

## BASIC SCIENCE REVIEW

## Basic Mechanisms of Antibiotic Resistance: Molecular Properties of Multidrug Transporters

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### INTRODUCTION

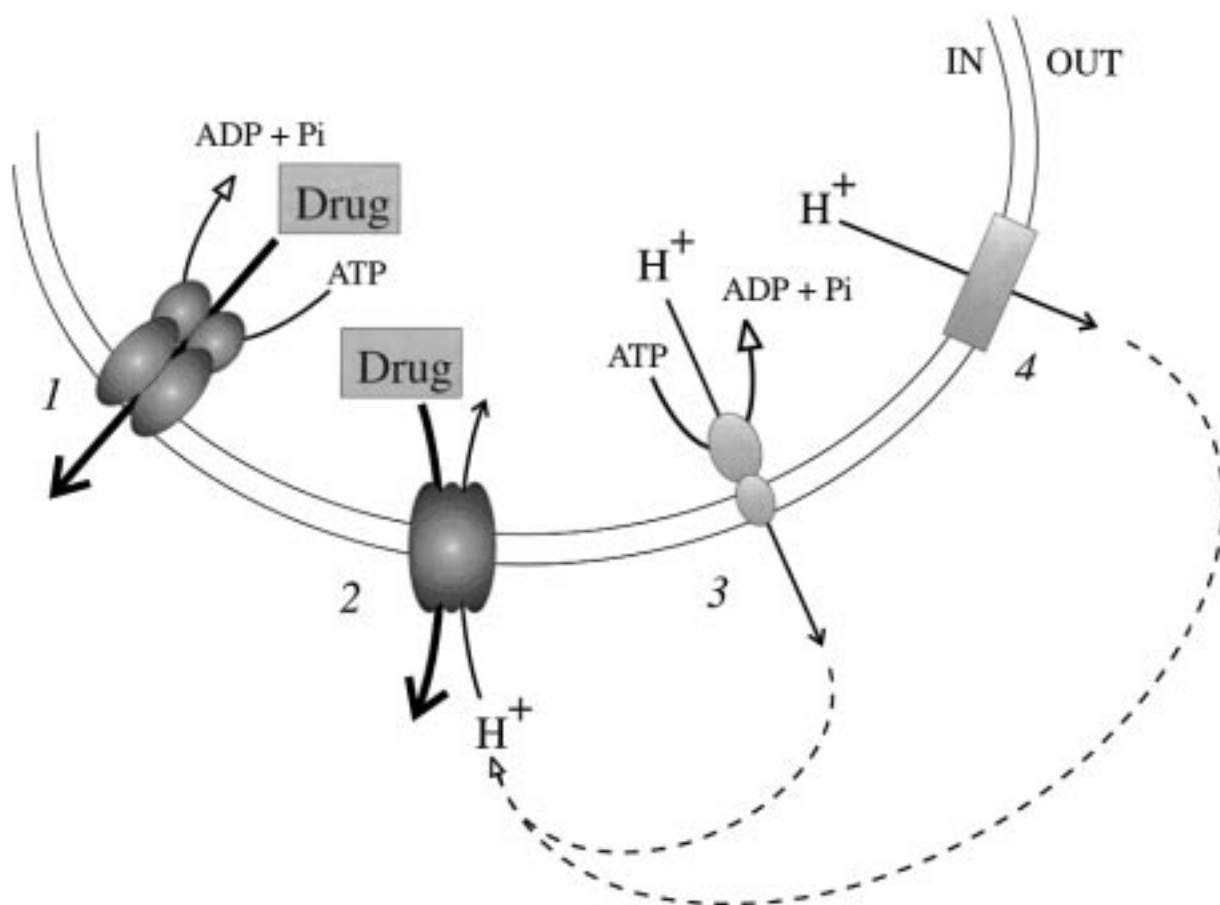
The selection of drug-resistant pathogenic microorganisms in hospitalized patients with serious infections such as pneumonia, urinary tract infections, skin infections, and bacteremia has generally been ascribed to the widespread use of antimicrobial agents (1,2). Likewise, nutritive and therapeutic treatment of farm animals with antibiotics, amounting to half of the world's antibiotic output, has selected for drug resistant microorganisms that contaminate the food produced (3). Issues of concern regarding gram-negative bacteria include the extended drug resistance spectrum of  $\beta$ -lactamase-producing *Escherichia coli*, *Salmonella typhimurium* and *Klebsiella pneumoniae*. Current concerns with gram-positive pathogens are increasing multidrug resistance in *Listeria monocytogenes*, methicillin-resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumoniae* (for review, see J. Kellner and D.E. Low in this issue of the *MJM*). Besides bacteria, parasitic protozoa are also responsible for some of the most devastating and prevalent diseases of humans and domestic animals, such as malaria (*Plasmodium* spp.), (muco)cutaneous and visceral leishmaniasis (*Leishmania* spp.), African sleeping sickness (*Trypanosoma brucei gambiense*, *Trypanosoma brucei rhodensiense*), South-American Chagas' disease (*Trypanosoma cruzi*), amoebic dysentery (*Entamoeba* spp.), and toxoplasmosis (*Toxoplasma* spp.) (for review see (4)). Chemotherapeutic treatment of infections by these parasites is being eroded by multidrug resistance.

