Structural Organization of the Microsomal Glutathione S-Transferase Gene (MGST1) on Chromosome 12p13.1–13.2

IDENTIFICATION OF THE CORRECT PROMOTER REGION AND DEMONSTRATION OF TRANSCRIPTIONAL REGULATION IN RESPONSE TO OXIDATIVE STRESS*

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The structure and regulation of the microsomal glutathione S-transferase gene (MGST1) are considerably more complex than originally perceived to be. The MGST1 gene has two alternative first exons and is located in the 12p13.1–13.2 region. Two other potential first exons were determined to be nonfunctional. The region between the functional first exons cannot direct transcription. Thus, one common promoter element directing transcription exists, and RNA splicing occurs such that only one of the first exons (containing only untranslated mRNA) is incorporated into each mRNA species with common downstream exons. MGST1 expression and regulation are therefore similar to those of other hepatic xenobiotic handling enzymes, which also produce mRNA species differing only in the 5′-untranslated regions to yield identical proteins. MGST1 was previously considered a “housekeeping” gene, as non-oxidant inducers had little effect on activity. However, the promoter region immediately upstream of the dominant first exon transcriptionally responds to oxidative stress. In this respect, MGST1 is similar to glutathione peroxidases that also transcriptionally respond to oxidative stress. The discovery that MGST1 utilizes alternative first exon splicing eliminates a problem with the first description of MGST1 cDNA in that it appeared that MGST1 expression was in violation of the ribosomal scanning model. The identification that the first exon originally noted is in fact a minor alternative first exon far downstream of the primary first exon eliminates this conundrum.

Glutathione S-transferases (GSTs); EC 2.5.1.18 are a complex gene superfamily of soluble and membrane-bound enzymes that catalyze the reaction of the tripeptide glutathione with a wide range of endogenous and xenobiotic lipophilic electrophiles (1–4). The classic membrane-bound GST is microsomal GST (also known as MGST1 or GST12), which was first noted in 1982 (5) and is a single-copy gene on chromosome 12 (6). Other members of the membrane-bound GST family include 5-lipoxygenase-activating protein, leukotriene C4 synthase (7, 8), and two new microsomal membrane-bound glutathione transferases named MGST2 and MGST3 (9, 10) as well as a recently discovered glutathione-dependent prostaglandin E synthase (11). Interestingly, the latter enzyme is the one most closely related to MGST1 (38% identity on the amino acid level).

A major difference between MGST1 and other GST enzymes is the ability of MGST1 to be activated (specific activity of the protein increased) post-translationally (12, 13). Activation of MGST1 occurs by a variety of mechanisms including exposure to sulfhydryl-reacting agents, thiol-disulfide interchange, proteolysis, and sulfhydryl oxidation to form dimers (14–17). The mechanism by which MGST1 activation proceeds is unclear, but is thought due to a higher rate of formation of the GSH thiolate anion within the activated enzyme (13).

MGST1 displays non-selenium glutathione-dependent peroxidase activity, and N-ethylmaleimide exposure (or activation) markedly increases this peroxidase activity (18). Lipid and fatty acid hydroperoxides (linoleic acid/ester hydroperoxide), lipid peroxidation products (4-hydroxynonen-2-enol), and oxidized phospholipids (linoleylpalmitoylphosphatidylcholine hydroperoxides and cholesterol linoleate hydroperoxides) are MGST1 substrates (19–22). In this respect, MGST1 demonstrates non-selenium-dependent hydroperoxide activity similar to the selenium-dependent hydroperoxide activity of the enzyme phospholipid hydroperoxide-glutathione peroxidase (GPX4).

MGST1 also conjugates a variety of potentially harmful xenobiotics, including halogenated hydrocarbons, activated esters, and unsaturated carbonyls (12, 13). Although GST reactions are generally a detoxification mechanism, the nephrotoxicity of several haloalkenes is attributed to hepatic microsomal GSH conjugate formation, metabolism of these GSH conjugates to corresponding cysteine S-conjugates, and subsequent translocation to the kidney, followed by bioactivation of the cysteine S-conjugates to a toxic intermediate (23). Chlorotrifluoroethene is a potent nephrotoxin that undergoes such bioactivation; and in rodent liver cells, 85% of the GSH conjugation with chlorotrifluoroethene (as a substrate) is catalyzed

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank/EMBL Data Bank with accession number(s) AF099926.

