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Towards understanding promiscuity in multidrug efflux pumps

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Drug export from cells is a major factor in the acquisition of cellular resistance to antimicrobial and cancer chemotherapy, and poses a significant threat to future clinical management of disease. Many of the proteins that catalyse drug efflux do so with remarkably low substrate specificity, a phenomenon known as multidrug transport. For these reasons we need a greater understanding of drug recognition and transport in multidrug pumps to inform research that attempts to circumvent their action. Structural and computational studies have been heralded as being great strides towards a full elucidation of multidrug recognition and transport. In this review we summarise these advances and ask how close we are to a molecular understanding of this remarkable phenomenon.

Mechanisms of multidrug resistance

All organisms require the ability to defend themselves against external toxic compounds. Although the initial evolutionary force for this was presumably the competition between organisms, the resulting effect is that cellular defence mechanisms against medicinal drugs are now ubiquitous. At the present time, public awareness of this phenomenon has come with recognition that the clinical treatment of infectious diseases and cancer can be prevented by drug resistance [1–4].

The mechanisms employed by cells to circumvent drug cytotoxicity are manifold but include the enzymatic metabolism of the drug (e.g., β-lactamase), the alteration of the target site to prevent drug binding (e.g., ribosome methylation), metabolic pathway alteration (e.g., reduced conversion of cyclophosphamide), increased repair of damage caused by the drug [e.g., O-6-methylguanine-DNA methyltransferase (MGMT)-mediated temozolomide resistance], and direct extrusion of the drug from the cell thus preventing cytotoxic concentrations from being reached. The latter mechanism has additional intrigue because it is carried out by 'multidrug' pumps, which undermine our understanding of substrate recognition in proteins. Rather than having a narrow substrate specificity, multidrug pumps show a lack of specificity that is staggering. For the best characterised, the list of potential substrates runs into the thousands. Furthermore, the promiscuity of multidrug pumps means that, even in normal human physiology, they play a significant role in the pharmacokinetics of many if not all prescribed drugs – as evidenced by regulations requiring transporter–drug interactions to be described as part of drug licensing processes [5]. It is therefore no surprise that the past 20 years have seen intensive effort directed towards a better understanding of the structures of these pumps, with a key aim being to understand substrate recognition and transport.

Multidrug pumps fall into one of five distinct families of membrane protein as characterised by Saier and colleagues [6], namely ATP-binding cassette (ABC) transporters, multidrug and toxic compound extrusion (MATE) transporters, major facilitator superfamily (MFS) transporters, resistance nodulation division (RND) transporters, and small multidrug resistance (SMR) transporters (Box 1) [6]. Because these are all membrane proteins, which are notoriously difficult to study structurally [7], successes are heralded as major breakthroughs. Importantly, in the past decade representative proteins from all five multidrug transporter families have been studied at medium-to-high resolution by structural biologists (Table 1, Figure 1). For some of these families we also have crystallographic data of the pump bound to drug substrate(s), which provide a foundation to explore similarities in drug recognition and drug export mechanisms and create an opportunity for future therapeutic inhibition of these transporters.

ABC transporters

Multidrug resistance (MDR) transporters of the ABC family are found in all organisms, but the human ABC transporters P-glycoprotein (Pgp/ABCB1), multidrug-resistance-associated protein 1 (MRP1/ABCC1), and breast cancer resistance protein (BCRP/ABCG2) are among the most studied due to their role in the development of MDR in cancer. Functional ABC exporters comprise two transmembrane domains (TMDs; three in the case of ABCC1), which are responsible for drug recognition and transport, and two cytosolic nucleotide binding domains (NBDs) where ATP is hydrolysed. Of all the MDR pumps, the pharmacology that is best understood is probably that of ABCB1. The structure and mode of action of the drugs that can be transported by ABCB1

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Box 1. Multidrug transporter families

ABC: ATP-binding cassette (ABC) transporters are multidomain proteins, and the only multidrug pump family that are primary active transporters; that is, transport is powered by the direct hydrolysis of ATP by the transporter itself. A small subset of ABC transporters can catalyse multidrug export in prokaryotes (Sav1866, MsbA) and eukaryotes (ABCB1, ABCC1, and ABCG2).

MATE: multidrug and toxic compound extrusion (MATE) family transporters are the most recently recognised drug exporter protein family, present in eukaryotes (hMATE1/2) and prokaryotes (NorM), and utilizing Na^+ or H^+ gradients in an antiport mechanism.

MFS: major facilitator superfamily (MFS) transporters are the largest family of membrane transporters in many organisms and, as with the ABC family, only a small minority of these transporters are multidrug pumps.

RND: resistance nodulation division (RND) proteins are part of tripartite pumps, and uniquely for multidrug transporters they mediate transport across the inner and outer bacterial membrane.

