Title:	Identification of residues critical for proton-coupled glutathione translocation in the yeast glutathione transporter, Hgt1p
Authors:	Zulkifli, M. (/jspui/browse?type=author&value=Zulkifli%2C+M.) Bachhawat, A.K. (/jspui/browse?type=author&value=Bachhawat%2C+A.K.)
Keywords:	Saccharomyces cerevisiae oligopeptide transporter family site-directed mutagenesis
Issue Date:	2017
Publisher:	Portland Press Ltd
Citation:	Biochemical Journal, 474 (11)
Abstract:	The proton gradient acts as the driving force for the transport of many metabolites across fungal and plant plasma membranes. Identifying the mechanism of proton relay is critical for understanding the mechanism of transport mediated by these transporters. We investigated two strategies for identifying residues critical for proton-dependent substrate transport in the yeast glutathione transporter, Hgt1p, a member of the poorly understood oligopeptide transporter family of transporters. In the first strategy, we tried to identify the pH-independent mutants that could grow at higher pH when dependant on glutathione transport. Screening a library of 269 alanine mutants of the transmembrane domains (TMDs) along with a random mutagenesis strategy yielded two residues (E135K on the cusp of TMD2 and N710S on TMD12) that permitted growth on glutathione at pH 8.0. Further analysis revealed that these residues were not involved in proton symport even though they conferred better transport at a higher pH. The second strategy involved a knowledge-driven approach, targeting 31 potential residues based on charge, conservation and location. Mutation of these residues followed by functional and biochemical characterization revealed E177A, Y193A, D335A, Y374A, H445A and R554A as being defective in proton transport. Further analysis enabled possible roles of these residues to be assigned in proton relay. The implications of these findings in relation to Hgt1p and the suitability of these strategic approaches for identifying such residues are discussed.
URI:	https://pubmed.ncbi.nlm.nih.gov/28389436/ (https://pubmed.ncbi.nlm.nih.gov/28389436/) http://hdl.handle.net/123456789/2471 (http://hdl.handle.net/123456789/2471)
Appears in Collections:	Research Articles (/jspui/handle/123456789/9)

Files in This Item:

File	Description	Size	Format	
Need to add pdf.odt (/jspui/bitstream/123456789/2471/1/Need%20to%20add%20pdf.odt)		8.63 kB	OpenDocument Text	View/Open (/jspui/bitstream/12345

Show full item record (/jspui/handle/123456789/2471?mode=full)
(/jspui/handle/123456789/2471/statistics)