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Review

Cell death

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MLKL trafficking and accumulation at the plasma membrane control the kinetics and threshold for necroptosis

Andre L. Samson, Ying Zhang, Niall D. Geoghegan, Xavier J. Gavin, Katherine A. Davies, Michael J. Mlodzianoski, Lachlan W. Whitehead, Daniel Frank, Sarah E. Garnish, Cheree Fitzgibbon, Anne Hempel, Samuel N. Young, Annette V. Jacobsen, Wayne Cawthorne, Emma J. Petrie, Maree C. Faux, Kristy Shield-Artin, Najoua Lalaoui, Joanne M. Hildebrand, John Silke, Kelly L. Rogers, Guillaume Lessene, Edwin D. Hawkins & James M. Murphy

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Molecular Cell

Review

The biochemical pathways of apoptotic, necroptotic, pyroptotic, and ferroptotic cell death

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REVIEW ARTICLE

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REVIEW ARTICLE

Targeting paraptosis in cancer: opportunities and challenges

Fangquan Chen[1,3], Hu Tang[1,3], Xiutao Cai[1], Junhao Lin[1], Limin Xiang[1], Rui Kang[2], Jiao Liu[1] and Daolin Tang[2]

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REVIEW ARTICLE OPEN

Diversity and complexity of cell death: a historical review

Wonyoung Park[1,2], Shibo Wei[3], Bo-Sung Kim[1,2], Bosung Kim[1,2], Sung-Jin Bae[4], Young Chan Chae[5], Dongryeol Ryu[6] and Ki-Tae Ha[1,2]

nature reviews molecular cell biology https://doi.org/10.1038/s41580-023-00689-6

Review article

A guide to cell death pathways

Junying Yuan[1,2] & Dimitry Ofengeim[3]

Cell death

Cell death supports **morphogenesis** during development and **homeostasis** after birth by removing damaged or obsolete cells.
It also curtails the spread of pathogens by **eliminating infected cells**.

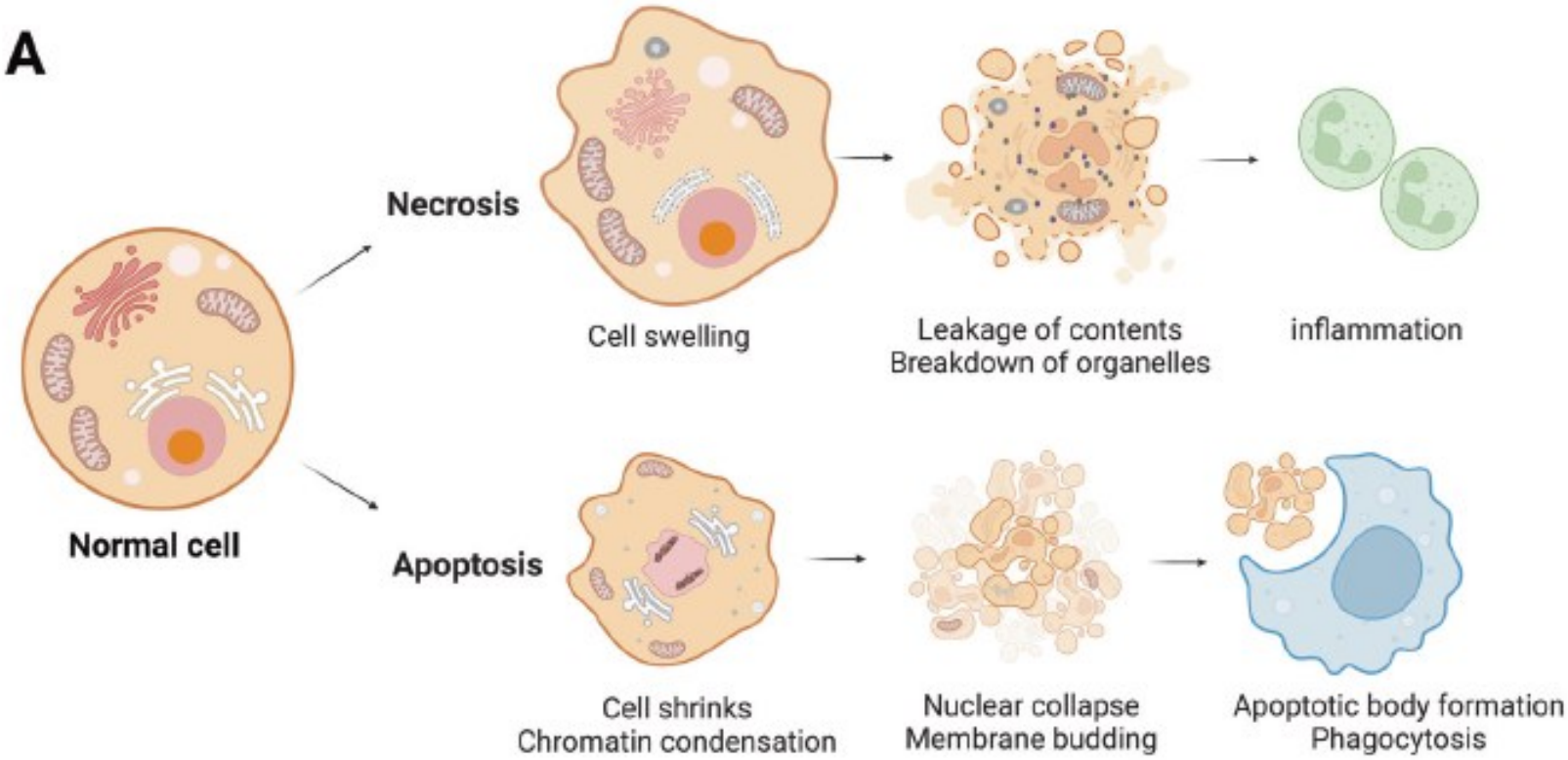
It is estimated that a staggering **10^{11} cells undergo programmed cell death each day** in an adult human, which is equivalent to our entire body weight in the course of a year.
Cell proliferation maintains the status quo as the body rids itself of old, dysfunctional, infected, or mutated cells.
Therefore, it makes intuitive sense that disrupting the homeostatic balance between cell proliferation and cell death will result in organismal dysfunction.

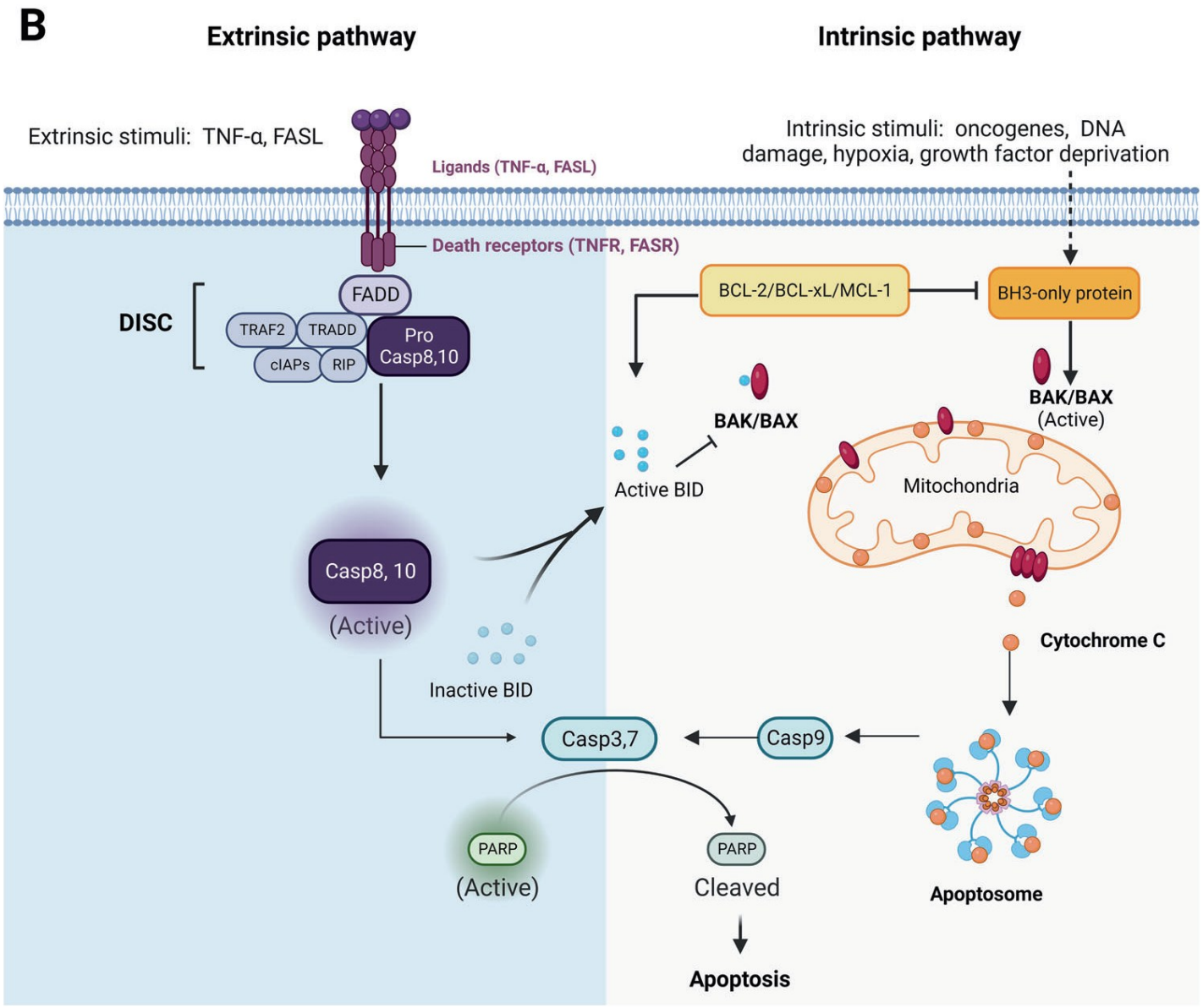
Indeed, too little cell death contributes to diseases of excess proliferation, such as cancer, and too much cell death to degenerative disorders, like neurodegenerative diseases.

We are now starting to decipher how cell death influences processes in addition to development, such as chemotaxis, phagocytosis, regeneration and **immunogenicity**.

Omeostasis of organisms as a balance between cell survival and cell death

- In 1842, Karl Vogt, while studying the metamorphosis of amphibians, realized that the resorption of the notochord and its replacement by vertebrae involved physiological cell death.
- The concept of 'programmed cell death' was conceived more than a century later, in 1964, when Lockshin and Williams described regulated cell death during insect metamorphosis.
- Schweichel and Merker **1973** were the first to report the presence of three distinct cell death morphologies in rat embryos, after exposure to toxins, which also occur with very low frequency in the developing mouse: type I cell death was associated with **heterophagy** ('eating of another'); type II cell death was associated with **autophagy** ('eating of itself'); and type III cell death **did not involve digestion**.
- Today, these cell death modes are referred to as **apoptosis**, cell death associated with **autophagy** and **necrosis**, respectively.
- We currently understanding cell death as a fundamental process that is regulated by multiple interconnected signalling pathways.


A
Necrosis
Cell swelling
Leakage of contents
Breakdown of organelles
inflammation
Normal cell
Apoptosis
Cell shrinks
Chromatin condensation
Nuclear collapse
Membrane budding
Apoptotic body formation
Phagocytosis


B
Extrinsic pathway
Intrinsic pathway
Extrinsic stimuli: TNF-α, FASL
Ligands (TNF-α, FASL)
Intrinsic stimuli: oncogenes, DNA damage, hypoxia, growth factor deprivation
Death receptors (TNFR, FASR)
DISC
FADD
TRAF2
TRADD
cIAPs
RIP
Pro Casp8,10
BCL-2/BCL-xL/MCL-1
BH3-only protein
BAK/BAX
BAK/BAX (Active)
Active BID
Mitochondria
Casp8, 10
(Active)
Inactive BID
Cytochrome C
Casp3,7
Casp9
PARP
(Active)
PARP
Cleaved
Apoptosome
Apoptosis

For two decades, **apoptosis was considered to be the standard cell death** form during development, homeostasis, infection and pathogenesis **whereas necrosis was mostly considered to be an 'accidental'** cell death that occurred in response to physico-chemical insults.

Recent evidence have greatly changed this view, and revealed the existence of multiple pathways of regulated necrosis (RN), including necroptosis, pyroptosis, and ferroptosis.

They all **converge** in a final step of the **plasma membrane disruption**, the subsequent **release of Damage-Associated Molecular Patterns** (DAMPs), and the induction of **inflammation** and activation of immune responses.

Although different forms of RN and apoptosis have their own defined machinery, they all communicate and cross-regulate. Therefore, the type of cell death predominating depends on the activation or deactivation of the molecular regulatory players involved in each pathway.

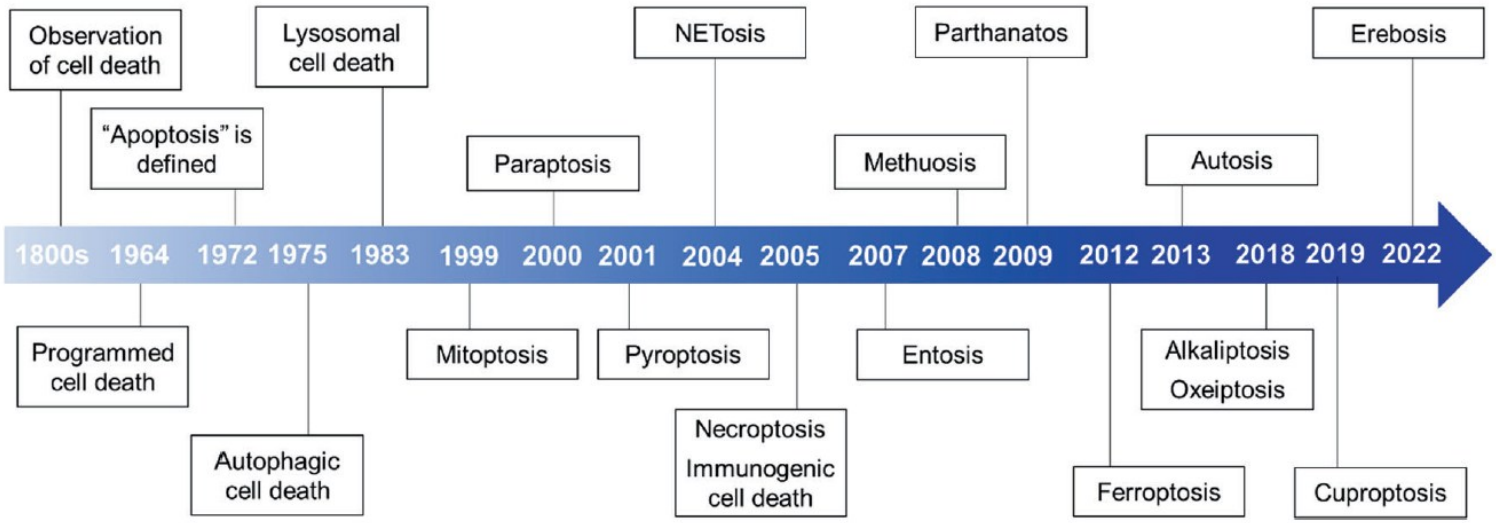


Fig. 1 Timeline of the discovery of cell death. This timeline depicts the important discoveries and advancements in cell death research, including the recognition of multiple forms of cell death.

- An apoptotic signal is transduced through the protease caspase-8, whereas **necroptosis**, a lytic form of cell death, may occur when **caspase-8 is inhibited**.
- **Necroptosis** probably evolved as **an anti-viral defense** mechanism because certain viruses, including cytomegalovirus, herpes simplex viruses, vaccinia virus, and adenovirus, encode inhibitors of caspase-8 to prevent apoptosis.
- Consistent with this notion, necroptosis-deficient mice are more susceptible than wild-type (WT) mice to infection with vaccinia virus.

Necroptosis

Necroptosis is a form of PCD that differs from necrosis and apoptosis in terms of morphology and biochemistry.

Necroptosis was first identified and described by Dr. Francis Chan et al. in 2005.

It is mediated by a signaling cascade involving the activation of receptor-interacting protein kinase 1 (RIPK1) and RIPK3 and the formation of a complex called the necrosome.

TNFα and TNFR1 ligation triggers a well-characterized necroptosis- inducing pathway. Under normal conditions, stress signals activate caspase-8, leading to the initiation of apoptosis. H

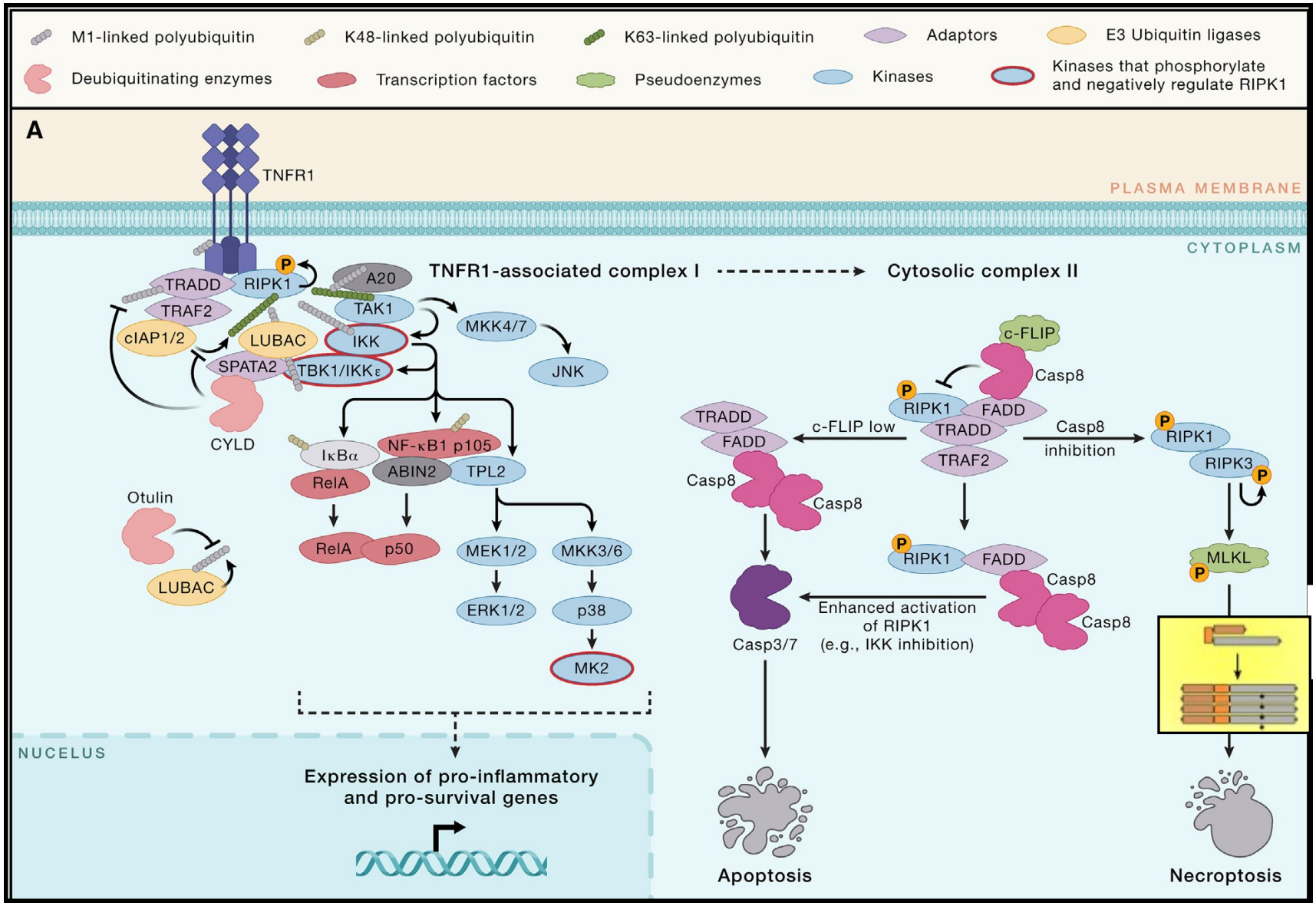
owever, **when caspase-8 activity is suppressed**, RIPK1 and RIPK3 are activated, leading to necroptosis. During TNF-induced necroptosis, RIPK1 can recruit RIPK3 through the RIP homotypic interaction motif (RHIM) to form necrosomes, which promote the oligomerization and phosphorylation of mixed lineage kinase domain-like protein (MLKL).

The oligomeric form of MLKL is translocated from the cytosol to the plasma membrane, leading to the formation of **membrane pores** and the subsequent **rupture of the plasma membrane**, resulting in the **release of DAMPs**.

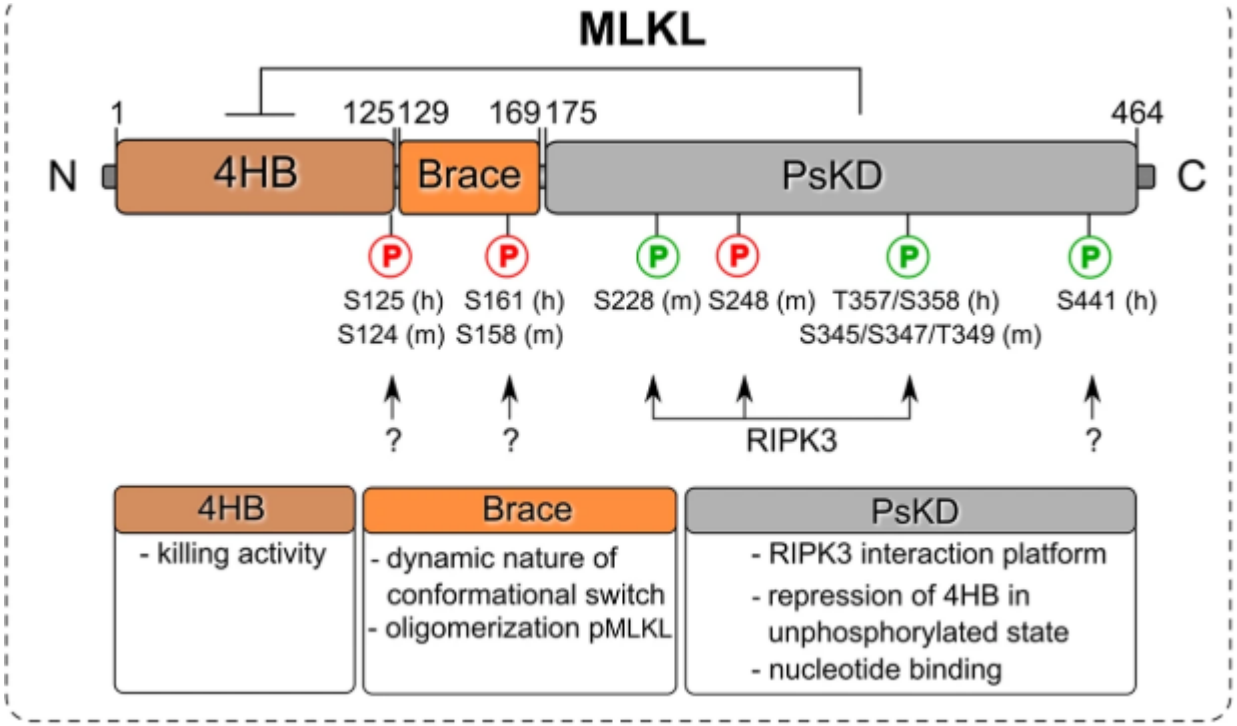
The released DAMPs are recognized by PRRs on immune cells, leading to the **activation of inflammatory responses**. This inflammatory response can contribute to the clearance of dead cells and the initiation of tissue repair processes. However, excessive or prolonged inflammation can cause tissue damage and contribute to the pathogenesis of various diseases.

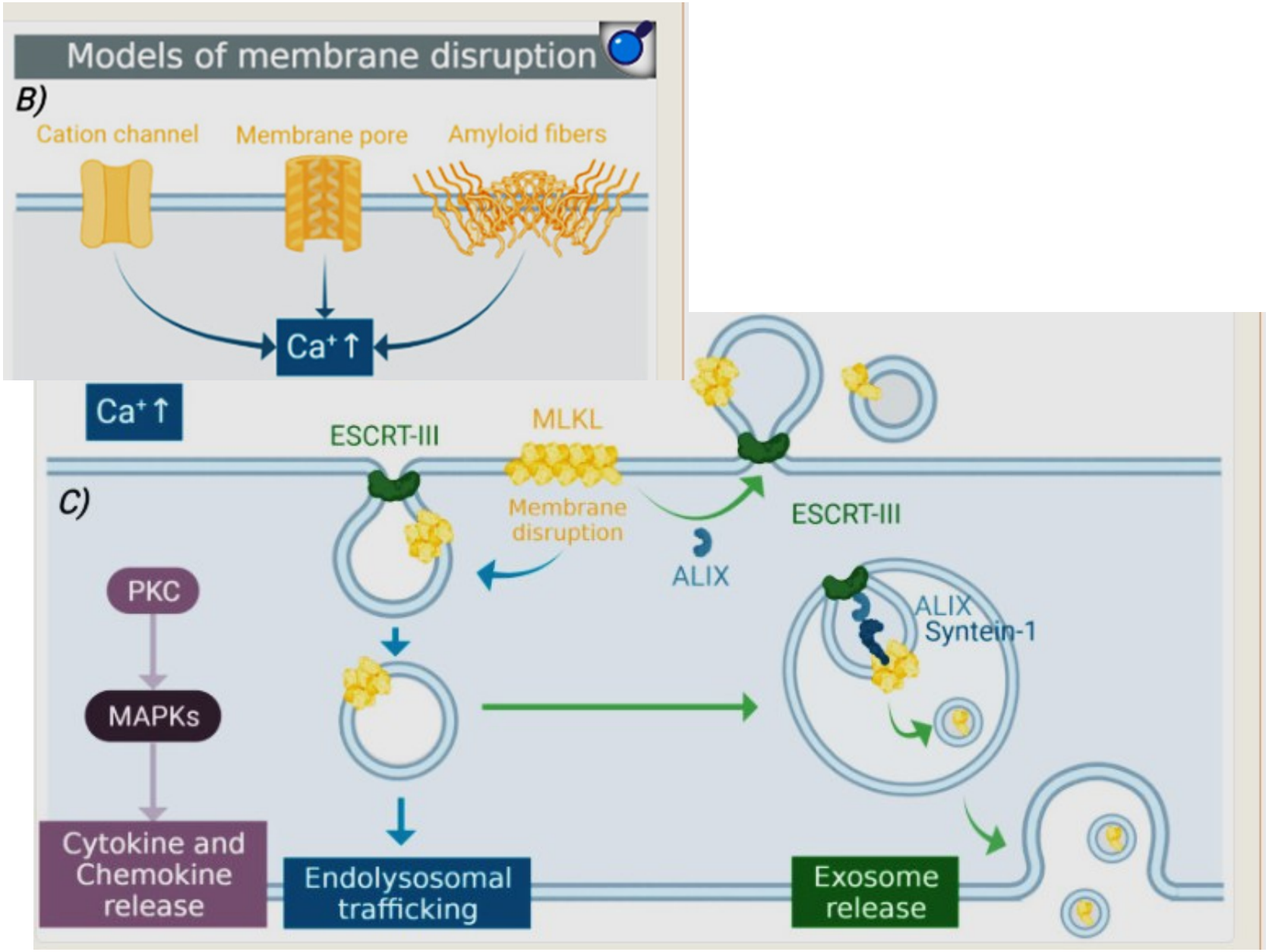
Research results suggest that necroptosis is involved in the pathogenesis of several diseases, including neurodegenerative diseases, viral infections, ischemic injury, and cancer.

