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# A ribosomal frameshifting error during translation of the *argI* mRNA of *Escherichia coli*

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**Abstract** Using fusions between the *Escherichia coli* genes *argI* and *lacZ*, it has been demonstrated that ribosomal frameshifting occurs at a frequency of between 3% and 16% within the *argI* mRNA, soon after the initiation codon. The frameshift involves a phenylalanyl-tRNA shifting into the + 1 frame at the sequence UUU-U/C. The shift does not occur if the in-frame phenylalanine codon UUU is replaced by UUC. The level of frameshifting is higher in dense cultures and is not dependent on phenylalanine starvation. In the wild-type *argI* gene this frameshifting event would be an error, leading to a truncated, non-functional protein. Therefore, it is unlike the numerous examples of required frameshifting events that have been described in other genes.

**Key words** Codon · Frameshifting · Ribosome · Translation

## Introduction

There are several different types of errors that can occur during translation. Some of these errors cause "premature termination", the production of truncated protein, and have been termed errors in processivity (Jørgensen and Kurland 1990). One type of processivity error is a ribosomal frameshift. Frameshifts in a typical mRNA lead to truncated proteins because after the frameshift the ribosome will encounter previously out-of-frame termination codons.

Unfortunately, for a number of reasons, it has been difficult to quantitate and analyze actual frameshift errors in vivo. First, of course, the truncated protein must be detected. A further problem is to determine exactly where the error occurred, since frameshifts at one of several different contiguous codons will yield a very similar product. A further problem will be to determine whether the truncated protein resulted from a frameshift or some other processivity error, such as ribosomal drop-off. To circumvent these problems, many studies have exploited suppression of frameshift mutations, and the error rate in translocation seems to vary widely (for review see Parker 1989, 1992). However, at most codons and contexts the average frequency of a translational frameshift error must be below $10^{-4}$ in order to make full-length protein from long mRNAs.

In spite of these difficulties, considerable progress has recently been made in understanding ribosomal frameshifting. However, for the most part this has come not from analyzing errors, but rather from studying transcripts from specific genes that require ribosomal frameshifts in order for a functional protein to be produced. These frameshifts, termed programmed frameshifts to distinguish them from frameshifting errors, range in efficiency from a few percent to over 50% (for review see Atkins et al. 1990; Jacks et al. 1988; Parker 1989, 1992). Programmed frameshifting seems to depend on two components within the mRNA: a site of action and a stimulatory signal (Gesteland et al. 1992). The site of action is known as the "shifty site" and shifting itself involves certain "slippery tRNAs". The stimulatory signal differs considerably among different types of programmed frameshifts, but it seems likely that in all cases the signal involves a ribosomal pause site.

In *Escherichia coli* a ribosomal frameshift is required to synthesize release factor 2 from the chromosomal

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gene *prfB* (Craigen et al. 1985; Craigen and Caskey 1986), and to synthesize the $\gamma$ subunit of DNA polymerase III, one of two products of the *dnaX* gene (Blinkowa and Walker 1990; Tsuchihashi and Kornberg 1990; Flower and McHenry 1990). In the case of the *prfB* mRNA, the stimulatory signal involves an in-frame termination codon, which is preceded by a Shine/Dalgarno-like sequence, both of which are important (Weiss et al. 1987; Curran and Yarus 1988; Weiss et al. 1988). The shifty site itself involves only CUU-U, which a $tRNA^{Leu}$ reads in the 0 frame and then shifts to the + 1 frame to read UUU. Frameshifting in the transcript of the *dnaX* gene involves the heptanucleotide sequence A-AAA-AAG, which is followed by a stem-loop. This frameshift involves simultaneous slippage of two tRNAs on the ribosome from the 0 to the − 1 frame at this site (Tsuchihashi and Brown 1992). The efficiency of the shift depends on the fact that the sole $tRNA^{Lys}$ species in *E. coli* has a stronger affinity for AAA than AAG (Tsuchihashi and Brown 1992). This mechanism is very like that programming the − 1 frameshifting observed in retroviruses (Chamorro et al. 1992; for reviews see Atkins et al. 1990; Parker 1992). Secondary structure in the mRNA is the stimulatory signal for this type of frameshifting (Brierley et al. 1989) and may well be involved in ribosomal pausing (Tu et al. 1992).

