APOPTOSIS

2011

Maestria en Biologia Molecular Medica

- 1842 VOGT DESCRIPCIÓN MORFOLÓGICA
- 1890 BOINET CÉLULAS MUEREN DURANTE EL TRANSCURSO NORMAL DE LA VIDA
- 1972 KERR APOPTOSIS
- 1980 HORNVITZ MUERTE CELULAR PROGRAMADA

DIVISION CELULAR

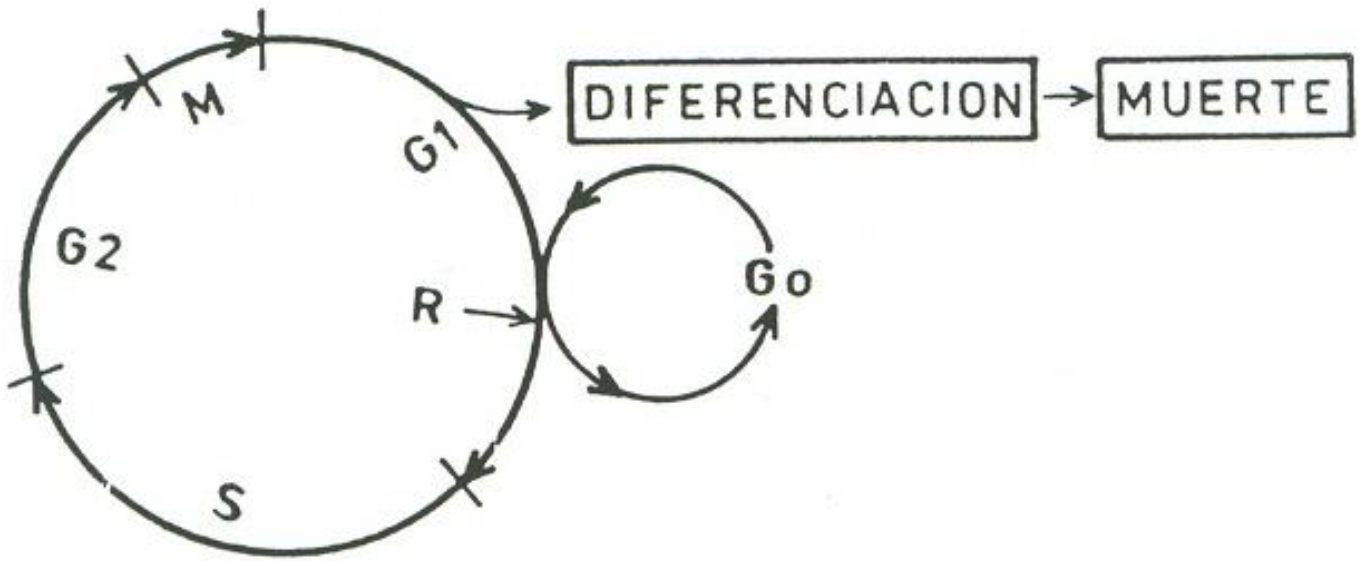
Número células

↑ División Celular

↓ Muerte Celular Programada
Apoptosis

División Celular > Apoptosis
Nº Células ↑

División Celular = Apoptosis
Nº Células constante



Esquema del Ciclo Celular

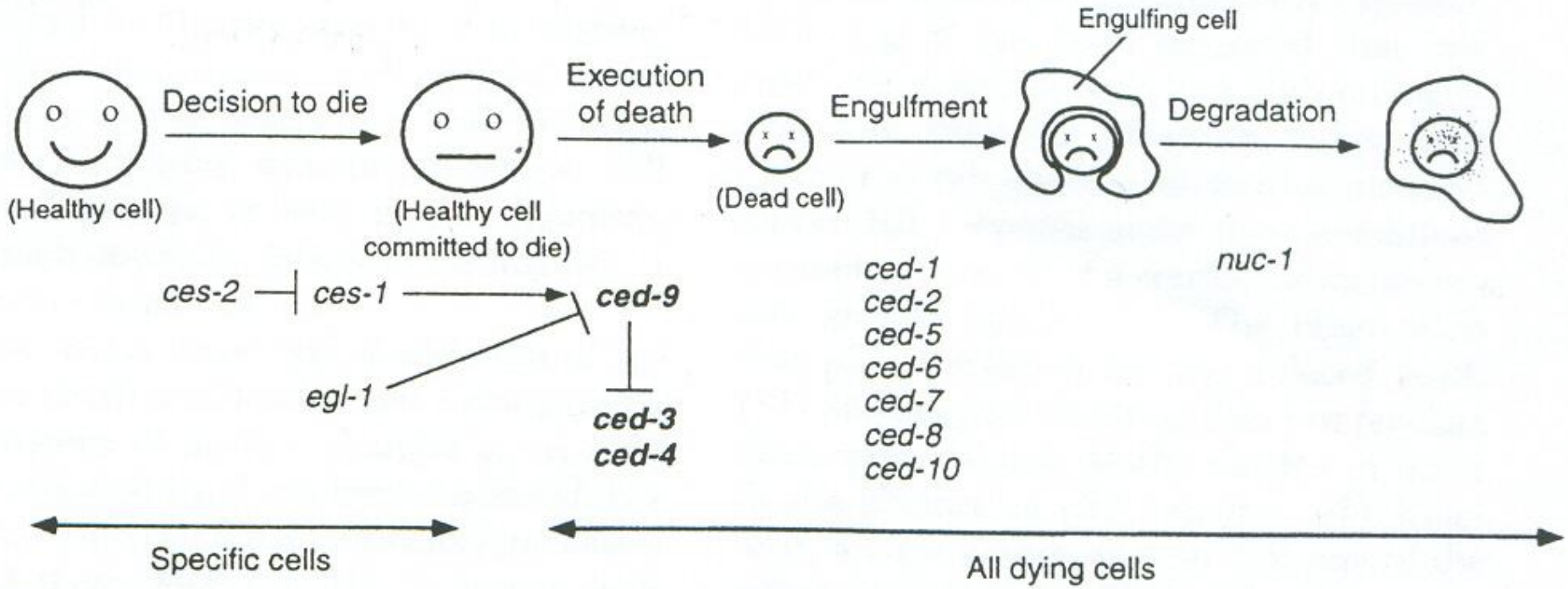


Fig. 1. Genetic pathway for programmed cell death in *C. elegans*. Mutations in 14 different genes have been isolated that affect specific stages of programmed cell death in *C. elegans*. Mutations that influence the decision to die affect only a small number of cells. In contrast, genes involved in all subsequent stages of cell death are common to all somatic cell deaths in this organism. Three genes, *ced-3*, *ced-4*, and *ced-9*, affect the execution of the death program. The activity of *ced-3* and *ced-4* promotes cell death, and *ced-9* activity prevents this process. The epistatic relationship among these genes has been deduced from double-mutant combinations. Symbols: → (positive regulation); ——| (negative regulation). [Adapted from (*14*)]

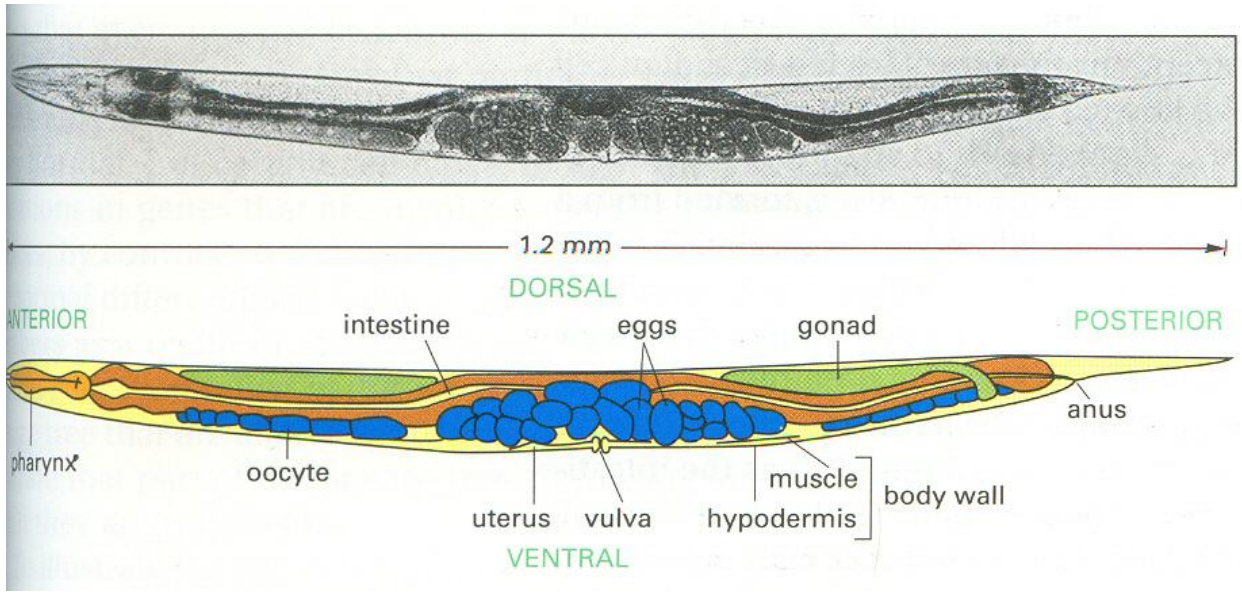


Figure 21–39 ***Caenorhabditis elegans.*** A side view of an adult hermaphrodite is shown. Note that the tissue called hypodermis in the nematode corresponds to the epidermis of other animals. (From J.E. Sulston and H.R. Horvitz, *Dev. Biol.* 56:110–156, 1977.)

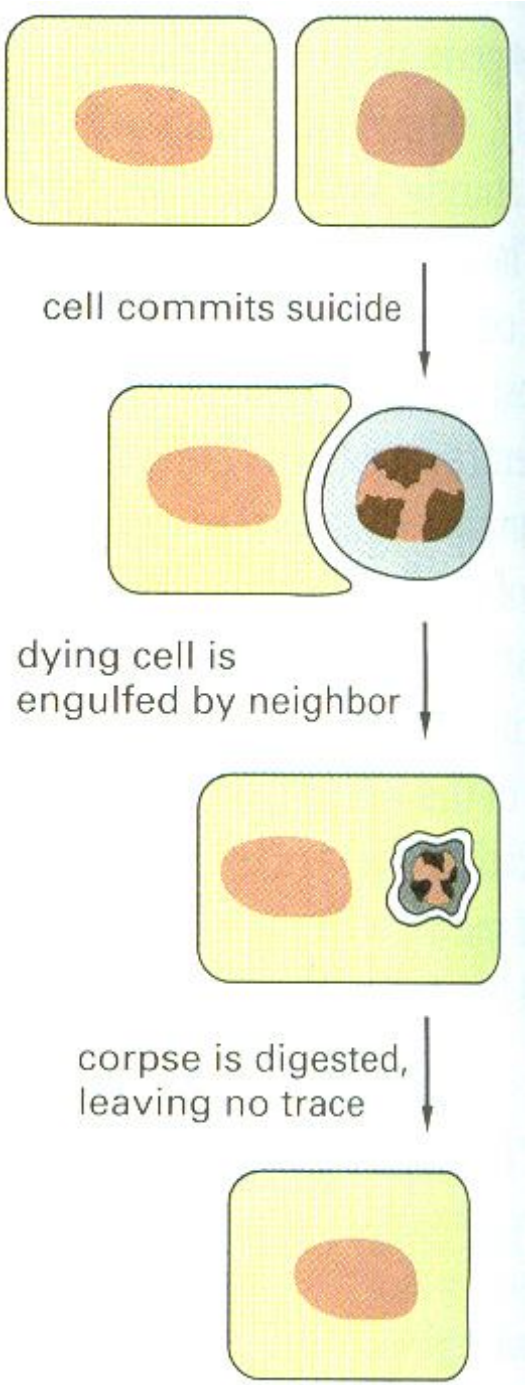
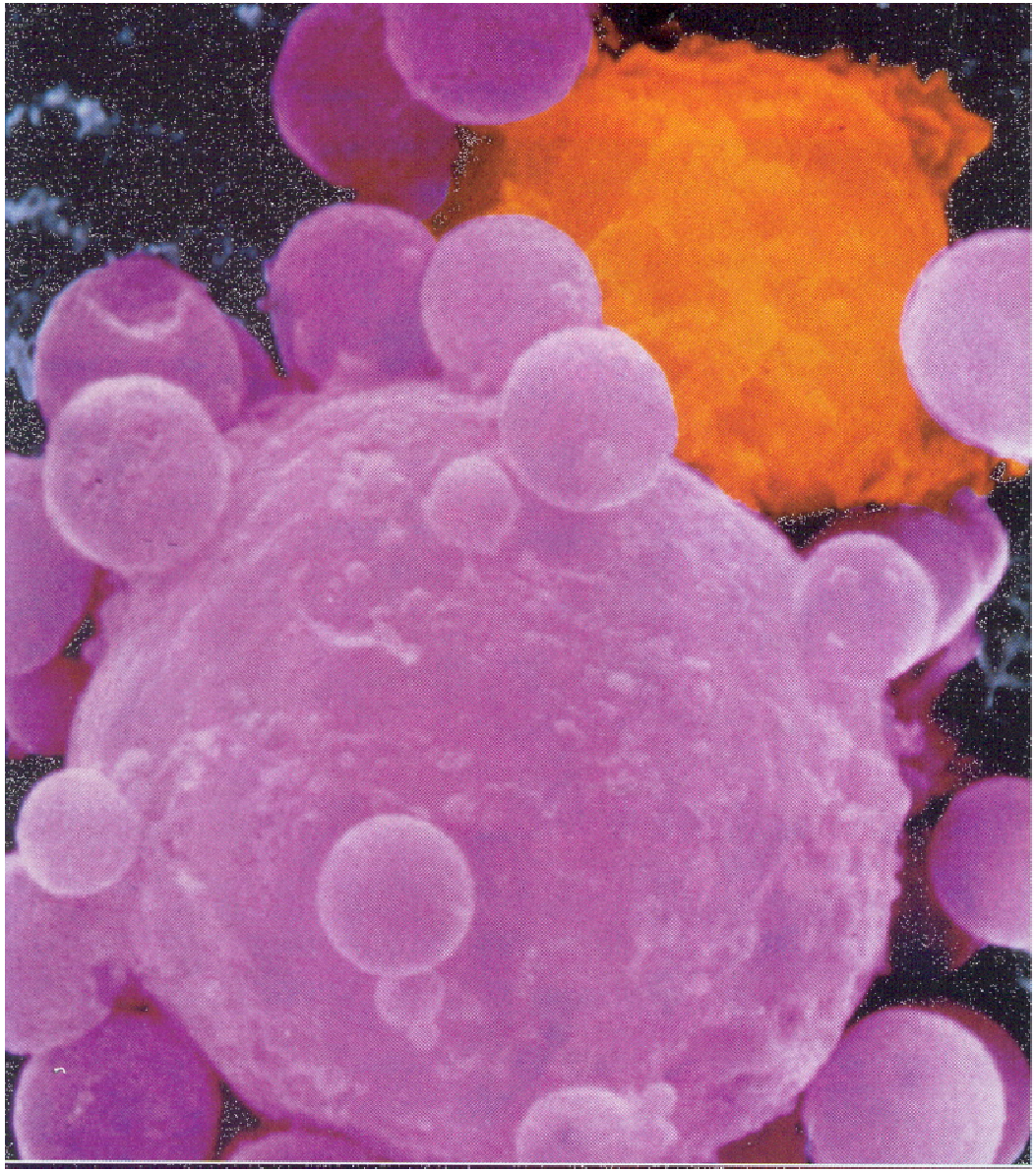


Figure 21–46 Apoptotic cell death in *C. elegans*. Death depends on expression of the *ced-3* and *ced-4* genes in the dying cell itself, whereas the subsequent engulfment and disposal of the remains depend on expression of other genes in the neighboring cells.



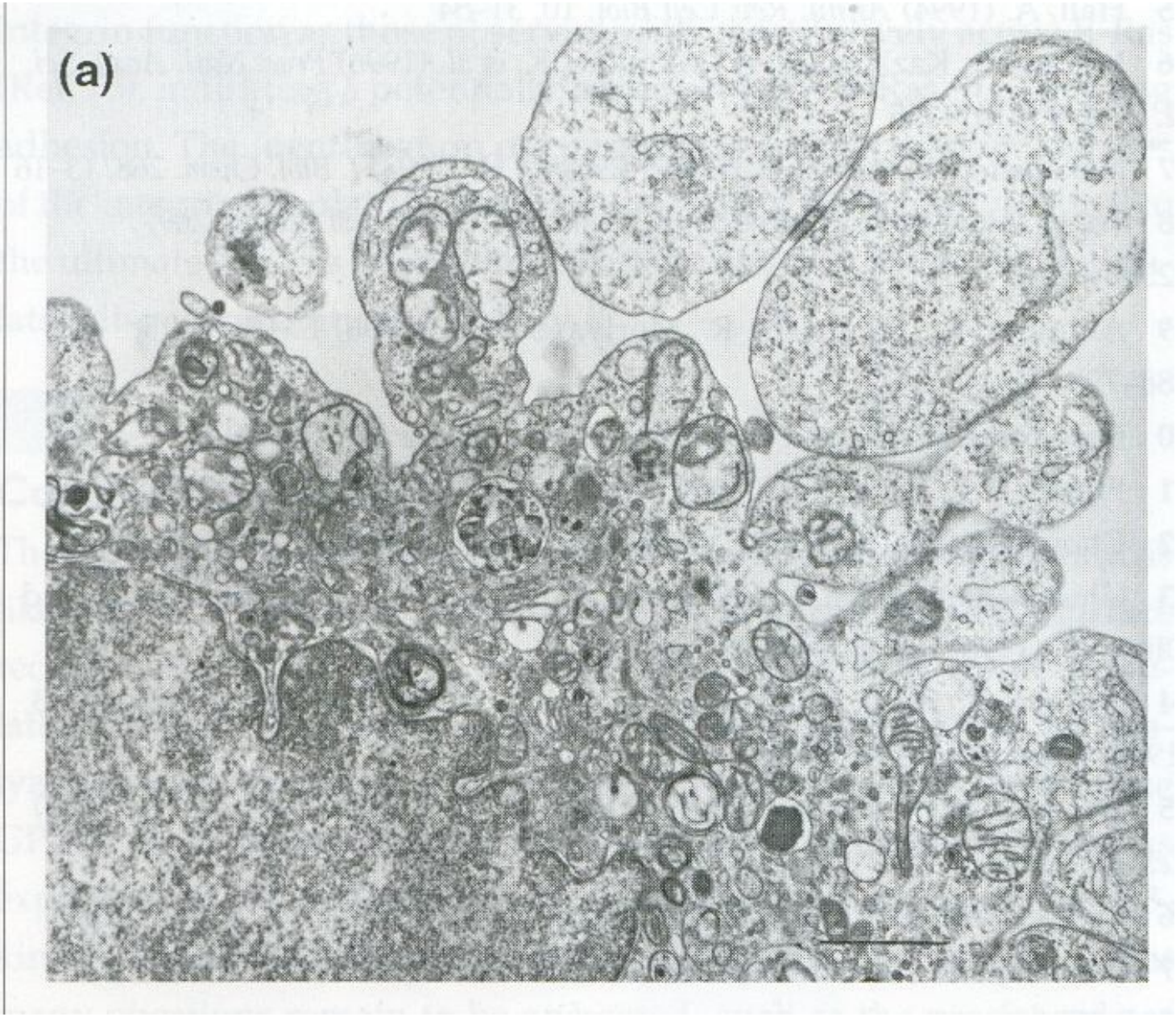
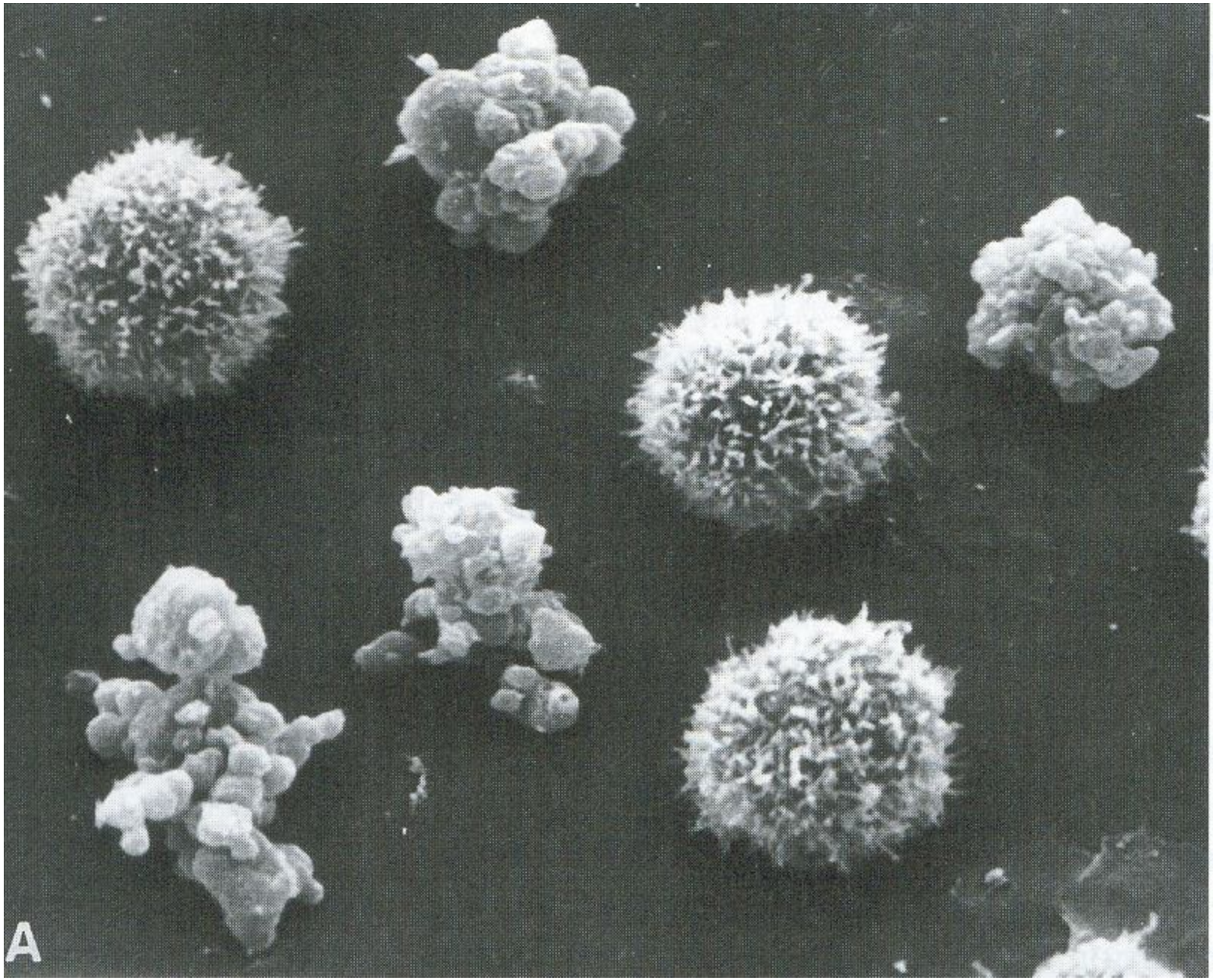


Fig. 1. *Apoptotic morphology in the cytotoxic targets of macrophages. (a) A mouse L cell displaying membrane blebbing typical of apoptosis*[6].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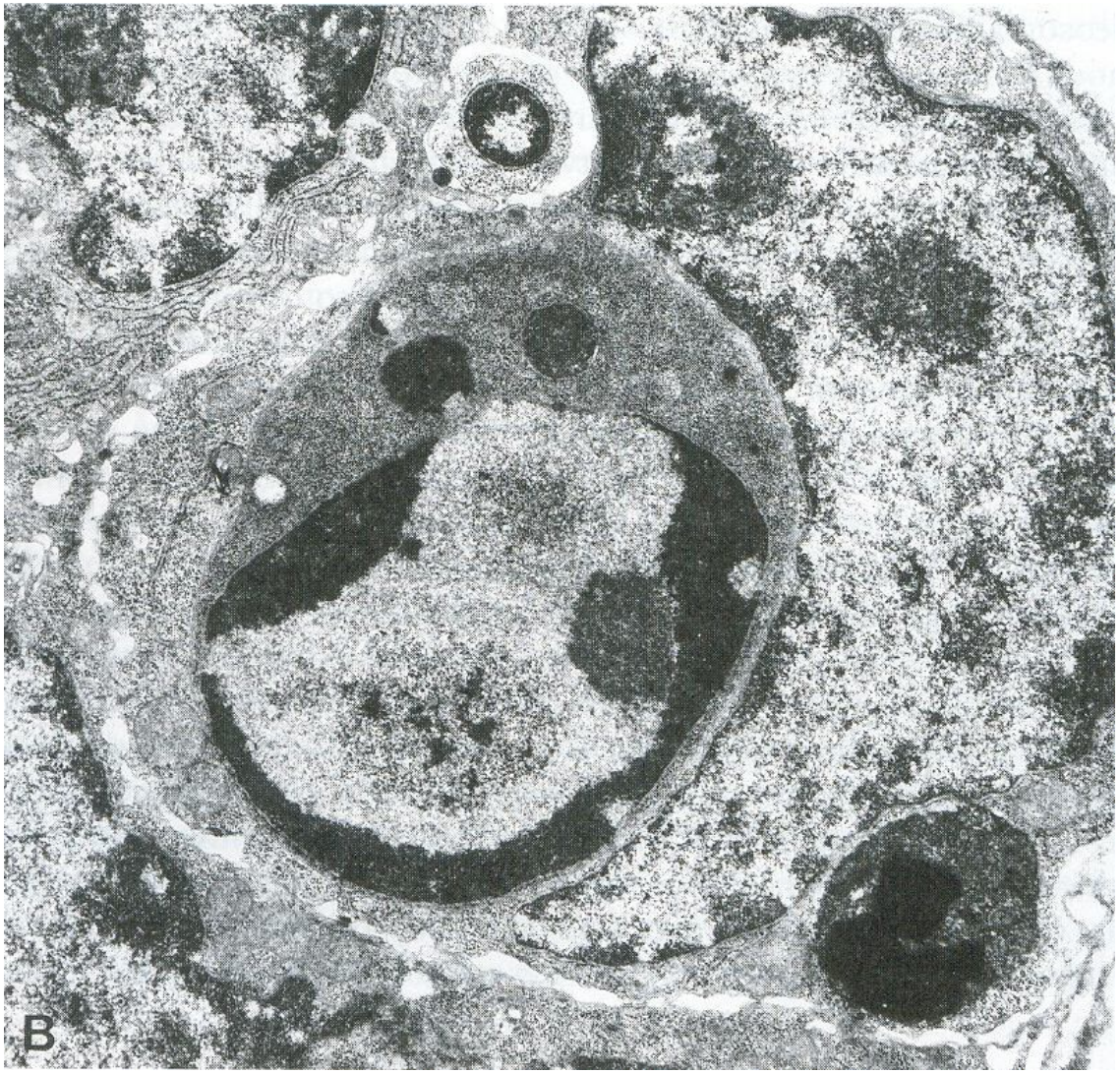
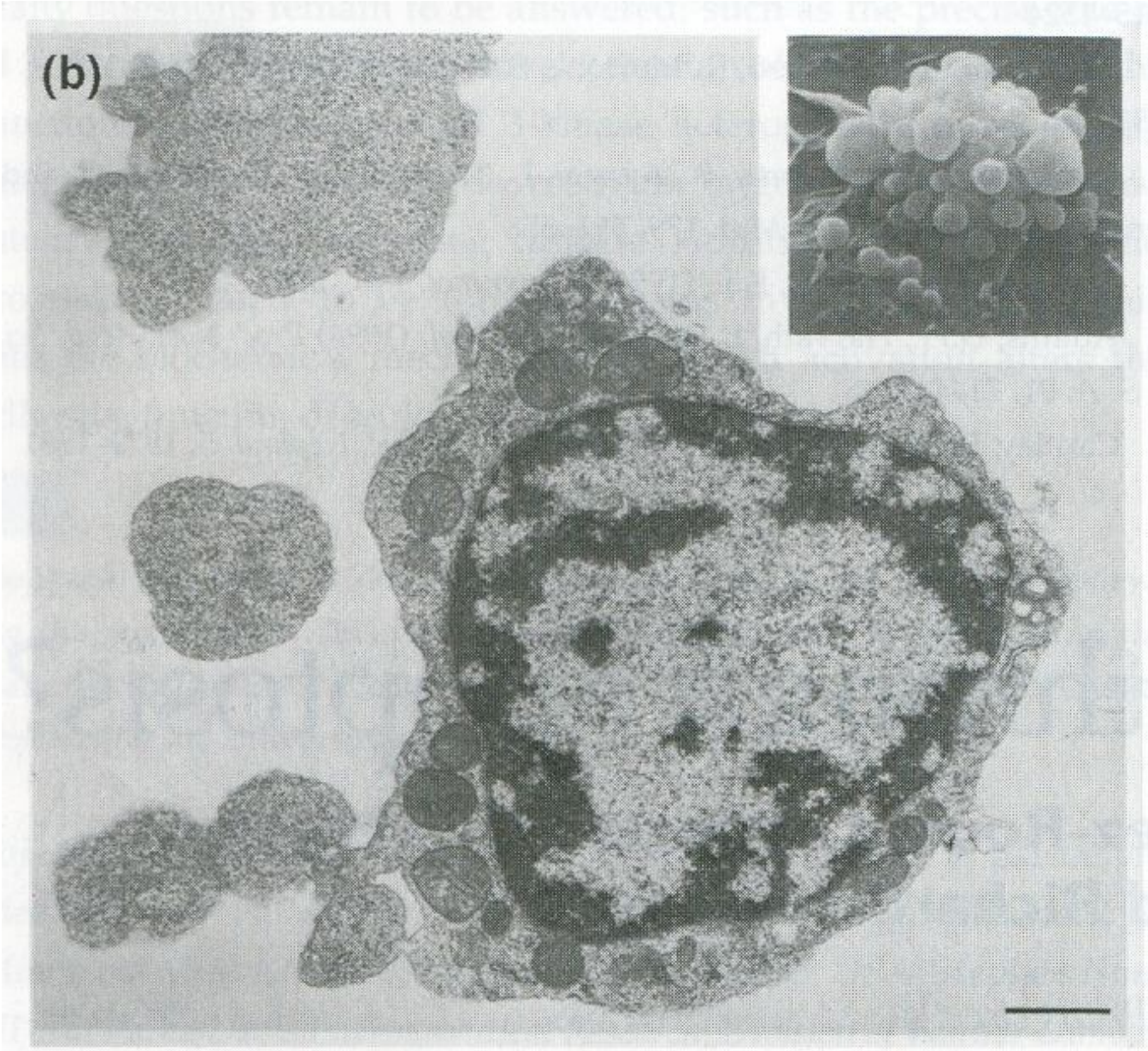


FIGURE 1 Cytoplasmic and nuclear changes characteristic of apoptosis. (A) Viable and apoptotic mouse sarcoma 180 cells growing as ascites tumors that were treated with actinomycin D 18 h previously. The viable, intact cells are covered with microvilli, whereas cells undergoing apoptosis display protuberances of the plasma membrane, or "blebbing," which are devoid of microvilli (courtesy of C. Bishop, published with permission of *Int. Rev. Cytol.*). (B) Apoptotic remnants of epithelial cells of mouse small intestinal crypts 4 h after exposure to 400 rads X rays which have been phagocytosed by an adjacent epithelial cell. The chromatin of the large nuclear remnant has condensed and collapsed along the nuclear periphery. Note also the characteristic nucleolar changes (courtesy of J. F. R. Kerr, published with permission from *Int. Rev. Cytol.*).



(b) SL2 lymphoma cell in a macrophage cytotoxicity assay with the chromatin condensation characteristic of apoptosis[11]; inset, SL2 lymphoma cell showing apoptotic surface convolutions[11].

