Novel UGA-suppressors in *Escherichia coli* K-12

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ABSTRACT

UGA-specific nonsense suppressors from *Escherichia coli* K-12 were isolated and characterized. One of them (Su⁺UGA-11) was identified as a mutant of the *prfB* gene for the peptide releasing factor RF2. It appears that in this strain, while peptide release at sites of UGA mutations is retarded, the UGA stop codon is read through even in the absence of a tRNA suppressor, exhibiting a novel type of passive nonsense suppression. Three suppressors (Su⁺UGA-12, -16 and -34) were capable of restoring the streptomycin sensitive phenotype in resistant bacteria (*strA*^r). Because of their drug-related phenotype, these are possibly mutations in the components of the ribosomal machinery, particularly those concerned with peptide release at UGA nonsense codons. A tRNA suppressor was also obtained which was derived from the tRNA^{Trp} gene. In this strain, a long region between *rrnC* (84.5 min) and *rrnB* (89.5 min) was duplicated and one of the duplicated genes of tRNA^{Trp} was mutated to the suppressor. The mechanism of UGA-suppression is discussed in terms of translation termination at the nonsense codon in both active and passive fashions.

1. INTRODUCTION

The well characterized nonsense suppressors are almost exclusively mutant tRNAs, which become capable of recognizing nonsense codons by a single-base substitution in one of the duplicated tRNA genes (see for a review, Ozeki et al., 1980). Single-base mutations in suppressor tRNAs are usually in the anticodon triplets, but they also can occur in some other parts of tRNAs. For instance, a mutation of G24 to A24 in the D-stem of tRNA^{Trp}, without altering the anticodon, makes this tRNA able to recognize the UGA codon (Hirsh, 1971; Buckingham and Kurland, 1977). In any case, a definitive mechanism of nonsense suppression has been established which is termed as "tRNA suppression", in which the introduction of a mutant tRNA into the cellular decoding system of genetic codons positively enables the reading of a nonsense codon as a sense codon, at least to an appreciable extent, during protein biosynthesis.

In contrast, one of the UGA-suppressors we have characterized here is

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apparently not a tRNA mutant but is rather a mutant of the *prfB* gene for one of the two peptide-chain release factors, RF2. We suggest that, while peptide release at a UGA mutation site is slowed by a mutation in *prfB*, the UGA codon is read through even in the absence of a known tRNA suppressor. If this is so, this type may be a novel mode of "passive suppression" in contrast to the active suppression by tRNA suppressors. Recently, Kawakami et al. (1988a) isolated and reported this type of UGA suppressors independently.

During the course of this study, we also detected another new type of UGA suppressors which are at the same time capable of restoring the streptomycin sensitive phenotype to resistant bacteria carrying a *strA* (*rpsL*) mutation in ribosomal protein S12. Although these suppressor genes have not yet been identified, their streptomycin related phenotype suggests that they are most likely to be mutants of components of the ribosomal machinery.

In this paper we report the isolation and characterization of new UGA suppressors, including these new types as well as the ordinary tRNA ones.

2. MATERIALS AND METHODS

(1) Bacteriological

Bacterial strains used in this study were derived from *Escherichia coli* K-12 and are listed in Table 1. Strains FTB1327 and FTB1918 were provided by Dr. Emanuel J. Murgola. Strain KK273 in which a Tn10 was inserted near *prfB* as well as plasmid pKK951 that contains the *prfB* gene were the kind gifts of Drs. K. Kawakami and Y. Nakamura prior to publication (Kawakami et al., 1988b). Media used are described in Inokuchi et al. (1979). Preparation and transformation of plasmid DNA were carried out as described by Maniatis et al. (1982). Mutagenesis was done with *N*-methyl-*N*-nitro-*N'*-nitrosoguanidine (NTG) (Adelberg et al., 1965).

