University of Massachusetts Medical School

eScholarship@UMMS

Open Access Articles

Open Access Publications by UMMS Authors

1989-11-15

Post-transcriptional regulation of glutathione peroxidase gene expression by selenium in the HL-60 human myeloid cell line

Sunil Chada
University of Massachusetts Medical School

Et al.

Let us know how access to this document benefits you.

Repository Citation

1989 74: 2535-2541

Post-transcriptional regulation of glutathione peroxidase gene expression by selenium in the HL-60 human myeloid cell line

S Chada, C Whitney and PE Newburger

---

Post-Transcriptional Regulation of Glutathione Peroxidase Gene Expression by Selenium in the HL-60 Human Myeloid Cell Line

By Sunil Chada, Constance Whitney, and Peter E. Newburger

We have used a cloned cDNA for the major human seleno-protein, glutathione peroxidase (GPx), to assess the mode of regulation of human GPx gene (GPX-1) expression by selenium. When the HL-60 human myeloid cell line is grown in a selenium-deficient medium, GPx enzymatic activity decreases 30-fold compared with selenium-replete cells. Upon return to a medium containing selenium in the form of selenite, GPx activity in the cells starts to increase within 48 hours and reaches maximal (selenium-replete) levels at 7 days. Steady-state immunoreactive protein levels corre-late with enzymatic activity. Cycloheximide inhibits the rise in GPx activity that accompanies selenium replenishment, indicating that protein synthesis is required for the increase. However, GPx mRNA levels and the rate of transcription of the human GPx gene change very little and thus appear to be independent of the selenium supply. Thus the human GPx gene appears to be regulated post-transcriptionally, probably cotranslationally, in response to selenium availability.

©1989 by Grune & Stratton, Inc.

GLUTATHIONE peroxidase (GPx; EC 1.11.1.9) is the most extensively characterized mammalian selenopro-tein.[1-3] The enzyme catalyzes the degradation of peroxides and hydroperoxides to the corresponding alcohol, using reduced glutathione as a specific hydrogen donor[4,5]

$$H_2O_2 + 2GSH \xrightarrow{\text{GPx}} GSSG + 2H_2O.$$
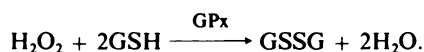
GPx serves as an important element of the cellular antioxi-dant defense system by detoxifying peroxides and hydroper-oxides that would otherwise damage cell membranes and DNA.[1,6,7]

The enzyme is a homotetramer, with each subunit contain-ing one atom of selenium.[8] Selenium resides in the protein in a catalytically active selenocysteine residue at amino acid 47.[9] The presence of selenocysteine in the active site raises the important question of how this unusual moiety is incorpo-rated into GPx. The two major possibilities are post-transla-tional addition of selenium into a common amino acid or direct cotranslational incorporation of selenocysteine.

Immunologic studies have failed to find any evidence for a GPx precursor protein or apoenzyme.[10,11] Cycloheximide and puromycin have been shown to inhibit the incorporation of radiolabeled selenium into GPx in isolated perfused rat liver.[12] Further studies have suggested that rat liver contains a species of tRNA that is specific for selenocysteine and that the aminoacylated tRNA is more active in an in vitro protein synthesis system than selenite, selenocysteine, or the deacyl-ated tRNA.[13] Isotope dilution studies have indicated that free selenocysteine is not an intermediate in the labeling of GPx by [75Se]-selenite.[12] Together, these data suggest, but do not directly demonstrate, a translational mechanism for selenocysteine incorporation into GPx. Sequence analysis of the murine glutathione peroxidase gene has revealed the selenocysteine residue to be encoded by a thymine-guanine-adenine (TGA) "termination" codon.[14] An identical codon, and overall similar sequences, have recently been reported for the human GPx (GPX1) gene in clones isolated from human liver cDNA,[15] kidney cDNA,[16] and genomic[17] libraries.

Using oligonucleotides directed against the bovine amino acid sequence, we have also isolated cDNA clones corre-sponding to the human GPx mRNA.[18] The present study used the cloned GPx cDNA to examine the regulation of the human GPX1 gene in response to selenium depletion and repletion, using the HL-60 human myeloid leukemia cell line[19] as an in vitro model system. This cell line can be cultured in defined medium with and without sodium selenite supplementation,[20] thus allowing selenium-dependent regu-lation of GPx activity in a homogeneous cell population free of the effects of whole organism nutrition and metabolism.

