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The antibiotic resistance ABC-F proteins: bringing target protection into the limelight

Liam K. Sharkey & Alex J. O'Neill*

Antimicrobial Research Centre and School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK

***Corresponding author. Mailing address: Antimicrobial Research Centre and School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds, LS2 9JT, United Kingdom. Phone +44 (0)113 343 5600, Fax +44 (0)113 343 5638, E-mail: a.j.oneill@leeds.ac.uk**

Members of the ATP-binding cassette (ABC)-F protein subfamily collectively mediate resistance to a broader range of clinically important antibiotic classes than any other group of resistance proteins, and are widespread in pathogenic bacteria. Following over 25 years' controversy regarding the mechanism by which these proteins work, it has recently been established that they provide antibiotic resistance through the previously recognised but underappreciated phenomenon of target protection; they bind to the ribosome to effect release of ribosome-targeted antibiotics, thereby rescuing the translation apparatus from antibiotic-mediated inhibition. Here we review the ABC-F resistance proteins with an emphasis on their mechanism of action, first exploring the history of the debate about how these proteins work and outlining our current state of knowledge, and then considering key questions to be addressed in understanding the molecular detail of their function.

Key words: protein synthesis inhibitors, ABC proteins, *lsa(A), msr(A), msr(D)*, *optrA, vga(A)*

Antibiotic resistance threatens to undermine our ability to treat bacterial infection, and therefore constitutes a global threat to human health [1]. Understanding the biology of this phenomenon – including the molecular mechanisms by which resistance is mediated - represents a vital facet of a comprehensive response to the problem. Aside from providing fundamental knowledge, elucidation of the mechanistic detail of antibiotic resistance offers useful strategic intelligence to guide rational improvement of existing antibiotic classes and/or the development of inhibitors of resistance mechanisms to rejuvenate the activity of antibacterial drugs whose clinical utility has become compromised by resistance. Our knowledge regarding the molecular mechanisms by which bacteria resist the effect of antibiotics is by now extensive, provided through nearly 80 years' of study; however, it is not yet comprehensive, and key gaps in our understanding remain.

This *Perspective* focusses on one such knowledge gap; a surprisingly large and long-standing gap given its clinical importance, and one that has only recently begun to be filled. Members of the ABC-F protein subfamily collectively mediate resistance to a broader range of antibacterial drug classes than any other single group of resistance proteins, and play a major role in clinically significant antibiotic resistance in pathogenic bacteria. Despite this, the fundamental way in which these proteins act to provide resistance was not clarified until 2016, at the conclusion of a debate that lasted over 25 years. As described below, it has now been established that proteins of this group mediate target protection, a category of resistance mechanism that had not previously been considered a leading cause of antibiotic resistance in the clinical setting. As the field now moves towards dissecting the molecular detail of this resistance mechanism, we review our current knowledge of these proteins with an emphasis on how they act to mediate antibiotic resistance, and reflect on why it took so long to gain this understanding. We also identify key unresolved questions that merit further investigation.

Nature of the ABC-F proteins and their role in antibiotic resistance

Members of the ATP-binding cassette (ABC) protein superfamily are found across all domains of life, with a considerable proportion of these proteins dedicated to energy-dependent transport of molecules across biological membranes [2]. This superfamily can be sub-divided into eight subfamilies (designated A-H), in line with a classification scheme originally derived from phylogenetic analysis of human ABC proteins [3]. Most of these subfamilies comprise canonical ABC transport proteins that consist of two ABC domains and two transmembrane domains (TMDs). However, members of the ABC-E and ABC-F subfamilies are not fused to

TMDs, nor are they genetically associated with TMDs in operons [3, 4]; instead, these proteins are composed of a single polypeptide that contains two ABC domains, separated by a linker of ~80 amino acids. Whilst only a single ABC-E protein is known (the eukaryotic protein, ABCE1), the ABC-F subfamily possesses a substantial membership that spans both eukaryotes and bacteria [3, 4]. To date, members of the ABC-F subfamily have been shown to participate in a variety of biological processes including DNA repair [5, 6], translational control [7-11] - and central to the present discussion, resistance to antibiotics that target bacterial protein synthesis [4].

