Promoter Analysis of Zfp-36, the Mitogen-inducible Gene Encoding the Zinc Finger Protein Tristetraprolin*

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The gene encoding the putative zinc finger protein tristetraprolin (TTP), Zfp-36, is rapidly induced by a variety of mitogens and growth factors. We show here that 77 base pairs 5' of the transcription start site are sufficient for full serum inducibility of the mouse Zfp-36 promoter. This region of the promoter includes consensus sequences for the binding of the transcription factors EGR-1, AP2, and Sp1. In addition, we have identified a previously undescribed element, TTP promoter element 1 (TPE1); this 10-base pair sequence includes a palindrome and is identical in the human, bovine, and mouse genes. Each of the three binding elements, EGR-1, AP2, and TPE1, contribute to the serum induction of Zfp-36 and can confer serum-inducible expression on a heterologous minimal promoter. Gel mobility shift assays demonstrate the formation of complexes consisting of this region of the promoter and cellular nuclear proteins and demonstrated that the extent of complex formation could be altered by treatment of the cells with serum or insulin. These results suggest that the response of Zfp-36 to serum and other mitogens is mediated by a series of cis-acting elements acting in concert to confer full inducible transcription of this gene.

An early response of many cells to serum and polypeptide growth factors is the activated transcription of specific genes in the absence of de novo protein synthesis. Many of these immediate-early response genes encode regulatory proteins that mediate growth responses, including transcription factors that modulate the expression of other genes. These genes encode a number of well studied transcription regulators such as the fos and jun families. One interesting member of the immediate-early response class of genes encodes tristetraprolin (TTP).1

1) Zfp-36, a zinc finger protein also known as Nup475 (2) and TIS11 (3). This protein, which is encoded by the gene Zfp-36 (2), is expressed at very low levels in quiescent fibroblasts but is rapidly induced by serum, polypeptide growth factors, and phorbol 12-myristate 13-acetate (PMA) (1, 3, 4). This expression is transient, with most mRNA disappearing after 2 h. In these cells, induction of Zfp-36 expression is independent of protein synthesis, and treatment with cycloheximide results in superinduction of the gene (1, 3–6). The mechanisms that control the activation of Zfp-36 transcription have not been defined.

To identify sequence elements involved in the transcriptional control of TTP synthesis, we have characterized the 5'-flanking region of Zfp-36 using site-directed mutagenesis and deletional analysis. Several potential binding sites for known transcription factors have been identified in this study as contributing to the serum-stimulated activity of this promoter. In addition, we have identified and characterized a previously unknown promoter element that appears to function as a transcriptional enhancer and that participates in the regulation of serum-induced Zfp-36 transcription.

EXPERIMENTAL PROCEDURES

Library Screening and Subcloning

A Balb/c mouse genomic library (Clontech, Palo Alto, CA) was screened using the mouse TTP cDNA (1) as a probe. Pure plaques positively hybridizing to the probe were subjected to Southern mapping analysis. Restriction fragments from two clones overlapping within the intron and 1 kb of TTP protein coding sequence (clone 13 extended 5', clone 7 extended 3' from the overlapping sequence) were subcloned into pBS2 (Stratagene, La Jolla, CA). An 8-kb SauI fragment from clone 13 was ligated into the SauI cloning site in pBS2, and another plasmid was made by ligating a 3-kb XhoI fragment from clone 7 into the SalI site of pBS2. The inclusion of the entire mRNA coding region was confirmed by dideoxy sequencing. Five kb of 5'-flanking region was also sequenced. The entire mouse genomic sequence has been deposited in GenBank (accession number L42317). A second mouse genomic library (129 sv, Stratagene) was also screened to obtain a 3.8-kb SauI-SallI fragment, which contained 0.9 kb of the 5'-flanking region, the entire mRNA coding sequence, and a single intron. DNA sequence analysis revealed only a 2-bp difference in the 5'-flanking region when compared with the clone from the Balb/c library. This clone was used for promoter activity analysis as well as for the construction of a gene-targeting vector to create knockout mice.2

