It has been suggested from in vivo and cryoelectron micrographic studies that the large ribosomal subunit protein L11 and its N-terminal domain play an important role in peptide release by, in particular, the class I release factor RF1. In this work, we have studied in vitro the role of L11 in translation termination with ribosomes from a wild type strain (WT-L11), an L11 knocked-out strain (ΔL11), and an L11 N-terminal truncated strain (Cter-L11). Our data show 4–6-fold reductions in termination efficiency ($k_{cat}/K_m$) of RF1, but not of RF2, on ΔL11 and Cter-L11 ribosomes compared with wild type. There is, at the same time, no effect of these L11 alterations on the maximal rate of ester bond cleavage by either RF1 or RF2. The rates of dissociation of RF2 but not of RF1 from the ribosome after peptide release are somewhat reduced by the L11 changes irrespective of the presence of RF3, and they cause a 2-fold decrease in the missense error. Our results suggest that the L11 modifications increase nonsense suppression at UAG codons because of the reduced termination efficiency of RF1 and that they decrease nonsense suppression at UGA codons because of a decreased missense error level.

L11 is a highly conserved ribosomal 14.8-kDa protein located at the base of the L7/L12 stalk of the ribosome, which is essential for several steps in protein synthesis (1–5). L11 binds to the nucleotides 1051–1108 of Escherichia coli 23 S rRNA, commonly called the L11 binding region (L11BR) (6), which constitutes the GTPase-associated center, an important sector of the bacterial ribosome, where all of the translational GTPases bind and hydrolyze GTP in the course of their action (7). This is also the site of action for the thiazole antibiotics thiostrepton and micrococin (8–10).

In addition to the GTPases, some other translational factors interact with L11 and the L11BR in functionally important ways. The class I release factors RF1 and RF2 belong to this group. RF1 recognizes the stop codons UAG and UAA, whereas RF2 recognizes the stop codons UGA and UAA in the A site of the ribosome (11). Recent cryo-EM studies with termination complexes containing RF2 (12, 13) and RF1 (14) show that these two factors acquire very similar overall conformations on the ribosome. Although they bind to the decoding center on the 30 S subunit and reach up to the peptidyltransferase center on the 50 S subunit to induce release of the nascent peptide chain, they interact with L11BR (12, 13) and L11 (15) with their flexible domain 1. These observations are in line with previous suggestions, based on biochemical and genetic experiments, that there are interactions between L11 and the release factors (4, 14–18).

The L11 protein consists of two domains, a tightly folded N-terminal domain (NTD), which is loosely connected to the large compact C-terminal domain (CTD). The CTD of L11 is in stable contact with the L11BR RNA, whereas the NTD can change its position and proximity with respect to the rest of the protein (19). Earlier biochemical and genetic studies indicate that depletion of L11 from the ribosome inhibits RF1-mediated translation termination on a UAG codon but facilitates RF2-mediated termination on a UGA codon (4, 6). Antibodies against the NTD of L11 inhibit RF1-mediated termination, indicating that this part of L11 is crucial for RF1 binding (14). In a bacterial strain, containing only the CTD of L11, efficient UAG suppression was seen as in the complete L11 knocked-out strain. This result shows that the NTD of L11 is required for proper functioning of RF1 (18).

Here, we have studied in vitro how ribosomes of L11 or its NTD affects RF1- and RF2-mediated release of the tetrapeptide fMet-Phe-Thr-Ile (MFTI) from pretermination ribosomes prepared from a cell-free system with E. coli components of high purity (20, 21). We have also studied the effects of L11 deletion or truncation on cognate and near cognate codon reading by tRNA. Taken together, our data on termination efficiency and missense error level explain why these L11 alterations lead to enhanced nonsense suppression at UAG codons, read by RF1, and decreased nonsense suppression at UGA codons, read by RF2 (4, 6).

EXPERIMENTAL PROCEDURES

Chemicals and Buffers

ATP, GTP, and radioactive amino acids were purchased from American Biosciences. Nonradioactive amino acids and other chemicals were from Sigma or Merck. All experiments were carried out in polyvinyl buffer, containing 5 mM ammonium chloride, 95 mM potassium chloride, 0.5 mM calcium chloride, 8 mM putrescine, 1 mM spermidine, 5 mM potassium phosphate and 1 mM 1–4-dithioerythritol (22).

