Control of mRNA Translation by Versatile ATP-Driven Machines

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Translation is organized in a cycle that requires ribosomal subunits, mRNA, aminoacylated transfer RNAs, and myriad regulatory factors. As soon as translation reaches a stop codon or stall, a termination or surveillance process is launched via the release factors eRF1 or Pelota, respectively. The ATP-binding cassette (ABC) protein ABCE1 interacts with release factors and coordinates the recycling process in Eukarya and Archaea. After splitting, ABCE1 stays with the small ribosomal subunit and emerges as an integral part of translation initiation complexes. In addition, eEF3 and ABCF proteins control translation by binding at the E-site. In this review, we highlight advances in the fundamental role of ABC systems in mRNA translation in view of their collective inner mechanics.

mRNA Translation and Surveillance

Genetic information is transcribed into mRNAs, which are translated via adaptors, called tRNAs, into proteins that decipher consecutive mRNA triplet codes into a covalently linked polypeptide chain. The fundamental process of translation is catalyzed by universally conserved rRNAs and ribosomal proteins that collectively form the small (40S in Eukarya; 30S in Archaea and Bacteria) and large (60S in Eukarya; 50S in Archaea and Bacteria) ribosomal subunits. During translation initiation, numerous initiation factors (eIFs) assist the assembly of an elongation competent ribosomal complex (80S in Eukarya; 70S in Archaea and Bacteria) comprising the small and large subunits, mRNA, and methionylated initiator tRNA. The conserved rRNA core and ribosomal proteins recruit elongation factors (eEFs) that coordinate tRNA binding and its transposition along the subunit interface. The ribosomal tRNA-binding sites are subdivided into the aminoacyl tRNA-carrying A-site, the peptidyl-tRNA-carrying P-site, and the exit E-site harboring deacylated tRNA. In a cyclic process, the GTPase elongation factor eEF1A guides the consecutive aminoacylated tRNA to the A-site. The nucleophilic attack of the α-amino group of the aminoacylated tRNA in the A-site on the carbonyl of the peptidyl-tRNA in the P-site is coordinated in the ribosomal peptidyl transferase center (PTC) [1]. Subsequently, the GTPase eEF2 translocates the elongated peptidyl-tRNA from the A-site to the P-site. GTPase activation of elongation factors is universally triggered by the sarcin–ricin loop of the large ribosomal subunit [2]. Canonically, protein elongation terminates at a stop codon, which is not recognized by a tRNA, but by the NIKS motif of the eukaryotic class I release factor eRF1 [3], which is delivered in complex with the GTPase class II release factor eRF3 [4–7] (Figure 1). A swing-out of the middle (M) domain in eRF1 positions a conserved GGQ motif in the P-site of the PTC that catalyzes the hydrolysis of the peptidyl-tRNA [8–11]. The prokaryotic release factors (RF1 and RF2) use the same mechanism of GGQ motif-driven termination but do not require a delivery factor and recognize different stop codons [12–15]. After release of the hydrolyzed peptide, the ribosomal complex is formally (post-)terminated with a bound tRNA in the P-site and the release factor in the A-site.
Evolution has responded to critical situations in mRNA surveillance with new mechanistic solutions and players. Translation may be 3'-stalled at stable secondary structures or 3'-truncated mRNAs, called a “no-go” stall [16]. In vacant ribosomes, the ribosomal subunits can be loosely associated if no mRNA is bound [18]. In hibernation mode, after starvation, the mRNA channel can be arrested by the suppressor protein Stm1 [17]. In these stalled and arrested ribosomes, the cell launches surveillance mechanisms via the no-go decay pathway for ribosome recycling (reviewed in [19,20]).

