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Recent structural studies on Dom34/aPelota and Hbs1/aEF1α: important factors for solving general problems of ribosomal stall in translation

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In the translation process, translating ribosomes usually move on an mRNA until they reach the stop codon. However, when ribosomes translate an aberrant mRNA, they stall. Then, ribosomes are rescued from the aberrant mRNA, and the aberrant mRNA is subsequently degraded. In eukaryotes, Pelota (Dom34 in yeast) and Hbs1 are responsible for solving general problems of ribosomal stall in translation. In archaea, aPelota and aEF1α, homologous to Pelota and Hbs1, respectively, are considered to be involved in that process. In recent years, great progress has been made in determining structures of Dom34/aPelota and Hbs1/aEF1α. In this review, we focus on the functional roles of Dom34/aPelota and Hbs1/aEF1α in ribosome rescue, based on recent structural studies of them. We will also present questions to be answered by future work.

Key words: structural biology, No-go decay, tRNA-mimicry, ribosome, aberrant mRNA

In the translation process, aminoacyl-tRNAs (aa-tRNAs) are delivered to the ribosomal A site by elongation factors, and translates mRNA codons into amino acids. Ribosomes then move on mRNA by one codon and incorporate newly delivered aa-tRNAs in the A site. By repeating this cycle, ribosomes move on mRNAs, incorporate specific amino acids via aa-tRNAs, and synthesize polypeptides correctly reflecting mRNA sequence. Usually, ribosomes finally reach stop codons on mRNA, and polypeptide release and ribosome recycling occur by release factors and recycling factors[1–3].

However, when ribosomes translate aberrant mRNAs, they are trapped at the aberrant region of mRNA and stall[4]. Such aberrant mRNAs should be eliminated because they trap functional ribosomes and produce truncated aberrant proteins harmful to cells. Eukaryotes have mRNA surveillance systems to eliminate aberrant mRNAs[4,5]. When a ribosome stalls on a premature stop codon, a stop codon upstream of authentic one, the problem is solved by nonsense mediated decay (NMD) pathway[6]. In NMD in multicellular organisms, release factors, Upf1 and SMG proteins detect ribosomes stalled on a premature stop codon. Then, the premature stop codon-containing mRNA is endonucleolytically cleaved by SMG6, followed by the degradation by exonucleases such as Xrn1 and exosome[6,7]. When a ribosome is blocked by the aberrant structure of mRNA such as stem loop and damaged nucleotides, the endonucleolytic cleavage and subsequent degradation of the aberrant mRNA and dissociation of stalled ribosomes occur by No-go decay (NGD) pathway[8–10].

NGD depends on two protein factors: Dom34 (Pelota) and Hbs1[8] (Table 1). Dom34 is related to eukaryotic release factor 1 (eRF1), which is delivered to the ribosomal A site by eRF3 and detects a stop codon in the translation termination[11–13] (Table 1). Hbs1 is related to translational GTPase proteins such as bacterial elongation factor (EF-Tu), eukaryotic/archaeal elongation factor (e/aEF1α), eRF3, and so on[14] (Table 1). Because Hbs1 forms a stable complex with Dom34[15], Hbs1 is considered to deliver Dom34 to the stalled

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Table 1 Names of factors involved in translation elongation, termination, No-go decay, and ribosome recycling

	Elongation		Termination		No-go decay		Ribosome recycling
	GTPase	Partner	GTPase	Partner	GTPase	Partner	
Eukaryotes (Yeast)	eEF1α	aa-tRNA	eRF3	eRF1	Hbs1	Pelota (Dom34)	ABCE1 (Rli1)
Archaea	aEF1α	aa-tRNA	aEF1α	aRF1	aEF1α	aPelota	aABCE1
Bacteria*	EF-Tu	aa-tRNA	–	–	–	–	–

* In bacterial, factors for elongation only are shown because the reaction mechanisms of other processes are different from those in eukaryotes and archaea.

ribosomal A site just as eRF3 carries eRF1 to the ribosomal A site harboring a stop codon[16] (Table 1). On the other hand, in archaea, archaeal homolog of Dom34 (aPelota) is widely conserved, whereas that of Hbs1 does not exist in the archaeal genome[17]. Instead, aEF1α interacts not only with tRNA but also with archaeal homolog of eRF1 (aRF1) and aPelota, and is therefore considered to perform three different functions: translation elongation, termination, and NGD[17] (Table 1).

However, recent studies revealed more extended functions of Dom34 and Hbs1 in translation. Ribosomes translating the aberrant mRNA lacking a stop codon reach the 3′ poly(A) tail of mRNA and translate it into polylysine sequence. The synthesized polylysine sequence also causes ribosomal stall, possibly because positively charged polylysine sequence forms the electrostatic interaction with the negatively charged ribosomal exit tunnel[5,18]. The binding of RACK1 and Hel2 proteins to ribosomes is required for this translation arrest[18,19]. In addition, the 5′ side of mRNAs truncated by NGD also cause ribosomal stall at their 3′ ends because they harbor neither stop codons nor poly(A) tail[20]. The degradation of such kinds of aberrant mRNAs and dissociation of stalled ribosomes from them were initially considered to be performed by nonstop decay (NSD) pathway involving Ski7 protein[21,22], but NSD was shown to involve Dom34 and Hbs1 and cause endonucleolytic cleavage of mRNAs[9,18,20]. Therefore the pathway of NSD seems to be essentially the same with that of NGD. Furthermore, Dom34 and Hbs1 were reported to be responsible for the elimination of defective ribosomes stalled on mRNAs, whose 18S rRNAs forming the decoding site in their 40S subunits harbor deleterious mutations (18S NRD)[23,24]. Therefore, Dom34/Pelota and Hbs1 are involved in solving broad range of problems of ribosomal stall in translation. However, in order to understand the functional roles of Dom34/Pelota and Hbs1, structural information of these factors is necessary. In this review, we focus on our understanding of the functional roles of Dom34/Pelota and Hbs1 in NGD obtained by recent structural studies, and present questions to be answered by future work.

Structures of aPelota/Dom34

Crystal structures of aPelota/Dom34 from archaea (aPelota) and yeast (Dom34) are shown in Figure 1A and 1B, respectively[11,25]. Both structures consist of three domains: domains A, B, and C, similarly to a/eRF1 structure (Fig. 1C)[17,26]. Although the relative orientation between each domain is different, the shape of each domain is similar between aPelota and Dom 34. The structures of domains B and C of aPelota/Dom34 are similar to those of a/eRF1, but there are some differences between them. Domain B of a/eRF1 harbors the long loop containing highly conserved GGQ motif, which catalyzes peptidyl-tRNA hydrolysis in translation termination[27] (Fig. 1C). The corresponding loop of aPelota/Dom34 (Loop C) does not harbor GGQ motif, while it was shown to play important role in the endonucleolytic cleavage of aberrant mRNAs in NGD[16]. In addition, the highly conserved unique motif of PGF/Q sequence exists in domain B of aPelota/Dom34 (Fig. 1A and B). PGF/Q motif was also shown to be important for that[16].

Furthermore, the structure of domain A is different between aPelota/Dom34 and a/eRF1 (Fig. 1A, 1B, and 1C), which may reflect the functional difference between them. Domain A of Dom34 was considered to interact with stalled ribosomes in the A site just as that of a/eRF1 recognizes a stop codon there[9,26]. This proposal was supported by the observation that the mutations of two loops at the tip of domain A of Dom34 (Loop A and B) (Fig. 1B) reduce the endonucleolytic mRNA cleavage efficiency in NGD[16]. Domain A of aPelota/Dom34 has weak similarity to Sm-fold proteins, which generally bind to single-stranded RNA[11,28]. The previous study reported the *in vitro* endoribonuclease activity of domain A of aPelota/Dom34[11], while other experimental data are against that observation[16,18]. The recent study reported that Dom34 and Hbs1 are involved in the endonucleolytic cleavage of mRNAs but not essential[18].