Components of the in Vitro Translation System

mRNAs and tRNAs—The mRNAs encoding the tetrapeptide fMet-Phe-Thr-Ile with any one of the UAA, UAG, or UGA stop codons were
prepared by *in vitro* T7 RNA polymerase transcription (23). The tRNA<sub>Met</sub> purification and subsequent charging and formylation reactions were performed as described previously (24), and the bulk tRNA was purified as described previously (25). The tRNA<sub>Met</sub> and tRNA<sub>Leu</sub> isoacceptors used for missense error measurements were purified by BD-Sepharose chromatography.

**Bacterial Strains and Plasmids**—The three different *E. coli* strains used for ribosome preparation (6, 18) (Table 1) carry a chromosomal knock-out of the *rplK* gene, which encodes the L11 protein. In the FTP6063 and FTP6066 strains the knock-outs were complemented with the whole L11 gene or its CTD cloned in the pΔCAT plasmid, which are referred to as pL11 and pL11Cter, respectively (18).

**Ribosomes**—Ribosomes from the bacterial strains were prepared using sucrose gradient zonal ultracentrifugation as described previously (26), with minor modifications. Prior to ribosome preparation we always checked the presence or absence of the whole gene or the gene for truncated L11 by PCR using primers specific for the upstream and downstream regions of *rplK* gene. We also had functional checks using methods described in Ref. 18. The ΔL11 ribosomes had minor contaminations of RF1 and RF2 causing release of tetrapeptides during prolonged incubations. These contaminations were removed by splitting the ΔL11 70 S ribosomes into 30 S and 50 S subunits, as described in Ref. 26, which were then used to reconstitute highly active 70 S ribosomes.

**tRNA Synthetases and Other Translation Factors**—The Ile-tRNA synthetase was isolated as described previously (27); the Phe-tRNA synthetase and the elongation factors EF-Tu, EF-Ts, EF-G were purified as described in Ref. 25; the Thr-tRNA synthetase was prepared according to Brunel et al. (28). The Met-tRNA synthetase was prepared as described in Ref. 24. RF1 and RF3 were purified following Ref. 27, and RF2-His-tagged was purified according to Ref. 29.

**Formation of Ribosome Pretermination Complex**

Pretermination ribosome complexes carrying fMet-Phe-Thr-Ile-tRNA<sup>Met</sup> in the P site and any one of the three stop codons UAA, UAG, or UGA at the A site were made with ribosomes containing the full-length L11 (WT-L11), only C terminus of L11 (Cter-L11), and no L11 (ΔL11). To compare the *k<sub>cat</sub>*/K<sub>m</sub> values for termination at the WT-L11, Cter-L11, and ΔL11 complexes programmed with a particular stop codon, these were allowed to compete in pairs against each other for RF1- or RF2-dependent peptide release. One competing pretermination complex carried [3H]Ile and the other [14C]Ile on the P site-bound peptidyl-tRNA for quantification of the amount of peptide released from each complex. Reaction mixes were prepared by combining the competing pretermination complexes to a final active concentration of 100 nM each. Preincubation of the complexes for 1 min was followed by addition of RF1 or RF2 to final concentrations between 0 and 400 nM or between 0 and 180 nM, respectively, in a reaction volume of 25 µL. After a 20-s incubation at 37 °C, a time sufficient for complete single round but prohibitive of

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**TABLE 1**

Plasmids and strains used in this study

| Plasmid and/or strain | Relevant characteristics | Reference source |
|-----------------------|-------------------------|------------------|
| pΔCAT                 | pACYC184 derivative with deletion of its 412-nucleotide PvuII segment | 18               |
| pL11                  | pΔCAT expressing wild-type *E. coli* L11 under the IPTG-inducible promoter P<sub>lac</sub> | 18               |
| pL11Cter              | pΔCAT expressing the 76-amino acid long CTD (residues 68–142) of *E. coli* L11 under P<sub>lac</sub> control | 18               |
| FTP6063               | ΔpIK/pL11/F<sub>trpA</sub>(UAG243) | Similar to NV003 in Ref. 18 |
| FTP6066               | ΔpIK/pL11Cter/F<sub>trpA</sub>(UAG243) | Similar to NV002 in Ref. 18 |
| FTP6027               | ΔpIK/pΔCAT/F<sub>trpA</sub>(UAG243) | Similar to NV004 in Ref. 18 |

