Genomic organization, 5′-flanking region and chromosomal localization of the human glutathione transferase A4 gene

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We have isolated and characterized a human glutathione transferase A4 (*hGSTA4*) subunit gene from a yeast artificial chromosome containing several other glutathione transferase alpha genes and pseudogenes. The homodimeric protein hGSTA4-4, is involved in the detoxification of 4-hydroxynonenal and other reactive electrophiles produced by oxidative metabolism, and may have a significant role in protecting intracellular components from oxidative damage. The *hGSTA4* gene spans nearly 18 kb, contains seven exons, maps onto chromosome 6p12, and lies in close proximity to the *7SK small nuclear RNA* gene in a head-to-tail orientation. The intron/exon borders conform to the standard rules, an open reading frame is present beginning at position 154 in exon 2, and the stop codon is at position 822 in exon 7. The transcription initiation site has been determined by primer extension analysis and is located 135 bp upstream of intron 1. Isolation and sequencing of the *hGSTA4* gene 5′-flanking region revealed it to be devoid of TATA or CCAAT boxes but it does contain an initiator element overlapping the transcription start site, a GC box and putative binding sites for transcription factors AP1, STAT, GATA1 and NF-κB. Reverse transcription-PCR analysis revealed that hGSTA4 mRNA was present in all the tissues tested, although in low amounts, suggesting that this subunit may be ubiquitously expressed.

INTRODUCTION

The cytosolic glutathione transferases (GSTs) form a supergene family of dimeric enzymes, which in humans is comprised of different multigene families referred to as alpha, mu, pi, theta, zeta and kappa [1–4]. These enzymes catalyse the conjugation reaction between reduced glutathione and a variety of electrophiles including carcinogens [1]. The alpha class GSTs are comprised of several genes and pseudogenes. To date, three distinct GST alpha genes coding for the hGSTA1, hGSTA2, and hGSTA3 subunits respectively have been isolated and characterized [5–8]. Several alpha class pseudogenes have also been identified [5,8–10]. *In situ* hybridization with GST alpha cDNA has demonstrated a high level of signal over band p12 of chromosome 6 [11] suggesting that this gene family is clustered in a small region. We isolated yeast artificial chromosome (YAC) clones that contained GST alpha genes and demonstrated the presence of several sequences that hybridized with a GST alpha gene probe within a 680 kb human genomic DNA fragment [9].

Additional alpha class subunits designated as hGSTA4 were isolated from various human tissues [12–14]. It has been suggested that this enzyme may have evolved for the detoxification of 4-hydroxynonenal and related endogenous electrophiles [12]. Thus the homodimeric protein hGSTA4-4 may play a significant role in the protection of intracellular components against the oxidative damage associated with aging, atherosclerosis, cataract formation, Parkinson's disease and Alzheimer disease, as well as other degenerative human diseases. The presence of several isoforms corresponding to this subunit seems to indicate the existence of multiple related genes in humans. However, to date, a single cDNA clone coding for a subunit *hGSTA4* has been recently isolated [15,16]. In the present study, we report on the isolation and the characterization of a gene encoding an alpha subunit of the human GST. The gene spans 18 kb and comprises seven exons separated by six introns. Nucleotide exon sequence analysis indicates that this sequence is equivalent to the cDNA clones, *hGSTA4*, isolated by Hubatsch et al. [15] and Board [16].

MATERIAL AND METHODS

Screening of a YAC library and construction of a phage sublibrary

A YAC clone containing a 680 kb human genomic DNA fragment (ICRFy900DO1128) was isolated from an Imperial Cancer Research Fund (ICRF) human YAC library [9]. This clone was identified from an early subset of the ICRF YAC library present on filter replicas [17] and contains GST alpha sequences as determined by hybridization with a *hGSTA1* cDNA probe. A phage sublibrary was constructed from the YAC ICRFy900DO1128 clone using a Lambda GEM12 vector [18]. Phage clones (2.2×10^4) were screened with total human DNA and 106 clones were purified. To facilitate clone identification, the clones were gridded and filter replicas of the matrix were then used for further analysis.

cDNA synthesis and phage library screening

A cDNA probe corresponding to the *hGSTA4* sequence [15,16] was generated by reverse transcription-PCR (RT-PCR) using total RNA from human liver. Oligonucleotides Exon 1 Forward

Abbreviations used: A, alpha; GST, glutathione transferase; RT-PCR, reverse transcription-PCR; YAC, yeast artificial chromosome.

