Review

Mechanism of recycling of post-termination ribosomal complexes in eubacteria: a new role of initiation factor 3

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Ribosome recycling is a process which dissociates the post-termination complexes (post-TC) consisting of mRNA-bound ribosomes harbouring deacylated tRNA(s). Ribosome recycling factor (RRF), and elongation factor G (EFG) participate in this crucial process to free the ribosomal subunits for a new round of translation. We discuss the overall pathway of ribosome recycling in eubacteria with especial reference to the important role of the initiation factor 3 (IF3) in this process. Depending on the step(s) at which IF3 function is implicated, three models have been proposed. In model 1, RRF and EFG dissociate the post-TCs into the 50S and 30S subunits, mRNA and tRNA(s). In this model, IF3, which binds to the 30S subunit, merely keeps the dissociated subunits apart by its anti-association activity. In model 2, RRF and EFG separate the 50S subunit from the post-TC. IF3 then dissociates the remaining complex of mRNA, tRNA and the 30S subunit, and keeps the ribosomal subunits apart from each other. However, in model 3, both the genetic and biochemical evidence support a more active role for IF3 even at the step of dissociation of the post-TC by RRF and EFG into the 50S and 30S subunits.

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1. Introduction

Once the translating ribosomes reach a termination codon, the binding of a class I release factor (RF1 or RF2) in response to the termination codon at the A-site triggers the release of nascent polypeptide chain from the P-site bound peptidyl-tRNA (Hershey 1983). Subsequent binding of the class II release factor, RF3, catalyses the recycling of RF1 and RF2 (Buckingham *et al* 1997; Freistroffer *et al* 1997; Pavlov *et al* 1997a), and RF3 itself dissociates from the ribosome following GTP hydrolysis (Zavialov *et al* 2001, 2002). The 70S ribosome and the tRNA remain bound to the mRNA to form the post-termination complex (post-TC). While there is evidence that in some cases such as the leaderless mRNA or the synthetic poly(U) mRNA, initiation utilizes 70S ribosomes (O' Donnell and Janssen 2002; Hirokawa *et al* 2004; Moll *et al* 2004; Udagawa *et al* 2004), in most cases initiation occurs with the 30S subunit (Hershey 1983; Kozak 1983). Therefore, it is imperative that the post-TC dissociates to release the tRNA, mRNA and the ribosomal subunits for a fresh round of protein synthesis. This process, termed ribosome recycling, is carried out by a concerted action of ribosome recycling factor (RRF) and elongation factor G (EFG) (Hirashima and Kaji 1972), and constitutes a vital step in eubacteria.

Biochemical and genetic studies have revealed that specific interactions between RRF and EFG are important for ribosome recycling. For instance, *Mycobacterium tuberculosis* RRF recycles *Escherichia coli* ribosomes with *M. tuberculosis* EFG but not with *E. coli* EFG (Rao and Varshney 2001). Similarly, *Thermus thermophilus* RRF requires the presence of *T. thermophilus* EFG for an

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Abbreviations used: EFG, Elongation factor G; IF 3, initiation factor 3; post-TC, post termination complex; RF, release factor; RRF, ribosome recycling factor.

efficient recycling of *E. coli* ribosomes (Ito *et al* 2002). Occurrence of specific interactions has been further elaborated by the isolation of suppressor mutations predominantly in domain II of *Tth*RRF, or in domain IV of *Eco*EFG which allow efficient recycling of *E. coli* ribosomes (Ito *et al* 2002). It was shown that although the wild type forms of *Tth*RRF and *Eco*EFG do not recycle *E. coli* post-TCs, they carry out a partial reaction of tRNA release *in vitro* (Raj *et al* 2005). Recently, elucidation of the three-dimensional structure of RRF (Selmer *et al* 1999; Kim *et al* 2000; Toyoda *et al* 2000; Yoshida *et al* 2001; Nakano *et al* 2003; Saikrishnan *et al* 2005); its complex with 70S ribosome or the 50S subunit (Agrawal *et al* 2004; Gao *et al* 2005; Wilson *et al* 2005); its chemical foot-printing on ribosome (Lancaster *et al* 2002), and the studies on the role of initiation factor 3 (IF3) in ribosome recycling (Karimi *et al* 1999; Hirokawa *et al* 2005; Peske *et al* 2005; Singh *et al* 2005; Zavialov *et al* 2005a) have added to our understanding of the mechanism of ribosome recycling and bridged the gap between the termination and the initiation steps. It is these aspects of recent research on ribosome recycling that are the major focus of this article.

