Apoptosis

Programmed cell death

APOPTOSIS

- Introduction
- History of apoptosis
- Apoptosis and necrosis
- Importance of apoptosis
- Apoptosis: Pathways
- Techniques for apoptosis detection

I. Introduction and history of apoptosis

- Cells are born, live for a given period of time and then die.

-Eventually, the term ***apoptosis*** had been coined in order to describe the morphological processes leading to controlled cellular self-destruction and was first introduced in a publication by Kerr, Wyllie and Currie [Kerr, 1972].

- ***Apoptosis*** is of greek origin, having the meaning "falling off or dropping off", in analogy to leaves falling off trees or petals dropping off flowers.

- **Apoptosis** is the process of programmed cell death (PCD) that may occur in multicellular organisms. Biochemical events lead to characteristic cell changes (morphology) and death. These changes include blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation.

-In the human body about 100,000 cells are produced every second by mitosis and a similar number die by apoptosis (Vaux and Korsmeyer, 1999)

(نحن قدرنا بينكم الموت وما نحن بمسبوقين * على أن نبدل أمثالكم وننشئكم في مالا تعلمون)

الواقعة 60 - 61.

(يخرج الحي من الميت ويخرج الميت من الحي)

development-during limb formation separate digits evolve by death of interdigital mesenchymal tissue

الَّذِي خَلَقَكَ فَسَوَّاكَ فَعَدَلَكَ (7) فِي أَيِّ صُورَةٍ مَا شَاءَ رَكَّبَكَ ﴿(8)
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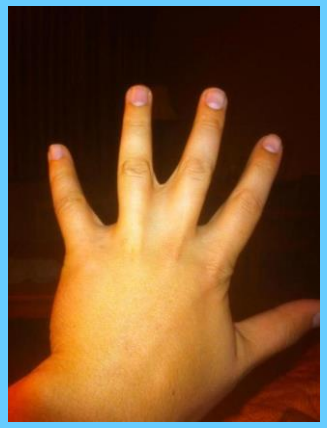
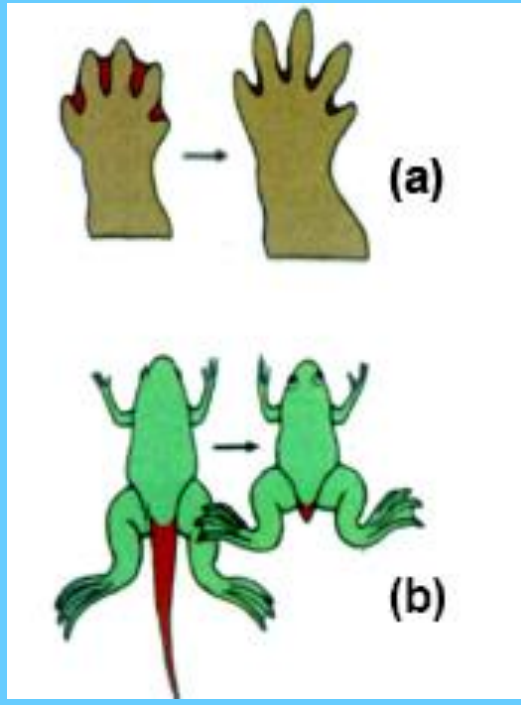
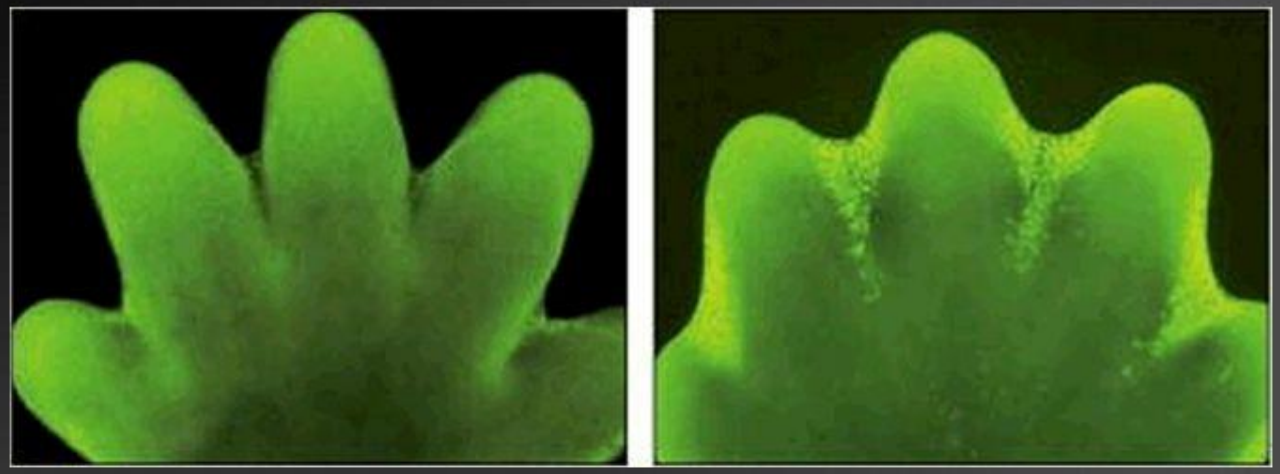


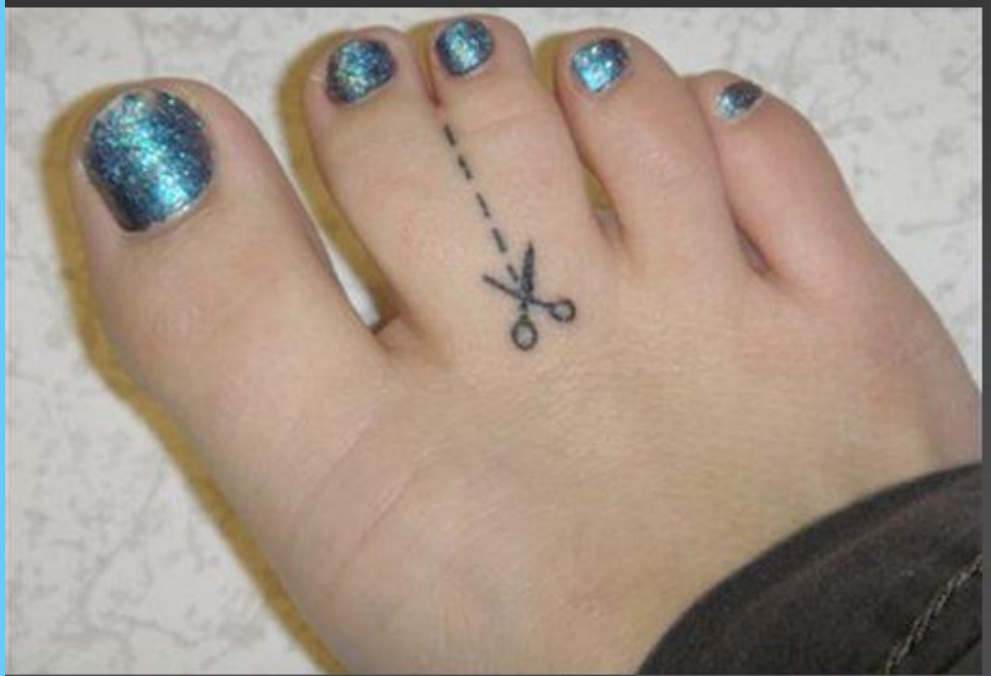


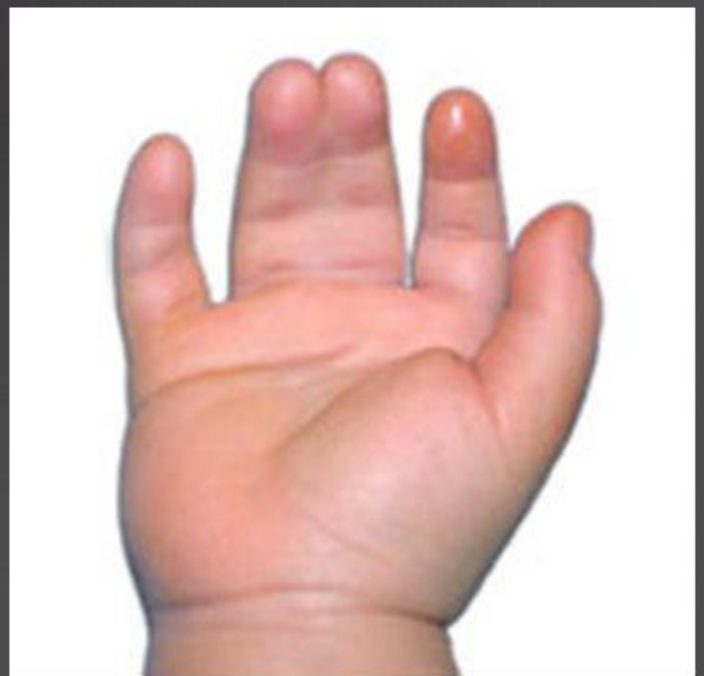
-Vogt described natural cell death as an integral part of toad development (Cotter and Curtin, 2003).

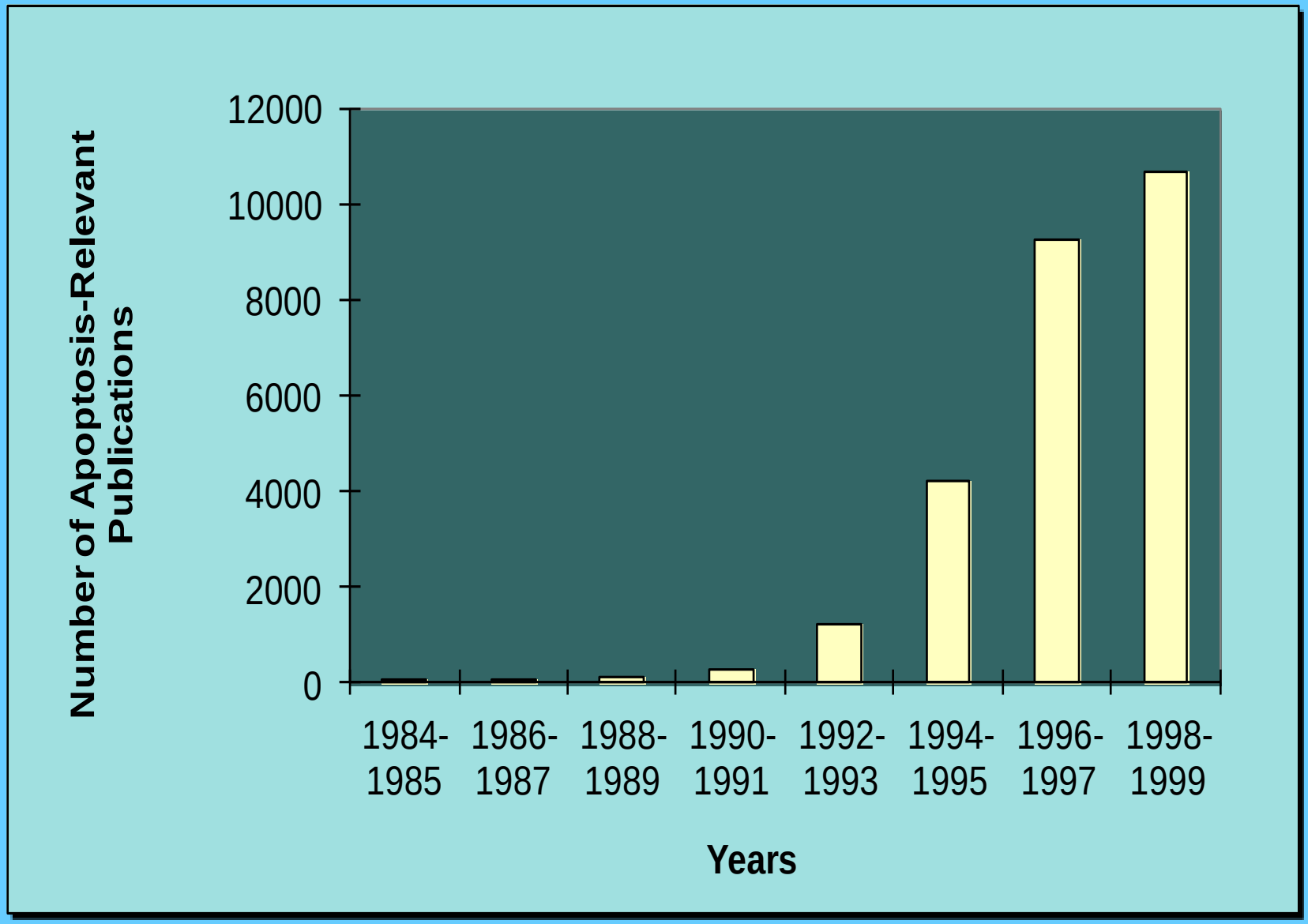
-Ablation of cells no longer needed such as the amphibian tadpole tail during metamorphosis.

Role of Apoptosis in the Hand Plate









2000-2014……..246000 article

History of cell death / apoptosis research

- 1800s — Numerous observation of cell death
- 1908 — Mechnikov wins Nobel prize (phagocytosis)
- 1930-40 — Studies of metamorphosis
- 1948-49 — Cell death in chick limb
- 1955 — Beginning of studies of lysosomes
- 1964-66 — Necrosis & PCD described
- 1971 — Term apoptosis coined
- 1977 — Cell death genes in *Caenorhabditis elegans*

(the first multicellular organism to have its whole genome sequenced in 2002)

- 1980-82 — DNA ladder observed & ced-3 identified
- 1989-91 — Apoptosis genes identified, including bcl-2, fas/apo1 & p53, ced-3 sequenced

(Richerd *et.al., 2001*)

الأميركي-البريطاني جون أوكيف والعالمة النرويجية ماي بريت موزر وزوجها إدفارد موزر فازوا جميعا بجائزة نوبل للطب لعام 2014 لاكتشافهم نظاما في الدماغ بمثابة جهاز داخلي لتحديد المواقع (.GPS)

https://www.youtube.com/watch?v=UwmnDI9GfGk

-Dysregulation of the apoptotic program is implicated in a variety of pathological conditions.

-**Defects in apoptosis** can result in cancer, autoimmune diseases and spreading of viral infections, **while** neurodegenerative disorders, AIDS and ischaemic diseases are caused or enhanced by excessive apoptosis [Fadeel, 1999].

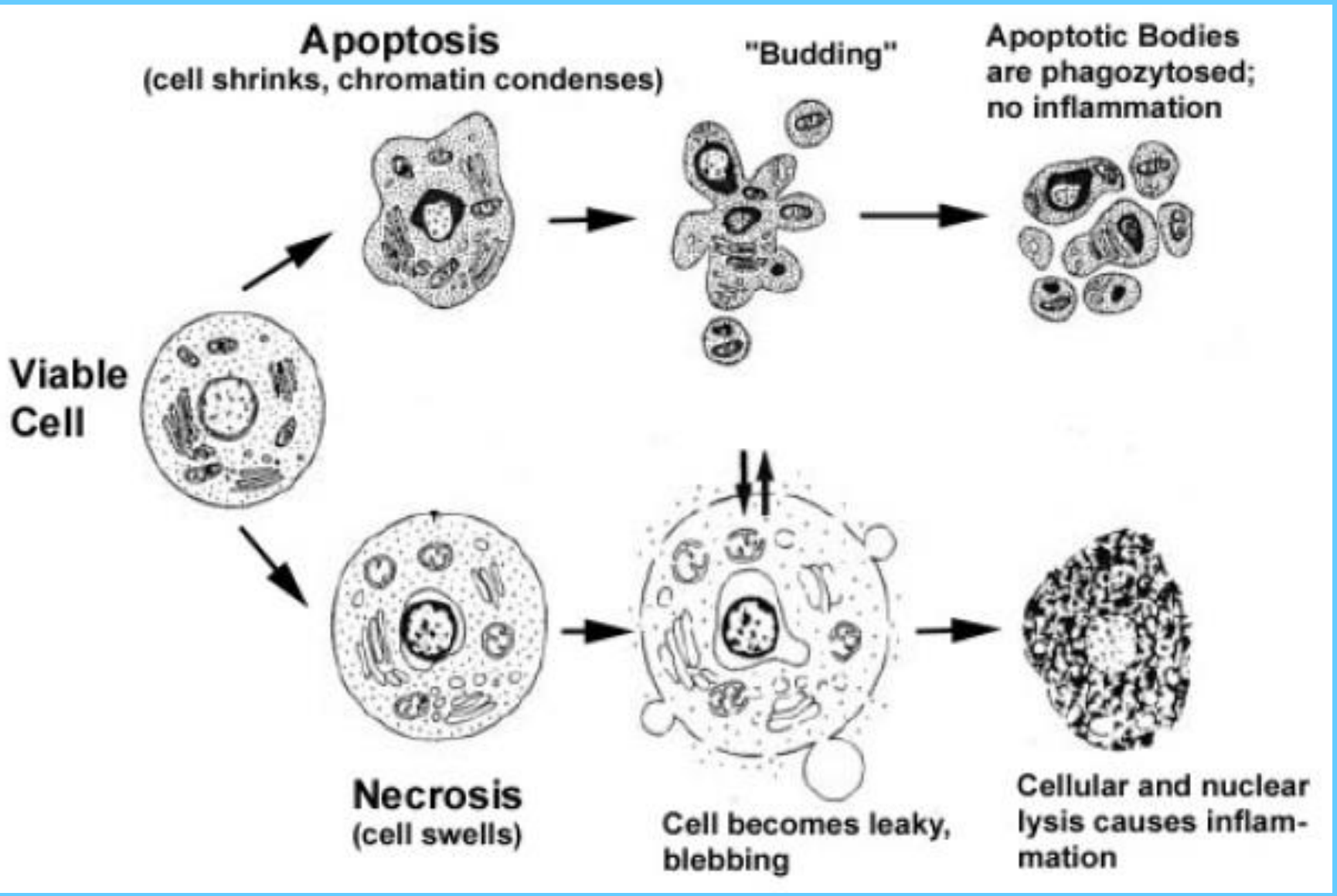
II- Apoptosis and necrosis

- Cells die by one of two mechanisms – **necrosis** or **apoptosis**
- Two physiologically different processes

