TITLE: The Mitochondrial Stress Response and the Communication of Stress Responses Between Subcellular Compartments

Protein misfolding in different cellular compartments can lead to an induction of unfolded protein response (UPR) (Credle et. al. 2005). For example, activation of HSF-1 in the cytoplasm will lead to a stress response (Morimoto, 1998), indicating that each compartment can mount a stress response for specific stresses rather than one global stress response. Compartment specific stress responses have been studied in some detail, as is the case in the heat shock response of the cytosolic compartment or in the UPR of the ER (Morimoto, 1998). Each compartment can sense an environmental change in its own protein folding environment and can mount a specific stress response. While these better understood responses exist, little research has focused on the mitochondria, a fundamentally important compartment in cell metabolism. Furthermore, little is known about the communication between stress responses of subcellular compartments. This research project focuses on the mitochondria's unfolding protein response and its crosstalk with other folding environments in the cell.

The first aim of this project seeks to determine how a specific folding stress in one compartment impacts the folding environment of other compartments. A second aim of this project is to elicit the mechanism by which the mitochondria respond to changes in its folding environment and how it transmits its stress response.

To address these questions, I will employ the use of the nematode, C. elegans, a model organism, which has several advantages for this proposed project. C. elegans is very amiable to genetic manipulation using gene deletions and RNA interference (RNAi) and has an extensive data bank of mutations that can be exploited. Furthermore, C. elegans has many identified mutations that correlate to specific behavioral phenotypes that can be easily followed. Together they allow me to study protein folding and function directly *in vivo* within the live animal, which allows me to address my biological question.

To monitor the protein folding environment we will use temperature sensitive (ts) mutations, which are mutations that display their phenotypes depending on the cellular folding environment (Gidalevitz et al. 2006). Ts mutations will be used to determine how a stress in one compartment affects another. We chose mutations that are specific to compartments within the cell with gas-1 being a mitochondrial ts mutation, unc-63 an ER mutation, and let-60 and unc-52 being cytosolic markers. In addition to ts mutations as markers of the folding environment, stress response reporters as well as RT-PCR methods will be used to further confirm the results

To induce a stress within C. elegans, we will use both pharmacological treatments and genetic manipulations (RNAi). A previous research paper has suggested that Ethidium bromide (EtBr) can cause a mitochondrial specific stress in C. elegans (Yoneda et al. 2004). Tunicamycin is known to cause an ER-specific stress (Marciniak et al. 2004). To induce stress in the cytoplasm, we will employ poly-Q mutants, proteins containing long sequences of glutamine repeats known to cause cytoplasmic stress (Morley et al. 2002). In addition, the use of RNAi to knock down various proteins specific to a compartment or a stress response will be used to both stress the cell and demonstrate the importance of a protein in a stress-response pathway of a compartment.

To test the hypothesis that ethidium bromide (EtBr) can induce a mitochondrial specific stress, I placed C. elegans worms expressing the gas-1 ts mutation on media containing EtBr at a concentration below the threshold required for inducing a stress response (Yoneda et al, 2004). Animals grown on 2.5 µg/ml EtBr exhibited an increase of 30% in phenotype penetrance versus animals grown on normal media, indicating the presence of stress. Ts mutants expressed in other compartments such as unc-63 and let-60 did not exhibit a phenotypic change when placed on the same low dosage of EtBr, demonstrating that the stress was mitochondrial specific. A tenfold increase in the dosage of EtBr resulted in similar phenotypic penetrance to low dose EtBr in gas-1 animals (30% increase), suggesting that the gas-1 effect is highly perceptive to mitochondrial stresses and was maximal at a low concentration of EtBr. Interestingly, the same increase in EtBr dosage caused a change in phenotype penetrance for let-60 animals, indicating that the higher mitochondrial stress from EtBr can elicit a response within another subcellular compartment. This preliminary data sugge[illegible]hat ts proteins can be used to sense specific subcellular folding stresses. Furthermore, it indicates that there is a cross-talk between different subcellular compartments and further experiments will be carried out to elucidate the nature of this response and how it is mediated.

My preparation for this project comes both from the classes I have taken and my experience working in the lab. Some influential classes include Biochemistry 309, Biochemistry/Molecular Biology 210-2, Genetics/Evolution 210-1, Cell Biology/Physiology 210-3, Introduction to Medicinal Chemistry (taken in England), Cell Regulation and Cancer (England), as well as the General Chemistry 101-103 sequence and the Organic Chemistry 210 sequence. These classes have familiarized me with the terminology of biology and the biology sequences in general have provided an excellent introduction in general information, biological lab techniques, and applications of these techniques.

In addition, I joined the laboratory of Dr. Morimoto in January 2007, and throughout the term I have learned how to work with the C. elegans nematodes and how to identify the phenotypes of the ts mutants I will be using (gas-1, unc-63, unc-52, and let-60). I have set up both the pharmacological assay and the knockout assay to induce subcellular compartment stresses and now have all the required assays for working in the lab. I have read the current research articles pertaining to the mitochondria and more generally to the stress responses of the ER and the cytosol. Finally, I will continue working on this project throughout spring quarter, giving me two quarters of experimental research before the summer quarter begins. Should I receive the grant, I would like credit for the Independent Research 399 class.

References

Credle, Joe J., Janet S. Finer-Moore, Feroz R. Papa, Robert M. Stroud, and Peter Walter. (2005). "On the Mechanism of Sensing Unfolded Protein in the Endoplasmic Reticulum." PNAS 102 : 18773-18784

Morimoto, R.I. (1998.) “Regulation of the Heat Shock Transcriptional Response: Cross Talk Between a Family of Heat Shock Factors, Molecular Chaperones, and Negative Regulators.” Genes Dev. 12. 3788-3796

Gidalevitz, Tali, Anat Ben-Zvi, Kim H. Ho, Heather R. Brignull, and Richard I. Morimoto. (2006.) "Progressive Disruption of Cellular Protein Folding in Models of Polyglutamine Diseases." Science. 311. 1471-1474

Yoneda, Takimari, Cristina Benedetti, Fumihiko Urano, Scott G. Clark, Heather P. Harding, and David Ron. (2004.) "Compartment-Specific Perturbation of Protein Handling Activates Genes Encoding Mitochondrial Chaperones." Journal of Cell Science. 117. 4055-4066

Marciniak, Stefan J., Chi Y. Yun, Seiichi Oyadomari, Isabel Novoa, Yuhong Zhang, Rivka Jungreis, Kazuhiro Nagata, Heather Harding, and David Ron. (2004.) "CHOP Induces Death by Promoting Protein Synthesis and Oxidation in the Stressed Endoplasmic Reticulum." Genes and Development. 18. 3066-3077

Top of Form Bottom of Form

Morley, James F., Heather R. Brignull, Jill J. Weyers, and Richard I. Morimoto. (2002). "The Threshold for Polyglutamine-Expansion Protein Aggregation and Cellular Toxicity is Dynamic and Influenced by Aging in Caenorhabditis Elegans." PNAS. 99. 10417-10422

Top of Form Bottom of Form

Overall comments:
Good quality proposal overall. The author clearly explains the aims and methods to carry out those aims.
Research question: Analysis of mitochondria's unfolding protein response and its crosstalk with other folding environments in the cell.
Compelling presentation of prior experience in courses and labs. Could include specific techniques learned.
Good use of citations and references
Suggestions:
Should add headings to make it more readable and add some structure.