Ribosome Rescue, Nearing the End

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In eukaryotes, Dom34 is involved in the rescue of ribosomes that stall on mRNAs during protein synthesis. Using ribosome profiling, Guydosh and Green reveal that, in addition to rescue of ribosomes stalled on truncated mRNAs, Dom34 also recycles ribosomes that are unexpectedly found in the 3′ untranslated regions of many cellular mRNAs.

During canonical translation, ribosomes terminate when they encounter a stop codon in the mRNA (Figure 1A). In eukaryotes, the stop codon is recognized by the eukaryotic release factor eRF1, which is delivered in complex with eRF3 (Dever and Green, 2012). Hydrolysis of GTP to GDP by eRF3 leads to dissociation of eRF3 from the ribosome, allowing binding of the ATP-binding cassette protein ABCE1. ABCE1 works in concert with eRF1 to catalyze release of the completed polypeptide chain and to dissociate and recycle the posttermination complex components for the next round of translation (Dever and Green, 2012). However, ribosomes that translate damaged or truncated mRNAs that lack a stop codon cannot enter into the canonical translation termination pathway and therefore become stalled at the 3′ end of the mRNA (Franckenberg et al., 2012). Studies using synthetic reporters have demonstrated that Dom34 rescues ribosomes stalled on these damaged mRNAs (Shao et al., 2013; Tsuboi et al., 2012); however, *bone fide* Dom34 mRNA substrates in vivo have not yet been identified. In a new study in this issue of *Cell*, Guydosh and Green (2014) employ ribosome profiling (Ingolia et al., 2009) to globally monitor the position, occupancy, and distribution of ribosomes on mRNAs in wild-type yeast (*S. cerevisiae*) as well as a yeast strain lacking Dom34 (*dom34*Δ). As expected, Dom34 is indeed observed to recycle ribosomes stalled at the 3′ end of truncated mRNAs, such as the *HAC1* mRNA. Unexpectedly, Dom34 also recycles ribosomes from the noncoding region of many cellular mRNAs.

In addition to truncated mRNAs, ribosomes can also stall during translation as a result of other factors, such as elevated levels of uncharged tRNAs (Hinnebusch, 2005) or encoded polyproline stretches (Gutierrez et al., 2013; Ude et al., 2013). While these stalling events are detected by Guydosh and Green (2014), the presence or absence of Dom34 does not influence the ribosome occupancy at these sites, suggesting that Dom34 does not play a general role in recycling all types of stalled ribosomes. A structural explanation for this is that binding of Dom34 to the ribosome (Becker et al., 2011) requires a free A site, which would be occupied by uncharged tRNA or Pro-tRNA, respectively, in the aforementioned stalling scenarios (Figure 1B). Consistently, ribosome stalling on the truncated *HAC1* mRNA, where the A site would be free, is shown to be rescued by Dom34 (Figure 1C) (Guydosh and Green, 2014). While very few additional examples of ribosome stalling on truncated mRNAs are found, this may simply reflect the lack of sensitivity to detect such infrequent and/or stochastic cleavage events. Further inspection does, however, reveal Dom34-mediated rescue of ribosomes stalled at sites on mRNAs that are subject to premature polyadenylation and nonstop decay (NSD), such as *SIR1*, a known target of Dom34 (Tsuboi et al., 2012). In this scenario, it remains unclear whether Dom34 can act directly on the poly(A)-stalled ribosomes and/or whether poly(A) stalling induces mRNA cleavage and generation of truncated mRNAs that are then targeted by Dom34.

By far the most surprising finding of the study of Guydosh and Green (2014) is the accumulation of stalled ribosomes observed within the noncoding region of many mRNAs in the *dom34*Δ strain. The stalled ribosomes are located at the end of the 3′ untranslated region (UTR) and often encompassed part of the poly(A) tail, but never more than 15 consecutive A's. So what is the origin of these ribosomes in the 3′ UTR? Ribosome run-off experiments reveal that ribosome occupancy decreases at the 5′ end of the coding region of the mRNA relative to the 3′ UTR (Guydosh and Green, 2014). Thus, one possible source for the stalled ribosomes might be that translating ribosomes manage to bypass the canonical stop codon of the mRNA, either by nonsense suppression or frameshifting, and then continue translating into the 3′ UTR. Alternatively, the translating ribosomes may terminate at the stop codon, but then reinitiate translation at the downstream AUG codon. To test whether the ribosomes in the 3′ UTR are translating, ribosome profiling was repeated using the *dom34*Δ strain but now treated with the drug 3-amino-1,2,4-triazole (3-AT), an inhibitor of histidine biosynthesis. As expected, these experiments reveal ribosome stalling at His residues within the coding region of mRNAs, but no increase in ribosome occupancy is observed in the 3′ UTRs, in any of the three reading frames. Moreover, the decrease in ribosome occupancy following stop codons that is observed in the coding region is not observed in the 3′ UTRs. Collectively, these data suggest that the majority of ribosomes in the 3′ UTR are of the nontranslating variety.

