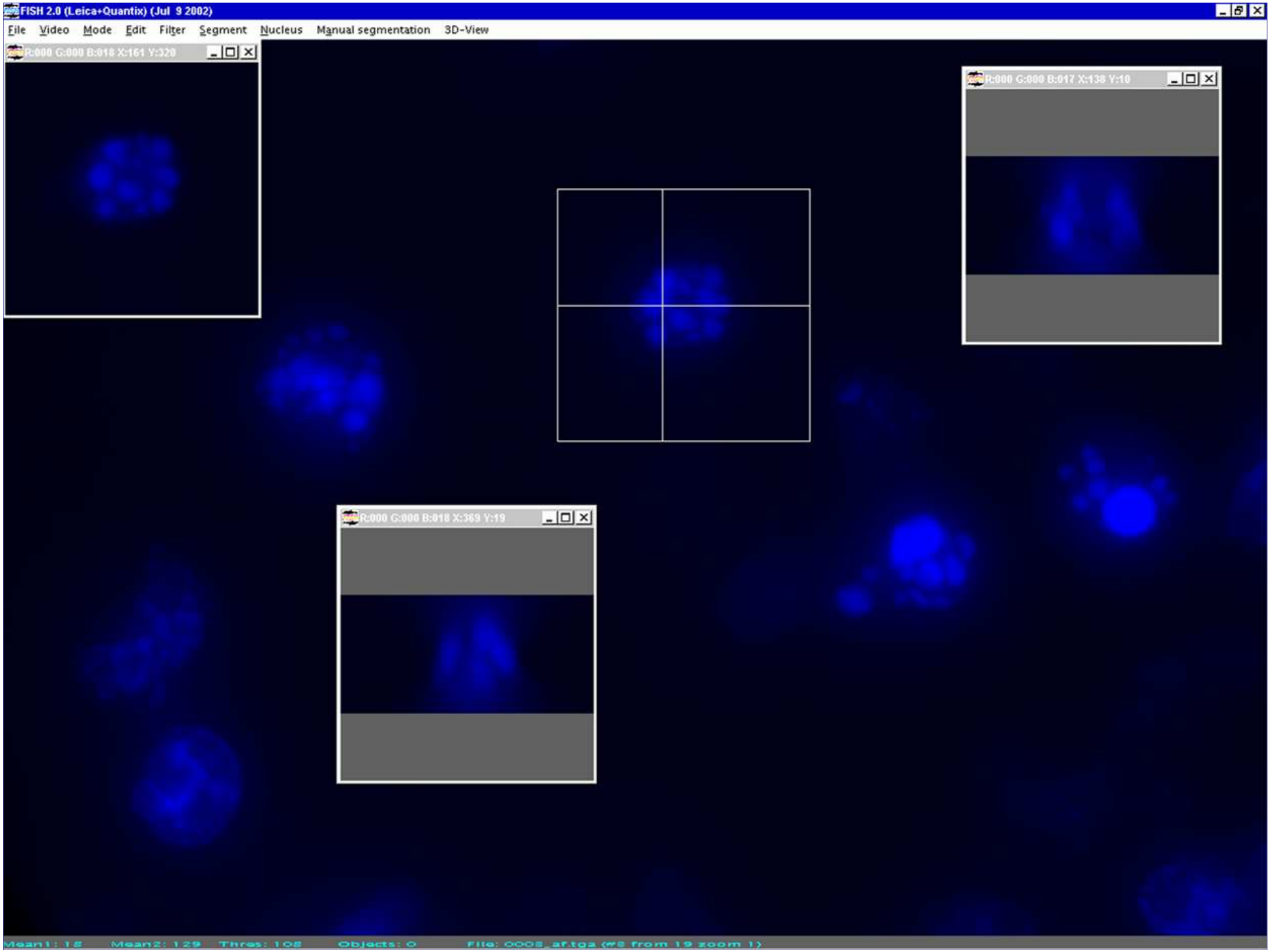

In situ nick translation
(template dependent)
nick
3'
5'
+ Terminal polymerase
+ X-dNTP ( )
3'
5'
In situ end labeling (TUNEL)
(template independent)
3'
5'
+ Terminal transferase
+ X-dNTP ( )
3'
5'

