The α-Isotype of the CCAAT/Enhancer-binding Protein Is Required for Mediating cAMP Responsiveness of the Phosphoenolpyruvate Carboxykinase Promoter in Hepatoma Cells*

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Cyclic AMP is the intracellular mediator of many extracellular stimuli and increases in the cellular concentration of this second messenger lead to the transcriptional activation of many genes. Distinct DNA sequences within gene promoters, termed cAMP response elements (CREs), have been shown to confer such cAMP responsiveness. CREs function by acting as binding sites for transcription factors which mediate the CAMP effect. These transcription factors include some members of the CREB/ATF-1 protein family (reviewed in Ref. 1), as well as activator protein 2 (2). However, in many, if not most, promoters examined to date, the maximal effect of CAMP on transcriptional activity is mediated by more than one cis-element. In these cases, either multiple CREs are present, or different cis-elements cooperate with the CRE to mediate the response, forming what has been termed a “CAMP response unit” (3). For example, the glycoprotein hormone (α-subunit) gene promoter contains two tandemly arranged CREs (4), the c-fos promoter contains four cis-elements which mediate the CAMP response (5), and the CAMP responsiveness of the tyrosine aminotransferase promoter is mediated by synergistic interactions between a CRE and a binding site for hepatic nuclear factor 4 (6). It has been hypothesized that utilization of several proteins to mediate the response may allow for a fine-tuned response of promoter activity to extracellular stimuli, as well as provide a mechanism for cell/tissue-specific responses (7).

The promoter for the gene that codes for the cytosolic form of phosphoenolpyruvate carboxykinase (PEPCK), the rate-limiting enzyme of gluconeogenesis, is strongly activated by CAMP in liver but poorly in kidney (8, 9). Footprinting experiments indicated that while both kidney and liver nuclear extracts contained CRE-binding activity, only extracts from liver footprinted a region upstream of the CRE (10). Subsequently, this liver-specific region was shown to contain several protein binding domains, all of which were critical for CAMP responsiveness of the promoter both in vivo and in vitro (3, 7, 11, 12). Thus, full responsiveness of this promoter to CAMP requires synergism between the CRE and the liver-specific region. Recently, we provided evidence that CREB mediates the response through the CRE (13). The factors which bind to the liver-specific region, however, have not yet been identified, although C/EBP protein members have been shown to bind to this region in vitro and overexpression of C/EBP proteins leads to transactivation of the promoter (14, 15).

Our goal is to identify and characterize the proteins which participate in mediating this liver-specific CAMP responsiveness of the PEPCK promoter in order to fully understand the molecular details of the response. In this study, we provide functional evidence in support of a role for C/EBPα in mediating the CAMP responsiveness of this gene synergistically with CREB.

EXPERIMENTAL PROCEDURES

Materials—DNA-modifying enzymes were purchased from New England Biolabs, Promega Corp., and U. S. Biochemical Corp. The abbreviations used are: CRE, cAMP response element; CREB, cAMP response element-binding protein; PEPCK, phosphoenolpyruvate carboxykinase; C/EBP, CCAAT/enhancer-binding protein; AP-1, activator protein 1; CAT, chloramphenicol acetyltransferase; PKA, protein kinase A; bZIP, basic region leucine zipper; LSR, liver-specific region; T₃, triiodothyronine.
C/EBPα Participates in Mediating cAMP Responsiveness

Poly(dI-dC) was purchased from Pharmacia Biotech Inc. [acetyl-3H]CoA (10 Ci/mmol) was purchased from DuPont NEN. Tissue culture supplies were from Life Technologies, Inc. HepG2 and JEG3 cells were acquired from American Type Culture Collection. Synthetic oligonucleotides were purchased from either the Regional DNA Synthesis Laboratory (University of Calgary) or Bio/Can Scientific. GAL4-specific cleotides were purchased from either the Regional DNA Synthesis Laboratory or Bio/Can Scientific. Synthetic oligonucleotides were acquired from American Type Culture Collection. An antisense to cAMP was purchased from Upstate Biotechnology, Inc.

Liver-specific region

**RESULTS**

A schematic of the cAMP response unit of the PEPCK promoter is shown in Fig. 1. It consists of two components: the CRE, to which CREB binds, and the liver-specific region, which consists of three sites to which C/EBP binds and an AP-1 site. All five of the binding sites shown are required for optimal responsiveness to cAMP.