2. RRF – An overview

RRF, a basic protein consisting of 185 amino acids (~20 kDa), was isolated and characterized by Hirashima and Kaji (1972) as a factor that catalyzed the breakdown of model post-TC when added along with EFG and GTP. As demonstrated in *E. coli*, the gene encoding RRF (*frr*) is essential (Janosi *et al* 1994) and its homologs have been found in all eubacteria, and the eukaryotic organelles harbouring protein synthetic machinery. Archaea do not appear to possess homologs of *frr*. *Mycoplasma genitalium*, with one of the smallest genomes, contains a copy of RRF gene, underscoring its importance in prokaryotic translation (Fraser *et al* 1995). RRF has been suggested to be an important target to develop newer antibacterial drugs (Kaji *et al* 1998). Using an *E. coli* strain temperature-sensitive for RRF, it has been shown that inactivation of RRF activity results in unscheduled translation reinitiation downstream of the stop codon (Janosi *et al* 1998). RRF has also been shown to play a role in preventing translation errors (Janosi *et al* 1996).

3. Structure of RRF

The three-dimensional structures of RRF from various organisms have revealed that it is a tRNA-like, L-shaped molecule consisting of 2 domains – a long three-helix bundle (domain I or the tail domain), and a three-layer $\beta/\alpha/\beta$ sandwich (domain II or the head domain) connected through a linker region (Selmer *et al* 1999; Kim *et al* 2000; Toyoda *et al* 2000; Yoshida *et al* 2001; Nakano *et al* 2003; Saikrishnan *et al* 2005). The linker region that connects the two domains makes the molecule very flexible, and a major difference between the three-dimensional structures of various RRFs corresponds to the angle between the two domains. The structures of *Aquifex aeolicus* (Yoshida *et al* 2001) and *M. tuberculosis* (Saikrishnan *et al* 2005) RRFs have highlighted two important motions of domain II: a rotation in the plane nearly perpendicular to the axis of domain I (the swinging door motion), and an internal rotation along its own axis (the screw motion). In spite of its overall similarity to tRNA, there are some architectural differences between the two, which include the flexible elbow of RRF (that has functional importance) compared to a rigid elbow in tRNA, and different surface electrostatic potentials between the two molecules (Toyoda *et al* 2000). Recent studies using directed hydroxyl radical probing (Lancaster *et al* 2002), as well as cryo-EM (Agrawal *et al* 2004; Gao *et al* 2005) have revealed that RRF contacts predominantly the 50S subunit, and its position on the ribosome is remarkably different from that of a tRNA. RRF bound to either empty ribosomes (Agrawal *et al* 2004) or to the post-TC (Gao *et al* 2005) makes extensive interactions with helices 69 and 71 of the 23S rRNA. These two helices participate in the formation of the two most prominent and conserved inter-subunit bridges, B2a and B3 respectively (Cate *et al* 1999; Gabashvili *et al* 2000; Yusupov *et al* 2001; Gao *et al* 2003). RRF binding results in a remarkable conformational change of these bridges. The bridge B2a is formed between 23S rRNA helix 69 and 16S rRNA helix 44. The surface of the 16S rRNA that contacts helix 69 in 23S rRNA also forms the binding site for IF3 (Moazed *et al* 1995; Dallas and Noller 2001). The X-ray crystallographic study of a complex of RRF with 50S ribosomal subunit (Wilson *et al* 2005) has also suggested that RRF binds in the interface canyon between the 50S and 30S subunits, and while its domain I establishes an extensive set of interactions with the 50S subunit, domain II is highly flexible and faces the 30S subunit. These observations have prompted the hypothesis that transient disruption of the inter-subunit bridges by the action of RRF and EFG, probably involving the inherent freedom of motion of the domain II of RRF, may allow access of IF3 to the 30S subunit and mediate subunit dissociation (Lancaster *et al* 2002). The anti-association activity of IF3 would thus have a role to play in ribosome recycling.

