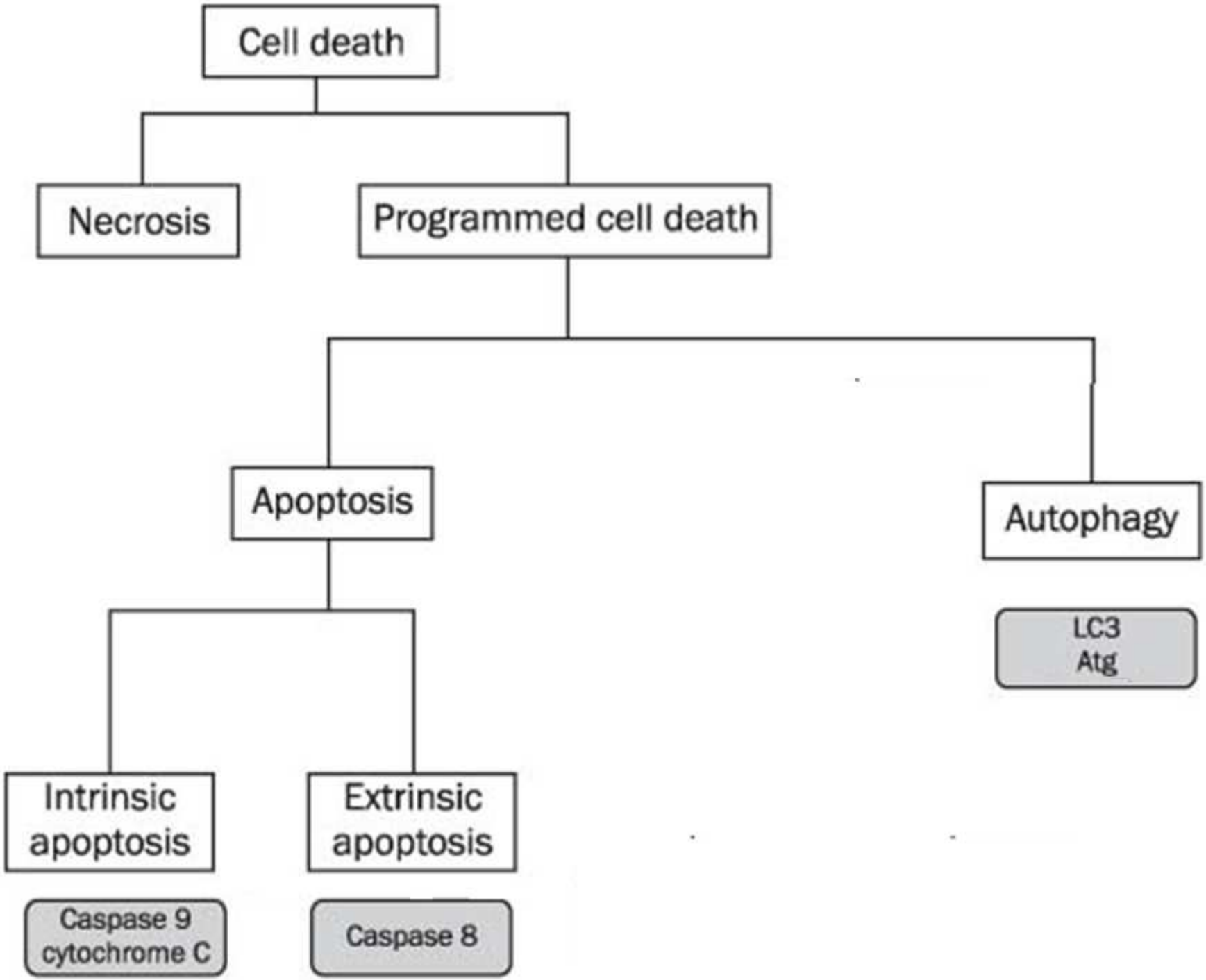
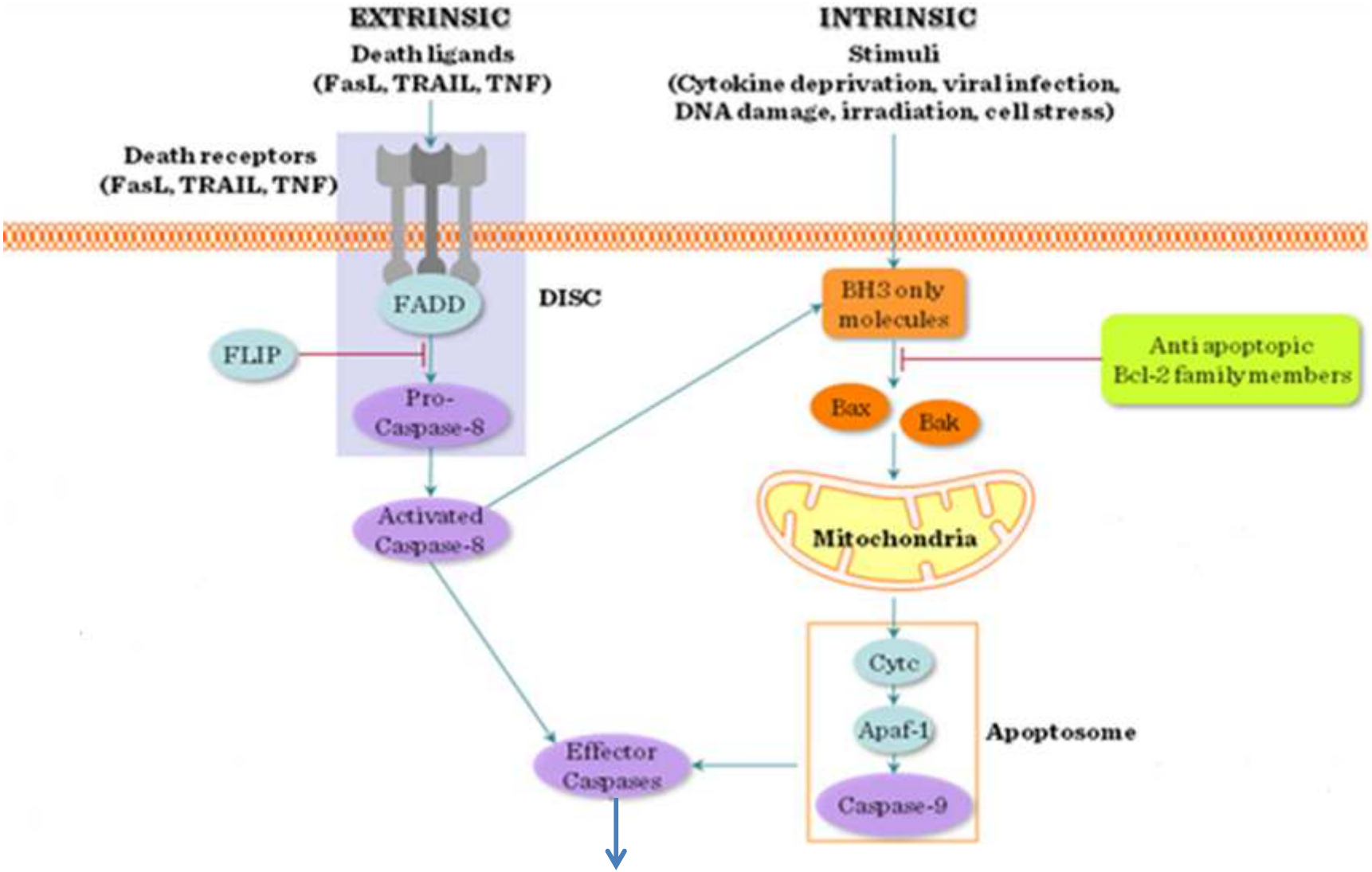
Necrosis	Apoptosis
Morphological features	
● Loss of membrane integrity	● Membrane blebbing, but no loss of integrity
	● Aggregation of chromatin at the nuclear membrane
● Begins with swelling of cytoplasm and mitochondria	● Begins with shrinking of cytoplasm and condensation of nucleus
● Ends with total cell lysis	● Ends with fragmentation of cell into smaller bodies
● No vesicle formation, complete lysis	● Formation of membrane bound vesicles (apoptotic bodies)
● Disintegration (swelling) of organelles	● Mitochondria become leaky due to pore formation involving proteins of the bcl-2 family.

Biochemical features	
● Loss of regulation of ion homeostasis	● Tightly regulated process involving activation and enzymatic steps
● No energy requirement (passive process, also occurs at 4°C)	● Energy (ATP)-dependent (active process, does not occur at 4°C)
● Random digestion of DNA (smear of DNA after agarose gel electrophoresis)	● Non-random mono- and oligonucleosomal length fragmentation of DNA (Ladder pattern after agarose gel electrophoresis)
● Postlytic DNA fragmentation (= late event of death)	● Prelytic DNA fragmentation ● Release of various factors (cytochrome C, AIF) into cytoplasm by mitochondria ● Activation of caspase cascade ● Alterations in membrane asymmetry (i.e., translocation of phosphatidylserine from the cytoplasmic to the extracellular side of the membrane)

Physiological significance	
● Affects groups of contiguous cells	● Affects individual cells
● Evoked by non-physiological disturbances (complement attack, lytic viruses, hypothermia, hypoxia, ischemica, metabolic poisons)	● Induced by physiological stimuli (lack of growth factors, changes in hormonal environment)
● Phagocytosis by macrophages	● Phagocytosis by adjacent cells or macrophages
● Significant inflammatory response	● No inflammatory response


Cell death
Necrosis
Programmed cell death
Apoptosis
Autophagy
LC3
Atg
Intrinsic apoptosis
Extrinsic apoptosis
Caspase 9
cytochrome C
Caspase 8



