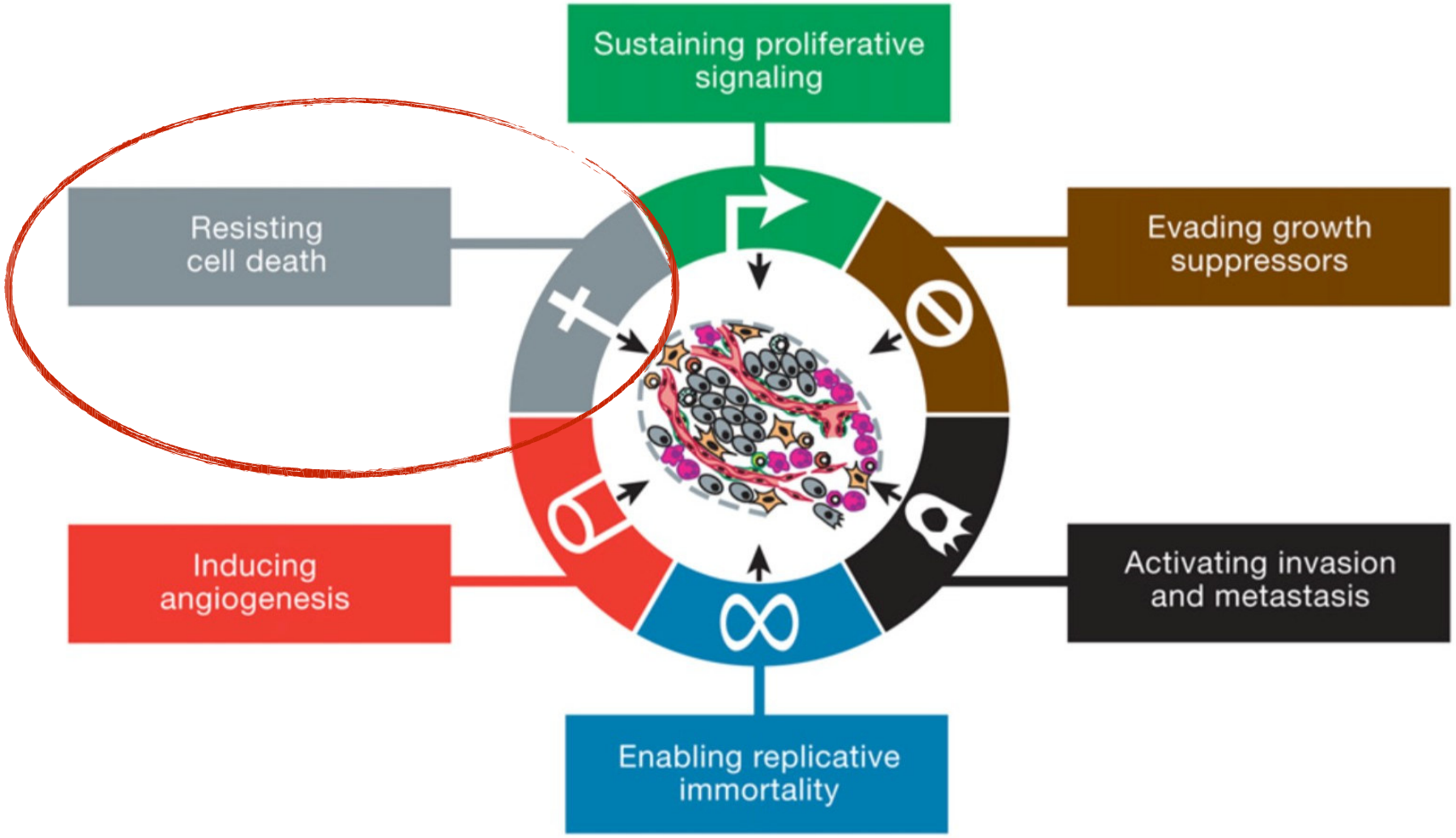
CELL DEATH

learning objectives

- key characteristiques of cell death mechanisms
- extrinsic and intrinsic pathways of cell death and their interactions
- necroptosis: signaling pathways and key proteins
- Immunogenic cell death
- necroptosis and inflammation: opportunity for anti-cancer treatment?

Resistance to Cell Death is one of the Hallmarks of Cancer



Cell *144*, March 4, 2011

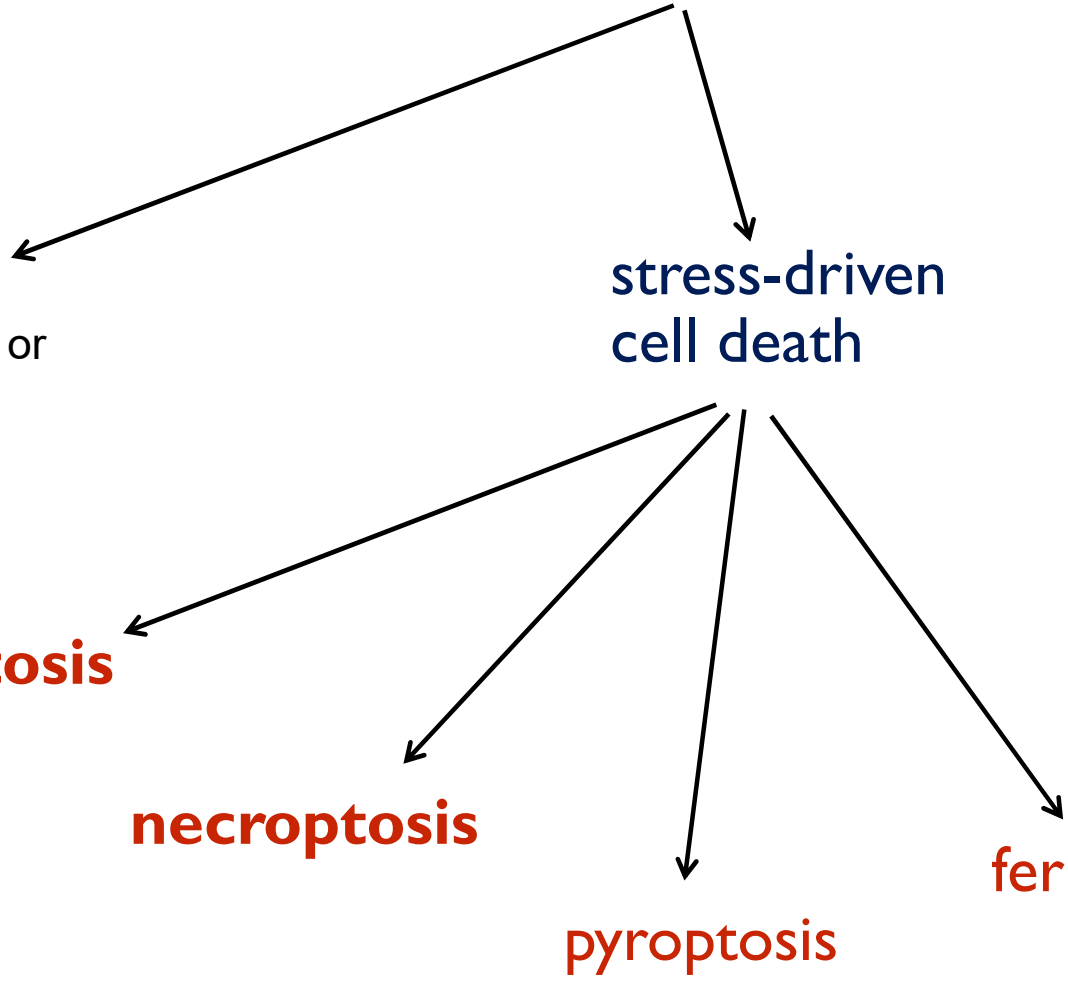
Classifications of Cell Death

accidental cell death

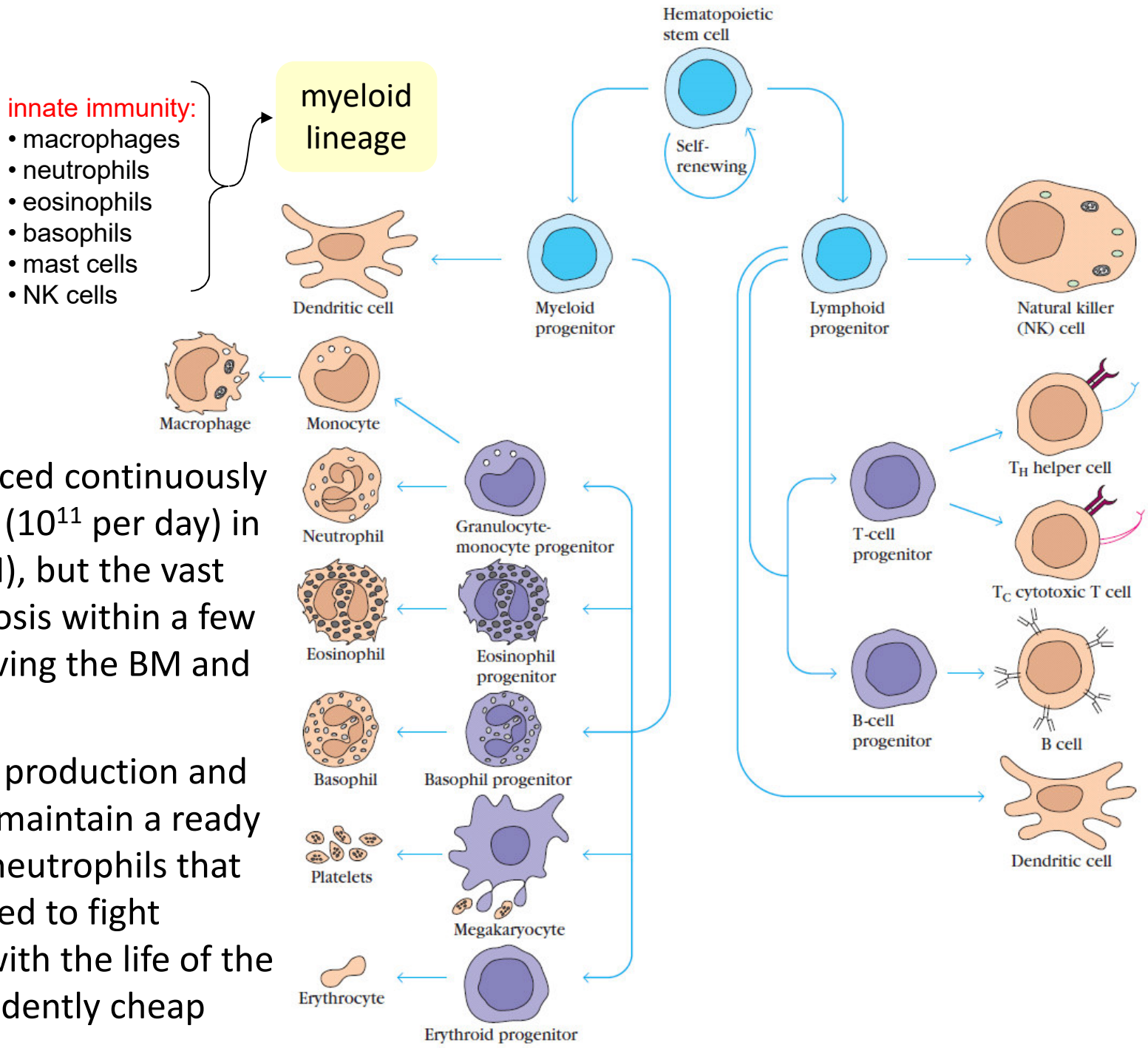
caused by severe physical, chemical or mechanical insults

regulated cell death **RCD**

relies on a molecular machinery

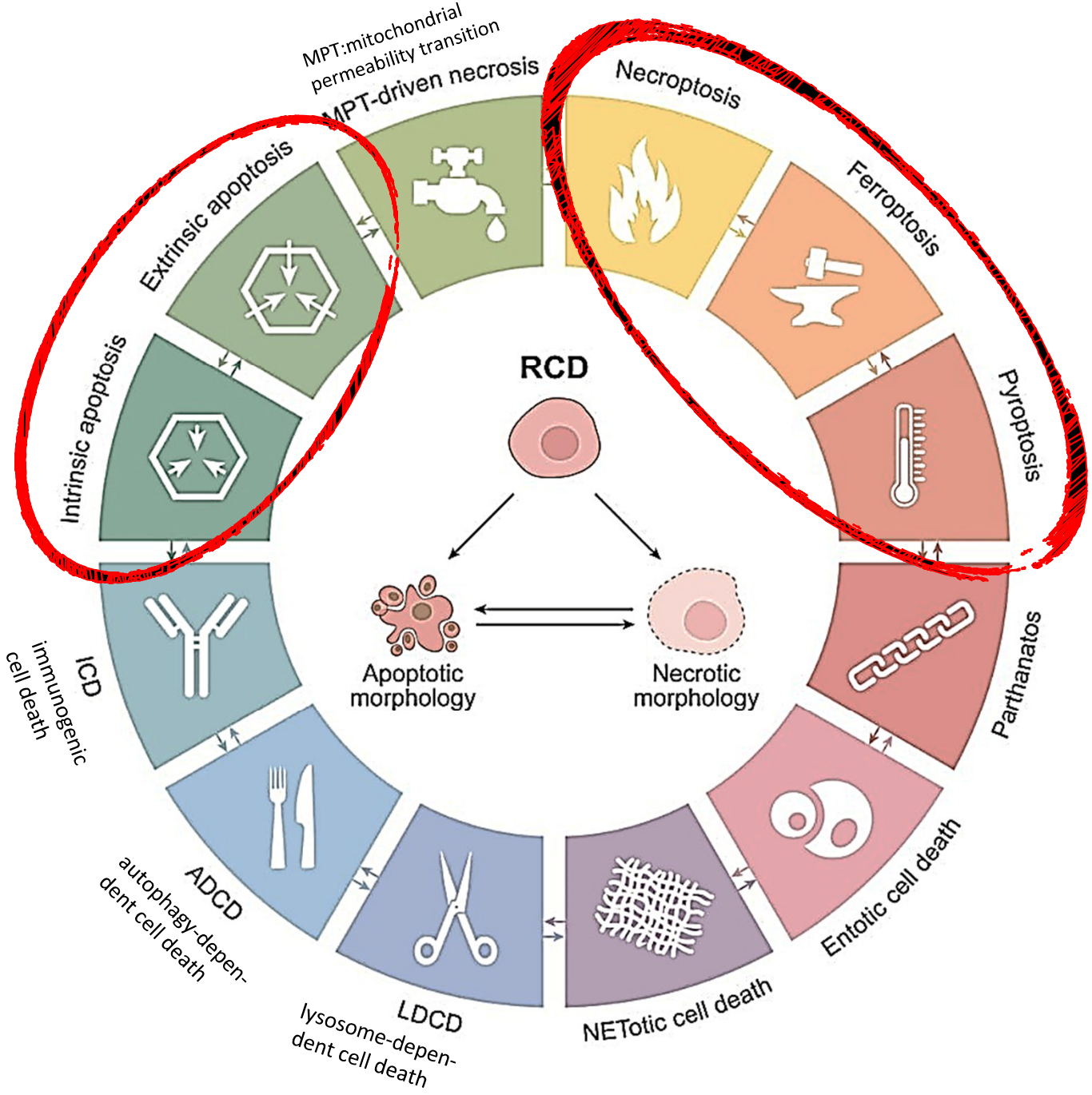


Hematopoiesis: homeostasis by massive apoptosis



- neutrophils are produced continuously in very large numbers (10^{11} per day) in the bone marrow (BM), but the vast majority die by apoptosis within a few days without ever leaving the BM and functioning
- this excessive cycle of production and destruction serves to maintain a ready supply of short-lived neutrophils that can be rapidly mobilized to fight infection; compared with the life of the organism, cells are evidently cheap

All currently known forms of Cell Death (CD)

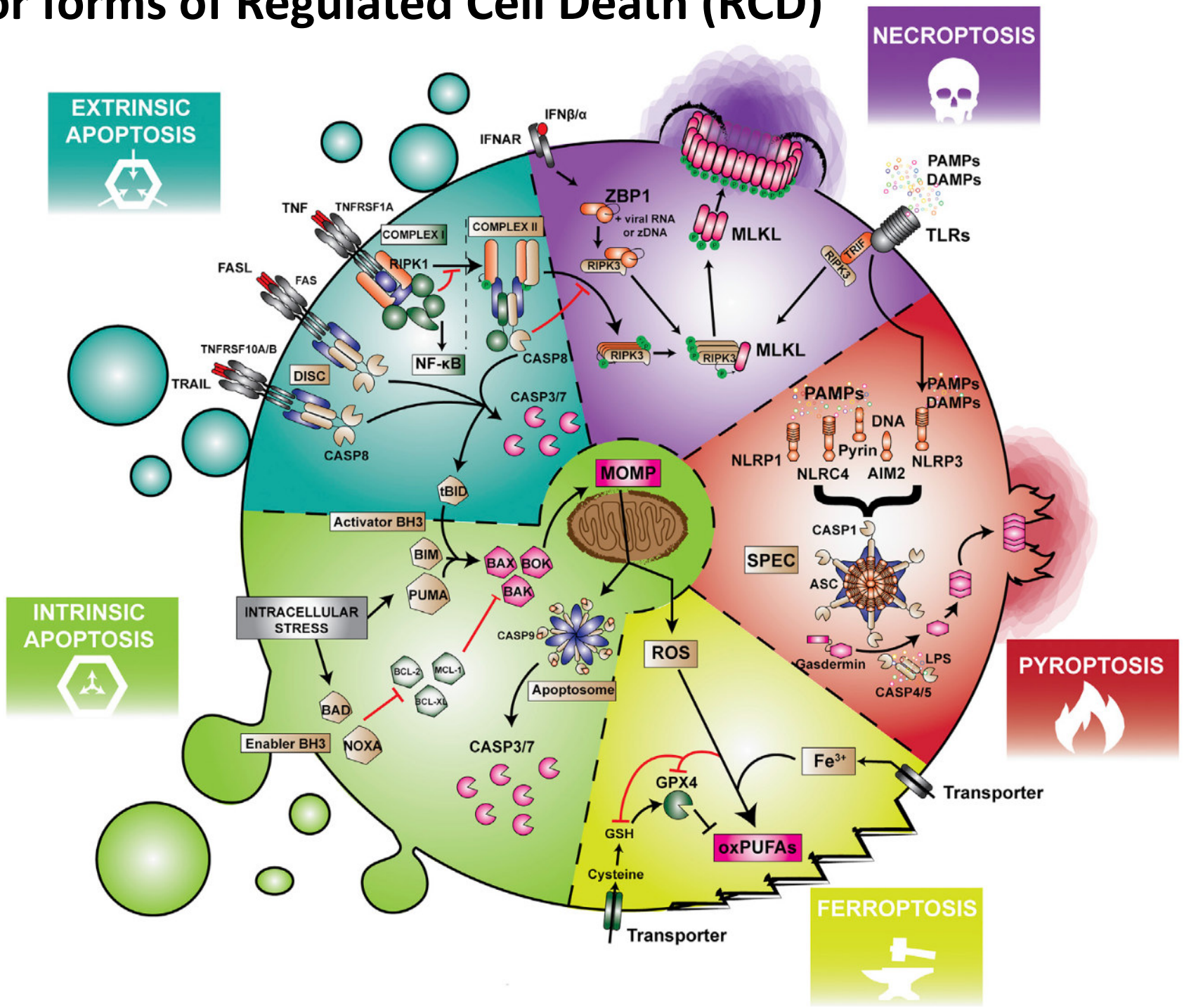


Each RCD mode is initiated and propagated by molecular mechanisms that exhibit a considerable degree of inter-connectivity.

Each type of RCD can manifest with an entire spectrum of morphological features ranging from fully necrotic to fully apoptotic.

Each type of RCD can exhibit an immunomodulatory profile ranging from anti-inflamatory and tolerogenic to pro-inflammatory and immunogenic.

Major forms of Regulated Cell Death (RCD)



Apoptosis

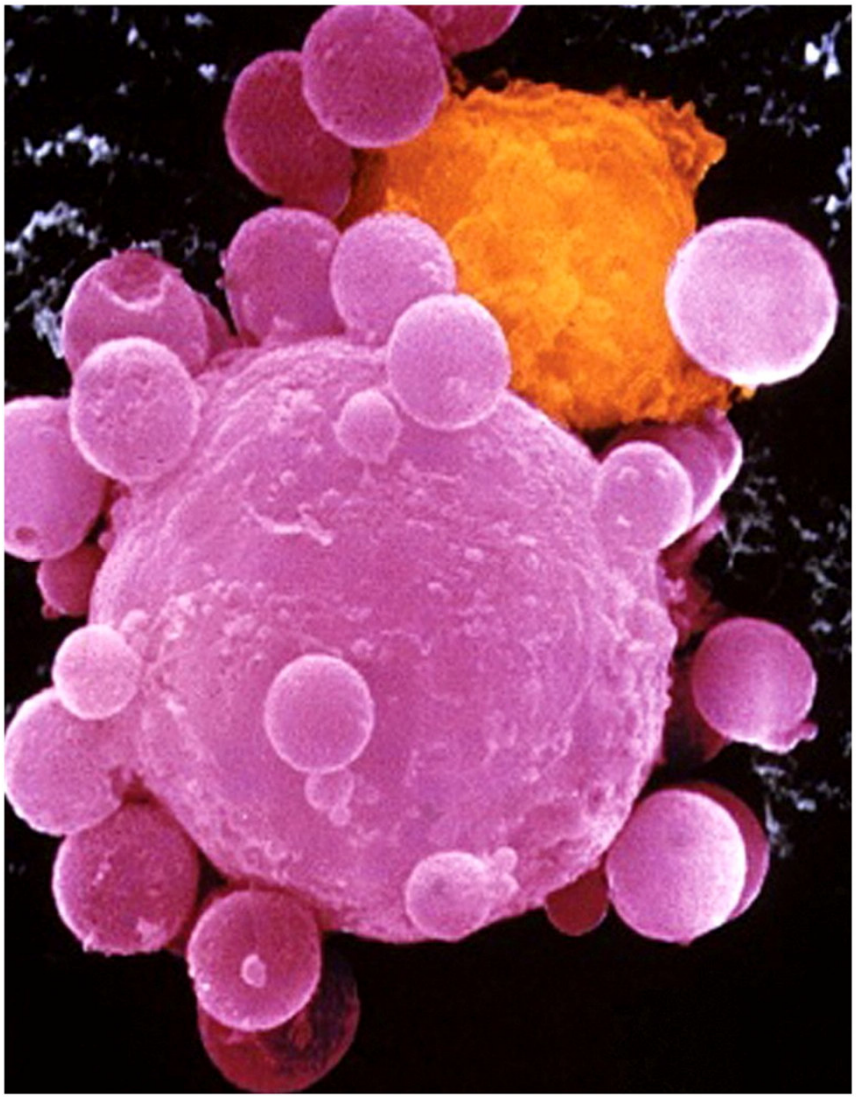
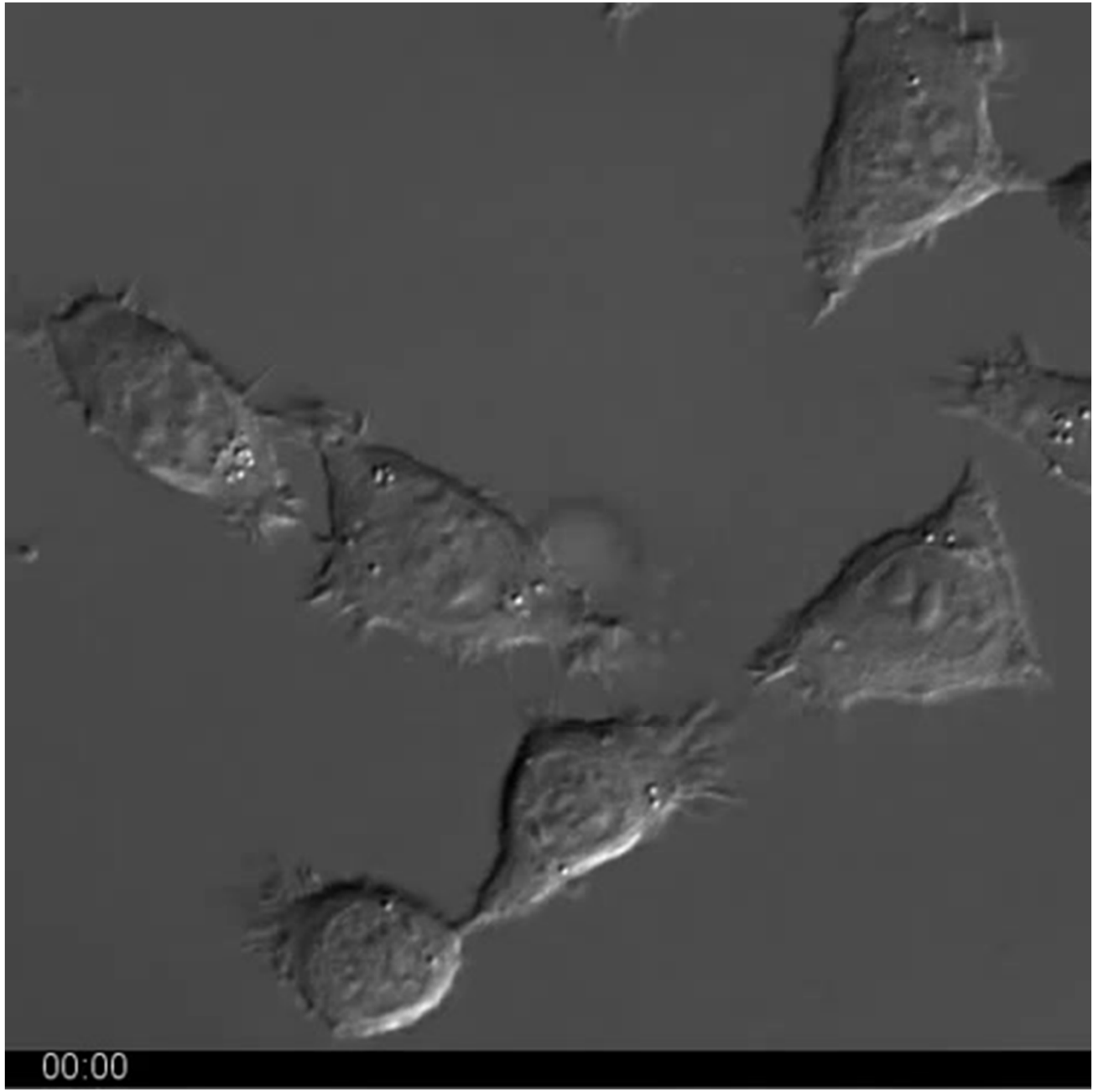
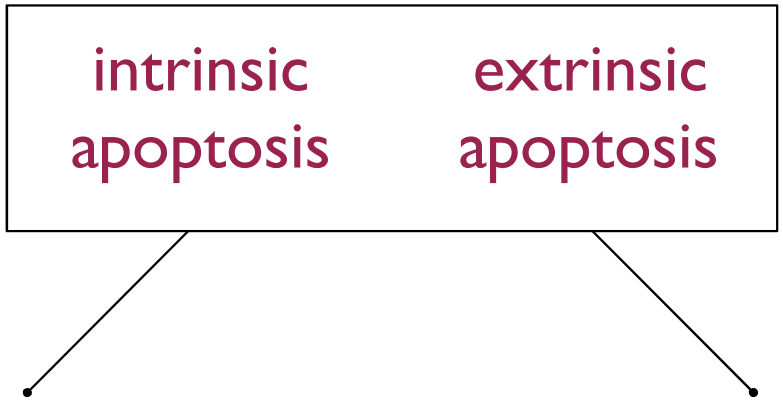