4. The role of EFG in ribosome recycling

EFG is required along with RRF to carry out ribosome recycling. The facts that EFG requirement is common to both the ribosome recycling, and the classical step of translocation during the elongation phase; and that RRF is a structural mimic of tRNA, provoked a speculation that RRF

binds to the ribosomal A-site and is translocated by EFG in a manner similar to tRNA. It was postulated that this translocation activity resulted in the release of tRNA, followed by the release of mRNA and 70S ribosome from the post-TC (Hirokawa *et al* 2002). However, the mechanistic details of such a 'dissassembly' process remained unclear. The fact that RRF competes with RF1 for ribosome binding (Pavlov *et al* 1997b) is consistent with the proposal that RRF binds to the A-site, or to an overlapping site on the ribosome. Several other observations such as, (i) inhibition of tRNA and mRNA release from the post-TCs by the inhibitors of translocation, (ii) inhibition of the release of mRNA but not tRNA from the complex in the presence of fusidic acid or a GTP analog, GMPPCP (which freezes EFG on to the ribosome but allows a single round of translocation to occur), and (iii) inability of some of the mutants of EFG (defective in their translocation activity) in recycling the model substrates and in the release of tRNA from them, etc. have been argued to support a translocation like role of EFG in ribosome recycling (Hirokawa *et al* 2002; Kiel *et al* 2003). On the contrary, the observations that, the position of mRNA relative to the ribosome does not change during the EFG-GTP and RRF mediated disassembly of post-TCs (Peske *et al* 2005), and the translocation and ribosome recycling efficiencies of several translocation defective EFG mutants do not correlate with each other, have been interpreted to mean that the role of EFG in ribosome recycling is different from its role in translocation (Fujiwara *et al* 2004). Further, based on the observations that, (i) RRF binding on ribosome occurs in a drastically different manner from that of a tRNA (Lancaster *et al* 2002) and, (ii) specific interactions between RRF and EFG are needed for ribosome recycling (Rao and Varshney 2001), it is clear that the role of EFG in ribosome recycling has to be different from its classical role in translocation. It is known that after GTP hydrolysis, EFG induces a conformational rearrangement of the ribosome which is necessary for tRNA-mRNA translocation (Rodnina *et al* 1997; Katunin *et al* 2002; Savelsbergh *et al* 2003). The parallel effects of the antibiotic inhibitors and the mutations in EFG on translocation and ribosome recycling (Kiel *et al* 2003) may indicate that these two processes share some of the EFG-induced conformational changes in ribosome (Rodnina *et al* 1999; Seo *et al* 2004).

In the experiments where binding of the factors to 50S subunits was analysed, EFG did not cause a release of RRF (Kiel *et al* 2003). In another study, the binding of RRF and EFG-GDPNP to the 50S subunits was found to be cooperative (Zavialov *et al* 2005a). On the other hand, the binding of RRF to EFG-GTP bound 70S ribosome, or EFG to 70S ribosomes in the presence of high amounts of RRF were found to be compromised (Kiel *et al* 2003; Zavialov *et al* 2005a). Taken together, these observations suggest that both of these factors share overlapping sites on the 70S ribosome but not on the 50S subunit, and that binding of these factors to 70S ribosomes in the presence of GTP would facilitate dissociation of 70S ribosomes into the two subunits. This interpretation is consistent with the observations wherein the elbow of RRF was found in an overlapping position at the junction of domains III, IV and V of EFG and the RRF domain II occupied an overlapping position with domain IV in the GDP state of EFG (Agrawal *et al* 2004). Further, it has been observed that the GTPase activity of EFG on the 70S ribosomes decreases with increasing amounts of RRF, but on the 50S subunits it increases with increasing amounts of RRF. However, EFG in the GDP form has low affinity for 50S even in the presence of RRF (Zavialov *et al* 2005a). Such an observation has relevance in the departure of the factors from the 50S subunit after GTP hydrolysis.