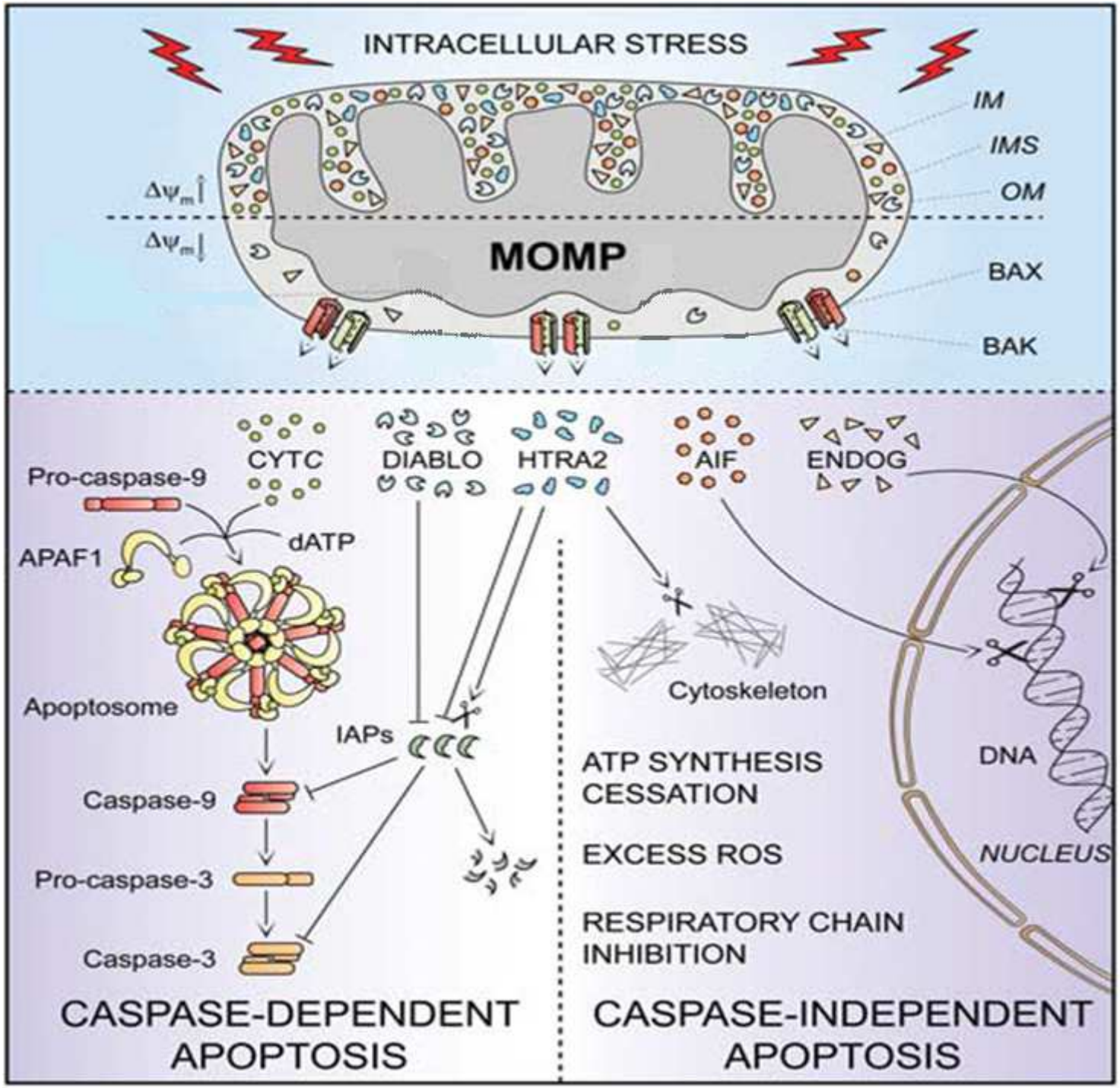
endonuclease activation → degradation of chromosomal DNA
protease activation → degradation of nuclear and cytoskeletal proteins → cytoskeletal reorganization

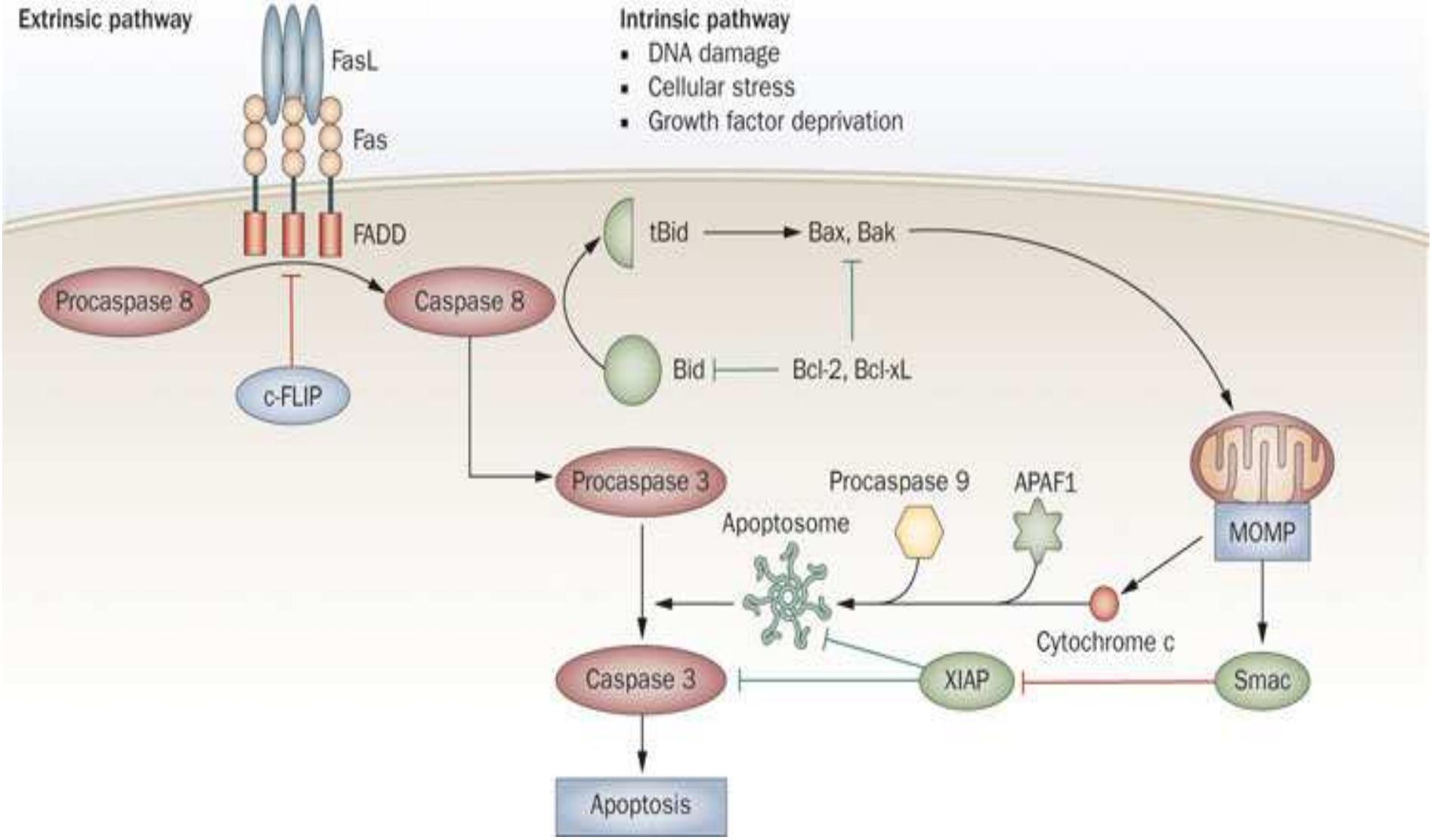
↓

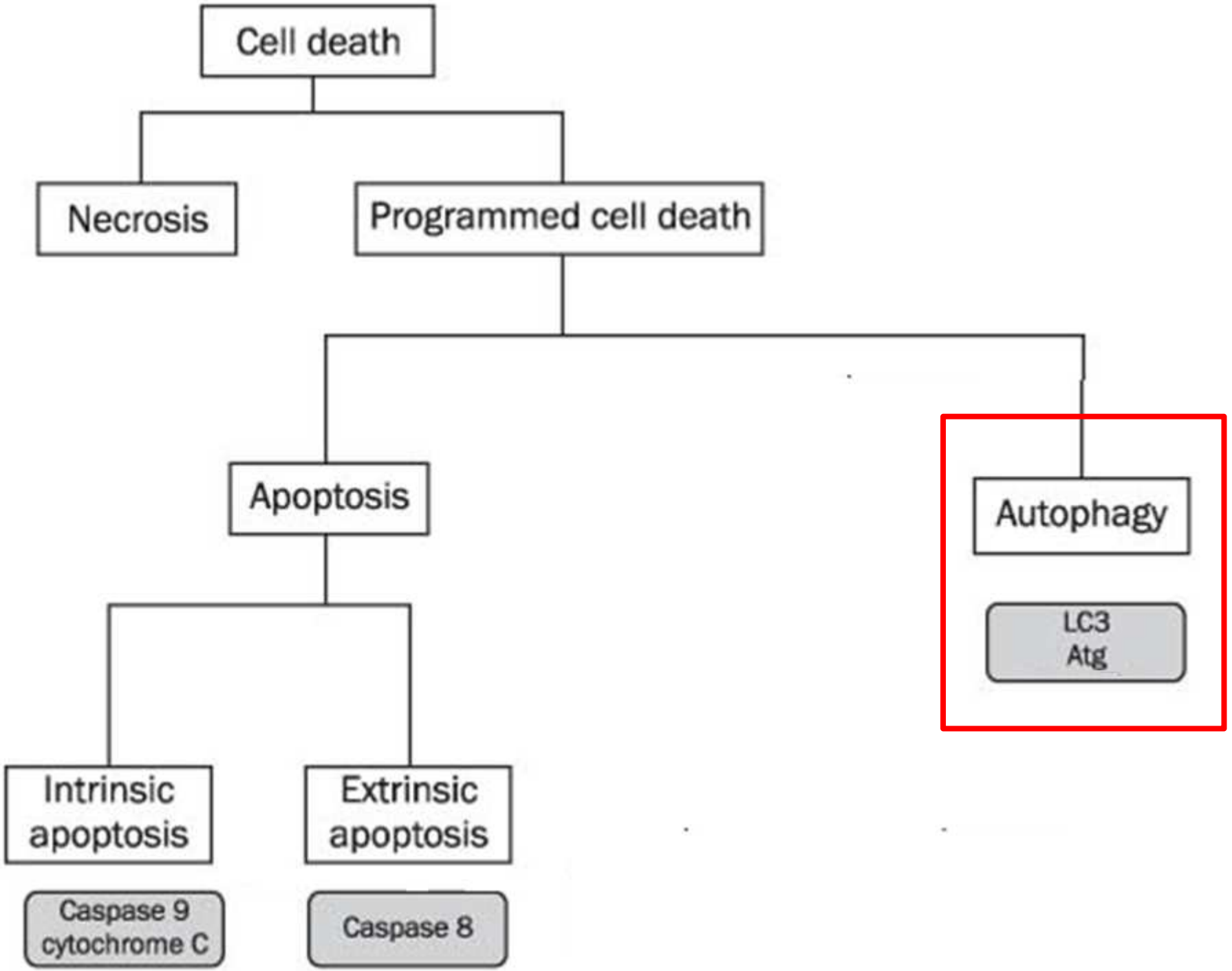
cytomorphological changes:
chromatin and cytoplasmic condensation, nuclear fragmentation, etc.

