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Frameshifting in the Expression of the *Escherichia coli trpR* Gene Is Modulated by Translation Initiation

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The *Escherichia coli trpR* gene encodes the 108-amino-acid-long Trp repressor. We have shown previously that a +1 frameshifting event occurs during the expression of *trpR*, resulting in the synthesis of an additional (+1 frame) polypeptide. Using *trpR-lac'Z* fusions, we have recently found that the transition from the 0 to the +1 frame occurs via the bypassing of a 55-nucleotide-long segment of the $trpR_{+1}$-*lac'Z* mRNA (I. Benhar, and H. Engelberg-Kulka, Cell 72:121–130, 1993). Here we show that the frequency of *trpR* frameshifting (or bypassing) can be regulated both in vivo and in vitro. This frequency is inversely proportional to the rate of initiation of translation of the *trpR* gene. Hence, modulating the level of translation initiation affects the frequency of frameshifting.

A translational frameshifting event permits an alternative use of the genetic code. It is manifested when at some point during the expression of a gene the reading frame is shifted backward or forward from that of the initiation codon. Frameshifting has been shown to be programmed by the sequence of the mRNA and sometimes also by its structure. Various components of the translational machinery have been found to be involved in the process (for reviews, see references 1, 2, and 20). Most examples of frameshifting have been described for eukaryotic and prokaryotic viral genes, particularly for retroviruses in which frameshifting is required for the synthesis of the *gag-pol* or the *gag-pro* polyproteins (for reviews, see references 20 and 25). In *Escherichia coli*, frameshifting has been found to be involved in the expression of three cellular genes: the *prfB* gene coding for protein release factor 2 (RF2) (9, 10), the *E. coli dnaX* gene, which codes for subunits τ and γ of DNA polymerase III (6, 12, 24), and the *trpR* gene coding for the Trp repressor (4, 5). Translational frameshifting provides a mechanism for gene expression that permits the synthesis of two different proteins from two separate reading frames of a single sequence of an mRNA molecule. It follows that the efficiency of the frameshifting process regulates the relative ratio of the different products of the gene. In most of the known examples of frameshifting, the level of this process has been reported as constant (20). In retroviruses, frameshifting varies from 5% in the Rous sarcoma virus *gag-pol* genes (17) to about 20% in mouse mammary tumor virus *gag-pro* genes (16). However, in each case there is a fixed ratio between the *gag* and the *gag-pol* or *gag-pro* proteins. In *E. coli*, frameshifting varies from about 5% in the *trpR* gene (5) to 50% in both the *prfB* gene (9, 10) and the *dnaX* gene (6, 12, 24). Until now, the only known example in which the frequency of frameshifting is regulated has been the *E. coli prfB* gene (10, 11).

Recently we have described a +1 frameshifting event involved in the expression of the *E. coli trpR* gene (5). We made the following observations. (i) The *trpR* gene directs the synthesis of two proteins, the expected 12-kDa frame 0 product and smaller amounts of an additional 10-kDa polypeptide. (ii) Immunoprecipitation experiments with peptide-directed antibodies revealed that these *trpR* products have identical N termini but different C termini. The C terminus of the shorter protein corresponds to the +1 frame of *trpR*. (iii) As shown by *trpR-lac'Z* fusions, the level of *trpR* +1 frameshifting varies from 2.7 to 6.5% under normal growth conditions. More recently, we have characterized the *trpR* frameshift site by introducing mutations and by amino acid sequence analysis (3). We found that *trpR* frameshifting does not occur because of slippage of a single nucleotide in the +1 direction. Rather, the transition from frame 0 to +1 of the fusion construct occurs via the bypassing of a 55-nucleotide-long segment of the coding mRNA. We identified two adjacent *cis* elements required for the process (3). Here we show that the level of *trpR* +1 frameshifting (or bypassing) is not constant. We found it to be inversely proportional to the rate of initiation of translation of the *trpR* gene.