Transfection Experiments—HepG2 cells were grown in Dulbecco’s modified essential medium containing 10% fetal calf serum. Four hours before transfection, the cells were plated to an approximate 30% confluence in 10-cm plates. DNA transfections were carried out by the calcium phosphate precipitate technique as described in Sambrook et al. (16). RSV-gal vector was co-transfected in all experiments to monitor transfection efficiency. The amount of DNA used in the various transfection experiments was maintained at 25 μg plate by the addition of the phagemid pTZ18R. RSV-gal vector is an expression vector for a dominant negative C/EBP polypeptide, driven by the RSV promoter, which preferentially forms heterodimers with C/EBP proteins (17). Expression vectors for GAL4-C/EBP proteins are detailed below. All other expression vectors and reporter plasmids used in this study, with the exception of −109/G3A1 (see below), have been described previously (3, 7, 13, 18).

A synthetic PEPCK promoter-CAT reporter plasmid was created to examine the transcriptional properties of the GAL4-C/EBP fusion proteins. This plasmid, called −109 PCK-CAT (3), consists of two components; the CRE, to which CREB binds, and the liver-specific region, which contains three binding sites for C/EBP and an AP-1 site. All five of the binding sites shown are required for optimal responsiveness to cAMP.

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activating the PEPCK promoter (−490 PCK-CAT), which contains the intact cAMP response unit, including the C/EBP binding sites, by approximately 40-fold. However, in the presence of expressed GBF-F, the activation by PKA was significantly reduced to about 9-fold. Experiments where increasing amounts of GBF-F were expressed indicated that maximum inhibition that could be achieved was a reduction to about 5-fold activation by PKA, consistent with the responsiveness mediated by the CRE alone (3). The specificity of this inhibitory effect was determined by examining the effect of GBF-F on the cAMP responsiveness of the α-subunit of glycoprotein hormone gene promoter in JEG-3 cells. The cAMP responsiveness of this promoter is mediated solely by CREB bound to two tandemly arranged CREs (18), thus this promoter should be relatively immune to the effects of the C/EBP repressor due to incompatible bZIP domains. As shown in Table I, the approximately 20-fold activation of the α-gene promoter by PKA was unaffected by the expression of GBF-F in JEG-3 cells.

The data above provide the first functional evidence that C/EBP proteins play a role in mediating the cAMP responsiveness of the PEPCK promoter in liver. In an effort to further test this hypothesis as well as to identify which isoform mediates the response, we created expression vectors for GAL4-C/EBPα fusion proteins and tested their activity on promoters which have the C/EBP binding sites replaced by binding sites for GAL4. As shown in Fig. 3, this approach allows the examination and characterization of C/EBP proteins in mediating the cAMP responsiveness. A, Gα1 contains the entire rat C/EBPα protein, minus the amino-terminal five amino acids, linked to the DNA-binding domain of the yeast transcription factor GAL4. Gα2 is similar to Gα1, except that amino acids 218–359, which contain the bZIP domain, have been deleted. Gβ1 contains all but the amino-terminal seven amino acids of human C/EBPβ (also called NFIL-6 (23)) linked to GAL4. Gβ2 is similar to Gβ1 except that the leucine zipper has been deleted, which disables the dimerization and thus DNA binding activity of this bZIP domain. Western blot analysis using a GAL4 antibody verified that all four proteins accumulated to approximately equivalent levels when expressed in HepG2 cells. B. The reporter plasmid −109/G3A1 consists of the −109 PCK-CAT 5′-deletion promoter (3), which contains the CRE, along with three GAL4-binding sites and an AP-1-binding site. The −109/G3 promoter is similar except that it lacks the AP-1 site. The −68/G3 promoter consists of the −68 PCK-CAT 5′-deletion promoter (3), which is a minimal promoter containing a TATA box, to which has been ligated three GAL4 binding sites. All three of these synthetic promoters drive expression of the CAT reporter gene. Refer to "Experimental Procedures" for details on plasmid construction.