A ribosomal frameshift also occurs in the *trpR* mRNA of *E. coli* at a frequency of about 5% (Benhar et al. 1992). This frameshift apparently occurs at codon 65 of the *trpR* mRNA, and at least in *trpR-lacZ* fusions involves translational bypassing of a 55 nucleotide segment of the mRNA (Benhar and Engelberg-Kulka 1993). The nature and function of the frameshifted *trpR* product is not known. If the product is without function, or does not serve a regulatory role, the frameshifting event would be considered an error.

Like programmed frameshifts that lead to required products, some frameshift errors may also happen at a high frequency and may also involve stimulatory signals, shifty sites, and slippery tRNAs. Some studies on the translational suppression of frameshift mutations have shown that frameshifting may happen at stop codons (Aulin and Hughes 1990), which must then be pause sites rather than efficient termination sites (Tate and Brown 1991), and can also be detected when ribosomes pause at certain codons made "hungry" by amino acid starvation (Kurland and Gallant 1986; Weiss and Gallant 1983, 1986). The direction of a frameshift ( + 1 or − 1) stimulated by amino acid starvation depends on the sequence of the mRNA surrounding the hungry codon (Lindsley and Gallant 1993).

Translation missense errors can also be induced by amino acid starvation. One such classic error is the substitution of leucine for phenylalanine. We have been able to detect and quantitate this error at both UUU and UUC during phenylalanine starvation (Parker and Precup 1986; Precup et al. 1989). However, in the *argI* mRNA of *E. coil* this missense error shows strong context dependence, being unmeasurably low at one position and occurring at a frequency of 0.6 at another, whichever phenylalanine codon was at these positions (Precup et al. 1989).

There is evidence that programmed frameshifting can occur at phenylalanine codons in *E. coli*. For example in the case of gene 10 of the coliphage T7 there is a high-level − 1 frameshift at the sequence G-GUU-UUC (Condron et al. 1991a, b). Because amino acid starvation induces ribosomal frameshifting as well as missense errors, it seemed possible that the context effect we saw with missense errors was the indirect result of a starvation-induced, context-specific frameshift error. Frequent frameshifting at a phenylalanine codon at a particular position in the *argI* mRNA would lower the probability of detecting a missense error at the same position.

Frameshifting early in the wild-type *argI* mRNA would result in an undetectable product because the shifted ribosome would very soon terminate at a previously out-of-frame stop codon. Therefore, we constructed a series of fusions between *argI* and *lacZ* in order to assay frameshifting by analysing the resulting fusion protein. Here we report that ribosomal frameshifting occurs with a high frequency at a phenylalanine codon early in the *argI* mRNA. However, this frameshifting is not dependent on phenylalanine starvation and occurs at a UUU but not a UUC codon. Since in the wild-type *argI* gene this frameshift would lead to the production of a short peptide, it seems clear that this high-level frameshift is an error.

## Materials and methods

### Strains, plasmids, and growth conditions

The host strain of *E. coli* K-12 used for growth and labeling experiments was JK601 (*asnA*, *asnB*, *lacI*$^q$, *lacZ*::Tn *9*, *pheA*::Tn *10*, *relA*, *thi*), which is derived from strain JK1, other derivatives of which have been used extensively in studies on translational fidelity (e.g. see, Parker et al. 1980). The plasmid pSKS107 (Casadaban et al. 1983), which contains a *lacZ* gene minus translation and transcriptional start sequences, and pKM-*tac*I (de Boer et al. 1983), which contains the *tac* promoter, were used in constructing plasmids for the frameshift assay.

