Nucleic Acids Research, Vol. 20, No. 13 3463–3469

Insertion (*sufB*) in the anticodon loop or base substitution (*sufC*) in the anticodon stem of $tRNA^{Pro}_2$ from *Salmonella typhimurium* induces suppression of frameshift mutations

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Received February 12, 1992; Revised and Accepted May 5 1992 EMBL accession nos X63776 and X63777

ABSTRACT

The dominant +1 frameshift suppressors *sufA6*, *sufB1* and *sufB2*, in *Salmonella typhimurium* act at runs of C and affect $tRNA^{Pro}_1$, $tRNA^{Pro}_2$ and $tRNA^{Pro}_2$, respectively. A recessive +1 frameshift suppressor, *sufC*, has a similar suppressor specificity (Riddle,D.L., and Roth,J.R., *Mol. Biol.* 66, 483 and 495, 1972). We show that *sufC* strains harbour two frameshift suppressors of which one, *sufX201*, is allelic to *sufB*. We cloned the $sufB^+$ wild type allele and by recombination *in vivo* the mutations *sufB1*, *sufB2* and *sufX201*. Determination of the DNA sequence revealed that the *sufB1* and *sufB2* mutations result in an extra G in the anticodon loop of the minor $tRNA^{Pro}_2$. The *sufX201* mutation results in a base substitution (G43 to A43) in the anticodon stem of this tRNA. Although the *sufB1* and *sufB2* mutations were earlier shown to be dominant, the $sufB^+$ wild type allele on multi copy plasmid inhibited the chromosomal *sufB1*, *sufB2* and *sufX201* mediated frameshift suppression but not that mediated by the dominant *sufA6* mutation. These results are discussed in view of the possible coding specificity of these mutated tRNAs. The DNA sequence showed a potential consensus promoter sequence upstream of the structural gene for $tRNA^{Pro}_2$ and downstream a dyad symmetrical structure followed by a T cluster, a possible rho-independent termination signal. The *Salmonella* $tRNA^{Pro}_2$ gene is identical to the *Escherichia coli* counterpart reported by Komine,Y. *et al.* (*J. Mol. Biol.* 212, 579–598, 1990). While the 5′ flanking sequence similarity between the two species is about 83%, the similarity of the 3′ flanking sequence is only 42%. Still, the *Salmonella* $tRNA^{Pro}_2$ gene has a rho-independent transcriptional termination signal similar to the one present in *E.coli* $tRNA^{Pro}_2$ gene.

INTRODUCTION

Nontriplet reading resulting in a shift in the reading frame has been shown to occur in both eubacteria and eukaryotes (1). Shifts in the normal reading frame are involved in leaky expression of genes, expression of overlapping genes resulting in two or more products from the same part of the mRNA, and in regulation of gene expression. The role of tRNA in such non-triplet reading was early established by the isolation of mutant tRNAs able to suppress certain frameshift mutations. The first suppressors of this kind to be characterized were the *sufA*, *sufB*, *sufC*, *sufD*, *sufE* and *sufF* (2). The *sufD42* was shown to be a mutated tRNA, with an anticodon CCCC that enables it to read GGGG (3). Protein sequencing of the suppressed product was first achieved for the *sufB2* mediated suppression of the *hisD3018* mutation (4). It was found that proline was inserted in the suppressed polypeptide consistent with the fact that the *sufB2* mutation also resulted in a changed chromatographic property of $tRNA^{Pro}_2$ (5). However, the nature of mutations at the *sufB* locus has so far not been established. This is also true for the recessive *sufC10* frameshift suppressor, which has a specificity of suppression reminiscent of that of *sufB1* and *sufB2* (2). To understand the mechanism by which a mutated tRNA can correct a reading frame error, it is necessary to know both the mRNA sequence at the frameshifting site and to identify the change in the tRNA molecule causing the frameshift to occur. This paper addresses the latter question with respect to *sufB1*, *sufB2* and *sufC10* mutations.

The two dominant frameshift suppressors, *sufA6* and *sufB2*, induce a changed chromatographic behaviour of $tRNA^{Pro}_1$ and $tRNA^{Pro}_2$, respectively (5,6). The *sufA6* and *sufB2* mutations are located at minutes 77 and 45, respectively, on the *Salmonella typhimurium* chromosome. Both *sufA6* and *sufB2* are +1 frameshift suppressors and act at runs of C (6). Although the molecule that mediates the frameshift suppression in *sufA6* and *sufB2* strains has not been identified, the facts cited above strongly suggest that they must be mutant derivatives of $tRNA_1^{Pro}$ and $tRNA_2^{Pro}$, respectively. The recessive *sufC10* mutation was initially located at min 15 on the *Salmonella* chromosome (7). However, we here report that the *sufC10* strain has a mutation, called *sufX201*, which is allelic to *sufB2*. The recessive nature of the *sufX201* mutation suggested that the suf^+ gene might encode a tRNA modifying enzyme. If so, the suppressing agent in *sufX201* cells might be an undermodified $tRNA^{Pro}$. Thus, on

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one hand the dominant nature of *sufB2* suggests a mutant tRNA, but on the other hand the recessive nature of the allelic *sufX201* mutation contradicts this. These apparently conflicting results further urged us to establish the identity of the dominant *sufB1* and *sufB2* mutations. We note, however, that recessive tRNA mutations have been characterized (see e.g. ref. 1).