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* IPTG, isopropyl 1-thio-β-D-galactopyranoside.
multiple round RF termination (27, 32), the reaction was quenched by the addition of 600 μl of ice-cold 5% trichloroacetic acid. The amount of released MFTI tetrapeptide in the supernatant was quantified by radiometry by utilization of the Beckman Coulter LS6500 Multi Purpose Scintillation Counter, which enabled simultaneous measurement of both $^3$H and $^{14}$C radioisotopes in separate windows that were corrected for minimal interference (27). The time evolution of the competing reactions are described by Equations 2 and 3,

$$\frac{dRC_1}{dt} = -[RF] \cdot \left( \frac{k_{cat}}{K_m} \right) \cdot RC_1 \quad (Eq. 2)$$

$$\frac{dRC_2}{dt} = -[RF] \cdot \left( \frac{k_{cat}}{K_m} \right)_2 \cdot RC_2 \quad (Eq. 3)$$

where [RF] is the concentration of RF1 or RF2. RC$_1$ and RC$_2$ are the amounts of the two competing pretermination complexes.

Because

$$\frac{1}{x} \frac{dx}{dt} = \frac{dlnx}{dt} \quad (Eq. 4)$$

dividing Equation 2 by RC$_1$ and Equation 3 by RC$_2$ leads to Equations 5 and 6.
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\[ \frac{d \ln(\text{RC}_1)}{dt} = -[RF] \cdot \left( \frac{k_{cat}}{K_m} \right)_{1} \]  
\[ \text{Eq. 5} \]

\[ \frac{d \ln(\text{RC}_2)}{dt} = -[RF] \cdot \left( \frac{k_{cat}}{K_m} \right)_{2} \]  
\[ \text{Eq. 6} \]

Division of Equation 5 by Equation 6 and integration over the 20-s incubation time leads to Equation 7,

\[ \ln(\text{RC})_1 - \ln([\text{RC}]_1)_{t=0} = r \cdot (\ln(\text{RC})_2 - \ln([\text{RC}]_2)_{t=0}) \]  
\[ \text{Eq. 7} \]

where

\[ r = \frac{\left( \frac{k_{cat}}{K_m} \right)_{1}}{\left( \frac{k_{cat}}{K_m} \right)_{2}} \]  
\[ \text{Eq. 8} \]

Rearranging Equation 7 then leads to,

\[ r = \frac{\left( \frac{(\text{RC})_1}{(\text{RC})_2} \right)_{t=0}}{\left( \frac{(\text{RC})_2}{(\text{RC})_1} \right)_{t=0}} = \frac{\ln \left( 1 - \frac{P_e}{P_{\text{max}}} \right)_{1}}{\ln \left( 1 - \frac{P_e}{P_{\text{max}}} \right)_{2}} \]  
\[ \text{Eq. 9} \]

where \( P_e \) is the amount of tetrapeptide released at a given concentration of release factor, \( P_{\text{max}} \) is the total amount of releasable tetrapeptide, and \( 1 - \left( \frac{P_e}{P_{\text{max}}} \right) \) is the peptide fraction remaining on peptidyl-tRNA in the pretermination complex after the incubation.

**Determination of the Rate Constant for Ester Bond Cleavage (k_{\text{cat}}) Induced by RF1 or RF2**

Pretermination complexes (RC_{\text{pre}}^{\text{MTT}}) made from WT-L11, Cter-L11, and ΔL11 ribosomes carrying MFTI-tRNA at the P site and different stop codons at the A site were prepared separately as described earlier. Peptide hydrolysis was initiated by rapid mixing of the RC_{\text{pre}}^{\text{MTT}} complex with the release factor cognate to the stop codon in a quench flow instrument (Chemical-Quench-Flow model QF-3, KinTek Corp.). The postmixing concentration of the termination complex was 200 nM, and the postmixing release factor concentrations were 0.5, 1.0, 2.0, and 3.0 mM. The reaction was quenched after varying incubation times with trichloroacetic acid at a final concentration of 5% trichloroacetic acid. As the extents of peptide release were quantified by radiometry as described above. At these concentrations of release factors, the rate-limiting step was ester bond hydrolysis. Accordingly, the time evolution of peptide release can be described by Equation 10.

\[ \frac{P_t}{P_{\text{max}}} = p(1 - e^{-kt}) + b \]  
\[ \text{Eq. 10} \]

Here, \( P_t \) and \( P_{\text{max}} \) are the amounts of released peptide at time \( t \) and at infinite time, respectively. The OriginPro® fitting software was used to obtain the best fit to Equation 10 by varying parameters \( p \) and \( b \).

