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The nucleotide sequence of the first externally suppressible −1 frameshift mutant, and of some nearby leaky frameshift mutants

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Nine mutants within a 23 nucleotide sequence of the *trpE* gene of *Salmonella typhimurium* have been characterized. *trpE91*, a mutant which is externally suppressible has a single base deletion. Eight (or nine) nucleotides upstream of this deletion, two independently isolated mutations have the same transversion. In combination with *trpE91* these mutations lead to partial restoration of synthesis of anthranilate synthetase in the absence of external suppressors. In the transversion the sequence A CA is changed to A AA and this new sequence may be the site where frameshifting occurs to allow leakiness. Leakiness is displayed by two further mutants of the same sign as *trpE91*, and one of the opposite sign, in the absence of any base substitution or external suppressors. Specific sequences, e.g., UUUC, may be especially prone to frameshifting and this sequence is created at the site of the +1 frameshift mutant which displays leakiness. In the new reading frame generated by the two −1 frame leaky mutants, a tryptophan codon is encountered. Leakiness is necessarily detected in the absence of tryptophan and under these conditions there will be a shortage of charged tryptophan tRNA. The possibility of such functional imbalance leading to frameshifting in these mutants is discussed.

Key words: context/fidelity/frameshift/ribosome/suppressor

Introduction

Frameshift mutations are known to be leaky, though in many instances this is only at a very low level. This leakiness is not a consequence of translation reinitiation close to the frameshift site, but is due to some ribosomes shifting reading frame (Atkins *et al.*, 1972). It has been presumed for a long time that this frameshifting occurred only or preferentially at certain sequences, but the identity of the sequences remained unknown. Recently, Fox and Weiss-Brummer (1980) sequenced several +1 and −1 leaky frameshift mutants in the yeast mitochondrial cytochrome oxidase gene. All were located within one TTTTC sequence. It was possible that the ribosomal frameshifting site was located close by and the occurrence of the mutations within this region reflected the mechanism of frameshift mutant origin, but more likely that the 'shifty' sequence was TTTTC itself. Here we show that a similar sequence may be responsible for the leakiness of a bacterial +1 frameshift mutant, and also that a run of As may be important for the leakiness of a −1 frameshift mutant. Interest in this phenomenon is provided by the accumulating evidence that certain viruses produce additional proteins as a result of frameshifting, and more intriguingly that frameshifting may be regulatory (Kastelein *et al.* 1982).

The above examples of frameshifting occurred with a wild-type (i.e., non-mutant) translation apparatus. However, mutants both of yeast and bacteria have been isolated which alter the decoding mechanism so that specific classes of frameshift mutants are suppressed (see Culbertson *et al.*, 1982; Bossi and Roth, 1981; Atkins, 1980). The best characterized suppressors were all shown to suppress +1 frameshift mutants. A sole exception giving suppression of a −1 frameshift mutant, or its functional equivalent, has been isolated. The suppressible −1 frameshift mutation is in the anthranilate synthetase gene of *Salmonella typhimurium* which is now designated *trpE* (formerly *trpA*). This mutant, *trpE91*, was isolated in the mid-1950s in Demerec's group at Cold Spring Harbor, and was first classified as a frameshift mutant by Bauerle and Margolin (1966). Sign-specific external suppressors for both *trpE91* and a nearby compensatory frameshift mutant of opposite sign, *trpE872*, were reported (Riyasaty and Atkins, 1968; Atkins and Ryce, 1974). However, at that time, only weak mutagenesis data indicated that it was *trpE91* rather than *trpE872* which was the −1 frameshift mutant. Here this inference is confirmed, and the identity of the mutant site established by DNA sequencing. This sequencing was facilitated by the previous determination of the complete *trpE* sequence from both *Escherichia coli* (Nichols *et al.*, 1981) and from *S. typhimurium* (Yanofsky and van Cleemput, 1982). The only *trp* frameshift mutants previously sequenced were some *trpA* frameshift mutants where the amino acid sequence in double frameshift mutants was determined (Brammer *et al.*, 1967).

