UGA Can Be Decoded as Tryptophan at Low Efficiency in *Bacillus subtilis*

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Replacement of *cat-86* codon 7 or 144 with the UGA codon permitted the gene to confer chloramphenicol resistance in wild-type *Bacillus subtilis*. UAA replacements of the same codons resulted in a chloramphenicol-sensitive phenotype in wild-type *B. subtilis* and a chloramphenicol-resistant phenotype in suppressor-positive strains. N-terminal sequencing showed that UGA at codon 7 was decoded as tryptophan in wild-type cells, at an efficiency of about 6%.

Two of the three known translation termination codons have been introduced by random or site-directed mutagenesis into *Bacillus subtilis* genes (3, 4, 8, 11, 13). The ochre (UAA) and amber (UAG) codons can block phenotypic expression when inserted within a coding sequence. Moreover, second-site mutations have been identified that suppress, at low efficiency, the primary UAA or UAG nonsense mutation. The amino acids inserted at ochre codons due to the action of three such nonsense suppressor mutations are shown in Table 1.

To our knowledge opal mutations, UGA, have never been introduced within coding sequences of *B. subtilis*. Thus, it is not surprising that suppressors of UGA have not been identified in this bacterium. On the other hand, it seems unusual that 30 years of mutagenesis and genetic analysis of *B. subtilis* have failed to reveal a UGA mutation in *B. subtilis*. One explanation is that UGA can be decoded, albeit at low efficiency, as an amino acid. To test this idea, codon 7 of *cat-86* was mutated from GAA (Glu) to UGA (Fig. 1). This mutant gene, *cat-86* UGA-7, specified measurable levels of chloramphenicol acetyltransferase (CAT) in four different strains of *B. subtilis*, represented by BR151, W23, ATCC 6633, and ATCC 7003 (Table 2). Each strain of *B. subtilis* carrying *cat-86* UGA-7 was resistant to 10 µg of chloramphenicol per ml. The same strains lacking a *cat* gene or containing a *cat-86* gene with an ochre mutation at codon 7, *cat-86* UAA-7, failed to grow on 10 µg of chloramphenicol per ml.

CAT-86 protein was purified from BR151 containing the *cat-86* UGA-7 gene. The sequence of the 12 N-terminal amino acids was the same as wild-type *cat-86* with the exception of amino acid 7, which was tryptophan in the mutant protein and glutamic acid in the wild-type protein.

The low CAT activity specified by *cat-86* UGA-7 was presumably a function of two parameters, the efficiency of insertion of tryptophan at UGA and the efficiency with which the corresponding protein functioned enzymatically. To estimate the contribution of the foreign amino acid at position 7 to the diminished CAT catalytic activity, codon 7

TABLE 1. Amino acids inserted at UAA due to three nonsense suppressors^a

Relevant genotype of <i>B. subtilis</i> host	<i>cat-86</i> codons and corresponding amino acid	
	Codon 7	Codon 9
Wild type	GAA/Glu	UAU/Tyr
Mutant		
<i>sup-3</i>	UAA/Lys	UAA/Lys
<i>sup-67</i>	UAA/Lys	UAA/Lys
<i>sup-44</i>	UAA/Leu	UAA/Leu

^a Proteins were purified from each cell type and the first 12 amino acids were sequenced by automated Edman degradation (7). The mutant alleles *sup-67* and *sup-44* were in strains CU48 and CU1965 obtained from S. Zahler (8). Data for *sup-3* were from reference 13. The *cat-86* version used in these studies was *cat-86C2*, a constitutively expressed gene, on pPL703-P4 (7). Plasmid isolation procedures were as in reference 9.

of *cat-86* was mutated to UGG, the codon for tryptophan. This mutation reduced the specific activity of CAT by 33% (Table 3). Thus, the major factor responsible for the low specific activity of CAT encoded by *cat-86* UGA-7 must be the decoding efficiency of UGA. We calculate this efficiency as about 6%, by comparing the specific activities of CAT encoded by *cat-86* UGG-7 and *cat-86* UGA-7.

To examine further the efficiency of UGA suppression in

TABLE 2. Specific activity of CAT-86 in *B. subtilis* strains containing wild-type or mutant *cat-86* genes

<i>B. subtilis</i> strain	CAT sp act (µmol/min/mg of protein) ^a		
	<i>cat-86</i> (wild type)	UGA-7	UAA-7
168 (BR151)	17.24	1.01	0.027
W-23	30.03	0.226	0.010
ATCC 6633	28.44	0.343	0.024
ATCC 7003	19.30	0.511	0.028

^a CAT was assayed at 25°C by the colorimetric procedure of Shaw (15), and protein was determined by the Bradford method (2).

