Ribosome recycling is the final – or first – step of the cyclic process of mRNA translation. In eukaryotes and archaea, dissociation of the two ribosomal subunits proceeds in a fundamentally different way than in bacteria. It requires the ABC-type ATPase ABCE1 [previously named RNase L inhibitor (Rli)1 or host protein (HP)68], but the reaction and its regulation remain enigmatic. Here, we focus on ribosome recycling in its physiological context, including translation termination and reinitiation. The regulation of this crucial event can only be described by a systems biology approach, involving a network of proteins modulating mRNA translation. The key role of ABCE1, and what is known about the structure and function of this versatile protein, is discussed.

Ribosome splitting must occur in distinct cellular pathways
Translation of mRNA takes place in four steps: initiation, elongation, termination, and ribosome recycling. During recycling, the ribosome is split into the small and large subunits (from 80S into 40S and 60S in eukaryotes; from 70S into 30S and 50S in archaea and bacteria) [1,2]. Ribosome recycling serves as the link between translation termination and initiation because a new round of translation is initiated by various factors on the released small ribosomal subunit [3]. Ribosome recycling can take place after successful elongation and termination, but it must also occur if polypeptide synthesis fails or if ‘empty’ (vacant) ribosomes are assembled. Ribosomes can be stalled by damaged or structured mRNA (e.g., hairpins), and they are recycled through a mechanism called ‘mRNA surveillance’ [4,5]. Vacant ribosomes accumulate during nutrition stress to prevent protein biosynthesis, and then they are split after stress release [6]. Intriguingly, ribosome biogenesis in eukaryotes involves the assembly and subsequent splitting of ‘80S-like’ ribosomal complexes as a means of quality control [7].

Ribosome splitting proceeds differently in eukaryotes and archaea than in bacteria. In eukaryotes and archaea it requires ABCE1 (also called Rli1p or HP68), an unusual member of the ATP-binding cassette protein family, together with the release factor 1 (e/ARF1) and the paralogous proteins Dom34 or eApelota. The reaction catalyzed by the ATPase ABCE1 remains poorly understood. Two crucial questions are beginning to be addressed after extensive research effort: what is the allosteric relation between ABCE1, the release factors, and the ribosome, and how does ABCE1 perform ribosome splitting at the molecular level? The regulation of ribosome recycling is fundamental for efficient protein biosynthesis as well as cell homeostasis and viability. Here, we review the mechanisms of ribosome recycling. We pay special attention to the structure and function of the crucial and enigmatic regulator ABCE1, which is also essential for HIV capsid assembly and for innate immunity against viral RNA.

Ribosome recycling: a comparison between the three domains of life
Although protein biosynthesis is generally conserved, there are striking differences between bacteria (Figure 1a), archaea (Figure 1b), and eukaryotes (Figure 1c). In all cases, translation termination and ribosome recycling involve numerous translation factors (Box 1). It is through these translation factors and a series of nucleotide exchange and hydrolysis events on the ribosome that translation termination and ribosome recycling are coupled and regulated [8]. In all domains of life, translation termination begins with recruitment of a ternary complex of a class I release factor and a GTPase with GTP to the ribosomal A site (step 1). In bacteria, binding of the class I release factor RF1/RF2 triggers peptide release. Subsequent GTP hydrolysis by the GTPase RF3 accelerates the dissociation of RF1/RF2•RF3 from the ribosomes (step 2), resulting in the post-termination complex (post-TC). By contrast, in eukaryotes and archaea it is GTP hydrolysis by the GTPase (eRF3 in eukaryotes and eRF1s in archaea) that ensures efficient peptide release by rearranging the conformation of the class I release factor eRF1 or aRF1, respectively. Afterwards, GDP-bound eRF3/eRF1α dissociates to yield a post-TC that still contains the class I release factor (step 1-2). However, peptide release is not a prerequisite for ribosome splitting [8].