When a *trpE91*-containing mutant strain is plated on minimal media lacking tryptophan, several types of spontaneous revertants to tryptophan independence can be selected (Riyasaty and Atkins, 1968). (i) A small proportion have growth rates indistinguishable from wild-type and are likely to be revertant at the frameshift mutant site restoring the wild-type sequence or its functional equivalent. (ii) Another small proportion, termed 'semi-fast', have very slightly retarded growth rates and several of these have been shown to have a nearby compensatory mutation. These correspond to the 'pseudo wild' category of Crick *et al.* (1961) in their classic paper on the nature of the genetic code. The compensatory mutants *trpE871* and *trp872* in two different semi-fast revertants were separated from *trpE91* in 1968, and the isolation of others is described below. (iii) The majority of *trpE91* revertants have a slower growth rate, and in *Salmonella* three different types of secondary alteration (suppressor) give this phenotype. (iiia) Two out of 384 of these suppressors examined have the compensatory mutation in the *trpE* gene close to the site of the *trpE91* mutation. One of these, termed, m_2, was characterized genetically and described in the 1968 paper, and the second, m_3, is described below. '*m*' stands for minute as these are amongst the very slowest growing revertants of *trpE91*. On genetic criteria it was deduced the m_2 was a base substitution which in the absence of *trpE91* had a phenotype similar to wild-type (the frameshift mutagen ICR 191, with a frequency characteristic of a single event, caused fast growing revertants of *trpE91* m_2 – presumably by reverting the *E91* mutation). The nucleo-

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tide sequencing studies have shown that m_2 is a base substitution. All the rest of the slower growing category of revertants of *trpE91* examined have the secondary compensatory mutation outside of the tryptophan operon and so are designated external suppressors. (iiib) Approximately 3% of the external suppresors of *trpE91*, mostly the weaker external suppressors, also suppressed UGA (Riyasaty and Atkins, 1968). All the suppressors of this type were shown to be alleles of the *supK* gene (Atkins and Ryce, 1974), a recessive UGA suppressor (Reeves and Roth, 1971, 1975). (iiic) 97% of the external suppressors of *trpE91* were frameshift specific and were the first frameshift external suppressors reported. The sequence of *trpE91* reported here sharply delimits the possible site of action of these suppressors. Previous work (Riyasaty and Atkins, 1968; and unpublished) have shown that none of ~30 other frameshift mutants tested were suppressed by this class of suppressors. These suppressors have been mapped and in *Salmonella* are alleles of a single gene (D. Hughes *et al.*, in preparation), termed *sufS* (see Atkins, 1980, for suppressor designations).

Results

Mutant isolation

The isolation of the two frameshift mutants *trpE871* and *trp872* was described previously (Riyasaty and Atkins, 1968). When either was put in combination with *trpE91* which is a frameshift mutant of opposite sign, the synthesis of anthranilate synthetase was restored to allow near wild-type growth rate on unsupplemented media. These combinations were termed 'semi-fast'. To isolate more mutants of the same sign as *trpE871* and *trpE872* five further spontaneous 'semi-fast' (SF) revertants of *trpE91* were isolated on minimal media. These were designated SF5-SF9. Separation of the compensatory mutants was performed by preparing donor transducing phage on SF5 to SF9, and having a deletion of the *trpD-A* genes in the transduction recipient strain (Figure 1). Transductants which acquired the wild-type *trp* operon, or both compensatory mutants were readily distinguishable from those which acquired either mutant alone by growth rate on minimal media supplemented with a trace amount of anthranilic acid. As we (Riyasaty and Atkins, 1968) and others (Guest and Yanofsky, 1966) have noted, a remarkably high frequency of double cross-over events can be detected when the segregation of very closely linked mutants is studied. Thus either compensatory mutant can be recovered from one transduction. The identity of the recovered *trpE91* mutants can be then established by use of the external suppressor for *trpE91*, *sufS609* (Table I). No new mutants were isolated from SF5, SF8 and SF9 but two new mutants designated *trpE874* and *trpE876*, respectively, were isolated from SF6 and SF7.