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The nucleotide sequence data reported will appear in the Genbank Nucleotide Sequence Databases under the accession numbers AF052051, AF050054–AF050059.

Table 1 Oligonucleotide primers used for sequencing and PCR

Name and location	Sequence
Exon 1 Forward	5′-CGC TGA CCT GGC GCT TTG TG-3′
Exon 1 Reverse	5′-CGG AGG CCT GGA GCC GCA-3′
Exon 2 Forward	5′-TCC CAA CGG AAG AGG CCG GA-3′
Exon 2 Reverse	5′-GCT AAA ACC CAT CTC ACG GAC TC-3′
Exon 3 Forward	5′-GAT GAA GAA TTT CTG GAA AC-3′
Exon 3 Reverse	5′-CAT CCT GCA ACT TGT ACA AC-3′
Exon 4 Forward	5′-GTA ACC ACC TGC TGT TCC AA-3′
Exon 4 Reverse	5′-AAG AGA TTG TGC TTG TCT GC-3′
Exon 5 Forward	5′-CAT GTA CGT GGA GGG GAC AC-3′
Exon 5 Reverse	5′-GAT CAT CTG GTT TTA AGA AA-3′
Exon 6 Forward	5′-AAG GGG TCA CGG ACA AAG CT-3′
Exon 6 Reverse	5′-TGC AGA CAG GAT ATT AGG AA-3′
Exon 7 Forward	5′-ATT AAG AGA TTC CTT GAA CCT GGC A-3′
Exon 7 Reverse	5′-TGG CCT AAA GAT GTT GTA GAC GG-3′

and Exon 7 Reverse served as primers for the PCR (Table 1). The cDNA was used as a radiolabelled probe for screening the 106 clones. The replica filters containing the 106 clones were pre-hybridized in a solution containing 0.5 M phosphate buffer (pH 7.2)/7 % (w/v) SDS/1 mM EDTA/1 % (w/v) BSA, and then hybridized overnight in the same solution containing the ^{32}P-labelled probe.

Determination of the gene structure and sequencing

To identify fragments containing exons, DNAs were digested with restriction enzymes, separated on a 1 % (w/v) agarose gel and transferred to a Hybond-N+ membrane (Amersham) with 0.4 M NaOH. Hybridizations with exon specific probes were carried out in the hybridization mixture as described in the screening procedure. Intron sizes were determined by restriction endonuclease mapping and PCR. Oligonucleotides which served as primers for the PCR are described in Table 1. Sequencing was performed on subcloned DNA fragments, or directly on phage clones, according to the method described by Sanger et al. [19].

Primer extension analysis

An 18-mer oligonucleotide primer (Exon 1 Reverse), located at the 3′-end of exon 1, was end-labelled with [γ-^{32}P]ATP, using T4 polynucleotide kinase, and hybridized to 30 μg of total RNA from liver. The primer was extended with AMV reverse transcriptase at 42 °C for 60 min and the reaction was terminated by incubation with RNase A. The extension products were treated with phenol/chloroform, ethanol-precipitated and analysed on a 6 % (w/v) acrylamide/7 M urea gel. A sequencing reaction was loaded on the same gel to determine the exact site of transcription initiation.

Fluorescence *in situ* hybridization

In order to map the *hGSTA4* gene, a fluorescence *in situ* hybridization was performed using the λ73 clone as a probe. The DNA was labelled with biotin-16-dUTP (Boehringer Mannheim) by nick-translation, hybridized to metaphase chromosomes prepared from normal blood lymphocytes, and detected with fluorescein isothiocyanate-avidin [20,21].