Increasing *trpR* translation initiation reduces the level of *trpR* frameshifting in vivo. The rate of translation initiation is a function of the interaction of the ribosomes with the ribosome binding site and the initiation codon on the mRNA (13, 15, 22). The *trpR* gene is known to be inefficiently translated because the gene has a very weak ribosome binding site; it does not carry a sequence resembling the Shine-Dalgarno consensus region (13, 18). In order to modulate the level of the initiation of translation of the *trpR* gene, we replaced the 12 nucleotides preceding its initiation codon (Fig. 1, sequence WT) with a 12-nucleotide-long synthetic segment identical to the Shine-Dalgarno region of the *lacZ* gene (Fig. 1, sequence SD1) and known to permit high levels of translation initiation (13). A *trpR* gene carrying an altered ribosome binding site was used to construct $trpR_0$-*lac'Z* (pIB49) and $trpR_{+1}$-*lac'Z* (pIB50) fusions which were otherwise identical to the wild-type fusion constructs pIB13 and pIB14 (Table 1). We compared the β-galactosidase activity directed by the plasmids carrying altered Shine-Dalgarno regions with the levels of activity directed by the respective wild-type fusion genes. As shown in Fig. 1B, substituting a strong ribosome binding site for the native *trpR* ribosome binding site increased $trpR_0$-*lac'Z* expression by almost 600%. However, $trpR_{+1}$-*lac'Z* expression was increased by only 50%. Thus, increasing the rate of *trpR* translation

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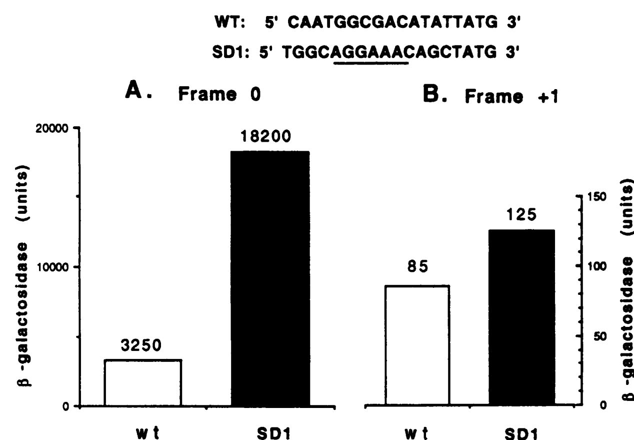

FIG. 1. Effect in vivo of an increase in translation initiation on frameshifting. The sequence preceding the wild-type *trpR* initiation codon (WT) and a synthetic sequence corresponding to the region preceding the *lacZ* initiation codon (SD1), which in this experiment was used to replace the wild-type *trpR* sequence, are shown at the top. *E. coli* SP361 cells were transformed by either plasmid pIB13 (panel A, wt) or pIB14 (panel B, wt), carrying the wild-type $trpR_0$-$lac'Z$ and $trpR_{+1}$-$lac'Z$ fusions, respectively, or alternatively by pIB49 (panel A, SD1) or pIB50 (panel B, SD1) (in both of which the wild-type ribosome binding sites were replaced with the SD1 sequence), carrying the $trpR_0$-$lac'Z$ and $trpR_{+1}$-$lac'Z$ fusions, respectively. Cells were grown in M9 minimal medium, and β-galactosidase activity was determined as described by us previously (5). The reported values were calculated from at least three independent experiments.

initiation sixfold decreased the level of expression of $trpR_{+1}$-$lac'Z$ relative to that of $trpR_0$-$lac'Z$ fourfold, from 2.7 to only 0.7%.

