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MEF2: a central regulator of diverse developmental programs

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The myocyte enhancer factor 2 (MEF2) transcription factor acts as a lynchpin in the transcriptional circuits that control cell differentiation and organogenesis. The spectrum of genes activated by MEF2 in different cell types depends on extracellular signaling and on co-factor interactions that modulate MEF2 activity. Recent studies have revealed MEF2 to form an intimate partnership with class IIa histone deacetylases, which together function as a point of convergence of multiple epigenetic regulatory mechanisms. We review the myriad roles of MEF2 in development and the mechanisms through which it couples developmental, physiological and pathological signals with programs of cell-specific transcription.

Introduction

The formation of specialized cell types and their integration into different tissues and organs during development requires the interpretation of extracellular signals by components of the transcriptional apparatus and through the subsequent activation of cascades of regulatory and structural genes by combinations of widely expressed and cell type-restricted transcription factors. The myocyte enhancer factor 2 (MEF2) transcription factor plays central roles in the transmission of extracellular signals to the genome and in the activation of the genetic programs that control cell differentiation, proliferation, morphogenesis, survival and apoptosis of a wide range of cell types.

Recent studies in mice and fruit flies have revealed upstream signaling systems that control MEF2 expression and activity, and downstream effector genes that mediate the actions of MEF2 throughout development, as well as in adult tissues. These studies point to MEF2 having a central role as a mediator of epigenetic regulatory mechanisms that involve changes in chromatin configurations and the modulation of microRNAs. Here we review the mechanisms that govern MEF2 activity and discuss commonalities in the functions of MEF2 as a regulator of differentiation of diverse cell types. The requirement of MEF2 for the differentiation of seemingly unrelated cell types from multiple lineages points to MEF2 being a key component of the regulatory codes that are required for metazoan development.

The MEF2 family

MEF2 proteins belong to the evolutionarily ancient MADS (MCM1, agamous, deficiens, SRF) family of transcription factors (Shore and Sharrocks, 1995). *Saccharomyces cerevisiae*, *Drosophila* and *Caenorhabditis elegans* possess a single *Mef2* gene, whereas vertebrates have four – *Mef2a*, *b*, *c* and *d*. The N-termini of MEF2 factors contain a highly conserved MADS-box and an immediately adjacent motif termed the MEF2 domain (Fig. 1), which together mediate dimerization, DNA binding, and co-factor interactions (Black and Olson, 1998; McKinsey et al., 2002a). The C-terminal regions of MEF2 proteins, which function as transcriptional activation domains, are subject to complex patterns of alternative splicing (Martin et al., 1994) and are divergent among family members (Fig. 1).

MEF2 proteins bind to the consensus DNA sequence YTA(A/T)$_4$TAR as homo- or heterodimers (Andres et al., 1995; Fickett, 1996; Gossett et al., 1989; Molkentin and Olson, 1996; Pollock and Treisman, 1991; Yu et al., 1992). Although MEF2 is a transcriptional activator, it relies on the recruitment of, and cooperation with, other transcription factors to drive the expression of its target genes. In addition, complex transcriptional, translational and post-translational mechanisms govern the functions of MEF2.

Yeast MEF2, referred to as Rlm1, binds the same DNA sequence as the vertebrate MEF2 proteins and functions as a downstream effector of the mitogen-activated protein (MAP) kinase pathway (Dodou and Treisman, 1997). Rlm1 regulates a cadre of genes that encode proteins involved in cell wall biosynthesis.

The four vertebrate *Mef2* genes display distinct, but overlapping, temporal and spatial expression patterns in embryonic and adult tissues, with highest expression in striated muscles and brain (Edmondson et al., 1994). However, in vertebrates, MEF2 is also expressed in lymphocytes, neural crest, smooth muscle, endothelium and bone (Arnold et al., 2007; Edmondson et al., 1994), and several reports claim that MEF2 proteins are ubiquitous (Black et al., 1997; Martin et al., 1993; McDermott et al., 1993; Pollock and Treisman, 1991; Yu et al., 1992). The expression of MEF2 proteins in many cell types, including in neurons, chondrocytes and muscle (cardiac, skeletal, and smooth), occurs concomitantly with the activation of their differentiation programs, and the balance between the transcription-activating functions of MEF2 and the repressive functions of class IIa histone deacetylases (HDACs) dictates the development of these tissues (Fig. 2) (Arnold et al., 2007; Chang et al., 2004; Chang et al., 2006; Lu et al., 2000; Verzi et al., 2007; Youn and Liu, 2000).

In adult tissues, MEF2 proteins act as a nodal point for stress-response and remodeling programs (for example, during cardiac hypertrophy and fiber-type switching in cardiac and skeletal muscle, respectively) (Potthoff et al., 2007b; Zhang et al., 2002). MEF2 proteins have also been implicated in cell survival, apoptosis and proliferation. In each of these settings, the spectrum of target genes activated by MEF2 depends on the specific post-translational modifications MEF2 undergoes and on its interaction with its co-factors.

Signaling to MEF2

MEF2 proteins serve as endpoints for multiple signaling pathways and thereby confer signal-responsiveness to downstream target genes (Fig. 2). MAP kinase signaling pathways converge on MEF2 factors in organisms ranging from yeast to humans (Dodou and

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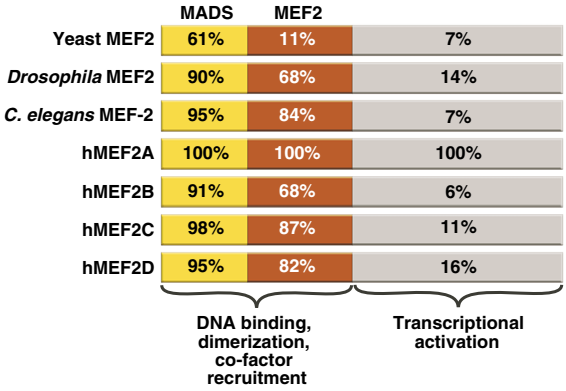

Fig. 1. Sequence conservation of MEF2. The percentage amino acid identity within the MADS, MEF2 and transcriptional activation domains of different MEF2 proteins from various organisms relative to human (h) MEF2A. N-termini are to the left.

