Glutamate cysteine ligase (GCL; also referred to as γ-glutamylcysteine synthetase, GCS) catalyzes the rate-limiting step of glutathione synthesis. The GCL holoenzyme is composed of a catalytic (GCLC; also called GCSα) and a modifier (GCLM; also called GCSβ) subunit, each encoded by a unique gene. Wild-type and mutant promotor/luciferase reporter transgenes containing the promoter region of each GCL subunit gene were transfected into A549 (lung carcinoma), HEK 293 (transformed embryonic kidney), HepG2 (hepatocellular carcinoma), and RD (skeletal muscle rhabdomyosarcoma) cells to examine potential cell-type related differences in transcriptional regulation. In A549, HepG2, and RD cells, maximal basal expression of the GCLC transgene required the full-length (~3802 bp) promoter. Maximal expression in HEK 293 cells was uniquely directed by cis-elements contained within the −2752 to −1286 bp fragment of the promoter. No differences in GLCM promoter function were detected among these 4 cell lines. GCL subunit induction in each cell line by pyrrolidine dithiocarbamate (PDTC), phenethyl isothiocyanate (PEITC), and β-naphthoflavone (β-NF) was examined by RNase protection assays. Although both genes were similarly induced in HepG2 cells by β-NF, PDTC, and PEITC, neither was induced by β-NF in A549, HEK 293, and RD cells. PDTC and PEITC induced GCLM to a much greater extent than GCLC in HEK 293 cells and failed to induce GCLC in RD cells. Neither subunit was induced by any of the agents in A549 cells. These studies indicate that the GCL subunit genes are independently regulated and display cell-type specific differences in both basal and inducible expression.

**Key Words:** glutathione; glutamate cysteine ligase; γ-glutamylcysteine synthetase; liver; lung; kidney; skeletal muscle; human cell lines; gene expression; tissue specific.

Glutathione (L-γ-glutamyl-L-cysteinylglycine; GSH) is an important intracellular antioxidant and is utilized as a cosubstrate in the detoxification of electrophilic xenobiotics and peroxides. GSH is synthesized in 2 sequential reactions catalyzed by glutamate-cysteine ligase (GCL; also known as γ-glutamylcysteine synthetase; GCS), and glutathione synthetase (GSS), the first of which is rate-limiting (Meister and Anderson, 1983). Levels of GSH vary considerably among tissue types. For example, the liver contains very high levels of GSH (~6–8 nmol/mg) compared to lung (~1–4 nmol/mg), kidney (~2 nmol/mg), and skeletal muscle (~0.77 nmol/mg) (Martenson et al., 1991; Meister, 1991) in rats and mice. GCL activity among these tissues also varies considerably, with the highest activity in the kidney (~8.3 nmol/min/mg protein), followed by the liver (~0.8 nmol/min/mg protein), lung, and skeletal muscle (both ~0.16 nmol/min/mg protein) in adult rats (Martenson et al., 1991). In humans, GCL activity is highest in the liver at ~3–10 nmols/min/mg protein whereas lung and kidney activities are both ~1–3 nmols/min/mg (Levonen et al., 2000). Human GCL exists as a heterodimer composed of a 73 kDa catalytic (GCLC, previously referred to as GCSα) and a 31 kDa modifier subunit (GCLM, previously referred to as GCSβ, which are encoded by separate genes present on chromosomes 6p12 and 1p21, respectively (Griffith, 1999; Wild and Mulcahy, 2000). The steady-state levels of GCL mRNA transcripts vary considerably among different human tissues (Gipp et al., 1995), and at different stages of development (Levonen et al., 2000). GCLC possesses all of the GCL catalytic activity and is the site of feedback inhibition by GSH. The importance of the catalytic subunit for GSH synthesis has been demonstrated by the absence of any detectable GSH in cells isolated from GCLC null mutant mouse embryos maintained in GSH-free medium (Shi et al., 2000). Although the GCLC subunit alone is able to ligate glutamate and cysteine to form γ-glutamylcysteine in vitro (Huang et al., 1993), experiments with recombinant rat and human GCLC and GCLM proteins suggest that the efficiency of the catalytic subunit is enhanced by dimerization with the modifier subunit to form the dimeric holoenzyme (Huang et al., 1993; Tu and Anders, 1998). In the case of the rat proteins it has even been suggested that the catalytic subunit alone would be nearly inactive under physiological conditions, becoming functional only when associated with the modifier subunit (Huang et al., 1993). The modifier subunit enhances the catalytic efficiency of GCLC in human cells as well (Tu and Anders, 1998), suggesting that it is an
important component of a functional holoenzyme. The requirement of both subunits for maximal catalytic efficiency fueled early speculation that common mechanisms were operative in induction of both GCL subunit genes. Identification of functional EpRE sequences in the promoters of both genes (Moinova and Mulcahy, 1998; Mulcahy and Gipp, 1995; Mulcahy et al., 1997) supported this early, simple model of GCL regulation.

