

Breaching the Barrier

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Transporter proteins are integral membrane proteins that selectively mediate the passage of molecules across the otherwise impermeable barrier imposed by the phospholipid bilayer that surrounds all cells and organelles. The identification of more than 360 families of transporters through biochemical and genomic analyses (1) highlights the importance of transport

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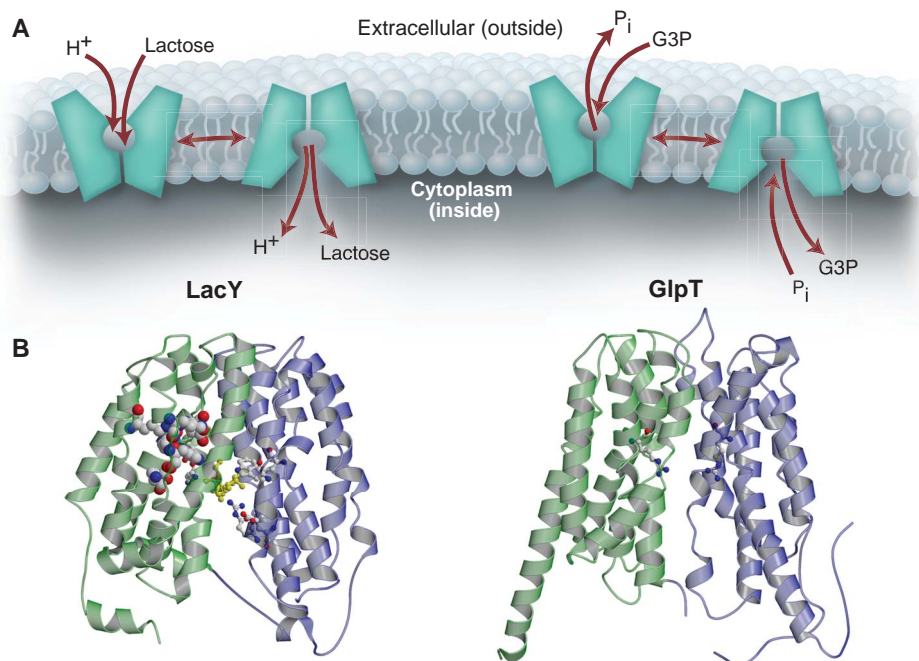
processes to cells. Among the most fascinating transporters are those that act as molecular pumps, translocating their substrates across membranes against a concentration gradient; this thermodynamically unfavorable process is powered by coupling to a second, energetically favorable process such as ATP hydrolysis or the movement of a second solute down a transmembrane concentration gradient. The latter mechanism is used by members of the major facilitator superfamily (MFS) of transporters, which includes the bacterial lactose permease LacY and the glycerol-3-phosphate transporter GlpT. LacY mediates the coupled cotransport of lactose and H⁺, whereas GlpT catalyzes the exchange of glycerol-3-phosphate for phosphate (see the figure). On pages 610 (2) and 616 (3) of this issue, two groups present their structural determinations of the *E. coli* LacY and GlpT transporters. The structures of LacY and GlpT, together with those of Ca²⁺-ATPase (4), MsbA (5), AcrB (6), and BtuCD (7), mark a new era in defining transport mechanisms in molecular detail.

The LacY and GlpT structures reveal that the expected 12 transmembrane helices in each protein are organized into two distinct domains composed of six helices in the amino terminus and six helices in the carboxyl terminus (see the figure). These domains have equivalent helix-packing arrangements and are approximately related by an intramolecular twofold rotation. This general arrangement had been anticipated by electron microscopy studies on the OxlT transporter (8). At the interface be-

tween the amino- and carboxyl-terminal domains, a crevice containing residues implicated in substrate binding is formed along the twofold axis. In both LacY and GlpT, this crevice opens into the cytoplasm, whereas access to the periplasm is blocked.

The LacY and GlpT structures support the "alternating access" mechanism of transport (9, 10) in which the protein undergoes a series of conformational transitions such that the ligand-binding site is alternatively accessible to one side of the membrane or the other, but not to both sides simultaneously. The key to successful operation of transporters is

to eliminate short-circuiting by preventing the uncoupled processes (that is, substrate translocation or ATP hydrolysis) from occurring individually in their energetically favorable directions. The challenge to biochemists has been to define the molecular details of a mechanism that goes beyond the cartoon level of resolution depicted in such general schemes as part A of the figure. Although still far from clear, this mechanistic analysis is most advanced for LacY, in large part owing to the experimental efforts of Kaback's group (11). The lactose- and proton-binding sites contain distinct sets of amino acids that are linked by critical residues. In the presence of the appropriate set of bound ligands, these residues apparently adopt different conformations that switch the transporter between inward- and outward-facing orienta-



Inward-looking membrane proteins. (A) The alternating-access model of transport for the bacterial transporters LacY (left) and GlpT (right). LacY catalyzes the coupled transport of lactose and H⁺ from one side of the membrane to the other, whereas GlpT mediates the exchange of glycerol-3-phosphate (G3P) for inorganic phosphate (P_i). The pair of structures for each transporter show the outward-facing conformation (exposed to the extracellular side) on the left, and the inward-facing conformation (exposed to the cytoplasm) on the right. The bottom surface of the membrane bilayer is depicted in contact with the cytoplasm; there is no standard convention in the transporter field for this top/bottom orientation, and different conventions are used in the LacY and GlpT papers (2, 3). (B) Ribbon representation of the polypeptide folds of LacY (left) and GlpT (right). Shown are the amino-terminal and carboxyl-terminal domains (blue and green, respectively); the substrate-binding site is located at their interface. (Left) Shown for LacY are a lactose analog (yellow), amino acids in the sugar-binding site (ball-and-sticks), and residues implicated in proton translocation (space-filling models). (Right) Shown for GlpT are arginine residues 45 and 269 (ball-and-sticks) at the proposed substrate-binding site. Bends and other irregularities in the α helices are indicated by deviations from ideally straight and continuous helical ribbons. The crystallographically determined structures for LacY and GlpT are both inward facing and would open into the cytoplasm. Part B of the figure was prepared with the program MOLSCRIPT (18).

