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Overproduction of Release Factor Reduces Spontaneous Frameshifting and Frameshift Suppression by Mutant Elongation Factor Tu

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Mutant forms of elongation factor Tu encoded by *tufA8* and *tufB103* in *Salmonella typhimurium* cause suppression of some but not all frameshift mutations. All of the suppressed mutations in *S. typhimurium* have frameshift windows ending in the termination codon UGA. Because both *tufA8* and *tufB103* are moderately efficient UGA suppressors, we asked whether the efficiency of frameshifting is influenced by the level of misreading at UGA. We introduced plasmids synthesizing either one of the release factors into strains in which the *tuf* mutations suppress a test frameshift mutation. We found that overproduction of release factor 2 (which catalyzes release at UGA and UAA) reduced frameshifting promoted by the *tuf* mutations at all sites tested. However, at one of these sites, *trpE91*, overproduction of release factor 1 also reduced suppression. The spontaneous level of frameshift "leakiness" at three sites in *trpE*, each terminating in UGA, was reduced in strains carrying the release factor 2 plasmid. We conclude that both spontaneous and suppressor-enhanced reading-frame shifts are influenced by the activity of peptide chain release factors. However, the data suggest that the effect of release factor on frameshifting does not necessarily depend on the presence of the normal triplet termination signal.

The accuracy of translation depends on two selections: selection of the correct aminoacyl tRNA and selection of the correct reading frame. The protein elongation factor Tu (EF-Tu) brings aminoacyl tRNA to the ribosome. EF-Tu function influences the accuracy with which the correct aminoacyl tRNA is selected on the ribosome. Thus, mutant forms of EF-Tu can increase the level of missense errors in vitro (9, 24). These mutant forms of EF-Tu also increase the level of nonsense suppression (7, 23, 28). These results support the view that EF-Tu has a role in the selection of the correct aminoacyl tRNA on the ribosome.

Somewhat surprisingly, these same mutations of EF-Tu also cause suppression of frameshift mutations (8, 27). Mutations in an equivalent protein, EF-1 alpha in the yeast *Saccharomyces cerevisiae*, have also been shown to cause frameshift suppression (21). Current models of translation do not suggest a direct role for EF-Tu in reading frame selection. An alternative possibility is that EF-Tu might influence reading frame selection or maintainance indirectly, through its role in the selection of the correct aminoacyl tRNA. It has been noted that the frameshift windows suppressed by the EF-Tu mutations studied here, *tufA8* and *tufB103*, end in the nonsense codon UGA (8). Thus, frameshifting may be coupled to mismatched codon-anticodon interactions at these UGA sites.

We tested the possibility that EF-Tu-mediated frameshifting is related to misreading at UGA. To do this, we introduced plasmids synthesizing either one of the release factors. Then we asked whether higher release factor concentrations influence the level of frameshifting. The data suggest that an excess of active release factor 2 (RF-2; recognizes UGA and UAA) reduces the level of frameshifting by mutant EF-Tu. In addition, overproduction of RF-2 also reduces readthrough of some leaky frameshift mutations. However, the expected negative results with RF-1 were not always found.

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MATERIALS AND METHODS

Bacterial and phage strains. All bacterial strains used in this study are listed in Table 1. *Salmonella typhimurium* strains are derived from strain LT-2. The high-frequency generalized transducing bacteriophage P22 mutant HT105/1, *int-201* (22) was used for all transductions. Tn*10* markers in *S. typhimurium* are originally from the collection of John Roth, University of Utah.

Construction of strains US811, US812, US813, US814, and 15. Strains TH401, TH402, TH403, TH405, and TH198 (D. Hughes collection), carrying the *tuf*-suppressible +1 frameshift mutations described below, were spread on plates, with selection for the loss of the tetracycline resistance encoded by *zee-1*::Tn*10* as described by Maloy and Nunn (11). The derivatives thus selected were named US811, US812, US813, US814, and US815, respectively.

Construction of strain US818. A P22 lysate made on strain TH378 (carrying *argH1823*::Tn*10* linked to *tufB103*) was used to transduce TH332 (*trpE91 tufA8 proBΔ4*; *proBΔ4* was made by selecting for excision of Tn*10* from *proB1657*::Tn*10*, resulting in a nonreverting *pro* mutation), with selection for tetracycline resistance. Transductants were screened for resistance to kirromycin (to ensure that both *tuf* mutations were present), and one such transductant was kept and named US836. A P22 lysate grown on strain LT-2 was used to transduce US836, with selection for arg^+. The resulting tetracycline-sensitive transductants were screened for kirromycin resistance, and one such clone was kept and named US818.

