

Human p300 Protein Is a Coactivator for the Transcription Factor MyoD*

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Human p300 protein is a cellular target of adenoviral E1A oncoprotein and a potential transcriptional coactivator. Both p300 and Rb family protein-binding regions of E1A are required for the repression of muscle gene expression, which is regulated by MyoD family transactivators. This implies that p300 is involved in MyoD-dependent transactivation. We show that the repression of MyoD-mediated E box (MyoD consensus) reporter activity by E1A is correlated with its interaction with p300, indicating that p300 participates in MyoD-dependent transactivation. In addition, p300 is able to interact both *in vivo* and *in vitro* with MyoD through a portion at the carboxyl-terminal cysteine/histidine-rich domain and associates with the components of the basal transcriptional complex through its two separate transactivation domains at the amino and carboxyl termini. Consistent with its role as a coactivator, p300 potentiates MyoD-activated transcription.

Human p300 protein was identified initially as a cellular target of adenoviral E1A oncoprotein (1–3). The region of E1A interacting with p300 is distinct from the one that interacts with tumor suppressor retinoblastoma protein (Rb) and Rb family proteins. The interactions of E1A with both p300 and Rb family proteins are required for cellular transformation. p300 protein is expressed ubiquitously, and its sequence is conserved from nematode to human (4), further implying the importance of its biological role.

The sequence of p300 reveals it to be a potential transcriptional coactivator (5). This potentiality is consistent with the fact that p300 has transcriptional activity (6) and forms a complex with TBP¹ *in vivo* (7). It was reported that human

protein p300 potentiates the transactivation by the cAMP-responsive element-binding protein (CREB), as does the homologous transcriptional coactivator, mouse CREB-binding protein (CBP) (8). However, the role and significance of this p300 function in cellular control and E1A transformation are unclear.

For transformation, E1A represses expression of muscle-specific genes and muscle differentiation (9), which are regulated by the basic helix-loop-helix protein MyoD and other MyoD family transactivators (10, 11). It was reported that this repression requires both regions of E1A to bind to p300 and Rb family proteins (11–13). Thus, it is possible that p300 is involved in MyoD-dependent transactivation.

We used E1A as an investigative tool to test this hypothesis. We studied the interaction between p300 and MyoD proteins and demonstrated that p300 potentiates MyoD-dependent transactivation. To examine the transcription potential of p300, we also identified the regions of the p300 protein containing transcriptional activities and their direct interactions with the components of the basal transcriptional complex.

MATERIALS AND METHODS

Cell Culture and Transient Transfection—U-2 OS human osteosarcoma cells were grown in Dulbecco's modified Eagle's medium with 10% fetal bovine serum (FBS). F9 teratocarcinoma cells were grown with 15% FBS, and C2C12 myoblasts were grown with 20% FBS. U-2 OS and F9 cells were transfected by the calcium phosphate precipitation method (14, 15). Total amounts of transfected DNA for each condition were balanced by pUC19 DNA. After a 12–13-h incubation in medium containing the precipitated DNA, the cells continued to grow in the same medium for 48 h before harvesting.

CAT Assay—CAT assays were carried out as described previously (14). The quantities of cell extracts used were normalized to β -galactosidase activity by cotransfection with 0.5–1 μ g pON260. In β -galactosidase assays, 10 out of 200 μ l of total cell lysate was incubated with 1 ml of 3 mM chlorophenol red β -D-galactopyranoside (Boehringer Mannheim) at 37 °C for 1 h; the reaction was stopped by chilling on ice, and β -galactosidase activity was measured for optical density at 570 nm. Thin layer chromatography was used for analysis in CAT assays. The results were quantitated as percent conversion with a PhosphorImager.

