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**At last, structures have been solved for different conformational states, facing in and facing out, for the same transporter protein of the major facilitator superfamily. The structural basis of alternating substrate access for binding and translocation can now be properly visualized.**



The D-xylose–$H^+$ transport protein XylE of *Escherichia coli* has long been recognized as a homolog of human GLUT transporters[1]. Both XylE and the GLUTs are members of the 'sugar porter' family, found in organisms from microbes to man, and they belong to the wider major facilitator superfamily[2,3] (MFS; **Fig. 1**). It is thought that a common set of conformational changes underlies translocation for all MFS members, regardless of their substrate and mode of operation: symport (in which two substrates, one usually a cation, move in the same direction), antiport (in which one substrate exchanges for another, both moving in opposite directions) or facilitated diffusion (in which one substrate moves in one direction)[4]. Long before structures of the transporters were available, researchers realized that at least four conformational states must participate in the translocation process: (i) open to one side of the membrane for substrate access, (ii) occluded, in which the usually hydrophilic substrate is inside the protein, inaccessible to either side of the membrane, (iii) open to the other side of the membrane for substrate release and (iv) unloaded, restoring the original state for repetition of the process. This is the 'alternating-access' model of membrane transport[5], which has been reinforced by the biochemical features of transport reversibility (reviewed in refs. 6–9) and the underlying symmetry within all the MFS proteins, which usually comprise 12 transmembrane helices (TMHs) divided into at least two duplicated subdomains (N-terminal TMH1–6 and C-terminal TMH7–12).

Until now, a picture of the structural basis of the alternating-access mechanism for the MFS has depended on comparing an available inward-facing structure of one protein to an available outward-facing structure of a different protein in a family of relatively little amino acid sequence similarity[9–11]. Despite the similarity of the overall fold of each transporter, the numerous subtle differences in structural details make interpretation of the actual mechanism open to uncertainty.

