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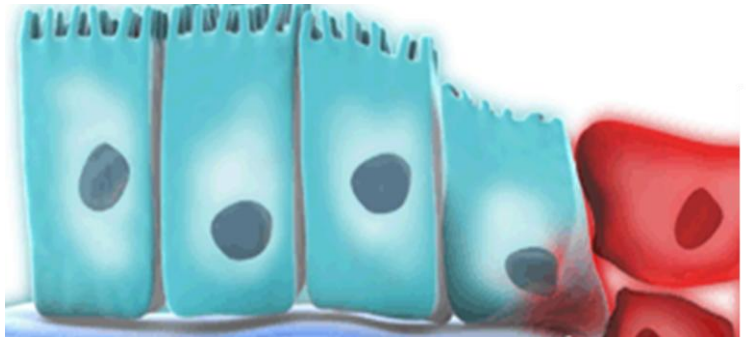
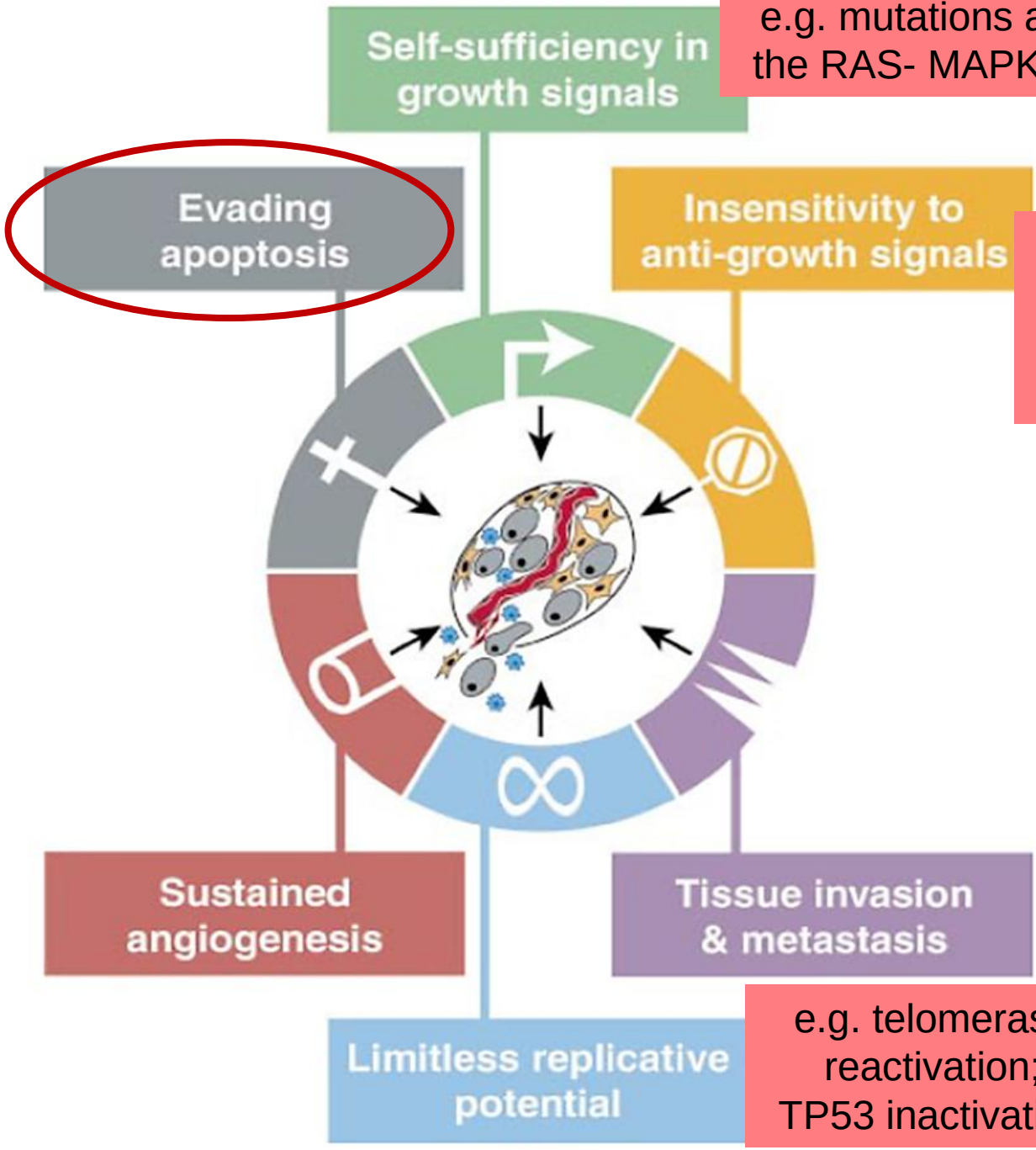


Oncobiology

Margarida Gama-Carvalho (DQB/FCUL) and Peter Jordan (INSA)

Apoptosis

The Hallmarks of Cancer
Cell 100, 57–70 (2000)
Hanahan & Weinberg



e.g. mutations activating the RAS- MAPK pathway

e.g. mutations inactivating the TGFβ-Smad pathway

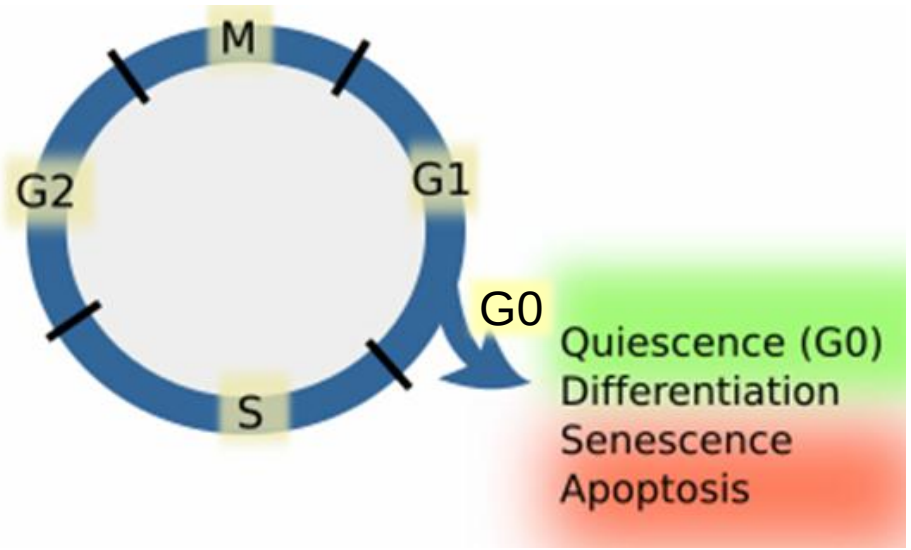
e.g. telomerase reactivation; TP53 inactivation

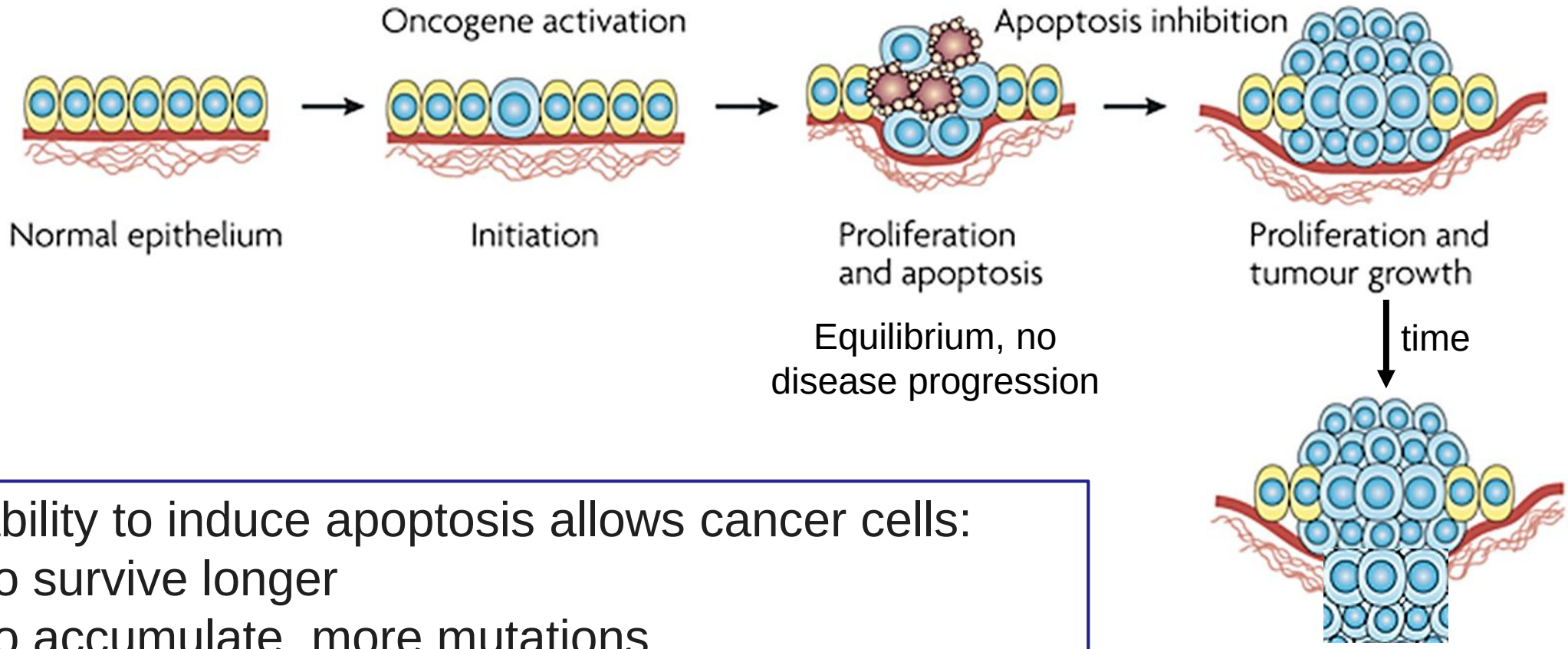
Defining Cancer *(see lecture 1)*

- Cancer is a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body.
- Cancer is caused by changes to genes that control the way our cells grow and divide.

tumour growth = Σ

- proliferation ↑
- differentiation ↓
- cell death ↓





Inability to induce apoptosis allows cancer cells:
- to survive longer
- to accumulate more mutations
- be resistant to certain chemotherapy agents

DOI:10.1038/nrc2254

Apoptosis
is a highly regulated
cellular programe
that leads to a controlled cell suicide

- Response to cellular stress or excessive genome damage
- Response to a virus infection in the cell
- Elimination of cells during embryogenic morphogenesis
- Elimination of self-reactive T cells
- ...

<u>concerning cancer:</u>

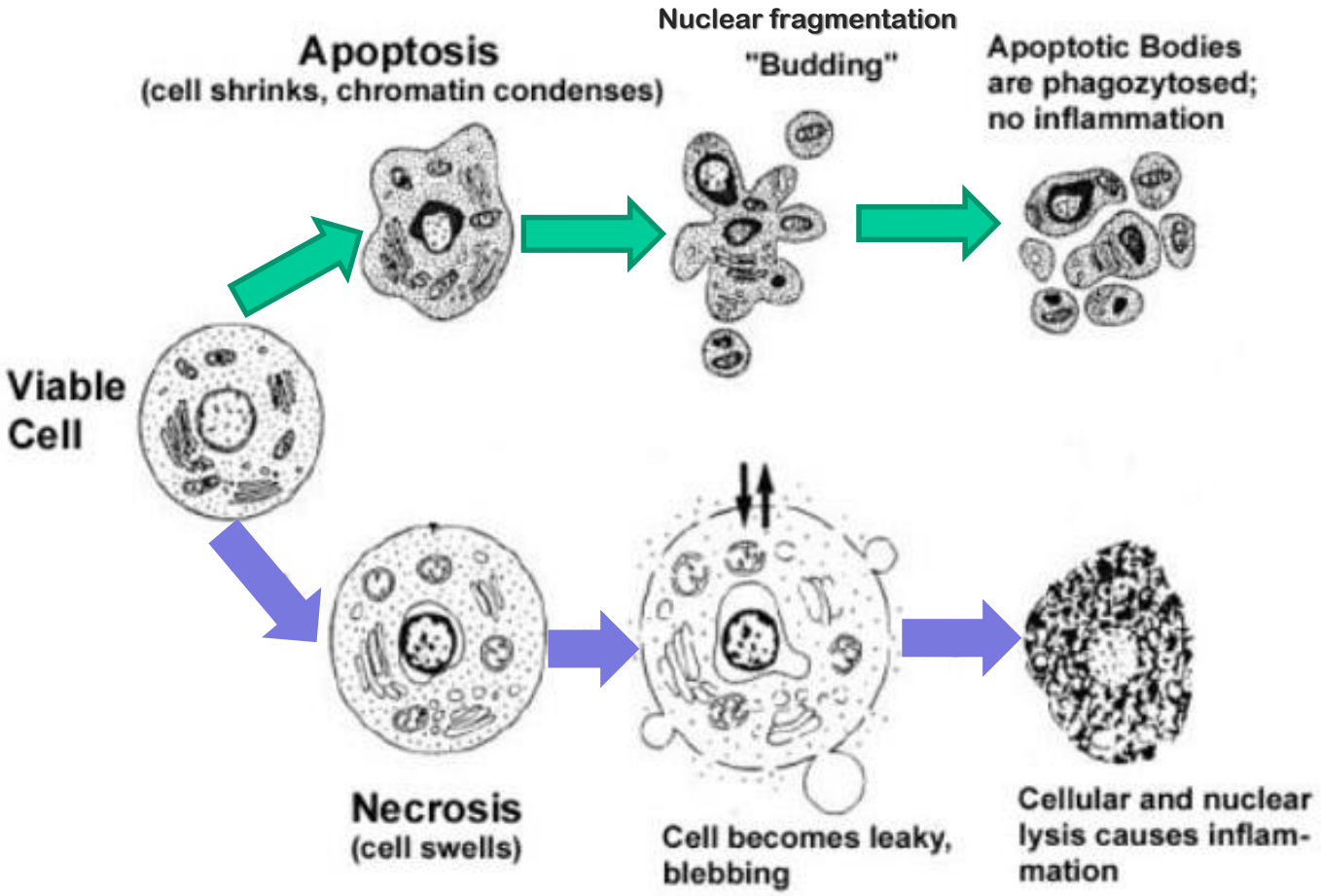
- Response to conflicting cell stimuli (*initiated tumour cells*)

In multicellular organisms, cell death is a critical and regulated process to maintain tissue homeostasis or eliminate potentially harmful cells.