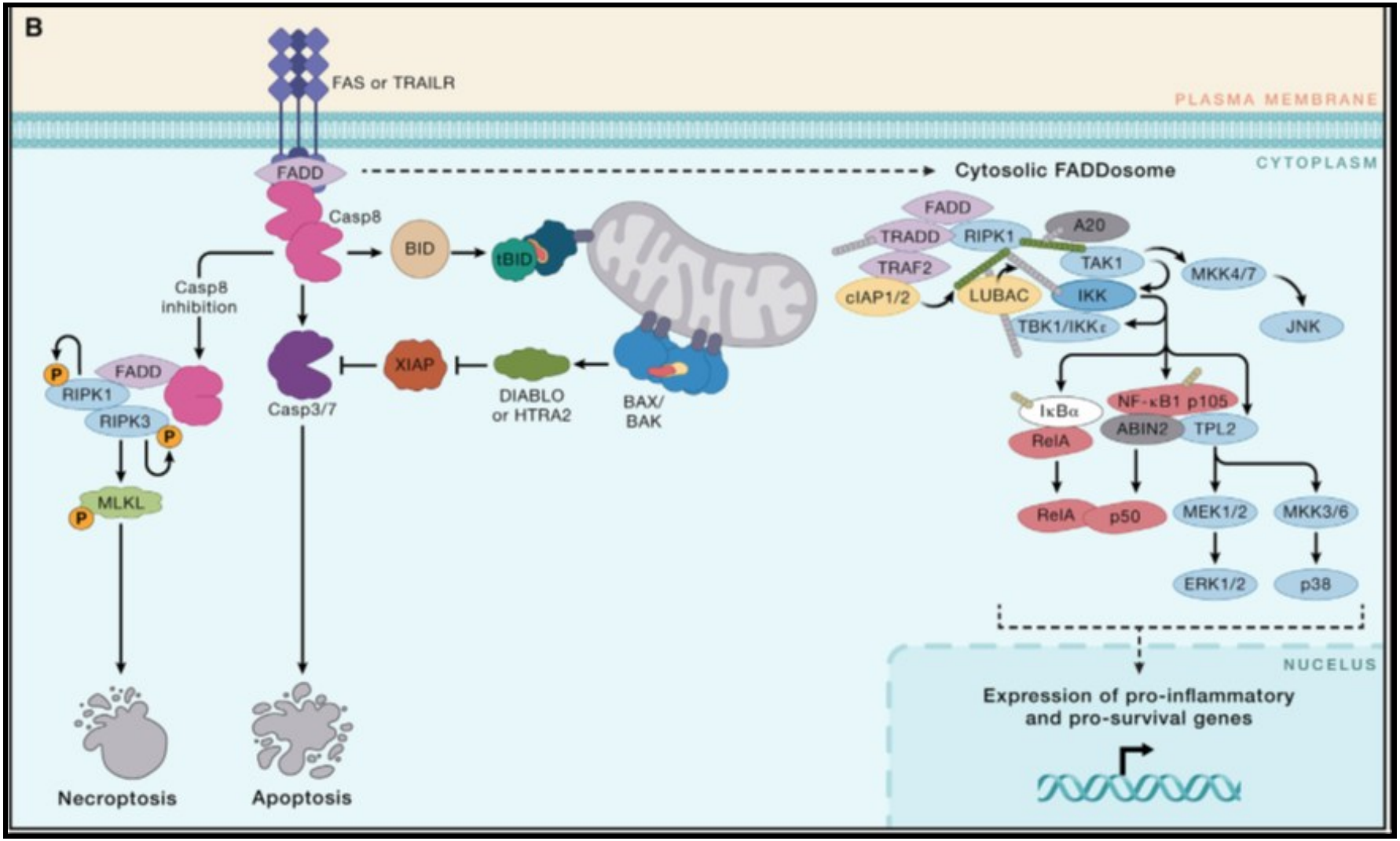
The inhibition of necroptosis has shown therapeutic potential in some disease models, making it an attractive target for drug development.



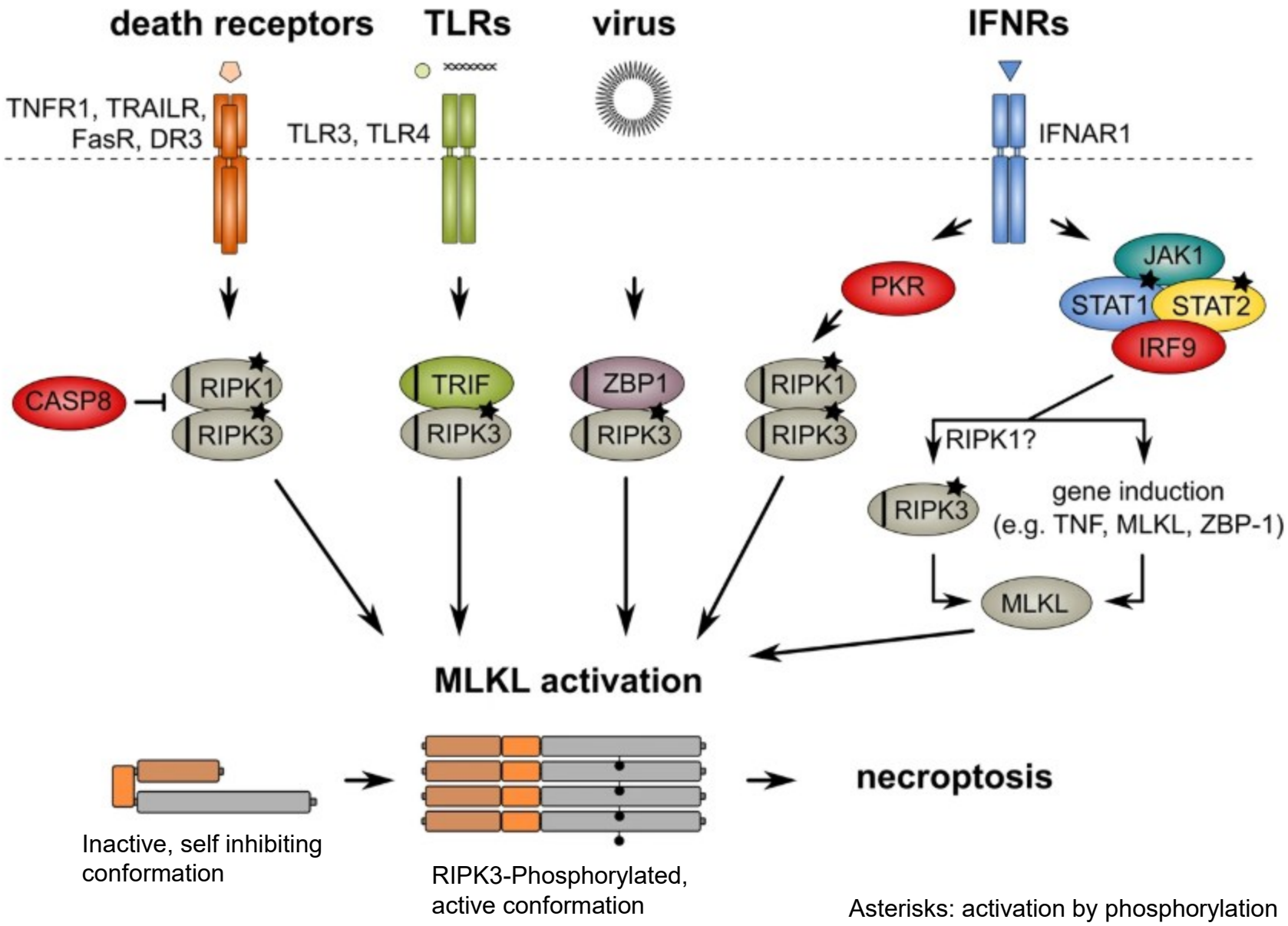
Necroptosis is a caspase-independent form of RN that relies on the activity of the pseudokinase Mixed Lineage Kinase domain-Like (MLKL) protein. Upon activation by Receptor-Interacting Protein Kinase 3 (RIPK3), MLKL forms oligomers and translocates to the plasma membrane, causing cell death

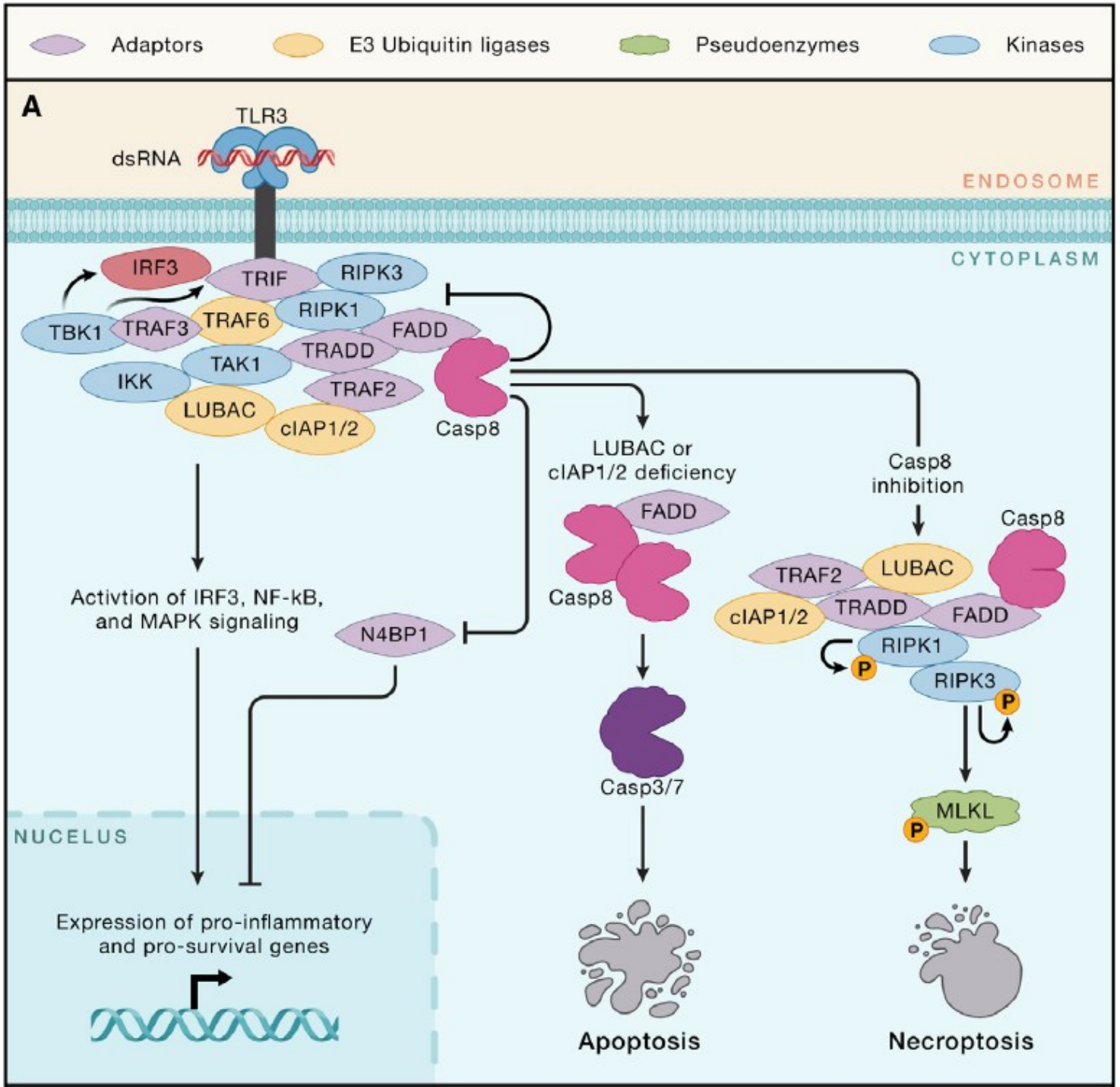

MLKL
1
125
129
169
175
464
N
4HB
Brace
PsKD
C
P
S125 (h)
S124 (m)
S161 (h)
S158 (m)
S228 (m)
S248 (m)
T357/S358 (h)
S345/S347/T349 (m)
S441 (h)
?
?
RIPK3
?
4HB
- killing activity
Brace
- dynamic nature of conformational switch
- oligomerization pMLKL
PsKD
- RIPK3 interaction platform
- repression of 4HB in unphosphorylated state
- nucleotide binding

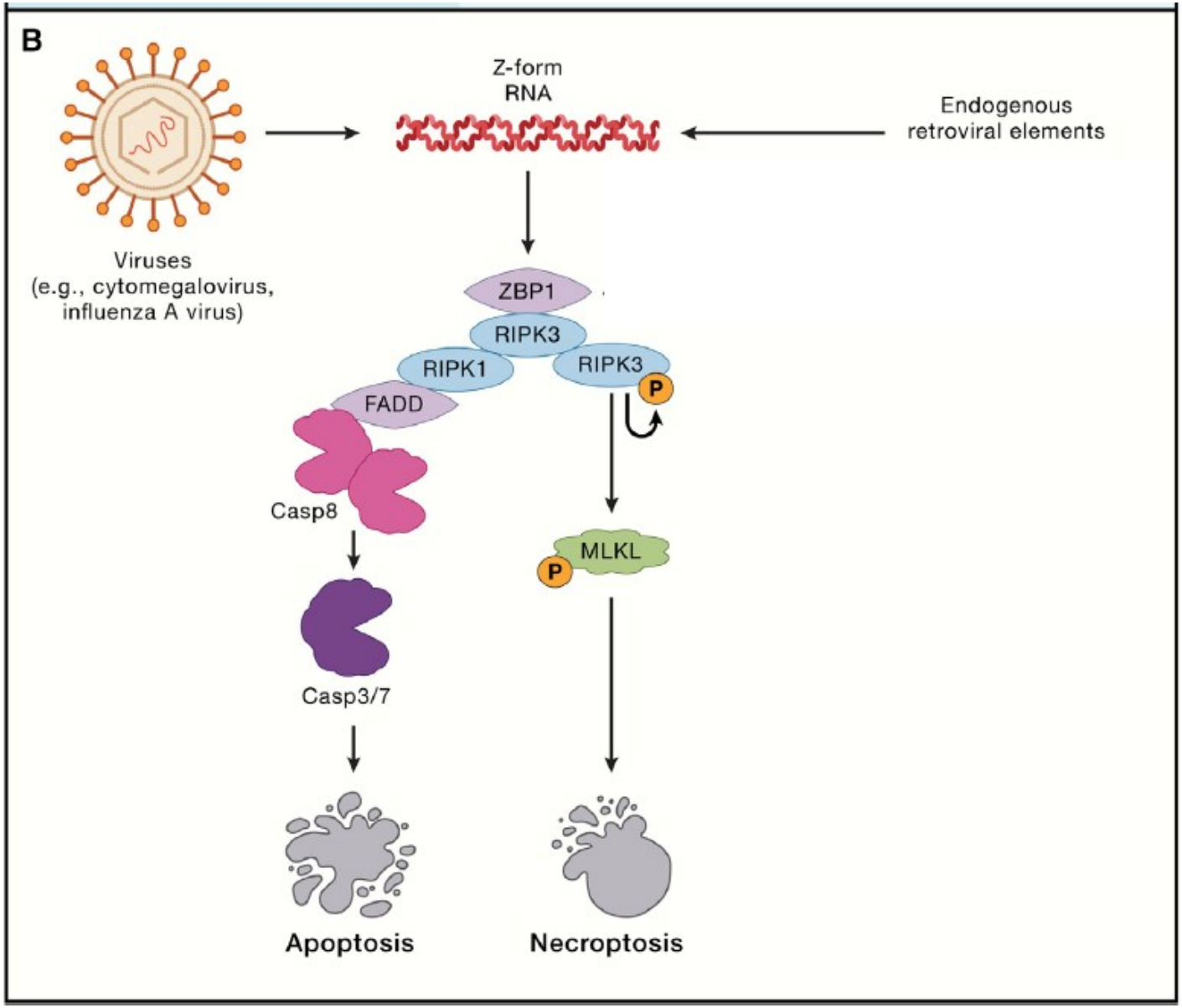

Models of membrane disruption
B)
Cation channel
Membrane pore
Amyloid fibers
Ca⁺ ↑
Ca⁺ ↑
ESCRT-III
MLKL
C)
Membrane disruption
ALIX
ESCRT-III
PKC
MAPKs
ALIX
Syntein-1
Cytokine and Chemokine release
Endolysosomal trafficking
Exosome release


B
FAS or TRAILR
PLASMA MEMBRANE
FADD
Cytosolic FADDosome
CYTOPLASM
Casp8
BID
tBID
Casp8 inhibition
FADD
RIPK1
RIPK3
MLKL
Casp3/7
XIAP
DIABLO or HTRA2
BAX/ BAK
TRADD
RIPK1
A20
TRAF2
cIAP1/2
LUBAC
TAK1
IKK
TBK1/IKKε
MKK4/7
JNK
IκBα
RelA
NF-κB1 p105
ABIN2
TPL2
RelA
p50
MEK1/2
MKK3/6
ERK1/2
p38
NUCELUS
Expression of pro-inflammatory and pro-survival genes
Necroptosis
Apoptosis

Multiple stimuli lead to necroptosis.




Adaptors
E3 Ubiquitin ligases
Pseudoenzymes
Kinases
A
TLR3
dsRNA
ENDOSOME
CYTOPLASM
IRF3
TRIF
RIPK3
RIPK1
TBK1
TRAF3
TRAF6
FADD
TRADD
TAK1
IKK
TRAF2
LUBAC
cIAP1/2
Casp8
Activtion of IRF3, NF-kB, and MAPK signaling
N4BP1
NUCELUS
Expression of pro-inflammatory and pro-survival genes
LUBAC or cIAP1/2 deficiency
FADD
Casp8
Casp3/7
Apoptosis
Casp8 inhibition
Casp8
TRAF2
LUBAC
cIAP1/2
TRADD
FADD
RIPK1
P
RIPK3
P
MLKL
P
Necroptosis



ZBP1 triggers RIPK3-dependent cell death after binding to Z-form nucleic acids (Z-RNA is a left-handed alternative conformation for the RNA double helix), including Z-RNAs made by certain viruses or endogenous retroviral elements.

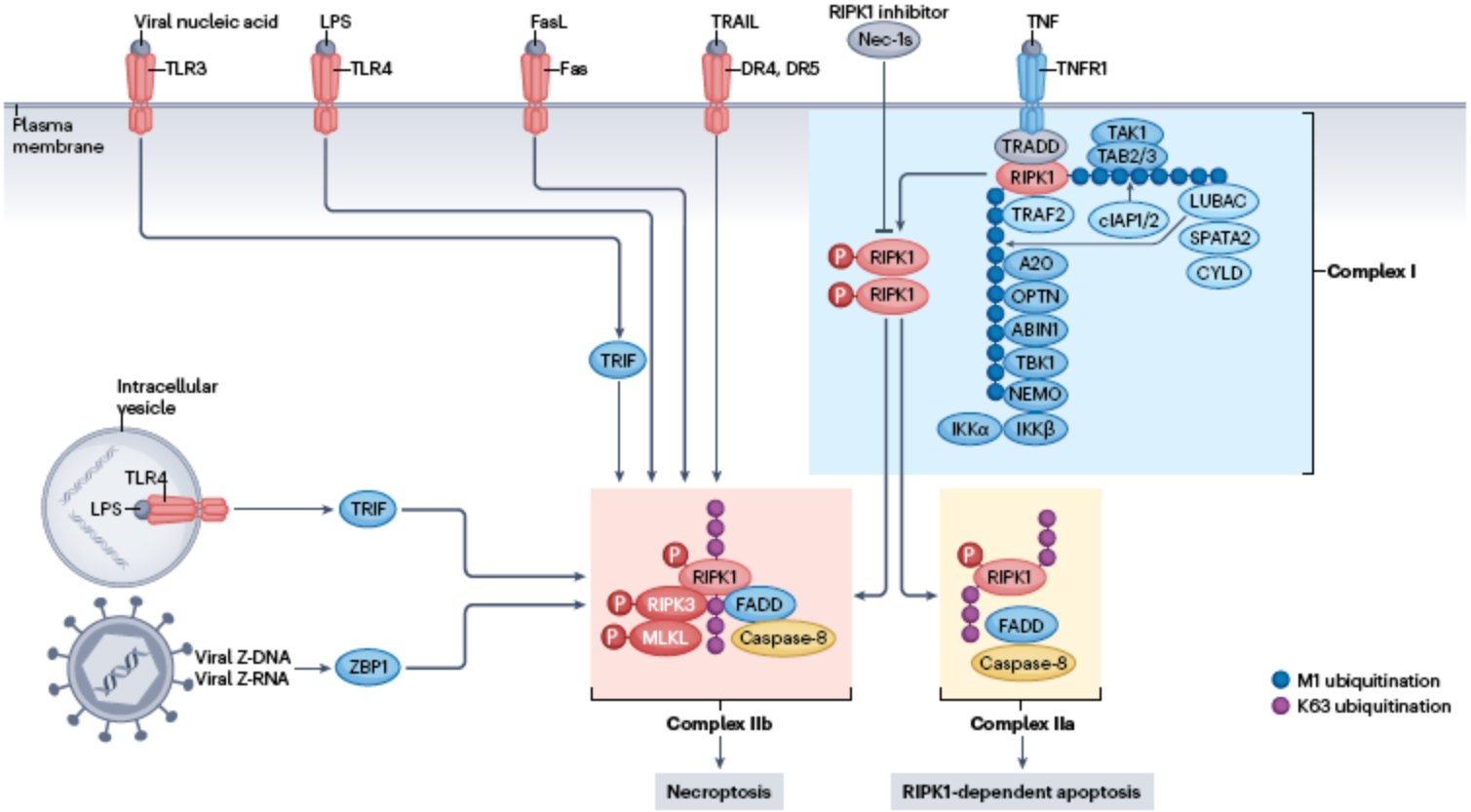


Fig. 2 | Necroptosis mediated by DD-containing receptors and pattern-recognition receptors. Upon activation by TNF, the cytoplasmic Death domain (DD) of trimerized TNFR1 mediates the formation of a transient intracellular complex, named complex I, which recruits adaptor protein TRADD, the DD-containing protein kinase RIPK1 and several E3 ubiquitin ligases, including TRAF2, cIAP1, cIAP2, the linear ubiquitin chain assembly complex (LUBAC), ubiquitin editing enzyme A20 and deubiquitylation enzyme cylindromatosis complex (CYLD and SPATA2) as well as multiple ubiquitin-binding proteins, including NEMO, ABIN1 and OPTN. In complex I, RIPK1 is rapidly polyubiquitylated by Lys63-linked and linear Met1-linked ubiquitin chains, which mediate the recruitment and activation of TAK1, TBK1 and IKK complexes. When RIPK1 kinase is not activated in living cells, the phosphorylation and subsequent ubiquitin–proteasome system-mediated degradation of IκB leads to the activation of NF-κB in the nucleus (not shown). Dysregulation of complex I promotes the activation of RIPK1 (as marked by pS166 RIPK1), which leads to the formation of two alternative cytosolic complexes, complex IIa or complex IIb, to mediate RIPK1-dependent apoptosis and necroptosis, respectively. Complex IIa includes the adaptor FADD protein, caspase-8 and RIPK1 to promote the activation of caspase-8, which in turn cleaves downstream caspases such as caspase-3, ultimately leading to apoptosis. When the activation of caspase-8 is inhibited, activated RIPK1 kinase binds to RIPK3 to form complex IIb. Activated RIPK3 in turn phosphorylates MLKL to mediate the execution of necroptosis by disrupting the integrity of the plasma membrane. The activation of Toll-like receptor (TLR3) and TLR4 by their cognate ligands, viral nucleic acids and bacterial lipopolysaccharides (LPS), respectively, in cells lacking caspase function can promote the binding of TIR domain-containing adaptor-inducing IFNβ (TRIF) to RIPK3 to mediate necroptosis. When activated by viral Z-DNA or Z-RNA, the binding of Z-DNA-binding protein 1 (ZBP1; also known as DAI) to RIPK3, mediated by their respective RHIMs (receptor-interacting protein homotypic interaction motifs), can also promote necroptosis in the absence of caspase function. The activation of DR4 or DR5 by TRAIL, Fas by FasL, and TLR4 by LPS has also been shown to promote necroptosis under certain conditions.

Pyroptosis involves inflammation and is mediated by caspase-1

Discovered in 2001; it is a form of **caspase-1- dependent Programmed Necrotic Cell Death in immune cells**, such as macrophages, monocytes and dendritic cells, that defends the body against intracellular pathogens.

Pyroptosis is initiated by the activation of pattern recognition receptors (PRRs, e.g. TLR) in response to pathogen-associated molecular patterns (PAMPs) or DAMPs, which trigger **inflammasome** assembly.

The inflammasome is a protein complex consisting of PRRs, adaptor proteins, and caspase-1, **with caspase-1 cleaving gasdermin D** (GSDMD) to produce an N-terminal GSDMD fragment that forms **membrane pores**.

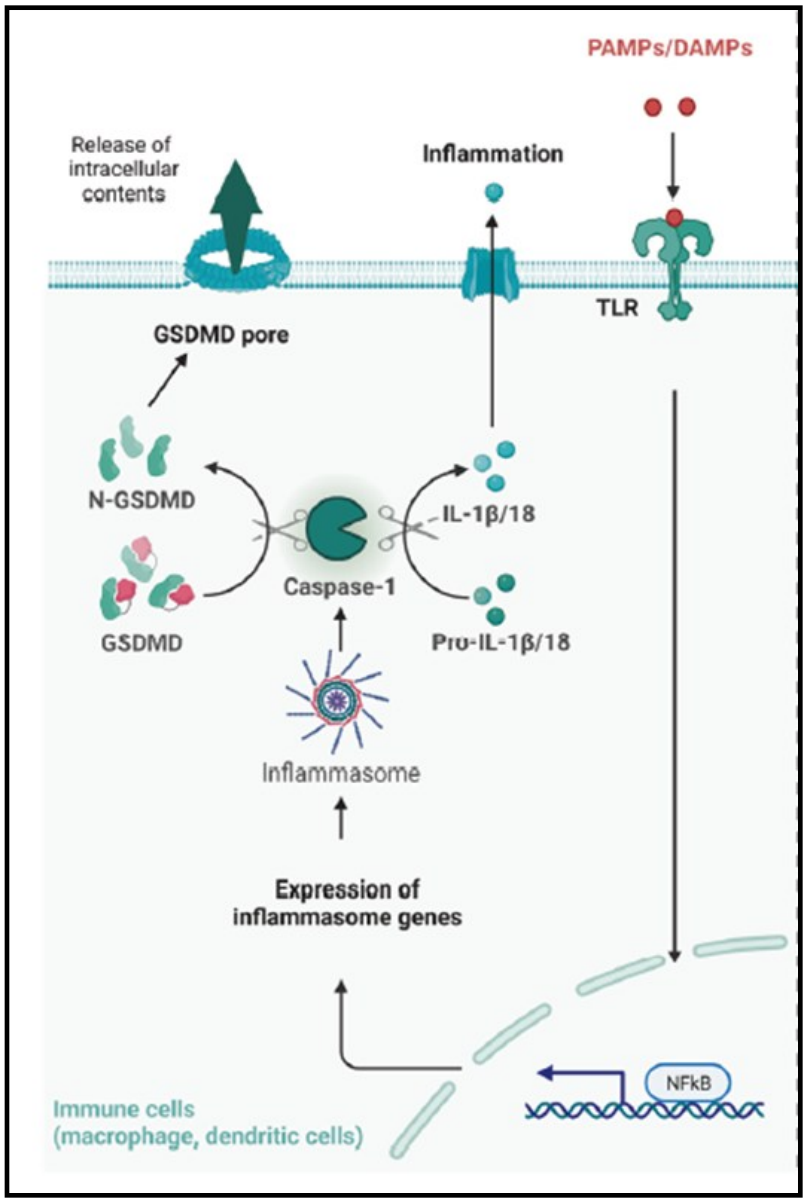
Caspase-1 also activates the proinflammatory interleukin-1 beta (IL-1β) and interleukin-18 (IL-18), via proteolysis.

The pores formed by GSDMD have an inner diameter of 10-14 nm, which can mediate the release of cytokines without signal peptides, such as mature IL-1beta.

Pyroptosis result in the release of proinflammatory cytokines and the recruitment of immune cells to the site of infection or injury.

Pyroptosis contributes to tissue damage in inflammatory disorders.

Pyroptosis has been implicated in several pathological conditions, including infectious diseases, autoimmune disorders, cancer, and neurodegenerative diseases.



