Isolation and characterization of regulators of oxidative stress induced apoptosis in yeast

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ABSTRACT

This project is a long-term project aimed at identifying possible mechanisms that connect the oxidative stress pathway to the apoptosis or cell death regulatory machinery in the budding yeast, *S. cerevisiae*. We have generated all the necessary yeast strains and gene expression systems, including a strain of yeast that expresses the mouse BCL-2 gene, an inhibitor of apoptosis. To increase the probability of isolating the appropriate mutants, the growth conditions for the genetic screen have been reworked over the past year to include an apoptosis inducer, hydrogen peroxide, in the growth medium. To date, all conditions for the screen have been established and tested. An exhaustive genetic screen will require analyzing nearly 250,000 yeast colonies. We have begun screening for the appropriate mutant cells and the identification of these mutants will be presented.

INTRODUCTION

Apoptosis or programmed cell death has been linked to the pathogenesis of many human diseases characterized by uncontrolled cell accumulations or loss including cancer, autoimmune diseases, neurodegenerative diseases, and AIDS.
In addition, apoptosis may contribute to the general decline of physiological function associated with aging.
Some elements of the apoptotic pathway are conserved in yeast and animals and are therefore, part of a basic, evolutionarily old mechanism.
Study of these mechanisms in yeast may be useful to trace the roots of apoptosis and solve some of the problems and apparent disagreements inherent in the current models of apoptosis. Research from a number of groups suggests that oxidative stress plays a major role in yeast apoptosis.

GROWTH CONDITIONS

Once the Bcl2 gene is in the yeast cells, they are mutagenized with UV light to approximately 15% survival, allowing DNA damage to induce mutations. The cells are then grown in the presence of hydrogen peroxide, which acts as a reactive oxygen species and initiates cell death.

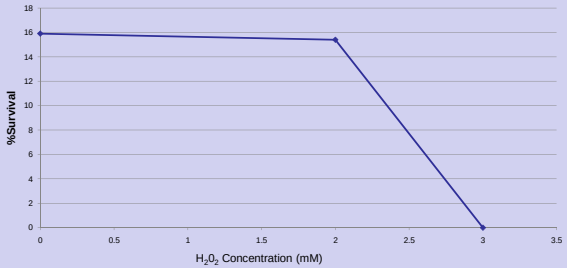

Figure 1: The percentage of UV treated yeast cells that survived when grown on different H_2O_2 concentrations (mM). As a control, untreated yeast cells were grown on equal H_2O_2 concentration (mM).

EXPERIMENTAL DESIGN

Figure 2: Diagram of possible colony colors due to presence or absence of plasmid. If ade3- ade2- yeast cells require Bcl2 to survive under oxidative stress they will form red colonies due to the expression of ADE3. Cells that do not require Bcl2 will be able to lose the plasmid (and thus lose expression of ADE3) to form white colonies.

Expression of Mouse Bcl2 in yeast

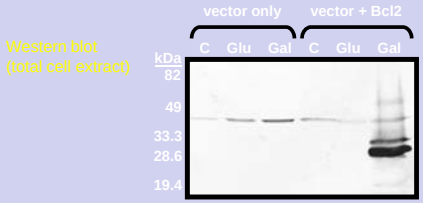

Figure 3: Represents a Western blot verifying the expression of a Bcl2 gene under induced conditions (galactose). This Western blot also included testing for vectors with and without a Bcl2 gene under different conditions including: controlled (C), glucose (glu) and galactose (gal) induced.

SCREENING

Colonies will be screened for non-sectoring red colonies, indicating a requirement for Bcl2 under oxidative stress. Red colonies form when the plasmid is present because it blocks the pathway at the point where it expresses ADE3 to produce red colored cells. If the yeast does not rely on the Bcl2 gene for survival they will eventually lose the plasmid and will begin to sector forming red and white sectoring colonies.

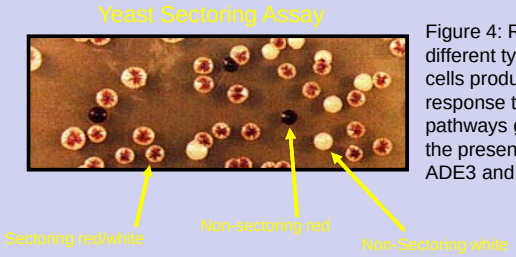

Figure 4: Represents different types of yeast cells produced as a response to biochemical pathways generated by the presence or lack of ADE3 and Bcl2 genes.

FUTURE DIRECTIONS

Fully characterize mutants isolated in the genetic screen
Clone wild-type yeast genes by complementation of mutant phenotype
Screen human cDNA libraries for those that complement the mutant phenotype