Infectious Diseases Diagnosis & Treatment

Saha C, et al. Infect Dis Diag Treat 7: 225.

www.doi.org/ 10.29011/2577- 1515.100225

www.gavinpublishers.com

OPEN ACCESS

GAVIN PUBLISHERS

Review Article

# Deciphering the Structural and Functional Properties of ABC-F ATPases

**Chiranjeet Saha[1†], Sujata Saha[2†], Asmita Chakraborty[2], Anjaneyulu Dirisala[3], Amit Ranjan Maity[1], Priyanka Bhowmik[4], Kunal Sikder[2], Soumyananda Chakraborti[5*], Arnab Basu[2*]**

[1]Amity Institute of Biotechnology, Amity University Kolkata, Kolkata, 700135, India

[2]JIVAN Department of Biomedical Science and Technology, Ramakrishna Mission Vivekananda Educational and Research Institute, Howrah, West Bengal-711202, India

[3]Innovation Center of NanoMedicine (iCONM), Kawasaki Institute of Industrial Promotion, Kawasaki 210-0821, Japan

[4]Department of Biological Sciences, School of Life Science & Biotechnology, Adamas University, Kolkata, West Bengal-700126, India

[5]National Institute of Malaria Research, Sector 8, Dwarka, New Delhi-110077, India

***Corresponding authors:** Soumyananda Chakraborti, National Institute of Malaria Research, Sector 8, Dwarka, New Delhi-110077, India.

Arnab Basu, JIVAN Department of Biomedical Science and Technology, Ramakrishna Mission Vivekananda Educational and Research Institute, Howrah, West Bengal-711202, India

**Citation:** Saha C, Saha S, Chakraborty A, Dirisala A, Maity AR, et al., (2023) Deciphering the Structural and Functional Properties of ABC-F ATPases. Infect Dis Diag Treat 7: 225. DOI: 10.29011/2577-1515.100225

**Received Date:** 17 July 2023; **Accepted Date:** 26 July 2023; **Published Date:** 31 July 2023

## Abstract

**Background:** The adenosine triphosphate (ATP)-binding cassette (ABC) superfamily of proteins belong to the group of ATP hydrolyzing enzymes (ATPases). They are involved in active transport with the help of their trans-membrane domains (TMDs). However, not all ABC ATPases are engaged in the membrane transport, for e.g., ABC-E and ABC-F ATPases lack TMDs, and they don't participate in the active transport. Interestingly, ABC-F ATPases are associated with ribosome and this family of protein protects ribosome in different living systems. Most importantly, bacterial ABCF ATPases confer resistance against various classes of antibiotics that act on the ribosome during the process of protein synthesis. **Main body:** Structurally, ABC-F protein carries dual nucleotide binding domain (ABC1 and ABC2), and domains are connected by a flexible linker (P-site tRNA interaction motif). ABC-F docks ribosome on the E site, and binding of ABC-F protein to ribosome triggers conformational changes in 50S ribosome and this conformational change is essential for smooth occurrence of translation. This mechanism of antibiotic resistance is exploited by different pathogenic bacteria as a part of their stress response mechanism. **Conclusion:** Our *in-silico* analysis reveals a strong yet dynamic interaction between the Energy-dependent Translational Throttle A (EttA, a member of ABC-F), and L1 ribosomal protein (largest protein found in the large ribosomal subunit), pivotal for the interaction with ribosome. Hence this review is important for understanding the structure-function relationship of the conserved ATPase and the general mechanism of antibiotic resistance in various domains of life.

**List of abbreviations:**

ATP: adenosine triphosphate; ABC: ATP-binding cassette; ATPases: ATP hydrolyzing enzymes; TMDs: trans-membrane domains; EttA: energy-dependent translational throttle A; ESKAPE: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter spp*.; NBD: nucleotide-binding domain; PTC: peptidyl transferase center; GCN: General control non-repressible; ARE: antibiotic resistance; E-site: ribosome exit site; NPET: nascent polypeptide exit tunnel; P-site: ribosome peptidyl-tRNA-binding site; PtIM: P-site tRNA interaction motif; CTD: C-terminal; RbbA: ribosome-bound ATPase; PRE: pre-translocation; A-site: aminoacyl-site; MS: macro-states; MD: molecular dynamics; $tRNA_i^{Met}$: methionyl-tRNA; ORF: open reading frame; hABCF: human ABCF; DEGs: differentially expressed genes; BGC: biosynthetic gene cluster; RPP: ribosome protection proteins; ARD: ARE domain; PLSA: pleuromutilin, lincosamide, and streptogramin A; LPS: lipopolysaccharide; AMP: antimicrobial peptides.

## Background

The proper functioning of a cell is often determined by its ability to perform protein translation under various physiological conditions. However, the detail molecular mechanism of protein translation is still elusive. Protein translation is a complex event and different ribosome-interacting proteins have been found responsible for the event [1, 2]. One of the key regulator of protein translation is adenosine triphosphate (ATP)-binding cassette (ABC)-F class of ATP hydrolyzing enzymes (ATPases) that directly influence in the structure-function regulation of ribosomes [3]. Interestingly, the entire cluster of ABC-F ATPase protein found functioning as a translation factor and represents the most prevalent family of soluble proteins within the ABC superfamily [4].

The ABC class of proteins are major drivers of energetic events in cellular systems. They function by hydrolyzing ATP into Adenosine diphosphate (ADP) and an inorganic phosphate (Pi) molecule. The naming of proteins within this family follows the nomenclature of human proteins, and therefore, they have been named alphabetically from ABC-A to ABC-H [3]. Structurally, ABC ATPases carries a pair of trans-membrane domains (TMD) coupled with a pair of ATP-binding domains known as nucleotide-binding domains (NBDs). Binding of ATP leads to NBD dimerization. Moreover, they provide energy for transporting hydrophobic materials across the membrane and various other functions in different classes [5]. The NBDs contain characteristic motifs (Walker A and B) homologous to those in the F1 ATPase and AAA+ ATPases found in different systems [6]. The Walker motifs are separated by approximately 90–120 amino acids. A signature sequence 'LSGGQ/E' or the 'C motif' is seen in between two walker motifs [4]. The unique structure of ABC-F proteins helps them to modulate diverse cellular processes ranging from DNA repair to protein translation [4].

The ABC-F proteins are widely distributed in bacteria and eukaryotes; however, they are absent in almost all archaea [3]. Earlier investigation showed that the ABC-F class of ATPases is involved in translational regulation by ATP-dependent conformational modulation of the peptidyl transferase center (PTC) in the ribosome [3]. In *Escherichia coli,* EttA acts as a ribosome translation factor [7]. The nearest eukaryotic homolog of EttA is eukaryotic elongation factor 3 (eEF3). The eEF3 is found in *Saccharomyces cerevisiae* and it functions in the translation elongation process [8]. Although there are various mechanisms of antibiotic resistance seen in bacteria, ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*) group of organisms frequently use ABC-F ATPases as a potential tool to alleviate antibiotic stress [4]. These pathogens utilize their ABC-F ATPases to nullify the action of 50S ribosomal subunit targeting drugs and peptides (e.g., ketolides, lincosamides, macrolides, oxazolidinones, phenicols, pleuromutilins, and streptogramins A/B) [3]. The gram positive *Staphylococcus aureus* and gram negative *Pseudomonas aeruginosa* use VgaA and MsrE respectively to confer antibiotics resistance [9, 10]. VgaA binds to tRNA on the E site protecting the PTC in an ATP dependent manner [10, 11]. MsrE binds to the E site protruding into the nascent polypeptide exit tunnel (NPET) [9]. To protect protein synthesis during antibiotic stress these ABC-F proteins induce conformational changes in ribosome [12, 13]. The objective of this review is to analyze the structure and function of bacterial ABC-F ATPases in minute details for better understanding their role physiology and stress response in various bacterial systems.

## Structure of ABC-F ATPases

A typical ABC ATPase is comprised of four domains, i.e., two TMDs and two NBDs (Supplementary Figure 1) [14]. The ABC transporters are assembled from 'half transporters' with either identical (homodimeric) or different (heterodimeric) halves [15]. In the case of the ABC-Fs, there is no TMD; therefore, this class of ATPases does not localise on the membrane and they are nor responsible for transport phenomena [3].

### Properties of NBDs

Among different domains of ABCF ATPase, NBDs are essential for ATP hydrolysis activities. In an earlier study, *Stauffacher* et al. described the structural features of NBD domain. According to them, NBDs can be further divided into N and C terminal [15]. In general, bacterial ABCF ATPase carries

two NBDs in its structure [15]. Sequence analyses of various prokaryotes and eukaryotes have shown a high percentage of sequence identity between two NBD domains; suggesting conserved functional activities across evolution. Detail inspection of the NBD domain of ABC-F ATPase revealed five highly conserved region. One of these regions is a phosphate-binding loop (P-loop) or Walker A motif which is responsible for binding of phosphates group of ATP/GTP. The motif carries a consensus sequence of GxxGxGKT/S (where G stands for glycine, K for lysine, T for threonine, S for serine and x representing any amino acid). The lysine plays a crucial role in nucleotide binding through electrostatic interaction [16, 17]. The glycine-rich Walker A motif is preceded by a β-strand and followed by an α-helix. Another region of interest is the Walker B motif that forms a β-strand has a consensus sequence of R/K-x-x-x-G-x-x-x-L-hydrophobic-hydrophobic-hydrophobic-hydrophobic-DE where R, K, G, L, D, and E denote arginine, lysine, glycine, and leucine followed by aspartate and glutamate residues, and x represents any amino acid. It coordinates and stabilizes $Mg^{2+}$ by providing a carboxylate residue [16]. Sir John Ernest Walker discovered Walker A and Walker B motif in ATP-binding proteins and same motif is also presented in many GTP-binding proteins and a variety of proteins with phosphorylated substrates [16]. Walker A motif found to be more conserved as compared to the Walker B across evolution. Another characteristic motif known as signature motif carries the consensus sequence of LSGGQ/E (where L, S, G, Q, and E stand for leucine, serine, glycine, glutamine, and glutamate). This motif is most likely responsible for γ phosphate sensing [18]. Apart from Walker and Signature motif, ABCF structure also carries Q loop (glutamine residue rich-loop) or 'lid' region this loop is also important for ATP binding through a water molecule [18]. Both the Q loop and the signature motif are found in the helical domain. ABCF structure also possesses a histidine rich loop, also known as the 'switch region' and found responsible for polarizing the attacking water molecule for hydrolysis [4].

