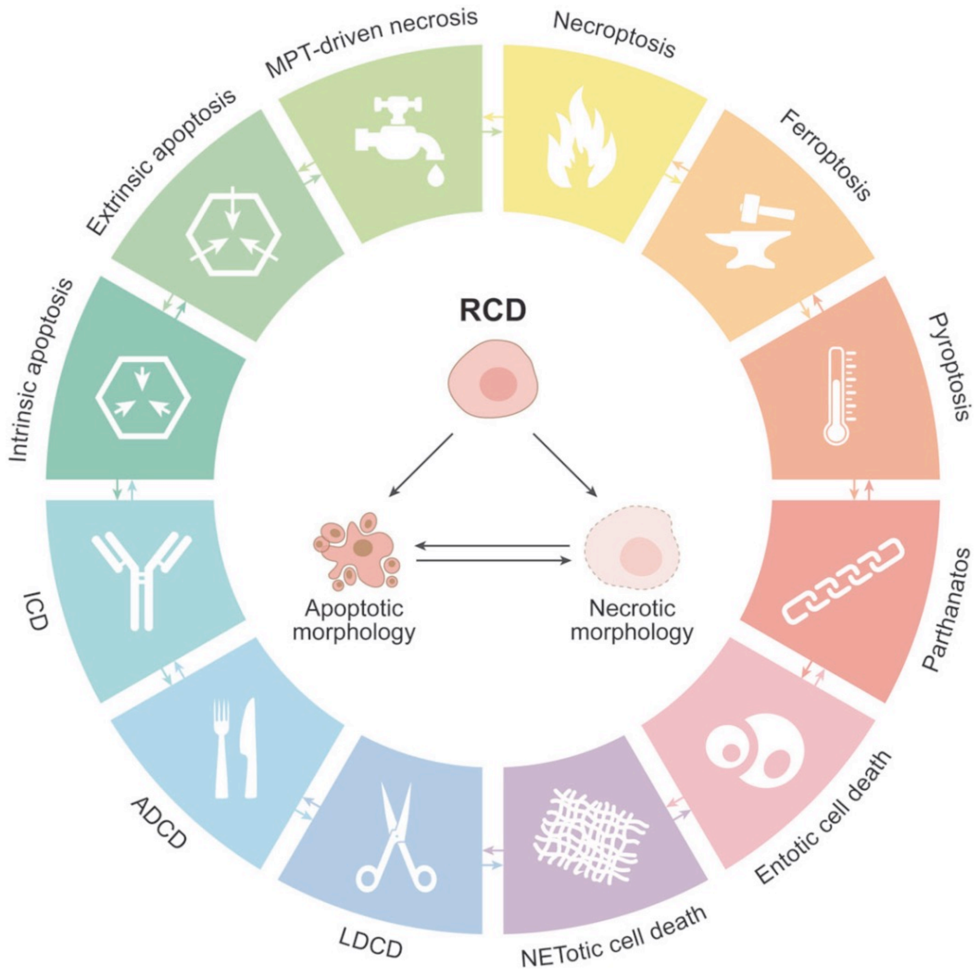
Regulated cell deaths and cancer



université PARIS-SACLAY
GRADUATE SCHOOL
Health and Drug Sciences

B. BENOIT, TU n°05, Paris Saclay, 2024-2025

M1 International, Cancer Cell Biology, TU n°05

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FACULTÉ DE PHARMACIE

Hallmarks of cancer : resistance to cell death

Adapted from Hanahan et Weinberg. Cell 2011

Different types of cell deaths

Accidental Cell Death (ACD)

- Extreme environmental conditions (pressure, T°C, osmotic imbalances, pH…)
- Instantaneous physical disassembly of the plasma membrane (PM)

Regulated Cell Deaths (RCD)

- Genetically regulated
- Morphological features
- Can signal damage to neighboring cells *via* DAMPs (*Damage-Associated Molecular Patterns*) / Alarmins

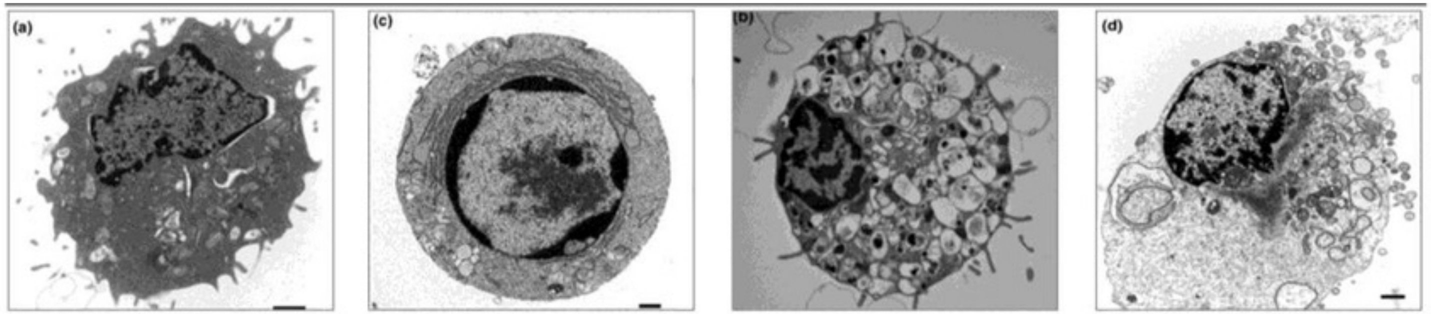
too eventually trigger a sterile inflammatory response

Galuzzi et al, Cell Death & Differ., 2018

Regulated cell deaths (RCDs)

- Different types of cell death : essential for organisms' life
- Elimination of superfluous, irreversibly damaged or potentially harmful cells
- Tissue homeostasis: balance between cell death and proliferation
- Initial classification: morphological alterations

	Cell death		
Live cell	Type I: Apoptosis	Type II: Autophagy	Type III: Necrosis



	1972, J. Kerr, J. Cormack	1974, C. De Duve	

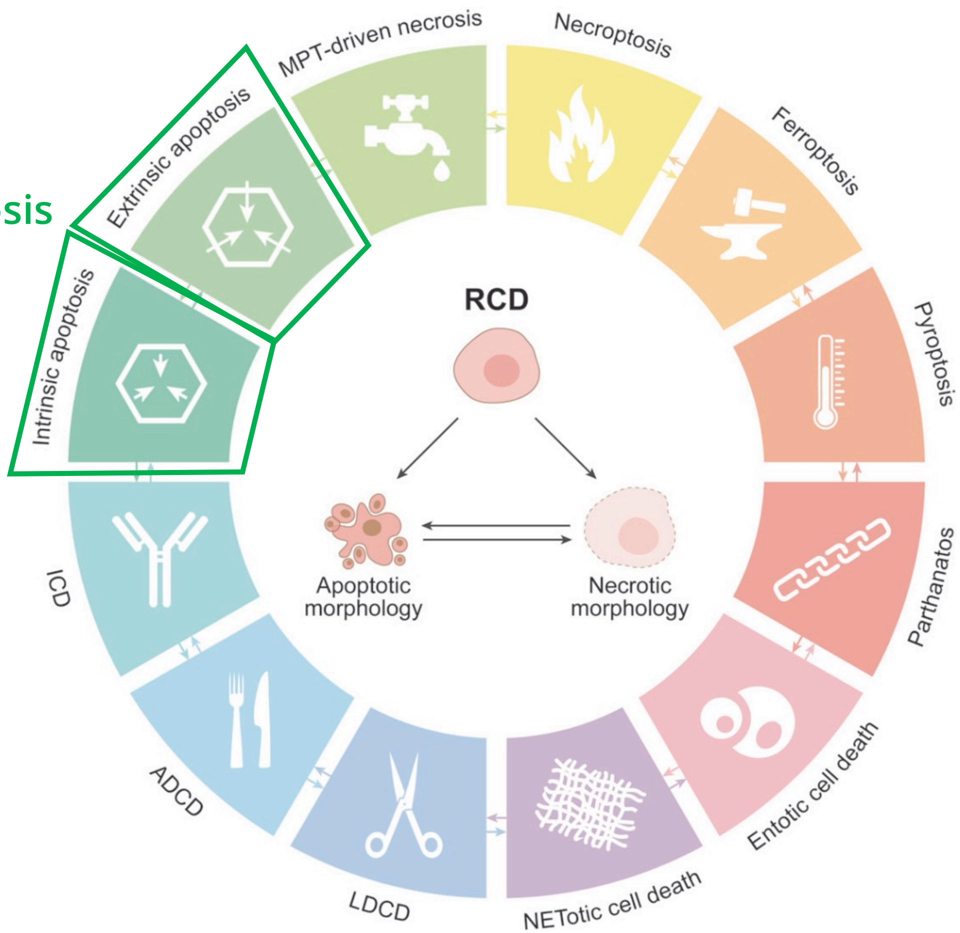
and many others …

Regulated cell deaths : apoptosis

Apoptosis

Nobel Prize in Physiology or Medicine 2002,
Brenner, Horvitz and Sulston

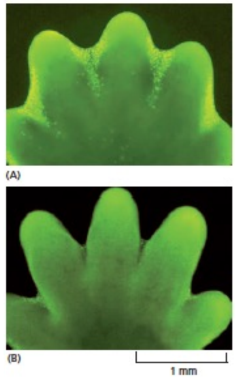
Genetic regulation of organ development and programmed cell death



Galuzzi et al, Cell Death & Differ., 2018

Apoptosis : physiological function

Interdigit space elimination



Beneficial for organisms, embryo development and adults

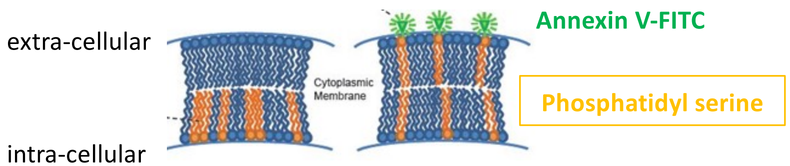
- Morphogenesis and structure
- Elimination of structures
- Immune system maturation: elimination of autoreactive T lymphocytes
- Nervous system maturation: elimination of neurons which do not reach their target
- Elimination of damaged and infected cells
- Tissue / organ size homeostasis (skin, blood, intestine)

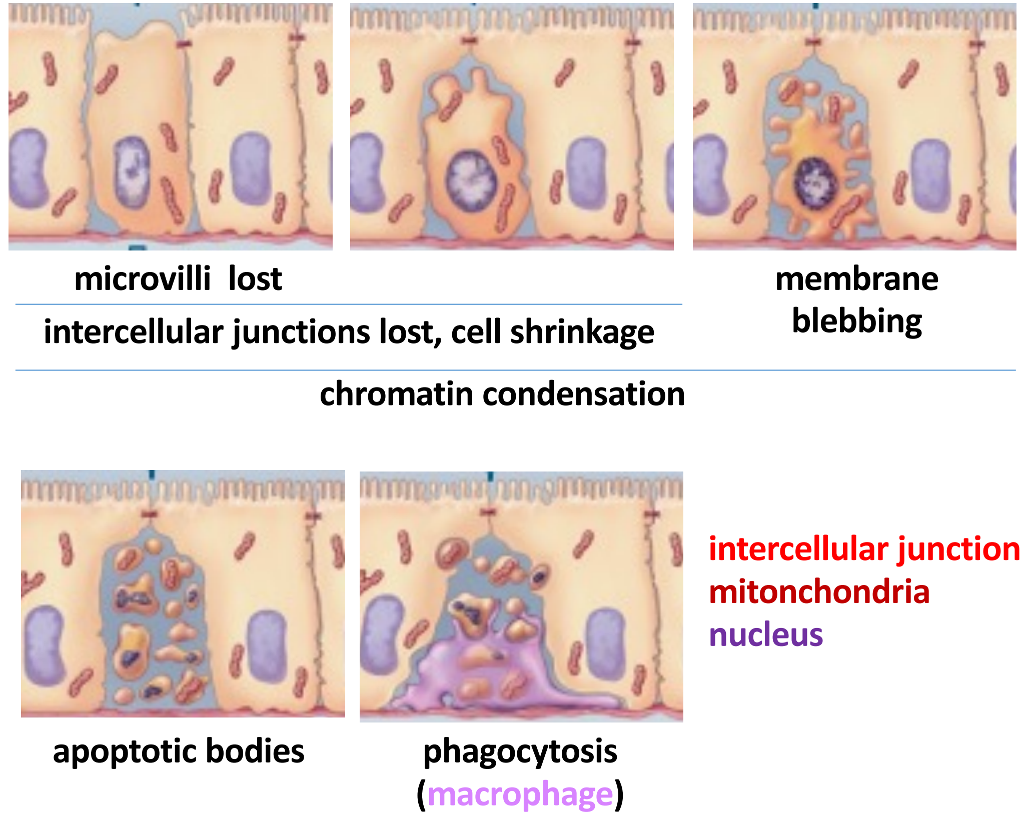
tail elimination

Apoptosis morphological and molecular features

Clean / silent cell death (no or low inflammation)

- Plasma membrane (PM) integrity preserved
- Loss of cell junctions
- PM asymmetry loss → PtdSer lipid externalization
- DNA and nucleus fragmentation
- Membrane budding and apoptotic bodies formation





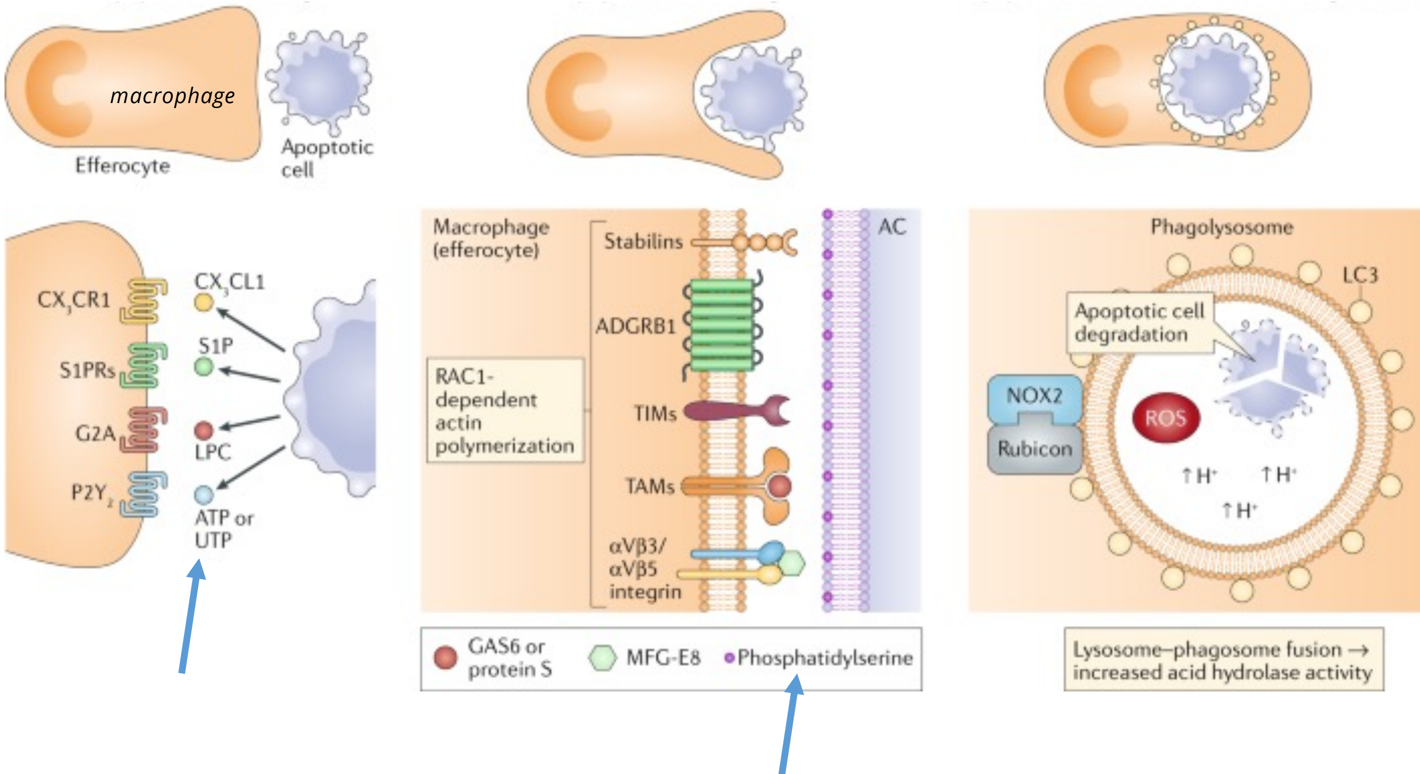
Adapted from Cell Biology third edition, T. Pollard, W. Earnshaw, Elsevier

A silent / clean cell death with elimination of apoptotic cells/bodies by phagocytes

« *Find-me* » Signals

« *Eat-me* » Signals

Lysosomal degradation
Anti-inflammatory response



No late apoptosis / secondary necrosis in an organism

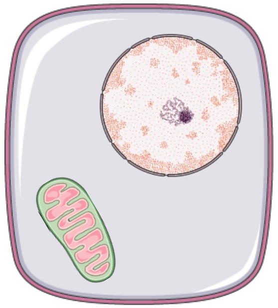
Doran et al, Nat Rev Immunol, 2019

The molecular program of apoptosis : 3 phases

INITIATION

- DNA damage
- Growth factor deprivation
- Cell cycle arrest
- **Anticancer agent**
- Death receptor activation

DECISION



Bcl-2 protein Family

EXECUTION

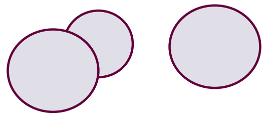
Caspase cascade

↓

Cell protein cleavage

↓

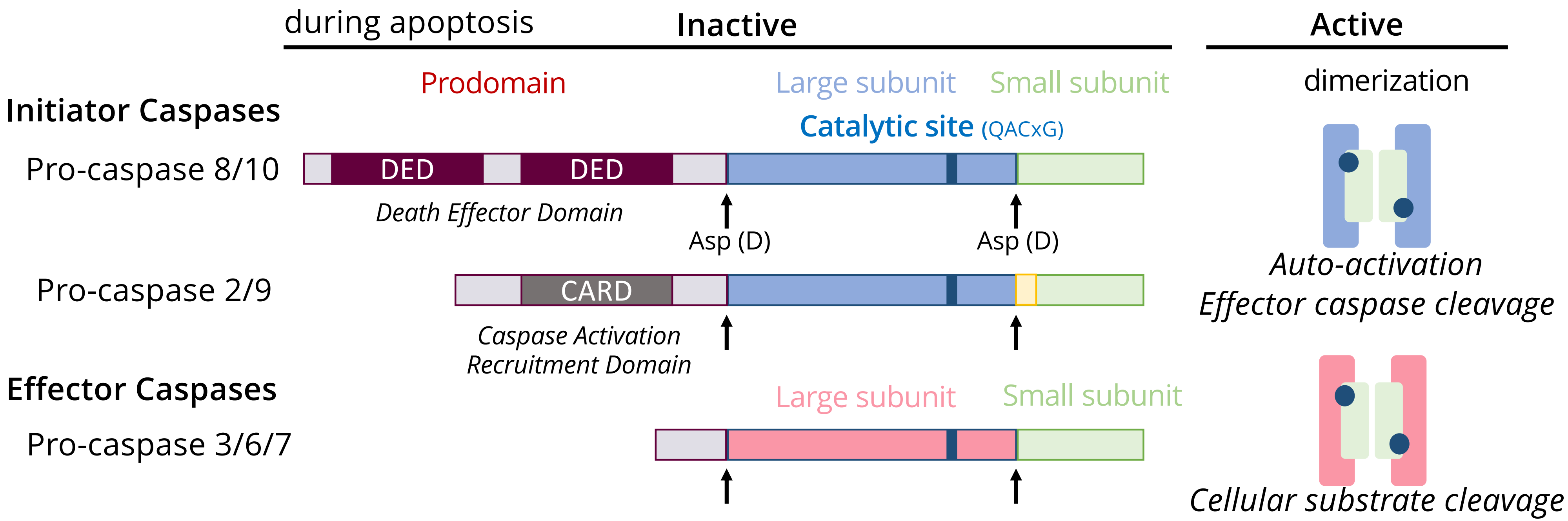
Apoptotic cell features



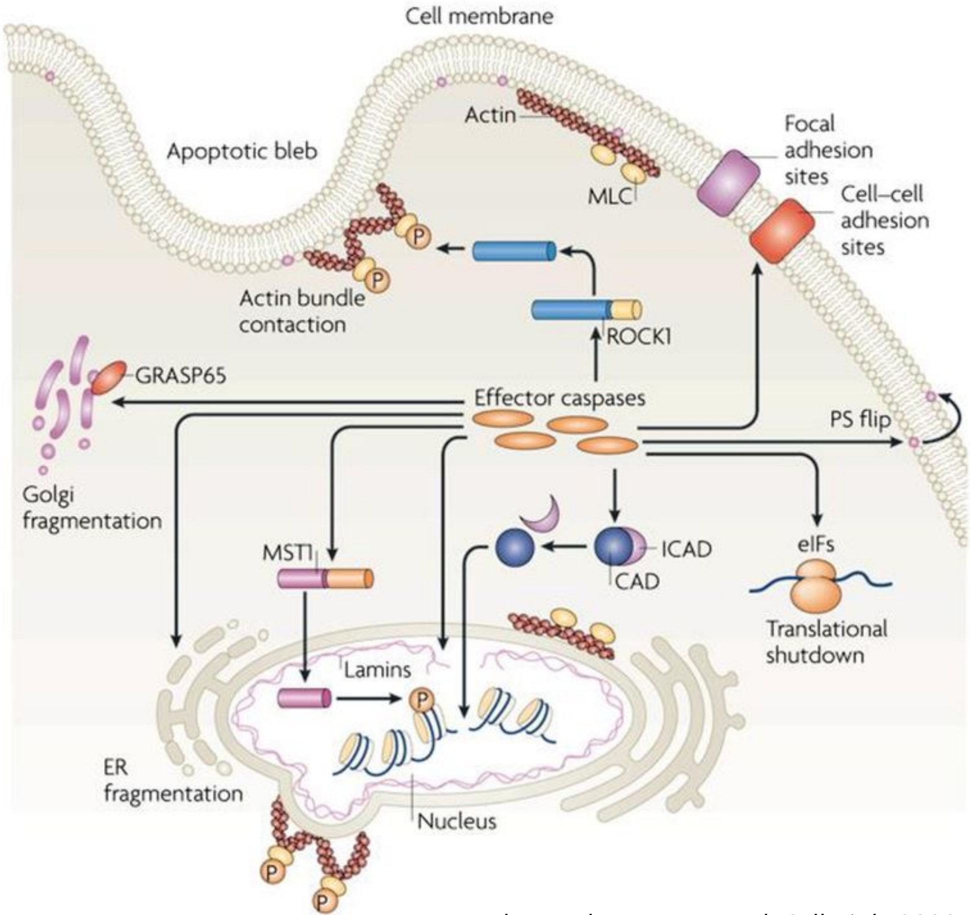
Caspases are key regulators / effectors of apoptosis