To recover different frameshift mutants of the same sign as *trpE91*, spontaneous semi-fast growing revertants of *trpE872* were isolated and designated SF11, SF13 and SF16. Donor phage was prepared on these revertants and transductions similar to those described above were performed again with the strain containing a deletion of the *trpD-A* region as recipient. The results are also presented in Table I. The identity of the recovered *trpE872* mutants can then be established by use of the external suppressor for *trpE872, sufT621*. New mutants were isolated from SF11 and 16 and designated *trpE873* and *trpE879*, respectively. The origin of all the frameshift mutants is summarized in Table I. Some of the mutants are leaky on minimal medium and this property is also indicated in Table I. *trpE91* itself is not detectably leaky on minimal medium but leakiness is revealed when *trp*E91 is present on a multicopy plasmid, or in the presence of excess levels of pooled amino acids omitting tryptophan.

trpE874 and *trpE876* cannot be genetically separated. Sequencing data (see below) show that these mutants occur within a single codon. Previously we were unable to obtain recombinants in crosses between *trpE871* and *trpE872*

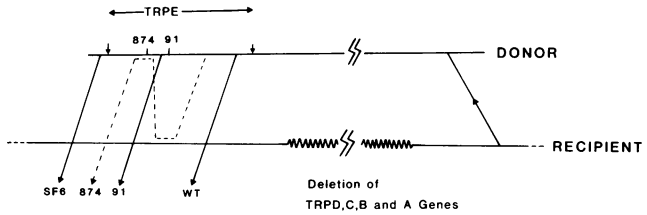

Fig. 1. Transductional separation of the *trpE91* and *trpE874* mutations in the semi-fast revertant (SF 6). Selection for $trpD^+$ demands that one cross-over be downstream of the deletion. The second cross-over can be either upstream of the *874* site in which case the SF6 donor is recovered, between the *874* and *91* sites in which case *91* itself is recovered, or downstream of *91* yielding wild-type (WT) recombinants. The quadruplet cross-overs indicated by the dotted lines allow the isolation of *trpE874*.

Table I. Transductional separation of the constituent mutations in semi-fast (SF) revertants

SF revertant of	SF designation (transduction donor)	Number of transductants	Number of $trpE^-$ recovered	Number not corresponding to starting mutant	Designation of new mutants	Leakiness on minimal medium
trpE91					*trpE871*	+
trpE91					*trpE872*	–
trpE91	SF 5	~20 000	2	0		
trpE91	SF 6	~20 000	8	3	*trpE874*	–
trpE91	SF 7	~20 000	3	1	*trpE876*	–
trpE91	SF 8	~20 000	1	0		
trpE91	SF 9	~20 000	1	0		
trpE872	SF 11	~23 330	8	4	*trpE873*	+
trpE872	SF 13	~33 200	0	–		
trpE872	SF 16	~14 000	6	1	*trpE879*	+

(Riyasaty and Atkins, 1968) and nucleotide sequencing studies (see below) have shown that these mutants are, at most, two nucleotides apart.

In our earlier study, we found one among 262 slow growing reversions of *trpE91* to have the secondary mutation close to *trpE91* and that this secondary mutation designated m_2 was likely to be a base substitution. We have now analysed 122 further slow growing revertants and found another mutant of this category, and it is designated m_3. The nucleotide sequence of both m_2 and m_3 is presented below.

Construction of recombinant plasmids

pKB5 was used as a source of wild-type *S. typhimurium* DNA, pBN61 in a derivative of pKB5 that was constructed by cleaving a mixture of pKB5 and pBR322 with *Eco*RI-*Sal*I and ligating the mixture of fragments. pBN61 was selected by transforming the ligated DNA into *E. coli* W3110 d(*trpLD102*) and selecting ampicillin-resistant tryptophan independent colonies. pBN61 contains a 5200-bp *S. typhimurium* $trpE^+D^+C^+$ fragment and 3710-bp pBR322 fragment.

pBN64 contains the *S. typhimurium trpE91* frameshift mutation. pBN64 was constructed by mixing *Eco*RI-*Sal*I-digested pBR322 and *Eco*RI-*Sal*I digested F′*trpE91* DNA and ligating the mixture of fragments. The ligation mixture was transformed into *E. coli* W3110 d(*trpLD102*) and $trpD^+$ colonies were selected by growth on anthranilate and ampicillin. The presence of the frameshift mutation in the recombinant plasmid was confirmed by the poor growth of colonies in the absence of anthranilate. pBN64 contains a 6100-bp *S. typhimurium* $trpE^-D^+C^+$ DNA fragment in pBR322.