Induction of GCL activity has been shown to be an important adaptive response to numerous cellular insults. Up-regulation of GCL activity in response to these challenges, including oxidative stress, appears to occur primarily through transcriptional up-regulation of the GCLC and GCLM genes, although post-translational modifications or changes in concentrations of substrates or inhibitors can also be important (Griffith, 1999; Wild and Mulcahy, 2000). Differences in the ability of cells to transcriptionally up-regulate the GCL subunit genes are expected to contribute to the susceptibility of different types of cells to toxic insults, as has been demonstrated in the case of methylmercury exposure in mice and rats (Li et al., 1997).

We therefore examined the regulation of GCLC and GCLM gene expression in transformed human cell lines derived from liver, lung, kidney, and skeletal muscle as a first step in the examination of possible cell-type related factors influencing basal and inducible expression of the 2 GCL subunit genes. Our studies have identified a fragment of the GCLC promoter capable of uniquely directing maximal expression in a kidney-derived cell line. These studies also demonstrate that GCL subunit gene expression in response to several inducing agents differs among the cell lines, and that even within individual cell lines the 2 GCL subunit genes are differentially induced. This latter observation provides evidence that transcriptional activation of the 2 subunit genes may involve distinct molecular mechanisms.

**MATERIALS AND METHODS**

**Cell culture and treatments.** Human lung carcinoma (A549, CCL-185), adenovirus transformed human embryonic kidney (HEK 293, CRL-1573), human hepatocellular carcinoma (HepG2, HB-8056), and human rhabdomyosarcoma (RD, CCL-136) cells were obtained from the American Type Culture Collection. HEK 293 and RD cells were maintained in high-glucose Dulbecco's modified Eagle's medium (DMEM), HepG2 in low-glucose DMEM, and A549 in α-minimal essential medium (αMEM). All media preparations were supplemented with 10% (v/v) fetal bovine serum and 50 μg/ml gentamicin. For induction studies, pyrroolidine dithiocarbamate (PDTC) was dissolved in double-distilled water to 100 mM, phenethyl isothiocyanate (PEITC) in ethanol to 10 mM and β-naphthoflavone (β-NF) in Me2SO to 25 mM. These solutions were then diluted 1000-fold in the appropriate medium prior to addition to cell cultures. The media on 60 mm tissue culture plates of A549, HEK 293, HepG2, and RD cells was replaced with fresh media containing 100 μM PDTC, 10 μM PEITC, 25 μM β-NF, or the appropriate vehicle control. Cells were exposed to PDTC or PEITC for 6 h or β-NF for 16 h before being rinsed with cold phosphate-buffered saline (PBS) then harvested for subsequent analyses. All cultures were approximately 90% confluent at the time of harvest.

