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VIEWPOINT

It takes two to tango: The dance of the permease

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The lactose permease (LacY) of Escherichia coli is the prototype of the major facilitator superfamily, one of the largest families of membrane transport proteins. Structurally, two pseudo-symmetrical six-helix bundles surround a large internal aqueous cavity. Single binding sites for galactoside and H^+ are positioned at the approximate center of LacY halfway through the membrane at the apex of the internal cavity. These features enable LacY to function by an alternating-access mechanism that can catalyze galactoside/H^+ symport in either direction across the cytoplasmic membrane. The H^+-binding site is fully protonated under physiological conditions, and subsequent sugar binding causes transition of the ternary complex to an occluded intermediate that can open to either side of the membrane. We review the structural and functional evidence that has provided new insight into the mechanism by which LacY achieves active transport against a concentration gradient.

Introduction

The lacY gene, the second structural gene in the lac operon (Müller-Hill, 1996), encodes lactose permease (LacY), the polypeptide solely responsible for β-galactoside transport in Escherichia coli. LacY (TCDB 2.A.1.5.1) is a member of the oligosaccharide/H^+ symporter subfamily of the major facilitator superfamily (MFS), and it recognizes disaccharides with a D-galactopyranosyl ring and D-galactose, with no affinity for glucopyranosides or glucose. The natural substrate of LacY is lactose, a disaccharide containing a galactopyranosyl and a glucopyranosyl motiety in a β-1→4 glycosidic linkage. LacY catalyzes reversible translocation of one galactoside with one H^+, and coupled translocation in the same direction is obligatory (symport).

The lacY gene is the first gene encoding a membrane transport protein to be cloned and sequenced (Büchel et al., 1980), the first symporter to be purified to homogeneity in a functional state (Newman et al., 1981) and the first symporter to have a structure determined (Abramson et al., 2003). LacY has 417 amino acid residues (65–70% of which are hydrophobic) and a molecular weight of 46,517 D, and each of the 417 residues has undergone Cys-scanning mutagenesis (reviewed in Frillingos et al., 1998) and site-directed alkylation (reviewed in Kaback et al., 2007; Nie et al., 2007) and/or other site-directed mutagenesis. Notably, only nine residues are essentially irreplaceable with regard to active lactose transport: Glu126 (helix IV), Arg144 (helix V), Trp151 (helix V), Tyr236 (helix VII), Glu269 (helix VIII), Asn272 (helix VIII), and His322 (helix X) form the galactoside-binding site (Kumar et al., 2014, 2015); and Arg302 (helix IX) and Glu325 (helix X) are essential for coupled H^+ translocation (Carrasco et al., 1989; Sahin-Tóth and Kaback, 2001). All nine side chains are located in or near the aqueous cavity in the approximate middle of the LacY molecule (Fig. 1 a and Fig. 2 a).

LacY is a chemiosmotic nano-machine that performs coupled transport of a galactopyranoside with an H^+ across the cytoplasmic membrane of E. coli (i.e., galactoside/H^+ symport). Active transport (i.e., transport against a concentration gradient) is achieved by transduction of the free energy released from energetically favorable movement of H^+ down the electrochemical H^+ gradient ($\Delta\tilde{\mu}_{H^+}$; interior negative and/or alkaline) as postulated by the chemiosmotic hypothesis of Mitchell (1967, 1968; Fig. 3 a). Conversely, downhill galactoside transport drives uphill H^+ flux and generation of $\Delta\tilde{\mu}_{H^+}$, the polarity of which depends upon the direction of the sugar concentration gradient (Fig. 3, b and c). Importantly, LacY also catalyzes transmembrane exchange of internal for external galactoside in a manner that is independent of $\Delta\tilde{\mu}_{H^+}$ (Fig. 3, d and e). Thus, the primary driving force for "alternating access" (Jardetzky, 1966) of the H^+ and sugar-binding sites to either side of the membrane is binding and dissociation of galactoside by protonated LacY. Without bound sugar, LacY cannot translocate H^+ with or without $\Delta\tilde{\mu}_{H^+}$, and unprotonated LacY cannot bind a galactoside because of low affinity. Therefore, binding of both sugar and H^+ is required for symport.

Despite a number of structures of MFS members, including 10 of LacY, mechanisms of these dynamic proteins are not completely understood. It has been demonstrated that sugar binding to highly dynamic protonated LacY triggers a global conformational change in which sugar- and H^+-binding sites

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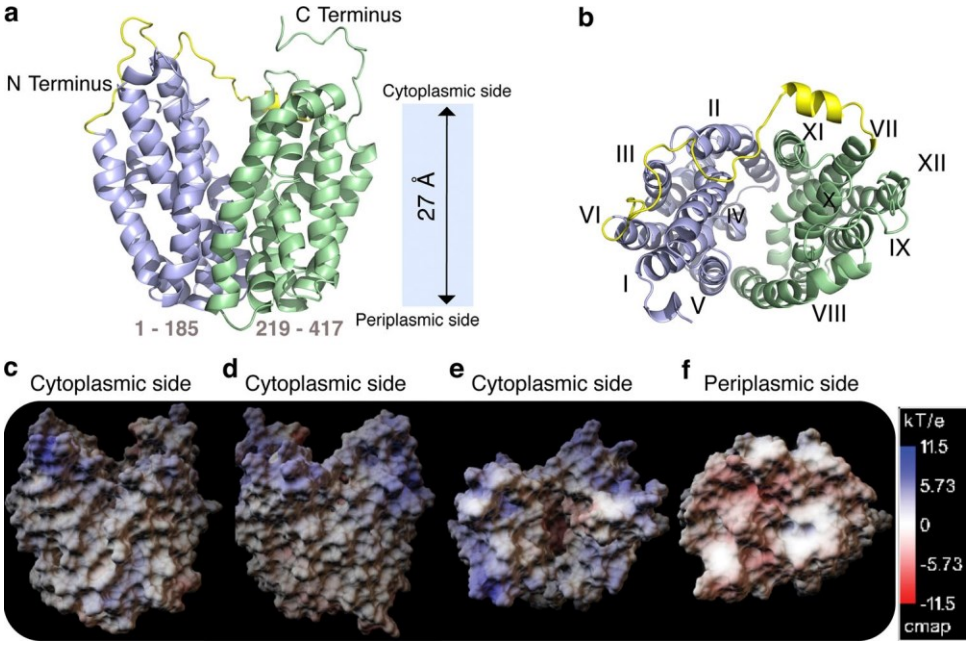

Figure 1. X-ray crystal structure of WT LacY (PDB accession no. 2V8N). LacY molecule in an inward-facing conformation. In the ribbon representations of LacY (a and b), the N- and C-terminal six transmembrane helices are colored in blue and green, and the middle cytoplasmic loop is colored yellow. (a) Viewed perpendicular to the membrane with helices V and VIII in front. The sidedness of the membrane is labeled. (b) Cytoplasmic view normal to the membrane. Helices are labeled in roman numerals. (c–f) Calculated electrostatic surface potential of LacY molecule. The scale indicates color-coded values of the electrostatic potentials (kT/e). (c and d) Viewed perpendicular to the membrane. (e and f) Viewed parallel to the membrane from cytoplasmic and periplasmic sides.

gain alternating access to either side of the membrane. Sugar binding and dissociation drive this conformational change through an induced-fit mechanism, while the proton electrochemical gradient accelerates the rate of deprotonation. LacY behaves much like an enzyme except that the transition state involves the protein rather than the substrate. X-ray structures of LacY inward- and almost occluded outward-facing conformations provide the structural basis for the alternating access mechanism. Alternating access has been documented almost unequivocally by applying pre–steady-state kinetics, as well as multiple biochemical and spectroscopic approaches, and by using kinetic data obtained in real time for several steps in the transport cycle. Thirty-one camelid nanobodies (Nbs) allow stabilization of LacY in different intermediate states that are providing an understanding of the structural changes underlying the symport mechanism.

