Opposing Actions of Fos and Jun on Transcription of the Phosphoenolpyruvate Carboxykinase (GTP) Gene

DOMINANT NEGATIVE REGULATION BY FOS*

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Jun homodimers and Fos/Jun heterodimers bind to the gene for phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32) (PEPCK) at three sites within the first 350 base pairs of the promoter. These include CRE-1 (−82 to −90), and P3(II) and P4 (−252 to −258 and −268 to −285, respectively). Over-expression of Jun in HepG2 cells resulted in a 10–15 fold increase in the level of transcription of a chimeric PEPCK (−490 to +73)-CAT gene, while expression of Fos decreased transcription and blocked the induction of transcription from the PEPCK promoter by Jun. The action of Fos and Jun on PEPCK gene transcription involved each of the Fos/Jun-binding sites and was modulated by additional transcriptional regulatory elements within the PEPCK promoter. The ability of Fos to inhibit PEPCK transcription was dependent upon P3(0), a region of the promoter which does not bind Fos/Jun heterodimers, but does bind members of the C/EBP family of transcription factors. Stimulation of PEPCK transcription by 8-Br-cAMP or by overexpression of the catalytic subunit of protein kinase A was inhibited by Fos expression. The inhibitory effects of phorbol esters and protein kinase C on PEPCK gene expression may be mediated through the action of Fos and Jun.

Since the initial discovery that Fos and Jun, products of the c-fos and c-jun proto-oncogenes, interacted with specific DNA sequences to modulate gene transcription, new information has suggested a central role for these proteins in the control of eukaryotic gene expression. Fos and Jun are rapidly induced in hepatic cells by insulin (Messina 1990; Mohn et al., 1990), and following partial hepatectomy (Mohn et al., 1990). In fibroblasts, Fos and Jun are induced by platelet-derived growth factor (Muller et al., 1984). In cells of the hippocampus, Fos and Jun levels increase in response to electrical stimulation or specific neurotransmitters (Morgan et al., 1987; Morgan and Curran, 1991; Sheng and Greenberg, 1990), and in aortic smooth muscle cells Fos and Jun are induced by angiotensin II (Nafillan et al., 1990). The levels of Fos and Jun activity are markedly increased by phorbol esters (Angel et al., 1988; Curran, 1988), agents which mimic the affect of diacylglycerol, an intermediate formed by phospholipase C, which induces the activity of protein kinase C (Schalasta and Doppler, 1990). Inhibitors of phospholipase C repress c-fos transcription (Schalasta and Doppler, 1990). Jun/Jun homodimers and Fos/Jun heterodimers as well as other members of the Jun and Fos families have been shown to modulate the transcription of a number of genes through a common sequence element termed an AP-1 site (activator protein 1) or TPA-responsive element (Angel et al., 1987a, 1987b; Lee et al., 1987a, 1987b; Nakabeppu et al., 1988; Rauscher et al., 1988a, 1988b). This sequence motif and the related CRE1 motif (cAMP response element) mediate the transcriptional response to multiple signal transduction pathways (Hai and Curran, 1991).

In addition to binding to AP-1 sites, Fos and Jun are each involved in interactions with other proteins. Jun forms a complex with the glucocorticoid receptor, thereby damping the ability of the receptor to bind DNA (Yang-Yen et al., 1990; Schule et al., 1990). Jun may bind to other steroid receptors, while Fos can form complexes with proteins other than Jun and acts through sequences other than the AP-1 element (Lucibello et al., 1990; Gius et al., 1990). An interaction between Jun and the steroid hormone receptors is likely to be involved in the regulation of genes of metabolic importance. For example, the concentration of Jun is markedly induced by deprivation of a single amino acid (Pohjampulo and Holtta, 1990) suggesting that it may play a role in regulating the cellular response to nutritional stress.

