The prokaryotic post-termination ribosomal complex is disassembled by ribosome recycling factor (RRF) and elongation factor G. Because of the structural similarity of RRF and tRNA, we compared the biochemical characteristics of RRF binding to ribosomes with that of tRNA. Unesterified tRNA inhibited the disassembly of the post-termination complex in a competitive manner with RRF, suggesting that RRF binds to the A-site. Approximately one molecule of ribosome-bound RRF was detected after isolation of the RRF-ribosome complex. RRF and unesterified tRNA similarly inhibited the binding of N-acetylphenylalanyl-tRNA to the P-site of non-programmed but not programmed ribosomes. Under the conditions in which unesterified tRNA binds to both the P- and E-sites of non-programmed ribosomes, RRF inhibited 50% of the tRNA binding, suggesting that RRF does not bind to the E-site. The results are consistent with the notion that a single RRF binds to the A- and P-sites in a somewhat analogous manner to the A/P-site bound peptidyl tRNA. The binding of RRF and tRNA to ribosomes was influenced by Mg²⁺ and NH₄⁺ ions in a similar manner.

Protein synthesis is terminated by the release of the completed polypeptide from the last tRNA by the action of release factors RF1–3 (1). Then the post-termination ribosomal complex is disassembled by ribosome recycling factor (RRF) and elongation factor G (EF-G) (for review see Refs. 2 and 3). RRF was first discovered in our laboratory (4), but Kung et al. (5) later independently discovered it also. Recent work (6) established that RRF is an essential factor for bacterial life, and the gene coding for RRF is found in all living organisms except for Archeons. Extensive genetic studies produced 12 temperature-sensitive and over 50 lethal mutants (7, 8). Crystal structures of RRF from three different organisms have been solved (9–11), and they showed that RRF is a near-perfect mimic of tRNA. On the basis of extreme similarity of the shape and dimension of RRF and tRNA, we postulated that RRF might also mimic tRNA functionally (9). An unusual flexibility of the relative positions of domains I and II was revealed by recent NMR studies. On this basis, we proposed that this flexibility might play an important role in the function of RRF (12). Because of the remarkable similarity of RRF and tRNA with regard to their interaction with ribosomes, as shown in this paper, a brief summary of the historical and recent work on tRNA binding to the ribosome pertinent to this work is given below.

In 1963, unesterified tRNA was reported to bind to non-programmed 70 S ribosomes, and the binding site was reported to be at the 30 S subunit but not at the 30 S subunit (13). In the same year, binding of cognate unesterified tRNA to programmed ribosomes was first observed by the isolation of a complex of tRNA, synthetic homopolynucleotide, and ribosome (14). This work together with the pioneering early work from the Lipmann laboratory (15) supported the fundamental concept that it is tRNA, not amino acid, that translates mRNA; amino acids can be inserted precisely in the polypeptide solely because of the codon-anticodon interaction (16). In the presence of low Mg²⁺ ion concentrations, aminoacyl-tRNA is mostly bound to the P-site, whereas both the P- and A-sites of programmed ribosomes were occupied at high Mg²⁺ (17).

The 30 S subunit, in the presence of mRNA, can bind cognate aminoacyl-tRNA to the site now known as the P-site (18, 19). On the other hand, the addition of 50 S subunits to the 30 S subunits created a second site for cognate aminoacyl-tRNA binding now known as the A-site (20). In addition to the A- and P-sites, 70 S ribosomes have a third binding site only for unesterified tRNA, called the E (exit)-site (21–24).

Binding of initiator tRNA to ribosomes has been extensively studied and reviewed recently (25). Interaction of tRNA with ribosomes during the elongation cycle has been studied in detail in vitro (reviewed in Refs. 26 and 27). X-ray crystallography of ribosomes with tRNA (28, 29), an analog of the anticodon stem loop of tRNA (30), and CCdA-phosphate-puromycin (an analog of the peptidyltransferase reaction substrate) (31) have been reported. Transfer RNA-ribosome interactions have also been visualized with cryo-electron microscopy (32, 33). These recent studies revealed that tRNA has interactions mostly with rRNA and some ribosomal proteins of both the 30 S and 50 S subunit portions of the A-, P-, and E-sites. The ribosome-bound messenger RNA that directs the cognate tRNA binding was shown to be around the neck of 30 S subunit (34–36).
In this paper, we focus our attention to the interaction of RRF with ribosomes in comparison with that of tRNA. We present evidence suggesting that one molecule of RRF is bound to the ribosome at the A- and P-sites. The significance of this finding for the mechanism of disassembly of the post-termination complex is discussed.

