

FUNCTIONS AND MUTATIONS OF THE PROAPOPTOTIC PROTEIN BAX IN YEAST CELLS

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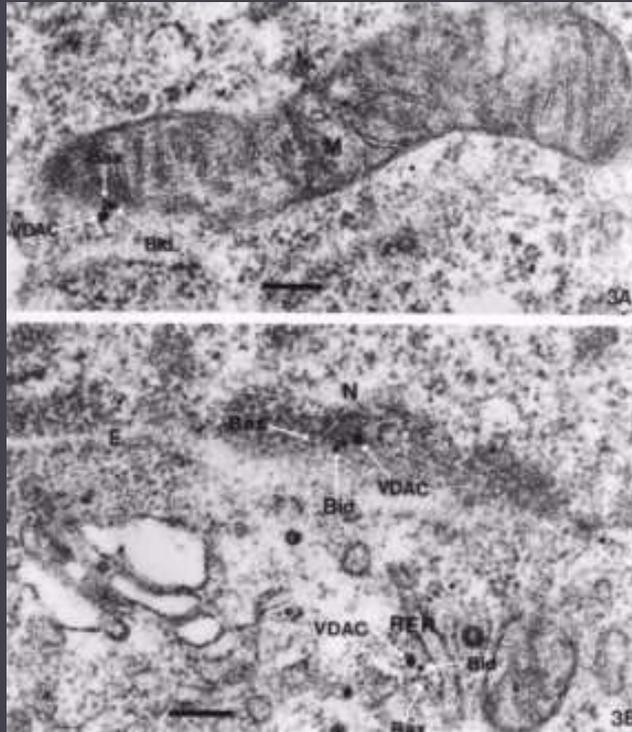
Project Goal

- To determine if changes in the interaction of the mitochondria and the endoplasmic reticulum (ER) will affect the activities of the bax protein

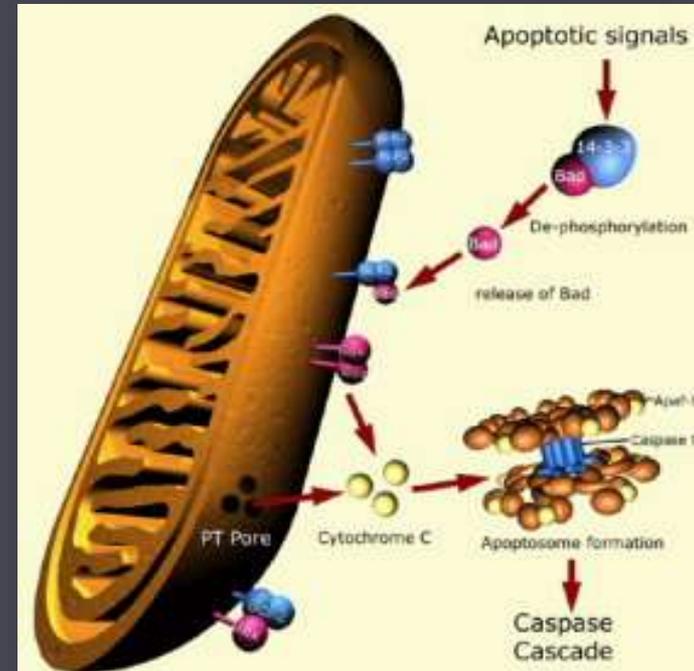
Apoptosis and the Bax Protein

- Apoptosis is the intentional programmed death of cells
- Bax is one of the main regulators of apoptosis in multicellular organisms.
- Apoptosis is used in development, to prevent the spread of infectious organism, or to halt the progression on cancer cells.

Apoptosis and the Bax Protein



An electron microscope picture of the Bax protein integrating into the outer mitochondrial membrane [1]



A diagram of the complete caspase cascade that leads to cell death [2]

Mechanism of Action

- ⦿ When apoptosis is needed, a signal cascade is activated leading to the activation of bax.
- ⦿ Bax facilitates cell death by punching holes in the mitochondrial membrane
- ⦿ Very little is known about how Bax inserts.
- ⦿ When this occurs the contents of the intermembrane space, mainly cytochrome c, are spewed out in to the cytoplasm and in turn setting off even more proapoptotic processes.

Mechanism of Action

- New research suggests that the endoplasmic reticulum and the mitochondrial outer membrane may be connected or at least very closely associated [3]
- This experiment was to explore if the bax protein was somehow using this connection to gain access to the membrane.

Saccharomyces cerevisiae

- ◎ Yeast
- ◎ A strain called JOY11 was used, which had been modified to produce bax
 - JOY11 contains a doxycycline-repressed promoter
 - Genotypic Marker- unable to grow on YPEG plates (ethanol is only carbon source) because the bax protein interacts with mitochondria, inhibiting oxidative phosphorylation, and interfering with energy production.

Saccharomyces cerevisiae

- ⦿ Yeast is a good model system for apoptosis because:
 - ⦿ it lacks any clear programmed cell death system of its own
 - ⦿ could be modified to produce the bax protein
 - ⦿ has an apoptotic response similar to multicellular eukaryotic organisms

Methods

- The goal was to see whether changing the interaction of the mitochondria and the endoplasmic reticulum (ER) will affect the bax protein.
- To determine this, a group of mutations, which affect the binding between the mitochondria and the ER, were selected and were crossed with the bax containing strain, JOY11.

