Functional Characterization of the Transcription Silencer Element Located within the Human Pi Class Glutathione S-Transferase Promoter*

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We have previously demonstrated enhanced transcriptional activity of the human Pi class glutathione S-transferase (GSTP1) promoter in a multidrug-resistant derivative (VCREMS) of the human mammary carcinoma cell line, MCF7 (Moffat, G. J., McLaren, A. W., and Wolf, C. R. (1994) J. Biol. Chem. 269, 16397-16402). Furthermore, we have identified an essential sequence (C1; –70 to –59) within the GSTP1 promoter that bound a Jun-Fos heterodimer in VCREMS but not in MCF7 cells. These present studies have examined the negative regulatory element (–105 to –86), which activated suppressor binding site to be located between nucleotides –97 and –90. In vitro DNA binding assays suggested that the repressor exerted its action by causing displacement of the essential non-AP-1-like MCF7 C1 complex. However, the addition of MCF7 nuclear extract did not disrupt binding of the VCREMS Jun-Fos C1 complex to the GSTP1 promoter. Furthermore, upstream insertion of the GSTP1 silencer element failed to inhibit activity of a heterologous promoter in MCF7 cells. These results highlighted the cell and promoter specificity of the GSTP1 transcriptional repressor and implicated a functional requirement for contact between the repressor and C1 complex. In this regard, the hypothesis that a direct interaction between the repressor and C1 complex was required to suppress GSTP1 transcription. Moreover, these findings suggest that cell-specific differences in the composition of the C1 nuclear complex may dictate repressor activity.

Glutathione S-transferases (GSTs) are important detoxification enzymes that catalyze the conjugation of a wide range of electrophilic compounds to glutathione. Mammalian GSTs have been classified into five distinct gene families: four cytosolic groups (Alpha, Mu, Pi, and Theta) and one microsomal form. The cytosolic proteins share a degree of homology and possibly have evolved from a common ancestral gene (2, 3). The human Pi class GST (GSTP1) gene is commonly overexpressed in neoplasia (4–7). However, a predictive correlation with tumor development has not been conclusively established. Indeed, Lee et al. (8) have recently shown a marked decrease in GSTP1 expression in 88 of 91 prostate cancers relative to normal prostatic tissue. Given the detoxification role of GSTs, it has been postulated in this case that negative regulation of GSTP1 gene expression in a subpopulation of cells may result in loss of protection against carcinogen exposure, thus leading to clonal expansion.

An involvement of GSTP1 in the development of antineoplastic drug resistance has also been implicated by elevated levels of GSTP1 mRNA in cell lines selected for resistance to a range of anticancer drugs (9–12). Furthermore, protection of cells against the cytotoxic effects of these drugs has been provided by the transfection of GSTP1 expression vectors (13–15). However, the molecular mechanisms responsible for changes in GSTP1 expression in these drug-resistant cells are poorly characterized.

In this regard, we have recently demonstrated significantly enhanced activity of the GSTP1 promoter in multidrug-resistant VCREMS cells compared with the parental human mammary carcinoma cell line, MCF7 (1). Furthermore, we have identified a promoter region, C1 (–70 to –59), that is essential for GSTP1 transcription in both cell lines but that binds members of the Jun and Fos protein families in VCREMS but not MCF7 cells.

An intriguing finding of these preliminary studies was the identification of a silencer element (–105 to –86), which acted to suppress GSTP1 transcription in the parental MCF7 cells but not in the drug-resistant VCREMS cell line. These present studies further localize the repressor binding site and provide an insight into the mechanistic pathways of GSTP1 transcriptional repressor action.

EXPERIMENTAL PROCEDURES

Cell Culture—The VCREMS cell line (a generous gift from Dr. Bridget Hill) was derived by selecting the human mammary carcinoma cell line, MCF7, for resistance (11-fold) to vincristine (16). In addition, VCREMS cells were also cross-resistant (3-fold) to adriamycin and etoposide. The VCREMS and MCF7 cell lines were both cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and supplemented with l-glutamine and a penicillin/streptomycin mixture (Life Technologies, Inc., Paisley, Scotland).

