Smads regulate transcription of defined genes in response to transforming growth factor-β (TGF-β) receptor activation. This process involves functional cross-talk of Smads with transcription factors at responsive DNA elements to achieve maximal transcription activation and specificity. TGF-β has been shown to induce transcription of the germ line (GL) Ig α constant region gene and to direct class switching to IgA antibodies. It has been shown that acute myeloid leukemia (AML) transcription factors cooperate with Smad3 to stimulate transcription from the GL Ig α constant region gene promoter. We report here that the TGF-β-induced transcription from this promoter requires DNA binding of cAMP-response element-binding protein (CREB) to the nearby ATF/cAMP-response element site and of Smads to a nearby Smad binding sequence. At these sites, Smad3/4 cooperates with CREB to activate transcription in response to TGF-β, and disruption of either binding sequence abolished TGF-β-induced transcription. In addition, AML1 or AML2 also binds to the promoter and cooperates with Smad3/4, and in this way further enhances the TGF-β-induced transcriptional activation of the GL Ig α promoter. Thus, whereas Smad3/4, CREB, and AML family members bind independently to the respective DNA sequences in the GL Ig α promoter, functional synergy of Smads with CREB and AML proteins results in maximal TGF-β-induced transcription.

During B cell differentiation, many B cells elaborate a process called class switching, whereby the initial synthesis of IgM antibodies is converted to the synthesis of IgD, IgG, IgE, or IgA antibodies of the same specificity. During the switch, the light chain and the variable region of the heavy chain of the antibody remain the same, but the heavy chain constant region changes, resulting in a change in effector function but not in specificity of the antibody. In embryonic cells, the genes for the constant regions of all kinds of heavy chains are located next to each other on the chromosome. Class switching occurs by an intrachromosomal recombination event that joins variable region genes with a gene for a constant region, Cδ, Cy, Ce, or Ca, to form a functional heavy chain gene for IgD, IgG, IgE, or IgA antibody, respectively. This process appears to be directed by transcription of unrearranged or germ line (GL)1 transcripts of heavy chain constant region genes before switch recombination (1).

Transforming growth factor-β (TGF-β) regulates a broad range of biological activities, including the immune response (2). In cultures of lipopolysaccharide-stimulated B cells, TGF-β induces transcription of the GL Ig α constant region gene and directs class switching to IgA antibodies (3–5). Previous work has shown that the promoter segment −128 to +46, relative to the first transcription initiation site for mouse GL Ig α transcripts, is sufficient for transcription and TGF-β-inducible expression in B cells (6). Within this segment resides an ATF/CRE site that is very important for constitutive expression of the promoter and a TGF-β response element (TβRE) that is required for induction of transcription by TGF-β. The TβRE contains two copies of the Smad3/4 binding site (GTCT or AGAC) and two copies of a binding site for the acute myeloid leukemia (AML) family of transcription factors, also known as core-binding factor A (CBFA) or polyomavirus enhancer-binding protein 2α (PDB-2α) (7, 8).

The signaling responses to TGF-β and related factors are mediated by a heteromeric cell surface complex of two types of transmembrane serine/threonine kinase receptors and their intracellular signal transducers, the Smad proteins (9–11). Upon TGF-β binding to the receptor complex, Smad3 and the closely related Smad2 are phosphorylated by the type I receptor kinase at their C-terminal SSXS sequence. This phosphorylation event activates these Smads and allows them to dissociate from the receptor and to associate with Smad4. The Smad heteromeric complex, presumably a trimer, then moves into the nucleus to activate the transcription of target genes. The ability of the Smad complex to activate transcription results from its ability to directly interact with DNA in the promoter sequences and to functionally cooperate with other transcription factors (12). This dual requirement confers selectivity to promoters that represent targets for TGF-β and Smad signaling.