Plasmid Constructs—The pGal4p300 fusion vector was made by inserting Gal4 1–147 sequence into p300 cDNA at the position for the 19th amino acid and subcloning the fusion unit into pCDNA3 (Invitrogen). The p300 deletions were made from pGal4p300 by digestion with restriction enzymes and religation. The junctions in each construct were checked by sequencing. p4RTKCAT is a CAT reporter construct driven by the TK core promoter and upstream four copies of the E box enhancer sequences (16). pCMV β p300 (5), pCMVMyoD (17), pON260, and pCMV12S (18) are expression vectors for p300, MyoD, β -galactosidase, and E1A 12S, respectively, and are driven by the CMV immediate early promoter. dl 2–36 is derived from pCMV12S bearing an amino-terminal deletion of E1A 12S between amino acid residues 2 and 36. pG5e1bCAT is a CAT reporter driven by the e1b TATA and upstream five copies of the GAI4-binding sites (19). pGEX-MyoD, pGEX-hTBP, and pGST-IIB (20) are bacterial expression vectors encoding the glutathione *S*-transferase (GST) fusions to MyoD, the TBP, and another

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¹ The abbreviations used are: TBP, TATA-binding protein; CREB, cAMP-responsive element-binding protein; CBP, CREB-binding protein; FBS, fetal bovine serum; CAT, chloramphenicol acetyltransferase; TK, thymidine kinase; CMV, cytomegalovirus; CAPS, 3-(cyclohexylamino)propanesulfonic acid.

general transcription factor, TFIIB.

Immunoprecipitation/Western Blotting—Whole cell extracts were prepared from C2C12 myoblasts and immunoprecipitated with an anti-p300 polyclonal antiserum, basically as described previously (21). The precipitates or the whole cell lysates containing equal amounts of total proteins were suspended in the SDS sample buffer without boiling (because the molecular weights of MyoD and immunoglobulin heavy chain are so close), separated on 10% SDS-polyacrylamide gel, and transferred onto polyvinylidene difluoride membrane (Millipore) for 1 h at a constant 70 volts, in a buffer containing 5% CAPS and 20% methanol. The membrane was blocked with 5% non-fat dry milk in TBS-T buffer (containing 20 mM Tris-Cl, pH 7.6, 137 mM NaCl, and 0.5% Tween 20) for 1 h, incubated with anti-MyoD antibodies (Santa Cruz) diluted in TBS-T with 3% the dry milk for 1 h, washed in TBS-T, further incubated for 1 h with a secondary antibody conjugated to horseradish peroxidase in TBS-T, and finally washed in TBS-T. The antigen-antibody interaction was visualized by incubation in a chemiluminescent reagent (DuPont NEN) for 1 min and exposure to x-ray film.

In Vitro Transcription and Translation—Labeled p300 proteins were produced from the Gal4p300 plasmid and its p300 deletion mutants in a TNT-coupled reticulocyte system with T7 RNA polymerase and [³⁵S]methionine (DuPont NEN), as described by the manufacturer (Promega).

GST Binding Assay—Expression from the pGEX-2T (Pharmacia Biotech Inc.), pGEX-MyoD, pGEX-hTBP, and pGST-IIB plasmids in *Escherichia coli* and immobilization of the GST fusion proteins are described by the manufacturer (Pharmacia). Binding assays were carried out basically as described previously (22, 23). Briefly, roughly equal molar amount of GST fusion proteins and GST alone bound to glutathione-agarose beads were mixed with roughly equal molar amounts of the labeled p300 and mutants in 0.5 ml of the binding buffer (20 mM Tris-Cl, pH 8.0, 100 or 250 mM NaCl, 5 mM MgCl₂, 0.2 mM EDTA, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 0.1% or 0.5% Nonidet P-40), incubated at room temperature for 1 h, and washed with the same buffer. The proteins bound to beads were eluted with 20 μ l of SDS sample buffer by boiling for 5 min and analyzed on 10% SDS-polyacrylamide gels followed by autoradiography.

RESULTS

p300 Protein Is Involved in MyoD-dependent Transactivation—We used an E box (MyoD consensus) reporter (p4RTKCAT) system to test the role of p300 in MyoD-activated transcription in U-2 OS human osteosarcoma cells. In this system, MyoD transactivated an appreciable level of E box reporter activity (Fig. 1A, lanes 2–4). This activity was specific to MyoD, because no transactivation was detected without it (Fig. 1A, lanes 1 and 5). This result indicates also that both endogenous and ectopic p300 alone cannot transactivate the E box reporter.

