Transcriptional Activation of the Alpha-1 Acid Glycoprotein Gene by YY1 Is Mediated by Its Functional Interaction with a Negative Transcription Factor

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ABSTRACT

Regulation of alpha-1 acid glycoprotein (AGP) gene expression involves both positive and negative transcription factors. We have previously identified two dominant factors: positive and negative transcription factors, AGP/EBP and factor B, respectively, involved in the transcription of AGP and other acute-phase response genes. In this report, we present evidence showing that the transcription of the AGP gene is positively regulated by a transcription factor, YY1. The activation of AGP gene by YY1 is mediated by a negative element B in the AGP promoter region. YY1 can also activate the B motif linked to a heterologous promoter. However, YY1 does not bind directly to the B motif *per se*. Rather, our data suggest that the activation of AGP gene by YY1 may be mediated by its functional interaction with factor B, which recognizes the B motif.

INTRODUCTION

ALPHA-1 ACID GLYCOPROTEIN (AGP) is one of the most abundant, acute-phase-responsive, liver-specific plasma proteins. The biological function of AGP is unknown, but there are indications that it may suppress the immune response (Bennett and Schmid, 1980). Some experiments suggest that AGP is a nonspecific antiinfection agent (Friedmann, 1983), and that it possesses nerve growth-promoting activity (Liu *et al.*, 1988). To investigate the regulation of AGP gene expression during physiological homeostasis and during perturbed physiological conditions (*i.e.*, acute inflammation), it is essential to study the complex interactions of various *trans*-acting factors in these different physiological states.

In rats and mice, the levels of liver AGP mRNA and plasma AGP protein increase 10- to 100-fold within 24 hr of experimentally induced inflammation (Baumann and Held, 1981; Baumann *et al.*, 1983; Kulkarni *et al.*, 1985; Baumann and Maquat 1986; Darlington *et al.*, 1986; Gauldi *et al.*, 1987; Klein *et al.*, 1988; Prowse and Baumann, 1988). The increase in mRNA is primarily attributed to transcriptional activation of the AGP gene (Kulkarni *et al.*, 1985; Baumann

and Maquat, 1986). We have previously identified four motifs recognized by a positive transcription factor, AGP/EBP, and a negative *cis* element recognized by a negative factor (*i.e.*, factor B) (Chang *et al.*, 1990; Lee *et al.*, 1993). During the acute-phase response, there is dramatic increase in the level of AGP/EBP, coupled to the decrease in factor B, resulting in the induction of AGP gene transcription (Lee *et al.*, 1993). In addition to AGP/EBP and factor B, glucocorticoids (Klein *et al.*, 1988) have also been shown to be involved in the induction of AGP gene. Despite the substantial progress in understanding the factors in regulating the activation of AGP gene during the acute-phase response, the regulation of this gene in normal physiological states remains to be investigated.

The upstream regulatory region of the AGP gene contains a sequence, GAACATT TT (–121 to –113) that is similar to a motif recognized by a transcription factor, YY1 (consensus sequence, CGACATTTT) (Shi *et al.*, 1991). YY1 is a human Krüppel-related protein that interacts with DNA elements (CGACATTTT, located at –50 to –70; CTC-CATTTT, located at the transcription initiation site) of the adeno-associated virus P5 promoter. These DNA elements are capable of repressing transcription directed by heterolo-

