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The ABC family of multidrug transporters in microorganisms

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Abstract

Multidrug transporters are membrane proteins that are able to expel a broad range of toxic molecules from the cell. In humans, the overexpression of the multidrug resistance P-glycoprotein (Pgp) and the multidrug resistance-associated protein MRP1 (MRP) is a principal cause of resistance of cancers to chemotherapy. These multidrug transporters belong to the ATP-binding cassette (ABC) family of transport proteins that utilize the energy of ATP hydrolysis for activity. In microorganisms, multidrug transporters play an important role in conferring antibiotic resistance on pathogens. In the last decade, homologs of human Pgp and MRP have been found in microorganisms such as *Plasmodium falciparum*, *Candida albicans*, *Saccharomyces cerevisiae* and, more recently, in *Lactococcus lactis*. In this review, we will summarize the current state of knowledge on three major aspects of microbial ABC-type multidrug transporters: (i) the functional and structural similarities among these proteins in prokaryotic and eukaryotic cells, (ii) the molecular mechanism of these transporters, and (iii) their potential physiological role. © 1998 Elsevier Science B.V.

Keywords: Multidrug resistance; ATP-binding cassette transporter; P-glycoprotein; MRP; LmrA

1. Introduction

Organisms ranging from bacteria to man have developed versatile mechanisms to resist cytotoxic drugs. Examples are the enzymatic degradation or inactivation of drugs [1], and the alteration of drug targets [2]. In addition, all cells possess membrane proteins which catalyse transmembrane drug transport, and hence are able to overcome cell cytotoxicity by lowering the cytoplasmic drug concentration [3–5]. Some drug transporters 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 transport proteins fall into a limited number of families [5]. One of the largest of these families belongs to the ATP-binding cassette (ABC) superfamily [6]. Well-known members are the human *MDR1* gene-encoded multidrug resistance P-glycoprotein (Pgp) [7] and the human multidrug resistance-associated protein MRP1 (MRP) [8] plasma membrane transporters, which catalyse the ATP-dependent extrusion of anti-tumor drugs during the chemotherapy of cancer cells.

The number of ABC-type multidrug transporters identified in microorganisms is vast and rapidly expanding. Based on (i) the alignment analysis of amino acid residues that comprise the nucleotide-

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binding domain(s), (ii) protein topology, and (iii) drug specificity, the ABC family of drug transporters can be divided into two major clusters: the Pgp cluster and the MRP cluster. In this review, we will give a comprehensive overview of these clusters using selected examples in pro- and eukaryotic cells. In addition, the review will focus on the remarkable conservation of function among ABC-type drug transporters, which suggests a common molecular mechanism of these proteins in all living cells.

2. ABC transporters

2.1. Pgp cluster

Members of the Pgp cluster play an important role in microbial resistance to neutral or positively charged, amphiphilic drugs (Table 1). These transporters function in human pathogens such as *Plasmodium falciparum* (pfMDR1) [9], *Entamoeba histolytica* (ehPgp) [10], or *Leishmania donovani* (ldMDR1) [11]. In addition, infections in immunodeficiency patients are often caused by the pathogenic yeast *Candida albicans* which expresses Cdr1p, an ABC transporter which confers antifungal resistance [12]. ABC proteins in the non-pathogenic *Saccharomyces cerevisiae* include the multidrug transporters Pdr5p [13] and Snq2p [14]. Pgp cluster members have also been identified in bacteria. In *Streptomyces* strains, dedicated transporters such as DrrAB [15] mediate the excretion of specific antibiotics to ensure self-resistance to the antibiotics that they produce. A true prokaryotic ABC-type multidrug transporter with significant sequence identity to Pgp in both the ABC and membrane domains has been found in *Lactococcus lactis* (LmrA) [16]. 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. Interestingly, LmrA shares significant sequence identity with the hop-resistance protein HorA in *Lactobacillus brevis* [17], and with the products of putative open reading frames in *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Helicobacter pylori*, *Haemophilus influenzae*, and *Mycoplasma genitalium* (Table 2). Thus, homologs of LmrA may play an important role in the multidrug resistance phenotype of pathogenic bacteria.

Table 1
Examples of ABC[a]-type multidrug transporters in microorganisms

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

[a]ATP-binding cassette superfamily [6].
[b]Accession number: GB, Genbank; EM, EMBL.

Like Pgp, a number of 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 domains are usually composed of six putative α-helical transmembrane segments (Fig. 1). Certain ABC proteins, such as lactococcal LmrA and *E. coli* α-hemolysin transporter HlyB [18], have half the size of Pgp with only a single transmembrane domain and ABC domain. The notion that two of these half-molecules must cooperate to the formation of a single transporter is, amongst others, supported by the observation that the independent expression of each half of the α-mating pheromone transporter Ste6p in yeast cells does not yield a functional transporter, while simultaneous expression of both halves does [19]. 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.