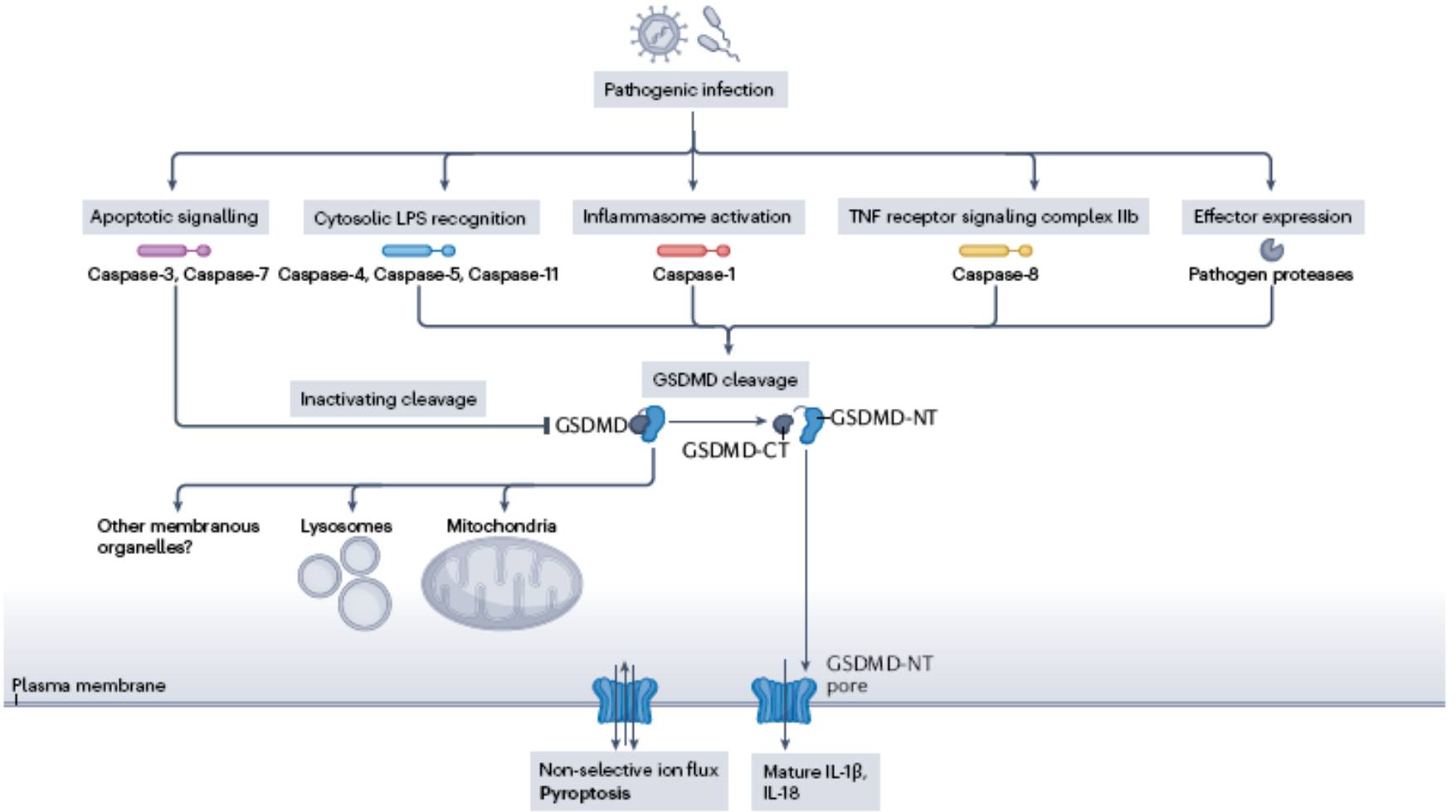
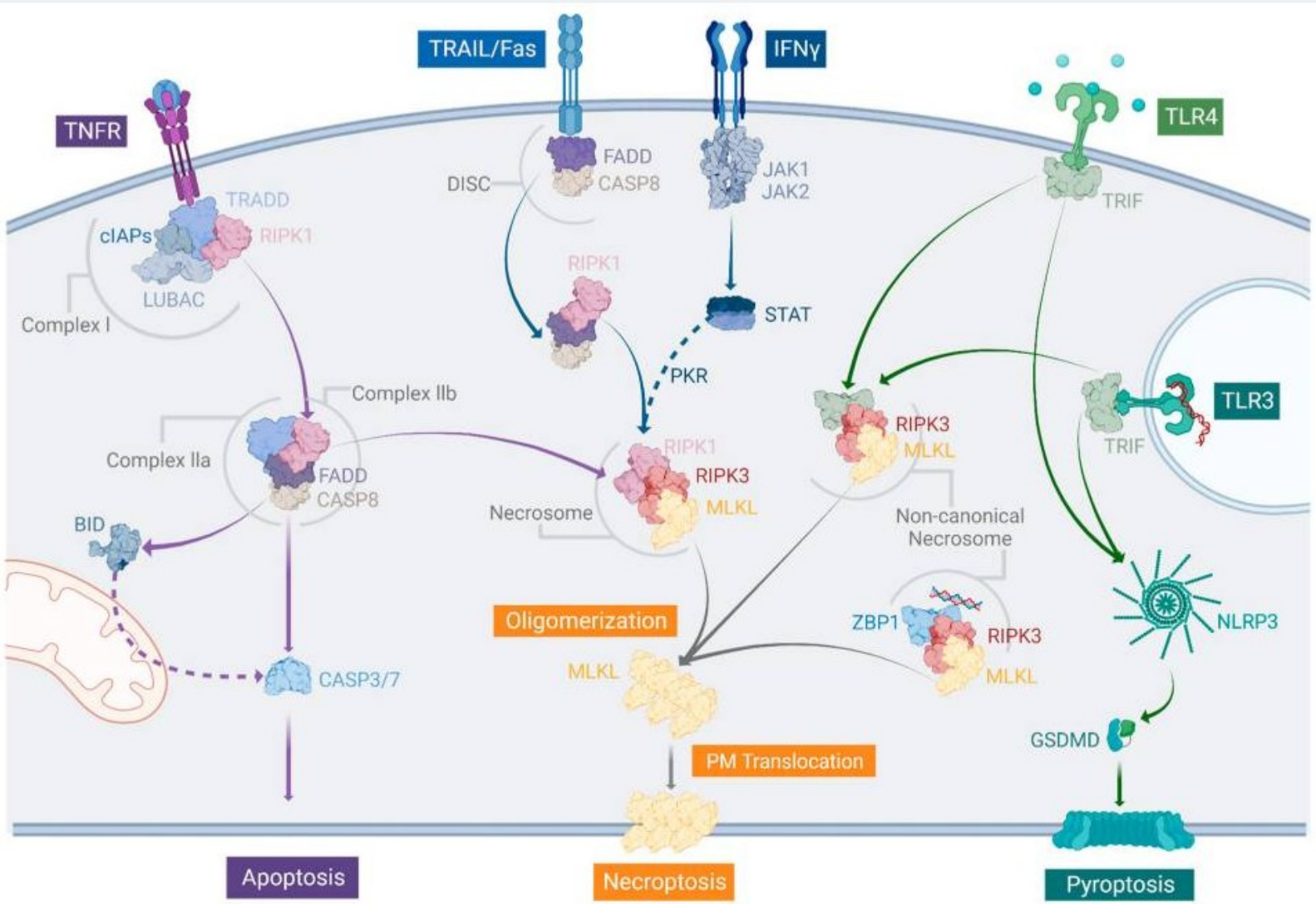


Fig. 3 | Pyroptosis induction and mature pro-inflammatory cytokine (IL-1β) release. Pathogenic infection by bacteria and viruses can promote the activation of caspase-1 and caspase-11 in mouse and caspase-1, caspase-4 and caspase-5 in human to mediate the cleavage of gasdermin D (GSDMD) to release the pore-forming N-terminal domain of GSDMD (GSDMD-NT) from its inhibitory C-terminal domain (GSDMD-CT). Activated caspase-8, caspase-3 and caspase-7 as well as pathogenic proteases can also mediate the cleavage of GSDMD. The pore formed by GSDMD-NT can mediate the secretion of mature IL-1β and IL-18 in living cells to promote inflammation and also promotes pyroptosis by cell lysis. LPS, lipopolysaccharides.

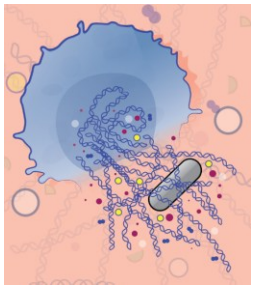
Necroptosis and its relationship with other cell death pathways



If caspase-8 is inhibited, RIPK1 is recruited to form the necrosome with RIPK3 and MLKL for the induction of necroptosis.

non-canonical necrosome can be formed via TRIF, after TLR3 and 4 activations, or when ZBP1 binds Z-DNA and Z-RNA intracellularly. TRIF can activate the NLRP3 inflammasome for the caspase-1-dependent cleavage of GSDMD to induce pyroptosis

NETosis (described by Volker Brinkmann et al. in 2004)



NETosis is characterized by the **release of neutrophil extracellular traps (NETs) into the extracellular space**.

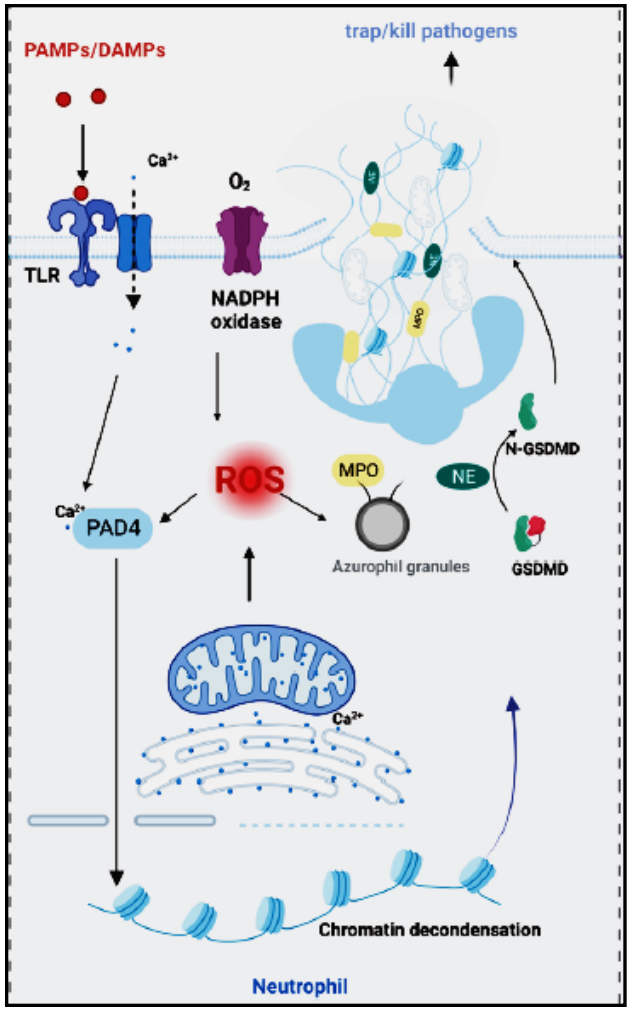
NETs are web-like structures composed of chromatin, histones, and granules of antimicrobial peptides (e.g peptide LL-37, mieloperossidase, proteinasi 3) that are released by neutrophils to trap and kill invading pathogens, including bacteria, viruses, and fungi.

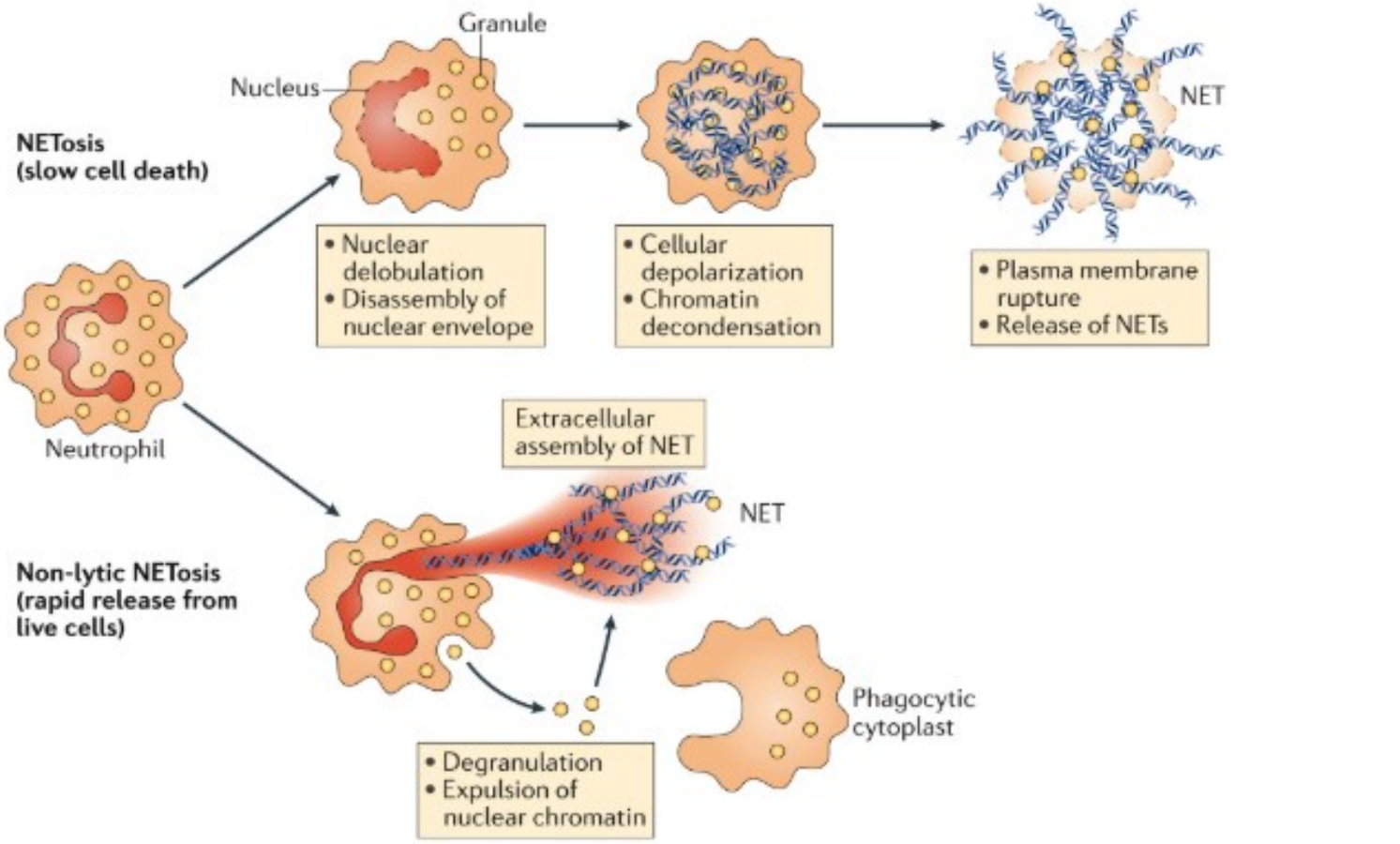
NETosis is initiated **by calcium release** from the neutrophil ER, which triggers the assembly of **the NADPH oxidase complex** and the generation of **ROS**. Mitochondrial ROS production also promotes NETosis. When ROS are activated, protein complexes known as “azurosomes” dissociate from azurophil granules and causes **Neutrophil elastase (NE)**, cathepsin G, azurocidin, and MPO to be released into the cytosol, where they contribute to **chromatin decondensation** and nuclear envelope disintegration.

Another important factor in NETosis is peptidyl-arginine deaminase 4 (**PAD4**), which is transferred from the cytoplasm to the nucleus to catalyze the **citrullination** of histones, leading to chromatin decondensation.

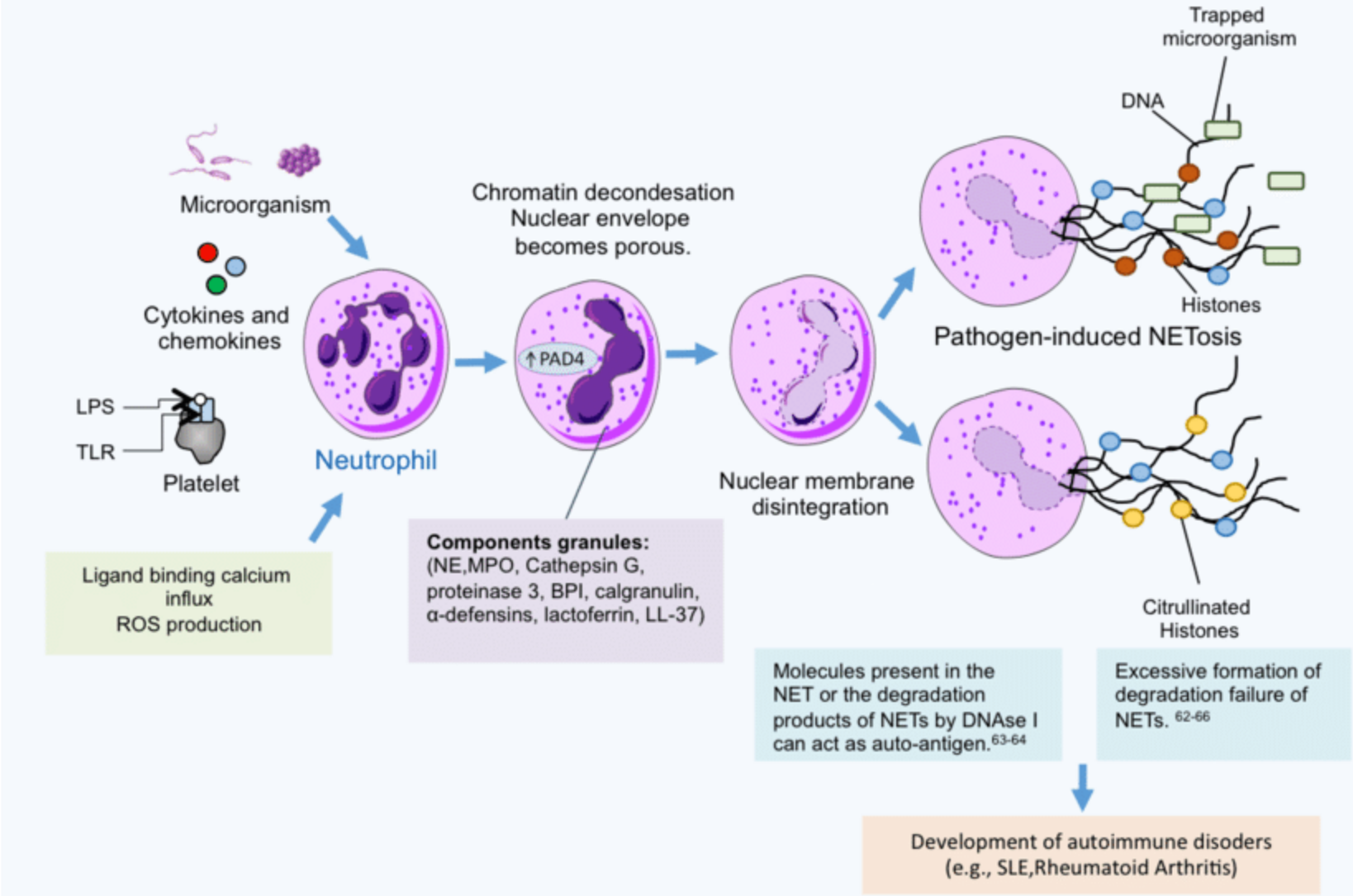
In the final stage, **pores are formed** in the plasma membrane, chromatin is released into the extracellular environment, and NETs are formed. **GSDMD** plays a critical role in the formation of these membrane pores. In contrast to pyroptosis, in which GSDMD is activated through caspase-induced cleavage, GSDMD is activated by NE.

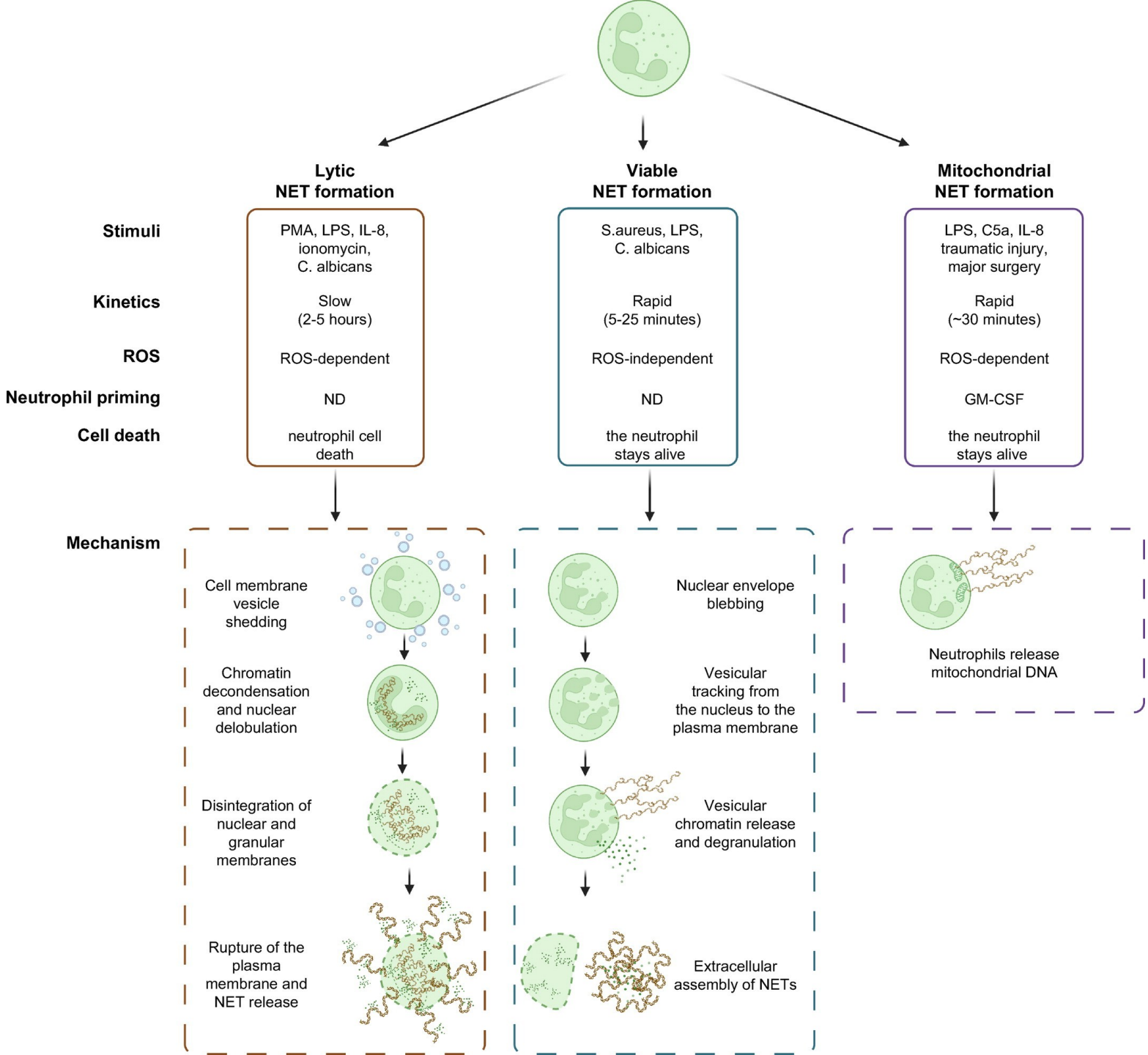
NETosis plays an important role in the innate immune response because it allows neutrophils to directly combat pathogens. However, excessive or inappropriate NETosis can contribute to the development of inflammatory and autoimmune diseases, such as sepsis, rheumatoid arthritis, lupus, and cancer.





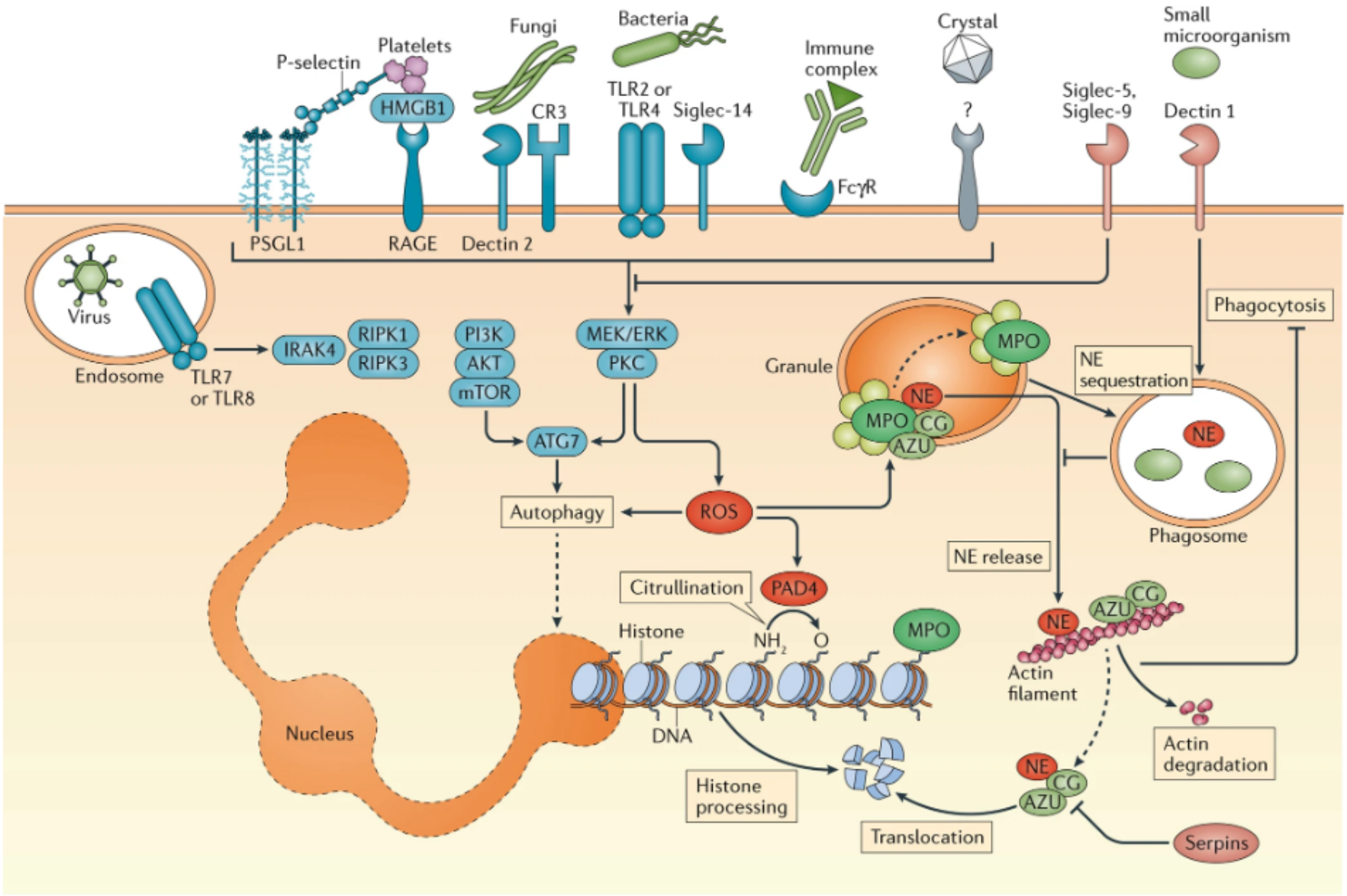
Neutrophil extracellular traps (NETs) form via two pathways. The first is a **cell death pathway** termed NETosis that begins with nuclear delobulation and the disassembly of the nuclear envelope and continues with loss of cellular polarization, chromatin decondensation and plasma membrane rupture. The second is **a non-lytic form of NETosis** that can occur independently of cell death and involves the secreted expulsion of nuclear chromatin (by nuclear envelop blebbing) that is accompanied by the release of granule proteins through degranulation. These components assemble extracellularly and leave behind active anucleated cytoplasts that continue to ingest microorganisms.


Trapped microorganism
DNA
Microorganism
Chromatin decondesation
Nuclear envelope
becomes porous.
Cytokines and chemokines
↑PAD4
LPS
TLR
Platelet
Neutrophil
Nuclear membrane disintegration
Pathogen-induced NETosis
Histones
Citrullinated Histones
Ligand binding calcium influx
ROS production
Components granules:
(NE,MPO, Cathepsin G, proteinase 3, BPI, calgranulin, α-defensins, lactoferrin, LL-37)
Molecules present in the NET or the degradation products of NETs by DNAse I can act as auto-antigen.[63-64]
Excessive formation of degradation failure of NETs. [62-66]
Development of autoimmune disoders (e.g., SLE,Rheumatoid Arthritis)

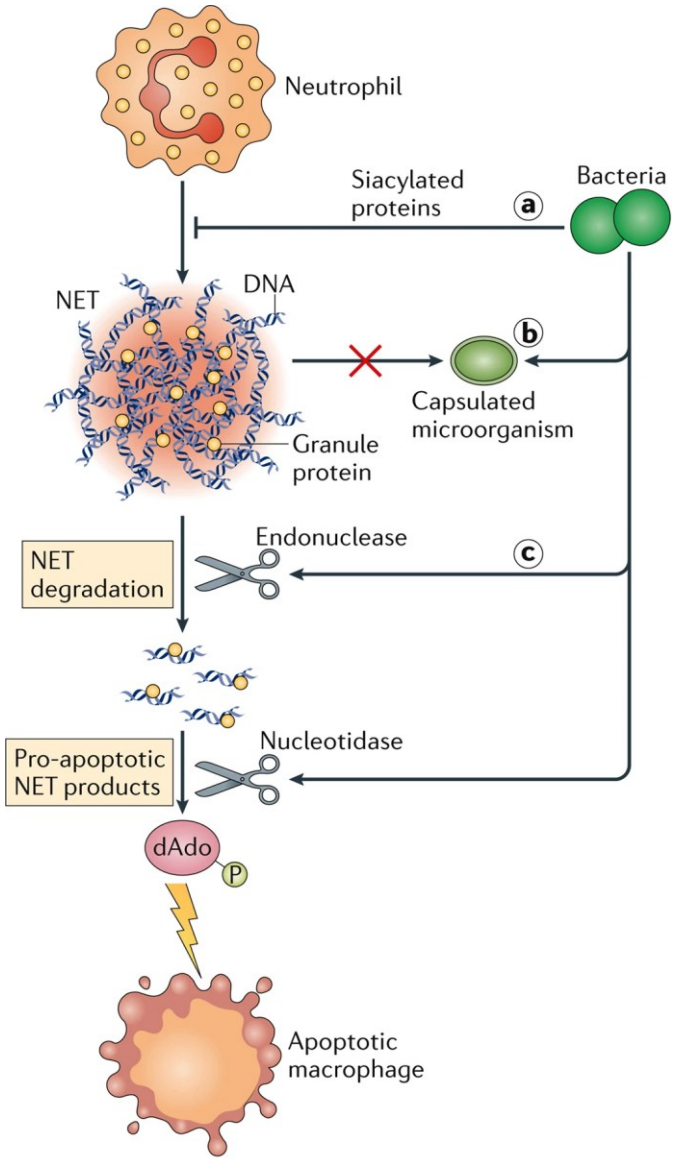

Lytic
NET formation
Viable
NET formation
Mitochondrial
NET formation
Stimuli
PMA, LPS, IL-8, ionomycin, C. albicans
S.aureus, LPS, C. albicans
LPS, C5a, IL-8 traumatic injury, major surgery
Kinetics
Slow (2-5 hours)
Rapid (5-25 minutes)
Rapid (~30 minutes)
ROS
ROS-dependent
ROS-independent
ROS-dependent
Neutrophil priming
ND
ND
GM-CSF
Cell death
neutrophil cell death
the neutrophil stays alive
the neutrophil stays alive
Mechanism
Cell membrane vesicle shedding
Chromatin decondensation and nuclear delobulation
Disintegration of nuclear and granular membranes
Rupture of the plasma membrane and NET release
Nuclear envelope blebbing
Vesicular tracking from the nucleus to the plasma membrane
Vesicular chromatin release and degranulation
Extracellular assembly of NETs
Neutrophils release mitochondrial DNA

Molecular mechanisms regulating NETosis.