Table 1. Bacterial strains

Strain	Genotype	Reference/source
CA274	HfrC, <i>lac</i> _{am125} , <i>trp</i> _{am}	Russell et al. (1970)
CAJ64	Su ⁺ 9 (UGA)	Sambrook et al. (1967)
LS653	F ⁻ , <i>lacZ</i> _{UGA659} , <i>leu</i> _{UGA} , <i>trpA</i> _{am9605} , <i>his</i> _{am29} , <i>ilv</i> ⁻ , <i>thyA</i> ⁻ , <i>metB</i> ⁻ , <i>argH</i> ⁻ , <i>rif</i> ^r , <i>strA</i> ^r (<i>rpSL</i>)	Soll (1974)
FTB1327	F' <i>tonB</i> ⁺ - <i>trpA</i> ⁺ / <i>glyT</i> (Su ⁺ UGA/G), <i>glyV</i> ₅₅ , Δ (<i>tonB</i> - <i>trpAB</i>), <i>val</i> ^r , ϕ 80 ^r	From E. J. Murgola
AB1157	<i>lac</i> _{UGA} , <i>his</i> _{oc} , <i>arg</i> _{oc} , <i>strA</i> 31	Our laboratory stock
HfrC		"
HfrH		"
KK273	<i>thr</i> ⁻ , <i>leu</i> ⁻ , <i>lac</i> ⁻ , <i>tonA</i> ⁻ , <i>thi</i> ⁻ , <i>supE</i> 44, <i>zgc-21</i> :Tn10	From K. Kawakami

(b) *Isolation of the UGA mutants of phage ϕ 80*

In order to confirm the suppressor activity and to test the suppression patterns of newly isolated UGA-suppressors of *E. coli*, we have isolated UGA nonsense mutants from phage ϕ 80. Phages grown in the presence of NTG (100 μ g) were plated on CAJ64 carrying the UGA-suppressor of tRNA^{Trp}, and the plaques formed were replicated on the lawns of CA274 (Su⁻) and CAJ64 (Su⁺). Fourteen UGA mutants were obtained and ten of them were chosen as a set of tester phages for the present work.

(c) *Biochemical*

Restriction enzymes used were purchased from Takara Shuzo, Inc., Kyoto, Japan. DNA probes were labeled with ³²P by using a nick translation kit (Amersham Japan Ltd, Tokyo, Japan). Southern hybridization experiment was performed by following the procedure of Wahl et al.(1979).

3. RESULTS

(1) *Isolation and classification of UGA suppressors*

Isolation of suppressors for the nonsense codon UGA was performed by using *E. coli* LS653 which carries UGA mutations in two genes, *lac* and *leu*. Mutagenized bacteria were plated on minimal-lactose plates lacking leucine, and the colonies were selected which showed the revertant phenotypes for both markers simultaneously, Lac⁺ and Leu⁺. Some sixty colonies were isolated and their suppressor activities were tested on the UGA mutants of phage ϕ 80 prepared above (MATERIALS AND METHODS). Among the 60 colonies tested, only 13 showed the suppressor activity, supporting growth of the tester phages. Many of the rest may also carry UGA suppressors, but may not insert appropriate amino acids at the UGA mutation site to suppress the ϕ 80 mutants used. This rather narrow range of suppression is presumably because the set of phages were isolated on the Su⁺9 Trp strain as described above (MATERIALS AND METHODS). Table 2 presents the suppression patterns of these 13 Su⁺UGA strains and based on these results they have been classified into six groups. One group contains eight strains while the other five were represented by only one for each group. For further analysis the following six strains were used, namely Su⁺UGA-3, -11, -12, -16, -23 and -34, which showed distinctive suppression patterns. Since the strain LS653 also carries two amber markers in *trp* and *his*, we tested the suppression on these markers. However, none of the Su⁺UGA strains showed amber suppressor activity. Similarly, no suppression was observed on amber or ochre mutants of T4 or lambda phages from our laboratory stock. Thus, all the six LS653 Su⁺UGA mutants were UGA-specific nonsense suppressors.

