The organization of the human GSTP1-1 gene promoter and its response to retinoic acid and cellular redox status

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High levels of expression of GSTP1-1 are associated with cell proliferation, embryogenesis and malignancy. Given the role of glutathione S-transferase (GST) in detoxication, it is possible that GSTP1-1 evolved specifically to protect proliferating cells and share regulatory mechanisms with other cellular genes which are involved in cell division and tumorigenesis. We have previously shown that the expression of *GSTP1* is suppressed by retinoic acid (RA) in the presence of the retinoic acid receptor (RAR) as a result of decreased transcription from its promoter. Through deletion analysis, we show here that the RA–RAR-dependent repression is mediated by the region −73 to +8. Further mutation analysis of this region indicates that the DNA sequence required for RA–RAR-dependent repression co-localizes with a consensus activator protein-1 (AP1) site essential for the promoter activity. The degree of repression correlates with the residual activity of the AP1 site. There are two adjacent G/C boxes. The one immediately downstream from the AP1 site is not essential for the promoter activity, but mutation of the second, further downstream, impairs the promoter. On the other hand, mutation of either of these two G/C boxes has little effect on RA–RAR suppression. We also show that the expression of *GSTP1* is regulated by the redox status of the cell. Using the chloramphenicol acetyltransferase assay system, we have demonstrated that treatment with H_2O_2 induced transcription from the promoter and that this effect can be blocked by pre-incubation with *N*-acetylcysteine (NAC). It was shown that the induction by H_2O_2 is mediated by *trans*-acting factor NF-κB (nuclear factor κB), via a putative NF-κB site, 'GGGACCCTCC', located from −96 to −86. Co-transfection with an NF-κB (p65) expression construct increased the promoter activity, an effect which could be blocked by co-transfection with an IκB (MAD-3) expression construct. Deletion of the NF-κB site abolished the effect of both H_2O_2 and co-transfection of NF-κB. Interestingly, NAC is also an inducer for *GSTP1*. The effect of NAC was shown to be mediated largely by the AP1 site, since mutation of this site abolished the induction by NAC.

INTRODUCTION

Glutathione transferases (GSTs) (EC 2.5.1.18) are a group of phase II drug-metabolizing enzymes involved in the detoxication of both cytotoxic and genotoxic electrophiles and free radicals [1,2]. The cytosolic forms of GST subunits can be grouped into four evolutionary classes, namely alpha, mu, pi and theta [3,4].

GSTP1-1 (formerly GST π, [5]), the only member of the human pi class of GSTs, is developmentally regulated [6]. High levels of GSTP1-1 are observed in proliferating fetal tissues and in the proliferative zones of many mature epithelia. This evidence implies that GSTP1-1 is associated with cell proliferation [7,8]. The expression of GSTP1-1 is regulated during the cell cycle with the highest amounts found in G_2 and S phases [9]. The level of GSTP1-1 expression is also elevated in tumours from a wide range of human tissues, for example, cancers of colon, stomach, oesophagus, cervix and bladder [10,11]; the amount of GSTP1-1 correlating with the degree of de-differentiation and malignancy.

The precise role of GSTP1-1 is not yet understood. Like most GSTs, GSTP1-1, has broad substrate specificities. It is possible that GSTP1-1 has evolved specifically to protect proliferating cells. It might also be important in the regulation of the process of cell proliferation by inhibiting the effects of reactive oxygen intermediates (ROIs) on cell division. On the basis of the effects of the GSTP1-1 inhibitor ethacrynic acid, a recent publication has suggested that GSTP1-1 facilitates cell proliferation and inhibits apoptosis, hence allowing the expansion of a population of initiated tumour cells [12].

We have characterized the basal promoter of *GSTP1*, the gene encoding GSTP1-1 [13,14]. It includes a consensus activator protein-1 (AP1) binding site which is essential for promoter activity [15,16]. A positive *cis*-acting element has been observed between nucleotides +8 and +72. This region is integral to the promoter as its function is position- and orientation-dependent. Proteins bound to this region appear to be titratable in cell culture and the element competes for proteins which are important for the basal promoter activity [14]. Our recent studies suggest that retinoic acid (RA) represses, whilst insulin induces, the expression of GSTP1-1 [16]. Here, we present some evidence that the RA repression is mediated by the AP1 site and that cellular redox status also regulates the expression of *GSTP1*, via both the AP1 site and a nuclear factor κB (NF-κB) site further upstream.

EXPERIMENTS

Materials

All tissue-culture media and their ingredients were from Gibco BRL LifeTechnologies (Renfrewshire, U.K.). RA, insulin, H_2O_2 and *N*-acetylcysteine (NAC) were obtained from Sigma (Dorset, U.K.). Acetyl-CoA was supplied by Boehringer-Mannheim Biochemicals (Penzberg, Germany). [^{14}C]Chloramphenicol was purchased from DuPont (U.K.). Oligonucleotides were synthesised

Abbreviations used: GST, glutathione S-transferase; NAC, *N*-acetylcysteine; RA, retinoic acid; RAR, retinoic acid receptor; RXR, retinoid receptor; ROI, reactive oxygen intermediate; CAT, chloramphenicol acetyltransferase; DMEM, Dulbecco's modified Eagle's medium; RARE, retinoic acid regulatory element; PMA, phorbol 12-myristate 13-acetate; AP1, activator protein-1; NF-κB, nuclear factor κB; IκB, inhibitory subunit of NF-κB.