In this report, we examine the possibility that Fos and Jun may be involved in mediating the rapid changes in PEPCK gene transcription. PEPCK catalyzes the rate-limiting step in gluconeogenesis, and its cellular concentration is regulated by rapid changes in the rate of gene transcription (Lamers et al., 1982; Graner et al., 1983) and by changes in mRNA stability (Hod and Hanson, 1988). Our initial interest in examining the role of Fos and Jun in PEPCK gene transcription was based on several observations. First, it has been shown that phorbol esters acutely inhibit transcription of the PEPCK gene (Chu and Graner, 1986). In addition, PEPCK gene transcription is altered under conditions in Fos and Jun expression are elevated, in response to insulin (Messina, 1990) or vanadate (Bosch et al., 1990) and during liver regeneration following partial hepatectomy (Milland et al., 1990; Mohn et al., 1990; Corral et al., 1985). The promoter region of the PEPCK gene also contains elements that resemble known Fos/Jun-binding sites, including a CRE, as well as additional

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upstream elements between −285 to −252 which are similar to the consensus AP-1 motif.

Transcription of the gene for PEPCK is acutely regulated by a variety of metabolic and hormonal signals (Liu and Hanson, 1991). Studies using transgenic animals which contain the PEPCK promoter linked to the bovine growth hormone structural gene have shown that a relatively small region of the PEPCK gene, extending from −450 to +73, contains the information required to provide appropriate hormonal regulation of transcription, as well as correct developmental and tissue/cell-specific expression in the liver (McGrane et al., 1988, 1990). Regulation of PEPCK transcription by cAMP depends upon the CRE and the multiple protein-binding sites within the P3-P4 region, particularly P3(I) (Liu et al., 1991). Induction of transcription by thyroid hormone involves binding of the thyroid receptor to sequences in the PEPCK promoter between −332 and −308 and an interaction with protein(s) bound at P3(I) (Giralt et al., 1991). P3(I), which has previously been shown to bind members of the C/EBP family of transcription factors, seems to play a central role in PEPCK promoter function and is involved in both thyroid and cAMP regulation, as well as the tissue-specific expression of the PEPCK gene in the liver. In addition, the inhibitory effect of insulin on glucocorticoid-stimulated transcription from the PEPCK promoter is mediated by elements within the region between −450 and −400 (O’Brien et al., 1990). In this study we report that the Jun homodimer and the Fos/Jun heterodimer bind specifically to several sites within the PEPCK promoter and modulate transcription from the PEPCK promoter.

**EXPERIMENTAL PROCEDURES**

**Materials**—DNA-modifying enzymes and poly(dI-dC) were purchased from Boehringer Mannheim. [γ-32P]ATP (6,000 Ci/mmol) and [32P]dCTP, dGTP, dATP, and dTTP were synthesized using an Applied Biosystems 380A DNA synthesizer. Oligonucleotides were chemically synthesized using an Applied Biosystems 380A DNA synthesizer. Recombinant Fos and Jun proteins were expressed as hexahistidine fusion proteins in Escherichia coli and were purified by nickel affinity chromatography (Abate et al., 1990). Recombinant Fos and Jun and mammalian expression vectors for wild type and mutant Fos and Jun proteins were the generous gift of Drs. Thomas Curran and Cory Abate of the Roche Institute of Molecular Biology, Nutley, NJ.

**DNase I Footprinting**—The DNA probes used in the footprinting assays were prepared by end-labeling the XbaI site of the −490 to +73 PEPCK promoter using T4 polynucleotide kinase and [γ-32P]ATP. Proteins were extracted from nuclei isolated from rat liver using the method of Gorski et al. (1988). The DNase I footprinting conditions have been described previously (Roesler et al., 1989). Conditions for Fos/Jun footprinting were as described in Abate et al. (1990).

**Construction of Vectors**—Block substitutions were introduced into specific protein-binding domains of the PEPCK promoter (−490 to +73) using a variation of the method of Kunkel (1985). The specific procedures used to introduce these block mutations into the PEPCK promoter have been described in detail previously (Liu et al., 1990). The expression vectors for the wild type and mutant Fos and Jun have been described previously (Gentz et al., 1989; Sonnenburg et al., 1989). These vectors include the cDNA of c-fos or c-jun (from rat) linked to the CMV (cytomegavia virus) promoter. Jun ΔL3 and Fos ΔL3 contain leucine to valine amino acid substitution at leucine 3 within the leucine zipper domain which have previously been shown to disrupt dimerization (Gentz et al., 1989). Fos ΔBR and Jun ΔBR contain amino acid deletions (amino acids 139-145 and 269-276, respectively) within the DNA-binding domain that disrupt DNA binding (Gentz et al., 1989). Fos 102-308 contains a deletion of 101 amino acids from amino-terminal and Fos 1-258 contains a carboxy-terminal truncation (Gius et al., 1990).