Decreasing *trpR* translation initiation increases the ratio of *trpR* +1-frame products to 0-frame products in vivo. In sodium dodecyl sulfate (SDS)-polyacrylamide gels, the *trpR* frame 0 product migrates as a polypeptide of 12 kDa (14, 19). We have previously shown that the +1 frameshift product of the *trpR* gene migrates as a polypeptide of 10 kDa (4, 5). Here we studied the in vivo synthesis of the *trpR* products under the condition of reduced translation initiation. The level of initiation was reduced by replacing the *trpR* initiation codon AUG with an AUC codon. In these experiments, the *trpR* gene was under the control of a T7 promoter on plasmids pCM64 (carrying the wild-type *trpR*) and pCM65 (carrying $trpR_{AUC}$, with a mutated initiation codon). These plasmids were introduced into cells carrying a thermoinducible T7 RNA polymerase gene on a pBR322-compatible plasmid (23). *trpR* transcription was induced at 42°C, and the synthesis of host proteins was inhibited by rifampin, as described by us previously (5). As shown in Fig. 2, expression of the wild-type *trpR* gene from the T7 promoter resulted in the production of two polypeptides: a major polypeptide of 12 kDa and a minor polypeptide of 10 kDa (lane 1). Both polypeptides remained stable during a 30-min chase (lane 2). The fact that the control plasmid pT7-5 (without *trpR*) directed neither of these polypeptides (Fig. 2, lanes 3 and 4) indicates that they are *trpR* products. We found by densitometric analysis of the autoradiograms of these two *trpR* products that the ratio of the amounts of the 10-kDa *trpR* +1-frame product to the 12-kDa *trpR* 0-frame product was about 0.2%. We also found that replacing the

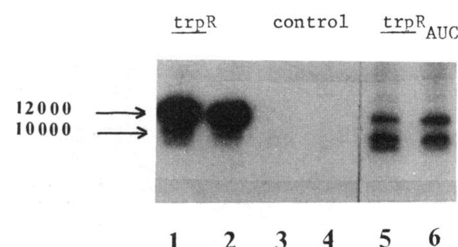

FIG. 2. Effect in vivo of a decrease in translation initiation on *trpR* frameshifting. *E. coli* K38 cells carrying a thermoinducible T7 RNA polymerase gene on plasmid pGP1-2 were transformed with plasmids pCM64 (lanes 1 and 2), pT7-5 (lanes 3 and 4), and pCM65 (lanes 5 and 6). Expression from the T7 promoter was induced at 42°C, rifampin was added, and the cells were labeled with [^{35}S]methionine for 5 min (lanes 1, 3, and 5). An excess of unlabeled methionine was added, and the reaction was terminated after an additional 30 min (lanes 2, 4, and 6). Samples were separated by Tricine-SDS-polyacrylamide gel electrophoresis (PAGE) and visualized by autoradiography, as described by us previously (5). Molecular masses are indicated in daltons.

TABLE 1. *E. coli* strains, bacteriophage, and plasmids used in this study

Strain, bacteriophage, or plasmid	Relevant genotype and characteristics	Source or reference
E. coli strains		
SP361	Δ*lacM25 nalR supF* Δ(*serB trpR*)*37-1*	7
K38	HfrC(λ)	21
Bacteriophage M13*trpR*	*trpR* in M13mp19	5
Plasmids		
pHEK	pKC30 with *trpR* under λP_L	5
$pHEK_{AUC}$	pHEK with mutation AUG→AUC in *trpR* initiation codon	This work[a]
pIB13	$trpR_0$-$lac'Z$ fusion	5
pIB14	$trpR_{+1}$-$lac'Z$ fusion	5
pIB49	pIB13 derivative carrying an altered ribosome binding site	This work[a]
pIB50	pIB14 derivative carrying an altered ribosome binding site	This work[a]
pGP1-2	Expresses T7 RNA polymerase	23
pT7-5	T7 promoter expression vector	23
pCM64	pT7-5 with *trpR* under T7 promoter	5
pCM65	pCM64 derivative with mutation AUG→AUC in *trpR* initiation codon	This work[a]

[a] Mutagenesis was carried out as described by us previously (3, 5).