Structures of Dom34·Hbs1 complex and aPelota·aEF1α·GTP complex

Following the structure determination of Dom34/aPelota, crystal structures of yeast Dom34·Hbs1 complex and archaeal aPelota·aEF1α·GTP complex were reported (Fig. 2A and 2B)[9,29]. In the aPelota·aEF1α·GTP complex structure, GTP-bound aEF1α recognizes domains B and C of aPelota through the groove formed among its three domains: domains 1, 2, and 3. GTP is bound to domain 1 of aEF1α through a magnesium ion (Fig. 2B). The structure of aPelota·aEF1α·GTP complex is similar to that of Dom34·Hbs1 complex, although domain 1 of Hbs1 does not interact with Dom34, probably because it is not bound to GTP (Fig.

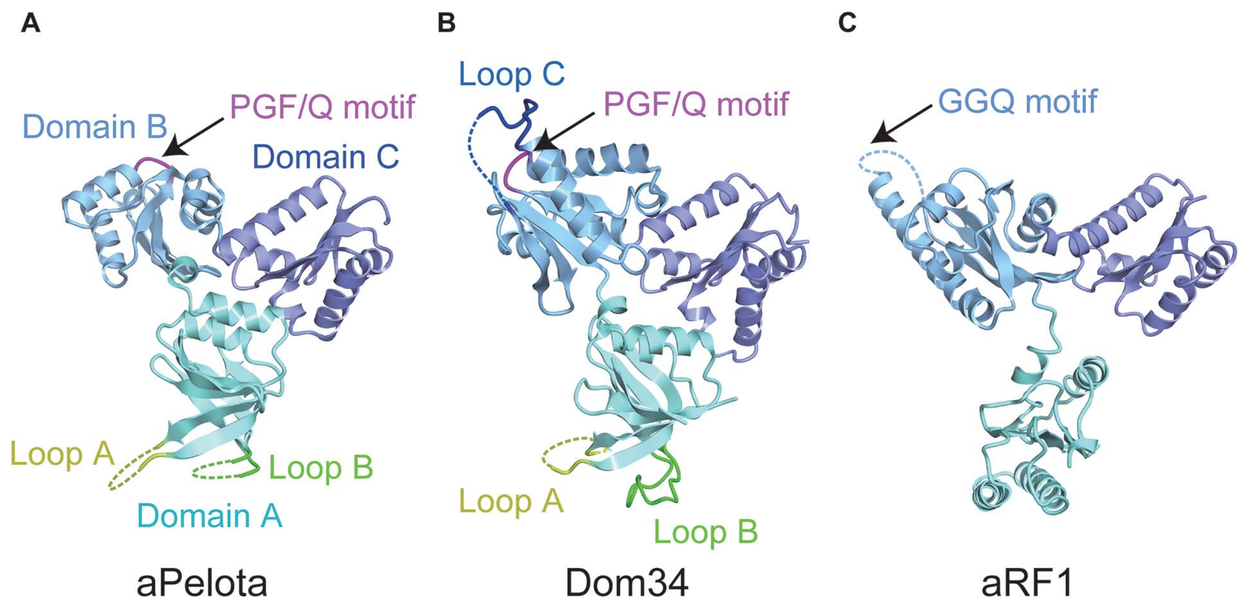

Figure 1 Crystal structures of (A) aPelota from *Thermoplasma acidophilum* (PDB ID: 2QI2), (B) Dom34 from *Saccharomyces cerevisiae* (PDB ID: 2VGM), and (C) aRF1 from *Aeropyrum pernix* (PDB ID: 3AGK). Domains A, B, and C are colored turquoise, light blue and purple, respectively. Disordered loops are represented as dashed lines. Loop A, B, C, and PGF/Q motif are colored yellow, green, blue, and pink, respectively.

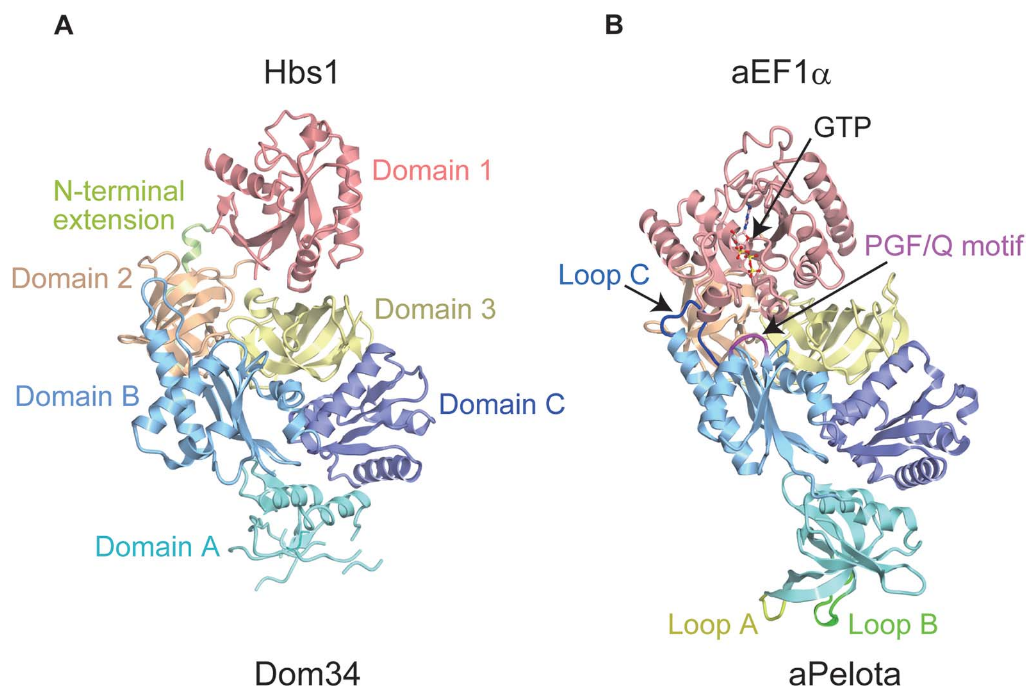

Figure 2 Crystal structures of (A) Dom34·Hbs1 complex (PDB ID: 3MCA) and (B) aPelota·aEF1α·GTP complex (PDB ID: 3AGJ). Domains 1, 2, and 3 of Hbs1/aEF1α are colored red, brown, and yellow, respectively. N-terminal extension of Hbs1 is colored light green. Dom34 and aPelota are colored as in Figure 1. GTP bound to aEF1α domain 1 is shown by ball-and-stick model.

2A and 2B). Hbs1 harbors N-terminal extension sequence absent in aEF1α, which is considered to stabilize the conformation of Hbs1 in complex with Dom34[29] (Fig. 2A). Therefore, the functional roles of Dom34/aPelota and Hbs1/aEF1α in NGD are considered to be essentially the same between archaea and eukaryotes.