Expression analysis

The human Multiple Tissue cDNA panel I (MTC™ from Clontech) was used to determine the tissues expressing hGSTA4 mRNA. This panel is a set of normalized first-strand cDNAs, generated using polyadenylated (poly A$^+$) RNA from different tissues. For each reaction 1 ng of cDNA prepared from brain, heart, kidney, lung, liver, pancreas, placenta and skeletal muscle respectively was used for PCR amplification with *hGSTA4*-specific primers (Exon 1 Forward and Exon 7 Reverse) and Taq DNA polymerase (Life Technology). PCR cycling parameters were: 38 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 90 s, and an additional 10 min at 72 °C for the final extension.

RESULTS

Structure of the *hGSTA4* gene

In a previous study, we isolated a YAC clone containing a 680 kb human genomic DNA fragment (ICRFy900DO1128) from an ICRF human YAC library [9]. A phage sublibrary was constructed from this clone using a Lambda GEM12 vector and 106 clones were purified after screening with total human genomic DNA. Among these 106 recombinant phages, we isolated several clones that contained sequences encoding *hGSTA1*, *hGSTA2*, *hGSTA3* and several GST alpha pseudogenes (results not shown).

A *hGSTA4* cDNA probe was synthesized by RT-PCR using total RNA from human liver. This *hGSTA4* sequence was used as a probe to screen the YAC phage sublibrary, revealing four positive clones called λ6, λ16, λ31 and λ73. Restriction maps of these clones are shown in Figure 1. The gene spans 18 kb and, like other human GST alpha genes, contains seven exons separated by six introns. All exons and exon/intron junctions were sequenced and are presented in Figure 2. The coding exons show 62 % similarity to *hGSTA1* and *hGSTA2* cDNAs and 65 % to mouse and rat *GSTA4*. The rules for the intron/exon junctions are fulfilled for all exons and an open reading frame is present starting at position 154 in exon 2, encoding for the first 29 amino acids of the hGSTA4 subunit. The sizes of the exons and introns are listed in Table 2. Exon 7 encodes amino acids 183 to 222 and encompasses the 3′-untranslated region of the corresponding mRNA. The polyadenylation site, which begins at 451 bp downstream of the stop codon, is of the common type AATAAA.

Characterization of the transcription initiation site

Primer extension analysis was performed to determine the transcription start site(s) of the human *hGSTA4* gene. An 18-mer oligonucleotide primer, complementary to the 3′-end of the *hGSTA4* exon 1, was radiolabelled and hybridized to total RNA from liver. The primer was extended with reverse transcriptase and the product was analysed on a sequencing gel, using a

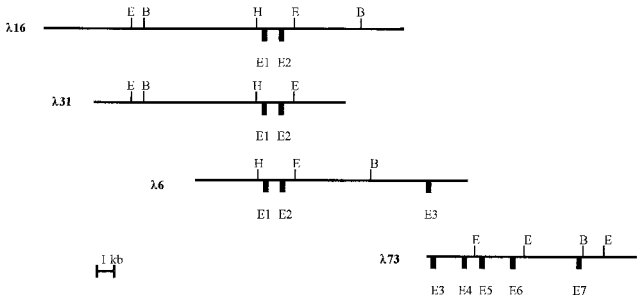

Figure 1 Restriction map of the λ6, λ16, λ31 and λ73 clones

Exons are denoted by black boxes, introns by thick dark lines. Restriction sites for the enzymes are shown as follows: E, *Eco*RI; B, *Bam*HI; H, *Hind*III.

Figure 2 Exon–intron organization of the *hGSTA4* structural gene

The DNA sequence of the *hGSTA4* exons was determined as described in the Materials and methods section. The initiation codon, ATG and the polyadenylation signals are boxed and the stop codon is underlined. Sequences of exons are shown in capital letters. Amino acid sequences are shown using the one-letter code.

sequence ladder of genomic DNA obtained with the same downstream primer that was used to generate the probe (Figure 3). The length of the major extended product was determined to be 123