One of the most striking differences between the three kingdoms of life is the absence of the ribosome-recycling factor (RRF) from eukaryotes and archaea. RRF is delivered to the bacterial post-TC by elongation factor EF-G•GTP (step 3). EF-G and RRF work together, in a GTP hydrolysis-driven process, to promote ribosome splitting (step 4) [9,10]. In eukaryotes and archaea, ABCE1 is instead recruited to the post-TC after dissociation of eRF3/eRF1α (step 3). In eukaryotes, a combination of ABCE1•ATP with release factors eRF1 and eRF3•GTP (or excess of eRF1) is needed to split post-TCs efficiently (step 4) [8,11]. Two different models are proposed for the mechanism of ribosome splitting by ABCE1 in eukaryotes.
Figure 1. Ribosome recycling in bacteria (a), eukaryotes (b), archaea (c), and mRNA surveillance (d). In (a–c), a stop codon (red) is accommodated in the A site at the end of the elongation phase. In (d), a secondary structure or no-stop end in mRNA prevents continuation of the elongation process. Step 1: A ternary complex of class I release factors, class II release factors and GTP binds to the A site. Bacterial release factor (RF)1 binds amber and ochre, whereas RF2 binds ochre andopal stop codons by conserved PAT and SPF motifs, respectively [76,77]. Class I release factors e/aRF1 in eukaryotes and archaea recognize all three stop codons by the NIKSI- and Y-X-C-X,F motifs in their N-terminal (N) domains [16]. In mRNA surveillance, the e/aRF1 paralogs Dom34 and e/aPe lottery bind mRNA and rRNA at the A site. The interaction is mediated by two loops in the N domain, containing one acidic and one basic motif [18,78]. Step 2: During the stop codon (or mRNA) sensing, a complex cascade of conformational changes is induced in the ribosome and its associated proteins. In bacteria, stop codon binding by RF1/2 triggers peptide release via the conserved GGG motif [79]. The subsequent hydrolysis of GTP by RF3 promotes dissociation of RF1/2/RF3 from the ribosome [80,81]. In eukaryotes and archaea, e/aRF1 induces GTP hydrolysis by eRF3:eEF1α. GTP hydrolysis unlocks the middle (M) domain of e/aRF1, which subsequently swings out 45 Å [45,82]. This conformational change enables e/aRF1 to hydrolyze the tRNA-peptidyl bond. In mRNA surveillance, the M domain of Dom34 and e/aPe lottery is not capable of peptide release, but swings in a similar fashion. Recently, ABC-type ATPase ABCE1 has also been proposed to trigger this movement [40]. The GDP-bound ATPase dissociates and the class I release factor remains at the A site. Peptide release is not a prerequisite for ribosome recycling, because it does not occur in mRNA surveillance. Step 3: In bacteria, ribosome splitting is initiated by the ribosome recycling factor RRF, which is delivered to the ribosomal A site by EF-G (ATPas) [83]. In eukaryotes and archaea, the post-termination complex (post-TC) recruits ABCE1. Step 4: Nucleotide hydrolysis by ABCE1 or EF-G promotes ribosome splitting in eukaryotes and bacteria, respectively [8,11,83]. In archaea, the binding of ATP to ABCE1 induces ribosome splitting [19]. After dissociation of the large ribosomal subunit, the order in which the P site tRNA, class I release factors, and mRNA detach is not clearly known. In bacteria, two models are proposed. In the first model, tRNA and mRNA release are catalyzed by RRF and EF-G. In the second model, initiation factor (IF)3 is required for tRNA release, leading to the spontaneous detachment of mRNA [84–86]. Step 5: In bacteria, IF3 binds first to the 30S and reinitiates translation [54,86]. In archaea, ABCE1 remains bound to the 30S until ATP hydrolysis occurs. Its interaction with initiation factors has not yet been investigated [19]. In eukaryotes, ABCE1 is proposed to disassociate after ribosome splitting [8,40], but it has also been shown to interact with several initiation factors [20,23,24].
and archaea (Box 2). In addition to ABCE1-dependent ribosome splitting, the eukaryotic elongation factors eIF1, elF1A, elF3, and their loosely associated subunit eIF3j can promote a passive splitting of the post-TC in vitro at low (nonphysiological) Mg2+ concentrations [12]. In eukaryotes and archaea, the order and kinetics of tRNA and mRNA dissociation, as well as the role of ABCE1 in translation reinitiation (step 4-5) have not yet been clarified.

ABCE1 functions not only at the post-TC, but also on stalled ribosomes. This pathway is called mRNA surveillance, which is not known in bacteria and represents another prominent disparity of translation-associated processes between the kingdoms of life.