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gous promoters. E1A not only relieves repression exerted by YY1 but also stimulates transcription through the YY1 binding site (Shi *et al.*, 1991). There is evidence that YY1 mediates both the repression and activation responses (Shi *et al.*, 1991). The ability of YY1 to mediate opposite effects depends on the intracellular milieu (*e.g.*, the presence or absence of E1A). YY1 is related to the GL1-Krüppel family of genes (Ruppert *et al.*, 1988). The Drosophila Krüppel protein can also repress or activate transcription, depending on the context of its binding site (Frasch and Levine, 1987; Ruppert *et al.*, 1988; Licht *et al.*, 1990). Two different domains in the Krüppel protein have been reported to exhibit repression activity when fused to a heterologous DNA-binding domain. It remains to be determined whether the Krüppel protein contains an activation domain. In addition to YY1, several transcription factors identical or homologous to YY1 have been identified and cloned. Among them are: protein delta, which binds to sequence elements (GCNGC-CATC) downstream of the transcriptional start sites in ribosomal protein genes and functions as an activator (Hariharan *et al.*, 1991); NF-E1, which binds to the IgK 3' enhancer (CCACCTCCATCTT) and functions as a repressor (Park and Atchison, 1991); UCRBP, which binds to the UCR core (CGCCATTTT) in the long terminal repeat of Moloney leukemia virus (which also functions as a repressor) (Flanagan *et al.*, 1992); and F-ACT1, which binds competitively to the most proximal serum response element (CGCCATATT) of the skeletal α -actin gene and functions as a repressor (Lee *et al.*, 1992). These data indicate that the binding specificity of YY1 or its homolog is unusually diverse. Furthermore, a negative regulatory domain in the human papillomavirus type 18 promoter was shown to be the target site of YY1 (Bauknecht *et al.*, 1992). Taken together, these data suggest that, depending on the context, YY1 can function as an activator, a repressor, or an initiator of transcription. For these reasons, we decided to investigate the possible involvement of the YY1-like motif in the regulation of AGP gene expression. In this study we examined the regulation of wild-type and a series of mutants of AGP promoter in transient transfection assays. We found that YY1 can activate AGP promoter by relieving the negative action of B element, located at -40 to -66. However, YY1 did not bind directly to this negative element. Instead, it relieves the repression of AGP gene by its functional interaction with factor B.

MATERIALS AND METHODS

Preparation of nuclear extract

Liver nuclear extract was prepared according to the procedures of Gorski *et al.* (1986) from 200- to 300-gram Wistar rats either untreated or treated with lipopolysaccharide (LPS) (10 μ g/gram body weight) for 4 hr. Detailed procedures were as described (Gorski *et al.*, 1986).

Plasmids and oligonucleotides

The AGP/CAT, AGP/EBP/CAT, and CMV-AGP/EBP were as described (Chang *et al.*, 1990; Lee *et al.*, 1993).

CMV-YY1 (kindly provided by Dr. T. Shenk) was detailed elsewhere (Shi *et al.*, 1991). Various CAT constructs containing the mutated AGP promoter sequence were generated essentially as described (Lee *et al.*, 1993). pCAT-promoter (CAT reporter gene controlled by SV40 promoter) and pSV- β -Gal (β -galactosidase reporter gene controlled by SV40 promoter) were obtained from Promega (WI). These two reporter vectors were used for constructing the recombinant plasmids containing the oligomeric B motif. All the constructs were verified by sequence analysis. The oligonucleotides used were as follows: B, TACTGTCCCTGGCT-TCAGTCCCATGCCCT; U (UCR), TAACGCCATTTTG-CAAGGCAT; mutant UCR (mt U); TAAATACATTTT-GCAAGGCAT.

Analysis of protein-DNA interaction

Gel mobility-shift and footprinting assays were as described (Lee *et al.*, 1993). Briefly, an end-labeled DNA fragment (-180 to +10 from AGP promoter region) (5 ng) generated by polymerase chain reactions (PCR) was added to a 20- μ l reaction mixture containing 50 mM NaCl, 20 mM Tris-HCl pH 7.6, 1 mM MgCl₂, 0.2 mM EDTA, 10% glycerol, 5 mM DTT, and 1 μ g poly(dI-dC). Nuclear extract or partially purified fraction containing factor B was preincubated with recombinant YY1 or partially purified YY1 for 5 min at room temperature followed by adding the probe and incubated for 90 min on ice. DNase I (30 μ g/ml, Sigma) freshly diluted in 10 mM MgCl₂ and 5 mM CaCl₂ was added to the reaction mixture (usually 1-3 μ l was used). Digestion was performed for 2-3 min on ice and stopped by the addition of 80 μ l of stop solution containing 75 μ g of yeast tRNA/ml, 20 mM EDTA, and 0.5% NaDodSO₄. The samples were extracted with phenol chloroform, precipitated with ethanol at -70°C. The pellets were dried and resuspended in sequencing solution (95% formamide, 1% xylene cyanol FF, and 1% bromphenol blue), heated at 95°C for 3 min, and loaded onto a sequencing gel.

