GENETIC CHARACTERIZATION OF THE *sufJ* FRAMESHIFT SUPPRESSOR IN *SALMONELLA TYPHIMURIUM*[1]

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ABSTRACT

A new suppressor of +1 frameshift mutations has been isolated in *Salmonella typhimurium*. This suppressor, *sufJ*, maps at minute 89 on the Salmonella genetic map between the *argH* and *rpo*(*rif*) loci, closely linked to the gene for the ochre suppressor *tyrU*(*supM*). The suppressor mutation is dominant to its wild-type allele, consistent with the suppressor phenotype being caused by an altered tRNA species. The *sufJ* map position coincides with that of a threonine tRNA(ACC/U) gene; the suppressor has been shown to read the related four-base codons ACCU, ACCC, ACCA.——The ability of *sufJ* to correct one particular mutation depends on the presence of a *hisT* mutation which causes a defect in tRNA modification. This requirement is allele specific, since other frameshift mutations can be corrected by *sufJ* regardless of the state of the *hisT* locus.——Strains carrying both a *sufJ* and a *hisT* mutation are acutely sensitive to growth inhibition by uracil; the inhibition is reversed by arginine. This behavior is characteristic of strains with mutations affecting the arginine-uracil biosynthetic enzyme carbamyl phosphate synthetase. The combination of two mutations affecting tRNA structure may reduce expression of the structural gene for this enzyme (*pyrA*).

FRAMESHIFT mutations are caused by the addition or removal of one or more nucleotide pairs (not multiples of three) in the sequence of a translated gene. As a consequence, the translation process loses its proper frame of reference and the synthesis of a missense polypeptide sequence ensues. It has been shown that some +1 frameshift mutations are correctable by informational suppressors (RIYASATY and ATKINS 1968; YOURNO *et al.* 1969; RIDDLE and ROTH 1970). All of the characterized frameshift suppressor mutations affect the function of some tRNA species, either by directly changing their primary structure or affecting the synthesis of minor bases (RIDDLE and CARBON 1973; RIDDLE and ROTH 1972). The first suppressors described act at codons with monotonous base runs (CCCC/U, GGGG). The specificity may be due to the fact that most frameshift and suppressor mutations studied were induced by the frameshift mutagen ICR191 which is known to cause addition or removal of base pairs in runs of G:C base pairs in DNA (reviewed by ROTH 1974).

[1] Abbreviations used: ICR191, 2-chloro-6-methoxy-9-(3-(2-chloroethyl) aminopropylamino) acridine dihydrochloride; Tet, tetracycline; Kan, kanamycin sulfate.

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To gain insight into the mechanism of frameshift suppression, new types of suppressors were sought that might act at sequences of the message other than monotonous runs (KOHNO, BOSSI and ROTH 1982); this was done by isolating suppressors of frameshift mutations that had been induced by mutagens other than ICR191. One such suppressor is *sufJ*. Evidence has been presented elsewhere that the *sufJ* suppressor can read the four-base codons ACCU, ACCC, and ACCA (BOSSI and ROTH 1981). This paper describes the genetic characterization and physiological properties of the *sufJ* suppressor.

MATERIALS AND METHODS

Media and growth conditions

Difco nutrient broth (0.8%) containing 0.5% NaCl was used as rich medium for routine cell growth. When required, this medium was supplemented with 25 μg/ml tetracycline (Sigma). The E medium of VOGEL and BONNER (1956) supplemented with 2% glucose was used as minimal medium. When required, this medium was supplemented with 0.1 mM (excess) or 0.005 mM (limiting) histidine, 0.5 mM (excess) or 0.01 mM (limiting) adenine, 0.5 mM uracil, and approximately 0.3 mM other amino acids. In E medium, tetracycline was used at 10 μg/ml final concentration, kanamycin sulfate was 120 μg/ml, and rifampicin (Sigma) was 10 μg/ml. For use in plates, medium was solidified by the addition of Difco agar to 1.5%.

Bacterial strains

The genotypes and the sources of the strains used are listed in Table 1. All strains are derivatives of *Salmonella typhimurium* strain LT2. The gene designations are those of the revised Salmonella genetic nomenclature (SANDERSON and HARTMAN 1978). The TT designation refers to strains in our collection that contain (or are derived from a strain that contains) a copy of translocatable drug resistance element.