A human genomic clone was obtained by screening a human placental genomic library (Clontech) with the human TTP cDNA (3) (GenBank accession number for the human genomic sequence: M19844). The bovine TTP cDNA was obtained from a bovine aorta epithelial cell cDNA library (Stratagene) using the human TTP cDNA as a probe (2). The bovine cDNA was then used to screen a bovine liver genomic library

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1 The abbreviations used are: TTP, tristetraprolin; PMA, phorbol 12-myristate 13-acetate; GH, growth hormone; CEF, chicken embryonic fibroblasts; FBS, fetal bovine serum; TPE1, TTP promoter element 1,
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(Stagrate). The bovine genomic sequence has been deposited in GenBank™ (accession number L42319).

Base pair numbers in this report refer to the transcription initiation site in the mouse genomic clone as determined by primer extension assays (data not shown). The position of the single intron was determined by sequence comparison of the genomic clones with the cDNAs and had the conventional sites for splice donors and acceptors. Southern mapping, subcloning, and DNA sequencing were performed by standard techniques (8).

Plasmid Constructions

Reporter Constructs—Zfp-36 promoter-driven human growth hormone reporter plasmids were created by ligation restriction fragments from genomic clone 13 to the 5′ end of a promoterless human growth hormone gene. Three unique restriction fragments extending upstream from the TTP mRNA translation start site (NcoI, 5 kb; XbaI-NcoI, 2.1 kb; and SstI-NcoI, 137 bp) were excised, filled in with dNTPs, and inserted into the blunt BamHI cloning site of pOGH (Nichols Institute Diagnostics, San Juan Capistrano, CA) to produce 5 kb GH, 2.1 kb GH, and the conventional sites for splice donors and acceptors (7).

Plasmids TTPpBS7.4S and TTPpBS7.4SInt, constructed in such a manner contained 1.7 kb and 77 bp, respectively, of 5′-flanking region, the intron, and the downstream coding sequence that includes the consensus polyadenylation signal and three consensus sequences for mRNA instability (2). Plasmids TTP1.7kb and TTP137bp, constructed by standard techniques (8), had the conventional sites for splice donors and acceptors (7), and had the conventional sites for splice donors and acceptors (7).

Southern mapping, subcloning, and DNA sequencing were performed by standard techniques (8). Site-specific deletions or substitutions in the mouse genomic clone as determined by primer extension assays (9, 10), EGR-1 (5′ GCCGCCCCATAAAAGG GCCCCCAGG 3′; Ref. 12), 2 and Zfp-36 as the “TTP promoter.” TTP sequences for DNA binding factors were used in this study. For EGR1-AP2, two complementary synthetic oligonucleotides were annealed to form a double-stranded oligonucleotide corresponding to mouse Zfp-36 nucleotides –77 to –37. A 5-base single-stranded tail (Sall site) was included at the 5′ end, and a 6-bp double-stranded EcoRV site was included at the other. For TPE1, two complementary synthetic oligonucleotides were annealed to form a 22-bp double-stranded oligonucleotide corresponding to bases –68 to –55 of mouse Zfp-36, with cloning sequences for HindIII and Sall site at either end.

To ensure sequence fidelity, each oligonucleotide pair was cloned into Bluescribe and sequenced. Double-stranded oligonucleotides were re-digested by ligating the plasmid with appropriate restriction enzymes, and the ends were filled in with dNTPs using the Klenow fragment of DNA polymerase. Competitor fragments containing putative binding sequences for EGR-1 (5′-GCGGCCCAGGCGCG) were synthesized and used in the experiment.

Component fragments containing putative binding sequences for EGR-1 (5′-GCGGCCCAGGCGCG) and TPE1 (5′-GCGGCCCAGGCGCG) were made by annealing the complementary strands and filling in the ends with dNTPs using the Klenow fragment of DNA polymerase (21).