Three major types of cell death programs exist:

- **Apoptosis,**
- **Autophagic cell death,**
- **Necrosis**

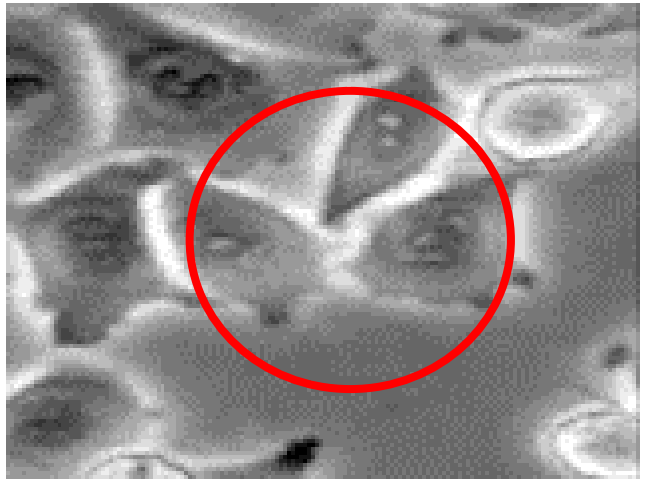
Morphologic distinction between apoptosis and necrosis



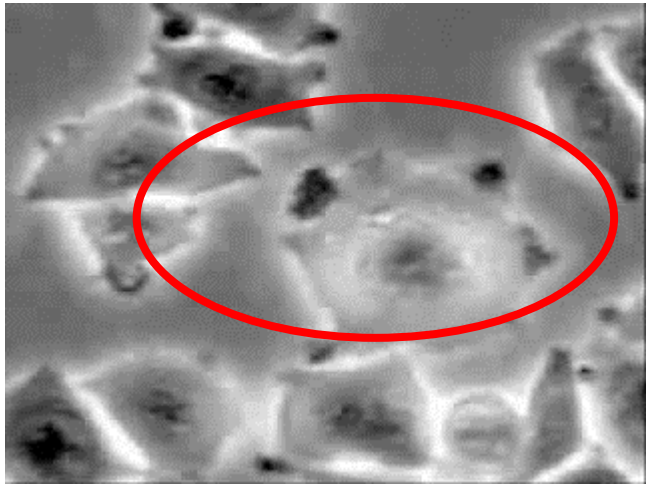
Apoptosis:
Cell shrinkage, cleavage of specific proteins, fragmentation of nuclei with degradation of genomic DNA, apoptotic vesicle formation, surface exposure of phosphatidylserine (PS)

Necrosis:
Cell swelling due to abnormally increased membrane permeability, followed by cell rupture and release of cellular contents

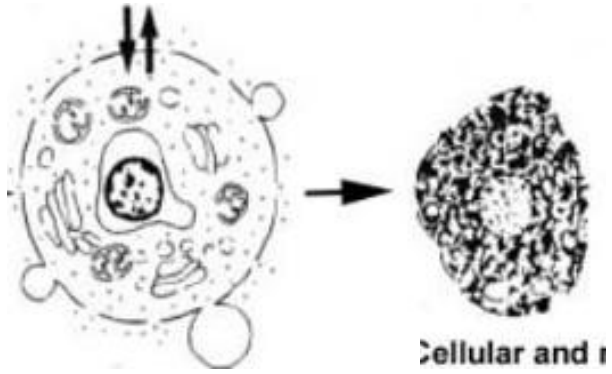
Apoptopedia: http://www.celldeath.de/encyclo/index.html



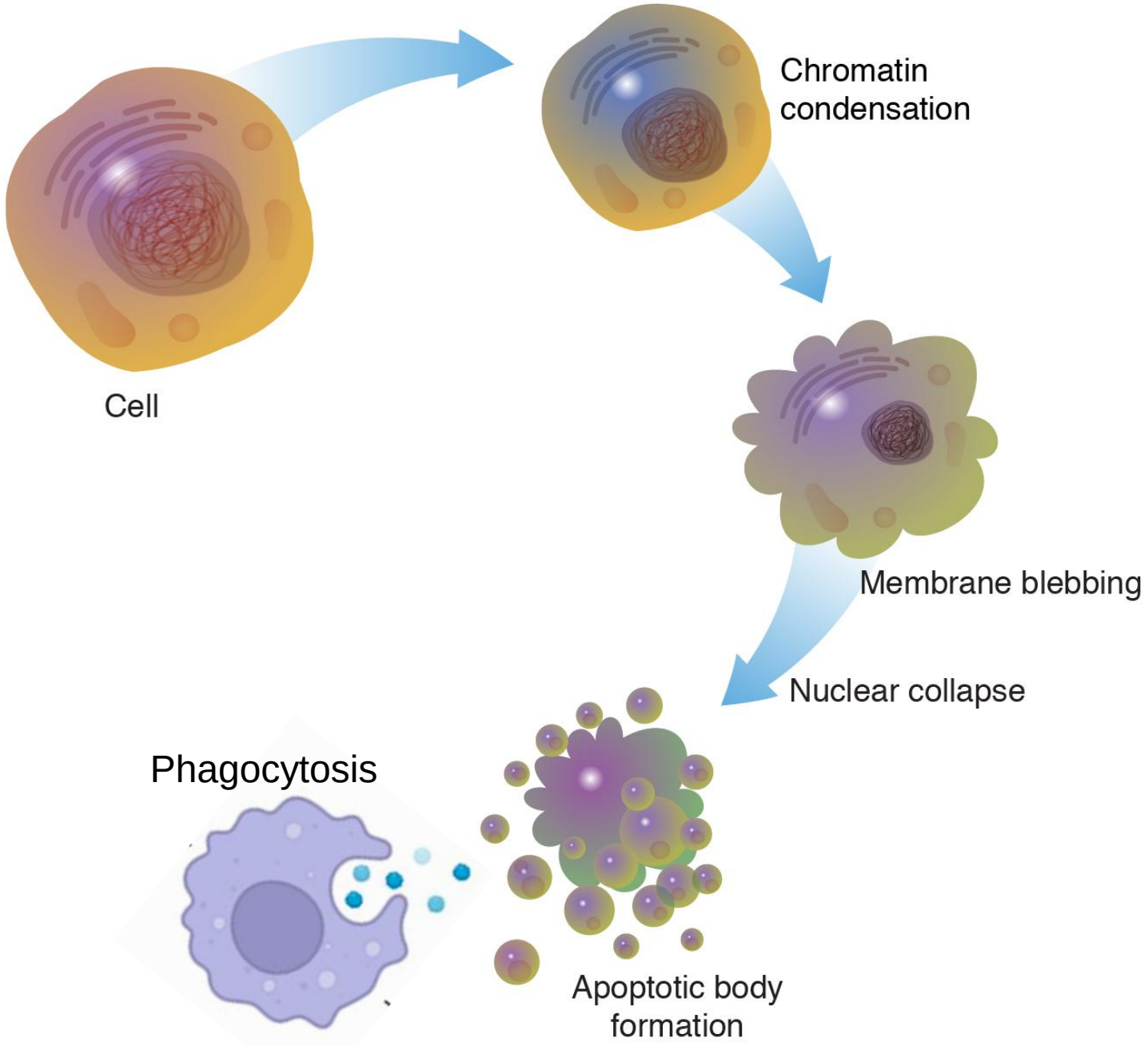
Apoptosis



Necrosis



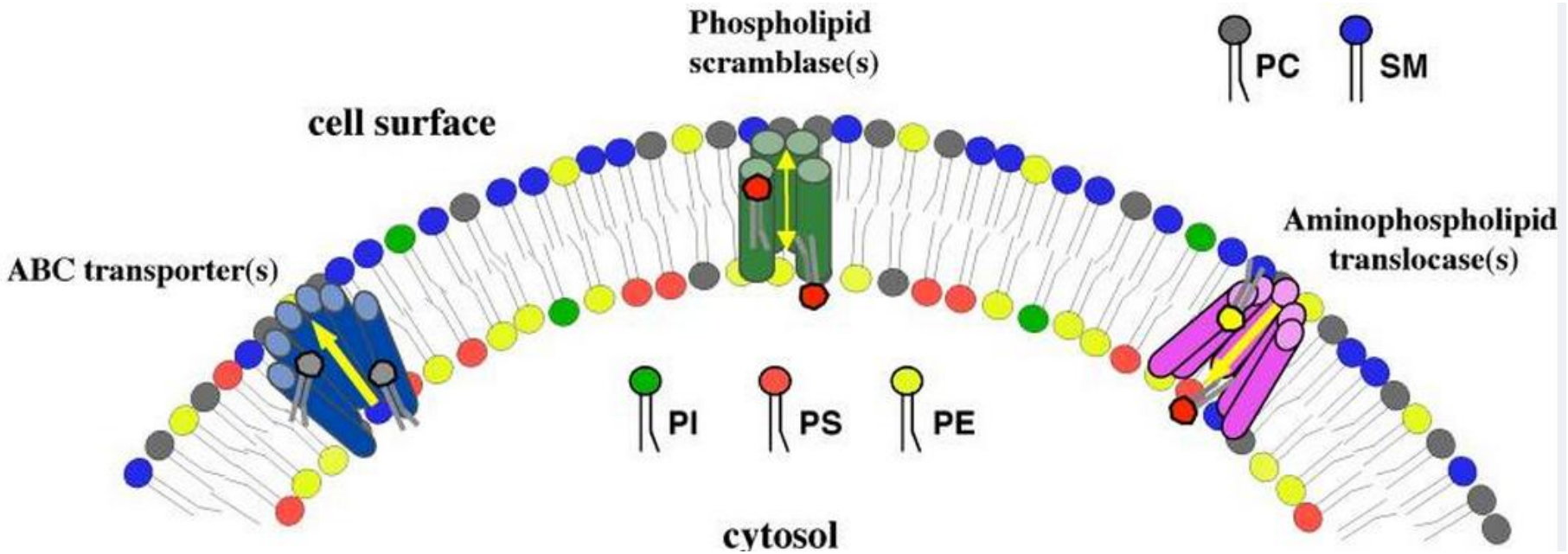
Videos de Igor Roninson, Ordway Research Institute, Albany NY



Visible steps during the apoptosis programe

Check out complementary vídeo no Moodle!

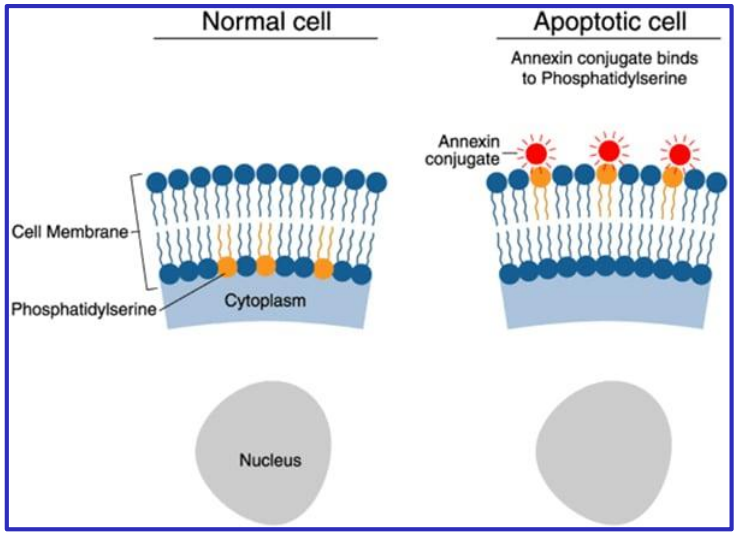
Plasma membrane lipids show an assymetric distribution



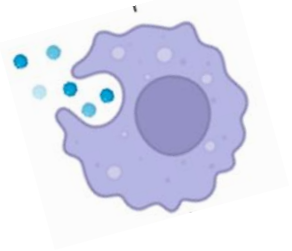
- PI generates second messengers (PLC: IP3 + DAG; PI3K: PIP3)

- PS at outer leaflet marks apoptotic cells;

used in annexin-based assays to detect or quantify the amount of apoptotic cells



recognition to be phagocytosed by macrophages



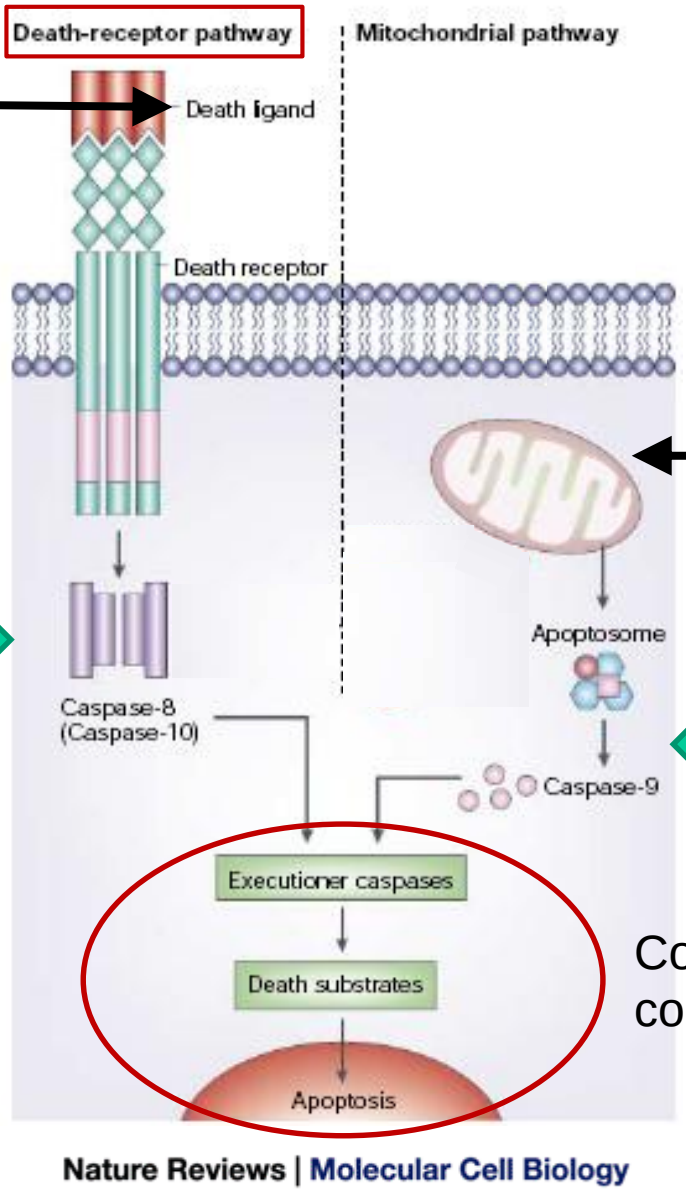
A regulated cellular program: two distinct pathways can induce apoptosis

extrinsic

tumor necrosis factor (TNF) family:

- TNFα,
- CD40 ligand,
- Fas ligand,
- TRAIL (TNF-related apoptosis inducing ligand)

intrinsic



Stress, infection, DNA damage

Caspase 8 activation by autoproteolysis after receptor-trimer induced procaspase proximity