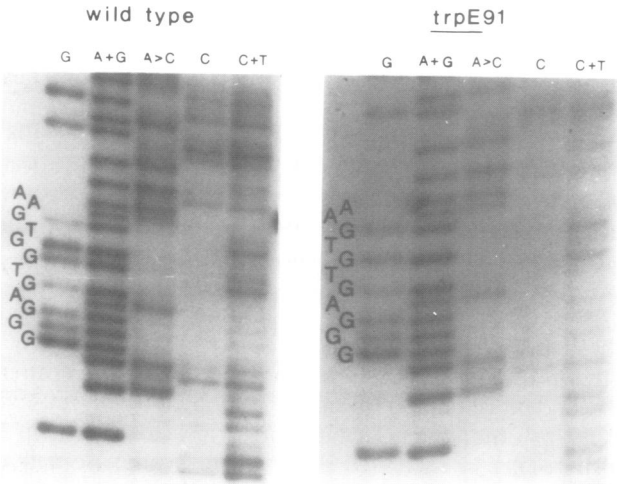

Fig. 2. Autoradiogram of a DNA sequencing gel showing the difference between wild-type (WT) and *trpE91*.

The remainder of the plasmids were constructed from *S. typhimurium* chromosomal DNA. 10 μg of chromosomal DNA from the *trpE872* mutant was digested with *Eco*RI and ligated with 5 μg of *Eco*RI, phosphatase treated pBR322. The ligation mixture was transformed into *E. coli* LE392 (R^-M^+) and ampicillin-resistant colonies were selected. Plasmid DNA was prepared from a pool of ~5000 ampicillin-resistant colonies, and transformed into *E. coli* W3110 d(*trpLD102*). $trpD^+$ colonies were selected on medium containing anthranilate and ampicillin. The resulting plasmid carried a 9000 bp *S. typhimurium Eco*RI fragment in pBR322. A 5900-bp *Eco*RI-*Sal*I $trpE^-D^+C^+$ DNA fragment was subcloned into pBR322, and the resulting plasmid designated pBN65.

The remaining *trpE* mutations were obtained by cloning the 5900-bp *Eco*RI-*Sal*I DNA fragment from chromosomal DNA. 50 μg of chromsomal DNA was digested with *Eco*RI and *Sal*I and electrophoresed on a 0.5% agarose gel. The portion of the gel corresponding to the size range of 5700−6000 bp was excised and the DNA eluted from the gel. This mixture of fragments was ligated with *Eco*RI-*Sal*I cut pBR322 and transformed into *E. coli* LE392, in order to modify the foreign DNA sequences appropriately. Plasmid DNA was prepared from the pooled colonies and transformed into *E. coli* W3110 d(*trpLD102*). $trpE^-D^+$ colonies were selected as described above.

DNA sequence analysis

Plasmid DNA was digested with *Hinc*II and the 626-bp fragment containing the first one-third of *trpE* was isolated. This fragment was then digested with *Hpa*II. The 215-bp fragment was isolated for DNA sequence analysis. For Maxam-Gilbert sequencing, the fragment was labelled at either the 5′ or 3′ termini, and the strands separated on an 8% polyacrylamide gel. For dideoxy sequencing, the fragment was cloned into the *Acc*I site of M13mp8 (Messing and Vieira, 1982). The sequence of both strands across the site of the mutation was obtained in all cases.