RESULTS

TTP Promoter Analysis—For convenience, we will refer to the 5′-flanking region of Zfp-36 as the “TTP promoter.” TTP promoter activity upstream of the translation start site was first tested by fusing 5-kb NcoI and 2.1-kb XbaI-NcoI fragments of the mouse genomic sequence to the human growth hormone gene, followed by assay of growth hormone (GH) secretion into the medium following transient transfection of the plasmids into HIR3.5 cells. Both the 5-kb and the 2.1-kb promoter sequences efficiently drove the expression of the GH gene (Fig. 1A). GH secretion from these constructs was 60–100-

RNA Preparation and Northern Blot Analysis

Total cellular RNA was prepared following the method of Chomczynski and Sacchi (18) with modifications by Xie and Rothblum (19).

Northern blots were prepared as described before (1). Blots were hybridized to random primed α-32P-labeled (Stagrate) mouse TTP cDNA, and the presence of TTP mRNA was quantified using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Nuclear Extracts and Gel Mobility Shift Assays

Nuclear extracts from HIR 3.5 cells and CEF cells were prepared for gel mobility shift assays as described previously (20). Briefly, 10 μl of nuclear extract buffer (10 mM Tris (pH 7.5), 1 mM EDTA, 5% glycerol) containing 1 m M dithiothreitol, 1 μg of poly(dI-dC) (Pharmacia Biotech Inc.), and 20 × 10^6 cpm of α-32P-labeled probe, was added to 5–12 μg of nuclear protein (as indicated in the figure legends), diluted to 10 μl in nuclear extract buffer (20 mM Tris (pH 7.9), 20% (v/v) glycerol, 100 mM KCl, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 μg ml^−1 NaF, 0.5 μg ml^−1 Na3VO4), and then subjected to binding reactions and electrophoresis (20).

The following synthetic oligonucleotides containing potential sequences for DNA binding factors were used in this study. For EGR1-AP2, two complementary synthetic oligonucleotides were annealed to form a double-stranded oligonucleotide corresponding to mouse Zfp-36 nucleotides –77 to –37. A 5-base single-stranded tail (Sall site) was included at the 5′ end, and a 6-bp double-stranded EcoRV site was included at the other. For TPE1, two complementary synthetic oligonucleotides were annealed to form a 22-bp double-stranded oligonucleotide corresponding to bases –68 to –55 of mouse Zfp-36, with cloning sequences for HindIII and Sall site at either end.

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Competitor fragments containing putative binding sequences for EGR-1 (5′-GCGGCCCAGGCGCG) were synthesized and used in the experiment. Component fragments containing putative binding sequences for EGR-1 (5′-GCGGCCCAGGCGCG) and TPE1 (5′-GCGGCCCAGGCGCG) were made by annealing the complementary strands and filling in the ends with dNTPs using the Klenow fragment of DNA polymerase (21).
Sequential deletions of 5'-proximal sequence produced a 9-fold greater than that of the promoterless plasmid pØGH. Sequential deletions of 5'- sequences from the 2.1-kb promoter were then made to determine the minimal promoter length conferring full expression activity on the reporter construct. A 137-bp fragment (StsI - Ncol) containing only 77 bp upstream of the transcription initiation site was found to promote GH expression in similar patterns of serum-inducible expression (data not shown). From these findings, we concluded that the mouse TTP promoter sequences that are essential for serum responsiveness are located within a 137-bp region 5'- proximal to the transcription start site or 77 bp 5'- of the transcription initiation site. The human, bovine, and mouse sequences were highly conserved in the proximal 5'-flanking region (Fig. 3A). Thus, the 5'-proximal sequence located 137 bp upstream from the translational start site revealed a consensus H2A (52 to +51) binding sequence for the eukaryotic core histone dimer H2A-H2B (21-22), and a consensus c-fos.5 binding site (from GCGCCACC to GGTCGACC) affected either the basal or serum-stimulated expression of mouse TTP mRNA (data not shown).