Table 2. Suppression patterns

Bacterial strain	ϕ 80 UGA mutant									
	1	4	5	6	18	19	34	42	44	45
CAJ64 (Su ⁺ 9-tRNA ^{Trp})	+	+	+	+	+	+	+	+	+	+
FTB1327 (Su ⁺ UGA-tRNA ^{Gly2})	—	—	—	+	+	—	—	—	—	—
LS653 Su [—]	—	—	—	—	—	—	—	—	—	—
Su ⁺ UGA-3	+	—	—	—	+	—	—	—	+	+
(-6, -7, -8, -14, -15, -21, & -40)										
Su ⁺ UGA-11	+	—	—	+	+	—	—	—	—	+
Su ⁺ UGA-12	+	+	+	+	+	—	+	—	—	—
Su ⁺ UGA-16	+	+	—	—	+	—	—	—	—	—
Su ⁺ UGA-23	+	+	+	+	+	+	+	+	+	+
Su ⁺ UGA-34	+	—	+	+	—	—	—	—	—	+

Suppression was tested by phage growth, spreading with a paper strip on a lawn of bacteria.
+ indicates confluent growth; +, partial; —, no growth.

(2) *Su⁺UGA-11, a mutant of the *prfB* gene for peptide release factor RF2*

A competitive relationship has been shown between nonsense suppressors and translational peptide release factors (RF): namely, RF1 (catalyzing termination at UAA and UAG) competes with UAG suppressors, and RF2 (catalyzing termination at UAA and UGA) competes with UGA suppressors (Caskey et al., 1984; Weiss et al., 1984). In order to test for this effect of RF2 on our UGA suppressors, we transformed plasmid pKK951, carrying a 3 kb *Eco*RI-fragment containing the *prfB* gene (Fig. 1), into the Su⁺UGA strains. In all the six strains tested, the suppression efficiency was reduced, judging from both colony growth on minimal-lactose plates lacking leucine and phage growth of ϕ 80UGA mutant (Table 3). Among these strains, the Su⁺UGA-11 was most affected by pKK951, as we could no longer detect the UGA suppressor activity in this strain.

Recently Kawakami et al. (1988b) have isolated an *E. coli* strain KK273 in which a Tn10 transposon (*tet^r*) is inserted at 62 min of the chromosome, which is very close to the *prfB* gene (Fig. 1). Using this strain as a donor, P1 transduction was performed with LS653 carrying various UGA-suppressors as the recipients, selecting for tetracycline resistant transductants. Among the Tn10 transductants thus obtained with the Su⁺UGA-11 strain, about half (25/49) became Su[—]; with other Su⁺UGA strains, no such Su[—] transductants were detected. This result indicated that the wild-type Su[—] allele of the Su⁺UGA-11 gene cotransduced with the Tn10 at a high frequency. Together with above observation with pKK951, we conclude that Su⁺UGA-11 is a mutant of *prfB* gene and its suppressor character is recessive to the wild-type allele.

Table 3. The effect of RF2-transformation on UGA suppression

Strains	No plasmid	Plasmid transformed	
		pACYC184	pKK951
LS653	—	—	—
LS653 Su ⁺ UGA-3	+	+	(+)
LS653 Su ⁺ UGA-11	+	+	—
LS653 Su ⁺ UGA-12	+	+	(+)
LS653 Su ⁺ UGA-16	+	+	(+)
LS653 Su ⁺ UGA-23	+	+	(+)
LS653 Su ⁺ UGA-34	+	+	(+)
CAJ64	+	+	(+)
FTB1327	+	+	(+)

pKK951 is a plasmid derived from pACYC184 which contains a 3 kb *EcoRI* fragment including the *prfB* gene, indicated in Fig. 1. The suppression activity of LS653 and its derivatives was tested by colony growth on minimal-lactose plates lacking leucine. The suppression activity of CAJ64 and FTB1327 was tested by the growth of ϕ 80 UGA mutants. +, well suppressed; (+), weakly suppressed; —, no suppression.

Fig. 1. The site of Tn10 in the *E. coli* chromosome [adapted after Kawakami et al. (1988b)]. An *EcoRI* fragment (3 kb) including the *prfB* gene has been cloned in pKK951 (see text).

