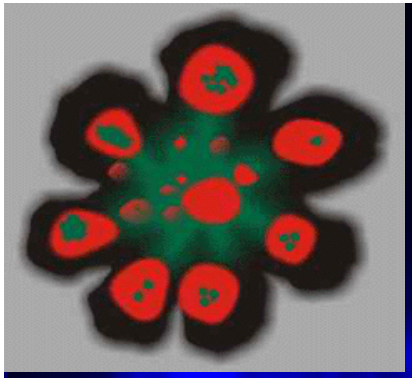
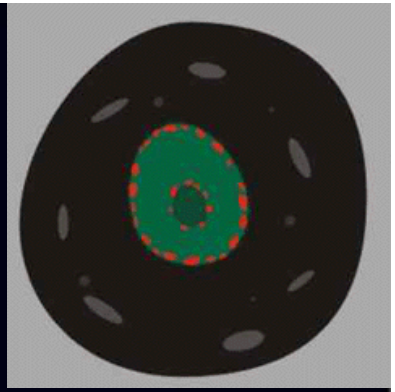
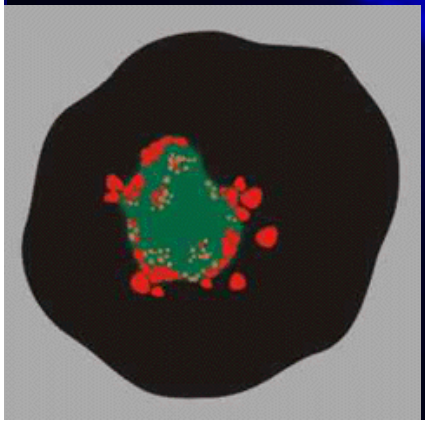


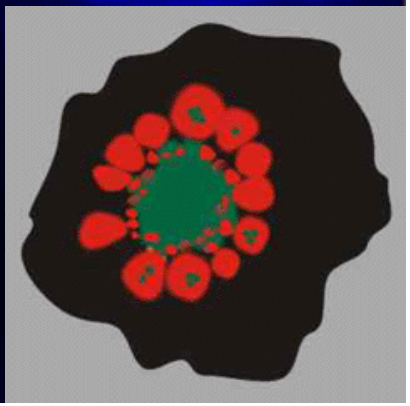


APOPTOSIS: ARCHITECTURE OF CHROMOSOMAL TERRITORIES IN APOPTOTIC CELLS

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 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-1β converting enzyme)] protease

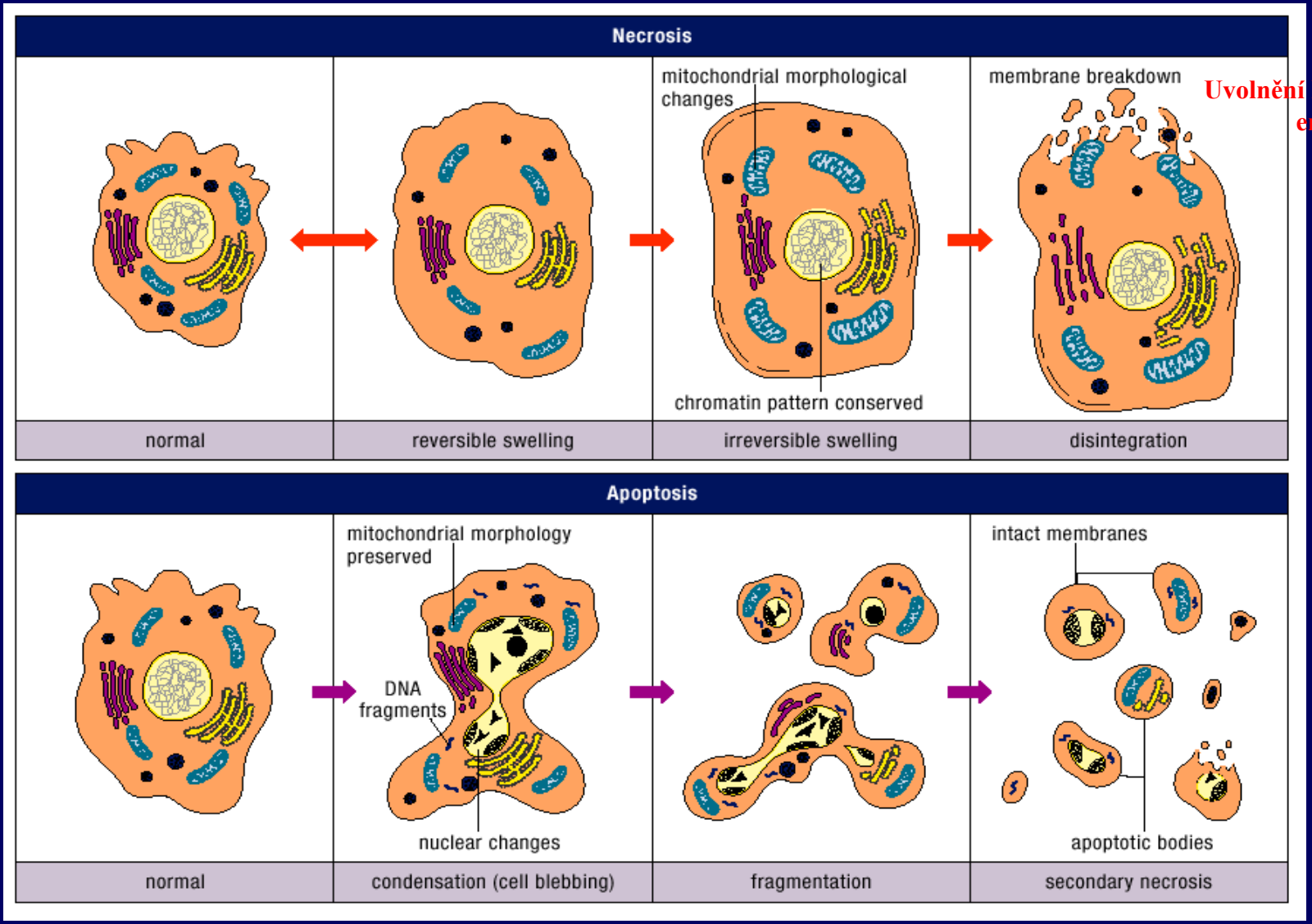
Discovery of new family of mammalian cysteine proteases - CASPASES

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

- **Development (embryonic, neuronal development)**
- **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

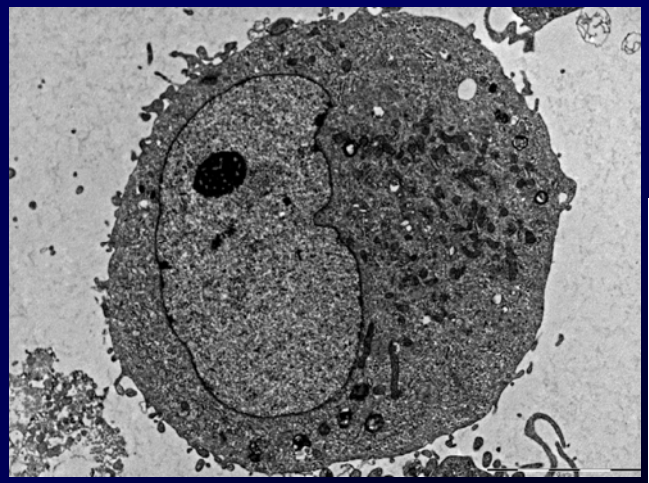
Apoptosis in contrast to necrotic cell death

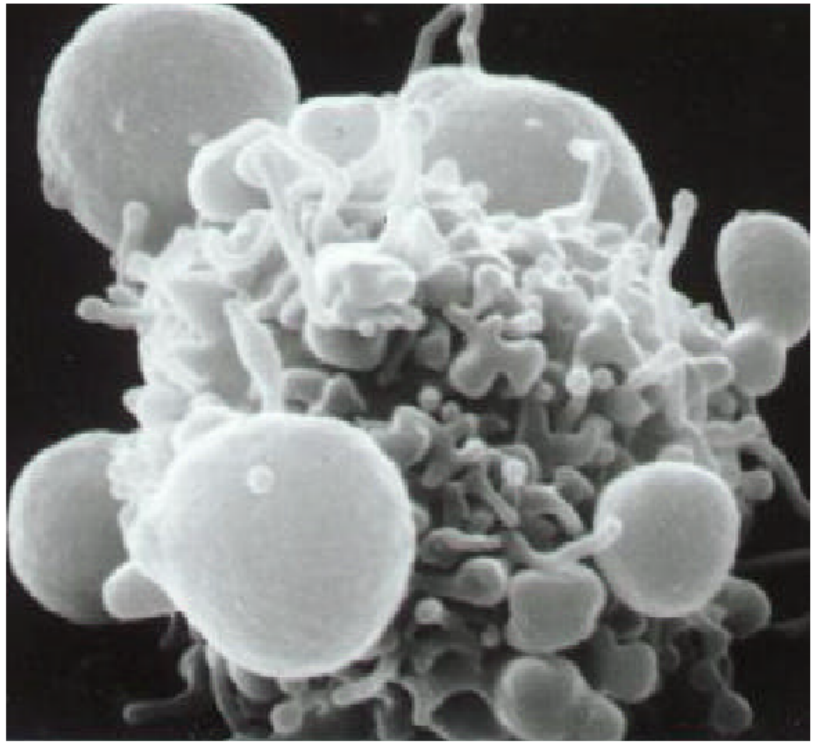


Uvolnění lysosomálních enzymů

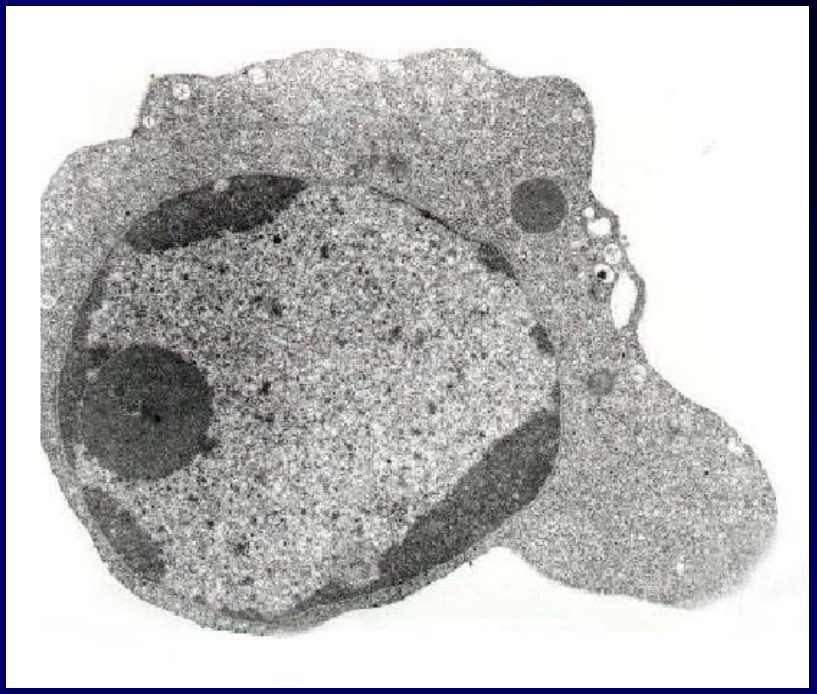
Roche: Cell Death - Apoptosis and Necrosis

Morphological features of apoptosis





Scanning electron micrograph

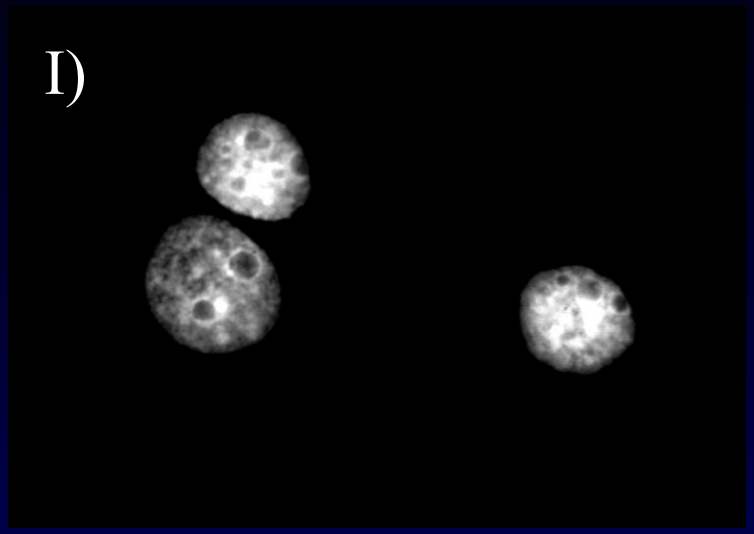


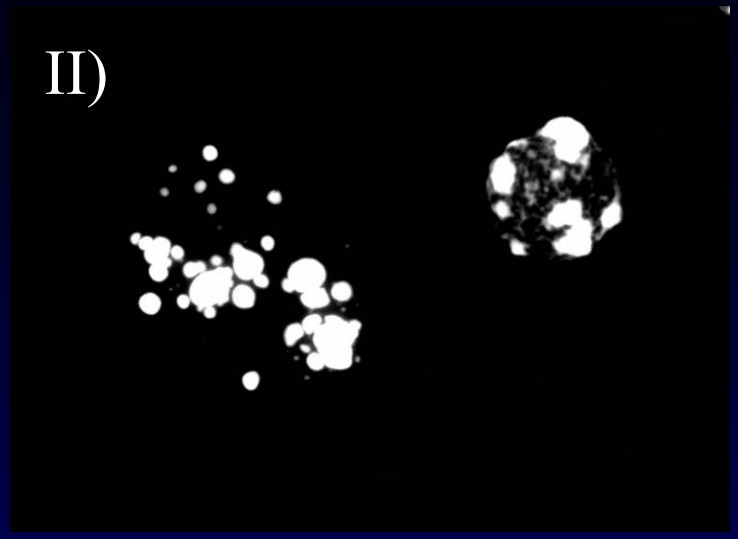
Transmission electron micrograph

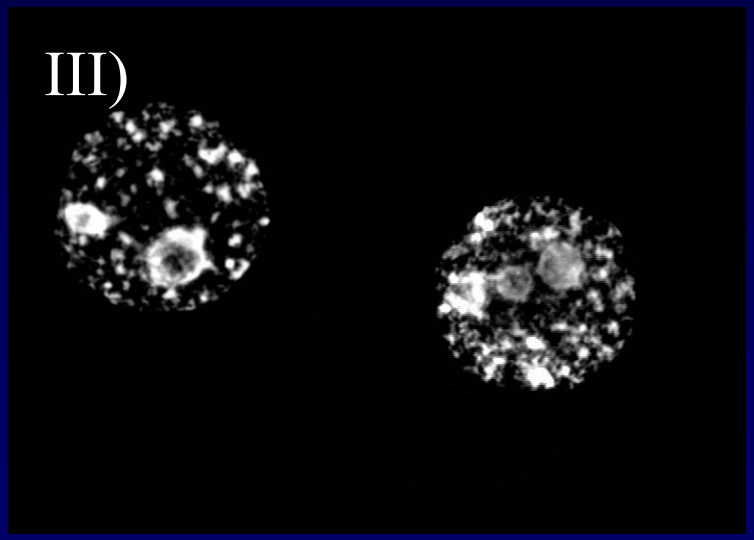
C-Knudson@uniowa.edu

Nuclear morfology in HL-60 cells

(P. Mlejnek 2001)