For gel mobility-shift assays, protein-DNA complexes were formed as described for the footprinting assays. After incubation for 20 min at room temperature, 1 μ l of 1% bromphenol blue was added, and the sample was loaded onto a 5% polyacrylamide gel (acrylamide/bisacrylamide = 30:1) containing 4% glycerol in Tris-Glycine buffer. Electrophoresis was performed at 150 V at room temperature with buffer circulation.

Recombinant YY1 and antibody to YY1

Recombinant YY1 was generated by cloning the *Nco*I-*Eco*RI fragment of YY1 cDNA (YY1 plasmid was kindly provided by Dr. T. Shenk, Princeton University) into plasmid pRSET (Invitrogen, San Diego, CA). The recombinant YY1 expressed in *Escherichia coli* [BL21 (DE3)] was purified by Ni column and detected by Western blot using rabbit polyclonal anti-YY1. Rabbit anti-YY1 was generated by immunizing the antigen expressed in *E. coli*. Briefly, a cDNA fragment corresponding to polypeptide of 232 amino acids containing the carboxy-terminal part of YY1 was cloned by PCR from human placenta cDNA library. The

sequence was verified by sequence analysis. The cDNA fragment was cloned into pRSET (Invitrogen, San Diego, CA) and expressed in BL21(DE3).

Cell cultures, DNA transfections, and CAT assays

Baby hamster kidney (BHK) cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. DNA transfections were performed by the calcium phosphate precipitation technique. For each 10-cm petri dish, the calcium phosphate-DNA precipitate contained 8 μ g of target plasmid, and 3 μ g CMV-YY1 or amino-terminal deleted YY1 (CMV-C250), which contains the carboxy-terminal 250 amino acids. Plasmid pGEM4 was used as a carrier to make the final DNA to be 15 μ g. Cells were harvested 48 hr post-transfection. Cell extract (30 μ g) was used for CAT assay. CAT activity was quantitated by densitometric scanning of the autoradiogram or by scanning of the TLC plate with BioRad Image analysis system.

Western blot analysis

Rat liver nuclear extracts were prepared as described (Chang *et al.*, 1990). Liver nuclear extracts were obtained from control and LPS-treated Wistar rats. Varying amounts of nuclear extracts were resolved on a 10% NaPodSO₄-polyacrylamide gel. Proteins were electroblotted to nitrocellulose membrane. The protein blot was subjected to standard Western blot development using rabbit anti-YY1 and goat anti-rabbit IgG alkaline phosphatase conjugate.

RESULTS

Transcriptional activation of AGP promoter by YY1

To investigate the possible involvement of YY1 in AGP gene regulation, we initiated functional and biochemical characterizations of YY1 on AGP regulation. BHK cells were used for the transfection assays because BHK and HepG2 cells behaved similarly as demonstrated by our previous studies (Lee *et al.*, 1993). Transfection of wild-type and several mutants of AGP-CAT together with CMV-YY1 showed that YY1 could activate AGP promoter (Fig. 1). Mutations in the sequence, GAACATTTT, do not have any effect on YY1's activation. These results indicate that YY1 can activate AGP transcription, but independently of the sequence, GAACATTTT. Further experiments by gel mobility-shift or footprinting assay using recombinant YY1 derived from *E. coli* have failed to show that YY1 could bind to this sequence (data not shown). Having demonstrated that YY1 could *trans*-activate AGP gene, we tested its possible interaction with the most prominent activator for AGP gene, AGP/EBP. It turned out that activation of AGP gene by YY1 and AGP/EBP is additive (Fig. 2). Therefore, the activation of AGP gene by YY1 and AGP/EBP are independent event. To understand the implications of these data further, we generated a series of mutants spanning from -140 to -94 and a B-element-truncated mutant (Lee *et al.*, 1993). The nucleotide sequence and the co-transfection data are shown in Fig. 3. YY1 could activate wild-type as well as every

FIG. 1. *Trans*-activation of the AGP gene by YY1. Cotransfection assays of CMV-YY1 expression vector and wild-type and mutated AGP sequences linked to CAT reporter gene. The upstream regulatory sequence (-122 to -155) of the AGP gene and the corresponding mutants are shown in the upper panel. The results of CAT assay are shown in the lower panel. CMV-YY1 (3 μ g) and 10 μ g of reporter gene were cotransfected into BHK cells. (-) Cells transfected with reporter gene without CMV-YY1; (+) cotransfected with CMV-YY1.