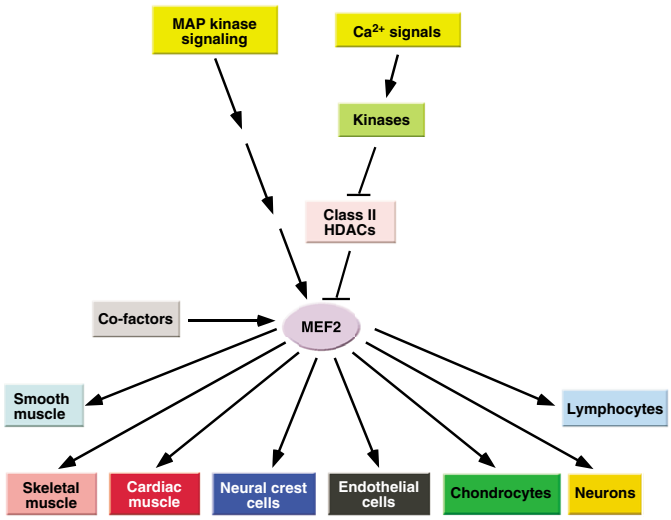

Fig. 2. MEF2 as a central regulator of differentiation and signal responsiveness. MAP kinase signaling activates MEF2. Calcium-dependent signals also activate MEF2 by stimulating calcium-dependent kinases that phosphorylate class II HDACs, thereby promoting their dissociation from MEF2 and derepressing MEF2 target genes. MEF2 recruits numerous co-factors to drive the differentiation of the various cell types shown. Although MAPK and HDAC signaling pathways have been implicated in the modulation of numerous MEF2-dependent developmental programs, these signaling pathways have not yet been shown to operate in all the cell types under MEF2 control.

Treisman, 1997; Han et al., 1997; Kato et al., 1997). Phosphorylation of the transcription activation domain of MEF2 by MAP kinases augments its transcriptional activity, and the MAP kinase ERK5 (also known as BMK1 and MAPK7) serves as a MEF2 coactivator through its signal-dependent direct association with the MEF2 MADS domain (Yang et al., 1998).

Calcium signaling pathways also modulate MEF2 activity through multiple mechanisms. In this regard, the activity of MEF2 is tightly governed by class IIa HDACs, which associate with the MADS domain and promote the formation of multiprotein repressive complexes on MEF2-dependent genes (Bertos et al., 2001; de Ruijter et al., 2003; McKinsey et al., 2001; McKinsey et al., 2002a; McKinsey et al., 2002b) (Fig. 2), such as myogenin (Cheng et al., 1993; Edmondson et al., 1992; Malik et al., 1995; Yee and Rigby, 1993), myoglobin (Bassel-Duby et al., 1992) and matrix metalloproteinase 10 (*Mmp10*) (Chang et al., 2006). Numerous calcium-regulated protein kinases, including protein kinase D (PKD) and calcium calmodulin-dependent protein kinases (CaMKs) phosphorylate class II HDACs on a series of conserved serine residues. This phosphorylation promotes the nuclear-to-cytoplasmic shuttling of these HDACs and the subsequent activation of MEF2 (McKinsey and Olson, 2005; Zhang et al., 2002). The regulated phosphorylation of class II HDACs thus provides a mechanism for the modulation of MEF2 target genes in response to physiological and pathological signaling.

MEF2: a central regulator of *Drosophila* myogenesis

MEF2 was first identified as a regulator of muscle gene expression (Gossett et al., 1989). The central role of MEF2 in orchestrating muscle development has been delineated most thoroughly in *Drosophila*. The single *Mef2* gene in *Drosophila* is expressed in early mesoderm and subsequently in different muscle cell lineages, where it is required for myoblast differentiation (Bour et al., 1995; Lilly et al., 1995; Ranganayakulu et al., 1995) (Fig. 3). A complex array of enhancers governs the transcription of *Mef2* in different cell types during *Drosophila* development. *Mef2* expression within the early mesoderm (Fig. 3A) requires a mesodermal enhancer that is directly activated by Twist (Cripps et al., 1998), a bHLH transcription factor required for mesoderm formation (Simpson, 1983). Twist and the zinc-finger transcription factor Lame duck act through separate enhancers to control *Mef2* transcription in specific sets of somatic muscle cells later in development (Fig. 3B) (Duan et al., 2001). MAD and Medea, downstream effectors of Decapentaplegic (DPP) signaling, also act directly on an *Mef2* enhancer to control its expression in the somatic muscle lineage (Nguyen and Xu, 1998). In addition, *Mef2* maintains its own transcription late in the muscle differentiation pathway by activating a distal *Mef2*-dependent autoregulatory enhancer (Cripps et al., 2004).

Within the cardiac lineage (Fig. 3C), the homeodomain protein Tinman directs *Mef2* transcription through a cardiac-specific enhancer that also contains essential binding sites for GATA factors (Cripps and Olson, 1998; Gajewski et al., 1997). Intriguingly, a mutation of the GATA sites switches the cell-type specificity of the enhancer from cardial to pericardial cells (Gajewski et al., 1998).

In *Mef2* mutant *Drosophila* embryos, somatic muscle founder cells are appropriately specified, but there is a complete block in myoblast fusion and in the expression of muscle differentiation markers (Bour et al., 1995; Lilly et al., 1995; Prokop et al., 1996; Ranganayakulu et al., 1995). Similarly, cardiac cells within the dorsal vessel, which functions as a heart, are patterned properly in *Mef2* mutant embryos, but cardiac contractile protein genes are not expressed.