Plasmid preparations were made from cultures grown in L broth (Lennox 1955) supplemented with 0.2% (w/v) glucose. The minimal medium used for growth experiments was M9 (Miller 1972) supplemented with 0.4% glucose, 10 mg/l thiamine, 50 mg/l L-asparagine, and 50 mg/l L-phenylalanine. Strains containing plasmids were grown in media containing 25 mg/l ampicillin. Bacteria were grown at 37° C and growth was monitored spectrophotometrically.

For experiments involving induction of the *tac* promoter, cells were grown in minimal medium and isopropyl-$\beta$-D-thiogalactopyranoside (IPTG) was added to 1 mM. When necessary cells growing in minimal medium were labeled by the addition of

[$^{35}$S]L-methionine to 10 $\mu$Ci/ml (ca. 1 Ci/mmol) and extracts prepared for electrophoresis as described (Parker et al. 1980).

### DNA manipulations and sequencing

Plasmids and DNA fragments were isolated and manipulated as described in Sambrook et al. (1989) or by the suppliers of the enzymes used. Restriction enzymes were purchased from New England Biolabs (Beverly, Mass.) and Promega (Madison, Wis). T4 DNA ligase was purchased from Gibco BRL (Gaithersburg, Md.). Oligonucleotides were synthesized using the Applied Biosystems Model 381A automated synthesizer. DNA sequencing using the dideoxy chain terminating method was performed using the Sequenase 2.0 kit from United States Biochemical (Cleveland, Ohio).

Construction of plasmids for frameshift assays was done by ligating duplexed oligomers, synthesized so as to have an *Eco*RI- and a *Bam*HI-compatible extension, between the *Eco*RI and *Bam*HI sites of pSKS107. The *tac* promoter from pKM-*tac* I was then ligated into the *Eco*RI site.

### Enzyme assay

The activity of $\beta$-galactosidase was assayed by the method of Miller (1972).

### Purification and amino acid sequencing of protein

Radioactive proteins were separated using two-dimensional polyacrylamide gel electrophoresis (O'Farrell 1975). Nonradioactive fusion protein for sequencing was isolated from 200 ml of an induced culture growing in minimal medium. The cells were pelleted, resuspended in cold buffer (100 mM TRIS-HCl, pH 7.4, 10 mM EDTA, 1 mM phenylmethylsulfonyl fluoride), and broken by sonication. Cell debris was removed by centrifugation and the soluble extract was diluted with 50 mM TRIS-HCl, pH 7.3, to approximately 4 mg/ml total protein. The extract was then loaded onto a ProtoSorb *lacZ* adsorbent immunoaffinity column (Promega) and fusion protein eluted as recommended by the manufacturer. The eluted protein was dialyzed extensively against distilled water, lyophilized, and then subjected to automated Edman degradation using an Applied Biosystems Model 477 protein sequencer at the Biotechnology Center of the University of Illinois. Each cycle was analyzed by an on-line amino acid analyzer.

## Results

Figure 1A shows the sequence of the mRNA at the beginning of *argI*, a gene encoding one of the ornithine transcarbamylase isozymes of *E. coli*. In our previous studies we have found that leucine misincorporation occurs at a high frequency at position 8 of ornithine transcarbamylase during phenylalanine starvation, but not at position 3 (Precup et al. 1989). Therefore, a number of different fusions of this region to *lacZ* were made in order to determine whether ribosomal frameshifting occurred at or near the codon at position 3. Ribosomal frameshifting can be detected in a particular region by fusing a frameshift window in different frames to a reporter gene (Weiss et al. 1987, 1990). To detect frameshifting the window is constructed so that the ribosome enters it in a frame different than that of the reporter gene. The 5′ end of the window constructed for this study was the initiation codon of *argI*. The 3′ end of the window is a termination codon in the same frame as that of the entering ribosome. Therefore, the ribosome must shift at some place between the initiation codon and this in-frame termination codon in order for active $\beta$-galactosidase to be produced.