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The abbreviations used are: GSTs, glutathione S-transferases; kb, kilobases(s); bp, base pair(s); PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; DMEM, Dulbecco’s modified Eagle’s medium; UTR, untranslated region; HNF, hepatocyte nuclear factor.

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by MGST1 (24).

The role of promoter induction or transcriptional regulation in increasing cytosolic GST expression is well documented. Although inducers of cytosolic GSTs have little effect on MGST1 activity, there are some exceptions. A consistent increase in MGST1 activity and protein occurs in mice treated with 2(3)-terbutyl-4-hydroxyanisole (25). A recent study noted that isoniazid, naphthoflavone, clofibrate, and isosafrole produce moderate increases in MGST1 activity and mRNA content (26), suggesting that transcriptional up-regulation plays a role in regulation of MGST1. Phorone (diospropylideneacetone) is another agent noted to increase MGST1 in vivo (27). As phorone reacts with sulfhydryl groups, it was originally believed that the in vivo induction of MGST1 was mediated either by disulfide interchange or covalent modification (activation by binding to the single cysteine). However, utilizing hepatocytes, it was subsequently demonstrated that these alternatives are less likely (28).

We previously determined the structural organization of the MGST1 gene (29) and assigned exons based on earlier work (30). However, further investigation, as described here and elsewhere (31), reveals that the MGST1 gene is considerably more complex than originally deduced (30), as alternative splicing is utilized, and in response to oxidative stress, the promoter or upstream 5′-nontranscribed region directs transcription.

EXPERIMENTAL PROCEDURES

Materials—HepG2 cells were obtained from the American Type Culture Collection (Manassas, VA). The pG2 enhancer, pGL3 enhancer, pGL2(5V) promoter, pSV-galactosidase, pGEM3, sequencing-grade Taq polymerase, and fmol™ thermocycling DNA sequencing kit were obtained from Promega (Madison, WI). The ALL lysis and luciferase reagents were obtained from Analytical Luminescence Laboratory (San Diego, CA). The Lipofectin and galactosidase reagents were obtained from Life Technologies, Inc. The P1 plasmid, with an insert of 75 kb containing the entire MGST1 gene, was isolated and purified as described previously (29).

PCR Analysis of Alternative Exon Expression in Human cDNA—The cDNA from human liver was a gift from Dr. Inger Johansson (Institute for Environmental Medicine, Karolinska Institute), and the pC-DG6367 cDNA library was a gift from Dr. Paul Berg (Stanford University, Palo Alto, CA). The following primers were used to study whether products corresponding to the alternative first exons were expressed: lower, 5′-TTGTCAATCTATAGAATGCAGTTGC-3′ (corresponding to the alternative first exon in GenBank™/EBI Data Bank accession number AA314967; exon 1C in Table I), and 5′-CTGGTATCATGAATTTGGA-3′ (corresponding to the common fourth exon) was was labeled with digoxigenin-dUTP by nick translation to produce the desired luciferase expression vector. All inserted genomic regions in the pGL2 expression vectors were bidirectionally resequenced using the dideoxynucleotide chain termination method of Sanger (32) to confirm identity.

Cell Culture—HepG2 cells were maintained in DMEM, 10% bovine serum, and 2 mM glutamine. As variation in GPX1 activity influences cellular response to paraquat (33, 34), the medium was supplemented with 50 ng/ml selenium. This eliminates variation in endogenous cellular cytosolic glutathione peroxidase (GPX1) (35) due to post-transcriptional regulation of GPX1 by selenium content (36, 37).