Plasmids. Plasmid transfers within *S. typhimurium* strains were by P22-mediated transduction, with selection for the appropriate antibiotic resistance. Plasmid constructions were made in the plasmid pUB16. pUB16 (Urban Johansson,

TABLE 1. *S. typhimurium* and *E. coli* strains used

Strain	Genotype	Source or reference
S. typhimurium		
TH147	*trpE91 hisG3720 tufA8 tufB103*	8
TH420	*trpE871* (+1 frameshift)	8
TH422	*trpE873* (−1 frameshift)	8
TH432	*trpE879* (−1 frameshift)	8
TH462	*trpE91 hisG3720*	8
US811	*trpE91 tufA8 tufB103 hisO1242 hisD3749*	This study
US812	*trpE91 tufA8 tufB103 hisO1242 hisD3749-S6*	This study
US813	*trpE91 tufA8 tufB103 hisO1242 hisD3749-S7*	This study
US814	*trpE91 tufA8 tufB103 hisO1242 hisD3749-S15*	This study
US815	*trpE91 tufA8 tufB103 hisD3018*	This study
US818	*trpE91 tufA8 tufB103 proBΔ4*	This study
E. coli US634	*ara argE* (UAG) Δ(*lac proB*) *nalA thi recA srl*	M. Rydén Aulin

Department of Molecular Biology, University of Uppsala, Uppsala, Sweden) is a derivative of pBR322 lacking the unique *Hin*dIII site, which has been filled in with the Klenow fragment. All plasmids used in this study are listed in Table 2.

Construction of plasmid pRUM10. Plasmid pKK951 (10) is derived from pACYC184 (20), has a 3-kb *Eco*RI fragment carrying the gene *prfB* for RF-2 cloned in the *Eco*RI site, and expresses tetracycline resistance. pKK951 was digested with *Eco*RI and ligated with *Eco*RI-cut pUB16. The ligation mixture was transformed to US634 and plated out on ampicillin plates. Transformants were picked, and minipreparations were analyzed to check that the 3-kb fragment was present in pUB16. Plasmids were also digested with *Sal*I to confirm the result and also to give the orientation of the 3-kb fragment. Thus, in pRUM10 the gene for RF-2, *prfB*, is transcribed in the same direction as the Tet gene.

Construction of plasmid pRUM12. Plasmid pRUM10 was digested with *Hin*dIII, thus cutting at a unique site in the RF-2 gene. This site was then filled in with the Klenow fragment, and the plasmid was ligated again. The ligation mixture was transformed into US634. Filling in a *Hin*dIII site introduces a frameshift mutation, and thus the plasmid pRUM12 should code for an inactive RF-2 (see Results).

Construction of pRUM11. Plasmid pRF1 (30) was cut with *Eco*RI, which generates three bands, one of which is approximately 2.7 kb, extending from the *Eco*RI site in pBR322 into the cloned fragment. The gene for RF-1, *prfA*, is encoded on this 2.7-kb fragment. This fragment was purified from an agarose gel and ligated into pUB16 as for RF-2 above. *prfA* is transcribed outward in the same direction as the Tet gene.

TABLE 2. Plasmids used in this study

Plasmids	Construction
pUB16	pBR322 but *Hin*dIII site filled in with the Klenow fragment
pRUM10	pUB16 with 3-kb *Eco*RI fragment carrying *prfB* from pKK951
pRUM11	pUB16 with 2.7-kb *Eco*RI fragment carrying *prfA* from pRF1
pRUM12	pRUM10 but RF-2 inactivated by filling in the unique *Hin*dIII site in *prfB*
pKK951	pACYC184 with 3-kb *Eco*RI fragment carrying pfrB (10)
pRF1	pBR322 derivative carrying *prfA* (30)

All plasmids were constructed in the strain *Escherichia coli* US634 and subsequently transformed into the restriction-negative strain *S. typhimurium* TR6717 (J. Roth collection). Phage P22 grown on these transformants was used to transduce the plasmids to other *S. typhimurium* strains.