**mRNA quality control: ribosome recycling in case of emergency**

The mRNA surveillance pathway is initiated when the ribosome is stalled and further elongation is prevented [4] (Figure 1d). The eukaryotic mRNA surveillance pathways, no-go decay (NGD) [13] and no-stop decay (NSD) [14,15], require ABCE1 [6,8]. The proteins Dom34 (yeast)/Pelota (mammals) and their interaction partner Hbs1 (GTPase) are paralogs of the eRF1–eRF3 system [16]. Similarly, these proteins are recruited to the A site of stalled ribosomes as a ternary complex (step 1). GTP hydrolysis by Hbs1 triggers conformational changes in Dom34/Pelota, but unlike translation termination after productive elongation, the peptidyl-tRNA remains intact (step 2). GTP hydrolysis and the subsequent dissociation of Hbs1 are required for binding of ABCE1 to Dom34/Pelota at the ribosomal complex (step 3) [12]. The molecular mechanisms of ribosome recycling after canonical termination and mRNA surveillance are considerably more similar in the subsequent steps. However, one distinction is that intact peptidyl-tRNA is released during ribosome splitting by ABCE1 and Dom34/Pelota [6,8]. Surprisingly, yeast Dom34 and Hbs1 do not require ABCE1 for the rescue of ribosomes stalled by NGD [17]. In archaea, the role of the ABCE1 interaction with aPelota and aEF1α during ribosome rescue is poorly understood [18]. However, ABCE1 plays a key role in catalysis and regulation of ribosome splitting at post-TCs and stalled ribosomes [19].

**Multitasking of cellular functions by ABCE1**

The strong sequence conservation of ABCE1 in eukaryotes and archaea (67% identity between human and yeast, 49% identity between human and the closest archaeal ortholog from *Methanocaldococcus fervens*) indicates a fundamental and essential function for this enzyme [20,21]. Initially, ABCE1 was discovered as RNase L inhibitor (Rli1) (Figure 2) [12]. RNase L plays a significant role in the inhibition of cellular protein synthesis and the resistance...
to viral infection. Double-stranded (viral) RNA activates the interferon-inducible 2'-5'-oligoadenylate synthase (OAS) pathway. Synthesized 2'-5'-oligoadenylates in turn activate RNAse L, which subsequently cleaves cellular single-stranded RNA as well as 18S and 28S rRNA [18,19], thus preventing mRNA translation in infected cells. The mechanism of RNAse L inhibition by ABCE1 is not clearly understood.

Growth and cell survival studies in Drosophila melanogaster were the first to point to a role for ABCE1 in translation regulation. Pixie, the ortholog of ABCE1 in Drosophila, was identified as an essential gene required for normal growth and translation [22,23]. Furthermore, the association of ABCE1 with initiation factors eIF2, eIF3, eIF5j (Hcr1 in yeast) and eIF5 suggests that ABCE1 may also play a role during translation initiation [20,23–26].

Interestingly, ABCE1 has been functionally implicated in an important link between ribosome recycling and ribosome biogenesis [7]. Ribosome biogenesis in eukaryotes involves the transcription of precursor rRNAs, their processing and folding, and finally binding of ribosomal proteins, which is catalyzed by several assembly factors. The early steps in ribosome biogenesis take place in the nucleolus, after which the immature ribosomes move to the cytosol [27,28]. Notably, ribosome maturation involves a translation-like cycle, in which eIF5B promotes assembly of the 60S ribosomal subunit with the pre-40S subunit to form 80S-like complexes in the absence of mRNA. 80S-like ribosomes are subsequently split by ABCE1 [7]. This cycle has been proposed to be a quality control event for 40S subunits before they enter the translation pool, because 80S-like complexes resistant to ABCE1 activity are rapidly degraded. Thus, ABCE1 provides a molecular link between ribosome recycling and ribosome biogenesis and quality control.

