Frameshift autoregulation in the gene for *Escherichia coli* release factor 2: partly functional mutants result in frameshift enhancement

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ABSTRACT

The regulation of release factor 2 (RF-2) synthesis in *Escherichia coli* occurs, at least in part, through autoregulatory feedback exerted at a unique frameshifting step required during RF-2 translation. We have constructed fusions between the genes for RF-2 and *E. coli trpE* which make direct measurement of frameshifting efficiency possible since both products of regulation, the termination product and the frameshift product, are stable. The addition of purified RF-2 to *in vitro* expressions of these fusion genes was found to result in decreased frameshifting and increased termination at the regulation site. The frameshifted *trpE*-RF-2 products synthesized from these fusions are unique with respect to their functional release factor activities; when tested in assays of two intermediate steps of translational termination, they were found to be partially active for the function of ribosome binding, but inactive for peptidyl-tRNA hydrolysis (release). These are the first examples of release factor mutants selectively active for only one of these functions. *In vivo* these chimeric proteins promote large increases in frameshifting at the RF-2 frameshift region, thereby reversing normal negative autoregulatory feedback and instead supporting fully efficient frameshifting in their own synthesis. This activity provides new evidence for the importance of ribosomal pausing in directing efficient frameshifting at the RF-2 frameshift region.

INTRODUCTION

The gene for the polypeptide chain release factor 2 (RF-2) in *Escherichia coli* contains an in-frame UGA stop at codon position 26 (1). Analysis of the amino acid sequence of native RF-2 protein indicates that for normal RF-2 production this stop is circumvented during translation by a +1 frameshift. The features of the region of RF-2 mRNA responsible for this activity have been extensively studied using site specific mutagenesis of synthetic RF-2 frameshift 'windows' located in a β-galactosidase reporter gene (2–4). Two principal elements are required for efficient frameshifting: the frameshift site itself, consisting of the leucine codon CUU next to a UGA stop, and an upstream sequence similar to a Shine-Dalgarno (SD) element, which is the feature of normal prokaryotic mRNAs which interacts with 16S rRNA during ribosome binding. Interaction of this SD-like sequence with the ribosome has been implicated as an important step in the frameshift mechanism (3).

This situation provides a unique opportunity for RF-2 protein to feed back on the level of its own synthesis with great specificity. Along with UAA, RF-2 also recognizes the stop codon UGA during its normal role in the termination of translation (5). Therefore, high levels of RF-2 protein should result in effective recognition of the internal UGA and increased premature termination of new RF-2 translation, thereby creating a functional autoregulatory circuit. The potential role of such a mechanism in regulating RF-2 synthesis has been supported by the finding that exogenous RF-2 can repress *in vitro* expression of the RF-2 gene (6).

This model for RF-2 regulation relies on the existence of a balanced competition between frameshifting and termination events at the RF-2 frameshift site. The occurrence of competition at this site *in vivo* has been supported by the demonstration that a suppressor tRNA directing continued translation in an unproductive frame can compete with frameshifting when using a synthetic RF-2 frameshift region in which UGA has been changed to UAG (4). But the occurrence of termination, which must be the normal result of a significant proportion of ribosomal passages through the frameshift site, has previously only been inferred, presumably because the small 25 amino acid product which results is unstable and rapidly degraded (7).

We set out to demonstrate and measure the actual partitioning of termination and frameshifting events at the frameshift site in RF-2 translation by making gene fusions which give a stable product upon termination. Using these we show that the effect of exogenously added RF-2 protein on *in vitro* RF-2 production is in fact mediated through modulation of the balance between these competing events. The RF-2 fusion proteins produced as a result of this strategy have however proven interesting in themselves, as they are the first examples of partly functional forms of a release factor capable of only one of the two main intermediate steps of release factor activity. This partial activity appears to make them capable of promoting increased efficiencies of frameshifting in RF-2 sequences *in vivo*.

