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Multifaceted roles of efflux proteins in prokaryotes

Irshad Ahmad[1], Nighat Nawaz[2], Sadeeq Ur Rahman[3], Abdul Sajid[3], Farhan A. Khan[4], Sher B. Khan[4], Mohammad Z. Mustafa[5] and Simon G. Patching[1]*

1 School of Biomedical Sciences, University of Leeds, Leeds, UK.
2 Department of Chemistry, Islamia College Peshawar, Peshawar, Pakistan.
3 College of Animal Husbandry and Veterinary Sciences, Abdul Wali Khan University, Mardan, Pakistan.
4 Department of Animal Health, The University of Agriculture, Peshawar, Pakistan.
5 Centre for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta, Pakistan.

E-mail : s.g.patching@leeds.ac.uk

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Abstract

Multidrug resistance is a globally increasing problem that has become an alarming threat to antibiotic therapy. A principal resistance mechanism that prokaryotic organisms have evolved is active multidrug efflux, whereby antibiotics and other xenobiotics are exported to the external environment by transport proteins in the cell membrane. Such proteins are especially abundant in Gram-negative bacteria that are responsible for a large proportion of hospital-acquired infections. Based on amino acid sequence similarity, substrate specificity and the energy source used for exporting substrates, prokaryotic organisms contain seven major families of distinct multidrug efflux transporters (ABC, MFS, RND, SMR, MATE, AbgT, PACE). Efflux proteins also have roles in biofilm formation, quorum sensing, resistance to heavy metals and biocides, cell homeostasis and in bacterial pathogenicity and virulence. Prokaryotic efflux proteins are therefore highly important targets for advancements in antibiotic therapy and for ongoing experimental characterisation of their structures, functions, molecular mechanisms and regulation.

Key words : active transport, biofilms, efflux proteins, Gram-negative bacteria, multidrug resistance

INTRODUCTION

All over the world, the rate of multidrug resistance is dramatically increasing and the mechanisms of resistance are varied and complicated. Diverse mechanisms contribute to intrinsic, acquired and phenotypic resistance to antimicrobial compounds. Active multidrug efflux systems are one of the major mechanisms of bacterial resistance to drugs and are an alarming threat to antibiotic therapy.[1-11] Gram-negative bacteria (e.g. *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*) are commonly intrinsically more resistant to many antibiotics and biocides as a result of their cell structure and the activity of multidrug efflux pumps[8,12-15] and they are responsible for over 30% of hospital-acquired infections.[16-20]Efflux pumps are proteinaceous transporters that can extrude antibiotics and other xenobiotics from the cytoplasm or surrounding membranes of cells to the outside environment. They are found in all microorganisms, including both Gram-negative and Gram-positive bacteria[21-24] and also in eukaryotic organisms.[25-32] The active transport mechanism of efflux pumps is driven by an energy source. In this respect, primary active transporters exploit ATP hydrolysis, whilst secondary active transporters use the electrochemical potential difference created by proton pumping or sodium ions. These transporters may be highly specialised for one compound or may be highly promiscuous, transporting a broad range of structurally dissimilar substrates.

FAMILIES OF MULTIDRUG EFFLUX PROTEIN

In the prokaryotic kingdom there are currently seven major families of distinct bacterial multidrug efflux transporters: adenosine triphosphate (ATP)-binding cassette (ABC) superfamily, major facilitator superfamily (MFS), resistance-nodulation-division (RND) family, small multidrug resistance (SMR) family, multidrug and toxic compound extrusion (MATE) family, p-aminobenzoyl-glutamate transporter (AbgT) family, proteobacterial antimicrobial compound efflux (PACE) family(Figure 1).These families are classified on the basis of their amino acid sequence similarity, substrate specificity and the energy source used to export their substrates. Protein structural organisation in the large majority of the families, including the number of transmembrane helices (TMH),has been confirmed by high resolution structure determination for at least one member.

