Int. J. Dev. Biol. 51: 315-320 (2007)
doi: 10.1387/ijdb.062260hz

Short Communication

Myoskeletin, a factor related to Myocardin, is expressed in somites and required for hypaxial muscle formation in *Xenopus*

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ABSTRACT ***Myoskeletin*** **was identified as a gene induced by activin in animal cap explants of** ***Xenopus laevis*****. This gene encodes a protein related to the transcription factor Myocardin. Whereas Myocardin is expressed in the heart and is known to be involved in heart and smooth muscle formation, Myoskeletin is expressed in the somites and in hypaxial muscle precursors as they migrate away from the somites during tadpole stages. Myoskeletin is required for hypaxial muscle formation, as reduction of its expression through injection of an antisense morpholino oligonucleotide leads to suppression of hypaxial muscle formation. In overexpression experiments in animal caps, Myoskeletin is capable of inducing multiple genes including skeletal muscle, cardiac muscle and smooth muscle-specific genes. We conclude that Myoskeletin is a somite and hypaxial muscle-specific member of the Myocardin family that is required for hypaxial muscle formation.**

KEY WORDS: *myocardin, skeletal muscle, hypaxial muscle, somite*

In the early development of the *Xenopus laevis* embryo, mesodermal cells are specified through inductive processes that depend on cell-cell signaling factors. Signaling by TGF-β-related factors of the nodal/activin group is essential in this process (Heasman, 2006; Wardle and Smith, 2006; Whitman, 2001). Some aspects of the complex processes that lead to mesoderm specification and patterning can be recapitulated in a culture system in which explants derived from the animal region of the blastula, so-called animal caps, are exposed to activin. While untreated animal caps develop as atypical ectoderm, exposure to activin leads to differentiation of multiple mesodermal derivatives (Asashima *et al.,* 1990; Smith, 1987; Smith *et al.,* 1990). We have made use of this system to survey the global changes in gene expression that take place during mesoderm induction by activin with the aid of the DNA microarray technology. Among the genes whose expression is strongly induced by activin in the animal cap system we identified a novel member of a transcription factor family that plays a role in the differentiation of muscle, a major derivative of the mesoderm.

Muscle formation has been extensively studied in multiple organisms. Several classes of transcription factors are involved in the process of the specification and differentiation of this tissue and in the elaboration of the various muscle subtypes such as skeletal, cardiac and smooth muscle. The joint action of varied combinations of factors from different families in the regulation of many muscle-specific genes provides a prime example for the combinatorial principle in gene regulation in general (Davidson, 2006). Several members of the basic helix-loop-helix (bHLH) class of factors are involved in muscle differentiation, including MyoD, Myf5, myogenin and MRF4 (Berkes and Tapscott, 2005; Pownall *et al.,* 2002; Tapscott, 2005). While early studies were strongly focused on the bHLH class of factors, other transcription factor families are likewise essential in this process. Mef2, a MADS-box (MCM-1, Agamous, Deficiens, SRF) family protein, is required for muscle formation and specifically for the transcriptional regulation of multiple muscle-specific genes (Naya and Olson, 1999). In addition, SRF (Serum Response Factor) has an important role in muscle formation, as reported in the *Xenopus* system some time ago (Mohun *et al.,* 1991). As the ubiquitous expression of SRF raises questions about its specific role in muscle cell differentiation, it was particularly interesting when a

Abbreviations used in this paper: bHLH, basic helix-loop-helix; MyoC, myocardin; MyoS, myoskeletin; SRF, serum response factor.

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0214-6282/2007/$30.00

Printed in Spain
www.intjdevbiol.com

cell type specific partner protein of SRF was discovered. Myocardin (MyoC), as this factor was named, binds SRF, leads to strong gene activation and has a specific role in the differentiation of cardiac and smooth muscle cells (Chen *et al.,* 2002; Small *et al.,* 2005; Teg Pipes *et al.,* 2006; Wang *et al.,* 2001; Wang and Olson, 2004). Clearly, SRF also has a role in skeletal muscle differentiation, but MyoC is not expressed in these cells and two related proteins, MRTF-A and MRTF-B, also do not appear to have a specific role in skeletal muscle (Teg Pipes *et al.,* 2006). Here we report the identification, among multiple genes induced by activin in animal caps, of an additional member of the MyoC family with strong and specific expression in skeletal muscle. During progress of this work we learned of related experiments by S. M. Meadows, A. S. Warkman, E. M. Small and P. A. Krieg (personal communication) and in agreement with these authors we name this protein Myoskeletin (MyoS). Our results suggest that MyoS is critical for the formation of the hypaxial muscles, a set of muscles that derive from the somites and migrate to form the body wall and limb musculature (Buckingham *et al.,* 2003). The discovery of MyoS fills an apparent gap in our understanding of the manner in which SRF controls the differentiation of various types of muscle cells.

