Genetic Characterization of Frameshift Suppressors with New Decoding Properties

DIARMAID HUGHES,† SHAHLLA THOMPSON, MICHAEL O’CONNOR, THÉRÈSE TUOHY, BRIAN P. NICHOLS, AND JOHN F. ATKINS

Department of Genetics, Trinity College, Dublin 2, Ireland; Department of Biochemistry, University College, Cork, Ireland; Howard Hughes Medical Institute and Department of Human Genetics, University of Utah Medical Center, Salt Lake City, Utah 84132; and Laboratory for Cell, Molecular and Developmental Biology, Department of Biological Sciences, University of Illinois at Chicago, Chicago, Illinois 60680

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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 S2 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 nonmonotonic 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 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*tyr* (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 *suF128*, 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*tyr*. Other *Salmonella* +1 frameshift suppressors in addition to *suFD* and *suFT* 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. *suFT2* 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*tyr*, and *suFG* is thought to affect tRNA*arg* (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′G37, 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 or 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, Geneties, 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, *suF2*, 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*tyr* (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.

* Corresponding author.
† Present address: Institute of Molecular Biology, The Biomedical Center, S-751 24 Uppsala, Sweden.
factor 2 (22), although a tRNA methylase deficiency is also found in supK mutants (36, 37). Both supK and EF Tu suppressors are weak relative to the majority of supS alleles and are best selected indirectly through special selective pressures (kirimycin resistance for tfu 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 antranilate (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 trpE91 or trpED24.

Identification of external suppressors at new map positions. External suppressors of trpE91 mapping to the supS 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 supS + -linked marker argH::Tn10 as a donor. Loss of suppression in 10 to 20% of Tet + transductants indicated that the suppressor was likely to be of the supS class, and retention of suppression in all Tet + recombinants indicated a position outside min 89. To identify supS alleles in E. coli, Trp + revertants were transduced to kanamycin resistance with phage grown on MC57 which carries a Tn10-derived Kan + element 47% linked to supS +. Kan + transductants were then scored for loss or retention of the suppressor.

The external suppressor supT621 of the +1 frameshift mutant trpE872 was tested for coselection of histidine +1 frameshift suppressors by sufA, B, C, D, E, F, G, J, or M suppressors. supT621 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 supA-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 Tn10 markers. Double mutants are easily constructed by the introduction of one mutation into a strain bearing the other by transduction via a linked Tn5 or Tn10 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::Tn10 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 histF-F644 deletion with a linked Tn10 (see 2::Tn10), followed by the introduction of his mutants by selection for growth of transductants on histidinol and retention of clones free of the linked Tn10.

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::Tn10, 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 rK- strain ST8612 bearing a trpED2424 deletion. Phage P1 grown on ST8612 F' trpE91 was used to transduce a CH6 derivative lacking srl::Tn10 and btuB markers to growth on anthranilic acid, thereby generating strain CH19.

Introduction of sufs, suft, suft, and histI loci into strains. The following linked antibiotic resistance markers here were used to transfer the above loci: argH::Tn10 or zii-614::Tn10 for sufs, rsl::Tn10 for suft, zii-614::Tn10 or argH::Tn10 for suft, and zeij-636::Tn5 for histI.

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 LCMB DNA synthesis Facility, University of Illinois) as a primer.

Transduction, Hfr crosses, 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 passage 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

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. This cross 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 tRNAY dependence 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, 52, 53, 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 putB12 (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 strict selective conditions. Among 243 revertants, 2 were external. Loss of the plasmid putB12 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. 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 B1) and screened for ptsH and hopR1. The frequency of the "quadruple crossover" class is interpreted to give the order hopR ptsH cysA.
hopR701 with a cysA::Tn10 marker as the donor, 11 (6%) of 179 Tet' 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\textsubscript{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::Tn10) as the donor, 103 (93%) of 120 Tet' transductants showed loss of the hopE phenotype. All 120 were nadA mutants, apart from 1 which was presumably due to a Tn10 transposition. These results suggest, but do not establish, that hopE1 may also be an allele of a gene for tRNA\textsubscript{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 (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 sufT621, and the donor strain ST106, which contains trpE872 sufT621, resulted in 40% loss of the suf::Tn10 on selection for suppression. A second transduction with ST106 as recipient and the suf::Tn10 recA-containing strain SGSC74 as donor indicated that recA was between suf::Tn10 and sufT621 (Table 2).

### 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 suf\textsuperscript{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 suf::Tn10; 38% of Tet', efficient malate-utilizing transductants exhibited the suppression phenotype, but none was UV sensitive. (One of the recA phenotypes, UV sensitivity, is not expressed in

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

<table>
<thead>
<tr>
<th>Classes of recombinants</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>srl::Tn10 rec\textsuperscript{+} sufT621</td>
<td>45 (34)</td>
</tr>
<tr>
<td>srl::Tn10 recA1 sufT621</td>
<td>25 (19)</td>
</tr>
<tr>
<td>srl::Tn10 recA1 suf\textsuperscript{+}</td>
<td>60 (46)</td>
</tr>
<tr>
<td>srl::Tn10 rec\textsuperscript{+} suf\textsuperscript{+}</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

*In the cross ST106 (trpE872 sufT621) × SGSC74 (srl::Tn10 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::Tn10 recA sufT621.*

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**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.**

**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.
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, hisD3580, hisD374987, 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 UCU (in hisG6609) or UCGU (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 CCGU. Since the sequence CCGU, shared by hisG6609 and trpE872, is a candidate for the suppression site, we attempted genotypically 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 as including tRNA{sup}_Ala (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 Tn5 from TT2835; 64% of the Kan' 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 Tn10 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 hopEl 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 hopRI and hopEl insert a single amino acid, valine, for the five-nucleotide sequence GUUUA. (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 repair of the tRNA to the overlined GUA (or GUG) triplet (13a). Supporting this interpretation are recent protein-sequencing results, which have shown that hopRI 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 a GUA-reading tRNA{val}A{sup}. The map position of hopE appears to correspond to another tRNA{val}A{sup} 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$^\text{Val}$, each suppressor is able to act in the presence of an abundance of WT tRNA$^\text{Val}$.

$sufT621$ 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. However, $sufT621$ affects a tRNA$^\text{Pro}$ gene (there are three $E. coli$ tRNA$^\text{Pro}$ 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$^\text{Leu}$ (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$^\text{Leu}$ may be reduced, with consequent enhanced suppression by $sufJ128$, when tRNA$^\text{Leu}$ is debulked 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 RNA reading CCGU is directly affected, or conceivably undermodified of the tRNA$^\text{Leu}$ 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