MATERIALS AND METHODS

Strains and Plasmids

Plasmids used for the construction and expression of *trpE* gene fusions (8), were of the pATH series created by T. J. Koerner and A. Tzagoloff. Two of these vectors, pATH10 and pATH11, were used depending on the reading frame required. Fragments of the gene for *E. coli* RF-2 were originally derived from a λgt10 clone carrying the RF-2 gene within a 3.4 kilobase *Eco*RI insert (7). These were combined to create a series of release factor-*trpE* hybrid (RFTH) genes as outlined in Fig. 1. The indicator plasmid p*lac*/RF-UGA was kindly supplied by Michael Yarus (4). It carries a modified version of the β-galactosidase gene into which the frameshift window of RF-2 has been inserted, such that active protein product occurs only as a result of a frameshift at this site. A control version of this plasmid, p*lac*/wt, carries a pseudowild-type version of the *lacZ* gene which was constructed by replacing the RF-2 frameshift window in p*lac*/RF-UGA with another fragment of similar size which does not necessitate a frameshift for active protein production.

The plasmids p*lac*/RF-UGA and p*lac*/wt were maintained in the *lac*$^-$ strain MY649 Δ*lac proXIII, ara, thi, recA56* (9). Other plasmids were maintained and expressed in strain JM101 (10). The RFTH plasmids are compatible with the indicator or control plasmids, and were each transformed together with either of them into MY649 for β-galactosidase assays.

Coupled Transcription-Translation *In Vitro*

In vitro expression of plasmid-borne genes was performed essentially as described (11), except that calcium acetate at 9.5 mM was substituted for calcium chloride in the salt mixtures (12). S30 extracts were prepared from log phase cultures of strain MRE600 (13). Products were labelled with 1 μCi [^{35}S]-methionine (Amersham) in expression reactions of 10 μl total volume, one fourth of which would normally be resolved on 13% polyacrylamide mini-gels (0.8×80×100 mm)(14). Gels were processed for fluorography and resulting exposures on preflashed Cronex X-ray film quantitated on an LKB Ultroscan XL laser densitometer. All quantities were corrected for the frequency of methionine in the products.

In Vivo Expression of Fusion Genes

Small 5 ml cultures were tested for expression using the method of Spindler *et al.* (8), by growing to an A_{600} of 0.4 in M9 media (15) containing 0.2% Casamino acids and 50 μg/ml ampicillin. Cultures were then split and shaken vigorously at 37°C after addition of 10 μg/ml indoleacrylic acid (IAA)(for derepression of the *trp* promoter), or 20 μg/ml tryptophan (for repression). After 2 hours, 1 ml aliquots were harvested by centrifuging for 1 min in an Eppendorf microfuge and resuspending the pellets in 50 μl of SDS sample buffer (14) containing 6M urea. Proteins were analyzed by electrophoresis on 13% polyacrylamide mini-gels followed by Coomassie blue staining.

Termination Reactions *In Vitro*

Preparation of release factors and 70S ribosomes from *E. coli* MRE600, as well as all other materials for termination assays has been described (16). RFTH fusion proteins for assay were isolated from induced cultures essentially by the method used for normal RF isolation (16,17), and then partially purified by separation over a Waters Protein Pak DEAE-5PW column on a Waters 650 chromatography system. The locations of authentic RF-2 and the fusion proteins in the elution profiles were determined by assaying termination activities (below) and/or visualization on polyacrylamide gels. The isolated proteins were stored in 50 mM KCl, 50 mM Tris-HCl (pH 8.0), and 1 mM DTT.