(3) *Su⁺UGA-12, -16 and -34 restore the streptomycin sensitive phenotype in the strA resistant mutants*

During the course of this study we found an unexpected effect of UGA-suppressors on the host bacteria. The *E. coli* strain LS653, which we used to select the UGA suppressors, is a streptomycin (Sm) resistant strain carrying a mutation in the *strA* gene (= *rpsL* for ribosomal protein S12). Nevertheless some of the Su⁺UGA mutants obtained became sensitive to the drug. Among the six strains tested, Su⁺UGA-12, -16 and -34 showed this phenotype. The following experiments confirmed that they still carry the *strA* allele and that introduction of these specific suppressors into other Sm-resistant strains also produces the Sm-sensitive phenotype.

Before we became aware of this peculiar phenomenon, we crossed the LS653 Su⁺UGA strains with HfrC or HfrH and attempted to select the recombinants on Sm-containing agar as usual; LS653 is an F⁻ strain carrying various markers, *leu*(UGA), *lacZ*(UGA), *trpA*(UAG), *his*(UAG), *thyA*, *ilv*, *metB*, *argH*, *rif^r* and

strA^r. In certain crosses, for instance between LS653 Su⁺UGA-34 and HfrC, Arg⁺, Met⁺ or Ilv⁻ recombinants were detected on the selective plates containing streptomycin, but all of them showed a Su⁻ phenotype (Leu⁻, Lac⁻), regardless of the recombinant types, as if the replacement of the Su⁺ gene with its Su⁻ wild-type allele was essential for the recombinants to grow on Sm-plates. We then checked the original Su⁺UGA-34 strain and found that this strain itself was phenotypically Sm-sensitive. Since all the Su⁻ recombinants became Sm-resistant, the suppressor mutation occurred in a gene other than *strA* which was still maintained in the Su⁺ strain, and replacement of Su⁺ with Su⁻ recovers the resistant phenotype. When the same Su⁺UGA strain was crossed with HfrH, Trp⁺ or His⁺ recombinants were hardly detectable on the selection plate containing streptomycin. In the crosses with HfrC, a majority of the Met⁺ or Arg⁺ recombinants were Ilv⁻. These results suggested that the UGA-34 suppressor gene was located around 85–100 min of the *E. coli* chromosome. Similar observations were made with the strains Su⁺UGA-12 and -16 whose phenotypes were also Sm-sensitive.

In order to see whether this phenomenon is specific to the *strA*^r allele in LS653, the suppressor gene of the UGA-34 strain was introduced by phage P1-mediated transduction to AB1157 *lac*_{UGA} *strA*-31, selecting for the Lac⁺ colonies. The suppressor activity of the transductants was confirmed by the growth of ϕ 80UGA mutants. All the 22 transductants tested showed a Sm-sensitive phenotype although the *strA* allele in AB1157 was supposedly different from that in LS653. The transduction of the same suppressor gene to its parental LS653 Su⁻ also gave the same result. Moreover, when phenotypic revertants of Sm-resistance were isolated from LS653 Su⁺-34 (phenotypically Sm-sensitive), usually became Su⁻ at the same time. All these results indicate that the suppressor itself is capable of rendering Sm-sensitivity to the *strA*^r bacteria in addition to UGA suppression. Although the three suppressors, UGA-12, -16 and -34, were isolated independently and each showed a different suppression pattern, they have the same consequences in this respect.

In a similar cross between Su⁺UGA-3 and HfrC, about two thirds of the Arg⁺ Sm^r recombinants were Su⁻; while a cross with HfrH, all the Trp⁺ Sm^r recombinants were still Su⁺ and no Su⁻ were detected. Thus the UGA-3 suppressor was roughly located around *argH* (90 min) on the *E. coli* chromosome.

(4) *Su*⁺UGA-23 is a *tRNA*^{Trp} suppressor

As shown in Table 2, the Su⁺UGA-23 was capable of suppressing all of the ϕ 80UGA mutants used, like CAJ64 carrying a known UGA suppressor of *tRNA*^{Trp}. Accordingly this suppressor was derived most likely from the *tRNA*^{Trp} gene (*trpT*). This possibility was confirmed in the following experiments. The *trpT* gene is located at the distal end of the ribosomal RNA operon *rrnC* (85.5 min) which is closely linked to the major *ilv* operon. First, we