Caspase = **C**ysteinyl-**Asp**artate-cleaving prote**ases** or **C**ysteine-**ASP**art**ASES**

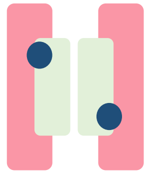
- Family of cytosolic proteases (11 in human cells)
- Cysteine residue in catalytic site
- Synthetized as inactive precursors and activated by cleavage and dimerization during apoptosis



Apoptosis triggers the cleavage of many substrates by effector caspases



Taylor et al., Nat. Rev. Mol. Cell Biol., 2008

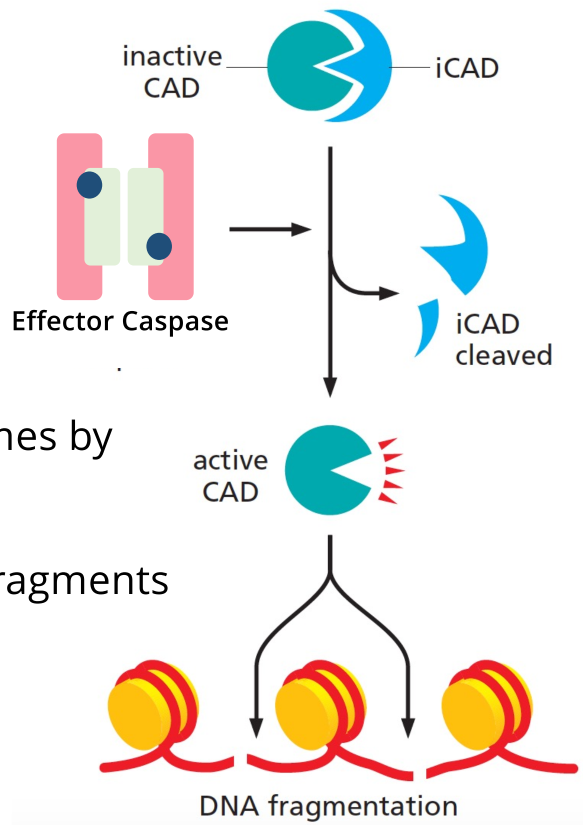


Effector Caspases
(3/6/7)

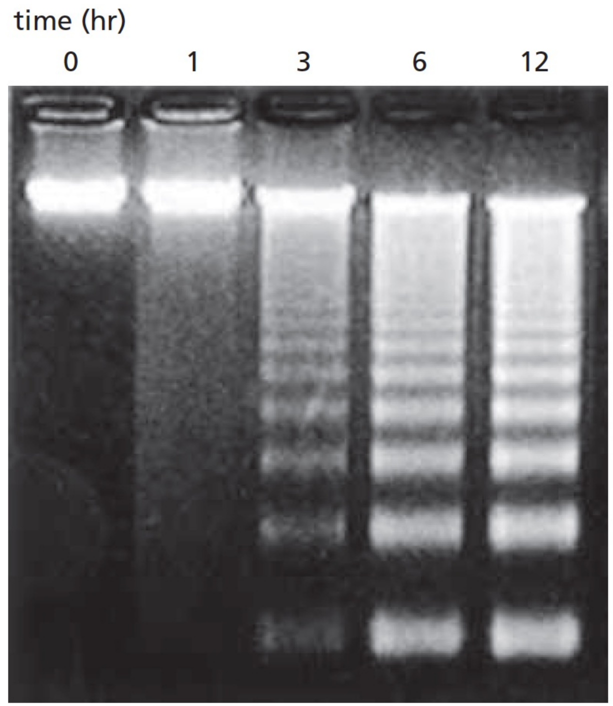
Cellular Substrate cleaved

- Cell adhesion proteins (junctions loss)
- Golgi protein (Golgi fragmentation)
- Translation protein eIF (stop translation)
- Rock1 / actin (apoptotic blebbing MP and NE)
- Lamins (nuclear fragmentation)
- MST1 (H2B histone kinase) : chromatin condensation
- ....

Apoptosis triggers the cleavage of many substrates by effector caspases



DNA cleavage between nucleosomes by
CAD (*Caspase Activated DNAse*)
"DNA ladder" multiple of 180 bp fragments

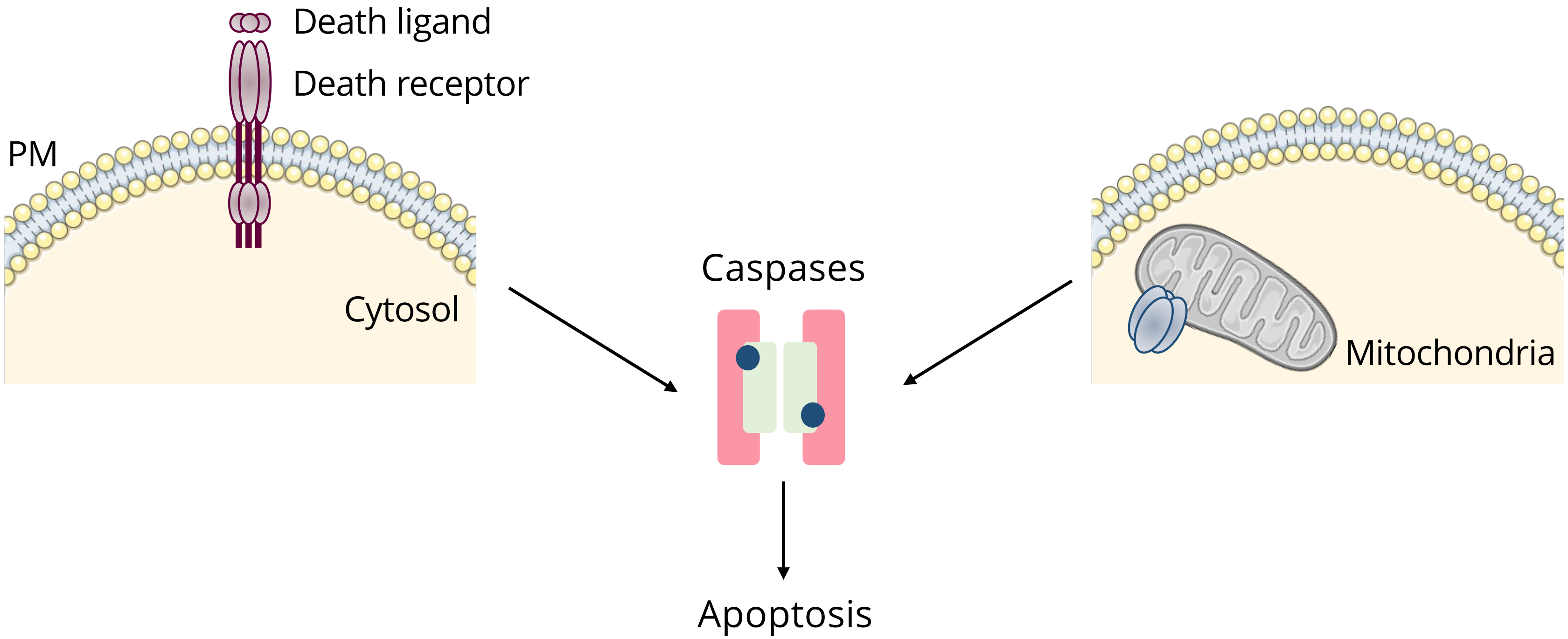


Molecular Biology of the Cell 6th edition

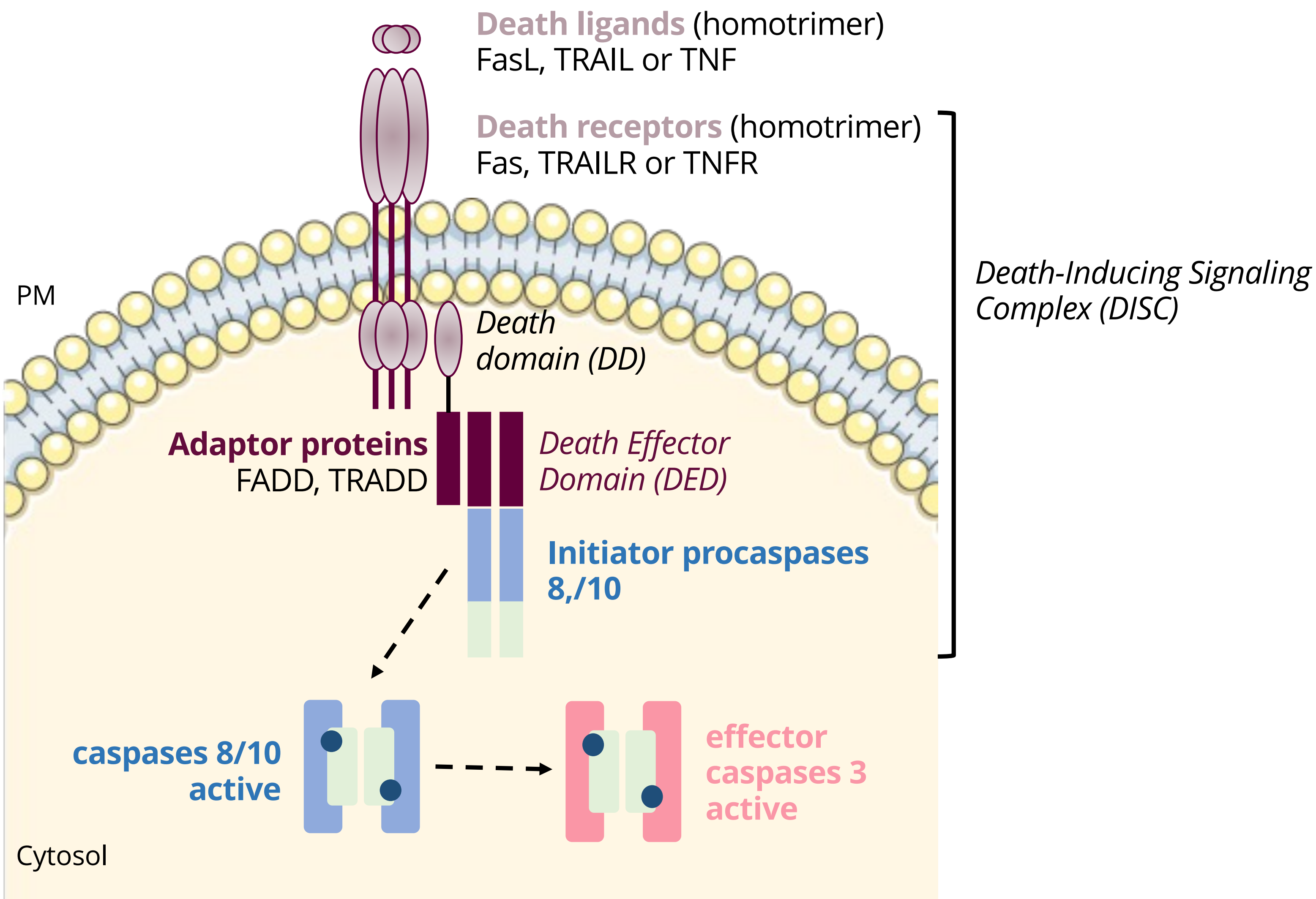
Two main apoptotic pathways

Extrinsic / death receptor pathway

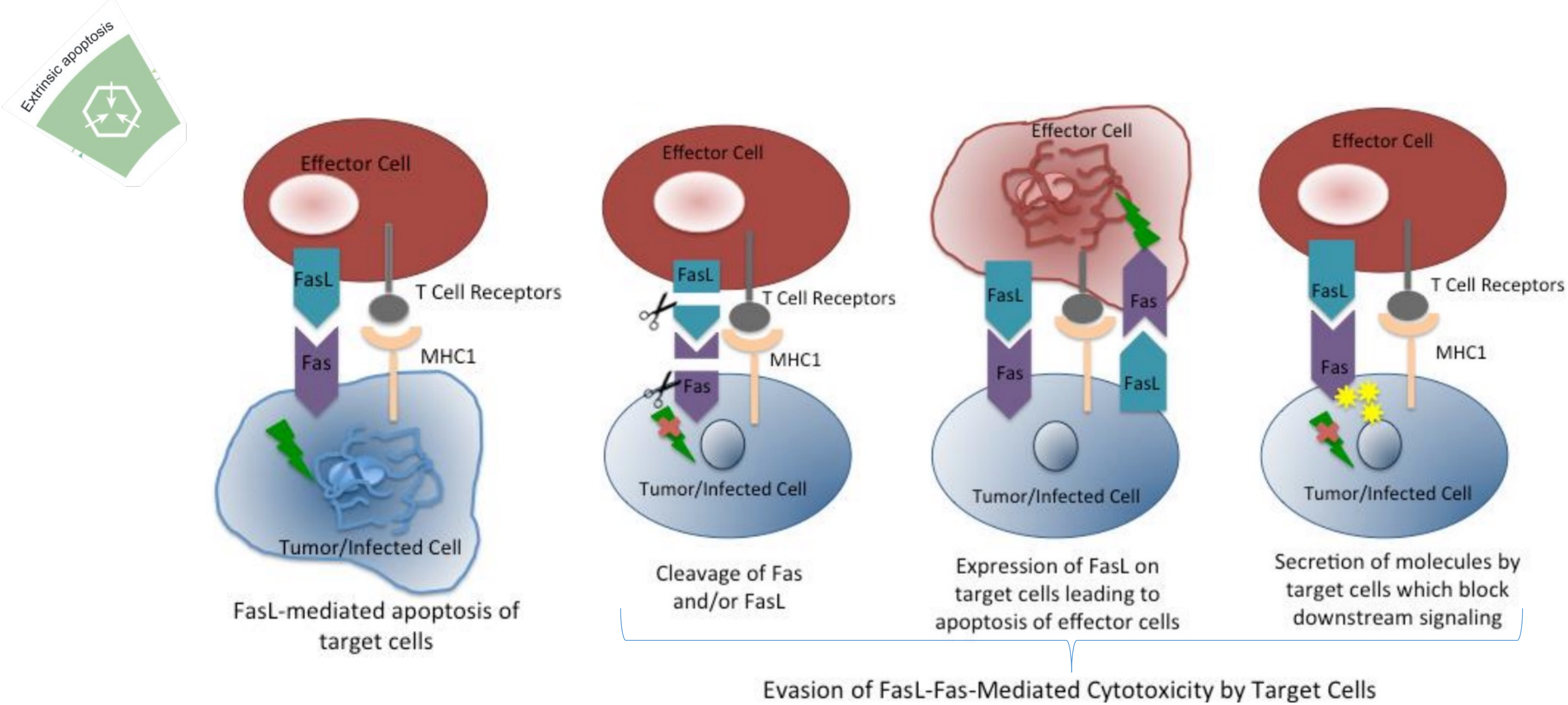
Intrinsic / mitochondrial pathway



Extrinsic / death receptor apoptotic pathway

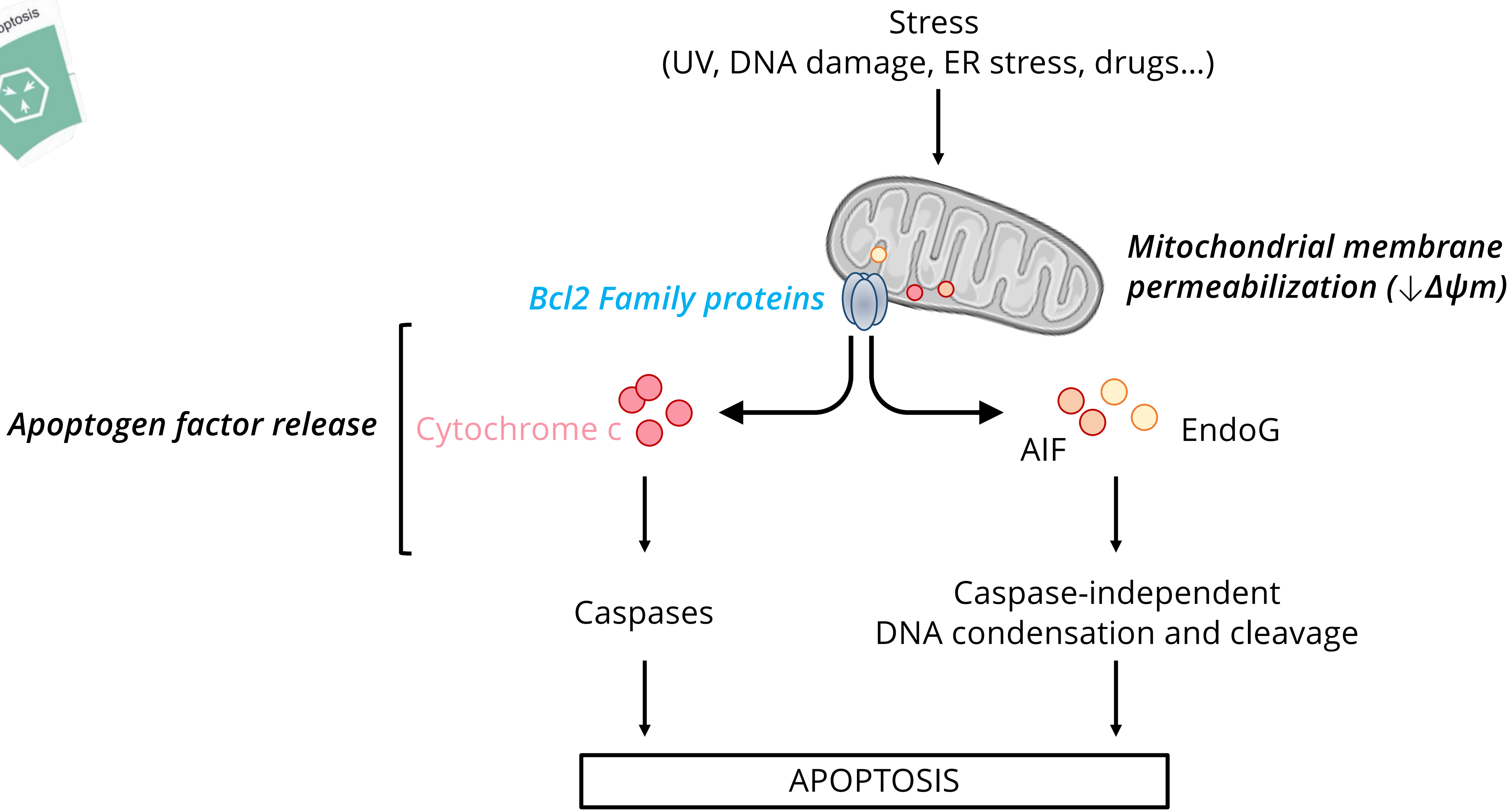


Fas/FasL in immune T cells and cancer cells



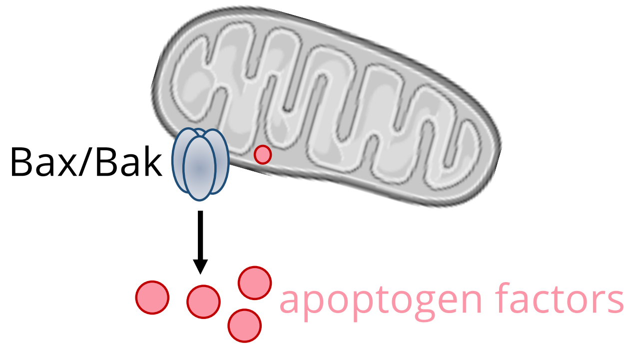
Kingfisher Biotech, 2015

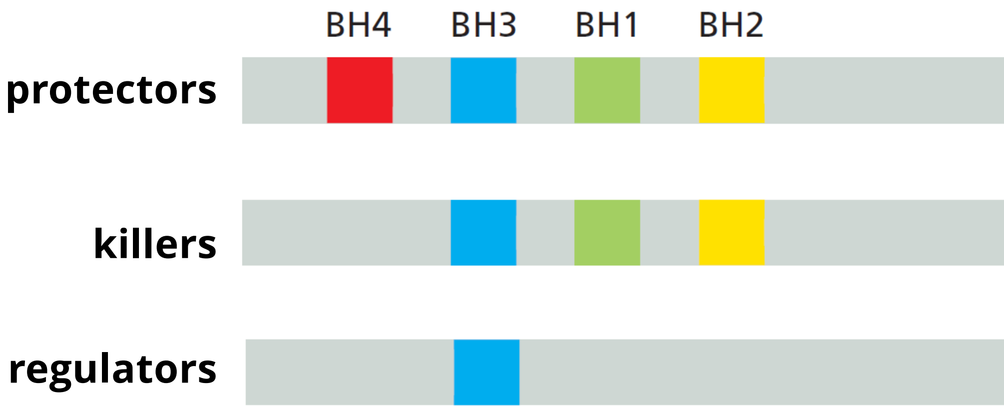
Intrinsic / mitochondrial apoptotic pathway



Intrinsic / mitochondrial apoptotic pathway : the Bcl2 family proteins

- 3 classes
- Regulate apoptogen factor release from mitochondria
- Pro- or anti- apoptotic functions
- Interactions modulate their activity