Assays for *in vitro* termination and codon directed complex formation with the ribosome were conducted as outlined in (16). For preparation of ribosomal complexes containing f[^{3}H]Met-tRNA in the P site, 50 μl of a mixture containing 20 mM Tris-HCl (pH 7.5), 150 mM NH_4Cl, 10 mM $MgCl_2$, 50 picomoles of 70S ribosomes, 2.5 nanomoles AUG, and 25 picomoles (approximately 100,000 cpm) of f[^{3}H]Met-tRNA were incubated for 30 min at 30°C. To measure *in vitro* termination, 5 μl of this substrate was mixed with the sample and 4 nanomoles of termination codon in 50 ml of 50 mM Tris-HCl (pH 7.5), 75 mM NH_4Cl, and 30 mM $MgCl_2$. After 30 min at 20°C the assay was stopped by addition of 250 μl 0.1 M HCl, and the free f[^{3}H]Met extracted into 1 ml of ethyl acetate and counted. To measure complex formation between release factor and ribosome, the sample was added to a 50 μl reaction containing 50 picomoles of 70S ribosomes, 400 picomoles of UA[^{3}H]A, 20 mM Tris-HCl (pH 7.5), 100 mM NH_4Cl, 20 mM $MgCl_2$, and 10% (v/v) ethanol. After 15 min at 4°C, complexes were collected on glass fibre filters and counted. Titrations of 0.1−2.5 μg of native RF-2 or RFTH fusion proteins were accommodated in each of these assays.

β-Galactosidase Activity Assays

Cells were prepared for assay by growing overnight in M9 media (15) supplemented with 0.1% Casamino acids, 10 μg/ml thiamine, 50 μg/ml proline, 25 μg/ml histidine, 25 μg/ml tryptophan, 30 μg/ml chloramphenicol and 50 μg/ml ampicillin. The overnight cultures were diluted 1:100 into fresh medium lacking antibiotics and tryptophan and grown to midlogarithmic phase at 37°C. Aliquots were assayed and activity calculated as described by Miller (18).

RESULTS

Fusions of the gene for release factor 2 with *trpE* were constructed using plasmids which contain a polylinker sequence immediately downstream of the 5′ region of the *trpE* gene as shown in Fig. 1 (19). Fragments of the RF-2 gene were made using the 3 sites shown in the figure and fused in-frame to *trpE* by blunt ligation. Two of the resulting sequences, RFTH2 and RFTH3, contain the RF-2 frameshift site, with the fusion points at different distances upstream from it. The third, RFTH4, is a control construct lacking the frameshift region. Expression of any of the fusions is thereby under the control of the *trp* promoter and ribosome binding site. The sequences of the fusion regions in each of the completed constructs was verified by sequencing using the dideoxy chain termination method (21).

In vitro transcription-translation of plasmids carrying the frameshift fusions results in 3 major protein products: β-lactamase from the vector, a large fusion protein resulting from frameshifting at the internal UGA, and a smaller fusion protein made up mostly of *trpE* sequences which results from termination at the internal stop (Table 1). The ratio of the latter two products reveals the efficiency with which ribosomal frameshifting has occurred. Such estimates for frameshifting obtained from expression of both the RFTH2 and RFTH3 proteins over many

