# Isolation and characterization of two mouse Pi-class glutathione S-transferase genes

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Pi-class glutathione S-transferases (GSTs) play an important role in the detoxification of chemical toxins and mutagens and are implicated in neoplastic development and drug resistance. In all species characterized to date, only one functional Pi-class GST gene has been described. In this report we have identified two actively transcribed murine Pi-class GST genes, Gst p-1 and Gst p-2. The coding regions of Gst p-1 and the mouse Pi-class GST cDNA (GST-II) reported by Hatayama, Satoh and Satoh (1990) (Nucleic Acids Res. **18**, 4606) are identical, whereas Gst p-2 encodes a protein that has not been described previously. The two genes are approximately 3 kb long and contain seven exons interrupted by six introns. In addition to a TATA box and a sequence motif matching the phorbol-ester-responsive element, the promoters of Gst p-1 and Gst p-2 exhibit one and two G+C boxes (GGGCGG) respectively. The cDNAs of the two genes were isolated from total liver RNA using reverse PCR. The peptide sequences deduced from the cDNAs share 97% identity and differ in six amino acids. Both genes are transcribed at significantly higher levels in male mouse liver than in female, and Gst p-1 mRNA is more abundant in both sexes than Gst p-2.

## INTRODUCTION

The cytosolic glutathione S-transferases (GSTs) form a family of dimeric enzymes which exhibit a number of catalytic activities, including the conjugation of glutathione to lipophilic electrophiles (Coles and Ketterer, 1990). On the basis of nucleotide and amino acid sequence similarities, mammalian cytosolic GSTs can be grouped into four subfamilies designated Alpha, Mu, Pi and Theta (Mannervik et al., 1985, 1992; Meyer et al., 1991). The Pi subfamily has attracted considerable attention following the observation by Sato and co-workers that the Pi-class GST enzyme is markedly elevated in rat liver tumour development (Sato et al., 1984a,b).

In the early stages of rat liver carcinogenesis, single cells and foci appear that exhibit a different phenotype from that of normal hepatocytes; these foci are believed to represent precursors of tumours. One of the most reliable markers for the identification of such (pre-)neoplastic foci is the Pi-class enzyme, GST-P (Sato, 1989). This protein is essentially absent from normal hepatocytes, whereas it is abundant in foci- or nodule-bearing livers. GST-P is expressed not only in foci or nodules induced by chemicals but also in spontaneously occurring hepatic (pre-)neoplastic lesions, without administration of any exogenous carcinogens (Sawaki et al., 1990).

In the human, there are several reports demonstrating a significant increase in the expression of the Pi-class enzyme, GST-$\pi$ (GSTP1-1), in a number of tumours including oral, colon, stomach and lung (Sato, 1989; Howie et al., 1990; Black and Wolf, 1991; Volm et al., 1991; Hirata et al., 1992). However, GST-$\pi$ levels are not increased in hepatocellular carcinomas (Hayes et al., 1991). In addition, Pi-class GST is expressed at elevated levels in cell lines made resistant to anticancer drugs and to chemical toxins in culture (Batist et al., 1986; Cowan et al., 1986; Wolf et al., 1990; Black and Wolf, 1991). Therefore, studying the function and regulation of Pi-class GSTs will lead to a better understanding of the mechanisms underlying carcinogenesis and drug resistance.

Studies in the mouse are of particular importance, as this species is genetically well characterized, and advances in transgenic and gene-targeting technology provide powerful tools for investigating gene regulation and function. Furthermore, the mouse GST Pi gene, unlike its rat counterpart is expressed constitutively in hepatocytes and is also subject to regulation by both hormones (Hatayama et al., 1986) and exogenous agents (McLellan and Hayes, 1989). In order to examine these GSTs, we have isolated and characterized two actively transcribed mouse Pi-class GST genes, as well as their corresponding cDNAs. We have also investigated relative hepatic mRNA levels of the two genes in male and female mice.

## MATERIALS AND METHODS

### Chemicals and reagents

Unless otherwise indicated, all chemicals were purchased from either Sigma Chemical Co., Poole, Dorset, U.K. or BDH, Glasgow, U.K., and were of analytical grade or better.