Isolation of the sufJ *suppressor*: The *sufJ* suppressor mutation characterized here was isolated as described by KOHNO, BOSSI and ROTH (1982). This was done by selecting His^+ revertants of strain TR5198 which carries the frameshift mutation *hisG6608* and also mutation *hisT1529*. A 0.2-ml aliquot of a fully grown nutrient broth culture of TR5198 was spread on unsupplemented minimal medium plates. Ten microliters of a 1-mg/ml solution of ICR191 (kindly donated by DR. H. J. CREECH) were spotted on the surface of each plate. Plates were incubated in the dark at 37° for 3 to 4 days. After this period several revertant colonies were seen around the ICR191 spot. Two different classes of revertants could easily be distinguished on the basis of colony size and morphology. One class formed large wrinkled colonies; the other formed small, smooth revertant colonies. Previous work has shown that true revertants, which completely remove polarity effects, form wrinkled colonies. Suppressor-carrying revertants retain most of the polarity of the suppressed mutation and, therefore, form smooth colonies. This phenomenon is described by RIDDLE and ROTH (1970) and by MURRAY and HARTMAN (1972). A number of the smooth revertant colonies were picked and purified. Most of the work described here involves one of these suppressor-carrying isolates (TR5532).

Isolation of a sufJ*-linked Tn*10 *insertion*: The isolation of a Tn*10* insertion genetically linked to the *sufJ* locus was carried out by taking advantage of the uracil sensitivity phenotype characteristic of strains carrying both *sufJ* and *hisT* mutations (see RESULTS). Strain TR5532(*hisG6608*, *sufJ*, *hisT*) was used as recipient in a transduction cross in which the donor is a mixture of 5000 pooled, random Tn*10* insertion mutants (KLECKNER, ROTH and BOTSTEIN 1977). Tetracycline-resistant transductants were selected and screened for those that had received associated donor material that conferred uracil resistance (*hisT*$^+$ or *sufJ*^{WT}) locus. Among about 1000 of these tetracycline-resistant colonies was one (TT2385) that had received a Tn*10* element linked to *sufJ*^{WT}. As expected, this strain had become phenotypically His^- due to loss of its *sufJ* allele. The location of the Tn*10* insertion near the *sufJ* locus was definitely established as described in the text (see RESULTS).

Transductional methods: For all transductional crosses, the phage used was P22 carrying the mutation *HT105/1* (SCHMIEGER 1972), which causes an increased frequency of generalized transduction, and mutation *int-201* (isolated by G. ROBERTS), which prevents stable lysogen formation.

TABLE 1

List of strains[a]

Strain	Genotype	Source
TR5198	*hisO1242 hisG6608 hisT1529*	This paper
TR5532	*hisO1242 hisG6608 hisT1529 sufJ128*	This paper
TT2385	*hisO1242 hisG6608 hisT1529 zii-614*::Tn*10*	This paper
TR2705	*hisO1242 hisD6580*	KOHNO and ROTH 1976
TT2798	*purD1835*::Tn5	Lab collection
TT4324	*hisO1242 hisD6580 purD1835*::Tn5	This paper
TT3681	*hisO1242 hisG6608 hisT1529 sufJ128 zii-614*::Tn*10*	This paper
TT3137	*hisO1242 hisD6580 argH1823*::Tn*10*	This paper
TK219	*aroD5 hisT1529 pro-710* (HfrK5)	Lab collection
TT4587	*aroD5 hisT1529 pro-710 zii-614*::Tn*10* (HfrK5)	This paper
TT4186	*hisO1242 hisD6580 rpo-43*	This paper
TT4190	*hisO1242 hisF3704 leu*-1110 *tyrU90* (*supM*)	This paper
TT4328-TT4329	*hisO1242 hisD6580* DUP381 [(*purD1835*::Tn5, *sufJ*, *argH*$^+$) (*purD*+, *sufJ*^{WT} *argH*$^+$)]	This paper
(independently-isolated strains containing tandem duplications that include the *purD*, *sufJ* and *argH* loci)		
TT4185	*hisO1242 hisD6580 sufJ128 argH1823*::Tn*10*	This paper
TR5643	*hisG6608 sufJ101 hisT1504 purF145*	This paper

[a] All strains are derivatives of *S. typhimurium* strain LT2. Strains are listed in the order of their appearance in the text. Gene designations are those of the revised Salmonella genetic nomenclature (SANDERSON and HARTMAN 1978). The nomenclature conventions for Tn*10* and Tn5 insertions has been described by CHUMLEY, MENZEL and ROTH 1979.

