MEF2 Protein Expression, DNA Binding Specificity and Complex Composition, and Transcriptional Activity in Muscle and Non-muscle Cells*

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Tissue-specific gene expression can be mediated by complex transcriptional regulatory mechanisms. Based on the dichotomy of the ubiquitous distribution of the myocyte enhancer factor 2 (MEF2) gene mRNAs compared to their cell type-restricted activity, we investigated the basis for their tissue specificity. Electrophoretic mobility shift assays using the muscle creatine kinase MEF2 DNA binding site as a probe showed that HeLa, Schneider, L6E9 muscle, and C2C12 muscle cells have a functional MEF2 binding activity that is indistinguishable based on competition analysis. Interestingly, chloramphenicol acetyltransferase reporter assays showed MEF2 site-dependent trans-activation in myogenic C2C12 cells but no trans-activation by the endogenous MEF2 proteins in HeLa cells. By immunofluorescence, we detected abundant nuclear localized MEF2A and MEF2D protein expression in HeLa cells and C2C12 muscle cells. Using immunogold shift analysis and also co-immunoprecipitation studies, we show that the predominant MEF2 DNA binding complex bound to MEF2 sites from either the muscle creatine kinase or c-jun regulatory regions in C2C12 muscle cells is comprised of a MEF2A homodimer, whereas in HeLa cells, it is a MEF2A:MEF2D heterodimer. Thus, the presence of MEF2 DNA binding complexes is not necessarily coupled with trans-activation of target genes. The ability of the MEF2 proteins to activate transcription in vitro correlates with the specific dimer composition of the DNA binding complex and the cellular context.

During muscle formation, the acquisition of the mature phenotype requires the activation of a vast number of unlinked cell-specific genes. Two families of transcriptional reporter assays have been shown to be critically involved in orchestrating this cell fate decision. These proteins are encoded by the basic helix-loop-helix myogenic factors (MyoD, MRF4, Myf5, and myogenin; reviewed in Refs. 1 and 2) and the genes encoding the myocyte enhancer factor 2 (MEF2A-D)† family (reviewed in Ref. 3).

MEF2 (also called RSRFs for related to serum response factor) genes encode nuclear proteins belonging to the MADS (MCM1, Agamous, Deficiens, Serum Response Factor) superfamily of DNA binding proteins (4, 5). The MEF2 proteins comprise a structurally distinct subfamily of MADS proteins distinguished from the other MADS proteins by their binding site specificity (C/T)TA(A/T)4 TA(G/A), to which they bind as homo- and heterodimers (4, 6, 7). The role of the MEF2 genes in the complex hierarchical regulation of muscle-specific gene expression has recently gained prominence. The presence of the MEF2 cis element in the regulatory regions of many muscle structural and metabolic genes (8–29), the role of MEF2 proteins in stabilizing the activation of myogenin gene expression during myogenesis (14), the physical synergistic interaction of the MEF2 proteins with members of the myogenic basic helix-loop-helix proteins (30, 31), the ability of the MEF2 proteins to activate myogenesis by forced expression in fibroblasts (30), and the requirement for the single Drosophila MEF2 homologue for normal muscle formation (32, 33) all point toward an evolutionarily conserved role of the MEF2 proteins during myogenesis.

Despite the persuasive data suggesting the important muscle-specific role of these factors during myogenesis, a controversy has emerged concerning the cell type and tissue specificity of the MEF2 proteins (4, 5, 16, 34–36). Although the MEF2 activity was originally characterized as a muscle-specific DNA binding activity (35), molecular cloning and tissue distribution studies of the factors (MEF2A-D) that bind to this site did not support this contention (4, 5, 34, 37–40). This work has documented that the mRNAs for the MEF2 genes are expressed quite ubiquitously, with MEF2C being the only gene that is tissue restricted in its expression pattern. A likely explanation for the disparity between MEF2 mRNA expression and activity is the existence of a mechanism for translational repression. Translational control of MEF2A expression in vascular smooth muscle cells has recently been reported, establishing this as a means of regulating the tissue specificity of MEF2 activity independent of mRNA expression (41). In support of this contention, we have also observed expression of MEF2C mRNA in non-muscle cells with a concomitant lack of detectable MEF2C protein. However, experiments showing a MEF2 DNA binding complex in non-muscle cells complicate this issue since this observation requires the presence of functional MEF2 proteins, thus negating the hypothesis of translational control. It has been postulated that the binding activity in non-muscle cells is distinct in terms of its protein composition (5) and also its sequence specificity (35). The precise nature of MEF2 complexes in vivo that are responsible for muscle-specific transcription have also not yet been defined.