### Screening of the mouse genomic library

An adult Balb/c mouse genomic library in $\lambda$ EMBL3 purchased from Clontech (Palo Alto, CA, U.S.A.) was screened using standard protocols (Benton and Davis, 1977). A 2.2 kb DNA fragment (MPCR2.2), generated by PCR, was radioactively labelled with [$\alpha$-$^{32}$P]dCTP (3000 Ci/mmol) by the method of Rigby et al. (1977), and used as a probe. MPCR2.2 was obtained using the oligonucleotides 5′-TGCCACCATACACCATTGTC-3′

Abbreviations used: GST, glutathione S-transferase; PMA, phorbol 12-myristate 13-acetate ('TPA'); PRE, PMA-responsive element; AMV, avian myeloblastosis virus.

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The mouse Pi-class GST nucleotide sequences, as well as the corresponding amino acid sequences, will appear in the GenBank/EMBL/DDBJ under the accession nos. X76143 and X76144.

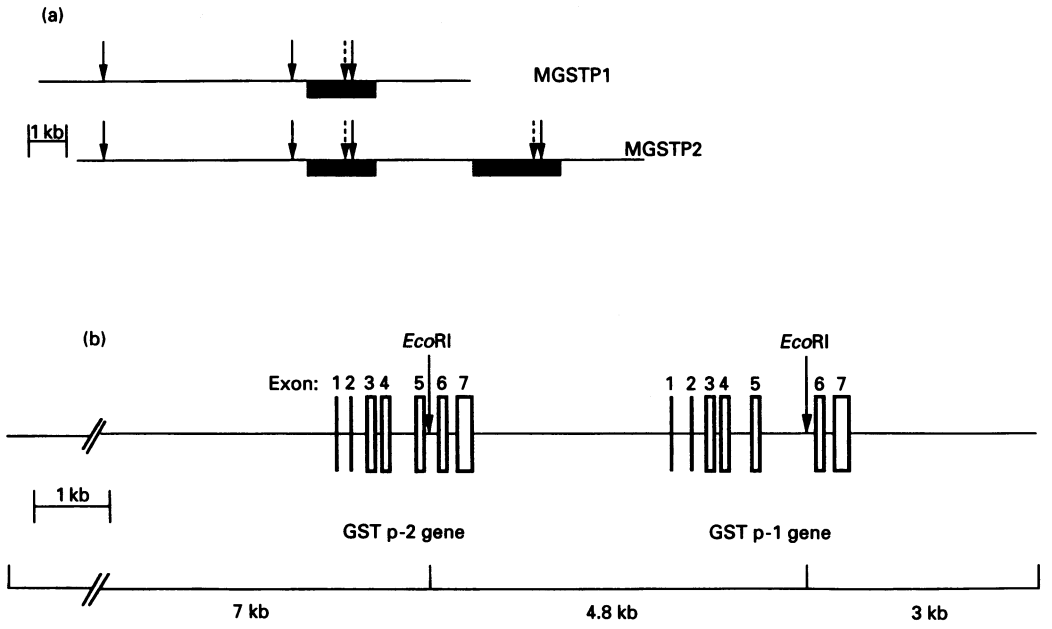


**Figure 1 Organization of the murine GST Pi locus**

(a) Restriction maps of the phage clones MGSTP1 and MGSTP2, containing one and two mouse Pi-class GST genes respectively. Solid and dashed arrows represent *Hin*dIII and *Eco*RI sites respectively; solid boxes indicate regions complementary to the probe MPCR2.2. (b) Restriction map and intron/exon arrangement of the two mouse Pi-class GST genes, Gst p-1, and Gst p-2, as contained in clone MGSTP2. Exons are shown as boxes numbered 1–7. Intron sizes were determined by DNA sequencing.

and 5′-TTTATTAGTGCTGGGAAAAC-3′ which hybridize to the 5′ end of exon 2 and to the 3′ end of exon 7 of the mouse gene, Gst p-1. The following temperatures/reaction times were used on a PHC-2 thermal cycler (Techne, Cambridge, U.K.): 2 min at 94 °C, followed by 30 cycles of 96 °C, 15 s; 60 °C, 1 min; 72 °C, 2.5 min. Hybridization was carried out in buffer containing 1 M NaCl, 50 mM Tris/HCl, pH 8.0, 10 mM EDTA, 5× Denhardt's solution [1 × Denhardt's = 0.02% BSA, 0.02% polyvinylpyrrolidone, 0.02% Ficoll], 0.1% SDS, 50 μg/ml denatured herring sperm DNA and $^{32}$P-labelled DNA probe MPCR2.2 at approx. $1 \times 10^6$ c.p.m./ml, for 16 h at 65 °C. The Hybond-N membranes (Amersham International plc, Amersham, Bucks., U.K.) were washed twice in 2 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M trisodium citrate)/0.1% SDS at room temperature and then twice in 0.2 × SSC/0.1% SDS at 65 °C, for 30 min on each occasion, before being exposed to Kodak XAR-5 autoradiograph film at −70 °C. Positive clones were plaque purified, and DNA was prepared by the method of Grossberger (1987). Inserts were excised from the clones by the restriction endonuclease *Sal*I, and separately digested with *Eco*RI and *Hin*dIII. The DNA fragments were individually cloned into the plasmid pGEM3Zf(+) (Promega, Madison, WI, U.S.A.).