**Determination of the Recycling Rate (q_d) of Release Factors without and with RF3**

The pretermination complexes prepared as described above with a final active concentration of 100 nM were mixed with 10 nM RF1 or RF2 depending on the stop codon used. For the reactions with RF3 the reaction mix also contained 200 nM RF3 along with 1 mM GTP, 2 mM phosphoenolpyruvate, 1 mM ATP, and 0.05 μg/μl pyruvate kinase in 1× polyvinyl buffer. After different times of incubation, aliquots were withdrawn, the reaction was quenched with 600 μl of ice-cold 5% trichloroacetic acid, and the released tetrapeptide was quantified from the supernatant by radiometry. The amounts of spontaneously released tetrapeptide in the absence of release factors were comparatively small and were subtracted as background. Under these conditions, the release factors had to cycle many times to remove all tetrapeptides from the pretermination complexes. The rates of recycling of the factors were estimated from the slopes of the straight lines in plots of the pmol (amount) of MFTI released/pmol of release factor versus time.

**Estimation of the Accuracy of Translation in Dipeptide Formation Assays**

Initiated ribosomes, containing [3H]fMet-tRNA_{\text{Met}} at the P site and a UUU codon at the A site, were prepared in an initiation mix, containing WT-L11, Cter-L11, or ΔL11 70 S ribosomes (2 μM active concentration), 10 μM [3H]fMet-tRNA_{\text{Met}}, 4 μM mRNA, IF1, IF2, and IF3 (2 μM each), and 1 mM GTP. Termination complexes consisting of EF-Tu, GTP, and amionic-tRNA were formed in an aminoacyl-tRNA, and the cognate reaction 2 μM tRNA_{\text{Phe}}, 2 μM EF-Tu, 0.2 μM EF-Ts, 0.5 unit/μl PhoRS, 400 μM phenylalanine, 2 mM ATP, 20 mM phosphoenolpyruvate, 1 mM GTP, 2 μg/ml pyruvate kinase, and 0.2 μg/ml myokinase. For the near cognate reaction the aminoacyl-tRNA mixture contained tRNA_{\text{Leu}} instead of tRNA_{\text{Phe}}. Ribosome and ternary complex mixes were preincubated for 10 min at 37 °C, mixed in equal volumes, incubated for varying times, and quenched by formic acid to a final concentration of 17%.

**RESULTS**

**Selective Effects of L11 Alterations on the Termination Efficiencies of RF1 and RF2**—We have compared the kinetic efficiencies (\( k_{\text{cat}}/K_m \)) of the release factors RF1 and RF2 in translation termination at UAA, UAG, and UGA codons in the A site of pretermination ribosomes, containing full-length L11 (WT-L11), NTD truncated L11 (Cter-L11), and no L11 (ΔL11). Pretermination complexes with the same stop codon but containing different variants of L11 were mixed in pairs and allowed to compete for RF1 (Fig. 1) or RF2 (Fig. 2) at varying concentrations. RF1 was used for complexes carrying either the UAA or UAG codon, whereas RF2 was used for complexes with the UAA or UGA codon. The reaction was stopped after 20 s, a time long enough for single round tetrapeptide release, but short enough to

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**TABLE 2**

Relative efficiencies of translation termination by RF1 and RF2

| RC1 vs. RC2 | RF1 | RF2 |
|-------------|-----|-----|
| UAA vs. UAG |     |     |
| WT-L11 vs. Cter-L11 | 4.2 ± 0.2 | 4.4 ± 0.3 |
| WT-L11 vs. ΔL11 | 5.8 ± 0.2 | 6.0 ± 0.2 |
| Cter-L11 vs. ΔL11 | 1.5 ± 0.1 | 1.4 ± 0.1 |

* RC pretermination complex.

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prevent multiple round peptide release. To differentiate between the tetrapeptides released from the two competing complexes, we have used \(^{14}\)C-labeled isoleucine (\([^{14}\)C]Ile) as the last amino acid in one complex and \(^{3}\)H-Ile in the other. In each set of experiments, the amount (pmol) of MFTI released was plotted as a function of the release factor concentration (A, C, and E in Figs. 1 and 2). For each release factor concentration we also plotted the negative (natural) logarithm of the fraction of tRNA-bound tetrapeptide on one ribosome complex versus the negative logarithm of the corresponding fraction on the competing ribosome. This generates a straight line with a slope that estimates the ratio between the \(k_{cat}/K_m\) values for termination at the two types of ribosome complex with high precision (B, D, and F in Figs. 1 and 2; data summarized in Table 2). Our experiments show that deletion of the N-terminal domain of L11 or of the whole L11 protein leads to a reduction of the RF1 termination efficiency at both UAA and UAG codons by a factor of 4 or 6, respectively (Fig. 1, B and D, and Table 2). When the Cter-L11 and \(\Delta L11\) complexes were competed for RF1, the ratio of \(k_{cat}/K_m\) values was 1.4 (Fig. 1, E and F, and Table 2), perfectly consistent with the \(k_{cat}/K_m\) ratios in the previous two cases, because 6/4 = 1.5, which is very close to 1.4. When similar competition experiments were performed for RF2, with different L11-containing pretermination complexes carrying