Phage was grown as described by HOPPE *et al.* (1979). Transductions were carried out by spreading selective plates with 10^8 to 10^9 phage particles and approximately 2×10^8 recipient cells. When selection was made for inheritance of a donor Tn*10* or Tn5 element, phage and bacteria were mixed and preincubated at 37° for 30 min in a nonselective liquid medium, before plating on drug-containing plates. Transductants were scored after 1 to 3 days' incubation at 37°. Transductants to be used further were purified by two to three sequential single colony isolations to remove phage and shown to be phage sensitive. At several stages in the course of this work, Tn*10* or Tn5 insertions in or near genes of interest have been used to selectively move genetic markers from one genetic background to another (KLECKNER, ROTH and BOTSTEIN 1977).

Conjugational methods: An overnight nutrient broth culture of the donor strain was diluted 1:20 in the same medium and grown with shaking for 2 hr. After this period, aliquots of the culture were mixed with a stationary phase culture of the recipient strain in a 1:2 ratio and incubated at 37° for 1 hr. From this mixture serial dilutions were made in nonselective medium and plated on selective plates. Exconjugants were scored after 1 to 3 days' incubation at 37°.

Use of chromosomal duplications in dominance studies: ANDERSON and ROTH (1979, 1981) have described a procedure to isolate tandem chromosomal duplications in virtually any region of the bacterial chromosome. Application of this method for dominance testing was described by JOHNSTON and ROTH (1980). A modified version of this procedure was used in this study to isolate duplications in the *purD* region of the Salmonella chromosome. Strain TR2705 (which contains *sufJ* suppressible

mutation *hisD6580*) was transduced to kanamycin resistance with a P22 phage lysate of strain TT2798 (which carries an auxotrophic Tn5 insertion in the *purD* gene). The resulting strain, TT4324, inherited, along with Tn5, the concomitant *purD* auxotrophy. When this strain was grown in liquid culture, it was expected that a small fraction of cells harbored spontaneous duplications in the *purD* region, resulting in the presence of two copies of the *purD*::Tn5 insertion mutation (ANDERSON and ROTH 1981). Such duplication-carrying strains were identified in the following way. A transductional cross was carried out using P22 grown on wild-type LT2 as donor and TT4324 as recipient. Selection was made for recombinants that are both prototrophic (*purD*$^+$) and kanamycin resistant. The vast majority of recipient cells cannot give rise to transductants under such a selection, since replacement of *purD*::Tn5 mutation with its wild-type allele results in the concomitant loss of the kanamycin resistance determinant. However, the fraction of recipient cells that carry pre-existing duplications in the *purD* region, can inherit a wild-type allele of *purD* in one copy of the duplicated region, while maintaining the *purD*::Tn5 insertion in the other copy. These cells give rise to Pur$^+$ Kan^R transductants. Two of these transductants were picked, purified by streaking twice on selective plates, and then tested for genetic stability. According to our hypothesis, these strains should be genetically unstable and should segregate both Pur$^-$ Kan^R and Pur$^+$ Kan^S cells due to homologous recombination between the two copies of the duplicated material. The stability test was performed by growing the two strains in nonselective liquid medium and then diluting and plating for single colonies on adenine-limiting E plates. Auxotrophic (Pur$^-$) segregants were identified by their smaller and paler colony morphology. The two duplication-carrying strains were then used in the dominance study of the *sufJ* mutation. As described in the text, this was achieved by placing a *sufJ* suppressor mutation in one copy of the duplication by means of a *sufJ*-linked Tn*10* element. The genetic stability of the resulting His$^+$ strain was tested on histidine-limiting E plates (0.005 mM histidine). On these plates, His$^-$ segregant colonies, which have lost their suppressor mutation, are easily recognized by their pale color and cone-shaped morphology.