ABC-F proteins that mediate antibiotic resistance (referred to hereafter as ARE [antibiotic resistance] ABC-F proteins) are widespread in Gram-positive bacteria, though have also been reported in Gram-negative species [12]. Members of this group provide self-protection in antibiotic-producing bacteria such as the streptomycetes [13, 14], as well as mediating both intrinsic [15, 16] and acquired [17-19] antibiotic resistance in medically important genera that include the staphylococci, streptococci, and enterococci (Figure 1). Collectively, the ARE ABC-F group confers resistance to the majority of antibacterial drug classes that target the large (50S) subunit of the bacterial ribosome, including the ketolides, lincosamides, macrolides, oxazolidinones, phenicols, pleuromutilins, and streptogramins of group A and B. No individual representative of the group confers resistance to all of these classes, however, and three phenotypic resistance profiles are currently distinguished in clinical isolates. Concurrent resistance to lincosamides, group A streptogramins (and sometimes pleuromutilins) is referred to as the LS_A (or LS_AP) phenotype and is conferred by *vga*-, *lsa*- and *sal*- type genes [15, 16, 18], combined resistance to macrolides, group B streptogramins (and sometimes ketolides) (MS_B phenotype) is provided by the *msr*-type determinants [17], and resistance to oxazolidinones and phenicols by the *optrA* gene [19](Figure 1).

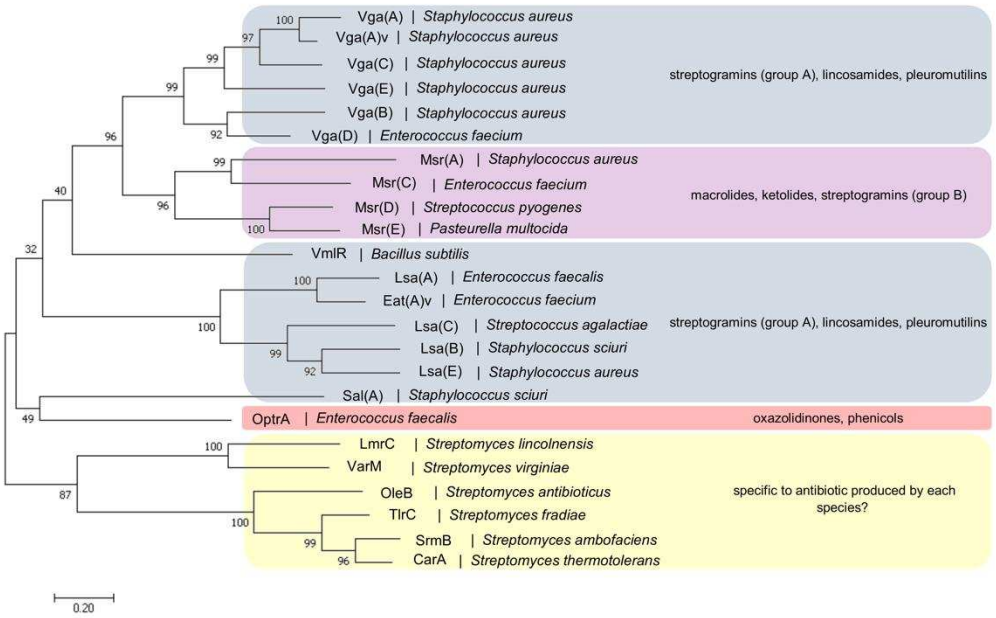

Figure 1. Phylogenetic tree and antibiotic resistance profiles of representative ARE ABC-F proteins found in pathogenic bacteria and antibiotic producer organisms. Antibiotic resistance phenotypes characteristic of the different sub-groups are shown at the right of the figure (though variations in individual resistance phenotypes within each subgroup are not). The tree was generated using the maximum likelihood method with the MEGA7 software package [20], with bootstrap values (500 replicates) indicated at nodes.

