Transcription enhancer factor-1 (TEF-1) has been implicated in transactivating a placental enhancer (CSEn) that regulates human chorionic somatomammotropin (hCS) gene activity. We demonstrated that TEF-1 represses hCS promoter activity in choriocarcinoma (BeWo) cells (Jiang, S. W., and Eberhardt, N. L. (1995) J. Biol. Chem. 270, 13609–13915), suggesting that TEF-1 interacts with basal transcription factors. Here we demonstrate that hTEF-1 overexpression inhibits minimal hCS promoters containing TATA and/or initiator elements, Rous sarcoma virus and thymidine kinase promoters in BeWo cells. Cotransfection of TEF-1 antisense oligonucleotides alleviated exogenous TEF-1-mediated repression and increased basal hCS promoter activity, indicating that endogenous TEF-1 exerts repressor activity. GST-TEF-1 fusion peptides fixed to glutathione-Sepharose beads retained in vitro-generated human TATA-binding protein, hTBP. The TEF-1 proline-rich domain was essential for TBP binding, but polypeptides also containing the zinc finger domain bound TBP with higher apparent affinity. TBP supershifted hTEF-GT-IIC DNA complexes, but TEF-1-inhibited in vitro binding of TBP to the TATA motif. Coexpression of TBP and TEF-1 in BeWo cells alleviated TEF-1-mediated transrepression, indicating that the TBP-TEF-1 interaction is functional in vivo. The data indicate that TEF-1 transrepression is mediated by direct interactions with TBP, possibly by inhibiting preinitiation complex formation.

Transcription enhancer factor-1 (TEF-1) appears to be a ubiquitous factor that has been implicated in directing the expression of a wide variety of genes, including the SV40 early promoter (Davidson et al., 1988; Xiao et al., 1991; Gruda et al., 1993; Hwang et al., 1993), human papillomavirus 16 E6 and E7 oncogenes (Ishii et al., 1992), muscle-specific genes (Shimizu et al., 1993; Kariya et al., 1993; Stewart et al., 1994), and the human chorionic somatomammotropin (hCS) gene enhancer (CSEn) function in placental cells (Walker et al., 1990; Jacquin et al., 1994; Jiang and Eberhardt, 1994). The mechanism of TEF-1 action has not been elucidated but appears to be complex. TEF-1 binds to sequences related to the general "enhancer core consensus," 5'-TGTGG(T/A)(T/A)(T/A)G-3' (Weiber et al., 1983; Kariya et al., 1993), and mutation of the binding sites for TEF-1 results in loss of transcriptional activation (Xiao et al., 1991). Nevertheless, when cells are cotransfected with TEF-1 expression vectors and promoter constructs containing the TEF-1 binding site, inhibition or squelching of promoter activity instead of activation is typically observed (Xiao et al., 1991; Shinji et al., 1992; Hwang et al., 1993; Jiang and Eberhardt, 1995). This has led to the concept that TEF-1 requires a limiting transcription factor for its transactivation function. In the case of the placenta-specific enhancer CSEn, we recently provided evidence that a factor, designated CSEF-1, which is distinct from TEF-1 and is abundant in choriocarcinoma and COS cells, bound to the GT-1IC and Sph-1/Sph-1 enhancers with binding specificity identical to that of TEF-1 (Jiang and Eberhardt, 1995). CSEn transactivation in COS and BeWo cells was correlated with the presence of CSEF-1, but not TEF-1, since COS cells contain very low levels, if any, of TEF-1. This raises the question of whether TEF-1 mediates transrepression but not transactivation in some cell types.