The eukaryotic class I release factor Pelota (Dom34 in yeast) is recruited to the stalled complexes [18,21–28]. Pelota is neither engaged in codon recognition nor able to hydrolyze the peptidyl-tRNA bond owing to a lack of NIKS and GGQ motifs, respectively and, thus, acts in a distinct manner compared with the termination factor eRF1 [25,29]. Consequently, peptide release does not occur in the no-go decay pathway. In Eukarya and Archaea, ribosomal complexes that are stalled in no-go decay, including complexes after canonical termination and hibernation, are resolved by splitting of the ribosome into its large and small subunits. Splitting is facilitated by the ABC protein of family E1 (ABCE1) in cooperation with class I eIFs (eRF1 or Pelota) [18,22,30,31]. By closing and opening its two head-to-tail-oriented nucleotide-binding domains (NBDs) elicited by ATP binding and hydrolysis, ABCE1 performs a tweezer-like motion [32]. The engagement of the two NBDs transmits...
mechanical power to associated domains and motifs [10,32–35]. ABCE1 harbors an N-terminal iron–sulfur (FeS) cluster domain, which interacts with class I eRFs [10,30,31,33–37]. The ribosome is split upon rotation of the FeS cluster domain, which pushes the class I eRFs into the intersubunit space [32,38]. In contrast to canonical termination, during no-go decay, the polypeptide tRNA remains in the exit tunnel of the large ribosomal subunit and represents the major discriminator of postsplitting ubiquitination and degradation of the aberrant polypeptide. Listerin and nuclear export mediator factor (NEMF), corresponding to Ltn1 and ribosome-associated quality control complex protein 2 (Rqc2) in yeast, recognize the blocked peptidyl-tRNA in the released 60S subunit to ubiquitinate the nascent polypeptide chain [39–41]. The ATPase Cdc48 can extract the ubiquitinated peptidyl-tRNA nascent chain, and the adaptor Vms1 eventually cleaves the peptidyl-tRNA bond via a conserved catalytic glutamine residue that is functionally analogous to the GGQ motif of release factors [42]. In addition, a stall in translation causes a ribosome collision that is sensed by the ubiquitin ligase ZNF598 (Hel2 in yeast) and leads to ubiquitination of the colliding ribosome at the 40S–40S interface [43]. Interestingly, the receptor of activated protein C kinase 1 (Rack1, Asc1 in yeast) of the stalled ribosome is an integral part of the di-ribosome interface and may explain its role in the detection of ribosome stalling [44,45].

Complete ribosome recycling requires the ejection of tRNA and the release of mRNA. In this review, we do not cover the mechanisms of tRNA and mRNA recycling. However, in summary, tRNA is ejected and mRNA is released by either canonical initiation factors [in combination eIF1, -1A, -3, and -3 (Hor1 in yeast)] or the noncanonical initiation factor eIF2D (also known as ligatin or Tma64 in yeast) or corresponding interacting proteins MCT-1–DENR (Tma20–22 in yeast) [30,46]. Release of tRNA and mRNA allows for subsequent canonical initiation. If the mRNA is not released, re-initiation on the same mRNA is possible. An upstream open reading frame (uORF) facilitates translation of the major ORF by re-initiation [47–49]. The key questions are how the ribosome discriminates between initiation and re-initiation, and how splitting and (re-) initiation are mechanistically linked to ensure energy conservation and an efficiently regulated handover. Notably, ABCE1 interacts with initiation factors and occupies ribosomal initiation complexes, providing a link between splitting and initiation [30–32,50–53].

In addition to ABCE1, the yeast ABC protein eEF3 splits the ribosome at the E-site and releases mRNA from the small subunit, allowing for a new round of initiation and translation [54,55]. Interestingly, upon antibiotic arrest of translating ribosomes, bacterial ABCF proteins release the stall and cause pathogenic resistance [56]. However, a range of conformational states remains to be elucidated for the ABC proteins of the subfamily F.