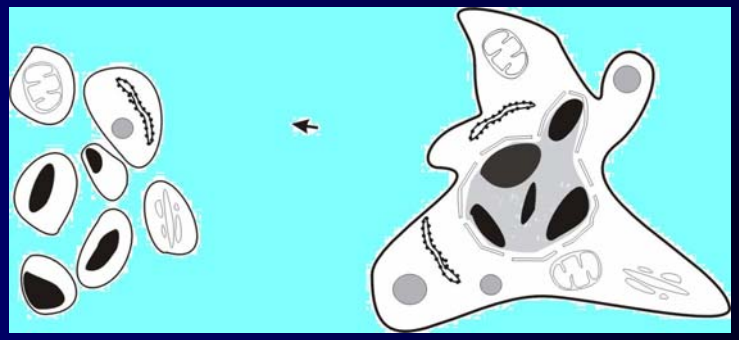
I) Control

II) Apoptosis

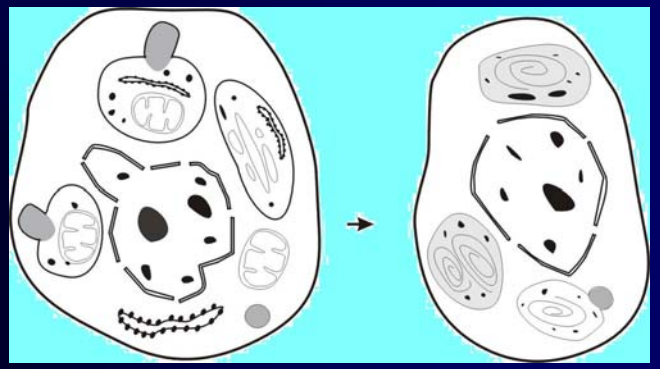
III) Necrosis

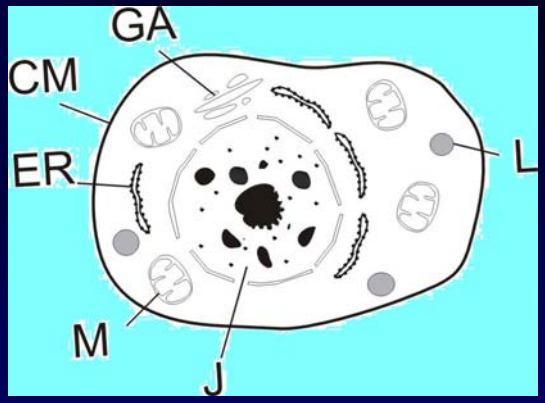
Cell death classification by Clarke

Apoptosis

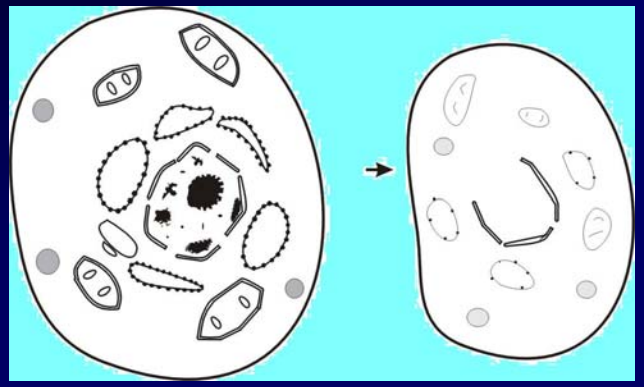


Autophagy





Nelysosomal disintegration



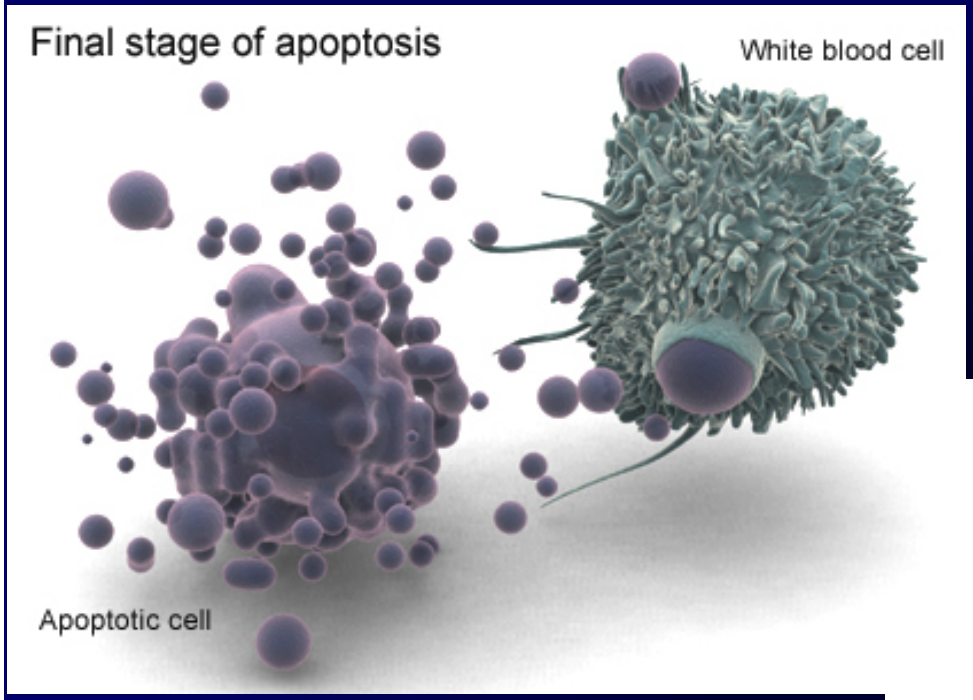
CM – cyt. membrane
J – nuclei
M – mitochondrion
ER – endopl. reticulum
GA – Golgy complex
L – lysosomes

Cell death classification by Clarke

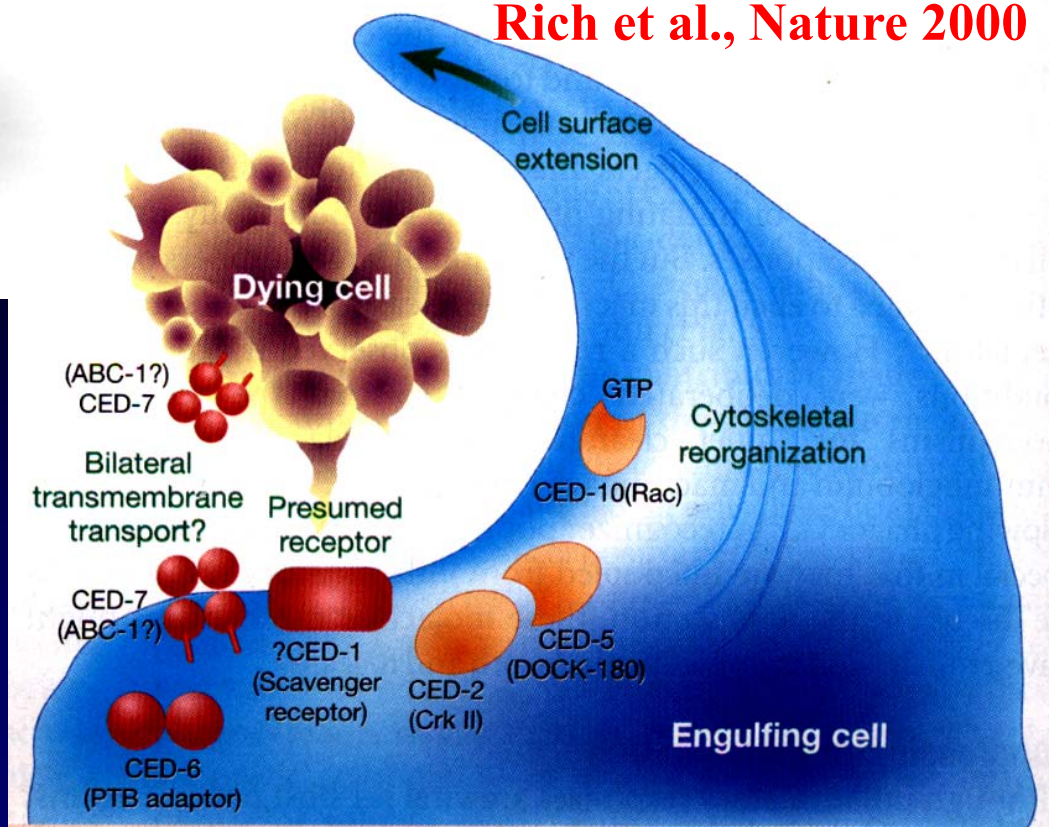
- Apoptosis - heterophagy, final cell destruction is done by lysosomes of other cells
- Autophagy - final cell destruction is done by its own lysosomes
- Nonlysosomal disintegration - cell destruction is mediated by unknown nonlysosomal proteases

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.

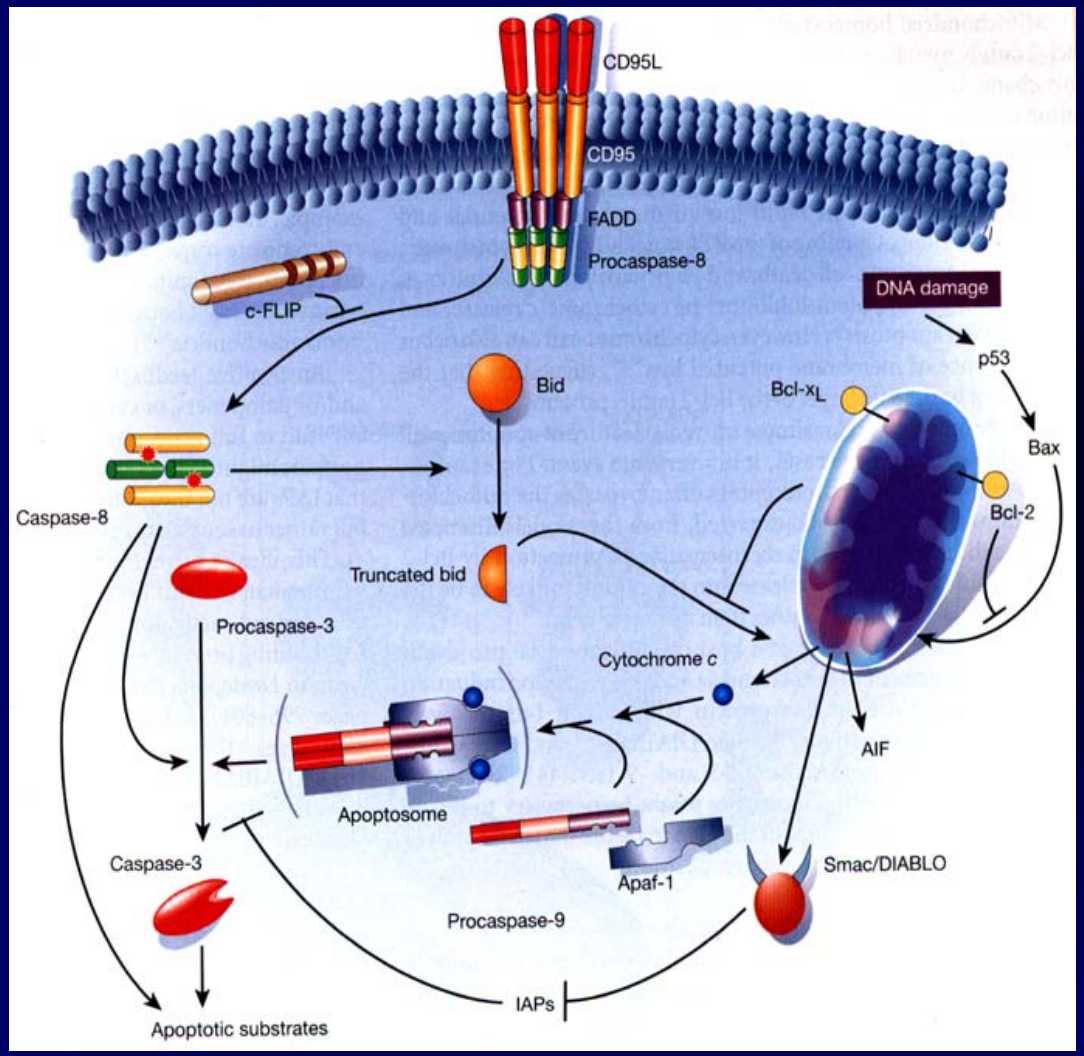
Apoptotic DNA degradation is followed by phogocytosis of apoptotic bodies



Rich et al., Nature 2000



Two major apoptotic pathways in mammalian cells

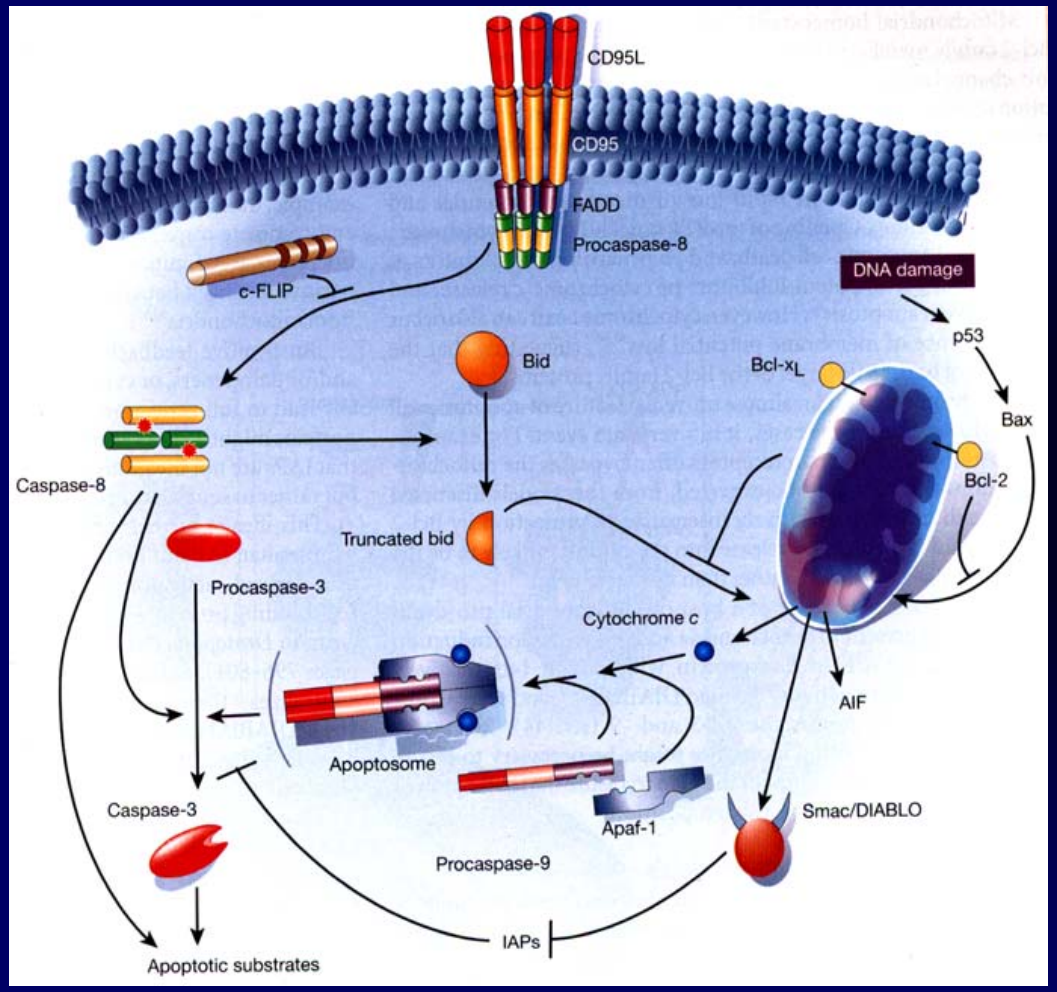


Death-receptor pathway:

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



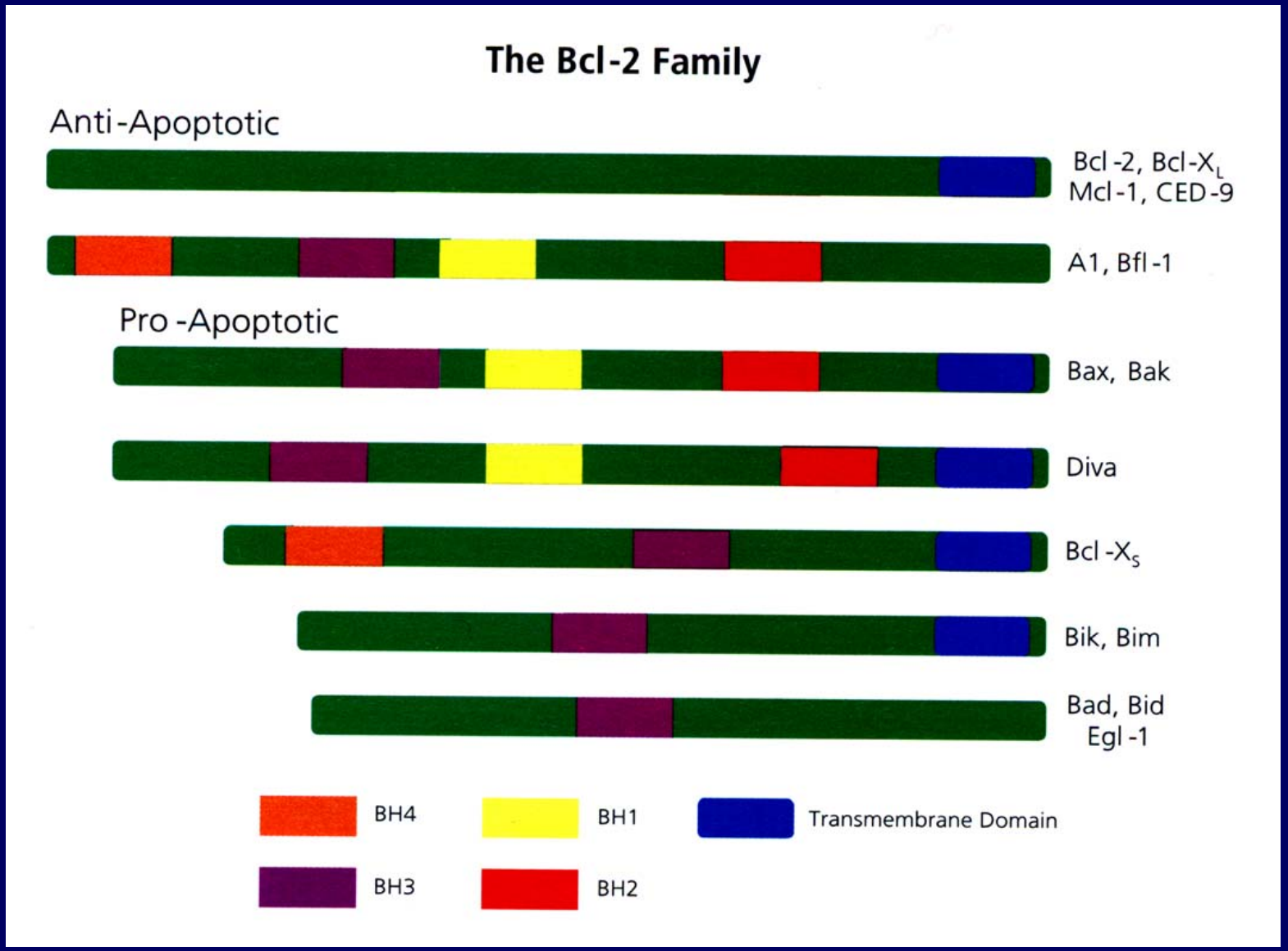
Hengartner M.O., Nature 2000

The mitochondrial pathway

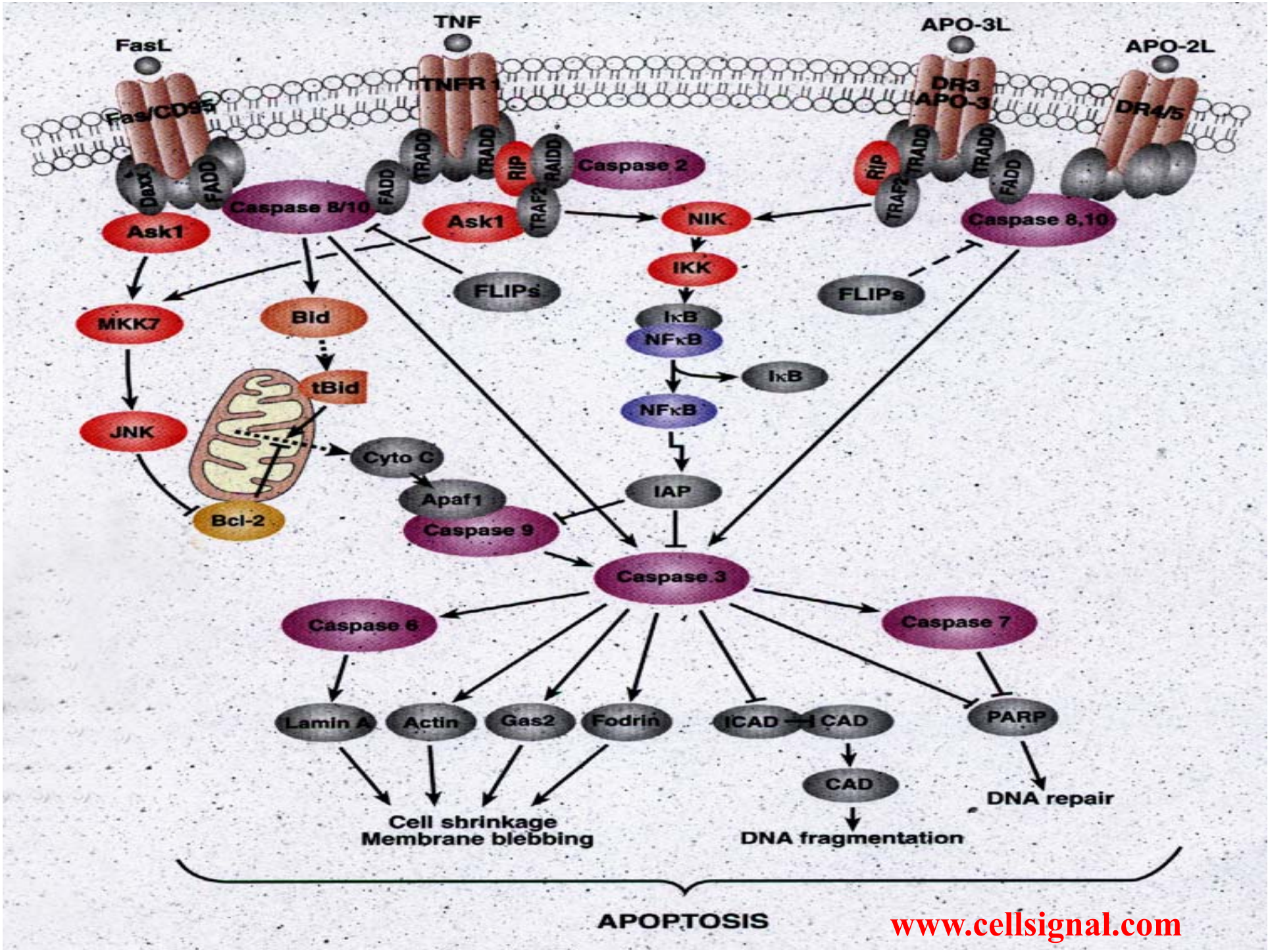
- activated after DNA damage
- proapoptotic members of Bcl-2 family, located on the surface of mitochondria, are activated
- Cytochrome c is released from mitochondria and forms complex with Apaf-1 and Procaspase 9.
- The complex is called APOPTOSOME.

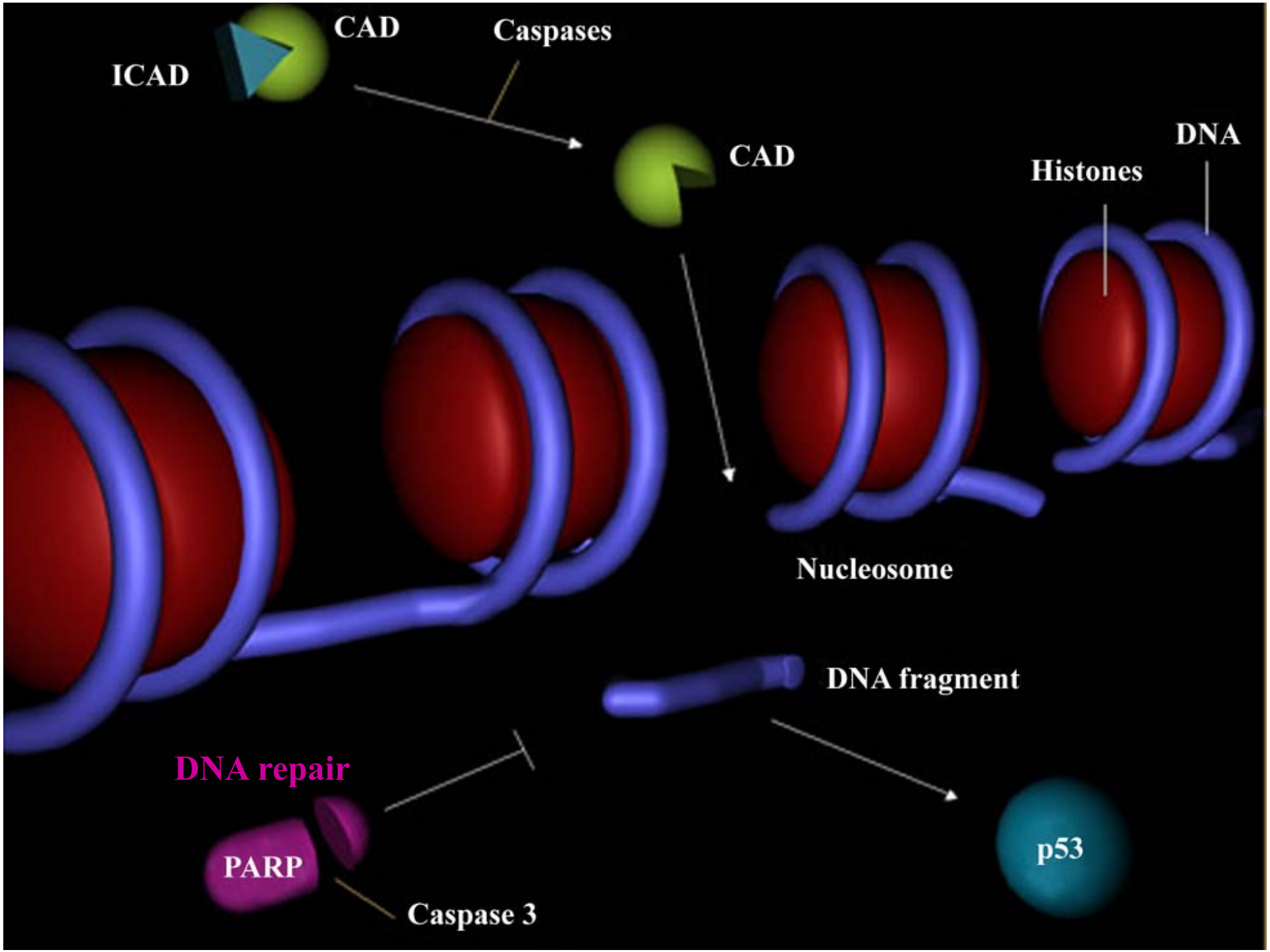
Both apoptotic pathways converge on the level of Caspase-3 activation

Caspase-3 activation is antagonized by IAP released from mitochondria



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
Ask1
NIK
IKK
FLIPs
MKK7
Bid
tBid
JNK
Cyto C
Apaf1
Caspase 9
Bcl-2
IκB
NFκB
IAP
Caspase 8,10
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