To address these issues, we reexamined the effects of overexpression of hTEF-1 on hCS promoter and enhancer function in choriocarcinoma cells (BeWo). Exogenous TEF-1 expression led to marked repression of basal hCS promoter activity without affecting relative enhancer-mediated stimulation of promoter activity. Interestingly, this repression appears to be independent of a GT-1IC binding site and was observed with several promoters, suggesting that basal transcriptional factors might be involved. Cotransfection of hTEF-1 antisense oligonucleotides not only reversed these effects, but also stimulated basal hCS promoter activity, indicating that endogenous TEF-1 was acting as a repressor in BeWo cells. Based on these studies we undertook an analysis of the ability of TEF-1 to bind to known components of the basal transcription apparatus. We found that TEF-1 bound to TATA-binding protein (TBP) and that the TEF-1 proline-rich and zinc finger domains were involved in this interaction. Since Hwang et al. (1993) demonstrated that these domains were required for both autostimulatory and stimulatory responses, our data suggest that TEF-1- TBP interactions were important for TEF-1 function in vivo. Interestingly, TEF-1 inhibited the binding of TBP to the hCS proximal promoter region containing the TATA box, whereas TBP supershifted hTEF-1-GT-1IC DNA complexes. Importantly, overexpression of TBP in BeWo cells alleviated the TEF-1-mediated inhibition of hCS promoter activity, indicating that TBP-TEF-1 interaction is functional in vivo. Our data are consistent with the hypothesis that TEF-1 transrepression may be the result of interference with the binding of TBP to the TATA element and that TEF-1-mediated transactivation requires additional factors. Alternatively, transactivation formally attributed to TEF-1 may be mediated by different factors. The latter possibility is strongly supported in the case of the placental

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TEF-1-TBP Interactions Mediate Transrepression

**EXPERIMENTAL PROCEDURES**

Materials-Oligonucleotides were synthesized by the Molecular Biology Core Facility, Mayo Clinic. Glutathione S-transferase (GST) gene fusion protein expression vectors (pGEX-4T-3), glutathione-Sepharose 4B, GST detection module, and poly(dI-dC) were purchased from Pharmacia Biotech Inc. Reduced glutathione was purchased from Sigma. [35S]Methionine (1,188 Ci/mmol) was obtained from Amersham Corp. [35S]-a-32P]ATP (5,000 Ci/mmol) and 5' triphosphosphate oligonucleotides were synthesized by the Molecular Biology Core Facility, Mayo Clinic. Glutathione Sepharose 4B (Pharmacia Biotech), biotinylated DNA (BIOTEC Inc.), and 2 mM L-glutamine (Life Technologies, Inc.) were purchased from ICN Biomedicals, Inc.

Cell Culture—BeWo cells (American Type Culture Collection) were maintained in RPMI 1640 (Life Technologies, Inc.), supplemented with 10% fetal bovine serum (Whitaker), 100 units/ml penicillin (Life Technologies, Inc.), and 2 mM L-glutamine (Life Technologies, Inc.). Cells were grown in monolayer at 37°C in an atmosphere containing 5% CO₂ and 100% humidity.

Cell Transfection and Luciferase Assay—Cell transfection by electroporation was performed as described previously (Jiang et al., 1995; Jiang and Eberhardt, 1995). Northern Blot Analysis—Triplicate transfections using 5 × 10⁵ BeWo cells and 15 ng of hCSP.LUC and either 500 ng of CMVp.gAL or CMVp.TEF-1 were performed as described previously (Jiang and Eberhardt, 1995). After various periods of incubation at 37°C, cellular RNA was isolated by the method of Xie and Rothblum (1991). RNA samples (50 µg) were subjected to standard conditions for Northern gel electrophoresis and transfer (Sambrook et al., 1989). Random primed DNA probes (1 × 10⁶ cpm/ml) generated from the luciferase coding region were hybridized at 45°C for 15 h according to Sambrook et al. (1989). The washed filters were exposed to Kodak x-ray film with intensifying screens at −70°C for 5 days.

Expression of TEF-1 in Escherichia coli—HB101 bacteria transformed with the various TEF expression plasmids were grown in 2 x YT-G medium (Sambrook et al., 1989) in the presence of 100 µg/ml ampicillin. The bacteria were induced by 100 mM isopropyl-1-thio-b-D-galactopyranoside treatment at 30°C for 4 h and then collected by centrifugation and resuspended in 1 x phosphate-buffered saline. After sonication on ice, Triton X-100 was added to a final concentration of 1% to aid the solubilization of proteins.