In this system E1A 12S repressed the MyoD-dependent E box activity in a dose-dependent manner (Fig. 1B, lanes 1–4). When we used an E1A 12S mutant bearing an amino-terminal deletion (dl 2–36) that loses its ability to bind to p300, but not to Rb family proteins (18, 24, 25), it repressed the reporter activity much less than the wild type (Fig. 1B, lanes 5–7), indicating that this repression by E1A may require its binding to p300.

To confirm this possibility, a p300 expression construct (pCMV β -p300) was cotransfected with E1A vector. Ectopic p300 largely released the E1A repression (Fig. 1C, lane 4 compared with lane 2). When the wild type p300 construct was replaced by a p300 mutant 1–1737 that removes the E1A-binding domain and carboxyl-terminal sequence, E1A did not repress any reporter activity (Fig. 1C, lane 6). Thus the E1A repression of reporter activity is associated with its ability to bind to p300, indicating that p300 is involved in MyoD-dependent transactivation. The stimulation of reporter activity by E1A in the presence of p300 mutant (Fig. 1C, lane 6) may be associated with E1A's ability to perturb p300 function indirectly, as shown by Arany *et al.* (6). In addition, p300 mutant 1–1737 that still contained transcriptional activity, but at a reduced level (data not shown), affected reporter activity in the absence of

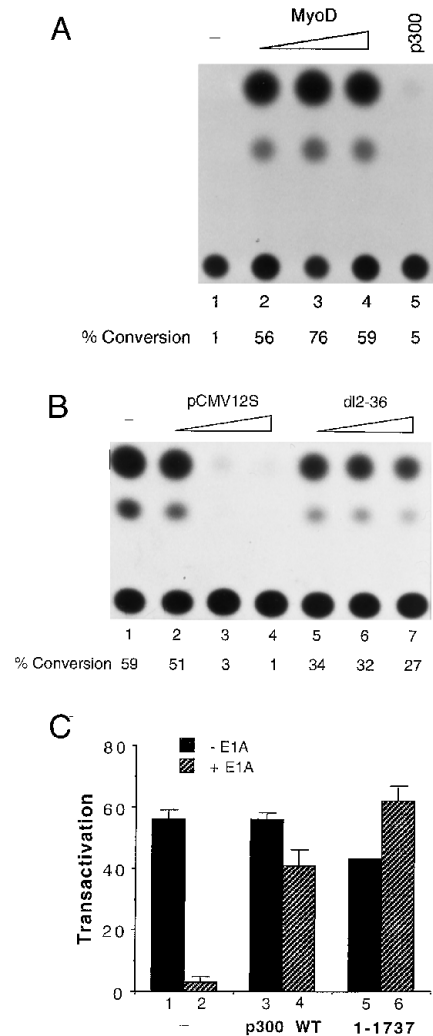


FIG. 1. p300 plays a positive role in MyoD-mediated transactivation. A, U-2 OS human osteosarcoma cells were transiently transfected with 4 μ g of p4RTKCAT, 0 μ g (lanes 1 and 5) or 1, 4, and 8 μ g (lanes 2–4) of pCMVMyoD, and 4 μ g of pCMV β -p300 (lane 5), followed by CAT assays as described under "Materials and Methods." A representative experiment with its percent conversion is shown. Means of percent conversion from three independent experiments \pm S.D. from lanes 1–5 are 1 \pm 0, 50 \pm 5.1, 74 \pm 12.5, 53 \pm 8.1, and 4 \pm 1.4. B, U-2 OS cells were transfected with 4 μ g of p4RTKCAT, 4 μ g of pCMVMyoD, 0, 0.5, 2, and 4 μ g of pCMV12S (lanes 1–4), and 1, 2, and 4 μ g of dl 2–36 (lanes 5–7), followed by CAT assays. A representative experiment with its percent conversion is shown. Means of percent conversion from three independent experiments \pm S.D. from lanes 1–7 are 62 \pm 4.2, 52 \pm 4.1, 5 \pm 2, 1 \pm 0, 33 \pm 1.9, 30 \pm 3.5, and 26 \pm 1.4. C, U-2 OS cells were transfected with 4 μ g of p4RTKCAT, 4 μ g of pCMVMyoD, 0 (lanes 1, 3, and 5) or 4 (lanes 2, 4, and 6) μ g of pCMV12S, and either blank (lanes 1 and 2), 4 μ g of pCMV β -p300 (lanes 3 and 4), or 4 μ g of p300 mutant vector 1–1737 (encoding a p300 fragment of amino acids 1–1737) (lanes 5 and 6), followed by CAT assays. "Transactivation" refers to the relative CAT activity (mean with S.D., $n = 3$) compared with the activity in the absence of MyoD.