![Image](image.png)

**FIG. 1.** GCLC and GCLM promoters used in luciferase reporter gene assays. The GCLC and GCLM promoters were cloned into the pG3 expression vector to create the –3802/GCLC 5′/luc and –1927/GCLM 5′/luc transgenes, respectively. These full-length transgenes were then digested with restriction enzymes and then re-ligated to create the series of promoter/reporter transgenes indicated. Numbering of the GCLC promoter is relative to the transcription start site (+1; arrow), while that for the GCLM promoter is relative to the translation start site. Consensus EpRE and Ap-1 sites indicated on the maps have been previously characterized (Moinova and Mulcahy, 1998; Wild et al., 1998).

**Luciferase reporter gene assays of promoter function.** PGL3 and PGL4 luciferase reporter constructs containing the GCLC and GCLM promoters shown in Figures 1, 2, and 3 have been described previously (Moinova and Mulcahy, 1998; Mulcahy et al., 1997; Wild et al., 1998). For transient transfections, A549 were plated at 2.5 × 10⁵ cells/35 mm dish, HEK 293 at 5 × 10⁵ cells/35 mm dish, HepG2 at 1 × 10⁵ cells/35 mm dish, and RD at 5 × 10⁵ cells/35 mm dish. These densities allowed the cultures to reach confluence 3 days later. The day after plating, equimolar amounts of GCL subunit promoter/luciferase reporter constructs were cotransfected with the β-galactosidase expression vector, pCMVβ (MacGregor and Caskey, 1989), using a standard calcium phosphate-glycerol shock protocol. A total of 2–5 μg of DNA was added to each 35 mm dish, depending on the length of the luciferase reporter construct and the empirically determined optimal transfection conditions for each cell line (data not shown). Two days later, cells were lysed using 300 μl reporter lysis buffer according to the manufacturer’s instructions (Promega, Madison, WI), and assayed for luciferase activity, β-galactosidase activity, and protein content. To assay for luciferase activity, 1–20 μl of lysate (depending on cell type) was added to luciferase reaction buffer (14 mM MgCl₂, 14 mM glycylglycine, 0.1 mg/ml bovine serum albumin, 18 mg/ml ATP, pH 7.8) to a total volume of 400 μl. An Analytical Luminescence Laboratory luminometer was programmed to inject 100 μl of 4 mg/ml luciferin in 10 mM Na₂CO₃, pH 6 into each sample and record luminescence as relative light units. β-galacto-
sidase activity was assayed as described by Rosenthal (Rosenthal, 1987). Briefly, cleavage of o-nitrophenyl-β-galactopyranoside was monitored colorimetrically, and units of β-galactosidase were expressed as (OD 420 - OD 380)/t where t = time in min and 380 converts OD 420 to moles of o-nitrophenyl-β-galactopyranoside cleaved. Protein content was determined according to Bradford (Bradford, 1976). Luciferase activity was normalized to transfection efficiency as determined by β-galactosidase activity after correcting for differences in the amount of lysate used for each assay.

**R**NA isolation and analysis. Total RNA was harvested from confluent cultures of A549, HEK 293, HepG2, or RD using TRI reagent (Molecular Research Center, Inc., Cincinnati OH) according to the manufacturer’s instructions.

For RNase protection assays, 20 μg of total RNA from each treatment group was analyzed by size on a 0.6% agarose/formaldehyde gel, then transferred to a charged nylon membrane (Hybond N+, Amersham Pharmacia Biotech Ltd., Buckinghamshire, UK). Prepared membranes were hybridized overnight with labeled DNA probes and washed according to the protocol provided by the manufacturer of the membrane. RNA was visualized using the PhosphorImager system. Preparation of probes for GCLC and GCLM analyses has been described previously (Gipp et al., 1995).

**Statistical analysis.** StatView statistical software (Abacus Concepts Inc., Berkeley CA) was used for statistical analyses. Results are presented as the means ± standard error. Statistical comparisons were made using ANOVA followed by Fischer’s PLSD. A value of p < 0.05 is considered statistically significant.