Protein Retention Assays—Protein-protein interaction experiments were performed with minor modifications of the procedure of Seto et al. (1992). Beads coated with GST-TEF fusion protein (20 µl) obtained from the batch purification step (manufacturer’s protocol) were washed three times with 400 µl of incubation buffer (50 mM KCl, 40 mM HEPES-HCl (pH 7.5), 2 mM MgCl₂, 0.5% nonfat milk, 0.5% Nonidet P-40, 1 mM dithiobis (1,1'-ethylenethiolether) and 1 mM phenylmethylsulfonyl fluoride). Aliquots (10 µl) of in vitro translated [35S]-labeled TBP, TFIIB, or TRβ proteins were mixed with the beads in 100 µl of incubation buffer. The beads were suspended by mechanical agitation at 4°C for 3 h, collected by centrifugation, and washed five times with cold incubation buffer. Bound proteins were recovered by brief boiling in 40 µl of SDS loading buffer (50 mM Tris-HCl (pH 6.8), 100 mM dithiobis (1,1'-ethylenethiolether) and 10% 2-mercaptoethanol and 20% glycerol) and analyzed by 10% SDS-PAGE along with prestained protein standards (Bio-Rad). After electrophoresis, the gels were dried and exposed to Kodak x-ray film at −70°C for 2-5 days with intensifying screens.

**RESULTS**

**Table I**

| Name            | Sequence                  | Purpose                          |
|-----------------|---------------------------|----------------------------------|
| 5'-pGEX         | GGCTGCGAAGCCACGGTTTG      | Sequencing                       |
| 3'-pGEX         | CGGGACGCTCAGTGGTCAGAGG    | Sequencing                       |
| GT-TEF-1 (+)    | GACACAGGGTGAAATGGTTGTC    | Gel shift probe                  |
| GT-TEF-1 (-)    | GACAGGACATCCACAGCTGTC     | Gel shift probe                  |
| GT-TEF-2 (+)    | GACAGCACGGTTGACTGCTTC     | Gel shift probe                  |
| GT-TEF-2 (-)    | GACAGCACGGTTGACTGCTTC     | Gel shift probe                  |
| AdML-TATA (+)   | GCCAGCGGCTTTATAGGCCTGAGG  | Gel shift probe                  |
| GST-TEF-1       | GGCGAGCGTTTATAGGCCTGAGG   | GST-TEF, GST-TEF-340/426 and GST-TEF-135/426 |
| GST-TEF-2       | GGCGAGCGTTTATAGGCCTGAGG   | GST-TEF, GST-TEF-340/426 and GST-TEF-135/426 |
| GST-TEF-3       | GGCGAGCGTTTATAGGCCTGAGG   | GST-TEF, GST-TEF-135/426         |
| GST-TEF-4       | GGCGAGCGTTTATAGGCCTGAGG   | GST-TEF-135/426                  |
| GST-TEF-5       | GGCGAGCGTTTATAGGCCTGAGG   | GST-TEF-135/426                  |
| GST-TEF-6       | GGCGAGCGTTTATAGGCCTGAGG   | GST-TEF-135/426                  |

Oligonucleotides used in current studies
activity in BeWo cells (Jiang and Eberhardt, 1995). This suggested that TEF-1-mediated inhibition of promoter activity was independent of a TEF-1 DNA binding site and might result from interactions of TEF-1 with basal transcription factors. To test this hypothesis, we cotransfected the CMVp.TEF expression plasmid with wild-type and mutated or CSEn-linked versions of the hCS promoter to ascertain whether TEF-1 function depended on a previously identified promoter element. Confirming our previous results (Jiang and Eberhardt, 1995), cotransfection of increasing amounts (40–1,080 ng) of cotransfected CMVp.TEF DNA resulted in progressive repression of basal hCS promoter activity (ANOVA, p < 0.0001) and total CSEn-stimulated activity (ANOVA, p < 0.0001). Panel B, relative enhancer-stimulated activity (fold stimulation) from the EnA hCSp.LUC gene was not affected by increasing amounts (40–1,080 ng) of cotransfected CMVp.TEF DNA (ANOVA, p < 0.2).

The hCS promoter is dominantly controlled by Sp1, TATA box, and initiator elements in BeWo cells (Jiang et al., 1995). We therefore analyzed CS promoters. RSV promoters (Fig. 2, B and C). Therefore, TEF-1-mediated transrepression results from interactions with the promoter and does not depend on GT-IIC sequences present in CSEn.

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TEF-1 overexpression. Increasing concentrations of TEF-1 function of endogenous TEF-1 was studied in the absence of antisense oligonucleotide was fully functional. Second, the control sense oligonucleotide (Fig. 4) in a manner indistinguishable from the hCS promoter. This repression was not due to the presence of excess promoter DNA, since cotransfection of large amounts of DNAs containing the CMV or TK promoters was without effect on hCS, RSV, or TK promoter activity (Fig. 2, A–C). These data further support the concept that overexpression of TEF-1 interferes with a common, basal transcription mechanism.

