Determining Cytosolic Calcium Responses to Ferrous Sulfate Concentrations in Mutant Strains of *Saccharomyces cerevisiae*

Jestrii Acosta, Jacqueline McCarter, and Pamela A. Marshall

Arizona State University West

Introduction

Bakers' yeast, *Saccharomyces cerevisiae*, is a eukaryotic microorganism that is used in basic biological research and is favored by most (2). For this reason, many people have looked into yeast and how it can be used as a model organism for a mammalian heart cell (1). Calcium is the main signaling molecule in all eukaryotic cells and it plays an important role in things like cell division in yeast (5). Iron was placed into the cell using a luminometer assay at different concentrations in order to see the effect it had on the calcium homeostasis in the yeast cell. Calcium is transported into the vacuole via mutants Vcx1p, Pmc1p, and Pmr1p are channels located in the yeast channel that maintain Ca^{2+} homeostasis. Vcx1p is a channel located on the vacuole of the yeast that that exchanges Ca^{2+} and H^{+} and pumps in a sudden pulse of Ca^{2+}into the vacuole (6). Pmc1p is located on the yeast vacuole and it mediates the high affinity transport of calcium (1). Pmc1p is an ATPase that is typically regulated by a slow calcium influx and it is crucial for the longer term of calcium homeostasis (1). Vcx1p exchanges Ca^{2+} and H^{+} that is regulated by vacuolar pH and by calcium through a presumably post-translational mechanism which is critical for short term homeostasis (1). Pmr1p is a channel that is located on the Golgi Apparatus that is something that responds in a normal physiological response to calcium stress (4). The calcium ion channel causes the influx of iron ions to enter into the vacuole. Pmr1p P-type ATPase that is regulated by a slow calcium dependent gene expression feedback control pathway (1). It mediates the high-affinity transport of Ca^{2+} and Mn^{2+} (1). What is being tested is the wild type, mutants Vcx1p, Pmc1p, and Pmr1p. The wild type yeast is a yeast cell with nothing being changed; this is what is used as the control. The mutants get the channel removed from the wild type cell, to see which of the channels is casing the most drastic change to calcium concentrations.

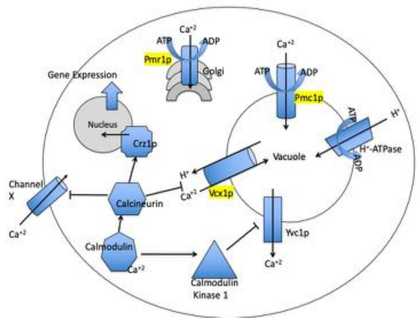

Figure 1. Calcium (Ca^{+2}) signaling and transport pathways in *Saccharomyces cerevisiae*. Calcium is transported into the cell via an unknown transport complex. It is then quickly sequestered away into the vacuole via Vcx1p and Pmc1p or Golgi via Pmr1p. Ca^{2+} can also enter the cytosol from the vacuole through Ycv1p, in response to certain stimuli. Calcium also stimulates gene expression through calmodulin, calcineurin, and then the transcription factor Crz1p. Modified from (16).

Hypothesis

High concentrations of iron can weaken or disrupt yeast cells, causing an influx of calcium into the vacuole of the yeast cell.

Why it's important?

Yeast has the same components, structure and function along with acting like mammalian heart cells' dynamic signaling as seen in mathematical models (1).Looking for at how metals affect the heart, iron is an element that is found in many households ranging from the drinking water to the dust around houses (3).

Experiment

Yeast needed to be prepared for the transformation, so it was grown in an overnight culture, containing YPD glucose and three to five colonies of cells in the incubator shaker (Figure 4) that was taken from a prepared plate of cells (Figure 7). Transformation of the normal yeast cells and three mutant cells using Frozen-EZ Yeast Transformation II. This was in order to insert the plasmid that contained the gene that encoded for aequorin protein.

