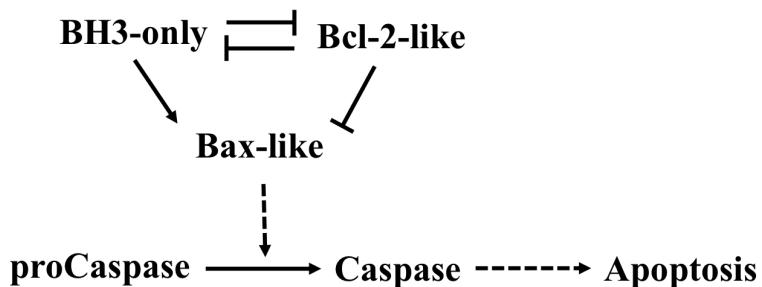


- Caspase act **downstream** of Bcl-2-like proteins to induce apoptosis
- high conc. of procaspase itself can induce the cleavage without any stimulation

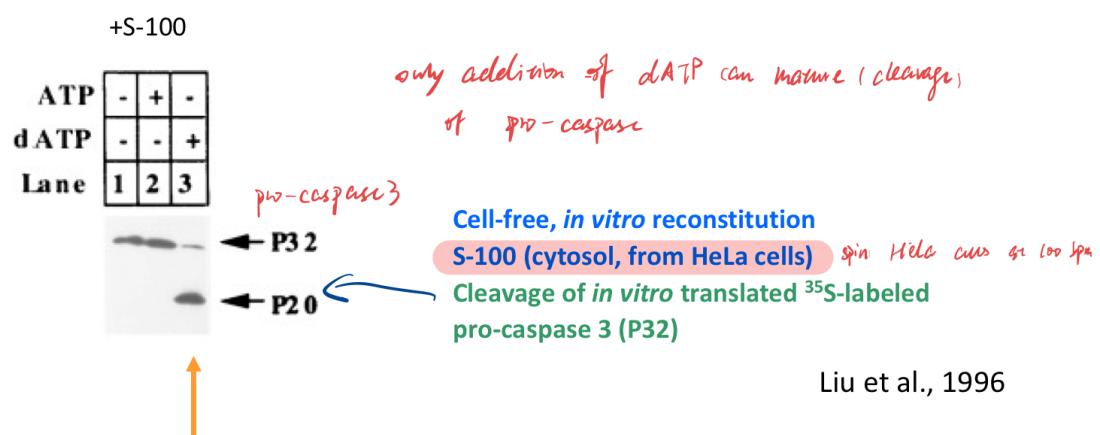


How are caspases activated?

The overexpression — [procaspase]↑ — so much so they can overrule themselves
w/ upstream signaling

