Effect of the Relative Position of the UGA Codon to the Unique Secondary Structure in the fdhF mRNA on Its Decoding by Selenocysteinyl tRNA in Escherichia coli*

Gia-Fen T. Chen, Li Fang, and Masayori Inouye‡

From the Department of Biochemistry, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, New Jersey 08854

The fdhF mRNA for formate dehydrogenase H of Escherichia coli contains a UGA codon at position 140. This termination codon is decoded by selenocysteinyl tRNA (the selC product) with the aid of its own specific elongation factor, SelB. For this decoding, a unique secondary structure immediately downstream of the UGA codon has been shown to be essential (Zinoni, F., Heider, J., and Böck, A. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4660–4664). We examined the positional effect of the UGA codon relative to the secondary structure on its decoding using a fdhF-lacZ fusion gene. When the UGA codon was separated by one codon (position -1) from the secondary structure, the UGA decoding, as measured by the β-galactosidase activity, dropped to approximately 78% of the normal level but was still as much as fully dependent upon selC and selenium in the culture medium as in the case of the UGA codon in the normal position (position 0). However, when the UGA codon was separated by two codons (position -2), the decoding level further dropped to 20% of the normal level, and in addition, became dependent only on selC but independent of selenium. When the UGA codon was further separated by three codons (position -3), the decoding level of UGA (-3) became higher than the decoding of UGA (-2) and was completely independent from selC and selenium, indicating that the UGA codon was nonspecifically suppressed. A similar nonspecific suppression was observed for the UGA codon at position -4, but at a lower level. When two UGA codons were tandemly placed at positions 0 and -1, they were still able to be decoded at 17% of the normal level in a selC- and selenium-dependent manner. In the absence of the SelB function, the decoding level of UGA(0) dropped to 1.6% of the normal level, whereas the UGA(-1) decoding dropped to 7.5%. These results indicate that the UGA codon at position 0 is not only most effectively decoded by selenocysteylinyl tRNA but also tightly blocked from its nonspecific suppression in the absence of any components required for the decoding.

A group of proteins are known to contain selenocysteine residues. Such selenoproteins are widely found in prokaryotic as well as eukaryotic cells (see Refs. 1–4 for reviews). In higher systems, the selenocysteine residue plays an essential role in the activity of glutathione peroxidase (5). In Escherichia coli, there are three isozymes of formate dehydrogenases, FDH1, FDH2 (6), and FDH3 (7), all of which contain a selenocysteine residue which has been shown to be essential for enzyme activity. The UGA codon, which normally functions as a terminator codon, has been shown to be used as the codon for selenocysteine residue in all selenoproteins so far characterized (see Refs. 1–4 for reviews).

It has been demonstrated that a putative secondary structure in the Escherichia coli fdhF mRNA, which encodes the 80-kDa subunit of formate dehydrogenase H, plays an important role for the decoding of the UGA codon at position 140 in the fdhF mRNA (8). The secondary structure exists immediately downstream of the UGA codon in the fdhF mRNA and is believed to be recognized by an alternative elongation factor, SelB (9). The SelB elongation factor is then able to decode the UGA codon with the aid of selenocysteinyl tRNA Sec (the selC product) (9, 10). The requirement of the secondary structure has been shown to be highly specific, including the primary sequence in the loop and stem region, the correct folding of the stem, and the size of the stem structure (11). The requirement of a similar secondary structure for the decoding of the UGA codon in the E. coli fdg gene has been demonstrated (12).

In the present report, we examined the effects of the distance between the UGA codon and the secondary structure on the efficiency and specificity of the decoding of the UGA codon in the fdhF mRNA by selenocysteinyl tRNA Sec. We demonstrated that when the UGA codon was moved to position -1, its decoding still required selB, selC, and selenium in the medium. However, the decoding became 78% of the normal level and nonspecific suppression of the UGA codon in the absence of selB, selC, or selenium became higher than in their presence. The decoding of the UGA codon at position -2 dropped to 20% of the normal level and was not dependent upon selenium, although it still required selC. The suppression of UGA codon at position -3 was higher than that of the UGA codon at position -2 and did not require selC and selenium. These results indicate that UGA codon at position 0 in the fdhF mRNA is most efficiently decoded by selenocysteinyl tRNA Sec, although UGA(-1) and UGA(-2) can be decoded by the tRNA Sec.