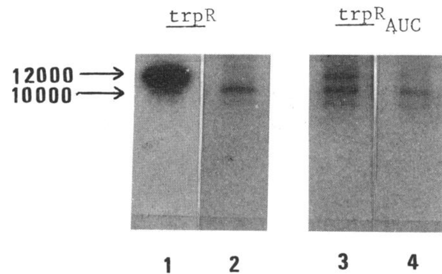

FIG. 3. Effect in vitro of a decrease in translation initiation on *trpR* frameshifting. Plasmids pHEK (lanes 1 and 2) and $pHEK_{AUC}$ (lanes 3 and 4) were used as templates in an *E. coli* in vitro transcription-translation system. The synthesized proteins were labeled with [^{35}S]methionine and immunoprecipitated with antibodies against the first 14 amino acids of the *trpR* product (lanes 1 and 3) or with antibodies against the last 16 amino acids of the C terminus of the +1 frame (lanes 2 and 4). Samples were separated by Tricine-SDS-PAGE and visualized by autoradiography. Detailed experimental procedures were as described by us previously (5). Molecular masses are indicated in daltons.

initiation codon of *trpR*, AUG, with AUC did not totally prevent the expression of the gene, since the expression of $trpR_{AUC}$ from plasmid pCM65 also resulted in the synthesis of both the 12-kDa and the 10-kDa *trpR* polypeptides (Fig. 2, lanes 5 and 6). However, note that this mutation caused a fivefold reduction of the amount of the 12-kDa polypeptide without affecting the amount of the 10-kDa polypeptide, so that the amounts of the two $trpR_{AUC}$ products were nearly identical. We also noticed that $trpR_{AUC}$ directed the synthesis of a small amount of an additional polypeptide of about 8 kDa, which disappeared during the chase (Fig. 2). We have no explanation for its appearance.

Decreasing *trpR* translation initiation increases the ratio of *trpR* +1-frame to 0-frame products in vitro. The effects of reducing the level of initiation of translation of *trpR* on its translation in two frames were also studied in an *E. coli* in vitro transcription-translation system. The wild-type *trpR* and the $trpR_{AUC}$ genes were under the control of the strong λP_L promoter on plasmids pHEK and $pHEK_{AUC}$ (Table 1). In these experiments, the *trpR* products were separated by gel migration and identified by their immunological specificities as described by us previously (5). We used antibodies prepared against two different domains of the polypeptides: (i) the first 14 amino acids (common to the two polypeptides) and (ii) the last 16 amino acids of the +1 frame. The results are shown in Fig. 3. The sample immunoprecipitated with antibodies against the common N termini of the polypeptides migrated as two bands: a major band of 12 kDa and a minor band of 10 kDa (Fig. 3, lane 1). By densitometric analysis of the autoradiograms, we determined that the ratio of these two *trpR* products was about 1 to 20. We have previously shown that when plasmid $pHEK_{ACC}$ was added to the extracts in place of pHEK, neither of the *trpR* products was synthesized (5), indicating that the two products are synthesized from the same initiation codon. Here we have shown that when plasmid $pHEK_{AUC}$ was added to the extracts in place of pHEK, the amount of the 12-kDa polypeptide was reduced about 20-fold, with no significant change in the amount of the 10-kDa polypeptide. As a result, similar amounts of the 12- and 10-kDa polypeptides were detected (Fig. 3, lane 3). The 10-kDa polypeptide must be a *trpR* +1-frame product, since it immunoprecipitated with the antibodies made against the C terminus of the +1 frame (Fig. 3, lanes 2 and 4).

