**The GTP-binding Release Factor eRF3 as a Key Mediator Coupling Translation Termination to mRNA Decay**

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GTP is essential for eukaryotic translation termination, where the release factor 3 (eRF3) complexed with eRF1 is involved as the guanine nucleotide-binding protein. In addition, eRF3 regulates the termination-coupled events, eRF3 interacts with poly(A)-binding protein (Pab1) and the surveillance factor Upf1 to mediate normal and nonsense-mediated mRNA decay. However, the roles of GTP binding to eRF3 in these processes remain largely unknown. Here, we showed in yeast that GTP is essentially required for the association of eRF3 with eRF1, but not with Pab1 and Upf1. A mutation in the GTP-binding motifs of eRF3 impairs the eRF1-binding ability without altering the Pab1- or Upf1-binding activity. Interestingly, the mutation causes not only a defect in translation termination but also delay of normal and nonsense-mediated mRNA decay, suggesting that GTP/eRF3-dependent termination exerts its influence on the subsequent mRNA degradation. The termination reaction itself is not sufficient, but eRF3 is essential for triggering mRNA decay. Thus, eRF3 is a key mediator that transduces termination signal to mRNA decay.

In eukaryotes, the process of translation is divided into at least three steps: initiation, elongation, and termination, and all the three steps are in common regulated by GTP-binding proteins (1–3). The structure of the GTP-binding proteins functioning at each step is well conserved from yeast to mammals, and these proteins are fundamental to living cells (4). In the initiation and elongation steps eIF2 and eEF1A, respectively, bring aminoacyl tRNAs to the A site of the ribosome (1, 2). While in the termination step, eRF3 was identified as the GTP-binding protein (5–8).

Translation termination is regulated by a heterodimeric release factor consisting of eRF1 and eRF3 (9–11). eRF3 directly recognizes all three termination codons to release completed polypeptide chain from the ribosome (9, 12), and eRF3 stimulates the termination reaction in a GTP-dependent manner (10, 13). In the yeast *Saccharomyces cerevisiae*, eRF1 and eRF3 are encoded by the essential genes SUP45 and SUP35 (9, 11). The eRF3 genes are conserved from yeast to mammals. The mammalian eRF3 gene, *GSPT*, was first identified by the ability to complement temperature-sensitive growth arrest phenotype of *sup35*/*gst1-1* mutation in *S. cerevisiae*, which is defective in G1 to S phase transition (14). Later, two subtypes of *GSPT* genes, *GSPT1* and *GSPT2*, were identified (15). Structural analyses revealed that eRF3 consists of two domains, the unique N-terminal region (N-domain) and the C-terminal region (C-domain) that contains eRF1A-like GTP-binding motifs. The C-domain of eRF3 is well conserved among several species, and eRF3 associates with eRF1 through the C-domain (15–18). Moreover, the C-domain of eRF3 is required and sufficient for the termination reaction (10).

In addition to translation termination, eRF3 functions in translation termination-coupled events. We previously showed that eRF3 interacts with poly(A)-binding protein (PABP) through its N-domain (19). The interaction is evolutionarily conserved from yeast to mammals (19–24). PABP binds to the 3'-poly(A) tail of mRNA and plays important roles in translation initiation and mRNA decay (25–27). Recently, we have reported in yeast that eRF3 regulates the initiation of normal mRNA decay at poly(A) tail-shortening step through the interaction with PABP in a manner coupled to translation termination (24). We also showed in mammals that eRF3 forms a complex with the initiation factor eIF4G through the interaction with PABP and contribute to the cap- and poly(A)-dependent translation suggesting that eRF3 mediates efficient recycling of ribosome to stimulate the next translation initiation in a manner coupled to translation termination (23).