## P-site tRNA interaction motif (PtIM) linker domain in ABC-F ATPase

ABC transporters are one of the largest and possibly one of the oldest gene families that are extensively distributed throughout all kingdom of life. Within ABC transporter family, InterPro database has reported over approximately 3 million sequences of ATP-binding domain (IPR003439) that are distributed across 52,000 species. An extension to this is the linker domain that joins the two NBD domains of ABC-F ATPase. This linker domain is known as ABC_tran_Xtn (IPR032781) and PtIM. The InterPro has reported over 124,000 sequences for PtIM that belong to more than 41,000 species. The PtIM motif is a 60-to-100 residue long stretch of polypeptide chain (Figure 1) that connects NBD1 and NBD2, also known as ABC1 and ABC2 i.e., the two subunits of ABC-F ATPase. It comprises of an α-helical hairpin structure with an inter-helical loop forming a 'Tip' of varying length as seen in various structures. The PtIM motif is a highly conserved and defining feature of the ABC-F protein family. This α-helical hairpin directly binds with the tRNA in the ribosome P-site [19]. ABCF1, a mammalian ABC ATPase that lacks TMDs but possesses two NBDs is one of the few mammalian ABC proteins whose crystal structure has been studied. The crystal structure of the ATP-ABCF1 complex (PDB ID 5ZXD) unveils the dependence of NBDs on ATPase activity essential for ribosome binding [20]. ABCF1, NBD1, and NBD2 are connected by the PtIM region and are alternately catalytic, i.e., have ATP-binding residues at different positions. ATP binding leads to large-scale conformational changes in the ABCF and as an outcome the mean distance between NBD1 and NBD2 reduced significantly, and structure become more compact. In the next step, ABCF1-ATP complex binds the ribosome using Arm region of ABCF. This binding trigger other conformational changes resulting in ATP hydrolysis. Interestingly, both NBD1 and NBD2 returns to their original conformation after releasing ATP [20].

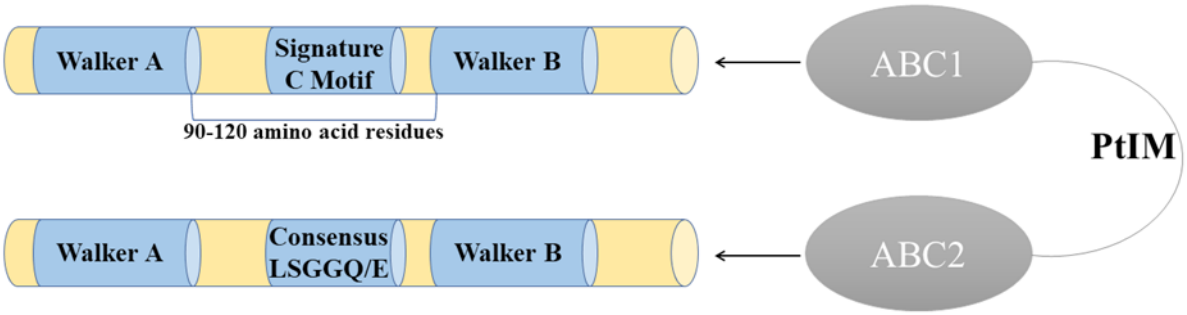


**Figure 1:** Adenosine triphosphate (ATP)-binding cassette F (ABC-F) protein.

Schematic showing two nucleotide-binding domains ABC1 and ABC2 of ABC-ATPase (marked in grey) along with Walker A and B motifs (in blue), the motifs A and B are separated by 90-120 amino acid residues. The signature C motif is positioned upstream of the Walker B motif. The domains are connected by a flexible α-helical hinge region P-site tRNA interaction motif (PtIM).

**Arm motif and C-terminal domain (CTD)**

Among different structural features presented withing ABCF, the Arm motif located at the C-terminal is very important for ribosome binding. Structurally Arm motif is an α-helical hairpin that contacts the L1 stalk in the 50S ribosomal subunit [7, 21]. Another structural feature specific to ABC-Fs is the C-terminal domain or CTD which was first seen in *E. coli* ABC-F ATPase Uup. This domain is annotated as ABC_tran_C (IPR032524) in the InterPro database (https://www.ebi.ac.uk/interpro/). The CTD has a coiled-coil structure with an unusual 3-10 helix in the α-hairpin region [22]. Its function is associated with DNA and RNA binding.

**ATPase-ribosome interaction**

ATPase and GTPases are indispensable factors for protein synthesis. During the elongation step, EF-Tu; a GTPase recruits activated aminoacyl-tRNA to the aminoacyl (A)-site, being conjugated with a ribosome-bound ATPase (RbbA). The association of RbbA results in fruitful recruitment of the aminoacyl-tRNA to the ribosomal A-site [23]. The subsequent activity of peptidyl transferase causes peptide bond formation in the A-site of the ribosome. RbbA from *Escherichia coli* displays intrinsic ATPase activity during translation and is triggered by 70S ribosomal subunit. The enhancement of the ATPase activity has a key role to play in protein-chain elongation and the release of deacyl-tRNA from ribosomes after peptide linkage synthesis takes place [24]. Studies on RbbA ATPase report the presence of Walker A and B motifs (similar to most ABC transporters), one RNA-binding motif, and six trans-membrane α-helices of specific function. Interestingly, RbbA binds directly to the ribosomal E-site and its hydrolysis activity is ATP-dependent [24].

Recycling of ribosome is one of the important steps in translation that plays pivotal role in cellular homeostasis. Prokaryotic recycling requires the presence of GTPase EF-G, that acts as a ribosome-recycling factor in bacteria. In contrast to that, the recycling of ribosome in eukaryotic and archaeal systems require ABC class of proteins such as ABCE1 [25-27]. The ABCE1 mediates the dissociation of 70S ribosomes in an ATP-dependent manner. The NBD1 and NBD2 domains of ABCE1 undergo a tweezer-like motion showing open and closed conformation in ATP-bound and ATP-hydrolyzed state respectively [28]. The ATP hydrolysis is shown to be the driving force of disassembly of 60S and 40S ribosomal subunits mediated by conformational changes [25].

ATP binding and hydrolysis are instrumental for the ABC-F-ribosome interaction, leading to structural changes in the peptidyl transferase center (PTC). Ribosome-bound EttA-$EQ_2$ is an ATPase-deficient EttA variant generated by simultaneous mutation of both glutamate residues to glutamine that results in an ATP-bound state of EttA. Cryo-EM studies have revealed the direct contact of ABC domains to the ribosomal E-site in an ATP-bound state shielding a tRNA in the adjacent P-site [21]. Other studies with different ABC-Fs (such as EttA, VmlR, and MsrE) have revealed that the PtIM region bind to the similar locations and orientations targeting the PTC on 23S rRNA of the 50S ribosomal subunit despite having a lack of sequence conservation [9,21,29]. These small but significant changes in local packing, interactions and binding geometry cause a drastic effect on the global conformation of the 70S ribosome.

## Functional significance of different ABC-F ATPases

Different ABC-F ATPases coordinate diverse functions across different domains of life by regulating protein translation

and ribosome protection. Some of their activities are explored below.

**Maneuvering protein translation**

The versatile *E. coli* ABC-F protein EttA, has been shown to 'throttle' the transition between elongation and earlier initiation stage, reported in various observation. Primarily EttA functions as a translational regulatory factor and is sensitive to the intercellular ATP/ADP ratio that controls its function [3]. The ATPase preferentially binds to the 70S ribosome initiation complex controlling the synthesis of the first peptide bond in the nascent peptide. Previous studies have confirmed that the EttA inhibits the synthesis of the first peptide bond in the reporter peptide in presence of increased concentration of ADP, whereas the addition of ATP reinstates its synthesis [7]. Thus, EttA controls the chain elongation during initial phase of polypeptide synthesis according to the environmental condition. Moreover, this conserved ATPase plays critical role in the overall elongation of the protein by modulating pre-translocation (PRE) complex function. The ribosomal pre-translocation (PRE) complex is required during translocation of the bound mRNA and the cognate tRNAs to the E- and P-sites respectively, from their earlier position in P- and A sites [21]. Cryo-EM studies have revealed that the PRE complex exists in a spontaneous equilibrium between two global conformational states (MS-I and MS-II) and plays a crucial role in the final step of translocation [21]. Chen et al. demonstrated that in an ATP-bound state, EttA-$EQ_2$ binds to the ribosomal E-site, and kinetically traps the PRE complex in the MS-I state; thereby modulating the ribosome and tRNA dynamics required for polypeptide elongation [21]. The mechanism of the action of EttA is depicted in (Figure 2). The crystal structure of ribosome-bound EttA (PDB ID 3J5S) is shown at a resolution of 7.50 Å [21]. Earlier work by Boël et al. on the EttA protein indicates that the ABC1–ABC2 complex represents the active form of EttA, and they are connected by a ~80-residue long inter-ABC-domain linker PtIM [7]. Both the ABC1 and ABC2 domains position near the 50S and 30S subunits and they interact with nearby ribosomal proteins [7]. Chen et al. have demonstrated that the ABC1 domain contacts helices 68 and 77 of the 23S rRNA, as well as ribosomal proteins L1 and L33 on the 50S subunit [21]. In contrast, the ABC2 domain contacts helices 41–42 of the 16S rRNA, ribosomal proteins L5 and L33 on the 50S subunit, and S7 on the 30S subunit [21]. Interfacial contact of EttA with nearby ribosomal proteins was performed using the PDBsum database (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/) and cryo-EM generated crystal structure (PDB ID 3J5S) from the PDB database (https://www.rcsb.org/). The result shows that EttA has maximum interfacial contacts (20 interfacial residues with an interface area of 1108 $Å^2$) with the L1 protein (23 interfacial residues making contact with an area of 1040 $Å^2$); owing to the non-bonded contacts followed by very few hydrogen bonds and electrostatic interactions (Table 1) [21,30]. The predicted binding affinity values ($\Delta G$ = -10.4 kcal $mol^{-1}$) and dissociation constant ($K_D$=$2.4\times10^{-8}$M) from the PROtein binDIng enerGY prediction (PRODIGY) web server (https://wenmr.science.uu.nl/prodigy/) at a default temperature of 25°C indicates strong interaction between EttA and 50S ribosomal protein L1 [31,32]. This dynamic interaction between the EttA and L1 protein is the driving force behind the necessary conformational changes of ribosome, thereby regulating the protein translation. Our analysis revealed that S7 ribosomal protein is the second most important interacting partner of EttA, having 14 interfacial residues contacting across an area of 709 $Å^2$. Similar to L1, the interaction with S7 is also resulted from the non-bonded contacts followed by very few hydrogen bonds and electrostatic interactions. Furthermore, the predicted binding affinity values ($\Delta G$ = -8.5 kcal $mol^{-1}$) and dissociation constant ($K_D$=$5.9\times10^{-7}$ M) are very close to that value of L1 (Table 1). The S7 ribosomal protein, which is located at the top of the 30S subunit, is one of the primary 16S rRNA-binding proteins responsible for initiating ribosome assembly [33]. In addition to that, S7 has also been shown to cross-link with tRNA molecules bound to the A- and P-sites of ribosome [34]. This strong interaction of EttA with S7 suggests its potential role in the protein translation process, a matter of future investigation. Molecular dynamics (MD) has been performed to study the motions and interactions between different ribosomal proteins and ATPase [35,36]. This computer based analysis is a powerful tool for detection of structural and functional alteration [37]. Here we have applied MD (a short, 10 ns simulation) to study the interaction between EttA and ribosomal proteins (Supplementary Figure 2), using CABS-flex 2.0 web server (http://biocomp.chem.uw.edu.pl/CABSflex2/index). According to the outcome of our MD analysis, the binding efficiency between EttA-L33 and EttA-S7 increases during the course of simulation (Table 1), while we have not observed any change in the case of EttA-L1 and EttA-L5 interaction.

