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Structures and Models of Transporter Proteins

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ABBREVIATIONS: 3-D, three dimensional; 5HTT, serotonin transporter; ABC, ATP binding cassette; AcrA, membrane fusion protein; AcrB, resistace-nodulation-cell division transporter; ADP, adenosine diphosphate; ATP, adenosine triphosphate; DAT, dopamine transporter; ECM, electron cryo microscopy; EmrE, bacterial multidrug transporter; GlcV, ATP binding cassette-ATPase subunit of glucose transporter; GlpT, glycerol-3-phosphate transporter; HAE1, Hydrophobe/amphiphile efflux-1 family; LacY, lactose permease; MDR, multidrug resistance; MFS, major facilitator superfamily; MsbA, lipid flippase; NBD, nucleotide-binding domain; NET, norepinephrine transporter; NhaA, Na^+/H^+ antiporter; NSS, neurotransmitter:sodium symporter; OFA, Oxalate:formate antiporter; OHS, Oligosaccaride:H^+ symporter; OPA, Organophosphate:P_i antiporter; OxlT, oxalate transporter; PDR, pleiotropic drug resistance; P-site, auto-phosphorylation site; RND, resistance-nodulation cell division; SERCA1a, skeletal muscle sarcoplasmic reticulum calcium ATPase; SMR, small multidrug resistance; TC, transporter protein classification system; TMH, transmembrane α-helix; TolC, outer membrane channel of multidrug exporter system.

ABSTRACT

Transporter proteins in biological membranes may be divided into channels and carriers. Channels function as selective pores that open in response to a chemical or electrophysiological stimulus, allowing movement of a solute down an electrochemical gradient. Active carrier proteins use an energy producing process to translocate a substrate against a concentration gradient. Secondary active transporters use the movement of a solute down a concentration gradient to drive the translocation of another substrate across a membrane. ABC transporters couple hydrolysis of adenosine triphosphate (ATP) to the translocation of various substrates across cell membranes. High-resolution 3-dimensional structures have now been reported from x-ray crystallographic studies of 6 different transporters, including 2 ABC transporters. These structures have explained the results from many previous biochemical and biological studies, and shed new light on their functional mechanisms. All these transporters have α-helical structures of the membrane spanning domains, as suggested from many previous studies, and some of the helices have irregular shapes with kinks and bends. Together these crystal structures demonstrate the large flexibility of transporter proteins and that substantial movements take place during the substrate translocation process, which to a certain extent may distinguish active carriers from channel proteins. These structures and other, low-resolution structures of membrane proteins, have served as a basis for construction of 3-dimensional protein models which have provided insight into functional mechanisms and molecular structures, and enabled formulation of new hypotheses regarding transporter structure and function which may be experimentally validated.

Twelve years ago a review article described the growth of information on transporters as a "transporter explosion" (Uhl and Hartig, 1992). Since then, genome sequencing has made information on transporters expand in magnitude at an even more rapid rate, and the amount of information on their structures and functional mechanisms seems to have grown exponentially. A transporter protein classification system (TC) proposed in 1999 was based on phylogenetic analysis and functional properties of about 250 families of sequence-related transporters identified in bacteria, archaea, and eukaryotes (Saier, 1999). Three years later, nearly 400 families were included in the TC classification system, which then had been adopted by the International Union of Biochemistry and Molecular Biology (Busch and Saier, 2002).

Phylogenetic analysis of integral membrane transport protein sequences has shown that families of transport proteins of similar topology have evolved independently of each other, at different times in evolutionary history, using different routes (Saier, 1999; Saier, 2000; Busch and Saier, 2002). Now that the detailed 3-dimensional molecular structures of some transporter proteins have been reported, the possible similarity in protein folding patterns between these and other transporters is of crucial importance. Similar folding patterns between any protein and one for which the crystal structure is known, would enable construction of a fairly accurate 3-dimensional protein model of the unknown structure using the related crystal structure as a template, and modern computational techniques.

However, while atomic resolution crystal structures of soluble proteins have been reported in an amazingly increasing number, such progress has not been made in terms of transporters and other membrane proteins, which have proven extremely difficult to crystallise for two main reasons. One is related to the amphipathic nature of their surface, with a hydrophobic area in contact with membrane phospholipids and polar surface areas in contact with the

aqueous phases on both sides of the membrane (Ostermeier and Michel, 1997). Secondly, the majority of medically important membrane proteins are present in tissues at very low concentrations, making overexpression a prerequisite for structural studies, and this often has proven to be very difficult. In spite of recent advances in overexpression technology, bacterial membrane proteins still are the most amenable for structure determination, simply because they often can be easily expressed in large quantities (Tate, 2001). As shown in Table 1, five out of the six currently reported high-resolution transporter structures are from *E. coli*.

Their inherent conformational flexibility may also contribute to the difficulties in obtaining stable crystals of transport proteins. Using inhibitors to rigidify their structure has therefore been proposed as a possible strategy for crystallization of flexible receptors and transporters (Tate, 2001). Lactose permease was only crystallized in a mutant form (Abramson et al., 2003) which shows greater thermal stability that the wild type and markedly reduced ligand-induced conformational changes (Smirnova and Kaback, 2003).