![Figure 2. Determination of the relative termination efficiency (\((k_{cat}/K_m)_{RC1}\) ratio versus \((k_{cat}/K_m)_{RC2}\) ratio) of RF2 on competing pretermination complexes (RC) with a UGA stop codon. The pretermination complexes containing WT-L11 (B), Cter-L11 (C), and the \(\Delta L11\) (E) were allowed to compete for RF2 in a pairwise manner to release the tetrapeptide fMet-Phe-Thr-Ile with time (A, C, and E). The relative termination efficiency of RF2 on the corresponding competing complexes was obtained from the slope of the linear curve when the negative natural logarithm of the fraction of tetrapeptide retained on one complex was plotted as a function of the same on the other of the competing pair (B, D, and F).]
either the UAA or UGA stop codon, the termination efficiency was reduced much less, only about 20 or 30% because of the N-terminal truncation and L11 deletion, respectively (Fig. 2 and Table 2).

**Determination of the Intrinsic Rate Constant for Ester Bond Hydrolysis**—The reduction of termination efficiency \(k_{cat}/K_m\) caused by N-terminal truncation of L11 or deletion of the whole protein (Table 2) could a priori be the result of (see “Experimental Procedures”) (i) a reduced intrinsic rate constant for ester bond hydrolysis \(k_c\); (ii) a reduced rate constant for factor association \(k_a\); or (iii) an increased rate constant for factor dissociation from the pretermination ribosome complex \(k_d\). To clarify this question, we determined \(k_c\) for cognate termination by RF1 or RF2 at a high concentration \((1–3 M)\) for all combinations of pretermination complexes \((200 nM)\) and stop codons (Table 3). For all of the pretermination complexes the \(k_c\) values for RF1- and RF2-mediated peptide release were estimated as \(\sim 0.55 s^{-1}\) and \(2.5 s^{-1}\), respectively (Table 3), in line with previous observations regarding wild type ribosomes (33). The natural logarithms of the fraction of tetrapeptide released against time result in linear fits as shown in Fig. 3, B and D. These results demonstrate that neither N-terminal truncation nor complete deletion of protein L11 affects the intrinsic rate constant for RF-induced ester bond hydrolysis during termination of translation.

**Termination of Protein Synthesis by Release Factors in Recycling Mode**—To study the rate of recycling of RF1 and RF2 during termination at the various pretermination ribosome complexes, either one of these was mixed in excess with a small amount of release factor. The recycling rate was then obtained from the slope of the straight line when the amount of released tetrapeptide per amount of active release factor was plotted as a function of time (Fig. 4 and Table 4). Under these conditions, the slow dissociation of release factor from the post-termination ribosome was rate-limiting and, accordingly, the recycling rate represented the rate constant \(q_d\) for dissociation of the release factor from the post-termination ribosome (Reaction 1). As a control, we also estimated the rate of spontaneous tetrapeptide release in the absence of any release factor from the pretermination complexes in an otherwise identical experimental setup, which was found negligible. Fig. 4 illustrates the recycling rates of RF1 (Fig. 4A) and RF2 (Fig. 4B) on pretermination complexes with a UAA codon in the A site. In the absence of RF3, RF1 recycled with a rate close to \(3 \times 10^{-3} s^{-1}\) for all the three ribosomal complexes (Fig. 4A and Table 4). There was essentially no difference when a UAG codon was present instead of a UAA codon (data not shown). For WT-L11 complexes programmed with a UAA codon recycled with a larger rate close to \(9 \times\)

### TABLE 3

| Rates of peptide cleavage \((k_c)\) by RF1 and RF2 |
|-----------------|----------------|----------------|
| Release complexes | RF1 | RF2 |
| UAA | UAG | UAA | UGA |
| WT-L11 | 0.55 ± 0.02 | 0.57 ± 0.02 | 2.4 ± 0.2 | 2.7 ± 0.2 |
| Cter-L11 | 0.53 ± 0.08 | 0.56 ± 0.06 | 3.0 ± 0.5 | 2.2 ± 0.2 |
| ΔL11 | 0.54 ± 0.04 | 0.55 ± 0.02 | 2.5 ± 0.1 | 2.5 ± 0.1 |