Methods

- ⦿ The strains were crossed by forcing them to sporulate and form diploids.
 - This was done by growing them on a minimal media and then selecting for diploids.
- ⦿ These diploid spores were then dissected, separated and then spaced apart from each other on a plate, using a tetrad dissecting scope.
 - Diploids will follow mendelian genetics. At least one of the spores should carry both traits.
- ⦿ The tetrad plates were then replica plated to various medias to identify double mutants.

Methods

- Once identified, the double mutants were plated on different medias, such as YPEG and YPD, and their growth was observed.
- Growth of the double mutants was evaluated for:
 - Whether double mutants regained the ability to use oxidative phosphorylation by growth on YPEG
 - different colony morphology or growth speed
 - Whether it effected growth at different temperatures (incubated at 15°C, 30°C and 37°C)

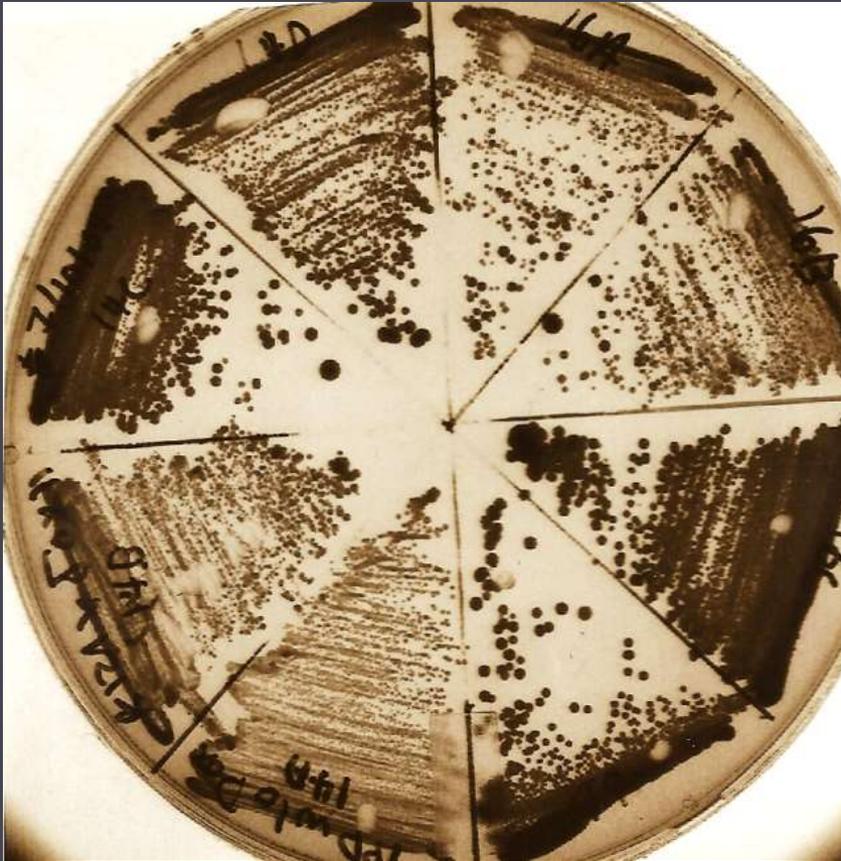
Marking MTY71 (VPS13-613)

- MTY71 was one of the strains crossed with JOY11.
- This strain contains a mutation in the VPS13 gene which produces a protein peripherally associated with membranes.
- In an effort to make this project simpler, an attempt was made to mark VPS13 with a URA3 marker.
- URA3 marker was inserted at the 3' end and then checked with PCR done with conformation primers.

Results

- Managed to create a double mutant for all the strains crossed
- On YPD there was no notable growth differences between the double mutant and the single mutations.
 - The double mutants growth was neither inhibited nor enhanced by the presence of mitochondrial changes.
- On YPEG there was no notable growth difference between the double mutant and the single mutations.

Results



- Example of plate containing double and single mutants
- No noticeable growth differences between strains

Results

- There was no noticeable growth differences between the strains grown at different temperatures.
- VPS13-613 was not marked with URA3 marker. Various transformations did not successfully insert the marker in the correct spot.

Problems Encountered

- ⦿ There were complications due to poor spore viability.
 - The spore could be located and were found to be ungerminated.
 - Initially, it was purposed this was caused by problems from combining bax with mitochondrial mutations. This theory was discarded after many plates were picked but there was no clear, discernible pattern as to which spores would not grow
 - It was found that the doxycycline-repressed promoter for the bax gene was not sufficiently turning off expression.
 - To solve this problem, the amount of doxycycline per plate was doubled to 150 μ l at 12.5 ng/ μ l

Problems Encountered

- ⦿ Attempts to create a marked VPS13-613 gene failed.
- ⦿ The transformation produced uracil positive yeast without the URA3 marker near the gene of interest.
- ⦿ It was proposed this was happening because the URA3 marker was unintentionally inserting itself back in the original URA3 site.
- ⦿ To fix this a different strain, one that had its URA3 site partially removed, was used to no success.

Discussion

- Based on my results I believe that my original hypothesis was incorrect.
- The effect of bax on yeasts cells ability to respire is not related to mutations that bind the mitochondria to the endoplasmic reticulum.
- By extension, bax point of entry into the mitochondrial outer membrane is probably not related to the connection between the mitochondrial outer membrane and the endoplasmic reticulum.

References

- [1] Gajkowska, B. Figures. Mossakowski Medical Research Centre. (2007)
- [2] Dash, P. Role of Mitochondria in Apoptosis. Reproductive and Cardiovascular Research Group. (2008)
- [3] Williamson, C.D., Colberg-Poleley, A.M. Access of viral proteins to mitochondria via mitochondrial-associated membranes, (2009)