5. Dissociation of ribosomes

The facts that RRF and EFG bind competitively to 70S ribosome but cooperatively to the 50S subunit, and the binding of RRF to 70S ribosomes results in conformational changes to the prominent intersubunit bridges B2a and B3, suggest that RRF and EFG dissociate the post-TC into the subunits. However, when the model substrates for ribosome recycling (puromycin treated polysomes) were processed with RRF and EFG, the release of 70S ribosomes, tRNA and mRNA was observed (Hirokawa *et al* 2002). It may be noted that in this setup, a direct release of 70S ribosomes could not be discriminated from the release of 50S and 30S subunits as the latter would reassociate to give rise to 70S ribosome. The recent evidence indicates that it is the 50S and 30S ribosomal subunits that are released upon ribosome recycling and kept apart by the anti-association activity of IF3 (Hirokawa *et al* 2005; Peske *et al* 2005; Zavialov *et al* 2005a). Whether, within the cellular milieu, IF3 merely keeps the dissociated subunits apart or has a more active role in ribosome recycling is still a matter of debate, and is discussed in detail as follows.

6. Mechanistic role of IF3 in ribosome recycling

While it is evident that IF3 participates in ribosome recycling, its exact role and the step at which it enters the ribosome recycling pathway is unclear. The currently available data have been interpreted to allow three different models (figures 1 to 3). Models 1 and 2 consider complete dissociation of the two subunits by RRF and EFG alone. However, they differ from each other at the steps where tRNA and mRNA are released. According to model 1, tRNA release is the first step that occurs after binding of RRF and EFG-GTP to the post-TC, this is then followed by mRNA release upon hydrolysis of GTP and subunit dissociation; and both of

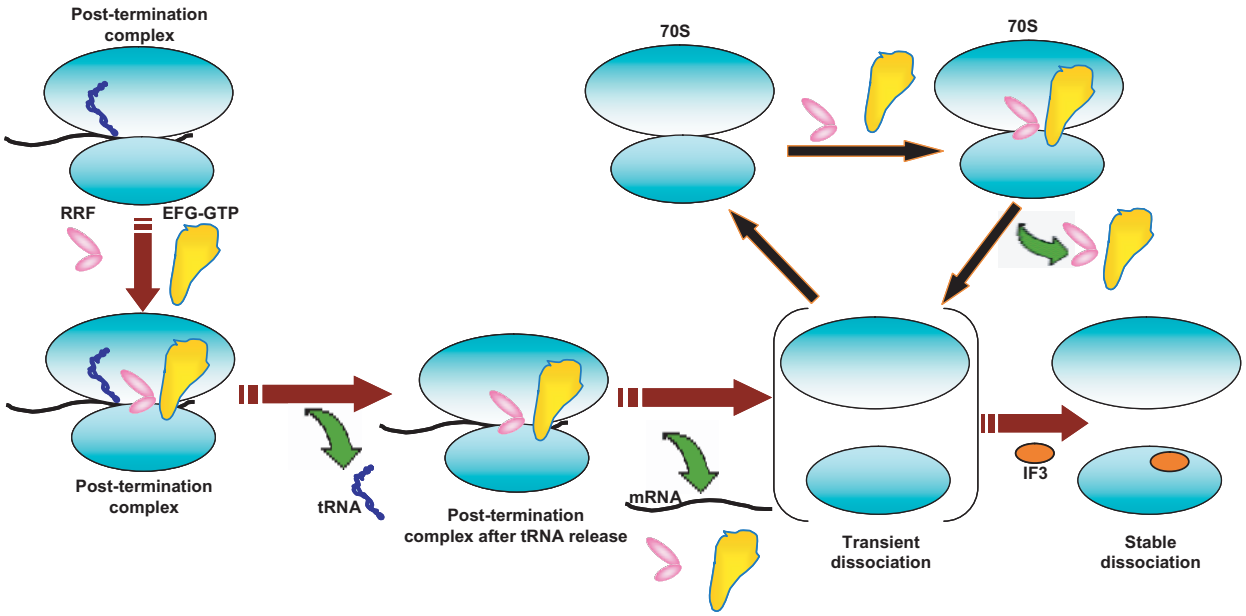

Figure 1. *Model 1:* RRF binds to the model post-TC (puromycin treated polysomes) containing deacylated tRNA in the P-site, followed by binding of EFG-GTP. The RRF is moved by EFG on the ribosome resulting in tRNA release. This is then followed by subunit dissociation and mRNA release. The transiently dissociated subunits are kept separated by IF3 binding to the 30S subunit. In the absence of IF3, the subunits reassociate to form 70S ribosomes that may again be acted upon by RRF and EFG to dissociate the subunits. Note that while only a single tRNA is shown bound to the post-TC, it may well be that two tRNAs are bound to this complex. In any case, all of the tRNA bound to the post-TC is released at the same step (see also the text).