BH = Bcl-2 Homology Domain

anti-apoptotic Bcl2 family

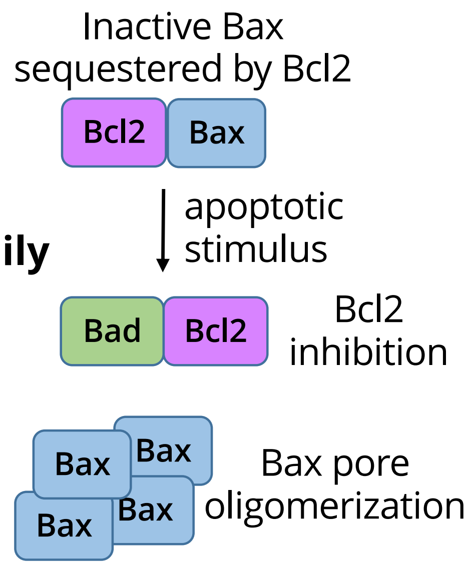
(Bcl2, BclX$_L$, MCL1…) Bcl2

pro-apoptotic effector Bcl2 family

(Bax, Bak, Bok) Bax

pro-apoptotic BH3-only family

(Bad, Bim, Bid, Puma, Noxa…) Bad



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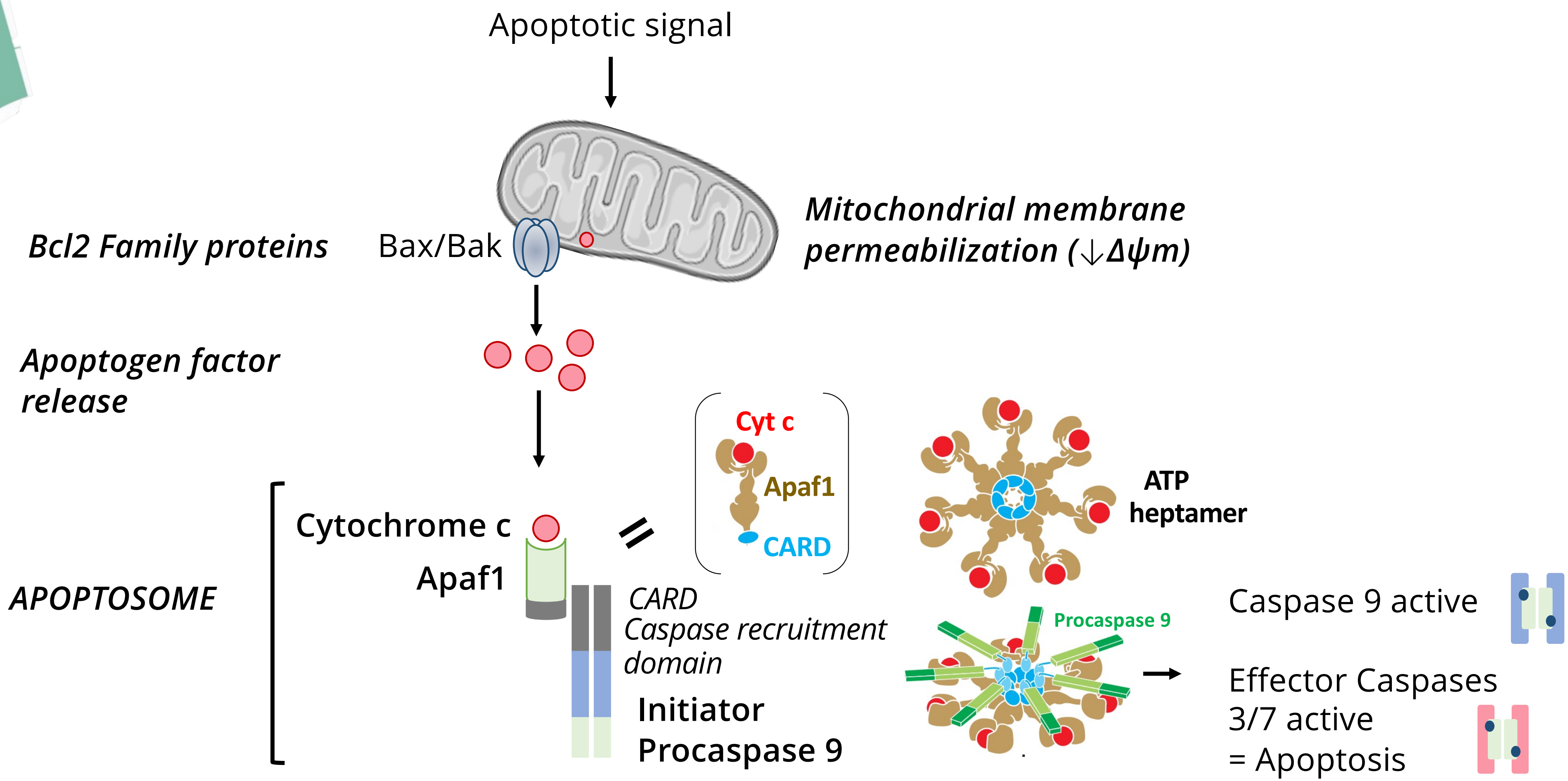
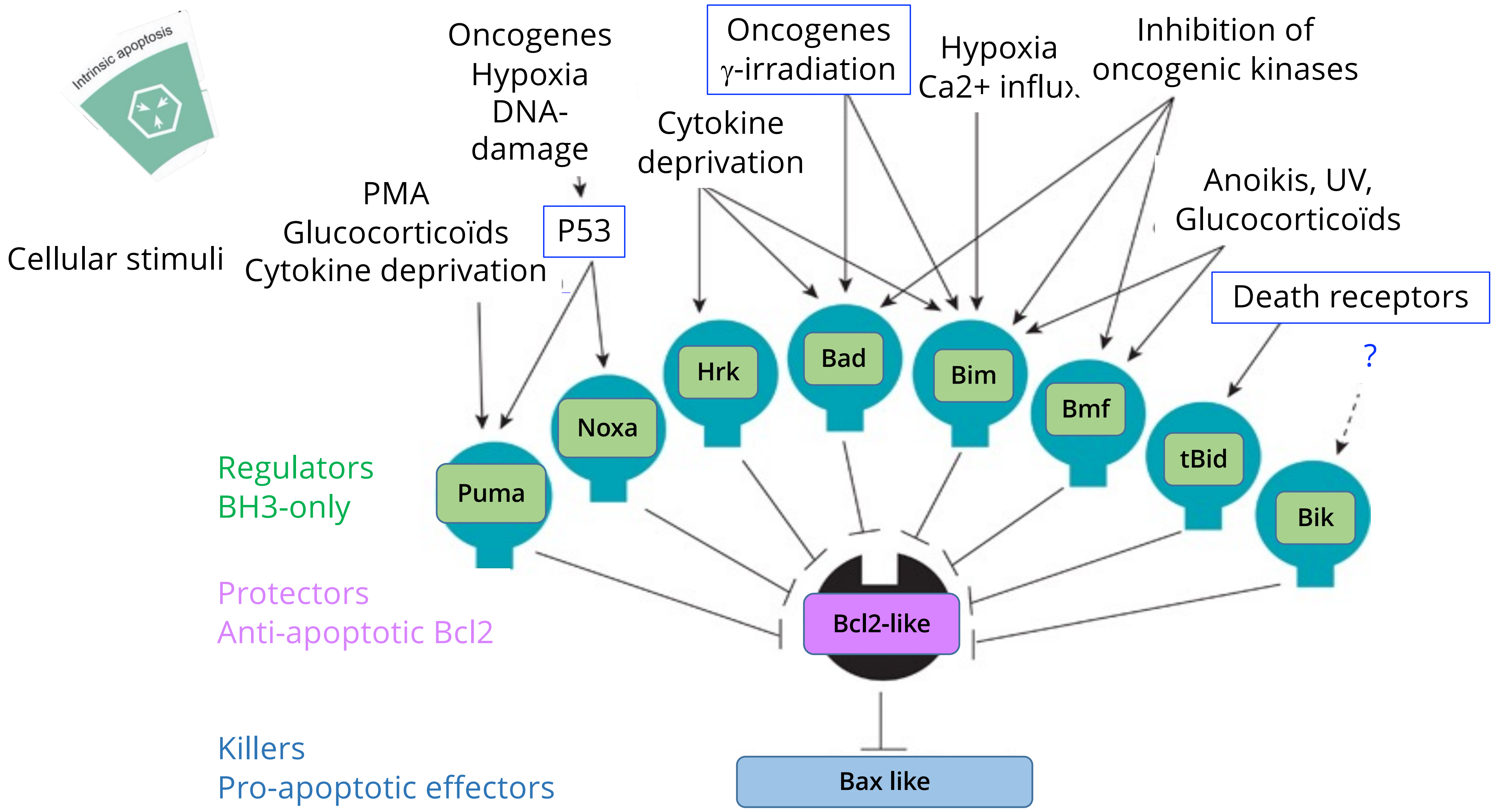
Intrinsic / mitochondrial apoptotic pathway : the apoptosome



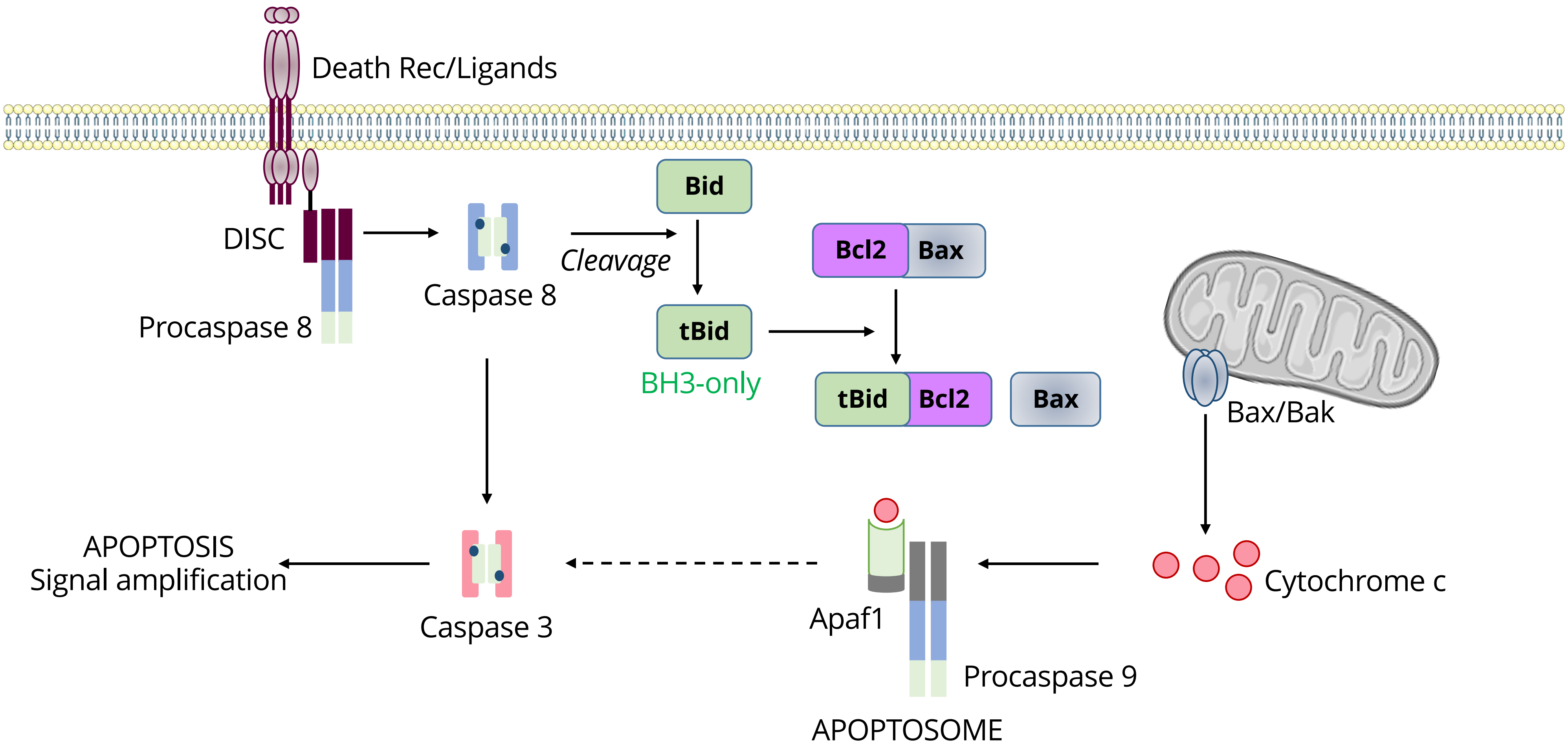
Molecular Biology of the Cell 6th edition

Intrinsic / mitochondrial apoptotic pathway : apoptotic stimuli

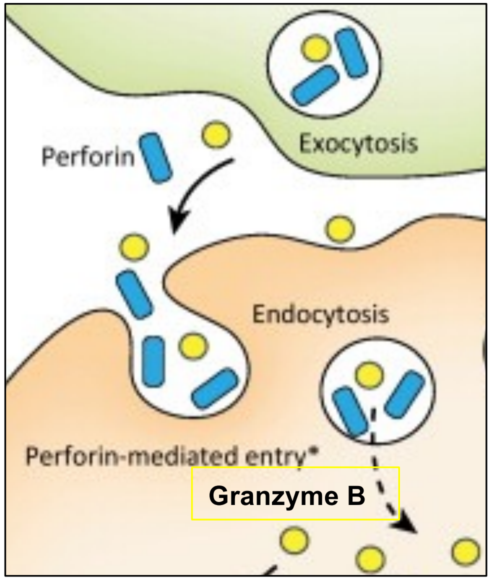


Kelly and Strasser, Cell Death and Diff., 2011

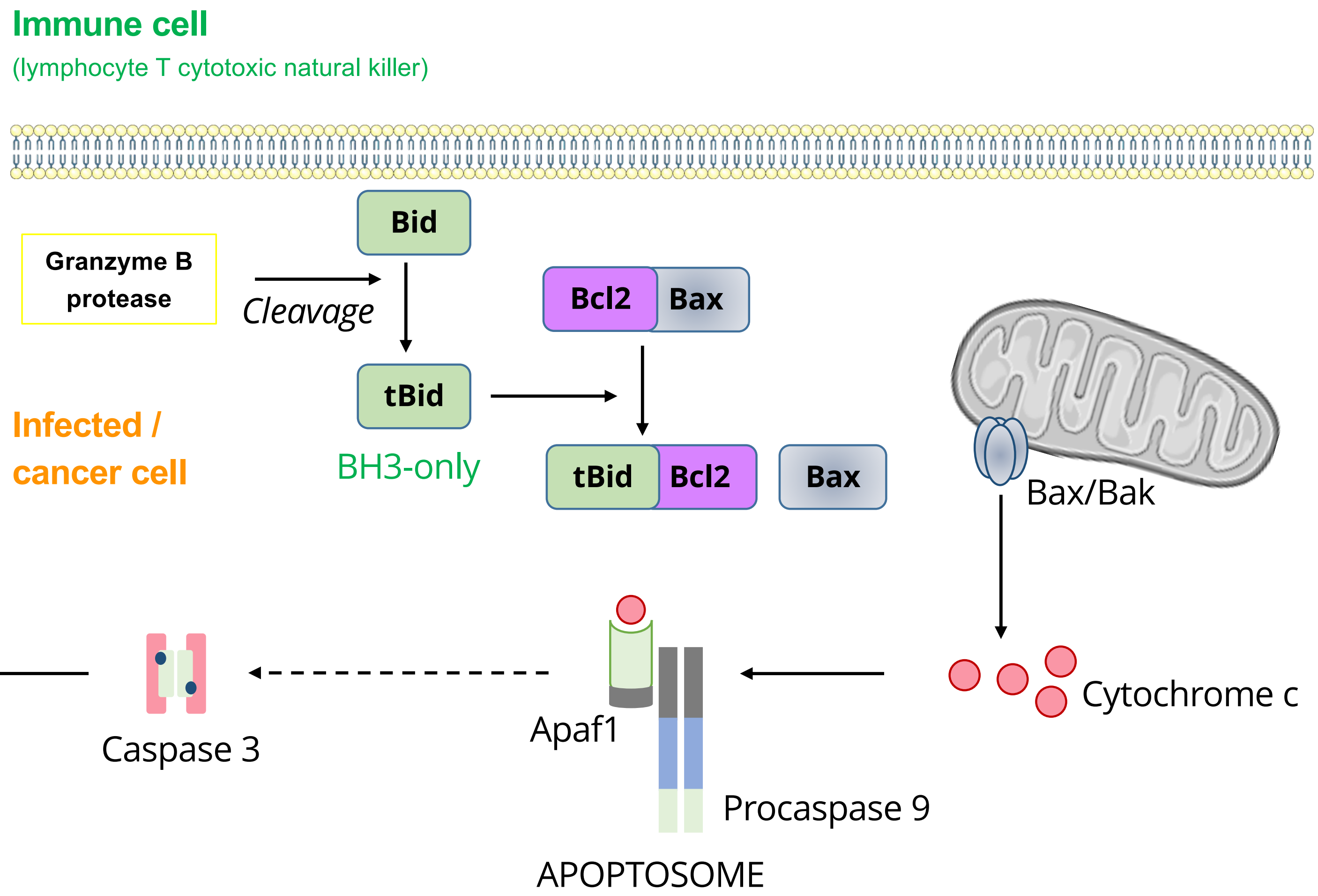
Link between the extrinsic & intrinsic apoptotic pathways



Intrinsic / mitochondrial apoptotic pathway and immunity



From Hiebert & Granville., Trends Mol Med., 2012



Apoptosis in pathologies

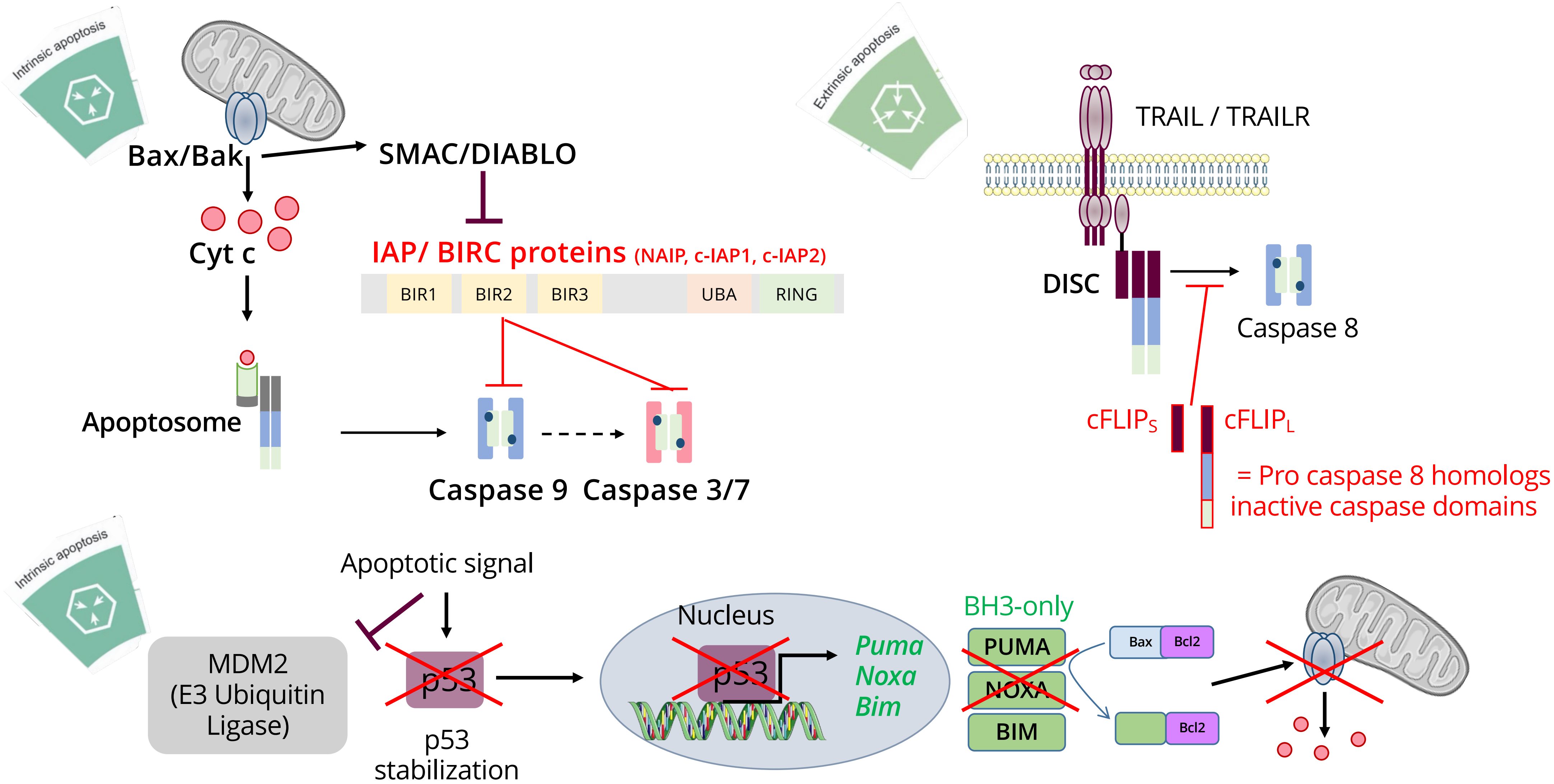
↳ ***Enhanced apoptosis*** *in AIDS, neurodegenerative diseases, fulminant hepatitis*

↳ ***Lack of apoptosis*** *in auto-immune diseases, virus infection*

↳ ***Lack of apoptosis = cancer therapy resistance***

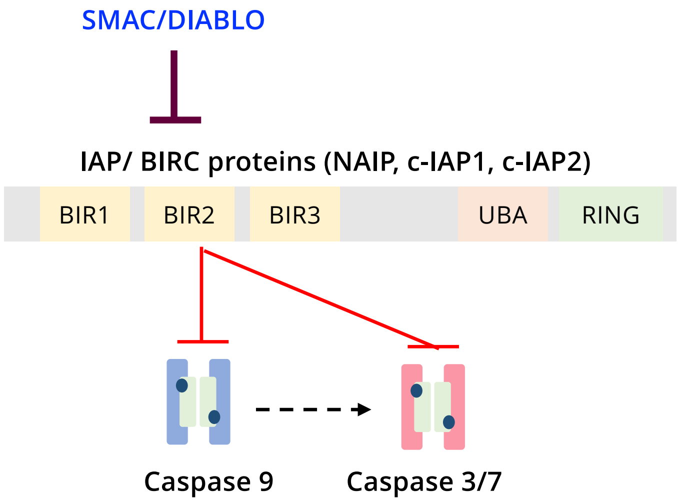
- *Activation of anti-apoptotic proteins (Bcl2, IAP/BIRC, cFLIP)*
- *Inhibition of pro-apoptotic proteins (death R, Bax)*
- *Inhibition of apoptotic signaling (p53)*