**RESULTS**

**Cell-Type Specific Differences in Constitutive Steady State GCLC and GCLM mRNA Levels**

Transcripts for both subunits were detected by Northern analysis in all cell lines examined (Fig. 4). As previously

![FIG. 2. Cell-type specific GCLC promoter function.](image)

- **A** Luciferase activity associated with transfection of individual GCLC promoter/luciferase transgenes detailed in Figure 1 for each of the 4 cell lines is shown. The results are normalized to the activity detected in individual cell lines transfected with the full –3802/GCLC 5’/luc reporter (dashed line at 1.0).
- **B** Sequence of mutations introduced into the Ap-1/EpRE motif at –3137 bp in –3802/GCLC 5’/luc.
- **C** Effect of point mutations in the Ap-1/EpRE site on –3802/GCLC 5’/luc luciferase activity. Results are expressed as the means ± standard error(s) of 3 independent determinations.

![FIG. 3. Cell-type specific GCLM promoter function.](image)

- **A** Luciferase activity associated with transfection of individual GCLM promoter/luciferase transgenes detailed in Figure 1 for each of the 4 cell lines is shown. The results are normalized to the activity detected in individual cell lines transfected with the full –712/GCLM 5’/luc reporter (dashed line at 1.0).
- **B** Sequence of mutations introduced into the Ap-1/EpRE motif at –1927 bp in –712/GCLM 5’/luc.
- **C** Effect of point mutations in the Ap-1/EpRE site on –712/GCLM 5’/luc luciferase activity. Results are expressed as the means ± standard error(s) of 3 independent determinations.

**DIFFERENTIAL GCL SUBUNIT EXPRESSION**

![Image]
Luciferase Reporter Gene Assays to Assess Cell-Type Specific Differences in Promoter Function

Maximal luciferase expression in A549, HepG2, and RD cells was achieved by transfection with a GCLC promoter/luciferase reporter transgene containing the full 3.8 kb of the GCLC promoter (−3802/GCLC 5′-luc). The −3802/GCLC 5′-luc promoter includes a consensus EpRE site at −3137 bp previously shown to be required for full constitutive expression and β-NF inducibility in HepG2 cells (Mulcahy et al., 1997; Wild et al., 1998). Transfection of reporter transgenes with progressively shorter fragments of the GCLC promoter supported reduced expression of luciferase in all 3 of these cell lines (Fig. 2A). In contrast, maximal reporter expression in HEK 293 cells was detected following transfection with the −2752/GCLC 5′-luc transgene. Inclusion of promoter sequences upstream of −2752 bp, which were critical for maximal expression in the other 3 cell lines, had no further effect on luciferase activity in HEK 293 cells. Transfection of the −1286/GCLC 5′-luc transgene into HEK 293 cells resulted in relative reporter gene expression similar to that detected in A549, HepG2, and RD cells, suggesting that one or more cis-elements between −1286 bp and −2752 bp were capable of directing maximal expression in the HEK 293 cells.

To further examine the role of the EpRE site at −3137 bp in GCLC expression in different cell types, each cell line was transfected with a −3802/GCLC 5′-luc transgene containing mutations that disrupted either the EpRE site, an Ap-1 site contained within the EpRE sequence, or both elements simultaneously (Fig. 2B). As shown in Figure 2C, a point mutation that disrupts both the EpRE and the embedded Ap-1 site (m1) reduced the promoter activity of the −3802/GCLC 5′-luc construct to a level comparable to that produced by the −2752/GCLC 5′-luc construct in A549, HepG2, and RD (p ≤ 0.05), indicating that this site was responsible for the basal promoter activity associated with the region between −2752 bp and −3802 bp of the GCLC promoter in these cell lines, as previously reported for HepG2 cells (Mulcahy et al., 1997). However, this mutation did not affect promoter activity in HEK 293 cells (p > 0.5). This observation is consistent with the interpretation that these cells utilize cis-elements between −1286 bp and −2752 bp of the GCLC promoter to direct full basal expression. In the presence of these as yet unidentified cis-elements, the Ap1/EpRE site located at −3137 bp is apparently functionally irrelevant in HEK 293 cells. Mutation of the embedded Ap1 site alone (m2) or the EpRE site alone (m3) decreased promoter activity in A549 and RD cells in a manner similar to that previously reported for HepG2 cells (Wild et al., 1998). In HEK 293 cells, the m2 mutation did not change promoter activity (p > 0.5), whereas reporter expression was actually increased slightly, but significantly (p < 0.05), in cells transfected with the m3 mutant.

