Selenoprotein H Is a Redox-sensing High Mobility Group Family DNA-binding Protein That Up-regulates Genes Involved in Glutathione Synthesis and Phase II Detoxification

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Selenoprotein H is a recently identified member of the selenoprotein family whose function is not fully known. Previous studies from our laboratory and others showed that Drosophila melanogaster selenoprotein H is essential for viability and antioxidant defense. In this study we investigated the function of human selenoprotein H in murine hippocampal HT22 cells engineered to stably overexpress the protein. After treatment of cells with 1-buthionine-(S,R)-sulfoximine to deplete glutathione, selenoprotein H-overexpressing cells exhibited higher levels of total glutathione, total antioxidant capacities, and glutathione peroxidase enzymatic activity than did vector control cells. Overexpression of selenoprotein H also up-regulated the mRNA levels of endogenous selenoprotein H, glutamylcysteine synthetase heavy and light chains, and glutathione S-transferases Alpha 2, Alpha 4, and Omega 1. The amino acid sequence of selenoprotein H contains four putative nuclear localization sequences and an AT-hook motif, a small DNA-binding domain first identified in high mobility group proteins. Chromatin immunoprecipitation using a green fluorescent protein-selenoprotein H fusion binding revealed binding to sequences containing heat shock and/or stress response elements. Thus, selenoprotein H is a redox-responsive DNA-binding protein of the AT-hook family and functions in regulating expression levels of genes involved in de novo glutathione synthesis and phase II detoxification in response to redox status.

Selenium has long been known for its antioxidant properties, and accumulated evidence indicates that many of the beneficial effects of this trace element in our diet are attributable to selenoenzymes. The functions of selenoenzymes include protecting cell membranes, proteins, and nucleic acids from cumulative oxidative damage and maintaining cellular redox balance. Selenium is highly retained in neuronal tissue during selenium deficiency (1), and the functions of selenoproteins in the brain are highlighted by the development of neurological defects in mice that underwent targeted disruption of selenoprotein P, a selenium transport protein whose functions may also include antioxidant defense and heavy metal chelation (2, 3). To date, 25 selenoprotein genes have been identified in the human genome (4), but the functions of many of them are yet to be fully defined. Selenoprotein H (SelH)3 was initially identified in the Drosophila melanogaster genome and subsequently in the human and mouse genomes, where expression is high in the brain. In previous studies we and others showed that D. melanogaster SelH is required for viability, and overexpression of the protein increased antioxidant capacity in Drosophila embryo-derived Schneider S2 cells when exposed to homocysteic acid-induced GSH depletion (5, 6).

Two recent studies have begun to investigate the functions of human SelH. We reported that overexpression of human SelH protects against UV-induced cell death via a decrease in superoxide levels (7). A study employing bioinformatic analysis and gene silencing identified a thioredoxin-fold motif in SelH and demonstrated a protective role for the protein against hydrogen peroxide-induced cell death (8). In the present study, we investigated the function of SelH in neuronal cells against oxidative stress induced by GSH depletion. Murine hippocampal neuronal HT22 cells were engineered to stably overexpress human SelH and subjected to 1-buthionine-(S,R)-sulfoximine (BSO) treatment to inactivate γ-glutamylcysteine synthetase (GCS), the rate-limiting enzyme in GSH synthesis. The effects of BSO treatment on antioxidant parameters and gene expression were assessed in SelH-overexpressing and vector control HT22 cells. These studies revealed that expression of SelH protected intracellular GSH and antioxidant levels and increased expression of key enzymes in GSH biosynthesis and in phase II detoxification.

3 The abbreviations used are: SelH, selenoprotein H; ABTS, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid); AOP, antioxidant potential; ARE/EpRE, antioxidant response elements/electrophile response elements; BSO, 1-buthionine-(S,R)-sulfoximine; FRAP, ferric-reducing ability of plasma; GCS, glutamylcysteine synthetase; GPx, glutathione peroxidase; GST, glutathione S-transferase; HMG, high mobility group; HSE, heat shock element; mGAPDH, mouse glyceraldehyde-3-phosphate dehydrogenase; PBS, phosphate-buffered saline; STRE, stress response element; Tetrox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TEAC, Tetrox equivalent antioxidant capacity; TPTZ, 2,4,6-tripyridyl-s-triazine; GFP, green fluorescent protein; ANOVA, analysis of variance.
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To gain insight into how SelH might up-regulate expression of these genes, the deduced amino acid sequence was analyzed using ExPASy analysis tools. The SelH sequence contains multiple nuclear localization signals, and we show that the protein localizes predominantly to the nucleus. Alignment with known members of the high mobility group (HMG) family of DNA-binding proteins revealed the presence of an AT-hook DNA-binding motif. Chromatin immunoprecipitation using a GFP-SelH fusion protein and antibodies to GFP revealed binding to sequences containing multiple heat shock or stress response elements. These findings identify SelH as a redox-sensing DNA-binding protein that up-regulates transcription of genes involved in antioxidant defense and phase II detoxification.

