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The *Xenopus* MEF2 gene family: Evidence of a role for XMEF2C in larval tendon development

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ABSTRACT

MEF2 transcription factors are well-established regulators of muscle development. In this report, we describe the cloning of multiple splicing isoforms of the XMEF2A and XMEF2C encoding genes, differentially expressed during *Xenopus* development. Using whole-mount in situ hybridization, we found that the accumulation of XMEF2C mRNA in the tadpole stages was restricted to intersomitic regions and to the peripheral edges of hypaxial and cranial muscle masses in contrast to XMEF2A and XMEF2D, characterized by a continuous muscle cell expression. The XMEF2C positive cells express the bHLH transcription factor, Xscleraxis, known as a specific marker for tendons. Gain of function experiments revealed that the use of a hormone-inducible XMEF2C construct is able to induce Xscleraxis expression. Furthermore, XMEF2C specifically cooperates with Xscleraxis to induce tenascin C and betaig-h3, two genes preferentially expressed in *Xenopus* larval tendons. These findings 1) highlight a previously unappreciated and specific role for XMEF2C in tendon development and 2) identify a novel gene transactivation pathway where MEF2C cooperates with the bHLH protein, Xscleraxis, to activate specific gene expression.

Introduction

The myocyte enhancer factor 2 (MEF2) family of MADS (MCMI, agamous, deficiens, serum response factor)-box transcription factors has four members in vertebrates, MEF2A, -B, -C and -D. Each MEF2 gene gives rise to multiple isoforms through alternative splicing (Black and Olson, 1998). MEF2 proteins bind to a conserved A/T-rich sequence known as a MEF2 site as homo- and hetero-dimers and combinatorially through protein–protein interactions with other transcription factors, to function as both positive and negative regulators of specific sets of target genes. The four MEF2 genes exhibit overlapping but distinct expression patterns in embryonic and adult tissues (Edmondson et al., 1994) and play a pivotal role in morphogenesis and myogenesis of skeletal, cardiac and smooth muscle cell differentiation. These transcription factors regulate also neuronal and immune cell differentiation (Potthoff and Olson, 2007). MEF2 proteins bind directly to the promoters or enhancers of the majority of muscle specific genes and interact with members of the MyoD family of basic helix–loop–helix (bHLH) proteins to activate the skeletal muscle differentiation program (Naya and Olson, 1999; Paris et al., 2004; Blais et al., 2005). Murine gene disruption studies provide genetic evidence for a discrete specific function of each MEF2 isotype. Thus, Mef2c null mice die from embryonic day 9.5 from abnormal cardiovascular development (Lin et al., 1997, 1998) whereas Mef2a null mice die perinatally from a spectrum of heart defects (Naya et al., 2002). However, distinct roles for each MEF2 gene still remain to be fully elucidated. In mice, two unexpected roles for MEF2C have been recently identified in both chondrocyte hypertrophy (Arnold et al., 2007) and craniofacial development (Verzi et al., 2007).

In *Xenopus*, two MEF2 cDNAs, SL1 (MEF2D) and SL2 (MEF2A), have been previously characterized (Chambers et al., 1994), but the function of MEF2C remains unknown. In this report, we describe the cloning of multiple splicing isoforms of the XMEF2A and XMEF2C encoding genes, differentially expressed during development and we show that the accumulation of XMEF2C mRNA in the tadpole stages was restricted to intersomitic regions and to the peripheral edges of hypaxial and cranial muscle masses. These cells express the bHLH transcription factor, Xscleraxis, a specific marker for tendons and ligaments (Schweitzer et al., 2001). Most of these cells express tenascin C and tenomodulin, two known structural markers for tendons (Tozer and Duprez, 2005), but also betaig-h3, a connective tissue expressed gene (Ferguson et al., 2003). We show that the use of a hormone-inducible XMEF2C construct is able to induce Xscleraxis expression. Furthermore, XMEF2C specifically cooperates with Xscleraxis to induce tenascin C and betaig-h3, two genes preferentially expressed in *Xenopus* larval tendons. These findings highlight a previously unappreciated and specific role for XMEF2C in tendon development and reveal a novel gene transactivation pathway where MEF2C cooperates with the bHLH protein, Xscleraxis, to activate specific gene expression.