From Diversified, Meandering Functions to Ribosome Recycling: A Brief History

While ABCE1 is one of the most conserved proteins among Eukarya and Archaea, diverse functions have been linked to it. The first report described ABCE1 as an RNase L inhibitor (Rli1) that is upregulated upon viral infection [57]. RNase L, encoded in higher vertebrates only, is activated by an interferon effector pathway via 2’-5’ oligoadenylation and cleaves single-stranded RNA upon viral infection [58–60]. ABCE1 has also been described as host protein 68 (HP68), required for HIV capsid assembly [61,62]. However, the mechanistic role of ABCE1 in these processes remains unclear. The evidence for a role in translation was found in the interaction of ABCE1 with the small ribosomal subunit and initiation factors, thus leading to the hypothesis that ABCE1 is involved in translation initiation [50,52,53]. ABCE1 was also discussed in the context of ribosome biogenesis because it localizes in the nucleus, stabilizes pre-rRNA and releases pre-80S-like complexes in a translation-like cycle for maturation of pre-40S subunits [53,63–66].
The universal and fundamental function of ABCE1 in ATP-driven ribosome splitting was determined through reconstitution assays in Eukarya and Archaea [22,30,31]. It is now well accepted that silencing of ABCE1 affects the entire translation cycle and the protein homeostasis of the cell. The effects, which are more severe for short-lived and less abundant proteins, can involve several pathways that are not directly linked to ABCE1 (reviewed in [67]). Over the past few years, ABCE1 has also emerged as a player in cancer development because its expression positively correlates with tumor progression [68–70]. Furthermore, the FeS clusters of ABCE1 have been discussed as a primary target for oxidative stress and are likely to be essential factors for the indispensable character of mitochondria and their FeS cluster biogenesis pathway in eukaryotes [66,71].

The Role of the Recycling Factor in Translation Termination

Canonical translation termination is defined by the hydrolysis of the peptidyl-tRNA bond by the G{\text{GQ}} motif in the M domain of eRF1 and subsequent peptide release [8–10,72,73]. In the no-go decay pathway, the eRF1 paralog Pelota (Dom34) is delivered to the A-site by the eRF3 paralog Hbs1 [19,20,74–77] (Figure 2A). In association with ABCE1 at the ribosome, Pelota adapts the same conformation as eRF1 in post-termination complexes with a swung-out M domain [10,33–35]. However, Pelota lacks the G{\text{GQ}} motif and, therefore, is deficient in peptidyl-tRNA hydrolysis and peptide release. Thus, we designate the conformation, in which the 80S-bound eRF1 or Pelota adjusts a swung-out displacement of the M domain to the peptidyl-tRNA, as the post-termination state (Figure 2B). This post-termination state was not observed in complexes occupied by class I and II eRFs [10,35,78,79]. Consistently, ABCE1 can bind to ribosomes only after eRF3/Hbs1 is released from the A-site, since all contact points for translational GTPases on the ribosome are used [10,30,33–35,80]. In addition, the M domain of eRF1 must be repositioned to allow for ABCE1 binding to the A-site in the presplitting state. If peptidyl-tRNA hydrolysis is prevented by mutation of the G{\text{GQ}} tip to AGQ or AAQ in eRF1, the 80S-eRF1-

Figure 2. Mechanisms of ABCE1 in Termination. (A) In the pretermination state, release factors of class I and II are bound to the ribosome. The middle (M) domain of eRF1 is not swung out towards the peptidyl-tRNA bond (PDB: SLZ2) [33]. (B) In the post-termination and presplitting state, ABCE1 is bound to class I eRFs (PDB: SLZ2) [33]. Structural rearrangements occur in the M and C-terminal (C) domains of eRF1. The M domain with the G{\text{GQ}} motif swings out towards the peptidyl-tRNA bond for hydrolysis. A superposition of these two states illustrates the clashes (red/blue line) between the hinge 1 motif and nucleotide-binding domain 2 (NBD2) of ABCE1 and eRF1 in the pretermination state (eRF1 in transparent blue). (C) In the postsplitting state, the iron–sulfur (FeS) cluster domain of ABCE1 is rotated towards helix 44 and clashes on this rotation trajectory (red/blue and red/green lines) with the M and C domain of eRF1 (transparent in background) as well as with UL14 of the large ribosomal subunit, facilitating ribosomal subunit splitting and subsequent anti-association, respectively (UL14 in transparent green) (PDB: 5LL6) [32].
ABCE1 complex is arrested before splitting in the post-termination state (Figure 3) \([30,34,35,72]\). In summary, the structurally resolved termination states indicate a role of ABCE1 in termination that was also suggested in functional studies based on observed accelerated peptide release with ABCE1 \([22]\).

