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REVIEW ARTICLE

Tripartite transporters as mechanotransmitters in periplasmic alternating-access mechanisms

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To remove xenobiotics from the periplasmic space, Gram-negative bacteria utilise unique tripartite efflux systems in which a molecular engine in the plasma membrane connects to periplasmic and outer membrane subunits. Substrates bind to periplasmic sections of the engine or sometimes to the periplasmic subunits. Then, the tripartite machines undergo conformational changes that allow the movement of the substrates down the substrate translocation pathway to the outside of the cell. The transmembrane (TM) domains of the tripartite resistance-nodulation-drug-resistance (RND) transporters drive these conformational changes by converting proton motive force into mechanical motion. Similarly, the TM domains of tripartite ATP-binding cassette (ABC) transporters transmit mechanical movement associated with nucleotide binding and hydrolysis at the nucleotide-binding domains to the relevant subunits in the periplasm. In this way, metabolic energy is coupled to periplasmic alternating-access mechanisms to achieve substrate transport across the outer membrane.

Keywords: ABC transporters; cryo-EM; drug efflux transporters; Gram-negative bacteria; membrane proteins; RND transporters; structural biology; tripartite transporters; X-ray crystallography

Multidrug resistance caused by drug efflux transporters is a severe problem in antibiotic therapy of numerous bacterial infections [1]. Bacterial transporters that mediate drug efflux are categorised into six different transporter families. These are the ATP-binding cassette (ABC) superfamily, the major facilitator superfamily (MFS), the resistance-nodulation-cell division (RND) superfamily, the small multidrug resistance family and related proteobacterial antimicrobial compound efflux family [2], and the multidrug and toxic compound extrusion family. Over the past two decades, the structures of transporters belonging to these transporter families were elucidated at high resolution [3–7]. These protein structures also helped to advance functional analysis by raising specific questions and hypotheses. The active transport of drugs from the cellular interior to the outside the cell involves drug movement across the membrane. The original theoretical model of how substrates are transported across the plasma membrane was proposed as the alternating-access mechanism by Jardetzky [8]. In this model, V-shaped protein embedded in the membrane interconverts its shape into an inverse V-shaped protein and vice versa. The substrate to be transported binds roughly in the middle of the membrane-embedded regions of the protein. To enable directional substrate movement, the transition between V-shape and inverted V-shape is associated with a change in

Abbreviations
ABC, ATP-binding cassette; CHs, coupling helices; MFS, major facilitator superfamily; NBDs, nucleotide-binding domains; OMC, outer membrane channel; PAP, periplasmic adaptor protein; RND, resistance-nodulation-cell division; TM, transmembrane.

binding affinity for the substrate. In drug exporters, substrate-binding site(s) in the inward conformation are thought to have a higher affinity for drugs than in the outward conformation, thus allowing the transporter to capture the substrate from the cellular interior. The outward conformation has a lower affinity for drugs to release them at the exterior of the cell. Most drug exporters have structural features in the membrane-embedded regions that are compatible with an alternating-access mechanism. They have the V-shape, inverse V-shape and the intermediate shape referred to as the occluded state [9,10]. In ABC-type drug transporters, these conformational changes are initiated by ATP binding and hydrolysis at the two nucleotide-binding domains (NBDs) that protrude at the cytosolic side of the molecule. Secondary-active drug exporters couple their conformational changes to the downhill movement of H^+ and/or Na^+ across the plasma membrane into the cell and catalyse ion/drug antiport.

Resistance-nodulation-drug-resistance exporters confer resistance to β-lactam drugs that target periplasmic penicillin-binding proteins (peptidoglycan transpeptidases), by mediating β-lactam extrusion from the periplasm [11,12]. Not only β-lactams but also other substrates of RND exporters are thought to interact with these efflux pumps from the periplasm [13]. The drugs then pass through the connecting periplasmic and outer membrane conduit that is composed of a periplasmic adaptor protein (PAP) [14] and the outer membrane channel (OMC) [15]. The RND exporter, PAP and OMC together comprise a tripartite transporter complex. AcrB in *E. coli* is one of the best-studied RND exporters, which cooperates with AcrA as PAP and TolC as OMC [16,17].

Not only RND transporters but also transporters in the ABC and MFS transporters can be part of tripartite complexes and share similar or identical components with RND tripartite complexes. In *E. coli*, EmrB and EmrY are the tripartite MFS exporters [18]. They cooperate with EmrA and EmrK as PAP, respectively, and TolC as OMC for both. The ABC tripartite transporter MacA-MacB-TolC contains the ABC protein MacB with MacA as PAP and TolC as OMC [19]. Among these three tripartite complexes, inner membrane MFS and ABC transporters utilise identical or homologous PAPs and OMCs. In *E. coli,* all three tripartite systems cooperate with the identical OMC, TolC. In other Gram-negative organisms, including *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and others, the plasma membrane component of the tripartite complexes cooperates with distinct OMC homologues. However, all these homologues share a remarkable structural similarity. Furthermore, the various PAPs in tripartite complexes also show structural conservation, even when associated with membrane-embedded components that belong to different transporter families [20,21]. Due to their structural similarities, some tripartite systems can form functionally active [22] and structurally stable heterologous assemblies [23]. For example, a complex of MexB from *P. aeruginosa* with AcrA and TolC from *E. coli* was transport-active. In contrast, a complex containing *P. aeruginosa* MexA-MexB and *E. coli* TolC was inactive [24].