RESULTS

sufJ: *isolation and suppression spectrum*

The *sufJ* suppressor was first isolated as an ICR191-induced extragenic revertant of frameshift mutant *hisG6608* by KOHNO, BOSSI and ROTH (1982), who demonstrated several surprising properties of the suppressor. It corrects several distinct classes of frameshift mutations, and it suppresses mutations *hisG6608* and *hisG6609* only when a *hisT* mutation is simultaneously present in the genetic background. The suppressor has been shown to suppress +1 frameshift mutations by reading the codons ACCU, ACCC and ACCA (BOSSI and ROTH 1981). We have further characterized the *sufJ* suppressor by genetic mapping, dominance tests and by checking its requirement for a secondary *hisT* mutation in order to correct certain frameshift mutations.

sufJ: *mapping*

The *sufJ* mutation was mapped by methods involving use of translocatable drug resistance elements (KLECKNER, ROTH and BOTSTEIN 1977). A tetracycline resistance element (Tn*10*) was inserted near the *sufJ* gene (see MATERIALS AND METHODS). Since tetracycline resistance is a directly selectable phenotype, mapping the Tn*10* insertion was simpler than mapping the suppressor mutation. Two-point transduction crosses were performed to determine the genetic distance between *sufJ* and Tn*10*. Data are shown in Table 2. There is a considerable difference in the *sufJ*-Tn*10* linkage, depending on whether Tn*10* is in the donor or in the recipient. This may reflect differences in the colony-forming efficiencies of various recombinant types; alternatively, the size (more than 9 kb) of the

TABLE 2

Transductional linkage between sufJ *and Tn10*[a]

Cross	Donor	Recipient	Selected donor marker	Unselected donor marker	Number of transductants scored	Coinheritance of donor markers (%)
1	*sufJ* Tet^S (TR5532)	Tet^R (TT2385)	*sufJ* (His^+)	Tet^S	142	37
2	*sufJ* Tet^R (TT3681)	Tet^S (TR5198)	Tet^R	*sufJ* (His^+)	256	68

[a] In the first cross, the donor carries *sufJ*; the recipient carries Tn*10* inserted near *sufJ*^{WT} and the suppressible frameshift mutation *hisG6608*. His^+ transductants were selected and scored for tetracycline resistance (inheritance of the recipient Tn*10* insertion). In the second cross, the donor contains Tn*10* inserted near *sufJ*; the recipient carries the suppressible frameshift mutation *hisG6608*. Tetracycline-resistant transductants were selected and scored for histidine-independence (His^+ = *sufJ*; His^- = *sufJ*^{WT}).

donor Tn*10* element may affect the frequency of the various transduced fragment types. Regardless of these differences, the data do demonstrate close linkage of Tn*10* to *sufJ*, making it reasonable to map this Tn*10* insertion in order to determine the chromosomal position of *sufJ*.

sufJ *is located between* argH *and* rpo (rif) *at 89 minutes of the Salmonella genetic map*: The approximate position of Tn*10* on the Salmonella map was determined in preliminary conjugation experiments. Tn*10* was transduced into the Hfr strain TK219. The resulting strain, TT4587, which mobilizes the chromosome in a counterclockwise direction starting from *his*, was mated with various recipients. Results suggested a location near the *metA* locus at minute 90 of the genetic map. Three-point conjugation crosses indicated the gene order: Tn*10*, *metA*, *purA*, *pyrB*. Therefore, various markers in the region of the map around 90 minutes were tested for transductional linkage with Tn*10*. No linkage was found between Tn*10* and either *metA* or *thy* (data not shown); however, about 20% cotransduction was found between Tn*10* and the *argH* locus at 89 minutes (see later). On the basis of its map position, the transposon insertion was designated *zii-614*::Tn*10* (CHUMLEY, MENZEL and ROTH 1979). The next step was to define the map position of *sufJ* more exactly.

Several features of *sufJ*-containing strains had to be taken into consideration in designing the transductional crosses to define the map position of the suppressor.

(1) Early experiments had shown that the efficiency of transduction is greatly reduced when *sufJ*-containing strains are used as recipients. This is apparently due to an increased sensitivity of *sufJ* strains to killing by P22 as judged by the fact that P22 plaques on *sufJ*-carrying strains are larger and clearer than plaques on *sufJ*$^+$ hosts.

(2) *sufJ*-containing strains grow slower than the *sufJ*^{WT} parent even on a rich medium. Therefore, cultures of these strains tend to accumulate cells that have lost the suppressor mutation (data not shown). These suppressor-free revertants greatly confuse mapping since they are indistinguishable from *sufJ*^{WT} recombinants.

