

DR K CHASES THE GREAT WHITE PERMEASE

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The lactose permease (LacY), a paradigm for membrane transport proteins and a member of the Major Facilitator Superfamily, catalyzes the coupled, stoichiometric translocation of a galactoside and an H^+ across the cytoplasmic membrane of *Escherichia coli*. Since transport is obligatorily coupled, sugar accumulation against a concentration gradient is driven by the free energy released from the downhill movement of H^+ with the electrochemical H^+ gradient ($\Delta\tilde{\mu}_{H^+}$; interior negative and/or alkaline). Moreover, downhill sugar translocation drives uphill H^+ translocation with the generation of $\Delta\tilde{\mu}_{H^+}$, the polarity of which depends on the direction of the sugar concentration gradient. X-ray crystal structures reveal an inward-facing conformation and confirm many conclusions from biochemical and biophysical experiments. LacY contains N- and C-terminal domains, each with 6 largely irregular transmembrane helices positioned pseudo-symmetrically and surrounding a deep water-filled cavity open to the cytoplasm only. Sugar- and H^+ - binding sites are located primarily in the N- and C-terminal helix bundles, respectively, at the apex of the cavity in the approximate middle of the molecule, and the periplasmic side is tightly sealed. Every residue in LacY has been mutagenized, and residues involved in sugar and H^+ binding have been identified. Surprisingly, those involved in H^+ binding and translocation are aligned parallel to the membrane at the same level as the sugar-binding site. Both sites are exposed reciprocally to water-filled cavities in the inward- or outward-facing conformation, thereby allowing sugar and H^+ release from either side of LacY via an alternating access mechanism. These features likely explain how LacY catalyzes lactose/ H^+ symport in both directions across the membrane utilizing the same residues. Site-directed alkylation, single molecule fluorescence energy transfer, double electron-electron resonance, thiol cross-linking and tryptophan quenching studies provide converging evidence for an alternating access mechanism. Furthermore, the primary driving force for the mechanism is sugar binding and dissociation. Finally, evidence for an 'occluded' intermediate will be discussed.