Figure 9-31c The Biology of Cancer (© Garland Science 2007)

cytotoxic T-lymphocyte
killing a tumor cell



Ways to die by Apoptosis

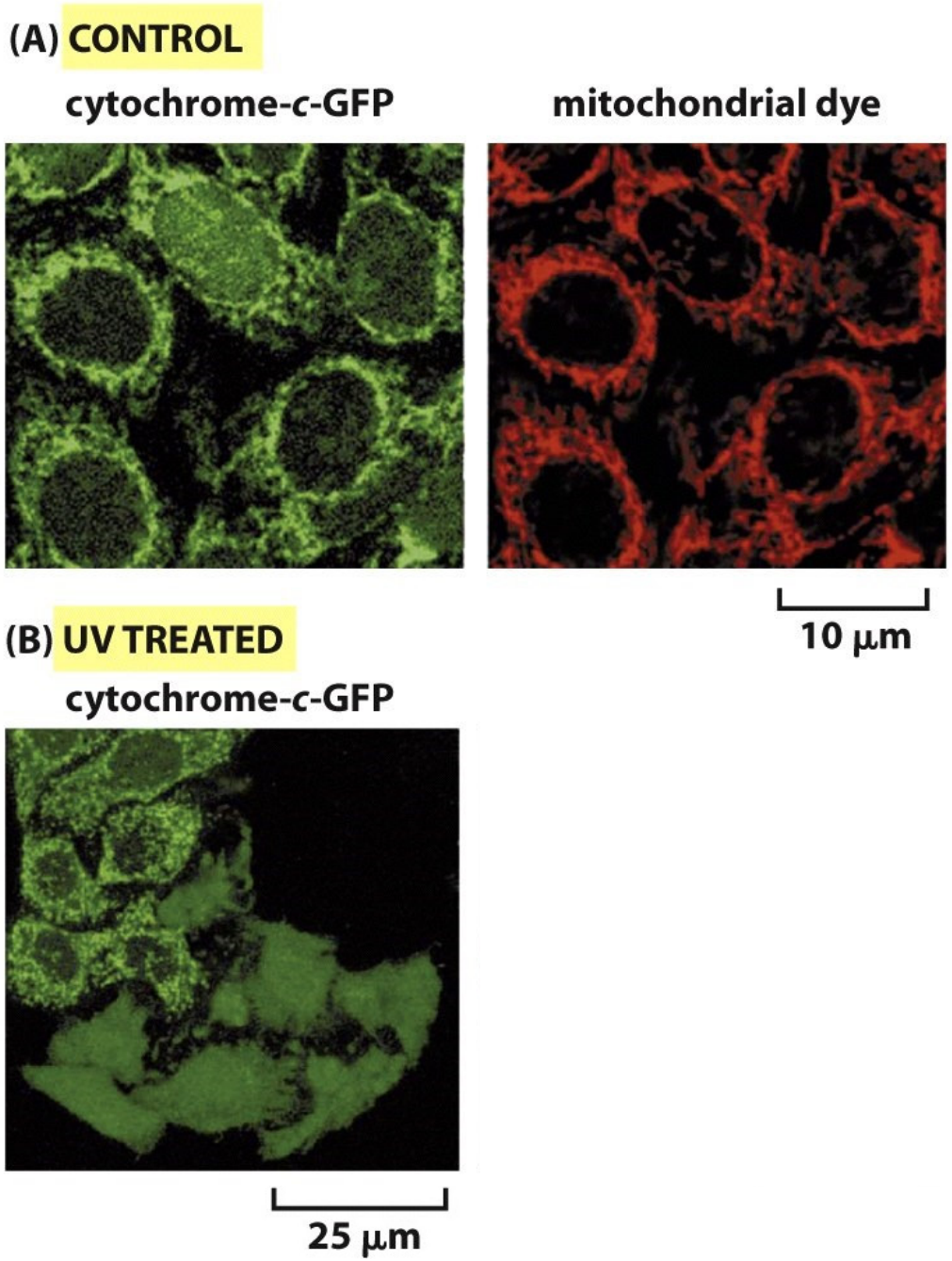


Intrinsic apoptosis: type of RCD initiated by perturbations of the extracellular or intracellular microenvironment, the crucial event is mitochondrial outer membrane permeabilization (MOMP) followed by activation of initiator caspases (CASP9) and executioner caspases, mainly CASP3.

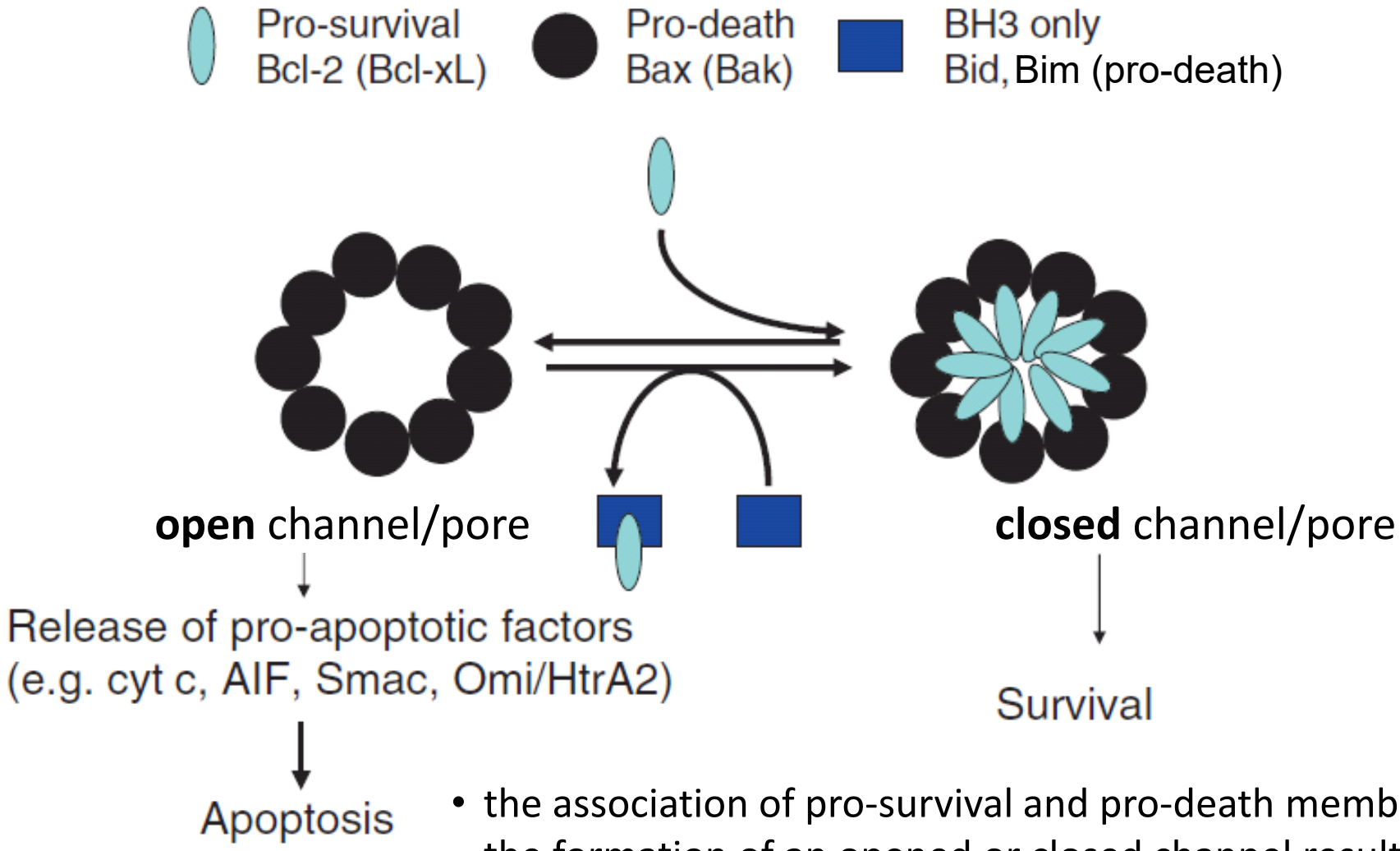
Extrinsic apoptosis: specific variant of RCD initiated by plasma membrane receptors, propagated by CASP8 and set in force by executioner caspases, mainly CASP3.

Cell Death & Differentiation (2018) 25:486–541 https://doi.org/10.1038/s41418-017-0012-4

The intrinsic pathway of apoptosis depends on mitochondria permeabilization

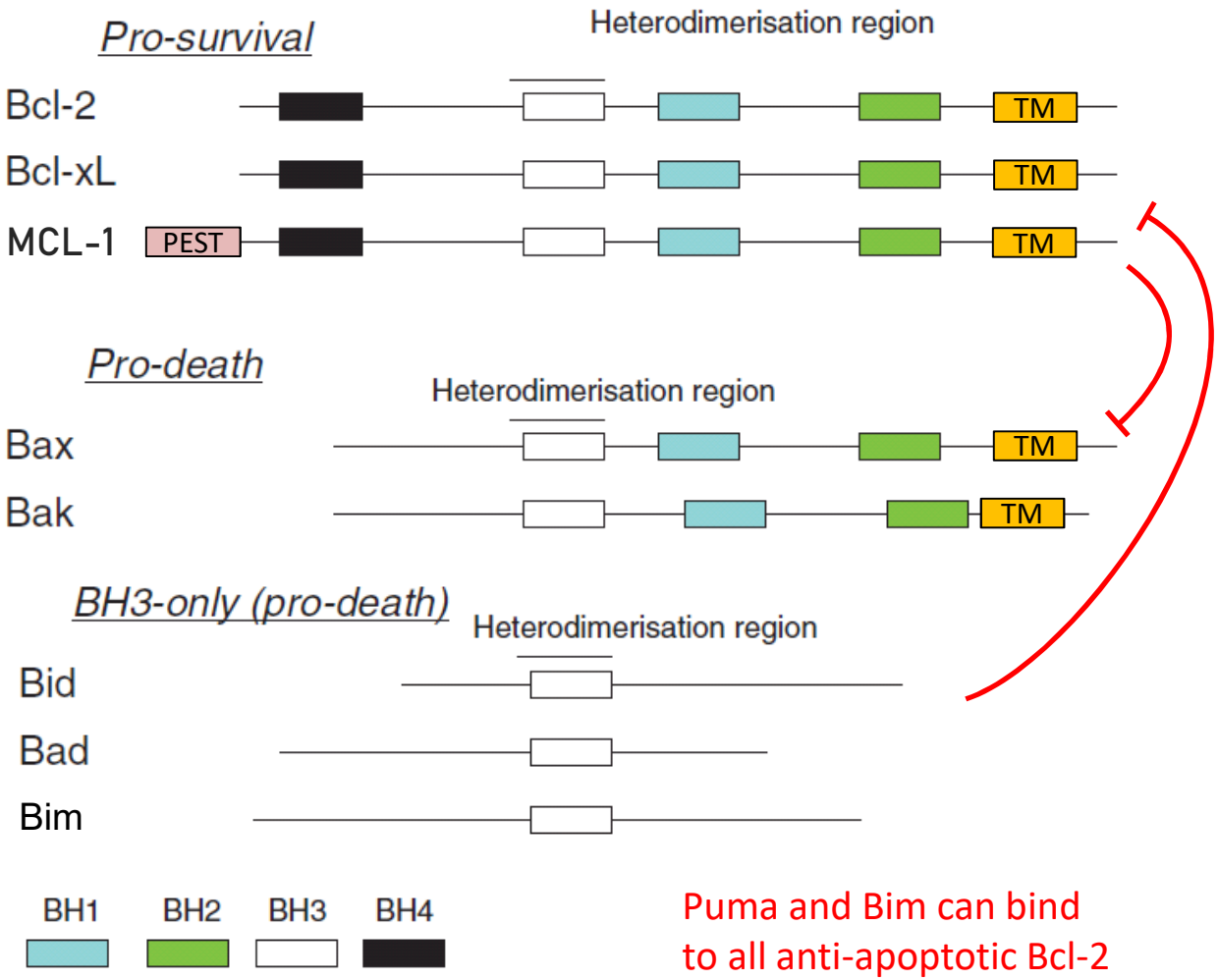


Model for regulation of mitochondrial permeability by proteins of the Bcl-2 family



- the association of pro-survival and pro-death members will result in the formation of an opened or closed channel resulting in either retention or release of pro-apoptotic factors from mitochondria
- BH3-only proteins, such as Bid or Bim, play a critical role in tipping the balance in favor of cell death

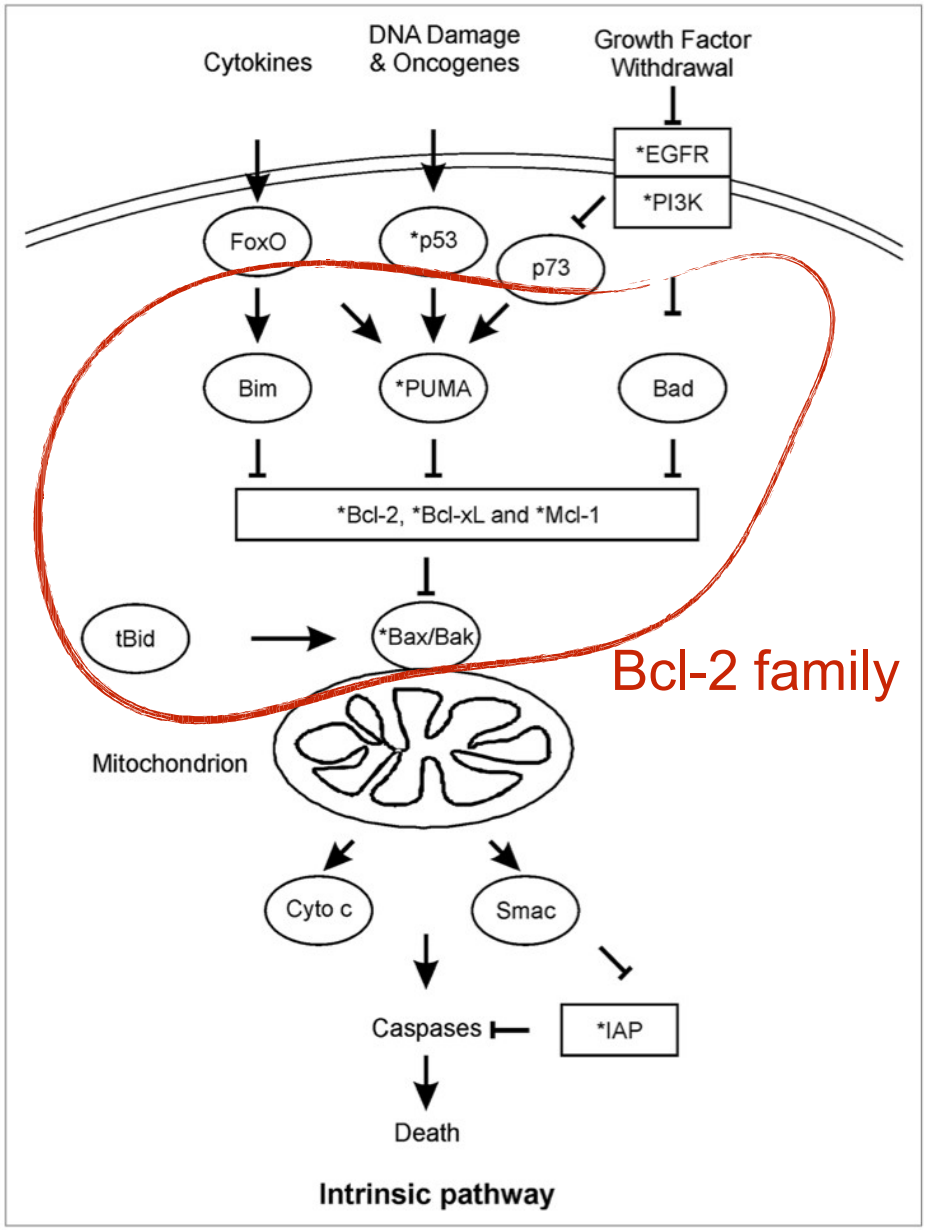
The BCL2 family



Puma and Bim can bind to all anti-apoptotic Bcl-2 members;
Bad interacts only with Bcl-2, Bcl-X_L, Bcl-w

- critical regulators of cell death induced by distinct apoptotic triggers
- contain a variable number of conserved domains termed Bcl-2 homology domains (BH domain) supporting homodimeric and heterodimeric interactions
- the BH3 only family acts pro-apoptotic by inhibiting the anti-apoptotic Bcl2-like family
- oligomerization of pro-apoptotic Bax and Bak allows to form a pore in the outer mitochondrial membrane
- both Bax and Bak also operate on the surface of the endoplasmic reticulum (ER); when activated in response to ER stress, they can release $Ca2^+$ into the cytosol, which helps activate the mitochondrial-dependent intrinsic pathway

The intrinsic pathway is often deregulated in tumors



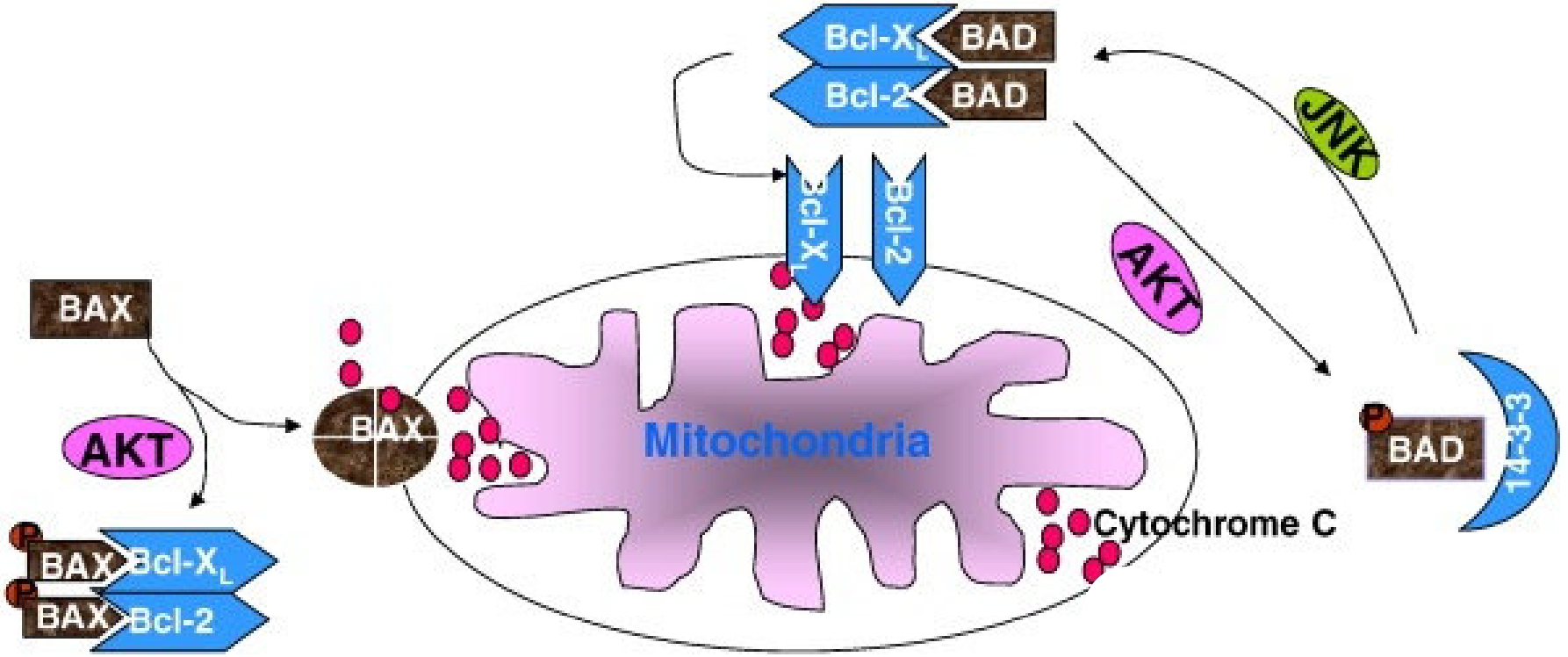
- p53 mutation/inactivation (>50%)
- BAX deletions or mutations (colorectal cancer)
- PUMA downregulation in melanomas and lymphomas
- phosphorylation and cytoplasmic sequestration of BAD by the PI3K pathway/Akt
- overexpression of anti-apoptotic Bcl-2 members
- transclocation (Bcl-2 in B-cell lymphomas)

<u>the consequences are:</u>

1- inhibition of normal apoptosis

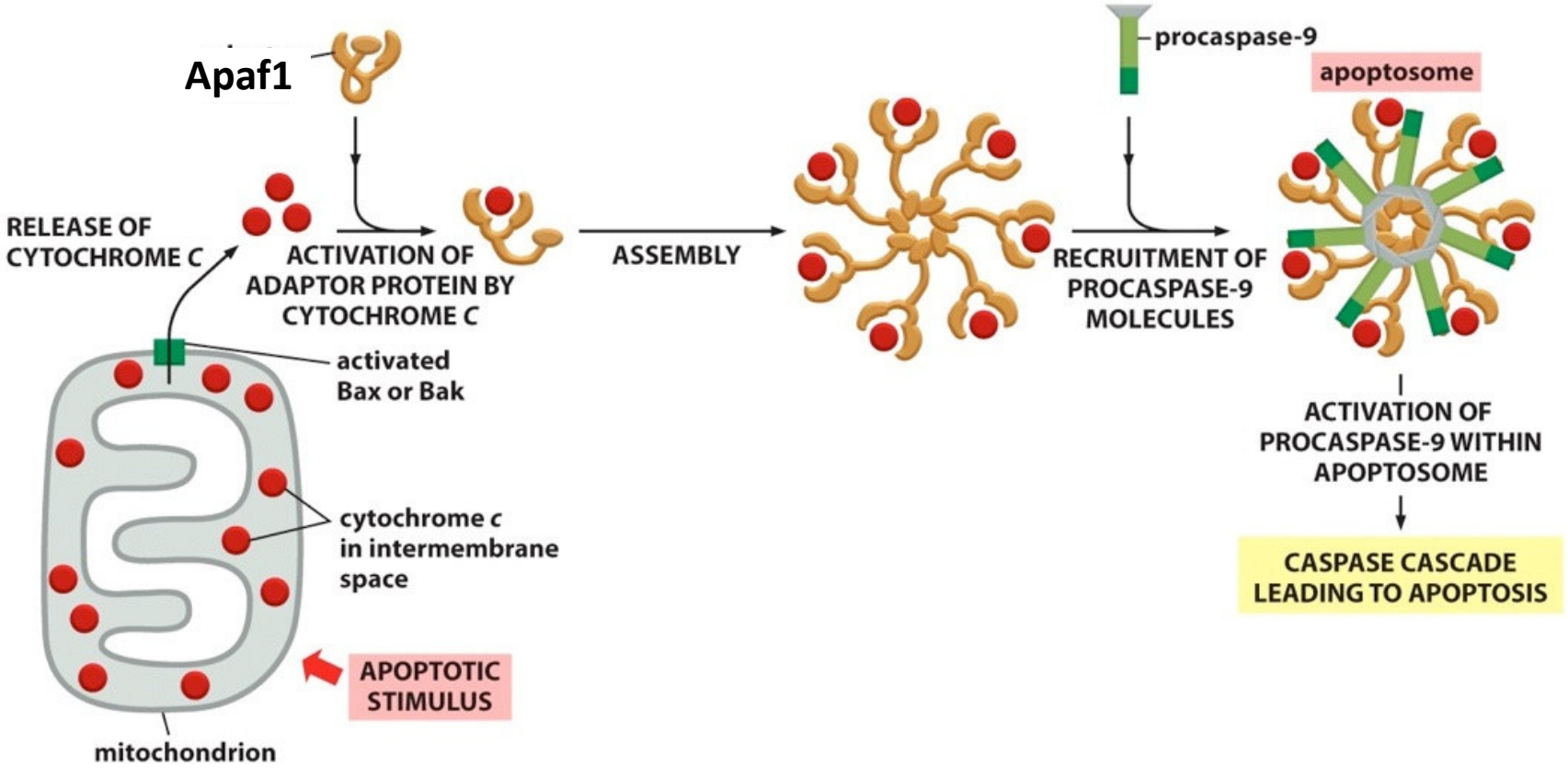
2. chemotherapy and radiotherapy resistance

Regulation of Bad/Bax by AKT



- strong pro-survival signals are mediated via AKT activation
- both Bad and Bax are inhibited as a result of phosphorylation by AKT
- phosphorylation of Bax by AKT blocks its translocation to the mitochondrial membrane where it would form pores for the release of cytochrome *c*
- when phosphorylated, Bad releases Bcl2/Bcl-X_L and becomes associated with 14-3-3, so that Bcl2/Bcl-X_L can block the release of cytochrome *c* and prevent cell death
- the stress-induced kinase Jun Kinase (JNK) blocks this effect of AKT by phosphorylating Bax on a different residue

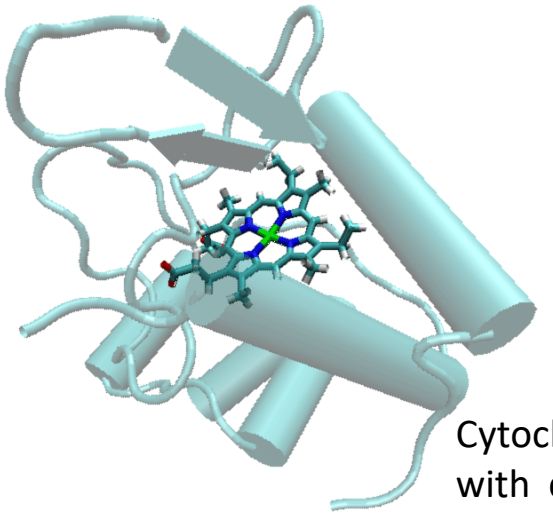
Cytochrome C mediated apoptosis induction