– Necrosis – death by injury

– Apoptosis – death by suicideانتحار

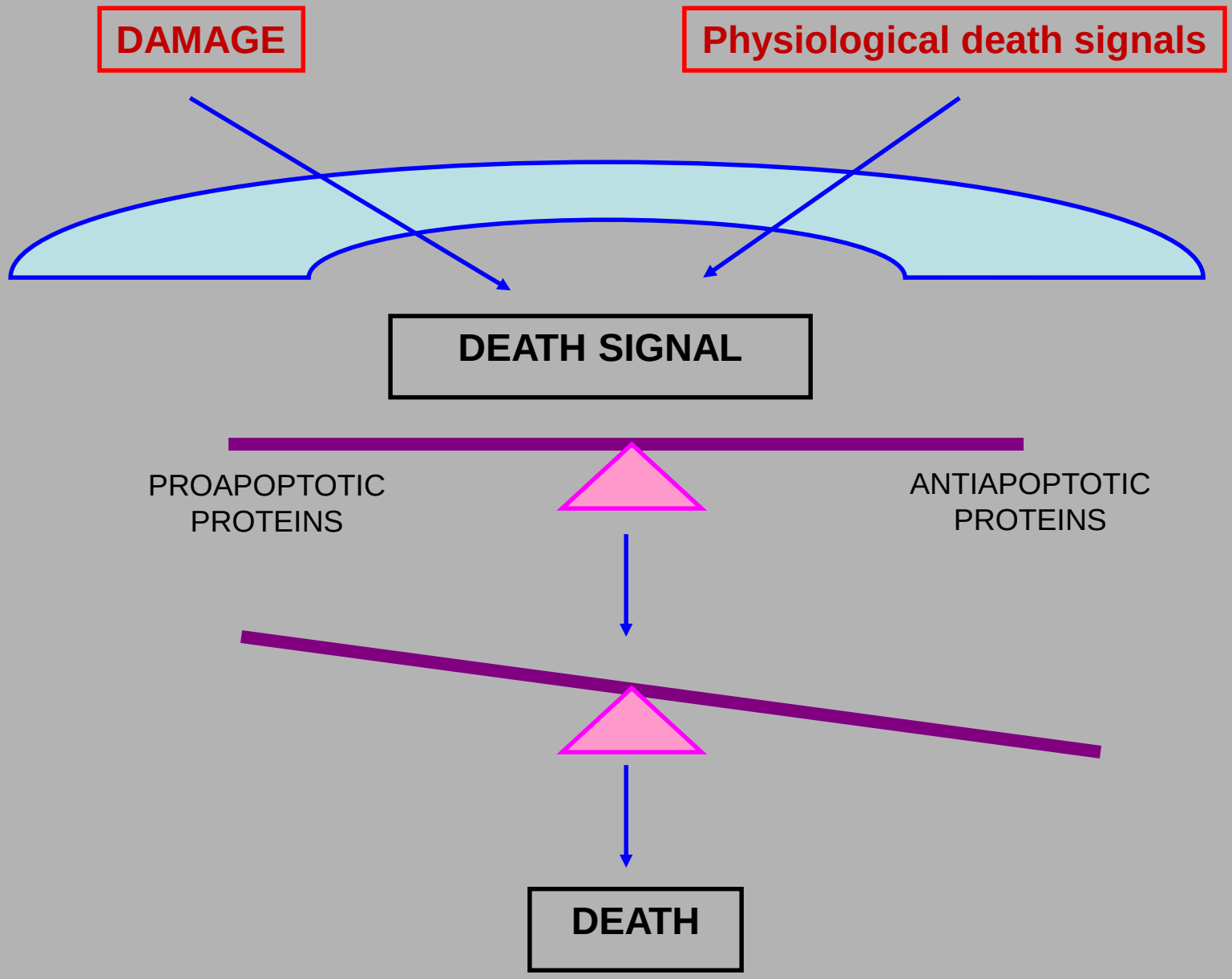
- Apoptosis and necrosis have different characteristics


Apoptosis
(cell shrinks, chromatin condenses)
"Budding"
Apoptotic Bodies
are phagozytosed;
no inflammation
Viable
Cell
Necrosis
(cell swells)
Cell becomes leaky,
blebbing
Cellular and nuclear
lysis causes inflam-
mation

Necrosis *vs.* Apoptosis

Necrosis	Apoptosis
• Cellular swelling • Membranes are broken • ATP is depleted • Cell lyses, eliciting an inflammatory reaction • DNA fragmentation is random, or smeared • In vivo, whole areas of the tissue are affected	• Cellular condensation • Membranes remain intact • Requires ATP • Cell is phagocytosed, no tissue reaction • Ladder-like DNA fragmentation • In vivo, individual cells appear affected

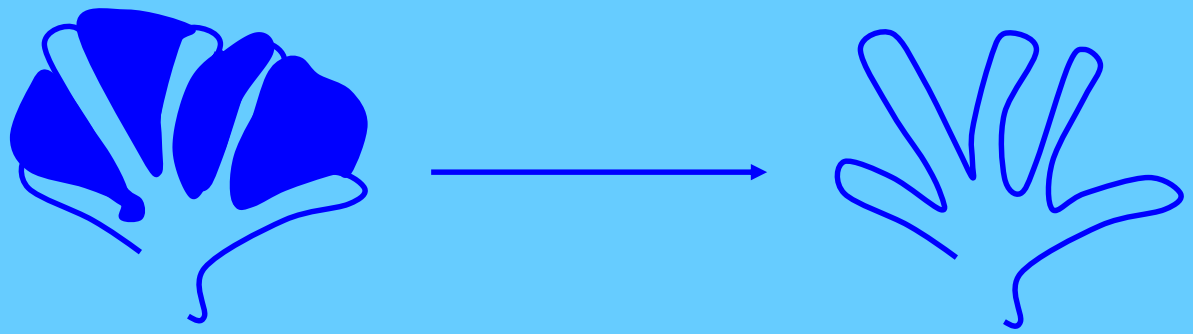
Cells are balanced between life and death



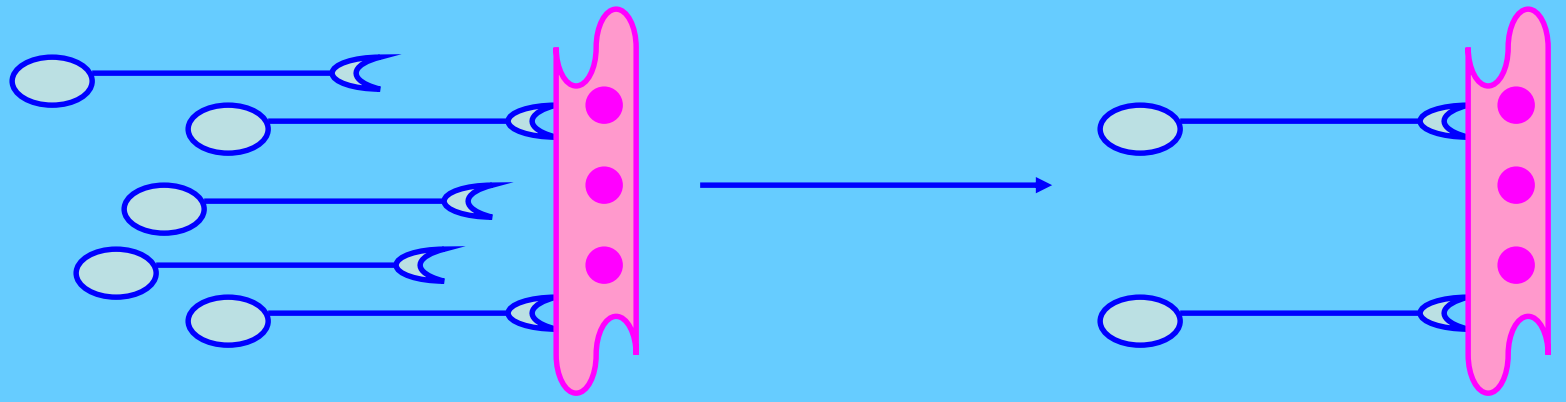
III- Importance of Apoptosis

APOPTOSIS: important in embryogenesis

Morphogenesis (eliminates excess cells):



Selection (eliminates non-functional cells):



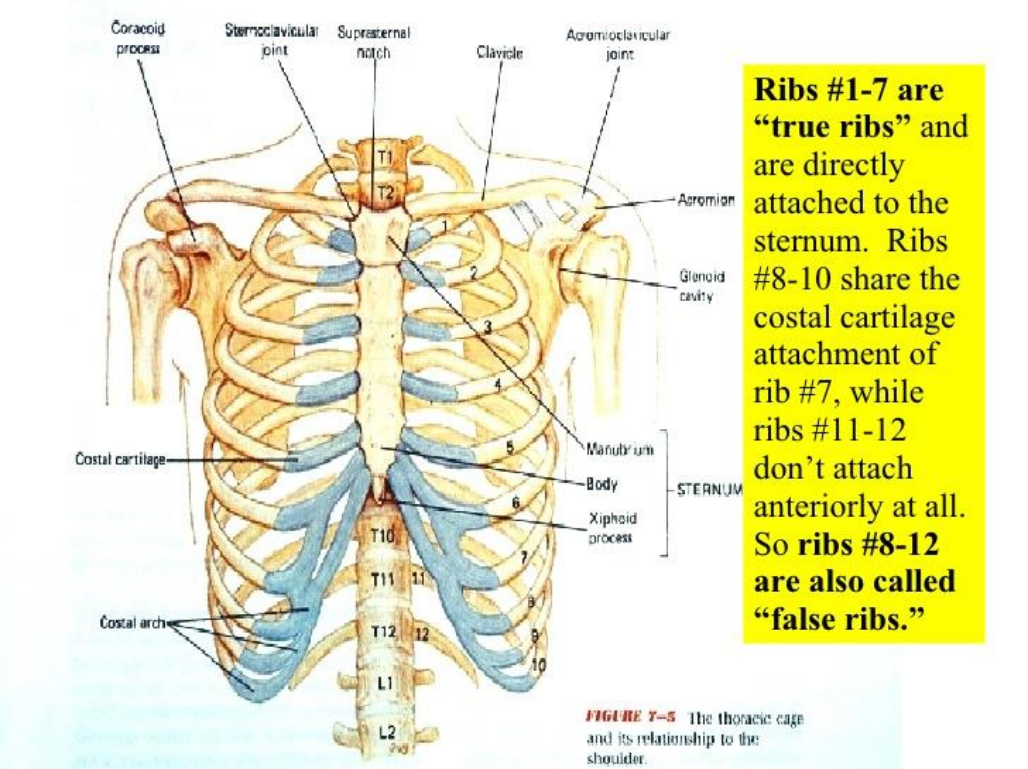
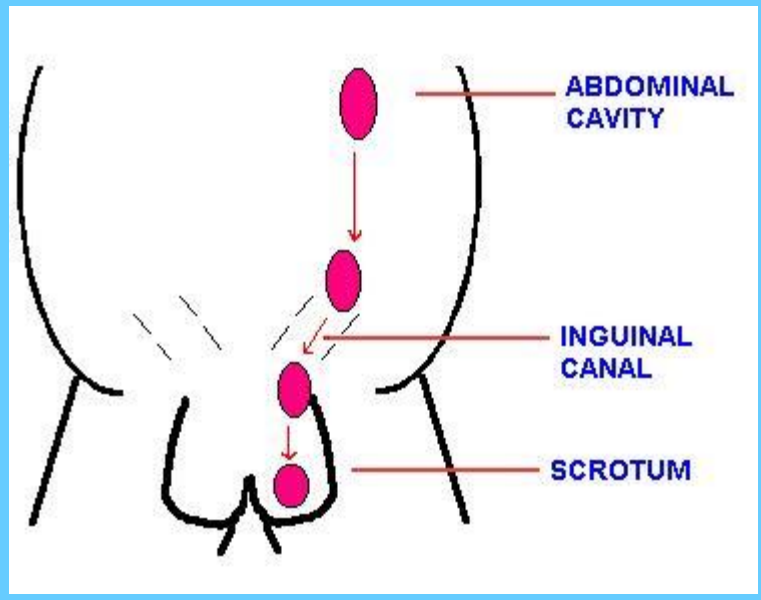
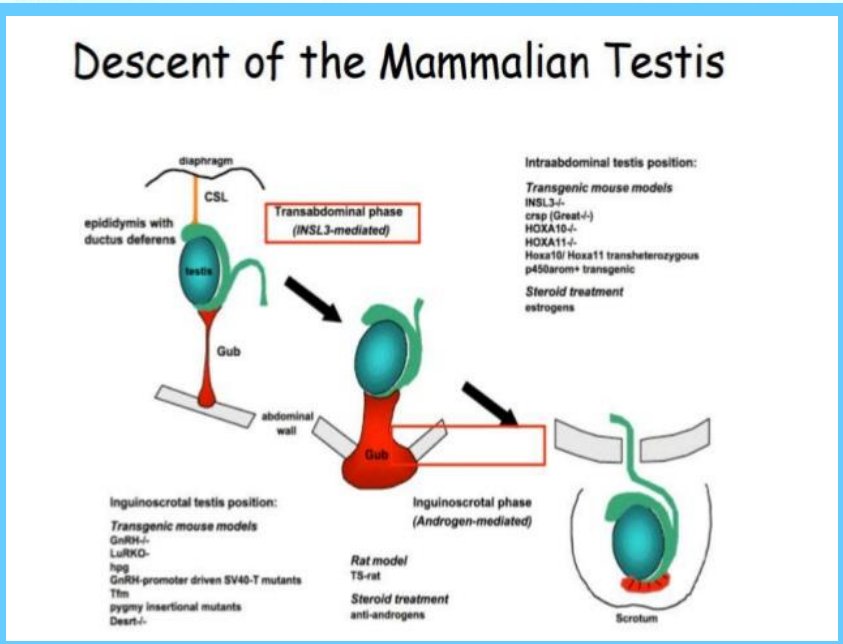


FIGURE 7–5 The thoracic cage and its relationship to the shoulder.

Ribs #1-7 are "true ribs" and are directly attached to the sternum. Ribs #8-10 share the costal cartilage attachment of rib #7, while ribs #11-12 don't attach anteriorly at all. So **ribs #8-12 are also called "false ribs."**

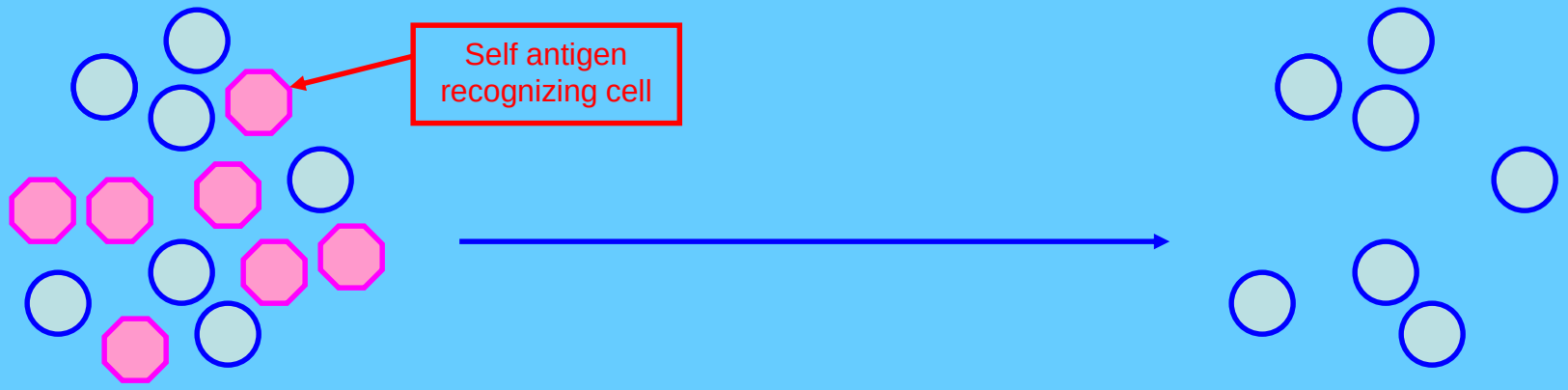


Cryptorchidism definition?????