Transfection—HepG2 cells were plated in 35-mm six-well plates at 100,000 cells/well and incubated overnight. The transfection mixture was freshly prepared by mixing equal amounts of solution A (2 μg of desired pGL2-derived plasmid, 3 μg of SV-galactosidase vector, and 100 μg of DMEM) and solution B (10 μl of Lipofectin in 100 μl of DMEM), incubating for 15 min at room temperature, and then adding 800 μl of DMEM. The pGL2-derived plasmids were always cotransfected with the SV-galactosidase vector to correct for variations in transfection efficiency. Cells were rinsed twice with phosphate-buffered saline and once with DMEM, and then the lipofection transfection solution (1 ml) was overlaid onto the cells. Cells were incubated for 5 h at 37 °C in a humidified incubator. The transfection solution was removed by washing with phosphate-buffered saline; and cells were removed by trypsinization, combined, and then replated to allow uniform distribution of transfected cells. Normal growth medium (2 ml) containing 50 ng/ml selenium, and the absorbance was determined at 420 nm. All values were corrected for spontaneous hydridization signals were detected by incubating hybridized filters with 4,6-diamidino-2-phenylindole. Luciferase Assay—The medium was removed; cells were washed three times with ice-cold phosphate-buffered saline; and 400 μl of 1.3 diluted ALL lysis buffer was added, followed by agitation for 15 min at 4 °C. The incubation and centrifugation were repeated to allow uniform distribution of transfected cells. Normal growth medium (2 ml) containing various quantities of paraquat was added, and cells were allowed to incubate for 18 h before luciferase and galactosidase assays.

β-Galactosidase Assay—The medium was removed; cells were washed three times with ice-cold phosphate-buffered saline; and 400 μl of diluted reporter buffer was added. Cellular debris was removed by scraping into a microcentrifuge tube, and the supernatant was collected. To 150 μl of supernatant was added 150 μl of 2× galactosidase assay buffer, and the mixture was allowed to incubate for 2 h at 37 °C. The reaction was stopped with 50 μl of sodium carbonate, and the absorbance was determined at 420 nm. All values were corrected for endogenous galactosidase cellular activity.

Chromosomal Localization—The chromosomal localization of P1 clone 2625 was determined by the fluorescence in situ hybridization technique as described previously (38). Briefly, the 17-kb SacI genomic clone containing MTG1 was labeled with digoxigenin-DUTP by nick translation. Labeled probe was combined with sheared human DNA and hybridized to normal metaphase chromosomes derived from phytohemagglutinin-stimulated peripheral blood lymphocytes. Hybridization reactions contained 50% formamide, 2× SSC, and 2× SSC. The specific hybridization signals were detected by incubating hybridized slides in fluoresceinated anti-digoxigenin antibodies, followed by counterstaining with 4,6-diamidino-2-phenylindole.

Sequence Analysis—Computer analysis of upstream MTG1 regions for potential transcription factor- and regulatory binding sites was by several approaches. The regions were analyzed by a nonmatrix method
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The sequence 1B describes the nucleotide sequence in cDNA clones isolated from human HepG2 and GM637 cells. The sequence 1D was previously identified (30) and is located in the MGST1 gene (29). The sequences 1A and 1C were obtained by analyzing expressed sequence tag submissions R86750 and AA14967 in the dBEST data bank of GenBank™.

| Variable exon 1 (contains only 5'-UTR) | Nonvariable exon 2 (contains coding) |
|--------------------------------------|--------------------------------------|
| 1A tcgtgacaaaagataattcctgtaatcagttggaaacc | attccagacccaatagttgaatATG (fetal liver, spleen) |
| 1B gcgacccggccgaacacgttcctctccctcgctctaccce | attccagacccaatagttaaATG (GM637, HepG2 cells; adult liver) |
| 1C aatcgatgcttttgaaaggaattgattttcctcgcgg | attccagacccaaatgaagATG (KCI cell line) |
| 1D gattcaagtctcaaaagctcagttggaaactactagatgacgaagtt | attccagaccaataaggggaATG (adult liver) |

including the WDNASIS® DNA motif Version 2.5 data base (latest version) and by matrix methods. Matrix methods included MacIn-spector® Version 2.2 software utilizing the Transfac® Version 3.3 data base (latest version) utilizing both normal homology scanning (core similarity at 0.80 and matrix at 0.85) and high homology scanning (core similarity at 1.00 and matrix similarity at 0.95). The matrix approach was also accomplished by accessing the Signal Scan MED Search Service of BIMAS using the MAMMALIAN mRNA database and the TFSSEARCH Program (Version 1.3) using the TFMATRIX data base (Release 3.3).

