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Minireview

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Rustless translation

Abstract: ATP binding cassette proteins are a large and diverse family of molecular machines and include transmembrane transporter, chromosome maintenance and DNA repair proteins, and translation factors. However, the function of the ABCE1, the only member of subfamily E of ABC proteins, remained mysterious for over a decade, even though it is perhaps the most conserved ABC protein in eukaryotes and archaea. Recent results have now identified ABCE1 as the ribosome-recycling factor of eukaryotes and archaea. Thus, two iron-sulfur clusters – the hallmark feature of ABCE1 – help catalyze an integral step of the translational cycle at the core of the protein synthesis machinery.

Keywords: ATP-binding cassette; iron-sulfur-cluster; ribosome; structural biology.

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Introduction

In this article, I review a decade of research on life's perhaps most central ATP binding cassette (ABC) enzyme: ABCE1. ABCE1 [also known as RNase-L inhibitor (Rli1) and host protein 68 kDa (HP68)] is the only member of subfamily E of ABC proteins, an otherwise large and diverse family of energy-converting molecular machines or switches. When I started my own laboratory in 2001, very little was known about this protein and its only function was a role in interfering with RNase-L activity (hence it is known as RNase-L inhibitor, Rli1) in the innate immune system (Bisbal et al., 1995). Shortly after, ABCE1 was found also as a 68 kDa host protein (named HP68 then) that was implicated in the assembly of immature capsids of human immunodeficiency virus (Zimmerman et al., 2002). However, ABCE1 is exceptionally conserved in evolution and must have a central, yet to be discovered function in the cell physiology of all eukaryotes and archaeal that goes beyond its relatively narrow role in RNase-L inhibition and HIV capsid assembly. Because of its difficult biochemistry and lack of identified substrates, this function remained mysterious for more than a decade. In the past years, however, with contributions from many laboratories (see below for references and details) ABCE1 emerged as a new translation factor and its substrate turned out to be the ribosome.

ABC enzymes: versatile molecular machines or switches

ABC enzymes include transmembrane channels and transporters such as cystic fibrosis transmembrane regulator (a gated chloride channel) or multidrug resistance protein (a pump for lipophilic small molecules), structural maintenance of chromosome proteins (e.g., the sister chromatid cohesion complex), DNA repair enzymes (e.g., Rad50, MutS/MSH or UvrA), and several translation factors (e.g., eEF3 or GCN20). For an overview see, for example, Holland et al. (2003).

Although ABC enzymes are very diverse with respect to their biological function and architecture, a central structural and mechanistic feature is widely conserved: they posses two nucleotide-binding domains (NBDs), either in the form of a homodimer, a heterodimer, or a tandem cassette on a single polypeptide chain (Figure 1A). During my postdoctoral work on the DNA repair enzyme Rad50, we unraveled a central aspect how ABC proteins function and showed that ABC type NBDs sandwich two ATP molecules in the NBD:NBD interface (Hopfner et al., 2000). ATP is recognized by Walker A and B motifs from one NBD, and by the ABC characteristic 'signature' motif (or 'C' motif) from the opposing NBD, and vice versa. ATP binding promotes NBD-NBD engagement and ATP-hydrolysis promotes disengagement of the two NBDs (Figure 1B). This conformational switch is the 'engine' of ABC enzymes.

Research by many colleagues and ourselves extended this analysis to complete ABC proteins (e.g., Lamers et al., 2000; Obmolova et al., 2000; Locher et al., 2002; Andersen et al., 2006; Dawson and Locher, 2006; Lammens et al.,