response, we took advantage of the development by Olive et al. (17) of a "designer" dominant negative C/EBP repressor molecule. This C/EBP repressor molecule, termed GBF-F, is a chimera containing a DNA-binding (basic) region from the plant bZIP protein GBF-1 and a modified leucine zipper which preferentially forms heterodimers with other C/EBP molecules rather than self-homodimers. Because of this preference for heterodimer formation, and because GBF-F lacks a transactivation domain, expression of this chimera in cells results in the formation of inactive heterodimers with all known members of the C/EBP family and thus repression of C/EBP transactivation (17). Initially, we tested the ability of GBF-F to inhibit the C/EBP-mediated activation of the PEPCK promoter in HepG2 cells. As shown in Fig. 2A, the 6-fold transactivation of the −490 PCK-CAT produced by C/EBPα overexpression was significantly inhibited by co-expression of GBF-F. In order to verify that the bZIP domain was required for this repressor effect, we performed a domain-swap experiment examining the ability of Gβ2, a C/EBPβ fusion protein that has the bZIP domain replaced by the DNA-binding domain of the yeast transcription factor GAL4 (see Fig. 3), to activate a PEPCK promoter derivative (−109/G3A1), which has the three C/EBP binding sites replaced by binding sites for GAL4. As shown in Fig. 2B, Gβ2 strongly transactivated the modified PEPCK promoter and was insensitive to the repressor effect of GBF-F. Thus, the repressor effects of GBF-F on the PEPCK promoter appear to be specific for the C/EBP bZIP domain.

We next examined the ability of GBF-F to inhibit the PKA responsiveness of the PEPCK promoter. As shown in Table I, expression of the catalytic subunit of PKA in HepG2 cells activated the PEPCK promoter (−490 PCK-CAT), which contains the intact cAMP response unit, including the C/EBP binding sites, by approximately 40-fold. However, in the presence of expressed GBF-F, the activation by PKA was significantly reduced to about 9-fold. Experiments where increasing amounts of GBF-F were expressed indicated that maximum inhibition that could be achieved was a reduction to about 5-fold activation by PKA, consistent with the responsiveness mediated by the CRE alone (3). The specificity of this inhibitory effect was determined by examining the effect of GBF-F on the cAMP responsiveness of the α-subunit of glycoprotein hormone gene promoter in JEG-3 cells. The cAMP responsiveness of this promoter is mediated solely by CREB bound to two tandemly arranged CREs (18), thus this promoter should be relatively immune to the effects of the C/EBP repressor due to incompatible bZIP domains. As shown in Table I, the approximately 20-fold activation of the α-gene promoter by PKA was unaffected by the expression of GBF-F in JEG-3 cells.

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ence by endogenous C/EBP proteins, as well as identification of the protein domain(s) which mediate the response. A schematic of the fusion proteins utilized in this study, shown in Fig. 3A, have either the entire protein or the transactivation domain (without a functional bZIP domain) linked to the DNA-binding domain of GAL4. It should be noted that only the α and β isoforms of C/EBP were examined, since the other major isoform expressed in liver, C/EBPδ, is not present in significant amounts in liver except under acute phase response conditions (24).

Initially, we examined the GAL4-C/EBP fusion proteins for their constitutive transcriptional activity by using a CAT reporter gene driven by a minimal promoter containing three GAL4 binding sites, termed −68/G3 CAT (see the schematic in Fig. 3B). As shown in Table II, the GAL4 DNA-binding domain alone (GAL4(1–147)) did not transactivate this promoter. Fusing the entire C/EBPα coding region to the GAL4 domain, producing G4.1, resulted in a protein that produced transactivation, although removal of the bZIP domain, forming G4.2, created a much stronger transactivator. The GAL4 fusion protein containing the entire C/EBPβ protein (Gβ1) did not demonstrate transactivation activity, although once again removal of the bZIP domain, forming Gβ2, resulted in a strong transactivator. We speculate that the poor transactivation activity of G4.1 and Gβ1 is related to the fact that these fusion proteins have two dimerization domains at each end of the polypeptide, one contributed by the GAL4 DNA-binding domain and the other by the C/EBPα bZIP domain, which could interfere with the transactivation properties of the fusion protein. However, we felt it important to test such constructs, since the bZIP domain has been shown previously to be important for the ability of human C/EBPβ to synergize with the transcription factor NF-κB (25). The activity of the GAL4-C/EBP fusion proteins was dependent upon the presence of GAL4 binding sites in the promoter; demonstrated by the observation that none of the fusion proteins significantly transactivated the promoter −68 PCK-CAT which lacks GAL4 binding sites (Table I).