RESULTS

Identification of Alternative Splicing in Human MGST1—We isolated the upstream region of MGST1 cDNA clones from λ cDNA libraries derived from human HepG2 hepatoma (liver tumor) and GM637 fibroblast cell lines by conventional molecular biology techniques. Sequencing of these clones revealed a marked difference in the 5'-UTR (Table I), which corresponded to the first exon 1B (30). The remaining mRNA sequence containing the coding region (exons 2–4) was identical in all isolated MGST1 cDNA clones, indicating that they all encode for an identical protein (Table I). However, the clones definitely had different 5'-UTR sequences than previously reported (30).

Examination of human expressed sequence tag submissions in the dbEST data bank of GenBank™ revealed that the majority of the submitted clones had a 5'-UTR, or first exon, identical to that detected in our GM637- or HepG2-derived clones. Examples of dbEST clones from other sources with a first exon (and thereby second exon) identical to ours clones include liver clones T86061 and N78024, and fibroblast clone H24621. There were two other submissions, clones R86750 and AA14967, with entirely different first exons. All of our clones and the GenBank™ submissions varied at the same exact point (exon 1/exon 2 junction) and were derived from different cDNA libraries, making it unlikely that numerous chimeric clones had been isolated by different investigators. Thus, there are at least four transcripts of MGST1 mRNA, with varying 5'-noncoding ends, expressed in humans (Table I).

Structural Organization of MGST1 and Identification of Alternative First Exon Splicing—Oligonucleotides specific for the different first exons (Table I) were used to directly sequence P1 clone 2625, previously demonstrated to contain the MGST1 gene (29). The P1 clones containing human gastrointestinal glutathione peroxidase (GPX2, chromosome 14) and glutathione reductase (GRD1, chromosome 8) were also directly sequenced, as negative controls, to ensure that we did not obtain erroneous sequencing results attributable to the P1 vector. When using the HepG2, adult liver, and lymphoid corresponding oligonucleotides, we obtained positive nucleotide sequencing results with the MGST1 clone (illustrating one of the advantages of using P1 clones with large inserts), but no results were obtained using these exon-specific oligomers with the negative controls (GPX2 and GRD1 P1 clones). These positive nucleotide sequencing results confirmed the presence of three potential alternative first exons that were in close proximity (~20 kb) to the MGST1 second exon. PCR amplification of the MGST1 P1 genomic clone using these exon-specific oligonucleotides in combination with an oligonucleotide complementary to the adult liver-specific exon first reported (30) revealed that these exons were located ~6 kb upstream. No PCR products were obtained with the GPX2 or GRD1 P1 clones (data not shown), indicating that the derived sequences were specific to the MGST1 gene.

It was therefore of interest to determine whether these mRNAs result from genetic variation or alternative splicing. Here we could demonstrate that PCR products of the expected size and sequence are produced when alternative exon 1B- and 1D-specific primers are used in combination with an exon 2 primer. The template was cDNA from a single human liver, and the results indicated alternative splicing. The strongest band was consistently obtained from exon 1B (Fig. 1), corresponding also to the most frequent expressed sequence tag clone.

Overlapping SacI and XbaI subclones of the MGST1-containing P1 phagemid clone were isolated and sequenced as described previously (29). Complete bi-directional nucleotide sequencing of these clones revealed that the predominant or primary first exon 1B was located ~6 kb upstream of the alternative or secondary first exon 1D that was originally described (Fig. 2). The locations of the nondetectable first exons 1A and 1C were also identified.