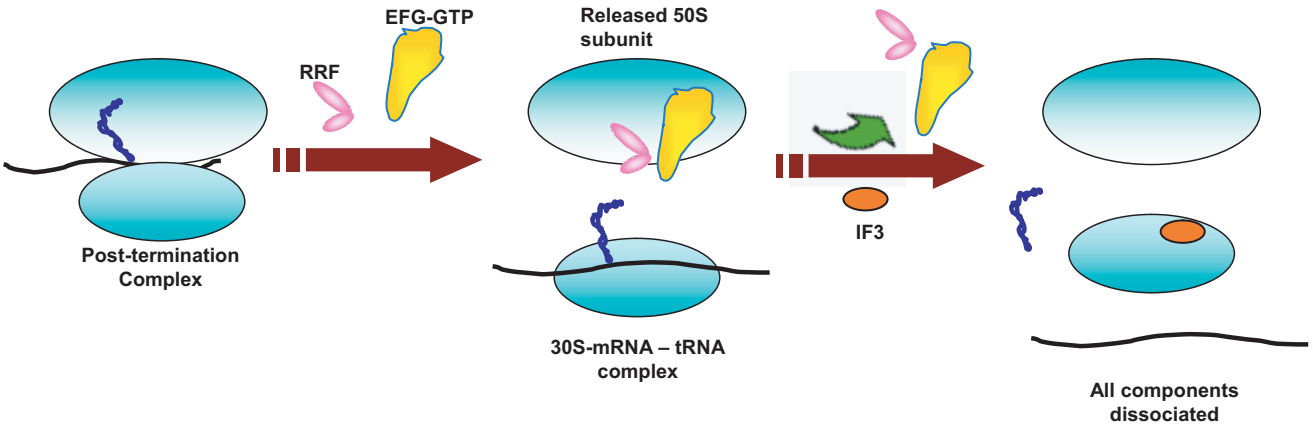

Figure 2. *Model 2*: RRF binds to the tRNA containing post-TC. Binding of EFG-GDP, followed by GTP exchange for GDP results in rotation of domain II of RRF. This is followed by GTP hydrolysis, and subunit dissociation. IF3 prevents the reassociation of the separated subunits, and also aids in the release of tRNA and mRNA from the 30S subunit. Note that the post-termination complex may possess two tRNAs. However, in this model one of the tRNAs is probably released upon RRF binding to the complex whereas the second one is released as shown.