Several approaches have been taken to identify MEF2 target genes in *Drosophila*. The use of chromatin immunoprecipitation (ChIP), followed by microarray analysis (ChIP on chip) on a tiling array that covers ~50% of the *Drosophila* genome, identified more than 200 direct target genes of MEF2 and over 650 regions of the genome that are bound by MEF2, highlighting the central role of MEF2 in the transcriptional hierarchy for myogenesis (Sandmann et al., 2006). Similar findings were made by an independent study that combined ChIP with spotted DNA microarrays that contain in-silico predicted cis-regulatory module targets (the so-called ChEST strategy) (Junion et al., 2005).

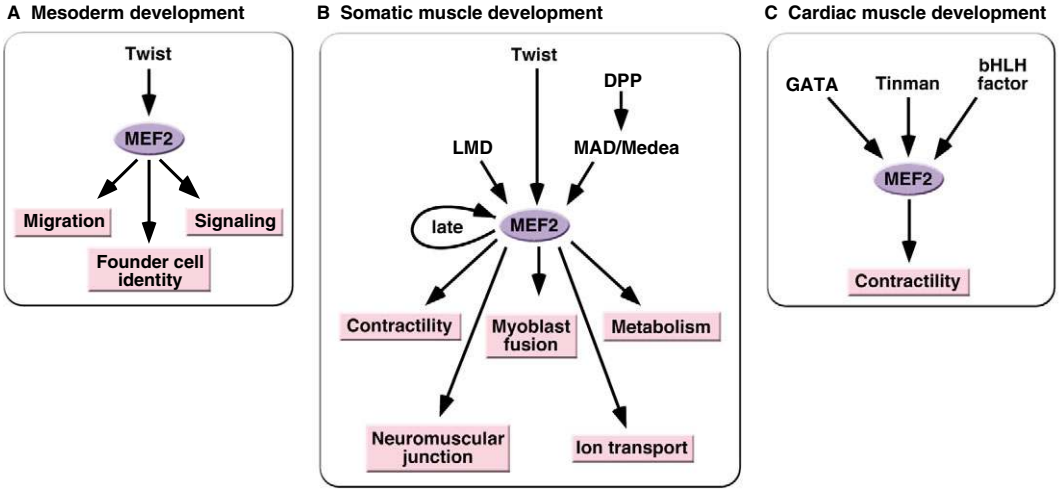

Fig. 3. Roles of MEF2 in *Drosophila* muscle development. (**A**) In the early mesoderm, MEF2 expression is activated by Twist, and MEF2 regulates downstream genes involved in cell migration, signaling and founder cell identity. (**B**) During differentiation of the somatic muscle lineage, Lame duck (LMD) activates MEF2 expression in a subset of muscle cells. DPP also regulates MEF2 expression via Twist and MAD/Medea. During late myogenesis, MEF2 autoactivates its own expression. MEF2 regulates hundreds of genes involved in contractility, neuromuscular junction formation, myoblast fusion, ion transport and metabolism. (**C**) In the developing dorsal vessel, MEF2 is regulated by GATA factors, Tinman, and by an as yet unidentified bHLH factor. MEF2 is essential for the expression of genes involved in cardiac contractility.

MEF2 exhibits three temporal patterns of enhancer binding during *Drosophila* muscle development. Although MEF2 is present at high levels early in development, it does not bind the enhancers of muscle differentiation genes until later in development, indicating the existence of mechanisms that govern target gene recognition by MEF2 (Sandmann et al., 2006). Twist, the central bHLH protein partner for MEF2 in the somatic muscle lineage in *Drosophila* (Cripps and Olson, 1998), binds with MEF2 to enhancers that are activated early in the mesodermal and myogenic lineages (Sandmann et al., 2007).

Drosophila MEF2 also regulates a striking number of genes that encode components of the Notch-Delta pathway, as well as of the Wingless (WNT), Hedgehog (HH), Fibroblast growth factor (FGF) and Epidermal growth factor (EGF) signaling pathways, which are involved in specifying the identities of somatic muscle founder cells (Sandmann et al., 2006). This type of function of MEF2 in the early stages of myogenesis coincides with the early, Twist-dependent phase of MEF2 expression and its regulation of genes in the early mesoderm (Taylor, 2000; Ruiz-Gomez et al., 2002).

Unexpectedly, MEF2 also regulates the enhancers of muscle identity genes, suggesting that it contributes to the robustness of myogenesis (Sandmann et al., 2006). At later stages of myogenesis, MEF2 regulates genes that are involved in muscle attachment, neuromuscular junction (NMJ) formation, ion transport, channel activity, metabolism and contractility (Sandmann et al., 2007) (Fig. 3B). These studies suggest that MEF2 regulates most, if not all, muscle genes, not just those encoding 'late' structural proteins of differentiated muscle, and thereby acts as a central regulator of myogenesis.

The ectopic expression of MEF2 in the epidermis of *Drosophila* results in the activation of skeletal muscle genes, such as *Tropomyosin 1* (*Tm1*), whereas ectopic expression of MEF2 in the nervous system does not activate these genes (Lin, M. H. et al., 1997), suggesting that the epidermis expresses a co-factor that cooperates with MEF2 to activate the muscle gene program. In this regard, a novel PAR-domain bZIP transcription factor, PDP1, which is expressed in cell types that are susceptible to MEF2-dependent muscle gene activation, has been shown to cooperate with MEF2 to activate muscle gene expression (Lin, S. C. et al., 1997).

In contrast to *Drosophila*, which contains a single *Mef2* gene, elucidation of the functions of mammalian *Mef2* genes has been comparably more difficult owing to the existence of four related genes that have overlapping expression patterns. In vertebrates, loss-of-function mutations frequently reveal only a subset of MEF2 functions in tissues in which the genes do not function redundantly. By generating conditional alleles of the different *Mef2* genes, we are now beginning to ascertain the importance of specific MEF2 proteins in various tissues through their combinatorial deletion (Arnold et al., 2007). Alternatively, the overexpression of chimeric MEF2 fusion proteins, such as the super-active MEF2-VP16 or super-repressive MEF2-engrailed, has been used to elucidate MEF2 function in different tissues while bypassing functional redundancy (Arnold et al., 2007; Karamboulas et al., 2006; Potthoff et al., 2007b).