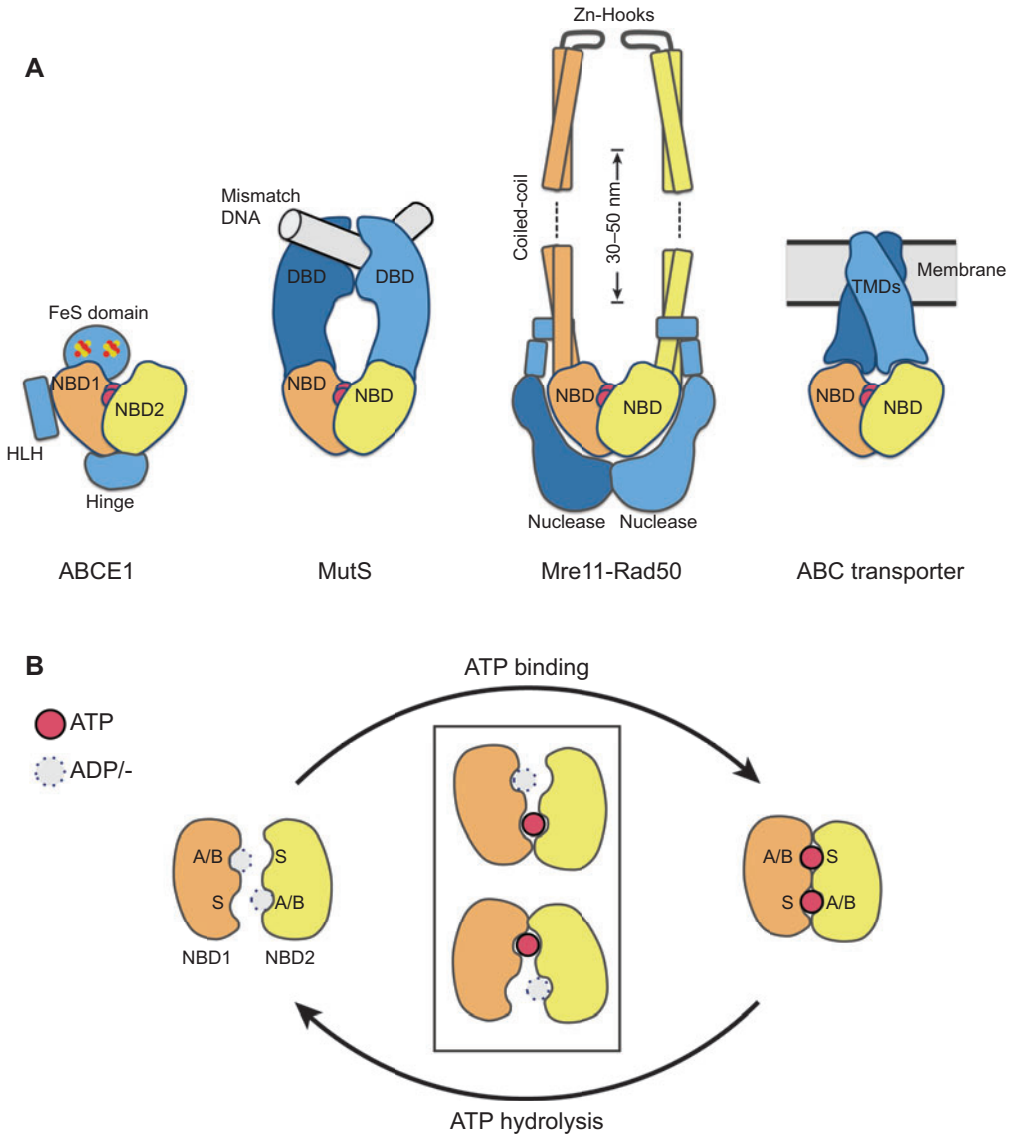

Figure 1 ABC protein architectures.
(A) Exemplary architectures of ABC proteins. ABCE1 has two nucleotide-binding domains (NBD1 and 2) along with helix-loop-helix (HLH), iron-sulfur cluster (FeS) and hinge domains. The DNA mismatch repair protein MutS forms a dimer where ATP dependent conformational changes of NBDs regulate mismatch binding by DNA binding domains (DBD with grey DNA). ATP binding to the Rad50 DNA double-strand break repair ABC protein regulates the Mre11 nuclease dimer. Conformational changes of NBDs in ABC transporters drive solute transport through transmembrane domains (TMDs). (B) ATP dependent engagement-disengagement of the two NDBs in ABC proteins: ATP binds to Walker A/B (A/B) motifs of one NDB and the signature (S) motif of the opposing NBD. The functional cycle might involve hybrid states where only one ATP binding site is engaged (bracketed).

2011; Lim et al., 2011), and there is now a good understanding of the principal architecture and ATP-dependent structural dynamics of a variety of ABC proteins (Figure 1A). A pair of NDBs undergoes conformational cycles by ATP driven engagement and disengagement, which impacts on the structure and substrate binding properties of the NBDs and its function-specific associated domains. The latter are, for example, DNA binding or nuclease domains in DNA repair enzymes, or transmembrane domains (TMDs) in ABC transporters, where the conformational cycles pump specific ligands across membranes.