Recently, it has been shown that Smads can bind directly to the TβRE and cooperate with AML proteins to stimulate transcription from the GL Ig α promoter (13, 14). In this report, we demonstrate that the ATF/CRE site, which flanks the TβRE, is required for TGF-β-induced transcription from the GL Ig α

1 The abbreviations used are: GL, germ line; TGF-β, transforming growth factor-β; AML, acute myeloid leukemia; TβRE, TGF-β response element; SBE, Smad binding element; ABE, AML binding element; GST, glutathione S-transferase; CRE, cAMP-response element; CREB, CRE-binding protein; CBP, CREB-binding protein; CBFA, core-binding factor A; PDB-2α, polyomavirus enhancer-binding protein 2α; HA, hemagglutinin.
Smad, CREB, and AML Regulate Ig α Gene Transcription

promoter. At the promoter, CREB and Smad3 bind to adjacent sequences and synergize with each other to mediate TGF-β-induced transcription. The transcriptional response is further enhanced through the additional cooperativity of AML proteins with Smad3/4. Our results suggest a model whereby Smads cooperate with both AML and CREB for maximal TGF-β-induced transcription from the GL Ig α promoter.

EXPERIMENTAL PROCEDURES

Expression Plasmids for TGF-β receptor, Smads, AMLs, and ATF/CREB—The pCMV-based expression plasmids for the constitutively active mutant TβRI (T202D) (15), the N-terminally FLAG-tagged Smad3, and the C-terminal Myc-tagged Smad4 have been described (16, 17). The expression plasmids pCMV5-HA-AML2B, pCMV5-AML2 were kindly provided by S. Hiebert. Plasmid pRK5-HA-AML2, encoding N-terminally HA-tagged AML2, was generated by subcloning the BamHI fragment from pCMV5-AML2 into the BamHI site of pRKHA, a pkK-derivative with the HA epitope tag (amino acids CYPVDVPDYASL) inserted into ClaI and EcoRI sites of pRK5. Plasmids pRSV and pRSV-CREB were provided by K. Yamamoto. The cytomegalovirus promoter-driven pRK-HA-CREB was generated by inserting a polymerase chain reaction-generated full-length cDNA sequence into the EcoRI and SalI sites of pRK-HA. pRK-HA-ATF1 and pRK-HA-ATF2 were similarly generated by subcloning EcoRI/HindIII and BamHI fragments from pSG424-ATF1 and pGEX3X-ATF2, respectively (gifts from M. Green), into pRKHA. The pRK5-based expression plasmid for HA-tagged-c-Jun has been described (17).

The bacterial expression plasmids pGEX-Smad3NL and pGEX-Smad4NL, used to express GST-fused Smad fragments, were described before (17), pGEX-CREB and pGEX-AML1, which express CREB or AML1 fused to GST, were constructed by subcloning the CREB or AML1 full-length cDNA sequence into the EcoRI/SalI site of pGEX-5X1 (Amersham Pharmacia Biotech).

Luciferase Reporter Plasmids and Functional Assays—pC179-Luc, containing the −179 to +46 region of the mouse germ line Ig α constant region gene promoter, was kindly provided by J. Stavnezer (6). TAuc, a minimal TATA box-containing promoter, was generated by replacing the HindIII/XbaI (2675–2797) fragment of pFR-Luc (Stratagene) with the oligonucleotide sequence AAGCTT CGGA TCTAGA. Reporter plasmid −132–94Ca TAuc and its mutant derivatives were generated by replacing the HindIII/XbaI (2675–2797) fragment of pFR-Luc with the oligonucleotide sequence AAGCTTCCAGCTATCTAG. Reporter plasmid −132–94Ca TAuc and its mutant derivatives were generated by replacing the HindIII/XbaI (2675–2797) fragment of pFR-Luc with the oligonucleotide sequence AAGCTTCCAGCTATCTAG. Reporter plasmid −132–94Ca TAuc and its mutant derivatives were generated by replacing the HindIII/XbaI (2675–2797) fragment of pFR-Luc with the oligonucleotide sequence AAGCTTCCAGCTATCTAG.

Transient transfections and luciferase assays were used to measure transcriptional activation from the promoter of interest. Transfections of HepG2 cells were done in six-well dishes using Fugene 6 (Roche Molecular Biochemicals). Transfection of A20.3 B lymphoma cells (pro-duced by J. Stavnezer) were done in six-well dishes using LipofectAMINE plus (Life Technology). Per transfection, 5 ng of each ex-pression plasmid (0.25 μg of vector DNA) was added. 12 h after transfection, cells were lysed, and luciferase expression from different reporters was measured as described (18). All luciferase values were normalized for transfection efficiency against the β-galactosidase levels.