Recently, structures of the entire complex of the tripartite RND transporters AcrA-AcrB-TolC from *E. coli* and MexA-MexB-OprM from *P. aeruginosa* and the tripartite ABC transporter MacA-MacB-TolC from *E. coli* have been elucidated by the cryoelectron microscopy [23,25–28]. When the tripartite RND and the tripartite ABC transporter complexes are compared (Fig. 1), the PAPs associated with these complexes share the same domain composition. However, due to different lengths of their α-coiled-coil domain, these external pathways can dock on the members of different transporter families, each with variations in the size of the periplasmic domain. Consequently, the different tripartite complexes have a similar connecting conduit through the periplasm and outer membrane. What are the differences and similarities of these tripartite transport systems below the homologous chimneys?

The tripartite ABC transporter, MacA-MacB-TolC, is widely distributed in Gram-negative bacteria and is an important efflux transporter that mediates not only the extrusion of macrolides [19] but also peptide toxins [29], virulence factors [30], siderophores [31], lipopolysaccharides [32] and protoporphyrins [33]. Three research groups solved the crystal structure of MacB from different microorganisms in 2017 and 2018 [34–36]. All these crystal structures are similar but do not yet provide consensus on the actual steps in the transport reaction that are initiated by ATP binding. MacB shares many similar structural features with standard ABC exporters. Dimeric MacB forms two structural wings as previously seen for ATP-bound outward-facing Sav1866 and MsbA [4,37]. Each monomer has the NBD at the cytosolic side. MacB has a noticeable periplasmic domain protruding into the periplasm, which is missing in Sav1866 and MsbA. Instead of membrane domains (MDs) with six transmembrane (TM) helices, the MacB-MD is a helix bundle with only four α-helices without swapping of a helical hairpin between the MDs in the two MsbA and Sav1866 half-transporters. As the inner membrane