Previous studies examining the basal activity of the GCLM promoter in HepG2 cells demonstrated that the first 712 bp upstream of the translation start site were sufficient to support full activity of a luciferase reporter gene (Moinova and Mulcahy, 1998). Consistent with this observation, maximal activity of the GCLM promoter/luciferase reporter transgene was achieved in all 4 cell lines with the −712 bp fragment. Results of transfections with other GCLM transgenes are presented in Figure 3A, normalized to the expression of −712/GCLM 5′luc. Low constitutive luciferase activity was associated with transfection of the −208/GCLM 5′luc transgene and intermediate activity was observed upon transfection with the −344/GCLM 5′luc transgene. The Ap1 and EpRE sites located between nucleotides −344 and −208 (Fig. 1) have been extensively characterized and shown to be key elements in both the basal promoter activity and inducibility in HepG2 cells (Galloway and McLellan, 1998; Moinova and Mulcahy, 1998; Mulcahy et al., 1997; Wild et al., 1998). GCLM promoter/luciferase reporter constructs containing mutations in 1 or both of these sites were therefore also transfected into each of the 4 cell lines to assess the role of these 2 cis-elements in basal GCLM expression in the various cell types (Figs. 3B and 3C). A point mutation that disrupted the EpRE site but left the upstream Ap1 site intact (m1) significantly reduced GCLM promoter activity in A549, HEK 293, and HepG2 cells (p ≤ 0.05). Similarly, a mutation that disrupts only the upstream
Ap-1 site (m4) also significantly reduced GCLM promoter activity in A549 and HepG2 cells ($p \leq 0.05$). Interestingly, individual mutations in either the Ap-1 or the EpRE did not significantly reduce expression in RD cells ($p > 0.1$). However, combining the 2 mutations (m1m4) significantly reduced GCLM promoter activity in all 4 cell lines ($p \leq 0.05$), the effect being significantly more profound in A549 cells when compared to the other 3 cell lines ($p < 0.05$).

**Cell-Type Specific Differences in the Inducibility of GCLC and GCLM by Different Agents**

Steady state levels of GCLC and GCLM mRNA were analyzed by RNase protection assays following exposure of each cell line to 100 μM PDTC for 6 h, 10 μM PEITC for 6 h, or 25 μM β-NF for 16 h (Fig. 3A), conditions previously shown to induce expression in HepG2 cells (Wild et al., 1999). In HepG2 cells, β-NF exposure resulted in an ~8 fold and ~11 fold increase in GCLC and GCLM expression, respectively (Fig. 5B). However, β-NF failed to induce expression of either subunit in the remainder of the other cell lines tested. Like β-NF, PDTC exposure induced both GCLC and GCLM expression to a similar degree in HepG2 cells. However, in the HEK 293 cells, GCLM expression was induced by PDTC to a much greater extent (~6 fold) than was GCLC expression (~2 fold). Differential induction of the 2 subunits was even more exaggerated in PDTC-treated RD cells. In these cells, PDTC treatment only induced GCLM ~6 fold but failed to alter expression of the GCLC subunit gene ($p > 0.5$). The pattern of GCL subunit induction following exposure to PEITC was similar to that observed with PDTC although the magnitudes of induction were uniformly more modest. Like PDTC, this agent also failed to induce expression of the GCLC subunit in RD cells. None of these agents significantly induced the expression of either subunit in A549 cells ($p > 0.3$).

