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Escherichia coli supH Suppressor: Temperature-Sensitive Missense Suppression Caused by an Anticodon Change in $tRNA_2^{Ser}$

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We describe the cloning and the DNA sequence of the *Escherichia coli supH* missense suppressor and of the *supD60*(Am) suppressor genes. *supH* is a mutant form of *serU* which codes for $tRNA_2^{Ser}$. The *supH* coding sequence differs from the wild-type sequence by a single nucleotide change which corresponds to the middle position of the anticodon. The CGA anticodon of wild-type tRNA and CUA anticodon of *supD* tRNA is changed to CAA in *supH* tRNA, which is expected to recognize the UUG leucine codon. We propose that the *supH* suppressor causes the insertion of serine in response to this codon. The temperature sensitivity caused by *supH* may be due to a conformation of the CAA anticodon in the *supH* $tRNA^{Ser}$ that is slightly different than that in the corresponding $tRNA^{Leu}$ species.

The *supH* suppressor was originally described as a suppressor of two independently isolated *ilvD* mutations and a *lac* mutation in *Escherichia coli* (9, 10). This suppressor was shown to cause temperature sensitivity of cell growth and bacteriophage multiplication. The temperature-sensitive phenotype is expressed both on minimal and nutrient media, and it is independent of the presence of a suppressible mutation. At temperatures of 42 to 43°C, cells carrying this suppressor form long filaments and their viability is severely reduced (9). The suppressor does not suppress amber or ochre mutations. Neither do amber or ochre suppressors act on mutations which are suppressible by the *supH* suppressor (9, 10). The suppressor is expected to be specific for missense mutations. Neither the suppressible codon nor the amino acid substitution mediated by the suppressor have been identified to date. *supH* mutations are readily induced by ethylmethanesulfonate (EMS) and diethylsulfate. They are of uniform phenotype. The *supH* gene maps at min 43 on the genetic map (2, 9).

The *supD* suppressor is known to suppress the amber codon UAG (35, 47, 49). Early studies showed that suppression by this suppressor is effected by a minor species of serine tRNA, a mutant form of $tRNA_2^{Ser}$ (1, 3, 7, 11, 42). This tRNA is encoded by the *serU* gene (2, 43). Both the wild-type form of this tRNA and an amber suppressor derivative have been sequenced (43). The suppressor tRNA, which is encoded by the *serU132* (*supD32*) allele, differs from the wild-type $tRNA_2^{Ser}$ by a single base substitution in the anticodon. The CGA anticodon of wild-type $tRNA_2^{Ser}$ is replaced by CUA in the suppressor tRNA. The nucleotide sequence of the *serU* gene has recently been determined (44). The *serU* gene has been mapped at min 43 (12, 18). Thus it is in the same chromosomal region as the *supH* gene. Approximate mapping indicates that other tRNA genes are also located in this region (21).

In this study we describe the molecular cloning and nucleotide sequence analysis of the *supH* allele and compare it with a *supD* allele.

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MATERIALS AND METHODS

Bacterial strains and phages. The bacterial strains used are described in Table 1. They are all derivatives of *E. coli* K-12. The origin of the *serU109* (*supD60*) mutation is CA5013 and is different from the *serU132* (*supD32*) mutation studied by Steege (43). Phages P1 and λ *c*I857 were used for transduction, and phages λ590 (34), M13mp2B (with a *Bam*HI site) (24), and M13mp8 (25) were used for molecular cloning of suppressor genes.

Bacteriological procedures. Bacterial cultures were routinely grown in L broth or on L agar plates. Minimal medium A used in this study was described by Miller (26). Cells infected with phage λ were grown in Bacto-Trypsin (BT) (10 g of Bacto-Trypsin and 5 g of NaCl per liter with 0.2 M $MgSO_4$). This medium was solidified with 1% agar when used for plates and with 0.6% agar for soft agar layers. The buffer used for dilution of phage λ contained 10 mM Tris-hydrochloride (pH 7.5) and 10 mM $MgSO_4$. Bacto-Trypsin without addition of $MgSO_4$ was used for growing M13 phage in liquid cultures. Plates used for growing M13 phage contained minimal agar with glucose and nutritional supplements as required by the host. Phage and bacteria were added in a 3-ml Bacto-Trypsin soft agar overlay. When required, Xgal and IPTG were added to the soft agar (22). Transduction with phage λ was performed by the method of Miller (26). Methods used for transduction with phage P1 and induction of mutations by EMS have been described by Thorbjarnardottir et al. (48). With the exception of the media used, techniques for growing phage M13 were as described by Zinder and Boeke (51).

