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


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 of great interest in biomedical science as it is involved in neoplastic events and viral infections as well as in neurodegenerative and cardiovascular diseases.

**Apoptosis 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:
  - Phenotypic fragment complementation assays
  - Drug-screening assays
  - Functional assays by heterologous expression of human proteins (humanized yeast)

**Yeast as a model organism**

- It is a unicellular system
- Many genes and proteins involved in cell death are conserved between yeast and mammals.
- Many biochemical mechanisms are conserved from yeast to human.
- Allows easy genetic manipulation
- Applications as an experimental tool include:
  - Phenotypic fragment complementation assays
  - Drug-screening assays
  - Functional assays by heterologous expression of human proteins (humanized yeast)

**Saccharomyces cerevisiae triggers an apoptotic phenotype**

Mutants in CDC48p show typical markers of apoptosis. This is the first indication of the apoptotic process in yeast.

- A yeast protein named Ychp harbours a BH3 domain similar to the pro-apoptotic proteins found in mammals.
- The expression of human disease associated proteins and anti-apoptotic proteins such as Bax and Bak triggers apoptosis in yeast.
- The interaction with BH3-only proteins activates Bax and Bak, leading to the formation of higher order oligomers.
- BH3-only proteins bind to Bcl-2 anti-apoptotic proteins and prevent their binding to Bak and Bax, triggering apoptosis.
- Growth viability assays in yeast reveal that BH3-only proteins are unable to directly potentiate the activation of effector pro-apoptotic proteins.
- BH3-only proteins are anchored to the MOM
- BH3-only proteins could activate Bak in the cytosol or at the MOM.

**Control of apoptosis by Bcl-2 family members**

- BH-only proteins induce a conformational change in Bax leading to its insertion into the MOM.
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**Bcl-2 family proteins**

- The BH3-only proteins 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 they associate with 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).

**Pro-apoptotic effector proteins**

- Bak is inserted in the MOM.
- Bax is translocated to the mitochondrion after the apoptotic signal.
- Bak and Bax both oligomerize and lead to the formation of a pore in the MOM through which cytochrome c is released.

**Pro-apoptotic BH3-only proteins**

- Bak, Bcl-x, Bim, Puma and Noxa are proteins constituting 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.

**Apoptosis and therapeutic agents**

- BH3 mimetic inhibitors like ABT-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.

**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 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 lead 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. BH3 mimetic 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 useful for activity and specificity evaluation of different drugs.

**References**