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Minireview

Multidrug resistance ABC transporters

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Abstract Clinical multidrug resistance is caused by a group of integral membrane proteins that transport hydrophobic drugs and lipids across the cell membrane. One class of these permeases, known as multidrug resistance ATP binding cassette (ABC) transporters, translocate these molecules by coupling drug/lipid efflux with energy derived from the hydrolysis of ATP. In this review, we examine both the structures and conformational changes of multidrug resistance ABC transporters. Together with the available biochemical and structural evidence, we propose a general mechanism for hydrophobic substrate transport coupled to ATP hydrolysis.

Key words: Multidrug resistance; ATP binding cassette transporter; Membrane protein; X-ray crystallography

1. Introduction

Multidrug resistance (MDR) is a significant challenge in the treatment of infectious diseases and cancer. The World Health Organization has reported that MDR bacteria can account for up to 60% of all hospital acquired infections globally [1]. Drug resistant bacterial strains that cause gonorrhea, pneumonia, cholera, and tuberculosis are widespread and are difficult to treat [2]. In humans, a similar mechanism of MDR is a major reason for the failure of several chemotherapeutics in the treatment of cancers. Tumor cells that are initially sensitive to a broad range of drugs can frequently reemerge and become resistant to entire families of anti-cancer drugs. Patients with these tumors often have severe relapse with few medical alternatives to improve their health condition.

2. The multidrug transporters

A major cause of bacterial and cancer drug resistance is attributed to a robust array of MDR transporters that extrude drug compounds out of the cell. MDR transporters can be divided into two classes based on their source of energy: Secondary transporters, which use proton gradients to facilitate an antiporter mechanism, and adenosine triphosphate (ATP) binding cassette (ABC) transporters that couple the hydrolysis of ATP to substrate transport across the cell membrane. Secondary MDR transporters are comprised of four superfamilies: the resistance/nodulation/division family, the multidrug/oligosaccharidyl-lipid/polysaccaharide flippase family, the drug/metabolite transporter superfamily, and the highly diverse major facilitator superfamily [3]. ABC transporters belong to one of the largest superfamilies of proteins and that either import or export a broad range of substrates that include amino acids, ions, sugars, lipids, and drugs [4–7]. The differences in their substrate specificities are reflected in their overall divergence in their transmembrane domains (TMDs). While bacteria genomes encode both classes of ABC proteins, eukaryotes have only exporters suggesting an early evolutionary divergence of their TMDs. In humans, 46 ABC transporters have been identified and play important roles in human diseases, which include cystic fibrosis, macular dystrophy, and several neurological disorders [8].

All ABC transporters are composed minimally of two nucleotide binding domains (NBDs) and two TMDs [9]. The NBD, which is also called an ABC, is the hallmark feature of this transporter family. The high-resolution structures of isolated NBDs have been multiply determined and are similar [10–13]. The role of the TMD is to recognize and mediate the passage of substrates across the cell membrane. For ABC transporters that permeate hydrophilic molecules, the TMD shields substrates from the lipids making up the bilayer by providing a hydrophilic pathway across the cell membrane. In most cases, these permeases have specific substrate specificities and may require a periplasmic binding protein to facilitate transport. The TMD for drug and lipid flippases, in contrast, are capable of recognizing and removing a large number of chemically unrelated lipids and toxins directly from the cell membrane. Many of these transporters translocate useful cytotoxins such as anti-cancer drugs [14].

Perhaps the most widely studied MDR-ABC transporter is the P-glycoprotein or human MDR1/ABCB1 [15]. First discovered in the early 1970s, this MDR-ABC transporter has been proposed to act as 'hydrophobic vacuum cleaner' because of its ability to remove both lipids and drugs as they intercalate and diffuse through the cell membrane [16,17]. The protein sequences of lipid and drug ABC transporters are similar and probably reflect a common transport mechanism. Human MDR3, for example, is a well-characterized phosphatidyl-choline flippase and has 73% protein sequence identity to human MDR1. In fact, human MDR1 is itself a lipid flippase transporting short chain phospholipids. Although the mechanism for ATP hydrolysis for most if not all ABC transporters is probably conserved, the details underlying the structural

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basis coupling this energy to substrate transport is likely different between ABC exporters that transport hydrophobic substrates and importers that permeate water-soluble molecules [18]. How do MDR-ABC transporters translocate drugs and lipids from the inner to the outer cell membrane leaflet?