E1A in the system (Fig. 1C, lane 5). This result also suggests that p300 plays a role in MyoD-dependent transactivation.

Direct Interaction between p300 and MyoD Proteins—Based on the proposed coactivator role of p300, its involvement in MyoD-dependent transactivation suggested that p300 might interact with MyoD directly. To test this possibility, C2C12 cells were lysed in the lysis buffer with either 250 or 150 mM NaCl, immunoprecipitated with anti-p300 antibodies, and then immunoblotted with anti-MyoD antibodies. An interaction between p300 and MyoD proteins was detected under the low stringency salt condition (Fig. 2, lane 5). Furthermore, in an *in*

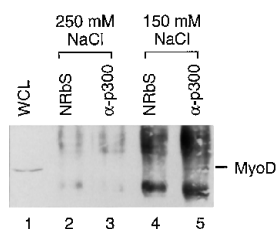


FIG. 2. **p300 associates MyoD *in vivo***. Immunoblot with an anti-MyoD antibody (Santa Cruz) following immunoprecipitation of whole cell extracts prepared from C2C12 myoblast cells in lysis buffer containing either 250 mM salt (*lanes 2 and 3*) or 150 mM salt (*lanes 1, 4, and 5*) with an anti-p300 polyclonal antiserum (5 μ l). *NRbS*, controls for immunoprecipitation with normal rabbit serum. *WCL*, whole cell extract without immunoprecipitation.

in vitro binding assay, the *in vitro* translated p300 protein also bound to the purified bacterial GSTMyoD fusion protein linked to glutathione-agarose beads (Fig. 3*B*, *lane 3*). This binding was associated with the specific affinity between p300 and MyoD, because p300 did not show any interaction with GST alone (Fig. 3*B*, *lane 2*).

Through deletion analysis, the portion of p300 containing the affinity to MyoD was detected in fragment 1514–2122 (Fig. 3*C*, *lane 1*) that covers the third cysteine/histidine-rich conserved domain (C/H3). The fragment 1737–2414, that contains a carboxyl portion of the C/H3 domain, was still able to bind to MyoD (*lane 2*), while the fragment 1945–2414, the most carboxyl-terminal region, was not (*lane 3*). These interactions between p300 and MyoD at the C/H3 region were specific, because no interactions were detected for GST alone and for the control protein (*lane 6*). This MyoD-binding portion of p300 is separate from the amino-terminal portion (1–663) that binds to CREB protein (8). Although the fragment 1–596 bound to GST-MyoD slightly (*lane 5*), a weak interaction between this amino-terminal fragment and GST alone was detected. The results obtained from the GST binding assays were repeatable when the same stringency conditions were used for binding and washing. The results were also essentially the same when the assays were carried out in lower stringency conditions (data not shown).

