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Review

Mef2 and the skeletal muscle differentiation program

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ABSTRACT

Mef2 is a conserved and significant transcription factor in the control of muscle gene expression. In cell culture Mef2 synergises with MyoD-family members in the activation of gene expression and in the conversion of fibroblasts into myoblasts. Amongst its *in vivo* roles, Mef2 is required for both *Drosophila* muscle development and mammalian muscle regeneration. Mef2 has functions in other cell-types too, but this review focuses on skeletal muscle and surveys key findings on Mef2 from its discovery, shortly after that of MyoD, up to the present day. In particular, *in vivo* functions, underpinning mechanisms and areas of uncertainty are highlighted. We describe how Mef2 sits at a nexus in the gene expression network that controls the muscle differentiation program, and how Mef2 activity must be regulated in time and space to orchestrate specific outputs within the different aspects of muscle development. A theme that emerges is that there is much to be learnt about the different Mef2 proteins (from different paralogous genes, spliced transcripts and species) and how the activity of these proteins is controlled.

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1. Discovery of the conserved Mef2 family

We write on the thirtieth anniversary of the publication of the paper describing the discovery of MyoD and its ability to transform a fibroblast into a myoblast ([1] Lassar, this special issue). It is difficult to overstate the impact of this discovery both to the field of muscle differentiation and also beyond, to cell differentiation programs in general and to cellular reprogramming. In mammals, MyoD was quickly identified as one of four structurally and functionally related transcription factors, the Myogenic Regulatory Factors (MRFs) [2]. They bind to specific DNA sequences found in the *cis*-regulatory modules (CRMs) that control the expression of many muscle genes, and together the MRFs have a critical role in muscle differentiation during animal development [3]. However, there is more to making muscle than the MRFs. For example, other DNA sequences in CRMs together with their cognate transcription factors are required for muscle gene expression. Indeed, MRFs can induce gene expression through DNA sequences that do not contain their binding site [4,5]. Mef2 quickly emerged as another significant player in the transcription of muscle genes.

Mef2 was first identified using mammalian cultured cells [6]. It was described as a protein activity that bound to a DNA sequence, distinct from the MRF-binding site, found in a CRM of the muscle creatine kinase (MCK) gene that is responsible for the expression of MCK during muscle cell differentiation. This Mef2-binding DNA sequence was subsequently found to be in the CRMs of nearly every known muscle-specific gene [5,7]. Molecular cloning of Mef2 [8,9] revealed that Mef2 is a member of the evolutionarily ancient MADS family of transcription factors [10,11]. This family also contains SRF, which itself has a role in the regulation of muscle gene expression [12] and references cited therein; [13].

Mef2 is highly conserved (Fig. 1). There are single *Mef2* genes in *Drosophila*, *C. elegans* and sea urchins, for example [7]. In contrast in the vertebrate genome, as with the MyoD family of MRFs, there are four closely-related *Mef2* genes: *Mef2a*, *-b*, *-c* and *-d* [7]. Different transcripts are generated from each gene by alternative splicing [7,9] (see 5.1 for functional significance). The encoded proteins bind DNA with the consensus sequence $CTA(A/T)_4TAG$ and can form both homo- and hetero-dimers [8]. It is unknown whether different Mef2 isoforms bind to variants of the Mef2 consensus sequence [14]. Key functional domains include the N-terminal MADS box and the adjacent Mef2 domain, that together are necessary and sufficient for DNA-binding and dimerisation, and these regions show extensive sequence similarity across all the Mef2 proteins from different species [7]. Point mutations in the conserved MADS box of *Drosophila* Mef2 result in lethality [15]. The C-terminal region is required for transcriptional activation [16], but the sequence has diverged considerably in different Mef2 proteins. Mef2 protein bends DNA on high affinity binding [17], and while the MADS box confers DNA-binding specificity [8], the Mef2 domain modulates the DNA-binding affinity [16]. Both crystal and solution structures for a homodimerised highly conserved MADS/Mef2 domain of MEF2A bound to DNA have been determined [18–20]. Many aspects of the Mef2 structure/function relationship remain to be analysed.

2. Mef2 gene expression

An initial step in the investigation of the possible role of *Mef2* in muscle differentiation was to determine its pattern of expression during embryonic development. Moreover, the expression pattern of individual genes, and indeed isoforms of each gene, continues to aid the interpretation of phenotypes and yield insights into the role of individual gene products. We will focus on Mef2 in skeletal muscle although, and in contrast to the skeletal muscle-specific MRFs, Mef2s are expressed and function in cardiac and smooth muscle, endothelium, brain neurons and many other tissues [21].

Invertebrates have a single *Mef2* gene. In *Drosophila*, *Mef2* is initially expressed throughout the mesoderm at gastrulation and subsequently almost exclusively in muscle. Both progenitors and differentiated cells of the somatic muscle (the equivalent of vertebrate skeletal muscle), heart and visceral muscle express *Mef2* [22]. The situation is more complex in vertebrate species with their separate *Mef2a*, *−b*, *−c* and *−d* genes. Mouse was the first species in which their expression patterns were described during development.

Murine *Mef2* genes are expressed at significant levels in a wide variety of tissues, but several show highest expression in muscle. *Mef2c* mRNA was first detected in the developing heart and shortly after in somitic muscle, and remained abundant in developing skeletal muscle at e14.5, being particularly concentrated at points of muscle attachment [23,24]. *Mef2d* showed a similar although weaker pattern in somitic muscle [23]. *Mef2a* is also highly expressed in somites containing differentiated muscle fibres [23]. *Mef2b* has the most divergent structure, the least obvious muscle-related expression and the least evidence for involvement in muscle development. The expression pattern of the *Mef2* genes during limb and head myogenesis is, to our knowledge, poorly described.

A striking feature of chick Mef2 expression is that it is biphasic. *Mef2b* and *Mef2c* show broad transient expression in nascent neck somites, prior to MRF expression [25]. In contrast later, in nascent trunk somites, *Mef2c* mRNA accumulates strongly prior to other Mef2 mRNAs in regions where both Myf5 and MyoD have become detectable [25]. *Mef2d* and *Mef2a* mRNAs are detected in each somite, a few hours after *Mef2c* [25,26]. At slightly later stages, when terminally differentiated fibres are present in significant numbers in somites, the location of *Mef2c* and *Mef2d* mRNAs appear distinct, with *Mef2c* mRNA located at the dermomyotomal lips where new myogenesis is on-going and *Mef2d* mRNA located centrally within the myotome where more mature fibres are located [25]. Thus, *Mef2c* and *Mef2d* display a spatial and temporal succession. This description reveals potentially interesting relationships both between the different Mef2 genes and to the MRFs, but the functional significance of this expression is at present unknown.

Zebrafish have six Mef2 genes, both *mef2a* and *mef2c* have two versions, and several are expressed during skeletal myogenesis [27]. Zebrafish *mef2d* is the first Mef2 gene detectably expressed, closely following the initiation of MRF expression in several distinct muscle precursor cell populations [28,29]. *Mef2ca* mRNA accumulates later, in parallel with myosin expression, in several distinct myocyte populations as each begins to elongate and assemble myofibrils [29]. *mef2aa* and *mef2cb* are expressed in skeletal muscle after *mef2d* [30–36], but are not fully described. Expression patterns