Oligomeric state

LacY is a monomer both in detergent and in the membrane. The polypeptide reconstitutes as a monomer, as shown by fluorescence anisotropy (Vogel et al., 1985) and by freeze-fracture EM in the absence or presence of an imposed $\Delta\tilde{\mu}_{H^+}$ (Costello et al., 1987). Initial rates of $\Delta\tilde{\mu}_{H^+}$–driven lactose transport in proteoliposomes reconstituted at various protein-to-lipid ratios vary linearly. The linearity of the data and particularly the complete lack of sigmoidicity at very low ratios are consistent with the conclusion that LacY functions as a monomer. Furthermore, an engineered fusion protein encoded by two tandemly fused lacY open reading frames exhibits good transport activity, and negative dominance is not observed with either mutations or chemical modification in either half of the covalent dimer (Sahin-Tóth et al., 1994). Finally, cross-linking of LacY singleCys mutants in the plane of the membrane is stochastic (Guan et al., 2002). Taken together, the findings indicate that LacY is both structurally and functionally a monomer (reviewed in Guan and Kaback, 2006).

3-D structure

LacY is organized into two six-helix bundles connected by a relatively long cytoplasmic loop between helices VI and VII, which exhibit twofold pseudo-symmetry when viewed parallel to the plane of the membrane (Fig. 1, a and b). Within each domain, there are two three-helix repeats with inverted topology. These structural features are observed in both inward- and outward-facing conformations. The protein is 86% α-helix in the x-ray structure (85% by circular

dichroism; Foster et al., 1983) and contains twelve transmembrane helices traversing the membrane in zigzag fashion connected by very short hydrophilic loops with both N and C termini on the cytoplasmic side of the membrane. Most of the helices are irregular and distorted with bends and kinks. Helices III, VI, IX, and XII are largely embedded in the membrane, and the cytoplasmic regions of helices I, II, IV, and V from the N-terminal domain and helices VII, VIII, X, and XI from the C-terminal domain line a hydrophilic cavity. The inwardfacing conformation may represent the resting state of the molecule (Nie and Kaback, 2010; Smirnova et al., 2011b; Kaback, 2015).

Four x-ray structures from LacY mutant G46W/G262W in a partially outward (periplasmic)–open conformation with a tightly sealed cytoplasmic side (Kumar et al., 2014, 2015, 2018; Jiang et al., 2016) have also been obtained (Fig. 2 a). Three of the structures (8, 9, and 27) are in an occluded conformation with a narrow periplasmic opening and a single galactoside molecule in the central sugar-binding site. The opening on the periplasmic side is too narrow to allow entrance or exit of the sugar (~3 A at˚ the narrowest point), and the bound galactoside is occluded. Mutant G46W/G262W is clearly able to open sufficiently to bind galactoside at rates approaching diffusion (Smirnova et al., 2013), and upon binding, the mutant tries

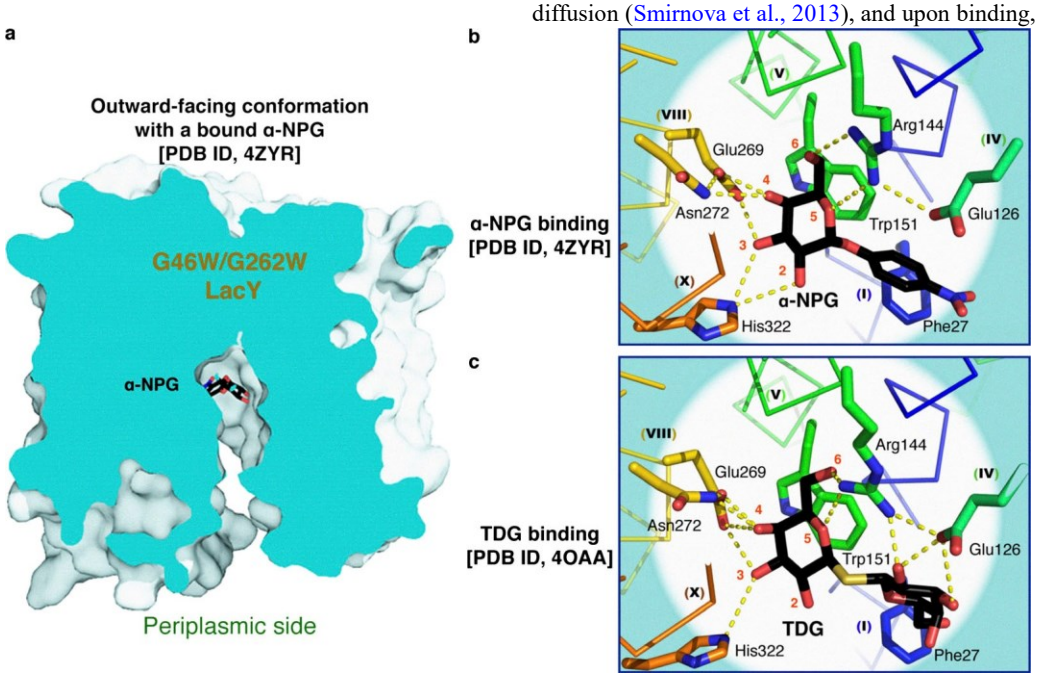

Figure 2. Mutant G46W/G262W and galactoside binding. Crystal structures of the LacY G46W/G262W mutant in a partially outward-facing occluded conformation with bound α-NPG (PDB accession no. 4ZYR) or TDG (PDB accession no. 4OAA). Both lactose analogues are shown in black. (a) Slab view of a surface representation of the LacY G46W/G262W molecule revealed by crystallography (PDB accession no. 4ZYR). An occluded α-NPG molecule is shown as sticks. (b) Binding interactions of α-NPG with LacY. (c) Binding interactions of TDG with LacY. The helices of LacY are colored in rainbow and labeled with roman numerals. Side chains that directly contact the sugar are shown as sticks. Dotted lines indicate interactions between the sugar and the protein, as well as between residues. The number in red indicates the hydroxyl group position in galactopyranosyl ring.

The initial structure was obtained with a conformationally constrained mutant, C154G LacY (Abramson et al., 2003), and a similar crystal structure was obtained for the WT a few years later (Guan et al., 2007). Viewed perpendicular to the membrane, both structures display heart-shaped molecules with a deep hydrophilic cavity open on the cytoplasmic side and sealed on the periplasmic side, an inward (cytoplasmic)–facing conformation (Fig. 1 a) with largest dimension of 60 × 60 A. Viewed˚ parallel to the plane of the membrane from the cytoplasmic side (Fig. 1 b), the molecules have a distorted oval shape that measures 30 × 60 A. Calculation of the electrostatic surface potential˚ reveals a positively charged ring around the cytoplasmic opening (Fig. 1, c–e; Yousef and Guan, 2009) and preferential distribution of negatively charged residues on the periplasmic side (Fig. 1 f). It is apparent that LacY conforms to the positive inside rule (von Heijne, 1992). Remarkably, LacY can be expressed in two fragments in vivo that find each other in the membrane and form a functional complex (Bibi and Kaback, 1990; Zen et al., 1994).

to transit into an occluded conformation. However, it cannot do so completely because the bulky Trp residues block complete closure. Thus, the mutant binds galactoside, which initiates transition into an occluded intermediate state, but it cannot complete the operation, which accounts for why the mutant is completely unable to catalyze transport of any type. It is also clear that an occluded intermediate is part of the transport cycle.