On the other hand, Upf1, which is a key component of the surveillance complex that recognizes and degrades aberrant mRNAs containing premature termination codons, was identified as a binding partner of eRF3 in yeast and humans (28). In nonsense-containing mRNA, translation termination is thought to occur by the termination complex eRF1-eRF3 at the premature termination codon. The eRF1-eRF3 associates with Upf1-Upf2-Upf3 to form a “surveillance complex” and triggers rapid mRNA decay via nonsense-mediated mRNA decay (NMD) pathway (28, 29).

These findings allowed us to speculate that eRF3 functions as a molecular switch in the process that couples translation termination to mRNA decay and/or re-initiation, where GTP-binding to eRF3 plays regulatory roles. In this study, we present the first evidence that GTP is essential for the association between eRF3 and eRF1 as well as for the termination reaction.
Although the binding of eRF3 to Pab1 and Upf1 is independent of the guanine nucleotides and not affected by a mutation in the GTP-binding motifs of eRF3, the mutation causes a defect in both normal and nonsense-mediated mRNA decay. Furthermore, eRF3 plays an indispensable role in the termination-coupled mRNA decay, and the termination reaction itself is not sufficient for triggering the mRNA degradation. These results provide a model for the mechanism whereby translation termination-coupled mRNA decay is regulated by the GTP-binding protein eRF3.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Growth Conditions**—The yeast strains used in this study are listed in Table I. The yeast cells were grown in standard culture media and transformed with DNA by the lithium acetate method. Deletion of the SUP35 gene was performed as described previously (24). Epitope tagging of SUP35, SUP45, PAB1, and UPF1 was performed by the one-step method described by Knop et al. (30). Transformants were checked by PCR for the correct integration. All epitope-tagged proteins expressed in this study were fully functional. The sequences of the oligonucleotides used for the epitope tagging are as follows: SUP35, GTA CCA CAA TAG CAA TTG GTA AAA TGT TTA AAA TGG CAG GTA CCG TGC AGG TCG AGC and GGT ATT GTG TTT GTA CTA ACT TAT GTT TGC AAG AAA TAT CAA TGA ATT CCG GCT CGT; SUP45, GAA TAT TAT GAC GAA GAT GAA GGA TCC GAC TAT GAT TCT ATT GCG CTC CAG GTC GAC and AAT TCT TTT CAA CAT TTT CTC CCC CTT TTA TTA TTT ATA TCG ATG; PAB1, GAG TCT TTC AAA AAG GAG CAA GGA CAA CAA ACT GAG CAA GCT CTT AGC CTG CAG GTC GAC and ATA GGT TTT AGT GAG GAA GAT GAA GAT TAC ATAG CAA TCG AGC; and UPF1, CAA AAG CAT GAA TTT CCA GAA AAC GAC TGC TTT TTT TTA ATT TAA CCA GCG TTC CAA GTC GAA ATT TTT GTA GGA GTG CAT GTC GAC and CAA GCC AGG TTT AAC ATT TTT TTA TAA CCA GCT TCA CCG AAA TCG ATG AAT TCG AGC TCG.

**Plasmid Construction**—pEUA was derived from YEpH195 by insertion of the yeast TRP5 terminator and ADC1 promoter. To obtain pEUA-Flag-SUP45, the PCR fragment of SUP45 was inserted into the region between XbaI and KpnI sites of pEUA. The XbaI site and FLAG sequence was inserted into the region between EcoRI and SalI sites of YCplac22. The BamHI site and FLAG sequence was inserted into the region between the StuI and NcoI sites of YCplac22-Flag-SUP35. To construct YEpH22-Flag-SUP35, the BamHI–SalI fragment of the vector (Promega), and N406I and D409N mutants were constructed by the one-step mutagenesis method (33). The BamHI–SalI fragment of the SUP35 gene including the promoter region was excised from pYK807 (32) and subcloned into the region between EcoRI and SalI sites of pGEM-T Easy. The EcoRI site was added to 5'-primer, and the SalI site was added to 3'-primer. The XbaI site and FLAG sequence was inserted into the region between XbaI and SalI sites of pGEM-T Easy. The BamHI–SalI fragment of the vector (Promega), and N406I and D409N mutants were constructed by the one-step mutagenesis method (33). To construct pURAGAL1-CL and CSL, CAT and luciferase genes were amplified with PCR from pCAT-control (Promega) and pGL2 (Promega), respectively. The EcoRI site was added to 5’-primer, and the XbaI site was added to 3’-primer for the CAT gene. The XhoI site was added to 5’-primer, and the SalI site was added to 3’-primer for the luciferase gene. Amplified DNA was subcloned into pURAGAL1. For CSL, a stop codon was inserted into 3’-primer for the CAT gene.