Cloning and analysis of DNA fragments. DNA was extracted from bacteria and from phage λ particles by established procedures (22, 39). In vitro packing into λ590 phage particles was carried out as described by Hohn and Murray (19). For extraction of DNA, lysates of λ590 were prepared on strain POP101 or ED8654 by the method of Blattner et al. (5). Phage M13 was routinely grown on strains JM101 or JM103. RF DNA of phage M13 was prepared by the method of Zinder and Boeke (51). Clones of phage M13mp8 or M13mp2B carrying suppressor genes were detected by their ability to form blue plaques on Xgal media, with indicator

TABLE 1. Bacterial strains

Strain no.	Description	Derivation (reference)
GE502	F⁻ *ilvD145 his-85 trpR55 trpA9605 pro-48 tsx-84*	Derived from strain DS100 (45) by introduction of *ilvD145* with phage P1 and curing for phage λ
GE510	F⁻ DE45 *ilvD145 lacZ53 trp-49 rpsL150 relA1? glnV44 (supE44) serU132 (supD32)* λ *c*I857	Derived from strain DS68 (45)
GE511	F⁻ DE45 *ilvD145 lacZ53 trp-49 rpsL150 relA1? glnV44 (supE44) supH111* λ *c*I857	*supH* mutation induced by EMS in GE510
GE515	F⁻ *ilvD145 his-85 trpR55 trpA9605 pro-48 tsx-84* λ *c*I857 λ *c*I857 *supH111*	Derived from GE502 by lysogenization with λ phage
GE543	F⁻ *ilvD145 his-85 trpR55 trpA9605 pro-48 tsx-84 serU109 (supD60)*	*serU109* from strain CSH56 (26) transduced by phage P1 into GE502
GE544	F⁻ *ilvD145 his-85 trpR55 trpA9605 pro-48 tsx-84 supH112*	*supH112* mutation induced by phage P1 from GE512 into GE502
GE545	F⁻ *ilvD145 his-85 trpR55 trpA9605 pro-48 tsx-84 lac-291*	*lac-291* mutation induced by EMS in strain GE502
GE845	*ilvD145 his-85 trpR55 trpA9605 pro-48 tsx-84 lac-291*/ F196 *his*⁺ *serU132 (supD32)*	F196 from strain WH-1 (18) introduced into GE545
GE884	F⁻ *ilvD188 his-85 trpR55 trpA9605 pro-48 tsx-84 ser-37* λ i^{434}	A strain derived from GE502 made lysogenic for λi^{434}
GE975	F⁻ *ilvD145 his-85 trpR55 trpA9605 pro-48 tsx-84 lac-291 serU109 (supD60)*	*serU109* introduced by P1 transduction into GE545
ED8654(NEM259)	F⁻ *lacY1* or *lac-3 galK2 galT22 metB1 trpR55 glnV44 (supE44) supF58 hsdR514* λ⁻	(6)
CSH39	Δ(*lac-pro*) *thi*/F′ *lacZ*(Am) *proAB*⁺	(26)
JM101	Δ(*lac-pro*) *supE thi*/F′ *traD36 proAB*⁺ *lacI*^{q}*ZM15*	(23)
JM103	Δ(*lac-pro*) *thi rpsL supE endA sbcB15 hsdR4*/F′*traD36 proAB*⁺ *lacI*^{q}*ZM15*	(24)
POP101	*HflA*	Obtained from N. E. Murray

strains carrying suppressible *lac* mutations. The methods for cleavage of DNA, ligation of DNA fragments, and their analysis on agarose or polyacrylamide gels were essentially as described by Maniatis et al. (22). DNA was sequenced by the dideoxy method (40).

