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Symmetric transporters for asymmetric transport

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Regardless of whether they are consumed in cans of soda or white bread, carbohydrates, such as glucose, are a central component of the Western diet. Indeed, the average adult metabolizes an extraordinary ~250 grams of glucose per day, half of which is utilized as an energy source for the brain alone. In order to benefit from these ingested carbs, they must first be broken down into glucose and absorbed through the epithelial cells of the intestine. Even more amazing is the glucose reabsorption process in the kidneys. In the fasting state, about 125 milliliters of plasma is filtered through the kidneys every minute, from which essentially all the 100 milligrams of glucose is reabsorbed by the proximal tubule, and the kidneys filter 180 liters of plasma per day! The molecular mechanism how such effective absorption is realized has fascinated physiologists for more than half a century. Previous work has shown that glucose is absorbed from the lumen into the epithelial cell by a Na^+ /glucose co-transporter, SGLT1 in the intestine, and by the related SGLT2 in the kidney (Figure A). The glucose is then exported by the glucose transporter GLUT2 to the basal side of the cell into the blood. Even though these transport proteins share the same substrate and all function as the so-called secondary membrane transporters, they are members of distinct protein families: SGLT1 and SGLT2 belong to the solute sodium symporters (SSS), whereas GLUT2 is a member of the major facilitator superfamily (MFS). The structure of a bacterial SSS protein reported by Faham and colleagues in the current issue of *Science* (1) represents a major advance in the mechanistic understanding of the sugar absorption process.

Secondary active membrane transporters couple “uphill” translocation of substrate across the membrane to the energetically favorable flow of ions down their concentration gradient, often of the proton in bacteria and Na^+ in eukaryotes. Both the substrates they transport, ranging from ions and sugars to lipophilic drugs, and their apparent protein architectures are diverse: over two hundred distinct families can be classified on the basis of primary structure. Nevertheless, biochemical, kinetic and, more recently, structural studies suggest that all secondary membrane transporters operate via a common alternating-access mechanism of transport (2). In this mechanism, the transporter is believed to have two major alternating conformations, inward-facing (C_i) and outward-facing (C_o). Interconversion between the two conformations facilitates the substrate translocation across the membrane. This kinetic scheme can be realized in physical space through two types of conformational changes: a rocker-switch type of movement of the two halves of the protein as proposed by Vidavsky (3) and, an alternating gates or gated-pore model proposed by Patlak (4) – a channel-like protein with two gates that open alternatively (Figure). The first type of transporters have been visualized in their C_i conformation at high-resolution by X-ray crystallography for two MFS proteins (5,6). Structures of several transporter proteins that presumably operate via the gated-pore model have also been determined, including the mitochondrial ADP/ATP exchanger (7), the bacterial glutamate transporter Glt_{Ph} (8) and leucine transporter LeuT (9), but none of these structures

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are of proteins that belong to the same transporter family. Therefore, it is still an open question whether any transporter indeed works as a gated pore.

Enter vSGLT. An SSS protein that shares 30% sequence identity with human SGLT1, vSGLT is a Na⁺-driven galactose transporter from *Vibrio parahaemolyticus*. Now Faham and colleagues report the 3.0 Å structure of vSGLT in its substrate-bound, inward-facing conformation (1). The structure consists of 14 transmembrane α -helices (TM1–r14). Interestingly, the core of the protein is made of two inverted, symmetric halves; TM2–6 and TM7–11 are related by a pseudo twofold symmetry around an axis in the membrane plane, with additional helices at both the N- and C-termini. The substrate, galactose along with a Na⁺ ion, is bound at the center of the core helices in the middle of the membrane. The substrate is closed off to the extramembrane space by a thick gate made of hydrophobic residues. However, the cytoplasmic gate is much thinner, consisting of only a few side chains, and a large cavity is found outside the gate. Therefore, the structure presents the substrate-bound, C_i conformation of the protein.

Strikingly, the vSGLT structure (1) is reminiscent of that of LeuT (1), a bacterial member of the neurotransmitter sodium symporter (NSS) family. While the core domain of vSGLT shares only 11% sequence identity with LeuT (1), significant sequence identity between some other SSS and NSS proteins, such as pig SGLT1 and the mouse GABA transporter GAT-2, have previously been recognized and a common ancestor for the two transporter families proposed (10). Besides sharing the same fold, both vSGLT and LeuT have equivalent helices that are unwound in the middle of membrane to form part of the substrate-binding site. Thus, this structural similarity allowed Faham and colleagues to deduce the unresolved Na⁺-binding site in the current vSGLT structure (1).