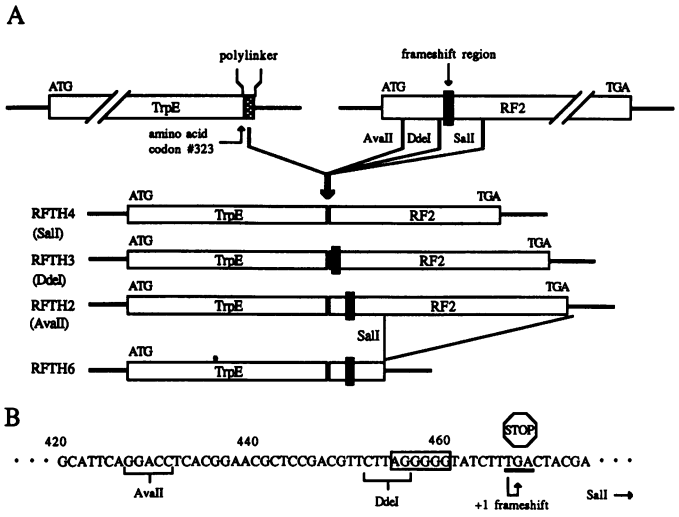

Fig. 1. Construction of *trpE*-RF-2 gene fusions. (A) Fragments of the gene for RF-2 were created by restriction with the enzymes indicated and repairing the ends with Klenow fragment. These were ligated with the *SmaI* site in the polylinker adjacent to the 5′ portion of the *trpE* gene in the vectors pATH10 or pATH11. Fusions RFTH2, 3, and 4 are the result. A further deletion in the RF-2 region of RFTH2 was created by recircularization of *SalI* digested plasmid, eliminating the fragment bounded by the internal *SalI* site and that remaining from the polylinker at the 3′ end of the gene. This fusion, RFTH6, should be terminated by an in-frame stop codon located about 50 nucleotides downstream of the deletion site. (B) The nucleotide sequence of the RF-2 gene in the frameshift region. The internal stop (TGA) is underlined, and the SD-like sequence is boxed. The positions of the sites used for constructing fusions (above), are as shown. The *SalI* site is located at residue 591 in the numbering of (1) and as used here.

experiments were always within the range of 35−50%, in agreement with earlier estimates made *in vivo* (4,6).

To show that the effects of exogenous RF-2 on its own synthesis *in vitro* are mediated through the frameshift event, purified RF-2 was titrated into *in vitro* expressions of the RFTH2 plasmid. Fig. 2(a) shows that the result of increasing RF-2 concentration is an increase in termination product (Mr 38,000) at the expense of frameshift product (Mr 76,000), with the larger of the two almost completely eliminated by higher concentrations of RF-2. A similar experiment performed using RF-1 (not shown), which is incapable of recognizing the UGA stop codon in the RF-2 gene, was used to demonstrate the specificity of the RF-2 effect on frameshifting. A plot of the effects on frameshifting observed above is shown in panel (b) of Fig. 2. These data demonstrate the effect of purified RF-2 on its own *in vitro* synthesis through modulation of frameshifting efficiency.

Expression of the fusion genes was also observed *in vivo* using the *trp* operon 'inducer' IAA. Whole cell extracts of cultures induced as described are shown in Fig. 3. Contrary to some other *trpE* fusion proteins (19,22) those with RF-2 did not partition appreciably into the insoluble fraction. The RFTH2 and RFTH3 fusions were unexpectedly both found to result in the production of high levels of large frameshifted fusion protein (Mr of 76,000 and 75,000 respectively in Fig. 3), with no detectable levels of the small Mr 38,000 termination product. The amounts of the large fusion product observed were roughly equal irrespective of whether or not the construct contained the frameshift region (RFTH4 compared with RFTH2 or RFTH3).

The contrast between the *in vivo* and *in vitro* efficiency values suggested to us that the hybrid gene products could be active in

Table 1. Relative molecular weights of peptide products of RFTH gene fusions

Gene fusion	Termination product (Mr)	Frameshift product (Mr)
RFTH2	38,007	76,226
RFTH3	37,208	75,428
RFTH4	–	70,117
RFTH6	38,007	44,909

