JOURNAL OF BACTERIOLOGY, July 1989, p. 3824–3830
0021-9193/89/073824-07$02.00/0

Vol. 171, No. 7

Suppression of a −1 Frameshift Mutation by a Recessive tRNA Suppressor Which Causes Doublet Decoding†

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Received 30 January 1989/Accepted 1 April 1989

***sufS* was found to suppress the only known suppressible −1 frameshift mutation, *trpE91*, at a site identified as GGA and mapped within the single gene of the only tRNA that can decode GGA in *Escherichia coli*. It mapped to the same gene in *Salmonella typhimurium*. *sufS* alleles were recessive, and dominant alleles could not be isolated. This is in contrast to all other tRNA structural gene mutations identified thus far that cause frameshift suppression. The recessiveness implies that all *sufS* alleles are poor competitors against their wild-type $tRNA_2^{Gly}$ counterparts. The base G immediately 5′ of the GGA suppression site influenced the level but was not critical for suppression by *sufS601*. From this result, it is inferred that *sufS601* causes frameshifting by doublet decoding.**

Numerous tRNA structural gene mutations that cause +1 frameshifting are known. These were either isolated directly as suppressors of +1 frameshift mutations (for a compilation, see references 28 and 50) or isolated in other selections and later shown to have this property (2; S. D. Tucker, E. J. Murgola, and F. T. Pagel, Biochimie, in press). In contrast, only two categories of −1 frameshift mutation suppressors are known to have alterations in tRNA structural genes. *hopR* and *hopE*, members of one category, are mutations of the four-copy gene for $tRNA_1^{Val}$ (28) and cause a single amino acid, valine, to be inserted at the five-base sequence GUGUG by a mechanism suggested not to involve quintuplet codon-anticodon base pairing (16). The other category is *sufS*, which, as shown here, comprises most of the original set of external suppressors (42) for the *Salmonella typhimurium* −1 frameshift mutation *trpE91* (3). Protein sequencing established that *sufS* does not lead to quintuplet translocation. It causes a −1 shift at the zero-frame sequence CAG GGA GUG (16), resulting in insertion of the amino acids Gln Gly Ser, with the Ser being decoded from the underlined AGU. This paper shows that *sufS* suppressors are alleles of the gene for $tRNA_2^{Gly}$, which decodes GGG and GGA.

Not all known −1 frameshift mutation suppressors are alleles of tRNA structural genes. The *supK* class of *trpE91* suppressors (4) is probably in the gene *prfB*, for polypeptide chain release factor 2 (31, 32), although a tRNA methylase deficiency is also found in *supK* mutants (39, 40). The other known class of −1 suppressors consists of alleles of either gene (*tufA* or *tufB*) for elongation factor Tu (27).

Many of the natural high-level reading frame shifts in normal decoding involve shifts to the −1 frame (6, 9, 15, 29, 51). The determinants known for specification of the shifts are programmed in the mRNA. However, it is not unlikely that further work will reveal the involvement of a particular subset of tRNAs in mediating the shifts. Studies of suppressor tRNAs are expected to highlight features important for such shifting as well as being of interest in their own right.

MATERIALS AND METHODS

Media and bacterial strains. Minimal medium, sugars, amino acids, antibiotics, and other media, including green plates for the recovery of P22-sensitive strains, were as described previously (13). Histidinol was used at 1 mM. Rifampin dissolved in methanol (20 mg/ml) was used at a final concentration of 50 μg/ml. *uvrB* and *recA* phenotypes were both sensitive to UV light. Bacterial strains, plasmids, and bacteriophages are listed in Table 1.

Isolation and identification of *sufS* suppressors of *trpE91*. Revertants resulting from external suppressors, both in *S. typhimurium* and in *Escherichia coli*, were isolated and identified as described elsewhere (28). In *S. typhimurium*, those mapping to the *sufS* region were identified by transduction with the *sufS*-linked marker *argH*::Tn*10* as the donor. Loss of suppression in 10 to 20% of Tet^r transductants indicated that the suppressor was likely to be a *sufS* allele. In *E. coli*, revertants identified as external were transduced to kanamycin resistance with phage grown on MC57 (28), which carries a Tn*10*-derived Kan^r element (47) 47% linked to $sufS^+$. Those that lost the *sufS* phenotype in a proportion of transductants were likely to be *sufS* alleles. (Transformation or transduction of *argH*-linked suppressors with plasmids pTuB11.1 and pTuB12 invariably gave the following result: loss of suppression in the presence of pTuB12 [functional tRNAs] and retention of suppression with pTuB11.1 [nonfunctional tRNAs]. *tufB* alleles are selectable as kirromycin resistant. *argH*-linked suppressors directly selected without the aid of kirromycin give the *sufS* pattern of results with the pTuB plasmids. The multicopy nature of pTuB plasmids does not allow an unequivocal distinction between recessive and dominant alleles of *sufS*. This distinction is made in merodiploids with F′111.)