**Mechanistic Basis and Structural Insights into Ribosome Splitting**

In recent years, several post-termination complexes have been reported in a similar conformation with bound ABCE1 and class I eRFs, eRF1 or Pelota \([10,33–35]\). A distinct splitting and quality control mechanism becomes apparent via either eRF1 or Pelota, based on the presence of the peptidyl-tRNA \([22]\) (Figure 3). Notably, a lack in peptidyl-tRNA hydrolysis is common between eRF1(AGQ) and Pelota, but Pelota-ABCE1 are able to split ribosomes.

**Figure 3. Mechanochemistry of ABCE1.** The translation progress is illustrated by the colored circles for the indicated release factors and nucleotides. The conformational and nucleotide-binding states of ABCE1 are indicated in the outer circle. eRF3 is released from the termination complex (1) and ABCE1 is free to bind at the A-site in an ATP-binding-dependent fashion; consequently, the post-termination state (2) is arrested by AMP-PNP. In addition, peptide release is stimulated by ATP binding in ABCE1. eRF1(AGQ) is impaired in peptidyl-tRNA hydrolysis and post-termination complexes (2) accumulate with bound ABCE1. Nucleotide-binding site II (NBS II) in ABCE1 occludes ATP and activates NBS I to bind and hydrolyze ATP until the iron–sulfur (FeS) cluster domain is rotated for 60S release during the splitting step (3). In the postsplitting state (4), the nucleotide-binding domains (NBDs) are closed and occlude two ATPs. Initiation factors (eIFs) bind to the postsplitting complex and form the preinitiation complex (5), in which ABCE1 is in the closed state with a rotated FeS cluster domain. The exact role of ABCE1 in the recruitment of initiation factors and its regulation are unknown (indicated by a dashed line). At a stop-codon in the elongation cycle (6), eRF1 is delivered by eRF3 in the pretermination state (7).
whereas eRF1(AGQ)-ABCE1 are arrested before splitting. In addition, splitting occurs preferentially on mRNAs with no or only short 3’ mRNA lengths after the last decoded sense codon [18,22]. Along the same line, the N-terminal domain of Pelota comprises a prolonged tip compared with eRF1, which fits into the free A-site because the mRNA is cleaved through no-go decay [21,25,35,81].

During ribosome splitting, ABCE1 must adapt conformations that tear the subunits apart. Strikingly, in the 4OS-ABCE1 postsplintering complex, the FeS cluster domain is rotated by 150° towards a cleft between helix 44 and uS12 [32] (Figures 2C and 3). The trajectory of the FeS cluster domain results in a collision with class I eRFs, which act as a wedge in the intersubunit space, pushing the subunits apart. A similar movement of the FeS cluster domain is evident from the structural analysis of the postsplintering complex in Archaea [38]. In a second step, the position of the FeS cluster domain collides with uL14 of the large ribosomal subunit and blocks re-association, which may serve as a timer or allosteric checkpoint for initiation factors to join the 40S subunit after splitting [32]. In the postsplintering state, the two NBDs of ABCE1 are fully closed, occluding two ATP molecules. This final snapshot sets the scope for conformational rearrangements of the ABC machinery between the presplintering and the postsplintering state. However, the structural switches in the presence of class I eRFs and the necessary structural reorganization for ABCE1 release remain to be elucidated.