MEF2 control of vertebrate skeletal muscle differentiation

Vertebrate skeletal muscle differentiation is regulated by the cooperative interactions of myogenic transcription factors with MEF2, and by signaling pathways that regulate MEF2 activity (Fig. 4). MEF2 factors alone do not possess myogenic activity but, in combination with bHLH transcription factors, drive and amplify the myogenic differentiation program (Molkentin et al., 1995; Wang et al., 2001). MEF2 also interacts with additional transcription factors that are required for proper muscle development. For example, the mastermind-like protein 1, MAML1, was recently shown to interact with MEF2C and to mediate crosstalk between Notch signaling and MEF2 in the regulation of myogenic differentiation (Shen et al., 2006). MEF2 has also been implicated in regulating skeletal myocyte survival through a CREB-dependent pathway (Berdeaux et al., 2007).

In addition to regulating numerous muscle structural genes, vertebrate MEF2 proteins regulate the expression of myogenic bHLH genes, such as myogenin, as well as other genes that encode

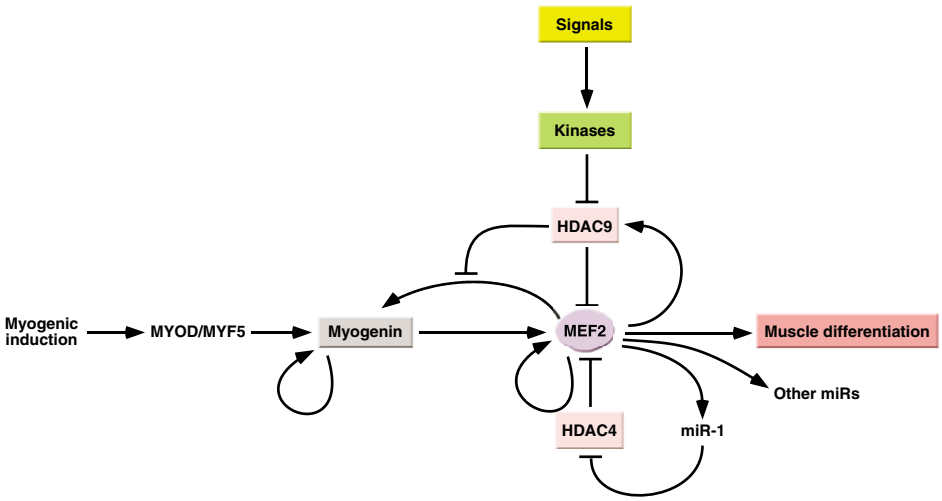

Fig. 4. MEF2 and mammalian skeletal myogenesis. Upstream inductive signals activate the expression of MYOD and MYF5, which activate the expression of myogenin in skeletal myocytes. Myogenin activates MEF2 expression, which feeds back on the myogenin promoter to amplify and maintain its expression. Myogenin and MEF2 also autoregulate their own promoters. MEF2, together with myogenic bHLH factors, activates genes involved in muscle differentiation. In addition, MEF2 activates HDAC9 expression, providing a negative-feedback loop that can be modulated by upstream signals that regulate HDAC9 phosphorylation. MEF2 also regulates the expression of the microRNA miR-1, which represses HDAC4 translation, thereby providing a positive-feedback loop for myogenesis.

transcription factors, thereby providing a positive feed-forward loop that perpetuates and amplifies the decision to differentiate (Cheng et al., 1993; Edmondson et al., 1992; Molkentin and Olson, 1996; Tapscott, 2005; Yee and Rigby, 1993). MEF2C has also been shown to positively regulate its own expression during mouse embryogenesis (Wang et al., 2001), consistent with the autoregulatory activity of *Drosophila* MEF2 (Cripps et al., 2004). Moreover, MEF2C activates the expression of the class IIa HDAC, HDAC9, thereby creating a negative-feedback loop that modulates and restrains MEF2 from excessive activity (Haberland et al., 2007) (Fig. 4). This type of negative-feedback loop also confers signal responsiveness to MEF2-dependent gene programs through the regulated phosphorylation of class IIa HDACs.

MEF2 establishes an additional level of myogenic regulation by regulating the expression of microRNAs (miRs), such as miR-1 and miR-133 (Sokol and Ambros, 2005; Zhao et al., 2005), that post-transcriptionally repress gene expression by binding the 3′ untranslated regions of mRNA targets and disrupting mRNA translation and stability (He and Hannon, 2004). Recently, several microRNAs were identified that affect skeletal muscle differentiation and proliferation (Boutz et al., 2007; Chen et al., 2006; Kim et al., 2006; Rao et al., 2006). Interestingly, miR-1 has been shown to target class II HDACs (such as HDAC4) (Chen et al., 2006) to establish a positive feed-forward mechanism for MEF2 activation and skeletal muscle differentiation (Fig. 4). This form of regulation, which enhances MEF2 activity, would oppose the direct activation of HDAC9 expression by MEF2, which represses MEF2 activity, illustrating the multifaceted mechanisms that exist to modulate MEF2. Presumably, these different regulatory loops are differentially controlled during various stages of skeletal muscle development and postnatal muscle remodeling.