We next tested the GAL4-C/EBP fusion proteins for their ability to mediate PKA responsiveness. Previously, we had shown that we could reconstitute the cAMP response unit of the PEPCk promoter by linking three C/EBP binding sites and the native AP-1 site of the PEPCk promoter to a S′ deletion of the PEPCk promoter containing the CRE, creating the −109/C3A1 promoter (3). This reconstituted PEPCk promoter mimicked the PKA-responsive characteristics of the native promoter. Thus, to test the ability of the GAL4-C/EBP fusion proteins to mediate PKA responsiveness, we simply replaced the C/EBP binding sites with GAL4 binding sites to form −109/G3A1 (Fig. 3B). This reporter vector, in the absence of GAL4 fusion protein expression, was activated approximately 3-fold by PKA expression (Table III), consistent with the PKA responsiveness mediated by the CRE alone (3). Expression of GAL4(1–147) in HepG2 cells provided no enhancement of this PKA responsiveness, and expression of G4.1, Gβ1, and Gβ2 produced the same negative result (Table IIII). However, when G4.2 was expressed in HepG2 cells, a 52-fold activation by PKA was observed, similar to the level of PKA activation observed using the intact PEPCk promoter in HepG2 cells (3). This finding indicates that the bZIP domain is not required for this activity of C/EBPα, which was confirmed by the observation that the PKA responsiveness mediated by G4.2 is not inhibited by the co-expression of GFB-F.2. The inability of G4.1 to mediate a PKA response may be due to the abnormal structure of this fusion protein as discussed above.

Previously, in characterizing the cAMP response unit of the PEPCk promoter, we showed that maximal responsiveness to PKA or cAMP analogs required all three components: the CRE, the three C/EBP binding sites, and the AP-1 site (3). Elimination of any one component significantly decreased the activity of the cAMP responsiveness. In order to further test our hypothesis that C/EBPα was the isoform involved, we tested the activity of G4.2 to mediate PKA responsiveness on promoters lacking one or more components of the cAMP response unit (see Fig. 3 for a schematic of these promoters). As shown in Table III, the synthetic promoter −68/G3, which contains the requisite three GAL4 binding sites but lacks the CRE and AP-1 sites (Fig. 3B), was unable to be significantly activated by PKA in the presence of G4.2. The small degree of activation obtained is consistent with previous observations from our laboratory which indicated that a promoter containing three C/EBP binding sites alone can mediate a weak PKA response (3). Additionally, the synthetic promoter −109/G3, which contains the CRE and the three GAL4 sites but lacks the AP-1 site (Fig. 3B), was not strongly responsive to PKA in the presence of G4.2 (Table III), again consistent with previous observations (3). Thus, the activity of G4.2 on the reconstituted PEPCk promoter (contain-
ing three GAL4 binding sites) requires the presence of the CRE and the AP-1 sites for full PKA-responsiveness, mimicking previous observations using the native C/EBP binding sites present in the promoter and the proteins native to HepG2 cells.

The requirement for C/EBPα in the cAMP response unit now provides a possible explanation for the weak activation of the PEPCK promoter by PKA or cAMP in most non-hepatoma cell lines, which typically do not express C/EBP proteins at significant levels. This hypothesis was tested by examining whether overexpression of C/EBPα could restore PKA responsiveness of the PEPCK promoter in JEG3 cells, a human placental cell line in which many studies on cAMP responsive promoters have been performed. Similar to previous observations, -490 PCK-CAT vector was not responsive to PKA in JEG3 cells, and overexpression of C/EBPα did not restore responsiveness (Fig. 4A). C/EBPα also did not transactivate the PEPCK promoter in JEG3 cells, even though C/EBPα accumulated in transfected JEG3 cells similar to the level achieved in transfected HepG2 cells as indicated by Western analysis. Since previous studies had indicated that the bZIP domain may modulate the activity of C/EBP proteins in non-hepatoma cells (26), we examined the ability of Gα2 to mediate PKA responsiveness on the synthetic promoter -109/G3A1. This promoter, in the absence of Gα2 expression, was activated less than 2-fold by PKA (Fig. 4B). Gα2 showed no constitutive transcriptional activity in JEG3 cells; however, it mediated a strong 52-fold activation in response to PKA. This “restored” PKA responsiveness was not related to some cryptic characteristic of the reconstituted promoter, since -109/C3A1, which is identical to that of -109/G3A1 except that it contains C/EBP binding sites instead of GAL4 sites (3), was not responsive to PKA in the absence or presence of C/EBPα expression (Fig. 4C). Thus the bZIP domain of C/EBPα appears to inhibit the PKA-mediating properties of this transcription factor in non-hepatoma cells.

