Molecular Neurobiology

ISSN 0893-7648/04/29(2): 155–165/$25.00

Myocyte Enhancer Factor-2 Transcription Factors in Neuronal Differentiation and Survival

Kim A. Heidenreich* and Daniel A. Linseman

Department of Pharmacology, University of Colorado Health Sciences Center and the Denver Veterans Affairs Medical Center, Denver, Colorado 80262

Abstract

Myocyte enhancer factor-2 (MEF2) transcription factors regulate genes that control critical cellular processes including proliferation, differentiation, and survival. Although MEF2 proteins were first identified as transcription factors that bound A/T rich DNA sequences and controlled muscle-specific genes during myogenic development, it is now apparent that MEF2 transcription factors are also highly expressed in neurons and are critical determinants of neuronal differentiation and fate. Here we focus our discussion on the role of MEF2 proteins in nervous tissue and the regulation of these transcription factors by calcium and phosphorylation signaling pathways.

Index Entries: Neuron; apoptosis; brain; MADS-box; protein kinases; calcium signaling; muscle; development.

MEF2 Family of Transcription Factors

The myocyte enhancer factor-2 (MEF2) proteins are members of the MADS (MCM1-agamous-deficiens-serum response factor) family of transcription factors *(1,2).* A hallmark of MADS-box proteins is their combinational association with other MADS domain factors and additional heterologous classes of transcriptional regulators *(3).* Vertebrate MEF2 proteins are encoded by four genes (MEF2-A, -B, -C, and -D), each of which gives rise to alternatively spliced transcripts *(1,4–7).* The MEF2 isoforms are expressed in distinct, but overlapping patterns during embryogenesis and in adult tissues. Posttranslational regulation is largely responsible for the functional pattern of expression of the MEF2 proteins *(1).*

MEF2 transcription factors contain a N-terminal 56 amino acid MADS-box domain that

Received 7/1/03; Accepted 10/1/03
* Author to whom all correspondence and reprint requests should be addressed. E-mail: Kim.Heidenreich@UCHSC.edu

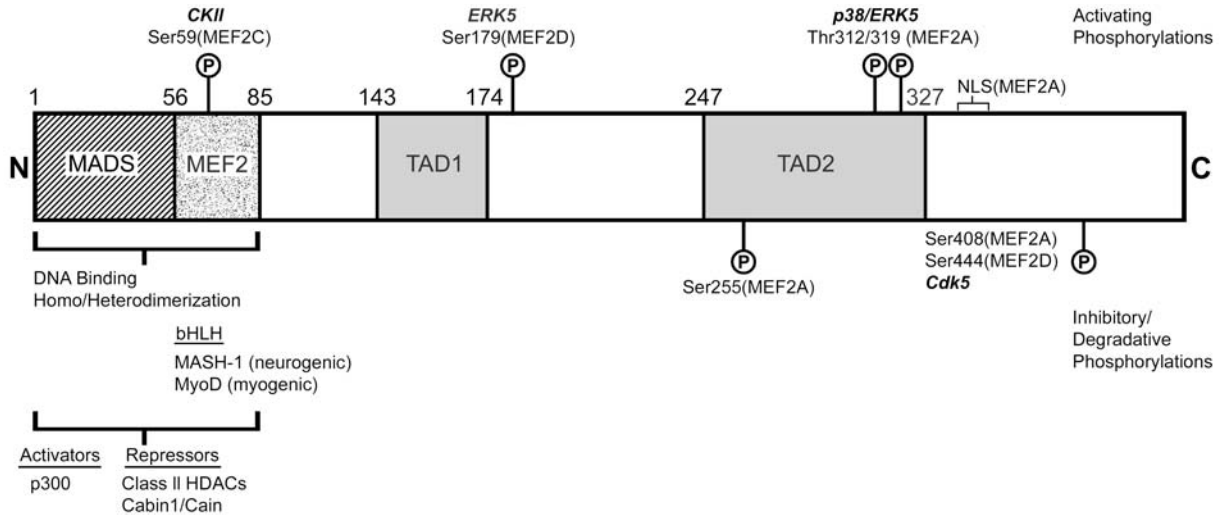

Fig. 1. Structural schematic of a MEF2 transcription factor. MEF2 proteins contain a highly conserved N-terminal MADS box and MEF2 domain. These elements are involved in DNA binding, dimerization with other MEF2 proteins and basic helix-loop-helix (bHLH) transcription factors, and interaction with various coactivators and repressors. The C-terminal region of MEF2 contains the transcriptional activation domains (TADs) as well as a nuclear localization sequence (NLS). MEF2 proteins are substrates for many protein kinases that regulate both DNA binding and transcriptional activity. For example, a conserved casein kinase II (CKII) site lies within the MEF2 domain and its phosphorylation enhances DNA binding. Multiple residues within the C-terminus are substrates for MAP kinase family members, p38 and/or ERK5, and phosphorylation of these sites generally enhances transcriptional activation. In contrast, phosphorylation at the extreme C-terminus by Cdk5 or at Ser255, by an as yet unidentified kinase, leads to inhibition of transcriptional activity and degradation, respectively.

serves as a minimal DNA-binding domain (*see* Fig. 1). The MADS box is a highly conserved structural motif involved in the regulation of homeotic fate, growth, and differentiation in many organisms *(1,8–10).* Adjacent to the MADS box is a 29 amino acid MEF2 domain that mediates high-affinity DNA binding and homo- and heterodimerization with other MEF2 proteins. The vertebrate MEF2 proteins share about 50% amino acid identity overall and about 95% similarity in the highly conserved MADS box and MEF2 domain *(11).* MADS box proteins generally bind A/T rich DNA sequences, whereas MEF2 binds preferentially to the consensus sequence CTA(A/T)$_4$TAG/A *(11).* The MADS box and MEF2 domain are necessary and sufficient for DNA binding but lack transcriptional activity on their own. The C-terminal half of MEF2 proteins contains the transcriptional activation domain, as well as, a number of regulatory domains including a nuclear localization sequence and multiple phosphorylation motifs *(12).* There is relatively low amino acid homology between the C-terminal regions of the various MEF2 isoforms and the C-terminus is subject to complex patterns of alternative splicing *(11).* The structural organization of MEF2 proteins allows them to receive and respond to multiple inputs from various intracellular signaling pathways. In this way, MEF2 activity is profoundly influenced by developmental cues and signals from the extracellular environment.