Promoter Deletion Constructs—The GSTP1 promoter deletion constructs p291CAT, p105CAT, p85CAT, p73CAT, and p65CAT were prepared as described previously (1) by ligating deletion fragments (with HindII and Sal I linkers) of the GSTP1 promoter (–291 to +36; –105 to +36; –85 to +36; –73 to +36, and –65 to +36) into HindII/SalI-digested pCAT.Basic vector (Promega, Southampton, UK).

The p105MR1–6CAT constructs were prepared by ligating each of the six mutated GSTP1 promoter fragments, –105 to +36 (as shown in Fig. 2A), with HindII and SalI linkers into HindII/SalI-digested pCAT.Basic. The pR1TKCAT construct was prepared by ligating the GSTP1 pro-

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motor fragment—105 to −74 (with HindIII and XbaI linkers at the 5′ and 3′ ends, respectively)—into the polylinker region of the HindIII/XbaI-digested pBlCAT vector, which contains a region of the Herpes simplex virus thymidine kinase promoter (−105 to +51) inserted upstream of the CAT reporter gene (17).

The splicing constructs pRL(30)CAT, pRL(26)CAT, pRL(20)CAT, and pRL(15)CAT were prepared by ligating the following GSTP1 promoter fragments; −117 to −74, −113 to −74, −107 to −80, and −102 to −85 (with HindIII linkers) in both orientations into HindIII-digested p3CAT.

\[
\begin{align*}
&\text{pRL(30)CAT (−117 to −74)} \\
&\text{pRL(26)CAT (−113 to −74)} \\
&\text{pRL(20)CAT (−107 to −80)} \\
&\text{pRL(15)CAT (−102 to −85)}
\end{align*}
\]

Following preparation, all constructs were sequenced by the dideoxy chain termination method (18).

**Transient Transfection Assay—** DNA transfections were performed by the calcium phosphate method (19) as described by Gorman (20) with the exception that glycercol was omitted. Cell extracts were assayed for protein content (21) and CAT activity was determined as described previously (22) and modified by Sambrook et al. (23). In all experiments, results were normalized for the activity of pCAT-Control (Promega), which contained the SV40 enhancer and promoter, whereas the promoterless pCAT Basic vector was used as a negative control.

**Electrophoretic Mobility Shift Assay—** Nuclear extracts were prepared from VCREMS and MCF7 cells using the method described by Dignam et al. (24). Electrophoretic mobility shift assays (EMSA) were performed using the reaction conditions described previously (1).

In competition experiments, the reaction mixture was preincubated for 20 min at room temperature with 100-fold molar excess of unlabelled DNA before the addition of radiolabeled probe. The following digonucleotides and their complementary sequences were used as probes and competitors: GSTP1 promoter fragment (−105 to −54) (5′-GGACGAGCGGGCGGGAGTCCGCGGGGCCGCAG-3′), GSTP1 promoter fragment, −85 to −54 (5′-AGGCCCGGCGGGAGTCCGCGGGGCCGCAG-3′), and the GSTP1 C1 promoter fragment, −73 to −54 (5′-GGCGGAGCTCATCCAGAAGAC-3′). For annealing, equal amounts of complementary digonucleotides were heated to 95 °C for 2 min and allowed to cool gradually to room temperature.

**RESULTS**

Identification of a Silencer Element in the GSTP1 Promoter—Previous studies in this laboratory have clearly demonstrated elevated levels of GSTP1 expression in VCREMS cells compared with the parental MCF7 cell line (1). Moreover, transient transfection assays utilizing GSTP1 promoter deletion constructs established significantly enhanced transcriptional activity of the GSTP1 promoter in VCREMS cells. These results are summarized in Fig. 1.

p291CAT and p105CAT were significantly more active (18.2- and 11.5-fold, respectively) in VCREMS cells, suggesting that augmented levels of GSTP1 promoter-derived transcription was the predominant regulatory mechanism responsible for GSTP1 overexpression in the drug-resistant line. In addition, deletion to position −65 abolished GSTP1 transcription in both cell lines.

