Title:	Charged/polar-residue scanning of the hydrophobic face of transmembrane domain 9 of the yeast glutathione transporter, Hgt1p, reveals a conformationally critical region for substrate transport
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Abstract:	Unraveling the mechanistic workings of membrane transporters has remained a challenging task. We describe a novel strategy that involves subjecting the residues of the hydrophobic face of a transmembrane helix to a charged/polar scanning mutagenesis. TMD9 of the yeast glutathione transporter, Hgt1p, has been identified as being important in substrate binding, and two residues, F523 and Q526, are expected to line the substrate translocation channel while the other face is hydrophobic. The hydrophobic face of TMD9 helix consists of residues A509, V513, L517, L520, I524, and I528, and these were mutated to lysine, glutamine, and glutamic acid. Among the 16 charged mutants created, six were nonfunctional, revealing a surprising tolerance of charged residues in the hydrophobic part of TMhelices. Furthermore, the only position that did not tolerate any charged residue was I524, proximal to the substrate binding residues. However, P525, also proximal to the substrate binding residues, did tolerate charged/polar residues, suggesting that mere proximity to the substrate binding residues was not the only factor. The I524K/E/Q mutants expressed well and localized correctly despite lacking any glutathione uptake capability. Isolation of suppressors for all nonfunctional mutants yielded second-site suppressors only for I524K and I524Q, and suppressors for these mutations appeared at G202K/I and G202K/Q, respectively. G202 is in the hydrophilic loop between TMD3 and TMD4. The results suggest that I524 in the hydrophobic face interacts with this region and is also in a conformationally critical region for substrate translocation.
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