The cell type specificity of the MEF2 activity may thus provide important clues as to the regulation and role of the MEF2 proteins in muscle and other cell types. Therefore, we under-

* This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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† The abbreviations used are: MEF, myocyte enhancer factor; CAT, chloramphenicol acetyltransferase; MCK, muscle creatine kinase; CIP, calf intestinal phosphatase; TK, thymidine kinase.
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took to systematically study MEF2 protein expression, DNA binding activity and complex composition, and transcriptional activity in a muscle and non-muscle cell line. We document that MEF2 protein expression and functional DNA binding specificity (by competition analysis) is indistinguishable in muscle (C2C12 and L6E9) and non-muscle (HeLa, Schneider, and NIH3T3) cells. Further analysis of C2C12 and HeLa cells as representative muscle and non-muscle cells, respectively, revealed that, despite the expression and functional DNA binding activity of the endogenous MEF2 proteins in these two cell types, the MEF2 proteins potently activate transcription in C2C12 muscle cells, but their trans-activation function is silent in HeLa cells. Immuno-gel shift analysis and co-immunoprecipitation experiments revealed a difference in the predominant MEF2 dimer complex in the two cell types. Thus, we have identified functional differences in the composition of MEF2 DNA binding complexes, and these differences correlate with trans-activation potential in muscle and non-muscle cells.

MATERIALS AND METHODS

Cell Culture, Plasmids, Antibodies, and DNA Transfections—For reporter assays, the appropriate reporter was transfected into HeLa cells or C2C12 myoblasts at 60% confluence by calcium phosphate coprecipitation. The cells were glycerol shocked after 16 h and then switched to differentiation media (Dulbecco’s modified Eagle’s medium supplemented with 5% heat-inactivated horse serum) and harvested 3 days after serum withdrawal. Each plate of cells was transfected with 10 μg of the appropriate CAT reporter construct and 3 μg of pSV β-galactosidase, which served as an internal control for the transfection efficiency. For the overexpression studies, 5 μg of an additional construct was transfected comprising either the MEF2A or MEF2B coding region in a pMT2 expression vector or the pMT2 vector alone as a control. Cell extracts were prepared, and CAT activity was determined as reported previously (39). The reporters consisted of the following: the embryonic myosin heavy chain promoter CAT gene downstream of two copies of the muscle creatine kinase (MCK) MEF2 sites, inserted in a concatemerized orientation at the −102 position of the embryonic myosin heavy chain promoter in plasmid PE102CAT. The same oligonucleotide binding sites were also cloned in front of a thymidine kinase promoter in the pSTKCAT vector (39). For studies using the anti-MEF2 antibodies, MEF2A, MEF2B, and MEF2D antibodies were provided by Ron Prywes (42). These antibodies have been extensively characterized previously (42). The MEF2C antibody used was prepared by us and recognizes the two MEF2 sites, the same oligonucleotide binding sites were cloned in front of a thymidine kinase promoter in the pSTKCAT vector (39). For studies using the anti-MEF2 antibodies, MEF2A, MEF2B, and MEF2D antibodies were provided by Ron Prywes (42). These antibodies have been extensively characterized previously (42). The MEF2C antibody used was prepared by us and characterizes a transcription factor that binds to the MEF2 sites, the same oligonucleotide binding sites were cloned in front of a thymidine kinase promoter in the pSTKCAT vector (39). For studies using the anti-MEF2 antibodies, MEF2A, MEF2B, and MEF2D antibodies were provided by Ron Prywes (42). These antibodies have been extensively characterized previously (42). The MEF2C antibody used was prepared by us and characterizes the two MEF2 sites, the same oligonucleotide binding sites were cloned in front of a thymidine kinase promoter in the pSTKCAT vector (39).