**Preparation of Yeast Lysate and Immunoprecipitation Assay**—Logarithmically growing yeast cells (1 × 10⁶) in standard yeast extract/peptone medium supplemented with glucose and adenine (YPD) were resuspended in 500 µl of a lysis buffer (50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 5 mM EDTA, 1 mM dithiothreitol, 0.1% Triton X-100, 10% glycerol, and protease inhibitors). The cells were mixed with glass beads (1 g) and disrupted by 12 cycles of vortexing for 30 s followed by incubation on ice for 1 min. After centrifugation at 15,000 × g for 20 min, the clear supernatant was used as the yeast lysate. The lysate was incubated at 4 °C for 30 min with protein G-Sepharose (Amersham Biosciences) and centrifuged at 3000 rpm for 10 s. The supernatant was mixed with 500 µl of a lysis buffer (50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 5 mM EDTA, 1 mM dithiothreitol, 0.1% Triton X-100, 2% glycerol, and protease inhibitors, and incubated with 1 mg/ml lysozyme at 4 °C for 30 min. The mixture was sonicated for 5 min on ice and centrifuged at 100,000 × g for 60 min. The expressed proteins were purified from the clear supernatant using glutathione-Sepharose 4B (Amersham Biosciences) and/or nickel-nitritriacetic acid-agarose (Qiagen) according to the manufacturer’s instructions. Flag-eRF1, Flag-Pab1, and Flag-Upf1 were expressed in yeast cells and purified using Anti-FLAG M2-agarose affinity gel (Sigma). Production of Recombinant Proteins—Various forms of eRF3 proteins were produced by the addition of 0.1 µl isopropyl-1-thio-β-D-galactopyranoside at 20 °C for 12 h in Escherichia coli JM109 containing the pGHS6-SUP35. The cells were resuspended in buffer A consisting of 50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.5 mM EDTA, 0.1% Triton X-100, 2% glycerol, and protease inhibitors, and incubated with 1 mg/ml lysozyme at 4 °C for 30 min. The mixture was sonicated for 5 min on ice and centrifuged at 100,000 × g for 60 min. The expressed proteins were purified from the clear supernatant using glutathione-Sepharose 4B (Amersham Biosciences) and/or nickel-nitritriacetic acid-agarose (Qiagen) according to the manufacturer’s instructions. Flag-eRF1, Flag-Pab1, and Flag-Upf1 were expressed in yeast cells and purified using Anti-FLAG M2-agarose affinity gel (Sigma).
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PAGE sample buffer by boiling for 5 min. The eluted proteins were separated by SDS-PAGE and immunoblotted with anti-GST, anti-FLAG (M2), and anti-Myc (9E10) antibodies. The binding experiments in immunoblot analysis (Fig. S1) were performed in the presence of 10 μg/ml of RNase A.

Read-through Assay—Yeast cells in the selective medium containing 2% galactose (2 ml) were grown at 26 °C to an A600 of ~0.6, and further incubated at 37 °C for 2 h. The cells were harvested and resuspended in 100 μl of the lysis buffer lacking Triton X-100. The cells were mixed with glass beads (0.1 g) and disrupted by 12 cycles of vortexing for 30 s followed by incubation on ice for 1 min. After centrifugation at 15,000 × g for 20 min, the 5 μl of clear supernatant was mixed with 45 μl of Bright-Glo luciferase assay regent (Promega), and luciferase activity was measured using a multivariate reader (Wallac).