Galactoside binding and specificity

There is one sugar-binding site in LacY that is selective for galactose or disaccharides containing a galactosyl moiety, and the symporter transports a single molecule per transport cycle. Two lactose analogues, β-D-galactopyranosyl 1-thio-β-D-galactopyranoside (TDG) and 4-nitrophenyl-α-D-galactopyranoside (α-NPG), as well as other homologues (Smirnova et al., 2018), bind to LacY with micromolar K_d. The natural substrate lactose has poor affinity with a K_d of ~1 mM (Guan and Kaback, 2004), and galactose is the most specific substrate for LacY with the lowest affinity. Various adducts

(particularly if they are hydrophobic) at the anomeric carbon (C_1) of the galactopyranosyl ring greatly increase affinity with little or no effect on specificity (Sahin-Tóth et al., 2000). Although glucose is an epimer of galactose, neither glucose nor glucosides are recognized by LacY. The C_4 OH on the galactopyranosyl ring is probably the most important determinant for specificity, with the C_5, C_3, and C_6 OH groups playing varying roles in determining affinity/specificity, while C_1 and C_2 OH are of little importance (Sahin-Tóth et al., 2000, 2001).

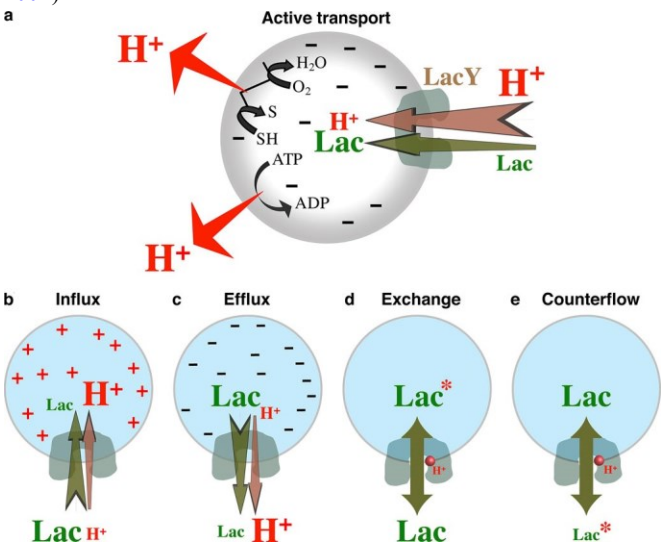

Figure 3. Transport reactions catalyzed by LacY. (a) Active transport. The electrochemical H^+ gradient ($\Delta\tilde{\mu}_{H^+}$) across the cytoplasmic membrane of E. coli is generated by efflux of H^+ via the respiratory chain or through the hydrolytic activity of F_1F_o ATPase. Free energy released from the energetically favored downhill movement of H^+ catalyzed by LacY (yellow) is converted to the uphill accumulation of lactose as indicated by the direction of the arrows and font size. (b) Influx. (c) Efflux. Energetically downhill lactose transport generates $\Delta\tilde{\mu}_{H^+}$, the polarity of which depends upon the direction of lactose concentration gradient (influx generates a $\Delta\tilde{\mu}_{H^+}$ that is interior positive and acid; efflux generates a $\Delta\tilde{\mu}_{H^+}$ that is interior negative and alkaline). (d) Equilibrium exchange. At equal intra- and extracellular lactose concentrations, lactose exchange across the membrane is catalyzed by protonated LacY. (e) Counterflow. At a high intracellular and low extracellular lactose concentrations, transient influx of radiolabeled external lactose or "counterflow" across the membrane is observed. The reaction is catalyzed by protonated LacY. Lac, lactose; SH, substrate in a reduced form.

Sugar-bound LacY in the inward-facing or partially outwardfacing conformation has been resolved crystallographically (Chaptal et al., 2011; Kumar et al., 2014, 2015, 2018). Both of the inward-facing and outward-facing structures contain a single galactoside that occupies the central cavity lined by the same residues regardless of the conformation of LacY, further confirming the conclusion that there is only a single sugar-binding site (Guan and Kaback, 2004). The site is located at the apex of the internal cavity in the middle of LacY, which enables transport in either direction.

The sugar-bound, inward-facing structure is from single-Cys V122C LacY with covalently bound methanethiosulfonylgalactoside (Chaptal et al., 2011), a suicide inactivator for the mutant (Guan et al., 2003b). Sugar-bound outward-facing structures have been obtained for the G46W/G262W mutant (Smirnova et al., 2013) with bound TDG or α-NPG (Kumar et al., 2014, 2015, 2018). The position of the galactosyl moiety in these structures is almost identical (Fig. 2, b and c), which provides strong evidence that it is indeed the galactosyl moiety that determines specificity.

Cys-scanning and site-directed mutagenesis have been used extensively to study the sugar-binding site in LacY (Frillingos et al., 1998), and the results from genetic engineering in combination with biochemical and biophysical characterization are largely confirmed crystallographically (Chaptal et al., 2011; Kumar et al., 2014, 2015, 2018). The LacY structures show that the galactopyranosyl ring of α-NPG or TDG stacks hydrophobically with Trp151 in helix V (Fig. 2, b and c), which is suggested to play important roles in orienting the galactopyranosyl ring and facilitating specific hydrogen bonding, in addition to increasing affinity (Guan et al., 2003a). Replacement of Trp151 with a Tyr or Phe residue decreases affinity for galactosides, but has little effect on lactose transport (Guan et al., 2003a; Vazquez-Ibar et al., 2003′; Smirnova et al., 2009b). Glu269 in helix VIII forms two hydrogen bonds with the C_4 and C_3 OH groups on the galactopyranosyl ring (Fig. 2, b and c), which explains why the Glu269 residue may be the primary determinant for sugar specificity (Weinglass et al., 2003). These important interactions are further stabilized by hydrogen bond interactions between the C_4 and C_3 OH with Asn272 (helix VIII) and between His322 (helix X) and the C_3 OH. The Asn272 side chain can be replaced with Gln exclusively without a significant loss of sugar-binding affinity. Glu269 and His322 are irreplaceable with respect to sugar-binding and transport activities. Two other irreplaceable residues, Arg144 and Glu126 in the N-terminal domain, form a salt bridge, and Arg144 serves directly as a hydrogen-bond donor to the C_5 and C_6 OH groups of the galactopyranosyl ring of TDG or α-NPG, and the C_{29} OH group in another galactopyranosyl ring. As described above, the glucopyranosyl ring in lactose can be replaced by various adducts that affect binding affinity only. Glu126, in addition to stabilizing the position of Arg144, also forms multiple hydrogen bonds with the C_{29} and C_{39} OH groups of TDG. Arg144 cannot be replaced with positively charged Lys, and Asp replacement for Glu126 also decreases affinity for galactosides significantly (Smirnova et al., 2009a).

Sugar binding and induced fit

Methanethiosulfonyl-galactose is a unique suicide substrate for a LacY mutant with Cys in place of Ala122 (Guan et al., 2003b). The x-ray structure (Chaptal et al., 2011) reveals that a molecule of methanethiosulfonyl-galactose is covalently bound to a Cys engineered at position 122 near the sugar-binding site, and the overall conformation of LacY remains in an inward-open conformation, although typically galactoside binding shifts LacY to the outward-open state (reviewed in Smirnova et al., 2011b). However, many side-chain interactions with the galactopyranoside ring are perturbed; for example, Asn272, His322, and Arg144 are no longer sufficiently close to interact with the galactopyranosyl ring. Therefore, although the binding site is partially occupied, critical interactions with these

important side chains are sufficiently disturbed that the interactions are insufficient to drive transition into the occluded state.