**Table 1:** Detail of interface interaction of energy-dependent translational throttle A (EttA; PDB ID 3J5S) with different ribosomal proteins.

The data was generated by extrapolating PDBsum database (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/) and Prodigy webserver (https://wenmr.science.uu.nl/prodigy/) results before and after undergoing molecular dynamics (MD) simulations.

| | Interaction of EttA with ribosomal proteins | No. of interface residues | Interface area (Å$^2$) | No. of salt bridges | No. of hydrogen bonds | No. of non-bonded contacts | Binding affinity $\Delta G$ (kcal mol$^{-1}$) | Dissociation constant $K_D$ (M) |
|---|---|---|---|---|---|---|---|---|
| **Before MD Simulation** | EttA-L1 | 20:23 | 1108:1040 | 1 | 5 | 276 | -10.4 | $2.4 \times 10^{-8}$ |
| | EttA-L5 | 5:5 | 263:245 | - | 1 | 28 | -4.5 | $5.1 \times 10^{-4}$ |
| | EttA-L33 | 1:1 | 178:176 | 1 | - | 3 | -4.9 | $2.7 \times 10^{-4}$ |
| | EttA-S7 | 14:14 | 701:709 | 4 | 3 | 186 | -8.5 | $5.9 \times 10^{-7}$ |
| **After MD Simulation** | EttA-L33 | 8:7 | 505:535 | 1 | 3 | 51 | -6.2 | $2.9 \times 10^{-5}$ |
| | EttA-S7 | 21:18 | 998:1017 | 4 | 5 | 275 | -11.4 | $4.3 \times 10^{-9}$ |

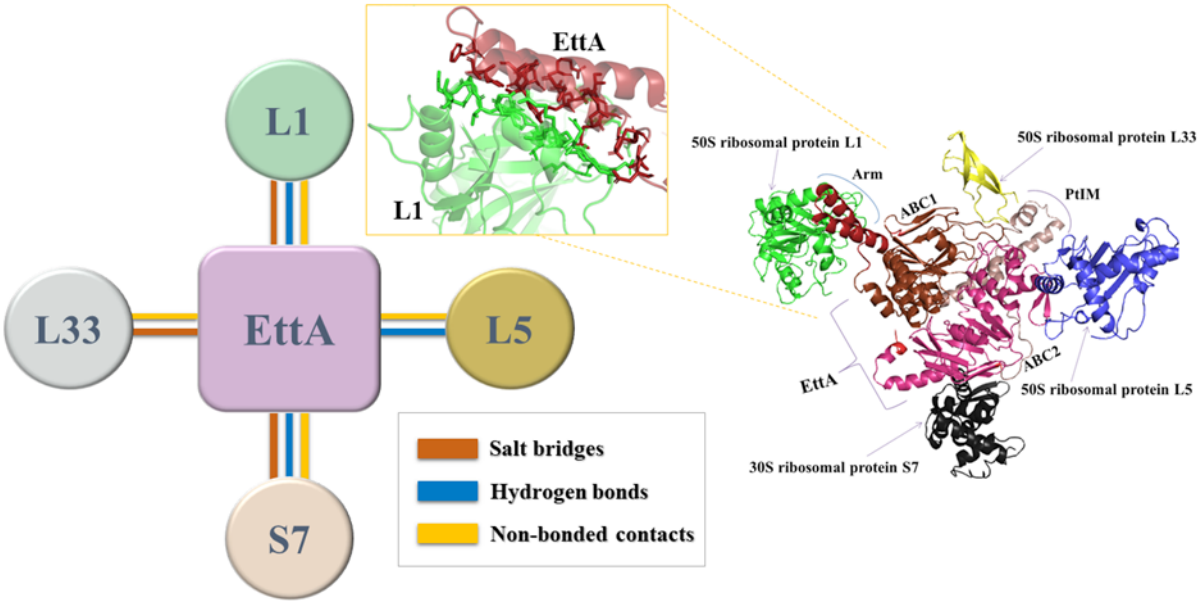


**Figure 2:** The various interactions of energy-dependent translational throttle A (EttA) with ribosomal proteins. Schematic diagram shwing salt bridges, hydrogen bonds and non-bonded contacts. Crystal structure of EttA (PDB ID 3J5S) highlighting its major domains; ATP-binding cassette (ABC)1, ABC2, Arm domain, and P-site tRNA interaction motif (PtIM) domain. The model of EttA was constructed based on the PDBsum database (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/).

The analysis of the EttA-$EQ_2$ mutant illustrates that EttA functionally interacts with the ribosome under both *in vivo* and *in vitro* condition [19]. ATP hydrolysis-deficient $EQ_2$ mutations trap the EttA in its ATP-bound state, thereby blocking its release from the ribosome [4]. Studies have shown that the native glutamate residues at positions 188 and 470, draw hydrogen atoms from the water molecule, which initiates the hydrolytic attack on the ATP γ-phosphate group. Mutations on these glutamate residues preserve the local stereochemistry but prevent activation of the hydrolytic water molecule [4, 7]. A study using N-terminal FLAG-TEV-$His_6$-tagged EttA (wild type and $EQ_2$ mutant), western blotting and radioactive $^{35}$S-methionine pulse-labeling assay has shown that the expression of $EQ_2$ mutants causes inhibition of protein translation and cell growth [3]. A 47-fold decrease in protein translation was reported for

the EttA-$EQ_2$ mutant, and similar trends have been observed for other EttA paralogues (15-fold, 7-fold, 6-fold decrease in YbiT-$EQ_2$ mutant, YheS-$EQ_2$ mutant and Uup-$EQ_2$ mutant respectively) [3]. We conducted a molecular docking study using both form of EttA (wild-type and $EQ_2$ mutant) and the two known ATP-binding sites available in the UniProt database (https://www.uniprot.org/) (P0A9W3 · ETTA_ECOLI) were used i.e., ATP-binding site 1 (GLNGAGKS at position 39-46) and ATP-binding site 2 (GPNGAGKS at position 356-363). Interestingly, EttA-$EQ_2$ mutant shows lower binding affinity for the ATP molecule than that wild-type counterpart (Figure 3). For the *in-silico* mutational analysis, the crystal structure of EttA at a resolution of 7.50 Å (PDB ID 3J5S) from the PDB database was used. The model of the EttA-$EQ_2$ mutant has been created using PyMOL software (https://pymol.org/2/). Further refinement of the structure was done by the GalaxyRefine (https://galaxy.seoklab.org/) online tool to exclude poor rotamers and to select the best model based on the Ramachandran plot. For the molecular docking, the CB-DOCK2 (https://cadd.labshare.cn/cb-dock2/php/index.php) online tool was used. Moreover, the binding affinity was calculated using the PRODIGY web server. Binding energy of the ATP-bound wild-type EttA at ATP-binding site 1 was found to be -5.00 kcal $mol^{-1}$ which is slightly higher than the EttA-$EQ_2$ mutant (-4.96 kcal $mol^{-1}$). Similar phenomenon was observed for ATP-binding site 2. Binding energy of the wild type EttA ($\Delta G$ = -5.08 kcal $mol^{-1}$) is higher than mutant ($\Delta G$ = -5.03 kcal $mol^{-1}$). Hence the ATP binding energy of wild type protein is higher than the mutant vindicates the weaker interaction with the ATP molecule. On the contrary, previous record showed that the EttA-$EQ_2$ mutant binds tightly to the ATP molecule thereby preventing its hydrolysis [3]. We therefore conclude that $EQ_2$ mutations neither have little any effect on binding with ATP molecule nor it can efficiently trap EttA in an ATP-bound state when ATP remains bound to a single ATP-binding site. In a nutshell, ATP hydrolysis-deficient mutant can trap EttA in an ATP-bound state only when both the ATP-binding sites (ABC1 and ABC2) are actively involved, preventing its release from the ribosome.

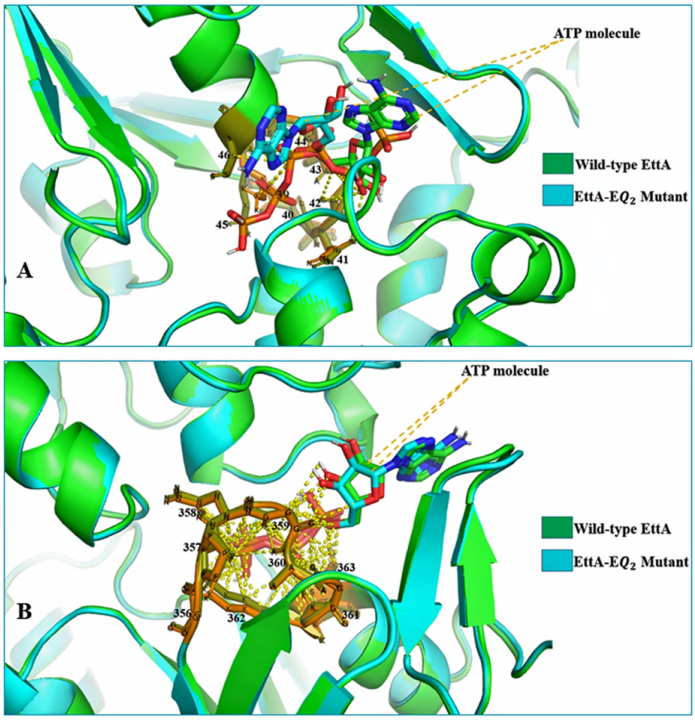


**Figure 3:** ATP-bound wild-type EttA and EttA-EQ2 mutant.

(A) ATP-bound wild-type EttA (green) and ATP-bound EttA-EQ2 mutant (cyan) at ATP-binding site 1 (GLNGAGKS at position 39-46). (B) ATP-bound wild-type EttA (green) and ATP-bound EttA-EQ2 mutant (cyan) at ATP-binding site 2 (GPNGAGKS at position 356-363).

