Sequences in the 3'-Untranslated Region of the Human Cellular Glutathione Peroxidase Gene Are Necessary and Sufficient for Selenocysteine Incorporation at the UGA Codon*

Qichang Shen, Fong-Fong Chu, and Peter E. Newburger††

From the Departments of Pediatrics and of Molecular Genetics/Microbiology, University of Massachusetts Medical School, Worcester, Massachusetts 01655 and the Department of Medical Oncology and Therapeutics Research, City of Hope National Medical Center, Duarte, California 91010

Glutathione peroxidase (EC 1.11.1.9) is one of a unique group of prokaryotic and eukaryotic enzymes that contain the unusual amino acid selenocysteine. The genes for these selenoproteins encode for the atypical amino acid at a TGA codon (UGA in the mRNA transcripts), which normally functions as a termination signal. The present studies analyzed the functional importance of sequences in the coding and 3'-untranslated regions of transcripts of the primary human cellular glutathione peroxidase gene (GPX1) to the insertion of selenocysteine at this UGA codon. Deletions in potential stem-loop or hairpin structures in the coding region did not substantially diminish incorporation of selenocysteine into glutathione peroxidase transiently expressed by the pCMV4 vector in COS-1 cells. However, selenocysteine insertion was completely abolished by deletion of four-nucleotide sequences in the 3'-untranslated region from within a conserved "selenocysteine insertion sequence" motif also found in the 3'-untranslated region of mammalian genes for other selenoproteins. Moreover, in constructs fusing the glutathione peroxidase 3'-untranslated region to the coding region of rab5b (an unrelated protein normally without any selenium moiety), the glutathione peroxidase 3'-untranslated region was sufficient to direct the translation of an opal (UGA) mutation into selenocysteine. Thus, our data directly demonstrate the importance of sequences in the coding and 3'-untranslated regions of transcripts from the primary human cellular GPx gene and specifically show that sequence elements in the 3'-untranslated region are both necessary and sufficient for translational insertion of selenocysteine at a UGA codon in eukaryotic mRNA.

Selenoproteins encompass a unique group of prokaryotic and eukaryotic polypeptides incorporating the unusual amino acid selenocysteine. This small class of proteins includes several enzymes, such as bacterial formate dehydrogenases (1, 2) and the mammalian glutathione peroxidase (GPX) family (3-6) and type 1 iodothyronine 5'-deiodinase (7), all of which contain a single selenocysteine moiety within the active site. Another common mammalian species, selenoprotein P, contains seven to ten selenocysteine moieties and may function as a transport protein or antioxidant (8). All of these proteins incorporate the selenocysteine cotranslationally at a UGA codon (9), formerly known only as a stop codon, and utilize a unique selenocysteine-charged tRNA containing the appropriate UCA anticodon (10, 11).

A critical question in the interpretation of this "extended genetic code" is how the ribosomal translation assembly can discriminate the special UGA codon in the open reading frame of a selenoprotein mRNA from the termination UGA codon in other mRNA species. Since the first identification of the UGA codon, speculation has focused on the possibility that one or more consensus sequences would function as a signal for insertion of selenocysteine, most likely by formation of specific mRNA secondary structures. Two major models have emerged.

In *E. coli* formate dehydrogenase mRNA, translation of the UGA codon as selenocysteine depends upon a 40-base stem-loop structure immediately downstream from the UGA and containing critical bases within the loop, 20 bases downstream from the UGA (2, 9). Very similar stem-loop structures were identified in the human GPx sequence, as well as in other bacterial selenoenzymes (2).

In contrast, in rat 5'-deiodinase mRNA, recognition of UGA as a selenocysteine, rather than a termination, codon depends upon a 200-nucleotide "selenocysteine insertion sequence" in the 3'-untranslated region (3'-UTR) (12). Sequence analysis of this region predicted a large stem-loop structure with unpaired UGAU and AAA sequences in the stem and loop, respectively. The sequence motif was conserved in the rat and human 5'-deiodinase 3'-UTR. However, these studies utilized relatively large deletions and did not specifically test the importance of the three- and four-nucleotide sequence elements in the 3'-UTR or of potentially important sequence elements in the coding region. The rat GPx 3'-UTR, although different in primary sequence, also contains a potentially similar stem-loop secondary structure with unpaired AAA and UGAU sequence pair in an analogous position (12); but the presence of this element has not been detected by sequence comparison and its functional role has never before been tested directly in GPx translation.

