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Three Repeated Glutathione S-Transferase Genes From a Marine Fish, the Plaice (*Pleuronectes platessa*)

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ABSTRACT

*The cytosolic glutathione S-transferases (GSTs) catalyse the transfer of glutathione to a variety of xenobiotic and toxic endogenous compounds. This results in detoxification of the offending chemical, and the resulting conjugate is able to enter the organism's excretion pathways. The major GST of plaice (*Pleuronectes platessa*) liver, GSTA, is structurally related to mammalian theta class GSTs and also to GSTs from plants and insects. GST genes are known to be induced in animals and plants by a wide range of xenobiotic chemicals and by oxidative stress, and our interest is in the regulation of GST genes from plaice. Screening of a plaice genomic DNA library with GSTA cDNA resulted in the isolation of two overlapping clones. Analysis of these clones revealed the presence of the gene for GSTA, designated* GSTA, *and also two more putative genes for closely related GSTs, designated* GSTA1 *and* GSTA2. *The exon structures of the three GST genes are very similar and the predicted amino acid sequences show 60–70% homology. Promoter analysis of the regions upstream of* GSTA *and* GSTA1 *were shown to have activity in a turbot fibroblast cell line, but the region upstream of* GSTA2 *was inactive in this system. The promoter active regions of* GSTA *contain sequence elements which have been shown to respond to oxidative stress in mammals, and the regions upstream of* GSTA1 *contain oestrogen and peroxisomal proliferator response elements. Thus we have shown that these two closely related genes are physically close together in the plaice genome but we believe them to be under separate control and to respond to different signals and stressors. Copyright © 1996 Elsevier Science Ltd*

The cytosolic glutathione S-transferases (GSTs) are a diverse family of enzymes found in all Phyla examined to date. Four gene families have been identified in mammals and are classed as alpha, mu, pi and theta. Each of these classes contain several distinct but structurally homologous isoenzymes. Individual GST isoenzymes have quite broad substrate specificities but a number have been shown to be relatively specific for particular

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reactions including the conjugation of certain drugs, aflatoxins and toxic endogenous cellular metabolites, for example hydroxyalkenals and purine and pyrimidine base-alkenals (Mannervik *et al.*, 1985; Meyer *et al.*, 1991; Berhane *et al.*, 1994). Substrates for GSTs are believed to share a common property: they are, or can give rise to, potentially reactive electrophiles, and when applied to cells many have been shown to cause oxidative damage, either directly, or by interacting with cellular redox systems (Spenser *et al.*, 1991). In addition some mammalian alpha class GST genes have specific DNA sequences within their promoter regions which are required for transcriptional up-regulation in response to xenobiotic electrophiles, such as β-naphthoflavone, *tert*-butylhydroxyquinone, and also H_2O_2. These sequences have been described as antioxidant response elements (AREs; Rushmore *et al.*, 1991) or electrophile response elements (EpREs; Friling *et al.*, 1992).

Very much less is known about GSTs from lower vertebrates and invertebrates. The major liver cytosolic GST from plaice, GSTA, appears to be structurally homologous to mammalian theta class isoenzymes and can be induced by chemicals which elicit induction in mammalian alpha class GSTs (Leaver *et al.*, 1993). GSTA, or structurally related forms, are found in a wide range of plaice tissues and, indeed, in many other fish species, and as part of the cellular protection system may have important functions in the metabolism of xenobiotics and the conjugation of endogenously derived toxins (George, 1994).

In order to study the regulation of plaice GSTA, a genomic DNA library was constructed in λFIXII (Stratagene) from plaice DNA, and screened with radiolabelled GSTA cDNA using standard methods (Sambrook *et al.*, 1989). After restriction digestion, subcloning and sequencing, two of the clones isolated, λgGSTA3 and λgGSTA29, were shown to overlap and contain the gene for GSTA, *GSTA*, and two other closely related genes, designated *GSTA1* and *GSTA2* (Fig. 1). The predicted exonic regions of these three genes were highly homologous and contained no translational termination codons. Comparison of the deduced amino acid sequences with that of GSTA showed that the product of *GSTA* was 97% identical, *GSTA1* 76% identical and *GSTA2* 64% identical. There were no significant homologies between the intronic, intergenic and flanking regions. In addition, 14 nucleotide differences between the GSTA cDNA and the GSTA gene coding regions which gave rise to six amino acid substitutions were noted, and the possibility that this is due to genetic polymorphism requires investigation since our libraries have been prepared from fish of different populations.