RNA Analysis—RNA isolation and Northern blot hybridization were performed as described previously (24). Radiolabeled probes for PGK1 and CYH2 mRNAs were prepared by random priming. SCR1 RNA was detected using an oligonucleotide probe (o77), TCT AGC CGC GAG GAA GGA. CYH2 and SCR1 mRNAs were prepared by random priming. SCR1 RNA was detected by using an oligonucleotide probe (o77), TCT AGC CGC GAG GAA GGA. The effects of various concentrations of guanine nucleotides (1 mM) and/or 10 mM Mg2++ on the association between eRF3 and eRF1 were investigated (Fig. 1C). These results support the above form. The effects of various concentrations of guanine nucleotides and Mg2++ were also investigated (Fig. 1C). The half-maximum eRF1 binding to eRF3 required 10–100 μM GTP (Fig. 1C, left), and the concentration is apparently higher than the dissociation constant (Kd) of eEF1A for GTP (36). Moreover, GTPγS-supported association between eRF3 and eRF1 was maximally observed at physiological concentrations (0.1–1 mM) of Mg2++ (Fig. 1C, right).

FIG. 1. The association between eRF3/Sup35 and eRF1/Sup45 requires GTP and Mg2++. A, cell extract was prepared from a yeast strain (YTK12) that expresses Myc-tagged eRF3 and protein A-tagged eRF1 under the control of their own promoters and immunoprecipitated with an anti-Myc antibody in a solution containing the indicated nucleotides (1 mM) and/or 10 mM Mg2++. Protein G-Sepharose was added to the mixture, and proteins retained in the resin were separated by SDS-PAGE and immunoblotted with anti-GST, anti-FLAG (M2), and anti-Myc (9E10) antibodies, as described under “Experimental Procedures.” B, the Sepharose resin retaining eRF3 and eRF1 was incubated with the indicated nucleotides (1 mM) in the presence of 10 mM Mg2++ and subjected to the immunoblot assay. One-twentieth of the volume of the lysate used for each assay was loaded on input lane. C, the Sepharose resin retaining eRF3 and eRF1 was further incubated with the indicated concentrations of GTP or GDP in the presence of 10 mM Mg2++ (left panel), or the indicated concentrations of Mg2++ in the presence of 1 mM GTP-S or GDP (right panel). After centrifugation and washing, eRF1 retained in the resin was analyzed by the immunoblot assay. The amounts of eRF1 bound to eRF3 are illustrated as percentages of the maximum values obtained with the highest concentration of GTP (left) or Mg2++ (right).

A GTP-binding Motif on the C-domain of eRF3/Sup35 Is Responsible for the Association to eRF1/Sup45—To confirm that GTP binding to eRF3 is required for the eRF1 association, we produced several eRF3 mutants that had been tagged with GST/His in E. coli. The mutant proteins were purified, mixed with extract from a yeast expressing FLAG-tagged eRF1, and subjected to pull-down assay with glutathione-Sepharose resin. In accordance with previous studies (15–18), the C-domain (the amino acid sequence of 254–685) of eRF3 but not its N-domain (1–253) associated with eRF1 (Fig. 2A). The GTP-dependent association between eRF3 and eRF1 was also observed in this assay system (Fig. 2B). eRF3 mutants, of which a GTP-binding motif (NKXKD) on the C-domain was replaced by Ile (N406I) or Asn (D409N), were subjected to the in vitro binding assay. The mutations in this motif of various GTP-binding proteins are demonstrated to result in reduced affinity for GTP (37, 38). As shown in Fig. 2C, eRF1 association was markedly impaired in these eRF3 mutants despite the presence of GTP. Furthermore, we confirmed the GTP-dependent association between eRF3 and eRF1 by pull-down assay using the purified proteins (see supplemental Fig. S1, A–C). These results support the above
idea that the GTP binding to eRF3 is necessary for its interaction with eRF1.