## Role in stress responses

Different ABC-F proteins are found to be associated with various stress responsive pathways in different organisms [4]. Strikingly, almost all of them are associated with ribosome and hence function through protein translation. That's why they are also referred to as translation factors [4]. Disruption of the gene

function not only transforms the organism to be more susceptible to the environmental stressors but also becomes prone to failure to strive under stressful conditions. Importance of ABC-F proteins in various biological systems are discussed below.

Plant *Arabidopsis thaliana* expresses five ABC-F paralogs: AtABCF1, AtABCF2, AtABCF3, AtABCF4, and AtABCF5. The AtABCF3 gene is involved in the development process of roots and its deletion from chromosome results in the formation of shorter roots in the mutant plant [38]. Environmental stressors such as high salinity, drought, free radicals, and heavy metals adversely affect the plants tissues. The ABC-F protein GCN20 in *Arabidopsis thaliana* promotes root elongation by modulating DNA damage repair [39]. Moreover, GCN20 is also involved in stomata closure during bacterial infection to protect *Arabidopsis thaliana* [40].

Interestingly, a recent genome-wide association study of salt-stressed plants (*Populus euphratica*) showed a possible crosstalk between ABC-F genes and saline tolerance [41]. Detail analysis illustrated that, ABCC2 (ABC-F ATPases), and respiratory burst oxidase homolog are responsible for salt stress-induced heterophylly [41]. This finding opens up a new research area on Osmo tolerance and its regulation by ABC-F proteins.

Protein synthesis in the eukaryotic cell can be down-regulated in presence of starvation, viral infection, or the presence of a defective aminoacyl-tRNA synthetase. Studies have shown that the GCN20-GCN1 association under limited amino acid condition activates GCN2 in the yeast cell [42]. The GCN2 is a protein kinase phosphorylates the α-subunit of eIF2 (eIF2α) to form eIF2B. The eIF2 is responsible for providing the initiator methionyl-tRNA ($tRNA_i^{Met}$) to the 40S ribosomal subunits in the form of eIF2/GTP/Met-$tRNA_i^{Met}$ ternary complex and is released as eIF2/GDP binary complex during the process of initiation. Under normal conditions, the ternary complex, together with four short upstream open reading frames (ORFs) from its 5' mRNA leader, act to attenuate GCN4 mRNA translation [43]. GCN4 is a transcriptional activator for amino acid biosynthetic enzymes that escalate the process of protein synthesis [42]. The formation of eIF2B reduces the formation of ternary complexes in the cell and stimulating the translation of GCN4 mRNA. Under amino acid starved conditions, GCN4 synthesis increases and eventually leads to activation of amino acid biosynthetic genes [43]. The GCN4 acts as important factor in oxidative stress by providing resistance against hydrogen peroxide in yeast [44]. Another important ABC-F protein is ABCF2. During hypotonic condition, human ABCF2 (hABCF) works with α-actinin-4 in the regulation of cell volume and anion channels in human epithelial cell. ABCF2 paralogue in yeast Arb1 (ABCF2) has been found to be involved in protein synthesis and ribosome biogenesis. Deletion of the gene results in delayed rRNA processing that causes problem in the biogenesis of 40S and 60S ribosomal subunits [45, 46].

The application of ABC-F ATPases is multifaceted. These multifunctional proteins help in conferring immunity to mosquitoes (*Aedes aegypti*) against arboviruses. The RNA sequencing data reveals the increased expression of cytosolic ABC transporter genes (E & F Subfamily) across the mosquito developmental stages, vindicating its importance in mosquito development [47]. In addition to that, the microarray dataset of mosquitoes infected with West Nile, yellow fever, and dengue viruses indicated elevated ABC-F subfamily genes, together with a small number of additional transporters [47]. These results strongly suggest the importance of the ABCF ATPase in the area of vector borne illness.

**Circumventing pesticide-induced stress**

The white-backed planthopper, *Sogatella furcifera* (*Horváth*) is one of the main agricultural pests invading rice paddies in Asia. The extensive use of pesticides to control pest proliferation has led to resistance against a wide variety of these pesticides. Transcriptome analysis of *S. furcifera* exposed to imidacloprid, deltamethrin, and triazophos stress elucidated the molecular mechanisms of pesticide resistance and regulatory role of ABC ATPase in this event [48]. Under imidacloprid stress, up-regulation of seven gene including P450s, GST, ABC-F ATPase, and heat shock proteins was observed [48]. Similar results were observed in the case of deltamethrin and triazophos stress, where induction of one or more ABC-F ATPase genes were reported [48]. Among these differentially expressed genes (DEGs), ABC-F4 ATPase is involved in the detoxification, metabolism, and translocation of insecticide. The same gene is most likely involved in changing epidermal permeability in *S. furcifera*; thereby contributing in insecticide resistance [48].

**Alleviation of antibiotic stress**

*Bacillus* and *Streptomyces* are the most abundant bacterial genera that are found in soil everywhere. *Streptomyces* is known to produce different types of antibiotics used in various clinical purposes. Interestingly, this particular bacteria carries a significant percentage of biosynthetic gene clusters (BGCs) found among all bacteria [49]. These BGCs are responsible for the synthesis of many specialized bioactive metabolites of pharmaceutical importance. The production of antibiotics in *Streptomyces sp.* is for defending their habitats from the competitors [50]. Interestingly, Ribosome-targeting antibiotics secreted by bacterial competitors at sub-inhibitory concentrations, act as elicitors (by inducing antibiotic and resistance-protein production). These induced resistance proteins provide protection against the inhibitory effect of their own produced antibiotics and the ribosome-targeting antibiotics secreted by their competitors. Additionally, various self-resistance mechanisms that are encoded within BGCs belong to ABC-F

ATPases [51]. One such example is seen in the case of soil actinobacterium *Streptomyces lincolnensis*. When *Streptomyces lincolnensis* faces antibiotic stress due to production of ribosome-targeting antibiotics (such as lincosamide, streptogramin A, or pleuromutilin) by their competitors, they promote lincomycin production in response. Primarily in the presence of antibiotic that works through ribosome-mediated attenuation, activates ABC-F ATPase LmrC (a lincomycin resistance protein that confers only moderate resistance but has a key role in antibiotic induced signal transduction). This is followed by the induction of LmbU (a transcriptional regulator), which helps in the expression of lincomycin BGC [52]. LmrA (a 23S rRNA methyl transferase) and LmrB get upregulated in this process that in turn inhibit the production of LmrC, as a consequence, the amount of the ribosome-bound lincosamide decreases, that alleviate antibiotic stress in *Streptomyces lincolnensis* [53].

**ARE and ribosome protection**

Ribosomes are one of the major targets of antibiotics and several clinically important antibiotics target bacterial ribosomes to inhibit protein synthesis [12]. Different antimicrobial drugs such as macrolides, ketolides, aminoglycosides, oxazolidinones, phenicols, lincosamides etc., work with a specific mechanism on the bacterial ribosome. They either target PTC in the large 50S subunit or the NPET adjacent to the PTC or the small 30S subunit of the ribosome [3]. However, the extensive use of antibiotics in the last few decades has led to the formation of numerous multi-drug-resistant superbugs. The diverse mechanisms of resistance against different classes of antibiotics are very much threatening to public health worldwide [54]. A common antibiotic resistance (ARE) mechanism that has been adopted by many bacterial families involves the induction of mutation or modification of the antibiotic binding site. This includes post-transcriptional modification of 23S rRNA by methyl transferases, resulting in decreased drug-binding efficiency [55]. Other mechanisms include antibiotic efflux and ribosome protection via ribosome protection proteins (RPP) that actively remove the drug compounds from the ribosome without changing its structure. For example, tetracycline removal mediated by TetM and TetO RPPs in a GTP-dependent manner [56]. Both antibiotic efflux and ribosome protection can be mediated by the members of ABC ATPases. The antibiotic efflux mechanism by ABC ATPases involves TMDs that use ATP to pump out the drug compounds from the cell [57]. The ABC-F ATPases, which lack the TMD, confer resistance by removing the PTC/NPET region targeting drug compounds by inducing conformational changes upon binding [12, 13]. These ABC-F ATPases are known as ARE ABC-F by virtue of conferring resistance against different antibiotics. The α-helical hairpin structure of PtIM that forms inter-helical loop of variable length is important for protection against antibiotics and popularly known as ARE domain (ARD) [12, 13]. Many of those ARE ABC-F proteins have an additional arm subdomain within NBD1 as well as an additional C-terminal extension [10]. ARE ABC-F proteins are extensively found among gram-positive bacteria but are rarely seen in gram-negative bacteria. They can be chromosomally and/or plasmid-encoded and are found in many clinically relevant pathogens, including ESKAPE group that has a major contribution in hospital-acquired infections [3]. Phylogenetic analyses indicate that the origin of ARE ABC-F may have originated from a convergent evolution. Based on the nature of conferring resistance, ARE ABC-Fs are classified into eight subfamilies with three distinguished resistance spectra [58].

1. PTC-binding antibiotics that confer resistance against pleuromutilin, lincosamide, and streptogramin A (PLSA): the ARE1, ARE2, ARE3, ARE5, and ARE6 subfamilies e.g., homologs of VmlR, VgaA, SalA, LmrC, and LsaA [59].
2. NPET-binding antibiotics contributing resistance against macrolide and streptogramin B belong to a subset of the ARE1 subfamily, and ARE4 e.g., MsrE homologs.
3. OptrA (ARE7) and PoxtA (ARE8) homologs providing resistance against phenicols and oxazolidinones; the mechanism of activity is not fully understood so far [13].

**ARE mechanism in VmlR**

The PLSA antibiotics inhibit translation by targeting the A-site of PTC with overlapping of the P-site and NPET. The VmlR protein belongs to the PLSA-class that binds to the E-site of the 70S ribosome during the interaction (Figure 4). The inter-domain linker PtIM (or ARD domain) allows these proteins to access the antibiotic-binding site in the PTC, which brings about conformational changes of the ribosome leading to drug disassociation. The detailed mechanism was unveiled by Crowe-McAuliffe et al. (2018), illustrates the structural analysis of *Bacillus subtilis* VmlR bound to a stalled ribosome complex [9]. The expression of VmlR was controlled by a multi-layered regulatory mechanism that include pausing of RNA polymerase, transcription - translation attenuation, and (p)ppGpp-mediated signaling to alleviate the antibiotic stress [60].