Four different plasmids were constructed, each containing a similar frameshift window and each containing a fusion of the early region of *argI* to *lacZ*. The differences between the plasmids lie in the nature of the junction sites of the fusions. The relevant portions of the mRNA from these constructs are shown in Fig. 1B. In pCFP1 the *argI* and *lacZ* segments were fused in-frame with no intervening stop codon. This plasmid would serve as a positive control, since active $\beta$-galactosidase should be synthesized in the absence of frameshifting. The construct in pCFP2 also has *argI* and *lacZ* in-frame but, in addition, has an intervening UAA. This construct could be used to detect both internal initiation in *lacZ* and leakiness of the UAA codon. The other two constructs have *argI* fused in the $-1$ frame (pCFP3) and the $+1$ frame (pCFP4) with respect to *lacZ*. In order to make fusion protein from the construct carried by pCFP3 the ribosome must frameshift into the $-1$ frame (which is most simply accomplished by shifting one base toward the 5′ end of the mRNA) somewhere between the initiation codon and the UAA codon. In pCFP4 the ribosome would have to shift into the $+1$ frame (a shift of one base in the 3′ direction) in the same region in order to make fusion protein.

A ribosomal frameshift from the normal (0) frame to either the $+1$ or $-1$ frame in the mRNA from a typical gene will usually result in synthesis of a truncated protein because of termination at a previously out-of-frame stop codon. In the wild-type *argI* mRNA there is a UAA in the $-1$ frame and a UGA in the $+1$ frame within the first ten codons (Fig. 1A). These out-of-frame stop codons were removed in the constructs for plasmids pCFP1–4. To accomplish this the histidine codon at position 5 was changed from CAU to CAC, and the leucine codon CUG at position 9 was changed to the isoleucine codon AUC. Although the latter change also involved an amino acid substitution, it avoided substituting an infrequently used leucine codon for the commonly used leucine codon CUG (Andersson and Kurland 1990).

After construction, and confirmation of DNA sequence, the plasmids were transformed into JK601. The level of $\beta$-galactosidase was measured in normally growing strains (no amino acid starvation) containing each of these four plasmids after induction with IPTG. The results of such experiments are given in Table 1. As expected pCFP1-containing cells produced $\beta$-galactosidase and those containing pCFP2 and 3 produced very low amounts. Most unexpectedly, cells containing