3. Low-resolution cryo-electron microscopy (EM) studies of MDR-ABC transporters

Historically, our understanding of drug transporter structure originated from single particle and 2D cryo-EM of human MDR1 [19]. In their work, a 25 Å resolution structure revealed a large aqueous chamber spanning the cell membrane with no evidence of close contacts between NBDs. Later, similar studies revealed human MDR1 trapped in distinct catalytic states showing dramatic rearrangements of the TMD during the transport cycle. Although the precise boundaries of the TMD and NBD components could not be resolved, the structures showed a substantial opening in the plane of the cell membrane leading into the chamber, suggesting that substrates could be directly accepted from the lipid bilayer. The existence of such a chamber within the bilayer supported a 'flippase' model for human MDR1 and other MDR-ABC transporters [20]. Since then, other EM structures of MDR-ABC transporters have demonstrated that these proteins can undergo significant conformational changes in both their TMD and NBD. The 3D cryo-EM structure of YvcC from *Bacillus subtilis* shows an 'open' conformation and compares well with the X-ray structure of Eco-MsbA [21]. The projection structure of the MRP1/ABCC1 reveals a dimer with each half of the monomer related by a pseudo two-fold axis in projection [22].

4. The MsbA MDR-ABC transporter homologs

The structures of the open and closed conformations of MsbA reveal large conformational changes and suggest a general transport mechanism for hydrophobic substrates [23,24]. MsbA is a lipid flippase that transports lipid A, a major component in the bacterial outer membrane [25]. Studies in vitro demonstrate that MsbA is an ATPase that is specifically stimulated by lipid A [26]. Loss of MsbA from the cell membrane or mutations that disrupt transport results in the lethal accumulation of lipid A in the inner cell membrane [27,28]. MsbA is the only essential ABC transporters in prokaryotes and is conserved in every bacterium with more than 30 orthologs identified. MsbA is a close bacterial homolog of human MDR1 by protein sequence homology and has overlapping substrate specificities with the MDR-ABC transporter LmrA from *Lactococcus lactis* [29]. MsbA from *Escherichia coli*, for example, is 36% identical to the NH_2-terminal half of human MDR1, suggesting a common evolutionary origin for the mechanics of hydrophobic substrate transport.

The overall structural organization of MsbA is similar to human MDR1, which has the TMD fused with the NBD into a single polypeptide. The MsbA gene, however, encodes only a half transporter that assembles to form a homodimer with a total molecular mass of ~130 kDa. The X-ray structures of both the open and closed conformation of MsbA validate the dimer configuration and reveals a chamber formed from 12 transmembrane α-helices. The openings of the chamber are defined by intermolecular contacts between TM2 from one monomer and TM5 from another. The chamber is lined with charged and polar residues that are likely solvated creating an energetically unfavorable microenvironment for hydro-

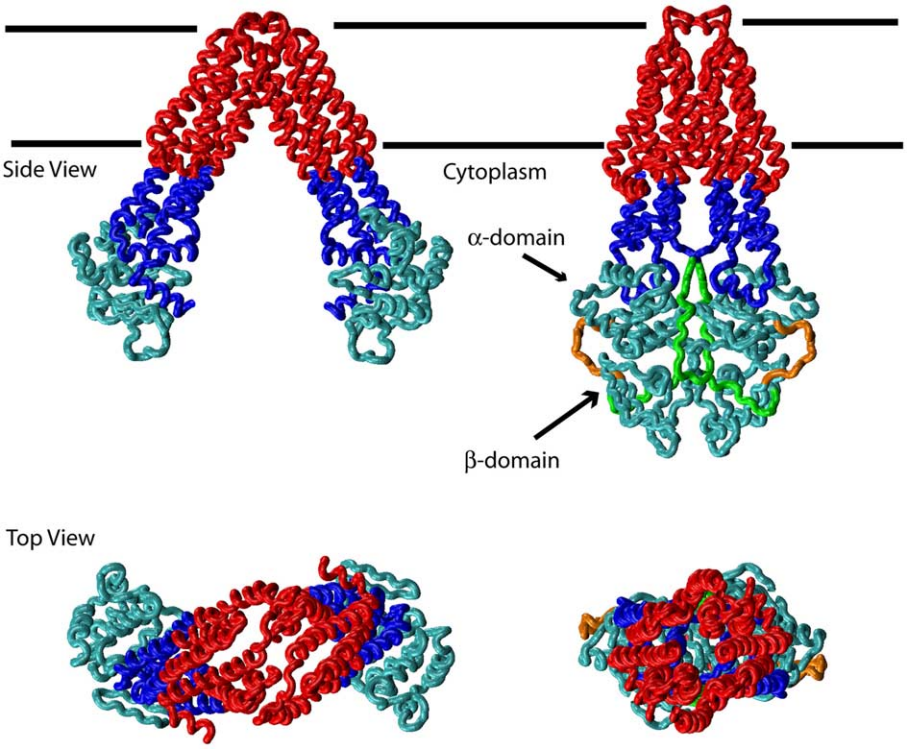

Fig. 1. Structure of MsbA. Views of MsbA in the open (left) and closed (right) conformation looking into the chamber opening (side view) and from the extracellular side (top view). The TMD, ICD, and NBD are colored red, dark blue, and cyan, respectively. The loop connecting the TMD with the ICD observed in the closed conformation from *V. cholerae* (right) is highlighted in green and the loop connecting the α and β domain of the NBD is shown in orange. The approximate position of the membrane bilayer is indicated by black lines.

phobic substrates. Faced with the charge and highly polar contribution of bound solvent, we propose that lipid A and other hydrophobic molecules 'flip' into an energetically more favorable position within the outer membrane leaflet.