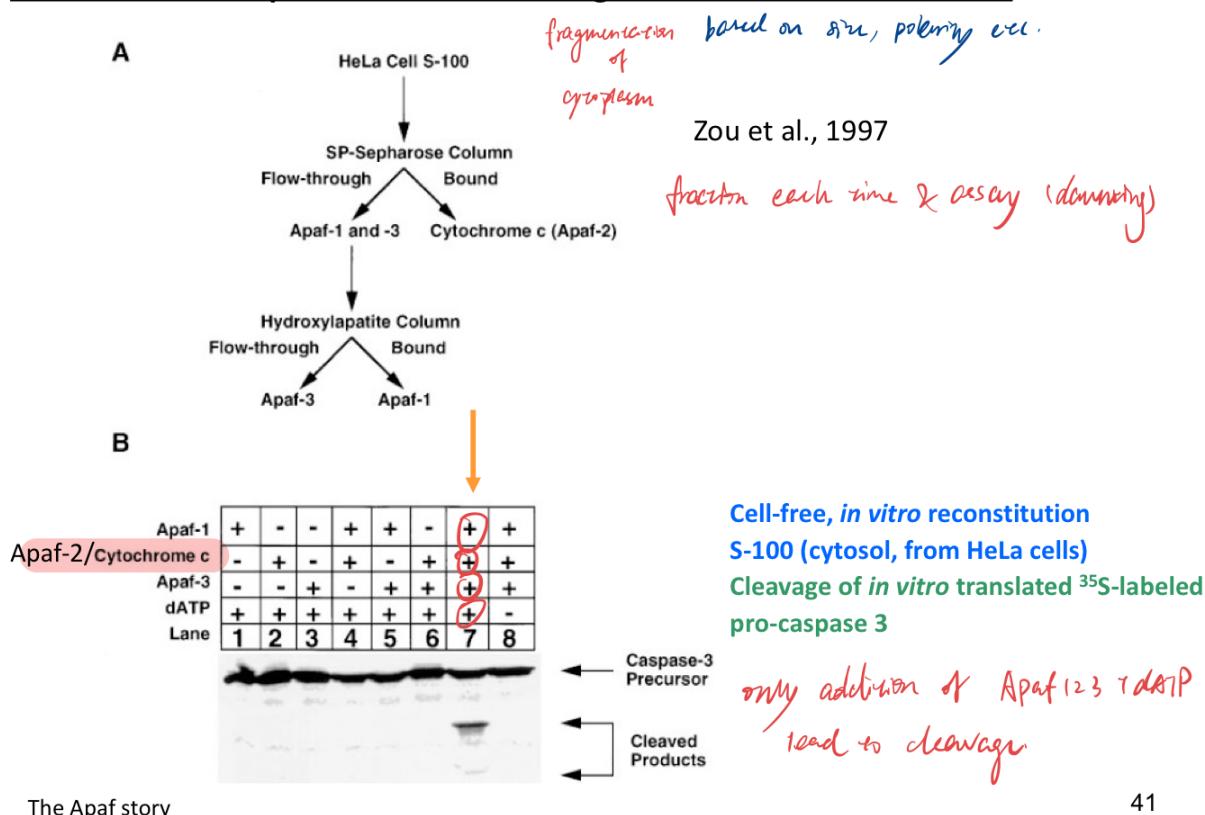
The Apaf role in apoptosis — found by using HeLa cells

In vitro Reconstitution of Caspase 3 Cleavage and Activation:



1. Only addition of dATP can activate caspase

Identification of Apaf 1-3 activities through biochemical fractionation:

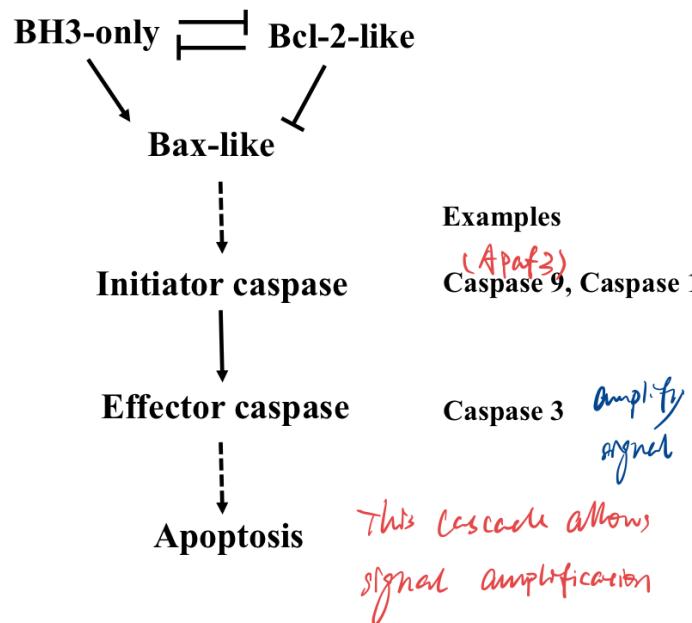


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2. Only addition of Apaf-1 Apaf2 (cytochrome C) Apaf-3 and dATP can activate caspase
3. Apaf-3 shares the same structure as cytochrome C, and it's identical to Caspase 9
 - Caspase 9 is the initiator caspase