Determination of mRNA Initiation Point—RACE analysis (39) to determine the 5'-cDNA termini was performed using an upper primer corresponding to the λ vector nucleotide sequence and lower primers corresponding to the different alternative first exons. The major product was only with the primer derived from the alternative first exon 1B. A faint band was detected from the primer corresponding to exon 1D. There was no detectable PCR product with primers corresponding to the alternative first exons 1A and 1C. Twenty-four exons were isolated from the exon 1B-derived PCR product. Nucleotide analysis of the clones revealed a common mRNA initiation site in the first exon 1B (Fig. 3), which matches a primer extension study (31).

Determination of the Correct Promoter Region—Computer analysis of the 5'-flanking region or common promoter element (Fig. 3) revealed the presence of a potential electrophile response element site (60–64) as well as numerous sites associated with hepatocyte-specific expression, including HNF-1, HNF-3, HNF-4, EBP40, and Oct-4A family members. A 568-bp insert corresponding to the region immediately upstream of the putative primary first exon 1B (region 6 in Fig. 2) and nucleotide sequence in Fig. 3 contained the putative ∆αRE site was inserted in the correct orientation into the pGL2 or pGL3 enhancer-luciferase reporter vectors to create the pGLMGST1(+) expression vector. The same insert was also inserted in the opposite or negative orientation into the pGL vectors to create the pGLMGST1(−) vector. As RACE and PCR analyses of liver cDNA suggested that exon 1D was functional, ...

3 M. J. Kelner, unpublished results.
although not to the same extent as exon 1B, several different length expression vectors were constructed by inserting different regions upstream of that exon (regions d–g in Fig. 2). These regions inserted into the expression vector ranged from 0.8 to 3.1 kb in size. Similarly, expression vectors were constructed by inserting the regions upstream of exons 1A and 1C (regions a and c in Fig. 2) into the pGL2 enhancer-luciferase expression vector (Fig. 2). All of the expression plasmids were resequenced to confirm nucleotide homology and correct orientation of the inserts.

Transfection of the pGL3MGST1(+) expression vector (containing the 568-bp insert upstream of the primary first exon 1B in the correct orientation; region b in Fig. 2) revealed that this region was capable of directing transcriptional expression of a heterologous gene product, as relative expression of luciferase by pGL3MGST1(+) was >1 million light units over background levels (~1000 light units) in the hepatic derived HepG2 cells. The directed expression of luciferase by the pGL3MGST1(+) vector was equivalent to that noted with the control SV-luciferase vector (data not shown). Expression of luciferase by pGLMGST1(−) (containing the 568-bp insert upstream of the primary first exon 1B in the opposite orientation) was not detected (<1000 light units). No expression was noted with any of the expression vectors derived from the region upstream of the first exon 1D originally described or from the regions upstream of exons 1A and 1C (~1000 light units). These studies confirm that the region between exons 1B and 1D functions solely as an intron.

Transcriptional Response to Oxidative Stress—The pGLMGST1(+) expression vector or the control SV-luciferase expression vector was transfected individually into HepG2 cells. After 18 h, the cells were exposed to varying concentrations of paraquat or menadione for 48 h. Analysis of luciferase expression revealed that the MGST1 promoter region responded to the oxidative stress induced by paraquat or menadione by regulating production of the luciferase reporter gene (Fig. 4).

Chromosomal Localization Studies—The initial fluorescence in situ hybridization experiment resulted in specific labeling of the mid-short arm of a group C chromosome believed to be chromosome 12 on the basis of size, morphology, and banding pattern. A second experiment was conducted in which a genomic probe, which had been previously mapped to the terminus of the long arm of chromosome 12, was cohybridized with the SacI genomic clone. This experiment resulted in the specific labeling of the distal long arm and the short arm of chromosome 12. Measurements of 10 specifically labeled chromosomes demonstrated that MGST1 is located at a position 59% of the distance from the centromere to the telomere of chromosome arm 12p, an area corresponding to band 12p13.1–13.2. A total of 80 metaphase cells were analyzed, with 75 exhibiting specific labeling. Thus, the fluorescence in situ hybridization chromosomal localization studies localized the human MGST1 gene to 12p13.1–13.2, in agreement with a previous report using somatic cell hybrids that localized MGST1 to chromosome 12 (6). During the course of this investigation, bacterial artificial chromosome clone AC007528 from human chromosome 12p was released and found to contain a portion of the MGST1 promoter region.