Gel Mobility Shift Assays—Gel mobility assays were done using the gel shift assay kit (Promega). The GST fusion proteins were expressed in Escherichia coli and purified using glutathione-Sepharose (Amersham Pharmacia Biotech). In vitro translated proteins were synthesized by coupled SP6-driven transcription and translation using rabbit reticulocyte lysate (TNT SP6 quick translation kit, Promega). Each gel shift reaction contained 0.1 μg of purified GST fusion protein in 1 μl of glutathione elution buffer or 1 μl of in vitro translated protein from the TNT reaction. After 10–15 min preincubation at room temperature, 1 μl of [32P]-labeled probe (35,000–50,000 cpm/0.35 pmol) was added to a 10-μl final volume and incubated for another 20–25 min. The complex was resolved on a 5% polyacrylamide gel in 0.5× TBE buffer and visualized by autoradiography. For gel mobility shifts and supershifts using nuclear extracts of transfected cells, 293 cells in 10-cm dishes were transiently transfected using LipofectAMINE (Life Technology) as described. Nuclear extracts were prepared as described (19). For each gel shift reaction, 2 μl of extract, containing approximately 1 μg/ml of protein, was used. For each supershift analysis, 1 μl of antibodies against Smad3 (H2), Myc (9E10), or CREB (240) from Santa Cruz Biotechnology, or HA (12CA4) from Roche Molecular Biochemicals was added to the reaction after incubation with [32P]-labeled probe for 20 min, and the incubation proceeded at 4 °C for 90 min.

RESULTS

The TGF-β Response Element and the ATF/CRE Sequence Are Both Required for TGF-β-induced Transcription from the GL Ig α Constant Region Gene Promoter—TGF-β induces transcrip-tion from the GL Ig α constant region gene promoter and increases class switching to IgA in splenic B cells and in sIgM+ B lymphoma cells (3–5). Previous studies have defined the mouse GL Ig α promoter segment between −128 and +46, relative to the first transcription initiation site, as sufficient for constitutive and TGF-β-induced transcription of a reporter gene in B cells (6). Within this segment, a TβRE has been identified as a sequence required for TGF-β-induced transcription. This 94-base pair sequence, shown in Fig. 1A, is located at positions −128 to −105 upstream from the first transcription start site and contains two copies of the consensus AML/CBF binding sites ACCGAGCA and two copies of the proposed optimal Smad3/4 binding site (GTCT or AGAC). The TβRE is proximally flanked with an ATF/CRE site TACAGTGG at positions −103 to −96, i.e. separated by only one base pair from the TβRE (Fig. 1A). This ATF/CRE site has been shown to be important for constitutive transcription from this promoter (6).
As a first step in defining the contributions of different transcription factors in TGF-β-induced transcription from this promoter, we inserted the GL Igα promoter sequence from position –132 to –94 immediately upstream from a minimal TATA box promoter and measured transcription activity of this reporter in HepG2 cells. Similarly to B cells, HepG2 cells express Smad3, Smad4, CREB, AML1, and AML2, as assessed by reverse transcription-polymerase chain reaction (data not shown). In the absence of TGF-β, this promoter in reporter plasmid –132/-94 TAluc was about 3-fold more active than the minimal promoter in TAluc. TGF-β strongly induced transcription from the –132/-94 TAluc promoter to a level that was comparable to pCa179, a luciferase reporter containing the GL Igα promoter segment from –179 to +77. However, the basal transcription level of the –132/-94 TAluc was lower than that of the –179 to +77 promoter segment (Fig. 1b). These data strongly suggest that the –132 to –94 promoter segment, which contains both the TβRE and the ATF/CRE sites, is sufficient to confer full TGF-β inducibility. To determine the roles of the TβRE and the ATF/CRE sites in TGF-β-induced transcription, we constructed two promoter/reporter plasmids that deleted or inactivated either the TβRE or the ATF/CRE site, respectively (Fig. 1A). As shown in Fig. 1b, deletion of the TβRE, without affecting the ATF/CRE site, in the –106/-94 promoter abolished the TGF-β response. Similarly, inactivation of the ATF/CRE site, without affecting the TβRE, also abrogated the TGF-β-induced responsiveness from the –132/-94mCRE promoter. We therefore conclude that both the TβRE and the ATF/CRE sites are required for TGF-β inducibility. This simultaneous requirement for two distinct and independent DNA binding elements suggests that Smad and ATF/CREB family members to ATF/CRE but not to TβRE. Gel shift assays were performed using in vitro translated proteins synthesized in rabbit reticulocyte lysate. Gel shifts were performed using probes containing the regulatory elements derived from the mouse GL Igα promoter (see Fig. 1A), flanked by AGCTT or CGGAT at the 5’ or 3’ end, respectively. A, effect of ATF/CREB family members on the TGF-β-induced transcription from the –132/-94 region of the GL Igα promoter in transfected cells. B, Smad3 and Smad4, but not Smad2, have the ability to bind DNA (26–29). The DNA binding is mediated by the N-terminal domain. As shown in Fig. 3A, both GST-fused Smad3NL and Smad4NL bound to the –132/–94 Igα promoter sequence. Consistent with published data, the Smad3NL-DNA complex migrated as a single band, whereas the Smad4NL-DNA complex separated as multiple bands (26). Whereas Smad3NL and Smad4NL bound to the TβRE sequence in the promoter, they did not bind to the ATF/CRE oligonucleotide.

