Coupling Multiple Stresses to the Activation of Akt-Kinase Signaling Pathway

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Cellular stress is caused by the exposure of cells to environmental stressors and is extremely harmful, e.g., oxidative stress is caused by free radicals, and is linked to cancer, ADHD, etc. The specific aim here is to investigate the role of SOD2 in mitigating different stresses. My previous study used computer modeling to show that the mitochondrial targeting of SOD2, for mitigating oxidative stress, is initiated by kinase AKT phosphorylating Hsp70 at either Serine 633 or Serine 631. This study identifies the correct location of these two, by employing molecular cloning techniques to create a Hsp70 S633A mutant and conduct Bradford protein assay. Radiolabeled gamma-32 ATP was then used to phosphorylate the two types of Hsp70 (wild type and mutant). A phosphorimager established that phosphorylation does not take place at Serine 633. A parallel lab study confirmed that phosphorylation takes place at Serine 631. The role of SOD2 in mitigating anaerobic stresses (endoplasmic reticulum, osmotic, and alcoholic stresses) is unknown and was explored with the model organism Saccharomyces cerevisiae. Computer modeling, including homology modeling and molecular dynamics simulation, confirmed that ySOD2 (yeast) is structurally and functionally similar to hSOD2 (human). As single node computers are too slow, a cluster computer was built at home to run the simulations. The complementation and spotting of deletion SOD2 strains on tunicamycin, ethanol, and glycerol plates discovered that ySOD2 stabilizes cells under all three stresses. These findings identify multiple new potential pathways to target SOD2 to reduce cellular stress with implications for drug design.

Awards Won:

Second Award of $1,500