Corrigendum

eFGF is required for activation of *XmyoD* expression in the myogenic cell lineage of *Xenopus laevis*

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Fig. 1B of this paper shows the expression of *Xmyf5* and not *XmyoD* as stated. As a consequence, the first paragraph of the Results section is inaccurate. The correct figure and text are given here and in the online versions.

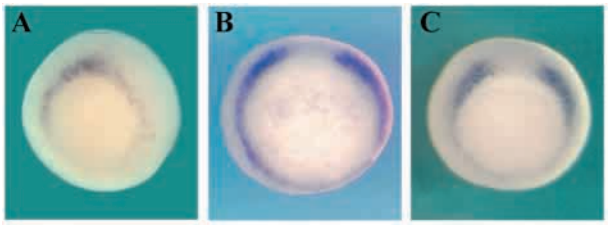


Fig. 1. The normal expression patterns of *eFGF* and *XmyoD* showing co-expression in the early mesoderm. Whole-mount in situ hybridisation showing expression of (A) *eFGF* at stage 10, (B) *XmyoD* at stage 10 and (C) *XmyoD* at stage 10 (+). Expression of *XmyoD* across the dorsal midline is rapidly excluded as Spemann's organiser signalling is established.

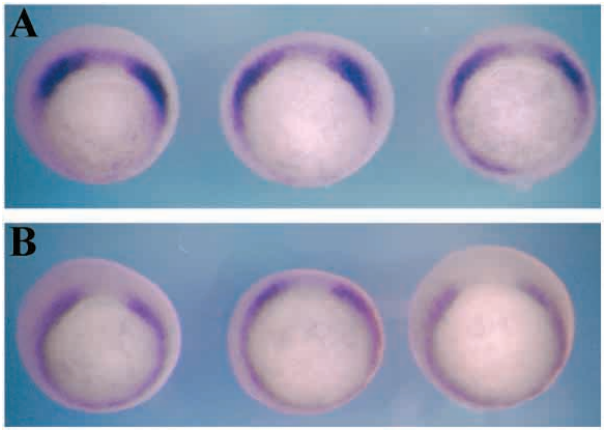
RESULTS

eFGF and XmyoD are co-expressed in the early mesoderm

As discussed above, there is evidence to suggest a role for FGF signalling during myogenesis. We show that the expression domains of *XmyoD* and *eFGF* overlap in the early mesoderm. Both genes are initially co-expressed within a region that encompasses much of the mesoderm (Fig. 1).

ADDITIONAL INFORMATION

The authors have supplied additional data to illustrate that the expression of these two genes is similar at this stage, as shown below.



The normal expression of *Xmyf5* and *XmyoD* at early gastrula stage 10. Whole-mount in situ hybridisation shows that *Xmyf5* expression (A) is strong dorsally and spans the dorsal midline. *XmyoD* (B) is expressed more evenly throughout the marginal zone but is excluded from the dorsal midline.

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eFGF is required for activation of *XmyoD* expression in the myogenic cell lineage of *Xenopus laevis*

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SUMMARY

This paper addresses the molecular mechanisms that regulate the transcriptional activation of the myogenic regulatory factor *XmyoD* in the skeletal muscle lineage of *Xenopus laevis*. Using antisense morpholino oligonucleotide-mediated inhibition, we show that the signalling molecule embryonic fibroblast growth factor (eFGF), which is the amphibian homologue of FGF4, is necessary for the initial activation of *XmyoD* transcription in myogenic cells. We demonstrate that eFGF can activate the expression of *XmyoD* in the absence of protein synthesis, indicating that this regulation is direct. Our data suggest that regulation of *XmyoD* expression may involve a labile transcriptional repressor. In addition, we show that eFGF is itself an immediate early response to activin, a molecule that mimics the endogenous mesoderm-inducing signal. We propose a model for the regulation of *XmyoD* within the early mesoderm, and discuss the relevance that these findings have for the understanding of myogenic specification in higher vertebrates.

Key words: Activin, FGF, Mesoderm, Muscle, MyoD, *Xenopus*

INTRODUCTION

An enormous breakthrough in our understanding of how the myogenic cell lineage is established came when the myogenic regulatory genes *MyoD*, *Myf5*, *myogenin* and *Mrf4* were identified. These genes encode bHLH transcription factors, known as MRFs (myogenic regulatory factors), which act as dominant regulators of myogenesis: expression of any one of these genes in many non-muscle cell types will convert those cells to myoblasts (reviewed by Weintraub, 1993). Moreover, mouse knockout experiments have shown that MRFs are essential for myogenesis in vivo (reviewed by Arnold and Winter, 1998). *MyoD* (*Myod1* – Mouse Genome Informatics) and *Myf5* in particular are important for early events during myogenesis, as mice deprived of both *Myod1* and *myf5* do not form any skeletal muscle precursor cells (Rudnicki et al., 1993). Consistent with a role in determining the skeletal muscle cell lineage, *MyoD* and *Myf5* are expressed in myogenic cells in all vertebrates but the precise regulation of gene expression varies among different vertebrates.