Lack of apoptosis in cancer cells

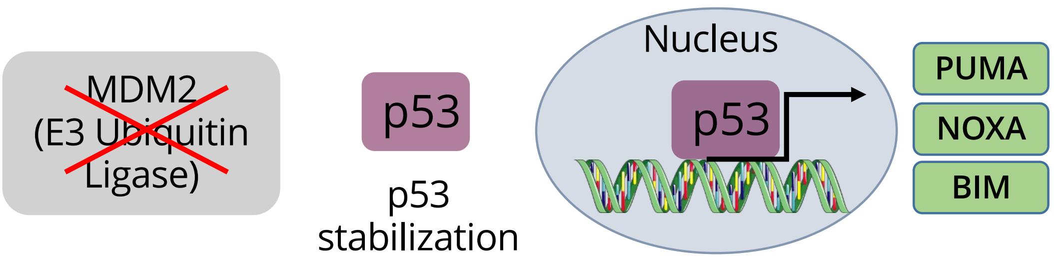


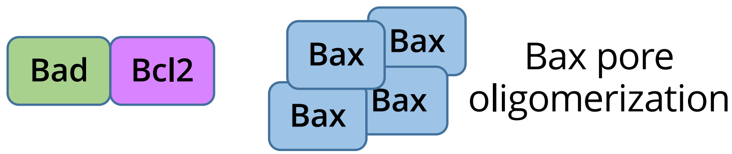
Examples of cancer therapies targeting apoptosis

↳ **To overcome resistance : need to rescue apoptosis (or to induce other cell death)**



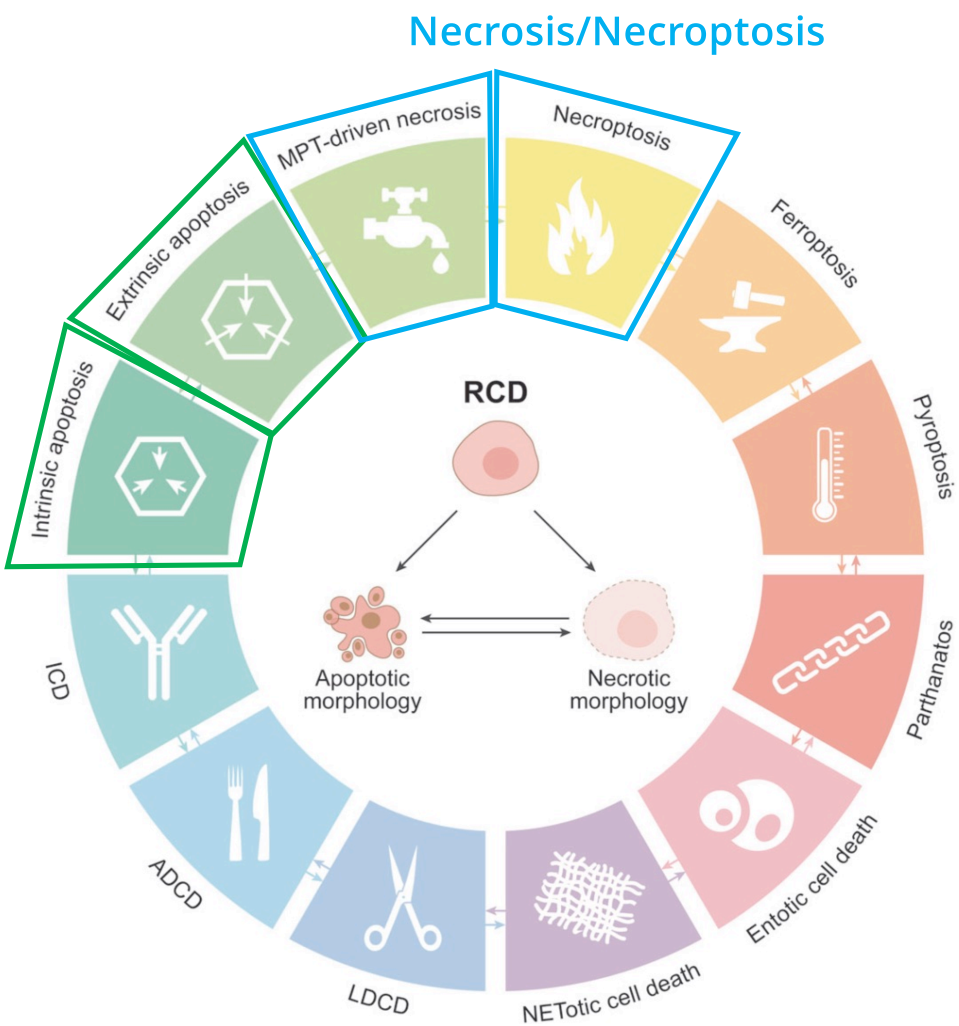
- **IAP inhibitors** : block anti-apoptotic proteins (LCL161, Birinapant) **SMAC mimetic**
- **Bcl2 (and BclXL) inhibitors** (Venetoclax, Navitoclax) **BH3 mimetic**
- **p53 stabilizing** (Idasanutlin) **MDM2 inhibitor**
- **Death receptor agonists** (GEN1029)





Carneiro & El-Deiry, Nat Rev Clin Oncol, 2020

Regulated cell deaths : necroptosis



Galuzzi *et al*, Cell Death & Differ., 2018

Necrosis & Necroptosis : physiological function

Necrosis upon violent circumstances and pathologic contexts

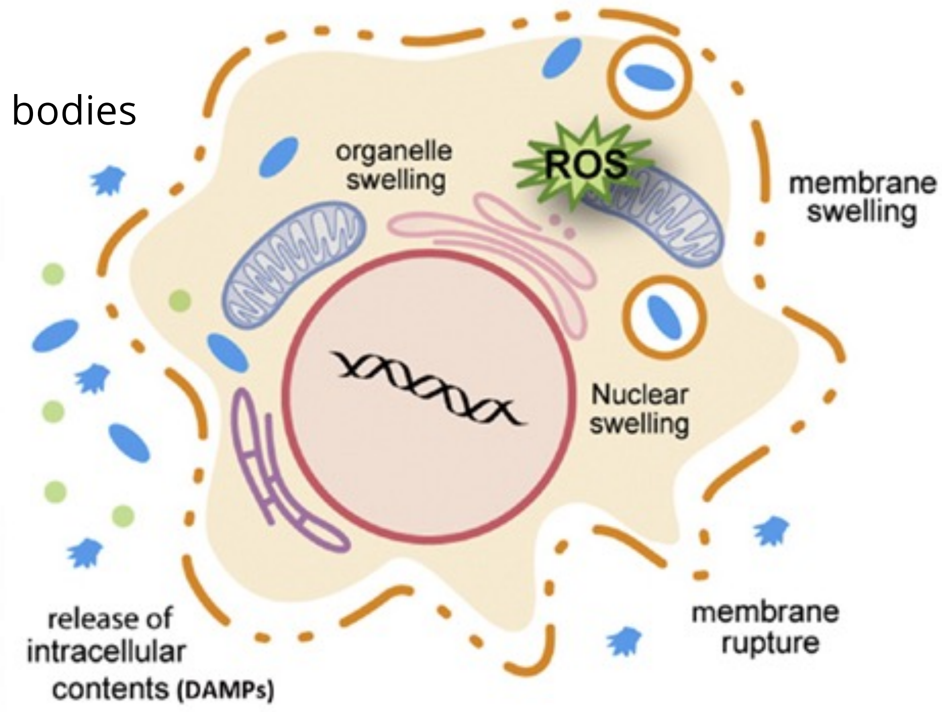
- **Hypoxia** (ischemia, heart attack, stroke, surgery, tumor)
- Chemotherapy/Radiations
- Extreme temperatures
- Infections (bacteria, lytic viruses)
- Toxins (acids, bases, venoms…)
- Physical trauma
- Acute pancreatitis

For a long time considered as accidental, random and uncontrolled = ACD (Accidental Cell Death) = necrosis

- BUT can be **inhibited by necrostatin** → signaling pathway
- Regulated Cell Death (RDC) = necroptosis

Necrosis & Necroptosis : morphological and molecular features

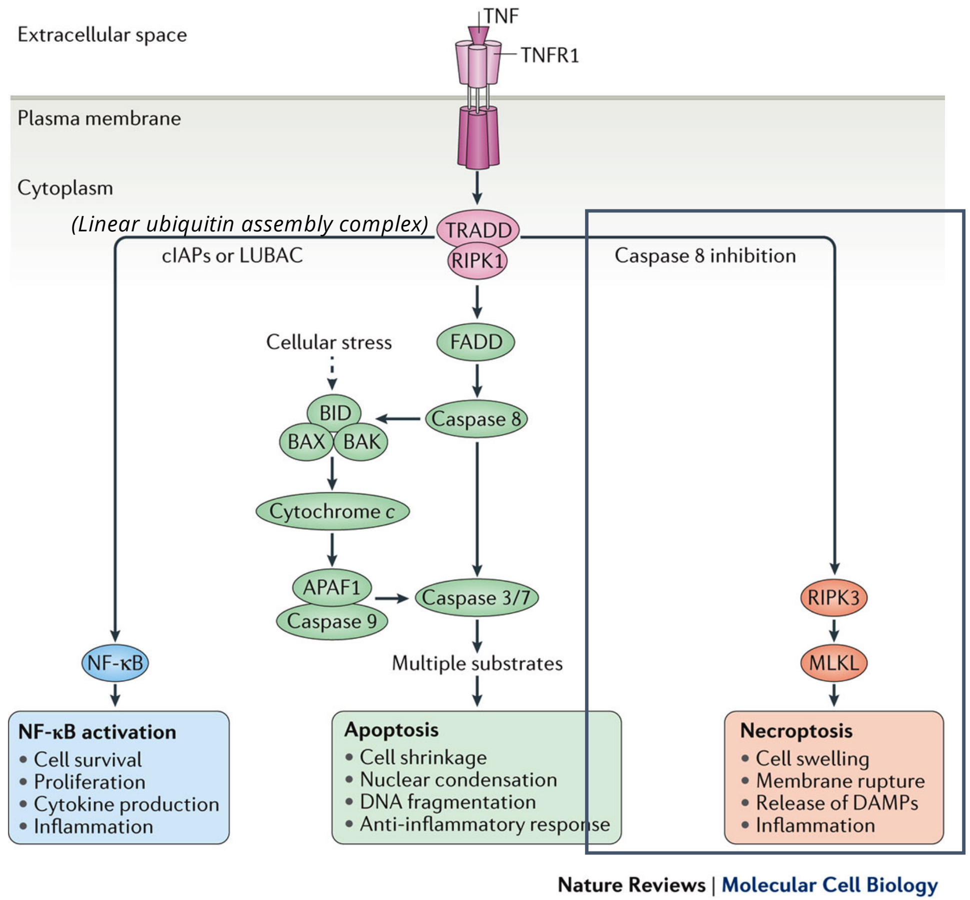
- Swelling of cells, organelles (mitochondria) and nucleus
- **Plasma membrane permeabilization/rupture**
- Cell content release in extracellular space (DAMPs → sterile inflammation)
- No membrane budding, no apoptotic bodies
- Random DNA fragmentation



Source: https://pathologia.ed.ac.uk/topic/necrosis/

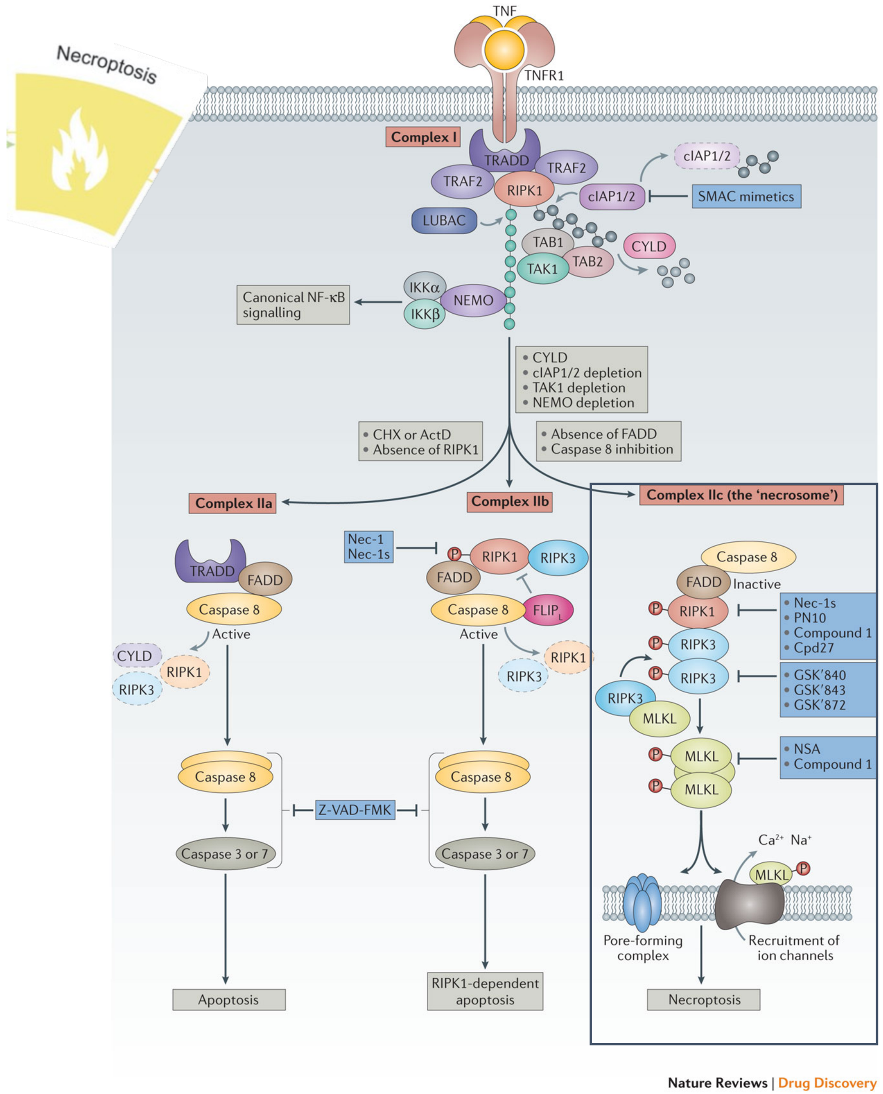
Necroptosis molecular pathway

- Induced by extracellular or intracellular perturbations detected by **death receptors** (FAS, TNFR, TLRs…)
- **Induced when apoptosis is blocked** (mutations, caspase inhibitor zVAD)
- **Best understood signaling pathway: TNFα /TNFR** (*Tumor Necrosis Factor*)



Weinlich *et al*, Nat Rev Mol Cell Biol, 2016

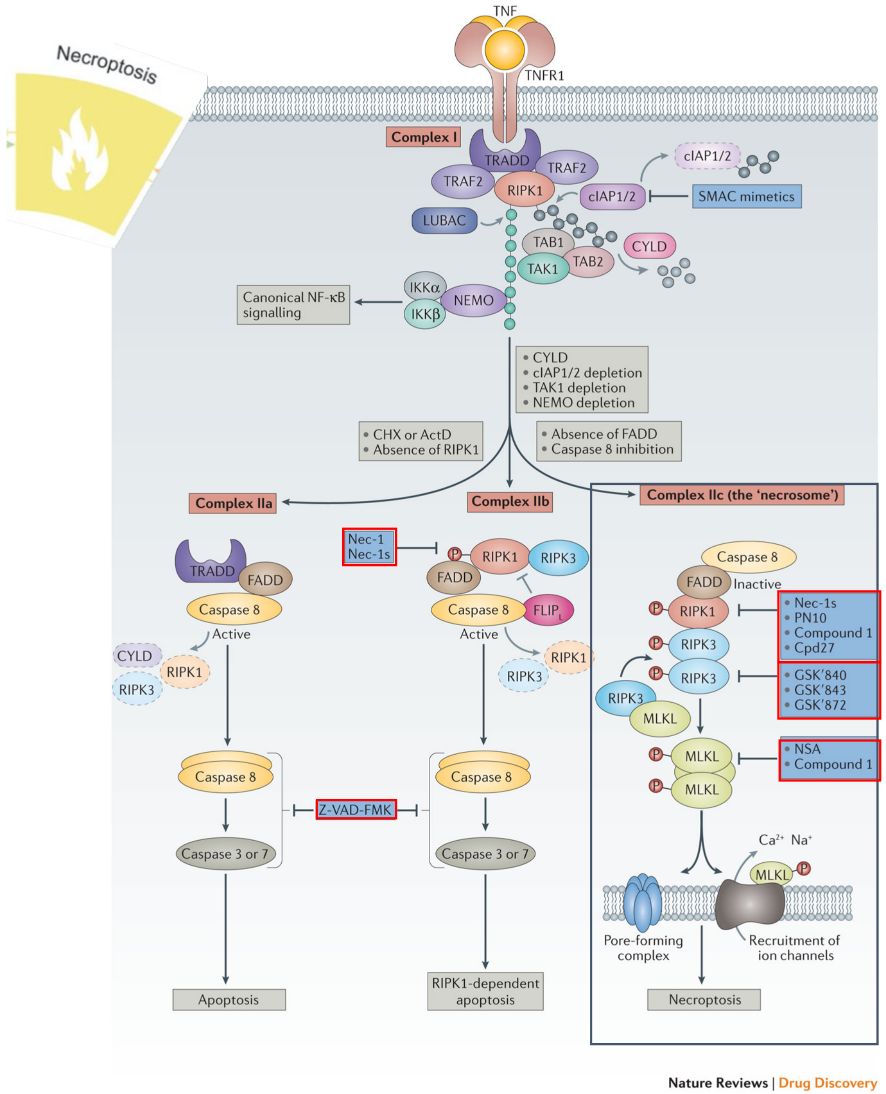
Necroptosis molecular pathway



- TNF/TNFR1 association
- **Complex 1: TRADD & RIPK1 recruitment**
- **RIPK1 de-ubiquitination (apoptosis or necroptosis)**
- Caspase 8 inhibition (= apoptosis inhibition, Z-VAD)
- **RIPK1 phosphorylation**
- **Complex IIc : Necrosome with phosphorylated RIPK1/RIPK3**
- MLKL **recruitment and phospho-activation → protein complex**
- **Pores in the plasma membrane** (DAMPs, inflammation)

Conrad et al, Nat Rev Drug Discov, 2016

Necroptosis molecular activators and inhibitors



Conrad et al, Nat Rev Drug Discov, 2016

- **Z-VAD : caspase / apoptosis inhibitor**

- Necrostatin : RIPK1 inhibitor
- GSK-872 : RIPK3 inhibitor
- NSA (necrosulfonamide) : MLKL inhibitor

Necroptosis in physio-pathology

↳ ***Role in tissue regeneration*** *(muscle, nerve remyelination),* ***antiviral defense***

↳ ***Enhanced necroptosis*** *in neurodegenerative disease*

↳ ***Anti-tumor***

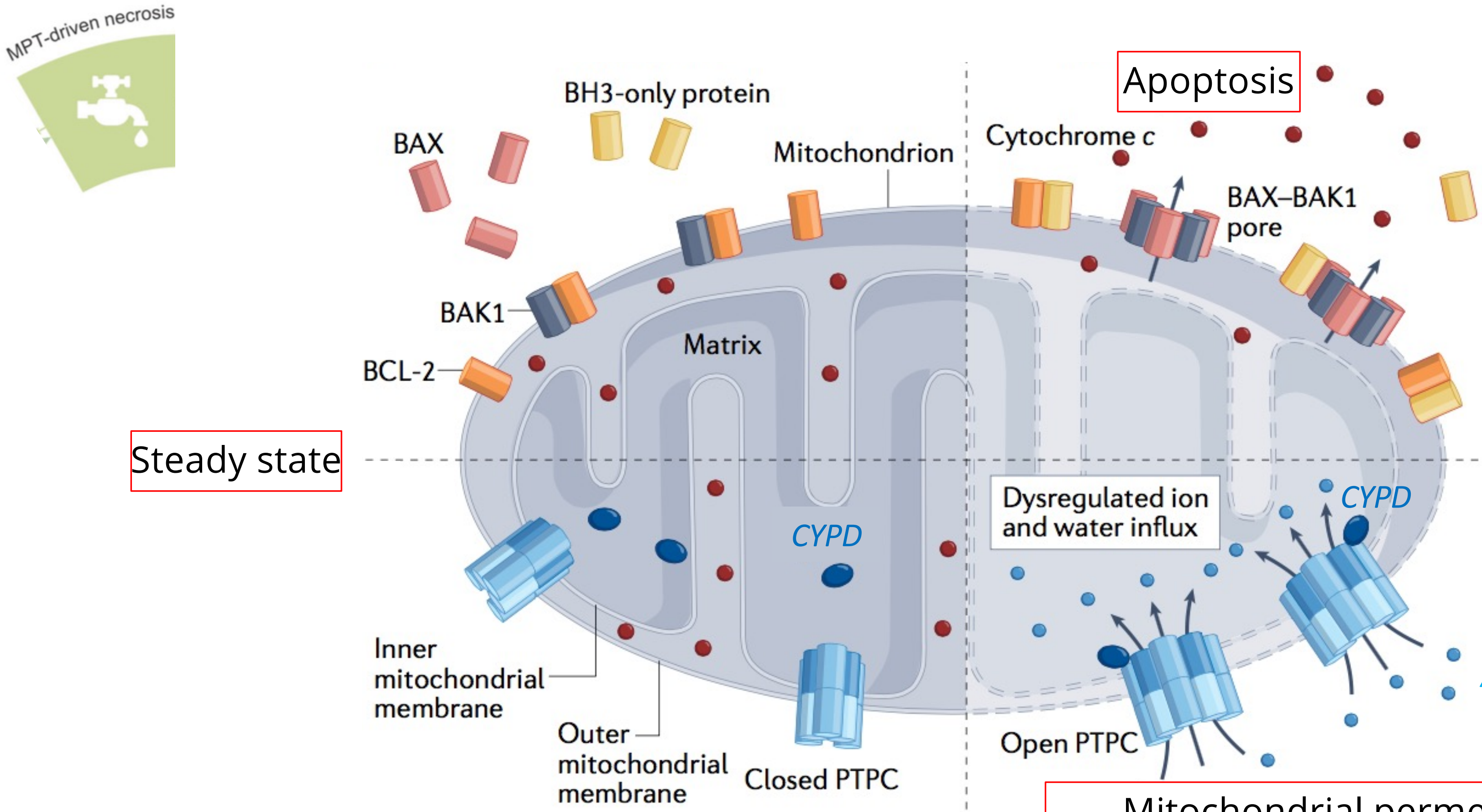
- *Second line of defense in tumor clearance if apoptotic deficiency*
- *Anti-tumor microenvironment (TME) : pro-inflammatory and immunogenic*