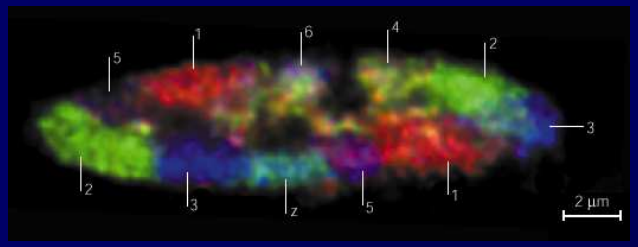
The results of TUNEL test



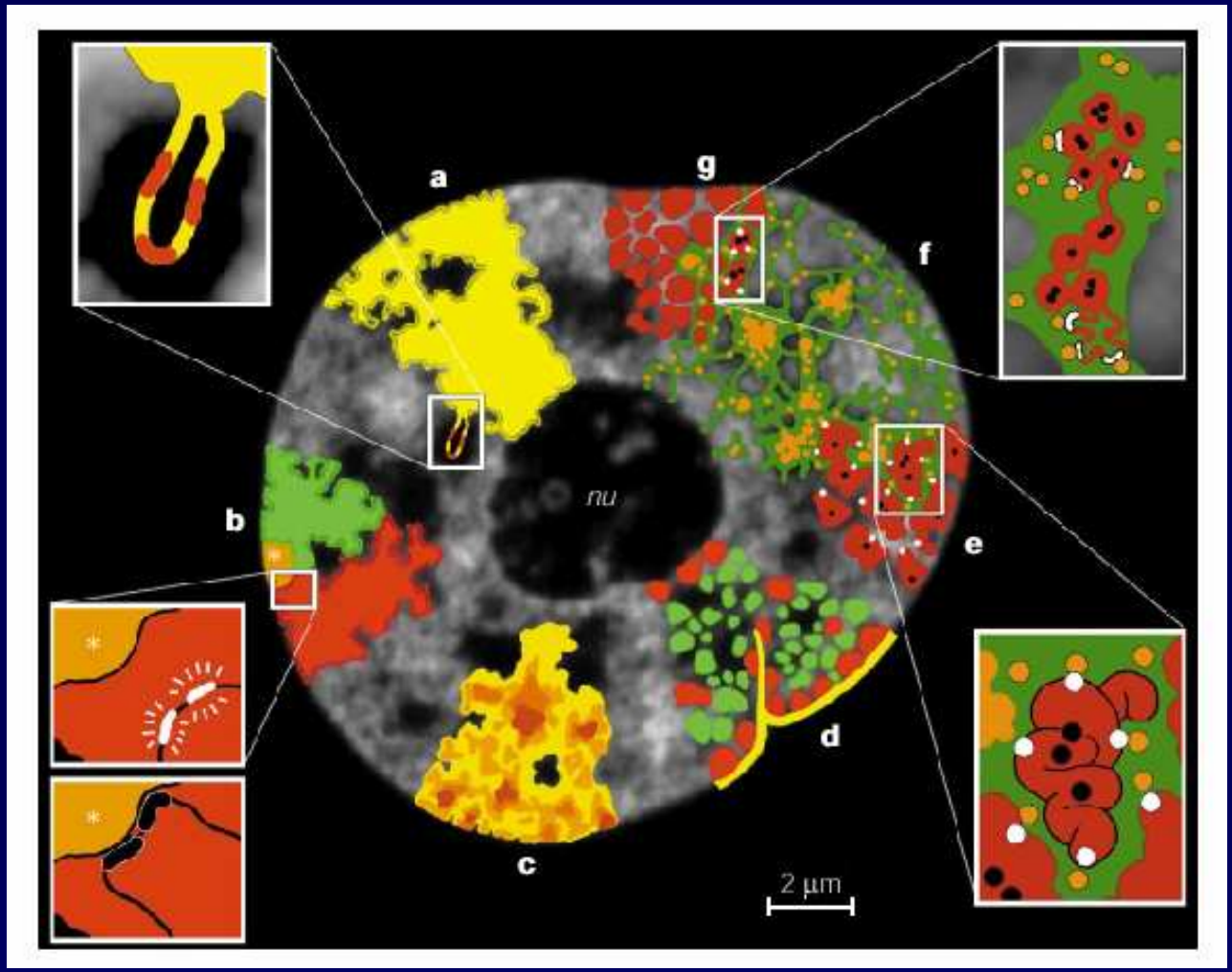




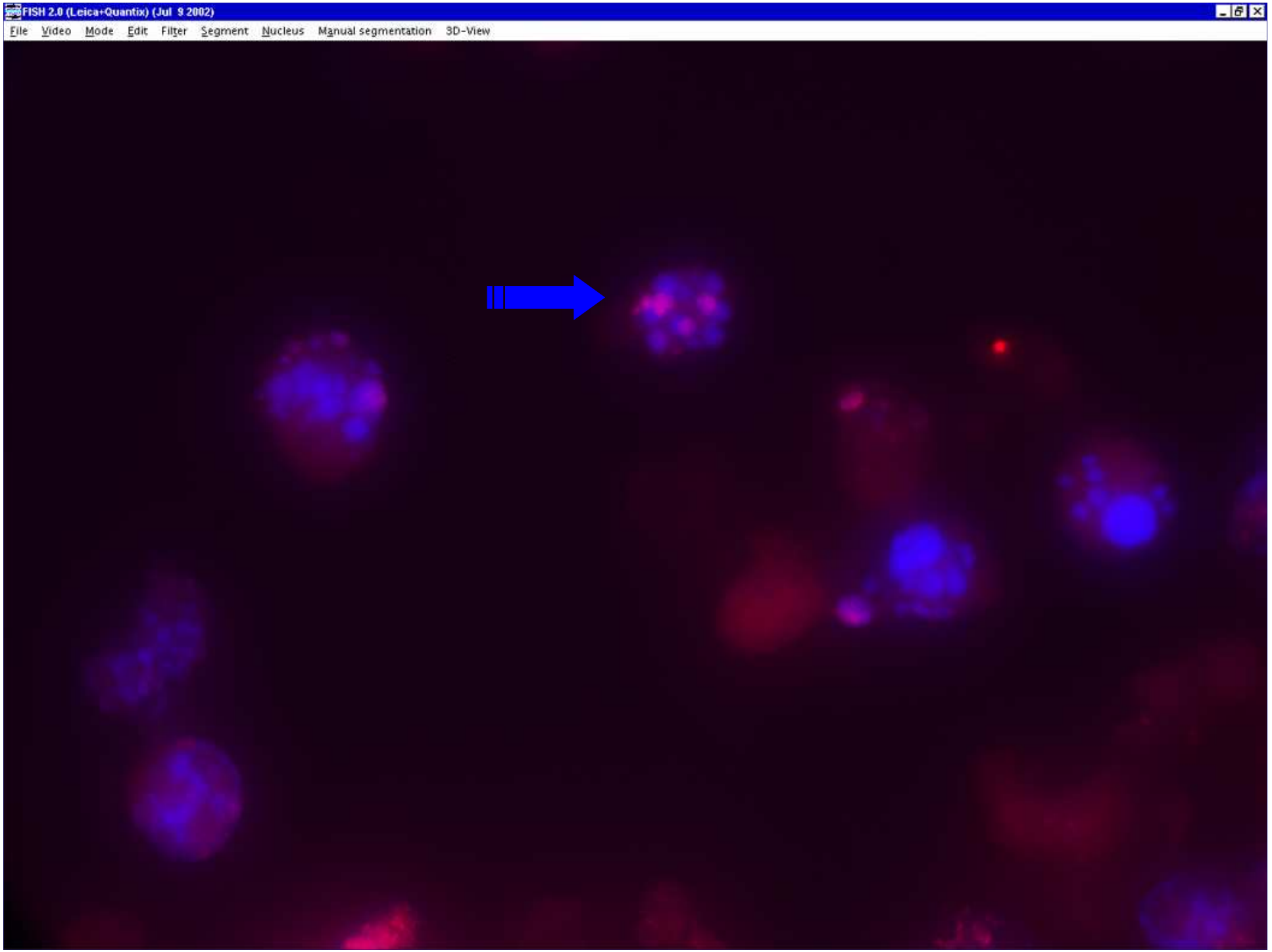

FISH 2.0 (Leica+Quantix) (Jul 9 2002)
File Video Mode Edit Filter Segment Nucleus Manual segmentation 3D-View
R:000 G:000 B:018 X:161 Y:320
R:000 G:000 B:017 X:138 Y:10
R:000 G:000 B:018 X:369 Y:19
Mean1: 15 Mean2: 129 Thres: 105 Objects: 0 File: 0005_af.tga (#8 from 19 zoom 1)



