Regulation of Human Thioredoxin Reductase Expression and Activity by 3'-Untranslated Region Selenocysteine Insertion Sequence and mRNA Instability Elements*

(Received for publication, March 30, 1999, and in revised form, May 20, 1999)

John R. Gasdaeka†, John W. Harney§, Pamela Y. Gasdaska‡, Garth Powis¶, and Marla J. Berry¶¶

From the †Arizona Cancer Center, Tucson, Arizona 85724 and §Thyroid Division, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts 02115

Thioredoxin reductases function in regulating cellular redox and function through their substrate, thioredoxin, in the proper folding of enzymes and redox regulation of transcription factor activity. These enzymes are overexpressed in certain tumors and cancer cells and down-regulated in apoptosis and may play a role in regulating cell growth. Mammalian thioredoxin reductases contain a selenocysteine residue, encoded by a UGA codon, as the penultimate carboxyl-terminal amino acid. This amino acid has been proposed to carry reducing equivalents from the active site to substrates. We report expression of a wild-type thioredoxin reductase selenoenzyme, a cysteine mutant enzyme, and the UGA-terminated protein in mammalian cells and overexpression of the cysteine mutant and UGA-terminated proteins in the baculovirus insect cell system. We show that substitution of cysteine for selenocysteine decreases enzyme activity for thioredoxin by 2 orders magnitude, and that termination at the UGA codon abolishes activity. We further demonstrate the presence of a functional selenocysteine insertion sequence element that is highly active but only moderately responsive to selenium supplementation. Finally, we show that thioredoxin reductase mRNA levels are down-regulated by other sequences in the 3'-untranslated region, which contains multiple AU-rich instability elements. These sequences are found in a number of cytokine and proto-oncogene mRNAs and have been shown to confer rapid mRNA turnover.

Mammalian thioredoxin reductases (TRRs) have many diverse cellular functions, ranging from the regulation of overall redox balance to generating reducing equivalents for ribonucleotide reductase, which is required for deoxyribonucleotide synthesis. These enzymes exhibit broad substrate specificity, reducing lipid hydroperoxides and lipoic acid, protein disulfide isomerase, and several low molecular weight disulfide substrates in addition to thioredoxin. The substrate thioredoxin functions in the proper folding and redox regulation of enzymes and transcription factors, including nuclear factor κB and transcription factor IIIC (1, 2), and in catalyzing electron transport to other reductive enzymes. Expression of both thioredoxin and TRR is elevated in a number of cancer cells and tumors (3, 4). Cells transfected with thioredoxin cDNA show increased tumor growth, and thioredoxin has been shown to contribute to the transformed phenotype of some human cancer cells (5). Finally, expression of TRR is down-regulated in apoptosis (4), and expression of the substrate, thioredoxin, inhibits apoptosis (6). Thus, the thioredoxin redox system is a major contributor to regulating cell growth and death.

Selenoenzymes have long been known to play important roles in protection from oxidative stress and the damaging effects of reactive oxygen species. The glutathione peroxidases, the best-characterized family of selenoenzymes, were thought to be solely responsible for the role of selenium in this protection. Selenium has also been implicated through nutritional and epidemiological studies in cancer prevention, and recent clinical studies have provided considerable support for such a role (7, 8), but the mechanisms are not known. The cloning of two human TRR genes (9–11) and the identification of these enzymes as selenoenzymes (12, 13) reveal a second pathway, in addition to the glutathione peroxidases, in which selenium plays a role in redox balance and protection from oxidative stress. Furthermore, the presence of selenium in the TRRs establishes a potential mechanistic link between this essential trace element and cancer.