The experimental obstacles involved in crystallization and structure determination of membrane proteins is illustrated by the fact that before determining the crystal structure of a lipid flippase (MsbA) from *Escherichia coli* the group cloned, over-expressed and purified more than 20 different bacterial ABC transporters, and tested 96 000 different crystallization conditions for several MDR-ABC transporters using about 20 different detergents (Chang and Roth, 2001).

Classification of transport proteins

In the TC classification system, transporters are divided into two main classes: channels and carriers (Saier, 2000; Busch and Saier, 2002), as indicated in Fig. 1. Channel proteins function as selective pores that open in response to a chemical or electrophysiological

stimulus, allowing movement of a solute down an electrochemical gradient. Active carrier proteins translocate substrates across a biological membrane *against* a concentration gradient. In order to do this, the transporter must couple the carrier process to another, energy producing process, and also be able to prevent that the two processes occur uncoupled in their energetically favorable directions.

Secondary Active Transporters use the movement of a solute down a concentration gradient to drive the translocation of an other substrate across a membrane against a concentration gradient, by symport or antiport mechanisms. Symporters (cotransporters) catalyse the transport of two or more molecular species in the same direction, and antiporters (exchange transporters) catalyse the exchange of one or more molecular species for another in opposite directions (Busch and Saier, 2002). Uniporters (facilitated diffusion carriers) transport a single molecular species down its concentration gradient. The uniporters, symporters and antiporters were previously regarded as members of one superfamily of transporters (Goswitz and Brooker, 1995). As more data have become available, these carrier proteins have since been classified in a large group of “porters” containing several transporter superfamilies (Busch and Saier, 2002). Secondary active transporters form a major part of the porter group.

The Major Facilitator Superfamily (MFS) includes more than 1000 evolutionarily related proteins, and is implicated in the transport of a variety of solutes and metabolites across the membranes of organisms ranging from bacteria to humans (Busch and Saier, 2002). The MFS includes both uniporters, symporters and antiporters. Many members of the MFS superfamily have medical or pharmacological relevance, as discussed by Huang and colleagues in their recent report on the GlpT structure (Huang et al., 2003).

It should be noted that a classification system based on transport mechanisms may not always be completely clear-cut. For example, the neurotransmitter transporters 5HTT, DAT and NET, classified as neurotransmitter:sodium symporters (Saier, 2000), not only mediate the transport of their substrates and Na in the same direction, but also transport some other ion (K^+ or H^+) in the opposite direction.

ATP-binding cassette (ABC) transporters are found in all taxa and form one of the largest transporter superfamilies, which contains both uptake and efflux transport systems (Saier, 2000). The ABC transporters are ubiquitous membrane proteins that couple hydrolysis of adenosine triphosphate (ATP) to the translocation of various substrates across cell membranes. Also the ABC transporter superfamily has more than 1000 members with known sequences. Members of this superfamily, which includes both importers and exporters, recognize substrates ranging from single ions to entire protein toxins. ABC transporters have a number of highly conserved ABC cassette motifs, many of which are involved in the binding and hydrolysis of ATP. It is generally assumed that all ABC cassettes bind and hydrolyse ATP in a similar way and use a common mechanism to provide energy for substrate transport through the membrane-spanning domains (Locher et al., 2002). When the substrate has traversed the membrane, the transporter returns to the resting state through dissociation of ADP and inorganic phosphate. Conformational changes that may have a role in the mechanism of energy-transduction and/or allosteric control of the ABC-ATPase activity has been reported from x-ray crystallographic studies of the ABC-ATPase subunit (GlcV) of a transporter mediating glucose uptake in the *Sulfolobus solfataricus* bacterium (Verdon et al., 2003).

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Three-Dimensional Transporter Structures

A list of the structures discussed below is given in Table 1, and their location in the TC classification system is indicated in Fig. 1.

Low-resolution structures

NhaA antiporter (NhaA). Na^+/H^+ antiporters play a major role in the pH and Na^+ homeostasis of cells from bacteria to mammals (Padan et al., 2001). The *Escherichia coli* Na^+/H^+ antiporter (NhaA), located in the bacterial inner membrane, uses an inwardly directed H^+ electrochemical gradient to expel Na^+ from the cytoplasm. A three-dimensional electron density projection map of the *Escherichia coli* NhaA, determined by electron cryo-microscopy, shows twelve cylindrical density features, assumed to represent membrane spanning domains (Williams, 2000). These results were significant because this was the first reported experimental 3-D structure of an ion-coupled transporter protein, confirming the conclusion from previous biological studies and sequence analysis that NhaA has 12 membrane-spanning domains. However, the reported electron density map did not indicate the order of the helices in the protein sequence, nor which sides of the map that represented the periplasmic and cytoplasmic sides of the transporter. Although being a low-resolution structure, it provided a basis for molecular modeling of the transporter when combined with results from other biochemical studies (Ravna et al., 2001).