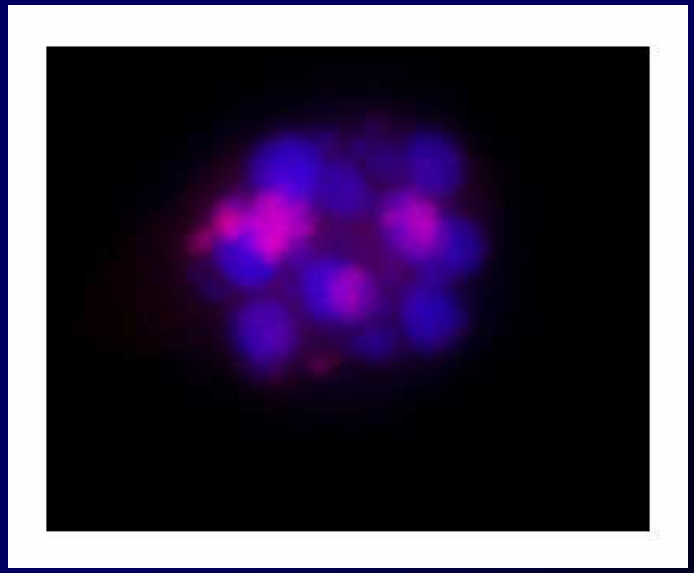
Nuclear organisation of chromosomal territories

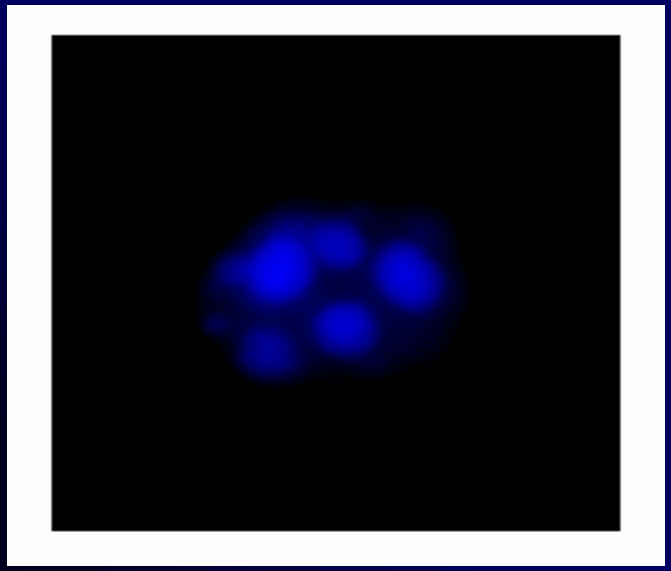


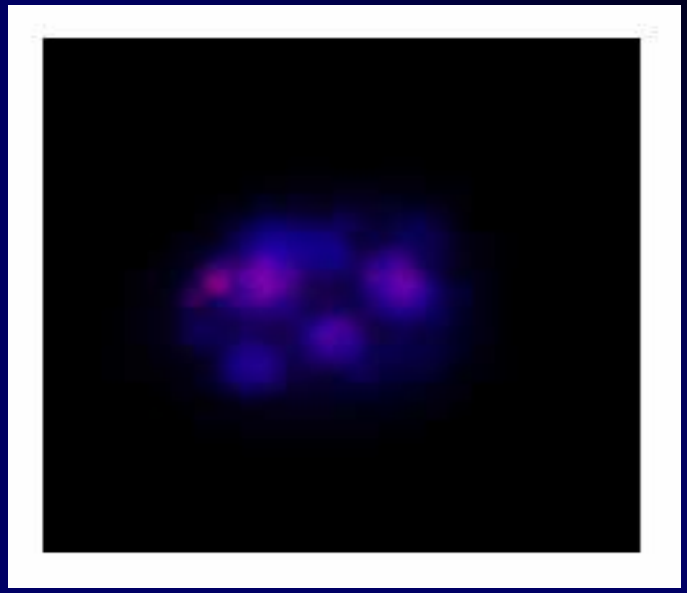
(Cremer T. and Cremer C., 2001)

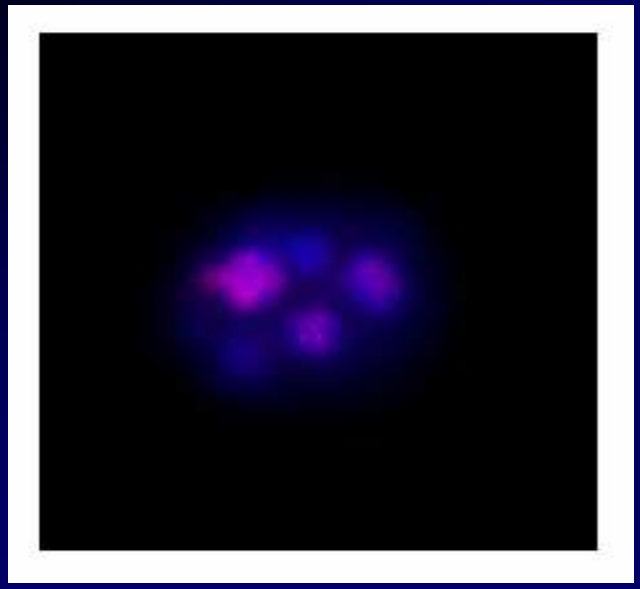

FISH 2.0 (Leica+Quantix) (Jul 9 2002)
File Video Mode Edit Filter Segment Nucleus Manual segmentation 3D-View