With few new drugs in the pipeline, prevention and circumvention of microbial multidrug resistance are medical and veterinary priorities, which require insight into the molecular basis of microbial multidrug resistance.

The presence of multidrug resistant pathogenic and non-pathogenic microorganisms suggests that multidrug resistance did not arise recently in pathogens in response to antimicrobial chemotherapy. Toxic compounds have always been part of the natural environment in which microorganisms dwell. The development of strategies for life in this habitat has been crucial for survival of the cell. As a result, microorganisms can use various mechanisms to resist cytotoxic drugs. Microorganisms can eliminate the drug target in the cell through the alteration or replacement of molecules that are normally bound by the antibiotic (5). Alternatively, microorganisms can reduce the intracellular concentration of drugs by: (i) synthesizing enzymes that degrade antibiotics or that chemically modify, and so inactivate, the drugs (6), (ii) eliminating entry ports for hydrophilic drugs, such as outer membrane porins in gram-negative bacteria (7), and (iii) manufacturing drug efflux systems that export lipophilic drugs before these compounds have the chance to find their cellular targets (4,8,9). Some of these drug efflux systems are fairly specific for a given drug or class of drugs, but the so-called multidrug transporters have specificity for compounds with very different chemical structures and cellular targets. Multidrug transporters can be amplified in drug resistant pathogenic microorganisms, and can shift their drug profiles, making them a menace to drug treatment.

Multidrug transporters are also found in mammals, in which they are a cause of multidrug resistance of tumor

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**Figure 1.** Schematic presentation of the two major classes of multidrug transporters in bacteria. (1) ABC-type multidrug transporters mediate the extrusion of drugs from the cell coupled to the hydrolysis of ATP. (2) Secondary multidrug transporters mediate the extrusion of drugs from the cell in antiport with protons (or sodium ions). The driving force for drug extrusion via proton-dependent drug antiport systems is supplied by the proton motive force, which is generated via proton extrusion by (3) the  $F_1F_0$   $H^+$ -ATPase, and/or (4) redox reaction-coupled primary  $H^+$  expulsion by the respiratory chain. IN and OUT refer to the inside and outside of the cytoplasmic membrane, respectively. ABC: ATP-binding cassette

cells. The structure and function of multidrug transporters is conserved from bacteria to man. On the basis of bioenergetic and structural criteria, multidrug transport systems can be divided into two major classes (Figure 1). ATP-binding cassette (ABC) transporters utilize the release of phosphate bond-energy by ATP hydrolysis, to pump drugs out of the cell (10). Secondary transporters mediate the extrusion of drugs from the cell in a coupled exchange with ions, in the absence of a chemical reaction (11). The driving force for transport is supplied by transmembrane electrochemical gradient of protons or sodium ions. In these latter cases, the transport systems are indicated as proton and sodium motive force-driven, respectively.

This paper represents a comprehensive review in which the current state of knowledge on three major aspects of drug efflux-based multidrug resistance in prokaryotic and eukaryotic cells will be summarized: (i) the functional and structural similarities among secondary and ABC-type multidrug transporters, (ii) the

molecular mechanism of these transporters, and (iii) their potential physiological role.

## SECONDARY MULTIDRUG TRANSPORTERS

The number of secondary multidrug transporters discovered in prokaryotic and eukaryotic cells is vast and rapidly expanding. Secondary multidrug transporters have been detected in pathogenic yeasts such as *Candida albicans* (caMDR1p) (12), and pathogenic bacteria such as methicillin-resistant *Staphylococcus aureus* (QacC) (13), *Mycobacterium smegmatis* (LfrA) (14), and *Neisseria gonorrhoeae* (MtrD) (15). Secondary multidrug transporters have also been detected in mammals, such as in rat kidney cells (OCT1) (16).

