ATP-dependent transport of reduced glutathione on YCF1, the yeast orthologue of mammalian multidrug resistance associated proteins.

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The transport systems involved in the export of cellular reduced glutathione (GSH) have not been identified, although recent studies implicate a role for some of the multidrug resistance associated proteins (MRP), including MRP1 and MRP2. The present study examined the hypothesis that the yeast orthologue of MRP, Ycf1p, mediates ATP-dependent GSH transport. [3H]GSH transport was measured in vacuolar membrane vesicles isolated from a control strain of Saccharomyces cerevisiae (DTY165), the isogenic DTY167 strain that lacks a functional Ycf1p, and in DTY167 transformed with a 2-micrometer plasmid vector containing YCF1. GSH transport in control vacuolar membrane vesicles was mediated largely by an ATP-dependent, low affinity pathway (Km = 15 +/- 4 mM). ATP-dependent [3H]GSH transport was cis-inhibited by substrates of the yeast Ycf1p transporter and inhibited by 4,4’-diisothiocyanatostilbene-2,2'-disulfonic acid, probenecid, and sulfipyrazone, inhibitors of MRP1 and MRP2, but was minimally affected by membrane potential or pH gradient uncouplers. In contrast, ATP-dependent GSH transport was not seen in vacuolar membrane vesicles isolated from the DTY167 yeast strain without a functional Ycf1p but was restored to near wild-type levels in the DTY167 strain transformed with YCF1 and expressing the vacuolar Ycf1p transporter. On the other hand, expression and functional activity of a bile acid transporter, Bat1p, and of the V-type ATPase were similar in all three yeast strains. These results provide direct evidence for ATP-dependent low affinity transport of GSH by the yeast Ycf1p transporter. Because of the structural and functional homology between Ycf1p and MRP1 and MRP2, these data support the hypothesis that GSH efflux from mammalian cells is mediated by these membrane proteins.