Title:	Understanding the Role of Oxidative Stress and Calcium Ions in the Regulation of the Yeast TRP Channel, Yvc1p
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Abstract:	Transient receptor potential channels are major sensory channels that are responsible for sensing pain, heat, chemicals, mechanical, and various other stimuli in mammals and other species. The Transient receptor potential channel, Yvc1p in Saccharomyces cerevisiae is the sole TRP channel of yeast. It is a voltage-gated ion channel present on the yeast vacuolar membrane. Yvc1p forms a homo-tetramer and is composed of six Transmembrane domains (S1-S6), with a pore region between S5-S6, crucial for ion permeation and selectivity. The principal divalent cation transported by Yvc1p is Ca 2+ . The Yvc1p is also regulated by calcium ions by a Calcium Induced Calcium Release mechanism (CICR). Oxidative stress regulation of Yvc1p is dependent upon glutathionylation of critical cysteines, carried out by a Glutathione S-Transferase, Gtt1p. In the first part of the study, we investigated how the regulation of Yvc1p under oxidative stress mediated through glutathionylation is dependent upon the regulation by calcium ions. We have demonstrated that the residues in the cytosolic calcium-binding region are important for channel activation under both oxidative and osmotic stress. The residues in the vacuolar lumen have been previously reported to be important for channel inhibition and mutation of the residues in the vacuolar lumen leads to hyperactivity under osmotic stress. We show that they are also important during oxidative stress. The yeast Glutathione S- transferase, Gtt1p, which has been previously shown to be important for oxidative stress regulation also shown to have an important role in the activity of hyperactive mutant. This was further validated by a combined mutant defective for glutathionylation as well as calcium binding mutants. From these observations, it appears three events are important for Yvc1p channel regulation. 1. Binding of calcium ions to cytosolic calcium-binding motif. 2. Release of inhibitory calcium from the luminal calcium-binding motif. 3. Glutahionylation of the channel by Gtt1p of three cysteines.In the second part, we have identified regions in Yvc1p that are important for binding to Gtt1p by using peptide mapping and protein-protein interaction studies. We found a direct interaction of Yvc1p with Gtt1p. Further, the cysteines that were critical for Yvc1p channel glutathionylation were not found to be important for binding to Gtt1p. Among several approaches that were undertaken only the peptide mapping approach yielded interactors to Yvc1p. The peptide motifs of Yvc1p 424 FDVFE 428 and 570 GYLD 573 were found to be strong interactors with Gtt1p. These motifs were then analyzed using mutational studies for protein-protein interaction with Gtt1p. Mutation of these motifs leads to loss of interaction with Gtt1p. The mutants also showed reduced functional activity. We have also attempted to identify regions in Gtt1p important for interaction with Yvc1p. In-silico approaches were used to model Gtt1p structure and Gtt1p putative G-site and H-site were identified. Although the mutational studies didn’t show these residues to be important for binding to Gtt1p. The functional assay, however, showed G-site residues important for Yvc1p channel activity under oxidative stress
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