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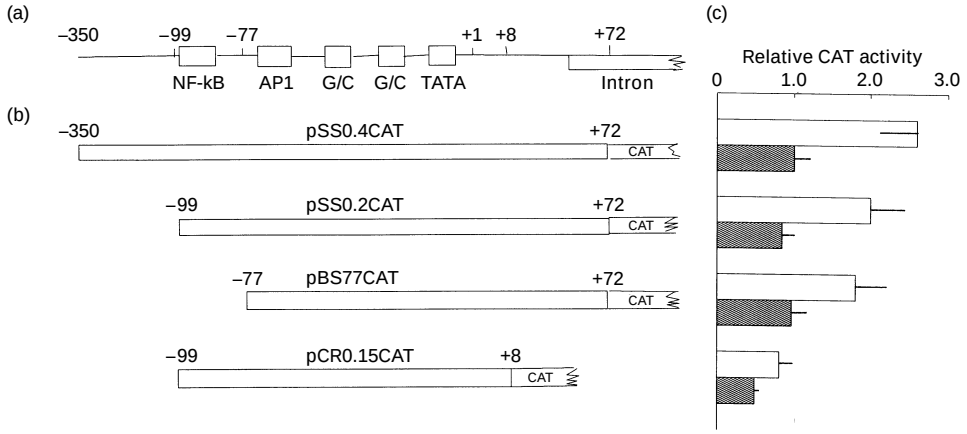

Figure 1 Transcription repression by RA in the presence of RAR

(**a**) Map of the *GSTP1* promoter, including an NF-κB site, an AP1 site, two G/C boxes and a TATA box. (**b**) Schematic view of the promoter–CAT constructs. (**c**) The effect of RA repression of the *GSTP1* promoter. HeLa cells co-transfected with hRARβ and pSS0.4CAT, pSS0.2CAT, pBS77CAT or pCR0.15CAT were treated with 1×10^{-6} M RA 16 h before harvesting, and CAT activities were measured. Each bar (▨, +RA; □, −RA) represents the mean of six to nine determinations.

by Oswel DNA Service (Edinburgh, Scotland, U.K.). The Transformer Site-Directed Mutagenesis kit was provided by CLONTECH (Cambridge, U.K.). Sequenase 2.0 sequencing kit was purchased from Cambridge BioScience (Cambridge, U.K.).

Cell culture

All media were supplemented with 10% (v/v) fetal-calf serum and 10 μg/ml gentamicin. Human bladder carcinoma cells (EJ), HeLa cells and human hepatoma cells (HepG2) were maintained in Dulbecco's modified Eagle's medium (DMEM). Human breast carcinoma cells (MCF7), a gift from Dr. P. Beverly (Imperial Cancer Research Fund, London, U.K.), were cultured in RPMI 1640 medium.

Plasmid constructs

Plasmid constructs pSS0.2CAT and pSS0.4CAT (where CAT is chloramphenicol acetyltransferase), which contain the promoter region of *GSTP1* (−99 to +72 and −350 to +72) respectively, have been described by Dixon et al. [15]. Plasmid pBS77CAT, which contains the promoter region from −77 to +72, and pCR0.15CAT, which contains the region −99 to +8, have been described by Xia et al. [14]. Plasmid pAP1M_nCAT and pGC_nM_nCAT were constructed using the Transformer Site-Directed Mutagenesis System with pSS0.2CAT as the template. The selection primer: 5′-GCTTCCTG**GGTACC**AGACAT-GAT-3′, complementary to the sequence from 3360 to 3384 bp of pSS0.2CAT, converted the unique *Bam*H1 site into a *Kpn*1 site. The mutagenic primer was designed to create each mutation:

pAP1M_1CAT, CAGTGCTGAGTCTCGGCGCCGGCC;
pAP1M_2CAT, CCCCAGTGCTGAGTAACGGCGCCG;
pAP1M_3CAT, CCCCAGTGCTGAGACACGGCGCCG;
pAP1M_4CAT, CCCCAGTGCTGATTCACGGCGCCG;
pAP1M_5CAT, CCCCAGTGCTGTGTCACGGCGCCG;
pAP1M_6CAT, CCCCAGTGCTTAGTCACGGCGCCG;
pAP1M_7CAT, CCCCAGTGCAGAGTCACGGCGCCG;
pGC_1M_1CAT, GCTCCGACCCAGTGCTGAGTC;
pGC_1M_2CAT, CCCCGCTCATCCCCAGTGCTGAGT;
pGC_2M_1CAT, CCCGACCCGCTCCGCCCCAGT;
pGC_2M_2CAT, TAAGGGTGGTCCATCCCCGCTCCG

Synthesis and selection of the mutant plasmids were carried out according to the manufacturer's instructions. Each plasmid was sequenced across the whole insert to verify the mutation.

The human retinoic acid receptor β expression vector, hRARβ, was a gift from Dr. P. Brickell (Department of Molecular Pathology, University College London, London, U.K.). Expression constructs for NF-κB (p65) and IκB (MAD3) were gifts from Professor Baeuerle (Institute of Biochemistry, University of Freiburg, Freiburg, Germany). pCH110, a β-galactosidase expression construct, was purchased from Pharmacia Biotech (Herts., U.K.). pSV_2CAT, a CAT construct which contains the SV40 promoter and enhancer, and pICCAT, a promoterless CAT construct, were prepared according to Dixon et al. [15].