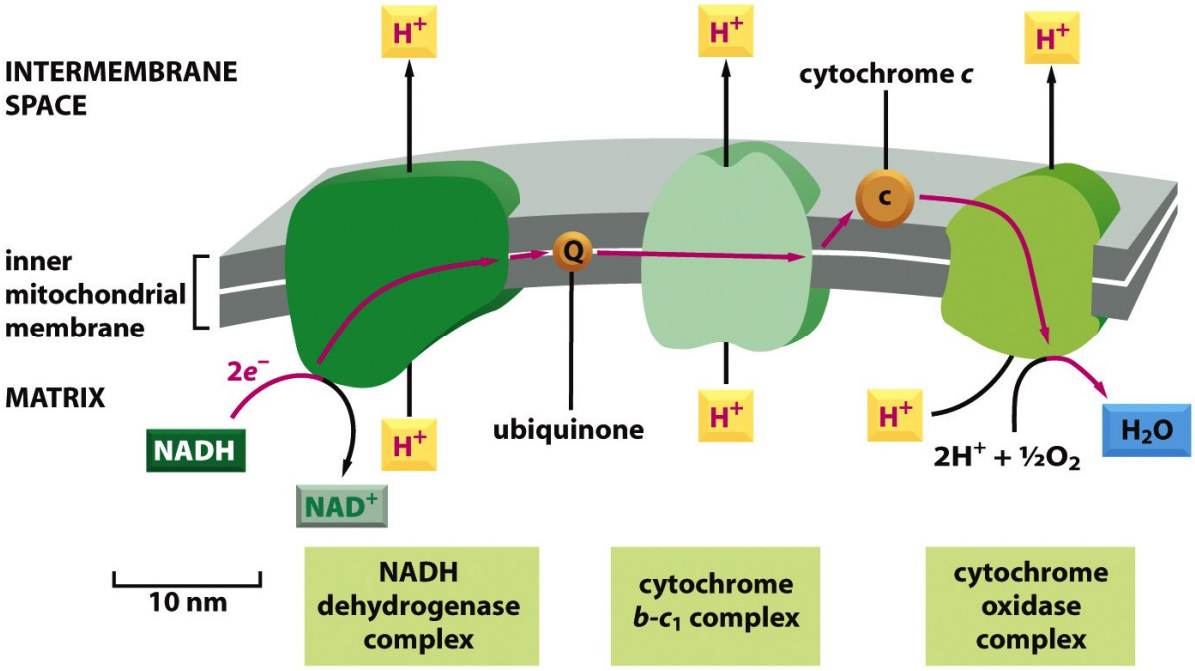
- cytochrome C, when released into the cytosol, binds together with dATP to a procaspase-activating adaptor protein called **Apaf1** (apoptotic protease activating factor-1)
- this causes Apaf1 to oligomerize into a wheel-like heptamer called an **apoptosome**
- Apaf1 proteins in the apoptosome then recruit **initiator procaspase-9**, which are activated by proximity to the other caspases in the apoptosome and its co-factor Apaf1
- activated caspase-9 then cleaves down stream executioner procaspases (3,7) to induce apoptosis

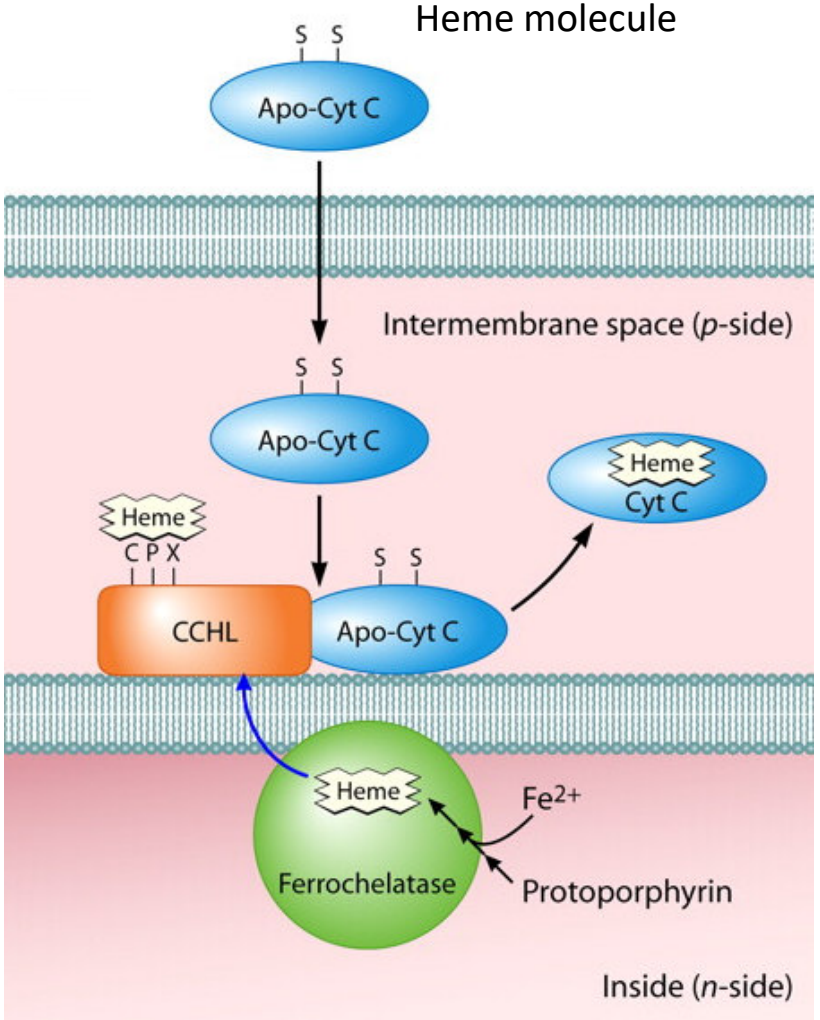
Normal role of Cytochrome C

- cytochrome C is the only soluble protein in the electron transport chain of mitochondria
- its release into the cytoplasm is detected as a major injury to the cell and triggers apoptosis
- to prevent apoptosis induction by the protein form synthetized in the cytoplasm (its a nuclear gene), only the processed form generated in mitochondria can trigger apoptosis

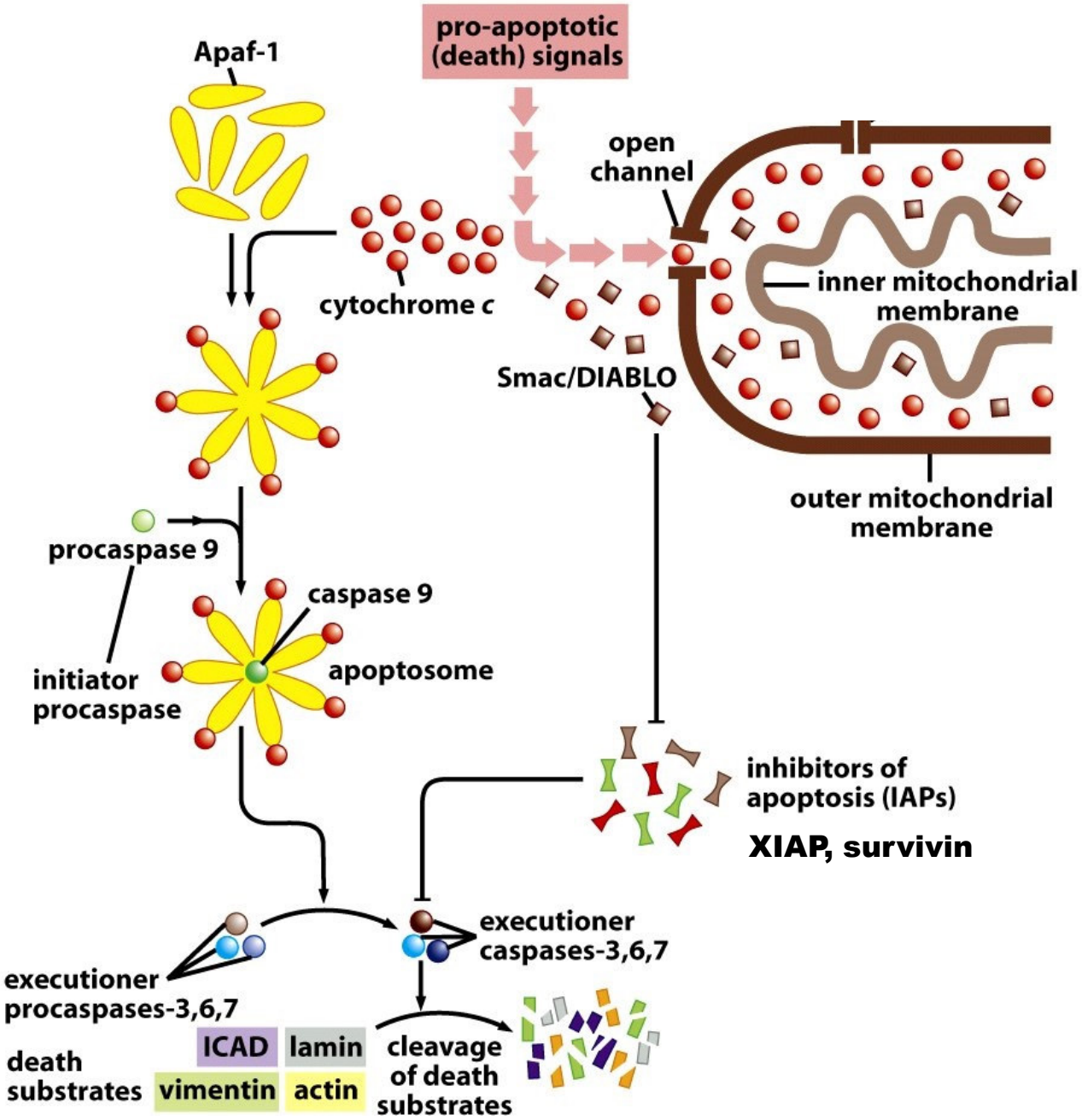


Cytochrome C (104aa) with central Heme molecule



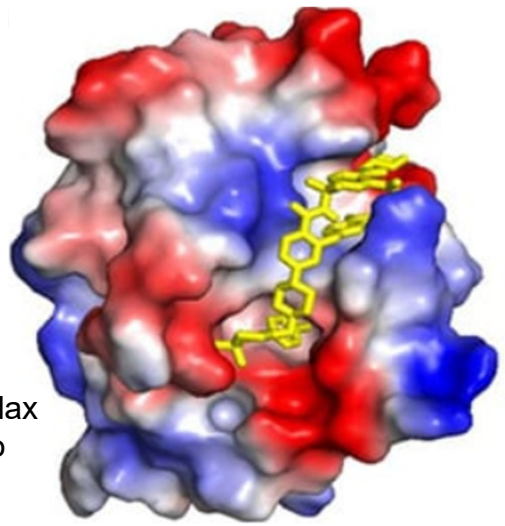


Amplification of initial signals ensures efficient execution of apoptosis: => feed-forward control

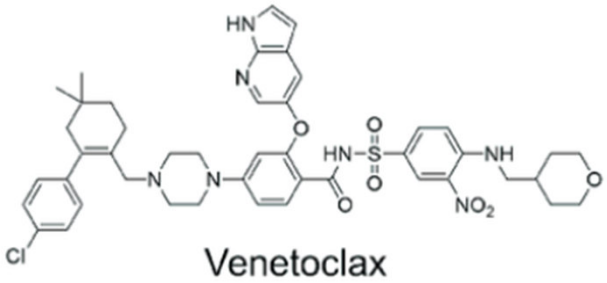


- in addition to cytochrome c, anti-IAPs such as Smac (Second Mitochondria-derived Activator of Caspases; also called DIABLO) are released from mitochondria
- Smac blocks IAPs (Inhibitors of Apoptosis Proteins) in the cytosol thereby promoting apoptosis
- IAPs such as XIAP normally operate to block caspase action in two ways:
  - direct binding to inhibit caspase proteolytic activity
  - marking caspases for ubiquitylation and degradation
- without the continued influence of IAPs, caspases are free to initiate the proteolytic cleavages that result ultimately in apoptosis

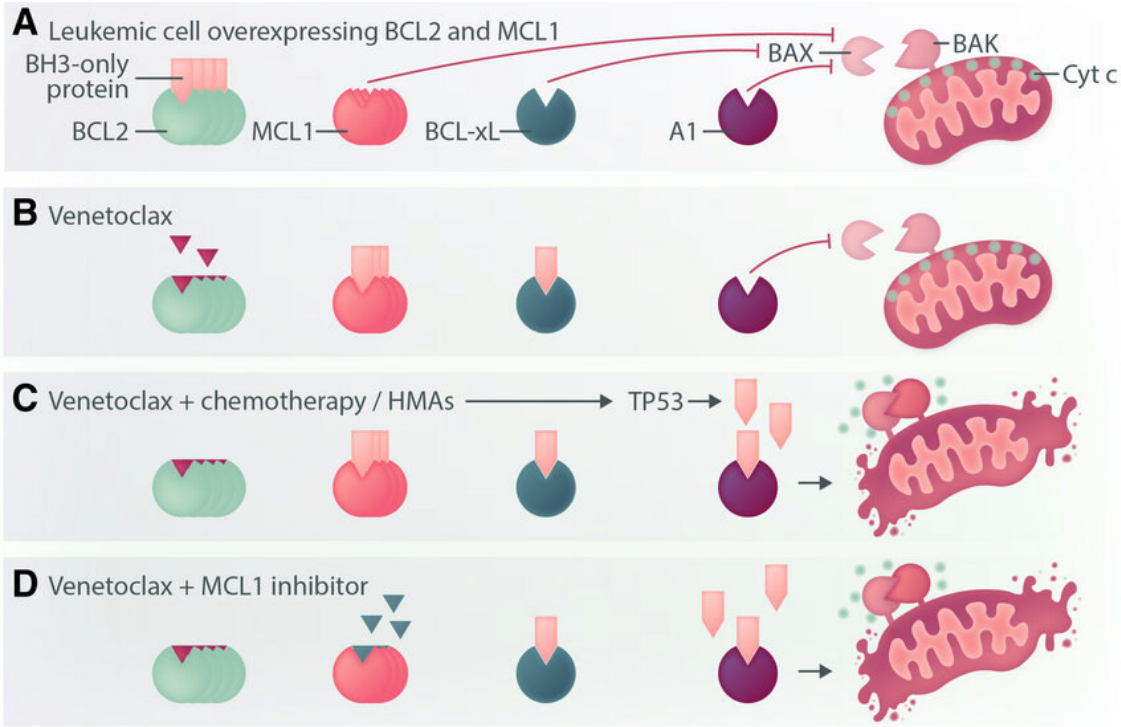
BH3 mimetics are drugs used to trigger tumor cell apoptosis



Venetoclax bound to Bcl-2



Venetoclax

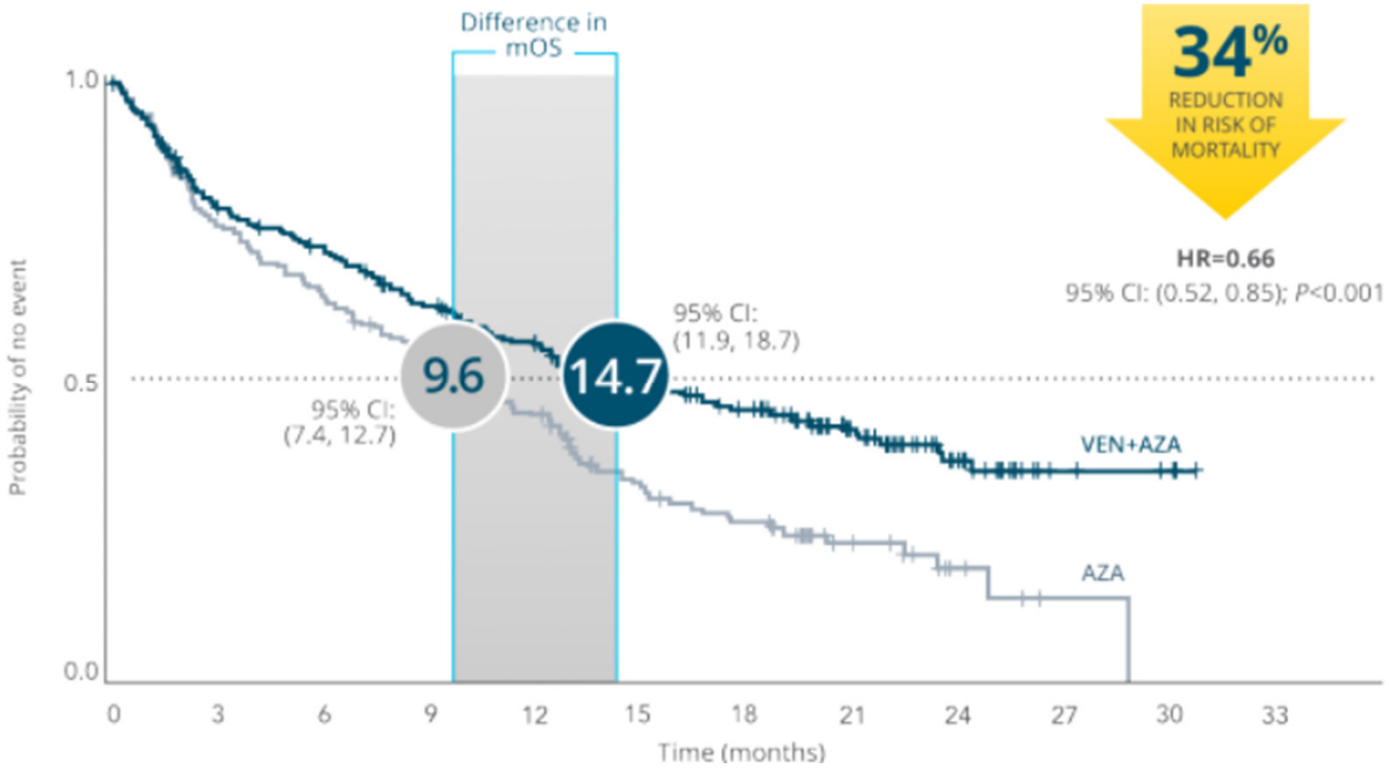


- Oncogene-driven increases in BH3-only protein burden are neutralized by an increased level of BCL2 family prosurvival proteins keeping BAX and BAK inactive
- Venetoclax blocks the capacity for BCL2 to neutralize endogenous BH3-only proteins
- Chemotherapy may further increase the total BH3-only protein and in combination with venetoclax can overwhelm the capacity of prosurvival proteins
- Combining venetolax with MCL1 inhibitors represents another approach to induce apoptosis in leukemic cells

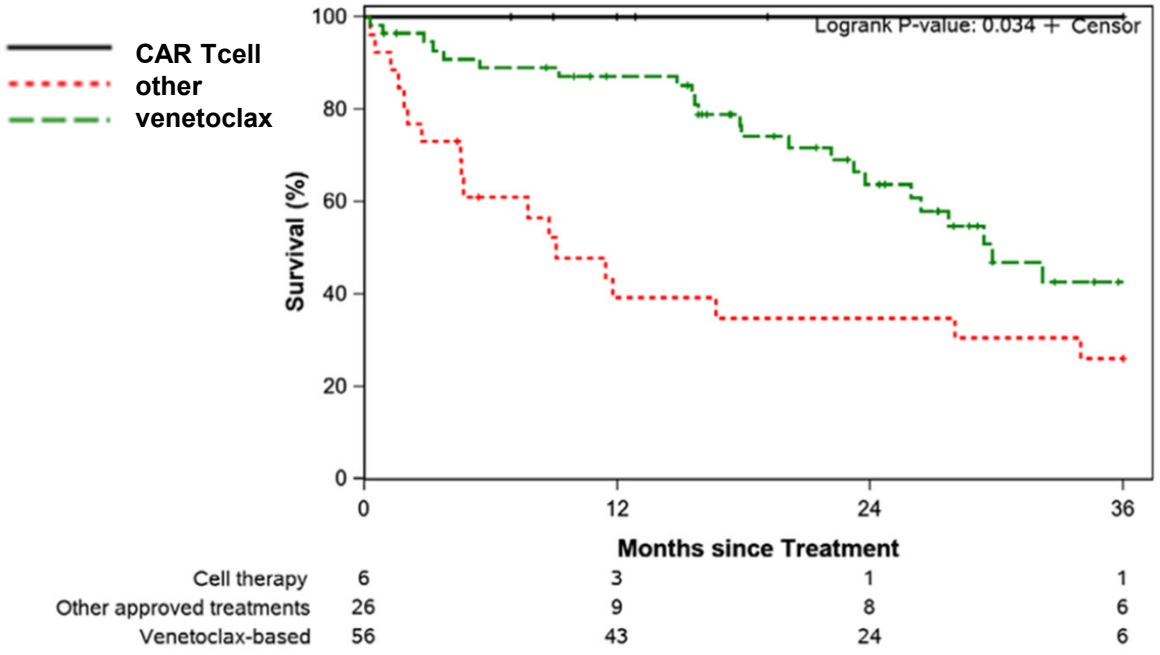
FDA approved drugs:

Venetoclax:	BCL-2 inhibitor, used to treat adults with chronic lymphocytic leukemia, small lymphocytic lymphoma, or acute myeloid leukemia.
PRT1419:	MCL-1 inhibitor, used to treat sarcoma, melanoma, lung and breast cancer
Xevinapant:	targeting cIAP1, cIAP2 and XIAP for degradation, used to treat head and neck cancer
Tolinapant:	cIAP1 and XIAP inhibitor, used to treat T cell lymphomas

Venetoclax for blood cancers

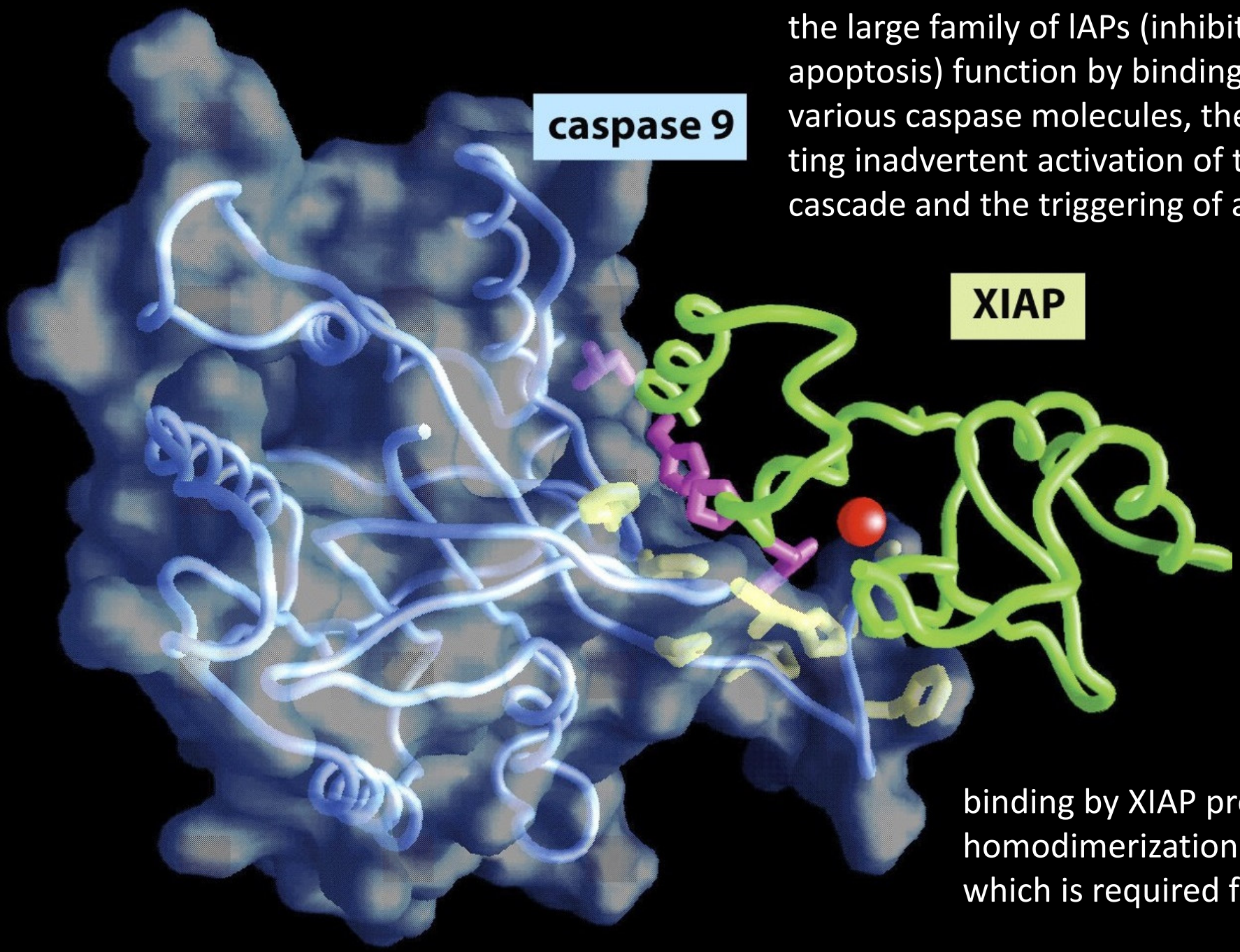


A randomized (2:1), double-blind, placebo-controlled, multicenter, phase 3 study that evaluated the efficacy and safety of venetoclax with azacitidine (VEN+AZA; N=286) vs placebo with azacitidine (PBO+AZA; N=145) in adults with newly diagnosed **AML** who were ≥75 years of age, or had comorbidities.



Overall survival between **CLL** patients treated with CAR T-cell therapy, venetoclax or other approved treatments after progression on ibrutinib (Bruton tyrosine kinase inhibitor).