1-methylguanosine is present on the 3′ side of the anticodon in all three $tRNA^{Pro}$ species of *S.typhimurium* (8). The structural gene *trmD*$^+$ for the enzyme tRNA(m^1G37) methyltransferase, which catalyses the formation of 1-methylguanosine (m^1G) in position 37 of the tRNA, is located at min 56 on the *Salmonella* chromosome. The recessive *trmD3* mutation causes a m^1G deficiency in $tRNA^{Pro}$ at 37°C and this results in frameshifting at many if not at all *his* operon frameshift mutation sites as those suppressed by the *sufA6* and *sufB2* mutations (9). Thus, undermodification can cause a suppressor phenotype. Although the *trmD3* mutation reduces the growth rate at 42.5°C, the mutant is still viable. The growth rate of *sufA6*, *sufB2* or *sufX201* mutants is not affected by high temperature. However, the double mutant *sufX201*, *trmD3* is unable to grow at 42.5°C on rich media. Since both the *sufX201* and *trmD3* mutations are recessive, it should be possible to complement either the *trmD3* or the *sufX201* mutation. Such a cell should have a temperature resistant phenotype. We have utilized this approach to clone the *sufX*$^+$, allele. Since the *sufX* mutation is allelic to *sufB*, the plasmid isolated by us harbours the wild type copy of the *sufB* gene. By recombination *in vivo* the *sufB1* and *sufB2* mutations were also cloned. This allowed an analysis of the *sufB* gene. Results presented in this paper show that both the *sufB1* and *sufB2* mutations result from an insertion of a G in the anticodon region of the $tRNA^{Pro}_2$ gene while *sufX201* is the result of a base substitution (G43 to A43) in the anticodon stem.

Table 1

Strains used *S.typhimurium*	Genotype
GT344	*hisO1242, hisD6404*
GT684	*hisO1242, hisC3737, sufA6*
GT782	*hisO1242, hisC3737, sufA6, trmD3*
GT784	*hisO1242, hisD3018, sufB2, trmD3*
GT833	*zee-2509*::Tn*10* (58% linked to *sufB*; 90% to *hisW*
GT850	*zee-2502*::Tn*10* (90% linked to *sufB*; 88% to *hisW*
GT853	*hisO1242, hisC3737*
GT854	*hisO1242, hisC3737, trmD3*
GT967	*hisO1242, hisC3737, sufX201, trmD3, srl-202*::Tn*10, recA1*
GT983	gene bank from strain GT344 in plasmid pLG339/*metA22, metE551, trpB2, ilv-452, xyl-404 rpsL120, fluA66, hsdL66, hsdA29, galE503, srl-202*::Tn*10, recA1*
GT1048	pUST17/*hisO1242, hisC3737, sufX201, trmD3, srl-202*::Tn*10, recA1*
GT1069	*hisO1242, hisC3737, sufC13 (sufY205, sufX02), zee-2502*::Tn*10*
GT1079	*hisO1242, hisC3737, sufC10 (sufY204, sufX01), zee-2502*::Tn*10*
GT1083	*hisO1242, hisC3737, sufC14 (sufY206, sufX203), zee-2502*::Tn*10*
GT1380	*hisO1242, hisC3737, sufX201, zee-2502*::Tn*10* (90% linked to *sufB*; 88% to *hisW*)
GT1434	*hisO1242, hisC3737, nag-1, tbf-99*::Tn*10* (90% linked to *nag*)
GT1525	*hisO1242, hisD3749-S15, sufX201, zee-2502*::Tn*10* (90% linked to *sufB*; 88% linked to *hisW*)
GT1547	*hisO1242, hisC3737, trmD3, sufX201, zee-2502*::Tn*10*
GT1684	*hisO1242, hisC3737, sufB1, trmD3*
TR935 (GT1402)	*hisO1242, hisD3018, sufB1*
TR936 (GT477)	*hisO1242, hisD3018, sufB2*
TR767 (GT1403)	*hisO1242, hisD3018*
TR1410 (GT944)	*hisO1242, hisC3737, sufC10*[a] *(sufX201, sufY204)*
TR1413 (GT945)	*hisO1242, hisC3737, sufC13*[a] *(sufX202, sufY205)*
TR1414 (GT946)	*hisO1242, hisC3737, sufC14*[a] *(sufX203, sufY206)*
ET3	*hisO1242, hisD3018, sufX201,*
ET4	*hisO1242, hisD3018*

[a]The strains TR1410 (*sufC10*), TR1413 (*sufC13*) and TR1414 (*sufC14*) do not harbour any frameshift suppressor at min 15, which were thought to be the location of the *sufC* gene (7). We show here that instead they harbour two frameshift suppressors, *sufX* and *sufY* of which one, *sufX* is allelic to *sufB*. The map location of *sufY* is not known but it is not located at min 15 (See text).

MATERIALS AND METHODS

Materials

Restriction enzymes were purchased from either Pharmacia, Sweden or New England Biolabs and used according to the manufacturer's instructions. Different oligonucleotides and radiochemicals were purchased from Symbicom AB, Umeå, Sweden and Amersham, respectively. All chemicals used were of analytical grade or better.

Difco nutrient broth was used as maximally supplemented medium (called rich medium). The medium E of Vogel and Bonner (10) supplemented with 2% of glucose was used as salt medium. Solid media contained 1.4% of agar. Medium E supplemented with 0.1 mM histidine was used when necessary.

Bacterial and phage strains and vectors

The bacterial strains used in this study are listed in Table 1. They all are derivatives of *Salmonella typhimurium* strain LT2. P22 phage HT*105/1* (*int-201*) (11) was used in all transductions. *Escherichia coli* strain TG1 (12) was used as the standard M13 host. Single-stranded DNA phages M13mp18 and M13mp19 were used as the cloning vehicle throughout this work (13). The low copy number plasmid pLG339 has been described (14).

Preparation of DNA

Plasmid DNA and replicative form DNA of phage M13 were prepared by the alkaline lysis procedure followed by equilibrium density centrifugation in cesium chloride as described by Sambrook *et al.*, (15). Single-stranded DNA of phage M13 was purified according to the Amersham sequencing handbook.

Detection of $tRNA_2^{Pro}$ by DNA-RNA hybridization

DNA fragments carrying wild-type and mutant phenotypes were fixed to the Hybond-N nylon membranes according to the procedure recommended by the manufacturer (Amersham) and then hybridized with 21-mer $tRNA^{Pro}_2$ probe (16).

