Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 3822–3826, May 1991
Developmental Biology

Transforming growth factor β represses the actions of myogenin through a mechanism independent of DNA binding

(myogenesis/Id)

THOMAS J. BRENNAN, DIANE G. EDMONDSON, LI LI, AND ERIC N. OLSON*

Department of Biochemistry and Molecular Biology, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Box 117, Houston, TX 77030

Communicated by Harold Weintraub, January 28, 1991 (received for review January 2, 1991)

ABSTRACT **Myogenin belongs to a family of regulatory factors that can activate myogenesis when transfected into nonmyogenic cells. A conserved DNA sequence, known as an E box, serves as the target for binding and trans-activation by myogenin. Using 10T½ fibroblasts that constitutively express a transfected myogenin cDNA, we show that myogenin accumulates in the nucleus but is unable to initiate myogenesis when cells are maintained with transforming growth factor β (TGF-β) or high serum. Although the final effect of TGF-β and high serum—inhibition of myogenesis—was the same, their effects on the DNA-binding properties of myogenin *in vitro* differed. TGF-β did not affect the ability of myogenin to bind DNA, whereas serum diminished the *in vitro* DNA-binding activity of myogenin. The helix-loop-helix (HLH) protein Id, postulated to inhibit DNA binding of other HLH proteins, was induced by high serum but not by TGF-β. The presence of Id correlated with the failure of myogenin to bind the muscle creatine kinase enhancer *in vitro*. These findings suggest that serum can inhibit myogenesis by attenuating the DNA-binding activity of myogenin, possibly as a consequence of Id protein expression, whereas TGF-β acts through a mechanism distal to DNA sequence recognition by myogenin and independent of Id.**

Expression of a differentiated skeletal muscle phenotype involves the generation of determined myoblasts from multipotential stem cells and the subsequent activation of an array of muscle-specific genes. Myoblast differentiation is negatively regulated by serum and peptide growth factors, such as transforming growth factor β (TGF-β) and fibroblast growth factor (1–5). How growth factor signals coordinately suppress a battery of unlinked muscle-specific genes is unknown.

A family of skeletal muscle-specific regulatory factors, MyoD (6), myogenin (7, 8), myf-5 (9), and MRF4/herculin/myf-6 (10–12), has recently been identified and shown to possess the ability to activate myogenesis when transfected into fibroblasts (for review see ref. 13). These factors share homology within a basic domain and an adjacent helix-loop-helix (HLH) motif that mediate DNA binding and dimerization, respectively (14–16). Each of these factors binds the DNA sequence CANNTG, known as an E box, which is present in the control regions of numerous muscle-specific genes (12, 14, 16–22). The affinity of myogenic HLH proteins for the E box consensus sequence is increased dramatically in the presence of the widely expressed *E2A* gene products (E12 and E47), with which they form heterodimeric complexes (14, 16, 17, 21, 23).

Although the myogenic HLH proteins can each activate muscle-specific genes in transfected fibroblasts, their ability to do so depends on withdrawal of serum and mitogenic factors from the medium (6, 7, 9, 24, 25). The HLH protein Id, which is down-regulated during myogenesis, has been postulated to mediate the inhibitory effects of serum on MyoD actions (26). Id can heterodimerize *in vitro* with E12 and MyoD, but it lacks a basic domain and consequently generates heterodimers that cannot bind DNA. Overexpression of Id in transfected cells can also inhibit trans-activation of the muscle creatine kinase (MCK) enhancer by MyoD (26). Whether Id inhibits binding of HLH proteins to their target sequences *in vivo* and whether it alone is sufficient to mediate the negative effects of serum and peptide growth factors on myogenesis, however, remain to be demonstrated.

In the present study, we show that high serum and TGF-β inhibit the ability of myogenin to activate myogenesis, but these inhibitors differentially affect the DNA-binding activity of myogenin measured *in vitro*. Although serum diminishes the DNA-binding activity of myogenin, possibly through induction of Id protein, TGF-β acts through a mechanism distal to DNA sequence recognition by myogenin and independent of Id.

MATERIALS AND METHODS

Cell Culture. C2 (27), 10T½, 10TFL2-3 (28), and BC_3H1 (29) cells were maintained in Dulbecco's modified Eagle's medium (DMEM)/20% fetal bovine serum [growth medium (GM)]. To initiate differentiation GM was replaced with DMEM containing 2% horse serum or 0.5% fetal bovine serum [differentiation medium (DM)].