**EXPERIMENTAL PROCEDURES**

- **Buffers**—The following buffers were used in this paper: buffer R (10 mM Tris-Cl, pH 7.4, 8.2 mM MgSO$_4$, 80 mM NH$_4$Cl, 0.14 mM DTT), buffer BD1 (100 mM Tris-Cl, pH 7.6, 10 mM MgSO$_4$, 3 mM KCl, 1 mM DTT, and various concentrations of NH$_4$Cl), buffer BD2 (100 mM Tris-Cl, pH 7.6, 20 mM MgSO$_4$, 3 mM KCl, 1 mM DTT, and various concentrations of NH$_4$Cl), buffer BD3 (100 mM Tris-Cl, pH 7.6, 30 mM KCl, 1 mM DTT, and various concentrations of MgSO$_4$), buffer BD4 (20 mM Tris-Cl, pH 7.4, 10 mM Mg(OAc)$_2$, 25 mM KCl), buffer BD5 (20 mM Tris-Cl, pH 7.4, 10 mM Mg(OAc)$_2$, 25 mM KCl), buffer BD6 (50 mM Tris-Cl, pH 7.6, 10 mM MgSO$_4$, 13 mM NH$_4$Cl, 18 mM KCl, 1 mM DTT), and buffer BD7 (60 mM Tris-Cl, pH 7.6, 15 mM MgSO$_4$, 20 mM KCl, 10 mM NH$_4$Cl, 1 mM DTT).

- **Disassembly of the Model Post-termination Complex by RRF and EF-G**—Disassembly of the model post-termination complex was followed by observing the release of ribosome from puromycin-treated polysome as described in (37). Briefly, A$_{260}$ unit of polysome isolated from tetracycline-treated Escherichia coli Q13 cells was incubated with RRF, EF-G (1 nmol), GTP (0.37 mM), and puromycin (275 µM) in 275 µl of buffer R at 30 °C for 15 min. Sedimentation profiles of the ribosome and remaining polysome in 15–30% sucrose density gradient centrifugation (Beckman SW50.1, 40,000 rpm, 75 min, 4 °C) were analyzed by $A_{260}$ measurement with an ISCO UA-6 spectrometer.

**RESULTS**

- **Unesterified tRNA Competitively Inhibits RRF for the Disassembly of the Model Post-termination Complex, Evidence for the A-site Binding of RRF**—Because of the near-perfect structural mimicry of tRNA by RRF, we first examined the possible inhibitory effect of a mixture of unesterified tRNA on the disassembly of the model post-termination complex by RRF and EF-G. The model substrate is a puromycin-treated polysome in which the E/E-site of most of the ribosomes are occupied by unesterified tRNA (40–43), and the A-site is empty as indicated previously (44). As shown in Fig. 1A, unesterified tRNA inhibited the conversion of polysome to monosome catalyzed by RRF and EF-G in a dose-dependent manner. Fifty percent inhibition of RRF-dependent disassembly was observed in the presence of a mixture of unesterified tRNA at a 5–10-fold molar excess over RRF. In Fig. 1B, a Lineweaver-Burk plot of the reaction inhibited by unesterified tRNA. Open circles, no tRNA added; closed circles, 2 nmol (7.3 µM) tRNA; open squares, 4 nmol (14.5 µM) tRNA; closed squares, 5 nmol (18.2 µM) tRNA; open triangles, 10 nmol (36.4 µM) tRNA. $K_m$ of RRF for the model post-termination complex calculated from these data was 0.32 × 10$^{-6}$ M. The $K_m$ values were variable, and we obtained lower values in some experiments. $K_s$ of the tRNA mixture was 3.6 × 10$^{-6}$ M.

- **Binding of [14C]Phe-tRNA, NAc-[14C]Phe-tRNA, f[35S]Met-tRNA$^f$Met, and [35S]RNA$^{65}$ to Ribosomes**—Washed ribosomes were prepared from E. coli Q13 or MRE600 cells as described previously (38). RRF (1 µM or otherwise specified) was incubated with ribosomes (0.2 µM) in buffer (indicated in each figure legend, 40 or 50 µl) at 30 °C for 10 min. The mixture was applied onto Microcon100 (Millipore) and centrifuged at 3,000–6,000 × g for 12 min. The micro-spin column was washed with 200 µl of the same buffer was added, and $A_{260}$ units of total tRNA (Sigma) was extracted once with equal volumes of phenol/chloroform and once with chloroform. After the addition of KAc to a final concentration of 0.3M, the tRNA was resuspended in 100 µl of buffer R at 30 °C for 15 min. Sedimentation profiles of the ribosome were analyzed by $A_{260}$ measurement with an ISCO UA-6 spectrometer.