Media. Luria broth and M9 salts supplemented with 0.2% glucose (14) were used as liquid media. Solid media contained 1% agar (Sicomol). Kirromycin resistance was checked on LC plates (26) containing 2 mM EDTA. Where appropriate, media contained tetracycline (20 μg/ml), ampicillin (200 μg/ml), kirromycin (100 μg/ml), histidine (200 μM), and tryptophan (100 μM).

Determination of suppression. Suppression of mutations in the *trp* or *his* operons was determined by streaking for single colonies on minimal media lacking histidine or tryptophan as appropriate and incubating at 37°C. Suppression of the *trp* and *his* auxotrophs used in this study, by *tufA8* and *tufB103*, allows growth in the absence of the normally required amino acid (8). The relative efficiency of this suppression is measured by the number of days taken to reach a colony size of 1 mm in the absence of the required amino acid at 37°C. Suppression of mutations in the *lacI* part (15) of the *lacIZΔ14* fusion (16) was measured as β-galactosidase activity (14).

RESULTS

Suppression of the frameshift mutation *trpE91* by *tufA8* and *tufB103* supports colony growth to a diameter of 1 mm on minimal medium lacking tryptophan after 4 days (8). A comparison with *sufS601* (a tRNA suppressor of *trpE91*) suggests that *tuf* suppression probably results in less than 2% of the in-frame level of protein (19). These *tuf* mutations are more efficient suppressors of UGA mutations (7, 8). Because the *trpE91* window ends in UGA, we asked whether misreading of the UGA site is important for the observed frameshifting. To answer this question we introduced plasmids carrying release factor genes capable of recognizing UGA or the other termination triplet codons. Our expectation is that the release factor and the mutant EF-Tu–tRNA complex will compete for interaction with the nonsense codon.

Suppression of the −1 frameshift mutation *trpE91* is reduced by introduction of the plasmid pRUM10 producing RF-2. The strains TH462 (*trpE91 hisG3720*) and TH147 (*trpE91 hisG3720 tufA8 tufB103*) are isogenic except for the *tuf* mutations. TH147 is resistant to kirromycin, and *tuf*-mediated suppression allows growth in the absence of either tryptophan or histidine. We introduced into each of these strains plasmids carrying either RF-2 (pRUM10), RF-1 (pRUM11), or inactivated RF-2 (pRUM12). As an additional control we also introduced the parental plasmid lacking an insert, pUB16. The resulting strains were streaked onto minimal media lacking tryptophan or histidine to test for suppression. The pattern of growth is shown in Fig. 1. As expected, there is no growth with any of the derivatives of the *tuf*$^+$ TH462. The presence of the vector pUB16 has no influence on suppression in TH147 (*tufA8 tufB103*), which grows in the absence of either amino acid as expected. However, the presence of pRUM10 (RF-2) reduces growth in the absence of either amino acid very significantly. Thus, readthrough of UGA (*hisG3720*) and frameshift suppression (*trpE91*) are reduced by the presence of pRUM10. The

