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Genetic Characterization of Frameshift Suppressors with New Decoding Properties

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Suppressor mutants that cause ribosomes to shift reading frame at specific and new sequences are described. Suppressors for *trpE91*, the only known suppressible −1 frameshift mutant, have been isolated in *Escherichia coli* and in *Salmonella typhimurium*. *E. coli hopR* acts on *trpE91* within the 9-base-pair sequence GGA GUG UGA, is dominant, and is located at min 52 on the chromosome. Its *Salmonella* homolog maps at an equivalent position and arises as a rarer class in that organism as compared with *E. coli*. The *Salmonella* suppressor, *hopE*, believed to be in a duplicate copy of the same gene, maps at min 17. The +1 suppressor, *sufT*, acts at the nonmonotonous sequence CCGU, is dominant, and maps at min 59 on the *Salmonella* chromosome.

The recent finding of high-level natural frameshifting at particular sequences has reawakened interest in frameshifting brought about by altered translational components. Mutants with this property have traditionally been selected as external suppressors of frameshift mutations. The earliest evidence that frameshift mutants were externally suppressible came from the finding that the *Salmonella typhimurium* mutant *trpE91* and another mutant, *trpE872*, were each externally suppressible (42). These *trpE* mutants when combined give the pseudo-wild-type phenotype (42). Sequencing of these alleles has since shown that *trpE91* is a −1 mutant (3) and *trpE872* is a +1 mutant (see below). In this paper we describe suppressors *hopR* and *hopE*, which act on *trpE91*, and *sufT*, which acts on *trpE872*. The decoding properties of all these suppressors are different from those of other characterized suppressors.

Several classes of mutants of translational components have been isolated as external suppressors for the many suppressible +1 frameshift mutants. These have been studied in *Saccharomyces cerevisiae* by Culbertson and colleagues (10, 11, 15, 31, 52, 53) and also in *Salmonella* species and *Escherichia coli* (2, 43) (see below). Many of the characterized +1 suppressors have tRNAs with enlarged anticodon loops (9–12, 15, 31, 38, 53). (At least one "normal" tRNA has an enlarged anticodon loop [28], but whether it is involved in natural frameshifting is unknown.) Study of the mutants with enlarged anticodon loops has been helpful in defining the role of tRNA in the translocation step size. There is current interest in understanding the decoding properties of such tRNAs, in particular the stacking (9, 12) and other (15) properties of the bases at the 3′ side of the anticodon loop in the process. To investigate this and related issues, an extensive set of insertion mutants of an amber suppressing $tRNA^{Trp}$ has been constructed in *E. coli* (12). These constructed mutants have been informative, but because of the large number of possible variants that need to be synthesized, characterization of genetically selected suppressors continues to be useful. The first sequenced frameshift suppressor, which was in *S. typhimurium*, had an extra base, C, in the CCC anticodon of $tRNA_1^{Gly}$ (38) in the suppressor *sufD42* (40). It and nearly all the published +1 suppressors act at a run of repeat bases such as GGGG. An exception is *sufJ128*, which probably reads all ACCN codons regardless of the fourth mRNA base (8). Bossi and Smith (9) have shown that it has an extra base, C, 5′ of the anticodon in the anticodon arm of $tRNA_3^{Thr}$. Other *Salmonella* +1 frameshift suppressors in addition to *sufD* and *sufJ* have been isolated by Roth and colleagues and are also utilized here in the *sufT* study even though they have not yet been characterized at the sequence level. *sufB2* leads to the insertion of proline (56), and it and *sufA* are presumed to affect proline tRNAs (40). Furthermore, *sufE* is thought to affect $tRNA^{Gly}$, and *sufG* is thought to affect $tRNA^{Lys}$ (24, 40). Tested alleles of these four suppressors are dominant (39). *sufC* and *sufF* are recessive and have been suggested to affect tRNA-modifying enzymes (39). One mutant, *E. coli trmD*, defective in the tRNA modification m^1G37, is known from the work of Björk (6) to cause +1 frameshifting at runs of C. The frameshift suppression properties of a *thyA* mutant have also been reported, but whether it acts via an effect on tRNA is unknown (17). Some +1 suppressors are not tRNA mutants but intriguingly have altered rRNA (51). Other suppressors have altered elongation factors (19; M. G. Sandbaken and M. R. Culbertson, Genetics, in press) or proteins related to elongation factors (52). The known suppressors of these types also suppress nonsense codons.

In contrast to the above available information on +1 mutant suppressors, −1 frameshift mutants other than *trpE91* have yet to be shown to be externally suppressible. Three distinct classes of suppressors of *trpE91* had been identified in *S. typhimurium* before the current study. One class, *sufS*, which includes most of the original external suppressors (42), comprises alleles of a tRNA gene *glyT* (33; D. O'Mahony et al., manuscript in preparation), the gene for $tRNA_2^{Gly}$ (1, 34). The other two classes are mutant in protein-coding genes comprising alleles of *supK* (4) and alleles of either gene *tufA* or *tufB* for elongation factor Tu (19). *supK* probably codes for polypeptide chain release