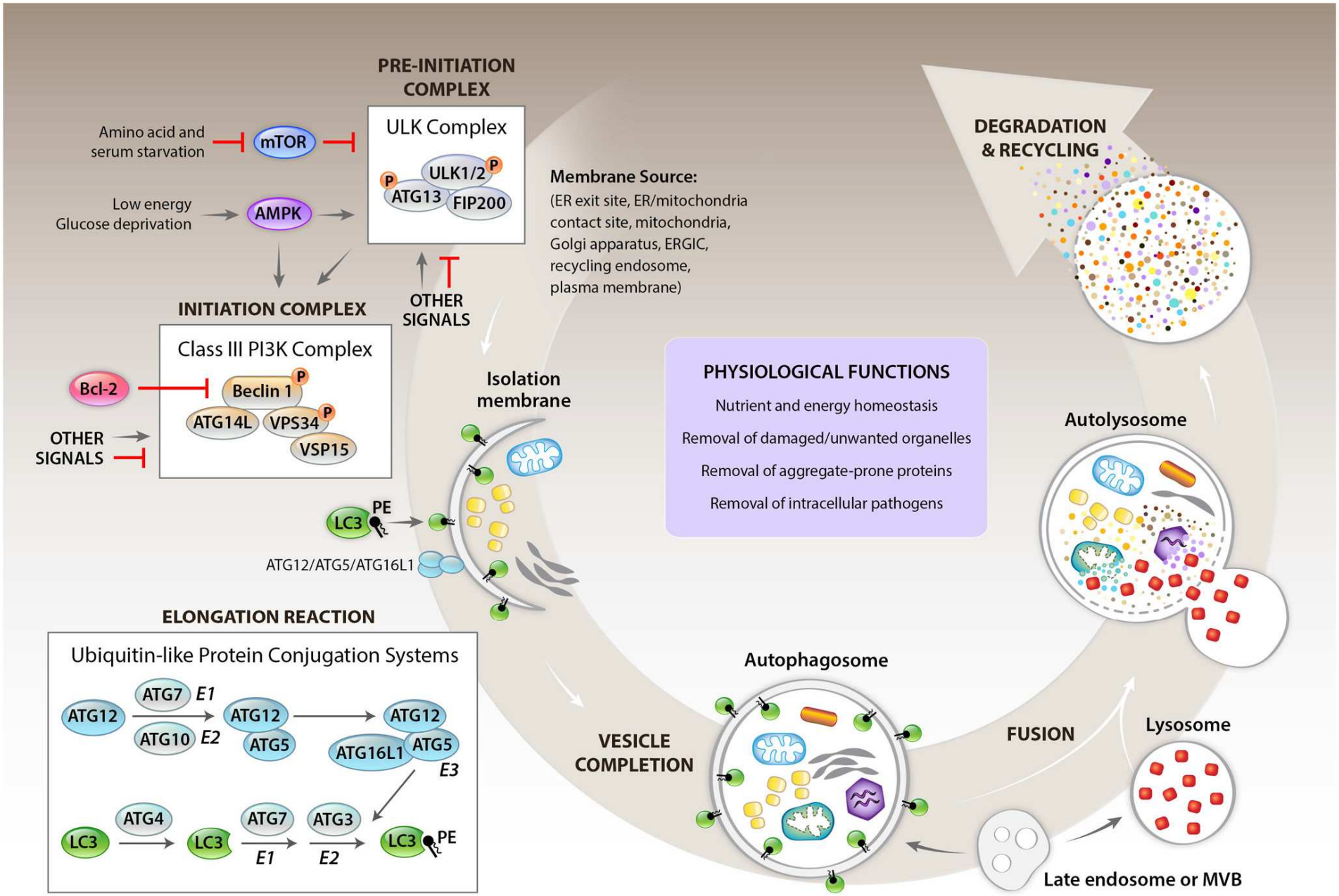
↓

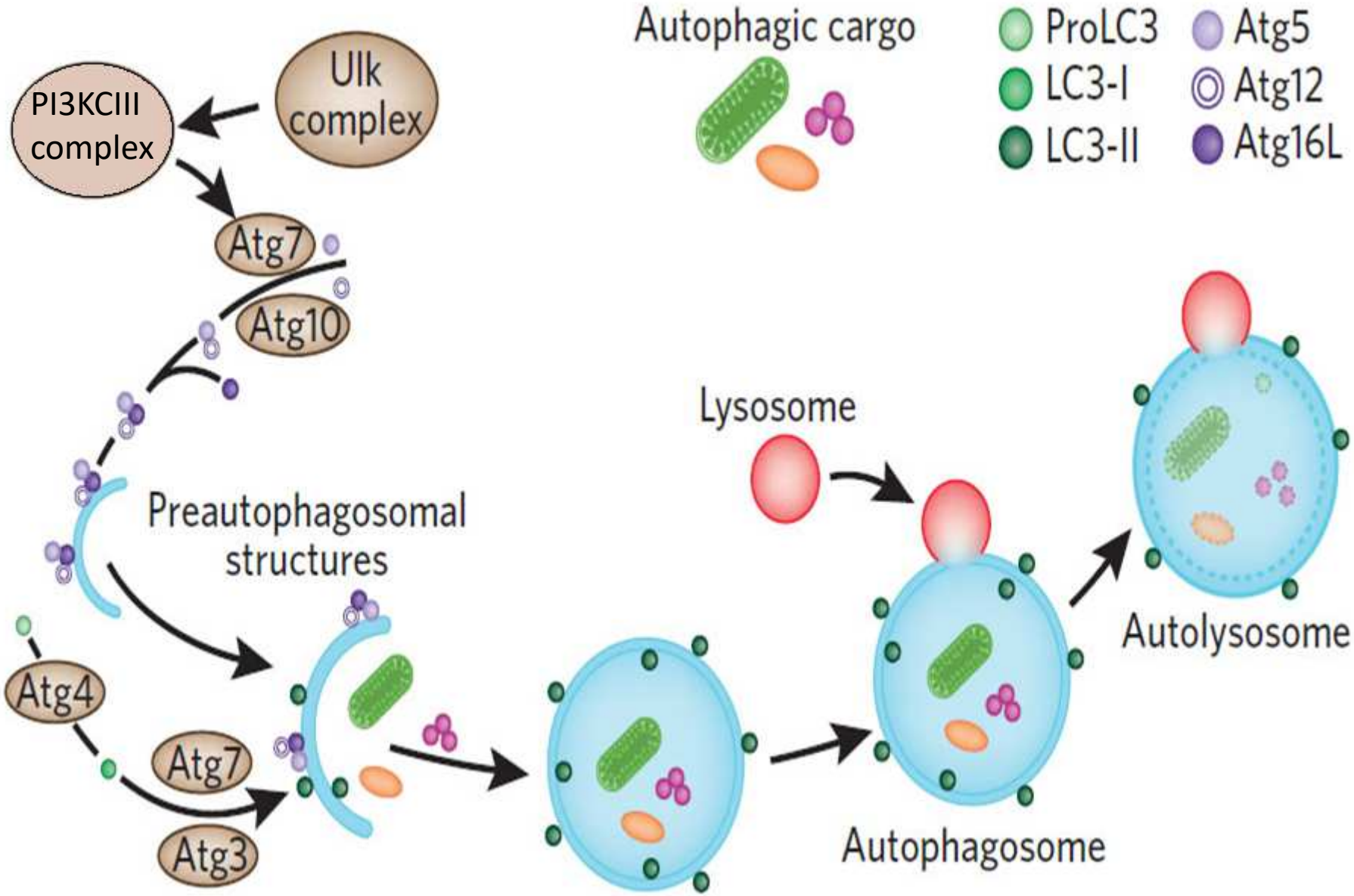
formation of apoptotic bodies


INTRACELLULAR STRESS
IM
IMS
OM
$\Delta\psi_m$
$\Delta\psi_m$
MOMP
BAX
BAK
CYTC
DIABLO
HTRA2
AIF
ENDOG
Pro-caspase-9
APAF1
dATP
Apoptosome
IAPs
Caspase-9
Pro-caspase-3
Caspase-3
Cytoskeleton
ATP SYNTHESIS CESSATION
EXCESS ROS
RESPIRATORY CHAIN INHIBITION
DNA
NUCLEUS
CASPASE-DEPENDENT APOPTOSIS
CASPASE-INDEPENDENT APOPTOSIS


Extrinsic pathway
FasL
Fas
FADD
Procaspase 8
Caspase 8
c-FLIP
Intrinsic pathway
• DNA damage
• Cellular stress
• Growth factor deprivation
tBid
Bax, Bak
Bid
Bcl-2, Bcl-xL
Procaspase 3
Procaspase 9
APAF1
Apoptosome
MOMP
Cytochrome c
Caspase 3
XIAP
Smac
Apoptosis


Cell death
Necrosis
Programmed cell death
Apoptosis
Autophagy
LC3
Atg
Intrinsic
apoptosis
Extrinsic
apoptosis
Caspase 9
cytochrome C
Caspase 8


PRE-INITIATION COMPLEX
Amino acid and serum starvation
mTOR
ULK Complex
ULK1/2
ATG13
FIP200
Low energy Glucose deprivation
AMPK
INITIATION COMPLEX
OTHER SIGNALS
Class III PI3K Complex
Bcl-2
Beclin 1
ATG14L
VPS34
VSP15
OTHER SIGNALS
Membrane Source:
(ER exit site, ER/mitochondria contact site, mitochondria, Golgi apparatus, ERGIC, recycling endosome, plasma membrane)
DEGRADATION & RECYCLING
PHYSIOLOGICAL FUNCTIONS
Nutrient and energy homeostasis
Removal of damaged/unwanted organelles
Removal of aggregate-prone proteins
Removal of intracellular pathogens
Isolation membrane
LC3
PE
ATG12/ATG5/ATG16L1
Autolysosome
ELONGATION REACTION
Ubiquitin-like Protein Conjugation Systems
ATG12
ATG7
E1
ATG10
E2
ATG12
ATG5
ATG12
ATG16L1
ATG5
E3
LC3
ATG4
LC3
ATG7
E1
ATG3
E2
LC3
PE
Autophagosome
VESICLE COMPLETION
FUSION
Lysosome
Late endosome or MVB


Autophagic cargo
ProLC3
LC3-I
LC3-II
Atg5
Atg12
Atg16L
PI3KCIII complex
Ulk complex
Atg7
Atg10
Preautophagosomal structures
Lysosome
Autolysosome
Atg4
Atg7
Atg3
Autophagosome

	Main biochemical features	Examples of inhibitory interventions[a]
Autophagic cell death	MAP1LC3 lipidation	AMBRA1, ATG5, ATG7, ATG12 or BCN1 genetic inhibition
Caspase-dependent intrinsic apoptosis	MOMP Irreversible $\Delta\psi_m$ dissipation	BCL-2 overexpression Z-VAD-fmk administration
Caspase-independent intrinsic apoptosis	Release of IMS proteins Respiratory chain inhibition	BCL-2 overexpression
Extrinsic apoptosis by death receptors	Death receptor signaling Caspase-8 (-10) activation BID cleavage and MOMP (in type II cells) Caspase-3 (-6,-7) activation	Genetic inhibition of caspases (8 and 3) Z-VAD-fmk administration