↳ ***But also pro-tumor***

- *Metastasis*
- *Peri-tumoral immune suppression*

↳ *Use necroptosis as a backup strategy in the event of apoptotic failure ?*

MPT-driven necrosis : Mitochondrial Permeability Transition Pore (MPTP)



Bax/Bak1 pore

Induced by ROS, Ca^{2+}
= Rapid ATP depletion
+ oxidative damage

ANT/VDAC pore / ATP synthase
CYPD/Cyclophilin D

Permeability transition pore complex

Mitochondrial permeability transition (MPT)-driven necrosis

Marchi et al, Nat Rev Drug Immuno., 2023

Regulated cell deaths : autophagy-dependent cell death (ADCD)

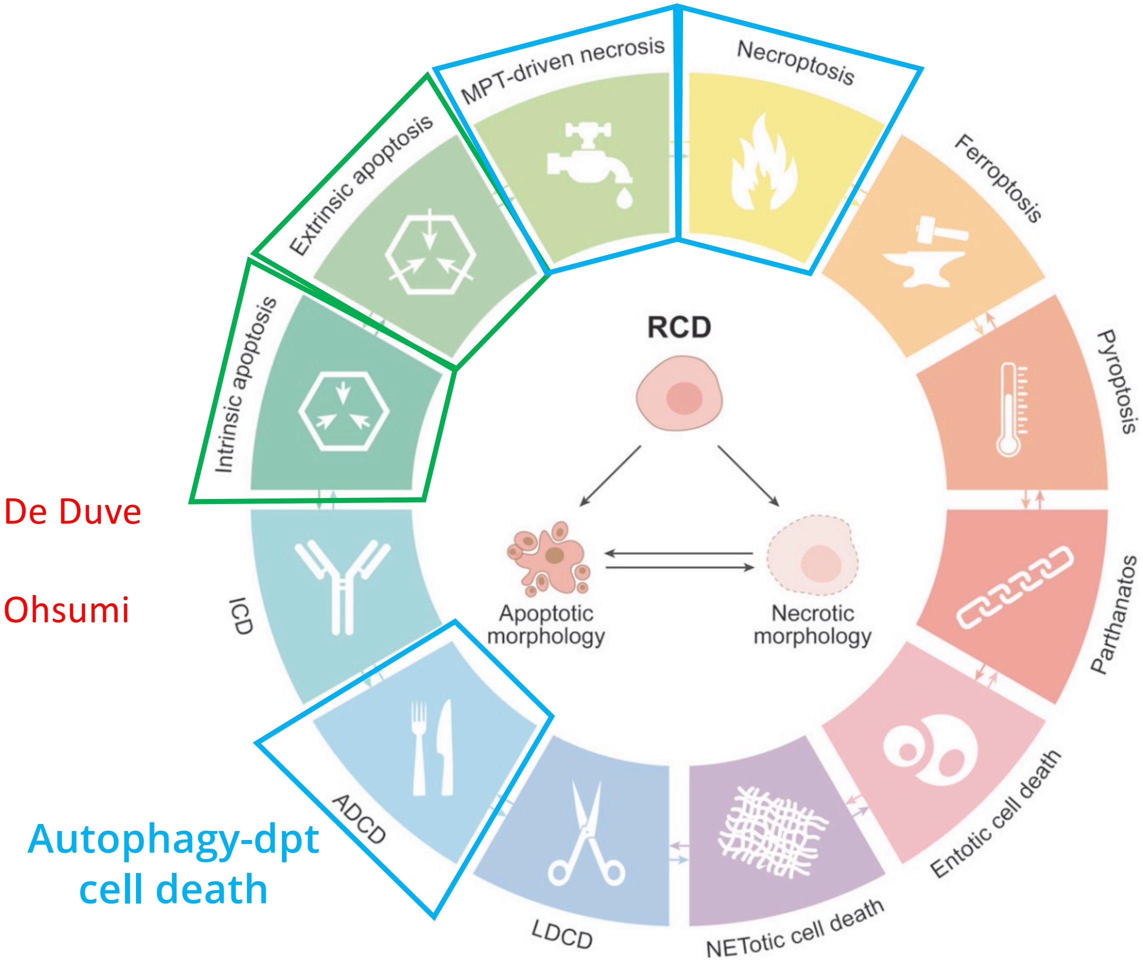
Nobel Prize in Physiology or Medicine 1974, De Duve

Lysosome (1955), autophagy (1963)

Nobel Prize in Physiology or Medicine 2016, Ohsumi

First yeast Atg = autophagy gene (1993)

Cloning (1997)



Autophagy : discovery of a self degrading and recycling cellular pathway

(Macro)Autophagy = self-eating process

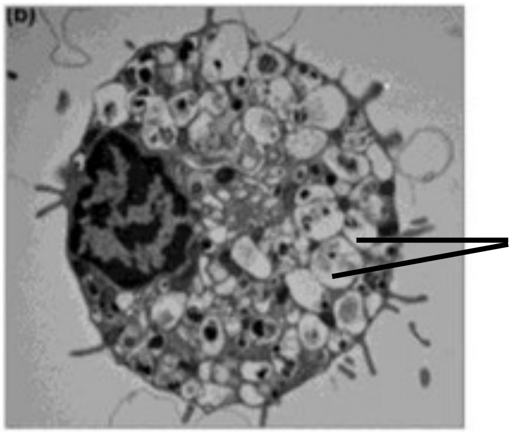
Lysosomal degradation of cellular macromolecules and organelles in autophagic vacuoles

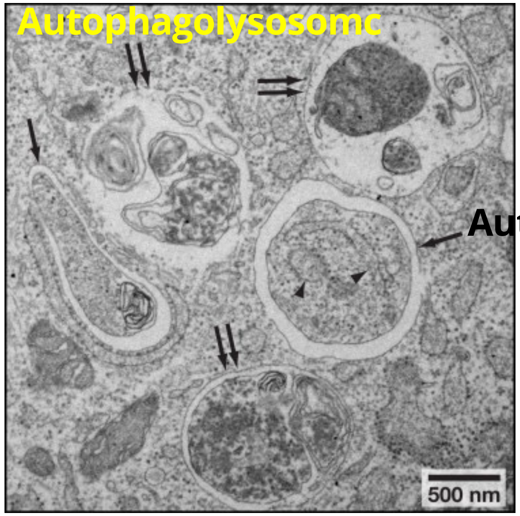
Autophagy-dependent cell death (ADCD)

Initially described as Type II cell death:

- massive accumulation of autophagic vacuoles
- extreme self-digestion
- Observed in *Drosophila melanogaster* and *Dictyostelium discoïdum*

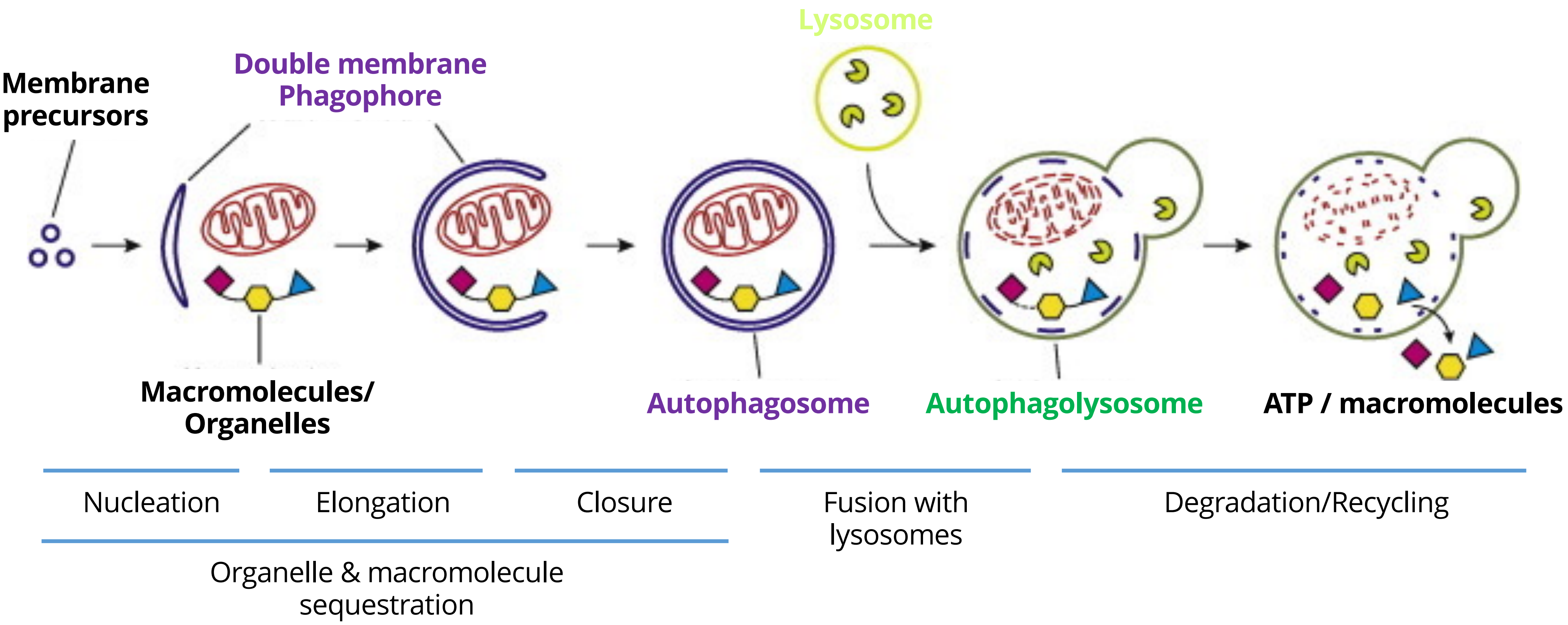
Autophagic cell death





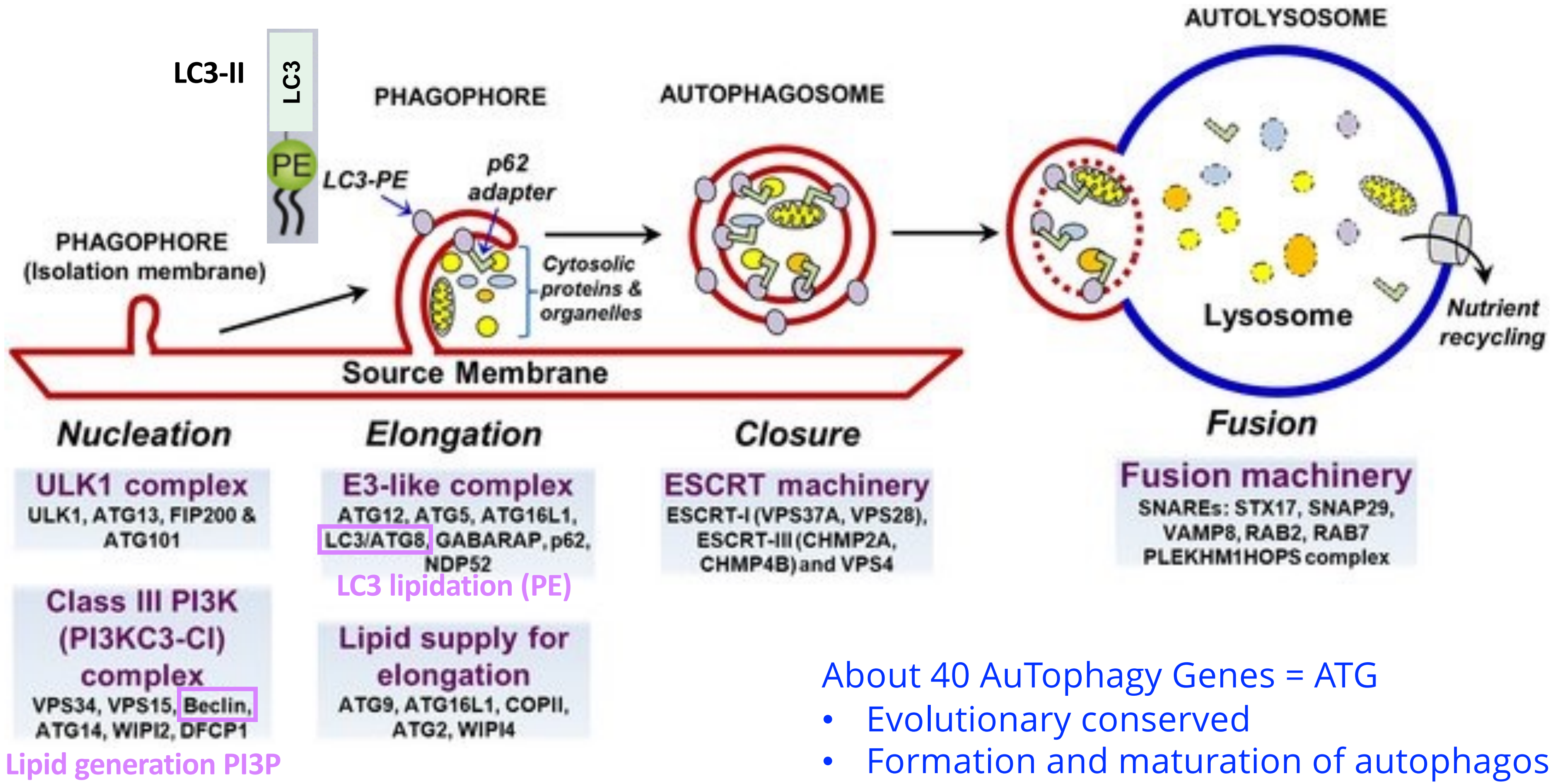
Mizushima et al, Cell, 2010

Autophagy morphological and molecular features



Zaffagnini & Martens, J Mol Biol, 2016

Autophagy molecular pathway



Saikia & Joseph, J. Mol. Med., 2021

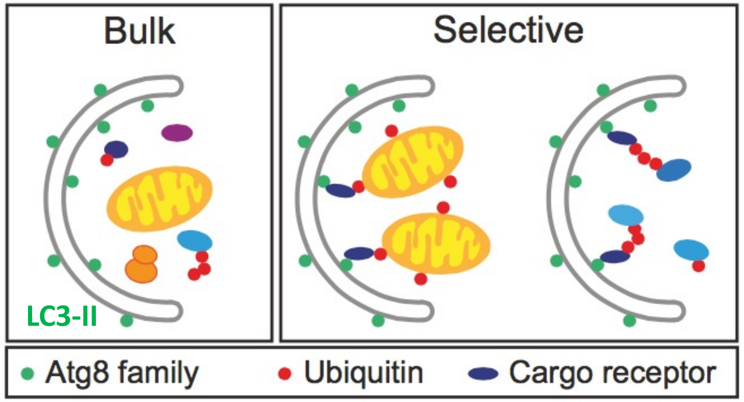
About 40 AuTophagy Genes = ATG

- Evolutionary conserved
- Formation and maturation of autophagosomes
- Regulation of autophagy

Bulk and selective autophagy

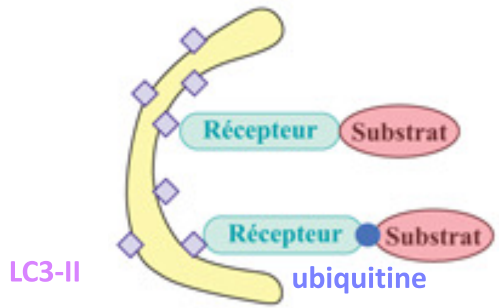
Bulk autophagy:

- Random sequestration of cytosolic components, unspecific cargos
- Basal or induced by limiting external nutrients/energy



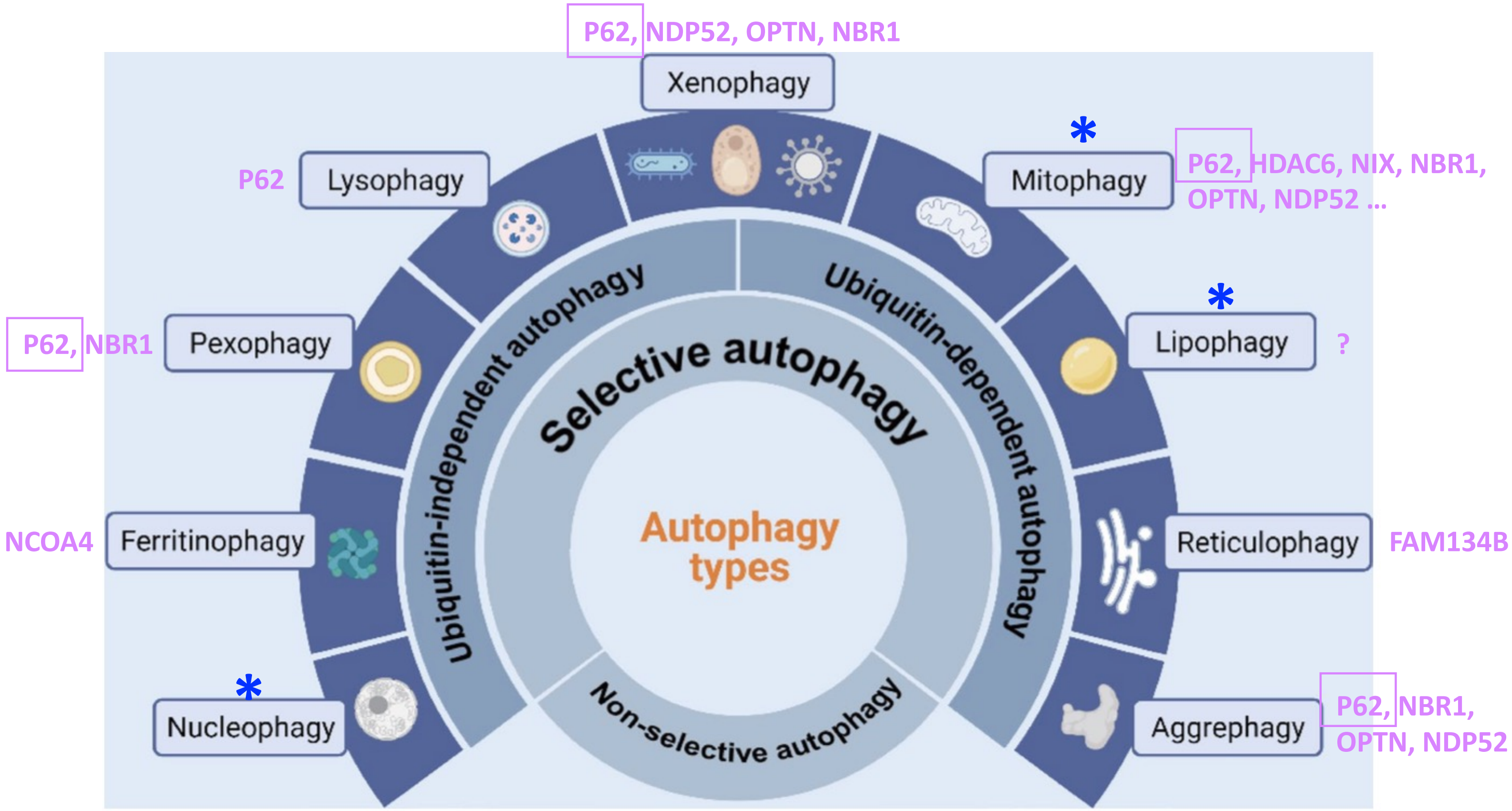
Selective autophagy:

- Specific cargo recognition: pathogens, damaged mitochondria, ER, protein aggregates…
- Autophagy receptors:
  - Recognize specific cargos
  - Bind LC3 (LC3-interacting region = LIR domain) on autophagosome



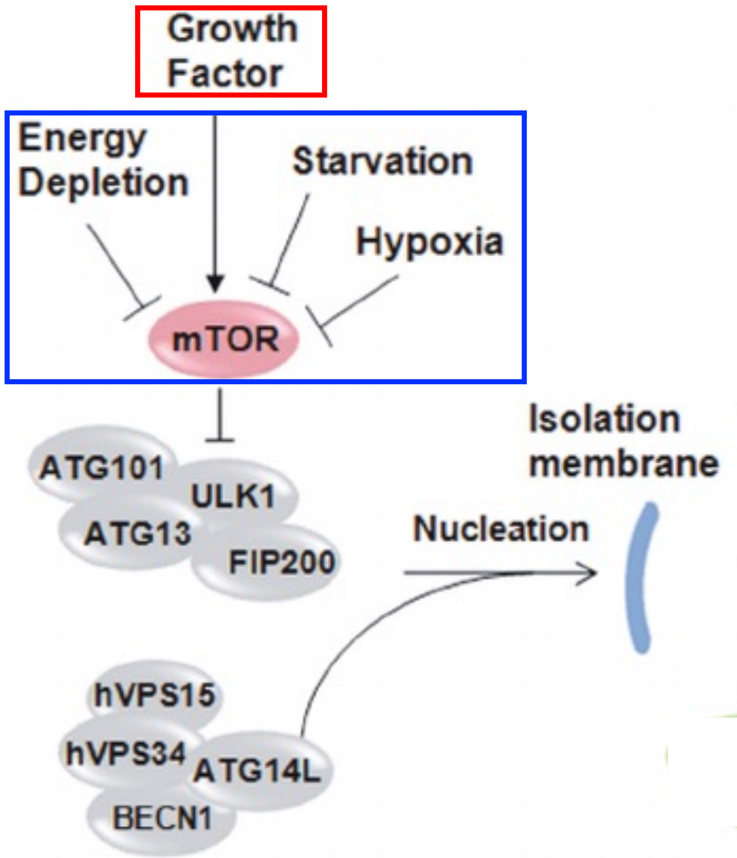
Denton & Kumar, Cell Death and Diff., 2018