Seeing is believing. By determining the vSGLT structure in the C_i conformation, the authors have provided strong evidence that a secondary membrane transporter can indeed operate like a gated pore. The LeuT crystal structure is in its substrate-bound, C_o conformation (9). Taking advantage of the structural homology between the two proteins, Faham and colleagues mapped the vSGLT sequence onto the LeuT structure and generated a C_o model for vSGLT (1). Comparison of the C_i and C_o conformations of vSGLT allowed the authors to propose a reasonable model for the Na⁺-coupled galactose transport across the membrane by vSGLT.

The symmetry between the vSGLT C_i structure and the LeuT C_o structure is however not perfect. What is missing on the cytoplasmic side of the vSGLT C_i structure (1) is a vestibule found between the gate and the tip of a hairpin loop on the symmetric, extracellular side of the LeuT C_o structure (9). Perhaps this vestibule can function as a reservoir for concentrating the substrate from the extracellular space for substrate import? Interestingly, tricyclic antidepressant molecules are found to bind at this site in LeuT and inhibit its transport activity (11,12). Mutagenesis in the human neurotransmitter transporters supports that this antidepressant-binding site is conserved in the human proteins (11). A recent paper in *Molecular Cell* (13) further shows that in LeuT substrate leucine can bind to the same antidepressant site and the binding triggers the cytoplasmic substrate release from the main site.

While both SSS-type gated pores and MFS-type of rocker switches are composed of two symmetry-related homologous domains, their interdomain association is distinct. Gated pores like SGLT are formed by the association of two V-shaped domains intertwined in an *antiparallel* manner. The substrate-binding site is located in the center of the membrane at two broken helices, one from each domain. The new vSGLT structure reveals that alternating access is achieved by rotation of the cytoplasmic portion of two broken helices from the substrate-binding site (Figure B). In contrast, In MFS, two *parallel* helical bundles associate to form a shared binding site at their interface. Substrate binding at this interface from one side of the

membrane drives domain closure, thereby alternately exposing the binding site to opposite sides of the bilayer.

The gated-pore and the rocker-switch models are kinetically identical as both utilize electrochemical gradient-driven membrane transport. Typically, the conformational changes required for transport in a gated pore are significantly smaller than that in a rocker-switch type of transporter, perhaps offering an advantage for crystallization experiments. The differences between proteins that operate via these two types of mechanisms do not stop here. A gated pore resembles an ion channel but with two gates instead of one and can often display uncoupled, channel-like ion conduction. Such uncoupled current has been observed in several NSS proteins (14). Likewise, in the absence of glucose, SGLT1 also displays channel-like behaviors (15).

The vSGLT structure fills in an important piece of the puzzle in understanding the molecular mechanism of sugar absorption in the human body. To completely understand this physiological process, however, there is an urgent need to identify and better characterize all of the conformational states in the transport cycle for a particular protein. The isolation of secondary transporters in any specific conformational state remains a major technical obstacle for structural studies. Unlike ion channels, which can be characterized by toxins and single-channel recording, or primary membrane transporters whose transport cycle can be defined with the help of non-hydrolyzable ATP analogs, secondary transporters are unamenable to such enzymological manipulation. As a result, their kinetic cycle is often defined by binding and transport assays only. However, with structural information for some of the conformational states in hand, increasingly valuable techniques, such as EPR spectroscopy and single molecule FRET can provide precise information about the nature and magnitude of the conformational states during the transport cycle. Only then can one succeed in solving the membrane transporter structure in all its states and not worry about any sugar loss during the sweet celebration!

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Figure. Gated Pores and Rocker Switches

(A) Net sugar transport across the intestinal epithelia is catalyzed by SGLT1 in the apical membrane, which couples Na^+ influx down its concentration gradient to glucose uptake, and GLUT2, which facilitates glucose transport across the membrane to the basal side of the cell. A similar transport pathway is responsible for glucose reabsorption in the kidneys. Gated pores like SGLT1 are formed by the association of two V-shaped domains intertwined in an *antiparallel* manner. In contrast, two *parallel* helical bundles associate to form a shared binding site at their interface in rocker switch GLUT2, a MFS protein. (B) In gated pores such as SGLT1, the rotation of two broken helices permits alternating access of the substrates to the opposing sides of the membrane, as seen by comparison of the vSGLT and LeuT structures. Rocker switches, exemplified by the glycerol-3-phosphate transporter (GlpT) (5) here, are thought to function by the rotation of two domains towards one another, alternately exposing

the substrate-binding site to each side of the membrane. The field anxiously awaits the visualization of a rocker switch in its C_o state.