Article Contents

• Multidrug Transporters (MDTs)
• Insights Into Structures of MDTs
• Substrate Recognition: Multiple Drug Binding Proteins
• Transport Mechanisms of MDTs
• Physiologic Roles and Natural Substrates

Drug Transport in Living Systems

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Living organisms are constantly assailed by a host of harmful chemicals from the environment. Because of the diversity of these “xenobiotics”, cellular survival mechanisms must deal with an immense variety of molecules. Polyspecific drug transporters supply one such strategy. They recognize a wide range of dissimilar substrates that may differ in structure, size, or electrical charge, and they actively remove them from the cytoplasm. As such, these transporters provide an essential survival strategy for the organism. However, given that the substrates of these multispecific transporters include many antibiotics as well as antifungal and anticancer drugs, they are associated with the phenomenon of multidrug resistance (MDR) that poses serious problems in the treatment of cancers and infectious diseases, and some of them are coined multidrug transporters (MDTs). As expected from their central role in survival, these transporters are ubiquitous, and in many genomes, several genes coding for putative MDTs have been identified. MDTs are found in evolutionary unrelated membrane transport protein families, which suggests that they might have developed independently several times during the course of evolution. In this review, we discuss some basic concepts regarding drug transport from bacteria to humans.

Drug elimination in living systems is a multistep process that may involve metabolism, binding to proteins in the circulatory system and binding to specific receptors and excretion processes. A common way by which living organisms, from bacteria to humans, protect themselves against the harmful effect of a toxic compound is the removal of these molecules from the cell, which thus reduces the amount of drug that reaches its target site of action. Membrane transport proteins recognize these drug molecules and in an energy-dependent manner transport them across the plasma membrane and away from the target, either out of the cell or into subcellular organelles. The scope of this review encompasses numerous biologic networks and a variety of transport systems operating in concert. We will only discuss some major systems involved in transport of so-called drugs across biologic membranes in organisms from bacteria to humans. We chose to focus on polyspecific drug transporters, and by examination of some of the best characterized, we extract some general concepts.

Multidrug Transporters (MDTs)

The discovery of P-glycoprotein (P-gp/MDR1) in the mid 1970s introduced the concept of a single protein that can confer resistance to a relatively large number of structurally diverse drugs (1). In the following decades it has become clear that all living cells, be it a bacterial cell avoiding the deleterious effect of an antibiotic, a kidney cell eliminating an environmental toxin such as nicotine from the body, or a cancerous cell evading chemotherapeutic agents, express multidrug efflux transporters. Multidrug transporters (MDTs) recognize a wide range of dissimilar substrates that may differ in structure, size, or electrical charge, and they actively remove them from the cytoplasm. As such, these transporters provide an essential survival strategy for the organism. However, given that the substrates of these multispecific transporters include many antibiotic, antifungal, and anticancer drugs, they are associated with the phenomenon of multidrug resistance (MDR) that poses serious problems in the treatment of cancers and infectious diseases (2–4).

The phenomenon of multidrug transport is an intriguing one because it seems to challenge the basic model of an enzyme...
binding specifically to a single substrate in an optimized set of interactions as a prerequisite to efficient catalysis. MDTs, on the other hand, are polyspecific proteins that recognize a remarkably broad array of substrates. The occurrence of MDTs in evolutionary unrelated membrane transport proteins families indicates that they have originated independently several times during the course of evolution (5, 6). MDTs have been identified in several families, based on their amino-acid sequence similarities (References (7) and (8), Table 1 (9–16)).

**ATP binding cassette (ABC) superfamily**

This superfamily contains primary active transporters that couple ATP binding and hydrolysis to substrate translocation across the lipid bilayer. This superfamily of transporters will be reviewed in a different chapter in this encyclopedia and will not be discussed here (see the article ATP binding cassette (ABC) Transporters in this encyclopedia).

**Multidrug and toxic compound extrusion (MATE) family**

A fairly newly identified family of MDTs called MATE includes representatives from all domains of life (17). These transporters contain 12 putative transmembrane helices. This family of MDTs was first identified with the characterization of NorM, which is a Na+/drug antiporter from *Vibrio parahaemolyticus* (18, 19).