**A**

```
                1   2   3   4   5   6   7   8   9   10
              Ser Gly Phe Tyr His Lys His Phe Leu Lys ...
GAUCU AUG UCC GGG UUU UAU CAU AAG CAU UUC CUG AAA ...
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**B**

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                                                    Ile
AAUUC AUG UCC GGG UUU UAU CAC AAG CAU UUC AUC AAA (AGC-lacZ          pCFP1
AAUUC AUG UCC GGG UUU UAU CAC AAG CAU UUC AUC AAA UAA (AGC-lacZ     pCFP2
AAUUC AUG UCC GGG UUU UAU CAC AAG CAU UUC AUC AAA UA(AGC-lacZ       pCFP3
AAUUC AUG UCC GGG UUU UAU CAC AAG CAU UUC AUC AAA UAA A (AGC-lacZ pCFP4
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**Fig. 1A, B** Messenger RNAs from *argI* and the *argI-lacZ* fusions. **A** A portion of the mRNA from the wild-type *argI* gene, including the region that encodes the first few amino acid residues of the protein. The bases in *italics* are out-of-frame termination codons. The amino acid residues and their numbered positions in the protein are shown above the mRNA. The initiating methionine is efficiently removed even when the protein is overproduced (Parker and Precup 1986) **B** The corresponding regions from the transcripts that are expected from the plasmid constructs pCFP1–4. The *underlined* bases are those that differ from the wild-type transcript. The in-frame termination codon in pCFP2–4 is shown in *italics*. The sequences before the *parentheses* also correspond to one strand of the oligodeoxynucleotides used to construct these plasmids. The other strand was offset to give the overlaps necessary for an *Eco*RI compatible end at the 5′ end and a *Bam*HI compatible end at the 3′ end. The AGC following the parentheses is an in-frame codon from the *lacZ* gene found on the vector pSKS107

**Table 1** β-Galactosidase activity in strains with frameshift plasmids

| Plasmid[a] | | Enzyme activity[b] | |
|---|---|---|---|
| | | low[c] | high[d] |
| pCFP1 | (0 frame) | 5140 | 5030 |
| pCFP2 | (0 frame plus stop) | 15 | 32 |
| pCFP3 | ( − 1 frame) | 15 | 49 |
| pCFP4 | ( + 1 frame) | 144 | 828 |

[a] All plasmids were in strain JK601 (*relA, lacI*$^q$, *lacZ*::Tn *9*)
[b] Enzyme activity is reported in Miller Units
[c] Data are averaged from three experiments in which the activity measurements were made using cultures with a reltively low cell density ( $\leqslant 3 \times 10^8$)
[d] Data are averaged from two experiments in which the activity measurements were made using cultures with a relatively high cell density ( $\geqslant 5 \times 10^8$)

pCFP4 produced easily measured amounts of β-galactosidase. Since this was not seen with pCFP2 or pCFP3, internal re-initiation could be ruled out. Therefore, it seemed that in pCFP4 a + 1 ribosomal frameshift occurred somewhere in the frameshift window at a high frequency in unstarved cells.

Some of the data in Table 1 are from experiments in which the cells were induced and harvested below a density of $3 \times 10^8$ cells/ml, when the culture was in mid-logarithmic phase. In these experiments the amount of β-galactosidase activity produced in cells containing pCFP4 was approximately 3% of that in cells with pCFP1. The remainder of the data in this table are from experiments in which the cells were assayed at higher cell density, when the culture was in late logarithmic phase. Here the relative level of activity from pCFP4 is higher. Figure 2 shows autoradiograms of two-dimensional gels containing proteins from cells that were labeled at a cell density of approximately $10^9$ ml. At the time of labeling the strain with pCFP1 had 3500 units of activity while the strain with pCFP4 had 670. In Fig. 2A the fusion protein produced from pCFP1 is marked by an arrow head. In Fig. 2D there is a protein of the same size, but slightly more acidic, that is produced in cells containing pCFP4.