Despite extensive studies of MEF2 in skeletal muscle in vitro, relatively little is known about the roles of MEF2 proteins in vertebrate skeletal muscle in vivo. During mouse embryogenesis, *Mef2c* is the first *Mef2* gene to be expressed in the somite myotome (~E9.0), with *Mef2a* and *Mef2d* expressed about a day later (Edmondson et al., 1994). Global deletion of *Mef2a* or *Mef2d* has little or no effect on skeletal muscle development (Potthoff et al., 2007a; Potthoff et al., 2007b). Since *Mef2c*-null mice die around E9.5 (Lin, Q. et al., 1997), its role in skeletal muscle was not examined until recently. Skeletal muscle deficient in *Mef2c* differentiates and forms myofibers during embryogenesis (Potthoff et al., 2007a; Potthoff et al., 2007b). However, on a C57BL/6 mixed genetic background, myofibers from mice with a skeletal muscle-specific deletion of *Mef2c* rapidly deteriorate after birth owing to the occurrence of disorganized sarcomeres and to the loss of integrity of the sarcomere M-line (Potthoff et al., 2007a). Interestingly, similar results have been observed in zebrafish following the combined knockdown of *mef2c* and *mef2d* (Hinits and Hughes, 2007). Notably, the muscle-specific overexpression of a super-active MEF2 protein in mice does not drive premature skeletal muscle differentiation (Potthoff et al., 2007b), consistent with previous in vitro studies that have demonstrated that MEF2 is not sufficient to drive skeletal muscle differentiation (Molkentin et al., 1995). These results reveal a key role for MEF2 proteins in the maintenance of sarcomere integrity and in the postnatal maturation of skeletal muscle.

Control of vertebrate heart development by MEF2

MEF2 regulates the expression of numerous cardiac structural and contractile proteins. Cardiac-specific overexpression of the repressive MEF2C-engrailed fusion protein under the control of the *Nkx2-5* enhancer (~E7.5) is sufficient to inhibit cardiomyocyte differentiation in vitro and in vivo. Moreover, the overexpression of MEF2C-engrailed downregulates the expression of GATA and NKX proteins in cardiomyocytes (Karamboulas et al., 2006), confirming the role of MEF2 as a regulator of the other core cardiac transcription factors that are required for cardiomyocyte differentiation.

Notably, cardiomyocyte development can still occur despite the loss of individual vertebrate MEF2 proteins. In the mouse and chick, MEF2C is the first MEF2 factor to be expressed, appearing initially in mesodermal precursors that give rise to the heart (Edmondson et

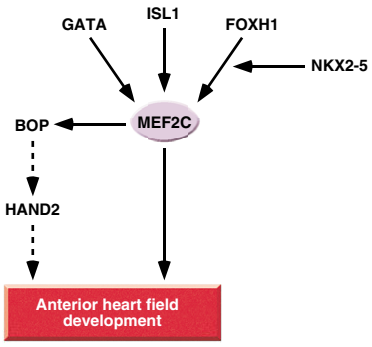

Fig. 5. Control of anterior heart field development by MEF2C. MEF2C expression in the anterior heart field is controlled by GATA4, ISL1 and FOXH1, together with NKX2-5. MEF2C directly activates the expression of BOP, which is required for expression of HAND2, an essential regulator of anterior heart field development. Solid lines indicate direct regulatory interactions and dotted lines indicate regulatory interactions for which the underlying mechanism has not yet been defined.

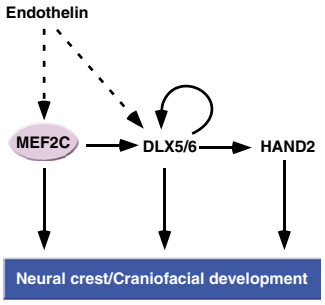

Fig. 6. Control of neural crest development by MEF2C. Signaling by endothelin 1 (EDN1) through the ET-A receptor (EDNRA) activates MEF2C expression in the neural crest. MEF2C directly activates the expression of DLX5 and DLX6, which regulate the expression of HAND2. Together, these factors regulate the expression of genes required for craniofacial development.

al., 1994). Shortly thereafter, the other *Mef2* transcripts are expressed. *Mef2c*-null mice die around E9.5 from cardiac looping defects (Lin, Q. et al., 1997), and *Mef2a*-null mice exhibit perinatal lethality from an array of cardiovascular defects (Naya et al., 2002). By contrast, *Mef2d*-null mice appear normal (Arnold et al., 2007). Although *Mef2c*-null mice exhibit early embryonic lethality, cardiomyocytes are still able to differentiate prior to the looping defects that occur (Lin, Q. et al., 1997). Interestingly, mice with a cardiac-specific deletion of *Mef2c*, which occurs at around E9.5 (*αMyHC*-cre), are viable (Vong et al., 2005), which demonstrates that *Mef2c* is dispensable in the heart after cardiac looping, probably owing to it being compensated for by other MEF2 factors.

Recent studies indicate that MEF2C is a nodal point in the development of the anterior heart field, which gives rise to the outflow tract and right ventricle of the heart (Black, 2007) (Fig. 5). In *Mef2c* mutant embryos, derivatives of the anterior heart field fail to form (Lin, Q. et al., 1997). Activation of *Mef2c* transcription in the anterior heart field is controlled by at least two separate enhancers: one that is activated directly by the forkhead DNA-binding transcription factor FOXH1, together with NKX2-5, which also confers TGF-β responsiveness to *Mef2c* (von Both et al., 2004); and a second enhancer that serves as a direct target of GATA4 and the LIM-homeodomain transcription factor ISL1, which is itself required for the formation of the anterior heart field (Dodou et al., 2004; Cai et al., 2003). Expression of the transcriptional repressor and putative histone methyltransferase BOP (also known as SMYD1) in the anterior heart field is controlled by the direct activation of an upstream enhancer by MEF2C (Phan et al., 2005), and the cardiac defects seen in *Bop* mutant embryos partially phenocopy those of *Mef2c* mutants, suggesting that *Bop* is an essential downstream mediator of the actions of MEF2C in the anterior heart field (Gottlieb et al., 2002). The bHLH transcription factor HAND2 appears to be a key target of BOP regulation, although the mechanistic basis for this regulation has not been resolved.