Gel-Mobility-Shift Assays. Preparation of nuclear extracts and gel-mobility-shift assays were done as described (17, 30). Unless indicated otherwise, 4 μg of protein was used in each assay. This amount corresponds to 2.2×10^5 nuclear equivalents for 10T½ cells, 1.0×10^5 nuclear equivalents for undifferentiated 10TFL2-3 and C2 cells, and 0.8×10^5 nuclear equivalents for differentiated 10TFL2-3 and C2 cells. The probe corresponds to the right, high-affinity, E box in the MCK enhancer (17, 18, 22, 31). The nonspecific oligomer used in competition assays corresponded to the region of the MCK enhancer encompassing the left, low-affinity, E box (17, 18, 31).

Immunoprecipiation and Immunofluorescence. Immunostaining for myosin was done as described (7). Myogenin immunofluorescence was measured as described (17) by using a mixture of affinity-purified antibodies directed against synthetic peptides corresponding to segments of myogenin. Labeling of cultures with [^{35}S]methionine, followed by immunoprecipitation of myogenin from nuclear extracts, was done as described (17) by using aliquots of extracts containing 1×10^7 cpm. Immune complexes were denatured in SDS sample buffer, resolved by SDS/PAGE, and processed for fluorography.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: DM, differentiation medium; GM, growth medium; HLH, helix-loop-helix; MCK, muscle creatine kinase; TGF-β, transforming growth factor type β; Tn-T, troponin T.

*To whom reprint requests should be addressed.

RNA Isolation and Analysis. RNA isolation and Northern analysis was done as described (3), using ^{32}P-labeled probes for rat troponin T (Tn-T) (32), mouse myogenin (7), mouse MyoD (6), canine MCK (33), and mouse Id (26). Levels of individual transcripts were quantitated by densitometry.

RESULTS

Serum and TGF-β Block Differentiation of 10T½ Cells that Constitutively Express Myogenin. We showed previously that myogenin-transfected 10T½ cells that were rapidly proliferating in the presence of high serum did not express endogenous muscle-specific genes until they became quiescent in low-serum medium, indicating that serum could override the actions of myogenin (7, 28). To further explore the mechanism(s) whereby growth signals suppressed myogenin actions, we selected the cell line 10TFL2-3, which was derived from 10T½ cells by stable transfection of a myogenin cDNA linked to the Moloney sarcoma virus long terminal repeat (7). The level of myogenin expression, measured by immunoprecipitation from nuclear extracts of [^{35}S]methionine-labeled cells, was equivalent in proliferating and differentiated 10TFL2-3 cells and was similar to that in differentiated C2 myotubes (Fig. 1). As reported (17), antibodies directed against peptide epitopes specific to myogenin recognized two polypeptides of M_r 32,000 and 34,000 in nuclear extracts (Fig. 1). The identity of these proteins as myogenin was confirmed by the ability of cognate peptides to compete for immunoprecipitation (Fig. 1).

To determine whether cell proliferation was required for growth factor-dependent repression of myogenin actions, we tested whether TGF-β, a nonmitogenic inhibitor of myogenesis (2, 4, 5, 34), could repress differentiation of 10TFL2-3 cells in the absence of serum. Fig. 2 shows that 10TFL2-3 cells form multinucleate myotubes that stain for myosin upon serum withdrawal. However, when these cells were exposed to low-serum medium containing TGF-β, they remained mononucleate, and there was no detectable expression of myosin or other muscle-specific gene products. Because the rate of myogenin synthesis in 10TFL2-3 cells was identical with and without TGF-β (data not shown), we conclude that TGF-β acting at the cell surface can silence myogenin activity and that repression does not require cell proliferation.

Serum and TGF-β Do Not Block Transport of Myogenin to the Nucleus. To determine whether serum or TGF-β suppressed the activity of myogenin by preventing its transport to the nucleus, we analyzed the subcellular distribution of myogenin in 10TFL2-3 cells by immunofluorescence. Nuclei of 10TFL2-3 cells maintained in GM (Fig. 3) or DM with

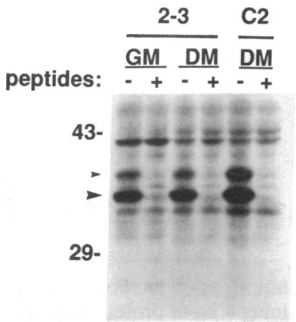


FIG. 1. Immunoprecipitation of myogenin from myogenin-transfected 10T½ cells and C2 myotubes. C2 or 10TFL2-3 cells were labeled with [^{35}S]methionine, and myogenin was immunoprecipitated from nuclear extracts with anti-myogenin antibodies without (−) or with (+) cognate peptides. Antigen–antibody complexes were resolved on 10% SDS gels followed by fluorography. Positions of the two forms of myogenin (arrowheads) and M_r markers are indicated; only the portion of the gel containing myogenin is shown.