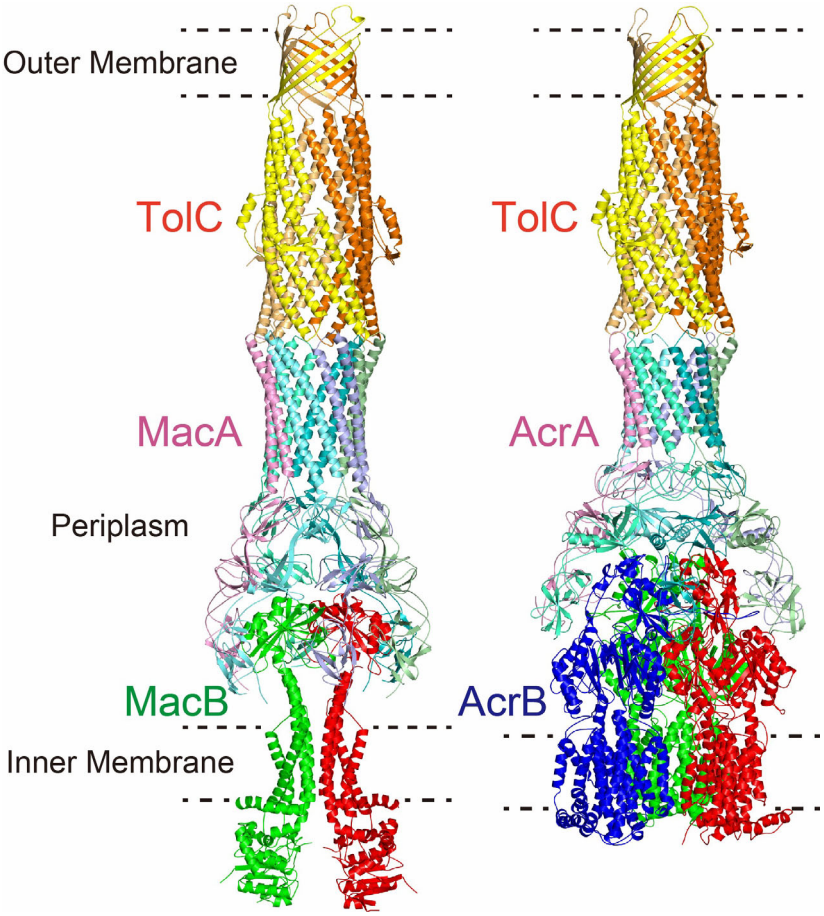

Fig. 1. Structural comparison between tripartite ABC transporter complex and tripartite RND transporter complex. Shown are MacA-MacB-TolC (PDB-ID: 5NIK) [25] and AcrA-AcrB-TolC (PDB-ID: 5NG5) [27] structures solved by the cryoelectron microscopy. Dotted lines indicate estimated membrane boundaries. Each protomer is coloured differently; (i) orange, light orange and yellow for the OMC TolC trimer, (ii) dark green, light green, cyan, dark cyan, light purple and pink for hexameric periplasmic adaptor protein MacA and AcrA, (iii) red and green for the MacB dimer and in red, green and blue for the AcrB trimer.

component of a mechanotransmitting tripartite efflux transporter complex, MacB is listed as a member of the type-VII transporters in the new classification of ABC transporters [38,39].

In this review, the basic architecture and mechanism of MacB are discussed as well as its role, and those of other tripartite systems, in maintaining homeostasis of the periplasmic environment in Gram-negative bacteria. The review aims to address the question of what the structural and functional similarities are between RND and ABC tripartite transporters that allow these systems to drive their periplasmic alternating-access mechanisms.

Tripartite ABC transporter

ATP-binding cassette transporters are very common in all kingdoms of life and share basic structural features. A functional unit is composed of two MDs and two NBDs that can be partially or fully linked together or expressed as separate proteins [40]. In many ABC exporters, the two MDs form a V-shape or an inversed V-shape in the functional unit. This conformational change is coupled to the ATP binding and dissociation of ADP and Pi following the hydrolysis of ATP at one or two nucleotide-binding sites at the NBD:NBD interface. This structural change is also directly coupled to substrate translocation across the plasma membrane through alternating access of the substrate-binding site, which is often located near the centre of the membrane at the MD:MD interface. The TM helices are extended at the cytosolic side and are connected by intracellular coupling helices (CHs). These CHs interact with NBDs and enable the transmission of conformational changes between the MDs and NBDs, which is referred to as 'coupling' [41]. The number of helices in the MDs depends on the class of ABC transporters [42]. The macrolide exporter MacB contains only four helices in each MD. Compared to other ABC exporters, this is the lowest number of helices known to date. The intracellular loop between TM2 and TM3 and the cytosolic C terminus are functional as CHs in MacB. The NBD of MacB has a similar structure as the NBDs in other ABC transporters.

Furthermore, MacB has a comparably large periplasmic domain which is responsible for the interactions with the periplasmic subunit MacA [34].

To survey the structural similarities in the MDs of the MacB dimer, the helix bundle of MacB was compared with that of other membrane proteins using the fold match program DALI [43,44]. Surprisingly, the first 20 significant hits all correspond to ABC transporters (Table 1). In particular, the arrangement of the four TM helix bundles in the MacB-MD shares conformational features with other ABC exporters (Table 1). According to the result of the search, the type-IV exporter ABCB1 (PDB-ID: 3G60, *Z*-score: 8.7) [45] is the top hit in addition to the MacB homologues from different organisms (Fig. 2A). The MDs of type-IV ABC proteins SUR1 (PDB-ID: 6C3O) [46], TmrAB (PDB-ID: 5MKK) [47] and PglK (PDB-ID: 5NBD) [48] also show conformational similarity with the MDs of MacB. Three helices (TM1-3) out of these six helices in the first MD of ABCB1 show comparably high structural similarity to the first three helices (TM1-3) of MacB. The next hits are the type-V transporters ABCA1 (PDB-ID: 5XJY, *Z*-score: 7.6) [49] and ABCG2 (PDB-ID: 5NJG, *Z*-score: 7.5; Fig. 2B,C) [50]. The statistics of structural matching is lower than that for ABCB1, but four helices (TM1-4) in the first MD superimpose on the four helices of MacB. On these superimposed structures, the dimer interface in MacB is not matched with the dimer interface of ABCB1 but matched with that of ABCA1 and ABCG2 (Fig. S1). Thus, three or four TM helices (TM1-3 or TM1-4) in type-IV, type-V and type-VII ABC transporters share common folds, but their overall architectures are quite different in the functional unit. These structurally conserved helices might play an essential role in the transmission of mechanical motion from NBD to MD. The remaining helices in ABCB1 (TM4-6), ABCA1 (TM5, TM6 and EH1, EH2) and ABCG2 (TM5, TM6), which do not match with those in MacB, form the putative substrate-binding cavities at their interface. Similar to MacB, ABCA1 has a large periplasmic domain between TM1 and TM2. The structures of the periplasmic domains do not match, but the periplasmic domains are thought to be important for transport activity in both ABC exporters [49]. In further investigations of the MDs with a cavity search program, there are no significant voluminous spaces in the MD regions of the MacB dimer [34].