ABCE1 may also be a potential target for therapeutic approaches because it is involved in HIV-1 Gag capsid assembly [29]. Immune depletion and complementation by recombinant ABCE1 (identified as HP68) have shown that the enzyme is essential for the formation of mature 750S HIV-1 capsids [29]. ABCE1 has been found to be transiently associated with Gag complexes during intermediate steps of capsid maturation, including 80S and 500S particles, but not 10S and 750S particles. ABCE1 also interacts with the viral proteins Gag-Pol and Vif. Interestingly, ABCE1 exhibits the same association pattern with HIV-2 and various simian immunodeficiency viruses. Thus, ABCE1 has been proposed to participate in ATP-dependent post-translational steps of Gag–capsid assembly, which are conserved among primate lentiviruses [30]. We speculate that simultaneous Gag translation and packing of viral RNA requires tight, local regulation by ABCE1. However, the mechanistic basis for this function of ABCE1 remains elusive.

In summary, active involvement of ABCE1 in various cellular processes highlights its role as a master key and further research is required to understand the structure and dynamics of this essential enzyme.

Structure and conformational dynamics of ABCE1
Several structures of ABCE1 have been resolved for an ABCE1 mutant lacking the FeS cluster domain from Pyrococcus furiosus (pfABCE1ΔFeS, 1YQT.pdb) and from Sulfolobus solfataricus (ssABCE1ΔFeS, 3OZJ.pdb) with bound ADP and Mg²⁺ at 1.9 and 2.0 Å resolution, respectively [19,31]. Importantly, a structure of a complete ABCE1 from Pyrococcus abyssi (paABCE1, 3BK7.pdb) was resolved to a resolution of 2.8 Å [32]. These structures have lent insights into the conserved structural elements of ABCE1.

Overall structure and conserved motifs of ABCE1
The overall structure of ABCE1 displays five regions, namely the FeS cluster domain at the N terminus, two nucleotide-binding domains (NBDs), and two hinge regions. The two NBDs are arranged in a head-to-tail orientation (Figure 3); an arrangement that is commonly found in ABC transporters and other ABC-type proteins [33,34]. Each NBD has the typical bilobed structure [35], where the first lobe harbors a Walker A, Walker B, and a Q-loop motif, and the second lobe the ABC signature motif. In addition, three other conserved motifs are present: the His-switch region (H loop), the D loop, and the aromatic A loop (also called the Y loop). In vitro activity assays and in vivo mutational studies have confirmed several other essential residues in the sequence of ABCE1 [19,24–26,31,36]. The conserved motifs are summarized in Table 1. Additionally, NBD1 contains a helix–loop–helix (HLH) structural motif, which is conserved in ABC1 but is absent from other ABC proteins. The two hinge regions are unusual compared to other ABC proteins and position the two NBDs in a ‘V’-like orientation (Figure 3b). Hinge 1 is formed by a small α/β-structure between the two NBDs, and hinge 2 is formed by the C-terminal stretch of ABCE1. A hydrogen bond network that involves several conserved arginine residues (R cluster) connects hinges 1 and 2. The mutations S558E and R573E in hinge 2 (Saccharomyces cerevisiae ABCE1) yield nonviable yeast cells [31], implicating a functionally important role of this region.

ATP binding causes conformational changes in ABCE1
A tweezers-like motion of the two NBDs, which is induced by ATP binding and hydrolysis, has been proposed for ABC proteins [34,37]. The hinge regions lie at the pivot point for such motions in ABCE1. However, it is still not clear how this motion is triggered and how it catalyzes ribosome recycling. Two ATPs can be bound at the interface of one NBD (Walker A and B) with the other NBD (ABC signature motif) (Figure 3c). ATP binding induces the closure of the gap between the two NBDs and occlusion of ATP, whereas ATP hydrolysis causes opening and release of inorganic phosphate and ADP [19]. Interestingly, in vitro analyses have further shown that the two ATP binding sites are structurally and functionally nonequivalent [19]. It is most likely that various allosteric signals trigger ATP binding and hydrolysis, which accounts for the expected conformational changes of the enzyme.