DISCUSSION

The PEPCK promoter offers a good model to examine how genes can respond to hormonal signals in a tissue-specific fashion. The cAMP responsiveness of this promoter is robust in liver-derived cells but weak in other cell types, and this liver-specific responsiveness is mediated by a complex cAMP response unit (3, 12). Consistent with the liver-specific nature of the response, three of the cis-elements required for maximal hormonal responsiveness bind factors that are enriched in liver (10). Results from in vitro DNA-protein binding assays suggested that this factor may be a member of the C/EBP family (3, 14, 15), although prior to the present study there has been no functional data in support of this hypothesis nor any data indicating what isoform may be involved. In the present study, we have obtained functional data in support of a role for C/EBP proteins, and, furthermore, using GAL4 fusion methodology, into JEG3 cells along with a 5 μg of C/EBPα expression vector and/or 2 μg of PKA expression vector per plate as described under “Experimental Procedures.” B, a transfection experiment was designed similar to that described in A except that the CAT reporter plasmid is -109/G3A1, which is a synthetic PEPCK promoter containing the CRE, the AP-1 site, and three GAL4 binding sites. Five μg of the Gα2 expression vector along with 2 μg of PKA expression plasmid were used. C, a transfection experiment in JEG3 cells was performed similar to that described for A and B, except that the CAT reporter plasmid was -109/C3A1, which is a synthetic PEPCK promoter containing the CRE, the AP-1 site, and three C/EBP binding sites (3). The values shown are the averages ± S.E. of at least three experiments and are expressed relative to the CAT activity obtained with transfection of the CAT reporter plasmid alone, which was set arbitrarily at 1.0.

3 W. J. Roessler and G. F. Davies, unpublished observations.
have identified that the α-isofrom mediates maximal cAMP responsiveness of this promoter. It should be noted that the findings of this study do not rule out the possibility that a combination of α and β isoforms bound to the three C/EBP binding sites could also mediate a cAMP response, only that three C/EBPα, but not three C/EBPβ, molecules bound to the promoter can mediate the response. It is also acknowledged that a role for C/EBPβ in the cAMP response, which was not explored in this study, cannot be formally eliminated as a possible candidate protein. However, given that 1) the transactivation domains of C/EBPα and δ share no apparent similarities, 2) the expression of C/EBPδ in liver is weak except under acute phase response conditions, and 3) the cAMP response is apparently mediated by a specific domain lying within the transactivation domain of C/EBPα as evidenced by the inactivity of the C/EBPβ transactivation domain, we feel that a role for C/EBPβ is unlikely.

Previous hypotheses as to what isofrom of C/EBPβ, if any, might be responsible for mediating the cAMP responsiveness of this promoter suggested C/EBPβα as a more likely candidate. This reasoning was based on several studies which linked the β-isofrom with the cAMP signaling system, which included the ability of C/EBP to (i) stimulate C/EBPβ gene expression (15) and (ii) to stimulate translocation of C/EBPβ from the cytosol to the nucleus (27), although this latter effect is likely cell type-specific. Recently, Darlington and co-workers (28) created knockout mice deficient in C/EBPα and δ, and they observed that PEPCK gene expression, which is normally turned on just prior to birth, was significantly reduced in these mice during the first few hours postpartum. While PEPCK gene expression in these knockout mice recovered to normal levels by 7–12 h postpartum, perhaps due to the presence of other C/EBP isoforms, it has not yet been determined whether these mice have the ability to respond to cAMP. The results of the present paper would suggest that, while basal expression of PEPCK gene expression is possible in these mice due to the many transcription factors which likely act on the promoter, including NF-1, HNF-1, HNF-4, CREB, AP-1, (10, 29–32), the lack of C/EBPα should significantly limit the extent to which this promoter can be activated by a rise in intrahepatocyte cAMP levels. In this respect, it is noteworthy that these knockout mice were severely hypoglycemic and could only be kept alive by glucose injections, implicating that there was an impairment in activating gluconeogenesis, the rate-limiting enzyme of which is PEPCK (33).