We next evaluated the ability of CREB to cooperate with Smad3 or Smad3/4. Smad3 expression activated transcription about 30-fold, and TGF-β enhanced this response to a 2000-fold level (Fig. 2C). Consistent with the central role of Smad4 as coactivator in signal transduction by receptor-activated Smads (24, 25), coexpression of Smad4 with Smad3 resulted in a much stronger transcriptional activation than the response to Smad3 or Smad4 individually. CREB was able to synergize with Smad3, and maximal transcription activity was observed when CREB, Smad3, and Smad4 were expressed together. Coexpression of c-Jun or ATF1 or ATF2 with CREB, Smad3, and Smad4 together decreased the CREB/Smad3/Smad4 activity (data not shown), which is consistent with the notion that CREB binds to the ATF/CRE site as a homodimer (21).
There are two copies of the proposed optimal Smad3/4 binding site (GTCT or AGAC) located in the T\(\beta\)RE sequence of the GL Ig\(\alpha\) promoter. Mutations that have been shown to inactivate Smad binding (26–29) were introduced into the SBE1 or SBE2 sequences, and the effects of these mutations were tested on TGF-\(\beta\)- and Smad3-induced transcription. As shown in Fig. 3B, inactivation of SBE1 caused a moderate decrease of the TGF-\(\beta\)- and Smad3-induced transcription. However, SBE2 inactivation severely decreased TGF-\(\beta\) responsiveness and abolished the transcriptional induction by Smad3, thus identifying the SBE2 site as the major Smad binding site. Although Smad3 and Smad4 did not bind to the ATF/CRE site (Fig. 3A), mutations of the ATF/CRE site also severely decreased Smad3-induced transcription (Fig. 3B), similar to the effect of this mutation on TGF-\(\beta\)-induced transcription (Fig. 1B). On the other hand, the ability of CREB to enhance TGF-\(\beta\)-induced transcription also required the Smad binding site. Mutation of SBE2 diminished TGF-\(\beta\)-induced transcription but not the transcription activation by CREB in the absence of TGF-\(\beta\) (Fig. 3B). Taken together, these results indicate that Smads cooperate with CREB to enhance the transcription from GL Ig\(\alpha\) promoter upon TGF-\(\beta\) stimulation and that DNA binding of Smad and CREB is required for their cooperation.