**Major facilitator superfamily (MFS)**

The MFS is one of the largest and most widespread families of transporters and is composed of numerous subfamilies of diverse functions. This superfamily consists of single-polypeptide transporters from bacteria to higher eukaryotes involved in the symport, uniport, or antiport of a variety of small solutes. The smallest known secondary active transporters belong to the drug metabolic transporter (DMT) superfamily (23). This family of small and tightly packed proteins of about 100 amino-acid residues, they are arranged as 12 transmembrane helices proteins with two large hydrophilic extra- cytoplasmic loops between helices 1 and 2 and helices 7 and 8. It has been postulated that these proteins developed from an internal gene duplication event. The members of the RND family are also secondary active transporters that catalyze the proton-motive-force driven transport of a range of substrates, including hydrophobic drugs, bile salts, fatty acids, heavy metals, and more (22).

**Small multidrug resistance (SMR) family**

One of the many families included in the MFS is the SLC (solute carrier) superfamily. The SLC superfamily represents approximately 900 genes in the human genome that encode for either facilitated transporters or secondary active symporters or antiporters. Members of the SLC superfamily transport various ionic and nonionic endogenous compounds and xenobiotics. The SLC22 family includes anion and cation transporters (Organic Anion Transporters, OATs; Organic Cation Transporters, OCTs). The organic anion and cation transporters of the SLC22 gene family share a common theme with the MDTs discussed above. These polyspecific transporters handle an impressive broad substrate range. Many substrates are toxicous to the organism, and these transporters actively remove them. The reasons they are not considered MDTs are therefore probably just historic.

**Resistance–nodulation–cell division (RND) superfamily**

RND is a large ubiquitous superfamily of transporters with representations in all domains of life. Composed typically of about 1000 amino-acid residues, they are arranged as 12 transmembrane helices proteins with two large hydrophilic extracytoplasmic loops between helices 1 and 2 and helices 7 and 8. It has been postulated that these proteins developed from an internal gene duplication event. The members of the RND family are also secondary active transporters that catalyze the proton-motive-force driven transport of a range of substrates, including hydrophobic drugs, bile salts, fatty acids, heavy metals, and more (22).

### Table 1  MDTs: families, examples, and selected references

<table>
<thead>
<tr>
<th>Superfamily</th>
<th>Examples</th>
<th>Recent reviews and additional reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC P-Glycoprotein</td>
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<td>the article ATP binding cassette (ABC) Transporters in this encyclopedia (9)</td>
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<tr>
<td>MFS MdrA OATs (SCL22 family) OCTs (SCL22 family)</td>
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<td>MATE NorM</td>
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<td>DMT EmrE (SMR family)</td>
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systems that function as heterooligomers are encoded by a pair of genes that belong both to the SMR family and are located in close proximity or even in an overlapping region in the genome.

The only heterodimer characterized thus far is the EmrAB transport system from *E. coli* (25).

Members of several RND, MFS, and ABC subfamilies have been proposed to function in combination with a periplasmic membrane fusion protein (MFP) and an outer membrane protein (OMP) to generate a single energy-coupled tripartite efflux system across both membranes of the gram-negative bacteria. The best characterized tripartite systems are MexAB-OprM from *Pseudomonas aeruginosa* and, by far, AcrAB-ToIC from *E. coli*.

**Insights Into Structures of MDTs**

Much research is directed toward deciphering the mechanisms by which MDTs confer resistance against cytotoxic drugs. Such understandings hold promise for tremendous clinical implications. Our current knowledge of the multidrug transport mechanism is based on a wealth of data obtained from extensive research using biochemical and genetic approaches and on recently achieved structural data. In our attempts to understand how these transporters confer multidrug resistance, several questions should be addressed. The first and most obvious question regarding this class of transporters is the question of substrate recognition: What is the architecture of a binding site that enables the binding of numerous dissimilar drugs? Next to be answered are questions regarding the drug translocation pathway across the membrane, the coupling of ion/drug fluxes and conformational changes that must occur throughout the transport cycle. Acquisition of high-resolution structural information on membrane proteins using X-ray crystallography is not an easy task, and so, to date, reliable high-resolution structural models were obtained only for a few ion-coupled transporters from the MFS and RND families. No structural information exists to date for transporters from the MATE family. A low-resolution (7 Å) projection map of the MFS transporter was supplied by the interpretation of 2-D crystals of EmrE is the closest to atomic resolution data that exists for the SMR class (26). Based on the combined evidence gathered up to date, several general perceptions can be drawn as to the function mechanism of MDTs.