RESULTS

Close linkage of *supH* and *supD* mutations. To determine whether the *supH* and *supD* genes are allelic, we first attempted to construct a strain carrying both suppressors. Strain GE502 has two mutations (*trpA9605* and *his-85*) which are suppressible by the *supD* suppressor and one mutation (*ilvD145*) which is suppressible by the *supH* suppressor. Derivatives of this strain, GE543 and GE544, were obtained, and they carried the *serU109* and *supH112* suppressor alleles, respectively. The *supH112* allele could readily be introduced by P1 transduction into the *serU109* strain and vice versa. However, selection for one suppressor invariably led to the loss of the other. When selection was made for both suppressors, very slowly growing colonies appeared which were unstable for either one of them. No stable transductants carrying both suppressors could be obtained. This result suggested that the two suppressor genes are allelic. This interpretation was confirmed when attempts were made to induce *supH* mutations by EMS or diethylsulfate in strain GE975, which carries two mutations (*ilvD145* and *lac-291*) suppressible by *supH* and two amber mutations (*trpA9605* and *his-85*) suppressible by *serU109*. The frequency of *supH* mutations after treatment with EMS was found to be about 100 times lower in this strain than in an isogenic strain (GE545) with the wild-type allele of the *supD* gene. All 13 of the *supH* mutants of the *supD* strain tested were found to have lost the *supD* suppressor. This result clearly indicated that the two suppressor mutations are allelic.

HFT λ phage carrying the *supH* gene. Steege and Low (45) described a strain which carries the *serU132* (*supD32*) suppressor allele and has λ *c*I857 inserted close to the *supD* locus. Transducing λ phages carrying the *serU132* allele were obtained from this strain. We introduced the *ilvD145* mutation into this strain, treated the resulting strain (GE510) with EMS, and selected for Ilv⁺ revertants. The reversion rate was very low. Among 16 Ilv⁺ revertants tested, only one was found to be due to *supH* mutation *supH111*. This revertant, strain GE511, no longer had an active *supD* suppressor, and the *serU132* allele could neither be recovered by induction of λ *c*I857 nor by P1 transduction. Although strain GE511 is lysogenic for λ *c*I857 it is not lysed on incubation for 2 to 3 h at 42 to 43°C. This is in accordance with the previously observed inhibitory effect of *supH* mutations on the multiplication of phage λ at 42 to 43°C (9). Strain GE510 is lysed under the same conditions. However, λ *c*I857 can be induced in strain GE511 by incubation for a short period (e.g., 10 min) at 42°C, followed by incubation at 37°C. In this way we obtained LFT lysates of transducing phages carrying the *supH111* allele. Such a lysate was used to lysogenize strain GE502. The resulting strain, GE515, which is lysogenic for both λ *c*I857 and λ *c*I857 *supH111*, was used for production of HFT lysates. Induction was carried out as described for strain GE511. Strain GE515 has the *supH*⁺ (wild type) allele on the chromosome. The effective suppression of the *ilvD145* mutation in this strain shows that suppression by *supH* is expressed as a dominant trait.

Cloning and DNA sequence of the *supH* and *supD* genes. DNA from a mixture of λ *c*I857 and λ *c*I857 *supH111* obtained after induction of strain GE515 was digested with *Sau*3A, and the fragments were ligated into the *Bam*HI site of M13mp2B. M13 phage carrying a functional *supH* gene were detected by the formation of blue plaques (on Xgal media) on strain GE845, which has the *supH*-suppressible *lac* mutation *lac-291*. Seventeen M13 *supH111* clones were selected for study. Fourteen of them were very unstable for the *supH111* allele. DNA was prepared from the remaining

three clones and cleaved with *Eco*RI for release of inserted fragments. One of these clones contained an insert of about 4,000 base pairs, which spontaneously shortened to a fragment of about 300 base pairs containing the functional *supH111* allele. The nucleotide sequence of this fragment is shown in Fig. 1.

DNA from strain GE543 which carries the *serU109* (*supD60*) suppressor allele was cleaved with *Hin*dIII, and fragments were ligated into the *Hin*dIII site of phage λ590. Recombinant phage were selected on strain POP101. Plate lysates were tested for the presence of *serU109* by infecting strain GE884, which is lysogenic for λi^{434} and has two mutations (*his85* and *trpA9605*) that are suppressible by *supD*. Selection was made for His^+ Trp^+ transductants on minimal agar plates. UV light induction of such transductants yielded λi^{434} and λ590 *serU109* phage. *Hin*dIII DNA fragments from a λ590 *serU109* phage were cloned into M13mp8 and tested for the presence of the *serU109* allele by their ability to form blue plaques (on Xgal media) on strain CSH39 (*lacZ* [Am]). One of the phages carried the suppressor on a 6-kilobase-pair fragment which had no *Hin*dIII or *Eco*RI restriction sites. After digestion with *Sau*3A, a fragment of about 650 base pairs carrying the suppressor was obtained. Its nucleotide sequence is shown in Fig. 1.