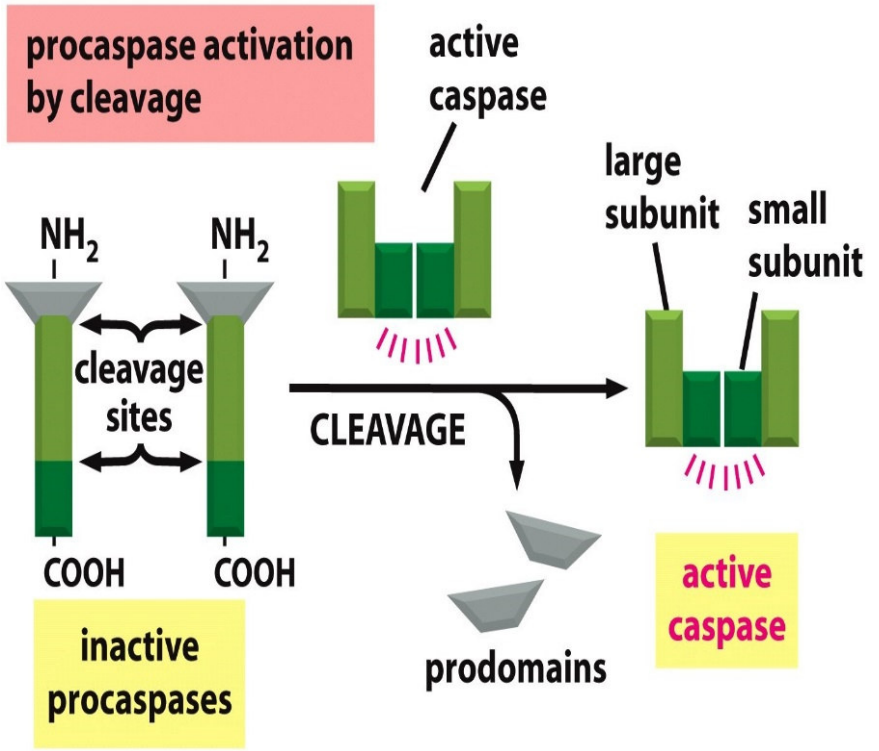
Binding of XIAP to caspase 9

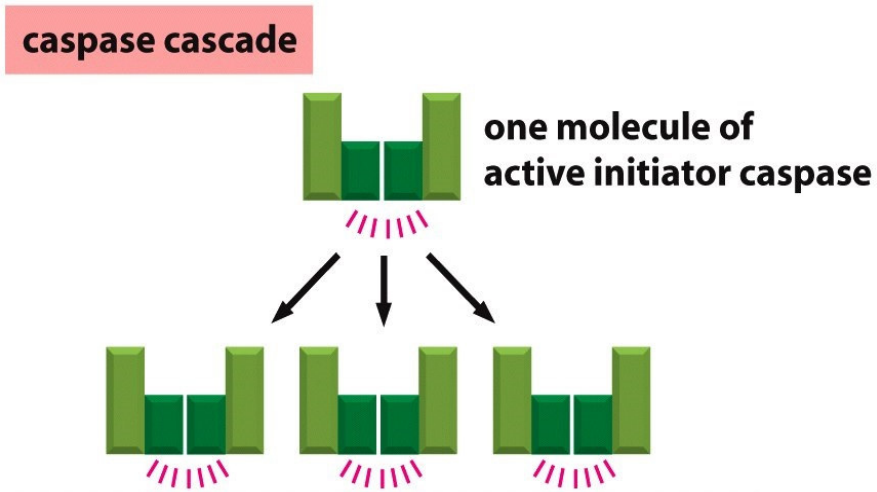


the large family of IAPs (inhibitors of apoptosis) function by binding and inhibiting various caspase molecules, thereby preventing inadvertent activation of the caspase cascade and the triggering of apoptosis

binding by XIAP prevents the homodimerization of caspase 9, which is required for its activity

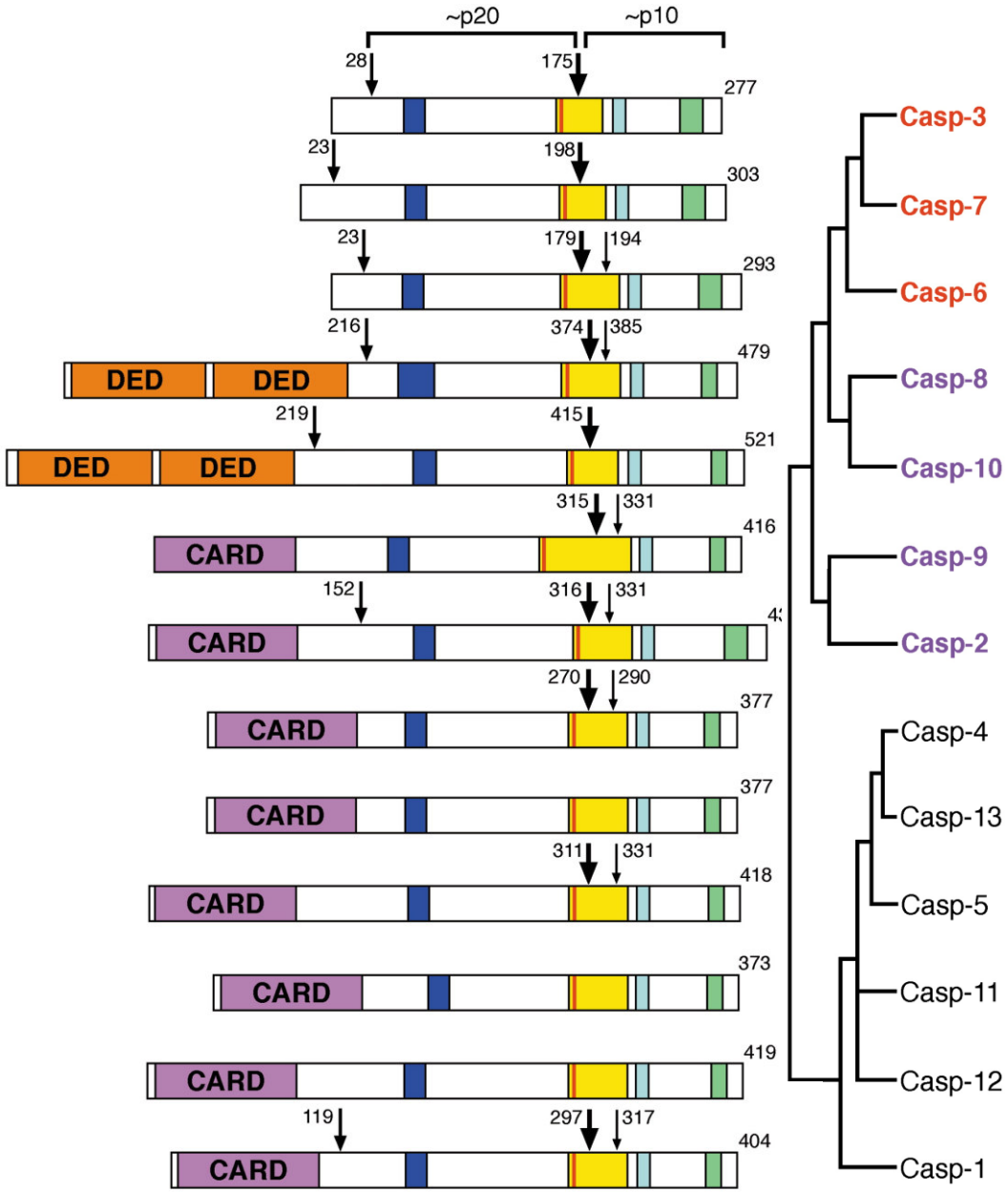
Cell Death induction often involves an intracellular proteolytic cascade





- **executioner caspases** are synthesized in the cell as inactive precursors (procaspases) which are typically activated by proteolytic cleavage by other, **initiator caspases**
- caspases have a cysteine at their active site and cleave their target proteins at specific aspartic acids, which is the reason they are called caspases
  (c for cysteine and asp for aspartic acid)
- the procaspase is split into a large (p20) and a small (p10) subunit that form a heterodimer, and two such dimers assemble to form the active tetramer
- once activated, caspases cleave, and thereby activate other procaspases, resulting in an **amplifying proteolytic cascade**
- this cascade is **irreversible**, so that once a cell reaches a critical point along the path to destruction, it cannot turn back
- not all caspases mediate apoptosis, some are involved in inflammation by releasing IL1β

The Caspase family



Phylogenetic analysis segregates caspases into three major subfamilies:

caspase3-like family lacking additional domains involved in the **execution phase** of apoptosis

caspase8-like family with a long prodomain (not shown) and DED or CARD domains for protein-protein interactions that are involved in the **initiation phase** of apoptosis

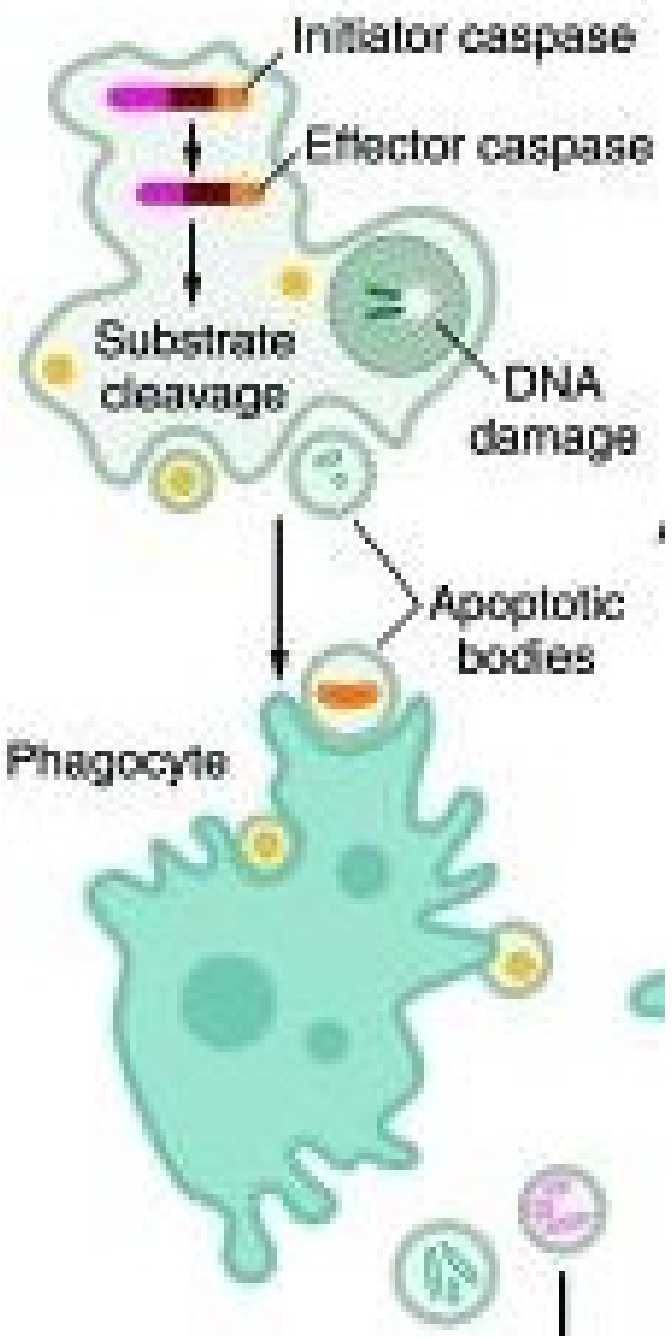
DED – death effector domain

CARD – caspase activation and recruitment domain

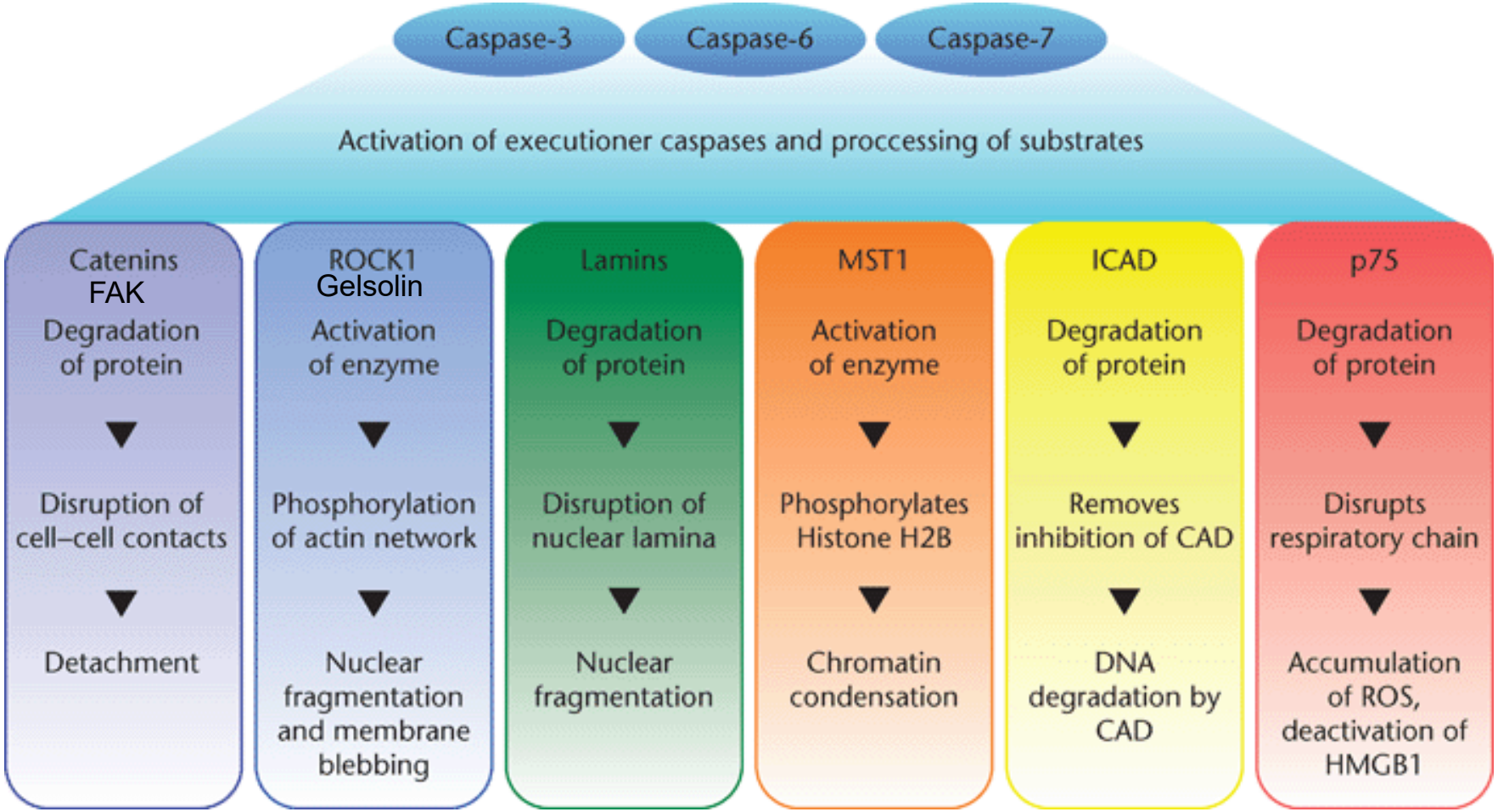
caspase1-like family that mediates cytokine maturation and release in **inflammation**

Key Characteristics of Apoptosis

- morphological changes
  - plasma membrane blebbing
  - nuclear compaction
  - chromatin condensation
  - cell body shrinkage
  - formation of membrane-covered apoptotic bodies
- biochemical changes
  - appearance of DNA fragments due to nucleosomal cleavage
  - flipping of phosphatidylserine from the inner leaflet to the outer leaflet of the plasma membrane
  - cleavage of other cellular proteins



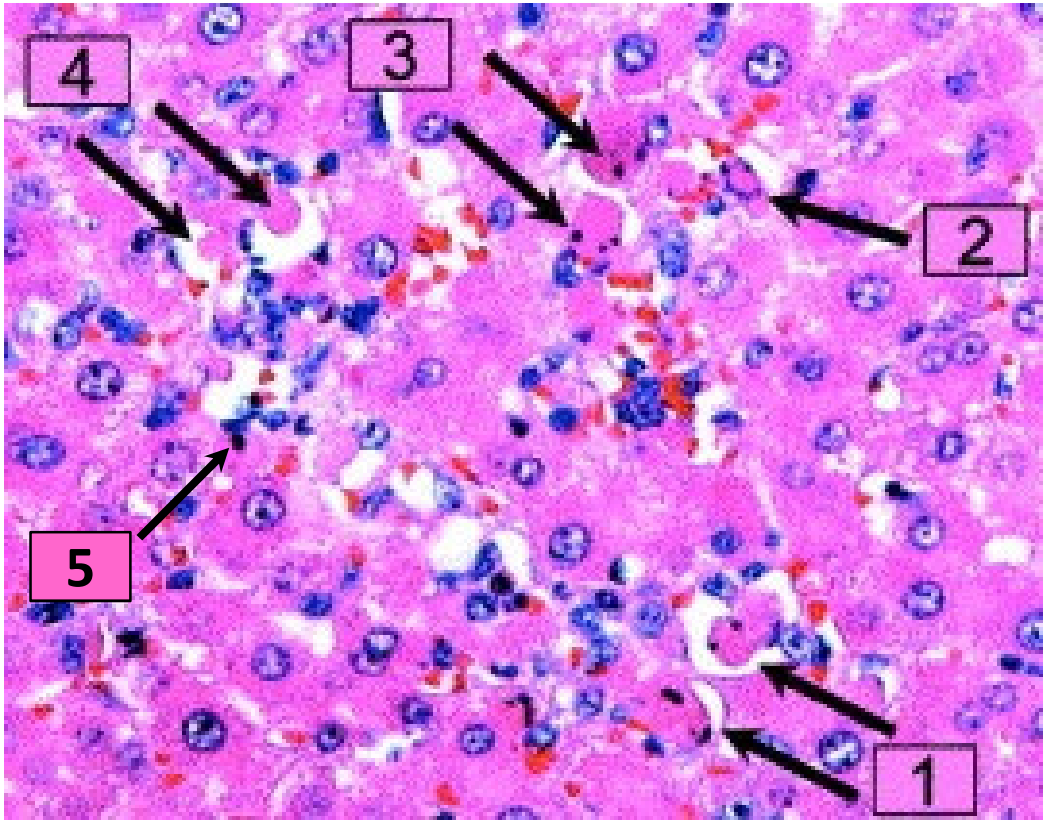
Caspase substrates



Caspase-mediated substrate cleavage has multiple effects summarized as:
(i) a halt of cell cycle progression, (ii) disabling of key metabolic steps (mRNA production, protein secretion) and repair mechanisms, (iii) disassembly of organelle structures, (iv) cell detachment, (v) DNA fragmentation and condensation (vi) maturation of cytokine precursors

Assays for detecting apoptosis

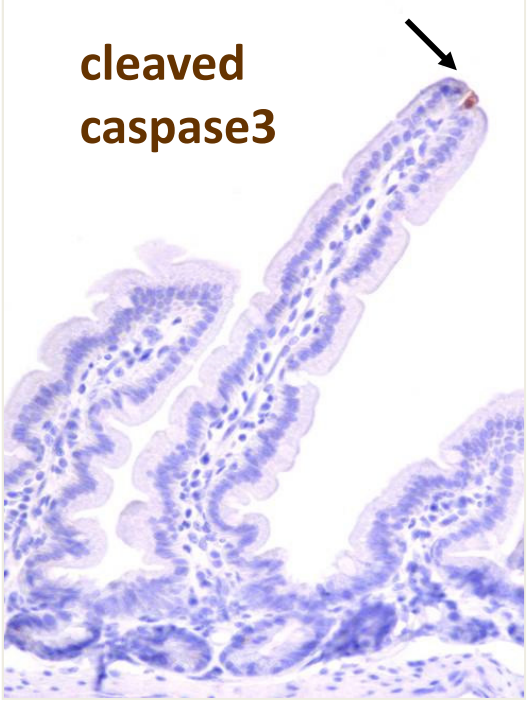
- Histological stains to identify condensed chromatin



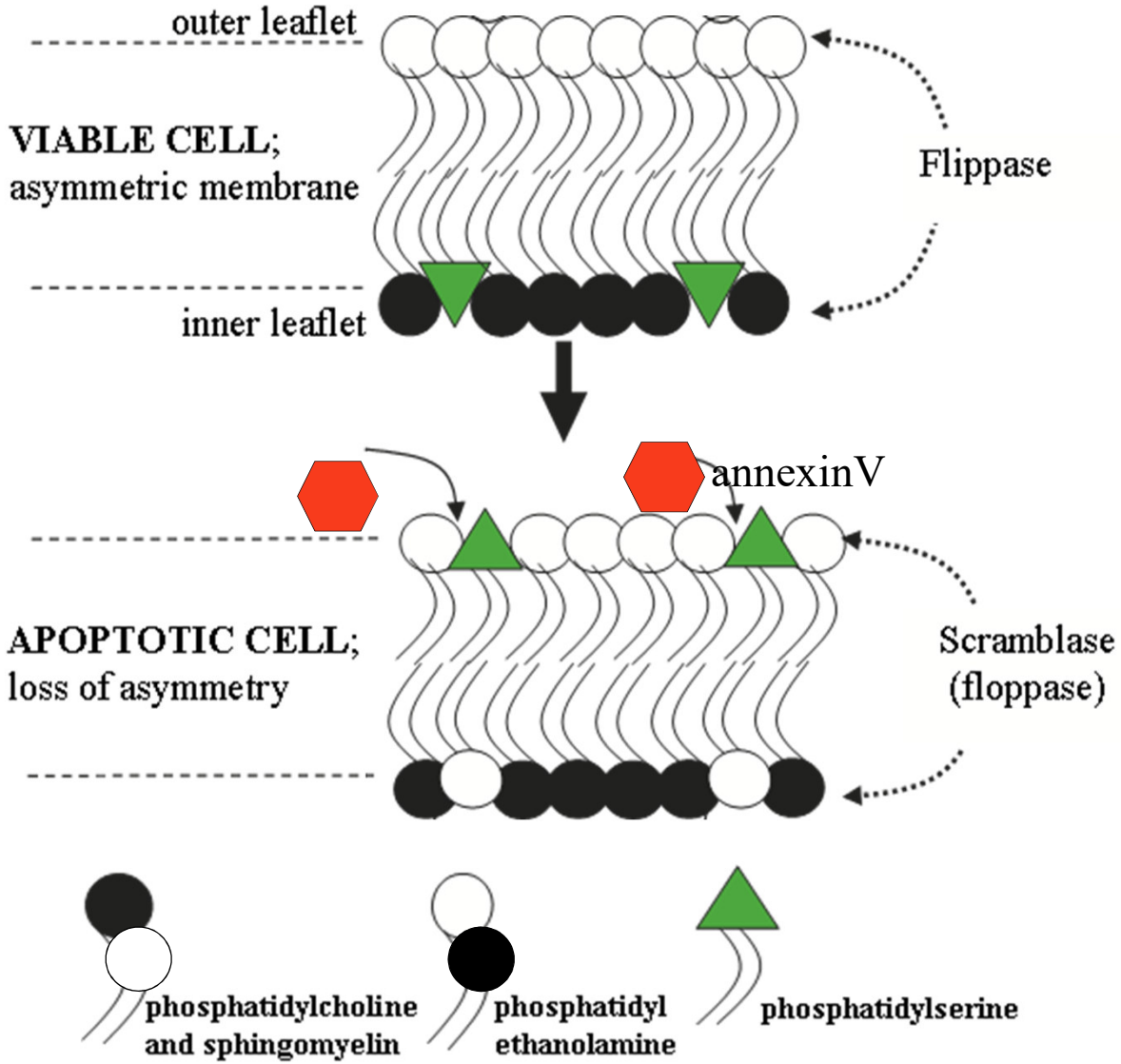
Liver histology of hepatocytes undergoing apoptotic cell death:

1) cell shrinkage
2) chromatin margination
3) chromatin condensation and fragmentation (karorrhexis)
4) formation of apoptotic bodies
5) pyknotic (shrunken and dark) nuclei

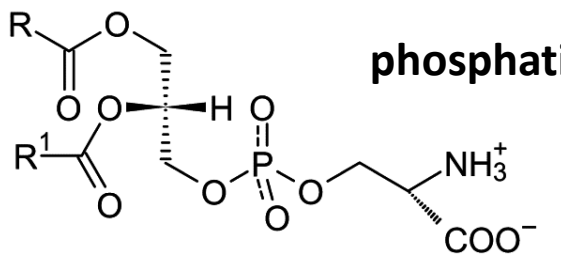
- DNA fragmentation assessed by gel electrophoresis or TUNEL assay
- annexin V staining - stains phosphatidyl serine in outer membrane
- cytochrome C release – assays measure cytochrome c in mitochondria vs cytosol
- caspase cleavage/activation – can be demonstrated by IHC or Western