Overexpression of TEF-1 Decreases hCSp.LUC Transcripts—TEF-1 transrepression in HeLa cells has been shown to be mediated at the level of transcription by in vitro transcription studies (Xiao et al., 1991; Hwang et al., 1993). We found that cotransfection of CMVp.TEF-1 inhibited hCSp.LUC mRNA transcripts in BeWo cells by analysis of Northern blots (Fig. 3). Low levels of hCSp.LUC transcripts were readily detected 3 h after transfection. No difference between control and TEF-1-overexpressing cells was observed up to 7 h after transfection. However, 18 h after transfection significantly lower levels of hCSp.LUC transcripts were observed in TEF-1-transfected cells. The time-dependent repression probably reflects the time required for TEF-1 to accumulate to sufficient levels to affect hCSp.LUC expression. These data are consistent with the concept that TEF-1 acts by a transcriptional mechanism in BeWo cells.

Cotransfection of TEF-1 Antisense Oligonucleotides Stimulates Basal hCS Promoter Activity—We demonstrated previously that TEF-1 levels in BeWo cells could be up- and down-regulated by transfection of a TEF-1 expression vector or TEF-1 antisense oligonucleotides (Jiang and Eberhardt, 1995). We utilized this approach to assess the physiological role of endogenous TEF-1 on transcription in BeWo cells. First, varying amounts of the sense and antisense oligonucleotides were cotransfected along with the fixed amounts of hCSp.LUC and CMVp.TEF genes. The low level of hCS promoter activity in the presence of the CMVp.TEF gene was derepressed with increasing concentrations of the antisense oligonucleotide, but not the control sense oligonucleotide (Fig. 4A), confirming that the antisense oligonucleotide was fully functional. Second, the function of endogenous TEF-1 was studied in the absence of TEF-1 overexpression. Increasing concentrations of TEF-1 antisense, but not sense, oligonucleotide resulted in a 2-fold increase in hCS promoter activity (Fig. 4B). The fact that TEF-1 antisense oligonucleotides almost completely reversed the effects of TEF-1 overexpression indicates that the oligonucleotide acts by inhibiting TEF-1 expression and not by another mechanism. It is noteworthy that the concentrations of antisense oligonucleotides at which the half-maximal effect occurs is left-shifted in the data in Fig. 4B (22.6 μM) versus that in Fig. 4A (28.5 μM). This dose-response sensitivity probably reflects the relatively higher concentration of TEF-1 mRNA (compare Fig. 3) in cells containing exogenous TEF-1 (panel A) than in cells that only contain endogenous TEF-1 (panel B). The inhibition of promoter activity observed at oligonucleotide concentrations greater than 40 μM with both the sense and antisense oligonucleotides appears to be due to nonspecific toxicity. These data indicate that endogenous levels of TEF-1 in BeWo cells inhibit hCS promoter activity and provide additional evidence that TEF-1 function in BeWo cells is dominantly inhibitory.

TEF-1 Binds to the TATA-binding Protein, TBP—The TATA box binding factor TBP and TFIIIB are frequently involved in
interaction with other transcriptional factors (Seto et al., 1992; Lin et al., 1991; Ing et al., 1992), and previous studies (Brou et al., 1993; Gruda et al., 1993) have suggested that components of the basal TFIID transcription complex might be involved with TEF-1 action. Since our studies suggested the possible TEF-1 involvement with basal transcriptional factor(s), we sought to determine whether TEF-1 interacts directly with hTBP or TFIIB. Initially a GST-TEF fusion protein was expressed in E. coli and purified using glutathione-Sepharose 4B beads. Aliquots (30 ng) of thrombin-cleaved, intact GST-TEF fusion protein and GST peptide were resolved by SDS-PAGE and visualized by silver staining. Panel B, 35S-labeled hTBP, hTFIIB, and hTRβ from in vitro translation. No 35S-labeled product was observed from control reactions lacking plasmid DNA (TNT). Panel C, recovery of 35S-labeled proteins after binding to GST-TEF-coated glutathione-Sepharose 4B beads.

