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Using yeast to reveal the function of the mammalian pro-apoptotic BAX molecule.
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Apoptosis is a critical process for the maintenance of tissue homeostasis in multicellular organisms. Pro-apoptotic proteins in the BCL-2 family (e.g. BAX) constitute a critical checkpoint in the apoptotic cascade by functioning as major executioners of a mitochondrial apoptotic program. However, the exact function of these molecules is still largely unknown. We are using the yeast *S. cerevisiae* as a model system to reveal the function of BAX, since a simple eukaryotic organism may facilitate our efforts. Expression of BAX in yeast results in its translocation to mitochondria, mitochondrial hyperpolarization, production of reactive oxygen species (ROS), and cell death. ROS production is important for BAX-induced yeast cell death, since adding the anti-oxidant N-acetylcystein to the culture medium reduces the levels of intracellular ROS, and inhibits the ability of BAX to kill yeast cells. Moreover, yeast cells become completely resistant to BAX under hypoxia conditions. To identify the downstream targets of BAX in yeast, we acquired the complete collection of yeast deletion strains (~5000 strains), each carrying a single non-essential gene knockout, and we are screening for strains which are resistant to BAX toxicity. Preliminary results indicate that BAX needs proteins involved in maintenance of mitochondrial morphology. Identifying the pathway triggered by BAX to induce cell death in yeast, could provide important insights to its mechanism of action in mammalian cells.

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Selection of longlived mutants of *S. cerevisiae*.
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The most important genetic avenue to analysis of the ageing process are long-lived mutants. This is because mutations in many different genes will cause a decrease in the overall fitness and therefore may also result in a shortened lifespan. This tells us nothing about the physiology that is specific to the ageing process. It has not been possible up to now to select directly for long-lived mutants in *S. cerevisiae*. This is because of the extreme scarcity of senescent cells in a yeast cell population and due to technical problems with physical separation of old and young cells. We are proposing here to generate a more complete set of long-lived mutants and to identify ageing relevant genes. We used a new mutant isolation and screening system based on strain K6001 (Bobola et al., Cell 84(5):699-709 (1996)). In the absence of galactose, mother cells maintain division and daughter cells cease to divide. The strain works since the essential gene CDC6 is deleted and replaced by a copy integrated under the HO promotor and one copy under the GAL1 promotor. If grown in glucose, the strain divides until the mother cells become senescent, then the population stops growing. The mothers can be followed throughout their lifespan without the experiment being overrun by progeny. This provides a tool for screening mutant strains for their average lifespan, which have been preselected for resistance to oxidants. Presently we are screening a large set of mutants resistant to oxidative stress.

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Effect of mild stress on survival in stationary phase in *Saccharomyces cerevisiae*.
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It has been shown many times that an exposure to a subletal stress can increase longevity (hormesis). In yeast, only one study has been devoted to this topic and showed that repeated mild heat shocks increased the number of divisions in *Saccharomyces cerevisiae*. In the present study, we investigated the effect of mild stress on survival of cells in stationary phase kept in distilled water. Populations were subjected either to 37°C for 90 minutes at the time of inoculation, or to 37°C for 10 hours or 5% ethanol for 1 hour after entry into stationary phase. Then daily, a small sub-sample of each population was taken, diluted and streaked on YPD plates. Three days later, the number of colonies was counted and used to estimate the number of living cells in each original population. The results showed that a mild heat shock before stationary phase did not alter subsequent survival in stationary phase. In contrast, an exposure to stress at the beginning of stationary phase led to a slightly increased survival of the cells. It seems that hormesis can be observed on survival in stationary phase of the cells subjected to the mild stress. In contrast, cells do not pass on to subsequent generations the beneficial effect they received from their exposure to stress, which would explain the lack of effect of the heat shock when applied early in the population growth.