**FIGURE 3.** Determination of the rate of peptidyl-tRNA hydrolysis \((k_c)\) by an excess amount of RF1 (A and B) and RF2 (C and D) \((3 \mu M)\) on pretermination complexes \((200 nM)\) programmed with the UAG and UGA stop codon, respectively. The fraction of MFTI tetrapeptide released is plotted as a function of time \((A \text{ and } C)\). The natural logarithm of the fraction of tetrapeptide remaining on these complexes is plotted in \(B\) and \(D\) as a function of time.
10^{-3} \text{s}^{-1} \) (Fig. 4B and Table 4). For the Cter-L11 and \( \Delta L11 \) complexes, RF2 recycled with significantly smaller rates close to \( 6 \times 10^{-3} \) and \( 3.5 \times 10^{-3} \text{s}^{-1} \), respectively, for UAA (Fig. 4B and Table 4) as well as for UGA codons (data not shown). When the recycling of RF1 and RF2 occurred in the presence of an excess amount of RF3 (see “Experimental Procedures”), both factors recycled much more rapidly, but the recycling rates responded to the L11 alterations in a way similar to that in the absence of RF3 (Table 4).

**Accuracy of mRNA Translation**—Studies of termination efficiency in living cells based on nonsense suppression (6, 18) are ambiguous in the sense that enhanced nonsense suppression may either be interpreted as reduced termination efficiency of a release factor or enhanced readthrough efficiency of the stop codon by tRNA. Therefore, we checked whether a complete or an N-terminal domain deletion of the L11 protein affects the accuracy of codon reading (i.e. the missense error level) by aminoacyl-tRNA. For this, we compared the effective association rate constants \( k_{cat}/K_m \) for a near cognate Leu-tRNAGAG in ternary complex with EF-Tu and GTP when interacting with wild type or L11-altered ribosome complexes programmed with UUU in the A site. The \( k_{cat}/K_m \) values were 143 ± 7.0 for the WT-L11, 82 ± 5.5 for the Cter-L11, and 78 ± 6.6 M^{-1} \text{s}^{-1} for the \( \Delta L11 \) ribosome variant. At the same time, \( k_{cat}/K_m \) for UUU codon reading by Phe-tRNAGAA was unaffected by the L11 alterations (see Fig. 5, A and B), suggesting about a 2-fold reduction in the missense error level by either one of the L11 changes.

**DISCUSSION**

L11 has been implicated in class I release factor-mediated termination since the 1970s, when scientists tried to understand the function of individual ribosomal proteins by neutralizing them with specific antibodies (34) or removing them from the ribosome with salt wash followed by reconstitution (3, 4). Removal of L11 from the ribosome appeared to reduce the activity of RF1 and, curiously, enhance the activity of RF2, and this scenario was reversed by readdition of L11 to the ribosomal core (4). Similar observations were made with ribosomes from three different mutant strains of *E. coli* lacking L11 (14, 35). When, furthermore, antibodies raised against the NTD of L11 (amino acids 1–64) were allowed to bind to wild type ribosomes, termination by RF1, but not RF2, was inhibited, suggesting that the NTD of L11 interacts directly with RF1, but not RF2 (14). In line with these observations, more recent in vivo studies demonstrated UAG suppression, growth deficiency, and temperature sensitivity for an L11 chromosomal knock-out strain (6). Overexpression of the L11-CTD from a plasmid partially reversed the growth defects but failed to reverse completely the UAG
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suppression (18). Despite these findings, the mechanistic role of L11 in termination of protein synthesis and its structural basis have remained obscure.

In the present study, we have obtained precise estimates of how the $k_{\text{cat}}/K_M$ values (efficiencies) for the interaction between the pretermination ribosome and class I release factors are affected by removal of the N-terminal domain from L11 as well as by depletion of the whole L11 protein from the ribosome. For RF1, we have found 4-fold or 6-fold reductions in the $k_{\text{cat}}/K_M$ values by L11 truncation or removal, respectively. For RF2, there were also reductions in the $k_{\text{cat}}/K_M$ values by these L11 alterations but much smaller and not exceeding 30% (Table 2). There was, in other words, a large reduction in the activity of RF1-dependent termination and a small but significant reduction in the activity of RF2-dependent termination. Another release factor-selective effect of these L11 alterations was an about 2-fold decrease in the rate constant for spontaneous release of RF2, but not of RF1, from the post-termination ribosome. Recent structural data on RFs in complex with pre- as well as post-termination ribosomes may have a bearing on our kinetic data.