**Cell Transfection and CAT Assays**—HepG2 cells, a human hepatoma cell line, were transfected with DNA using the calcium phosphate precipitation procedure (Maniatis et al., 1982). Each transfection contained 5 μg of CAT vector, 2.5–10 μg of either wild type CMV-Fos or CMV-Jun (as indicated in the figure legends), and 2 μg of a vector containing the β-galactosidase gene driven by the Rous sarcoma virus promoter (pRSV-β-gal) which was cotransfected at a 4 × 10^6 ratio with trypsin and added to the precipitated DNA, and the mixture was divided between two plates. Two days after transfection, the cells were harvested and lysed by freeze-thawing. Analysis of CAT activity was performed as described by Ausubel et al. (1989) using an equal amount of protein from each assay (Bradford, 1976). [γ-32P]Chloramphenicol and butyryl-SCoA were added to each extract of the hepatoma cells. The butyryl chloramphenicol was extracted by xylene and the radioactivity determined by scintillation counting. The difference in transcription efficiency between various samples was corrected by dividing the percentage of butyryl chloramphenicol by the β-galactosidase activity. The extent of conversion of chloramphenicol to butyryl chloramphenicol was between 1 and 20%. Each transfection experiment was performed in duplicate.

**RESULTS**

**Binding of Fos and Jun to the PEPCK Promoter**—To investigate the possibility that the PEPCK promoter contains binding sites for Fos and Jun, DNase I footprint assays were conducted using purified Fos and Jun. A segment of the PEPCK promoter extending from −490 to +73 was end-labeled and incubated with Fos and Jun. Protein binding was observed at three regions of the PEPCK promoter which had been shown previously to bind proteins present in rat liver nuclei (Fig. 1A). These regions include the CRE, a cAMP-responsive element which has also been shown to bind both C/EBP and cAMP regulatory binding protein (CREB), as well as P2 (−200 to −164), and the P3(II)-P4 region (−285 to −252). P4 also binds members of the C/EBP family of transcription factors. Examination of the nucleotide sequence within the P3(II) and P4 regions of the PEPCK promoter indicated the presence of two distinct sequence elements which were similar to the AP-1 consensus binding sequence. This suggested that the observed footprint pattern was the result of two adjacent binding sites. To determine whether this region contained two AP-1-binding sites, DNase I footprint assays were conducted using the Fos/Jun promoter (−490 to +73) containing specific block substitutions which disrupted the potential AP-1 sites. The PEPCK promoter which contained a mutation in P3(II) retained Fos and Jun binding within the P4 region and vice versa (Fig. 1B), thus the P3(II)-P4 region contains two adjacent AP-1 sites.

To determine the relative binding affinity of Fos and Jun for specific elements in the PEPCK promoter, DNase I footprinting was conducted over a range of protein concentrations (Fig. 2). The relative order of the binding affinities for Fos and Jun to these sites is CRE-1 > P3(II) > P4 > P2. Binding to P2 is weak, and the sequence within this region does not bear any obvious similarity to the AP-1 consensus binding sequence. Jun is known to bind to DNA as a homodimer, albeit with lower apparent affinity than the Fos/Jun heterodimer (Nakabeppe et al., 1988; Halazonetis et al., 1988), due in part to greater stability of the Fos/Jun heterodimer than the Jun homodimer (Rauscher et al., 1988a, 1988b). In agreement with these observations, we noted that Jun bound to the PEPCK promoter at the same locations as Fos/Jun, but with markedly lower affinity (Fig. 2). Subsequent to these footprint studies, we observed that the apparent binding activity of Fos and Jun could be markedly enhanced by addition of carrier protein, such as bovine serum albumin, to the DNA binding reaction. This is probably a nonspecific effect since we also observed this phenomenon with several other transcription factors. The affinity with which Fos/Jun interacts with CRE-1 was determined by Scatchard analysis of the binding observed in gel retardation assays conducted
Fos/Jun regulates PEPCK gene transcription