In view of the breadth of antibacterial drug classes to which determinants of the ABC-F subfamily confer resistance, and the nature of the bacteria in which they are found, it follows that they represent an important source of clinically-significant antibiotic resistance in Gram-positive pathogens. The *msr(D)* gene (also known as *mel*) is found in the majority of macrolide-resistant *Streptococcus pneumoniae* isolates in parts of the world, including North America and the UK [21]; this same determinant is also prevalent amongst macrolide-resistant *Streptococcus pyogenes* [22]. Whilst such strains also harbor an additional macrolide-resistance gene (*mef(A)/ mef(E)*), the *msr(D)* determinant appears to play the predominant role in conferring macrolide resistance (at least in *S. pyogenes*) [23]. In staphylococci, where ribosomal methylation is generally the most prevalent mechanism underlying clinical resistance to macrolide and streptogramin B antibiotics, *msr*-type determinants (particularly *msr(A)*) are nonetheless responsible for resistance in up to a third of strains exhibiting an MS_B phenotype [24, 25]. ABC-F determinants are also an important source of pleuromutilin resistance in staphylococci; Gentry *et al.* [26] found the closely-related *vga(A)* and *vga(A)v* genes to account

for all instances of resistance to retapamulin in nearly 6000 isolates tested. Amongst the enterococci, the *optrA* gene is a prevalent cause of oxazolidinone resistance in China (the country in which it was first detected) [27], and has recently been detected in North America and Europe [28-30]. Significantly, this gene, whose presence has also been reported in staphylococci [31], is to date the sole known horizontally-transmissible determinant capable of conferring resistance to tedizolid [19], a second generation oxazolidinone that was only approved by the FDA in 2014. OptrA is the most recent addition to the ARE ABC-F protein catalogue, first identified only 3 years ago [19], and it seems likely that further members of the ABC-F subfamily mediating clinically-relevant antibiotic resistance remain to be discovered. A recent phylogenetic analysis of ABC-F proteins encoded within sequenced genomes supports this idea, reporting as-yet uncharacterized bacterial ABC-F subfamily members that cluster with known groups of ARE ABC-F proteins [32].

The mechanism by which ABC-F proteins mediate antibiotic resistance – a history of controversy

In 2016, we demonstrated that the ARE ABC-F proteins mediate antibiotic resistance through target protection, a mechanism wherein a resistance protein physically associates with an antibiotic target to safeguard the function of the latter in the face of an antibiotic challenge. In other words, and as discussed further below, these proteins bind to the ribosome to effect release of ribosome-targeted antibiotics and thereby rescue the translation apparatus from inhibition [33]. That these proteins act in this manner was not a new idea, but represented the lesser favoured hypothesis in a long-standing debate over their mechanism. We consider it instructive to briefly review this controversy here, both to explain how an incorrect hypothesis emerged and came to prominence, but also to help avoid similar pitfalls in future studies seeking to characterize antibiotic resistance mechanisms.

The predominant hypothesis in the scientific literature had long been that the ARE ABC-F proteins effect antibiotic efflux, mediating resistance by exporting antibiotics out of the cell. That this remained a hypothesis – and indeed, the concept that there was any controversy over the mechanism of resistance – was not widely appreciated outside of the immediate field, and large swathes of the literature simply refer to these proteins as ABC transporters. The efflux hypothesis originated in the study that first characterized the *msr(A)* determinant in 1990 [17], and was built on two key observations. The first of these was that the rudimentary bioinformatic tools available at the time revealed homology between Msr(A) and the ABC

domains of transport proteins; since roles for ABC proteins outside the realm of transport had not been defined at that juncture, this similarity appeared to implicate efflux. Though Msr(A) and other ARE ABC-F proteins lack the TMDs that enable ABC transporters to translocate their substrates across biological membranes, it was reasoned that they might co-opt components of the native transport machinery to produce a complex competent for antibiotic efflux. The second observation – demonstrated in both the original work on Msr(A) and in subsequent studies/ with other ARE ABC-F group members [17, 34-36] – was that these proteins mediate an energy-dependent reduction in intracellular antibiotic accumulation, an effect that would at first glance appear to be synonymous with efflux.