introduced the *ilv*⁺ gene by transduction with phage P1 from CA274 (*ilv*⁺ Su⁻) to LS653 (*ilv*⁻ Su⁺UGA-23) and tested the suppressor activity of *ilv*⁺ transductants. As expected, 44/48 *ilv*⁺ transductants were Su⁻, supporting the above possibility. Second, a transducing phage ϕ 80dSu⁺7 was lysogenized into the LS653 Su⁺UGA-23. ϕ 80dSu⁺7 is a defective transducing phage carrying an amber suppressor of tRNA^{Trp} (Su⁺7) whose prophage is formed usually by integration into the *E. coli* chromosome by homology with the transducing fragment. Since LS653 Su⁺UGA-23 contained two amber markers (*trp* and *his*, Table 1), such a lysogen carrying ϕ 80dSu⁺7 prophage was effectively selected. By utilizing this property, the transducing phages carrying the UGA-23 suppressor instead of the original Su⁺7 amber-suppressor were easily obtained via lysogenization of ϕ 80dSu⁺7, being followed by induction with wild-type ϕ 80 as helper. This type of marker-exchange experiment indicates that the UGA-23 is allelic to the Su⁺7 tRNA^{Trp}, being derived from a Trp tRNA gene.

It is known that only one copy of the tRNA^{Trp} gene is present in the *E. coli* genome (Fournier and Ozeki, 1985). Accordingly, the mutation of this gene to a

Fig. 2. Southern hybridization with a DNA probe of the tRNA^{Trp} gene. DNA prepared from the suppressor strains were digested with restriction enzymes, *Mlu*I and *Eco*RI. Southern hybridization was performed with a Trp tDNA probe. M represents *Mlu*I; E, *Eco*RI. The lanes 11, 23 and 34 are for strains LS653 Su⁺UGA-11, -23 and -34, respectively.

suppressor may require its duplication prior to the mutation in order to maintain the translation of the normal UGG codon for tryptophan. Indeed, the first instance of a tRNA^{Trp} suppressor was successfully isolated after the introduction of an F'*ilv* carrying this gene (Soll and Berg, 1969). In the present experiment, however, we have obtained the UGA-23 suppressor without such an operation to deliberately duplicate the *trpT* gene. In order to clarify the situation, we analyzed the copy number of *trpT* in the Su⁺UGA strains by Southern hybridization using tRNA^{Trp} DNA as probe. Fig. 2 presents the result. Evidently the *trpT* gene was duplicated in UGA-23, while not in the UGA-11 and -34. Further analysis revealed that a rather long region (about 5 min) was duplicated in tandem which apparently occurred by unequal recombination between *rrnC* and *rrnB* (Fig. 3). Details of this analysis will be described elsewhere (Chang et al., in preparation).

Fig. 3. A schematic presentation of an unequal cross-over occurring between *rrnB* and *rrnC*, resulting in the duplication of the *trpT* gene. A five min region (84.5–89.7 min) indicated by an arrow is duplicated; all the *rrn* operons in this figure are transcribed in the direction indicated by the arrow. The *trpT* on the left is the suppressor UGA-23 (see text).

We interpret that in the *E. coli* population there is always a minor fraction in which *trpT* is duplicated as above, and, only in such a cell, one of two *trpT* genes is capable of mutating to a suppressor. This duplicated situation may be unstable and tend to reverse towards the singlet state, unless the Su⁺ bacteria are kept to be selected. Without needing such a selection, however, the suppressor in LS653 Su⁺UGA-23 is rather stable. This suggests that the left *trpT* in Fig. 3 is more likely to be the Su⁺ gene than the one on the right, since the latter is flanked by a long homologous region and is expected to be unstable, but if this is the wild type *trpT*, it is essential for viability.