Computer-based sequence analyses have revealed that secondary drug transporters belong to one of three distinct families of transport proteins: the Major Facilitator Superfamily (MFS) (17), the Resistance-Nodulation-Cell Division (RND) family (18), and the

**Table 1.** Examples of secondary multidrug transporters in microorganisms.

Transporter Family	Protein	Organism	Accession No. <sup>a</sup>
MFS family	caMDR1p	<i>Candida albicans</i>	SW P28873
	LmrP	<i>Lactococcus lactis</i>	GB X89779
	Bmr	<i>Bacillus subtilis</i>	SW P33449
	NorA	<i>Staphylococcus aureus</i>	SW P21191
	QacA	<i>Staphylococcus aureus</i>	EM X56628
	EmrB	<i>Escherichia coli</i>	SW P27304
RND family	AcrB	<i>Escherichia coli</i>	EM U00734
	MexB	<i>Pseudomonas aeruginosa</i>	GB L11616
	MtrD	<i>Neisseria gonorrhoeae</i>	W P43505
SMR family	QacC	<i>Staphylococcus aureus</i>	SW P14319
	QacE	<i>Klebsiella aerogenes</i>	PR S25583
	EmrE	<i>Escherichia coli</i>	SW P23895

<sup>a</sup> Accession number: GB, Genbank; SW, SwissProt; EM, EMBL; PR, PIR. **MFS**: Major Facilitator Superfamily (17); **RND**: Resistance-Nodulation-Cell Division family (18); **SMR**: Small Multidrug Resistance family (19).

Small Multidrug Resistance (SMR) family (19) (Table 1). Bacterial examples of each type of secondary drug transporter are *Lactococcus lactis* LmrP (MFS family) (20), *S. aureus* QacC (SMR family) (13), and *Pseudomonas aeruginosa* MexB (RND family) (21). LmrP and QacC have specificity for an exceptionally wide range of amphiphilic, cationic drugs including antibiotics, quaternary ammonium compounds, aromatic dyes, and phosphonium ions. MexB confers resistance to amphiphilic anionic compounds such as antibiotics, basic dyes and detergents. In Gram-negative bacteria, transport of drugs from the interior of the cell to the external medium requires the translocation of solutes across the cytoplasmic and outer membrane. Therefore, some drug transporters (e.g., MexB) in such organisms are found in association with an accessory protein which spans the periplasmic space and interacts with a porin in the outer membrane. These auxiliary proteins belong to the Membrane Fusion Protein family (18) and Outer Membrane Factor family (22).

### Energetics of Drug Transport

Bacterial secondary transporters are located in the cytoplasmic membrane across which a proton motive force (interior negative and alkaline) and sodium motive force (interior negative and low) exists (Figure 1). Based on (i) the structural similarity between secondary drug transport proteins and known proton or sodium motive force-dependent transport systems, and (ii) the sensitivity of drug transport to agents that dissipate the proton and sodium motive force, it is assumed that secondary drug transporters function as drug/ion antiporters. The direct involvement of the proton motive force as driving force was demonstrated for the *E. coli* tetracycline transporter TetA(B) (MFS

family), which mediates an electroneutral exchange reaction of a metal<sup>2+</sup>-tetracycline<sup>1-</sup> complex with one proton (23), and *L. lactis* LmrP (MFS family) (24) and *E. coli* EmrE (SMR family) (25) which both mediate an electrogenic drug/*n*H<sup>+</sup> antiport reaction (*n* = 2).

### Structure-Function Relationships

Analysis of the topography of secondary drug transporters suggests the presence of either 12 or 14 membrane-spanning segments in members of the MFS family, and 12 membrane-spanning segments in members of the RND family. The transmembrane segments are most likely in  $\alpha$ -helical configuration, and are connected by hydrophilic loops protruding into the cytoplasm or periplasmic space. The structure of TetA(B) has been studied most extensively by limited proteolysis (26), site-directed antibody binding (27), PhoA-fusion analysis (28), and site-directed chemical labeling (29). A relatively large cytoplasmic loop in the middle of the protein separates TetA(B) in an N- and C-terminal half, each containing 6 transmembrane segments. Both halves are evolutionarily related, presumably by a gene duplication event, and are well conserved among TetA proteins of various classes (30). The N- and C-terminal halves of TetA(B) represent separate domains in the drug transporter (31). The functional interaction between these domains is suggested by (i) the intragenic complementation between mutations in the first and second halves of the *tetA(B)* gene (32), (ii) the ability of hybrid tetracycline efflux transporters containing N- and C-terminal halves of different classes of tetracycline efflux proteins to confer resistance (33) and (iii) the functional reconstitution of tetracycline resistance upon co-expression of the N- and C-terminal halves of TetA(B)

as separate polypeptides (34). Members of the SMR family contain four putative transmembrane  $\alpha$ -helices. This topological model has recently been confirmed for QacC by genetic fusion using alkaline phosphatase and  $\beta$ -galactosidase as reporters of subcellular localization (35), and for *E. coli* EmrE by transmission fourier-transform infrared spectroscopy (36). In view of the 12 transmembrane models proposed for members of the MFS and RND families, members of the SMR family may function as a homotrimer.