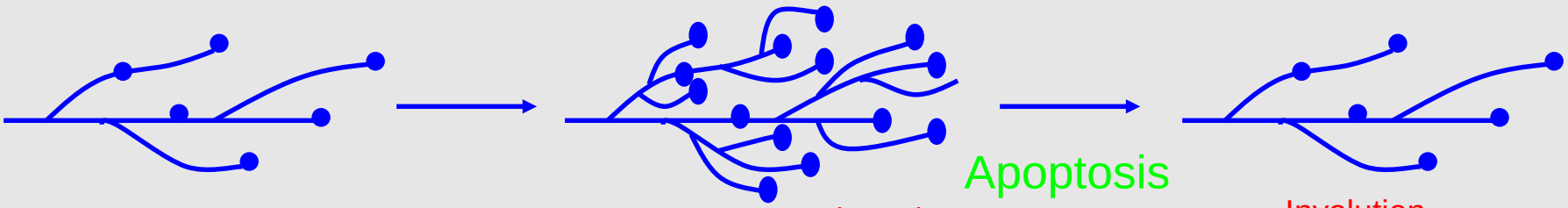
APOPTOSIS: important in embryogenesis

Immunity (eliminates dangerous cells):



APOPTOSIS: important in adults

Tissue remodeling (eliminates cells no longer needed):



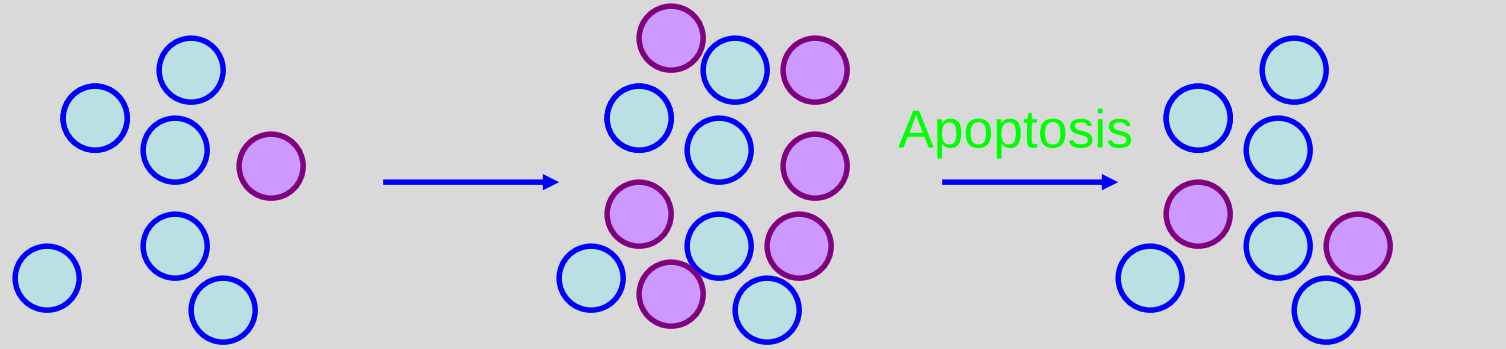
Virgin mammary gland

Late pregnancy, lactation

Involution
(non-pregnant, non-lactating)

APOPTOSIS: important in adults

Tissue remodeling (eliminates cells no longer needed):

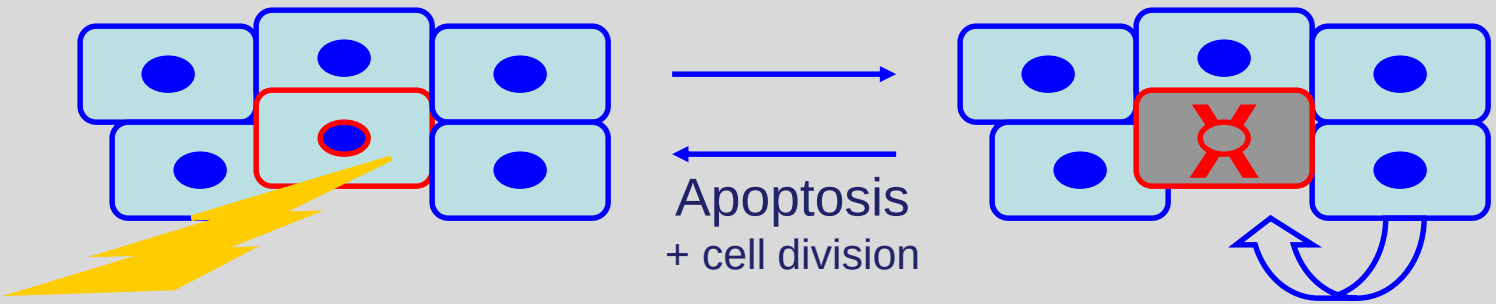


Steroid immunosuppressants: kill lymphocytes by apoptosis

Lymphocytes poised to die by apoptosis

APOPTOSIS: important in adults

Maintains organ size and function:



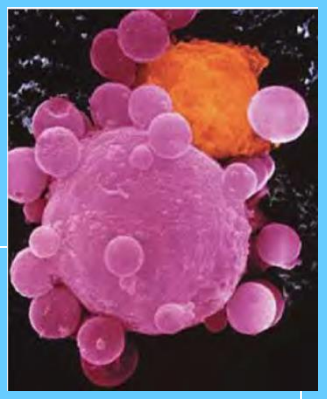
Cells lost by apoptosis are replaced by cell division

(remember limited replicative potential of normal cells restricts how many times this can occur before tissue renewal declines)

IV- Apoptosis: Pathways

Signaling to PROGRAM cell death (Apoptosis)

Signaling to PROGRAM cell death (Apoptosis)



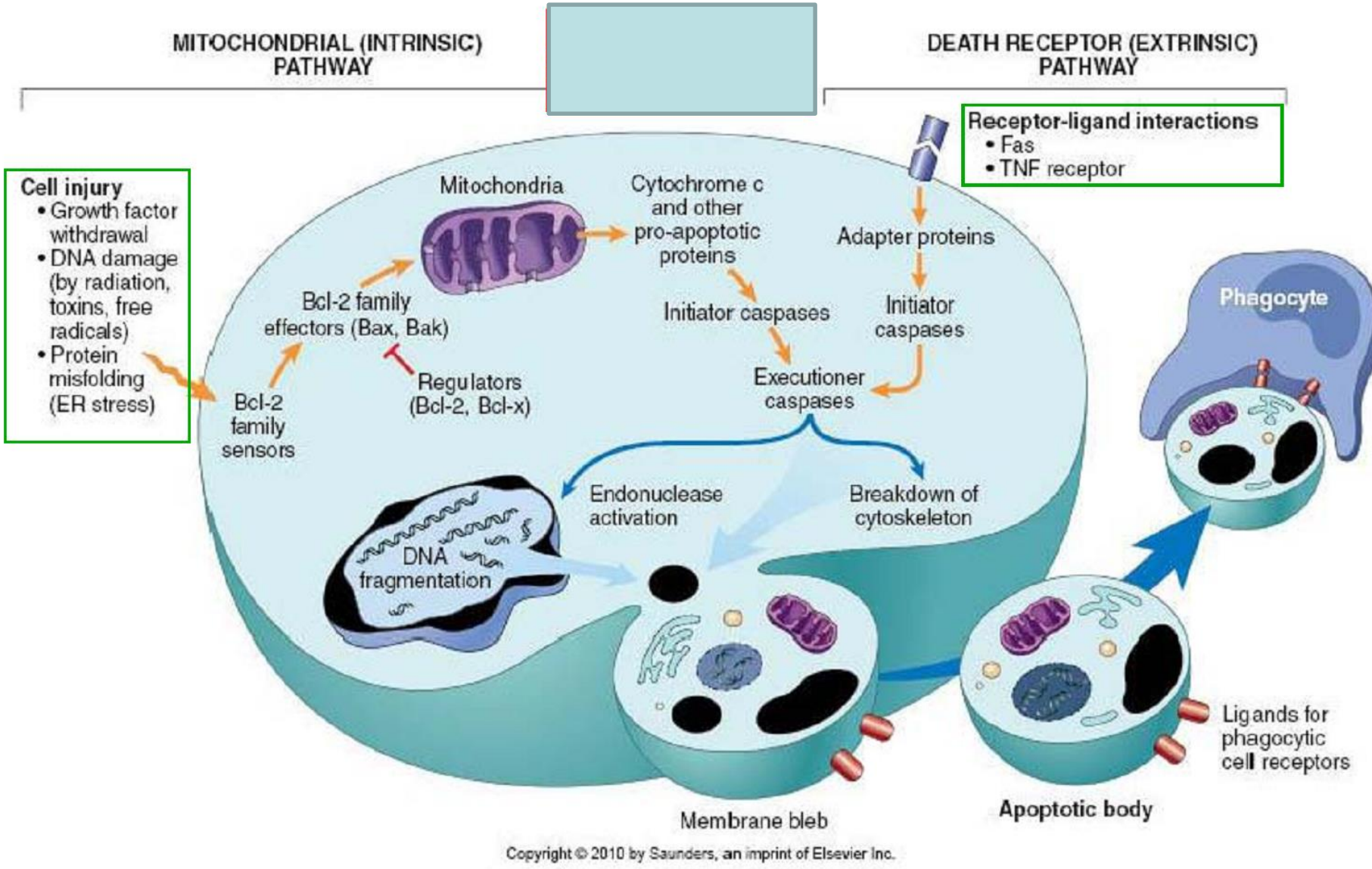
Factors that induce apoptosis:

Internal stimuli: abnormalities in DNA
External stimuli: removal of growth factors, addition of cytokines (Tumor Necrosis Factor or TNF)

Signal transduction pathways leading to apoptosis:

There are two major pathways:

1- Intrinsic pathway (mitochondria-dependent)
2- Extrinsic pathway (mitochondria-independent)(death receptor pathway).



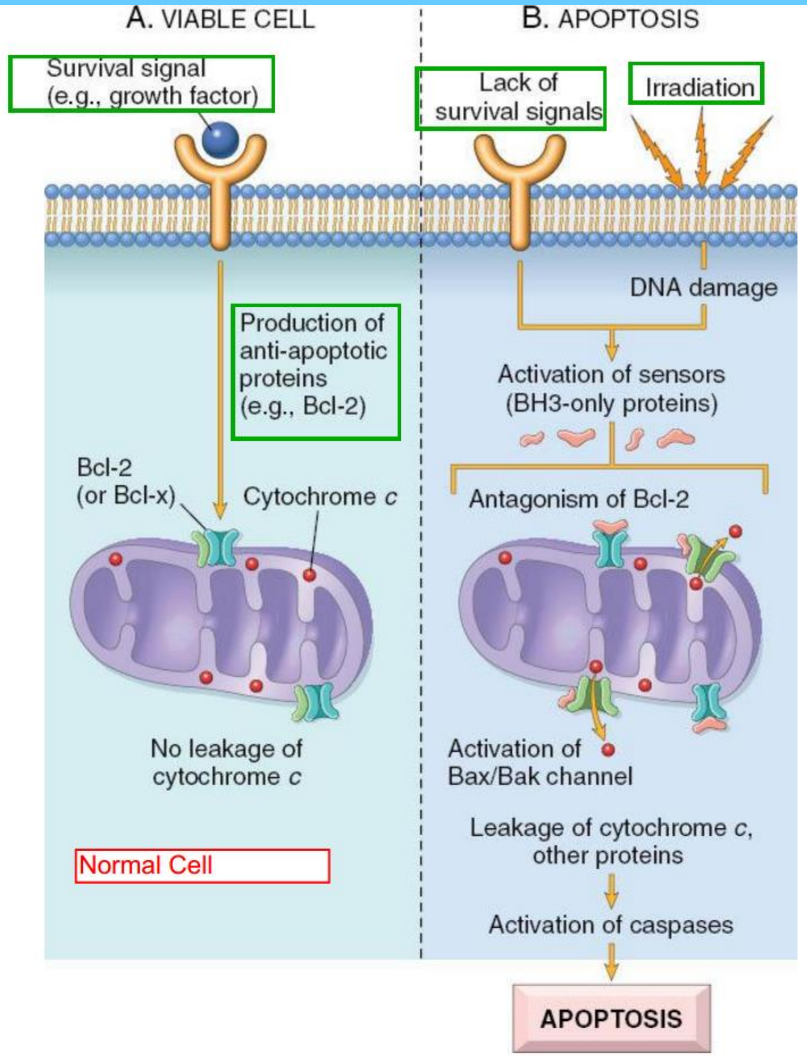
Execution Phase

The intrinsic and extrinsic pathways converge to a caspase activation cascade.

Caspases (*cysteine-aspartic-acid-proteases)* are conserved across species.

Synthesized as inactive precursors; activated by proteolytic cleavage.

Family of at least 12 proteases, a few of which are involved in inflammation, and many of which are involved in apoptosis



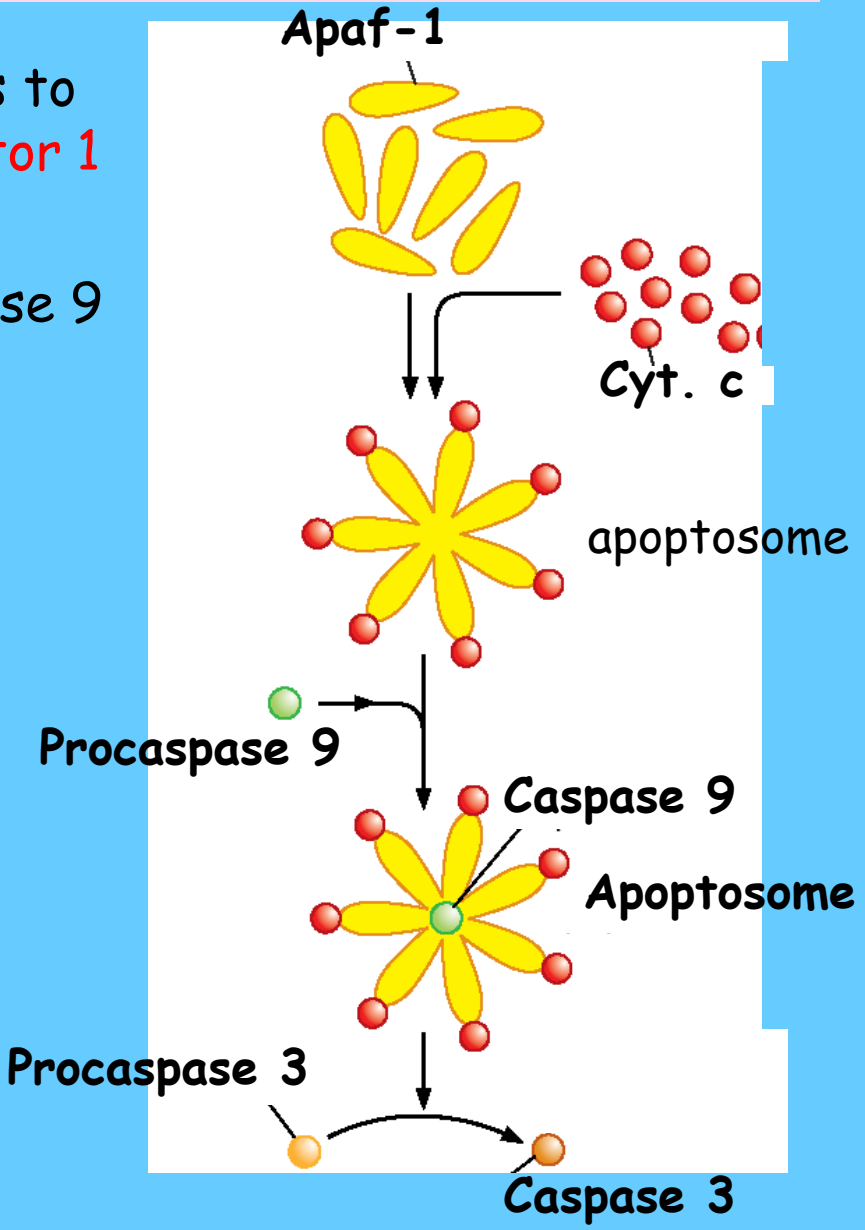
Intrinsic (Mitochondrial) Pathway of Apoptosis

Pro-apoptotic and protective molecules that regulate mitochondrial permeability and the release of death molecules sequestered in the mitochrondria are maintained in balance normally.

- **BAX= Bcl-2-associated X protein**
- **BAK= Bcl-2 homologous antagonist/killer**

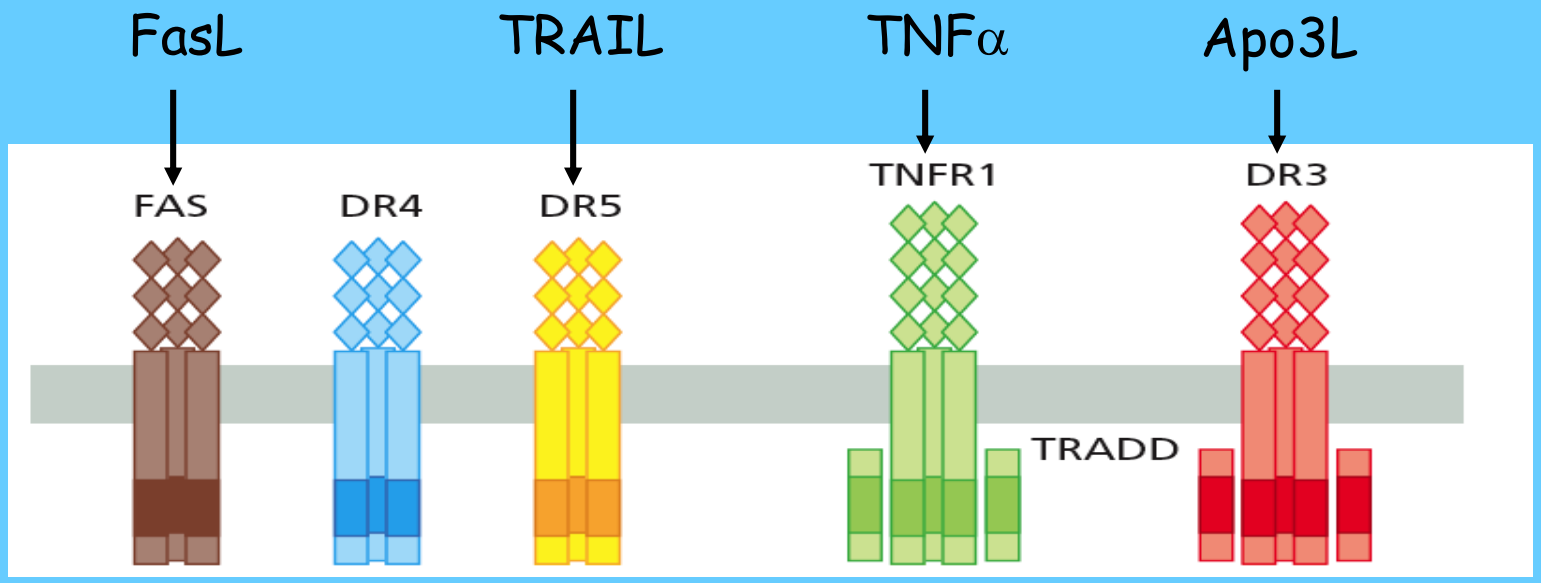
Cytochrome c translocation initiates caspase cascade

- In the cytosol, cytochrome c binds to Apoptotic protease activating factor 1 **(Apaf-1)** to form apoptosome
- The apoptosome recruits procaspase 9 to generate the active caspase 9
- Caspase 9 then activates the executioner caspase 3, 6, 7



Death receptor pathway

- The death receptor pathway is activated by external cytokines and is mitochondria-**independent**
- The ligands of the death receptors are members of the tumor necrosis factor (TNF) family of proteins, including TNFα, Fas ligand (FasL), TRAIL/Apo2L, Apo3L



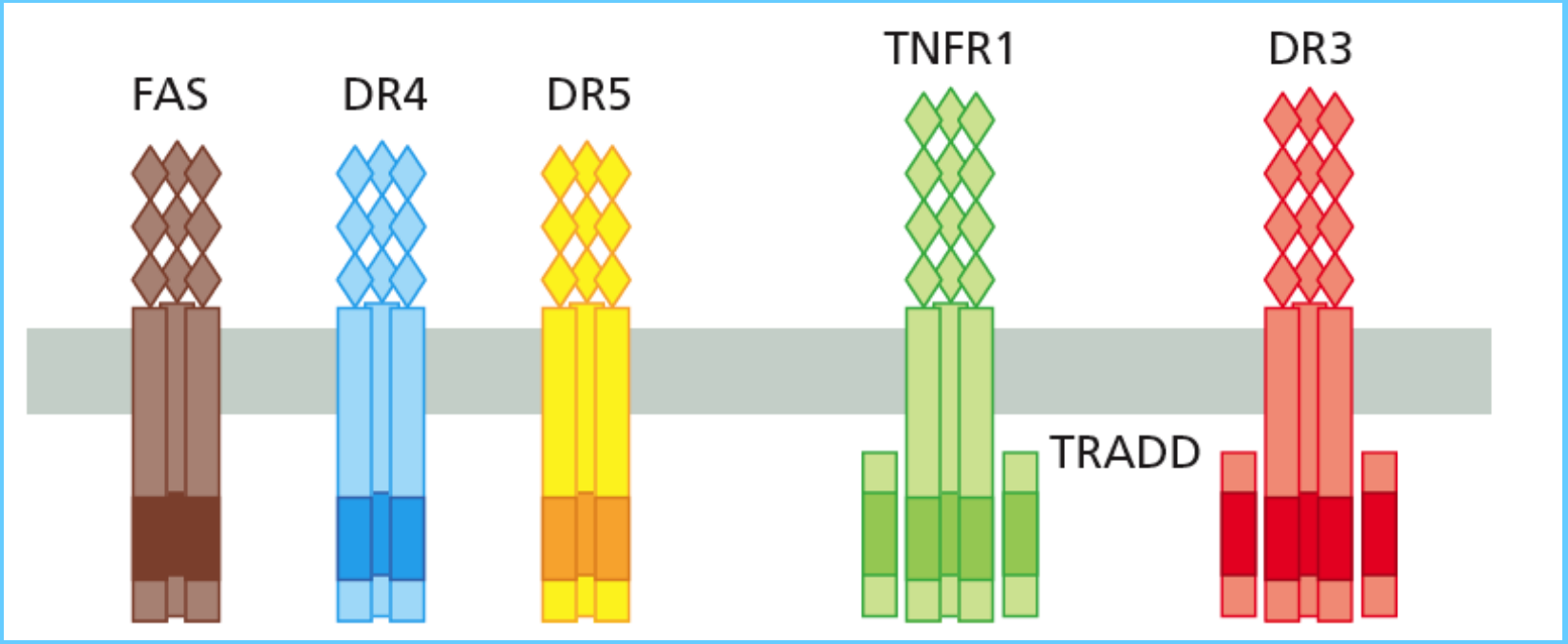
Fas ligand (FasL or CD95L) is a type-II transmembrane protein that belongs to the tumor necrosis factor (TNF) family.