Isolation and characterization of MyoS

In an effort to identify novel genes involved in the induction of mesodermal tissues in the *Xenopus* embryo we compared the transcriptome of control animal caps with that of animal caps that were treated with activin, using DNA microarray technology. A large number of genes were upregulated in this experiment, among them a gene with sequence similarity to the previously identified *Xenopus MyoC* gene (Small *et al.,* 2005); the new gene is named *MyoS* (see above). The increase in *MyoS* expression after activin induction was about 20-fold in four repeats of the microarray assay.

The predicted MyoS protein shows highest overall sequence similarity to *Xenopus* MyoC, with somewhat lower similarity to the related proteins MRTF-A and MRTF-B (Table 1). An alignment of the four MyoC family members in *Xenopus* is shown in Figure 1. Much of the sequence identity is concentrated in putative conserved domains including basic, glutamine(Q)-rich, SAP (SAF-A/B, Acinus and PIAS) and leucine zipper domains; the basic and Q-rich domains are embedded in a larger region of high sequence conservation. MyoS contains most of the conserved functional domains of the MyoC family including the basic and Q-rich domains that mediate interactions between MyoC and SRF (Teg Pipes *et al.,* 2006). MyoS thus is likely to represent a new functional member of the Myocardin factor family.

Embryonic expression of MyoS

MyoS RNA is present as a maternal component in the egg, decreases during early to mid gastrula and subsequently increases through tailbud and tadpole stages (Fig. 2A). The maternal *MyoS* expression does not show a distinct pattern so that *in situ* hybridization through gastrulation is not effective in visualizing this RNA (Fig. 2B). Staining becomes intense in the forming somites during neurula stages and intensifies in somites and presomitic mesoderm at least up to stage 41 (Fig. 2C-E). At about stage 37/38, MyoS expression is also seen clearly in the hypaxial muscles and in discrete regions in the head (Fig. 2E). The hypaxial muscles, which will form the body wall and limb musculature, arise in *Xenopus* from about stage 26 in the ventro-lateral areas of the somites, become more clearly defined by stage 28/29 and begin to migrate from the somites during subsequent development (Martin and Harland, 2001; Martin and Harland, 2006). Because of the strong expression of *MyoS* in the somites, initial stages of its expression in hypaxial precursors cannot be distinguished, but *MyoS* RNA is clearly visualized when these cells leave the somites (Fig. 2D). The expression pattern of

```
                                                                       60
xMyoS_    --------------------------MTLLASERSMLIRS--KFRSVLQLRMQHRRSQEQ