Loop C and PGF/Q motif of aPelota

aPelota stabilizes GTP-bound domain 1 of aEF1α through

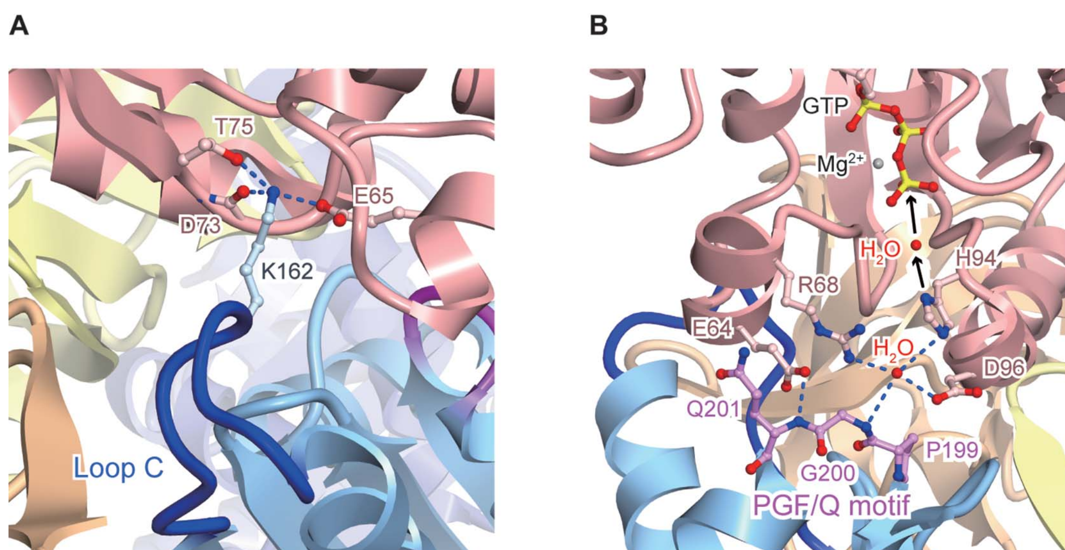

Figure 3 Detailed interaction manner between GTP-bound aEF1α and aPelota through (A) Loop C and (B) PGF/Q motif of aPelota. aEF1α and aPelota are colored as in Figure 2. Water molecules and the magnesium ion are depicted by red and gray spheres, respectively. Hydrogen bonds are indicated by dashed blue lines. The catalytic pathway of the nucleophilic attack to the γ-phosphate of GTP caused by the activation of the water molecule by His94 of aEF1α is shown by arrows.

several interactions with it, which is consistent with the previous observation that Dom34 increases the affinity of Hbs1 to GTP[25]. Lys162 of Loop C of aPelota interacts with Glu65, Asp73, and Thr75 of aEF1α domain 1 (Fig. 3A). Furthermore, the highly conserved PGF/Q motif of aPelota (from Pro199 to Gln201) forms a small bulge and interacts with the GTPase catalytic site of domain 1 of aEF1α (Fig. 3B). Pro199 of aPelota seems to contribute to form the bulge, whose shape is complementary to that of the GTPase catalytic site of aEF1α. In addition, the main chain nitrogen of Gly200 of aPelota interacts with the side chain of His94 through the water molecule and sequesters it from the γ-phosphate of GTP (Fig. 3B). His94 of aEF1α, which is highly conserved among translational GTPase family proteins, is considered to be responsible for the hydrolysis of GTP by activating the catalytic water molecule behind the γ-phosphate of GTP on ribosomes[30,31], although the actual role of this histidine residue remains controversial[32,33]. Therefore, if His94 of aEF1α actually activates the catalytic water molecule, the small bulge formed by PGF/Q motif of Dom34/aPelota is considered to stabilize the GTP bound to Hbs1/aEF1α by preventing the activation of the catalytic water molecule. These observations suggest that mutations of Loop C and PGF/Q motif of Dom34 destabilize GTP-bound domain 1 of Hbs1 and reduce intermolecular contacts between Dom34 and Hbs1, which may reduce the efficiency of the endonucleolytic mRNA cleavage in NGD.

A/T state tRNA-mimicry by aPelota

The overall structure of aPelota·aEF1α·GTP complex resembles that of aa-tRNA·EF-Tu·GDPNP complex[34], similarly to aRF1·aEF1α·GTP complex structure[35], with domains A, B, and C of aPelota corresponding to anticodon arm, acceptor stem, and T stem of tRNA, respectively (GDPNP is a nonhydrolyzable analog of GTP.) (Fig. 4A, 4B, and 4C). Therefore, Dom34/aPelota is considered to be delivered to the A site of stalled ribosomes in NGD, just as aa-tRNA is delivered to that of ribosomes harboring a sense codon in translation elongation, and a/eRF1 is delivered to that of ribosomes harboring a stop codon in translation termination.

The question is how Dom34·Hbs1·GTP/aPelota·aEF1α·GTP complex recognizes stalled ribosomes and causes NGD. The decoding mechanism by aa-tRNA·EF-Tu·GTP complex in bacteria is well studied[30,31]. The aa-tRNA delivered to the ribosomal A site by GTP-bound EF-Tu forms the codon-anticodon paring by bending its anticodon arm approximately 30°. In this conformation, called A/T state, the aa-tRNA interacts with 16S rRNA nucleotides forming the decoding center (DC) of the ribosomal 30S subunit, such as G530, A1492, and A1493. The interaction between aa-tRNA and DC causes the conformation change of the ribosomal 30S subunit, which ultimately leads to the GTP-hydrolysis by EF-Tu and the release of EF-Tu from the ribosome. After the release of EF-Tu, aa-tRNA is fully accommodated into the ribosomal A site. Therefore, adopting A/T state conformation is responsible for the decoding by tRNAs because tRNAs can interact with DC through the correct codon-anticodon paring only when they adopt this conformation. Actually, it is reported that the miscoding efficiency by aa-tRNAs is drastically enhanced by enabling them to access the A/T state more easily by increasing the flexibility of tRNA body[36].

Interestingly, the present structure of aPelota bound to

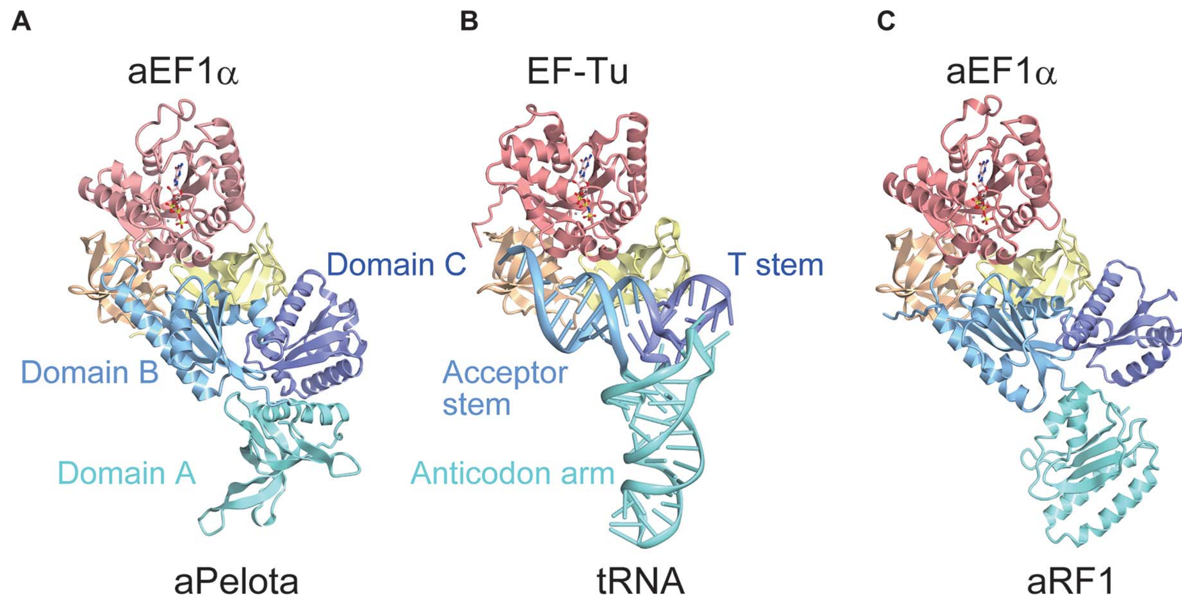

Figure 4 Crystal structures of (A) aPelota·aEF1α·GTP complex (PDB ID: 3AGJ), (B) aa-tRNA·EF-Tu·GDPNP complex (PDB ID: 1TTT), and (C) aRF1·aEF1α·GTP complex (PDB ID: 3VMF). Domains are colored as in Figure 2. The anticodon arm, acceptor stem, and T-stem of tRNA are colored turquoise, light blue and purple, respectively.