Phosphatidyl serine as a marker of apoptotic cells



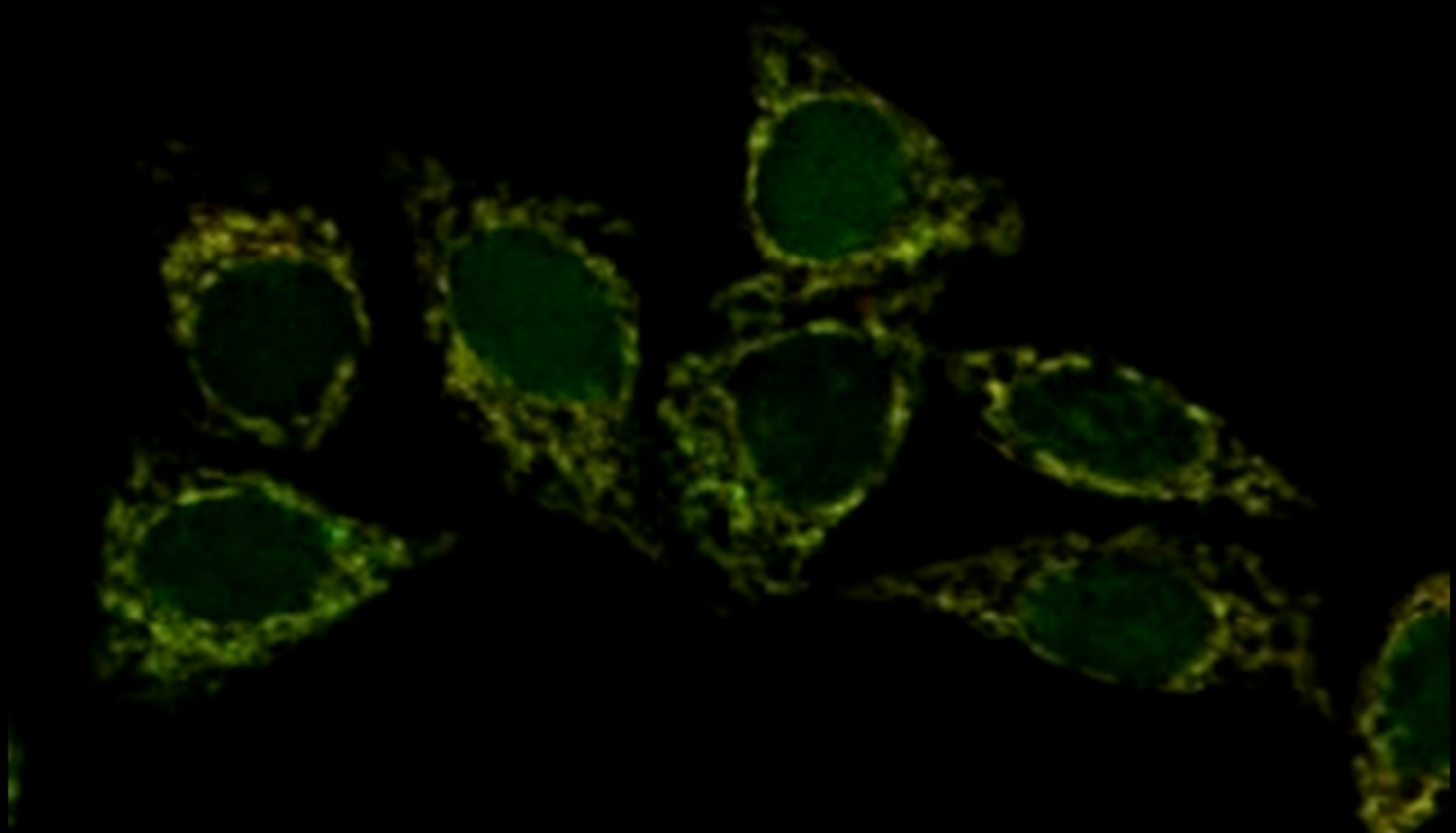
- in membrane lipid bilayers, the compositions of the inner and outer membrane leaflets are different, with the inner (cytoplasmic) leaflet largely composed of phosphatidyl ethanolamine, **phosphatidyl serine** and phosphatidyl inositol and the outer (extracellular) leaflet based on phosphatidyl choline, sphingomyelin and a variety of glycolipids
- maintaining this asymmetric composition is an energy dependent process involving the activity of enzymes, termed '**flippases**'
- apoptotic cells activate the enzyme '**scramblase**' (floppase) which transfers phosphatidyl serine to the outside of the membrane



phosphatidyl serine

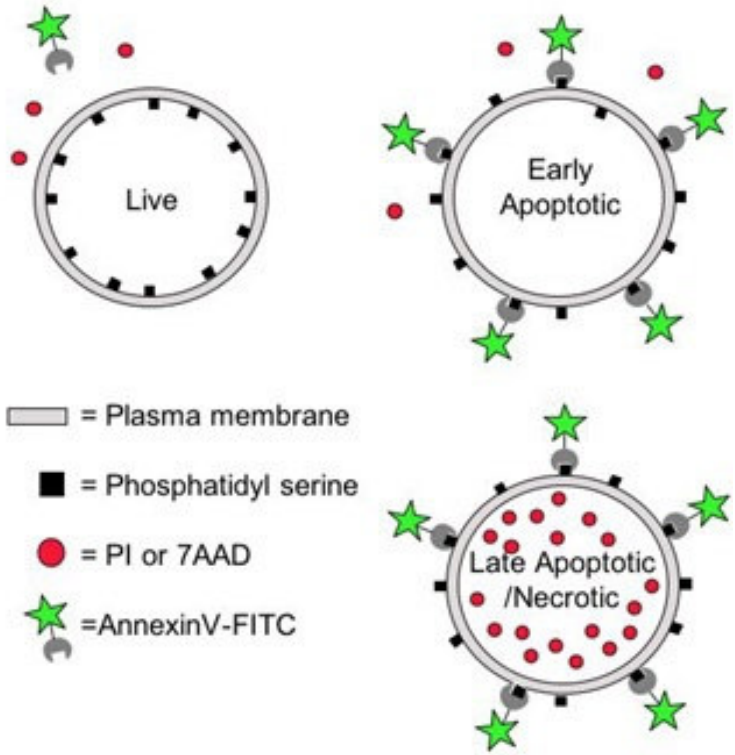
Apoptosis

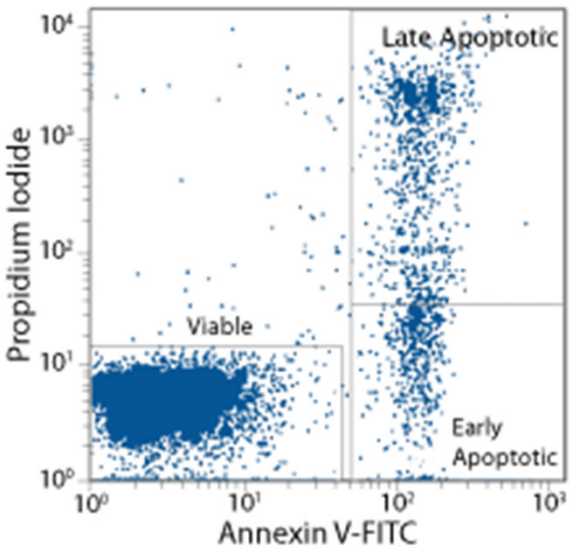
cytochromeC
phosphatidyl-
serine
DNA

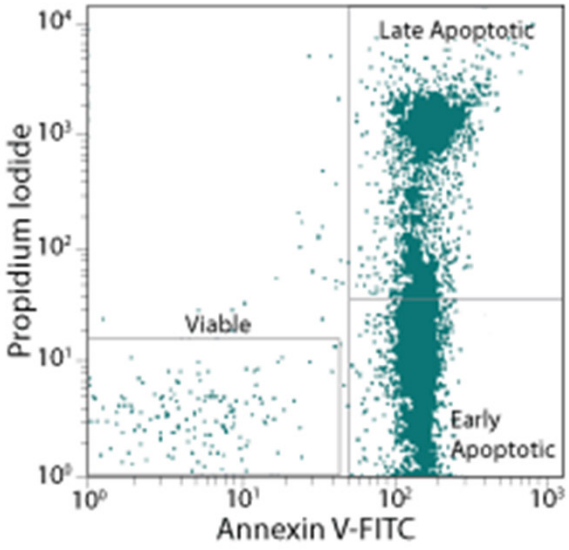


Apoptosis detection by FACS

- externalisation of the lipid **phosphatidyl serine (PS)** from the inner to the outer plasma membrane can be detected by **AnnexinV** that specifically binds PS
- fluorescent labelling of annexinV enables the flow cytometric detection of apoptotic cells
- when used in conjunction with a live/dead cell discriminator that measures membrane integrity (such as propidium iodide, PI), early apoptotic cells (annexinV positive only) can be distinguished from late apoptotic/necrotic cells (annexinV and PI positive)
- the early apoptotic phase can be quite rapid, making it often necessary to perform a time course experiment to prove that cells are in fact traversing through early apoptosis before reaching late apoptosis/necrosis

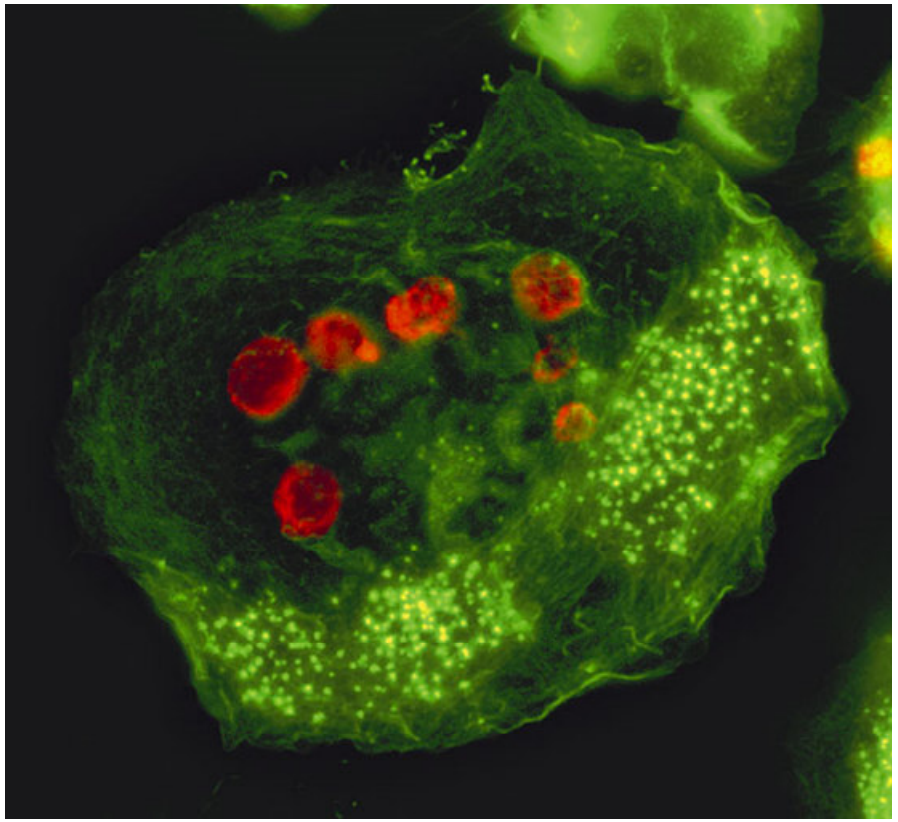






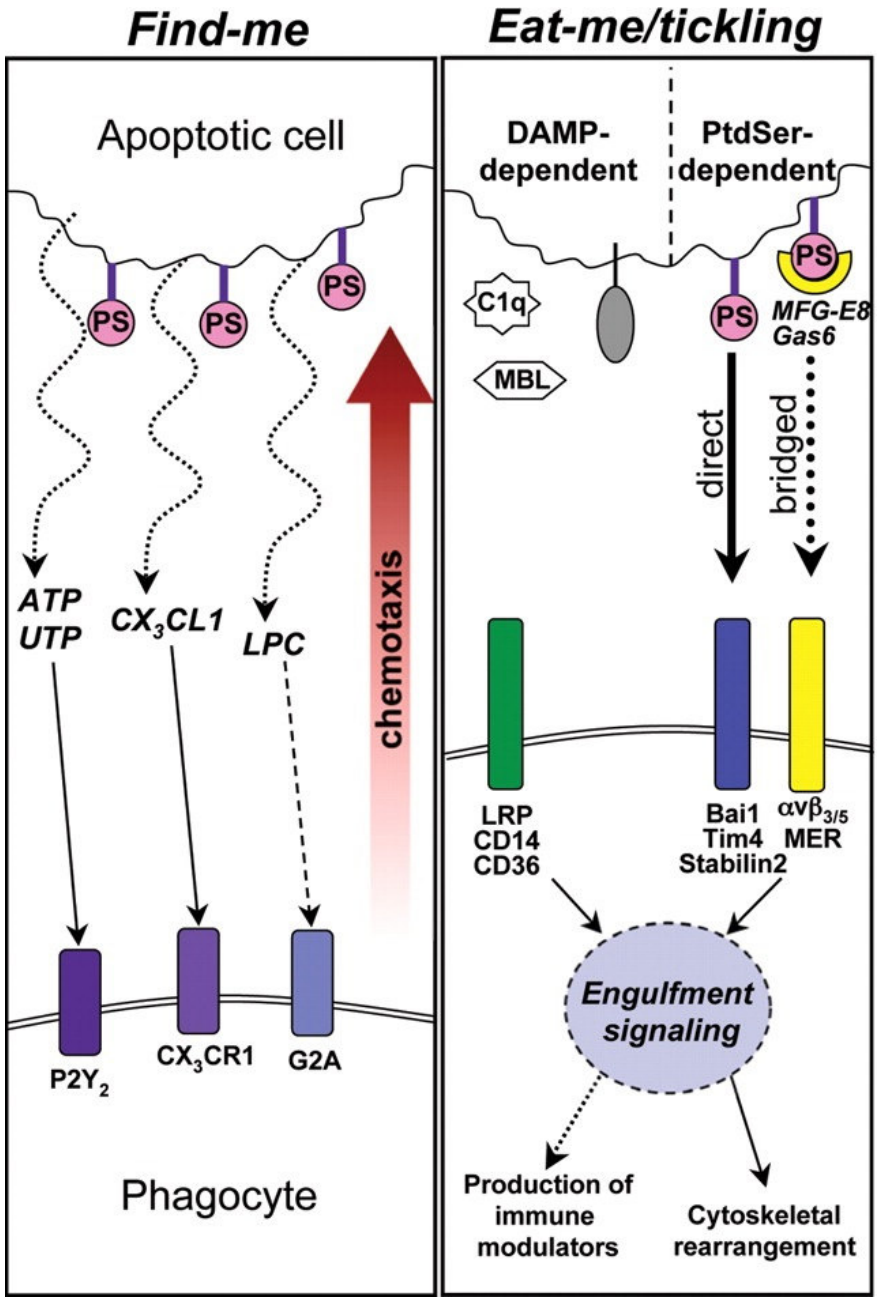
Corpse clearing: apoptotic cell phagocytosis

- an important advantage of apoptotic cell death is that the dying cell is cleared before it can release pro-inflammatory molecules or damage nearby cells
- cells undergoing apoptosis show changes in their plasma membrane expressing "eat me" signals such as phosphatidyl serine that are recognized by phagocytes and other cells
- phagocytes (mostly macrophages) ingesting apoptotic cells release anti-inflammatory and immunosuppressive cytokines such as transforming growth factor-beta (TGF-β) and IL-10
- epithelial or mesenchymal cells ingesting apoptotic cells release growth and migration factors to facilitate tissue repair
- in contrast, phagocytes ingesting necrotic cells will release pro-inflammatory mediators such as TNFα and IL1β



Lymphoma cells were labelled in red and irradiated to induce apoptosis. Macrophages were stained with FITC-phalloidin to identify actin filaments. *Nature (407) pp 784-788*.

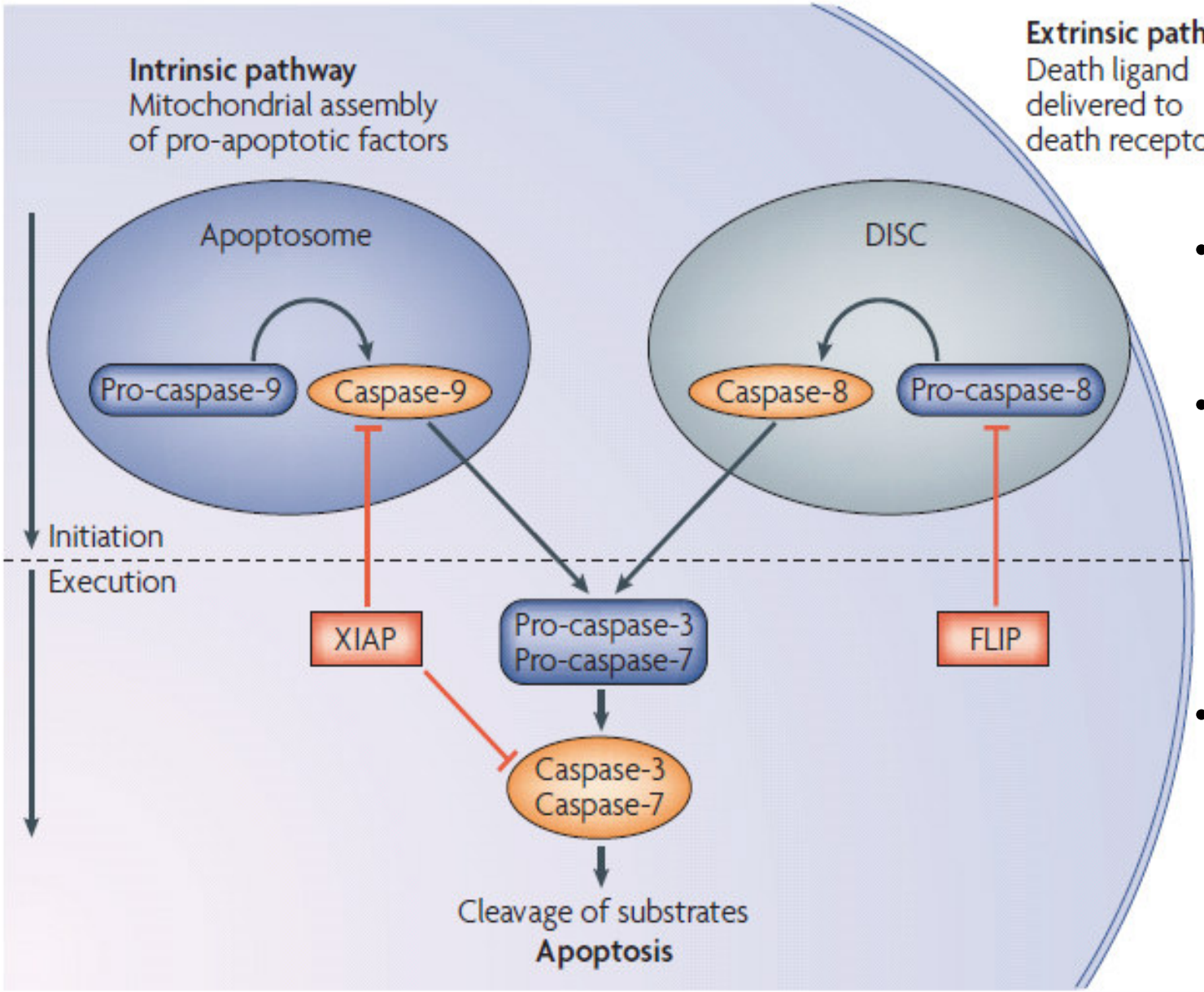
Stages of apoptotic cell engulfment



- **“find-me” step**
  apoptotic cells release soluble chemoattractants that promote chemotaxis of phagocytes via corresponding receptors on the phagocyte

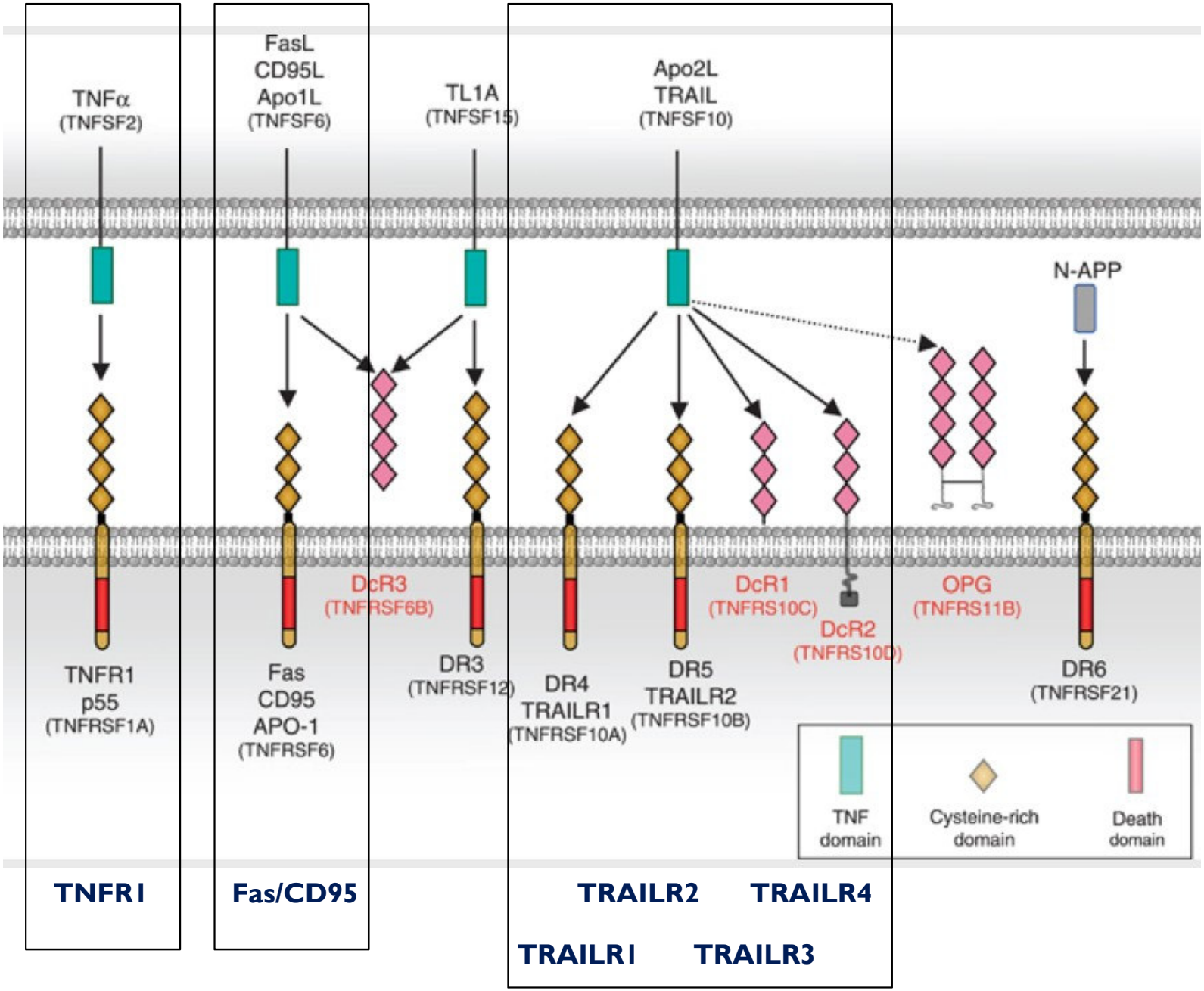
- **“eat-me” step**
  appearance of ligands on the surface of the dying cell mark it as a target to be engulfed by phagocytes bearing appropriate DAMP or PS recognition receptors

Intrinsic and extrinsic activation of apoptosis

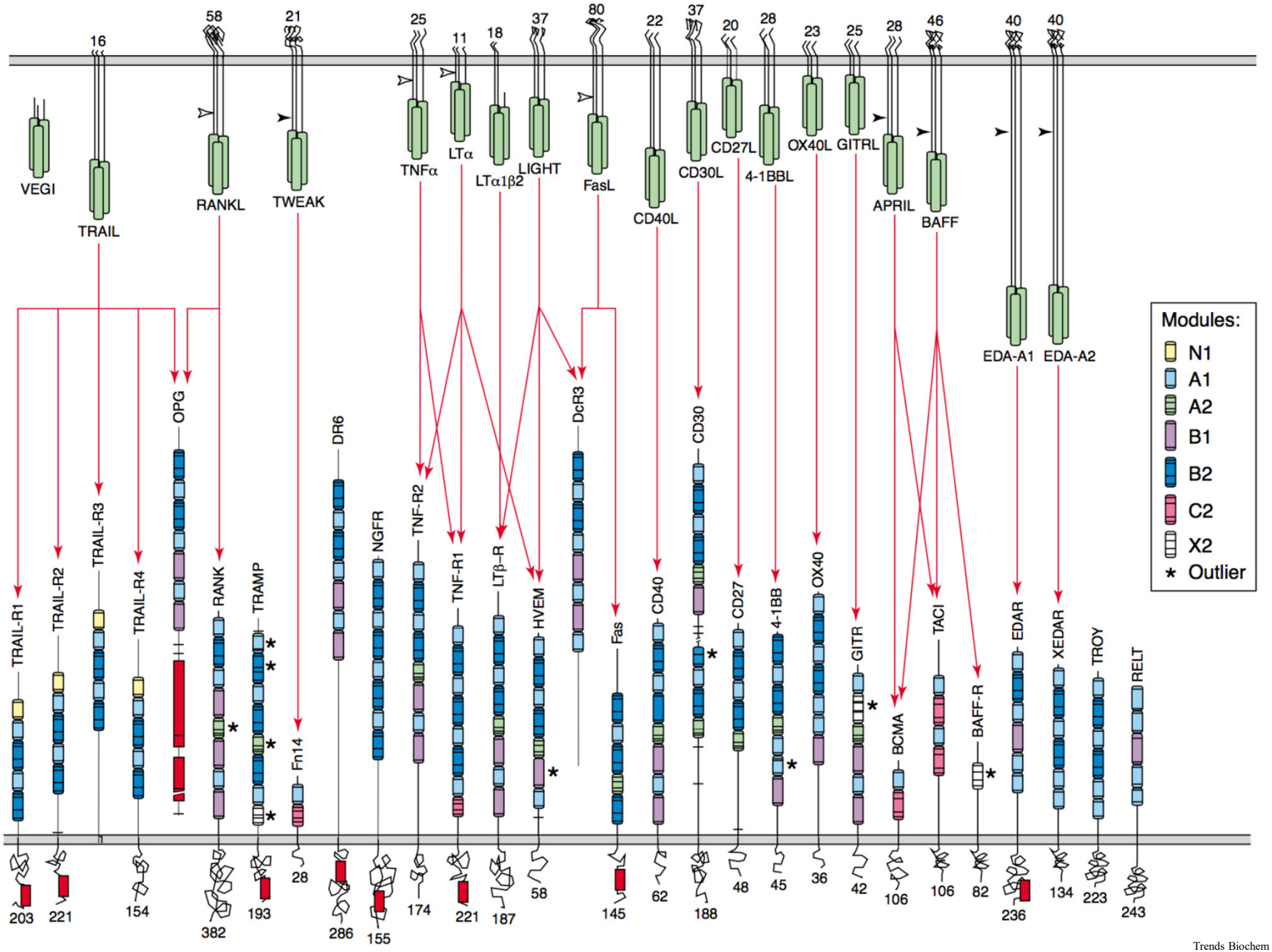


- two main pathways, the intrinsic and the extrinsic can activate apoptosis
- both use their own **initiator caspases (9 or 8)** and activation complexes but result in activation of the same executioner caspases (3 and/or 7)
- both have unique inhibitors (**XIAP**, **FLIP**) which block caspases and are essential to ensure tight, <u>all-or-nothing</u> regulation