MATERIALS AND METHODS

Cells. HL-60 cells (originally obtained from Dr R. Gallo[19]) were maintained in RPMI 1640 medium, supplemented with either insulin, transferrin, and selenium (ITS; ITS premix, Collaborative Research, Inc, Lexington, MA) containing insulin (5 μg/mL), transferrin (5 μg/mL) and selenium as sodium selenite (5 ng/mL); or insulin and transferrin (IT) only.[20]

In order to assess whether selenium deprivation caused severe generalized biological consequences, selenium-replete cells (cultured for 30 days in ITS medium) and selenium-deficient cells (day 20 in IT medium) were examined for their ability to differentiate morpho-logically and functionally. Table 1 shows cell morphology, assessed by differential counting of Wright-Giemsa–stained cytocentrifuge preparations of HL-60 cells cultured for 3 weeks in ITS or IT medium and then treated with 80 mmol/L dimethylformamide (DMF) to induce granulocytic differentiation.[21] Selenium-deficient and selenium-replete cells developed equally well into the expected pattern of distribution into progressively more mature myeloid cell types. Cells grown in IT and ITS also exhibited similar levels of functional differentiation as assayed by reduction of nitroblue tetrazolium dye,[22] used as a measure of respiratory burst function (Table 2). When ITS, IT, or serum-supplemented cells were treated with phorbol myristate acetate (10^{-7} mol/L) to induce macrophagic differentiation,[23] they also differentiated similarly, as assayed by morphology and adherence (data not shown).

Table 1. Effect of Selenium on Morphologic Differentiation of HL-60 Cells

Cells	Granulocytic Differentiation Stage				
	Promyelo	Myelo	Meta	Band	PMN
Day 0					
IT	83	9	2	1	0
ITS	87	9	4	0	0
Day 2					
IT	49	50	1	0	0
ITS	51	36	7	4	2
Day 4					
IT	5	71	20	4	0
ITS	9	50	30	7	5
Day 6					
IT	2	41	36	16	5
ITS	1	35	41	24	9

HL-60 cells were grown in selenium-replete (ITS) or -deficient (IT) medium as indicated. Granulocytic differentiation was induced by the addition of dimethylformamide (DMF) for the times indicated, and the percentages of differentiated cells assayed morphologically.

Abbreviations: Promyelo, promyelocyte; Myelo, myelocyte; Meta, metamyelocyte; Band, band neutrophil; PMN, polymorphonuclear leukocyte.

GPx activity. GPx enzymatic activity was monitored using an adaptation of the coupled peroxidase-reductase method of Beutler.[24] Cells were adjusted to 2×10^7/mL in Dulbecco's phosphate-buffered saline (PBS) pH 7.4. Both sample and reference cuvettes contained 10^6 cells, 0.05% Triton X-100, 0.2 mmol/L reduced nicotinamide-adenosine dinucleotide phosphate (NADPH), 2 mmol/L reduced glutathione (GSH), and 1 U/mL glutathione reductase. The oxidation of NADPH upon addition of t-butyl hydroperoxide to the sample cuvette was followed spectrophotometrically at 340 nm.[20]

Protein separation and detection (Western blotting). Postnuclear supernatant fractions from 3×10^6 cells, pretreated with 1 mmol/L diisopropyl fluorophosphate, were prepared as described,[25] electrophoresed under reducing conditions on a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel, transferred to nitrocellulose by standard procedures,[26] detected with polyclonal anti-GPx antiserum, and stained with a goat–anti-rabbit alkaline-phosphatase–coupled second antibody.

cDNA clones. Human GPx cDNA[18] or its restriction fragments (digested according to the endonuclease supplier's instructions) were

Table 2. Functional Differentiation of HL-60 Cells Grown in Selenium-Replete, Selenium-Deficient, or Fetal Calf Serum-Containing Medium

Cells	Medium	NBT Reduction (% Positive)
HL-60	ITS	87
HL-60	IT	89
HL-60	FCS	88
Granulocytes	—	98

HL-60 cells were cultured as indicated in RPMI 1640 medium containing ITS, IT, or 10% fetal calf serum (FCS) and harvested after 6 days of further incubation in the same medium containing 60 mmol/L DMF. Granulocytes were isolated from human peripheral blood and superoxide-generating capacity was determined as the percentage of cells capable of NBT dye reduction, as described in Materials and Methods.

gel purified, then cleared of contaminating agarose and salt by glass beads (GENE-CLEAN; Bio 101, Inc, La Jolla, CA) and ethanol precipitation. When necessary, the cDNA was radiolabeled to a specific activity of 0.5 to 2×10^9 cpm/μg using random oligonucleotide primers.[27] Other cDNA clones included human tubulin[28] (provided by Dr P. Dobner), heavy chain of phagocyte NADPH oxidase b-cytochrome,[29] phosphoglycerate kinase[30] (provided by Dr S.H. Orkin, The Children's Hospital, Boston, MA), heavy and light chains of ferritin[31] (provided by Dr H. Munro, Tufts University, Boston, MA), and chicken β-actin[32] (provided by Dr R. Singer, University of Massachusetts Medical School, Worcester).

RNA preparation and analysis. Whole-cell RNA was extracted using the guanidine-HCl method[33] and polyadenylated RNA isolated by passage over an oligo-dT cellulose column using standard methods.[34] Whole cell or polyadenylated RNA was quantitated spectrophotometrically, denatured, electrophoresed in a 1.2% agarose-formaldehyde gel, and then transferred to nitrocellulose or nylon filters by standard methods.[34] Slot blots from similarly prepared RNA were performed using a Schleicher & Schuell Minifold II apparatus according to the instructions of the manufacturer (Keene, NH). Procedures for prehybridization, hybridization, filter washes, and filter stripping were performed as described by Gatti et al.[35] Control RNA from the cellular slime mold *Dictyostelium discoideum* was provided by Dr Alan Jacobson.