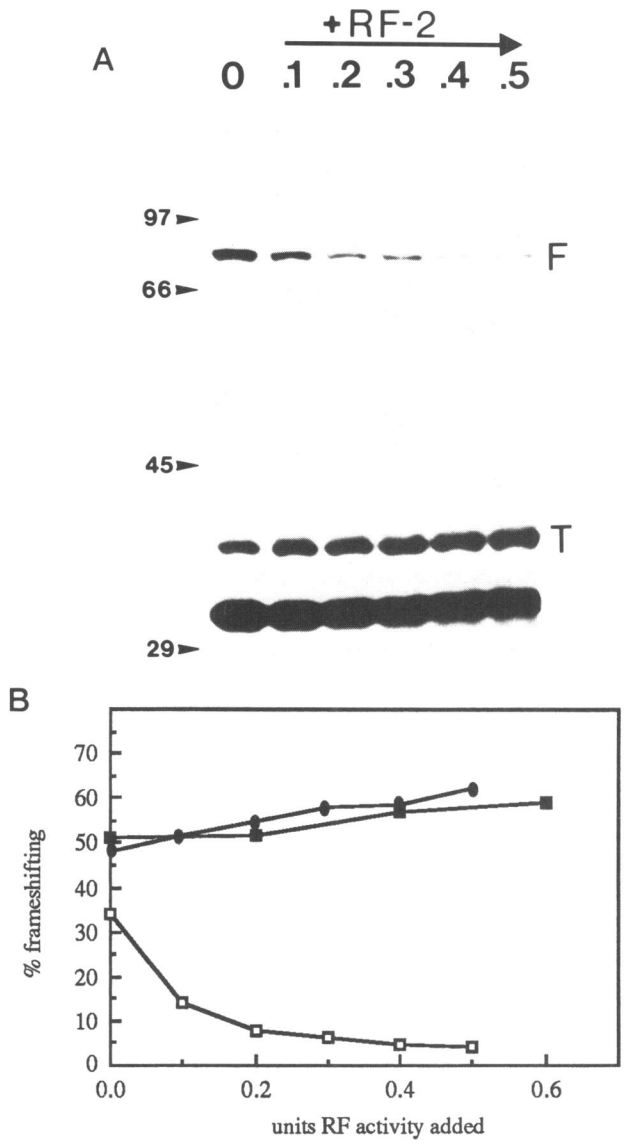

Fig. 2. Titration of release factor proteins into *in vitro* transcription-translations of the RFTH2 fusion gene. To 10 μl expression reactions containing pRFTH2 a variable quantity of purified release factor was added before incubation. Individual release factor preparations usually yield protein of varying specific activity. Therefore, added factor was equalized for relative activity. One unit of activity releases 0.5 picomoles of fMet in 30 min from 1 picomole of substrate in our standard assay (20). [^{35}S]-Labelled products were resolved on polyacrylamide gels which were processed and exposed to Cronex X-ray film. (A) Titration of purified RF-2 protein; unit quantities added are indicated. β-Lactamase precursor (Mr approximately 32,000) is encoded by the plasmid vector. The remaining two products result from termination (T) or frameshifting (F) at the internal stop codon of the RFTH2 fusion (see Table 1). The relative positions of marker proteins (Amersham Rainbow Markers) are shown at the left. (B) Plot of percent frameshifting relative to added RF activity. The frameshifting efficiency is derived from the ratio of the terminated and frameshifted products observed. (□) RF-2, (●) RF-1, (■) buffer only control.

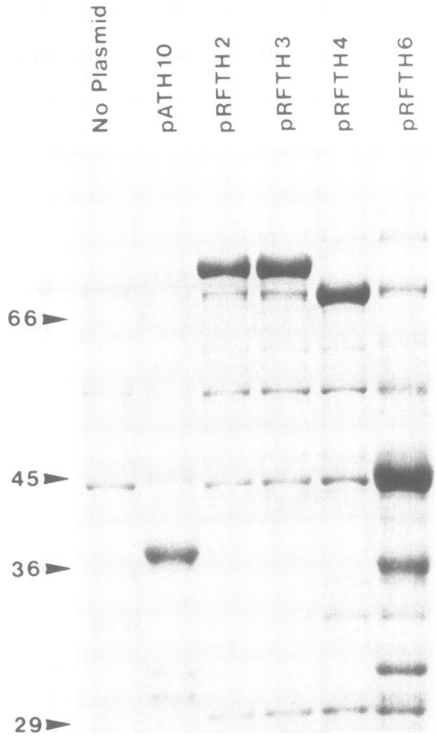

Fig. 3. Total cell extracts from strain JM101 carrying various RFTH plasmids. Total cell extracts from 5 ml cultures were made as described to analyze expression of fusion gene products *in vivo*. Aliquots were resolved by PAGE and stained with Coomassie blue. The gel shows samples from IAA induced cultures only, and the sizes of the expected products are found in Table 1. The vector plasmid pATH10 is expected to produce a peptide of approximate Mr 38,500 upon induction. The remaining lanes are as marked. The relative positions of marker proteins (Sigma Dalton Mk VII) are shown at the left.