The death receptors

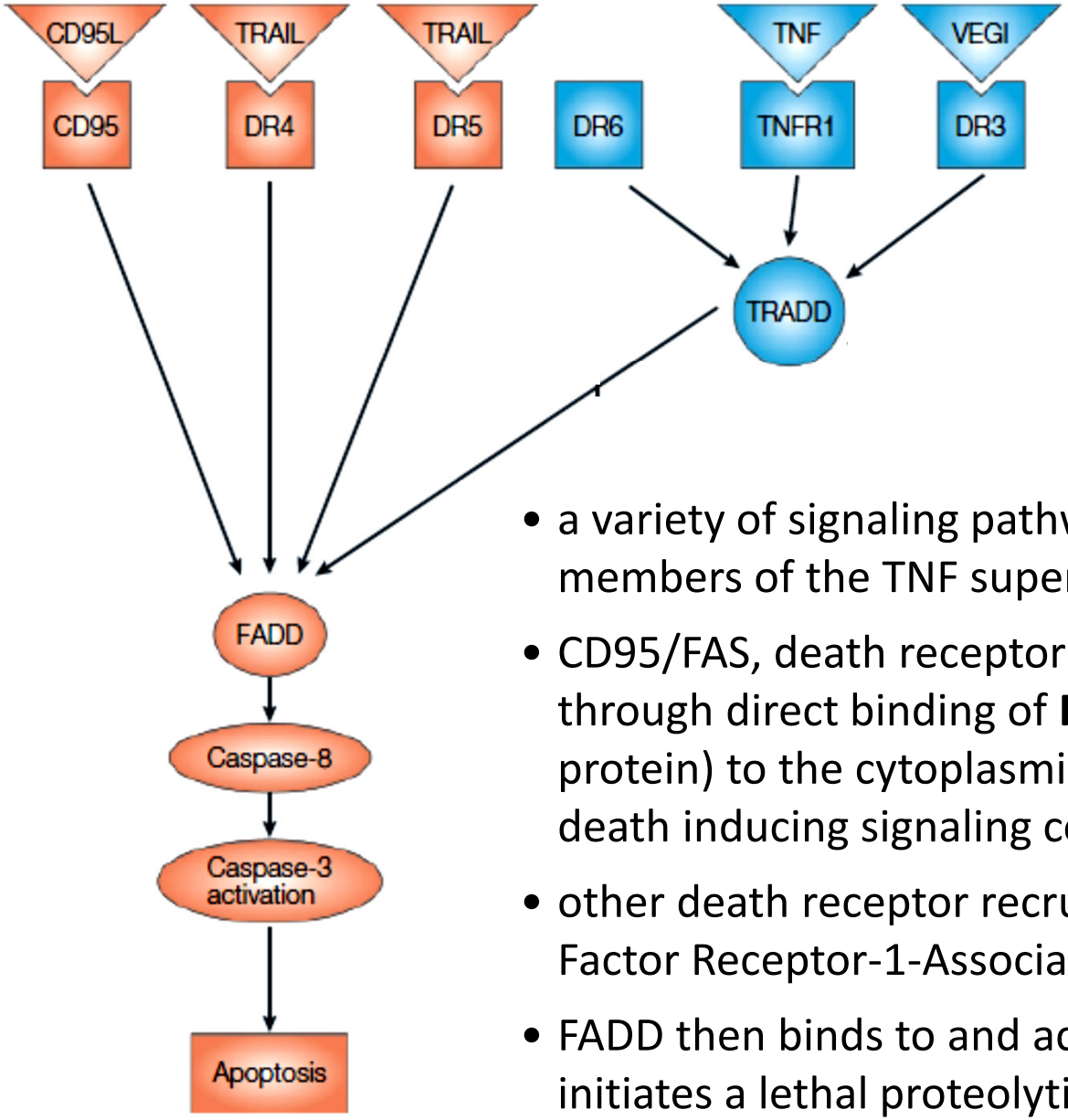


The TNF and TNFR superfamilies



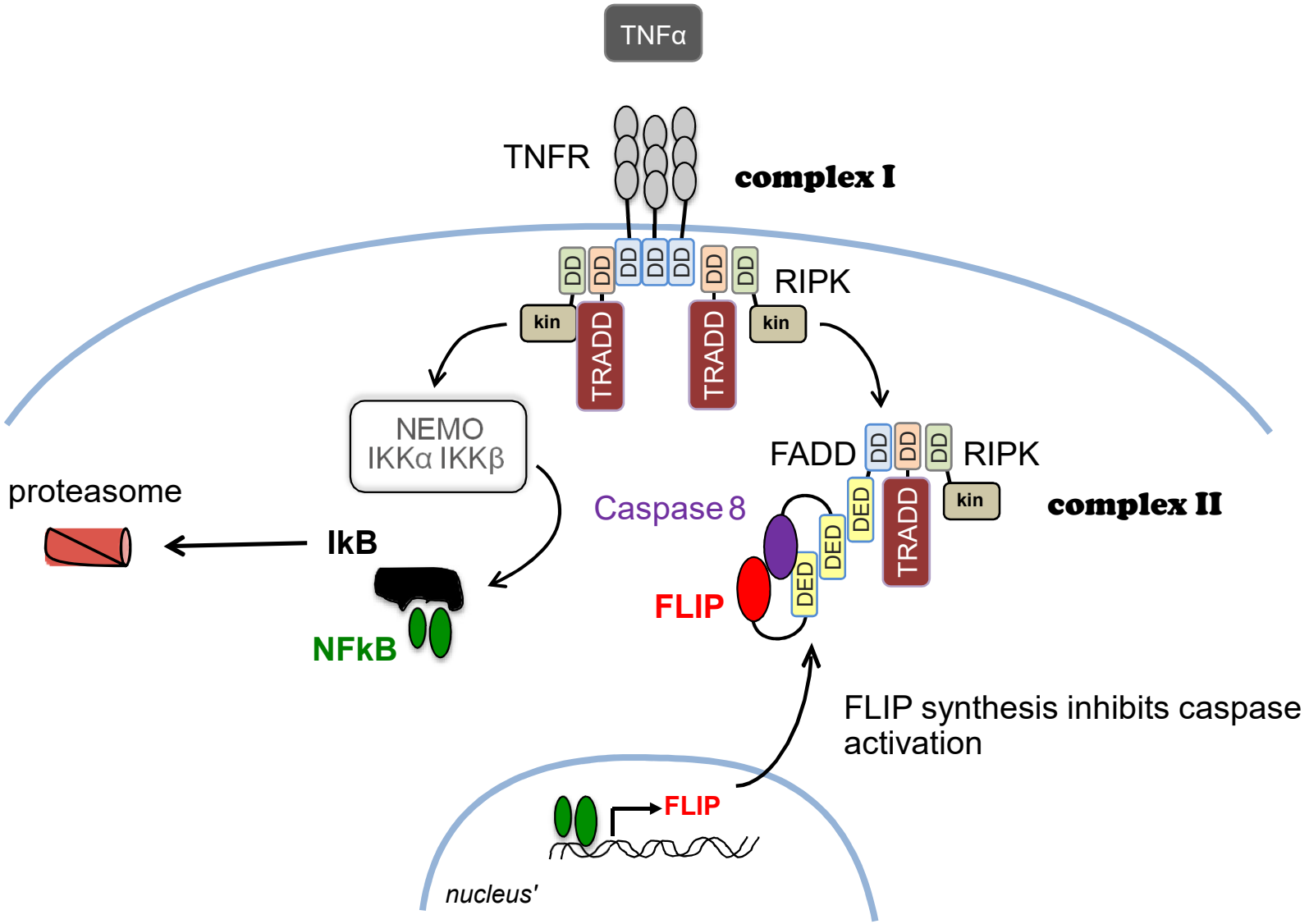
Trends Biochem Sci. 2002 Jan;27(1):19-26.

TNFR signaling: Main downstream events

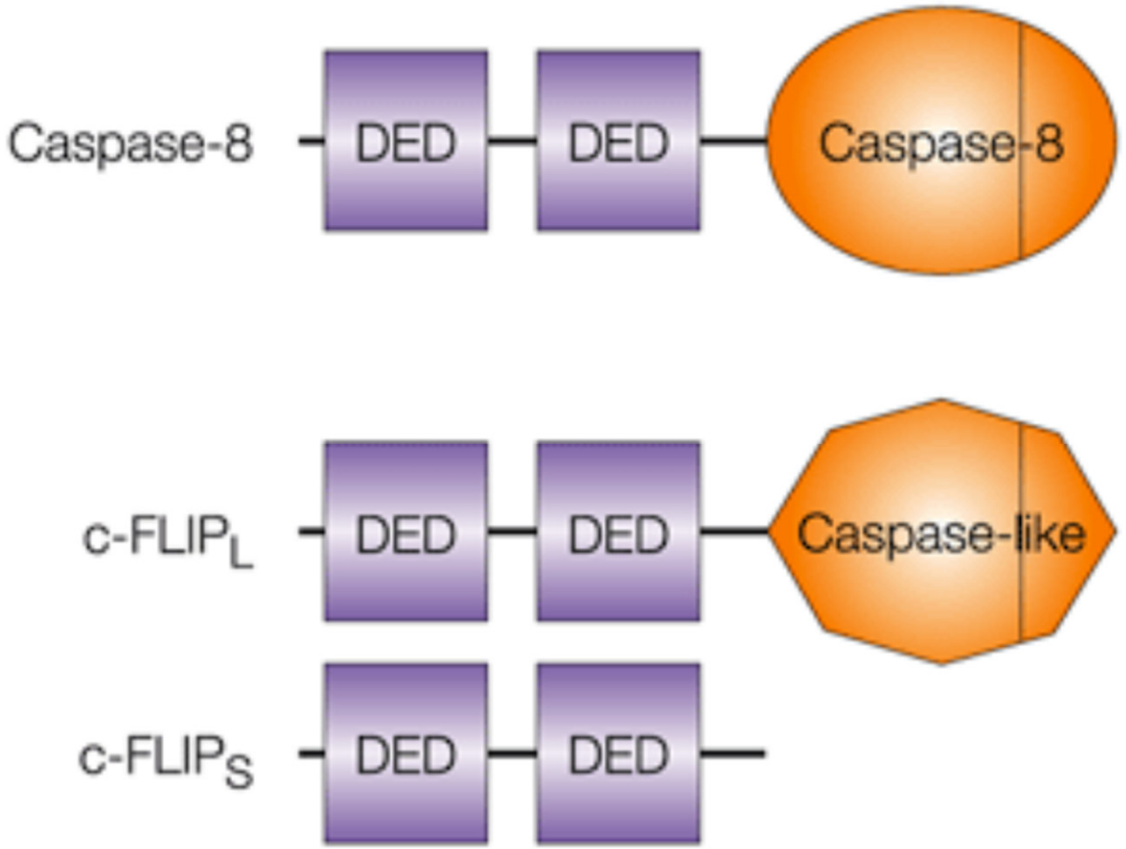


- a variety of signaling pathways can be activated in response to members of the TNF superfamily
- CD95/FAS, death receptor 4 (DR4) and DR5 activate apoptosis through direct binding of **FADD** (Fas Associated Death Domain protein) to the cytoplasmic tail of the receptor and assembly of the death inducing signaling complex (**DISC**)
- other death receptor recruit the adaptor TRADD ((Tumor Necrosis Factor Receptor-1-Associated Death Domain) to link to FADD
- FADD then binds to and activates **caspase 8** (or caspase10) and initiates a lethal proteolytic cascade via activation of **caspase 3**, which in turn induces apoptosis

TNF-induced NF-kB signaling: prevention of apoptosis through up-regulation of FLIP



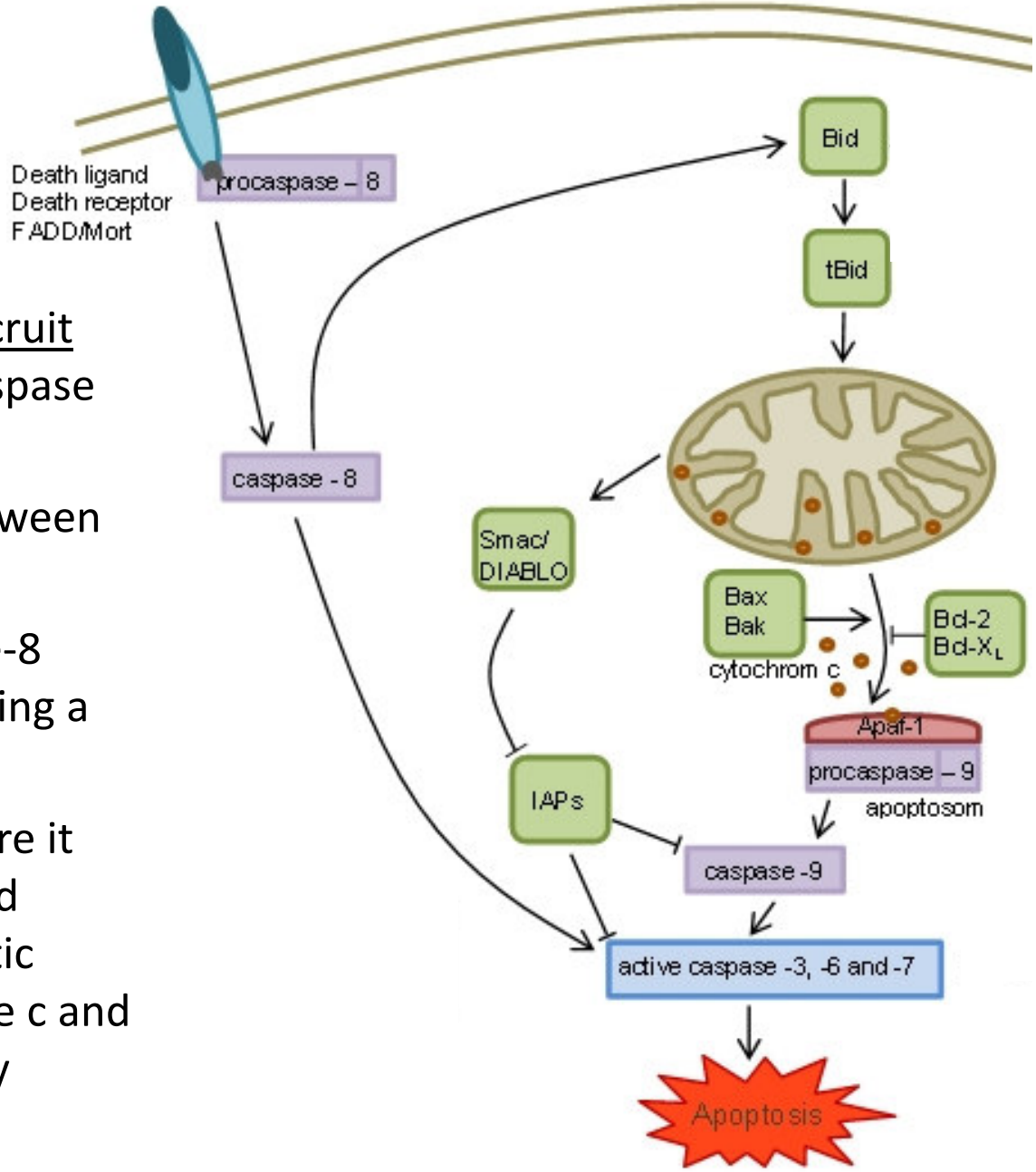
FLIPs are cellular inhibitors of caspases



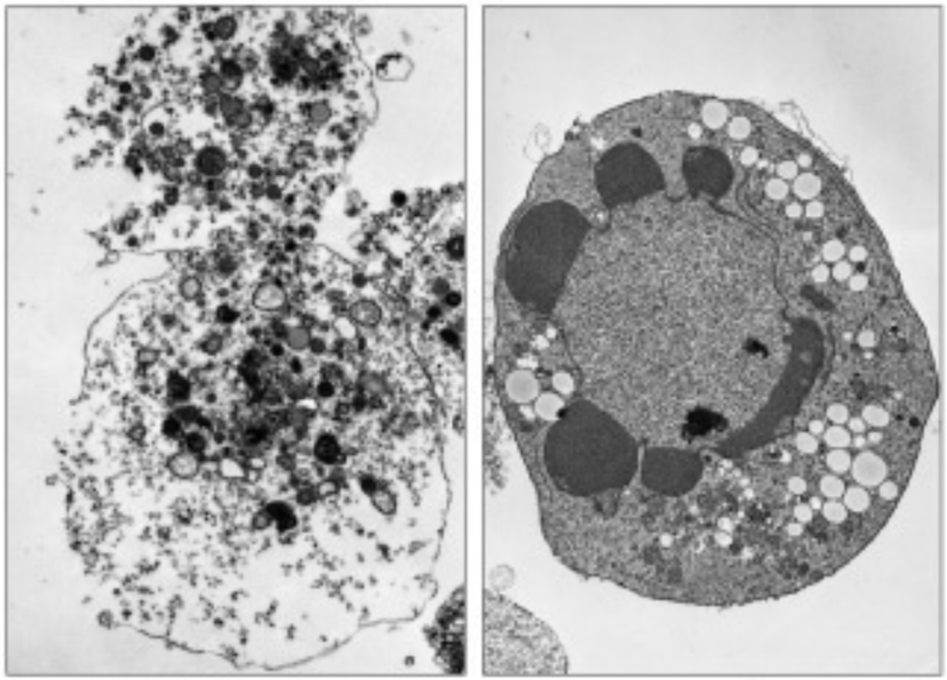
Nature Reviews Immunology 1, 50-58 (October 2001)

Links between intrinsic and extrinsic pathways

- the <u>extrinsic apoptotic pathway can recruit the intrinsic pathway</u> to amplify the caspase cascade to kill the cell
- the BH3-only protein **Bid** is the link between the two pathways
- the extrinsic pathway activates caspase-8 which cleaves and activates Bid producing a truncated form of Bid called **tBid**
- tBid translocates to mitochondria, where it inhibits anti-apoptotic Bcl2 proteins and triggers the aggregation of pro-apoptotic Bak/Bax proteins to release cytochrome c and other intermembrane proteins, thereby amplifying the death signal

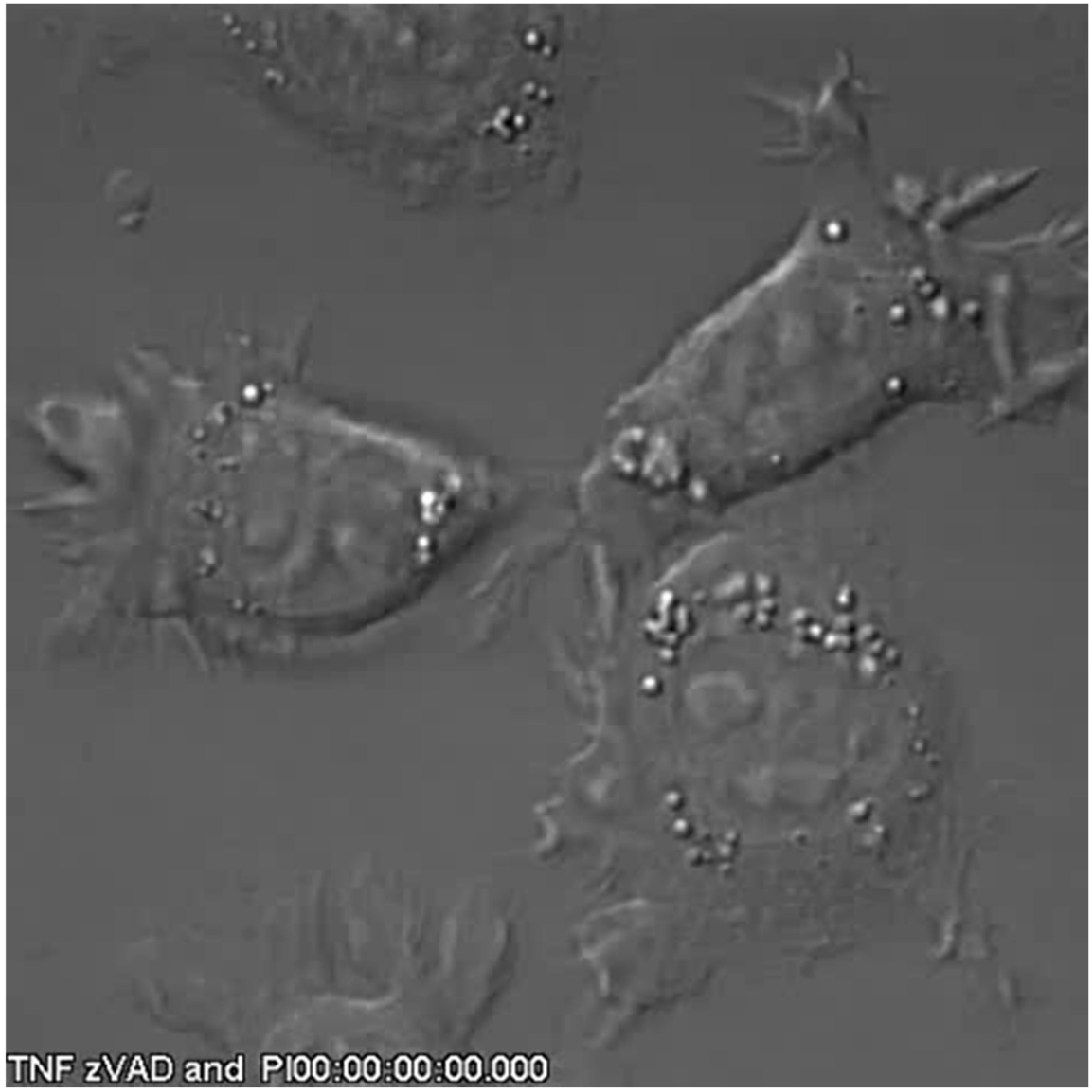


Characteristics of Necroptosis



necroptosis **apoptosis**

- extensive vacuolation of the cytoplasm
- chromatin appears coarse and clumpy
- breakdown of cell organelles
  - mitochondrial swelling
  - dilation of the endoplasmic reticulum
  - karyolysis (disintegration of the nucleus)
- cell swelling and rupture
- release of intracellular components followed by activation of inflammatory responses



propidium iodide staining

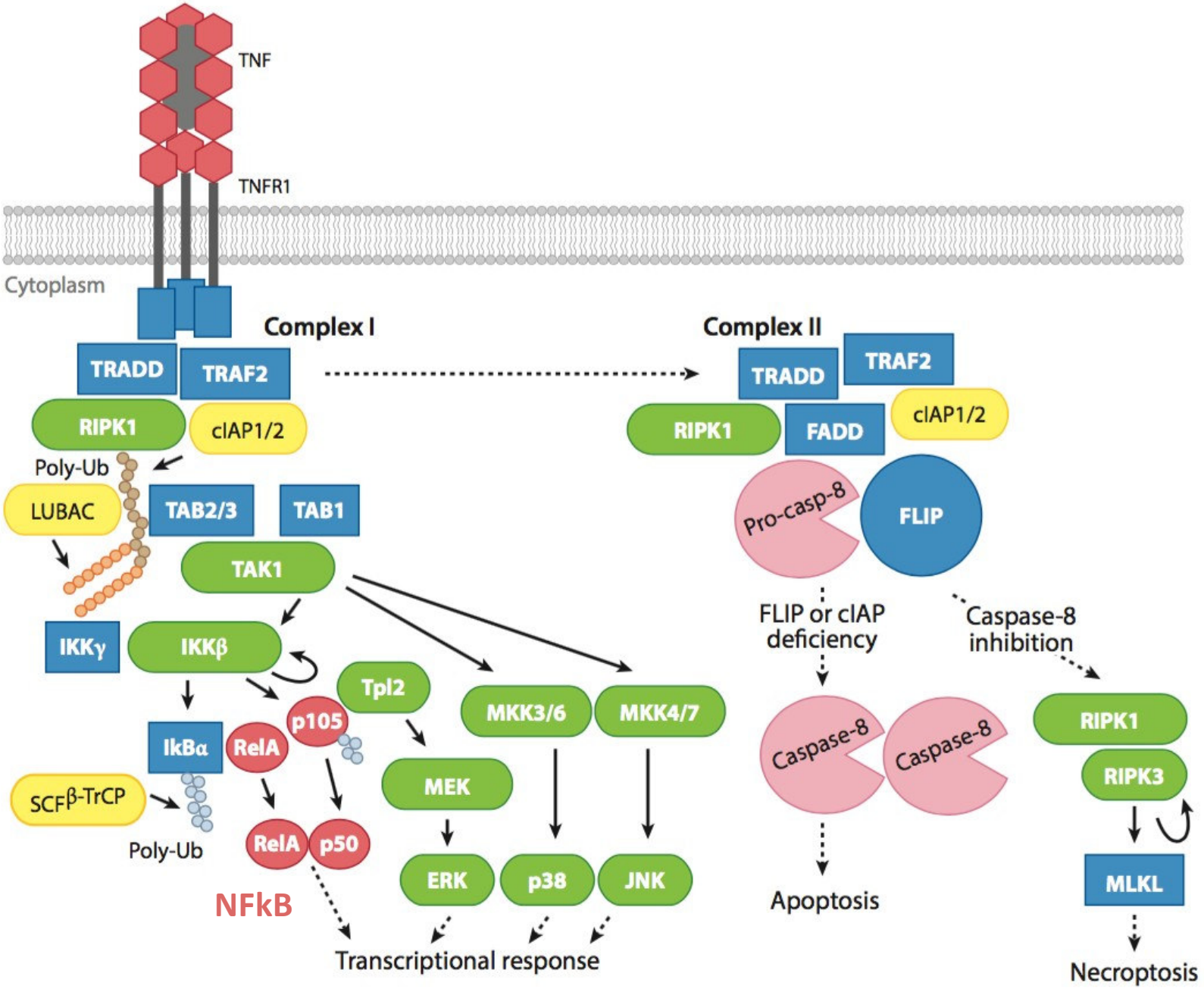
Morphological and biochemical differences between apoptosis and necroptosis

Features	Apoptosis	Necroptosis (a major form of regulated necrosis)
Cytoplasmic shrinkage	Yes	No
Chromatin condensation	Yes	Mild
Nuclear fragmentation	Yes	No
Membrane blebbing	Yes	No
Shedding of apoptotic bodies	Yes	No
Swelling of organelles	No	Yes
Lysosomal membrane permeabilization	No	Yes
Plasma membrane permeabilization	No	Yes
Caspase activation	Yes	No
Key regulators in pathway	Bid, Bax/Bak; cytochrome *c*; Apaf-1; caspase-9; caspase-8/10; FADD; RIP1	RIP1, RIP3, MLKL
Executors of cell death	Caspase-3; caspase-7	MLKL trimer and ion channels formation
Inhibitors	zVAD	Nec-1 (RIP1 kinase inhibitor); GSK-843, GSK-872 and GSK-840 (RIP3 kinase inhibitors); NSA (MLKL inhibitor)
Physiological and pathological roles	Controlling cell numbers during embryogenesis and homeostasis, immune regulation, and pathogen defense. Inhibition of apoptosis may result in cancers, autoimmune diseases, inflammatory diseases, and viral infections	Virus infection, TNF-mediated hypothermia and systemic inflammation, ischemic reperfusion injury, neurodegeneration, Gaucher's disease, progressive atherosclerotic lesions, and cancers

Abbreviations: Nec-1, necrostatin-1; NSA, necrosulfonamide
Note: Necroptosis is a major and most well-studied form of regulated necrosis, but regulated necrosis may also include other forms, such as parthanatos, oxytosis, ferroptosis, NETosis, pyronecrosis, and pyroptosis[2]

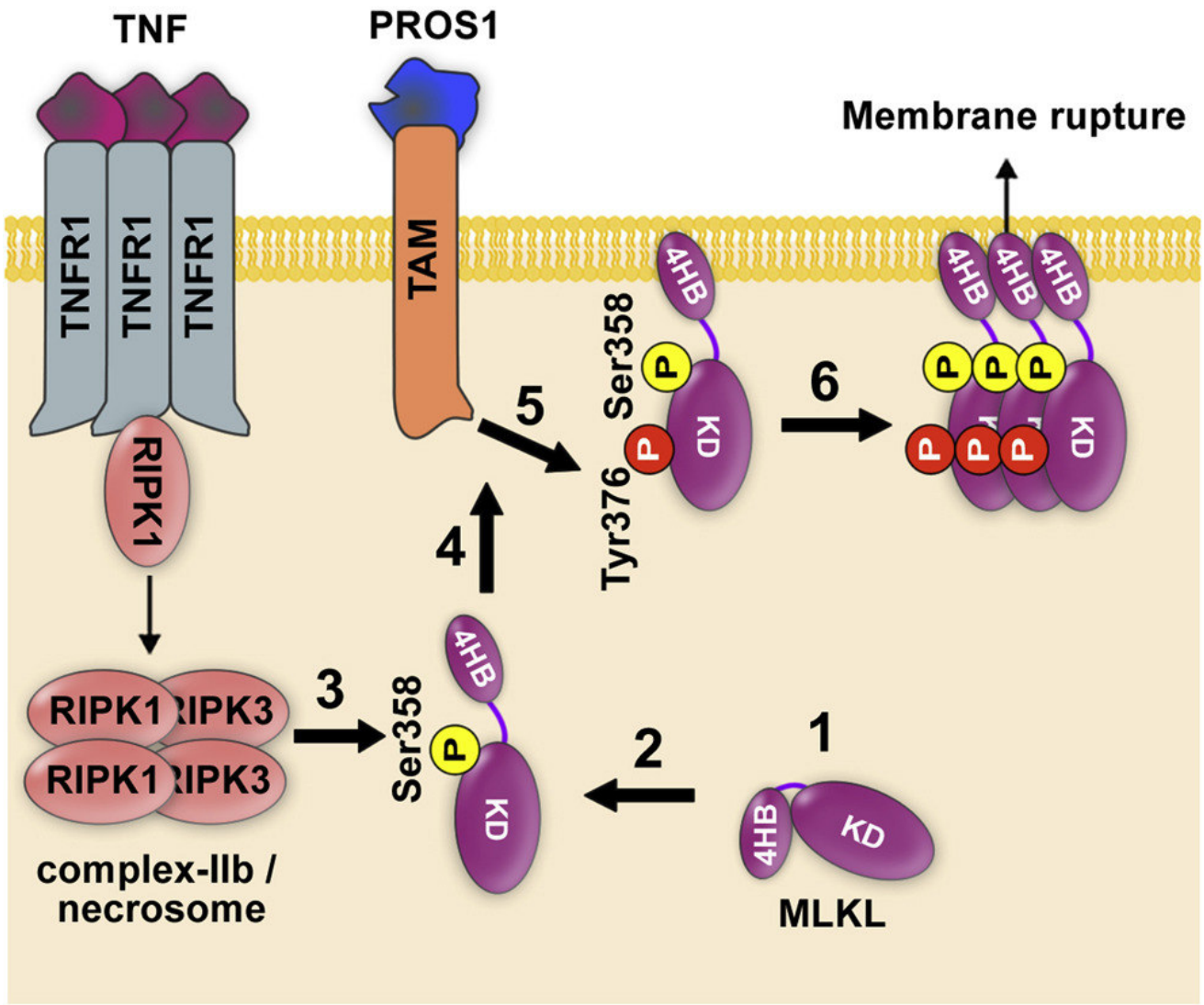
Cell Death and Differentiation (2016), 1–9