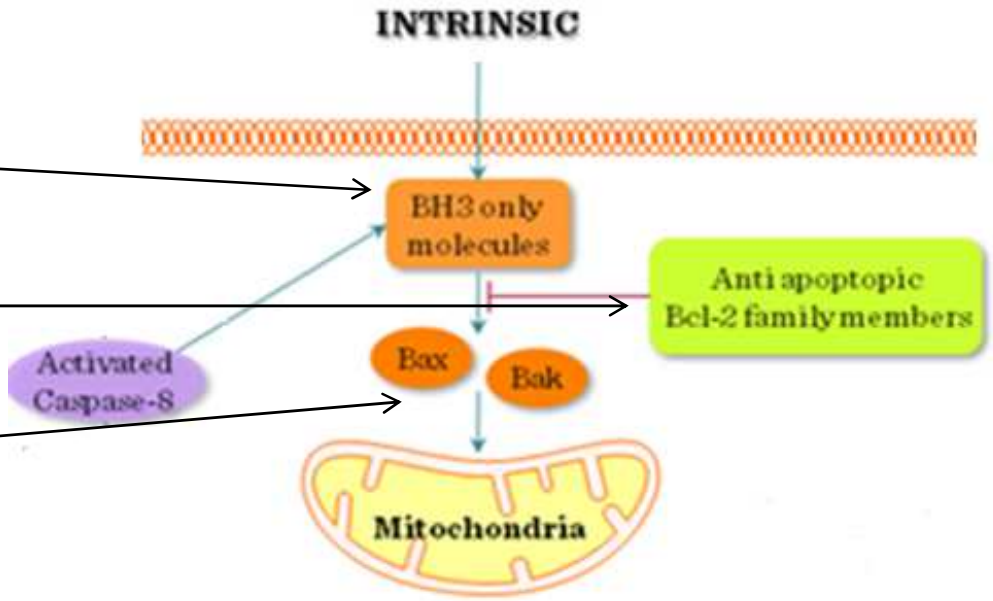
Initiators: BH3-only proteins
(BIM, PUMA, BAD, NOXA, BIK, HRK, BMF and tBID)

Guardians: multi-domain pro-survival proteins
(BCL-2, BCL-X_L, BCL-W, MCL1, A1 and BCL-B)

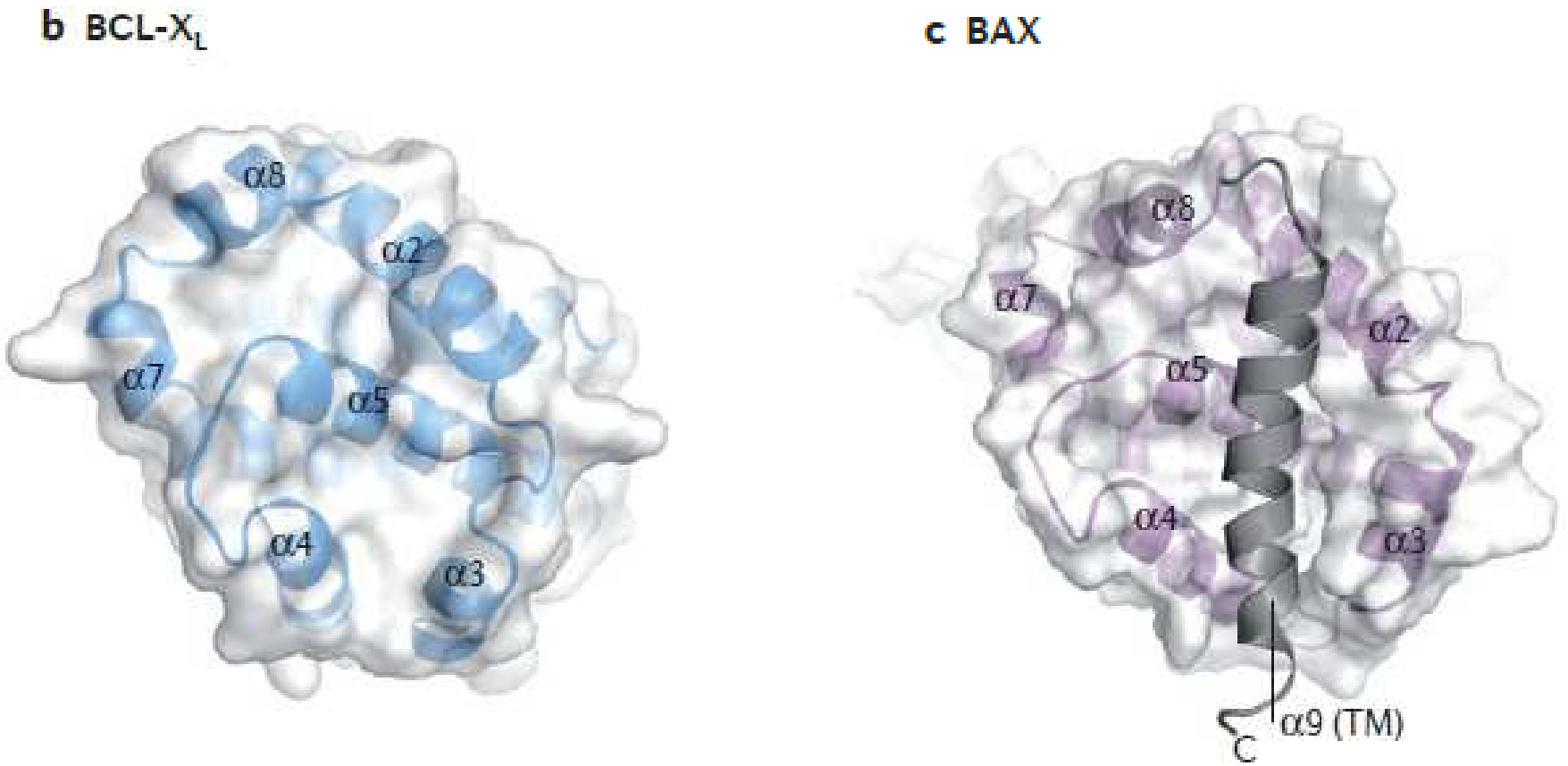
Effectors: multi-domain pro-apoptotic proteins
(BAX, BAK and BOK)

The Bcl-2 family

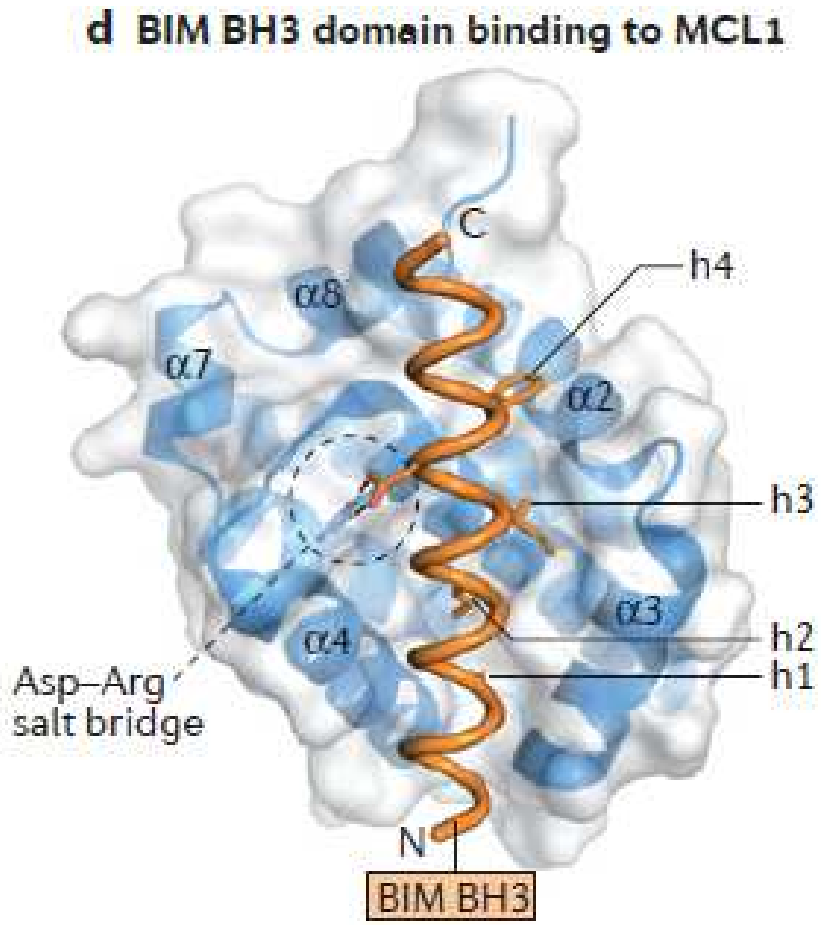
- Attivatori
- Anti-apoptotici
- Pro-apoptotici