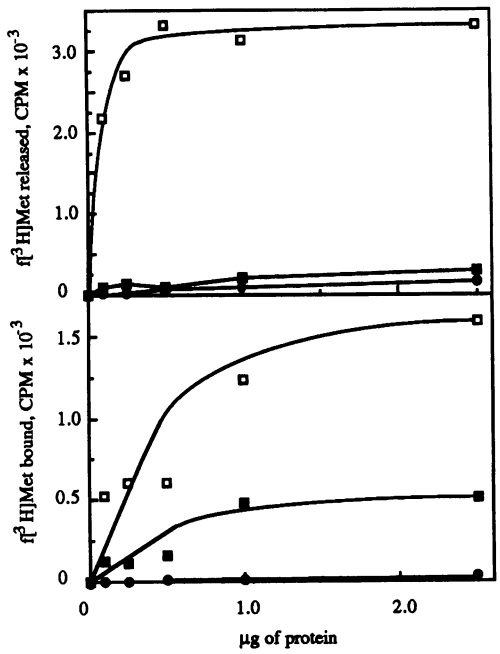

Fig. 4. *In vitro* termination activities of partially purified RFTH fusion protein. Variable amounts of protein were assayed for activity in two assays of termination function (16): peptidyl-tRNA hydrolysis (release) and codon directed complex formation with the ribosome (binding). (□) native RF-2, (■) RFTH2, (●) RFTH6.

Table 2. β-Galactosidase activities of strain MY649 carrying p*lac*/RF-UGA or p*lac*/wt in the presence of various RFTH fusion plasmids.

Plasmid	p*lac*/RF-UGA β-Galactosidase activity (units)	p*lac*/wt
–	5,564	14,556
pATH10	7,292	20,658
pRFTH6	7,917	22,042
pRFTH2	16,900	17,185
pRFTH3	15,922	15,854

Overnight cultures of strain MY649 carrying the indicated plasmids were diluted 1:100 in fresh media (Methods) lacking antibiotics and tryptophan (repressor), and grown with shaking at 37°C to midlogarithmic phase. b-Galactosidase activity was determined by the method of Miller (18). Values are the averages of triplicate determinations from quadruplicate cultures. β-Galactosidase levels in p*lac*/RF-UGA carrying cultures depend on the rate of frameshifting at the RF-2 frameshift window. p*lac*/wt serves as a control for when a frameshift is not required. RFTH fusion plasmids are described in Fig. 1.

some way at the frameshift site when overexpressed *in vivo*. Therefore, the large frameshifted fusion proteins were partially purified from induced cultures carrying the relevant plasmids, isolating them from endogenous RF-2 activity of the host strain JM101 and the smaller terminated peptides consisting mainly of *trpE* sequences. The isolated proteins were then tested for function in assays of two intermediate steps of termination: peptidyl-tRNA hydrolysis (release assay) and termination codon dependent ribosome binding (binding assay)(16). Both the RFTH2 and RFTH3 hybrid proteins were found to bind to the ribosome in a codon dependent manner, as shown for RFTH2 in Fig. 4 (lower panel). The activity was significant, although lower than that shown by native RF-2. Neither of these hybrids, however, showed any significant ability to catalyze the release step when compared with RF-2 (also shown for RFTH2 in the upper panel of the figure). The RFTH4 polypeptide, which contains less of the amino terminal sequence of RF-2, was found to be even less active than the other fusions in the binding assay (not shown). These chimeric proteins are the first characterized examples of release factor mutations active for only one of the two main release factor functions.