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tions. At a minimum, this rearrangement must reposition the amino- and carboxyl-terminal domains through reconfiguration of helix-helix interactions along the interface surrounding the substrate-binding site. Both groups describe plausible models for this transition (2, 3).

Beyond mechanistic insights, the new structures provide an opportunity to evaluate extensive biochemical studies on LacY that were designed to define functionally important residues and helix-packing interactions (11). In general, the biochemical studies correctly identified mechanistically critical residues in the LacY ligand-binding site. However, an additional important observation from the structures of LacY, GlpT, and other membrane proteins is that transmembrane helices can be bent or exhibit other types of irregular features (see the figure). Together with the conformational flexibility inherent in these transporters, which is necessary for their activity, deviations from ideal α helices have confounded attempts to unambiguously establish helix-helix interactions through biochemical studies, such as cross-linking (12). The mechanistic importance of helical distortions and irregularities for transport processes is underscored by the role these features have been proposed to play during substrate translocation by the Ca^{2+} -ATPase (13), as well as gating transitions in K^{+} (14) and acetylcholine receptor channels (15).

Why did the LacY and GlpT structure determinations succeed? Although the terse method sections may imply otherwise, "magic" was not involved; rather, both structures are the result of extended and committed efforts to surmount the innumerable experimental barriers encountered in these projects. Perhaps even more daunting was the necessity to survive promotion and funding hurdles long enough to see the structures through to completion. The LacY and GlpT structure determinations illustrate different emphases in tackling the membrane protein structure problem. In the case of LacY, decades of experimental studies on *E. coli* LacY dictated that this protein would be the target of structural inquiry. Ultimately, suitable crystals were prepared of a mutant form of LacY trapped in the inward-facing conformation to minimize conformational dynamics. In contrast, the GlpT structure was the result of a more general desire to crystallographically characterize a member of the MFS family. The *E. coli* GlpT was identified through the screening of a number of homologs to find the most suitable candidate for structural work (16). In both cases, the structures could only have been solved through ready access to synchrotron x-ray sources and their staffs to surmount the limitations of modest diffraction quality and substantial crystal-to-crystal variability. Because LacY and GlpT were both crystallized in the inward-facing conformation, a high priority for future studies

will be to develop approaches to trap each protein in the outward-facing state to define the endpoints of the transporter mechanism.

The LacY and GlpT structures provide significant advances in our understanding not only of the structure and mechanism of an important class of transporters, but also of membrane proteins in general. The development of new approaches encourages the hope that 18 years after the determination of the photosynthetic reaction center structure (17), the crystallography of membrane proteins is coming of age. With sufficient effort, the major barriers to the structural determinations of membrane proteins are finally being breached.

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PHYSICS

Getting Entangled in Free Space

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Entanglement is a property of quantum theory that allows two particles (such as photons) to be much more strongly correlated than is possible in classical physics. Conventional quantum theory sets no limit on the range or duration of these correlations. Over the past 20 years, many optical experiments have demonstrated these apparently nonlocal effects in the laboratory (1–4) and more recently over ranges of up to 10 km in optical fibers (5–7).

On page 621 of this issue, Aspelmeyer *et al.* (8) report the latest of these demonstrations. In contrast to earlier studies, they study entanglement not in optical fibers but in free space. The authors show that strong correlations can be maintained in

free space over a separation of 600 m. The optical losses were comparable to those in a putative space-based experiment, in which a satellite would send correlated photon pairs to two separate observers on Earth about 600 km away.

A simplified version of the experimental setup used by Aspelmeyer *et al.* is shown in the figure. Key to the experiment is a source that creates entangled pairs of photons with anticorrelated polarizations. If the source emits a horizontal photon in direction 1, a vertical photon is emitted in direction 2, and vice versa. This anticorrelation holds for any polarization direction. The photons are sent in opposite directions to two well-separated polarizers and single-photon detectors. The polarizers, which can be rotated to any angle, allow those photons to pass that are polarized parallel to this angle, while blocking all photons polarized at 90° to this angle.

The detectors fire when photons are detected, producing a series of clicks that measure the arrival times of photons. Separate measurements in arm 1 or arm 2 show no change in the click rate when the polarizer angle is changed. Hence, there is no favored polarization emitted by the source. But when both detectors fire simultaneously, indicating the detection of photon pairs, the click rate increases when the polarizers are set at 90° to each other: For instance, a detector click in channel 1 behind a vertical (*V*) polarizer immediately implies a click in channel 2 when using a horizontal (*H*) polarizer.

In a classical (or "local realistic") view, we would make the naïve assumption that photons have a real (locally labeled) polarization when they leave the crystal. For instance, an *H*-polarized photon emitted in direction 1 would imply a *V*-polarized photon emitted in direction 2. After emission, we could set the angle for the polarizer to 45° in channel 1 (–45° in channel 2), such that only half the intensity of horizontally or vertically polarized light will pass through the polarizer. Each individual *H* or *V* photon then has a 50% chance of passing

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