DNA Binding Assays—The DNA binding assays were carried out as described previously (39). Complementary oligodeoxyribonucleotides were synthesized with an Applied Biosystems synthesizer. The preparation of extracts for binding assays was carried out as described previously (39). For the DNA binding assays with various cell extracts, the incubation reaction contained equivalent amounts of protein (based on a Bradford total protein assay), 0.2 ng of probe, 0.45 μg of poly(dI-dC), and 100 ng of single-stranded oligonucleotide in a total volume of 20 μl. The bound fraction was separated from the free probe by electrophoresis on a 4.5% polyacrylamide gel (acrylamide:bis, 29:1) at 4 °C. The core nucleotide sequences of probes and competitor DNAs used in the binding assays were as follows: MEF2, 5′-cgctgtaaaaataaccc-3′; MEF2mt1, 5′-cgctgtaaaaataaccc-3′; MEF2mt2, 5′-cgctgtaaaaataaccc-3′; MEF2mt3, 5′-cgctgtaaaaataaccc-3′; MEF2mt4, 5′-cgctgtaaaaataaccc-3′; MEF2mt5, 5′-cgctgtaaaaataaccc-3′; MEF2mt6, 5′-cgctgtaaaaataaccc-3′; CArg, 5′-gagacgacagcagacag-3′; and c-jun MEF2, 5′-cgctgtaaaaataaccc-3′.

Nucleotides in the underlined print conform to the consensus sequence of the MEF2 site, mutated nucleotides are shown in uppercase letters. Where competitor probes were added to the reaction, they were added at a 50-fold molar excess over the labeled MEF2 site. For the immuno-gel shift analysis, where appropriate, 1 μl of antiserum or preimmune serum was added to the incubation reaction (in all cases, 0.1 μg of anti-serum in Dulbecco’s modified Eagle’s medium supplemented with 5% heat-inactivated horse serum) and incubated for another 3–4 days. Cells were washed with phosphate-buffered saline (pH 7.4) three times and fixed for 5 min in cold methanol at −20 °C. Before staining, nonspecific binding sites were blocked with 10% fetal bovine serum/Dulbecco’s modified Eagle’s medium supplemented with 5% horse serum for 1 h. The cells were then washed with phosphate-buffered saline, and cells were counterstained for nuclei using ethidium bromide at 2 μg/ml for 2–5 min. After washing with phosphate-buffered saline, coverslips were mounted and treated with anti-fade media (Molecular Probes) and examined using a Bio-Rad MRC 1000 laser scanning confocal imaging system fitted to a Nikon diaphot microscope. The system is equipped with the Bio-Rad COMOS operating software. Ethidium bromide labeling was detected at the 588-nm excitation laser setting using the 522/35 emission filter attached to the green band epifluorescence. Images were recorded on Kodak TMAX 100 black-and-white film.

RESULTS

MEF2 DNA Binding Activity in Muscle and Non-muscle Cells

Initially, we tested the capacity of endogenous MEF2 proteins from three different cell types to bind to a double-stranded oligodeoxyribonucleotide, comprising the previously characterized MCK enhancer MEF2 site (35). The specificity of the protein-DNA complex (as determined by using several synthetic oligonucleotides as competitors. The results of these experiments using L6E9 (Fig. 1A, lanes 1–7), Schneider (Fig. 1B, lanes 8–10), and HeLa (Fig. 1C, lanes 14–19) cell extracts are consistent with the known specificity of the consensus binding site (CTTT(A/T)2/TA(N)2; L6E9 and Schneider cells bind similarly to C2C12 myoblasts in terms of their MEF2 DNA binding activity (C2C12 binding activity was published previously). Competition analysis with oligonucleotides that have
been shown previously to discriminate between MEF2 binding and ubiquitous factors (35) (Fig. 1) did not show any differences between the different cell types, suggesting that the proteins bound to this site in all three cell types are bona fide MEF2 proteins and that the composition of the complexes was similar based on their mobility (the mobility of the complex in the different cell types was the same when they were run side by side on the same gel (data not shown)).

**MEF2 Proteins Activate Transcription in C2C12 Myotubes but Not in HeLa Cells**—Because DNA binding by transcription factors is not always coordinated with transcriptional activation, we asked if, in both muscle and non-muscle cell types, the endogenous MEF2 proteins could activate a reporter construct in a MEF2 site-dependent manner. Therefore, the following reporter constructs were transfected into HeLa and C2C12 muscle cells under identical culture conditions: (a) the basal embryonic myosin heavy chain promoter (PE102CAT); and (b) the herpes simplex virus TK promoter (TK-CAT). Each promoter/reporter construct was transfected either with or without two copies of the MEF2 binding site attached upstream of the promoter. As shown in Fig. 2A, trans-activation of the reporter construct in C2C12 cells was dependent on the presence of the intact MEF2 sites, since the reporter without the MEF2 site or the reporter containing a mutated MEF2 site (same sequence as mutant 1, see “Materials and Methods”) was not activated. Conversely in HeLa cells, despite the presence of the MEF2 DNA binding activity, there was no activation of the reporter by the endogenous proteins (Fig. 2A). This data was essentially repeated when the TK promoter was substituted for the embryonic myosin heavy chain promoter (Fig. 2B). However, overexpression of MEF2 proteins in HeLa cells can overcome this trans-activation inhibition since cotransfection of the appropriate reporters with MEF2A or MEF2C expression plasmids (pMT2 MEF2A or MEF2C) activated transcription in a MEF2 site-dependent manner (Fig. 2C). Thus, the cellular context of the MEF2 proteins is crucial in determining whether the endogenous MEF2 proteins are transcriptionally active.