**Fig. 1.** DNase I footprint analysis of Fos- and Jun-binding sites in the PEPCK promoter. Panel A, the XbaI-BglII fragment of the PEPCK promoter (−490 to +73) was isolated from PEPCK-CAT and end-labeled on the noncoding strand at the XbaI site. The footprint assays were conducted using 20,000 cpm of end-labeled DNA with [γ-32P]ATP (6,000 Ci/mmol). The left lane is the DNase I digestion pattern of the DNA in the absence of protein. The middle lane is the pattern of protection obtained in the presence of 10 pg of proteins isolated from rat liver nuclei. Nuclear extracts were prepared from adult male rats according to the method of Gorski et al. (1986). The right lane is the protection pattern obtained following incubation of the labeled DNA with 1 μM Fos and Jun (1:1 mixture). The binding sites are outlined by boxes at the right. Panel B, XbaI-BglII fragments from PEPCK promoters containing block mutations within the P3(II) and the P4 region were labeled as in panel A. These block mutations were produced by site-directed mutagenesis as previously described (Liu et al., 1990). The DNase I footprinting pattern obtained in the absence of protein and in the presence of 1 μM Fos and Jun (1:1 mixture) using the specific end-labeled segment of the PEPCK promoter is shown.

**Fig. 2.** DNase I footprint analysis of the relative binding affinity of Jun homodimers and Fos/Jun heterodimers to the PEPCK promoter containing block mutations. A PEPCK promoter fragment (XbaI-BglII) containing either the intact promoter or the promoter containing block mutations at specific protein-binding domains was labeled as in Fig. 1A and incubated with the indicated amounts of Fos and Jun. The specific conditions of the binding reaction were as described by Abate et al. (1990).

With a constant concentration of Fos/Jun (with bovine serum albumin) and a range of concentrations of the CRE oligonucleotide. The observed $K_0$ of $3.6 \times 10^9$ M$^{-1}$ is approximately equal to that observed for the binding of CREB to CRE-1.$^3$

**Activation of Transcription from the PEPCK Promoter by Fos and Jun—**To examine whether the binding of Fos and Jun to the PEPCK promoter was of functional significance, PEPCK-CAT was cotransfected into HepG2 cells, together with expression vectors encoding Fos or Jun cDNA. These vectors (CMV-Fos and CMV-Jun) contain the entire open reading frame of either Fos or Jun, transcribed from the CMV promoter. Increasing amounts of CMV-Jun markedly increased transcription from the PEPCK promoter (Fig. 3). In contrast, the transfection of an expression vector containing the chimeric CMV-Fos gene inhibited transcription from the PEPCK promoter and blocked the expected increase in transcription caused by the transfection of CMV-Jun, in a concentration dependent manner (Fig. 3). The role of each of the protein-binding domains of the PEPCK promoter in the stimulation of transcription by Jun was determined by transfecting the PEPCK promoter containing defined block mutations together with CMV-Jun (Fig. 4). Mutation of the CRE, P3(II), or P4 greatly reduced the ability of Jun to stimulate transcription from the PEPCK promoter. These are the same regions of the PEPCK promoter which bound Jun in the DNase I assay. Interestingly, P3(II), a C/EBP-binding domain immediately 3′ to P3(II), was also required for full

$^3$ E. A. Park, A. L. Gurney, and R. W. Hanson, unpublished observations.
activation of PEPCK transcription by Jun and for Fos inhibition. Transcription from the promoter containing a block mutation in the P3(I) was increased in a synergistic manner by the combined action of Jun and Fos. Transcription from PEPCK promoters containing mutations in P1 or P2 was stimulated by Jun; Fos inhibited this stimulation.

The binding of Fos/Jun to the CRE of the PEPCK pro-

Fig. 3. Alterations in transcription from the of PEPCK pro-

moter in hepatoma cells by expression of Jun and Fos. HepG2 cells were transfected with 5 μg of PEPCK-CAT, 2 μg of RSV-β-gal, and the indicated amounts of CMV-Fos and CMV-Jun. The total amount of DNA in each transfection experiment was maintained constant at 20 μg/plate by addition of carrier DNA (pTZ18R or pBR322). After 48 h, the cells were harvested and the CAT activity was measured as described under "Experimental Procedures." The quantity of cell extract used was sufficient to maintain a percent conversion of chloramphenicol to butyrated chloramphenicol of between 1 and 20%. The results shown are corrected for β-galactosidase activity and are the mean ± the standard error of the mean for at least three separate DNA transfection experiments (carried out in duplicate).