**How Does ABCE1 Power Ribosome Splitting?**

ABC proteins are evolutionarily conserved chemomechanical devices that enforce conformational switches for processes including chromosome segregation, DNA repair, membrane transport, and, in the case of ABCE1 and ABCF proteins, remodeling of the ribosome and controlling mRNA translation. All ABC systems include two NBDs oriented head-to-tail, forming two composite sites for nucleotide binding and hydrolysis. Each NBD harbors a RecA-like and an α-helical subdomain. The RecA-like fold is common to a large class of ATPases, including helicases of superfamilies I, II, and III, AAA+–ATPases, F-type and V-type ATPases, and ABC proteins [32]. The nucleotide-binding motifs are the Walker A (P-loop) and B motifs present on each NBD. In addition, the NBDs comprise a signature motif (C loop) that is provided in trans by the opposite NBD. An aromatic loop positions the nucleotide base by π–π stacking. The Q-loop and His-switch are involved in Mg(II) and γ-phosphate coordination [83–85]. The two nucleotide-binding sites (NBS I and II) become asymmetric in the case of nonidentical NBDs or due to auxiliary motifs, domains, or interaction partners. In the open state, nucleotides can freely exchange and bind to each NBS. In ABC proteins, ATP binding triggers the engagement of the two NBDs and nucleotide occlusion, while ATP hydrolysis causes opening of the NBDs. Substitution of the catalytic glutamate residue close to the Walker B motif of ABCE1 with either glutamine or alanine impairs ATP hydrolysis, and promotes NBS closure and binding to the small ribosomal subunit [31,86]. Remarkably, if ATP hydrolysis in NBS II of ABCE1 is blocked, hydrolysis in NBS I is enhanced tenfold, indicating a control mechanism [31,86] (Figure 3). This effect can be explained by a regulatory mechanism in NBS II, which is likely facilitated by ABCE1 binding to the ribosome.

At first, ribosome splitting appears to be dependent on ATP hydrolysis because splitting was blocked by AMP-PNP [22,30] (Figure 3). However, the twin ATPases occlude two AMP-PNP molecules in the postsplintering state, indicating that ATP binding drives splitting [32]. The discrepancy between these observations can be filled with a refined model, which suggests several rounds of ATP binding and hydrolysis in the hyperactivated NBS I that transmits mechanical power to the FeS cluster domain until the final postsplintering state is reached [86] (Figure 3). In this case, the splitting efficiency is dependent on the stability of subunit
association that correlates with the presence of the peptidyl-tRNA bond. Notably, eRF1 (AGQ) cannot hydrolyze the peptidyl-tRNA bond and ribosome splitting is prevented. Thus, energy consumption is increased, likely due to repeated rounds of NTPase in ABCE1 [30]. If splitting is not possible, ABCE1 will occupy stalled ribosomes and may help to mediate binding of yet unidentified interaction partners of defective nascent protein and mRNA decay pathways. For example, stalled translation at the outer mitochondrial membrane recruits Pelota, ABCE1, and NOT4, which ubiquitinates ABCE1 and triggers mitophagy [87]. Thus, RNA and protein quality control are linked to organelle quality control, which opens an exciting new research avenue.

What Is the Role of the Recycling Factor in Translation (Re-)Initiation?

Translation initiation on the small ribosomal subunit requires the recognition of the start codon of mRNA (AUG) by initiator tRNA (tRNA) and eIFs (reviewed in [47,88]). The 43S preinitiation complex (40S-eIF1α/-1A/3/5) is loaded with tRNA, by the ternary eIF2(GTP)-tRNA complex. The eIF4F multiprotein factor and the poly(A)-binding protein (PABP) both assemble a messenger ribonucleoprotein particle (mRNP) that activates mRNA by a closed loop between the 3'-poly(A) tail and the 5'-7-methylguanosine cap. The 43S preinitiation complex binds near the 5'-cap of the mRNP and scans the mRNA in a 5’-to-3’ direction for an AUG start codon. After positioning of the start codon at the P-site by base-pairing with the tRNA, the 48S initiation complex is formed. Furthermore, eIF5 triggers GTP hydrolysis in eIF2, which is subsequently released. Initiation factors dissociate and eIF5B(GTP) mediates 60S joining and forming the 80S initiation complex. The complex is then ready to start elongation at the tRNA, in the P-site.