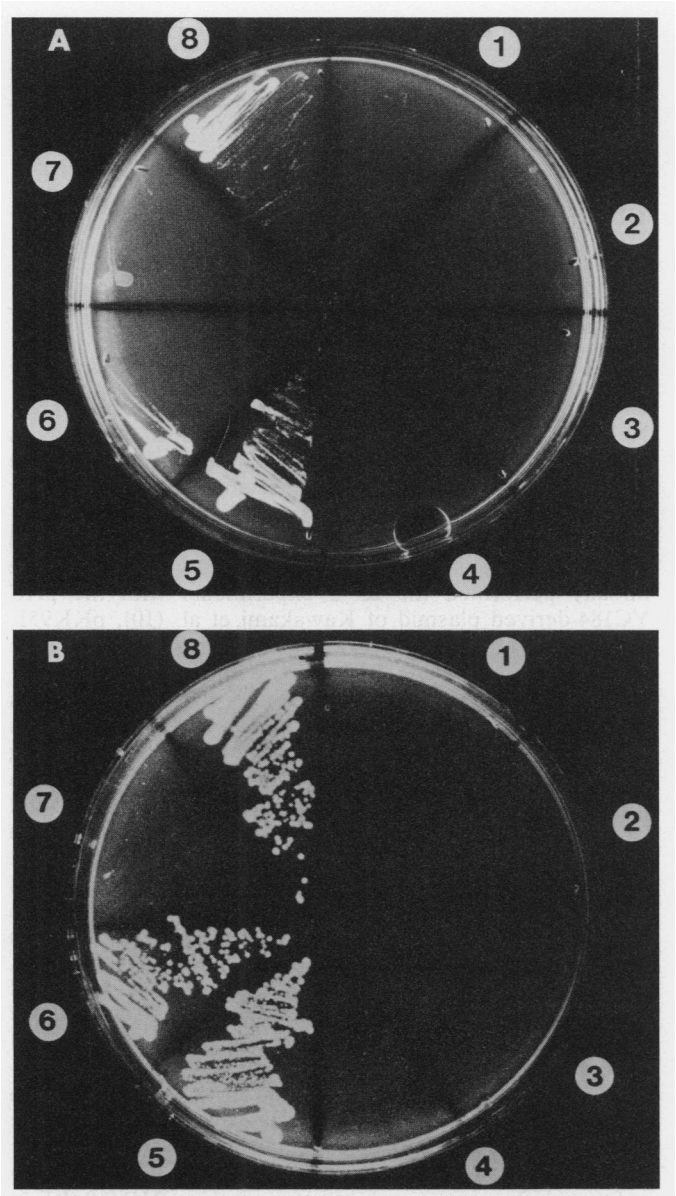

FIG. 1. (A) Effect of release factor plasmids on the suppression of *trpE91* (−1 frameshift) by *tuf* mutations. (B) Effect of release factor plasmids on the suppression of *hisG3720* (UGA) by *tuf* mutations. Sections 1 through 4 are streaked with TH462 (*trpE91 hisG3720*); sections 5 through 8 are streaked with TH147 (*trpE91 hisG3720 tufA8 tufB103*). Plasmids in each strain (sections): 1 and 8, pUB16 (vector); 2 and 7, pRUM10 (RF-2); 3 and 6, pRUM11 (RF-1); 4 and 5, pRUM12 (RF-2 inactive).

control plasmid pRUM12, carrying an inactivated RF-2 gene, does not affect suppression of either mutation. Surprisingly, the presence of the plasmid pRUM11 (RF-1) also significantly reduces suppression of the frameshift mutation *trpE91* (but not UGA *hisG3720*). The reduction in *tuf*-mediated suppression of UGA (*hisG3720*) associated with the presence of the RF-2 plasmid (pRUM10) acts as a control that this release factor is being overproduced and acts at UGA as expected. The naive conclusion is that the associated reduction in suppression of the frameshift mutation *trpE91* is also due to RF-2 recognition of UGA, in this case at the end of the frameshift window. This does not explain the effect of RF-1 on suppression. An alternative model to explain this result and the influence of RF-1 is discussed below (see Discussion).

Influence of release factor plasmids on frameshift suppression at +1 sites. *tufA8* and *tufB103* suppress several +1 frameshift mutations in the *his* operon. Suppression of these mutations is weaker than the *tuf*-mediated suppression of *trpE91*, as judged by growth in the absence of histidine (8). We introduced our four test plasmids into a series of strains (US811, US812, US813, US814, US815) carrying frameshift mutations in the *his* operon. Each of these frameshift mutations is suppressed by *tufA8* and *tufB103* and has a window ending in UGA (8). Suppression of *trpE91* in these strains serves as a control on the plasmid phenotypes and in each strain gave the expected results (see above).

The presence of the plasmids reduces (the already slow) growth in the absence of histidine for two of the strains (US811, US812) such that it is no longer possible to measure suppression. In the other three strains (US813 *hisD3749-S7*, US814 *hisD3749-S11*, US815 *hisD3018*), frameshift suppression by *tufA8* and *tufB103* is measurable. The level of suppression of each of these mutations is reduced significantly by the presence of pRUM10 (RF-2) but is not influenced by either the vector (pUB16) or the inactivated RF-2 (pRUM12). pRUM11 (RF-1) causes a small reduction in suppression of one of these mutations (*hisD3749-S7*). We conclude that the RF-2 plasmid reduces *tuf*-mediated suppression of both −1 and +1 frameshift mutations.