Fig. 4. The effect of Fos and Jun on transcription from PEPCK promoter-containing block mutations. HepG2 cells were transfected with PEPCK-CAT vectors containing specific block substitutions within the protein-binding domains of the PEPCK promoter. The block mutations in the PEPCK promoter used in these experiments have been described previously (Liu et al., 1990). The region of the PEPCK promoter which contains a mutation is indicated on the horizontal axis. The presence of CMV-Jun or CMV-Fos expression vectors, cotransfected with the specific PEPCK-CAT vector, is indicated below each column. The activity of CAT noted for each PEPCK promoter mutation was normalized to the activity of the mutant promoter transfected in the absence of Fos or Jun. The HepG2 cells were transfected with 5 μg of the indicated PEPCK-CAT vector, 2 μg of RSV-β-gal, and (where indicated) 2.5 μg of CMV-Jun and/or 10 μg of CMV-Fos, as outlined in Fig. 3. CAT activity was determined as outlined under "Experimental Procedures" and Fig. 3. The results shown are the mean ± the standard error of the mean for at least three independent transfection experiments, performed in duplicate.
be capable of binding to the CRE of the PEPCK promoter which bind to Fos and Jun are known to be involved in the stimulation of transcription when introduced into hepatoma cells. The specific regions of the PEPCK promoter that can provide a clue to the major regulatory interaction occurring between cAMP and insulin in the transcription of the PEPCK gene.

A major question is whether Fos contributes to the effect of insulin on the PEPCK gene. Insulin has been described as the major and dominant regulatory agent in controlling transcription of the PEPCK gene (Granner et al., 1983). Other, non-physiological compounds such as vanadate (Bosch et al., 1990), phorbol esters (Chu and Granner, 1986), and lithium (Bosch et al., 1992) also block transcription of the PEPCK gene in hepatoma cells. Recently, we have shown that protein kinase C, when transfected into hepatoma cells, blocks the stimulatory effect of 8-Br-cAMP on transcription from the PEPCK promoter. It is interesting to note that all of these agents have also been shown to increase c-fos gene expression (Bosch et al., 1990; Curran, 1988; Schalasta and Doppler, 1990) suggesting a possible link between Fos and the action of insulin on PEPCK gene expression.

The gene for c-fos is known to be rapidly induced in hepatic cells by insulin (Messina, 1990; Mohn et al., 1990), which is consistent with Fos playing a role in the regulation of PEPCK transcription. The induction of c-fos is the most rapid known effect of insulin on gene transcription (Messina, 1990). However, c-fos gene expression can also be induced by cAMP (Squinto et al., 1989), the major positive regulator of PEPCK gene expression. In explaining this apparent contradictory effect of c-fos, it is necessary to separate the short and long term effects of cAMP on PEPCK gene expression. The administration of cAMP to animals results in rapid stimulation of PEPCK gene transcription. However, there is a marked attenuation of the stimulatory effect of the cyclic nucleotide

**DISCUSSION**

The ability of Jun and Fos/Jun to bind to the PEPCK promoter was predicted based on the presence of several sites in the promoter which have homology to the AP-1-binding sites present in the promoters for the genes coding for collageenase (Angel et al., 1987a, 1987b), SV40 (Lee et al., 1987), metallothionein IIA (Lee et al., 1987a, 1987b), and proenkephalin (Sonnensberg et al., 1989). Transcription of these four genes is stimulated by phorbol esters (Angel et al., 1987; Comb et al., 1988). In contrast, PEPCK gene transcription is markedly inhibited by phorbol esters (Chu and Granner, 1986). A unique aspect of the regulation of PEPCK gene transcription by Jun is the fact that Fos completely blocks the stimulatory effect of Jun without diminishing its binding to the PEPCK promoter. In fact, the Fos/Jun heterodimer has a higher affinity for specific elements in the PEPCK promoter than does the Jun/Jun homodimer. Jun is one of several transcription factors which have now been shown to be capable of binding to the CRE of the PEPCK promoter and also to stimulate transcription when introduced into hepatoma cells. The specific regions of the PEPCK promoter which bind to Fos and Jun are known to be involved in the regulation of PEPCK gene transcription by hormones, most notably by cAMP and thyroid hormone (Giralt et al., 1991). The negative effect of Fos on transcription from the PEPCK promoter could provide a clue to the major regulatory interaction occurring between cAMP and insulin in the transcription of the PEPCK gene.