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Genotype or relevant characteristics	Source or reference
E. coli		
CH6	CSH57 derivative, *gal*$^+$ *trpBE9 srl*::Tn*10 btuB*	This study
CH19	CSH57 derivative, *gal*$^+$ *trpBE9 trpE91* (chromosomal integration)	This study
CSH57	*ara leu lacY purE gal trp his argG rpsL mal xyl mtl ilv thi metA/B*	Cold Spring Harbor (32)
MCI	CSH57 derivative, *gal*$^+$ *trpBE9 trpE91 hopR1 cysA*	This study
Xac	*ara* Δ(*lac-pro*) *thi argE*(Am) *rpoB*	J. Miller
Strains carrying F'142, 143, and 198		B. Bachmann (18)
MC57	*thr leu thi lacY ara-14 xyl-5 mtl-1 proA2 his-4 rpsL31 tsx-33* Tn*10*-*ptac*-mini-Kan (47% linked to *sufS*$^+$)	This study
S. typhimurium		
TH38	*hisO1242 hisG6609 trpE872 sufJ128 srl-202*::Tn*10 sufT621*	This study
TH44	*HfrA purC*, deletion of *uvrB*, a UV^s derivative of SR305	This study
TH71	*metA22 leu-151 proB401 trpE872 sufT621 hisO1242 hisC3737*	This study
TH72	*trpE872 srl-202*::Tn*10*	This study
TT98	*trp*::Tn*10*	J. R. Roth
TT398	*nadA*::Tn*10*	J. R. Roth
TT2835	*hisT1504 zej-636*::Tn*5*	J. R. Roth
ST105	*trpE872*	S.T. and J.F.A. collection (42)
ST106	*trpE872 sufT621*	S.T. and J.F.A. collection (42)
ST107	*trpE872 hisO1242 sufB2*	This study
Strains carrying *sufA-F*, *-G*, *-J*, and *-M*		J. R. Roth
SGSC74	*srl-202*::Tn*10 recA1 rpsL*	K. E. Sanderson
SR305	*HfrA purC*	K. E. Sanderson
ptuB12 plasmid	pBR322 clone carrying functional *glyT*	L. Bosch

factor 2 (22), although a tRNA methylase deficiency is also found in *supK* mutants (36, 37). Both *supK* and EFTu suppressors are weak relative to the majority of *sufS* alleles and are best selected indirectly through special selective pressures (kirromycin resistance for *tuf* and simultaneous suppression of frameshift and UGA for *supK*). To reveal further tRNA classes of suppressors, these or other selective conditions were not applied in the current study. The experiments reported here led to the recovery of two new classes of *trpE91* suppressors, *hopR* and *hopE*, which have proved to be very different from other known suppressors.

MATERIALS AND METHODS

Media and bacterial strains. Minimal (E) medium, sugars, amino acids, antibiotics, and other media including green plates for the recovery of P22 phage-sensitive strains were as described previously (13). L-Malate and sorbitol as sole carbon sources were at a concentration of 0.2%. Histidinol was used at 1 mM final concentration. The bacterial strains and plasmids used are listed in Table 1.

Isolation and identification of external revertants of *trpE91* and *trpE872*. Independently arisen *trpE*$^+$ revertants, isolated at 37°C on minimal medium, were used as donors to transduce a *Salmonella* strain carrying an extensive deletion of the *trp* operon (*trpEDC130*) to growth on indole. If the resulting transductants required anthranilate (the phenotype of *trpE* mutants), the original revertant was concluded to be due to an external suppressor. In *E. coli* the revertants were transduced into the *trp* deletion strain *trpBE9* or *trpED24*.

Identification of external suppressors at new map positions. External suppressors of *trpE91* mapping to the *sufS* locus at min 89 were identified and eliminated to reveal possible new suppressor loci both in *S. typhimurium* and *E. coli*. In *S. typhimurium*, either revertant was isolated in the presence of the ptuB12 plasmid (see Results), or each externally suppressed revertant was used as a transductional recipient with the *sufS*$^+$-linked marker *argH*::Tn*10* as a donor. Loss of suppression in 10 to 20% of Tet^r transductants indicated that the suppressor was likely to be of the *sufS* class, and retention of suppression in all Tet^r recombinants indicated a position outside min 89. To identify *sufS* alleles in *E. coli*, Trp$^+$ revertants were transduced to kanamycin resistance with phage grown on MC57 which carries a Tn*10*-derived Kan^r element 47% linked to *sufS*$^+$. Kan^r transductants were then scored for loss or retention of the suppressor.

The external suppressor *sufT621* of the +1 frameshift mutant *trpE872* was tested for cosuppression of histidine +1 frameshift mutants suppressible by *sufA*, *B*, *C*, *D*, *E*, *F*, *G*, *J*, or *M* suppressors. *sufT621* was transduced into strains containing *trpE872* and each histidine +1 frameshift mutation with selection for *trpE872* suppression and subsequent screening of the histidine requirement. Two mutants, *hisG6609* and *hisG2804*, showed suppression (see below). The introduction of *sufA-F*, *-G*, *-J*, or *-M* into the same doubly mutant strains containing *trpE872* and *his* mutants with selection for histidine suppression and subsequent scoring of Trp requirements showed retention of *trpE872* auxotrophy and, hence, nonsuppression by these suppressors.

Construction of *trpE his* double mutants free of linked Tn*10* markers. Double mutants are easily constructed by the introduction of one mutation into a strain bearing the other by transduction via a linked Tn*5* or Tn*10* marker. When the retention of the antibiotic marker was not desired, the following procedures were followed. *trpE* mutations were always introduced into *hisD* mutants in two steps, first by

the introduction of a *trp*::Tn*10* marker that generated Trp requirement, followed by the introduction of *trpE* mutations by selection for growth on the Trp intermediate anthranilic acid. Histidine mutants other than *hisD* could be introduced into *trpE* strains in two steps, first by the introduction of the *hisG-F644* deletion with a linked Tn*10* (*zee-2*::Tn*10*), followed by the introduction of *his* mutants by selection for growth of transductants on histidinol and retention of clones free of the linked Tn*10*.

Replacement of one *trpE* allele by another in the same strain. To replace *trpE91* by *E873* or *E879* and to replace *trpE872* by *E880*, a two-step procedure was followed. First, the original *trpE* mutation was replaced by *trp*::Tn*10*, generating antibiotic resistance and Trp requirement. Next, the desired *trpE* allele was transduced by selection on anthranilic acid, and its anthranilate requirement was confirmed.