- **Binding of RRF to Ribosomes**—Washed ribosomes were prepared from E. coli Q13 or MRE600 cells as described previously (38). RRF (1 µM or otherwise specified) was incubated with ribosomes (0.2 µM) in buffer (indicated in each figure legend, 40 or 50 µl) at 30 °C for 10 min. The mixture was applied onto Microcon100 (Millipore) and centrifuged at 3,000–6,000 × g for 12 min. The micro-spin column was washed with 200 µl of the same buffer was added, and $A_{260}$ units of total tRNA (Sigma) was extracted once with equal volumes of phenol/chloroform and once with chloroform. After the addition of KAc to a final concentration of 0.3M, the tRNA was resuspended in 100 µl of buffer R at 30 °C for 15 min. Sedimentation profiles of the ribosome were analyzed by $A_{260}$ measurement with an ISCO UA-6 spectrometer.

**DISCUSSION**

Unesterified tRNA competes with RRF for the release of tRNA from the model post-termination complex. The disassembly of the model post-termination complex by RRF and EF-G was examined in the presence of various amounts of a mixture of unesterified tRNA (Sigma). A, the % increase of 70 S ribosomes formed from puromycin-treated polysome by RRF (200 pmol (0.73 µM) and EF-G (1 nmol (3.6 µM)) in 15 min at 30 °C was plotted against the amount of added tRNA. B, Lineweaver-Burk plot of the reaction inhibited by unesterified tRNA. Open circles, no tRNA added; closed circles, 2 nmol (7.3 µM) tRNA; open squares, 4 nmol (14.5 µM) tRNA; closed squares, 5 nmol (18.2 µM) tRNA; open triangles, 10 nmol (36.4 µM) tRNA. $K_m$ of RRF for the model post-termination complex calculated from these data was 0.32 × 10$^{-6}$ M. The $K_m$ values were variable, and we obtained lower values in some experiments. $K_s$ of the tRNA mixture was 3.6 × 10$^{-6}$ M.

**One RRF Binds Per One 70 S Ribosome, Lack of mRNA Effect**—In the experiment shown in Fig. 2A, the binding of RRF to ribosomes was examined by isolating the complex of RRF and 70 S ribosomes or 30 S ribosomal subunits using a microfiltration technique. It is clear that no significant binding to the 30 S subunits was observed at low concentrations of RRF. Only slight binding was observed at the maximum concentration of RRF (5 µM). As can be seen from this figure, dose-response curves of RRF binding to non-programmed 70 S ribosomes revealed that the $K_m$ of RRF to ribosomes is 0.59 × 10$^{-6}$ M (shown in Fig. 2B). This $K_m$ value for the binding of RRF measured in this fashion is of the same order of magnitude as the $K_m$ value determined in Fig. 1 (0.32 × 10$^{-6}$ M). It appears, therefore, that the A-site binding discussed in Fig. 1 is the same binding as...
that observed with the micro-filtration technique. To estimate the number of RRF molecules bound per ribosome, a total of 17 measurements, as described in Fig. 2A, was performed at the saturation point, and the value of 1.01 molecules per ribosome was obtained with an S.D. of 0.25. It should be noted in Fig. 2A that the binding of RRF to the ribosomes or to the 30 S subunits is not influenced by poly(U). This is in sharp contrast to the effect of mRNA inducing cognate tRNA binding to ribosomes (14) or to 30 S subunits (18, 19).

Binding of RRF to Ribosomes Under Various Ionic Conditions, Similarity to tRNA Binding to Ribosomes—Fig. 3 shows the effect of NH4Cl concentration on the binding of RRF compared with that on tRNA binding to ribosomes. Under the physiological mono-valiant ion condition (150 mM NH4Cl (27)), RRF binds to the ribosome, but the extent of binding at these physiological conditions was 30% of the maximum binding observed at 30 mM NH4Cl.

As shown in Fig. 4, divalent ion concentration also affected the binding of RRF to ribosomes. The affinity of RRF to the ribosome was decreased at lower Mg2+ concentrations. When we superimpose this curve with that of NAc-Phe-tRNA (48) or Phe-tRNA binding (49), it is clear that these curves are almost identical.