Selenocysteine is encoded by UGA codons, which typically serve as signals for termination of protein synthesis. Recoding of UGA as a selenocysteine codon occurs in the presence of specific mRNA secondary structures, termed selenocysteine insertion sequence (SECIS) elements, located in the 3'-untranslated regions (3'-UTRs) of selenoprotein mRNAs (14). All selenoproteins characterized to date for which functions are known are redox enzymes, with selenocysteine in the active center. The selenocysteine residue in mammalian TRRs is the penultimate amino acid from the carboxyl terminus of the protein and is not part of the predicted catalytic site (15). Instead, selenocysteine has been proposed to carry reducing equivalents from the active site to substrates (16). In support of an essential role for selenocysteine in TRR activity, removal of the carboxyl terminus by either carboxypeptidase treatment (17) or trypsin digestion (16) or alklylation of the selenocysteine residue (18) all resulted in inactivation of the enzyme.

Due to the critical functions of the TRR enzymes and the unusual location of the selenocysteine residue, we undertook the present study to express the human wt selenoenzyme and a cysteine mutant analog to investigate the role of selenocysteine in catalytic activity and to characterize the SECIS element. Our studies utilized the first mammalian TRR cDNA,
which was cloned from a human placenta library and is now termed TR-α (10) or TrxR1 (11). We have expressed the wild-type, cysteine mutant, and UGA-terminated enzymes in mammalian cells and overexpressed the cysteine mutant and UGA-terminated protein in insect cells. We demonstrate that substitution of cysteine for selenocysteine decreases the $k_{cat}$ for thioredoxin by 2 orders of magnitude, confirming the requirement for selenocysteine for maximal activity. Our studies also reveal that TRR expression is repressed by sequences in the 3'-UTR. This repression occurs at the mRNA level and is likely due to the presence of a series of AUUUZ instability elements (AU-rich elements), regulatory sequences typically found in growth factor, cytokine, and proto-oncogene mRNAs. Regulation of expression of TRR by these AU-rich elements may be crucial in maintaining tight control on this important enzyme and the cellular processes it affects.

**EXPERIMENTAL PROCEDURES**

**SECIS Element Constructs**—All TRR sequences are derived from human placental TRR cDNA (9), now designated TR-α or TrxR1. The type 1 deiodinase (D1) coding + D1 SECIS construct used as a control and the parent vector for the D1 construct has been described previously (construct 1536 in Ref. 19). The TRR SECIS element was generated by designing complementary overlapping PCR oligonucleotides to synthesize the predicted TRR element, with unique restriction sites (HindIII and NotI) on either end. The sequence of the upstream oligonucleotide is 5'-CCAGGTTTATGATCCCACTGACATGCTTCAGTCTCAAGGCCCCATGTGGTGG-3', and the sequence of the downstream oligonucleotide is 5'-CCGGGCCGCTGTTCCATACGCGCTACACCATGGGTTG-3'. Regions of complementarity are underlined, and restriction sites are shown in italics. The PCR product was digested to create unique HindIII and NotI fragments. This fragment was subcloned downstream of the D1 coding region in place of the D1 SECIS element. The resulting construct was verified by dideoxy sequencing.

**Mammalian Expression Constructs**—The selenocysteine to cysteine mutant was generated using the Quickchange site-directed mutagenesis kit (Stratagene). TRR clone 30B (9) was the template for this mutagenesis reaction. The TRR mammalian expression constructs were generated by subcloning the wt TRR cDNA (9) or the cysteine mutant into pCMX vector (20) via KpnI and BamHI sites in the polylinker. Deletion constructs were generated using the unique restriction sites XhoI (nt 2014), PstI (nt 2482), DraIII (nt 3326), and BamHI (nt 3690) or by PCR (nt 1933). The native TRR polyadenylation signal (beginning at nt 3776) was deleted in all TRR constructs, thus the first polyadenylation site is in the vector, ~0.6 kilobase downstream of the polylinker. A weak polyadenylation signal (UAUAAA at nt 3137) is present in the 3326 and 3690 constructs, possibly accounting for the additional RNA band detected by Northern blotting. This signal is deleted in the shorter constructs.