[il extracellular traps in immunity and disease]



NET evasion mechanisms

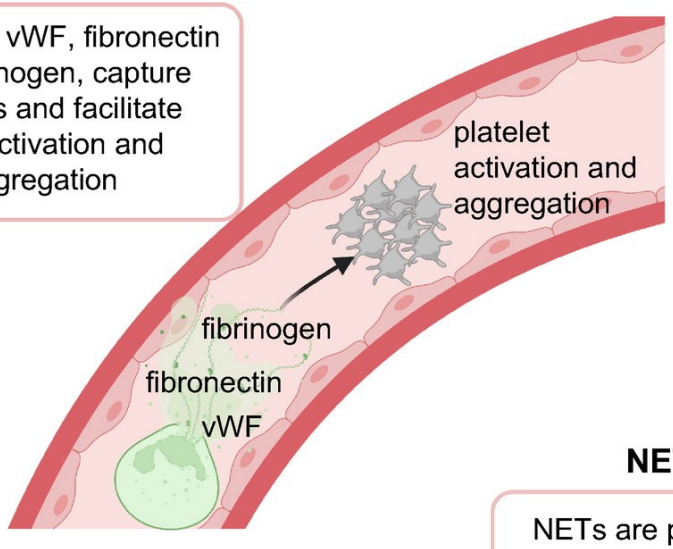


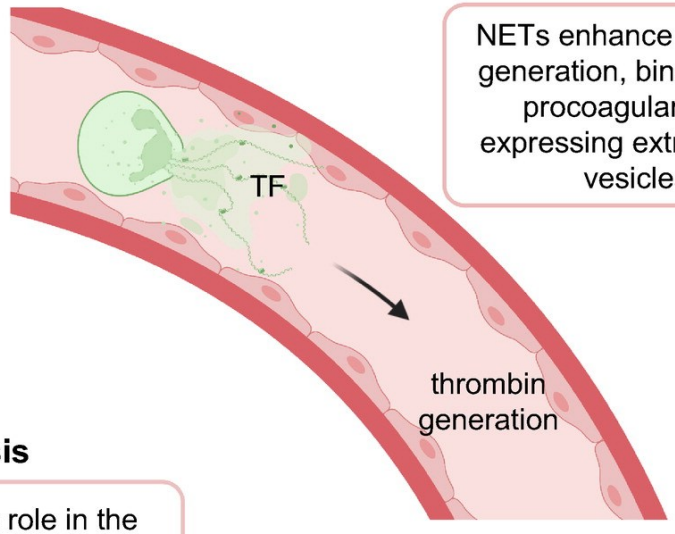
Nature Reviews | Immunology

NETs might also have a **deleterious effect** on the host, because the extracellular exposure of histone complexes could play a role during the development of **autoimmune diseases** like systemic lupus erythematosus (SLE).
NETs could also play a role in **inflammatory diseases**. NETs have also been reported in the colon mucosa of patients with the inflammatory bowel disease ulcerative colitis.
NETs have been shown to contribute to **thrombosis**

Preclinical research suggests that NETs are jointly responsible for **cancer-related pathologies** like thrombosis, organ failure and **metastasis** formation.

NETs bind vWF, fibronectin and fibrinogen, capture platelets and facilitate their activation and aggregation



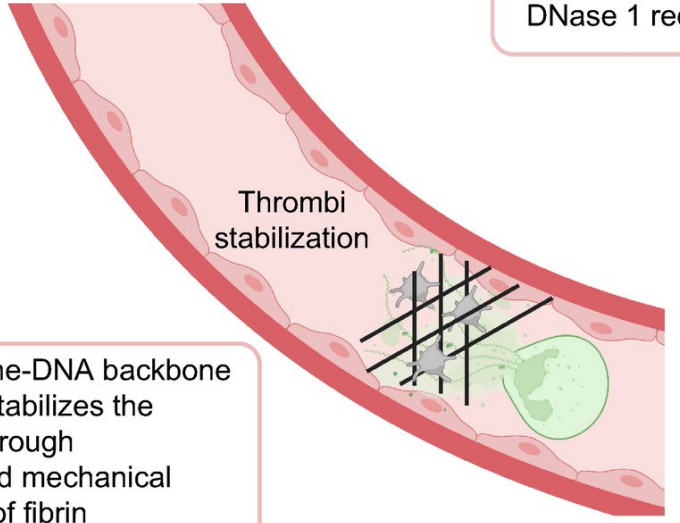


NETs enhance thrombin generation, bind TF and procoagulant TF-expressing extracellular vesicles

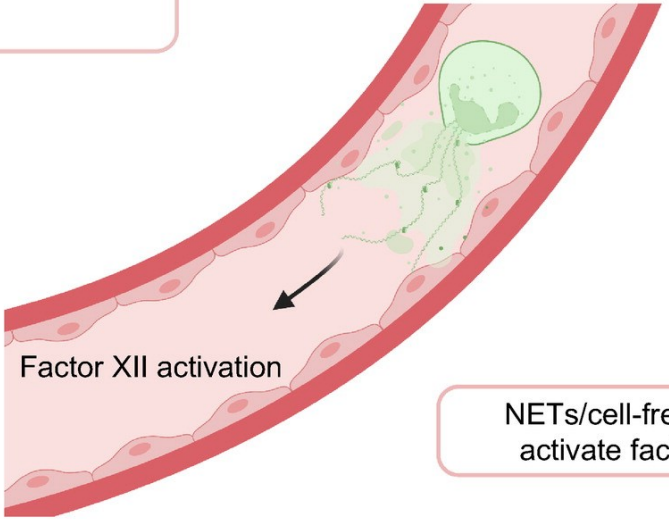
NETs in thrombosis

NETs are present and play a role in the formation and stabilization of
- Venous thrombosis
- Arterial thrombosis
- Cancer-associated thrombosis

Deficiency of PAD4 or administration of DNase 1 reduces thrombosis



The histone-DNA backbone of NETs stabilizes the thrombi through
- increased mechanical stability of fibrin
- anti-fibrinolysis



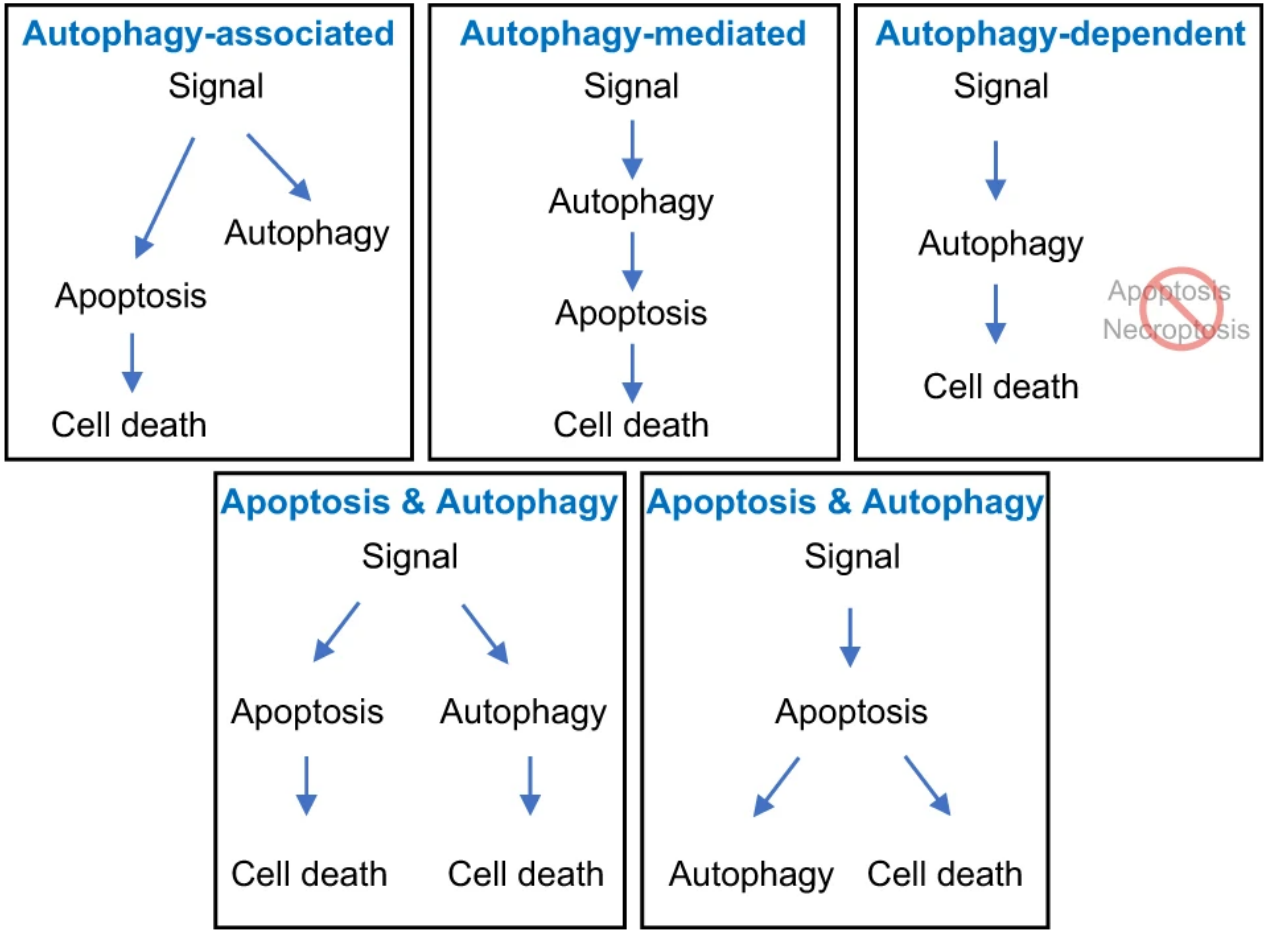
NETs/cell-free DNA activate factor XII

Autophagy

Autophagy is a conserved process that **delivers components of the cytoplasm to lysosomes for degradation**. While originally identified as a cell survival mechanism, autophagy plays highly context-specific roles in mediating cell death.

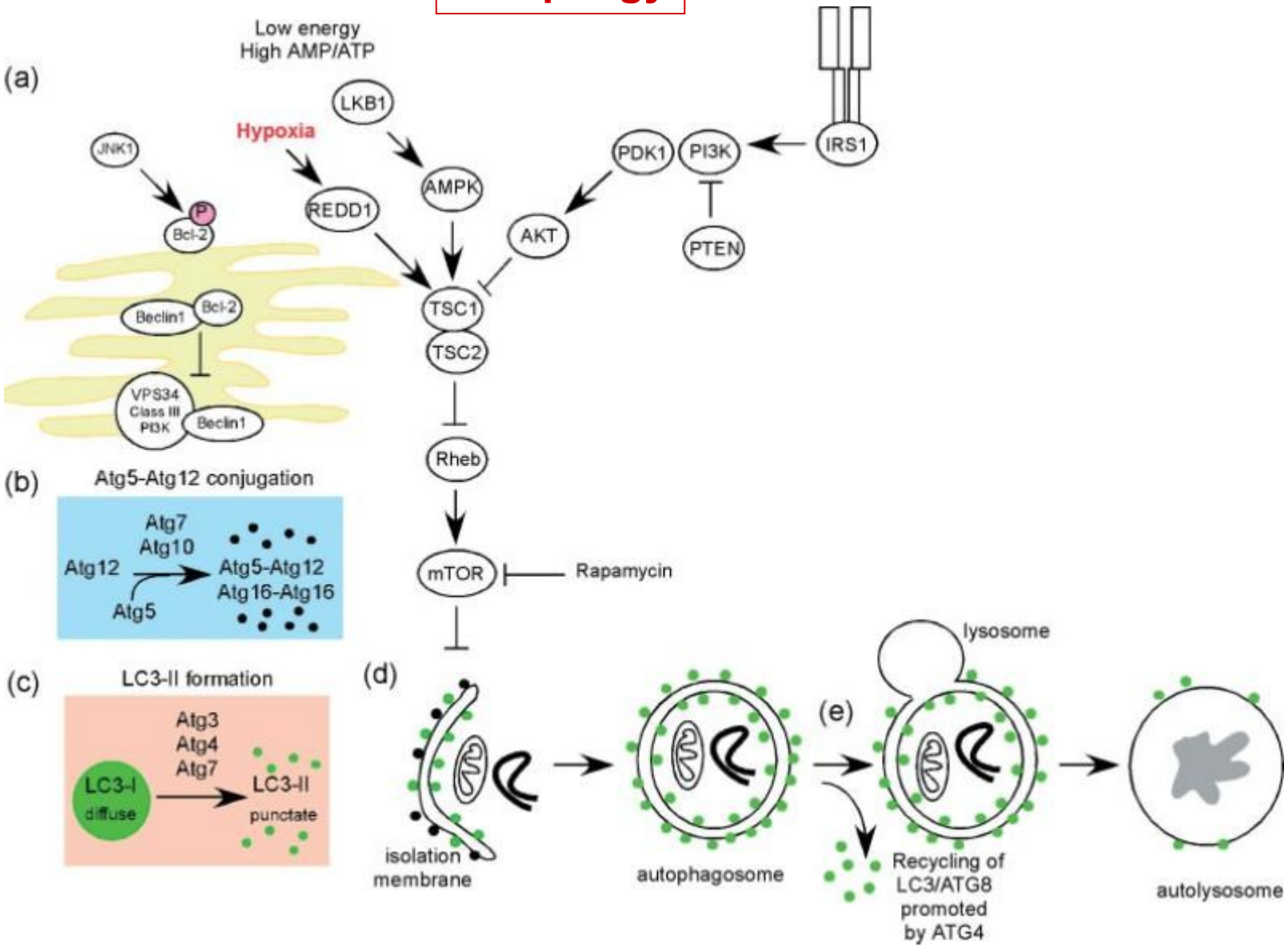
There are three main forms of autophagy, known as macroautophagy (herein referred to as autophagy), microautophagy and chaperone-mediated autophagy (CMA). Microautophagy involves the transfer of cytosolic cargo components directly into the lysosome through membrane invaginations. CMA involves the selective translocation of proteins containing the KFERQ-like motif across the lysosomal membrane.

Roles of autophagy in cell death.



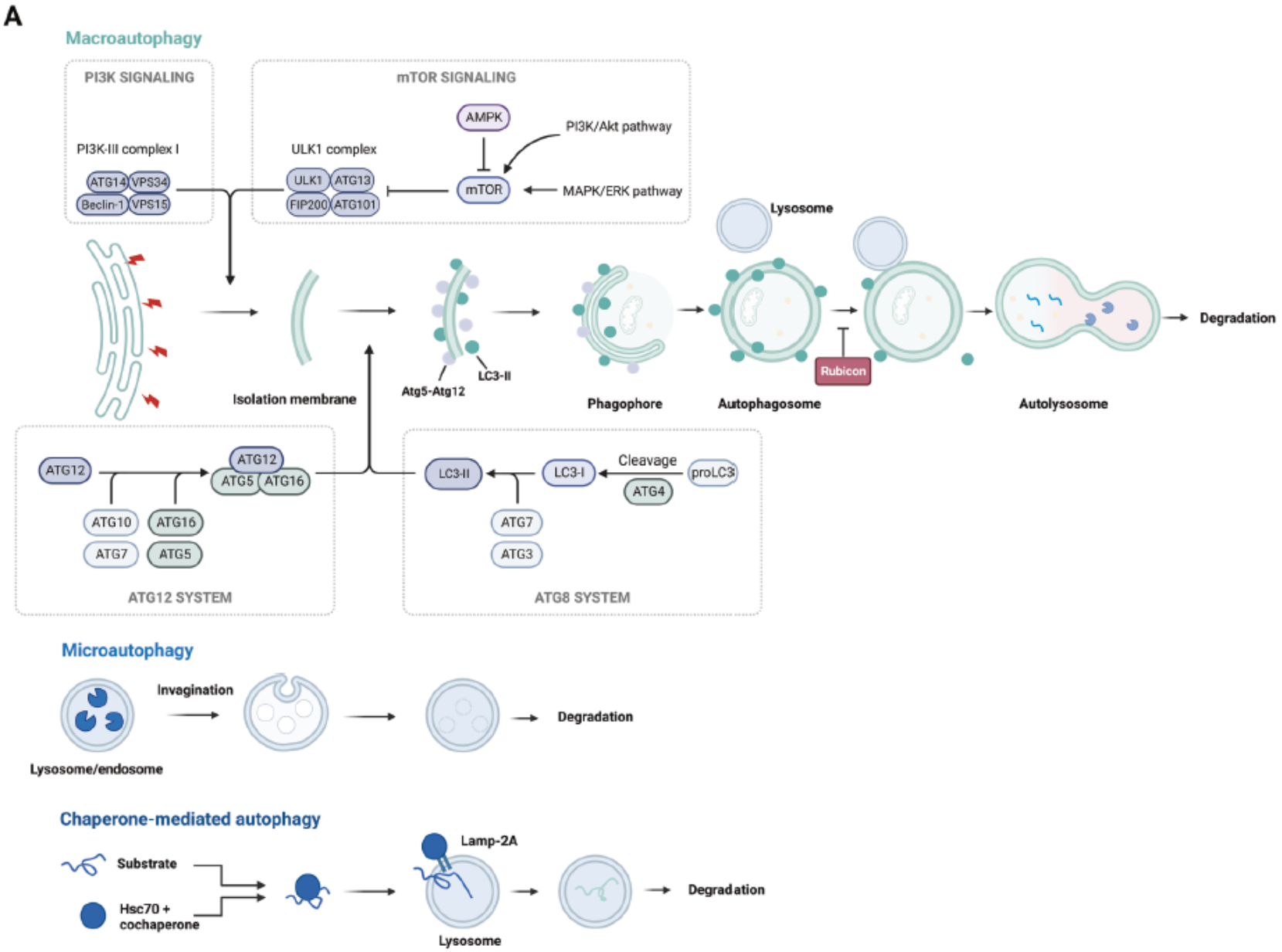
The role of autophagy in cell death can be: autophagy-associated cell death, where the induction of autophagy accompanies apoptosis (or other cell death pathways); autophagy-mediated cell death, where the autophagy pathway activates apoptosis (or other cell death modalities); and autophagy-dependent cell death, which occurs independently of apoptosis or necrosis (e.g. Drosophila larval midgut degradation). An additional context-specific mode of cell death involves the coordinated action of both apoptosis and autophagy in parallel (e.g. Drosophila salivary gland degradation), and Bax- and Bak-mediated induction of both apoptosis and autophagy.

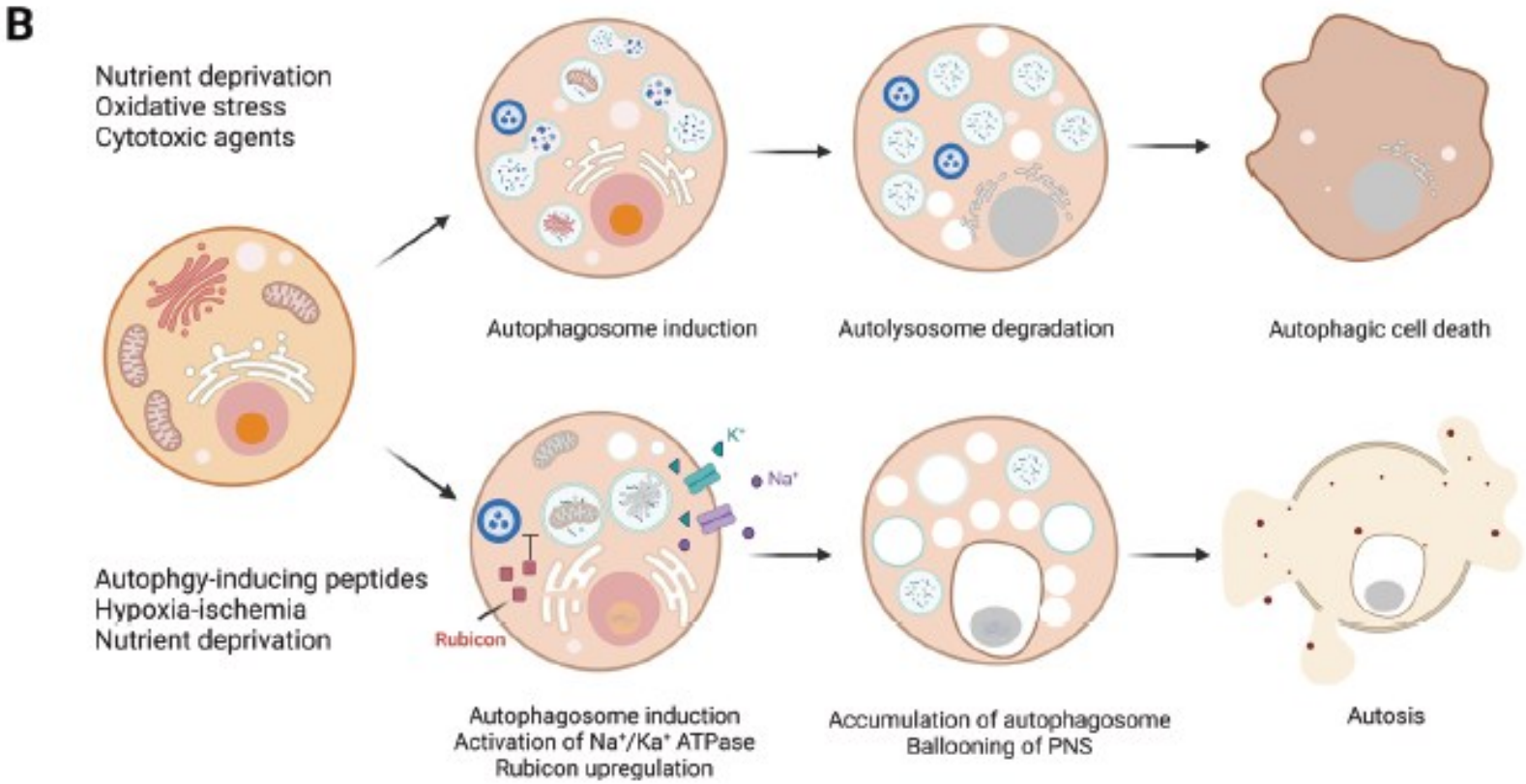
autophagy

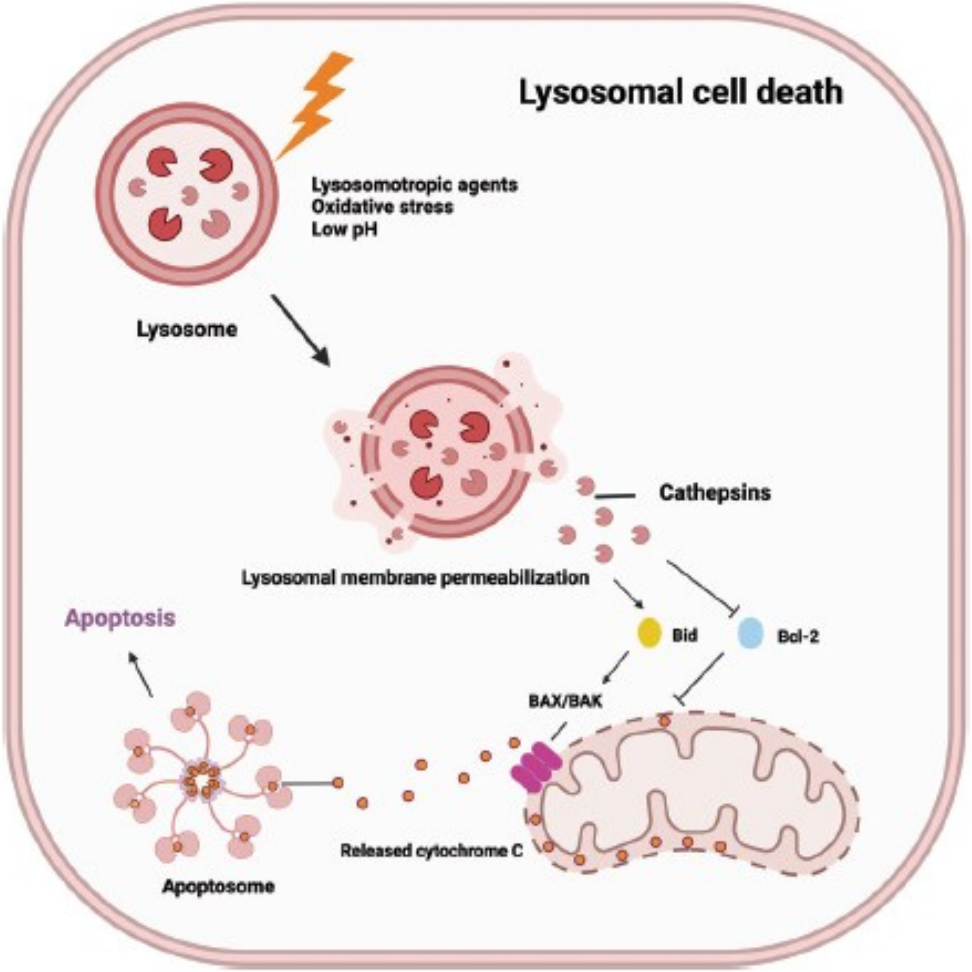

(a)
Low energy
High AMP/ATP
LKB1
Hypoxia
JNK1
REDD1
AMPK
P
Bcl-2
PDK1
PI3K
IRS1
AKT
PTEN
Beclin1
Bcl-2
TSC1
TSC2
VPS34
Class III
PI3K
Beclin1
Rheb
(b)
Atg5-Atg12 conjugation
Atg7
Atg10
Atg12
Atg5-Atg12
Atg16-Atg16
Atg5
mTOR
Rapamycin
(c)
LC3-II formation
Atg3
Atg4
Atg7
LC3-I
diffuse
LC3-II
punctate
(d)
lysosome
(e)
isolation
membrane
autophagosome
Recycling of
LC3/ATG8
promoted
by ATG4
autolysosome

Autophagy-deficient mouse models and human diseases linked to defects in specific autophagy genes

Autophagy gene and function (human/ mouse)	Human disease linked to mutation/ inactivation	Mouse model phenotype	Refer
ATG16L/Atg16L			3
Atg16L complexes with conjugated Atg5–Atg12 to promote expansion and curvature of the nascent phagophore	*T300A* mutation in *ATG16L* linked to Crohn's disease, discovered by GWAS	Loss of Atg16L1 inhibits autophagy in Paneth cells, reducing secretion of granules of antimicrobial peptides that influence intestinal microbiota and causing increased inflammation	
BECN1/Becn1			1
Beclin1 regulates the kinase activity of Vps34 at the ER; complex includes regulatory components UVRAG, Atg14L, Rubicon and Ambra	*BECN1* is mono-allelically deleted in breast, ovarian and prostate cancer	*Becn1*-null mice are embryonic lethal, showing a defect in cavitation of the blastocyst. *Becn1* heterozygotes are predisposed to lymphoma, hepatocellular carcinoma and other cancers	
UVRAG/Uvrag			
UVRAG complexes with Beclin1 and Vps34 at the ER to promote autophagy	*UVRAG* is mono-allelically deleted in colon cancer	N/A	
IRGM/Irgm			