Transfection and CAT assay

All plasmids used for transfection were purified by two successive CsCl density-gradient centrifugations. Cells were seeded at $2–8 \times 10^5$ cells/dish and transfected by the calcium phosphate co-precipitation technique [17] in DMEM 24 h later. Promoter–CAT constructs (5 μg), alone or with 1 μg of expression vectors for hRARβ, or NF-κB (p65) or IκB (IκBα/MAD3), were used in each transfection. Cells were exposed to RA, H_2O_2 or NAC, 18–24 h after transfection. In each case, 16 h later, cell extracts were prepared for CAT assays as described by Gorman et al. [17]. The efficiency of each transfection was assessed by co-transfection with pCH110 (1 μg).

RESULTS

RA–RAR-dependent repression of the *GSTP1* promoter is mediated by the AP1 site

We have previously shown that the expression of *GSTP1* is suppressed by RA in the presence of hRARβ as a result of decreased transcription from the promoter [14]. To characterize further the region involved in RA–RAR-mediated repression, *GSTP1* promoter–CAT constructs pSS0.4CAT (−350 to +72), pSS0.2CAT (−99 to +72), pBS77CAT (−77 to +72, which lacks the putative NF-κB site) and pCR0.15CAT (−99 to +8, which lacks the intronic *cis*-acting element) were transfected together with hRARβ into the HeLa cells, and stimulated with

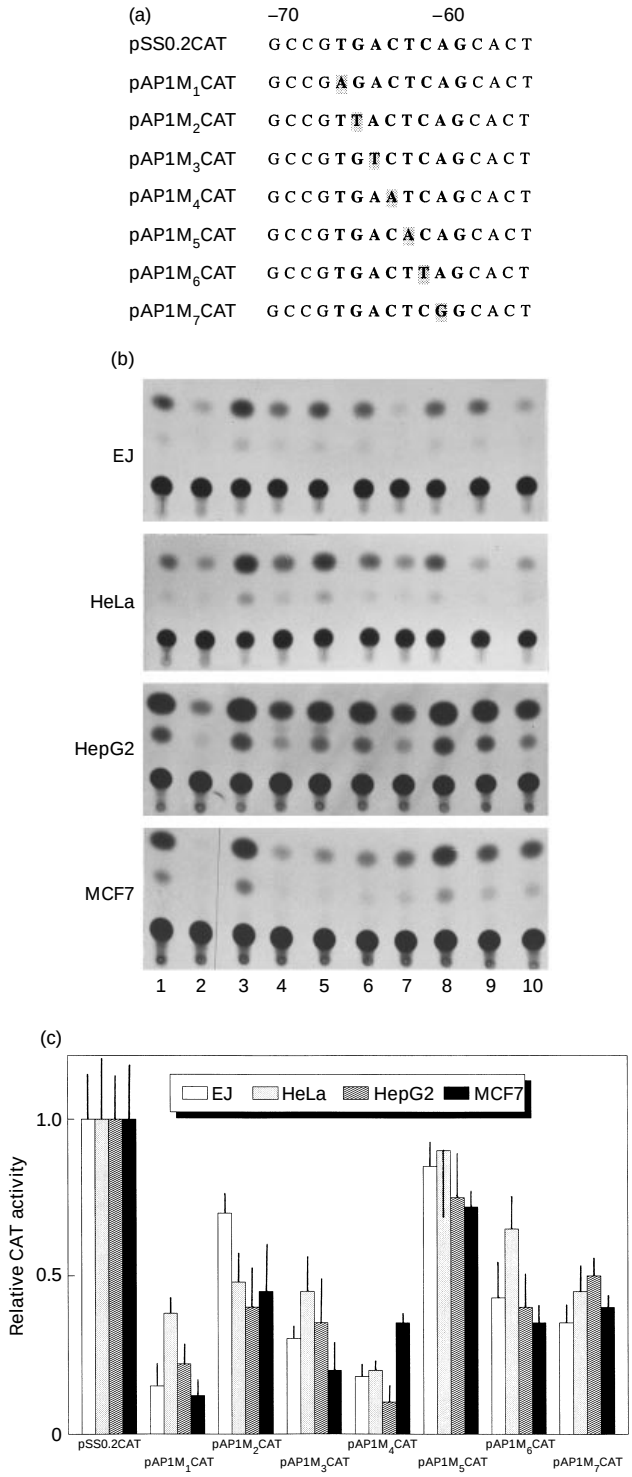

Figure 2 Mutational analysis of the AP1 site

(**a**) Schematic representation of the point mutation of the AP1 site. (**b**) CAT assay with EJ, HeLa, HepG2 and MCF7 cells transfected with *GSTP1* promoter–CAT constructs. Lane 1, positive control pSV$_2$CAT; lane 2, negative control pICCAT; lane 3, pSS0.2CAT; lane 4, pAP1M$_1$CAT; lane 5, pAP1M$_2$CAT; lane 6, pAP1M$_3$CAT; lane 7, pAP1M$_4$CAT; lane 8, pAP1M$_5$CAT; lane 9, pAP1M$_6$CAT; and lane 10, pAP1M$_7$CAT. (**c**) CAT activities were calculated relative to that of pSS0.2CAT. The results are means of six to nine assays.