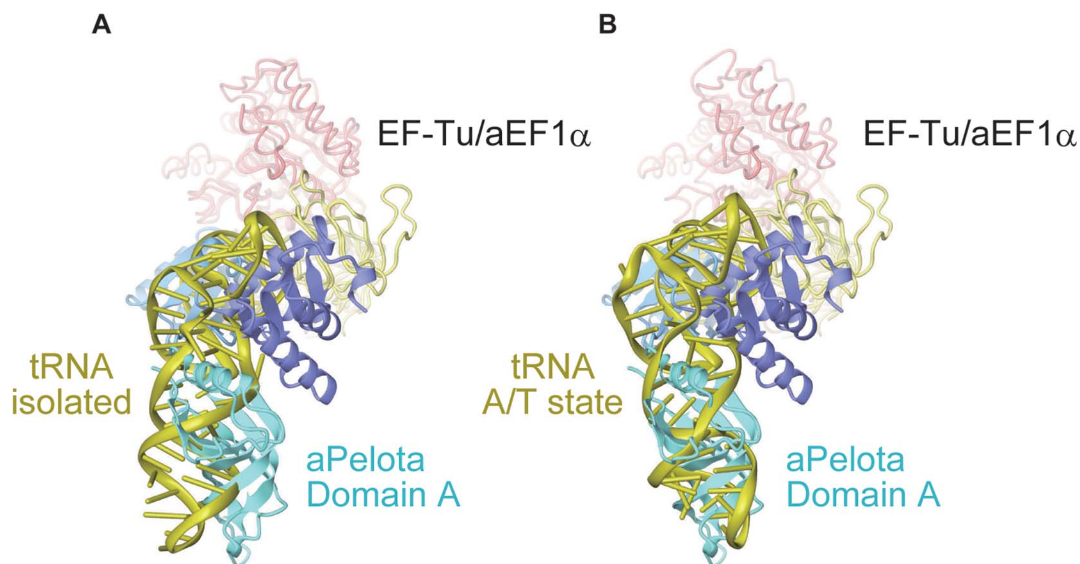

Figure 5 Structural comparison of aEF1α-bound aPelota (PDB ID: 3AGJ) with (A) tRNA bound to EF-Tu (PDB ID: 1TTT) and (B) A/T state tRNA bound to EF-Tu and mRNA in the ribosomal A site (PDB ID: 2XQD). aPelota and aEF1α/EF-Tu are colored as in Figure 2, while tRNA is colored yellow. This figure was cited and modified from ref. 9.

aEF1α is superposed better on the A/T state tRNA than on the tRNA in the isolated aa-tRNA·EF-Tu·GDPNP complex (Fig. 5A and 5B). Domain A of aPelota is rigidly fixed in this conformation through several interactions with domain C. This observation implies that aPelota mimics ribosome-bound A/T state tRNA in advance. Therefore, aPelota delivered to the A site of the stalled ribosomes by aEF1α was proposed to directly recognize DC of them without codon recognition and cause NGD[9]. This proposal is consistent with the function of Dom34/aPelota in NGD. Because random or no codon exists in the A site of the stalled ribosome, Dom34/aPelota should recognize the ribosomal A site in a codon-independent manner.

Implications for NGD

Based on these observations, the docking model of ribosome in complex with aPelota·aEF1α·GTP was con-

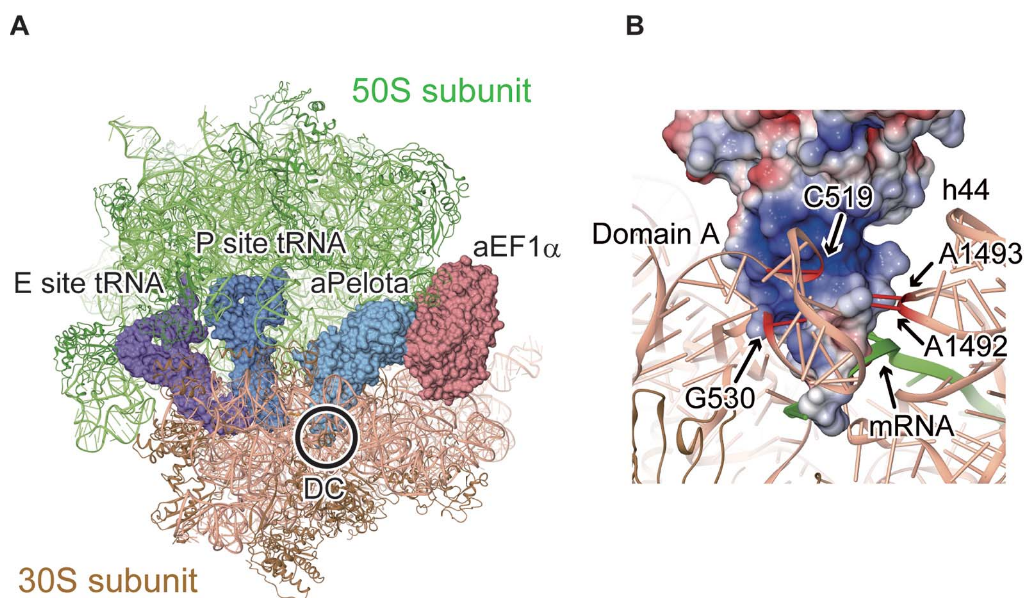

Figure 6 The docking model of the ribosome in complex with aPelota·aEF1α·GTP. (A) The overall view of the docking model. The 50S and 30S subunits of the ribosome (PDB ID: 2WRR and 2RWQ) are colored green and brown, respectively. tRNAs in the E and P sites, aPelota, and aEF1α are shown in surface representations colored purple, blue, light blue, and red, respectively. (B) Close up view around DC of this model. aPelota is shown in the surface representation with positively charged regions and the negatively charged regions colored blue and red, respectively. The intensity of the color is proportional to the local potential (range −10 kT/e to +10 kT/e). mRNA is colored green. 16S rRNA nucleotides seemed to interact with aPelota domain A are colored red. This figure was cited and modified from ref. 9.

structured[9] (Fig. 6A). In this model, aPelota directly interacts with phosphates of nucleotides forming DC through its positively charged residues on its domain A (Fig. 6B). Mutations of counterpart residues of Dom34 reduced its *in vivo* efficiency of both endonucleolytic mRNA cleavage and dissociation of ribosomes stalled at the 3′ end of mRNA in NGD[9]. Mutations of Loop A and Loop B of Dom34 brought a similar effect[9]. These results strongly support that domain A of Dom34/aPelota recognizes the A site of the stalled ribosomes in a codon-independent manner in NGD.

