EXPRESSION OF MRF4 IN CRANIAL REGIONS OF *XENOPUS LAEVIS* EMBRYOS

Myogenic regulatory factors (MRFs) are transcription factors that regulate gene expression in skeletal muscle cells. In mammals, expression of the four genes that make up the MRF family, MyoD, Myf5, myogenin and MRF4, is largely confined to the skeletal muscle cell lineage. The frog *Xenopus laevis* is an attractive model system for studying MRF expression and the roles of these genes during embryogenesis. The embryonic cell populations that express these genes, particularly MRF4, are not, however, precisely known in *Xenopus*. Published *in situ* hybridization results have shown MRF4 mRNA in somites, but studies in our laboratory also show MRF4 expression in the anterior region, possibly in the eye primordia and the brain. To confirm this, we dissected embryos into ventral, anterior, and dorsal regions and assessed MRF4 mRNA from each region by reverse transcription polymerase chain reaction (RT-PCR). Our results indicate that MRF4 is expressed in the anterior region at a level comparable to that found in the dorsal region where the somites are located. This suggests that MRF4 may be involved more broadly in cell differentiation in *Xenopus laevis* than it is in mammals.

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BACKGROUND

MRFs control gene expression during specific cellular events (eg, myoblast specification, myofiber differentiation, and myofiber maintenance, hypertrophy, repair, or regeneration) but their individual roles in these events remain incompletely understood.[1] This gene family is conserved throughout the vertebrate classes, yet the individual expression patterns of each gene differ among representatives of the classes that have been examined. Several observed differences between rodents and *Xenopus* indicate that the MRF gene regulatory network must function somewhat differently in *Xenopus* than it does in mammals.[2]

While studying the regulation of MRF4 expression in *Xenopus laevis*, we have consistently obtained results by whole-mount *in situ* hybridization for XMRF4 mRNA in a pattern consistent with its presence in forebrain, eyes, and other neural structures, in addition to the somites, at neurula through tailbud stages. Della Gaspera et al,[3] however, have recently published *in situ* hybridization data for XMRF4 describing expression only in the somites. To test whether our *in situ* hybridization results are correct, we turned to reverse transcription polymerase chain reaction (RT-PCR) as an independent measure of XMRF4 mRNA in the anterior embryo.

METHODS

Ovulation was induced in gravid females and eggs were fertilized by standard methods.[4] Embryos were maintained in 0.1× MMR at room temperature or at 16°C and the jelly coat was removed with 2% cysteine in 0.4× MMR, pH 8. At desired stages, vitelline membranes were removed manually and the embryos were dissected into three regions, dorsal, ventral, and anterior. Care was taken to ensure that the anterior regions lay entirely rostral to the first somite. RNA was prepared using RNAwiz reagent (Ambion) according to the manufacturer's directions. Concentrations of total RNA were determined with spectrophotometry, verified by gel electrophoresis, and adjusted to 0.5 μg/μL. First-strand cDNA was prepared from 0.5 μg total RNA with MMLV reverse transcriptase (Epicentre Biotechnologies) using random primers. One-tenth of a cDNA reaction was used as the template for PCR. Primers used for PCR were:

XMRF4: up, TCCACCAGGACTACAACCCC; down, ATGGTCAGGAATATGGTGCC
EF1α up, CAGATTGGTGCTGGATATGC; down, -ACTGCCTTGATGACTCCTAG

These XMRF4 primers amplify a sequence region that spans the first intron of the gene to ensure that genomic DNA contamination cannot be the source of the signal. For PCR we used JumpStart ReadyMix (Sigma) reagents in 50-μL reactions for either 30 or 35 cycles according to the manufacturer's directions. Relative amounts of PCR products were visualized by loading 10 μL onto 2% agarose gels. Following electrophoresis and ethidium bromide staining, gels were photographed with a Kodak EDS290 system.

