Title:	Redox regulation of the yeast voltage-gated Ca2+ channel homolog Cch1p by glutathionylation of specific cysteine residues
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Keywords:	Redox regulation voltage-gated Ca2+ glutathionylation
Issue Date:	2017
Publisher:	Company of Biologists Ltd
Citation:	Journal of Cell Science, 130 (14)
Abstract:	Cch1p, the yeast homolog of the pore-forming subunit α1 of the mammalian voltage-gated Ca2+ channel (VGCC), is located on the plasma membrane and mediates the redox-dependent influx of Ca2+. Cch1p is known to undergo both rapid activation (after oxidative stress and or a change to high pH) and slow activation (after ER stress and mating pheromone activation), but the mechanism of activation is not known. We demonstrate here that both the fast activation (exposure to pH 8–8.5 or treatment with H2O2) and the slow activation (treatment with tunicamycin or α-factor) are mediated through a common redox-dependent mechanism. Furthermore, through mutational analysis of all 18 exposed cysteine residues in the Cch1p protein, we show that the four mutants C587A, C606A, C636A and C642A, which are clustered together in a common cytoplasmic loop region, were functionally defective for both fast and slow activations, and also showed reduced glutathionylation. These four cysteine residues are also conserved across phyla, suggesting a conserved mechanism of activation. Investigations into the enzymes involved in the activation reveal that the yeast glutathione S-transferase Gtt1p is involved in the glutathionylation of Cch1p, while the thioredoxin Trx2p plays a role in the Cch1p deglutathionylation.
URI:	https://jcs.biologists.org/content/130/14/2317 (https://jcs.biologists.org/content/130/14/2317) http://hdl.handle.net/123456789/1897 (http://hdl.handle.net/123456789/1897)
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