2.2. MRP cluster

Members of the MRP cluster contain an N-terminal membrane-bound domain consisting of five putative α-helical transmembrane segments, which is followed by two homologous halves each with six putative α-helical transmembrane segments and an ABC domain (Fig. 1) [20–22]. Like MRP1, MRP homologs in microorganisms mediate the extrusion of

Table 2
Homologs of *lmrA*[a] in bacteria

Organism	Gene	Predicted length (aa)	Identity (%)	Ref.
Lactobacillus brevis	*horA*[b]	583	53	[17]
Bacillus subtilis	*yvcC*	589	43	[53]
	ywjA	575	35	
	yknV	604	31	
	yfiC	604	30	
Staphylococcus aureus	*abcA*	575	33	—[c]
Escherichia coli	*msbA*	582	30	[54,55]
	mdlA	590	28	
	mdlB	593	29	
	cydC	573	27	
	cydD	588	25	
Helicobacter pylori	HP1082	552	27	[56]
Haemophilus influenzae	HI0060	587	28	[57]
	HI1051	614	26	
Mycoplasma genitalium	MG015	589	25	[58]

[a]LmrA is a 590-aa polypeptide with six transmembrane segments in the N-terminal hydrophobic domain, followed by the ABC domain [16,35].
[b]HorA functions as a multidrug transporter [17].
[c]Unpublished data, Genbank accession no. U29478.