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

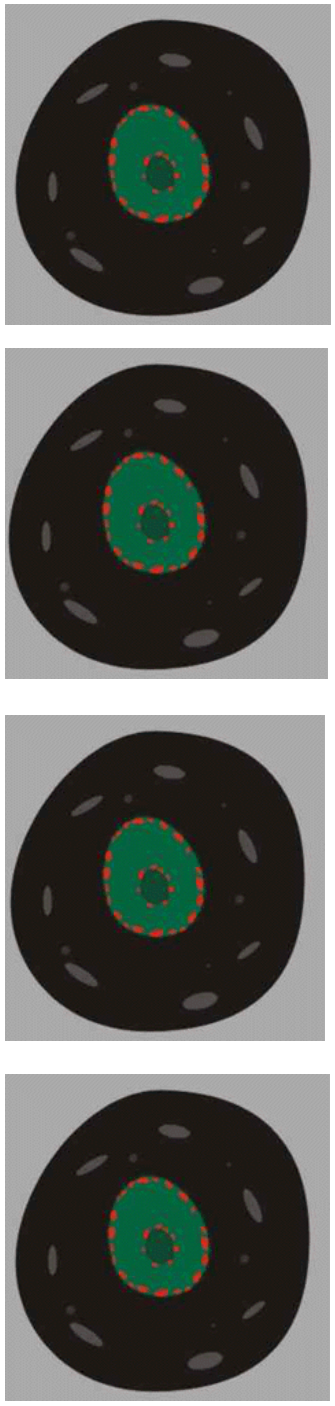
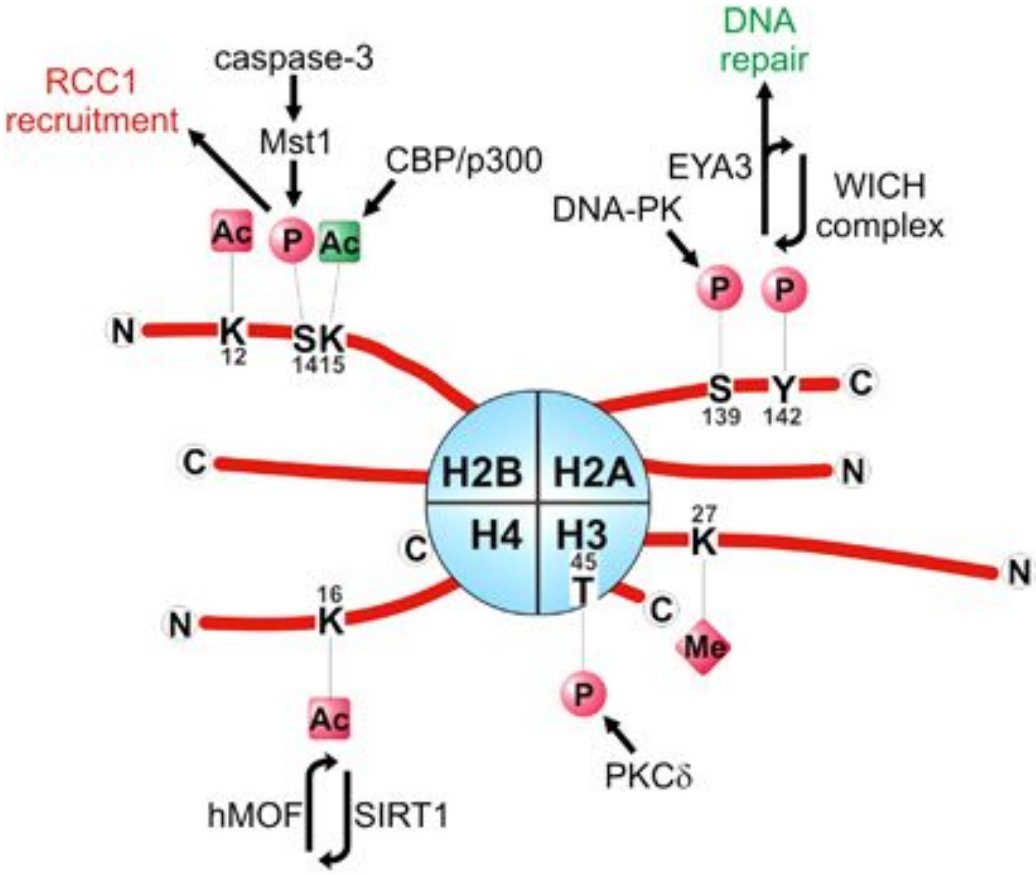
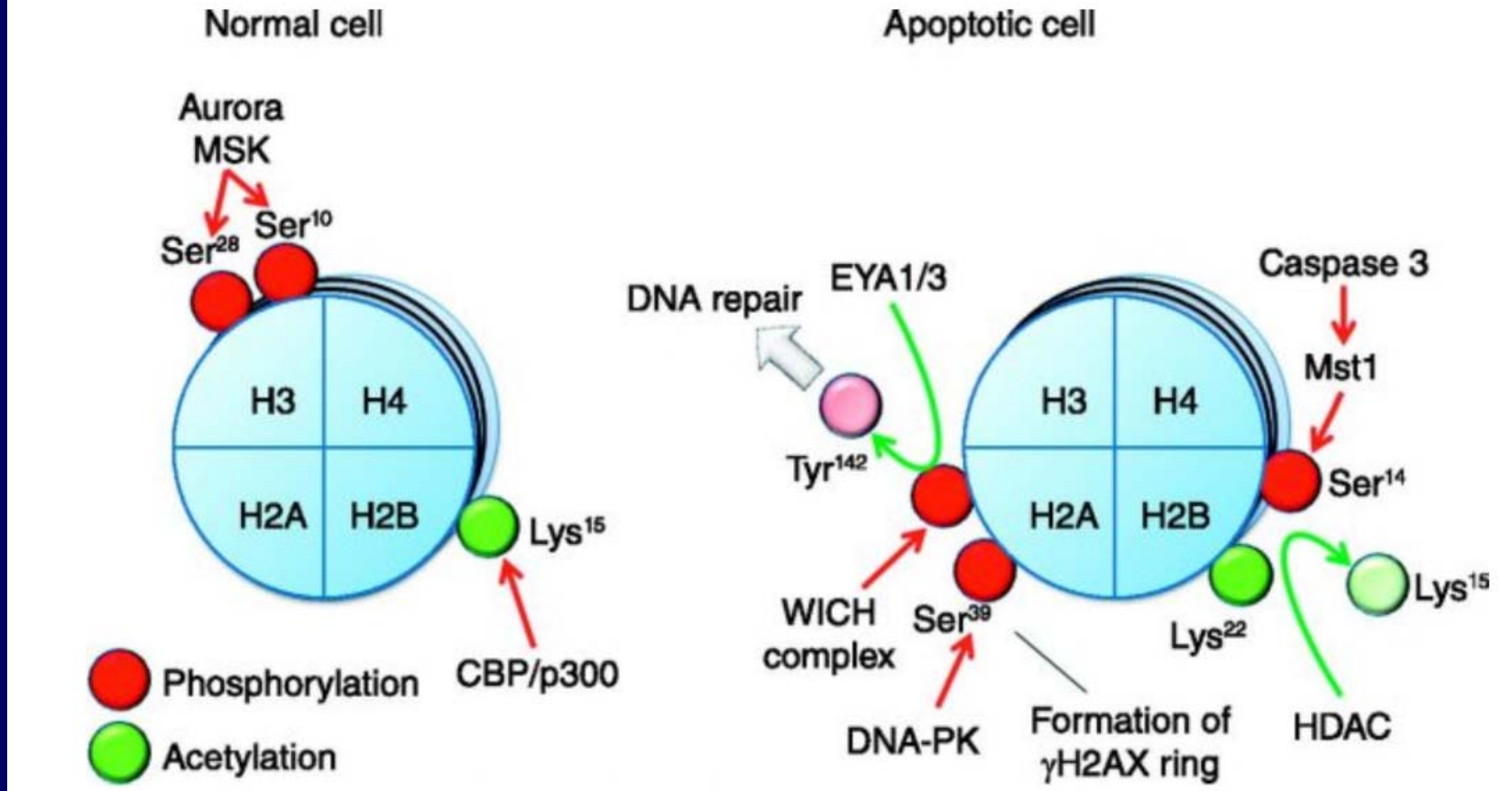
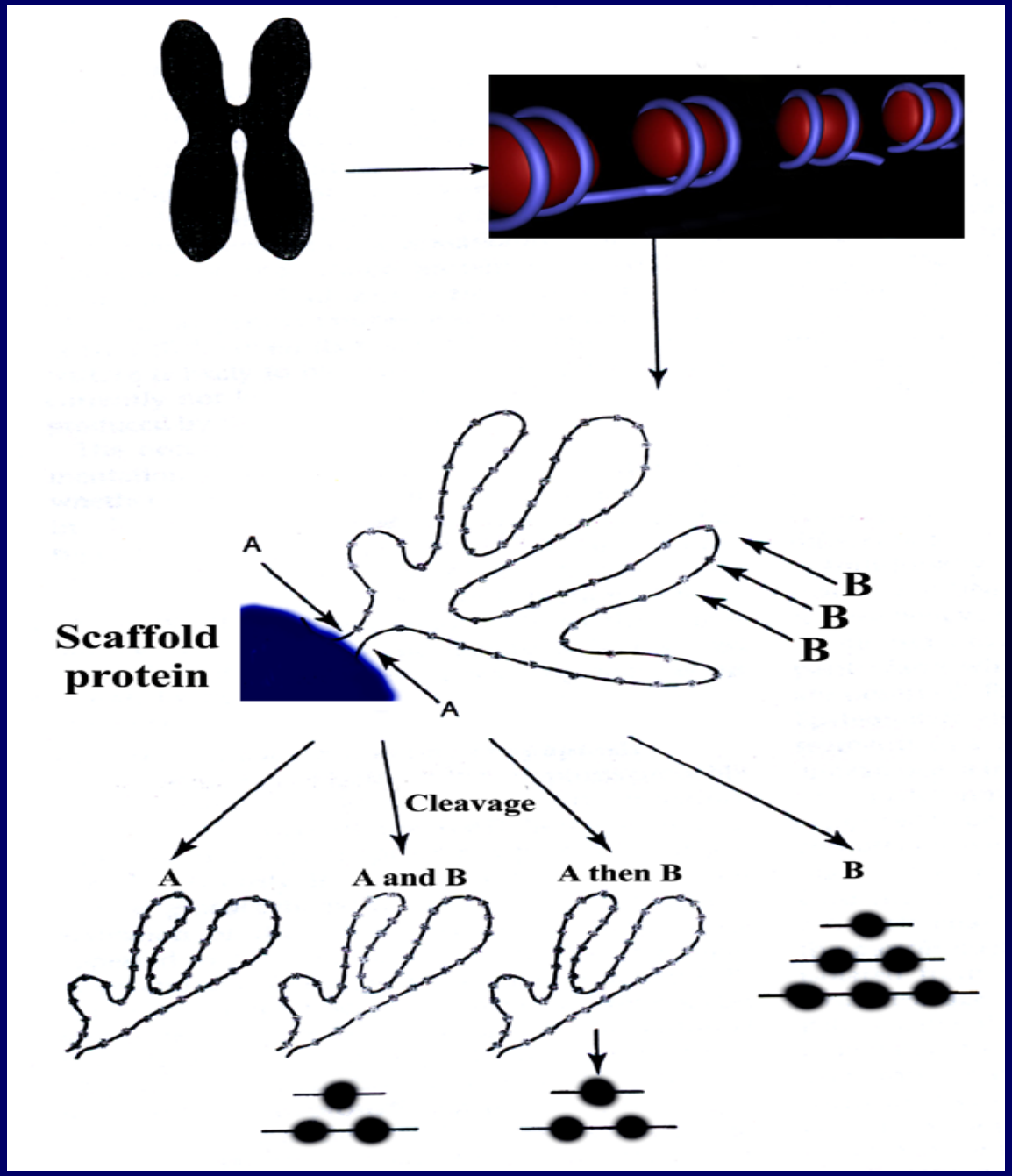


Figure 2



Apoptotic histone code. Specific histone modifications that have been shown to occur in apoptotic cells are shown in red whereas the H2B-K15ac mark, a hallmark of non-dying cells is shown in green. The enzymes reported to carry out these modifications are shown in black. ac, acetylated; me, methylated; ph, phosphorylated; ub, ubiquitinylated


Normal cell
Aurora
MSK
Ser28
Ser10
H3
H4
H2A
H2B
Lys15
CBP/p300
Phosphorylation
Acetylation
Apoptotic cell
DNA repair
EYA1/3
Caspase 3
Mst1
Tyr142
Ser14
WICH
complex
Ser39
Lys22
Lys15
DNA-PK
Formation of
γH2AX ring
HDAC

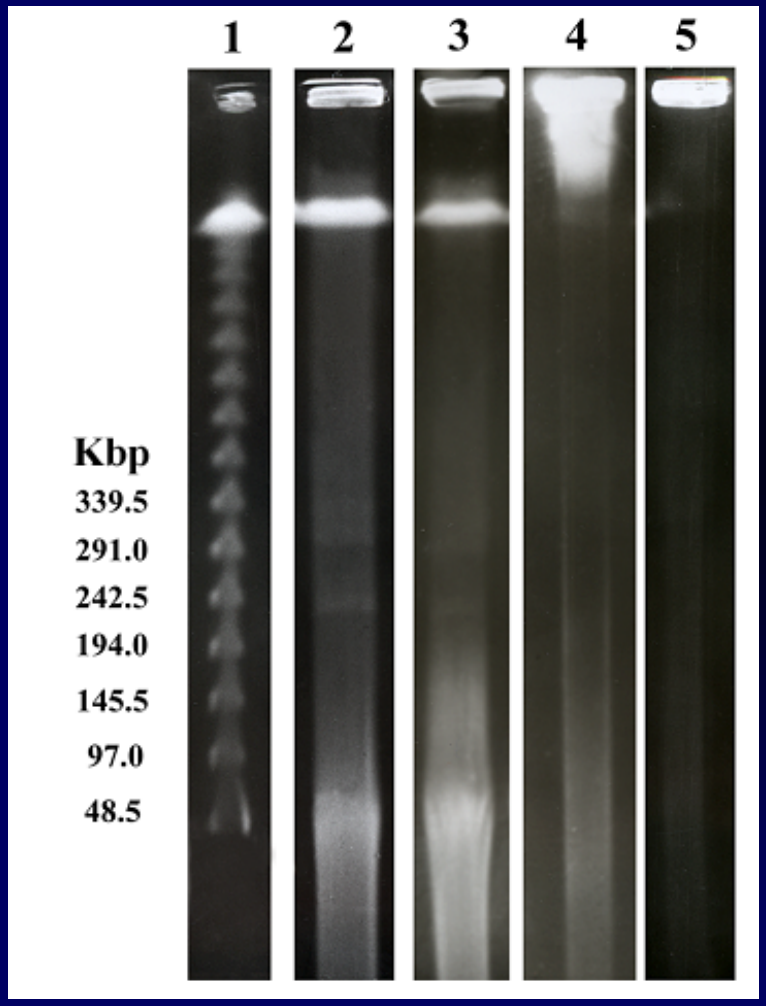


DNA fragmentation during apoptosis

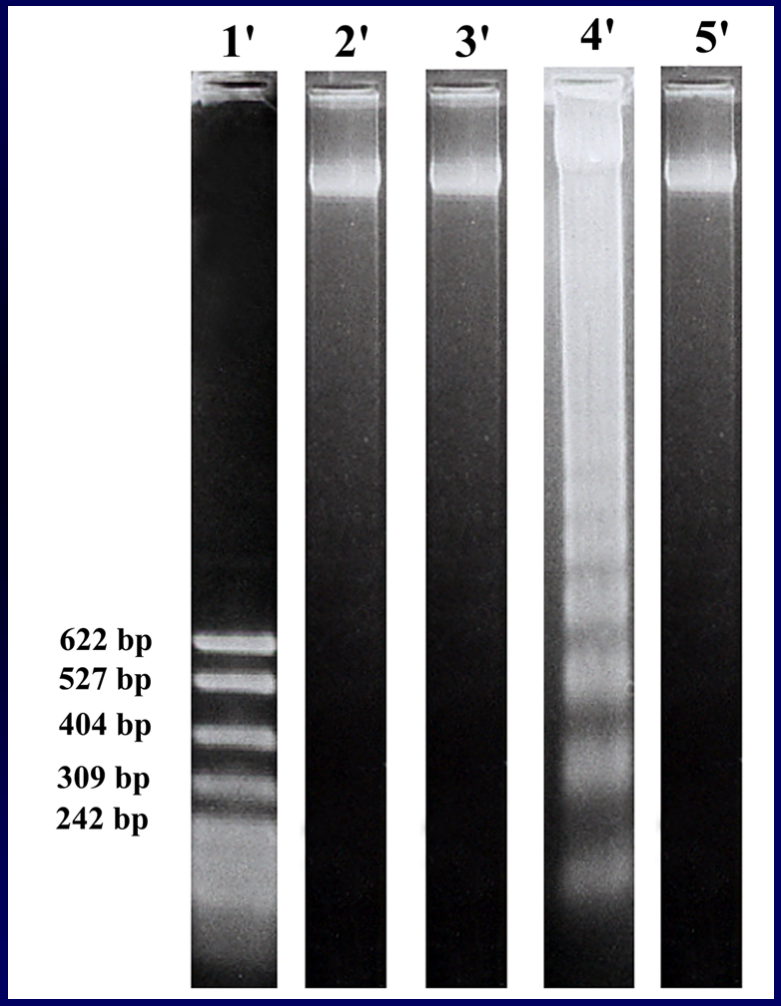
1. High molecular weight DNA fragmentation (50-300 kbp)
2. Oligonucleosomal DNA fragmentation (180-200 bp)
3. Single- strand cleavage

Bortner C.D. et al., 1995

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

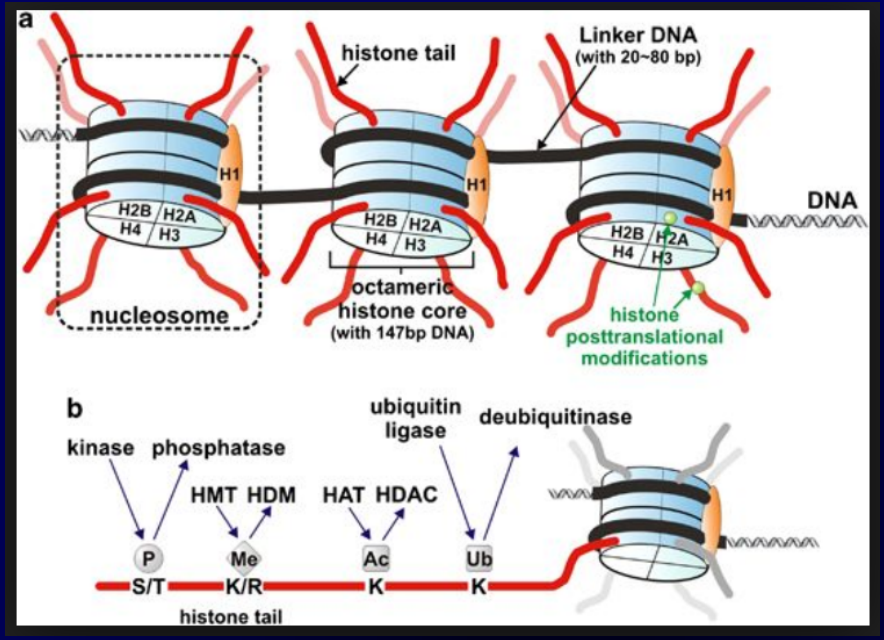


Field inversion electrophoresis (FIGE)