p300 Protein Communicates with the Basal Transcription Complex through Its Two Separate Transactivation Domains—The transactivation domain of the highly related CBP protein resides at the carboxyl-terminal glutamine-rich region (23). Deletion of the corresponding region of p300 as in the mutant 1–1737 did not abolish its transcriptional ability. To determine which portion of p300 allowed transactivation, we fused p300 and its deletion mutants to the Gal4 DNA-binding domain (1–147) (Fig. 4) and used them for transient transfection assays in U-2 OS cells. As shown in Fig. 4, p300 wild type transactivated an appreciable level of Gal4-consensus reporter activity. This induction by the fusion protein must relate to p300 function, because Gal4 1–147 alone did not show any transactivation. The most carboxyl-terminal glutamine-rich portion of p300 (1945–2414) contributed a weak transactivation. When this portion was extended, as in the construct 1737–2414, the induction was increased by 8-fold. The further addition of an amino-terminal sequence 19–242 to the construct (dl 242–1737) allowed a nearly 1-fold higher induction, but this amino-terminal sequence in the construct (1–242) had no transactivation itself. The middle portions (964–1922 and 1514–1922) did not show any transactivation. The amino-terminal portion of p300 (1–596) induced a level of transactivation similar to the carboxyl-terminal portion (1737–2414) and about 3-fold higher than the wild type. The addition of 148 amino acids in the construct (1–743) allowed an increase in transactivation by more than 1-fold.

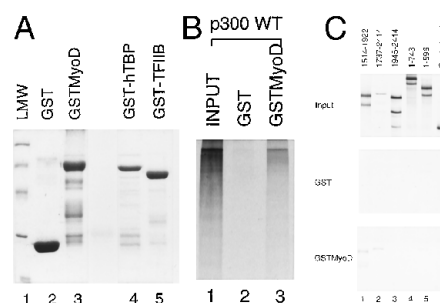


FIG. 3. **p300 specifically binds MyoD *in vitro* at the carboxyl-terminal cysteine/histidine-rich domain**. *A*, GSTMyoD, GSThTBP, and GSTTFIIB fusion proteins and GST alone were expressed from pGEX-MyoD, pGEX-hTBP, pGEX-IIB, and pGEX-2T (Pharmacia) vectors in *E. coli*, purified by being immobilized on glutathione-agarose beads (Pharmacia), and analyzed on 15% SDS-polyacrylamide gel. *LMW*, low molecular weight markers (Bio-Rad: 112, 84, 53.2, 34.9, and 28.7 kDa). *B* and *C*, the purified bacterial GST-MyoD fusion protein or GST alone was incubated with roughly equal molar amount of labeled p300, either wild type (*WT*) in *B* or fragments indicated by amino acids in *C*, as described under "Materials and Methods." The binding assay in *C* was carried out in a higher stringency condition with 250 mM NaCl and 0.5% Nonidet P-40 in binding buffer. Input refers to *in vitro* translation products used in the binding assays. Roughly equal volume amount of each *in vitro* translation product was used in the "Input" panel in *C*.