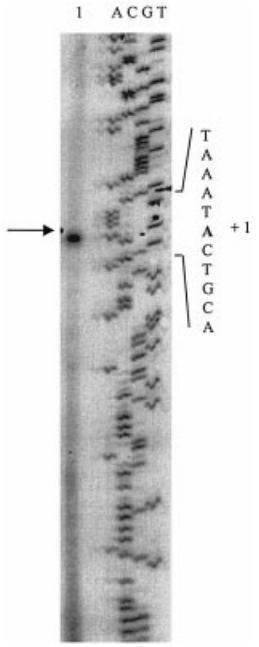

Figure 3 Primer extension analysis of the *hGSTA4* gene

An 18-mer oligonucleotide primer (Exon 1 reverse) located at the 3′-end of exon 1 was end-labelled and hybridized to 30 μg of total RNA from human liver. The annealed template/primer was extended with reverse transcriptase and the product was analysed on a 6% (w/v) acrylamide/7 M urea denaturing gel (lane 1). The arrow denotes the potential transcription start site. Marker lanes A, C, G and T indicate sequencing ladders of the human *hGSTA4* gene using the same primer.

nucleotides indicating that the adenine residue marked +1 in Figure 4 may be the transcription start site.

Sequence analysis of the 5′-flanking region

The phage clones λ6, λ16 and λ31 contain sequences upstream of exon 1. Figure 4 shows 2319 bp of this 5′-flanking region. Searches of GenBank/EMBL DNA data showed that the human *7SK small nuclear RNA* gene lay upstream of the *hGSTA4* exon 1 in a head-to-tail orientation. Moreover, a CA dinucleotide repeat sequence was found 1734 bp upstream of the *hGSTA4* transcription start site. The putative promoter region upstream of the start site possesses neither CAAT nor TATA boxes, but

Table 2 Size of exons and introns in the human *GSTA4* gene

The sizes of all exons were determined by DNA sequence analysis. The approximate sizes of introns were determined by restriction endonuclease mapping and by PCR.

	Exon (bp)							Intron (kb)						
Gene	1	2	3	4	5	6	7	1	2	3	4	5	6	Reference
GSTA4	135	105	52	133	142	132	582	1	7	1.85	0.8	1.8	3.85	
GSTA1	36	117	52	133	142	132	198	4.5	1.6	1.3	2.0	1.1	1.0	[7]
GSTA2	36	117	52	133	142	132	317	4.1	1.7	1.1	2.0	1.3	1.0	[6]
GSTA2	36	117	52	133	142	132	317	5.6	1.6	1.2	2.0	1.2	0.85	[5]
GSTA3		117	52	133	142	132	317		2.5	1.3	2.4	2.1	1.2	[8]

A.