The human, bovine, and mouse sequences were highly conserved in the proximal 5'-flanking region (Fig. 3A). Sequence analysis revealed consensus motifs for several transcription factors in the 5'-proximal region of all three TTP promoters, including sites for the TATA binding protein and Sp1 binding protein (Fig. 3A). Deletion of these sites from the mouse construct containing the 137-bp 5'-proximal sequence produced a predictable inhibition of TTP mRNA expression (Fig. 3B).

Consensus sequences for the binding of transcription factors EGR-1
abolish, serum-induced expression, we examined whether any deletion or mutation of each single element reduced, but did not confers serum responsiveness to the silent human gene (10). Using transient transfection in CEF cells, we tested the ability of these putative elements, EGR-1, TPE1 and AP2, to confer serum responsiveness on the silent human \( \beta \)-globin promoter. The expression of Glo48TTP plasmids containing each of the test sequences was induced by serum as compared with the plasmid lacking these sequences (Fig. 5), both in the presence and absence of cycloheximide. In addition, the presence of

![Fig. 3. Deletion analysis of the mouse TTP promoter. Panel A, sequences of the first 390 bp of TTP promoter from human, bovine, and mouse are compared. The consensus sequences for the EGR-1, TPE1, AP2, and \( \beta \)-globin gene are shown. The TTP intron is present in the mouse and bovine promoter sequences. Panel B, TTP plasmids containing 77 bp of genomic sequence 5' of the TTP mRNA transcription start site (TTP, 137 bp) deleted of the TTP intron (-Int), EGR-1, or AP2 sequences were transfected into CEF cells. 24 h after transfection, the cells were deprived of serum for 24 h and then treated with control conditions (C) or 10% FBS (S) for 60 min. Northern blot analyses were performed as described in the legend to Fig. 2.

The above results suggest that the EGR-1, TPE1, and AP2 elements within the first 77 bp upstream of the mouse TTP transcription start site all contribute to transcriptional activation of Zfp-36 by serum in CEF cells (Table I, Group 2). Since deletion or mutation of each single element reduced, but did not abolish, serum-induced expression, we examined whether any of these elements was able to impart serum-responsiveness to
TABLE I
Summary of TTP promoter activity after deletion or mutation of promoter elements

| Group 1 | CEF cells were transfected with ΔTPE1 mutants of the corresponding wild-type constructs TTP1.7kb or TTP0.9kb. Group 2, CEF cells were transfected with TTP1.7kb, with the indicated deletions (d) and mutation (D). For both groups, the cells were serum-deprived and treated with control conditions or 10% FBS for 60 min. The indicated values were obtained by comparing quantified PhosphorImager values from northern blots of TTP mRNA bands transcribed from the mutant plasmids compared with the corresponding control plasmid in each transfection experiment. The quantified PhosphorImager values were in turn normalized for transfection efficiency by analysis of a co-transfected plasmid, pXGH5. The values shown are means ± S.D. from individual transfection experiments. See the text for further details.

| Group 1 | n | Percentage of wild-type ± S.D. |
|---------|---|-------------------------------|
| TTP1.7kb, ΔTPE1 | 2 | 9.4 ± 1.3 |
| TTP0.9kb, ΔTPE1 | 3 | 25.3 ± 1.6 |

| Group 2 | n | Percentage of TTP1.7kb ± S.D. |
|---------|---|-------------------------------|
| dEGR-1 | 4 | 35.4 ± 13.5 |
| dAP2 | 2 | 23.2 ± 3.7 |
| dTPE1 | 9 | 26.3 ± 8.7 |
| ΔTPE1 | 3 | 25.8 ± 3.2 |

all three putative enhancer elements together resulted in a 1.5-fold increase in expression compared with the sum of expression from each element individually (Fig. 5). Finally, the reverse orientation of the TPE1 element resulted in a similar increase in serum-induced expression to that of the forward construct (data not shown).