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FIG. 1. Mutations in the *cat-86* gene used to analyze nonsense suppression. All mutations were generated by oligonucleotide-directed mutagenesis (16, 18). The version of *cat-86* used in these studies was a constitutively expressed gene designated C2 (7).

B. subtilis, codon 144 of *cat-86* was mutated from the tryptophan codon, UGG, to UGA (Fig. 1). This mutant gene, *cat-86* UGA-144, specified a CAT specific activity of 0.26 $\mu\text{mol}/\text{min}/\text{mg}$ of protein in *B. subtilis* (Table 3). Thus, UGA at codon 144 of *cat-86* is decoded at an efficiency of about 1%, which is less than the decoding efficiency at codon 7; this difference may reflect the sequence of the regions flanking codons 7 and 144 (1, 12). A subsequent mutation that replaced UGG-144 with UAA (the ochre codon) resulted in a CAT specific activity of 0.01 $\mu\text{mol}/\text{min}/\text{mg}$ of protein (Table 3), and BR151 (*cat-86* UAA-144) could not grow on 10 μg of chloramphenicol per ml, whereas *cat-86* UGA-144 conferred resistance to this level of the antibiotic. Thus, an authentic stop codon placed at position 144 in *cat-86* led to a CAT-negative phenotype.

cat-86, *cat-86* UGA-7, and *cat-86* UAA-7 were introduced into protoplasts of *Staphylococcus aureus* RN450 (5). Fifty neomycin-resistant transformants resulting from each transformation were tested for chloramphenicol resistance. Only the transformants containing *cat-86* UAA-7 were chloramphenicol sensitive (Fig. 2). These results suggested that the UGA codon was also suppressed in *S. aureus*. To ensure that the UGA mutation was retained after the plasmid transformation of *S. aureus*, plasmid was isolated from a *cat-86* UGA-7 transformation of *S. aureus* and the sequence across the region expected to contain the UGA codon was determined. The UGA codon was present. The CAT specific activities in *S. aureus* RN450 containing *cat-86*C2, *cat-86*C2 UGA-7, and *cat-86*C2 UAA-7 were 1.26, 0.055 and 0.009 $\mu\text{mol}/\text{min}/\text{mg}$ of protein, respectively.

The basis for UGA suppression in *B. subtilis*, and presumably also *S. aureus*, is most likely due to a tryptophan tRNA which can decode UGA (6). The predicted anticodon for the tryptophan codon UGG is 3'ACC, and the anticodon for

UGA is 3'ACU. Possibly the anticodon for a tryptophan tRNA species has been altered from 3'ACC to 3'ACU. The latter should recognize UGA as primary codon but 3'ACU should also recognize the authentic tryptophan codon UGG, through wobble.

The gene for the presumed suppressor tRNA probably resides in the *B. subtilis* chromosome since our inspection of the nucleotide sequence of pUB110, the vector for *cat-86*, indicates that the plasmid does not encode a tRNA (10). Interestingly, a single strain of *B. subtilis* that is reportedly cured of the prophage SP β (strain YB886) also suppressed UGA-7, suggesting that the prophage was not responsible for the observed suppression (data not shown). However, data available for *B. subtilis* indicate that the genome contains only a single gene that specifies tryptophan tRNA (17). If this were correct, it would indicate that this single tRNA species decodes 5'UGG at high efficiency and 5'UGA at low efficiency. This is inconsistent with the conventional rules of wobble, but might be attributed to a physical and therefore functional heterogeneity among the products of a single gene, e.g., differences in posttranscriptional modification (14). It is also possible that *B. subtilis* does contain two genes for tryptophan tRNA: one gene encoding a species that decodes only UGG and a second gene encoding a rare tRNA species that decodes both UGA and UGG.

TABLE 3. Specific activity of the CAT-86 enzyme in *B. subtilis* strain BR151 containing wild-type or mutant *cat-86* genes

Version of <i>cat-86</i>	Amino acid inserted	CAT sp act ($\mu\text{mol}/\text{min}/\text{mg}$ of protein)	%
GAA-7 (wild type)	Glu	24.9	100
UAA-7 (stop)	None	0.027	0.1
UGA-7 (stop/Trp)	Trp	1.06	4.2
UGG-7 (Trp)	Trp	16.7	66.9
UGG-144 (wild type)	Trp	24.9	100
UAA-144 (stop)	None	0.016	0.06
UGA-144 (stop/Trp)	Trp	0.263	1.05

FIG. 2. *S. aureus* and transformants containing wild-type or mutant *cat-86* genes streaked onto brain heart infusion agar containing 10 μg of chloramphenicol per ml.

This investigation was supported by Public Health Service grant GM-42925 from the National Institutes of Health and grant DMB-8802124 from the National Science Foundation.

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