## Southern blot analysis

Balb/c mouse liver DNA was prepared by standard methods (Sambrook et al., 1989), and 20 μg was digested to completion with the restriction enzyme *Eco*RI, and subjected to electrophoresis in a 0.8% agarose gel. After alkali treatment and neutralization, the DNA was transferred to a Hybond-N membrane (Amersham International) according to the manufacturer's instructions. Hybridization was carried out as described for the plaque hybridization. The membrane was washed twice in 2 × SSC/0.1% SDS at room temperature and then twice in 0.1 × SSC/0.1% SDS at 65 °C, for 30 min each time, before being exposed to autoradiographic film as described above.

## Isolation of total RNA

Total RNA was isolated from the liver of adult male and female Balb/c mice fed *ad libitum* on standard laboratory diet by the method of Chomczynski and Sacchi (1987).

## Reverse PCR

### Isolation of the mouse Pi-class cDNAs

Total male mouse liver RNA (2 μg) was reverse-transcribed in a total volume of 20 μl using 1 × PCR buffer (Promega), 2 mM of each dNTP, 1 unit of RNasin (Promega), 100 pmol of oligo(dT) and 24 units of avian-myeloblastosis-virus reverse transcriptase (Boehringer, Mannheim, Germany). After 10 min at room temperature, the samples were incubated for 60 min at 42 °C. In order to denature the RNA–cDNA hybrids and to inactivate the reverse transcriptase, the reaction mixture was heated to 95 °C for 5 min and then cooled on ice. Then 80 μl of 1 × PCR buffer containing 50 pmol each of upstream and downstream primer and 2.5 units of *Taq* polymerase (Promega) were added. Then 35 cycles were performed as follows: 20 s denaturing at 96 °C, annealing for 1 min at 60 °C, and primer extension for 1 min at 72 °C. The primer pairs used for the PCR reactions were 5′-CCTCACTCTGAGTACCCCTCTGTCTACG-3′ and 5′-TTTATTAGTGCTGGGAAAAC-3′ for Gst p-1, and 5′-CCATCCCTGAGACACCTCTCTGACTATT-3′ and 5′-TTTATTAGTGCTGGGAAAAC-3′ for Gst p-2.

### mRNA levels of the mouse Pi-class GST genes

Reverse PCR was carried out as described above with the exceptions that 1 μg of total RNA and 100 units of Moloney