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-2319  CCAGGTTCAAGCGATTCTCCCGCCTCAGCCTCCTGAATAGCTGGGACTACAGGAACAGGCCACCACGCCCGACTA
-2244  ATTTTGTATTTTTAGTGGAAACAGGGTTTCACCATGTTGGCCTCGCTGGTCTTGAACTCCTGACCTCAGGTGATC
-2169  CGCCCGCCTCAGCCTCTCAAAGTGCTGGGATTACAGGCGTGAGCCACTGCGCCCAGCCCTCAATGGCTTTTTCAA
-2094  CTGTCTAGTGGGTATTTCCAGCTGGATGGTTCATAGCAACAAAAGACAAGTCAACCCATTTCCCCAAACTTTGTC
-2019  TTCCTTCTCCGCTCTTTATGGAACTAGAAACCAAAGCATTGCTCATTCATTCATTCAACAAGTGTTCTCTGAATA
-1944  CCTACTCTATACTAACCAGGATGTACAAAAGGAAAACCAAAAGCGCAAAAATCCTCGATCTCGTGGAATTTACAT
-1869  TCTAGGAATCTACAGACAAATAAATAAAAAAGTCCTGTTAGATGACAATACATGTTATTAAAAAAAAGACAAGAA
-1794  GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTTGGACAGGGCATCATTC
-1719  TAGACTTTTCTTCCTCCTCCCTTCCCTTTGATCTGATTAGCAATTGCTTTACCACCTACCTCCTTAGTATTTCAC
-1644  CTTTTCTTACTCATATTCTTCAAACTCATGGTGCTACTTTCCTTTCCACAGACACAAGAGGTTTCTTTCTCTACC
       .............................827 base pairs................................
-742   TGTAATCCCAGCTACTCGGGAGGCTGAGTCAGGACAATCGCTTGAACCCGGGAGGTGGAGGTTACAGTGAGCCGA
-667   GATCCGTCTCAAAAATAAAAAATAAATAATGAAAAAAATTAAAAATTTAAAAATTAAAAAACATTTAAAAAAGCA
-592   TCTAACTTAGATGGGTAATGGGTCAAAAGAAAGGCAGACTGCCACATGCAGCGCCTCATTTGGATGTGTCTGGAG
-517   TCTTGGAAGCTTGACTACCCTACGTTCTCCTACAAATGGACCTTGAGAGCTTGTTTGGAGGTTCTAGCAGGGGAG
-442   CGCAGCTACTCGTATACCCTTGACCGAAGACCGGTCCTCCTCTATCGGGGATGGTCGTCCTCTTCGACCGAGCGC
                                                              NFκB
-367   GCAGCTTCGGGAGGGACGCACATGGAGCGGTGAGGGAGGAAGGGGACACCCGCCTAGCCAGCCAGATCAGCCGAA
                            AP1
-292   TCAACCCTGGCGATCAATGGGGTGACAGATGTCGCAGCCAGATCGCCCTCACATCCGAGGTACCCAAGCGGCCCA
-217   CAAGCTATATAAACCTGAAGGAAGTCTCAACTTTACACTTAGGTCAAGTTGCTTATCGTACTAGAGCTTCAGCAG
                                                        STAT               GATA
-142   GAAATTTAACTAAAATCTAATTTAACCAGCATAGCAAATATCATTTATTCCCAAAATGCTAAAGTTTGAGATAAA
                                                                         +1
                              AP1                          Sp1           Inr
-67    CGGACTTGATTTCCGGCTGTTTGACACTATCCAGAATGCCTTGCAGATGGGTGGGGCATGCTAAATACTGCAGT
                              Exon 1
+9     CTCCATTGGTGAGGTCGTCCCGGAGCCTCGCCCAGCTCCCGCGCGCTAGAGCCGCCGCCTGCTGGTCTCACCCAG
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B.

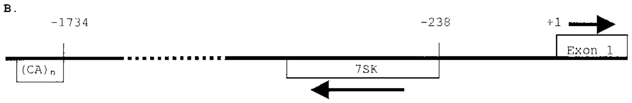

Figure 4 5′-flanking region of the *hGSTA4* gene

(**A**) Nucleotide sequence (approx. 2.3 kb) upstream of exon 1 of the *hGSTA4* gene. The putative transcription initiation site for *hGSTA4* is marked as +1. Underlines show transcription factor recognition sequences. Dashed underlines show the localization of the *7SK small nuclear RNA* gene present on the complementary strand. The CA repeat sequence is boxed. Inr, initiator element. (**B**) Schematic diagram showing localization of the *hGSTA4* exon 1, *7SK small nuclear RNA* gene and CA repeat. Arrows indicate the direction of transcription.

contains a sequence which has some similarity to the initiator sequence (YYANT/AYY) overlapping the transcription initiation site [22]. A potential binding site for the transcription factor Sp1 has been identified with the sequence 5′-TGGGTGGGGC-3′ in positions −10 to −19, which resembles the consensus sequence 5′-(G/T)GGGCGG(G/A)(G/A)(C/T)-3′ [23]. Upstream of this region, the sequence revealed putative binding sites for transcription factors AP1, GATA1, STAT and NFκB.

Chromosomal localization of *hGSTA4*

The human *GSTA4* gene was mapped to 6p12 by fluorescence *in situ* hybridization using clone λ73 as a probe. A representation of the data is shown on Figure 5. This experiment, as well as the presence of the *hGSTA4* gene in the YAC ICRFy900DO1128 clone, confirmed the location of the gene in the human GST alpha gene cluster. Moreover we clarified the exact location of the *7SK small nuclear RNA* gene on chromosome 6 band p12. In a previous study this gene was mapped to human chromosome 6