FIG. 2. The activation of AGP promoter function by AGP/EBP and YY-1 is additive. AGP/CAT, CMV-YY1, and CMV-AGP/EBP were detailed in the previous section or elsewhere. BHK cells were used for the transfection assays. AGP/CAT (10 μ g) and 1 μ g of CMV-YY1 and/or CMV-AGP/EBP were used for the transfection assay.

FIG. 3. Trans-activation of AGP gene by YY1 is mediated by a B motif. Cotransfection assays of CMV-YY1 expression vector and mutated upstream regulatory sequence (spanning from -140 to -94) of the AGP gene linked to CAT reporter gene. The mutated sequences are shown in the upper panel. dB is the deleted mutant of the B motif linked to CAT, as reported in our earlier publication (Lee *et al.*, 1993). The amounts of plasmid and the cells used for the transfection are the same as in Fig. 1. (- and +) Transfection in the absence and in the presence of CMV-YY1.

mutated AGP/CAT except the B-truncated mutant (Fig. 3, lower panel, dB). These data indicate that YY1 is unlikely dependent on a single motif spanning from -140 to -94 of AGP promoter for the activation. However, when the B-motif-deleted mutant was co-transfected with CMV-YY1, the activation was abolished (Fig. 3, dB). As detailed in our earlier publication (Lee *et al.*, 1993), the B motif is a negative element that is recognized by factor B. The CAT activity of B-motif-deleted mutant was substantially elevated as compared with the wild-type or other mutated AGP/CAT, consistent with the negative effect of the B motif. Having observed that YY1 could activate the AGP promoter but failed to do so when the B element was deleted, we initiated studies on the possible recognition of the B motif by YY1. Extensive experiments using a number of oligonucleotide probes and recombinant YY1 or YY1 purified from HepG2 failed to show that YY1 can bind to the B motif (data not shown). These data suggest that the observed activation of YY1 on the AGP promoter is unlikely to be mediated by direct interaction of YY1 and the B element; rather, a functional interaction may be the likely explanation.

FIG. 4. Transfection assays demonstrate that the activation of AGP promoter function by YY1 is through the B motif. CAT reporter constructs containing the wild-type AGP promoter, deletion mutants of B motif (dB), and upstream of -71 (-71), oligomeric B motif (B10) linked to SV40 promoter-CAT (oligo B), and SV40 promoter-CAT (pCAT-P) were used for transfection (-) or cotransfection with CMV-YY1 (+) into BHK cells. Reporter plasmid (10 μ g) and 3 μ g of CMV-YY1 were used.

YY1 can activate the B motif linked to heterologous promoter

The above experiments showed that the activation of YY1 on the AGP promoter is mediated by the B element, but not by direct protein-DNA interactions. To address further the issue of how the B motif might mediate the activation of the AGP promoter by YY1, we constructed a heterologous promoter (SV40) containing oligomeric B sequences as well as -71/AGP (upstream of -71 of AGP promoter was deleted) promoter linked to CAT (Lee *et al.*, 1993). Wild-type and -71/AGP promoter CAT could be activated by CMV-YY1 (Fig. 4). YY1 could also activate oligomeric B-linked heterologous promoters. In striking contrast to these data, YY1 had no effect on promoters that do not contain the B element. Consistent with the conclusion that B is a negative element from previous studies, heterologous promoters containing oligomeric B (B_{10}) element showed reduced activity. To rule out the possibility that there is a difference in transfection efficiency between experiments, we used B_{10} -linked-pSV- β -Gal for the transfection and co-transfection assays. The B_{10} -linked pSV- β -Gal was much weaker than the pSV- β -Gal when staining with X-Gal (data not shown). However, when YY1 was co-transfected with B_{10} -pSV- β -Gal, the β -Gal activity was elevated (data not shown). This increase in activity is not due to the increase in transfection efficiency when YY1 was present, as evident by the apparent number of cells positive in X-Gal staining are relatively constant (data not shown). These data suggest that YY1 is involved in modulating the factor B activity either by direct protein-protein or functional interaction with B factor or by repressing the expression of the B factor gene.