We next analyzed gel shift experiments with TEF and the GT-IIC enhanson in the presence or absence of TBP. When purified TBP was added to gel shift assays using different amounts of in vitro-generated TEF-1, a weak but visible super-shift was observed (Fig. 6). Since neither GST protein nor TBP binds to the GT-IIC probe, the supershift represents the TBP-TEF-GT-IIC complex. This result confirms the TEF-1 interaction and indicates that this protein-protein interaction does not affect the ability of TEF-1 to bind DNA.

TEF-1 Inhibits In Vitro Binding of TBP to the TATA Element—Since TBP also has specific DNA binding activity, we analyzed the effects of TEF-1 on the ability of TBP to bind to the TATA element. Surprisingly, the addition of increasing amounts of GST-TEF inhibited the binding of TBP to the proximal (nucleotides −40/−10) HCS promoter region that contains the TATA element (Fig. 7). The inhibition was not due to the presence of GST on the fusion protein, since GST had no observable effect on TBP binding to the TATA element (Fig. 7).
These data suggest that TEF-1 might inhibit promoter action by inhibiting preinitiation complex formation. Also, if TEF-1 has any transactivation function in BeWo cells, the data support the concept that additional factors would be required for such activity.

The TEF-1 Proline-rich and Zinc Finger Domains Are Required for Binding TBP—Our data suggest that the TEF-TBP interaction may be essential for TEF function. Three TEF-1 domains have been identified which are required for its transcriptional activation and autointerference activities in HeLa cells (Hwang et al., 1993). These domains include a COOH-terminal zinc finger, STY-, and proline-rich domains (Fig. 8).

To ascertain whether any of these domains might be important for the TEF-1-TBP interaction, we analyzed the TEF-1 domains that were required for TBP binding. Several in-frame GST-TEF-1 deletion mutants (Fig. 8 and Fig. 9, A and B) were expressed in E. coli (Fig. 9B), and glutathione-Sepharose beads coated with truncated GST-TEF-1 were incubated with in vitro-generated $^{35}$S-labeled TBP and washed, and the bound TBP was eluted and analyzed by gel electrophoresis (Fig. 9B). All of the fusion polypeptides were expressed at similar levels except GST-TEF-1(133-426), which was more abundantly expressed (Fig. 9B) and which did not bind TBP appreciably (Fig. 9C). All of the polypeptides that contained the proline-rich domain (amino acids 143–204) bound TBP, indicating that this region was essential for TBP binding. Those polypeptides that included the region downstream of the proline-rich domain, particularly the zinc finger domain, bound TBP with the highest apparent affinity. However, the zinc finger domain alone did not bind TBP. These data suggest that
hCS

TEF-1 is proposed to transactivate a variety of eukaryotic enhancers and promoters, including the SV40 (Davidson, 1988; Xiao et al., 1991; Hwang et al., 1993) and hCS (Walker et al., 1990; J acquemin et al., 1994; J iang and Eberhardt, 1994, 1995) enhancers, muscle-specific genes (Shimizu et al., 1993; Kariya et al., 1993; Stewart et al., 1994), human papillomavirus-16 E6 and E7 oncogenes (Ishii et al., 1992), and mouse early developmental genes (Melin et al., 1993). Since overexpression of TEF-1 in various cells results in transcriptional squelching (Xiao et al., 1991; Ishii et al., 1992; Hwang et al., 1993; J iang and Eberhardt, 1995), it has been proposed that other limiting transcription factors along with TEF-1 are required for transactivation.

Recently we found that another factor, CSEF-1, possibly unrelated to TEF-1, is correlated with CSEn transactivation in choriocarcinoma cells (BeWo). In these cells TEF-1 overexpression failed to activate the hCS enhancer and repressed hCS promoter activity in the absence of the enhancer (J iang and Eberhardt, 1995). These results reflect those found with the myosin heavy chain β gene, in which two distinct factors, a ubiquitous mouse TEF-1 homolog and an unrelated muscle-specific factor, bind to the GT-IIC-related element, and the ubiquitous TEF-1 homolog failed to transactivate myosin heavy chain β gene expression in mouse skeletal muscle cells (Shimizu et al., 1993). Also, Stewart et al. (1993) have shown that GAL4 chimeras containing a novel isoform of chicken TEF-1 which has 13 additional COOH-terminal amino acids can transactivate GAL4-dependent reporter genes, whereas chimeras corresponding to the ubiquitous hTEF-1 isoform only exhibit squelching activity. These studies raise the possibility that the dominant function of the ubiquitous form of TEF-1 is a repressor.