Nuclear runon assay for transcription rates. Nuclear runons were performed with minor modifications of the method developed for HL-60 cells by Linial et al.[36] HL-60 cells were harvested, washed once in cold PBS and once in reticulocyte standard buffer (10 mmol/L Tris, pH 7.4; 10 mmol/L NaCl; 3 mmol/L $MgCl_2$), and then lysed with 0.5% NP-40 in reticulocyte standard buffer. Nuclei were collected by centrifugation at $50 \times g$, washed twice in reticulocyte standard buffer, and resuspended in nuclear freezing buffer (40% glycerol; 50 mmol/L Tris, pH 8.3; 5 mmol/L $MgCl_2$; 0.1 mmol/L EDTA) before immediate use or freezing at $-70°C$. At the time of the assay, the nuclear suspensions receive additions of 20 μL of ^{32}P-UTP (3,000 Ci/mmol/L; 10 μCi/μL) and 60 μL of runon buffer (25 mmol/L Tris, pH 8.0; 12.5 mmol/L $MgCl_2$; 750 mmol/L KCl; 1.25 mmol/L each of adenosine triphosphate [ATP], guanosine triphosphate [GTP], and cytodine triphosphate [CTP]; 1 μmol/L uridine triphosphate [UTP]; 100 μg creatine phosphokinase; 1 mmol/L dithiothreitol; and 20 mmol/L phosphocreatine). After incubation for 30 minutes at 26°C, the reaction was stopped by addition of DNase I and $CaCl_2$. Newly synthesized RNA was then extracted, precipitated, and finally resuspended in hybridization buffer. Trichloroacetic acid–precipitable cpm were determined and equal counts for each sample within an experiment, usually 3 to 20 × 10^6 cpm, were then used for hybridizations. Specific sequences synthesized in the reaction were detected by hybridization to nonlabeled cDNA probes that were denatured by boiling and applied to filters in a slot-blot apparatus. The filters were baked, prehybridized, and hybridized with the labeled, newly synthesized, RNA for 36 to 48 hours at 65°C. The filters were washed, dried, and exposed to roentgenographic film for autoradiography.

Probes for the nuclear runoffs included a 350 bp fragment containing the 5′ portion of the GPx cDNA, a 600 bp clone from the central region of the GPx cDNA, and a 150 bp restriction fragment representing the 3′UT region of the GPx cDNA; M13 phage (not shown) and pBR322 plasmid served as negative controls for nonspecific hybridization.

Densitometry of Northern blots and nuclear runoffs were performed on a Helena Laboratories QuickScan (Beaumont, TX) and areas under the curves determined by the weight of cut-out chart paper. For Northern blots, results for the GPx probe were normalized to the relative densities of the β-actin or phosphoglycerate kinase signals.

RESULTS

Effect of selenium on GPx activity and protein. We have used the HL-60 cell line[19] as a model system to study the regulation of expression of the human GPx gene by selenium. This cell line may be maintained in a defined medium consisting of RPMI 1640 plus insulin and transferrin, with or without supplemental selenium (IT and ITS media, respectively).[20]

To examine the effects of selenium depletion, HL-60 cells grown in ITS medium were pelleted and resuspended in IT. Alternatively, cells cultured in IT medium for 2 to 4 weeks to deplete them of selenium were selenium replenished by transfer to ITS medium. Aliquots were removed at various timepoints during these treatments. As shown in Fig 1, cells from selenium-replete medium (ITS point at day 1) contained substantial amounts of GPx enzymatic activity (slightly less than 250 nmol/L NADPH oxidized/min/10[7] cells). When these cells were transferred to selenium-deficient medium (ITS → IT curve), a time-dependent decrease in enzymatic activity occurred. After 20 days in selenium-deficient medium, the cells contained only 4% of the GPx activity of the initial selenium-replete cells. When selenium-deficient cells were returned to selenium-replete medium (IT → ITS), enzymatic activity increased 25-fold to the level of fully replete cells over approximately seven days. Thus, the exogenous selenium supply appears to control the enzymatic activity of GPx in these cells. The rate of change of enzyme activity with selenium replenishment is slower than might be expected if selenium were incorporated into a pre-existing stable apoenzyme.

Total cellular proteins isolated from selenium-replete and selenium-deficient HL-60 cells were analyzed using a polyclonal antibody raised against human erythrocyte GPx.[10] The Western blot shown in Fig 2 indicates that selenium depletion causes a rapid decrease in cellular GPx immunoreactive protein, with negligible levels being observed after seven days in IT medium. When these cells were returned to selenium-replete (ITS) medium (ie, day 20 in IT was day 0
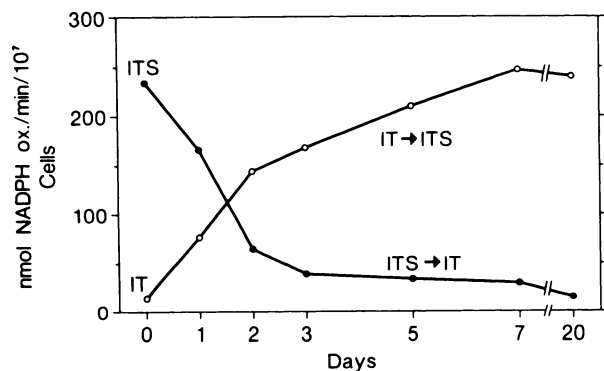