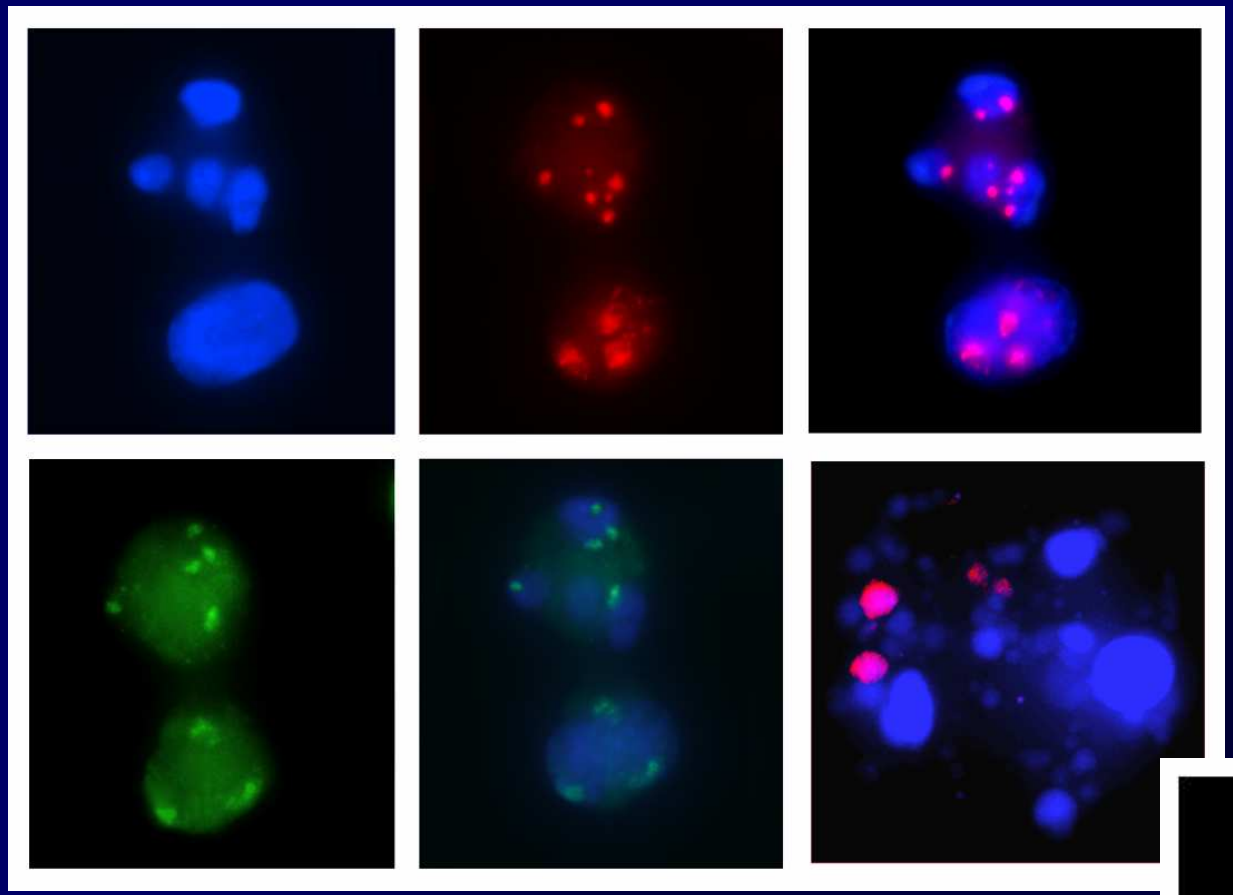
Apoptotic territory of chromosome 3





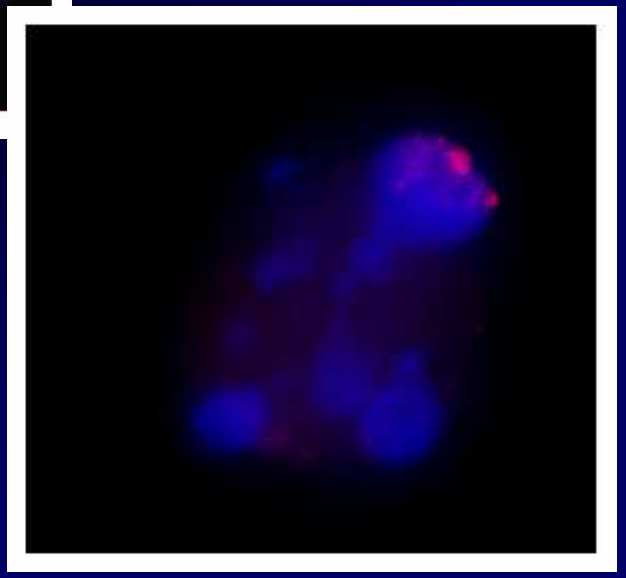




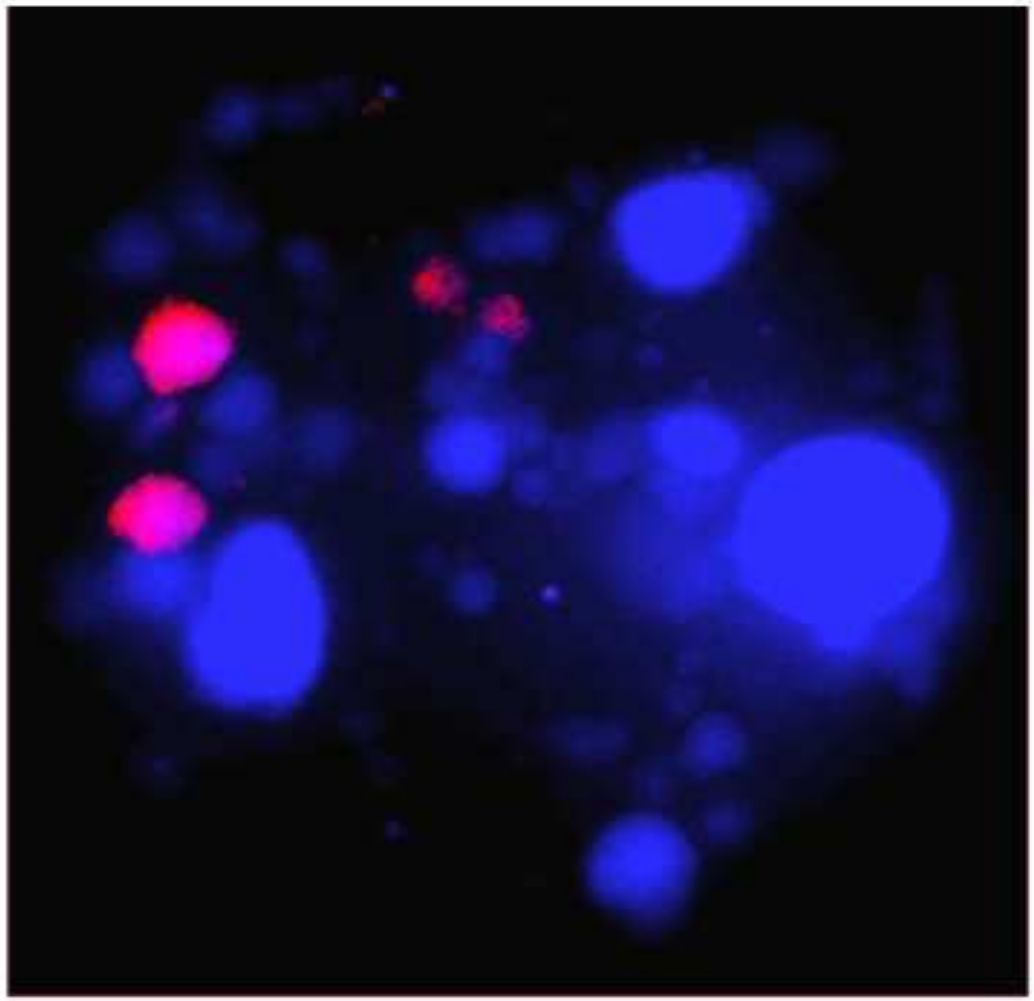


Territory of chromosome 11 and 17

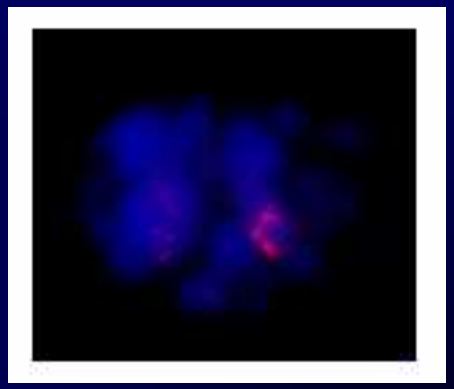