Integration of *trpE91* into the *E. coli* chromosome. The *S. typhimurium trpE91* mutation was transferred onto an F′ *trp* plasmid of *S. typhimurium* origin (3). This F′ *trpE91* was introduced into the *E. coli* r k$^-$ strain ST8612 bearing a *trpED24* deletion. Phage P1 grown on ST8612 F′ *trpE91* was used to transduce a CH6 derivative lacking *srl*::Tn*10* and *btuB* markers to growth on anthranilic acid, thereby generating strain CH19.

Introduction of *sufS*, *sufT*, *sufJ*, and *hisT* loci into strains. The following linked antibiotic resistance markers here were used to transfer the above loci: *argH*::Tn*10* or *zii-614*::Tn*10* for *sufS*, *srl*::Tn*10* for *sufT*, *zii-614*::Tn*10* or *argH*::Tn*10* for *sufJ*, and *zej-636*::Tn*5* for *hisT*.

trpE mutations were cloned onto pBR322 and prepared for DNA sequence analysis as previously described (3). Restrictions, ligations, and transformations were performed as previously described (30). DNA sequencing reactions were done with Sequenase reagents purchased from United States Biochemicals. DNA sequencing of plasmids was carried out by a published procedure (25), with the synthetic 18-mer GTGTGGCAGCGCTTCAAA (obtained from the LCMDB DNA synthesis Facility, University of Illinois) as a primer.

Transduction, Hfr crosses, F′ and plasmid interspecies transfer, and plasmid curing. In transductions the *Salmonella* phage P22HT and the *E. coli* phage P1 *virA* were handled as described previously (30, 32), as were F′ transfers and Hfr crosses (32). Interspecies transfers of F′ were by conjugation and those of plasmids were by transformation and achieved by initial passaging through restriction-deficient derivatives. F′ and plasmid elimination from strains was by repeated subculturing under nonselective conditions.

Scoring of *ptsH*, *uvrB*, and *recA* phenotypes. *ptsH* mutants were scored by their inability to utilize sorbitol or mannitol as a sole carbon source. *uvrB* and *recA* were both sensitive to UV light. *cysA*-containing strains were isolated as chromate-resistant mutants (35).

RESULTS

External suppressors in *E. coli* of the −1 frameshift mutant *trpE91*. In *S. typhimurium* the overwhelming majority of external suppressors for *trpE91* isolated were of the *sufS* class. In contrast, while attempting to isolate *sufS* suppressors in *E. coli*, we discovered that more than half of the suppressors did not map in the *sufS* region of the chromosome. The reason for this disparity is unknown. To isolate suppressors in *E. coli*, *trpE91* was transferred into *E. coli* to generate strain CH19 (see Materials and Methods). Strain CH19 has an internal deletion, *trpBE9*, of part of the *E. coli trp* operon and, at an unknown but different location, the

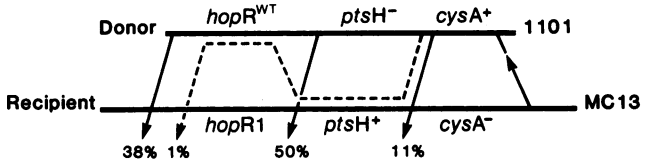

FIG. 1. Transductional mapping of *hopR* with respect to the *ptsH* gene. Cys$^+$ transductants (475) were selected on minimal medium containing anthranilic acid (plus galactose and B_1) and screened for *ptsH* and *hopR1*. The infrequency of the "quadruple crossover" class is interpreted to give the order *hopR ptsH cysA*.

Salmonella trp operon (containing the *trpE91* mutation). Such merodiploids are frequently encountered in *S. typhimurium-E. coli* intergeneric crosses (27). Trp$^+$ revertants of strain CH19 were isolated, and those containing external suppressors were identified. One such suppressor, designated *hopR1* (see below), which was distinct from *sufS*, was mapped in Hfr crosses with the strains (32) CSH62, 64, 70, 74, and 77 to between map positions 42 and 58 and in preliminary P1 transductions close to *cysA* at position 52 (5). The linkage of *hopR1* to *cysA* and a nearby marker *ptsH* was analyzed in the cross shown in Fig. 1. These crosses showed 40% linkage of *hopR* to *cysA*, and the frequency of the "quadruple-crossover" class, *cysA*$^+$ *ptsH*$^+$ *hopR*$^+$, indicated the order *cysA ptsH hopR*. The dominance of *hopR1* was tested by the introduction, with selection for Cys$^+$, of F′142 and F′198 into *E. coli* strain MC1, which contains *cysA trpE91 hopR1*. The retention of the Trp$^+$ phenotype in exconjugants indicated the dominance of *hopR1* but did not establish it due to the possibility of F′ fragmentation and loss of the *hopR*$^+$ gene. An amber suppressor, *supN* (48), maps at position 51.5 on the same side of the *ptsH* as *hopR*. Both F′142 and F′198 carry a suppressor allele of *supN*, and introduction of these F′ factors into *E. coli* Xac, which has an *argE* amber mutation, confirmed the presence of the suppressor as evidenced by the Arg$^+$ phenotype of Xac/F′. This result provides supporting evidence for the dominance of *hopR1* (see Discussion). The dominance of *hopR* was subsequently used in the selection of clones containing alleles of *hopR*, which were shown to be mutant in one or other of the three genes, designated *valU*, for $tRNA_1^{Val}$ at min 52 (M. O'Connor, manuscript in preparation). Alleles of *hopR* are not difficult to obtain in *E. coli*, and we have isolated from 24 *trpE91* revertants seven further alleles designated *hopR11*, *15*, *16*, *512*, *513*, *523*, and *530*, all of which are dominant.