Procedure to seek dominant alleles of *sufS*. A two-step procedure was used to identify dominant *sufS* alleles. In the first step, independently derived revertants were sought in haploid cells, analyzed by transduction, and separated into three groups: a (linked to *trpE91* [internal revertants]); b (linked to *argH*::Tn*10* [presumptive *sufS* or *tufB* suppres-

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TABLE 1. Bacterial strains, plasmids, and phages

Strain, plasmid, or phage	Genotype or relevant characteristic	Source or reference
Bacterial strains		
S. typhimurium		
SGSC10	*galE*	K. Sanderson
SR305	HfrA *purC*	K. Sanderson (43)
SL4213	*hsdL6 hsdSA29 galE496 metA22 metE551 ilv xyl-404 rpsL120* H1-b H2-e, n, x Fels 2 Nml	J. R. Roth
TT2385	*hisT159 hisO1242 hisG6608 zii-614*::Tn*10*	J. R. Roth
TH42	*metA22 leu-151 proB401 trpE91 sufS601 hisO1242 hisC3737*	This study
TH44	Derivative of SR305 with deletion of *uvrB* and *gal*, UV^S	28
TH51	*trpE91 sufS601 rpoB argH*::Tn*10 galE btuB*	This study
TH66	*trpE91 sufS601 argH*::Tn*10* (Tn*10*-*sufS* ca. 15% linked)	This study
ST108	*trpE91 metF96* F'111 Rif^D	This study
Strains carrying *sufJ128*		J. R. Roth
79 strains carrying *his* frameshift mutations		J. R. Roth
E. coli		
SU1675	CSH26 *rec*	M. O'Connor
CSH57	*ara leu lacY purE gal trp his argG rpsL mal xyl mtl ilv thi metA/B*	J. Miller
CGSC4258	Strain carrying F'111, including region *metA* to *metF*	B. Bachmann
CH19	CSH57 derivative, gal^+ *trpBE9 Salmonella trpE91* (chromosomal integration)	28
MC57	*thr leu thi lacY ara-14 xyl-5 mtl-1 proA2 his-4 rpsL31 tsx-33* Tn*10*–p*tac*–mini-Kan ($sufS^+$ 47% linked)	28
Plasmids		
pTuB11.1 with Tet^r	pBR322 clone carrying functional *tufB*-deleted tRNAs	L. Bosch
pTuB12 with Tet^r	pBR322 clone carrying functional tRNAs and translationally nonfunctional *tufB*	L. Bosch
Phages		
P22HT		Collection of S. Thompson and J. F. Atkins
MG-178-1		Collection of S. Thompson and J. F. Atkins
P1 *virA*		Collection of S. Thompson and J. F. Atkins
BF23		Collection of S. Thompson and J. F. Atkins
C21		Collection of S. Thompson and J. F. Atkins

sors]); and c (linked to neither [presumptive *tufA*, *supK*, *hopR*, *hopE*, or unknown suppressors]). Their growth rates were then matched against that of *sufS601* and the category to which they belonged. A total of 710 independent revertants were tested in this way. All revertants with a faster growth rate than, and four revertants indistinguishable from, *sufS601* were internal (category a; total, 661). Two revertants indistinguishable from *sufS601* and 44 slower-growing revertants were linked to *argH* (category b; total, 46). Three revertants among the 710, all slower growing than *sufS601*, were in category c. This result showed that revertants unequivocally faster growing than *sufS601* are unlikely to contain *sufS* alleles. *sufS601* and the phenotypically indistinguishable *607* and *609* alleles are the most efficient (i.e., fastest growing on minimal media) *sufS* alleles we have ever isolated. They are recessive, as are the *sufS* suppressors in any of the slower-growing revertants tested (see Results). On the basis of this finding, in the second step, dominant alleles were sought in merodiploids with F'111 (23) as follows. Revertants of *trpE91* selected in ST108 under continuous selection for Rif^D (dominant) and Met^+ phenotypes, to ensure the presence of two copies of the $sufS^+$ gene, were compared with *sufS601* mutants, and all mutants except those clearly of faster growth than *sufS601* were retained. To test for dominant alleles on the F', F'111 Rif^D was transferred from each revertant to a *trpE91 argH*::Tn*10* strain, selecting for Tet^r and Rif^r to seek F'-ductants with Trp prototrophy. To test for chromosomal dominant alleles, a Tn*10* marker linked to *sufS* was introduced into each revertant by transduction, and loss of prototrophy was sought in a proportion of the transductants.