However, p85CAT and p73CAT, unlike p291CAT and p105CAT, were only marginally more active in VCREMS cells (1.1- and 2.5-fold, respectively). This observation is best explained by the 6.7-fold increase in GSTP1 promoter activity in MCF7 cells following deletion from position −105 to position −85, indicating the presence of a negatively acting regulatory element (−105 to −86) that acts to repress GSTP1 transcription in the MCF7 cell line. Indeed, the magnitude of GSTP1 transcriptional induction in MCF7 cells following removal of the silencer element implied that suppressed transcription of the GSTP1 gene in MCF7 cells plays the major role in contributing to the observed cell-specific pattern of GSTP1 expression.

Localization of the Repressor Binding Site—To further define the repressor binding site within the GSTP1 promoter, six sets of 4-bp clustered mutations were introduced into the silencer element (−105 to −86), as shown in Fig. 2A. These oligonucleotides (MR1-6) were utilized to prepare six mutated GSTP1 promoter fragments (−105 to +36) by polymerase chain reaction, which were then subbonded upstream of the CAT reporter gene, to generate p105MR1CAT–p105MR6CAT.

Transient transfection assays were performed in MCF7 cells using these constructs, and their CAT activities were compared with p105CAT and p73CAT (Fig. 2B). Changing nucleotides −97 to −94 from GGAC to TTGC (p105MR3CAT) completely restored GSTP1 promoter activity. In addition, substituting nucleotides −93 to −90 from CCTC to AGGA (p105MR4CAT) increased GSTP1 transcriptional levels to 65% of optimal activity. However, the remaining four sets of mutations did not enhance GSTP1 promoter activity. Therefore, this experiment shows that the repressor binding site resided between nucleotides −97 and −90, although the upstream half of this element appeared to be more functionally important. Interestingly, this region of the GSTP1 promoter is homologous to the known response element for the NFκB family of transcription factors (25, 26).

The GSTP1 Transcriptional Repressor Caused Displacement of the Essential C1 Complex in MCF7 Cells—To examine the molecular mechanisms responsible for these silencing effects on GSTP1 promoter activity, we prepared nuclear extracts from MCF7 and VCREMS cells. EMSAs were then used to analyze the nuclear proteins bound to the GSTP1 promoter fragments, −105 to −54, −85 to −54, and −73 to −54 (C1), and these results are shown in Fig. 3. A single DNA-protein complex was formed using the C1 probe with VCREMS nuclear extract, whereas the same amount of protein from MCF7 nuclear extract produced a similar band shift pattern. Binding specificity of the C1 complex to the GSTP1 promoter in both VCREMS and MCF7 cells has been demonstrated previously (1). However, despite similar mobility, we have clearly demonstrated significant composition differences in the C1 complex, namely J un-Fos in VCREMS but not MCF7 cells (1). Identical results were found using the −85 to −54 probe.

However, inclusion of the silencer element (−97 to 90) yielded an intriguing result. As shown for both the C1 and −85 to −54 probes, the −105 to −54 fragment bound a single complex in VCREMS nuclear extract. In contrast, the essential MCF7 C1 complex, which bound strongly to both the C1 and −85 to −54 probes, was unable to bind to the −105 to −54 GSTP1 promoter fragment. These results suggested that the GSTP1 transcriptional repressor in MCF7 cells exerts its suppressive effects by causing displacement of the essential C1-bound complex, thus inhibiting GSTP1 transcription.

Cell-specific Nature of GSTP1 Transcriptional Repressor Activity—The results shown above suggested that GSTP1 repressor activity in MCF7 cells was mediated by displacement of the C1 complex, which we have demonstrated to be required for GSTP1 transcription. Furthermore, our CAT assay data have suggested that a DNA binding requirement is likely for repressor function and that the repressor binds between nucleotides −97 and −90. However, the EMSA experiments presented in Fig. 3 were unable to detect DNA binding of either the repressor or C1 complex to the GSTP1 promoter fragment, −105 to −54.
These results suggested that competition with an oligomer spanning the repressor binding site may restore binding of the MCF7 C1 complex to the –105 to –54 probe. However, despite the presence of a 100-fold molar excess of either the –105 to –86 or –105 to –74 oligomers, binding of MCF7 nuclear proteins to the –105 to –54 probe was still undetectable (data not shown). Consistent with this data, using the –105 to –74 oligomer as an EMSA probe, binding of nuclear proteins was not detected. These results implied that in addition to the requirement for an intact silencer element, the transcriptional repressor was targeted to the GSTP1 promoter by interacting with proteins within the MCF7 C1 complex.