H^+ binding

Unexpectedly, LacY exhibits a perturbed apparent pK of ~10.5 with respect to galactoside-binding affinity, and the pHdependent apparent pK is abrogated when Glu325 (helix X) is replaced with a neutral side chain (Smirnova et al., 2008; Fig. 4). Mutant E325A binds sugar with an affinity similar to WT, but affinity is independent of pH up to at least pH 11. Even more

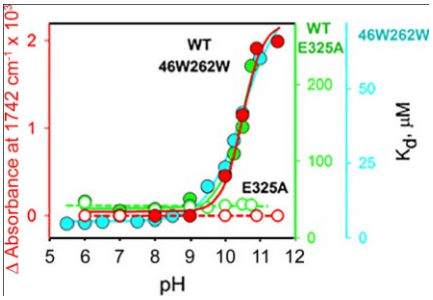

Figure 4. pK_a of Glu325 in LacY. The pH dependence of Δ-IR intensity change at 1,742 cm^{-1} was measured with the LacY G46W/G262W mutant in the absence or presence of α-NPG (filled red circles) or E325A LacY (open red circles). The K_d values for α-NPG binding to WT LacY (filled green circles), E325A LacY (open green circles), or LacY G46W/G262W mutant (filled cyan circles) were calculated as the ratio of rate constants (k_{off}/k_{on}) measured by stopped-flow fluorescence.

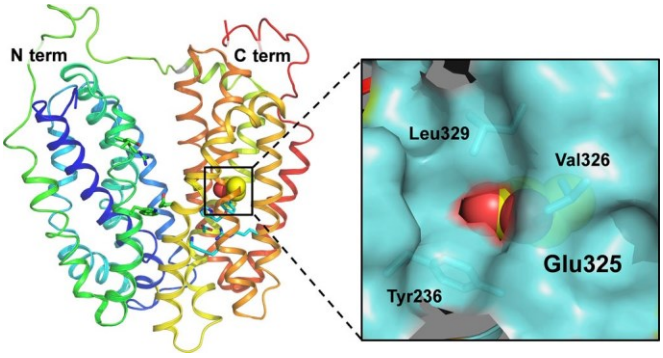

Figure 5. Position of Glu325 in WT LacY (PDB accession no. 2V8N). LacY is presented as rainbow-colored backbone (from blue to red for helices I to XII) with a hydrophilic cavity open to the cytoplasmic side. The side chain of Glu325 in helix X is shown as spheres. The area around Glu325 is enlarged with the hydrophobic environment displayed as a space-filled cartoon (cyan).

remarkably, Glu325 also exhibits a pK_a of 10.5 by direct measurement with surface enhanced infrared absorption spectroscopy (SEIRAS; Grytsyk et al., 2017; Fig. 5). The perturbed pK_a is due to location in a hydrophobic pocket between helices IX and X (Figs. 5 and 6). Therefore, Glu325 is clearly the primary (and possibly the only) functional H^+-binding site in LacY. A pK_a value of 10.5 translates to a K_d for H^+ of less than ~30 pM, so Glu325 is completely protonated over a very broad pH range. The overall conclusion is that the galactoside-binding site in LacY is remarkably stable to alkaline pH, but in order to elicit this property, Glu325 must be replaced with a neutral side chain.

Of potential importance, Glu325 is in close proximity to an electrostatic/hydrogen-bond network involving His322 and Lys319 on helix X, Tyr236 and Asp240 on helix VII, and Arg302 on helix IX (Fig. 6). Glu269 and particularly His322, initially thought to be involved in H^+ translocation, are both ligands for the galactopyranosyl moiety in the sugar-binding site (Fig. 2, b and c). Mutation of Glu269, His322, or Tyr236 causes a marked decrease in sugar-binding affinity (Smirnova et al., 2008). Another salt bridge (Asp237 in helix VII and Lys358 in helix XI) also lies in the cavity, but does not make a direct contact with either site and is not involved in sugar or H^+ binding. However, this salt bridge is essential for the insertion of LacY into the membrane and for maintaining protein stability (Kaback et al., 2001).

Since transmembrane exchange does not involve $\Delta\tilde{\mu}_{H^+}$, it is apparent that alternating access is driven by binding and dissociation of sugar, and does not involve turnover of H^+ (Kaback, 2015). Because the driving force for accumulation against a gradient is $\Delta\tilde{\mu}_{H^+}$ and E. coli has a stable internal pH of 7.6 (Padan et al., 1976; Ramos et al., 1976; Zilberstein et al., 1979), a pK_a of 10.5 for LacY (Smirnova et al., 2008; Grytsyk et al., 2017) implies that decreasing the pK_a by three orders of magnitude to cytoplasmic pH would release only ~50% of the transported lactose from LacY. How to increase the rate of deprotonation without decreasing the H^+ concentration of the E. coli cytoplasm? Arg302 is relatively close to Glu325 (~6 A), and mutants R302Aor R302S˚ cannot perform active transport, but catalyze transmembrane exchange (Sahin-Tóth et al., 2001; Weinglass et al., 2002). A recent SEIRAS study (Grytsyk et al., 2019) on the effect of mutating residues in the immediate vicinity of Glu325 shows that only mutation R302K alters the Glu325 pK_a, causing over a 2-pH U acid shift. Overall, the findings support the idea that H^+ is extracted from Glu325 by spatial fluctuations in the position of Arg302, expelling H^+ by moving near Glu325 (Weinglass et al., 2001) or vice versa.

Although nothing more is known at present, neutral replacements for specific carboxyl residues in the E. coli melibiose (Ethayathulla et al., 2014), fucose (Dang et al., 2010), and xylose permeases (Sun et al., 2012), as well as the Staphylococcus epidermidis glucose permease (Iancu et al., 2013), behave functionally in a manner similar to neutral replacement mutants for Glu325 in LacY. Thus, each of these sugar permease mutants is defective with respect to active transport (i.e., H^+/sugar symport), but still able to catalyze transmembrane exchange reactions. At the least, the findings indicate that these MFS family members likely have an ordered kinetic mechanism similar to that found with LacY.

Alternating access and the symport mechanism

An alternating-access type of mechanism is widely accepted to explain membrane transport; thus, H^+ symporters expose both H^+- and cargo-binding sites alternatively to either side of the membrane through conformational changes. In LacY, both the sugar- and H^+-binding sites are located at the approximate middle of the molecule at the apex of the cavity, providing a structural basis for such an alternating-access mechanism.

The periplasmic- and cytoplasmic-open conformations with bound galactoside and H^+ reflect static states. However, LacY must undergo dynamic conformational changes to expose both the sugar- and H^+-binding sites to either side of the membrane. Multiple independent experimental methods (Smirnova et al., 2011a), e.g., site-directed alkylation of single-Cys mutants; thiol cross-linking; single molecule FRET; double electron–electron resonance spectroscopy; and time-resolved FRET techniques to detect conformational change, sugar-binding kinetics, and nanobodies, have been used to examine sugar-induced conformational changes which provide independent converging evidence that periplasmic and cytoplasmic cavities open

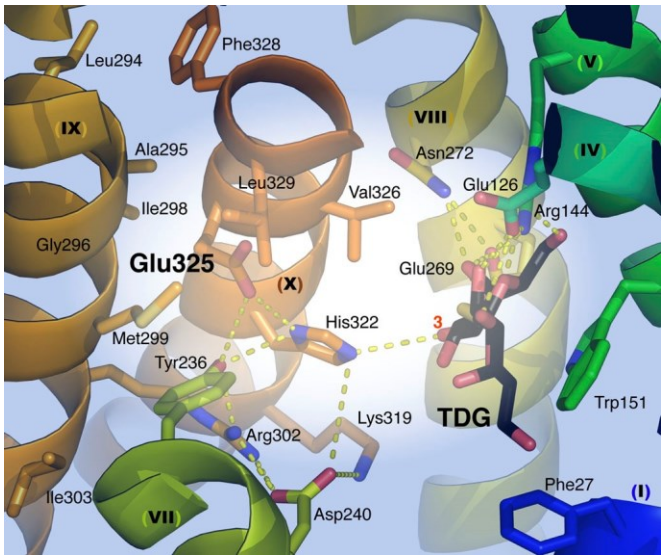

Figure 6. Environment of Glu325. Crystal structure of the LacY G46W/ G262W mutant in an outward-facing partially occluded conformation (PDB accession no. 4OAA). Helices of LacY are colored in rainbow and labeled with roman numerals. Dotted lines indicate polar/charged side chains/OH groups that are in close proximity and may form salt-bridge/H-bond interactions. TDG is shown in black, and C_3 OH is indicated. Glu325 is in helix X.