xMyoC_    --------------------------MTLLGSEHSLLIRN--KFRSVLQLRLQQRRSREH
xMRTF-A   --------------------------MTLLDTEQALL-----AIHTVLQLKLQQRRTREE
xMRTF-B   MIDSSKKQQQQPPQHYSFADILSAGDLDPLKEKEWLDPGSQKSLKEVLQLRLQQRRTREQ

                                                                      120
xMyoS_    MSERNLIPSPVVKKTPAPFPEQNGNLGQNKVEDFMKIKGQNKLHKVAALKMHFPED----
xMyoC_    LVSQGLMP-PL--KNPAAFQEQRKNVDRAKAEDYLKHKIRSRP---EILNMQILQDPANE
xMRTF-A   LENQGIMP-PL--KSPAAFHEQRRSLERARTEDYLKRKIRSRPERAELVRMHILEETSAE
xMRTF-B   LVDQGIMP-PL--KSPAAFHEQIKSLERARTENFLKHKIRSRPNRSELVRMHILEETLAE

                                                                      180
xMyoS_    ------QLQIS-----------------P-------S-----------------S----D
xMyoC_    SSAQAAQIKLKRARLADDLNERIALRPGPLELVEKNIIPVESTVKEVFKGNQVNFSKSVD
xMRTF-A   PSLQAKQIKLKRARLADDLNEKISQRPGPMELVVKNILPVETSLKEVIID--VDYPEVVD
xMRTF-B   PSLQATQLKLKRARLADDLNEKIAQRPGPLELVEKNILPVDLSVKEAITVSQTNLPENLD

                                                                      240
xMyoS_    AFSFEDDIS----SSS-SSSTSSSPRFAPSPGLSLNLSPTSTNTVFQLDLPQIIEVNQPN
xMyoC_    AFAFEEDSSNDGLSPEKEPSENSPVLNKASFQETKDLETLN-SLHSGLTQNHSQEHDSDA
xMRTF-A   NSSFDEDSS-DALSPE-QPASQESQGSIPSPIENRPSETTQIPALSPSHAFSCVQFGTDA
xMRTF-B   TLSFDEDSS-DALSPE-QPASQESQGSAASPGEMKTSDSSS-PVSNTTIQCQTVSSPLPD

                                                     basic domain  300
xMyoS_    VRTV-TEAETLVTSRPATHNPTQASSTVPKVTVKPSDVGKIQRPKKPKDTKPKVKKLKYH
xMyoC_    QDGSSIQSHSCSLHSESQLSPSMSASAAVKSKSPI--DVKN-RHKKTKDIKPKVKKLKYH
xMRTF-A   FNQDSLQSTAITISNGLTASICKSLPALVKQSQPKPSFEKSQRIKKPKEPKPKVKKLKYH
xMRTF-B   FFKP-VPTADLTTRSPLSCIVSKPGPALIKQTQPK-HTEKP-RSKKSKDPKPRVKKLKYH

                                        Q-rich domain                 360
xMyoS_    QYIPPDQKAEKAPVAMDAAYSRLLQQQQIFLQLQILNQQQN-PTFCVQTVHPLTTS-IPA
xMyoC_    QYIPPDQKAEKSPPPMDSAYARLLQQQQLFLQLQILSQQQQQQHFGYTGMHHSQLK-LPN
xMRTF-A   QYIPPDQK-QKGTPAMDSSYAKLLQQQQLFLQLQIINQQQH-QHYNYQTILPAPPKPLPD
xMRTF-B   QYIPPDQKGEKIEEEMDSNYARLLQQQQLVLQLQILSQ----QHSTLTTSRKSYPAPLKS

                                                                      420
xMyoS_    DQVISFTGAPPSSAPAINLSPAPGTAAVTTAPTSTVPSPMKTEMLPANIDDLTVSELRQH
xMyoC_    DLIRNS-NTSSVNSPS--LSP---VKTTFSGQAN-VSMK--AGLLPSNLDDLKVSELRQQ
xMRTF-A   QQNTNSSSTTTVRSMST-VAPSTLATPTITRQNSNVAVGGRTGPLPHNLDEMKVAELKLE
xMRTF-B   QKKQNTINTTICNGNAG-APP---AQCSVNRQNS-VPCK-KTGPLPSSLDDMKVAELKME

            SAP domain                                                480
xMyoS_    LRKRGLPVSGTKPSLLERLRPYQIPRAK----TIPAPIQSAGLMTPIIELSAFPKQSVCD
xMyoC_    LRIRGLPVSGTKTSLMERLRPFQECSGN-TVPTYGEITTVTFPVTPNGTLSGYQSHASAG
xMRTF-A   LKHRGLPVSGTKIDLIERLKASQDPST-----ATAASAKPT-PVQQAKPPEVVPIVSSSC
xMRTF-B   LKLRGLPVSGTKMDLIERLKPFQDFSSNGVSPSSANTVNITNPACNTTDDATTAFSTSAL

                                                                      540
xMyoS_    STVPTLCTFQTVPS----------P-PSGEVPQETS---------E-----T--ACSMPE