The findings of the present study indicate that the βZIP domain of C/EBPα is not required for its ability to mediate cAMP responsiveness in hepatoma cells. Structure-function studies of this protein indicate that the βZIP domain is also not required for its constitutive transactivation function, which instead is mediated by several regions in the amino terminus, including a proline-rich domain (26, 34, 35). While it is not yet known whether the domain(s) mediating the C/EBPα co-resides with the constitutive transactivation domains, the ability of Ga2 to mediate PKA responsiveness in JEG3 cells, without demonstrating any constitutive transactivation function (Fig. 4B), suggests that different regions of the protein may mediate cAMP-inducible and constitutive activities, similar to that which has been observed for the transcription factor CREB (36, 37).

The βZIP domain may, however, play an important role in modulating the activity of C/EBPα in non-hepatoma cells. Our studies using JEG3 cells suggest an important role for the βZIP domain of C/EBPα in mediating the transactivation properties of C/EBPα. Removal of the βZIP domain allowed unmasking of the PKA-mediating activity of C/EBPα, although no constitutive activity was detectable (Fig. 4, A and B). These results suggest that the βZIP domain exerts a strong inhibitory effect on the PKA-mediating activity of C/EBPα in JEG3 cells. Nerlov and Ziff (26) also showed that the βZIP domain exerted a strong inhibitory effect on the ability of C/EBPα to transactivate the albumin promoter in HeLa cells. Whether this masking effect occurs through modulation of the DNA binding activity or the transactivation properties is not clear, although it is interesting to note that the DNA-binding activity of C/EBPα can be attenuated by phosphorylation of serine 299 (38). The “scissors-grip” model of the tertiary structure of the βZIP domain (39) predicts that serine 299, which lies within the basic region, interacts with the major groove of the DNA. Phosphorylation of this serine residue, which has been shown to be a good substrate for protein kinase C (38), could alter the contact between C/EBPα and the negatively charged DNA binding site. Whether the observed “masking” of C/EBPα’s transactivation properties is mediated by PKC in vivo remains to be determined. However, if this turns out to be the case, then the masking phenomenon would also be expected to be operational in liver-derived cells that contain protein kinase C activity. Studies examining the effect of protein kinase C on C/EBPα’s participation in the cAMP responsiveness of the PEPCK promoter are currently under way.

A question that arises from this study is how does C/EBPα get specifically recruited to the PEPCK promoter? In vitro binding assays indicate that both α and β isoforms bind, with identical relative affinities, to the same sites on the promoter (14, 15), and the similarities in their βZIP domains as well as results from comparative in vitro binding assays (40, 41) suggest that their absolute binding affinities to the three C/EBP binding sites in the PEPCK promoter would be similar. Thus, differences in DNA binding affinities are unlikely to explain the specific recruitment of the α-isofrom to the promoter. Another model for strategic positioning of proteins involves the specific “fitting” of transcription factors to promoters, using protein contact surfaces of adjacent and bound factors in addition to DNA sequences (42). In support of this model, studies from our laboratory have shown that while the AP-1 alone or in conjunction with CREB has no effect on cAMP responsiveness, it greatly augments the weak synergism displayed between the three C/EBP proteins and CREB in mediating cAMP responsiveness (3). This “augmenting activity” of AP-1 could take the form of recruiting C/EBPα to the promoter, at least to one of the C/EBP binding sites and possibly all three. Such an activity would likely require protein-protein interactions, and it is noteworthy that not only does AP-1 bind to the promoter in a region where it is closely surrounded by the three C/EBP molecules, but one of the C/EBP binding sites is closely juxtaposed to the AP-1 site such that an uninterrupted DNase I footprint extends over both sites on the sense strand using liver nuclear extracts (10). However, since AP-1 is also required for maximal cAMP responsiveness in the studies using Ga2 (Table III), where no specific recruitment is necessary due to the GAL4 domain, AP-1 must possess additional functions besides the putative recruiting role.

The findings of this study have important implications for other regulatory aspects of the PEPCK promoter. Thyroid hormone (T3) stimulates transcription of this gene, and T3 responsiveness is mediated by a C/EBP-binding site that resides within the LSR along with a typical thyroid hormone response element (43, 44). While it is still unknown as to whether C/EBPα is involved in this response, it seems possible that this transcription factor could serve to integrate information from different signaling pathways along with tissue-specific responses. In fact, the involvement of C/EBPα in both the cAMP
and thyroid hormone responses may provide a mechanism for the synergistic activation of PEPCK gene transcription elicited by these two signals (43). Studies examining the role of C/EBPα in the T₃ response, along with characterization of the specific domains that mediate the various responses, should provide interesting information on the various functions mediated by this protein.

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