DNA sequencing

The dideoxy nucleotide sequencing method (17) was used. The accession numbers at the EMBL data library for *S.typhimurium* $tRNA^{Pro}_2$ gene and the *sufB1/2* mutants are X63776 and X63777, respectively.

RESULTS

Cloning of the *sufC*$^+$ allele

The recessive *trmD3* mutation induces suppression of several frameshift mutations, which are not only suppressed by the dominant *sufA6* and *sufB2* suppressors but also by the recessive

sufC10. Cells harbouring any of the latter three mutations grow quite well at high temperature. Cells harbouring the *trmD3* mutation also grow at 42.5°C on rich medium, although at reduced rate (9, 18). Combination of the *trmD3* mutation with any of the *sufA6, sufB2* or *sufC10* mutations in the same cell renders the cell unable to grow at 42.5°C. The molecular mechanism behind the temperature sensitivity of the double mutants is not known. However, the combined strength of two frameshift suppressors may be too much for the cell to sustain especially if both mutations affect the same molecule mediating the frameshifting ability. The *sufA6* and *sufB2* are dominant mutations while *sufC10* like *trmD3* is recessive (2, 9). Since the double mutant (*trmD3, sufC10*) is temperature sensitive for growth and both mutations are recessive, we utilized this double mutant to select for plasmids harbouring chromosomal fragments, which enable the cell to grow at high temperature. In theory, two types of plasmids should be obtained — one harbouring the $trmD^+$ gene and another harbouring the $sufC^+$ gene. Strain GT983 contains a *S.typhimurium* gene bank from strain GT344 in plasmid pLG339, which confers kanamycin resistance (Km^R). This gene bank was transferred by phage P22 to strain GT967 (*sufC10, trmD3, hisC3737*), which is temperature sensitive (Ts) for growth on rich medium and able to grow in the absence of histidine (His^+ phenotype) at permissive temperature due to the presence of *sufC10*, and to a lesser extent to the presence of the *trmD3* mutation. Kanamycin resistant (Km^R) colonies were selected at 30°C, replica plated on minimal, minimal supplemented with histidine, and rich media. Minimal and minimal plus histidine media agar plates were incubated at 30°C, 37°C and 41°C. Rich media agar plates were incubated at 42.5°C. Screening for colonies with plasmids conferring antisuppression activity (i.e. His^- phenotype) and temperature resistance (T^R) at 42.5°C was performed. One His^- T^R, Km^R clone was isolated among 3×10^4 Km^R clones. This clone (strain GT1048) contained a plasmid designated pUST17. Phage P22 was grown on this isolate, the plasmid was transduced back to strain GT967 (*sufC10, trmD3*) and selection for Km^R was performed. The His^- T^R phenotype was 100% (200 Km^R clones tested) linked to the Km^R phenotype.

sufC10 strain contains two frameshift suppressors of which one, *sufX201*, is located close to *sufB2*

The *sufC10* mutation was initially located close to the *nag* gene at min 15 on the *Salmonella* chromosome (7). However, we noticed (see below) that plasmid pUST17 ($sufC^+$) also inhibited the dominant *sufB2* (located at min 45) mediated frameshift suppression. This urged us to map the frameshift suppressor in strain GT944 (*sufC10, hisO1242, hisC3737*). Phage P22 was grown on this strain and Nag^+ transductants were selected using strain GT1434 (*hisO1242, hisC3737, nag-1, zbf-99*::Tn*10*; this Tn*10* is 90% linked to *nag*) as a recipient. Among 100 Nag^+ transductants, none were His^+ but 90% had become tetracycline sensitive (Tc^s). Thus, strain GT944 (*sufC10*) as well as strains GT945 (*sufC13*) and GT946 (*sufC14*) (data not shown) do not contain any frameshift suppressor closely linked to *nag-1* able to correct the *hisC3737* frameshift mutation. We have therefore renamed the frameshift suppressors present in these strains *sufX201, sufX202* and *sufX203*, respectively. Since plasmid pUST17 ($sufC^+$) inhibited the *sufB2* mediated suppression, we asked whether the *sufX* mutations were located in the *sufB* area of the chromosome. Two Tn*10* insertions, *zee-2502*::Tn*10* and *zee-2509*::Tn*10* are located close to *sufB* (data not shown). Strain GT833 harbouring the *zee-2502*::Tn*10* insertion was used as donor and strains GT944 (*sufX201, hisC3737*), GT945 (*sufX202, hisC3737*) and GT946 (*sufX203, hisC3737*) as recipients. Among 100 tetracycline resistant (Tc^R) transductants 20–40% were His^{++} (visible growth on plates lacking histidine after 1 day, i.e. similar His^{++}-phenotype as the recipients) and 60–80% were His^+ (visible growth after 2 days). However, no His^- transductants (no growth after two days) were obtained. Similar results were also obtained using strain GT856 (*zee-2509*::Tn*10*) as a donor. The fact that two different His^+-phenotypes were obtained suggests that indeed these *sufX* mutations are cotransducible with both Tn*10* insertions. However, since no His^- recombinant were found, we concluded that the recipient strains (GT944 (*sufC10*), GT945 (*sufC13*), and GT946 (*sufC14*) in addition to *sufX* also contain another weaker suppressor (here denoted *sufY*). If so, the suppressor linked to these Tn*10* insertions