![Figure 5](image-url)  
**Fig. 5.** Effect of Fos on the induction of transcription for the PEPCK promoter caused by the C subunit of protein kinase A and 8-Br-cAMP. Panel A, HepG2 cells were transfected with PEPCK-CAT (5 μg), CMV-Fos (10 μg) as in Fig. 3. Forty h after transfection, 8-Br-cAMP (0.5 mM) was added to the media, and the cells were incubated for an additional 6 h. CAT activity was determined as described under "Experimental Procedures" and in Fig. 3. The results shown are the mean ± the standard error of the mean for at least three separate experiments, performed in duplicate. Panel B, HepG2 cells were transfected with 5 μg of PEPCK-CAT, 2 μg of RSV-β-gal, and the indicated amounts of CMV-Fos and SRα-protein kinase A. SRα-protein kinase A is an expression vector for the C subunit of protein kinase A (Muranatso et al., 1989). The cells were harvested after 48 h, and CAT activity was measured. The results are the mean ± the standard error of the mean for at least three independent transfection experiments, each performed in duplicate.

102–308, lacking the first 101 amino acids, or the carboxyl terminus (Fos 1–258, lacking the last 50 amino acids) of Fos did not eliminate its ability to reduce Jun-stimulated transcription from the PEPCK promoter. Thus, the DNA-binding and leucine zipper domains of both Fos and Jun are required to mediate their effects on PEPCK transcription.

![Figure 6](image-url)  
**Fig. 6.** Effect of mutations in Fos and Jun on their ability to alter PEPCK-CAT gene transcription. HepG2 cells were transfected with 5 μg of PEPCK-CAT, 2 μg of RSV-β-gal, and 5 μg each of expression vector for the indicated mutant form of Fos or Jun. The expression vectors for the wild type and mutant Fos and Jun proteins have been described previously (Gentz et al., 1988, Sonnenburg et al., 1989). These vectors include the cDNA of c-fos or c-jun (from the rat) linked to the CMV promoter. Jun ΔL3 and Fos ΔL3 contain leucine to valine amino acid substitution at leucine 3 within the leucine zipper domain. Fos ΔBR (deletion of amino acids 139–145) and Jun ΔBR (deletion of amino acids 260–266) contain amino acid deletions within the DNA-binding domain of Fos or Jun which disrupt their binding to DNA. Fos 102–308 contains a deletion of 101 amino acids from amino-terminal and Fos 1–258 contains a carboxyl-terminal truncation which removes a "repressing" region identified by Gius et al. (1990). The results are presented as the mean ± the standard error of the mean for at least three independent transfection experiments, each performed in duplicate.
on transcription with continued exposure to the cyclic nucleotide (Lamers et al., 1982). In fact, transcription of the PEPCk gene becomes refractory to added cAMP (Sasaki et al., 1984). It is possible that this attenuation in PEPCk gene transcription is related to the induction of Fos within the cell. In this paper we demonstrate that Fos expression in hepatoma cells will totally block the induction of transcription from the PEPCk promoter by cAMP, the c subunit of protein kinase A, and Jun. Fos is the first and only known transcription factor which has been linked to the negative regulation of the PEPCk promoter. The full mechanism of insulin action on PEPCk transcription is complex and is partly mediated by elements upstream of the Fos-Jun-binding domains (O'Brien et al., 1990). Nonetheless, the inhibition of PEPCk transcription by Fos could provide a model for studying the inhibition of this highly regulated gene.