**Nuclear Localized Expression of MEF2A and MEF2D in C2C12 and HeLa Cells**—Given that we had observed a functional MEF2 DNA binding complex in both HeLa and C2C12 cells with distinct differences in the ability of these complexes to activate transcription, we next addressed two questions to try to account for these differences: (a) which MEF2 proteins are expressed in the two cell types? and (b) what is the cellular localization of these proteins? Immunofluorescence analysis using four previously well characterized antibodies specific for MEF2A-D (MEF2A, MEF2B, and MEF2D) characterizations as reported by Han and Prywes (42) and MEF2C as reported by McDermott et al. (39)) revealed that MEF2A and MEF2D were present in the nuclei of both HeLa cells and C2C12 (Fig. 3, A, B, F, and G), whereas MEF2B and MEF2C were not detectable in either cell type (data not shown). Further analysis with anti-myogenin, myosin heavy chain, and desmin antibodies confirmed the nature of the two different cell types in that no positive staining for myogenin, desmin, or myosin heavy chain was observed in HeLa cells (Fig. 3, H, I, and J), whereas C2C12 myotubes were positively stained for these proteins (Fig. 3, C, D, and E). Therefore, at the protein level, the expression and cellular localization of the MEF2 factors was indistinguishable between the two cell lines.

**Heterogeneous Composition of MEF2 Dimers Bound to the MEF2 Site in C2C12 Myotubes and HeLa Cells**—In an attempt to understand the discrepancy between DNA binding and trans-activation by the MEF2 proteins in the two cell types, we were next interested in determining whether the DNA binding complexes in the two cell types might differ in their composition. Since the similar molecular weight of the MEF2 proteins gives rise to similarly sized DNA binding complexes, it is not possible to discern heterogeneous complex composition by the mobility of the binding complex alone. Therefore, to determine this, we used electrophoretic mobility shift analysis in conjunction with specific antibodies against the four MEF2 proteins. Fig. 4 shows that in C2C12 cells, the predominant dimer combination indicated by the immuno-gel shift analysis was a MEF2A homodimer since the majority of the complex was shifted by the MEF2A antibody (Fig. 4A, lane 2). A small amount of the complex was also shifted by the MEF2D antibody, indicating the probable presence of a small number of MEF2A:MEF2D heterodimers (this was not due to a limiting amount of the antibody since the same results were obtained if the reaction contained 0.1 or 1 μl of the MEF2D antiserum; Fig. 4A, lane 5). We, therefore, contend that the major complex formed in C2C12 muscle cells is a MEF2A homodimer. Conversely, this analysis in HeLa cells revealed that either the MEF2A or MEF2D antibodies supershifted the whole MCK MEF2 DNA binding complex, indicating the presence of these proteins as a MEF2A:MEF2D heterodimer (Fig. 4B, lanes 7 and 10). Since the MCK MEF2 site is a low affinity MEF2 binding site, 2 we also carried out a similar analysis with a high affinity MEF2 site (the MEF2 site from the c-jun promoter (42) (Fig. 4)). In HeLa cells, there was no difference in complex composition on the c-jun MEF2 site as compared to the MCK MEF2 site (Fig. 4B, lanes 6–10 and 11–15, respectively), confirming that independent of the specific MEF2 site used (MCK or c-Jun), the composition of the MEF2 protein complex binding to it was the same. This analysis was repeated for C2C12 cells, and the results were identical to those obtained with the MCK MEF2 site (data not shown). Taken together, our interpretation of these experiments is that the predominant MEF2 dimer formation in HeLa cells comprises a MEF2A:MEF2D heterodimer, whereas the complex in C2C12 myotubes is predominantly a MEF2A homodimer.