Control of neural crest development by MEF2

Neural crest cells are multipotent, migratory cells that originate between the dorsal neural tube and epidermis of the embryo (Knecht and Bronner-Fraser, 2002; Trainor, 2005). In response to specific signaling cues, neural crest cells undergo an epithelial-to-mesenchymal transition, and then migrate to different parts of the embryo to give rise to a variety of cell types, including neurons, skeletal and smooth muscle, chondrocytes, osteocytes, melanocytes, hormone-producing cells, and many more (Knecht and Bronner-Fraser, 2002). Loss of *Mef2c* in neural crest cells results in craniofacial defects and neonatal lethality caused by an upper airway obstruction (Verzi et al., 2007); in zebrafish, loss of *mef2ca* produces similar craniofacial defects (Miller et al., 2007). MEF2C directly activates the expression of the homeodomain transcription factors DLX5 and DLX6, two transcription factors that are necessary for craniofacial development, and MEF2C acts synergistically with these factors to direct craniofacial development (Miller et al., 2007; Verzi et al., 2007) (Fig. 6).

The peptide hormone endothelin controls a diverse array of developmental processes, including neural crest migration and differentiation during craniofacial development (Clouthier et al., 1998; Kurihara et al., 1994). DLX6 and the transcription factor HAND2 are important regulators of branchial arch development and require endothelin signaling for their expression (Charitè et al., 2001; Clouthier et al., 2000; Thomas et al., 1998). Interestingly, aspects of endothelin signaling in zebrafish require Mef2, including activation of the endothelin target genes *hand2*, *dlx5* and *dlx6* (Miller et al., 2007) (Fig. 6). Therefore, MEF2 plays a crucial role in neural crest development by activating the expression of endothelin signaling-dependent transcription factors that are required for proper development.

Control of bone development by MEF2

During embryonic development, bones develop through intramembranous or endochondral ossification. Endochondral ossification involves a cartilaginous intermediate, whereas intramembranous ossification occurs through the direct conversion of mesenchymal tissue into bone (Hall and Miyake, 1995). During endochondral ossification, mesenchymal precursor cells become committed to cartilage cells, forming a template for future bone. These committed mesenchymal cells differentiate into chondrocytes, proliferate rapidly to form a template for osteoblasts (committed bone precursor cells), secrete a cartilage-specific extracellular matrix, and then stop dividing and undergo hypertrophy (Bruder and

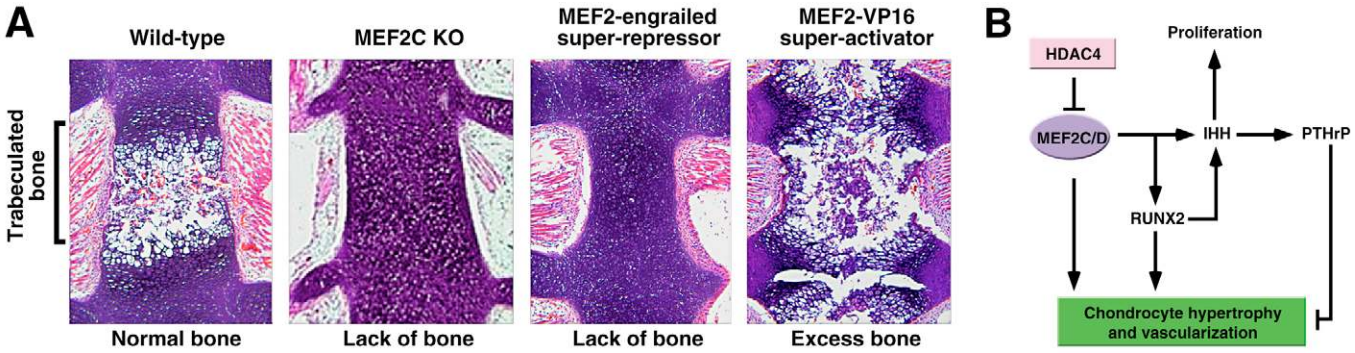

Fig. 7. Regulation of bone development by MEF2. (**A**) High-magnification frontal view of Hematoxylin and Eosin-stained sections of mouse sternum. Left, wild-type trabeculated bone. MEF2C KO, chondrocyte-specific deletion of a conditional *Mef2c* allele, which results in a lack of bone owing to failure in chondrocyte hypertrophy. MEF2-engrailed super-repressor, when expressed in the cartilage of transgenic mice, also prevents ossification, whereas expression of a MEF2-VP16 super-activator results in the formation of excessive bone. (**B**) MEF2C and MEF2D promote chondrocyte hypertrophy and vascularization of developing bones by activating a network of transcription factors and signaling molecules involved in bone development. HDAC4 imposes negative control over the network by repressing the activity of MEF2 [adapted from Arnold et al. (Arnold et al., 2007)]. IHH, Indian hedgehog; PTHrP, parathyroid hormone-related peptide; RUNX2, runt related transcription factor 2.

Caplan, 1989). Signaling coordinated by Indian hedgehog (IHH) and parathyroid hormone-related peptide (PTHrP; also known as PTHLH) regulates the hypertrophy of chondrocytes, which is necessary for bone vascularization, osteoblast differentiation and endochondral ossification. IHH produced by prehypertrophic chondrocytes induces the expression of PTHrP, which regulates the rate at which chondrocytes undergo hypertrophy (Karaplis et al., 1994; Lanske et al., 1996; St-Jacques et al., 1999; Vortkamp et al., 1996; Weir et al., 1996).

Recently, MEF2 proteins were shown to be necessary and sufficient to drive chondrocyte hypertrophy (Arnold et al., 2007) (Fig. 7A). Interestingly, this function of MEF2 is tightly regulated by the opposing function of HDAC4 (Arnold et al., 2007; Vega et al., 2004). Genetic deletion of *Hdac4* (Vega et al., 2004) or the chondrocyte-specific overexpression of a constitutively activated form of MEF2C – the MEF2C-VP16 fusion protein – in mice is sufficient to drive premature bone formation (Arnold et al., 2007) (Fig. 7A). Conversely, the genetic deletion of *Mef2c*, or the overexpression of a MEF2-engrailed repressor in mouse chondrocytes, prevents chondrocyte hypertrophy and endochondral ossification (Arnold et al., 2007) (Fig. 7A). MEF2 functions, at least in part, by directly activating collagen 10a1 (*Col10a1*) expression, a specific marker of chondrocyte hypertrophy, and *Runx2*, a transcription factor necessary for chondrocyte hypertrophy (Arnold et al., 2007) (Fig. 7B). Undoubtedly, however, there are additional downstream targets and upstream regulators of MEF2 in developing chondrocytes that remain to be defined.