The first structural evidence that approaches atomic resolution of an MFS transporter was supplied by the interpretation of the three-dimensional structure of the oxalate transporter, OxlT, obtained at the low resolution of 6.5 Å (27, 28). Later on, structures of two proteins from this superfamily were achieved at higher resolution: LacY, the lactose permease (29), and GlpT, the glyceral-3-phosphate transporter (30). While LacY and GlpT are both MFS members, they share a mere 23% sequence identity and differ in their mechanism of function. Although LacY acts as a symporter of protons and β-galactosides, GlpT is an organic phosphate/inorganic phosphate (Pi) antipporter. Surprisingly, despite these differences, both structures present a highly similar overall fold. In addition, also the general overall structure of OxlT seems to be very similar to the LacY and GlpT structures. The finding that the fold might be better conserved than the sequence suggests the possibility that a general architecture exists for the 12 transmembrane MFS proteins and that the specific function of each transporter is achieved by subtle changes in the substrate binding site and translocation pathway. Following this line of thought, various structural representations of MFS transporters have been generated based on structural homology to LacY and GlpT, among which we can find models of VMA T (31), TetAB (31), MdtA (32), and dOCT1 (33).

Thus, although neither one of the solved MFS structures is a multidrug transporter, it is reasonable to assume that general insights can still be extracted and applied.

Recently, another structure of an MFS multidrug transporter, the structure of EmrD from *E. coli*, solved to a 3.5 Å resolution, was published (34). Regrettably, the structure was solved without any substrate bound, and, to date, no biochemical data exist to support the mechanism speculated by the authors.

On the other hand, two newly solved structures of AcrB, which is a well-characterized RND multidrug transporter from *E. coli*, with and without bound substrates, provide valuable information regarding transport mechanisms of tripartite multidrug efflux systems, alongside newly arising questions (35, 36). Functional implications rising from these publications will be discussed in more detail.

**Substrate Recognition: Multiple Drug Binding Proteins**

Substrate recognition by MDTs is the first and most obvious puzzle that arises when considering the remarkable broad-ranged substrate specificity of these proteins. How can a binding pocket accommodate so many structurally dissimilar substrates? This characteristic of MDTs has long been considered paradoxical because of its ostensible contradiction to basic dogmas of enzyme biochemistry (37, 38).

**Soluble regulatory proteins**

The first structural information to shed light on the nature of the multi-specific binding pocket came from the solved 3-D structures of soluble multidrug-recognizing proteins. As membrane proteins are not easy to crystallize, the focus of structural analysis attempts has been directed also to the soluble drug-binding proteins that regulate the expression of MDR proteins. The underlying rationale is that the regulatory proteins and the transporters share some substrates, and so the information obtained from these structures would be applicable to the binding sites of the MDTs. BmrR, which is a transcription regulator of *Bacillus subtilis*, promotes the expression of Bmr, which is an MDR transporter of cationic lipophilic drugs. The structure of BmrR bound to TPPh+, which is an analog of the coactivator TPP+, reveals the drug-binding domain. The drug-binding pocket contains hydrophobic and aromatic residues that interact with the drug molecule. The phenyl rings of the substrate molecule interact via van der Waals and stacking interactions with aromatic residues, and an essential electrostatic interaction takes place between a negatively charged residue and the...
positively charged substrate. Complementary electrostatic interactions are found between TPSβ and carbohydrate groups of several acidic residues positioned in close vicinity (39). Valuable information was also achieved from the solved structures of QacR, which is a Staphylococcus aureus multidrug binding protein that represses the transcription of the QacA multidrug transporter gene. Structures of QacR were obtained in complex with six different lipophilic drugs, both monovalent and bivalent cations and many of them substrates of QacA (40). The structure of QacR exhibits a large drug-binding site with several aromatic residues forming stacking interactions with the substrate’s rings, and several polar residues that mediate drug-specific interaction through hydrogen bonds. Four glutamate residues within the binding pocket maintain electrostatic interactions with the substrate’s positively charged moiety. The different structures of QacR–drug complexes reveal a large, multifaceted drug-binding pocket in which different drugs can bind to different and partially overlapping sets of residues.