ABCE1 harbors two unequal FeS clusters
The FeS cluster domain of ABCE1 from S. solfataricus has been extensively studied by biophysical, biochemical, and yeast genetic approaches [32,36]. Two dissimilar, diamagnetic [4Fe-4S]²⁺ clusters, which are buried in a hydrophobic
cocoon, are stable at low redox potential and sensitive to oxidation (Figure 3d). The FeS 1 cluster is unique to ABCE1, whereas FeS 2 is a ferredoxin-type cluster (Table 1). In yeast, mutational studies have revealed that iron coordination in the ferredoxin cluster can switch between cysteine C56 and C68 [36]. The residue C56 is not essential because the FeS 2 cluster is also stable and functional in its paramagnetic [3Fe-4S]2+ state. Recent data indicate that oxidative stress inhibits cell growth in part through inhibition of ABCE1 function [36,38]. Whether this results from structural disturbance of the FeS cluster domain or from electron transfer activity remains unclear. However, the typical FeS cluster function of electron transfer has not yet been shown for ABCE1, although the distance between the two FeS clusters (12 Å center-to-center distance) makes it possible. Notably, electron transfer is coupled to conformational changes induced upon ATP/ADP binding by the two NBDs of the nitrogenase enzyme [39]. Still, the most probable function of the FeS clusters in ABCE1 is considered to be a structural one [19,36,40]. The FeS domain extends into the cleft between the two NBDs and is presumably pushed out of this cleft when the protein adopts a ‘closed’ conformation [32].

The structural dynamics of ABCE1 and its allosteric regulation are central for ribosome recycling, but the detailed mechanism remains elusive. Recently published results provide insight into the structural basis for ribosome splitting by ABCE1.

A first snapshot of the ribosome recycling complex

A recent breakthrough in ribosome research was provided by the cryo-electron microscopy (EM) reconstruction of stalled complexes from yeast (the 80S ribosome with Dom34 and srABCE1) and P. furiosus (the 70S ribosome with aPelota and p/ABCE1) at a resolution of around 7 Å [40]. These structures show that ABCE1 occupies the position of translational GTPases (eRF3/Hbs1) and is potentially capable of inducing peptide release (Figure 4). The FeS domain of ABCE1 appears to bind to the C-terminal domain (C domain) of Dom34/aPelota. Another three putative ABCE1 binding sites have been mapped to the ribosome. The results confirm a previously reported ribosomal binding site of ABCE1. Arresting eRF3/Hbs1 at the ribosome with the nonhydrolysable GTP analog (GMPPNP) in mammals and yeast prevents ribosome splitting by ABCE1 [6,8,11]. Thus, it has been
The FeS domain (brown) with its two \( [4Fe-4S]^{2+} \) clusters extends into the cavity between the two NBDs, in close proximity to ATP site I. Two ADP molecules are bound on the inner side of the V cavity in the crystal structure. Hinges 1 and 2 are pivotal for the tweezers-like motions. (d) ADP binding in \( \text{paABCE1} \). Conserved motifs (colors according to (a)) along the gap between the two NBDs are shown. Conserved residues \((G)^{106}, E^{190}\) and the His switch \((H^{272})\) point towards the \( \gamma \) phosphate (not shown) of ATP for its hydrolysis. The signature motif of NBD2 is positioned in close proximity to the Walker A motif, resulting in its interaction with the \( \gamma \) phosphate of ATP. The role of the Y loop in fixing the ribose and adenine moiety is also presented. (d) The FeS domain of \( \text{paABCE1} \). The \( [4Fe-4S]^{2+} \) clusters (Fe brown, S yellow) are coordinated by three cysteines of the one motif and one of the other.

**Table 1. Domains and conserved motifs of \( \text{paABCE1}^a \)**

<table>
<thead>
<tr>
<th>Name</th>
<th>Consensus sequence</th>
<th>Residues (region 1)</th>
<th>Residues (region 2)</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>4Fe-4S (ABCE1-type, FeS 1)</td>
<td>C-XPX_{2}-C-X_{3/4}C-X_{6/7}-CP</td>
<td>C15, 20, 25</td>
<td>C65</td>
<td></td>
</tr>
<tr>
<td>4Fe-4S (ferredoxin-type, FeS 2)</td>
<td>C-PX_{4}-C-X_{3/4}C-X_{6/7}-C</td>
<td>C29</td>
<td>C55, 58, 61</td>
<td></td>
</tr>
<tr>
<td>NBD</td>
<td></td>
<td>70–313</td>
<td>338–558</td>
<td></td>
</tr>
<tr>
<td>Hinge region</td>
<td></td>
<td>314–337</td>
<td>559–593</td>
<td>[91]</td>
</tr>
<tr>
<td>Y loop (A loop)</td>
<td>YGVNAP/YGSF</td>
<td>87–92</td>
<td>353–356</td>
<td>[91]</td>
</tr>
<tr>
<td>HLH motif</td>
<td></td>
<td>135–160</td>
<td>–</td>
<td>[91]</td>
</tr>
<tr>
<td>Q loop</td>
<td>PQYV/PQYI</td>
<td>168–171</td>
<td>409–412</td>
<td>[91]</td>
</tr>
<tr>
<td>ABC signature motif</td>
<td>LSGGELO</td>
<td>215–221</td>
<td>458–464</td>
<td>[91]</td>
</tr>
<tr>
<td>Walker B (catalytic Glu) and D loop</td>
<td>DEPSYSYLD/DEPSAYLD</td>
<td>239–246</td>
<td>482–489</td>
<td>[91]</td>
</tr>
<tr>
<td>H loop (His switch)</td>
<td>VVHDL/VEHHDVL</td>
<td>269–274</td>
<td>514–519</td>
<td>[91]</td>
</tr>
</tbody>
</table>