Caspase 9 activation by autoproteolysis after apoptosome-heptamer induced pro-caspase 9 proximity

Common pathway components

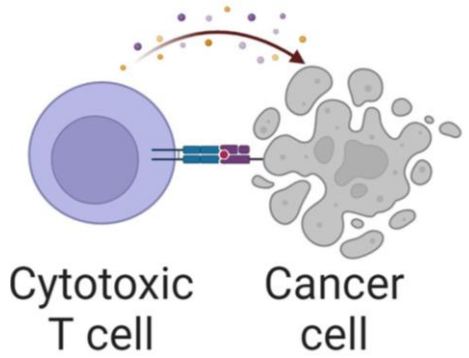
Igney & Krammer (2002) Nat Rev Mol Cell Biol 2, 277-288

One example: Fas Ligand-induced apoptosis

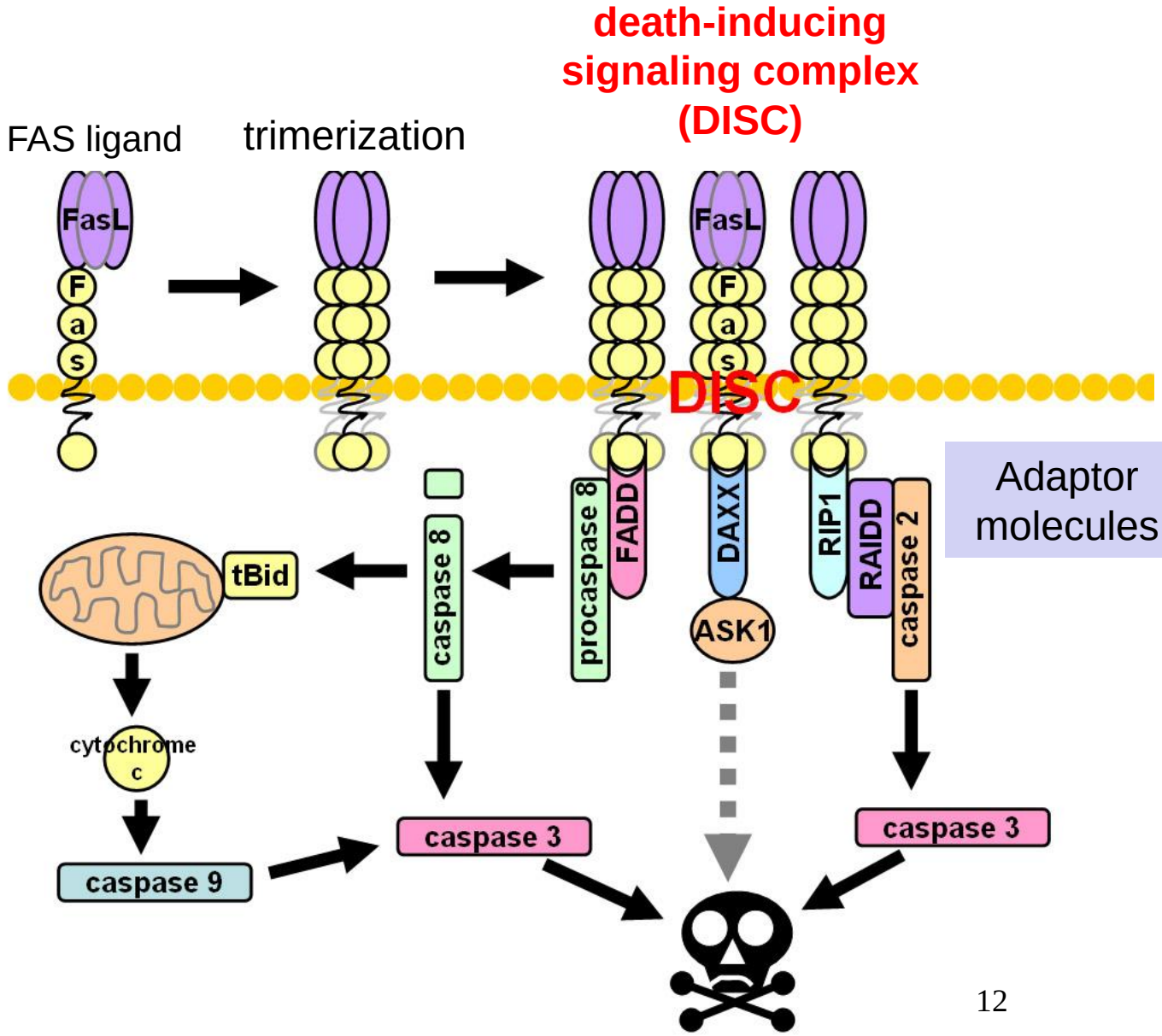
extrinsic

"Cell murder"

- Elimination of autoreactive T-cells;
- Cytotoxic T-cell-mediated killing;

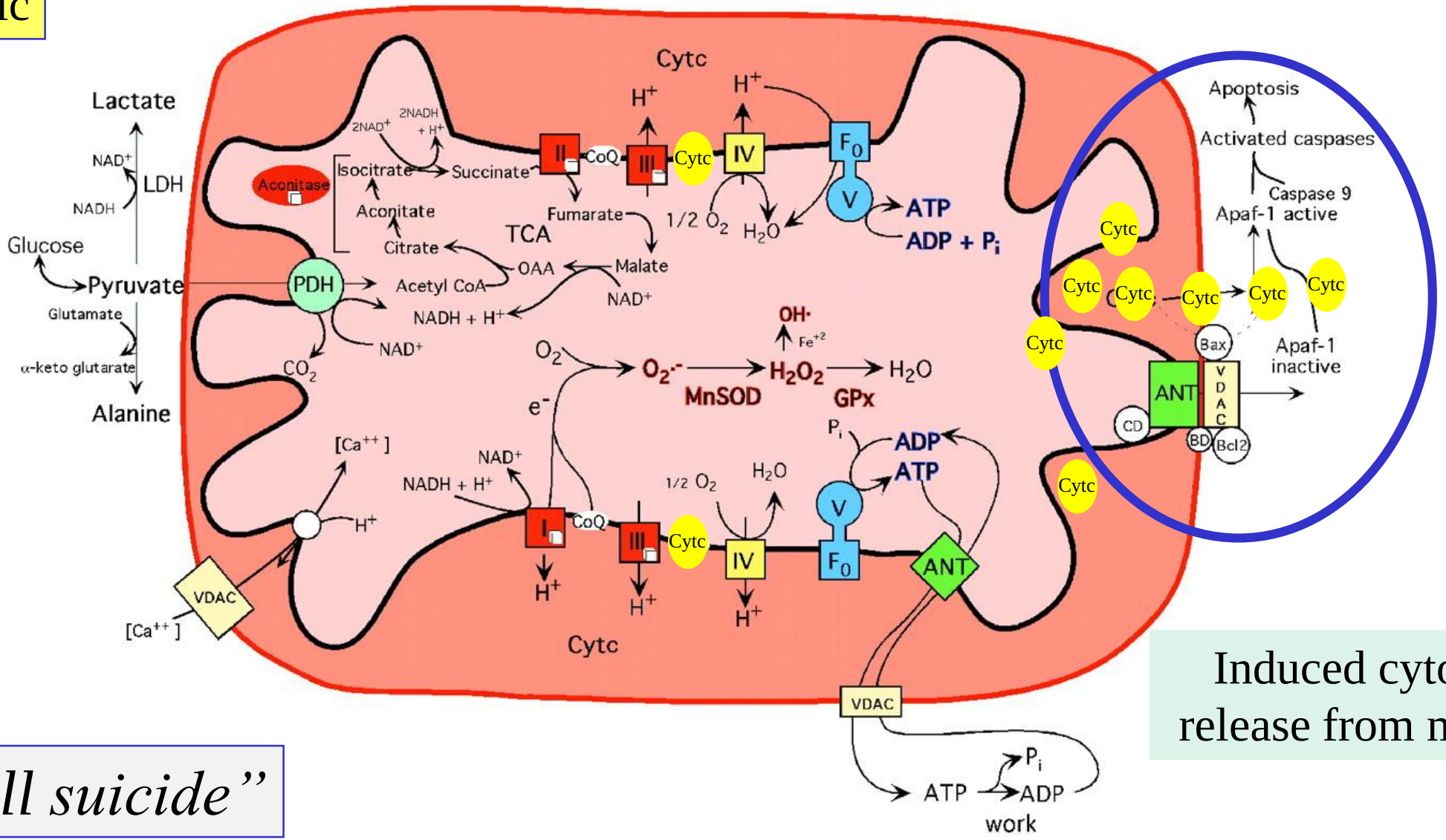


- Tumour cell-induced apoptosis of infiltrating lymphocytes (immune escape)



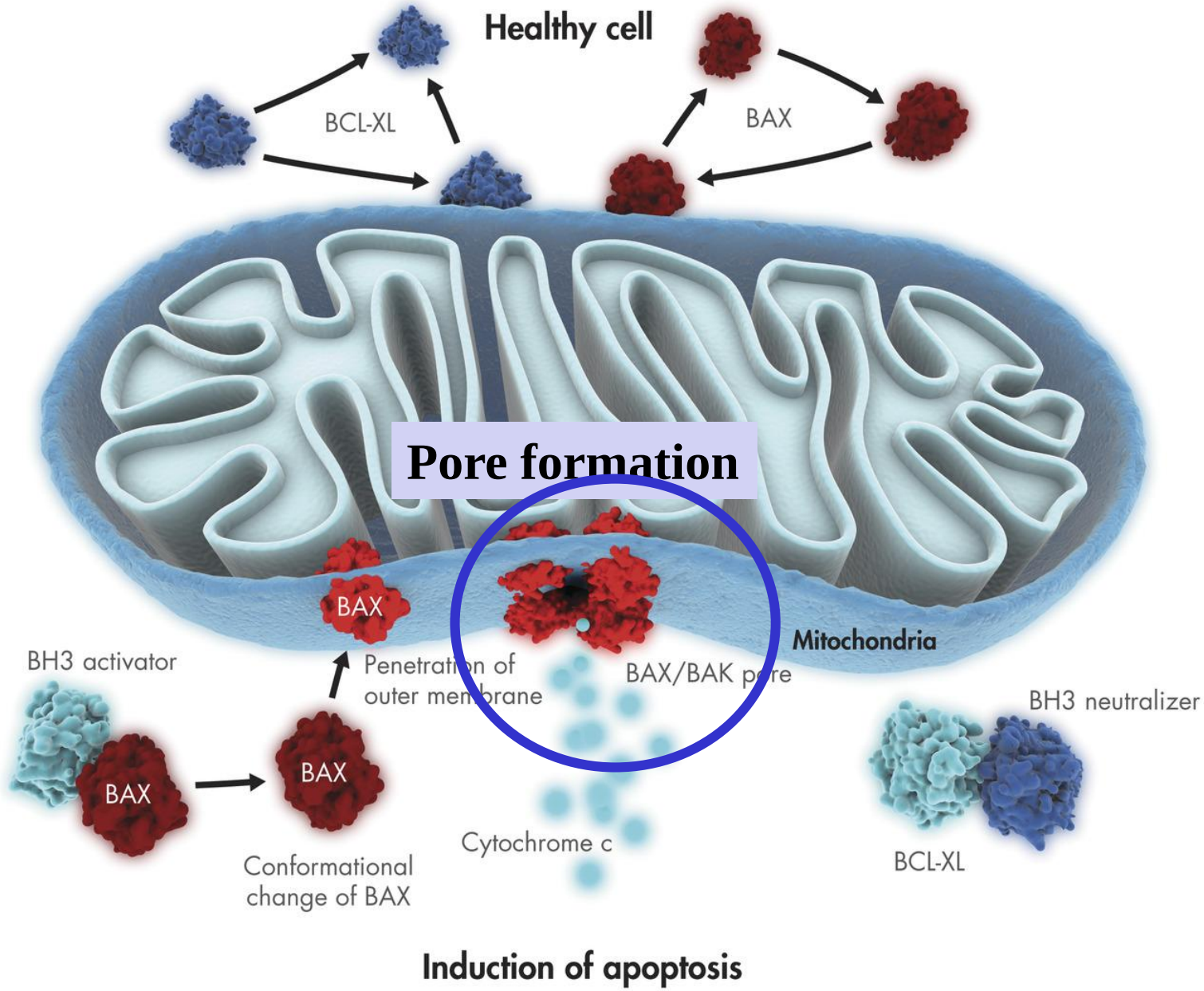
The intrinsic pathway: central role of mitochondria

intrinsic



Induced cytochrome C release from mitochondria

"Cell suicide"



BAK or BAX proteins can form a membrane pore

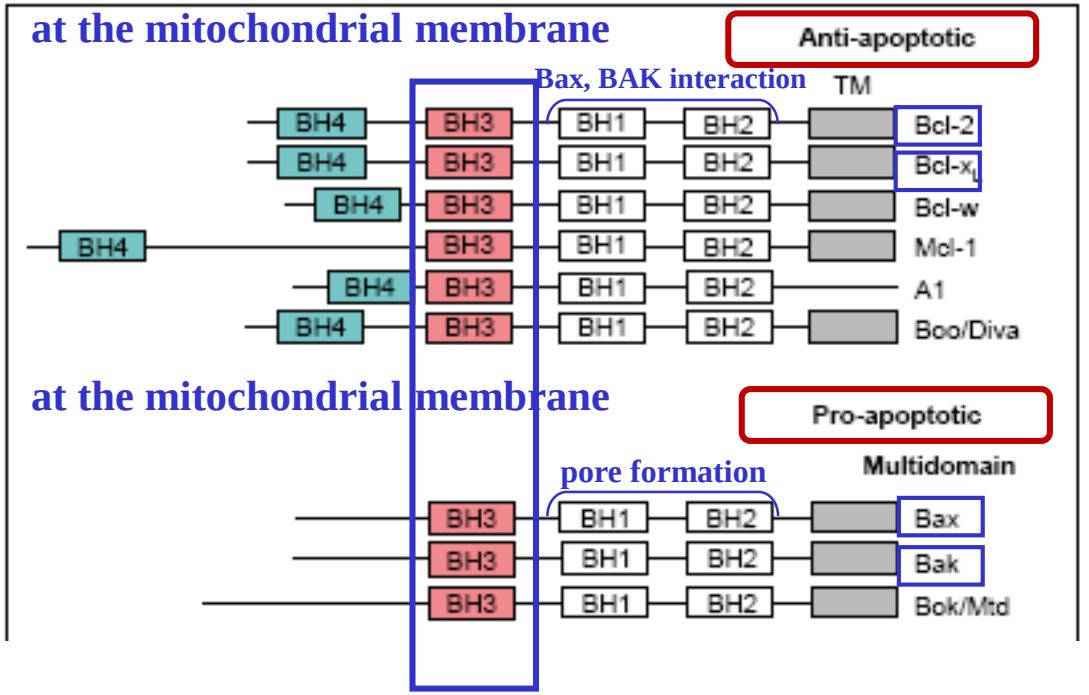
BAX= BCL2 associated X, apoptosis regulator
BAK= BCL2 antagonist/killer 1

Image: https://www.qiagen.com/~/media/nextq/image%20library/illu/03/41/illu_0341_rt2profiler/1_5.ashx?la=en&hash=BF639C1F0C3B0A3D70DD3FE2EA7A12740F98957F

The family of BCL-2 proteins are regulators of the intrinsic apoptosis pathway

The BCL proteins protect the cell from apoptosis by promoting the release of BAX or BAK proteins from the mitochondrial membrane into the cytosol.