DISCUSSION

A comparison of the nucleotide sequences of the *supH* and *supD* suppressor genes (Fig. 1) shows that their coding sequences differ by a single nucleotide. This base change corresponds to the middle position of the tRNA anticodon. The *serU109* allele sequence is identical to the *serU132* gene sequence determined by Steege (43). Therefore, *supH111*, *serU132*, and *serU109* are all mutations of the same gene, *serU*. The CGA anticodon of wild type $tRNA_2^{Ser}$ is changed to CUA by the *supD* mutations ($tRNA_2^{Ser}$ *supD*) and to CAA by the *supH111* mutation ($tRNA_2^{Ser}$ *supH*). The CUA anticodon recognizes the UAG amber codon, but the CAA anticodon is expected to recognize the UUG leucine codon (Fig. 2).

In addition to having a different anticodon, the *supH111* allele differs from both *serU109* and *serD132* by an A—C change in the presumed promoter sequence (Fig. 1). Possibly, this difference arose when the *supH111* allele was derived from the *serU132* (*supD32*) allele by treatment with EMS. However, both the anticodon mutation and the promoter mutation are transversions, which are only induced at a low frequency by EMS (8). It is not clear whether this change affects the efficiency of expression of the *serU* gene. An analysis of known procaryotic promoter sequences (15) shows that T is strongly conserved as the first base of the −10 hexamer (the position of the change in *supH*). Of 112

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                   -35                    Pribnow
                  Region                    Box
supH  5'-GGGACTGTTAAAATGCCAAATTTCCTGGAATCATGGCAACCATCTGAACGG
supD     ----------------------------C----------------------

supH     AGAGATGCCGGAGCGGCTGAACGGACCGGTCTCAAAAACCGGAGTAGGGGC
supD     ---------------------------------T-----------------

supH     AACTCTACCGGGGGTTCAAATCCCCCTCTCTCCGCCACTTTATCAATG-3'
supD     ------------------------------------------------
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FIG. 1. Nucleotide sequence of the *supH* and *supD* alleles. The sequence presented covers the essential parts of the gene. Additional sequences have been sent to GenBank. The putative promoter regions are indicated. The anticodon is overlined. Changes in *supD* are given, and conserved bases are indicated by a dash.

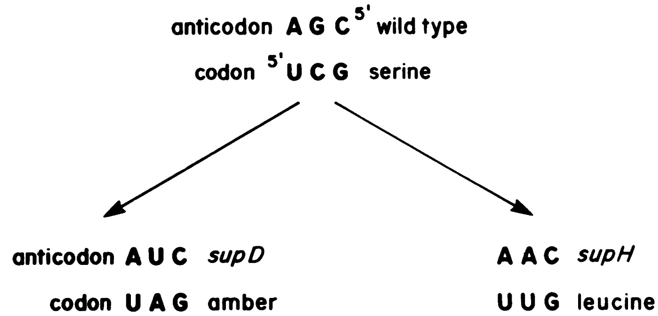

FIG. 2. Scheme of the mutations relating *serU* to *supH* and *supD*.

promoter sequences compared, 89 had T at this position, 11 had G, 10 had C, and only 2 had A. Substitutions of C or A for T have been found to reduce promoter activity, but no substitutions of A for C or C for A have been reported at this position (15). As suppression is effected by the finished tRNA product, it is very unlikely that the observed promoter change directly affects suppressor function. The contention that action of the *supH* suppressor is independent of the observed promoter change is further supported by the observation that *serU109* can be converted to *supH* at a frequency which is too high to be accounted for by double transversions (G. Eggertsson, unpublished data). Most of the *supH* alleles thus derived must be identical to the *serU109* allele in their promoter sequences.

It is noted that the sequence between the Pribnow Box and the origin of the $tRNA_2^{Ser}$ coding sequence differs from that of other tRNA genes which have been sequenced (41) in not being particularly rich in GC base pairs.