xMyoC_    MLSNGFYQFGSTSS---------TP-PISPASSDFS-VSG---S-LPDTFSDG-PMSSPQ
xMRTF-A   LTTREPIKLCSTSS---------TP-PGSPCPSEVSVVSMDEVSMISDALGETVACPVTQ
xMRTF-B   INSSSPTPSVSIGNNQTMLDGINSPLPMSPTPSEQSNFSSEDTN-ITDTFAEILTMMSPS

                                                        zinc finger   600
xMyoS_    STEMPAT---VQDK---DTP--------MQDIE--DDHVLMEKQKVIENLTWKLKQEQKQ
xMyoC_    FGLHPSPIHLSAEESLMNSMNSGTYQVELEGIDAERDKMLVEKQKVINELTWKLRHEQRQ
xMRTF-A   QVQ-QNP---AAEK----SP----P----DARD--KDLMLREKDRQIEELTQRLKQKQEL
xMRTF-B   QFMNTSPL-KVNEDSMGATPGN-TPNVELDAVE--KDRKLQEKEKQIEELKRKLEQEQKL
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MyoS is thus distinct from that of other members of the family, none of which is expressed in somites and hypaxial muscles (Teg Pipes *et al.,* 2006). Further, the heart, which is the site of strong and specific *MyoC* expression (Small *et al.,* 2005), is entirely negative for *MyoS* at all stages examined.

Interference with MyoS expression inhibits hypaxial muscle formation

To obtain insight into the function of MyoS in embryonic development we used an antisense morpholino oligonucleotide (MO) designed to bind to the region surrounding the initiation codon of the protein (see Experimental Procedures). MyoS-MO or a control MO (C-MO) was injected into the equatorial region of both 2-cell stage blastomeres. Injection of 20 ng MyoS-MO into each blastomere led to absence or strong reduction in the formation of hypaxial muscles as visualized by the antibody 12/101 (Kintner and Brockes, 1984), whereas the C-MO-injected embryos were normal (Fig. 3A,B). No substantial inhibition on the formation of somites was observed. As *MyoS* is strongly expressed in the hypaxial muscles we used this gene as an additional marker for the differentiation of these groups of cells. As seen in Figure 3C,D, injection of MyoS-MO, but not C-MO, strongly inhibited the expression of *MyoS* in the hypaxial muscles. An attempt was made to rescue hypaxial muscle differentiation after MyoS-MO injection by coinjection of mRNA in which the recognition site was modified (see Experimental Procedures). In this experiment, in which 20ng MO was injected into one blastomere at the 2-cell stage, the fraction of embryos that developed hypaxial muscles was substantially increased when mutated mRNA was coinjected with MyoS-MO (Fig. 4A,B).

TABLE 1

SEQUENCE SIMILARITY IN MYOCARDIN FAMILY PROTEINS

	MyoS	MyoC	MRTF-A	MRTF-B
MyoS		30	26	24
MyoC	30		30	31
MRTF-A	26	30		38
MRTF-B	24	31	38	

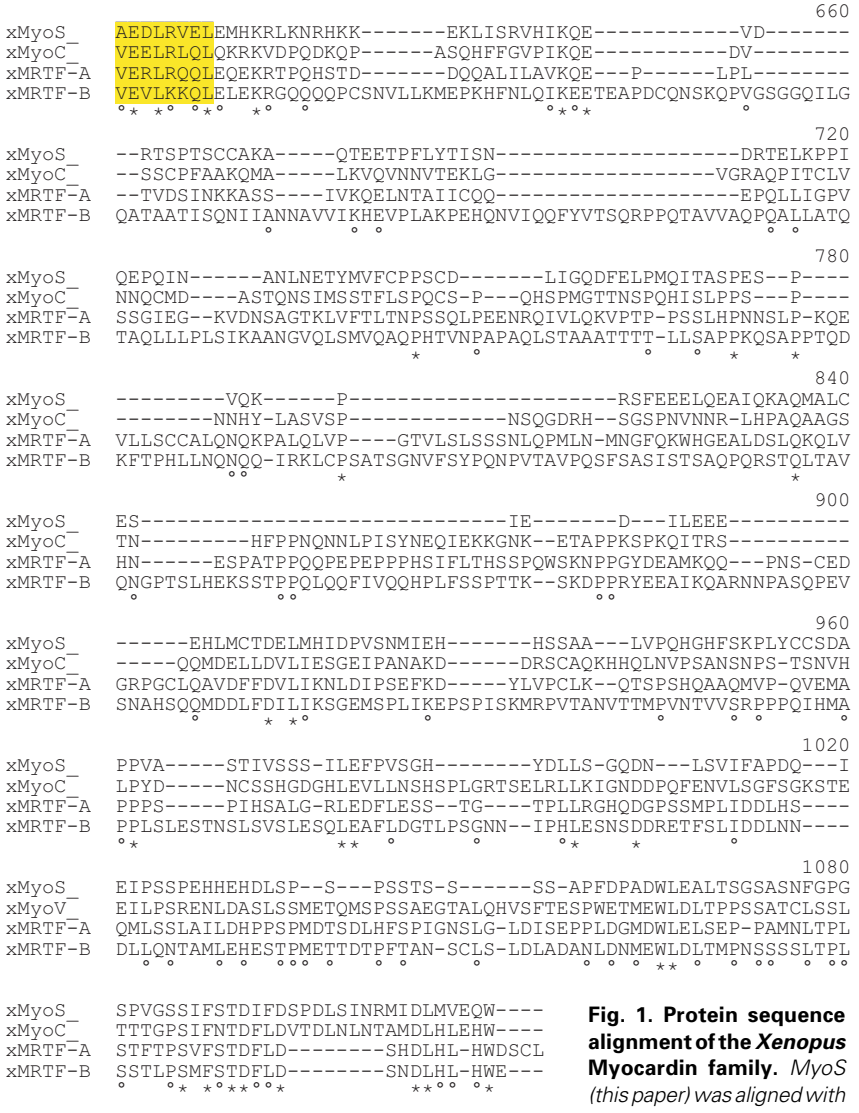


Fig. 1. Protein sequence alignment of the *Xenopus* Myocardin family. *MyoS (this paper) was aligned with MyoC (AAT90392) (Small* et al., *2005), MRTF-A (Q8AYC2) and MRTF-B (Q8AYC1). Residues conserved in all sequences are indicated by an asterisk, those conserved in three sequences by a small circle. Conserved motifs are indicated, including the basic, Q-rich, SAP and Zinc Finger domains.* [The first part of this fig. begins on the previous page.]