**ARE mechanism in MsrE**

MsrE confers resistance from NPET-targeting antibiotics, such as macrolide. Upon sensing the drug, it binds into the E-site of the 70S ribosome in an ATP-dependent manner (Figure 4). MsrE operates through its long inter-domain linker PtIM (or ARD domain) that extends into the NPET after passing through PTC [29]. MsrE binding leads to conformational changes of ribosome which is necessary for dissociation of drug from it. Su et al. (2018) described the first ever structural insight of ABC-F-mediated

ribosome protection mechanism using cryo-EM based analysis [29].

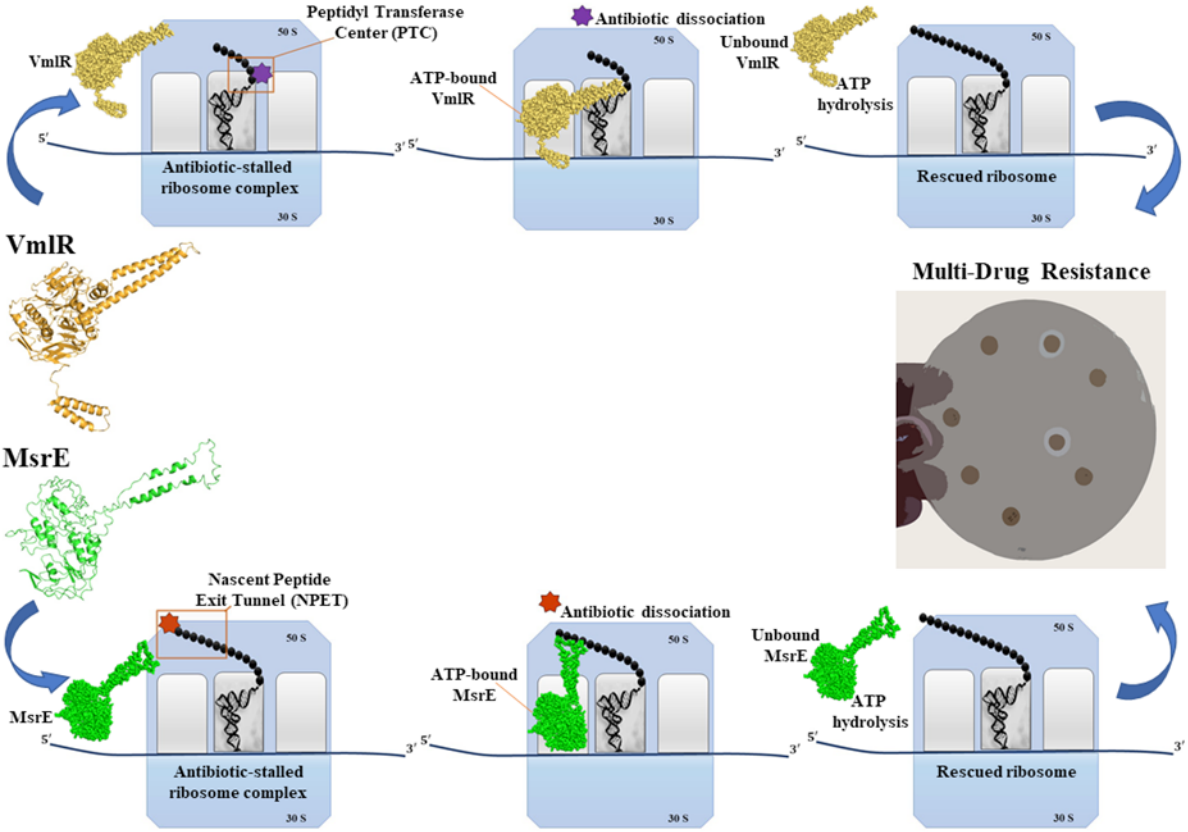


**Figure 4:** Stages of ribosomal rescue by VmlR and MsrE adenosine triphosphate (ATP)-binding cassette F (ABC-F) proteins.

Schematic diagram showing of antibiotic drug targeting centre (the peptidyl transferase center PTC; purple star) and the 50S nascent peptide exit tunnel (NPET) adjacent to the PTC (red star). ATP-bound VmlR enters the ribosome exit (E)-site, and binding of VmlR with the ribosome induces a shift in tRNA conformation in the ribosome peptidyl-tRNA-binding (P)-site. VmlR's antibiotic resistance domain (ARD) accesses the PTC, ultimately leading to dissociation of the antibiotic drug. Similarly, ATP-bound MsrE enters the E-site; the long-extended loop helps it to project deeper into the NPET initiating conformational changes in the PTC region and slightly widening the NPET around the drug-binding site; ultimately leading to dissociation of the antibiotic drug. The rescued ribosome gets separated from VmlR/MsrE upon ATP hydrolysis, and thereby providing multi-drug resistance in the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp)* organisms.

## Effect in ribosome biogenesis

The proper fitness of ribosomes is necessary for executing protein synthesis in living systems. Since ribosomes are macromolecules, proper association of different small and large subunit proteins and ribosomal RNA is important for it function. Hence, ribosome biogenesis plays supreme importance in the context of protein translation and is instrumental for cellular function. Different proteins and molecular chaperones are directly or indirectly associated with ribosome biogenesis. Arb1 is an essential yeast protein from *S. cerevisiae* and closely related to the ABC superfamily that interacts with ribosomes and functions in ribosome biogenesis [61]. Depletion of Arb1 from the genetic background of yeast cells leads to impairment of 40S subunits due to lack of processing of 35S pre-rRNA [45]. Additionally, delayed processing of mature 18S rRNA, and defects in nuclear export of pre-40S subunits were observed. As Arb1 shuttles between the nucleus and cytoplasm and is physically associated with 40S, 60S, and 80S/90S ribosomal species, loss of ATPase function results in the drastic change of ribosomal component. The maturation of 60S subunits is also affected by the absence of this indispensable ATPase that stimulates multiple steps in its biogenesis. Another ABC-F class of ATPase known as New1 helps in maintaining

ribosomal integrity in yeast and absence of this factor causes ribosomal assembly defects, manifested by increased susceptibility towards different environmental stressors. Perturbation of New1 function also causes the premature halt of protein translation, which is very much deleterious to the organism [62].

## Functions of ABC-Fs in humans

In *Homo sapiens*, three distinct paralogs of ABC-F have been identified: ABCF1 (ABC50), ABCF2, and ABCF3. ABC50 has been extensively studied among them and found to be the first human ABC protein that lacks TMDs. ABC50 is associated with initiation of translation in mammalian cells [63]. Similarly, like GCN20, ABC50 interacts with eEF2 and stimulates the formation of the eIF2/GTP/Met-$tRNA_i^{Met}$ ternary complex. Accurate recognition of the start codon by ABC50 was reported by Stewart et al [64]. Furthermore, ABC50 is associated with tumor necrosis factor-α-stimulated synoviocytes and leukemia cells [65]. Similar to ABC50 (ABCF1), ABCF2 is associated with numerous forms of human cancer [4]. Human ABCF3 (hABCF3) is preferentially found in the cytoplasm, unlike its paralog hABCF1. Moreover, it is highly expressed in vital organs like heart, liver, and pancreas [66]. The hABCF3 protein plays a positive role in cell proliferation that may be a consequence of its strong interaction with the TPD52L2 (tumor protein D52-like 2) protein [66].

### ABC-F-mediated immune-modulation in humans

Although several ABC-F ATPases have been extensively studied for their role in controlling immune responses and their relationship to a wide range of human diseases, the underlying mechanism of their interaction remains unknown in many cases. The hABCF1 protein has been characterized as an autoimmune disease modulator and its association with different autoimmune diseases have been reported [4]. The ABCF1 controls mortality rates in lipopolysaccharide (LPS)-induced sepsis by repressing hypotension-induced renal circulatory dysfunction. ABCF1 possesses class IV E2 ubiquitin enzyme activity that modulates the activity of LPS toll-like receptor-4. As a consequence, it acts as a molecular switch that regulates the transition from inflammatory phase of sepsis to the endotoxin-tolerance phase. It further influences cytokine storm and production of interferon-β-dependent by controlling immunotherapeutic mediator, Sirtuin 1 [67]. Since ABCF1 is involved with many autoimmune diseases, it is an interesting target site for designing new drug compounds. Dicarboximides have been reported to act as anticancer therapeutics that preferentially downregulates ABC50 (ABCF1) activity in leukemia cells [65]. ABCF2 has been identified as an anti-apoptotic factor which is seen to be associated with various types of cancers such as adenocarcinoma, hepatocellular carcinoma etc [4]. Previous analysis has suggested that ABCF2 acts as a potential biomarker to detect different cancers at their early stages [4]. The biochemical characterization of the mouse ABCF3 protein illustrates, how the lipid-stimulated ATP hydrolysis may be a contributing factor for the reduction of viral RNA that leads to flavivirus resistance. Since humans and mice share nearly 90 percent of genes, similar results are to be expected in genetic screening using hABCF3 [68].