The present studies analyzed the functional importance of potential selenocysteine incorporation sequences in the open reading frame and the 3'-UTR to the translation of mRNA transcripts from the primary human cellular GPx gene, GPX1. Our data demonstrate that segments of the 3'-UTR, spesific
Selenocysteine Incorporation Sequences in GPx 3'-UTR

**TABLE I**

| Nucleotide sequence (5' → 3') | Function |
|------------------------------|----------|
| GAAAGACCACTAGGACAT | Flanking primers for all GPx deletion subclones |
| GAAAACGACCGCCAGTG | Primers directing deletion in ORF-D1 |
| AATTCGACCCCTCTGTGAGATC | Primers directing deletion in ORF-D2 |
| Complementary to 3 | Primers directing deletion in ORF-D3 |
| 5' | Primers directing deletion in ORF-D4 |
| AAGGACCCCGCCTGCTGAGATC | Primers directing deletion in ORF-D5 |
| Complementary to 4 | Primers directing deletion in ORF-D6 |
| 3' | Primers directing deletion in UTR-D4 |
| Complementary to 6 | Primers directing deletion in UTR-D5 |
| Complementary to 7 | Primers for epitope sequence tagging |
| Complementary to 8 | Flanking primers for rab5b constructs |
| Complementary to 9 | Primers directing an open mutation in rab5b |
| Complementary to 10 | Primers directing an open mutation in rab5b |
| Complementary to 11 | Primers directing an open mutation in rab5b |

sequence were constructed by conventional DNA recombination techniques. In brief, the subclone UTR-D3, in which the entire GPx 3'-UTR was deleted, was constructed by excision of a 250-nt AccI-SpeI fragment from the epitope-tagged GPx subclone GPxEPI in pBluescript KS and then religating the remaining large fragment. Subclone UTR-D2 was constructed by excision of the AeqII-Xhol fragment and re-ligation of the remaining large fragment in the GPx 3'-UTR sequence from GPxEPI-containing pBluescript KS with the plasmid Xhol site eliminated. The subclone UTR-D1 was obtained by inserting GPxEPI containing fragment with a sticky Clal end and an end-filled Xhol end, excised from the construct GPxEPI in pBluescript KS, into the expression vector pCMV4 via the Clal and Smal polylinker restriction sites.

EXPERIMENTAL PROCEDURES

Construction of GPx and rab5b Subclones—GPx subclone GPxR in the vector pBluescript KS (Stratagene) was used as a common template for constructing all GPx deletion subclones. It was derived by inversion of the orientation of a GPx1 cDNA (13) in the same vector. DNA sequencing of this clone (using standard dideoxy sequencing techniques with a "Sequenase" kit (United States Biochemical Corp.) showed one additional GCG codon immediately upstream of the previously reported codon 11 (GCC) (4, 14). The plasmid pMT2, carrying a 1.6-kilobase pair rab5b cDNA clone, was obtained as a kind gift from D. B. Wilson. Construct rab5b(opal)GPx3'-UTR contained a fusion product of the rab5b coding region with an opal (UGA) mutation at codon 63, fused with the GPx 3'-UTR sequence. The oligonucleotide sequence of the flanking and mutagenesis primers are listed in Table I. The 3'-PCR flanking primer sequence resulted in the removal of the native rab5b TGA termination codon, and substitution of the last three codons of the GPx open reading frame, including its TAG stop codon. The resultant rab5b(opal) mutant was inserted into pBluescript KS containing the entire GPx 3'-UTR sequence derived from the Clal-AeqII double digestion of the native GPxR clone in pBluescript KS. The gene fusion product was inserted into pCMV4 as above. The same strategy was also used to construct rab5b(WT)GPx3'-UTR, except in this case, conventional PCR was applied using only the flanking primers, and the fusion product (WT, i.e. wild type without the opal mutation) was inserted into pCMV4. The construct rab5b(opal), which contains the coding region opal mutation but the native rab5b 3'-UTR, was constructed by fusion of the approximately 900-nt NheI-EcoRI fragment of rab5b 3'-UTR sequence with the rab5b(opal)GPx3'-UTR subclone, from which the GPx 3'-UTR sequence had been deleted as an AeqII-EcoRI fragment. The resulting rab5b(opal) sequence was then inserted into pCMV4 as above.