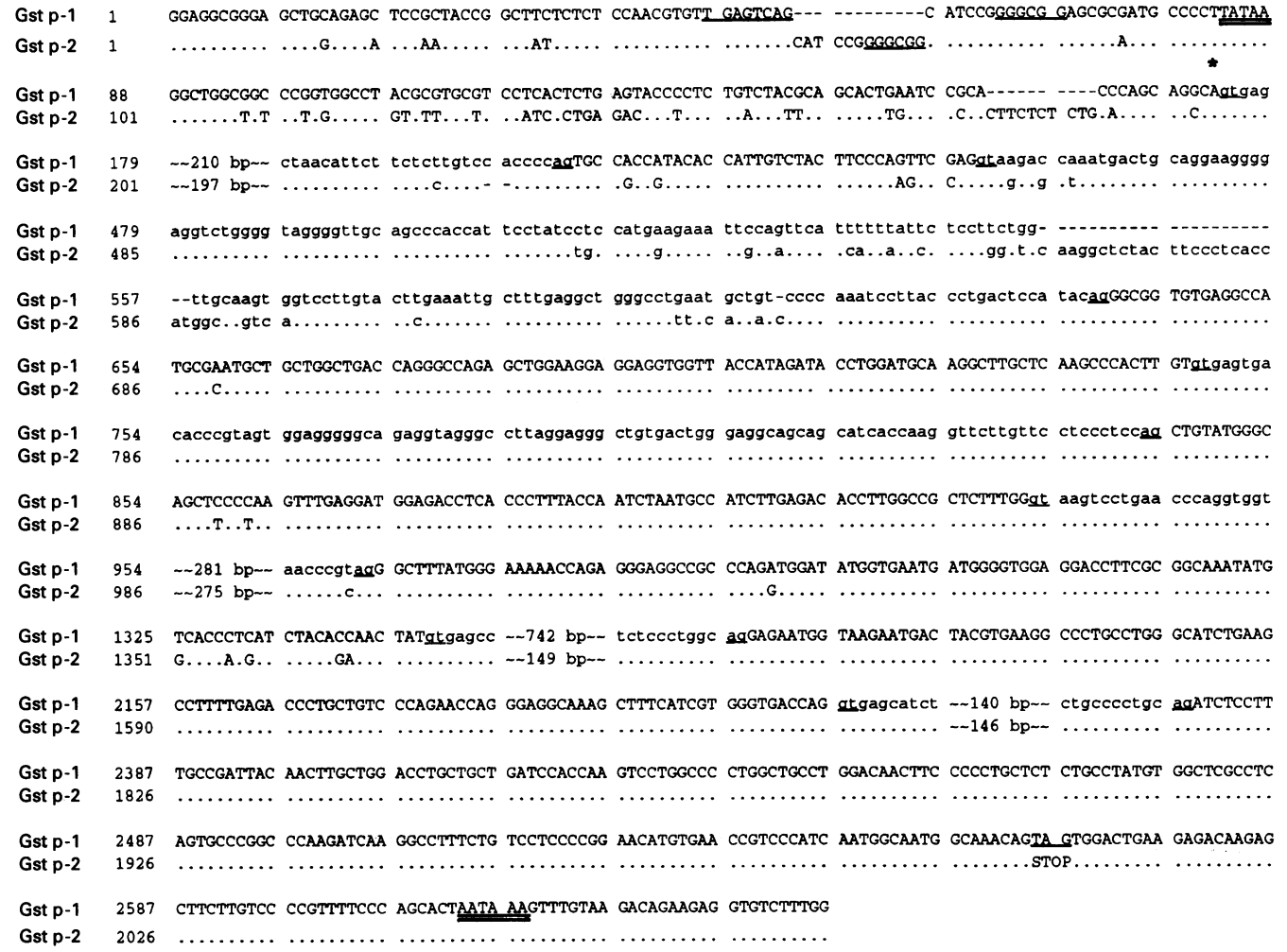


**Figure 2 Nucleotide sequences of the mouse Pi-class GST genes, Gst p-1 and Gst p-2**

The nucleotide sequence of gene Gst p-1 is shown with substitutions in gene Gst p-2 indicated below. Intronic sequences are presented in lower case. Dashes indicate gaps inserted in the sequences to optimize the similarity between the two genes. The TATA box and the polyadenylation signal are double, and the GC boxes, the phorbol-ester-responsive-element motif, the GT/AG splice sites and the stop codon are single underlined. An asterisk indicates the translational start site.

murine leukaemia virus reverse transcriptase (Gibco/BRL, Paisley, U.K.) were used instead of 24 units of AMV reverse transcriptase (Boehringer) per reaction.

In order to assess whether the two pairs of oligonucleotides differ in their affinity toward their target DNA, we compared the yields of the following PCRs. Known amounts (0.1 ng) of the subcloned cDNAs, Gst p-1 or Gst p-2, were used separately together with the corresponding primer pairs, and 10, 15, 20, 25 and 30 PCR cycles were performed. Equal amounts of the two PCR products (Gst p-1 and Gst p-2 cDNAs), generated by the same number of cycles, were electrophoresed on an agarose gel, visualized with ethidium bromide and yields were compared. No difference was detected (results not shown), indicating that the two pairs of oligonucleotides do not vary in their ability to anneal to their target DNA.

Various numbers of PCR cycles (10, 15, 20, 25, 30) were performed to determine conditions under which the template DNA represents the limiting factor in the amplification reaction, so that the generation of the PCR product is directly proportional to the template present. In the experiment shown in Figure 4, this was the case for cycle numbers 10, 15 and 20, 25 for Gst p-1 and Gst p-2. The assay was carried out in triplicate and each experiment gave identical results.

### DNA sequencing

Three *Eco*RI (3 kb, 4.8 kb and 7 kb) and two overlapping *Hin*dIII (1.4 kb and 4.8 kb) restriction fragments of clone MGSTP2, which each hybridized to the MPCR2.2 probe, were cloned into the plasmid pGEM3Zf(+). Sequence data were determined by the dideoxy chain termination method of Sanger et al. (1977). All of the sequence data presented were determined from both strands of DNA.