The present studies expand our previous observation that TEF-1 represses hCS promoter activity independently of the presence of GT-IIC motifs (J iang and Eberhardt, 1995). Consistent with the concept that TEF-1 acts at the level of transcription, steady-state levels of luciferase transcripts were diminished in BeWo cells cotransfected with the hCSp.LUC and CMVp.TEF-1 genes (Fig. 3). Repression was not restricted to the hCS promoter, since it occurs with the heterologous TK and RSV promoters (Fig. 2, B and C), which have not been previously known to respond to TEF-1. Furthermore, TEF-1 inhibits transcription from the minimal hCS promoter (Fig. 2A), which contains a TATA box and an initiator element (J iang et al., 1995). Equivalent repression was observed with constructs lacking either the TATA or initiator element (Fig. 2A). Thus TEF-1-mediated repression may involve interactions with basal transcriptional factors that can function with both TATA and initiator elements. The TEF-1-mediated repression could be overcome by cotransfecting antisense oligonucleotides (Fig. 4). More importantly, the data demonstrate that endogenous levels of TEF-1 negatively regulate hCS promoter activity in BeWo cells, since antisense oligonucleotides increased hCS promoter activity in the absence of cotransfected CMVp.TEF-1 (Fig. 4). This finding suggests that TEF-1 does not function as a transactivator in these cells.

Previous studies suggested that TEF-1 might interact with TBP. First, in studies primarily directed at SV40 T antigen-TEF-1 interactions, it was observed that TBP could bind to TEF-1 (Gruda et al., 1993). Second, Brou et al. (1993) had shown that the activity of GAL-TEF-1 chimeras appeared to be mediated by at least two distinct classes of TFIID complexes. Using GST-TEF-1 pull-down assays we confirmed the observation of Gruda et al. (1993), demonstrating that TEF-1 specifically interacts with hTBP, but not hTFIIB or as a control the.
human TRβ receptor (Fig. 5C). Interestingly, TBP was able to
form supershifted complexes with TEF-1 and DNA containing
the GT-IIC enhancer (Fig. 6); however, TBP inhibited the
transcriptional activity. Drosophila p230 subunit of TFIID inhibits the TBP TATA
binding activity and represses transcription (Kokubo et al., 1994), activities that are shared by TEF-1.

Importantly, the TEF-1-mediated repression of the hCS promoter is alleviated by the cotransfection of increasing amounts of TBP expression plasmids (Fig. 10), indicating that TBP and TEF-1 functionally interact in vivo. This result provides strong support for the concept that the in vitro TBP-TEF-1 interactions observed in these studies represents a physiologically relevant interaction and suggests, but does not prove, that TEF-1 can interact with hCS TFIID. It is noteworthy that when overexpressed in eukaryotic cells, like TEF-1, many of the TBP-binding factors (Table II) cause strong squelching effects on heterologous promoters. Tax1 transactivates several viral and cellular promoters and enhancers through specific DNA elements. Tax1 interacts specifically with TBP and exerts strong squelching effects on transcription (Caron et al., 1993). Like the TBP-mediated inhibition of TEF-1 repression (Fig. 10), overexpression of TBP was able to stimulate the transactivation of GAL4-Tax1 chimeras and partially alleviates Tax1-mediated squelching (Caron et al., 1993). Also, c-Fos- and FosB-mediated inhibition of transcription can be partially relieved by overexpression of TBP (Metz et al., 1994a, 1994b). These studies suggest that TBP levels may be limiting, in which case squelching mechanisms involving additional factors are considered unlikely to explain the transcription inhibition. However, although TBP overexpression increased p65/NFκB-mediated transactivation, neither TBP nor TFIIB overexpression was able to alleviate p65/NFκB-mediated squelching (Schmitz et al., 1995), suggesting that additional proteins were required for transactivation in this case. Also, in BeWo cells, overexpression of TBP itself results in reduced promoter activity, suggesting that other limiting factors may