MEF2 Transcription Factors in Muscle and Immune Cells

The MEF2 family of genes is highly expressed in cells of muscle lineage, where they have been

shown to be important regulators of gene expression during development of skeletal, cardiac, and smooth muscle *(6,7,13).* In these tissues, MEF2 proteins interact with myogenic basic helix-loop-helix (bHLH) transcription factors such as Myo D to activate myogenesis *(14–16).* MEF2 factors are thought to activate transcription by binding to individual E-box or MEF2 consensus sites or via adjacent E-box/MEF2 elements. During myogenesis, MEF2 transcription factors activate the expression of structural genes, as well as maintain and amplify the expression of other transcription factors that initiate muscle differentiation *(17).* Loss-of-function mutations of the mouse MEF2C *(18)* and the Drosophila MEF2 gene *(19,20)* have demonstrated an essential role of MEF2 proteins in myogenesis and morphogenesis of striated and nonstriated muscle cell types. In addition to their critical role in muscle development, MEF2 proteins are involved in adult cardiac hypertrophy *(21–23).*

MEF2 proteins are also present in cells of the immune system where they mediate cell fate decisions. T-cell activation leads to MEF2-mediated transcriptional activation of Nur77, an orphan nuclear steroid receptor that activates apoptosis in these cells *(24).* MEF2 binds directly to regulatory elements in the Nur77 gene and also cooperates with NFAT to drive Nur77 expression *(25).* The increase in MEF2 transcriptional activity on the Nur77 regulatory element occurs in the absence of changes in DNA binding, implicating other regulatory mechanisms such as cofactor binding and/or changes in phosphorylation of the transactivation domain.

MEF2 Transcription Factors in Brain

MEF2 family members are also highly expressed in neurons of the central nervous system *(5,6,26–29).* In vertebrates, each MEF2 isoform shows a unique temporal expression pattern in different regions of the brain. The timing of MEF2 expression in neurons is consistent with a role of MEF2 transcription factors in neuronal differentiation and maintenance. In human brain, MEF2C is preferentially expressed in certain neuronal layers of the postnatal cerebral cortex in a temporal manner declining from postnatal d 2 to adult *(5,6).* These immunocytochemical data suggest that MEF2C regulates the laminar differentiation of central neurons. Of note, is the absence of MEF2C in dividing neurons in the subventricular zones *(6).* An alternative splice variant of MEF2C is found exclusively in the brain and contains a unique SEDVDLLL peptide sequence in the transactivation domain that may serve to mediate tissue-specific protein–protein interactions *(26).*

In the mouse brain where more extensive regions have been examined by *in situ* hybridization, the timing of MEF2 gene expression is also associated with neurons exiting the cell cycle and entering differentiation *(27).* Mef2C mRNA is first detected in the mouse telencephalon at postnatal d 11.5. This region of the brain is one of the first to begin neuronal differentiation. At postnatal d 13.5, Mef2C is expressed in a layer of cells in the intermediate zone of the frontal cortex and in the olfactory bulb. At this time MEF2 transcripts are also localized in different regions of the neural tube. Mef2A is distributed in a gradient with highest levels in the dorsal portion and lowest levels in the ventral portion. Mef2C is expressed only in the dorsal region, whereas, Mef2D is distributed throughout the neural tube. At postnatal d 14.5, Mef2C and Mef2D are detected in frontal cortex, hippocampus, amygdala, midbrain, olfactory bulb, and cerebellum. Mef2B is abundant in frontal cortex, present at lower levels in the hippocampus, midbrain, and amygdala, and absent in cerebellum. At postnatal d 16.5, low levels of Mef2A overlap with high levels of Mef2C in the hippocampus, midbrain, and frontal cortex. Mef2B is also present in both frontal and midbrain cortex. Mef2D is the most widely distributed of the four genes at this time. At birth, the expression pattern of Mef2B and Mef2C overlap in frontal cortex and olfactory bulbs. Mef2B transcripts appear in the cerebellum. In the 2 wk postnatal brain, all four gene transcripts are present in dendate gyrus.

Mef2C, -D, and -A appear in neurons of the horn of Ammon. Mef2C and Mef2D are expressed equally throughout the layers of the frontal cortex. Between 2 and 6 wk after birth, Mef2 transcripts show a striking pattern of differential expression in the mature cerebellum. Mef2A and Mef2D are found predominantly in the granule layer of the cerebellum. Mef2C is expressed primarily in Purkinje neurons and Mef2B is very low or absent. This dynamic pattern of mRNA expression during pre- and postnatal development in the mouse suggests that different MEF2 isoforms may perform unique roles at different stages of neuronal maturation.