Construction of *lac* frameshift mutants. Oligonucleotides were synthesized by an ABI 380A or -B synthesizer, and the crude material was cloned directly. On annealing, the complementary oligonucleotides had overhanging 5' and 3' ends designed for insertion into *Hin*dIII and *Apa*I restriction targets, respectively. Construction of the pBR322-based plasmid vector with a functional *bla* gene, a *tac* promoter for the included *lacZ* gene, and multiple cloning sites, including *Hin*dIII and *Apa*I, is described elsewhere (48). After ligation, an *E. coli rec* derivative of CSH26 (Su1675; Table 1) was transformed by standard procedures (34). The sequences of the plasmid inserts were checked by the dideoxy method of sequencing on double-stranded DNA (11). Transfer to *S. typhimurium* was via transformation into the restriction-minus strain SL4213 (12). From this strain, plasmids were transformed into the suppressor-containing and isogenic parental strains. The dependence of *lac* suppression on the *sufS* allele was confirmed by replacing *sufS* with its wild-type $sufS^+$ via a linked Tn*10* from strain TT2385. The same transductants that became Trp^- because of the loss of *sufS* were now Lac^-. Whole-cell β-galactosidase assays were based on the procedure of Miller (35), with the minor modifications described elsewhere (48). Assay values were also determined on cultures grown in minimal medium and

found to be higher but proportionally very similar. The units presented are those from cells grown in LB broth.

Rif^r, Rif^D, and BF23^r selection. Rif^r mutants were selected for resistance to 50 μg of rifampin per ml in haploid strains (30). Many of these mutants are recessive and arise at a frequency of 10^{-8}. Rif^D mutants (38) were sought in the same way but in merodiploid strains with F'111. One mutant resulting from a change on the plasmid-borne copy of *rpoB* was distinguished from those with alterations of the chromosomal copy. F'111 fragmentation led to loss of the F'-borne Rif^D unless selection was maintained. Phage BF23 grows on all *E. coli* strains but only on *galE* mutants of *S. typhimurium* (20). Selection for BF23 resistance in *galE Salmonella* strains yields mutations in the *btuB* gene (20). Up to 100 colonies can appear in the area of lysis of a spot of 10^9 BF23 particles on a lawn of *Salmonella* cells because of either loss of *galE* or mutations in *btuB*. Loss of *galE* is identifiable by resistance to phage C21.

Other methods. Integration of *trpE91* into the *E. coli* chromosome, introduction of *sufS* and *sufJ* into desired strains, construction of *trpE his* double mutants, intraspecies transfer of F' and plasmids, and F' and plasmid elimination were carried out as described previously (28). Hfr and intraspecies F' transfers and P1 transduction in *E. coli* were done as described by Miller (35). Transduction in *S. typhimurium* was performed as described elsewhere (13, 44).

RESULTS

Mapping of *Salmonella sufS* suppressors. Initial mapping was performed with one of the stronger members, *sufS601*, of the original set of suppressors for the −1 frameshift mutation *trpE91*. (*sufS601* in the earlier report [42] had been designated *sup-601*.) The approximate location of *sufS* was determined by Hfr mapping. The origin of HfrA in *Salmonella* strain TH44, which also has deletions in *uvrB* and *gal*, is at min 81, and the chromosome is transferred clockwise. Interrupted mating between TH44 and the *metA leu sufS601* strain TH42, using *uvrB* as a counterselective marker, resulted in 30% of donor *metA*$^+$ (min 89.5) recombinants coinheriting the wild-type *sufS*, whereas only 8% of *leu*$^+$ (min 2.8) recombinants were *sufS*$^+$. This procedure located *sufS* closer to min 89. The *rpoB* locus maps at min 88.5. P1 transduction crosses between a rifampin resistance allele of *rpoB* in a derivative of TH44 and a *galE* derivative of TH42 demonstrated a closer linkage of *sufS* to *rpoB* (88%) than to *metA* (35%). Three-point test crosses (see Materials and Methods) indicated the order