However, viewed through the lens of biological knowledge acquired since these early studies, neither of these original observations provide even indirect support for efflux. Crucially, it has been demonstrated that such antibiotic accumulation experiments are unable to distinguish between a mechanism of resistance entailing efflux and one involving displacement of the antibiotic from the drug target. For example, Erm-type methylases that mediate resistance by modifying ribosomal RNA to reduce binding of macrolides to the ribosome also prompt a decrease in intracellular macrolide concentration [37, 38]. Similarly, in transport experiments using radiolabelled erythromycin, addition of an excess of unlabelled macrolide or streptogramin B antibiotic (to chase the radiolabelled drug from the ribosome) results in decreased intracellular drug accumulation [39]. The reason for this is as follows. In a bacterium not expressing an antibiotic resistance mechanism, an antibiotic will enter the cell and a proportion of this intracellular antibiotic fraction will become bound to its cognate target. By binding antibiotic, the drug target acts as a 'sink', shifting the equilibrium in free antibiotic concentration between the inside and the outside of the bacterium, and establishing a concentration gradient that promotes further antibiotic ingress [40]. Any factor that disrupts this sink – including a resistance protein that prevents binding of the antibiotic to the target or drives release from the target – will therefore serve to reduce the total intracellular antibiotic concentration. Consequently, reduced intracellular accumulation of an antibiotic cannot be taken as evidence of efflux, and caution is therefore urged for those employing similar accumulation experiments to define the mechanism of newly-discovered antibiotic resistance proteins.

Ribosomal protection by the ARE ABC-F proteins: circumstantial and direct evidence

Whilst the early studies on the mechanism of the ABC-F proteins emphasized the efflux hypothesis, there was nonetheless a recognition that these observations did not unequivocally point to antibiotic efflux. Indeed, the authors of the original study that explored the mechanism of Msr(A) concluded their paper with the comment that "...the involvement of ribosomal binding in the MS (MS_B) resistance phenotype has not been ruled out." [17], and in a later review reiterated that the efflux hypothesis "…should not be etched in tablets of stone just yet." [39]. In spite of their understandable caution, the case for efflux appeared sufficiently strong to convince the majority, and subsequently became widely accepted.

However, in recent years a body of circumstantial but compelling evidence began to amass suggesting that the ABC-F proteins more likely provide antibiotic resistance through a mechanism involving ribosomal protection. First, through an improved understanding of the ABC protein superfamily and a dramatic expansion in the size of the protein databases, it became evident that Msr(A) and other ARE ABC-F proteins in fact exhibit the greatest degree of similarity not to ABC transporters, but to other ABC proteins that bind the ribosome to regulate translation [4, 9, 10, 41]. Second, as a structural understanding of antibiotic binding to the ribosome emerged, and as new ABC-F resistance determinants were identified and characterized, it became apparent that the antibiotic classes to which a given ARE ABC-F protein mediates resistance have overlapping binding sites on the 50S ribosomal subunit [42] (Figure 2). Proteins belonging to the Vga- and Lsa- subgroups yield resistance to antibiotics that overlap both the A-site and P-site of the ribosome (e.g. group A streptogramins and pleuromutilins) or the A-site/ entrance to the peptide exit tunnel (lincosamides) (Figure 2B). In contrast, Msr-type proteins mediate resistance to 14- and 15-membered macrolides, ketolides and group B streptogramins, all of which bind to overlapping sites in the nascent polypeptide exit tunnel (Figure 2C). OptrA gives resistance to oxazolidinones and phenicols, which share an overlapping binding site at the ribosomal A-site (Figure 2D). That overlap in binding site on the ribosome determines which antibiotic classes a given ARE ABC-F protein mediates resistance to - rather than common chemical composition as might be anticipated for an efflux-based mechanism of resistance – is strongly suggestive of a resistance mechanism operating at the drug target to protect it from antibiotics.

Direct evidence for ribosomal protection by these proteins has now been obtained [33]. This was achieved in the first instance by examining the ability of the ARE ABC-F proteins to rescue

translation from antibiotic-mediated inhibition *in vitro* using a staphylococcal transcription/translation (TT) assay. Two phylogenetically distinct ABC-F proteins (Lsa(A) and Vga(A); Figure 1) were purified and tested in this system, and both were found to provide dose-dependent protection of translation specifically against antibiotics that fall within the resistance spectrum of these respective proteins [33]. To confirm that these *in vitro* results were representative of the resistance mechanism as it occurs in whole cells, several key phenotypic properties of these proteins were subsequently recapitulated within the TT assay. Thus, we demonstrated that (i) Vga(A) requires two functional ABC domains to protect translation, (ii) mutations in the inter-domain linker of Vga(A) alter specificity in respect of antibiotic resistance profile, and (iii) neither Vga(A) nor Lsa(A) is able to protect the *Escherichia coli* translation apparatus from antibiotic-mediated inhibition [33]. Subsequently, we conducted a binding study using staphylococcal ribosomes and radiolabelled lincomycin, demonstrating that Lsa(A) is capable of displacing pre-bound lincomycin from the ribosome [33]. Collectively, these results - achieved under *in vitro* conditions where transport cannot occur - provided clear evidence that ABC-F proteins do indeed mediate antibiotic resistance through ribosomal protection.