Multiple alignment of the amino acid sequences of the MFS family members reveals the presence of two conserved sequence motifs that are located at similar positions within the putative secondary structure of the proteins. Motif A (GXXXDRXGR(K/R)) is found in many members of the MFS family (17) and is present in the cytoplasmic loop between transmembrane segment 2 and 3. Motif A may be of structural importance by mediating opening and closing of the translocation pathway. Motif B (GXXXGXXGG), the drug extrusion consensus sequence, is found at the end of transmembrane segment 5, and is typical for LmrP and other drug export systems of the Major Facilitator superfamily (20,37). At present, the role of motif B in drug recognition or binding is unclear.

It has been suggested that amino residues within transmembrane segments of the human multidrug resistance P-glycoprotein MDR1 (Pgp) play a role in drug specificity (38). Observations on secondary drug transporters are consistent with this notion. The MFS members QacA and QacB in *S. aureus* are homologous proteins (98% identical amino acid residues). QacB confers resistance to monovalent organic cations but differs from QacA by conferring no resistance to the divalent organic cations pentamidine isethionate and propamidine isethionate. This phenotypic difference between QacA and QacB is solely due to the presence of the acidic Asp323 within the putative transmembrane segment 10 of the QacA protein, compared to the neutral Ala323 in QacB (39). The ability of the MFS member Bmr in *Bacillus subtilis* to interact with reserpine and other drugs is strongly affected by substitutions of Val286 (40), and of Phe143 or Phe306 (41), which are located within transmembrane segments. Thus, neutral, charged and aromatic residues located within the phospholipid bilayer appear to interact with drugs and may be involved in drug recognition or binding. Interestingly, it has been shown that quaternary ammonium compounds and other cations can bind to the  $\pi$  face of the aromatic ring structures of tyrosine, phenylalanine or tryptophan residues in the hydrophobic environment of the membrane (42,43).

## ABC-TYPE MULTIDRUG TRANSPORTERS

Transport proteins belonging to the ABC transporter superfamily are involved in the tolerance to a wide diversity of cytotoxic agents in both prokaryotes and eukaryotes (10). Included in this superfamily are the human multidrug resistance P-glycoprotein (Pgp) (44) and human multidrug resistance-associated protein MRP1 (MRP) (45) plasma membrane transporters, which catalyze the extrusion of anti-tumor drugs during the chemotherapy of cancer cells. Based on (i) the alignment analysis of amino acid residues that comprise the nucleotide binding domain(s) of ABC proteins, (ii) protein topology, and (iii) transport mechanism, the ABC transporter superfamily can be divided into two major clusters: the Pgp cluster and MRP cluster.

### Pgp Cluster

Members of the Pgp cluster play an important role in microbial resistance to neutral or positively charged, amphiphilic drugs (Table 2). These transporters function in human pathogens such as the malaria parasite *Plasmodium falciparum* (pfMDR1) (46), the *Entamoeba histolytica* (ehPgp) protozoan responsible for human amoebiasis (47), or the *Leishmania donovani* (ldMDR1) protozoan responsible for visceral leishmaniasis (48). In addition, infections in immunodeficient patients are often caused by the pathogenic yeast *Candida albicans* which expresses Cdr1p, an ABC transporter which confers antifungal resistance (49). ABC proteins in the non-pathogenic *Saccharomyces cerevisiae* include the multidrug transporters Pdr5p (50) and Snq2p (51). Pgp cluster members have also been identified in bacteria. In *Streptomyces* strains, dedicated transporters such as DrrAB (52,53) mediate the excretion of specific antibiotics to ensure self-resistance to the antibiotics that they produce. A true prokaryotic multidrug transporter with significant sequence identity to Pgp in both the ABC and membrane domains has been found in *L. lactis* (LmrA) (54).

