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Apoptosis: yeast as a model for the study of Programmed Cell Death

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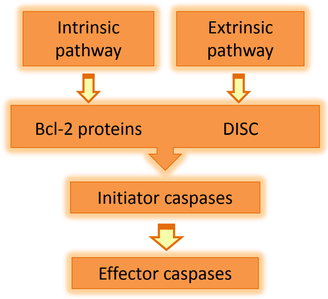
Apoptosis

Apoptosis is an extremely regulated cell death program which is essential during development and for the maintenance of cell turnover in adult tissues. The study of this process is currently of great interest in biomedical science as it is involved in neoplastic events and viral infections as well as in neurodegenerative and cardiovascular diseases.



Yeast as a model organism

- It is an eukaryotic system
- Several genes involved in human disease have yeast orthologs.
- Many biochemical mechanisms are conserved from yeast to human.
- Allows easy genetic manipulation
- Applications as an experimental tool include:
  - Protein-fragment complementation assays
  - Drug-screening assays
  - Functional assays by heterologous expression of human proteins (humanized yeast)

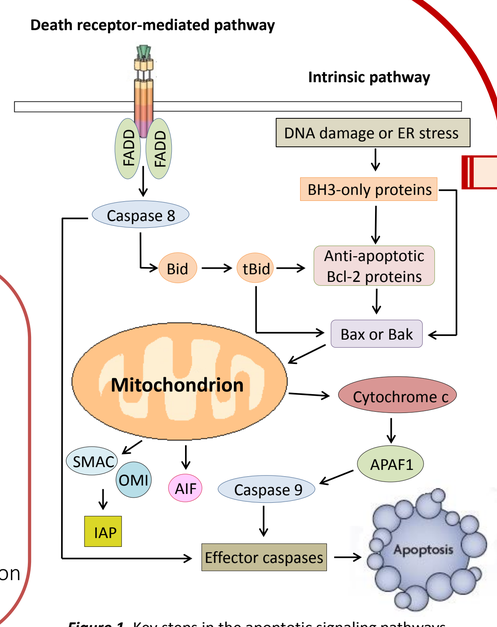


Figure 1. Key steps in the apoptotic signaling pathways.

The Bcl-2 family of proteins

- The members of the Bcl-2 family are the main regulators of the intrinsic pathway of cell death.
- These proteins control the efflux of cytochrome c and other intermembrane proteins from mitochondrion to the cytosol where the first one associates to Apaf1 and to pro-caspase 9 to form the apoptosome complex.
- This complex activates pro-caspase 9 which in turn activates effector caspases.
- Bcl-2 pro-apoptotic members activation leads to Mitochondrial Outer Membrane permeabilization (MOMP).

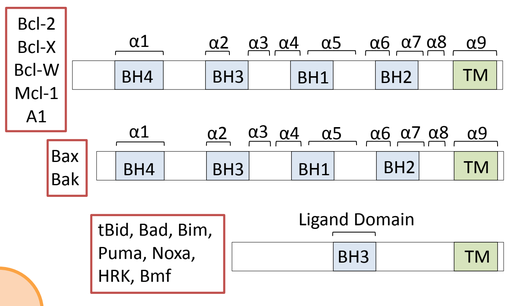


Figure 2. Homology domains of Bcl-2 family of proteins.

Pro-apoptotic effector proteins

- Bak is inserted in the MOM.
- Bax is translocated to the mitochondrion after the apoptotic signal.

Bax and Bak both oligomerize and lead to the formation of a pore in the MOM through which cytochrome c is released.

Pro-survival proteins

Bcl-2, Bcl-X, Bcl-W, Mcl-1 and A1 are anti-apoptotic proteins. They avoid formation of the pore by association with Bax or Bak.

Pro-apoptotic BH3-only proteins

Bid, Bad, Bik, Bim, Puma and Noxa are proteins constituted by a unique BH3 domain.
Its role in the cell can be explained by two different models :

- **Direct model**: Bax and Bak are activated by BH3-only proteins.
- **Indirect model**: Pro-survival proteins are inhibited by BH3-only proteins.