RESULTS

RT-PCR with 30 cycles of amplification clearly showed dorsal and anterior XMRF4 expression in mid-neurula

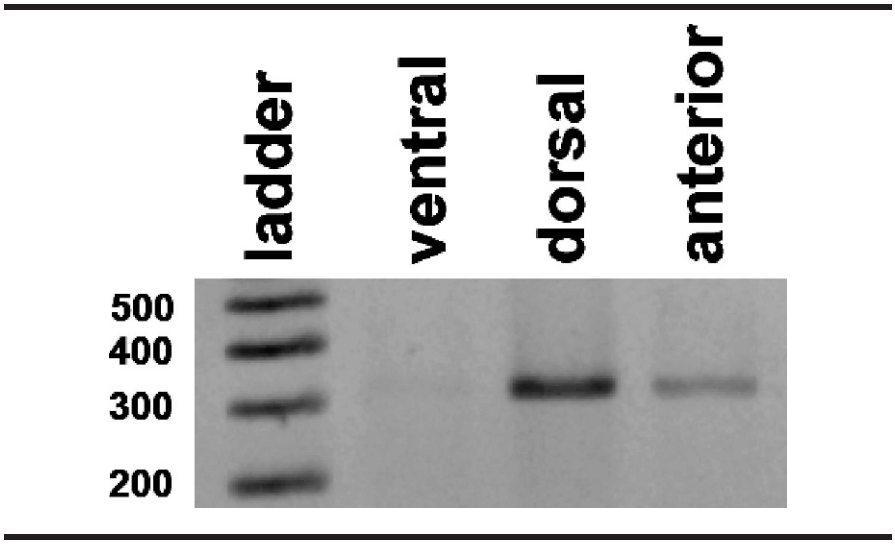

Fig 1. Dorsal and anterior XMRF4 expression in mid-neurula stage embryos

stage embryos (Figure 1). Based on comparison of fluorescence intensities after 25 cycles (not shown), 30 cycles and 35 cycles (Figure 2), we judged 30 cycles to be within the range of logarithmic amplification. Assuming that equal amounts of total RNA were used, XMRF4 mRNA appeared to be somewhat less abundant in the anterior region of these embryos than in the dorsal region. Interestingly, the ventral region showed a very low-level signal as well.

We repeated the PCR with 35 cycles to improve visualization of the ventral-region signal, and we also performed PCR for elongation factor 1 α (EF1α), a ubiquitously expressed message, to normalize for the amount of RNA used. In this situation, it appeared that the relative amount of MRF4 to EF1α was similar for the dorsal region and the anterior region (Figure 2). By comparison, the ratio of XMRF4 to EF1α in ventral tissue was far lower.

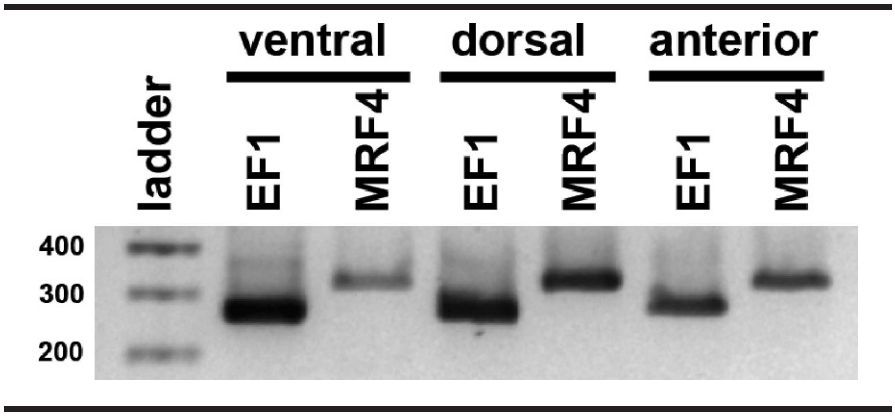

Fig 2. MRF4 to EF1α comparison for the dorsal region and the anterior region

CONCLUSION

MRF4 expression in the anterior region of *Xenopus laevis* has not previously been reported. Based on our *in situ* hybridization results, however, we believed it may be expressed there. We have now verified the presence of XMRF4 mRNA in the anterior region through an independent method, RT-PCR. Additional experiments are underway to confirm that dorsal somitic cells were not inadvertently included in the dissected anterior regions, by RT-PCR for somitic markers such as skeletal muscle actin and MyoD. Histological studies will demonstrate whether XMRF4 is expressed in structures of the neural ectoderm such as the eyes and brain. If the XMRF4 message is demonstrated in these cells, it will suggest that this gene may have unanticipated roles in the development of neural structures in *Xenopus*.

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