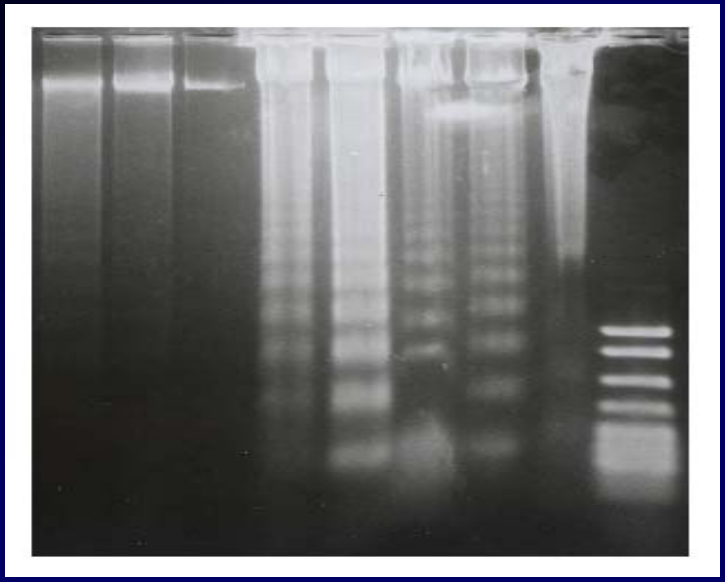


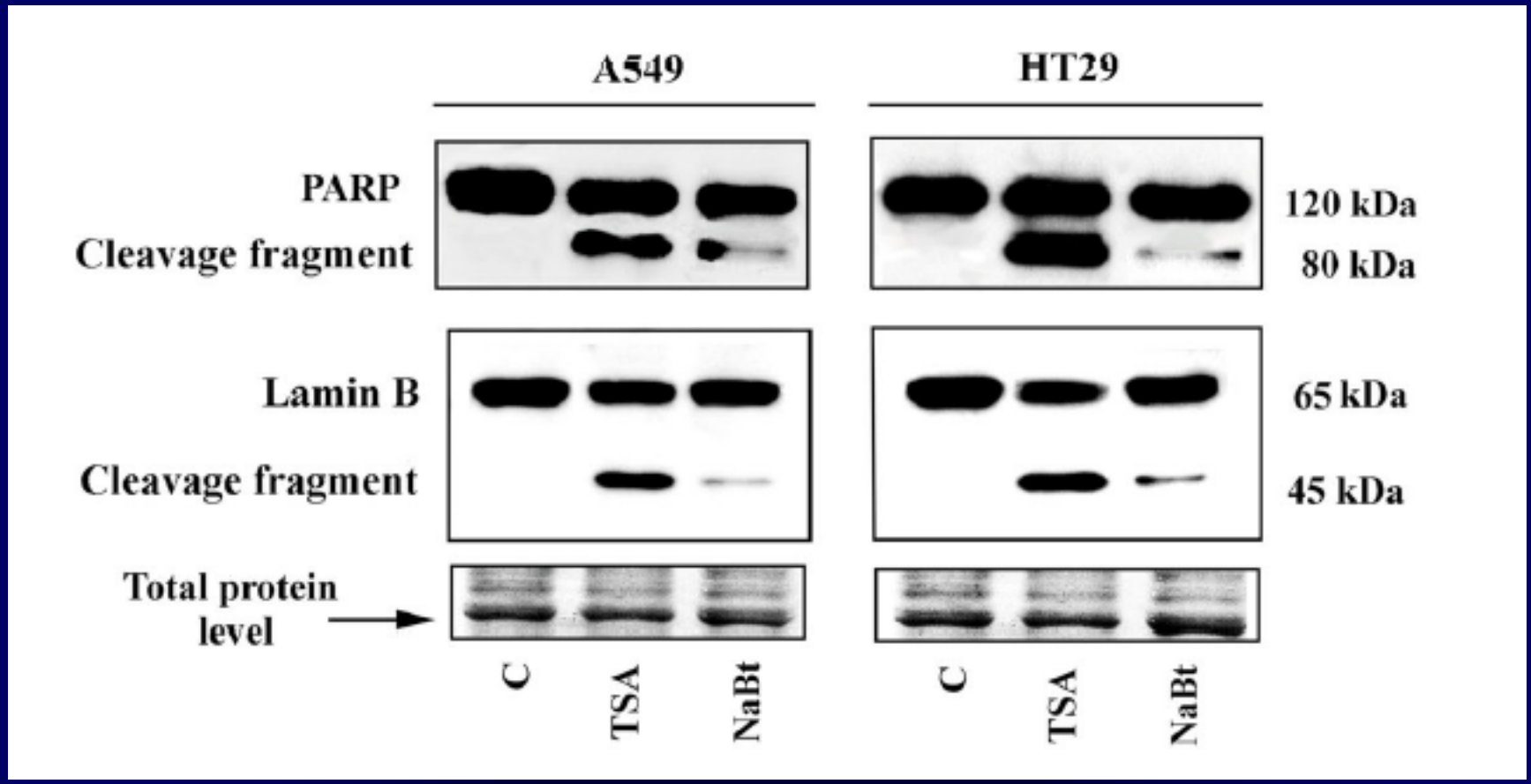
DNA fragmentation test

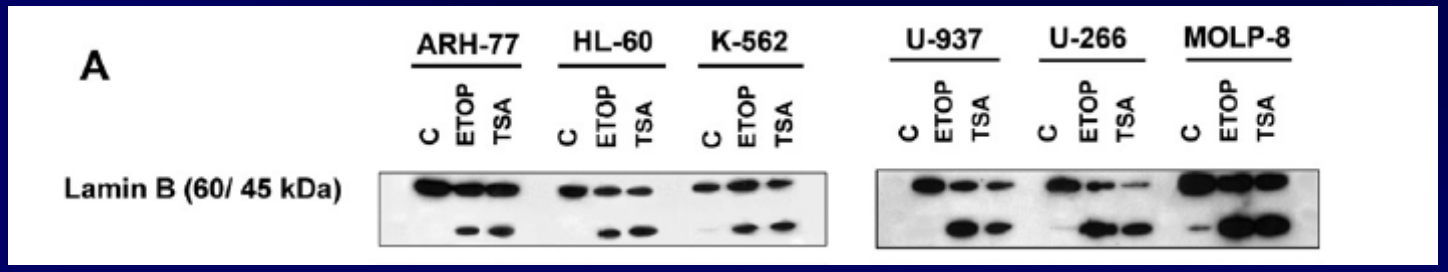
APOPTOSIS DETECTION

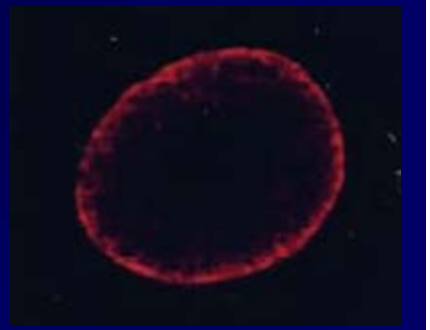


DNA fragmentation test

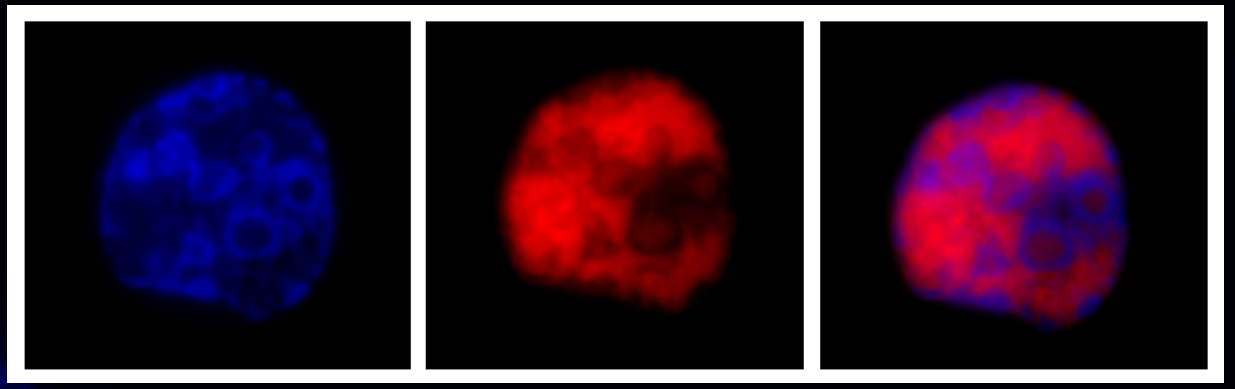


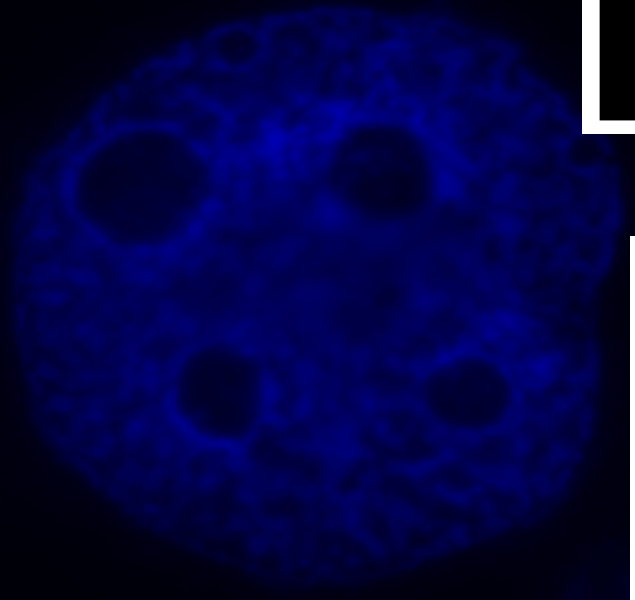

A549
HT29
PARP
Cleavage fragment
120 kDa
80 kDa
Lamin B
Cleavage fragment
65 kDa
45 kDa
Total protein level
C
TSA
NaBt
C
TSA
NaBt

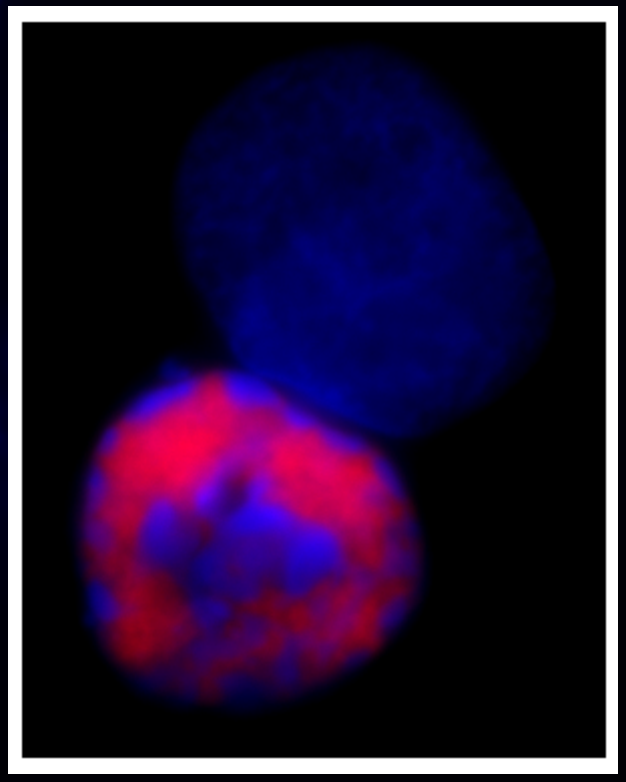

A
ARH-77
HL-60
K-562
U-937
U-266
MOLP-8
C
ETOP
TSA
Lamin B (60/ 45 kDa)