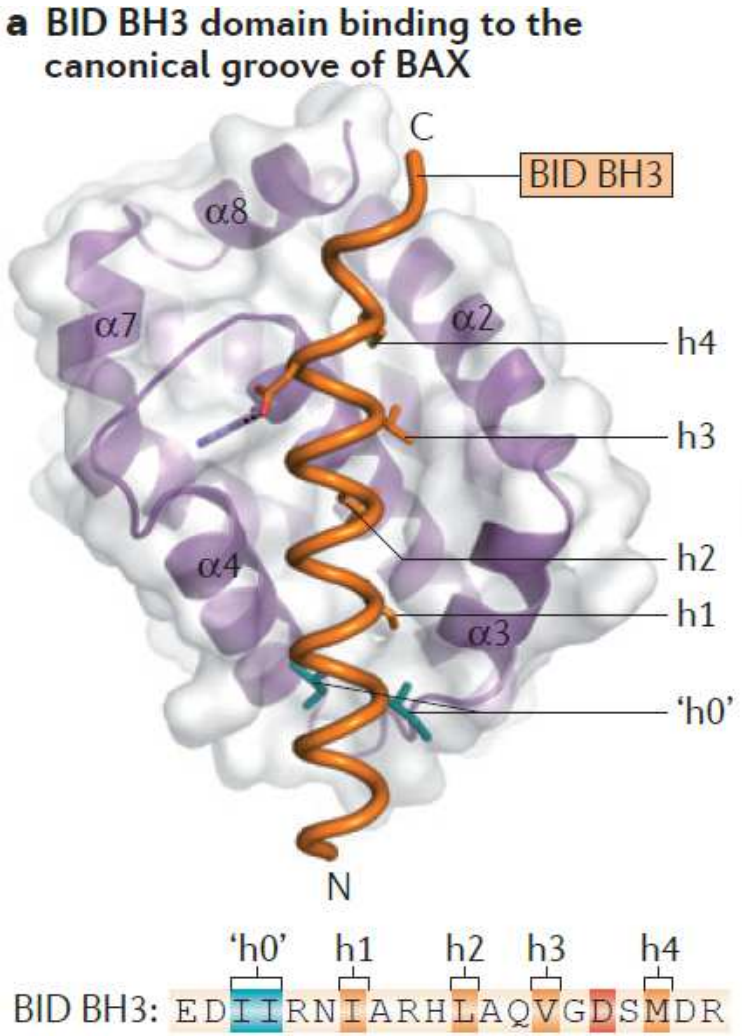


Globular structure with a central BH3-binding hydrophobic groove



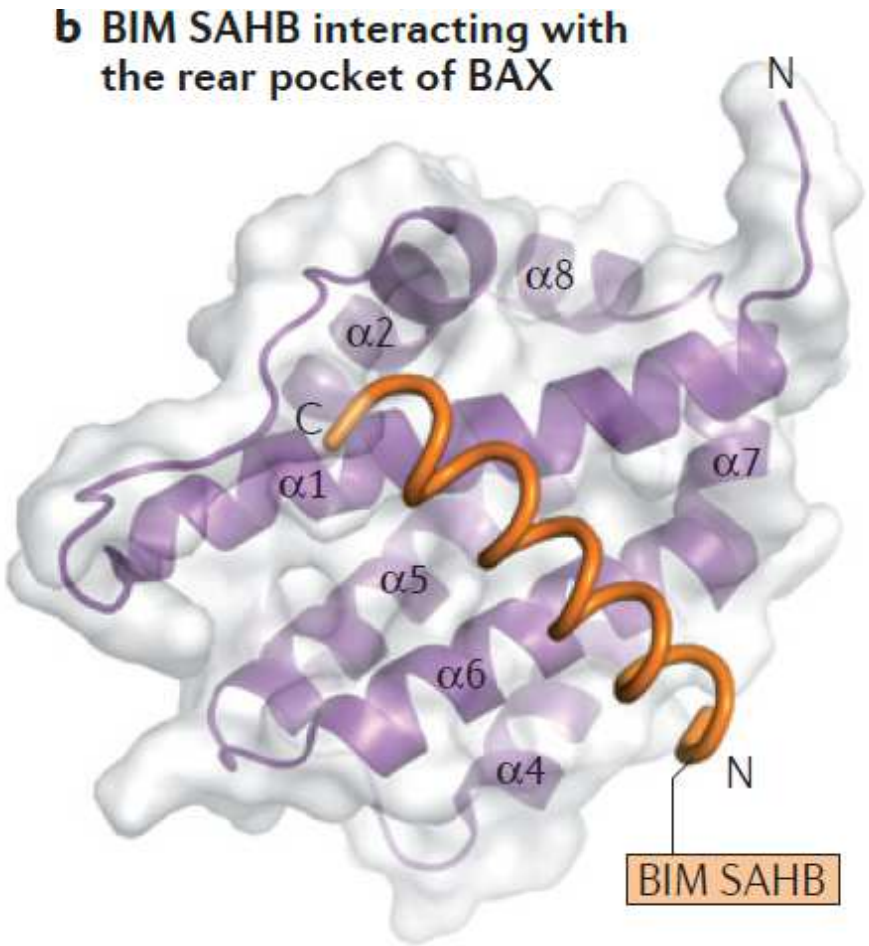
- Amphipathic helix
- Activators conformational disorder(and the Bid exception)
- Hydrophobic residues(h0→h4) and salt bridge

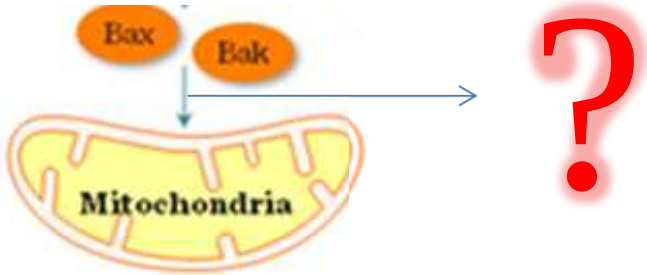




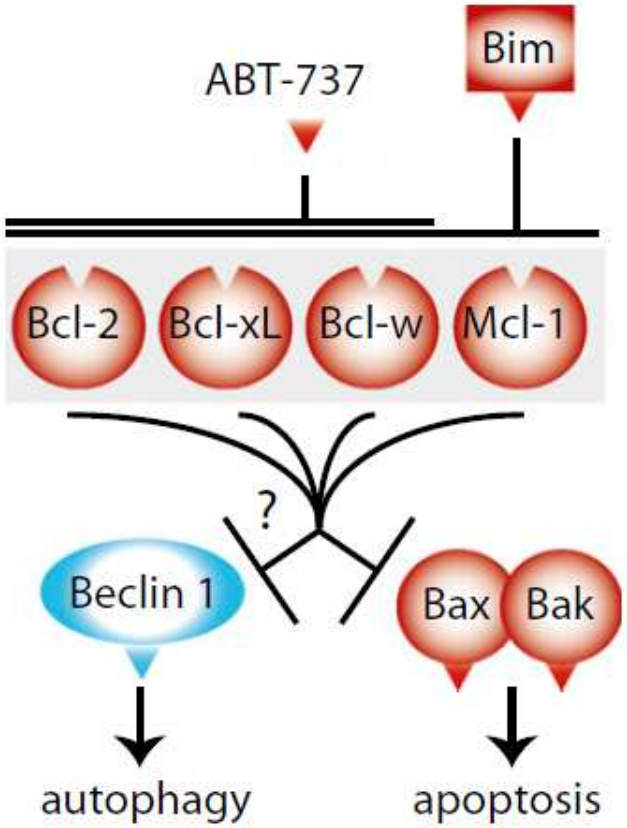
Second activation site for Bax

Alpha 9 extrusion trigger

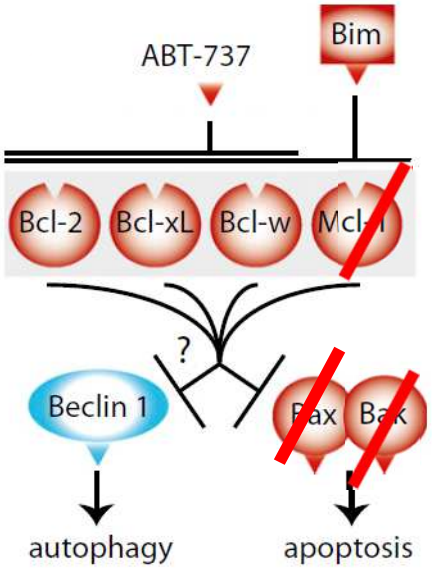




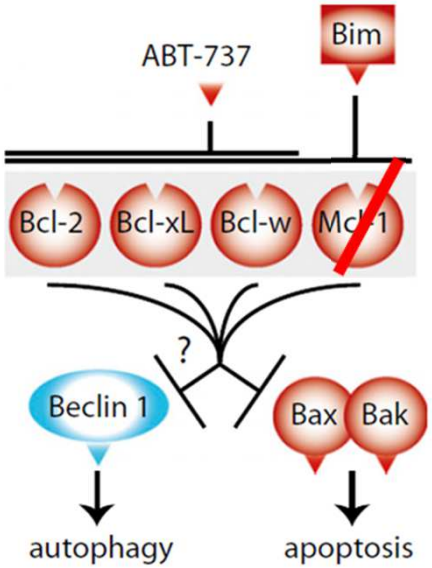
It is widely accepted that Bcl-2 family members not only inhibit apoptosis but also negatively regulate autophagy by binding to Beclin 1



Lisa M. Lindqvist et al.,2014 PNAS



Bax-/-Bak-/-Mcl-1-/-



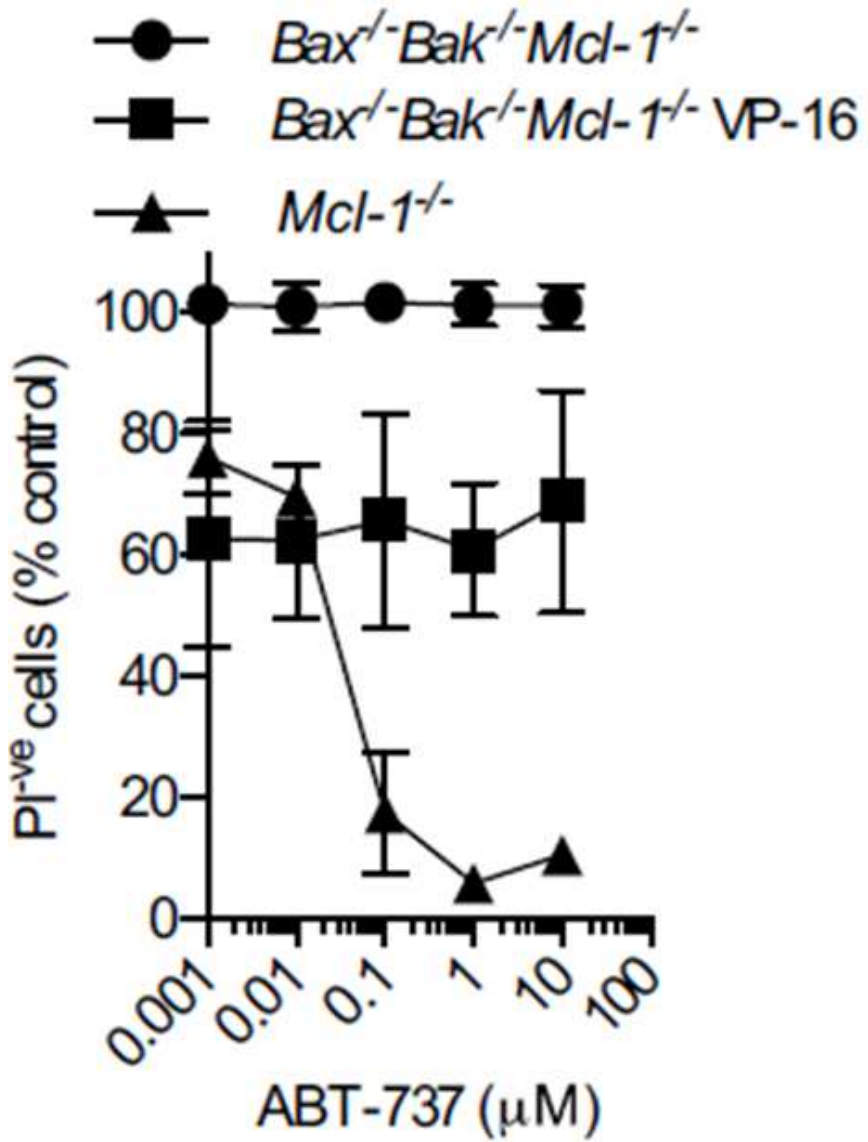
Bax-/-Bak-/-Mcl-1-/- VP-16

VP-16 chemotherapeutic agent etoposide, inducer of autophagy

Mcl-1-/-

Lisa M. Lindqvist et al.,2014 PNAS

Q: Does inhibiting anti-apoptotic Bcl-2 members interfere with non-apoptotic death?