Release factor plasmid reduces frameshift leakiness. The previous results show that plasmid pRUM10 (RF-2) and sometimes plasmid pRUM11 (RF-1) reduce *tufA8 tufB103* suppression of frameshift mutations. We asked whether the spontaneous level of frameshifting typical of "leaky" frameshift mutations could also be reduced by these plasmids. Three leaky frameshift mutations in the *trp* operon were tested. Each of these mutations, *trpE871* (+1) *trpE873* (−1), and *trpE879* (−1), has a frameshift window ending in UGA (1, 31). The four test plasmids were introduced into each strain (TH420, TH422, TH432), and growth in the absence of tryptophan was assayed. The results (Fig. 2) clearly show that the RF-2 plasmid pRUM10 reduces the leakiness of all three mutations. The other plasmids (RF-1, inactivated RF-2, and vector) do not influence the leakiness. We conclude that the presence of the plasmid producing active RF-2 can reduce the level of spontaneous frameshifting.

Level and specificity of nonsense readthrough in the plasmid-containing strains. We reason that, since *tuf*-mediated suppression of the UGA at *hisG3720* and at several other positions in the *his* operon (data not shown) is reduced by the presence of pRUM10, at least some overproduction of RF-2 occurs. To quantify this effect and to assess the effect of RF-1, we assayed readthrough of nonsense codons in a more direct way. This was done by using F′ factors with a fused *lacIZ* gene carrying various nonsense mutations in the *lacI* part of the fusion. Suppression of these nonsense mutations gives active β-galactosidase, which can be measured by a standard assay (14), and thus allows an estimate of the level of nonsense readthrough.

Into strain US818 (*proΔ4 trpE91 tufA8 tufB103*) we introduced F′ *lacIZΔ14* carrying either a UGA (position 189 or 280) or a UAG (position 181, 189, or 220) mutation in *lacI* and each of the four test plasmids (pUB16, pRUM10, pRUM11, pRUM12). β-Galactosidase activity was measured in each strain (Table 3).

Readthrough of UGA at position 189 is slightly reduced by the presence of pRUM10 (RF-2) but not by any of the other

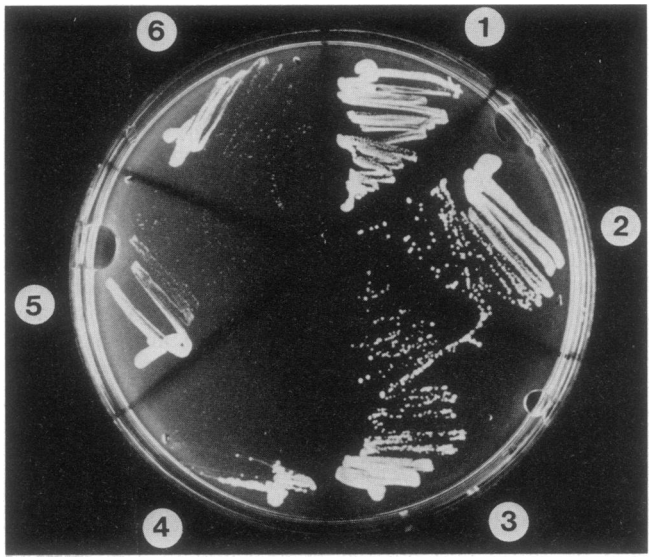

FIG. 2. Effect of release factor plasmids on the spontaneous suppression of three leaky frameshift mutations. The alleles are *trpE871* (sections 1 and 6), *trpE873* (sections 2 and 5), and *trpE879* (sections 3 and 4). The strains in sections 1 through 3 carry pUB16 (vector; pRUM11 and pRUM12 gave the same result), those in sections 4 through 6 carry pRUM10 (RF-2).

plasmids. Readthrough of UGA280 is reduced almost threefold in the strain carrying pRUM10 (RF-2). Neither RF-1 nor inactivated RF-2 reduces readthrough at this position. The high level of readthrough at position UGA189 (2 to 3%) is dependent on the *tufA8* and *tufB103* mutations, whereas the low level of readthrough of UGA280 is the spontaneous base level (7). Thus, the additional RF-2 due to the presence of pRUM10 is apparently sufficient to reduce the low spontaneous level of readthrough of UGA280 but has little influence on the high-level, *tuf*-dependent readthrough of UGA189.

At two of the three UAG positions tested (181 and 189), readthrough has been increased four- to fivefold over the base level by the *tuf* mutations (7). Readthrough of UAG at these two positions is reduced approximately twofold by the plasmid pRUM11 (RF-1). Readthrough of UAG220 is unaffected by either the *tuf* mutations or the plasmid pRUM11. Readthrough of the UAG sites is not decreased by the other plasmids tested, although strangely pRUM10 (RF-2) actually increases readthrough of UAG189. The basis of this effect is unknown.