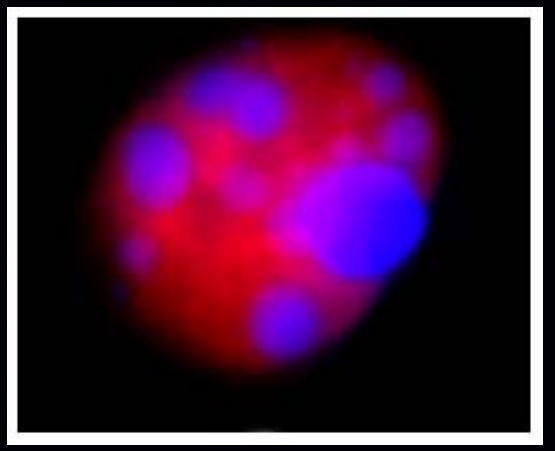


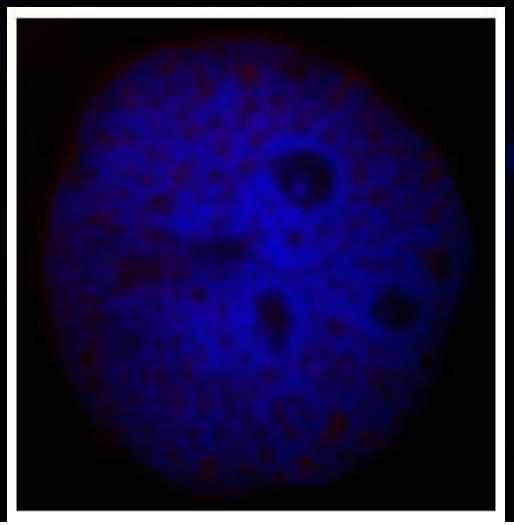
Anti-PARP p85
fragment pAb







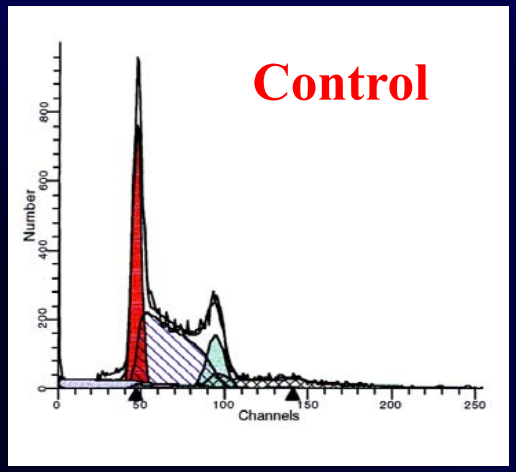


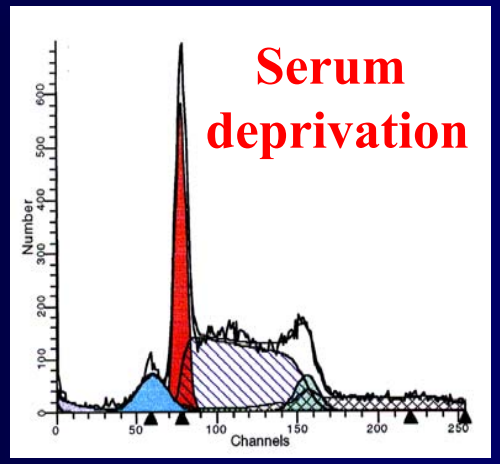


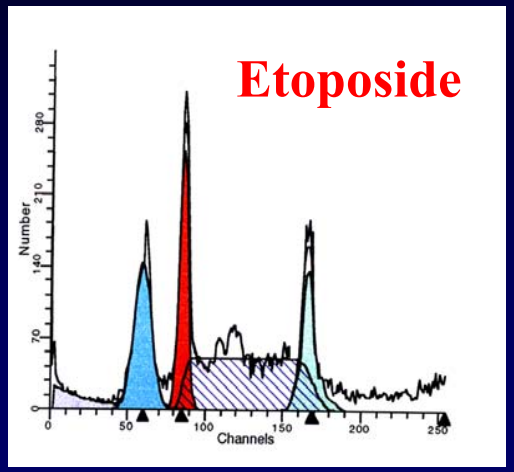
Poly(ADP-ribosyl)ation
and apoptosis

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



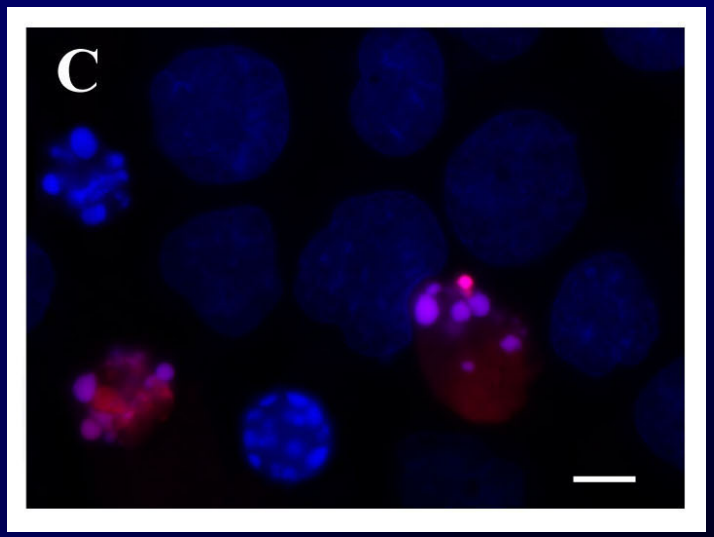


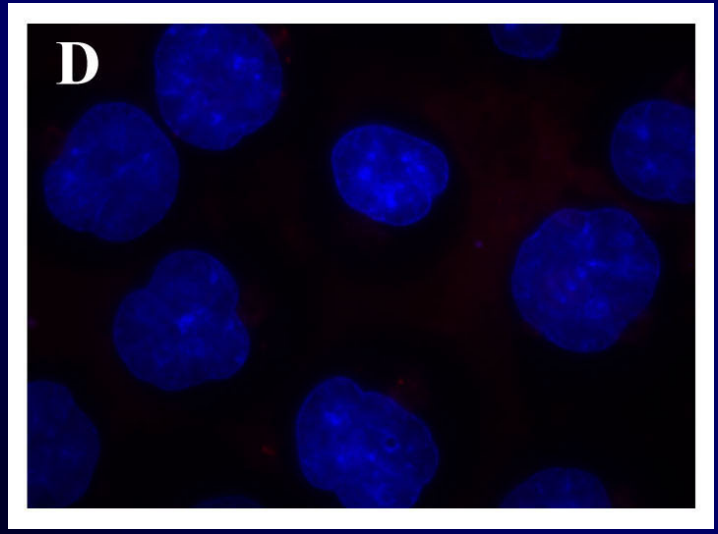


Hoechst33342 / PI

Etoposide

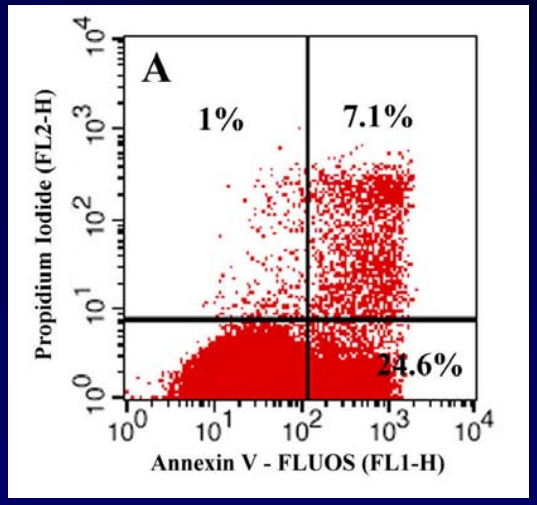
Control

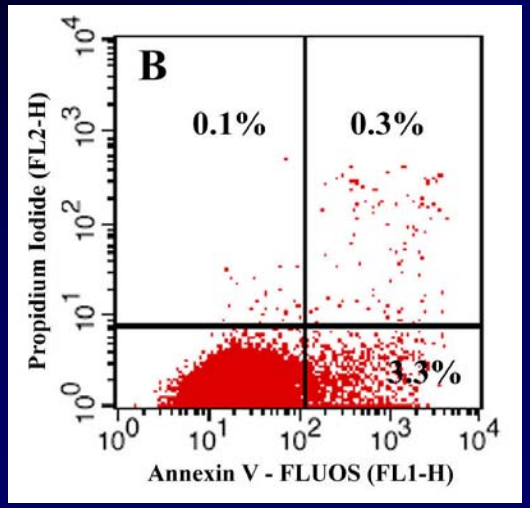




Anexin V binds to phosphatidylserines that are traslocated from the inner side od the plasma membrane to the cell surface soon after the induction of apoptosis