Beclin 1, formation of a complex with the mammalian PI3K Vps34
and nucleation of the autophagosome membrane

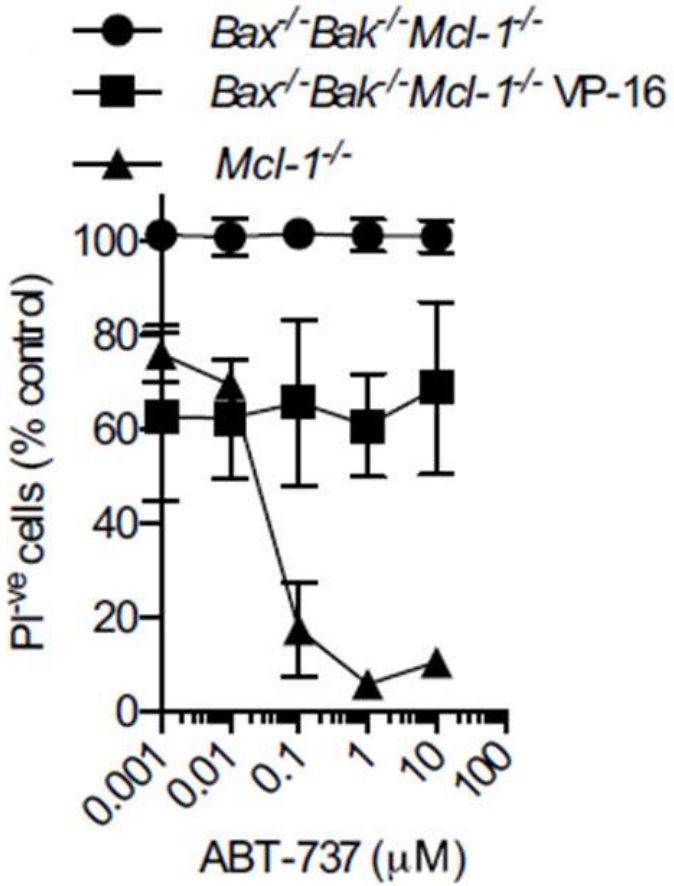
When nutrients are abundant, Bcl-2 and the related proteins Bcl-xL and Mcl-1 bind to the Beclin 1's BH3 domain and thereby inhibit induction of autophagy

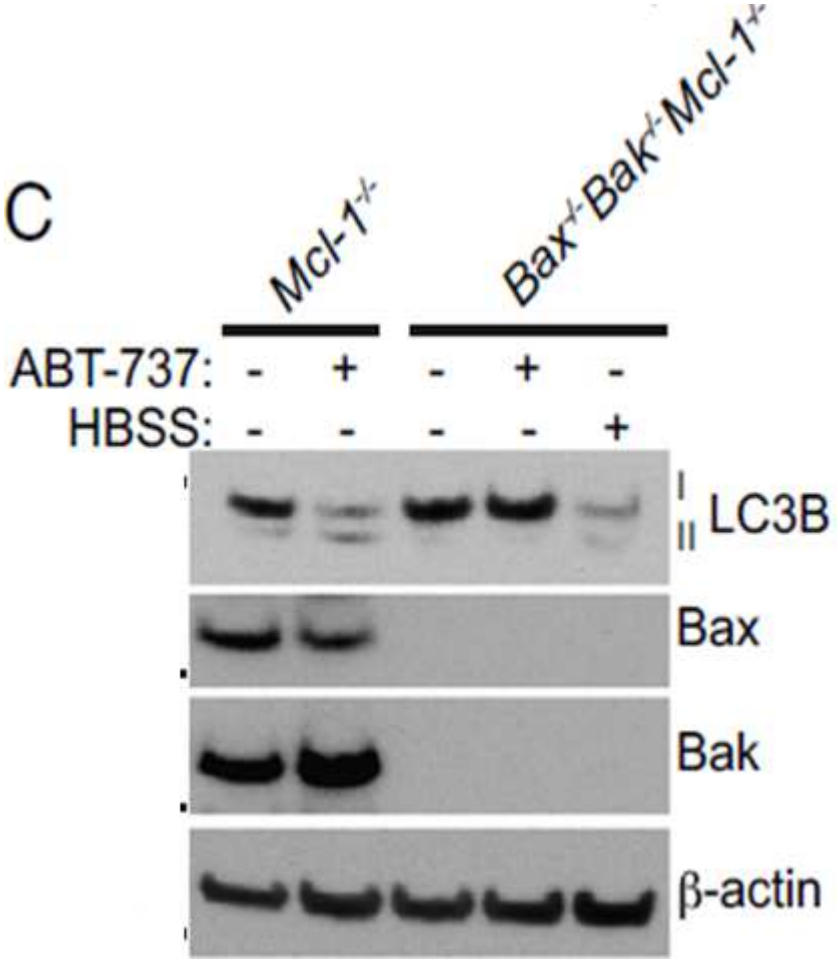
When nutrients are scarce, Bcl-2 is phosphorylated by JNK1, which prevents its binding to Beclin 1 and allows it to initiate formation of autophagosomes

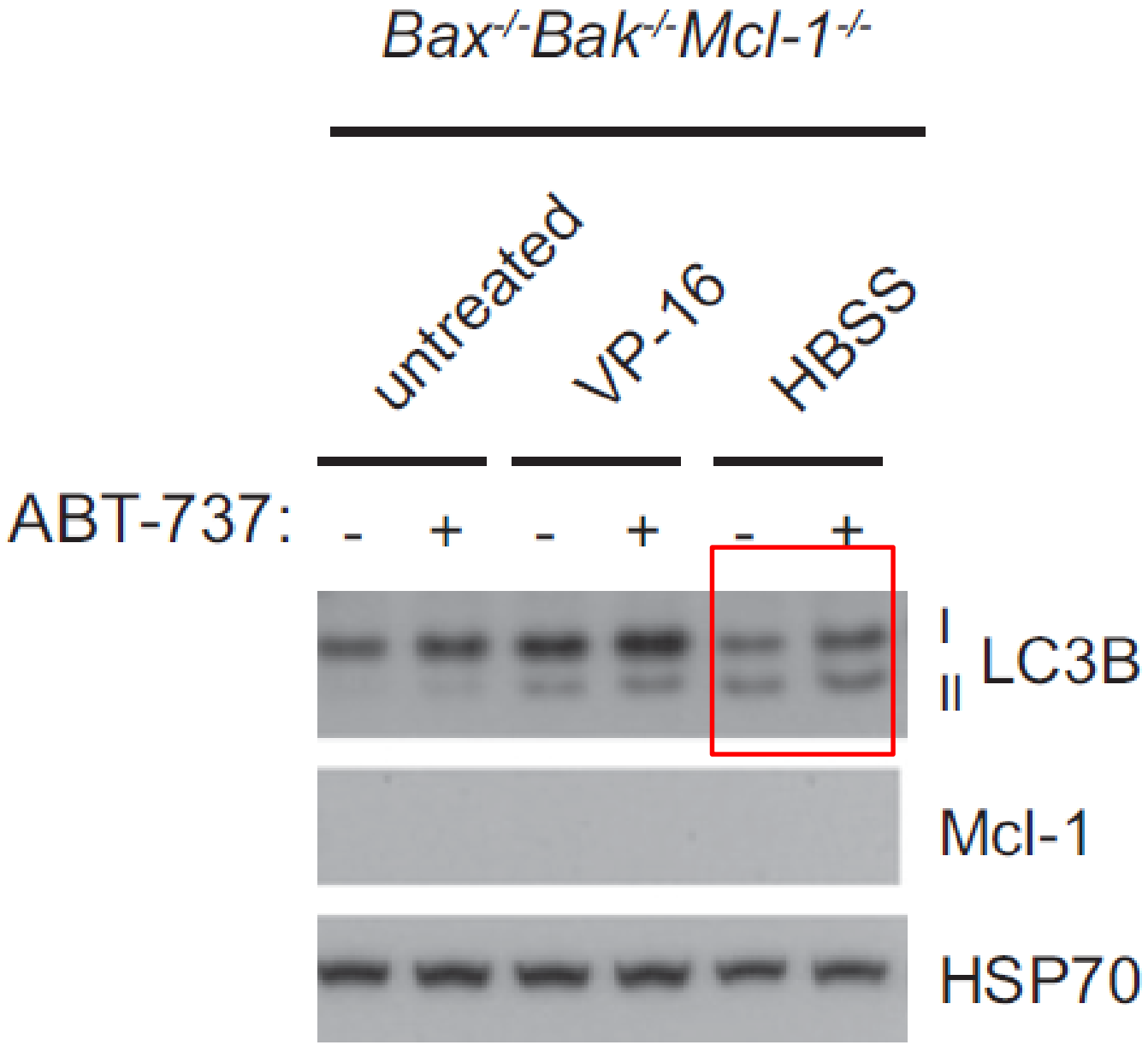
BH3 mimetic ABT-737

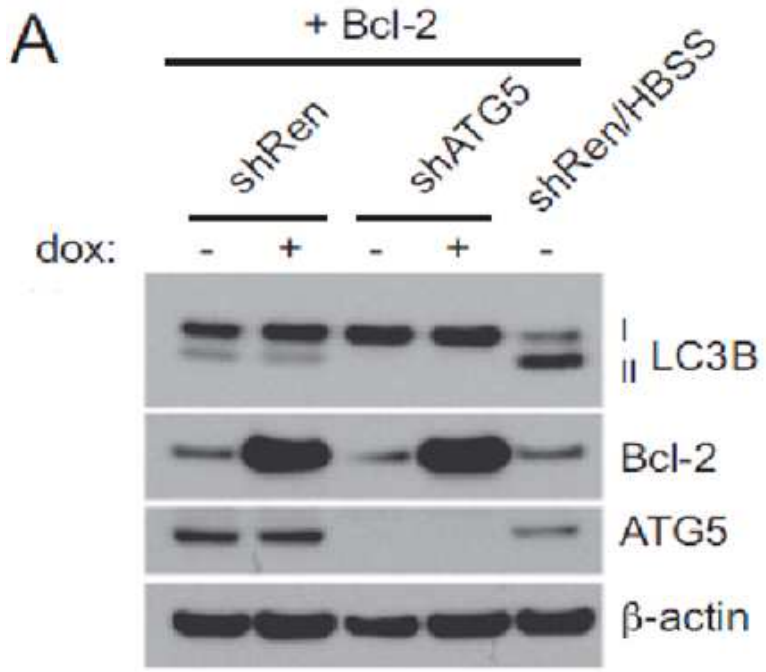
VP-16 chemotherapeutic agent etoposide, inducer of autophagy, including the () or nutrient starvation by culturing in HBSS, potent inducer of apoptosis

Q: Does it affect autophagy?