On the other hand, a cavity-like structure can be found in the helical extension of the TMs in MacB that protrude into the periplasm. Thus, the TM regions lack the structural features that would be expected for a transporter operating by an alternating-access mechanism across the plasma membrane. In conclusion, the conservation of architectural features between MacB and type-IV and type-V ABC transporters is essential to capture metabolic energy from ATP binding and hydrolysis and to transmit mechanical movements in the NBDs to the MD. However, the additional helices that form substrate-binding sites and translocation pathways in the MDs of type-IV and type-V exporters are not present in MacB and other type-VII – 'mechanotransmitter' ABC transporters. Instead, these transporters contain two elongated TM helices that enable substrate binding and translocation in the periplasm, and that facilitate a 'periplasmic alternating-access mechanism' (Fig. 3). An inner membrane ABC transporter LolCDE in *E. coli* translocates lipoproteins to the outer membrane and is a close homologue of MacB [34]. LolCDE collects lipoproteins at the outer leaflet of the plasma membrane, where they are delivered as a precursor by the Sec translocon [51]. After the precursors are processed to mature lipoproteins, the LolCDE complex grabs the lipoproteins from the outer leaflet. This is similar to the mechanism by which the tripartite ABC transporter MacB can bind macrolide drugs and peptide toxins.

RND transporters

The RND transporters also form a large family across species. The RND subfamily HAE-1 contains major multidrug efflux transporters in Gram-negative bacteria [52,53]. These transport systems are proton-coupled transporters that cooperate with a PAP and OMC to form the tripartite transporter complex [11,17,26]. Some HAE-1 subfamily members, such as VexF from *Vibrio cholerae*, use Na^+ as the coupling ion rather than H^+ [54]. Both the structure and function of *E. coli* AcrB have been well-studied [55]. AcrB is functional as a homotrimer and was crystallised in different crystal forms [3,56–58]. In some of these forms, the AcrB trimer shows an asymmetric conformation. Each protomer in the trimer has a different conformation in the periplasmic domains termed 'access (A)', 'binding (B)' and 'extrusion (E)' [56] or by analogy to the functional rotation in the F_OF_1-ATPase [59], the 'loose (L)', 'tight (T)' and 'open (O)' conformation [57]. These conformations each correspond to a particular monomer conformation in the substrate translocation process. In the access state, the substrate gets access to the substrate translocation pathway through the entrance or vestibule [60,61]. In the binding state, substrates are transferred into the expanded substrate-

Table 1. Structural homologues of the four-helix bundle of MacB (TM1:282–321 and TM2-4:521–664, including two coupling helices). Top 20 hits from DALI search ranked by *Z*-score, show the structural motif conservation. The following abbreviations are used. PDB Description: the COMPND (compound) record from the PDB entry. *Z*-score: Statistical significance of the similarity between protein-of-interest and other homologous proteins. RMSD: RMS deviation of Cα atoms in the least-squares superimposition of the structurally equivalent Cα atoms. lali: number of structurally equivalent residues. # residue: the total number of amino acid residues in the hit proteins. %id: percentage of identical amino acids over all structurally equivalent residues. PDB-ID and Chain ID: PDB accession code and chain identifier.

Rank	Protein	Type	Protein description	*Z*-score	RMSD	lali	# residue	% id	PDB ID	Chain ID
1	ABCB1	Type-IV	Multidrug resistance protein 1A	8.7	5.4	159	1182	9	3G60	B
2	SUR1	Type-IV	ATP-sensitive inward rectifier potassium channel	8.1	4.2	152	1290	5	6C3O	F
3	SUR1	Type-IV	ATP-sensitive inward rectifier potassium channel	8.1	4.2	153	1290	5	6C3O	H
4	ABCB1	Type-IV	Multidrug resistance protein 1A	8.1	6.0	168	1188	9	4M1M	A
5	SUR1	Type-IV	ATP-sensitive inward rectifier potassium channel	8.1	4.2	152	1290	5	6C3O	E
6	ABCB1	Type-IV	Multidrug resistance protein 1A	8.0	7.4	160	1182	9	4Q9H	A
7	ABCB1	Type-IV	Multidrug resistance protein 1A	7.8	6.5	157	1180	7	4M1M	B
8	ABCB1	Type-IV	Multidrug resistance protein 1	7.7	5.0	156	1182	8	6QEX	A
9	SUR1	Type-IV	ATP-sensitive inward rectifier potassium channel	7.7	4.8	153	1359	6	6C3P	E
10	TmrAB	Type-IV	Multidrug resistance ABC transporter ATP-binding	7.7	5.6	154	593	12	5MKK	A
11	SUR1	Type-IV	ATP-sensitive inward rectifier potassium channel	7.7	4.1	148	1359	5	6C3P	G
12	ABCA1	Type-V	ATP-binding cassette sub-family A member 1	7.6	5.1	156	1901	10	5XJY	A
13	ABCB1	Type-IV	Multidrug resistance protein 1A	7.6	5.1	154	1182	8	4KSC	A
14	ABCB1	Type-IV	Multidrug resistance protein 1A	7.5	4.7	152	1182	7	4KSD	A
15	PglK	Type-IV	WLAB protein	7.5	6.3	153	564	8	5NBD	A
16	ABCG2	Type-V	ATP-binding cassette sub-family G member 2	7.5	3.9	149	286	6	5NJG	B
17	ABCB1	Type-IV	Multidrug resistance protein 1A	7.4	5.0	155	1182	8	4KSB	A
18	SUR1	Type-IV	ATP-sensitive inward rectifier potassium channel	7.4	4.2	152	1290	5	6C3O	G
19	YbtPQ	Type-IV	Inner membrane ABC-transporter	7.3	5.3	145	571	8	6P6J	A
20	ABCB1	Type-IV	ATP-binding cassette, sub-family B, member 1	7.3	5.2	152	588	7	3WMF	A