In this regard, we have previously shown that Jun-Fos proteins comprise the C1 complex in VCREMS but not MCF7 cells. Given these significant composition differences and the possible targeting mechanism cited above, nuclear extracts prepared from both cell lines were mixed to test whether the MCF7 GSTP1 transcriptional repressor activity was able to displace the Jun-Fos C1 complex in VCREMS cells.

VCREMS nuclear extract was incubated with the end-labeled GSTP1 promoter fragment, –105 to –54 for 20 min. Increasing amounts of MCF7 nuclear extract were then added. However, as shown in Fig. 4, the repressor activity in MCF7 cells failed to displace the VCREMS C1 complex. Moreover, identical results were obtained when both MCF7 and VCREMS nuclear extract were added simultaneously (data not shown) or when the MCF7 nuclear extract was added 20 min before the VCREMS nuclear proteins (Fig. 4).

**Fig. 1. Identification of a silencer element in the GSTP1 promoter.** A, schematic representation of the GSTP1 promoter fragments ligated upstream of the CAT reporter gene. B, summary of CAT assay results. Each GSTP1 promoter deletion construct was transfected into MCF7 and VCREMS cells. The cells were harvested 48 h later, protein concentrations were determined, and CAT assays were performed. pCAT.Basic (contains no enhancer/promoter sequences) and pCAT.Control (contains SV40 enhancer and promoter) were used as negative and positive controls, respectively. All results were expressed relative to the activity of p105CAT in MCF7 cells (given an activity value of 1.0) and were compared between cell lines by correcting for pCAT.Control activity levels.
These results further demonstrated a possible interaction between the repressor and C1 complex in MCF7 cells. Indeed, this experiment provided evidence that cell-specific differences in the composition of the C1 complex in VCREMS and MCF7 cells rendered the repressor inactive in the resistant cell line. However, an alternative explanation for these results could be the presence of repressor modification activity within VCREMS nuclear extract that directly inhibited repressor function.

GSTP1 Promoter Specificity of the Repressor—As suggested by the results shown above, the composition of the MCF7 C1 complex bound to the GSTP1 promoter fragment (−105 to +36) and was then ligated into pcAT. Basic to generate six mutant constructs p105MR1CAT–p105MR6CAT. These constructs were transfected into MCF7 cells, and their relative CAT activities were compared with p105CAT and p73CAT.

![Fig. 2. Localization of the repressor binding site. A, the six sets of 4-bp clustered mutations (MR1–MR6) introduced into the GSTP1 silencer element (−105 to −86). B, each of the six sets of mutations was incorporated into the GSTP1 promoter fragment, −105 to +36, and was then ligated into pcAT. Basic to generate six mutant constructs p105MR1CAT–p105MR6CAT. These constructs were transfected into MCF7 cells, and their relative CAT activities were compared with p105CAT and p73CAT.](image)

![Fig. 3. The GSTP1 transcriptional repressor caused displacement of the essential C1 complex in MCF7 cells. EMSAs demonstrating the nuclear complexes from VCREMS and MCF7 cells (10 μg of nuclear extract/reaction) that bound to three different GSTP1 promoter fragments, −73 to −54, −85 to −54, and −105 to −54.](image)

![Fig. 4. Cell-specific nature of the GSTP1 transcriptional repressor. Nuclear extracts from MCF7 and VCREMS cells were mixed to assess the ability of the GSTP1 transcriptional repressor activity in MCF7 cells to displace the Jun-Fos VCREMS C1 complex from the GSTP1 promoter fragment, −105 to −54.](image)
element within the GSTP1 promoter. Moreover, our data have indicated that in addition to its DNA recognition motif, GGACCCTC, the repressor was targeted to the promoter by an interaction with the C1 complex. To investigate this mechanism in more detail, we examined this region of the GSTP1 promoter in terms of DNA topology.