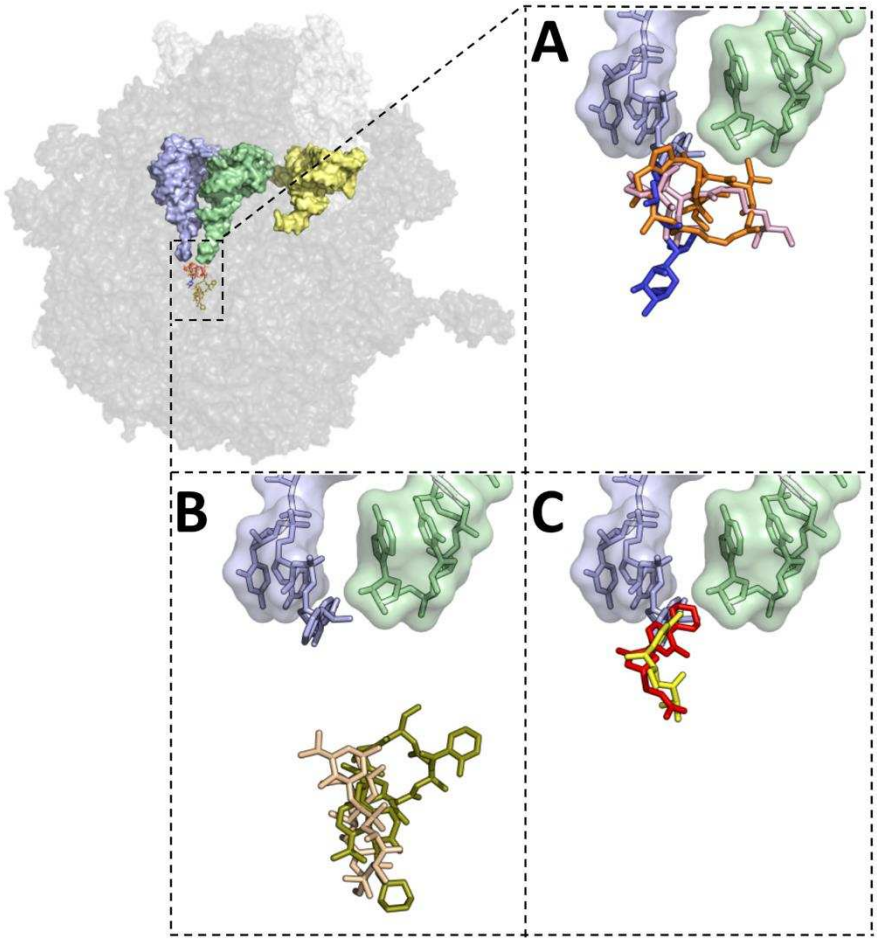

Figure 2. Overlap in binding site on the 50S subunit of the ribosome determines which antibiotic classes a given ARE ABC-F protein mediates resistance to. The *Thermus thermophilus* ribosome is shown with tRNA occupying the A-site (blue), P-site (green) and E-site (yellow) (co-ordinates from PDB 4V5C [43]), with boxes A, B and C providing a magnified view of antibiotics binding at the peptidyl-transferase centre and peptide exit tunnel. **(A)** Binding sites of antibiotics to which Vga-type and Lsa-type ARE ABC-F proteins mediate resistance; dalfopristin (streptogramin A, orange, PDB 1SM1 [44]) retapamulin (pleuromutilin, pink, PDB 2OGO [45]), and clindamycin (lincosamide, blue, PDB 4V7V [46]). **(B)** Binding sites of antibiotics to which Msr-type proteins mediate resistance; erythromycin (14-membered macrolide, salmon, PDB 4V7U [46]) and quinupristin (streptogramin B, mustard, PDB 1SM1 [44]). **(C)** Binding sites of antibiotics to which OptrA mediates resistance; chloramphenicol (phenicol, yellow, PDB 4V7T [46]) and linezolid (oxazolidinone, red, PDB 3DLL [47]).