Binding of TPE1 by Nuclear Extracts—Our transient transfection analysis of the 77-bp minimal TTP promoter identified three DNA elements that contribute to its serum responsiveness other than the intron and general factor binding sites such as the TATA box. These include two known DNA-protein binding motifs (EGR-1 and AP2) as well as a previously undescribed element (TPE1), all of which are highly conserved in three mammalian species (Fig. 3A). To determine if nuclear proteins could associate with these elements, we performed DNA mobility shift analyses using nuclear extracts from both serum- and insulin-treated and quiescent cells. When a radiolabeled probe (EGR1-AP2) containing the EGR-1, TPE1, and AP2 elements was used in gel shift assays, four major protein-probe complexes were identified (Fig. 6A). One of these complexes (C4) showed a reproducible decrease in intensity following 10 min of treatment of the cells with insulin or serum, whereas complexes C1 and C2 increased following insulin or serum stimulation. Double-stranded oligonucleotides encoding the individual EGR-1, TPE1, and AP2 sites were then used as competitors in gel shift assays with the EGR1-AP2 probe. No specific DNA-protein complex was displaced by the EGR-1 oligonucleotide (not shown). The top three complexes (C1, C2, and C3) were decreased in the presence of the AP2 competitor, although higher concentrations of the AP2 competitor were required to completely abolish C3. The TPE1 competitor oligonucleotide specifically abolished C4. We also observed that the intensity of C1 and C2 increased when C4 was competed by the TPE1 oligonucleotide competitor.

Wild-type and mutant TPE1 double-stranded oligonucleotides were next radiolabeled for use as probes in gel shift assays. Nuclear extracts from both serum-treated and control cells produced a single DNA-protein complex with the wild-type TPE1 probe, which corresponded in electrophoretic mobility to complex C4 seen with the EGR1-AP2 probe. As with the EGR1-AP2 probe, formation of this complex was decreased in extracts from serum-treated cells. A mutant ΔTPE1 oligonucleotide competitor containing base changes from TCCCGGA to TAACTTA had no effect on formation of the TPE1 complex (Fig. 6B). The mutant ΔTPE1 oligonucleotide probe was also directly radiolabeled and shown to be unable to form this complex (Fig. 6C). These results suggest that the TPE1 element was recognized by one or more specific nuclear proteins. The small but consistent decrease in the intensity of the TPE1 complex following serum treatment, seen with both the EGR1-AP2 (C4) and the TPE1-specific probe, suggests that serum treatment might modify these binding proteins in such a way as to decrease their binding to the TPE1 element. Similar decreases in intensity of complex C4 were seen when HIR3.5 cells were treated with either insulin (70 nm) (Fig. 6A) or PMA (1.6 µM) (not shown) for 10 min.

Figure 5. Effect of TTP promoter elements on the activity of a silent promoter. CEF cells were transfected with Glo48TTP alone (–), or with Glo48TTP constructs containing a single copy of the indicated TTP promoter elements or the c-fos SRE. Cells were deprived of serum for 24 h after 24 h of recovery from transfection; FBS (10%) was then added to the treatment group (S) for 60 min at 37°C, compared with control conditions (C). The position of the TTP mRNA is indicated. Other details describing the Northern blotting are contained in the legend to Fig. 2.

