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Title: Glutathione degrading enzymes (Chac1 and Chac2) and their turnover in yeast

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Abstract:

ChaC enzymes are known to degrade glutathione in cytoplasm and maintain the redox status of the cell. Two major members of this family, ChaC1 and ChaC2 have been described till date. ChaC1 is known to be upregulated during various stress conditions like ER-UPR stress, amino acid starvation, infection, while ChaC2 is found to have a basal expression. ChaC2 is considered to be the primitive form and has been found to be present in all organisms ranging from prokaryotes to lower and higher eukaryotes, while ChaC1 has been reported to be present only in higher eukaryotes. In this work, degradation mechanism of ChaC enzymes has been investigated. The human ChaC1, human ChaC2, mouse ChaC1 and yeast ChaC (GCG1) were cloned under a strong constitutive promoter (TEF) and a regulatory promoter (Gal). The expression of these proteins was confirmed by western blotting. However, human ChaC2 under the gal promoter could not be detected. Half – life of these enzymes has been found by performing Cycloheximide Pulse Chase experiment. GCG1 and mouse ChaC1 were observed to be stable proteins for 6 hours while human ChaC1 was found to be stable up to 3 hours. To augment our understanding further, elucidating the degradation mechanism using proteasomal mutants would be done.

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