To rule out further any possible artefacts resulting from these cotransfection experiments, we constructed a YY1 expression vector (CMV-C250) in which only protein containing the carboxy-terminal 250 amino acids could be expressed. The results from the cotransfection experiments

FIG. 5. The *trans*-activation of the AGP gene by YY1 requires the full-length YY1 molecule. The upper panel shows the construction of YY1 and the truncated YY1 expression vectors, CMV-YY1 and CMV-C250. The lower panel shows the *trans*-activation of AGP/CAT by CMV-YY1 but not by CMV-C250. Cotransfection experiments were detailed in the legends of Figs. 1 and 2. The numbers on top of the lower panel indicate the fold stimulation relative to the basal CAT activity of each CAT reporter construct (normalized to 1). (–) Transfection with the CAT reporter plasmid alone; (YY) cotransfection with CMV-YY1; (C) cotransfection with CMV-C250. Wild-type (wt) or mutant AGP-CAT (G1, G2, and G3) are shown on the bottom line of the lower panel.

demonstrated that when the amino-terminal portion of YY1 was deleted, it failed to activate the AGP gene (Fig. 5). Taken together, the activation of the AGP gene by YY1 not only requires the B motif but also needs the amino-terminal portion of YY1 molecule.

YY1 does not interfere with the binding of B factor to its cognate motif

Having demonstrated that YY1 can activate the AGP gene through the B motif, we would like to know whether YY1 interacted with the B factor physically. Purified YY1 (from liver) or recombinant YY1 was incubated with rat liver-derived B factor. Purified YY1 did not interfere with the B factor binding to its motif in footprinting analysis (data not shown) or in gel mobility-shift assay in the presence or absence of specific antibody to YY1 (data not shown). Purified AGP/EBP or protein derived from other column fractions behaved similarly. Furthermore, recombinant YY1 did not inhibit the footprinting activity of B factor. These data indicated that YY1 did not form a protein–protein complex with factor B; rather, it interacted with B factor functionally without interfering with the DNA-binding activity of B factor.

The level of YY1 does not change during the acute-phase response

We have shown previously that the levels of AGP/EBP (the most prominent positive factor) and B factor (a negative factor) are induced and reduced during the acute-phase

reaction (Change *et al.*, 1990; Lee *et al.*, 1993). This may account in part for the net induction of the acute-phase-response genes. Because we have now shown that YY1 can activate the transcription of AGP gene, it would be interesting to examine the change in the protein level as well as the binding activity of YY1 during the acute-phase response. Liver nuclear extracts were prepared from untreated or LPS-treated rats and used for gel retardation assays (using UCR oligonucleotide, which also contains an overlapping AGP/EBP-binding site) and Western blot analysis. Both the binding activity and the protein level of YY1 remains constant whether pre- or post-acute-phase response (Fig. 6). AGP/EBP was increased during the acute-phase response, which was a nice internal control. Taken together, these data suggest that the protein level of YY1 does not change; however, its functional activity for interaction with B factor may be elevated (*e.g.*, through post-translational modification) during acute inflammation.