'Initiator' and 'effector' caspases are activated sequentially

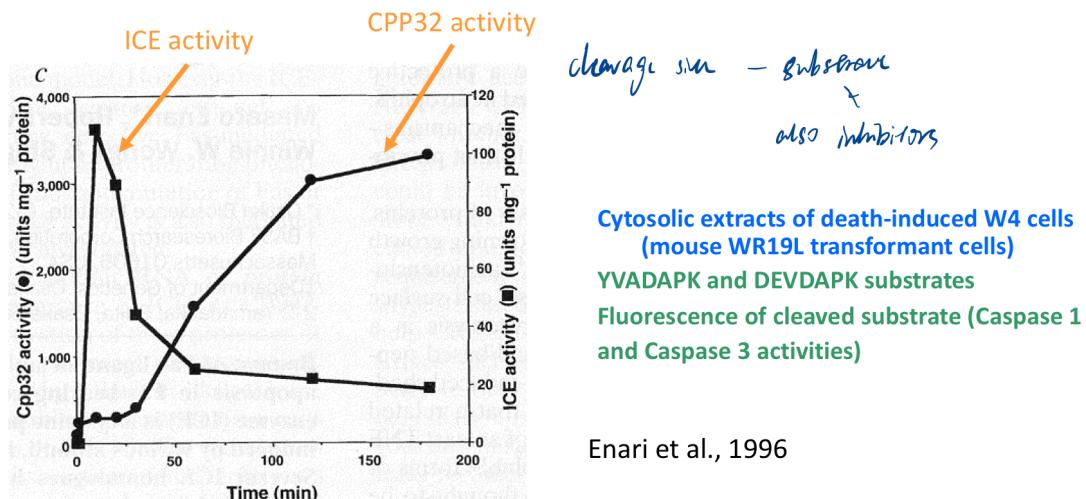
Mammals



- Cascade: initiator caspase → effector caspase; purpose: signal amplification

This found by the time-lapse activity of different caspase

ICE/Caspase 1 activation occurs before CPP32/Caspase 3 activation



- YVADAPK is a **specific substrate for caspase-1** because it contains the amino acid sequence recognized by this caspase. When caspase-1 is active, it **cleaves the peptide bond between the aspartic acid (D) and alanine (A) residues** in the YVAD sequence. This cleavage releases a fluorophore or a chromophore, which can be measured spectrophotometrically or fluorometrically, allowing quantification of caspase-1 activity.
- DEVDAPK acts a **specific substrate fro caspase-3**
- Cpp32 is often referred to **Caspase-3**

▼ To find where Apafs are involved in the regulation: Apaf-2 and Cytochrome C

1. Cytochrome C is located at the **inner membrane of mitochondria**
 - a. transfer electrons from complex III to complex IV

Apaf-2 is identical to Cytochrome c:

Apaf-2

Cytochrome c

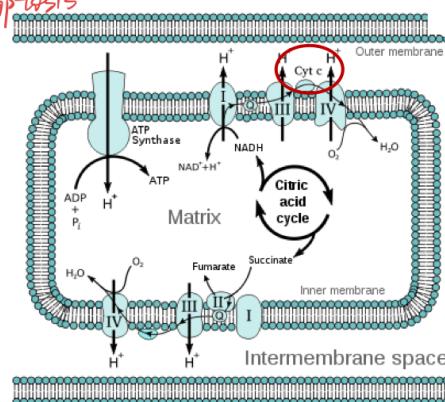
Apaf-2 is structurally identical to cytochrome c

- is an essential component of the mitochondrial electron transport chain
Can transfer e⁻ between Complex III & Complex IV
- resides in the intermembrane space loosely associated with the inner mitochondrial membrane

Cytochrome c can induce apoptosis

Liu et al., 1996

Where does cytochrome c come from?



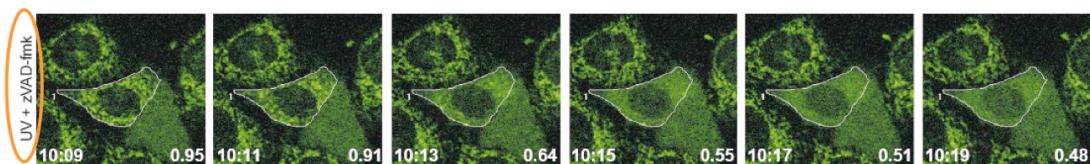
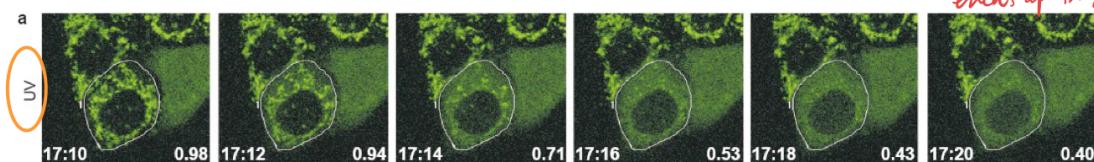
2. Upstream / downstream of caspase cleavage?