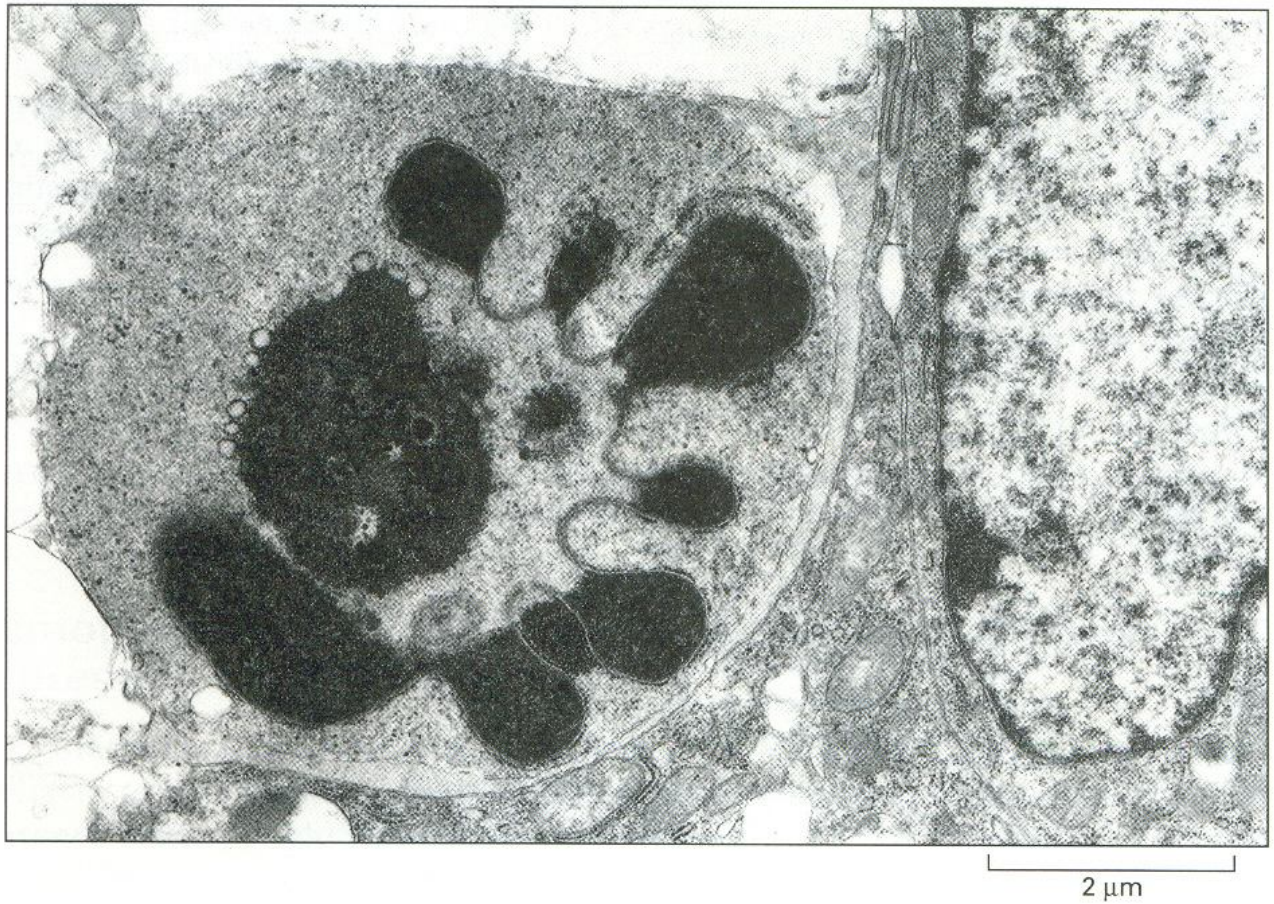


Figure 22–35 Cells dying by apoptosis. The electron micrograph shows an apoptotic cell in the mammary gland. Apoptotic cell death is a normal occurrence here, balancing the proliferation of mammary epithelial cells that occurs in each menstrual cycle. Note the disintegrating nuclear envelope and the dark clumps of condensed chromatin. For comparison, part of a normal cell is visible to one side of the picture. (Courtesy of David Ferguson.)

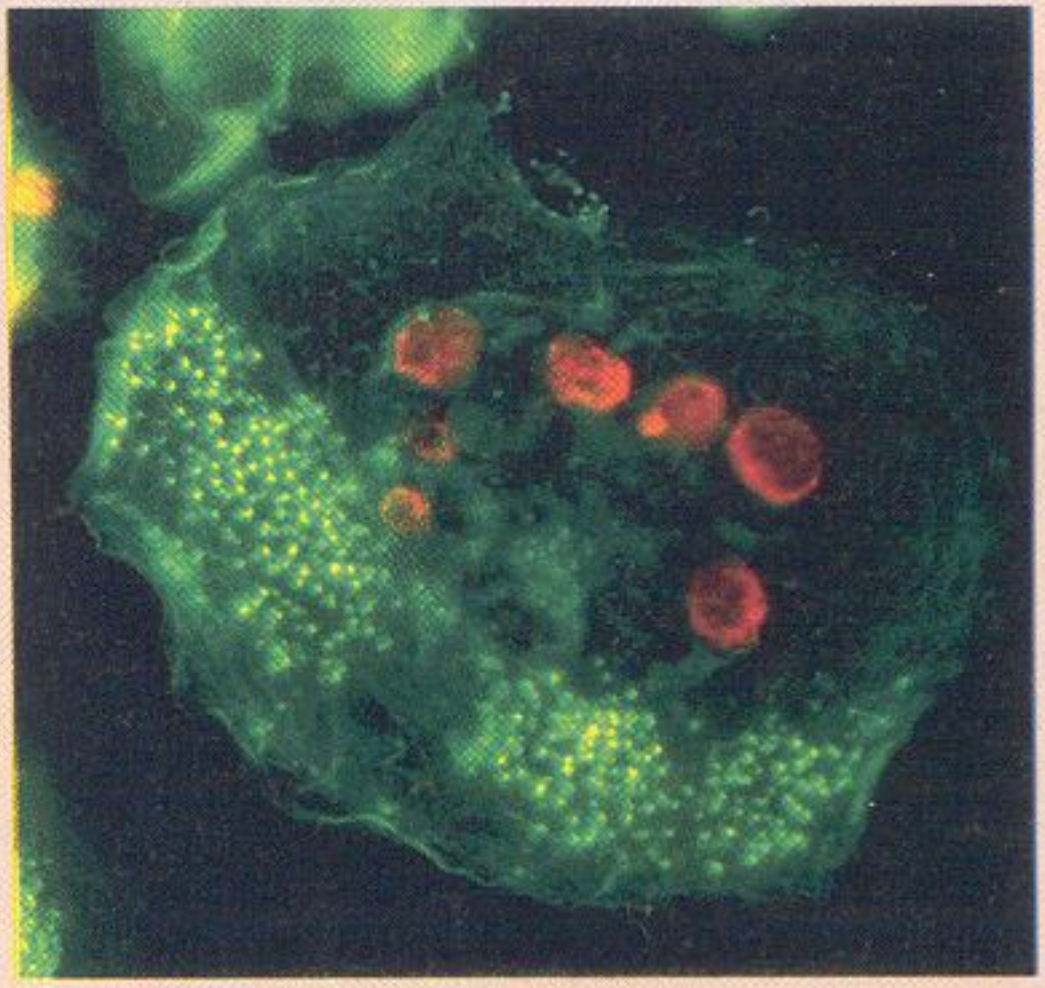


Figure 2 A human monocyte-derived macrophage ingests multiple apoptotic bodies. Jurkat T-cell targets were labelled with 5-(and 6)-carboxytetramethylrhodamine succinimidyl ester and irradiated to induce apoptosis. The macrophages were stained with fluorescein isothyocyanate-conjugated phalloidin to identify actin filaments. (Photograph courtesy of M. Janes and P. Henson).

APOPTOSIS
MUERTE CELULAR PROGRAMADA

- CONDENSACIÓN NUCLEAR Y CITOPLASMÁTICA
- ALTERACIONES EN LA ESTRUCTURA DE LA MEMBRANA
- VARIACIÓN EN LA COMPOSICIÓN DE LÍPIDOS.
  TRANSLOCACIÓN DE LA FOSFATIDILSERINA
- BURBUJEO O BLEBBING DE LA MEMBRANA CELULAR
- RÁPIDA DESTRUCCIÓN DEL GENOMA
- ACTIVACIÓN DE ENDONUCLEASAS QUE CLIVAN EL DNA GENÓMICO EN MÚLTIPLES FRAGMENTOS INTERNUCLEOSOMALES
- FRAGMENTACIÓN DE LA CÉLULA EN CUERPOS APOPTÓTICOS
- ELIMINACIÓN DE LOS FRAGMENTOS CELULARES POR CÉLULAS FAGOCÍTICAS

NECROSIS
DAÑO IRREVERSIBLE Y MUERTE RÁPIDA

- DILATACIÓN O SWELLING DEL NÚCLEO
- DILATACIÓN DE ORGANELAS CITOPLASMÁTICAS
- RUPTURA DE LA MEMBRANA PLASMÁTICA
- DESINTEGRACIÓN CELULAR
- LIBERACIÓN DE MATERIAL CITOPLASMÁTICO
- RESPUESTA INFLAMATORIA

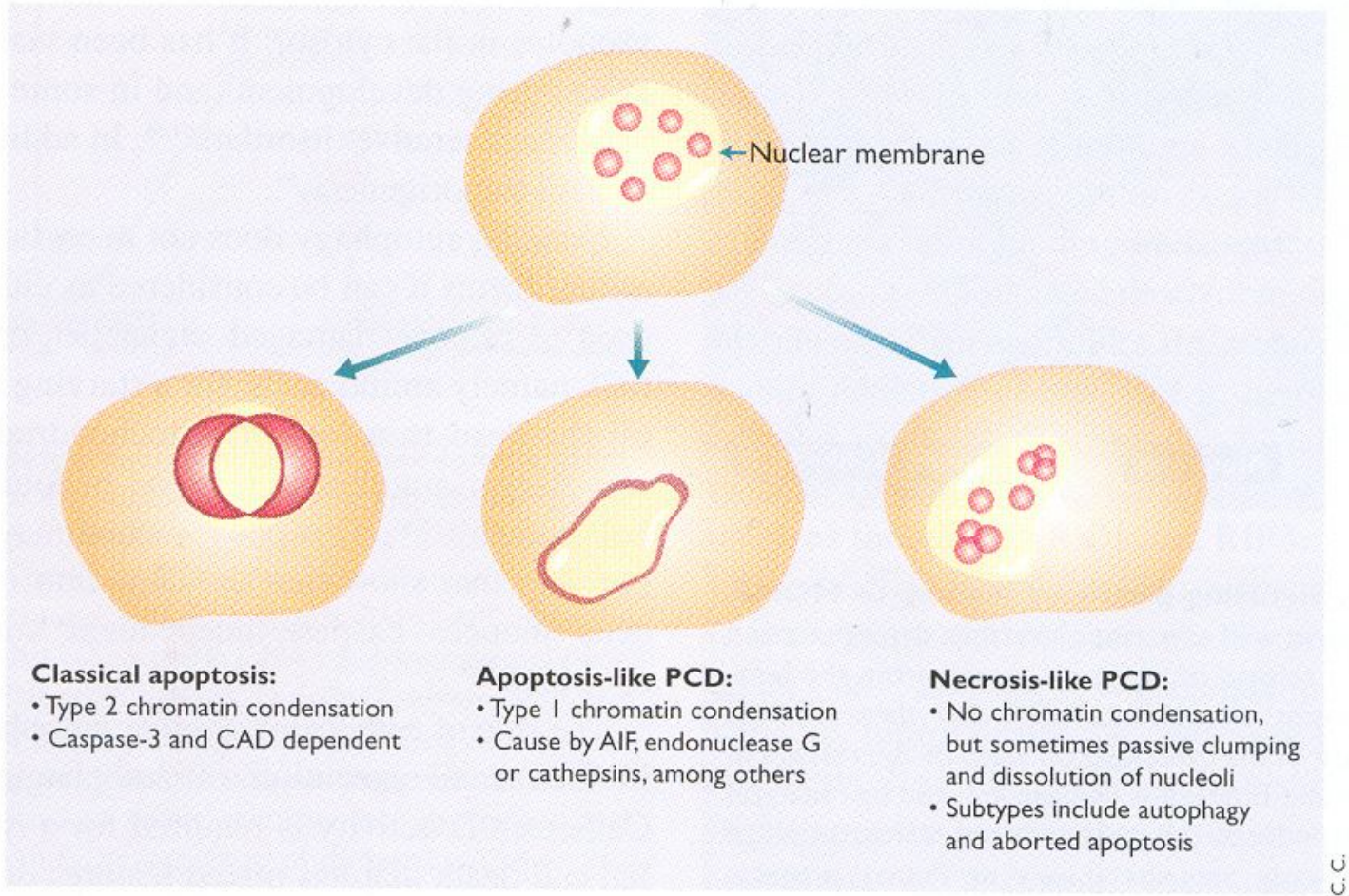


Figure 1. Chromatin condensation as a criterion to distinguish apoptosis from apoptosis-like and necrosis-like PCD. Control chromatin is speckled, showing areas of euchromatin and heterochromatin and mostly one or several more-condensed nucleoli (top). Caspase-dependent chromatin compaction and fragmentation leads to crescent or spherical masses at the nuclear periphery (left). Caspase-independent chromatin margination is triggered directly by the microinjection of AIF or by the activation of noncaspase proteases in several models of apoptosis-like PCD (middle); many intermediate forms and also transitions to necrosis are possible. Necrotic morphology—that is, a lack of chromatin condensation—is observed in models in which caspases are inhibited before apoptosis is completed (right).

Nature Immunology

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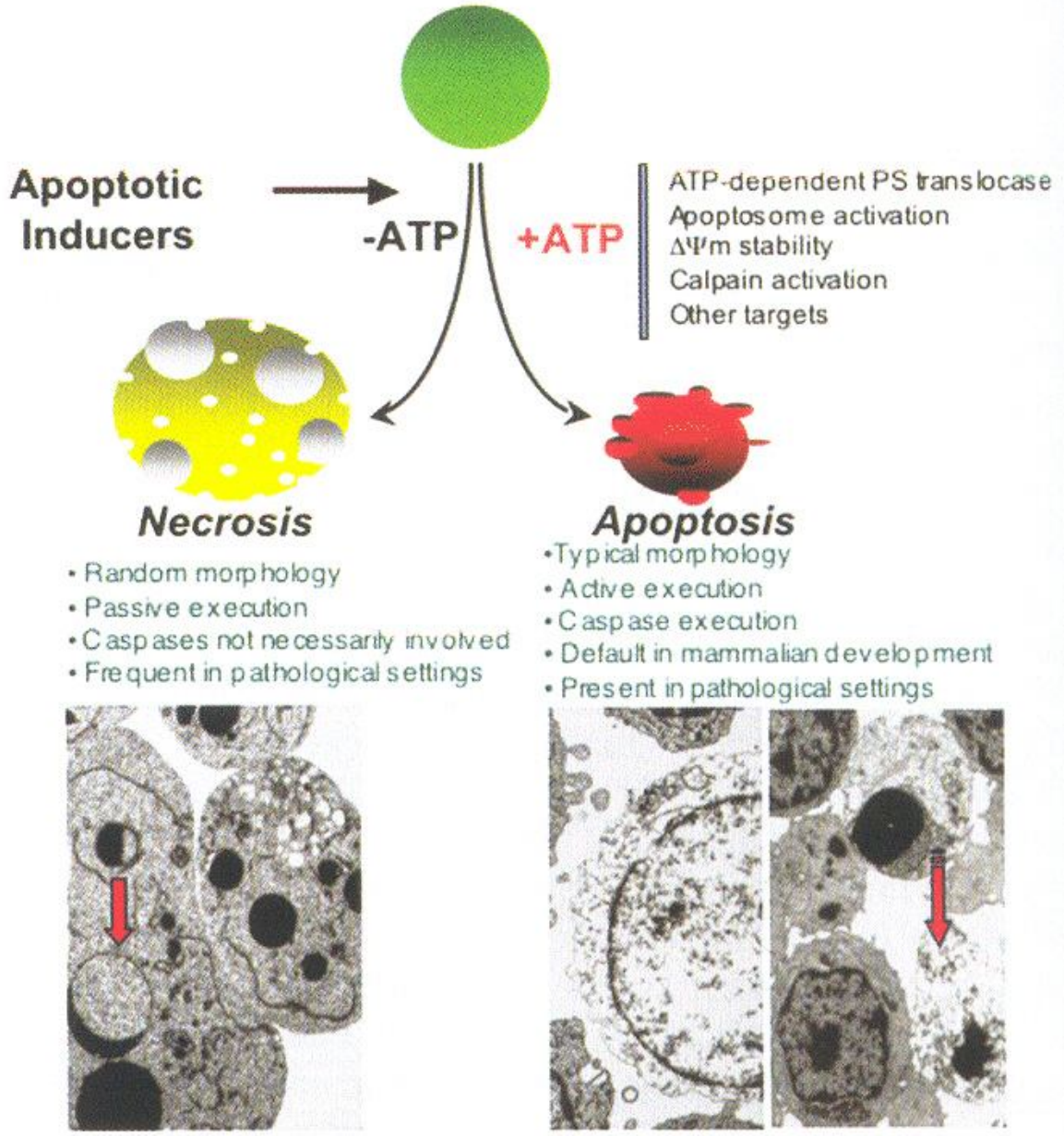


Figure 1 Apoptosis–necrosis switch by ATP. Switch of the morphology of cell death, shown by electron microscopy, and its regulation by the intracellular ATP concentration levels

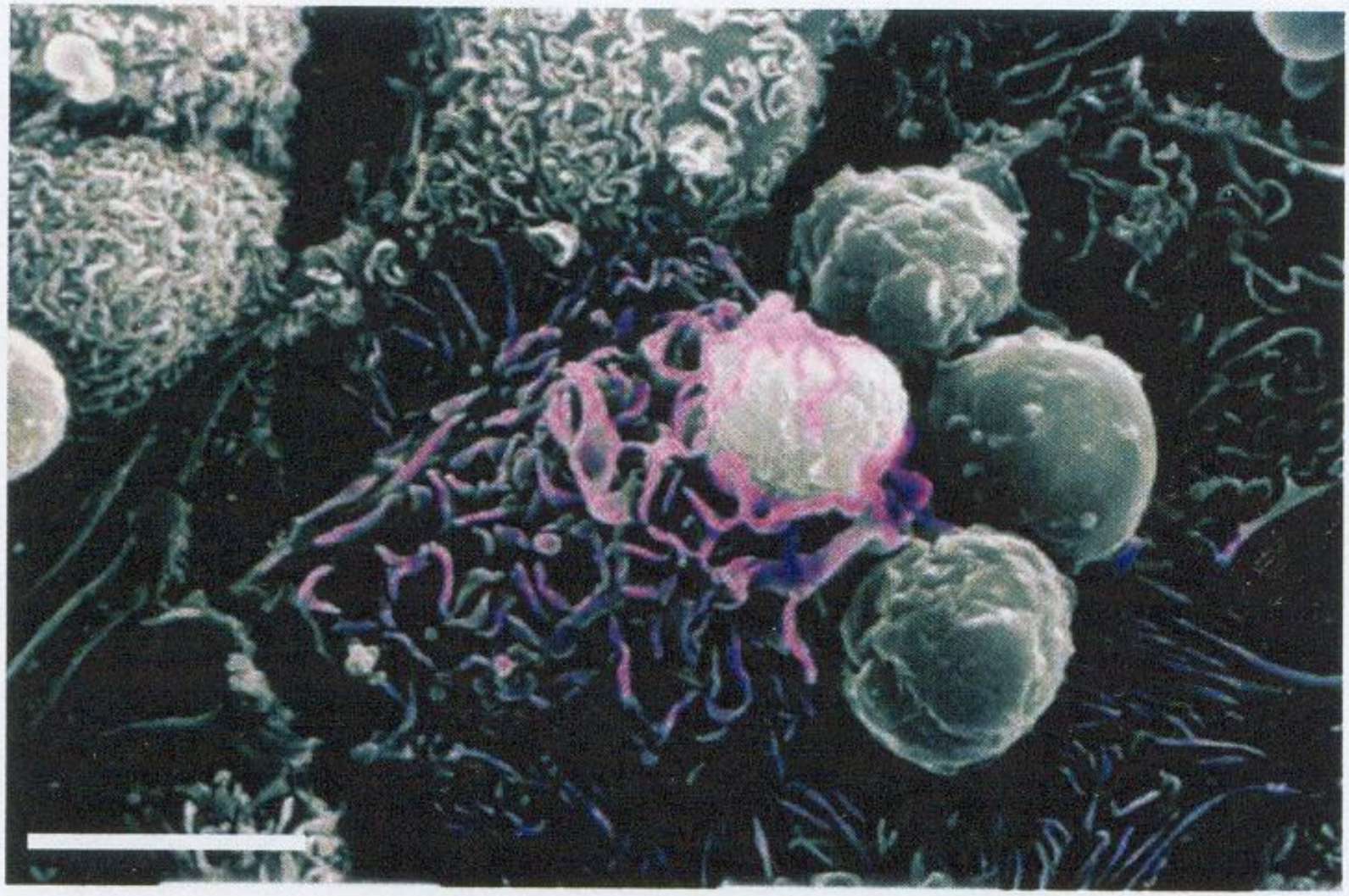
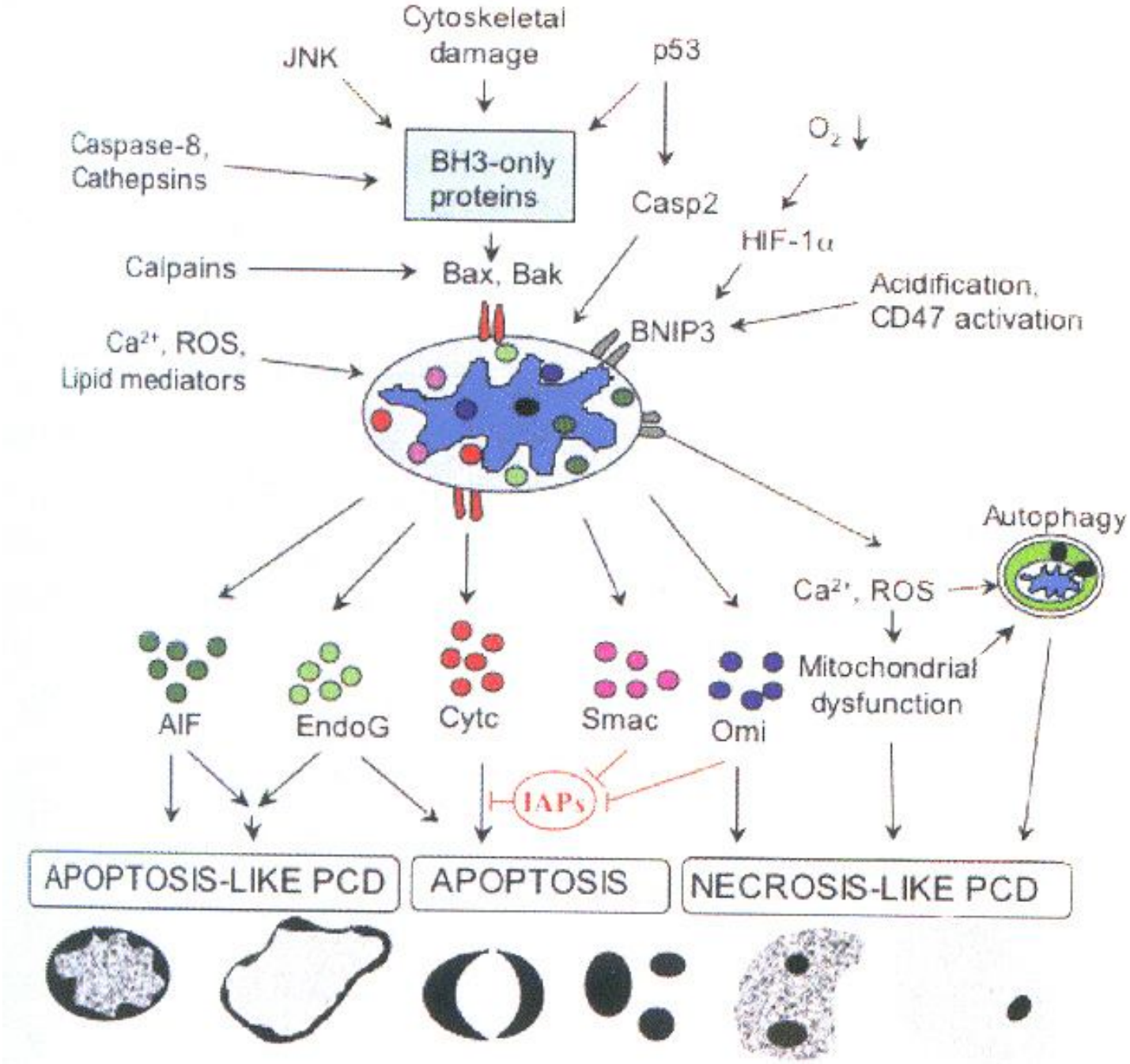
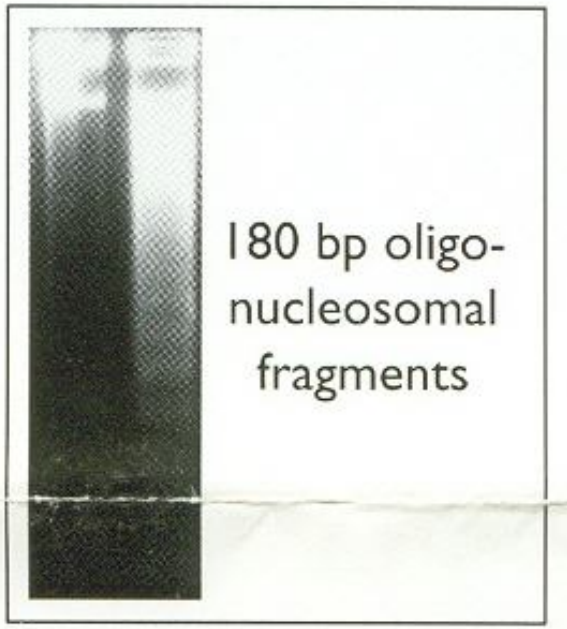
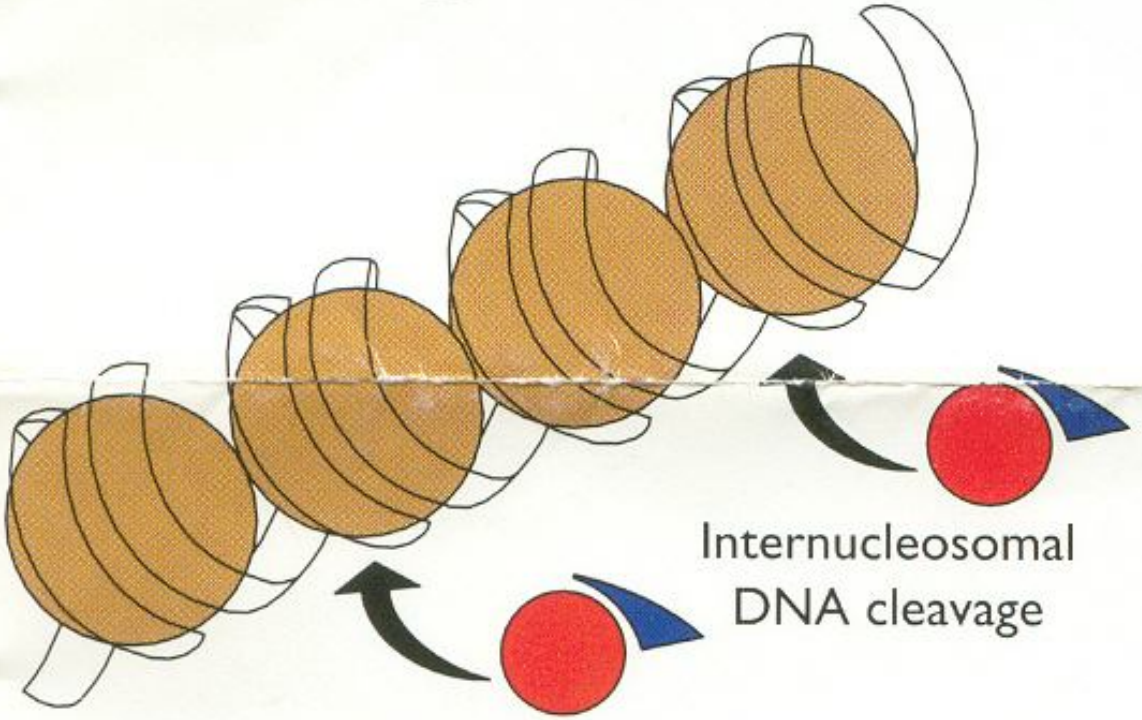


Figure 2 | **Virtual colour-scanning electron micrograph of phagocyte sampling of the surface of an apoptotic cell.** Macrophage plasma-membrane ruffles and processes that palpate apoptotic cells are indicated in cerise. The white bar represents 10 μm. Image courtesy of M. Clarke and S. Mitchell, University of Edinburgh, UK.


