Understanding how MitoNEET Contributes to Oxidative Stress

Sydney Oliver[1,2], Dr. Mary Konkle[1], Dr. Eric Rubenstein[2],
Department of Chemistry, Department of Biology, Ball State University

Introduction

MitoNEET is an outer mitochondrial membrane protein that contains a [2Fe-2S] cluster in a unique ligation of 3Cys-1His residues. If the metal cluster is lost it can cause oxidative stress by formation of ROS which can then go and cause the formation of RSS. Reactive species can cause damage to proteins, lipids or DNA. three genes of the protein family have been identified as *CISD1* (mitoNEET), *CISD2* (NAF-1) and *CISD3* (Miner 2). MitoNEET and NAF-1 play a role in resistance to oxidative stress and, are possible drug targets for cancer cell, diabetes, and Parkinson's Disease.

TLC

1.) Let contents react for an hour at room temp, open to air
2.) Heat at 97℃ for 10 minutes
3.) Spin for 1 minute
4.) Spot plate and place in chamber with mobile phase for 40 minutes
5.) Stain with ninhydrin

Yeast-2 Hybrid (Y2H)

1.) Serially dilute yeast containing bait & prey Y2H plasmids
2.) Spot serial dilutions of yeast onto plasmid-selective & interaction-selective agar media
3.) Incubate at 30℃ for 2-7 days and image

Results

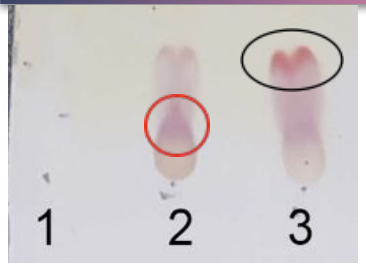
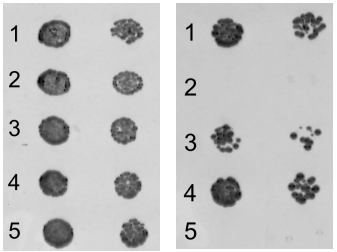


Figure 1. TLC of mitoNEET (1), mitoNEET+cysteine (2) and cysteine (3). The black circle shows spot containing cysteine. The red circle shows new spot that appeared when mitoNEET and cysteine were present.



Plasmid Selective | Interaction Selective

Figure 2. Y2H growth assay using CISD2 as bait. Y2H positive control (1), Y2H negative control (2), CISD2 x CISD1 (3), CISD2 x CISD2 (4), and CISD2 x CISD3 (5). There was interaction between CISD2 and both CISD1 and CISD2.

Conclusion and Future

<u>Thin Layer Chromatography (TLC):</u>
MitoNEET is consuming cysteine and a new unknown product is being made. For future studies, I want to see if mitoNEET will also convert sulfur-containing biochemicals homocysteine, glutathione, and cysteamine in a similar way.

<u>Yeast Two Hybrid:</u>
Interaction was observed between CISD2 and both CISD1 and CISD2. In the future, I plan to quantitatively assay Y2H interactions by performing growth curves using liquid media. I will then test the effects of oxidative stress on Y2H interaction.

Acknowledgements

-Ball State University Departments of Biology and Chemistry
-NIH R15 Grant to ER
-NSF #1806266 (RUI - Konkle)
-NSF HRD-1618408 (LSAMP - Salazar)

NSF

NIH
National Institutes of Health