**Transport proteins**

With MDTs the only detailed structural evidence is provided by AcrB. The recent structure of AcrB in the presence of substrates reveals a substrate binding pocket in each of the three protomers (35). Bound antibiotic-drug molecules, minocycline and doxorubicin, are observed only in one of the three protomers. The substrate binding pocket is rich in aromatic amino-acid residues, mostly Phe residues. Such aromatic side chains may interact with the drug molecules through hydrophobic or aromatic interactions. Several polar residues located in close vicinity may form hydrogen bonds with the drug molecules. The structure implies a voluminous binding pocket in which different sets of residues are used for binding of the different kinds of substrates. Such a strategy is similar to that previously observed in the regulatory protein QacR.

The critical role of aromatic side chains in the binding of lipophilic substrates is also demonstrated by site-directed mutagenesis studies of other MDTs. Three aromatic residues (Tyr40, Tyr60, and Tyr63) in EmrE, which is an extensively characterized SMR transporter from Escherichia coli, play a role in substrate recognition (41, 42). The importance of aromatic side chains in the equivalent positions has been demonstrated for other SMR proteins as well (43).

A distinguishing feature of MDTs that recognize cationic drugs seems to be the presence of a membrane-embedded negatively charged residue, namely glutamate or aspartate, that neutralizes the positively charged moiety of the drug. EmrE, which is a proton-coupled SMR transporter, has only a single membrane embedded charged residue, Glu14. Through site-directed mutagenesis studies, Glu14 has been shown to be an essential residue (35). In LmrP, which is an MFS multidrug transporter from Lactococcus lactis, two acidic residues have been shown to be essential for the recognition of the monovalent cation etidithium (38). Also in the soluble regulatory proteins the equivalent of such a bared residue has been visualized. Glu253 of the transcription factor BmrR contributes to the protein–drug interaction through its carbohydrate group (39–47).

In QacR, which is another regulatory protein, four partially buried glutamates in the drug-binding site exist, at positions 57, 58, 90, and 120, that surround the drug-binding pocket and interact with the positively charged moiety of the substrates (40).

Another structure of the soluble regulatory protein QacR, this time in complex with bivalent diamidine substrates, reveals a novel manner of drug binding through electrostatic interactions. In these structures the electrostatic neutralization of the positively charged substrate was achieved through drug interactions with the negative dipoles of several oxygen atoms from nearby side chains and the phosphate backbone, and not through interactions with the carbohydrate of an acidic residue (48). QacA and QacB are both MFS transporters from Staphylococcus aureus that are closely related. Although QacA confers resistance to both monovalent and bivalent cations, QacB confers resistance to monovalent cations only. Site-directed mutagenesis studies provided evidence that the substrate specificity differences are caused by the presence of an acidic residue at position 323 in QacA that plays a critical role in conveying resistance to bivalent cations (8).

In MlaA, however, an MFS multidrug transporter, it has been demonstrated that no single acidic residue plays an irreplaceable role, and although efficient transport of positively charged substrates does require a negative charge at position 26 (either Glu or Asp), neutralization of this position does not necessarily abolish the interaction of MlaA with cationic drugs (49, 50). In the recently solved structure of AcrB in complex with minocycline and doxorubicin, no acidic residues seem to participate in cationic-substrate binding, and this finding may be a reflection of the fact that substrates of AcrB can be also uncharged and negatively charged (55).