(3) Mapping of the genes in question is complicated by the fact that this region of the chromosome is frequently duplicated. The genes lie between ribosomal RNA (*rrn*) loci and, therefore, are included in duplications generated by recombination between these rrn loci (HILL *et al.* 1977; ANDERSON and ROTH 1981). If a recipient cell carries two copies of the region being analyzed, it is impossible to score inheritance of recessive donor markers.

To avoid all of these problems in the work described here, crosses are designed so that dominant alleles are placed in the donor. (The dominant nature of the *sufJ* suppressor will be described later.) By placing *sufJ* mutations in the donor, one minimizes the problems of phage killing. The problem of accumulation of revertants that have lost *sufJ* is also avoided since one scores acquisition of the suppressor, and very little inaccuracy is introduced by a few suppressor revertants in the donor. When the *sufJ* mutation is in the recipient, one scores linkage as loss of the suppressor, and revertants that have lost the suppressor are indistinguishable from recombinants and give the false impression that the suppressor is linked to any recipient marker tested. Inheritance of dominant donor markers can be unambiguously scored even when a subset of the recipient population is diploid for the region in question.

Two three-point transductional crosses were performed to determine the position of *sufJ* relative to the other markers at 89 minutes of the map. These experiments are shown in Tables 3 and 4. The data in Table 3 is best explained by the order: *argH*-Tn*10*-*sufJ*; from the data in Table 4, the order: Tn*10*-*sufJ*-*rpo* is inferred. Therefore, *sufJ* must be located between *argH* and *rpo*. This conclusion was confirmed by an additional three-point cross involving these three markers (data not shown). The order: *argH*, Tn*10*, *sufJ* was confirmed by measuring the linkage between *argH* and *sufJ* with and without Tn*10* present in the donor. If Tn*10* is located between these markers, its size should reduce the likelihood of including both the outside markers in the same transduced fragment. Without Tn*10*, linkage was found to be 12%; with Tn*10*, linkage was reduced to 5%. This is consistent with the central location of Tn*10*. Summarizing, the order of markers is: *argH*, *zii-614*::Tn*10*, *sufJ*, *rpo*.

TABLE 3

Mapping of sufJ *with respect to* argH *and* zii-614::*Tn10*[a]

Donor:	TT3681	*argH*$^+$	*Tet*^{R}	*sufJ*
		1 2	3	4
Recipient:	TT3137	*argH*$^-$	*Tet*^{S}	*sufJ*^{WT}
Selected donor marker	Recombinant class	Unselected markers inherited	Regions of required crossover	Percentage of recombinants in the class
argH$^+$	I	*Tet*^{R} *sufJ*	1,4	4
	II	*Tet*^{R} *sufJ*^{WT}	1,3	15
	III	*Tet*^{S} *sufJ*^{WT}	1,2	80
	IV	*Tet*^{S} *sufJ*	1,2,3,4	1

[a] The donor carries the *sufJ* suppressor and a linked Tn*10* insertion (*zii-614*::Tn*10*). The recipient carries an *argH* mutation and the suppressible mutation *hisD6580*. The Arg$^+$ phenotype was selected and recombinants were scored for inheritance of the donor Tn*10* (tetracycline resistance) and the donor *sufJ* mutation (histidine independence). A total of 150 recombinants were scored.

TABLE 4

Mapping of sufJ *with respect to* rpo (rif) *and* zii-614::Tn10[a]

Donor:	TT3681	Tet^R	*sufJ*	$rpo^+(rif^S)$	
		1	2	3	4
Recipient:	TT4186	Tet^S	$sufJ^{WT}$	$rpo^-(rif^R)$	
Selected donor marker	Recombinant class	Unselected markers inherited		Regions of required crossover	Percentage of recombinants in the class
Tet^R	I	*sufJ* rpo^+		1,4	5
	II	*sufJ* rpo^-		1,3	37
	III	$sufJ^{WT}$ rpo^-		1,2	58
	IV	$sufJ^{WT}$ rpo^+		1,2,3,4	0

[a] The donor carries *sufJ* and the linked Tn*10* insertion. The recipient carries the *sufJ*-suppressible mutation *hisD6580* and a rifampicin-resistance mutation *rpo*. Tetracycline-resistant recombinants were selected and scored for rifampicin sensitivity; inheritance of *sufJ* was scored by determining whether recombinants require histidine for growth. A total of 150 recombinants were scored.

sufJ *is closely linked to the ochre suppressor* tyrU (supM): The tyrosine-inserting ochre suppressor *tyrU* (*supM*) is also located in the *argH-rpo* region of the chromosome (ORIAS *et al.* 1972; WINSTON, BOTSTEIN and MILLER 1979). Transductional experiments were carried out in order to define the relative positions of the two suppressors. In the crosses to be described, we were unable to detect recombination between the two suppressors; this suggests that the two mutations are very closely linked (>99%) and are probably within several hundred base pairs of each other (WU 1966).