Fig 1. GPx activity of HL-60 cells during selenium depletion and replenishment. Selenium-replete cells (ITS) were transferred to selenium-deficient medium (ITS → IT) and GPx activity assayed at the indicated number of days therein (abscissa). Selenium-deficient cells (IT) were transferred to selenium-replete medium (IT → ITS) and GPx activity similarly assayed. GPx activity was measured spectrophotometrically as the oxidation of NADPH, as described in Materials and Methods.

in ITS), GPx protein was detectable after 2 days, and was substantially higher after 30 days of selenium replenishment. These changes in immunoreactive GPx protein roughly correlate with GPx enzymatic activity, and thus further support the model of selenium regulation of GPx synthesis, as opposed to insertion into a pre-existing apoenzyme. The findings also confirm our previous studies using the same antibody in a radioimmunoassay.[10]

Inhibition of protein synthesis by cycloheximide (CYX) was used to investigate whether the increase in GPx activity with selenium is due to de novo protein synthesis in selenium-replenished cells. As illustrated in Fig 3, selenium-deficient cells (equilibrated in IT medium) showed a 4.6-fold increase in GPx activity 24 hours after transfer to ITS medium. However, when the ITS also contained CYX 50 μg/mL (ITS + CYX), the rise in activity was nearly abolished, with only a 1.6-fold rise evident. CYX treatment of selenium replete cells (ITS + CYX in the lower panel of Fig 3) caused a decrease in GPx activity similar in magnitude to that observed when the replete cells were transferred to selenium-deficient medium (IT; lower panel). The combination of CYX and selenium depletion (IT + CYX) did not diminish GPx activity significantly more than either treatment alone. These results indicate that the increase in activity observed with selenium replenishment requires protein synthesis.

CYX treatment of ITS cells produced a decrease in activity similar to that observed when selenium was removed from replenished cells. This finding suggests a rapid inhibition of synthesis of GPx protein and is consistent with the model of a specific translational block in the absence of selenium.

Effect of selenium on GPx gene expression. We next examined GPX mRNA levels in selenium-replete cells (grown in ITS medium for 30 days) or selenium-deficient cells (grown in IT medium for 20 days). Figure 4 shows an autoradiograph of a slot blot from such an experiment. The indicated amounts of total cellular RNA from ITS cells and IT cells were probed with the cDNA for GPx. RNA from the cellular slime mold *D discoideum* served as a negative control. Densitometric scans of this blot and Northern blots from similar experiments (not shown) demonstrated a range of only 1.2- to 2.3-fold more GPX mRNA in selenium-replete than in selenium-deficient cells, relative to the levels of constitutively expressed control transcripts (β-actin and phosphoglycerate kinase). On the slot blot, the ratios were similar at each RNA amount loaded (range, 1.36 to 1.99). Thus, selenium depletion caused a decrease in steady-state levels of GPx mRNA, but the change was not nearly sufficient to explain the 25-fold difference in enzyme activity and content.

In order to test whether selenium depletion affects GPx gene expression at the level of transcription, nuclear runon experiments were performed to examine transcription rates of the GPx gene in selenium replete (ITS) or deficient (IT) cells. Radiolabeled runoff RNA from ITS and IT cells was hybridized with filters bearing slots with immobilized cDNA fragments representing the 5' end, the middle, and the 3' end of the GPx transcription unit. The results are shown in Fig 5
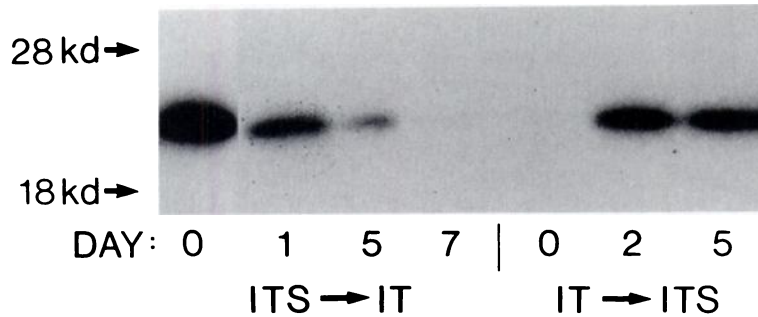
Fig 2. Immunoreactive GPx protein in HL-60 cells during selenium depletion and replenishment, analyzed by Western blotting. Selenium-replete cells were transferred to selenium-deficient medium (IT) and protein extracted at days 1, 5, 7, and 20 (as indicated) of selenium depletion. The resultant selenium-deficient cells were transferred to selenium-replete medium (ITS, day 20 in IT becomes day 0 in ITS) and protein extracted at days 2, 5, and 30 (as indicated) of selenium replenishment. Western blot analysis using a polyclonal anti-GPx antibody was performed as described in Materials and Methods; the size markers on the left indicate the positions of 28-Kd and 18-Kd molecular weight standards.