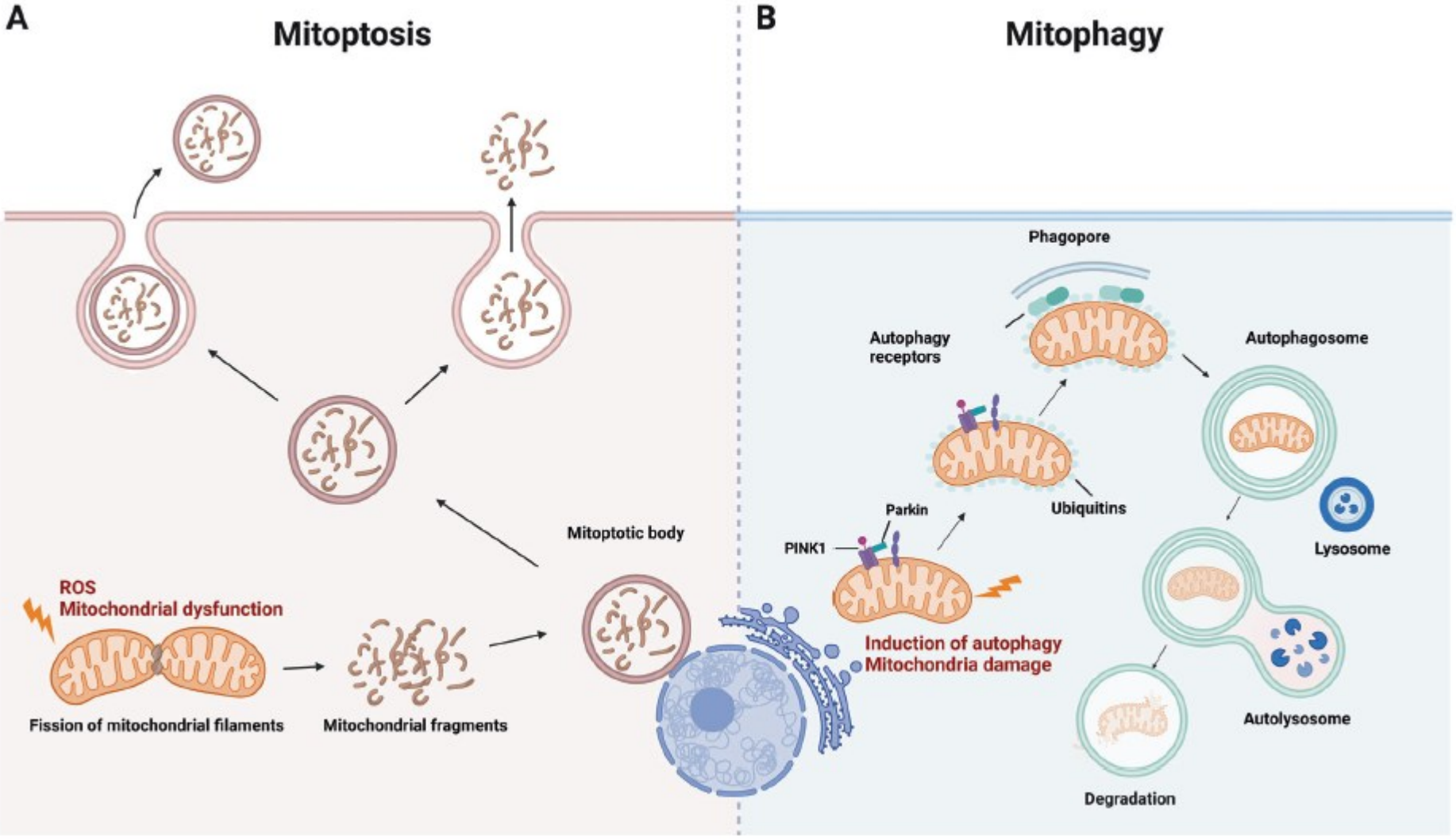

A
Macroautophagy
PI3K SIGNALING
mTOR SIGNALING
AMPK
PI3K/Akt pathway
PI3K-III complex I
ULK1 complex
ATG14
VPS34
Beclin-1
VPS15
ULK1
ATG13
FIP200
ATG101
mTOR
MAPK/ERK pathway
Lysosome
Isolation membrane
Atg5-Atg12
LC3-II
Phagophore
Autophagosome
Rubicon
Autolysosome
Degradation
ATG12
ATG12
ATG5
ATG16
ATG10
ATG16
ATG7
ATG5
ATG12 SYSTEM
LC3-II
LC3-I
Cleavage
proLC3
ATG4
ATG7
ATG3
ATG8 SYSTEM
Microautophagy
Invagination
Degradation
Lysosome/endosome
Chaperone-mediated autophagy
Substrate
Hsc70 +
cochaperone
Lamp-2A
Lysosome
Degradation


B
Nutrient deprivation
Oxidative stress
Cytotoxic agents
Autophagosome induction
Autolysosome degradation
Autophagic cell death
Autophgy-inducing peptides
Hypoxia-ischemia
Nutrient deprivation
K+
Na+
Rubicon
Autophagosome induction
Activation of Na+/Ka+ ATPase
Rubicon upregulation
Accumulation of autophagosome
Ballooning of PNS
Autosis



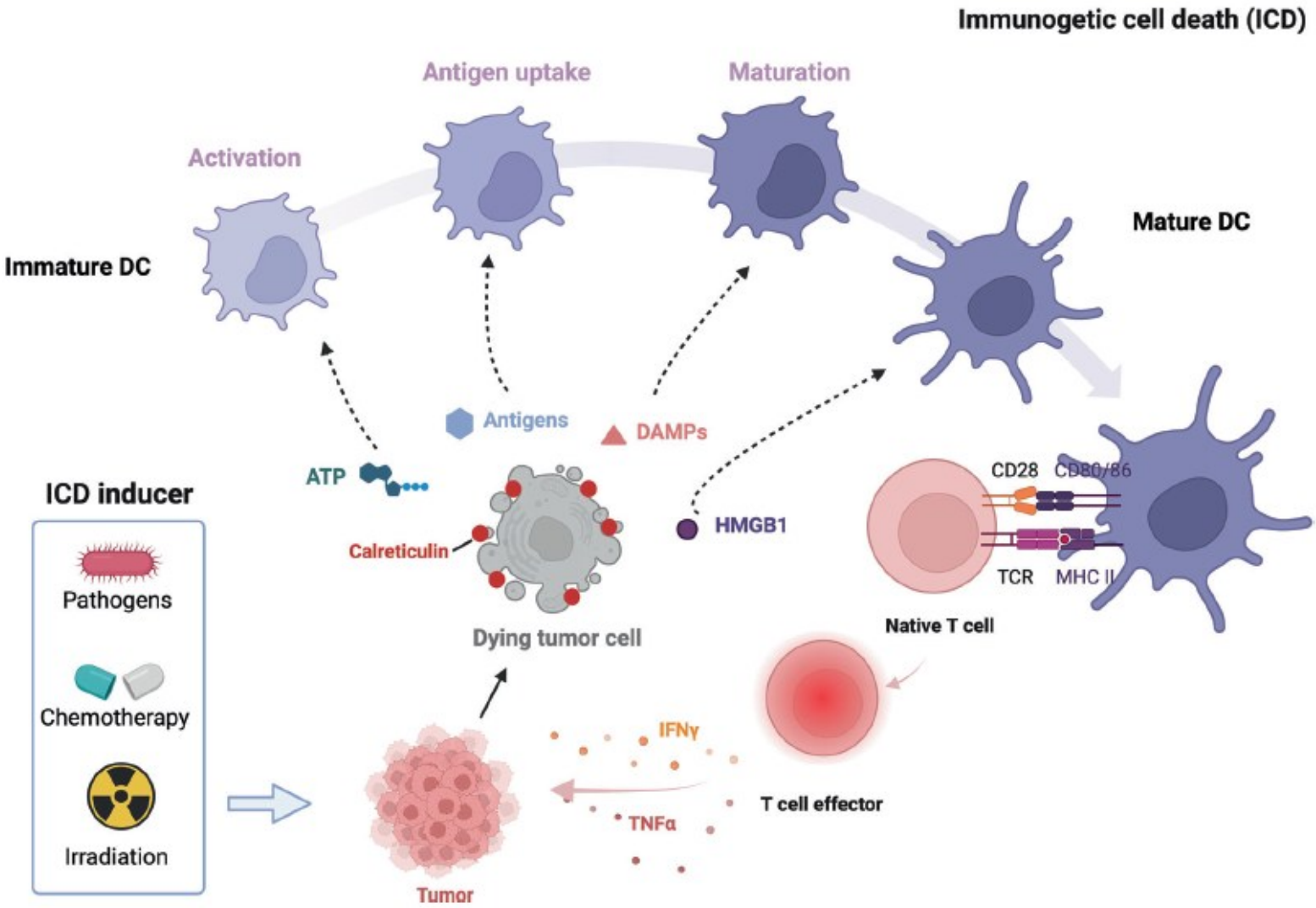
Mechanism of **lysosomal cell death.** This figure illustrates lysosomal cell death caused by lysosomal membrane permeabilization and the release of lysosomal enzymes into the cytoplasm, leading to the activation of apoptotic cell death pathways.

Lysosomal cell death can be induced by stimuli, such as changes in lysosomal pH, oxidative stress, and lysosomotropic agents. The release of lysosomal proteases, such as cathepsins, activates the lysosomal apoptotic pathway by cleaving Bid and degrading antiapoptotic Bcl-2 homologs.


A
Mitoptosis
B
Mitophagy
Mitoptotic body
ROS
Mitochondrial dysfunction
Fission of mitochondrial filaments
Mitochondrial fragments
Phagopore
Autophagy receptors
Autophagosome
Ubiquitins
Parkin
PINK1
Lysosome
Induction of autophagy
Mitochondria damage
Autolysosome
Degradation

Immunogenic cell death

- Immunogenic cell death (ICD) is a type of PCD in which an immune response is triggered by the release of damage associated molecular patterns (DAMPs) from dying cells, which attract immune cells to the site of cell death.
- The ICD was first proposed by the group led by Guido Kroemer and Laurence Zitvogel in 2005.
- During ICD, dying tumor cells express calreticulin on their surface, which functions as an “eat me” signal to dendritic cells (DCs) and other phagocytic cells.
- This signaling promotes phagocytosis of the dying cells by the DCs, leading to the activation of an immune response. ICD also involves the release of DAMPs, such as ATP, high-mobility group box 1, and heat shock proteins (HSPs), from dying cells.
- These DAMPs activate DCs and other immune cells, thereby promoting antigen presentation and immune activation.
- Moreover, IFNγ and TNFα released by effector T cells attract and activate other immune cells, including natural killer cells and macrophages, which detect and eradicate cancer cells.
- ICD has emerged as a promising strategy for cancer therapy. It potentially enhances the effectiveness of cancer treatments, such as chemotherapy and radiotherapy, which in turn induce ICD in cancer cells.
- ICD-based therapies provide long-lasting protection against cancer recurrence and metastasis by promoting immune responses against cancer cells.


Immunogetic cell death (ICD)
Antigen uptake
Maturation
Activation
Immature DC
Mature DC
Antigens
DAMPs
ATP
ICD inducer
Calreticulin
HMGB1
CD28
CD80/86
TCR
MHC II
Native T cell
Pathogens
Chemotherapy
Irradiation
Dying tumor cell
IFNγ
TNFα
T cell effector
Tumor

Cuproptosis

Cuproptosis is triggered by copper (Cu). This was first described by Tsvetkov, P. et al. in 2019.

Cuproptosis differs from other types of oxidative stress-related cell death, such as apoptosis and ferroptosis, and is characterized by **mitochondrial stress** caused by the aggregation of **lipoylated mitochondrial enzymes and the loss of Fe–S cluster proteins**.

Copper plays vital roles in various biological processes, including oxygen transport, energy production, and antioxidant defense. However, excess copper can be toxic to cells and tissues, leading to a condition known as **copper overload or copper toxicity**.

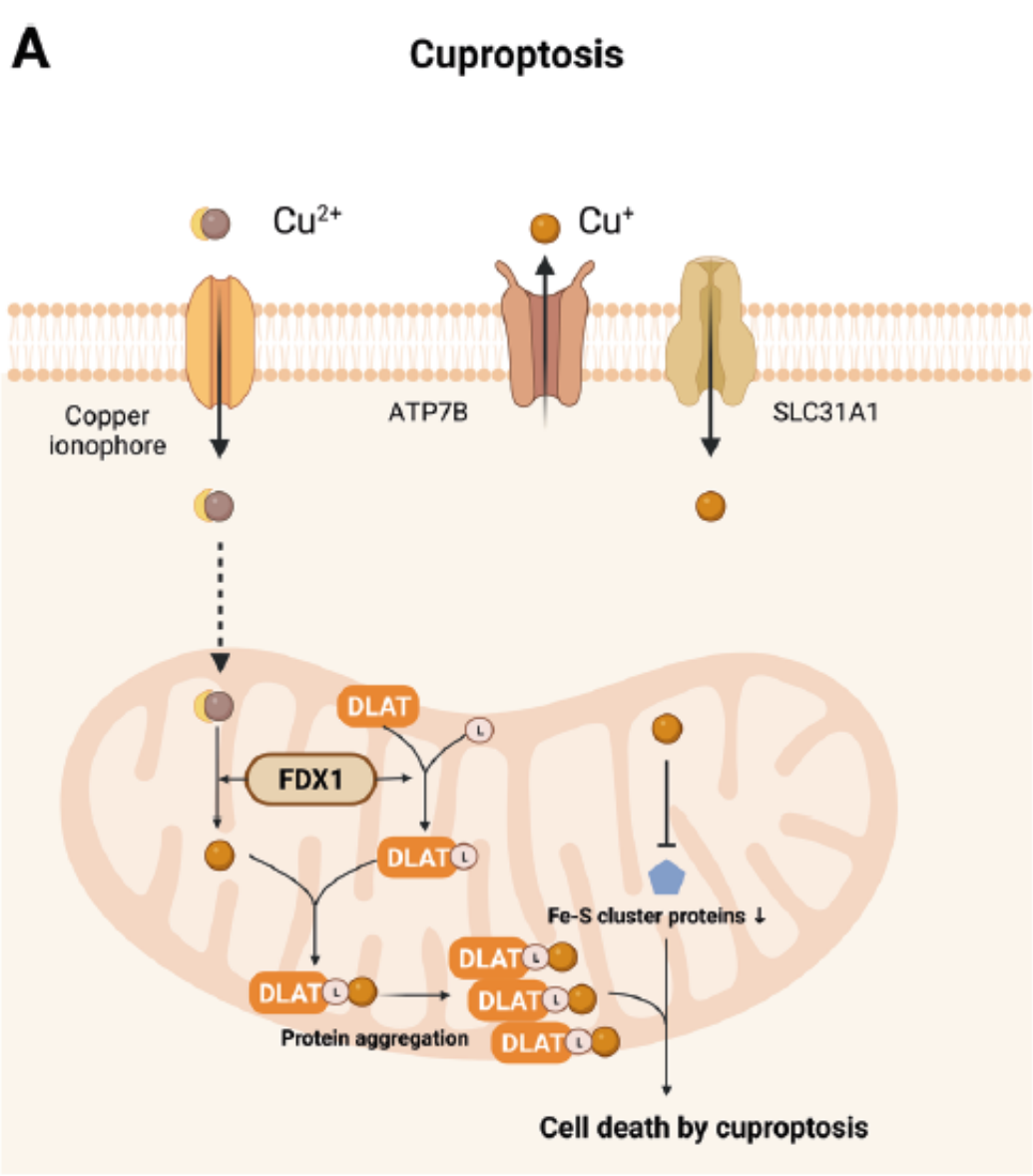
Two mitochondrial proteotoxic stress pathways mediate cuproptosis:

-The mitochondrial matrix reductase ferredoxin 1 (FDX1) catalyzes the reduction of ES–Cu2+ to Cu+, releasing it into mitochondria. FDX1 has also been identified as a novel effector of lipoylation that contributes to the accumulation of toxic lipoylated dihydrolipoamide S-acetyltransferase (DLAT). Cu+ binds to lipoylated DLAT, promoting the disulfide bond-dependent aggregation of lipoylated DLAT, which leads to the accumulation of toxic lipoylated DLAT and subsequent cuproptotic cell death.

This type of cell death depends on the amount of copper in cells and the lipoylation status of tricarboxylic acid (TCA) cycle enzymes.

Cuproptosis has been implicated in several pathological conditions, such as Wilson’s disease, a genetic disorder characterized by the accumulation of copper in the liver, brain, and other organs.

A


Cuproptosis
Cu2+
Cu+
Copper ionophore
ATP7B
SLC31A1
DLAT
FDX1
DLAT
DLAT
Fe-S cluster proteins ↓
DLAT
DLAT
DLAT
Protein aggregation
Cell death by cuproptosis

Ferroptosis

Ferroptosis is an iron-dependent form of PCD (described in 2012 by Brent Stockwell) that involves the accumulation of lipid peroxides and oxidative stress leading to membrane damage.

Ferroptosis is initiated by the **accumulation of lipid peroxides** generated through the **oxidation of polyunsaturated fatty acids via lipoxygenases or other enzymes**.

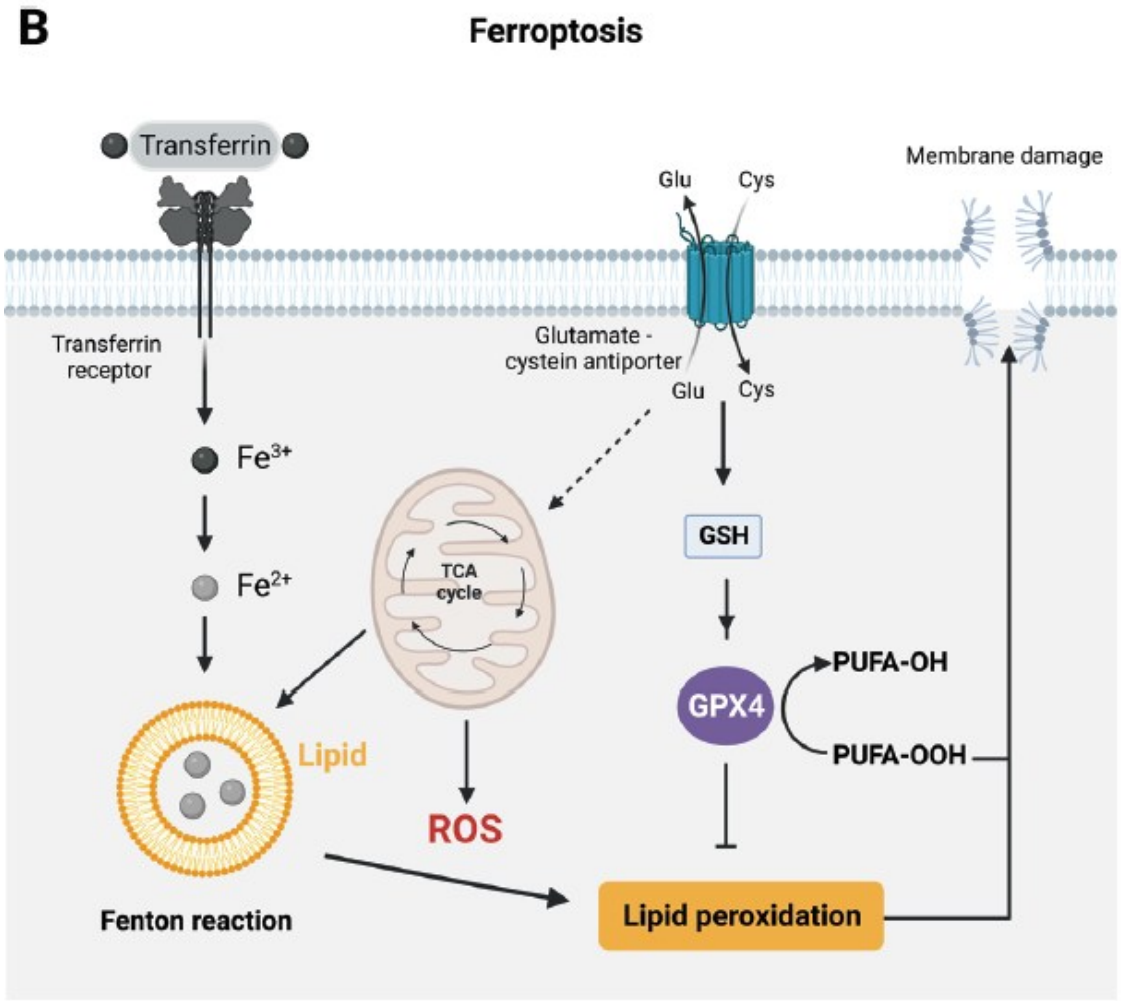
Lipid peroxides accumulate through the oxidation of polyunsaturated fatty acids, a process that can be further amplified via the iron-catalyzed Fenton reaction, generating ROS and hydroxyl radicals that attack and damage cellular components, particularly the cell membrane, resulting in cell death.

The cystine/glutamate antiporter system imports cystine, a precursor of the antioxidant glutathione, and thus plays a significant role in regulating the accumulation of lipid peroxides and iron. Glutathione neutralizes free radicals and ROS and protects cells from oxidative stress and lipid peroxidation.

Ferroptosis is **characterized by the depletion of intracellular glutathione and decreased activity of glutathione peroxidase 4 (GPX4),** leading to the **accumulation of unmetabolized lipid peroxides and the production of high levels of ROS**.

Other factors that can regulate ferroptosis include iron metabolism; the activity of lipid metabolism enzymes, such as acyl-CoA synthetase long-chain family member 4 (ACSL4); and the expression of genes involved in cell stress response pathways, such as the p53 pathway.

In cancer treatment, inhibition of the cystine/glutamate antiporter system induces ferroptosis. In addition, the use of ferroptosis-inducing agents, such as erastin and RSL3, may become a novel approach to cancer therapy. Ferroptosis is thought to play a role in various physiological processes, including ischemia, cancer, and neurodegeneration.



Ferroptosis is characterized by the depletion of intracellular glutathione and decreased activity of glutathione peroxidase 4 (GPX4), which leads to the accumulation of unmetabolized lipid peroxides and increased ROS production. Membrane damage is also a result of lipid peroxidation

Paraptosis

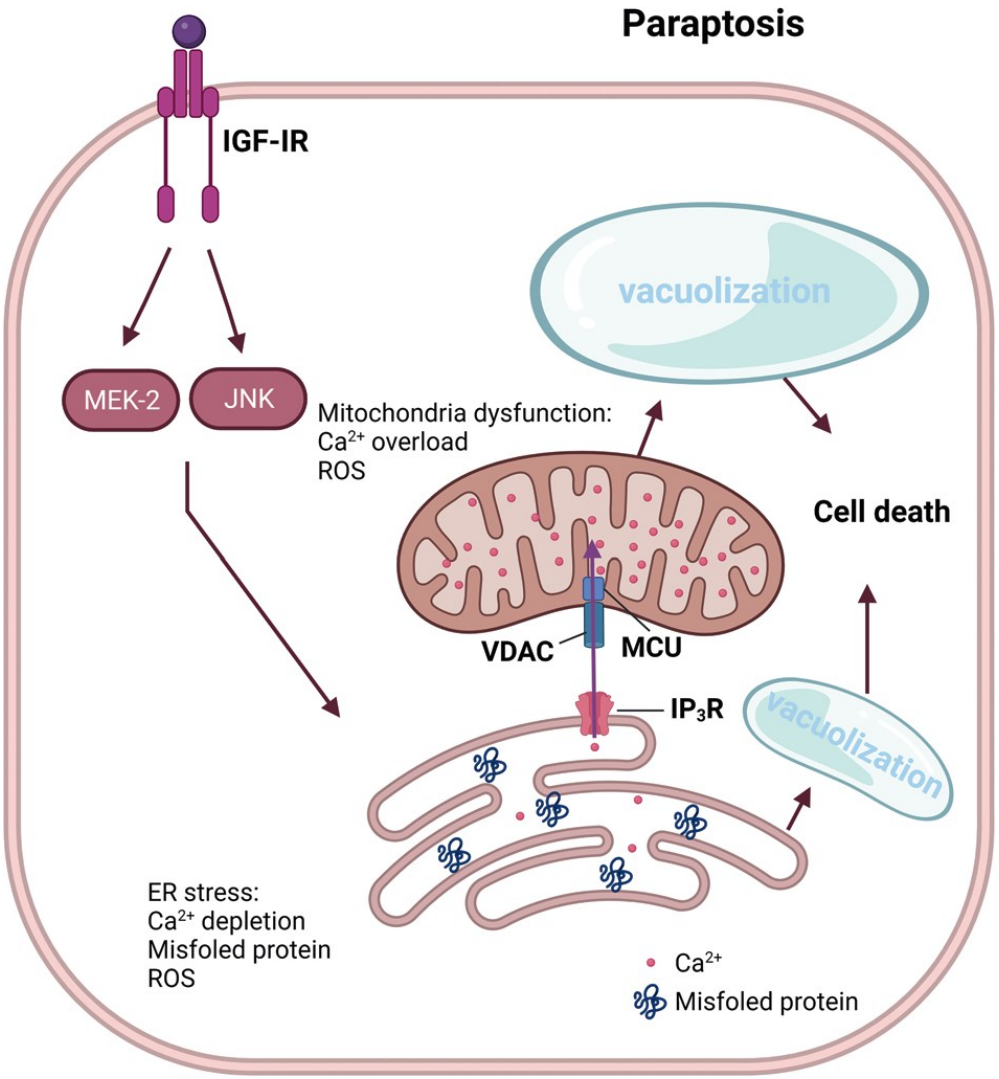
Paraptosis, the name of which is derives from the combination of “para”, meaning next to or related to, and “apoptosis,” is a type of PCD that was initially discovered by Sabina Sperandio et al. in 2000.

Paraptosis and apoptosis are typically induced simultaneously in cells. Paraptosis does not involve caspase activation or DNA fragmentation and is characterized by the swelling and vacuolization of the ER and mitochondria, resulting in the formation of large cytoplasmic vacuoles.

Multiple mechanisms can trigger paraptosis. Impaired proteostasis due to proteasomal inhibition or altered protein thiol homeostasis, as well as unbalanced ion homeostasis, can lead to paraptosis. Paraptosis is characterized by cytoplasmic vacuolization resulting from swelling of the ER and mitochondria. The accumulation of misfolded proteins within the ER lumen leads to the development of an osmotic force that causes water to be drawn away from the cytoplasm, causing ER distension .

ER stress and dilation can contribute to the release of Ca2+ from the ER, which can cause mitochondrial Ca2+ overload via an intracellular Ca2+ flux mechanism located at the ER-mitochondrial axis and thus mitochondrial dilatation. Stimulation of the MEK-2 and JNK pathways by IGF-IR, as well as its inhibition mediated by AIP-1/Alix, is known to promote paraptosis.

Paraptosis is believed to play a role in various physiological and pathological processes, including embryonic development, neurodegeneration, and cancerogenesis. In cancer cells, paraptosis is induced by various chemotherapeutic agents, including the proteasome inhibitor bortezomib and histone deacetylase (HDAC) inhibitor suberoylanilide hydroxamic acid (SAHA)



Paraptosis is characterized by the development of large vacuoles in the endoplasmic reticulum (ER) and mitochondria, ultimately leading to the formation of large cytoplasmic vacuoles. Impaired proteostasis, altered ion homeostasis, and ER stress cause paraptosis, resulting in the discharge of Ca2+ from the ER and accumulation of Ca2+ in mitochondria. Paraptosis can be facilitated by the activation of mitogen-activated protein kinase (MAPK) signaling pathways via IGF-IR and inhibited by AIP-1/Alix.

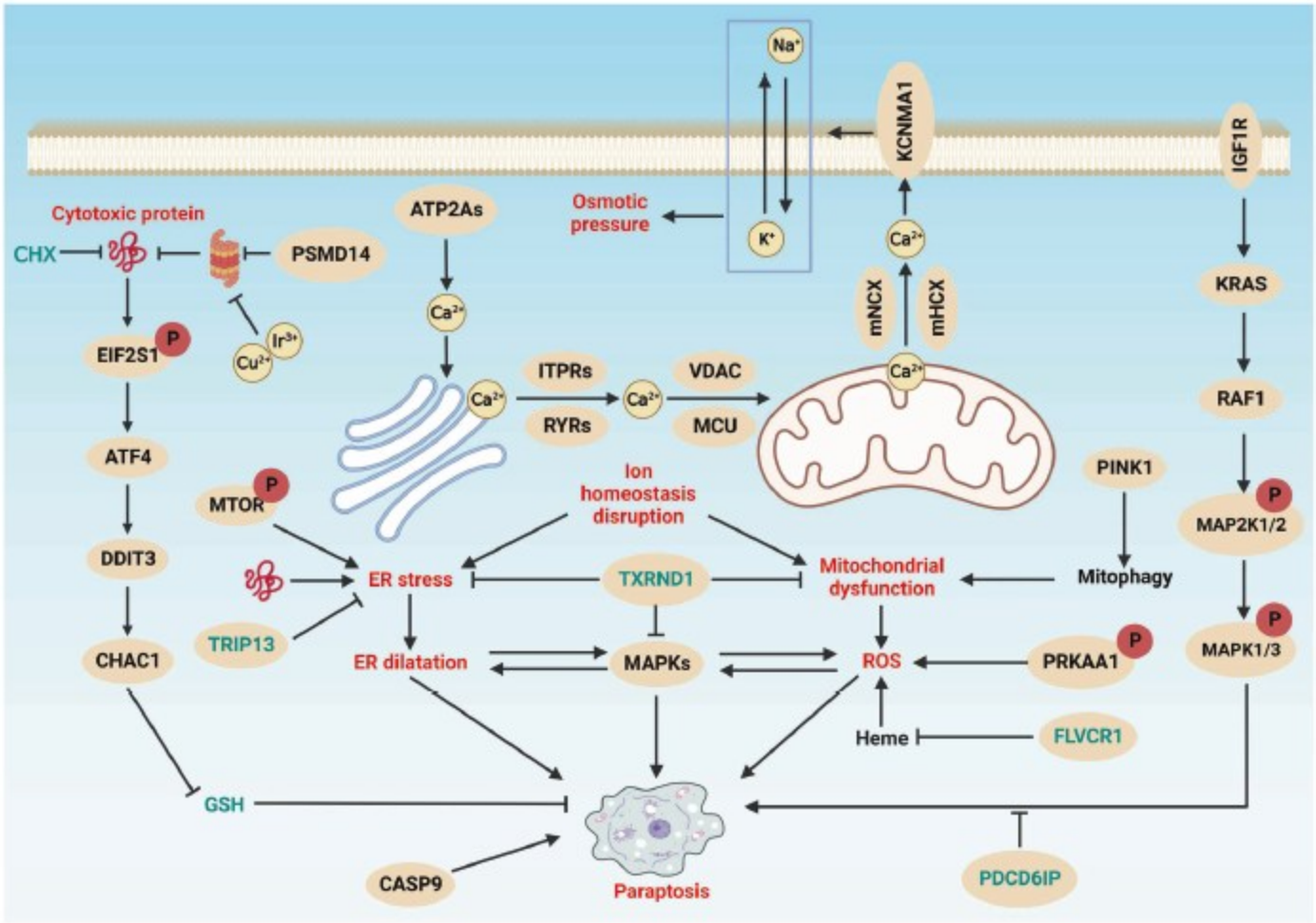


Fig. 1 Core molecular machinery and signaling regulation of paraptosis. Paraptosis can be initiated through multiple major pathways, including MAPK activation by IGFIR, ER stress induced by proteasome inhibition or other mechanisms, osmotic stress resulting from ROS production, and aberrant mitochondrial function triggering BKCa channel activation. Additionally, imbalances in ion homeostasis can lead to ER and mitochondrial swelling. Conversely, certain key genes, such as PDCD6IP, FLVCR1, and TXRND1, inhibit paraptosis.