Conclusion and discussion. The *E. coli trpR* gene encodes the Trp repressor, which is involved in the regulation of tryptophan metabolism (7, 14, 27). Here we studied the effects of modulating the level of initiation of translation of the *trpR* gene of *E. coli* on the rate of frameshifting events. We increased the level of translation initiation by replacing the ribosome binding site of the *trpR* gene with a more efficient ribosome binding site (Fig. 1). We decreased the level of translation initiation by replacing the *trpR* AUG initiation codon with an AUC codon. Varshney and Raj-Bhandary (26) showed that in *E. coli* extracts the AUC codon is a weak initiator. Here we confirmed their observation in the case of *trpR* (Fig. 3) and also found that an AUC codon can be a weak initiator in the in vivo T7 promoter-polymerase system (Fig. 2).

The results of our experiments revealed that, unlike the rates of most of the known examples of frameshifting, the frequency of *trpR* frameshifting is not a constant value but, rather, can be regulated. We found this frequency to be inversely proportional to the rate of translation initiation. Increasing the level of the translation initiation of *trpR-lac'Z* fusions resulted in a decrease in the frequency of +1 frameshifting (Fig. 1). On the other hand, decreasing the level of translation initiation resulted in an increase in the rate of +1 frameshifting, as determined by the ratio of the *trpR* +1-frame and 0-frame products, both in vivo (Fig. 2) and in vitro (Fig. 3). It seems that changing the level of translation initiation has a profound effect on the expression of the *trpR* gene in the 0 frame while only slightly affecting the expression in the +1 frame (Fig. 1 through 3). Thus, the effect on *trpR* frameshifting of modulating translation initiation is manifested by a change in the ratio of the products of the alternative frames of the gene rather than by an increase in the frameshifting process itself.

As mentioned above, in most of the reported examples of frameshifting the level of the process and hence the ratio of the products have constant values (as reviewed in reference 20). The only other known example of regulatable frameshifting is in the expression of the *E. coli prfB* gene which codes for release factor 2. In that case, the UGA stop codon was found to be part of a regulatory circuit for frameshifting (9, 10). The product of *prfB* frameshifting, the RF2 protein, is required for translation termination at the UGA stop codon. Since the alternative to *prfB* frameshifting is translation termination at the UGA codon, which forms the slippery site for frameshifting, high levels of RF2 reduce *prfB* frameshifting (9). In contrast to *prfB* frameshifting, which is regulated by the end product of the regulated gene, *trpR* frameshifting seems to be regulated by the level of translation initiation. The frequency of frameshifting in other systems has not been found to be modulated by translation initiation. This may be, however, because this problem has not been addressed directly in most cases in which frameshifting has been characterized. The problem was considered in the case of T7 gene *10* frameshifting, in which reducing the level of translation initiation did not affect the efficiency of frameshifting. It is important, however, to take into account that the signals for the translation initiation of T7 gene *10* are probably the strongest known in *E. coli* systems (8). The fact that translation initiation of the *trpR* gene is very inefficient may in fact permit regulation of *trpR* frameshifting by the modulation of the rate of translation initiation (13, 18). Alternatively, frameshifting resulting from the slippage of a single nucleotide, as most commonly

described so far, might not be affected by modulating the rate of translation initiation. Instead, it might affect only the special form of frameshifting which occurs in *trpR*. As we have shown recently, in *trpR-lac'Z* fusions, the transition from the 0 frame to the +1 frame is the result of the bypassing of a long, untranslated segment of the mRNA (3). We have suggested that this bypassing event occurs by the looping out of a 55-nucleotide-long mRNA segment and that this looping out may be facilitated by a presumed interaction between the bypassed mRNA segment and a ribosomal protein(s). It may be that such an interaction is possible only when the density of ribosomes on the mRNA template is low, as is presumably the case when the rate of translation initiation is low. On the other hand, when the rate of translation initiation is high, the density of ribosomes on the mRNA template is higher, and this may decrease the probability for the occurrence of the interaction required for the bypassing event.

The possible physiological significance of the described modulation of *trpR* bypassing is currently under our investigation.

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