1×10^{-6} M RA. Figure 1 shows that these constructs produced different basal activities, but their responses to RA repression were similar. From the above results, it was concluded that for

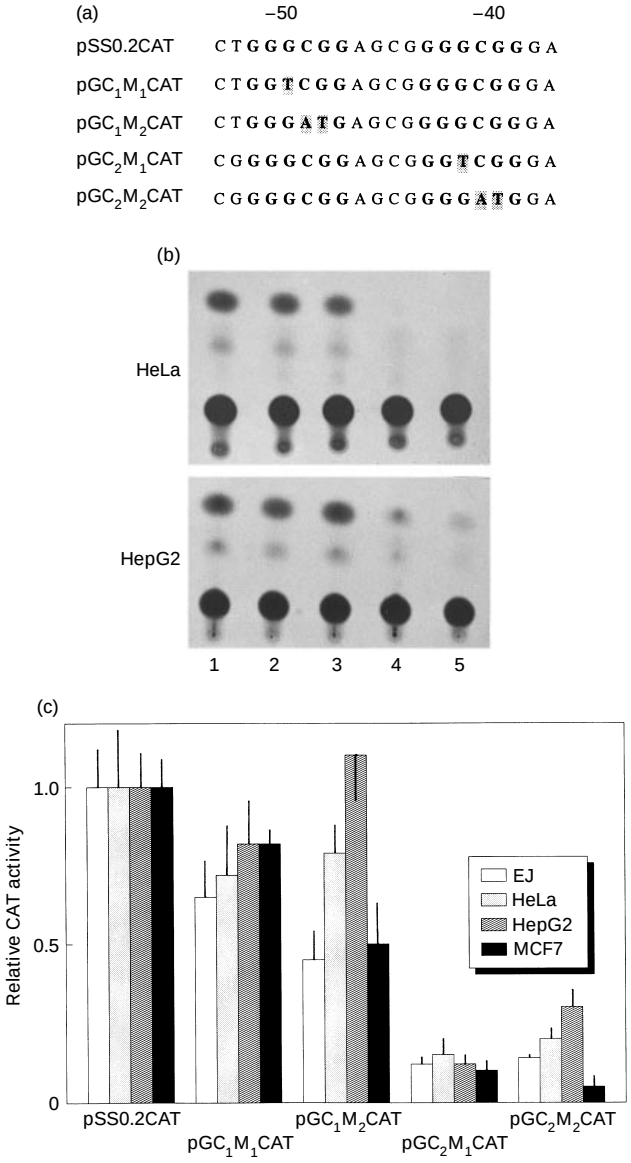

Figure 3 Mutational analysis of the two G/C boxes

(**a**) Schematic representation of the point mutation of each G/C box. (**b**) CAT assay with HeLa and HepG2 cells transfected with *GSTP1* promoter–CAT constructs. Lane 1, pSS0.2CAT; lane 2, pGC$_1$M$_1$CAT; lane 3, pGC$_1$M$_2$CAT; lane 4, pGC$_2$M$_1$CAT; and lane 5, pGC$_2$M$_2$CAT. (**c**) CAT assays with EJ, HeLa, HepG2 and MCF7 cells with the G/C box mutant constructs. The CAT activities were calculated relative to that of pSS0.2CAT and the results are means of six to nine assays.

the RA–RAR-dependent repression of *GSTP1* the nucleotide sequence between -77 and $+8$ is required.

The region from -77 to $+8$ of the promoter consists of an AP1 site, two G/C boxes and a TATA box (Figure 1). To characterize the involvement of the AP1 site in RA–RAR-dependent repression, a set of *GSTP1* promoter–CAT constructs, pAP1M$_n$CATs, with single-point mutation at each residue of the AP1 site were made (Figure 2a) using the Transformer Site-Directed Mutagenesis system. Mutant promoter–CAT constructs were transfected into EJ, HeLa, HepG2 and MCF7 cells and the residual promoter activities were determined by measuring the CAT reporter-enzyme activity. Similar results were obtained in