Then, the role of GTP hydrolysis by Hbs1 in NGD was analyzed. Hbs1 mutants deficient in the GTPase activity showed a complete loss of ribosome dissociation activity, whereas moderately reduced mRNA cleavage activity[9]. This result implies that GTP hydrolysis by Hbs1 is essential for the ribosome dissociation but not for the mRNA cleavage, which is consistent with the previous observation that Hbs1 is not essential for the mRNA cleavage[18]. It was also reported that GTP hydrolysis by aEF1α is repressed on ribosomes in the presence of aPelota[17]. Therefore, Dom34/aPelota is considered to delay ribosome dissociation by repressing GTP hydrolysis activity of Hbs1/aEF1α on ribosomes in NGD, which may ensure the putative endonucleolytic cleavage of mRNA before ribosome dissociation.

Based on these observations, the following NGD mechanism was proposed[9]. First, ribosomes stalled on aberrant mRNAs are detected by Dom34·Hbs1·GTP/aPelota·aEF1α·GTP complex. Dom34/aPelota is inserted into ribosomal A site and directly recognizes DC by mimicking A/T state tRNA. Secondly, the putative endonuclease is recruited and cleaves the aberrant mRNA. Dom34/aPelota may enable the endonuclease to cleave mRNA before ribosome dissociation by repressing GTP hydrolysis by Hbs1/aEF1α. Finally, GTP is hydrolyzed by Hbs1/aEF1α, which would induce the dissociation of the stalled ribosome.

ABCE1: an essential factor for the ribosome dissociation

Consistent with the proposed NGD mechanism, Dom34·Hbs1·GTP complex was shown to detect the A site of stalled ribosomes and promotes subunit dissociation and peptidyl-tRNA drop off in an A site codon-independent manner *in vitro*[37]. It was also reported that Dom34 and Hbs1 enables exosome to degrade mRNAs by dissociating stalled ribosomes[20]. Furthermore, the Cryo-EM image of the yeast stalled ribosome in complex with Dom34·Hbs1·GDPNP was reported[38]. The structure of ribosome-bound Dom34·Hbs1·GDPNP complex is similar to that of aPelota·aEF1α·GTP complex[9], and domain A of Dom34 directly interacts with the DC. Therefore, it was shown that Dom34 directly recognizes the A site of stalled ribosomes by mimicking A/T state tRNA in advance. As a result, Dom34·Hbs1·GTP complex may dissociate the stalled ribosome and drop off the peptidyl-tRNA in a codon-independent manner. Because Dom34 and Hbs1 interact with rRNA and ribosomal proteins forming an mRNA stabilizing network, they were suggested to destabilize mRNA by disrupting this network[38].

However, additional factors are considered to be required for the ribosome dissociation in NGD under physiological conditions because the efficiency of ribosome dissociation by Dom34·Hbs1·GTP complex is low[38]. As a factor responsible for the eukaryotic ribosome dissociation in NGD, ABCE1 protein, which is highly conserved in eukaryotes and archaea (Rli1 in yeast and aABCE1 in archaea) was identified[39] (Table 1). ABCE1 is a member of the ATP-binding cassette superfamily, which facilitates mechanochemical work by its conformation change induced by ATP binding and hydrolysis[40]. After translation termination is completed, aABCE1/ABCE1 interacts with a/eRF1 in ribosomes and promotes the dissociation of ribosomes into subunits in an ATP-dependent manner[41–43]. Moreover, *in vitro* reconstitution analysis showed that mammalian Pelota·Hbs1·GTP complex efficiently dissociates ribosomes on mRNAs lacking stop codon only in the presence of ABCE1 and ATP[39]. Therefore, aABCE1/ABCE1 is considered to be responsible for the ribosome dissociation in both translation termination and NGD.

Structures of ribosome in complex with Dom34 and Rli1, and that with aPelota and aABCE1

The cryo-EM image of yeast ribosome in complex with Dom34 and Rli1, and that of archaeal ribosome in complex with aPelota and aABCE1 showed that Rli1/aABCE1 occupies the intersubunit space of ribosomes, where translational GTPases such as EF-Tu and Hbs1 also bind, and forms multiple contacts with both ribosomal subunits via all domains of Rli1/aABCE1[44]. In addition, the iron-sulphur cluster (FeS) domain of Rli1/aABCE1, which contains two [4Fe-4S] clusters, interacts with domain C of Dom34/aPelota. Therefore, the dissociation of Hbs1/aEF1α from ribosomes caused by GTP hydrolysis may precede Rli1/aABCE1 binding. As for Dom34/aPelota, a notable conformation change is observed in its domain B. Compared with their Hbs1/aEF1α-bound conformation, they rotated by approximately 140° toward P site tRNA and interacts with the acceptor stem of it through the Loop C (Fig. 7A and 7B) (Movies explaining this conformation change are available from ref. 44.). Therefore, the Loop C of Dom34/aPelota may be involved in the recognition of both Hbs1/aEF1α and P site tRNA, which further explains the important role of the Loop C in NGD[16]. Furthermore, Dom34/aPelota domains form a broad interaction network with both ribosomal subunits, as well as with P-site tRNA. Therefore, these observations imply that ATP-driven conformation change of Rli1/aABCE1 may be transmitted through the contact between FeS domain and Dom34/aPelota to P site tRNA and ribosomal subunits, which may ultimately dissociate ribosomes by destabilizing ribosomal intersubunit bridges and P site tRNA. The functional role of the FeS domain as the bridge between Rli1/aABCE1 and Dom34/aPelota is supported by the previous report that the deletion of this domain of aABCE1 abolishesd its ribosome dissociation activity[42]. However, it is also possible that the ATP-driven conformation change of Rli1/aABCE1 directly disrupts ribosomal intersubunit bridges.

Based on these insights, the following ribosome dissociation mechanism in NGD was proposed[44]. After Dom34/aPelota recognizes the stalled ribosome, Hbs1/aEF1α hydrolyzes GTP and is released from it. Subsequently, Dom34/aPelota domain B is rotated and interacts with the acceptor stem of P site tRNA. Then, Rli1/aABCE1 binds to the canonical GTPase binding site of the ribosome between

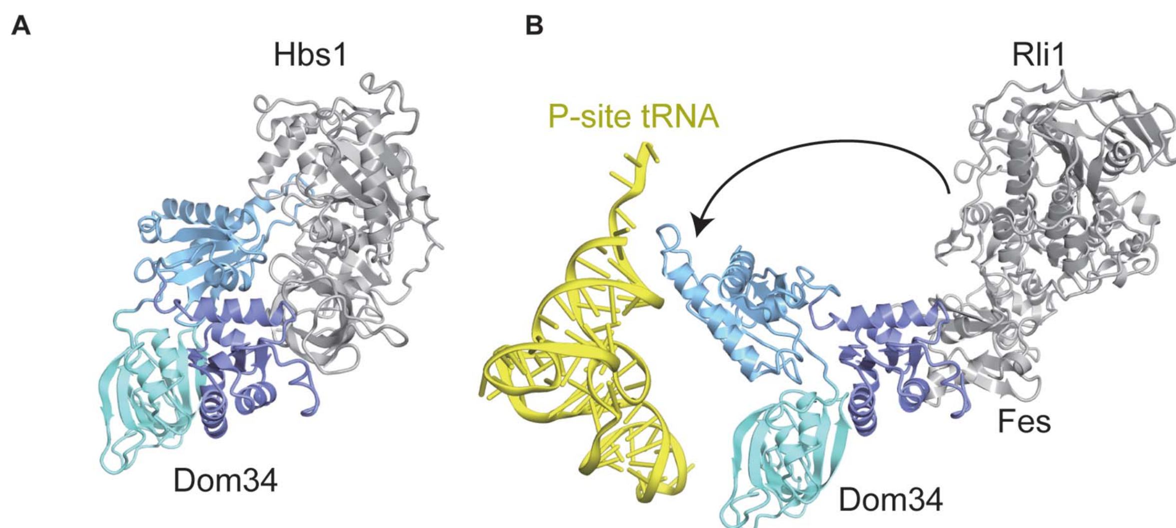

Figure 7 Structural comparison between GDPNP·Hbs1-bound Dom34 and Rli1-bound Dom34. (A) Structure of Dom34·Hbs1·GDPNP complex in ribosomal A site (PDB ID: 3IZQ). (B) Structure of Dom34·Rli11 complex in ribosomal A site (PDB ID: 3J16). The movement of domain B of Dom34 from its Hbs1-bound position is indicated as an arrow. Dom34 is colored as in Figure 1. Hbs1 and Rli1 are colored gray. P-site tRNA is colored yellow.