4. DISCUSSION

Among the UGA-suppressors newly isolated from LS653, only one was assigned

positively as a tRNA suppressor of *trpT*. A single-base substitution in the anticodon region may be the simplest and satisfying way to create a tRNA suppressor, although an instance is known in which a mutation at the D-stem (A24) of Su^{+9} tRNA^{Trp} is enough to provide the UGA-suppressor activity. As possible candidates for UGA suppressors, the following tRNA genes (having an anticodon in parentheses) may be considered; Trp(CCA), Cys(GCA), Arg(UCU), Gly(UCC), Ser(UGA) and Leu(UAA). As an additional requirement for a tRNA gene to become suppressor, the gene must be duplicated in the *E. coli* genome, at least temporarily like as the *trpT* gene shown in Fig. 3. Murgola and Childress (1980) have isolated a UGA-suppressor of tRNA^{Gly2}(UCC) which was also used in the present work (Table 2). Since this tRNA gene (*glyT*) is not duplicated, they first changed the anticodon of one of the Gly₃(GCC) tRNA genes to the Gly₂ type and then selected for the UGA suppressor of Gly₂ tRNA. This type of UGA suppressor was not obtained in our experiment judging from the suppression pattern in Table 2. Since, however, the *glyT* gene is located between two rRNA operons, *rrnB* and *rrnE* (at 90 min), this gene may duplicate by unequal crossing over, as we observed in the case of *trpT*. The genes for Arg(UCU) and Ser(UGA) tRNAs have been identified as mutants for DNA replication [*dnaY*; (Mullin et al., 1984)], and for cell division [*divE*; (Tamura et al., 1984)], respectively, and therefore they are presumably not duplicated and may not be considered as suppressor candidates. Recently, by using a set of DNA clones which covers the entire chromosome of *E. coli* (Kohara et al., 1987), we have elucidated all the detectable tRNA genes in the genome (Komine, Adachi, Inokuchi and Ozeki, 1990). Through this work we confirmed that none of the above six genes, including those for Cys(GCA) and Leu(UAA) which had not been identified, is duplicated in the genome. Accordingly, UGA-suppressors may not be readily obtained from tRNA genes by a single mutation. Presumably, therefore, when we forced the selection of UGA-suppressors from LS653, passive suppressors predominated rather than the tRNA suppressors. In the case of amber and ochre suppressors, in contrast, most of the candidate tRNA genes are naturally in a duplicated state, and therefore tRNA suppressors are predominantly isolated by genetical procedures (Ozeki et al., 1980).

It has been shown that the polypeptide releasing factors can compete with tRNA suppressors (Weiss et al., 1984). The situation in UGA-11, however, is different from these, because in this case there is no apparent suppressor tRNA which can read through the UGA codon. It has been generally considered that the UGA stop codon is relatively leaky compared to UAG or UAA. The reasons for this are not known, but the tRNAs for Cys(GCA) or Trp(CCA) may read UGA to certain extent. Moreover, recently a new minor species of tRNA inserting selenocysteine was found in *E. coli* under anaerobic conditions, which has an anticodon for UGA (Leinfelder et al., 1988). These potential UGA-suppressor tRNAs, however, are evidently not enough to suppress UGA mutants; otherwise

one could not isolate UGA mutants in *E. coli*. But, if the peptide releasing efficiency is reduced by mutations in RF2 or other factors required for release, the effect of these potential tRNA suppressors might become more pronounced. Alternatively, even without a tRNA suppressor, the UGA codon may be circumvented and translation continues by some other mechanism, such as by skipping the stop codon. Generally a frame-shift occurring at UGA may be a possible mechanism. However, it is rather unlikely in the present case, because many $\phi 80$ UGA mutants can be restored their function by shifting the frame.

The dual phenotype of UGA-34 (-12 and -16), affecting Sm resistance and suppression, is interesting but puzzling. It is now generally accepted that the target of antibiotic binding is ribosomal 16S RNA rather than the ribosomal proteins, although mutations in some 30S ribosomal proteins are well known to effect the drug sensitivity. Recently, Murgola et al.(1988) discovered a novel UGA suppressor in *E. coli*, namely a mutant 16S RNA provides the suppressor activity specific to UGA. Accordingly, a mutation in 16S RNA gene might possibly provide the dual phenotype we found. Similarly, a mutation in a ribosomal protein may also affect both suppression and drug sensitivity. In either case, the codon specific suppression for UGA suggests that the suppression mechanism appears to be related particularly to the translation termination by peptide releasing at UGA (and perhaps at UAA) by RF2, but not by RF1. Identification of these suppressor genes would help to elucidate the components of ribosomes, RNA and/or proteins, which may interact with RF2 for translation termination.

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