TRAIL: TNF-related apoptosis-inducing ligand. It is a cytokine

Apo2 ligand or tumour necrosis factor-related apoptosis-inducing ligand (Apo2L/TRAIL) is one of the several members of the tumour necrosis factor (TNF) gene superfamily that induce apoptosis through engagement of death receptors (DRs).

Activation of the death receptors

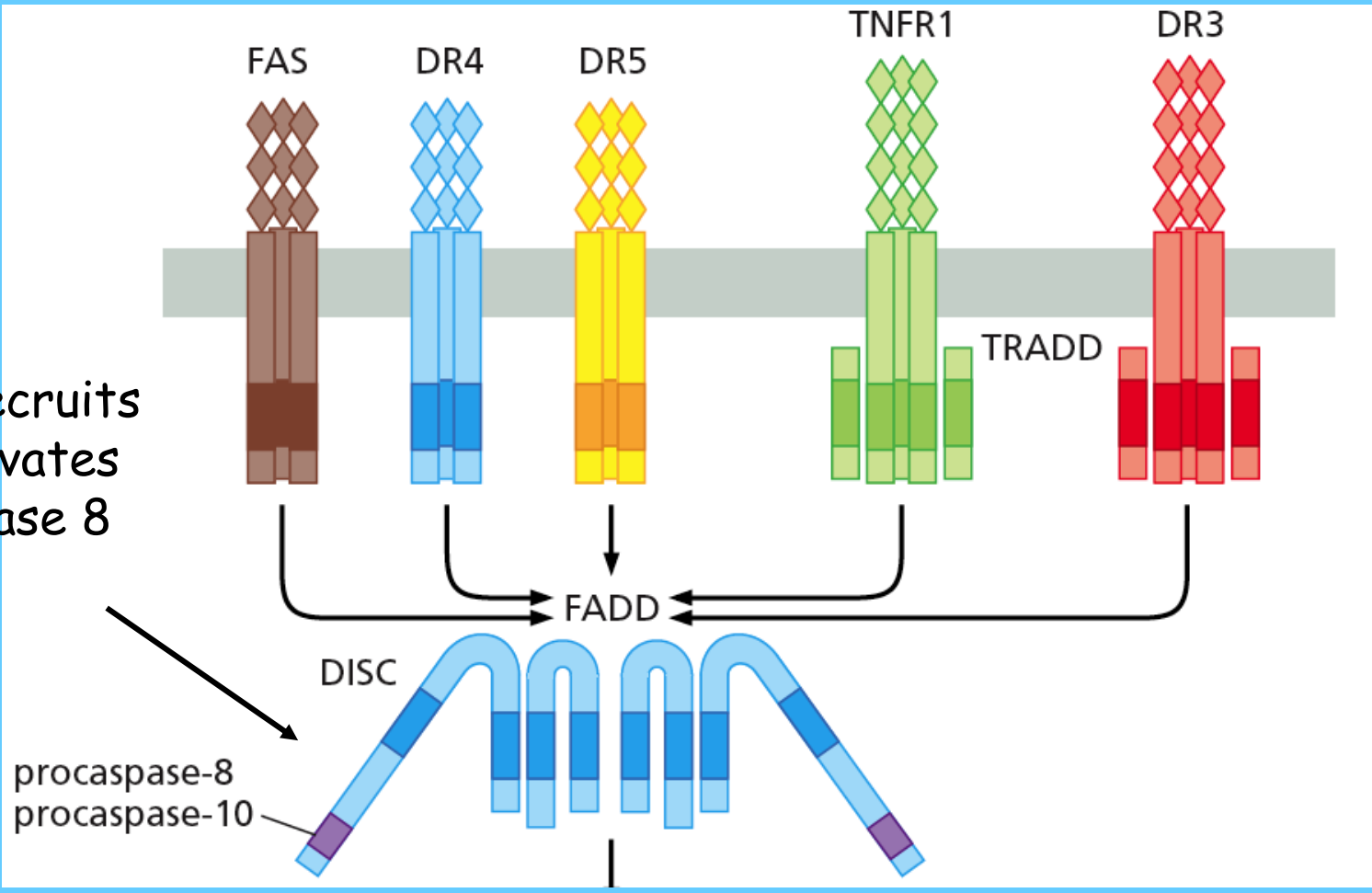
- Binding of ligand to the death receptors results in homo trimerization of the receptors
- The death receptors contain a death domain in the cytoplasmic region that is required for apoptosis signaling



Activation of the death receptors

- Trimerization of the receptor death domains allows binding and activation of FADD (Fas-associated death domain protein) and formation of death-inducing signaling complex (DISC)

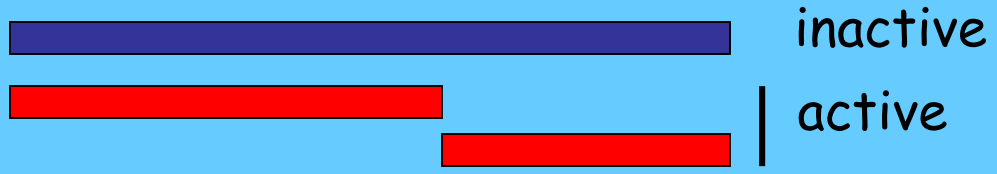
DISC recruits and activates Procaspase 8 and 10



Activation of procaspases

- Caspases are a family of cysteine aspartyl-specific proteases that are activated at an early stage of apoptosis and are responsible for triggering most, if not all, of the changes observed during apoptosis.

- In the absence of stimulation, caspases exist in the cell as an inactive precursor. **Activation of caspases by DISC** results in enzymatic removal of portions of the caspase precursors and release of smaller fragments that are active.



- The active caspase then diffuses into the cytoplasm and cleaves target proteins.

Two major classes of caspases

- **Initiator caspases**: initiate the onset of apoptosis by activating the executioner caspases

  Caspase 8, caspase 9, caspase 10, etc.

- **Executioner caspases**: destroy actual targets in the cell to execute apoptosis

  Caspase 3, caspase 6, caspase 7

Some cellular targets of caspases

FAK (focal adhesion kinase): inactivation of FAK disrupt cell adhesion, leading to detachment of the apoptotic cell from its neighbors.

Lamins: important component of the nuclear envelope. Cleavage of lamins (filaments and membrane associated proteins) leads to disassembly of the nuclear lamina.

Proteins required for cell structure: actin, intermediate filaments, etc. Cleavage of these proteins lead to changes in cell shape and the surface blebbing.

Endonuclease (as Caspase-activated DNase (CAD)): responsible for chromosome fragmentation. CAD cuts DNA into small fragments. CAD normally binds to an inhibitor protein. Caspases cleaves the inhibitor protein and activates CAD.

Summary of death receptor pathway

ligands

↓

Death receptors

↓

FADD/DISC

↓

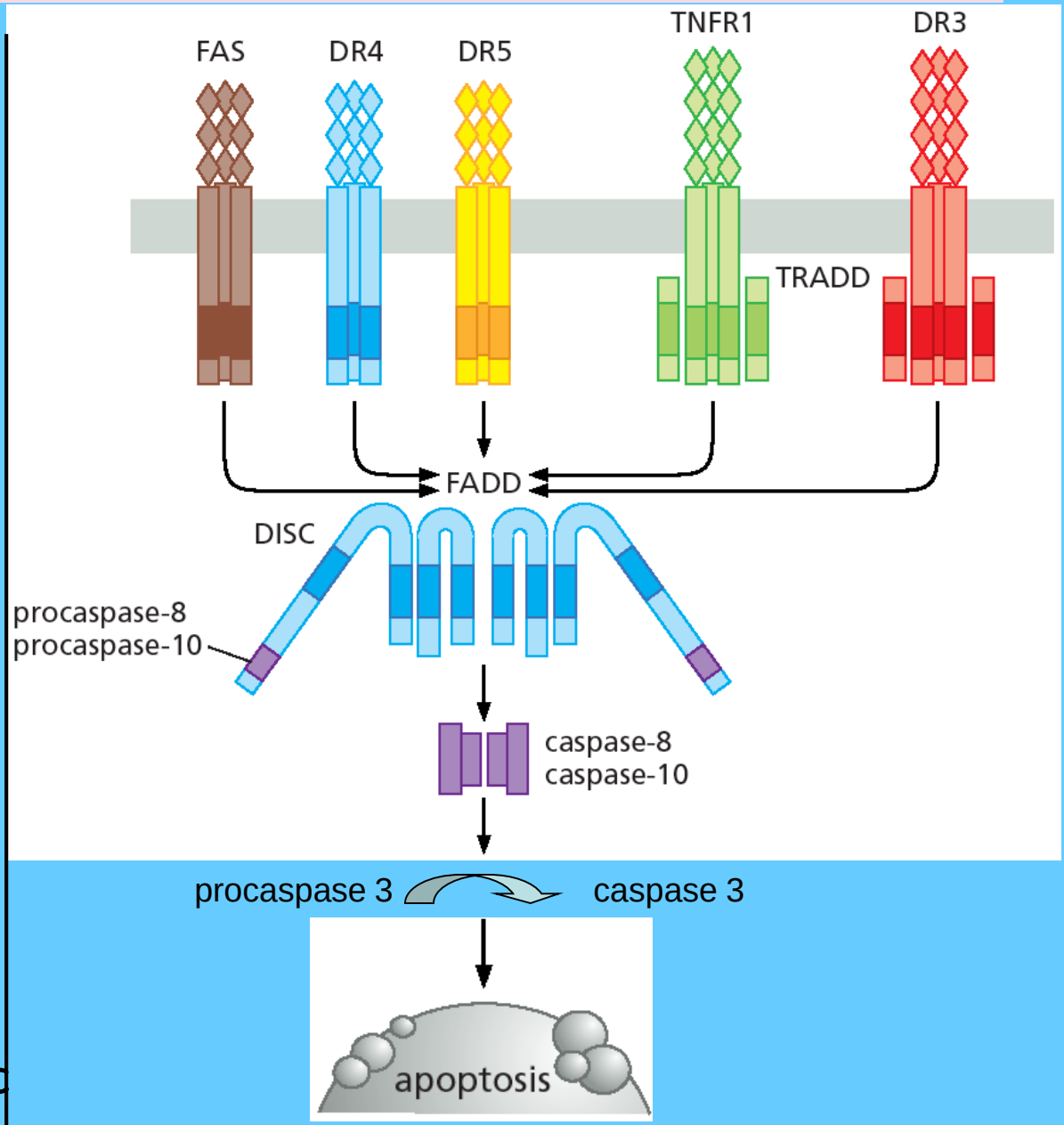
Caspase 8, 10

↓

Caspase 3, 6, 7

↓

Lamin, CAD,actin,
Adhesion molecules, etc



The bcl-2 family of proteins

pro-survival 6 proteins

Bcl2,
Bcl-x, A1,
Bcl-w, Mcl1

pro-apoptosis 18 proteins

Bax,
Bak, Bok

Bid

Bim,
Bik, Bad,
Bmf, Hrk,
Noxa,
Puma

BH: bcl-2 homology

The bcl-2 family proteins homo- and hetero-dimerize

- The Bcl-2 family members can form homo- or hetero-dimers through the BH3 domains.
- The pro-apoptotic Bax homo-dimers promotes apoptosis.
- Bcl-2 forms hetero-dimers with Bax, leading to the inhibition of the apoptotic activity of Bax.

Thus, the relative levels of pro-survival and pro-apoptosis Bcl-2 family proteins determine cell survival or apoptosis.

How do these proteins regulate apoptosis?

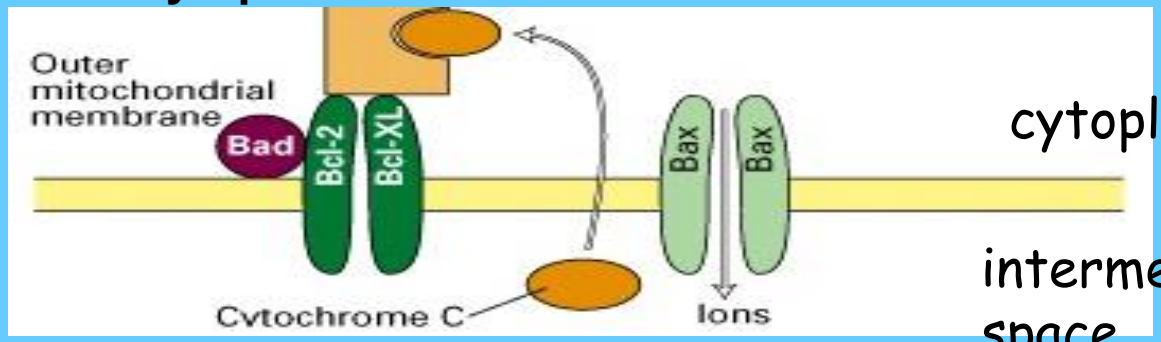
Bcl-2 family proteins control cytochrome c translocation

Bcl-2 (B-cell lymphoma 2), encoded in humans by the *BCL2* gene, is the founding member of the Bcl-2 family of regulator proteins that regulate cell death (apoptosis), by either inducing (pro-apoptotic) or inhibiting (anti-apoptotic) apoptosis.

Apoptosis regulator BAX, also known as **bcl-2-like protein 4**, is a protein that in humans is encoded by the *BAX* gene. *BAX* is a member of the Bcl-2 gene family

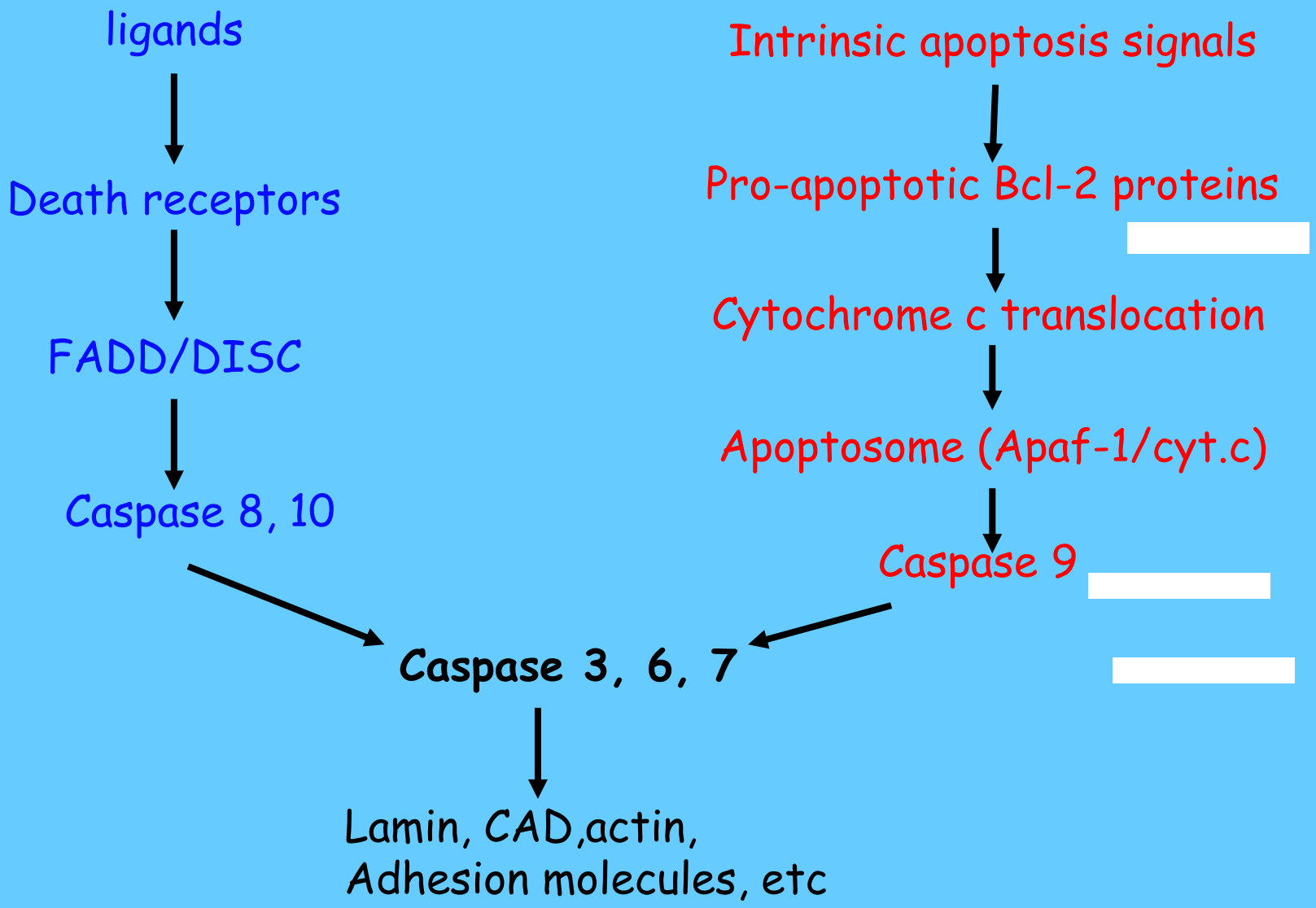
Bax forms homo-dimers in the presence of apoptotic signals.