Each round of mRNA translation is linked to the next to ensure efficient regulation of the cycle and energy conservation. Thus, it is not surprising that translation factors share recycling and initiation functions, such as eIFs 1/1A/3/3j and Ligatin or MCT-1–DENR, which mediate the release of mRNA and tRNA and facilitate the formation of the 43S preinitiation complex [30,46,89]. Importantly, the recycling of mRNA and tRNA by initiation factors requires the ribosomes to be split by ABCE1 [30,46,90]. Hence, we hypothesize that ABCE1 is closely linked to initiation, since it directly interacts with initiation factors and catalyzes the high-level formation of preinitiation complexes [50,52,53]. Cryo-EM reconstruction of native pull-outs of ABCE1 with AMP-PNP revealed a 43S preinitiation complex with bound ABCE1 in the postsplitting state [32]. After start-codon recognition, ABCE1 was found to be part of the late 48S initiation complex [51]. Intriguingly, the FeS cluster domain of ABCE1 in the postsplitting state may fit well between the ribosome and the eIF3 b-g-i module in initiation complexes [91] (Figure 4A). In addition, start-codon recognition by eIF2 could be affected by the FeS cluster domain interacting with helix 44 [123]. The preinitiation complex with ABCE1 showed eIF2γ released from helix 44 and the tRNA locked in the P-site [51]. Hence, the interaction of the FeS cluster domain and eIF2γ with helix 44 appears mutually exclusive or requires a processive mechanism (Figure 4B). Nevertheless, direct interactions of ABCE1 with initiation factors have not yet been observed in structural studies. Alternatively, ABCE1 could serve as an antiassociation timer to ensure sufficient initiation factor recruitment [32,92].

In higher eukaryotes, initiation may also occur after a 5’-uORF, which regulates the translation efficiency of the major ORF upon re-initiation (reviewed in [93]). After splitting by ABCE1, the 40S subunit remains bound to mRNA and can re-initiate at nearby 5’-AUG start codons [49]. ABCE1 depletion resulted in re-initiation of post-terminated 80S ribosomes at sense codons in the 3’-untranslated region [94]. Importantly, re-initiation at AUG codons requires preceding ABCE1-driven ribosome splitting [30,46,49]; mRNA footprinting of ORF 3’-ends revealed 40S recycling intermediates by longer reads that were linked to 40S-ABCE1-eIF complexes [124]. Hence, ABCE1 may have an additional role after splitting that links termination and re-initiation.
Recently, the re-initiation complex 40S-eIF2D (ligatin) was structurally described [95]. The WH domain of eIF2D would clash with the FeS cluster domain of ABCE1 in the postsplitting state. Hence, these two factors mutually exclude each other in the resolved conformations, suggesting two distinct pathways of re-initiation or a step-by-step mechanism (Figure 4C). Further studies are needed to reveal the effect of ABCE1 on recycling and re-initiation within specific mRNA populations.