binding pocket along the substrate translocation pathway. In the extrusion state, the expanded pocket is compressed to squeeze out the bound substrate to the exit of the transporter. The exit is connected to the continuing pathway composed by the PAP hexamer. As each protomer changes its conformations from access to binding, from binding to extrusion and from extrusion back to access, the three conformers always exist in the same trimer. This working model has been presented as a functionally rotating mechanism of RND transporters [56,62]. In this mechanism, the access state corresponds to the inward conformation that attracts substrate into the translocation pathway. Furthermore, the extrusion state corresponds to the outward conformation that expels the substrate through the exit pathway. Surprisingly, this mechanism operates just external to the outer leaflet of the plasma membrane. Thus, similar to the tripartite ABC transporter MacAB-TolC, this RND mechanism grabs substrates from the outer leaflet of the plasma membrane and directly from the periplasmic space, and transports these across the periplasm and outer membrane. For the ability of AcrB to confer resistance to membrane-active compounds such as detergents and amphiphilic antibiotics, each protomer contains a substrate-entry pathway from the outer leaflet of the plasma membrane. In response to the decrease in the concentration of substrates in the outer leaflet of the plasma membrane, the substrate concentration in the inner leaflet of the plasma membrane and cytoplasm will decrease by the redistribution of substrate to the outer leaflet. This redistribution of substrate is also actively mediated by nontripartite multidrug transporters in the plasma membrane [63]. Crystal structures of the AcrB homologues show similar structures in which bound drugs are observed in the periplasmic domains that are formed by the external loop regions [60,64,65]. In contrast to nontripartite transporters,