Cytoskeletal damage
JNK
p53
Caspase-8, Cathepsins
BH3-only proteins
O_2
Casp2
HIF-1α
Calpains
Bax, Bak
Acidification, CD47 activation
BNIP3
Ca^{2+}, ROS, Lipid mediators
Autophagy
Ca^{2+}, ROS
Mitochondrial dysfunction
AIF
EndoG
Cytc
Smac
Omi
IAPs
APOPTOSIS-LIKE PCD
APOPTOSIS
NECROSIS-LIKE PCD

DNA fragmentation





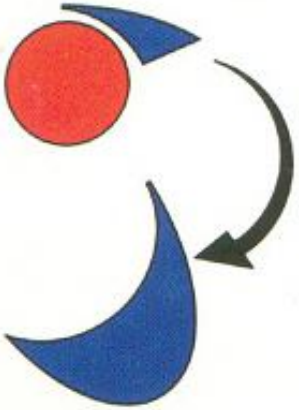
Internucleosomal
DNA cleavage

Active
caspase-3

iCAD/
DFF45

CAD/
DFF40/
CPAN

Active
nuclease



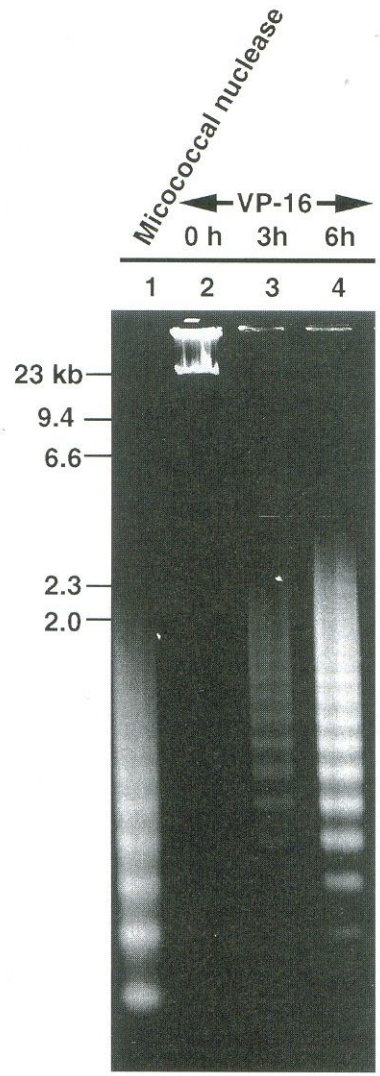
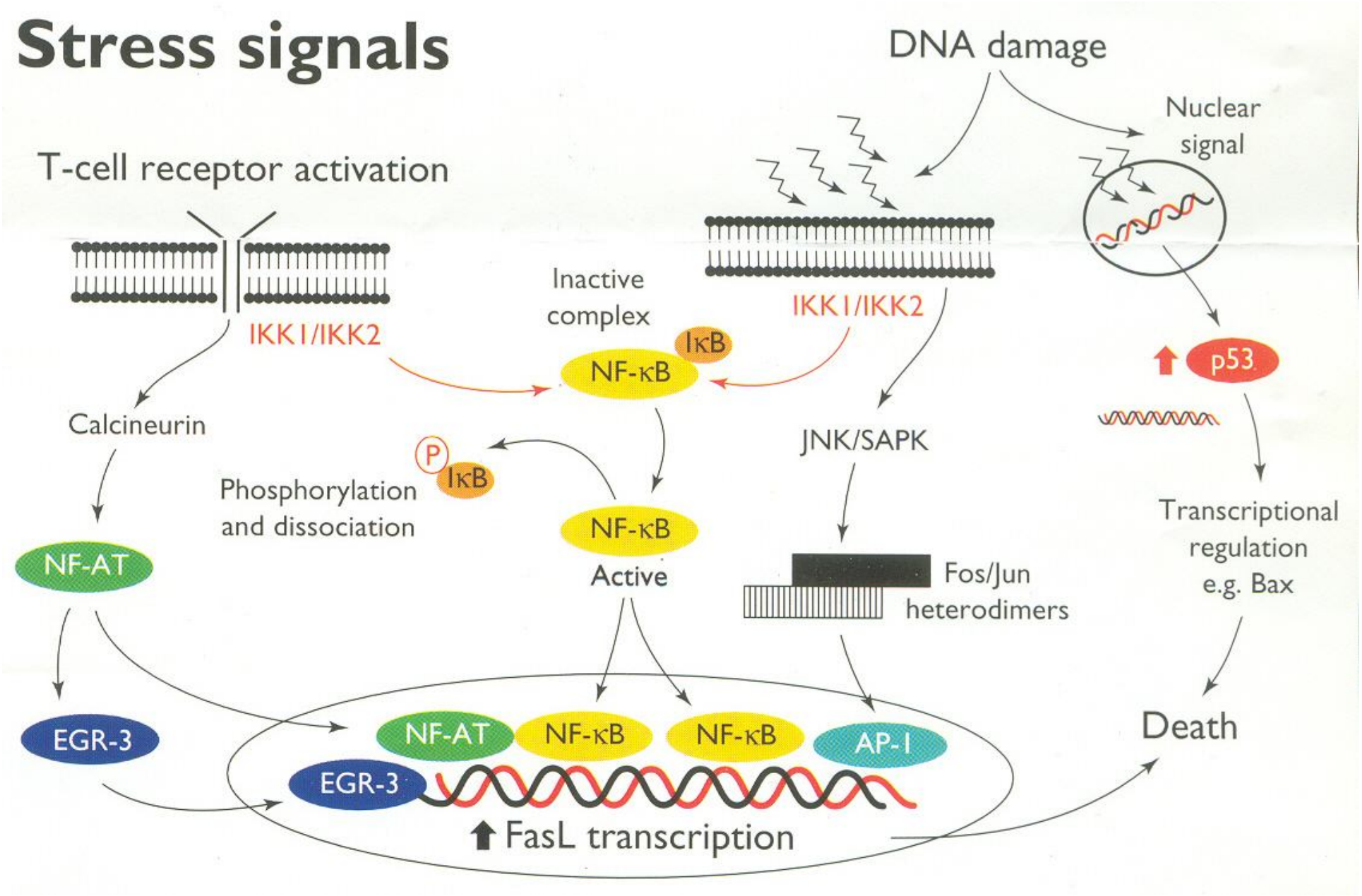
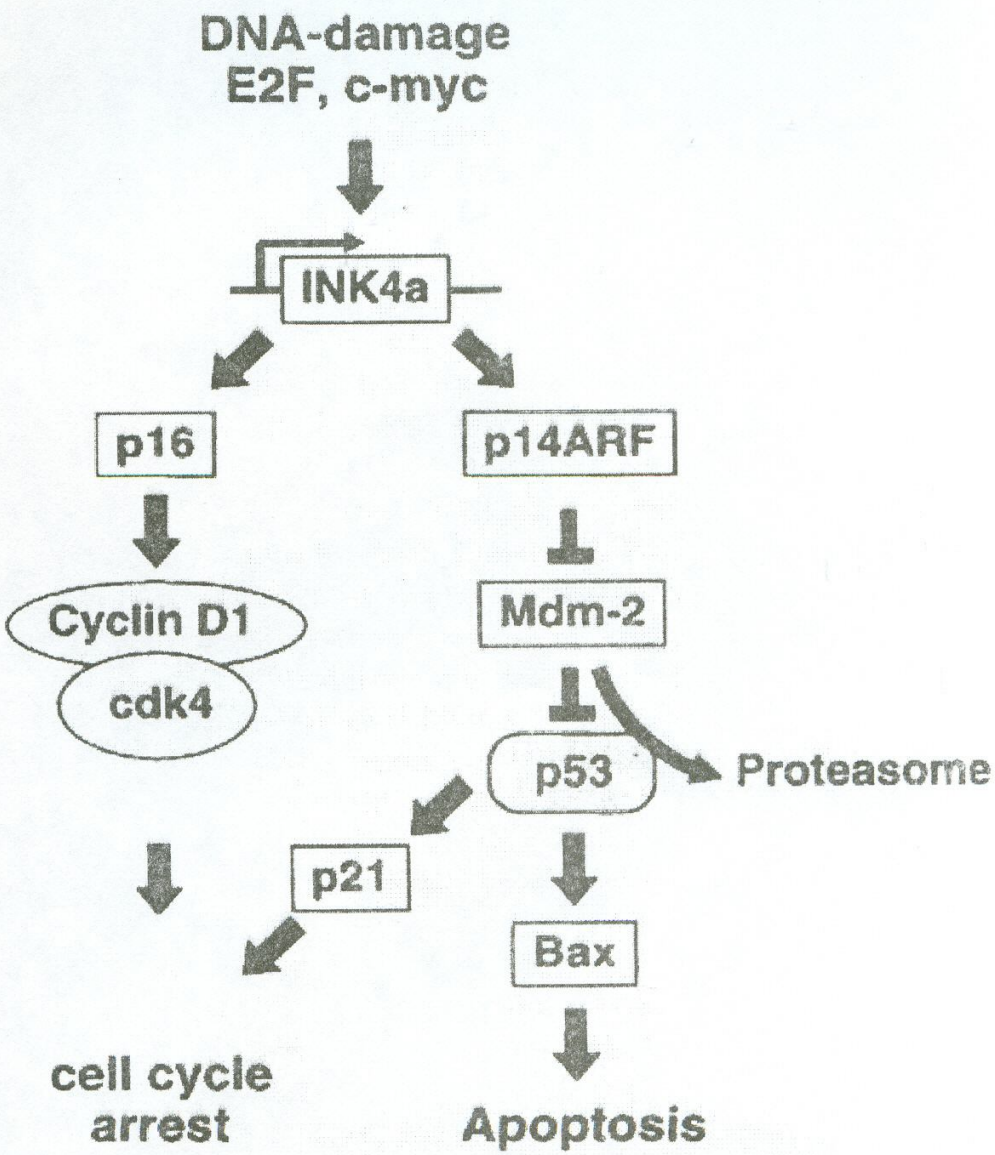


FIGURE 2 Detection of internucleosomal DNA fragmentation by conventional agarose gel electrophoresis. HL-60 cells were treated with 68 μM etoposide (VP-16) for 0–6 h as indicated, then lysed in 500 m*M* Tris–HCl (pH 9.0 at 21°C) containing 2 m*M* EDTA, 10 m*M* NaCl, 1% sodium dodecyl sulfate, and 1 mg/ml proteinase K. After a 48-h incubation at 48°C, samples were extracted twice with 1 : 1 chloroform : phenol and once with phenol. Aliquots containing DNA from 5×10^5 cells were digested with 300 μg/ml RNase A for 1 h at room temperature, then applied to adjacent wells of a 1.2% (w/v) agarose gel containing 40 m*M* Tris–acetate and 1 m*M* EDTA. After electrophoresis at 1.5 V/cm for 15 h, gels were stained with 0.1 μg/ml ethidium bromide and photographed under UV light. Lane 1 contains DNA obtained by treating nuclei of control HL-60 cells with micrococcal nuclease [102]. Note the similarity of the degradation patterns in lanes 1 and 4.

Stress signals



APOPTOSIS Y CICLO CELULAR



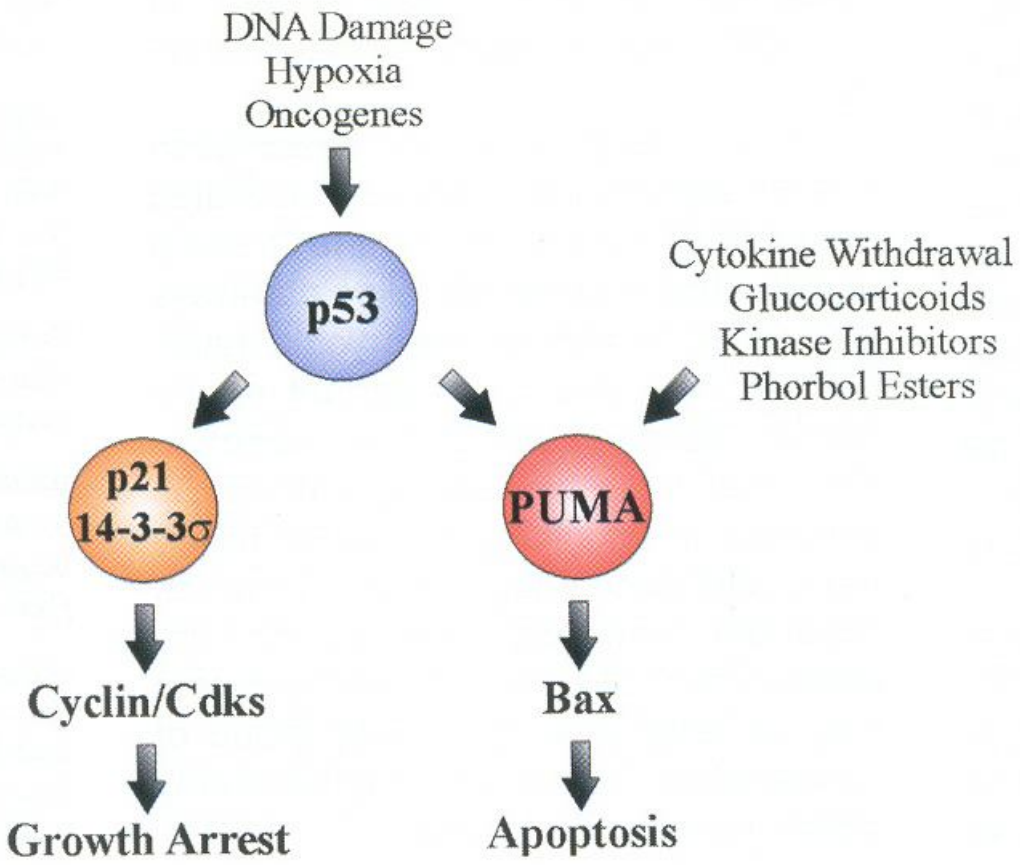


Figure 1. PUMA is essential for p53-dependent and -independent apoptosis

p53 induces either cell cycle arrest or apoptosis depending on cell type and subcellular context. PUMA is required for p53-dependent apoptosis induced by DNA damage, hypoxia, and oncogenes. PUMA is also necessary for apoptosis induced by p53-independent stimuli including serum withdrawal, glucocorticoids, kinase inhibitors, and phorbol esters. p53-dependent cell cycle arrest is mediated by p21 and 14-3-3σ.

Phagocytosis

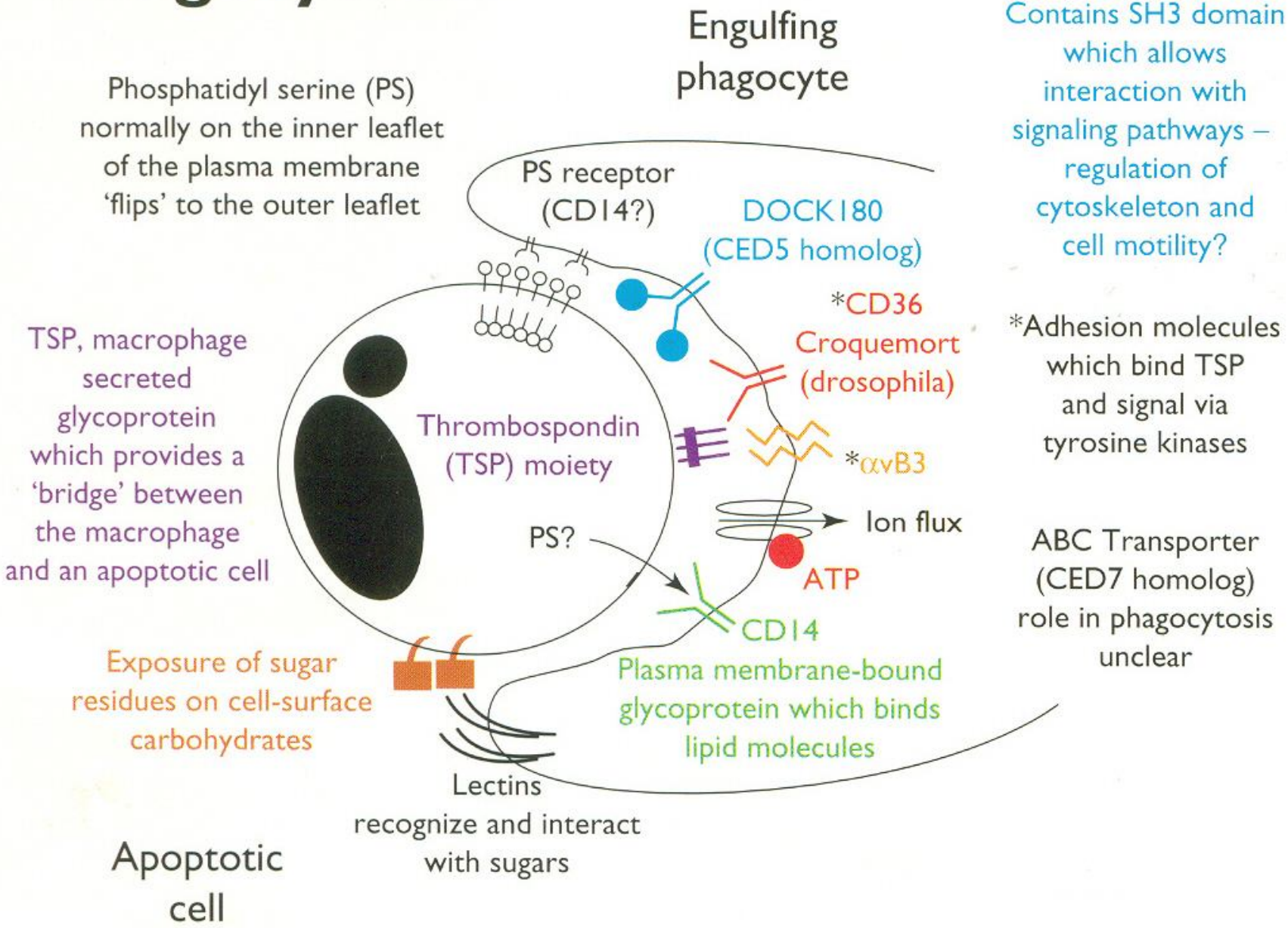
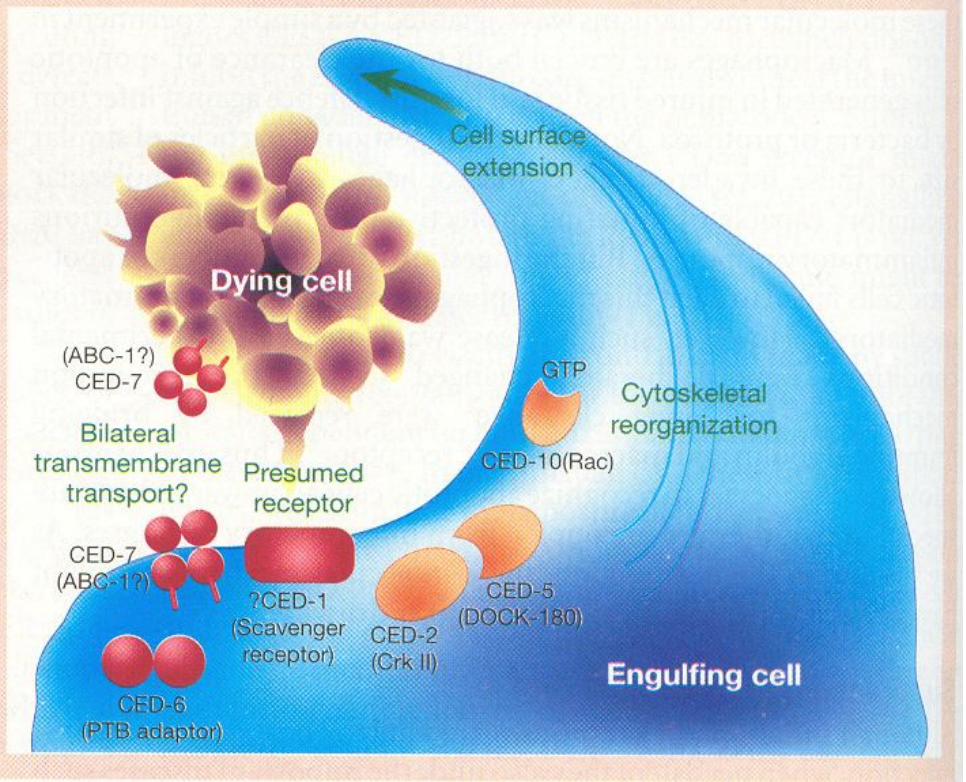
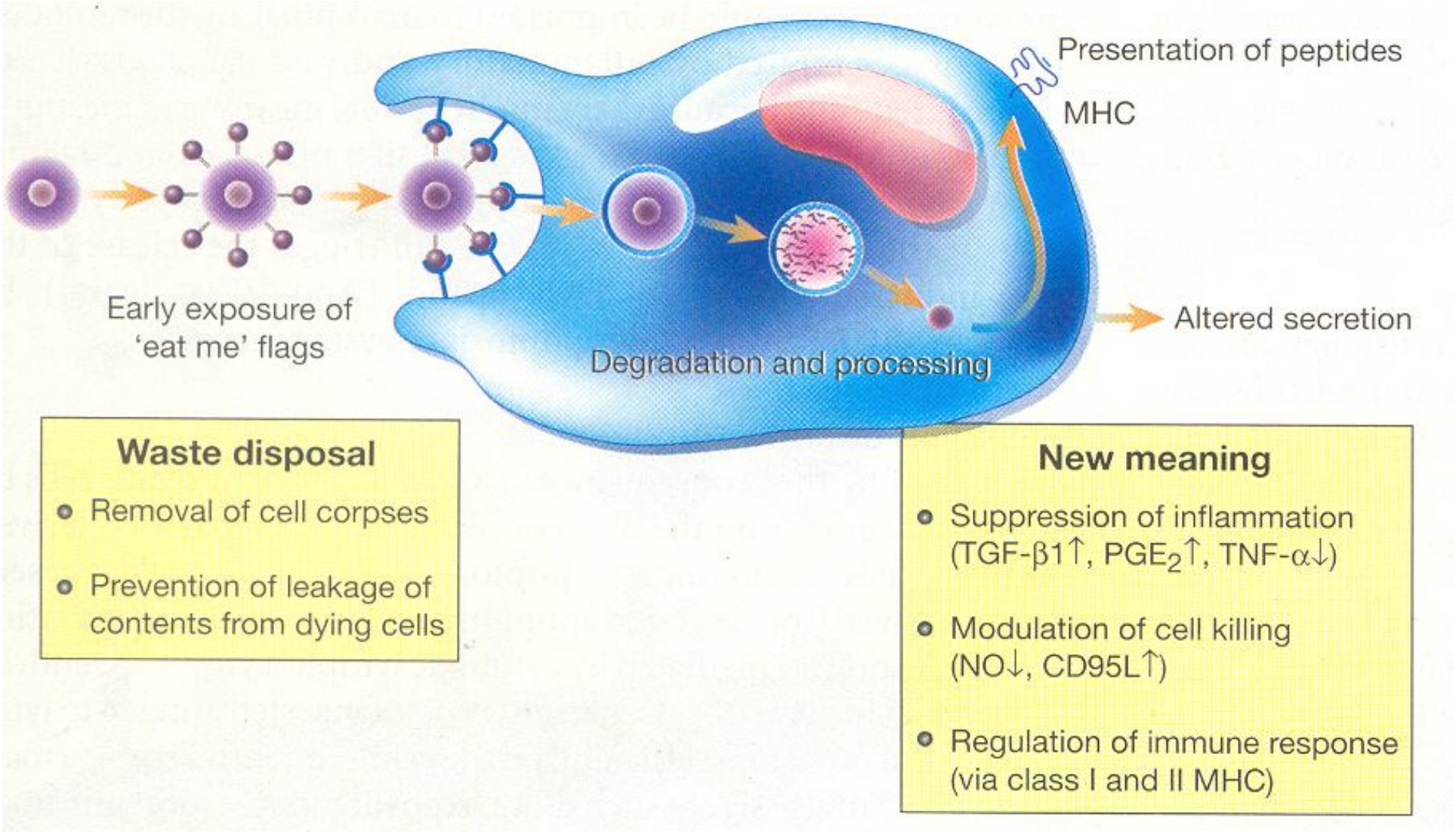
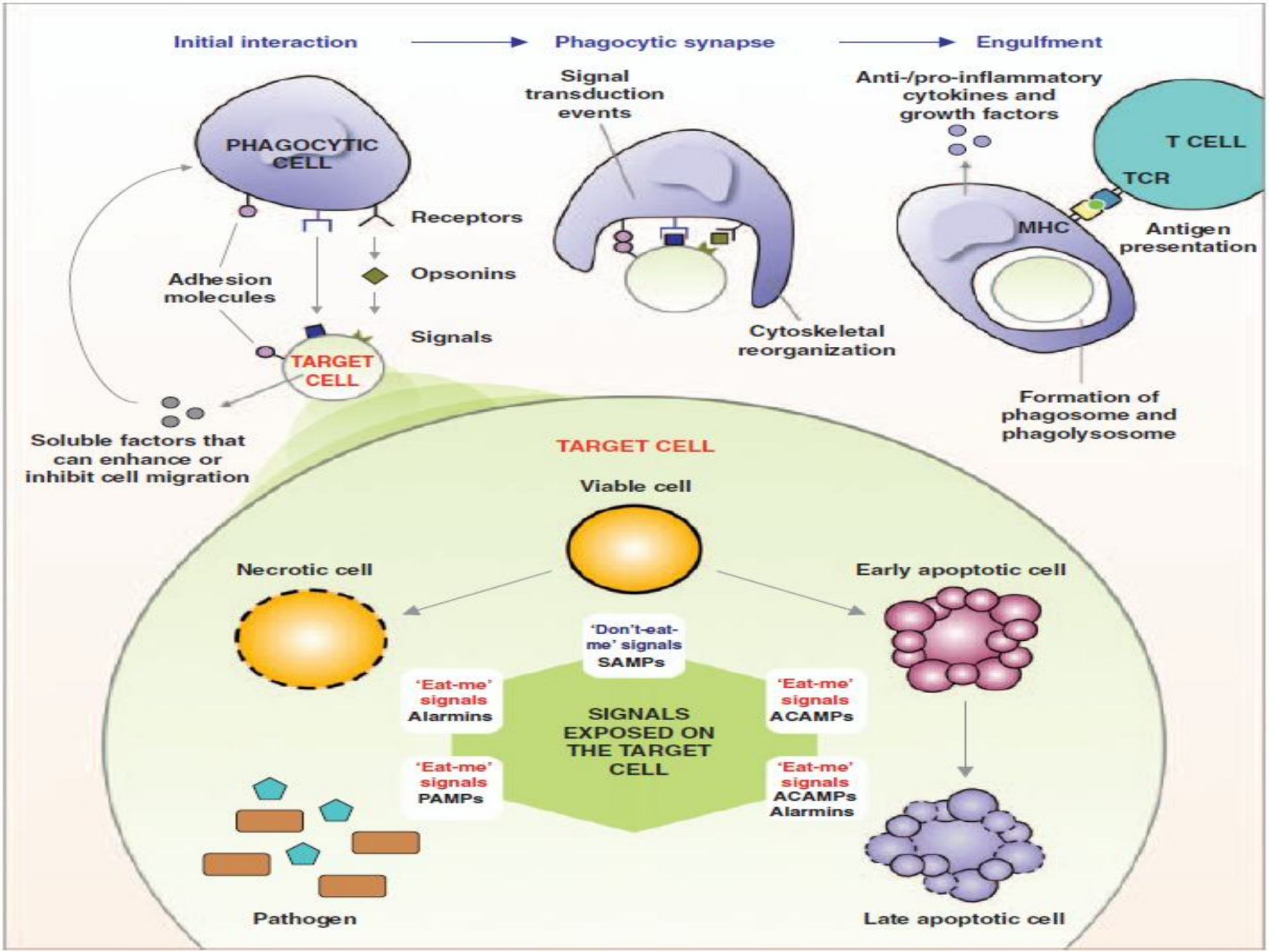
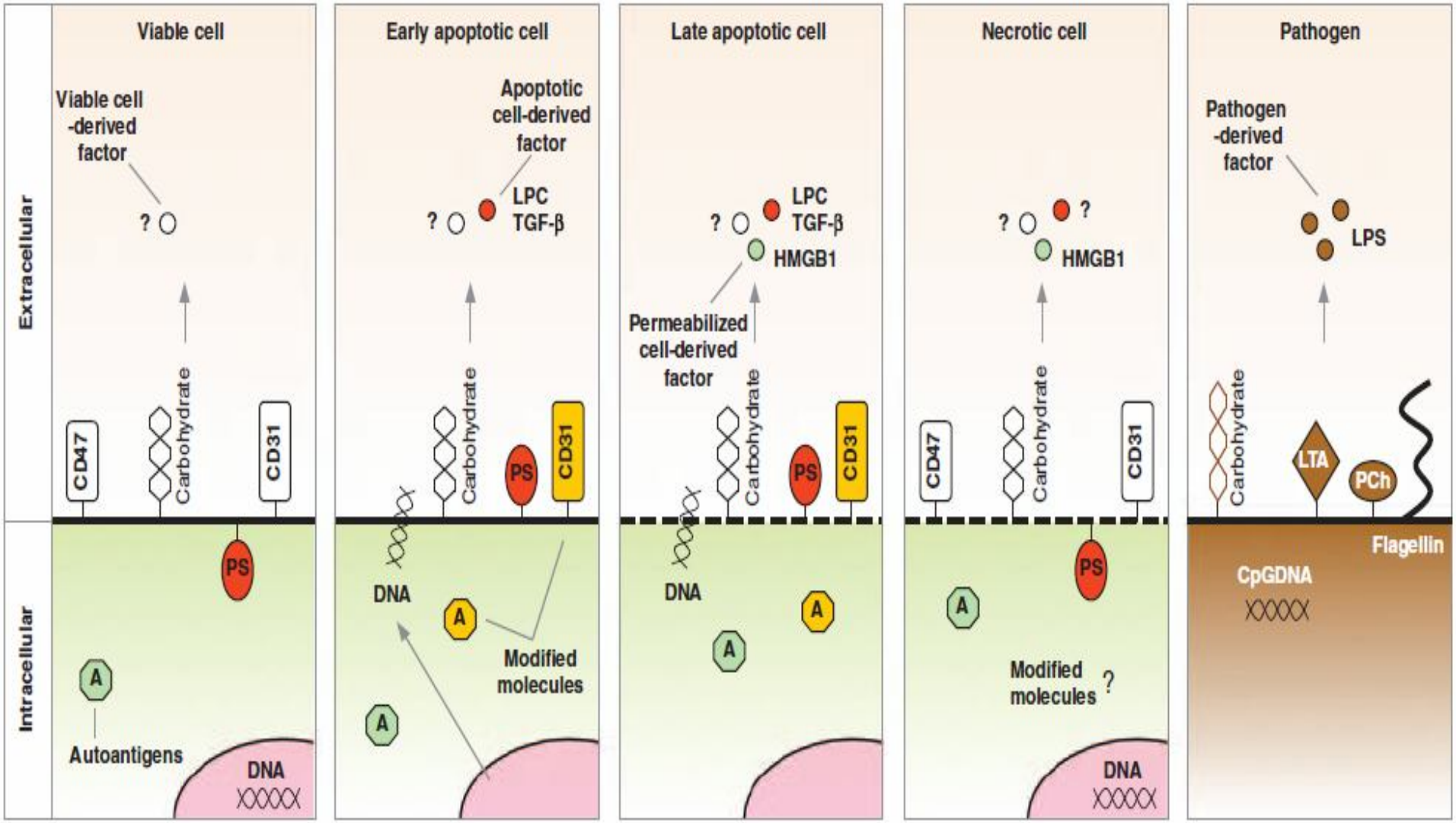


Figure 3 Signalling the engulfment of dying cells in *Caenorhabditis elegans*. Mutations in six genes are known to affect the engulfment of cell corpses by non-professional neighbouring cells in this nematode. CED-2, CED-5 and CED-10 intracellular proteins signal in a manner comparable to their respective mammalian homologues CrkII, DOCK 180 and Rac, mediating the cytoskeletal reorganization and extension of the engulfing cell surface around the dying cell. CED-7, homologous with mammalian phagocyte ABC-1, acts in both dying and engulfing cells[6], possibly in transmembrane lipid transport. We speculate that CED-1, yet to be characterized, is analogous to mammalian scavenger receptors; CED-7 and CED-1 probably promote engulfment by interacting with the signalling adaptor protein CED-6. PTB, phosphotyrosine binding.