The organic anion (OATs) and cation (OCTs) and multidrug transporters share a fundamental trait of polyspecificity. It is interesting to compare, from the knowledge that has accumulated so far, the strategy adopted by each such group of multispecific transporters that allows for broad-range substrate recognition.

The OATs transport various small amphiphilic organic anions, uncharged molecules, and even some organic cations that are usually around the molecular weight of 400–500 Da and include clinically relevant drugs such as anti-HIV therapeutics, anti-tumor drugs, antibiotics, anti-hypertensives, and anti-inflammatory in (11). OCTs substrates are organic cations and weak bases that are positively charged at physiologic pH, as well as noncharged compounds, with molecular mass of < 500 Da. Among transported substrates of OCTs are endogenous compounds, drugs, and xenobiotics (12).

Many SLC22 family members are expressed in the boundary epithelia of the kidney, liver, and intestines and play a major role in drug absorption and excretion. Substrate translocation by most OATs is energized by coupling the uptake of an organic anion into the cell to the extrusion of another organic anion from the cell; thus, OATs use the existing intracellular versus extracellular gradients of anions, such as α-ketoglutarate, as a driving force (11). Similarly, OCT1-3 from the SLC22 family are exchangers of organic cations (12). Several OAT members have been identified and cloned from various eukaryotic origins. Human OAT1-4 and UMT1 were more thoroughly studied...
than others, and so knowledge regarding substrate specificity, drug transport, regulation, and overall characteristics has accumulated. In contrast, a description regarding the functional characteristics of OAT3-9 is still lacking.

In the organic anion transporter rOAT3, site-directed mutagenesis studies lead to the proposal that rOAT3 contains a large binding pocket with several interaction domains responsible for the high-affinity binding of structurally diverse substrates. Two essential membrane-embedded basic residues (Arg 454 and Lys 370) attract negatively charged substrates through electrostatic interactions, but their role in determining specificity is not identical. Interestingly, replacement of the basic amino acids at positions 370 and 454 with the corresponding residues of OCTs, thus generating the mutant rR54D/K370A, changed the charge selectivity of the protein that could now transport the cation MPP+ (41). Several conserved aromatic residues that have been shown to be important for the transport of different substrates by rOAT3 are assumed to mediate substrate recognition through aromatic interactions that could include π-π interactions with ring containing substrates (42). A conservative replacement of Asp475 in rOCT1 to glutamate yielded a protein that exhibited higher affinities for some of its cationic substrates but also impaired transport rates. The authors suggested that the mutation at position 475 from Asp to Glu alters the structure of the cation binding site and harms the translocation step, thus altering the selectivity of rOCT1 (53). Additional studies of the binding site of rOCT1 reveal six more amino acids critical for substrate affinity, among which are aromatic and polar amino acids (33).

The general feature emerging is that of a multifaceted binding pocket, in which different drugs bind to separate, yet overlapping, sets of residues, many of them aromatic.

Transport Mechanisms of MDTs

AcrB

Structures of the RND transporter AcrB (35, 36) suggest a possible mechanism for this class of transporters. AcrB functions together with the auxiliary MFP AcrA and the OMP ToLC as a tripartite transport system (Fig. 1). The AcrB structures reveal an asymmetric trimer, in which each protomer adopts a different conformation according to its proposed role in the catalysis of the transport reaction (Fig. 2) (54). The "binding" protomer is occupied by bound substrate, and the "extrusion" protomer is outwardly open in a way that suggests it is the form present just after the extrusion of the substrate through the ToLC funnel; the "access" protomer seems to be in the state just before substrate binding. The three different protomer conformations are suggested to represent consecutive states of the transport cycle that result in the guiding of the substrate through AcrB toward ToLC. How is the proton gradient coupled to the drug efflux and to the conformational changes that shift the protoners between states? In AcrB it seems that the proton translocation pathway is separate from that of the substrate translocation pathway. Site-directed mutagenesis studies in AcrB and MexB, which is an AcrB homolog from Pseudomonas aeruginosa, show that any replacements of either of the three transmembrane charged residues Asp407, Asp408, and Lys940 of AcrB completely abolishes the ability of the protein to confer drug resistance (55, 56). No other charged residues reside within the transmembrane domains making this triplet the most likely proton translocation pathway. The structure reveals that in the "access" and "binding" protomers Lys940 is coordinated by salt bridges with Asp407 and Asp408, whereas in the "extrusion" protomer, Lys940 is turned toward Thr978 and the salt bridges are eliminated. Additional studies identify also Thr978, which is close to the essential Asp-Lys-Asp network, as important for AcrB’s function and as a putative part of the proton relay network (57). Some unanswered questions remain on the way to grasp fully the AcrB transport mechanism, among which is how this movement caused by protonation and deprotonation is transmitted to the large conformational changes that must occur throughout the transport cycle. Also, the essential role of AcrA and the interaction with ToLC have yet to be decoded.