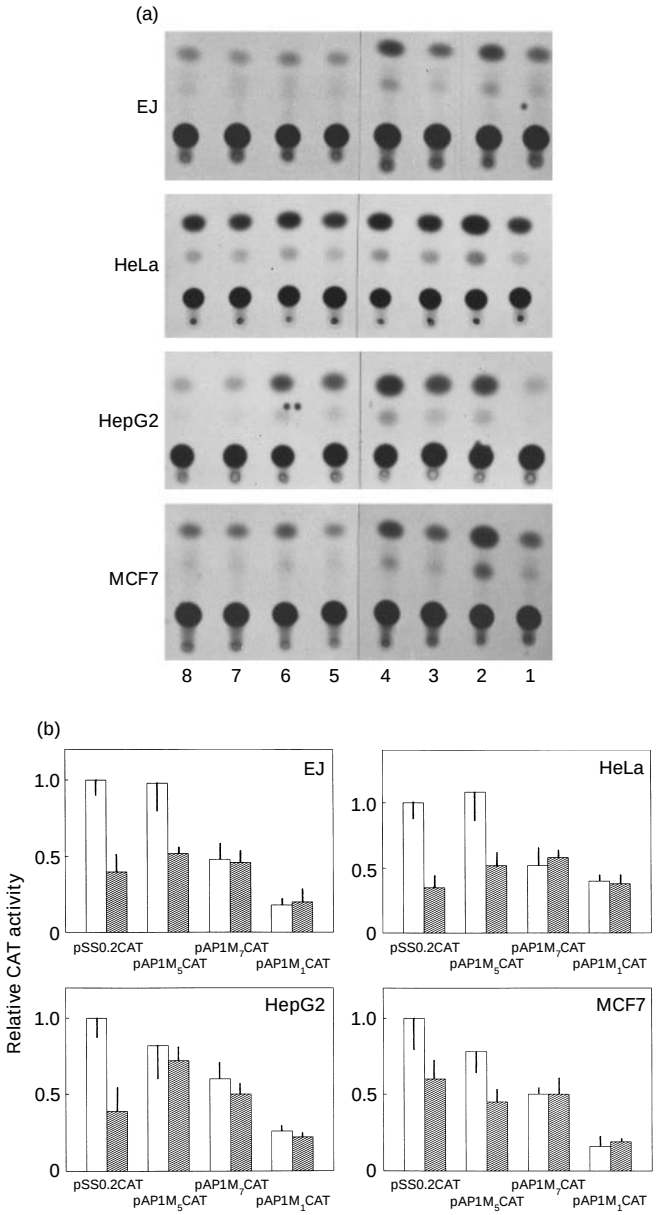

Figure 4 Effect of mutation of the AP1 site on RA response

(**a**) EJ, HeLa, HepG2 and MCF7 cells were co-transfected with hRARβ and *GSTP1* promoter–CAT constructs. Lanes 1 and 2, pSS0.2CAT; lanes 3 and 4, pAP1$_5$CAT; lanes 5 and 6, pAP1$_7$CAT; and lanes 7 and 8, pAP1$_1$CAT. Cells were treated with (lanes 1, 3, 5 and 7) or without (lanes 2, 4, 6 and 8) 1×10^{-6} M RA 16 h before harvesting and CAT activities were measured. (**b**) The CAT activities were calculated relative to that of pSS0.2CAT. Each bar (▨, +RA; □, −RA) represents the mean of six to nine determinations.

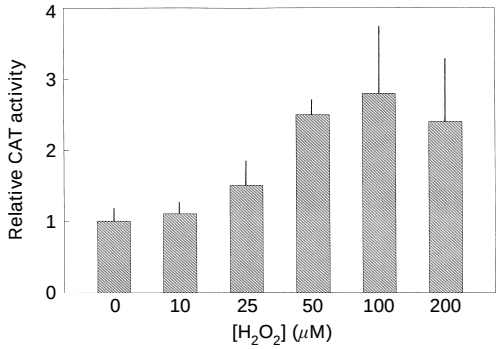

Figure 5 Effect of H_2O_2 on *GSTP1* promoter activity

HeLa cells transfected with pSS0.4CAT were treated with H_2O_2 at concentrations ranging from 10 to 200 μM 16 h before harvesting, and CAT activities were measured. The result represents the means of three determinations.

all four cell lines tested (Figures 2b and 2c). Mutation at '**T**'GACTCAG (pAP1M$_1$CAT) and TGA'**C**'TCAG (pAP1M$_4$CAT) almost totally abolished the activity of the AP1 site, whereas mutation of TGAC'**T**'CAG (pAP1M$_5$CAT) retained full activity. Other mutant promoters showed smaller losses of activity depending on the mutation.

To analyse the involvement of the two G/C boxes downstream from the AP1 site in RA–RAR-mediated repression of expression, a set of *GSTP1* promoter–CAT constructs, pGC$_n$M$_n$CATs, with mutation at each G/C box, were constructed using the Transformer Site-Directed Mutagenesis system (Figure 3a). Constructs with these mutations were transfected into HeLa, HepG2, EJ and MCF7 cells and the residual activities were measured. As shown in Figures 3b and 3c, mutation of the G/C box immediately downstream from the AP1 site (pGC$_1$M$_1$CAT and pGC$_1$M$_2$CAT) has little effect on the promoter activities in all four cell lines analysed, whereas mutation of the G/C box further downstream (pGC$_2$M$_1$CAT and pGC$_2$M$_2$CAT) caused up to 80% loss of activity.

To prove that the AP1 site is essential for the RA–RAR-dependent repression, plasmid pSS0.2CAT and pAP1M$_n$CATs were co-transfected with hRARβ into EJ, HeLa, HepG2 and MCF7 cells. 24 h after transfection, cells were exposed to 1×10^{-6} M RA and CAT assays were carried out after 16 h. Similar results were observed with all cell lines except HepG2. CAT activities for plasmids pSS0.2CAT (wild-type promoter), pAP1M$_1$CAT (which lost 80% activity), pAP1M$_5$CAT (which retained full activity) and pAP1M$_7$CAT (which retained about 50% activity) are shown in Figure 4. These results showed that the DNA sequence required for RA–RAR-dependent repression co-localizes with the AP1 site. In EJ, MCF7 and HeLa cells, mutation of the AP1 site resulting in loss of part or full promoter activity abolished the RA–RAR-dependent repression. On the other hand, mutation of the AP1 site without loss of activity retained RA–RAR responsiveness. In contrast, mutation of any residue of the AP1 site impairs the RA–RAR responsiveness in HepG2 cells (Figure 4).