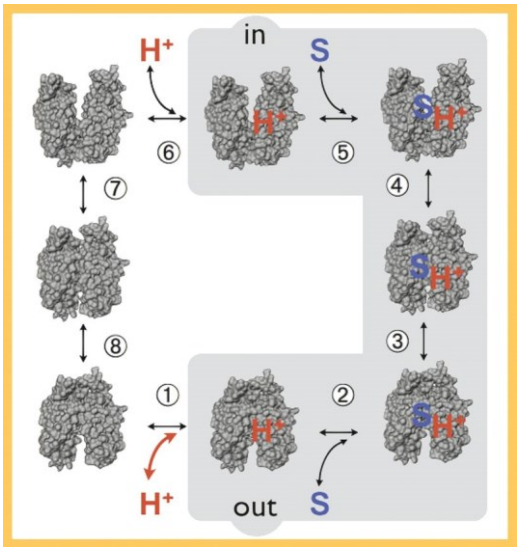

Figure 7. The H^+-lactose symport mechanism. There are eight kinetic steps as indicated. H^+ and galactoside bind in the middle of the molecule, and LacY moves around the binding sites to release the sugar and the H^+ by an ordered mechanism on either side of the membrane as indicated by the arrows. The alternating-access process involves the shaded steps only (2–5). S, substrate; H^+, proton.

reciprocally and that opening of the periplasmic cavity controls closing of the cytoplasmic cavity (Kaback et al., 2011; Smirnova et al., 2017). In the absence of a galactoside, LacY may favor an inward-facing conformation, the resting state, as indicated by the crystal structure (Fig. 1), but galactoside binding causes LacY to populate intermediate occluded and outward-open conformations.

The LacY transport cycle includes alternating-access and several other steps that are explained by a simplified eight-step kinetic scheme (Fig. 7). Based upon an ordered-binding model (Kaczorowski et al., 1979), different transport modes are explained in detail below.

Active transport

Active transport of lactose against a lactose concentration gradient is driven by $\Delta\tilde{\mu}_{H^+}$ (interior negative and/or alkaline). LacY is completely protonated in the outward-open state, which primes it for sugar binding with relatively high affinity (1). Binding of galactoside from the periplasm (2) to protonated LacY induces the formation of an occluded intermediate (3), in which sugar and H^+ are bound and both solvent accessible pathways from either side of protein are closed. The intermediate with a bound sugar and H^+ then opens to the cytoplasm (4), sugar dissociates (5) followed by deprotonation of Glu325 (6) to the cytoplasm, unloaded LacY returns to the outward-facing conformation by steps (7 and 8), and Glu325 is protonated immediately (1), ready for the next transport cycle. Notably, the ordered mechanism, with H^+ on first and off second, makes it particularly difficult to develop a H^+ leak.

LacY drives accumulation of lactose against a 50- to 100-fold concentration gradient (Robertson et al., 1980) with a turnover number of ~20 s^{-1}. Both ΔΨ (interior negative) and ΔpH (interior alkaline) have quantitatively the same effect on transport, with a 50- to 100-fold decrease in K_m and the same thermodynamic equilibrium. In opposition to time-honored conjecture, the K_d for galactosides on either side of the membrane is essentially the same in the absence or presence of $\Delta\tilde{\mu}_{H^+}$ (Guan and Kaback, 2004). In the absence of $\Delta\tilde{\mu}_{H^+}$, there is threeto fourfold inhibition of lactose transport in the presence of deuterium oxide (D_2O), indicating that deprotonation is rate limiting (Viitanen et al., 1983; Garcia-Celma et al., 2009, 2010; Gaiko et al., 2013). However, when there is a driving force on the H^+ in the presence of $\Delta\tilde{\mu}_{H^+}$, deprotonation is no longer ratelimiting, and the transport rate is unaffected by D_2O. It is particularly noteworthy that a number of experimental findings indicate that $\Delta\tilde{\mu}_{H^+}$ functions kinetically as a driving force on the H^+, but alternating access itself is driven by galactoside binding and dissociation and independent of $\Delta\tilde{\mu}_{H^+}$ (Guan and Kaback, 2006).

Transport down a concentration gradient

Inwardly directed flux (influx) or outwardly directed flux (efflux) down lactose concentration gradients (Fig. 3, b and c) occurs in a similar fashion, starting at step 1 for influx and step 6 for efflux and

proceeding around the circle. Both transport modes generate $\Delta\tilde{\mu}_{H^+}$, the polarity of which depends on the direction of the flux, and the limiting step involves deprotonation of Glu325. Both reactions exhibit deuterium isotope effects; i.e., influx and efflux are inhibited by D_2O.

Transmembrane exchange

Equilibrium exchange and counterflow represent alternating access of the galactoside-binding site to alternative sides of the membrane. In either reaction, LacY binds a sugar molecule on the external side of the membrane (unlabeled if equilibrium exchange; labeled if counterflow) and exchanges that sugar molecule for its opposite member on internal side of membrane. With equilibrium exchange, the concentrations are the same on both sides of the membrane; with counterflow, the internal concentration of unlabeled galactoside is much higher than the external concentration of labeled sugar. Under both conditions, the H^+ does not dissociate from LacY, and both reactions are unaffected by ambient pH or $\Delta\tilde{\mu}_{H^+}$and do not exhibit a deuterium isotope effect. Only steps 2 through 5 in Fig. 7 are engaged. For counterflow, the initial 1/1 exchange of internal unlabeled substrate for external labeled galactoside leads to a rapid increase in internal radioactivity until all of the internal unlabeled sugar inside is exchanged for external labeled galactoside. At this point, the increase in radioactivity stops, and the internal radioactivity decreases with time as the concentrations equilibrate and the specific activity of the external labeled substrate decreases, thereby causing the typical "overshoot" profile that is observed.

Ordered binding and release of H^+ and galactoside are strongly supported by the behavior of neutral replacement mutants in Glu325 that do not catalyze any reactions involving net H^+ translocation (i.e., electrogenic transport), but catalyze transmembrane exchange (i.e., electroneutral transport) at the same rate as WT LacY (Carrasco et al., 1989).

Outward-facing conformers stabilized by Nbs

Single-domain camelid Nbs raised against a LacY mutant immobilized in an outward-open conformation bind to LacY completely inhibiting transport and stabilizing the openoutward conformers of the WT (Smirnova et al., 2014). Using site-directed, distance-dependent Trp quenching/unquenching of fluorescent probes on opposite surfaces of LacY, conformational states of LacY complexed with each of eight unique, highaffinity Nbs that bind exclusively to the periplasmic side and block transport, but increase accessibility of the sugar-binding site, were examined (Smirnova et al., 2015). Each Nb induces quenching with three pairs of cytoplasmic Trp/bimane probes, indicating closure of the cytoplasmic cavity. In reciprocal fashion, the same Nbs induce unquenching of fluorescence in three pairs of periplasmic probes due to opening of the periplasmic cavity. Since the extent of fluorescence change with various Nbs differs and the differences correlate with changes in the stopped-flow rate of sugar binding, it is also concluded that the Nbs stabilize several different outward-open conformations of LacY. Thus, Nb binding clearly involves conformational selection of LacY molecules, which has been confirmed by thermodynamic studies with isothermal titration calorimetry (Hariharan et al., 2016). X-ray crystal structures of three Nb/LacY complexes, one apo structure (Jiang et al., 2016) with no bound sugar substrate, and two with bound lactose analogues (Kumar et al., 2015, 2018), confirm the biochemical data indicating that the Nbs bind stoichiometrically to the periplasmic face of the C-terminal sixhelix bundle with nanomolar affinities.