Even without caspase cleavage - still apoptosis

Cytochrome c is released from mitochondria in apoptotic cells

relocal.

ends up in cytoplasm



Functional Cytochrome c-GFP fusion protein

Expression in HeLa Cells

UV treatment to induce apoptosis

+/- zVAD-fmk (caspase inhibitor) — Cytochrome C upstream from Caspase

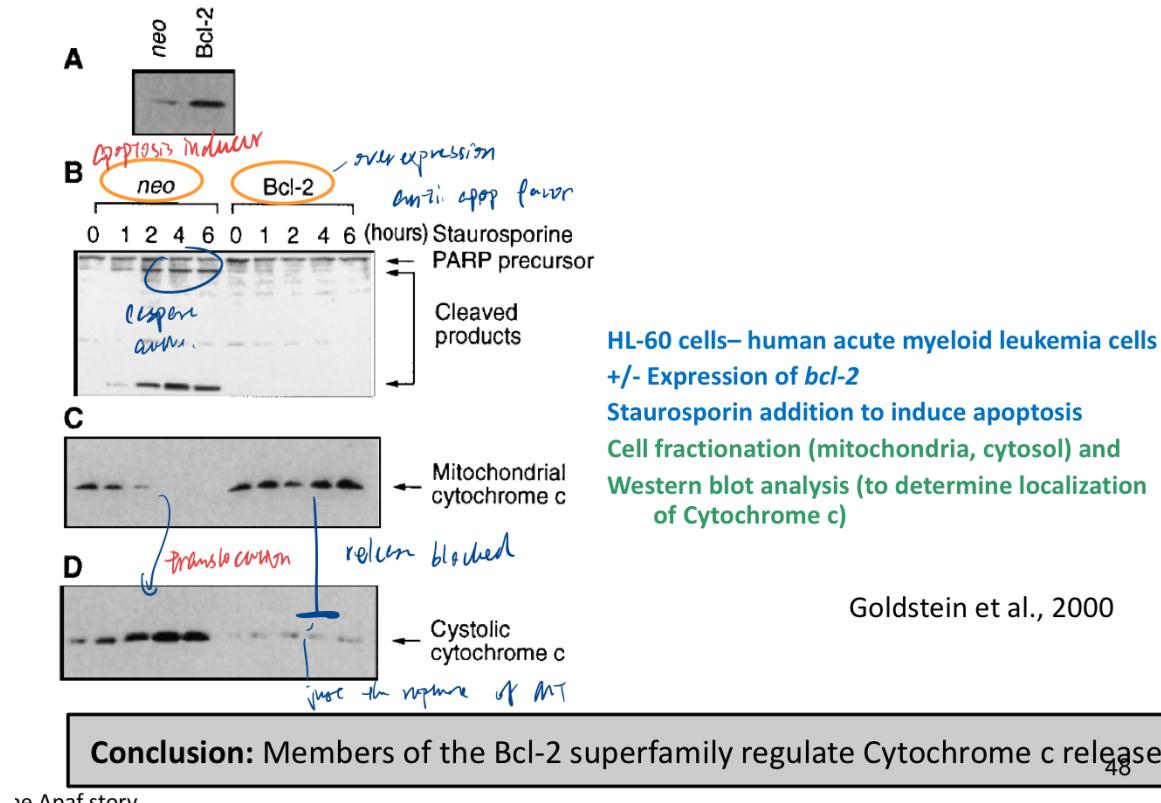
Time-lapse, fluorescence microscopy (Cytochrome c relocalization)

Same efflux : Goldstein et al., 2000

Conclusion: Cytochrome c release occurs before caspase activation

- By using **caspase inhibitor**, the mitochondria cytochrome C still translocates from **mitochondria to cytosol**
- cytochrome c release occurs before caspase activation
- **Upstream or downstream of Bcl-2?**