In these crosses, the frequent duplication of the region is a particular problem. In crosses between a *sufJ*-containing strain and a *supM*-containing strain, it is possible to obtain recombinants that carry both suppressors without the necessity of recombination in the small region between the mutations. This occurs when the recipient strain carries a duplication of the region including the suppressors. When the recipient carries one suppressor (*e.g.*, *supM*) and the donor carries another (*e.g.*, *sufJ*), the donor can replace the recipient marker *in one of the two copies of the duplicated region.* This leaves a recombinant that carries one of the suppressors in each copy of its duplicated region. Such recombinants occur with high frequency and falsely suggest that recombination between the mutations has occurred.

This problem was minimized by using the Tn*10* insertion as a selective marker and scoring the inheritance of the suppressors as unselected markers, allowing time for segregation to occur before scoring. The Tn*10* element used is approximately 50% linked to both suppressors. In the crosses, the donor strain (TT3681) carried the Tn*10* insertion (*zii-614*::Tn*10*) and *sufJ*. The recipient strain (TT4190) carried *supM* and two suppressible auxotrophic markers which permit scoring of each suppressor. Of 200 tetracycline-resistant recombinants scored, 86 inherited the donor *sufJ* allele without *supM*; 107 recombinants inherited the reciprocal combination (*supM* without *sufJ*); seven carried *supM* in one copy of the region and *sufJ* in the other copy. No recombinants were recovered that inherited both suppressors in the same copy of the region or that had inherited

neither suppressor. Thus, no recombination between the two suppressors was observed.

sufJ: *dominance studies*

To test dominance of a given mutation in bacteria, one must construct strains that are merodiploid for the region of interest. This is traditionally done using F′ plasmids which carry the chromosomal region in question. For bacteria like *S. typhimurium*, for which the number of available F's is limited, this method necessitates the use of *E. coli*-derived episomes. Use of tandem duplications avoids this complication and permits construction of strains with two copies of the region of interest; mutations of interest can be introduced into these copies by transduction. This method has two advantages over use of F′ episomes. (1) The gene dosage of the two alleles being tested is nearly equal. (2) The merodiploid strains generate segregants of *both* haploid types, thus permitting verification of the composition of both copies of the diploid region. Schemes have been described for isolating and mapping duplications in virtually any region of the chromosome (ANDERSON and ROTH 1981).

Dominance of the *sufJ* suppressor was demonstrated using two independently isolated strains (TT4328 and TT4329) containing duplications of the *sufJ* region. These were isolated by selecting for duplication of the nearby *purD* locus (see MATERIALS AND METHODS). As isolated, the strains carry a *purD*::Tn*5* insertion in one copy of the duplicated region and a *purD*$^+$ allele in the other copy. The duplication can be maintained by selection for Pur$^+$ and kanamycin resistance (Kan^R); haploid segregants are identified as Pur$^-$ Kan^R or Pur$^+$ Kan^S derivatives. The *sufJ* suppressor was introduced into the duplication strains by transduction using strain TT4185 (*argH*::Tn*10*, *sufJ*) as donor. Each Tet^R transductant receives the Tn*10* element and associated material in one of the two copies of the duplication. These transductants remain phenotypically Arg$^+$; they have received the *argH*::Tn*10* mutation in one copy but have retained an *argH*$^+$ allele in the other. Since *sufJ* is closely linked to the *argH*::Tn*10* insertion (see Table 3), many of these transductants will have acquired *sufJ* in one copy and retained *sufJ*^{WT} in the other. Expression of *sufJ* can be scored by suppression of an appropriate *his* mutation which is present in the recipient strain. Results of the cross show that His$^+$ recombinants do occur; these are presumed to reflect the activity of an inherited *sufJ* mutation which is detected despite the presence of its wild-type allele. To check this, haploid segregants were recovered from the duplication strain. Some of these haploids remained His$^+$, others became His$^-$. This demonstrates that the His$^+$ duplication strain carried both the *sufJ* and the *sufJ*^{WT} alleles. The *sufJ* suppressor is clearly dominant to its wild-type allele.