A model for ABC-F mediated antibiotic resistance

Having established that ARE ABC-F proteins directly protect the bacterial ribosome from antibiotics, the next question is *how*? A compelling hypothesis regarding the molecular mechanism of protection, first articulated by Lenart and colleagues [41] prior to our demonstration of ribosomal protection, derives from the relatively recent characterization of a bacterial ABC-F protein that has a role outside of antibiotic resistance. EttA (energy-dependent translational throttle A) associates with the *E. coli* ribosome to restrict translation activity in response to reduced cellular ATP levels, with structural studies revealing that EttA binds into the E-(exit) site to modulate the conformation of the peptidyl transferase centre (Figure 3) [9, 10]. EttA achieves this via an inter-domain linker region (Figure 3) designated the P-site tRNA interaction motif. In the majority of ARE ABC-Fs, the inter-domain linker is extended by approximately 30 amino acids relative to EttA [41]. Based on this, a model has been proposed in which ARE ABC-F proteins sense the vacant E-site of antibiotic-stalled ribosomes and bind in a similar mode to EttA; the linker of these proteins acts in an analogous fashion to that of EttA, but the extended ARE ABC-F linker affords deeper penetration towards the peptidyltransferase centre, where it can prompt dissociation of bound antibiotic either via direct steric displacement of the drug, or through prompting allosteric conformational change in the drug binding sites (Figure 3). Since the presence of tRNA in the P-site would appear to prevent direct access of an ARE ABC-F protein located in the E-site to a bound antibiotic molecule at the peptidyltransferase centre/ peptide exit tunnel [48], the latter option – involving an allosteric modulation of the drug binding site resulting from interaction with rRNA and/or P-site tRNA to effect antibiotic release – seems the more plausible. In potential support of this overall model, it has been demonstrated that the inter-domain linker of ABC-F proteins plays a crucial role in mediating resistance, with mutagenesis of this region of Vga(A) leading to alterations in the spectrum of antibiotics against which the protein provides protection [41].

This model implies parallels between the mechanism of the ARE ABC-F proteins and that of the canonical example of target protection provided by the tetracycline ribosomal protection proteins (TRPPs), such as Tet(M). Like the ARE ABC-F proteins, the TRPPs share homology with translation factors (EF-G and EF-Tu [49]). This homology underlies the ability of the TRPPs to bind the ribosome at the same site recognised by these elongation factors [50, 51], an event that serves to chase tetracycline from its binding site and thereby to protect the ribosome from antibiotic-mediated inhibition.

Whilst we consider that the EttA-type model currently represents the most plausible explanation for the action of the ARE ABC-F proteins, it raises questions as to how their interaction with the ribosome is coordinated to ensure efficient protein synthesis in the presence of antibiotic. Since translation cannot occur with an ABC-F protein bound permanently into the E-site of the ribosome, it would imply that a repeated cycle of ABC-F-mediated antibiotic release - each cycle entailing association/dissociation of the ABC-F protein and the ribosome - would be required to achieve translation of an entire protein. Within a given cycle, it is not obvious what would prevent an antibiotic from immediately rebinding the ribosome upon dissociation of an ABC-F protein [48]. For the TRRPs, it has been proposed that antibiotic rebinding might be prevented as a result of a TRRP-induced conformational change in the ribosomal RNA that persists following dissociation of the resistance protein [52-55]; a similar mechanism may be at play in ABC-F mediated antibiotic resistance.