**Predominance of MEF2A:MEF2D Heterodimers in HeLa Cells and MEF2A Homodimers in C2C12 Cells**—To confirm this heterogeneous cell type-regulated dimer formation by a different approach, we used immunoprecipitation/immunoblot analysis to assess the interaction of the MEF2 proteins in C2C12 and HeLa cells. These experiments revealed that when
the MEF2A antibody was used to immunoprecipitate MEF2A from both cell types, immunoblot analysis of the immunoprecipitate using the MEF2A antibodies revealed the presence, as expected, of two MEF2A isoforms (Fig. 5, top panel). However, when the MEF2A immunoprecipitate was probed with the MEF2D antibody, MEF2D immunostaining was abundantly present in the MEF2A immunoprecipitate from HeLa cells, whereas a barely detectable amount was present in C2C12 cells (Fig. 5A, middle panel). Thus, the existence of a MEF2A homodimer in muscle cells and a MEF2A:MEF2D heterodimer in HeLa cells is further supported in this experiment. When the converse experiment was done and the anti-MEF2D antibody was used for immunoprecipitation from the two cell types (Fig. 5B), the subsequent immunoprecipitate was positive for MEF2D (Fig. 5B, top panel) but positive for MEF2A only in HeLa cells (Fig. 5B, middle panel), thus indicating the coprecipitation of MEF2A:MEF2D dimers in HeLa and not in C2C12. Further analysis of the MEF2A and MEF2D immuno-

2xMEF2 mt CAT (mt1, see “Materials and Methods”); or no binding sites. An overexpression study was also carried out (C) in which pMT2 MEF2A or pMT2 MEF2C expression vectors were cotransfected with the PE102 CAT reporter containing: two MCK MEF2 sites (PE102CAT-2xMEF2 or TK 2xMEF2 CAT); mutated MEF2 sites (PE102CAT-2xMEF2mt or TK 2xMEF2 mt CAT). The experiments were performed at least twice with two different DNA preparations. Each data point is a mean of triplicate samples. The S.E. was not more than 9% of the mean value for any of the data points.
precipitates from HeLa and C2C12 cells revealed that the precipitated MEF2 protein was phosphorylated on serines as determined by a commercially available phosphoserine-recognizing antibody by immunoblot (Fig. 5, A and B, bottom panels). Incubation of extracts and probe with the specific MEF2A–MEF2D immune sera was carried out to test whether the endogenous MEF2 complex was supershifted by the antibodies in C2C12 (lanes 1–5) or HeLa cells (lanes 6–15) as well as a nonimmune serum control (PI).

**DISCUSSION**

**Tissue-specific Regulation of MEF2 Transcriptional Activity Can Be Accomplished by Posttranslational Control**—Tissue-specific expression of transcriptional regulatory proteins is a critical component of cell fate specification. However, it is also recognized that tissue-specific gene expression can be mediated by complex regulatory mechanisms involving ubiquitously expressed transcription factors which, in combination, specify a unique signal for tissue-restricted gene expression. The results presented here support the latter case for the MEF2 transcription factor family, which are quite ubiquitously distributed despite the fact that their activity is confined to certain cell types. Here we present evidence showing that the presence of the MEF2 proteins and DNA binding activity is not necessarily correlated with their capacity for transcriptional activation through the MEF2 site. Moreover, we also show differences in the composition of the MEF2 DNA binding complex in different cell types that may modulate its trans-activational potency. These data thus illustrate the first evidence for cell type-specific posttranslational regulation of the MEF2 proteins.

**Potential Complexity of MEF2 Homo- and Heterodimer Combinations in Vivo**—As stated previously, the MEF2 proteins can form both homo- and heterodimers that interact with the dyad symmetrical MEF2 binding site by each protein of the dimer recognizing a half site (43, 44). This binding is mediated by the N-terminal MADS/MEF2 domain, which is present in all
of the MEF2 proteins and is responsible for DNA binding and dimerization (43, 44).