To date, there have not been extensive immunocytochemical studies performed to localize the specific MEF2 proteins in the brain partly due to the scarcity of high affinity isoform-specific antibodies. The data thus far indicate that MEF2 protein expression in the brain correlates with mRNA expression *(30).* For example, in the developing mouse cerebellum (postnatal d 7), MEF2A protein expression occurs primarily in the internal granule layer where the final differentiation and maturation of granule neurons occurs. Levels of MEF2A are very low in the external granule layer where granule neuron precursors are still dividing. This pattern of expression matches that of mRNA localization *(27,31).* In the developing rat cerebral cortex, MEF2C protein expression is detectable at embryonic d 17 and peaks around E21 *(29).* MEF2C is expressed in the cortical plate but not in the ventricular zone where dividing neuronal precursors reside. Cortical cells expressing MEF2C also express β-tubulin type III, but not glial fibrillary acidic protein, indicating that MEF2C expression is restricted to neurons. Again, the pattern of expression of MEF2C protein in cerebral cortex correlates with the pattern of expression of Mef2C mRNA. The immunocytochemistry data taken together with the *in situ* hybridization studies indicate that MEF2 transcription factors are primarily expressed in differentiating neurons but not in dividing neuronal precursors. Distinct MEF2 isoforms may mediate similar functions in different populations of neurons, but because of their structural variations they might be regulated in cell type-specific and isoform-specific manners.

In Vitro Studies of Neuronal MEF2

Studies of primary neuronal cultures support the hypothesis that MEF2 transcription factors regulate neuronal survival and differentiation. In cultures of cerebral cortical neurons where proliferating precursor cells and postmitotic differentiating neurons can be distinguished, MEF2C is selectively expressed in newly generated postmitotic neurons and is not detectable in BrdU-positive precursor cells *(31).* Blocking the function of MEF2C in the postmitotic cortical neurons, by introducing a dominant-interfering form of MEF2, results in apoptotic cell death. Similar results have been obtained in cultures of cerebellar granule neurons where expression of a dominant-interfering form of MEF2 reduces the survival of granule neurons grown in the presence of depolarizing potassium *(32).* Conversely, expression of a constitutively active form of MEF2 promotes the survival of granule neurons grown in the absence of depolarizing potassium, conditions that normally trigger apoptosis *(32).* Additional evidence that MEF2 regulates activity-dependent survival of granule neurons comes from gene silencing experiments demonstrating that RNA interference (RNAi) of MEF2A in primary cerebellar cultures markedly decreases the survival of granule neurons *(33).* The loss of MEF2 activity also contributes to the death of cortical neurons subjected to excitotoxic stress *(34).* Exposure of primary cortical neurons to excitotoxic concentrations of NMDA leads to caspase-mediated cleavage of MEF2 that yields dominant-interfering foms of MEF2 *(34).* Caspase-mediated cleavage of MEF2A and MEF2D also contributes to the death of granule neurons when deprived of trophic support *(32).*

In P19 neuronal precursor cells, expression of MEF2C induces a mixed neuronal/myogenic phenotype *(35).* During retinoic acid-induced

neurogenesis of these cells, a dominant-negative form of MEF2C enhances apoptosis but does not affect cell division. On the other hand, P19 cells induced to undergo apoptosis can be rescued from cell death by expression of constitutively-active MEF2C. In addition, overexpression of MEF2C in P19 cells results in induction of neurofilament protein, the nuclear antigen NeuN, and MASH-1, a neural-specific bHLH transcription factor known to interact with MEF2s *(36).* These data suggest that MEF2 proteins regulate neuronal development not only by promoting survival but by also inducing differentiation.

Other examples of a differentiation role of MEF2 during neuronal development include MEF2C activation of the NMDA receptor subunit 1 (NR1) gene in cerebral cortical cultures *(37)* and the role of the MEF2B/2C heterodimer to mediate adhesion related kinase repression of the GnRH gene in GnRH neuronal cells *(38).* In the former case, MEF2C interacts with Sp1 proteins to activate transcription. This mechanism of transcriptional activation represents a nonclassical mode of gene regulation by MEF2 proteins that may not involve bHLH binding partners. In the case of the GnRH promoter, the putative homeodomain partner interacting with MEF2B/2C heterodimer has not yet been identified.

Activation of MEF2 Proteins by Protein Kinases

Direct phosphorylation of MEF2 proteins plays an important role in the regulation of MEF2 function. A casein-kinase II (CKII) phosphorylation site is conserved in the MEF2 domain of all MEF2 proteins. Phosphorylation of this site by CKII enhances DNA binding of MEF2 *(39).* This site appears to be constitutively phosphorylated in vivo with no evidence for its regulation. It is likely that phosphorylation by CKII induces a conformational change that enhances the ability of the MADS box and MEF2 domain to contact DNA.

Phosphorylation of the transactivation domain of MEF2 transcription factors has also been shown to increase MEF2 activity. At least two isoforms of p38 MAP kinase (p38α and p38β) activate MEF2A and MEF2C by phosphorylating residues located in their activation domains *(40,41).* In the case of MEF2A, p38-mediated phosphorylation of T312 and T319 within the transactivation domain is required for transcriptional activation in muscle cells *(42).* Additional serine residues including S355, S453, and S479 are phosphorylated in vitro by p38 but their relevance to transcriptional activation is questionable *(40,41,43).* Activation of MEF2C by p38 seems more complex than for MEF2A and is subject to cell-type specific regulation. Three prominent residues (T293, T300, and S387) in the activation domain of MEF2C are phosphorylated by p38 in vitro *(41).* Phosphorylation of these residues has been shown to be important for MEF2C activation by p38 in T lymphocytes *(44).* However, only phosphorylation of T293 is induced in differentiating myocytes and is required for MEF2C activation by p38 during muscle differentiation *(45).* Introduction of dominant-interfering mutants of p38 in primary neurons and differentiating P19 neurons has been shown to reduce MEF2-dependent transcription and induce apoptosis *(31,35),* suggesting that p38 mediates the activation of MEF2 in neurons. The isoforms of MEF2 that are phosphorylated by p38 in neurons and the sites that are critical for enhanced transactivation are not yet understood. Moreover, whether activation of Mef2 proteins by p38 is a common mechanism shared by diverse types of neurons is unclear.