Control of vascular integrity by MEF2

The development of the vasculature occurs through two stages, termed vasculogenesis and angiogenesis. Vasculogenesis is the de novo formation of blood vessels from mesodermal progenitor cells, and angiogenesis is the expansion of a capillary plexus by the formation of additional branches from pre-existing vessels (Patan, 2000). MEF2 proteins are expressed in developing endothelial and smooth muscle cells (Lin et al., 1998) and are required for vascular development and for the maintenance of vascular integrity. *Mef2c* expression in the developing endothelium is controlled by a conserved endothelial-specific enhancer that binds ETS-family factors and drives expression as early as E8.5 in all endothelial cells of the mouse embryo and yolk sac (De Val et al., 2004). Endothelial cells are specified and differentiated in *Mef2c*-null mice, but they are unable to organize properly (Lin et al., 1998).

MEF2 proteins have been implicated in maintaining vascular integrity by promoting endothelial cell survival (Hayashi et al., 2004; Olson, 2004) (Fig. 8). The MAP kinase ERK5 is necessary for endothelial cell survival and proliferation; its conditional deletion from endothelial cells in mice results in vascular death and embryonic lethality (at E9.5-10.5) due to apoptosis and a failure of endothelial cells to proliferate (Hayashi et al., 2004). The introduction of MEF2C-VP16 into ERK5-deficient endothelial cells is sufficient to partially protect the cells from apoptosis, whereas the removal of ERK5 from endothelial cells eliminates the serum-stimulated activation of MEF2 in these cells (Hayashi et al., 2004).

Recently, we demonstrated an unexpected role for MEF2-HDAC signaling in the maintenance of vascular integrity (Fig. 8). HDAC7 is expressed specifically in endothelial cells during development, and global deletion of *Hdac7* results in embryonic lethality due to blood vessel rupture caused by defects in cell-cell adhesion, a phenotype that is recapitulated by the endothelial-specific deletion of *Hdac7* (Chang et al., 2006). Knockdown of HDAC7 in human endothelial cells in vitro results in a similar loss in cell adhesion, accompanied by upregulation of MMP10, a secreted endoproteinase that degrades the extracellular matrix, and downregulation of its inhibitor, tissue inhibitor of metalloproteinase 1 (TIMP1). MEF2 proteins directly activate the expression of MMP10, and HDAC7 is sufficient to repress this activation (Chang et al., 2006).

Abnormalities in growth and integrity of the vascular endothelium lead to a variety of cardiovascular disorders (for example, atherosclerosis and aneuryisms). During development, MEF2 may be involved in angiogenesis by promoting cell survival (Hayashi et al., 2004) and vascular remodeling (Chang et al., 2006). In response to stress signals (for example, oxidative or fluid shear stress), MEF2 activation may actually promote vascular remodeling at the site of injury. In this regard, ERK5 has been demonstrated to be atheroprotective, as it displays increased activation in response to fluid shear stress and oxidative stress (Pi et al., 2004). Therefore, if MEF2 becomes activated at local sites of injury, which results in

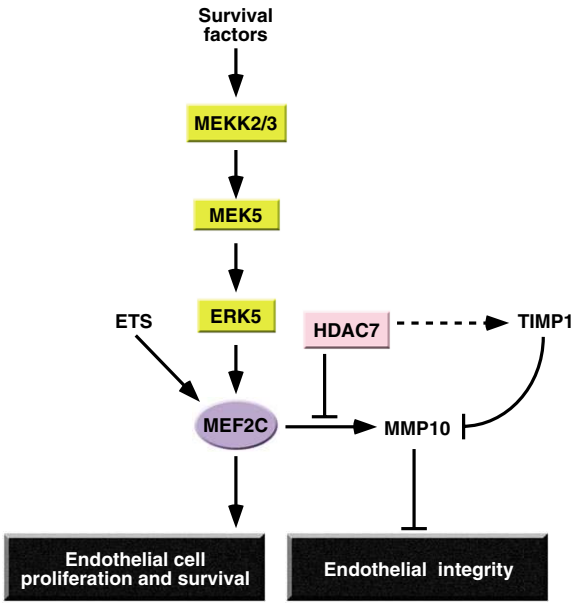

Fig. 8. MEF2 functions in the endothelium. During embryogenesis, MEF2C expression in the endothelium is dependent on ETS factors, which bind an endothelial cell-specific enhancer. MEF2 activity is also modulated in the endothelium by survival factors, which act through the MAP kinase pathway [MEKK2/3 (also known as MAP3K2/3); MEK5 (also known as MAP2K5)], culminating on ERK5 (MAPK7), which associates with MEF2 directly to enhance transcriptional activity. MEF2 activates transcription of the *Mmp10* gene, which encodes a matrix metalloproteinase that degrades endothelial cell junctions. HDAC7, which is expressed specifically in the developing endothelium, represses *Mmp10* expression via MEF2. In the absence of HDAC7, MMP10 is upregulated and its inhibitor, TIMP1, is downregulated, leading to a loss in vascular integrity.

blood vessel remodeling, then removal of MEF2 repression by deletion of *Hdac7* might explain the global vascular rupture that is seen in *Hdac7*-null embryos.