\(^a\)Abbreviations: hydrophobic residue; X, any residue; n, integer number; conserved residues in bold.
suggested that the binding site of ABCE1 is formed after eRF3 release [2].

**FeS domain of ABCE1 binds to class I release factor**

The putative contacts between ABCE1 and the ribosomal complex are illustrated in Figure 5. The cooperative action of ABCE1 and Dom34 in ribosome recycling has been described in a recent kinetic analysis of post-translational events in yeast [8]. The cryo-EM reconstruction indicates that the FeS domain of ABCE1 interacts with the C domains of Dom34 and aPelota (Figure 5a) [40]. aPelota [18] and Dom34 [41] engage the GTPase (aEF1α and Hbs1, respectively) via their C and middle (M) domains. However, the contacts with ABCE1 are established exclusively by the C domain [40]. Despite the relatively high resolution, the cryo-EM reconstruction cannot reveal detailed contacts.

Interestingly, different contact sites between ABCE1 and the class I release factor are possible in the absence of assembled ribosomes compared to the ribosome-bound structures. Yeast-two-hybrid analysis and coimmunoprecipitation studies have indicated that only NBD2 of ABCE1 is important for the interaction with eRF1 (Sup45 in yeast) [42]. Complex formation and rearrangement of eRF1 and ABCE1 is still an open question for research.

**Ribosome contact sites of ABCE1**

ABCE1 exhibits four major contact sites with the post-translation complex (Figure 5). Extensive contacts with the small subunit of the ribosome are established by unique structural elements of ABCE1: the HLH motif and the hinge region. The HLH in NBD1 putatively binds to the 18S rRNA and to the ribosomal protein rpS24-A (Figure 5b). The hinge regions likely bind rRNA in close proximity to helices h8 and h14 in the 40S/30S subunits (Figure 5c).

![Figure 4](image_url)

**Figure 4.** Ribosome binding sites of ABC-type ATPase ABCE1. (a) Overall architecture of the yeast 80S ribosome and the position of ABCE1. The cryo-electron microscopy results (emd-2008; 3J16.pdb) are superpositioned with the crystal structure of the yeast ribosome (3U5P-L.pdb) [97]. ABCE1 binds at the intersection between the large and the small ribosomal subunit and is seen interacting with Dom34 (both shown in surface representation). The neighboring 18S rRNA (blue), 28S rRNA (purple), and the tRNA at the P site (lime green) are shown in cartoon representation. (b) Zoomed-in: ABCE1, Dom34, and P site tRNA are arranged in a row between the 40S and 60S ribosomal subunits. The FeS domain of ABCE1 binds to the C-terminal (C) domain of Dom34 (left box, see details in Figure 5a). The helix-loop-helix (HLH) motif in nucleotide-binding domain (NBD)1 binds to 18S rRNA (right box, see details in Figure 5b). The N-terminal (N) domain of Dom34 is buried in the A site, whereas the middle (M) domain is swung out into the direction of the peptidyl transferase center (PTC) to contact tRNA. They constitute a network, which likely transmits conformational changes during ribosome splitting. Neighboring ribosomal proteins are illustrated in cartoon representation.