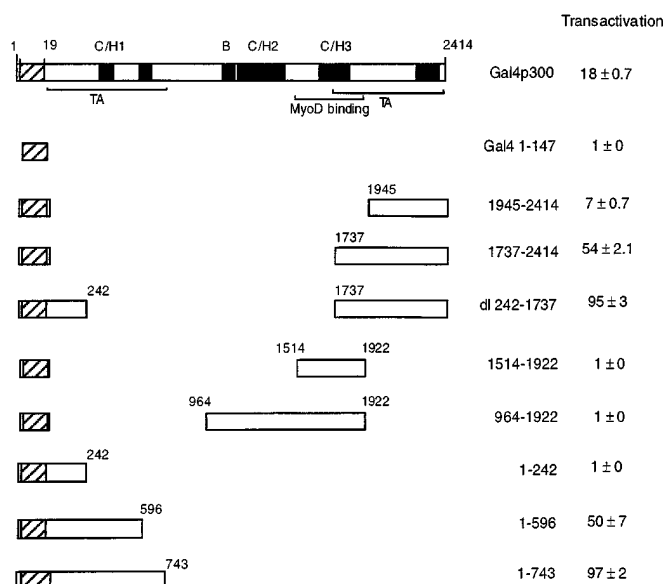


FIG. 4. **p300 contains two separate transactivation domains**. Diagrams showing p300 and its deletions fused to Gal4 DNA-binding domain. The striped box refers to the Gal4 DNA-binding domain. The filled box represents the conserved regions of p300. The number in the diagram indicates the amino acid positions of p300. The cysteine/histidine-rich regions are indicated as C/H1–3. *B*, bromodomain. *TA*, regions containing transcriptional activity. The values in the *Transactivation* column are the relative CAT activity (mean ± S.D., *n* = 3) compared with the activity of Gal4 1–147 alone. For CAT assays, U-2 OS cells were transiently transfected with 4 μ g of pG5e1bCAT and 4 μ g of either Gal4 1–147, Gal4p300, or one of the p300 deletion vectors, as described under "Materials and Methods."

An additional question is whether the amino- and carboxyl-terminal transactivation domains of p300 interact with the basal transcription complex directly. Previous reports showed that p300 formed a complex with TBP *in vivo* (7), and CBP interacted with TFIIB at the conserved C/H3 region (1680–1812) (23). By using *in vitro* binding assay with TBP and TFIIB fused to GST, we detected a specific affinity between the carboxyl-terminal transactivation domain of p300 (1737–2414) containing a C/H3 region and either TBP or TFIIB (Fig. 5*A*). The amino-terminal portion of p300 (1–743), inducing a high

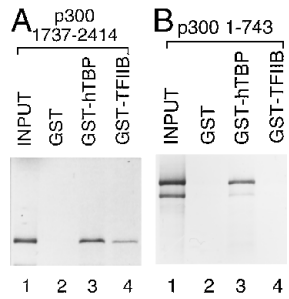


FIG. 5. p300 communicates with the components of the basal transcriptional machinery through its transactivation domains. *A* and *B*, the purified bacterial fusion proteins GSTTBP and GSTTFIIB and GST alone (Fig. 3*A*) were incubated with labeled p300 fragments indicated by amino acids and analyzed for binding, as described under "Materials and Methods." The binding assays were performed in the binding buffer containing 100 mM NaCl and 0.1% Nonidet P-40. *Input* refers to *in vitro* translation products used in the binding assays.

level of transactivation, also bound to TBP but not to TFIIB (Fig. 5*B*).

p300 Protein Modulates MyoD-dependent Transactivation—The involvement of p300 in MyoD-activated transcription and its ability to bind to MyoD suggested that p300 might mediate MyoD-dependent transactivation directly. To test this hypothesis, we performed transient transfection assays in F9 teratocarcinoma cells by using p300 and MyoD expression constructs and measuring the E box reporter activity. We cotransfected increasing amounts of p300 expression vector with and without MyoD vector. As shown in Fig. 6*A*, p300 potentiates the transcriptional activity of MyoD. This function of p300 requires the presence of MyoD. Similar results were obtained when the assay was conducted in U-2 OS cells (Fig. 6*B*, lanes 1–3), although a large amount of ectopic p300 relative to the level of MyoD could repress the MyoD-dependent transactivation.

To confirm the role of p300 in MyoD-dependent transactivation, we selected p300 mutants that interfered the transcription activity of endogenous p300 protein in transfected U-2 OS cells. As shown in Fig. 6*B*, two mutants markedly affected the MyoD-dependent transactivation, one (dl 242–1737) positively (lanes 6 and 7) and the other (1514–1922) negatively (lanes 4 and 5). We believe that the effects of these p300 mutants are related to their disturbing the endogenous p300 function, because they did not show relevant effects on the activity of the promoter driving the ectopic MyoD expression (data not shown) or the MyoD level in the transfected cells (Fig. 6*C*, lanes 3 and 4).