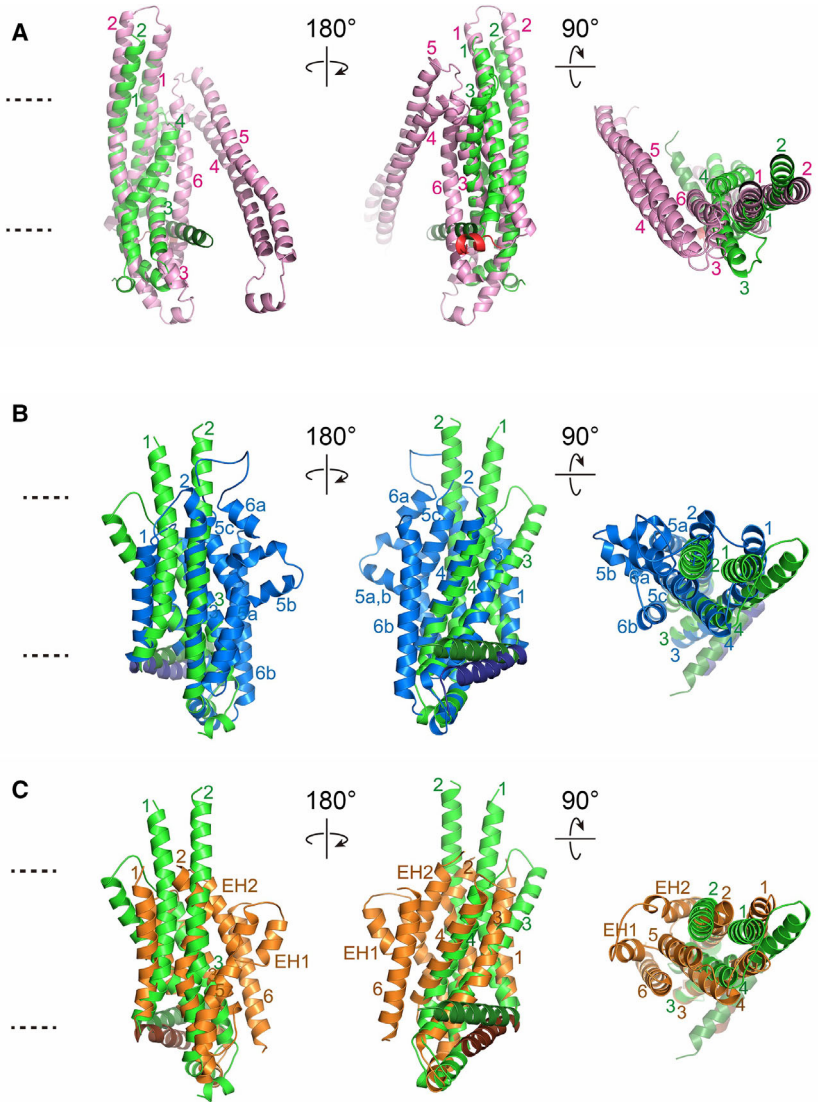

Fig. 2. Superimposition of TM region of ABCB1 (type-IV), ABCG2 (type-V) and ABCA1 (type-V) exporters vs MacB (type-VII) according to the DALI search results. (A) Comparison between MacB (PDB-ID; 5GKO, green) [34] vs ABCB1 (PDB-ID: 3G60, pink) [45], (B) MacB vs ABCG2 (PDB-ID: 5NJG, blue) [50] and (C) MacB vs ABCA1 (PDB-ID: 5XJY, orange) [49]. Elbow helices of each protein are coloured in their darker colour (dark green, red, dark blue, brown, respectively). Right panels of each set represent the top view. Dotted lines depict the approximate membrane boundaries.

RND drug exporters have no known substrate translocation pathway that allows the direct transport of drugs from the cytoplasm across the plasma membrane. The TM regions of AcrB and others contain salt-bridged titratable residues in the middle of the helix bundle [66]. These residues form the essential part of the proton translocation pathway. Thus, current structural data suggest that the TM region of tripartite RND drug transporters does not participate in substrate translocation across the plasma membrane. But to enable proton translocation across the membrane, the MDs in each protomer also utilise an alternating-access mechanism. In the 'Access' conformation, the MDs are open to the inside, in the 'Binding' conformation, they are open to the outside, and in the 'Extrusion' conformation, they form an occluded state [67]. The MDs harvest the electrochemical potential of protons and transduce this form of metabolic energy into mechanical motions of TM helices. These motions are transmitted to the periplasmic domains, where a periplasmic alternating-access mechanism in each protomer changes the conformation of the substrate-binding pocket by a mechanism of functional rotation. This, in turn, generates an inwardly directed substrate gradient across the outer membrane. In this way, RND drug exporters act as inner membrane components of mechanotransmitting tripartite complexes, which facilitate the transduction of the electrochemical proton (or Na^+) potential into the chemical potential of the substrate. In this process, the MDs in each protomer might act as a proton (or Na^+) uniporter that mediates an electrogenic proton