***trpE91* suppressors in *S. typhimurium*.** The ease of isolation of *hopR* suppressors in *E. coli* contrasted with our previous failure to detect a class mapping in an equivalent region of *S. typhimurium*. This in turn prompted a more extensive search in *S. typhimurium*. Several of the selections were performed with a *trpE91*-containing strain, whereas the *sufS* class was excluded by the presence of the plasmid ptuB12 (49). (This plasmid carries the wild-type [WT] allele of *glyT* to which *sufS* mutants are recessive [O'Mahony et al., in preparation].) By excluding *sufS* suppressors, revertants would be expected to have secondary changes within *trpE*, unless new classes of external suppressors arise, since the efficient recovery of *supK* and *tuf* classes requires special selective conditions. Among 243 revertants, 2 were external. Loss of the plasmid ptuB12 did not interfere with the suppression phenotype of either of the two. On the basis of subsequent results, one was designated *hopR701* and the other was designated *hopE1*. In a P22 transduction cross of

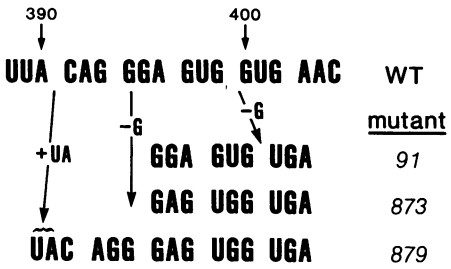

FIG. 2. Nucleotide changes in the *trpE* mutants (3) used to delimit the site of *hopE1* and *hopR701* suppression. The codons encountered in translating the three mutants are the same except for those shown.

hopR701 with a *cysA*::Tn*10* marker as the donor, 11 (6%) of 179 Tet^r transductants showed loss of *hopR701* phenotype. This result also maps the *Salmonella hopR* to the 50-min (52 min in *E. coli*) region of the chromosome.

Since *hopR701* is an allele of *valU* (O'Connor, in preparation) and there is another gene encoding $tRNA_1^{Val}$ at position 17 (54), we tested the second suppressor, *hopE1*, for its linkage to *nadA*, which is located at position 17 (45). In a P22 transduction cross of *trpE91 hopE1* with TT398 (which contains *nadA*::Tn*10*) as the donor, 103 (93%) of 120 Tet^r transductants showed loss of the *hopE* phenotype. All 120 were *nadA* mutants, apart from 1 which was presumably due to a Tn*10* transposition. These results suggest, but do not establish, that *hopE1* may also be an allele of a gene for $tRNA_1^{Val}$.

To delimit the site of action of the *Salmonella* suppressors, we tested *hopE1* and *hopR701* suppression of *trpE873* and *trpE879*. These −1 frameshift mutants have their mutation sites 4 and 8 nucleotides, respectively, 5′ of the site of the *trpE91* mutation (Fig. 2). Neither *trpE873* nor *trpE879* was suppressed by *hopE1* or *hopR701*; since their 5′ codons up to each mutant site are in common with those found in *trpE91*, the site of *hopE1* and *hopR701* suppression must be within the "Gly Val stop" coding sequence GGA GUG UGA.

The +1 frameshift mutant *trpE872* and its suppressor *sufT*. *trpE872* was cloned on pBR322 from the strain ST106 (see Materials and Methods) and sequenced. It is a +1 frameshift mutant (Fig. 3). None of the known +1 suppressors (*sufA*, *B*, *C*, *D*, *E*, *F*, *G*, *J*, and *M*) studied by Roth and colleagues suppressed *trpE872*. *trpE872* is externally suppressible by *suf-621* (4), later designated *sufT621* (2, 3). As shown below, *sufT621* is distinct from the suppressors (see Introduction) studied by others. Conjugation crosses between TH71, containing *trpE872 sufT621*, and the Hfr strains su418, SA970, SA534, SA486, SH462, and SW1403 (44) and TH44 (Table 1) suggested that *sufT621* maps between min 58 and 62 on the *Salmonella* chromosome. This region contains the sorbitol operon (*srl*) and the *recA* gene (45). Transduction crosses between TH72, which contains *trpE872* and *srl*::Tn*10*, and the donor strain ST106, which contains *trpE872 sufT621*, resulted in 40% loss of the *srl*::Tn*10* on selection for suppression. A second transduction with ST106 as recipient and the *srl*::Tn*10 recA1*-containing strain SGSC74 as donor indicated that *recA* was between *srl*::Tn*10* and *sufT621* (Table 2).

TABLE 2. Transductional mapping of *sufT621* with respect to *srl* and *recA* genes[a]

Classes of recombinants	No. (%)
srl::Tn*10 rec*$^+$ *sufT621*	45 (34)
srl::Tn*10 recA1 sufT621*	25 (19)
srl::Tn*10 recA1 suf*$^+$	60 (46)
srl::Tn*10 rec*$^+$ *suf*$^+$	1 (1)

[a] In the cross ST106 (*trpE872 sufT621*) × SGSC74 (*srl*::Tn*10 recA*), the least frequent class (*srl rec*$^+$ *suf*$^+$) is taken as indicative of a "quadruplet crossover," and the results are interpreted to give the gene order *srl*::Tn*10 recA sufT621*.