subunits and recognizes Dom34/aPelota domain C. Finally, the conformation change of Rli1/aABCE1 caused by ATP binding and hydrolysis induces ribosome dissociation into subunits directly or indirectly through conformation changes of Dom34/aPelota. However, further studies are required for understanding how Rli1/aABCE1 dissociates ribosomes into subunits in an ATP-dependent manner.

Similarities and differences between NGD and translation termination

NGD is analogous to translation termination. First, GTP-bound Hbs1 delivers Dom34 to the A site of stalled ribosomes, whereas GTP-bound eRF3 delivers eRF1 to the A site of ribosomes harboring a stop codon (Table 1). Dom34 and Hbs1 are related to eRF1 and eRF3, respectively. Although the crystal structures of Dom34·Hbs1·GTP complex and eRF1·eRF3·GTP complex are not determined, those of aPelota·aEF1α·GTP complex and aRF1·aEF1α·GTP complex are similar[9,35].

However, the shapes of domain A, which may be involved in the recognition of ribosomes are different between aPelota and aRF1. aPelota is considered to directly recognize the DC of stalled ribosomes in an A site codon-independent manner by rigidly fixing its domain A and mimicking the shape of A/T state tRNA. In contrast, domain A of aRF1, which harbors stop codon recognition sequences, seems to be flexible because it does not interact with its domains B and C. The flexibility of aRF1 domain A may mimic the anticodon arm flexibility of tRNA. As described above, the flexibility of tRNA anticodon arm plays an important role in the correct decoding because it enables tRNAs to interact with DC by adopting the A/T state conformation only when they form the correct codon-anticodon paring[30]. Similarly to decoding by tRNA, translation termination by e/aRF1 should occur in a stop codon-dependent manner. Therefore, the flexibility of aRF1 domain A may enable it to interact with DC and terminate translation only when it recognizes a stop codon[35].

Second, GTP hydrolysis by Hbs1/eRF3 on ribosomes enhances NGD/translation termination process. It may be required for the conformation change of domain B of Dom34/eRF1 in ribosomes and the release of GDP-bound Hbs1/eRF3 from ribosomes. Given the similarity of domains B and C between Dom34 and eRF1, domain B of eRF1 may adopt the conformation similar to that of Dom34 and interact with the acceptor stem of tRNA after the release of GDP-bound eRF3[44]. Then, because the loop of eRF1 corresponding to Loop C of Dom34 harbors GGQ motif, which is responsible for the hydrolysis of peptidyl-tRNA, it may be ideally positioned to contact the polypeptide-bound CCA end of P site tRNA in the peptidyl transferase center[44]. Therefore, in the translation termination process, the rearrangement of eRF1 domain B may be responsible for the hydrolysis of peptidyl-tRNA in the P site.

Third, following the conformation change of Dom34/eRF1, Rli1 interacts with it and finally dissociate ribosomes into subunits in an ATP-dependent manner. Therefore, Dom34·Hbs1·GTP complex is responsible for the dissociation of ribosomes stalled on mRNA, whereas eRF1·eRF3·GTP complex is for the dissociation of those harboring a stop codon.

Conclusion and Perspective

Dom34 and Hbs1 were initially considered to be involved in the cleavage and subsequent degradation of mRNAs harboring the stable stem loop structure[8]. However, recent studies revealed that they enhance the degradation of other aberrant mRNAs such as those lacking stop codons, 5′ side of mRNAs truncated by NGD, and so on[20]. Dom34 and Hbs1 are considered to dissociate stalled ribosomes into subunits, which enables exosome to degrade mRNAs[20]. Although the ribosome dissociation from mRNAs lacking stop codons and subsequent degradation of them were initially considered to involve the C-terminal domain of Ski7 in yeast[22], recent studies revealed that it is involved in the degradation of those mRNAs but not in ribosome dissociation[20]. In addition, the recent study reported that Pelota and Hbs1 facilitate the degradation of mRNAs lacking stop codons in mammalian cells lacking Ski7[45]. Therefore, further studies are required to reveal the functional role of Ski7 in NGD. Moreover, Dom34 and Hbs1 are responsible also for the detection of stalled ribosomes on mRNA because of the defect in their 18S rRNA[23]. It was also reported that Dom34 and Hbs1 are involved in the clearance of ribosomes which stalled on the endoplasmic reticulum surface in the process of the co-translational translocation of secretory proteins[46]. Therefore, Dom34 and Hbs1 are considered to be responsible for solving the general problems of ribosomal stall in translation. Structural studies proposed that Dom34 is delivered to the A site of stalled ribosomes by GTP-bound Hbs1, and that subsequent GTP hydrolysis and release of GDP-bound Hbs1 allow Rli1 to bind ribosomes and to dissociate them into subunits in an ATP-dependent manner.

However, at least two questions about the function of Dom34 and Hbs1 in NGD remain to be answered. The first question is how the endonucleolytic cleavage of aberrant mRNAs occurs in NGD. Although Dom34 and Hbs1 stimulate the endonucleolytic cleavage of aberrant mRNAs, that is reported to occur independent of them[18]. The endo- and exonuclease activity of exosome is responsible for the rapid degradation of mRNAs lacking stop codons, but the endonuclease activity of it is not required for the endonucleolytic cleavage of mRNAs containing stem loops[47]. Therefore, how aberrant mRNAs are endonucleolytically cleaved and how Dom34 and Hbs1 stimulate that in NGD remain to be elucidated.

The second question is concerned with the interaction between Dom34 and ribosomes. Although Dom34 was re-

ported to interact with the A site of ribosomes stalled in the middle of mRNAs[37], *in vitro* reconstitution analyses showed that the ribosome dissociation efficiency by Dom34/Pelota·Hbs1·GTP complex in concert with Rli1/ABCE1 depends on the length of mRNA downstream of the ribosomal P site[43]. Dom34/Pelota·Hbs1·GTP complex cannot efficiently dissociate ribosomes containing long mRNA nucleotides downstream of the P site even in the presence of Rli1/ABCE1[39,43]. Hbs1 is considered to confer the dependency on the length of mRNA in NGD[43]. Therefore, the main target of Dom34/Pelota·Hbs1·GTP complex may be ribosomes stalled at the 3′ end of mRNAs. Then, ribosomes following the first stalled one will be efficiently dissociated by Dom34/Pelota·Hbs1·GTP complex because the cleavage of mRNA by the first stalled ribosome will lead all following ribosomes to ultimately stall at the 3′ end of mRNA. However, how are ribosomes stalled in the middle of mRNA, for instance by the stem loop structure of mRNA or poly(A) tail, dissociated? If the endonucleolytic cleavage occurs in the vicinity of A site downstream the stalled ribosomes, they may be dissociated by Dom34/Pelota·Hbs1·GTP complex because they will ultimately stall at the 3′ end of mRNA. However, it was reported that the endonucleolytic cleavage occurs mainly at the 5′ side of stalled ribosomes[20]. In order to answer these questions, further structural and functional studies of Dom34/Pelota and Hbs1 are required.