BAK or BAX proteins can oligomerize and form a membrane pore

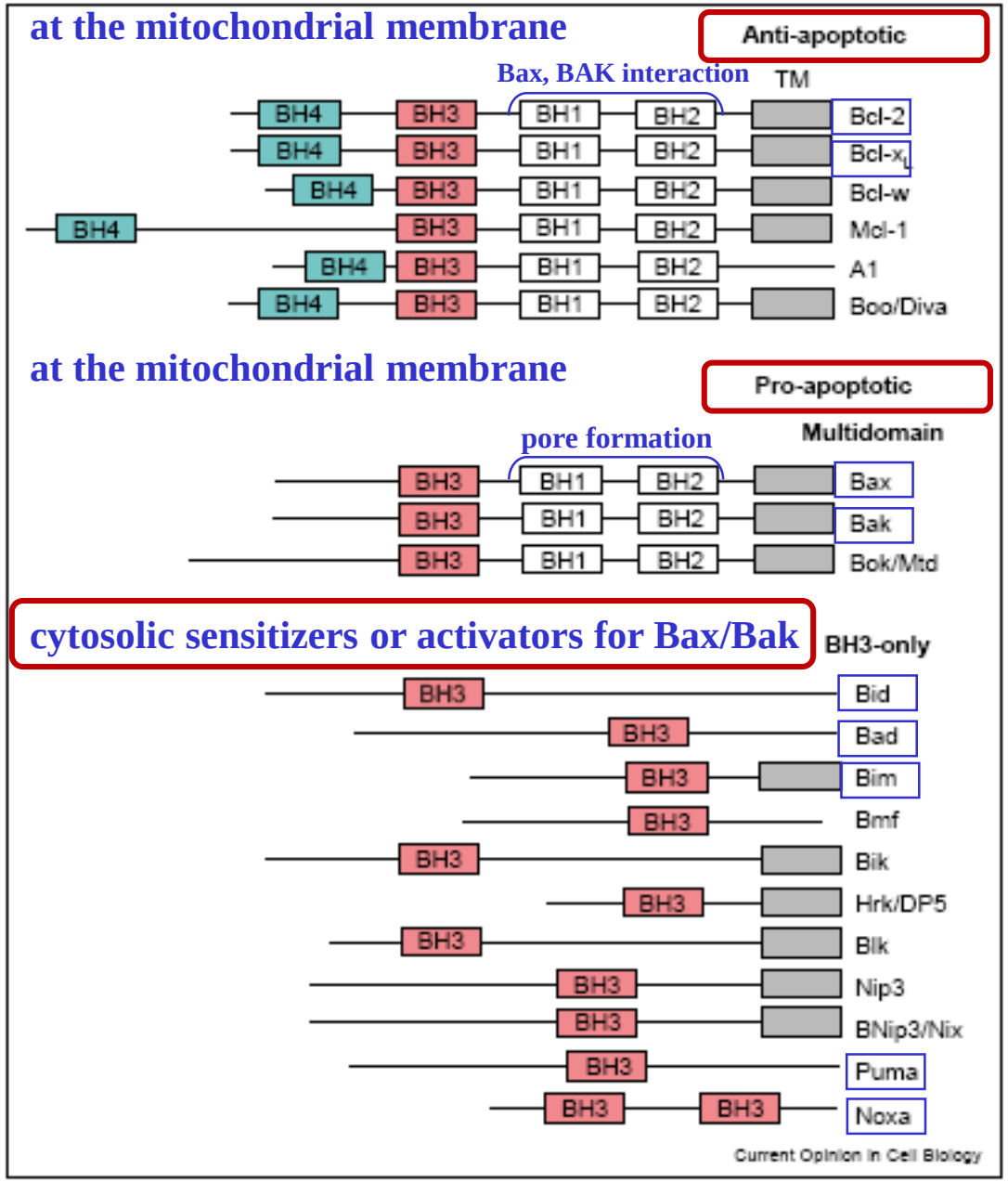


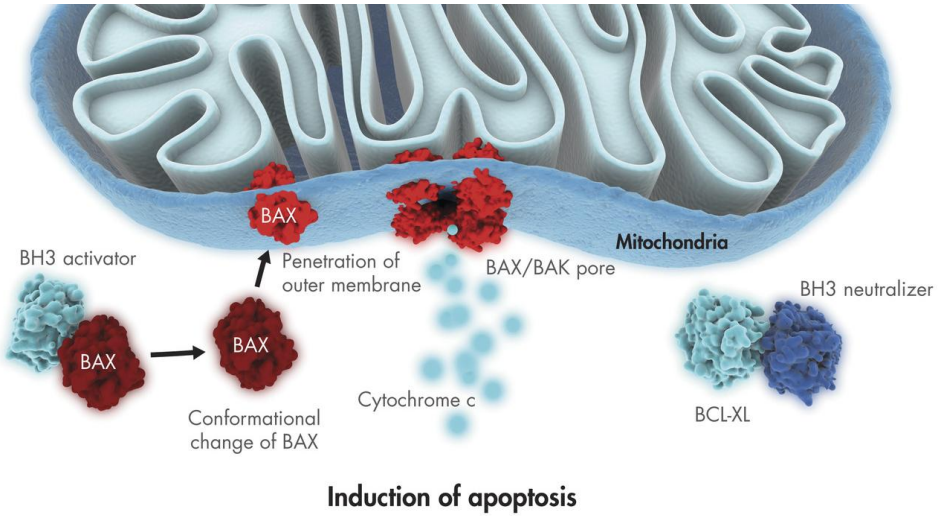
Direct physical interactions between the BCL-2 family proteins depend on their BCL-2 homology 3 (BH3) regions.

The family of BCL-2 proteins– regulators of the intrinsic apoptosis pathway

Whether BAK/BAX pores can form depends on:

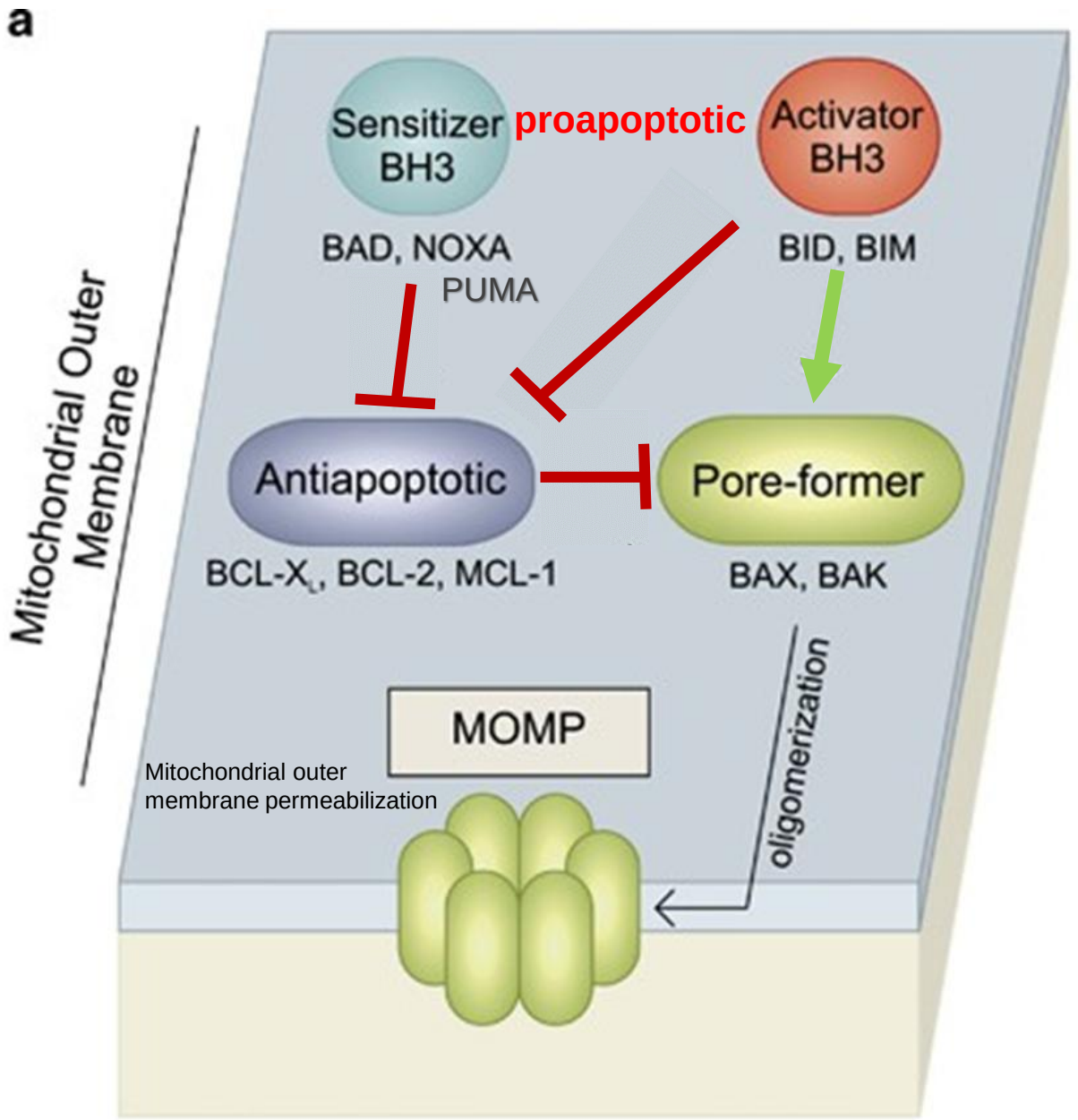
- differential expression levels of the various BCL-2 members,
- their subcellular localization,
- their post-translational modifications (e.g. phosphorylation by AKT)



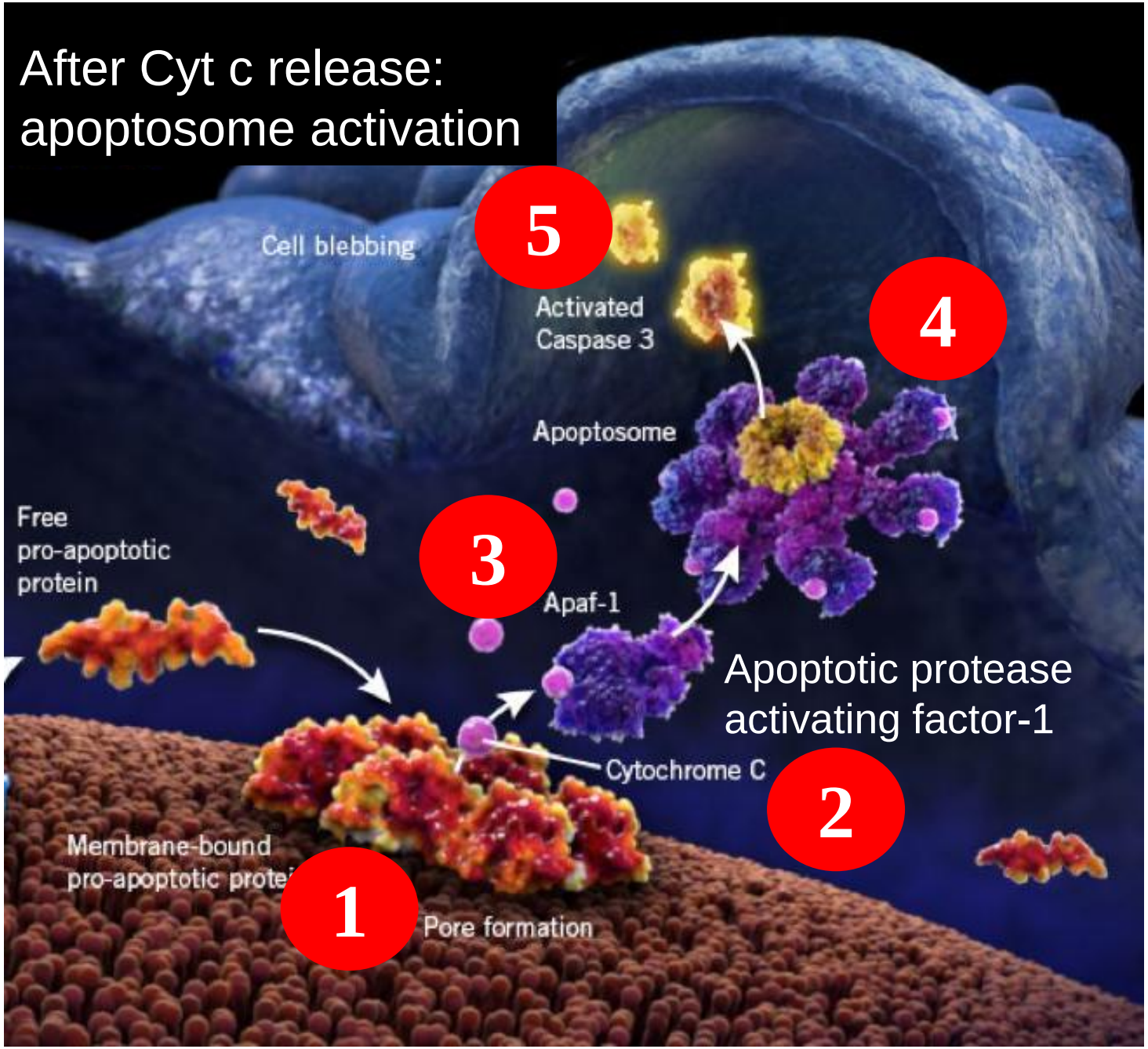


Induction of apoptosis

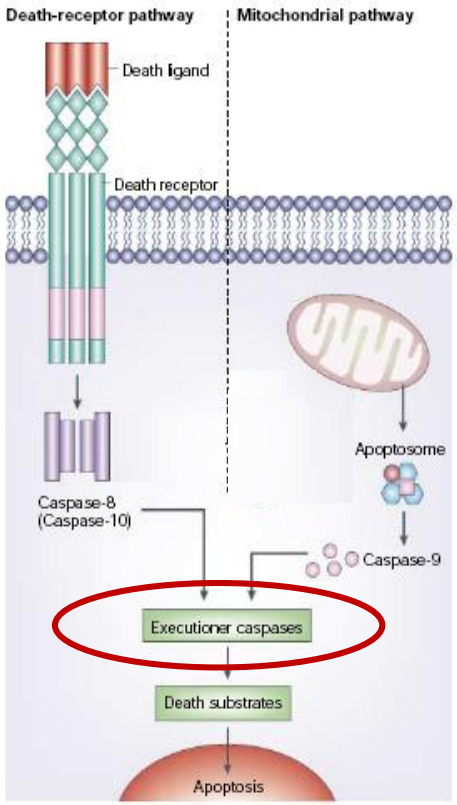
All BCL-2 family proteins are small 18 -37 kD proteins



