Saccharomyces cerevisiae AS A MODEL ORGANISM TO STUDY Hog1 AND MAMMALIAN HOMOLOGOUS p38 PATHWAY.

ANDREA CAMÍ BONET

ESCOLA MARE DE DÉU DE LA SALUT

Introduction

Saccharomyces cerevisiae is a model organism deeply applicable nowadays, in this research work it will be used to study the cellular response and adapting processes upon osmotic stress, which are controlled by the Hog1 pathway in yeast and mammalian homologous p38. The p38 pathway is particularly interesting because of its dual role as both tumor promoter and tumor suppressor depending on cancer's stage. Through different experiments, the hypotheses of the project will be tested and finally, it will be proven that the Hog1 pathway ensures cell survival upon stress, and therefore, that its deletion or mutation affects the adapting response leading to fatal outcomes. Moreover, phosphorylation of the protein in the cytoplasm is paramount for its entrance to the nucleus and there, perform its catalytic activity.

Hypotheses

The Hog1 pathway is responsible for an adapting response that ensures cell survival upon osmotic stress conditions. Thus, the deletion of the *HOG1* gene will result in cell death because of the lack of adapting response, and its mutation of will cause deficiencies in its efficiency, and this will also cause cell death. Finally, the activation of Hog1 will cause a movement of the protein from the cytoplasm to the nucleus.

Goals

The aim of this work is to determine and understand the role of Hog1 in the following situations. First of all, the differences between the behavior of the cell in normal and stress conditions will be studied. Secondly, the repercussions of eliminating *HOG1* will be looked into in both a normal medium and upon stress. Moreover, it is also of high importance to see the repercussions of mutating *HOG1* instead of eliminating it, in both conditions. These mutations will be directed to the phosphorilative part of Hog1 and the amino acids responsible for its catalytic activity. Lastly, the changes in the movement from the cytoplasm to the nucleus when mutating Hog1 will also be studied.

Methodology

This research work can be divided into two different parts, a more theoretical part, that helped me gain the necessary knowledge to understand the pathway and the osmotic response. And an experimental part. The strains used for the experiments were Wild-type (wt, with the allele at the normal locus), *HOG1Δ* (*Δ,* represents the absence of the gene), *HOG1 TA/YA* (*TA/YA,* mutant that cannot be phosphorylated), and *HOG1 KM* (*KM,* mutant without catalytic activity).

Following the steps of the scientific method, in order to achieve the goals and confirm the hypotheses of this research, three different experiments were conducted, a spotting assay to see cell survival in different conditions (YPD and YPD+0,8M NaCl) growth curves, to analyze sell proliferation of the different strains and how much time it took the cells to get a stabilized growth. Moreover, a Western Blot to study phosphorylation of Hog1 at different time points upon stress. Finally, the results of an immunofluorescence microscopy that had already been published (Ferrigno et al., 1998) were extracted to study the protein's movement.

Results

The spotting assay showed that only the wt strain can survive under stress conditions. In the growth curves, similar behavior is seen, cell proliferation is almost non-existent in the *Δ, TA/YA* and *KM* strains, we can also observe the time it takes for the cell growth to stabilize, if it does.

The western blot shows that Hog1 gets phosphorylated upon stress in both wt and *KM* strains, and after long exposure, this phosphorylation ceases only in wt. For the *Δ* and *TA/YA* strains there is no phosphorylation at any point.

Finally, the results of the immunofluorescence microscopy showed that the protein enters the nucleus and gets concentrated for the wt and *KM* strain, but does not in the *TA/YA* mutant.

Conclusions

From the experiments, it can be concluded that Hog1 is essential for the adaptation process upon osmotic stress. Thus, the absence or mutation of *HOG1* results in cell death in mediums with high NaCl concentration. It has also been seen that it is paramount that the signal of the protein enters the nucleus and performs its catalytic activity, articulating a proper response. In order to do this, phosphorylation of Hog1 in the cytoplasm is required. Overall, the hypotheses were correct.

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