We next assessed the ability of Smad3/4 and CREB to bind to the TGF-\(\beta\)-responsive –132/–94 GL Ig\(\alpha\) promoter sequence in gel shift assays using nuclear extracts prepared from transfected cells. TGF-\(\beta\)-receptor activation induced the formation of a gel-shifted band with a slightly slower mobility than the constitutive band (Fig. 3C). This inducible band was supershifted by an antibody against Smad3 and further supershifted in the presence of both anti-Smad3 and anti-Smad4(MyC) antibodies, thus indicating that the Smad3/4 complex interacts with the promoter segment. Addition of CREB-specific antibodies supershifted primarily the DNA-protein complex that was not induced by TGF-\(\beta\) receptor activation, without major effect on the TGF-\(\beta\)-inducible complex. Addition of anti-Smad antibody did not result in an additional shift of the anti-CREB antibody-supershifted complex (Fig. 3C). These results suggest that both Smad3/4 and CREB bind independently to the promoter segment. The DNA binding of Smad3 and CREB was also analyzed using purified GST fusion proteins. As shown in Fig. 3D (left) and as expected, Smad3NL and CREB both bound to the promoter segment. The DNA binding of Smad3 and CREB did not result in competition for each other's binding or in the formation of an additional band. However, when an excess amount of Smad3NL was used in the experiment, the formation of an additional, slower migrating complex was observed (Fig. 3D, right). This result suggests that Smad3 and CREB can bind simultaneously to the DNA, but their binding does not involve a high affinity physical association between Smad3 and CREB. This is consistent with our inability to coprecipitate Smad3 with CREB in transfected cells (data not shown) using conditions that allowed coprecipitation of c-Jun with Smad3 (17).

AML1/2 Binding to the T\(\beta\)RE Contributes to TGF-\(\beta\)-induced Transcription—In addition to Smad binding sites, the T\(\beta\)RE contains two proposed binding sites (ABE1 and ABE2) for the AML family of transcription factors, also known as CBFA or PEBP2\(\alpha\) (7, 8). This family consists of three highly conserved proteins, AML1 (or CBFA2/PEBP2\(\alpha\)), AML2 (or CBFA3/PEBP2c\(\alpha\)), and AML3 (or CBFA1/PEBP2\(\alpha\)) (30). Although all AML proteins can bind and activate transcription through the same promoter binding sequence, the expression pattern of the different AML proteins depends on the cell type. AML3 (CBFA1) is most abundant in osteoblasts, plays an essential role in osteoblast differentiation, and is able to activate osteoblast-specific genes in a Smad-independent manner (22).
role in osteoblast-specific gene expression, and is not expressed in pre-B and surface IgM (sIgM) B cell lines (31, 32). In contrast, AML1 is a key regulator of hematopoiesis (33), and both AML1 and AML2 are present in B cells, with AML2 being more abundant (32, 34).

It has recently been shown that AML1, AML2, or both bind to their proposed binding sites in the TbRE and form a complex with Smad3 to stimulate transcription from the GL Igα promoter in a cooperative manner (13, 14). Consistent with previous observations, expression of AML1 or AML2 individually enhanced TGF-β-induced transcription without a major effect on basal transcription (Fig. 4A). Coexpression of AML1 or AML2 with Smad3 or Smad3/4 resulted in a strong transcriptional synergy, indicating that AMLs functionally cooperate with Smad3 and Smad3/4. However, coexpression of CREB had little effect on the transcriptional activity induced by AML1 or AML2. This suggests that CREB and AMLs do not directly cooperate with each other, even though they both functionally synergize with Smad3.

AML1 could bind directly to the TbRE sequence (7, 14) but not to the ATF/CRE sequence (Fig. 4B). DNA binding of AML1 to the –132/–94 sequence was independent of DNA binding of Smad3, as co-incubation of Smad3NL and AML did not interfere with or enhance the DNA binding of either one (Fig. 4B). Inactivation of the ABE1 in the –128/–94 segment had little effect on the TGF-β-induced transcription from the promoter, whereas inactivation of ABE2 strongly decreased the TGF-β-induced transcription (Fig. 4C, left panel) and the ability of overexpressed AML1 to activate transcription in response to TGF-β (Fig. 4C, right panel). We therefore conclude that even though both ABE sites can bind AML1 (14), the ABE2 site, but not the ABE1 site, is the functional AML/CBF binding site that contributes to the TGF-β response. Although the ABE2 site overlaps with the first Smad binding site (SBE1), the ABE2 sequence most likely functions as an AML binding site. This conclusion is supported by the fact that AML proteins have a higher affinity for DNA binding than the affinity of Smad3 for its binding site (29, 35) and on the observation that the overlapping SBE1 mutation had only a moderate effect on the TGF-β/Smad3 response compared with the SBE2 mutation (Fig. 3B).