https://www.nature.com/articles/cdd2017186



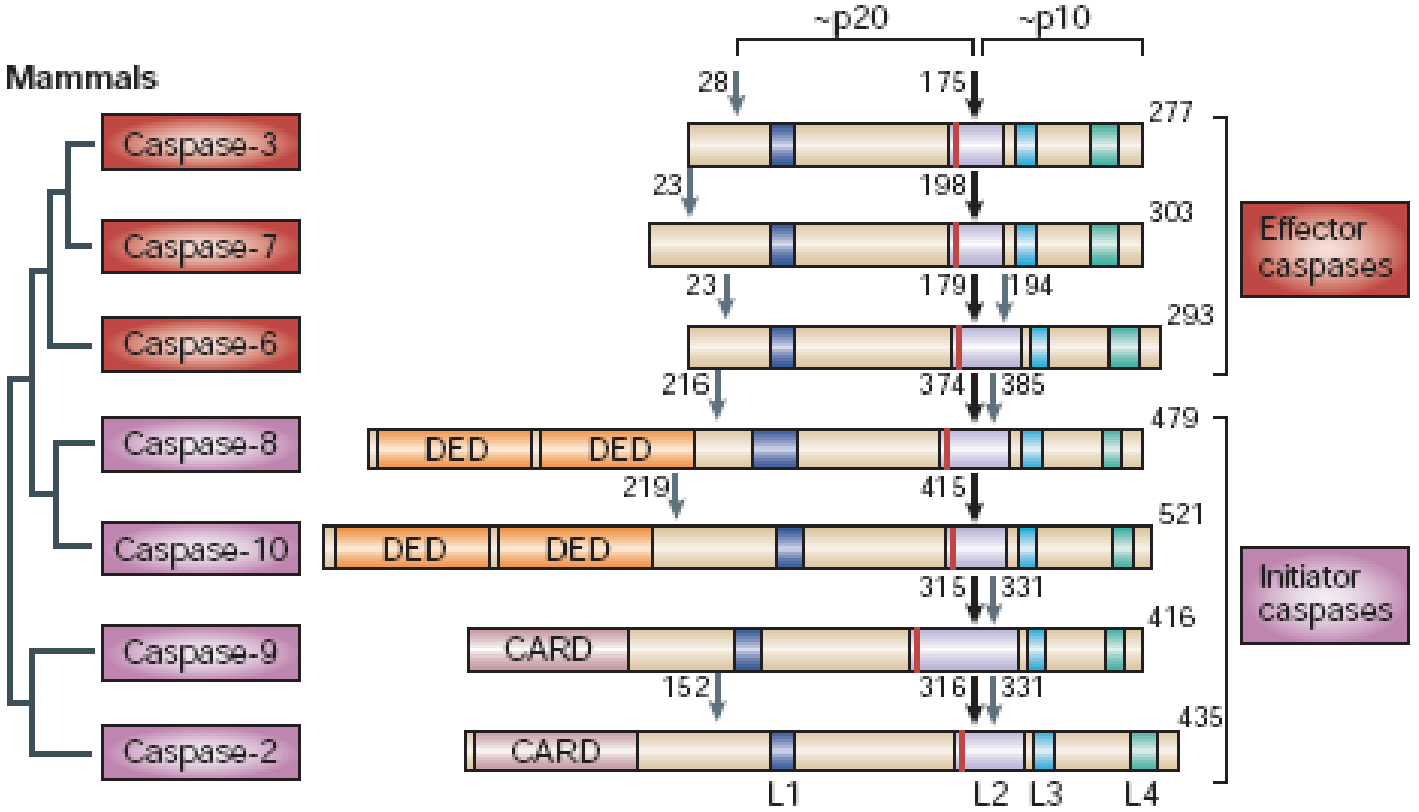
https://www.genentechoncology.com/pathways/cancer-tumor-targets/bcl-2.html



Executing the apoptotic programe:

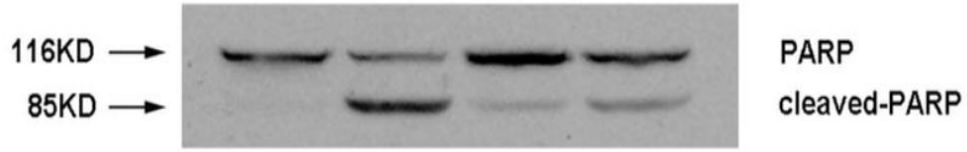
a cascade of activation of intracellular proteases

(aspartate-specific cysteine protease = caspase)



Nature Reviews Molecular Cell Biology 5, 897f (2004)

Effector caspases selectively cleave key cellular proteins

Nuclear Lamins	**Chromatin condensation and nuclear shrinkage**
Inhibitor of DNase CAD, PARP DNA repair protein	
Cytoskeleton regulatory proteins	**Blebbing and fragmentation of the cell and formation of apoptotic bodies**
Phosphatidyl-serine flippase*	**Increased PS levels at the surface of apoptotic bodies as an ‘eat-me’ signal for phagocytes**

* https://doi.org/10.1158/2159-8290.CD-RW2014-131

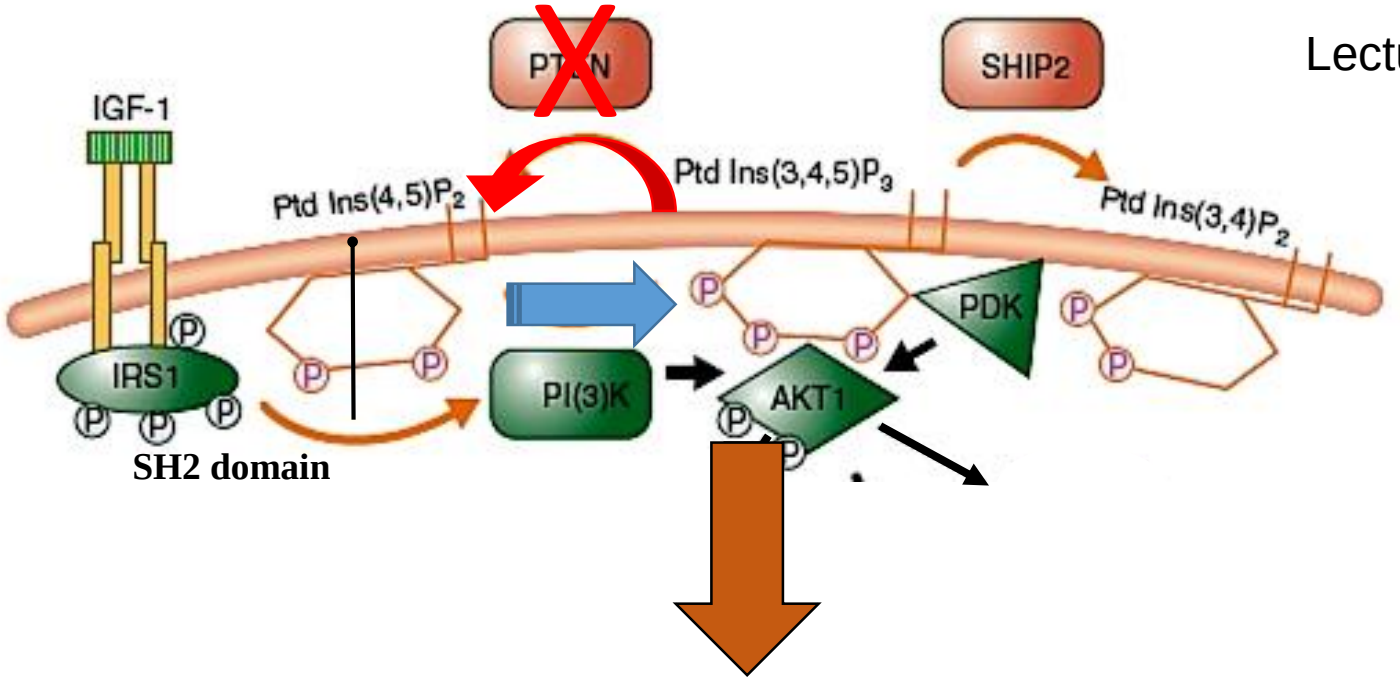
Anti-apoptotic signalling- 1: downstream of PI3K