EXPERIMENTAL PROCEDURES

Cell Maintenance and Sample Harvesting—All of the cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 15 μg/ml gentamicin, 50 μg/ml ampicillin, with 4 mm L-glutamine supplement at 5% CO₂ and ~50% relative humidity. The selenium concentration in the serum-supplemented medium used in this study was ~56 nm, as determined by inductively coupled plasma mass spectrometry analysis of the serum. Cells in 100 mm cell culture dishes were subjected to BSO treatment when the optical confluency reached ~90%. After treatment, the dishes were transferred onto ice, and the medium was aspirated. The cells were washed once with cold phosphate-buffered saline (PBS), trypsinized, and then suspended in 2 ml of fetal bovine serum-free Dulbecco’s modified Eagle’s medium. 10 μl of cell suspension was taken out in duplicate for counting cell numbers, and 1900 were maintained in Dulbecco’s modified Eagle’s medium. 10

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hSelH Transfection—The hSelH clone was purchased from Invitrogen and subcloned into pMSCVpuro retroviral vector purchased from BD Biosciences. Packaging cells (RetroPack PT67, BD Biosciences) were plated in 60-mm dishes at 70% optical confluency 12 h before transfection. The cells were transfected with FuGENE 6 transfection reagent (Roche Applied Science) mixed with 2 μg of hSelH-MSCV retroviral vector or empty MSCV vector. Culture medium was aspirated 4 h after transfection. PT67 cells were washed twice with PBS and allowed to grow for 24 h in 3 ml of complete medium. The cells were selected in puromycin, with the concentration of puromycin optimized to kill the nontransfected control PT67 cells in 7–10 days. Transfected PT67 cells that survived the puromycin selection stably produced virus. For viral infection of target cells, HT22 cells were plated 12 h before infection at ~70% optical confluency. The medium from transfected PT67 cells containing virus was collected, filtered through a 0.45-μm filter, and added to the HT22 cells in the presence of 4 μg/ml Polybrene. Virus-containing medium was replaced after 24 h of incubation. After infection for 48 h, the target cells were subjected to puromycin selection, with uninfected cells as selection control. Selection lasted for ~10 days, until all of the uninfected control cells were killed. The cells that survived the selection made up the HT22-hSelH and HT22-vector cell lines.

75Se Labeling of SelH—HT22-hSelH and HT22-vector cell lines were labeled by the addition of neutralized [75Se]selenious acid (3 μCi/ml; specific activity, 1,000 Ci/mmol), followed by incubation for 36 h. The cells were harvested, and nuclear and cytoplasmic lysates were prepared using a compartment protein extraction kit (Chemicon) according to the manufacturer’s instructions. The proteins were separated on a 10–14.5% gradient SDS-polyacrylamide gel and transferred to nitrocellulose Hybrid C-extra membranes (Amersham Biosciences), followed by phosphorimaging using a Cyclone™ Storage phosphor screen (PerkinElmer Life Sciences).

Reverse Transcription Real Time PCR—cDNAs were synthesized from RNA extracts using SuperScript III First-Strand synthesis system (Invitrogen). Sequences of forward and reverse primers for real time PCR of the housekeeping genes, glyceraldehyde-3-phosphate dehydrogenase (mGAPDH) and hypoxanthine phosphoribosyl-transferase (mHPRT) and for target genes are given in Table 1. All of the PCRs were performed in 10-μl reaction volumes using a Platinum SYBR Green qPCR SuperMix-UDG kit from Invitrogen. Amplification and detection were carried out using a LightCycler 2.0 real time PCR machine (Roche Applied Science).