| Factor          | Source                   | Reference         |
|-----------------|--------------------------|-------------------|
| VP16            | Herpes simplex virus     | Stringer et al., 1990 |
| EIA protein     | Adenovirus               | Harikoshi et al., 1991 |
| tat             | Human immunodeficiency virus | Kashanishi et al., 1994 |
| IE1/I2 protein  | Human cytomegalovirus    | Hagemeier et al., 1992 |
| protein X       | Hepatitis B virus        | Qadri et al., 1995 |
| AD1             | Yeast                    | Auble and Hahn, 1993 |
| Oct/Oct2        | Human POU homeodomain proteins | Zwingler et al., 1994 |
| p230            | Drosophila TFIID subunit | Kukubo et al., 1994 |
| Dr1             | HeLa cells               | Inostroza et al., 1992 |
| DBF1-DBF4       | HeLa cells               | Meisterernst et al., 1991 |
| Tax1            | HTLV-1 transactivator    | Caron et al., 1993 |
| P53             | Human oncogene          | Seto et al., 1994 |
| MDM2            | Human oncogene          | Leng et al., 1995 |
| Pu.1            | Human transcription factor | Hagemeier et al., 1993 |
| FosB            | Cellular Fos oncogene    | Metz et al., 1994a |
| c-Fos           | Cellular Fos oncogene    | Metz et al., 1994b |

Fig. 11. Comparison of identified TBP-binding domains among transcription factors, whose interaction with TBP is essential for transcriptional activity.
be involved in mediating hCS promoter function.

Metz et al. (1994b) have provided evidence for the existence of a TBP binding motif that is shared by VP16, E1A, and c-Fos. We therefore compared the essential proline-rich TEF-1 domain and a number of other transcription factors that were known to bind to TBP and whose TBP binding domain had been narrowly mapped. Alignment of the sequences around the TBP binding motif, (F/L)V(F/L)D, indicates that TEF-1 shares some homology with these TBP-binding proteins (Fig. 11). Interestingly, TEF-1, p53, and VP16 show a region of homology just downstream of the putative TBP binding motif in a proline-rich domain, suggesting that these factors are more closely related, and the proline-rich region might constitute part of a conserved TBP binding domain.

Although TEF-1 blocks the formation of TBP-TATA complexes, this does not exclude the possibility that TEF-1 can serve as a transactivator in certain cell types. In such cells, additional cofactors may prevent TEF-1 inhibition of TBP-TATA complex formation in a manner similar to TFIIA inhibition of ADI-mediated disruption of the TBP-TATA complex (Auble and Hahn, 1993). However, the data do support the concept that TEF-1 may be an exclusive repressor in some cell types. This conclusion is strengthened by our recent findings that a 30-kDa GT-IIC-binding factor, apparently unrelated to TEF-1, is correlated with hCS enhancer activity in COS-1 cells, which express very low levels of TEF-1 (Jiang and Eberhardt, 1995). Accordingly, it is possible that TEF-1 provides a counterregulatory stimulus to the actions of other factors that mediate transactivation through the GT-IIC enhancer.