**RRF and tRNA<sup>Phe</sup> Similarly Compete with N-Acetylphenylalanyl-tRNA for Binding to the P-site of Non-programmed Ribosomes, Evidence for RRF Binding to the P-site—**We next addressed whether RRF binds to the P-site of non-programmed ribosomes. NAc-Phe-tRNA, an analog of peptidyl-tRNA, is known to bind almost exclusively to the P-site of non-programmed ribosomes (24, 50). Thus, RRF binding to the P-site can be examined by following its effect on the binding of NAc-Phe-tRNA to non-programmed ribosomes (Fig. 5A). As shown in this figure, RRF inhibited effectively NAc-Phe-tRNA binding to non-programmed ribosomes. An ~10-fold molar excess of RRF inhibited the binding of NAc-Phe-tRNA almost completely.

It is known that unesterified tRNA binds to the non-programmed ribosomal P-site (21–24). It follows then that tRNA should inhibit the binding of NAc-Phe-tRNA (24). As shown in Fig. 5A, this is exactly the case. A striking finding was that the inhibition of NAc-Phe-tRNA binding by tRNA was almost identical to that by RRF.

As shown in Fig. 5, B and C, the binding of NAc-Phe-tRNA to poly(U)-programmed ribosomes or the binding of N-formyl-methionyl-tRNA (fMet-tRNA) to AUGUUU-programmed ribosomes was not affected by the presence of RRF. The data presented in Fig. 5, B and C, indicate that RRF does not compete with cognate peptidyl-tRNA for the P/P-site when added alone. This is important because otherwise RRF would interfere with the normal elongation step under physiological conditions. It should be noted that, with the normal elongation process, the P/P-site is always under the influence of mRNA that strengthens the binding of tRNA or peptidyl tRNA. Based on Fig. 5, we suggest that RRF covers the P-site (see “Discussion”).

**RRF Blocks 50% of the Binding of tRNA<sup>Phe</sup> to Non-programmed Ribosomes, Evidence for Lack of RRF Binding to the E-site—**We further examined the binding of RRF to the E-site. In Fig. 6, the binding of unesterified tRNA (tRNA<sup>Phe</sup>) to non-programmed ribosomes was examined in the presence of various amounts of RRF. Under the experimental conditions used in Fig. 6 (10 mM Mg(OAc)<sub>2</sub>, 25 mM KCl, no polyamine), unesterified tRNA<sup>Phe</sup> is known to bind to the P- and E-(E2-)sites of non-programmed ribosomes (22–24, 27). As shown in this figure, RRF inhibited the binding of tRNA<sup>Phe</sup> only about 50% but should be pointed out that the affinity of tRNA to non-programmed ribosomes.
Fig. 5. A. RRF and unesterified tRNA similarly inhibit NAc-Phe-tRNA binding to the P-site of non-programmed ribosomes. Binding of NAc-[14C]Phe-tRNA (9.0 µM, 80 cpm/µmol) to ribosomes (3.0 µM) was examined in the presence of various amounts of either RRF (open circles) or unesterified tRNA (closed circles) in 40 µl of buffer BD4 (10 mM MgCl₂). In the absence of RRF or unesterified tRNA, 0.65 pmol of NAc-[14C]Phe-tRNA per 1 pmol of ribosome was bound. B, lack of inhibitory effect of RRF on NAc-Phe-tRNA binding to poly(U)-programmed ribosomes. Binding of NAc-[14C]Phe-tRNA (1.0 µM, 160 cpm/µmol) to poly(U)-programmed (0.5 mg/ml) ribosomes (0.38 µM) was examined in the presence of various amounts of RRF in 40 µl of buffer BD6 (10 mM MgCl₂). In the absence of RRF, 0.18 pmol of NAc-[14C]Phe-tRNA per 1 pmol of ribosome was bound. C, absence of the inhibitory effect of RRF on fMet-tRNA binding to AUGUUU-programmed ribosomes. Binding of f[35S]Met-tRNA (3.4 µM, 11.1 x 10⁵ cpm/µmol) to ribosomes (0.21 µM) was examined in the presence of AUGUUU (64 µM) and various amounts of RRF in 50 µl of buffer BD7 (15 mM MgCl₂). In the absence of RRF, 0.19 pmol of f[35S]Met-tRNA per 1 pmol of ribosome was bound.