Impact on Ribosome Homeostasis and Ribosomopathies

Ribosome splitting by ABCE1 regulates the availability of ribosomal subunits, which is crucial for cell homeostasis. In the ‘ribosome concentration hypothesis’, limiting levels of translating ribosomes cause preferential translation of efficiently initiating mRNAs and lead to disorders in cell homeostasis [67,96]. In the case of inefficient recycling at stop codons, translation continues into the 3′-untranslated region, which may evoke the generation of immunogenic peptides [27,94,97,98]. Surveillance mechanisms, such as nonsense-mediated decay at
premature stop codons, discharge into the no-go decay pathway due to mRNA cleavage and recycling by ABCE1 [19,99]. The mRNA levels of ABCE1 and Hbs1 are significantly downregulated in brain tissues of patients with Parkinson’s disease [87]. Interestingly, a loss of ABCE1 is complemented by increasing levels of Pelota, as described in primary reticulocytes for hemoglobin production during hematopoiesis [97]. This indicates alternative recycling pathways involving Pelota, which manifest as physiological disorders upon knockout of the gene encoding Pelota (PELO) [100]. Global translation is upregulated and short ribosome footprints accumulate, resulting in abnormal differentiation and hyperproliferation of epidermal stem cells [100]. Undoubtedly, ABCE1 interplay with release factors has a crucial role in ribosomopathies, which are associated with translation efficiency and ribosome availability [67].

Ribosome Remodeling by the Fungal ABC-Type Elongation Factor eEF3
In addition to the two canonical elongation factors eEF1A and eEF2, the fungal-specific ABC-type elongation factor eEF3 is essential for tRNA release at the E-site [55,101,102]. In addition, eEF3 drives ribosome splitting at the E-site [54,55] and antagonizes translational repression by the starvation factor Stm1 [103]. eEF3 and eEF1A can occupy the ribosome simultaneously and may coordinate the expulsion of tRNA at the E-site and the loading of aminoacylated tRNA at the A-site [104,105]. The N-terminal HEAT and 4-helix bundle domains correspond to the FeS cluster domain in ABCE1 and are similarly linked by long unstructured linker regions that contain in one state a short helix, which can unwind and allow for rotation, analogous to that seen in ABCE1 [105]. In the presence of AMP-PNP, eEF3 binds to the 80S ribosome with a tRNA in the P-site. In the cryo-EM structure, the NBDs of eEF3 are dimerized and most likely occlude ATP, as seen in the postsplitting state of ABCE1 (Figure 5A,B). The conformational switch of the associated HEAT and chromo domains in the ABC protein remains elusive. Based on X-ray structures of eEF3 [105], NBDs must perform a tweezer-like motion that opens the ABC system at the ribosomal E-site upon ATP hydrolysis, followed by ribosome splitting [54]. This indicates a modus operandi different from ABCE1, which is in a closed state after splitting.

Antibiotic Resistance and Translational Control
Multidrug-resistance mediated by ABC proteins is of key medical relevance for anticancer therapies and treatments for pathogen-associated infections. The largest group comprises ABC transporters, which expel drugs and toxins out of the cell via the associated transmembrane domains [85,106]. The ribosome is the major target for antibiotics [56]. Strikingly, ABC proteins of the F subfamily displace drugs from bacterial ribosomes [107]. In a working model, ABCF proteins bind to the ribosomal E-site, as structurally elucidated for the Escherichia coli ortholog Etta (YijK). Etta comprises two NBD promoters with additional arm and linker domains, which interact with L1 of 60S and the P-site tRNA, respectively. Etta binds to the ribosome in an ATP-dependent manner as an NBD dimer in a closed conformation [108] (Figure 5C). Substitution of the catalytic glutamate arrests translation in vivo after initiation complex assembly and formation of the first peptide bond [109]. Etta blocks translation progression into the elongation cycle by occupying the E-site, which was validated by a lack of tripeptide formation and polypeptide loss after induction of a catalytically inactive variant. The arrest was related to Etta sensing the low-energetic state at starvation. As soon as energy levels in the cell returned, Etta facilitated the restart of cell growth [109]. However, the link between the ATPase cycle of Etta and the conformational switches of the associated domains leading to tRNA interaction remains unclear.