Transfection, Labeling, and Lysis of COS-1 Cells—COS-1 cells were transfected for transient expression of GPx or rab5b subclones by modified calcium phosphate precipitation or electroporation methods (19) and then cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 5 mg/ml sodium selenite, and 1 x penicillin-streptomycin-fungizone (GIBCO/BRL). All experiments were performed two to four times.

As a control for transfection efficiency, COS-1 cells were cotransfected with 2 μg of plasmid pXGH5 included in a human growth hormone transient expression assay system supplied by Nichols Institute. Human growth hormone secreted into the medium was de-
tected by radioimmunoassay using the Crystal Multidetector Radioimmunoassay System (United Technologies Packard).

For $^{35}$Se labeling, 10 µCi of $^{35}$Se as selenenic acid (ICN) was mixed with 100 ng of total cell RNA, 10 µCi of yeast tRNA, and 1 ml of riboprobe (400,000 trichloroacetic acid-precipitable cpm/µl) were performed by standard techniques (19).

For $^{35}$S labeling, 5 or 1 µl, respectively, of $^{35}$S as selenous acid diluted in nitric acid, with an original specific activity of 750-1000 Ci/mmol, was added to the plate. After 20-min incubation shaking at 4 °C, the lysed cell suspension was transferred into a microcentrifuge tube and spun at 1,000 x g for 10 min to remove the cell debris. SDS was added to the supernatant to a final concentration of 0.5%, and the cell lysate was heated in boiling water for 5 min and then cooled on ice.

**RESULTS**

**Role of the Nucleotide Sequence around the UGA Codon—** We first explored the possibility that nucleotide sequences within the open reading frame of GPx mRNA might serve as a signal for selenocysteine insertion. Sequence analysis of GPx mRNA has revealed no conserved sequences common to prokaryotic and eukaryotic selenoprotein mRNAs but has predicted two possible loop structures around the UGA codon (UGA142) in the open reading frame of the E. coli formate dehydrogenase gene (2). Another, incorporating the UGA142 codon at the tip of a "hairpin" (shown in Fig. 1C), is conserved among several mammalian GPx isoforms (including the 3'- UTR of the UGA142 codon). ORF-D1 through ORF-D5 and shown on Fig. 1.

The first four deletion subclones were located within the putative stem-loop region immediately downstream of the UGA142 codon. ORF-D1 lacked a sequence from codon 49 through codon 53; ORF-D2, codons 65 through 69; ORF-D3, codons 54 through 63; and ORF-D4, codons 71 through 74. The sequences deleted from ORF-D1, ORF-D2, and ORF-D3 corresponded, respectively, to the 5' part of the stem, the 3' part of the stem, and the most (29 of 31 nt) of the loop of the putative stem-loop structure (2) in the open reading frame region of GPx mRNA. ORF-D4 represented a 12-nt sequence immediately downstream of the putative stem-loop structure and also important to selenocysteine translation in E. coli formate dehydrogenases (2). GPx subclone ORF-D5 was a deletion of codon 47, located immediately upstream of the UGA142 codon of the GPx mRNA and forming part of the stem of the alternative putative hairpin loop structure (21).

These deletion subclones, carried by the eukaryotic expression vector pcMV4, were individually transfected into COS-1 cells and GPx expression detected by $^{35}$Se labeling, immunoprecipitation, SDS-polyacrylamide gel electrophoresis, and autoradiography.

As shown in Fig. 2A, COS-1 cells transfected by the vector alone (lane 1) demonstrate a background level of $^{35}$Se-containing polypeptide (most likely the native monkey cellular GPx) with a 23-kDa size similar to that of human GPx. Transient expression of the native human GPx cDNA and of deletions ORF-D1 through ORF-D5 (lane 2 and lanes 3-6, respectively) all show high levels of $^{35}$Se incorporation into GPx protein. These deletions resulted in a slight, but not substantial, decrease in GPx expression. Repeated experiments (including the creation of identical deletions in the epitope-tagged construct) also showed slightly diminished expression (data not shown). Similarly, as shown in Fig. 2B, deletion ORF-D5 produces little or no diminution of selenocysteine insertion into GPx. Thus, the putative open reading frame mRNA loop structures may slightly modulate GPx expression, but neither one appears absolutely necessary for translation of the UGA142 codon as selenocysteine in human GPx.