Figure 6. Gel mobility shift assay for nuclear proteins binding to the TTP promoter. Panel A, nuclear extracts (12 µg of protein) from HIR3.5 cells treated for 10 min with 70 nm insulin (I) or control conditions (C) were then allowed to bind to the EGR1-AP2 probe containing -35 to –77 of the mouse TTP promoter. These assays were performed with or without specific oligonucleotide competitors comprising either the AP2 or TPE1 sites, as indicated at the top of the gel. C1-C4 represent DNA-protein complexes 1-4 as described in the text. Panel B, nuclear extracts (5 µg of protein) from CEF cells treated for 10 min with 10% FBS (S) or control conditions (C) were allowed to bind to the TPE1 probe. Panel C, nuclear extracts (5 µg of protein) from HIR3.5 cells treated with control conditions (C) were allowed to bind to the wild-type or the mutant (ΔTPE1) TPE1 probes. All lanes contained 20 × 10^6 cpm of 32P-labeled double-stranded oligonucleotide, in the presence of poly(dI-dC) at a final concentration of 50 µg/ml. The unlabeled competitor oligonucleotides were present at 1 µg/reaction when indicated. Assay conditions are described under “Experimental Procedures.” The sequences of the probes used were EGR1-AP2, ctagCCGGGGGCGCCTCGGG-GAAGCTCTCTAGTGGCCACGCCCCCAGGCgatatc; TPE1, tcgacGTC- CCGGAAGCGtcga; ΔTPE1, tcgacTGAAATTAAGCGCtca, where the underlined bases indicate the core sequences of the consensus protein binding sites, and the lowercase bases indicate portions of the restriction sites used for subcloning.
These studies establish that the first 77 bp 5' upstream of the transcrip
tional start site are sufficient for maximal serum induction of the mouse TTP gene (Zip-36) when expressed in CEF cells; deletions 3' of this point dramatically decrease serum inducibility of this gene. Within this minimal effective promoter, we have also identified several putative transcription factor binding sites in the mouse TTP promoter, all of which are present in the human and bovine genes. The presence of each is necessary for the full, serum-inducible expression of the gene. Finally, we have identified binding proteins in cell nuclear extracts that bind specifically to some of these DNA motifs and whose binding is altered by prior treatment of the cells with mitogens. These studies have begun to evaluate the mechanisms by which serum and other mitogens rapidly and dramatically stimulate the transcription of this immediate-early response gene.

One conserved transcription factor binding site identified in the present study is the Sp1 site, located at -35 to -30 5' of the transcription start site in the mouse promoter. Sp1 is a well characterized zinc-finger-containing transcription factor, which enhances transcription by RNA polymerase II from promoters that contain at least one properly positioned GGCGCG hexanucleotide (for review, see Ref. 26). Sp1 has been shown to regulate transcription of certain proto-oncogenes (27, 28) and growth factor genes (29). When the consensus binding sequence for Sp1 was deleted from the TTP137bp plasmid, an 80% decrease in TTP expression resulted, indicating that the TTP promoter is Sp1-responsive (Fig. 4B); however, we have not demonstrated directly that Sp1 binds to its hexanucleotide binding site in the TTP promoter. The TTP promoter construct with the Sp1 site deleted retained serum-responsive but to a lesser extent than the wild-type construct, implicating other promoter elements in the serum-induced expression of TTP mRNA.

EGR-1 is another zinc-finger-containing transcription factor, also known as NGF1-A (30), KROX24 (31), TIS-8 (4), and Zif268 (32). It binds to a GC-rich consensus sequence, GCAGGGGCG, that is found in the 5'-flanking regions of many genes involved in cell growth such as proto-oncogenes and genes encoding mitogens and mitogen receptors. Several immediate-early response genes also have EGR-1 binding sites in their promoters (33). Deletion of the EGR-1 binding sequence from the TTP promoter decreased its serum-stimulated expression by 65%. Although we found no direct evidence of EGR-1 binding to the TTP promoter in our gel shift assays, our deletion analysis indicates that EGR-1 may contribute to the regulation of TTP expression.