GSH and Total Antioxidant Capacity Measurements—Total intracellular GSH assays were carried out using the GSH/ GSSG-412™ assay kit from Oxis following the instructions of the manufacturer. The Fe³⁺-reducing ability was measured by the ferric-reducing ability of plasma (FRAP) procedure with modification (9). The reagent contained 0.83 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) and 1.67 mM ferric chloride in 0.1 M acetate buffer (pH 3.6). A 30-μl volume of sample was mixed with 370 μl of reagent and incubated at room temperature for

| TABLE 1 |
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| Reverse transcription real time-PCR primers used for cDNA amplification |
| mGAPDH forward | 5'-GTCATCTGGAAGCAGTCTGACG-3' |
| mGAPDH reverse | 5'-CCTGTGTCGCTGAGGCTCTTC-3' |
| mHPRT1 forward | 5'-GGCTGTTATCATGCTGACATC-3' |
| mHPRT1 reverse | 5'-GCTTCCAGTAAGGAGGAGGAGG-3' |
| mSelH forward | 5'-GACAACAAAATCCCAACACAGG-3' |
| hSelH reverse | 5'-GGACTTCTCTCTCTCTCTCTC-3' |
| mGCS-HC forward | 5'-GGATCTACAGTAAAGCGAGG-3' |
| mGCS-HC reverse | 5'-ATTGATGCTTAAGGACGTGG-3' |
| mGCS-CL forward | 5'-ATGACTGCTCAGAGGACGTGG-3' |
| mGCS-CL reverse | 5'-ATGACTGCTCAGAGGACGTGG-3' |
| mGSTO1 forward | 5'-GAACAGACCTTGGCTCTCAGA-3' |
| mGSTO1 reverse | 5'-GCTGACAGAGGACGAGGACG-3' |
| mGSTO2 forward | 5'-GCTGACAGAGGACGAGGACG-3' |
| mGSTO2 reverse | 5'-GCTGACAGAGGACGAGGACG-3' |
| mGSTA4 forward | 5'-GCTGACAGAGGACGAGGACG-3' |
| mGSTA4 reverse | 5'-GCTGACAGAGGACGAGGACG-3' |

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10 min. Reduction of Fe$^{3+}$-TPTZ to Fe$^{2+}$-TPTZ was monitored by the absorbance increase at 595 nm by spectrophotometry. Trolox, a water-soluble vitamin E analogue, was used as standard. The results are expressed as Trolox equivalent antioxidant capacity (TEAC). The 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging ability was measured by the ABTS method described by Pellegrini et al. (10). To prepare the ABTS radical, an ABTS solution was oxidized in water by potassium persulfate (molar ratio 1:0.35) for 12–16 h in the dark and then diluted in PBS (pH 7.4) prior to assay, giving an absorbance of 0.70 ± 0.02 at 730 nm. A 2-μl volume of sample was added to 398 μl of reagent, followed by incubation at room temperature for 10 min. Scavenging of the ABTS radical was then monitored by the absorbance decrease at 730 nm. Trolox was used as standard, and the results were expressed as TEAC. The Cu$^{2+}$-reducing ability of samples was measured using an antioxidant potential (AOP) assay kit (Oxis). The manufacturer’s instructions were followed except that Trolox was used as standard, and the results were expressed as TEAC.

Antioxidant Enzyme Activity Assays—GPx activity assay was based on the classical principle (11) with optimization to the HT22 cell lysate. The peroxide used in this study was t-butyldihydroperoxide (0.32 mM), the concentration of GSH was reduced to 1.88 mM, and the pH of the assay was increased to 7.6. The unit of GPx activity was defined as 1 milliunit = 1 nmol of NADPH oxidized per min. Glutathione reductase (GR) activity assay measured the GR-catalyzed reduction of GSSG to GSH, using NADPH as the electron donor. A 390-μl volume of reagent containing GSSG in 0.1 M potassium phosphate, 5 mM EDTA buffer at pH 7.4 was mixed with 10 μl of sample and incubated at 37 °C for 3 min, and then NADPH was added to initiate the reaction. The final concentrations of GSSG and NADPH were 0.94 and 0.25 mM, respectively. The decrease of absorbance at 340 nm was recorded for 3 min, and the slope was calculated. The unit of GR activity was defined as that of GPx.

Fluorescence Microscopy—Cells on slides were mounted in mounting medium containing 4’,6’-diamino-2-phenylindole (Vector Laboratories) to visualize nuclei and rhodamine-phalloidin (Molecular Probes) to visualize filamentous actin. Epifluorescence was performed using an Olympus IX71 microscope with an Olympus U-CMAD3 camera and MicroFire 1.0 software (Optronics).