ABC superfamily proteins[33-36] are the only primary transporters, usingenergy from ATP hydrolysis to expel substrates. They are comprised of a transmembrane domain that forms the extrusion pathway and a cytoplasmic nucleotide-binding domain that binds and hydrolyses ATP. Ahomodimeric structural organisation (2 x 6TMH) in bacterial ABC proteins is represented by multidrug efflux transporter Sav1866 from *Staphylococcus aureus*(PDB 2HYD).[37]

All of the other families are secondary transporters that use the proton or sodium gradient as the energy source in an antiport manner. The MFS proteins[38-43]have 12 or 14 TMH and are structurally described by *E. coli* multidrug resistance transporter MdfA (PDB 4ZOW), which confers resistance to chloramphenicol.[44] MFS transporters are proton-driven and typically contain a 12TMH core consisting of two pseudo-symmetrical six-helix domains. A central cavity between these two domains forms the substrate-transport path and the protein undergoes a rocker-switch mechanism that involves cycling between outward-facing and inward-facing conformations. RND family proteins[45-49]are proton-driven and function in a tripartite assembly with an outer membrane protein and a periplasmic adapter protein that connects them together. Structural organisation in RND proteins is represented by AcrB from *E. coli* (PDB 2DRD),[50-52] which functions in complex with AcrA and TolC. Each AcrB protomer consists of a 12TMH

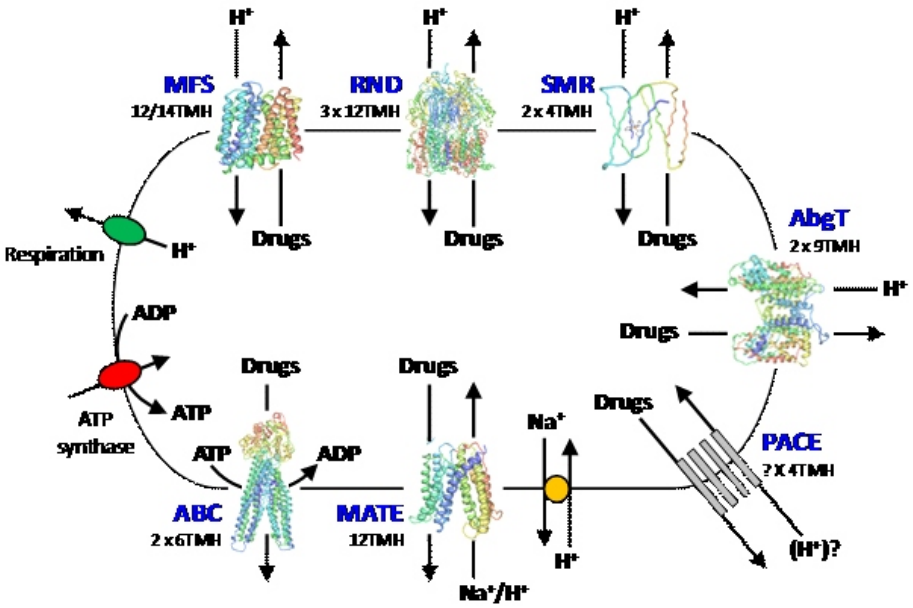

Fig. 1: Schematic illustration for the energisation and structural organisation of seven distinct families of multidrug efflux protein found in bacteria. The large oval represents a bacterial cell. An electrochemical H^+ gradient (proton-motive-force) across the cytoplasmic membrane is generated by respiration (*green*). Energy from the proton-motive-force drives secondary active transport proteins, such as those in the MFS, RND, SMR and AbgT (super)families. PACE family transporters are also likely to be driven by the H^+ gradient. Sodium/proton antiporters (*orange*) exploit the proton-motive-force to generate a Na^+ gradient that drives transport by other multidrug efflux proteins, including those in the MATE family. ATP production by ATP-synthase (*red*) is also driven by the proton-motive-force and ATP is used to drive transport by primary active transporters of the ABC superfamily. The structural organisation in each family is illustrated by a picture of a crystal structure of a representative protein as follows: ABC Sav1866 from *Staphylococcus aureus* (PDB 2HYD); MFS MdfA from *Escherichia coli* (PDB 4ZOW); RND AcrB from *Escherichia coli* (PDB 2DRD); SMR EmrE from *Escherichia coli* (PDB 3B5D); MATE NorM from *Vibrio cholerae* (PDB 3MKT); AbgT MtrF from *Neisseria gonorrhoeae* (PDB 4R1I); PACE no structure available;. Structures were drawn using the relevant PDB entry and PDB Workshop 3.9. The number of TMH based on structural characterisation is also given.