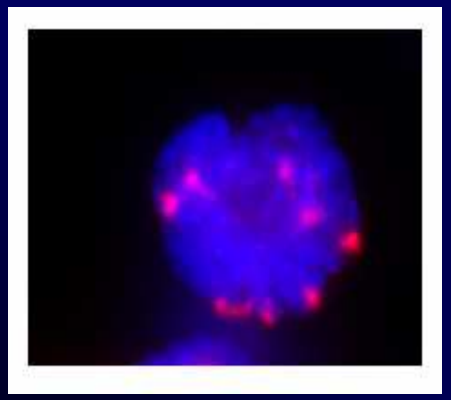
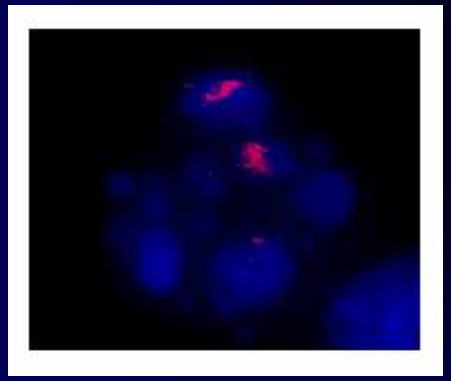
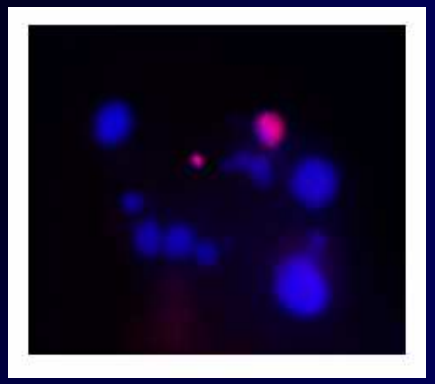
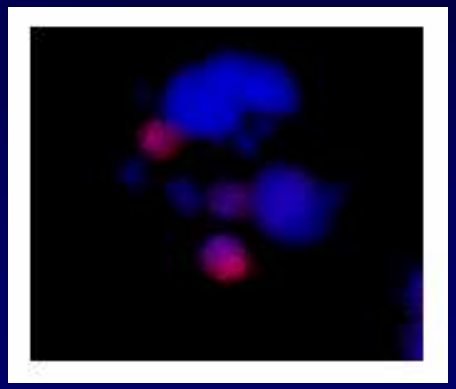
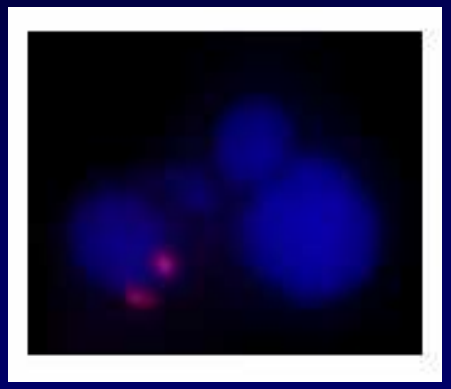
Territory of chromosome 3