**Cell Culture and Transfections**—Human embryonic kidney HEK-293 cells were grown and maintained by standard tissue culture techniques in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Transient transfections were carried out by calcium phosphate precipitation as described previously (21).

**Deiodinase Activity Assays**—Cells sonicated were assayed for the presence of 5'-deiodinase activity using 125I-reverse 3,5,3'-triiodothyronine (9) as the substrate, as described previously (21).

$^{75}$Se Labeling—$^{75}$Se labeling was carried out for 24 h, beginning 1 day after transfection. $^{75}$Se (specific activity, $\sim$1000 Ci/mg) was added at 4 mCi/60-mm dish in 2 ml of Dulbecco’s modified Eagle medium + 10% fetal bovine serum. Cells were harvested in phosphate-buffered saline, washed once in the same, sonicated briefly, and resuspended in electrophoresis buffer containing 1.25% SDS and 0.5 M $\beta$-mercaptoethanol. Samples were boiled for 3 min and electrophoresed on 10% polyacrylamide gels, followed by autoradiography.

**Baculovirus Expression**—A PCR fragment (base pairs 440–1954) containing the cysteine mutant described above was cloned into the BamHI site of the pFASTBAC1 vector (Life Technologies, Inc.). The UGA-terminated TRR was generated by cloning a PCR fragment (base pairs 440–1933) into the BamHI and XhoI sites of the pFASTBAC1 vector (Life Technologies, Inc.). Recombinant TRR bacmid DNA isolated from transformed DH10Bac cells was used to generate recombinant baculovirus particles from Sf21 cells according to the manufacturer’s instructions (Bac-to-Bac Baculovirus Expression System; Life Technologies, Inc.). The virus was amplified to a final titer of 1–108 plaque-forming units/ml. Sf9 cells were infected with recombinant baculovirus particles with a multiplicity of infection of 10 and harvested after 3 days.

**Purification of Thioredoxin Reductase**—Human placental thioredoxin reductase was purified to homogeneity as described previously (22). Baculovirus-derived TRR protein was purified to homogeneity, as assessed by silver staining, using an adaptation of the protocol described above in combination with chromatography steps as described by Gladyshev et al. (13). Briefly, Sf9 cells were resuspended in 100 mM Tris, 5 mM EDTA, and 5 mM phenylmethylsulfonyl fluoride (pH 7.6) and sonicated, and particulate material was removed by centrifugation at 20,000 $g$. The supernatant was loaded onto 1.25% ADP-Sepharose column (Sigma) pre-equilibrated with 50 mM Tris and 1 mM EDTA, pH 7.6 (Buffer A). A yellow band containing recombinant protein was eluted from the column with Buffer A containing 200 mM sodium phosphate and 1 M ammonium sulfate at pH 7.4. The column was washed with sequential washes of 20 mM sodium phosphate containing 0.75, 0.5, 0.25, and 0 M ammonium sulfate. TRR was eluted in the last wash.

**Western Blotting**—Sonicates from transfected cells were electrophoresed on 10% polyacrylamide denaturing gels and electroblotted to Immobilon membrane (Millipore). Western blot analysis utilized a polyclonal antiserum (M2098, used at 1:2,500 dilution) directed against a human TRR peptide (amino acids 435–454; VVGFHVLGPNAGEVTQGFAA) (9) and a donkey anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:2,000 dilution). This peptide lies 44 amino acids upstream of the selenocysteine or the corresponding mutant cysteine codon; thus, antibody reactivity would not be predicted to be affected by the presence of selenocysteine rather than cysteine or termination. Signals were visualized by enhanced chemiluminescence using the Renaissance ECL system (NEN Life Science Products) and quantitated by scanning densitometry using a Molecular Dynamics laser densitometer and ImageQuant software. Protein levels in Table II are from a single experiment but are representative of results from three separate transfections.