## RESULTS AND DISCUSSION

### Isolation of genomic clones

The amino acid sequences deduced from the mouse Pi-class GST cDNA reported by Hatayama et al. (1990) and the rat Pi-class

**Table 1 Sequence identities of the mouse Gst p-1 gene with mouse Gst p-2, rat GST-P and human GST-π genes**

The values given in parentheses represent identity comparison with the mouse Gst p-1 sequence. The sequences for rat GST-P and human GST-π (GST P1–1) were taken from Okuda et al. (1987) and Marrow et al. (1989) repectively.

| | Number of nucleotides (bp) | | | |
|---|---|---|---|---|
| | Gst p-1 | Gst p-2 | GST-P | GST-π |
| Exon 1* | 1 | 1 (100%) | 1 (100%) | 1 (100%) |
| Intron 1 | 242 | 226 (80%) | 209 (69%) | 288 (38%) |
| Exon 2 | 36 | 36 (86%) | 36 (94%) | 36 (86%) |
| Intron 2 | 188 | 212 (75%) | 185 (60%) | 293 (36%) |
| Exon 3 | 107 | 107 (99%) | 107 (92%) | 107 (78%) |
| Intron 3 | 98 | 98 (100%) | 101 (83%) | 114 (48%) |
| Exon 4 | 88 | 88 (98%) | 88 (94%) | 88 (81%) |
| Intron 4 | 312 | 306 (94%) | 329 (75%) | 365 (40%) |
| Exon 5 | 104 | 104 (93%) | 104 (88%) | 104 (85%) |
| Intron 5 | 761 | 168 (14%)† | 716 (75%) | 862 (37%) |
| Exon 6 | 108 | 108 (100%) | 108 (96%) | 108 (87%) |
| Intron 6 | 162 | 168 (96%) | 156 (80%) | 177 (54%) |
| Exon 7* | 186 | 186 (100%) | 186 (95%) | 186 (85%) |

* Only the number of coding nucleotides of exons are given.
† The low identity is due to the different intron sizes of Gst p-1 and Gst p-2

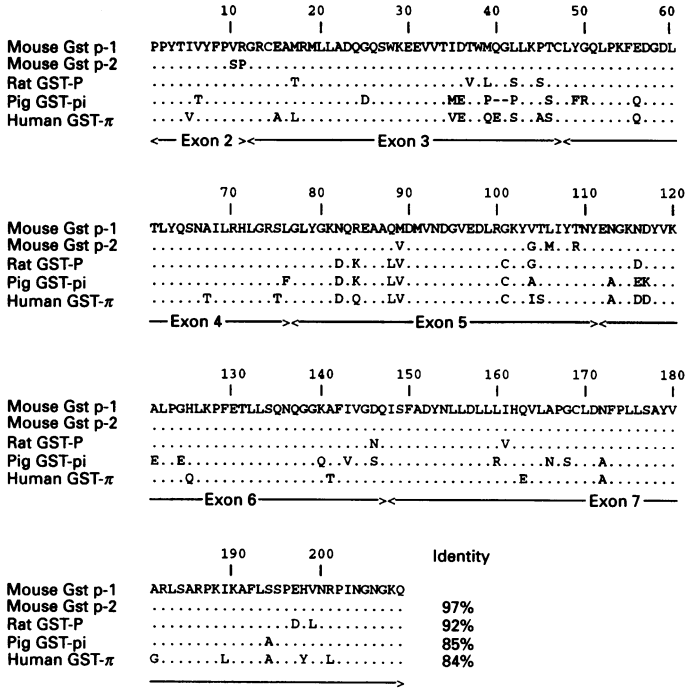


**Figure 3 Alignment of amino acid sequences of mammalian Pi-class GSTs**

The Pi-class GST peptide sequences of rat GST-P, pig GST-pi and human GST-π were taken from Okuda et al. (1987), Dirr et al. (1991) and Kano et al. (1987). Dots represent amino acids identical with those in mouse Gst p-1. Dashes indicate gaps inserted in the sequences to optimize the similarity between the sequences. The percentage identities between mouse Gst p-1 and other Pi-class GSTs are shown in the last column. Exons corresponding to peptide sequences are indicated for the mouse, the rat and the human sequences only, as the exon/intron junctions of the pig Pi-class GST have not been determined.