Another distinct feature of MDR-ABC transporters is a conserved region located between the TMD and NBD, which we collectively call the intracellular domain (ICD). The structures of MsbA reveal that this region is helical and likely functions to couple the TMD to the NBD. ICD1 (residues 97–139) is in contact with the NBD and forms a U-like structure consisting of three α-helices. The second helix of ICD1 serves as a conserved pivot about which the NBD could rotate. The vitamin B12 importer BtuCD also has an analogous contact mediating energy transduction from the NBD to the TMD [30]. The tether joining the TMD with the NBD is clearly resolved in the closed conformation structure of MsbA (highlighted in green, Fig. 1). This peptide linker allows the NBD to disassociate and dimerize to form an ATP-competent state.

5. Possible general mechanism for MDR-ABC transporters

MDR-ABC transporters are unique because they extract lipid/drug molecules as they diffuse through the cell membrane and either flip them to the outer leaflet or expel them into the extracellular milieu. In order to accomplish this, they must first recognize substrates, and then sequester it from the membrane in a microenvironment that preferentially favors its movement to the outer membrane leaflet. The 'flipping chamber' formed from the two halves of the transporter spanning the cell membrane is seen in both the open and closed conformations of MsbA, giving merit to the notion that the TMDs are a structurally conserved architecture. The position of NBDs between the two structures, however, is rotated substantially while sharing the same point of contact in the TMD (residues 113–119). An analogous contact is observed in the vitamin B12 ABC importer and probably represents a fundamental feature in all ABC transporters.

The structures of MsbA from *Vibrio cholerae* and *E. coli* demonstrate that the NBDs can sample a large conformational space in the absence of nucleotides and is consistent with cross-linking studies of human MDR1 suggesting that TM6, which is physically tethered to the NBD, rotates during the catalytic cycle [31]. For example, recent cryo-EM studies of the MsbA homolog YvcC reveal that the NBD can disengage from the ICD during the catalytic cycle [32]. The 3D reconstruction of the yeast MDR-ABC transporter Pdr5p reveals a closely packed arrangement and a structural organization such that the orientations of the two NBDs are perpendicular within a monomer [33]. The existence of different angular positions of the NBDs with respect to the Pdr5p's TMDs suggests large rotational movements during the transport cycle. Unlike importers, ABC exporters have evolved to fuse the TMD with the NBD, to ensure a physically tethered catalytic domain. The presence of the peptide tether would allow the NBD to dissociate from ICD1 in the absence of substrate. Upon substrate binding, conformational changes in ICD1 may increase its affinity for the NBD such that it greatly favors an orientation that promotes NBD dimerization driving chamber closure and ATP hydrolysis. This conformational transition would ensure substrate recognition and ATP hydrolysis, which is tightly regulated.

Conformational changes in the NBD may add another level of control for ATP hydrolysis. The recent X-ray structures of the glucose ABC domain from *Sulfolobus solfataricus*, for example, show significant rearrangement of domains within the NBD between the nucleotide-bound and nucleotide-free states [34]. While the β domain of the NBD was disordered in the open conformational MsbA structure, it is clearly resolved in the VC-MsbA crystal form (Fig. 1). This structure, however, reveals that in the absence of nucleotides, the α and β domains are loosely associated but folded. The α and β domains are connected by a loop region that is less conserved among ABC proteins (highlighted in orange, in Fig. 1). This conformation has not been observed in previously solved structure of isolated NBD and gives rise to a couple interpretations. One possibility is that the separation of the α and β domains may represent a potential NBD conformation in the absence of nucleotides and can occur within the context of the TMDs. Clearly, the separation of the α and β domains is possible for the NBD alone. The NBD of the Rad50 ATPase enzyme, for example, is formed from α and β domains that are from two separate polypeptides that fold independently and associate to form a catalytically active NBD [35]. This raises the intriguing possibility that the interactions of NBD with the TMD upon substrate binding could act to stabilize the association of the domains favoring the ATP-bound state. An alternative possibility is that the separation of the domains is a consequence of crystal packing. Crystals of VC-MsbA, however, are sensitive to several non-hydrolyzable nucleotide analogs at micromolar concentrations. The disruption in the crystal lattice contacts is likely due to the association of the α and β domains to form the ATP-competent state (see Fig. 2). The structures of additional MsbA structures will reveal other possible structural configurations.