Dominance of *sufT*. To determine whether *sufT621* is dominant or recessive, we isolated, in a strain carrying the WT allele of *sufT*, duplications of the region of the chromosome spanning the *sufT* gene and substituted one of the WT copies with *sufT* to generate a *sufT*^{WT} *sufT621* merodiploid strain (Fig. 4). The duplication was accomplished by selection in a *trpE872*-containing strain (ST105) for mutants with improved utilization of L-malate as a sole carbon source. Mutants arising in such a selection normally result from tandem duplication of one-third of the chromosome spanning map position 59 (46). We introduced *sufT621* and *recA* by transduction, with selection for a linked *srl*::Tn*10*; 38% of Tet^r, efficient malate-utilizing transductants exhibited the suppression phenotype, but none was UV sensitive. (One of the *recA* phenotypes, UV sensitivity, is not expressed in

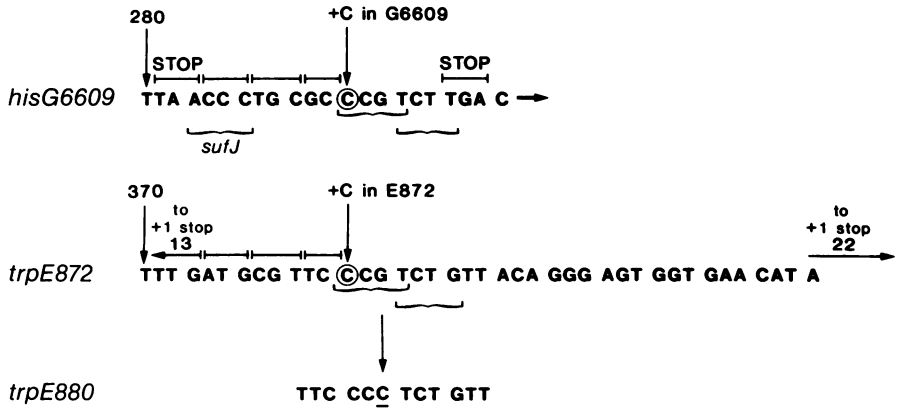

FIG. 3. Suppression window within which suppressors must act to return translation to the WT frame and avoid premature termination. The two codons that occur within the *hisG6609* window and that also occur close to the site of the *trpE872* mutation have a bracket under them in the *trpE872* sequence. *trpE880* is derived from *trpE872*, and the single base change from *trpE872* is underlined.

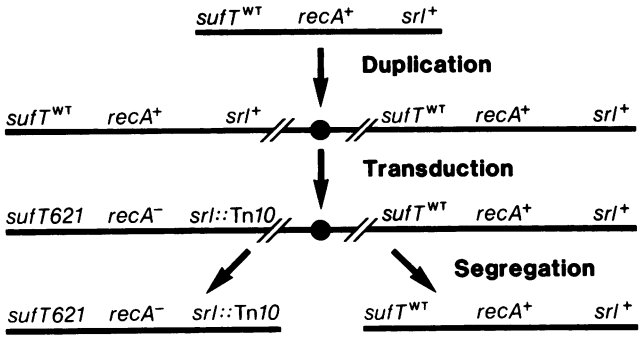

FIG. 4. Duplication strategy for determining whether *sufT621* is dominant or recessive.

merodiploids carrying the WT *recA*$^+$ because *recA* is recessive.) In the absence of malate selection pressure, these transductants subsequently segregated two classes, nonsuppressing Rec$^+$ and suppressing Rec$^-$ (Fig. 4). We conclude that *sufT621* is dominant. This conclusion was supported by a study with a relevant F′ factor, F′143 (18, 29).

Site of *sufT* action. The *trpE872* sequence shows that *sufT621* could potentially act at a significant distance from the site of the *trpE872* mutation (Fig. 3). To delimit the site of suppressor action, we tested over 30 *his* frameshift mutants (16) for suppression by *sufT621*. Two mutants, *hisG6609* and *hisG2804*, were suppressed, although suppression of the latter was very weak. Although both of these mutants are *sufJ* suppressible (23), we found that other *sufJ*-suppressible mutants, *hisD6580*, *hisD3749S*7*, and *hisD3018* (8), are not suppressible by *sufT*. Also, as noted above, *trpE872* is not suppressible by *sufJ*. The suppression window for *hisG6609* is very short, but it shares two potential targets with the sequences flanking *trpE872*, namely, CCGU and either UCUU (in *hisG6609*) or UCUG (in *trpE872*) (Fig. 3). *hisG2804* has not been sequenced, but the sequence around its likely position in *hisG*, based on its map location, includes the sequence CCGC. Since the sequence CCGU, shared by *hisG6609* and *trpE872*, is a candidate for the suppression site, we attempted genetically to change this site in *trpE872* and checked for continued suppressibility by *sufT621*. This was done by constructing a strain (ST107) with *trpE872* and *sufB2*. *sufB2*, which suppresses at CCCU (55), does not suppress *trpE872*. Starting with strain ST107 we isolated a derivative of *trpE872*, designated *trpE880*, which was now suppressed by *sufB2*. *trpE880* is not suppressible by *sufT621*. *trpE880* was cloned onto pBR322 and sequenced (see Materials and Methods). The sequencing showed that the TTC CCG TCT sequence in *trpE872* is TTC CCC TCT in *trpE880* (Fig. 3). From this result we deduced that *sufT621* acts at the sequence CCGU.

Effect of *hisT* on *sufT* suppression. We found that suppression of *trpE872* by *sufT621* was adversely affected by the presence of the *hisT1504* mutation. The *hisT*$^+$ gene product is responsible for the formation of pseudouridine in the anticodon of certain tRNAs including $tRNA_1^{Leu}$ (47). We first noted that attempts to transduce *sufT621* into strains containing *trpE872* were only successful when the recipient was *hisT*$^+$. In addition, *hisT1504* was transduced into TH38, which contains *hisG6609*, *trpE872*, and *sufT621*, by selection for the kanamycin resistance property of a linked Tn*5* from TT2835; 64% of the Kan^r transductants had lost their ability to suppress *trpE872* efficiently. The same *hisT1504* mutation had no effect on *sufT* suppression of *hisG6609*.

