Protein synthesis meets ABC ATPases: new roles for Rli1/ABCE1

Marina V. Rodnina

The essential, conserved NTPase Rli1/ABCE1—a member of the ABC (ATP-binding cassette) superfamily of ATPases—has been implicated in translation initiation, ribosome biogenesis and human immunodeficiency virus capsid assembly. Two recent papers by the Krebber and Pestova groups—the former published in this issue of *EMBO reports*—suggest new important roles of Rli1/ABCE1 in translation termination and ribosome recycling in eukaryotes (Khoshnevis *et al*, 2010; Pisarev *et al*, 2010).

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Protein synthesis is divided into four phases—initiation, elongation, termination and ribosome recycling—which are catalysed by several translation factors. The fundamental reactions of protein synthesis, such as mRNA decoding, peptide bond formation and tRNA translocation, follow the same basic principles in prokaryotes and eukaryotes. However, some steps are quite different and require a larger set of factors in eukaryotes. The best-studied example of eukaryotic complexity is the initiation of protein synthesis. In prokaryotes, initiation is catalysed by only three factors—IF1, IF2 and IF3—whereas in mammals it requires at least 13. Two recent papers shed new light on termination and ribosome recycling in the yeast and mammalian systems, suggesting that these two steps are also different in eukaryotes and prokaryotes (Khoshnevis *et al*, 2010; Pisarev *et al*, 2010).

In prokaryotes, translation termination is promoted by three release factors: RF1, RF2

and RF3. RF1 and RF2 recognize the three stop codons and catalyse the hydrolysis of the peptidyl-tRNA. RF3, a GTP-binding protein that is not essential in bacteria, does not participate in peptide release but, at the expense of GTP hydrolysis, promotes the dissociation of RF1 and RF2, thereby accelerating their turnover (Kisselev *et al*, 2003). To free the ribosome for initiation on another mRNA (a process known as recycling), the post-termination ribosome is disassembled in a step that requires ribosome recycling factor (RRF) and one of the elongation factors, the GTPase EF-G. Together, these factors promote the dissociation of the post-termination complex into subunits. The subsequent dissociation of tRNA and mRNA from the small ribosomal subunit is promoted by initiation factors, in particular IF3 (Peske *et al*, 2005).

In eukaryotes, translation termination is mediated by only two factors: eRF1 recognizes all three termination codons and triggers the hydrolysis of peptidyl-tRNA, whereas eRF3 accelerates the process in a GTP-dependent manner (Fig 1; Alkalaeva *et al*, 2006). Unlike prokaryotic RF1 or RF2—which have no measurable affinity for RF3—eRF1 binds tightly to eRF3, and it is probably the complex of the two proteins that enters the ribosome. The mechanism of guanine nucleotide exchange on eRF3 is also different from that on prokaryotic RF3, suggesting that termination in eukaryotes and prokaryotes differs in almost every detail except, probably, the mechanism of peptidyl-tRNA hydrolysis itself. Nevertheless, the identification of an additional factor that facilitates termination was unexpected. In this issue of *EMBO reports*, Khoshnevis and colleagues use the power of yeast genetics to show that a protein named Rli1 (RNase L inhibitor 1) interacts physically with the termination factors eRF1 (known as Sup45 in yeast) and, to a

lesser extent, eRF3 (Sup35; Khoshnevis *et al*, 2010). The downregulation of Rli1 expression increases stop codon read-through in a dual reporter system, indicating a lower efficiency of termination. Conversely, upregulation of Rli1 partly suppresses the increased read-through caused by certain mutations of eRF1. Although the mechanism by which Rli1 affects translation termination is not understood, the results of the Krebber lab provide strong evidence that Rli1 mediates the function of eRF1 and eRF3 *in vivo* (Fig 1).

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Surprisingly, modulating the efficiency of termination seems not to be the only function of Rli1 in translation. In a parallel study, Pestova and co-workers show that in higher eukaryotes, the homologue of Rli1—ABCE1—strongly enhances ribosome recycling (Pisarev *et al*, 2010). Eukaryotes lack a homologue of bacterial RRF and thus have to use other factors to disassemble the post-termination ribosome. Ribosome recycling can be brought about to some extent by eIF3, eIF1 and eIF1A (Pisarev *et al*, 2007), which is reminiscent of the IF3/IF1-mediated slow ribosome recycling that seems to occur in some conditions in bacterial systems. In eukaryotes, the initiation-factor-driven recycling operates only in a narrow range of low Mg²⁺ concentrations, probably because the affinity of the subunits to one another increases steeply with Mg²⁺ (Pisarev *et al*, 2010). By contrast, ABCE1 seems to catalyse efficient subunit dissociation in various