The ability of Fos to inhibit PEPCk expression depends upon the presence of P3(I), a region which does not bind Fos-Jun heterodimers. P3(I) binds to members of the C/EBP family and expression vectors for C/EBPα transactivate transcription from the PEPCk promoter through a promoter region. Recent work has shown that C/EBPβ (Cao et al., 1991) also known as LAP (Descombes et al., 1990) and NF-IL6 (Akira et al., 1990), also binds to the PEPCk promoter with similar sequence specificity to C/EBPα.4 Work is currently under way to determine the identity and relative contribution of each of the proteins which bind to this region of the PEPCk promoter. It is not clear how Fos interacts with the factors which bind to P3(I) to block PEPCk transcription. Both Fos and the members of the C/EBP family contain the leucine zipper structural motif, so it is possible that Fos forms heterodimers with a protein(s) which binds directly to the P3(I) region. Alternatively, Fos may bind to a protein(s) in such a manner as to prevent subsequent DNA binding or may act in a less direct way to alter the levels or activities of transcription factors available for binding to the PEPCk promoter. This could be similar to recent models proposed for the interaction of the glucocorticoid receptor with Jun (Yang-Yen et al., 1990; Schule et al., 1990), in that Fos may function as an adaptor to modify the function of pre-existing factors.

Fos has been shown to repress expression of a variety of genes (Gius et al., 1990; Wilson and Treisman, 1988). Analysis of the repression by Fos of transcription of the Egr-1 gene, an immediate early gene from adenosivirus, that is inducible by growth factors identified a target site which was distinct from the consensus AP-1 sequence (Gius et al., 1990). Jun did not appear to be involved in the repression of Egr-1 expression by Fos. The ability of Fos to inhibit transcription was dependent upon the COOH-terminal 40 amino acids, and did not depend upon the DNA-binding domain or the leucine zipper region. This differs from the results presented here in that the DNA-binding and leucine zipper regions of Fos are clearly required for its inhibition of PEPCk transcription.

Both Fos and Jun are members of a family of related transcription factors, containing the leucine zipper motif and can interact with each other to form homo- or heterodimers (Nakabeppu et al., 1990; Cohen and Curran, 1988; Cohen et al., 1989; Nishina et al., 1990; Zerial et al., 1989). In addition to Fos and Jun, it is possible that other members of this family may also regulate PEPCk gene transcription. However, Jun D and Fra-1, (Fos-related antigen 1) did not alter transcription from the PEPCk promoter in experiments in which expression vectors for these proteins were introduced into hepatoma cells together with the PEPCk promoter.

Other leucine zipper transcription factors, including C/EBPα, C/EBPβ and CREB, can also bind to the CRE of the PEPCk promoter, and the possibility exists that these proteins may function as heterodimers.

The levels of Fos and Jun can be regulated by phospholipase C activity through changes in the level of diacylglycerol which allosterically activates protein kinase C (Schalasta and Dopp, 1990). Both the cAMP and the diacylglycerol-phosphodiesterase inositol second messenger systems can alter PEPCk transcription through specific cis-acting elements located within a few hundred base pairs upstream of the transcription start site. These two pathways interact at the PEPCk promoter through competition for shared cis-acting sequences, such as the CRE, which are capable of binding to a surprisingly large number of distinct proteins. There are reported examples of cross-talk between the signal transduction pathways. Examples include the cAMP-induced translocation of protein kinase C into the nucleus (Chambert et al., 1987) and the phorbol ester-induced phosphorylation of adenylate cyclase (Yoshimasa et al., 1987). The pivotal role of P3(I) in mediating both the stimulation of transcription by cAMP and thyroid hormone and the inhibition of transcription by Fos suggests that there are likely to be additional levels of interaction including direct protein-protein contacts between proteins and to distinct regions of the PEPCk promoter or competition for utilization of specific transcription factors at elements in the promoter. Future efforts will be directed toward elucidating the mechanisms by which the multiple protein binding regions are able to act synergistically to establish the rate of PEPCk gene transcription.

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REFERENCES


4 E. Park, A. Gurney, S. Nizinski, and R. Hanson, unpublished observation.
Fos/Jun Regulates PEPCK Gene Transcription

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Lucibello, F. C., Slater, E. P., Jooes, K. U., Beato, M., and Muller, R. (1990) EMBO J. 9, 2827-2834