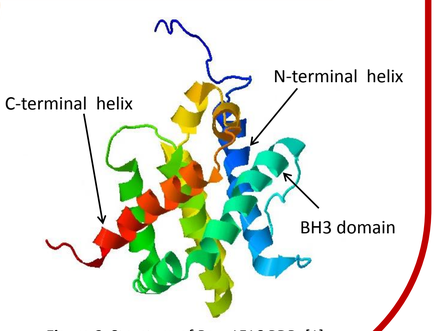


Figure 3. Structure of Bax. 1F16 PDB. [1]

Saccharomyces cerevisiae triggers an apoptotic phenotype

Saccharomyces cerevisiae

- Mutants in CDC48p show typical markers of apoptosis. This is the first indication of the apoptotic process in yeast.
- A yeast protein named Ybh3p harbours a BH3 domain similar to the pro-apoptotic proteins found in mammals.
- The expression of human disease associated proteins and pro-apoptotic proteins such as Bax and Bak, triggers apoptosis in yeast.
- Caspase activity: *S.cerevisiae* has a metacaspase (YCA1).
- Deletion of the ASF1/CIA histone chaperone in yeast leads to cellular cycle arrest at G2/M stage and cellular death with apoptotic traits.
- HtrA/Omi y AIF, two of the proteins that leave mitochondrion after the apoptotic signal, have yeast homologs: Nma111p and Aif1p.

Control of apoptosis by Bcl-2 family members

1 Evidences of two different models of activation

Direct Model

- BH3-only proteins induce a conformational change in Bax leading to its insertion into the MOM.
- The interaction with BH3-only proteins activates Bax and Bak, leading to the formation of higher order oligomers.

Indirect Model

- BH3-only proteins bind to Bcl-2 anti-apoptotic proteins and prevent their binding to Bax and Bak, triggering apoptosis.

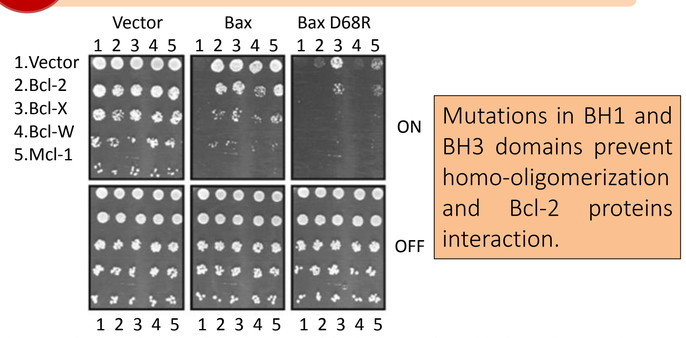
Growth viability assays in yeast reveal that BH3-only proteins are unable to directly potentiate the activation of effector pro-apoptotic proteins.

3 BH3-only proteins are anchored to the MOM

➢ BH3-only proteins could activate Bax in the cytosol or at the MOM.

In vitro mitochondrial import assays suggest integral membrane insertion of these proteins by its C-terminal end.

2 Bcl-2 proteins interact via their BH3 domain



Mutations in BH1 and BH3 domains prevent homo-oligomerization and Bcl-2 proteins interaction.

Figure 4. **The BH3 domain of Bax is essential for its interaction with the anti-apoptotic proteins.** Yeast are co-transformed with the corresponding Bcl-2 prosurvival protein and Bax or mutant Bax D68R under an inducible GAL promoter. ON (in the presence of galactose), OFF (in the presence of glucose). [2]

Bax and Bak induce pore formation

➢ With activation of the intrinsic pathway, the mitochondrial membrane loses its integrity and permeabilizes. Some evidences suggests that this is due to pore formation in the MOM.

➢ Different models describing this process have been proposed:
- Formation of proteinaceous channels.
- Lipidic pore formation induced by Bax and Bak.
- Pore formation influenced by Bax, Bak and mitochondrial membrane lipids.
- Increase in permeability of an existing channel induced by Bax and Bak.