The results of preliminary Northern blotting experiments with juvenile plaice indicate that GSTA1 mRNA is present at higher levels in intestine and kidney than liver, whereas GSTA mRNA, which has a similar transcript size of around 1200 nucleotides, is present at higher levels in liver than in other tissues (results not shown and Leaver *et al.*, 1993). GSTA2 mRNA does not appear to be expressed in any of the tissues tested so far.

Transcriptional start sites have yet to be mapped for *GSTA1* and *GSTA2*, but in plaice liver *GSTA* appears to be transcribed from 60 nucleotides upstream of its translation initiation codon (Leaver & George, 1995). Upstream and intergenic regions up to, but not including, the translation initiation codons for *GSTA*, *GSTA1* and *GSTA2* were subcloned into pCATBasic (Promega Corp, Fig. 1) and these constructs were transfected (by the calcium phospate co-precipitate technique) into TF cells, a cell line derived from turbot fin fibroblasts (George *et al.*, 1992). After exposure to the DNA/$CaPO_4$ precipitate for 16 h the cells were washed, fresh media was applied and, after a further 30 h, the cells were harvested. Chloramphenicol acetyltransferase was assayed with ^{14}C-labelled substrate, chlorampenicol metabolites separated by TLC plates and then quantitated by liquid

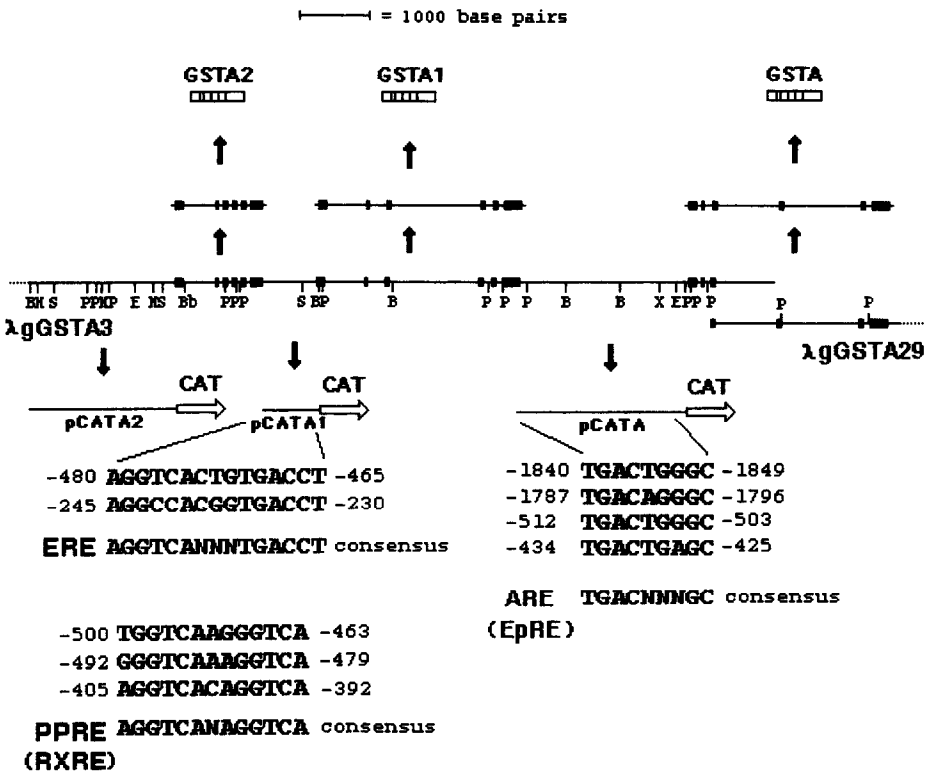

Fig. 1. Map of λgGSTA3 and λgGSTA29 clones containing plaice GST genes. Restriction sites are indicated: B, BamHI; Bb, BbuI; E, EcoRI; H, HindIII; P, PstI; S, SalI; and X, XbaI. Also indicated are the positions of putative exons and structures of transcripts (above map). The regions tested for promoter activity, by their ability to drive CAT expression, are indicated below the map and the sequence elements referred to in the text are given.