Like Pgp, some ABC transporters in microorganisms are expressed as single multifunctional polypeptides containing two homologous halves, each with an ABC domain and a membrane domain. The membrane domain is usually composed of six putative  $\alpha$ -helical transmembrane segments. Other ABC proteins, such as lactococcal LmrA and the *Escherichia coli*  $\alpha$ -hemolysin transporter HlyB (55), are half the size of Pgp with only a single transmembrane domain and ABC domain (Figure 2). The notion that two of these half-molecules must cooperate to form a single transporter is supported by the observation, amongst others, that the independent expression of each half of the *a*-mating pheromone

**Table 2.** Examples of ABC-type multidrug transporters in microorganisms.

	Protein	Organism	Accession No. <sup>a</sup>
Pgp cluster	LmrA	<i>Lactococcus lactis</i>	GB U63741
	pfMDR1	<i>Plasmodium falciparum</i>	GB A32547
	ehPgp	<i>Entamoeba histolytica</i>	GB M88599
	ldMDR1	<i>Leishmania donovani</i>	GB L01572
	Cdr1p	<i>Candida albicans</i>	GB X77589
	Pdr5p	<i>Saccharomyces cerevisiae</i>	GB L19922
	Snq2p	<i>Saccharomyces cerevisiae</i>	GB Z48008
MRP cluster	Ycf1p	<i>Saccharomyces cerevisiae</i>	GB Z48179
	Yor1p	<i>Saccharomyces cerevisiae</i>	GB Z73066
	ltPgpA	<i>Leishmania tarentolae</i>	GB A34207
	ceMRP1	<i>Caenorhabditis elegans</i>	EM 466260

<sup>a</sup> Accession number: GB, Genbank; EM, EMBL. **ABC**: ATP-binding cassette superfamily (10)

transporter Ste6p in yeast cells does not yield a functional transporter, while simultaneous expression of both halves does (56). In drug transporters such as DrrAB, the two membrane domains are fused into a single polypeptide which is associated with a second polypeptide containing the two ABC domains.

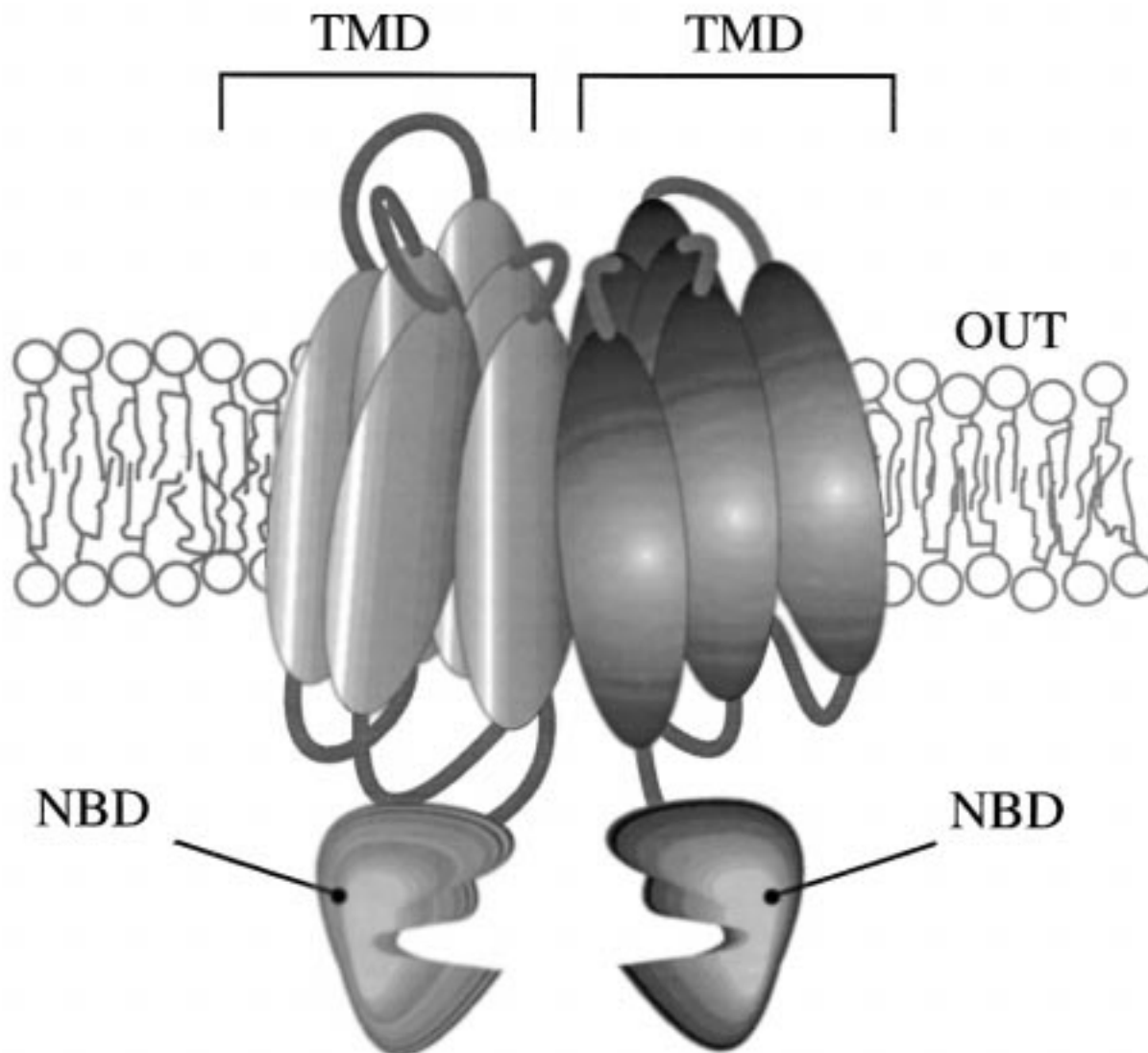
Interestingly, the sequence conservation between LmrA and Pgp includes particular regions (e.g., the first cytoplasmic loop and the region comprising transmembrane segments V and VI) that have been implicated as determinants of drug recognition and binding by Pgp. LmrA also shares significant sequence identity with the hop-resistance protein HorA in *Lactobacillus brevis* (57) and ABC proteins in *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Helicobacter pylori*, *Haemophilus influenzae*, and *Mycoplasma genitalium* (58).