To set the perspective for a discussion about selective effects of L11 on the kinetics of RF1 and RF2, we note that L11 is located at the GTPase-associated center of the ribosome at the base of the ribosomal stalk (36, 37) quite distant from the decoding and peptidyltransferase centers, believed to be the major points of action of class I release factors (38). However, domain I of the release factors is seen in close proximity to L11 and the L11BR in recent cryo-EM reconstructions. Domain I is important for RF interaction with RF3 (13), and when it was removed or swapped between the RFs, this resulted in a general loss of guanine nucleotide exchange on RF3 (29) and in selective effects on the efficiencies of RF1 and RF2; although the efficiency of RF1 remained unaltered when domain I was removed, the efficiency of RF2 decreased considerably (29). These data suggest that the interaction of domain I with the GTPase-associated center is different for the two factors, in line with recent cryo-EM observations; that is, despite an overall similarity in the trilobed cryo-EM density of RF1 and RF2 on the ribosome, there is an arc-like extra density, seen close to domain I of RF1, which is absent in the cryo-EM structure of RF2 (13), and attributed to the NTD of L11. In contrast, domain I of RF2 appears to interact with the CTD of L11 and L11BR (13). These differences in the way the two release factors interact with L11 as well as L11BR may explain their different kinetic responses to L11 truncation or deletion. However, establishment of firmer links between structural data on RFs in ribosomal complexes and the present kinetic results will require refined modeling approaches based on structures at much higher resolution than are available today.

The results of our study are compatible with in vivo data showing increased read-through of the RF1-specific UAG codon in an L11 knock-out (6) and an L11 truncated (18) E. coli strain, compared with wild type. Our results seem, at the same time, to be incompatible with in vivo data, demonstrating decreased read-through of the RF2-specific UGA codon by the same L11 alterations (6, 18). However, the outcome of such in vivo experiments depends on the competition between an RF and a tRNA for stop codon reading. Therefore, increased read-through of a stop codon may either be explained by decreased efficiency of an RF or increased ability of a tRNA to read the stop codon. Decreased read-through may, according to the same logic, be explained as enhanced efficiency of a release factor or decreased ability of a tRNA to read the stop codon. We have found a 2-fold decrease in the efficiency by which Leu-tRNA$_{\text{GAG}}$ reads the near cognate UUU, suggesting that the efficiency of near cognate stop codon reading by tRNAs was reduced by about the same factor in these read-through experiments. If correct, this would predict increased read-through of UAG and decreased read-through of UGA stop codons by the L11 alterations because the 2-fold decrease in the efficiency of near cognate tRNA reading is smaller than the 4–6-fold decrease in termination efficiency by RF1, but larger than the 30% decrease in termination efficiency by RF2. The results of our study are also compatible with in vitro experiments suggesting that RF1-dependent termination is inhibited by removal of L11 from the ribosome, but in apparent contradiction to the further observation that RF2-dependent termination is stimulated by L11 depletion (4). It may, however, be borne in mind that these early experiments were performed with ribosome complexes bound to separate AUG and UGA triplets, which we now know is a poor model system for pre-termination ribosomes (31, 39).