Presentation of peptides
MHC
Early exposure of
'eat me' flags
Degradation and processing
Altered secretion
Waste disposal
Removal of cell corpses
Prevention of leakage of contents from dying cells
New meaning
Suppression of inflammation (TGF-β1↑, PGE_2↑, TNF-α↓)
Modulation of cell killing (NO↓, CD95L↑)
Regulation of immune response (via class I and II MHC)


Initial interaction
Phagocytic synapse
Engulfment
PHAGOCYTIC CELL
Receptors
Opsonins
Signals
Adhesion molecules
TARGET CELL
Soluble factors that can enhance or inhibit cell migration
Signal transduction events
Cytoskeletal reorganization
Anti-/pro-inflammatory cytokines and growth factors
T CELL
TCR
MHC
Antigen presentation
Formation of phagosome and phagolysosome
TARGET CELL
Viable cell
Necrotic cell
Early apoptotic cell
'Don't-eat-me' signals SAMPs
'Eat-me' signals Alarmins
'Eat-me' signals ACAMPs
SIGNALS EXPOSED ON THE TARGET CELL
'Eat-me' signals PAMPs
'Eat-me' signals ACAMPs Alarmins
Pathogen
Late apoptotic cell


Extracellular
Intracellular
Viable cell
Viable cell -derived factor
?
CD47
Carbohydrate
CD31
PS
A
Autoantigens
DNA
Early apoptotic cell
Apoptotic cell-derived factor
LPC
TGF-β
?
Carbohydrate
PS
CD31
DNA
A
Modified molecules
A
Late apoptotic cell
LPC
TGF-β
?
HMGB1
Permeabilized cell-derived factor
Carbohydrate
PS
CD31
DNA
A
A
Necrotic cell
?
HMGB1
CD47
Carbohydrate
CD31
PS
A
Modified molecules ?
DNA
Pathogen
Pathogen -derived factor
LPS
Carbohydrate
LTA
PCh
Flagellin
CpGDNA

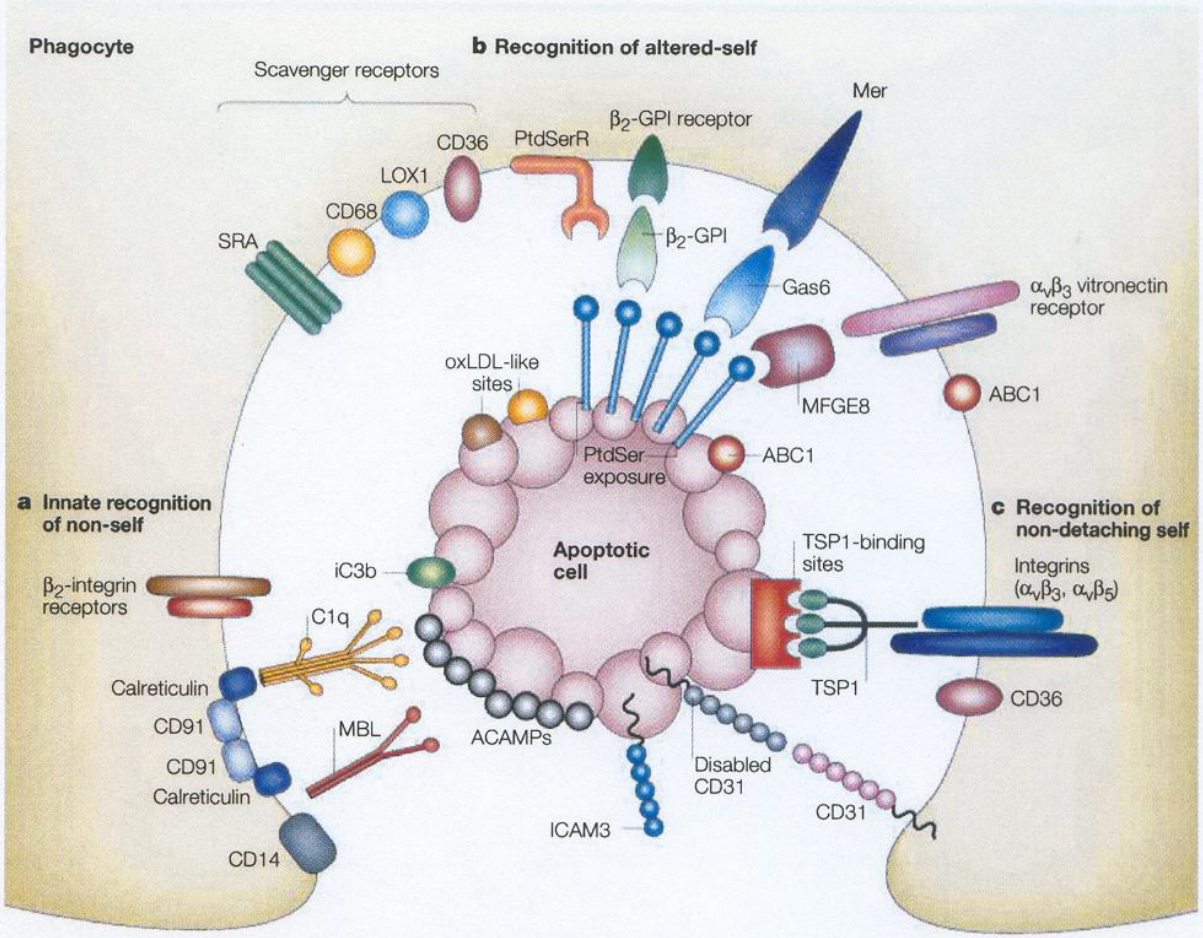


Figure 1 | **Three classes of mechanism for the recognition of apoptotic cells by phagocytes. a** | Innate recognition of non-self involves phagocyte CD14, β_2-integrins (which bind the opsonic complement fragment inactivated C3b, iC3b) and the CD91–calreticulin complex (which can bind the first component of complement, C1q, and mannose-binding lectin, MBL, which recognizes pathogen-like apoptotic-cell-associated molecular patterns, ACAMPs). **b** | Recognition of altered-self involves an array of scavenger receptors, including the class-A scavenger receptor (SRA), CD68, LOX1 (oxidised low-density lipoprotein receptor 1) and CD36, which recognize oxidised sites on apoptotic cells that mimic oxidised low-density lipoprotein (oxLDL). Exposure of phosphatidylserine (PtdSer) on the surface of apoptotic cells is a key 'eat-me' flag. It is detected by phagocyte phosphatidylserine receptor (PtdSerR), receptors for the bridging plasma-protein β_2-glycoprotein I (β_2-GPI), the Mer kinase receptor for the bridging protein Gas6, and $\alpha_v\beta_3$ integrin (vitronectin receptor), which binds the bridging protein milk-fat globule epidermal growth factor 8 (MFGE8). Rearrangement of plasma-membrane lipids in both the dying cell and the phagocyte by the ATP-binding cassette transporter ABC1 can contribute to this type of recognition. **c** | Recognition of non-detaching self involves disabling the detachment signals that are conferred by apoptotic-cell CD31 and, possibly, similar alterations in another immunoglobulin-superfamily member, intercellular adhesion molecule 3 (ICAM3). Disabled apoptotic-cell CD31 binds tightly to phagocyte CD31, which may promote binding of the bridging protein thrombospondin-1 (TSP1) by phagocyte integrins.

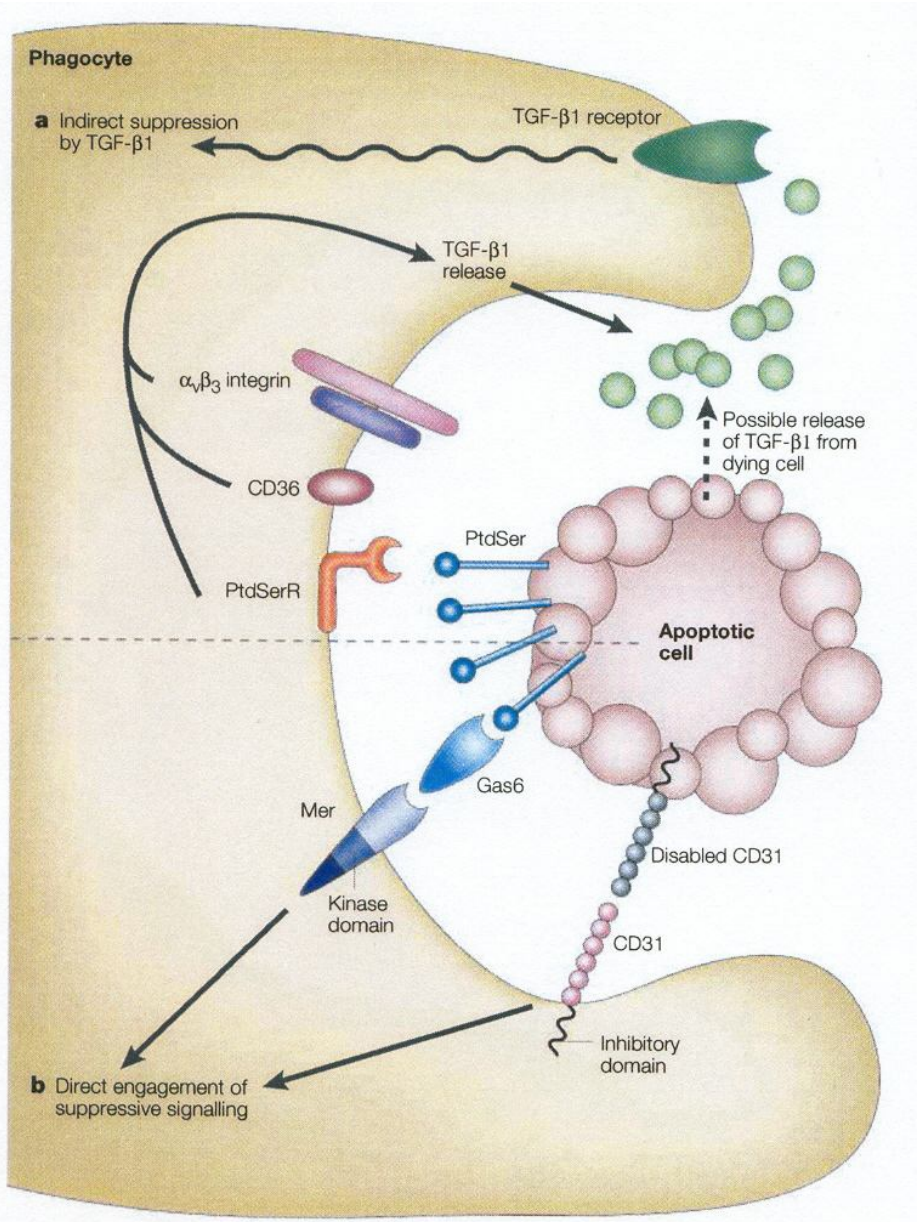
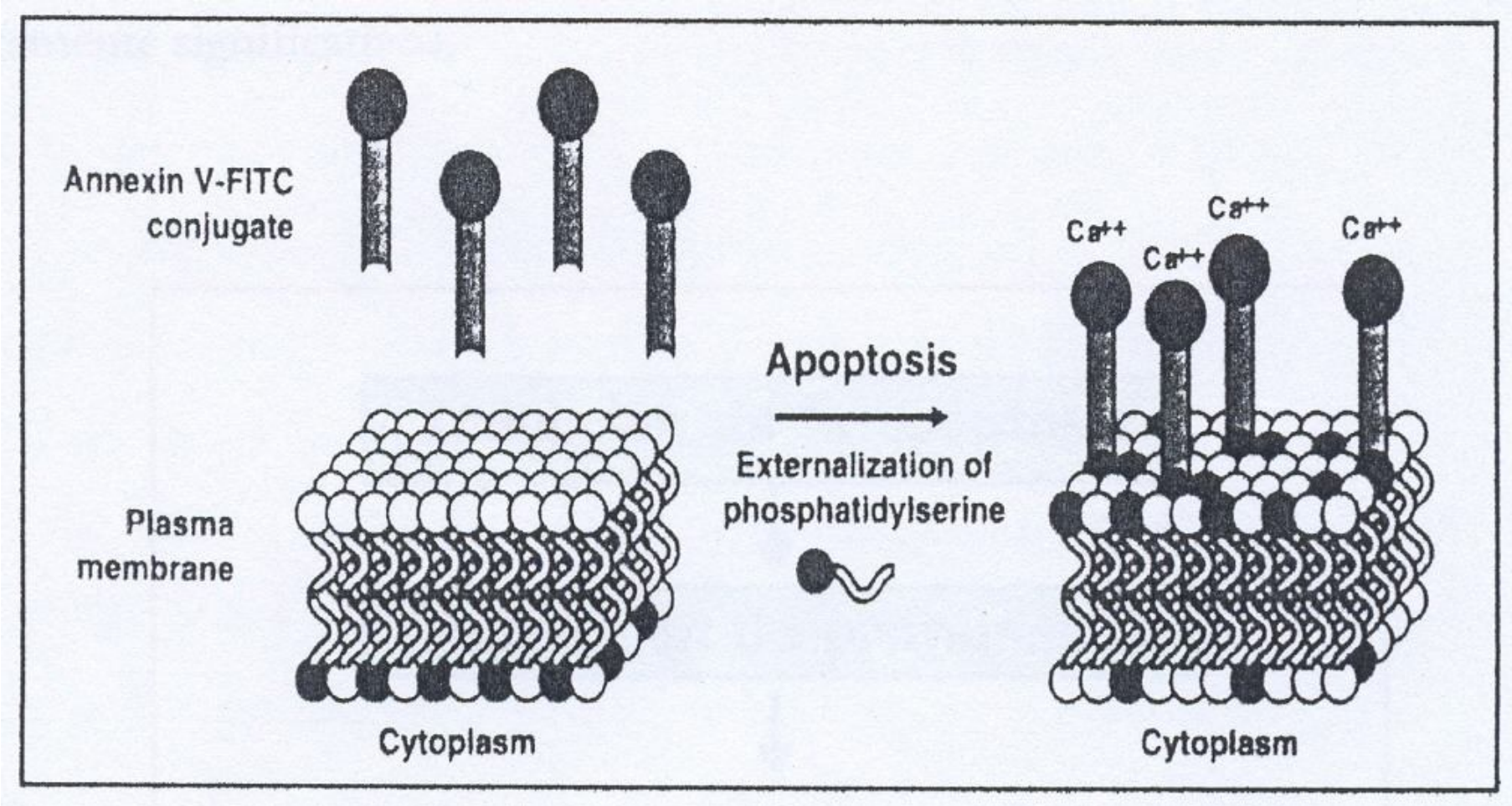


Figure 3 | **Two classes of mechanism for apoptotic-cell suppression of phagocyte pro-inflammatory responses. a** | Indirect suppression by transforming growth factor-β1 (TGF-β1) derived from macrophages that have ingested apoptotic cells is triggered by ligation of the macrophage receptor for phosphatidylserine (PtdSerR), CD36 and $\alpha_v\beta_3$ integrin, possibly supplemented by the direct release of TGF-β1 from dying cells. Similar mechanisms might apply for interleukin-10 (not shown). **b** | Direct suppressive signalling could arise through the kinase domain of Mer and the tyrosine inhibitory domain of CD31 and, possibly, related inhibitory receptors.


Annexin V-FITC
conjugate
Plasma
membrane
Cytoplasm
Apoptosis
Externalization of
phosphatidylserine
Ca++
Ca++
Ca++
Ca++
Cytoplasm

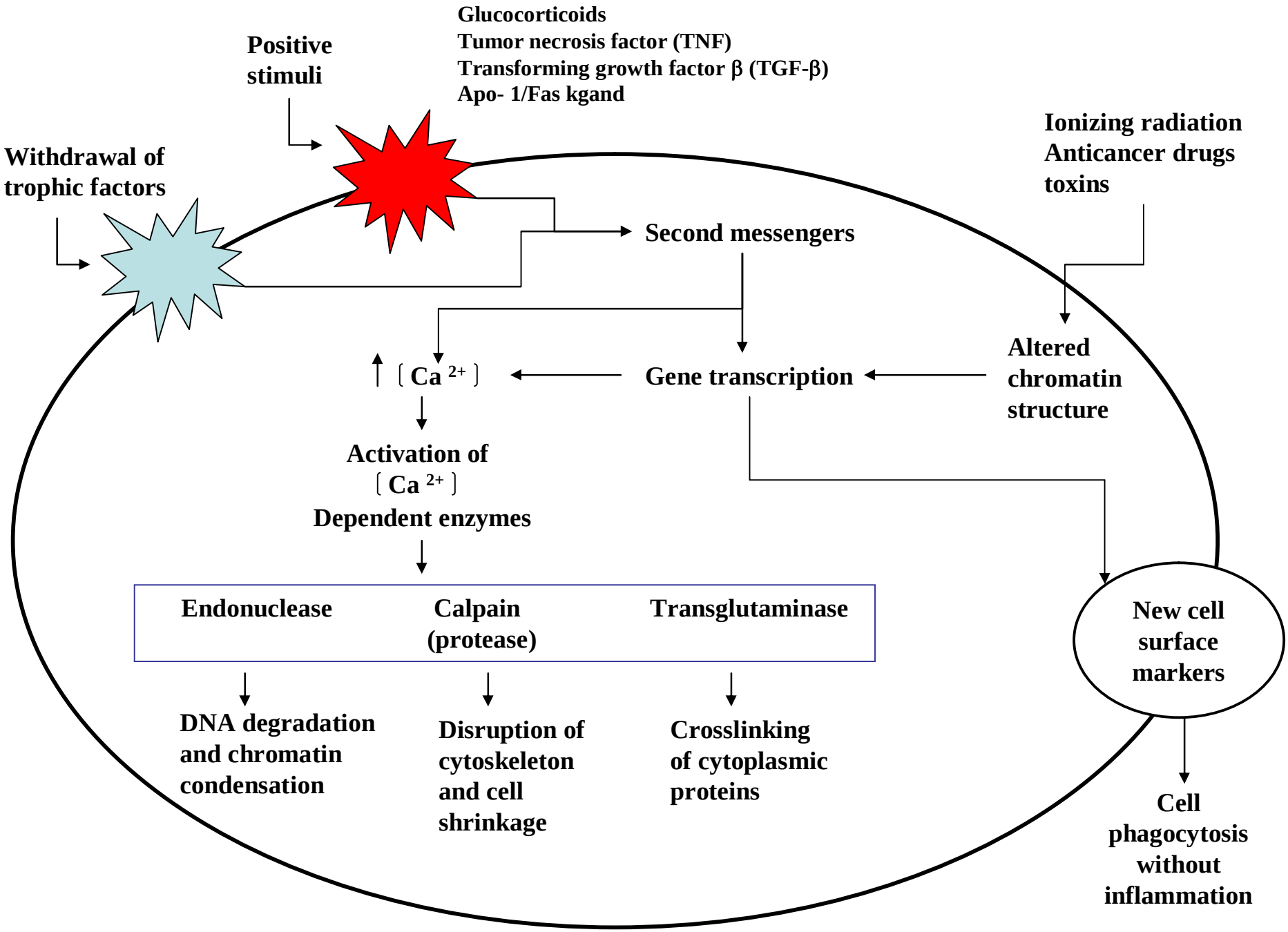
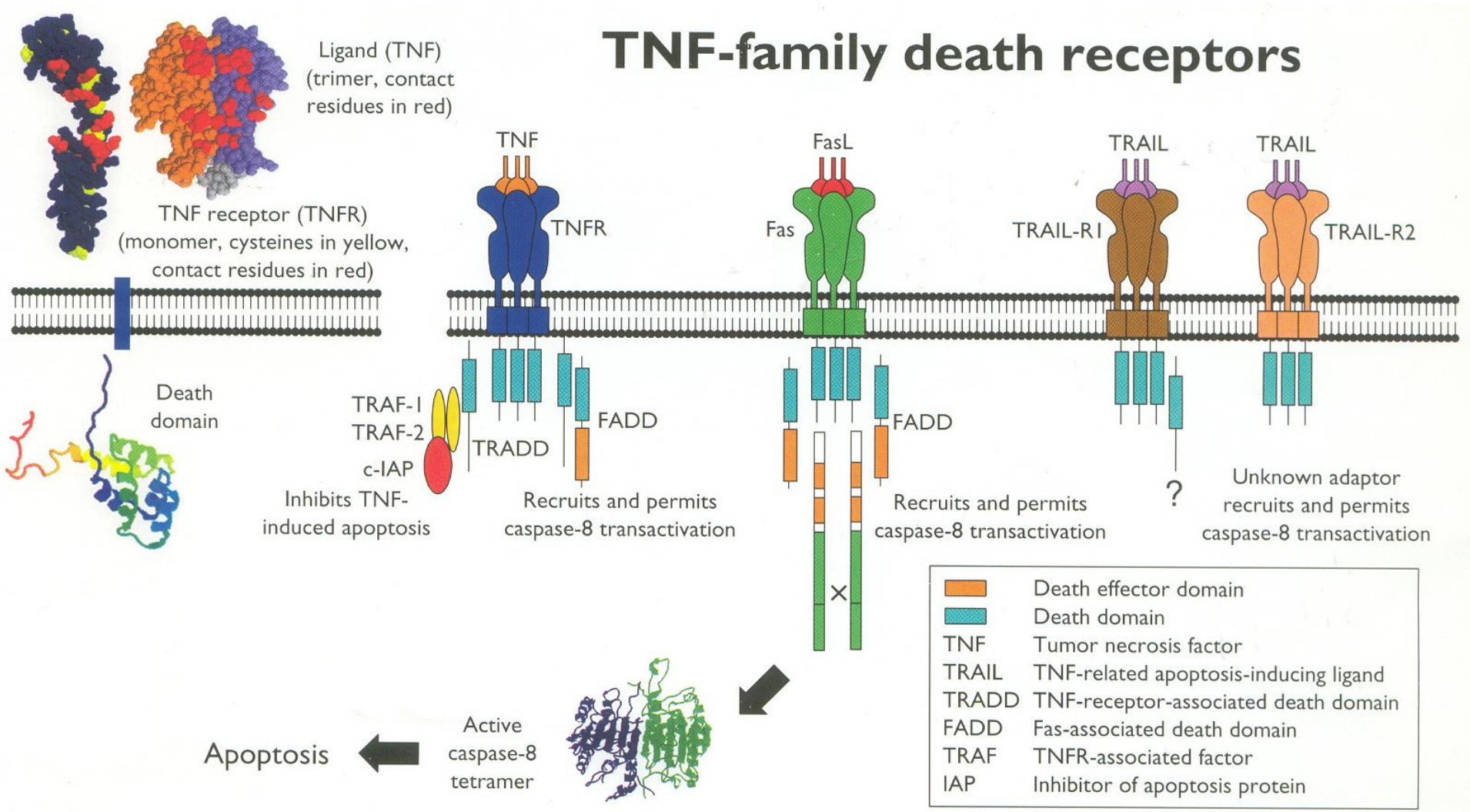


Fig-1 Common metabolic events in apoptosis


TNF-family death receptors
Ligand (TNF)
(trimer, contact
residues in red)
TNF receptor (TNFR)
(monomer, cysteines in yellow,
contact residues in red)
Death
domain
TNF
TNFR
FasL
Fas
TRAIL
TRAIL-R1
TRAIL
TRAIL-R2
TRAF-1
TRAF-2
c-IAP
TRADD
FADD
FADD
Inhibits TNF-
induced apoptosis
Recruits and permits
caspase-8 transactivation
Recruits and permits
caspase-8 transactivation
?
Unknown adaptor
recruits and permits
caspase-8 transactivation
Death effector domain
Death domain
TNF Tumor necrosis factor
TRAIL TNF-related apoptosis-inducing ligand
TRADD TNF-receptor-associated death domain
FADD Fas-associated death domain
TRAF TNFR-associated factor
IAP Inhibitor of apoptosis protein
Active
caspase-8
tetramer
Apoptosis

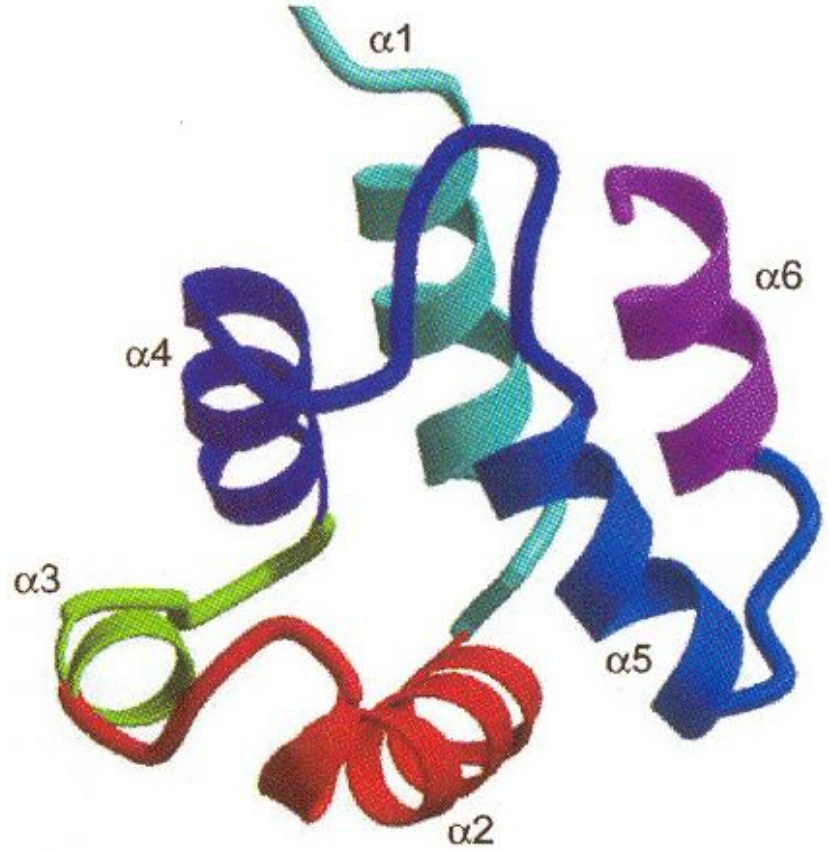
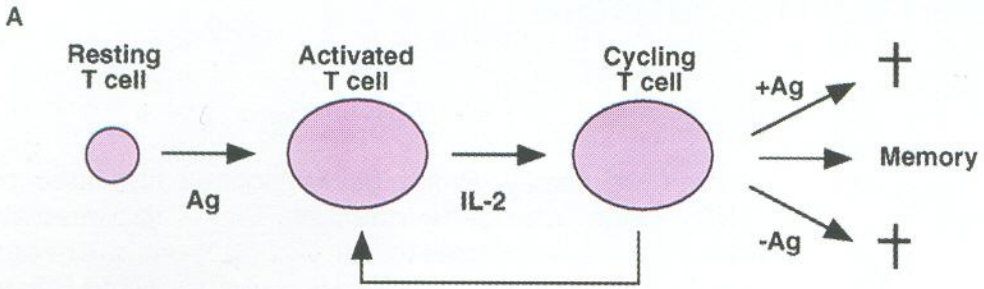


Figure 1. Ribbon diagram of the hexahelical structure of the DED. The DED consists of six antiparallel, amphipathic α-helices, which are numbered and highlighted here in different colors, connected by short loops of irregular structure[13]. The only exception is the loop connecting helices α4 and α5, which contains a β-turn. A similar structure has been observed for the DD and CARD protein domains.



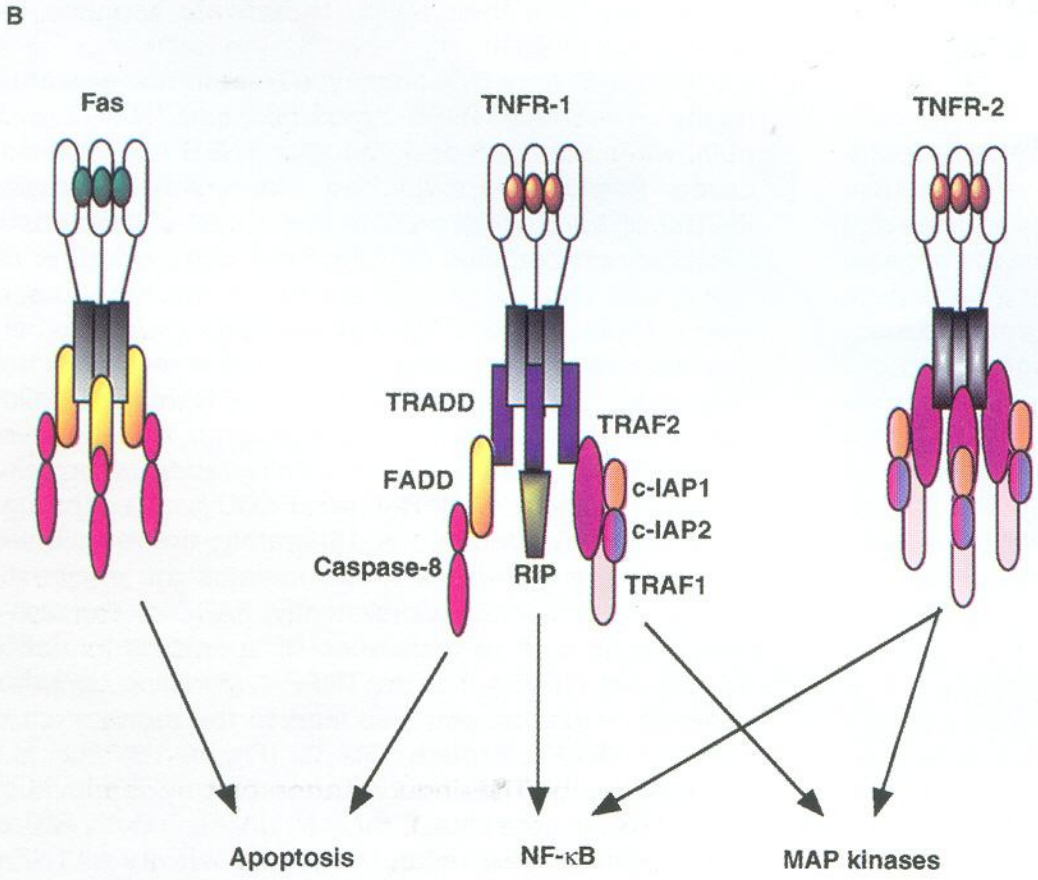


Figure 1. TNFR Signaling and T Cell Homeostasis

(A) Propriocidal and lymphokine-withdrawal death in T cell homeostasis. Antigen (Ag)-activated T cells are driven into cell cycle by cytokines like IL-2. Restimulation of the same T cells by antigen leads to propriocidal apoptosis mediated by death cytokines. Removal of antigen stimulation results in death receptor-independent, lymphokine withdrawal death. (B) Proximal components of the Fas, TNFR-1, and TNFR-2 signal transduction pathways.