DISCUSSION

Mechanism of activation of AGP gene by YY1

In this paper we have demonstrated that YY1 activates transcription directed by the AGP promoter or heterologous (SV40) promoter containing the negative element B. YY1 represses activity of the SV40 enhancer/promoter containing the YY1-binding motif (Shi *et al.*, 1991). The fundamental difference between the YY1 motif-mediated and the B-motif-mediated activity of YY1 is the involvement of direct YY1-DNA interaction in the former case and only functional interaction of YY1 and B-motif in the latter case. Two lines of evidence suggest that the B motif of AGP promoter mediates YY1's activation of AGP gene: (i) the activation of AGP promoter by YY1 depends on the presence of B sequence; (ii) YY1 can activate B-motif-mediated repression of SV40 promoter when the B motif is inserted into this promoter. An extensive search failed to reveal any DNA element in the AGP promoter region that can be recognized by YY1. How can the B sequence mediate the action of YY1 without direct DNA–protein interaction? Because the B element was shown to be recognized by a negative transcription factor B (Lee *et al.*, 1993), four possible mechanisms may exist: (i) direct YY1 and factor B interaction at the protein level resulted in the reduced repressor activity of B factor; (ii) YY1 could repress the expression of the B factor, resulting in a reduced protein level of factor B; (iii) there is only a functional interaction between YY1 and factor B without direct protein–protein interaction, *e.g.*, modulating of factor B's activity by YY1; (iv) YY1 interacts with factor B functionally *via* an intermediary. For the first possibility, it could be a physical interaction between YY1 and factor B. For the second possibility, YY1 may bind to the cognate motif in the regulatory region of the factor B gene and result in repression of expression of factor B. For the third possibility, YY1 may affect factor B's activity by modification of factor B post-translationally. For the fourth possibility, there may exist a corepressor for the interaction of YY1 and factor B.

Two lines of evidence indicate that YY1 interacts with B factor functionally, but not physically (*i.e.*, complex forma-

FIG. 6. Determination of the protein level and the DNA-binding activity of YY1 in the nuclear extracts prepared from normal and LPS-treated liver. **A.** Western blot analysis showing the relative constant protein level of YY1 in normal rat liver nuclear extract (N) and extract prepared from LPS-treated liver (LPS). Rabbit antibody to YY1 was used for the blot analysis. The AGP/EBP level (indicated by a 35 and arrow) was used as a positive control to show its increase during the acute-phase response. **B.** Gel mobility-shift assay showing the constant level in binding activity of YY1 in normal liver nuclear extract and extract from liver of the acute-phase response (lanes 1–6 contain 10, 8, 7, 6, 4, and 2 μ g of nuclear extract, respectively). The arrow indicates the YY1-DNA complex; the arrow head indicates the AGP/EBP- or AGP/EBP-related factors.

tion): (i) both purified and recombinant YY1 cannot inhibit the footprinting activity of factor B, and antibody to YY1 failed to pattern of complex formation in a bandshift assay; (ii) although YY1 cannot bind to the B motif, it can activate genes that contain a B motif. The following examples show that YY1 could affect gene expression through a third proteins: (i) YY1 repression of the P5 promoter is relieved by adenovirus E1A protein (Shi *et al.*, 1991). (ii) A fusion protein of YY1 and the GAL4 DNA binding domain represses transcription of a thymidine kinase promoter with GAL4 binding sites (Seto *et al.*, 1991). (iii) c-Myc can form complex with YY1 and thus modulates the activity of YY1 (Shrivastava *et al.*, 1993). A simple model to explain the functional versatility of YY1 is that its interaction with different cellular proteins may alter its activity. During the acute-phase response, the binding activity of factor B decreases while both the protein level and the DNA-binding activity of YY1 do not change. The activity of YY1 can be modulated during the acute inflammation in such way so that its functional interaction with factor B is enhanced, while the DNA-binding activity of factor B is unaffected. However, this modification of YY1's activity, which ultimately affects the activity of factor B, may not involve in the formation of YY1-B complex. Alternatively, factor B is modified during the acute-phase response and results in the enhanced func-

tional interaction with YY1. Furthermore, both of these possibilities exist. Future experiments should be designed to resolve this issue.