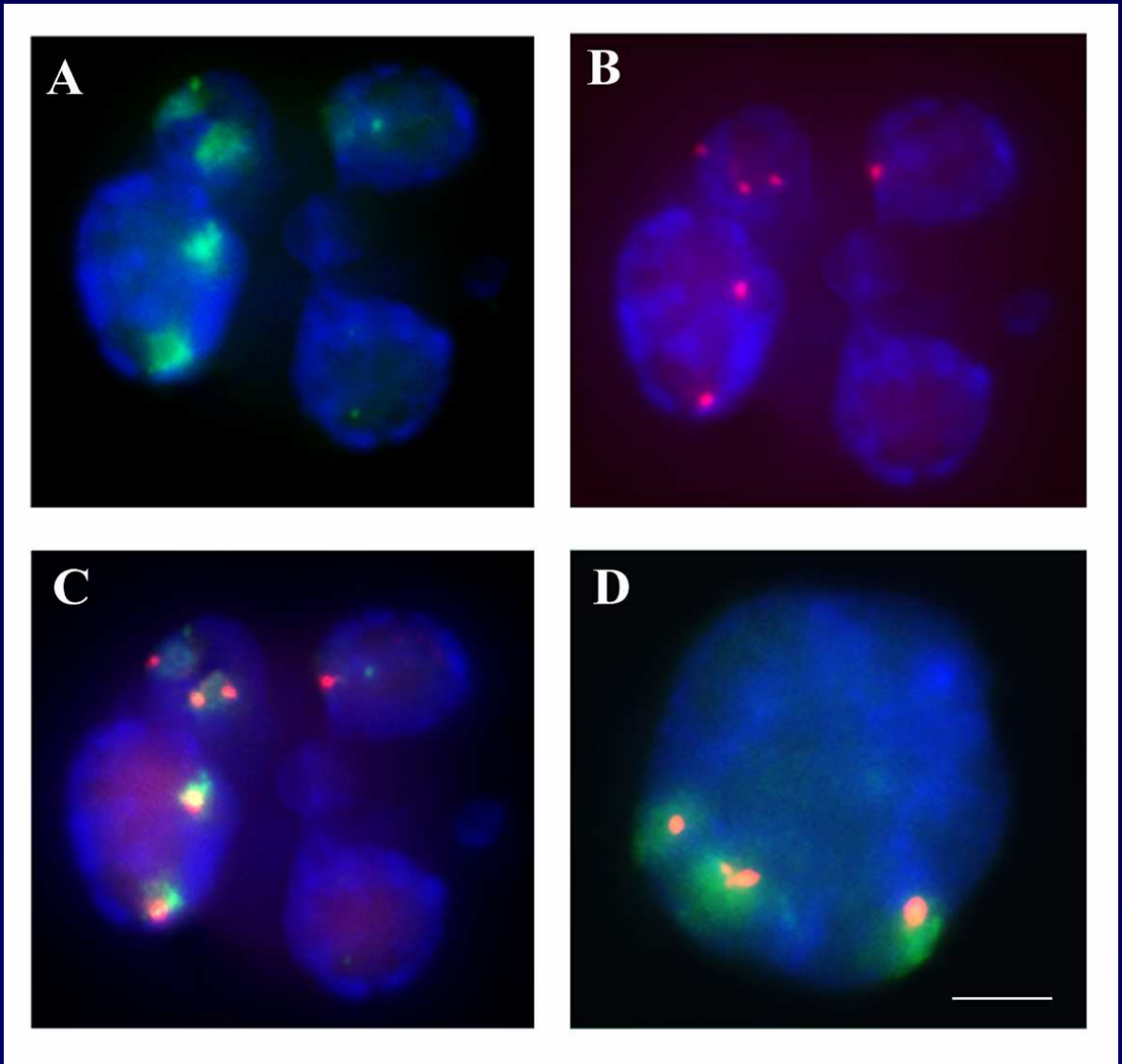
Arcitecture of chromosomal territories during apoptosis