Annexin V / PI

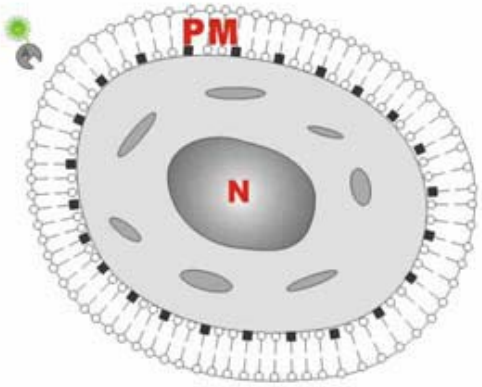




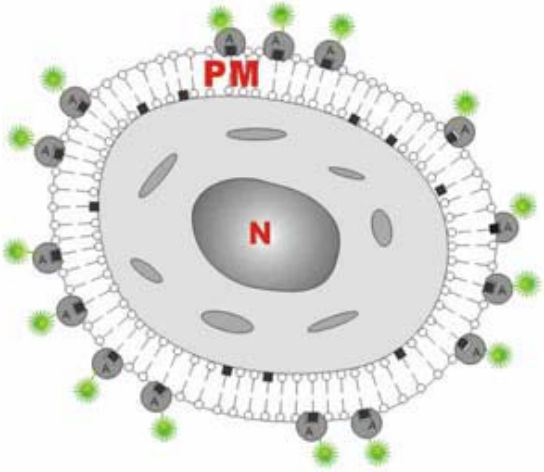
Phospholipid

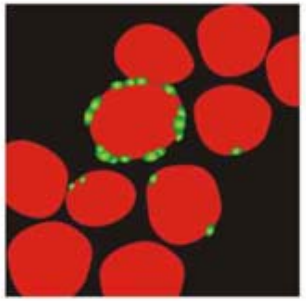
Phosphatidylserin

Annexin
with a green fluorescent label



NORMAL, HEALTLHY CELL



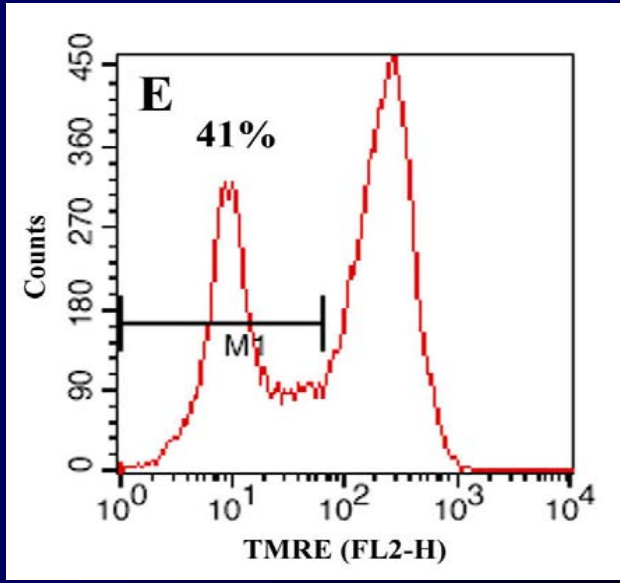


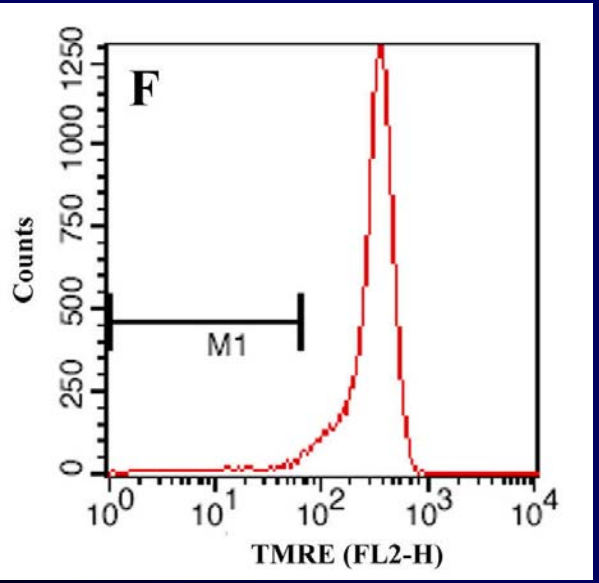
APOPTOTIC CELL

TMRE

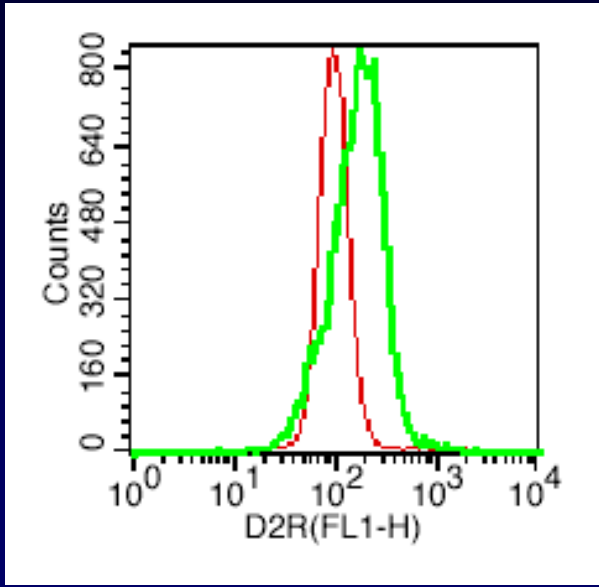
Etoposide

Control





CaspSCREEN (tm)
BioVision kit



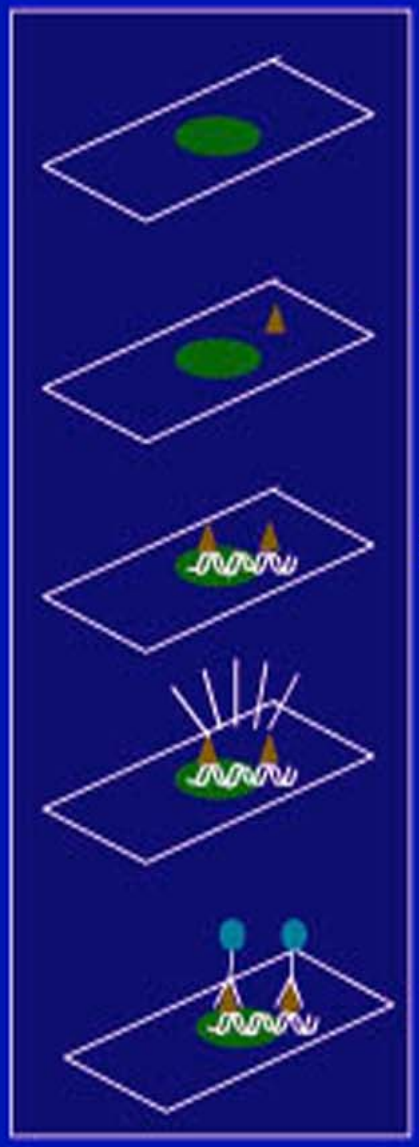

Esc
Ext
Enter
Power
Leica

DNA Fragmentation - TUNEL

In Situ Cell Death Detection Kit - Test Principle

Roche

Diagnostics



cells on slide

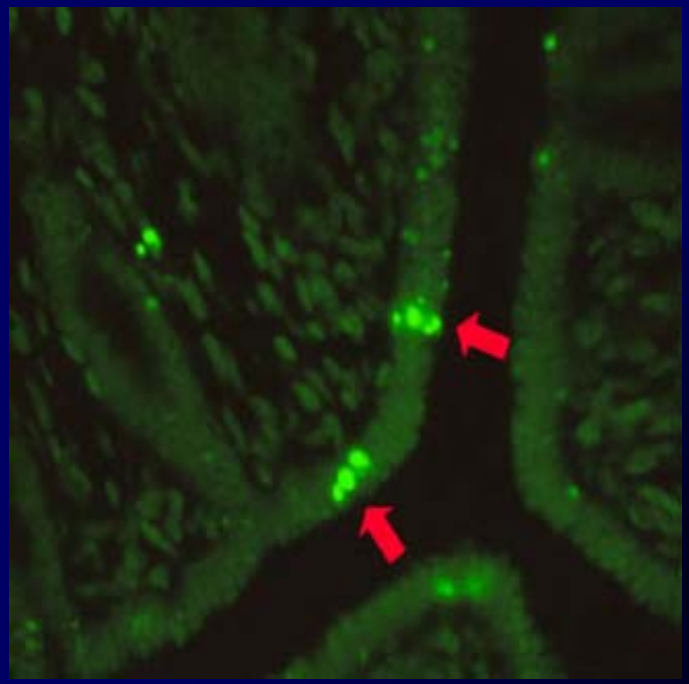
Add TdT and FITC-dUTP to cells

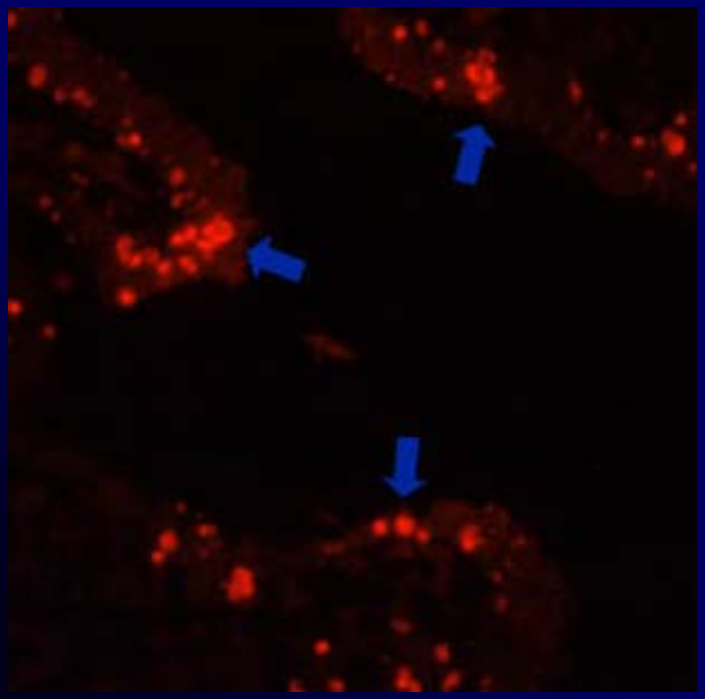
FITC-dUTP incorporated into DNA

Fluorescence microscopy

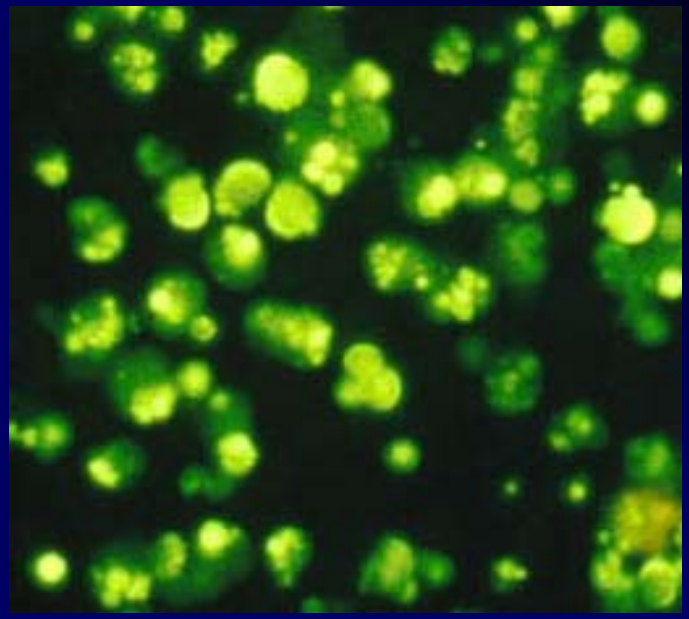
<FITC>POD or <FITC> AP

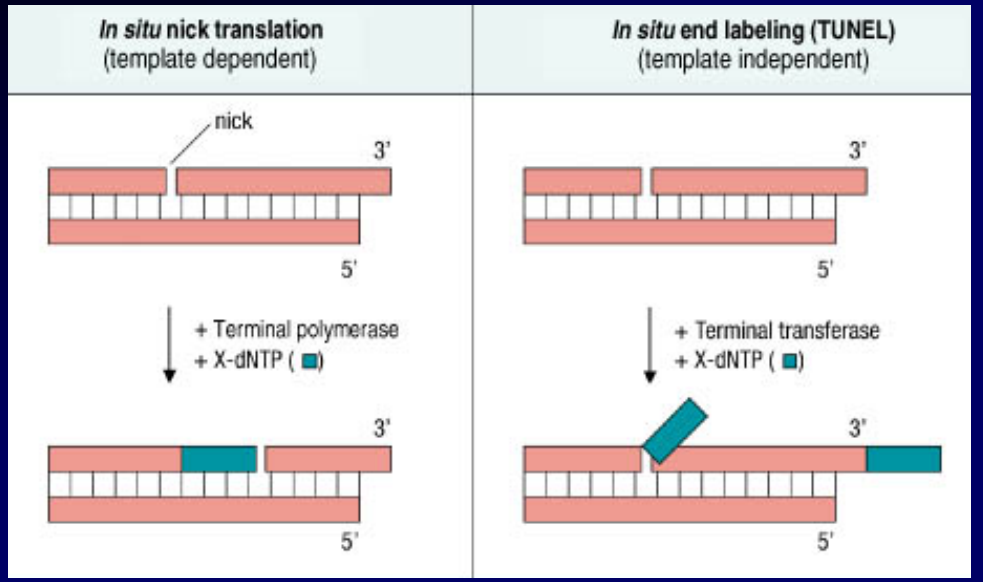
Light microscopy

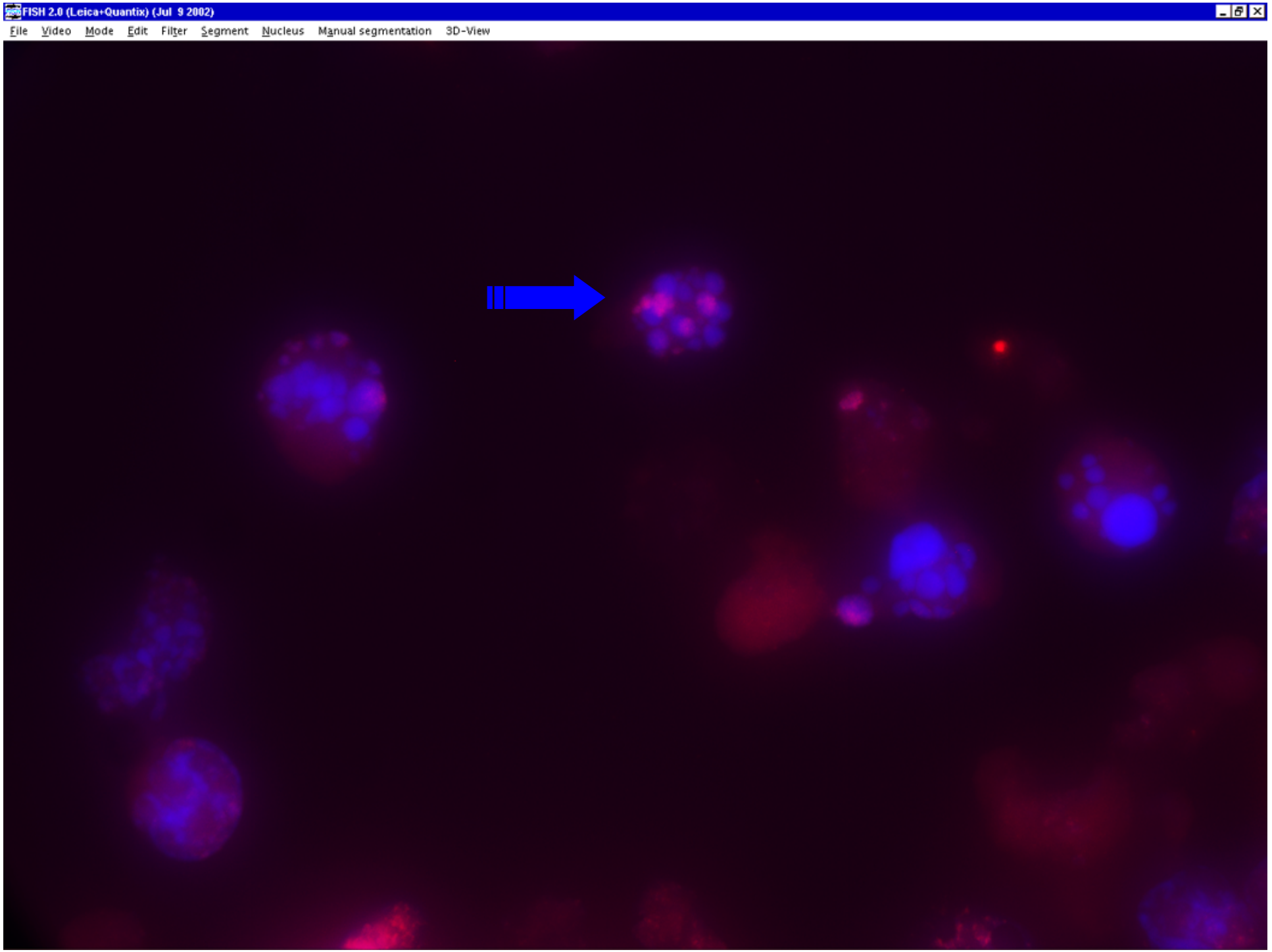


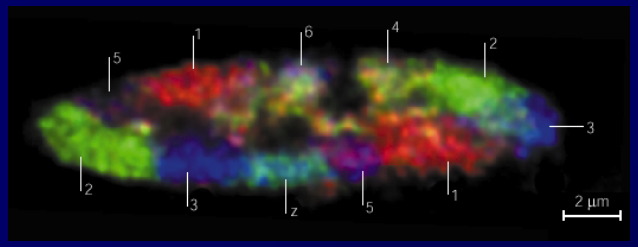


Roche

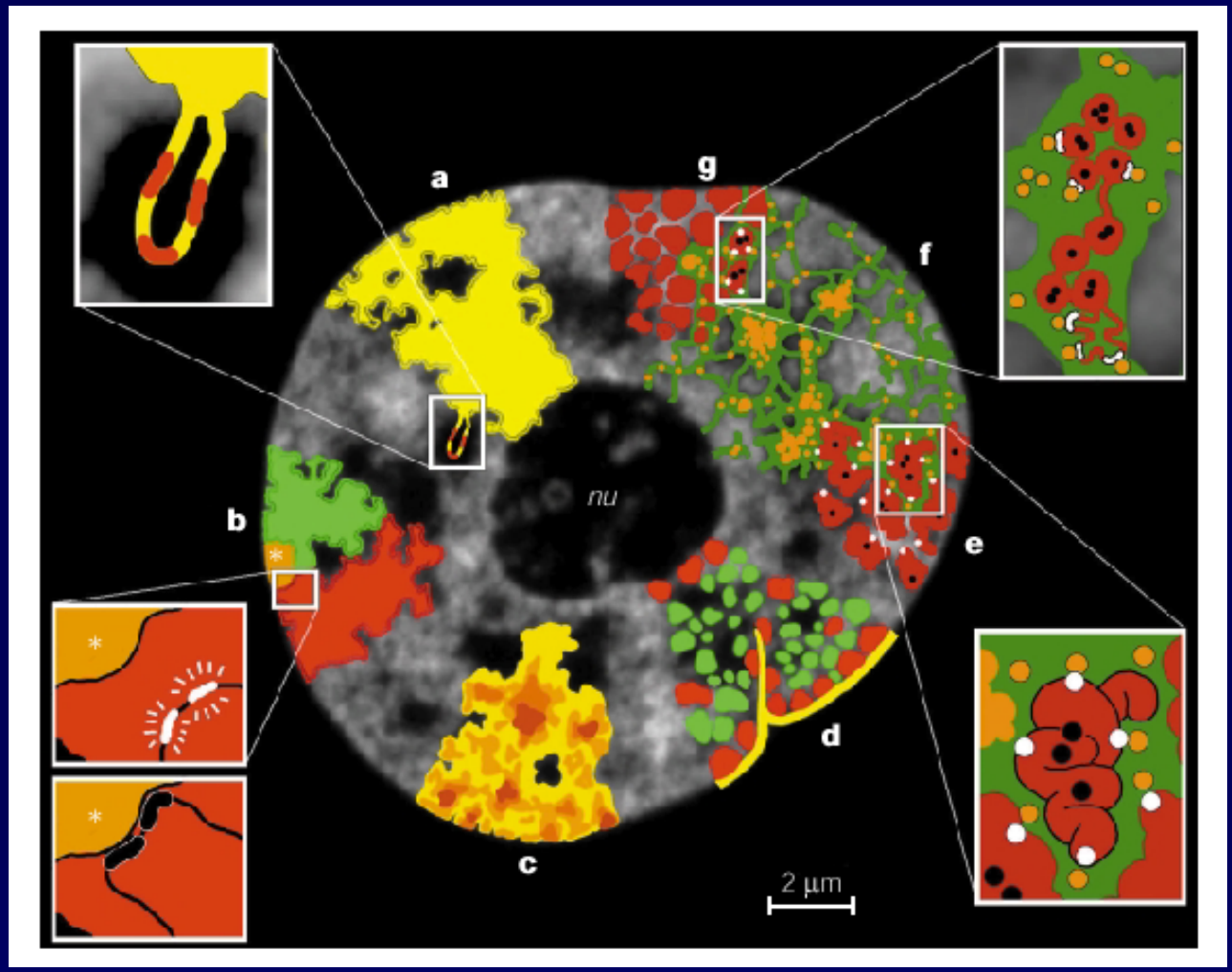



In situ nick translation
(template dependent)
nick
3'
5'
+ Terminal polymerase
+ X-dNTP ( )
3'
5'
In situ end labeling (TUNEL)
(template independent)
3'
5'
+ Terminal transferase
+ X-dNTP ( )
3'
5'

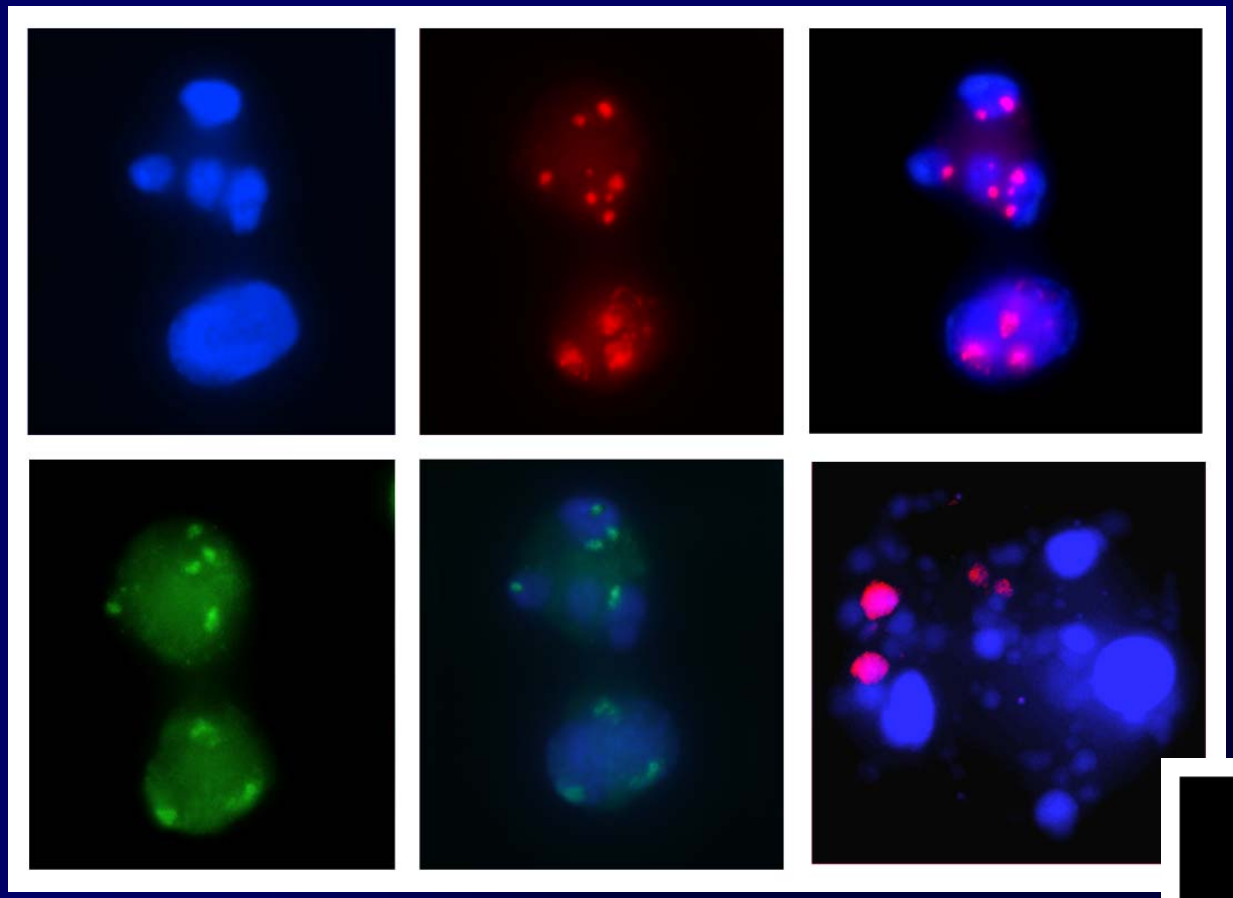

FISH 2.0 (Leica+Quantix) (Jul 9 2002)
File Video Mode Edit Filter Segment Nucleus Manual segmentation 3D-View



Nuclear organisation of chromosomal territories

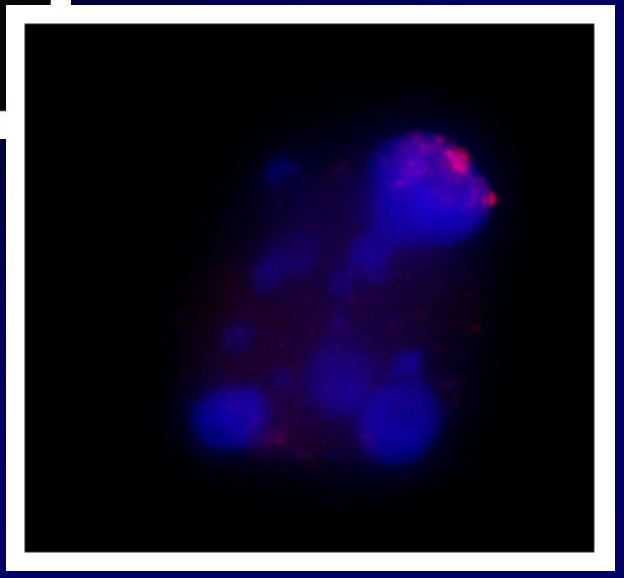


(Cremer T. and Cremer C., 2001)