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Experimental procedures

Animals

Adult *Xenopus laevis* were maintained at 20 °C in tap water and fed twice a week.

Microinjection and dexamethasone induction

All pSP64T plasmids used for microinjection were linearized with Xba I except for the plasmid coding for nuclear beta-galactosidase which was linearized with Xho I. Capped mRNAs were produced in vitro from linearized plasmids using the SP6 message machine kit (Ambion). *Xenopus* embryos were injected unilaterally at the two-cell stage at the marginal zone level with 100 to 500 pg of synthetic mRNA. For X-gal staining, embryos were coinjected with synthetic mRNA coding for nuclear beta-galactosidase. When embryos were injected with hormone-inducible XMEF2C or XMEF2D mRNAs, the induction by 10 μM dexamethasone (Sigma) was performed at stage 20 until embryos were fixed at stages 30–32.

Cloning of XMEF2 isoforms

Total RNA was extracted with a mini RNAeasy kit (Quiagen) and the first strand cDNA was synthesized by Impront II reverse transcriptase (Promega) at 37 °C for 1 h with oligo(dT) priming from 1 μg total RNA from stage 15 and 40 embryos. PCR was performed using a Taq and Pfu polymerase mix. Xscleraxis cDNA was amplified with sens 5′-GAGAG-TTCCCAGCAGTTGGCA-3′ and antisens 5′-AGGAATGTCCATTCTTCCC-TCA-3′ primers which were designed from *Xenopus tropicalis* genomic sequence. The MEF2 isoforms were obtained with the following primers (sense and antisense respectively): 5′-AAAGTTGCTG-TAGCTGGCAA-3′ and 5′-TACACTGAGGCCTAATGCAT-3′ for XMEF2A, 5′-GGTGCCCAAGTAGAGCAGAG-3′ and 5′-GGTCTGCCACTTTCGGGCAA-3′ for XMEF2C and 5′-TTCACTGCAAGGTCTTCGCT-3′ and 5′-TTTGAG-CAAGAGGTAAGGCA-3′ for XMEF2D. PCR products were ligated into

