Title:	Mutations in the N-terminal region of the Schizosaccharomyces pombe glutathione transporter pgt1+ allows functional expression in Saccharomyces cerevisiae
Authors:	Bachhawat, A.K. (/jspui/browse?type=author&value=Bachhawat%2C+A.K.)
Keywords:	OPT family Heterologous expression Hydroxylamine mutagenesis
Issue Date:	2013
Publisher:	John Wiley & Sons, Ltd.
Citation:	Yeast, 30(2), pp. 45-54.
Abstract:	Pgt1p encodes a glutathione transporter in Schizosaccharomyces pombe, orthologous to the Saccharomyces cerevisiae glutathione transporter, Hgt1p. Despite high similarity to Hgt1p, Pgt1p failed to display functionality during heterologous expression in S. cerevisiae. In the present study we employed a genetic strategy to investigate the reason behind the non-functionality of pgt1+ in S. cerevisiae. Functional mutants were isolated after in vitro mutagenesis. Several mutants were obtained and four mutants analysed. Among these, three yielded different point mutations in the N-terminal region (301–350 bp) of the transporter before the first transmembrane domain, while one mutant contained a deletion of 42 nucleotides within the same region. The mutant pgt1+ proteins not only expressed and localized correctly, but displayed high-affinity glutathione transport capabilities in S. cerevisae. Comparison of wild-type pgt1+ with the functional mutants revealed that a loss in protein expression was responsible for lack of functionality of wild-type pgt1+ in S. cerevisiae. The mRNA levels in wild-type and mutants were comparable, suggesting that the block was in translation. The formation of a strong stem–loop structure appeared to be responsible for inefficient translation in pgt1+ and disruption of these structures in the mutants was probably permitting translation. This was confirmed by making silent mutations in this region of wild-type pgt1+, which led to their functionality in S. cerevisiae. This genetic strategy to relieve functional blocks in expression should greatly facilitate the study of these and other transporters from more intractable genetic organisms in a heterologous expression system.
Description:	Only IISERM authors are available in the record.
URI:	https://onlinelibrary.wiley.com/doi/full/10.1002/yea.2939 (https://onlinelibrary.wiley.com/doi/full/10.1002/yea.2939) http://hdl.handle.net/123456789/3004 (http://hdl.handle.net/123456789/3004)
Appears in Collections:	Research Articles (/jspui/handle/123456789/9)

Show full item record (/jspui/handle/123456789/3004?mode=full)

(/jspui/handle/123456789/3004/statistics)