Selective autophagy : a diverse set of receptors



Chen et al, J Biomed. Sci., 2023

Physiological and artificial regulators of autophagy

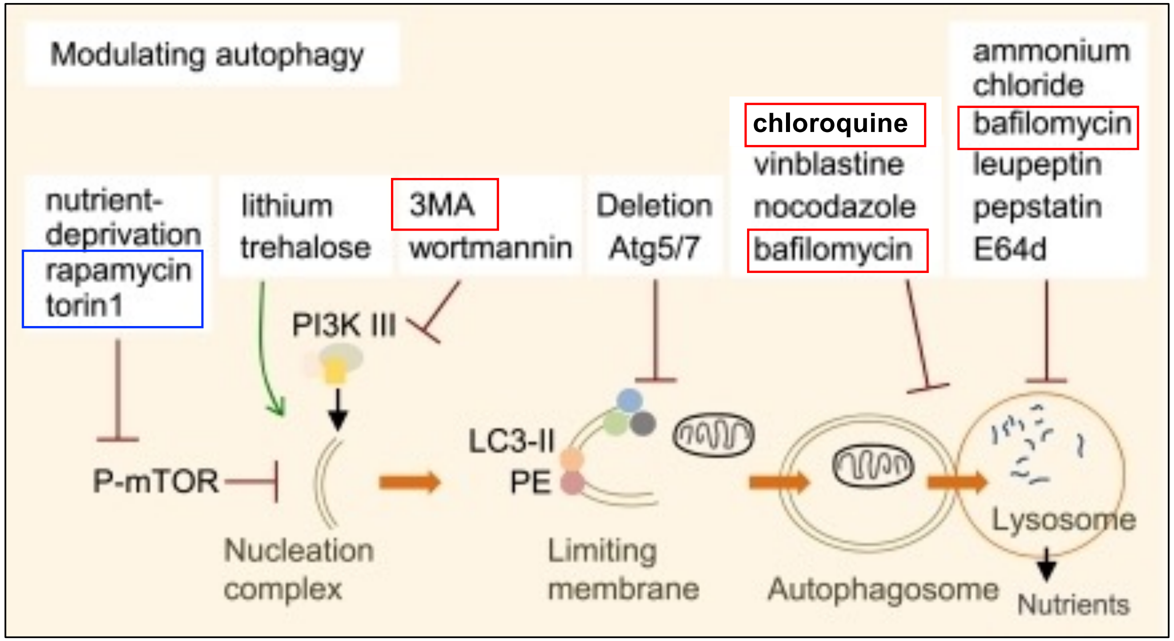


From Karakas & Gozuacik, Turkish Journal of Biology, 2014

Rapamycine, Torin 1 : autophagy induction by mTOR inhibition

3-MA : Vps34 / PI3K III inhibitor

Bafilomycin A1 : flux inhibitor (inhibits H+ v-ATPase / lysosome acidification)



Yamada & Singh, Diabetes, 2012

Basal and stress-induced autophagy are key for cell survival

Basal autophagy

Removal of protein aggregates and damaged organelles

Cytosol quality control

Limits ROS production, maintains cell homeostasis

Stress-induced autophagy

- Metabolic, hypoxia (birth)
- Protein aggregate
- Oxidative (ROS)
- Mechanic
- Therapy
- UV
- Infection (degradation, immunity)

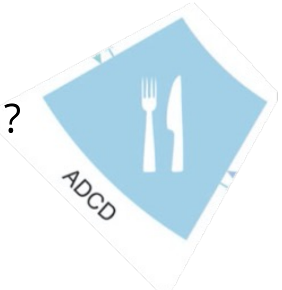
Use of cellular components for new macromolecule and ATP biosynthesis

Adaptation to stress (supply in energy, nutrients)

CELL SURVIVAL

Autophagy and cell deaths

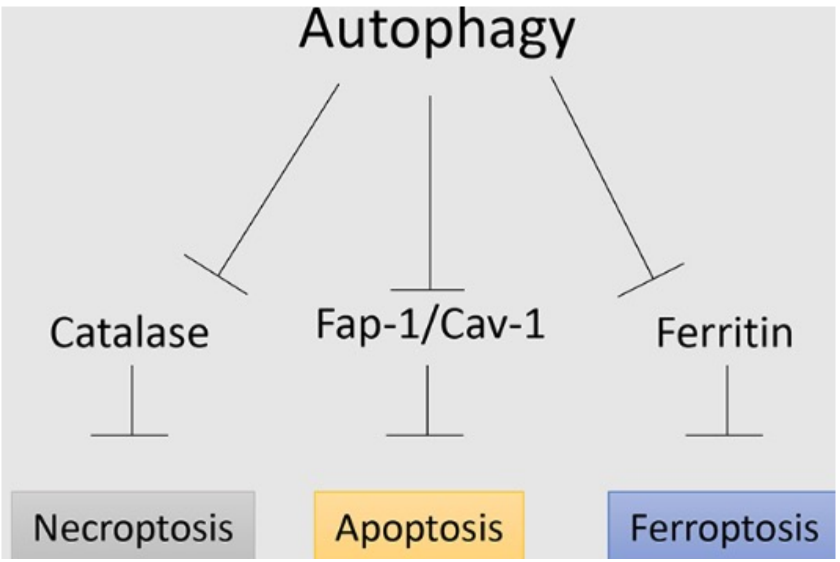
Not universal ?



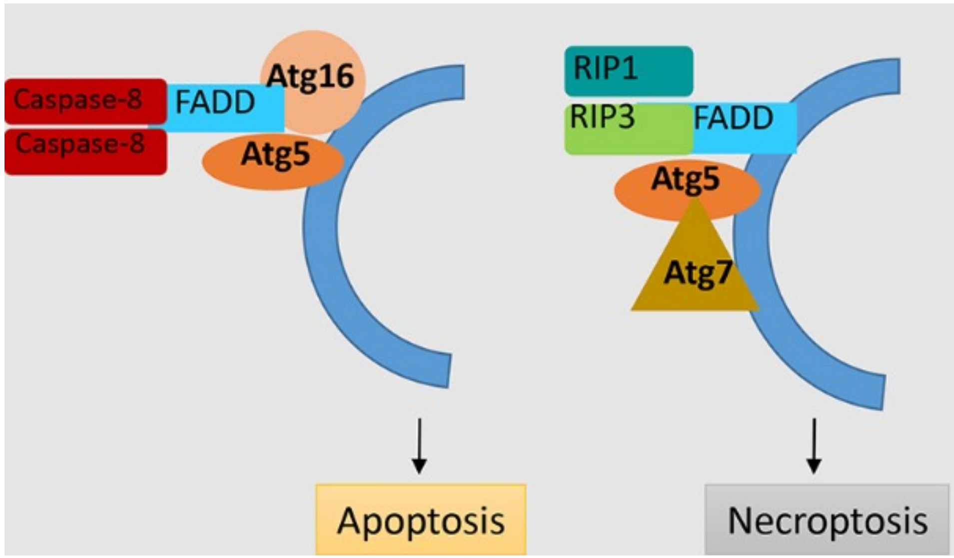
- massive accumulation of autophagic vacuoles
- extreme self-digestion
- Observed in *Drosophila melanogaster* and *Dictyostelium discoïdum*

But …

Degradation of vital components

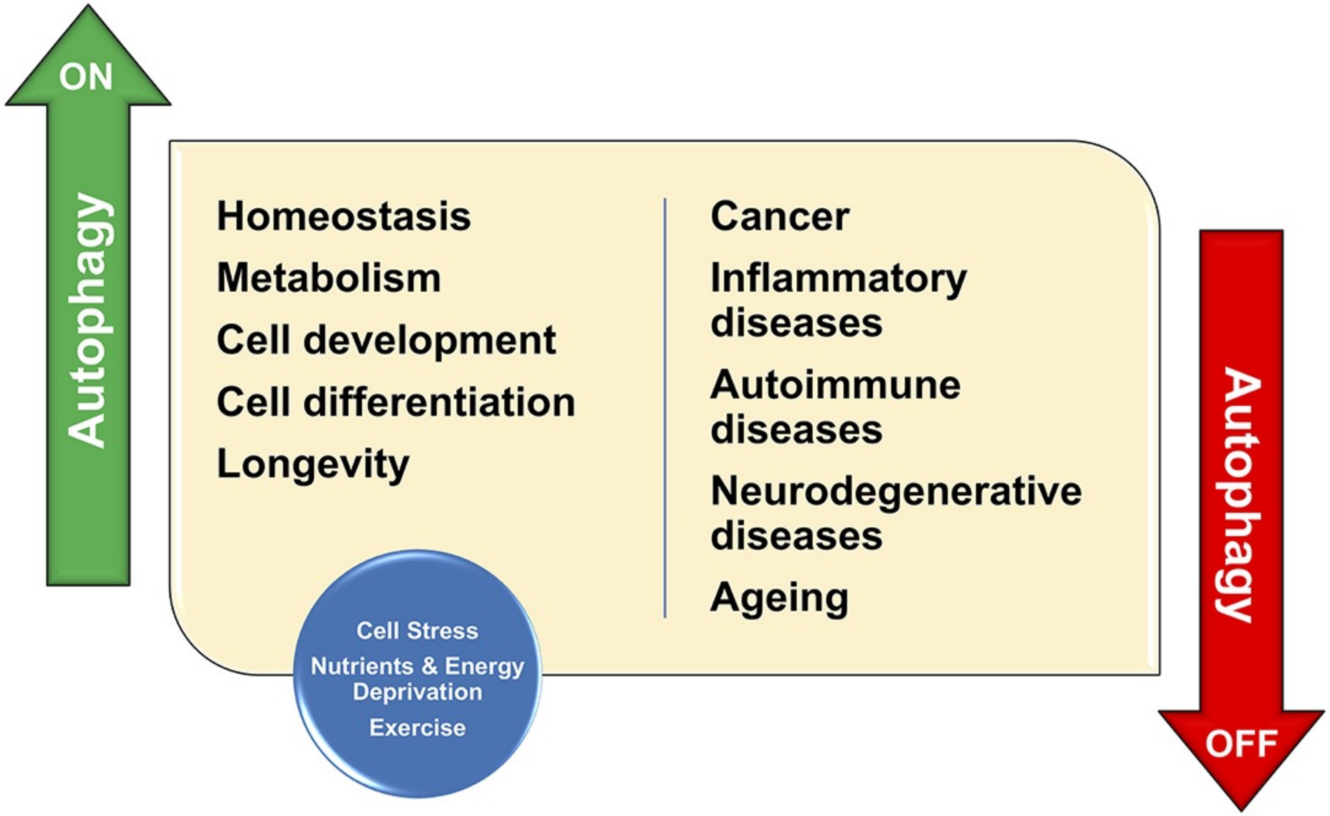


Atg5, 7,16 are scaffold proteins for regulated cell death



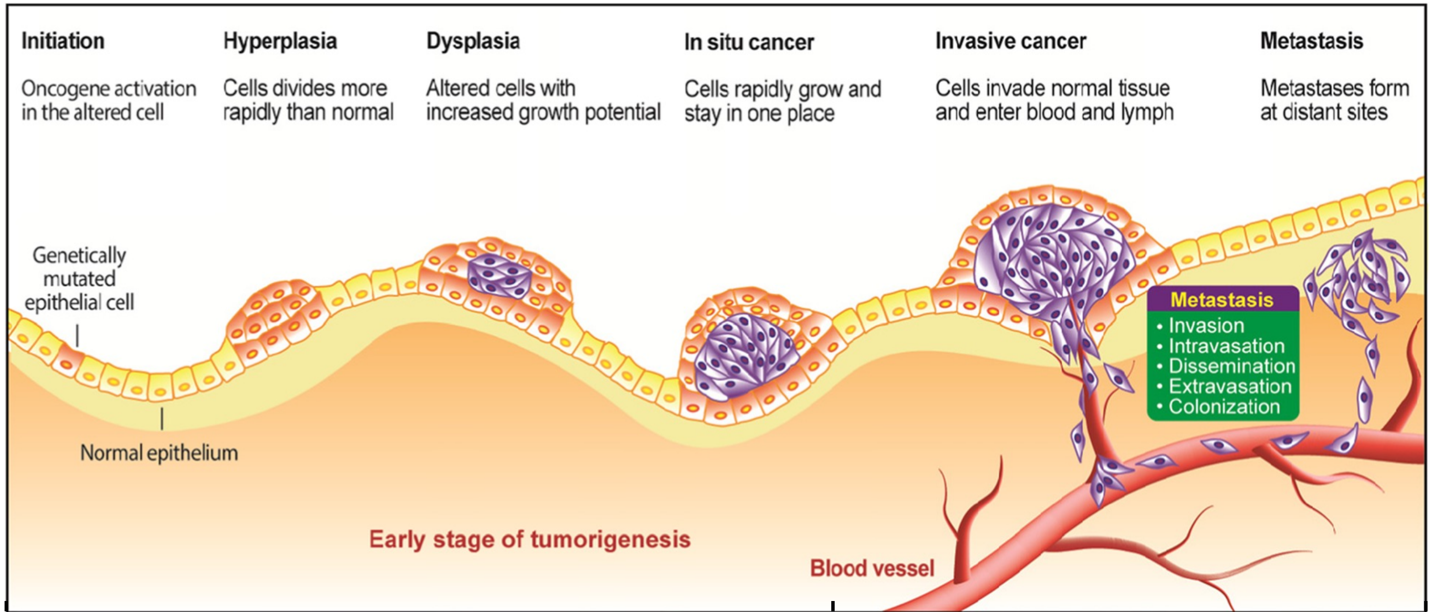
Doherty & Baehrecke, Nat. Cell Biol., 2022

Autophagy in physiopathology



Flati et al, Front Cell Dev Biol, 2021

Dual role of autophagy in cancer : a matter of timing



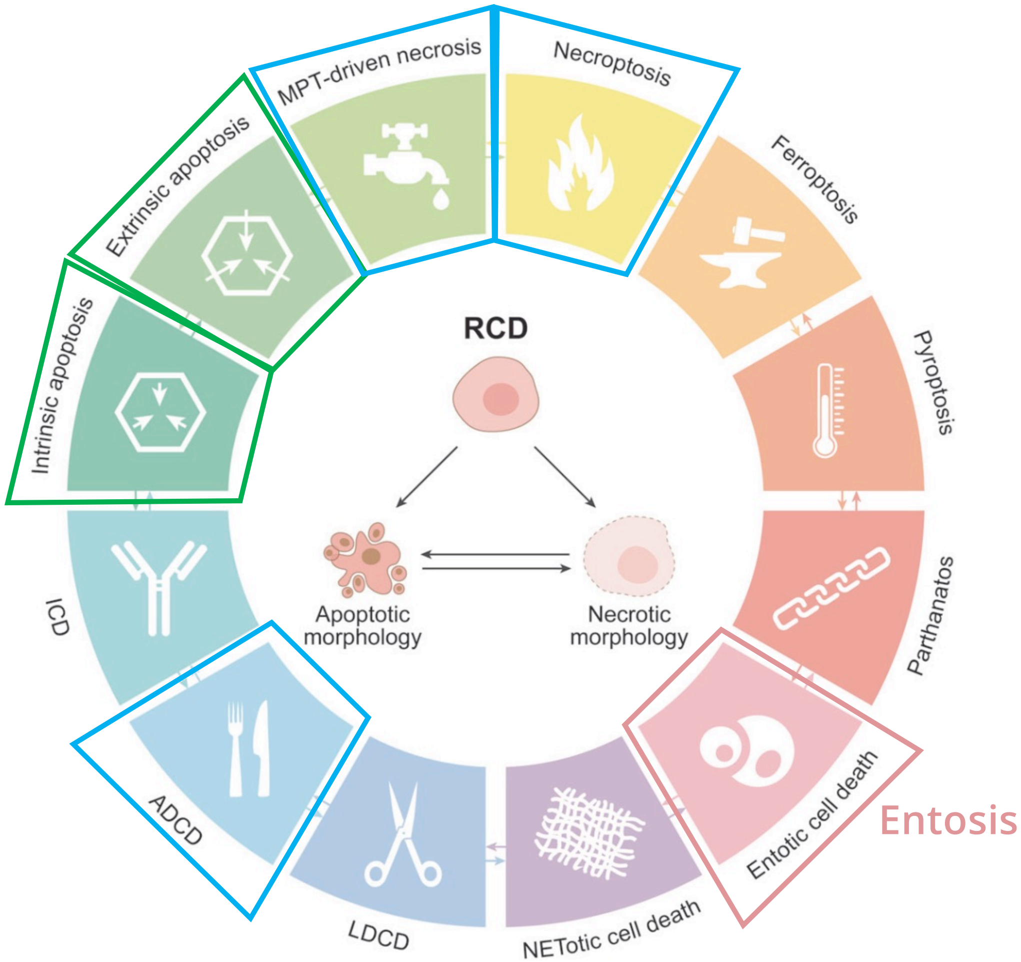
Normal cells	Tumor-suppressing in tumor initiation and invasion	Cancer-promoting in cell invasion and metastasis
- Energetic homeostasis - Quality control cytoplasm	- Genomic stability - Low ROS, low DNA-protein-organelle damage - Low necrosis, inflammation - Clearing pathogens - Anti-tumor immunity	- Anoïkis resistance, stemness, metastasis, - Energy supply - Cancer cell dormancy - Drug resistance

Protection against stress and damage — **Anti-tumor**

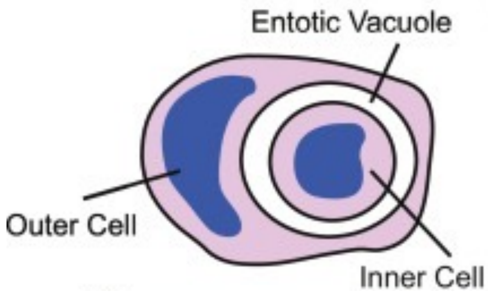
Pro-tumor — Survival in harsh condition

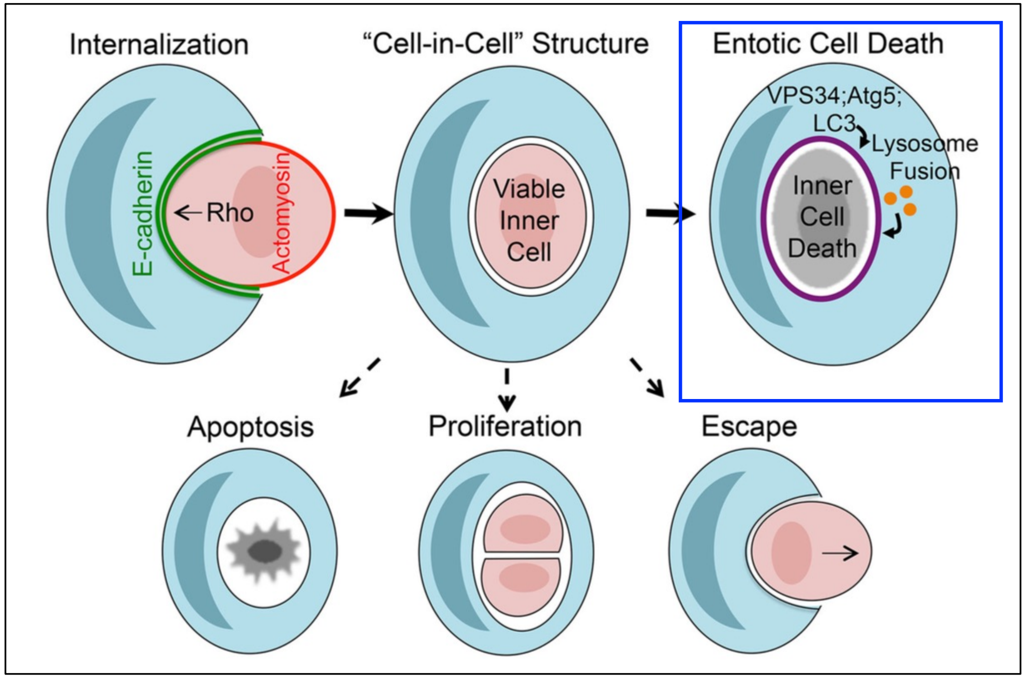
Li et al, Mol. Cancer, 2020

Regulated cell deaths : Entosis



Entosis : live cell-in-cell structure that can lead to cell canibalism / entotic cell death



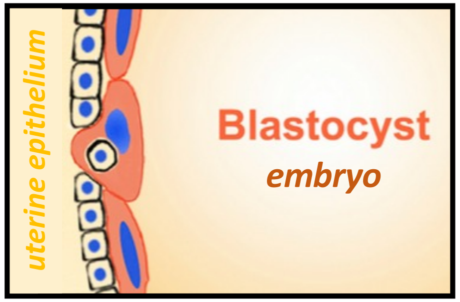


- Between live detached epithelial cells : competition mechanism
- Requires E-cadherin and adherens junctions
- Entotic death of the inner cell relying on autophagy genes + lysosomes

Durgan & Florey, BBA - Mol Cell Res, 2018
Krishna & Overholtzer, Cell. Mol. Life Sci. 2016

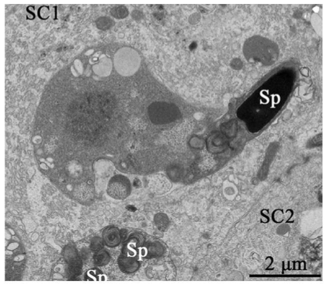
Entosis and entotic cell death in physiopathology

Embryonic implantation :
Digestion of the uterine epithelium
by embryonic trophoblast cells



Li et al. Cell Reports, 2015

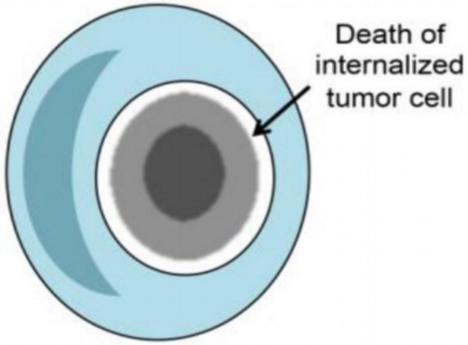
Sperm removal during turtle hibernation by Sertoli cells