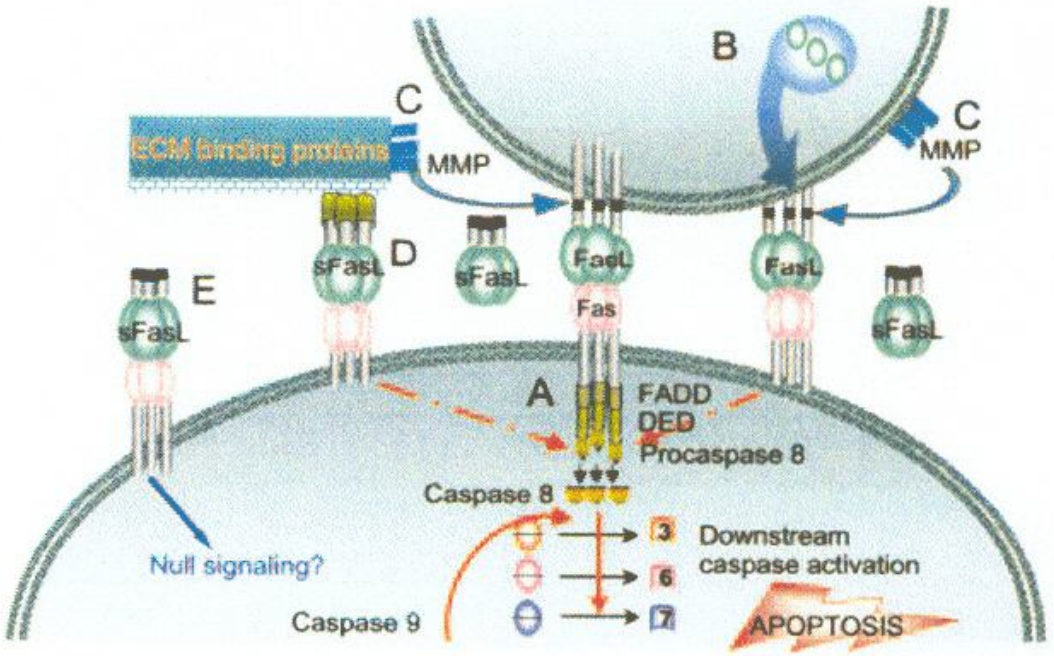
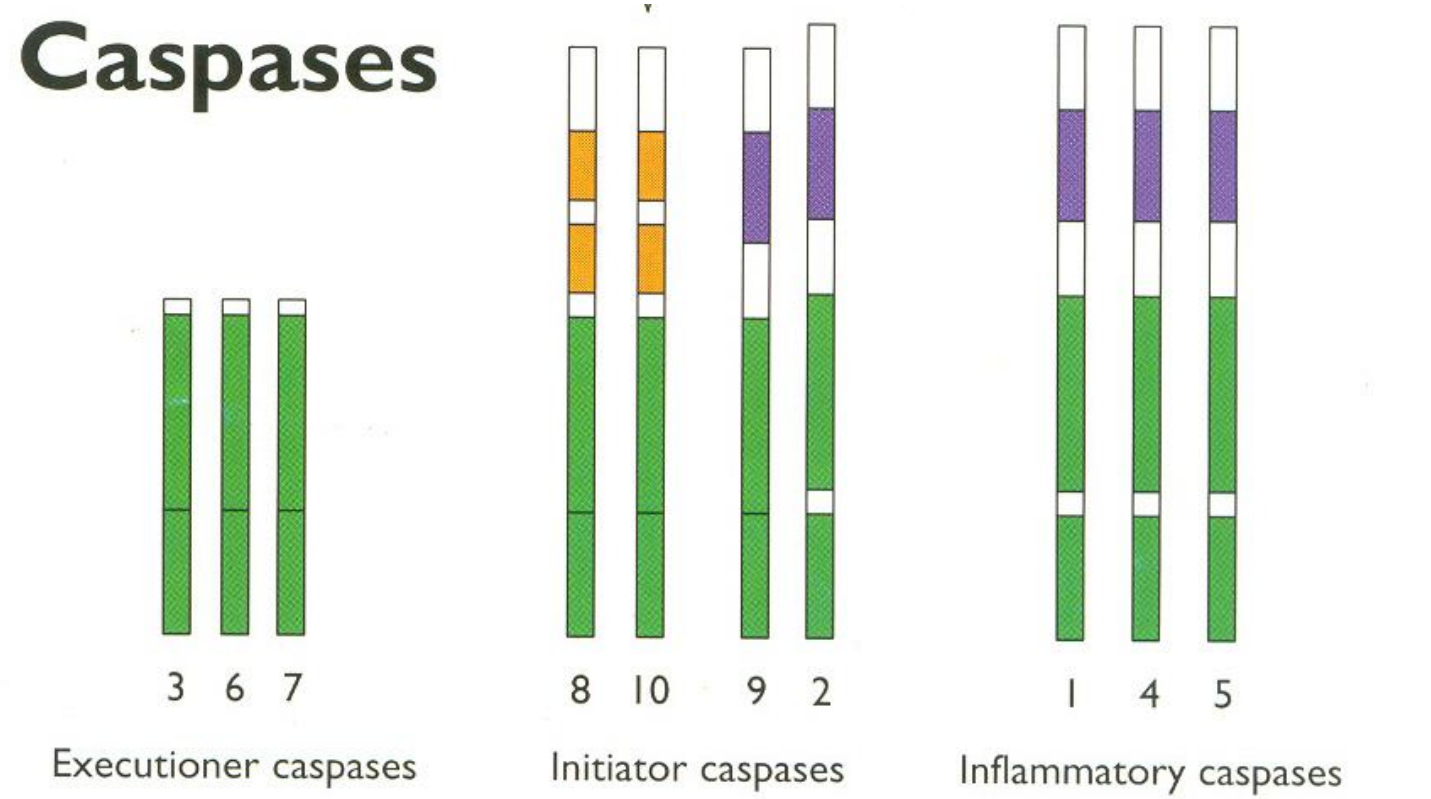
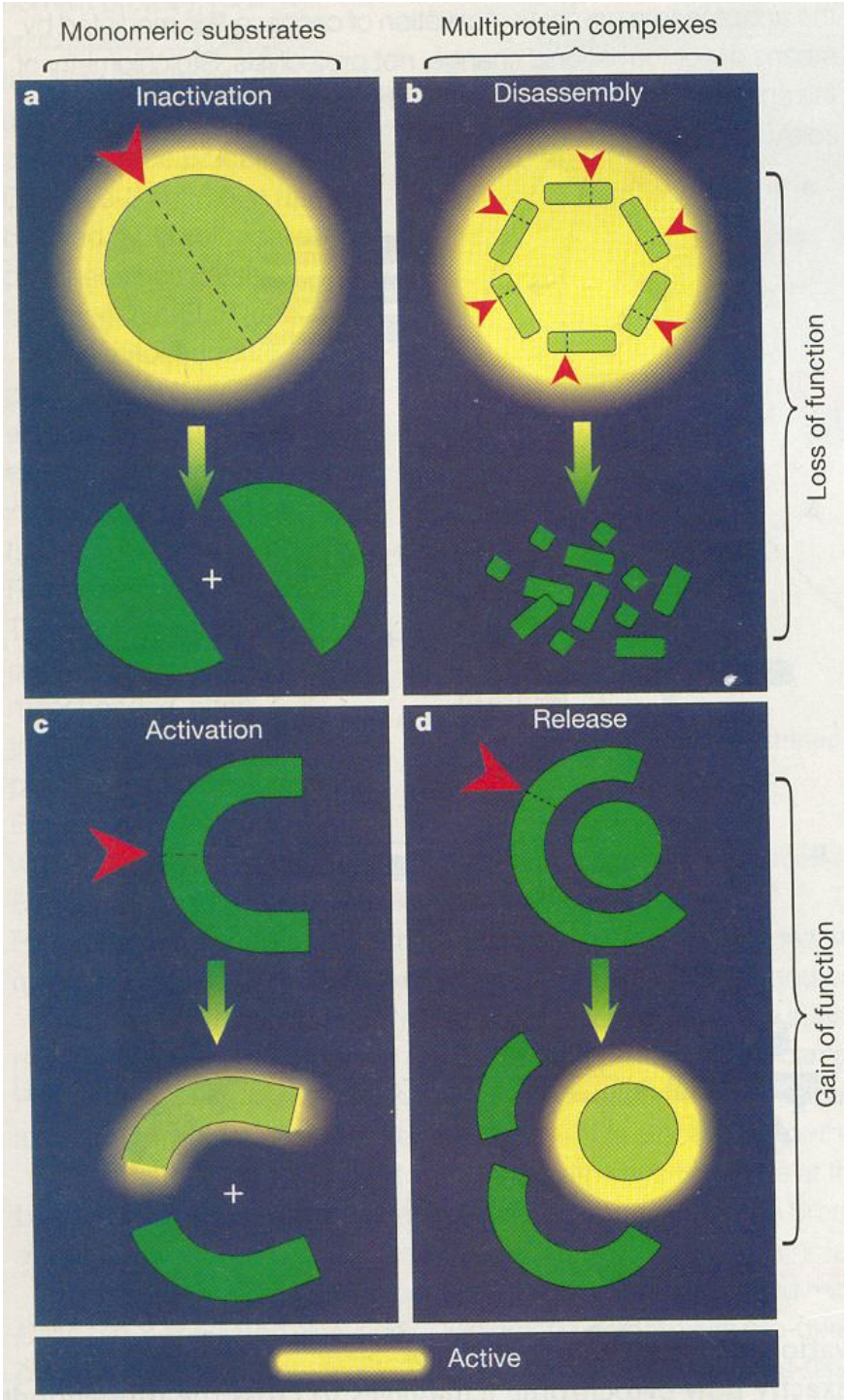
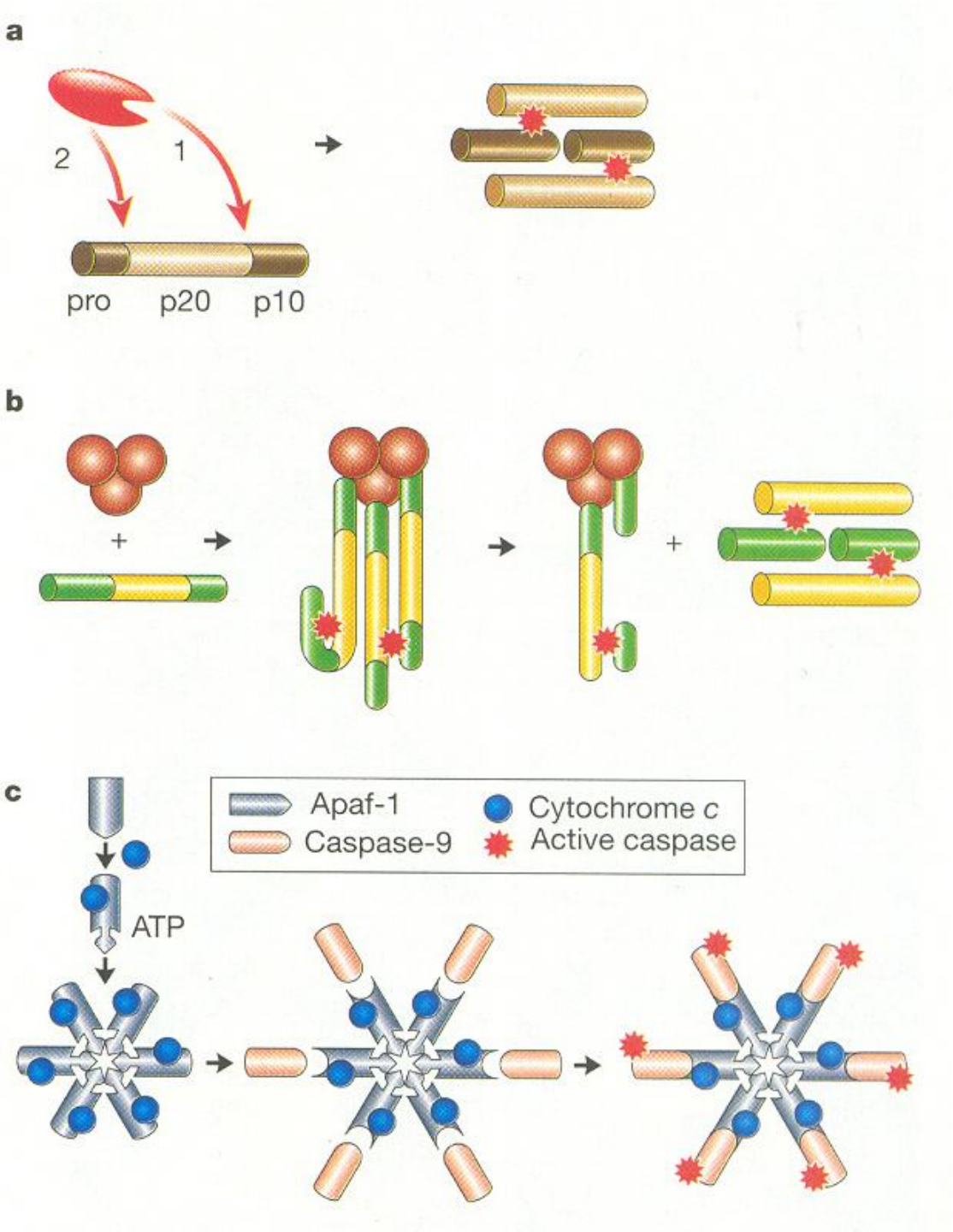


Figure 2. Fas/FasL-mediated apoptosis. (A) Binding of 3 FasL molecules with Fas leads to cell surface oligomerization, recruitment of the adapter protein FADD (Fas-associated protein with death domain), and procaspase-8 via its death effector domain (DED) into a death-inducing signaling complex (DISC). Activation of procaspase-8 by autocatalysis results in the initiation of extrinsic apoptosis by converting inactive effector procaspases-3, -6, and -7 into active enzymes via transproteolysis and intrinsic apoptosis via cleavage of Bid, release of cytochrome c, and activation of caspase-9 (not shown). (B) FasL is synthesized and stored as a membranous protein in vesicles by selected cell types. Upon activation by various physiologic stimuli, these vesicles are excreted from the cell and cause apoptosis of Fas-positive cells. (C) Wild-type FasL is cleaved from the cell surface by matrix metalloproteinases (MMPs) and accumulates as a soluble protein (sFasL). (D) sFasL may transiently interact with proteins on the cell surface or the extracellular matrix (ECM) to form oligomeric structures with apoptotic activity. (E) sFasL as a soluble homotrimer cannot induce oligomerization of Fas and as such blocks apoptosis by competing with the membranous form for Fas binding.

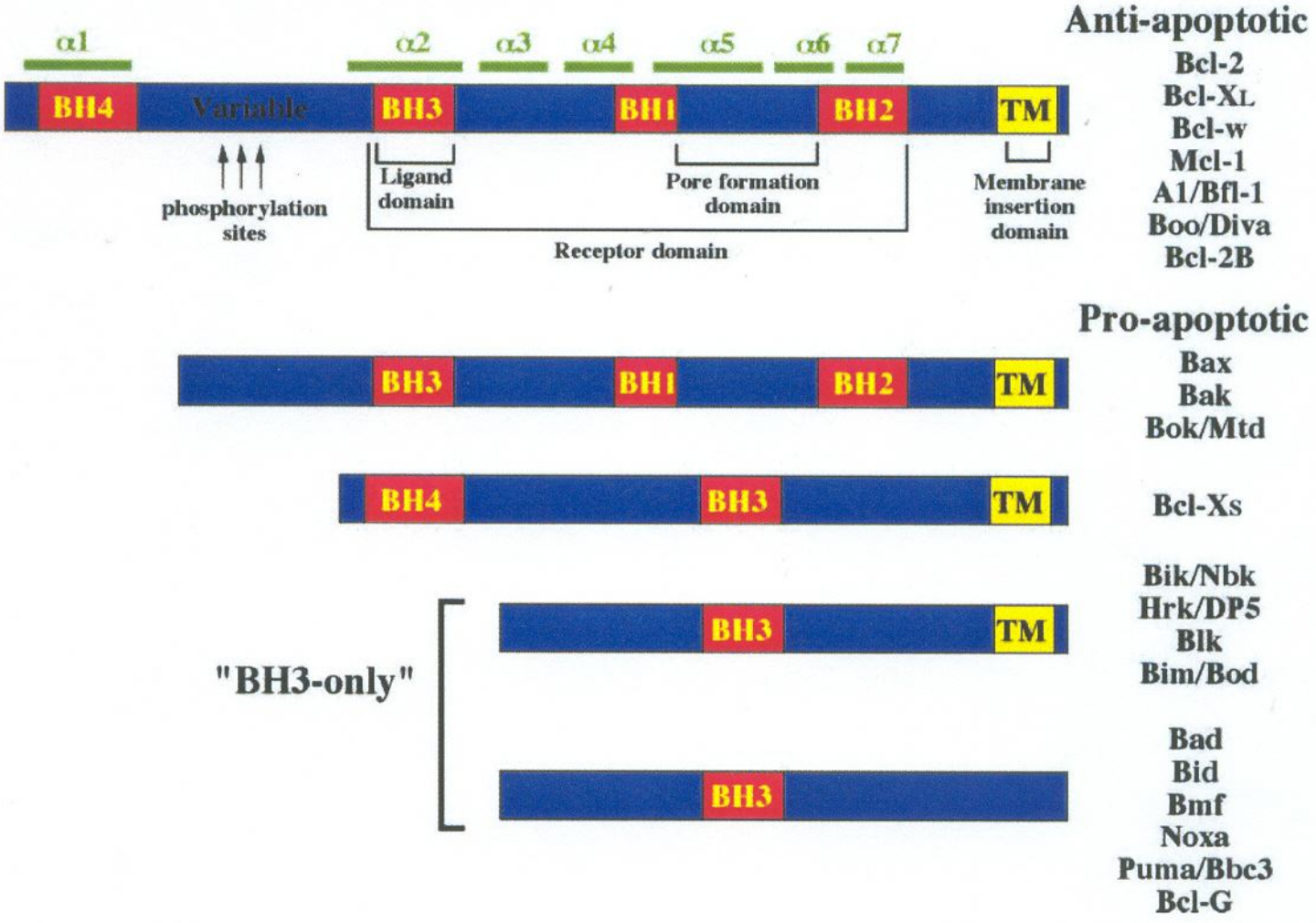
Caspases


3 6 7
8 10 9 2
1 4 5
Executioner caspases
Initiator caspases
Inflammatory caspases
Death effector domain
Caspase recruitment domain
Prodomain
Protease subunits
Adaptors
Initiator caspase
Executioner caspase
Transactivation
Active caspase tetramer (2 heterodimers)


Monomeric substrates
Multiprotein complexes
a
Inactivation
b
Disassembly
Loss of function
c
Activation
d
Release
Gain of function
Active


a
2
1
pro
p20
p10
b
+
+
c
Apaf-1
Caspase-9
Cytochrome c
Active caspase
ATP

Box 1 Proteins from the Bcl-2 family. Proteins from the Bcl-2 family share homology with Bcl-2 in the Bcl-2 homology regions (BH1 to BH4). Two classes of Bcl-2 family members can be distinguished. Anti-apoptotic Bcl-2 homologs possess all four BH1 homology regions. These proteins are primarily localized in mitochondria and stabilize their membranes. Pro-apoptotic Bcl-2 homologs can lack the BH4 domain (Bax, Bak, Bok/Mtd), BH2 (Bcl-XS), or BH1, BH2, and BH4. These latter proteins are referred to as 'BH-3-only' proteins and can possess a C-terminal transmembrane (TM) region.



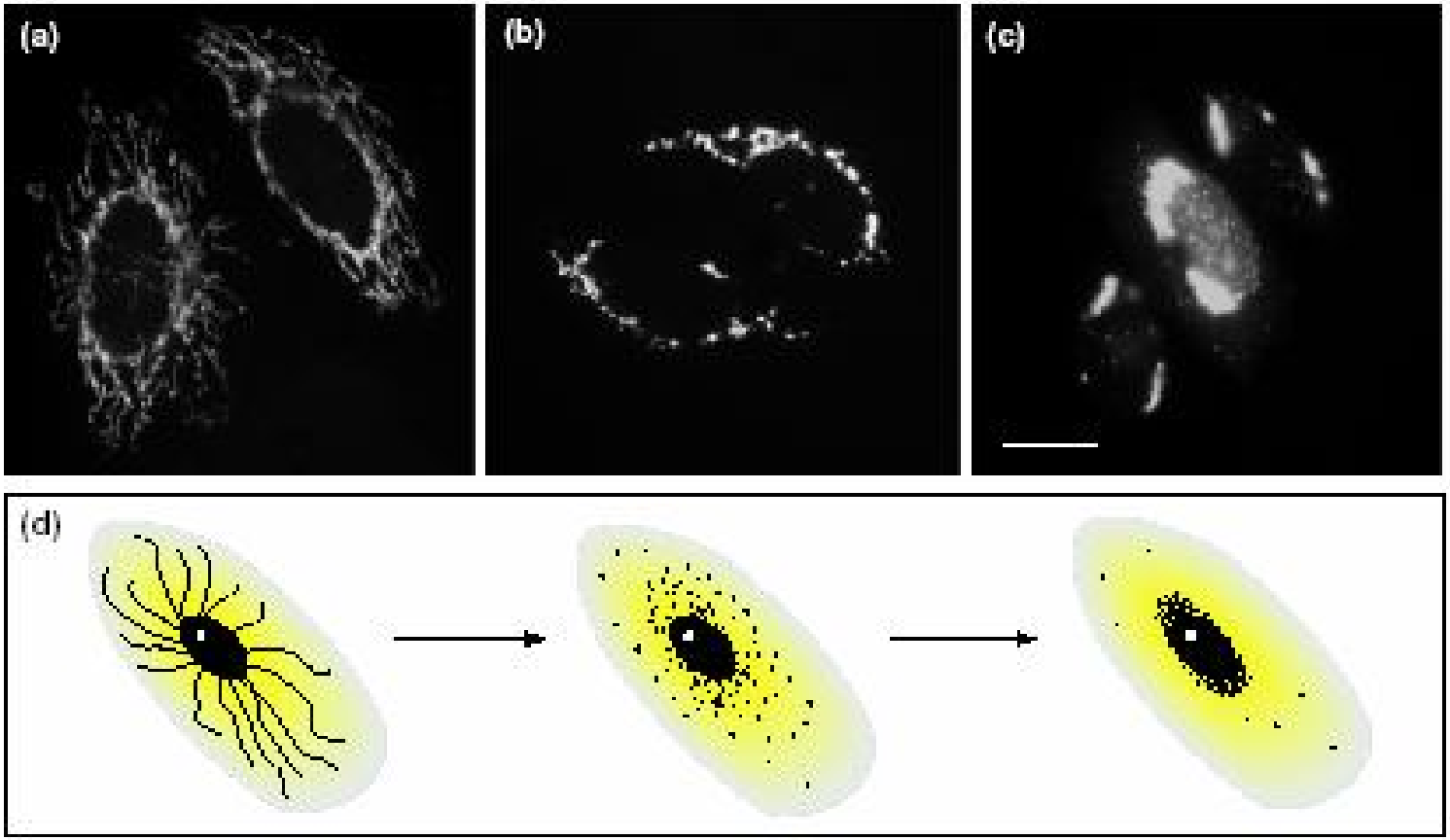


FIGURE 1

Morphological changes and redistribution of mitochondria in HeLa cells overexpressing Bax. (a) Control HeLa cells immunostained with an antibody against mitochondrial Hsp70 in order to reveal mitochondria. (b,c) Mitochondria from HeLa cells labelled with the same antibody, 15 h after transfection with a Bax-encoding cDNA in the presence of the peptide caspase inhibitor z-VAD to prevent apoptosis. Normal cells display 'worm-like' mitochondria (a), but cells overexpressing Bax (assessed by Bax immunostaining, not shown) display either punctiform mitochondria dispersed throughout the cell (b) or mitochondria that have disappeared from the cell periphery to form aggregates around the nucleus (c). These changes are independant of caspase activity. (d) A likely explanation for this is that, following Bax insertion into the outer mitochondrial membrane, mitochondria first condense ('pyknotic mitochondria'), possibly fragment, and then cluster around the nucleus. The condensed mitochondria have lost their cytochrome c (A. Osen-Sand and J-C. Martinou, unpublished.) Bar, 15 μm.

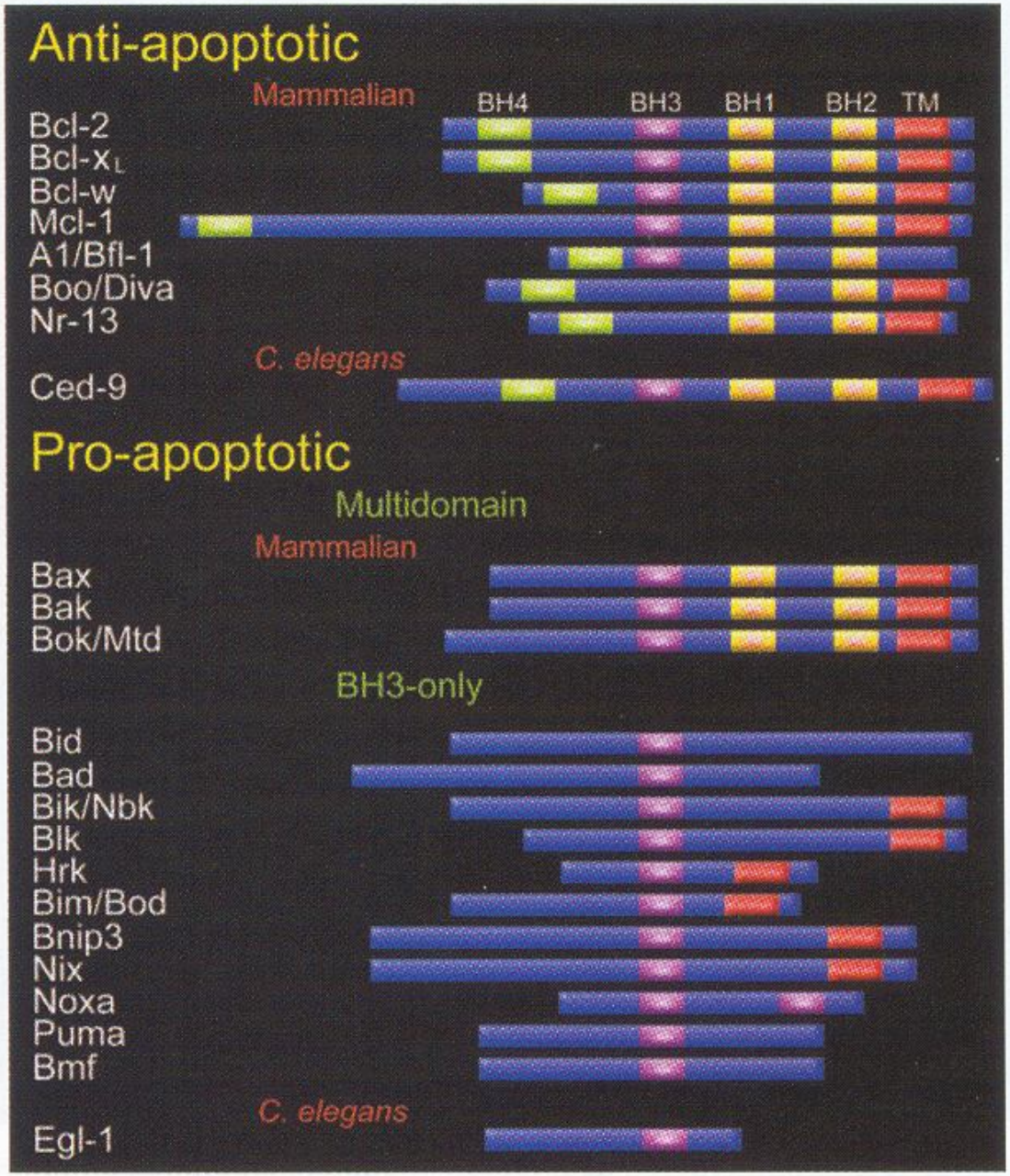


Figure 1. The Bcl-2 family.

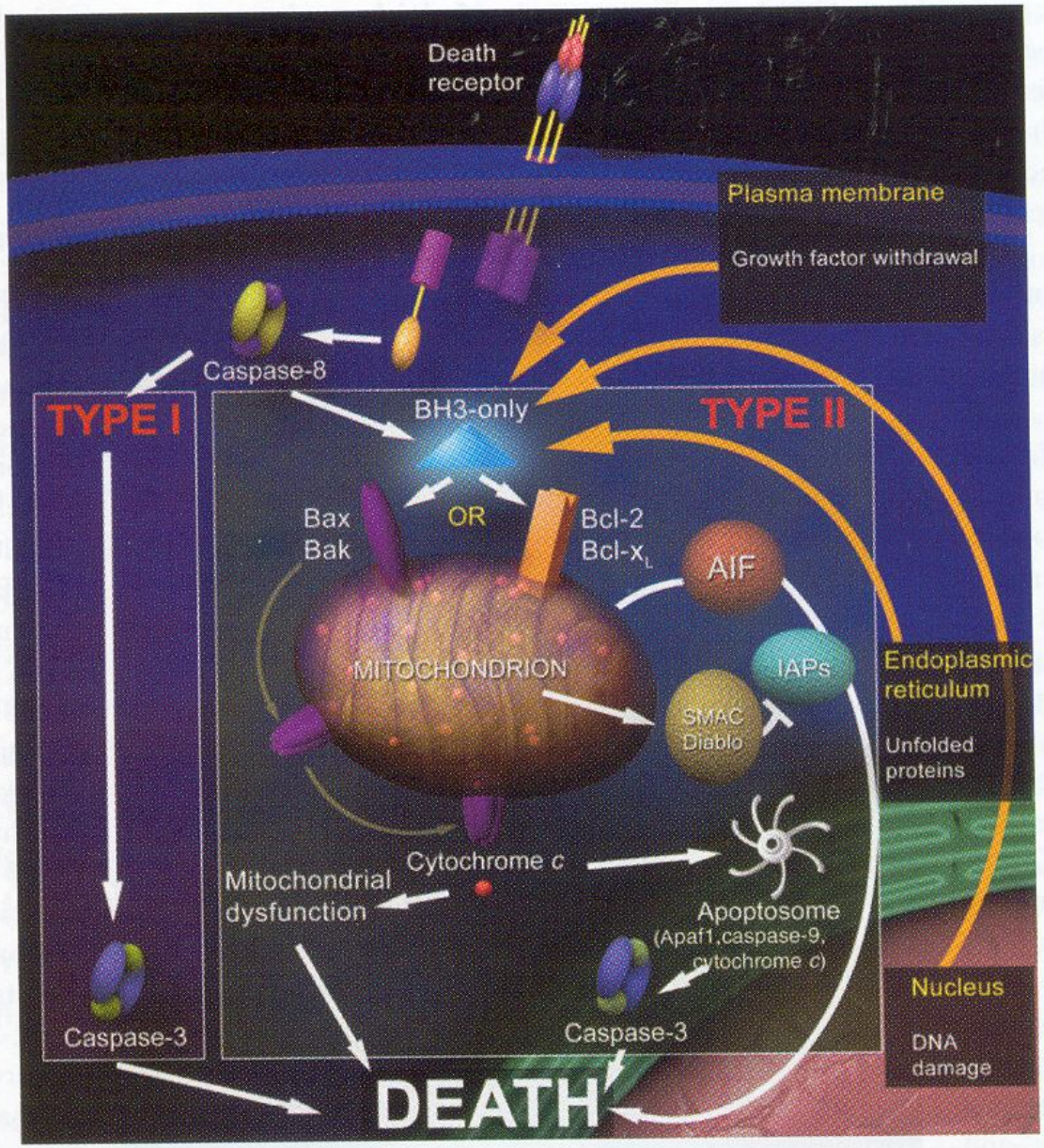


Figure 2. Cell death pathways. Two principal apoptotic execution programs follow death receptor signals: the caspase pathway and mitochondrial dysfunction. Whether a cell will live or die is determined by a balance of positive *versus* negative regulators from the proximal death signals to the core apoptotic pathway.

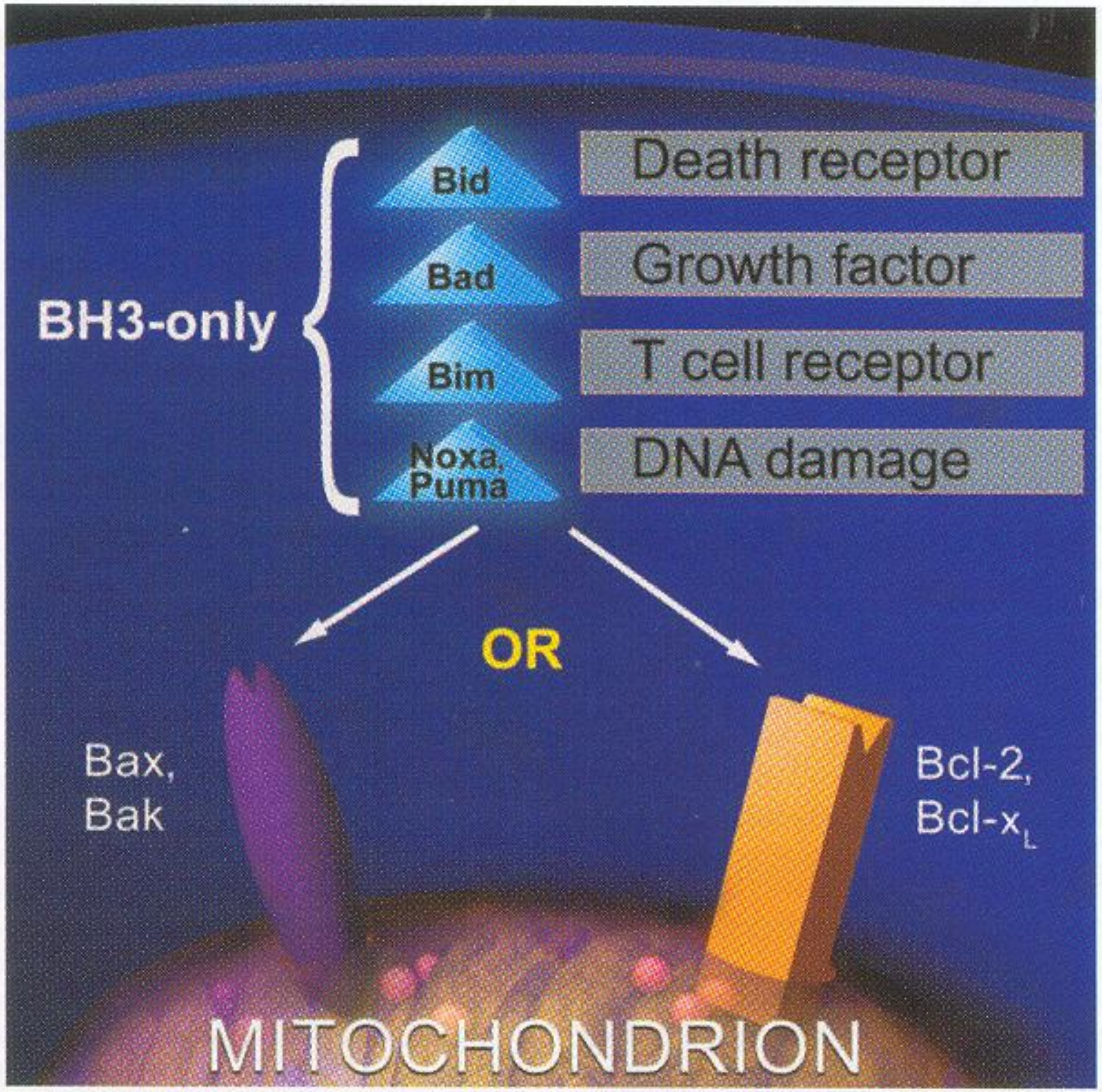
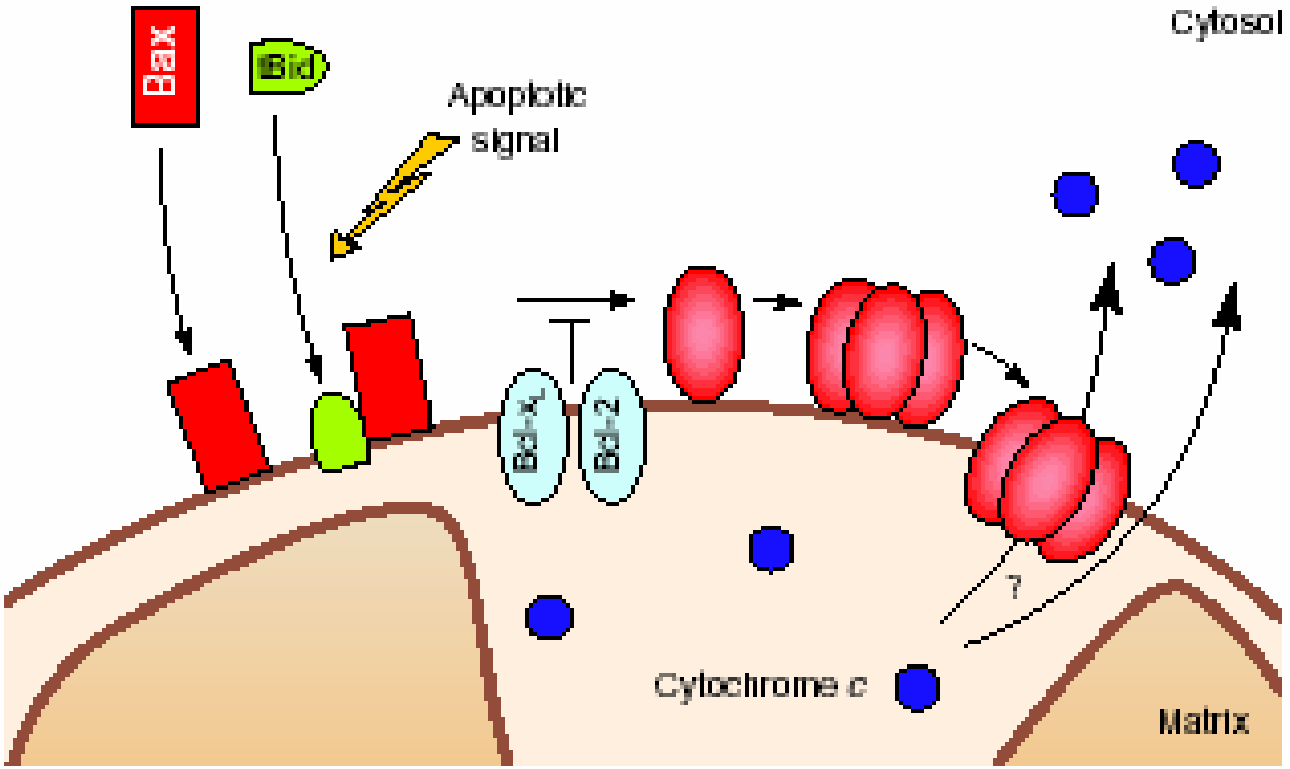


Figure 3. Detail of the mitochondrial apoptotic pathway. The BH3-only molecules Noxa, Puma, Bid, Bim and Bad all activate and require the multidomain pro-apoptotic proteins Bax and Bak to release cytochrome *c* and kill cells. In contrast, anti-apoptotic Bcl-2 or BCL-x_L sequesters BH3-only molecules and blocks the proapoptotic cascade.