Using the CAT assay system, it was demonstrated that mutation of each of the two G/C boxes downstream from the AP1 site has little effect on RA–RAR suppression (results not shown).

Regulation of *GSTP1* redox status, requirement for NF-κB and AP1 sites

HeLa cells were transfected with the pSS0.4CAT reporter construct, which contains the −350 to +72 region of *GSTP1*, and treated with 10–200 μM H_2O_2 for 16 h. *GSTP1* promoter activity was increased 2- to 4-fold (Figure 5). Owing to the increased toxicity of H_2O_2 at higher concentrations, 100 μM was used in subsequent experiments.

Reactive oxygen intermediates, such as H_2O_2, have been shown to activate a number of genes via the *trans*-acting factor NF-κB

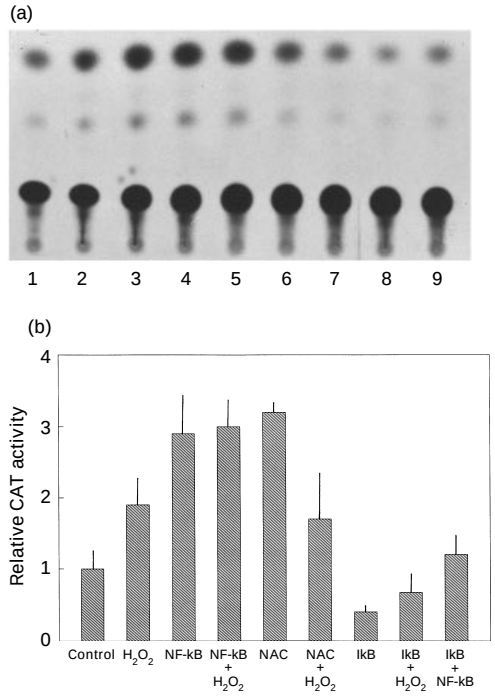

Figure 6 Effect of co-expression of NF-κB (p65) or IκB (IκBα/MAD3) and treatment with H_2O_2 or NAC on transcription from the *GSTP1* promoter

(**a**) HeLa cells were transfected with pSS0.4CAT alone or co-transfected with NF-κB (p65) or IκB (IκBα/MAD3) expression vectors. Cells were treated with or without 10 mM NAC for 1 h, followed by treatment with or without 100 μM H_2O_2, 16 h before harvesting. Lane 1, control; lane 2, H_2O_2; lane 3, NF-κB; lane 4, NF-κB + H_2O_2; lane 5, NAC; lane 6, NAC + H_2O_2; lane 7, IκB; lane 8, IκB + H_2O_2; lane 9, IκB + NF-κB. (**b**) CAT activities are means of six to nine determinations.

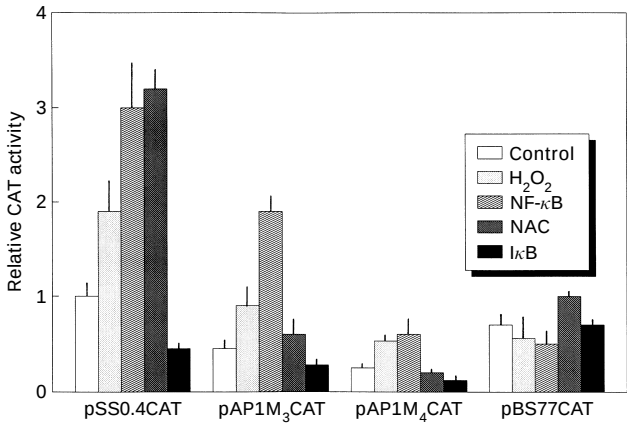

Figure 7 Effect of mutation of AP1 or NF-κB site on the response of the *GSTP1* promoter to treatment with NAC or H_2O_2, or co-expression of NF-κB (p65) or IκB (IκBα/MAD3)

HeLa cells were transfected with pSS0.4CAT, pAP1M$_3$CAT, pAP1M$_4$CAT or pBS77CAT alone, or co-transfected with NF-κB (p65) or IκB (IκBα/MAD3) expression vectors. Cells transfected with promoter–CAT constructs alone were treated with either 10 mM NAC or 100 μM H_2O_2 16 h before harvesting and CAT activities were measured. The results are means of six determinations.

[18]. To determine whether NF-κB is involved in *GSTP1* induction by oxidants such as H_2O_2, the expression vector for NF-κB (p65) was co-transfected with pSS0.4CAT into HeLa cells. As shown in Figure 6, co-transfection of NF-κB (p65) caused a 3-fold increase in the transcription. Stimulation with 100 μM H_2O_2 did not augment this effect. On the other hand, co-expression of IκB (IκBα/MAD3) inhibited the basal promoter activity and blocked the induction by both H_2O_2 and co-expression of NF-κB (p65) (Figure 6).