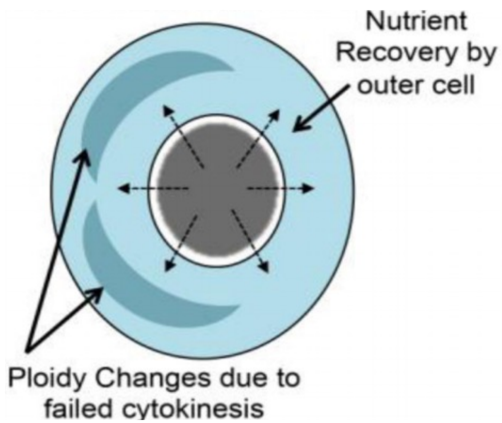
Entotic sperm (sp)
Sertoli cells (SC)

Ahmed et al. Front. In Physio., 2017

Observed in carcinomas for more than a century

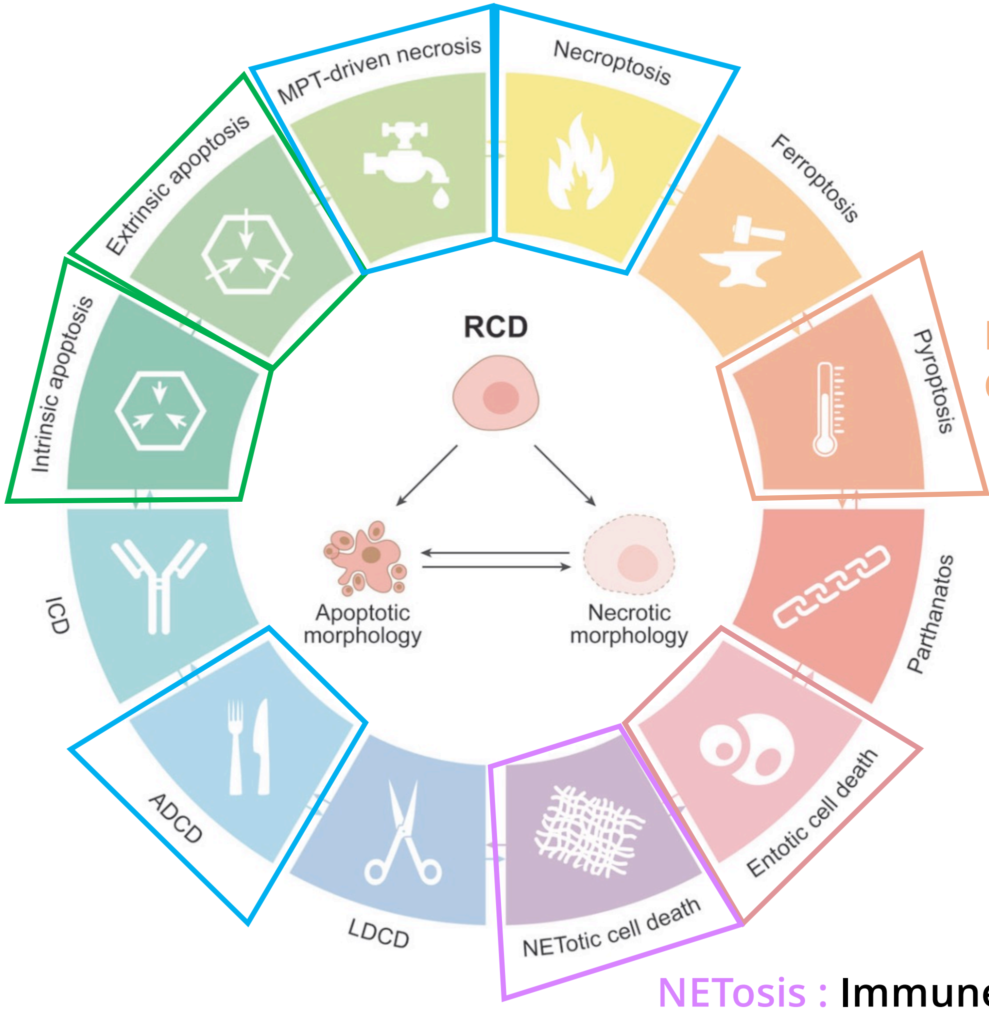


Inner cell
- Death (anti-metastasis)
- Division **(pro-tumor)**
- Escape **(pro-tumor)**



Outer cell
- Aneuploidy **(pro-tumor)**
- Nutrient **(pro-tumor)**

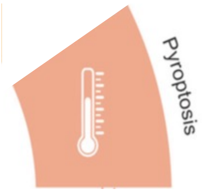
Regulated cell deaths : pyroptosis and NETosis

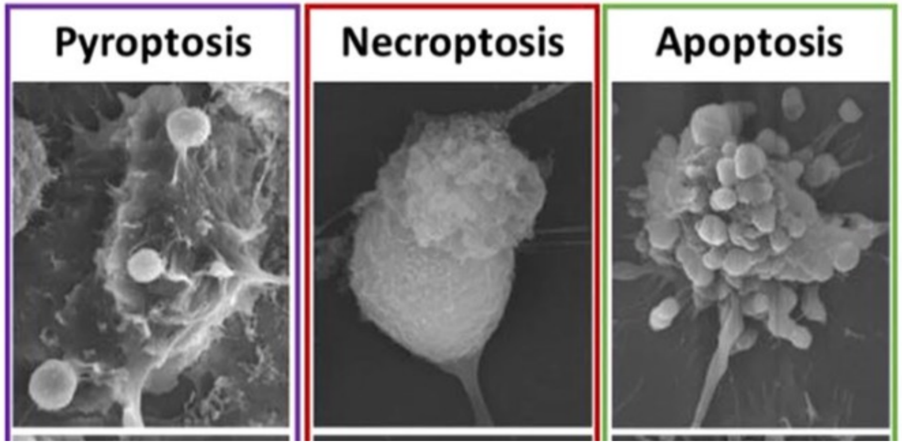


Pyroptosis : Immune factor mediated
Caspase-1 mediated GSDMD pore formation

NETosis : Immune factor mediated / Formation of NETs

Pyroptosis morphological and molecular features





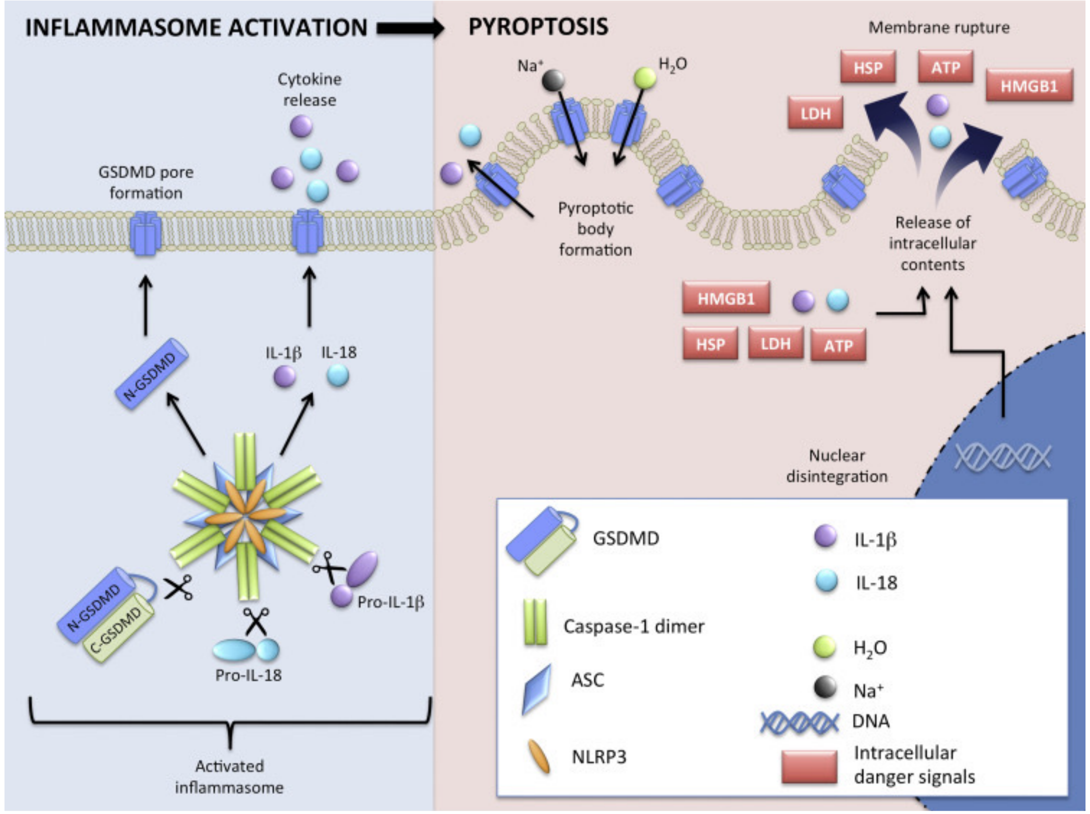
ME, Zhang et al., Cell Res., 2018

- Defense mechanism against intracellular infection of macrophages
- Hallmarks : gasdermin (GSDMD) pore in the plasma membrane (PM), cell swelling, pyroptotic bodies, osmotic lysis
- Kill the infected cell and release pro-inflammatory signals (cytokines)

Inflammasome-activated pyroptosis (IAP) molecular features in immune cells

- **PAMPs, DAMPs (bacterial, virus, HSP..) recognition**
- **Intracellular inflammasome formation (**NLRP, ASC, **caspase-1**)
- **Caspase-1** activation (not involved in apoptosis)
- **Gasdermin (GSDMD) cleavage : GSDMD pore formation in the PM**
- Pro-IL cleavage → **pro-inflammatory IL** released through gasdermin pores

- **Pyroptotic body formation : ion/water flux, cell swelling**
- **PM rupture : cell death with DAMPs release**

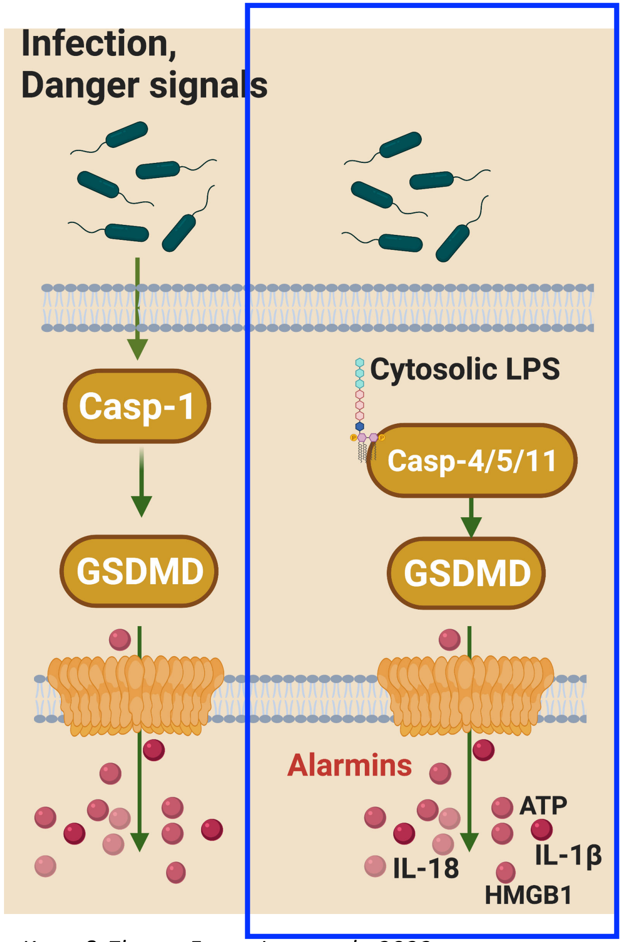


McKenzie et al, Trends Neurosci., 2020

Non canonical pyroptosis molecular features

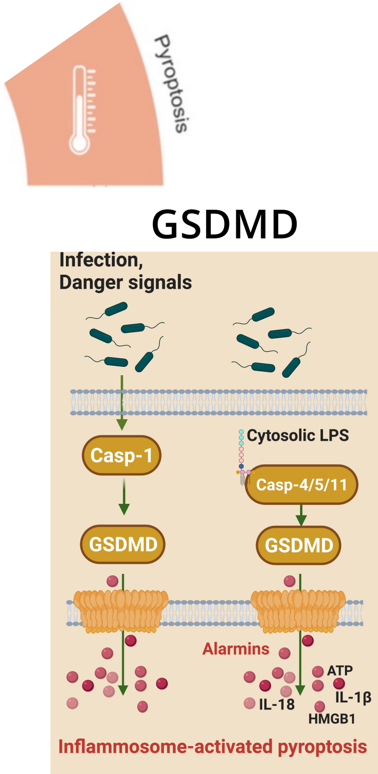
Found in infected epithelial cells (no inflammasome)

- **Cytosolic LPS (lipopolysaccharide) recognition**
- **Caspase-4/5 activation** (not involved in apoptosis)
- **GSDMD pore formation for IL release**

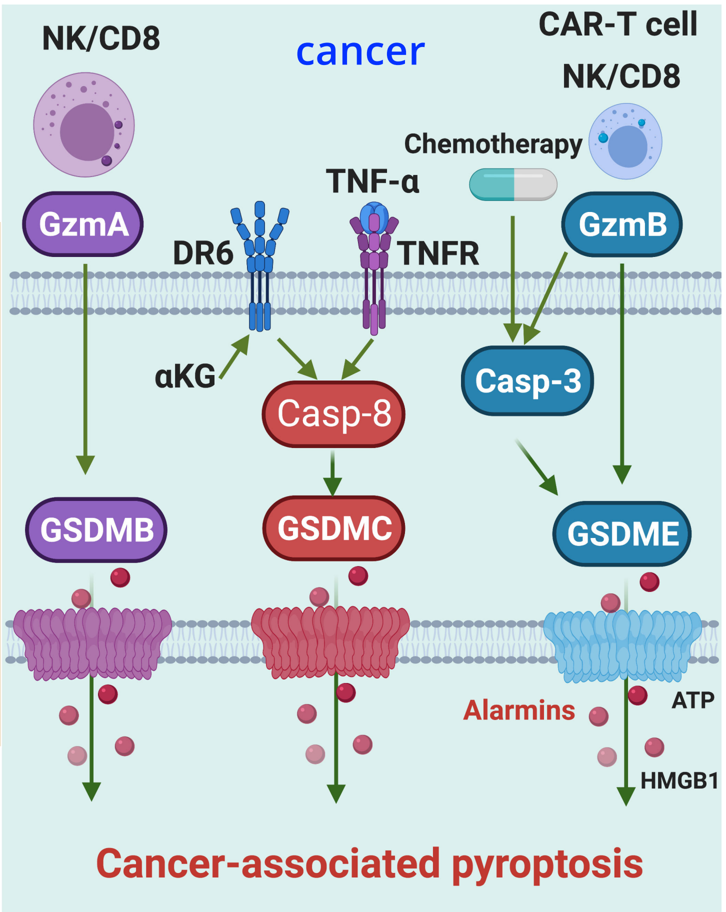


Kong & Zhang, Front. Immunol., 2023

Cancer-associated pyroptosis (CAP) molecular features



Activation of other GSDMs in



Kong & Zhang, Front. Immunol., 2023

Chemotherapy can trigger cancer cell pyroptosis

- Cancer GSDM pores formation and pyroptosis

Role in anti-tumor immunity

- DAMPs release : anti-cancer immune cells recruitment in the tumor microenvironment (TME)
- Cytotoxic immune cells release granzyme proteases and TNF toward cancer cells : induce CAP

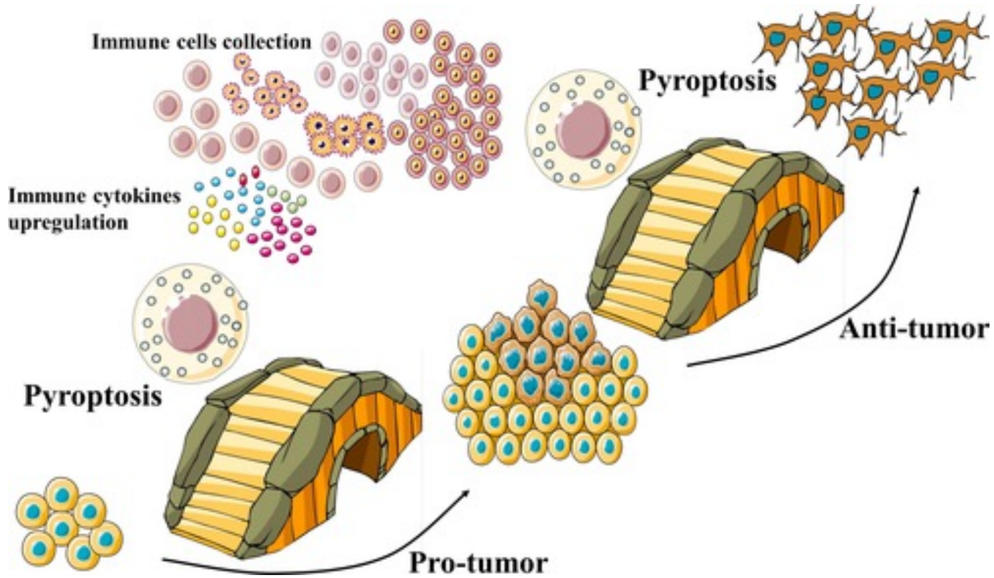
- GSDM downregulation found in many cancers

But pyroptosis can be pro-tumor in some cases

- **Sustained inflammation** due to prolonged inflammasome pyroptosis can fuel **tumor progression**
- **High level of GSDM (B, C or D)** is associated with a **high metastasis** rate and a low survival rate in breast cancer, melanoma, non-small cell lung cancer (NSCLC)

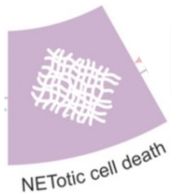
Pyroptosis
= bridge between immune system and tumor

Released cytokines
influences the tumor immune microenvironment
(pro/anti-tumor)



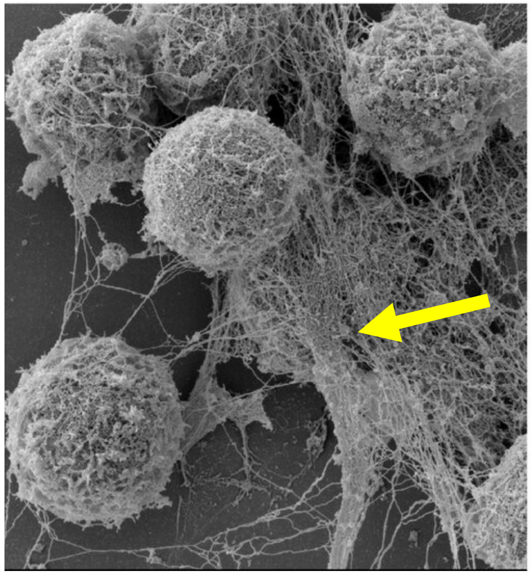
Xia et al, Cell Detah Dis., 2019
Li et al, Cancer Sci., 2021

NETosis : a granulocyte neutrophil death

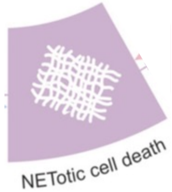


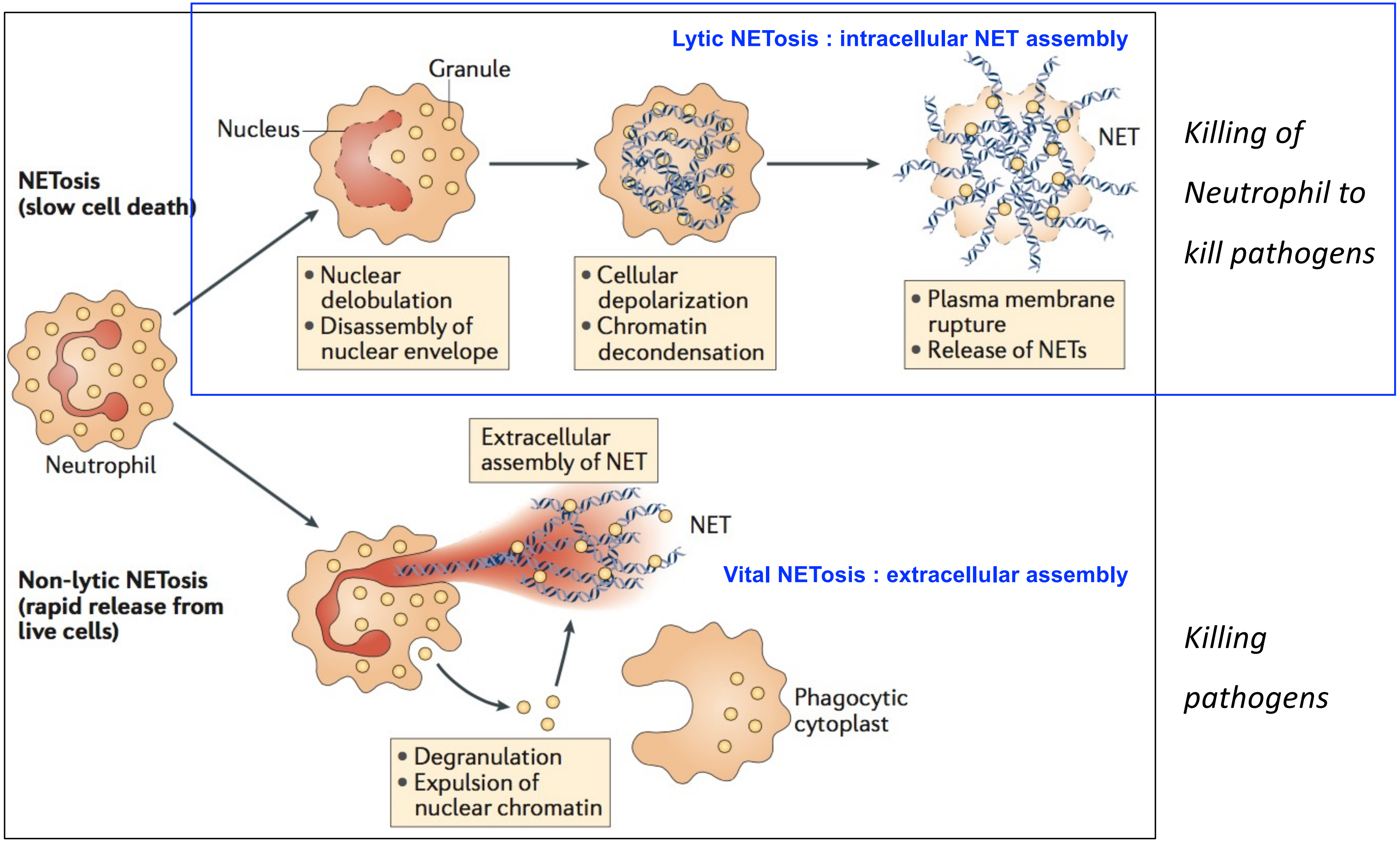
- Death of **granulocyte neutrophils** to release an **extracellular trap** (NET) that **entangles and eliminates pathogens** in the **blood stream** or **infected tissues** (innate immunity)
- In the NET : decondensed chromatin + elastase (protease) + myeloperoxydase (MPO) + citrullinated histones

  = **cytotoxic = kill pathogens**



Lytic NETosis *versus* vital NETosis



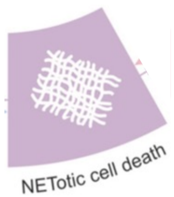


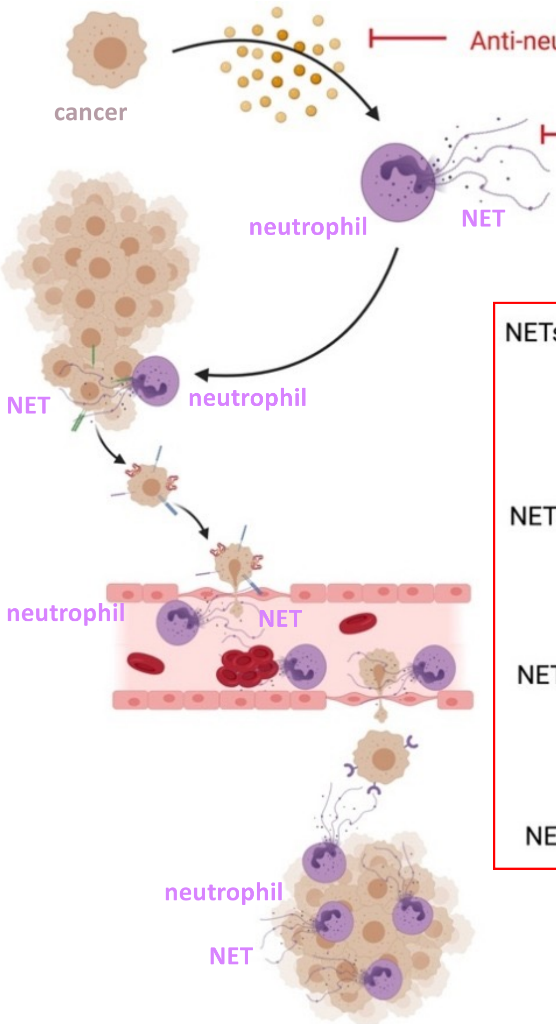
Killing of Neutrophil to kill pathogens

Killing pathogens

Papayannopoulos, Nat. Rev. Immuno., 2018

NETosis is pro-tumoral and induces cancer-associated systemic effects





Anti-neutrophil recruitment therapy

Anti-NET therapy

Anti-NET therapy, to treat cancer ?