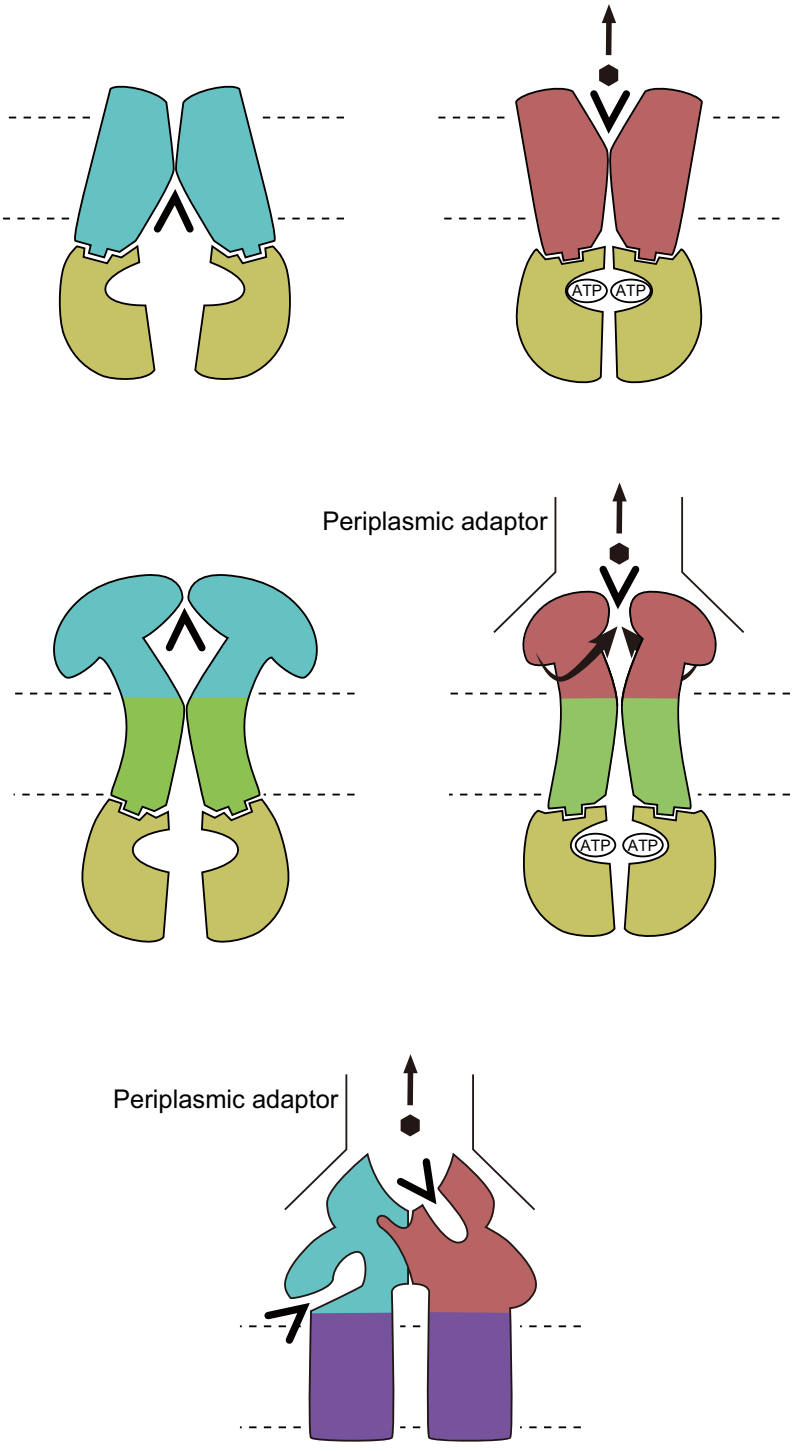

Fig. 3. Schematic comparison of the conventional alternating-access model for ABC transporters vs the periplasmic alternating-access model for tripartite ABC and RND transporters. (A) Nontripartite type ABC transporter, (B) tripartite ABC transporter and (C) tripartite RND transporter. The inward conformation and the outward conformation in the alternating-access mechanisms are coloured in cyan and brown, respectively, in each transporter. The NBD domain of ABC transporters is coloured in dark yellow. TM region of tripartite ABC and RND is coloured in green and purple, respectively. TM region of nontripartite ABC transporter is involved in the alternating-access mechanisms enabling transport across the plasma membrane (A). The periplasmic domain in the inner membrane subunit of tripartite transporters is engaged in a periplasmic alternating-access mechanism (B, C). Periplasmic adaptor forms a funnel-like pathway for substrate translocation across the outer membrane (B,C).

(or Na^+) import reaction independent of whether the substrate is anionic, cationic, twitter-ionic or neutral. This is different from ion-coupled plasma membrane transporters such as the MFS transporters MdfA and LmrP. MdfA mediates electrogenic drug/H^+ antiport for neutral drugs, but electroneutral antiport for monovalent cationic drugs [68]. In LmrP, the number and location of proton-binding catalytic carboxylates have a direct influence on the electrogenicity of the drug/H^+ antiport reaction for the same substrate [69].

The structures of the other RND subfamilies were also solved recently. SecDF (PDB-ID: 3AQP) [70]