Dependence on hisT *for suppression of mutations* hisG6608 *and* hisG6609

The *sufJ* suppressors were first isolated as revertants of mutations *hisG6608* and *hisG6609* (KOHNO, BOSSI and ROTH 1982). These two mutations map at the same site (HOPPE *et al.* 1979) and have identical sequence changes (W. BARNES, personal communication). The *sufJ*-carrying revertants of these mutants were only seen in strains that carry an unlinked *hisT* mutation in addition to the suppressible mutation; no suppressor-carrying strains were present among His$^+$ revertants of these mutants when the *hisT* mutation was absent. The *hisT* gene

encodes a tRNA modification enzyme responsible for generating pseudouridine in the anticodon loop of many tRNAs (CORTESE *et al.* 1974; TURNBOUGH *et al.* 1979). It appears that two tRNA changes may be needed to permit correction of this mutant site, one change is caused by the suppressor mutation itself and one by the *hisT* mutation. Results in the accompanying paper show that the dependence of *sufJ* on the *hisT* mutation is specific for these two mutations. All other suppressible *his* mutations can be corrected by *sufJ* with or without a *hisT* mutation present in the genetic background (KOHNO, BOSSI and ROTH 1982).

The *hisT* dependence has been confirmed by construction of strains carrying various combinations of the mutations involved. The amber mutation *hisT2890* serves to permit suppression; the temperature-sensitive mutation *hisT1535* permits suppression only at high temperatures. A particularly clear demonstration of the *hisT* effect can be seen when a $hisT^+$ allele is transferred to a strain depending on a $hisT^-$ mutation for its His^+ phenotype. This can be done using the phenotypically His^+ strain TR5643 (*hisG6608, sufJ, hisT1504, purF145*). When this strain receives the F'_{32} $hisT^+$ episome, it becomes phenotypically His^-. Thus, the enhancement of suppression by *hisT* is a recessive property of the *hisT* mutation and is probably due to a deficiency in *hisT* function.

Physiological effects of sufJ

In the course of characterizing *sufJ*, some interesting pleiotropic phenotypes of the suppressor mutation were noted. The slower growth and increased sensitivity to P22 killing were mentioned earlier. In addition to these effects, it was found that strains simultaneously carrying a *sufJ* suppressor and a *hisT* mutation are unable to grow in the presence of 0.1 mM uracil (a concentration normally used to supplement uracil auxotrophs). The growth inhibition caused by uracil can be reversed by addition of arginine (or citrulline, but not ornithine). Thus, it appears that the presence of uracil causes arginine limitation in *sufJ-hisT*-carrying strains. Uracil sensitivity was occasionally seen also in strains containing *sufJ* alone ($hisT^+$), however, the effect was much less pronounced. Strains carrying only a *hisT* mutation (suf^{WT}) are not uracil sensitive. Thus, the combination of the two mutations is required for the maximum uracil sensitivity.

That the *sufJ* suppressor mutation is responsible for the *ura*-sensitive phenotype is further demonstrated by the finding that when *ura*-resistant revertants are isolated, most of them turn out to be histidine auxotrophs. (They have apparently lost the *sufJ* suppressor.) In other resistant revertants, the activity of *sufJ* is somewhat reduced as judged by the much slower growth rate in absence of histidine relative to the initial strain. This suggests that uracil sensitivity is due to the action of the suppressor tRNA rather than to a lack of the normal tRNA. Involvement of tRNA in causing the uracil sensitivity is also supported by the requirement for a *hisT* mutation; this mutation leaves cells unable to form pseudouridine in the anticodon loops of many tRNAs. A possible explanation for the uracil sensitivity phenotype will be discussed.

DISCUSSION

The frameshift mutation *sufJ* has been genetically mapped at minute 89 on the Salmonella chromosome. The dominance of the *sufJ* mutation is consistent

with its affecting the primary structure of a tRNA molecule. The map position of *sufJ* coincides with the location of ochre suppressor *tyrU* (*supM*) at a genetic locus where a cluster of tRNA genes (tyrosine, glycine and threonine) has been identified in *E. coli* (SQUIRES *et al.* 1973). The threonine tRNA encoded in this gene cluster normally reads ACC. Since *sufJ* suppressor has been shown to read the related four-base codons ACCU, ACCC and ACCA (BOSSI and ROTH 1981), we think it is likely that the suppressor will prove to affect the threonine tRNA in this cluster.