IIA^{Glc} regulation of lactose utilization by catabolite repression

Transcription of the lacY gene and LacY transport activity are down-regulated by the phosphoenolpyruvate:glucoside phosphotransferase system (PTS; Postma et al., 1993; Park et al., 2006). A phosphotransfer protein IIA^{Glc} of the PTS, the most abundant cytosolic protein in E. coli, plays a key role in the regulating carbohydrate metabolism by binding to various families of sugar transporters and many other soluble proteins, modulating their activities (Deutscher et al., 2014; Hariharan and Guan, 2014; Vogt et al., 2014; Hariharan et al., 2015). Binding measurements (Hariharan et al., 2015) show that unphosphorylated IIA^{Glc} binds to LacY in the absence or presence of galactoside. Glucose transport by PTS-mediated vectorial phosphorylation (Kaback, 1968) leads to preponderance of unphosphorylated IIA^{Glc}, which binds to LacY and inhibits galactoside binding and subsequent inducer formation. By this means, synthesis of inducer is prevented so that the cell preferentially utilizes glucose via the PTS. The phenomenon is known as "inducer exclusion."

Summary and future prospects

Studies with LacY have provided new insights into the mechanism of H^+/galactoside symport as follows. (1) High-resolution structural information of at least two important conformations of LacY in apo and substrate-bound forms have been obtained. (2) Detailed understanding of galactoside and H^+ binding and symport, much of which was unanticipated, has been obtained. (3) Although chemiosmosis is the driving force for the overall process by which lactose is accumulated against a concentration gradient, the basic alternating access conformational change itself is independent of $\Delta\tilde{\mu}_{H^+}$. (4) Active transport does not involve a change in affinity for galactoside on either side of the membrane. Rather, there is a marked change in affinity for H^+ driven by $\Delta\tilde{\mu}_{H^+}$. And perhaps most surprisingly, (5) The mechanism of coupling between H^+ and galactoside binding is relatively simple—in order to bind/transport galactoside, Glu325 must be protonated.

Despite these advances, several fundamental questions remain unsolved, some of which are the following: How generalized are the findings with LacY with respect to other permeases? Why does a negative charge on Glu325 block galactoside binding? Is Glu325 the only side chain in LacY directly involved in coupled H^+ translocation? Although there is no change in K_d for galactosides, why kinetically is there is a decrease in K_m in the presence of $\Delta\tilde{\mu}_{H^+}$? What structural changes resulting from release of sugar during the transport cycle cause LacY deprotonation? Is an increase in water accessibility involved with deprotonation of Glu325? Does the transport mechanism in other symporters involve a carboxyl side chain with a perturbed pK_a that can be detected by SEIRAS? By using

Nbs, can cryo-EM be applied successfully to a protein the size of LacY?

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References

Abramson, J., I. Smirnova, V. Kasho, G. Verner, H.R. Kaback, and S. Iwata. 2003. Structure and mechanism of the lactose permease of Escherichia coli. Science. 301:610–615. https://doi.org/10.1126/science.1088196

Bibi, E., and H.R. Kaback. 1990. In vivo expression of the lacY gene in two segments leads to functional lac permease. Proc. Natl. Acad. Sci. USA. 87: 4325–4329. https://doi.org/10.1073/pnas.87.11.4325

Büchel, D.E., B. Gronenborn, and B. Müller-Hill. 1980. Sequence of the lactose permease gene. Nature. 283:541–545. https://doi.org/10.1038/283541a0

Carrasco, N., I.B. Püttner, L.M. Antes, J.A. Lee, J.D. Larigan, J.S. Lolkema, P.D. Roepe, and H.R. Kaback. 1989. Characterization of site-directed mutants in the lac permease of Escherichia coli. 2. Glutamate-325 replacements. Biochemistry. 28:2533–2539. https://doi.org/10.1021/bi00432a028

Chaptal, V., S. Kwon, M.R. Sawaya, L. Guan, H.R. Kaback, and J. Abramson. 2011. Crystal structure of lactose permease in complex with an affinity inactivator yields unique insight into sugar recognition. Proc. Natl. Acad. Sci. USA. 108:9361–9366. https://doi.org/10.1073/pnas.1105687108

Costello, M.J., J. Escaig, K. Matsushita, P.V. Viitanen, D.R. Menick, and H.R. Kaback. 1987. Purified lac permease and cytochrome o oxidase are functional as monomers. J. Biol. Chem. 262:17072–17082.

Dang, S., L. Sun, Y. Huang, F. Lu, Y. Liu, H. Gong, J. Wang, and N. Yan. 2010. Structure of a fucose transporter in an outward-open conformation. Nature. 467:734–738. https://doi.org/10.1038/nature09406

Deutscher, J., F.M. Ake, M. Derkaoui, A.C. Z´ ebr´ e, T.N. Cao, H. Bouraoui, T.´ Kentache, A. Mokhtari, E. Milohanic, and P. Joyet. 2014. The bacterial phosphoenolpyruvate:carbohydrate phosphotransferase system: regulation by protein phosphorylation and phosphorylation-dependent protein-protein interactions. Microbiol. Mol. Biol. Rev. 78:231–256. https://doi.org/10.1128/MMBR.00001-14

Ethayathulla, A.S., M.S. Yousef, A. Amin, G. Leblanc, H.R. Kaback, and L. Guan. 2014. Structure-based mechanism for Na(+)/melibiose symport by MelB. Nat. Commun. 5:3009. https://doi.org/10.1038/ncomms4009

Foster, D.L., M. Boublik, and H.R. Kaback. 1983. Structure of the lac carrier protein of Escherichia coli. J. Biol. Chem. 258:31–34.

Frillingos, S., M. Sahin-Tóth, J. Wu, and H.R. Kaback. 1998. Cys-scanning mutagenesis: a novel approach to structure function relationships in polytopic membrane proteins. FASEB J. 12:1281–1299. https://doi.org/10.1096/fasebj.12.13.1281

Gaiko, O., A. Bazzone, K. Fendler, and H.R. Kaback. 2013. Electrophysiological characterization of uncoupled mutants of LacY. Biochemistry. 52: 8261–8266. https://doi.org/10.1021/bi4013269

Garcia-Celma, J.J., I.N. Smirnova, H.R. Kaback, and K. Fendler. 2009. Electrophysiological characterization of LacY. Proc. Natl. Acad. Sci. USA. 106: 7373–7378. https://doi.org/10.1073/pnas.0902471106

Garcia-Celma, J.J., J. Ploch, I. Smirnova, H.R. Kaback, and K. Fendler. 2010. Delineating electrogenic reactions during lactose/H+ symport. Biochemistry. 49:6115–6121. https://doi.org/10.1021/bi100492p

Grytsyk, N., J. Sugihara, H.R. Kaback, and P. Hellwig. 2017. pK_a of Glu325 in LacY. Proc. Natl. Acad. Sci. USA. 114:1530–1535. https://doi.org/10.1073/pnas.1621431114

Grytsyk, N., A.F. Santos Seiça, J. Sugihara, H.R. Kaback, and P. Hellwig. 2019. Arg302 governs the pK_a of Glu325 in LacY. Proc. Natl. Acad. Sci. USA. 116: 4934–4939. https://doi.org/10.1073/pnas.1820744116