**Role of the 3'-UTR—** To test the role of the 3'-UTR in selenocysteine insertion, we constructed GPx subclones containing deletions of various lengths in that region. Epitope tags (17) were incorporated into these subclones in order to improve the resolution of the transiently expressed human GPx products from the COS-1 background. As diagrammed in Fig. 1A, we replaced the first four codons of GPx with a 50-nt sequence encoding an ATG start codon and a 9-amino acid epitope of human influenza hemagglutinin protein (17). The unambiguous discrimination of the transiently expressed epitope-tagged GPx was possible, because the tagged GPx migrated slowly enough on SDS-polyacrylamide gel electrophoresis that its band resolved at a position detectably higher than that of the untagged GPx. This difference of mobility corresponded, respectively, to the 5' part of the stem, the 3' part of the stem, and the most (29 of 31 nt) of the loop of the putative stem-loop structure (2) in the open reading frame region of GPx mRNA. ORF-D4 represented a 12-nt sequence immediately downstream of the putative stem-loop structure and also important to selenocysteine translation in E. coli formate dehydrogenases (2). GPx subclone ORF-D5 was a deletion of codon 47, located immediately upstream of the UGA142 codon of the GPx mRNA and forming part of the stem of the alternative putative hairpin loop structure (21).
Selenocysteine Incorporation Sequences in GPx 3'-UTR

A schematic diagram of human cellular glutathione peroxidase cDNA and mRNA sequences. A, structure and modifications of GPx cDNA constructs. The open reading frame (ORF) and 3'-untranslated region (3'-UTR) are indicated by a wide bar and plasmid elements and 5'-untranslated region, by flanking lines. Nucleotide numbering starts at the beginning of the open reading frame; the ATG initiation codon is at nt 1-3, the TGA selenocysteine codon is at nt 142-144, and the TAG termination codon is at nt 607-609. Arrows above the schematic show the positions of indicated restriction endonuclease sites. Lines below the schematic show the positions of the indicated deletions; the hatched bar below the schematic shows the position of insertion of the epitope tagging sequence and above it the region of cDNA replaced.

B, potential secondary structure in the coding region of human Gpx mRNA. The figure represents the UGA selenocysteine codon with an immediately downstream potential stem-loop structure and diagrams the positions of deletions ORF-D1 through ORF-D4.

C, alternative potential secondary structure in the coding region of human Gpx mRNA. The figure represents the UGA selenocysteine codon within a potential hairpin structure and diagrams the position of deletion ORF-D5.

GPx subclone GPxEPI, which served as a positive control for the transient expression of the GPx 3'-UTR deletion constructs. These deletions are also mapped in Fig. 1A.

We first tested the effects of three large deletions of the 3'-UTR on [75Se]selenocysteine incorporation into GPx in transfected COS-1 cells, as shown in Fig. 3. Lane 1 demonstrates the background GPx signal in cells transfected with vector alone. The slightly larger epitope-tagged GPx is expressed by the GPxEPI construct with its 3'-UTR intact (lane 2) and easily distinguished from the endogenous COS-1 background. Deletion of the distal 100 nt of the 3'-UTR (UTR-D1, lane 3) did not diminish expression of the transfected GPx. However, deletion of the proximal 129 nt (construct UTR-D2, lane 4) or the entire 3'-UTR (construct UTR-D3, lane 5) completely eliminated detectable 75Se incorporation into GPx. The distal and entire 3'-UTR deletions did not result in a GPx mRNA without a 3'-UTR, since the 3'-UTR sequence of the human growth hormone gene is built into the pCMV4 vector, so as to fuse to the inserted sequence (if not separated by a transcription termination site, as in the other GPx constructs).