PTEN

Gene deletion or gene silencing

↓

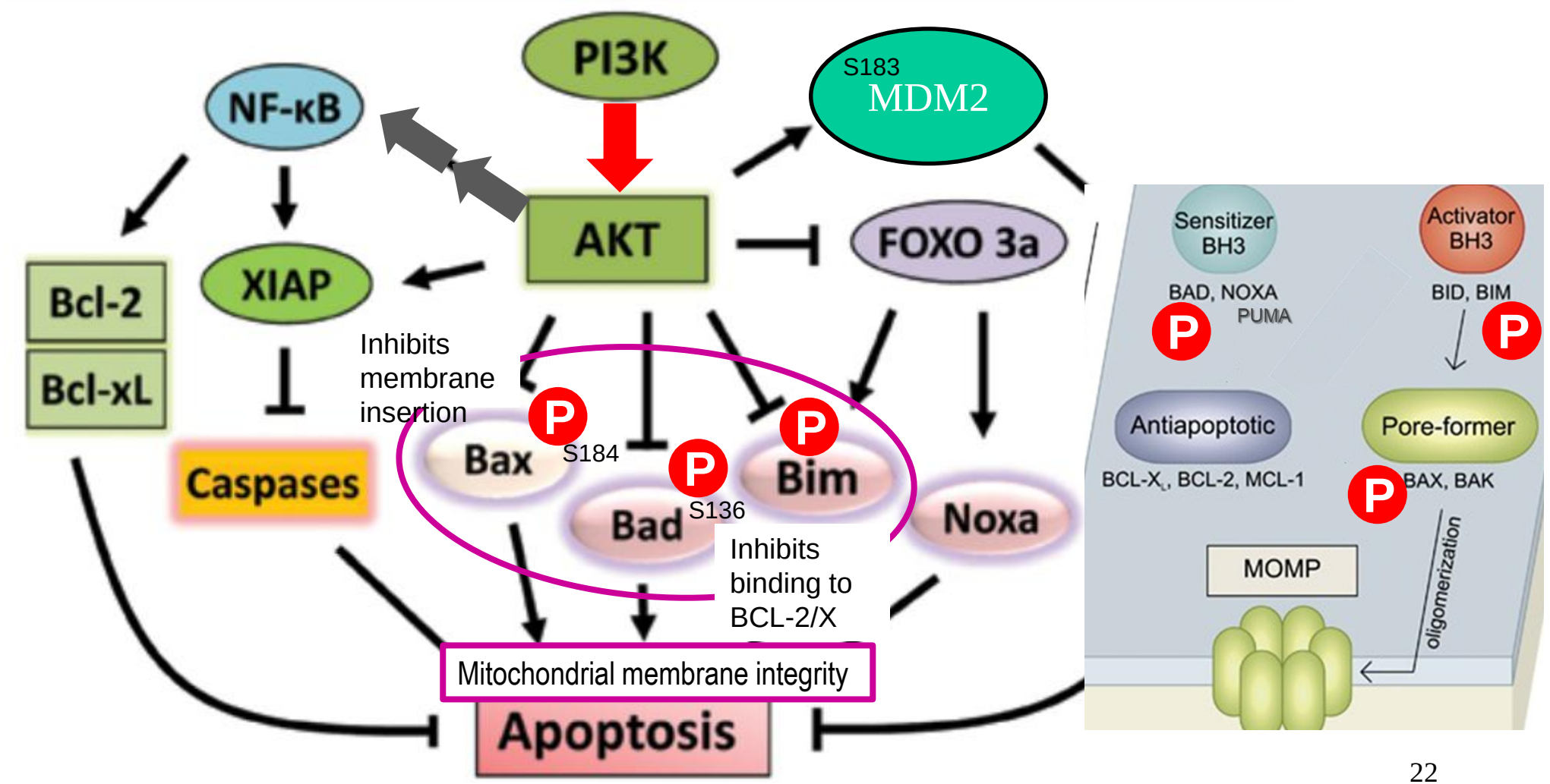
Brain and breast tumours



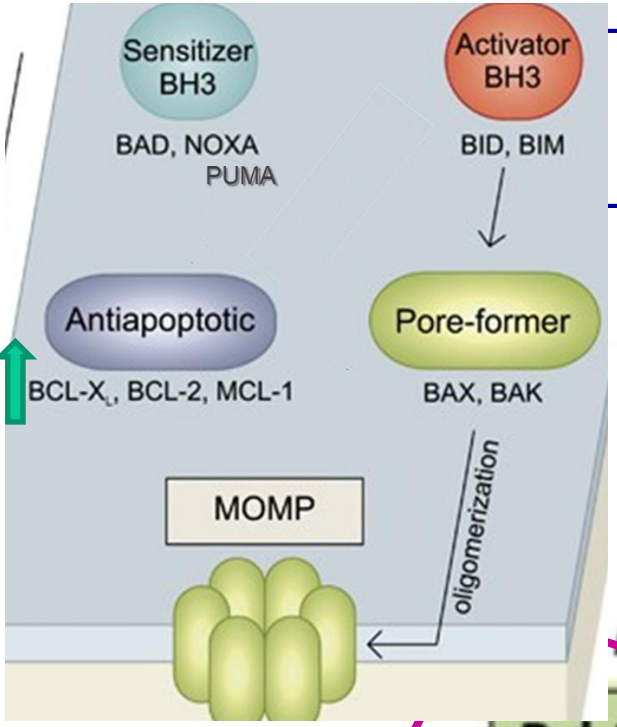
Lecture cell signalling- part 2

Inhibition of apoptosis-inducing proteins

Anti-apoptotic signalling- 1: downstream of PI3K



Anti-apoptotic signalling- 1: downstream of PI3K



Transcription factors

Increased nuclear import

PI3K

NF-κB

AKT

P S183 MDM2

FOXO 3a

XIAP

Bcl-2

Bcl-xL

Caspases

Ub Ub Ub

p53

P Bax S184

P Bad S136

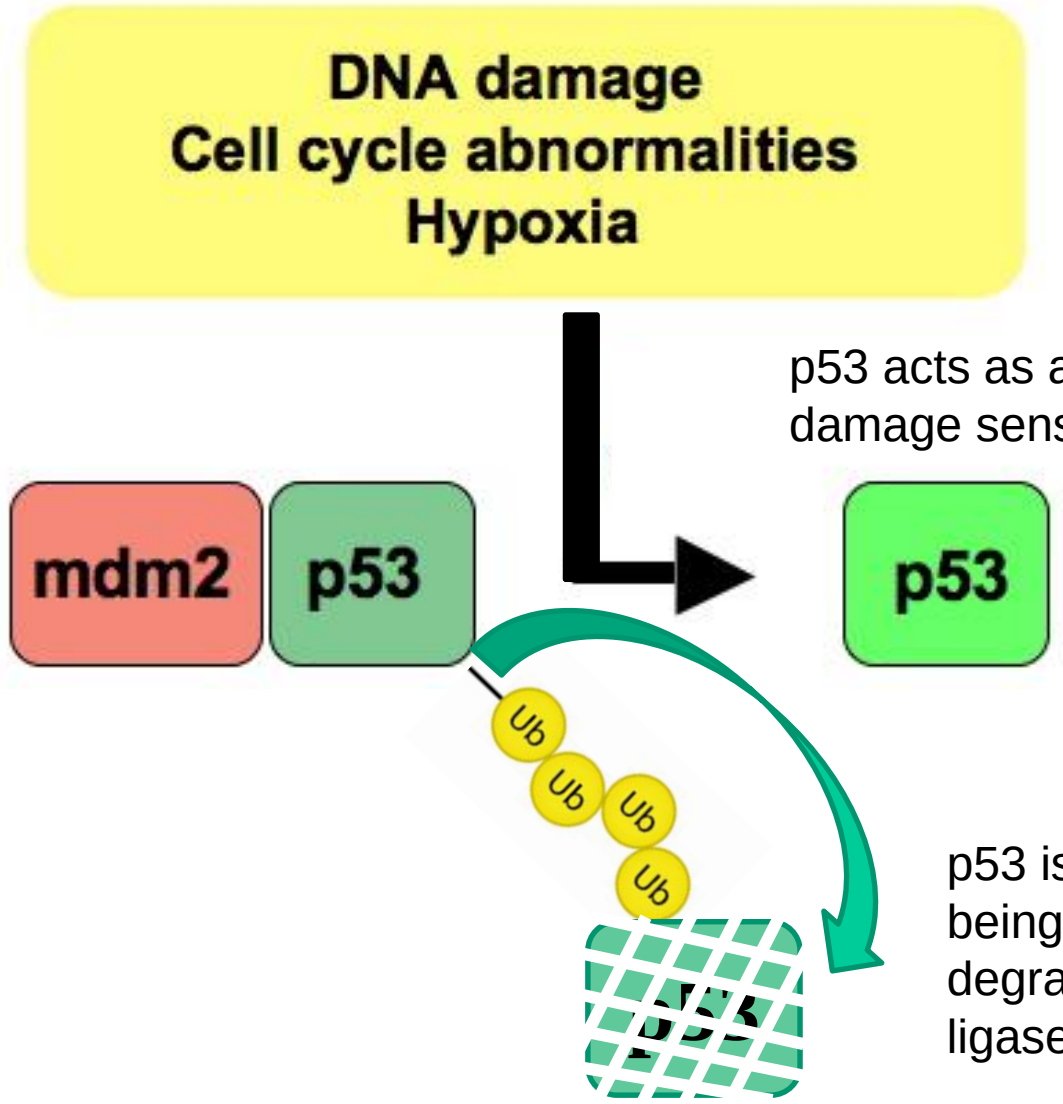
P Bim

Noxa

Mitochondrial membrane integrity

Apoptosis

Anti-apoptotic signaling by p53



p53 acts as a cellular stress and DNA damage sensor that can induce apoptosis

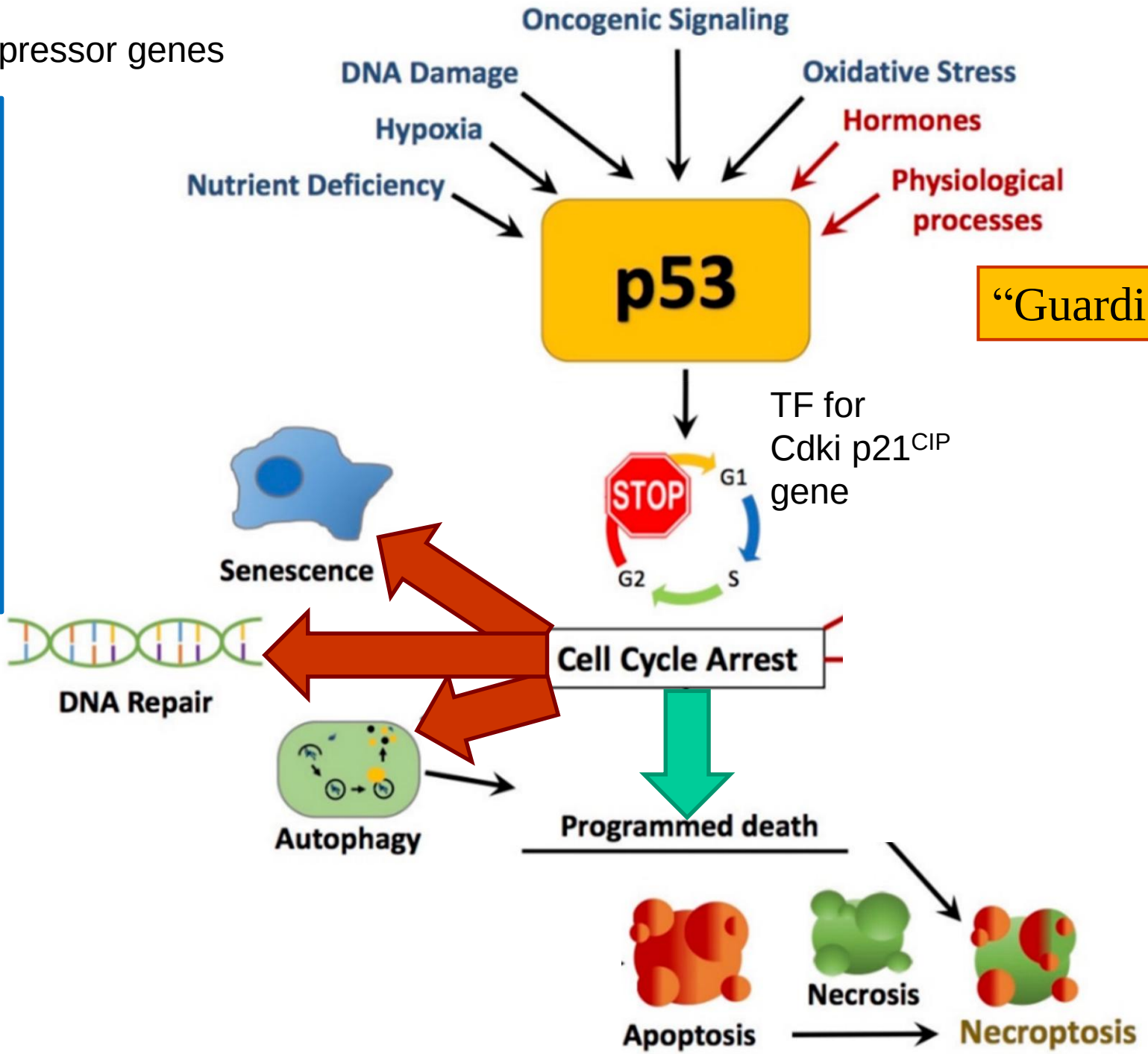
p53 is normally kept at low levels by being constantly marked for degradation by the E3 ubiquitin ligase protein MDM2.

TP53

Gene deletion or point mutations

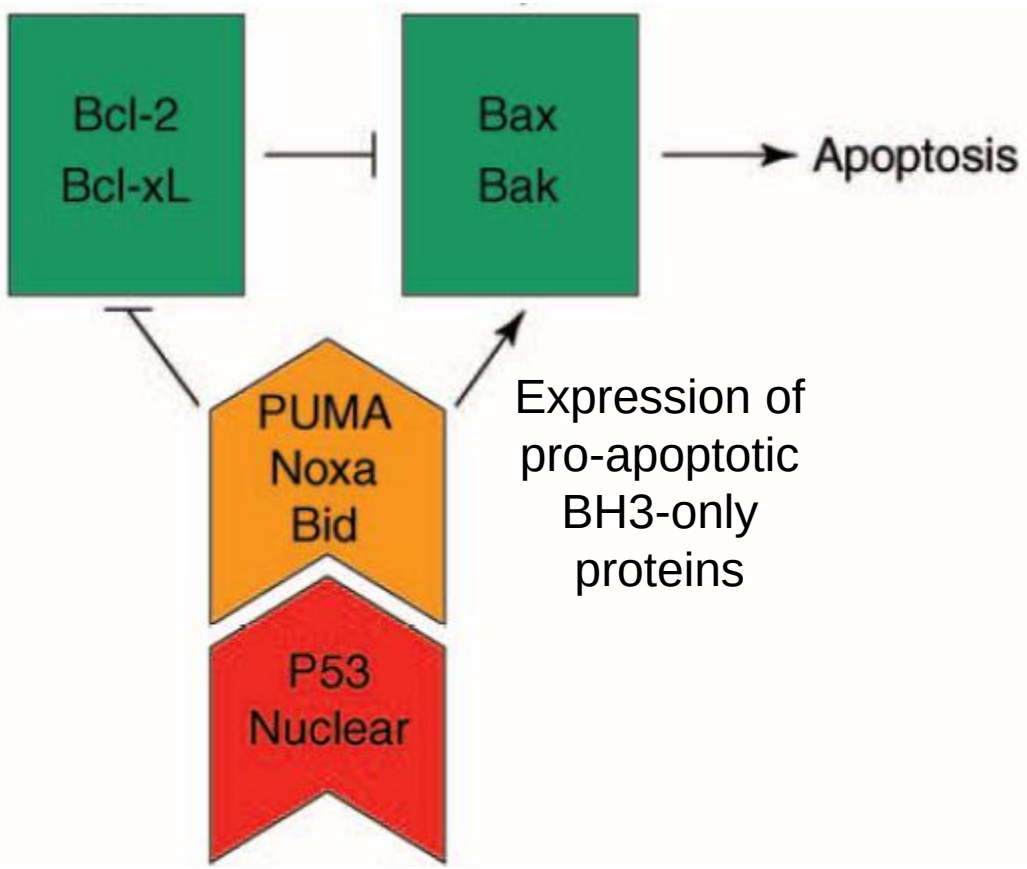
↓

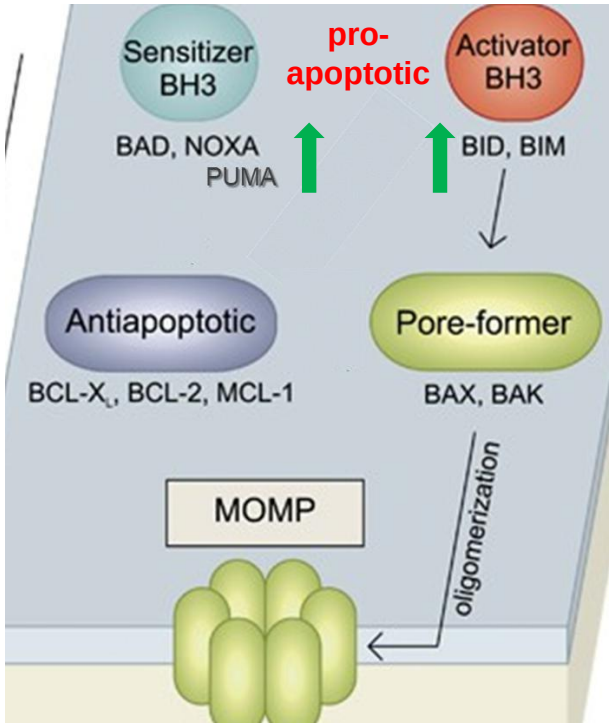
Li-Fraumeni syndrome; most cancer types



"Guardian of the genome"

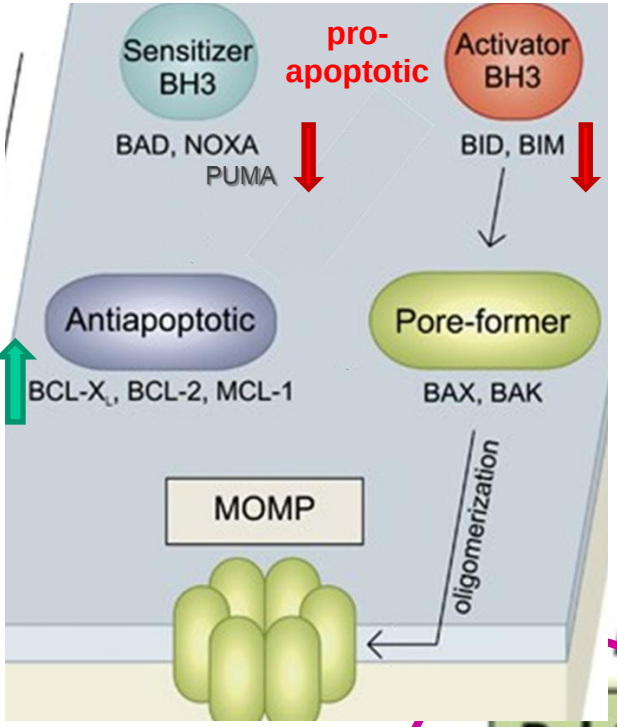
p53 promotes apoptosis by controlling the transcription of proapoptotic genes from the BCL-2 family that promote BAX/BAK pore formation