DISCUSSION

A new type of transcriptional regulator has been classified recently as the transcriptional coactivator that communicates between transactivators binding on enhancer DNA and the basal transcriptional complex formed on the promoter near the transcription initiation site (26). This communication is required for transcriptional activation by the transactivators. p300 protein contains a bromodomain that is a hallmark of certain transcriptional adapter proteins (5). A sequence at the C/H3 domain of p300 has homology to the yeast ADA2 protein (27), a putative transcriptional coactivator (28). These p300 properties suggest that p300 is a potential transcription coactivator. This potentiality is supported by evidence demonstrating that p300, like its homologous protein, can interact with the transactivator CREB and potentiate its transactivation (8), that a fusion of E2 DNA-binding domain to p300 transactivates an E2-binding site reporter (6), and that p300 can form a complex *in vivo* with TBP, a component of the basal transcriptional complex (7).

We demonstrated that p300 is a transcriptional coactivator

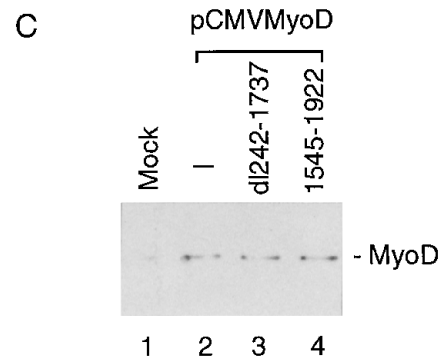
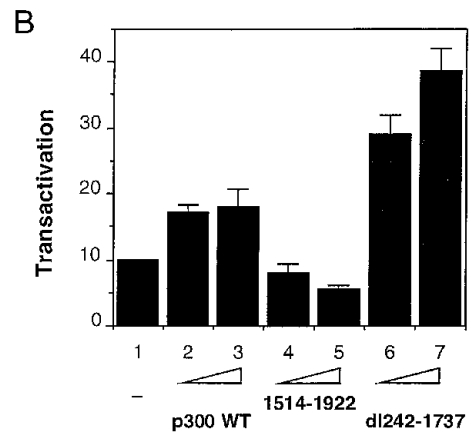
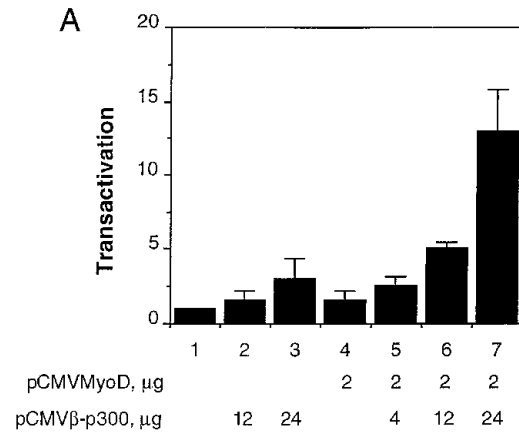


FIG. 6. p300 potentiates MyoD-activated gene expression. *A*, F9 teratocarcinoma cells were cotransfected with p4RTKCAT (4 μg) and other vectors as indicated, followed by CAT assays as described under "Materials and Methods." *Transactivation* refers to the relative CAT activity (mean with S.D., $n = 3$) compared with activity in absence of ectopic MyoD and p300. *B*, U-2 OS cells were cotransfected with 4 μg of p4RTKCAT, 2 μg of pCMVMyoD, and 0, 2, and 8 μg of either pCMVβ-p300 (*p300 WT*) or one of two p300 deletion mutants as indicated, followed by CAT assays as described under "Materials and Methods." *Transactivation* represents the relative CAT activity (mean with S.D., $n = 3$) compared with the activity in the absence of ectopic p300. *C*, Western blot analysis of U-2 OS cells transfected with 4 μg of pCMVMyoD and 0 (lane 2) or 8 μg of either p300 mutant dl 242–1737 (lane 3) or 1545–1922 (lane 4). Control cells received no transfection (lane 1).

for MyoD. The analysis with E1A and the potentiation of MyoD-dependent transactivation by p300 indicate that p300 participates in transactivation mediated by the interaction between the E box enhancer and MyoD. The interactions between MyoD and p300 proteins, and between p300 and either TBP or TFIIB, suggest that p300 can communicate directly between the E box-binding MyoD and the basal transcriptional complex

during transactivation. This feature of p300 defines it as a transcriptional coactivator for MyoD.