## Evolutionary relationship within ABC-F ATPases

The evolution of different ABC-F ATPases has unique characteristics across different domains of life. To understand the evolutionary relatedness among common members of ABC-F ATPases, a maximum likelihood-based phylogenetic tree was created (Figure 5). 12 different ABC-F protein sequences from diverse origin were used to construct the phylogenetic tree. The two major clades clearly differentiate the prokaryotic and eukaryotic ABC-F ATPases. The prokaryotic ABC-F ATPases include PTC- and NPET-targeting ARE ABC-Fs together with EttA [58]. The eukaryotic sequences were derived from yeast and humans with diverse functions. Among the prokaryotic ABC-Fs, a high bootstrap confidence score grouping was seen between VgaA and MsrE, suggesting a common lineage within ARE class. The only difference exists in case of interaction with ribosome as the mechanism of action is different. VgaA acts on the A-site of the PTC whereas MsrE binds to NPET while excluding the drug. Despite having different targeting sites, they both belong to the ARE1 subgroup of ABC-F proteins [58]. Phylogenetic analysis indicates MsrE has slight sequence divergence from VgaA; inferring that it has evolved from a common ancestor but gradually diverse over time. LsaA possesses the longest evolutionary distance from its kin VmlR; therefore, they belong to different ARE subgroups [58]. Both proteins target the PTC while excluding pleuromutilins from the ribosome. The relative distribution of different ARE classes of ABC-F ATPases actually indicates the convergent evolution across different ESKAPE organisms showing multi-drug resistance. Among different eukaryotic ABC-F ATPases, New1 and eEF3 (eEF3A) are nested together (supported by a high bootstrap confidence score); implying that they are descendants of a common ancestor. Interestingly, unicellular eukaryotic yeast ABC-F protein eEF3 shows significant sequence divergence from New1. The divergence in the protein sequence as well as in the evolution process may be due to the differences in their functions. As it is well predicted that eEF3 acts as a ribosome translation factor, whereas New1 is proposed to be involved in ribosome biogenesis; helping protein translation indirectly [3, 4]. The GCN pathway is conserved across different eukaryotes and the proteins within this group play critical roles in the stress-response pathway reported in *A. thaliana* and *S. cerevisiae* [69]. Our phylogenetic analysis also shows that GCN20 is a close relative of yeast Arb1 and human ABCF1. Under amino acid starved condition in yeast, GCN20 is shown to sense situation, whereas Arb1 is involved in ribosome biogenesis [3, 4]. Previously conducted studies suggested

that GCN20 is closely related to ABC proteins identified in *Caenorhabditis elegans*, *Oryza sativa,* and humans. It implies the function of GCN20 is conserved among diverse eukaryotic origins. Sequence analysis of ABCF1 (ABC50) revealed of its similarity with GCN20. The phylogenetic divergence of GCN20 from its next kin in the clade may be a consequence of functional heterogeneity mentioned in the literature. Two ABC domains of the ABC-F family may have had different evolutionary histories that are another cause of divergence in the phylogenetic tree within closely related ABC-F proteins [3]. The possibility of divergence due to recombination has been ruled out as the experiments suggest no evidence of the process being involved in the evolution.

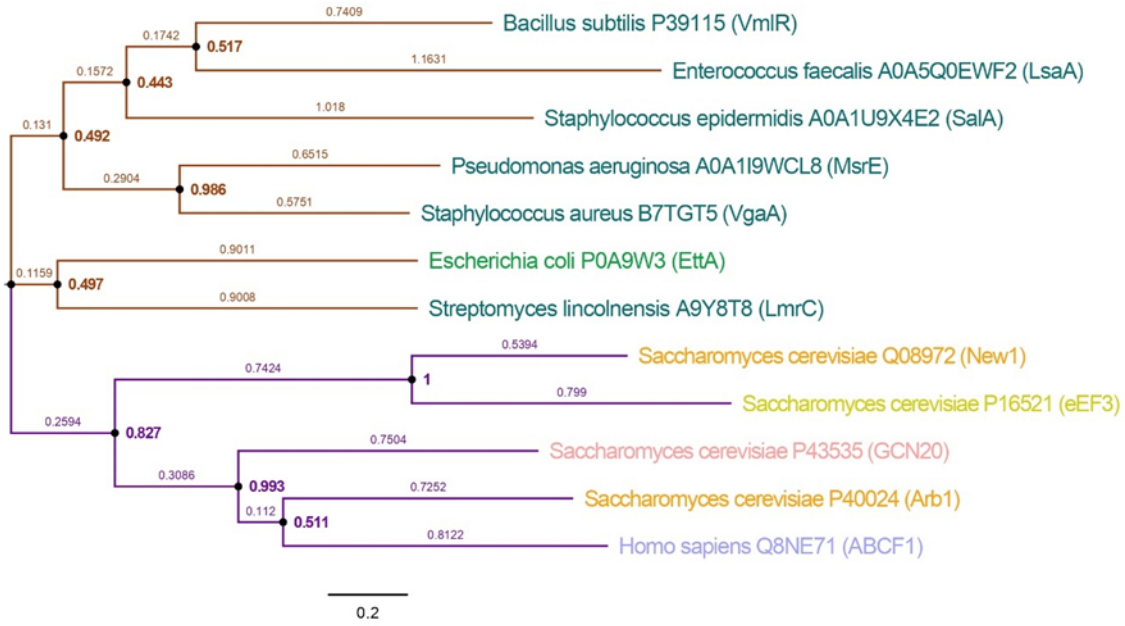


**Figure 5:** Phylogenetic tree of ABC ATPase generated based on maximum likelihood (ML) method and Jones-Taylor-Thornton (JTT) matrix-based model.

Branch support from 1000 bootstrap replicates. Analysis involved twelve different (ATP)-binding cassette F (ABC-F) proteins. Confidence scores less than 0.5 were not taken into account (bootstrapping cut off value was set to 50%). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pair-wise distances estimated using the JTT model, and then by selecting the topology with the superior log likelihood value. Horizontal branches are proportional to substitution per site as per the inset scale bar. Evolutionary analyses were conducted using MEGA11 (https://www.megasoftware.net/) and FigTree v1.4.4 software (http://tree.bio.ed.ac.uk/software/figtree/).

## ABC-F ATPase and drug designing

The rapid emergence of resistant bacteria is occurring worldwide, thereby endangering the efficacy of antibiotics, which have created a profound effect on the public health. Like other mechanisms, the member of ABC-F class of proteins collectively mediate resistance to a broader range of clinically important antibiotics seen in different pathogenic organisms (Figure 6). Since many antibiotics target ribosome, this ribosome-bound ATPase has seminal importance in conferring resistance in various ways [70-72]. The ATPase domains play a crucial role in the interaction with ATP, which is the driving force behind their function. The ATP-binding site of these ATPase can be manipulated, or the ATPase function can be perturbed using selective inhibitors [73]. Different chemical molecules show intrinsic binding properties with the ATPase domain; thereby masking the ATP binding. This actually helps in inhibiting the function of the ATPase as it can no longer exclude the antibiotics from the ribosome during the protein translation. The theory of drug repurposing is also applicable here. The effectivity of the drug can be augmented using chemical modification. As a possible effect, the chemically modified drug can bind to the ATP-binding pocket more efficiently than its native counterpart. Prior knowledge of the chemical structure and implementation of molecular dynamic simulations can be effective for augmenting drug-ATPase interaction [74-76].

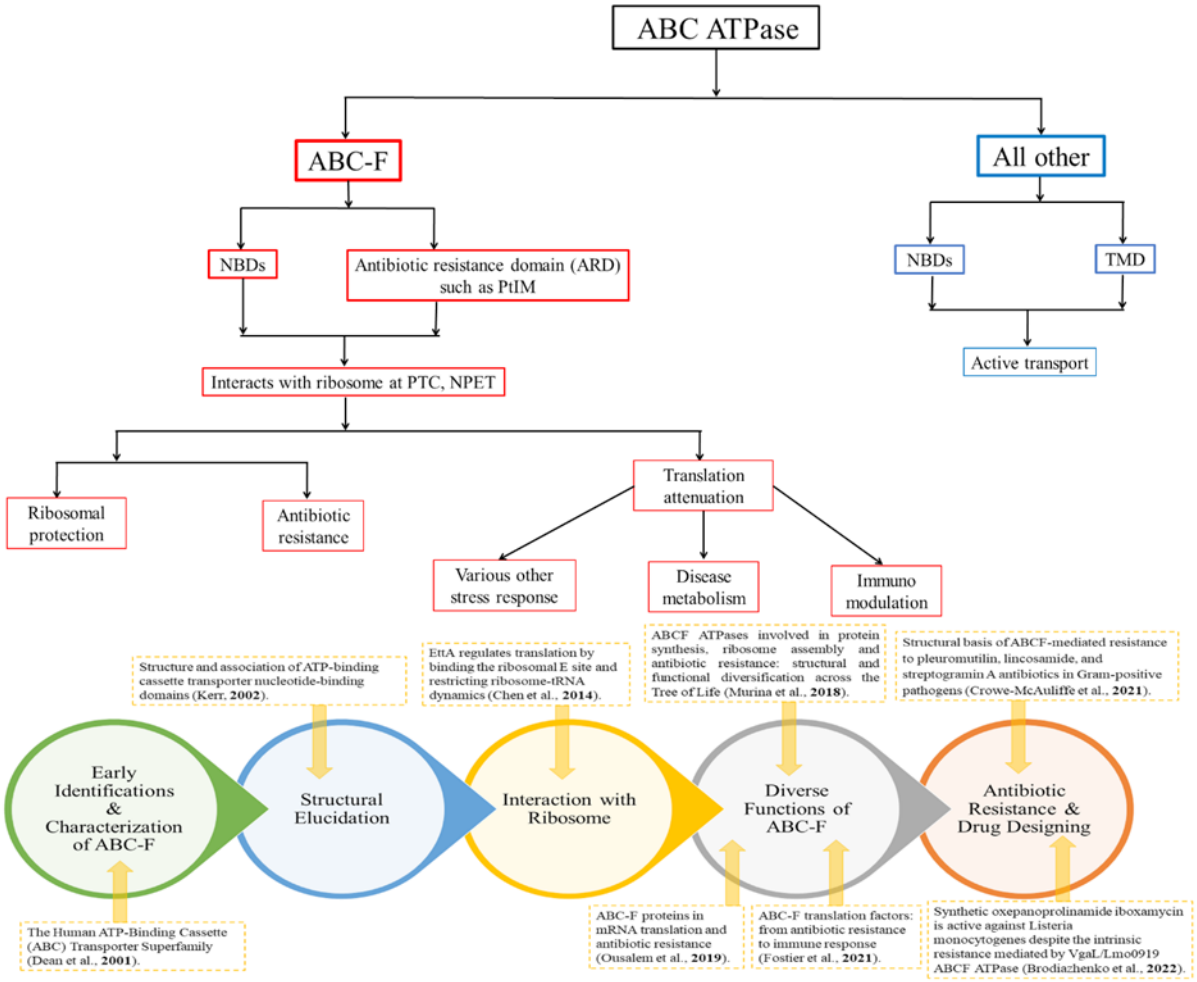


**Figure 6:** Schematic diagram illustrating structural and functional divergence of ABC-Fs.

(Upper panel) Flow chart of structural and functional aspects of adenosine triphosphate (ATP)-binding cassette (ABC) ATPase. (Lower panel) Major landmarks in the field of ABC-F ATPase.