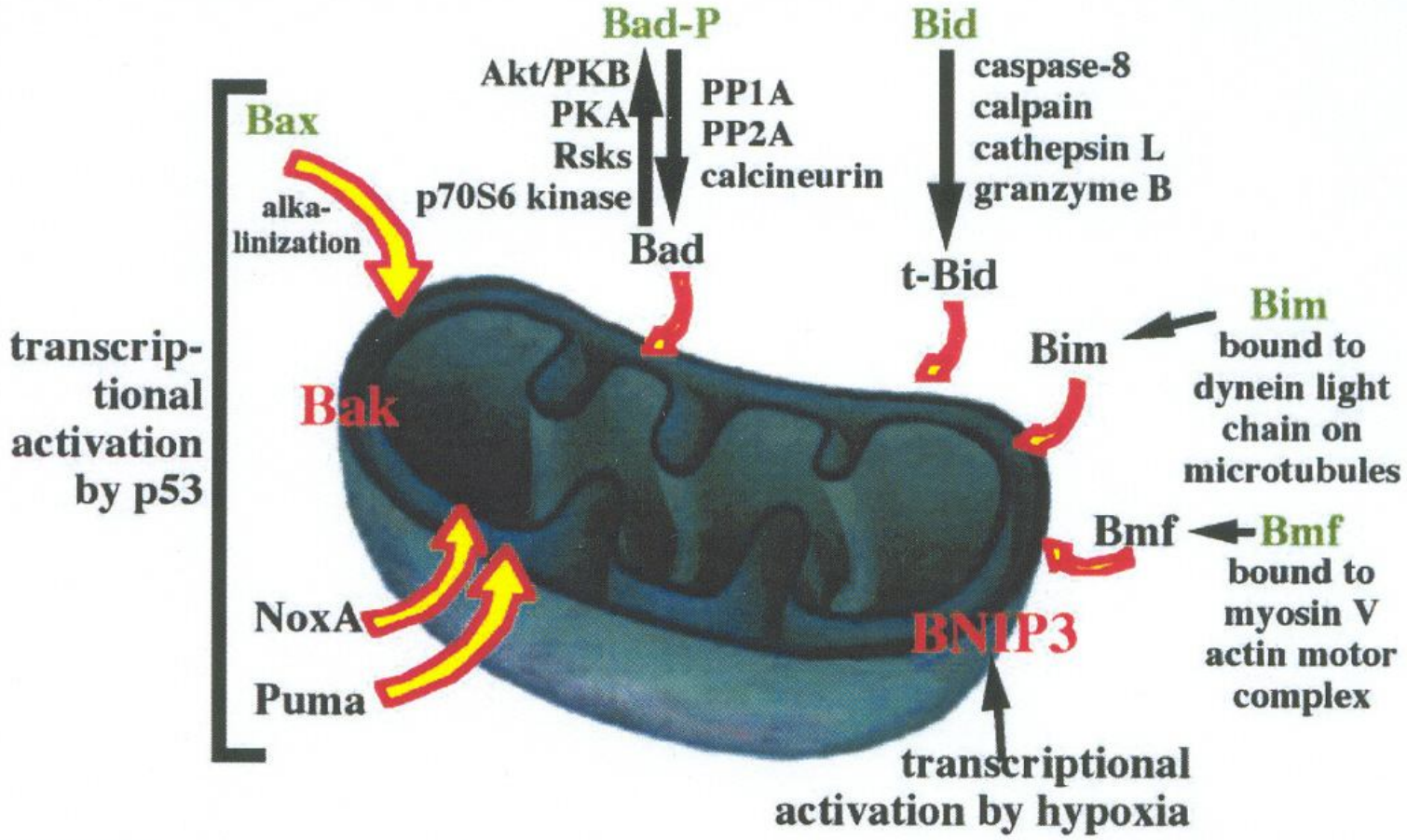


Trends in Cell Biology

FIGURE 3

After an apoptotic signal, Bax moves from the cytosol to the mitochondria. In some cell types, Bax is already loosely attached to the organelles and this translocation cannot be detected. After this, Bax undergoes a conformational change, oligomerizes and inserts into the outer mitochondrial membrane. This is rapidly followed by cytochrome-c release. It is possible that Bax inserts into the membrane before it oligomerizes. All these events can be induced by either full-length Bid or caspase-8-cleaved Bid (tBid) and prevented by Bcl-2 or Bcl-x_L, probably by direct interaction with Bax[33,37]. Another Bax-like protein, Bak, is activated through similar mechanisms[33,78].

During apoptosis induction pro-apoptotic Bcl-2 family members can translocate from an extra-mitochondrial to mitochondrial membranes, causing their permeabilization. This applies to Bax, which translocates from the cytosol to mitochondria as a result of cytosolic alkalinization. Bid is also normally found in the cytosol and translocates to mitochondria upon digestion by proteases (in particular by caspase-8 but also by calpain, cathepsin L, or granzyme B), yielding truncated Bid (t-Bid). Similar translocation reactions have been described for Bad (which is cytosolic when phosphorylated, for instance by the pro-survival kinase Akt/PKB or other kinases and mitochondrial when dephosphorylated), Bim (which is normally associated with the dynein light chain and hence associated with microtubuli), Bmf (normally associated with the myosin V actin motor complex), as well as NoxA and PUMA (all transcriptionally activated by p53, as Bax). Bak and BNIP3 (which is induced by hypoxia) are constitutively present in mitochondrial membranes.



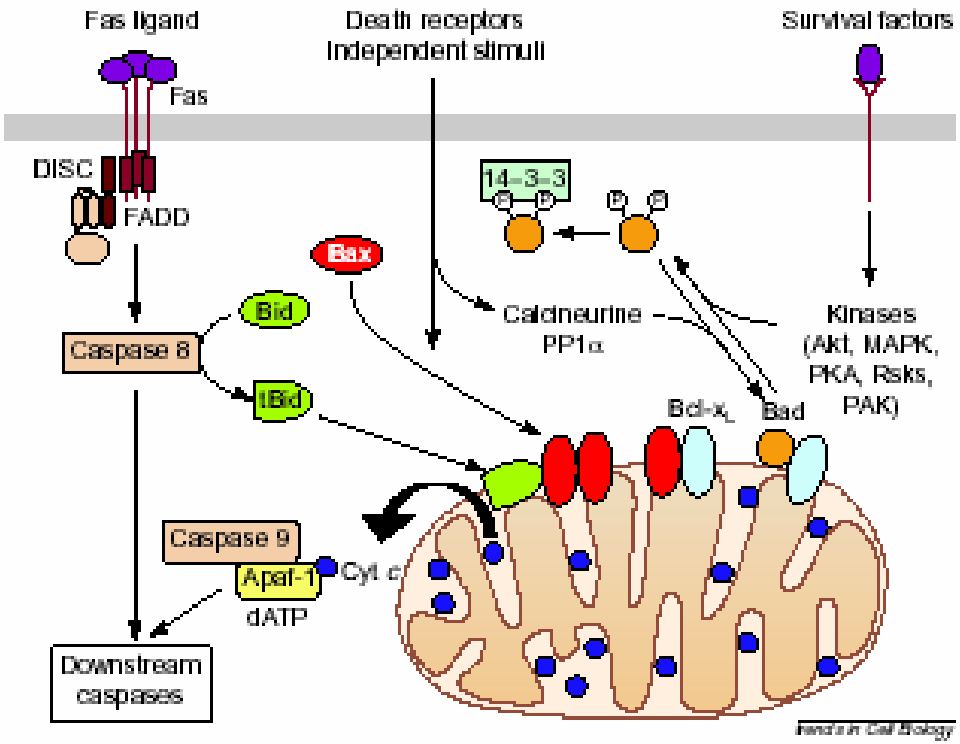
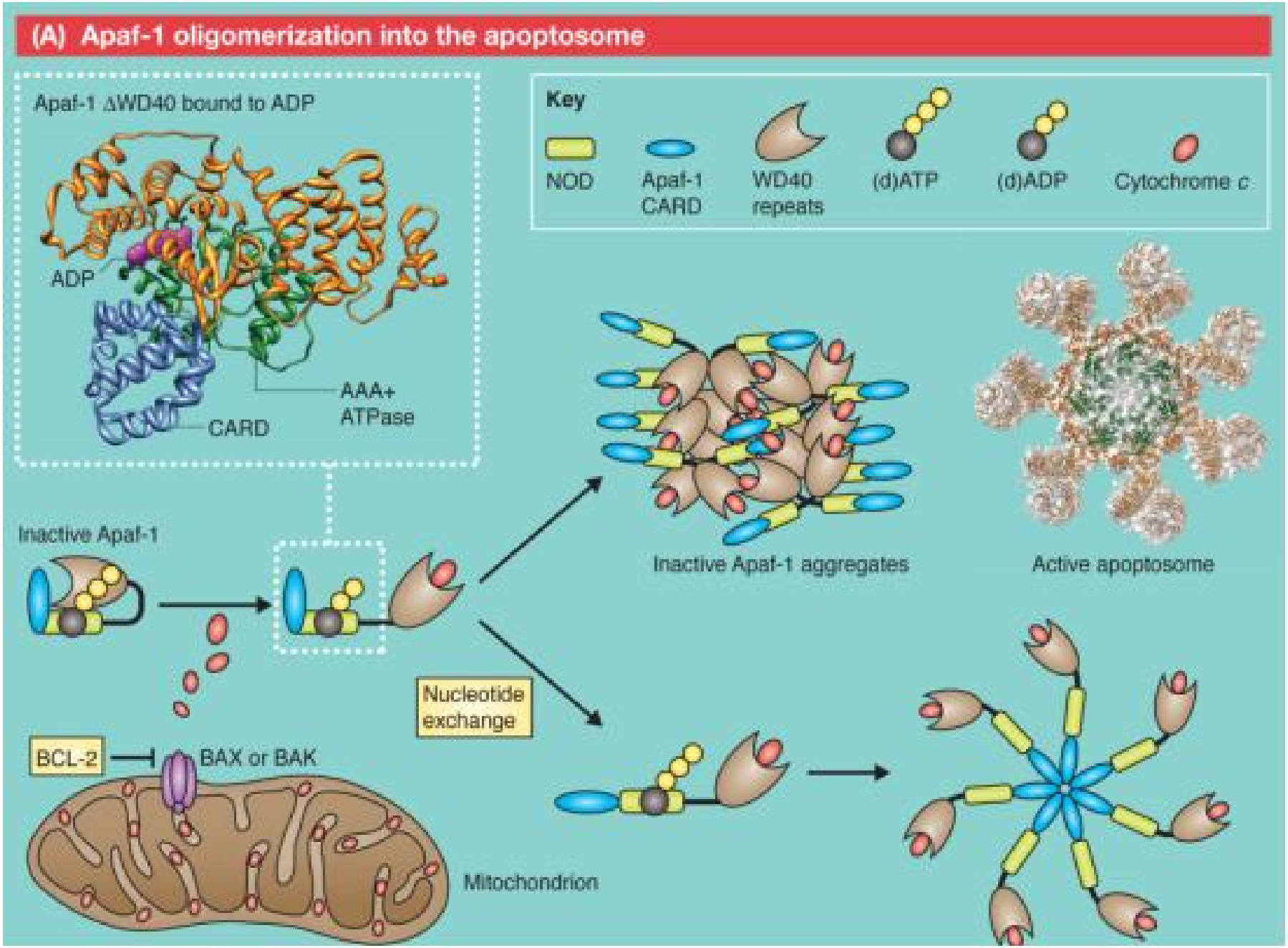
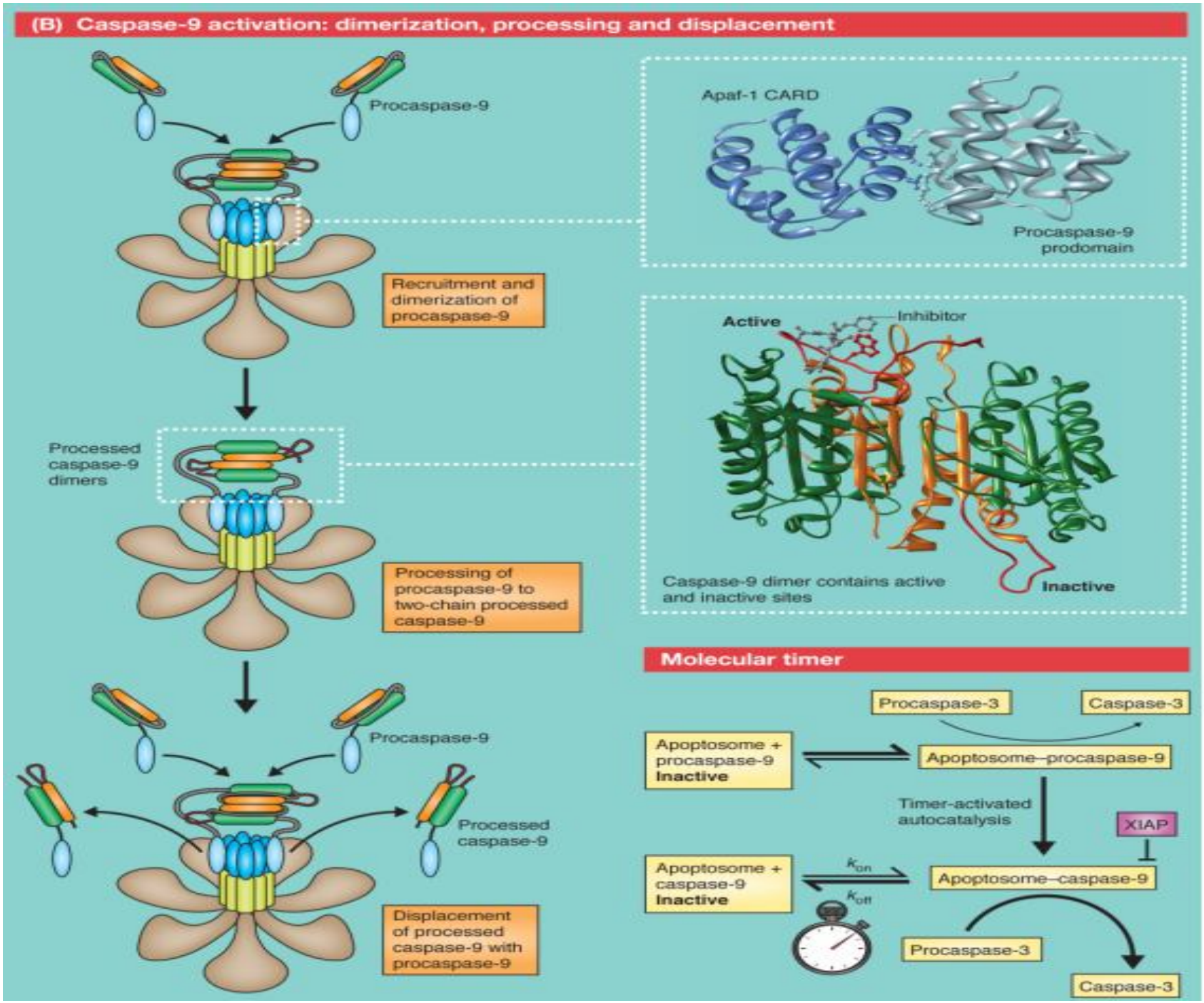
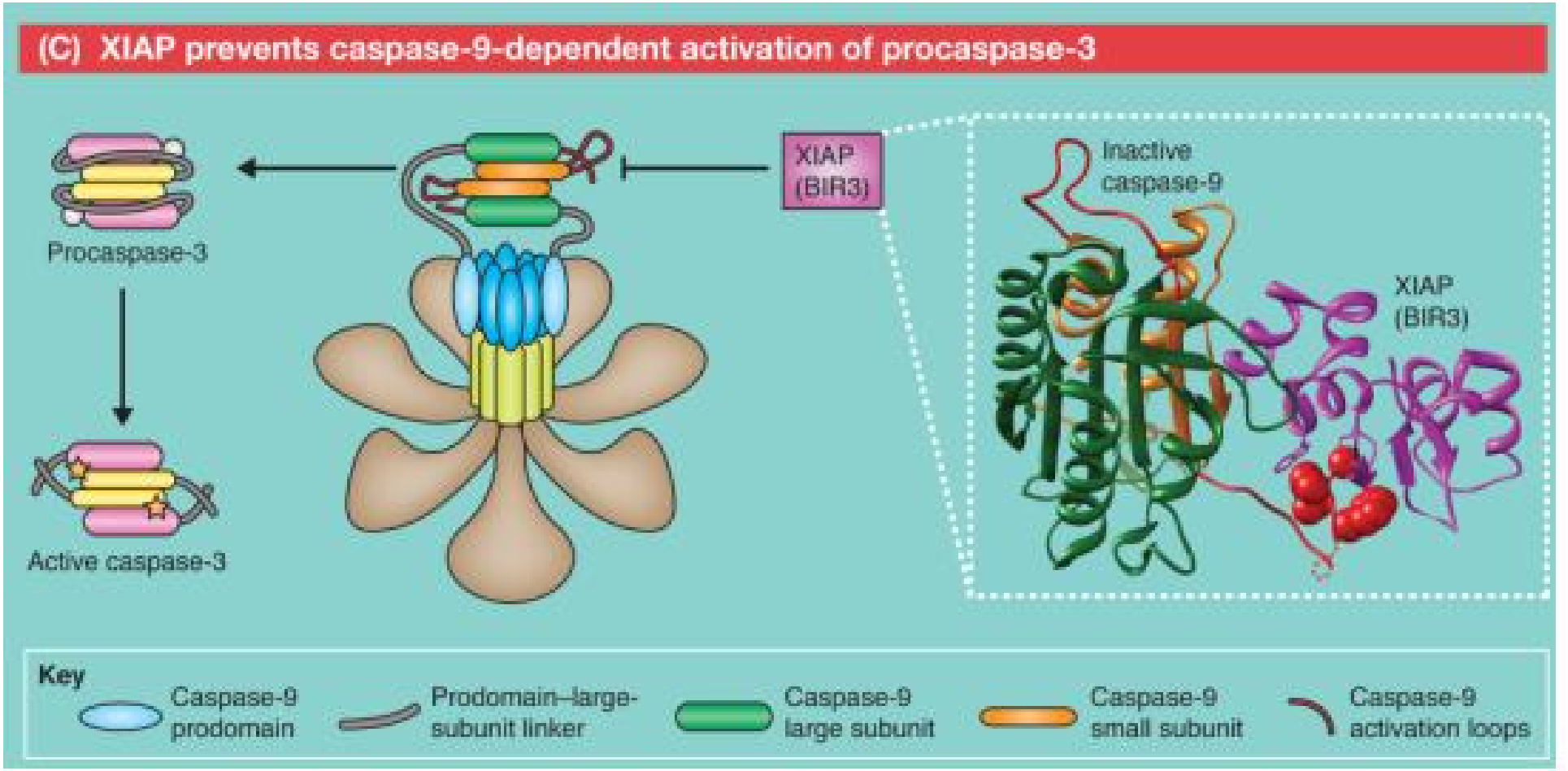


FIGURE 2

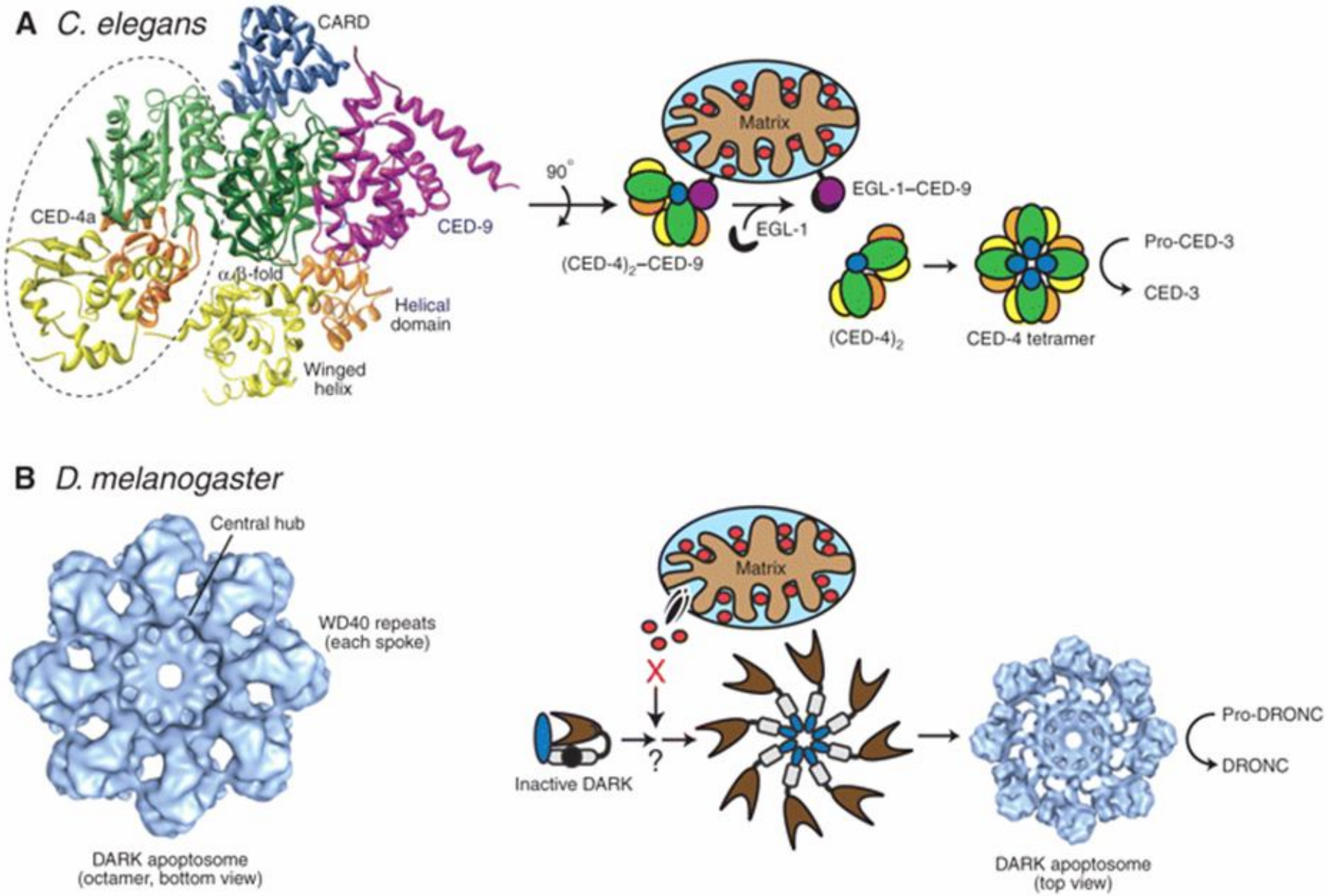
Many death signals converge onto mitochondria and are mediated through members of the Bcl-2 protein family called 'BH3-only' proteins, such as Bid and Bad. These proteins are recruited to specific pathways. In some cells, binding of Fas ligand to its receptor Fas leads to the trimerization of Fas and to the formation of the death-inducing signalling complex (DISC). This complex is formed by association of the cytoplasmic region of Fas, the adaptor protein FADD (Fas-associating protein with death domain) and procaspase 8, which is proteolytically cleaved to generate the active enzyme[7]. Caspase 8 then cleaves Bid, whose C-terminal fragment (tBid) translocates to mitochondria, where it activates Bax or Bax-like proteins and results in cytochrome-c (cyt *c*) release. tBid might also act on its own to trigger cytochrome-*c* release. Once in the cytosol, cytochrome *c* activates caspase 9 by binding to Apaf-1 and dATP. The physiological relevance of this pathway has recently been shown by the resistance of Bid-deficient mice to Fas hepatotoxicity[69]. Caspase 8 can also initiate a direct signalling pathway that is independent of mitochondria by cleaving and activating downstream caspases. Death-receptor-independent stimuli and growth-factor deprivation can trigger apoptosis by inducing translocation of Bax or Bad to mitochondria. In healthy cells, Bad can be phosphorylated in response to survival factors by several kinases, including Akt, mitogen-activated protein kinase (MAPK), Erk, protein kinase A (PKA), Rsks (MAPK-activated kinases) and p21-activated kinase 1 (PAK). The two serine residues that are phosphorylated are embedded in a 14–3–3 consensus site. Phosphorylation of either residue or both results in the sequestration of Bad in the cytosol through its binding to 14–3–3. During apoptosis, Bad is dephosphorylated by the Ca^{2+}-sensitive phosphatase calcineurin or the protein phosphatase 1α (PP1α) and translocates to mitochondria, where it binds to Bcl-x_L. This displaces Bcl-x_L from Bcl-x_L–Bax heterodimers, thereby inhibiting the death-repressor activity of Bcl-x_L.


(A) Apaf-1 oligomerization into the apoptosome
Apaf-1 ΔWD40 bound to ADP
ADP
AAA+
ATPase
CARD
Key
NOD
Apaf-1
CARD
WD40
repeats
(d)ATP
(d)ADP
Cytochrome c
Inactive Apaf-1
Inactive Apaf-1 aggregates
Active apoptosome
Nucleotide
exchange
BCL-2
BAX or BAK
Mitochondrion


(B) Caspase-9 activation: dimerization, processing and displacement
Procaspase-9
Recruitment and dimerization of procaspase-9
Processed caspase-9 dimers
Processing of procaspase-9 to two-chain processed caspase-9
Procaspase-9
Processed caspase-9
Displacement of processed caspase-9 with procaspase-9
Apaf-1 CARD
Procaspase-9 prodomain
Active
Inhibitor
Caspase-9 dimer contains active and inactive sites
Inactive
Molecular timer
Procaspase-3
Caspase-3
Apoptosome + procaspase-9 Inactive
Apoptosome–procaspase-9
Timer-activated autocatalysis
XIAP
Apoptosome + caspase-9 Inactive
k_{on}
k_{off}
Apoptosome–caspase-9
Procaspase-3
Caspase-3


(C) XIAP prevents caspase-9-dependent activation of procaspase-3
Procaspase-3
Active caspase-3
XIAP (BIR3)
Inactive caspase-9
XIAP (BIR3)
Key
Caspase-9 prodomain
Prodomain–large-subunit linker
Caspase-9 large subunit
Caspase-9 small subunit
Caspase-9 activation loops

Box 1. Evolutionary conservation of apoptosome complexes



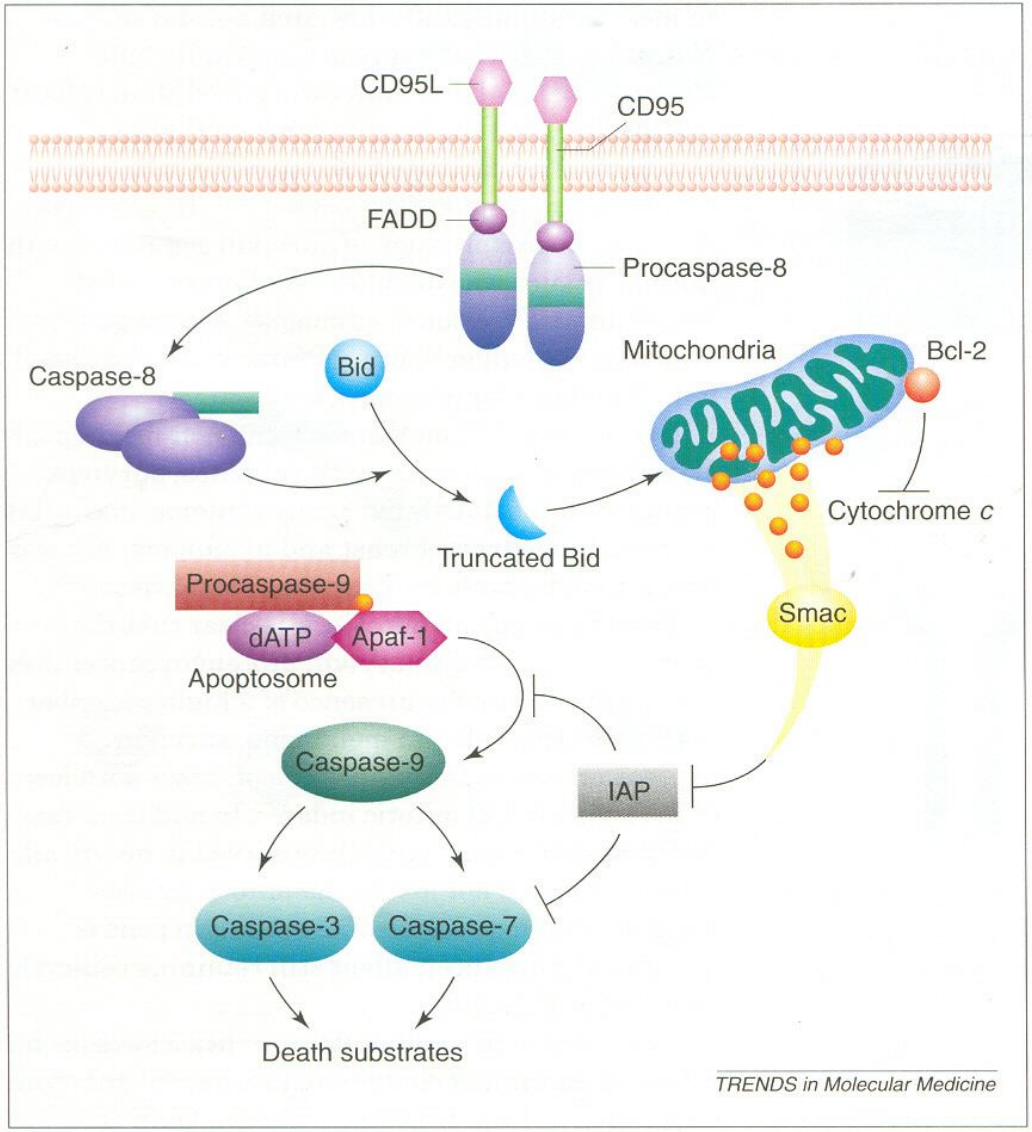
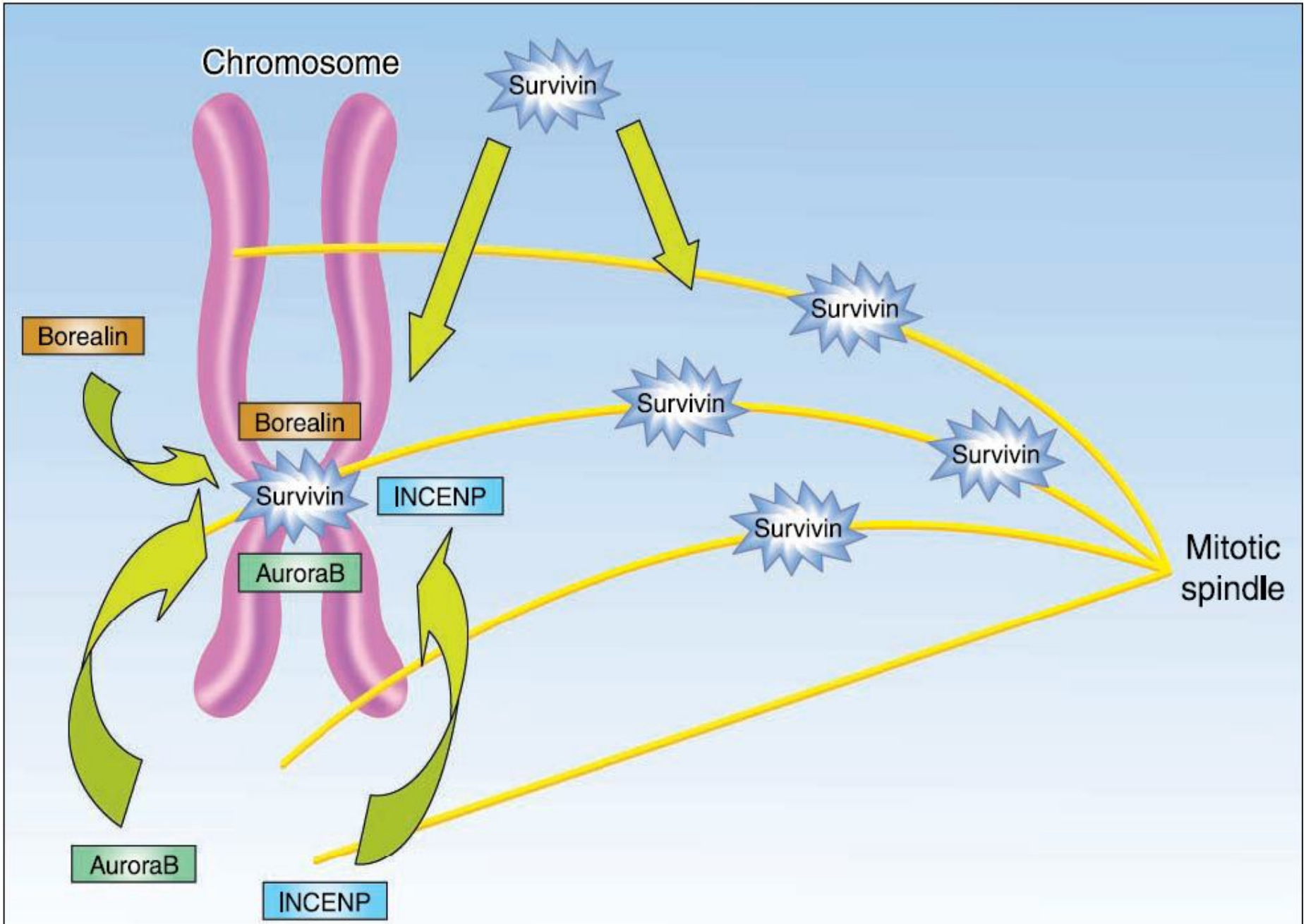


Fig. 1. General apoptotic pathways and modulation by inhibitor of apoptosis protein (IAP) family members. Cell-death pathways can be initiated by ligation of death receptors (e.g. CD95), resulting in activation of upstream caspase-8, or by release of mitochondrial cytochrome *c* in the cytoplasm. Cross-talk between the two pathways is provided by caspase-8-dependent cleavage of the Bcl-2 family member, Bid. The ability of IAP molecules to counteract the processing/function of upstream caspase-9 and effector caspase-3 and -7 is shown. Smac, a mitochondria-released protein, opposes IAP-dependent cytoprotection by releasing the bound caspase, favoring downstream completion of apoptosis.