Oxalate Transporter (OxlT). The bacterial oxalate transporter (OxlT) is an MFS antiporter that catalyses the exchange of oxalate for formate across the cytoplasmic membrane. A 3-D electron density projection map of OxlT from *Oxalobacter formigenes*, determined by cryo electron microscopy, indicates that OxlT has 12 membrane spanning helices surrounding a single central cavity, presumably along the pathway of substrate

transport (Hirai et al., 2002). In the oxalate-bound state, the 12 helices form a symmetrical structure that displays a well-defined pseudo twofold axis perpendicular to the membrane plane and two less pronounced, mutually perpendicular pseudo twofold axes in the plane of the membrane, passing through the center of the molecule.

Bacterial multidrug transporter (EmrE). The three-dimensional structure of the *Escherichia coli* multidrug transporter EmrE, an H^+ antiporter involved in antibiotic drug resistance in bacteria, was determined to 7.0 Å resolution by electron cryo microscopy of two-dimensional crystals (Ubarretxena-Belandia et al., 2003). The EmrE structure shows an asymmetric homodimer forming a bundle of eight transmembrane α-helices, with one substrate molecule bound near the centre. The substrate binding chamber is formed by six α-helices and is accessible both from the aqueous phase and laterally from the lipid bilayer.

MDR1 P-glycoprotein (P-gp*)*. The P-glycoprotein encoded by the *mdr1* gene hydrolyses ATP and extrudes cytotoxic dugs from mammalian cells. A low-resolution structure of the mammalian MDR1 P-glycoprotein shows two hydrophobic transmembrane domains, each spanning the membrane 6 times, and two hydrophilic nucleotide-binding domains (NBD) located at the cytoplasmic face of the membrane (Rosenberg et al., 2001). The 12 membrane spanning segments were assumed to be α-helices.

MDR3 P-glycoprotein (P-gp). A low-resolution structure of the mouse MDR3 P-gp shows two domains related by a pseudo 2-fold symmetry axis through the middle of the molecule, perpendicular to the membrane plane. The two domains were interpreted as the N- and C-terminal halves of the molecule (Lee et al., 2002).

Multidrug efflux transporter (Pdr5p.) Pdr5p is a multidrug exporter in the yeast *Saccharomyces cerevisiae* and a member of the ATP-binding cassette (ABC) superfamily.

Pdr5p shares similar mechanisms of substrate recognition and transport with the human MDR1 P-gp. The Pdr5p structure determined at 25 Å resolution showed a dimeric organization (Ferreira-Pereira et al., 2003). Each monomer was composed of three sub-regions corresponding to a membrane region that joins two well separated protruding stalks, each ending with a cytoplasmic nucleotide-binding domain (NBD) lobe. The two NBDs appeared oriented perpendicularly within a monomer.

Multidrug transporter (YvcC). A low-resolution structure of a bacterial multidrug resistance transporter, YvcC, showed a homodimeric organisation with each subunit composed of a transmembrane region, a "stalk" of about 40 Å in height and 20 Å in diameter, and a cytoplasmic lobe identified as the nucleotid-binding domain (Chami et al., 2002). 3-D reconstruction of YcvC embedded in a membrane revealed an asymmetric organisation of the two NBD sites within the homodimer, and a dimeric interaction between two homodimers.

High-resolution structures

Lactose permease symporter (LacY). Lactose permease belongs to the MFS superfamily and is a member of the oligosaccaride:proton symport family (Fig. 1). LacY mediates the coupled co-transport of lactose and H^+, using the movement of H^+ down a concentration gradient as energy source. A high-resolution crystal structure of the protonated Cys154→Gly mutant of LacY from *Escherichia coli,* in an inward-facing conformation and with bound substrate, shows two almost identical protein domains related by an approximate two-fold symmetry axis (Abramson et al., 2003). Each domain has 6 membrane-spanning α-helices, and the substrate binding site was located at their interface at a similar distance from each side of the membrane. This structure, and the glycerol-3-phosphate transporter (GlpT) structure from *Escherichia coli* which was published at the same time (Huang et al., 2003), both support the "alternating access" mechanism of transport, in which the protein undergoes

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. Each of the eight helices that form the surface of the hydrophilic substrate-binding cavity in LacY contains several proline and glycine residues, and was heavily distorted by kinks and bends.

Glycerol-3-phosphate transporter (GlpT). A member of the MFS transporter superfamily and the organophosphate:P_i antiporter family (Fig. 1), GlpT, catalyses the exchange of glycerol-3-phosphate for inorganic phosphate using the movement of phosphate down a concentration gradient as energy source. A high-resolution crystal structure of GlpT from *Escherichia coli* in an inward-facing conformation shows two similar protein domains related by a central pseudo two-fold symmetry axis perpendicular to the membrane plane, and a substrate binding site at their interface. Each domain has 6 membrane-spanning α-helices (Huang et al., 2003), as also observed for LacY.