A peculiar feature of the new frameshift suppressor is its unique suppression spectrum. The finding that *sufJ* is able to simultaneously correct frameshift mutations in runs of C, G and A (KOHNO, BOSSI and ROTH 1982) led to the conclusion that the suppressor does not read sites containing any of these particular residues but rather exerts its corrective action by reading particular sites removed from these original frameshift sites. The frequency of this cross-suppression furthermore suggests that the site of *sufJ* action is quite common in messages. The ability of *sufJ* to read ACCU, ACCC and ACCA suggested that it might read a three-base codon and force the translation apparatus to ignore the fourth base. This probably accounts for the frequency with which *sufJ* suppressor sites are encountered; these three-base sites would be expected to occur more frequently than the four-base sites required for other frameshift suppressors.

The *hisT* mutation, causing a defect in tRNA modification, affects two aspects of *sufJ*'s behavior. The uracil-sensitive phenotype of *sufJ* is seen only in strains that also carry a *hisT* mutation, and suppression of mutations *hisG6608* and *hisG6609* requires the presence of both *sufJ* and a *hisT* mutation. Other suppressible mutations are corrected by *sufJ* regardless of the state of the *hisT* locus. Since $tRNA^{Thr}$ does not contain a pseudouridine residue in its anticodon loop, these phenomena cannot be explained by a direct effect of the *hisT* mutation on the structure of the suppressor tRNA. We suggest that the *hisT* mutation improves *sufJ* efficiency at certain sites by reducing the activity of a tRNA that interferes with suppression at those sites. This fits with the observation that *hisT* mutations do reduce the efficiency of certain nonsense suppressor tRNAs that normally contain anticodon loop pseudouridine (BOSSI and ROTH 1980).

A detailed possibility for explaining the effect of *hisT* on *sufJ* is suggested by considering the mRNA sequence at the site in question. Mutation *hisG6608* has the sequence ACCCUGC at the site of suppressor action. We suggest that *sufJ* tRNA (reading ACCC) competes with $tRNA^{Leu}_{CUG}$ for occupancy of the fourth C residue. This competing leucine tRNA has two pseudouridines in its anticodon stem loop and is likely to be rendered less efficient by a *hisT* mutation. When the CUG-reading tRNA is weakened by *hisT*, then there is a higher probability that the four-base codon ACCC can be occupied by *sufJ* tRNA and that the next tRNA to bind to the ribosome will read the UGC codon thereby shifting the reading frame and causing suppression.

The uracil sensitivity of *sufJ-hisT* double mutant strains is sufficiently unique to permit some suggestions as to mechanism. Mutants with the identical phenotype have been found to affect the structural gene for carbamyl phosphate

synthetase (*pyrA*) (ABD-EL-AL and INGRAHAM 1969a, b). This enzyme catalyzes the formation of carbamyl phosphate, an intermediate in the biosynthesis of both arginine and pyrimidines. It has been shown that *pyrA* expression is under cumulative repression by arginine and a uracil compound. Moreover, the enzyme is subject to feedback inhibition by UMP and is activated by ornithine, an arginine precursor. The uracil-sensitive *pyrA* mutants are thought to make an altered enzyme which is no longer activated by ornithine. When the residual activity is inhibited by UMP, insufficient activity remains for synthesis of arginine, leading to an arginine requirement. It seems likely that any impairment of *pyrA* gene expression or carbamyl phosphate synthetase enzymatic activity would lead to this uracil sensitivity phenotype. We propose that the *sufJ, hisT* combination interferes with *pyrA* gene expression. This could occur if regulation involved an attenuator mechanism with a leader peptide that included an ACC codon and codons read by tRNAs that contain pseudouridine in their anticodon loops. This possibility seems likely since C. TURNBOUGH has obtained evidence suggesting that a different pyrimidine biosynthetic gene, the *pyrB* gene, does indeed seem to be regulated by an attenuation mechanism. Alternatively the *pyrA* gene could be unusually rich in ACC codons; the frameshifting induced at these sites by *sufJ* could reduce the level of gene product sufficiently to result in the observed uracil-sensitive growth.

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