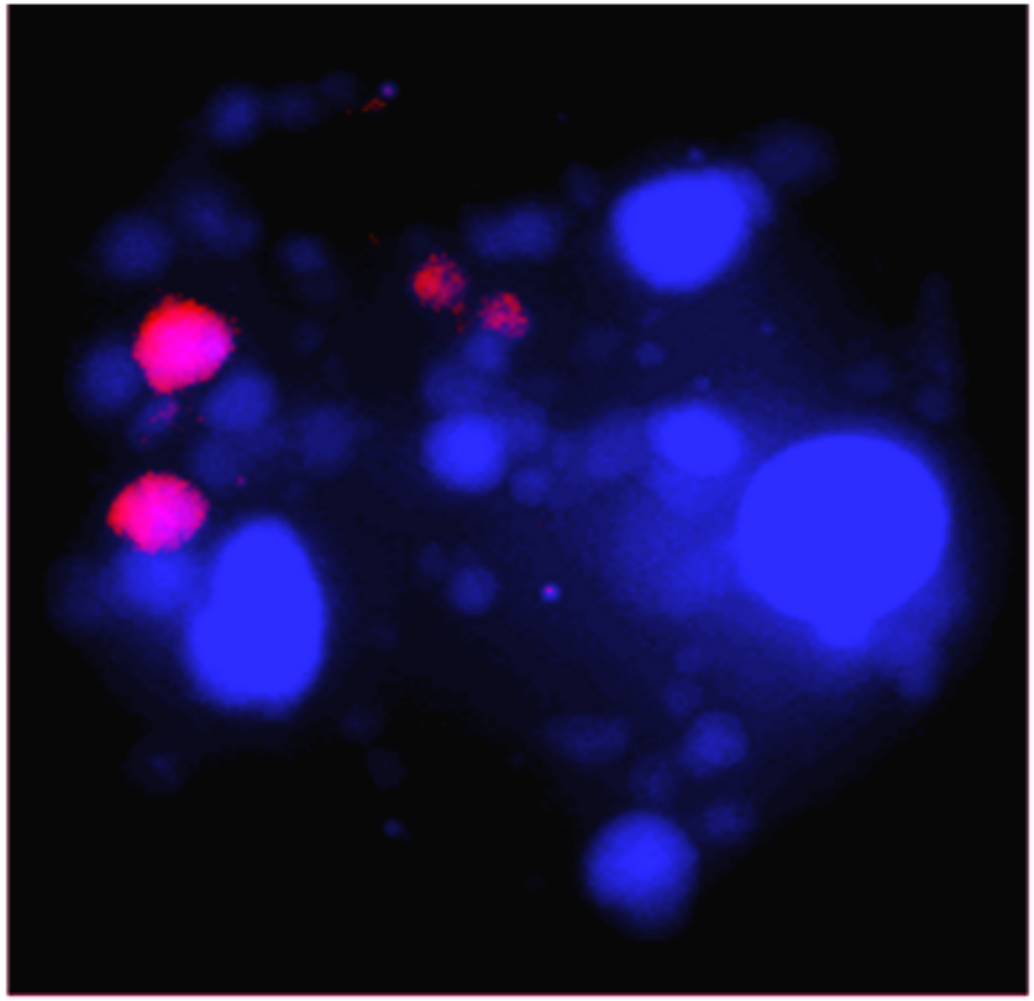


Territory of chromosome
11 and 17

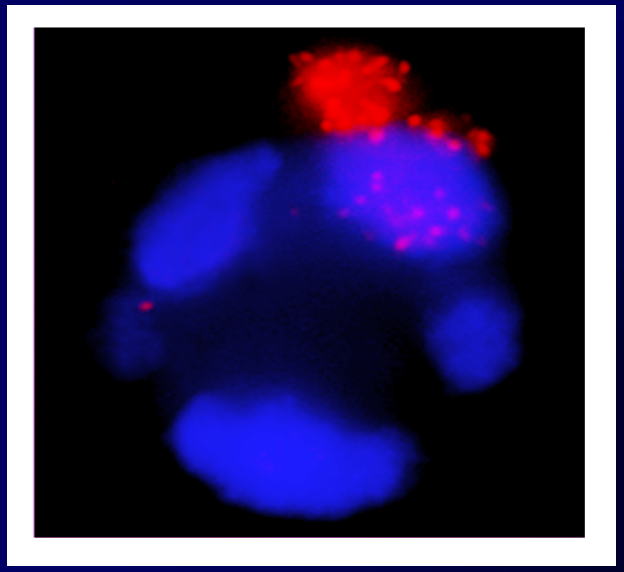
Territory of chromosome 3

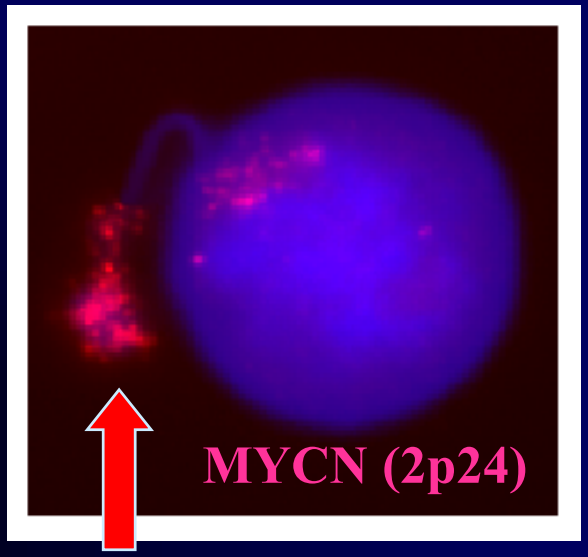


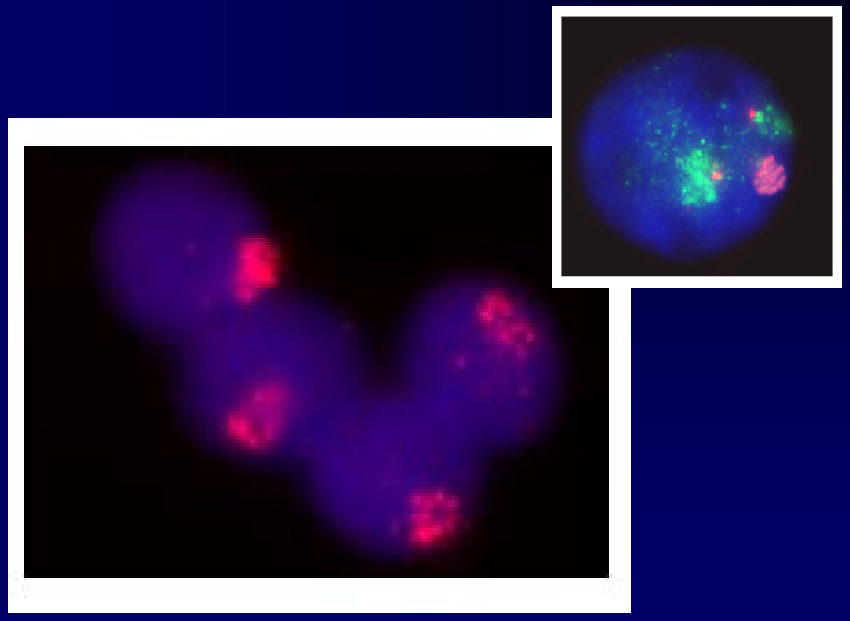
Arcitecture of chromosomal territories during apoptosis

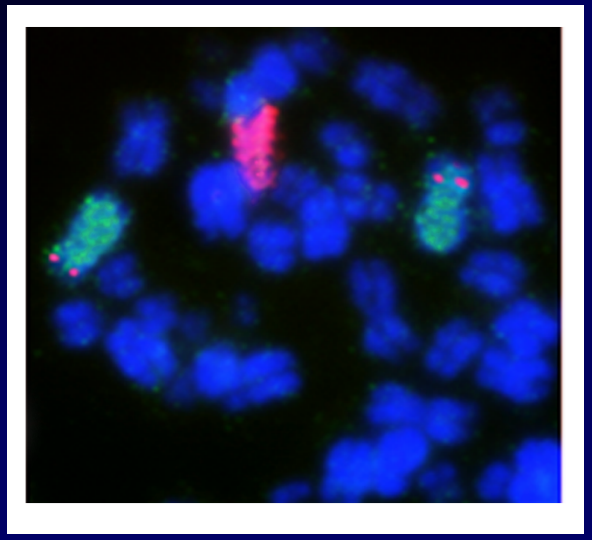


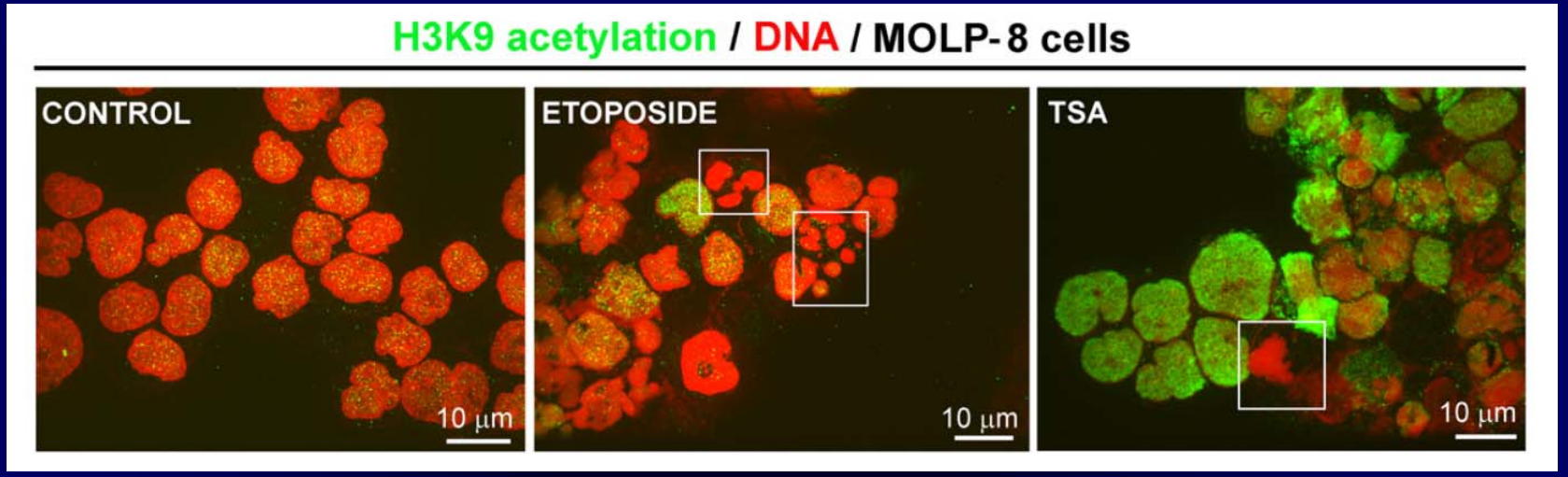
Retinoblastoma Y79 cells and HSR

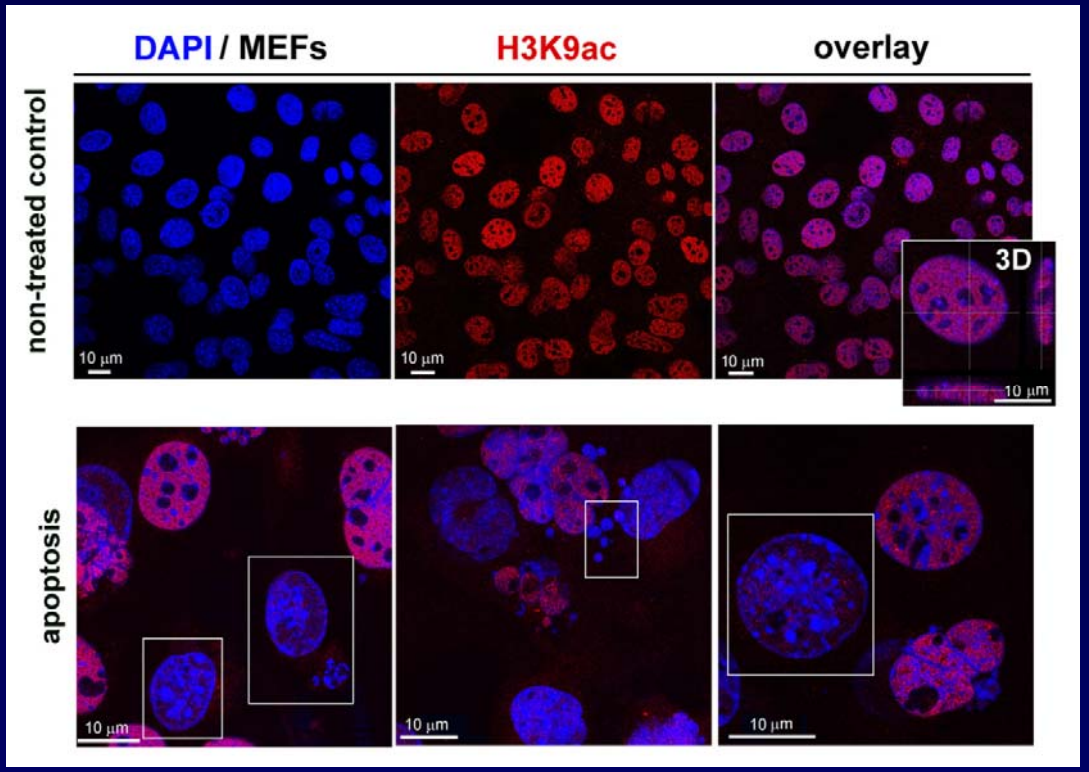




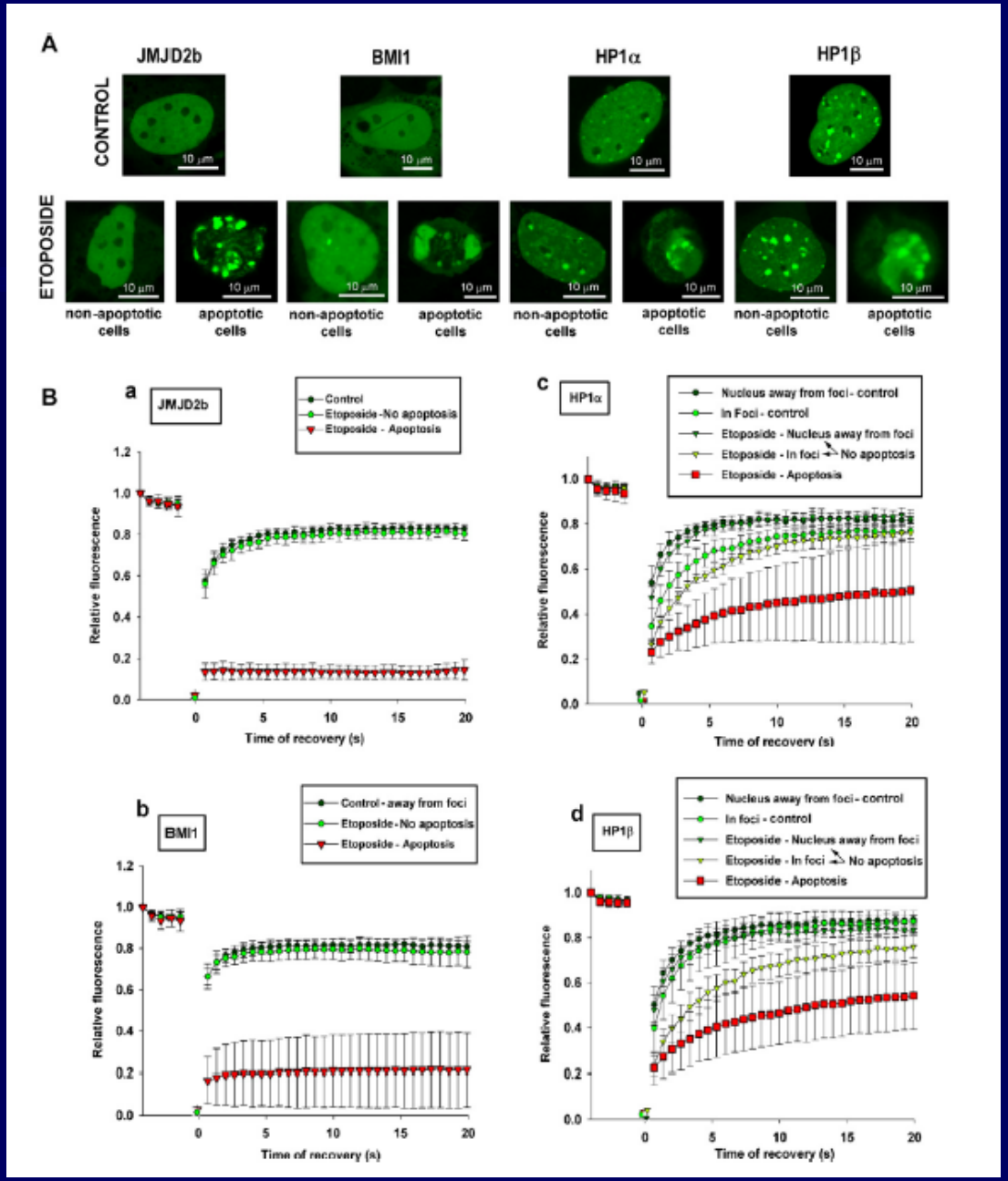




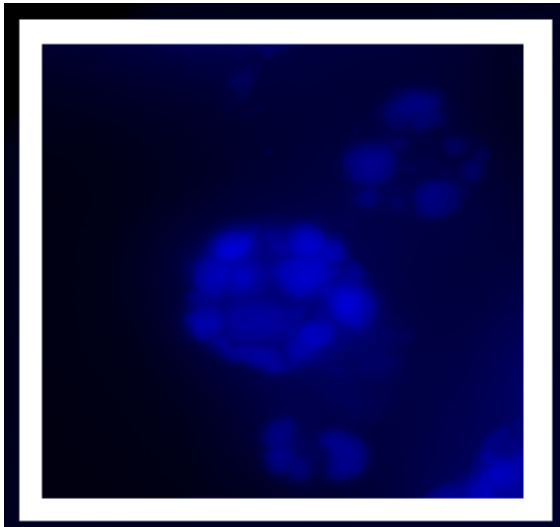




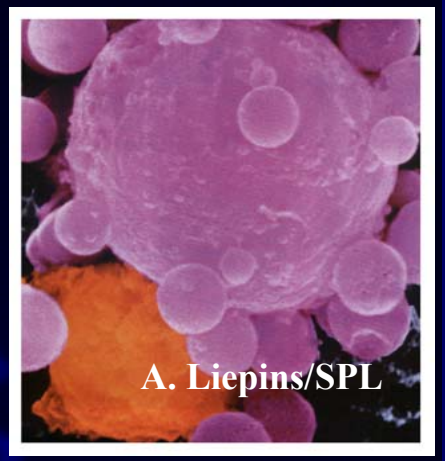
Legartová et al. (2013)



Legartová et al. (2013)



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.