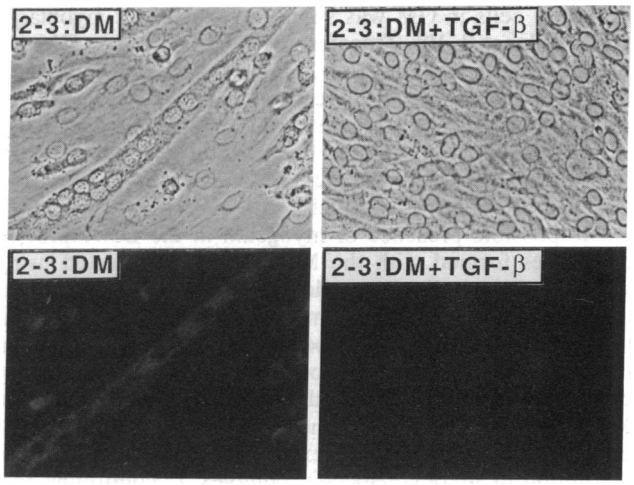


FIG. 2. TGF-β blocks differentiation of myogenin-transfected 10T½ cells. 10TFL2-3 cells were transferred to DM with or without TGF-β at 5 ng/ml, as indicated. Four days later, cultures were fixed and stained for myosin heavy chain. (*Upper*) Phase-contrast photomicrographs. (*Lower*) Immunofluorescence photomicrographs.

TGF-β (data not shown) showed a similar pattern of nuclear immunostaining to nuclei of C2 myotubes, indicating that serum and TGF-β do not block the actions of myogenin by preventing its transport to the nucleus.

Serum and TGF-β Differentially Affect the Ability of Myogenin to Bind DNA. To further investigate the mechanism through which growth factor signals blocked the actions of myogenin, we used gel-mobility-shift assays to examine whether serum or TGF-β altered the ability of myogenin to interact with its target sequence in the MCK enhancer (17, 21). Fig. 4*A* shows that a DNA probe encompassing the high-affinity E box from the MCK enhancer gave rise to a major DNA–protein complex, designated complex 1 (lanes 1–3) with extracts from C2 myoblasts in GM. Upon transfer to DM, complex 1 reproducibly diminished in intensity, and a second major complex appeared (designated complex 2, lanes 4–6). We also observed minor complexes, primarily with extracts from differentiated cells, that migrated as diffuse bands above complex 1. The pattern of DNA–protein complexes generated with extracts from proliferating

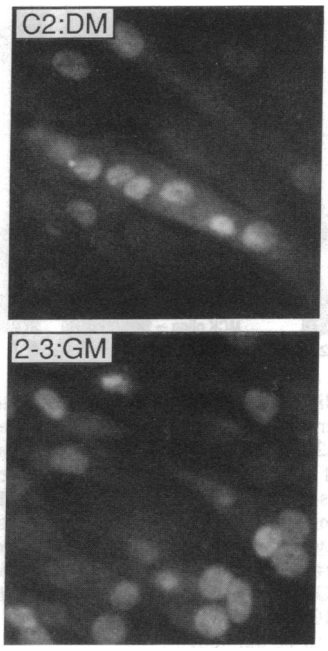


FIG. 3. Myogenin nuclear localization in myogenin-transfected 10T½ cells and differentiated C2 myotubes. C2 cells were exposed to DM for 4 days, during which extensive myotubes were formed (*Upper*). 10TFL2-3 cells were maintained in GM under conditions that prevented expression of muscle-specific genes (*Lower*). Cultures were fixed and analyzed for myogenin expression by indirect immunofluorescence.

10TFL2-3 cells in GM was similar to that of C2 myoblasts (Fig. 4*A*, lanes 8–10). Transfer of 10TFL2-3 cells to DM led to the appearance of complex 2 (Fig. 4*A*, lanes 11–13, and *C*, lane 2). Extracts from 10T½ cells gave rise only to complex 1 in GM and DM (Fig. 4*A*, lane 7; ref. 7). Sequence-specificity of binding was confirmed by competition experiments with homologous and heterologous DNA used as competitors (denoted S and N, respectively, in Fig. 4*A*).

The possible presence of myogenin in the DNA–protein complexes formed with the MCK probe was tested using anti-myogenin antibodies. Addition of the antibodies to extracts from C2 or 10TFL2-3 myotubes resulted in a diminution of complex 2 and the appearance of a minor complex at the top of the gel, which appears to represent a tertiary complex between the antibodies and complex 2 (Fig. 4*B*). Myogenin peptides corresponding to the antibody epitopes eliminated the tertiary complex and interfered with antibody disruption of myocyte-specific complex 2, confirming that myogenin is a component of both complexes. Addition of anti-myogenin antibodies to the gel-mobility-shift assay had no effect on the formation of complex 1, indicating that it does not contain myogenin (Fig. 4*B*, lanes 1 and 2).