The yeast then needed to be prepared for the luminometer assay, the strains that were transformed where then grown overnight in culture, containing YNB glucose, yeast cells, and the appropriate nutritional supplements. A back dilution was necessary in order to get cells to be at the desired concentration of 0.4 (A600nm) which was measured in spectrophotometer (Figure 8), and the colonies were put into the incubator shaker (Figure 4) for two hours. A coelenterazine treatment was then done to activate aequorin in each of the strain colonies with centrifuging the colonies in a centrifuge(Figure 5) in tubes and then in a microcentrifuge (Figure 6). The microfuge tubes were then covered with aluminum to keep light out.

For the luminometer assay, the luminometer (Figure 9) was primed with iron which will pulse through the cells at the 30 second point of the calcium kinetics assay. The samples were put into the instrument in 200 microliter samples in each of the strains of cells and measured over a 3 minute 40 second time. Additionally, a 56 second Lmax assay was carried out for each of the strains as well with a detergent solution put in before the rest was run. Using the highest Lmax value and the values of the calcium kinetics assays graphs were created using formulas that give a total cytosolic calcium ion concentration. The Lmax is used to normalize the data gathered. Once the data was collected these values were put into the y-axis and then the time was put into the x- axis over the time.

Figure 4. Incubator Shaker

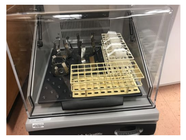

Figure 5. Centrifuge

Figure 6. Microcentrifuge

Figure 7. Yeast Culture

Figure 8. Spectrophotometer

Figure 9. Luminometer

Results

Cytosolic Calcium Levels

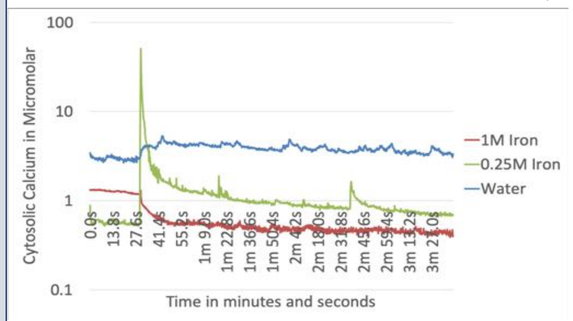

Figure 10. Cytosolic calcium levels, in the logarithmic scale, in Water (blue), 1 Molar (red), and 0.25 Molar (Green) concentrations of iron added to the wild type yeast cell in minutes and seconds.

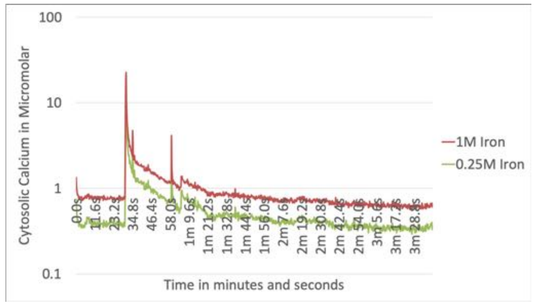

Figure 11. Cytosolic calcium levels, in the logarithmic scale, in mutant strain lacking *VCX1* in 1 Molar (red) and 0.25 Molar (Green) concentrations of iron added to the wild type yeast cell in minutes and seconds.

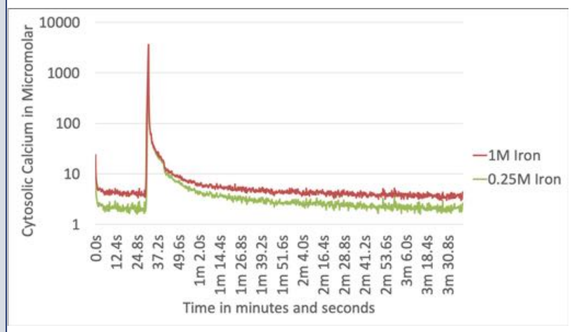

Figure 12. Cytosolic calcium levels, in the logarithmic scale, in mutant strain *PMC1* in 1 Molar (red) and 0.25 Molar (Green) concentrations of iron added to the wild type yeast cell in minutes and seconds.