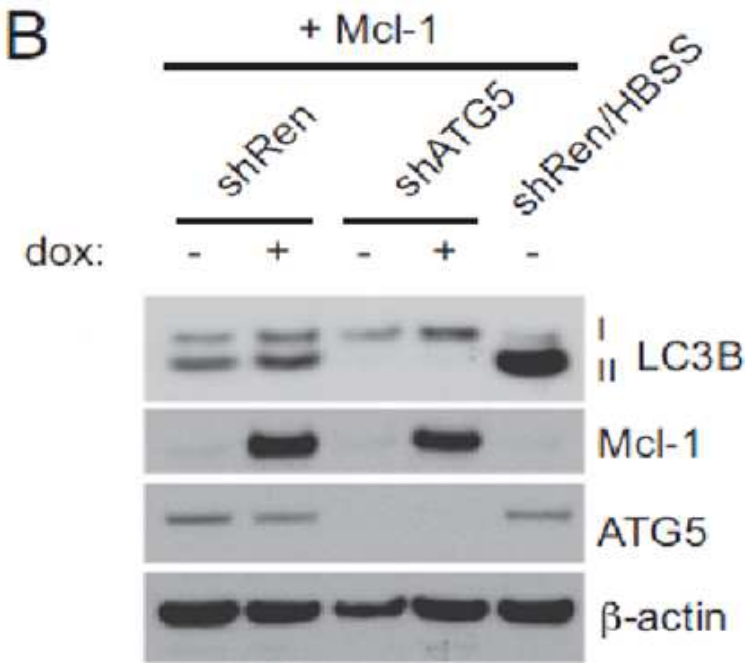


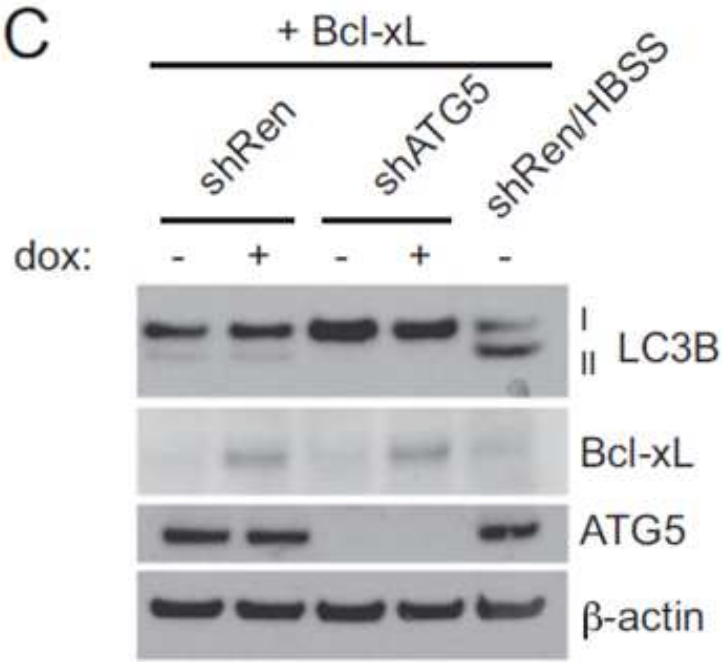


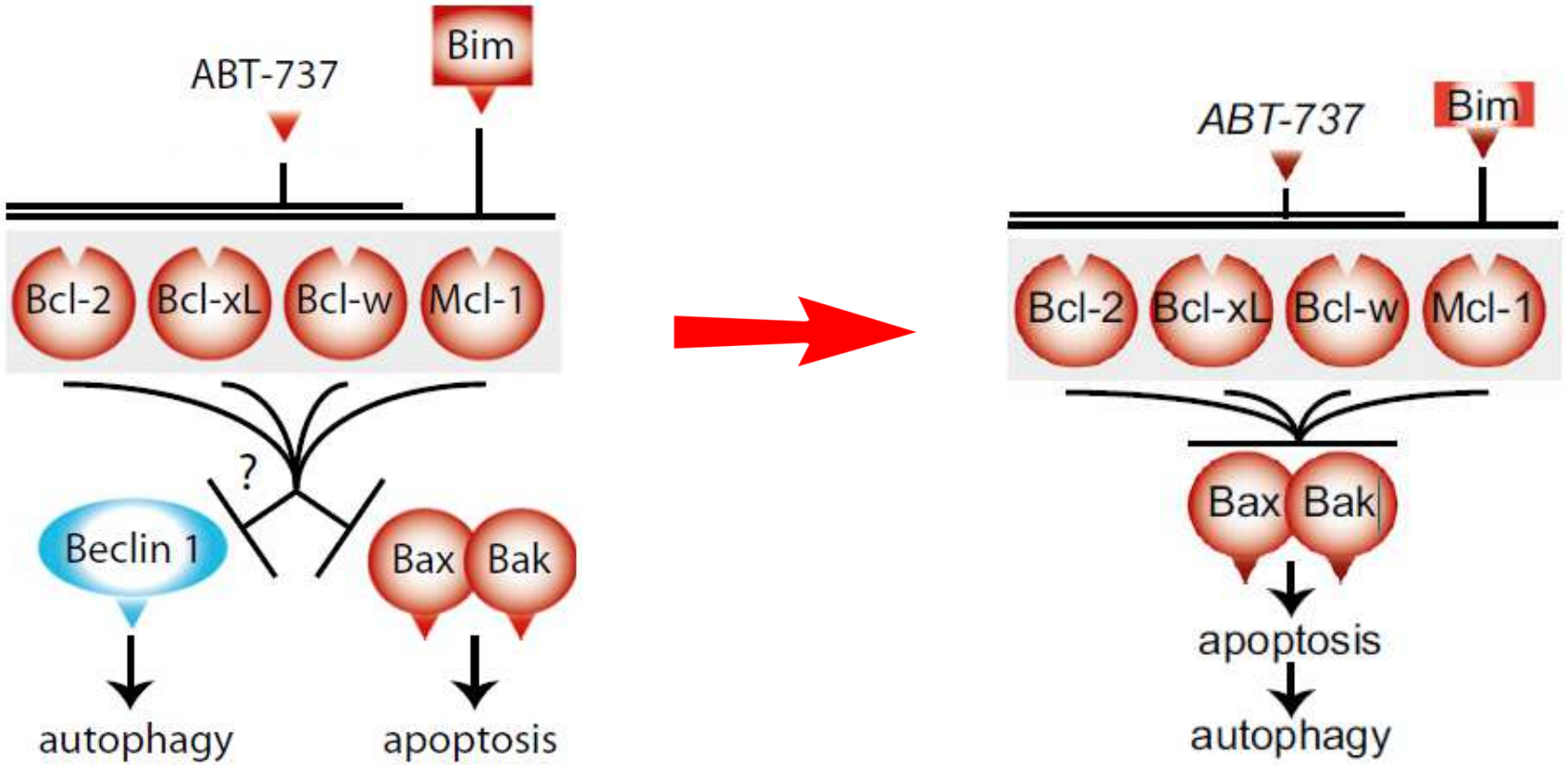

Bax-/-Bak-/-Mcl-1-/-
untreated
VP-16
HBSS
ABT-737: - + - + - +
I
II
LC3B
Mcl-1
HSP70



Q: How about overexpressing them?







the effects of Bcl-2 on autophagy are instead an indirect consequence of its inhibition of apoptosis mediators Bax and Bak.

- None of the prosurvival Bcl-2 family members bind to Beclin-1 under physiological circumstances or they do not significantly inhibit its function

- In conclusion, the data demonstrate that the prosurvival Bcl-2 family of proteins does not directly regulate autophagy, but any impact they have on autophagy is indirect, via Bax and Bak activation

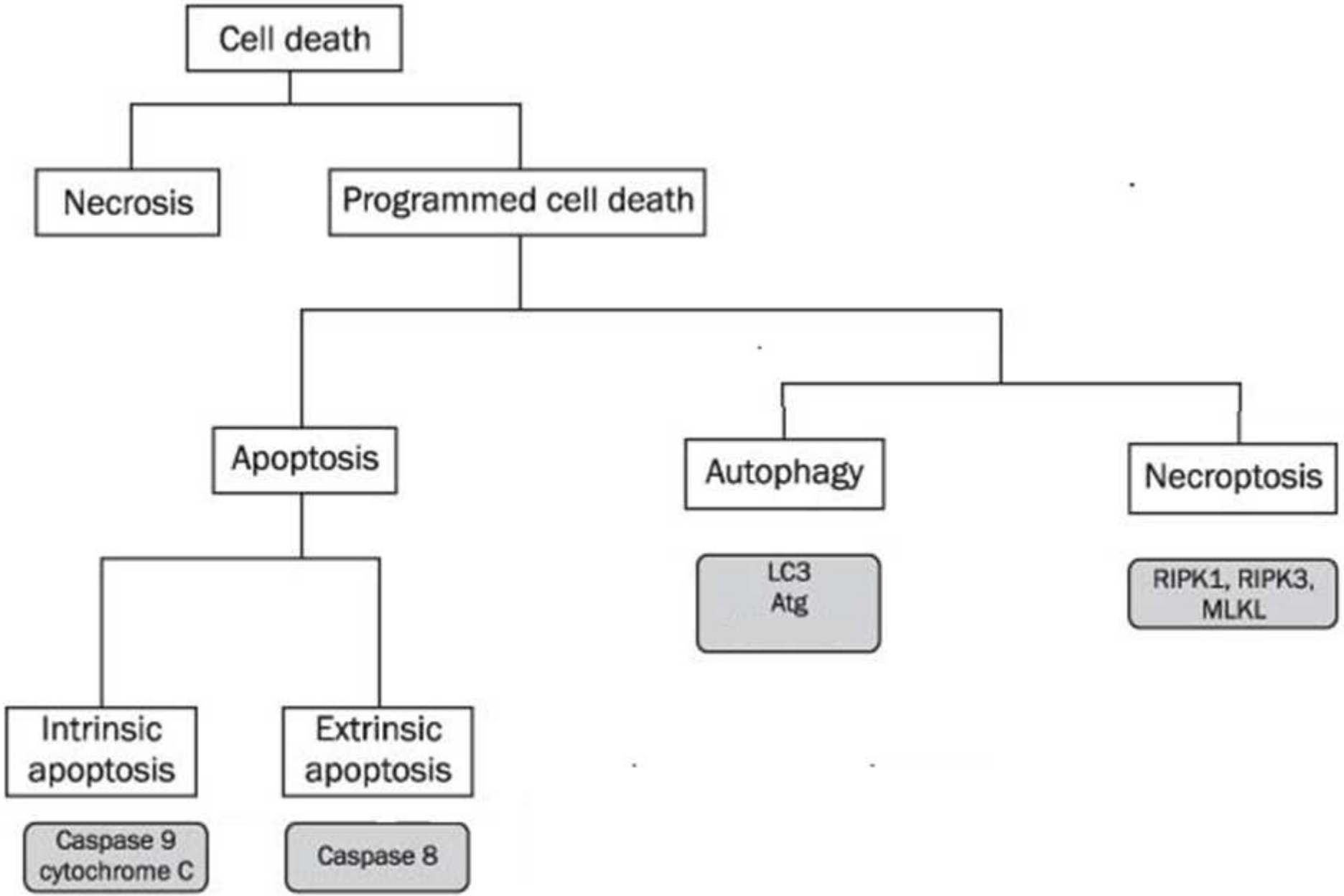
It is widely accepted that Bcl-2 not only inhibits apoptosis but also negatively regulates autophagy by binding to Beclin 1.

We provide genetic and biochemical evidence that the effects of Bcl-2 on autophagy are instead an indirect consequence of its inhibition of apoptosis mediators Bax and Bak.

We show that in the absence of Bax and Bak, antagonizing or altering the levels of Bcl-2 has no detectable impact on autophagy.

Because several inhibitors of both autophagy and Bcl-2 are in clinical trials for the treatment of cancer, it is important to understand the cross-talk between these pathways.