Table 2. Specificity of *sufX201, sufB2* and *trmD3* mediated frameshift suppression

his-allele	*sufX201*		*sufB2*		*sufC10*[2]	*trmD3*[3]	
	30°	37°	30°	37°	37°	30°	37°
hisB6480	(+)[1]	+	(+)	+	n.d.	+	+
hisC3060	−	−	−	−	−	−	−
hisC3072	−	−	−	−	n.d.	−	−
hisC3734	(−)	(+)	++	++	+	(−)	(+)
hisC3737	(+)	+	++	++	+	−	(−)
hisD3018	(+)	+	++	++	−	(−)	+
hisD3068	−	−	−	−	n.d.	−	−
hisD3702	(+)	+	++	++	n.d.	−	−
hisD3749	+	+	++	++	n.d.	(−)	+
hisD6580	−	−	−	−	n.d.	−	−
hisD6610	(+)	−	++	+	n.d.	−	−
hisD3749-S6	−	−	++	++	n.d.	(−)	(+)
hisD3749-S7	(+)	+	++	++	n.d.	(−)	+
hisD3749-S11	−	−	−	−	n.d.	−	−
hisD3749-S15	++	++	++	++	n.d.	(−)	+
hisF2439	−	−	−	−	−	+	+
husF6527	−	−	−	−	n.d.	−	−

[1]growth character on minimal medium: − lack of growth, (−) growth after 2.5 days, (+) growth after 2 days, + growth after 1 day, ++ growth after 0.5 day.
[2]according to Riddle and Roth (2); n.d. not determined.
[3]Data from Björk *et al.*, (9).

should be transferable to a recipient strain not containing any frameshift suppressor. Therefore, transductants possessing a strong His^{++} phenotype and *zee-2502*::Tn*10* were purified (strain GT1079 (*sufX201*), GT1069 (*sufX202*), GT1083 (*sufX203*). These strains were used as donors and strain GT853 (*hisO1242, hisC3737*) as a recipient in the next transduction experiment. Among 100 Tc^R transductants, 90%, 58% and 90% were also His^+ with strains GT1079, GT1069, and GT1083 as donors, respectively. Thus, the *sufX201, sufX202* and *sufX203* are cotransducible with *zee-2502*::Tn*10*, which is also cotransducible with *hisW* (88%) and *sufB2* (90%). We conclude that the original *sufC10, sufC13* and *sufC14* strains do not contain any frameshift suppressor close to *nag* (15 min) as earlier proposed (7). Instead these strains contain two frameshift suppressors (here denoted *sufX* and *sufY*). We further conclude that the *sufX201, sufX202* and *sufX203* mutations are located in the same region as the *sufB2* allele. Below we show that indeed the *sufX201* mutation is allelic to *sufB1* and *sufB2* mutations. Therefore, the plasmid pUST17 isolated as being $sufC^+$ is indeed phenotypically $sufB^+$, as well as $sufX^+$. No further analysis of the *sufX202, sufX203* and the *sufY* mutations have been done.

Specificity of the *sufX201* mediated suppression

The specificity of the *sufB* mediated suppression has been established by Riddle and Roth (2). We have also earlier analyzed the frameshift suppression specificity mediated by the *trmD3* mutation (9). The latter induces m^1G37 deficiency in several tRNAs including $tRNA^{Pro}_2$. The specificities of the frameshifting suppression mediated by *sufX201, sufB2* and *trmD3* were compared. Table 2 shows that the *sufX201* induces a frameshift suppression pattern similar but not identical to that of *sufB2* and *trmD3*. Note that *sufX201* is unable to suppress *hisD3749-S6*, which is suppressed by *sufB2* and weakly by *trmD3*.

Cloning of the *sufB1, sufB2,* and *sufX201* alleles by recombination *in vivo*

The plasmid pUST17 (isolated as being $sufC^+$ but is indeed also $sufB^+$) was used for isolation of the *sufB1, sufB2*, and *sufX201* alleles from *S.typhimurium* chromosome by recombination *in*

Table 3. Recombination of the *sufB1, sufB2*, and *sufX201* alleles onto plasmid pUST17

Donor	Recipient	Exp	Km^R	No. of His^+	Frequency
pUST17($sufB^+$)/GT1402	GT1403($sufB^+$)	1	6300	51	0.8×10^{-2}
sufB1)		2	1254	9	
pUST17($sufB^+$)/GT477	GT1403($sufB^+$)	1	17008	131	0.8×10^{-2}
(*sufB2*)		2	11792	97	
pUST17($sufB^+$)/GT1403	GT1403($sufB^+$)	1	9040	0	$<1\times10^{-4}$
($sufB^+$)		2	1808	0	
pUST17/GT1525 (*sufX201*)	GT1525(*sufX201*)	1	11924	21	1.8×10^{-3}
pUST17/GT1526 ($sufX^+$)	"	1	5460	0	$<1.8\times10^{-4}$
pUST17/ET3 (*sufX201*)	GT1525(*sufX201*)	1	12920	21	1.6×10^{-3}
PUST17/ET4 ($sufX^+$)	"	1	6935	0	$<1.4\times10^{-4}$

Strains GT1402 (*sufB1*), GT477 (*sufB2*) or GT1403 ($sufB^+$), all containing plasmid (pUST17 ($sufB^+$)) were grown in rich media to allow recombination between the wild type $sufB^+$ allele present on plasmid pUST17 and the respective alleles on the chromosome. Phage P22 was grown on these stains, plasmids were transferred into recipient strain GT1403 ($sufB^+$, *hisO1242, hisD3018*, Phenotypically His^-) and Km^R transductants were selected. Such Km^R transductants were screened by replica plating for His^- phenotype. His^+ phenotype indicated that a plasmid harbouring the dominant frameshift suppressors (*sufB1* or *sufB2*) was present in the recipient strain GT1403. His^+ transductants in strain GT1525 indicated that a plasmid harbouring the recessive frameshift suppressor *sufX201* existed in the recipient strain GT1525.

Table 4. Tests of phenotypes of relevant *S.typhimurium* mutants after transduction of plasmids containing *sufB sufB1* or *sufB2* alleles.