Multidrug efflux transporter (AcrB). Multidrug efflux pumps cause serious problems in cancer chemotherapy and treatment of bacterial infections. AcrB is a resistance-nodulation cell division (RND) transporter that cooperates with a membrane fusion protein (AcrA) and an outer membrane channel (TolC), to form a multidrug exporter system that transports cationic, neutral and anionic substances out of bacteria. The crystal structure of AcrB from *Escherichia coli* shows three protomers organized as a homotrimer (Murakami et al., 2002). Each protomer has a transmembrane region with a protruding headpiece opening at the top like a funnel, where TolC is docked directly into AcrB (Fig. 2). A pore formed by three α-helices, one from each TolC protomer unit, connects the funnel with a central cavity located at the bottom of the headpiece. A long hairpin structure (~35 Å) protrudes from each TolC protomer into that of the next, interlocking the three protomers in an arrangement that holds the headpiece together. The transmembrane region has three protomers oriented around a tree-

fold symmetry axis. Each protomer has twelve membrane spanning α-helices, oriented about a pseudo two-fold symmetry axis such that the six N-terminal helices are symmetrically arranged with the six C-terminal helices, as also observed for LacY and GlpT. The structure implies that substrates translocated from the cell interior through the transmembrane region, and from the periplasm through the vestibules, are collected in the large central cavity and then actively transported through the pore into the TolC tunnel (Murakami et al., 2002). The structure of ligand-bound AcrB from *Escherichia coli* was later solved, and demonstrated that three ligand molecules may bind simultaneously to the central cavity (Yu et al., 2003).

Lipid flippase (MsbA). MsbA is a member of the multidrug resistance (MDR) exporter family, which belongs to the ATP binding cassette (ABC) superfamily of transporters (Busch and Saier, 2002). MsbA uses the energy of ATP hydrolysis for substrate transport, and has high sequence similarity to several mammalian P-glycoproteins involved in multidrug resistance. The crystal structure of MsbA from *Escherichia coli*, determined by x-ray crystallography, shows a homodimer with each subunit containing six transmembrane α-helices and a nucleotide-binding domain at the cytoplasmic face of the membrane (Chang and Roth, 2001). An asymmetric distribution of charged residues lining a central chamber suggests a general mechanism for substrate translocation by MsbA and other MDR-ABC transporters. The MsbA structure demonstrates that this is not a pore through the cell membrane but a “molecular machine scanning the lower bilayer leaflet substrates, accepting them laterally, and flipping them to the outer leaflet” (Chang and Roth, 2001). The cytoplasmic NBDs did not seem to contribute to the transmembrane substrate pathway or to the “gate” that opens and closes this pathway.

Vitamin B_{12} transporter (BtuCD). The crystal structure of the *Escherichia coli* BtuCD protein, an ABC transporter mediating vitamin B12 uptake, shows two ATP-binding cassettes

(BtuD) in close contact with each other, and two membrane-spanning subunits (BtuC), each with 10 membrane-spanning α-helices (Locher et al., 2002). The BtuC subunits thus have a total of 20 transmembrane helices, which are grouped around a translocation pathway that is closed to the cytoplasm by a gate region. This arrangement of the transmembrane domain is distinct from that observed for the *E. coli* lipid flippase MsbA (Chang and Roth, 2001). A prominent cytoplasmic loop of BtuC forms the contact region with the ATP-binding cassette and appears to represent a conserved motif among the ABC transporters.

The authors suggest that the variability in the number of transmembrane passes among different ABC transporters may reflect diverse architectures of the membrane-spanning domains, where the sequence similarities are generally low between different transporters, or the need for more transmembrane passes to translocate larger substrates such as vitamin B12 (Chang and Roth, 2001). Alternatively, difficulties in predicting membrane-spanning segments may have led to an underestimation of their number for other ABC transporters (Chang and Roth, 2001). The authors also note that the 10 transmembrane helices found per BtuC subunit are packed together in a rather intricate way that does not resemble the arrangement of the six helices in an MsbA monomer.

Calcium ATPase (SERCA1a). In skeletal muscle cells, calcium ions are pumped from the cytoplasm into the sarcoplasmic reticulum against a concentration gradient by a P-type calcium ATPase (SERCA1a). P-type phosphorylases are ion pumps that are auto-phosphorylated during the reaction cycle, and the group also includes Na^+K^+-ATPase and gastric H^+K^+-ATPase. The crystal structure of SERCA1a has been determined with two Ca^{2+} ions bound in the transmembrane domain, after growing crystals in the presence of 10 mM Ca^{2+} (Toyoshima et al., 2000). The structure of the transporter in a calcium-free state was later reported from the same group (Toyoshima and Nomura, 2002). The transporter has ten

membrane spanning α-helices, some of which were unwound or kinked at the middle of the membrane. The cytoplasmic region consists of three well-separated domains, with the phosphorylation site in the central catalytic domain and the adenosine-binding site on another domain. The auto-phosphorylation site was located more than 25 Å away from the bound nucleotide. This and other studies (Huang and Squier, 1998) indicate that binding of Ca^{2+} induces large-scale domain motions, and that the nucleotide binding and phosphorylation sites get closer to each other during ATP hydrolysis.