The origins of the head musculature are complex. As described in the chick, most of these muscles arise from non-segmented head mesoderm (Noden and Francis-West, 2006). In *Xenopus*, Martin and Harland (2005) showed that certain head muscles arise from cells that migrate from anterior somites in a similar way as hypaxial cell precursors. The partial sensitivity of head muscles to MyoS-MO may be due to their multiple developmental origins.

The experiments described above indicate that MyoS function is required for the formation of hypaxial muscles in the *Xenopus* embryo. The lack of effect of the MyoS-MO on somitic muscle differentiation might indicate that hypaxial muscles are more sensitive to diminished MyoS concentrations, while somitic muscle may require only low levels of this factor which might persist in the MO-injected embryos.

MyoS can activate many muscle-specific genes in animal explants

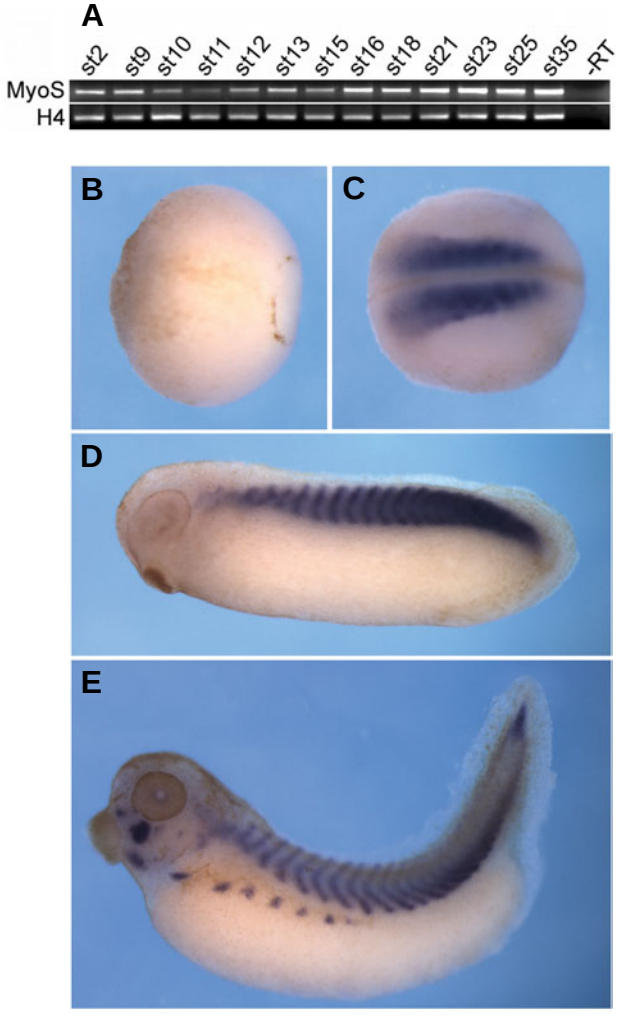
Animal explants are suitable for testing the ability of a transcription factor to activate the expression of various genes. We used this system to examine the ability of MyoS to induce the expression of muscle-specific genes that are not expressed in untreated animal caps. As seen in Fig. 5, injection of 1 or 2ng *MyoS* RNA into the animal region of the embryo led to the induction of SRF, the putative partner of MyoS. This activation suggests that a positive feedback loop is activated by *MyoS* expression. Further, some regulatory genes involved in

muscle formation were induced including *Nkx2.5* and, rather weakly, *Mef2A*. In contrast, *MyoD* and *Myf5* were not induced at a detectable level. Likewise, other members of the MyoS family, *MyoC*, *MRTF-A* and *MRTF-B* were not induced. Genes characteristic for differentiating muscle cells of different types were activated, including skeletal and cardiac *actin*, cardiac *troponin* and smooth muscle genes including *actin* and *SM22*. In contrast several *myosin* chain genes were induced weakly or not at all. It is notable that cardiac and smooth muscle genes were induced effectively even though MyoS is not expressed in the heart or in smooth muscle cells, at least not during embryogenesis up to stage 41. In distinction to these results, MyoC activated smooth muscle and cardiac muscle, but not skeletal muscle genes after ectopic expression in animal explants (Small *et al.,* 2004). Our observations may imply that under conditions of overexpression, MyoS cross activates target genes specific for the related factor MyoC. Similar observations have been reported for other members of the Myocardin family (Du *et al.,* 2004; Selvaraj and Prywes, 2004; Wang *et al.,* 2002).