**Northern Blotting**—Total RNA was prepared from transfected cells using Trizol reagent (Life Technologies, Inc.) according to the manufacturer’s specifications. 10 μg of RNA were analyzed on 1.2% agarose-formaldehyde gels in 4-mM sodium phosphate–containing nitrocellulose was blotted to Nitran nylon membranes by vacuum blotting. A TRR cDNA probe (a XhoI fragment spanning nucleotides 1010–2470) was prepared by random priming (Prime It; Stratagene). Blots were hybridized at 65°C and washed in 0.1× SSC at 65°C. Hybridization signals were quantitated by scanning densitometry using a Molecular Dynamics laser densitometer and ImageQuant software.

**RESULTS**

**Characterization of the Thioredoxin Reductase SECIS Element**—Incorporation of selenocysteine at UGA codons in eukaryotes has been shown to require the presence of a stem-loop or hairpin structure termed the SECIS element (14). SECIS elements are characterized by a small number of conserved nucleotides at specific positions in the stem-loop, notably the sequences AUGA and GA opposite each other on the 5' and 3' arms of the stem, respectively, and two or three adenosines in either the terminal loop or an internal bulge. We examined the 3'-UTR of the human TRR sequence for the presence of these nucleotides and their potential to form the consensus stem-loop structure. We identified a single region fitting these criteria located between nucleotides 2184 and 2227 (numbering according to Ref. 9). The position of the SECIS element relative to the coding region and the rest of the 3'-UTR is shown in Fig. 1A. The predicted SECIS element is shown in Fig. 1B.

During the course of these studies, predicted structures of this putative SECIS element were reported elsewhere (10, 17). However, demonstration of the ability to direct selenocysteine incorporation and quantitation of SECIS activity relative to
other elements have not been reported. We synthesized the predicted TRR SECIS element by PCR and subcloned it into a mammalian expression vector downstream of the coding region of another selenoprotein, the D1 (19). We have previously shown that a functional SECIS element is required for synthesis of active deiodinase enzyme (14, 25), and that SECIS elements from a variety of different eukaryotic selenoproteins could function in this capacity (26). After transient transfection of the chimeric D1-TRR construct into HEK-293 cells, the TRR element efficiently directed selenocysteine incorporation into D1, as assessed by the production of D1 activity (Fig. 1 C). The activity of the TRR SECIS element was approximately 50% greater (mean of five independent transfections) than that of the D1 element. Because most other elements fall in the same range of activity as the D1 element (26), the TRR SECIS is among the more potent elements identified.

Selenoproteins have been proposed to follow a hierarchy for selenium supply in that the amounts of certain selenoproteins decrease more rapidly in selenium deficiency and reappear more slowly after selenium repletion. A previous study suggested that this was due in part to differences in the SECIS elements of different proteins (27). We examined the effects of selenium supplementation on the relative activities of the TRR and D1 SECIS elements. Whereas in the absence of added selenium, the TRR SECIS was 1.5-fold more potent than that of D1, the addition of 100 nm selenium resulted in a greater increase in D1 SECIS activity relative to the TRR SECIS (Fig. 1D). This effect was seen with cells cultured in either 1% or 10% serum. Thus, in 10% serum, the addition of 100 nm selenium increased activity with the D1 SECIS element by 4-fold, versus a 2-fold increase for TRR. In 1% serum with the same addition (100 nm selenium), the TRR SECIS element activity increased by ~7-fold, versus a 16-fold increase for D1.

Expression of Thioredoxin Reductase in Mammalian Cells—We next examined the expression of TRR and the incorporation of 75Se into the protein after transient transfection of the human TRR cDNA in mammalian cells. Expression constructs either containing or lacking the entire 3'-UTR were transfected, 75Se was added to the media 1 day later, and cells were harvested for protein analysis the following day. Transfection of the full-length TRR-UTR construct resulted in an increase in labeling of TRR above the endogenous protein levels seen in vector-transfected cells (Fig. 2, lane 2 versus lane 4). Upon transfection of the TRR-UTR construct, the level of 75Se-labeled TRR corresponded to that seen with vector alone (Fig. 2, lane 3 versus lane 4), presumably reflecting only the 75Se-labeled product from the endogenous cellular TRR gene. No additional 75Se-labeled TRR is produced from the TRR construct lacking the 3'-UTR. Transfection of a full-length construct in which the selenocysteine codon was mutated to a cysteine codon also produced no additional 75Se-labeled TRR, confirming that this codon serves to direct selenocysteine in-