![Figure 5](image_url)

**Figure 5.** Interaction sites of ABC-type ATPase ABCE1 with the ribosomal complex. (a) The interface between the FeS domain of ABCE1 and the C-terminal (C) domain of Dom34. The ribosomal protein rpl11 is in close proximity (nomenclature according to [97]). The Hbs1-binding motif CILKY (teal) and the conserved C-terminal acidic stretch of Dom34 is also found in eRF1 of eukaryotes and some archaea, but is absent in bacteria [16]. A conserved basic patch is present on the surface of the FeS domain of ABCE1 as a counterpart to acidic residues in Dom34 and eRF1 [32]. (b) The helix-loop-helix (HLH) motif in nucleotide-binding domain (NBD1) binds to 18S rRNA. The C-terminal helix of ribosomal protein rpS24-A extends into the ribosomal binding site of HLH. (c) The two hinge regions are proposed to play a critical role for the conformational dynamics and allosteric regulation of ABCE1. The binding site consists of 18S rRNA, rpS6-A, and rpl23-A (UniProtKB P0CX41). It is similar to that of the GTPase switch-regions of Hbs1 and other translational GTPases [43,44]. (d) The structurally diverse region of NBD2 (SDR, dark orange) [31] participates in 28S rRNA binding in close proximity to rpl9-A.
These regions on the bacterial ribosome are suggested to play a role in EF-G-mediated ratchet-like subunit rearrangement during elongation [43]. Interestingly, toe-printing experiments have indicated that ABCE1 is capable of inducing conformational changes in post-TCs [11]. The structural basis for this observation is still elusive, but the interaction with h14 might be a starting point for further investigation. Hinge 2 contacts the ribosomal protein rpS6-A in close proximity to rpL23-A in yeast and rpL14 in *F. furiosus*. In contrast to the GTPases, which extensively contact the sarcin–ricin loop (helix H95) [43], NBD2 binds to proteins rpL9-A in both species and to a minor extent to 28S rRNA and rpP0 in yeast (Figure 5d).

**ABCE1 promotes peptide release**

ABCE1 promotes peptide release by eRF1, independent of ATP hydrolysis and without enhancing recruitment of eRF1 to the ribosome [8]. Importantly, peptide release is not a prerequisite for subunit dissociation and does not occur during mRNA surveillance or splitting of vacant ribosomes. A model of the post-TC with eRF1 has been postulated based on the high structural similarity of eRF1 and Dom34. This model shows a conformation of eRF1 that is proposed to be optimal for peptide release, where the N-terminal (N) domain is bound to the A site, the C domain is in contact with the GTPase (or ATPase in case of ABCE1), and the M domain protrudes into the peptidyl transferase center (PTC) [44,45]. Dom34 adopts a similar conformation in the post-TC (Figure 4). Earlier cryo-EM studies of the Dom34•Hbs1•GMPPNP complex have indicated that the M domain of Dom34 remains bound to Hbs1 and does not swing out towards the PTC [44]. This movement likely occurs in concert with GTP hydrolysis and conformational changes in the ribosome, which are not triggered in presence of GMPPNP [46,47]. Small angle X-ray scattering (SAXS) data do not indicate such domain rearrangement during eRF1•eRF3 complex formation in solution [45]. We speculate whether there is some conserved structural basis in the actions of eRF3 and ABCE1 for peptide release.

**Allosteric control of ABCE1**

Considering the biological function of ABCE1 as a ribosome splitting factor by virtue of ATP binding/hydrolysis, it seems logical that this protein would be allosterically regulated by components of the translation system, analogous to the ribosome dependence of the GTPase of class II release factors [48]. Structural evidence for allosteric regulation of translational GTPases is available for eukaryotes [44] and bacteria in a recently published crystal structure of the 70S termination complex with RF3•RF3•GMPPNP at 3.3 Å resolution [47]. For yeast ABCE1, incubation with Dom34 and 80S ribosomes increases its ATPase activity [8]. However, its regulation has not yet been investigated at the structural level and the present cryo-EM analysis is the first step in understanding this process [40]. The allosteric regulation of ABCE1 by the ribosome could be via its unique structural features: the HLH and the hinge region. The sequence of the HLH that binds the ribosome is conserved among ABCE1 proteins from different species, and the HLH is near the catalytic Q loop in ATP site I. Therefore, it might act as an allosteric sensor for the regulation of ATP binding and/or hydrolysis. Another mechanism of allosteric regulation might be through the conserved hinge region, which might regulate the ATP site II. It binds close to 18S rRNA helix h14, which has been shown to stabilize the switch I region in the GTPase domains of EF-G [43] and Hbs1 [44] and is important for their activation. Hinge 1 of ABCE1 occupies a very similar position.