Guan, L., and H.R. Kaback. 2004. Binding affinity of lactose permease is not altered by the H+ electrochemical gradient. Proc. Natl. Acad. Sci. USA. 101:12148–12152. https://doi.org/10.1073/pnas.0404936101

Guan, L., and H.R. Kaback. 2006. Lessons from lactose permease. Annu. Rev. Biophys. Biomol. Struct. 35:67–91. https://doi.org/10.1146/annurev.biophys.35.040405.102005

Guan, L., F.D. Murphy, and H.R. Kaback. 2002. Surface-exposed positions in the transmembrane helices of the lactose permease of Escherichia coli determined by intermolecular thiol cross-linking. Proc. Natl. Acad. Sci. USA. 99:3475–3480. https://doi.org/10.1073/pnas.052703699

Guan, L., Y. Hu, and H.R. Kaback. 2003a. Aromatic stacking in the sugar binding site of the lactose permease. Biochemistry. 42:1377–1382. https://doi.org/10.1021/bi027152m

Guan, L., M. Sahin-Tóth, T. Kalai, K. Hideg, and H.R. Kaback. 2003b. Probing´ the mechanism of a membrane transport protein with affinity inactivators. J. Biol. Chem. 278:10641–10648. https://doi.org/10.1074/jbc .M211355200

Guan, L., O. Mirza, G. Verner, S. Iwata, and H.R. Kaback. 2007. Structural determination of wild-type lactose permease. Proc. Natl. Acad. Sci. USA. 104:15294–15298. https://doi.org/10.1073/pnas.0707688104

Hariharan, P., and L. Guan. 2014. Insights into the inhibitory mechanisms of the regulatory protein IIA(Glc) on melibiose permease activity. J. Biol. Chem. 289:33012–33019. https://doi.org/10.1074/jbc.M114.609255

Hariharan, P., D. Balasubramaniam, A. Peterkofsky, H.R. Kaback, and L. Guan. 2015. Thermodynamic mechanism for inhibition of lactose permease by the phosphotransferase protein IIAGlc. Proc. Natl. Acad. Sci. USA. 112:2407–2412. https://doi.org/10.1073/pnas.1500891112

Hariharan,P., M. Andersson,X.Jiang, E.Pardon, J.Steyaert,H.R.Kaback, andL. Guan. 2016. Thermodynamics of Nanobody Binding to Lactose Permease. Biochemistry. 55:5917–5926. https://doi.org/10.1021/acs.biochem.6b00826

Iancu, C.V., J. Zamoon, S.B. Woo, A. Aleshin, and J.Y. Choe. 2013. Crystal structure of a glucose/H+ symporter and its mechanism of action. Proc. Natl. Acad. Sci. USA. 110:17862–17867. https://doi.org/10.1073/pnas.1311485110

Jardetzky, O. 1966. Simple allosteric model for membrane pumps. Nature. 211: 969–970. https://doi.org/10.1038/211969a0

Jiang, X., I. Smirnova, V. Kasho, J. Wu, K. Hirata, M. Ke, E. Pardon, J. Steyaert, N. Yan, and H.R. Kaback. 2016. Crystal structure of a LacY-nanobody complex in a periplasmic-open conformation. Proc. Natl. Acad. Sci. USA. 113:12420–12425. https://doi.org/10.1073/pnas.1615414113

Kaback, H.R. 1968. The role of the phosphoenolpyruvate-phosphotransferase system in the transport of sugars by isolated membrane preparations of Escherichia coli. J. Biol. Chem. 243:3711–3724.

Kaback, H.R. 2015. A chemiosmotic mechanism of symport. Proc. Natl. Acad. Sci. USA. 112:1259–1264. https://doi.org/10.1073/pnas.1419325112

Kaback, H.R., M. Sahin-Tóth, and A.B. Weinglass. 2001. The kamikaze approach to membrane transport. Nat. Rev. Mol. Cell Biol. 2:610–620. https://doi.org/10.1038/35085077

Kaback, H.R., R. Dunten, S. Frillingos, P. Venkatesan, I. Kwaw, W. Zhang, and N. Ermolova. 2007. Site-directed alkylation and the alternating access model for LacY. Proc. Natl. Acad. Sci. USA. 104:491–494. https://doi.org/10.1073/pnas.0609968104

Kaback, H.R., I. Smirnova, V. Kasho, Y. Nie, and Y. Zhou. 2011. The alternating access transport mechanism in LacY. J. Membr. Biol. 239:85–93. https://doi.org/10.1007/s00232-010-9327-5

Kaczorowski, G.J., D.E. Robertson, and H.R. Kaback. 1979. Mechanism of lactose translocation in membrane vesicles from Escherichia coli. 2. Effect of imposed delata psi, delta pH, and Delta mu H+. Biochemistry. 18: 3697–3704. https://doi.org/10.1021/bi00584a010

Kumar, H., V. Kasho, I. Smirnova, J.S. Finer-Moore, H.R. Kaback, and R.M. Stroud. 2014. Structure of sugar-bound LacY. Proc. Natl. Acad. Sci. USA. 111:1784–1788. https://doi.org/10.1073/pnas.1324141111

Kumar, H., J.S. Finer-Moore, H.R. Kaback, and R.M. Stroud. 2015. Structure of LacY with an α-substituted galactoside: Connecting the binding site to the protonation site. Proc. Natl. Acad. Sci. USA. 112:9004–9009. https://doi.org/10.1073/pnas.1509854112

Kumar, H., J.S. Finer-Moore, X. Jiang, I. Smirnova, V. Kasho, E. Pardon, J. Steyaert, H.R. Kaback, and R.M. Stroud. 2018. Crystal Structure of a ligand-bound LacY-Nanobody Complex. Proc. Natl. Acad. Sci. USA. 115: 8769–8774. https://doi.org/10.1073/pnas.1801774115

Mitchell, P. 1967. Translocations through natural membranes. Adv. Enzymol. Relat. Areas Mol. Biol. 29:33–87.

Mitchell, P. 1968. Chemiosmotic coupling and energy transduction. Glynn Research Ltd, Bodmin, UK.

Müller-Hill, B. 1996. The lac Operon: A Short History of a Genetic Paradigm. Walter de Gruyter, Berlin, NY. https://doi.org/10.1515/9783110879476

Newman, M.J., D.L. Foster, T.H. Wilson, and H.R. Kaback. 1981. Purification and reconstitution of functional lactose carrier from Escherichia coli. J. Biol. Chem. 256:11804–11808.

Nie, Y., and H.R. Kaback. 2010. Sugar binding induces the same global conformational change in purified LacY as in the native bacterial membrane. Proc. Natl. Acad. Sci. USA. 107:9903–9908. https://doi.org/10.1073/pnas.1004515107

Nie, Y., N. Ermolova, and H.R. Kaback. 2007. Site-directed alkylation of LacY: effect of the proton electrochemical gradient. J. Mol. Biol. 374:356–364. https://doi.org/10.1016/j.jmb.2007.09.006

Padan, E., D. Zilberstein, and H. Rottenberg. 1976. The proton electrochemical gradient in Escherichia coli cells. Eur. J. Biochem. 63:533–541. https://doi.org/10.1111/j.1432-1033.1976.tb10257.x

Park, Y.H., B.R. Lee, Y.J. Seok, and A. Peterkofsky. 2006. In vitro reconstitution of catabolite repression in Escherichia coli. J. Biol. Chem. 281: 6448–6454. https://doi.org/10.1074/jbc.M512672200

Postma, P.W., J.W. Lengeler, and G.R. Jacobson. 1993. Phosphoenolpyruvate: carbohydrate phosphotransferase systems of bacteria. Microbiol. Rev. 57: 543–594.