scintillation counting of the radioactive zones using standard techniques (Sambrook *et al.*, 1989). There was less than 10% variation within any treatment between triplicate transfections. The putative promoter regions for *GSTA* and *GSTA1* were active in this system as was a positive control construct containing SV40 promoter and enhancer elements (pCATControl, Promega Corp.) whereas the region upstream of *GSTA2* was inactive (Fig. 2). The *GSTA* promoter contains four consensus sequences for AREs (EpREs) and the *GSTA1* promoter contains consensus sequences for several elements recognized by peroxisomal proliferators (PPREs; Issemann & Green, 1990), as well as two oestrogen response elements (EREs; Martinez *et al.*, 1987). The region upstream of *GSTA2* contained no recognizable promoter elements.

Although the direct participation of these sequence elements in expression of the plaice GSTA gene family has yet to be tested, their presence is highly suggestive. First, it may be expected that GSTA is regulated in a similar fashion to mammalian alpha class GST genes, via a number of AREs, and may thus be inducible by a range of electrophilic xenobiotics. Second, the GSTA1 gene may be regulated by oestrogen and by peroxisome proliferators, a class of compounds which includes hypolipidemic agents such as fibrates and certain fatty acids. Therefore, although GSTA and the putative GSTA1 protein are

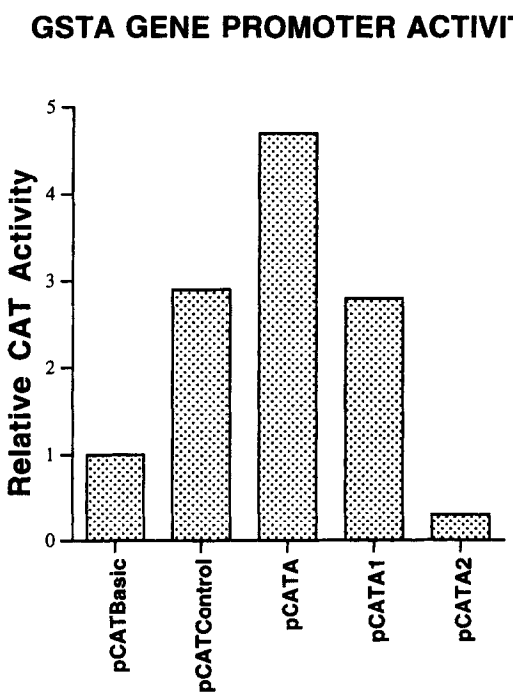

Fig. 2. Relative promoter activity of GSTA gene upstream regions. The upstream regions of *GSTA*, *GSTA1* and *GSTA2* were cloned into pCATBasic (pCATA, pCATA1 and pCATA2, respectively) and tested for their ability to drive CAT expression in a fish cell line. pCATControl is a positive control containing SV40 virus promoter and enhancer elements.

structurally very similar, it is likely that they are under distinct and separate transcriptional controls. GSTA may have a role in general cellular defence as indicated by the broad range of tissue expression and its potential for induction by electrophilic toxins via AREs. In contrast, the EREs in the promoter region of *GSTA1* suggest a role in sexually maturing female plaice and the PPREs as part of a battery of genes that, in mammals at least, include fatty acid metabolizing enzymes such as the microsomal cytochrome P4504A6 (Tugwood *et al.*, 1992) and peroxisomal acyl-CoA oxidase (Muerhoff *et al.*, 1992).

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