Current Opinion in Cell Biology 2005, 17:631–636

Anti-apoptotic signaling downstream of PI3K



Transcription factors

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P FOXO 3a

XIAP

Bcl-2

Bcl-xL

Caspases

P Bax S184

P Bad S136

P Bim

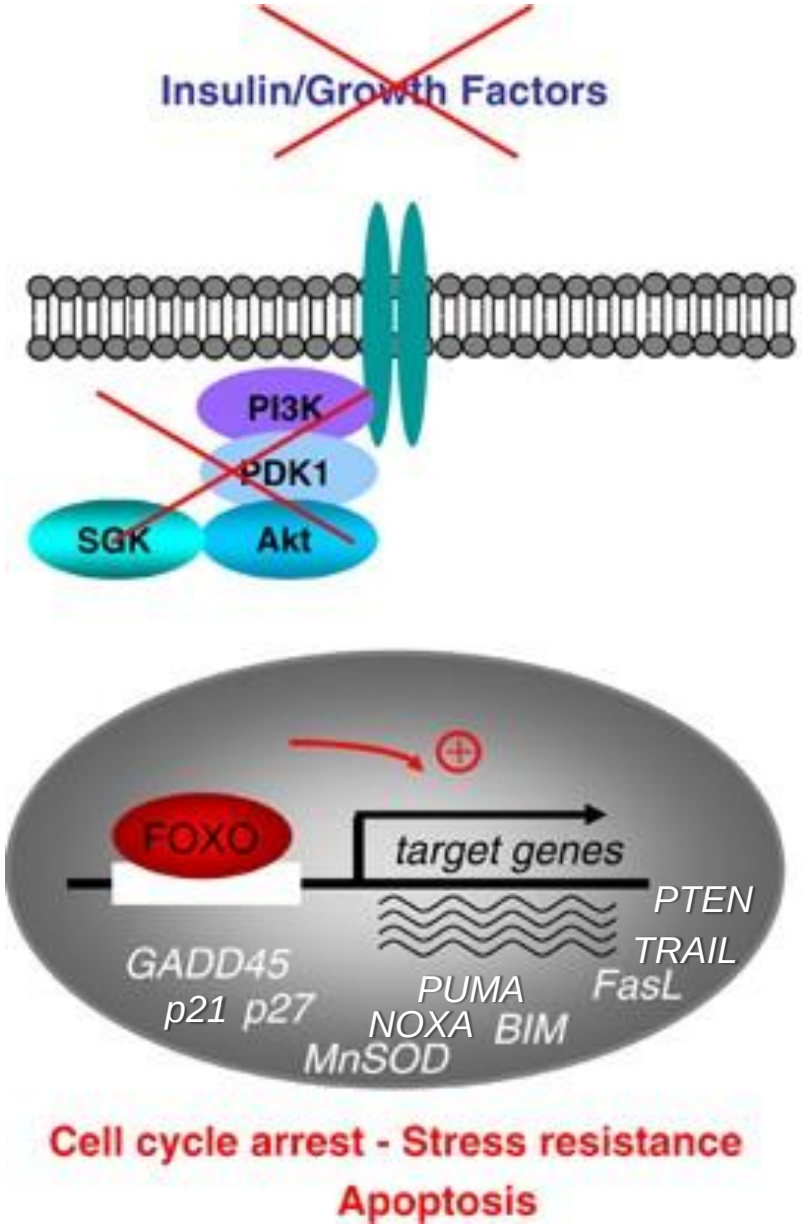
Noxa

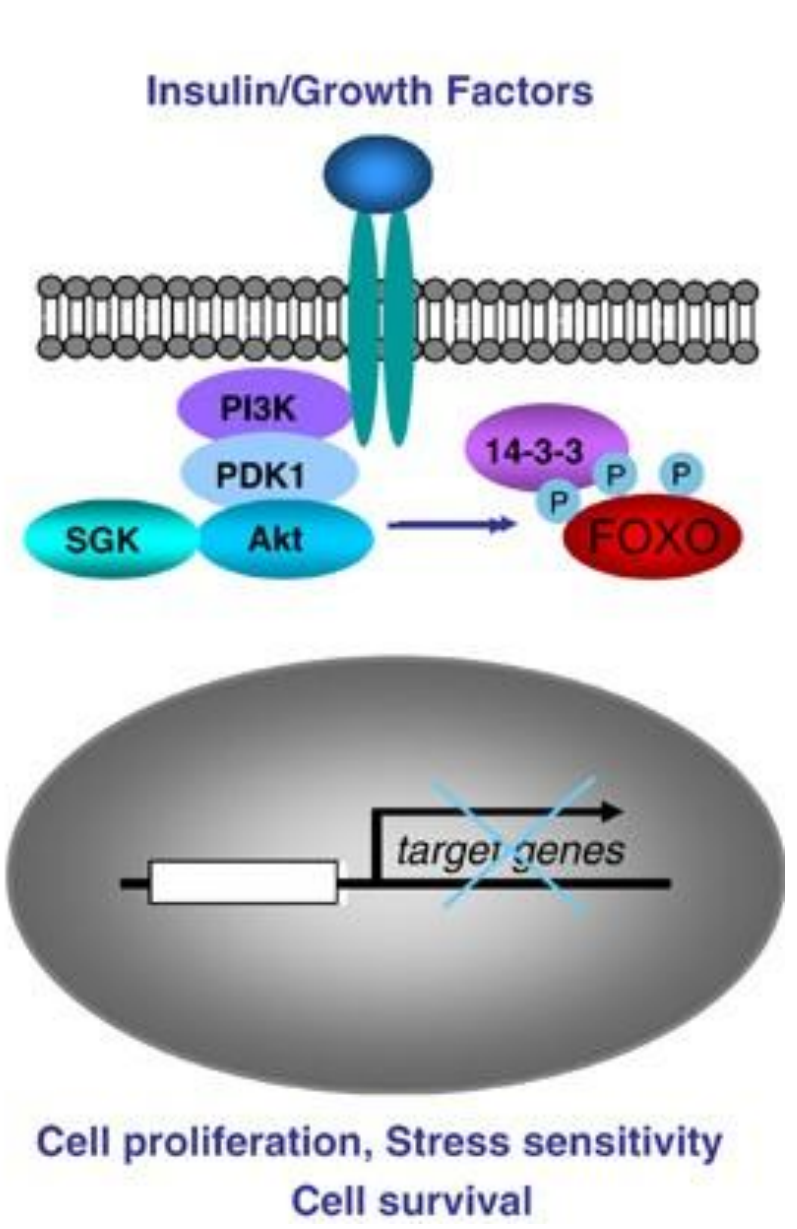
Ub Ub Ub

p53

Mitochondrial membrane integrity

Apoptosis





PI3K/AKT/Foxo

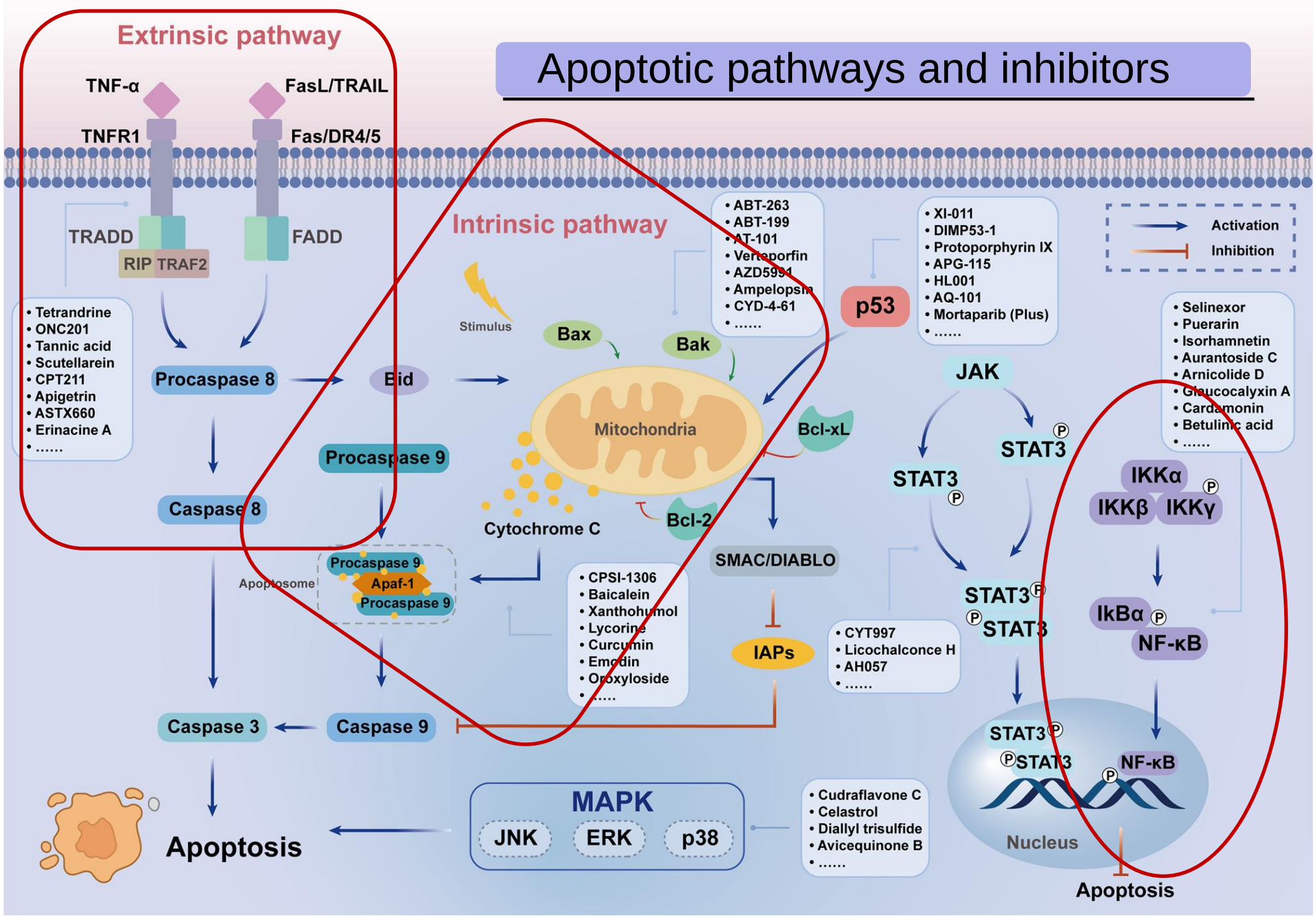
FOXO transcription factors

- are activated in normal cells by diverse cell stress conditions to promote cell cycle arrest, stress resistance, or apoptosis

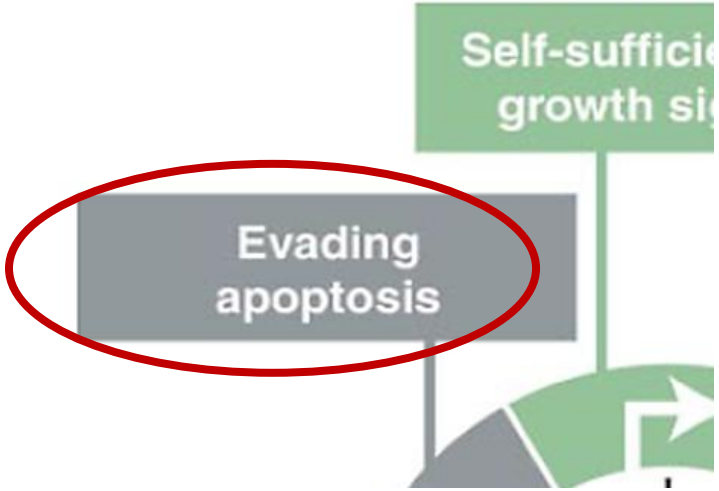
- are inactivated through phosphorylation by AKT, which impedes shuttling of FOXO proteins into the nucleus;

Tumour suppressor function

Apoptotic pathways and inhibitors

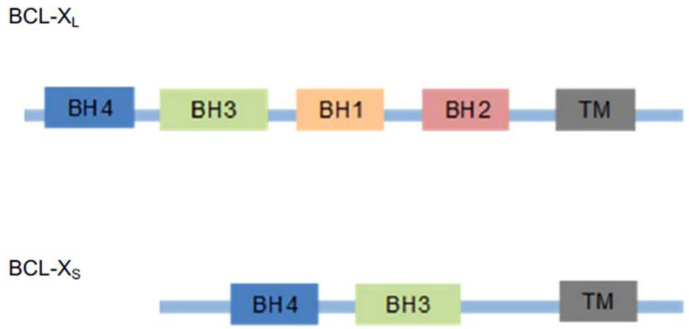


DOI 10.1038/s41392-022-01110-y



How do cancer cells escape death by apoptosis?

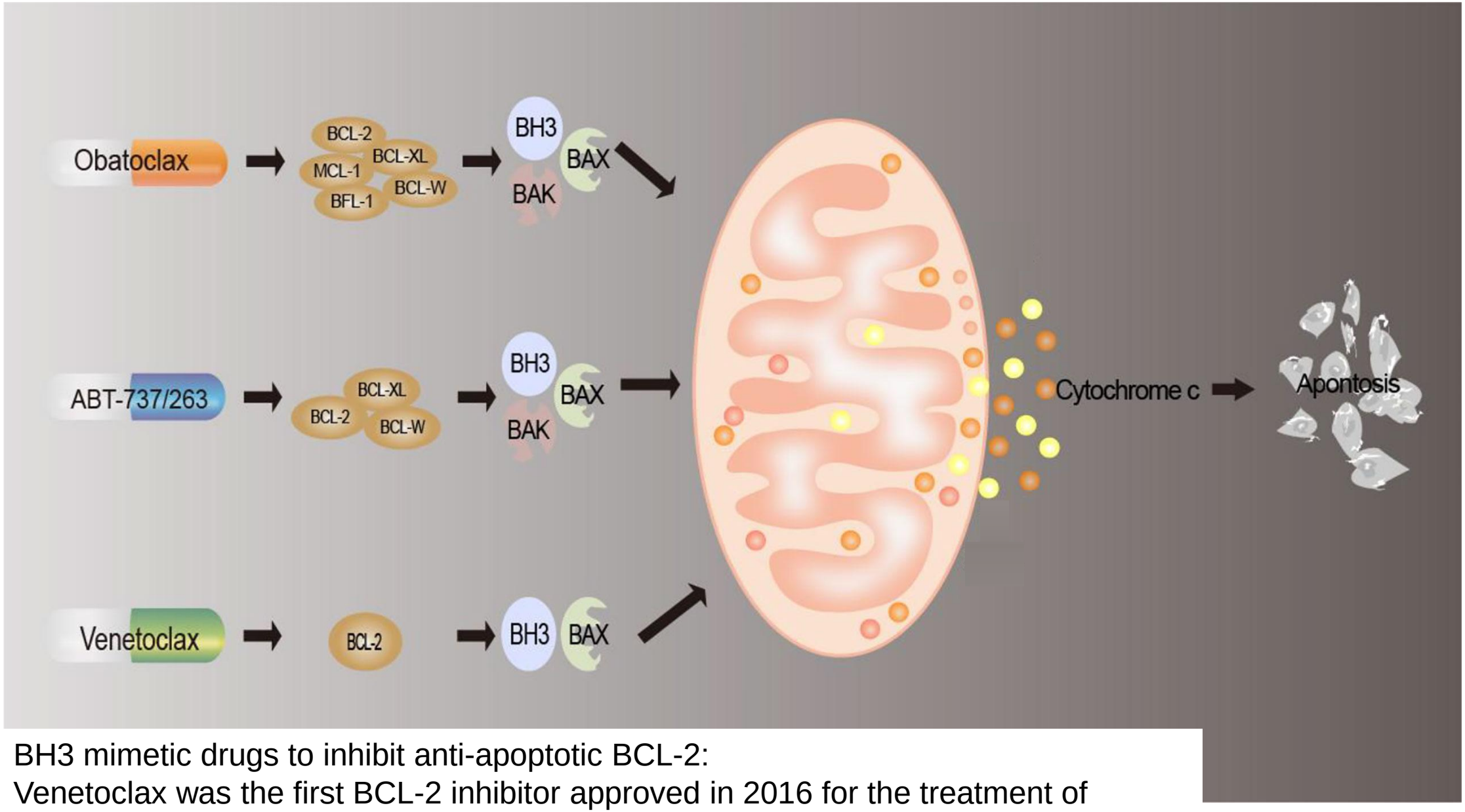
- Loss of the apoptosis gatekeeper, the protein P53 (mutation, deletion, sequestration by oncogenic viral proteins);
- Overexpression of anti-apoptotic proteins such as BCL-2, or BCL-XL alternative splicing variant:



Doi: 10.1038/s41419-019-1407-6

$BCL\text{-}X_L$ exerts its anti-apoptotic regulation by formation of heterodimers with both BAX and BAK pore-forming proteins; BCL-Xs does not form these heterodimers

- Transcriptional downregulation of pro-apoptotic pore-forming proteins BAX and BAK
- Inactivating phosphorylation of pro-apoptotic proteins by AKT due to activation of PI3K pathway

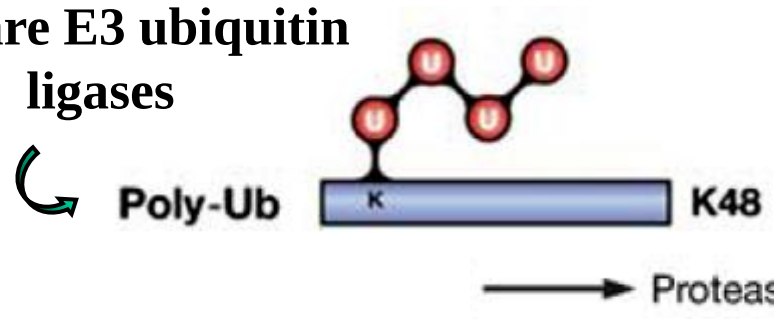


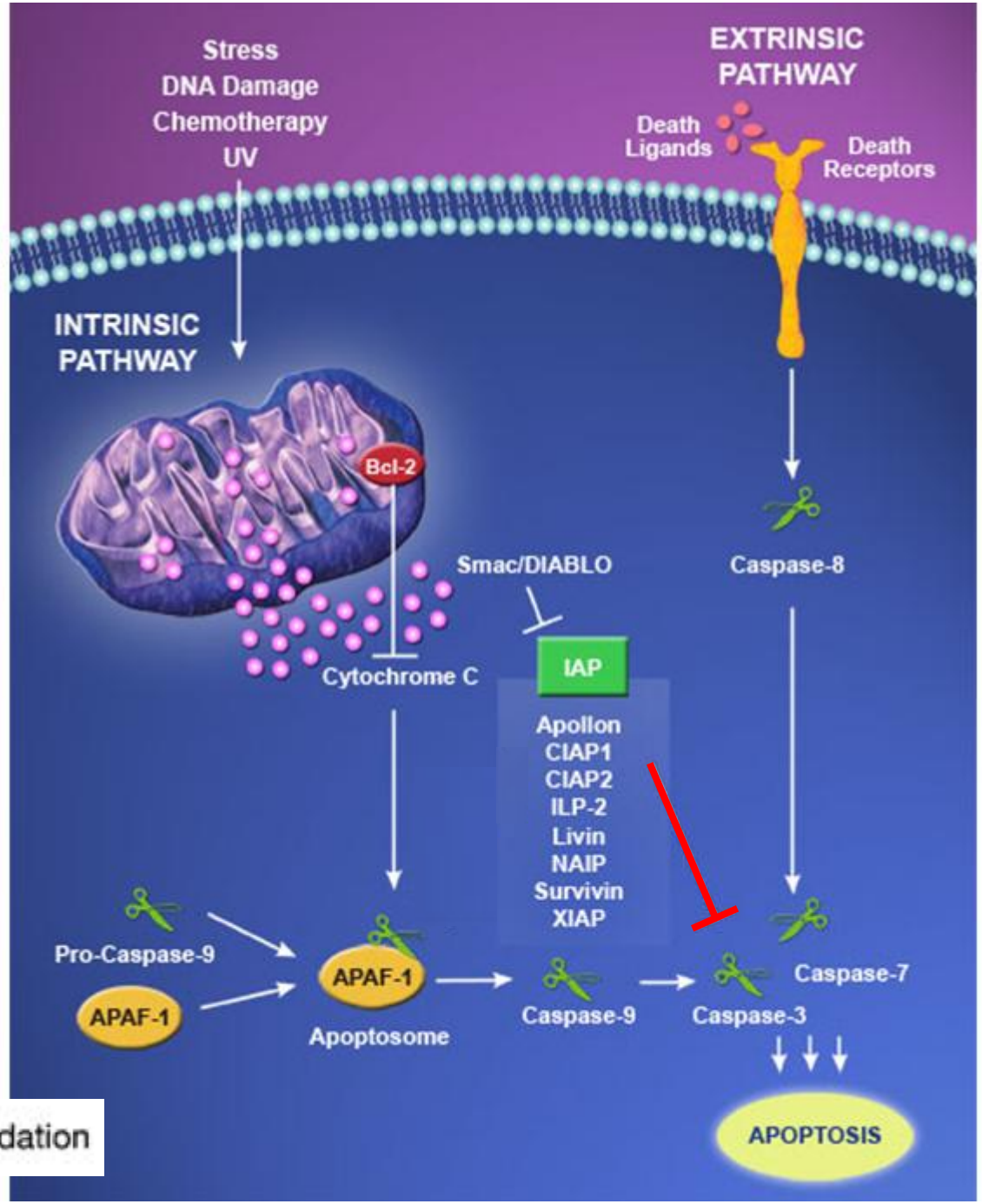
BH3 mimetic drugs to inhibit anti-apoptotic BCL-2:
Venetoclax was the first BCL-2 inhibitor approved in 2016 for the treatment of chronic lymphocytic leukemia and acute myeloid leukemia without thrombocytopenia

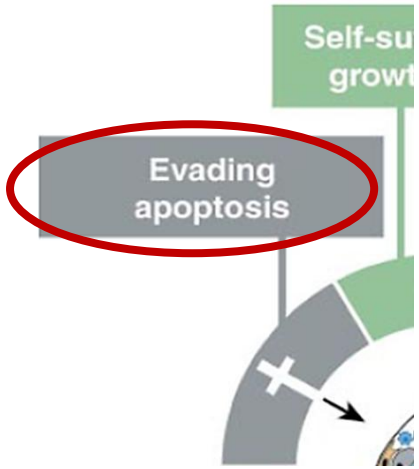
Last break: preventing caspase activation

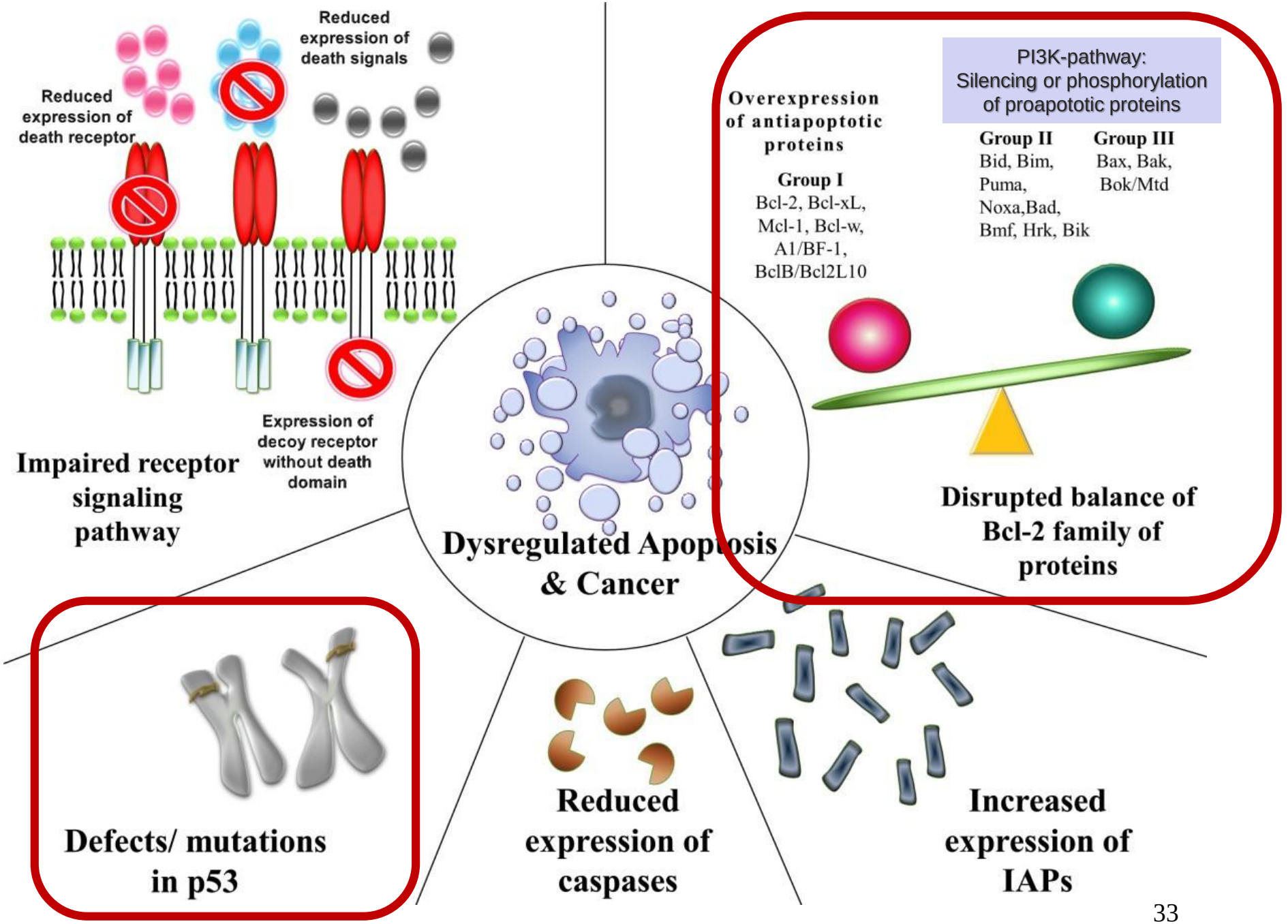
IAP family proteins
(inhibitor of apoptosis)
directly inhibit
the catalytic action of caspases;
=
higher cell tolerance
against
pro-apoptotic stimuli

IAPs are E3 ubiquitin ligases









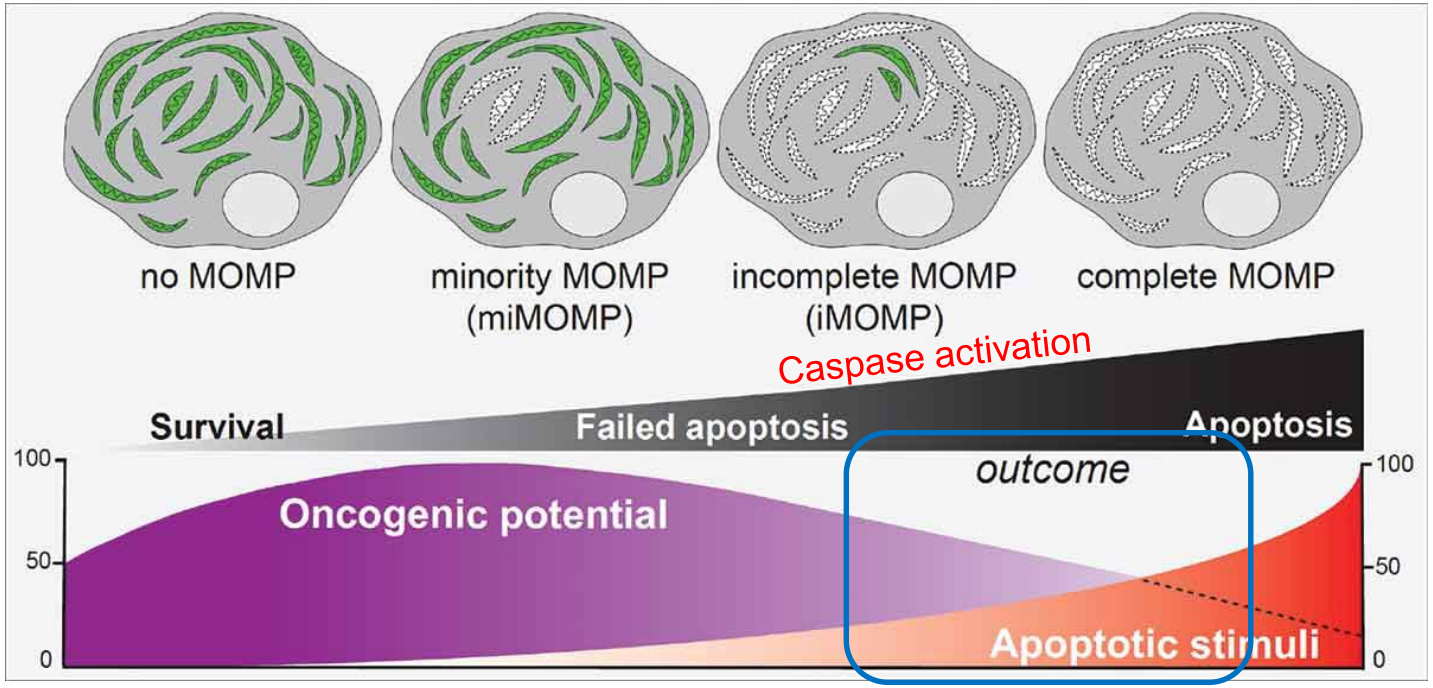
https://doi.org/10.1186/1756-9966-30-87

Apoptosis is a highly regulated cellular programe that leads to a controlled cell suicide

pro-apoptotic signalling

anti-apoptotic signalling

https://doi.org/10.1080/23723556.2020.1797430



Cell plasticity vs ‘point-of–no-return’

Lecture 8- Some take-home concepts

- **Apoptosis is a highly regulated cellular program controlled by signaling pathways;**
- **An extrinsic pathway can be triggered by ligand proteins like TNFα that bind plasma membrane death receptors, whereas an intrinsic pathway senses cellular stress or DNA damage and modulates members of the BCL2 family of proteins that affect mitochondrial membrane permeability;**
- **Both pathways lead to activation of caspases that degrade cellular components and structures;**
- **The relative expression levels or phosphorylation status of members of the BCL2 family determine whether mitochondrial pores are formed to release cytochrome c;**
- **Cancer cells can escape from apoptosis by activation of the PI3K/AKT pathway, loss of the TP53 tumour suppressor gene, or overexpression of anti-apoptotic BCL-2 family members.**