In order to determine whether an antioxidant, such as NAC, can block the induction of *GSTP1* by H_2O_2, HeLa cells transfected with pSS0.4CAT were pre-incubated with 10 mM NAC [18] for 1 h before stimulation by 100 μM H_2O_2. Interestingly, incubation with NAC alone induced the promoter activity 3-fold (Figure 6). On the other hand, cells pre-incubated with NAC followed by addition of H_2O_2 resulted in CAT activity 1.6-fold that of the control, which was lower than those from cells stimulated alone with either H_2O_2 or NAC (Figure 6). This implies that NAC antagonizes the induction of *GSTP1* by H_2O_2.

We have observed a putative NF-κB-binding site, 'GGGACCCTCC', located between −96 and −86 of the promoter region. Deletion of this region resulted in a loss of 50% of the activity [14] (Figure 1). Antioxidants, such as NAC, have been shown to induce transcription from the chimeric thymidine kinase promoter with three copies of AP1 sites. [19,20]. In order to determine the involvement of the NF-κB and AP1 sites of *GSTP1* in response to oxidant and antioxidant, constructs pSS0.4CAT (wild-type promoter), pAP1M$_3$CAT (mutation at AP1 site causing loss of 50% activity in HeLa cells), pAP1M$_4$CAT (mutation at the AP1 site causing loss of 80% activity in HeLa cells) and pBS77CAT (deletion of the NF-κB site) were used in CAT assays. HeLa cells were transfected with each of these constructs alone or co-transfected with NF-κB (p65) or IκB (IκBα/MAD3). Cells transfected with these constructs alone were subjected to stimulation by either H_2O_2 or NAC. The results are shown in Figure 7. Deletion of the NF-κB site abolished both induction by H_2O_2 and the effects of co-expression of NF-κB (p65) as well as the inhibition of co-expression by IκB (IκBα/MAD3), whereas mutation of the AP1 site did not alter the effects of H_2O_2 on induction. On the other hand, while mutation of the AP1 site abolished NAC response, deletion of NF-κB site only reduced the sensitivity of NAC responsiveness. This implies that the response of *GSTP1* to oxidants is mediated by NF-κB via the NF-κB site, whereas the response to antioxidants is mediated largely by the AP1 site. Therefore, the regulation of the expression of *GSTP1* according to cellular redox status appears to result from co-operation between the NF-κB and the AP1 sites.

DISCUSSION

RA, a metabolite of vitamin A (retinol), has numerous effects on cell growth and differentiation [21]. These effects are believed to be mediated through nuclear receptors, namely the retinoic acid receptors (RARα, β and γ) and the retinoid receptors (RXRα, β and γ), which are encoded by at least six genes [22]. The RARs and RXRs are a distinct subclass of the nuclear receptor superfamily [23]. They influence gene expression through retinoic acid regulatory elements (RAREs), composed of 'hormone response element half-site sequences' (consensus sequence AGG/TCA) and arranged as direct repeats separated by a variable number of residues [22].

However, the present results show that the sequence in *GSTP1* essential for repression by RA is not RAREs but is

coincident with the AP1 site. A similar situation has been observed in both the human collagenase and rat stromelysin promoters [24–26]. The AP1 site has a consensus sequence TGAC/GTCA that in most cases is capable of conferring phorbol ester [phorbol 12-myristate 13-acetate (PMA)] inducibility upon heterologous promoters and the binding of AP1, which is a heterodimeric complex composed of Jun and Fos, encoded by the proto-oncogenes, *c-jun* and *c-fos*, and other related proteins such as Maf, Nrl and the AP1-like proteins [27–29]. The AP1 site of *GSTP1* is essential for the promoter activity, and yet, unlike typical AP1 sites, it is not responsive to either PMA stimulation in HeLa, HepG2 or MCF7 cells [15] or co-transfection of *c-fos* or *c-jun* expression vectors [30]. The mutational analysis of the AP1 site suggested that the activity of the AP1 site is contextual, since the mutation of TGAC'**T**'CAG, which abolished the activity in collagenase promoter, retained full activity in the *GSTP1* promoter. Nevertheless, Jun and Fos or Jun/Fos-like proteins are involved in the regulation of *GSTP1* in VCREMS cells, a multidrug resistant MCF7 cell line, via the AP1 site, since anti-Jun or anti-Fos antibodies abolished the binding of proteins to the AP1 site in gel-shift assays [31]. Therefore, the function of the AP1 site of *GSTP1* is cell-type dependent.

From these findings, we can conclude that RA–RAR represses the transcriptional activation of *GSTP1* by blocking the function of AP1 or AP1-like proteins. This conclusion is similar to earlier proposals in that the inhibition of the collagenase and stromelysin promoters by RA is not mediated by binding of the RAR–RA complex to DNA but appears to be due to direct protein–protein interaction between RARs and AP1, or AP1-like proteins [26]. It is interesting to note that RXR has no effect on the repression of *GSTP1* expression by RA and does not augment the action of RAR [16]. Recently, RXRs have been shown to act as auxiliary proteins by interacting directly with, and enhancing, the DNA-binding activities of RARs [32], providing further evidence that the RAR-mediated RA repression of the *GSTP1* promoter may occur through a mechanism that does not involve the binding of an RAR–RA complex to DNA. The effect of RA–RAR repression via the AP1 site is contextual, as mutation of the AP1 site (pAP1M_5CAT) retained RA–RAR-mediated repression in EJ, HeLa and MCF7 cells, but not in HepG2 cells. It is possible that other factors may be involved in the formation or regulation of formation of the functional RA–RAR–AP1 protein complex.