A fusion protein that resulted from a frameshift at position 3 in pCFP4 would be more acidic than the normal fusion protein because the frameshifted product would have three fewer basic amino acid residues than the 0-frame produce (see Fig. 3). However, in order to determine precisely where the frameshift occurred it was necessary to sequence the protein produced by pCFP4. Therefore, the fusion protein was isolated from a strain carrying pCFP4 and analyzed by automated Edman degradation. The residues determined by this analysis are shown in Fig. 3. Note that although this sequence is consistent with a + 1 frameshift at the sequence UUU-U, it is also consistent with a + 1 frameshift at the sequence GGG-U, or a simultaneous frameshift. Such a simultaneous shift by tRNAs occupying both the P-site and the A-site of the ribosome would be similar to the mechanism of frameshifting at the heptanucleotide sequences found in retroviruses, but in the opposite direction. In order to establish more precisely the mechanism of the frameshift in *argI*, constructs were made that were similar to pCFP4, but with changes in the bases in and around the frameshift site. These constructs, and the results we obtained by assaying β-galactosidase levels in strains containing them, are shown in Fig. 4.

The results are perfectly consistent with a + 1 slippage of a Phe-tRNA$^{Phe}$ reading the codon UUU to either an overlapping UUU codon (pCFP4 and 9) or an overlapping UUC codon (pCFP8). Changes that would prevent normal pairing by this tRNA in either the

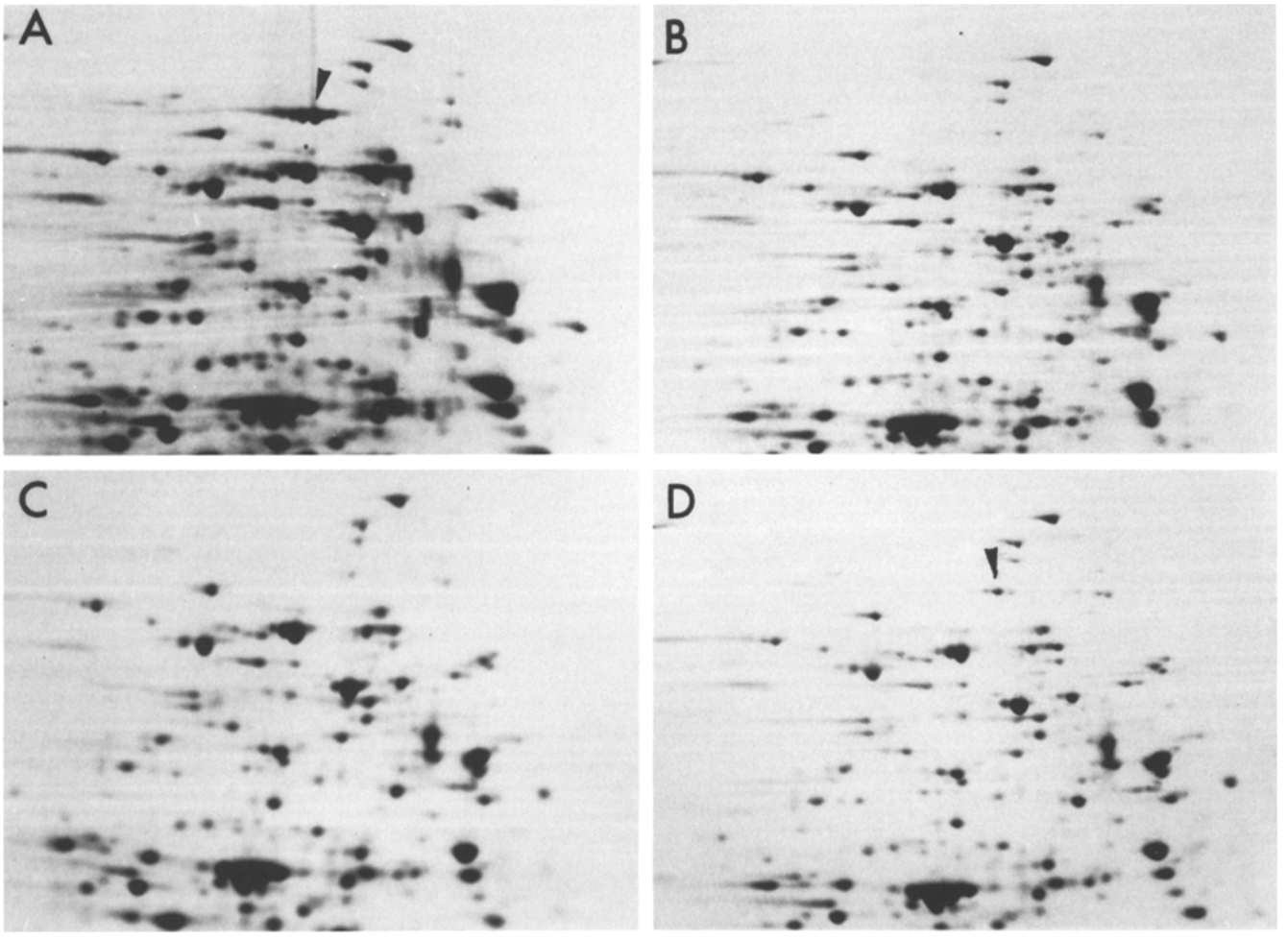


**Fig. 2A–D** Autoradiograms of two-dimensional gels containing proteins from strains with the fusion plasmids. Strains of JK601 each containing a different plasmid were grown in minimal medium and isopropyl-β-D-thiogalactopyranoside (IPTG) was added to 1 mM when the cell density was approximately $4 \times 10^8$ cells/ml. After 120 min the cells were labeled with [$^{35}$S]L-methionine for 5 min and harvested. Extracts were prepared and proteins separated by two-dimensional gel electrophoresis as described in Materials and methods. The acidic proteins are toward the right, and the high $M_r$ proteins at the top of each panel. Proteins from cells with: **A** pCFP1; **B** pCFP2; **C** pCFP3, and **D** pCFP4. The *arrow heads* identify the fusion proteins

0 frame or the + 1 frame abolish frameshifting (pCFP5, 6 and 7). A change in the upstream GGG codon that should similarly effect slippage by a Gly-tRNA has no effect (pCFP9).

## Discussion

Unexpectedly we have found that in normally growing cells ribosomes can frameshift at a high frequency at a sequence nearly identical to that found at the beginning of the *argI* mRNA. The + 1 shift we observed seems to involve the slippage of Phe-tRNA$^{Phe}$ from one UUU codon to another at the sequence UUU-U. The shift also occurs at the sequence UUU-C, but does not occur at a measurable frequency if UUC is used as the in-frame phenylalanine codon, nor if the sequence is changed to UUU-A (see Fig. 4). The data indicate that base-pairing between the codon and the anticodon must be maintained at each position after the shift. Further, changing the GGG codon upstream from the shifty site to a GGC had no effect. This would indicate that the mechanism of frameshifting does not involve simultaneous slippage of two tRNAs as has been observed for the − 1 frameshift that occurs in retroviral mRNA (for reviews see Atkins et al. 1990; Parker 1992) and also occurs in the *dnaX* gene of *E. coli* (Tsuchihashi and Brown 1992) and gene 10 of coliphage T7 (Condron et al. 1991b).