Since ATP is essential for the ABC-F protein to mediate resistance against antibiotics; ATP-binding domain can be an easy target for abating its function [12]. The manipulation of the ATP- or $Mg^{+2}$-binding site in the NBDs, or the linker region in between can be changed to attenuate the function of the ATPase [77]. The ABC-F ATPase makes contact with specific sites of the ribosome and employs its ARD or loop region to exert resistance [12]. Therefore, if the contact point between ATPase and ribosomes is hindered, the interaction will be lost [12]. Similarly, other molecules such as antimicrobial peptides (AMP) can attain a similar structure to the ATPase during their interaction with the ribosome. As a result, AMP can exclude the ATPase from the site of infection, thereby eradicating the resistance. Since these ABC-F classes of ATPases, like any other proteins, are not rigid molecules, and their internal motion and conformational changes play an important role in their function. *In-silico* simulations provide a link between their structure and dynamics by exploring the conformational energy landscape accessible to the protein molecule. This may also allow us to know about the effect of various perturbations such as binding or removal of a ligand, mutations, protonation, phosphorylation, or any such modification that alters their functional characteristics. The recent development of synthetic lincosamide iboxamycin that has been found to be effective against the gram-positive bacterium *Listeria monocytogenes* overcomes the intrinsic lincosamide resistance mediated by VgaL/Lmo0919 ABCF ATPase. This recent advancement can be considered as a pioneering event in the field of drug development to fight against multi-drug resistant superbugs [78].

## Conclusion

The ABC-F ATPases are ubiquitous in most of the life forms except Achaea. These unique classes of ATPases are involved in diverse functions associated with protein translation. Various studies have indicated their role as translation factors modulating the PTC or NPET site of mature ribosomes. Their activity causes temporary conformational changes in the ribosome prompted by the ATP binding and hydrolysis. The advanced structural analysis has helped us to determine the 3D structure of these proteins and their interaction with the ribosome. These interactions are instrumental in understanding their responses against various environmental stresses and conferring ARE to the multi-drug resistant 'superbugs'. *In-silico* analysis has elucidated the interaction of EttA with other ribosomal proteins, providing the insight of regulation of translation under energy-deprived conditions. This multifaceted ATPase controls wide range of cellular functions such as DNA repair, modulation of immunity apart from their fundamental role of ribosome protection. Structural and phylogenetic analysis suggests that, despite having diverse functions, they may have evolved from common ancestors displaying similar structural features across different species. In a nutshell, this review tried to explore the structure-function relationship of ABC-F ATPase which could be very important for the development of novel drugs overcoming the problem of antibiotic resistance in different pathogens.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

The dataset(s) supporting the conclusions of this article is(are) included within the article.

### Competing interests

The authors declare that they have no competing interests.

### Funding

Not applicable.

### Authors' contributions

AB, SC designed the manuscript; CS, SS, AC, AB, SC analysed the data; CS, AB wrote the manuscript; AB, SC, ARM, PB, KS, and AD involved in proofreading. All authors reviewed the manuscript.

### Acknowledgement

AB is thankful to Department of Biotechnology (DBT) Govt. of India for providing Ramalingaswami Re-Entry Fellowship (BT/RLF/Re-entry/43/2019). SC thankfully acknowledges the Ramalingaswami Fellowship (BT/RLF/Re-entry/09/2019). ARM is grateful to the Department of Biotechnology (DBT) for the same fellowship, reference no: BT/RLF/Re-entry/53/2019.

## References

1. Wilson DN, Nierhaus KH (2007) The weird and wonderful world of bacterial ribosome regulation. Crit Rev Biochem Mol Biol 42:187-219.
2. Schmeing TM, Ramakrishnan V (2009) What recent ribosome structures have revealed about the mechanism of translation. Nature 461:1234-1242.
3. Murina V, Kasari M, Takada H, Hinnu M, Saha CK, et al., (2019) ABCF ATPases Involved in Protein Synthesis, Ribosome Assembly and Antibiotic Resistance: Structural and Functional Diversification across the Tree of Life. J Mol Biol 431:3568-3590.
4. Fostier CR, Monlezun L, Ousalem F, Singh S, Hunt JF, et al., (2021) ABC-F translation factors: from antibiotic resistance to immune response. FEBS Lett 595:675-706.
5. Dean M, Rzhetsky A, Allikmets R (2001) The human ATP-binding cassette (ABC) transporter superfamily. Genome Res 11:1156-1166.
6. Krishnan A, Burroughs AM, Iyer LM, Aravind L (2020) Comprehensive classification of ABC ATPases and their functional radiation in nucleoprotein dynamics and biological conflict systems. Nucleic Acids Res 48:10045-10075.
7. Boël G, Smith PC, Ning W, Englander MT, Chen B, et al., (2014) The ABC-F protein EttA gates ribosome entry into the translation elongation cycle. Nat Struct Mol Biol 21:143-151.
8. Andersen CBF, Becker T, Blau M, Anand M, Halic M, et al., (2006) Structure of eEF3 and the mechanism of transfer RNA release from the E-site. Nature 443:663-668.
9. Crowe-McAuliffe C, Graf M, Huter P, Takada H, Abdelshahid M, et al., (2018) Structural basis for antibiotic resistance mediated by the Bacillus subtilis ABCF ATPase VmlR. Proc Natl Acad Sci U S A 115:8978-8983.
10. Murina V, Kasari M, Hauryliuk V, Atkinson GC (2018) Antibiotic resistance ABCF proteins reset the peptidyl transferase centre of the ribosome to counter translational arrest. Nucleic Acids Res 46:3753-3763.
11. Vimberg V, Cavanagh JP, Novotna M, Lenart J, Thi Ngoc BN, et al., (2020) Ribosome-Mediated Attenuation of vga(A) Expression Is Shaped by the Antibiotic Resistance Specificity of Vga(A) Protein Variants. Antimicrobial agents and chemotherapy 64.
12. Ero R, Kumar V, Su W, Gao Y (2019) Ribosome protection by ABC□F proteins—Molecular mechanism and potential drug design. Protein Sci 28:684-693.
13. Wilson DN, Hauryliuk V, Atkinson GC, O'Neill AJ (2020) Target protection as a key antibiotic resistance mechanism. Nat Rev Microbiol 18:637-648.

14. Zhang Z, Liu F, Chen J (2018) Molecular structure of the ATP-bound, phosphorylated human CFTR. Proc Natl Acad Sci U S A. 115:12757-12762.

15. Kerr ID (2002) Structure and association of ATP-binding cassette transporter nucleotide-binding domains. Biochim Biophys Acta 1561:47-64.

16. Walker JE, Saraste M, Runswick MJ, Gay NJ (1982) Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J 1:945-951.

17. Hanson PI, Whiteheart SW (2005) AAA+ proteins: have engine, will work. Nat Rev Mol Cell Biol 6:519-529.

18. Hopfner KP, Karcher A, Shin DS, Craig L, Arthur LM, et al., (2000) Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily. Cell 101:789-800.

19. Ousalem F, Singh S, Chesneau O, Hunt JF, Boël G (2019) ABC-F proteins in mRNA translation and antibiotic resistance. Res Microbiol 170:435-447.

20. Qu L, Jiang Y, Cheng C, Wu D, Meng B, et al., (2018) Crystal Structure of ATP-Bound Human ABCF1 Demonstrates a Unique Conformation of ABC Proteins. Structure 26:1259-1265.e3.

21. Chen B, Boël G, Hashem Y, Ning W, Fei J, et al., (2014) EttA regulates translation by binding the ribosomal E site and restricting ribosome-tRNA dynamics. Nat Struct Mol Biol 21:152-159.

22. Carlier L, Haase AS, Burgos Zepeda MY, Dassa E, Lequin O (2012) The C-terminal domain of the Uup protein is a DNA-binding coiled coil motif. J Struct Biol 180:577-584.

23. Xu J, Kiel MC, Golshani A, Chosay JG, Aoki H, et al., (2006) Molecular localization of a ribosome-dependent ATPase on Escherichia coli ribosomes. Nucleic Acids Res 34:1158-1165.

24. Babu M, Aoki H, Chowdhury WQ, Gagarinova A, Graham C, et al., (2011) Ribosome-dependent ATPase interacts with conserved membrane protein in Escherichia coli to modulate protein synthesis and oxidative phosphorylation. PLoS One 6:e18510.

25. Imai H, Abe T, Miyoshi T, Nishikawa S-I, Ito K, et al., (2018) The ribosomal stalk protein is crucial for the action of the conserved ATPase ABCE1. Nucleic Acids Res 46:7820-7830.

26. Nürenberg-Goloub E, Heinemann H, Gerovac M, Tampé R (2018) Ribosome recycling is coordinated by processive events in two asymmetric ATP sites of ABCE1. Life Sci Alliance 1:e201800095.

27. Gouridis G, Hetzert B, Kiosze-Becker K, de Boer M, Heinemann H, et al., (2019) ABCE1 Controls Ribosome Recycling by an Asymmetric Dynamic Conformational Equilibrium. Cell Rep 28:723-734.e6.

28. Nürenberg□Goloub E, Kratzat H, Heinemann H, Heuer A, Kötter P, et al., (2020) Molecular analysis of the ribosome recycling factor ABCE1 bound to the 30S post□splitting complex. EMBO J 39:e103788.

29. Su W, Kumar V, Ding Y, Ero R, Serra A, et al., (2018) Ribosome protection by antibiotic resistance ATP-binding cassette protein. Proc Natl Acad Sci U S A. 115:5157-5162.

30. Laskowski RA, Jabłońska J, Pravda L, Vařeková RS, Thornton JM (2018) PDBsum: Structural summaries of PDB entries. Protein Sci 27:129-134.

31. Vangone A, Bonvin AM (2015) Contacts-based prediction of binding affinity in protein-protein complexes. Elife 4: e07454.

32. Xue LC, Rodrigues JP, Kastritis PL, Bonvin AM, Vangone A (2016) PRODIGY: a web server for predicting the binding affinity of protein-protein complexes. Bioinformatics 23:3676-3678.

33. Wimberly BT, White SW, Ramakrishnan V (1997) The structure of ribosomal protein S7 at 1.9 Å resolution reveals a β-hairpin motif that binds double-stranded nucleic acids. Structure 5:1187-1198.

34. Hosaka H, Nakagawa A, Tanaka I, Harada N, Sano K, et al., (1997) Ribosomal protein S7: a new RNA-binding motif with structural similarities to a DNA architectural factor. Structure 5:1199-1208.

35. Singharoy A, Chipot C, Moradi M, Schulten K (2017) Chemomechanical coupling in hexameric protein-protein interfaces harness energy within V–type ATPases. J Am Chem Soc 139:293-310.

36. Gupta C, Khaniya U, Chan CK, Dehez F, Shekhar M, et al., (2020) Charge Transfer and Chemo-Mechanical Coupling in Respiratory Complex I. J Am Chem Soc 142:9220-9230.