MFS transporters

In the case of MFS transporters (Fig. 1) it is tempting to speculate that they may be acting by an alternative access mechanism similar to that suggested originally by Jardetzky (58) and recently supported for the extensively characterized MFS transporter LacY, which is a lactose permease that cotransports protons and sugar (29). The substrate and proton binding sites and translocation pathways are proposed to be separate, and the coupling is transmitted by a series of conformational changes induced on substrate binding. The sugar-binding site located in the approximate middle of the molecule at the apex of a deep hydrophilic cavity has alternating access to either side of the membrane as a result of reciprocal opening and closing of hydrophilic cavities on either side of the membrane.

EmrE

The simplest coupling mechanism between drug and proton transport has been described for the MDTs from the SMR family (Fig. 1). A single membrane-embedded charged residue, Glu14, in the case of EmrE, an E. coli SMR, is evolutionarily conserved throughout the SMR family and is essential for function. This residue provides the core of the coupling mechanism because its deprotonation is essential for substrate binding (59, 60). Conversely, substrate induces proton release, and both reactions (substrate binding and proton release) have been observed directly in the detergent solubilized preparation of EmrE (61). The estimated pKₐ for Glu14 is unusually increased (about 8.3–8.5 compared with 4.25 for the same carboxyl in aqueous environment) (54, 59). The fine-tuning of the pKₐ is essential because replacement of Glu14 with Asp results in a decrease in the pKₐ of the carboxyl and generates a protein that at physiological pH has already released the previously bound protons, can still bind substrate but cannot longer couple the substrate flux to the proton gradient (44, 61).
SMR proteins function through a binding site shared by protons and substrates, which can be occupied in a mutually exclusive manner and provide the basis for the simplest coupling of two fluxes (15, 59).

**Physiologic Roles and Natural Substrates**

MDTs provide a survival strategy for living organisms that are constantly assailed by a host of harmful chemicals from the environment. Because of the diversity of these “xenobiotics”, cellular survival mechanisms must deal with an immense variety of molecules. MDTs supply one such strategy. The patterns of abundance of drug efflux systems in different organisms do not correlate directly with the above-suggested role. Although MDTs are highly abundant in the soil and environmental bacteria, they exist in relatively large numbers in other organisms as well, such as intracellular bacteria (5). It is not clear, however, whether this is the primary function of all of those identified as MDTs. It is also not clear why so many different ones are required and why there is such a high redundancy in substrate specificities among different MDTs within the same genome. This may be from the necessity to cover a range as wide as possible of substrates, and overlapping provides a backup strategy in case of failure of one of the systems.

In some cases, a direct correlation is found between MDTs and adaptation to specific environments. The natural environment of an enteric bacterium such as *E. coli* is enriched in bile salts and fatty acids. Bile acids are amphipatic molecules that...
Figure 2  The structure of the AcrB–drug complex and proposed mechanism of drug transport (from Reference 54). (a) The complex is observed from the side, with the drug shown as a red hexagon. The dotted line indicates a possible pathway for substrates moving from the cytoplasm. The complex contains three molecules of AcrB, AcrA accessory proteins, and the TolC channel to the exterior. The drug is proposed to enter AcrB when it is in the access (A) conformation before binding more closely to the porter domain of AcrB in the binding (B) conformation. It is then transported to the opposite face and is released from the extrusion (E) conformation of AcrB. Transport of the xenobiotic is powered by the proton (H⁺) gradient across the membrane. (b) The proposed ordered multidrug binding change mechanism of the three-unit AcrB complex.