The average structure of DNA in the genome is found in the B-form, i.e. 10.4 base pairs per helical turn with a 34.6° angle of rotation between adjacent nucleotides. It follows that DNA sequence motifs of similar size and spaced by a multiple of 10.4 nucleotides will align on the same face of the DNA double helix.

Clearer definition of the silencer element (−97 to −90) revealed that the repressor binding site and the essential C1 sequence (−70 to −59) were separated by 20 bp (Fig. 6A). Therefore, given the above information, it seemed reasonable to assume that the binding sites for these two regulatory complexes were located on the same face of the helix, thus facilitating the proposed interaction between the repressor and C1 complex.

This hypothesis was directly tested by the introduction of half-helical turns between these two elements. If a direct interaction was required for repressor function, then suppressed activity of the GSTP1 promoter would only be observed when the two elements were found on the same face of the DNA double helix, i.e. spaced by a multiple of 10 bp. For this purpose, four constructs were prepared, pRC1(30)CAT, pRC1(26)CAT, pRC1(20)CAT, and pRC1(15)CAT, which spaced the two elements by 30, 26, 20, and 15 bp, respectively (Fig. 6A).

Results presented in Fig. 6B, clearly show that GSTP1 promoter activity was suppressed in the pRC1(30)CAT and pRC1(20)CAT constructs but not in pRC1(26)CAT. Therefore, the repressor was functional when the two elements were spaced by 30 and 20 bp but not when separated by 26 bp, i.e. only when the two elements were on the same face of the DNA helix. This finding provided strong evidence that an interaction between the repressor and the C1 complex was absolutely required for repressor function.

A corollary to these results was the finding that pRC1(15)CAT also permitted repressor activity despite containing an element spacing of 15 bp. However, it is possible that steric interference induced by the closer proximity of the two motifs in this construct may overcome the unfavorable positioning of the two complexes imposed by the double helical structure.

It should also be noted that the data presented in Fig. 6B describe results obtained when the silencer element was ligated in the reverse orientation. Identical results were obtained when the repressor binding site was inserted in the correct orientation (data not shown) with the exception of pRC1(30)CAT, which failed to exhibit repressor activity. The significance of this finding is discussed later in this report.

Proposed Model for the Transcriptional Regulation of the GSTP1 Gene in MCF7 and VCREMS Cells—To summarize our findings, we have proposed a model for the regulation of GSTP1 transcription in MCF7 and VCREMS cells (Fig. 7). Clearly, displacement of the MCF7 C1 complex was only observed using an in vitro EMSA approach, and therefore its physiological importance remains to be ascertained. However, our data have provided strong evidence that cooperative effects involving the presence of an intact silencer element and the influence of protein:protein interactions with the C1 complex direct the repressor complex to the GSTP1 promoter. Therefore, it is proposed that this dual mechanism mediates negative regulation of GSTP1 transcription in MCF7 cells. This model includes recent data from this laboratory, which have outlined the functional importance of the distal Sp1 binding site (−57 to −49) in the GSTP1 promoter (27).

DISCUSSION

Our previous studies have established the importance of transcriptional mechanisms in regulating differential expression of the GSTP1 gene in MCF7 cells and the drug-resistant derivative cell line, VCREMS. These present data highlight the existence of a silencer element, which acts to suppress GSTP1 transcription in MCF7 cells. Indeed, the magnitude of this effect implies that repression of GSTP1 transcription is the predominant mechanistic pathway utilized to produce the observed cell-specific pattern of GSTP1 expression.