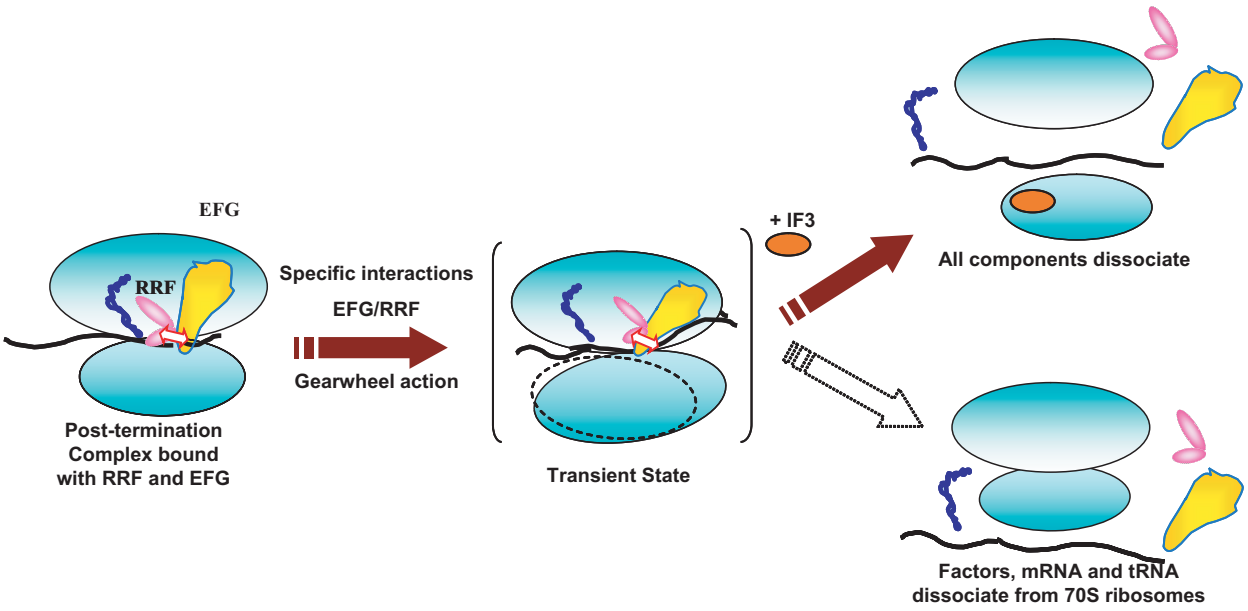

Figure 3. *Model 3*: Post-TC harbouring deacylated tRNA binds RRF, and EFG. A dynamic set of specific interactions between RRF and EFG may result in a gearwheel action between RRF and EFG leading to a transient state of the post-TC exposing the IF3 binding site on the 30S subunit. At this stage, a productive binding of IF3 to the exposed site on the 30S subunit leads to dissociation of all components, and an occasional failure of IF3 to bind 30S subunits may result in the release of 70S ribosomes from mRNA. The tRNA(s) may be released at a stage before and/or after IF3 binding.

these steps occur independent of IF3 function (Hirokawa *et al* 2006). According to model 2, release of both the tRNA and mRNA occurs from the 30S subunit upon binding of IF3 (Zavialov *et al* 2005a). It is possible that the reaction conditions and the differences in the substrates used (such as the *in vitro* assembled post-TCs using short mRNAs containing Shine Dalgarno sequence versus the puromycin treated polysomes) could be responsible for at least some of the difference in the models 1 and 2. In any case, neither of these models incorporates the aspects of specific interactions between RRF and EFG. Model 3, on the other hand, incorporates both the *in vivo* and *in vitro* observations, such as the specific interactions between RRF and EFG, and the effect of IF3 inclusion in ribosome recycling assays. Additionally, this model is consistent with the data that have allowed proposal of models 1 and 2. It may be noted that it is likely that the puromycin treated model post-TCs possess both an E-site, and a P-site bound deacylated tRNAs (Remme *et al* 1989). The release of these tRNAs has not been investigated in any specific sense. In the studies that led to proposal of model 1, all of the ribosome bound tRNA is released at the same step (Hirokawa *et al* 2002). On the other hand, in the model 2, while the E-site bound tRNA is probably released upon binding the RRF (Zavialov *et al* 2005a), the P-site bound tRNA is released after subunit dissociation. However, for the sake of simplicity, in all the three models that we discuss here, a single tRNA has been shown to be present on the complexes.

6.1 *Model 1 – IF3 merely keeps the dissociated subunits apart*

According to this model (figure 1), RRF binding to the post-TC is followed by binding of EFG-GTP, leading to a movement of RRF by a translocation-like activity, which is in turn followed by tRNA release (Hirokawa *et al* 2002). Further, a heterologous combination of factors such as *Tth*RRF and *Eco*EFG releases tRNA, but not the RRF and mRNA (Raj *et al* 2005). Thus, the tRNA release could occur as the first step in ribosome recycling in a manner which is independent of both the GTP hydrolysis and the release of RRF and mRNA from the post-TC. It may be noted that the conditions used in these experiments are such that the tRNA release could occur either from the P or both the P- and E-sites (Remme *et al* 1989; Gao *et al* 2005).