Cytochrome c release is blocked by Bcl-2 over-expression

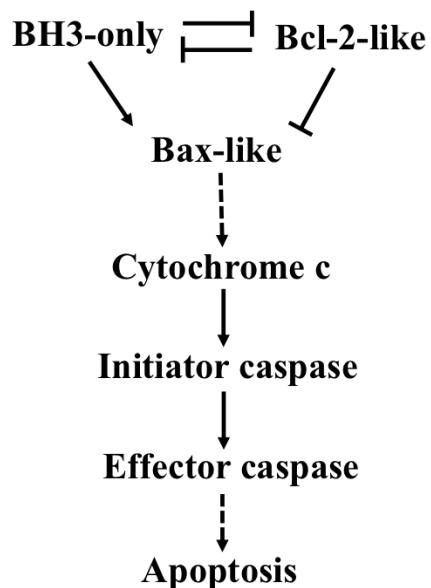


ie Apaf story

1. without Bcl-2 & with apoptosis inducing factor:
 - a. there are cleaved caspase
 - b. decreased mitochondria cytochrome c → increased cytosolic cytochrome c: translocation
2. with the overexpression of Bcl-2:
 - a. no cleaved caspse → anti-apoptotic effect
 - b. fewer translocated /released cytochrome c
 - **Therefore, this proofs that Bcl-2 acts upstream of Apaf!**

The Bcl-2 superfamily controls the release of cytochrome c

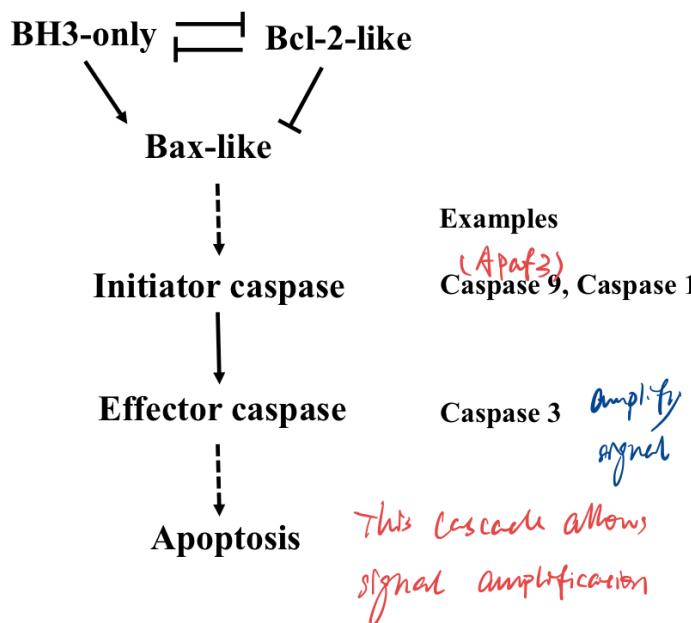
Mammals



▼ Apaf-1 function (similar to Ced-4 in C. elegans)

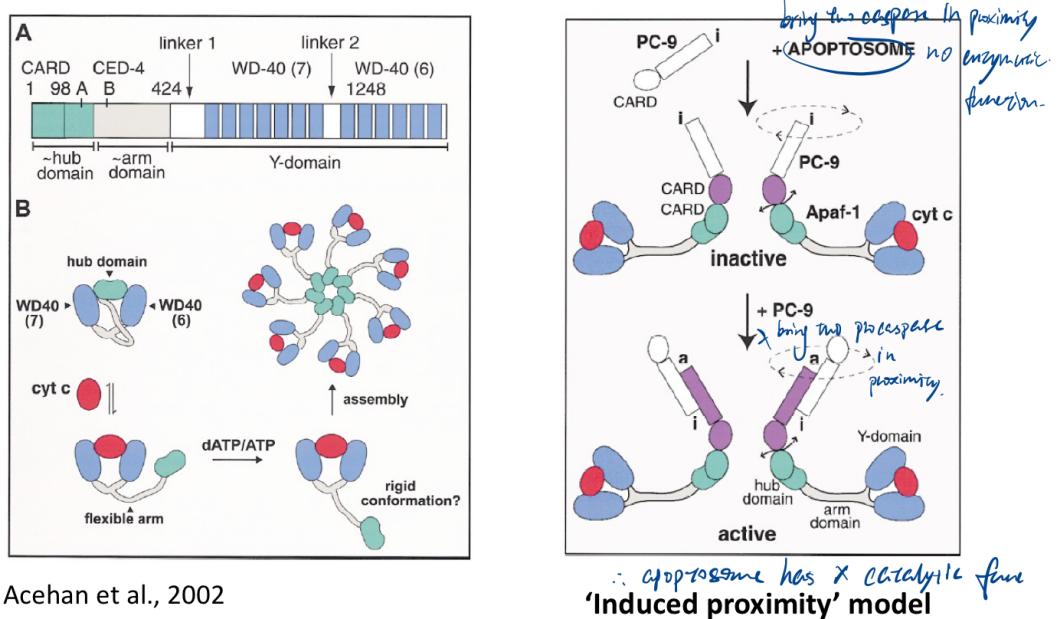
'Initiator' and 'effector' caspases are activated sequentially