In addition, in the absence of selB, selC, and/or selenium, UGA(0) is very effectively prevented from its nonspecific suppression by other tRNAs.

EXPERIMENTAL PROCEDURES

Bacteria Strains and Growth Conditions—E. coli FM433 (lacZ::selC::Tn5), WL81460 (lacZ::selC::Tn5), and WL50153 (lacZ::selC::Tn5-amp') (13) were used for transformation with various plasmids described below. The transformed cells were grown in a selenium-deficient medium (14) in the presence or absence of 5 μM Na2SeO3 as described previously (15).

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‡ To whom correspondence should be addressed. Tel.: 908-235-4115; Fax: 908-235-4783.
Isopropyl-1-thio-β-galactopyranoside (IPTG) was used at a final concentration of 2 mM to induce the fdhF-lacZ genes. β-Galactosidase was assayed using a culture of late stationary phase according to Miller.

**Plasmid Constructions**—All the constructions except for ptT662 contained a DNA fragment from the fdhF coding region encompassing from codon 123 to 156. This fragment was amplified by polymerase chain reaction (PCR) with the following primers: 5'TTAAAGCTTTGCG-TACCGA3' for the 5' end and 5'AAATGGATCCGCGCCCAT-TACCGA3' for the 3' end. In the case of the ptT662 construct, the region from codon 123 to codon 144 was amplified using the following primers: for the 5' end primer, the same primer described above was used, and for the 3' end primer, 5'AAATGGATCCGCGCCCATGTC3'. The primers created a HindIII site at the 5' end and a BamHI site at the 3' end. The resultant PCR fragments were digested with HindIII and BamHI and inserted into the HindIII-BamHI site of PIT-lac1(17). Plasmid p2182 contained the wild-type fragment (codon 123-156) possessing the UGA codon corresponding to the 140th codon of the fdhF mRNA. The position of this UGA codon was assigned as 0 and the codon immediately upstream of the UGA codon as -1. In the following plasmids, p111, p112, p113 and p145, the UGA codon at position 0 was changed to UCA, and the codons at positions -1, -2, -3, and -4 were altered to UGA, respectively. Plasmid p421 was created by changing the codon at position -1 to UGA in p111, so that it contained two tandemly repeated UGA codons at position 0 and -1. In p927, the UGA codon at position 0 was simply changed to UCA, without any other alteration.

All the plasmids described above contained the bla gene for ampicillin resistance. When strain WLS60153 (amp') was used, the bla gene in p2182 and p111 were replaced with the cat gene for chloramphenicol resistance. These plasmids were digested with PstI, followed by alkaline phosphatase treatment (fragment 1). Plasmid pN-III-ompA3-Cm' (18) was also digested with PstI, and the resulting 2.5-kb fragment containing the cat gene was isolated. This fragment was ligated to the fragment 1 to yield p2182 Cm' and p111 Cm'.

**Enzymes and Chemicals**—Restriction enzymes were obtained from New England Biolabs or BRL Life Technologies, Inc. IPTG was purchased from Sigma. The DNA oligomers were synthesized on an Applied Biosystems 380B DNA synthesizer.

### RESULTS AND DISCUSSION

**TGA Codon at Different Positions in the fdhF Gene**—On the basis of the very stringent requirement of the secondary structure immediately downstream of the UGA codon, the entire secondary structure is likely to be essential for the function of the UGA-specific elongation factor, SelB. It has been shown that shortening of the stem structure by three base pairs at the bottom of the stem completely abolished the UGA decoding, whereas the addition of 3 base pairs in the stem structure reduced the UGA decoding by 50%. In the latter case, 7S selB-like incorporation into the product was still detected.