transmembrane domain and a headpiece that protrudes into the periplasm. SMR family proteinsare proton-driven and highly hydrophobic.[53-56] They have a homodimeric structural organisation (2 x 4TMH),described by EmrE from *E. coli* (PDB 3B5D),[57,58]in which a single membrane-embedded and highly conserved charged residue (Glu14 in TM1) is essential for transportactivity. MATE family proteins[59-61]are driven by either the sodium or proton gradient and their 12TMH structural organisation is represented by NorM from *Vibrio cholerae* (PDB 3MKT) or by DinF from *Bacillus halodurans*(PDB 4LZ6).[62-65] The two most recently identified types of bacterial multidrug efflux transporters are from the AbgT family, which confer resistance to sulfonamide antimetabolite drugs[66-67] and from the PACE family, which confer resistance to the bisbiguanide antiseptic chlorhexidine.[68-70] AbgT transporters are proton-driven and their homodimeric structural organisation(2 x 9TMH) has been described by crystal structures of YdaH from *Alcanivorax borkumensis* (PDB 4R0C) and MtrF from *Neisseria gonorrhoeae* (PDB 4R1I).[71-73] PACE family proteins are likely to be proton-driven and they putatively contain 4TMH that probably function as an oligomer. High-resolution structural information is yet to be obtained for any member of the PACE family.

Simultaneous expression of members from all seven families in the same organism is entirely feasible and a significant challenge for overcoming multidrug resistance.

ABUNDANCE AND MULTIPLE ROLES OF EFFLUX PROTEINS

Up to30% of all the genes in most sequenced genomes encode membrane proteins.[74-76] In prokaryotic genomes, up to 50% of these are transport proteins[77]and a large proportion of these encode efflux proteins, further reflecting their importance. Efflux proteins not only play a key role in drug resistance but also perform many other physiological functions in bacteria.[9,78-80]Efflux proteins are highly active in bacterial biofilms and they are involved in the formation and maintenance of biofilms.[81-87] For example, there is a potential role of the AdeFGH efflux pump in the synthesis and transport of autoinducer molecules during biofilm formation of *Acinetobacter baumannii*.[88] Related to biofilm formation, there is interdependence between efflux proteins and bacterial quorum sensing. Efflux proteins can transport out quorum sensing molecules that can induce expression of genes relating to processes such as biofilm formation and virulence.[89-93] For example, active efflux influences the potency of quorum sensing inhibitors in *Pseudomonas aeruginosa*.[94] Efflux proteins also contribute to heavy metal resistance in prokaryotes.[95,96] This is exemplified by the structurally characterised RND-type *E. coli* CusC(F)BA complex, which exports and confers resistance to copper(I) and silver(I) ions involving a methionine shuttle.[97-100] There is a developing understanding about co-selection of resistance

mechanisms in bacteria to antibiotics, biocides and metals that includes efflux proteins.[101-104] Other transport proteins capable of exporting metal ions also contribute to metal ion homeostasis in prokaryotes[105-114] and this includes members of the ABC, RND, CDF (cation diffusion facilitator),[115-117] MntX (transporter mediating manganese export)[118] and CorA (cobalt resistance protein A)[119-122]transporter families. Efflux proteins also have wider roles in bacterial pathogenicity and virulenceto humans, other animals and plants.[80, 123]

CONCLUSION

The high importance of prokaryotic efflux proteins, not least in the global problem of antibiotic resistance, makes them prime targets for drug development and this requires a rigorous ongoing characterisation and understanding of their structures, functions, molecular mechanisms and regulation using genetic approaches and by application ofchemical, biochemical, biophysical and computational techniques. Proper high-resolution three-dimensional structure elucidation(using X-ray crystallography, NMR spectroscopy, electron microscopy) in the first instance requires successful gene cloning and amplified expression of the target protein in *E. coli*,[124-132] followed by production of sufficient quantities of high quality membranes and/or stable and active purified protein. Such studiescontribute information that could lead to increased susceptibility to antibiotics (e.g. by inhibition of multidrug efflux pumps), development of new antibiotics or the reversal of bacterial resistance mechanisms.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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