Mammals



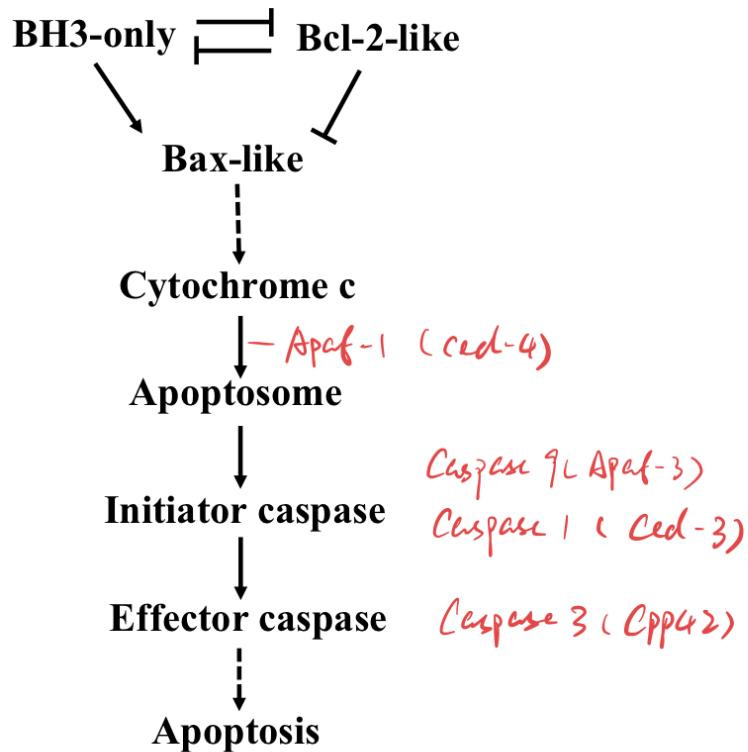
- **Caspase 9 (Apaf-3); Caspase 1 (ICE, Ced-3); Cytochrome c (Apaf-2); Apaf-1 (Ced-4) ; are all required for activation of Caspase 3 (Cpp32)**
- ▼ Apaf-1 (Ced-4) → Apaf-2 → Apaf-3 (Caspase 9) & Ced-3 (Caspase 1) → Effecter caspase (Caspase 3 - Cpp32)
 - **Apaf-1 and cytochrome C form apoptosome**
 - Apaf-1 self-assembly into monomer
 - When cytochrome C is released into cytosol: recruited to Apaf-1
 - addition of dATP causes conformational change
 - form **7-subunit** heptameric polymers
 - procaspse 9s bind, bring two in close proximity → transactivation
 - Therefore, apoptosome does not have catalytic ability → just bring two molecules in close proximity

...to which proCaspase 9 (PC-9) molecules can bind, thereby enabling their maturation and activation