In our previous work, we presented a putative mechanism where upon binding lipid A, MsbA adopts a closed conformation that traps it within the chamber. This rearrangement could be driven by the ATP-mediated dimerization of the NBDs. There is significant biochemical evidence from studies of human MDR1 and LmrA indicating their NBDs interact during the transport cycle [36,37]. Indeed, the structures of the Rad50 and MJ0796 show the formation of a stable dimer upon binding ATP [38]. Biochemical studies of MsbA and human MDR1 indicate that substrate recognition stimulates ATP binding and hydrolysis by the NBD. In fact, ATP binding and hydrolysis are thought to be independent events and have been shown to be associated with distinct conformation states of human MDR1 [39,40]. Therefore, it is reasonable to propose that binding of ATP to the NBDs triggers their dimerization and results in the movement of transmembrane α-helices. The formation of the NBD dimer may represent the 'power stroke' of MsbA that drives chamber closure to 'flip' the lipid from the inner to the outer membrane leaflet. Upon doing so, the chamber then opens to release the substrate (Fig. 2). Indeed, recent cryo-EM studies of MsbA from *V. cholerae* in the presence of ADP show the opening of TMD allowing substrate expulsion into the outer leaflet of the bilayer (personal communication from A. Ward and R. Milligan, to be published).

The structure of the open and closed conformation of MsbA represent two snapshots of an ABC exporter and further substantiates the notion that MDR-ABC transporters are molecular machines that scan the lower bilayer leaflet for

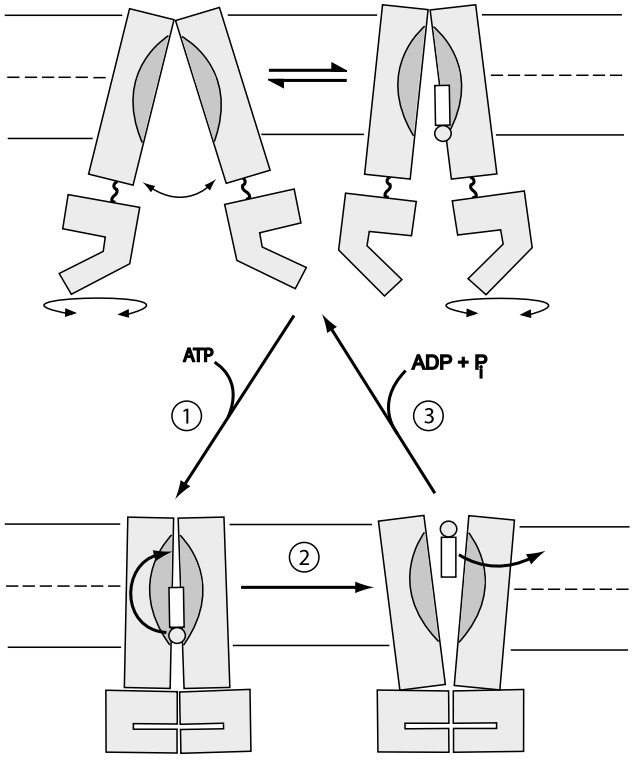

Fig. 2. In the nucleotide-free resting state, MsbA oscillates between conformational states where the tethered NBDs dissociate from ICD1 and are free to rotate relative to the TMD. Substrate recognition at the chamber opening induces nucleotide binding by the NBDs with an equal probability for either site as indicated (1). The binding of nucleotide changes their conformation and promotes the formation of an NBD dimer. Dimerization of the NBDs is the 'power stroke' of the transport cycle and drives recruitment of the substrate into the chamber, where it flips spontaneously to its new location. The NBD dimer then cooperatively hydrolyzes ATP to form the high-energy ADP+P_i intermediate (2). Conformational shifts in the NBD dimer upon nucleotide hydrolysis are relayed through the ICD causing the TMD to open, releasing the substrate into the outer membrane leaflet. Relaxation of the high-energy intermediate (3) causes the NBDs to disassociate and resets the TMD to the resting state. The figure is reproduced with permission [24].

substrates, accept them laterally into a chamber, and flip them to the outer membrane leaflet. Both structures give new insights into the conformational possibilities and provide a structural basis for the mechanism associate with lipid/drug 'flip–flop'. Taken together with the available biochemical evidence and EM studies, the X-ray structures of MsbA shed light on the molecular structural basis of drug transport by MDR-ABC transporters. Clearly, more high-resolution structures of MsbA and other MDR-ABC transporters are needed to fully understand the structural mechanisms underlying drug/lipid transport across the cell membrane.

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