The role of the AML proteins on the functional cooperation between Smad3 and CREB was also examined using the ABE2 inactivating promoter mutant construct. Whereas inactivation of ABE2 strongly inhibited the synergy between Smad3 and AML1, it only slightly decreased the ability of overexpressed Smad3 or Smad3/CREB to activate transcription (Fig. 4C). These results are consistent with the observation that the DNA binding of Smad3 was independent of DNA binding by AMLs.
Smad, CREB, and AML Regulate Ig α Gene Transcription

The results shown in the present study revealed a mechanism of functional cooperativity between AML proteins, Smads, and CREB at the GL Ig α promoter to mediate a high level transcriptional induction in response to TGF-β. Recent results have revealed transcriptional cooperativity between AML proteins and Smad3/4 at the T6RE element (13, 14). Although we confirmed this synergy, we also established the essential role of the adjacent ATF/CRE site in the mechanism of TGF-β-dependent transcription from this promoter. We showed that the integrity of this DNA binding site is required for transcriptional induction, thus indicating that CREB binding is essential for the TGF-β response. In addition, Smad binding to an adjacent site is also required, and Smad3/4 functionally synergizes with CREB to induce transcription from the GL Ig α promoter in response to TGF-β. The combined requirement of both a CRE and a Smad binding sequence is reminiscent of the regulation of transcription from the c-Jun promoter, and from the Drosophila Ubx promoter (36, 37). TGF-β-induced transcription from the c-Jun promoter requires both a Smad3/4 binding sequence and the AP-1/CRE binding site, and inactivation of either sequence results in complete or severe reduction in TGF-β-induced transcription activation (36). In the Drosophila Ubx promoter, both a CRE and a Mad binding site have genetically been shown to be required for induction of expression by Dpp, a Drosophila homolog of the TGF-β-related BMP-2 and BMP-4 (37).

We were unable to detect a direct interaction between Smad3 or Smad4 and CREB (data not shown), and we did not see cooperativity in DNA binding between Smad3 and CREB (Fig. 3, C and D). These findings suggest that the transcriptional synergy between Smad3/4 and CREB requires additional cooperating factors, which bring these different factors together in a complex. It is known that the transcriptional coactivator CBP/p300 can form multiprotein complexes with several transcription factors and activate transcription through the recruitment of basal transcription factors and chromatin acetylation (38–40). CREB interacts with an N-terminal segment of CBP/p300 (41), whereas Smad3 associates with a C-terminal segment of CBP (42, 43), and Smad4 stabilizes this interaction (42). Because the interactions of CREB and Smad3/4 with CBP/p300 are therefore most likely not mutually exclusive, CBP/p300 may be the coactivator that mediates the synergy between CREB and Smad3.