Genotypes[1] of host strains		Growth at 30°C on plates lacking histidine and on rich media at 42.5°C			
suf	*trmD*	Vector only	$sufB^+$ insert[2]	*sufB1* or *sufB2* insert[2]	*sufX201* insert[2]
+	+	−,R[3]	−,R	+,R	−,R
+	*D3*	(−),R	−,R	+,S	(−),R
B[4]	+	+,R	−,R	+,R	+,R
B[4]	*D3*	+,S	−,R	+,S	+,S
A6	+	+,R	+,R	+,R	n.d.
A6	*D3*	+,S	+,R	+,S	+,S
X201	+	+,R	−,R	+,R	n.d.
X201	*D3*	+,S	−,R	+,S	+,S

[1]All strains harbour the following mutations: *hisO1242* and *hisD3018* (+1C) or *hisC3737* (+1C); + = wild-type allele.
[2]Three different chromosomal fragments from either wt ($sufB^+$) or mutants (*sufB1, sufB2* or *sufX201*) were inserted into the vector pLG339. A 9 kb chromosomal fragment is present in plasmids pUST17 ($sufB^+$), pUST32 (*sufB1*), pUST34 (*sufB2*) and pUST36 (*sufX201*). A 4.4 kb chromosomal fragment is present in plasmids pUST80 ($sufB^+$), pUST82 (*sufB1*), pUST83 (*sufB2*) and pUST84 (*sufX201*) and a 1 kb chromosomal fragment is present in plasmids pUST92 ($sufB^+$), pUST95 (*sufB1*) and pUST97 (*sufX201*). The same results were obtained irrespectively of the size of the chromosomal insert.
[3]Growth characteristics on the minimal medium: −lack of growth, (−) growth after 4 days, + growth after 1 day incubation. Growth on the rich medium at 42.5°C: S = temperature sensitive, R = temperature resistant, growth after 1 day.
[4]Strains having *sufB1* or *sufB2* mutations gave the same results.

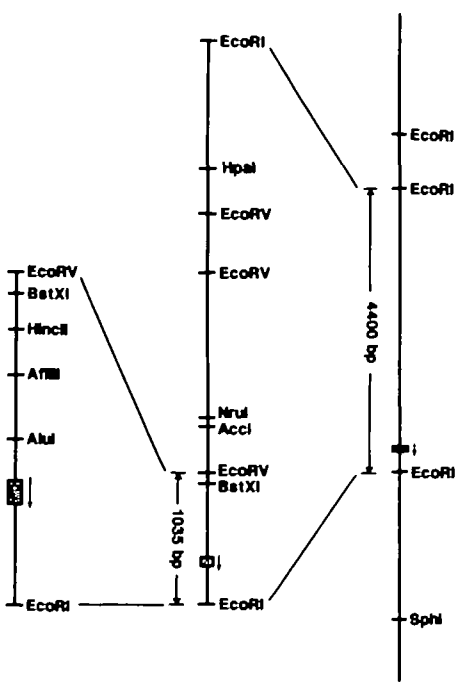
vivo. This plasmid was introduced into strains GT1402 (*sufB1*), GT477 (*sufB2*), and GT1525 (*sufX201*). Growth in rich medium for several generations allowed recombination between the plasmid wild type allele and the chromosomal *sufB1*, *sufB2*, and *sufX201* mutations. Next the plasmids were transferred to strain GT1403 (*sufB*$^+$, *hisO1242*, *hisD3018*) and recombinant plasmids (harbouring the dominant *sufB1* or *sufB2* mutation) were identified as being His$^+$. The *sufX201* mutation is recessive (see below). Therefore, we used as a recipient strain GT1525 (*sufX201*, *hisO1242*, *hisD3749-S15*). In this strain a plasmid harbouring the *suf*$^+$ allele is phenotypically His$^-$ but His$^+$ when harbouring a recombinant plasmid (*sufX201*). Mutations *sufB1*, *sufB2* and *sufX201* were recombined onto the plasmid at a frequency of $10^{-2}-10^{-3}$ (Table 3). Plasmids harbouring the *sufB1* mutation (pUST32), the *sufB2* mutation (pUST34) and *sufX201* mutation (pUST36) were further analyzed.

Phenotypic characterization of the cloned chromosomal fragments harbouring the *sufB*$^+$ and its mutant alleles

The pUST17 (*sufB*$^+$) plasmid was introduced into several different strains (Table 1) and the ability to suppress *his* frameshift mutations and to permit growth on rich medium at high temperature were analyzed (Table 4). The pUST17 (*sufB*$^+$) plasmid caused temperature resistance and inhibited suppression of the *hisC3737* mutation in strain GT1547 (*hisC3737*, *sufX201*, *trmD3*). It also made strain GT782 (*sufA6*, *trmD3*) and strain G1784 (*sufB2*, *trmD3*) temperature resistant. Furthermore, it inhibited the frameshifting ability of not only the recessive *sufX201* mutation but also of the dominant *sufB1* and *sufB2* mutations. Note also that the *sufX201* mutation is recessive when present on a multicopy plasmid. However, inhibition of the frameshifting ability was not observed in the case of the dominant *sufA6* mutation in strain GT684 (*sufA6*, *hisO1242*, *hisC3737*, *trmD3*) or strain GT782 (*sufA6*, *hisO1242*, *hisC3737*) (Table 4). The pUST32 (*sufB1*) and pUST34 (*sufB2*) plasmids did not complement the temperature sensitive phenotype of strains GT782 (*sufA6*, *trmD3*), GT784 (*sufB2*, *trmD3*) or GT1547 (*sufX201*, *trmD3*). However, they induced the ability to suppress the *hisC3737* mutation (Table 4). These results are consistent with the fact that the *sufB1* and *sufB2* mutations are located on the indicated plasmids.