**DISCUSSION**

The cell lines used in this report are homogeneous cultures of transformed, immortalized cells. Although these cells do not mimic their normal counterparts in all respects, they do maintain varying degrees of lineage specific traits. For example, HepG2 cells, derived from a human hepatocellular carcinoma, display morphological and biochemical features of hepatocytes (Knowles et al., 1980) and have been utilized as an in vitro model of liver cells for decades. A549 cells are derived from a human lung carcinoma and retain characteristics of type II alveolar epithelial cells (Lieber et al., 1976), while rhabdomyosarcoma-derived RD cells retain some biochemical characteristics of skeletal muscle cells, such as the presence of myoglobin and myosin ATPase (McAllister et al., 1969). The HEK 293 cells were derived from human embryonic kidney epithelial cells that had been transformed by an adenovirus in vitro (Graham et al., 1977) and have been shown to contain kidney-specific transcription factors (Cederberg et al., 1999; Ernstsson et al., 1996). While direct extrapolation from these studies to regulation of GCL gene expression in specific cell types in vivo is not intended, the observed variations in GCL subunit gene expression are indicative of the existence of differential regulatory mechanisms which may segregate in a cell-type specific manner.

As has been documented for normal human tissues (Gipp et al., 1995; Levonen et al., 2000), differences in steady state mRNA levels of GCLC and GCLM, as well as in the ratios of the 2 transcripts, were detected among the different cell lines (Fig. 4). Differences among the cell lines in transcriptional control of GCL subunit expression were also evident in experiments employing GCL subunit promoter/luciferase reporter transgenes. An EpRE site at ~3137 bp in the GCLC promoter, which is critical for maximal expression in A549, HepG2 and RD cell lines, is expendable in the HEK 293 cells. In these cells, maximal constitutive expression of the GCLC promoter transgene was directed by elements within the region of the
promoter between position −1286 and −2752 bp. This region of the promoter did not enhance expression in any of the other cell lines examined (Fig. 2). Sequence analysis of this region revealed the presence of several cis-elements that may direct cell-type specific enhancer activity, including putative Ap-1 and C/EBPβ sites, as well as several E-boxes. Since HEK 293 cells are transformed embryonic kidney cells, it is unclear whether this differential expression reflects transformation-related, kidney-specific, or development-dependent effects on GCLC gene transcription. Further studies to examine these possibilities are in progress. Nevertheless, these studies clearly suggest the existence of 1 or more previously unrecognized cis-elements in the GCLC promoter that are capable of enhancing gene expression specifically in the HEK 293 cells.

Specific differences were even more evident when evaluating induction of GCLC and GCLM by β-NF, PDTC, or PEITC. Among the differences observed, 3 were particularly noteworthy: (1) induction of either subunit following exposure to β-NF was only observed in HepG2 cells; (2) none of the agents tested significantly induced GCLC or GCLM subunit expression in A549 cells; and (3) exposure to PDTC and PEITC resulted in differential induction of GCLM subunit gene expression in HEK 293 and RD cells, without any induction of the GCLC gene in the case of the latter.

The ability of β-NF to induce GCL subunit expression only in HepG2 cells is most likely attributable to the ability of these cells to metabolize planar aromatic compounds to reactive intermediates which then initiate transactivation through the EpRE. β-NF was chosen for study because it was one of the inducing agents originally used to identify the EpRE (Rushmore et al., 1991; Rushmore and Pickett, 1990). In the original studies it was shown that induction of genes by β-NF through this element did not occur in mouse hepatoma cells that lacked either a functional Aryl hydrocarbon receptor (AhR) or a functional Cytochrome p450 1A1 (Cyp1A1) (Rushmore and Pickett, 1990). Cyp1A1 expression can be induced in HepG2 cells by the classical AhR ligand 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) (Kress and Greenlee, 1997) indicating that HepG2 cells express both Cyp1A1 and Ah receptor genes.