In addition to p38 MAP kinase, the serine/threonine kinase ERK5 is capable of phosphorylating the transactivation domains of MEF2A, -C, and -D resulting in increased transcriptional activity *(46–49).* Interestingly, ERK5 has also been shown to possess a transactivation domain and may stimulate MEF2-dependent transcription by functioning as a coactivator that facilitates recruitment of the basal transcription machinery *(50).* In T lymphocytes,

ERK5-MEF2 interactions are stimulated by increases in intracellular calcium and inhibited by Cabin *(50).* Recent studies indicate that ERK5 activation of MEF2 plays an important role in BDNF-mediated survival of primary cortical neurons *(51).* Similarly, the temporal survival effects of BDNF in cerebellar granule neurons appear to be mediated by an ERK5/MEF2 signaling pathway that induces transcription of neurotrophin-3 *(52).*

Inactivation of Neuronal MEF2 Proteins by Phosphorylation and Regulation by Calcium Signaling

MEF2 transcription factors in muscle cells are sensitive to calcium signals that act through multiple mechanisms to modulate transcriptional activity *(12).* Recent work suggests that MEF2 activity in neurons is similarly regulated by intracellular calcium. Calcineurin is a serine/threonine phosphatase that is activated by the binding of calcium and calmodulin. Recent studies have shown that calcineurin promotes the DNA binding and activity of MEF2 transcription factors in neurons that depend on depolarization-mediated calcium influx for their survival *(29,31,32).* Cultured cerebellar granule neurons survive in the presence of high intracellular calcium, a condition that mimics activity-dependent neuronal survival during development. The neurons are cultured in elevated (25 m*M*) potassium that promotes an influx of calcium by opening L-type voltage sensitive calcium channels resulting in persistent activation of calcineurin. Removal of depolarization induces hyperphosphorylation of MEF2D and MEF2A on serine/threonine residues, resulting in decreased DNA binding and susceptibility to caspase cleavage *(29,32).* The subsequent loss of MEF2 dependent gene transcription leads to apoptosis of granule neurons (*see* Fig. 2). The hyperphosphorylation of MEF2 is mimicked by the addition of calcineurin inhibitors to depolarizing medium, indicating that calcineurin maintains MEF2 in a hypophosphorylated state that has enhanced DNA binding and transcriptional activity *(29,32).* The kinase(s) responsible for phosphorylating the sites that calcineurin keeps dephosphorylated in healthy neurons is unknown. Recent findings, however, indicate that a MEF2 kinase activated during neuronal apoptosis is lithium-sensitive but is distinct from glycogen synthase kinase-3 (GSK-3) *(53).* Additional studies, discussed in the following section, have shown that Cdk5 phosphorylates and inactivates MEF2 in cerebral cortical neurons undergoing apoptosis *(54).* Whether or not Cdk5 regulates MEF2 phosphorylation in granule neurons during removal of depolarization signals remains to be determined.

In primary cortical neurons, phosphorylation of MEF2 by Cdk5 results in inhibition of MEF2 transcriptional activity *(54).* The Cdk5 phosphorylation site is conserved in MEF2A, -C, and -D and lies within the transactivation domain of MEF2. Oxidative stress and excitotoxic concentrations of glutamate increase nuclear Cdk5 activity leading to Cdk5-dependent phosphorylation of MEF2 and inhibition of function. Moreover, MEF2 mutants that are resistant to Cdk5 phosphorylation rescue neurons from neurotoxin-induced apoptosis. In light of accumulating evidence that Cdk5 activity is dysregulated in a number of neurodegenerative disorders including, Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease *(55–58),* these data raise the possibility that decreased function of the survival-promoting transcription factor MEF2 contributes to the neuronal loss observed in neurodegenerative diseases.

The mechanism by which phosphorylation by Cdk5 inhibits MEF2 function is not understood. Phosphorylation by Cdk5 may target MEF2 for cleavage and/or degradation. Whether or not Cdk5 phosphorylation of MEF2 is related to the caspase cleavage of MEF2 proteins that occurs during neuronal apoptosis remains to be investigated. Another possibility is that phosphorylation changes the conformation of MEF2 which in turn alters its