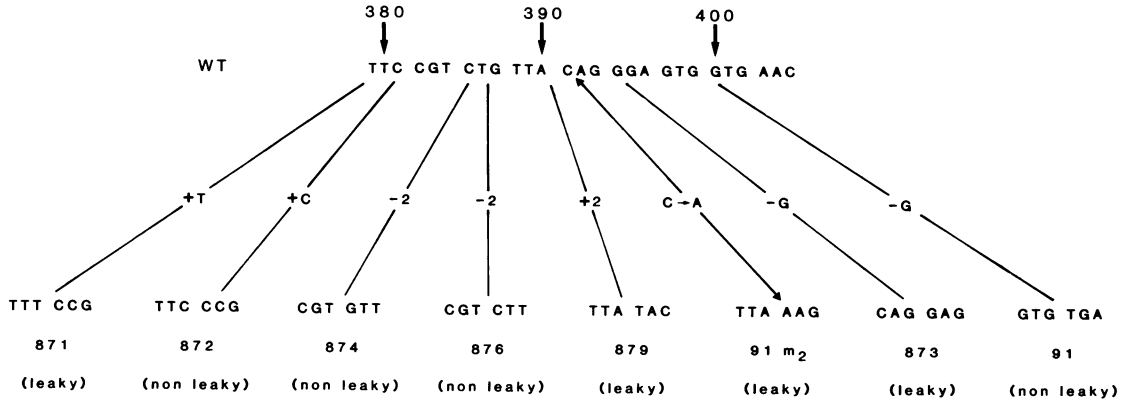

Fig. 3. The nucleotide changes in the *trpE* mutant sequences. m_3 has the same alteration as m_2. The mutants were classified as leaky or non-leaky depending on their ability to grow or not on minimal medium lacking tryptophan.

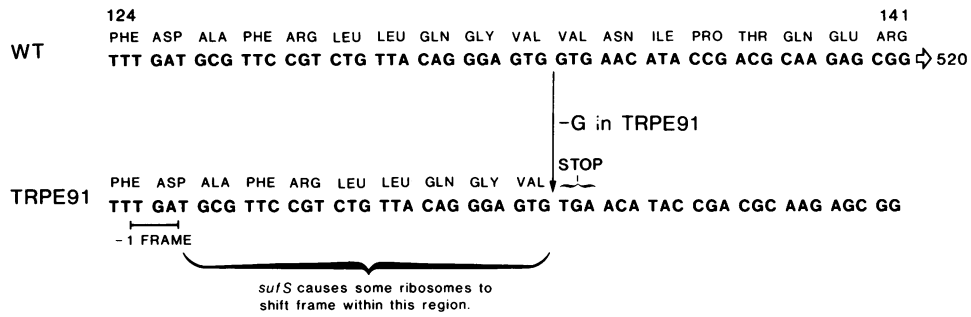

Fig. 4. *trpE91* has a single base deleted from the wild-type sequence of the anthranilate synthetase gene. The first translation stop signal encountered in the new reading frame created by the deletion is the next codon, (TGA). *SufS* causes some ribosomes to shift into the appropriate out-of-phase reading frame somewhere between the first stop codon in the −1 frame preceding the site of the deletion (9.33 codons upstream), and the downstream TGA. The new reading frame is that which restores the ribosomes to the original wild-type frame on passing the deletion site.

Some DNA sequencing results are displayed in Figure 2. The wild-type sequence agrees with that reported by Yanofsky and van Cleemput (1982). *trpE91* results from a single base pair deletion (a G residue) at position 399 or 400 (Figure 3). The mutations m_2 and m_3 are identical. These result from a base substitution (A for C) at position 391 in the sequence (Figure 3). *trpE873* is a deletion of one of the G residues at positions 393−395.

trpE879 is a frameshift of the −1 sign that is the result of a 2-bp insertion. The resulting sequence may have arisen in three different ways, either by the insertion of a TA dinucleotide sequence between residues 388 and 389 or by the insertion of an AT dinucleotide sequence between residues 389 and 390, or by the insertion of a TA dinucleotide sequence between residues 390 and 391.