However, there are still open questions for many ABC systems, in particular allosteric regulation of the ATPase by ligand binding or the question whether both ATP molecules in the NBD-NBD sandwich are hydrolyzed simultaneously or rather sequentially (Figure 1B). ABCE1, apart

from its emerging exciting biology, also revealed interesting new insights into allosteric regulation and sequential ATP binding and hydrolysis.

A brief history of the early research on ABCE1

ABCE1 is a soluble, highly unique ABC protein consisting of an N-terminal domain with iron-sulfur (FeS) cluster binding sites and an NBD tandem cassette. It was described in 1995 as RNase-L inhibitor (RLI1) on the basis of its co-immunoprecipitation with RNase-L and inhibitory effect on RNase-L mediated RNA degradation (Bisbal et al., 1995). RNase-L is an interferon-dependent polysome-associated antiviral defense enzyme that is activated by 2′-5′ oligoadenylates and degrades mRNA and single-stranded viral RNA in virus infected cells (Silverman, 2007), but also has a variety of additional functions (Chakrabarti et al., 2011). In 2002, ABCE1 was found to be required for the assembly of immature HIV-1 capsids and it has been suggested that it accounts for the ATP dependence of the rather poorly understood capsid assembly process (Zimmerman et al., 2002). While these early results made ABCE1 an interesting target for biomedical research and therapeutic intervention, the cellular function of ABCE1 remained unclear. In particular, the 2′-5′-oligoadenylate synthetase/RNase-L antiviral defense pathway is only found in vertebrates, while ABCE1 is highly conserved in all archaea and eukaryotes although absent in bacteria (Kerr, 2004). The phylogenetic distribution suggested that ABCE1 is involved in a rather fundamental process in life that is conserved in archaea and eukaryotes, but mechanistically distinct in bacteria. In support of a core role in eukaryotic and archaeal cell physiology, ABCE1 turned out to be essential in all organisms tested (Dong et al., 2004; Zhao et al., 2004; Coelho et al., 2005; Karcher et al., 2005; Kispal et al., 2005; Yarunin et al., 2005).

First insights into its core function emerged, when the Baillie, Dean, Hinnebusch, Leevers and Marygold labs found ABCE1 (Rli1p) to be involved in the assembly of the translation pre-initiation machinery, to interact with the translation initiation machinery and polysomes in yeast and to be important for translation and in worms, flies and vertebrates (Dong et al., 2004; Zhao et al., 2004; Coelho et al., 2005; Chen et al., 2006). At the same time, the Lill and Hurt labs found a functional link between the iron-sulfur clusters of ABCE1 and ribosome biogenesis (Kispal et al., 2005; Yarunin et al., 2005) while we could crystallize and determine the crystal structure of the twin ABC cassette of archaeal ABCE1 bound to ADP (Karcher et al., 2005) (Figure 2). Studies in flies then showed that ABCE1 (known as Pixie in Drosophila) binds the 40S ribosome in an ATP-dependent manner and depletion leads to an increase in in empty 80S ribosomes (Andersen and Leevers, 2007). ABCE1 interacts with the translation initiation factor eIF3 through the Hcr1 component in yeast (Dong et al., 2004),

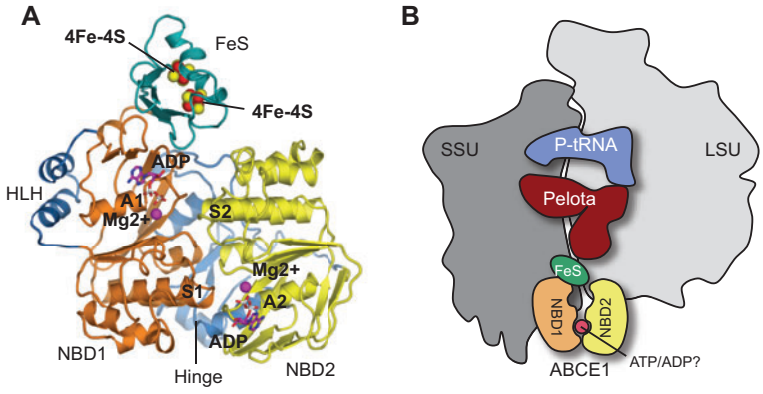

Figure 2 Structure and ribosome interaction of ABCE1.
(A) Crystal structure of *Pyrococcus furiosus* ABCE1, shown as ribbon model. Magnesium-ADPs (magenta) and the two 4Fe-4S clusters (yellow-red) are highlighted. The two nucleotide binding sites are formed by opposing Walker A (A1 and A2) and signature (S1 and S2) motifs. (B) ABCE1 binds to the intersubunit cleft (SSU, small subunit; LSU, large subunit) of stalled ribosomes in an asymmetric conformation with a closed second ATP binding site. The asymmetric conformation could be induced by ATP at the closed second ATP binding site (as modeled here), but the role of nucleotides in each active site need to be addressed in future studies. While NBD2 and 1 predominantly interact with SSU and LSU, respectively, FeS binds the surveillance factor Pelota. ATP driven conformational changes in ABCE1 could directly split ribosomes or split ribosomes by modulating the conformation of Pelota.