That extracts from 10TFL2-3 cells maintained in GM showed little evidence of the myogenin-containing complex (complex 2), despite equivalent levels of myogenin protein to extracts prepared from differentiated 10TFL2-3 cells, suggests that myogenin accumulates in the nucleus of proliferating 10TFL2-3 cells in a form that does not bind efficiently to the MCK enhancer until serum has been withdrawn. In contrast to the apparent absence of the myogenin-dependent complex in extracts from proliferating 10TFL2-3 cells in GM, nuclear extracts from 10TFL2-3 cells exposed to DM containing TGF-β showed levels of the myogenin-dependent complex (complex 2) equivalent to extracts from differentiated 10TFL2-3 cells (Fig. 4*C*, lanes 2–6). Thus, myogenin can bind to its target sequence in extracts from cells that are blocked from differentiating by TGF-β. The ability of myogenin to bind DNA, therefore, appears to be differently affected in proliferating and quiescent cells arrested in the differentiation pathway by serum or TGF-β, respectively.

Expression of Id Protein Inversely Correlates with the Ability of Myogenin to Bind the MCK Enhancer. Because the HLH protein Id has been postulated to mediate the negative effects of serum on myogenesis by inhibiting the ability of MyoD to bind DNA (26), we examined whether the pattern of Id expression in 10TFL2-3 cells was consistent with its possible involvement in growth factor-dependent repression of myogenesis. Myogenin mRNA was expressed at a high level in 10TFL2-3 cells independent of differentiation (Fig. 5). In contrast, Id mRNA was expressed in proliferating 10TFL2-3 cells in GM and was down-regulated upon transfer to DM. The extent of down-regulation of Id expression after transfer to low-serum medium did not change in the presence of TGF-β. Thus, Id mRNA expression was inversely correlated with the ability of myogenin to bind DNA—i.e., in the presence of high serum, Id levels were high and the DNA-binding activity of myogenin was low; in the presence of low serum, Id levels were low, and the DNA-binding activity of myogenin was high. The decline in Id expression in quiescent, undifferentiated 10TFL2-3 cells in the presence of TGF-β suggests that down-regulation of Id is linked to cell-cycle withdrawal rather than activation of the differentiation program and that TGF-β does not block the actions of myogenin through an Id-dependent mechanism.