Necroptosis induction by TNF signaling



Annu. Rev. Biochem. 2016. 85:4.1–4.21

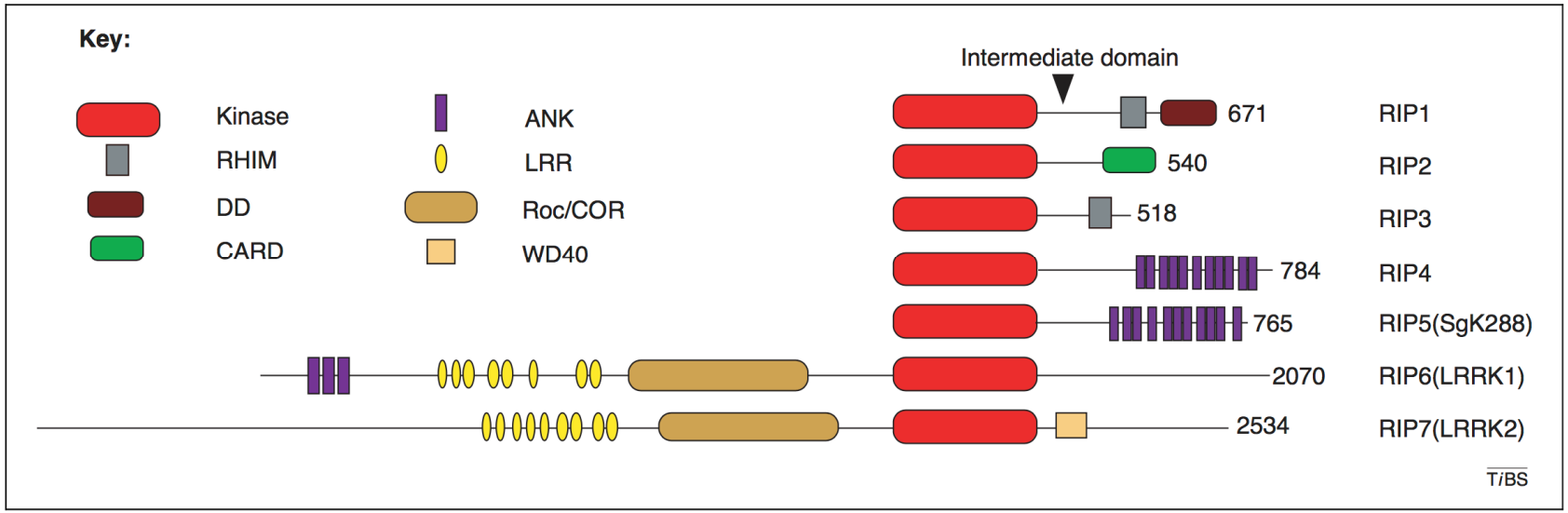
Membrane rupture by MLKL



- TAM (Tyro3, Axl, and Mer) kinase activity is involved in mediating necroptosis
- TAM receptor ligands are Gas6, protein S and phosphatidylserine (PtdSer)-exposed membranes which have implicated the receptors in innate immune-mediated cell clearance
- TAM kinases phosphorylate MLKL to promote oligomerization and lytic pore formation
- Pharmacologic or genetic targeting of TAM kinases results in a potent inhibition of necroptotic cell death, resistance to systemic inflammatory response syndrome and accumulation of apoptotic cell debris

Najafov, Mol Cell 2019

Primary Structure of RIP kinases

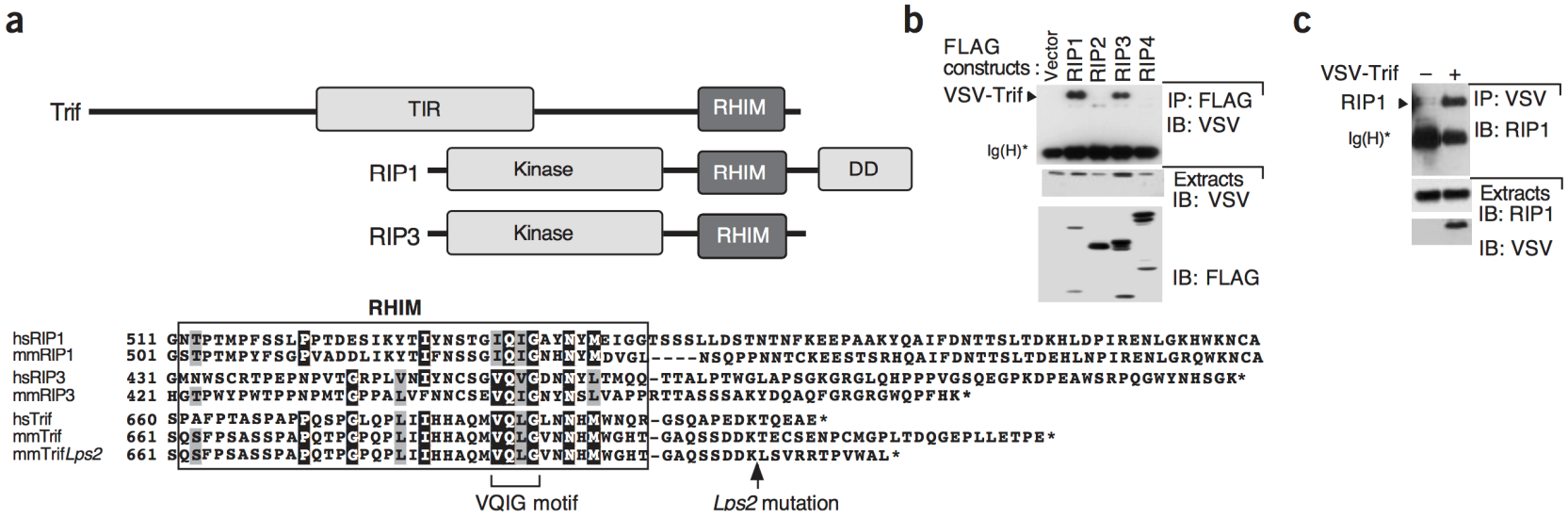


RIPK1 and RIPK3 share a common domain, the RHIM domain (RIPK homology interacting motif) which allows heterodimerization of the two kinases

Meylan E, Tschopp J. Trends Biochem Sci. 2005 Mar;30(3):151-9.

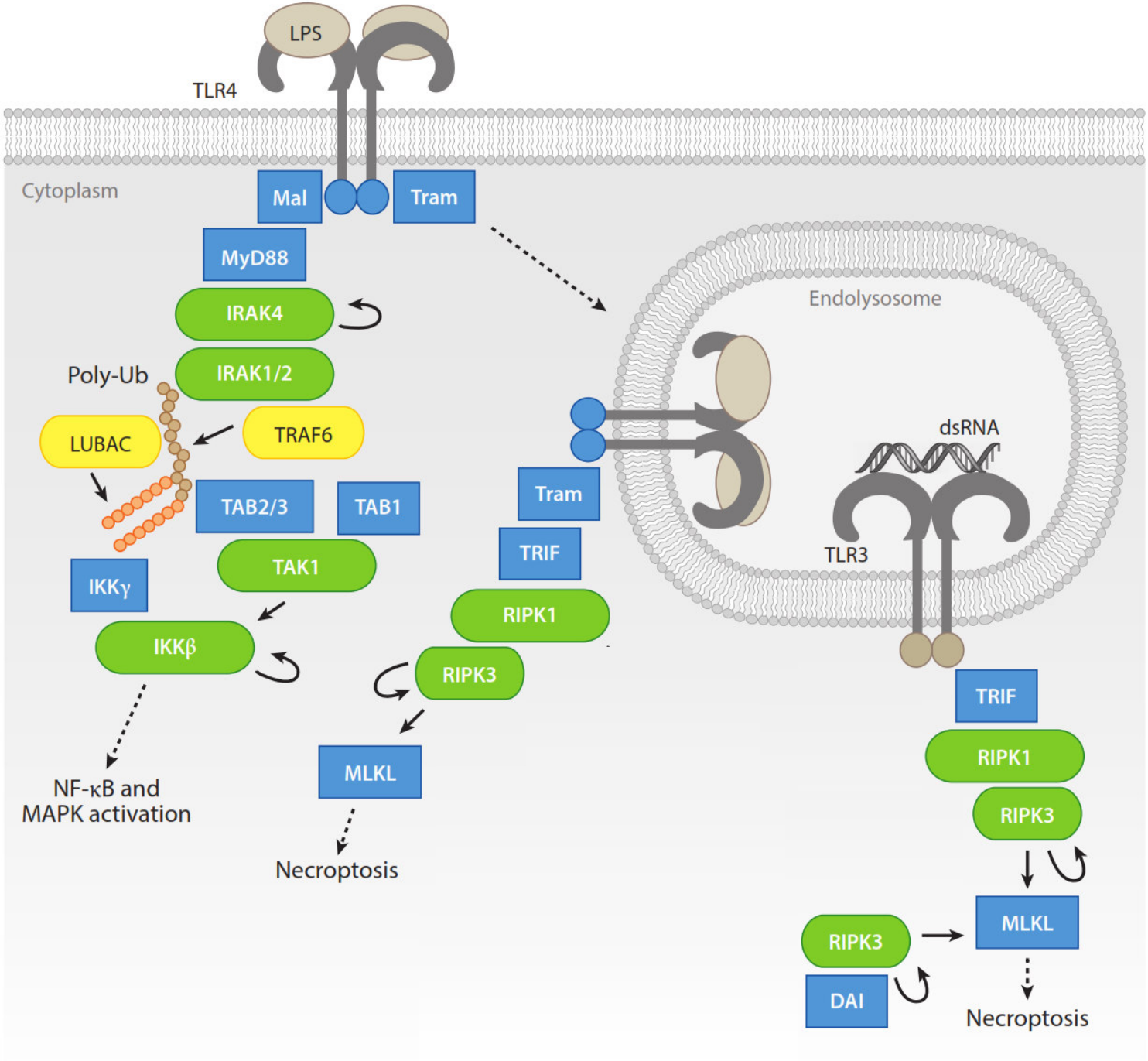
How can TLRs induce necroptosis?

a common motif is found in RIP1, RIP3 and TRIF, the TLR3 adaptor:



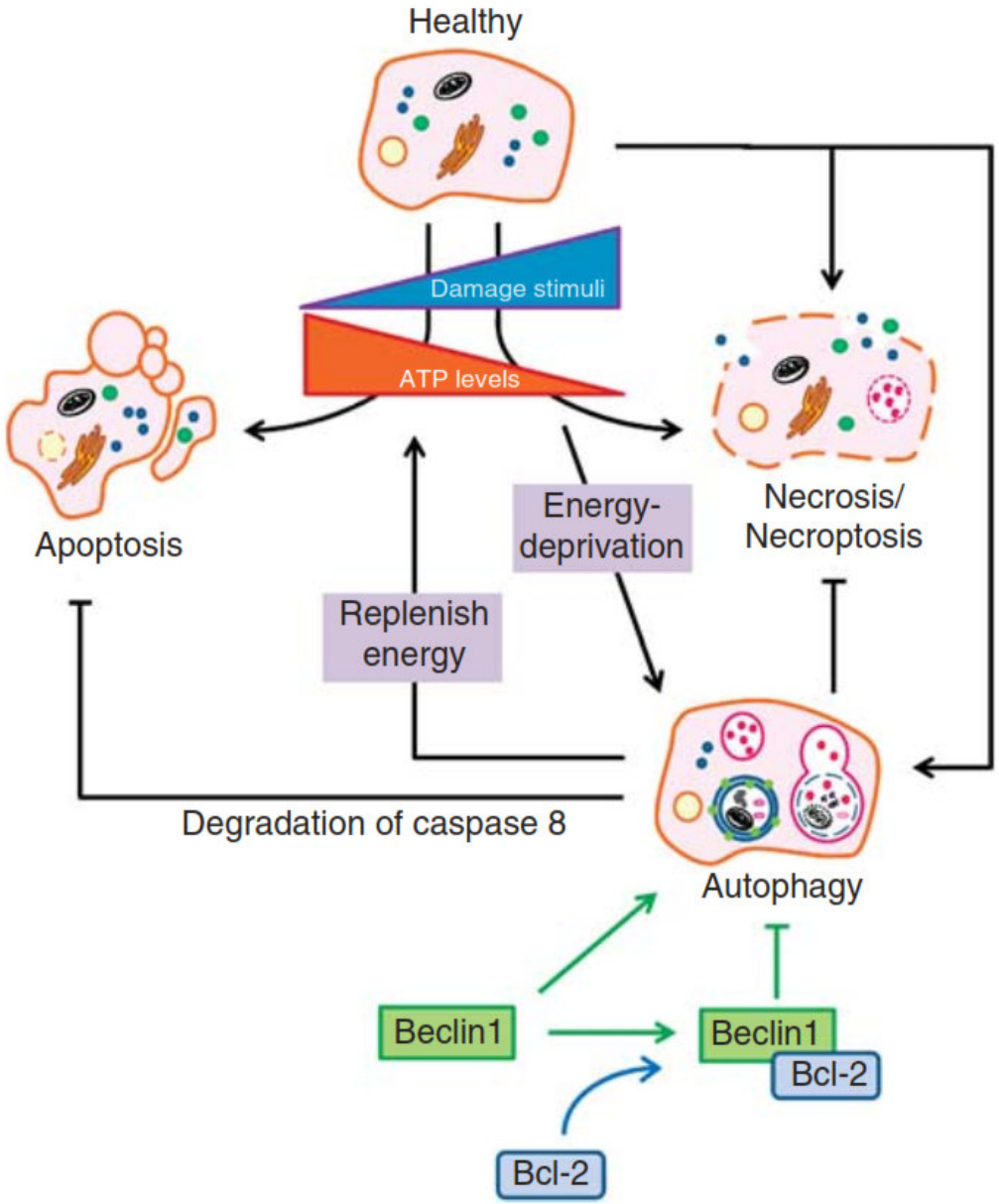
Meylan E, et al., Nat Immunol. 2004 May;5(5):503-7.

Necroptosis induction by TLRs



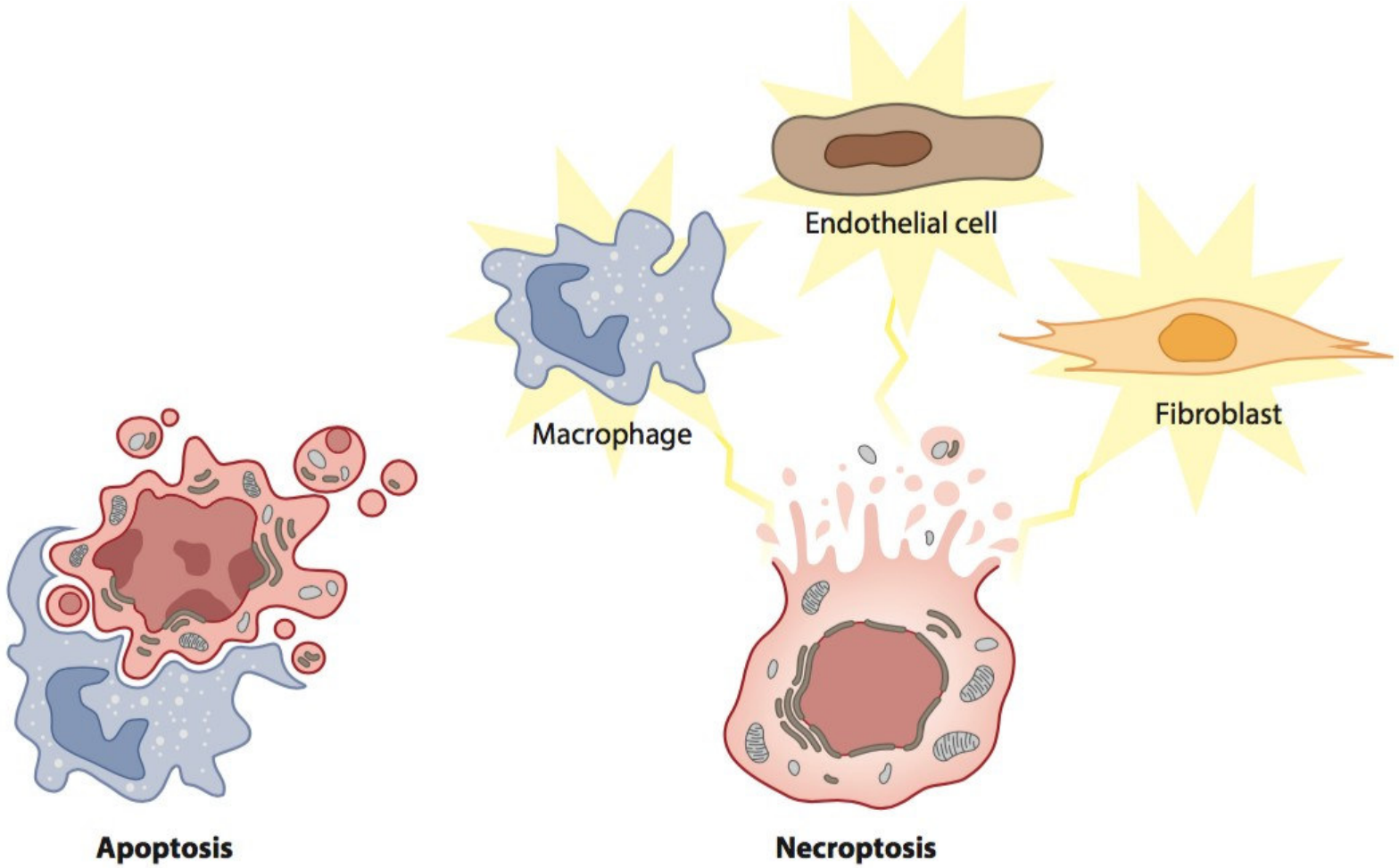
Newton, Annu. Rev. Biochem. 2016:85

Energy requirements of apoptosis vs. necroptosis



- the decision taken by a cell to undergo apoptosis or necroptosis is governed by various factors including energy/ATP availability and the amplitude of damage or stress
- cells die from necroptosis rather than apoptosis when intracellular ATP is depleted prior to otherwise apoptotic stimuli
- ATP is required for the formation of cytochromeC/Apaf-1/procaspase-9 complexes and for chromatin condensation
- intracellular ATP in apoptotic cells is ultimately depleted and/or released to mediate phagocyte attraction
- low ATP levels often stimulate autophagy to sustain cell viability and prevent necroptosis
- apoptosis regulates autophagy through Bcl-2 mediated sequestration or caspase-dependent cleavage of Beclin 1 while autophagy impedes apoptosis by degrading caspase-8

Immunogenic cell death

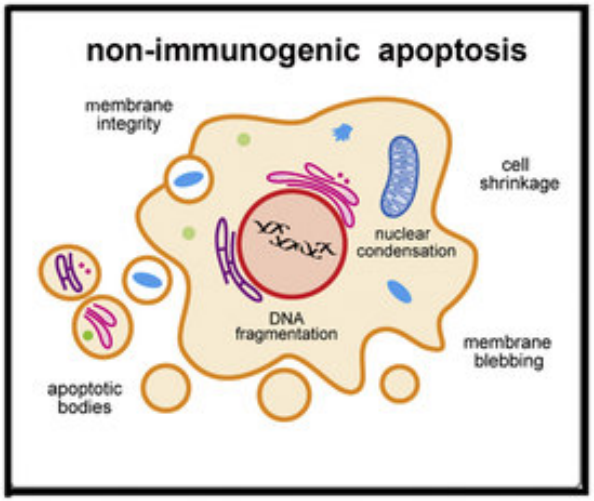


Cells dying by apoptosis are often dismantled into membrane enveloped fragments that are cleared rapidly by phagocytic cells.

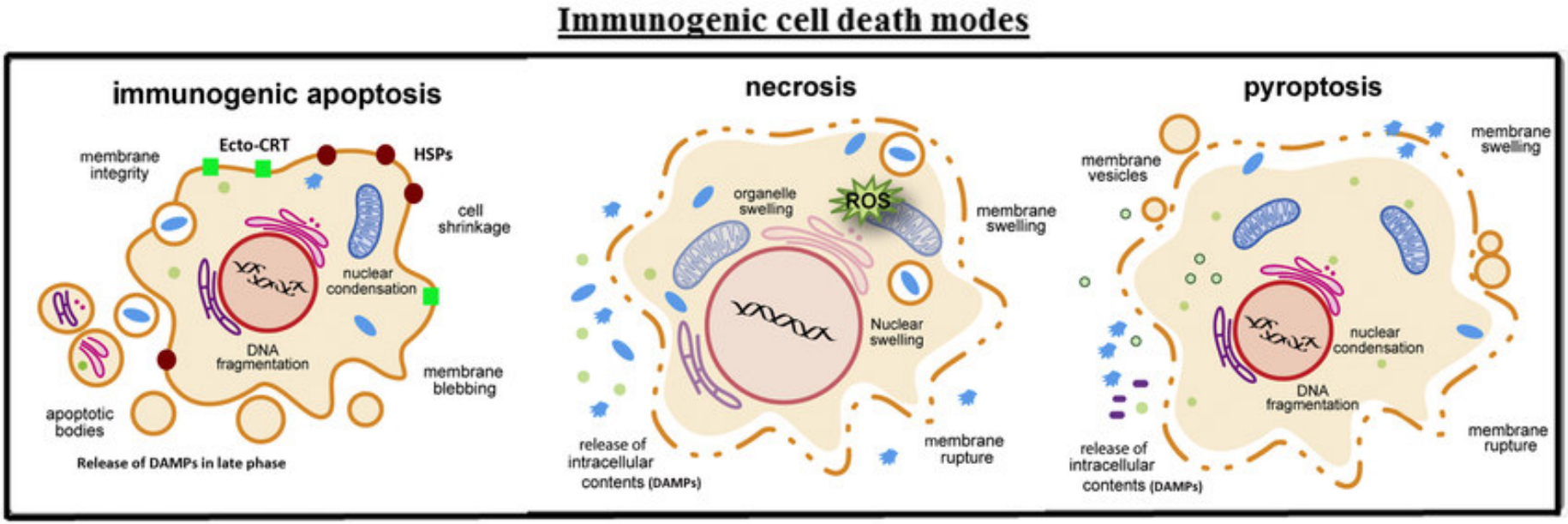
Cells dying by necroptosis rupture and release intracellular content that can be sensed as damage signal by macrophages, fibroblasts, epithelial and endothelial cells and can elicit an innate immune response.