Chromatin Immunoprecipitation—Chromatin immunoprecipitation was carried out using an adaptation of the method of Kuo and Allis (12). SelH was fused to GFP in the pEGFP-C3 vector (BD Biosciences). GFP-SelH or GFP-vector was expressed in HEK-293 cells by transient transfection using Trans IT-LT1 reagent (Mirus-Bio Company). Twenty-four hours post-transfection the cells were cross-linked with 1% formamide at 37 °C for 10–15 min, and the reactions were terminated with 0.125 M glycine buffer at room temperature for 5 min. After washing with ice-cold PBS, the cells were harvested and lysed with 1 ml of lysis buffer (20 mM HEPES, pH 7.4, 25% glycerol, 0.42 M NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol) supplemented with complete protease inhibitor (Roche Applied Science). The lysates were incubated for 10–15 min on ice with pipetting to aid in release of nuclei. The pelleted nuclear fraction was resuspended in nuclear lysis buffer (50 mM Tris-HCl, pH 8.1, 10 mM EDTA, 1% SDS) plus complete protease inhibitor (Roche Applied Science) and incubated on ice for 10 min. The nuclear lysates were sonicated for four pulses of 10 s and 40% amplitude, resulting in fragmentation of DNA to a size range of ~100–500 bp. Nuclear extract was used for chromatin immunoprecipitation with anti-GFP rabbit polyclonal antibodies (Molecular Probes; catalogue number 11122) using 0.2 μg of antibody/pull-down. For negative antibody control, an aliquot of cross-linked chromatin was immunoprecipitated with normal rabbit serum instead of a specific antibody. Immunoprecipitated DNA was purified by phenol/chloroform extraction, ethanol-precipitated, and used for cloning in pJET vector (Fermentas).

Statistical Work—GraphPad Prism 4 software was used for t test and two-way ANOVA assay. A p value ≤ 0.05 was considered as significant.

RESULTS

Expression Level of hSelH in Stably Transformed Cells after Retroviral Infection—The expression levels of hSelH and mSelH were monitored by reverse transcription real time PCR in HT22-hSelH and HT22-vector cells growing in normal culture conditions, and the results are shown in Fig. 1A. The hSelH gene was highly expressed following stable transfection, with expression levels ~34-fold higher than the expression levels of endogenous mSelH. As expected, the mRNA level of hSelH in HT22-vector cells was not detectable. The mRNA level of endogenous mSelH is ~10% higher in HT22-hSelH cells than in HT22-vector cells. Protein expression as detected by labeling with [75Se]selenious acid is shown in Fig. 1B.

Total Intracellular GSH Level—When cells were maintained in normal culture conditions at optical confluence of 80–90%, the total intracellular GSH level of HT22-hSelH was 7.76 ± 0.23 nmol/million cells, and that of HT22-vector cells was 9.06 ± 0.82 nmol/million cells (average ± S.D. from three independent experiments). No significant difference was detected. However, after treatment with concentrations of BSO ranging from 0.03 to 10 mM for 15 h, the GSH level was better maintained in the HT22-hSelH cells than in the HT22-vector cells (Fig. 2A).

Antioxidant Enzyme Activities—GPxs are selenoproteins that reduce peroxides using GSH as the electron donor, whereas GR is an enzyme that reduces GSSG to GSH. The influence of SelH expression level on the activities of these two GSH-related enzymes was investigated. Reverse transcription real time PCR analysis indicates that the mRNAs for Gpx1 and Gpx4 are abundant in HT22 cells, whereas the mRNAs for Gpx2, Gpx3, and Gpx6 are below the limits of detection (data not shown). Thus, the majority of the GPx activity measured in the present study likely derives from Gpx1 and Gpx4. The baseline GPx activities in HT22-hSelH and HT22-vector cells did not significantly differ from one another. After treatment with BSO for 15 h, the GPx activity in hSelH-HT22 increased to 109.7 ± 1.3% of the untreated control at 0.03 mM BSO, whereas that in vector-HT22 cells decreased to 89.3 ± 9.4% (average ± S.D. from three independent experiments, p value = 0.0204). GPx activities in the hSelH-HT22 versus vector-HT22 cells

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The expression level of hSelH in stably transformed cells after retroviral infection was monitored by reverse transcription real time PCR in HT22-hSelH and HT22-vector cells growing in normal culture conditions, and the results are shown in Fig. 1A. The hSelH gene was highly expressed following stable transfection, with expression levels ~34-fold higher than the expression levels of endogenous mSelH. As expected, the mRNA level of hSelH in HT22-vector cells was not detectable. The mRNA level of endogenous mSelH is ~10% higher in HT22-hSelH cells than in HT22-vector cells. Protein expression as detected by labeling with [75Se]selenious acid is shown in Fig. 1B.