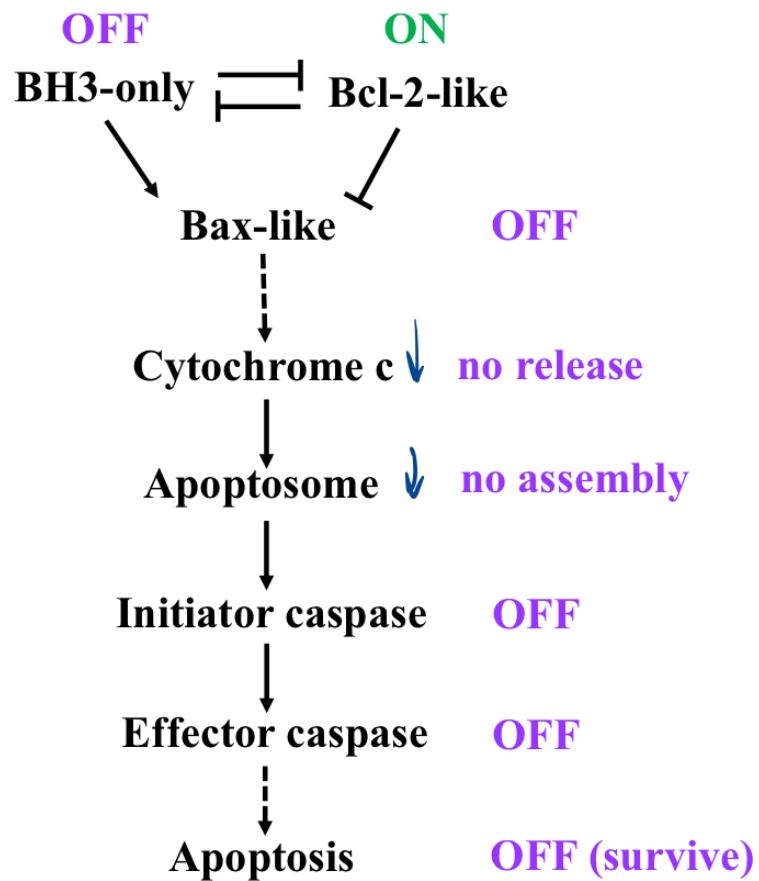
Acehan et al., 2002

Cytochrome c-dependent apoptosome assembly is required for caspase activation



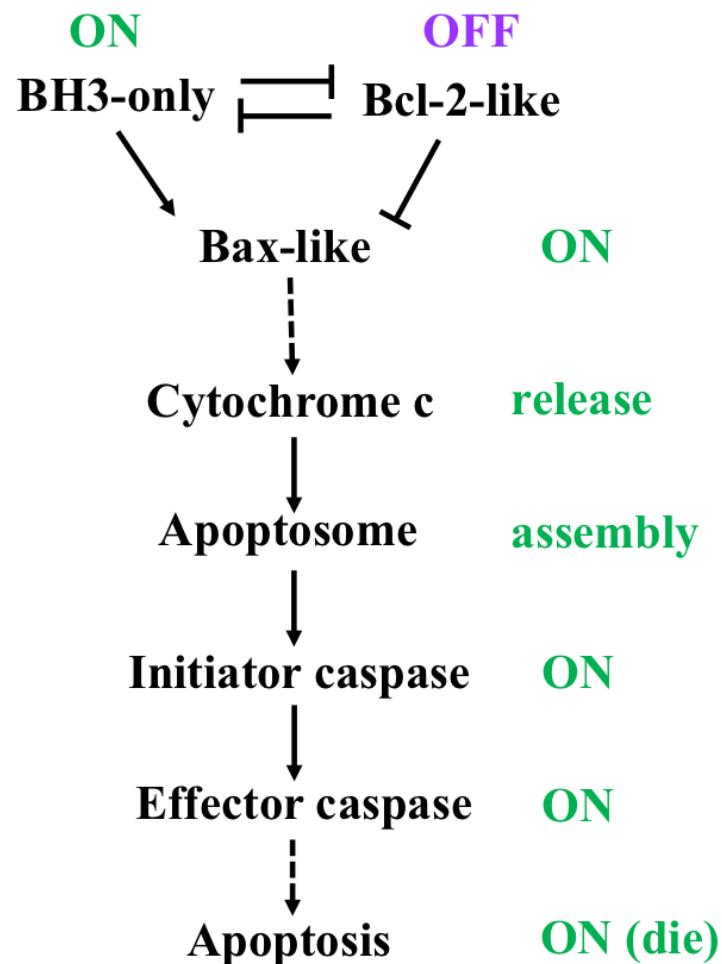
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When BH3-only proteins are 'OFF', cells survive



...
...