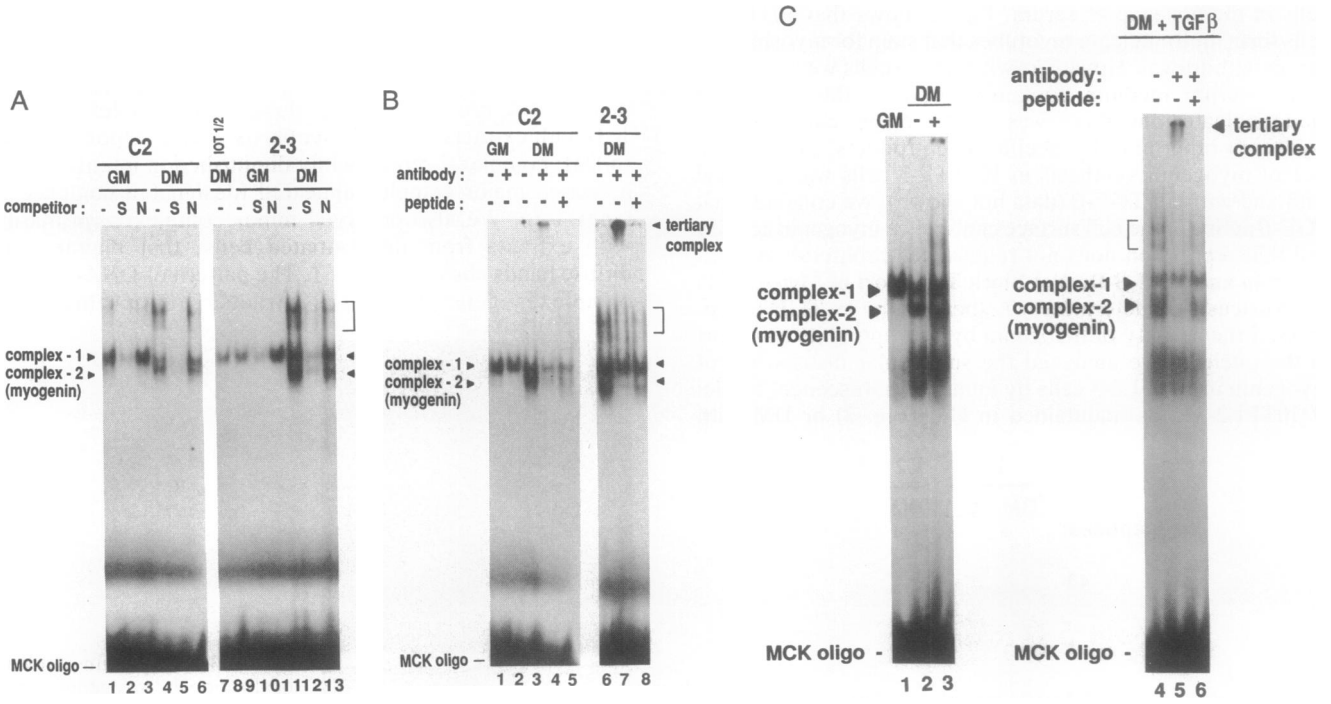


FIG. 4. Analysis of DNA binding activity of myogenin in nuclear extracts. Gel-mobility-shift assays were done with an end-labeled probe encompassing the right E box from the MCK enhancer (17). Aliquots of nuclear extract containing 4 μg of protein were used for 10T½ and C2 cells, and 8 μg was used for 10TFL2-3 cells. (*A*) Specificity of factor binding to the labeled oligomer was determined in the absence (−) or presence (+) of a 100-fold molar excess of unlabeled homologous (designated S) or heterologous (designated N) oligomer. Lanes: 1–3, C2 myoblast extracts; 4–6, C2 myotube extracts; 7, extracts from 10T½ cells in DM; 8–10, extracts from 10TFL2-3 cells in GM; 11–13, extracts from 10TFL2-3 cells in DM. (*B*) The effect of anti-myogenin antibodies on formation of DNA–protein complexes with the labeled probe was tested in the absence (−) or presence (+) of cognate myogenin peptides. Complex 1, which is ubiquitous, and complex 2, which contains myogenin, are indicated. Lanes: 1 and 2, C2 myoblast extracts; 3–5, C2 myotube extracts; 6–8, extracts from 10TFL2-3 cells in DM. (*C Left*), nuclear extracts were prepared from 10TFL2-3 cells in GM or DM with and without TGF-β for 72 hr, as specified, and used in gel-mobility-shift assays as described. (*C Right*) Nuclear extracts from 10TFL2-3 cells in DM with TGF-β were used in the gel-mobility-shift assay. Brackets indicate diffuse slowly migrating complexes that remain to be defined.

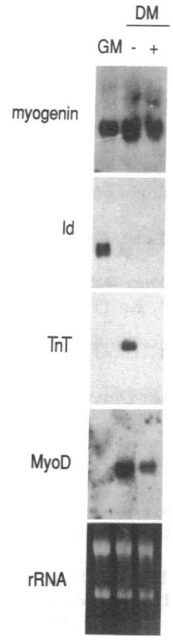


FIG. 5. Id mRNA is expressed at a high level in the presence of serum but not TGF-β. Total cellular RNA was isolated from 10TFL2-3 cells in GM or after transfer to DM with (+) and without (−) TGF-β for 72 hr. Expression of the indicated mRNAs was determined by Northern analysis. Ethidium bromide staining of the gel (*Bottom*) confirmed that equivalent quantities of RNA were applied to each lane.

We also examined the expression of MyoD in 10TFL2-3 cells. As reported (28, 40), MyoD mRNA was not expressed in the presence of high serum but was up-regulated upon transfer to DM (Fig. 5). It is interesting to note that MyoD was also expressed in the presence of TGF-β, whereas genes associated with terminal differentiation, such as Tn-T (Fig. 5) and myosin (Fig. 2) were not. These results suggest that TGF-β selectively suppresses "downstream" muscle-specific genes and has a less dramatic effect on regulatory interactions between myogenin and MyoD (see below).

TGF-β Can Uncouple Myogenin Expression from Expression of Other Muscle-Specific Genes. We also asked whether TGF-β was also able to block the actions of myogenin in other established muscle cell lines. Fig. 6 shows that myogenin mRNA was rapidly induced in the BC_3H1 muscle cell line after serum withdrawal and was followed by induction of MCK and Tn-T mRNAs. In the presence of TGF-β, myogenin expression was reduced, but myogenin mRNA eventually increased. Myogenin protein was induced in parallel with myogenin mRNA (data not shown). In contrast, MCK and Tn-T mRNAs remained at low levels in the presence of TGF-β, despite high levels of myogenin expression. These results show that TGF-β can uncouple myogenin expression from induction of other muscle-specific genes.