Relevance of 3-D transporter structures. Two of the most widely prescribed drugs in the world, fluoxetine and omeprazole, have a transporter protein as site of action, and high-resolution transporter structures have a large potential value in drug discovery. As pointed out in a commentary to the reported LacY and GlpT structures (Locher et al., 2003), one prerequisite for the solving of these high-resolution transporter structures has been the access to synchrotron x-ray sources and their staffs to surmount the limitations of modest diffraction quality and substantial crystal-to-crystal variability. Due to the availability of such techniques, high-resolution structures of more than 90 membrane proteins have now been reported (*http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html*), and the number is increasing steadily.

In their report of the high-resolution SERCA1a structure, the authors note that their crystal structure "explains many more experimental results than we can describe here" (Toyoshima et al., 2000). This may indeed have been the case for all the recently reported high-resolution transporter structures, which have been preceded by a wealth of biochemical and biological data.

It interesting to note, for example, that all high-resolution transporter structures have confirmed the α-helical structures of the membrane spanning domains (Table 1), suggested

from a many previous studies. The high-resolution structures also show that α-helixes lining the presumed substrate translocation pathway often have irregular shapes with kinks and bends.

Together these crystal structures demonstrate the large structural flexibility of transporter proteins, and that substantial movements take place during the substrate translocation process. Recent data indicate that this feature may not be unique to transporters, but may be a property shared with certain ion channels (Jiang et al., 2003a; Jiang et al., 2003b).

Molecular modeling of transporter proteins

Molecular modeling may be used to simulate 3-D structures for proteins where no high-resolution structural data are available, and dynamic structural rearrangements related to functional mechanisms. Model building of proteins with an unknown 3-D structure may be performed by using a traditional homology modeling approach, or by using indirect and low-resolution structural knowledge from experimental studies. The latter approach is referred to as “knowledge-based modeling” in the present article.

Knowledge-based modeling usually starts with an initial model of conserved regions based on information from a low-resolution structure, amino acid sequence alignment, and information from biochemical and biophysical studies. The accuracy and reliability of such protein models depend on the accuracy of the structural data that the model is based on, and the resemblance of the modeled structure with the one or those used as a template for the initial model.

In homology modeling, the initial model is based on a high-resolution structure of a homolog protein, and amino acid sequence alignments. In general, a higher accuracy may be expected by homology-based than by knowledge-based modeling. However, also the accuracy

of homology-based models is limited by the validity of the sequence alignment of the modeled molecule to the template, the accuracy of the template, and by the accuracy of the modeling of inserted or non-aligned regions. This is particularly important when a protein from a bacteria is used as template to model a related mammalian membrane protein, which may have substantially larger intra- and extracellular loops between the transmembrane α-helices. This is illustrated in Fig. 2, which shows the crystal structures of LacY, GlpT and AcrB from *E. coli* and a molecular model of the human DAT.

ABC transporters. ABC transporters are associated with many different diseases including intrahepatic cholestasis, cystic fibrosis, and failure of cancer chemotherapy, and represent potential targets for therapeutic drugs in various therapeutic areas. Molecular modeling of ABC transporters therefore has considerable interest in drug discovery.

ABC transporters may undergo major conformational changes during the transport cycle, as demonstrated by biochemical assays and visualized at low resolution for the human multi-drug resistance P-glycoprotein encoded by the *mdr1* gene (Rosenberg et al., 2001). The most significant rearrangement is believed to occur as the substrate is shuttled across the membrane and ATP is hydrolyzed. Molecular modeling of ABC transporters would, in principle, provide an excellent tool for simulation of such rearrangements, working hand in hand with experimental biochemical studies.

However, some ABC transporters translocate hydrophobic substances while other translocate highly hydrophilic substances including sugars and inorganic ions. As discussed in a commentary to the reported MsbA structure (Higgins and Linton, 2001) it is not apparent, therefore, how for instance the crystal structure of MsbA, which transports hydrophobic substances from the bacterial inner membrane leaflet, can be extrapolated to other ABC transporters.

Comparative sequence analyses, motif search and secondary structure prediction indicated that each of the two halves of the human MDR1 P-glycoprotein is structurally similar to the monomer structure of the *Escherichia coli* MsbA lipid transporter, and suggested that the open dimer structure may be used as a valid model for the P-glycoprotein (Seigneuret and Garnier-Suillerot, 2003). Homology modeling was therefore used to predict the structure of P-glycoprotein, using MsbA as a template. Significant differences between P-glycoprotein and MsbA in size and charges of the internal chamber open to the inner leaflet and the inner medium were ascribed to the differences of the transported substrates. The authors note that the modeled structure provided insight into structural relationships between the transmembrane and nucleotide binding domains that seem important for transport. The interactions between the intracellular domain and the nucleotide binding domain of the modeled structure suggested that these contacts are involved in mediating the coupling between conformational changes of the nucleotide binding domain and reorientation of transmembrane α-helices during the transport process (Seigneuret and Garnier-Suillerot, 2003).