DISCUSSION

As described here and in the previous studies cited above, several classes of mutants of translation components act to suppress the −1 frameshift mutant *trpE91*. This means that contrary to earlier perceptions (14), when sought, such suppressible −1 frameshift mutants may not be difficult to find. However, at least one other −1 frameshift mutant, *hisD3052*, is not externally suppressible (20; S. Thompson, unpublished observations), nor indeed is another frameshift mutant which is a +1 insertion (21).

hopR, the designation given to the *E. coli* class of *trpE91* suppressors described in this paper, was also used to designate the *Salmonella* suppressor allele *701*, even though the cotransduction frequencies of the *Salmonella* and *E. coli* suppressors with the flanking *cysA* marker are not the same (see Results). However, since different phages are used for transduction in *E. coli* and *S. typhimurium*, the *Salmonella* cross has a Tn*10* insertion within *cysA*, and since there are some differences in this chromosomal region between *E. coli* and *S. typhimurium* (41), the results are not inconsistent with the two genes corresponding. In fact, cloning and sequencing work, which took advantage of the dominance relationships reported here, shows that the two genes correspond (M. O'Connor, in preparation). Kohno et al. (23) have previously mapped the unstable dominant +1 frameshift suppressor *sufH* to the *cysA* region of the chromosome in *S. typhimurium* near where *hopR* maps. The linkage of *sufH* to *hopR* has not been tested, but the possibility cannot be ruled out that the two genes correspond. As detailed in Results, nonsuppression of *trpE873* by *hopR701* or *hopE1* restricts the site of suppression to the nine-nucleotide sequence GGA GUG UGA (Fig. 2). There is only a single gene encoding GGA-reading tRNA (33), and as noted above, the recessive *trpE91* suppressors *sufS*, which are quite distinct from *hopR* or *hopE*, are alleles of this gene. Thus the genetic evidence hints at the site of *hopR* and *hopE* reading being confined to the sequence GUG UGA.

Protein-sequencing work shows that *hopR1* and *hopE1* insert a single amino acid, valine, for the five-nucleotide sequence GUGUA. (Similar results were obtained with GUGUG, but because of the complications of internal translation initiation within the *lac* constructs utilized, the results were less clear [13a].) These results were interpreted to be due to detachment of the tRNA from underlined GUG but retention of the tRNA within the ribosome while the message slipped forward by two bases with subsequent repairing of the tRNA to the overlined GUA (or GUG) triplet (13a). Supporting this interpretation are recent protein-sequencing results, which have shown that *hopR1* causes a single valine to be inserted for the "Val stop Val" coding sequence GUG UAA GUU (O'Connor, in preparation). To highlight this hopping property of these mutants and because of the analogy to stop codon hopping by WT tRNAs (50), we have used *hop* rather than *suf* designations. Whether tRNA hopping is naturally utilized is unknown, but some possible examples have been discussed (50). DNA sequencing of seven alleles of *E. coli hopR* and of *Salmonella hopR701* (M. O'Connor, in preparation) shows that they are alleles of a gene designated *valU* which encodes GUG, GUA and GUU decoding $tRNA_1^{Val}$. As the map position of *hopE* appears to correspond to another $tRNA_1^{Val}$ gene (*valT*) (54) at a different position, min 17 (*hopR* is at min 52), it is likely that *hopR* and *hopE* encode the same tRNA. Also, as noted above, protein sequencing shows that both suppressors insert a single valine at the site of the same five nucleotides. In any case,

since there are several genes encoding $tRNA_1^{Val}$, each suppressor is able to act in the presence of an abundance of WT $tRNA_1^{Val}$.

sufT621 is a new dominant +1 suppressor. Like *sufJ*, but unlike the other suppressors cited above, it acts at a nonmonotonous sequence. However, that +1 mutant suppression at nonmonotonous sites may not be uncommon is shown by recent studies of Tucker et al. (S. D. Tucker, E. J. Murgola, and F. T. Pagel, manuscript in preparation) (33). These authors have isolated a number of *E. coli* tRNA suppressors in selections for nonsense and missense mutants and later shown that they also suppress +1 frameshift mutants by acting at nonrepeat sequences. For all published +1 suppressors, the amino acid inserted is the one that is normally decoded by the first three bases of the quadruplet. On this basis *sufT621* should insert proline at a CCGU sequence. *sufT621* maps at a different position, 59, from any known tRNA gene including *sufA* and *sufB* (39), which are thought for *sufA* (40) or known for *sufB* (55) to insert proline. Whether *sufT621* affects a $tRNA^{Pro}$ gene (there are three $tRNA^{Pro}$ species [26]) cannot be inferred from the present work. However, preliminary protein-sequencing results indicate that arginine, rather than proline, is inserted at the CCGU sequence (T. Tuohy, unpublished observations). (CGU is an arginine codon.)

The presence of *hisT* results in lack of the pseudouridine modification in the anticodon loop of several tRNAs including $tRNA_1^{Leu}$ (47). Interestingly, *sufJ* requires *hisT* for its suppression of some frameshift mutants, such as *hisG6609* (7), but not for others (23). The sequence ACCC, read by *sufJ* in *hisG6609*, occurs as ACC CUG (7). Competition for the underlined C by CUG-reading $tRNA_1^{Leu}$ may be reduced, with consequent enhanced suppression by *sufJ128*, when $tRNA_1^{Leu}$ is debilitated by lack of pseudouridine in a *hisT1504* background (7, 9). *sufT621* is presumed to read CCGU in the *trpE872* sequence CCG UCU G. In the presence of *hisT1504* suppression of *trpE872* by *sufT621* is markedly reduced, in contrast to the *sufJ* result above. Perhaps the tRNA reading CCGU is directly affected, or conceivably undermodification of the $tRNA_1^{Leu}$ that reads the next codon (CUG) in the new frame causes it to be slow to decode and reduces fixation of the new frame. The possible effect of the latter may be analogous to a rare codon in the new frame.