DISCUSSION

The correct structural organization and chromosomal localization of the human MGST1 gene have now been determined. The MGST1 gene is located in the 12p13.1–13.2 region, in agreement with previous results that determined that the gene was on chromosome 12 (6). There are two alternative first exons (1B and 1D) utilized by the MGST1 gene, and the upstream or 1B exon appears to be the predominate exon in the liver. This alternative first exon 1B also gives rise to the predominate mature mRNA transcript in most human tissues (40). The region between these two alternative first exons is not capable of directing transcription. Thus, MGST1 cDNA clones can display a variable 5′-UTR (first exon 1B or 1D), but common or nonvariable other exons (exons 2–4). In this respect, MGST1 appears to function by the “type B” pattern of alternative splicing or “mutually exclusive exon splicing” (41). In this pattern, there is one common promoter element, and RNA splicing occurs such that only one type of exon 1 is incorporated.
into each mRNA species. The other potential pattern, "type F" (41) or the "alternative promoter pattern of alternative RNA splicing," requires two (or more) distinct promoter elements that individually regulate each type of mRNA by regulating the first exon. As the region between the two alternative exons cannot direct transcription, the \textit{MGST1} gene must display the type B pattern of alternative splicing and utilize exons 1B and 1D. With regard to the potential first exons 1A and 1C, these appear to be artifactual and nonfunctional based on the findings that we cannot detect their presence by PCR; only two clones contain these exons, so they are relatively rare submissions in GenBank\textsuperscript{TM} (compared with numerous clones with exon 1B); and the upstream 5'-flanking regions cannot function to direct transcription.

Computer analysis of the common promoter element, or 5'-flanking region to the first exon 1B, revealed numerous potential transcription factor- and regulatory sites of high homology (100% core similarity and 95% matrix similarity) are \textit{underlined}. \textit{HNF-A}, histone nuclear factor-A; \textit{EpRE}, electrophile response element; \textit{C/EBP}\textsubscript{\alpha}, \textit{CAAT/enhancer-binding protein-\alpha}.

The \textit{EpRE} site (60–64) located at approximately –500 bp, based on the menadione stimulation study, appears functional and can confer transcriptional regulation upon the \textit{MGST1} gene in response to oxidative stress.

There is precedence for this process of alternative first exon expression as several other "liver-associated" xenobiotic metabolism or detoxification enzymes are regulated in this manner. The rat \textit{g}-glutamyltransferase gene uses four alternative promoters, in conjunction with splicing, to produce different mRNAs, each encoding a unique 5'-UTR leader sequence, but encoding for identical proteins (44–46). Similarly, the flavin-containing monooxygenase family (\textit{FMO1} and \textit{FMO2}) utilizes alternative promoters and splicing for tissue-dependent expression (47, 48). The UDP-glucuronosyltransferase gene differentially uses six nested promoters, resulting in transcripts containing a unique exon 1, but common exons 2–4. Family 19 (\textit{CYP19}) of the cytochrome P450 gene superfamily uses numerous different promoters to generate numerous untranslated first exons in a tissue-specific fashion (49). A common splice junction located upstream of the start of translation is used in all splicing events. Thus, the coding regions of the transcripts (and hence the protein) are identical regardless of the tissue site of expression. What differs (in a tissue-specific fashion) for \textit{CYP19} and \textit{\gamma}-glutamytransferase transcripts is the 5'-end ter-

\textbf{FIG. 3.} Nucleotide sequence of the 5'-flanking region to the first exon 1B. The horizontal arrows mark the region used to construct the heterologous luciferase expression vectors. The mRNA initiation site as determined by RACE from a normal liver Marathon cDNA library is marked with a vertical arrow. Nucleotides transcribed in the first exon 1B are italicized. Potential transcription and regulatory sites of high homology (100% core similarity and 95% matrix similarity) are \textit{underlined}. \textit{HNF-A}, histone nuclear factor-A; \textit{EpRE}, electrophile response element; \textit{C/EBP}\textsubscript{\alpha}, \textit{CAAT/enhancer-binding protein-\alpha}.
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MGST1 is similar to γ-glutamyltransferase and CYP19 in that there are several different mRNA transcripts with different 5′-UTR ends, all spliced at a common site just upstream of the start codon to yield an identical protein. Tissue-specific DNase I-hypersensitive sites are often associated with specific promoters in select tissue (50, 51). It remains to be determined for MGST1 whether tissue-specific DNase I-hypersensitive sites exist.