89.5 88.5
metA.....*rpoB*.....*sufS*

An additional transduction, using the P22-like phage MG178-1, showed 17% linkage of *sufS* to *argH*::Tn*10*, and three-point test crosses indicated the order

89.5 88.5 88.1
metA.....*rpoB*.....*sufS*.....*argH*

In *E. coli*, *btuB* is known to map between *rpoB* and *argH*, approximately 3 kilobase pairs (kbp) from *argH* (20). We mapped an equivalent gene in *S. typhimurium*, also between *rpoB* and *argH* and close to the latter. The results of four-point test crosses (Fig. 1 and Table 2), together with the above data, gave the order *metA*....*rpoB*....*sufS*....*btuB*.... *argH*.

In addition to *sufS601*, we isolated 22 other suppressors with similar map positions. The gene for tRNA$_3^{Thr}$ (*thrT*) is located between *rpoB* and *btuB* (1, 5, 25). Transduction crosses between strains containing a *sufJ* allele of *thrT* (*sufJ128*) and *sufS601* yielded recombinants containing either *sufS* or *sufJ* but not both, which indicated tight linkage of the two markers. We isolated spontaneous *sufS* alleles in the presence of *sufJ128* to demonstrate that they are not incompatible.

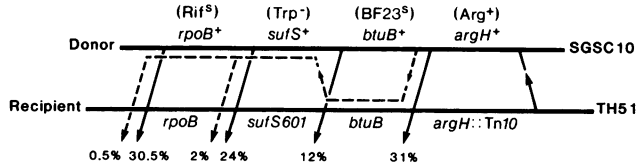

FIG. 1. Transductional mapping of *sufS*. Selection was for Arg$^+$, and transductants were scored for resistance to phage BF23 and rifampin and for the presence of *sufS*. The relative order of *sufS* and *btuB* depicted is that which best fits the data of Table 2, in which the frequency of the predicted quadruple-crossover classes 5 and 6 (class 7 is not shown), as expected, is low.

These data, taken together, indicated a position of *sufS* between min 88.1 and 88.5 tightly linked to the *thrT* locus. This region in *E. coli* contains the *tufB* operon (comprising four tRNA genes in the order *thrU tyrU glyT thrT tufB*) flanked by *rrnB* and *rpl* gene clusters (1, 25). In *S. typhimurium*, the *thrT* and *tufB* genes were known to be in the same positions as in the *E. coli* counterparts, with the *tufB* gene starting 115 bp downstream from the 3' end of the tRNA gene cluster (5, 26; L. Bossi and D. Dunn, personal communication). This is very similar to the 114-bp spacing in *E. coli*. Only six nucleotides separate *glyT* from *thrT* in *E. coli* (1, 10, 25). It is now known that the *glyT* gene is also very close to *tufB*, being separated from it by only 197 bp in *S. typhimurium* (5; D. O'Mahony, unpublished data). A complementation test was used to determine whether *sufS* was likely to be in the four-tRNA-gene cluster, in *tufB*, or in neither. An earlier study (27) had shown that some alleles of *tufB* are *trpE91* suppressors; therefore, although the *sufS* alleles are largely phenotypically distinguishable from the *tufB* alleles by growth rate, the possibility that the *sufS* suppressors constituted a different class of *tufB* alleles had to be considered seriously. Since *sufS* is recessive (see below), we considered the possibility that introduction of plasmid-borne wild-type copies of either *tufB* or the tRNA gene cluster might mask the phenotype of this gene.

Plasmids pTuB11.1 and pTuB12, constructed by J. van Delft and L. Bosch, are pBR322 derivatives carrying a cloned *tufB* operon. pTuB11.1 has a functional *tufB* but an

TABLE 2. Result of a four-point test cross to establish the position of *sufS601* relative to *btuB* and *argH*[a]