This homodimer presumably promotes the opening of a channel that controls the translocation of cytochrome c from the intermembrane space to cytoplasm.



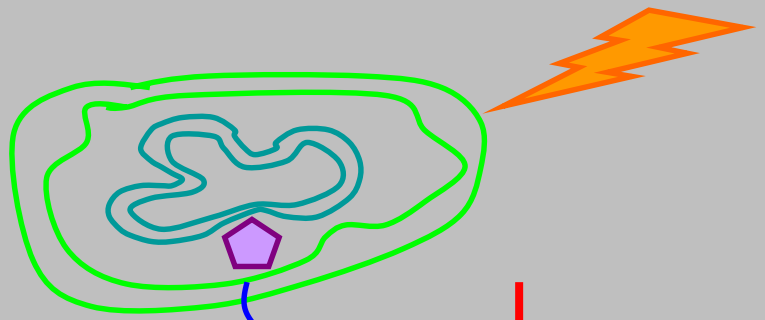
Bcl-2 interferes with Bax function by forming a hetero dimer with Bax. This leads to the closing of the channel and inhibition of cytochrome c translocation.

Extrinsic and intrinsic pathways converge at executioner caspases



APOPTOSIS: control

Intrinsic pathway (damage):



Mitochondria

Cytochrome c release

Pro-caspase 9 cleavage

Pro-execution caspase (3) cleavage

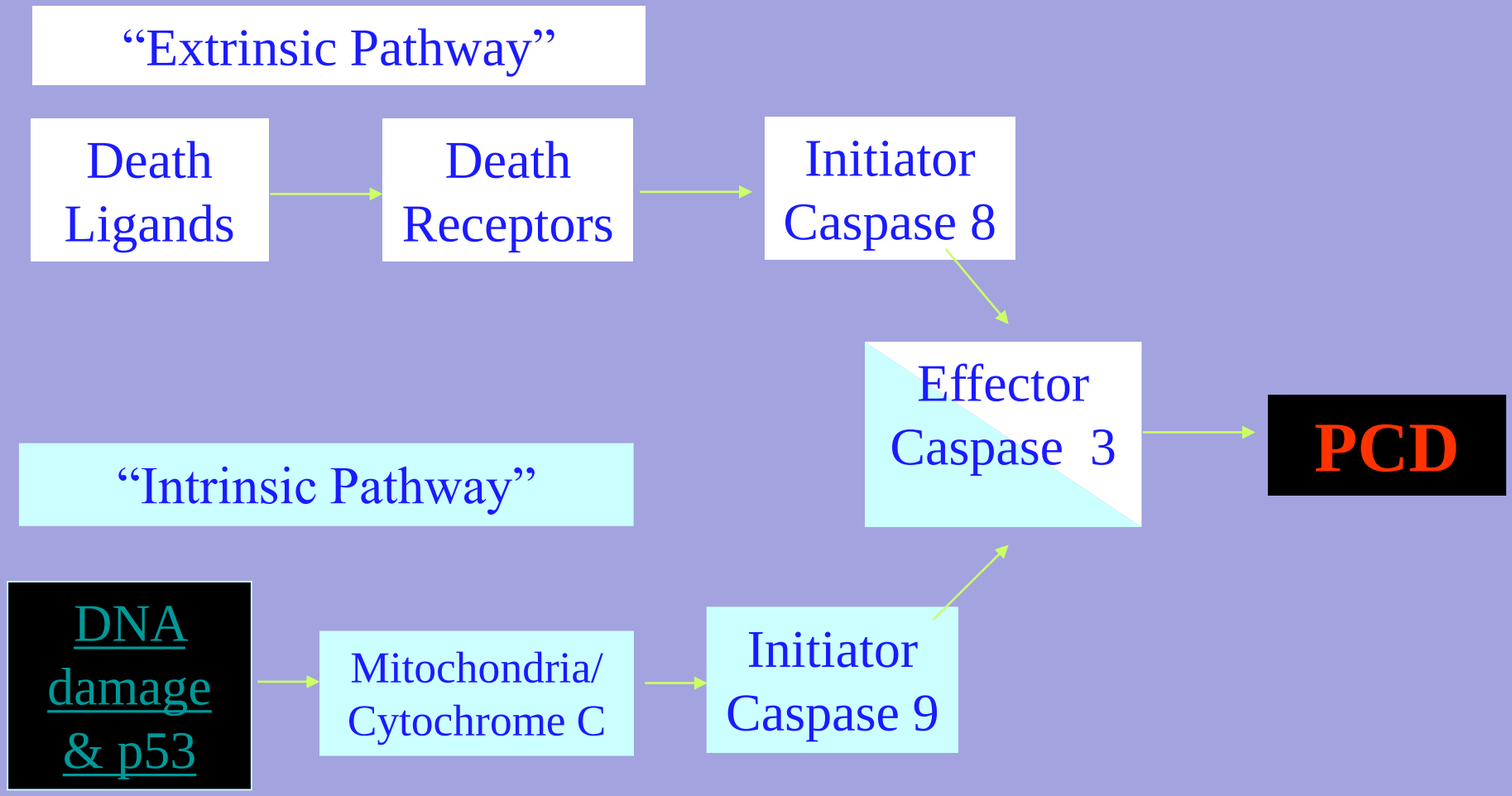
Caspase (3) cleavage of cellular proteins,
nuclease activation,
etc.

Death

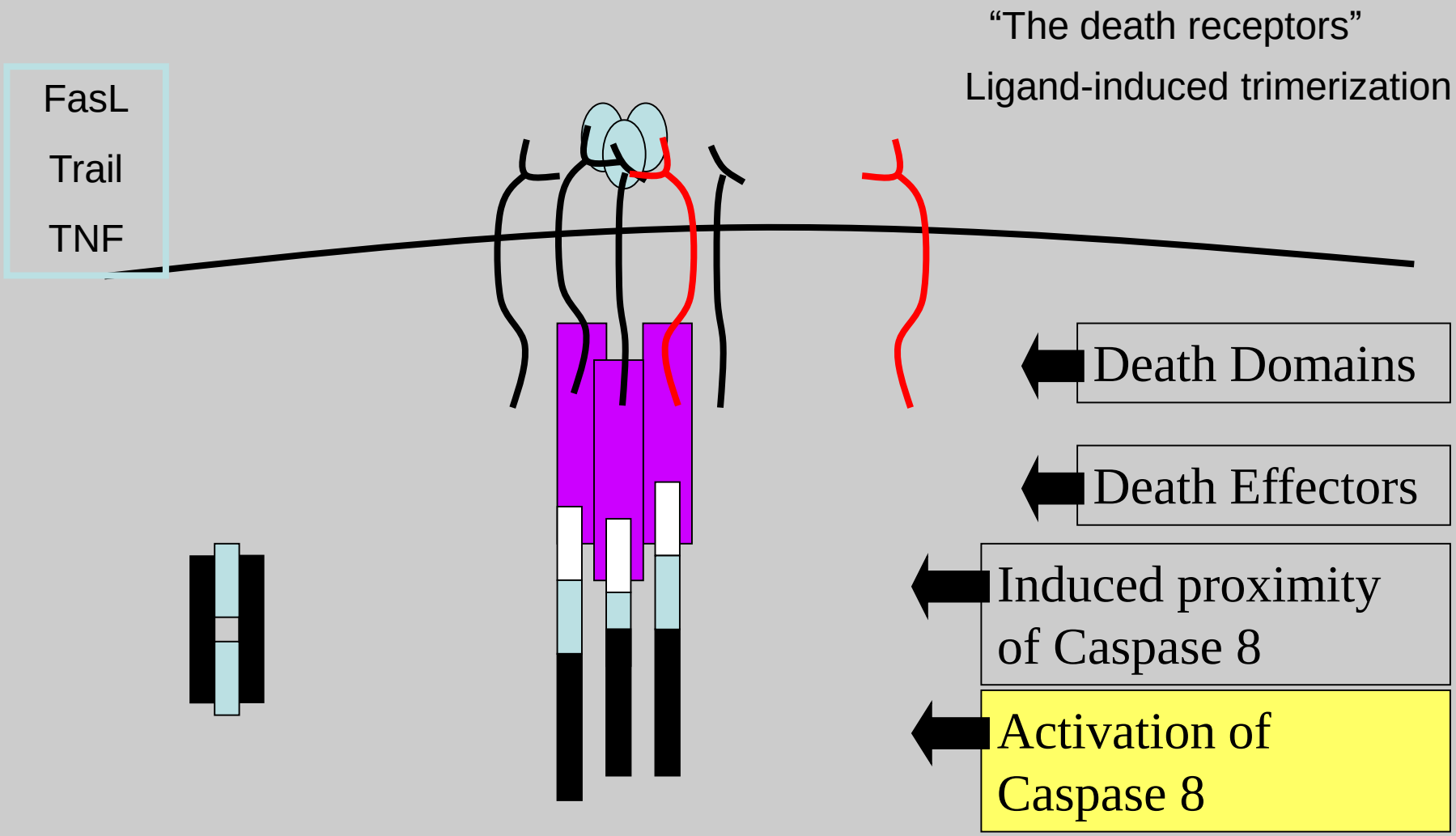
BAX
BAK
BOK
BCL-Xs
BAD
BID
B IK
BIM
NIP3
BNIP3