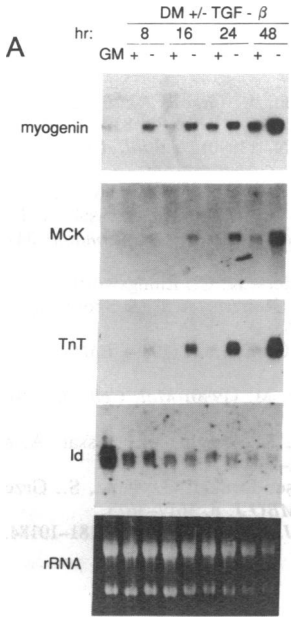
As observed in 10TFL2-3 cells, Id mRNA was expressed at a high level in proliferating BC_3H1 myoblasts in GM. Upon transfer to DM, Id mRNA was down-regulated to a basal level within 8 hr. TGF-β had no effect on the pattern of Id expression. Thus, down-regulation of Id expression is an early event in the differentiation program that precedes the expression of genes that are known targets for activation by myogenin.

DISCUSSION

Our results show that myogenin can accumulate in the nuclei of cultured cells in a functionally inactive form when the cells are maintained in the presence of TGF-β or serum. Although the final effect of TGF-β and serum—inhibition of myogenesis—is the same, these inhibitors appear to affect the *in vitro* DNA-binding activity of myogenin in different ways. In extracts from proliferating cells maintained in high serum, the ability of myogenin to bind the MCK enhancer was impaired. In contrast, myogenin was able to bind the MCK enhancer in extracts from quiescent cells arrested in the differentiation pathway by TGF-β. These results suggest that TGF-β inhibits the actions of myogenin through a mechanism independent of DNA sequence recognition, whereas high serum may block myogenesis through inhibition of the DNA-binding activity of myogenin.

The apparent inability of myogenin to bind DNA in extracts from proliferating myoblasts that express Id at high levels and the acquisition of DNA-binding activity when Id levels declined upon exposure to low-serum medium is consistent with the postulated role of Id as a negative regulator of DNA binding (26). Moreover, cells that were arrested in the differentiation pathway by TGF-β did not express significant levels of Id mRNA and contained a species of myogenin capable of binding DNA *in vitro*. The absence of Id, combined with the ability of myogenin to bind DNA in the presence of TGF-β, argues against a role for Id in the mechanism whereby TGF-β inhibits myogenesis.

The failure of myogenin to bind the MCK enhancer in extracts from proliferating myoblasts is consistent with the behavior of the myocyte enhancer binding factor MEF-1, which shares antigenicity with MyoD and is only detectable in myotubes (18, 22). Similarly, *in vivo* footprinting experi-



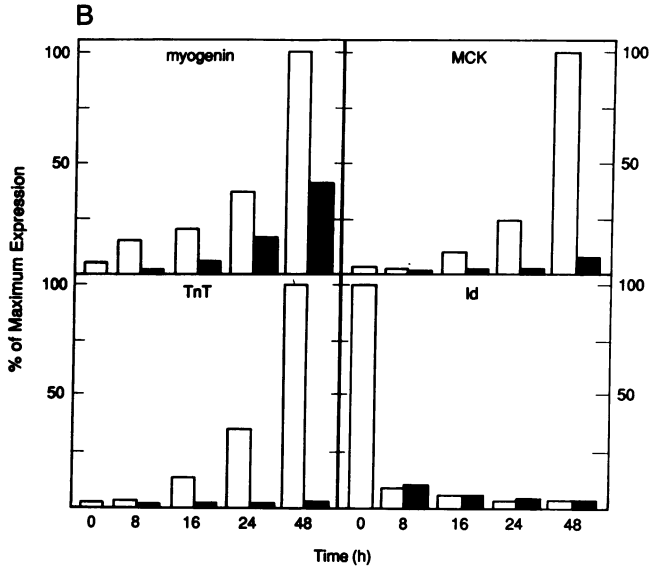


FIG. 6. TGF-β blocks the actions of myogenin in BC_3H1 cells but does not maintain Id expression. (*A*) Proliferating BC_3H1 cells were transferred from GM to DM with and without TGF-β (5 ng/ml) for the indicated times. Total cellular RNA was isolated from cultures under each condition, and expression of the indicated mRNAs was determined by Northern (RNA) analysis. (*B*) Levels of expression of each mRNA under each condition were quantitated by densitometry. Values are expressed relative to the maximal level of expression of each individual transcript. Open bars indicate GM at time zero or DM without TGF-β; filled bars indicate DM with TGF-β.

ments have shown that the high-affinity E box in the MCK enhancer is occupied only in myotubes, despite high levels of MyoD expression in myoblasts (35). Paradoxically, we observed an additional enhancer-binding complex (complex 1) with extracts from myoblasts and 10T½ cells. We do not know the identity of the protein(s) contained in this complex, but it is conceivable they could compete for occupancy of the E box *in vivo*. The failure to detect this binding activity by *in vivo* footprinting (35) suggests that *in vivo* and *in vitro* DNA-binding assays may not always correlate.