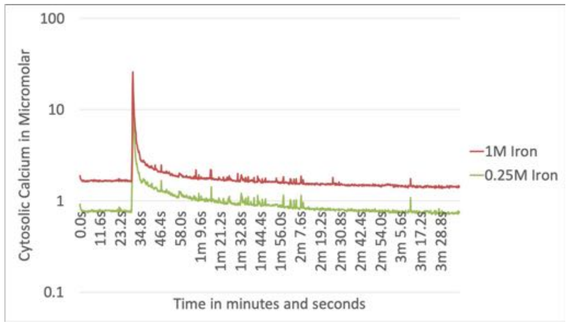

Figure 13. Cytosolic calcium levels, in the logarithmic scale,in mutant strain *PMR1* in 1 Molar (red) and 0.25 Molar (Green) concentrations of iron added to the wild type yeast cell in minutes and seconds.

Conclusions

The yeast proteins tested posses the same functional similarities as cardiac myocytes. Vcx1p (yeast) is compared to the cardiac protein *NCX1*(cardiac). Both proteins are exchangers that mediate the removal of Ca^{2+}. Pmc1p (yeast) displays similarities with *PMCA1* (cardiac), these proteins have ATPases that mediate the removal of cytoplasmic Ca^{2+}. Pmr1p (yeast) and *SERCA2* (cardiac) proteins mediate the sequestration of cytoplasmic Ca^{2+} into the ER/SR and are regulated by cytosolic Ca^{2+}. Yeast and mammalian cardiac myocytes have calcium transport proteins that can impact homeostasis.

What was determined from this research is that the higher the concentrations of ferrous sulfate showed there is an influx of calcium that enters the yeast vacuole. In order to fully comprehend which transport pathways are being triggered from stress, further research needs to be conducted. Next steps will need to include the use of mutants that are specifically for ferrous sulfate ion channels. Such as, Ftr1p, Fet3p, Fre1/2p, and Fet4p.

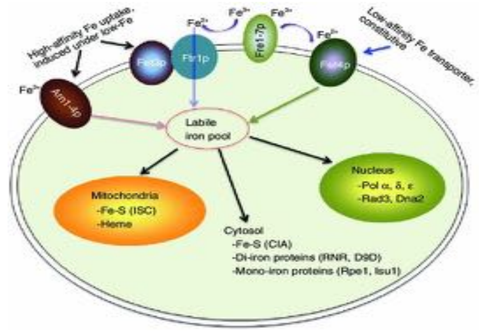

Figure 14. Iron uptake and utilization inside the cell. (copyright Caiguo Zhang)

References

1. Cui, J., Kaandorp, J. A., Sloot, P. M., Lloyd, C. M., & Filatov, M. V. (2009). Calcium homeostasis and signaling in yeast cells and cardiac myocytes. FEMS yeast research, 9(8), 1137-1147.
2. Dujon, B. (1996). The yeast genome project: what did we learn?. Trends in Genetics, 12(7), 263-270.
3. Hassan, S. K. M. (2012). Metal concentrations and distribution in the household, stairs and entryway dust of some Egyptian homes. Atmospheric Environment, 54, 207-215.
4. Kellermayer, R., Aiello, D. P., Miseta, A., & Bedwell, D. M. (2003). Extracellular Ca2+ sensing contributes to excess Ca2+ accumulation and vacuolar fragmentation in a pmr1Δ mutant of S. cerevisiae. Journal of Cell Science, 116(8), 1637-1646.
5. Kepler, J. L. (2013). Deletion mutation analysis identifies the inhibitory action of calmodulin kinase 1 on the vacuole Ca2 exporter during the process of intracellular calcium homeostasis in Saccharomyces cerevisiae. 1-53.
6. Miseta, A., Kellermayer, R., Aiello, D. P., Fu, L., & Bedwell, D. M. (1999). The vacuolar Ca2+/H+ exchanger Vcx1p/Hum1p tightly controls cytosolic Ca2+ levels in S. cerevisiae. FEBS letters, 451(2), 132-136.
7. Feldmann, H. (2013). *Yeast: Molecular and Cell Biology*. Place of publication not identified: Wiley VCH Imprint.

Acknowledgments

Research reported in this poster was supported by the National Institute of Environmental Health Sciences of the National Institutes of Health under award number 1R25ES030238-01. (2019)

RESEARCH POSTER PRESENTATION DESIGN © 2015
www.PosterPresentations.com