No Requirement of GTP for the Association between eRF3/Sup35 and Pab1—Recently, we and others have identified a PABP that binds to the 3′-poly(A) tail of eukaryotic mRNA as a new partner binding to eRF3 (19–24). In yeast, eRF3 associates with Pab1 (yeast PABP) to mediate mRNA decay through the regulation of deadenylation (24). Therefore, we investigated whether the association between eRF3 and Pab1 is regulated by GTP. The recombinant eRF3 proteins fused with GST/His were mixed with the lysate of a yeast strain (yTK3) expressing C-terminal epitope-tagged Pab1 and subjected to the in vitro binding assay. The full-length and N-domain (the amino acid sequences of 1–253) of eRF3 interacted with Pab1 (data published in Ref. 24). We next investigated the effect of GTP on the interaction between full-length eRF3 and Pab1. In sharp contrast to eRF1, Pab1 binding to eRF3 was not dependent on the presence of GTP (Fig. 3A). Similar results were also obtained from pull-down assays using the purified proteins (see supplemental Fig. S1, A, B, and D). Furthermore, Pab1 associated with the eRF3 mutants (N406I and D409N) almost equivalent to the wild-type eRF3 (Fig. 3B). These results suggested that the association of Pab1 with the N-domain of eRF3 is not affected by GTP binding to the C-domain of eRF3.

No Requirement of GTP for the Association between eRF3/Sup35 and Upf1—Recent studies have revealed that eRF3 interacts with Upf1, which is a component of the surveillance complex. Because Upf1 has been reported to bind to the C-domain (the amino acid sequence 254–465) of eRF3 (29), it was supposed that the association of eRF3 with Upf1 might be regulated by guanine nucleotides. We constructed a yeast strain (yTK13), in which the chromosomal copies of eRF3, eRF1, and Upf1 were tagged with nine Myc, protein A, and three HA epitopes. As shown in Fig. 4A, no incubation, eRF3 co-immunoprecipitated with Upf1, in addition to eRF1, in the yeast lysate. We next investigated whether GTP is required for the interaction. Although eRF1 was detected only when GTP or GTP·S was added to the incubation mixture (Fig. 4A, right panels, top lane), the precipitated amount of Upf1 was not modified by the presence or absence of guanine nucleotides (middle lane).

The interaction was further examined by in vitro binding assay with the mutant forms of eRF3 and lysate of a yeast expressing C terminally HA-tagged Upf1. As shown in Fig. 4B, the full-length eRF3 associated with Upf1 (lane 1). However, neither its N-domain nor C-domain associated with Upf1 under the present conditions (Fig. 4B, lanes 2 and 3). The association of the full-length eRF3 with Upf1 was still observed after incubation without GTP (Fig. 4C). We confirmed that the interaction of Upf1 with eRF3 was independent of the presence of GTP in pull-down assay using the purified proteins (see supplemental Fig. S1, A, B, and E). Moreover, wild-type eRF3 and its mutants (N406I and D409N) associated equivalently with Upf1 (Fig. 4D). These results suggested that Upf1 binds to both GTP- and GDP-bound forms of eRF3.

GTP Binding to eRF3/Sup35 Is Required for Translation Termination and mRNA Decay—To elucidate whether GTP-dependent eRF3 binding to eRF1 is necessary for translation...
transcription was inhibited by the addition of thiolutin (4 μg/ml). At the non-permissive temperature (37 °C for 4 h), the cell extracts were incubated with Bright-Glo luciferase assay reagent, and luciferase activity was measured using a multiplate reader. CSL/CL values are illustrated after normalization with the concentration of total protein in lysate. D, the yeast strains that had been grown at 26 °C (A<sub>0.6</sub>) were further incubated at 37 °C for 1 h, and transcription was inhibited by the addition of thiolutin (4 μg/ml). At the indicated times, the cells were harvested, and extracted RNA was analyzed on a 1% formaldehyde-agarose gel. Northern blotting was performed using the indicated probes. E, the yeast strains grown at 26 °C were further incubated at 37 °C for 4 h. The cells were subjected to Northern blot analysis as described in D.