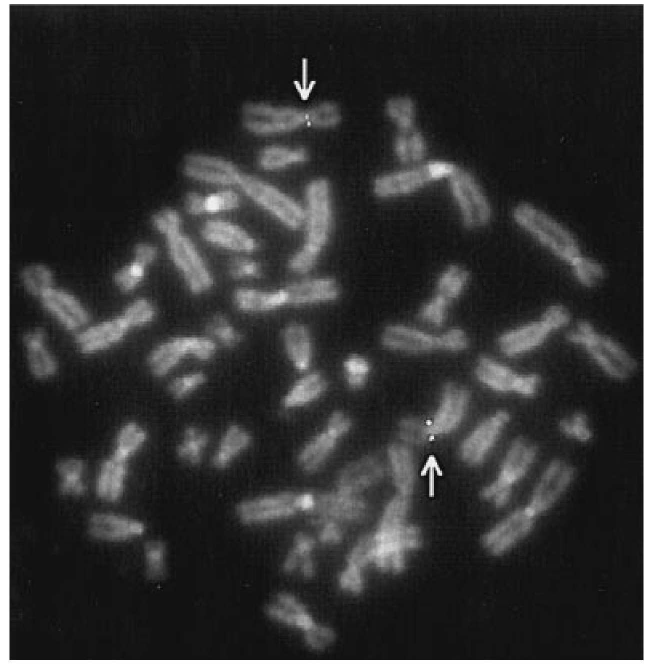

Figure 5 Fluorescence *in situ* hybridization analysis of *hGSTA4*

The arrows indicate the specific signal of *hGSTA4* DNA on the short arm of chromosome 6 on band p12.

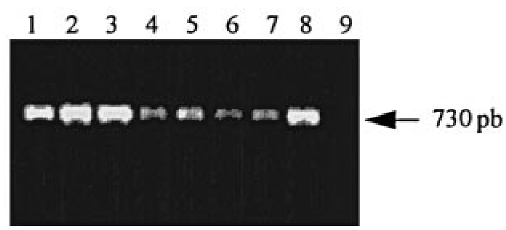

Figure 6 Expression of hGSTA4 mRNA in various tissues

The expression levels of hGSTA4 mRNAs in heart (lane 1), brain (lane 2), placenta (lane 3), lung (lane 4), liver (lane 5), skeletal muscle (lane 6), kidney (lane 7) or pancreas (lane 8) were estimated after 38 cycles of amplification by PCR. Lane 9 contains water instead of cDNA.

by analysing Northern blots derived from a panel of somatic cells that contained single human chromosomes [24].

Expression of hGSTA4 mRNA in human tissues

In order to determine the expression of hGSTA4 mRNA in various tissues, we screened a human tissue cDNA panel by using semiquantitative PCR. The hGSTA4 mRNA was found to be expressed in brain, pancreas, heart, liver, placenta, kidney, lung and skeletal muscle (Figure 6). At least 31 cycles of PCR amplification were necessary to detect the PCR product (730 bp), indicating that the hGSTA4 mRNA was present in low amounts in these tissues.

DISCUSSION

We have isolated a new gene encoding an alpha class GST subunit corresponding to hGSTA4. The corresponding cDNA has been cloned and the heterologous expression in *Escherichia*

coli showed that this enzyme possesses a high catalytic efficiency in the conjugation of 4-hydroxynonenal and other genotoxic products of lipid peroxidation [15,16]. The *hGSTA4* gene is approximately 18 kb in length and contains seven exons. The coding region of this gene shows 62 % similarity to the other alpha class subunit cDNAs and the deduced amino acid sequence shows 53 % similarity to the major GST alpha enzymes hGSTA1 and hGSTA2. We have isolated and characterized this gene from a YAC clone containing a 680 kb human genomic DNA fragment. Several other alpha GST genes (i.e. *hGSTA1*, *hGSTA2* and *hGSTA3*) and pseudogenes were present in the YAC clone thus indicating that the human alpha class GST locus, located on chromosome 6p12, contained at least four separated genes and several pseudogenes. *hGSTA1* and *hGSTA2* cDNAs were isolated previously and it has been shown that the two subunits share very similar sequences and differ in only 11 amino acids [11,25,26]. The corresponding genes have been isolated [5–7] and are both 12–13 kb in length. The *hGSTA3* gene and cDNA have also been isolated [8,16]. The deduced amino acid sequence of the *hGSTA3* gene shows 19 and 26 amino acid substitutions compared to *hGSTA1* and *hGSTA2* respectively.