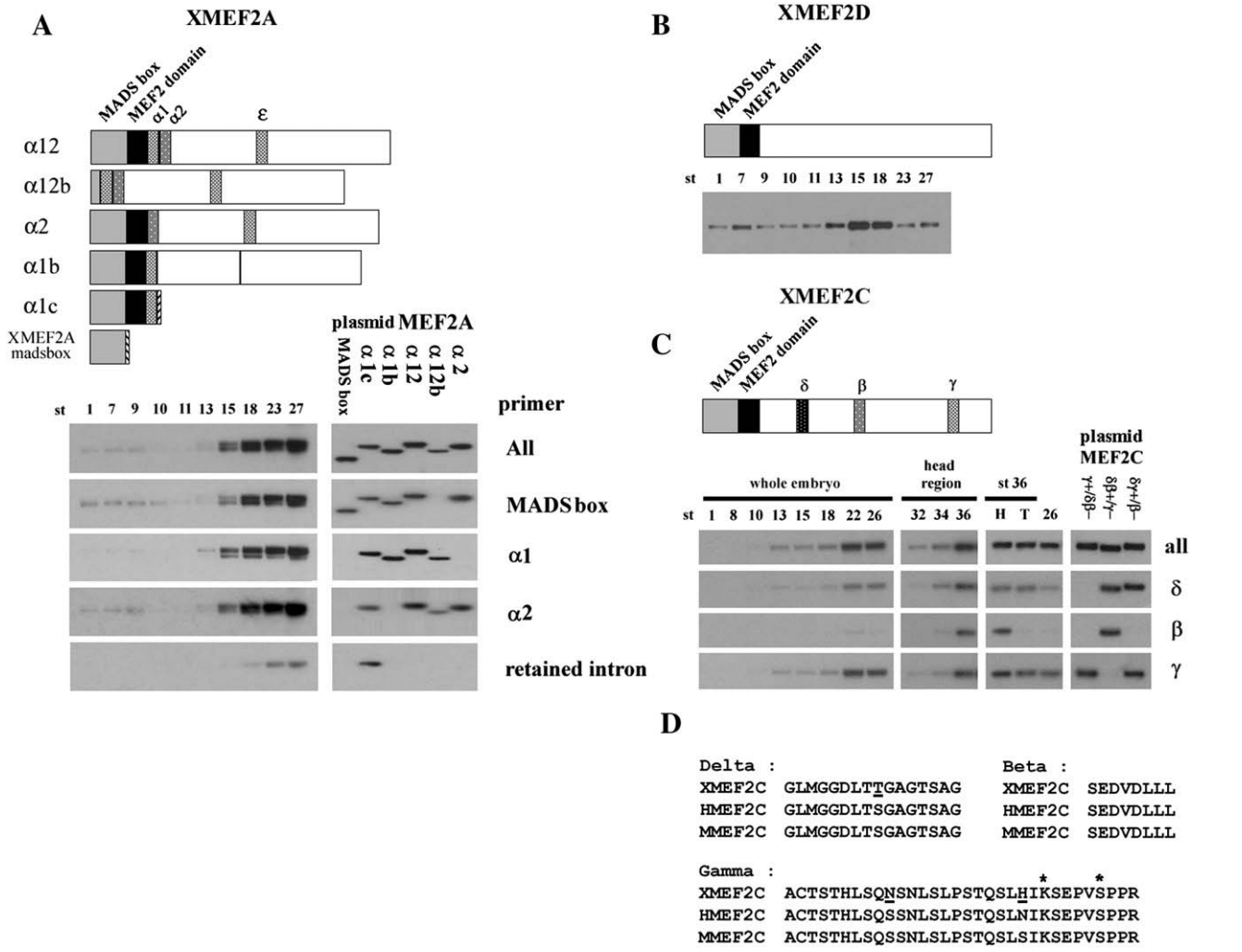

Fig. 1. Developmental expression profile of different alternatively spliced exons of XMEF2A, XMEF2D and XMEF2C. (A) Schematic structure of different alternatively spliced XMEF2A gene products and developmental expression profile of different alternatively spliced exons (MADS box, α1, α2, retained intron) by semi-quantitative PCR, submitted to Southern blot. ODC was used as an internal standard control of quantification, no variation between different stages was observed after hybridization (data not shown). The primer "all" recognizes the first exon which is not alternatively spliced. Plasmid PCR products were used to check the hybridization reaction specificity and as internal standard for comparison of signal intensity after hybridization with the different labeled primers. (B) Schematic structure of the XMEF2D gene product and developmental expression profile of XMEF2D mRNA. (C) Schematic structure of different alternatively spliced XMEF2C gene products and developmental expression profile of different alternatively spliced exons (δ, β and γ) in whole embryo, head (H) or trunk and tail regions (T). The primer "all" recognizes the MADS box sequence which is not alternatively spliced. Since the PCR reactions were not exactly performed under the same conditions, no relevant comparison could be drawn on the basis of the hybridization signal intensity between distinct blots for XMEF2C. Comparison between different primers can be made since plasmid control can be used as internal control for signal intensity. The numbers refer to the developmental stages. st, stage. (D) Comparison of amino acid sequences for *Xenopus* (X), human (H) and mouse (M) alternatively spliced exons of MEF2C. Variations in amino acid sequence are underlined. Asterisks designs lysine 391 and Serine 396 in exon γ of human sequence.