Methuosis

Methuosis (first described by Overmeyer et al. in 2008) is characterized by the accumulation of vacuoles derived from macropinosomes, which are large endocytic vesicles.

The term methuosis is derived from the Greek word for “methuo,” meaning to drink to intoxication, and refers to the fact that the vacuoles in methuotic cells appear to be filled with an unknown substance.

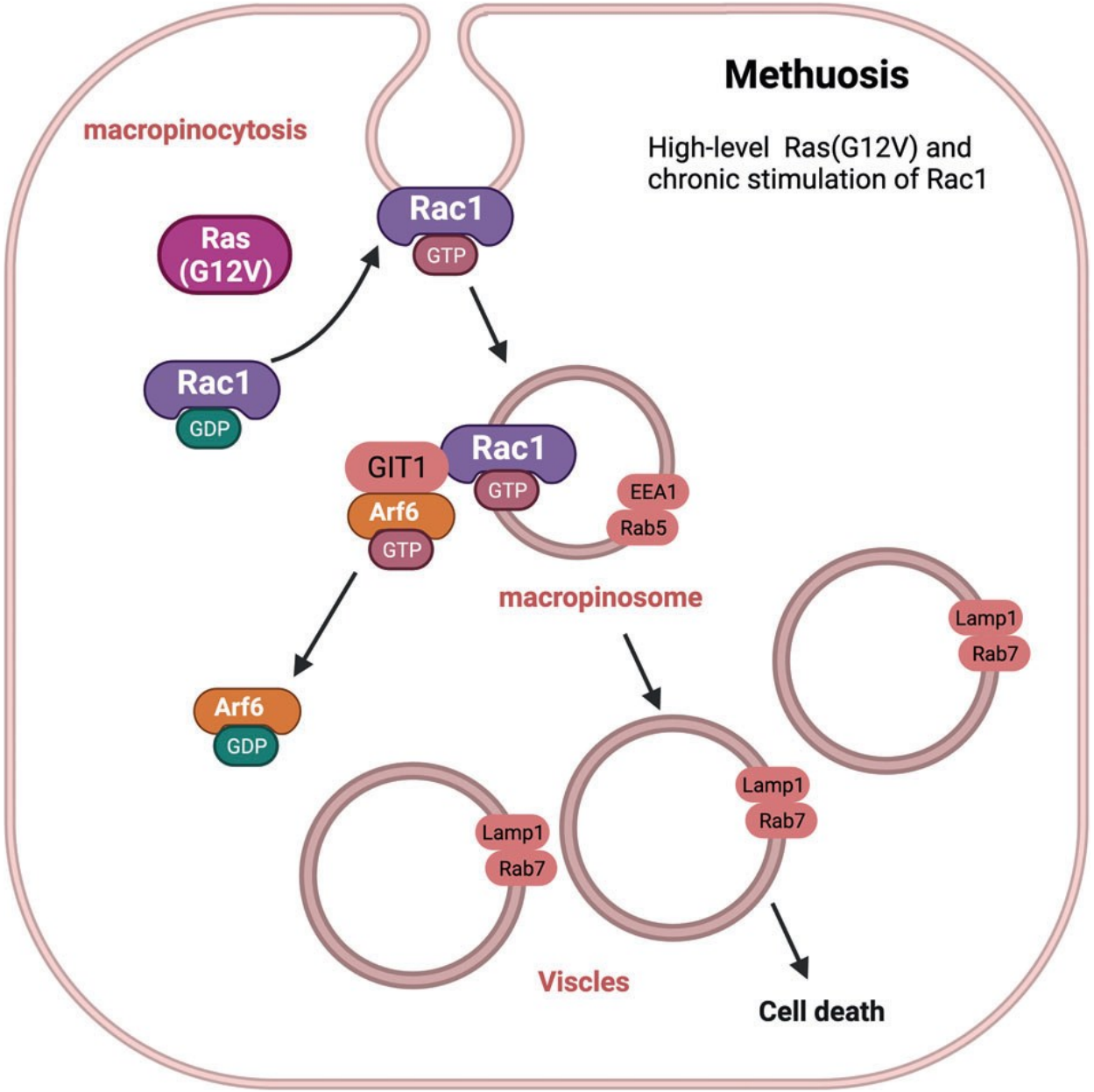
Methuosis **is triggered by sustained high-level expression of the activated form of Ras (G12V) and chronic stimulation of Rac1.**

This stimulation increases the rate of macropinocytic, which is the process of molecules uptake into the extracellular fluid through the formation of large vesicles called macropinosomes.

However, methuosis impairs macropinosome recycling by decreasing the pool of active Arf6, which is a protein involved in vesicle trafficking. Thus, macropinosomes accumulate and fuse to form large vacuoles that displace the nucleus and other organelles in a cell.

Eventually, the vacuoles become sufficiently large to rupture the cell membrane, leading to cell death. However, the exact molecular mechanisms underlying this form of cell death are not fully understood.

Methuosis has been observed in various cancer cell lines and has been proposed to be a potential therapeutic target for cancer treatment.


Methuosis
High-level Ras(G12V) and chronic stimulation of Rac1
macropinocytosis
Rac1
GTP
Ras (G12V)
Rac1
GDP
GIT1
Rac1
GTP
Arf6
GTP
EEA1
Rab5
macropinosome
Arf6
GDP
Lamp1
Rab7
Lamp1
Rab7
Lamp1
Rab7
Viscles
Cell death

Entosis

Entosis (described by Overholtzer et al. in 2007) is a nonapoptotic form of cell death in which one living cell detach from the extracellular matrix (ECM) and actively internalizes and degrades another living cell.

The internalization of a living cell by another cells leads to the formation of a double-membrane vesicle called the entotic vacuole.

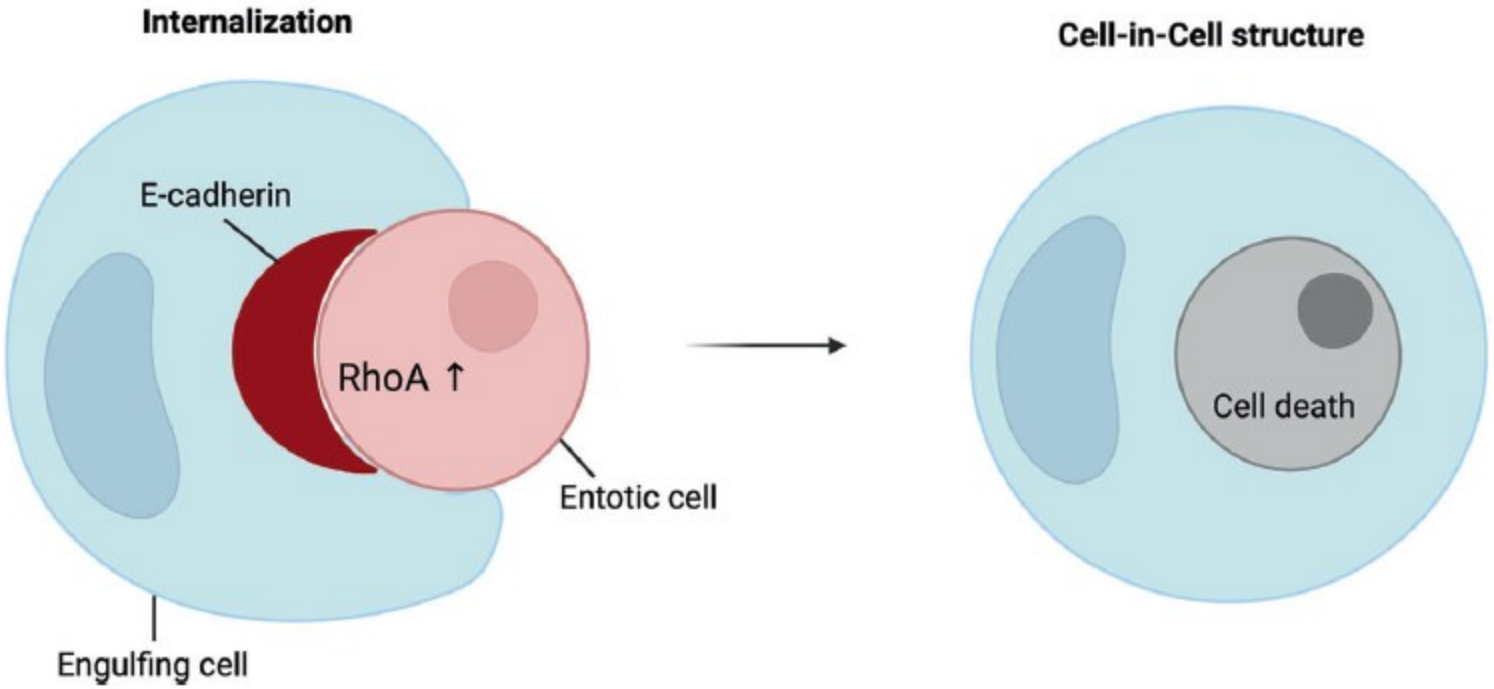
One of the crucial pathways involved in entosis is the Rho/Rho-associated protein kinase (ROCK)/actomyosin pathway, which regulates actin and myosin II activities and is essential for cell engulfment.

This pathway involves the activation of the Rho family of small GTPases, which activate downstream effectors, such as ROCK. In turn, ROCK activates myosin II, a motor protein that generates contractile forces by interacting with actin filaments. During entosis, an invading (engulfed) entotic cell forms an actin-rich structure that protrudes into the cytoplasm of the engulfing cell. Myosin II is recruited to this structure and contracts it, pulling the invading cell into the engulfing cell. The internalized cells are subsequently degraded by lysosomes within the engulfing cell. Entosis is induced by various stimuli, such as nutrient starvation and ultraviolet radiation. Nutrient starvation, particularly glucose starvation, plays a pivotal role in inducing entosis by activating AMP-activated protein kinase (AMPK) in internal cells, while ultraviolet radiation induces JNK and p38 stress-activated kinase signaling to activate entosis. **Entosis differs from anoikis, which is triggered by a lack of cell attachment to the ECM but not by matrix detachment.** Entosis shares more similarities to cell invasion than to a cell engulfment mechanism.

During normal development, entosis is thought to play a role in the removal of excess cells and in shaping tissues and organs. In cancer, entosis has been shown to contribute to tumor growth and tumor cell invasion by facilitating the engulfment of neighboring cells. During normal development, entosis is thought to play a role in the removal of excess cells and in shaping tissues and organs. In cancer, entosis has been shown to contribute to tumor growth and tumor cell invasion by facilitating the engulfment of neighboring cells.

Entosis

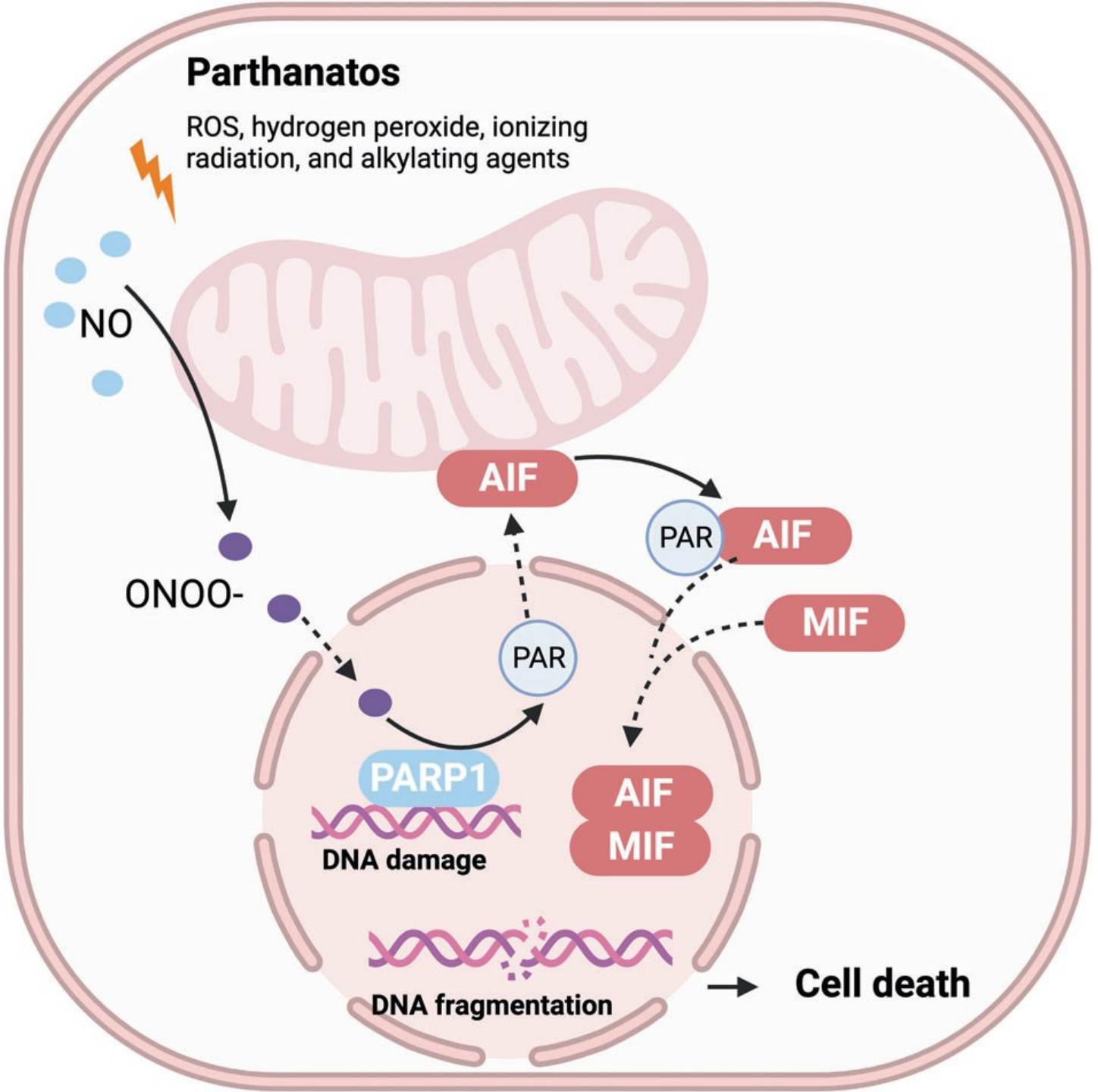
detachment from extracellular matrix



Parthanatos

Parthanatos (discovered by Karen Kate David in 2009) is a form of PCD that is mediated by the activation of poly ADP-ribose polymerase (PARP) . This type of cell death was first identified in neurons and plays an important role in several neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases.

Parthanatos is triggered by various agents, such as ROS, hydrogen peroxide, ionizing radiation, and alkylating agents. When DNA damage is mild, PARP-1 recruits DNA damage repair proteins to repair damaged DNA. Severe DNA damage leads to PARP-1 overactivation and PAR polymer formation. Accumulated PAR polymers bind to apoptosis-inducing factor (AIF) and mediate AIF release from mitochondria. AIF interacts with MIF to form the AIF/ MIF complex, which is translocated to the nucleus, causing DNA fragmentation and leading to parthanatos. Overall, parthanatos is a complex form of PCD that plays an important role in several disease processes. Further research is needed to fully understand the mechanisms underlying parthanatos and its potential therapeutic applications.


Parthanatos
ROS, hydrogen peroxide, ionizing radiation, and alkylating agents
NO
ONOO-
AIF
PAR
AIF
MIF
PAR
PARP1
DNA damage
AIF
MIF
DNA fragmentation
Cell death

Alkaliptosis

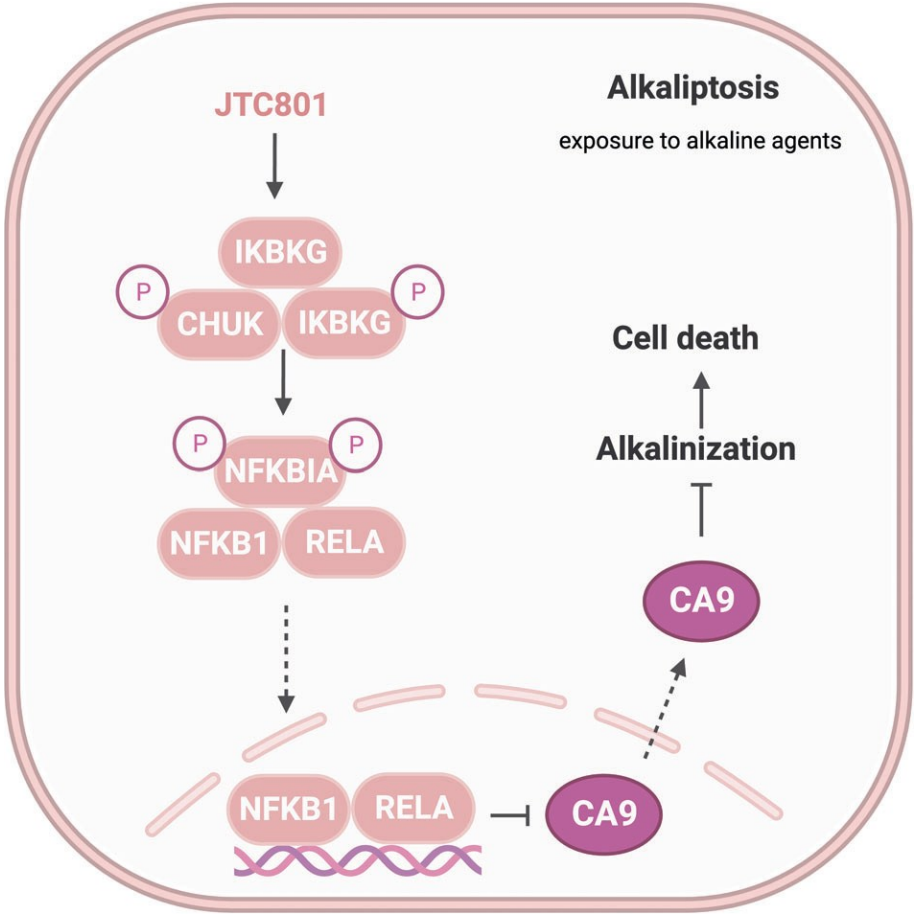
Alkaliptosis (Daolin Tang in 2018) is a recently discovered form of regulated necrosis **triggered by exposure to alkaline agents**, such as ammonia, sodium hydroxide, or high-pH buffers.

Alkaliptosis can be activated by the upregulation of nuclear factor-kappa B (NF-κB) pathways and subsequent downregulation of carbonic anhydrase 9 (CA9). CA9 is a member of the carbonic anhydrase family that plays a role in regulating pH levels. NF-κB negatively regulates CA9 activity, which in turn inhibits alkaliptosis. Depletion of CA9 can restored the sensitivity of cancer cells that lack functional NF-κB to alkaliptosis. Another study showed that ACSS2-mediated NF-κB activation promoted alkaliptosis in human pancreatic cancer cells.

ACSS2 has been found in the nucleus and cytoplasm and **provides AcCoA**, which is important for lipogenesis and histone acetylation.

ACSS2 plays a role in alkaliptosis by maintaining NF-κB activation and increasing the pH value via histone acetylation in human PDAC cells.

Alkaliptosis is a promising strategy for cancer therapy because cancer cells have a profoundly unbalanced pH, and their proliferation, metastasis, and metabolic adaptation are determined by their pH sensitivity.



JTC-801 is a selective antagonist for the nociceptin receptor, also known as the ORL-1 receptor.[3] This was the fourth opioid receptor to be discovered and is still the least understood. The nociceptin receptor has complex effects which are involved in many processes involved in pain and inflammation responses, and activation of this receptor can either increase or reduce pain depending on dose

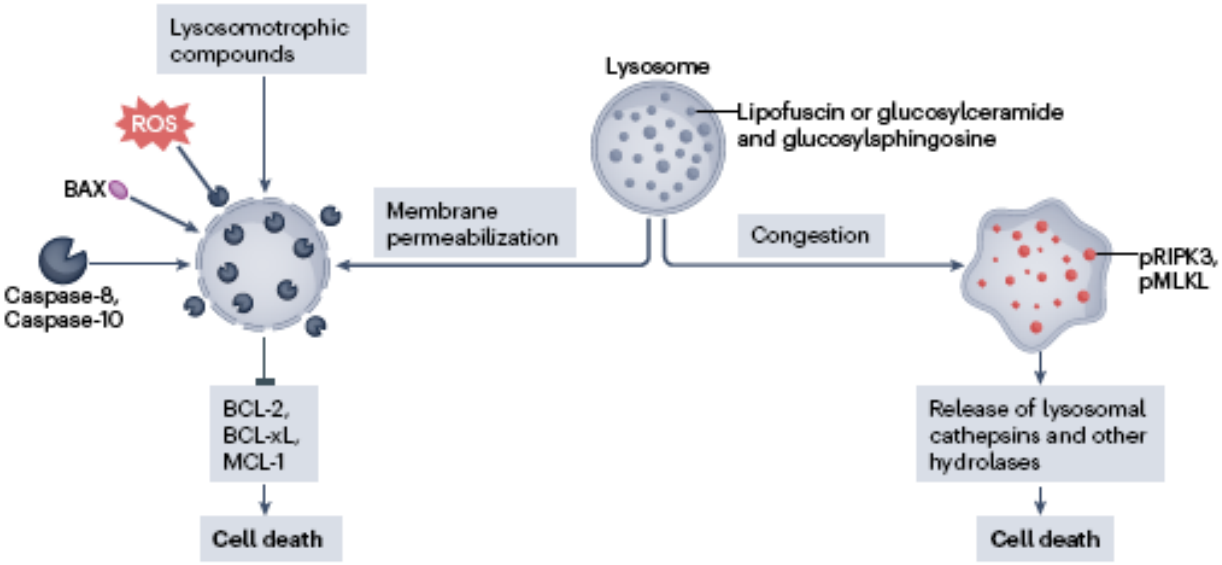


Fig. 4 | Cell death mediated by disruption of lysosomal function. Lysosomal membrane permeabilization (left), which may be induced by reactive oxygen species (ROS), lysosomotrophic compounds and activated BAX and caspase, leads to the release of cathepsins and other hydrolases from the lysosomal lumen to the cytosol. Lysosomal congestion (right), induced by the accumulation of glucosylceramide and glucosylsphingosine in Gaucher disease and of lipofuscin in Stargardt and dry age-related macular degeneration, can lead to the activation of RIPK3 and MLKL. pMLKL, phosphorylated MLKL; pRIPK3, phosphorylated RIPK3.

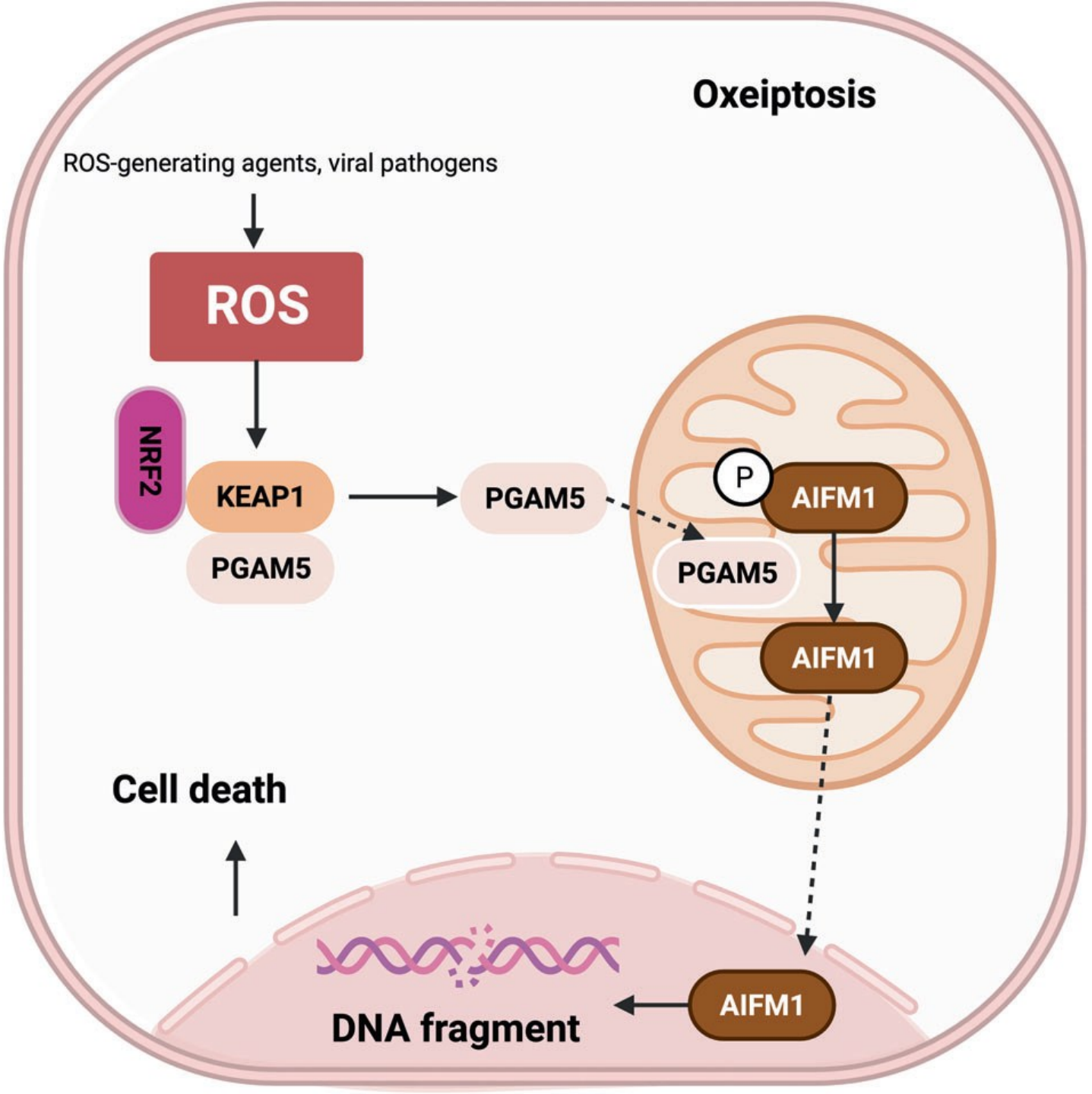
Oxeiptosis

The term “oxeiptosis” was coined by Holze et al. in 2018 to describe a caspase-independent, ROS-sensitive, and noninflammatory cell death pathway that protects against inflammation induced by ROS or ROS-generating agents, such as viral pathogens.

Oxeiptosis is characterized by the **activation of the KEAP1/PGAM5/AIFM1 signaling pathway**. Under oxidative stress conditions, AIFM1 is dephosphorylated, and its activity is regulated by KEAP1 and PGAM5. Dephosphorylated AIFM1 is translocated from mitochondria to the nucleus, where it induces chromatin condensation and DNA fragmentation, leading to cell death.

Activation of the KEAP1/PGAM5/AIFM1 signaling pathway is a hallmark of apoptosis and differs from other cell death pathways, such as the apoptosis and necrosis pathways. The mechanisms underlying oxeiptosis are not yet fully understood, but it is thought to involve the activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway.

Many conditions, such as allergies, autoimmune diseases, allograft rejection, cancer, and infection with pathogens, lead to ROS production, indicating that oxeiptosis may be triggered under various pathological conditions. Notably, excessive or dysregulated oxeiptosis can lead to tissue damage and contribute to disease development. Understanding the mechanisms underlying oxeiptosis and its role in health and disease is an active area of research that may identify new therapeutic intervention targets.


Oxeiptosis
ROS-generating agents, viral pathogens
ROS
NRF2
KEAP1
PGAM5
PGAM5
P
AIFM1
PGAM5
AIFM1
Cell death
DNA fragment
AIFM1

Erebosis

In 2022, Sa Kan Yoo et al. reported a novel form of cell death called erebosis during the natural turnover of gut enterocytes, which are the cells that make up the gut epithelium.

The term “erebosis” is derived from the Greek word “erebos,” meaning complete darkness.