We also measured CYH2 pre-mRNA levels in these strains to monitor NMD, because it has been shown that inefficiently spliced CYH2 pre-mRNA containing a premature termination codon is degraded by NMD pathway (39). After a shift to a non-permissive temperature (37 °C for 4 h), the strains were harvested and analyzed by Northern blot analysis. CYH2 pre-mRNA accumulated in the mutant N406I strain, showing its defect in NMD (Fig. 5E). These results suggested that GTP-binding to eRF3 is required not only for normal mRNA decay but also for NMD.

**eRF3/Sup35 Plays an Indispensable Role in Translation Termination-coupled mRNA Decay**—The low translation termination activity observed in the sup35 mutant N406I (Fig. 5C) could be explained by the finding that the mutant has low affinity to eRF1 (Fig. 2C). However, the sup35 mutant also shows defects in normal and nonsense-mediated mRNA decay (Fig. 5, D and E), although there was no apparent change in the Fab1- or Up1-binding activity of eRF3 (Figs. 3C and 4D). These results supported the notion that the mRNA decay pathways are coupled to translation termination, and this is in good agreement with previous work (24). Interestingly, we found that extra copies of SUP45 suppress the temperature-sensitive sup35/gst1-1 mutation (Fig. 6A). Therefore, translation termination activity was examined by the read-through assay in the SUP45-expressing strain (Fig. 6B). The frequency of the read-through was rather high in the sup35/gst1-1 mutant, and the defect in translation termination was effectively suppressed by the extra copies of SUP45 to a level almost equivalent to the wild-type control strain. We further analyzed mRNA-decay processes in these strains, where the extra copies of SUP45 suppressed the defect in translation termination caused by the
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Sup35 null mutation. As shown in Fig. 6C, the slow decay rate of PGK1 mRNA observed in the sup35 mutant was not restored by the extra copies of SUP45. Moreover, CYH2 pre-mRNA still accumulated in sup35 mutant even when an overdose of SUP45 was present (Fig. 6D). These results indicated that extra copies of SUP45 are not capable of suppressing the defect of both normal and nonsense-mediated mRNA decay in sup35. This indicates that the translation termination reaction itself is not sufficient for triggering mRNA decay, and eRF3 plays an indispensable role for the coupling between translation termination and mRNA decay.

**DISCUSSION**

As described above, the GTP-binding protein eRF3 interacts with eRF1, which directly recognizes the termination codons to perform translation termination. Moreover, eRF3 mediates normal and nonsense-mediated mRNA decay through its association with Pab1 and Upf1. However, it has not been fully elucidated the way in which GTP binding to eRF3 exerts its influence on the multiple functions mediated by eRF3. We initially attempted to measure the binding constants of eRF3 for guanine nucleotides by filtration methods, but failed to determine the parameters because of low-affinity characteristics of this GTP-binding protein. This is consistent with a recent report showing that the affinity of eRF3 C-domain (196–662) from Schizosaccharomyces pombe is quite low (K_d = 100 μM) for a GTP analog, GDPNP, in the presence of millimolar order Mg^{2+} (40). Nevertheless, we could successfully observe the proper effects of guanine nucleotides to evaluate the protein interaction by using yeast cell lysates and purified components. In the present study, we also took advantage of yeast genetics and mutational analysis to investigate the GTP/eRF3-sensitive steps. The yeast proteins involved in the termination and mRNA-decay machineries were epitope-tagged by a chromosomal tagging method (30) and physiologically produced under the control of their own promoters. Furthermore, a single-point mutation (N406I) in the GTP-binding motifs of the yeast eRF3, which was biochemically characterized as a "loss-of-function" mutation in terms of the GTP-dependent eRF1-binding activity, allowed us to study the coupling between translation termination and mRNA decay.