The signals obtained from all three GPx probes were only slightly higher in the replete relative to the deficient cell nuclei. Similar ITS to IT labeling ratios were observed for the GPx probe at the 5' and 3' ends of the mRNA, indicating that there was no interruption of transcription[37] between the two exons in selenium-deficient cells. The other genes studied in this experiment (β-actin, phagocyte cytochrome b heavy chain [X-CGD], and ferritin heavy and light chains) displayed a similar, slight difference in transcription rates in selenium-deficient and -replete cell nuclei. The observed transcription rates parallel the steady-state levels of the transcripts, indicating a small but general transcriptional enhancement in the selenium-replete state (or inhibition in selenium deficiency). Overall, these results support the inference that the major degree of regulation of GPx expression by selenium is not mediated at the level of gene transcription.

DISCUSSION

We have used the human HL-60 myeloid cell line as a model system to study the relationship between selenium supply and the expression of the human gene for GPx, an unusual mammalian selenoprotein incorporating selenocysteine in its active site.[9] GPx enzymatic activity was approxi-

mately 30-fold higher in selenium-replete than -deficient cells. When replete cells were deprived of selenium, enzymatic activity decreased rapidly, reaching selenium-deficient baseline levels after approximately 10 days. Replenishment of deficient cells with selenium led to a marked increase in activity (to 25% of selenium-replete) within 24 hours and full activity after 7 days. Steady-state levels of GPx protein correlated with enzymatic activity. CYX studies showed that the increase in GPx activity in response to selenium required protein synthesis, and that the decrease in activity upon selenium deprivation may be mimicked by blocking protein synthesis. However, steady-state levels of GPx mRNA and the rate of transcription of the GPx gene were essentially independent of the selenium supply. These studies show that the availability of selenium controls human GPx activity and that regulation is exerted at a post-transcriptional level.

The relationship between selenium supply and GPx enzyme activity in vivo has received extensive investigation both experimentally in animals[38] and clinically in humans.[39,40]

Fig 4. GPx mRNA expression in selenium-replete (ITS) and -deficient (IT) HL-60 cells. The cells were cultured for 3 weeks in the indicated medium before harvesting for extraction of total cellular RNA and slot blot analysis as described in Materials and Methods. Total cellular RNA from the cellular slime mold *D discoideum* served as a negative control. Each slot contained the amount of RNA indicated to the right.
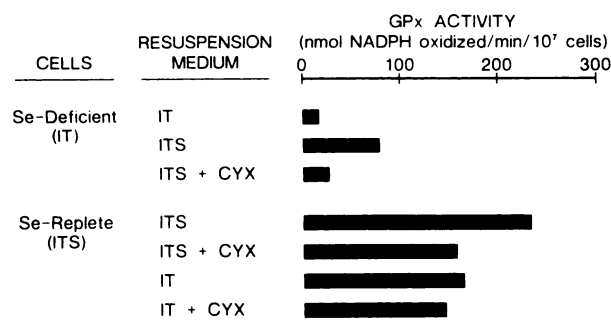

Fig 3. Effect of CYX on GPx activity during selenium replenishment. Selenium-deficient or -replete HL-60 cells were pelleted and resuspended in either IT or ITS medium, with CYX 50 μg/mL in the indicated groups. GPx enzyme activity, shown as the horizontal bars, was measured spectrophotometrically 24 hours later.
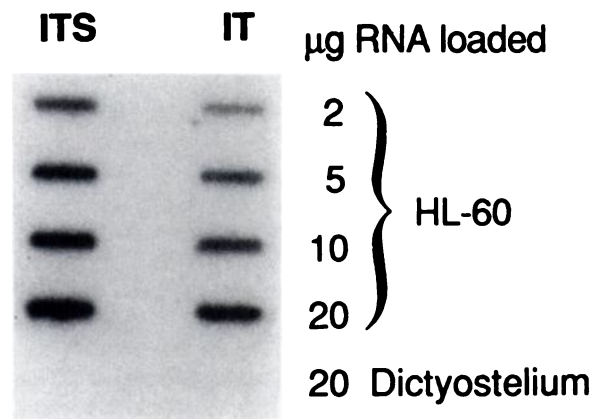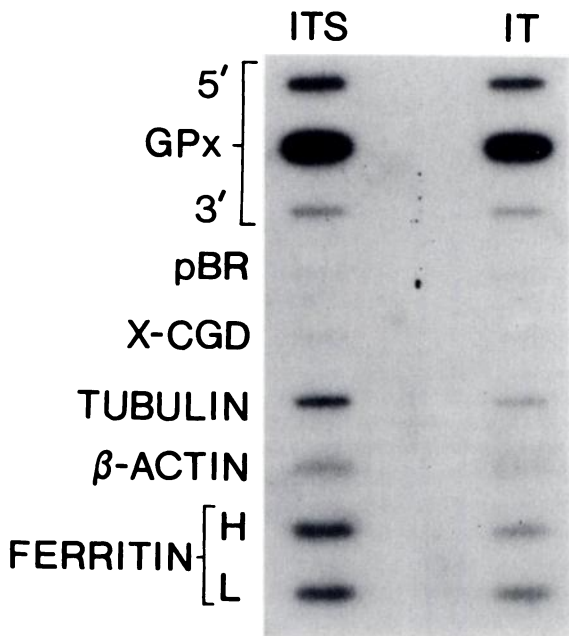
Fig 5. Transcription of GPx mRNA in nuclei from selenium-replete and -deficient HL-60 cells. The autoradiograph shows the amount of RNA transcribed in vitro by nuclei from cells grown in ITS or IT medium. The labels on the left indicate the cDNA probes used to identify the newly synthesized mRNA: GPx probes, including full length cDNA (center) and restriction fragments from its 5′ and 3′ ends as indicated; pBR (plasmid negative control); the phagocyte cytochrome b heavy chain (X-CGD); tubulin; β-actin; and ferritin heavy (H) and light (L) chains.