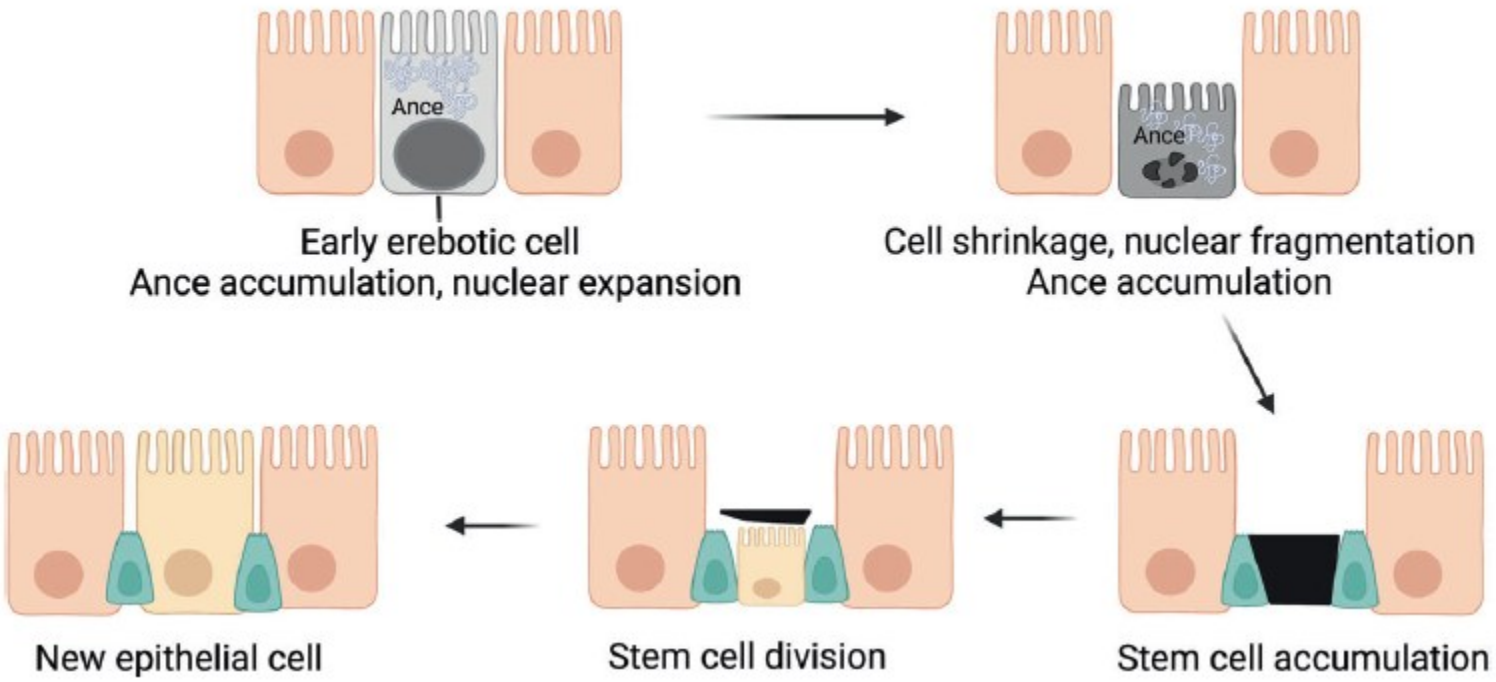
Erebotic cells undergo membrane blebbing and actin cytoskeleton changes, eventually leading to cell disintegration.

This process is marked by the loss of cell adhesion, organelles, and fluorescence emitted from labeled proteins and the accumulation of angiotensin-converting enzyme (ACE). In contrast to cell undergoing apoptosis, necrosis, and autophagic cell death, cells undergoing erebosis do not exhibit distinguishing features.

Notably, apoptosis inhibition does not affect apoptosis or gut cell turnover. The authors suggested that erebosis may play a vital role in maintaining gut barrier function through the natural shedding of gut enterocytes.

In contrast, abnormal erebosis may contribute to the development of gastrointestinal conditions, such as inflammatory bowel disease

Erebosis



the process of erebosis, a novel form of cell death observed during the natural turnover of enterocytes that constitute the gut epithelium.
Nuclear expansion and accumulation of **angiotensin-converting enzyme (ACE)** are observed in the early stages of erebosis. Subsequently, **cell shrinkage and nuclear fragmentation** are observed. Late erebotic cells are surrounded by stem cells that eventually undergo division to generate new epithelial cells, contributing to the replenishment of the gut epithelium.

PANoptosis

PANoptosis: an emerging and complex form of cell death In addition to the different types of cell death that share the same molecular pathways, it has recently been shown that different types of cell death are mediated simultaneously in a single cell. This phenomenon was first described by Kanneganti et al. in 2016.

PANoptosis is triggered by the formation of a protein complex called the **PANoptosome**, which is composed of several proteins, including **RIPK1, RIPK3, caspase-8, NLRP3, and ASC**.

This complex activates various cell death pathways, including pyroptosis, apoptosis, and necroptosis, resulting in an inflammatory cell death response.

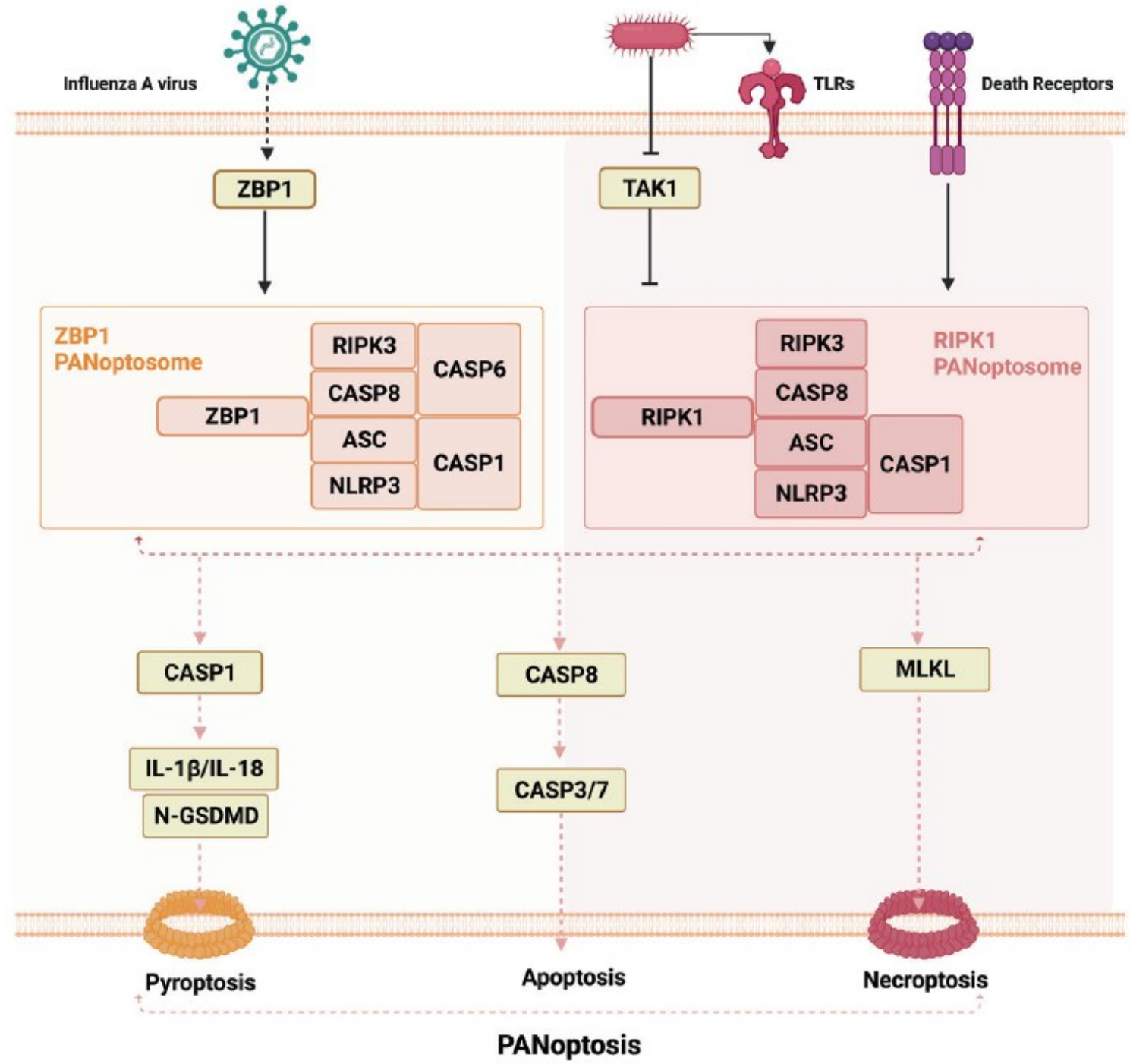
During influenza A virus (IAV) infection, Z-DNA-binding protein (ZBP1) plays a critical role in activating PANoptosis by recognizing viral ribonucleoproteins and inducing the formation of the ZBP1-dependent PANoptosome.

This complex consists of ZBP1 (the sensor), RIPK3, RIPK1, NLRP3, ASC, caspase-1, caspase-8, and scaffold caspase-6. TGF-β-activated kinase 1 (TAK1) is a crucial regulator of PANoptosis that negatively controls its initiation.

Notably, bacterial infections can interrupt PANoptosis suppression via the action of the Yersinia T3SS effector YopJ, leading to PANoptosome formation.

When TAK1 was inhibited and signaling through TLRs or death receptors was activated, RIPK1- dependent PANoptosomes were formed.

During PANoptosis, the activation of caspase-1 or caspase-8 leads to the cleavage and activation of downstream effector proteins, such as gasdermin D and RIPK3, which drive pyroptosis and necroptosis, respectively. In addition, activated caspase-8 can cleave and activate caspase-3, leading to apoptosis.


Influenza A virus
TLRs
Death Receptors
ZBP1
TAK1
ZBP1
PANoptosome
RIPK3
CASP8
ZBP1
ASC
NLRP3
CASP6
CASP1
RIPK1
PANoptosome
RIPK3
CASP8
RIPK1
ASC
NLRP3
CASP1
CASP1
IL-1β/IL-18
N-GSDMD
CASP8
CASP3/7
MLKL
Pyroptosis
Apoptosis
Necroptosis
PANoptosis

The complexity of cell death classification

The categorization system for cell death is intrinsically complexity. Initially, cell death was classified primarily based on morphological characteristics, as described below2. Type 1 cell death, known as apoptosis, is characterized by cytoplasm shrinkage, chromatin condensation, membrane blebbing, and the formation of apoptotic bodies, which are cleared through phagocytosis and lysosomal degradation by the surrounding cells210. Type 2 cell death is characterized by intense autophagy or cytoplasmic vacuolization, leading to phagocytosis by neighboring cells and degradation via lysosomes210. Type 3 cell death, or necrosis, results in cell death without triggering phagocytosis or lysosomal degradation and is not characterized by any of the features used to identify type 1 and 2 cell death modalities210. As more forms of cell death were identified, type 4 cell death modalities were classified, and these types of cell death includes those that cannot be classified into one of the three previously defined categories211. However, the current classification system of cell death, based on morphological changes, is limited because newly discovered forms of cell death cannot be integrated into it. Furthermore, the existing classification system does not account for the increasingly recognized importance of molecular pathways, specifically their greater importance than morphological changes, in identifying forms of cell death210. Thus, there is a need for more comprehensive guidelines that are based on genetic, biochemical, and functional criteria.

To address this need, the NCCD issued guidelines on the “classification of cell death” in 2005212, 2009213, and 20182. The 2018 classification system aimed to establish a more comprehensive system based on genetic, biochemical, and functional criteria, not merely morphological features (Fig. 16)2. However, this system also has several limitations. First, certain forms of cell death, namely, paraptosis, methuosis, alkaliptosis, oxeiptosis, cuproptosis, and erebosis, were not classified. Second, although pyroptosis, NETosis, and necroptosis are categorized as different types of cell death67, these modalities all involve immunogenic cell death98. Finally, the current classification system may not account for the potential interplay and crosstalk among different forms of cell death because cell death processes are mediated via a complex network of interactions contributing to cellular processes that may not be fully reflected in the classification system. Complexity of the interconnectedness among different types of cell death The interconnectedness among the various forms of cell death is an important factor that contributes to cell death complexity (Fig. 17). Necroptosis, a regulated form of necrosis, shares similarities with necrosis and apoptosis131,133.

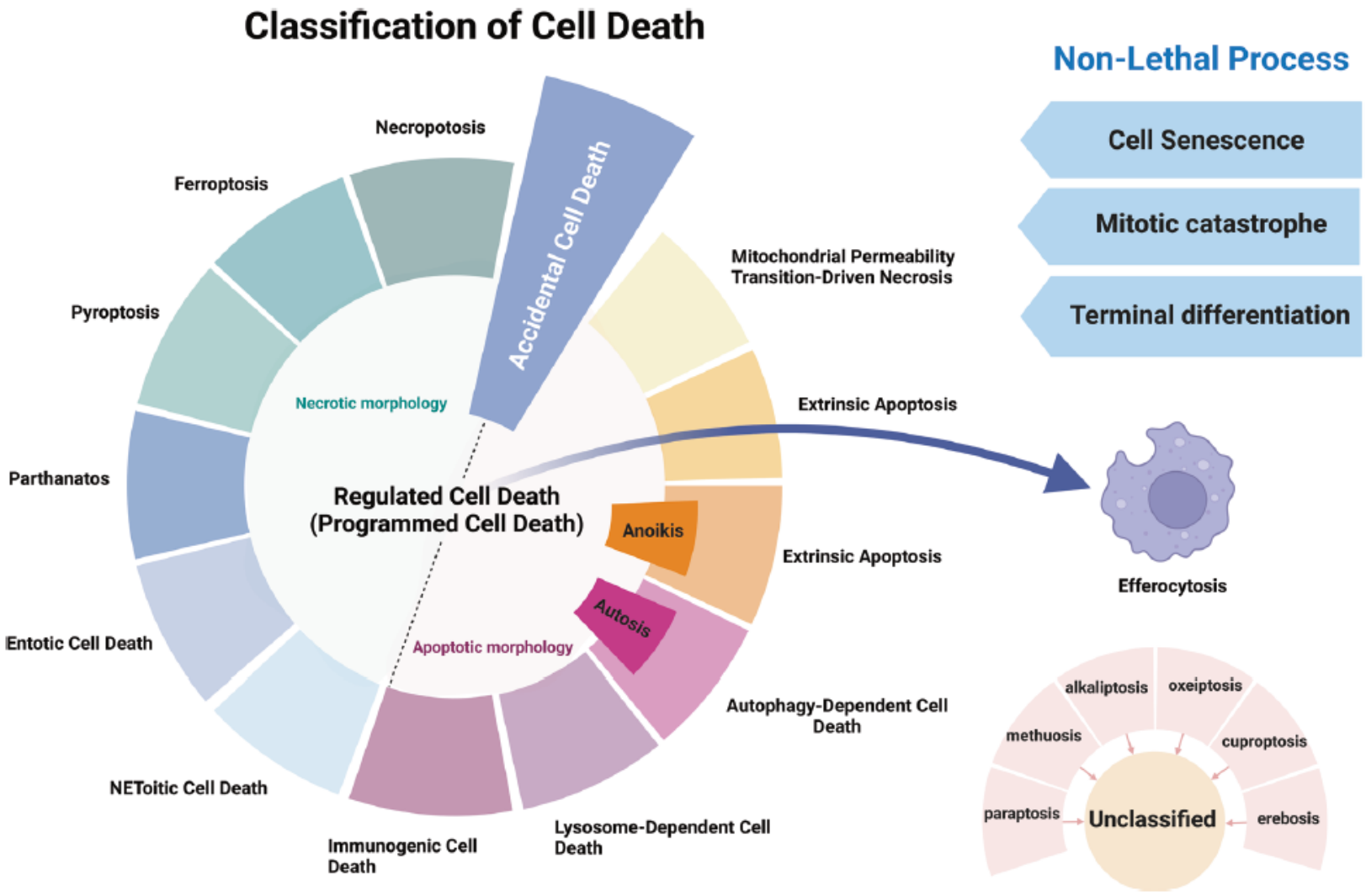
Autophagy is required for both autosis and autophagic cell death programs, which are triggered via different molecular pathways53,75. Similarly, cells undergoing parthanatos display morphological and cytological characteristics of both apoptosis and necrosis26. Recent studies have suggested that autophagy and ferroptosis pathways interact in a complex manner. Autophagy has been suggested to regulate ferroptosis by eliminating damaged mitochondria and peroxidized lipids214. However, excessive or prolonged autophagy can lead to ferritin degradation, which can trigger ferroptosis215. Cell death is influenced by various factors, including cellular organelles and environmental conditions. Mitochondria play crucial roles in different types of cell death, including apoptosis, NETosis, paraptosis, parthanatos, and oxeiptosis216. For example, apoptosis is triggered by mitochondrial outer membrane permeabilization (MOMP), which leads to the release of cytochrome c and activation of the caspase cascade216,217. In cells undergoing NETosis, mitochondria produce mitochondrial ROS (mtROS), whereas in cells undergoing paraptosis, Ca2+ released from the ER causes mitochondrial Ca2+ overload and mitochondrialdilation218,219. Both parthanatos and oxeiptosis involve mitochondrial release of AIF, which is translocated from the mitochondria to the nucleus, resulting in cell death30,195. Lysosomes play several roles in cell death. During lysosomal cell death, lysosomal membrane permeabilization results in the release of cathepsins, which activate apoptotic pathways83. During necrosis, lysosomal membrane permeabilization causes the release of lysosomal hydrolases and ROS, which cause cell damage and inflammation [183]. In cells undergoing autophagic death, lysosomes fuse with autophagosomes to degrade cellular components, leading to cell death220. Lysosomal exocytosis has been implicated in the induction of pyroptosis, a type of inflammatory cell death221. In addition, endothelial cells undergo lysosomal degradation182. Similar to lysosomal factors, ROS trigger cell death in various ways. For example, ROS play a crucial role in activating NADPH oxidase, which is required for the degradation of azurophilic granules that trigger NETosis116. Moreover, ROS can cause lipid peroxidation, leading to ferroptosis27. Additionally, ROS can cause ER stress by inducing the accumulation of unfolded proteins, triggering paraptosis222. ROS production is also the primary cause of oxeiptosis initiation30. Furthermore, a rapid increase in the cytosolic pH vale can induce both lysosomal cell death and alkaliptosis17,29.

Some types of cell death, such as paraptosis and methuosis, are caused by the formation of large vacuoles, highlighting the importance of organelle dysfunction in regulating cell death223. Overall, the interconnectedness among the different types of cell death and their regulation via diverse signaling pathways and environmental factors highlight the complexity of cell death. Understanding the interplay among different signaling pathways and the impact of the cell context on the cell death modality is crucial for developing new therapeutic strategies that target cell death pathways for the treatment of various diseases. Further research is needed to fully characterize and differentiate among the various forms of cell death and their roles in health and disease.

Table 1. Characteristics of the major types of cell death.

Classification	Type	Morphological features	Key regulator molecules	Major inhibitors (target)
Non-RCD	Necrosis	Cell swelling, plasma membrane rupture, loss of organelle	-	-
RCD-apoptotic	Apoptosis (Anoikis)	Cell shrinkage, nuclear condensation, apoptotic body fragmentation, nuclear membrane rupture, plasma membrane blebbing, loss of positional organization of organelles	Death receptors and their ligands, Bax, Bak, Bcl-2, AIF, caspase-3, caspase-8, caspase-9, TP53	Z-VAD-FMK, emricasan, Q-VD-OPh, Z-VAD(OH)-FMK (pancaspase), Z-DEVD-FMK (caspase-3, -6, -7, and -10), Z-VDVAD-FMK (caspase-2), ivachtin (caspase-3), Ac-DEVD-CHO (caspase-3 and -7), Z-IETD-FMK (caspase-8), Q-LEHD-OPh (caspase-9)
RCD-vacuole presenting	Autophagic cell death (Autosis)	Autophagic vacuolization, plasma membrane blebbing, organelle enlargement, depletion of cytoplasmic organelles	AMPK, mTOR, ULK1, PI3KIII, BECN1, ATGs, LC3, Na^+/K^+-ATPase	Chloroquine (lysosome), bafilomycin A1, concanamycin A (H^+-ATPase), 3-methyladenine, wortmannin (PI3K), spautin 1(USP10 and USP13)
	Entosis	Cell-in-cell structure	RhoA, ROCKI/II, E-cadherin, α-catenin, actomyosin, LC3, ATGs	C3-toxin (RhoA), Y-27632 (ROCK), blebbistatin (myosin)
	Methuosis	Accumulation of large fluid-filled single-membrane vacuoles, cell swelling, plasma membrane rupture	Ras, Rac1, Arf6, LAMP1, Rab7	SP600125 (JNK)
	Paraptosis	Accumulation of large fluid-filled single-membrane vacuoles, dilation of the ER or mitochondria	mHCX, mNCX, MCU, VDAC, PyR, IP_3R, SERCA, MAPKs	Actinomycin D, cycloheximide (ER stress)
RCD-mitochondria dependent	Mitoptosis	Mitochondria disappearance, decomposition of the mitochondrial reticulum into small spherical organelles	Bax, Bak, TIMM8a (DPP), Drp1	NAC, trolox (ROS)
	Parthanatos	Chromatic condensation, large DNA fragment formation, lack of apoptotic bodies and small-scale DNA fragments, loss of membrane integrity, lack of cell swelling	PARP, AIF	BYK204165, AG-14361, iniparib (PARP1)
RCD-metal dependent	Ferroptosis	Small mitochondria, reduced number of mitochondrial cristae, elevated mitochondrial membrane density, increased rate of mitochondrial membrane rupture	System X_C^-, GPX4, lipid ROS	Deferoxamine, ciclopirox, deferiprone (Fe), ferrostatin-1, liproxstatin-1, β-mercaptoethanol, vitamin E, β-carotene, NAC, XJB-5-131, zileuton, CoQ10, baicalein (ROS), vildagliptin, alogliptin, linagliptin (DPP4), thiazolidinedione, rosiglitazone (ACSL4), selenium (GPX4)
	Cuproptosis	Mitochondrial shrinkage	SLC31A1, ATP7B, FDX1	GSH (chelate Cu), UK5099 (MPC), rotenone, antimycin A (ETC)
RCD-immunogenic	Necroptosis	Cell swelling, plasma membrane rupture, moderate chromatin condensation	Death receptors, TLRs, TCR, RIPKs, MLMK	Tetrahydroisoquinolines, lactoferrin, DNase (NETs), cl-amidine (PADI4)
	Lysosomal cell death	Lysosomal and plasma membrane rupture	Cathepsins, STAT3, TP53, NF-κB, and MCOLN1	CA-074Me (CTSB), deferoxamine (Fe), NAC (ROS)
	Pyroptosis	Lack of cell swelling, plasma membrane rupture, bubbling, moderate chromatin condensation and fragmentation	NLRs, ALRs, caspase-1, caspase-11, GSDMD	Disulfiram, LDC7559, Ac-FLTD-CMK, Polyphyllin VI (GSDMD), morroniside (MMP2/9)
	NETosis	Plasma membrane rupture, nuclear membrane collapse, chromatic fiber release	NOX4, PADI4	Tetrahydroisoquinolines (NETs), cl-amidine (PADI4)
Other	Alkaliptosis	Necroptosis-like morphology	NF-κB, IKBKB, CA9	NAC, *N*-acetyl alanine acid (pH), IMD0354, CAY10657, SC514 (IKBKB)
	Oxeiptosis	Apoptosis-like morphology	KEAP1, PGAM5, AIFM1	NAC (ROS)
	Erebosis	Loss of proteins, organelles, and nuclear contents; ACE accumulation; shortened microvilli; fragmented nuclei	Unknown	Unknown

RCD regulated cell death, *ACE* angiotensin-converting enzyme, *GSDMD* gasdermin D.


Classification of Cell Death
Non-Lethal Process
Cell Senescence
Mitotic catastrophe
Terminal differentiation
Necropotosis
Ferroptosis
Pyroptosis
Parthanatos
Entotic Cell Death
NETotic Cell Death
Immunogenic Cell Death
Lysosome-Dependent Cell Death
Autophagy-Dependent Cell Death
Extrinsic Apoptosis
Mitochondrial Permeability Transition-Driven Necrosis
Accidental Cell Death
Necrotic morphology
Regulated Cell Death (Programmed Cell Death)
Apoptotic morphology
Anoikis
Autosis
Extrinsic Apoptosis
Efferocytosis
alkaliptosis
oxeiptosis
methuosis
cuproptosis
paraptosis
Unclassified
erebosis

Regulated necrosis

- Regulated necrosis is defined as a genetically controlled cell death process that eventually results in cellular leakage, and it is morphologically characterized by cytoplasmic granulation, as well as organelle and/or cellular swelling ('oncosis').
- Attempts to define and classify forms of cell death and their underlying pathways have resulted in multiple neologisms, such as necroptosis, parthanatos, oxytosis, ferroptosis, ETosis, NETosis, pyronecrosis and pyroptosis; all of these processes are characterized by a particular aspect of the cell death process

classified the mechanistic steps

- a trigger (level 1) activates
- an initiator mechanism (level 2). This subsequently activates
- several mediators that propagate the signal (level 3) and ultimately relay it to
- overlapping biochemical mechanisms (executioners) that cause necrotic cell death (level 4).

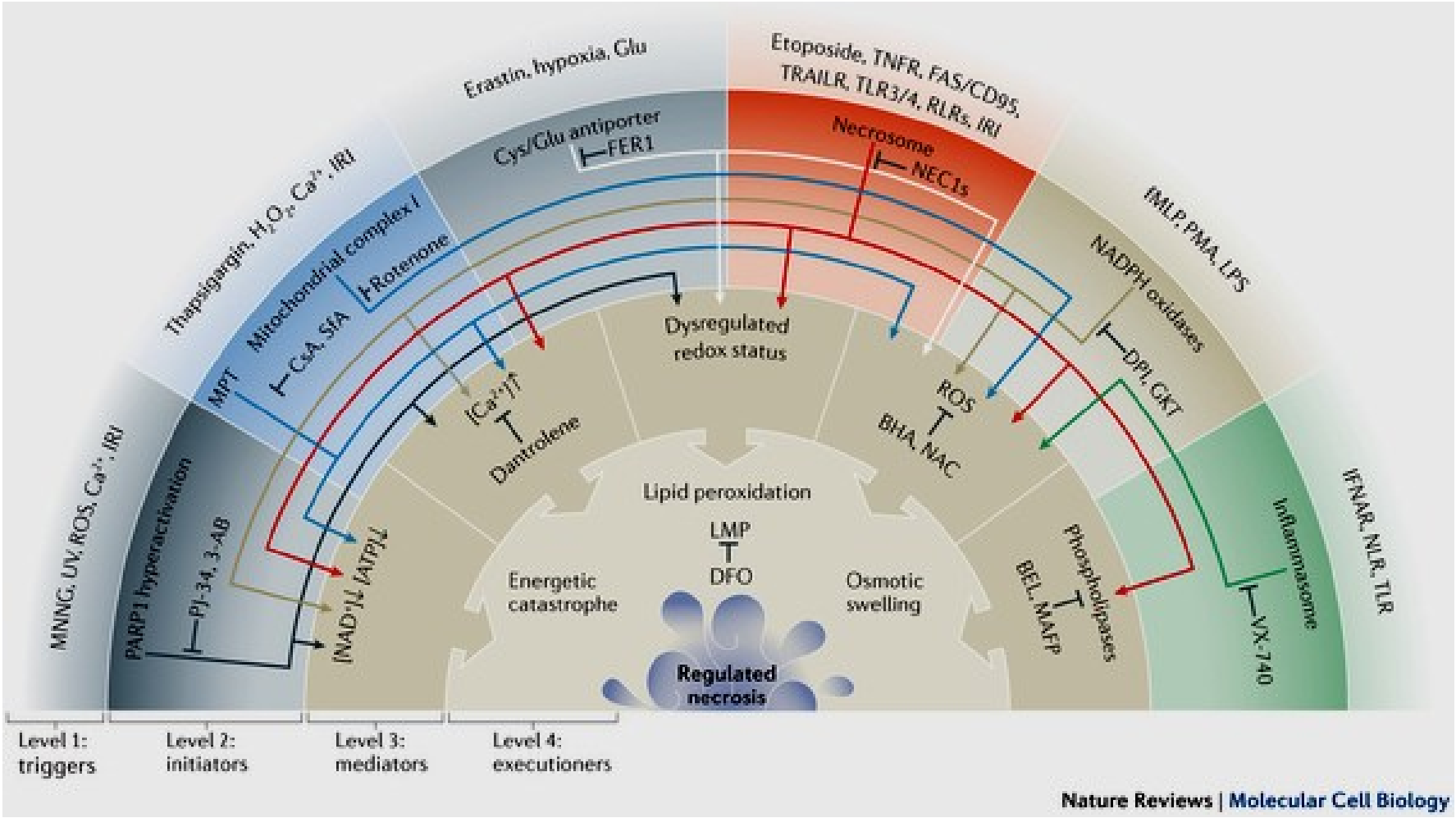


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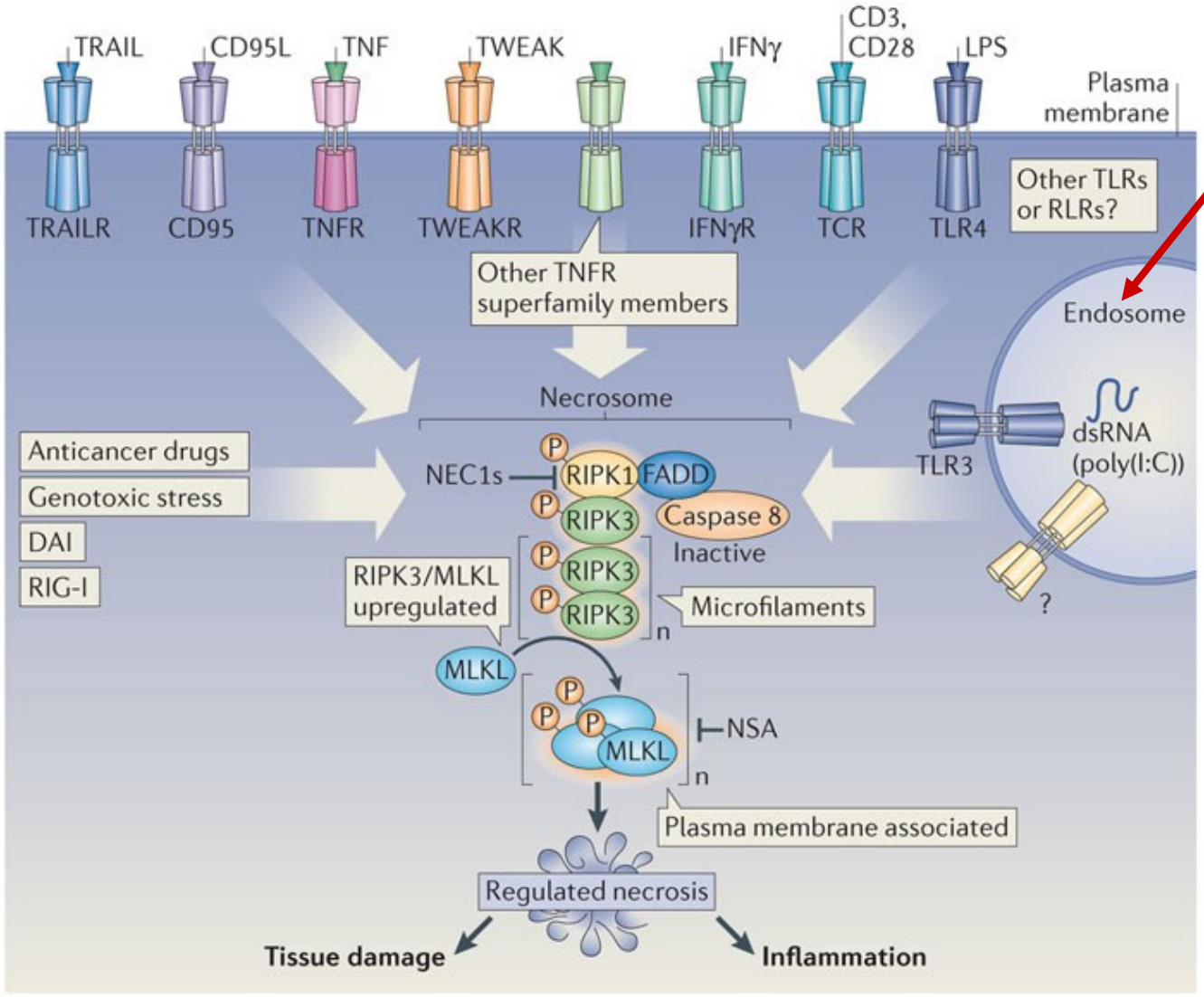
Table 2. List of cell death-related diseases.