![Fig. 1](image-url) Functional identification of the TRR SECIS element. A, a schematic of the TRR cDNA showing positions of the open reading frame ( ), UGA selenocysteine codon, UAA termination codon, SECIS element (stem-loop structure), and AU-rich elements (asterisks) is shown. B, predicted structure of the human thioredoxin reductase SECIS element. Nucleotides conserved in all vertebrate SECIS elements are shown in bold. Non-Watson-Crick, non-wobble (G-U) base pairs are indicated by filled ovals. C, activity of the TRR SECIS element. Constructs containing the D1 coding region and either the TRR SECIS element, the D1 SECIS element, or no SECIS element were assayed for expression of D1 activity in HEK-293 cells. Activity is expressed as the mean + S.E. of duplicate assays from five independent transfections, normalized to hGH in the media from a co-transfected hGH-expressing plasmid. D, the TRR SECIS is moderately responsive to added selenium. Activities are expressed as described above, normalized to activity with the D1 SECIS element in the absence of added selenium, which is assigned an activity of 1.0.
corporation (data not shown).

Expression of Thioredoxin Reductase in the Baculovirus System—We were interested in studying the contribution of the selenocysteine residue to TRR enzyme activity. We and others have previously shown that substitution of cysteine for selenocysteine in iodothyronine deiodinase (28) or formate dehydrogenase (29) decreased the $k_{cat}$ by ~300-fold in each case. We wished to express cysteine mutant and UGA-terminated TRR proteins to compare with the wt enzyme. However, even if we could achieve overexpression of the cysteine mutant by 2 orders of magnitude relative to the endogenous wt enzyme, the predicted 2 order of magnitude decrease in specific activity with the cysteine substitution would result in the endogenous TRR enzyme contributing significantly to the total measured activity. Therefore, we undertook expression of the mutant enzymes in the baculovirus system, which allows for higher levels of expression than mammalian cells. Constructs encoding either a selenocysteine to cysteine mutant or a UGA-truncated version of TRR were subcloned into the pFASTBAC baculovirus system, and recombinant baculovirus stocks were generated. Mutant proteins were expressed after viral infection of SF9 insect cells, purified to homogeneity, and assayed for reduction of thioredoxin and DTNB. We and others have found that in both the baculovirus system and transfected mammalian cells, synthesis of selenoproteins is very inefficient (30). Therefore, we used human placenta as a source of wt enzyme. The native placentar TRR enzyme was purified to homogeneity, as described previously (22). The wt and mutant enzyme activities are shown in Table I. The $k_{cat}$ for the reduction of thioredoxin was 135-fold higher for the wt enzyme than for the cysteine mutant. No activity was detectable with the UGA-terminated protein. With DTNB as substrate, the $k_{cat}$ was ~10-fold higher for the selenoenzyme than it was for either the cysteine mutant or UGA-terminated protein. The $K_m$ values were only minimally affected by the cysteine substitution, increasing by a factor of ~2 for both substrates.