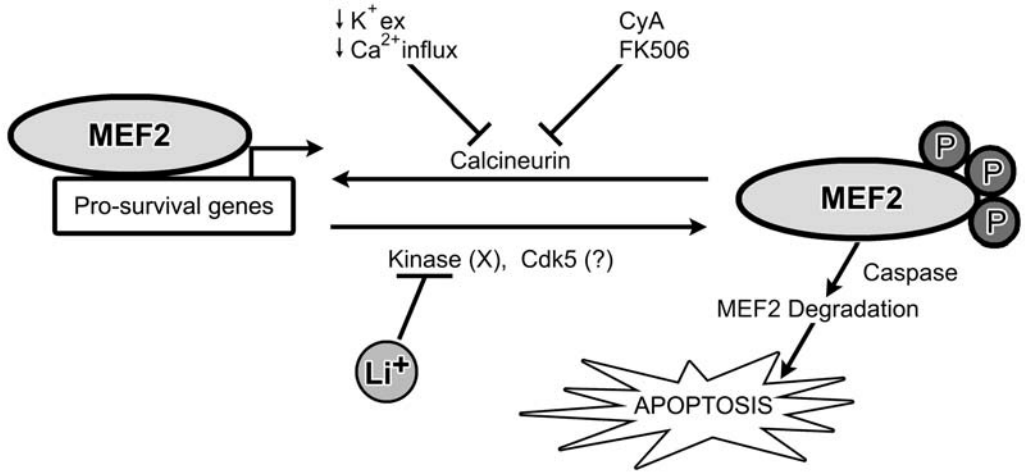

Fig. 2. Regulation of neuronal MEF2 function by calcium signaling. Primary cultures of cerebellar granule neurons require depolarization-stimulated MEF2 activity for their survival. Removal of extracellular depolarizing potassium leads to a decrease in calcium influx and subsequent inactivation of calcineurin phosphatase activity. The loss of calcineurin activity acts in a coordinated manner with activation of a pro-apoptotic MEF2 kinase (X), resulting in a marked hyperphosphorylation of MEF2 proteins. Hyperphosphorylation of MEF2 decreases its DNA binding and enhances its susceptibility to caspase-mediated degradation. The loss of MEF2-dependent transcription of pro-survival genes contributes to granule neuron apoptosis. The hyperphosphorylation of MEF2 can be partially mimicked under depolarizing conditions by addition of calcineurin inhibitors (cyclosporin A and FK506), demonstrating a key role for calcineurin in maintaining MEF2 proteins in a hypophosphorylated (DNA-bound) state in healthy neurons. Neither the sites of hyperphosphorylation induced on MEF2 during neuronal apoptosis, nor the kinase responsible, are currently known. However, the MEF2 kinase (X) is a lithium-sensitive kinase distinct from the known lithium target, glycogen synthase kinase-3. Whether or not Cdk5, a pro-apoptotic kinase that phosphorylates and inactivates MEF2 in cortical neurons, plays a role in the hyperphosphorylation of MEF2 in cerebellar granule neurons remains to be investigated.

interaction with cofactors or the basal transcription machinery.

Indirect Mechanisms of MEF2 Regulation by Protein Kinases

In addition to the direct phosphorylation of MEF2 proteins that regulate their activity, there are also indirect mechanisms by which protein kinases influence MEF2 function. In particular, serine phosphorylation of two repressors of MEF2 activity, class II histone deacetylases (HDACs) and MEF2-interacting transcription repressor (MITR), leads to their nuclear export and association with cytoplasmic scaffolding proteins of the 14-3-3 family *(59–62)*. In this manner, a serine kinase acts indirectly to derepress MEF2 activity. In all of the studies cited above, overexpression of active calcium/calmodulin-dependent kinases (CaMKs) I and IV was utilized to stimulate phosphorylation of the HDACs in muscle cells and nonmuscle cells (e.g., COS or NIH3T3 fibroblasts). However, the identity of the endogenous kinase responsible for phosphorylation of HDACs in muscle is currently unknown. We have recently shown that class II HDACs (HDAC4 and HDAC5) are excluded from the nuclei of cerebellar granule neurons cultured in depolarizing medium *(63)*. Either removal of the depolarization stimulus or addition of CaMK

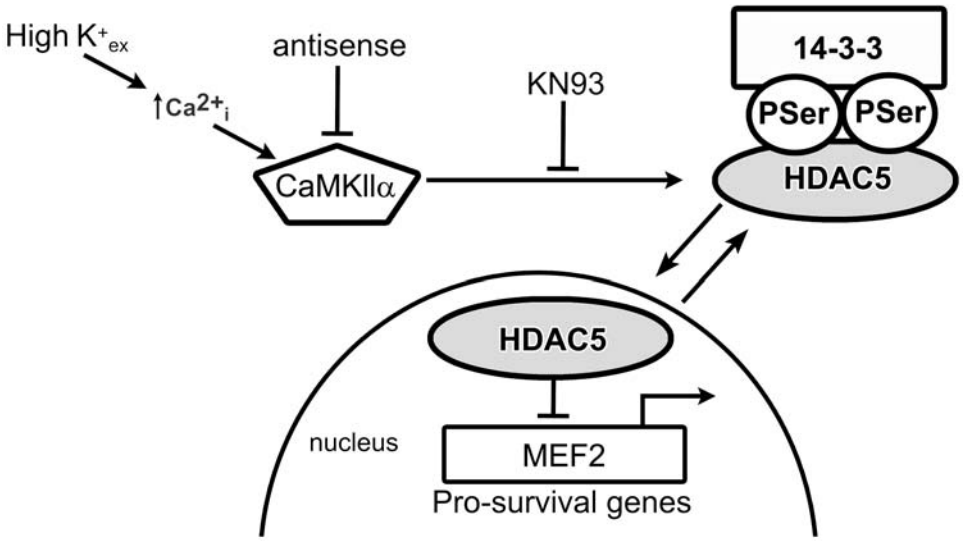

Fig. 3. Derepression of neuronal MEF2 activity by CaMKII-mediated phosphorylation of HDAC. In cerebellar granule neurons cultured in depolarizing extracellular potassium, the MEF2 repressor histone deacetylase-5 (HDAC5), is excluded from the nucleus. Under these conditions it is retained in the cytoplasm in a phosphorylated state, likely associated with scaffolding proteins of the 14-3-3 family. Removal of the depolarization stimulus induces a rapid translocation of HDAC5 into the nucleus where it interacts with MEF2 to repress its transcriptional activity. The nuclear translocation of HDAC5 can also be stimulated under depolarizing conditions either by the addition of a calcium/calmodulin-dependent kinase (CaMK) inhibitor, KN93, or by the antisense-mediated depletion of CaMKIIα. These data suggest a mechanism by which depolarization-mediated calcium influx acts through CaMKII to induce phosphorylation and nuclear export of HDAC5, resulting in derepression of MEF2 activity in healthy neurons.