In the present experiment, we attempted to examine the effects of the position of the UGA codon relative to the secondary structure on the UGA decoding. For this purpose, the stem-loop structure of the fdhF mRNA (see Fig. 1) was kept intact, and the UGA codon was moved upstream codon by codon. As shown in Fig. 1A, the UGA codon at position 140 was first changed to UCA for Ser and codons at positions -1, -2, -3, and -4 relative to the UGA (position 0) codon were individually altered. As shown in Fig. 1B, in p387, the UGA codon was replaced with UCA without any other mutations and in ptT662, the entire secondary structure downstream of the UGA codon was removed by deleting the sequence downstream of codon 144 (see the figure legend for details). All the constructs except for ptT662 contained a DNA fragment from the fdhF coding region encompassing from codon 123 to 156 (codon 140 is the wild-type fdhF gene). These fragments were amplified by PCR with the following primers: 5'TTAAAGCTTTGGCG-

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1 The abbreviations used are: IPTG, isopropyl-1-thio-β-galactopyranoside; PCR, polymerase chain reaction.

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**FIG. 1.** The secondary structure immediately downstream of the UGA codon at position 140 in the E. coli fdhF gene (8). A, the UGA codon corresponds to the 140th codon from the initiator codon of fdhF. In the figure, the position of the UGA (or UCA) codon is assigned 0, and the codons upstream of the UGA codon are assigned -1 to -4. The lacZ gene was fused in frame after codon 156 (GCC) using a BamHI site created by PCR as described in the text. Plasmid p2182 contains the wild-type fragment, and the plasmids, p111, p112, p113, and p145, the UGA codon was changed to UCA, and the codons at positions -1, -2, -3, and -4 were altered to UGA, respectively. In plasmid p421, in addition to UGA(0) of the wild-type fdhF mRNA, the codon (−1) was also altered to a UGA codon. The + signs indicate the requirement of the factors for the lacZ expression. B, p387 the same structure as shown in A except that the UGA codon at position 140 is replaced with UCA (circled), a codon for Ser. In ptT662, the entire secondary structure was removed by deleting the sequence after codon 144. The UGA codon is circled. In both A and B, the BamHI sites created by PCR are underlined.
transformed were grown in a selenium-deficient medium (14) in the presence or absence of 5 μM Na₂SeO₃ as described previously (15). IPTG was also added at the concentration of 2 mM in order to induce the fdhF-lacZ genes. For each construct, four β-galactosidase assays were carried out as shown in Fig. 2. In case of the wild-type construct (set 1), β-galactosidase activity was highly dependent upon the selC gene as well as selenium added in the medium. The activity in the selC⁻ cells was reduced to less than 3.3% (column 2) of that of the selC⁺ cells (column 1). In the absence of selenium, the activity was reduced to approximately 3.7% regardless of the cells, SelC⁺ or SelC⁻ (columns 3 and 4, respectively).

When the UGA codon was moved one codon upstream (set 2 in Fig. 2), the activity was reduced by approximately 24% in the selC⁺ cells in the presence of selenium (compare column 1 of set 1 with that of set 2). This activity was still highly dependent upon the selC gene and reduced to 8.8% in the selC⁻ cells (column 2). The activity was also highly dependent upon selenium in both selC⁺ and selC⁻ (columns 3 and 4, respectively). When the UGA codon was further moved up to the −2 position (set 3 in Fig. 2), the β-galactosidase activity (column 1) dropped to 20% as that of the wild-type (column 1 of set 1). However, the activity was still dependent upon the selC gene; in the selC⁺ cells (column 2), the activity reduced to approximately a quarter of that of the selC⁺ cells. Most notably, however, this mutant lost the dependence on selenium in the medium (column 3 in set 3); the activity in the selC⁺ cells in the absence of selenium was as high as that in the presence of selenium (column 1). This activity was still dependent upon the selC gene (compare column 3 with 4 of set 3) as in the presence of selenium (column 1 versus 2). This result indicates that the UGA at position −2 can still be decoded by tRNA<sub>Sec</sub>, whereas the stringent requirement of selenocysteine to be charged on tRNA<sub>Sec</sub> for the decoding is lost. It appears that seryl-tRNA<sub>Sec</sub>, a precursor of selenocysteiny-l-tRNA<sub>Sec</sub>, can be used to decode the UGA codon equally well as selenocysteiny-tRNA<sub>Sec</sub>, probably because the SelB elongation factor on UGA(−2) is unable to discriminate seryl-tRNA<sub>Sec</sub> from selenocysteinyl tRNA<sub>Sec</sub>.