Subcloning of the *sufB* gene

The pUST17 plasmid was subjected to restriction enzyme analysis (Fig. 1). A 4.4 kb *Eco*RI fragment was subcloned into plasmid pLG339 (14). All the phenotypes associated with pUST17 were also present on this 4.4 kb *Eco*RI fragment (Table 4). Since earlier results (See introduction) have shown that the *sufB1* and *sufB2* mutations affected the tRNA$^{Pro}_2$ we also monitored the presence of the tRNA^{Pro} gene by hybridization to a 21-mer oligonucleotide complementary to tRNA$^{Pro}_2$ from base G20 in DHU-loop to base G39 in the anticodon loop. The 9 kb, the 4.4 kb, and 1.03 kb fragments contain the tRNA$^{Pro}_2$ gene, inhibit *sufB2* mediated suppression and induce temperature resistance of strain GT784 (*sufB2*, *trmD3*). Similar subclonings were performed starting with plasmids pUST32 (*sufB1*), pUST34 (*sufB2*) and pUST36 (*sufX201*). Table 4 shows that the phenotypes associated with the original plasmids were associated with the corresponding 4.4 kb and 1.03 kb *Eco*RV-*Eco*RI DNA fragments (Figure 1). Thus, the *sufB*$^+$ gene and the gene encoding tRNA$^{Pro}_2$ are present on the same 1.03 kb DNA fragment. The *Eco*RI-*Eco*RV fragments carrying the *sufB1*, *sufB2* or *sufX201* mutations were similar in size.



Plasmids	Size of DNA-fragment	Phenotypes present	Hybridization signal
pUST 17, 32, 34	9.0 kb	+	+
pUST 80, 82, 83	4.4 kb	+	+
pUST 92, 95, –	1.0 kb	+	+

Figure 1. Restriction map of the *sufB* region. Summary of the subcloning procedure of the 9 kb-DNA fragment from 45 min of *S.typhimurium* chromosome. DNA fragments giving respective phenotypes (see Table 4) were hybridized with the 21-mer oligonucleotide complementary to the anticodon arms of the tRNA$^{Pro}_2$ described in Materials and Methods.

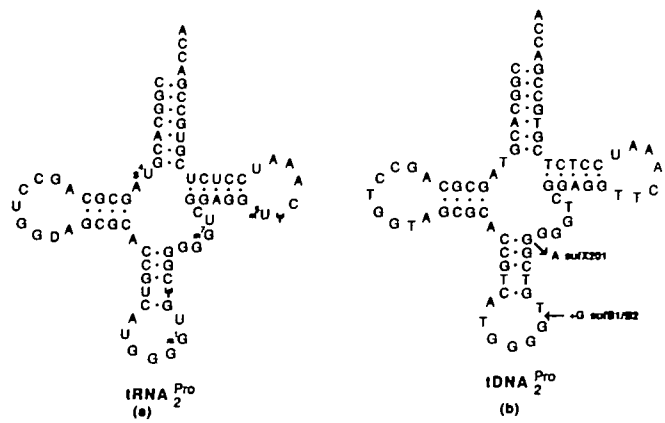


Figure 2. Nucleotide sequences of *Salmonella* tRNA$^{Pro}_2$ (a) and its gene (b). The *sufB1* and *sufB2* mutants contained an insertion of a G residue into the anticodon region of the tRNA$^{Pro}_2$ gene and the *sufX201* a base substitution (G43 to A43) as indicated by an arrow.

Sequence of the $tRNA^{Pro}_2$ gene from the wild type of *Salmonella typhimurium*

In order to characterize the *sufB1* and *sufB2* mutations in the $tRNA^{Pro}_2$ gene, we determined the total nucleotide sequence of the 1.03 kbp *Eco*RV-*Eco*RI fragment from the wild type and the two *sufB* mutants of *S.typhimurium*. This fragment carrying the $tRNA^{Pro}_2$ gene from pUST17 was subcloned into pTZ phagemid vector. The complete sequence of the 1.03 kb fragment was determined by the dideoxy nucleotide sequencing method. The gene for $tRNA^{Pro}_2$ was found between nucleotides 648 and 724. The sequence of the *Salmonella* $tRNA^{Pro}_2$ gene reported here is identical to the sequence of the *E.coli* $tRNA^{Pro}_2$ gene reported by Komine *et al.* (19). The previous sequence of *Salmonella* $tRNA^{Pro}_2$ reported by Kuchino *et al.* (8) had miss-identifications at three residues. The revised $tRNA^{Pro}_2$ sequence that coincided completely with the DNA sequence of the tRNA gene is shown in Fig. 2. At positions 37 and 14 upstream from the 5′ terminus of the mature tRNA sequence, TTGCAT and TAGTATT sequences were present. These sequences are both consistent with the −35 and −10 consensus promoter sequences of eubacterial genes. At position 16 downstream from the 3′ terminus of the tRNA molecule, there was a dyad symmetrical structure followed by a T cluster, which is a typical rho-independent transcriptional termination signal. The nucleotide sequence similarity of the 5′ flanking region between *Salmonella* and *E.coli* $tRNA^{Pro}_2$ genes was approximately 83%. By contrast, the 3′ flanking region of the *Salmonella* $tRNA^{Pro}_2$ gene showed only 42% sequence similarity with the corresponding region of the *E.coli* $tRNA^{Pro}_2$ gene, although the same rho-independent transcription termination signal was present in *Salmonella*.

Sequence of the $tRNA^{Pro}_2$ gene from the *sufB1*, *sufB2*, and *sufX201* mutant strains

Based on the sequence of the wild type *Salmonella* $tRNA^{Pro}_2$ gene, various 20 nucleotide primer DNA fragments were chemically synthesized and used for sequence analysis of the 1.03 kbp *Eco*RV-*Eco*RI fragments from the *sufB1* and *sufB2* mutant strains. The *sufB1* mutation and *sufB2* result from an insertion of a G residue into the anticodon region of the $tRNA_2^{Pro}$ gene (Figure 2) and no other differences were observed in the 1 kb fragment when compared to the wild type DNA sequences. In contrast, the *sufX201* mutation resulted in a G43 to A43 base substitution.