Regulation of TRR Expression by Sequences in the 3′-UTR—During the course of our mammalian expression studies, quantitation of expression levels revealed a potentially interesting regulatory mechanism involving inhibitory sequences in the 3′-UTR. We therefore returned to the transfected cell system to pursue this finding. Because the expressed TRR protein lacking selenocysteine could not be detected by selenium labeling, we monitored expression of the UGA-terminated product from the TRR-UTR construct by Western blotting. The UGA codon in TRR lies two codons upstream from the authentic termination codon; thus, the UGA-terminated product and the selenocysteine incorporation product would differ in size by only two amino acids. The mobilities of the proteins would not be distinguishable on standard SDS-polyacrylamide gels; they would comigrate. Endogenous TRR would also be detected by Western blotting, but increased levels of expression of truncated protein from the transfected plasmid would be predicted. The low level of endogenous TRR protein in these cells is seen in Fig. 3, lane 1 (vector-transfected cells). After transfection with the TRR expression plasmid containing the entire 3′-UTR, the level of TRR protein increased 8- to 12-fold (Fig. 3, lane 2). The additional antibody-reactive protein seen in lane 2 versus lane 1 would presumably derive from a combination of expressed full-length TRR containing selenocysteine and any protein that terminated at the UGA codon due to inefficient selenocysteine incorporation. Transfection of the TRR construct in which the 3′-UTR was deleted produced a surprising result: the levels of expressed protein were dramatically increased by this deletion (Fig. 3, lane 3). Quantitation of TRR expression in the absence of the 3′-UTR revealed an additional ~8-fold increase over expression in the presence of the 3′-UTR or an increase of 60- to 100-fold over endogenous levels. The TRR protein in lane 3 presumably derives from the UGA-terminated product from the transfected plasmid as well as the minor contribution from endogenous TRR. The presence of a selenocysteine codon was not required for this 3′-UTR effect because cysteine mutant constructs plus or minus the 3′-UTR exhibited the same patterns of expression as the selenocysteine-encoding constructs. Furthermore, expression levels from the full-length constructs or deletion constructs were not significantly affected by supplementation of the media with 100 nM selenium.

Decreased mRNA Steady-state Levels Conferring by AU-rich Elements—To investigate the site of action of this regulatory effect, we next examined the levels of TRR mRNA produced in the presence or absence of the 3′-UTR. The TRR + UTR mRNA was barely detectable by Northern blotting, whereas the TRR − UTR mRNA was abundant (see below). This result suggested a possible role for sequences in the 3′-UTR in mRNA stability, although other stages of expression, such as RNA processing, nuclear export, or localization, could also be affected. Examination of the sequence of this region revealed the presence of multiple AUUUA sequences, a motif present in the 3′-UTRs of growth factor and oncogene mRNAs undergoing rapid turnover. These include three individual AUUUA sequences and a tandem repeat, AUUUAUUAAUUUAA. To investigate the possible role of these AU-rich elements in the decrease in mRNA levels, we generated additional constructs in which portions of the 3′-UTR were deleted. The end points for these deletions and the number of AUUUA repeats present

| Table I | Kinetics of thioredoxin and DTNB reduction by TRR selenoenzyme, cysteine mutant, or UGA-terminated protein |
|---------|----------------------------------------------------------------------------------------------------------------|
|         | Thioredoxin                                                                                   | DTNB                                      |
|         | $k_{cat}$                        | $K_m$       | $k_{cat}$                       | $K_m$         |
| TRR wt  | 661 min$^{-1}$                      | 0.55 mM    | 1770 min$^{-1}$                 | 0.365 mM     |
| Cys mutant | 4.9 min$^{-1}$                      | 1.06 mM    | 174 min$^{-1}$                  | 0.83 mM      |
| UGA-terminated protein | ND                                  | ND         | 145 min$^{-1}$                  | 1.27 mM      |

Fig. 2. Expression of TRR in mammalian cells. Autoradiograph of 75Se-labeled cell extracts after transfection of TRR expression constructs. Lane 1, 14C molecular weight marker (M, 45,000); lane 2, TRR + UTR-transfected cells; lane 3, TRR − UTR-transfected cells; lane 4, pCMV vector-transfected cells. The arrowhead indicates the position of TRR, and the bar indicates the position of the molecular weight marker.
Regulation of TRR by Sequences in the 3′-UTR