### MRP Cluster

Members of the MRP cluster contain an N-terminal membrane-bound domain consisting of five  $\alpha$ -helical transmembrane segments, which is followed by two homologous halves each with six  $\alpha$ -helical transmembrane segments and an ABC domain (59). Like MRP, MRP homologs in microorganisms mediate the transport of a wide variety of organic anions, such as carboxyfluorescein derivatives, taurocholate, bis(glutathionate)-cadmium and other glutathione S-conjugates. The best characterized members of this cluster include: (i) the yeast cadmium factor (Ycf1) (60,61) and Yor1 protein (62) in *S. cerevisiae*, (ii) transporters associated with heavy metal resistance in *Leishmania* species (ImPgpA) (63,64) and the nematode *Caenorhabditis elegans* (ceMRP1) (65), and (iii) the bile acid transporter Bat1p in *S. cerevisiae* (66) (Table 2). The BCECF (2',7'-bis-(2-carboxyethyl)-5-(and 6)-carboxyfluorescein) transporter in *L. lactis* may be the first prokaryotic example of this cluster (67).

### Structure-Function Relationships

The effects of amino acid substitutions on the substrate specificity of ABC-type multidrug transporters has been studied most extensively using Pgp. In general, Pgp mutants can be classified into three groups. The first group is of mutations Gly141-to-Val, Gly185-to-Val, and Gly187-to-Val, and others, all in the first cytoplasmic loop (68-70); Gly228-to-Val in the second cytoplasmic loop (71); Gly812-to-Val and Gly830-to-Val in transmembrane segment 4 (71); Phe335-to-Ala and Val338-to-Ala in transmembrane segment 6 (72,73). These mutations increase the ability of Pgp to confer resistance to colchicine and doxorubicin, and decrease the ability of Pgp to confer resistance to vinblastine. The second group consists of mutations Pro223-to-Ala in transmembrane segment 4 (74); Gly341-to-Val in transmembrane segment 6 (73); Pro866-to-Ala in transmembrane segment 10 (74); Phe978-to-Ala in transmembrane segment 12 (72); Ser939-to-Phe, Tyr949-to-Ala, and Phe953-to-Ala in transmembrane segment 11 of mouse *mdr1* (75,76); and Ser941-to-Phe in transmembrane segment 11 of mouse *mdr3* (77). These mutations decrease the ability of Pgp to confer resistance to colchicine and doxorubicin, and increase or do not affect the ability of Pgp to confer resistance to vinblastine. Interestingly, Pgp mutants with amino acid substitutions at position 61 in transmembrane segment 1 fall into the first or the second group of mutants, depending on the size of the side chain (78). Hence, this transmembrane segment is also important in substrate specificity. The third group of mutations that affect substrate specificity of Pgp fall in the ATP-binding domains: Lys536-to-Arg in the ABC signature sequence, and mutations at positions 522-525 and 578 near the Walker B region of the N-terminal ATP-binding domain (79,80). In summary, amino acid substitutions which affect the substrate specificity of Pgp are found scattered throughout the