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REFERENCES

Auble, D. T., and Hahn, S. (1993) Genes & Dev. 5, 844–856
Brou, C., Chaudhary, S., Davidson, I., Lutz, Y., Wu, J., Egy, J. M., Tora, L., and Chambon, P. (1993) EMBO J. 12, 489–499
Caron, C., Rouset, R., Beraud, C., Moncolin, V., Egy, J. M., and Jalinot, P. (1993) EMBO J. 12, 4269–4278
Chatterjee, S., and Struhl, K. (1995) Nature 374, 820–822
Davidson, I., Xiao, J. H., Rasael, R., Staub, A., and Chambon, P. (1988) Cell 54, 931–942
Gruda, M. C., Zablotny, J. M., Xiao, J. H., Davidson, I., and Alwine, J. C. (1993) Mol. Cell. Biol. 13, 961–969
Hagenmeier, C., Walker, S., Caswell, R., Kozarides, T., and Sinclair, J. (1992) J. Virol. 66, 4452–4456
Hagenmeier, C., Bannister, A. J., Cook, A., and Kozarides, T. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 1580–1584
Horikoshi, N., Maquire, K., Krall, A., Maldonado, E., Reinberg, D., and Weinmann, R. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5124–5128
Hwang, J. J., Egly, J. M., and Davidson, I. (1993) EMBO J. 12, 2373–2348
Ing, N. H., Beekman, J. M., Tsai, S. Y., Tsai, M. J., and O'Malley, B. W. (1992) J. Biol. Chem. 267, 17617–17623
Inostroza, J. A., Meruelstein, F. H., Ha, I., Lane, W. S., and Reinberg, D. (1992) Cell 70, 477–489
Ishiji, T., Lance, M. J., Parkkinnen, S., Anderson, R. D., Haugen, T. H., Cripe, T. P., Xiao, J. H., Davidson, I., Chambon, P., and Turek, L. P. (1992) EMBO J. 11, 2271–2281
Jacquemin, P., Oury, C., Peers, B., Morin, A., Belaey, A., and Matar, J. A. (1994) Mol. Cell. Biol. 14, 93–103
Jiang, S.-W., and Eberhardt, N. L. (1994) J. Biol. Chem. 269, 10384–10392
Jiang, S.-W., and Eberhardt, N. L. (1995) J. Biol. Chem. 270, 13906–13915
Jiang, S.-W., Shepard, A. R., and Eberhardt, N. L. (1995) J. Biol. Chem. 270, 3683–3692
Kariya, K., Farrant, I. K., and Simpson, P. C. (1993) J. Biol. Chem. 268, 26658–26662
Kashanchi, F., Piras, G., Radonovich, M. F., Fattaey, A., Chiang, C. M., Roeder, R. G., and Brady, J. N. (1994) Nature 367, 295–299
Klages, N., and Strubin, M. (1995) Nature 374, 822–823
Kokubu, T., Yamashita, S., Horikoshi, M., Roeder, R. G., and Nakatani, Y. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5320–5324
Leng, P., Brown, D. R., Deb, S., and Deb, S. P. (1995) Int. J. Oncol. 6, 251–259
Lin, Y. S., Ha, I., Maldonado, E., Reinberg, D., and Green, M. R. (1991) Nature 353, 569–571
Meisterernst, M., Roy, A. L., Lieu, H. M., and Roeder, R. G. (1993) Cell 66, 981–993
Melin, F., Miranda, M., Montreau, N., DePamphilis, M. L., and Blangy, D. (1993) EMBO J. 12, 4657–4666
Metz, R., Kozarides, T., and Bravo, R. (1994a) EMBO J. 13, 3832–3842
Metz, R., Bannister, A. J., Sutherland, J. A., Hagemeier, C., O'Rourke, E. C., Cook, A., Bravo, R., and Kozarides, T. (1994b) Mol. Cell. Biol. 14, 6021–6029
Qadri, I., Maquire, H., and Siddiqui, A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1003–1007
Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., pp. 7.26–7.29, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Schmitz, M. L., Steiger, G., Altmann, H., Meisterernst, M., and Baxevarel, P. A. (1995) J. Biol. Chem. 270, 7219–7226
Seno, E., Usheva, A., Zambetti, G. P., Momand, J., Horikoshi, N., Weinmann, R., Levine, A. J., and Shenk, T. (1992) Proc. Natl. Acad. Sci. U. S. A. 90, 1208–1209
Shimizu, N., Smith, G., and Izumo, S. (1993) Nucleic Acids Res. 21, 4103–4110
Snedeker, G. W., and Cochran, W. G. (1980) Statistical Methods, 7th Ed., p. 116, Iowa State University Press, Ames, IA
Stewart, A. F., Larkin, S. B., Farrant, I. K., Mar, J. H., Hall, D. E., and Oridahl, C. P. (1994) J. Biol. Chem. 269, 3147–3150
Stringer, K. F., Ingles, C. L., and Greenblatt, J. (1990) Nature 345, 783–786
Walker, W. H., Fitzpatrick, S. L., and Saunders, G. F. (1990) J. Biol. Chem. 265, 12940–12948
Weiser, H., Konig, M., and Gruss, P. (1983) Science 219, 626–631
Xiao, J. H., Davidson, I., Matthes, H., Garner, J. M., and Chambon, P. (1991) Cell 65, 551–568
Xie, W., and Rothblum, L. I. (1991) BioTechniques 11, 325–327
Yokomori, K., Zeidler, M. P., Chen, J. L., Verrijzer, C. P., Midzdek, M., and Tijan, R. (1994) Genes & Dev. 8, 2313–2323
Zwillinger, S., Annweller, A., and Wirth, T. (1994) Nucleic Acids Res. 22, 1655–1662
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