Type	Disease	Reference
Necrosis	Inflammation and damage to surrounding tissues	37
Apoptosis (Deficient)	Cancer, autoimmune disorders, and viral infections	231
Apoptosis (Excessive)	Ischemic heart disease, stroke, neurodegenerative diseases, sepsis, and multiple organ dysfunction syndrome	231
Autophagic cell death	Cancer, neurodegeneration, ischemic injury, and heart disease	70–74
Autosis	Severe liver disease	82
Entosis	Cancer	191,192
Methuosis	Cancer	180,181
Mitoptosis	Mitochondrion-associated human diseases	232
Pyroptosis	Infectious diseases, autoimmune disorders, cancer, and neurodegenerative diseases	112,113
NETosis	Inflammatory and autoimmune disease	11,12
Necroptosis	Neurodegenerative diseases, viral infections, ischemic injury, and cancer	141–144
Parthanatos	Smoke-related lung diseases, macular degeneration, Parkinson's disease, and oxidative stress-related hearing disorders	233–236
Ferroptosis	Acute kidney injury, cancer, cardiovascular disorders, neurodegenerative conditions, and hepatic diseases	237
Cuproptosis	Wilson's disease	154
Oxeiptosis	Allergies, autoimmunity, allograft rejection, cancer, and infection with pathogens	208

Triggers of necroptosis and its links to tissue damage and inflammation



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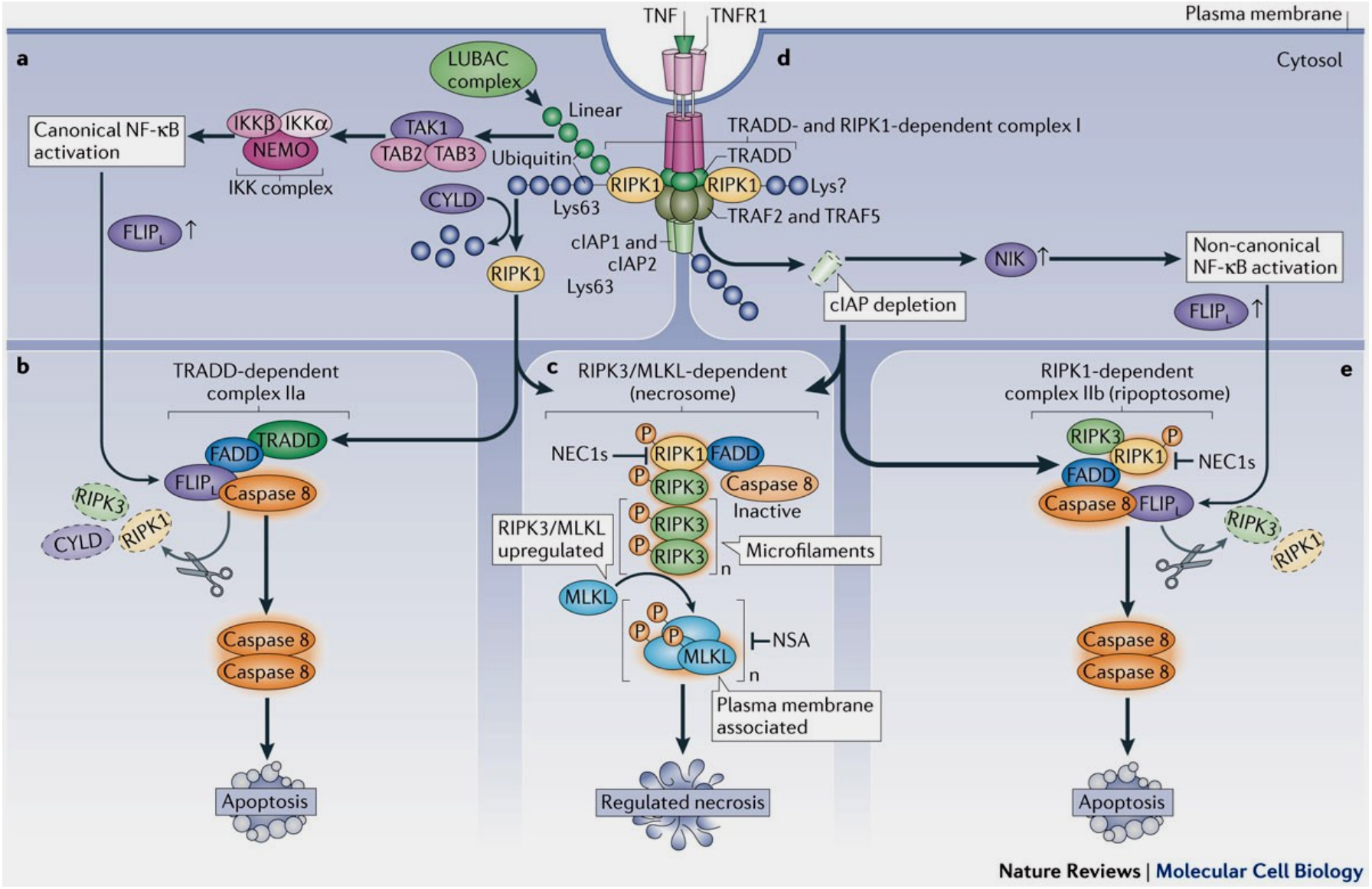
PAMPs (pathogen-associated molecular patterns) such as: polycytidylic acid (poly(I:C))-mediated activation of Toll-like receptor 3 (TLR3); retinoic acid inducible gene I (RIG-I)-like receptors (RLRs), such as RIG-I or MDA5 (melanoma differentiation-associated protein 5); or LPS (lipopolysaccharide) - mediated activation of TLR4

- Typically, necroptosis is induced upon caspase 8 inhibition, as was originally observed *in vitro,* and confirmed in caspase 8-deficient mice and FADD-deficient mice. Of note, necroptotic damage can be reversed by receptor-interacting protein kinase 3 (RIPK3) deficiency in caspase 8-knockout mice, and by RIPK3 or RIPK1 deficiency in FADD-deficient mice.
- The pharmacological inhibition of necroptosis — for example, by using necrostatin 1 (NEC1) — or the genetic ablation of RIPK3 or mixed lineage kinase domain-like (MLKL) has shown that necroptosis can also occur in the absence of caspase inhibition in various pathological settings, such as ischaemic or traumatic brain injury, myocardial infarction, retinal ischaemia, photoreceptor cell loss, renal ischaemia–reperfusion injury, experimental pancreatitis, skin necroptosis, *Salmonella enterica* infection, TNF-induced systemic inflammatory response syndrome and atherosclerosis.
- Finally, low levels of MLKL were recently found to be associated with decreased overall survival in patients with early-stage resected pancreatic adenocarcinoma, which indicates a correlation between chemo-sensitivity and the induction of regulated necrosis.

- FLIPL, which is upregulated by NF-κB, and pro-caspase 8 form heterodimers that cleave and inactivate RIPK1, RIPK3 and CYLD to prevent necroptosis
- when caspase 8 is inhibited by chemical caspase inhibitors or virally encoded proteins such as CrmA (cytokine response modifier protein A) or vIRA (viral inhibitor of RIP activation), the RHIM (RIP homotypic interaction motif) domains of RIPK1 and RIPK3 associate in microfilament-like complexes called necrosomes. The auto- and transphosphorylation of RIPK1 and RIPK3 and the recruitment of mixed lineage kinase domain-like (MLKL) initiate necroptosis. Several events could mediate necroptosis, including GRIM19 (gene associated with retinoic and interferon-induced mortality 19)-mediated ROS (reactive oxygen species) production, JUN N-terminal kinase (JNK) activation or the translocation of the necrosome to mitochondria-associated membranes.


TNF
TNFR1
Plasma membrane
Cytosol
a
d
LUBAC complex
Linear
Ubiquitin
Lys63
Canonical NF-κB activation
IKKβ
IKKα
NEMO
IKK complex
TAK1
TAB2
TAB3
CYLD
FLIPL ↑
RIPK1
TRADD- and RIPK1-dependent complex I
TRADD
RIPK1
RIPK1
Lys?
TRAF2 and TRAF5
cIAP1 and cIAP2
Lys63
cIAP depletion
NIK ↑
Non-canonical NF-κB activation
FLIPL ↑
b
TRADD-dependent complex IIa
TRADD
FADD
FLIPL
Caspase 8
RIPK3
CYLD
RIPK1
Caspase 8
Caspase 8
Apoptosis
c
RIPK3/MLKL-dependent (necrosome)
NEC1s
P
RIPK1
FADD
P
RIPK3
Caspase 8
Inactive
RIPK3/MLKL upregulated
P
RIPK3
P
RIPK3
n
Microfilaments
MLKL
P
P
P
MLKL
NSA
n
Plasma membrane associated
Regulated necrosis
e
RIPK1-dependent complex IIb (ripoptosome)
RIPK3
P
RIPK1
NEC1s
FADD
Caspase 8
FLIPL
RIPK3
RIPK1
Caspase 8
Caspase 8
Apoptosis
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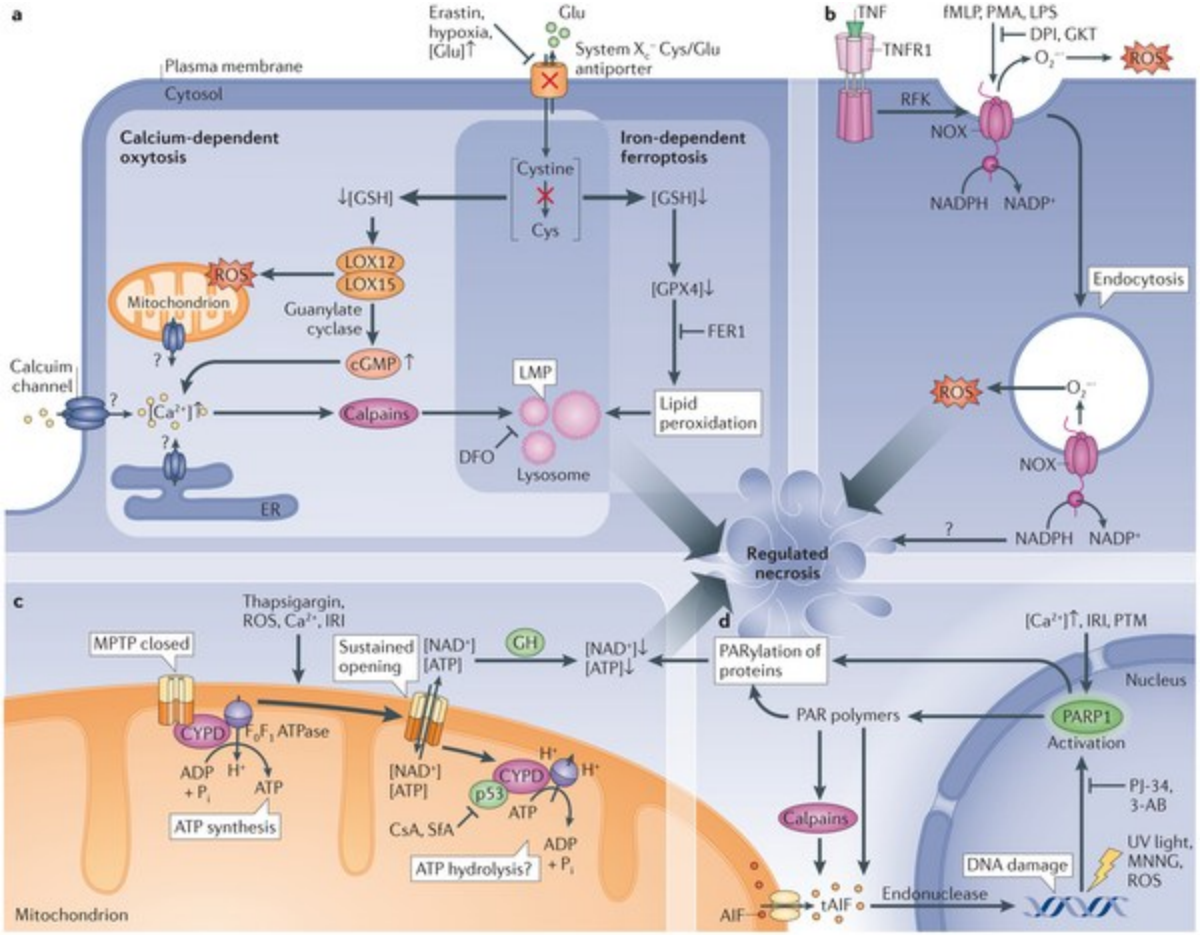
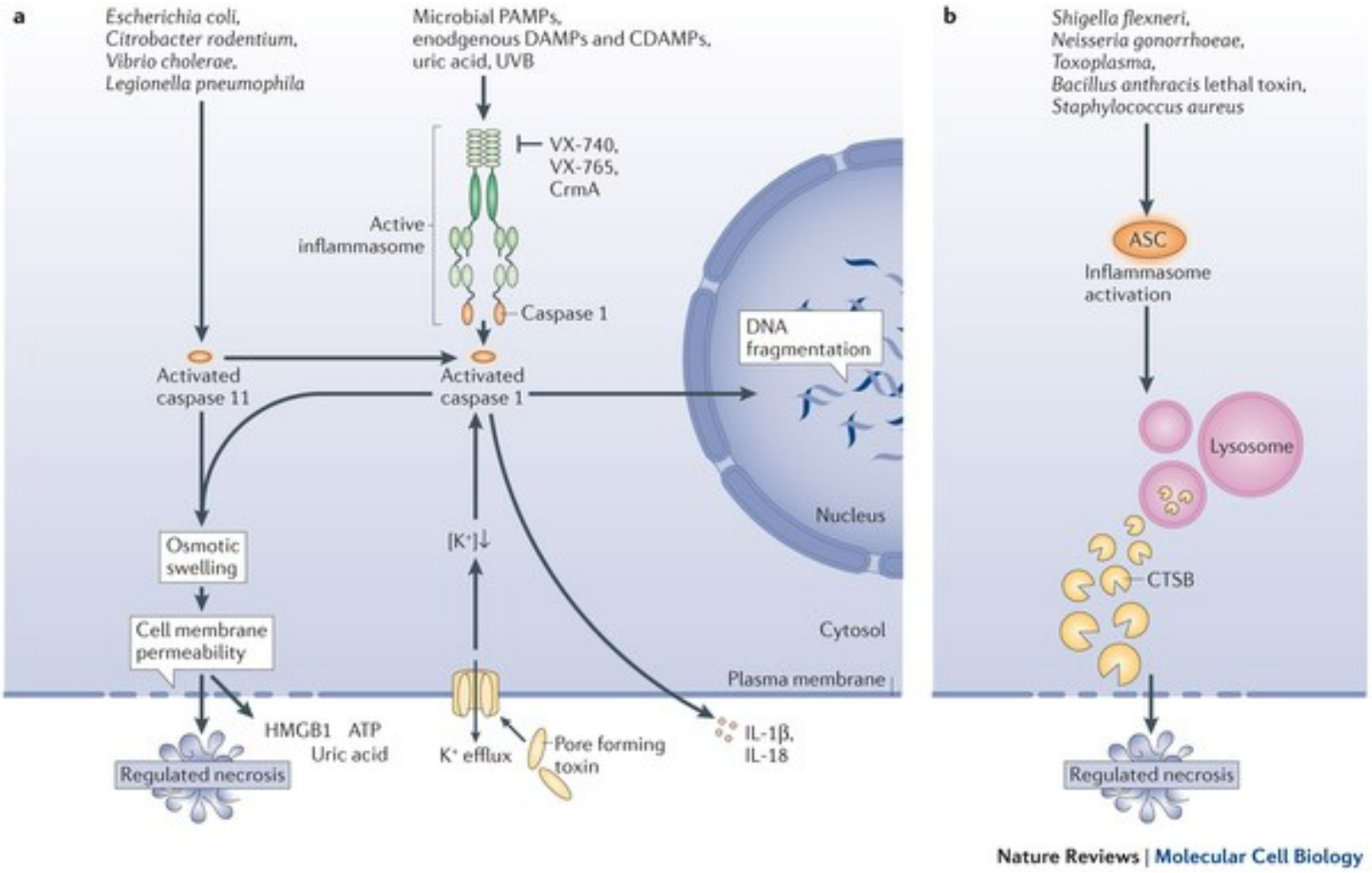
Necroptosis in pathology.

- Mice deficient for RIPK3 or MLKL, the central inducers of necroptosis, did not show defects in development or during homeostasis, which illustrates that necroptosis is not required during these processes. Interestingly, viruses can interfere with necroptosis, highlighting its physiological relevance as an antiviral mechanism, as is demonstrated by the fact that RIPK3-deficient mice die after challenge with vaccinia virus. Some viruses, such as cytomegalovirus, have developed a strategy to interfere with this host antiviral response. For example, the viral inhibitor of RIPK1 activation (vIRA) and the viral inhibitor of caspase 8 activation (vICA) prevent the activation of both necroptosis and apoptosis, respectively by TNF. In many settings necroptosis dysregulation contributes to tissue damage and inflammation, and has thus been found to contribute to several pathologies.


a
Erastin, hypoxia, [Glu]↑
Glu
System X_c^- Cys/Glu antiporter
Plasma membrane
Cytosol
Calcium-dependent oxytosis
Iron-dependent ferroptosis
Cystine
Cys
↓[GSH]
[GSH]↓
LOX12
LOX15
ROS
Mitochondrion
Guanylate cyclase
cGMP ↑
[GPX4]↓
FER1
Calcuim channel
$[Ca^{2+}]$↑
Calpains
LMP
Lipid peroxidation
DFO
Lysosome
ER
b
TNF
TNFR1
fMLP, PMA, LPS
DPI, GKT
$O_2^{\cdot-}$
ROS
RFK
NOX
NADPH
$NADP^+$
Endocytosis
ROS
$O_2^{\cdot-}$
NOX
?
NADPH
$NADP^+$
Regulated necrosis
c
Thapsigargin, ROS, Ca^{2+}, IRI
MPTP closed
Sustained opening
[NAD^+] [ATP]
GH
[NAD^+]↓ [ATP]↓
CYPD
F_0F_1 ATPase
ADP + P_i
H^+
ATP
ATP synthesis
[NAD^+] [ATP]
p53
CsA, SfA
ATP hydrolysis?
ADP + P_i
Mitochondrion
d
$[Ca^{2+}]$↑, IRI, PTM
PARylation of proteins
Nucleus
PAR polymers
PARP1
Activation
PJ-34, 3-AB
Calpains
UV light, MNNG, ROS
DNA damage
Endonuclease
AIF
tAIF



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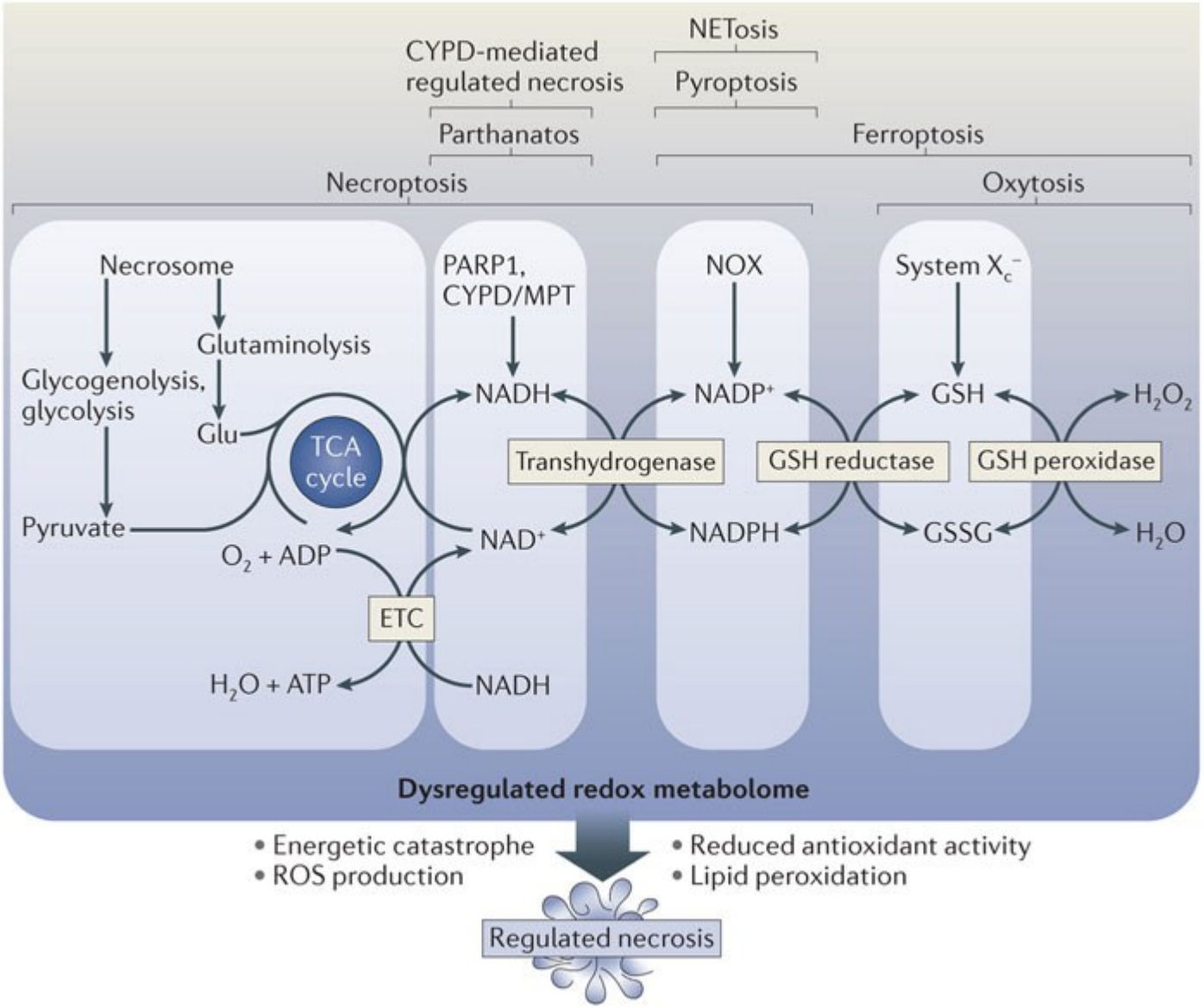
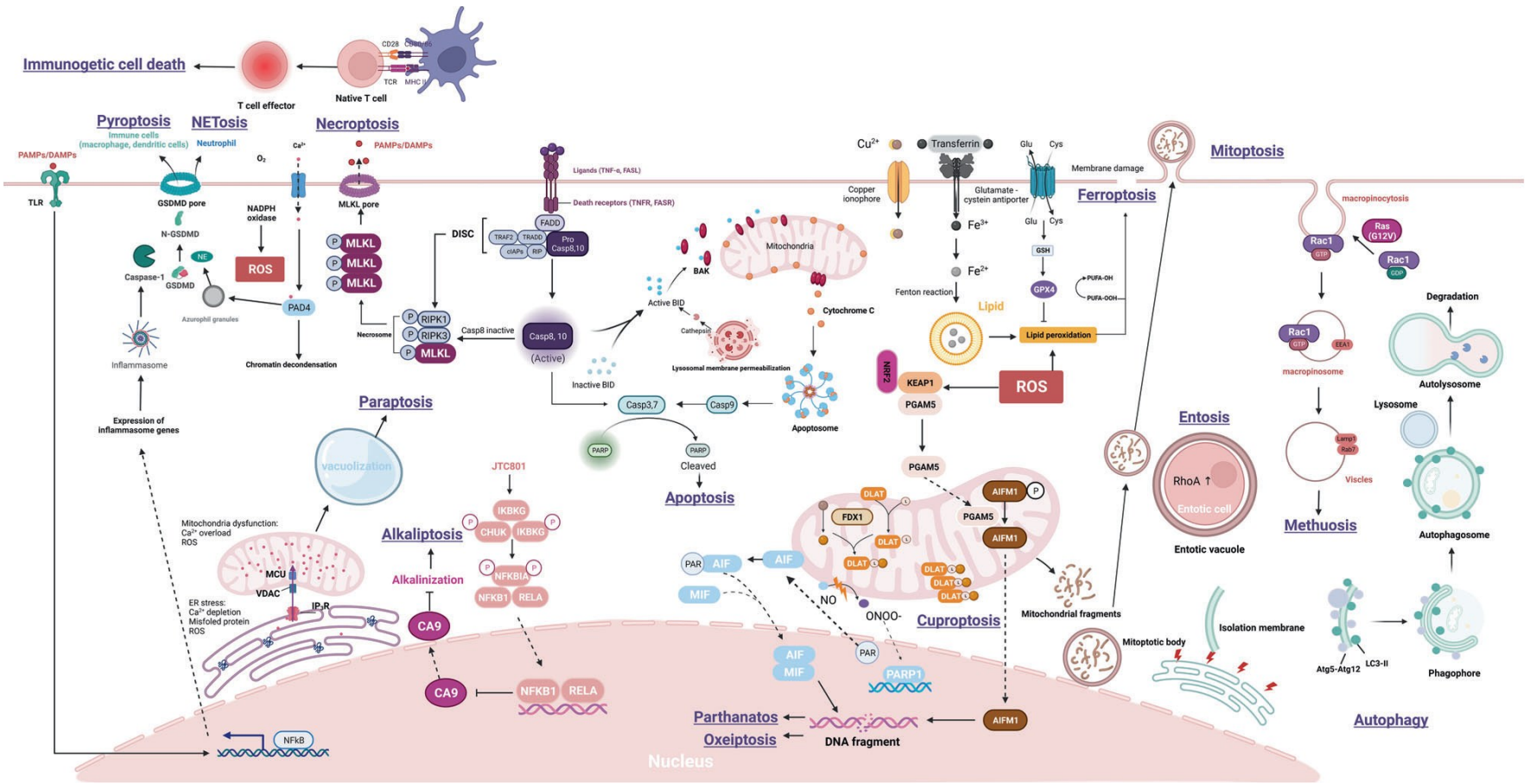

NETosis
CYPD-mediated regulated necrosis
Pyroptosis
Parthanatos
Ferroptosis
Necroptosis
Oxytosis
Necrosome
Glutaminolysis
Glycogenolysis, glycolysis
Glu
TCA cycle
Pyruvate
O_2 + ADP
ETC
H_2O + ATP
NADH
PARP1, CYPD/MPT
NADH
NAD+
NOX
NADP+
NADPH
System X_c^-
GSH
GSSG
H_2O_2
H_2O
Transhydrogenase
GSH reductase
GSH peroxidase
Dysregulated redox metabolome
• Energetic catastrophe
• ROS production
• Reduced antioxidant activity
• Lipid peroxidation
Regulated necrosis

Complexity of cell death. Mechanisms by which different types of cell death pathways interact and influence each other and the ways in which they can be regulated by various signaling pathways and environmental factors



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