inhibitors (KN93 or KN62) stimulates a rapid translocation of HDACs into the nucleus, resulting in repression of MEF2 activity and induction of cerebellar granule neuron apoptosis. Furthermore, HDAC nuclear translocation and apoptosis are induced by antisense-mediated depletion of CaMKIIα, a neuronal specific CaMK isoform. These results are the first to identify an endogenous kinase capable of regulating HDAC localization and suggest a mechanism by which depolarization-mediated calcium influx acts through CaMKII to phosphorylate HDAC, promoting its nuclear export and derepression of MEF2 activity (*see* Fig. 3). In further support of this mechanism in neurons, Chawla et al. *(64)* recently showed that CaMK inhibitors similarly attenuate HDAC nuclear export in hippocampal neuronal cultures. Thus, CaMK-mediated inactivation of HDACs may act in a cooperative manner with kinases that directly phosphorylate and activate MEF2 proteins to regulate this important transcription factor in neurons.

Summary and Future Directions

The MEF2 family of transcription factors act as effectors of diverse signaling pathways that regulate fundamental cellular processes. It seems paradoxical that MEF2 could control such diverse and opposing functions as proliferation, differentiation, and apoptosis. A likely explanation lies in the identity of tissue-specific DNA-binding partners and coactivators, as well as, cell-type and isoform-specific regulation by

a variety of protein kinases. The identities of the MEF2 target genes that mediate many of the cellular responses, particularly in neurons, remain to be determined. Future studies using conditional gene inactivation to assess the in vivo functions of specific MEF2 isoforms in neuronal development will further our understanding of MEF2 function in the nervous system.

Acknowledgments

This work was supported by a Department of Veterans Affairs Merit Award (Kim A. Heidenreich), a Department of Defense Grant DAMD17-99-1-9481 (K.A.H.), an NIH Grant NS38619-01A1 (Kim A. Heidenreich), and a Department of Veterans Affairs Research Enhancement Award Program (Kim A. Heidenreich and Daniel A. Linseman).

References

1 Yu Y.T., Breitbart R.E., Smoot L.B., Lee Y., Mahdavi V., Nadal-Ginard B. (1992) Human myocyte-specific enhancer factor 2 comprises a group of tissue-restricted MADS box transcription factors. *Genes Dev.* **6,** 1783–1798.

2 Naya F.S., Olson E. (1999) MEF2: a transcriptional target for signaling pathways controlling skeletal muscle growth and differentiation. *Curr. Opin. Cell Biol.* **11,** 683–688.

3 Shore P., Sharrocks A.D. (1995) The MADS-box family of transcription factors. *Eur. J. Biochem.* **229,** 1–13.

4 Breitbart R.E., Liang C.S., Smoot L.B., Laheru D.A., Mahdavi V., Nadal-Ginard B. (1993) A fourth human MEF2 transcription factor, hMEF2D, is an early marker of the myogenic lineage. *Development* **118,** 1095–1106.

5 Leifer D., Krainc D., Yu Y.T., et al. (1993) MEF2C, a MADS/MEF2-family transcription factor expressed in a laminar distribution in cerebral cortex. *Proc. Natl. Acad. Sci. USA* **90,** 1546–1550.

6 McDermott J.C., Cardoso M.C., Yu Y.T., et al. (1993) hMEF2C gene encodes skeletal muscle- and brain-specific transcription factors. *Mol. Cell. Biol.* **13,** 2564–2577.

7 Martin J.F., Miano J.M., Hustad C.M., Copeland N.G., Jenkins N.A., Olson E.N. (1994) A Mef2 gene that generates a muscle-specific isoform via alternative mRNA splicing. *Mol. Cell. Biol.* **14,** 1647–1656.

8 Moore S., Vrebalov J., Payton P., Giovannoni J. (2002) Use of genomics tools to isolate key ripening genes and analyse fruit maturation in tomato. *J. Exp. Bot.* **53,** 2023–2030.

9 Ng M., Yanofsky M.F. (2001) Function and evolution of the plant MADS-box gene family. *Nat. Rev. Genet.* **2,** 186–195.

10 Yun K., Wold B. (1996) Skeletal muscle determination and differentiation: story of a core regulatory network and its context. *Curr. Opin. Cell Biol.* **8,** 877–889.

11 Black B.L., Olson E.N. (1998) Transcriptional control of muscle development by myocyte enhancer factor-2 (MEF2) proteins. *Annu. Rev. Cell. Dev. Biol.* **14,** 167–196.

12 McKinsey T.A., Zhang C.L., Olson E.N. (2002) MEF2: a calcium-dependent regulator of cell division, differentiation and death. *Trends Biochem. Sci.* **27,** 40–47.

13 Molkentin J.D., Firulli A.B., Black B.L., et al. (1996) MEF2B is a potent transactivator expressed in early myogenic lineages. *Mol. Cell. Biol.* **16,** 3814–3824.

14 Molkentin J.D., Olson E.N. (1996) Combinatorial control of muscle development by basic helix-loop-helix and MADS-box transcription factors. *Proc. Natl. Acad. Sci. USA* **93,** 9366–9373.

15 Ornatsky O.I., McDermott J.C. (1996) MEF2 protein expression, DNA binding specificity and complex composition, and transcriptional activity in muscle and non-muscle cells. *J. Biol. Chem.* **271,** 24927–24933.

16 Puri P.L., Sartorelli V. (2000) Regulation of muscle regulatory factors by DNA-binding, interacting proteins, and post-transcriptional modifications. *J. Cell. Physiol.* **185,** 155–173.