gene, GST-P (Okuda et al., 1987), share 92% identity. On the basis of the conserved intron/exon organization of homologous mammalian genes, we predicted the intron/exon boundaries of the mouse Pi-class gene from the rat gene sequence reported by Okuda et al. (1987). A pair of oligonucleotides to sequences in exon 2 and 7 were then used to amplify part of the mouse Pi-class GST gene using PCR (Saiki and Gelfand, 1989). Exon 1 was omitted as it only contains the A of the ATG initiation codon. An approx 2.2 kb PCR product (MPCR2.2) was obtained which was cloned into the plasmid pGEM3Zf(+) and partially sequenced. The sequence over the coding region was identical with the murine Pi-class cDNA described by Hatayama et al. (1990). This clone, MPCR2.2, was then used as a probe to screen a mouse Balb/c liver genomic library in λEMBL3. Four positive clones were isolated, three of which were identical and represented MGSTP2. The two distinct clones, MGSTP1 and MGSTP2, contained inserts of approx. 12 kb and approx. 15 kb respectively. The restriction maps of these clones are shown in Figure 1(a). The maps also show the region that hybridized to MPCR2.2.

## Sequence analysis of clone MGSTP2

The approximately 15 kb insert of clone MGSTP2 was digested separately with the endonucleases *Eco* RI and *Hin* dIII to generate smaller overlapping DNA fragments which were individually cloned into the plasmid pGEM3Zf(+). Restriction mapping, hybridization analysis and DNA sequencing showed that clone MGSTP2 contains two closely related genes, Gst p-1 and Gst p-2, which have not been described previously (Figures 1b and 2). They are adjacent and only separated by about 3 kb of intervening DNA. The coding regions of Gst p-1 and Gst p-2 share 100% and 98% identity with the sequence of the mouse Pi-class cDNA reported by Hatayama et al. (1990). The positions of splice junctions were deduced by comparison of the nucleotide sequence of the genes with that of the murine Pi-class cDNA reported previously (Hatayama et al., 1990). Both genes were sequenced completely and shown to contain seven exons interrupted by six introns. Gst p-1 is about 2.7 kb in length, whereas Gst p-2 is about 2.1 kb long because of a smaller-sized intron 5 (Table 1).

The nucleotide sequence at all splice junctions is consistent with the canonical GT/AG rule of Breathnach and Chambon (1981), indicating that the exonic segments within each gene transcript can be spliced accurately to form functional mRNA. The polyadenylation signal, AATAAA, occurs in the seventh exon of the two genes, which is further evidence that both gene transcripts can be processed to mature mRNAs.

Each gene possesses an open reading frame of 630 nucleotides encoding a protein of 210 amino acids including the initiator methionine. The calculated molecular masses of the proteins encoded by Gst p-1 and Gst p-2 are 23.6 kDa and 23.5 kDa respectively. As found in the rat and human homologues, the start codon, ATG, is split by the first intron in both mouse genes (Figure 2). The coding nucleotide sequences of the two genes differ in 14 positions (Figure 2); five of these substitutions are neutral and the remaining nine result in the replacement of six amino acids (Figure 3). The substitutions at positions 10 and 11 allow the protein encoded by Gst p-1 to be identified as the major Pi form purified from mouse liver (Phillips and Mantle, 1993).

Two of the amino acid differences, namely $Met^{89}$/Val, and $Leu^{106}$/Met, are conservative (Miyata et al., 1979). The crystal structure of porcine Pi-class GST (Reinemer et al., 1991), which shares 85% and 83% identity with Gst p-1 and Gst p-2 respectively, suggests that the non-conservative changes, $Arg^{11}$/Pro, $Val^{104}$/Gly and $Thr^{109}$/Arg are distant from the active site. $Val^{104}$ is not conserved in mammalian Pi-class GSTs isolated so far (rat, pig and human; Figure 3), which indicates that the amino acid at position 104 is not critical for enzyme activity. However, the Ser for Val difference at the tenth amino acid may be of significance, as $Val^{10}$ is conserved in all Pi-class GSTs

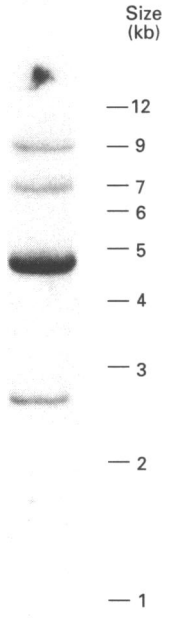


**Figure 4 Complexity of the murine GST pi-gene cluster**

Genomic DNA (20 $\mu$g) was digested with *Eco*RI and separated on a 0.8% agarose gel. After transfer to a Hybond-N filter (Amersham), hybridization was carried out with a $^{32}$P-labelled MPCR2.2 probe using the conditions described in the Materials and methods section. The approx. 4.8 kb *Eco*RI fragment gives the strongest hybridization signal, because it contains parts of both Pi genes (see Figure 1), whereas the approx. 2.5 kb, approx. 4.8 kb, and approx. 9 kb fragments contain only a part of one of the pi genes.