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REFERENCES

1. Tate, W. P., McCaughan, K. K., Ward, C. D., Sumpter, V. G., Trotman, C. N., Stoffler-Melleicke, M., Malv, P., and Brimacombe, R. (1986) J. Biol. Chem. 261, 2289–2293
2. Kazemier, M. (1975) Eur. J. Biochem. 58, 501–510
3. Armstrong, I. L., and Tate, W. P. (1978) J. Mol. Biol. 120, 155–166
4. Tate, W. P., Schulze, H., and Niehaus, K. H. (1983) J. Biol. Chem. 258, 12186–121820
5. Moazed, D., Robertson, J. M., and Noller, H. F. (1988) Nature 334, 362–364
6. Van Dyke, N., Xu, W., and Murgola, E. J. (2002) J. Mol. Biol. 319, 329–339
7. Bourne, H. R., Sanders, D. A., and McCormick, F. (1991) Nature 349, 117–127
8. Wollenzen, P., Expert-Bezancon, A., and Favre, A. (1991) Biochemistry 30, 1788–1795
9. Ryan, P. C., Lu, M., and Draper, D. E. (1991) J. Mol. Biol. 221, 1257–1268
10. Ryan, P. C., and Draper, D. E. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 6308–6312
11. Scolnick, E., Tompkins, R., Caskey, T., and Nirenberg, M. (1968) Proc. Natl. Acad. Sci. U.S.A. 61, 768–774
12. Klafholz, B. P., Pape, T., Zavialov, A. V., Myasnikov, A. G., Orlova, E. V., Vestergaard, B., Ehrenberg, M., and van Heel, M. (2003) Nature 421, 90–94
13. Rawat, U. B., Zavialov, A. V., Sengupta, J., Valle, M., Grassucci, R. A., Linde, J., Vestergaard, B., Ehrenberg, M., and Frank, J. (2003) Nature 421, 87–90
14. Tate, W. P., Dognia, M. J., Noah, M., Stoffler-Melleicke, M., and Stoffler, G. (1984) J. Biol. Chem. 259, 7317–7324
15. Moffat, G. I., Timms, K. M., Trotman, C. N., and Tate, W. P. (1991) Biochimie (Paris) 73, 1113–1120
16. Jerniolo, D. K., Pagel, F. T., and Murgola, E. J. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 12309–12313
17. McCaughan, K. K., Poole, E. S., Pel, H. J., Mansell, J. B., Mannon, S. A., and Tate, W. P. (1998) Biochim. Biophys. Acta 1379, 857–866
18. Van Dyke, N., and Murgola, E. J. (2003) J. Mol. Biol. 330, 9–13
19. Conn, G. L., Draper, D. E., Lattman, E. E., and Gittis, A. G. (1999) Science 284, 1171–1174
20. Pavlov, M. Y., and Ehrenberg, M. (1996) Arch. Biochem. Biophys. 328, 9–16
21. 1005–1014
22. Jelenc, P. C., and Kurland, C. G. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 3174–3178
23. Pavlov, M. Y., Freistdroffer, D. V., MacDougall, J., Buckingham, R. H., and Ehrenberg, M. (1997) EMBO J. 16, 4314–4314
24. Seno, T., Kobayashi, M., and Nishimura, S. (1968) Biochim. Biophys. Acta 169, 80–94
25. Ehrenberg, M., Bilgin, N., and Kurland, C. (1990) in Ribosomes and Protein Synthesis: A Practical Approach (Spedding,G., ed.) pp. 101–129, IRL Press, Oxford, U.K.
26. Rodnina, M. V., and Wirtzfelder, M. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 1945–1949
27. Freistdroffer, D. V., Pavlov, M. Y., MacDougall, J., Buckingham, R. H., and Ehrenberg, M. (1997) EMBO J. 16, 4126–4133
28. Brune, C., Romby, P., Moine, H., Caillet, J., Grunberg-Manago, M., Springer, M., Ehresmann, B., and Ehresmann, C. (1993) Biochimie (Paris) 75, 1167–1179
29. Mor, L., Zavialov, A., Ehrenberg, M., and Buckingham, R. H. (2003) Mol. Microbiol. 50, 1467–1476
30. Pedersen, K., Zavialov, A. V., Pavlov, M. Y., Elf, J., Gerdes, K., and Ehrenberg, M. (2003) Cell 112, 131–140
31. Zavialov, A. V., Mor, L., Buckingham, R. H., and Ehrenberg, M. (2002) Mol. Cell 10, 789–798
32. Pavlov, M. Y., Freistdroffer, D. V., Heurgue-Hamard, V., Buckingham, R. H., and...
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33. Freistroffer, D. V., Kwiatkowski, M., Buckingham, R. H., and Ehrenberg, M. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 2046–2051
34. Tate, W. P., and Caskey, C. T. (1975) J. Mol. Biol. 93, 375–389
35. Stoffler, G., Cundliffe, E., Stoffler-Meilicke, M., and Dabbs, E. R. (1980) J. Biol. Chem. 255, 10517–10522
36. Agrawal, R. K., Linde, J., Sengupta, J., Nierhaus, K. H., and Frank, J. (2001) J. Mol. Biol. 311, 777–787
37. Ban, N., Nissen, P., Hansen, J., Moore, P. B., and Steitz, T. A. (2000) Science 289, 905–920
38. Kisselev, L., Ehrenberg, M., and Frolova, L. (2003) EMBO J. 22, 175–182
39. Zavialov, A. V., Buckingham, R. H., and Ehrenberg, M. (2001) Cell 107, 115–124