Suppression by the *supH* suppressor can be explained as recognition of the leucine codon UUG by the CAA anticodon of $tRNA_2^{Ser}$ *supH*. Unless this base change would cause tRNA mischarging (for review see reference 46), this would lead to substitution of serine for leucine at the mutant site. The possibility of mischarging must also be considered. It is clear that mischarging of $tRNA_2^{Ser}$ *supH* by leucine could neither explain the suppression nor the temperature sensitivity. However, misacylation with other amino acids could explain the temperature sensitivity caused by *supH*, as such mischarging would lead to the insertion of a noncognate amino acid in response to the UUG codon. This codon is expected to be read by one relatively abundant tRNA species $tRNA_{NAA}^{Leu}$ (4, 20, 50) and by a minor $tRNA_{CAA}^{Leu}$ (4, 16). In a cell carrying the *supH* suppressor there will therefore be competition between this leucine tRNA and $tRNA_2^{Ser}$ *supH* for reading of the UUG codon. As $tRNA_2^{Ser}$ is produced in small amounts (1, 42), suppression is expected to be inefficient. The efficiency of the *supH* suppressor has not been determined directly. However, the very slow growth of *supH*-carrying strains when suppression is required for growth (9) probably reflects a correspondingly low efficiency of suppression. According to our explanation, suppression by the *supH* suppressor is a typical case of missense suppression. Many missense suppressors have been described in *E. coli*, and several of them have been well characterized (14, 17, 27–33, 37, 38). All of the latter have involved glycine tRNA with the exception of a suppressor of UGG which was derived from the *supD* suppressor (28). Conversion of the *supD* suppressor into a suppressor of ochre (UAA) mutations has also been described (29, 36).

The wild-type $tRNA_2^{Ser}$ is expected to read the UCG serine codon. As this codon will also be read by $tRNA_1^{Ser}$ (anti-

codon UGA) the dispensibility of the wild-type $tRNA_2^{Ser}$ is not surprising. The possibility that there is a second copy of the $tRNA_2^{Ser}$ gene is unlikely. First, all *supH* and *supD* mutations which have been mapped are located at min 43 (2, 9). Second, we find that the frequency of induction of *supH* mutations by EMS is about 100 times lower in a *supD*-carrying strain than in an isogenic strain carrying the wild-type allele of the *serU* gene. If two $tRNA_2^{Ser}$ genes were present in the cell, the frequency of induction of *supH* mutations would be expected to be only halved by the presence of a *supD* mutation in one of these genes. Third, we find that *supH* derivatives of strains carrying a *supD* suppressor have invariably lost the *supD* mutation. It is noted that a GC—AT transition is required to change wild-type $tRNA_2^{Ser}$ into $tRNA_2^{Ser}$ *supH*, whereas a TA—AT transversion is needed to change $tRNA_2^{Ser}$ *supD* into $tRNA_2^{Ser}$ *supH*. The difference in frequency found between these two mutational changes after treatment with EMS is in agreement with the known mechanism of action of this mutagen (8).

The temperature sensitivity of growth caused by the *supH* suppressor is not fully understood. It has been suggested that it is due to an increased frequency of misreading by the suppressor tRNA at high temperatures (9). According to this hypothesis the $tRNA_2^{Ser}$ *supH* would compete more efficiently at higher temperature (42 to 43°C) with two $tRNA^{Leu}$ species which recognize UUA. This would lead to a higher rate of misreading of the UUG codon and substitution of serine for leucine in a variety of proteins, with lethal effects for the cell. A possible explanation lies in the "context effect" of mRNA reading (46) in which the nucleotide sequence of the anticodon loop/stem and the state of nucleotide modification may impart a different conformation to the same anticodon in different tRNAs. Comparison of the anticodon stem/loop sequences shows that the CAA anticodon in $tRNA_2^{Ser}$ *supH* has better stacking interaction (13) than the anticodon in the $tRNA_{CAA}^{Leu}$ and $tRNA_{NAA}^{Leu}$ species. This may result in differences in codon-anticodon interaction by the three tRNAs when the temperature is varied. It should be noted that the presence of *supH* also affects growth rates (to a lesser extent) at 30 and 37°C (9).

Temperature-resistant mutants of *supH*-carrying strains can easily be obtained without loss of suppressor activity (9). The high frequency and frequent instability of such mutants indicates that they may be caused by duplications or other chromosomal rearrangements. It is suggested that at least some of these mutants are due to duplications of a gene coding for UUG-reading leucine tRNA. Increased production of such tRNA as a consequence of the duplication might effectively reduce the rate of misreading by $tRNA_2^{Ser}$ at 42 to 43°C, which was suggested above for strains carrying the *supH* suppressor.

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