However, the effect of selenium deprivation on GPx gene expression in vivo has been studied only recently by several groups reporting differing results. Saedi et al,[41] using a murine GPx cDNA, and Yoshimura et al,[42] using a rat GPx cDNA, found on Northern blot analysis that liver from selenium-deficient rats contained much lower GPx mRNA levels than liver from selenium-replete rats. However, Reddy et al,[43] using a rat GPx cDNA in similar experiments, found virtually equal levels of GPx transcripts in selenium-deficient and -replete liver extracts. Our results, in a defined in vitro system, are more consistent with the latter findings, which are also suggestive of post-transcriptional regulation. However, varying control mechanisms may operate in studies involving different species, cell lineages, and experimental designs.

A likely mechanism of post-transcriptional regulation would be cotranslational insertion of selenocysteine into GPx. Recently, analyses of the murine[14] and human[15,16,18] GPx cDNA sequences have demonstrated the very unusual occurrence of the TGA "terminator" codon at the position encoding selenocysteine. The carbon backbone of selenocysteine in GPx has recently been shown to be derived from serine[44] rather than cysteine. Taken together with Tappel's previous demonstration of a selenocysteyl-tRNA,[13] these findings suggest the following cotranslational model for insertion of selenocysteine into GPx: a uracil-guanine-adenine (UGA)-recognizing tRNA is charged with serine, which is then enzymatically altered to generate a selenocysteyl-tRNA, which in turn incorporates selenocysteine directly at the UGA codon occur-

ring in the appropriate codon context. Such a cotranslational mechanism for selenocysteine incorporation at a UGA codon has recently been directly demonstrated in the synthesis of formate dehydrogenase in *Escherichia coli*.[45]

Possible candidate tRNAs for this process are the opal (UGA) suppressor tRNA species that have been characterized by Hatfield et al in mammalian, avian, and *Xenopus* tissues; they are the only known naturally occurring suppressor tRNAs in higher eukaryotes.[46] They are aminoacylated by seryl-tRNA synthetase and then phosphorylated to form phosphoseryl-tRNA. Their unique features and extreme conservation suggest that they may be used in specific biochemical processes requiring suppression of terminator codons within specific sequence contexts.[46] The insertion of selenocysteine into GPx may represent one such condition, in that the modified amino acid is derived from serine and the sequence context of the UGA is unusual and perhaps conducive to selective secondary structure.[18]

Thus, regulation could proceed by control of the translation process at the mRNA UGA triplet that can function either as the codon for selenocysteine or as a terminator. Selenium incorporated into a selenocysteyl-tRNA could allow translational read-through whereas, in the absence of selenium, the selenocysteine tRNA could remain unacylated and the UGA codon would then function in its more usual terminator capacity. Alternatively, translation in the absence of selenium could proceed at a normal or somewhat reduced rate, but with misincorporation of a different amino acid (eg, phosphoserine[46]). In that case, the resultant inactive protein would also have to be very unstable in order to escape detection by the polyclonal antisera used in our western blots (Fig 2) and radioimmunoassays.[10] The Western blot also failed to detect any truncated GPx polypeptide, the translation product that would be expected if termination were taking place at the UGA codon in selenium-deficient cells. However, such a short (46-amino acid) peptide may be unstable in the cytoplasmic milieu and rapidly degraded.

Alternatively, a post-translational mechanism of control would provide a consistent, but less attractive, model for the present studies. Direct insertion of selenium into the completed 22 Kd GPx polypeptide has been proposed.[47,48] The latter study suggested that selenocysteine was generated by a modification reaction between the side chain of cysteine in the polypeptide and a precursor selenium compound. However, such post-translational insertion of selenium into a stable apoenzyme is unlikely in view of the relatively slow kinetics and dependence on protein synthesis for rise in GPx activity after selenium replenishment (Figs 1 and 3), as well as the absence of immunoreactive GPx protein on Western blotting (Fig 2). The existence of a labile apoenzyme cannot be ruled out. However, it would have to be either very unstable in the absence of selenium or not be recognized by the anti-GPx antibody used in this study. The latter possibility is unlikely since the antibody, generated against purified human erythrocyte GPx, is polyclonal and recognizes both native and SDS-denatured GPx protein. However, selenium could be required to stabilize a labile GPx apoenzyme in the manner of metal-binding proteins, such as ferritin,[49] that may be protected against degradation by the prosthetic group.