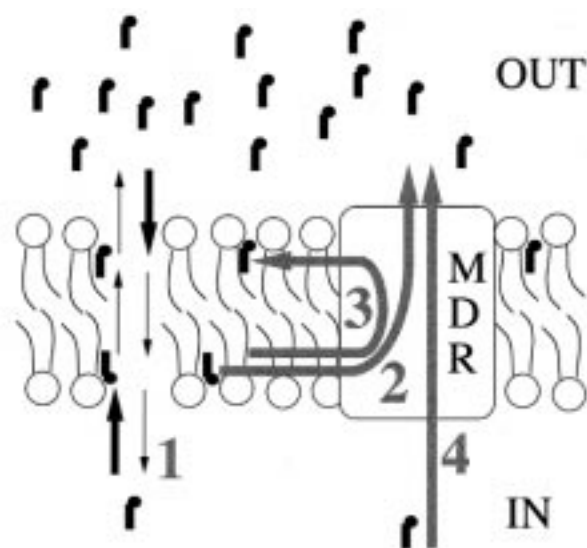


**Figure 2.** Structural model of a homodimer of the ABC-type multidrug transporter LmrA of *Lactococcus lactis*. LmrA is predicted to contain a transmembrane domain (TMD) with six transmembrane  $\alpha$ -helices (depicted as ellipses), and a nucleotide-binding domain (NBD) with the ABC signature and Walker A/B sequences (10). The structure of LmrA is very similar to that of the mammalian multidrug resistance P-glycoprotein (Pgp). Pgp contains two covalently linked homologous halves, each with 6 transmembrane segments and an ATP-binding domain. In view of the twelve-transmembrane model proposed for Pgp, LmrA may function as a homodimeric complex. **IN** and **OUT** refer to the inside and outside of the cytoplasmic membrane, respectively.

molecule in transmembrane domains, cytoplasmic loops and ATP binding domains.

A limited set of chimeric Pgp proteins has been constructed using mammalian P-glycoproteins, such as human MDR2, as partner protein to identify proteins segments and amino acids in Pgp implicated in drug recognition (81-83). Human MDR2 (often called MDR3), a phosphatidylcholine-specific translocase in the bile canicular membrane of hepatocytes, shares about 77% identity with Pgp. However, overexpression of human MDR2 or mouse *mdr2* in drug-sensitive cells does not confer MDR (84,85). Greenberger and

coworkers have replaced regions with the greatest sequence diversity between Pgp and MDR2, from transmembrane segment 10 to the C-terminus of Pgp, by the corresponding sequences from MDR2 (83). Only a replacement limited to transmembrane segment 12 of both proteins results in a functional hybrid Pgp, which was markedly impaired in conferring resistance to actinomycin D, vincristine, and doxorubicin, but not to colchicine. The drug resistance phenotype was associated with an impaired ability to photoaffinity label the hybrid protein with iodoaryl azidoprazosin. Thus, amino acids within transmembrane segment 12 may



**Figure 3.** Possible mechanisms of drug transport across the cytoplasmic membrane. (1) Drugs enter the cell by passive diffusion via the outer and inner leaflet of the phospholipid bilayer. Due to their lipophilicity, a significant amount of drug molecules will be retained in the membrane. Therefore, drugs may be expelled from the cell through: (2) extrusion from the inner leaflet to the external water phase (hydrophobic vacuum cleaner model), and/or (3) extrusion from the inner leaflet of the phospholipid bilayer to the outer leaflet (flippase model). (4) Alternatively, a drug molecule may be moved back to extracellular environment by extrusion from the internal water phase to the external water phase (aqueous pore model). Partitioning of drugs between the water phase and phospholipid bilayer is indicated by a black arrow. Protein-mediated drug transport is indicated by a gray arrow. IN and OUT refer to the inside and outside of the cytoplasmic membrane, respectively. MDR: multidrug resistance protein

compose part of a drug binding pocket of Pgp and are in close proximity to a photoaffinity drug-labeling domain.

Taken together, a number of amino acid substitutions in Pgp have been found to affect the drug specificity of the protein, but particular domains in Pgp involved in drug recognition, binding and translocation have not yet been identified.

### Functional Complementation

The structural similarity between ABC-type drug transporters can result in functional similarity as the mouse Pgp-homolog *mdr3* (86) and *Plasmodium* *pfMdr1* (87) can complement yeast *Ste6p*, thus restoring mating in a  $\Delta ste6$  sterile yeast strain. Likewise, MRP can functionally complement the Ycf1 protein in a  $\Delta ycf1$  cadmium-sensitive strain of *S. cerevisiae* (88), and partially complement *Ste6p* in the sterile  $\Delta ste6$  null mutant of this organism (89). The functional substitution of one ABC-type drug transporter by another is not only confined to eukaryotic transport proteins. Bacterial LmrA was able to functionally complement human Pgp in human lung

fibroblast cells (90). Surprisingly, LmrA was targeted to the plasma membrane. The pharmacological characteristics of LmrA and Pgp-expressing lung fibroblasts were very similar, and the affinities of both proteins for vinblastine and magnesium-ATP indistinguishable. Blockers of P-glycoprotein-mediated multidrug resistance also inhibited LmrA-dependent drug resistance. Kinetic analysis of drug dissociation from LmrA expressed in plasma membranes of insect cells, revealed the presence of two allosterically-linked drug binding sites indistinguishable from those of P-glycoprotein (90,91). The remarkable conservation of function between ABC-type drug transporters suggests a common fundamental molecular mechanism of these proteins in prokaryotic and eukaryotic cells.