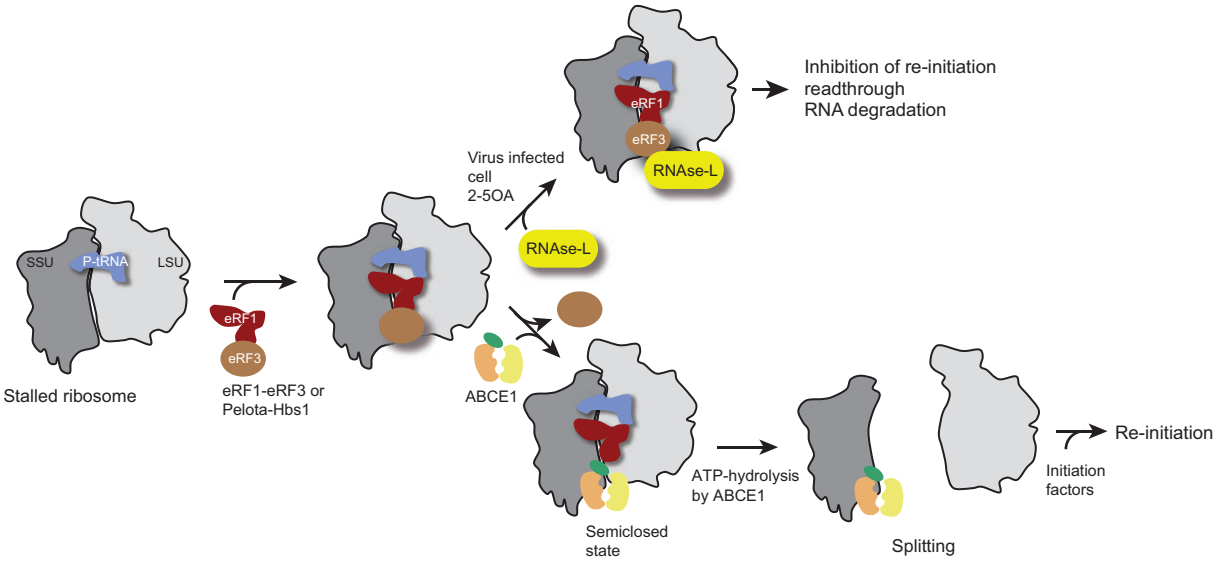

Figure 3 Model for ABCE1 function in ribosome recycling and competition with the RNase-L pathway.

and with eIF2 and eIF5 in vertebrates (Chen et al., 2006). Hcr1 or eIF3 have additional functions, including also the coordination between termination and initiation, ribosomal subunit dissociation and rRNA maturation (Valasek et al., 2001), which made the functional interpretation of these interactions difficult. Taken together, however, it became clear that ABCE1 functions in a ribosome-associated process although its mechanism was still mysterious on the basis of the pleiotropic effects ranging from defects in ribosome biogenesis to translation.

Structure of ABCE1

While the biochemical mechanism of ABCE1 remained entirely unclear – for example the interacting partners found in yeast have no counterpart in archaea where ABCE1 also highly conserved – the structural analysis made significant progress. The Lill laboratory revealed that ABCE1 binds iron and requires the iron-sulfur-cluster biosynthesis machinery, while the Tampé laboratory showed that ABCE1 contains two diamagnetic, non-equivalent [4Fe-4S]$^{2+}$ clusters (Kispal et al., 2005; Barthelme et al., 2007). At the same time, we crystallized full length ABCE1 using an anoxic chamber that enabled us to *in vitro* reconstitute FeS clusters in overexpressed ABCE1 and prevent deterioration of the oxidation sensitive FeS clusters during crystallization.