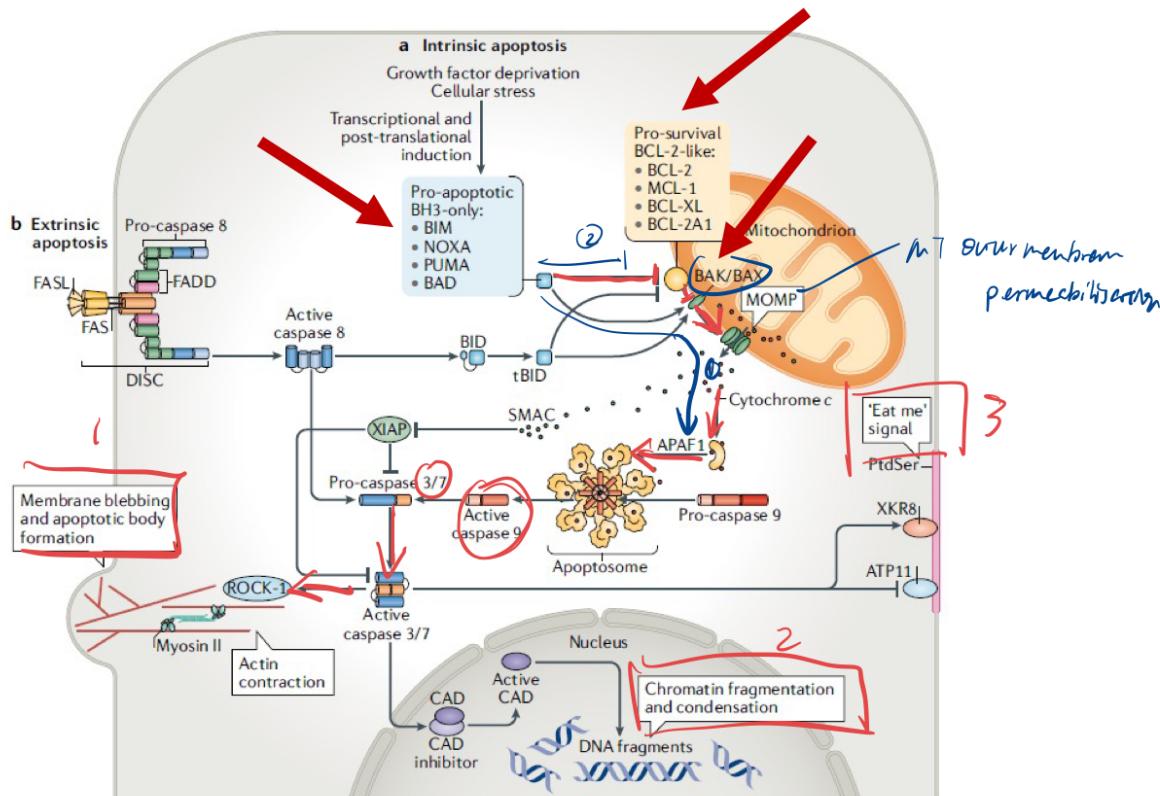
When BH3-only proteins are ‘ON’, cells die



Apoptosis cellular activation (Intrinsic)

Molecular mechanisms of apoptosis pathway activation

LOOK FOR: Bcl-2 superfamily



osis pathway

Bedoui, Herold, Strasser, 2020

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1. Pro-apoptotic **BH3-only protein** (including BAD) inhibit **Bcl-2 like pro-survival protein**
2. Leads to the **disinhibition of BAK/BAX**
3. Activation of **MOMP** (mitochondria outer membrane permeabilisation)
4. **release cytochrome C to cytosol**
5. cytochrome C binds to **ApaF-1**
6. activation of procapase 9
7. initiator caspase (caspase 9)activates effector **caspase 3**
 - a. Active caspase 3 activates Rho-Rock pathway → actomyosin contraction → membrane blebbing

- b. Active caspase 3 activates CAD in the nucleus → DNA fragmentation and condensation
- c. Active Caspase 3 activates XKR8 (as a scramblase?) & inhibits ATP11 to overall flip PS to outer leaflet & inhibit ATP11

▼ Active ongoing questions

- 1. How is the activity of BH3-only proteins controlled, via growth factor deprivation, cell stress, transcriptional and PTM induction?
- 2. How do Bax-like proteins cause cytochrome c release?
 - a. might have a domain with channel formation ability to form pores at outer membrane