Annu. Rev. Biochem. 2016. 85:4.1–4.21

Immunogenic vs. non-immunogenic forms of cell death

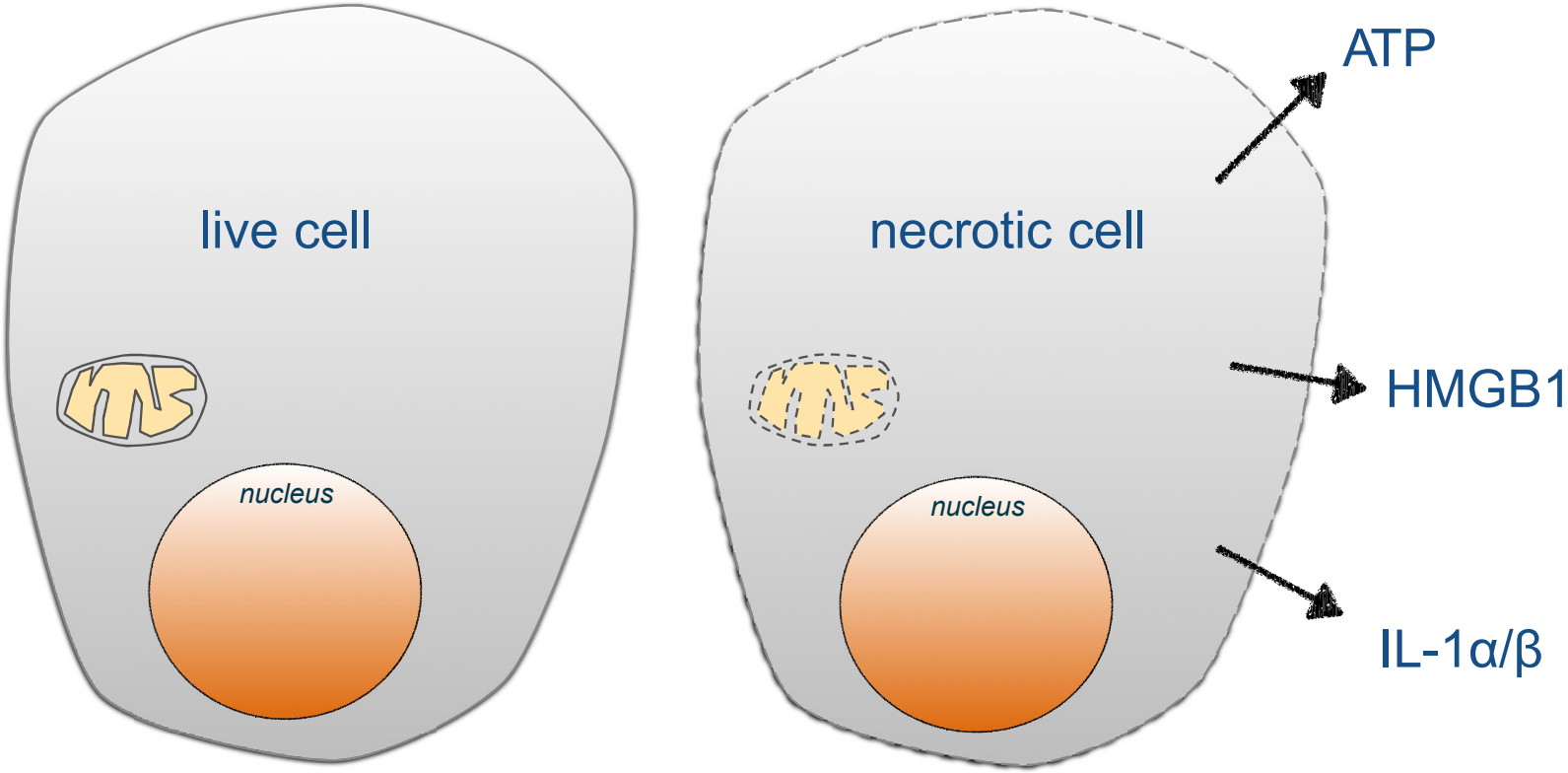


- in <u>apoptosis</u>, retention of plasma membrane integrity and formation of apoptotic bodies render it a non-immunogenic cell death
- in <u>necroptosis</u>, secretion of pro-inflammatory cytokines and release of cytoplasmic content, including DAMPs (ATP, HMGB1, and uric acid, etc.) trigger inflammation
- However, immunogenicity is difficult to predict and immunogenic forms of apoptosis and non-immunogenic forms of necrosis have been observed
- Irradiation and certain chemotherapies (e.g. oxaliplatin but not cisplatin) lead to an immunogenic apoptosis with surface exposure of calreticulin (ecto-CRT) and heat-shock proteins (HSPs) prior to apoptosis, and other DAMPs released in the later phase

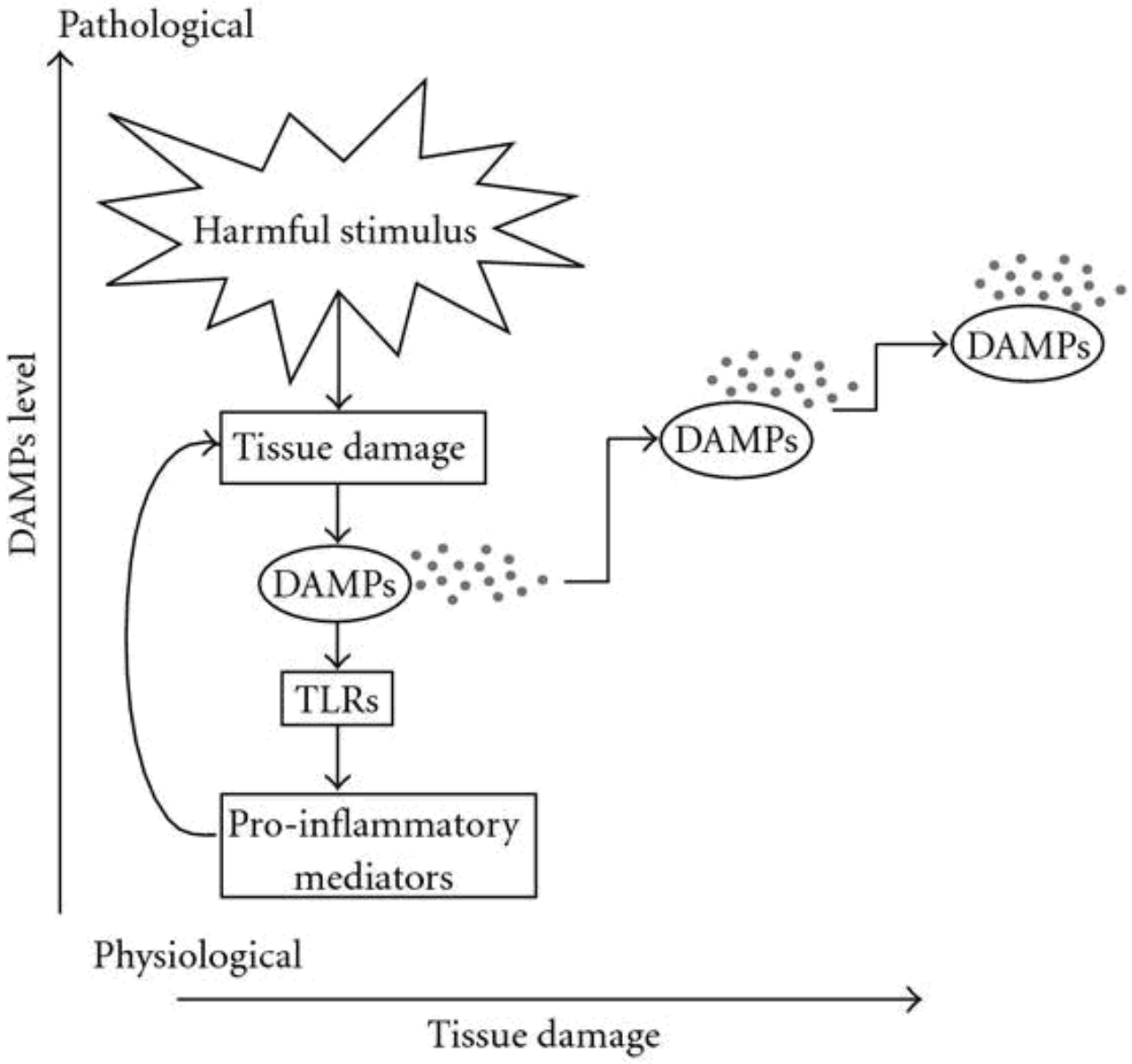


Guo et al. Frontiers Oncol 2014

Release of DAMPs (damage-associated molecular pattern)



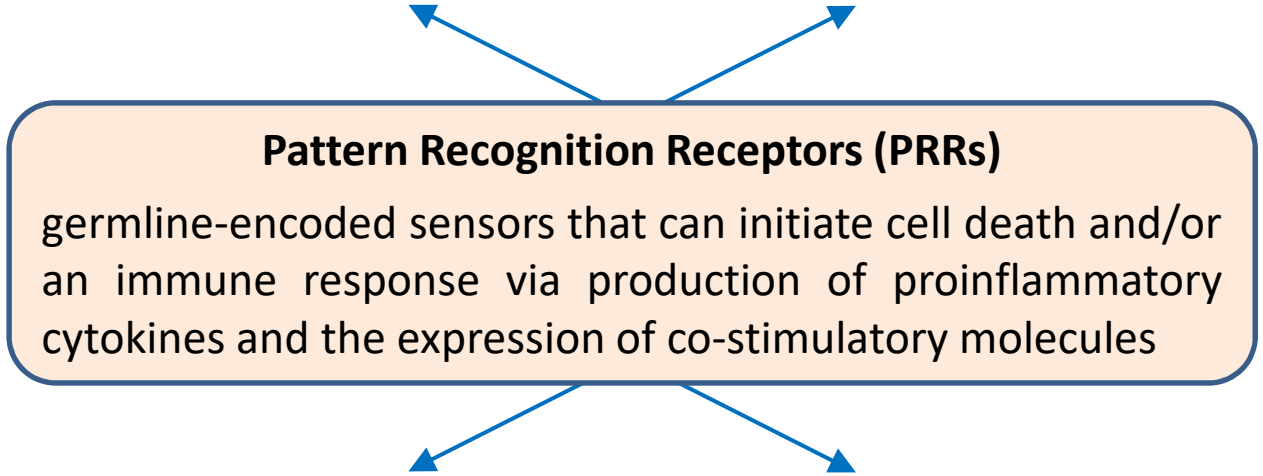
Release of DAMPs can trigger a cascade of activity enhancing tissue damage



Pattern Recognition Receptors (PRRs)

Pathogen associated Molecular Patterns (PAMPs)
highly conserved molecules typical of many pathogens
e.g. dsRNA (TLR3), LPS (TLR4), flagellin (TLR5), non-methylated CpG (TLR9), lipoteichoic acids on gram-positive bacteria (SRA1/MSR1/CD204)

Damage Associated Molecular Patterns (DAMPs)
Endogenous ligands released by distressed or damaged cells (Sterile Inflammation)
e.g. ATP, HMGB1, F-actin



PRRs
- Toll- like receptors (TLRs)
- NOD- like receptors (NLRs)
- retinoic acid-inducible gene I (RIG- I)-like receptors (RLRs)
- C- type lectin receptors (CLRs)
- intracellular DNA sensors

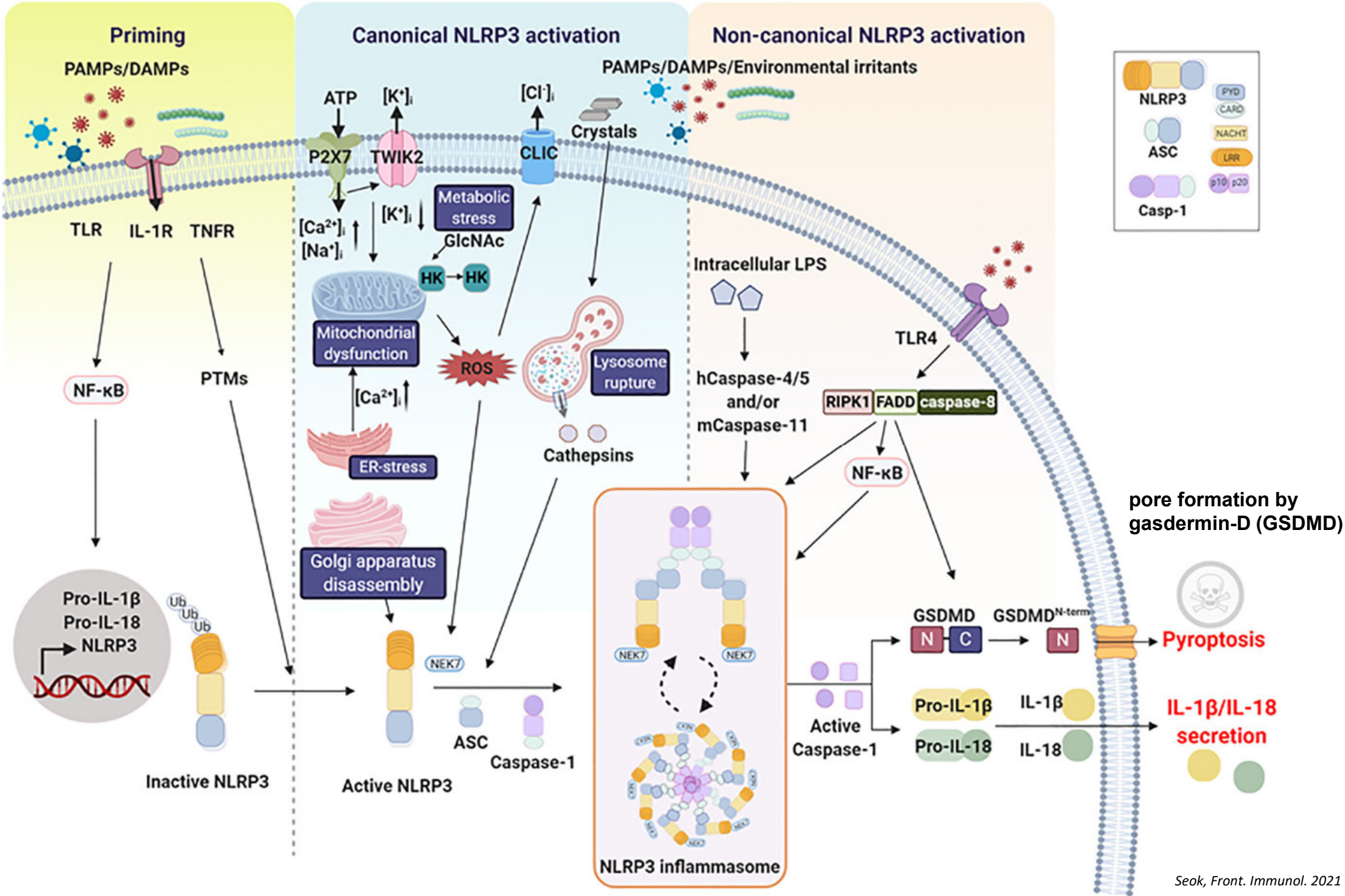
non-classical PRRs
- receptor for advanced glycation end products (RAGE)
- triggering receptors expressed on myeloid cells (TREMs)
- certain G- protein-coupled receptors (GPCRs)
- ion channels

PAMPs/DAMPs and their receptors

Sensing receptor	PAMPs/DAMPs	Pro-inflammatory functions
TLRs		
TLR2	HMGB1, several HSPs, SNAPIN, versican, biglycan, decorin, eosinophil-derived neurotoxin, surfactant protein A/D, β-defensin 3, histone, SAA, Aβ, β2-glycoprotein I	Promotes the production of pro-inflammatory cytokines and chemokines
TLR3	mRNA	
TLR4	HMGB1, tenascin-C, several HSPs, S100s, HMGN1, biglycan, decorin, heparin sulfate, hyaluronic acid, fibrinogen, fibronectin, β-defensin 2, surfactant protein A/D, lactoferrin, neutrophil elastase, peroxiredoxin, histone, SAA, ox-LDL	Promotes the production of pro-inflammatory cytokines, chemokines and IFN-I
TLR7	IgG–ribonucleoprotein complex, microRNAs	Promotes the production of IFNα and other cytokines and chemokines
TLR9	IgG–chromatin complex, mtDNA, HMGB1	Promotes the production of IFNα and other cytokines and chemokines
CLRs		
DNGR1	F-actin	Promotes DC antigen cross-presentation, inhibits IL-10 production
MINCLE	Sin3A-associated protein 130, β-glucosylceramide	Promotes pro-inflammatory cytokine production
Dectin-1	N-glycans	Promotes IRF5-dependent gene expression
NLRs		
NLRP3	MSU, glucose, cholesterol crystals, Aβ, ATP, oxPAPC, Alu-RNA	Promotes IL-1β and IL-18 secretion and initiates pyroptosis
RLRs		
RIG-I	Endogenous 5′ppp RNA	Promotes the production of IFN-I and other cytokines and chemokines
MDA5	Unedited long self-dsRNA, endogenous retroviral RNA	Promotes the productionof IFN-I and other cytokines
Cytoplasmic DNA sensors		
cGAS	Cytoplasmic DNA	Promotes the production of IFN-I and other cytokines
AIM2	Cytoplasmic DNA, damaged DNA in the nucleus	Promotes IL-1β and IL-18 secretion and initiates pyroptosis

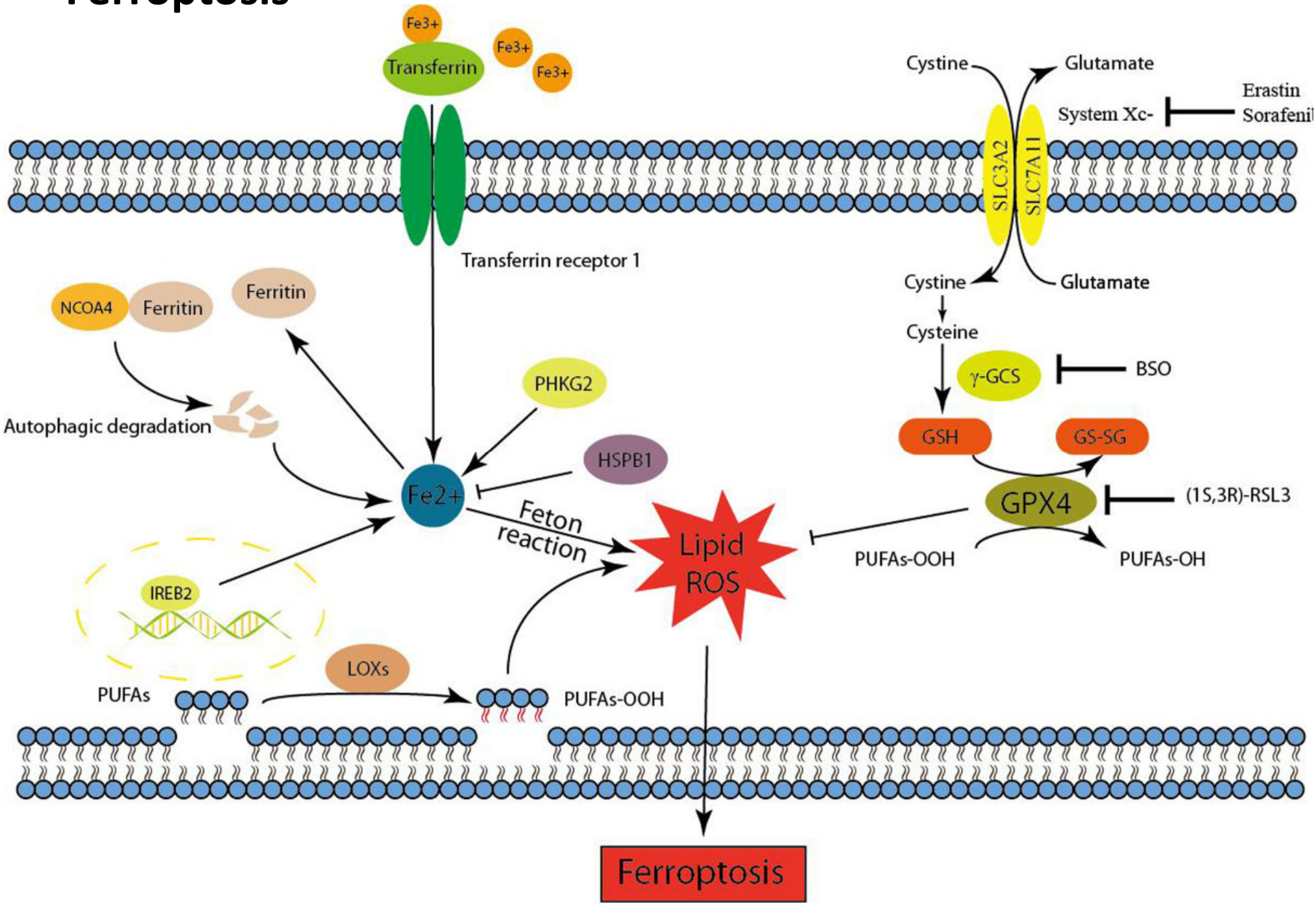
Sensing receptor	PAMPs/DAMPs	Pro-inflammatory functions
RAGE		
RAGE	AGEs, HMGB1, S100s, Aβ, DNA	Promotes the expression of pro-inflammatory genes, as well as cell migration, proliferation and apoptosis
TREMs		
TREM1	HMGB1, HSP70, PGLYRP1, actin	Myocardial infarction, atherosclerosis, RA, ureteralobstruction, cancer
TREM2	PA, PC, PE, PG, PI, PS, CL, SF, SM, APOA1, APOA2, APOB, APOE, APOJ, LDL, HDL, VLDL, Lp(a), HSP60	Modulates cell differentiation, survival, phagocytosis, chemotaxis
GPCRs		
FPR1	N-formylated peptides, cathepsinG, FAM19A4, annexin 1	Promotes chemotaxis of neutrophils and monocytes/macrophages
FPR2	Aβ42, SAA, oxLDL, LL-37 and other peptidesATP, UTP	Promotes chemotaxis of neutrophils and monocytes/macrophages
P2Y2R	ATP, UTP	Promotes migrationand activation of various immune cells
P2Y6R	UDP	Promotes proliferation and cytokine and chemokine production in stromal cells
P2Y12R	ADP	Promotes platelet activation and Th17 differentiation
CaSR	Ca2+	Promotes monocyte/macrophage recruitment and NLRP3 activation
GPRC6A	Ca2+	Promotes NLRP3 activation
Ion channels		
TRPM2	ROS	Promotes chemokine production and NLRP3 activation
Other TRPs	ROS	Promotes the production of inflammatory neuropeptides
P2X7R	ATP	Promotes cytokine andchemokine production, NLRP3 inflammasome activation and T cell activation

Pyroptosis



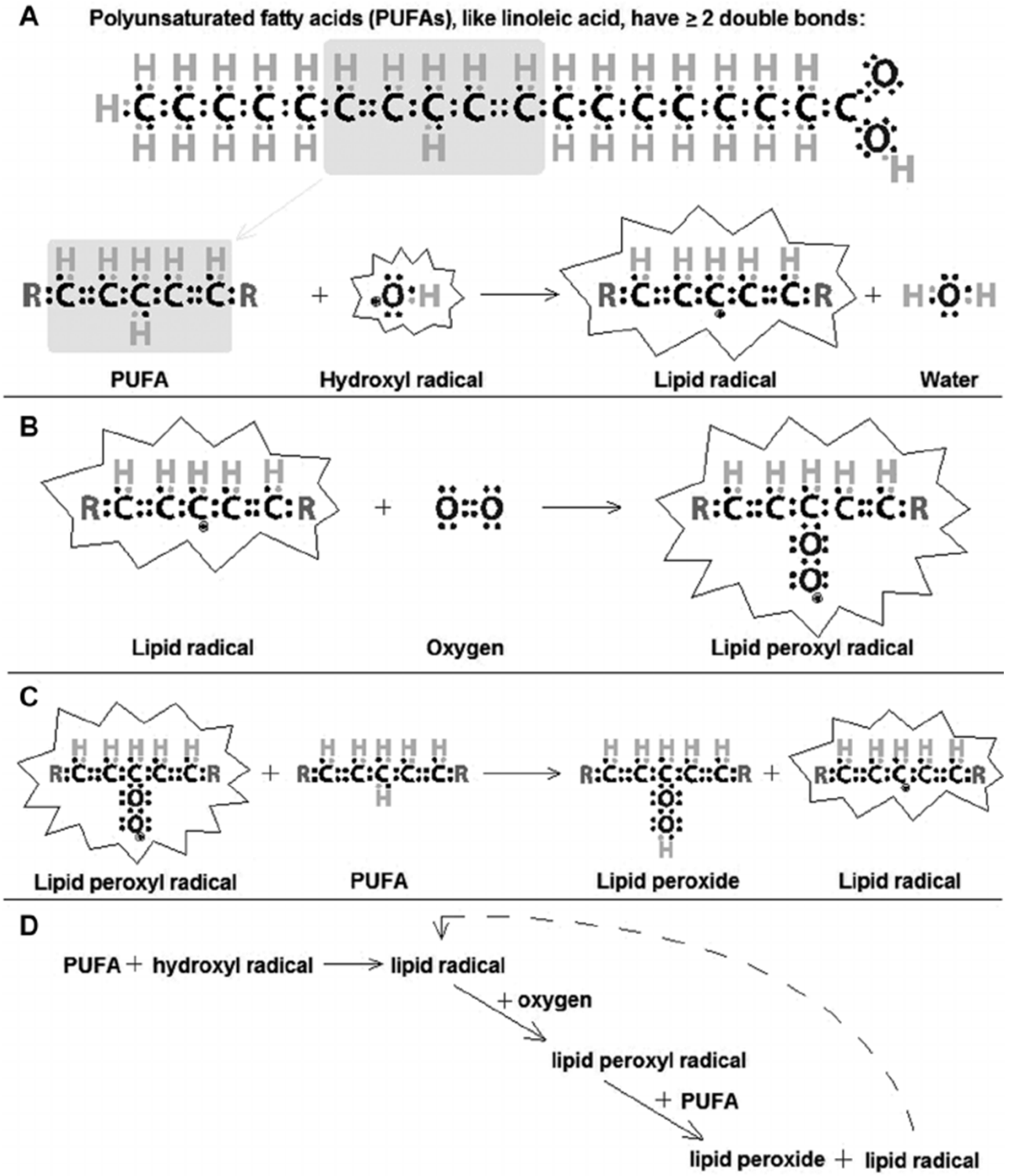
Seok, Front. Immunol. 2021

Ferroptosis

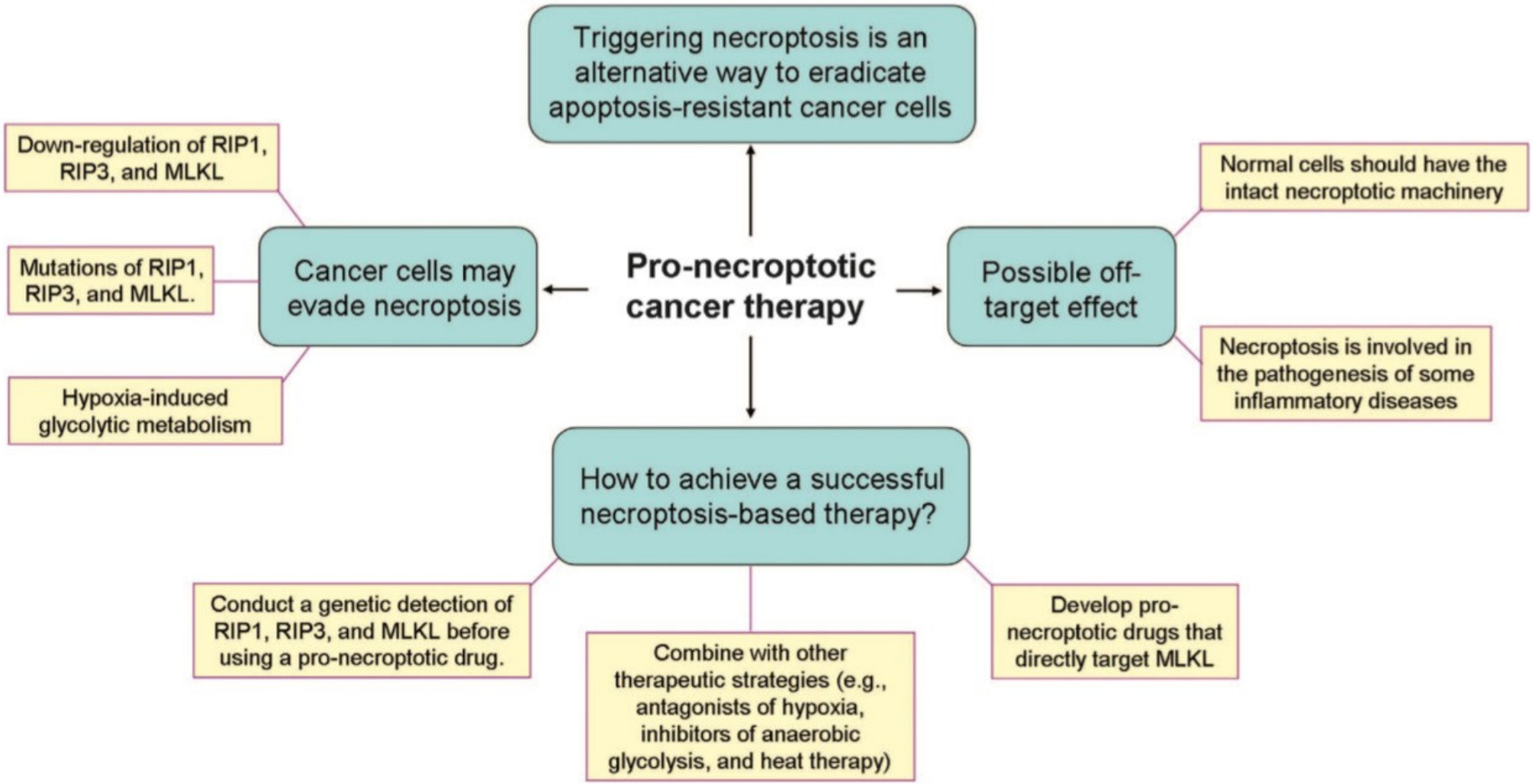


Lu, Front. Pharmacol, 2018

Lipid radicals



Does necroptosis induction offer a therapeutic opportunity in cancer ?



Cell Death and Differentiation (2016), 1–9