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were not significantly different after treatment with higher BSO concentrations (data not shown). However, when the incubation time was decreased to 4, 8, or 12 h, 0.1 mM BSO treatment resulted in higher GPx activities in HT22-hSelH cells than in HT22-vector cells (Fig. 2B).

GR is the enzyme that reduces GSSG to GSH, the reducing substrate for GPx. Similar to GPx, the base-line activities of GR were comparable in the two cell lines. After treatment with 0.03 mM BSO for 15 h, the GR activity in hSelH-HT22 cells increased to 111.7 ± 3.7% of the untreated control, whereas that in the vector-HT22 cells was 99.1 ± 6.5% of the untreated control (average ± S.D., three independent experiments, \( p = 0.0432 \)).

Total Antioxidant Capacities—The FRAP method measures the ferric-reducing ability of samples at pH 3.6, when GSH and other thiol compounds are inactivated as antioxidants, and most proteins are precipitated. Thus, the FRAP value reflects the total antioxidant capacity from nonthiol, low molecular weight compounds. Under normal cell culture conditions, the FRAP values of HT22-hSelH and HT22-vector cells were not significantly different. However, when the cells were challenged with 0.03, 0.1, 1, or 10 mM BSO for 15 h, the FRAP values of the hSelH-HT22 increased to 125, 108, 106, and 106% of the untreated control level, whereas those of the HT22-vector cells decreased to 99, 89, 90, and 80% of the untreated control level (data not shown).

The ABTS assay reflects the potential of a sample to scavenge ABTS radicals. When cultured in the absence of BSO, no significant difference in the ABTS values of the two cell lines was observed. When GSH synthesis was blocked with 0.03–10 mM BSO for 15 h, the ABTS value gradually decreased as the BSO concentration increased and maintained slightly (∼5%) but consistently higher in HT22-hSelH cells in comparison with the vector control (data not shown).

AOP value is a measure of Cu(II)-reducing ability of cell lysates. In the absence of BSO, no difference in AOP value was detected between the two cell lines. After treatment with 0.03–10 mM BSO for 15 h, the AOP value of the HT22-hSelH cells was decreased to ∼80% of the untreated control level throughout the dosage range, whereas that of HT22-vector was decreased to 74–67% when the BSO concentration was increased from 0.03 to 10 mM (Fig. 3A). Treatment with 0.1 mM BSO for 4–15 h produced a decrease in AOP value in the HT22-hSelH cells to the range of 86–76% of untreated control, whereas that of the HT22-vector cells was maintained at ∼70% of the control level (Fig. 3B).

Overexpression of SelH Induced the Expression Levels of Other Genes—GCS is the rate-limiting enzyme involved in de novo GSH synthesis. It consists of GCS-LC and GCS-LC, the catalytic and regulatory subunits, respectively. GCS-LC and GCS-LC mRNA levels were measured in the HT22-SelH and HT22-vector cell lines after treatment with varying concentrations of BSO for 15 h (Fig. 4A and B). Overexpression of SelH resulted in increases in the mRNA levels for both subunits throughout the range of BSO concentrations.

GSTs are important enzymes involved in phase II detoxification. We examined the influence of SelH overexpression on the mRNA levels of GST Alpha and Omega subfamilies. GSTO1 expression levels in HT22-hSelH cells were more than twice the levels in HT22-vector cells but were not influenced by treatment with 0–1 mM BSO (Fig. 4C). The mRNAs for GSTO2 and GSTO3 were not significantly different in the two cell lines during 4–15 h of 0.1 mM BSO treatment (two-way ANOVA, \( p < 0.05 \)).