Tumour-derived factors induce NETosis

↓

NETs induce tumour cell proliferation and foster an immunosuppressive enrivonment

↓

NETs favour tumour cell mobilization and vascular permeability

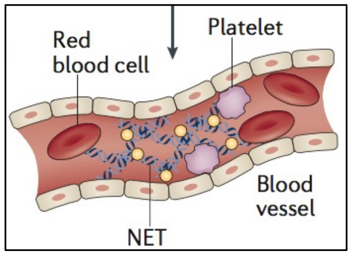
↓

NETs capture circulating tumour cells and promote their egress from the vasculature

↓

NETs mediate the establishment of metastases

NETosis and cancer-associated systemic effect

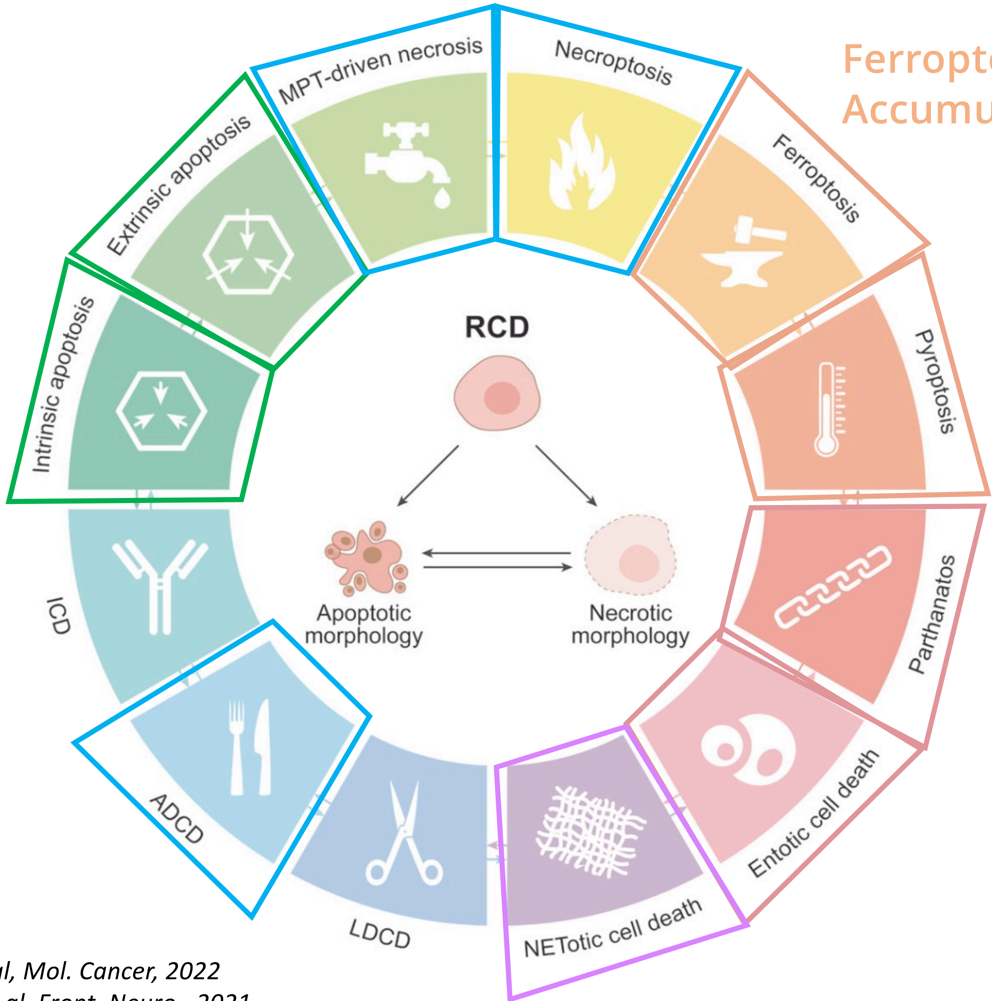


Tumor-associated thrombosis

Blood vessel occlusion
Inflammation
Organ failure

De Meo et al, Sem. Immuno., 2021
Cedervall et al., Cancer Res., 2016
Papayannopoulos, Nat. Rev. Immuno., 2018

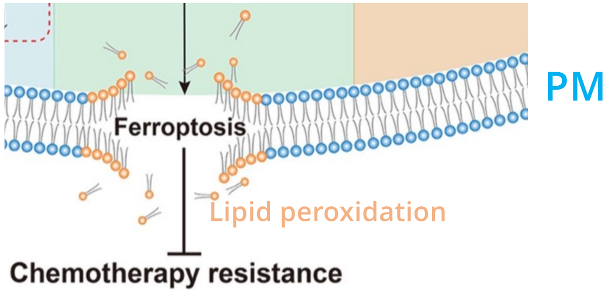
Regulated cell deaths : ferroptosis and parthanatos



Zhang et al, Mol. Cancer, 2022
Koehler et al, Front. Neuro., 2021

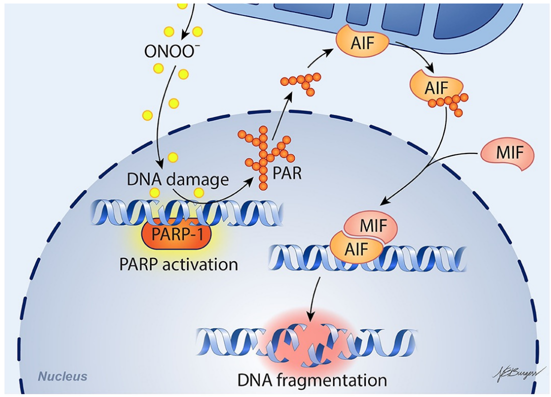
Ferroptosis : iron mediated death

Accumulation of cellular iron and lipid peroxidation



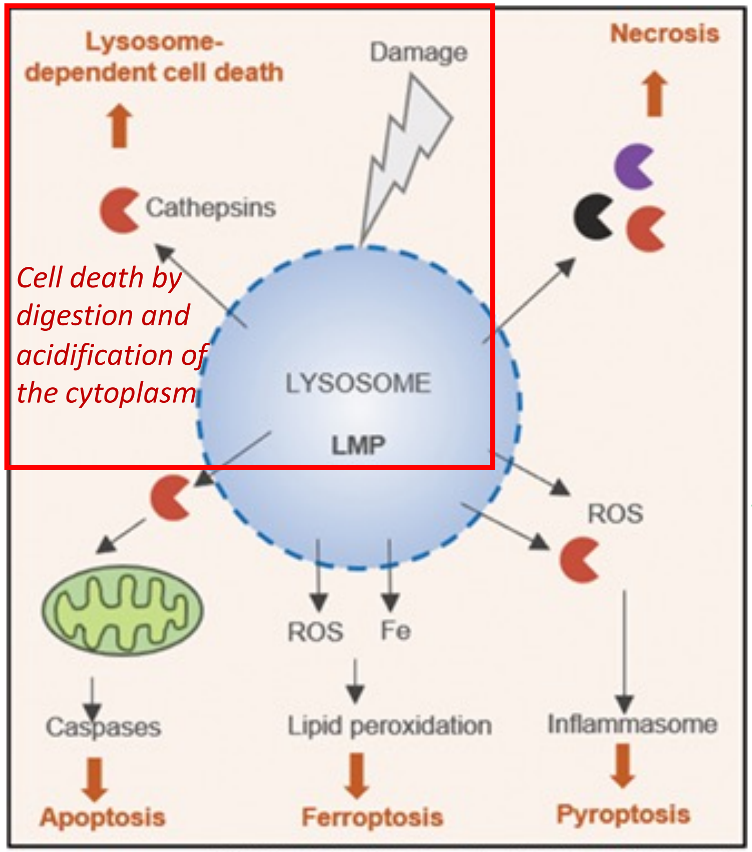
Parthanatos : mitochondria mediated death

Hyperactivation of the DNA repair PARP1

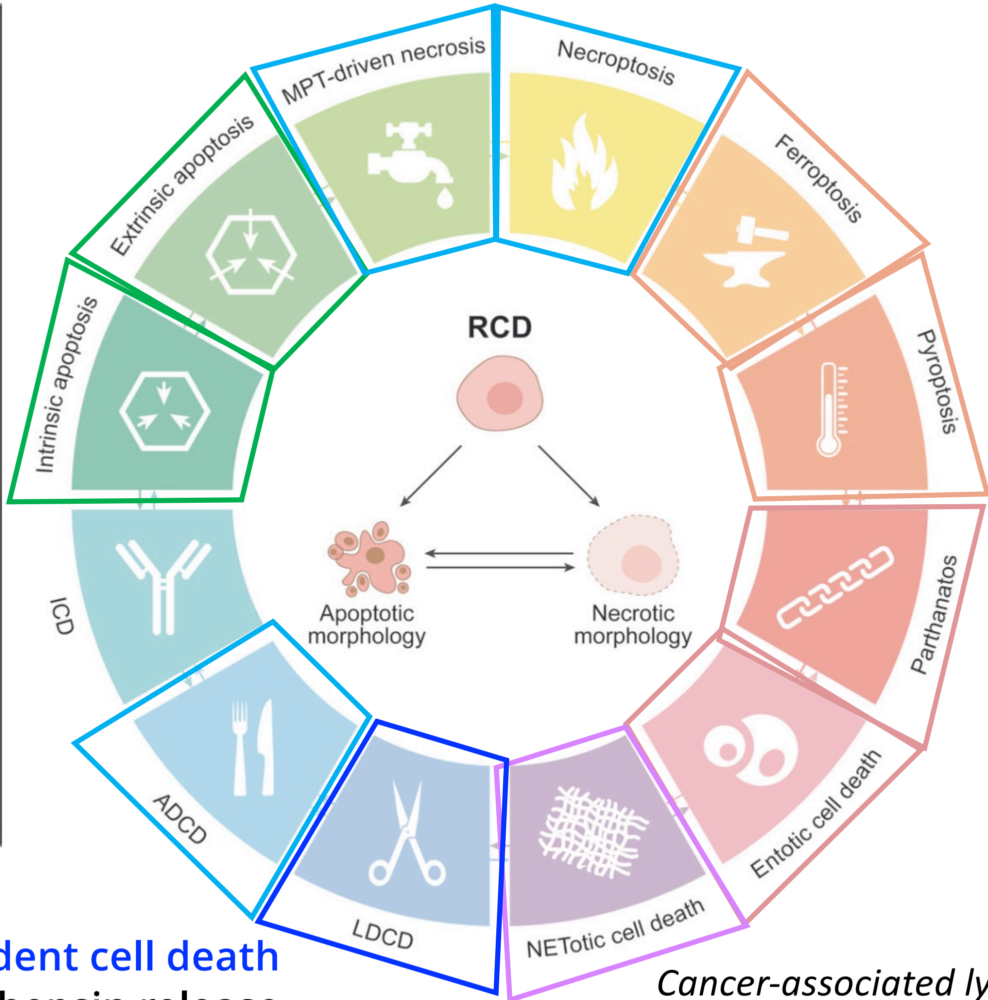


PARP1 overexpression is frequent in cancer (without parthanatos)

Regulated cell deaths : lysosomal-dependent cell death (LDCD)



Wang et al, Traffic, 2018

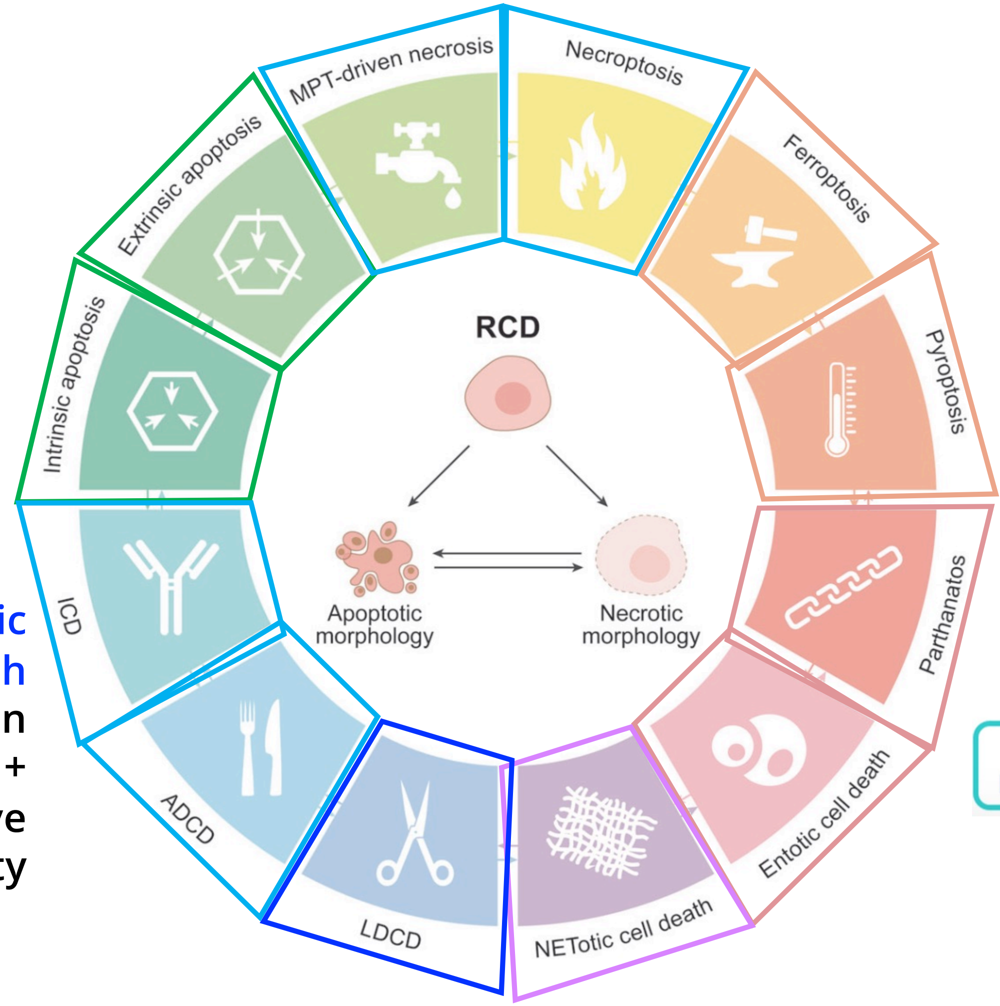


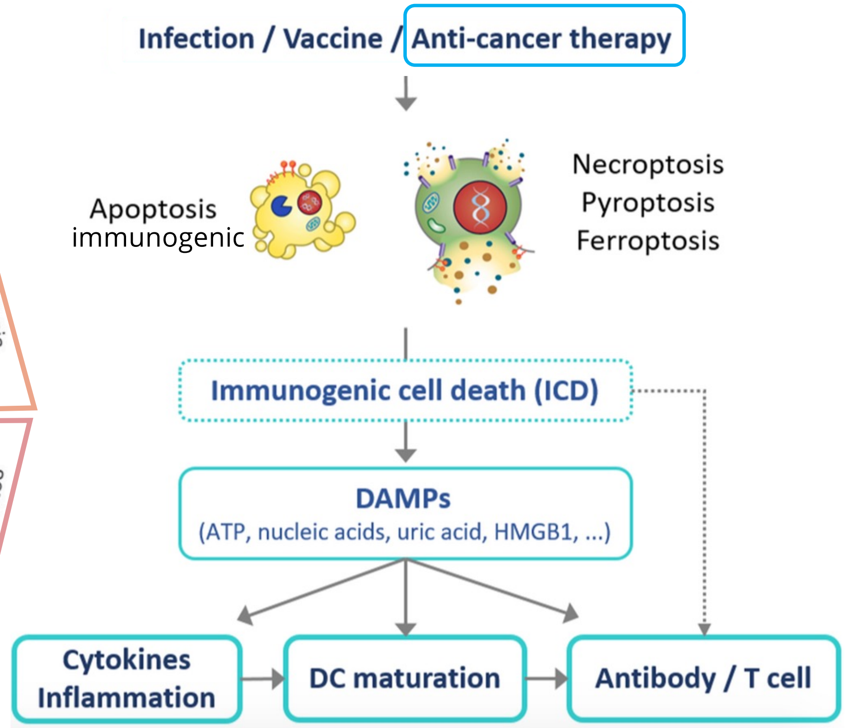
Lysosomal-dependent cell death
lysosome permeabilization, cathepsin release

Cancer-associated lysosomes
Suitable for specific LDCD induction ?

Regulated cell deaths : immunogenic cell death (ICD)

Immunogenic cell death
Inflammation
+
adaptive immunity



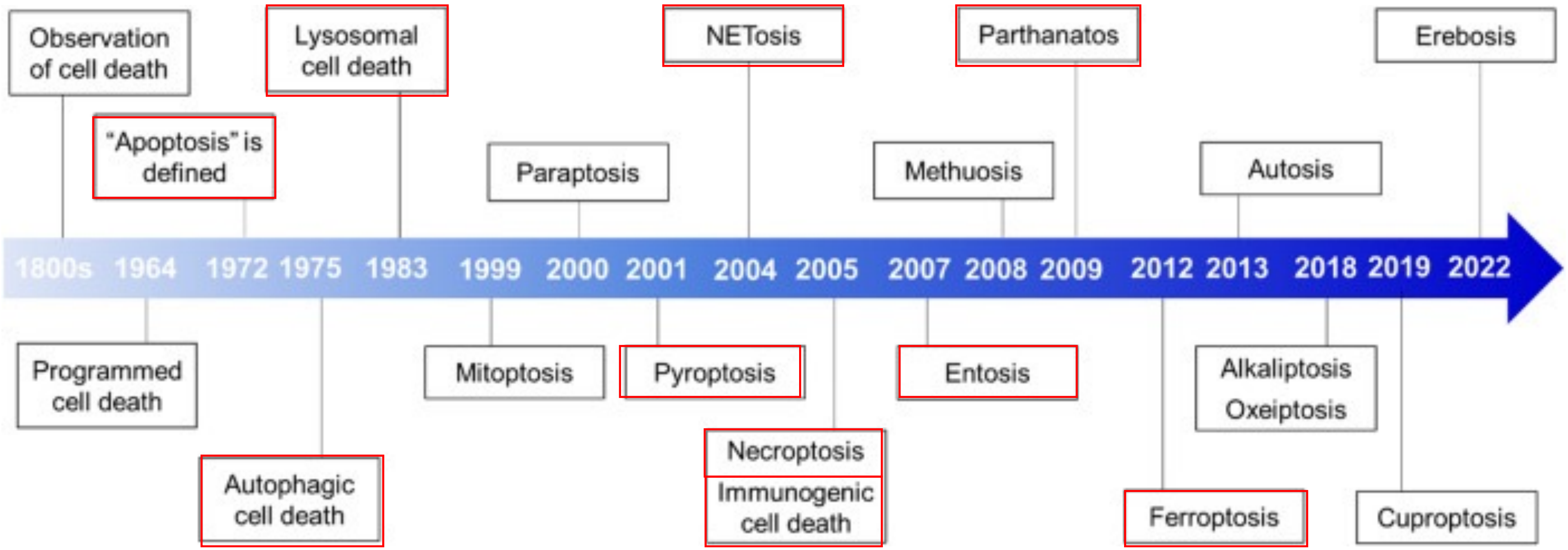


= immunogenic response /
anti-tumor immunity after therapy

Agressive cancers often resist ICD : cause of relapse

Lee et al, Viruses, 2021

Timeline of the discovery of the programmed cell deaths



Park et al, Experim. Mol. Med., 2023