The crystal structure of BtuCD (Locher et al., 2002) may be combined with previous biochemical and structural data to simulate the structural rearrangements associated with vitamin B12 transport. However, judged from the diversity of the structural architecture of the membrane spanning and other domains of the ABC transporters included in Table 1, it is appears that the BtuCD structure may not readily be extrapolated to a larger part of the ABC transporter superfamily.

MFS transporters. The reported OxlT electron density map did not permit direct assignment of the helices to specific membrane segments. However, analysis of extensive biochemical data on this and related proteins indicated that helices 3, 6, 9 and 12 most likely

are located at the periphery of the transporter, and facing the lipid bilayer (Hirai et al., 2002). Information from the 6.5 Å resolution OxlT structure was later combined with sequence information from other members of this protein family to construct models of the arrangement of the transmembrane α-helices in the MFS superfamily (Hirai et al., 2003). A probable model for the relative spatial arrangement for the 12 helices was proposed, consistent both with the structural findings from the cryo-electron microscopic study and with previous biochemical studies on members of this superfamily (Hirai et al., 2003).

Glut 1 uniporter. A three-dimensional model of the human facilitative glucose transporter (Glut1) has been constructed by knowledge-based methods, since its structure and function had been extensively studied an no detailed 3-dimensional template was available (Zuniga et al., 2001). The Glut1 models had two channels, one traversing the structure completely, lined by many solvent-accessible residues, which may serve as the substrate transport pathway. The structure was consistent with previous mutagenesis and biochemical studies.

NhaA cation antiporter. A three-dimensional molecular model of the NhaA antiporter was constructed by interactive molecular graphics and energy calculations, based on the three-dimensional electron density projection map from *Escherichia coli* NhaA and information from previous biochemical and biophysical studies (Ravna et al., 2001). The model suggests that the pH dependent activity of NhaA may be explained by charge changes in the intracellular loop between TMH8 and TMH9 that alter the positions of TMHs 4, 5 and 11relative to each other, such that a pore area of the transporter protein is opened.

The neurotransmitter:sodium symporter family. Members of the neurotransmitter:sodium transporter family are the target of action of many currently used psychotropic drugs and several substances of abuse. The serotonin transporter (5HTT) and the norepineprine transporter (NET) are the main sites of action of antidepressant drugs, and the

dopamine transporter (DAT) is the main site of action of cocaine and other substances of abuse. We have used knowledge-based methods to construct molecular models of DAT, NAT and 5HTT, since no detailed crystal structure of any homolog protein has been available (Ravna et al., 2003a; Ravna et al., 2003b). There are examples that a single point mutation may convert a symporter into a uniporter (Busch and Saier, 2002), which suggests a similarity in their overall three-dimensional molecular structures. Although many data have indicated that there is a certain structural similarity between the antiporter NhaA on one side, and the symporters DAT, 5HTT and NET on the other side, it has not been proven that these share a common architecture of the membrane spanning domains.

However, based on the hypothesis that this was indeed the case, modeling of the 12 membrane spanning α-helices in DAT, NET and 5HTT were based on the low-resolution NhaA structure (Williams, 2000) like our previous NhaA model, in addition to site-directed mutagenesis and other biological data on DAT, 5HTT, NhaA, and LacY (Ravna et al., 2003b). Shortly after publication of the DAT and 5HTT models, the high-resolution LacY structure was reported (Abramson et al., 2003). A relatively good resemblance between the membrane spanning domains of LacY and our NhaA model, as shown in Fig. 3, demonstrates that knowledge-based modeling may give a reasonably good model of a membrane protein, provided sufficient biochemical data are available in addition to a low-resolution structure. Neurotransmitter transporter models may therefore predict ligand binding mechanisms and provide new insight into the structure and functional mechanisms of such transporters. Computational methods based on accurate molecular transporter models represent one among other useful tools in the discovery of safer and more efficient drugs acting on membrane transporters.

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Footnotes

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Legends for figures

Fig. 1. Classification according to (Saier, 2000) of the neurotransmitter:sodium symporter (NSS) family and the crystallized transporters included in Table 1. High-resolution crystal structures are indicated in boldface. For a more complete transporter classification see *http://tcdb.ucsd.edu/tcdb/background.php*.

Fig. 2. Crystal structures of AcrB (Murakami et al., 2002), GlpT (Huang et al., 2003), LacY (Abramson et al., 2003) from *E. coli*, and a mammalian DAT model (Ravna et al., 2003b), viewed in the membrane plane.

Fig. 3. Localisation of TMHs in LacY (Abramson et al., 2003) and in a previous molecular NhaA model (Ravna et al., 2001), viewed perpendicular to the membrane plane.