The conclusion we draw from these results is that the RF-1- and RF-2-producing plasmids reduce readthrough of UAG and UGA sites, respectively, in some but not all cases. Neither the vector alone nor the inactivated RF-2 influences readthrough. The quantitatively small effect of the release factor plasmids on the level of readthrough may reflect controls on the level of release factor in the cell.

TABLE 3. Effect of release factor plasmids on suppression or readthrough of nonsense mutations in *lacI*[a]

Position in *lacI*	β-Galactosidase activity (%) with plasmid:			
	pUB16	pRUM10	pRUM11	pRUM12
UAG 181	0.10	0.11	0.04	0.12
UAG 189	0.09	0.16	0.04	0.11
UAG 220	0.16	0.12	0.16	0.18
UGA 189	2.3	1.7	2.2	2.6
UGA 280	0.35	0.12	0.32	0.48

[a] β-Galactosidase activity is expressed as a percentage of that from a nonmutated *lacIZΔ14* fusion in the same strain background. Each result is an average of four to five independent experiments.

TABLE 4. Growth rates of strains with release factor plasmids[a]

Strain	Generation time (min)
TH462(pUB16)	49
TH462(pRUM10)	51
TH462(pRUM11)	50
TH462(pRUM12)	51
TH147(pUB16)	63
TH147(pRUM10)	67
TH147(pRUM11)	62
TH147(pRUM12)	60

[a] Each result is an average of four independent measurements.

Reduced suppression of *trpE91* and *hisG3720* is not caused by a specific plasmid-related growth rate reduction. We initially performed the RF-2 experiments with the pAC YC184-derived plasmid of Kawakami et al. (10), pKK951. This plasmid has an even more dramatic effect than the pBR322-derived plasmid pRUM10 on both UGA and *trpE91* suppression. *tuf*-mediated suppression of each mutation is abolished or very significantly reduced by pKK951, as judged by the absence of any growth on plates lacking tryptophan or histidine. However, we find that the selection for tetracycline required for the maintainance of this plasmid causes a significant lag before growth (when measured in liquid culture), and this may contribute to the apparent reduction in suppression on solid media. To avoid this complication we have constructed all of our plasmids in a pBR322-derived background (see Materials and Methods), in which plasmid maintainance is by selection for ampicillin, which does not cause a lag before growth. We measured growth rates for TH462 (*tufA*$^+$ *tufB*$^+$) and TH147 (*tufA8 tufB103*) carrying each of the four test plasmids in minimal medium supplemented with tryptophan, histidine, and ampicillin. The results (Table 4) show a growth rate difference associated with the *tuf* mutations, as expected (8), but there is no significant additional effect related to any particular plasmid. Thus, we conclude that the reduced frameshifting or frameshift suppression in strains carrying pRUM10 (RF-2) or pRUM11 (RF-1) is related to the presence of an additional active *prf* gene and is not an effect of the plasmid on overall growth rate.

DISCUSSION

We initiated this study to determine whether the suppression of frameshift mutations by mutant EF-Tu was coupled to errors of termination or nonsense readthrough. It has previously been noted that each of the frameshift mutations suppressed by *tufA8 tufB103* has a window ending in UGA (8), a termination codon that is fairly efficiently suppressed by these *tuf* mutations (7). Our interest in the possibility of coupled nonsense and frameshift errors arises because it is not obvious from the current data that EF-Tu has any direct influence on reading frame selection. We began with the hypothesis that the reading of UGA as a sense codon, promoted by mutant EF-Tu, might result some of the time in a reading frame shift (possibly because of the incorrect tRNA-ribosome geometry or the nonstandard codon-anticodon interaction). This hypothesis predicts that additional

RF-2 might reduce the level of this frameshifting by increasing the efficiency of a competing termination reaction at UGA. If, however, frameshifting occurs prior to the termination codon, then the UGA will not appear in frame and release factor should not influence the outcome of the event.