How do nontranslating ribosomes enter the 3′ UTR of some mRNAs? One possibility is that translating ribosomes undergo termination at the canonical stop codon, but that ribosome recycling by ABCE1 is inefficient, which allows ribosomes to scan into the 3′ UTR (Figure 1D). Consistent with this hypothesis is the

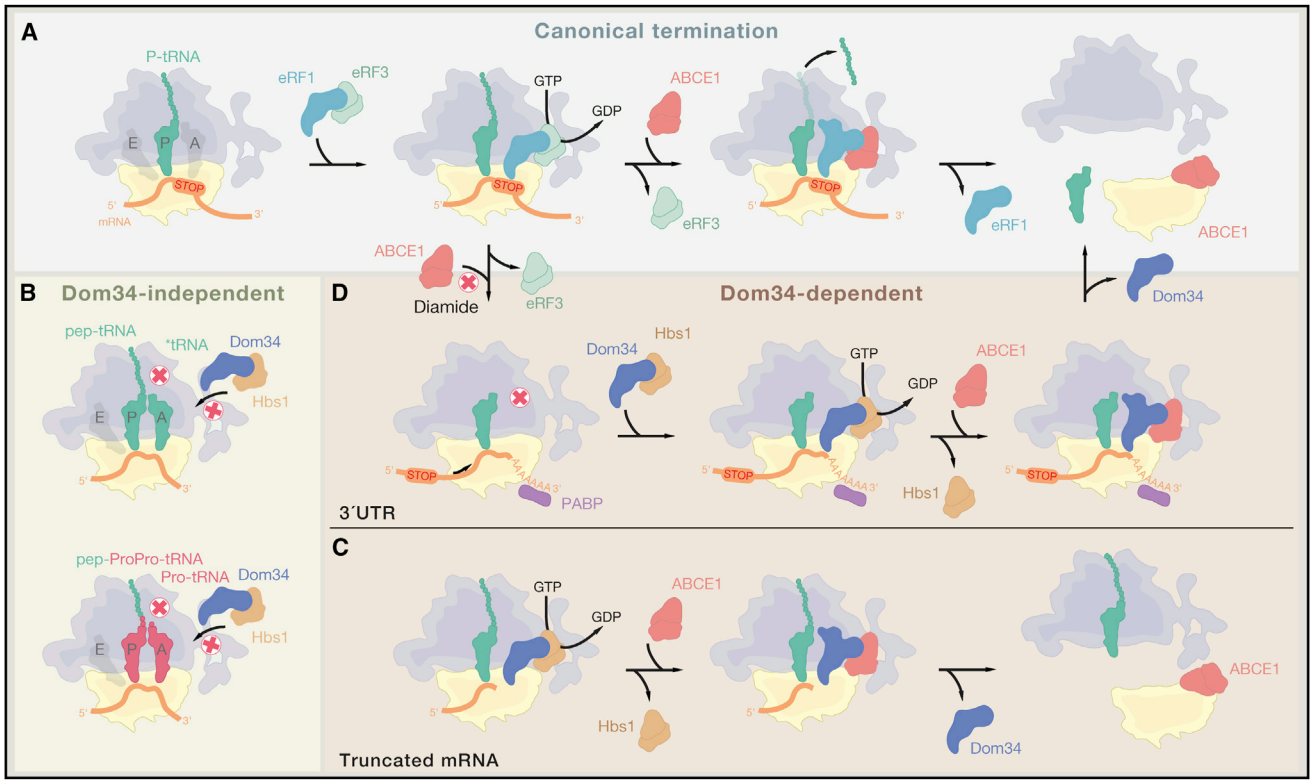

Figure 1. Ribosome Termination and Recycling in Eukaryotes

(A) Scheme for canonical termination by eRF1-eRF3 and ribosome recycling by eRF1 and ABCE1.

(B) Ribosome stalling with an occupied A site, as in the case of translation under conditions of amino acid starvation or of polyproline stretches, is not rescued by Dom34.

(C) Dom34-dependent rescue of ribosomes stalled on truncated mRNAs.

(D) Inefficient recycling by ABCE1, for example in the presence of diamide, allows ribosomes to scan into the 3′ untranslated regions (UTR) of the mRNA. Ribosome scanning into the poly(A) tail may be prevented by the presence of PABP. The stalled ribosomes are recycled by the consecutive action of Dom34-Hbs1 and ABCE1.

observation that treatment of yeast with the oxidizing agent diamide, which inactivates the FeS-cluster proteins such as ABCE1, leads to increased occupancy of ribosomes in the 3′ UTR (Guydosh and Green, 2014). Nevertheless, it remains unclear why an increased occupancy of ribosomes is found in the 3′ UTR of some mRNAs and not others. Comparison of ribosomal protein genes reveals duplicate pairs with identical or near-identical coding sequences, yet differing 3′ UTRs and ribosome occupancies. Thus, it is tempting to speculate that the 3′ UTR modulates the ribosome accumulation, possibly by influencing the efficiency of termination and/or recycling. Yet the lack of any obvious common feature between 3′ UTRs with the highest ribosome occupancy provides us with little clues as to how this is mediated. Elucidating which, if any, protein factors are associated with these 3′ UTRs and whether they exert an influence on ribosome recycling and scanning may provide some initial hints. In this respect, the accumulation of ribosomes adjacent to the poly(A)-tail may arise due to the presence of the poly(A)-binding protein (PABP), which prevents further scanning of the ribosomes along the mRNA (Figure 1D). Finally, the large number of transcripts (>10%) with ribosomes scanning into the 3′ UTR raises the questions as to whether these non-translating ribosomes serve a specific purpose, possibly by regulating the pool of free ribosomes in the cell or more directly regulating the gene expression of the associated mRNAs in some manner.

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