The association between p300 and MyoD was detected only in a low stringency salt condition *in vivo*. This result is consistent with the fact that interactions of transcription factors are generally weak and instant, and it is difficult to detect them *in vivo* except by use of a two-hybrid system. The association between MyoD and Rb was detected also in a similar low stringency salt condition (29). This condition is created by a lysis buffer containing a 150 mM rather than a 250 mM salt concentration and is closer to the physiological salt condition in the cell.

A transcription factor communicates directly or indirectly with the basal complex through its transactivation domain. The transactivation activities of p300 were detected in separate amino- and carboxyl-terminal regions. The interactions between the amino- and carboxyl-terminal regions of p300 and either TBP or TFIIB *in vitro* indicate that p300 can associate with the basal complex directly. However, these results cannot rule out an indirect interaction of p300 with the basal transcriptional complex occurring simultaneously, as was reported by Abraham *et al.* (7) when they found that the interaction between p300 and TBP may be mediated by two 64- and 59-kDa polypeptides. It is also possible that p300 interacts with and is mediated by other transcriptional factors during transactivation (30, 31).

The dominant negative and dominant positive mutants of p300 were shown to affect endogenous p300 function in MyoD-dependent transactivation of the E box reporter in this study. Because the dominant negative mutant 1514–1922 is able to bind to MyoD but has no transcriptional activity, its negative role may be associated with sequestering MyoD from the wild type p300 in cells. The dominant positive mutant dl 242–1737, while it has a high transactivation ability, it lacks a large middle portion, including the bromodomain and the second cysteine/histidine-rich region. Results of other Gal4 reporter assays imply a negative role for this middle portion of p300 in transactivation,² and thus the mutant dl 242–1737 could bypass repressor involvement. It also contains the MyoD-binding domain, it is able to interact with MyoD (data not shown), and it could therefore deliver its higher transactivation potential to the E box via MyoD. These data further support the contention that p300 serves as a coactivator for the myogenic transactivator MyoD.

All MyoD family transactivators can transactivate the E box enhancer. Under the condition of muscle differentiation, MyoD induces the expression of myogenin. In order to distinguish the E box activity mediated by MyoD from that mediated by other myogenic transactivators, we carried out experiments in non-muscle cells under nondifferentiation conditions. However, we discovered that the dominant positive and negative mutants of p300 affected E box reporter activity during MyoD-induced differentiation in a way similar to that detected in nondifferentiation conditions.² The effect of p300 mutants on E box activity without ectopic MyoD was observed also in C2C12 myoblast and human rhabdomyosarcoma cells under low serum differentiation conditions.² It appears that p300 modulates transactivation by MyoD, and possibly by other MyoD family transactivators, under myogenic differentiation. Consistent with this, we observed that the p300 mutant 1–1737 bypassed E1A repression of myogenin-induced E box reporter activity,² indicating a potential role for p300 in myogenin-dependent transactivation.

The function of p300 as a coactivator for MyoD, and possibly

for other MyoD family transactivators, suggests one of the mechanisms by which it can be involved in the regulation of muscle differentiation. This mechanism probably plays an important role because, for efficient repression of muscle-specific gene expression and differentiation, E1A oncoprotein must have the ability to attack both p300 and Rb family proteins. This does not rule out the possibility that the repression of the muscle-specific gene by E1A also is correlated with its direct interaction with myogenin (32), which could be another pathway for E1A to inhibit the myogenic process. It has been shown that the p300 protein is involved, during keratinocyte differentiation, in the induction of the cyclin-dependent kinase inhibitor p21 (33), which is regulated by MyoD and which is required for permanent cell cycle withdrawal during muscle differentiation (17, 34). This result further supports the proposition that p300 participates in myogenesis through modulating MyoD-dependent transcription.

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² W. Yuan, G. Condorelli, M. Caruso, A. Felsani, and A. Giordano, unpublished data.