These aspects are directly connected to the antibiotic-resistance mechanism of ABCF proteins. Several antibiotics target the PTC and inactivate its transpeptidase reaction. Recently, the
ABCF protein VgaA was reported to reset peptidyl-transferase activity after antibiotic treatment that was followed by restored puromycin reactivity, which mimics an aminoacyl-tRNA and prematurely terminates translation by puromycylation of the nascent chain [110]. Importantly, an ATPase-silenced variant did not restore the peptidyl-transferase activity but rather inhibited it independently of antibiotic presence. This indicates that ATP hydrolysis is the trigger for VgaA release. The ABCF protein and macrolide-resistance factor MsrE utilizes the same mechanism for displacement of the streptotagmin B drug class [111]. In its ATP-bound form, MsrE positions an extended loop that corresponds to the linker domain of Etta in the PTC. The loop shifts the P-site tRNA position and bends its acceptor stem towards the A-site [111], releasing the drug. VmlR is a resistance factor for the streptotagmin A class of antibiotics and binds in a similar conformation to the 70S ribosome for drug displacement [112]. It differs from MsrE and Etta in an additional C-terminal extension that interacts with the small ribosomal subunit at its Shine-Dalgarno cavity. It is still pending whether the length of nascent peptide influences drug displacement by ABCF proteins and whether the translation continues after resetting.

Figure 5. ABC-Type Machines at the Ribosome. (A) ABCE1 (PDB: 5LL6) [32] bound to the 40S subunit in a closed conformation with a rotated iron–sulfur (FeS) cluster domain that interacts with helix 44 and u512. (B) eEF3 bound to the 80S ribosome nascent chain complex in the post-translational state (PDB: 2IX8) [105]. The chromo domain interacts with rpl5/11 and 5S rRNA. The HEAT domain interacts with the 40S head rpSX2 and helix 39 of 60S. (C) Etta (YjjK, ABCF family) bound to the bacterial 70S ribosome (PDB: 3J5S) [108]. The linker domain interacts with the P-site tRNA and stabilizes it. The arm domain binds the L1 stalk. The ABC systems are shown in similar orientation and in the ATP-occluded state.
Concluding Remarks and Outlook

Tightly controlled at all stages, translation is crucial for the quality control of nascent polypeptide chain and mRNA. ABCE1 bridges translation termination, splitting, and initiation by acting as a structural and functional linker [32,113]. Remarkably, the nature of the interaction of ABCE1 is transient and must be applied to various situations, which is solved by the introduction of auxiliary factors, such as eRF1 and Pelota in the case of termination and mRNA surveillance, respectively. Moreover, intramolecular communication between the two asymmetric NBSs may trigger diverse events in the recycling process that are mediated by ABCE1 [86]. In addition, ABCF proteins and eEF3 show distinct modes of chemomechanical action at the ribosome. The interaction between ABCE1 and various partners in translation initiation is enigmatic and may depend on specific mRNAs and initiation pathways (see Outstanding Questions). In accordance with the ‘specialized ribosome hypothesis’, physiological disorders based on ribosomopathies may only be visualized in a subset of translating ribosomes or in specific tissues [67,87,114]. Furthermore, specific states and interactions may be populated only for a short time or might only be discernable by an acceleration in kinetic studies upon addition of ABCE1, but cause disorders in sensitive processes, such as embryogenesis and morphogenesis, as shown for ABCE1 mutants in Drosophila melanogaster and Caenorhabditis elegans [115,116] or selective autophagy of organelles [87].

ABCF proteins modulate ribosome-associated kinases (GCN20) [117], influence ribosome biogenesis (Arb1) [118], are involved in transposon excision (Usp) [119–121], and promote translation initiation (ABC50) [122]. The diversity of functions driven by ABC-type systems is built on the nucleotide-based control of associated domains. Without doubt, we are only beginning to understand how these sophisticated molecular machines are constructed, how they allosterically couple modular domains to the dimerization of the NBDs, and how they control the recruitment and release of interaction partners in many fundamental processes. In future studies, trapping of transition states will be the key challenge to understand the inner mechanics of ABC systems.

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