BCL-2
BCL-XL
BCL-W
MCL1
BFL1
DIVA
NR-13
Several viral proteins

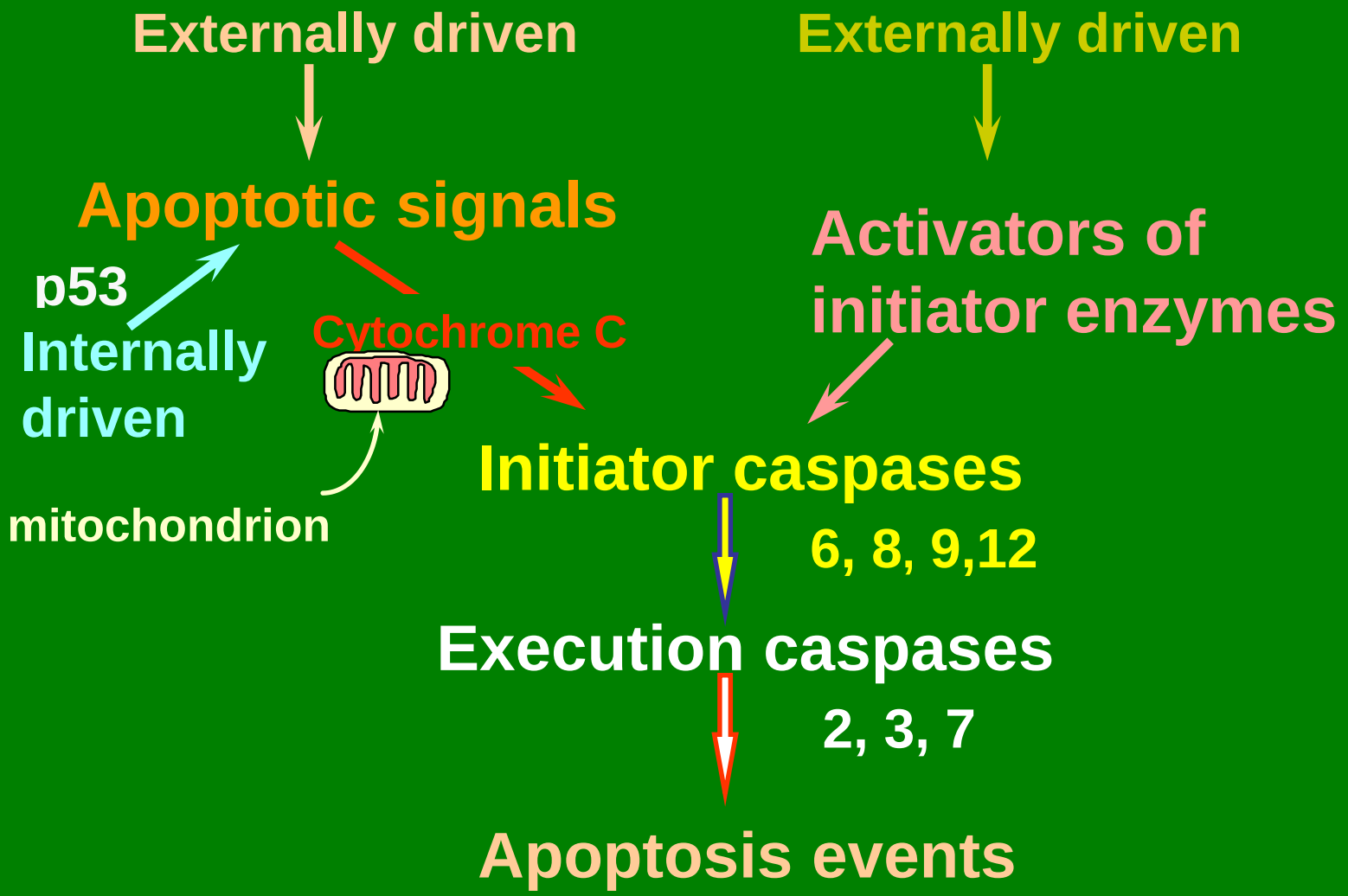
Apoptosis: Pathways



Ligand-induced cell death

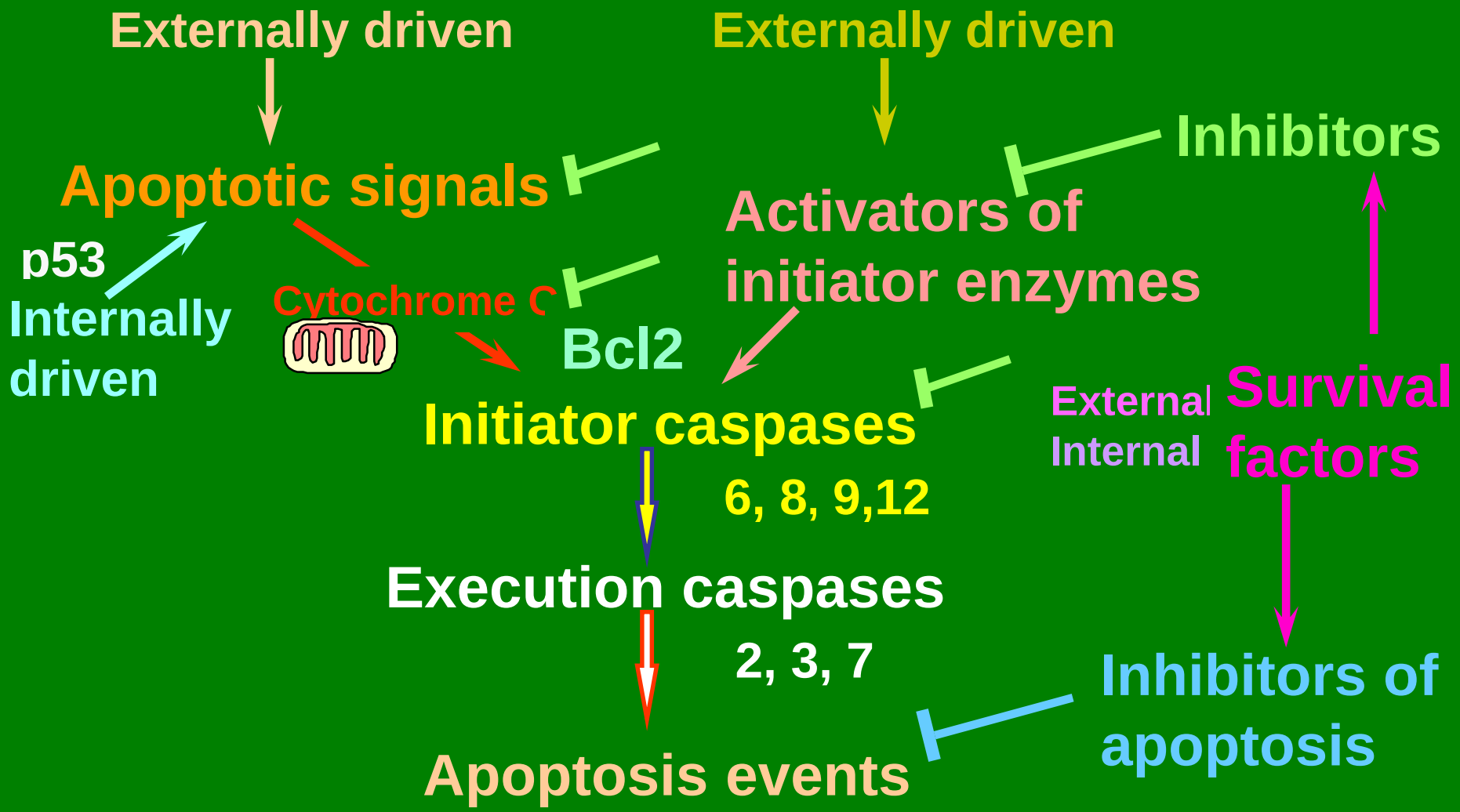


APOPTOSIS: Signaling & Control pathways I

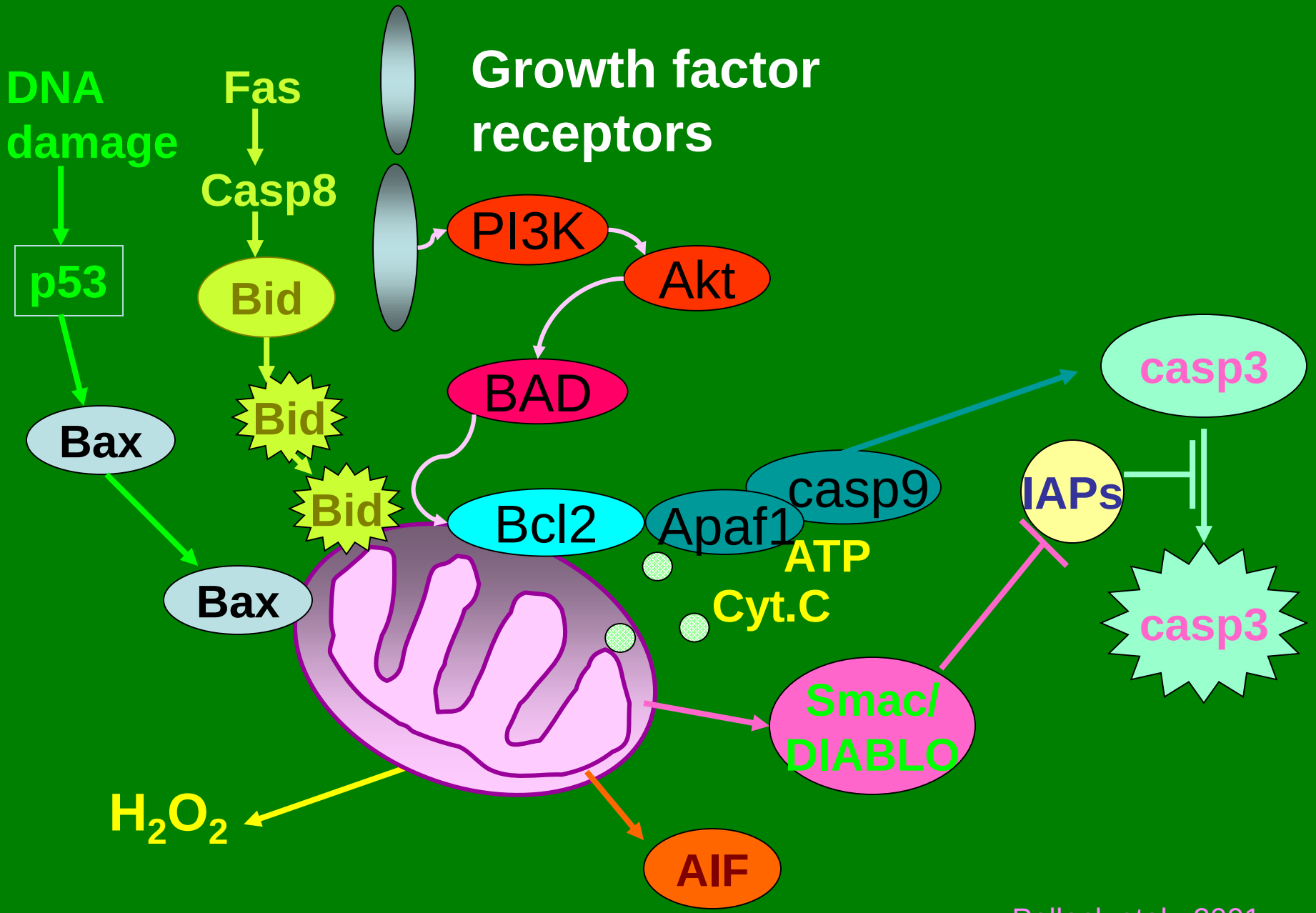


Activation

APOPTOSIS: Signaling & Control pathways II

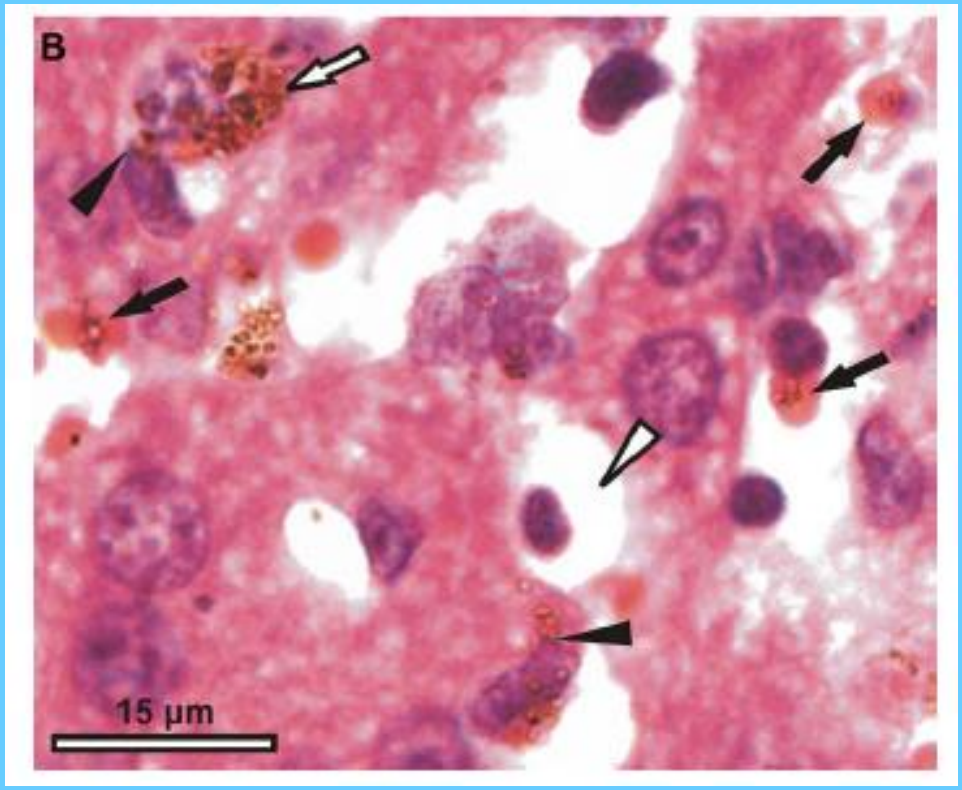


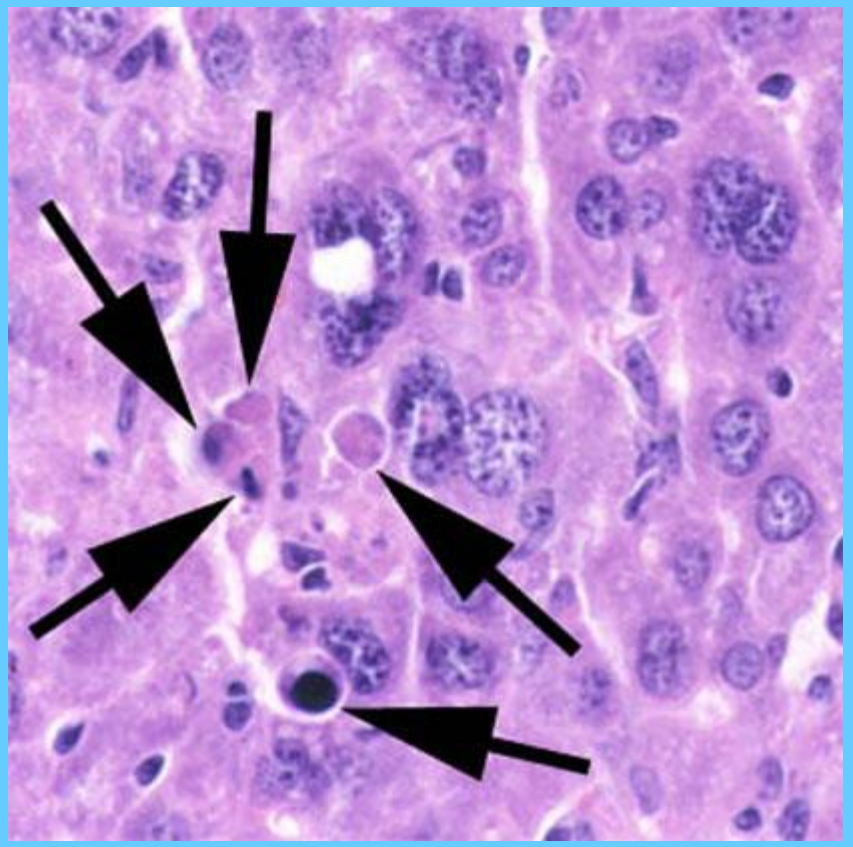
The mitochondrial pathway



Pollack etal., 2001

V- Techniques for apoptosis





Paraffin sections stained with Haematoxylin and eosin

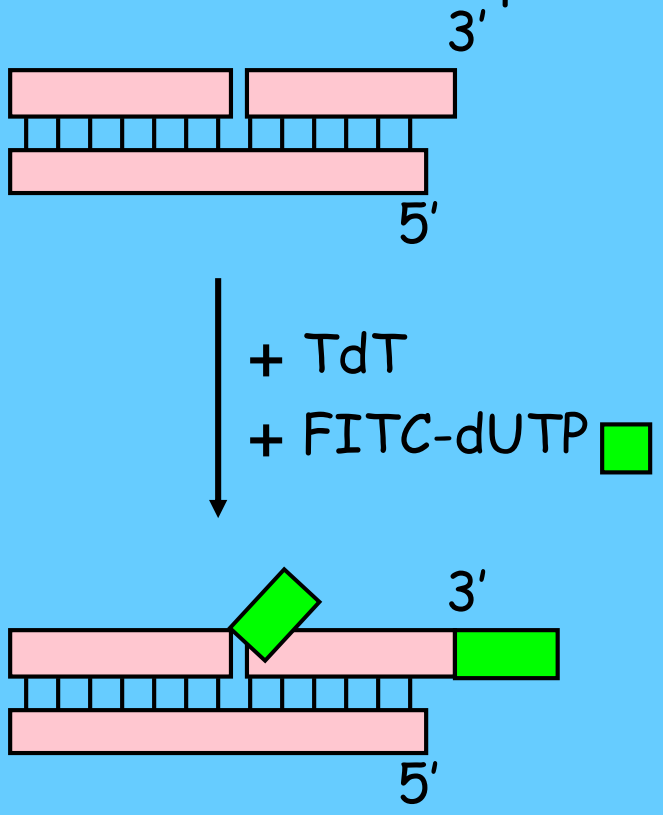
Methods to measure apoptosis

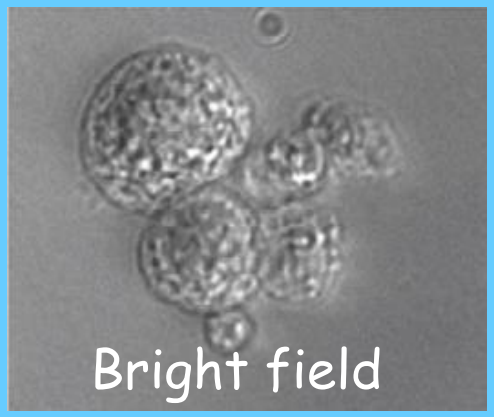
TUNEL assay: measure DNA fragmentation

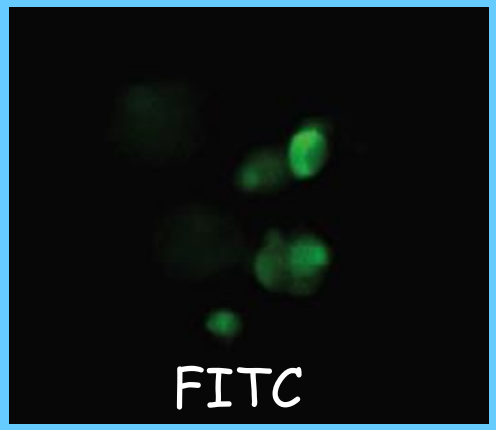
TUNEL= Terminal deoxynucleotidyl transferase dUTP nick end labeling.

TdT (terminal deoxynucleotidyl transferase):

adds dNTPs to the 3′ end of DNA molecules in the absence of a template







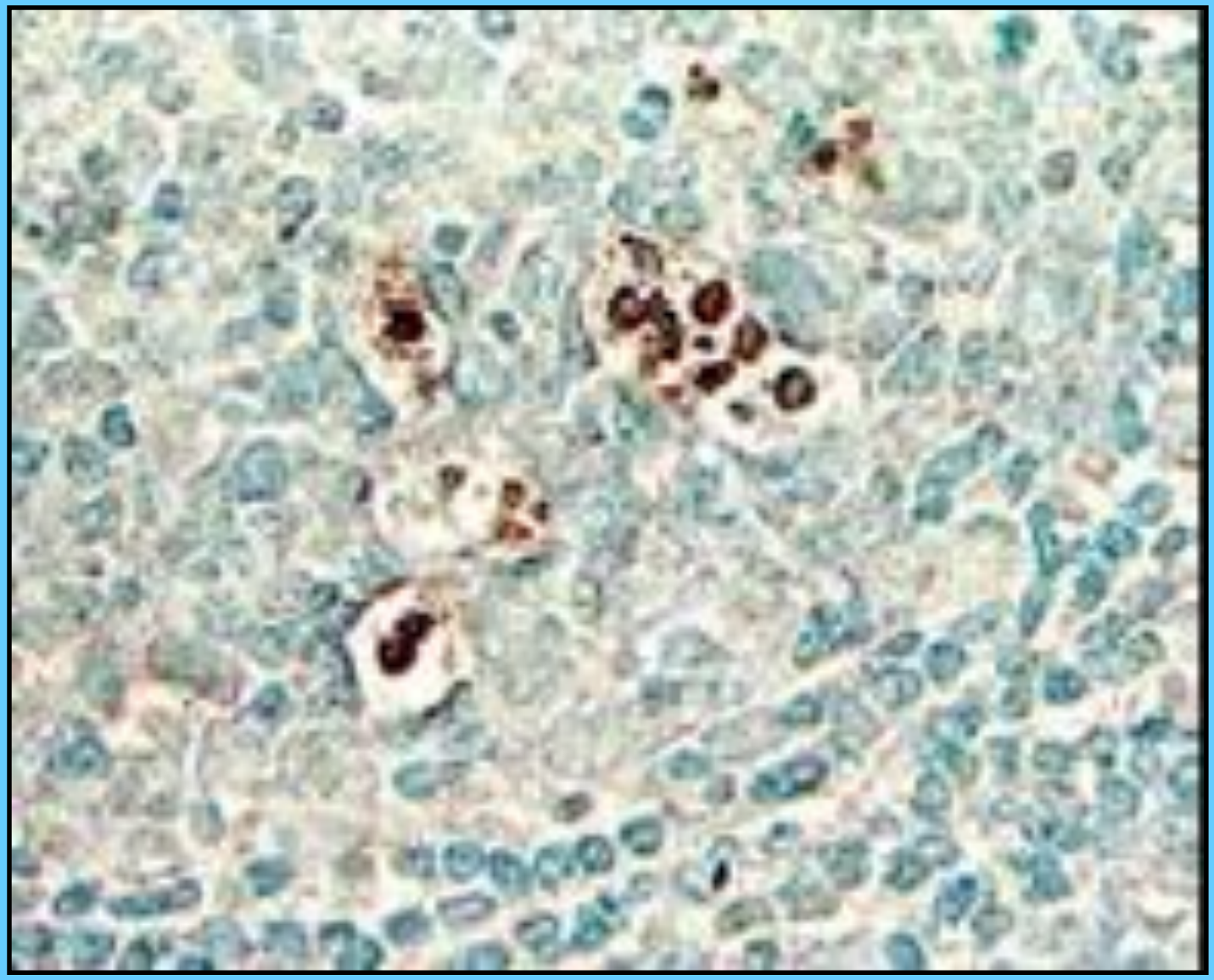


Figure 5.16. Detection of apoptotic cells in human lymph node Tunel reaction. The tissue was fixed in 10% neutral-buffered formalin and paraffin-embedded.

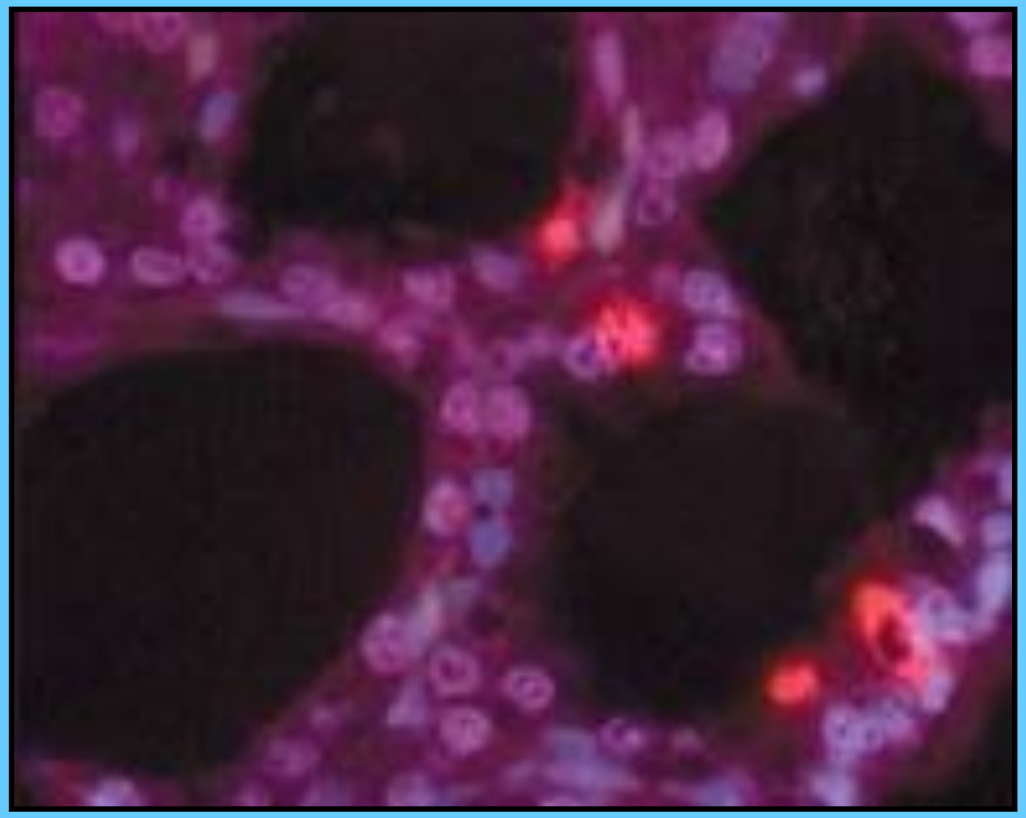


Figure 5.18. Detection of apoptotic cells in human lymph node using Fluorescein Direct *In Situ* Apoptosis Detection method.