![Graph A](image1)

**DISCUSSION**

In this paper, we propose that RRF binds to the ribosome at the A/P-site. We first discuss the evidence suggesting that the binding site of RRF covers at least a portion of the A-site. The model substrate that is presumably similar to the natural substrate of RRF has an empty A-site and P/E-sites that are occupied with unesterified tRNA (40, 41). The only available tRNA-binding site is the A-site. On the basis of the competitive inhibition of the RRF reaction by unesterified tRNA (Fig. 1), we suggest that the initial binding site of RRF covers the A-site. This is consistent with an earlier suggestion that RRF competes with RF1 for the ribosomal binding (51). The Kₘ value of RRF to the ribosome calculated from the data was 0.52 x 10⁻⁶ M. This value agrees with the value calculated from the kinetic data obtained by other laboratories (0.37 x 10⁻⁶ M, see Fig. 2A and the legend where the authors measured the rate of ribosome recycling with various RRF concentrations (52)). The Kₘ value obtained in Fig. 1 is similar to the dissociation constant of RRF from the non-programmed ribosome (0.59 x 10⁻⁶ M, Fig. 2). It should be noted that the affinity of cognate EF-Tu-GTP-aminoacyl-tRNA to the A-site is much stronger (10¹⁴ M⁻¹ (46)), and therefore RRF would not interfere with the initial binding of cognate aminoacyl-tRNA during normal physiological protein chain elongation.

We now discuss evidence that the ribosome-bound RRF occupies the P-site as well as the A-site. There are two experimental evidences that suggest that RRF binds to the P-site. First, RRF inhibited the binding of NAc-Phe-tRNA to the P-site of non-programmed ribosomes. Second, the mode of inhibition of NAc-Phe-tRNA binding to the P-site by RRF is identical to that by tRNA. Because tRNA and NAc-Phe-tRNA are known to bind to the P-site under these conditions, we interpret these two observations as a strong indication that the ribosome-bound RRF covers the P-site. In support of this conclusion, our recent hydroxyl radical probing of the position of the bound RRF obtained in collaboration with the Noller group (5) indicates that the ribosome-bound RRF indeed covers part of the P-site of ribosomes. An important conclusion from Fig. 5 is that the affinity of RRF to the P-site is identical to that of tRNA to non-programmed ribosomes (less than 10⁸ M⁻¹ at 10 mM MgCl₂ (46)). This is about 1/100 of the affinity calculated from the data obtained from Fig. 1 and 2A. In fact, the affinity of cognate N-blocked aminoacyl-tRNA to the mRNA-coded P/P-site (40, 41) is so strong (1.1 x 10¹⁰ M⁻¹ (46)) that RRF with a weak affinity to the P-site will not displace it (Fig. 5B). This suggests that the portion of RRF that is responsible for the binding to the A-site plays the major role in the interaction with the ribosome. It is therefore possible that, under certain circumstances, RRF may only bind to the A-site. Because only one molecule of RRF binds to one ribosome, we must conclude that the ribosome-bound RRF covers both the A- and P-sites.

Because of the structural similarities, one would expect that functional similarities exist between tRNA and RRF. First of all, as discussed above, the fact that the bound RRF covers both the A- and P-sites is one example of functional similarity. This is reminiscent of the A/P binding of peptidyl tRNA right after peptide bond formation (41, 42). In addition to this functional similarity, the response of the binding of RRF and the ribosomal tRNA binding to ionic conditions is amazingly similar

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(Figs. 3 and 4). This is perhaps due to the influence of these ions on the structure of ribosomes as was observed during the reconstitution of ribosomes (53–55). The effect of Mg$^{2+}$ and NH$_4^+$ ions on the ribosomal structure has been documented (56, 57). These ribosomal structural changes probably influence the binding of these two molecules in a similar manner. In addition, Fig. 3 suggests that the electrostatic interactions between the positive charge of RRF and the phosphate backbone of tRNA can be displaced by positive ammonium ions as is the case with initiation factor I (58). As for the interaction of tRNA with ribosomes, the data presented suggest that NH$_4^+$ ions displace the interaction between the negative charge of the phosphate backbone of tRNA and the positive charge of ribosomal proteins (29).