Mechanisms of transcriptional repression generally fall into two categories; passive repressors, which compete for the DNA binding site of a positive regulator, and active repressors, which directly prevent transcriptional initiation (for review see Ref. 28). Examples of passive repression include AP-1-mediated repression of basal and retinoic acid-inducible transcription of the human osteocalcin gene by competing with the retinoic acid receptor for overlapping DNA binding sites (29) and repression of estrogen-induced expression of the mouse lactoferrin gene through competition for the estrogen receptor DNA binding site by the transcription factor, COUP (30). However, this type of mechanism would not appear to account for GSTP1 transcriptional repressor action, whose binding site (−97 to −90) does not overlap the C1 element (−70 to −59). Indeed, repression of GSTP1 transcription was only observed in the presence of the intact silencer element.

In addition, there are several examples of transcriptional activators being down-regulated by inhibitory proteins with which they form protein complexes with reduced DNA binding affinity. Such a mechanism would appear more relevant to our observations on the GSTP1 promoter. For example, heterodimerization of CCAAT/enhancer-binding protein with CCAAT/enhancer-binding protein-homologous protein, which lacks a functional basic domain required for DNA binding, inhibits the interaction of CCAAT/enhancer-binding protein with DNA (31).

Although we have been unable to demonstrate directly an interaction of the repressor with the GSTP1 promoter, our data

![Fig. 5. GSTP1 promoter specificity of the repressor.](image-url)
have provided strong evidence that binding of the repressor to the GSTP1 promoter is absolutely required for silencing activity. This conclusion was based on the use of GSTP1 promoter deletion constructs, which exhibited negative regulation of GSTP1 transcription only in the presence of an intact repressor binding site, 297 to 290. Indeed, the same criteria holds for the C1 displacement activity observed in our EMSA experiments. Hence, the presence of an intact silencer element within the GSTP1 promoter was absolutely required for repressor activity.

However, despite this apparent requirement for DNA binding, we were unable to show binding of the repressor to the GSTP1 promoter using band shift assays. To explain this phenomenon, we have proposed that when located in close proximity to each other, the repressor and C1 complex form an unstable interaction with the GSTP1 promoter in MCF7 cells, which in turn mediates repression of GSTP1 transcription.

Consistent with this hypothesis, our results have suggested that a direct interaction between the repressor and the C1 complex was necessary for suppressed activity of the GSTP1 promoter. The most compelling evidence to support this conclusion was provided by the preparation of GSTP1 promoter constructs containing additional half-helical turns between the binding sites for these two protein complexes. These experiments clearly demonstrated that repressor activity was only observed when the two sequence motifs were aligned on the same face of the DNA double helical structure, i.e., separated by a multiple of 10 base pairs, thus facilitating a direct interaction.

Interestingly, when the elements were spaced by 30 nucleotides, negative regulation of GSTP1 promoter activity was detected only when the silencer element was inserted in the

**Fig. 6.** Repressor function was dependent upon a direct interaction with the C1 nuclear complex in MCF7 cells. A, the GSTP1 repressor binding site (−97 to −90) flanked by different lengths of adjacent sequence was ligated into the p73CAT construct to generate pRC1(30)CAT, pRC1(26)CAT, pRC1(20)CAT, and pRC1(15)CAT, which spaced the silencer element (R1) and C1 element by 30, 26, 20, and 15 bp, respectively. B, pRC1(30)CAT, pRC1(26)CAT, pRC1(20)CAT, and pRC1(15)CAT were transfected into MCF7 cells, and their CAT activities were compared with those of p105CAT and p73CAT.
reverse orientation. However, mutational analysis of the negative regulatory region indicated that the repressor binding site may not span the entire 8-bp silencer element GGACCCTC (−97 to −90). Indeed, our data suggest that the upstream half of this recognition motif is functionally more important because p105MR3CAT was significantly more active than p105MR4CAT. It follows then that the elements may only be aligned on the same face of the double helix in pRC1(30)CAT, i.e. spaced by 30 bp, when the silencer element was ligated in the reverse orientation.

With regard to the interaction between the repressor and C1 complex, our results have indicated that the GSTP1 transcriptional repressor operates in a position-dependent manner. Such position dependence has been reported for a number of other transcriptional repressors, e.g. the Wilm's tumor gene product, WT1, and human YY1, which is related to the Drosophila developmental regulator factor, Kruppel.