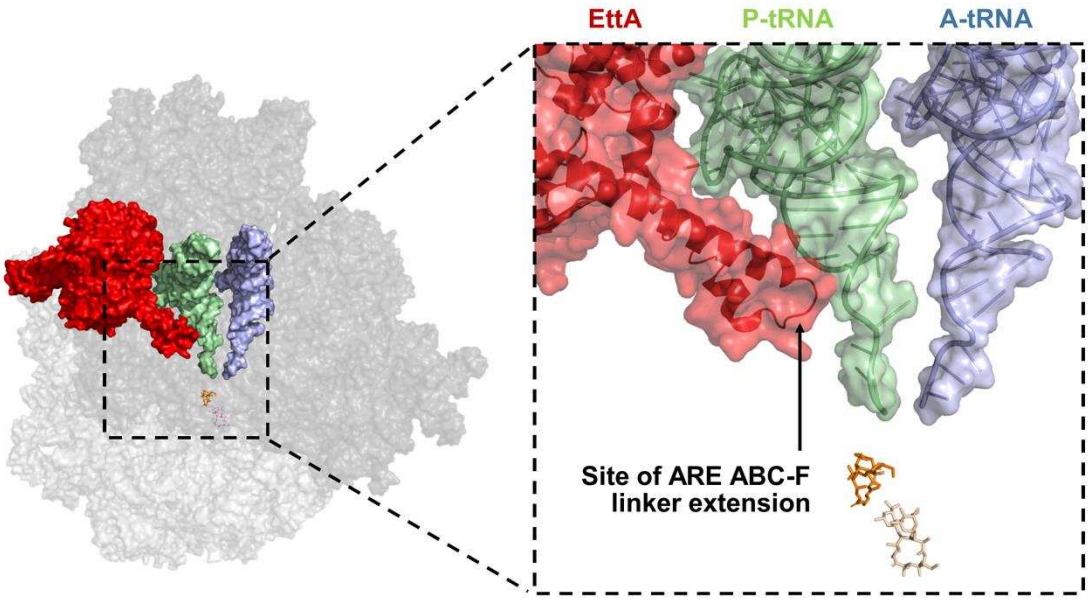

Figure 3. Interaction of EttA with the ribosome offers a potential basis for understanding the molecular mechanism of action of the ARE ABC-F proteins. EttA (red) binds at the E-site of the 70S ribosome, where its inter-domain linker is inserted towards the peptidyltransferase centre and makes contacts with the acceptor arm of the P-site tRNA (PDB 3J5S [10] and 4V6G [56]). The majority of ARE ABC-F proteins possess an extended inter-domain linker that should afford deeper penetration towards the antibiotic binding sites in the peptidyltransferase centre and peptide exit-tunnel; the binding sites of the macrolide, erythromycin (light pink, PDB 4V7U [46]) and the group A streptogramin, dalfopristin (orange, PDB 4U24 [57]) are shown. Figure based on that of Lenart *et al.*[41] with permission from the American Society for Microbiology.

Towards an improved mechanistic understanding of the ARE ABC-F proteins

As will likely be apparent from the preceding discussion, a crucial step in elucidating the molecular mechanism of these resistance proteins will be to achieve structural characterization of representatives of this group bound to the ribosome. Such information would not only establish the site and mode of binding of these proteins on the drug target, but would also shed light on whether resistance indeed occurs via allosterically-induced conformational change of the drug binding site. Moreover, it should reveal the basis for the apparent specificity of many of these proteins for the Gram-positive ribosome; Msr(A), Vga(A) and Lsa(A) have all been shown incapable of protecting *E. coli* ribosomes from antibiotic-mediated inhibition [17, 33].

Structural studies of this sort will require the isolation of a stable ARE ABC-F•ribosome complex. To achieve structural characterization of the non-ARE protein EttA in complex with

the ribosome, Chen *et al.* [10] utilised an engineered mutant protein, EttA-EQ_2, that is able to bind - but not hydrolyse – ATP; since release of EttA from the ribosome is contingent on ATP hydrolysis, EttA-EQ_2 forms a stable complex with the ribosome. Isolation of this EttA-EQ_2•ribosome complex was achieved by virtue of a polyhistidine tag engineered onto the former, enabling an "*in-vivo* pull-down" of the complex directly from *E. coli* cell lysates via metal affinity chromatography [10]. In our hands, analogous pull-down experiments using EQ_2 mutants of Vga(A) and Lsa(A) expressed in *Staphylococcus aureus* have to date not proven successful in recovering ARE ABC-F•ribosome complexes (*data not shown*). Perhaps then, an alternative approach analogous to that used to study the TRPPs will be required [58, 59], involving the use of non-hydrolysable nucleotide analogues to trap the ARE ABC-F proteins in their ribosome-bound configurations and enable *in vitro* reconstitution of the complex.