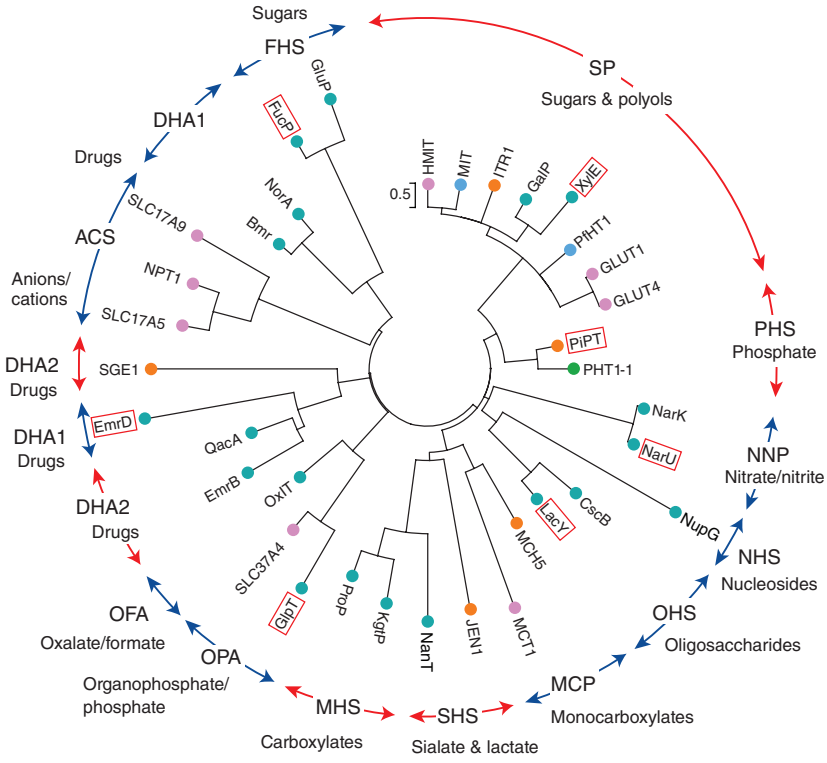


**Figure 1** The sugar porter (SP) family and its relationship to other families in the major facilitator superfamily. Transport proteins are indicated by their acronyms, and those of known structure are marked with red boxes. The different families are marked with blue or red double-headed arrows around the circumference, with their substrates indicated; red arrows indicate families with a predicted conserved salt bridge between TMH1 aspartate and TMH4 arginine residues (with the exception of equilibrative transporters such as GLUT1, GLUT4 and PfHT1), which is proposed to be involved in the translocation of protons. Colored dots indicate the organisms in which the proteins appear: teal, bacteria; orange, fungi; green, plants; blue, protozoa; purple, humans.

Now Nordlund and coworkers[12] have resolved two structures of the inward-facing form of XylE, a partially occluded inward-facing conformation and an open conformation, presented in this issue of *Nature Structural & Molecular Biology*. Taken together with the structure of XylE in an outward-facing, partially occluded form with bound substrate, recently solved by Sun *et al.*[10], Nordlund and colleagues[12] deduce a sequence of events (**Fig. 2**) in which the protein, initially oriented toward the outside, closes around the ligand to yield a form occluded to both sides that then opens to the inside. Now, for XylE at least, a plausible marriage of structural and biochemical data can be undertaken, and molecular dynamics simulations can be used to probe further the thermodynamics of transport.

Despite the relatively low resolution of the new XylE structures[12], the conformational changes accomplishing the alternating-access transport of the substrate from outside to inside (or its reverse) are described by the authors as "mainly due to a rigid body movement of the two subdomains with respect to each other," rather than as due to differences within each subdomain (**Fig. 2**). Specifically, the subdomains undergo a tilting motion resulting in tighter interactions at the periplasmic side and a wide opening appearing at the cytoplasmic side in the inward open

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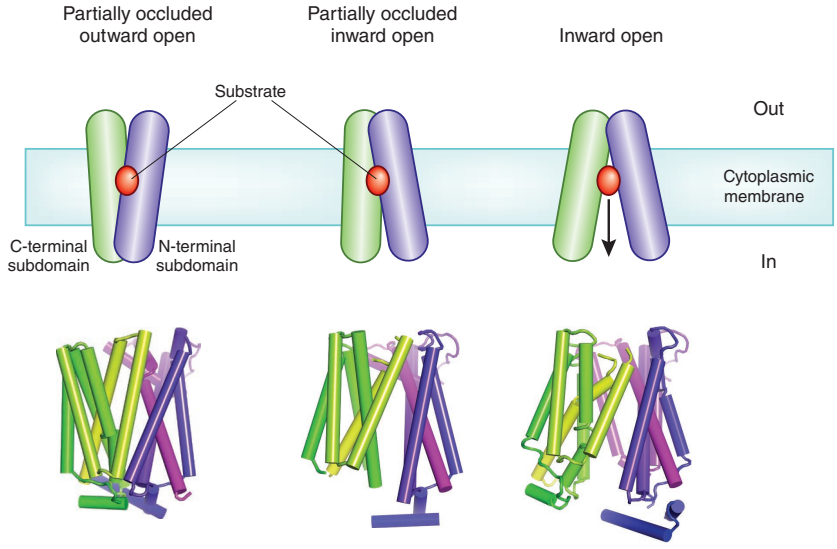


**Figure 2** The three structures of XylE are represented from the plane of the membrane. The occluded forms would contain bound substrates, whereas the inward-facing open conformation would release substrates. The structures in the bottom correspond to PDB 4GBY (partially occluded outward open), 4JA3 (partially occluded inward open) and 4JA4 (inward open); models generated by M. Breidenbach.

state. In addition, in the outward-facing XylE structure, a helix bundle, containing motifs that are highly conserved in the sugar porter family on the inside face of the proteins[1,10,12], interacts with the TMHs mainly through salt bridges, closing the exit of the substrate to the inside of the cell[10]; in the inward-facing forms of XylE, these interactions are seen to be broken[12] (**Fig. 2**), thus highlighting the important contribution of the motifs to the transport mechanism.