The easiest way to test whether the activities of the functional hybrids were in fact responsible for the *in vivo*–*in vitro* disparity was to eliminate any residual function by further deletion of their RF-2 regions. Therefore a 3′ deletion of the RF-2 coding segment extending upstream to the *Sal*I site of RFTH2 was made (designated RFTH6–see Fig 1). This construct produces a smaller frameshift peptide of approximate Mr 45,000, which can still be easily separated from the 38,000 Mr product of termination (Table 1), and is deleted at both the N and C terminal regions of the RF-2 protein sequence. This peptide was found to have no detectable binding (or release) activity *in vitro*. As can be seen in Fig. 3, expression *in vivo* of this construction results in significant levels of termination as well as frameshifting at the internal UGA, in contrast with RFTH2 and RFTH3. This suggests that the partial activity of the chimeric release factors is itself responsible for their increased frameshifting *in vivo*.

To provide a better test of the fusion protein's ability to affect frameshifting, the frameshift indicator plasmid p*lac*/RF-UGA (4) was expressed in strains also carrying the RFTH fusion plasmids. This plasmid contains a copy of the β-galactosidase gene into

which the RF-2 frameshift region has been inserted, such that the production of functional enzyme is dependent on the occurrence of a frameshift at that site. In the control plasmid p*lac*/wt, the frameshift window has been replaced such that a frameshift is not required for β-galactosidase translation. Table 2 displays the results of β-galactosidase assays of strain MY649 carrying either of these plasmids in combination with each of the RFTH fusion plasmids. All of the cultures were grown for assay in the absence of tryptophan to allow RFTH expression.

The most striking feature of the data (Table 2), is the markedly higher activity shown with the frameshift indicator plasmid p*lac*/RF-UGA when paired with either of the partly functional RFTHs (RFTH2 or RFTH3). These values are each experimentally equivalent to their counterparts with the control β-galactosidase plasmid p*lac*/wt, indicating frameshifting occurs in these cultures with virtually 100% efficiency. By comparison, the inactive fusion (RFTH6) and the fusion vector (pATH10), both caused smaller increases in the activities of *lac*/RF-UGA strains, which were mimicked by their *lac*/wt control counterparts. These increases are consequently not frameshift specific, and are most likely due to effects on plasmid or mRNA levels. The increases found with either RFTH2 or RFTH3 in the presence of the p*lac*/wt control were not however as large, and this is most likely due to their slower growth rates when grown in the absence of repressor. In sum, these data demonstrate the specific effect of RFTH expression in increasing the efficiency of frameshifting at the RF-2 frameshift site.

DISCUSSION

The gene for polypeptide release factor 2 contains an in-frame stop codon which must be circumvented by a +1 frameshift for production of normal RF-2 protein (1). Ribosomal passages which instead result in termination at the internal stop, should produce a small 25 amino acid peptide. This peptide has previously not been observed and experiments with a synthetic product of the same sequence have shown it to be very unstable in cell extracts (7). However, current views on the regulation of RF-2 synthesis (23) rely on the existence of a balanced competition between the alternative possibilities of frameshifting and termination at this site. For RF-2 protein to autoregulate its own production at this point there should be significant levels of the terminated RF-2 peptide fragments as a natural by-product. To make it possible to measure the relative rates of these competing events it was necessary to stabilize the product of termination as a fusion with the N terminal portion of the *E. coli trpE* protein and thereby allow its quantitation. *In vitro* expression of *trpE*-RF-2 fusion genes revealed the presence of the products resulting from both termination and frameshifting at the internal stop codon as predicted. The ratios of the two products indicate an *in vitro* frameshifting efficiency in the range of 35−50%.

Craigen and Caskey first showed that exogenous RF-2 was capable of suppressing expression from a plasmid-borne RF-2 gene *in vitro* (6). On the basis of this and further *in vivo* data showing frameshifting normally occurs with roughly 50% efficiency at the in-frame stop, they suggested that the frameshift event was the step through which RF-2 protein was acting to regulate synthesis. However, this approach could not directly show such alterations in frameshifting efficiency, because the product of the competing termination event could not be measured, nor could it say whether some of the effects observed *in vitro* occurred at the level of transcription.