Finally, more complex mechanisms could account for the post-transcriptional regulation of human GPx expression by selenium. For example, further analogies may be drawn to the regulation of ferritin synthesis by iron, mediated both by iron-responsive mRNA elements[50] and by protection of the protein from proteolytic degradation.[49] Selenium could also play a role in the recruitment of GPx mRNA onto polysomes or in the regulation of its translational initiation.

The present studies show that the human GPx gene is regulated post-transcriptionally by selenium. The data are most consistent with, but do not directly demonstrate, cotranslational insertion of selenocysteine by specific suppression of a UGA codon in selenium replete cells and termination of translation in selenium deficiency. The studies provide a basis for the further elucidation of the precise control mechanisms involved and for the determination of the role of the UGA codon and its surrounding structure in the synthesis of GPx and other selenoproteins.

REFERENCES

1. Flohé L: The glutathione peroxidase reaction: Molecular basis of the antioxidant function of selenium in animals. Curr Top Cell Regul 27:473, 1985

2. Behne D, Hilmert H, Scheid S, Gessner H, Elger W: Evidence for specific selenium target tissues and new biologically important selenoproteins. Biochim Biophys Acta 966:12, 1988

3. Danielson KG, Medina D: Distribution of selenoproteins in mouse mammary epithelial cells in vitro and in vivo. Cancer Res 46:4582, 1986

4. Mills GC: Glutathione peroxidase: An erythrocyte enzyme which protects hemoglobin from oxidative breakdown. J Biol Chem 229:189, 1957

5. Tappel AL, Hawkes WC, Wilhelmsen EC, Motsenbocker MA: Selenocysteine-containing proteins and glutathione peroxidase. Methods Enzymol 107:602, 1984

6. Fridovich I: The biology of oxygen radicals. Science 201:875, 1978

7. Schraufstätter I, Hyslop PA, Jackson JH, Cochrane CG: Oxidant-induced DNA damage of target cells. J Clin Invest 82:1040, 1988

8. Tappel AL: Selenium-Glutathione peroxidase: Properties and synthesis. Curr Top Cell Regul 24:87, 1984

9. Forstrom JW, Zakowski JJ, Tappel AL: Identification of the catalytic site of rat liver glutathione peroxidase as selenocysteine. Biochemistry 17:2639, 1978

10. Takahashi K, Newburger PE, Cohen HJ: Glutathione peroxidase protein. Absence in selenium deficiency states and correlation with enzymatic activity. J Clin Invest 77:1402, 1986

11. Yoshimura S, Komatsu N, Watanabe K: Purification and immunohistochemical localization of rat liver glutathione peroxidase. Biochim Biophys Acta 621:130, 1980

12. Sunde RA, Hoekstra WG: Incorporation from selenite and selenocysteine into glutathione peroxidase in the isolated perfused rat liver. Biochem Biophys Res Commun 93:1181, 1980

13. Hawkes WC, Tappel AL: In vitro synthesis of glutathione peroxidase from selenite. Translational incorporation of selenocysteine. Biochim Biophys Acta 739:225, 1983

14. Chambers I, Frampton J, Goldfarb P, Affara N, McBain W, Harrison PR: The structure of the mouse glutathione peroxidase gene: The selenocysteine in the active site is encoded by the "termination" codon, TGA. EMBO J 5:1221, 1986

15. Fallon HJ, Frei E III, Davidson JD, Trier JS, Burk D: Leukocyte preparations from human blood: Evaluation of their morphologic and metabolic state. J Lab Clin Med 59:779, 1962

16. Sukenaga Y, Ishida K, Takeda T, Takagi K: cDNA sequence coding for human glutathione peroxidase. Nucleic Acids Res 15:7178, 1987

17. Ishida K, Morino T, Takagi K, Sukenaga Y: Nucleotide sequence of a human gene for glutathione peroxidase. Nucleic Acids Res 15:10051, 1987

18. Chada S, Whitney C, Newburger PE: Isolation and regulation of the human glutathione peroxidase gene, in Cerutti P, Fridovich I,

McCord J (eds): Oxy-Radicals in Molecular Biology and Pathology. New York, NY, Liss, 1988, p 273

19. Collins SJ, Gallo RC, Gallagher RE: Continuous growth and differentiation of human myeloid leukaemic cells in suspension culture. Nature 270:347, 1977

20. Speier C, Baker SS, Newburger PE: Relationships between in vitro selenium supply, glutathione peroxidase activity and phagocytic function in the HL-60 human myeloid cell line. J Biol Chem 260:8951, 1985

21. Collins SJ, Ruscetti FW, Gallagher RE, Gallo RC: Terminal differentiation of human promyelocytic leukemia cells induced by dimethyl sulfoxide and other polar solvents. Proc Natl Acad Sci USA 75:2458, 1978

22. Newburger PE, Chovaniec ME, Greenberger JS, Cohen HJ: Functional changes in human leukemic cell line HL-60: A model for myeloid differentiation. J Cell Biol 82:315, 1979

23. Rovera G, Santoli D, Damsky C: Human promyelocytic leukemia cells in culture differentiate into macrophage-like cells when treated with phorbol ester. Proc Natl Acad Sci USA 76:2779, 1979