Western blot analysis of TRR expressed in the presence or absence of the 3′-UTR sequence (nt 1933–3690), removing all six AU-rich elements, resulted in a 10- to 15-fold increase in TRR mRNA levels relative to those obtained with the wt construct. Deletion of the three upstream AU repeats (nt 1975–3360), retaining the downstream tandem repeats, increased mRNA levels 10- to 12-fold relative to the full-length construct, i.e. to the same extent as deletion of all six AU repeats. This indicates that the downstream tandem repeats do not, by themselves, function efficiently to decrease mRNA levels. A deletion removing the three downstream tandem AU repeats (nt 3326–3690) but retaining the upstream ones resulted in TRR mRNA levels ~3-fold higher than those of the full-length construct. Interestingly, a larger deletion removing all six AU repeats but retaining a short stretch of the upstream 3′-UTR sequence (nt 2014–3690) also produced intermediate levels of the mRNA, suggesting the presence of additional non-AU-rich destabilizing sequences in the region between nt 1933 and 2014. Although these constructs also differ regarding the presence or absence of the SECIS element, the effects on RNA levels are independent of selenocysteine incorporation because the same patterns were observed with constructs containing the selenocysteine codon or the cysteine mutation (data not shown). Close correlation was seen between protein and RNA quantities with all 3′-UTR deletion constructs, indicating that the observed regulation occurs primarily at the RNA level.

**DISCUSSION**

In this report, we describe the expression and characterization of wt and mutant forms of TRR and functional characterization of the SECIS element of this enzyme. We also show that TRR expression is regulated by sequences in the 3′-UTR that exert stringent control over TRR mRNA levels. Sequences in the 3′-UTRs of mRNAs can confer regulation of expression by mechanisms as diverse as affecting mRNA turnover, translation initiation, subcellular localization, or, in the case of selenoenzymes, dictating the choice between incorporation of an amino acid or the termination of protein synthesis. The 3′-UTR of TRR exhibits at least two of these regulatory functions: (a) a SECIS element to direct selenocysteine incorporation and modulate the response to selenium supply, and (b) the regulation of mRNA levels by AU-rich elements. The TRR 3′-UTR sequence contains seven AU repeats, six of which are present in the cDNA used in the present studies. Shyu and co-workers have proposed that AU-rich elements can be divided into three different classes. mRNAs bearing either the class I AUUUAA element or the class III non-AUUUA element display synchronous poly(A) shortening, whereas class II AU-rich element-containing mRNAs are deadenylated asynchronously with the formation of poly(A) intermediates (31). A cluster of five or six copies of AUUUAA motifs, as in the TRR mRNA, is the key feature that dictates the choice between synchronous versus asynchronous deadenylation. All class II AU-rich elements identified to date come from cytokine-like mRNAs (32). Inactivation of AU-rich elements in growth factors and proto-oncogenes has been linked to promotion of cellular transformation and oncogenesis. For example, stabilization of c-myc mRNA due to the deletion of AU-rich sequences promotes oncogenic transformation in vitro and is associated with a human T-cell leukemia (33).

Because overexpression of TRR correlates with cellular transformation and tumorigenesis, the AU-rich elements in this gene may serve to maintain stringent control of TRR expression levels to prevent the deleterious effects of TRR excess. Cloning of a second TRR gene has recently been described by two groups. This isoform was reported to localize to mitochondria in one study (11), but a second study showed expression in both the mitochondria and cytosol (10). Interestingly, the sequence of this clone does not contain AU-rich elements in the 3′-UTR, but the presence of other regulatory elements was not examined. One other selenoprotein, the type 2 iodothyronine deiodinase (34), has been shown to contain AU-rich elements in the 3′-UTR. Like TRR, type 2 deiodinase mRNA has a short half-life (~2 h; Ref. 35). The effects of deleting the AU-rich elements in this gene have not been reported.