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LITERATURE CITED

1. **An, G., and J. D. Friesen.** 1980. The nucleotide sequence of tufB and four nearby tRNA structural genes of *Escherichia coli*. Gene **12**:33–39.
2. **Atkins, J. F.** 1980. Non-triplet tRNA-mRNA interactions, p. 439–449. *In* D. Söll, J. Abelson, and P. Schimmel (ed.), Transfer RNA. Part 2: biological aspects. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
3. **Atkins, J. F., B. P. Nichols, and S. Thompson.** 1983. The nucleotide sequence of the first externally suppressible −1 frameshift mutant, and of some nearby leaky frameshift mutants. EMBO J. **2**:1345–1350.
4. **Atkins, J. F., and S. Ryce.** 1974. UGA and non-triplet suppressor reading of the genetic code. Nature (London) **249**:527–530.
5. **Bachmann, B. J.** 1987. Linkage map of *Escherichia coli* K-12, edition 7, p. 807–876. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
6. **Björk, G. R.** 1987. Modification of stable RNA, p. 719–731. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
7. **Bossi, L., T. Kohno, and J. R. Roth.** 1983. Genetic characterization of the *sufJ* frameshift suppressor in *Salmonella typhimurium*. Genetics **103**:31–42.
8. **Bossi, L., and J. R. Roth.** 1981. Four-base codons ACCA, ACCU, and ACCC are recognized by frameshift suppressor *sufJ*. Cell **25**:489–496.
9. **Bossi, L., and D. M. Smith.** 1984. Suppressor *sufJ*: a novel type of tRNA mutant that induces translational frameshifting. Proc. Natl. Acad. Sci. USA **81**:6105–6109.
10. **Cummins, C. M., M. R. Culbertson, and G. Knapp.** 1985. Frameshift suppressor mutations outside the anticodon in yeast proline tRNAs containing an intervening sequence. Mol. Cell. Biol. **5**:1760–1771.
11. **Cummins, C. M., T. F. Donahue, and M. R. Culbertson.** 1982. Nucleotide sequence of the *suf2* frameshift suppressor gene of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **79**: 3565–3569.
12. **Curran, J. F., and M. Yarus.** 1987. Reading frame selection and transfer RNA anticodon loop stacking. Science **238**:1545–1550.
13. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

13a. **Falahee, B., R. B. Weiss, M. O'Connor, S. Doonan, R. F. Gesteland, and J. F. Atkins.** 1988. Mutants of translational components that alter reading frame by two steps forward or one step back. J. Biol. Chem. **263**:18099–18103.

14. **Freifelder, D.** 1978. Molecular biology and biochemistry, p. 275. W. H. Freeman and Co., San Francisco.
15. **Gaber, R. F., and M. R. Culbertson.** 1982. The yeast frameshift suppressor gene *suf16-1* encodes an altered glycine tRNA containing the four base anticodon 3′-CCCG-5′. Gene **19**:163–172.
16. **Hartman, P. E., Z. Hartman, R. C. Stahl, and B. N. Ames.** 1971. Classification and mapping of spontaneous and induced mutations in the histidine operon of *Salmonella*. Adv. Genet. **16**: 1–34.
17. **Herrington, M. B., A. Kohli, and M. Faraci.** 1986. Frameshift suppression by *thyA* mutants of *Escherichia coli* K12. Genetics **114**:705–716.
18. **Holloway, B., and K. B. Low.** 1987. F-prime and R-prime factors, p. 1145–1153. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
19. **Hughes, D., J. F. Atkins, and S. Thompson.** 1987. Mutants of elongation factor Tu promote ribosomal frameshifting and nonsense readthrough. EMBO J. **6**:4235–4239.
20. **Isono, K., and J. Yourno.** 1974. Chemical carcinogens as frameshift mutagens: *Salmonella* DNA sequence sensitive to mutagenesis by polycyclic carcinogens. Proc. Natl. Acad. Sci. USA **71**:1612–1617.
21. **Isono, K., and J. Yourno.** 1974. Non-suppressible addition frameshift in *Salmonella*. J. Mol. Biol. **82**:355–360.
22. **Kawakami, K., Y. H. Jönsson, G. R. Björk, H. Ikeda and Y. Nakamura.** 1988. Chromosomal location and structure of the operon encoding peptide-chain-release factor 2 of *Escherichia*