Transductant class	Phenotype	No.	%
1	Arg$^+$ BF23^r Trp$^+$ (*sufS*) Rif^r	68	31
2	Arg$^+$ BF23^s Trp$^+$ (*sufS*) Rif^r	26	12
3	Arg$^+$ BF23^s Trp$^-$ (*sufS*$^+$) Rif^r	52	24
4	Arg$^+$ BF23^s Trp$^-$ (*sufS*$^+$) Rif^s	66	30.5
5	Arg$^+$ BF23^r Trp$^-$ (*sufS*$^+$) Rif^r	4	2
6	Arg$^+$ BF23^r Trp$^-$ (*sufS*$^+$) Rif^s	1	0.5
7	Arg$^+$ BF23^s Trp$^+$ (*sufS*) Rif^s	0	0

[a] Results from the cross shown in Fig. 1. Both donor and recipient strains are *galE*, which confers phage BF23 sensitivity. BF23 resistance results from an additional mutation in the *btuB* gene. Rifampin resistance arises from mutations in the *rpoB* gene.

approximately 400-bp deletion spanning the tRNA genes (46). In the isogenic pTuB12, in contrast, the four tRNA genes are functional but there is a 240-bp deletion within *tufB* (45). Introduction of these plasmids into 22 *sufS Salmonella* strains, linked to *argH* at min 88, and into a *sufS601* strain resulted in the retention of *sufS* expression in the presence of pTuB11.1 (nonfunctional tRNAs) but loss of expression in the presence of pTuB12 (functional tRNAs). In contrast to *sufS*, alleles of *tufB* showed loss of expression in the presence of the elongation factor EF Tu-encoding plasmid pTuB 11.1. Segregants of each strain with *sufS* (pTuB12) lacking pTuB12 regained *sufS* expression. These results, together with the transduction mapping data, indicated that *sufS* is allelic either to one of the four tRNA genes or to a tightly linked gene adjacent to the tRNA gene cluster whose expression is masked in the presence of multiple copies of the tRNA genes.

E. coli sufS. As part of a previous study (28), *trpE91* was transferred into the chromosome of an *E. coli* strain with a deletion of the *E. coli* tryptophan operon to give strain CH19. In that study, the *hopR* category of *trpE91* suppressors was analyzed and found to constitute a higher proportion of the suppressors than in *Salmonella* strains, in which they are rare. Two of the non-*hopR* suppressors, *suf-3* and *suf-519*, noted among the Trp^+ revertants were used for the study described here. Because of the mapping results for *S. typhimurium* (see above), *suf-3* was tested for linkage to the *rpoB-btuB* region of the *E. coli* chromosome. In P1 transductions, the results showed 77% linkage to *rpoB*, 78% linkage to *btuB*, and 37% linkage to *argΔBC* (contiguous with *argH*). *suf-519* mapped to the same region. Both were established as presumptive alleles of *sufS* by the plasmid complementation test and designated *sufS3* and *sufS519*. Expression of each allele was masked by the presence of plasmid pTuB12 (expressed tRNAs) but not by the presence of pTuB11.1. The importance of finding a *sufS* class of suppressors in *E. coli* similar to the *Salmonella* counterpart becomes apparent (see Discussion), as more is known in *E. coli* of the singularity of the gene to which *sufS* is allelic.

***sufS* is recessive, and dominant alleles could not be isolated.** The *E. coli* F′ factor F′111 includes the region *metA* to *argH*. *Salmonella* strain TH66, which is *argH*::Tn*10* and contains *trpE91 sufS601*, is Arg^+ but tryptophan requiring in the presence of F′111, which indicates that *sufS* is recessive. Fortuitously, F′111 fragments readily. Loss of the $sufS^+$ gene results in segregants of TH66/F′111 regaining the *sufS601* phenotype. Such segregants arise at an extremely high frequency unless selection is maintained for F′ material spanning *sufS* on both sides. A derivative, F′111 Rif^D, was isolated by selection for a dominant rifampin resistance allele of *rpoB* (80 to 88% linkage to *sufS*). When maintenance of F′ material from *argH* to *rpoB* was forced in TH66/F′111 Rif^D, *sufS* expression was completely masked and segregants regaining the *sufS* phenotype did not arise. $MetA^+$ selection was substituted for Rif^r selection in another strain, with comparable results. Similar experiments were done with the *sufS* alleles *605*, *607*, *609*, *617*, *625*, and *627*, with the same result. All of the *sufS* alleles tested in *S. typhimurium* were recessive. In contrast, F′111 Rif^D did not affect the phenotype of a *hopE* allele. For the equivalent experiment in *E. coli*, in which the same F′ could recombine with the chromosome at a much higher frequency since it was of *E. coli* origin, a *recA* derivative of a strain with *sufS3* was used. F′111 Rif^D was introduced. *sufS3* was also found to be recessive, with haploid segregants regaining the *sufS* phenotype. At one time, it was considered likely that a recessive property of frameshift suppressors indicated that the suppressors were likely to be allelic with a tRNA modification enzyme gene rather than with a tRNA structural gene (41), but the results presented above show that this is not always necessarily so.