Activation of several muscle-specific genes in animal explants in response to *MyoS* RNA injection did not lead to elongation of the explants (Fig. 6). Elongation is usually observed when mesoderm is induced by signaling factors such as activin and involves the activation of the *Xbra* gene, which is not activated by *MyoS* injection (Fig. 5A). Thus *MyoS* overexpression appears to activate only a portion of the genetic program that leads to full tissue differentiation.

MyoS is a novel transcription factor involved in muscle differentiation

The results reported in this paper indicate that Myoskeletin is a novel member of the transcription factor family of which the founding member is Myocardin. As the expression of *MyoS* and *MyoC* is non-overlapping in the *Xenopus* embryo it is likely that these two related factors have similar functions in the regulation of muscle gene expression in different types of muscle. As the role of MyoC in cardiac and smooth muscle differentiation has been well documented (Chen *et al.,* 2002; Small *et al.,* 2005; Teg Pipes *et al.,* 2006; Wang *et al.,* 2001; Wang and Olson, 2004), the question arose whether skeletal muscle can be formed without participation of a factor of this class. It is now clear that somites and hypaxial precursors express a member of the MyoC family, MyoS, during their differentiation, suggesting that MyoS is involved in the regulation of muscle-specific gene expression in these cells. This suggestion could be supported by the finding that a morpholino antisense oligonucleotide targeting MyoS can suppress the formation of hypaxial muscles in the embryo. The reason why we did not observe a reduction in somite formation in the MO-injected embryos is not presently clear. It is possible that low levels of MyoS that persisted in the Mo-injected embryos were sufficient for somite differentiation. Alternatively an additional, as yet undiscovered factor in the MyoC family, might compensate for the reduction of MyoS levels in the somites after MyoS-MO



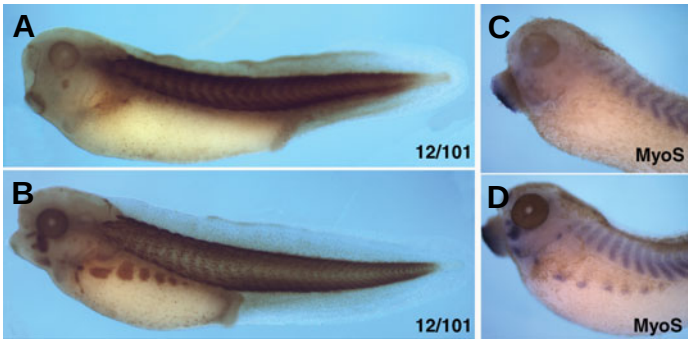
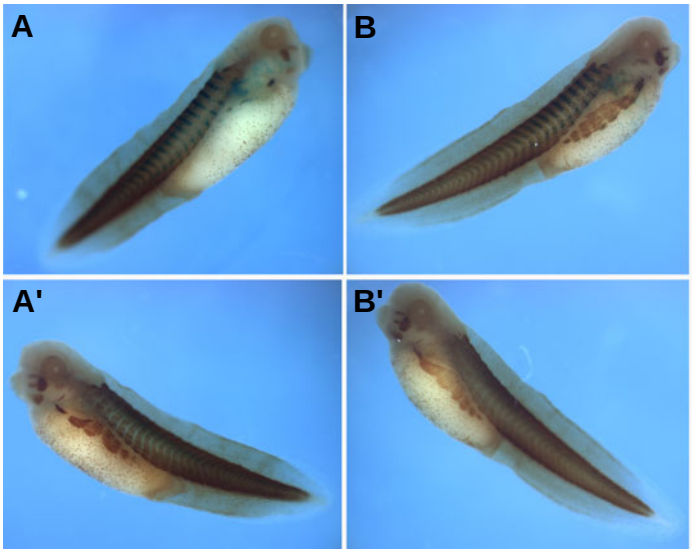


Fig. 2 (Left). Expression pattern of *MyoS*. (A) MyoS *expression assayed by RT-PCR. (B-E)* In situ *hybridization. No pattern of expression is seen at stage 10.5* **(B)**, *but strong expression in the somites is apparent as stages 18* **(C)**, *24* **(D)** *and 37/38* **(E)**. *At stage 37/38, strong expression is also seen in hypaxial muscles and in head musculature.*