A number of systems in which protein-protein interactions resulted in altered functions of a specific transcription factor have been demonstrated (Lee *et al.*, 1993; Nishio *et al.*, 1993; Stein *et al.*, 1993). The functions of YY1 are mediated mainly by its binding to a specific DNA motif (Hariharan *et al.*, 1991; Gualberto *et al.*, 1992; Montalvo *et al.*, 1991; Park and Atchison, 1991; Seto *et al.*, 1991; Shi *et al.*, 1991; Bauknecht *et al.*, 1992; Flanagan *et al.*, 1992; Lee *et al.*, 1992). Therefore, YY1 may also modulate the expression of factor B transcriptionally through its binding to the regulatory regions of factor B gene. Definitive answers must wait until the factor B gene is isolated and cloned. Taken together, our present data and the rapid reaction during the acute inflammation suggest that the functional interaction of YY1 and factor B, rather than the transcriptional mechanism is the more likely mechanism in modulating the factor B activity. However, despite vigorous tests (performed by anti-YY1 antibody depletion followed by footprinting assay or the use of anti-YY1 antibody in gel mobility-shift assay), we have failed to demonstrate that the YY1 and factor B complex exists.

One of the most dramatic features of AGP gene regulation

FIG. 7. Schematic representation of the regulation of AGP gene by various transcription factors. Cytokines stimulate AGP/EBP- or AGP/EBP-related *trans*-activators and result in the induction of AGP during the acute-phase response. YY1 participates in the AGP regulation by repressing the functional activity of a negative factor, B. Thus, YY1 activates AGP gene indirectly by functional interaction with factor B.

is its induction during the acute-phase response. We have previously shown the level of AGP/EBP increased several fold during the acute-phase response. We also documented a decrease in the binding activity of factor B. In this report, we presented evidence for the activation of AGP gene by YY1, including that (i) this activation is mediated by B motif; (ii) the activation of AGP gene by YY1 requires the amino-terminal portion of YY1 molecule; and (iii) YY1's function in activating AGP gene is independent of AGP/EBP.

Both the protein level and the binding activity of YY1 do not change in rat liver during the acute-phase response (Fig. 6). Because the mechanism of reduction of factor B's binding activity during the acute-phase response remains to be solved, it is highly possible that some post-translational mechanisms are involved in regulating factor B's activity, such as interacting with YY1. If this indeed happens, then the functional activity of YY1 under normal physiological conditions must be different from that during the acute-phase response. The regulatory role of YY1 on factor B's level or activity offers an attractive model for gene regulation: Regulation of a negative factor by another negative/positive (Yin-Yang-1) factor. A schematic mode summarizing the regulation of AGP gene is presented in Fig. 7.

Additive effect of activation by YY1 and AGP/EBP on the AGP promoter

We have shown previously that AGP/EBP could activate AGP transcription and that AGP/EBP is the key nuclear factor in conveying the signals [*i.e.*, cytokines like interleukin-1 (IL-1), IL-6, or leukemia inhibitory factor] to their target genes (*e.g.*, AGP) during the acute-phase response. To

determine whether the activation effects of AGP/EBP and YY1 on AGP are independent events, we performed transfection experiments using wild-type AGP/CAT, CMV-AGP/EBP, and CMV-YY1. As shown in Fig. 2, AGP/EBP and YY1 could activate the AGP promoter, and the effect of these two expression vectors seems to be additive. These data suggest that AGP/EBP and YY1 do not interact with one another to activate the AGP gene, but rather act independently in activating the AGP gene. These results are consistent with the data that the activity of YY1 depends on the B motif whereas AGP/EBP depends on other motifs. Taken together, YY1, AGP/EBP, C/EBP- α , and factor B are important *trans*-acting factors in regulating AGP gene transcription. The data present in this paper are particularly important in the potential modulation of YY1's activity during the acute inflammation and its subsequent interaction with factor B. Further understanding along these lines may clarify not only factor B's regulation but also YY1's mode of action and regulation.

ACKNOWLEDGMENTS

We thank Dr. Tung-Tien Sun for a critical review of the manuscript, Mr. Tso-pang Yao for performing the PCR of YY1 cDNA, Dr. Wuh-Liang Hwu for technical help, Wen-Hai Tsai for nuclear extracts, and Drs. T. Shenk for CMV-YY1 and K. Ozato for the expression vector of UCRBP. This research was supported by grants NSC82-0412-B002-251 from the National Science Council.

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Received for publication March 22, 1994, and in revised form May 24, 1994.