Ramos, S., S. Schuldiner, and H.R. Kaback. 1976. The electrochemical gradient of protons and its relationship to active transport in Escherichia coli membrane vesicles. Proc. Natl. Acad. Sci. USA. 73:1892–1896. https://doi.org/10.1073/pnas.73.6.1892

Robertson, D.E., G.J. Kaczorowski, M.L. Garcia, and H.R. Kaback. 1980. Active transport in membrane vesicles from Escherichia coli: the electrochemical proton gradient alters the distribution of the lac carrier between two different kinetic states. Biochemistry. 19:5692–5702. https://doi.org/10.1021/bi00566a005

Sahin-Tóth, M., and H.R. Kaback. 2001. Arg-302 facilitates deprotonation of Glu-325 in the transport mechanism of the lactose permease from EscherichiacoliProc. Natl. Acad. Sci. USA. 98:6068–6073. https://doi.org/10 .1073/pnas.111139698

Sahin-Tóth, M., M.C. Lawrence, and H.R. Kaback. 1994. Properties of permease dimer, a fusion protein containing two lactose permease molecules from Escherichia coli Proc. Natl. Acad. Sci. USA. 91:5421–5425. https://doi.org/10.1073/pnas.91.12.5421

Sahin-Tóth, M., K.M. Akhoon, J. Runner, and H.R. Kaback. 2000. Ligand recognition by the lactose permease of Escherichia coli: specificity and affinity are defined by distinct structural elements of galactopyranosides Biochemistry. 39:5097–5103. https://doi.org/10.1021/bi0000263

Sahin-Tóth, M., M.C. Lawrence, T. Nishio, and H.R. Kaback. 2001. The C-4 hydroxyl group of galactopyranosides is the major determinant for ligand recognition by the lactose permease of Escherichia coli. Biochemistry. 40:13015–13019. https://doi.org/10.1021/bi0112331

Smirnova, I.N., V. Kasho, and H.R. Kaback. 2008. Protonation and sugar binding to LacY. Proc. Natl. Acad. Sci. USA. 105:8896–8901. https://doi.org/10.1073/pnas.0803577105

Smirnova, I., V. Kasho, J. Sugihara, J.Y. Choe, and H.R. Kaback. 2009a. Residues in the H+ translocation site define the pKa for sugar binding to LacY. Biochemistry. 48:8852–8860. https://doi.org/10.1021/bi9011918

Smirnova, I., V. Kasho, J. Sugihara, and H.R. Kaback. 2009b. Probing of the rates of alternating access in LacY with Trp fluorescence. Proc. Natl. Acad. Sci. USA. 106:21561–21566. https://doi.org/10.1073/pnas .0911434106

Smirnova, I., V. Kasho, and H.R. Kaback. 2011a. Lactose permease and the alternating access mechanism. Biochemistry. 50:9684–9693. https://doi.org/10.1021/bi2014294

Smirnova, I., V. Kasho, J. Sugihara, and H.R. Kaback. 2011b. Opening the periplasmic cavity in lactose permease is the limiting step for sugar binding. Proc. Natl. Acad. Sci. USA. 108:15147–15151. https://doi.org/10.1073/pnas.1112157108

Smirnova, I., V. Kasho, J. Sugihara, and H.R. Kaback. 2013. Trp replacements for tightly interacting Gly-Gly pairs in LacY stabilize an outward-facing conformation. Proc. Natl. Acad. Sci. USA. 110:8876–8881. https://doi.org/10.1073/pnas.1306849110

Smirnova, I., V. Kasho, X. Jiang, E. Pardon, J. Steyaert, and H.R. Kaback. 2014. Outward-facing conformers of LacY stabilized by nanobodies. Proc. Natl. Acad. Sci. USA. 111:18548–18553. https://doi.org/10.1073/pnas.1422265112

Smirnova, I., V. Kasho, X. Jiang, E. Pardon, J. Steyaert, and H.R. Kaback. 2015. Transient conformers of LacY are trapped by nanobodies. Proc. Natl. Acad. Sci. USA. 112:13839–13844. https://doi.org/10.1073/pnas .1519485112

Smirnova, I., V. Kasho, X. Jiang, and H.R. Kaback. 2017. An Asymmetric Conformational Change in LacY. Biochemistry. 56:1943–1950. https://doi.org/10.1021/acs.biochem.7b00134

Smirnova, I., V. Kasho, X. Jiang, H.M. Chen, S.G. Withers, and H.R. Kaback. 2018. Oversized galactosides as a probe for conformational dynamics in LacY. Proc. Natl. Acad. Sci. USA. 115:4146–4151. https://doi.org/10.1073/pnas.1800706115

Sun, L., X. Zeng, C. Yan, X. Sun, X. Gong, Y. Rao, and N. Yan. 2012. Crystal structure of a bacterial homologue of glucose transporters GLUT1-4. Nature. 490:361–366. https://doi.org/10.1038/nature11524

Vazquez-Ibar, J.L., L. Guan, M. Svrakic, and H.R. Kaback. 2003. Exploiting´ luminescence spectroscopy to elucidate the interaction between sugar and a tryptophan residue in the lactose permease of Escherichia coli Proc. Natl. Acad. Sci. USA. 100:12706–12711. https://doi.org/10.1073/pnas.1835645100

Viitanen, P., M.L. Garcia, D.L. Foster, G.J. Kaczorowski, and H.R. Kaback. 1983. Mechanism of lactose translocation in proteoliposomes reconstituted with lac carrier protein purified from Escherichia coli. 2. Deuterium solvent isotope effects. Biochemistry. 22:2531–2536. https://doi .org/10.1021/bi00279a034

Vogel, H., J.K. Wright, and F. Jahnig. 1985. The structure of the lactose per-¨mease derived from Raman spectroscopy and prediction methods. EMBO J. 4:3625–3631. https://doi.org/10.1002/j.1460-2075.1985.tb04126.x

Vogt, A.D., N. Pozzi, Z. Chen, and E. Di Cera. 2014. Essential role of conformational selection in ligand binding. Biophys. Chem. 186:13–21. https:// doi.org/10.1016/j.bpc.2013.09.003

von Heijne, G. 1992. Membrane protein structure prediction. Hydrophobicity analysis and the positive-inside rule. J. Mol. Biol. 225:487–494. https://doi.org/10.1016/0022-2836(92)90934-C

Weinglass, A.B., I.N. Smirnova, and H.R. Kaback. 2001. Engineering conformational flexibility in the lactose permease of Escherichia coli: use of glycine-scanning mutagenesis to rescue mutant Glu325-->Asp. Biochemistry. 40:769–776. https://doi.org/10.1021/bi002171m

Weinglass, A.B., M. Sondej, and H.R. Kaback. 2002. Manipulating conformational equilibria in the lactose permease of Escherichia coli. J. Mol. Biol. 315:561–571. https://doi.org/10.1006/jmbi.2001.5289

Weinglass, A.B., J.P. Whitelegge, Y. Hu, G.E. Verner, K.F. Faull, and H.R. Kaback. 2003. Elucidation of substrate binding interactions in a membrane transport protein by mass spectrometry. EMBO J. 22: 1467–1477. https://doi.org/10.1093/emboj/cdg145

Yousef, M.S., and L. Guan. 2009. A 3D structure model of the melibiose permease of Escherichia coli represents a distinctive fold for Na+ symporters. Proc. Natl. Acad. Sci. USA. 106:15291–15296. https://doi.org/10.1073/pnas.0905516106

Zen, K.H., E. McKenna, E. Bibi, D. Hardy, and H.R. Kaback. 1994. Expression of lactose permease in contiguous fragments as a probe for membranespanning domains. Biochemistry. 33:8198–8206. https://doi.org/10.1021/ bi00193a005

Zilberstein, D., S. Schuldiner, and E. Padan. 1979. Proton electrochemical gradient in Escherichia coli cells and its relation to active transport of lactose. Biochemistry. 18:669–673. https://doi.org/10.1021/bi00571a018