Moreover, recent studies reported that Dom34 plays a functional role in the area other than translation. In the maturation process of ribosomal 40S subunits, 60S subunits join pre-40S subunits to form 80S-like ribosomes[48]. Following this, Dom34 and Rli1 dissociate 80S-like ribosomes to prepare mature 40S subunits for the translation initiation[48]. Consistent with this observation, 80S monosomes accumulate in yeast *dom34*Δ strain, suggesting the repression of translation initiation[49,50]. These observations suggest that Dom34 plays important roles not only in translation but also in ribosome maturation. Therefore, further functional studies of Dom34 and Hbs1 may reveal novel functions of them as well as the molecular mechanism of NGD.

References

1. Petry, S., Weixibaumer, A. & Ramakrishnan, V. The termination of translation. *Curr. Opin. Struct. Biol.* **18**, 70–77 (2008).
2. Zhou, J., Korostelev, A., Lancaster, L. & Noller, H.F. Crystal structures of 70S ribosomes bound to release factors RF1, RF2 and RF3. *Curr. Opin. Struct. Biol.* **22**, 733–742 (2012).
3. Dever, T.E. & Green, R. The elongation, termination, and recycling phases of translation in eukaryotes. *Cold Spring Harb. Perspect. Biol.* **4**, a013706 (2012).
4. Inada, T. Quality control systems for aberrant mRNAs induced by aberrant translation elongation and termination. *Biochim. Biophys. Acta* **1829**, 634–642 (2013).
5. Shoemaker, C.J. & Green, R. Translation drives mRNA quality control. *Nat. Struct. Mol. Biol.* **19**, 594–601 (2012).
6. Kervestin, S. & Jacobson, A. NMD: A multifaceted response to premature translational termination. *Nat. Rev. Mol. Cell Biol.* **13**, 700–712 (2012).
7. Kashima, I., Jonas, S., Jayachandran, U., Buchwald, G., Conti, E., Lupas, A.N. & Izaurralde, E. SMG6 interacts with the exon junction complex via two conserved EJC-binding motifs (EBMs) required for nonsense-mediated mRNA decay. *Genes Dev.* **24**, 2440–2450 (2010).
8. Doma, M.K. & Parker, R. Endonucleolytic cleavage of eukaryotic mRNAs with stalls in translation elongation. *Nature* **440**, 561–564 (2006).
9. Kobayashi, K., Kikuno, I., Kuroha, K., Saito, K., Ito, K., Ishitani, R., Inada, T. & Nureki, O. Structural basis for mRNA surveillance by archaeal Pelota and GTP-bound EF1α complex. *Proc. Natl. Acad. Sci. USA* **107**, 17575–17579 (2010).
10. Gandhi, R., Manzoor, M. & Hudak, K.A. Depurination of brome mosaic virus RNA3 in vivo results in translation-dependent accelerated degradation of the viral RNA. *J. Biol. Chem.* **283**, 32218–32228 (2008).
11. Lee, H.H., Kim, Y.S., Kim, K.H., Heo, I., Kim, S.K., Kim, O., Kim, H.K., Yoon, J.Y., Kim, H.S., Kim, D.J., Lee, S.J., Yoon, H.J., Kim, S.J., Lee, B.G., Song, H.K., Kim, V.N., Park, C.M. & Suh, S.W. Structural and functional insights into Dom34, a key component of No-go mRNA decay. *Mol. Cell* **27**, 938–950 (2007).
12. Cheng, Z., Saito, K., Pisarev, A.V., Wada, M., Pisareva, V.P., Pestova, T.V., Gajda, M., Round, A., Kong, C.G., Lim, M., Nakamura, Y., Svergun, D.I., Ito, K. & Song, H. Structural insights into eRF3 and stop codon recognition by eRF1. *Genes Dev.* **23**, 1106–1118 (2009).
13. Taylor, D., Unbehaun, A., Li, W., Das, S., Lei, J.L., Liao, H.Y., Grassucci, R.A., Pestova, T.V. & Frank, J. Cryo-EM structure of the mammalian eukaryotic release factor eRF1-eRF3-associated termination complex. *Proc. Natl. Acad. Sci. USA* **109**, 18413–18418 (2012).
14. Inagaki, Y., Blouin, C., Susko, E. & Roger, A.J. Assessing functional divergence in EF-1 alpha and its paralogs in eukaryotes and archaebacteria. *Nucleic Acids Res.* **31**, 4227–4237 (2003).
15. Carr-Schmid, A., Pfund, C., Craig, E.A. & Kinzy, T.G. Novel G-protein complex whose requirement is linked to the translational status of the cell. *Mol. Cell Biol.* **22**, 2564–2574 (2002).
16. Passos, D.O., Doma, M.K., Shoemaker, C.J., Muhlrad, D., Green, R., Weissman, J., Hollien, J. & Parker, R. Analysis of dom34 and its function in No-go decay. *Mol. Biol. Cell* **20**, 3025–3032 (2009).
17. Saito, K., Kobayashi, K., Wada, M., Kikuno, I., Takusagawa, A., Mochizuki, M., Uchiumi, T., Ishitani, R., Nureki, O. & Ito, K. Omnipotent role of archaeal elongation factor 1 alpha (EF1α) in translational elongation and termination, and quality control of protein synthesis. *Proc. Natl. Acad. Sci. USA* **107**, 19242–19247 (2010).
18. Kuroha, K., Akamatsu, M., Dimitrova, L., Ito, T., Kato, Y., Shirahige, K. & Inada, T. Receptor for activated C kinase 1 stimulates nascent polypeptide-dependent translation arrest. *Embo Rep.* **11**, 956–961 (2010).
19. Brandman, O., Stewart-Ornstein, J., Wong, D., Larson, A., Williams, C.C., Li, G.W., Zhou, S., King, D., Shen, P.S., Weibezahn, J., Dunn, J.G., Rouskin, S., Inada, T., Frost, A. & Weissman, J.S. A ribosome-bound quality control complex triggers degradation of nascent peptides and signals translation stress. *Cell* **151**, 1042–1054 (2012).
20. Tsuboi, T., Kuroha, K., Kudo, K., Makino, S., Inoue, E., Kashima, I. & Inada, T. Dom34:Hbs1 plays a general role in quality-control systems by dissociation of a stalled ribosome at the 3′ end of aberrant mRNA. *Mol. Cell* **46**, 518–529 (2012).
21. Frischmeyer, P.A., van Hoof, A., O′Donnell, K., Guerrerio,

A. L., Parker, R. & Dietz, H. C. An mRNA surveillance mechanism that eliminates transcripts lacking termination codons. *Science* **295**, 2258–2261 (2002).