Chromosome
Survivin
Survivin
Survivin
Survivin
Survivin
Survivin
Borealin
Borealin
Survivin
INCENP
AuroraB
AuroraB
INCENP
Mitotic spindle

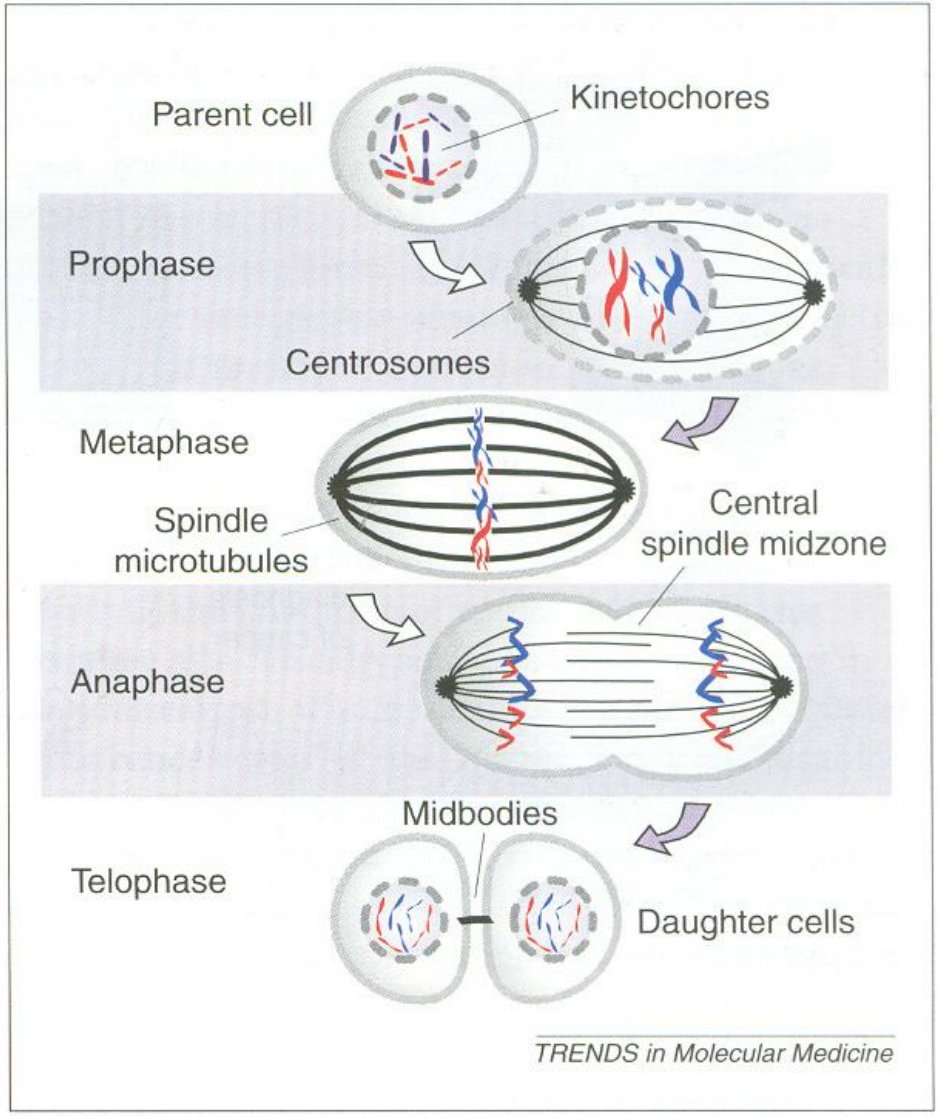
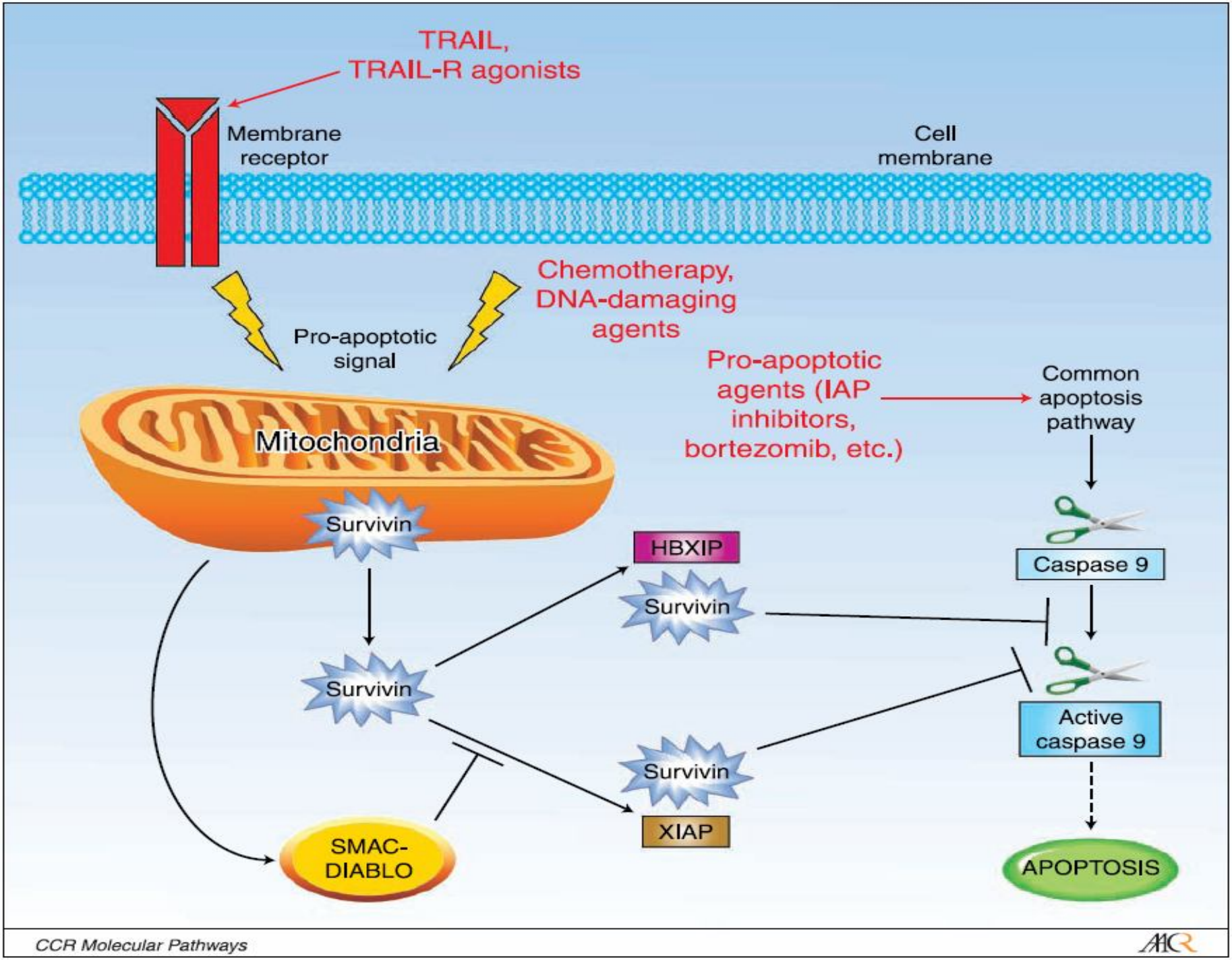


Fig. 2. Subcellular localization of survivin during mitosis. Survivin localizes to kinetochores, centrosomes (microtubule-organizing centers), spindle microtubules, central spindle midzone and midbodies. Approximately 80% of the total cellular survivin content in mitotic cells is bound to centrosomes and microtubules of the metaphase and anaphase spindle.


TRAIL,
TRAIL-R agonists
Membrane receptor
Cell membrane
Chemotherapy, DNA-damaging agents
Pro-apoptotic signal
Pro-apoptotic agents (IAP inhibitors, bortezomib, etc.)
Common apoptosis pathway
Mitochondria
Survivin
HBXIP
Survivin
Caspase 9
Survivin
Active caspase 9
Survivin
XIAP
SMAC-DIABLO
APOPTOSIS

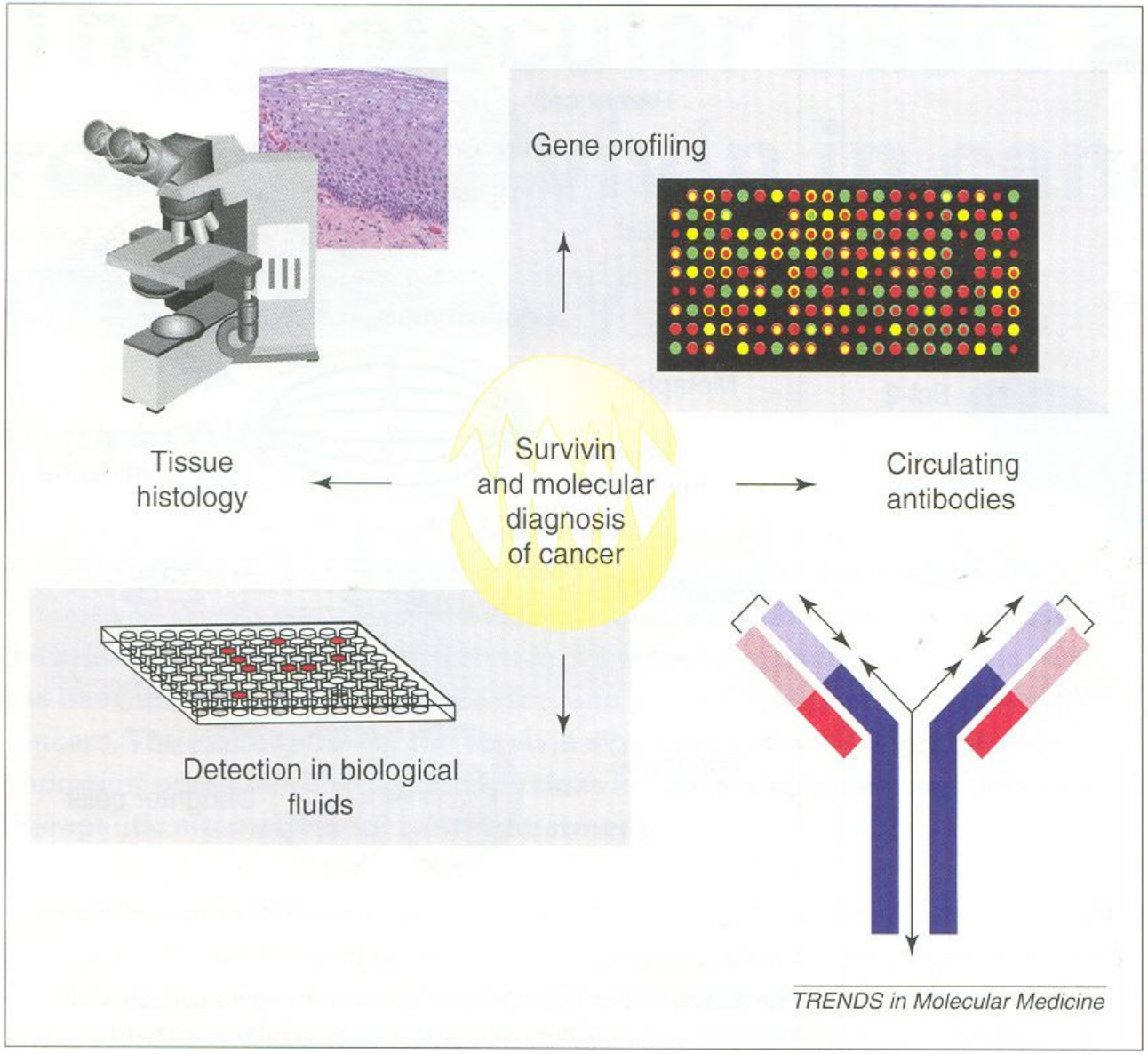


Fig. 3. Molecular diagnosis of cancer by survivin detection. Potential diagnostic applications of survivin in cancer patients includes immunohistochemical detection of survivin in tissue biopsies, microarray-based gene-profiling studies, direct detection in biological fluids (e.g. urine, serum, sputum), and determination of a cancer-specific immune response by assaying circulating antibodies to survivin.

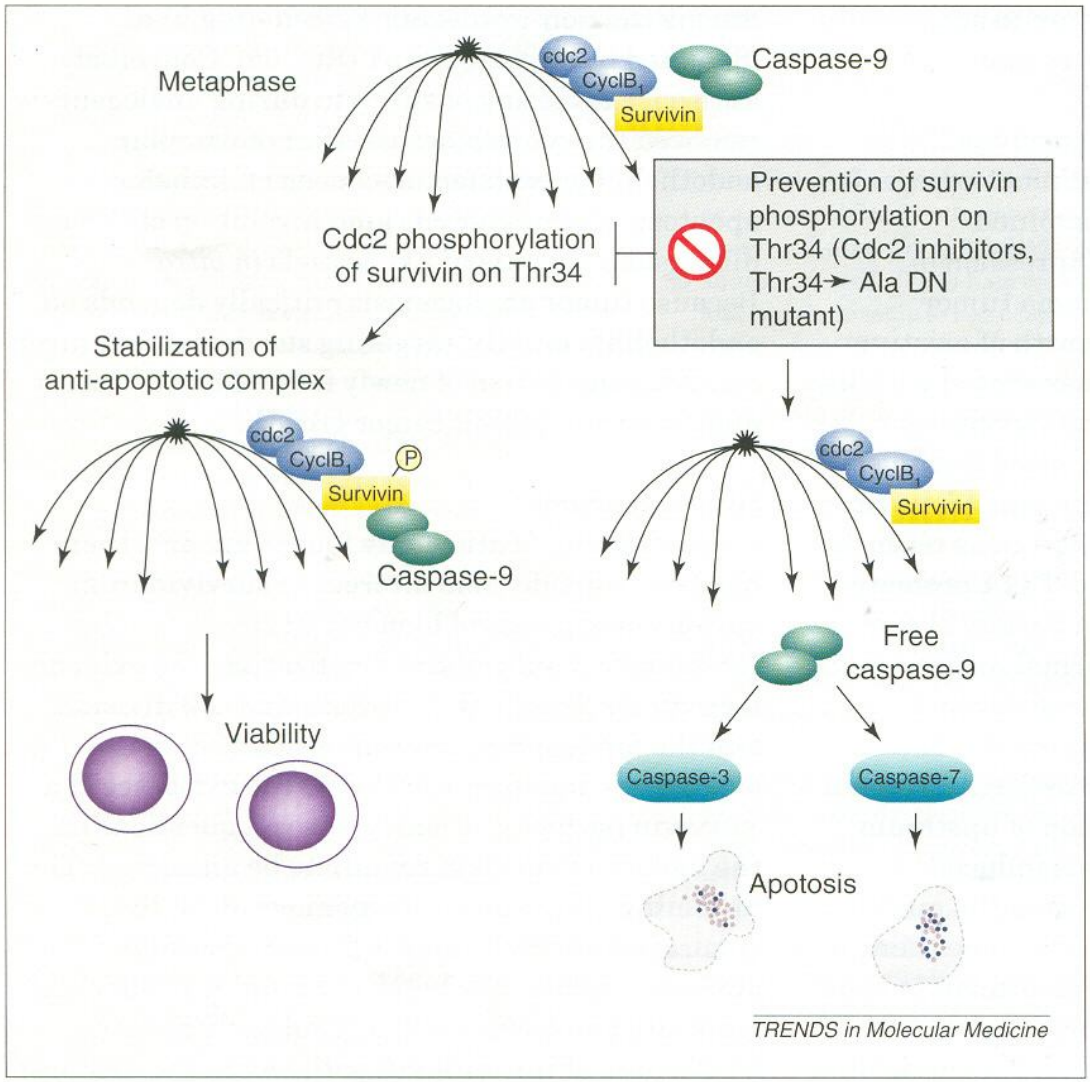


Fig. 4. Phosphorylation on Thr34 as a crucial requisite for survivin-dependent cytoprotection in cancer. A potential pathway of survivin-dependent cytoprotection in cancer cells requires mitotic phosphorylation on Thr34. At prometaphase, survivin complexes with the main mitotic kinase $p34^{cdc2}$-cyclin B1 on the mitotic apparatus, and is phosphorylated during mitosis by $p34^{cdc2}$-cyclin B1 on Thr34. Interference with survivin phosphorylation on Thr34 by expression of a phosphorylation-defective survivin Thr34→Ala dominant-negative (DN) mutant or by treatment with a cyclin-dependent kinase inhibitor results in the dissociation of a survivin–caspase-9 complex, mislocalization of caspase-9 from the mitotic apparatus and caspase-dependent apoptosis of cells traversing mitosis.

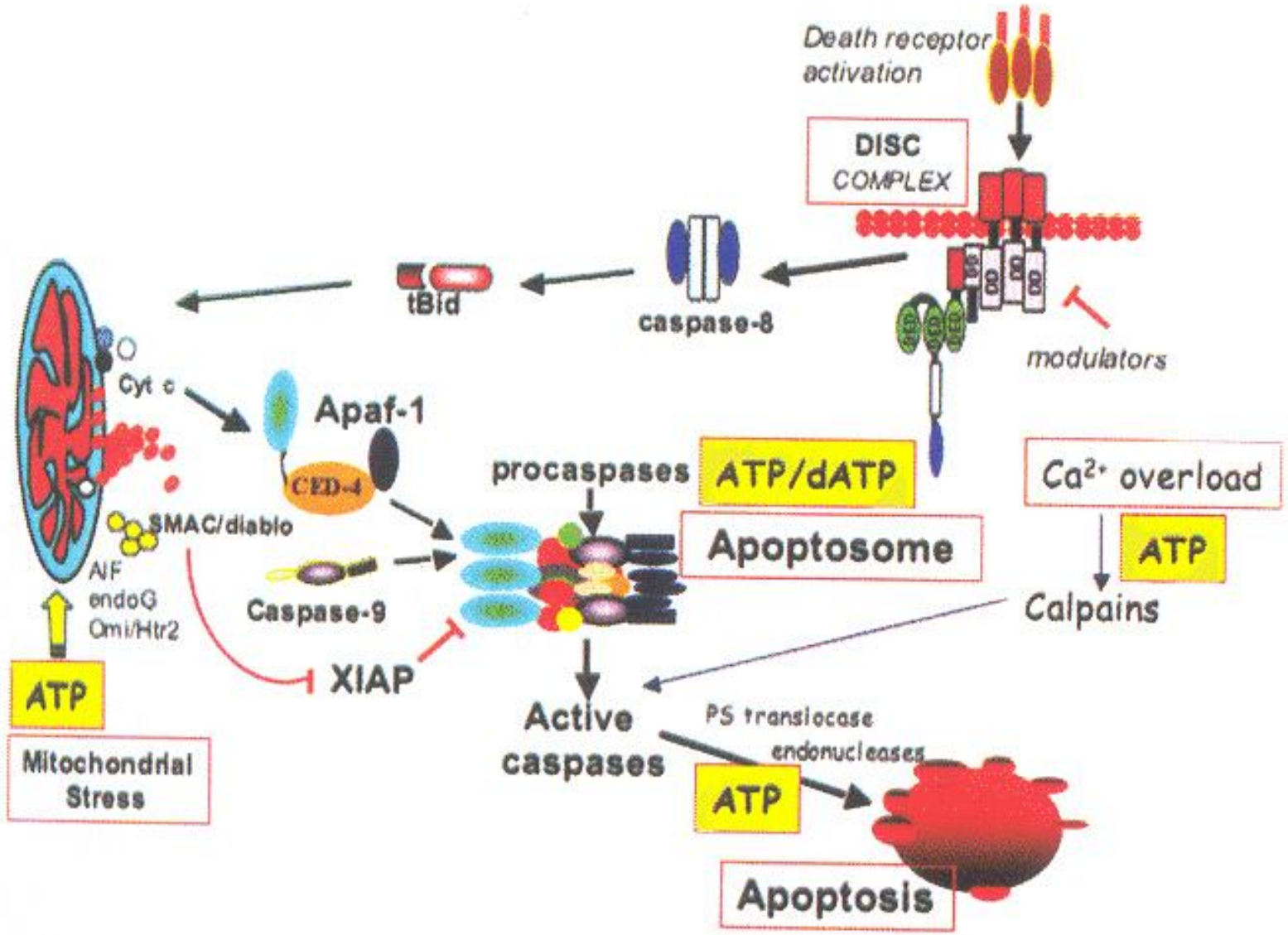
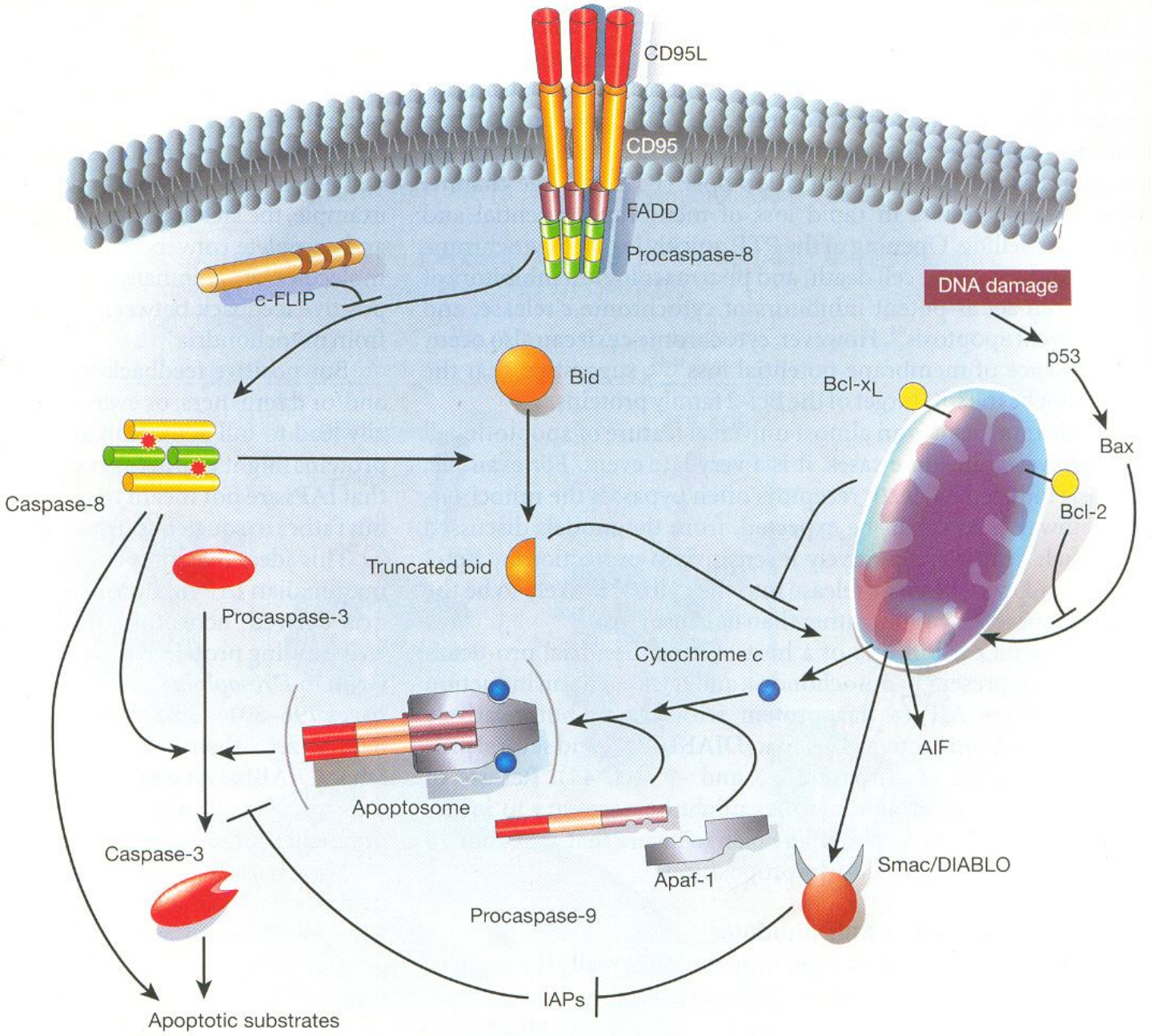


Figure 2 ATP-dependent steps in apoptosis. Potential regulation of the cell death machinery by ATP


CD95L
CD95
FADD
Procaspase-8
c-FLIP
DNA damage
p53
Bid
Bcl-xL
Bax
Caspase-8
Bcl-2
Truncated bid
Procaspase-3
Cytochrome c
AIF
Apoptosome
Caspase-3
Apaf-1
Smac/DIABLO
Procaspase-9
IAPs
Apoptotic substrates

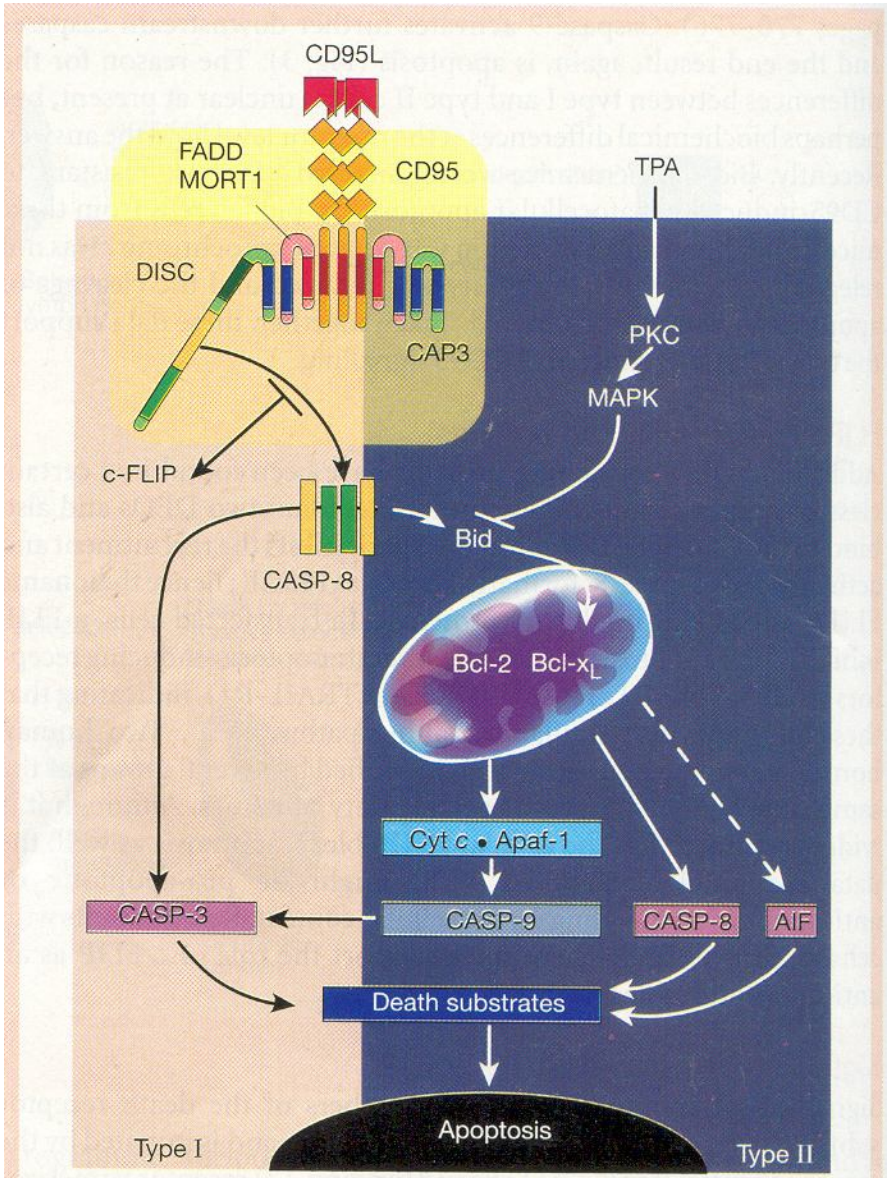


Figure 3 Signalling pathways induced by CD95. CD95 signalling pathway (including DISC formation) used in type I and type II cells (see text). TPA, 12-*O*-tetradecanoylphorbol-13-acetate; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; CASP, caspase; AIF, apoptosis-initiating factor; CAP3, cytotoxicity-dependent Apo-1-associated protein 3).