Our results demonstrate that the activities of myogenic HLH proteins are profoundly influenced by extracellular signals. There is evidence to suggest that this is not simply a tissue culture phenomenon but rather may reflect regulatory mechanisms operative during embryogenesis. In the developing somite, for example, myogenin is expressed 2 days before other muscle-specific genes (8, 36). TGF-β is present in embryonic somites before muscle formation (37) and could potentially inhibit certain of myogenin's activities at this stage of embryogenesis, thus delaying induction of "downstream" muscle-specific genes. This action would allow the population of committed myogenic precursors to be amplified before terminal differentiation. Similarly, MyoD is expressed during *Xenopus* embryogenesis much earlier than other muscle-specific genes, suggesting that MyoD activity is subject to negative control (38, 39).

It is intriguing that TGF-β completely suppressed expression of genes associated with terminal differentiation, even in the presence of high levels of exogenous myogenin, whereas TGF-β inhibited endogenous myogenin expression only partially in BC_3H1 cells, and it allowed expression of MyoD in 10TFL2-3 cells. These results suggest that the TGF-β pathway discriminates between the myogenic regulatory factor genes and downstream muscle-specific genes that are targets for activation by myogenic HLH proteins. Perhaps binding of myogenin to the control regions of the regulatory factor genes is sufficient to activate the autoregulatory loop (28, 40, 41) in the presence of TGF-β, whereas binding to the control regions of genes associated with terminal differentiation is not sufficient. Suppression of myogenin and MyoD expression by TGF-β has been observed in some muscle cell lines, but the extent of suppression seems to vary depending on the cell line and exact culture conditions (24, 42).

Our results show that TGF-β can block the actions of myogenin through a mechanism independent of DNA binding and Id, but these experiments do not indicate how this repression is achieved. Given the ability of TGF-β to coordinately suppress a large array of genetically unlinked muscle-specific genes, we favor the hypothesis that the effects of TGF-β are directed at the myogenin protein itself or at a component of the transcriptional machinery required by myogenin to activate its target genes. Indeed, recent identification of a transcription-activation domain near its carboxyl terminus (J. Schwartz, T. Chakraborty, and E.N.O., unpublished work) may provide a mechanism for modulating the transcription-activating potential of myogenin independent of DNA binding.

We thank M. E. Perry and S. Jasser for technical assistance and E. Madson for preparation of the manuscript. We thank M. Perry, B. Klein, and M. Schneider for critically reading the manuscript. We are grateful to A. Lassar and H. Weintraub for the MyoD and Id cDNAs; A. Strauss for the MCK cDNA; and B. Nadal-Ginard for Tn-T cDNA. This work was supported by grants from the National Institutes of Health and the American Cancer Society. E.N.O. is an Established Investigator of the American Heart Association. D.E. and L.L. are Rosalie B. Hite Predoctoral Fellows, and T.J.B. is a National Institutes of Health predoctoral fellow.