Our measurements of nonsense readthrough suggest that the presence of the RF-1 and RF-2 plasmids results in an increase in the level of the relevant release factor in the cell, as expected. The specificity of action of plasmid-borne release factor genes is crucial for our experiments and has been demonstrated both here and previously (13). One of the principal results of this study is to show that the presence of a plasmid carrying an active *prfB* (RF-2) gene reduces the level of spontaneous and mutant *tuf*-mediated frameshifting at frameshift windows ending with UGA. Neither the vector itself nor a control plasmid carrying an inactive RF-2 has an effect on frameshift suppression or leakiness. Thus, these results appear to support our hypothesis that these instances of frameshifting are related to errors at the UGA codon. However, another prediction of our hypothesis is that RF-1 should not influence events occurring at UGA codons. Contrary to expectation, a plasmid carrying an active *prfA* (RF-1) also reduces suppression at at least one of these sites (*trpE91*). This result is not predicted by the current data on release factor action (6). The possible significance of this positive result with RF-1 is discussed below. We would have liked to test frameshift mutations with windows ending with other terminators, but unfortunately all of the sequenced suppressible or leaky mutations known to us in *S. typhimurium* end in UGA.

It has been noted previously that mutations in *supK* (probably identical to the structural gene for RF-2, *prf2* [10]) increase the level of spontaneous UGA readthrough and also of frameshift leakiness of *trpE91* and *hisD3018* (2). These results are not inconsistent with ours. Although it is uncertain how the *supK* mutants act, a reasonable model is that a reduction in enzyme activity caused by the mutation reduces the probability of translational termination at UGA, thus enhancing the level of readthrough. The increased leakiness of the frameshift mutations *trpE91* and *hisD3018* in *supK* strains suggests that the activity of the release factor (RF-2) is important in preventing frameshifting at these sites. Our results support this hypothesis.

There are other data suggesting that termination codons can be associated with frameshifting even in the presence of single-copy wild-type release factor genes. An early study of phase shift mutants in phage T4 by Barnett et al. (3) suggested a link between a UGA codon and an increased level of frameshifting. These authors noted a high level of frameshifting within a particular segment of the rII B cistron that had to occur before a "barrier" (now known to be a UGA codon). To test whether the barrier itself had anything to do with the shifting, they removed it and found that in its absence the phaseshifting disappeared. More recently, Weiss et al. (29) have reported that frameshifting on "shifty sequences" is often greatly enhanced if the sequence is immediately bounded at its 3′ end by a nonsense codon (preferentially UGA or UAA). The reasons for this enhancement are currently unknown. Results from the same laboratory (18) show that reading frame shifts caused by a mutant tRNA, *hopR1*, are greatly enhanced when the "take-off" site is followed by a stop codon (UAA and UAG were tested). Each of these studies (3, 18, 29) suggests that nonsense codons can be associated with enhanced levels of frameshifting.

In summary, the present data suggest that both the signals and effectors of translational termination have properties that influence the maintainance of the correct reading frame. Our results show that RF-2 influences frameshifting on windows terminating in UGA. The unexpected effect of RF-1 on suppression of *trpE91* remains to be explained. If the RF-1 result is shown to be more than just a singular exception to the accepted rules, it raises questions about the nature of the signals that are recognized by the release factors. With regard to this point, we note the recent report that the stop signal for RF-2 may be a tetranucleotide rather than a trinucleotide codon (4). The evidence is that each release factor catalyzes translational termination in a nonsense-codon-dependent manner (6). Evidence that the release factors directly recognize the termination codons is weak (5). Recent results have localized a domain on the ribosome overlapping the base of the L7/L12 stalk with which the release factors interact (25). As yet, however, there is little information on the signals that promote this binding, particularly those determining which of the two release factors will bind. Intriguing results with rRNA mutants suggest that it may be the ribosome itself that is the agent of termination codon recognition, at least for UGA (17). This prompts us to suggest a model in which the ribosome, upon encountering a termination codon, signals for a specific release factor, which, upon interaction with the ribosome, catalyzes termination. Such a model could explain release factor action in the absence of the usual termination codons. It may be that such ribosomal signals, normally induced by an encounter with a specific termination codon, can also be induced as a product of translational errors, including those associated with reading frame shifts (for example the unusual tRNA-ribosome geometry associated with incorrect codon-anticodon interactions). Thus, it is of interest that close to 30% of ribosomes translating *lacZ* mRNA fail to complete translation (12; F. Jørgensen and C. G. Kurland, J. Mol. Biol., in press) and that most of this loss of processivity occurs at the level of translation. Whether release factors are involved in this premature translational termination is currently unknown. An alternative possibility to explain RF-1 activity at *trpE91* (extrapolating from the results of Tate's group [4]) is that this frameshift mutation site carries both an RF-2 and a previously unrecognized RF-1 termination signal.

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