Although AML binding is not required for TGF-β-dependent transcription from the GL Ig α promoter, AML proteins cooperate with Smad3/4 in a TGF-β-dependent manner and enhance the TGF-β-induced synergy of CREB and Smad3. These results, together with an examination of the role of the ABE and SBE sites, strongly suggest that efficient and maximal transcription in response to TGF-β requires all three types of transcription factors. AML proteins have also been shown to interact with CBP/p300, and the sequence in CBP/p300 that mediates this interaction is distinct from the CREB- and Smad-interacting sequences (38). This raises the distinct possibility that all three types of transcription factors, i.e., AML, Smad3, and CREB, may interact with CBP in a complex. Such a hypothetical model would provide a role of CBP/p300 in integrating the activities of AML1 or AML2, Smad3/4, and CREB and a role as transcriptional coactivator. This model could then explain the cooperativity between the three types of transcription factors. This model would also predict that increased levels of CBP/p300 enhance TGF-β-induced transcription from the GL Ig α promoter. Accordingly, increased CBP expression enhanced in a dose-dependent manner the transcriptional activation of the Ig α promoter by TGF-β (data not shown).
The transcriptional cooperativity of AML, CREB, and Smad proteins at the GL Igα promoter and the binding of these three types of transcription factors, which are in close proximity of each other, to the promoter DNA resemble a previously proposed mechanism whereby AML1 facilitates the activity of other adjacent transcription factors. At the myeloperoxidase gene promoter, AML1, c-Myb, and C/EBP bind noncooperatively to adjacent sites, and CBP/p300 may mediate their functional synergy by interacting with all three factors (38). Accordingly, AML1 synergizes with c-Myb to activate transcription of the myeloperoxidase gene and the T cell receptor promoter (44–46). Somewhat similarly, p300 has been shown to bring Smad1 and STAT3 together, and this protein complex may be the basis for the functional synergy of Smad1 with STAT3 in the transcriptional activation of the glial fibrillary acidic protein promoter (47). Further studies of the effects of p300/CBP and other transcription coactivators on the TGF-β-dependent transcription from the GL Igα promoter should contribute to our understanding of the complex mechanism of transcriptional regulation by TGF-β.

Acknowledgments—We thank X.-F. Wang, J. Stavnezer, S. Hiebert, M. Green, and K. Yamamoto for valuable reagents and the “regulatory complex group” at UCSF for valuable discussions.