The crystal structure of *Pyrococcus abyssi* ABCE1 at 2.8 Å resolution revealed a relatively compact particle with five structural domains (Karcher et al., 2008) (Figure 2). Two NBDs are arranged in a V shape by a hinge domain, similar to our previously determined structure of *P. furiosus* ABCE1-ΔFeS. In all our structures, we found Mg-ADP moieties stably bound to both active sites and the structures represent a 'symmetric' post-hydrolysis state of the enzyme. Remarkably, ADP was retained at the protein from the cells throughout purification and could not be exchanged to ATP or non-hydrolysable derivatives in our hands. Related structures form other organisms from ourselves and others revealed a similar ADP-state and even mutating the Walker B motif to prevent ATP hydrolysis still resulted in ADP-bound active sites (Karcher et al., 2005; Karcher et al., 2008; Barthelme et al., 2011). Unfolding followed by refolding of the protein in the presence of AMP-PNP removed ADP in favor of AMP-PNP, but rather than a structural change in the protein, AMP-PNP was forced to adopt an unusual ADP-like conformation with misplaced γ-phosphate (unpublished). This put forward the argument that ABCE1's ADP conformation is rather stable in the absence of physiological binding partners and ADP→ATP exchange is likely allosterically regulated. In any case, comparison with ABC proteins in ATP-bound states argued that the two NBDs in ABCE1 undergo a tweezers-like movement in the functional cycle of the protein (Chen et al., 2003). The structures also revealed other notable motifs, such as a helix-loop-helix (HLH) motif, which are often involved in protein-nucleic acid interactions.

The most unique and notable feature of ABCE1 compared to other ABC proteins is the FeS domain. It is

situated at the lateral opening of the ATP binding cleft, where ATP-dependent conformational changes between the NBDs might impact on the position of the FeS domain. The structure is in agreement with previous biophysical data and showed two 4Fe-4S clusters bound to eight cysteins in a ferredoxin-like fold (Barthelme et al., 2007; Karcher et al., 2008). All cysteines except one are essential in yeast. However, an additional cysteine next to the non-essential cysteine might rescue the FeS cluster in yeast (Barthelme et al., 2007). In any case, mutational analysis revealed that both ATP binding sites and both FeS clusters are essential for ABCE1 function.

FeS clusters most prominently function in electron transfer reactions but can also have structural roles or poorly understood functions in the recognition of nucleic acids. In fact, more and more nucleic acid associated proteins are found to contain FeS clusters, yet the functions of these clusters in the vicinity of the nucleic acid remain to be clarified (Kuo et al., 1992; Wu and Brosh, 2012). It is possible that the positive charge on the clusters plays a functional role in the electrostatic recognition of the nucleic acid, or the clusters stabilize the protein to provide a rigid fold in ribosome splitting functions (see below). In ABCE1, both FeS cluster are well shielded from solvent (Karcher et al., 2008), ruling out direct contacts of the irons to an interaction partner or a direct participation in a catalysis reaction, and they are electronically very stable (Barthelme et al., 2007). The redox potential is also not changed upon ribosome binding (Barthelme et al., 2011). Thus, on the basis of present data, it is unlikely that the FeS clusters have a role in electron transfer. On the other hand, twin clusters in other proteins (e.g., ferredoxins) typically function in electron transfer reactions. The distance between the two clusters in ABCE1 is with 12 Å center to center ideal for electron transfer, so the function of the FeS clusters in ABCE1 needs to be further studied.

ABCE1 is a eukaryotic and archaeal termination and ribosome-recycling factor

The biochemical function of ABCE1 in translation became clearer recently. A first set of studies found that ABCE1 is involved in translation termination. Translation has four phases: initiation, elongation, termination and ribosome recycling. Termination in eukaryotes was initially thought to be mediated solely by eRF1 and eRF3: the eRF1-eRF3 complex binds to the stopped ribosome, eRF3 dissociates upon guanine nucleotide exchange, while eRF1 recognizes the termination codons and triggers hydrolysis of peptidyl-tRNA. Unexpectedly, work from the Krebber and Ficner labs found that ABCE1 physically interacts with eRF1 and is required for proper stop codon recognition (Khoshnevis et al., 2010). At the same time the Pestova lab showed that ABCE1 functions in mammalian ribosome recycling by studying ABCE1 promoted ribosome splitting in biochemical assays (Pisarev et al., 2010). Here, Pestova and colleagues found that the ATPase activity of ABCE1 is stimulated by post-termination complexes and that ABCE1 splits ribosomes using ATP hydrolysis (Pisarev et al., 2010). In parallel, the Green and Tampé labs demonstrated that ABCE1 is the ribosome recycling factor in yeast and archaea, respectively, establishing that ABCE1's role in ribosome recycling is evolutionary conserved (Barthelme et al., 2011; Shoemaker and Green, 2011).