The capacity for heterodimerization between the MEF2 proteins can potentially create a bewildering diversity of dimer complexes. Taking into account that each of the MEF2 genes gives rise to multiple transcripts and subsequently protein isoforms by alternative pre-mRNA splicing, the number of isoforms and their potential interaction with the other MEF2 proteins could generate a vast array of heterogeneous MEF2 DNA binding complexes. Since the splicing events occur in the trans-activation domain and some of the splice variants exhibit tissue specificity, there may be differences in transcriptional activation and tissue-specific function of the different heterodimer complexes. A further layer of complexity could also be added by the interaction of other proteins with the alternatively spliced exons, since some of these exons do contain predicted secondary structure that may mediate protein-protein interactions (39). The exact number and the extent of heterodimer formation between the different MEF2 proteins in vivo is not currently known, but the capacity for fine tuning the transcriptional activation of target genes containing this cis element is enormous. In this study, we provide evidence that there is a difference in MEF2 dimer formation between two different cell types and that this heterogeneous dimer formation is correlated with either transcriptional activation or quiescence. This is the first observation of differential formation of MEF2 dimer combinations in vivo.

**MEF2D Expression, in Vivo, Is Correlated with Abrogated Transcriptional Activation through the MEF2 cis Element**—In our studies, the presence of MEF2D in the MEF2 DNA binding complex is associated with a lack of transcriptional activation by the complex. Moreover, we have also found that overexpressed MEF2D is a very weak transcriptional activator compared to the other MEF2 proteins. Consideration of the temporal pattern of expression of the MEF2 proteins during myogenesis further supports the notion of the trans-activation quiescence of MEF2D. MEF2D is expressed first in myoblasts, but it does not activate muscle target genes until the myogenic program is initiated (34). The mechanism that represses MEF2D in myoblasts remains to be determined. Immediately after serum withdrawal and the induction of myogenes, MEF2A (5) accumulates, followed several days later by MEF2C (39). Our explanation of this pattern of expression and its functional consequences based on our current data is that it is the accumulation of MEF2A and the subsequent displacement of MEF2D from the target sites that activate the transcription of muscle-specific genes during the myogenic cascade. If there is a negatively acting factor that interacts with MEF2D, then it is likely that it could be induced by serum and down-regulated upon differentiation. If there is an important role played by the relative levels and thresholds for transcriptional activation by the different MEF2 factors, it is plain to see why overexpression studies have not elucidated this level of regulation.

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3 O. Ornatsky and J. McDermott, unpublished observation.
tively (35, 47, 48). The MRF4 gene also contains overlapping MEF2/TATA binding sites, as does the myoglobin gene (8, 22, 49), and MEF2 protein can interact with this sequence (8, 48). It is, therefore, possible that MEF2 proteins might play a role at certain promoters independent of its more “traditional” role as an enhancer binding protein. This is a possibility since it has been known for some time that the structure and composition of proteins assembled at a “basal” promoter can differ (50).

In summary, two models are proposed for MEF2 regulation in muscle and non-muscle cells. In the first (Fig. 7), which we favor, MEF2D-containing complexes occupy the MEF2 sites in many cell types, and the trans-activation function of this binding complex is inactive. Activation through the MEF2 cis element is, therefore, dependent on the displacement of this binding activity by other more potent MEF2 dimer combinations, such as the MEF2A homodimer, which is prevalent and active in differentiating muscle cells. This model implies that the relative levels of the MEF2 proteins and their respective dimerization partners may be critical in reaching threshold concentrations for activation of target genes. The results presented here are consistent with this hypothesis.

A second more remote possibility is that MEF2D is a target for a negatively acting accessory factor present in non-muscle cells, which by protein-protein interaction can inhibit trans-activation by the complex. The displacement or inactivation of this negative interactor would then allow the existing MEF2 factors to activate transcription of target genes. It is possible that a putative negative factor could be inactivated or displaced by a signaling pathway since serum stimulation has been reported to activate MEF2D transcriptional activation. However, the lack of transcriptional activation by MEF2D in proliferating myoblasts in the presence of serum argues against this possibility. Thus, based on our results, we propose a model for activation of MEF2 target genes, in which transcriptional activation is dependent on the threshold levels of a specific MEF2 dimer complex and the displacement of a functionally inactive complex.

Acknowledgments—We thank Ron Prywes for kindly providing anti-MEF2A, MEF2B, and MEF2D antibodies and the c-Jun binding site probe. We also thank Andre Bedard for helpful advice during the course of these studies and for suggestions on the manuscript.

Addendum—While this manuscript was in preparation, Dodou et al. (Dodou, E., Sparrow, D. B., Mohun, T., and Treisman, R. (1995) Nucleic Acids Res. 23, 4267–4274) reported that non-muscle MEF2 complexes contain MEF2A and another unidentified MEF2 protein. They also reported the nuclear localized expression of MEF2A in non-muscle cells, including HeLa cells.