Computer analysis of either the entire GPx mRNA or its 3'-UTR sequence using the FOLD program of the University of Wisconsin Computer Group software (22) revealed a potential secondary structure consisting of a long stem with two small loops (Fig. 4), similar to that found in the 3'-UTR of rat and human 5'-deiodinase and rat Gpx genes (12). Moreover, two 4-nt sequences within the loops (UAAA in the first and UGAU in the second, indicated in Fig. 4) were identical to those at the same positions within the selenocysteine insertion sequence motif in the 5'-deiodinase gene (12).

In order to test whether these two short sequences were necessary for selenocysteine insertion into GPx, we constructed two small deletion mutants, UTR-D4 and UTR-D5 (diagrammed on Fig. 1), that specifically eliminated each sequence. Fig. 5 shows that either of these short deletions (lanes 3 and 4) completely abolished detectable selenocysteine incorporation into epitope-tagged GPx in transfected COS-1 cells.

In order to rule out the possibility that the deletion mutations affect the level of GPx transcripts, we measured levels of GPx mRNA in transfected COS-1 cells by RNase protection assays. As shown in Fig. 6, untransfected COS-1 cells (lane 2) contained no mRNA detectable by a riboprobe spe-
Selenocysteine Incorporation Sequences in GPx 3' - UTR

11467

A

kD

29 - 18.4

B

kD

29 - 18.4

FIG. 2. Effects of open reading frame deletions on selenocysteine incorporation into GPx. A, autoradiograph of SDS-polyacrylamide gel of immunoprecipitated 75Se-labeled COS-1 cell extracts after transfection with: pCMV4 vector (lane 1), native GPx (lane 2), and deletion mutants ORF-D1 through ORF-D4 (lanes 3-6, respectively). B, autoradiograph of SDS-polyacrylamide gel of immunoprecipitated 75Se-labeled COS-1 cell extracts after transfection with: pCMV4 vector (lane 1), native GPx (lane 2), and deletion mutant ORF-D5 (lane 3).

FIG. 3. Effects of large 3'-UTR deletions on selenocysteine incorporation into GPx. Autoradiograph of SDS-polyacrylamide gel of immunoprecipitated 75Se-labeled COS-1 cell extracts after transfection with: pCMV4 vector (lane 1), epitope-tagged GPx (lane 2), deletion mutants UTR-D1 through UTR-D3 (lanes 3-5, respectively).

FIG. 4. Effects of deletions of short sequence elements in the 3'-UTR on selenocysteine incorporation into GPx. Autoradiograph of SDS-polyacrylamide gel of immunoprecipitated 75Se-labeled COS-1 cell extracts after transfection with: pCMV4 vector (lane 1), epitope-tagged GPx (lane 2), deletion mutant UTR-D4 (lane 3), and deletion mutant UTR-D5 (lane 4).

specific for the epitope-tagged GPx transcript, indicating multiple sequence differences between the human and COS-1 (monkey) GPx transcripts. COS-1 cells transfected with epitope-tagged wild-type GPx (lane 3) contain the same amount of transcript as those transfected with the UTR-D4 and UTR-D5 deletions (lanes 4 and 5). Deletions in the open reading frame (ORF-D1, -D2, and -D3) also produced no detectable change in GPx transcript levels (data not shown). Transfection efficiency, assayed by cotransfection with a vector encoding human growth hormone, was also similar from group to group in these experiments (data not shown).

The GPx 3' - UTR Is Sufficient for Selenocysteine Insertion—Having demonstrated that sequences in the 3'-UTR of GPx mRNA are necessary for translational insertion of selenocysteine, we next investigated whether this 3' - UTR would be sufficient to direct the same process at a UGA codon in an unrelated coding sequence. The chosen target gene, rab5b, encodes a 25-kDa GTP-binding protein, a member of Ras-related GTPase superfamily (18). This gene was used for three constructs. rab5b(opal) had a codon 63 UGU (cysteine) modified to a UGA (opal) mutant, with the native rab5b 3' - UTR gene fusion construct rab5b(opal)GPx 3'-UTR consisted of the rab5b(opal) coding sequence fused to a 3' portion of GPx cDNA incorporating the last three codons of the GPx coding region, including its stop codon (UAG), and the entire GPx 3'-UTR; and rab5b(wt)GPx 3'-UTR was also a rab5b-GPx fusion product but carried the wild type codon 63 rather than the opal mutation. The fusion constructs placed the UGU (cysteine) or UGA (potential selenocysteine) codon the...
same number of nt upstream from the GPx 3'-UTR as in native GPx transcripts.