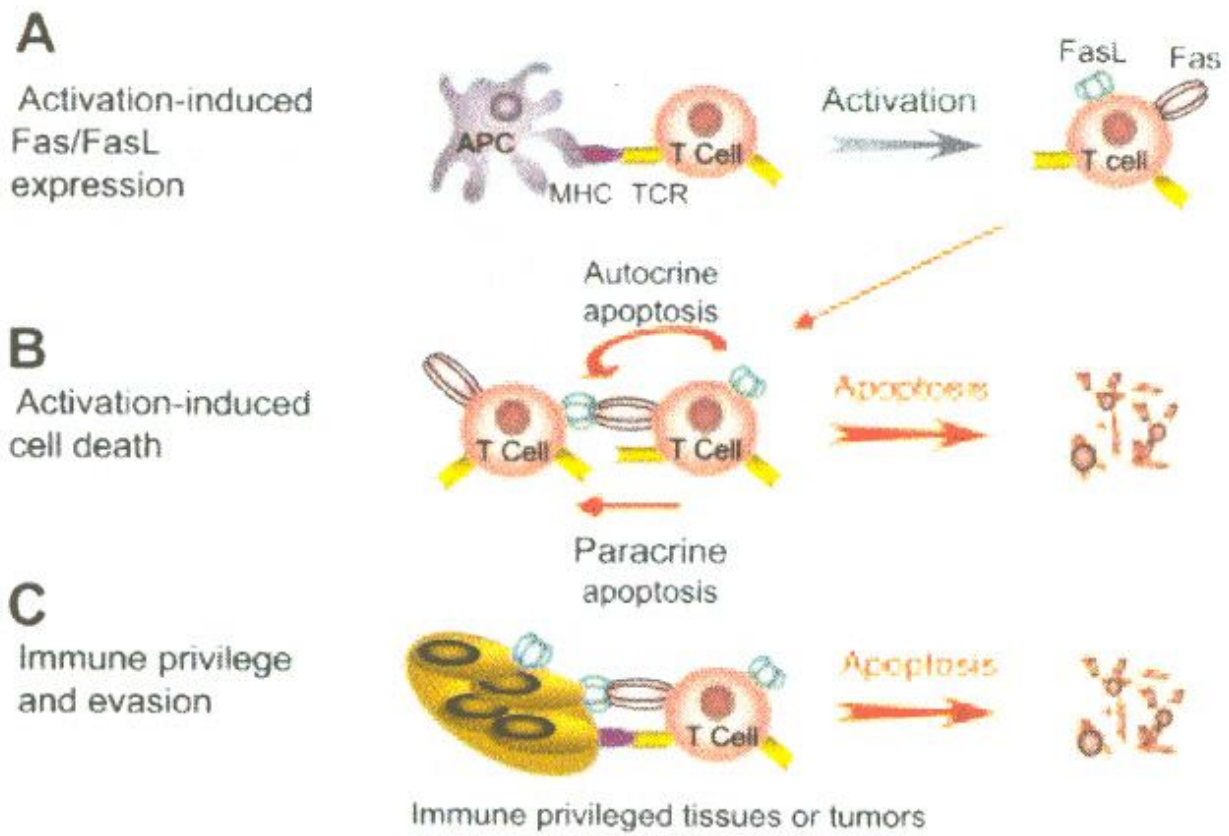


Figure 1. Physiologic regulation of immune responses by Fas/FasL-induced apoptosis. (A) Activation of T cells by the engagement of T-cell receptors (TCR) with the peptide/major histocompatibility complex (MHC) bimolecular complex in conjunction with the transduction of secondary signals (not shown) leads to the up-regulation of both Fas and FasL expression. (B) Upon repeated antigenic stimulation, T cells become sensitive to Fas/FasL-mediated apoptosis and undergo activation-induced cell death (AICD) via either autocrine or paracrine apoptosis. (C) Immune privileged tissues, such as the eye and testis, express FasL that triggers apoptosis in lymphocytes expressing Fas as a mechanism to prevent massive exacerbation of inflammatory reactions. Similarly, many tumor cells express FasL during disease progression and eliminate tumor reactive T cells as a means of immune evasion.

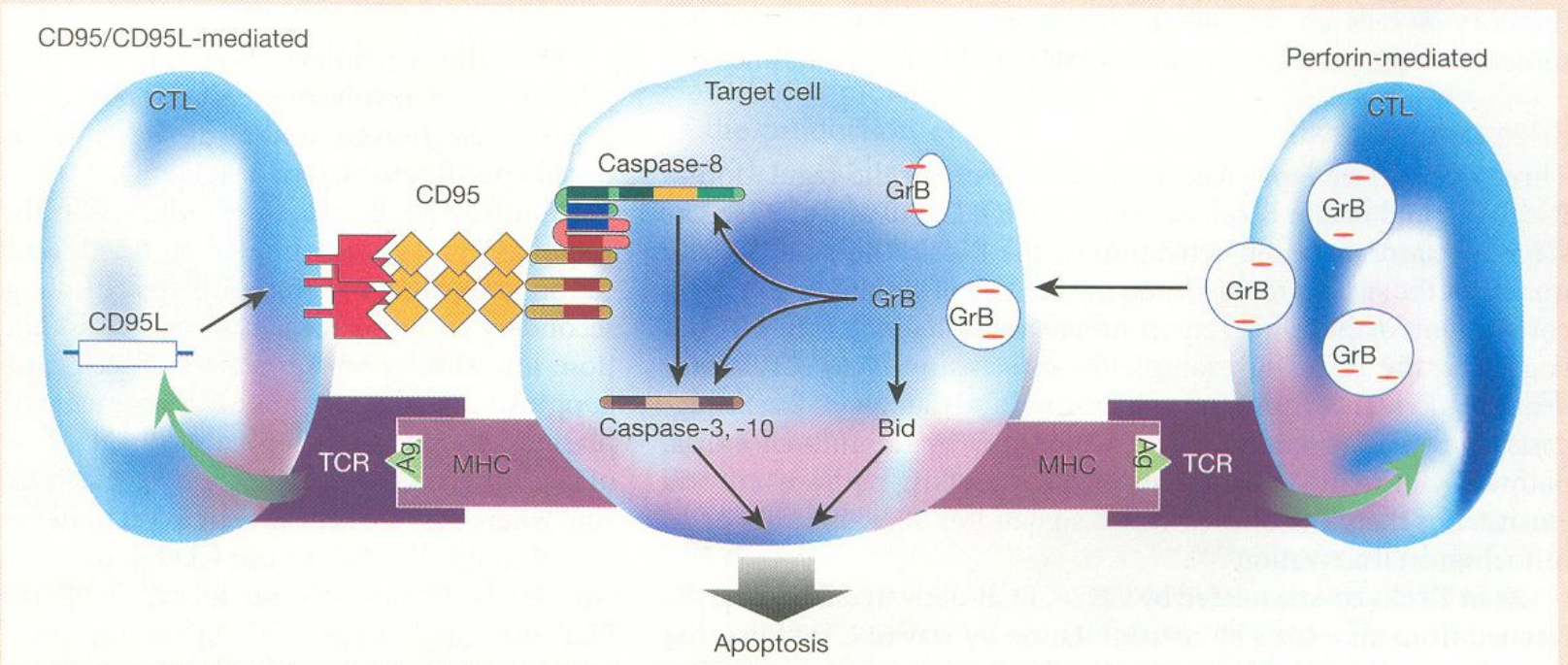
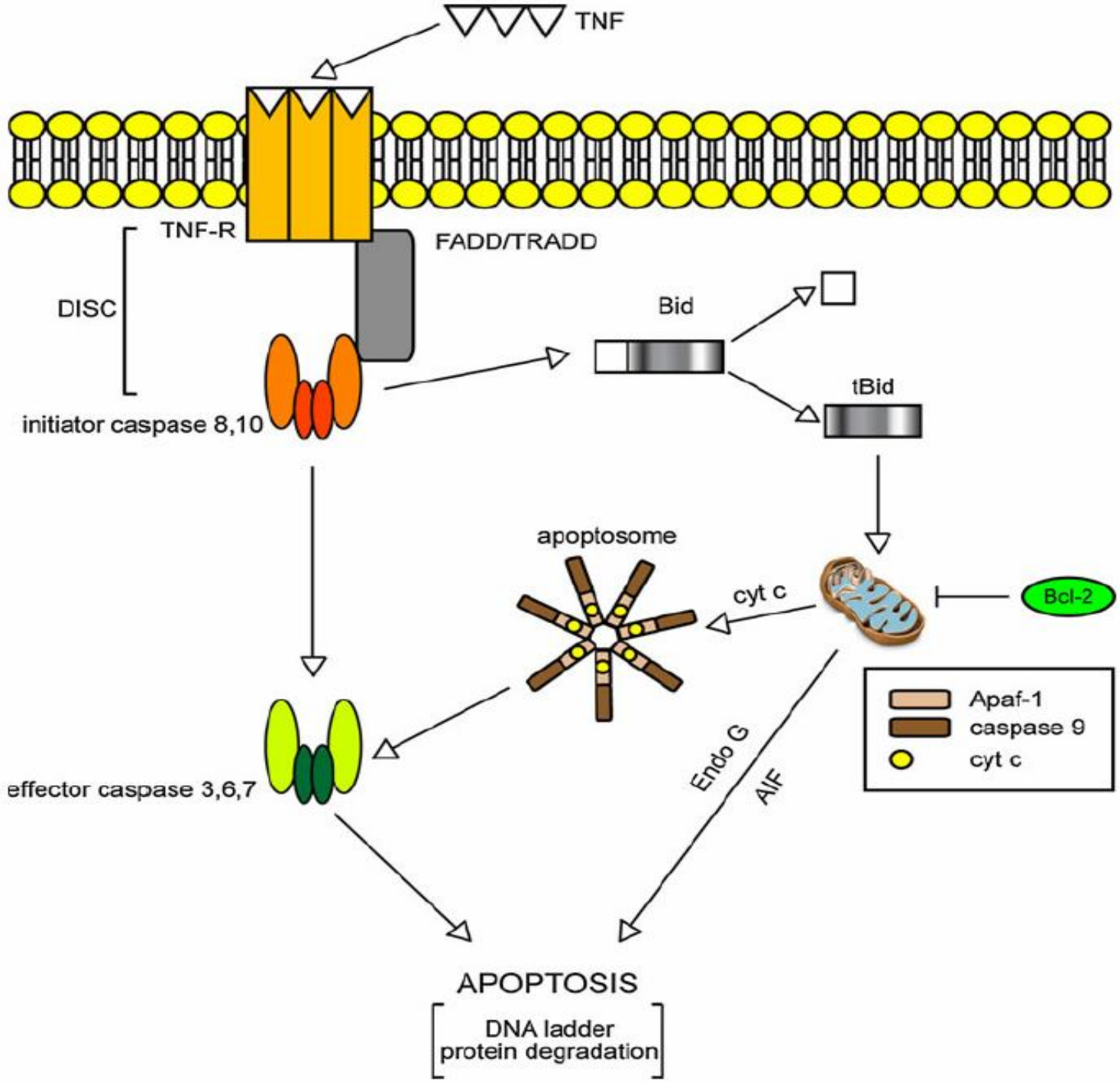
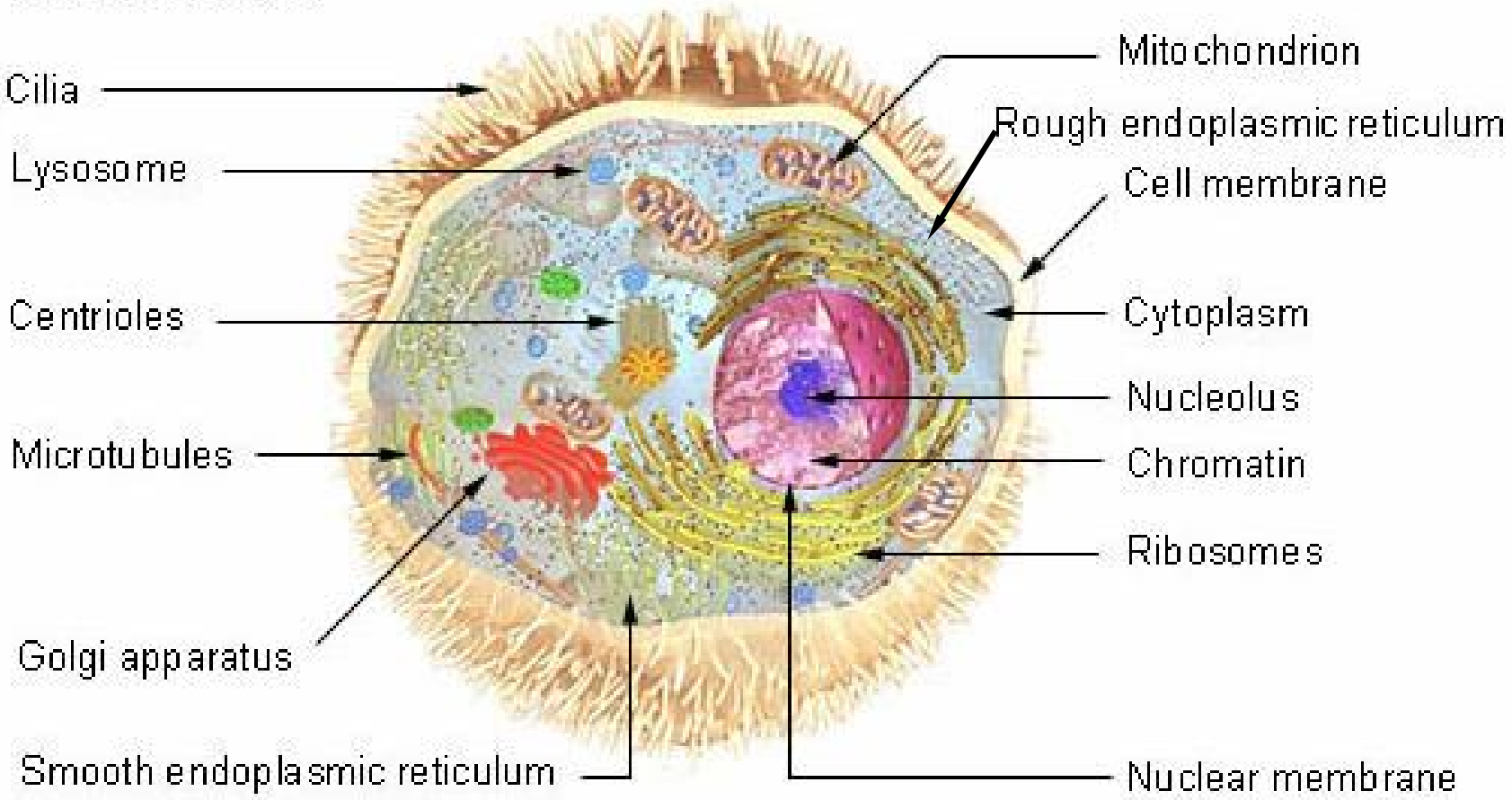
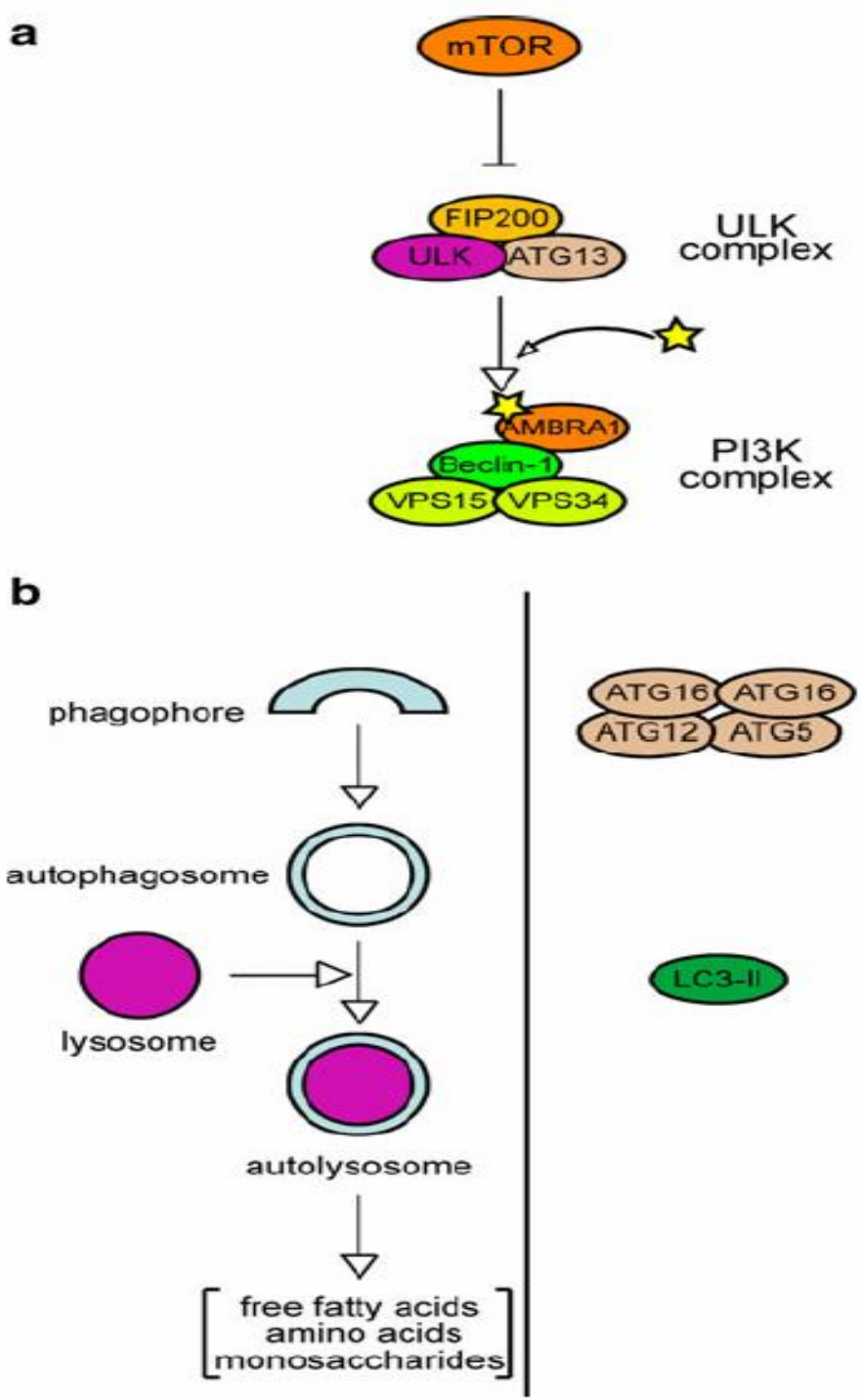


Figure 2 Cytotoxic T lymphocytes (CTLs) can kill target cells by the CD95 (yellow)/CD95L (red) system (left) or by the perforin/granzyme B (GrB) system (right). CD95 signalling involves a caspase cascade (see text). The entry of GrB into target cells and its release involve perforin. GrB cleaves and activates caspases in the target cell. The target cell dies by apoptosis through either a CD95 or a GrB signalling event.


TNF
TNF-R
FADD/TRADD
DISC
initiator caspase 8,10
Bid
tBid
apoptosome
cyt c
Bcl-2
Apaf-1
caspase 9
cyt c
effector caspase 3,6,7
Endo G
AIF
APOPTOSIS
DNA ladder
protein degradation

Cell Structure




a
mTOR
FIP200
ULK
ATG13
ULK complex
AMBRA1
Beclin-1
VPS15
VPS34
PI3K complex
b
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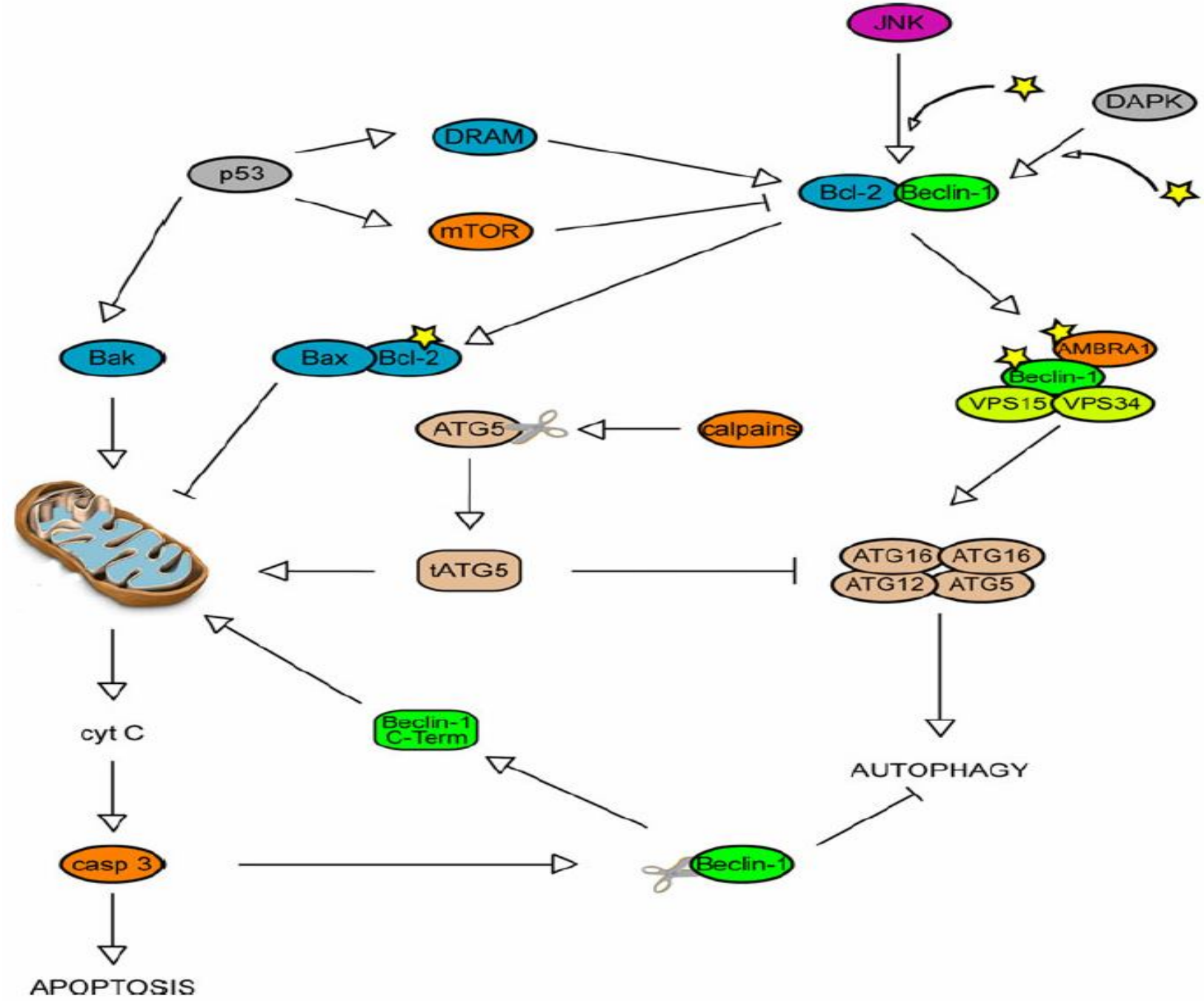
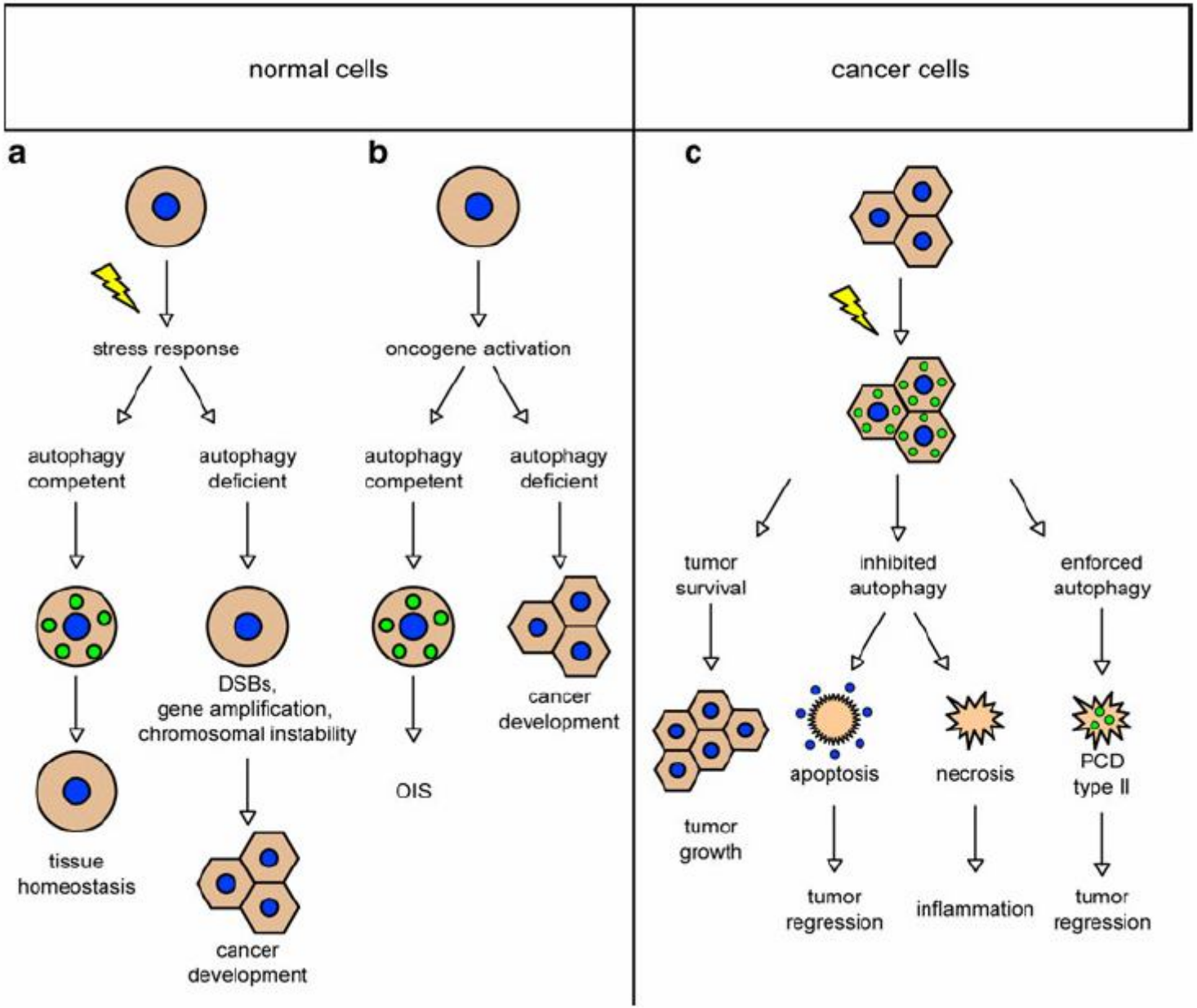
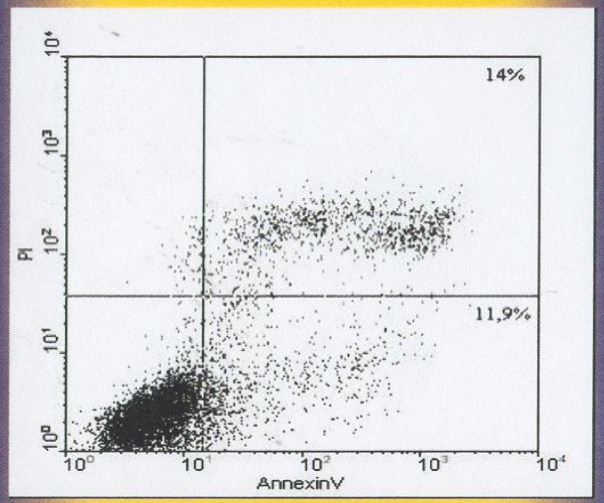
ATG16
ATG16
ATG12
ATG5
LC3-II


JNK
DAPK
DRAM
p53
Bcl-2
Beclin-1
mTOR
Bak
Bax
Bcl-2
AMBRA1
Beclin-1
VPS15
VPS34
ATG5
calpains
tATG5
ATG16
ATG16
ATG12
ATG5
cyt C
Beclin-1
C-Term
AUTOPHAGY
casp 3
Beclin-1
APOPTOSIS

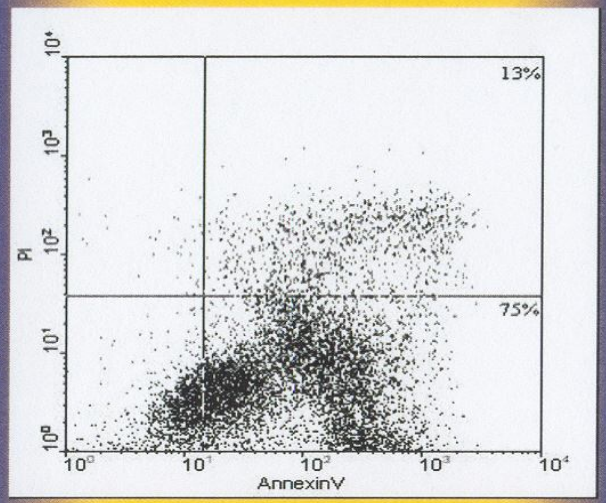

normal cells
cancer cells
a
b
c
stress response
oncogene activation
autophagy competent
autophagy deficient
autophagy competent
autophagy deficient
tissue homeostasis
DSBs, gene amplification, chromosomal instability
OIS
cancer development
cancer development
tumor survival
inhibited autophagy
enforced autophagy
tumor growth
apoptosis
necrosis
PCD type II
tumor regression
inflammation
tumor regression

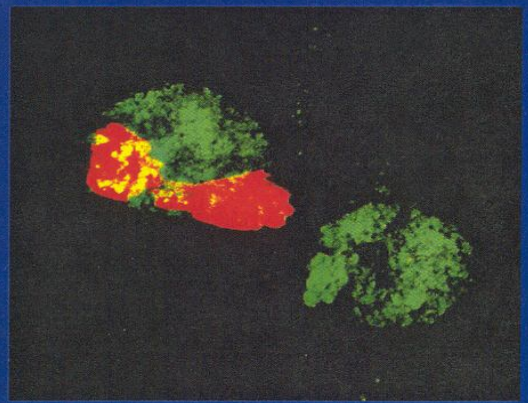
Células Apoptóticas

No irradiadas

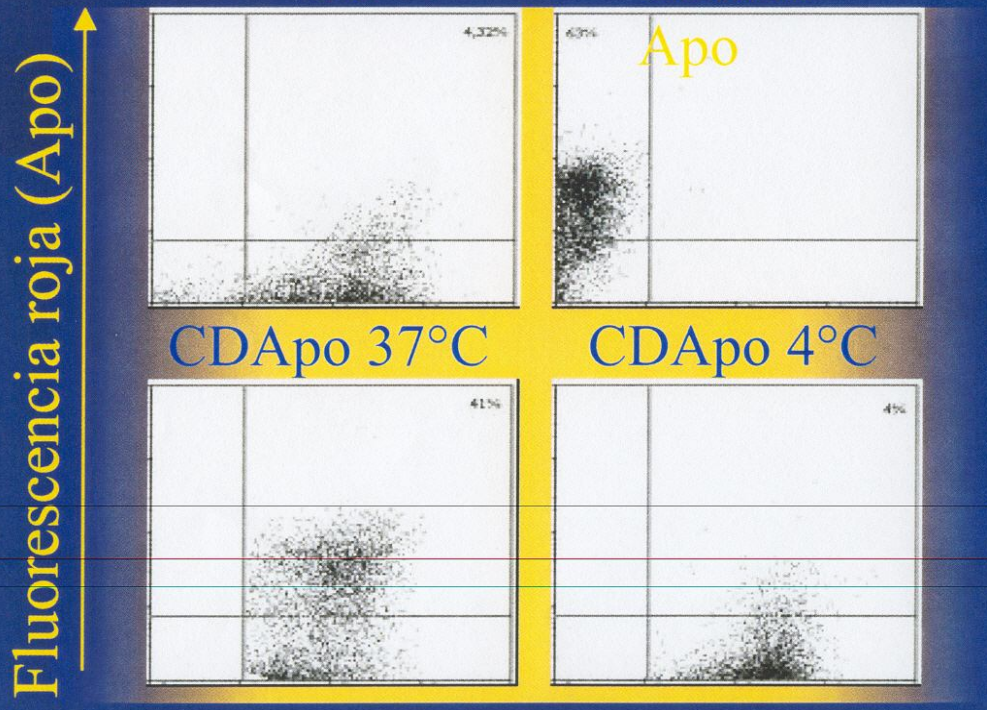


Irradiadas





Cocultivo de células dendríticas y apoptóticas



CDApo