The *trpE871* and *872* mutations are the result of single base pair insertions. *trpE871* contains the insertion of a T residue to yield a run of three Ts at position 379−380, and *872* is the insertion of a C residue to yield a run of three Cs at position 381−382.

trpE874 and *876* are both frameshift mutations of the +1 sign that are actually the result of 2-bp deletions. In each case, the deletion may have occurred in two different ways to result in the final sequence. *trpE874* may have arisen by the deletion of either the TC dinucleotide at positions 384−385 or the CT dinucleotide at positions 385−386. Similarly, the deletion in *trpE876* may have occurred by deleting either the TG at positions 386−387 or the GT at positions 387−388.

Discussion

As shown in Figure 4, the proximity of barriers (translation stop codons) sharply delimits the sequence in which the suppressors of *trpE91* must act. Further delimitation within this region can be achieved by the construction *in vitro* of mutants where the UUA codon preceding the *91* site is changed to the stop codon UAA and subsequent alteration of nucleotides in the target area.

The predicted size of the relevant tryptic fragment from the protein derived from *sufS* suppression of *trpE91* is small, and there is also the possibility of changing the CUG codon preceding the *91* site to AUG (methionine codon) to create a new cyanogen bromide cleavage site in the protein. These studies are being initiated and should help identify the site of suppression.

Several explanations are formally possible for the leakiness of *trpE91*m_2 and *trpE91*m_3. The codon altered in m_2 and m_3 is CAG. If CAG were to exert a downward modulating effect, removal of this codon could increase the translation through the region. The downstream terminator is UGA which is the only one of the three terminators which is significantly leaky (~3%) (Model *et al.*, 1969; Roth, 1970; Hirsh and Gold, 1971). Hence, a more favourable codon-anticodon interaction at m_2 would result in less ribosomes stalling before reaching the UGA and more would then pass it and be available for frameshifting back to the wild-type frame downstream of the UGA. In that case one would expect that an efficient UGA suppressor would also allow the downstream frameshifting to occur. However, this is not the case as the recessive lethal *supR* suppressor of Miller and Roth (1971) does not suppress *trpE91*. In addition, CAG does not appear to be a modulating codon (see Ikemura, 1981; Sanger *et al.*, 1982; Konigsberg and Godson, 1983). Another possibility is that the ribosomal frameshifting occurs upstream of m_2 and m_3, and that m_2 and m_3 are context mutants (c.f., Bossi and Roth, 1980) in this new frame which is later returned to the wild-type frame by the *91* mutation. However, this sequence (ACA) is read in the combination of *trpE871* or *trpE872* and *trpE91* and these combinations give only slightly impaired growth. This possibility is unlikely. A further possibility in which the frameshifting occurs upstream of m_2 and m_3 is that it is the amino acid substitution caused by the m_2/m_3 mutations (Lys in m_2/m_3 instead of Gln in *91*) which results in increased anthranilate synthetase activity. Again, on account of the high level of anthranilate synthetase activity in the combination of *trpE871* or *trpE872* and *trpE91*, where five amino acids in this region are altered, we do not favour this possibility. The possibility we favour is that the ribosomal frameshifting occurs at the m_2 (m_3) site and that part of the Leu-Lys sequence UUA AAG is responsible for shifting ribosomes into the AAA frame. We plan to test this prediction using synthetic DNA sequences and, if the result is positive, to quantitate the level of frameshifting at this and derivative sequences.

trpE873 and *trpE879* share the same −1 frame as *trpE91* and, in contrast to *trpE91*, are leaky on minimal medium without the m_2/m_3 base substitution. The only codons that are different in *trpE873* and *trpE91* mRNA are GAG UGG in *trpE873* and GGA GUG in *trpE91*. In *trpE879* there is an additional UAC AGG codon difference from *trpE91*. The presence of the tryptophan codon UGG is noteworthy since *trpE873* and *trpE879* being tryptophan-requiring mutants are deficient in charged tryptophan tRNA on minimal medium. In studies on the *r11* region of phage T4, Weiss and Gallant (1983) have shown that when there is a scarcity of tryptophan