When the UGA codon was moved further up to the −3 position, it was still quite effectively decoded as shown in column 1 in set 4 in Fig. 2. The decoding level was approximately 30% of the full activity in column 1 in set 1 and notably higher than that for UGA(−2) (column 1 in set 3). However, most importantly, the UGA suppression was hardly dependent upon selC or selenium (see columns 2, 3, and 4 in comparison with column 1 in set 4). The read-through or nonspecific suppression of the UGA codon is known to be much higher than UAA and UAG codons (19). It is interesting to note that when the UGA codon was placed at position −4 (set 5 in Fig. 2), the nonspecific suppression became less effective. However, it is still higher than the suppression without the secondary structure (set 8 in Fig. 2), indicating that the secondary structure is still effective for the read-through of the UGA codon.

These results indicate that the position of the UGA codon just prior to the stable secondary structure enhances nonspecific suppression of the nonsense codon. This is probably due to stalling of a ribosome on the mRNA because of the secondary structure. The stalling of a ribosome on or nearby a termination codon probably enhances the misincorporation of a nonspecific amino acid residue at the termination codon. This present result suggests that a UGA codon 9 bases upstream (UGA(−3)) of a stable secondary structure is most effective for the nonspecific suppression. It should be also noted that in the cases of UGA(−3) and UGA(−4), the suppression seems to be slightly higher in the selC⁺ cells than in the selC⁻ cells (see columns 1 and 3 versus columns 2 and 4 in sets 4 and 5 in Fig. 2), suggesting that selenocysteine may be still incorporated at least partially at the UGA codons.

When UGA at position 0 was simply altered to UCA for a codon for serine without any other mutations, the lacZ expression (set 7 in Fig. 2) was as high as in the case of UGA at position 0 in the selC⁺ cells in the presence of selenium (column 1 of set 1), and the lacZ expression became completely independent from selC as well as selenium in the medium (columns 2, 3, and 4 of set 7).

**Requirement of the selB Function**—The same plasmids used above were used to examine the effect of the selB function on the UGA decoding. However, since the selB⁻ cells (WL50183) used was ampicillin-resistant, the plasmids (p2182 and p111) were converted to chloramphenicol resistant by inserting the gene for chloramphenicol acetyltransferase into the bla gene of the plasmids as described under “Experimental Procedures.” These plasmids (p2182 Cm⁻ and p111 Cm⁻) were transformed into E. coli FM433 (selB⁺) and WL61460 (selB⁻) (13), and β-galactosidase activity was measured. With UGA(0), the activity in the selB⁻ cells dropped to 1.6% of that in the selB⁺ cells, clearly indicating that the selB function is absolutely required for the UGA(0) decoding. In the case of UGA(−1), the β-galactosidase activity was 23% of that of the UGA(0) construct in the selB⁺ strain and dropped to 7.5% in selB⁻ strain. This indicates that the decoding of UGA(−1) still requires the selB function. However, it appears that the selB-independent suppression of the UGA codon substantially increases in UGA(−1) in comparison with UGA(0).

**Conclusions**—The present results indicate that the UGA decoding is most effective in the fdhF mRNA by placing the UGA codon immediately upstream of the specific secondary structure. In addition, this arrangement in the fdhF mRNA was found to be important to block nonspecific selB⁻ and selC⁻ independent suppression of the UGA codon. It remains to be elucidated how this low nonspecific suppression level can be achieved in the wild-type fdhF mRNA in comparison with the
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UGA codon placed further upstream of the specific secondary structure.

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