The recessive nature of all of the *sufS* alleles tested prompted a search for dominant *sufS* suppressors. F′111 Rif^D was introduced into the suppressor-free *Salmonella* strain *trpE91 metF96* to generate ST108. Continuous selection for *rpoB* (Rif^D) and $metF^+$ genes, which span $sufS^+$ on F′111 Rif^D, ensures that ST108 contains two copies of the wild-type $sufS^+$ gene. Any *sufS* allele isolated should therefore be dominant. A total of nearly 1,000 Trp^+ revertants from 100 independent cultures were selected and tested (see Materials and Methods). No *sufS* alleles were found. The results were taken to indicate that all retained revertants of ST108 were attributable to reversions either within *trpE* or in genes other than *sufS* and that dominant alleles are extremely rare or do not occur. The possibility that multiple mutations could give rise to a dominant *sufS* allele cannot, of course, be excluded.

Specificity of *sufS601*. Previous results (42) showed that *sufS607* and *sufS609* did not suppress the 8 *hisC* frameshift mutations *13*, *122*, *146*, *377*, *540*, *855*, *856*, and *857* and that *sufS607* did not suppress 18 amber, 6 ochre, or 2 UGA mutations. Since that time, several suppressible +1 frameshift mutations have been reported (cited in references 28 and 50). The suppressible +1 frameshift *Salmonella* mutations *hisD3749*, *hisC3734*, *hisC3737*, *hisF3704*, *hisC3072*, *hisC3736*, *hisD6580*, *hisG6609*, *hisD3749S7*, and *trpE872* were studied mainly by J. Roth and colleagues (see reference 28). For the study presented here, these mutations were tested for suppression by *sufS601* and 25 other *sufS* alleles. No suppression was detected. *sufS601* was then tested with 69 other known *Salmonella his* frameshift mutations (18, 19, 24, 33, 49), the entire number in the collection of J. Roth, and again no suppression was detected.

To delimit the site of suppression and determine its specificity, we then turned to synthesizing *lacZ* mutants by using recently developed methods (48). Complementary DNA oligonucleotides that recreated the *trpE91* suppression window (Table 3) were cloned just downstream of the start codon of a plasmid-borne *lacZ* gene such that ribosomal frameshifting within the window was required to give β-galactosidase. Initial experiments first showed that the suppression site was in the second half of the window and subsequently that the sequence CAG GGA sufficed (construct a in Table 3). Note that the serine AGU codon overlapping the GGA in *trpE91* is not necessary. GGA is crucial to suppression. Changing the first or last nucleotide of the GGA to C or G, respectively (constructs b and c in Table 3), abolished suppression. The levels of β-galactosidase with these constructs were indistinguishable in the *sufS601* mutant or in its isogenic suppressor-free parent. This result is noteworthy when the last nucleotide is changed to generate GGG; its significance is considered in Discussion. In additional constructs, with sequences similar to that of construct c, which has the in-frame GGG, different codons, including AGA (which, like AGG, is a rare codon and so may be slow to decode [7]), were placed immediately 3′ of the GGG codon. These also showed no suppression by *sufS601* (data not shown). The CAG codon immediately 5′ of the GGA was found not to be necessary for suppression, but the identity of the nucleotide 5′ of the GGA did influence the level of suppression (Table 3). G at this position (construct a) gave approximately twice the level of suppression of A, U,

TABLE 3. Specificity of *sufS601* suppression assayed on synthetic *lacZ* mutants[a]

	Construct	Frameshift required	Sequence	β-Galactosidase activity (U)	
				sufS601	*sufS* (wild type)
a		−1	AGC UUU AAC | CAG GGA ACC | UAA ACU CGG GCC	616	38
		−1	AGC UUU AAC | CAG NGN ACC | UAA AUC CGG GCC		
b			C A	18	18
c			G G	21	20
		−1	AGC UUU AAC | CAN GGA ACC | UAA ACU CGG GCC		
d			A	281	42
e			U	328	36
f			C	353	32
g		+1	AGC UUA AAC | CAG GGA ACC | UAA AUC GGG CC	30	35
h		0 (stop hop)	AGC UUC GGA UAA GGG AAC GGC C	19	12
	trpE91 suppression window		UUU GAU GCG UUC CGU CUG UUA | CAG GGA GUG | UGA		