Deletion of the acrAB operon leads to increased susceptibility to bile acids and fatty acids, such as decanate, which suggests that efflux of these compounds may be one of the physiologic roles of the AcrAB efflux system in *E. coli*. Consistent with that, the expression of AcrAB was increased by growth in the presence of 5 mM decanate (62). In another study, both bile and mammalian steroid hormones (estradiol and progesterone) have been shown to be substrates of the two major efflux systems of *E. coli*: AcrAB-TolC and the MFS EmrAB-TolC (63). CmeABC, which is a tripartite efflux pump from *Campylobacter jejuni*, was also shown to contribute to the bile resistance of the bacterium (64) and so have similar efflux systems from other bacteria, including *Salmonella typhimurium* (65) and *Vibrio cholerae* (66). In some cases, deletion of MDTs decreases the pathogenicity of given microorganisms, most likely because of their impaired adaptability (67). It has been suggested that some bacterial MDTs might be involved in export of signals for cell–cell communication (68). For some MDTs, a specific function has been well documented. BLT, which is an MFS multidrug transporter of *Bacillus subtilis*, has been shown to function as a substrate-specific transporter of the polyamine spermidine, which is a natural cellular constituent. BLT is encoded in an operon with BltD, a spermidine/spermine acetyltransferase, which emphasizes its possible physiologic role in downregulating cellular spermidine levels through efflux, in concert with an enzyme that chemically modifies spermidine (69).

The vesicular monoamine transporters (VMA Ts) from the SLC18 family provide another example of polyspecific proteins with a very defined physiologic role. These essential proteins are involved in the storage of monoamines in the central nervous system and in endocrine cells in a process that involves exchange of 2H⁺ with one substrate molecule. The VMA Ts interact with various native substrates and clinically relevant drugs, and they display the pharmacologic profile of multidrug transporters (70).

More recently possible roles in supporting pH homeostasis and alkali tolerance have been suggested for TetL, which is a tetracycline efflux protein from *Bacillus subtilis*, and the multidrug transporter MdfA (71).

In higher multicellular organisms, the strategy for drug elimination is a multistep process that may involve metabolism, binding to proteins in the circulatory system and binding to specific receptors and excretion processes. A variety of polyspecific transporters (from the ABC, MATE, RND, and MFS superfamilies) are expressed throughout the organism. Some of these transporters provide mechanisms that protect the brain and other sanctuaries from exposure to toxins, both endogenous and exogenous. Others are found in the boundary epithelia of kidney, liver, and intestine and play a major role in drug absorption and excretion.

The discussion of the role of MDTs is tightly connected with the question of the evolution of polyspecificity. One can suggest two possible paradigms of multidrug transporter evolution: The first one states that because MDTs confer broad-range drug

**Drug Transport in Living Systems**

- TolC
- AcrA
- AcrB
- AB
- TolC-docking
- Porter
- Transmembrane

- Periplasm
- Cytoplasm
resistance, they evolved to protect cells from environmental “xenobiotics.” A second possible scenario is that each MDT has evolved as a substrate-specific transporter, and its ability to transport toxins is only an opportunistic side effect (72).

These issues and the others discussed above provide us with a glimpse of this fascinating and complex world of polyspecific proteins. These ubiquitous proteins in many cases play central roles in survival of organisms by providing the means to remove “xenobiotics” away from their targets. On the other hand, in some cases, they present a serious threat for treatment of drug-resistant cancers and infectious diseases. Understanding the molecular mechanisms that underly the mechanism of action of these proteins is therefore highly relevant.

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Drug Transport in Living Systems


Further Reading
Membrane_Proteins.html.

See Also
ATP-Binding Cassette (ABC) Transporters
Bacterial Resistance to Antibiotics
Ion Transport
Transport: Antiporters and Symporters