Abstract

The human cystinosin (CTNS) protein is a lysosomal cystine transporter with 7 transmembrane domain that effluxes cystine from the lysosome to cytosol. The yeast Saccharomyces cerevisiae has a homologue of CTNS, ERS1. Although indirect experiments have suggested that ERS1 might be the functional homologue of ERS1, a direct demonstration of ERS1 to efflux cystine has not been made. In this thesis I have attempted to examine whether ERS1 is indeed involved in cystine transport. To facilitate such studies the approach was to mislocalize ERS1 to the plasma membrane and use a growth assay already developed in the lab, to examine the ability of yeast strains that are organic auxotroph to grow on cystine as a sulphur source. An In vitro mutagenesis of ERS1 was first explored to isolate mislocalized ERS1. A second strategy was to identify a putative consensus motif at the C-terminal domain by sequence alignment of other ERS1 homologues, followed by deletion of the motif to examine for mislocalization. Finally a series of domain swaps between ERS1 and CTNS (GYDQLΔ) were created to examine for trafficking to the plasma membrane. The first 2 approaches did not yield any insights on the function of ERS1. The third strategy has been initiated and is still ongoing.