RA has been demonstrated to inhibit or reverse malignant transformation in pre-neoplastic and neoplastic cells [33]. It has been proven to be able to prevent human oral cancers [34] and to be an effective therapeutic agent in the treatment of human carcinomas of the cervix and skin [35,36]. High levels of expression of *GSTP1* are associated with cell proliferation, embryogenesis and malignancy. It is possible that GSTP1-1 may have evolved specifically to protect proliferating cells and share regulatory mechanisms with other cellular genes which are involved in cell division and tumorigenesis. Therefore, *GSTP1* provides us with a model system to study the regulatory mechanisms of cell growth and differentiation, by RA, especially during malignant progression.

In this paper, we have also presented experimental evidence that cellular redox changes influence the expression of *GSTP1*. Eukaryotic cells continuously produce ROIs as side products of electron-transfer reactions [37]. Major ROIs are superoxide (O_2^-), H_2O_2 and organic hydroperoxides, hydroxyl radicals ($OH^\bullet$) and singlet oxygen ($O_2^\bullet$). Above normal levels of ROIs are said to cause oxidative stress. This occurs frequently in cells exposed to high levels of O_2, heavy burdens of xenobiotic metabolism and attack by neutrophils and macrophages generated during an immune response. Oxidative stress can also result from exposure to exogenous agents such as high-energy radiation [38,39]. Oxidative stress can cause irreversible damage to DNA, proteins and lipids, and is associated with carcinogenesis, both during initiation and promotion stages [40], and may affect the control of cell division [41]. In addition, there is evidence that oxidative stress is involved in the induction of apoptosis [42–44].

Oxidative stress triggers a detoxication system which counteracts ROIs and ROI-induced damage [19]. This involves the induction of enzymes, such as GSH peroxidase and GSTs, which reduce peroxides and detoxify or decompose the products of peroxidation. GSTP1-1 has been shown to detoxify lipid- and DNA-hydroperoxides and their derivatives, such as hydroxyalkenals, malondialdehye and base propenals [45,46]. In addition, GSTP1-1 can react directly with ROIs via a very sensitive SH group, and cause inactivation by disulphide formation that can be reversed by glutathione [47]. Therefore, it has a specific response to oxidative stress.

NF-κB, a heterodimeric transacting factor composed of two subunits, p50 and p65 [18,48], can be regarded as an oxidative-stress responsive factor, as its induction by H_2O_2 and a variety of other agents such as phorbol esters can be blocked by a variety of chemically distinct antioxidants [49]. In most cell types, NF-κB resides in an inactive IκB-complexed form, located in the cytoplasm [50] (IκB is the inhibitory subunit of NF-κB). Its activation involves dissociation of IκB from the NF-κB heterodimer followed by nuclear translocation and the activation of expression of downstream genes which contain NF-κB sites [51,52]. The induction of *GSTP1* by H_2O_2 is mediated by NF-κB, as over-expression of p65 enhances the promoter activity, whereas mutation of the NF-κB site abolishes the induction by both H_2O_2 and p65 expression. On the other hand, Moffatt et al. have shown that the NF-κB site functioned as a suppressor of the *GSTP1* promoter in VCREMS cells [53]. These results again suggest that the mechanism of transcriptional regulation of *GSTP1* expression is cell-type dependent.

H_2O_2 is known to mimic the growth-promoting effects of insulin and related growth factors [54]. However, our results suggest that the effect of H_2O_2 on *GSTP1* promoter activity is distinct from that of the insulin (C. Xia, unpublished work).

AP1 is also a redox-responsive protein [19,20]. In contrast to NF-κB, DNA binding and transactivation by AP1 are strongly enhanced by antioxidants such as NAC and pyrrolidine dithiocarbamate [19]. Therefore, the AP1 site represents a potential antioxidant-responsive element, which has been observed in NAD(P)H:quinone reductase (NQO_1) and GST1b genes [55,56]. It is an apparent paradox that AP1 mRNA can also be induced by factors causing a pro-oxidative state of cells, such as H_2O_2 [57] and UV light [58]. However, the activity of AP1 is low in these cases. It thus appears that under both antioxidant and pro-oxidant conditions, AP1 genes are induced, but that more active AP1 protein is produced under antioxidant conditions. Under pro-oxidant conditions, AP1 protein exists in a latent form which is only active when cells regain the hypoxic state. This allows the production of AP1 under very diverse conditions, but restricts its biological activity to a defined state of the cell [19,20].

We have demonstrated here that the induction of *GSTP1* transcription by NAC is mediated by the AP1 site, since this effect can be abolished by mutation of the AP1 site. Interestingly, deletion of the NF-κB site reduces the sensitivity of *GSTP1* in response to NAC stimulation. These results suggest possible interactions between proteins that bind to the AP1 and NF-κB sites. Recently, Stein et al. [59] have demonstrated that AP1 functions synergistically with NF-κB, through its direct interaction with the p65 subunit of NF-κB. As antioxidants strongly induce AP1 but inhibit NF-κB activation [19,20], NF-κB would

be an ideal target for synergistic activation by AP1 under such conditions, and vice versa. In view of these findings, the regulation of *GSTP1* provides us with a perfect model to study the competition between, or integration of, two oxidative response pathways represented by NF-κB and AP1.

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