17 Puri P.L., Wu Z., Zhang P., et al. (2000) Induction of terminal differentiation by constitutive activation of p38 MAP kinase in human rhabdomyosarcoma cells. *Genes Dev.* **14,** 574–584.

18 Lin Q., Schwarz J., Bucana C., Olson E.N. (1997) Control of mouse cardiac morphogenesis and myogenesis by transcription factor MEF2C. *Science* **276,** 1404–1407.

19 Bour B.A., O'Brien M.A., Lockwood W.L., et al. (1995) Drosophila MEF2, a transcription factor that is essential for myogenesis. *Genes Dev.* **9,** 730–741.

20 Prokop A., Landgraf M., Rushton E., Broadie K., Bate M. (1996) Presynaptic development at the Drosophila neuromuscular junction: assembly and localization of presynaptic active zones. *Neuron* **17,** 617–626.
21 Kolodziejczyk S.M., Wang L., Balazsi K., DeRepentigny Y., Kothary R., Megeney L.A. (1999) MEF2 is upregulated during cardiac hypertrophy and is required for normal post-natal growth of the myocardium. *Curr. Biol.* **9,** 1203–1206.
22 Passier R., Zeng H., Frey N., et al. (2000) CaM kinase signaling induces cardiac hypertrophy and activates the MEF2 transcription factor in vivo. *J. Clin. Invest.* **105,** 1395–1406.
23 Zhang C.L., McKinsey T.A., Chang S., Antos C.L., Hill J.A., Olson E.N. (2002) Class II histone deacetylases act as signal-responsive repressors of cardiac hypertrophy. *Cell* **110,** 479–488.
24 Youn H.D., Sun L., Prywes R., Liu J.O. (1999) Apoptosis of T cells mediated by Ca2+-induced release of the transcription factor MEF2. *Science* **286,** 790–793.
25 Youn H.D., Chatila T.A., Liu J.O. (2000) Integration of calcineurin and MEF2 signals by the coactivator p300 during T-cell apoptosis. *Embo J.* **19,** 4323–4331.
26 Leifer D., Golden J., Kowall N.W. (1994) Myocyte-specific enhancer binding factor 2C expression in human brain development. *Neuroscience* **63,** 1067–1079.
27 Lyons G.E., Micales B.K., Schwarz J., Martin J.F., Olson E.N. (1995) Expression of mef2 genes in the mouse central nervous system suggests a role in neuronal maturation. *J. Neurosci.* **15,** 5727–5738.
28 Ikeshima H., Imai S., Shimoda K., Hata J., Takano T. (1995) Expression of a MADS box gene, MEF2D, in neurons of the mouse central nervous system: implication of its binary function in myogenic and neurogenic cell lineages. *Neurosci. Lett.* **200,** 117–120.
29 Mao Z., Wiedmann M. (1999) Calcineurin enhances MEF2 DNA binding activity in calcium-dependent survival of cerebellar granule neurons. *J. Biol. Chem.* **274,** 31102–31107.
30 Lin X., Shah S., Bulleit R.F. (1996) The expression of MEF2 genes is implicated in CNS neuronal differentiation. *Brain Res. Mol. Brain Res.* **42,** 307–316.
31 Mao Z., Bonni A., Xia F., Nadal-Vicens M., Greenberg M.E. (1999) Neuronal activity-dependent cell survival mediated by transcription factor MEF2. *Science* **286,** 785–790.
32 Li M., Linseman D.A., Allen M.P., et al. (2001) Myocyte enhancer factor 2A and 2D undergo phosphorylation and caspase-mediated degradation during apoptosis of rat cerebellar granule neurons. *J. Neurosci.* **21,** 6544–6552.
33 Gaudilliere B., Shi Y., Bonni A. (2002) RNA interference reveals a requirement for myocyte enhancer factor 2A in activity-dependent neuronal survival. *J. Biol. Chem.* **277,** 46442–46446.
34 Okamoto S., Li Z., Ju C., Scholzke M.N., Mathews E., et al. (2002) Dominant-interfering forms of MEF2 generated by caspase cleavage contribute to NMDA-induced neuronal apoptosis. *Proc. Natl. Acad. Sci. USA* **99,** 3974–3979.
35 Okamoto S., Krainc D., Sherman K., Lipton S.A. (2000) Antiapoptotic role of the p38 mitogen-activated protein kinase-myocyte enhancer factor 2 transcription factor pathway during neuronal differentiation. *Proc. Natl. Acad. Sci. USA* **97,** 7561–7566.
36 Skerjanc I.S., Wilton S. (2000) Myocyte enhancer factor 2C upregulates MASH-1 expression and induces neurogenesis in P19 cells. *FEBS Lett.* **472,** 53–56.
37 Krainc D., Bai G., Okamoto S., et al. (1998) Synergistic activation of the N-methyl-D-aspartate receptor subunit 1 promoter by myocyte enhancer factor 2C and Sp1. *J. Biol. Chem.* **273,** 26218–26224.
38 Allen M.P., Xu M., Linseman D.A., et al. (2002) Adhesion-related kinase repression of gonadotropin-releasing hormone gene expression requires Rac activation of the extracellular signal-regulated kinase pathway. *J. Biol. Chem.* **277,** 38133–38140.
39 Molkentin J.D., Li L., Olson E.N. (1996) Phosphorylation of the MADS-Box transcription factor MEF2C enhances its DNA binding activity. *J. Biol. Chem.* **271,** 17199–17204.
40 Yang S.H., Galanis A., Sharrocks A.D. (1999) Targeting of p38 mitogen-activated protein kinases to MEF2 transcription factors. *Mol. Cell. Biol.* **19,** 4028–4038.
41 Zhao M., New L., Kravchenko V.V., et al. (1999) Regulation of the MEF2 family of transcription factors by p38. *Mol. Cell. Biol.* **19,** 21–30.
42 Wu Z., Woodring P.J., Bhakta K.S., et al. (2000) p38 and extracellular signal-regulated kinases regulate the myogenic program at multiple steps. *Mol. Cell. Biol.* **20,** 3951–3964.
43 Ornatsky O.I., Cox D.M., Tangirala P., et al. (1999) Post-translational control of the MEF2A transcriptional regulatory protein. *Nucleic Acids Res.* **27,** 2646–2654.