In addition to endothelial cells, MEF2 has important functions in smooth muscle cells. Whereas endothelial cells differentiate in *Mef2c*-null embryos, smooth muscle cells fail to properly differentiate (Lin et al., 1998). This function of MEF2 occurs, at least partly, through myocardin, a serum response factor (SRF) and MEF2 transcriptional coactivator that is necessary for smooth muscle differentiation (Wang et al., 2004; Wang et al., 2003). We recently showed that MEF2 recruits a specific isoform of myocardin, which together with MEF2 coactivates the myocardin gene through a positive-feedback loop (Creemers et al., 2006). Interestingly, MEF2 is upregulated in activated smooth muscle cells (Firulli et al., 1996), which suggests that MEF2 functions in the smooth muscle stress response after injury or in pathological states (e.g. artherosclerosis).

Control of neuronal differentiation and survival by MEF2

MEF2 proteins are highly enriched in neurons and exhibit distinct patterns of expression in different regions of the brain, with highest levels being present in the cerebellum, cerebral cortex and hippocampus (Ikeshima et al., 1995; Leifer et al., 1993; Lin et al., 1996; Lyons et al., 1995). MEF2 protects neurons from apoptotic death (Mao et al., 1999; Mao and Wiedmann, 1999; Okamoto et al., 2000), which contrasts with its pro-apoptotic function in thymocytes (Woronicz et al., 1995). The ability of MEF2 to regulate neuronal-specific transcriptional programs may occur through DNA-binding site selection. MEF2 that is expressed in neurons shows optimal DNA-binding constraints for specific nucleotide sequences that flank the MEF2 site, and this is not observed with MEF2 factors from other cell types (Andres et al., 1995).

Recently it was shown that MEF2 proteins regulate dendrite morphogenesis, differentiation of post-synaptic structures (Shalizi et al., 2006) and excitatory synapse number (Flavell et al., 2006). Sumoylation of MEF2A promotes the post-synaptic differentiation of neurons by repressing the expression of the NUR77 transcription factor (Shalizi et al., 2006), a negative regulator of dendritic differentiation (Scheschonka et al., 2007). In addition, dephosphorylation of MEF2 by calcineurin regulates the expression of activity-regulated cytoskeletal-associated protein (*Arc*) and synaptic RAS GTPase-activating protein (*synGAP*; also known as *Syngap1*) (Flavell et al., 2006). ARC and synGAP play important roles in synaptic disassembly by promoting the internalization of glutamate receptors (Flavell et al., 2006) and by inhibiting Ras-MAP signaling (Vazquez et al., 2004), respectively. In response to activity-dependent calcium signaling, calcineurin dephosphorylates MEF2 at Ser408, signaling a switch from the sumoylation to the acetylation of its residue Lys403. This change restricts the numbers of synapses that form (Flavell et al., 2006) and inhibits dendritic claw differentiation (Shalizi et al., 2006) through the activation of the orphan nuclear receptor *Nur77* and of *Arc* and *synGAP*.

Thus, specific signaling events modulate gene expression by post-translationally modifying MEF2 to control synapse development and plasticity. The functions of individual MEF2 proteins and their roles in synaptic differentiation and disassembly in vivo have yet to be examined. Based on recent literature, however, MEF2 appears to play a role in synaptic plasticity, suggesting an important role for these proteins in learning and memory.

Control of T-cell development by MEF2

The development and activation of thymocytes (T-cells) is a highly regulated process that requires multiple signaling cascades to direct changes in gene expression that alter T-cell state or fate. Calcium signaling pathways play important roles in T-cell selection during development and in T-cell receptor (TCR)-induced apoptosis (Woronicz et al., 1995). NUR77 is a crucial mediator of TCR-induced apoptosis, and TCR-induced expression of NUR77 is mediated through two MEF2 sites in the *Nur77* promoter (Youn et al., 1999). In unstimulated T-cells, MEF2 is associated with transcriptional co-repressors, such as HDAC7 and Cabin1, which inhibit *Nur77* expression (Dequiedt et al., 2003; Youn and Liu, 2000; Youn et al., 1999).

Following TCR activation, HDAC7 becomes dissociated from MEF2 through nucleocytoplasmic shuttling. Phosphorylation of HDAC7 by PKD1 recruits 14-3-3 and translocates HDAC7 to the cytoplasm, allowing the activation of MEF2 (Parra et al., 2005). Conversely, HDAC7 is dephosphorylated by protein phosphatase 1β (PP1β; also known as PPP1CB) and myosin phosphatase targeting subunit 1 (MYPT1; also known as PPP1R12A), which are components of the myosin phosphatase complex that promote HDAC7 nuclear localization and repression of NUR77 expression (Parra et al., 2007). Therefore, regulation of MEF2 activity by association with transcriptional repressors is highly regulated in T-cells, and demonstrates the importance of MEF2-HDAC signaling in T-cell development, differentiation and thymocyte selection (Kasler and Verdin, 2007).

Conclusion

MEF2 is an ancient mediator of signal-dependent transcription and cell differentiation, and predates most of the transcription factors with which it cooperates to control metazoan development. In virtually every cell type in which its functions have been investigated, MEF2 has been found to serve as a central component of differentiation and development through its ability to potentiate the activities of other regulators, and we speculate that MEF2 will be found to regulate the differentiation of additional cell types, in which its functions have yet to be investigated. In addition to its central role in tissue-specific gene expression and differentiation, MEF2, through its responsiveness to upstream signaling pathways and through its association with other signal-dependent activators and repressors, such as class II HDACs, also serves as a key intermediary in the transmission of extracellular signals to the genome. This function brings signal-dependence to its downstream programs of gene expression. How MEF2 engages its myriad partner proteins in different cell types to activate different and often opposing programs of gene expression, and why such a diversity of cell types and gene programs evolved with a reliance on MEF2, are interesting questions for the future.

Understanding the mechanism of action of MEF2 has provided not only a window into the logic of development, but has also revealed basic mechanisms of numerous diseases. Armed with these insights, it should be possible to modulate complex developmental and disease phenotypes through the manipulation of MEF2 activity.

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