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**FIGURE 1.** Quantitation of hSelH and mSelH mRNA and hSelH protein levels in HT22-hSelH and HT22-vector cells. A, mRNA levels were quantitated by reverse transcription real time PCR and normalized to mRNAs for housekeeping genes as described under “Experimental Procedures.” The averages ± S.D. are shown (\( n = 2 \)). The inset shows reverse transcription real time PCR amplification curves of hSelH, mSelH, and mGAPDH genes in HT22-hSelH cells and mSelH and mGAPDH genes in HT22-vector cells. B, HT22-vector and HT22-hSelH cells were labeled with \(^{[35}S\)selenious acid, and nuclear-enriched lysates were analyzed by SDS-PAGE and phosphorimaging. Lane 1, HT22-vector; lane 2, HT22-hSelH. Molecular weight markers are indicated on the left, and the position of SelH is indicated by the arrow.

**FIGURE 2.** Total intracellular GSH and GPx activities in HT22-hSelH and HT22-vector cells. A, total GSH levels in the two cell lines after 15 h of BSO treatment. Significance in t test is as follows: * represents \( 0.01 < p \leq 0.05 \); ** represents \( 0.001 < p \leq 0.01 \); and *** represents \( p \leq 0.001 \). B, GPx activities in the two cell lines during 4–15 h of 0.1 mM BSO treatment (two-way ANOVA, \( p = 0.0156 \)). The averages ± S.E. are shown for three independent experiments for each assay.
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The GCS-HC is well known to be regulated by HSE and STRE elements in its promoter (14), and alignment of these sequences with the HSE and STRE sequences pulled down by GFP-SelH revealed significant conservation in these elements (Fig. 6), supporting a direct role for SelH in regulating expression of GCS-HC via these elements.

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DISCUSSION

HT22 is a neuronal cell line that is highly sensitive to GSH depletion (15). The expression level of endogenous mSelH was relatively low in HT22 cells; thus, introducing the hSelH gene resulted in significant overexpression of SelH. In the absence of induced oxidative stress, no significant differences in total intracellular GSH, total antioxidant capacity, and GPx and GR activities were observed between SelH-overexpressing and vector control cells, possibly because of homeostatic control of critical factors that influence the redox status of the cells, such
as GSH. Regulation of the enzymatic activity of GCS, the key enzyme involved in GSH synthesis, occurs through multiple mechanisms. The heavy chain is regulated by both transcriptional and post-transcriptional mechanisms, including post-translational regulation (16). Build-up of GSH also acts to feedback-inhibit the enzymatic activity of GCS, thereby helping to ensure homeostatic control over GSH synthesis (17).

The intracellular antioxidants consist of a large variety of molecules. Assessing total antioxidant capacity requires evaluating multiple contributing compounds in the system. Therefore, we used multidimensional assays (18). SelH overexpression maintained higher total antioxidant capacity values during BSO treatment, but the profiles of the change of total antioxidant capacity differed, indicating that SelH overexpression had a different influence on different categories of antioxidant under BSO-induced oxidative stress.

This study reveals that SelH exerts profound gene regulatory function and that this function is influenced by cellular redox status, i.e., BSO-mediated GSH depletion. As shown in Fig. 4 (A and B), the expression levels of GCS-HC and -LC are influenced...
by both SelH overexpression and BSO-induced oxidative stress. The transcriptional regulation of GCS-HC occurs via several response elements, including AP-1, NF-κB, HSE, STRE, xenobiotic response element, and antioxidant response elements, also termed electrophile response elements (ARE/EpRE) (19). Similarly, GCS-LC is also regulated by AP-1 and ARE/EpRE (20).

The identification of an AT-hook in SelH, and the demonstration via chromatin immunoprecipitation that SelH pulls down HSE and STRE-containing sequences, in conjunction with the up-regulation of GCS-HC and LC by SelH in response to BSO, supports a function for SelH as a redox-responsive DNA-binding protein of the HMG family. The AT-hook motif binds to the minor groove of DNA via a semi-conserved 9-amino acid sequence. Proteins containing this motif are implicated in chromatin structure and as transcription accessory factors. Thus, SelH may potentially serve as a cellular redox sensor that functions in conjunction with some of the many transcription factors that respond to changes in redox status.

GSH depletion and general oxidative stress have been widely reported in the pathogenesis of numerous chronic diseases, including diabetes, cardiovascular disease, Parkinson disease, and cancers. Selenium supplementation was found to sustain the GSH levels in the striatum in mice treated with 6-hydroxydopamine (21). Our unpublished data show that the mRNA levels of mSelH in HT22 cells are highly up-regulated by selenium supplementation; therefore, a possible contribution of the gene regulatory function of SelH should be considered in interpreting potential beneficial effects of selenium supplementation in chronic diseases of oxidative stress.

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