**Roles of Guanine Nucleotides in Translation Termination—**

Caskey and co-workers (41, 42) reported in 1970s that translation termination is a GTP-dependent step in eukaryotes, and later Sup35 was identified as the entity (eRF3) conferring the GTPase property on eRF3 (43). In this sense, it was speculated that the nucleotide-dependent conformational change also occurs in the C-terminal region of eRF3. Quite recently, three-dimensional structures of GTP- and GDP-bound forms of eRF3 from S. pombe have been reported (40). Surprisingly, no apparent change in the overall structures was observed between them, although the structures are largely similar to EF-Tu. The authors used N terminally truncated eRF3 (residues 196–662), termed eRF3c, and unexpectedly found that the extra N-terminal residues 236–236 bound to the potential eRF1-binding site on eRF3 in the absence of eRF1. Therefore, it is possible that a steric hindrance by the N-domain, rather than a conformational change in the C-terminal region of eRF3 is responsible for the GTP-dependent regulation of eRF1 binding.

Several lines of evidence revealed the differences between eRF3 and RF3. First, eRF3 is essential for cell growth, whereas RF3 is dispensable. Second, in contrast to eRF1/eRF3, no significant binding has been demonstrated between free RF1/2 and RF3. Third, RF3 has structural homology with EF-G, whereas eRF3 is homologous to eRF1, it was speculated that the nucleotide-dependent conformational change also occurs in the C-terminal region of eRF3. Quite recently, three-dimensional structures of GTP- and GDP-bound forms of eRF3 from S. pombe have been reported (40). Interestingly, no apparent change in the overall structures was observed between them, although the structures are largely similar to EF-Tu. The authors used N terminally truncated eRF3 (residues 196–662), termed eRF3c, and unexpectedly found that the extra N-terminal residues 215–236 bound to the potential eRF1-binding site on eRF3 in the absence of eRF1. Therefore, it is possible that a steric hindrance by the N-domain, rather than a conformational change in the C-terminal region of eRF3 is responsible for the GTP-dependent regulation of eRF1 binding.

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**Boles of Guanine Nucleotides in eRF3/Sup35-mediated mRNA Decay—**

eRF3 also mediates mRNA decay through its interaction with several factors in a manner dependent on translation termination. Aberrant mRNAs containing premature termination codons are recognized and degraded via the
NMD pathway, in which the translation termination reaction is thought to occur by the termination complex eRF1-eRF3 on the premature termination codons (28, 29). The termination complex associates with Upf1-Upf2-Upf3 to form a surveillance complex and triggers rapid degradation of the aberrant mRNA. On the other hand, eRF3 also mediates normal mRNA decay through its interaction with Pab1 and Upf1, and these interactions are independent of the presence of guanine nucleotides, which is in sharp contrast to the interaction with eRF1. The association of the N-domain of eRF3 with Pab1 is not affected by the nucleotide binding to its C-domain (see Fig. 3), suggesting that the Pab1-binding region on the N-domain is not significantly affected by guanine nucleotide binding to the C-domain. On the other hand, Upf1 binds to the GTPase domain (the amino acid sequence 254–465) of eRF3 (29). This led us to speculate that the interaction might be regulated by guanine nucleotides. However, Upf1 binding was also not affected by the guanine nucleotide forms of eRF3 (see Fig. 4). Thus, the guanine nucleotide-dependent regulation appears to occur only at the C-terminal side of the C-domain of eRF3.

In this study, we showed that guanine nucleotide binding to eRF3 is required for both normal and nonsense-mediated mRNA decay, although the guanine nucleotide dependence was not observed for the interaction between eRF3 and Pab1/Upf1. These results strongly suggested that the GTP-eRF3-dependent translation termination exerts its influence on the subsequent mRNA decay, because the mRNA decay occurs in a manner coupled to translation termination. Consistent with this, mRNA decay is shown to be aberrant in the sup35 null mutant in which extra copies of SUP45 suppressed the defect of translation termination to a level equivalent to the wild-type strain. Thus, eRF3 has indispensable roles in coupling translation termination to mRNA decay.