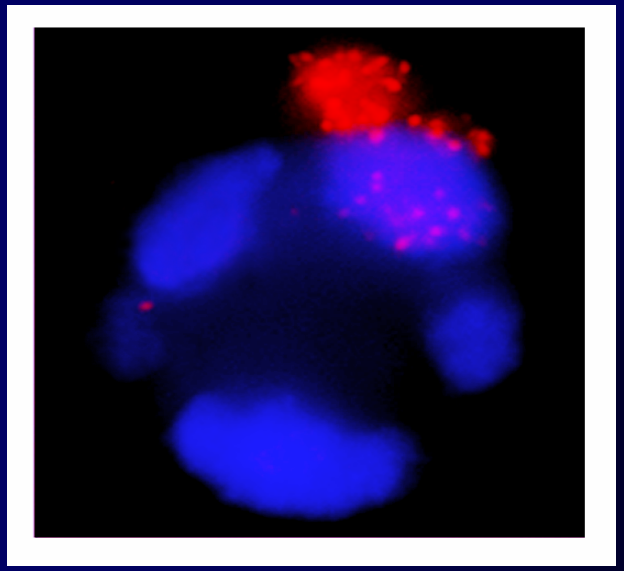
Apoptosis and HSA 21 in K-562 cells

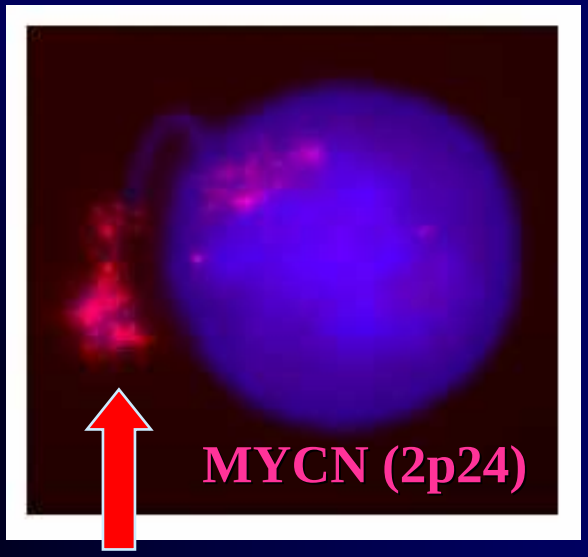







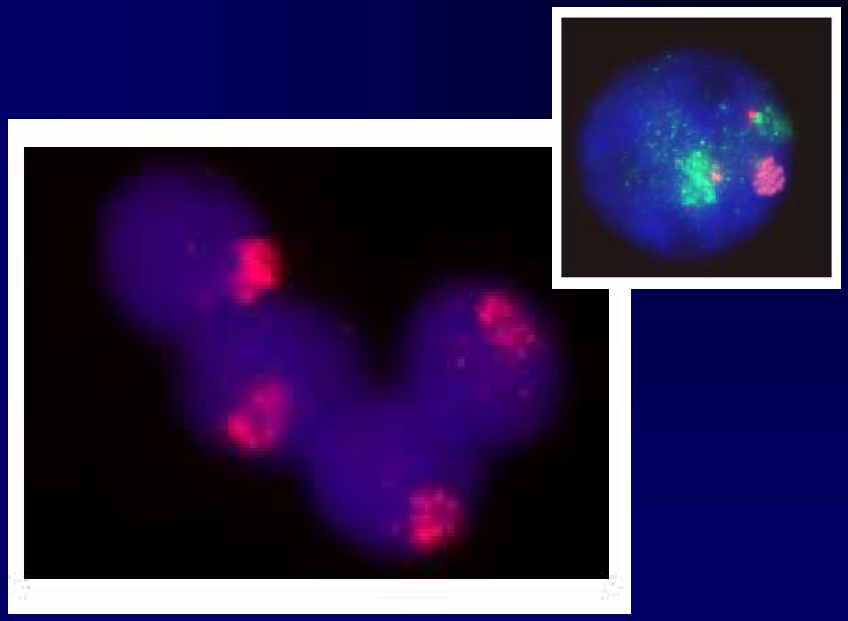
Apoptosis and chromosomal territory and centromeric region of HSA 11 in K- 562 leukemic cells