[a] The sequences shown are those of one strand of the oligonucleotides inserted six nucleotides 3′ of the start of the *lacZ* gene. In the −1 and +1 frame constructs, ribosomes are required to shift frame in the window delimited by the overlined stop codons to enter the zero frame of the main body of the downstream *lacZ* gene. With the zero-frame construct, the ribosomes are required to maintain frame but to bypass or read through the UAA stop codon to produce β-galactosidase. Units of β-galactosidase are as defined by Miller (35). The frameshift window of the *trp* mutant suppressible by *sufS* is shown for comparison.

or C (construct d, e, or f). The observed level of suppression was directly related to the presence of *sufS601*. Replacement with *sufS*$^+$ (by transduction) in the plasmid-bearing strains reduced the β-galactosidase level to that of background. (The different background levels of β-galactosidase between constructs b and c on one hand and those in constructs a, d, e, and f very likely resulted from the effect of the U three bases 3′ of the stop codon in constructs a, d, e, and f on a very low level of internal translation initiation at the triplet UCG, which was not previously known to be capable of acting as an initiator.) We estimate the level of suppression with construct a to be of the order of 2% of the in-frame level of β-galactosidase. (This value is approximate because of the unhealthy state of cells with very high levels of β-galactosidase from the *tac* promoter on the multicopy plasmid and some variation in in-frame control values with different sequences.)

No +1 frame suppression by *sufS601* was apparent, at least with the sequence in construct g (Table 3). These results on the high degree of specificity of *sufS* are not inconsistent with the lack of suppression of the *his* mutants tested (see above). A striking feature of the other class of tRNA suppressors of *trpE91*, *hopR*, is their ability to cause hopping. With *hopR1*, flanking of a UAA stop codon by its target codon (e.g., GUG UAA GUG) results in suppression, with one amino acid being inserted for the nine nucleotides (M. O'Connor et al., manuscript in preparation; 16). With a sequence analogous to that which worked for *hopR*, no suppression by *sufS601* was detected (construct h in Table 3).

DISCUSSION

Previous protein-sequencing results had shown that *sufS601* causes the sequence CAG GGA GUG to be decoded as Gln Gly Ser, with the Ser being encoded by the underlined AGU (16). The genetic analysis presented here maps *sufS* to a cluster of four adjacent tRNA genes, one of which is *glyT*, the gene for $tRNA_2^{Gly}$ which decodes GGA and GGG. Results of *sufS601* suppression of the *lac* constructs delimit the suppression site to GGA, with GGG being nonsuppressible. At least for normal in vivo conditions, GGA is decoded only by $tRNA_2^{Gly}$, for which only one gene, *glyT*, exists in *E. coli* (22, 36, 37). The lack of suppression of a GGG site by *sufS601* is compatible with *sufS* being allelic with *glyT* since, unlike GGA, GGG is also decoded by $tRNA_1^{Gly}$ (22, 36). A $tRNA_2^{Gly}$ encoded by a mutant *sufS* may not be competitive with wild-type $tRNA_1^{Gly}$ for GGG reading, or the interaction of the mutant $tRNA_2^{Gly}$ with GGG may be better than with GGA and therefore not prone to cause frameshifting. We conclude that *sufS* alleles are mutations of *glyT*. Our collaborators B. H. Mims and E. J. Murgola and their colleagues have determined the altered tRNA sequence and base modification pattern of $tRNA_2^{Gly}$ encoded by several *sufS* alleles, and DNA sequencing has pinpointed the changes at the *glyT* gene level in the same and other *sufS* alleles (D. J. O'Mahony, B. H. Mims, S. Thompson, E. J. Murgola, and J. F. Atkins, manuscript in preparation). Since earlier results have shown that in *E. coli* there is only one gene for the sole tRNA that decodes GGA (22, 36, 37), we conclude that *sufS* alleles retain triplet-decoding ability.