Therefore, our data have strongly indicated that direct interaction between the repressor and C1 complex is a prerequisite for transcriptional repression of the GSTP1 gene. One can draw parallels with this model and the well documented interaction of the retinoblastoma protein with the E2F transcription factor. The retinoblastoma gene is one of the best characterized tumor suppressor genes and encodes a protein that binds the E2F/DRTF family of transcription factors. E2F is of particular importance because it has been shown to be involved in the regulation of several proliferation-associated genes. Interestingly, Arroyo and Raychaudhuri have shown that retinoblastoma-mediated repression of E2F-dependent transcription requires a direct interaction of retinoblastoma with E2F.

However, unlike the examples described above, our data have suggested an essential role for both DNA binding and protein-protein interaction in determining the GSTP1 transcriptional repressor function. Indeed, our data suggest that the GSTP1 transcriptional repressor is targeted to the GSTP1 promoter by the MCF7 C1 nuclear complex.

A similar mechanism has been proposed for the prokaryotic CytR/cAMP-cAMP receptor protein (CRP) regulatory system. CytR is mediated by the formation of protein complexes in which CytR interacts with DNA and cAMP-CRP.

CytR is a member of the Lac/GalR family of DNA binding proteins but unlike other members of this protein family, CytR lacks the ability to locate its DNA binding site with a sufficiently high affinity to repress transcription. However, in the presence of cAMP-CRP, DNA binding of CytR is enhanced 1000-fold. Furthermore, cooperative binding of CytR and cAMP-CRP is mediated by direct interactions between the proteins. Therefore, it has been proposed that the promoter specificity of CytR is mediated by the cAMP-CRP complex.

Repression of GSTP1 transcription and the associated mechanisms of derepression have obvious implications for the regulation of GSTP1 levels in its role as a detoxification enzyme and in the development of antineoplastic drug resistance. Interestingly, negative regulation of the rat Pi class GST (GST-P) gene has previously been reported. The silencer region of the rat GST-P promoter (located between nucleotides −400 and −140) is comprised of multiple elements and is active not only in rat nonhepatoma and hepatoma cells but also in human and mouse cell lines. Moreover, at least two proteins have been shown to bind to this region, one of which has been partially purified. As stated above, our results have indicated that the human GSTP1 transcriptional repressor binds between nucleotides −97 and −90. This region shares strong homology (75%) with one of the protected elements (−166 to −159) within the rat GST-P silencer. Therefore, it is possible that a regulatory protein responsible for down-regulation of rat GST-P gene expression performs a similar function on its human counterpart.

To gain further insight into the negative regulatory mechanism of GSTP1 transcription, it is clearly imperative to establish the identity of both the repressor complex and the MCF7 C1 complex. Interestingly, the C1 element, which does not bind a Jun-Fos heterodimer in MCF7 cells, also shares homology with the antioxidant response element (GTGACNNNGC) found in the promoters of the rat GST Ya and quinone reductase genes.

Despite strong similarity with the known binding site for AP-1, the antioxidant response element motifs located...
in the rat GST Ya or quinone reductase promoters do not contain high affinity binding sites for Jun-Fos proteins (55, 56). Recent evidence has indicated that members of the Maf family of transcription factors may bind to the antioxidant response elements in the rat and human quinone reductase, the rat GST Ya, and rat GST-P promoters (57). The possibility that Maf-like proteins may contribute to the C1 complex bound to the GSTP1 promoter in MCF7 cells is currently under investigation.

Consistent with its implicated role in drug resistance, GSTP1 and GSTs in general have been proposed to play a role in protecting cells against the genotoxic effects of electrophilic carcinogens (3, 58). Intriguingly, a recent report described a remarkable decrease in GSTP1 expression that accompanied human prostatic carcinogenesis (8). This finding led the authors to postulate that perhaps a subpopulation of low expressing GSTP1 prostate cells undergo clonal expansion following carcinogen exposure. Therefore, understanding the molecular mechanisms of negative regulation of GSTP1 transcription may have important clinical implications for the control of GST gene expression and their contribution to chemoprevention.

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