Another striking similarity between tRNA and RRF is their binding to ribosomal subunits. RRF binds to the 50 S subunit with the dissociation constant of $1.9 \times 10^{-6}$ M (59). Similarly, early work showed that unesterified tRNA binds to the 50 S subunit (13). On the other hand, RRF does not appreciably bind to the 30 S subunit (Fig. 2A). Likewise, tRNA does not bind to the non-programmed 30 S subunit (13, 18, 19). However, the 30 S subunit must contribute somehow to the binding of RRF to the non-programmed 70 S ribosomes because the affinity to the 70 S ribosomes ($K_d = 0.59 \times 10^{-6}$ M) is clearly higher than that to the 50 S subunit. Likewise, the addition of 50 S subunits to the programmed 30 S subunits doubles the ribosomal capacity of cognate tRNA binding (20).

Despite the above-mentioned similarities, we found several important differences between RRF and tRNA binding to the ribosome.

First, the data presented in Fig. 6 are consistent with the notion that tRNA but not RRF binds to the E-site of non-programmed ribosomes. Although the binding of tRNA to the non-programmed E-site is under dispute (21–24), under the buffer conditions used in our experiment shown in Fig. 6, unesterified tRNA must bind to the E2-site (27). RRF does not appear to bind to the E2 site.

Second, the affinity of RRF to non-programmed and programmed ribosomes is almost identical (Fig. 2A), whereas our early work established that the affinity of cognate tRNA to ribosomes is greatly increased by the presence of mRNA (14). In Fig. 2A, we showed that RRF does not appreciably bind to the 30 S subunit even in the presence of poly(U). In contrast, our early work (18) and work by Pestka and Nirenberg (19) demonstrated that tRNA or aminoacyl-tRNA binds to the programmed 30 S subunit. The lack of response of RRF to mRNA is consistent with the observation that there is no amino acid in the RRF sequence corresponding to the third nucleotide of the anticodon region of tRNA (3, 9). It also agrees with the observation that RRF and EF-G disassemble the model post-termination complex regardless of the codon in the A-site (44).

Third, the number of RRF molecules bound to empty ribosomes was estimated to be about one per ribosome (Fig. 2). This is in sharp contrast to the binding of two unesterified tRNA to non-programmed ribosomes. In fact, it is well known that there are three sites for ribosomal tRNA binding (21–24), whereas only two sites (A- and P-sites) are available for single RRF binding (Figs. 1, 5, and 6). Most importantly, whereas RRF binds to the A/P-site, unesterified tRNA never takes the position of the A-site. Unesterified tRNA binds to the E/E- and P/E-site but not to the A-site of non-programmed ribosomes (21–24, 40, 41). The only time tRNA occupies the A-site is after the peptide bond is formed (40, 41). Therefore, despite similarities between RRF and tRNA, the mode of ribosomal binding of RRF must be quite different from that of tRNA. Indeed, our recent collaborative work with the Noller group$^3$ suggests that the mode in which RRF binds to the A/P-site is quite different from that of tRNA binding to the A/P-site.

The natural substrate of RRF is the complex of mRNA and ribosome with unesterified tRNA at the P/E-site. We therefore postulate that RRF binds to the A/P-site as indicated in this paper and then translocated to release tRNA (37, 59). The exact position of RRF after the translocation remains obscure. The release of mRNA by RRF is probably dependent on the specific interaction with EF-G (61). A relatively high dissociation constant of RRF from the P-site may help RRF to dissociate from the ribosome after releasing mRNA and tRNA. The release of RRF from the ribosome is aided by EF-G.$^3$ The flexibility of the two domains of RRF revealed by the solution structure of RRF (12) and the crystal structures of three different RRFs (9–11) probably plays an important role in this last step.

It should be mentioned that the scheme discussed above might apply to a typical post-termination complex that is not close to the canonical initiation signal or other components, which may increase the affinity of the mRNA to the ribosome. At the border between the lysis protein gene and the coat protein gene of GA phage, the termination codon overlaps with the initiation codon. In this case, RRF releases ribosomes from the termination codon, but a portion (about 25%) of the released ribosome is “recaptured” precisely at AUG (62). Without RRF, the ribosome stays on mRNA and slides downstream along the mRNA and randomly initiates translation (7), resulting in an inactive, truncated lysis protein (62). In an in vitro system with a short synthetic mRNA having a strong Shine-Dalgarno sequence, no ribosome is released from mRNA by RRF, but subunit dissociation takes place leaving the 30 S subunit on the mRNA (60). Under these special conditions, the mode of ribosomal binding of RRF may be different from what is proposed above.

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Binding of Ribosome Recycling Factor to Ribosomes, Comparison with tRNA
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