Note that this shift occurs at a high frequency in the absence of phenylalanine starvation. In fact the frameshifted fusion protein was not detected in phenylalanine-starved cells (Fu, unpublished results). This may seem obvious since both the in-frame and + 1 codons are phenylalanine codons, but Gallant and Lindsley (1992) have demonstrated that lysine deprivation increased frameshifting from one lysine codon to another. The fact that an in-frame UUC is not shifty (see

**Message of *argI-lacZ* fusion from pCFP4**

AUG UCC GGG UUU UAU CAC AAG CAU UUC AUC AAA UAA A AGC ...

**Predicted sequence of products from pCFP4**

(Met) Ser Gly Phe Tyr His Lys His Phe Ile Lys (Stop)

(Met) Ser Gly Phe Ile Thr Ser Ile Ser Ser Asn Lys Ser ...

**Actual sequence of aminoterminal region of purified product of pCFP4**

Ser Gly Phe Ile Thr Ser Ile Ser ? Asn Lys Ser

**Fig. 3** Amino acid sequence of the fusion protein made from pCFP4. The *upper line* shows the sequence of the mRNA that would result from the fused gene carried by pCFP4 beginning at the initiation codon. The UUU in the + 1 frame is *underlined*. Shown below this are two possible translational products. The upper sequence is the peptide that would be obtained if the ribosome did not shift reading frames after initiation. The next sequence is that that would be obtained if the ribosome made a + 1 shift at the UUU codon. The final sequence shown is that obtained from sequencing purified fusion protein from pCFP4 as described in Materials and methods. The *question mark* at residue 9 indicates that the identity of this residue was not established in either of two separate experiments

Fig. 4) would seem to indicate that this frameshift is not the reason we fail to detect missense errors at position 3 of *argI* even when the codon at this position is UUC (Precup et al. 1989). However, the codon UUC was not checked in any other construct. Therefore, it is conceivable that when this codon is found in the mRNA there is a − 1 frameshift, or some other unexpected translational event, such as ribosome drop-off.

The level of "spontaneous" frameshifting we observed (from 2% to well over 10%) seems extraordinarily high considering that there is no evidence that this is anything other than an error. If this frameshift occurs in normal *argI* mRNA, termination will take place only a few codons downstream. There are no arginine codons in either reading frame that could conceivably be used to control the frequency of frameshifting or stimulate the ribosome to shift back into the correct frame. Therefore, there is no reason to believe the shift has regulatory significance. There is evidence in the literature that other frameshift errors may happen at high frequency. A collection of mutants containing *lacZ* frameshift mutations were found to be phenotypically leaky at frequencies ranging up to only 0.06% of wild type (Atkins et al. 1972), but mutations with potentially higher levels of leakiness (Newton 1970) were not examined. In addition many synthetic frameshift windows also show considerable shiftiness (Weiss et al. 1987). However, the data presented here with *argI* are the first that show that sequences within a normal reading frame can lead to a high level, and apparently erroneous, shift out of that frame. Since this kind of processivity error could have very serious consequences for the cell, it will be important to determine the nature of such sites and what controls the frequency of frameshifting at them.