Fig. 3 (Right). MyoS is required for hypaxial muscle differentiation. *MyoS-MO* **(A,C)** *or Control (C)-MO* **(B,D)** *were injected at a level of 20 ng into each blastomere at the 2-cell stage. (A,B) Staining with 12/101 antibody at stage 39. (C,D)* In situ *hybridization with* MyoS *probe at stage 33/34. Strong reduction or complete absence of antibody staining was seen in 31/31 MyoS-MO-injected embryos* **(A)**, *while 34/37 C-MO-injected embryos showed intense staining equivalent to that in uninjected embryos* **(B)**; *three separate experiments were carried out. Using* MyoS *staining, 27/27 embryos showed strong suppression* **(C)**, *while 35/36 C-MO-injected embryos showed normal expression* **(D)**.



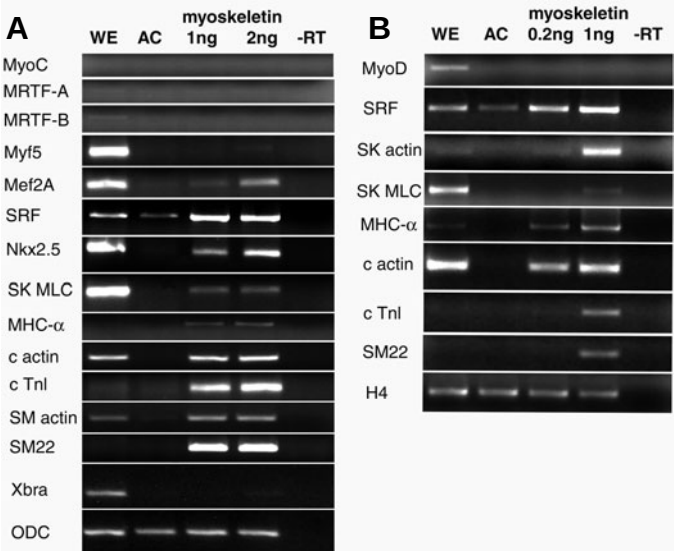


Fig. 4 (Left). Rescue of hypaxial muscle differentiation. *Embryos were injected into one side with MyoS-MO (20 ng) or MyoS-MO plus* MyoS *mRNA (0.5 ng); the injected side was marked by* lacZ *RNA. Embryos were stained with 12/101 antibody and for β-galactosidase.* **(A)** *MyoS-MO, injected side (3/16 or 20% of embryos showed medium to strong staining, 13/16 weak to negative staining);* **(A')** *uninjected side (16/16 strong staining).* **(B)** *MyoS-MO plus mut-*MyoS *mRNA, injected side (8/13 or 60% medium to strong staining, 5/13 weak to negative);* **(B')** *uninjected side (13/13 strong staining).*

Fig. 5 (Right). MyoS induces muscle genes in animal explants. MyoS *RNA was injected into the animal region of 2-cell embryos, animal caps were dissected at blastula, cultured to equivalent stage 12.5 and the expression of various genes was assayed by RT-PCR. In (A), 1 and 2ng mRNA was injected per embryo, in (B) the doses were 0.2 and 1ng. AC, uninjected animal caps; -RT, without reverse transcriptase.*

injection, but might be absent or insufficient in hypaxial muscle precursors. Irrespective of these considerations it is likely that MyoS does have a role in the differentiation of muscle cells that arise in the somites during *Xenopus* embryogenesis.

Experimental Procedures

For microarray analysis, animal caps from stage 8/9 embryos were cultured with or without addition of 500pM activin until sibling embryos reached stage 11.5, for about 4hr. The explants were homogenized with Stat-60 (TEL-TEST), RNA was precipitated by isopropanol, purified using the RNeasy (Qiagen) system and treated with DNase I. Biotinylated probe was prepared with the Enzo RNA Transcript Labeling Kit following the protocol from Affymetrix. The probes were hybridized to Affymetrix *Xenopus* chips and the results analyzed using the GCOS1.1 and JMP5.1 software. Labeling and hybridization were repeated with four independent preparations of activin-treated and control RNA. Details of these experiments will be presented elsewhere.

The sequence of *myoskeletin* is based on the Image cDNA clone number 4959565, Est BM179219. The *myosleletin* sequence has been submitted to GeneBank under Accession number EF175167.