Based on these and previous findings, we propose a model for the translation termination-coupled mRNA decay (Fig. 7). This model includes the following: (i) GTP-bound form of eRF3 associates with eRF1, Upf1, and Pab1 to form a termination complex and enters the A site of the ribosome to recognize the stop codon, which results in the release of the completed polypeptide chain from the ribosome, (ii) the ribosome and eRF1 stimulate the intrinsic GTPase activity of eRF3, and the GTP-bound eRF3 is converted to GDP-bound form, and (iii) the GDP-bound eRF3 dissociates from eRF1, although it remains to be associated with Upf1 and Pab1. Although the precise mechanism triggering mRNA decay is not clear at present, it is tempting to speculate that the GDP-bound form of eRF3 is directly involved in the mechanism. Wang et al. (29) reported that Upf2/Upf3 and eRF1 compete with each other for interacting with eRF3 in in vitro binding assay. Therefore, it is reasonable to assume that Upf2 and/or Upf3 binds to the GDP-bound eRF3 to form the surveillance complex after translation termination. This would trigger a rapid decay of aberrant mRNA by NMD. In the case of normal mRNA decay, we have identified PAN as the mRNA deadenylase that is involved in eRF3-mediated mRNA decay (24). Because eRF3 interacts with PAN deadenylase, the GDP-bound form of eRF3 might recruit PAN after translation termination to mediate poly(A) shortening and mRNA decay.

Evolution of the Linkage between Translation Termination and mRNA Decay—In Archaea bacteria, archival release factor 1 (aRF1) related to the eRF1 has been identified, whereas the eRF3 homologue has not been identified so far. Consistent with this, aRF1 does not have a C-terminal region corresponding to the eRF3-binding site of eRF1. Moreover, in *Giardia lamblia*, which diverged early from the rest of the eukaryotes, the eRF3 contains only the region corresponding to the eRF1A-like domain and lacks the N-domain corresponding to the PABP-binding domain of eRF3 in other eukaryotes (50). Thus, the translation termination system appears to have evolved to be coupled to mRNA decay systems, first by adding the eRF3-binding domain to eRF1 and acquiring eRF3 with the eEF1A-like GTPase domain and secondly by adding the N-domain to the eRF3 for its interaction with PABP. In the present study, we have demonstrated that as is the case in Archaea bacteria, a cell can be viable and the translation termination system can be restored without eRF3 when eRF1 is overexpressed in *S. cerevisiae*. Thus, it seems reasonable to suppose that during the course of evolution, the expression level of eRF1 had been lowered, and by developing the GTP-binding regulatory protein eRF3, both efficient translation termination and GTP-dependent coupling between translation termination and mRNA decay had been acquired.

![Fig. 7. A model for the processes from translation termination to mRNA decay mediated through eRF3. A, GTP-bound form of eRF3 associates with eRF1, Pab1, and Upf1 to form a termination complex. This complex enters the A site of ribosome and recognizes stop codon, resulting in the release of the completed polypeptide chain. In the ribosome, GTP bound to eRF3 is hydrolyzed by the activation of intrinsic GTPase to form GDP-bound eRF3. B, the GDP-bound eRF3 dissociates from eRF1 and remains to be associated with Pab1 and Upf1 for mRNA degradation. In normal mRNA decay, the association of eRF3 with Pab1 is responsible for poly(A) shortening. On the other hand, aberrant mRNA is degraded by the NMD pathway, which is triggered by the formation of the surveillance complex consisting of eRF3 and Upf1-Upf2-Upf3.](http://www.jbc.org/content triệu vi phạm hệ thống)

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