The AP2 consensus binding site GCCNNNGGC (34) is present in the minimal effective promoter of TTP from all three animal species tested. This sequence, when bound by AP2 homodimers, has been identified as a control element for several viral and cellular genes (24, 34). AP2 mediates regulation of gene expression in response to a number of different signal transduction pathways (35). The activity of AP2 is increased in response to treatment of cells with phorbol esters and agents that elevate cAMP levels (34-36). When the AP2 consensus sequence in the mouse TTP promoter was deleted, induction of TTP expression by serum was decreased by 72%. We have previously shown that PMA could induce TTP expression (1), making it possible that the AP2 binding site was involved in PMA-induced TTP expression. However, when the AP2 binding sequence was deleted from the TTP137bp construct, PMA still induced TTP expression to a similar extent as the serum-induced response (data not shown). These results indicate that the PMA-stimulated TTP expression does not depend solely upon the consensus AP2 binding sequence in the TTP promoter.

We also identified a previously undescribed promoter element at -66 to -60 5' of the cap site in the mouse gene that we have called TPE1. It contains a palindromic element with dyad symmetry, TCC(C/G)GGA. It is perfectly conserved in the TTP promoter from all three animal species we have studied. Deletion or mutation of this element led to a 75% decrease in serum-induced TTP expression. Mutation of the TPE1 palindrome also severely impaired serum responsiveness of the TTP promoter when introduced into longer promoter constructs. Both orientations of the TPE1 element could also confer serum responsiveness to the silent promoter Glo48, indicating that this element behaves as a transcriptional enhancer.

The TPE1 palindrome also appears to represent a binding site for a nuclear protein, as demonstrated by gel shift analysis. Mutations within the palindrome that impaired serum responsiveness of the promoter also abolished binding of this nuclear protein. In addition, nuclear extracts from cells stimulated with serum or other mitogens for 10 min showed a small but consistent decrease in band intensity in the gel shift assays. This suggests, but does not prove, that the TPE1 element binding protein is a target of the signaling cascade responsible for serum induction of the TTP promoter.

We have located consensus TPE1 binding sites in the promoters of a number of genes, including c-her1 (27), mdm2 (37), and fos (38). fos is induced by serum with early response gene characteristics. It will be interesting to investigate the possibility that this motif is involved in the regulated expression of these and other genes.

Because the serum-responsive region in the TTP promoter contains multiple potential promoter elements, it is likely that a number of nuclear proteins interact in its regulation. The DNA mobility shift assay using the EGR1-AP2 probe provides evidence to suggest that several proteins interact with the -77 to -35 sequence to mediate the serum response. We observed small but consistent changes in the intensity of a number of protein-DNA complexes using nuclear extracts from serum-treated and control cells. Serum-induced transcriptional activation of TTP is likely to result from post-transcriptional modification of pre-existing nuclear protein complexes, since TTP gene transcription is very rapidly stimulated by insulin or serum, even when cells have been pretreated with the protein synthesis inhibitor cycloheximide (1). Post-transcriptional modification of such factors following serum treatment could result in changes in the affinity of these factors for their DNA binding sites. Isolation and characterization of the TPE1 protein should lead to a better understanding of the transcription factor interactions that are involved in the regulation of TTP expression.

Our results suggest cooperative interactions among nuclear proteins binding to closely positioned cis-acting elements in the TTP promoter. For example, the EGR-1, TPE1, and AP2 elements from the TTP promoter each conferred only low levels of serum-inducible expression of β-globin-TTP hybrid constructs, but together they produced greater than additive expression. The gel mobility shift data also suggest the possibility of cooperative interactions among several transcription factors; for example, there was a reciprocal increase in the binding of nuclear proteins to the AP2 binding site and decreased protein binding to the TPE1 site. Finally, the presence of the single intron in the TTP gene markedly enhanced serum induction of the gene; we suspect this is due to enhanced transcription, although we cannot exclude effects of the intron on processing or stability of the mRNA (39). Further experimentation will be necessary to determine whether the intron and/or proteins that
bind to it interact in some way with the other serum-responsive elements of the TTP promoter.

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