Previous studies investigating the role of selenocysteine in TRR activity examined the effects of either removing carboxy-terminal residues, thus disrupting the structure, or alkylating the selenocysteine residue. Because some isoforms of TRR and the related glutathione reductase enzymes contain cysteine at the corresponding position (see Fig. 4 in Ref. 11), our goal was to determine the potential biochemical advantage of selenocysteine over cysteine in this enzyme. However, we and others have previously shown that both transfected mammalian cells and the baculovirus system synthesize selenoproteins ineffi-

![Fig. 3. Regulation of TRR expression by sequences in the 3′-UTR. Western blot analysis of TRR expressed in the presence or absence of the 3′-UTR by transient transfection in mammalian cells. Lane 1, pCMV vector-transfected cells; lane 2, TRR + UTR-transfected cells; lane 3, TRR – UTR-transfected cells. Proteins were detected with a human TRR peptide antibody as described under “Experimental Procedures.”](image-url)

**TABLE II**

| 3′-UTR deletion | SECIS | AUUUA sequences retained | RNA levels | Protein levels |
|------------------|-------|--------------------------|------------|---------------|
|                  |       |                          | A          | B             |
| 1933–3690        | –     | 0                        | 14.5       | 10.8          | 11.4          |
| 1975–3360        | –     | 3 (downstream)           | 10.1       | 11.6          | 10.3          |
| 3326–3690        | +     | 3 (upstream)             | 2.9        | 3.0           | 0.7           |
| 2014–3690        | –     | 0                        | 2.9        | 3.8           | 2.5           |
| None (wt)        | +     | 6                        | 1.0        | 1.0           | 1.0           |

RNA and protein levels were determined by scanning densitometry of Northern or Western blots, respectively, as described under “Experimental Procedures.” RNA levels were determined from two preparations, A and B, from paired transfected dishes. Relative densities are normalized to those obtained with the wt construct.
Fig. 4. TRR RNA steady-state levels are decreased by sequences in the 3′-UTR. Northern blot analysis of mRNA from cells transfected with TRR constructs containing deletions in the 3′-UTR. Upstream deletion end points are indicated above the lanes. The downstream end point for all constructs is at 3690.

cently. Kim et al. (30) reported expression of a wt selenoprotein in the baculovirus system at ~3% of the level of the corresponding cysteine mutant. We observed a similar inefficiency in expression of wt versus cysteine mutant type I deiodinase in the baculovirus system. These findings are in agreement with the 20- to 400-fold differences in wt versus cysteine mutant expression we observed in transfected cells (28, 36). Thus, we considered the substitution of cysteine for selenocysteine in yeast peroxidase. These findings are consistent with the proposed role of one or more selenoproteins in the 3′-UTR. Northern blot analysis of mRNA from cells transfected with TRR constructs containing deletions in the 3′-UTR. Upstream deletion end points are indicated above the lanes. The downstream end point for all constructs is at 3690.

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active under standard cell culture conditions but is less responsive to selenium supplementation than the D1 SECIS element. Thus, TRR levels would be better preserved when the selenium supply is low, but protein levels would not increase as dramatically under conditions of selenium excess.

Selenoenzymes are critical for normal mammalian growth and development, as indicated by the important biological roles and processes in which they function. Attesting to the essential role of one or more selenoproteins is the report that targeted disruption of the rRNAsec gene in mice resulted in embryonic lethality apparent from the third day in utero (37). The specific selenoprotein or proteins responsible for this lethality have not been identified, but some of the known selenoenzymes can be ruled out. Targeted disruption of the cytoplasmic GPX gene resulted in mice that were viable and apparently normal; thus, cGPX is not essential for life (38). The iodothyronine deiodinases may also be nonessential at this early stage because maternal thyroid hormones cross the placental barrier during early gestation in animals, and deiodinase expression is only detected in the fetus at later stages of gestation (39). Whereas no information exists on whether TRR is essential, targeted disruption of the thiorodoxin gene in mice produced early embryonic lethality (40). Because the TRR enzymes are the only known reductants of thiorodoxin, these enzymes may present the strongest candidates identified to date for selenoproteins that are essential for viability in mammals.

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