Previously, a human GSTA4 (named hGST5.8) was isolated from liver, heart, pancreas, brain and ocular tissues by an immunoadsorption chromatography procedure using antibodies raised against the mouse protein [12–14]. This hGSTA4 enzyme isolated from different tissues showed high specific activity for 4-hydroxynonenal and other lipid peroxidation products. Partial sequencing of cyanogen bromide-peptides of hGSTA4 proteins, isolated from different tissues, revealed the existence of several distinct isoforms differing in their primary structures, suggesting that there could be several closely related genes encoding hGSTA4 subunits. However, it is worth noting that the cDNA corresponding to the gene we isolated differs in terms of amino acid sequence compared to all peptide sequences obtained by Singhal et al. [13]. Further analysis will be necessary to characterize and localize genes encoding other hGSTA4 subunits.

Using human-liver total RNA, we determined the transcription start site by primer extension and showed a major band located 135 bp upstream of intron 1. Sequence analysis of the 5′-flanking region upstream of the transcription start site of the *hGSTA4* gene revealed that this gene lacks a TATA box and a CAAT sequence at the usual positions. Nevertheless, a sequence present around the putative transcription start site (+1) has some similarity to an initiator element. This initiator sequence has been proposed to function as an alternative transcription factor IID-binding site in genes lacking a TATA box [22]. An Sp1 site, which is important for the initiator activity, is present upstream (−10 to −19) of the transcription start site. The very low levels of hGSTA4 mRNA detected in the various tissues analysed by RT-PCR are in accordance with the fact that initiator-containing TATA-less promoters slowly produce continuous transcripts [27]. Moreover weak promoter activity was demonstrated when a 630 bp fragment (from −510 to +120), recombined with a promoterless luciferase vector, was transfected into HepG2 cells (results not shown). The promoter also contains putative binding sites for transcription factors AP1, GATA1, STAT and NFκB. Interestingly, comparison of the 5′-flanking region sequence with GenBank/EMBL DNA data showed that a human 7*SK small nuclear RNA* gene lies upstream of exon 1 of *hGSTA4* in a head-to-tail orientation. The *7SK small nuclear RNA* gene is highly conserved in mammals, exists as part of an abundant ribonucleoprotein [28] and is transcribed by RNA polymerase III [29]. While its function is as yet unknown, sequence and structural analyses suggest that 7SK small nuclear RNA is involved in pre-mRNA processing [28]. The human gene for *7SK small nuclear RNA* has been isolated and its regulation studied [30–34]. Although the exact localization of the sequence encoding this RNA was unknown, it had been mapped to human chromosome 6 by analysing Northern blots derived from a panel of somatic cells that contained single human chromosomes [24]. Finally we report on the existence of a 58 base purine–pyrimidine dinucleotide repeat (CA repeat) 1737 bp upstream of the *hGSTA4* transcription start site.

In conclusion, we herein demonstrate that the human *hGSTA4* gene is comprised of seven exons and spans nearly 18 kb. This gene corresponds to a recently isolated cDNA [15,16] and its coding region shows 62 % similarity to the other human alpha class subunit cDNAs. Analysis of the 5′-flanking region shows that this gene is TATA-less but possesses several putative binding sites. Further studies are required to determine whether *hGSTA4* expression is inducible by exogenous and/or endogenous compounds, and whether variation in expression could result in interindividual differences in the susceptibility to oxidative damage.