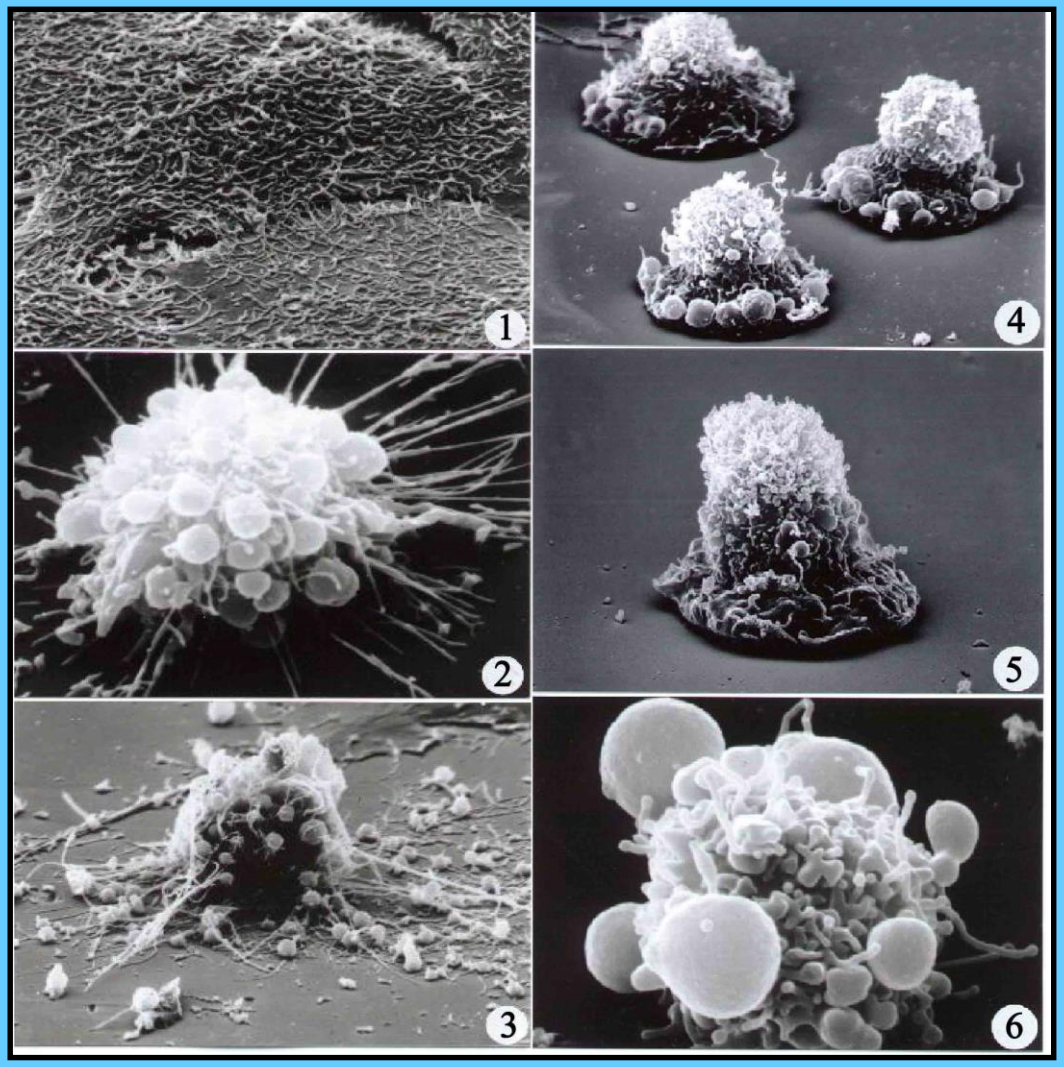


Figure 5.20. Scanning electron microscopy (5-10). Epithelial cells. Different stages. Flat cells (1) undergo different forms of rounding, surface blebbing and cell retraction (2-5) preceding the typical apoptotic figure shown in (6).

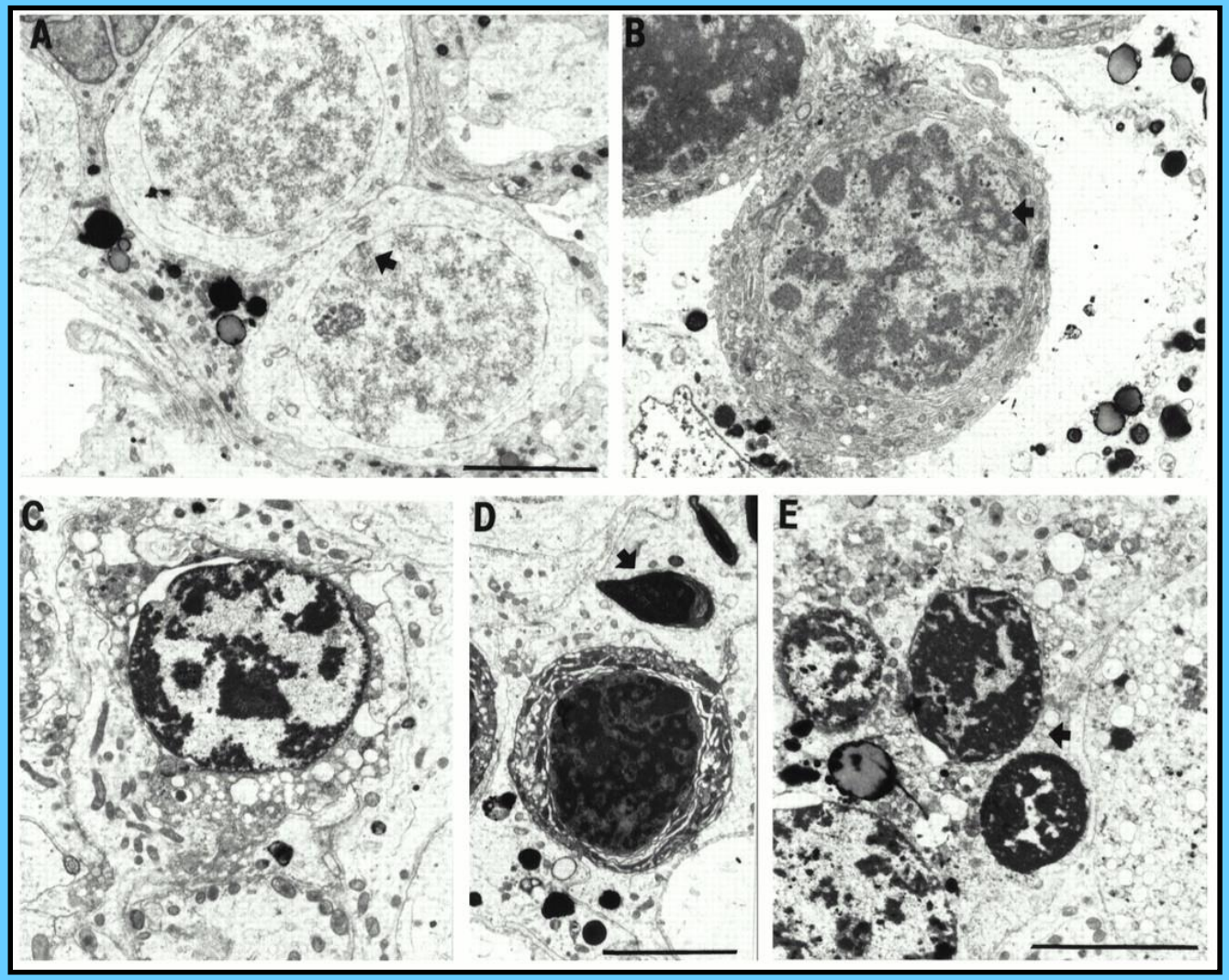


Figure 5.21. Electron micrographs of cells from seminiferous tubules of human testis. A, Two normal pachytene primary spermatocytes. Characteristic of these cells is the synaptonemal complex (*arrow*). B, Early apoptosis of pachytene spermatocyte, with chromatin beginning to condense (*arrow*). Most organelles in the cytoplasm retain a normal appearance. At the *upper left* is another apoptotic spermatocyte, with more advanced chromatin condensation. C–E, Further along in the apoptotic process, chromatin as well as cytoplasm condenses, and cytoplasmic organelles cease to be identifiable, with finally only small dense pycnotic bodies seen (*arrow* in E indicates late spermatid nucleus).

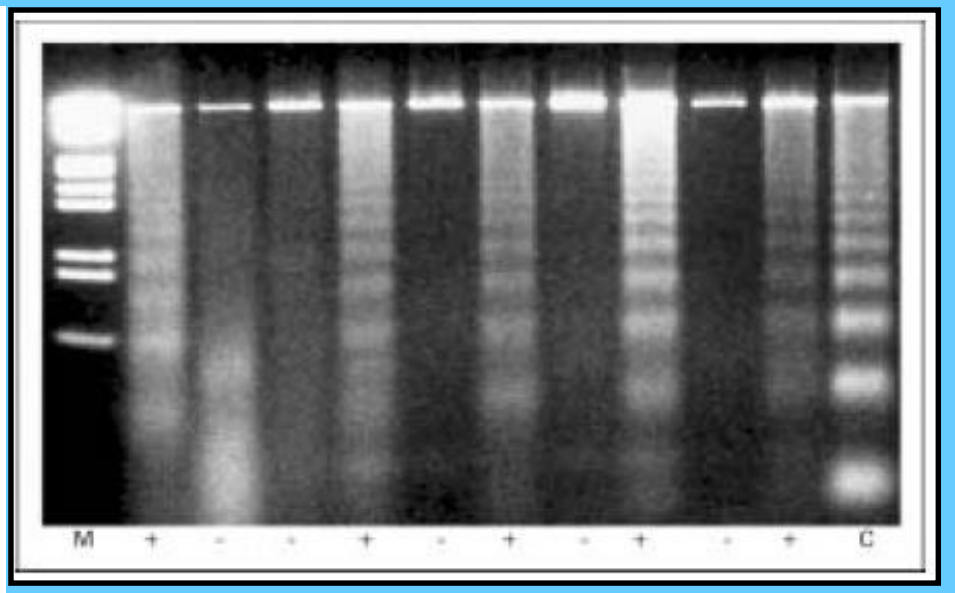


Figure 1. DNA ladder assayed with apoptotic DNA ladder kit. M (siye marker), - (control cell without camptothecin), + (cells treated with camptothecin, c (positive control from kit).

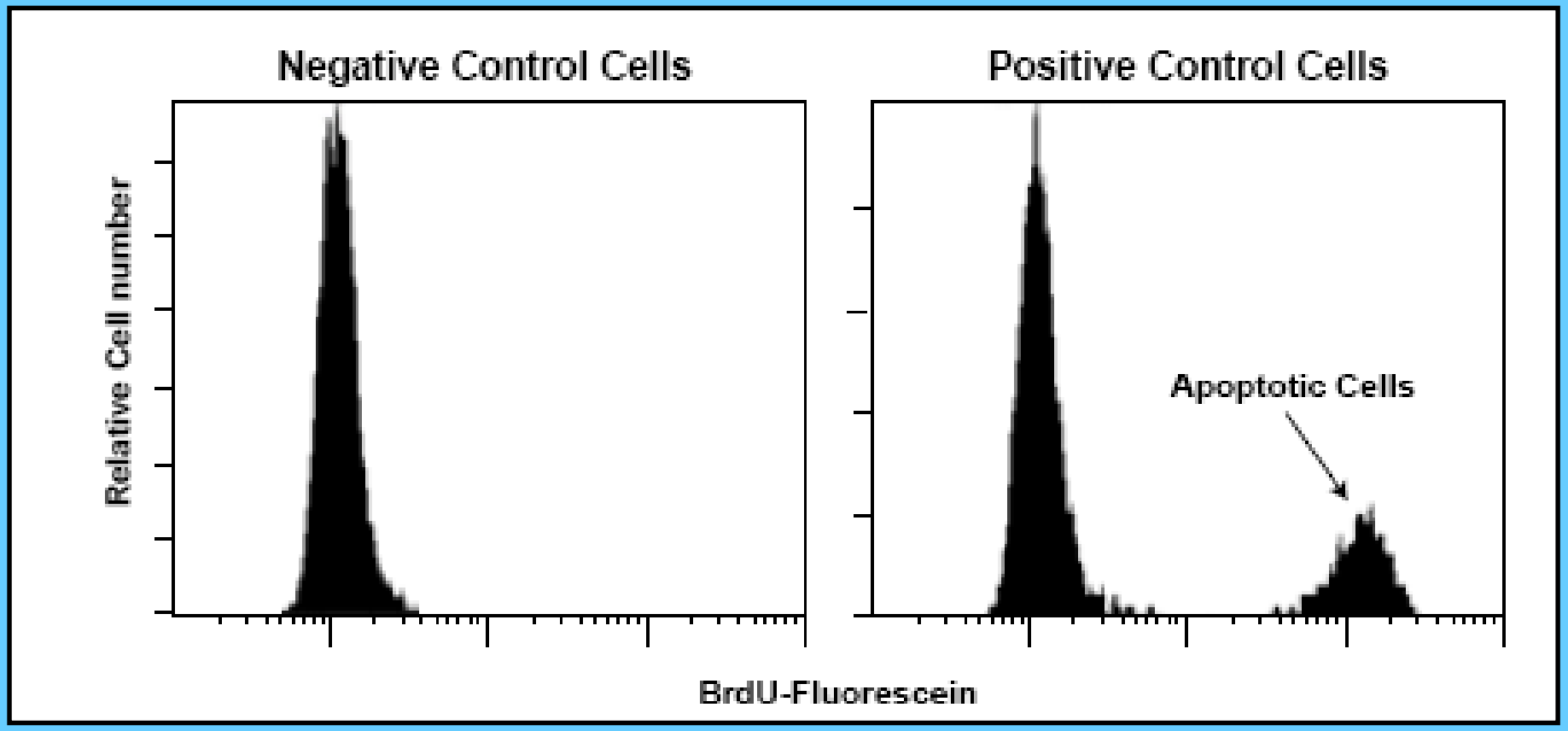
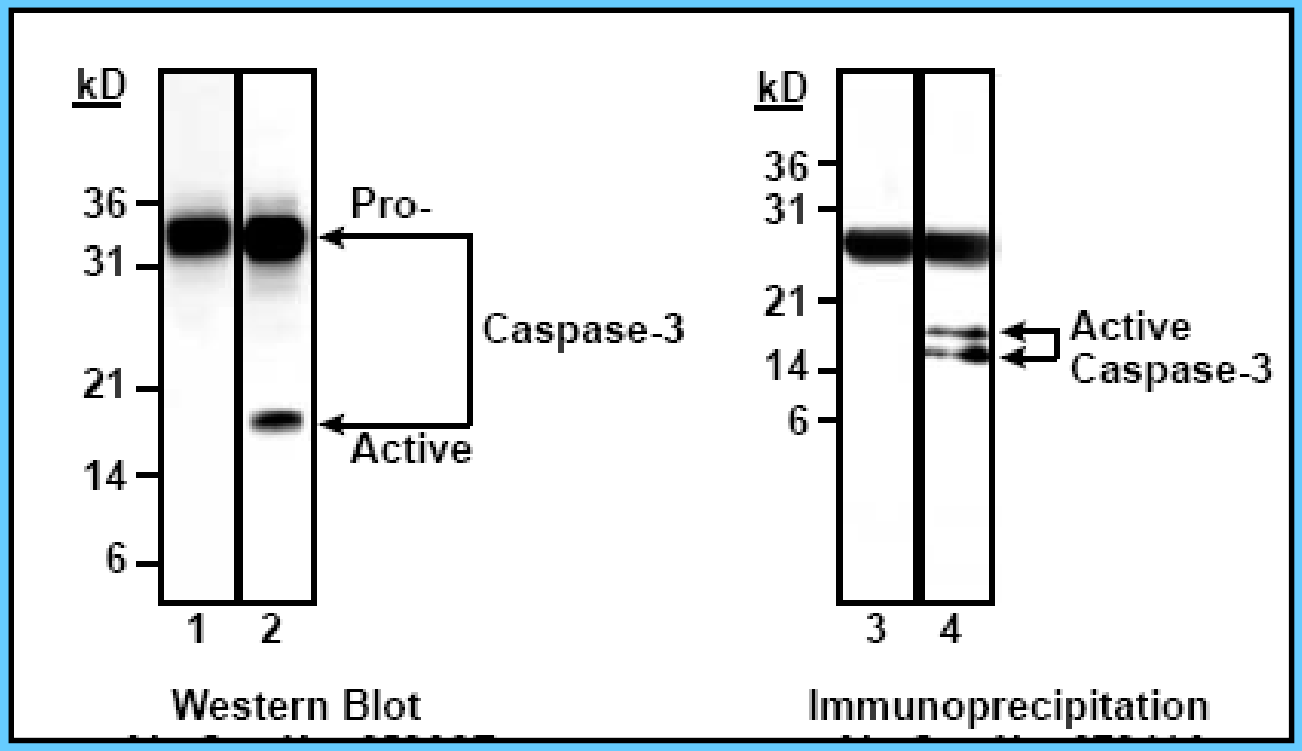
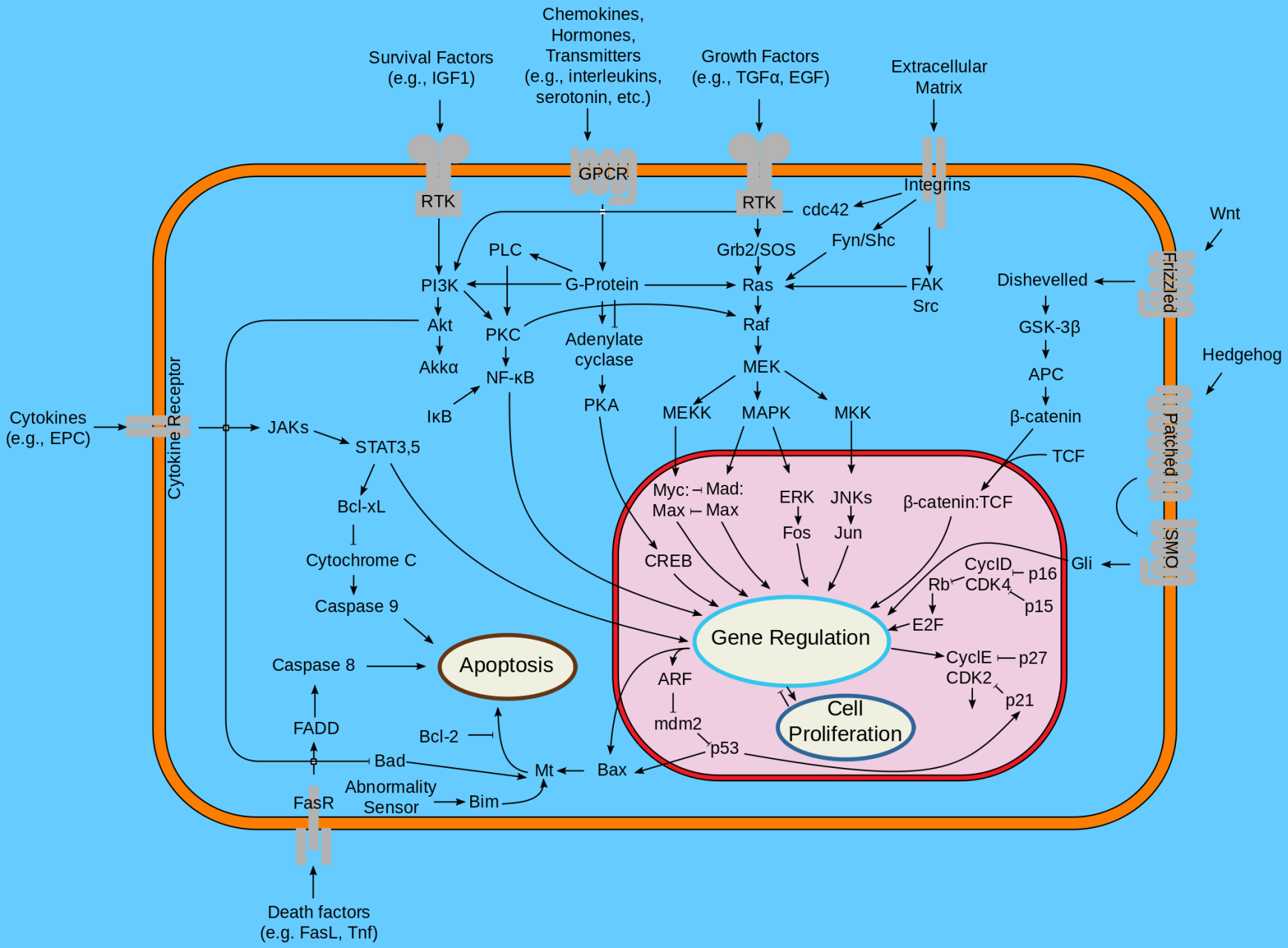
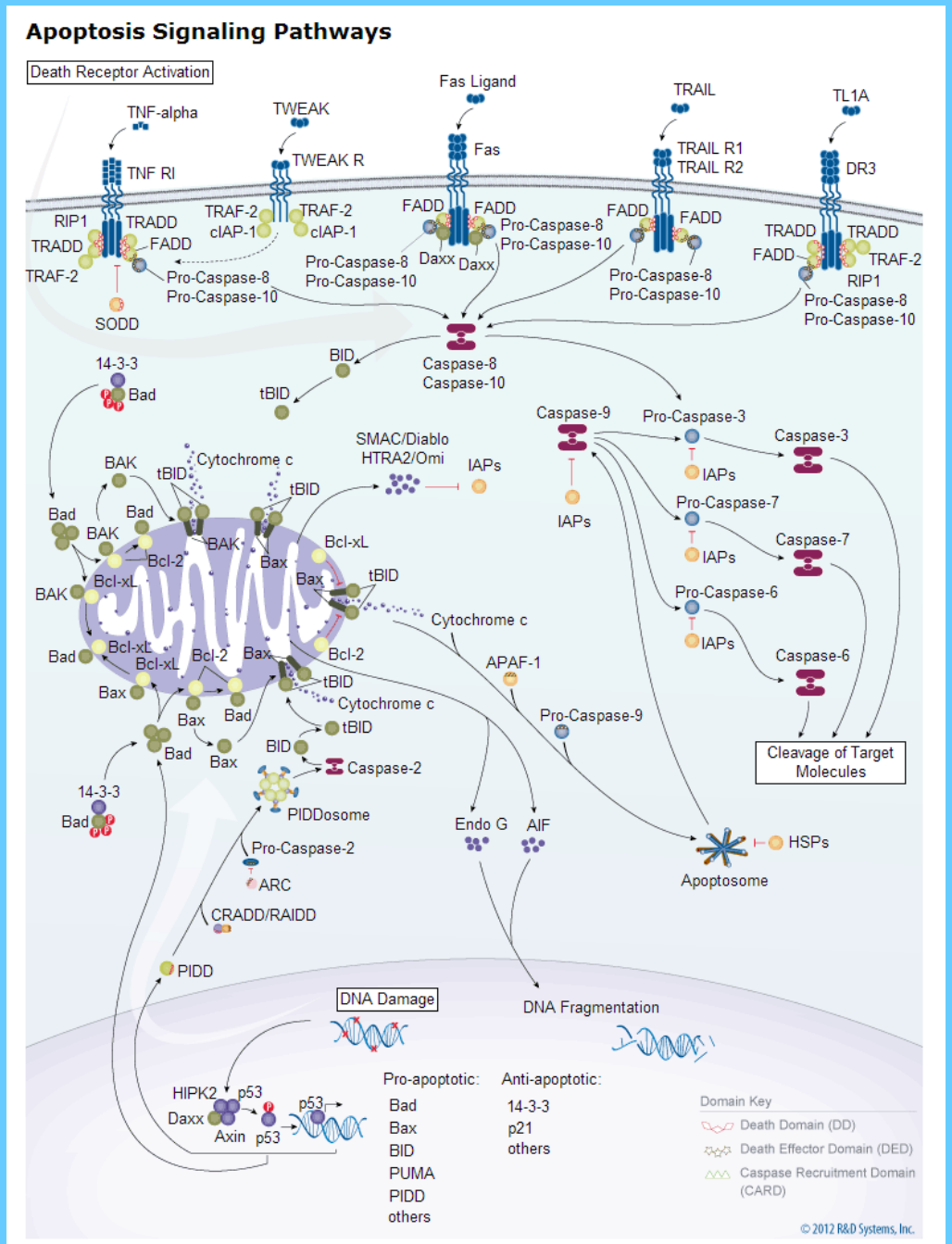