The failure of any of the agents examined to induce expression of either GCL subunit gene in A549 cells may be related to high endogenous GSH levels and GCL activity (Mulcahy, unpublished data) relative to the other cell lines in this study. The relatively high abundance of GCL mRNA and the pronounced effect of the mIm4 mutant −1927/GCLM S′-lac on constitutive expression of the reporter gene are also consistent with the possibility that these cells are already expressing active GCL at high levels. Nevertheless, the current experimental data are insufficient to define specific mechanisms responsible for the lack of induction seen in A549 cells.

The current studies demonstrate that GCL subunit gene expression in response to the xenobiotics examined varies considerably within, as well as among, the different cell lines, suggesting that distinct mechanisms might be involved in the induction of the 2 GCL subunit genes. This possibility is also suggested by other studies that have noted differential induction of either the GCLC or GCLM genes. In cultured rat hepatocytes, for example, only GCLC mRNA was up-regulated by hydrocortisone or insulin, while treatment with BSO or the oxidants tert-butyl hydroquinone (tBHQ) and diethyl maleate (DEM) increased expression of both subunits to a similar extent (Cai et al., 1997). Similarly, in cultured rat lung epithelial cells, expression of both subunits is up-regulated by the specific GCL inhibitor buthionine sulfoximine (BSO) (Tian et al., 1997) and by the redox-cycling quinone, 2,3-dimethoxy-1,4-naphthoquinone (DMNQ) (Shi et al., 1994; Tian et al., 1997). In HepG2 cells, preferential up-regulation of the GCLC gene has been observed in cells incubated under hypoxic conditions or in the presence of the iron chelator desferrioxamine (Wild and Mulcahy, unpublished data). Differential subunit regulation apparently extends to down-regulation of expression as well. In mice constitutively expressing the HIV-1 encoded transactivator protein, Tat, GCLM mRNA levels are lower than in controls in some tissues, while levels of GCLC mRNA are not affected (Choi et al., 2000). While many possible mechanisms could be invoked to explain these differences in the regulation of GCL subunit expression, none have yet been verified experimentally. One intriguing possibility is that cellular GCL enzyme activity in some cell types (i.e., HEK 293 and RD) is limited by low abundance of one of the subunits (i.e., GCLM). Up-regulation of that particular subunit gene might then be required to affect increased enzyme activity. In other cell types (i.e., HepG2) increased enzyme activity may require increased expression of both subunit genes. Investigation of these possibilities requires further characterization of the relative levels of the 2 subunit proteins in various cell and tissue types.

Several cis-elements located in the promoters of GCLC and GCLM genes have been implicated in transcriptional regulation in HepG2 cells, including Ap-1 and EpRE sites. Nrf2, in association with as yet unidentified binding partners belonging to the bZip family of transcription factors, has been shown to play a prominent role in the regulation of expression of both GCL subunit genes (Wild and Mulcahy, 2000), as well as other EpRE regulated genes, including heme-oxygenase 1, several glutathione S-transferases, and NADPH quinone oxidoreductase (Alam et al., 1999, 2000; Chan and Kan 1999; Hayes et al., 2000; Ishii et al., 2000; Itoh et al., 1997; Nguyen et al., 2000; Venugopal and Jaiswal 1998, 1996). Cell or tissuespecific differences in distribution and function of relative transcription factors such as Nrf2, small Maf, Jun, or other bZip members might therefore contribute to the cell-type specific variations in GCL subunit gene expression observed in this study.

Although many details remain elusive, regulation of expression of the human GCL subunit genes is clearly more complex than suggested by the prevailing simple model of coordinate subunit regulation through EpRE sequences. Further elucidation...
tion of the molecular events involved in GCS gene expression is warranted as such effects could shed light on important differences in the susceptibility of specific cells and tissues to oxidative stress and xenobiotic challenges and their differential abilities to mount GSH-related adaptive responses.

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