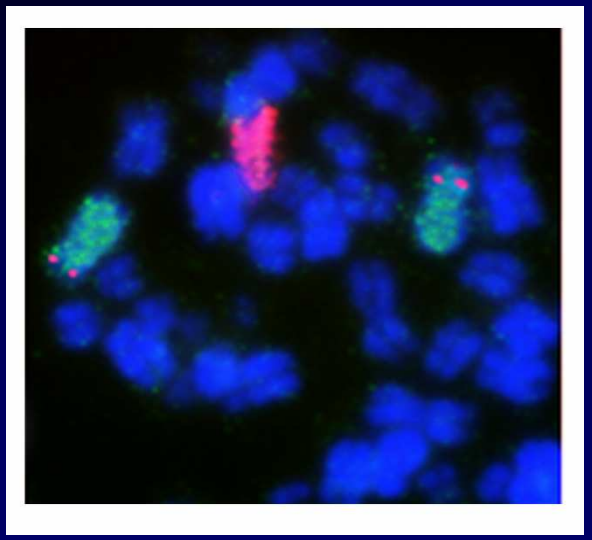


Retinoblastoma Y79 cells and HSR

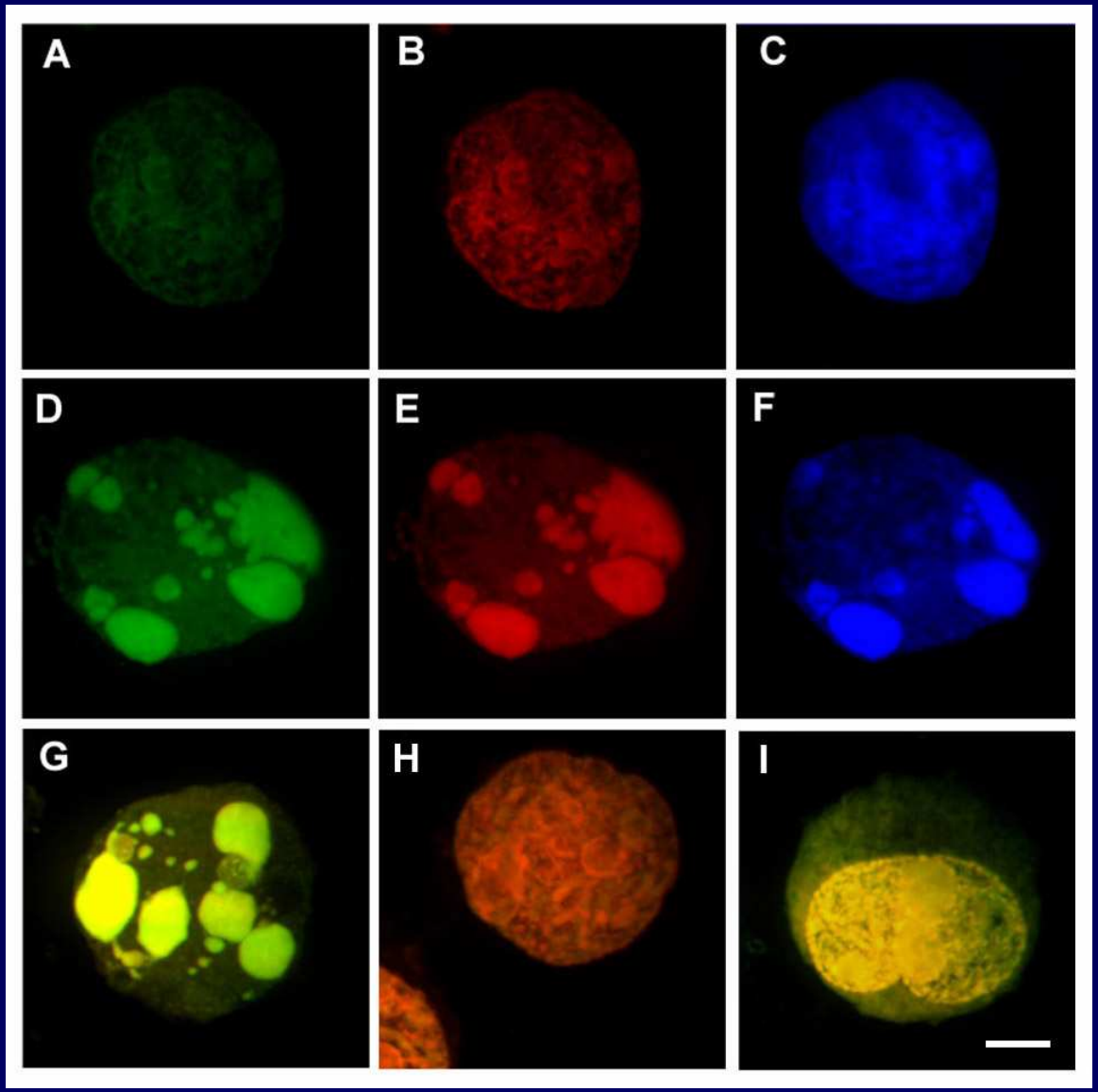




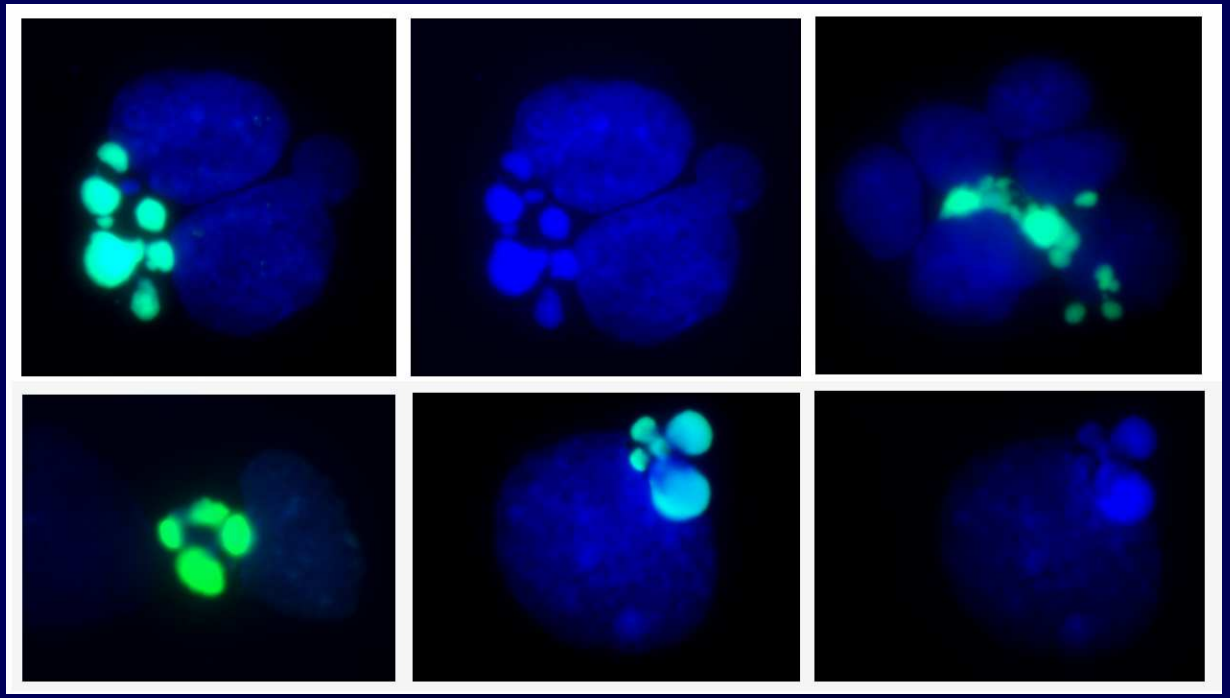


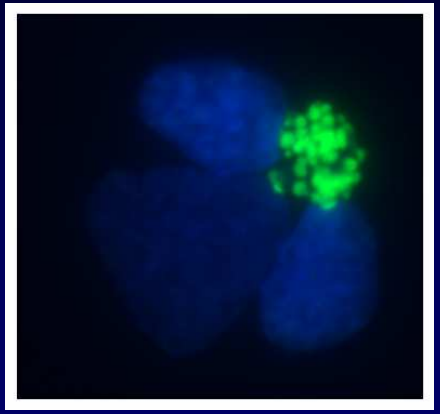


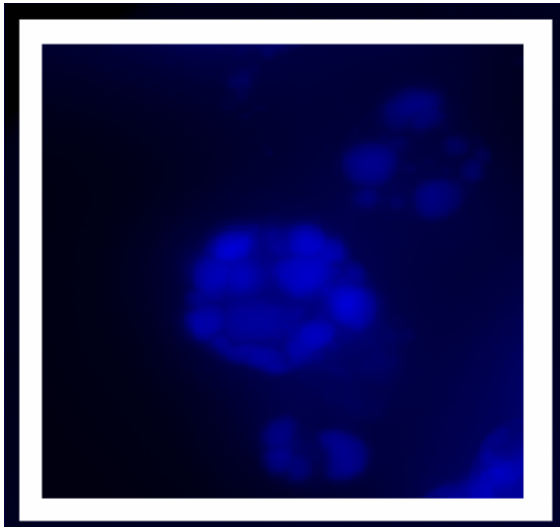
TUNEL and PI staining of fixed cells



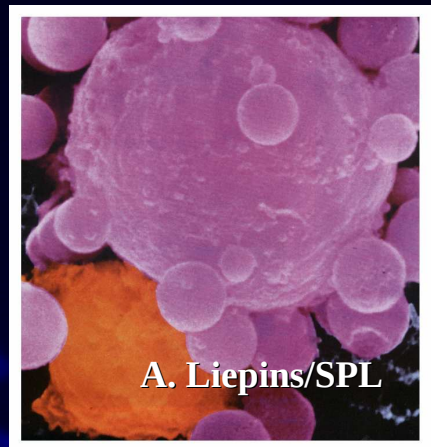
Apoptosis in patient suffering from retinoblastoma
TUNEL and DAPI staining







Conclusions



* 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.