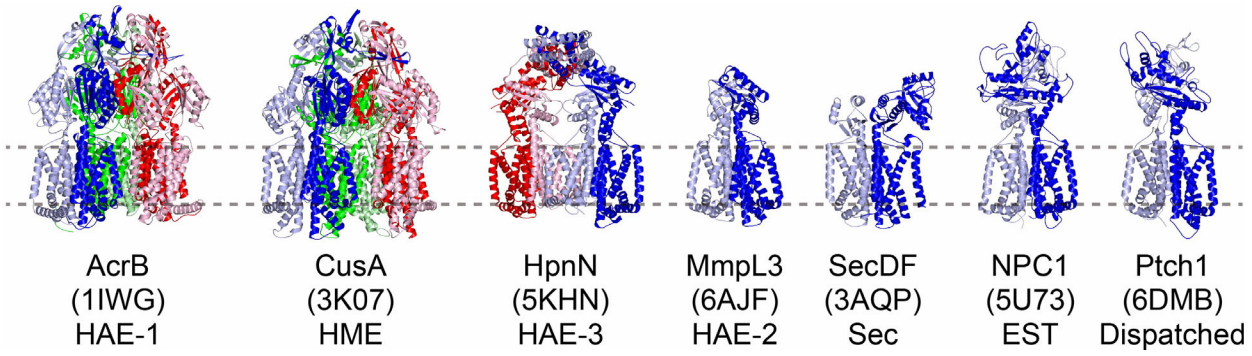

Fig. 4. Structures of RND superfamily transporters belonging to different subfamilies. (from left to right) AcrB (HAE-1 family, PDB-ID: 1IWG) [3], CusA (HME family, PDB-ID: 3K07) [71], HpnN (HAE-3 family, PDB-ID: 5KHN) [73], MmpL3 (HAE-2 family6AJF) [72], NPC1 (EST family, PDB-ID: 5U73) [74] and Ptch1 (dispatched family, PDB-ID: 6DMB) [75] are shown. Trimeric AcrB and CusA are individually coloured in blue, red and green. Dimeric HpnN is coloured in blue and red. Monomeric MmpL3, SecD, NPC1 and Ptch1 are coloured in blue. Each monomer has pseudo-twofold symmetry. N-terminal and C-terminal halves of each monomer are coloured in a dark and pale colour, respectively. Dotted lines depict the approximate membrane boundaries.

forms part of the protein secretion machinery and shares structurally similar TM region with AcrB and other HAE-1 subfamily transporters. However, SecDF is functional as a monomer with a periplasmic domain that is different from AcrB. But, by analogy to AcrB, MacB and LolCDE, the periplasmic domain of SecDF is thought to change its conformation dynamically to grab its substrate, here, a nascent protein chain that is pulled through the TM pathway in the SecYEG translocon. In this system, the major mechanical work to pull up the nascent chain is carried out by the periplasmic domain of SecDF, which couples this mechanical motion to downhill proton translocation by the MDs of SecDF. CusA (HME family, PDB-ID: 3K07) [71], MmpL3 (HAE-2 family6AJF) [72], HpnN (HAE-3 family, PDB-ID: 5KHN) [73], NPC1 (EST family, PDB-ID: 5U73) [74] and Ptch1 (dispatched family, PDB-ID: 6DMB) [75] all belong to RND subfamilies different from HAE-1. All have similar MDs as AcrB and other members of the HAE-1 subfamily (Fig. 4). CusA is a biocidal RND exporter for Cu(I) and Ag(I) ions. In CusA, ion binding is observed in the periplasmic domain. However, structurally important methionine residues for ion binding in the MDs were also found to be important in functional assay with methionine substitution mutants. Thus, substrate entry into translocation pathways in CusA might proceed from the cytosol as well as the periplasm [71]. Accordingly, all these RND proteins transduce the proton or sodium motive force into mechanical movements of TM regions and transmit these movements to the other domains or subunits to drive the translocation of the substrate to the extracellular side of the tripartite complex. Most ABC and RND tripartite complexes appear to transport their substrates from the plasma membrane and/or periplasm to or across the outer membrane *via* a periplasmic alternating-access mechanism (Fig. 3).

Conclusions and Perspectives

The periplasm is a cellular compartment that is highly exposed to physicochemical fluctuations in the external environment. To extrude xenobiotics that enter the periplasm and to deliver lipidic and proteinous materials to the outer membrane, unique types of transport machinery developed in Gram-negative bacteria in the course of evolution. As ATP is not available in the periplasm and the outer membrane does not sustain electrochemical ion gradients, the periplasmic pumping mechanisms utilise chemiosmotic energy at the plasma membrane or the free energy of nucleotide binding and hydrolysis in the cytosol. For this requirement, MDs in tripartite transporters acquired ion translocation pathways or NBDs, respectively. The captured metabolic energy is transduced into mechanical movement in TM helices, which is transmitted to the large external loop regions comprising the substrate-binding pocket. Thus, if type-VII ABC transport complexes are recognised as mechanotransmitters, then RND transporters, and especially HAE-1 drug exporters, could be categorised in a similar way (Fig. 3).

In 1993, the periplasmic vacuum cleaner hypothesis was first proposed as a mechanism for ABC multidrug transporters [76] and subsequently also demonstrated for MFS multidrug transporters [77]. In these models, the substrate is bound by the transporter from the inner leaflet of the plasma membrane and effluxed out of the cell at the expense of metabolic energy (ATP or

ion motive force). The mechanisms of tripartite ABC and RND transporters are complementary to the original hydrophobic vacuum cleaner as it sucks up unwanted substances from the periplasm and outer leaflet of the plasma membrane. In this way, conventional MFS and ABC multidrug transporters cooperate with tripartite ABC and RND transporters to achieve drug efflux from the cytoplasm, plasma membrane and periplasm across the outer membrane in Gram-negative bacteria. Therefore, ABC, RND and most likely also MFS tripartite transporters are important components of detoxification mechanisms in Gram-negative bacteria.

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Author contributions

SM and HWV wrote the manuscript with input from UO.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Comparison of two-fold symmetry axes at the centre of the functional unit of ABCB1 (type-IV), ABCG1 (type-V) and ABCA1 (type-V) vs MacB (type-VII) according to the DALI search results for comparison of TM regions.