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REFERENCES

1 Hayes, J. D. and Pulford, D. J. (1995) Crit. Rev. Biochem. Mol. Biol. **30**, 445–600
2 Pemble, S. E., Wardle, A. F. and Taylor, J. B. (1996) Biochem. J. **319**, 749–754
3 Board, P. G., Baker, R. T., Chelvanayagam, G. and Jermiin, L. S. (1997) Biochem. J. **328**, 929–935
4 Mannervik, B., Alin, P., Guthenberg, C., Jensson, H., Tahir, M. K., Warholm, M. and Jornvall, H. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 7202–7206
5 Klone, A., Hussnatter, R. and Sies, H. (1992) Biochem. J. **285**, 925–928
6 Rohrdanz, E., Nguyen, T. and Pickett, C. B. (1992) Arch. Biochem. Biophys. **298**, 747–752
7 Rozen, F., Nguyen, T. and Pickett, C. B. (1992) Arch. Biochem. Biophys. **292**, 589–593
8 Suzuki, T., Johnston, P. N. and Board, P. G. (1993) Genomics **18**, 680–686
9 Morel, F., Schulz, W. A. and Sies, H. (1994) Biol. Chem. Hoppe–Seyler **375**, 641–649
10 Lorper, M., Schulz, W. A., Morel, F., Warskulat, U. and Sies, H. (1996) Biol. Chem. Hoppe–Seyler **377**, 39–46
11 Board, P. G. and Webb, G. C. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 2377–2381
12 Singhal, S. S., Zimniak, P., Awasthi, S., Piper, J. T., He, N. G., Teng, J. I., Petersen, D. R. and Awasthi, Y. C. (1994) Arch. Biochem. Biophys. **311**, 242–250
13 Singhal, S. S., Zimniak, P., Sharma, R., Srivastava, S. K., Awasthi, S. and Awasthi, Y. C. (1994) Biochim. Biophys. Acta **1204**, 279–286
14 Singhal, S. S., Awasthi, S., Srivastava, S. K., Zimniak, P., Ansari, N. H. and Awasthi, Y. C. (1995) Invest. Ophthalmol. Visual Sci. **36**, 142–150
15 Hubatsch, I., Ridderstrom, M. and Mannervik, B. (1998) Biochem. J. **330**, 175–179
16 Board, P. G. (1998) Biochem. J. **330**, 827–831
17 Lehrach, C. T., Drmanac, R., Hoheisel, J., Larin, Z., Lennon, G., Monaco, A. P., Nizetic, D., Zehetner, G. and Poutska, A. (1990) in Genome Analysis, vol. 1, Genetic and Physical Mapping (Davies, K. E. and Tighman, S. M., eds.), pp. 39–81, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York
18 Frischauf, A. M., Lehrach, H., Poustka, A. and Murray, N. (1983) J. Mol. Biol. **170**, 827–842
19 Sanger, F., Nicklen, S. and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U.S.A. **74**, 5463–5467
20 Taiar, N., Qumsiyeh, M. B., Croteau, S., Rollet, J. and Benkhalifa, M. (1995) Ann. Genet. **38**, 102–105
21 Viegas-Pequignot, E., Dutrillaux, B., Magdelenat, H. and Coppey-Moisan, M. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 582–586
22 Smale, S. T., Schmidt, M. C., Berk, A. J. and Baltimore, D. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 4509–4513
23 Briggs, M. R., Kadonaga, J. T., Bell, S. P. and Tjian, R. (1986) Science **234**, 47–52
24 Driscoll, C. T., Darlington, G. J. and Maraia, R. J. (1994) Nucleic Acids Res. **22**, 722–725

25 Rhoads, D. M., Zarlengo, R. P. and Tu, C. P. (1987) Biochem. Biophys. Res. Commun. **145**, 474–481
26 Tu, C. P. and Qian, B. (1986) Biochem. Biophys. Res. Commun. **141**, 229–237
27 Yean, D. and Gralla, J. (1997) Mol. Cell. Biol. **17**, 3809–3816
28 Wassarman, D. A. and Steitz, J. A. (1991) Mol. Cell. Biol. **11**, 3432–3445
29 Zieve, G., Benecke, B. J. and Penman, S. (1977) Biochemistry **16**, 4520–4525
30 Moon, I. S. and Krause, M. O. (1991) DNA Cell Biol. **10**, 23–32
31 Murphy, S., Di Liegro, C. and Melli, M. (1987) Cell (Cambridge, Mass.) **51**, 81–87
32 Murphy, S., Tripodi, M. and Melli, M. (1986) Nucleic Acids Res. **14**, 9243–9260
33 Kleinert, H., Bredow, S. and Benecke, B. J. (1990) EMBO J. **9**, 711–718
34 Kleinert, H., Gladen, A., Geisler, M. and Benecke, B. J. (1988) J. Biol. Chem. **263**, 11511–11515

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