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TABLE 1

Three-dimensional transporter structures

Protein	Organism	Transmembrane domains			Other domains	Structure determination		Reference
		Number	THM (segments)/ domain	TMH (segments)/ transporter		Method*	Resolution	
Na^+/H^+ antiporter, **NhaA**	*E. coli*	1	12	12		ECM	7 - 14 Å	(Williams, 2000)
Oxalate transporter, **OxlT**	*Oxalobacter formigenes*	1	12	12		ECM	6.5 Å	(Hirai et al., 2002)
Multidrug transporter, **EmrE**	*E. coli*	2	4	8		ECM	7 Å	(Ubarretxena-Belandia et al., 2003)
MDR1 P-glycoprotein, **P-gp**	Chinese hamster ovary cells	2	6	12	2 NBD	ECM	10 Å	(Rosenberg et al., 2001)
MDR3 P-glycoprotein, **P-gp**	Mouse MDR3 P-gp expressed in *Picia partoris*	2				ECM	22 Å	(Lee et al., 2002)
Multidrug efflux transporter, **Pdr5p**	*Saccharomyces cerevisiae*	2			2 "stalk" formed, 2 NBD	ECM	25 Å	(Ferreira-Pereira et al., 2003)
Multidrug transporter, **YvcC**	*Bacillus subtilus* YvcC expressed in *E. coli*	2			2 "stalk" formed, 2 NBD	ECM	25 Å	(Chami et al., 2002)
Lactose permease, **LacY**	*E. coli*	2	6	12		X-ray	3.5 Å	(Abramson et al., 2003)
Glycerol-3-phosphate transporter, **GlpT**	*E. coli*	2	6	12		X-ray	3.3 Å	(Huang et al., 2003)
Multidrug efflux transporter, **AcrB**	*E. coli*	3	12	36	3 AcrA, 3 TolC	X-ray	3.5 Å	(Murakami et al., 2002)
							3.7 Å (with ligands)	(Yu et al., 2003)
Lipid flippase, **MsbA**	*E. coli*	2	6	12	2 NBD	X-ray	4.5 Å	(Chang and Roth, 2001)
Vitamin B_{12} transporter, **BtuCD**	*E. coli*	2 (BtuC)	10	20	2 NBD (BtuD)	X-ray	3.2 Å	(Locher et al., 2002)
Calcium ATPase, **SERCA1a**	Skeletal muscle sarcoplasmic reticulum	1	10	10	1 NBD, 1 P-site	X-ray	2.6 Å (with Ca^{2+})	(Toyoshima et al., 2000)
							3.1 Å	(Toyoshima and Nomura, 2002)