DISCUSSION

In this report both *sufB1* and *sufB2* mutations in *S.typhimurium* were shown to result in an insertion of a G residue in the anticodon loop of $tRNA^{Pro}_2$. In a previous report both mutations were shown to be dominant using an F′ plasmid harbouring the wild type allele $sufB^+$ (6). However, if the wild type allele is present on multicopy plasmids, these mutations are recessive (Table 4). A double mutant *sufB1*, *trmD3* is unable to grow at high temperature on rich media. This phenotype can be complemented by a plasmid harbouring the wild type $sufB^+$ allele but not by a plasmid harbouring the *sufB1* or *sufB2* mutations (Table 4). Thus, the *sufB1* and *sufB2* mutations are dominant when the wild type allele is on a low copy plasmid (F′ plasmid) but recessive when the wild type allele is on a multicopy plasmid. This phenomenon can be explained by a more efficient competition by the wild type tRNA when present in higher concentration, as a result of a higher gene dosage. We cannot discount that undermodification of the *suf* tRNA contributes to its frameshifting activity.

The *sufC* strains earlier characterized (2) were shown to contain two frameshift suppressors, *sufX* and *sufY*. None of these mutations are located close to the *nag* gene as earlier suggested (7). However, it is clear that the *sufX* mutations are located in the same region as *sufB2* and is indeed allelic to *sufB1* and *sufB2*. Sequence determination revealed that a G43 to A43 base substitution of the $tRNA^{Pro}_2$ gene had occurred in the *sufX* strain. Since we have earlier shown that lack of m^1G37 induces frameshift suppression (9), the G43 to A43 base substitution may also affect the ability for tRNA(m^1G37)methyltransferase to methylate $tRNA^{Pro}_2$. If so, this rRNA would induce frameshift suppression due to m^1G deficiency. Indeed, $tRNA^{Pro}_2$ from a *sufX201* strain is deficient in m^1G37 (Qian and Björk, unpublished). Since, it is known that m^1G37 deficiency of $tRNA^{Pro}$ results in frameshifting at CCCU sequences, the *sufA201* mediated suppression may be completely or partly due to m^1G deficiency. Note, however, that while the *trmD3* mutation induces the ability to suppress *hisD3749-S6*, the *sufX201* mutation does not. Therefore, the *sufX201* mediated suppression is likely to be partly due to m^1G deficiency but also to other structural features induced by the G43 to A43 base substitution. A quantitative comparison of the efficiency of *trmD3* (results in m^1G deficiency) and *sufX201* (results in both m^1G deficiency and G43 to A43 base change) mediated suppression may reveal the extent to which the different structural changes contribute to the suppression observed in *sufX201* strain.

The *sufA6* mediated temperature sensitivity of the double mutant *sufA6, trmD3* is complemented by the wild type $sufB^+$ allele, while the *sufA6* mediated suppression is not (Table 4). Although the frameshifting specificity is overlapping between *sufA6, sufB1* and *sufB2* (2) the mutations affect different tRNAs ($tRNA^{Pro}_1$ in the case of *sufA6* and $tRNA^{Pro}_2$ in the case of *sufB* mutations) with different coding capacities. Wild type $tRNA^{Pro}_1$ has the anticodon U33-CGG-m^1G37 and $tRNA^{Pro}_2$ U33-GGG-m^1G37. According to the wobble rules (20), these tRNAs read codons CCG and CCC/U, respectively. Both *sufA6* and *sufB2* frameshift suppressors are known to suppress at the sequence CCC-U, which is present in the mutations *hisD3018* and *hisD3749* (21, 22). This frameshifting specificity of *sufB2* is expected if the *sufB2* tRNA makes a quadruplet anticodon-codon pairing, i.e. the wobble base of *sufB2* $tRNA^{Pro}_2$ is able to make a basepair with U in the sequence CCC-U. This is not the case for *sufA6* provided it still has a C in the wobble position. If so, it is reasonable that $sufB^+$ on the plasmid does not inhibit the *sufA6* mediated suppression and suggests that the mechanism by which *sufA6* mediates suppression of CCC-U is not the same as that used by the *sufB2*. However, since the $tRNA^{Pro}_1$ (affected by *sufA6*) is a major $tRNA^{Pro}$ and $tRNA^{Pro}_2$ is a minor, the reason for the inability of $sufB^+$ to compete successfully with *sufA6* mediated suppression might be merely due to a concentration effect. Note that the $sufB^+$ on a plasmid complements the temperature sensitivity of the double mutant *sufA6, trmD3*, indicating some competition between wild type $tRNA^{Pro}_2$ and the *sufA6* $tRNA^{Pro}_1$. Since the *sufA6* mutation has not been characterized a specific mechanism for the incomplete inhibition of the *sufA6* mediated suppression by the $sufB^+$ plasmid may exist.

Culbertson and collaborators have characterized several

suppressor mutations in the yeast *Saccharomyces cerevisiae*, which affect $tRNA^{Pro}$ (23). One group (SUF7, SUF8, SUF9, SUF11 and TRN1) all affect the yeast $tRNA^{Pro}$ with anticodon UGG. This tRNA would be the yeast counterpart of $tRNA^{Pro}_3$ in *S.typhimurium* with the anticodon-cmo^{5}UGG (cmo^{5}U being uridine-5-oxyacetic acid). All these yeast mutants have a base substitution at position 39, which disrupts anticodon stem pairing and results in a 9 base loop. So far no frameshift suppressor of $tRNA^{Pro}_3$ in *S.typhimurium* has been characterized. The other group (SUF2, SUF10) of yeast frameshift suppressors contains a mutated $tRNA^{Pro}$ with anticodon IGG (I, inosine), and would be the yeast counterpart of *S.typhimurium* $tRNA^{Pro}_2$ with the anticodon GGG. All frameshift suppressors of yeast $tRNA^{Pro}_{IGG}$ characterized have a base insertion in the anticodon loop. Interestingly, these yeast frameshifting tRNAs would be similar to the *sufB1* or *sufB2* frameshift suppressor of *S.typhimurium*, which also are due to a base insertion in the anticodon loop.