These data established a function of ABCE1 in the recycling of ribosomes that are stalled at termination codons. Subsequent studies extended the role of ABCE1 also to the recycling of stalled elongation complexes (Pisareva et al., 2011): ribosome elongation complexes that are stalled for instance at mRNA with secondary structures or at mRNAs that lack a proper stop codon trigger no-go decay or non-stop decay surveillance pathways, upon which malfunctioning mRNAs are degraded in eukaryotes (van Hoof and Wagner, 2011). Analogous to the eRF1-eRF3 system in normal translation termination, the stalled ribosomes are recognized by Pelota (Dom34 in yeast, a homolog of eRF1) and Hbs1 (a homolog of eRF3), resulting in the release of peptidyl-tRNA (Saito et al., 2010; Tsuboi et al., 2012). ABCE1 also works together with Dom34/Pelota, suggesting that ABCE1 is a universal ribosome splitting/recycling factor in eukaryotic and archaeal translation termination that works not only release factor dependent termination but also on Dom34/Pelota dependent recognition of stalled ribosomes (Pisareva et al., 2011) (Figure 3).

A hybrid view of the structure of ABCE1 bound to the ribosome

A recent breakthrough on the structural mechanism came from Roland Beckmann's laboratory where in collaboration with Rachel Greens laboratory and us the structure of yeast and archaeal ABCE1-ribosome complexes were studied by cryo-electron microscopy and visualized by cryo-electron microscopy and single particle reconstruction (Becker et al., 2012) (Figure 2B). Here, ribosome nascent chain complexes (RNCs) stalled

by an mRNA that contained a synthetic stem loop were incubated with Pelota/Dom34 and ABCE1 in the presence of ADPNP (a non-hydrolysable ATP analog). The exceptional quality of the resulting EM densities for both *S. cerevisiae* and *P. furiosus* systems (7.2 and 6.6 Å, respectively) allowed unambiguous interpretation of the EM density with an atomic model of ABCE1 derived from the crystal structure of the *P. abyssi* protein and even revealed the two electron-dense FeS clusters directly in the electron densities (Becker et al., 2012).

ABCE1 binds both the archaeal and eukaryotic ribosome at the intersubunit space, showing that its interaction and mode of activity is highly conserved in evolution. HLH and NBD1 interact with the small subunit while NBD2 and hinge predominantly interact with the large subunit. Thus, ABCE1 populates the same site at the ribosome as the elongation and termination GTPases (EF-Tu and EF-G in bacteria; eEF2, Hbs1 and eRF3 in eukaryotes) (Gao et al., 2004; Klaholz et al., 2004; Spahn et al., 2004; Schmeing et al., 2009; Tsuboi et al., 2012). Since both NBDs have multiple contacts to the ribosomal subunits, ATP driven conformational changes between the NBDs could have important roles both in recognition of ribosomes and ribosome splitting (Becker et al., 2012). Remarkably, however, the FeS domain had not direct contacts to the ribosome but instead binds and induces a large conformational shift in Pelota. Although not directly visualized by EM yet, an analogous shift in the Pelota homolog eRF1 by ABCE1 would bring the GGQ motif of eRF1 into close proximity to P-site tRNA for peptidyl-tRNA hydrolysis, suggesting that ABCE1 could modulate eRF1 to trigger peptide release prior to splitting (Frolova et al., 1999; Becker et al., 2012). These results argue that a key function of the FeS domain is recognition of ribosomes with Pelota and eRF1 on one side, and inducing a structural change to prepare recycling on the other side.

In any case, from the biochemical and structural studies ABCE1 emerged as a universal recycling factor for translationally terminated and stalled ribosomes, and in both eukaryotes and archaea.

Asymmetry and allosteric regulation: a new chapter in ABC protein structural research

An unexpected outcome of the EM studies on ABCE1 on stalled RNCs was the observation that the conformation of ABCE1 at the ribosome is somewhat distinct from the crystallographically observed state with Mg^{2+}-ADP bound to both NDBs (Karcher et al., 2008; Becker et al., 2012). Although the nucleotide state cannot be directly deduced from the EM maps, the conformation of the two NBDs most closely match a novel hybrid state, where nucleotide binding site 1 (NBS1: Walker A/B from NBD1 and signature motif from NBD2) is 'open' and either ADP bound or nucleotide free, and NBS2 (NBD1 signature motif and NBD2 Walker A/B motifs) is closed and presumably bound to ADPNP (Figure 2B). Such a 'hybrid' nucleotide binding state with an ATP bound and ADP bound or empty NBS has not been structurally observed before, although other ABC enzymes such as the DNA mismatch sensor MutS and also ABC transporters displayed other types of asymmetry (ADP and empty NBSs) (Lamers et al., 2000; Obmolova et al., 2000; Hvorup et al., 2007).