*ECM: Electron cryo microscopy, X-ray: X-ray crystallography

Fig. 1

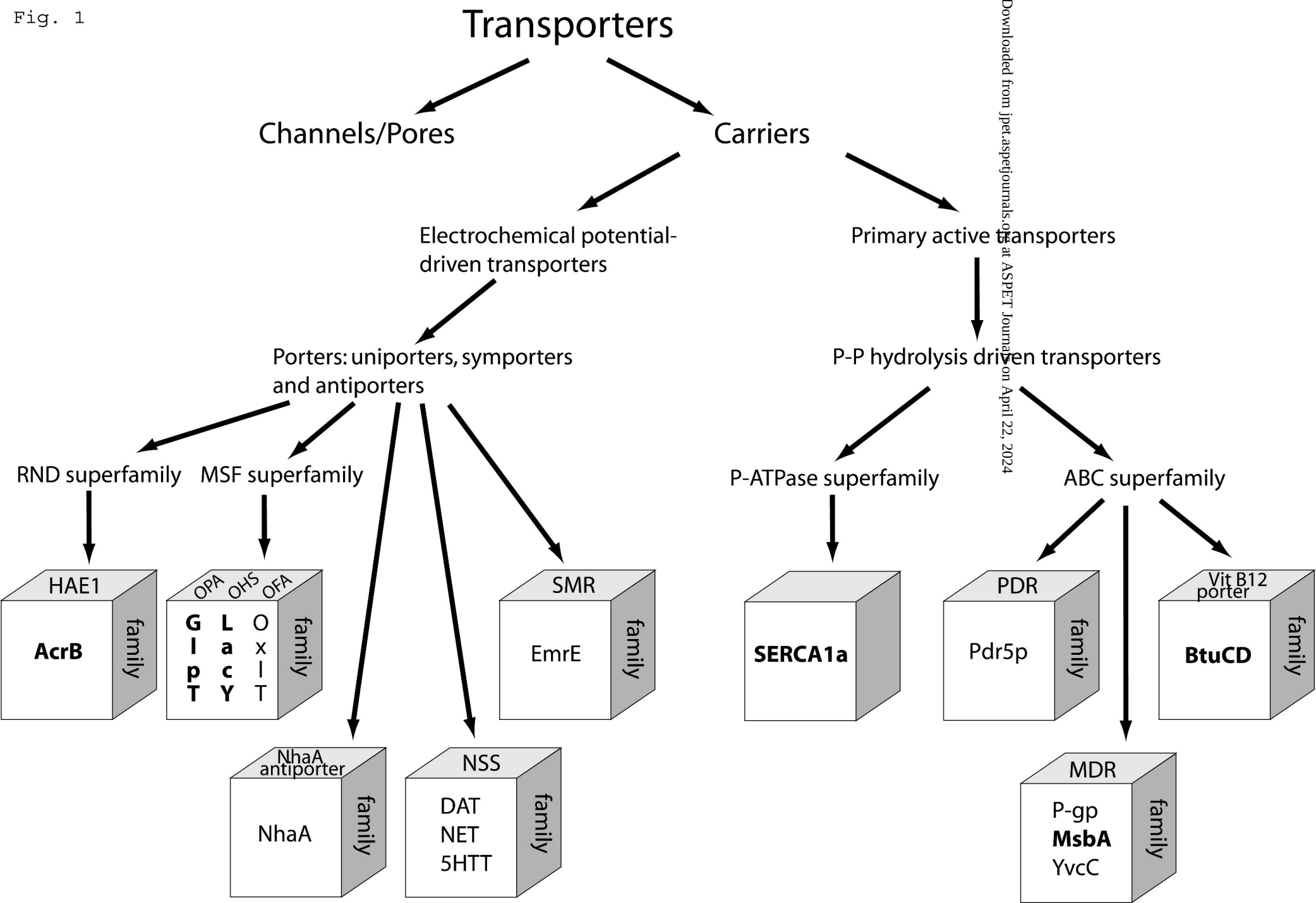

Fig. 2

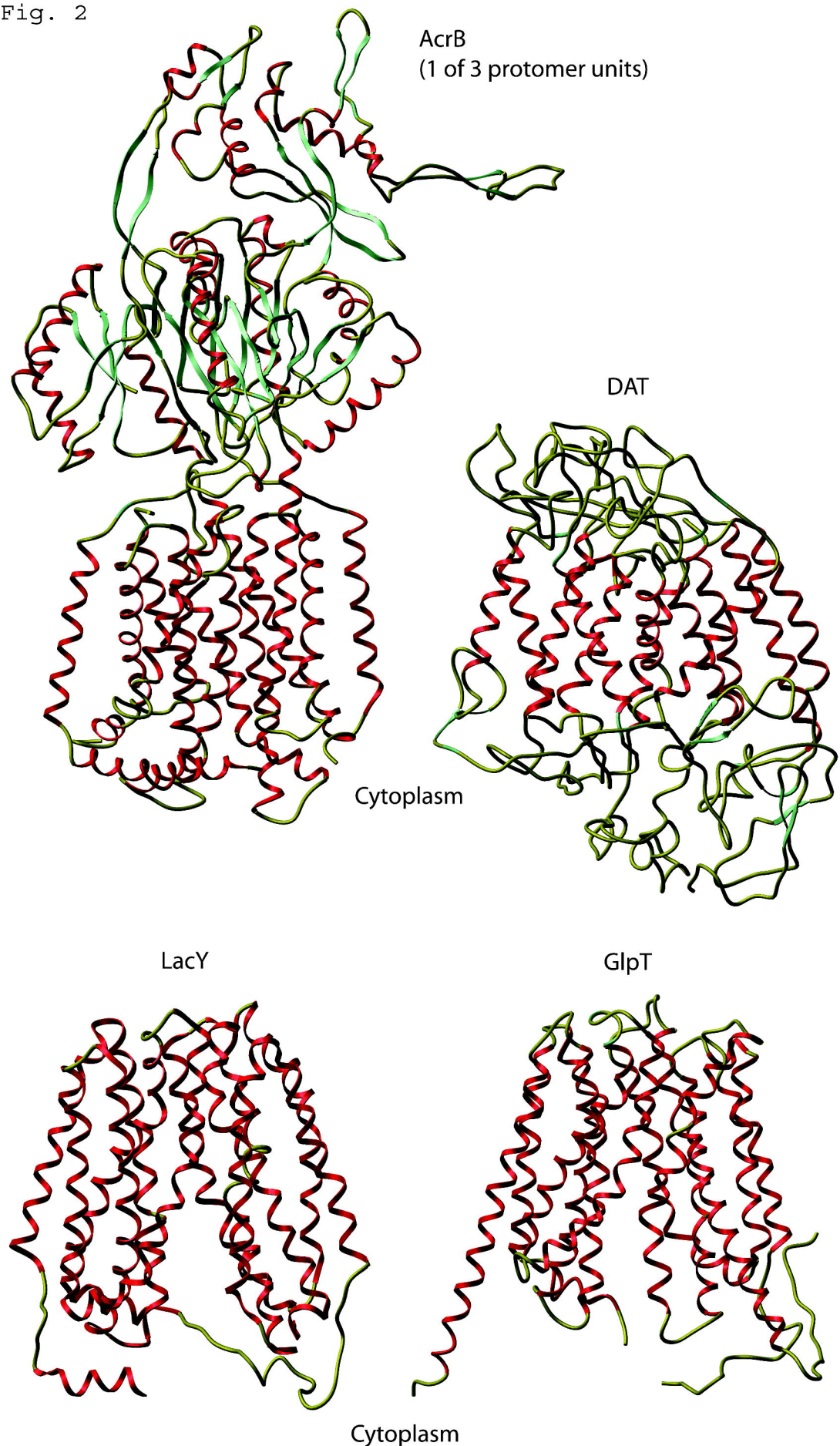
AcrB
(1 of 3 protomer units)
DAT
Cytoplasm
LacY
GlpT
Cytoplasm

Fig. 3