Fig 1 | New roles of Rli1/ABCE1 in translation termination and ribosome recycling in eukaryotes. During termination, translating ribosomes contain peptidyl-tRNA (peptide is shown in dark blue and tRNA in dark red) in the P site and expose a stop codon in the A site. The stop codon is recognized by termination factor eRF1, which enters the ribosome together with eRF3-GTP. After GTP hydrolysis, catalysed by eRF3, the peptide is released from the peptidyl-tRNA with the help of eRF1. The point at which Rli1/ABCE1 binds to the ribosome is unknown, but the order shown is consistent with the effect of the factor on both termination and recycling. After NTP hydrolysis by Rli1/ABCE1, the 60S subunit and factors dissociate from the 40S subunit. Finally, tRNA and mRNA are released from the 40S subunit with the help of initiation factors (not shown). ABCE1, ATP-binding cassette, sub-family E member 1; eRF, eukaryotic release factor; Rli1, RNase L inhibitor 1.

conditions. To bind to the ribosome, ABCE1 requires the presence of eRF1, which is thought to induce a conformational change of the ribosome that unmarks the binding site for ABCE1. Subunit dissociation requires NTP (ATP, GTP, CTP or UTP) hydrolysis by ABCE1 (Fig 1). Subsequently, the dissociation of mRNA and tRNA from the small ribosomal subunit is promoted by initiation factors, which also inhibit the spontaneous reassociation of the subunits. Thus, the sequence of events during ribosome recycling in the eukaryotic system is remarkably similar to that in prokaryotes, and ABCE1 and eRF1 (possibly together with eRF3) seem to act as genuine ribosome recycling factors, similar to bacterial RRF/EF-G, despite the lack of any similarity in sequence or structure.

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Rli1/ABCE1 is a member of the ABC ATPases and comprises four structural domains (Karcher *et al*, 2008). Two nucleotide-binding domains (1 and 2) are connected by a hinge and arranged in a head-to-tail orientation. In contrast to other ABC enzymes, ABCE1 has an amino-terminal iron-sulphur (Fe-S) cluster domain, which is located in close proximity to, and presumably interacts with the nucleotide-binding loop of domain 1. Thus, there is a potential link

between Fe-S domain function and NTP-induced conformational control of the ABC tandem cassette. Interestingly, although Khosnevis and colleagues map the eRF1 binding site on the second, carboxy-terminal ATPase domain, the Fe-S cluster is required for the function of Rli1/ABCE1 in termination and recycling (Khosnevis *et al*, 2010). One might speculate that NTP hydrolysis is coupled to splitting the ribosome into subunits, in analogy to the prokaryotic recycling factors RRF/EF-G that couple the free energy of GTP hydrolysis and phosphate release into subunit dissociation (Savelsbergh *et al*, 2009). Kinetic experiments measuring single-round rates of subunit dissociation and NTP hydrolysis would be required to establish the existence and nature of such coupling.

Another intriguing question is the role of the Fe-S cluster in Rli1/ABCE1. Fe-S protein biogenesis is the only known function of mitochondria that is indispensable for the viability of yeast cells (Lill, 2009). As yeast mitochondria do not contain essential Fe-S proteins, the essential character of the mitochondrial Fe-S protein assembly machinery could be attributed to its role in the maturation of extra-mitochondrial Fe-S proteins, such as Rli1/ABCE1, which is essential in all organisms tested.

Another interesting finding by the Krebber group is that Rli1 can bind to Hcr1 (known as eIF3j in higher eukaryotes; Khosnevis *et al*, 2010). Hcr1/eIF3j is an RNA-binding subunit of initiation factor eIF3, which is involved in initiation and required for Rli1/ABCE1-independent ribosome recycling.

The fact that Rli1/ABCE1 binds to both eRF1 and Hcr1/eIF3j might indicate a functional or regulatory link between the termination, recycling and initiation machineries eukaryotes. It is unclear why eukaryotes require termination and recycling machinery that is so different from that of prokaryotes. One possibility is that Rli1/ABCE1, in contrast to its prokaryotic counterparts, not only acts in termination and recycling but also provides a platform for the recruitment of initiation factors to the ribosome, thereby acting as an additional checkpoint for translational control. Thus, the results of the Krebber and Pestova labs open a new, exciting avenue of research on eukaryotic protein synthesis.

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Marina V. Rodnina is at the Department of Physical Biochemistry, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany.
E-mail: rodnina@mpibpc.mpg.de

Published online 12 February 2010

EMBO reports (2010) **11**, 143–144.
doi:10.1038/embor.2010.25