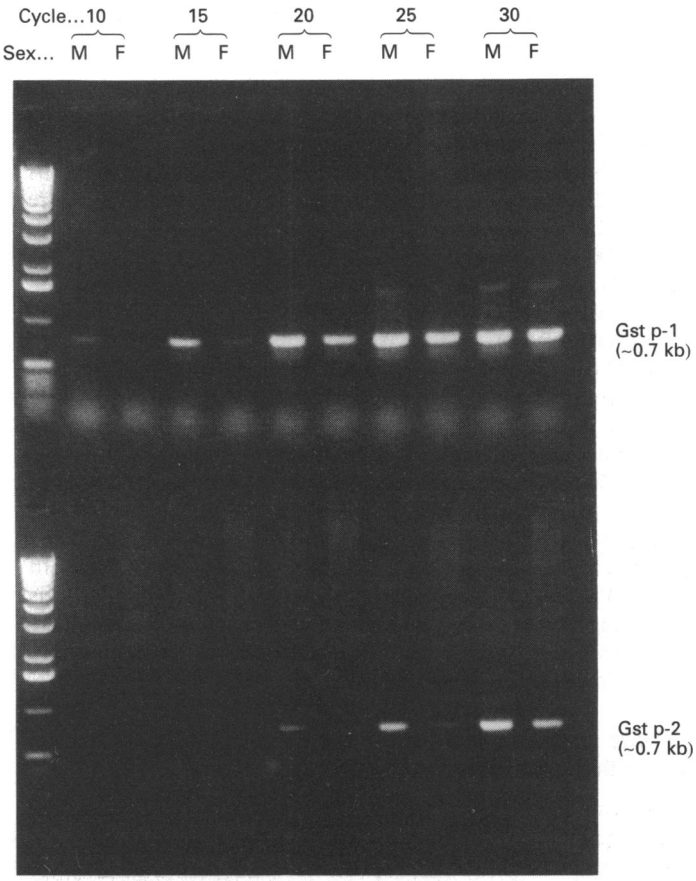


**Figure 5 Relative levels of Gst p-1 and Gst p-2 mRNA in male and female mouse liver**

Reverse PCR (10, 15, 20, 25, 30 cycles) was carried out using total mouse liver RNA to assess the relative expression of Gst p-1 and Gst p-2 in male (M) and female (F) mice as described in the Materials and methods section: 20 $\mu$l of the PCR product was subjected to electrophoresis in a 0.8% agarose gel and visualized with ethidium bromide. The first, unlabelled, lane contained size markers.

isolated to date, with the exception of murine Gst p-2 (Figure 3). Reinemer et al. (1991) proposed three possible locations in the active site to which the electrophilic substrate binds (H-site). One of them implicates the participation of amino acid Val$^{10}$. The catalytic activities of Gst p-1 and Gst p-2 remain to be characterized.

## Analysis of the promoter regions

A TATAA sequence and a possible CCAAT element (CCAAC) can be found in the 5′ flanking regions of both genes (Figure 2). These elements, which are present in most eukaryotic promoters, are associated with the accurate initiation and promotion of transcription (Maniatis et al., 1987). Furthermore, a GC box, which precisely matches the consensus sequence 5′-(G/T)GGGCGG(G/A)(G/A)(C/T)-3′ for the binding site of transcription factor SP1, forms part of the promoter of the two genes (Kadonaga et al., 1986). Gst p-2 has an additional GC box, 5′-GGGGCGGCAT-3′, which differs from a perfect SP1-binding site in only one nucleotide, and is missing in Gst p-1. Immediately upstream of the GC boxes in both genes is the sequence 5′-TGAGTCAG-3′ which corresponds to the consensus sequence found in promoters of genes that are responsive to phorbol esters [e.g. phorbol 12-myristate 13-acetate (PMA; 'TPA')]. This motif has been called a PMA-responsive element (PRE). In comparison, the human and the rat Pi-class genes also possess two and one GC boxes and a PRE (Cowell et al., 1988; Okuda et al., 1987). Muramatsu and co-workers have shown that a PRE is involved in the high level of transcription of GST-P observed during rat hepatocarcinogenesis (Diccianni et al., 1992). In addition, the 5′ untranslated regions of both genes exhibit various substitutions and deletions. The overall differences between the 5′ non-coding regions of the two mouse genes suggest that they are regulated differently. Comparison of the intron sequences of Gst p-1 with Gst p-2, rat GST-P and human GST-$\pi$ shows that all the introns have diverged to a similar extent from each other indicating that a specific intron does not play an important role in the regulation of the genes (Table 1).