The recessive property of *sufS* indicates that the tRNA conformational states that promote frameshifting are poor competitors against their wild-type tRNA counterpart. The inability to isolate dominant alleles confirms that single-step mutations in $tRNA_2^{Gly}$ that render the suppressor tRNA more competitive with the wild type at the frameshift site do not occur or occur exceedingly rarely. This finding is somewhat surprising. The 6% level of *sufS* alleles among all *trpE91* revertants found in *Salmonella* haploid cells is reduced to zero in merodiploids. This is without the contraints of having to retain normal triplet decoding by one of the two (or three) available *glyT* alleles. Why are *sufS* alleles recessive to their wild-type *glyT* counterpart? The aminoacylation ability of several missense suppressor mutants of $tRNA_2^{Gly}$ is greatly reduced (21, 36), and Mims and Murgola (O'Mahony et al., in preparation) have found that this is also the case for the $tRNA_2^{Gly}$ encoded by some, but not others, of the *sufS* alleles. Thus, decreased aminoacylation ability cannot be the only explanation for *sufS* recessivity. It is possible that the mutant charged $tRNA_2^{Gly}$ is defective in its interaction with

elongation factor Tu or is discriminated against at the proofreading stage.

The experiment in which the base 5′ flanking to the GGA suppression site was varied permits a distinction to be made between the two main models for suppression, originally suggested by the protein-sequencing results and the correspondence of *sufS* to *glyT*. *sufS* alleles were isolated as suppressors of *trpE91*, which has the sequence CAG GGA at the suppression site. In one model, the *sufS* mutant form of $tRNA_2^{Gly}$ pairs with GGA less stably than would wild-type $tRNA_2^{Gly}$; it disengages from the GGA but remains within the ribosome. On occasions when the message slips back one base, it re-pairs with the underlined GGG in a triplet interaction. The A of the GGA would then be available to be the first nucleotide of the next AGU codon, with a resultant shift to the −1 frame. An analogous model appears to be the explanation for the other category, consisting of *hopR* and *hopE*, of tRNA suppressors for *trpE91* except that the shift there is +2 rather than −1 (16). Since certain wild-type tRNAs also have this capability (48), the phenomenon may be of general interest. In the second model, the pairing of the first two bases of the GGA codon to the anticodon bases 36 and 35 does not get disrupted. In a specific version of this model, the third codon base is occluded by its anticodon counterpart, base 34, most but not all of the time; i.e., the wobble base 34 wafts close to and away from the third codon base, as dictated perhaps by a conformational flux of the tRNA (third-position "waffle"). When it is distant from the third codon base, the latter is available to become the first nucleotide of a −1 frame AGU codon. Thus, frameshifting is caused by doublet decoding. This model is somewhat distinct from the only previous examples, in which doublet codon-anticodon interaction was the cause of frameshifting (8, 14, 17). In the prior example, which was in vitro, there was no hint of occlusion of the third codon base (8, 14, 17) and a noncognate codon-anticodon interaction was involved, in contrast to the cognate interaction involved with *sufS*.

An influence of the base 5′ flanking to the GGA codon on the suppression mediated by *sufS601* is apparent from the results presented here. The disengagement–re-pairing model requires a G in the 5′ position. However, while suppression is best with G in the 5′ position, it also occurs at a high level with A, C, or U. We interpret the latter result as indicating that the second model operates in any case. Whether the influence of the 5′ G represents some context effect on a mechanism that does not involve disengagement or whether the disengagement–re-pairing is also operative at a certain level has not been resolved. Different *sufS* alleles have their alterations from wild type at several different positions in *glyT* (O'Mahony et al., in preparation). When the characterization of all alterations in *glyT* that give rise to suppressors is complete, it will be worthwhile to check an example of each type for its specificity of suppression. Not all alleles may use the same mechanism, and such information may provide a clue to the reason for the different mechanisms of suppression by *sufS601* and *hopR1*.

ACKNOWLEDGMENTS

We thank Dymphna Hill for spirited assistance, Thérèse Tuohy and Michael O'Connor for performing some experiments and for comments on the manuscript, John Roth for generous access to his culture collection, Leen Bosch for the invaluable pTuB plasmids and hospitality to J.F.A., and Bob Weiss for major contributions to the construct approach. We thank Ray Gesteland for traditionally generous support and Ray Gesteland and Manny Murgola for comments on the manuscript.

This work was supported by grants from the Irish National Board for Science and Technology/Eolas (to S.T. and J.F.A.), by National Science Foundation grant DMG-8408649 (to Ray Gesteland and J.F.A.), and by the Howard Hughes Medical Institute.

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