END


Cell death
Necrosis
Programmed cell death
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Necroptosis
LC3
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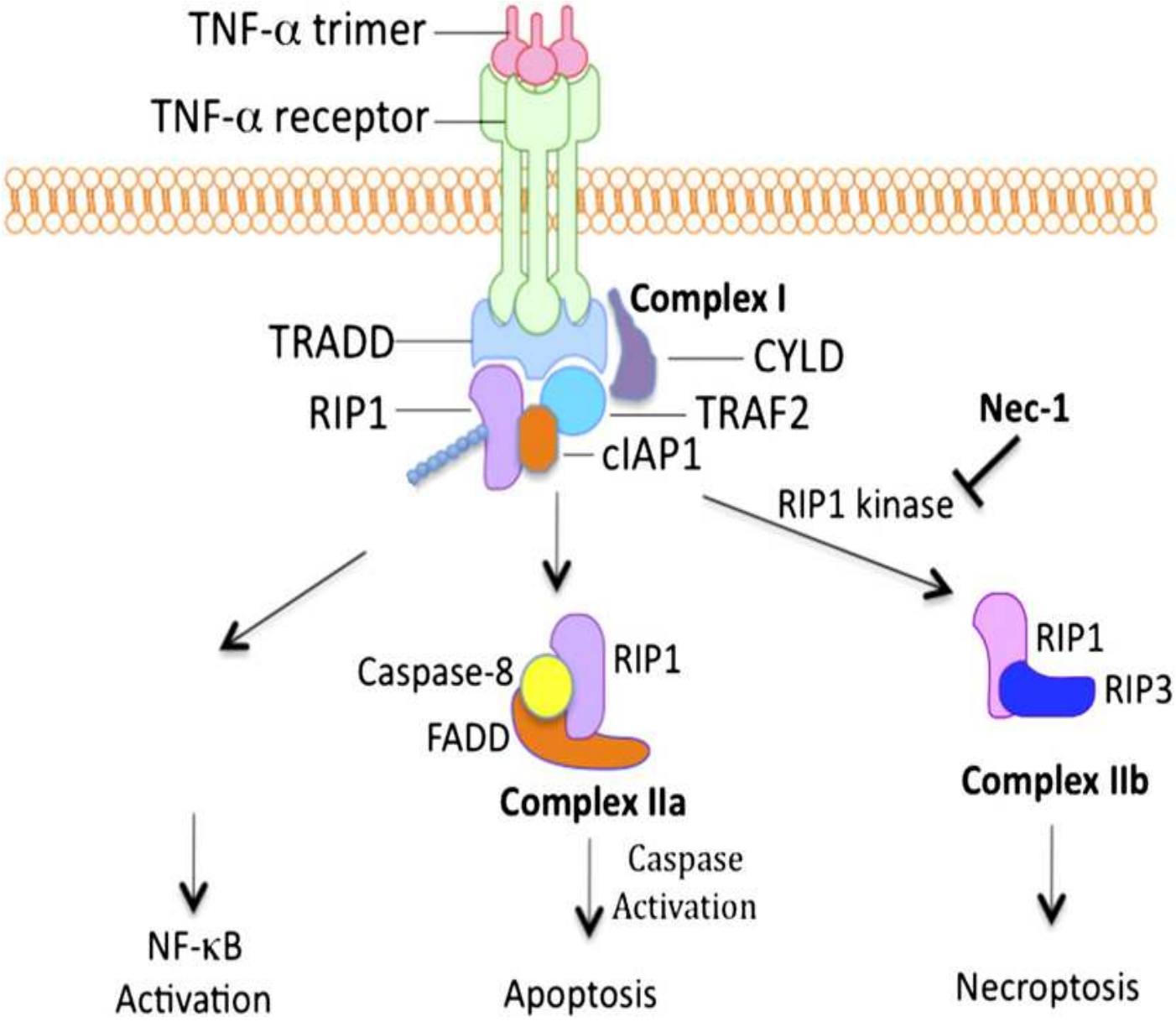
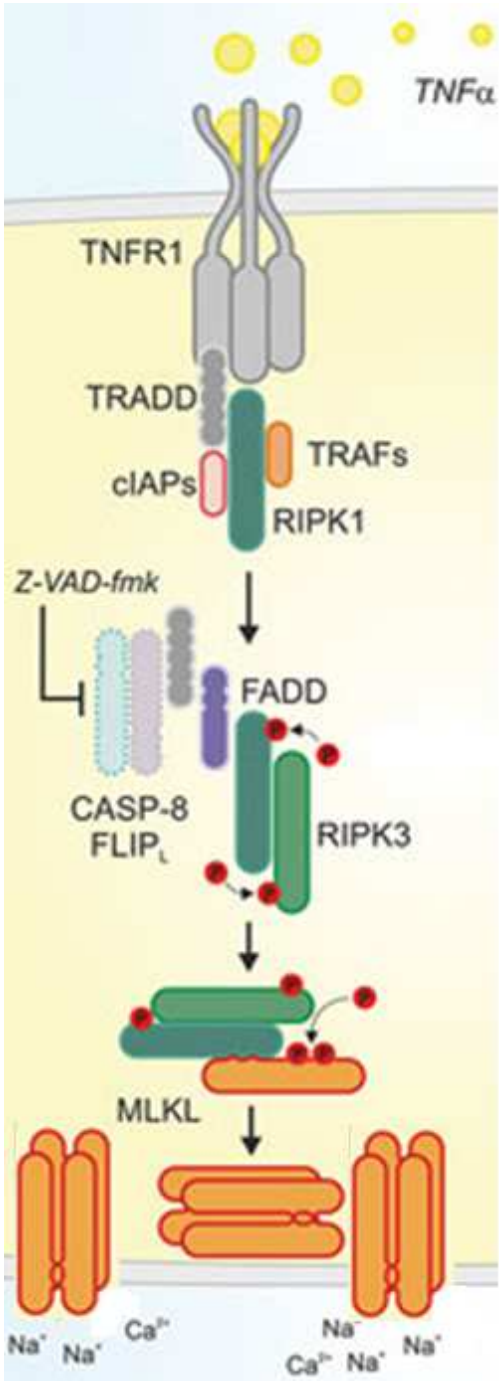
RIPK1, RIPK3,
MLKL
Intrinsic
apoptosis
Extrinsic
apoptosis
Caspase 9
cytochrome C
Caspase 8

NECROPTOSIS





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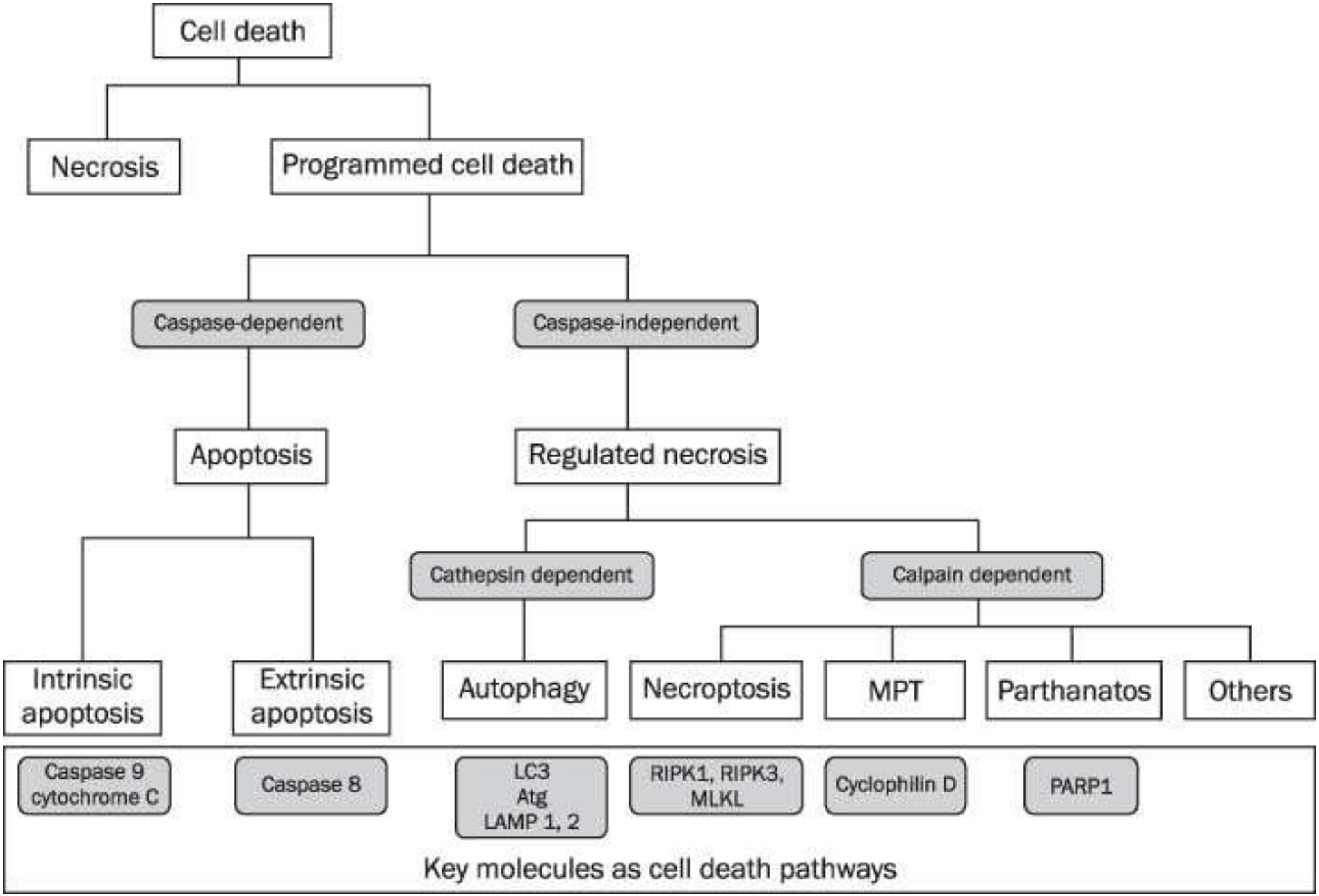
Extrinsic apoptosis by death receptors	Death receptor signaling Caspase-8 (-10) activation BID cleavage and MOMP (in type II cells) Caspase-3 (-6,-7) activation	Genetic inhibition of caspases (8 and 3) Z-VAD-fmk administration
Necroptosis	Death receptor signaling Caspase inhibition RIP1 and/or RIP3 activation	Administration of necrostatin(s) Genetic inhibition of RIP1/RIP3


Cell death
Necrosis
Programmed cell death
Caspase-dependent
Caspase-independent
Apoptosis
Regulated necrosis
Cathepsin dependent
Calpain dependent
Intrinsic apoptosis
Extrinsic apoptosis
Autophagy
Necroptosis
MPT
Parthanatos
Others
Caspase 9 cytochrome C
Caspase 8
LC3 Atg LAMP 1, 2
RIPK1, RIPK3, MLKL
Cyclophilin D
PARP1
Key molecules as cell death pathways