tRNA the only tryptophan codon, UGG, is read by a leucine tRNA, and leads to a shift in the reading frame such that a +1 frameshift mutant can be corrected. In a similar experiment, Weiss and Gallant (personal communication) have recently not detected frameshifting at a G UGG U sequence leading to the correction of the formal equivalent of a −1 frameshift mutant (the combination of the two +1 frameshift mutants, FC_0 and FC_{38}). This is the same sequence which occurs in the vicinity of *trpE873* and *trpE879*. Whether the difference between the two systems is an absolute one or in part a quantitative one remains to be determined. There are several features which differ in the two systems, and among them is the nature of the codons as opposed to merely the single nucleotides which flank the UGG. In *trpE873* and *trpE879* the UGG codon is directly followed by UGA, whereas in the *r11* system it is followed by the more efficient stop codon UAA. It is conceivable that limitation of charged tryptophan tRNA may allow frameshifting at a sequence including the first two nucleotides of the UGA. It could entail reading of the wild-type frame GUG in the sequence UGG UGA. This possibility and that of non-cognate decoding at UGA is absent in the *r11* system.

Another factor is the nature of the tRNA reading the 0 frame upstream codon. With *trpE873* and *trpE879* any reading of the underlined 0 frame GUG in the sequence GAG UGG by its cognate tRNA might be influenced by the tRNA reading the GAG codon, whereas the equivalent tRNA in the *r11* system decodes AAG. Limitation of charged tryptophan tRNA might facilitate reading of GUG, but it is also possible that if aberrant reading of the UGG codon was responsible for the frameshifting it might be sensitive to the identity of the tRNA decoding the adjacent 5′ codon. The original proposal about non-cognate codon-anticodon interaction causing frameshifting was made by Kurland (1979), but distinction between the possible mechanisms for the frameshifting observations with the *trpE* mutants awaits further work. Situations where there is a shortage of charged tryptophan tRNA for decoding the tryptophan biosynthetic operon are minimized by several of the genes, including *trpE* and *trpA*, not containing any in-frame tryptophan codons (see Somerville and Yanofsky, 1964; Nichols *et al.*, 1981).

The wild-type (RNA) sequence G UUC C is changed to G UUU CC in the +1 frameshift mutants *trpE871* (see Results), and it is changed to G UUC CC in the +1 frameshift mutant *trpE872* (results of B.N. to be published in a study of the external suppressors, *sufT*, of *trpE872*). *TrpE871* is leaky whereas *trpE872* is not (Riyasaty and Atkins, 1968). Perhaps some ribosomes shift to the +1 frame at a sequence upstream of the *871* site. In this event the sequence given will be read in the frame GU UUC C in *871* and GU UCC C in *872*. It is possible that the lack of detectable leakiness of *872* is due to the Phe→Ser substitution in the resulting protein. The functionally +1 frameshift mutants *trpE874* and *trpE876* are also non-leaky and could be similarly explained if the frameshifting is upstream. The ribosomal frameshifting does not occur downstream of the *871* site since the codons encountered in *871* and *872* would be the same. Thus, the alternative is that the leakiness of *trp*E871 is due to ribosomal frameshifting at the UUU C sequence created by the additional U in *871* and this is absent in the other three mutants. Intriguingly this same sequence occurs where Fox and Weiss-Brummer (1980) found the leaky mitochondrial frameshift mutants and in the region where Kastelein *et al.* (1982) showed that frameshifting occurred to regulate the expression of phage MS2 lysis gene. The same sequence occurs at an appropriate position in two phage T7 late genes where Dunn and Studier (1983) find additional products which may be due to frameshifting. Whether it is significant that the run of U is followed by C rather than for instance G remains to be seen. In any event it is interesting that both in this case and with m_2/m_3 a monotonous run of repeat bases occurs and this is likely to be significant in permitting reading frame slippage by presenting two synonymous codons with each nucleotide able to form only two hydrogen bonds.