44 Han J., Jiang Y., Li Z., Kravchenko V.V., Ulevitch R.J. (1997) Activation of the transcription factor MEF2C by the MAP kinase p38 in inflammation. *Nature* **386,** 296–299.
45 Wu H., Naya F.J., McKinsey T.A., et al. (2000) MEF2 responds to multiple calcium-regulated signals in the control of skeletal muscle fiber type. *Embo J.* **19,** 1963–1973.
46 Kato Y., Kravchenko V.V., Tapping R.I., Han J., Ulevitch R.J., Lee J.D. (1997) BMK1/ERK5 regulates serum-induced early gene expression through transcription factor MEF2C. *Embo J.* **16,** 7054–7066.
47 Yang C.C., Ornatsky O.I., McDermott J.C., Cruz T.F., Prody C.A. (1998) Interaction of myocyte enhancer factor 2 (MEF2) with a mitogen-activated protein kinase, ERK5/BMK1. *Nucleic Acids Res.* **26,** 4771–4777.
48 Kato Y., Zhao M., Morikawa A., et al. (2000) Big mitogen-activated kinase regulates multiple members of the MEF2 protein family. *J. Biol. Chem.* **275,** 18534–18540.
49 Marinissen M.J., Chiariello M., Pallante M., Gutkind J.S. (1999) A network of mitogen-activated protein kinases links G protein-coupled receptors to the c-jun promoter: a role for c-Jun NH2-terminal kinase, p38s, and extracellular signal-regulated kinase 5. *Mol. Cell. Biol.* **19,** 4289–4301.
50 Kasler H.G., Victoria J., Duramad O., Winoto A. (2000) ERK5 is a novel type of mitogen-activated protein kinase containing a transcriptional activation domain. *Mol. Cell. Biol.* **20,** 8382–8389.
51 Liu L., Cavanaugh J.E., Wang Y., Sakagami H., Mao Z., Xia Z. (2003) ERK5 activation of MEF2-mediated gene expression plays a critical role in BDNF-promoted survival of developing but not mature cortical neurons. *Proc. Natl. Acad. Sci. USA* **100,** 8532–8537.
52 Shalizi A., Lehtinen M., Gaudilliere B., et al. (2003) Characterization of a neurotrophin signaling mechanism that mediates neuron survival in a temporally specific pattern. *J. Neurosci.* **23,** 7326–7336.
53 Linseman D.A., Cornejo B.J., Le S.S., et al. (2003) A myocyte enhancer factor 2D (MEF2D) kinase activated during neuronal apoptosis is a novel target inhibited by lithium. *J. Neurochem.* **85,** 1488–1499.
54 Gong X., X. T, Wiedmann M, Wang X, et al. (2003) Cdk-5 mediated inhibition of the protective effects of transcription factor MEF2 in neurotoxicity-induced apoptosis. *Neuron* **38,** 33–46.
55 Grant P., Sharma P., Pant H.C. (2001) Cyclin-dependent protein kinase 5 (Cdk5) and the regulation of neurofilament metabolism. *Eur. J. Biochem.* **268,** 1534–1546.
56 Bajaj N.P. (2000) Cyclin-dependent kinase-5 (CDK5) and amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord.* **1,** 319–327.
57 Maccioni R.B., Otth C., Concha I.I., Munoz J.P. (2001) The protein kinase Cdk5. Structural aspects, roles in neurogenesis and involvement in Alzheimer's pathology. *Eur. J. Biochem.* **268,** 1518–1527.
58 Takahashi M., Iseki E., Kosaka K. (2000) Cyclin-dependent kinase 5 (Cdk5) associated with Lewy bodies in diffuse Lewy body disease. *Brain Res.* **862,** 253–256.
59 Lu J., McKinsey T.A., Zhang C.L., Olson E.N. (2000) Regulation of skeletal myogenesis by association of the MEF2 transcription factor with class II histone deacetylases. *Mol. Cell* **6,** 233–244.
60 McKinsey T.A., Zhang C.L., Lu J., Olson E.N. (2000) Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. *Nature* **408,** 106–111.
61 Kao H.Y., Verdel A., Tsai C.C., Simon C., Juguilon H., Khochbin S. (2001) Mechanism for nucleocytoplasmic shuttling of histone deacetylase 7. *J. Biol. Chem.* **276,** 47496–47507.
62 Zhang C.L., McKinsey T.A., Olson E.N. (2001) The transcriptional corepressor MITR is a signal-responsive inhibitor of myogenesis. *Proc. Natl. Acad. Sci. USA* **98,** 7354–7359.
63 Linseman P.A., Bartley C.M., Le S.S., et al. (2003). Inactivation of the myocyte enhancer factor-2 repressor histone deacetylase-5 by endogenous Ca(2+)/calmodulin-dependent kinase II promotes depolarization-mediated cerebellar granule neuron survival. *J. Biol. Chem.* **278,** 41472–41481.
64. Chawla S., Vanhoutte P., Arnold F.J., Huang C.L., Bading H. (2003) Neuronal activity-dependent nucleocytoplasmic shuttling of HDAC4 and HDAC5. *J. Neurochem.* **85,** 151–159.