Figure 5.5. Flow Cytometry Data of APO-BRDU™ Negative and Positive Control cells. Negative and positive control cells were incubated with Br-dUTP in the presence of TdT enzyme in order to incorporate Br-dUTP into exposed 3'-OH DNA ends. Br-dUTP sites are detected with a fluorescein labeled anti-BrdU mAb. Non-apoptotic cells do not incorporate significant amounts of Br-dUTP due to the lack of exposed 3'-OH ends, and consequently have relatively little fluorescence compared to apoptotic cells which have an abundance of 3'-OH ends.


kD
36
31
21
14
6
Pro-
Caspase-3
Active
1
2
Western Blot
kD
36
31
21
14
6
Active
Caspase-3
3
4
Immunoprecipitation


Survival Factors
(e.g., IGF1)
Chemokines,
Hormones,
Transmitters
(e.g., interleukins,
serotonin, etc.)
Growth Factors
(e.g., TGFα, EGF)
Extracellular
Matrix
RTK
GPCR
RTK
Integrins
cdc42
Fyn/Shc
Grb2/SOS
PLC
PI3K
G-Protein
Ras
FAK
Src
Wnt
Frizzled
Dishevelled
GSK-3β
APC
β-catenin
Hedgehog
Patched
SMO
TCF
Akt
PKC
Adenylate
cyclase
Raf
Akkα
NF-κB
IκB
MEK
PKA
MEKK
MAPK
MKK
Cytokines
(e.g., EPC)
Cytokine Receptor
JAKs
STAT3,5
Bcl-xL
Cytochrome C
Caspase 9
Caspase 8
Apoptosis
FADD
Bcl-2
Bad
Mt
Bax
FasR
Abnormality
Sensor
Bim
Death factors
(e.g. FasL, Tnf)
Myc: ⊣ Mad:
Max ⊢ Max
ERK
JNKs
Fos
Jun
β-catenin:TCF
CREB
Gli
CycID
p16
Rb
CDK4
p15
E2F
Gene Regulation
CycIE
p27
CDK2
p21
ARF
mdm2
p53
Cell
Proliferation


Apoptosis Signaling Pathways
Death Receptor Activation
TNF-alpha
TNF RI
TWEAK
TWEAK R
Fas Ligand
Fas
TRAIL
TRAIL R1
TRAIL R2
TL1A
DR3
RIP1
TRADD
FADD
TRAF-2
cIAP-1
Pro-Caspase-8
Pro-Caspase-10
Daxx
SODD
Caspase-8
Caspase-10
BID
tBID
14-3-3
Bad
BAK
Bax
Bcl-2
Bcl-xL
Cytochrome c
SMAC/Diablo
HTRA2/Omi
IAPs
Caspase-9
Pro-Caspase-3
Caspase-3
Pro-Caspase-7
Caspase-7
Pro-Caspase-6
Caspase-6
APAF-1
Pro-Caspase-9
Apoptosome
HSPs
Cleavage of Target Molecules
Caspase-2
PIDDosome
Pro-Caspase-2
ARC
CRADD/RAIDD
PIDD
Endo G
AIF
DNA Damage
DNA Fragmentation
HIPK2
p53
Axin
Pro-apoptotic:
Bad
Bax
BID
PUMA
PIDD
others
Anti-apoptotic:
14-3-3
p21
others
Domain Key
Death Domain (DD)
Death Effector Domain (DED)
Caspase Recruitment Domain (CARD)
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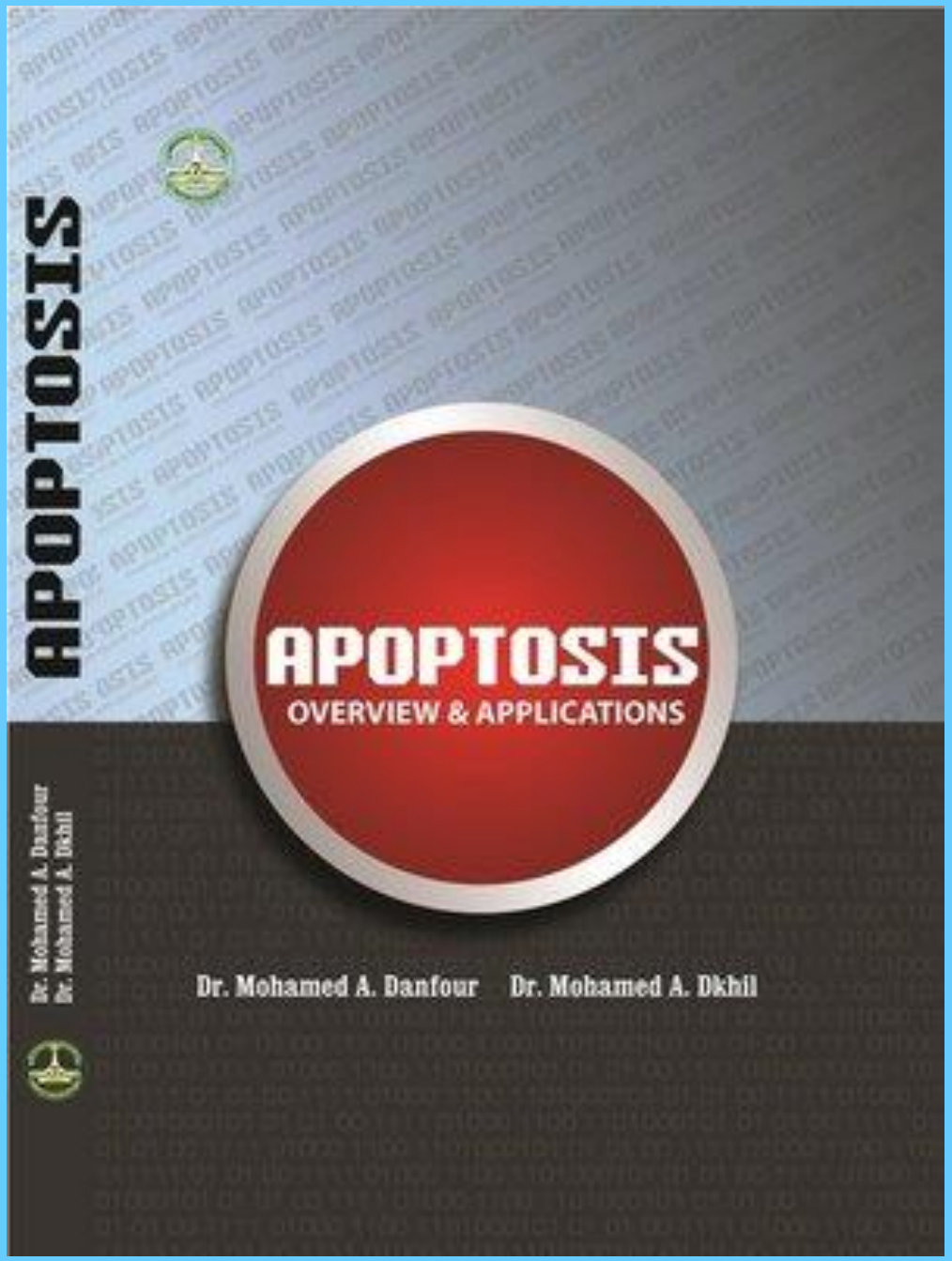
APOPTOSIS
APOPTOSIS
OVERVIEW & APPLICATIONS
Dr. Mohamed A. Danfour
Dr. Mohamed A. Dkhil
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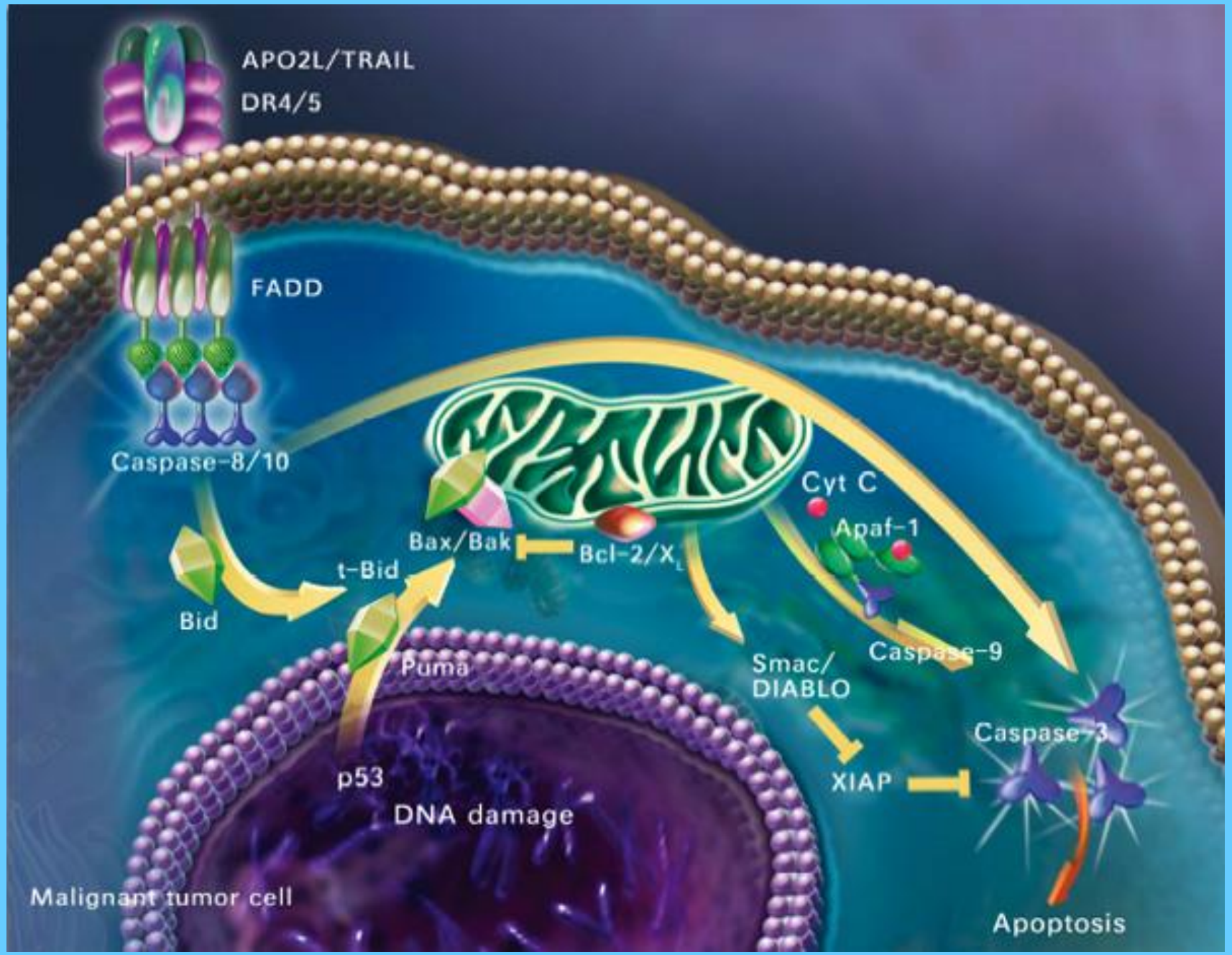
Dr. Mohamed A. Dkhil

Molecular mechanisms of apoptosis signalling pathways

A- Various death signals activate common signaling pathways

Apoptosis can be triggered by various stimuli from outside or inside the cell, e.g. by ligation of cell surface receptors ,by DNA damage as a cause of defects in DNA repair mechanisms, treatment with cytotoxic drugs or irradiation, by a lack of survival signals, contradictory cell cycle signalling or by developmental death signals. Death signals of such diverse origin nevertheless appear to eventually activate a common cell death machinery leading to the characteristic features of apoptotic cell death.


APO2L/TRAIL
DR4/5
FADD
Caspase-8/10
Bid
t-Bid
Bax/Bak
Bcl-2/X$_L$
Cyt C
Apaf-1
Caspase-9
Smac/
DIABLO
XIAP
Caspase-3
Apoptosis
Puma
p53
DNA damage
Malignant tumor cell

Clinical Applications of Apoptosis Research

Many diseases as cancers, autoimmune diseases and neurodegenerative diseases, including Alzheimer's demonstrate either a failure of apoptosis to eliminate harmful cells or the inappropriate activation of apoptosis leading to loss of essential cells.