The events described above are in agreement with the general ideas about MFS translocation. However, the distinct functional features of each family within the MFS, in terms of substrate recognition, cation involvement and kinetics, must surely have been the selective forces driving their evolution from a primordial congenitor[2,3] (**Fig. 1**). During the evolution of plants, for example, a steadily increasing variety of sugars and other materials was incorporated into plants' metabolism and the architecture of their cell walls, from D-glucose in cellulose to D-xylose in xylans (**Fig. 3**). For primitive microorganisms living on decaying plant remains, there would be a reward in survival for evolving transport systems and metabolic enzymes to sequester these new nutrients. Although the mechanistic events of translocation remained similar across all MFS families[6,7,9], the ligand-recognition elements must have changed. Inevitably, the free-energy differences resulting from binding a different ligand would have needed to be compensated by changes in the amino acid sequence that eventually led to discrete families within the superfamily.

One important unknown that can now be addressed is the nature of the proton-binding site in XylE. This must be different from that of the lactose–$H^+$ symporter LacY because the residues involved in $H^+$ recognition by LacY[13] are not found in XylE and other members of the sugar porter family. Functional characterization of mutants at the corresponding positions in the *E. coli* galactose/glucose transporter GalP[14] and the *Leishmania donovani* myo-inositol transporter MIT[15] suggests that Asp27 in TMH1 is a prime candidate. A highly conserved acidic residue is found at this position in TMH1 of all cation-linked transporters in the sugar porter and several other MFS families (indicated with red arrows in **Fig. 1**), but it is typically replaced by an amide in facilitated diffusion systems, such as human GLUT1 and GLUT4, and PfHT1 of the malarial parasite *Plasmodium falciparum*. In the three XylE structures now available, the aspartate residue is predicted to be involved in a salt bridge with the highly conserved TMH4 residue Arg133. From models of the fully open-outward conformation of XylE, based on the equivalent structure of the *E. coli* fucose transporter FucP, we hypothesize that protonation of that aspartate breaks the bridge and triggers the transition to the fully open-outward state. Re-formation of the bridge after xylose binding and transition to the inward-facing conformation would be associated with release of the proton to the cytoplasm, perhaps through a tunnel akin to that recently described in the fungal phosphate transporter PiPT[16]. Testing of this hypothesis on coupling between proton and sugar transport awaits determination of the structure of a fully open-outward conformation of XylE or a close homolog. Identification of the proton-binding residue(s) would help explain why most GLUT proteins and PfHT1 do not recognize cations and thus catalyze facilitated diffusion rather than energized active transport. This should resolve the structural basis for active transport versus facilitated diffusion. In addition, more parochial explanations are needed for why D-glucose is not transported by XylE even though it is very similar to D-xylose (**Fig. 3**) and is bound by XylE, and for why XylE is not inhibited by the antibiotics cytochalasin B or forskolin, both of which inhibit GLUT1 (ref. 17). Answers to these questions might aid in the design of drugs to modify sugar uptake specifically into cancer cells, for example[7].

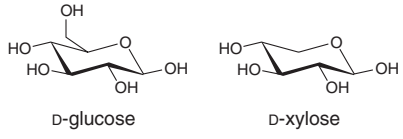


**Figure 3** The cyclic forms of D-glucose and D-xylose in solution.

Much is made of the relevance of results from studies of XylE toward understanding human GLUTs[10,12]. Of similar biological, agricultural and medical importance, of course, is the panoply of homologous sugar transport proteins found in yeasts, plants and protozoan parasites (**Fig. 1**). In fact, D-xylose metabolism has long been of interest for conversion of wood to biofuel, which generally involves incorporation of the transport protein and metabolic enzymes into a fermentation host such as yeast[18]. Understanding the precise structure-activity relationships of these transporters and the energetics of the translocation process will be informative for such aspirations.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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