Embryo handling, whole mount *in situ* hybridization and antibody staining followed standard procedures. RNA for microinjection was prepared by the mMessage mMachine kit (Ambion) and MOs were obtained from Gene Tools. The sequence of the MyoS-MO is CGCTCTGAGGCCAGCAGGGTCATCT, the Control-MO is CCTCTTACCTCAGTTACAATTTATA. To generate mut-*MyoS* mRNA for use in rescue experiments, the sequence of the first 23 nts of the ORF was changed from ATGACCCTGCTGGCCTCAGAGCG to ATGACACTTCTGGCGTCCGAGCG. These changes generate four mismatches with the MyoS-MO but do not change the encoded amino acid sequence. MO against *MyoS* or an unrelated control (C) MO was injected into the equatorial region of both blastomeres at the 2-cell stage at a level of 20ng per cell. For RNA co-injection, MyoS RNA was mutated to abolish the target region while leaving the coding capacity unchanged; 20ng MyoS-MO or C-MO plus 0.5ng RNA were injected into one blastomere at the 2-cell stage together with 100ng β-galactosidase RNA to mark the injected side. Embryos were analyzed by *in situ* hybridization with MyoS at stages 33/34 and 39 and by staining with monoclonal antibody 12/101 (Developmental Studies Hybridoma Bank, University of Iowa) at stages 39-40.

For explant studies, RNA was injected into the animal region at the 2-cell stage, animal caps dissected at stage 9 and the explants harvested at equivalent stage 12.5; incubation time was approximately 6 hr. RNA was extracted as described above and assayed by RT-PCR analysis. PCR primers used in this assay were those described by (Small *et al.,* 2005), except for *cardiac α-actin* (Niehrs *et al.,* 1994), *MyoD* (Schohl and Fagotto, 2003) and *H4*, *ODC*, *Xbra* and *Myf5* (http://www.hhmi.ucla.edu/derobertis/index.html).

Acknowledgment

We thank Stryder Meadows and Paul Krieg for making information available before publication. This work has been supported by the

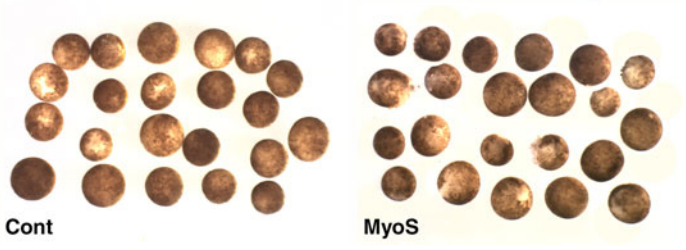


Fig. 6. Appearance of control explants and explants injected with 1 ng *MyoS* RNA. *The animal explants were cultured in the same way as the explants in Fig. 5. No elongation was seen in these explants.*

intramural research program of the National Institute of Child Health and Human Development, National Institutes of Health.

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Received: 12th December 2006
Reviewed by Referees: 12th January 2007
Modified by Authors and Accepted for Publication: 21st February 2007
Published Online: 4th May 2007

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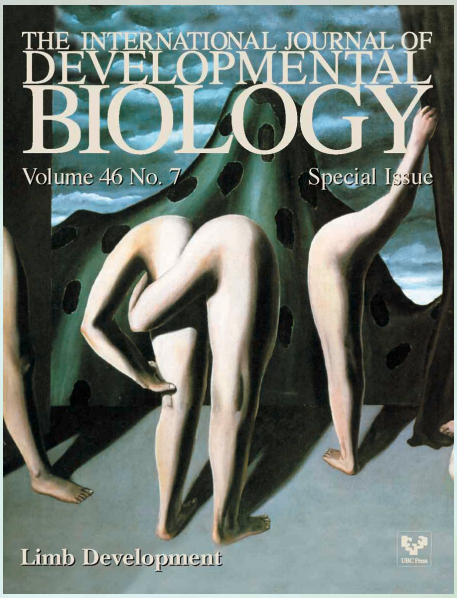

Central motif of a magic realist painting portraying animate, isolated limbs as individuals. This picture entitled "Intermission" (1928) was painted by the Belgian surrealist René Magritte (1898-1967). Oil on canvas. Private collection.

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