The finding that MGST1 utilizes alternative splicing alleviates a theoretical problem noted with the first description of the MGST1 cDNA structure (30). This first description of MGST1 cDNA (30) described only the alternative exon 1D (Fig. 1), which contained several unusual features, and these present a theoretical problem with regard to expression. The first feature was the presence of an upstream TGA stop codon in frame with the initiating ATG codon, and this eliminated the possibility of a cleavable signal peptide sequence directing membrane localization. There was also an out-of-frame ATG codon upstream of the initiating ATG codon, which should result in expression of a heptadecapeptide (30). According to the scanning model for eukaryotic translation initiation (52, 53), such an arrangement should depress translation initiation at the authentic ATG codon. To alleviate the inhibition expected in MGST1 synthesis due to the heptadecapeptide open reading frame, it was postulated that the upstream termination codon (from the authentic ATG start codon) would need to be used for production of a mini-cistron that began with another ATG farther upstream. This would allow for ribosomal reinitiation at the downstream (or correct) start codon (54). Our subsequent work on the structural organization of human MGST1 failed, however, to identify such an upstream codon. Thus, how MGST1 attained such a high expression, in violation of the ribosomal scanning model, was an unexplained quandary. Our identification of the original first exon 1D as a minor alternate exon, located downstream of the correct MGST1 promoter region, eliminates this problem.

The use of alternative promoters and splicing has precedence as a mechanism for regulation of tissue-specific transcription. Alternative mRNA splicing can lead to tissue-specific patterns of gene expression by generating multiple forms of mRNA to produce different protein products with distinct functions and regulation. Alternative splicing can also function as an on/off switch. In one mode, it can produce a functional protein; but in an alternative mode, it can produce a mRNA that lacks an open reading frame (41, 55, 56) or, as described here, produce an alternate exon (such as exon 1B) in which translation would be suppressed due to the presence of upstream alternative start or stop codons. The extent to which human MGST1 utilizes this process to control expression in different tissues and during fetal development will need to be defined in future experiments.

MGST1 has been considered to be a “housekeeping” gene because most inducers of cytosolic GSTs such as phenobarbital have a minimal effect on MGST1 activity. The exceptions were with pro-oxidants such as 2-(3)-terbutyl-4-hydroxyanisole (25) and phorone (diisopropylidineacetone) (27). Indeed, it was previously noted that MGST1 displays non-selemium glutathione-dependent peroxidase activity toward a variety of lipid and fatty acid hydroperoxides and lipid peroxidation products (19–22). Some oxidized phospholipids (linoleoylpalmitylphosphatidylincholine hydroperoxides and cholesterol linolate hydroperoxides) are substrates for MGST1, but not for cytosolic GSTs (19–22). Thus, MGST1 demonstrates hydroperoxide-reducing activity similar to that noted with glutathione peroxidase enzymes. GPX1 displays transcriptional up-regulation upon exposure to paraquat (57). In retrospect, it is not surprising that MGST1 is also capable of transcriptionally responding to paraquat or menadione as we demonstrated here. Indeed, our finding that MGST1 can respond transcriptionally to exogenous agents may explain (in part) the alterations in MGST1 previously noted upon exposure to butylated hydroxyanisole, cobalt chloride, t-butyl hydroperoxide, and naphthyl isocyanate (25, 28, 58). This ability of MGST1 to respond transcriptionally may also explain the hormonal dependence and variation of MGST1 previously noted (59).

In summary, our findings define the promoter region of MGST1 that, interestingly, responds to oxidative stress. Thus, further insights into genes that are regulated by oxidative stress are now possible. MGST1 appears unique in its ability to respond to stress at both the gene and protein levels (activation).

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