### MOLECULAR MECHANISM

Several transport models have been postulated for Pgp and LmrA pump function to explain the broad specificity for chemically unrelated compounds (Figure 3). Drug translocation may involve substrate transport from the cytoplasm to the exterior (conventional transport hypothesis (92)) which would require an enormous flexibility of an 'enzyme-like' substrate recognition site. Alternatively, the multidrug transporters could recognize the lipophilic drugs by their physical property to intercalate into the lipid bilayer, and transport drugs from the lipid bilayer to the exterior (vacuum cleaner hypothesis (93)), or from the inner leaflet to the outer leaflet of the lipid bilayer (flippase hypothesis (94)). A property common to all known Pgp and LmrA substrates is their ability to intercalate between the phospholipids of biological membranes. This notion has led to the suggestion that these transporters recognize substrates within the membrane (24,44,95). Drug recognition within the membrane is supported by a number of observations: (i) photoaffinity analogs of Pgp-substrates label Pgp predominantly in or near the transmembrane helices 4-6, and 11-12 (96-98), (ii) point mutations resulting in the alteration in drug specificity of Pgp are frequently found in transmembrane helices (38), (iii) acetoxymethyl esters of several fluorescent probes accumulate less in Pgp or LmrA-expressing cells, despite the fact that the ester moieties are rapidly cleaved by intracellular esterases and the resulting carboxylates are not substrates for Pgp and LmrA (95, 99), (iv) the kinetics of ATP-dependent transport of Hoechst 33342 by Pgp and of TMA-DPH by LmrA in membrane vesicles is consistent with transport of the compounds from the inner, but not from the outer leaflet of the lipid bilayer (95,100,101). Interestingly, these latter observations have also been made for the secondary lactococcal multidrug transporter LmrP (24). Thus, a main determinant of specificity would be the

ability of a substrate to be intercalated into the lipid bilayer.

This transport mechanism is likely to be a more general mechanism for ABC transporters with hydrophobic substrates. The human *MDR2* gene-encoded P-glycoprotein transports phosphatidylcholine from the cytoplasmic leaflet of the bile canicular membrane of hepatocytes into the bile (84,102). In addition, the *E. coli*  $\alpha$ -hemolysine transporter HlyB most likely binds the transport signal sequence of  $\alpha$ -hemolysine, when the signal sequence forms an amphiphilic helix that binds to the cytoplasmic leaflet of the plasma membrane (103,104).

### PHYSIOLOGICAL ROLE

It has been proposed that Pgp participates in the protection of human cells against hydrophobic xenobiotics by active excretion of these compounds from the membrane into bile, urine, or the intestinal lumen, preventing their accumulation in critical organs such as the brain (105). Likewise, a defense function can be envisaged for multidrug transporters in microorganisms. These organisms encounter numerous hydrophobic compounds in their habitat which will accumulate in phospholipid bilayers (for review see 106). It is noteworthy that the natural environment of enteric microorganisms is enriched in bile salts and fatty acids, and that these compounds are substrates for various multidrug transporters (107,108). In addition, some microbial multidrug transporters may play a role in the transport of endogenous substrates, such as lipids (84,102,109,110), pheromones (111,112), or lipid-linked precursors of peptidoglycan (for review see (113)) or other extracellular biopolymers.

### CONCLUSION

Infectious disease caused by multidrug resistant microorganisms is an increasingly complex clinical and public health problem. Prevention and control strategies will require the application of epidemiological and behavioral approaches, as well as research technologies aimed at the basic mechanisms of drug resistance. This review summarizes the molecular properties of multidrug transporters in prokaryotic and eukaryotic cells, and shows that the microbial systems are very similar in structure and function to those found in mammalian tumor cells. Interestingly, multidrug transporters have an exceptionally broad drug specificity, and can shift their specificities in the course of drug treatment. The structure-function relationships that dictate drug recognition by multidrug transporters will represent an intriguing new area of inquiry, and may allow the rational development of inhibitors of these proteins, and of new drugs which are not extruded from the cell.

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