Use of a gene fusion approach eliminates any potential contributions of transcriptional or the more traditional translational controls (such as blocking of translational initiation by mRNA binding) to RF-2 regulation because all expression signals are those of the *trpE* gene. This has allowed us to visualize the autoregulation of RF-2 synthesis *in vitro* by modulation of frameshifting efficiency. It has also made it possible to detect the effects of RF-2 on this process using nanogram quantities of factor (not shown), greater than 10 times the sensitivity of previous measurements (6), and at a level of added protein low enough to avoid general depression of protein synthesis.

Comparing the rates of frameshifting we observed *in vitro* (35−50%), and *in vivo* (100%), first suggested to us that the fusion proteins themselves might be capable of acting at the frameshift site when overexpressed *in vivo*. An alternative possibility, that the product of early termination (Mr 38,000) was absent *in vivo* because it was being selectively degraded due to some signal in the 25 amino acid N terminus of RF-2 is unlikely for two reasons. First, the amounts of the fusion products resulting *in vivo* were found to be essentially the same whether the frameshift region was present or not (RFTH2 or RFTH3 compared with RFTH4 in Fig. 3). And second, the same truncated product was clearly observed resulting from expression of the construct in which further RF-2 sequences were deleted (RFTH6 in Fig. 3).

There is now growing evidence that a pause in translation, irrespective of its cause, may be capable of providing an opportunity for ribosomal slippage on a 'slippery' mRNA sequence (4). Documented cases of the causes for such pausing are variable, from a shortage of charged cognate tRNA for the A site codon (24), to the presence of secondary structural elements in the mRNA (25,26). In the case of RF-2, the presence of a sub-optimal stop codon (27) may provide an opportunity for the tension of an improperly spaced SD-type interaction to propel the pausing ribosome ahead 1 base on a slippery run of U's. It may also be relevant that the codons directly preceeding this stop are likely to be translated quite slowly, as the codon adaptation index (28) for this region shows them to be very poor choices for rapid aminoacyl-tRNA selection.

If therefore the existence of a pause at the in-frame UGA can provide the opportunity for the occurrence of a frameshift, then that event should be maximized by exaggerating the pause for as long as possible. A partly active RF molecule would provide the ideal means for this if it were capable of binding to the ribosome-mRNA complex when UGA is in the A site, while being unable to complete termination and release the truncated N terminus of RF-2. Neither of these two release factor functions has however previously been separated from the other in a single partially active molecule. Therefore, when the isolated RFTH fusion proteins were found to be capable of codon dependent ribosome binding in the absence of any release function, it suggested to us that these partially disabled release factors could themselves be causing the increased frameshifting we observed. In effect, by changing the functional activities of the final product, we have reversed the direction of the normal autoregulatory circuit and instead produced a positive regulatory feedback through enhanced frameshifting. The ability of the fusion proteins to positively enhance frameshifting at the RF-2 site was also verified using a β-galactosidase indicator construction on an independent plasmid.

In vitro, the concentration of fusion protein produced is presumably inadequate to significantly affect the rate at which

frameshifting occurs during its own production, because of its much lower binding activity compared with native RF-2 (Fig. 4). *In vivo*, however, the effective levels of the induced protein can be much higher (Fig. 3), and the activity of the protein could be superior inside the intact cell (we have found the binding activity of these proteins can be very easily lost when purifying and/or concentrating them). This residual binding activity of the chimeric proteins also provides some insight into the locations of functional regions in the RF-2 molecule. In particular, it shows that release activity is apparently dependent on the integrity of an N terminal domain, of which the ribosome binding function is at least partly independent. However, the most significant consequence of this partitioning of the two release factor functions in the RFTH fusions is their apparent ability to effect increased rates of frameshifting at the RF-2 frameshift site. This ability provides further evidence that ribosomal pausing plays a central role in the processes which direct an efficient frameshift event in RF-2 translation.

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