Interestingly, biochemical studies on archaeal ABCE1 showed that a mutation in NBS2 Walker B-aimed at slowing ATP hydrolysis in NBD2-induces increased ATP hydrolysis activity in NBS1, while the equivalent mutation in NBS1 reduced ATPase activity of ABCE1 by half (hence not stimulating NBS2) (Barthelme et al., 2011). These remarkable results biochemically identified asymmetry between ABCE1s NBS1 and 2 before the structural analysis and suggested that ATP binding to NBS2 stimulates ATP binding and/or hydrolysis of NBS1.

In summary, the recent observations argue that ABCE1 is allosterically controlled by the ribosome. A plausible model is that ABCE1 acts as a two-step engine. In step 1, ABCE1 (likely in the crystallographically observed ADP form) binds to the stalled ribosome and allosteric regulation by ribosome/Pelota/eRF1 enables ADP->ATP exchange in NBS2. NBS2 closes and NBS1 remains open. In this conformation, Pelota and possibly eRF1 are structurally modulated and eRF1 could trigger peptide release. ATP binding to NBS1 might then induce a further conformational change to split the ribosome. Some differing data exist with respect to the necessity of ATP binding versus ATP hydrolysis in this splitting reaction. While in one study on the archaeal system a non-hydrolysable ATP analog triggered splitting (Barthelme et al., 2011), in two other studies on the eukaryotic and archaeal systems, hydrolysis competent ATP was necessary (Pisarev et al., 2010; Becker et al., 2012). However, the stability of ribosomes is sensitive to buffer conditions (especially magnesium), which may account for these discrepancies. Perhaps in a physiological context, repeated ATP-binding and hydrolysis cycles are necessary for splitting. Alternatively, upon ATP binding to NBS1, ATP hydrolysis in NBS2 might generate an asymmetric state with open NBS2 and closed NBS1 to fully promote splitting.

Although we have a first structural snapshot, the intermediates and the conformation of ABCE1 on the small subunit after splitting need to be structurally addressed in future studies. For instance, it will be interesting to see whether the FeS domain has more direct functions in splitting as well and/or interaction with the small subunit after splitting. Because ABCE1 also interacts with initiation factors and especially the HCR1 component in yeast (Dong et al., 2004), the functional role of ABCE1 on recycled small subunits is another interesting road of investigation.

In any case, the directly observed asymmetry in the two ATP binding sites of ABCE1 when bound to its substrate is a very important step forward in our understanding of ABC proteins and of high relevance also for studies on other ABC systems.

Innate immune and virus associated functions of ABCE1 revisited

Given the role of ABCE1 in ribosome recycling, how can one reconcile its functions in RNase-L pathway and the assembly of HIV capsids? With respect to inhibition of RNase-L, the original data found a diminished binding of 2-5ApCp to RNase-L when ABCE1 was overexpressed in reticulocyte extracts and the activity of RNase-L against degradation of 18S rRNA was reduced (Bisbal et al., 1995). Given the core function of ABCE1 in ribosome splitting and interaction with ribosomal small subunits, it is possible that ABCE1 prevents RNase-L cleavage of 18S rRNA simply by steric hindrance. However, RNase-L appears to also have a more direct function in translation termination itself: it interacts with eRF3, reduces translation termination and increases +1 frameshift read-throughs at premature stop codons (Le Roy et al., 2005). Thus, RNase-L appears to oppose the function of ABCE1 also in translation termination and recycling. As both proteins interact with the eRF3-eRF1 machinery, it is important to clarify whether RNase-L and ABCE1 have overlapping binding sites with the translation apparatus or whether they bind simultaneously. In any case, as the function of RNase-L is to cleave viral mRNA, recruitment of RNase-L to the ribosome in virus-infected cells and cleavage of actively translated mRNA is presumably of advantage, as is prevention of ribosome recycling and re-initiation to hinder production of new viral mRNAs. Therefore, from a biological and evolutionary point of view RNase-L might be considered as an 'ABCE1 inhibitor' and not the other way around (Figure 3).