SMR: small multidrug resistance (SMR) proteins are restricted to bacteria and are exemplified by *Escherichia coli* EmrE. At just 100–120 amino acids they are the smallest multidrug resistance (MDR) pumps in terms of primary sequence.

is well known, and includes anthracyclines, vinca alkaloids, taxanes, peptides, and steroids [8]. In addition to transported substrates there are also many more molecules that can bind to ABCB1 and inhibit or modulate its function. A conceptual model for ABCB1–drug interaction – the hydrophobic vacuum cleaner model – has been proposed in which drugs partition into the inner leaflet of the membrane and are then flipped to the outer leaflet in a nonspecific manner. Although there is physicochemical and biophysical evidence for substrate interaction with ABCB1 through the inner leaflet [9,10], the recognition of substrates is more complicated because ABCB1 can detect subtle structural variations in ligand [11]. Consequently it remains challenging to predict effectively whether a ligand will be transported by ABCB1, inhibit it, or have no effect (discussed later). Ligand-binding and transport assays have shown that ABCB1 has at least four pharmacologically distinct binding sites that are allosterically coupled [12,13], an effect that is also seen with other ABC MDR transporters such as ABCC1 [14] and ABCG2 [15]. Although pharmacologically distinct, it may be that the binding sites on ABCB1 are not spatially separate. Indeed, extensive mutagenesis, single cysteine labelling, and photoaffinity labelling combined with mass spectrometry of ABCB1 have shown that residues from all 12 transmembrane helices appear to be involved in drug binding and that a large drug-binding cavity is consistent with the pharmacology data (Table S1 in the supplementary material online). Structures of other MDR pumps (see MATE and RND sections below) confirm that large drug-interaction surfaces to which drugs bind and make a distinct set of interactions could be a hallmark of MDR pump pharmacology.

The involvement of human ABC transporters in cancer MDR has prompted vast efforts to understand their structure. To date, several ABC MDR transporter structures have been reported (Table 1) from prokaryotes and eukaryotes. However, no human ABCB1 structure has been reported, prompting numerous attempts to model this important protein computationally (see below). The 'ATP switch' [16] mechanism for ABC exporters, in which nucleotide-driven interaction of the NBDs causes reorientation of the TMDs and reduces drug affinity [17,18], may be reflected in the inward- and outward-facing structures of *Caenorhabditis elegans* ABCB1 and Sav1866, respectively [19,20]. Of all the ABC MDR transporter structures determined, only one (the murine ABCB1a structure) contains a drug substrate [21]. However, these data have recently been significantly revised [22,23], undermining our confidence in using the murine ABCB1a structure as a basis for understanding drug binding. Fortunately, the field benefits from decades of experimental work to identify the

Table 1. Crystal structures of multidrug efflux pumps

Protein	Family	Resolution (Å)	Drugs present	Refs	PDB code
NorM	MATE	4.2	None	[26]	3MKT
		3.5 to 3.8	Apo, EtBr, Rh, TPP	[27]	4HUK to 4HUN
PfMATE		2.1 to 3.0	Apo Peptide inhibitors	[28]	3VVN,3VVO,3W4T 3VVP to 3VVS
Sav1866	ABC	3.0	None	[19]	2HYD
ABCB1 *Caenorhabditis elegans*		3.4	None	[20]	4F4C
ABCB1a mouse		3.8 to 4.2	None	[22]	4KSB to 4KSD
TM 287–288		2.9	None	[63]	3QF4
EmrE	SMR	4.5 4.5	None TPP	[64]	3B62 3B61
AcrB	RND	2.5 2.9 3.3 to 3.5 2.8 to 3.3 1.9, 2.25 3.05	None None Apo, Rif, Ery, Rif, and Min Apo, Min, Dox Min, Dox inhibitor	[65] [48] [46] [45] [49] [51]	2J8S 2GIF 3AOA to 3AOD 2DHH,2DRD,2DR6 4DX5, 4DX7 3W9H
MexB		3.0	DDM	[47]	1T5E
EmrD	MFS	3.5	None	[32]	2GFP

The word 'to' is used to indicate a series of structures with PDB codes differing in the last letter only. For the sake of brevity, the unrevised ABCB1a structures and MsbA (which may be able to function as a multidrug resitance pump but which is a lipid A transporter) are omitted, as are symmetrical AcrB trimer structures.

Drug abbreviations: Apo, drug-free structure determined in a study that also included a drug-bound state; EtBR, ethidium bromide; Rh, rhodamine; TPP, tetraphenylphosphonium; Rif, rifampicin; Ery, erythromycin; Min, minocycline; Dox, doxorubicin. Detergent abbreviation: DDM, dodecylmaltoside. Family abbreviations: ABC, ATP-binding cassette; MATE, multidrug and toxic compound extrusion; MFS, major facilitator superfamily; RND, resistance nodulation division; SMR, small multidrug resistance.

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