REFERENCES
1. Stavnezer, J. (1996) Curr. Opin. Immunol. 8, 199–205
2. Letterio, J. J., and Roberts, A. B. (1998) Annu. Rev. Immunol. 16, 137–161
3. Lehman, D. A., Nomura, D. Y., Coffman, R. L., and Lee, F. D. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 3962–3966
4. Lehman, D. A., Lee, F. D., and Coffman, R. L. (1990) J. Immunol. 144, 952–959
5. Shockett, P., and Stavnezer, J. (1991) J. Immunol. 147, 4374–4383
6. Lin, Y. C., and Stavnezer, J. (1992) J. Immunol. 149, 2914–2925
7. Shi, M. J., and Stavnezer, J. (1998) J. Immunol. 161, 6751–6760
8. Xie, X. Q., Pardali, E., Holm, M., Sideras, P., and Grundstrom, T. (1999) Eur. J. Immunol. 29, 488–498
9. Derynck, R., and Feng, X.-H. (1997) Biochem. Biophys. Acta 1333, F105–F150
10. Heldin, C. H., Miyazono, K., and ten Dijke, P. (1996) Nature 380, 465–471
11. Massagué, J. (1996) Annu Rev. Biochem. 67, 753–791
12. Derynck, R., Zhang, Y., and Feng, X. H. (1998) Cell 95, 737–740
13. Hanai, J., Chen, L. F., Kanno, T., Ohtani-Fujita, N., Kim, W. Y., Gao, W.-H., Imamura, T., Ishidou, Y., Fukushi, M., Shi, M. J., Stavnezer, J., Kawabata, M., Miyazono, K., and Ito, Y. (1999) J. Biol. Chem. 274, 31577–31582
14. Pardali, E., Xie, X.-Q., Tsapogas, P., Itoh, S., Arvanitidi, K., Heldin, C.-H., ten Dijke, P., Grundstrom, T., and Sideras, P. (2000) J. Biol. Chem. 275, 3552–3560
15. Choy, L., and Derynck, R. (1998) J. Biol. Chem. 273, 31455–31462
16. Zhang, Y., Feng, X. H., Wu, R. Y., and Derynck, R. (1996) Nature 383, 168–172
17. Zhang, Y., Feng, X. H., and Derynck, R. (1998) Nature 394, 909–913
18. Feng, X. H., and Derynck, R. (1997) EMBO J. 16, 3912–3923
19. Liu, F., Pouponnot, C., and Massague, J. (1997) Genes Dev. 11, 3157–3167
20. Ziff, E. B. (1990) Trends Genet. 6, 69–72
21. Dwaraki, V. J., Montminy, M., and Verma, I. M. (1990) EMBO J. 9, 225–232
22. Hai, T., and Curran, T. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3720–3724
23. Karin, M., and Smeal, T. (1992) Trends Biochem. Sci. 17, 418–422
24. Lagana, G., Hata, A., Hemmati-Brivanlou, A., and Massague, J. (1996) Nature 383, 832–836
25. Zhang, Y., Museci, T., and Derynck, R. (1997) Curr. Biol. 7, 270–276
26. Zawel, L., Dai, J. L., Buckhaults, P., Zhou, S., Kinzler, K. W., Vogelstein, B., and Kern, S. E. (1998) Mol. Cell 1, 611–617
27. Yingling, J. M., Datto, M. B., Wong, C., Frederick, J. P., Liberati, N. T., and Wang, X. F. (1997) Mol. Cell. Biol. 17, 7019–7028
28. Dennler, S., Ihu, S., Vivien, D., ten Dijke, P., Huet, S., and Gauthier, J. M. (1998) EMBO J. 17, 3091–3100
29. Shi, Y., Wang, Y. F., Jayaraman, L., Yang, H., Massague, J., and Pavletich, N. P. (1998) Cell 94, 585–594
30. Levanon, D., Negreanu, V., Bernstein, Y., Bar-Am, I., Avivi, L., and Groner, Y. (1994) Genomics 23, 425–432
31. Ducy, P., Zhang, R., Geoffrey, V., Ridall, A. L., and Karsenty, G. (1997) Cell 89, 747–754
32. Meyers, S., Lenny, N., Sun, W., and Hiebert, S. W. (1996) Oncogene 13, 303–312
33. Okuda, T., van Deursen, J., Hiebert, S. W., Grosveld, G., and Downing, J. R. (1996) Cell 84, 321–330
34. Bae, S. C., Yamaguchi-Iwai, Y., Ogawa, E., Maruyama, M., Inuzuka, M., Kagohama, H., Shigesada, K., Satake, M., and Ito, Y. (1998) Oncogene 16, 809–814
35. Crute, B. E., Lewis, A. F., Wu, Z., Bushweller, J. H., and Speck, N. A. (1996) J. Biol. Chem. 271, 26251–26260
36. Wang, C., Rouger-Chapman, E. M., Frederick, J. P., Datto, M. B., Liberati, N. T., Li, J. M., and Wang, X. F. (1999) Mol. Cell. Biol. 19, 1821–1830
37. Szuts, D., Eresh, S., and Blenz, M. (1998) Genes Dev. 12, 2022–2035
38. Kamei, Y., Xu, L., Heinzl, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S. C., Heyman, R. A., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. (1996) Cell 85, 403–414
39. Merika, M., Williams, A. J., Chen, G., Collins, T., and Thonas, D. (1998) Mol. Cell 1, 277–287
40. Parkin, D., Ferreri, K., Nakajima, T., LaMorte, V. J., Evans, R., Koerber, S. C., Hoeger, C., and Montminy, M. R. (1996) Mol. Cell. Biol. 16, 694–703
41. Feng, X. H., Zhang, Y., Wu, R. Y., and Derynck, R. (1998) Genes Dev. 12, 2153–2163
42. Janknecht, R., Wells, N. J., and Hunter, T. (1998) Genes Dev. 12, 2114–2119
43. Hernandez-Munain, C., and Kranigel, M. S. (1995) Mol. Cell. Biol. 15, 3090–3099
44. Britos-Bray, M., and Friedman, A. D. (1997) Mol. Cell. Biol. 17, 5127–5135
45. Zhang, D. E., Hetherington, C. J., Meyers, S., Rhoades, K. L., Larson, C. J., Shi, M.-J., Stavnezer, J., Kawabata, M., Miyazono, K., and Ito, Y. (1999) J. Biol. Chem. 274, 232–238
Transcriptional Regulation of the Transforming Growth Factor-β-inducible Mouse Germ Line Ig α Constant Region Gene by Functional Cooperation of Smad, CREB, and AML Family Members
Ying Zhang and Rik Derynck

J. Biol. Chem. 2000, 275:16979-16985.
doi: 10.1074/jbc.M001526200 originally published online March 23, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M001526200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 47 references, 24 of which can be accessed free at http://www.jbc.org/content/275/22/16979.full.html#ref-list-1