Based on the biochemical experiments wherein factor binding to 70S ribosomes has been analysed, it has been suggested that EFG converts an initial high affinity binding of RRF to ribosome to a low affinity binding. This change in the affinity of RRF binding has been interpreted as EFG mediated movement of RRF on ribosome by a “piston like activity” (Kiel *et al* 2003). It may be possible that this

movement of RRF leads to tRNA release, disruption of the inter-subunit bridges (B2a, B3), mRNA release and dissociation of the two ribosomal subunits, which are then kept separated by binding of IF3 to the 30S subunit (Hirokawa *et al* 2005). Also, it was observed that dissociation of 70S ribosomes increased in the presence of IF3, indicating that IF3 shifts the equilibrium more towards dissociation. Thus, in this model, while IF3 is not required for RRF and EFG to dissociate the 70S ribosomes into subunits, its presence is needed to keep the dissociated subunits apart.

6.2 *Model 2 – IF3 is needed for release of tRNA and mRNA from 30S subunit and to keep it separated from the 50S subunit*

As in model 1, the first step in this model (figure 2) is the binding of RRF to the post-TC. The structure of the post-termination ribosome in complex with RRF, as determined by cryo-EM, shows the ribosome in a 'twisted' conformation and the tRNA in the P/E site. Such a ribosomal conformation has been recently proposed to serve as the guanine nucleotide exchange factor for EFG (Gao *et al* 2005; Zavialov *et al* 2005b). Accordingly, the free EFG in its GDP form (EFG-GDP) is converted to EFG-GTP upon association with the RRF bound ribosome (Zavialov *et al* 2005b). Since RRF and EFG-GTP destabilize each other's binding to the 70S ribosome, the domain II of RRF is expected to undergo a large rotation with respect to the domain I, in order to accommodate both the factors on 70S ribosome. Such a rotation is expected to disrupt several inter-subunit bridges. This is followed by GTP hydrolysis, Pi release, dissociation of the 50S subunits from the remainder of the post-TC (tRNA and mRNA on 30S subunit), and the release of RRF and EFG. The IF3 stabilizes the dissociated subunits, and aids in the release of tRNA and mRNA from the 30S subunit (Zavialov *et al* 2005a). Further, in rapid kinetics assays, it was shown that while RRF and EFG were seen to promote the dissociation of 50S subunits from the post-TC without IF3; IF3 was needed subsequently for the release of tRNA and mRNA from the small subunit (Peske *et al* 2005). It was also shown that the step of tRNA release is slower than the subunit dissociation, and hence subunit dissociation must occur prior to tRNA release (Peske *et al* 2005). In fact, such a model for ribosome recycling was discussed in an earlier study as well (Karimi *et al* 1999).

6.3 *Model 3 – Active role of IF3 in promoting subunit dissociation*

Using genetic and biochemical approaches, it was recently shown that IF3 plays a more active role in recycling ribosomal complexes in *E. coli* (Singh *et al* 2005). In these experiments, overexpression of IF3 in *E. coli* LJ14 (temperature sensitive for RRF) allows a heterologous RRF from *T. thermophilus* (*Tth*RRF) to complement *E. coli* LJ14, as opposed to in the absence of its (IF3) overexpression. Further, conversion of model post-TCs (polysomes) to monosomes, which constitutes an *in vitro* assay for ribosome recycling, occurs by *Tth*RRF and *Eco*EFG only if IF3 was included in the reaction. Interestingly, studies by Raj *et al* (2005) showed that tRNA release from such model substrates occurred with *Tth*RRF and *Eco*EFG even in the absence of IF3. Taken together, these observations suggest that in the presence of *Tth*RRF and *Eco*EFG, ribosomes are converted into a state that is compatible to bind IF3, which in turn could lead to the release of 50S and 30S subunits from mRNA. It is very clear that ribosome recycling with homologous factors (e. g. *Eco*RRF and *Eco*EFG on *E. coli* ribosomes) occurs efficiently even in the absence of IF3 **(**Rao and Varshney 2001; Hirokawa *et al* 2002; Zavialov *et al* 2005a). However, it is also evident that treatment of the post-TCs with RRF and EFG passes through an intermediate state (as inferred from the treatment of post-TCs with *Tth*RRF and *Eco*EFG) which is compatible to be acted upon by IF3 (figure 3). Thus, we suggest that within the cellular milieu, IF3 would participate even at the step of subunit dissociation by RRF and EFG and make the process more efficient. In fact, the role of IF3 in recycling of the ribosomes, at this step, becomes quite apparent when one examines the recycling of pre-termination stalled complexes (pre-TC, harbouring peptidyl-tRNA as opposed to deacylated tRNA) with *Eco*RRF and *Eco*EFG. In such complexes, recycling occurs only if IF3 is present along with RRF and EFG, both *in vivo* and *in vitro* (Singh *et al* 2005). Requirement of IF3 for RRF and EFG mediated dissociation of 70S ribosomes has also been highlighted in a more recent study (Umekage and Ueda 2006).