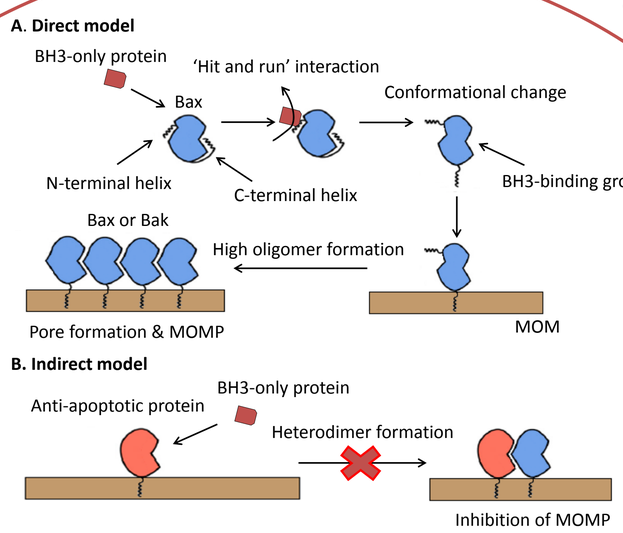


Figure 5. Mechanisms of Bax and Bak activation and Bcl-2 sensitization triggered by BH3-only proteins. [3]

Apoptosis and therapeutic agents

➢ BH3 mimetic inhibitors like ATB-737 or ABT-263, which antagonize the anti-apoptotic proteins, exhibit a great potential for cancer therapy.

➢ Like BH3-only proteins, these peptides bind to the anti-apoptotic proteins and prevent apoptosis inhibition.

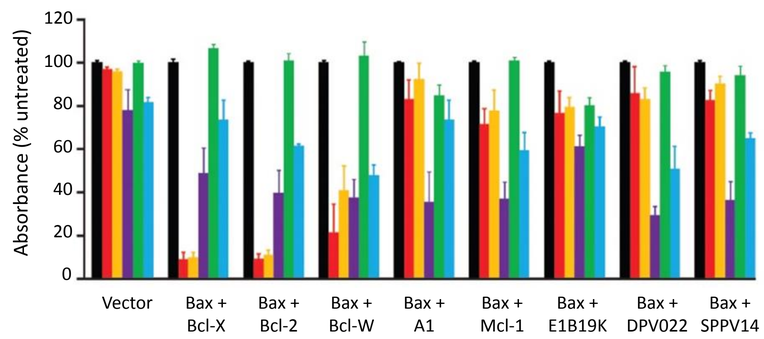


Figure 6. **Impact of different drugs on yeast expressing Bax with Bcl-2 anti-apoptotic members.** E1B19K, DPV022 and SPPV14 are viral Bcl-2 proteins. This graph displays the absorbance of each drug-treated culture when the corresponding untreated culture was closest to 0.5. DMSO (black), ABT-737 (red), ABT-263 (orange), TW-37 (violet), HA14-1 (green) and Obatoclax (blue). [4]

➢ *S.cerevisiae* can also be used to identify:
- Caspase activators
- IAP antagonists (anti-inhibitors of apoptosis)

Conclusions

Suitability of the yeast as a model for apoptosis:

- ✓ It has an apoptotic machinery similar to that present in mammals.
- ✓ It is a low-complexity model that allows study of individual interactions between the molecules involved in this pathway.
- ✓ It is useful for screening inhibitors of anti-apoptotic proteins.
- ✓ It is suitable for testing new drugs prior to its use in mammalian cell lines.
- ✘ It lacks the anti and pro-apoptotic molecules observed in mammals.
- ✘ The results cannot be extrapolated to a multicellular organism and they must be validated in animal models.
- ✘ Recent and major improvements in mammalian cell culture media leave this model aside.

Applications and perspectives:

Many diseases are linked to apoptotic processes. Understanding how the process is regulated in a simple model like the one herein presented might help develop effective therapies against these pathologies. *S.cerevisiae* is a useful tool for studying the mechanism of action of the Bcl-2 family of proteins and for the comprehension of its function in the cell. It can also be used for the identification of new therapeutic targets and for activity and specificity evaluation of different drugs.

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