22. van Hoof, A., Frischmeyer, P. A., Dietz, H. C. & Parker, R. Exosome-mediated recognition and degradation of mRNAs lacking a termination codon. *Science* **295**, 2262–2264 (2002).
23. Cole, S. E., LaRiviere, F. J., Merrikh, C. N. & Moore, M. J. A convergence of rRNA and mRNA quality control pathways revealed by mechanistic analysis of nonfunctional rRNA decay. *Mol. Cell* **34**, 440–450 (2009).
24. van den Elzen, A. M. G., Henri, J., Lazar, N., Gas, M. E., Durand, D., Lacroute, F., Nicaise, M., van Tilbeurgh, H., Seraphin, B. & Graille, M. Dissection of Dom34-Hbs1 reveals independent functions in two RNA quality control pathways. *Nat. Struct. Mol. Biol.* **17**, 1446–1452 (2010).
25. Graille, M., Chaillet, M. & van Tilbeurgh, H. Structure of yeast Dom34—A protein related to translation termination factor eRF1 and involved in No-Go decay. *J. Biol. Chem.* **283**, 7145–7154 (2008).
26. Song, H. W., Mugnier, P., Das, A. K., Webb, H. M., Evans, D. R., Tuite, M. F., Hemmings, B. A. & Barford, D. The crystal structure of human eukaryotic release factor eRF1—Mechanism of stop codon recognition and peptidyl-tRNA hydrolysis. *Cell* **100**, 311–321 (2000).
27. Frolova, L. Y., Tsivkovskii, R. Y., Sivolobova, G. F., Oparina, N. Y., Serpinsky, O. I., Blinov, V. M., Tatkov, S. I. & Kisselev, L. L. Mutations in the highly conserved GGQ motif of class 1 polypeptide release factors abolish ability of human eRF1 to trigger peptidyl-tRNA hydrolysis. *RNA* **5**, 1014–1020 (1999).
28. Khusial, P., Plaag, R. & Zieve, G. W. LSm proteins form heptameric rings that bind to RNA via repeating motifs. *Trends Biochem. Sci.* **30**, 522–528 (2005).
29. Chen, L. M., Muhlrad, D., Hauryliuk, V., Cheng, Z. H., Lim, M. K., Shyp, V., Parker, R. & Song, H. W. Structure of the Dom34-Hbs1 complex and implications for No-go decay. *Nat. Struct. Mol. Biol.* **17**, 1233–1240 (2010).
30. Schmeing, T. M., Voorhees, R. M., Kelley, A. C., Gao, Y. G., Murphy, F. V., Weir, J. R. & Ramakrishnan, V. The crystal structure of the ribosome bound to EF-Tu and aminoacyl-tRNA. *Science* **326**, 688–694 (2009).
31. Voorhees, R. M., Schmeing, T. M., Kelley, A. C. & Ramakrishnan, V. The mechanism for activation of GTP hydrolysis on the ribosome. *Science* **330**, 835–838 (2010).
32. Liljas, A., Ehrenberg, M. & Aqvist, J. Comment on "The Mechanism for Activation of GTP Hydrolysis on the Ribosome". *Science* **333**, 37 (2011).
33. Voorhees, R. M., Schmeing, T. M., Kelley, A. C. & Ramakrishnan, V. Response to comment on "The Mechanism for Activation of GTP Hydrolysis on the Ribosome". *Science* **333**, 37 (2011).
34. Nissen, P., Kjeldgaard, M., Thirup, S., Polekhina, G., Reshetnikova, L., Clark, B. F. C. & Nyborg, J. Crystal structure of the ternary complex of Phe-tRNAPhe, EF-Tu, and a GTP analog. *Science* **270**, 1464–1472 (1995).
35. Kobayashi, K., Saito, K., Ishitani, R., Ito, K. & Nureki, O. Structural basis for translation termination by archaeal RF1 and GTP-bound EF1α complex. *Nucleic Acids Res.* **40**, 9319–9328 (2012).
36. Schmeing, T. M., Voorhees, R. M., Kelley, A. C. & Ramakrishnan, V. How mutations in tRNA distant from the anticodon affect the fidelity of decoding. *Nat. Struct. Mol. Biol.* **18**, 432–436 (2011).
37. Shoemaker, C. J., Eyler, D. E. & Green, R. Dom34:Hbs1 promotes subunit dissociation and peptidyl-tRNA drop-off to initiate No-go decay. *Science* **330**, 369–372 (2010).
38. Becker, T., Armache, J. P., Jarasch, A., Anger, A. M., Villa, E., Sieber, H., Motaal, B. A., Mielke, T., Berninghausen, O. & Beckmann, R. Structure of the No-go mRNA decay complex Dom34-Hbs1 bound to a stalled 80S ribosome. *Nat. Struct. Mol. Biol.* **18**, 715–720 (2011).
39. Pisareva, V. P., Skabkin, M. A., Hellen, C. U. T., Pestova, T. V. & Pisarev, A. V. Dissociation by pelota, Hbs1 and ABCE1 of mammalian vacant 80S ribosomes and stalled elongation complexes. *EMBO J.* **30**, 1804–1817 (2011).
40. Karcher, A., Schele, A. & Hopfner, K. P. X-ray structure of the complete ABC enzyme ABCE1 from Pyrococcus abyssi. *J. Biol. Chem.* **283**, 7962–7971 (2008).
41. Pisarev, A. V., Skabkin, M. A., Pisareva, V. P., Skabkina, O. V., Rakotondrafara, A. M., Hentze, M. W., Hellen, C. U. T. & Pestova, T. V. The role of ABCE1 in eukaryotic posttermination ribosomal recycling. *Mol. Cell* **37**, 196–210 (2010).
42. Barthelme, D., Dinkelaker, S., Albers, S. V., Londei, P., Ermler, U. & Tampe, R. Ribosome recycling depends on a mechanistic link between the FeS cluster domain and a conformational switch of the twin-ATPase ABCE1. *Proc. Natl. Acad. Sci. USA* **108**, 3228–3233 (2011).
43. Shoemaker, C. J. & Green, R. Kinetic analysis reveals the ordered coupling of translation termination and ribosome recycling in yeast. *Proc. Natl. Acad. Sci. USA* **108**, E1392–E1398 (2011).
44. Becker, T., Franckenberg, S., Wickles, S., Shoemaker, C. J., Anger, A. M., Armache, J. P., Sieber, H., Ungewickell, C., Berninghausen, O., Daberkow, I., Karcher, A., Thomm, M., Hopfner, K. P., Green, R. & Beckmann, R. Structural basis of highly conserved ribosome recycling in eukaryotes and archaea. *Nature* **482**, 501–506 (2012).
45. Saito, S., Hosoda, N. & Hoshino, S. The Hbs1-Dom34 protein complex functions in non-stop mRNA decay in mammalian cells. *J. Biol. Chem.* **288**, 17832–17843 (2013).
46. Izawa, T., Tsuboi, T., Kuroha, K., Inada, T., Nishikawa, S. & Endo, T. Roles of Dom34:Hbs1 in nonstop protein clearance from translocators for normal organelle protein influx. *Cell Rep.* **2**, 447–453 (2012).
47. Schaeffer, D. & van Hoof, A. Different nuclease requirements for exosome-mediated degradation of normal and nonstop mRNAs. *Proc. Natl. Acad. Sci. USA* **108**, 2366–2371 (2011).
48. Strunk, B. S., Novak, M. N., Young, C. L. & Karbstein, K. A translation-like cycle is a quality control checkpoint for maturing 40S ribosome subunits. *Cell* **150**, 111–121 (2012).
49. Davis, L. & Engebrecht, J. Yeast dom34 mutants are defective in multiple developmental pathways and exhibit decreased levels of polyribosomes. *Genetics* **149**, 45–56 (1998).
50. Bhattacharya, A., McIntosh, K. B., Willis, I. M. & Warner, J. R. Why Dom34 stimulates growth of cells with defects of 40S ribosomal subunit biosynthesis. *Mol. Cell Biol.* **30**, 5562–5571 (2010).