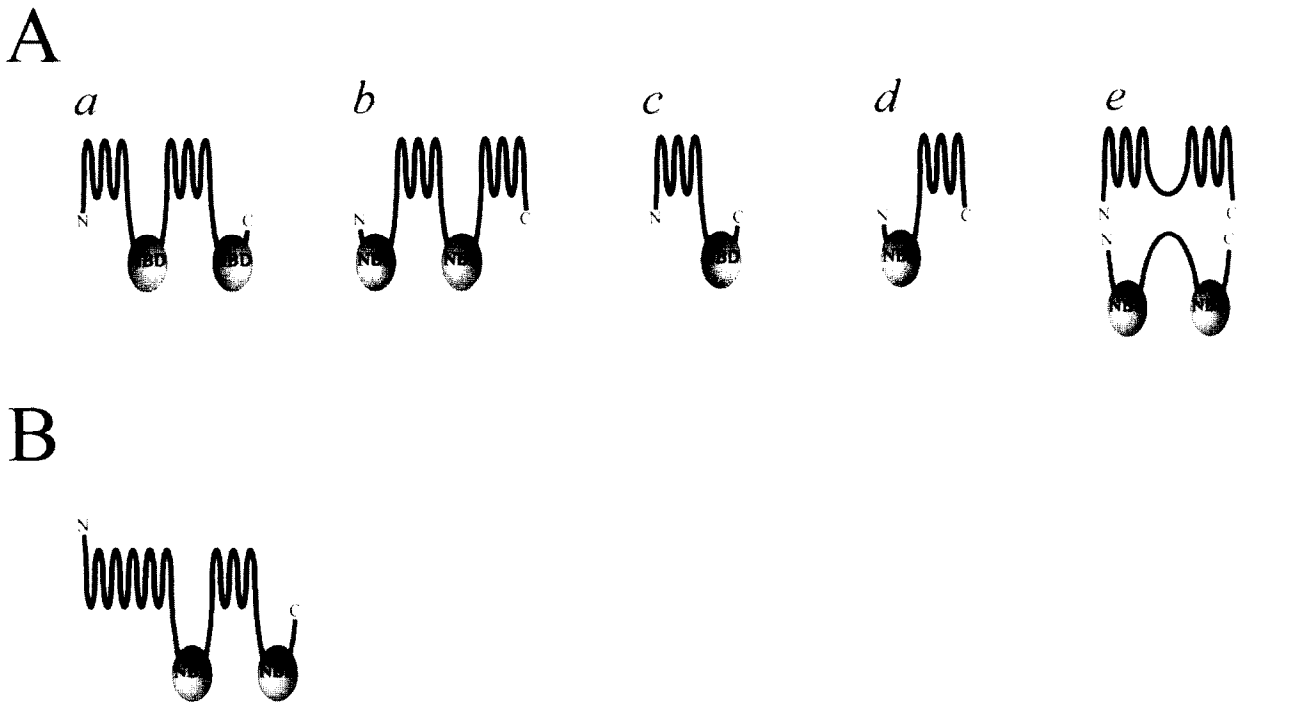

Fig. 1. Predicted topology of ABC-type multidrug transporters in microorganisms. (A) Pgp cluster. The typical ABC transporter of this cluster contains four membrane-associated domains. Two of these domains are highly hydrophobic and each consist of six putative transmembrane segments in α-helical configuration. These transmembrane domains (TMD) form the pathway through which substrate crosses the membrane, and are believed to determine, in part, the substrate specificity of the transporter. The other two domains are nucleotide-binding domains (NBD) which are peripherally located at the cytoplasmic face of the membrane, and which contain the Walker A and B motifs and the ABC signature [6]. The individual domains are frequently fused into a single multifunctional polypeptide with a $\{TMD–NBD\}_2$ configuration (a) or $\{NBD–TMD\}_2$ configuration (b) or expressed on two separate polypeptides with $\{TMD\}_2\{NBD\}_2$ configurations (c). 'Half-size' transporters with a {TMD–NBD} topology (d) or {NBD–TMD} topology (e) are also frequently found. Examples of the various ABC transporters are (a) *Plasmodium falciparum* pfMDR1 [9], (b) *Saccharomyces cerevisiae* Pdr5p [13] and Snq2p [14], (c) *Streptomyces peuceticus* DrrAB [15], (d) *Lactococcus lactis* LmrA [16], and (e) *S. cerevisiae* ADP1p [59]. (B) MRP cluster. The N-terminal transmembrane domain consists of five putative α-helical transmembrane segments, and is followed by two homologous halves each with six putative α-helical transmembrane segments and a NBD. Representative members of this cluster are mentioned in the text.

a wide variety of organic anions, such as carboxyfluorescein derivatives, bis(glutathionate)cadmium, and other glutathione S-conjugates. The best characterized members of this cluster include: (i) the yeast cadmium factor (Ycf1p) [23,24] and Yor1 protein [25] in *S. cerevisiae*, (ii) transporters associated with heavy metal resistance in *Leishmania* species (lmPgpA) [26,27] and the nematode *Caenorhabditis elegans* (ceMRP1) [28], and (iii) the bile acid transporter Bat1p in *S. cerevisiae* [29] (Table 1). The BCECF (2′,7′-bis-(2-carboxyethyl)-5(and 6)-carboxyfluorescein) transporter in *L. lactis* [30] may be the first prokaryotic example of this cluster.

3. Functional similarity

The structural similarity between ABC-type drug transporters can result in functional similarity. Albeit at low efficiency, the mouse Pgp-homolog mdr3 [31] and *Plasmodium* pfMdr1 [32] can complement yeast Ste6p, thus restoring mating in a Δ*ste6* sterile yeast strain. MRP1 can fully complement the Ycf1 protein in a Δ*ycf1* cadmium-sensitive strain of *S. cerevisiae* [33], and partially complement Ste6p in the sterile Δ*ste6* null mutant of this organism [34].

The functional substitution of one ABC-type drug transporter by another is not only confined to eukaryotic transport proteins. The bacterial LmrA protein was able to functionally complement human Pgp in human lung fibroblast cells [35]. Surprisingly, LmrA was even 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 [36,37].

4. Transport mechanism

The remarkable conservation of functional properties between ABC-type drug transporters suggests a common molecular mechanism of transport by these proteins in pro- and eukaryotic cells. Several models have been postulated for the pump function of multidrug transporters to explain their broad specificity for chemically unrelated compounds. Drug translocation may involve substrate transport from the cytoplasm to the exterior via an aqueous pore with a flexible 'enzyme-like' substrate recognition site (conventional transport hypothesis) [38]. Alternatively, 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) [39], or from the inner leaflet to the outer leaflet of the lipid bilayer (flippase hypothesis) [40].

Recent evidence suggests that bacterial LmrA [41] and human Pgp [42,43] recognize drugs by their hydrophobic properties, and that both proteins transport these compounds from the cytoplasmic leaflet of the plasma membrane. 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 [44,45]. In addition, the *E. coli* α-hemolysin transporter HlyB most likely binds the transport signal sequence of α-hemolysin, when the signal sequence forms an amphiphilic helix that binds to the cytoplasmic leaflet of the plasma membrane [46,47].

5. Concluding remarks

Multidrug transporters are present in all living cells. Appreciation of the mechanism and physiological function of these transport proteins is crucial for the development of effective new drugs able to suppress multidrug resistance in clinical settings. 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, and by preventing their accumulation in critical organs such as the brain [48]. Likewise, a defense function can be envisioned for multidrug transporters in microorganisms. These organisms encounter numerous hydrophobic compounds in their habitat

which will accumulate in phospholipid bilayers (for a review on the membrane toxicity of various lipophilic compounds, see Sikkema et al. [49]). It is noteworthy that the natural environment of enteric microorganisms is enriched in toxic bile salts and fatty acids, and that these compounds are substrates for various multidrug transporters [50]. Alternatively, multidrug transporters may play a specific role in the transport of a common endogenous substrate, such as lipid [44,45,51,52], which remains to be established. Studies on multidrug transporters in microorganisms could bring valuable information about the general molecular properties of these transport systems in prokaryotic and eukaryotic cells, and their implications in human disease.

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