Fig. 7 presents the results of a representative transient expression experiment with these constructs in COS-1 cells, transfected in these experiments by electroporation. The expression of rab5b was detected by an affinity-purified rabbit antibody against a synthetic peptide sequence, following either 35S (lanes 1–4) or 75Se (lanes 5–8) radioiodotope labeling. COS-1 cells transfected with the vector alone (lanes 1 and 5) showed no detectable immunoreactive protein at the appropriate 25-kDa molecular mass for rab5b. All three constructs directed the synthesis of a 35S-labeled polypeptide of approximatively 25 kDa, at detectable but widely differing levels; but only rab5b(opal)GPx3'-UTR, the fusion product of the rab5b with theopal mutation coupled to the GPx 3'-UTR, incorporated 75Se (lane 6). The rab5b(opal) transfectants expressed only a very low level of 35S-labeled protein, probably reflecting the existence of an alternativeopal nonsense suppression mechanism (23) in the COS-1 cells. No 75Se was detectable even on very long exposures (data not shown), indicating that no selenocysteine insertion occurred at the UGA codon in the presence of the rab5b 3'-UTR. No truncated polypeptide was detectable on 35S-labeled immunoprecipitates, suggesting that the short polypeptide product was unstable or not immunoreactive with the antisera. Transfection with the rab5b (wt)GPx3'-UTR fusion construct resulted in expression of immunoreactive protein without any detectable 75Se incorporation, as expected for the wild type rab5b coding region. For these experiments, transfection efficiency was again confirmed by cotransfection with a vector encoding human growth hormone and measurement of secreted growth hormone (data not shown).

**DISCUSSION**

The present studies analyzed the functional importance of mRNA sequence elements in the coding region and the 3'-UTR of human GPX1 transcripts to the translation of the UGA42 as the unusual amino acid selenocysteine, rather than as a termination signal. Two different models have been proposed for the direction of selenocysteine incorporation in GPx, based upon findings in other selenoprotein genes. The GPx mRNA sequence contains a potential stem-loop configuration immediately downstream from UGA42 and homologous to a structure essential to selenocysteine incorporation in E. coli formate dehydrogenases (2, 9). However, the GPx 3'-UTR also contains AAA and UGAU sequence elements in an analogous position to those found within a selenocysteine insertion sequence motif in rat type I iodothyronine 5'-deiodinase (12). Our data demonstrate that these conserved short sequences in the 3'-UTR, but not the potential stem-loop or hairpin structures in the coding region, are essential for selenocysteine translation in human GPx. Moreover, our data indicate for the first time that the GPx 3'-UTR alone is sufficient to signal the translation, as selenocysteine, of an opal mutation (UGA) in the open reading frame of an unrelated non-selenoprotein, rab5b.

As shown in Fig. 4, the two critical short sequences in the selenocysteine insertion motif lie within a potential stem-loop that is structurally conserved in gene sequences for several other eukaryotic selenoproteins (12). These unpaired AAA and UGAU segments had been identified previously as conserved elements by sequence analysis in a study that also demonstrated the importance of the 3'-UTR to selenocysteine incorporation in 5'-deiodinase (12). However, those experiments utilized relatively large deletions and rearrangements and did not specifically test the importance of the small three- and four-nucleotide sequence elements. The present studies have demonstrated the importance of the selenocysteine insertion motif in GPx for the first time and have specifically shown the necessity of the AAA and UGAU sequence segments. Further investigation will be necessary to determine whether the surrounding secondary structure or other sequence elements within the 200-nt motif are also necessary for its function in GPx or 5'-deiodinase. The conservation of the stem-loop structure among different selenoprotein genes in rat and human suggests that some such additional elements are necessary for this unique function of these very short sequences that must commonly occur in the 3'-UTR of other gene transcripts.