coli. Proc. Natl. Acad. Sci. USA **85**:5620–5624.
23. **Kohno, T., L. Bossi, and J. R. Roth.** 1983. New suppressors of frameshift mutations in *Salmonella typhimurium*. Genetics **103**: 23–29.
24. **Kohno, T., and J. R. Roth.** 1978. A *Salmonella* frameshift suppressor that acts at runs of A residues in the messenger RNA. J. Mol. Biol. **126**:37–52.
25. **Kraft, R., J. Tardiff, K. S. Kranter, and L. A. Leinwald.** 1988. Using mini-prep plasmid DNA for sequencing double stranded templates with sequenase. Biotechniques **6**:544–547.
26. **Kuchino, Y., Y. Yabusaki, F. Mori, and S. Nishimura.** 1984. Nucleotide sequences of three proline tRNAs from *Salmonella typhimurium*. Nucleic Acids Res. **12**:1559–1562.
27. **Lehner, A. F., and C. W. Hill.** 1985. Merodiploidy in *Escherichia coli-Salmonella typhimurium* crosses: the role of unequal recombination between ribosomal RNA genes. Genetics **110**: 365–380.
28. **Li, M., and A. Tzagoloff.** 1979. Assembly of the mitochondrial membrane system: sequences of yeast mitochondrial valine and an unusual threonine tRNA gene. Cell **18**:47–53.
29. **Low, K. B.** 1972. *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. **36**:587–607.
30. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Mendenhall, M. D., P. Leeds, H. Fen, L. Mathison, M. Zwich, C. Sleiziz, and M. R. Culbertson.** Frameshift suppressor mutations affecting the major glycine transfer RNAs of *Saccharomyces cerevisiae*. J. Mol. Biol. **194**:41–58.
32. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Murgola, E. J.** 1988. *trpA* and its protein: something old, something new . . ., p. 195–209. *In* M. F. Tuite, M. Picard, and M. Bolotin-Fukuhara (ed.), Genetics of translation, new approaches. Springer-Verlag, Berlin.
34. **Murgola, E. J., and F. T. Pagel.** 1980. Codon recognition by glycine transfer RNAs of *Escherichia coli in vivo*. J. Mol. Biol. **138**:833–844.
35. **Ohta, N., P. R. Galsworthy, and A. B. Pardee.** 1971. Genetics of sulfate transport by *Salmonella typhimurium*. J. Bacteriol. **105**:1053–1062.
36. **Pope, W. T., and R. H. Reeves.** 1978. Purification and properties of the tRNA methylase from *Salmonella typhimurium*. J. Bacteriol. **136**:191–200.
37. **Reeves, R. H., and J. R. Roth.** 1975. Transfer ribonucleic acid methylase deficiency found in UGA suppressor strains. J. Bacteriol. **124**:332–340.
38. **Riddle, D. L., and J. Carbon.** 1973. Frameshift suppression: a nucleotide addition in the anticodon of a glycine transfer RNA. Nature (London) New Biol. **242**:230–234.
39. **Riddle, D. L., and J. R. Roth.** 1972. Frameshift suppressors. II. Genetic mapping and dominance studies. J. Mol. Biol. **66**: 483–493.
40. **Riddle, D. L., and J. R. Roth.** 1972. Frameshift suppressors. III. Effects of suppressor mutations on transfer RNA. J. Mol. Biol. **66**:495–506.
41. **Riley, M., and S. Krawiec.** 1987. Genome organization, p. 967–981. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
42. **Riyasaty, S., and J. F. Atkins.** 1968. External suppression of a frameshift mutant in *Salmonella*. J. Mol. Biol. **34**:541–557.
43. **Roth, J. R.** 1981. Frameshift suppression. Cell **24**:601–602.
44. **Sanderson, K. E., and P. R. MacLachlan.** 1987. F-mediated conjugation, F^+ strains and Hfr strains of *Salmonella typhimurium* and *Salmonella abony*, p. 1138–1144. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
45. **Sanderson, K. E., and J. R. Roth.** 1983. Linkage map of *Salmonella typhimurium*, edition VI. Microbiol. Rev. **47**:410–453.
46. **Straus, D. S., and G. R. Hoffmann.** 1975. Selection for a large genetic duplication in *Salmonella typhimurium*. Genetics **80**: 227–237.
47. **Turnbough, C. L., R. J. Neill, R. Landsberg, and B. N. Ames.** 1979. Pseudouridylation of tRNAs and its role in regulation in *Salmonella typhimurium*. J. Biol. Chem. **254**:5111–5119.
48. **Uemura, H., S. Thorbjarnardóttir, V. Gamulin, J. Yano, O. S. Andrésson, D. Söll, and G. Eggertsson.** 1985. *supN* ochre suppressor in *Escherichia coli* codes for $tRNA^{Lys}$. J. Bacteriol. **163**:1288–1289.
49. **van Delft, J. H. M., D. S. Schmidt, and L. Bosch.** 1987. The tRNA-*tufB* operon transcription termination and processing upstream from *tufB*. J. Mol. Biol. **197**:647–657.
50. **Weiss, R. B., D. M. Dunn, J. F. Atkins, and R. F. Gesteland.** 1987. Slippery runs, shifty stops, backward steps and forward hops: −2, −1, +1, +2, +5, and +6 ribosomal frameshifting. Cold Spring Harbor Symp. Quant. Biol. **52**:687–693.
51. **Weiss-Brummer, B., H. Sakai, and F. Kaudewitz.** 1987. A mitochondrial frameshift-suppressor (+) of the yeast *S. cerevisiae* maps in the mitochondrial 15S rRNA locus. Curr. Genet. **11**:295–301.
52. **Wilson, P. G., and M. R. Culbertson.** 1988. *suf12* suppressor protein of yeast. A fusion protein related to the EF-1 family of elongation factors. J. Mol. Biol. **199**:559–573.
53. **Winey, M., M. D. Mendenhall, C. M. Cummins, M. R. Culbertson, and G. Knapp.** 1986. Splicing of a yeast proline tRNA containing a novel suppressor mutation in the anticodon stem. J. Mol. Biol. **192**:49–63.
54. **Yoshimura, M., M. Kimura, M. Ohno, H. Inokuchi, and H. Ozeki.** 1984. Identification of transfer RNA suppressors in *Escherichia coli*. III. Ochre suppressors of lysine tRNA. J. Mol. Biol. **177**:609–625.
55. **Yourno, J., and T. Kohno.** 1972. Externally suppressible proline quadruple CC^U. Science **175**:650–652.
56. **Yourno, J., and S. Tanemura.** 1970. Restoration of inphase translation by an unlinked suppressor of a frameshift mutation in *Salmonella typhimurium*. Nature (London) **225**:422–426.