The early characterized +1 frameshift suppressors both in bacteria (Yourno and Tanemura, 1970; Riddle and Roth, 1972; Yourno, 1972; Kohno and Roth, 1978) and in yeast (Culbertson *et al.*, 1977; Donahue *et al.*, 1981) suppressed frameshift mutants occurring in runs of repeat bases, either CCCU, GGGG or AAAA or derivatives. Suppressors for a run of U repeat bases have not yet been characterised. This may be because frameshift mutants with this sequence have not been studied so much due to their leakiness. Whether the use of a restrictive str^R allele in the background would diminish the leakiness of *trpE871* to enable selection for suppressors which do not also suppress *trpE872* remains to be seen.

There is an emerging pattern of the involvement of specific sequences (UUUC and probably also a run of As) and functional imbalance in the frameshifting in suppressor free cells. This pattern is being provided by the studies on the *E. coli* phages and the mitochondrial mutants referred to above, and the studies on the phenotypic suppression of frameshift mutants on specific amino acid limitation (Gallant and Foley, 1980; Weiss and Gallant, 1983). In the latter studies three of the six amino acids whose imbalance exacerbates frameshifting are Phe, Lys and Trp so there is some overlap in the results from the different approaches. It will be interesting to test for frameshifting in eukaryotic cytoplasmic protein synthesis and to determine whether it is utilized by any eukaryotic viruses or organelles.

Materials and methods

Bacterial genetics

The techniques used, the media and the source of several of the strains were described in detail previously (Riyasaty and Atkins, 1968). The anthranilate synthetase gene of *S. typhimurium* is now designated *trpE* to comply with the *E. coli trp* gene nomenclature. *E. coli* CSH57 has the genotype F^- *arg met ile val leu his trp Δ BE9 ade thi ara lac mal xyl mtl* str^r and was obtained from the Cold Spring Harbor strain kit. *E. coli* CSH57/F′ *S. typhimurium trpE91* was constructed using the following technique (W. Brammar, personal communication): an F′ *S. typhimurium* $cysB^-$ trp^+ plasmid was introduced into a $cysB^-$ strain also carrying a deletion of the tryptophan operon. The strain was then transduced to cysteine independence using chromosomal $cysB^+$ *trpE91* as donor. Transductants which were still Trp^- were then checked for the presence of *trpE91* on the plasmid by transfer into a tryptophan deletion strain carrying an external suppressor of *trpE91*. F′*trpE91* was transferred into CSH57 by mating. Plasmid pKB5 (Bennett *et al.*, 1978) and *E. coli* W3110 (*dtrpLD102*) were obtained from the laboratory of C. Yanofsky.

Enzymes

Restriction endonucleases were either purchased from New England Biolabs or purified according to published procedures. Digests were according to supplier's or publisher's recommendations. When fragments were being digested in preparation for 5′ end-labelling, 0.4 units of calf alkaline phosphatase (Boehringer-Mannheim) were included in the restriction endonuclease incubation.

T4 polynucleotide kinase was purchased from PL Biochemicals. *E. coli* DNA polymerase I (Klenow fragment) was purchased from Boehringer-

Mannheim. [γ-^{32}P]ATP (2000 Ci/mmol) and [α-^{32}P]dCTP (800 Ci/mmol) were purchased from Amersham.

5′-Terminal labelling was done as described by Maxam and Gilbert (1980). 3′-Terminal labelling was done as described by Nichols and Donelson (1978).

Ligation reactions were carried out at DNA concentrations of 20–50 μg/ml in 10 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 0.5 mM ATP, and sufficient T4 DNA ligase to convert all the DNA fragments to higher mol. wt. forms in 1 h at 12°C.

DNA isolation and transformation

Chromosomal DNA was isolated by the method of Marmur (1963). Plasmids were isolated by the SDS-cleared lysate method as described in Gunsalus *et al.* (1979). CsCl-ethidium bromide gradients were performed when necessary. The rapid plasmid isolation described by Birnboim and Doly (1979) was used for small preparations of plasmid and F′ DNA.

Transformations were carried out according to Mandel and Higa (1970).

DNA sequence analysis

DNA sequences were determined by the method of Maxam and Gilbert (1980) or Sanger *et al.* (1977). The polyacrylamide gel electrophoresis system described by Sanger and Coulson (1978) was employed.

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