**Ribosome recycling connects translation termination and initiation**

Ribosome recycling connects two processes that have been separated in research for several decades: termination and initiation. The principles and mechanism of translation initiation and its regulation have been described in several recent reviews [49–51]. The first stage of translation initiation directly results from ribosome recycling, and so provides a mechanistic link between termination and initiation.

Translation initiation begins with the formation of preinitiation complexes. In eukaryotes, the ternary complex of initiator tRNA-Met•eIF2•GTP and initiation factors eIF3, eIF1, eIF1A, and probably eIF5 bind to the small ribosomal subunit (43S preinitiation complex) [51–57]. In the next step, mRNA decorated with several other initiation factors is recruited [51,58–62] and forms start codon–anticodon base pairs in the P site of the small ribosomal subunit (48S preinitiation complex) [51,63–67]. Finally, eIF2 stimulates GTP hydrolysis by eIF2 [51,68,69]. Rearrangement of other initiation factors allows assembly with the large ribosomal subunit, leading to an elongation–competent 80S [51,70–72]. Mechanisms of translation initiation differ between eukaryotes and archaea. Only five initiation factors are known in archaea (aIF1, aIF1A, aIF2/5B, aIF2, and aIF6) and their functional spectrum is different from their eukaryotic homologs [50]. The mechanistic link between translation termination and ribosome recycling opens new prospects to investigate the function of initiation factors and overall mechanisms of translation initiation.

ABCE1 interacts with the translation initiation factors eIF2, eIF3, and eIF5 in yeast as shown by communoprecipitation and in vitro formaldehyde crosslinking [24]. A decrease in 48S formation, and significantly lower binding of eIF2 and eIF3 (and to a lesser degree eIF5) to 40S subunits, has been observed in ABCE1-depleted cells, as well as with ATPase-deficient ABCE1 mutants. The function of ABCE1 in ribosome splitting is fully consistent with these results but cannot explain the observed interactions with initiation factors. e/aIF2 is a three-subunit GTPase (35–50 kDa each in eukaryotes), which recruits Met–tRNA to the ribosome. e/aIF2 dissociates after GTP hydrolysis, which is initiated by eIF5 after start codon recognition. eIF3, a large complex of 13 subunits (~800 kDa) in eukaryotes, binds to 40S subunits, where it initiates 43S formation. It also promotes the subsequent mRNA attachment and start codon recognition by tRNA-Met. Additionally, eIF3 has been shown to prevent the joining of small and large ribosomal subunits [51]. Interestingly, no homologs of eIF3 and eIF5 have been identified among the five known initiation factors from archaea [50]. Structural
information on translation initiation complexes is rare [73]. eIF3 has been proposed to position close to the E site, opposite to the ABCE1 ribosomal binding site, based on biochemical data [74] and low resolution (30 Å) cryo-EM analysis [57,75]. However, little is known about the position of other initiation factors on the small ribosomal subunit. Undoubtedly, more information is needed to evaluate the possible role of ABCE1 for translation reinitiation (Box 3).

Concluding remarks
Translation termination and ribosome recycling differ largely between the kingdoms of life. The most striking difference is the presence of the highly conserved ATPase ABCE1 in euukaryotes and archaea instead of the bacterial RRF. Besides ribosome recycling, ABCE1 is involved in several interesting cellular events. The mechanism of ribosome recycling and translation reinitiation by ABCE1 is still puzzling. To date, only the first step of ribosome recycling has been described at the structural level. It is expected that various intermediate steps occur between ABCE1 recruitment to post-TCs and initiation factor accretion on the freshly released small ribosomal subunit. Further studies on the function and molecular dynamics of ABCE1 promise to give new insights into the biological mechanism of protein biosynthesis in euukaryotes and archaea. The molecular interaction of ABCE1 with different ligands, its allosteric properties, and the functional asymmetry of its ATP-binding sites are fundamental to the understanding of protein biosynthesis. In this review, the differences between euukaryotes/archaea and bacteria provide an intriguing perspective.

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