The importance of the distance between the UGA codon and the selenocysteine insertion sequence also remains unknown. Our target for induced selenocysteine incorporation, rab5b, is similar in amino acid number to GPx, and the codon mutated to UGA is a similar distance (550 nt) from the 3'-UTR as the UGA42 in GPx. However, in 5'-deiodinase the span is approximately 1200 nt, so the distance between these elements necessary for selenocysteine incorporation is probably not critical.

Notwithstanding the critical role of the 3'-UTR in the direction of selenocysteine incorporation, other sequences upstream from the UGA codon probably play a regulatory role in selenoprotein translation. Recently, Mizutani et al. (24) have suggested that the 5'-UTR of the human GPx mRNA is essential, and areas of the coding region are important for expression of the gene in transfected COS-7 cells. They found hardly any expression of a construct containing only 10 nt of the 5'-UTR and better expression with the full native 311-nt 5'-UTR than with 257- or 408-nt constructs. However, we obtained excellent expression of GPx with only 5 nt of 5'-UTR, perhaps due to differences in the vector or the host cells. They also reported decreased, but not absent, GPx expression in mutants changed in unspecified ways in the coding region upstream of the UGA42. Although we did not observe any substantial effect of the upstream deletion ORF-D5, we did not extensively investigate the role of the upstream sequence in selenocysteine incorporation. However, in preliminary experiments, we attempted to use bacterial neomycin phosphotransferase as a tag by inserting a nearly full-length neor gene between the GPx fifth and sixth codons. Transient expression of this construct resulted primarily in the translation of a neor'-GPx fusion polypeptide truncated at the selenocysteine position, plus some (10–20% production) of a full-length fusion protein incorporating selenocysteine.
Selenocysteine Incorporation Sequences in GPx 3′-UTR

In both prokaryotes and eukaryotes, the expansion of the genetic code to include the unusual amino acid selenocysteine has utilized the opal termination codon UGA. Also necessary to this process has been the evolution of a signal mechanism to indicate whether an individual UGA encodes for selenocysteine or for termination. Our data fit an emerging pattern in mRNA stability in response to cytokine stimulation (28). This difference might relate to a requirement for post-transcriptional regulation by elements within individual coding regions of polycistronic mRNAs. More likely it reflects the evolution, in eukaryotes, of a functional or regulatory role for the 3′-UTR and its polyadenylated tail. The latter plays a role not only in mRNA stability (26) but also in a newly described regulation of maternal transcripts during oocyte development (27). Specific sequences within the 3′-UTR determine the regulation of mRNA stability in response to cytokine stimulation (28) and to iron availability (29). The direction of codon translation in selenoproteins by the selenocysteine incorporation sequence further expands the known repertoire of the 3′-UTR in post-transcriptional gene regulation.

Acknowledgments—We thank Dr. David Wilson for the rabb cDNA clone and Carolyn Padden for technical assistance.