However, these approaches assume that ATP hydrolysis is required to effect release of ARE ABC-F proteins from the ribosome, an assumption that has not been verified. Indeed, the role played by ATP (and potentially, other nucleotide triphosphates) in the mechanism of these proteins, and how that role varies across the ARE ABC-F group, is another area that will require experimental dissection. The essential nature of both ABC systems for activity in proteins such as Vga(A) – in particular, the observation that inactivation of a single Walker B domain abrogates resistance [60]- implies a requirement for ATP hydrolysis in the resistance mechanism. This idea is corroborated by the observation in antibiotic accumulation experiments that the resistance phenotype associated with Vga(A) and Msr(A) is energy-dependent [17, 34-36]. As discussed by Wilson [48] in his commentary on our original paper [33], it seems reasonable to suppose that these proteins utilise ATP either to drive release of antibiotic from the ribosome, or to effect their own subsequent dissociation from the ribosome. However, not all ARE ABC-F proteins appear to have a strict requirement for functional ATPase domains, pointing to differences in the role that ATP plays in the mechanism of different representatives of the group. For example, deletion of either the N- or C-terminal ABC systems of the Ole(B) protein is associated with only modest reduction in oleandomycin resistance [61], indicating that this resistance mechanism is functional in the presence of a single ABC domain. More striking is the report that macrolide resistance mediated by the LmrC protein appears to occur independently of the protein's ability to even bind ATP; replacement of the critical lysine residues within both of the Walker A motifs of LmrC had no significant impact on its ability to mediate tylosin resistance [62]. Clearly, future studies to understand the molecular detail of

these resistance proteins will need to proceed alive to this apparent lack of mechanistic homogeneity across the ARE ABC-F group.

In the final stages of compiling this article, Murina *et al*. reported a detailed survey of ABC-F subfamily members across all species for which genomes have been sequenced [32]. This study distinguished seven groups of ARE ABC-Fs, and revealed the lack of a clear sequence signature for ARE versus non-ARE ABC-F proteins. These observations may suggest that antibiotic resistance has arisen on several occasions amongst the ABC-F subfamily, and in multiple ways, which underscores the comments above regarding a potential lack of uniformity in the detail of their molecular mechanisms. It also highlights the question: to what extent have the ARE ABC-F proteins evolved specifically to provide resistance to antibiotics? In the case of the ARE ABC-F proteins that provide self-protection in antibiotic-producing bacteria, it seems reasonable to suppose that their evolved cellular role is to protect the translation machinery from antibiotics. However, for ARE ABC-F determinants of the kind found in bacterial pathogens, the ability to confer resistance to antibiotics – though a function that will undoubtedly be driving their selection now - may well be coincidental to their primary evolved function as translation factors. This is particularly the case for ARE ABC-F genes that are an intrinsic component of a particular species (e.g. *lsa(A)* in enterococci [8]); intuitively, it seems more plausible that their *raison d'etre* in this setting is to provide additional functionality to the translation machinery rather than to offer intrinsic resistance to antibiotics that the species is unlikely to encounter in its natural habitat. Thus, part of achieving a comprehensive mechanistic understanding of the ARE ABC-F proteins will include an interrogation of their cellular function(s) aside from antibiotic resistance.

Concluding remarks

Target protection as a mechanism of antibiotic resistance was first described in the context of tetracycline resistance nearly three decades ago. Despite the subsequent demonstration that this phenomenon also underlies resistance to fusidic acid (via the FusB-type proteins [63, 64]) and reduced susceptibility to the fluoroquinolones (via Qnr [65]), this mechanistic paradigm has nevertheless remained an unusual foot-note alongside the better-known means through which bacteria resist the effect of antibiotics (e.g. enzymatic inactivation or efflux of antibiotic, permanent physical alteration of the antibiotic target). The recent demonstration that ARE ABC-F proteins act to protect the ribosome against a broad swathe of antibacterial drugs in important bacterial pathogens now reveals target protection as a key mechanistic player in antibiotic resistance in the clinic. Further study of the ARE ABC-F proteins along the lines

described here will not only shed light on the specific mechanism of these proteins and evolve our understanding of the broader paradigm of target protection, but also offers the potential to inform structure-guided design of small molecules to evade or inhibit the action of these resistance proteins.

Author information

Corresponding author

*E-mail: a.j.oneill@leeds.ac.uk

ORCID

Liam Sharkey: 0000-0001-9324-8678

Alex O'Neill: 0000-0003-3222-5493

Conflict of Interest

None to declare.

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