Results presented in this paper show that the dominant frameshift suppressors *sufB1* and *sufB2* are identical and both are the result of an insertion of a G in the anticodon region of $tRNA^{Pro}_2$ (Figure 2). The suppressor tRNA therefore possesses a 4G anticodon resulting in the potential for a four base pair anticodon-codon interaction. At the translocation step this 'extended' base pair could force the ribosome to move 4 bases instead of 3 and thereby shift into the +1 reading frame. The protein sequence of a product of extragenic suppression was first obtained by suppression of *hisD3018* by *sufB2* (4). The result was consistent with a quadruplet base pairing and the results presented in this paper are also consistent with this suggestion, ie. the *sufB2* $tRNA^{Pro}_2$ may be able to make a four base anticodon−codon interaction. Thus, the *sufB1* and *sufB2* mutations resemble the frameshift mutation *sufD42*, which is an insertion of a C in the anticodon of $tRNA^{Gly}$ (3). This mutant $tRNA^{Gly}$ has an anticodon of 4 C's and it was presumed to act by a 4 base pair anticodon−codon interaction. Also Gaber and Culbertson (24) have characterized a yeast frameshift suppressor with the same anticodon as *sufD42*, ie. -CCCC-. Although the potential for pairing of the four bases enhances the efficiency of suppression, it is not required (24). Therefore, in the case of $tRNA^{Gly}$ with an extra nucleotide in the anticodon loop, frameshifting is promoted by sterically hindrance of binding of the next tRNA, rather than by quadruplet anticodon−codon pairing (24, 25). Weiss *et al.* (25) have shown that this is also true for *sufD41* in *S.typhimurium*. Thus, more studies concerning the specificity of the suppression mediated by the *sufB1* and *sufB2* mutations must be performed before the mechanism by which they act can be established.

ACKNOWLEDGEMENTS

This work was supported by the Swedish Cancer Society (project 680 to G.R.B.), the Swedish Natural Science Foundation (project BBU 2930 to G.R.B.), the Swedish Board for Technical Development (grant 4206 to G.R.B.) and by the Ministry of Education, Science and Culture of Japan (to Y.K.). We thank Kerstin Jacobsson and Gunilla Jäger for skillful technical assistance and Kerstin Thontors-Olsson for excellent typing of the manuscript. The critical reading and linguistic improvement of the manuscript by Martin Buck, Sussex, England and Claes Gustafsson, Tord Hagervall, Britt Persson, and K.Kjellin-Stråby and Qiang Qian all Umeå are gratefully acknowledged.

REFERENCES

1. Atkins,J.F., Weiss,R.B., Thompson,S. and Gesteland,R.F. (1991) *Annu. Rev. Genet.* **25**, 201−228.
2. Riddle,D.L. and Roth,J.R. (1970) *J. Mol. Biol.* **54**, 131−144.
3. Riddle,D.L. and Carbon,J.C. (1973) *Nature New Biol.* **242**, 230−234.
4. Yourno,J. and Tanemura,S. (1972) *Nature* **225**, 422−426.
5. Riddle,D.L. and Roth,J.R. (1972) *J. Mol. Biol.* **66**, 495−506.
6. Riddle,D.L. and Roth,J.R. (1972) *J. Mol. Biol.* **66**, 483−493.
7. Sandersson,K.E. and Hurley,J.A. (1987) In Neidhardt,F.C. (ed.), *Escherichia coli and Salmonella typhimurium. Cellular and Molecular Biology*. American Society for Microbiology, Washington, DC. pp. 877−918.
8. Kuchino,Y., Yabusaki,Y, Mori,F. and Nishimura,S. (1984) *Nucleic Acid Res.* **12**, 1559−1562.
9. Björk,G.R., Wikström,P.M. and Byström,A.S. (1989) *Science* **244**, 986−989.
10. Vogel,H.J. and Bonner,D.M. (1956) *J. Biol. Chem.* **218**, 97−106.
11. Schmieger,H. (1972) *Mol. Gen. Genet.* **119**, 75−88.
12. Gibson,J. (1984) Ph.D. Thesis. Cambridge University, England.
13. Yaniseh-Peron,C., Vieira,J. and Messing,J. (1985) *Gene* **33**, 103.
14. Stoker,N.G., Fairweather,N.F. and Spratt,B.G. (1982) *Gene* **18**, 335−341.
15. Sambrook,J., Fritsch,E.F. and Maniatis,T. (1982) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
16. Southern,E.M. (1975) *J. Mol. Biol.* **98**, 503−517.
17. Sanger,F., Nicklen,S. and Coulson,A.R. (1977). *Proc. Natl. Acad. Sci. USA* **74**, 5463−5467.
18. Hagervall,T.G., Ericson,J.U., Esberg,B., Li,J.N. and Björk,G.R. (1990) *Biochim. Biophys. Acta* **1050**, 263−266.
19. Komine,Y., Adachi,T., Inokuchi,H. and Ozeki,H. (1990) *J. Mol. Biol.* **212**, 579−598.
20. Crick,F.C.H. (1966) *J. Mol. Biol.* **19**, 548−555.
21. Yourno,J. and Kohno,T. (1972) *Science* **175**, 650−652.
22. Bossi,L. and Roth,J.R. (1981) *Cell* **25**, 489−496.
23. Culbertson,M.R., Leeds,P., Sandbaken,M.G. and Wilson,P.G. (1990) In: *The Ribosome. Structure, function and evolution*. Eds. Hill,W.E., Dahlberg,A., Garret,R.A., Moore,P.B., Schlessinger,D. and Warner,J.R. pp. 559−570.
24. Gaber,R.F. and Culbertson,M.R. (1984) *Mol. Cell. Biol.* **4**, 2052−2061.
25. Weiss,R.B., Dunn,D.M., Atkins,J.F. and Gesteland,R.F. (1990) *Progr. Nucl. Acids Res. Mol. Biol.* **39**, 159−183.