The role of ABCE1 in HIV capsid assembly is more difficult to reconcile. Here, ABCE1 interacts with the Gag polypeptide, which is the only viral protein required to assemble and release virus like particles (Zimmerman et al., 2002; Dooher and Lingappa, 2004; Lingappa et al., 2006; Klein et al., 2011). After its synthesis from translation of full length viral genomic RNA, 3000–5000 Gag polypeptides assemble in a stepwise process at the plasma membrane, forming buds that produce immature capsids (Briggs and Krausslich, 2011). Later, the capsids mature in a process that involves proteolysis of Gag into several polypeptides (Ross et al., 1991). Analysis of capsid assembly in cell free systems indicated an energy-dependent step and an important role of ABCE1. ABCE1 is found in assembly intermediates at the plasma membrane but not in mature capsids (Zimmerman et al., 2002; Lingappa et al., 2006). Hereby, the nucleocapsid (NC) domain of Gag binds viral genomic RNA and is also required for interaction with ABCE1, albeit indirectly (Klein et al., 2011).

The mechanistic role of ABCE1 in capsid assembly of HIV-1 and -2 is obscure. Although ABCE1 undergoes ATP-driven conformational changes that split ribosomes and could in principle also chemo-mechanically function in capsid assembly, a different scenario is perhaps more likely. Full length (unspliced) HIV-1 RNA has two functions: it both acts as a template for Gag and Gag-Pol translation and as genomic RNA that is packaged into virions (Ganser-Pornillos et al., 2012). The packaging signal that is recognized by Gag is in the 5′ untranslated region where translation initiation factors also bind (Hayashi et al., 1992; Zeffman et al., 2000). It is likely that there is a competition between Gag translation and Gag mediated packaging. In fact, Gag regulates its own translation, with low concentrations stimulatory and higher concentrations inhibitory (Waheed and Freed, 2012). Thus, it is plausible that the interaction of Gag with ABCE1 helps to regulate and coordinate the molecular conflict between translation and packaging of full length viral RNA. Hereby, functions of ABCE1 in both termination and splitting, but also its interactions with translation factors, could be modulated or exploited by the virus. Because the interaction of Gag with ABCE1 requires Gag dimerization (Klein et al., 2011), formation of large assembly intermediates could specifically sequester ABCE1 to downregulate initiation in *cis*, but other models are also possible. Therefore, it will be interesting to investigate whether, for example, Gag and ABCE1 interact at ribosomes, at ribosomal subunits or alternatively whether Gag prevents binding of ABCE1 to ribosomes or its interaction with translation factors. In any case, the central role of ABCE1 in ribosome recycling and the available structural information of ABCE1 bound

to ribosomes, offers a whole new framework to specifically address its function in host-pathogen interactions.

Concluding remarks

The combination of biochemical and cell biological analysis with structural biology hybrid methods identified ABCE1 as a new evolutionary core translation factor and visualized it bound to its substrate, the ribosome (Figure 3). However, our understanding of ABCE1 is far from being complete. Clearly, additional functional states of ABCE1 need to be visualized at the ribosome or ribosomal subunits to reveal the role of ATP-driven conformational changes in ribosome recycling. In addition the molecular role of ABCE1 in HIV capsid assembly and RNase-L inhibition can now be better addressed and it should be clarified whether ABCE1 in these instances acts through its core function in translation or has additional activities. Finally, the role of the iron-sulfur clusters is, in my opinion, not understood. Although no evidence for a redox function has been described so far, the twin clusters of iron-sulfur clusters are costly for the cell. It is possible that the functional interaction with Pelota and eRF1 and conformational changes impose very peculiar architectural constraints on the system that require iron-sulfur clusters. However, at least bacteria have found a different solution and it remains to be shown why none of the archaeal and eukaryotic species replaced the FeS domain with a simpler motif. Alternatively, the electronic properties and charge distribution of the clusters could be critical. Indeed Barthelme et al. (2011) observed that ABCE1 with deficient FeS clusters does not bind to the ribosomal small subunit any longer under conditions where the WT protein bound. It is possible that even without electron transfer, the stable charge of the clusters and electrostatic potential is critical for the interaction with ribosomes or their splitting. In fact, such a function could also be important for FeS clusters in DNA repair and replication enzymes. In any case, we look forward to the next years of exciting research on this new translation factor.

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