Although it is clear what the shifty site is in the case of these *argI-lacZ* constructs, it is not at all clear what represents the stimulatory site. This frameshift does not involve ribosomal pausing at a stop codon or at a hungry codon. The UAU codon following the shifty site is read by a reasonably abundant tRNA in *E. coli* (Ikemura 1981) and, therefore, pausing at a rarely used codon, which can lead to frameshifting (Belcourt and Farabaugh 1990; Xu and Boeke 1990), also does not seem to be involved. There does seem to be an increase in the frameshifting when cells are grown to high density (see Table 1). Some types of translational errors, including frameshifting, are known to be sensitive to the metabolic state of the cell (Bogosian et al. 1990).

**Fig. 4** Frameshift assays using plasmids with mutations at or near the frameshift site. Plasmids pCFP5–9 were constructed as described for the construction of pCFP4 except that the oligodeoxynucleotides were synthesized to contain single base-pair mutations. The figure shows the sequence of pCFP4 and the relevant sequence of each of the mutant plasmids; the single base differences are *underlined*. The amino acid sequence that would result from a + 1 shift in any of the plasmids, if the shift occurred at the same site, it shown above the mRNA sequence. To the right of each sequence are shown the average levels of β-galactosidase activity obtained from two experiments.

| Plasmids | Sequences | LacZ Activity |
|---|---|---|
| | Ser Gly Phe Ile Thr Ser ... | |
| pCFP4 | AUG UCC GGG UUUU AUC ACA AGC ... | 362 |
| pCFP5 | UUCU | 49 |
| pCFP6 | UCUU | 50 |
| pCFP7 | UUUA | 42 |
| pCFP8 | UUUC | 323 |
| pCFP9 | GGC | 250 |

However, the level of frameshifting we observe is quite high under all conditions we have examined. This would seem to indicate that the metabolic state of the cell is not the primary stimulatory mechanism for this frameshift, although the shift is sensitive to some culture conditions.

Frameshifting sites, and sites for other types of reading alternatives (for review see Atkins et al. 1992), often involve secondary structure in mRNA. Using computer analysis, we have examined both the wild-type *argI* mRNA and the mRNA from the *argI-lacZ* fusion for possible secondary structure (note that every little of the *argI* mRNA is contained in the *argI-lacZ* fusions and even this has several point mutations). In neither case is there a possible stem-loop structure immediately following the slippery site, although in both cases extensive secondary structure is possible in this general region of the mRNA (results not shown). The stimulatory site for frameshifting at the overlapping phenylalanine codons in gene 10 of T7 may involve complex structure in the mRNA rather than a simple secondary structure (Condron et al. 1991b) Further analysis will be required before it is possible to understand the mechanism that stimulates the high-level, but apparently erroneous frameshift that occurs in the *argI* mRNA.

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