1. Clegg, C. H., Linkhart, T. A., Olwin, B. B. & Hauschka, S. D. (1987) *J. Cell Biol.* **105**, 949–956.
2. Olson, E. N., Sternberg, E., Hu, J. S., Spizz, G. & Wilcox, C. (1986) *J. Cell Biol.* **103**, 1799–1805.
3. Spizz, G., Roman, D., Strauss, A. & Olson, E. N. (1986) *J. Biol. Chem.* **261**, 9483–9488.
4. Florini, J., Roberts, A. B., Ewton, D. Z., Falen, S. L., Flanders, K. D. & Sporn, M. B. (1986) *J. Biol. Chem.* **261**, 16509–16513.
5. Massague, J. T., Cheifetz, S., Endo, T. & Nadal-Ginard, B. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8206–8210.
6. Davis, R. L., Weintraub, H. & Lassar, A. B. (1987) *Cell* **51**, 987–1000.
7. Edmondson, D. G. & Olson, E. N. (1989) *Genes Dev.* **3**, 628–640.
8. Wright, W. E., Sassoon, D. A. & Lin, V. K. (1989) *Cell* **56**, 607–617.
9. Braun, T., Buschhausen-Denker, G., Bober, E., Tannich, E. & Arnold, H. H. (1989) *EMBO J.* **8**, 701–709.
10. Rhodes, S. J. & Konieczny, S. F. (1989) *Genes Dev.* **3**, 2050–2061.
11. Miner, J. H. & Wold, B. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1089–1093.
12. Braun, T., Bober, E., Winter, B., Rosenthal, N. & Arnold, H. H. (1990) *EMBO J.* **9**, 821–831.
13. Olson, E. N. (1990) *Genes Dev.* **4**, 1454–1461.
14. Davis, R. L., Cheng, P.-F., Lassar, A. B. & Weintraub, H. (1990) *Cell* **60**, 733–746.
15. Murre, C., McCaw, P. S. & Baltimore, D. (1989) *Cell* **56**, 777–783.
16. Murre, C., McCaw, P. S., Vaessin, H., Caudy, M., Jan, L. Y., Jan, Y. N., Cabrera, C. V., Buskin, J. N., Hauschka, S. D., Lassar, A. B., Weintraub, H. & Baltimore, D. (1989) *Cell* **58**, 537–544.
17. Brennan, T. & Olson, E. N. (1990) *Genes Dev.* **4**, 582–595.
18. Lassar, A. B., Buskin, J. N., Lockshon, D., Davis, R. L., Apone, S., Hauschka, S. D. & Weintraub, H. (1989) *Cell* **58**, 823–831.
19. Piette, J., Bessereau, J.-L., Huchet, M. & Changeux, J.-P. (1990) *Nature (London)* **345**, 353–355.
20. Wentworth, B. M., Donoghue, M., Engert, J. C., Berglund, E. B. & Rosenthal, N. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1242–1246.
21. Chakraborty, T., Brennan, T. & Olson, E. N. (1991) *J. Biol. Chem.* **266**, 2878–2882.
22. Buskin, J. N. & Hauschka, S. D. (1989) *Mol. Cell. Biol.* **9**, 2627–2640.
23. Braun, T., Winter, B., Bober, E. & Arnold, H. (1990) *Nature (London)* **346**, 663–665.
24. Vaidya, T. B., Rhodes, S. J., Taparowsky, E. J. & Konieczny, S. F. (1989) *Mol. Cell. Biol.* **9**, 3576–3579.
25. Yutzey, K. E., Rhodes, S. J. & Konieczny, S. F. (1990) *Mol. Cell. Biol.* **10**, 3934–3944.
26. Benezra, R., Davis, R. L., Lockshon, D., Turner, D. L. & Weintraub, H. (1990) *Cell* **61**, 49–59.
27. Yaffe, D. & Saxel, O. (1977) *Nature (London)* **270**, 725–727.
28. Brennan, T. J., Edmondson, D. E. & Olson, E. N. (1990) *J. Cell Biol.* **110**, 929–937.
29. Schubert, D., Harris, A. J., Devine, C. E. & Heinemann, S. (1974) *J. Cell Biol.* **61**, 398–413.
30. Gossett, L., Kelvin, D., Sternberg, E. & Olson, E. N. (1989) *Mol. Cell. Biol.* **9**, 5022–5033.
31. Sternberg, E. A., Spizz, G., Perry, W. M., Vizard, D., Weil, T. & Olson, E. N. (1988) *Mol. Cell. Biol.* **8**, 2896–2909.
32. Garfinkel, L., Periasamy, M. & Nadal-Ginard, B. (1982) *J. Biol. Chem.* **257**, 11078–11086.
33. Roman, D., Billadello, J., Gordon, J., Grace, A., Sobel, B. & Strauss, A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8394–8398.
34. Li, L., Hu, J. S. & Olson, E. N. (1990) *J. Biol. Chem.* **265**, 1556–1562.
35. Mueller, P. R. & Wold, B. (1989) *Science* **246**, 780–786.
36. Sassoon, D., Lyons, G., Wright, W. E., Lin, V., Lassar, A. B., Weintraub, H. & Buckingham, M. (1989) *Nature (London)* **341**, 303–307.
37. Heine, U. I., Munoz, E. F., Flanders, K. C., Ellingsworth, L. R., Lam, H.-Y. P., Thompson, N. L., Roberts, A. B. & Sporn, M. B. (1987) *J. Cell Biol.* **105**, 2861–2876.
38. Hopwood, N. D., Pluck, A. & Gurdon, J. (1989) *EMBO J.* **8**, 3409–3417.
39. Scales, J., Olson, E. N. & Perry, M. (1990) *Mol. Cell. Biol.* **10**, 1516–1524.
40. Thayer, M., Tapscott, S., Davis, R., Wright, W., Lassar, A. & Weintraub, H. (1989) *Cell* **58**, 241–248.
41. Braun, T., Bober, E., Buschhausen-Denker, G., Kotz, S., Grzeschik, K. & Arnold, H. (1989) *EMBO J.* **8**, 3617–3625.
42. Heino, J. & Massagúe, J. (1990) *J. Biol. Chem.* **265** 10181–10184.