## Southern-blot analysis

The number of Pi-class GST genes in the mouse genome was estimated by Southern-blot analysis. Figure 4 shows that four *Eco* RI fragments of mouse DNA, approx. 2.5 kb, approx. 4.8 kb, approx. 6.8 kb and approx. 9 kb in size, hybridize to probe MPCR2.2. On the basis of the restriction map of clone MGSTP2 (Figure 1b), this suggests that there are at least three Pi-class GST-related sequences in the mouse genome. Clone MGSTP2 accommodates three of these hybridizing fragments, approx. 4.8 kb, approx. 6.8 kb and approx. 9 kb in length. However, the approx. 2.5 kb band is not present in any of the positive clones isolated. This fragment requires further characterization to establish whether it is a pseudogene or a further functional Pi-class Gst gene. Pi-class GST pseudogenes have been found in rat and human (Okuda et al., 1987; Board, 1993).

### Evidence for transcription of the mouse Pi-class GST genes Gst p-1 and Gst p-2

In order to assess whether the two mouse Pi-class GST genes are transcribed correctly, we attempted to clone the corresponding cDNAs by reverse PCR. The nucleotide sequences of the Gst p-1 and Gst p-2 gene are identical in the 3′ untranslated regions (Figure 2). However, differences in the 5′ non-coding regions are sufficient to allow the design of two pairs of oligonucleotides (5′-CCTCACTCTGAGTACCCCTCTGTCTACG-3′ and 5′-TTTATTAGTGCTGGGAAAAC-3′ for Gsp p-1, and 5′-CCATCCCTGAGACACCTCTCTGACTATT-3′ and 5′-TTTATTAGTGCTGGGAAAAC-3′ for Gst p-2) which are specific for each gene. These oligonucleotides were used together with total liver RNA from male mice to perform reverse PCR. This procedure resulted in two DNA fragments of approx. 700 bp. The two fragments were cloned individually into the plasmid vector pGEM3Zf(+) and sequenced. As anticipated, these PCR fragments represented the two mouse Pi-class cDNAs, with coding regions identical with those found in the genes, Gst p-1 and Gst p-2. The isolation of these cDNA clones clearly shows that both mouse Pi-class GST genes are transcribed. This observation is intriguing in view of the finding that only one active Pi-class GST gene has been reported in both human and rat (Okuda et al., 1987; Cowell et al., 1988; Board et al., 1993).

### Relative mRNA levels of Gst p-1 and Gst p-2 in male and female mouse liver

Using the same oligonucleotides that enabled us to amplify and isolate the Gst p-1 and Gst p-2 cDNAs, we developed a reverse PCR assay to estimate the expression levels of the two genes in mouse liver. Conditions under which reverse PCR was performed were identical for each reaction and as described in the Materials and methods section. When a primer pair was used together with the non-compatible subcloned Gst p cDNA as a template, no PCR product was generated (results not shown), demonstrating that the primer pairs were specific for the amplification of the corresponding cDNA. Using this analysis procedure, we found that both of the murine Pi-class GST genes are expressed at significantly higher levels in male compared with female liver (Figure 5). Moreover, mRNA encoding Gst p-1 is much more abundant than that of Gst p-2 in both male and female mouse liver (Figure 5). It remains to be established whether the two homologous proteins encoded by Gst p-1 and Gst p-2 have similar substrate specificities. This work is currently in progress.

### Conclusions

Unlike other species characterized to date, the mouse contains at least two functional Pi-class GST genes. These genes appear to have certain regulatory characteristics common to previously reported Pi-class GST genes, in that both of their promoters contain a PRE. The proteins encoded by these genes differ in six amino acids. On the basis of data obtained from the crystal structure of porcine Pi-class GST, the replacement of $Ser^{10}$ by Val is most likely to cause a difference in the enzymic activity. Further work will establish the relative tissue distribution, substrate specificity and mode of regulation of these two proteins, and whether they have common or distinct functions.

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