24. Beutler E: Red Cell Metabolism (ed 3). Philadelphia, PA, Grune & Stratton, 1984, p 74

25. Dinauer MC, Orkin S, Brown R, Jesaitis AJ, Parkos CA: The glycoprotein encoded by the X-linked chronic granulomatous disease locus is a component of the neutrophil cytochrome b complex. Nature 327:717, 1987

26. Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc Natl Acad Sci USA 76:4350, 1979

27. Feinberg AP, Vogelstein B: Addendum to a technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137:266, 1984

28. Hall JL, Dudley L, Dobner PR, Lewis SA, Cowen NJ: Identification of two human beta-tubulin isotypes. Mol Cell Biol 3:854, 1988

29. Royer-Pokora B, Kunkel LM, Monaco AP, Goff SC, Newburger PE, Baehner RL, Cole FS, Curnutte JT, Orkin SH: Cloning the gene for an inherited disorder—chronic granulomatous disease— on the basis of its chromosomal location. Nature 322:32, 1986

30. Michelson AM, Markham AF, Orkin SH: Isolation and DNA sequence of a full-length cDNA for human X chromosome-encoded phosphoglycerate kinase. Proc Natl Acad Sci USA 80:472, 1983

31. Aziz N, Munro HN: Both subunits of rat liver ferritin are regulated at a translational level by iron induction. Nucleic Acids Res 14:915, 1986

32. Cleveland D, Lopata MA, MacDonald R, Cowan N, Rutter W, Kirschner M: Number and evolutionary conservation of alpha- and beta-tubulin and cytoplasmic actin genes using specific cloned cDNA probes. Cell 20:95, 1980

33. Ginsburg D, Handin RI, Bonthron DT, Donlon TA, Bruns GAP, Latt SA, Orkin SH: Human von Willebrand factor: Isolation of complementary DNA clones and chromosomal location. Science 228:1401, 1985

34. Maniatis T, Fritsch EF, Sambrook J: Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, 1982, p 197

35. Gatti RA, Concannon P, Salser W: Multiple use of southern blots. Biotechniques 2:148, 1984

36. Linial M, Gunderson N, Groudine M: Enhanced transcription of c-myc in bursal lymphoma cells requires continuous protein synthesis. Science 230:1126, 1985

37. McCachren SS Jr, Salehi Z, Weinberg JB, Niedel JE: Transcription interruption may be a common mechanism of c-myc regulation during HL-60 differentiation. Biochem Biophys Res Commun 151:574, 1988

38. Baker SS, Cohen HJ: Altered oxidative metabolism in selenium-deficient rat granulocytes. J Immunol 130:2856, 1983

39. Van Rij AM, Thompson CD, McKenzie JM, Robinson MF: Selenium deficiency in total parenteral nutrition. J Clin Nutr 32:2076, 1979

40. Cohen HJ, Chovaniec ME, Mistretta D, Baker SS: Selenium repletion and glutathione peroxidase—differential effects on plasma and red blood cell enzyme activity. Am J Clin Nutr 41:735, 1985

41. Saedi MS, Smith CG, Frampton J, Chambers I, Harrison PR, Sunde RA: Effect of selenium status on mRNA levels for glutathione peroxidase in rat liver. Biochem Biophys Res Commun 153:855, 1988

42. Yoshimura S, Takekoshi S, Watanabe K, Fujii-Kuriyama Y: Determination of nucleotide sequence of cDNA coding rat gluta-thione peroxidase and diminished expression of the mRNA in selenium-deficient rat liver. Biochem Biophys Res Commun 154:1024, 1988

43. Reddy AP, Hsu BL, Reddy PS, Li N, Thyagaraju K, Reddy CC, Tam MF, Tu C-PD: Expression of glutathione peroxidase I gene in selenium-deficient rats. Nucleic Acids Res 16:5557, 1988

44. Sunde R, Evanson J: Serine incorporation into the selenocysteine moiety of glutathione peroxidase. J Biol Chem 262:933, 1987

45. Leinfelder W, Zehelein E, Mandrand-Berthelot MA, Böck A: Gene for a novel tRNA species that accepts L-serine and cotranslationally inserts selenocysteine. Nature 331:723, 1988

46. Lee BJ, de la Pena P, Tobian JA, Zasloff M, Hatfield D: Unique pathway of expression of an opal suppressor phosphoserine tRNA. Proc Natl Acad Sci USA 84:6384, 1987

47. Yasumoto K, Iwami K, Yoshida M: Vitamin B6–dependence of selenomethionine and selenite utilization for glutathione peroxidase in the rat. J Nutr 109:760, 1979

48. Diplock AT: Metabolic and functional defects in selenium deficiency. Philos Trans R Soc Lond [Biol] 294:105, 1981

49. Drysdale JW, Munro HN: Regulation of synthesis and turnover of ferritin in rat liver. J Biol Chem 241:3630, 1966

50. Casey JL, Hentze MW, Koeller DM, Caughman SW, Rouault TA, Klausner RD, Harford JB: Iron-responsive elements: Regulatory RNA sequences that control mRNA levels and translation. Science 240:924, 1988