REFERENCES

1. Zinoni, F., Birkmann, A., Stadtmann, T. C., and Böck, A. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 4650-4654
2. Zinoni, F., Heider, J., and Böck, A. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4660-4664
3. Chambers, I., Franpton, J., Goldfarb, P., Affara, N., McBeain, W., and Harrison, P. R. (1986) EMBO J. 5, 1221-1227
4. Mullensbach, G. T., Tabrizi, A., Irvine, B. D., Bell, G. I., and Hallevoll, R. A. (1987) Nucleic Acids Res. 15, 5484
5. Esworthy, R. S., Chu, F. F., Paxton, R. J., Ackman, S., and Doroshow, J. H. (1991) Arch. Biochem. Biophys. 286, 380-386
6. Takahashi, K., and Cohen, H. J. (1986) Blood 68, 640-645
7. Berry, M. J., Banu, L., and Larsen, P. R. (1991) Nature 349, 438-440
8. Read, R., Bellow, T., Yang, J.-G., Hill, K. E., Palmer, I. S., and Burk, R. F. (1990) J. Biol. Chem. 265, 17909-17915
9. Böck, A., Forchhammer, K., Heider, J., and Baron, C. (1991) Trends Biochem. Sci. 16, 463-467
10. Hawkes, W. C., Lyons, D. E., and Tappel, A. L. (1982) Biochim. Biophys. Acta 699, 183-189
11. Lee, B. J., Worland, P. J., Davis, J. N., Stadtmann, T. C., and Hatfield, D. L. (1989) J. Biol. Chem. 264, 9724-9727
12. Berry, M. J., Banu, L., Chen, Y. Y., Mandel, S. J., Kieffer, J. D., Harney, J. W., and Larsen, P. R. (1991) Nature 353, 273-275
13. Chu, F. F., Esworthy, R. S., Ackman, S., and Doroshow, J. H. (1990) Nucleic Acids Res. 18, 1681-1689
14. Chada, S., Le Beau, M. M., Casey, L., and Newburger, P. E. (1990) Genomics 6, 268-271
15. Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K., and Pesse, L. R. (1989) Gene (Amst.) 77, 51-59
16. Andersson, S., Davis, D. L., Dahlback, H., Jornvall, H., and Russell, D. W. (1988) J. Biol. Chem. 263, 8222-8229
17. Chada, S., Whitney, C., and Newburger, P. E. (1989) Blood 74, 2535-2541
18. Wilson, D. B., and Wilson, M. P. (1992) J. Clin. Invest. 89, 996-1005
19. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
20. Ginsburg, D., Handin, R. I., Bonthron, D. T., Denton, T. A., Bruns, G. A. P., Latt, S. A., and Orkin, S. H. (1985) Science 228, 1401-1406
21. Chada, S., Whitney, C., and Newburger, P. E. (1988) in Oxy-Radicals in Molecular Biology and Pathology (Cerutti, P., Frldovlch, I., and McCord, J., eds) pp 273-288, Alan R. Liss Inc., New York
22. Devereux, J., Haehner, P., and Smithies, O. (1984) Nucleic Acids Res. 12, 387-395
23. Hatfield, D. (1985) Trends Biochem. Sci. 10, 201-204
24. Kursta, H., Kamoshihata, K., Kawai, E., Sukenaga, Y., and Mizutan, T. (1987) FEBS Lett. 232, 10-14
25. Rocher, C., Fauchee, C., Herve, F., Benicourt, C., and Lalanne, J. L. (1991) Gene (Amst.) 88, 193-200
26. Brawerman, G. (1981) Crit. Rev. Biochem. 10, 13-38
27. Sallés, F. J., Darrow, A. L., O'Connell, M. L., and Strickland, S. (1992) Genes & Dev. 6, 1202-1212
28. Akashi, M., Shaw, G., Gross, M. A., Saito, M., and Koefler, H. P. (1991) Blood 78, 2005-2012
29. Casey, L., Hentze, M. W., Koeller, D. M., Caugman, S. W., Rouault, T. A., Klausner, R. D., and Harford, J. B. (1988) Science 240, 924-928

(data not shown). Also, we found a minor, albeit inconsistent, effect of downstream open reading frame deletions on selenocysteine insertion (Fig. 2A). Thus, disruption of the 5′-UTR or of the coding region neighboring the UGA recurrently may also diminish GPx expression. These effects could be mediated by multiple regulatory pathways, of which diminished translation of the UGA recurrently codon is only one of many possible mechanisms.

In both prokaryotes and eukaryotes, the expansion of the genetic code to include the unusual amino acid selenocysteine has utilized the opal termination codon UGA. Also necessary to this process has been the evolution of a signal mechanism to indicate whether an individual UGA encodes for selenocysteine or for termination. Our data fit an emerging pattern in mRNA stability in response to cytokine stimulation (28). This difference might relate to a requirement for post-transcriptional regulation by elements within individual coding regions of polycistronic mRNAs. More likely it reflects the evolution, in eukaryotes, of a functional or regulatory role for the 3′-UTR and its polyadenylated tail. The latter plays a role not only in mRNA stability (26) but also in a newly described regulation of maternal transcripts during oocyte development (27). Specific sequences within the 3′-UTR determine the regulation of mRNA stability in response to cytokine stimulation (28) and to iron availability (29). The direction of codon translation in selenoproteins by the selenocysteine incorporation sequence further expands the known repertoire of the 3′-UTR in post-transcriptional gene regulation.

Acknowledgments—We thank Dr. David Wilson for the rabb cDNA clone and Carolyn Padden for technical assistance.