Can we use *Saccharomyces cerevisiae* as a sugar sensor ?

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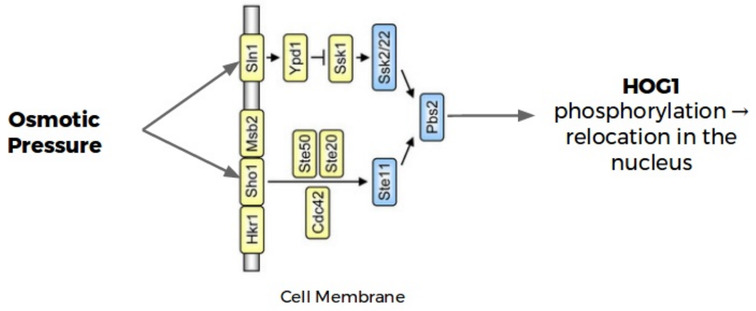
How did we choose our project?

For this final week of Biosensors, we wanted to observe the reaction of Saccharomyces cerevisiae to different concentrations of sugar.

Higher concentrations in the media than the inner cell's concentration of sugar create an **hyperosmostic pressure** in yeast, and we want to observe what happens in the cell during this **osmotic stress**. The pathway which allows the cell to survive to osmotic stress is called HOG pathway.

The protein HOG1, involved in the pathway, move from the cytoplasm into the nucleus during an osmotic stress.



To observe the movement of this protein, we chose to work with the GFP strain of HOG1 for Saccharomyces cerevisiae.

Sources:

Hersen, Pascal, Megan N. McClean, L. Mahadevan, and Sharad Ramanathan. ***"Signal Processing by the HOG MAP Kinase Pathway."*** Proceedings of the National Academy of Sciences 105, no. 20 (May 20, 2008): 7165-70.

Nagiec, Michal J., and Henrik G. Dohlman. ***"Checkpoints in a yeast differentiation pathway coordinate signaling during hyperosmotic stress."*** PLoS Genetics 8.1 (2012)

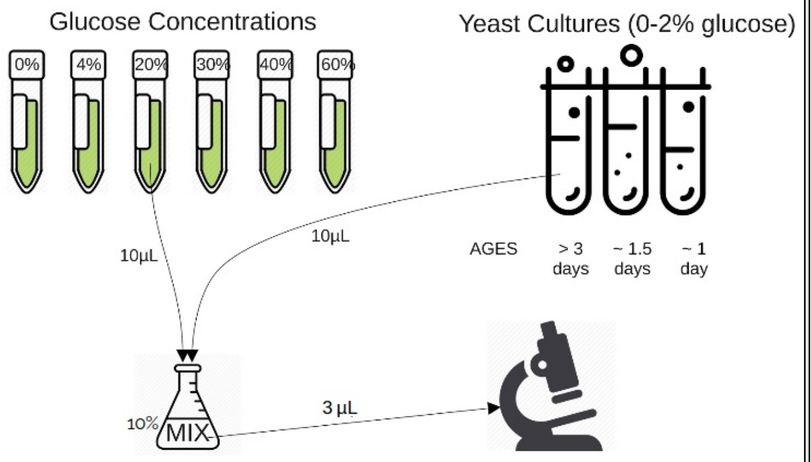
O'Rourke, Sean M., Ira Herskowitz, and Erin K. O'Shea. ***"Yeast go the whole HOG for the hyperosmotic response."*** Trends in Genetics 18.8 (2002) : 405-412.

Saito, Haruo, and Francesc Posas. ***"Response to hyperosmoticstress."*** Genetics2012:289-318.

Hohmann, Stefan. ***"Osmotic stress signaling and osmoadaptation in yeasts."*** Microbiology and molecular biology reviews : MMBR 66.2 (2002) : 300-372.

Our protocol

We experiment with five different concentration, and three cultures with different age/stade because the stade of the culture can change the metabolism of the yeast.

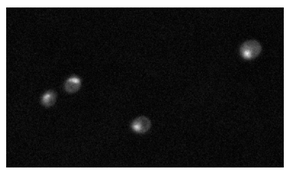


After the mix of the YPD with glucose we need to take less than 10 minute to take the pictures, because proteins leave the nucleus afterwards.

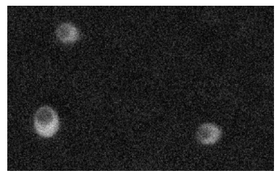
We use a fluorescent microscope to take pictures of our fluorescent protein, these pictures are then transformed in different shade of grey.

How did we analyze our results ?

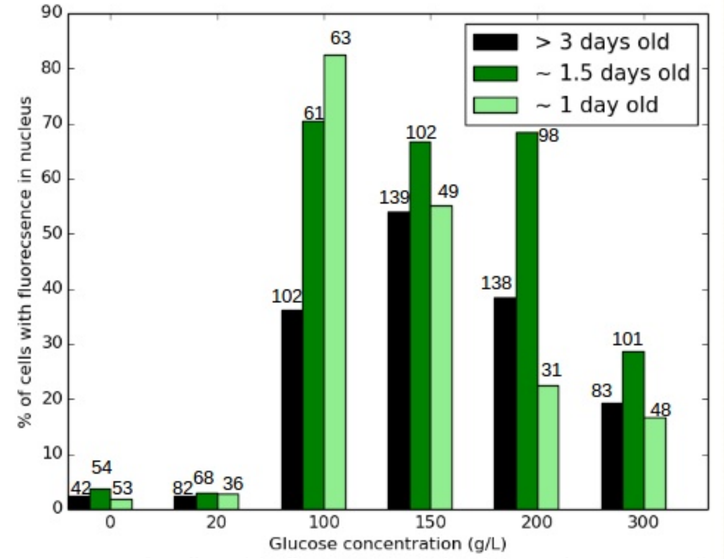
To analyze the pictures taken with the microscope we use **ImageJ**, thanks to this we count the numbers of cells (only non budding cells) with fluorescence in the cytoplasm or in the nucleus.



Cells with fluorescent proteins in the nucleus



Cells with fluorescent proteins in the cytoplasm.



Percentage of cells with fluorescence in the nucleus according to the media's concentrations of glucose and ages of the cultures

We observe that:

No fluorescence in the nucleus for 0g/L and 20g/L of glucose.

⇨ No response of the cells

Fluorescence in the nucleus for 100g/L and higher

⇨ Clear response of the cells to osmotic pressure

But we observe too, that percentage of cells with fluorescence in the nucleus **decrease** between 100g/L and 300g/L, maybe because a lot of cells began to die.

We observe too, that the "old" culture's cells (>3 days) react less than the two others cultures' cells, maybe because this culture is stagnant, and cells' metabolism respond slower than the two others cultures to hyperosmotic pressure

Conclusion :

This project sets a basis for exploring the ability of HOG1–GFP strains of Saccharomyces cerevisiae to sense hyperosmotic pressure. It's only a basis because we lacked the control on our objectivity, but also the timeline of the experiment and the noise of the instruments. However, thanks to our project, we can say that yeast cells reacted to hyperosmotic pressure with a relocation of HOG1 protein into the nucleus.