How do RRF and EFG effect subunit dissociation? We discussed that domain II of RRF undergoes two major motions: a rotation in the plane nearly perpendicular to the plane of domain I (the swinging door motion) and an internal rotation along its own axis (the screw motion) (Saikrishnan *et al* 2005**)**. The structure of a complex of RRF with ribosome showed that two of the major inter-subunit bridges, B2a and B3 are displaced upon RRF binding **(**Agrawal *et al* 2004**)**. Although, the RRF and EFG destabilize each others binding to the 70S ribosomes (Kiel *et al* 2003; Zavialov *et al* 2005a), the genetic evidence strongly suggests occurrence of specific functional interactions between domain II of RRF and domain IV of EFG (Rao and Varshney 2001; Ito *et al* 2002). Taken together, it can be envisaged that the binding of RRF and EFG to the post-TC is followed by a dynamic set of specific interactions between RRF and EFG, which results in a gearwheel action of the two factors on the ribosome. Such an activity of RRF

and EFG could convert the post-TC into a state compatible to be acted upon by IF3 and its dissociation into subunits (Singh *et al* 2005). Biochemical and biophysical studies have shown that a high affinity binding of RRF to the ribosome is converted to a low affinity state upon EFG binding (Kiel *et al* 2003; Seo *et al* 2004). It is not clear whether these affinity differences arise because RRF binds to a different site in the ribosome due to a piston like movement by EFG (Kiel *et al* 2003), or due to a change in the contact points between RRF and ribosome because of the gearwheel action between RRF and EFG. While both of these proposals are consistent with the changes in the fluorescence measurements (Seo *et al* 2004), the latter model accommodates both the swinging door and screw motions of RRF domain II, and is also consistent with genetic studies using heterologous RRF and EFG in *E. coli*. The degree of gearwheel action between RRF and EFG (which would be governed by the extent to which the interactions between the two factors are compatible) may determine the extent to which the inter-subunit bridges are distorted to expose the elements of IF3 binding to the 30S subunit. While a stable binding of IF3 to 30S subunit would result in complete dissociation of all the components, occasionally, a non-productive approach of IF3 to bind the intermediate may, upon exit from mRNA, lead to re-establishment of 50S and 30S subunit interactions for release as 70S ribosome, useful for translation of leaderless mRNA (Moll *et al* 2004; Hirokawa *et al* 2004). It is interesting to speculate that a critical balance of events occurring at the intermediate step would also feed the ribosomes to translation reinitiation in polycistronic mRNAs.

7. Future prospects

While several aspects of the ribosome recycling step in protein biosynthesis are becoming explicable, there are still several questions that need to be addressed. The step at which tRNA release occurs and the step for which the energy of GTP hydrolysis is utilized remains a matter of debate. Further, RRF and EFG are also known to recycle pre-TCs and effect peptidyl-tRNA release from them (Heurgue-Hamard *et al* 1998; Rao and Varshney 2001; Singh *et al* 2005). The mechanistic details of recycling of pre-TCs are not understood. It is unclear as to how RRF binds to such complexes. Considering that structures of RRF bound 70S ribosomes have revealed that it (RRF) occupies a site which overlaps the CCA arms of the A- and P-site bound tRNAs (Agrawal *et al* 2004), it would be interesting to check whether the initial binding of RRF to pre-TCs (harbouring peptidyl-tRNA) occurs at a site different from that seen in the empty ribosomes. The exact details of the specific interactions between RRF and EFG which are responsible for recycling of both the pre- and post-TCs on mRNAs also remain to be explored. Interestingly, the partially recycled complexes (as obtained upon treatment of *E. coli* post-TCs with *Tth*RRF and *Eco*EFG) may provide the structure biologists with a functionally relevant intermediate in ribosome recycling to allow a better understanding of this process. Clearly, such knowledge would be important in exploiting RRF as a target for newer drugs.

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