37. Zhong X, Xiang H, Wang T, Zhong L, Ming D, et al., (2018) A novel inhibitor of the new antibiotic resistance protein OptrA. Chem Biol Drug Des 92:1458-1467.

38. Kato T, Tabata S, Sato S (2009) Analyses of expression and phenotypes of knockout lines for Arabidopsis ABCF subfamily members. Plant Biotechnology 26:409-414.

39. Han T-T, Liu W-C, Lu Y-T (2018) General control non-repressible 20 (GCN20) functions in root growth by modulating DNA damage repair in Arabidopsis. BMC Plant Biol 18:274.

40. Zeng W, Brutus A, Kremer JM, Withers JC, Gao X, et al., (2011) A genetic screen reveals Arabidopsis stomatal and/or apoplastic defenses against Pseudomonas syringae pv. tomato DC3000. PLoS Pathog 7: e1002291.

41. Fu Y, Li F, Mu S, Jiang L, Ye M, et al., (2021) Heterophylly Quantitative Trait Loci Respond to Salt Stress in the Desert Tree Populus euphratica. Front Plant Sci 12:692494.

42. Garcia-Barrio M, Dong J, Ufano S, Hinnebusch AG (2000) Association of GCN1–GCN20 regulatory complex with the N-terminus of eIF2α kinase GCN2 is required for GCN2 activation. EMBO J 19:1887-1899.

43. You T, Stansfield I, Romano MC, Brown AJP, Coghill GM (2011) Analysing GCN4 translational control in yeast by stochastic chemical kinetics modelling and simulation. BMC Syst Biol 5:131.

44. Mascarenhas C, Edwards-Ingram LC, Zeef L, Shenton D, Ashe MP, et al., (2008) Gcn4 is required for the response to peroxide stress in the yeast Saccharomyces cerevisiae. Mol Biol Cell 19:2995-3007.

45. Dong J, Lai R, Jennings JL, Link AJ, Hinnebusch AG (2005) The novel ATP-binding cassette protein ARB1 is a shuttling factor that stimulates 40S and 60S ribosome biogenesis. Mol Cell Biol 25:9859-9873.

46. Ando-Akatsuka Y, Shimizu T, Numata T, Okada Y (2012) Involvements of the ABC protein ABCF2 and α-actinin-4 in regulation of cell volume and anion channels in human epithelial cells. J Cell Physiol 227:3498-3510.

47. Kumar V, Garg S, Gupta L, Gupta K, Diagne CT, et al., (2021) Delineating the Role of Aedes aegypti ABC Transporter Gene Family during Mosquito Development and Arboviral Infection via Transcriptome Analyses. Pathogens 10:1127.

48. Zhou C, Yang H, Wang Z, Long G, Jin D (2018) Comparative transcriptome analysis of Sogatella furcifera (Horváth) exposed to different insecticides. Sci Rep 8:8773.

49. Mukherjee S, Seshadri R, Varghese NJ, Eloe-Fadrosh EA, Meier-Kolthoff JP et al., (2017) 1,003 reference genomes of bacterial and archaeal isolates expand coverage of the tree of life. Nat Biotechnol 35:676-683.

50. Abrudan MI, Smakman F, Grimbergen AJ, Westhoff S, Miller EL, et al., (2015) Socially mediated induction and suppression of antibiosis during bacterial coexistence. Proc Natl Acad Sci U S A. 112:11054-11059.

51. Wu J, Li L, Deng Z, Zabriskie TM, He X (2012) Analysis of the mildiomycin biosynthesis gene cluster in Streptoverticillum remofaciens ZJU5119 and characterization of MilC, a hydroxymethyl cytosyl-glucuronic acid synthase. Chembiochem 13:1613-1621.

52. Hou B, Zhu X, Kang Y, Wang R, Wu H, et al., (2019) LmbU, a Cluster-Situated Regulator for Lincomycin, Consists of a DNA-Binding Domain, an Auto-Inhibitory Domain, and Forms Homodimer. Front Microbiol 10:989.

53. Koberska M, Vesela L, Vimberg V, Lenart J, Vesela J, et al., (2021) Beyond Self-Resistance: ABCF ATPase LmrC Is a Signal-Transducing Component of an Antibiotic-Driven Signaling Cascade Accelerating the Onset of Lincomycin Biosynthesis. mBio. 12: e0173121.

54. Wendlandt S, Kadlec K, Feßler AT, Schwarz S (2015) Identification of ABC transporter genes conferring combined pleuromutilin-lincosamide-streptogramin A resistance in bovine methicillin-resistant Staphylococcus aureus and coagulase-negative staphylococci. Vet Microbiol 177:353-358.

55. Liu M, Douthwaite S (2002) Activity of the ketolide telithromycin is refractory to Erm monomethylation of bacterial rRNA. Antimicrob Agents Chemother 46:1629-1633.

56. Nguyen F, Starosta AL, Arenz S, Sohmen D, Dönhöfer A, et al., (2014) Tetracycline antibiotics and resistance mechanisms. Biol Chem 395:559-575.

57. Thaker TM, Mishra S, Zhou W, Mohan M, Tang Q, et al., (2022) Asymmetric drug binding in an ATP-loaded inward-facing state of an ABC transporter. Nat Chem Biol 18:226-235.

58. Crowe-McAuliffe C, Murina V, Turnbull KJ, Kasari M, Mohamad M, et al., (2021) Structural basis of ABCF-mediated resistance to pleuromutilin, lincosamide, and streptogramin A antibiotics in Gram-positive pathogens. Nat Commun 12:3577.

59. Mohamad M, Nicholson D, Saha CK, Hauryliuk V, Edwards TA, et al., (2022) Sal-type ABC-F proteins: intrinsic and common mediators of pleuromutilin resistance by target protection in staphylococci. Nucleic Acids Res 50:2128-2142.

60. Takada H, Mandell ZF, Yakhnin H, Glazyrina A, Chiba S, et al., (2022) Expression of Bacillus subtilis ABCF antibiotic resistance factor VmlR is regulated by RNA polymerase pausing, transcription attenuation, translation attenuation and (p)ppGpp. Nucleic Acids Res 50:6174-6189.

61. Su T, Izawa T, Thoms M, Yamashita Y, Cheng J, et al., (2019) Structure and function of Vms1 and Arb1 in RQC and mitochondrial proteome homeostasis. Nature 570:538-542.

62. Kasari V, Pochopien AA, Margus T, Murina V, Turnbull K, et al., (2019) A role for the Saccharomyces cerevisiae ABCF protein New1 in translation termination/recycling. Nucleic Acids Res 47:8807-8820.

63. Paytubi S, Wang X, Lam YW, Izquierdo L, Hunter MJ, et al., (2009) ABC50 promotes translation initiation in mammalian cells. J Biol Chem 284:24061-24073.

64. Stewart JD, Cowan JL, Perry LS, Coldwell MJ, Proud CG (2015) ABC50 mutants modify translation start codon selection. Biochem J 467:217-229.

65. Cieślak M, Kaźmierczak-Barańska J, Królewska-Golińska K, Napiórkowska M, Stukan I, et al., (2019) New Thalidomide-Resembling Dicarboximides Target ABC50 Protein and Show Antileukemic and Immunomodulatory Activities. Biomolecules 9:446.

66. Zhou J, Lin Y, Shi H, Huo K, Li Y (2013) hABCF3, a TPD52L2 interacting partner, enhances the proliferation of human liver cancer cell lines in vitro. Mol Biol Rep 40:5759-5767.

67. Arora H, Wilcox SM, Johnson LA, Munro L, Eyford BA, et al., (2019) The ATP-Binding Cassette Gene ABCF1 Functions as an E2 Ubiquitin-Conjugating Enzyme Controlling Macrophage Polarization to Dampen Lethal Septic Shock. Immunity 50:418-431.e6.

68. Peterson E, Shippee E, Brinton MA, Kaur P (2019) Biochemical characterization of the mouse ABCF3 protein, a partner of the flavivirus-resistance protein OAS1B. J Biol Chem 294:14937-14952.

69. Pochopien AA, Beckert B, Kasvandik S, Berninghausen O, Beckmann R, et al., (2021) Structure of Gcn1 bound to stalled and colliding 80S ribosomes. Proc Natl Acad Sci U S A. 118: e2022756118.

70. Marks J, Kannan K, Roncase EJ, Klepacki D, Kefi A, et al., (2016) Context-specific inhibition of translation by ribosomal antibiotics targeting the peptidyl transferase center. Proc Natl Acad Sci U S A. 113:12150-12155.

71. Choi J, Marks J, Zhang J, Chen D-H, Wang J, et al., (2020) Dynamics of the context-specific translation arrest by chloramphenicol and linezolid. Nat Chem Biol 16:310-317.

72. Beckert B, Leroy EC, Sothiselvam S, Bock LV, Svetlov MS, et al., (2021) Structural and mechanistic basis for translation inhibition by macrolide and ketolide antibiotics. Nat Commun 12:4466.

73. Wang J, Jiao H, Meng J, Qiao M, Du H, et al., (2019) Baicalin Inhibits Biofilm Formation and the Quorum-Sensing System by Regulating the MsrA Drug Efflux Pump in Staphylococcus saprophyticus. Front Microbiol 10:2800.

74. Svetlov MS, Vázquez-Laslop N, Mankin AS (2017) Kinetics of drug-ribosome interactions defines the cidality of macrolide antibiotics. Proc Natl Acad Sci U S A. 114:13673-13678.

75. Syroegin EA, Flemmich L, Klepacki D, Vazquez-Laslop N, Micura R, et al., (2022) Structural basis for the context-specific action of the classic peptidyl transferase inhibitor chloramphenicol. Nat Struct Mol Biol 29:152-161.

76. Tsai K, Stojković V, Lee DJ, Young ID, Szal T, et al., (2022) Structural basis for context-specific inhibition of translation by oxazolidinone antibiotics. Nat Struct Mol Biol 29:162-171.

77. Lenart J, Vimberg V, Vesela L, Janata J, Balikova Novotna G (2015) Detailed Mutational Analysis of Vga(A) Interdomain Linker: Implication for Antibiotic Resistance Specificity and Mechanism. Antimicrob Agents Chemother 59:1360-1364.

78. Brodiazhenko T, Turnbull KJ, Wu KJY, Takada H, Tresco BIC, et al., (2022) Synthetic oxepanoprolinamide iboxamycin is active against Listeria monocytogenes despite the intrinsic resistance mediated by VgaL/Lmo0919 ABCF ATPase. JAC Antimicrob Resist 4: dlac061.