In mice, *Myf5* is the earliest expressed MRF and is initially found in the epaxial domain of the epithelial somite at 8dpc, while *MyoD* expression is detected in somites significantly later at 10.5 dpc (Ott et al., 1991). In avians, high expression of both *Myf5* and *MyoD* is closely associated with somitogenesis (Hirsinger et al., 2001; Pownall and Emerson, 1992). However, other recent studies have detected expression of *Myf5* (Kiefer and Hauschka, 2001) and *MyoD* (Gerhart et al., 2000) in pre-segmented mesoderm and gastrula stage embryos. Transcription of *Myf5* has also been detected in mouse pre-segmented mesoderm by RT-PCR (Lin-Jones and Hauschka, 1996) and in pre-segmented mesoderm of transgenic mice where *lacZ* has been knocked into the *Myf5* locus (Cossu et al., 1996). The relevance of these low levels of MRF gene expression in early chick and mouse mesoderm is at present unknown, however, in frogs and fish *MyoD* and *Myf5* are expressed at high levels in the mesoderm prior to somitogenesis (Chen et al., 2001; Hopwood et al., 1991; Hopwood et al., 1989; Weinberg et al., 1996). The exact nature of the signals that regulate MRF gene expression in the early mesoderm remains to be determined.

XmyoD is expressed dynamically during *Xenopus* development: there are maternal *XmyoD* transcripts present throughout the egg and when zygotic transcription begins, a low level of transient, ubiquitous *XmyoD* expression initiates (Harvey, 1990; Harvey, 1991; Rupp and Weintraub, 1991). Expression of *XmyoD* in the myogenic lineage is activated during early gastrula stages, where high levels of expression are detected in the cells of the marginal zone and *XmyoD* continues to be expressed in myogenic cells throughout somitogenesis (Hopwood et al., 1989). The skeletal muscle specific expression of *XmyoD* is activated in response to endogenous mesoderm inducing signals (Hopwood et al., 1989) and the growth factors bFGF and activin have also been shown to activate *XmyoD* expression (Harvey, 1991). In *Xenopus*, it is possible to determine whether the expression of a particular gene is a direct response to mesoderm inducing signals (Cascio and Gurdon, 1987; Rosa, 1989; Smith et al.,

1991). It has been reported that the expression of *XmyoD* in response to factors such as activin, which mimic the endogenous mesoderm inducing signal, is a delayed response, requiring protein translation (Harvey, 1991; Steinbach et al., 1998), rather than a direct, immediate early response, as has been shown for *Mix1* (Rosa, 1989) and *Xbra* (Smith et al., 1991).

FGF signalling has been shown to be required for the expression of many mesodermal genes (Amaya et al., 1991; Amaya et al., 1993) and notably to directly regulate the *Xenopus* homologue of the T-box gene *brachyury* (*Xbra*) (Isaacs et al., 1994; Schulte-Merker and Smith, 1995; Smith et al., 1991) as well as the *caudal* homologue *Xcad3* (Isaacs et al., 1998; Pownall et al., 1996). Previous work also suggests a role for FGF signalling in regulating *XmyoD*: (1) *XmyoD* expression is activated in animal cap explants treated with bFGF (Harvey, 1990) and the induction of *XmyoD* in explants by activin requires a functional FGF signalling pathway (Cornell and Kimelman, 1994); (2) in whole embryos, blocking FGF signalling results in a dramatic downregulation of *XmyoD* expression (Isaacs et al., 1994) and loss of skeletal muscle (Amaya et al., 1991; Amaya et al., 1993); and (3) a community effect among muscle precursor cells is required for myogenic differentiation (Gurdon et al., 1993) and has recently been shown to be mediated by eFGF (Standley et al., 2001).

We present evidence that eFGF is necessary for the initial activation of *XmyoD* expression during *Xenopus* myogenesis. We show that during early gastrula stages, *XmyoD* and *embryonic FGF* (*eFGF*), which is the *Xenopus* homologue of *Fgf4*, are co-expressed in the nascent mesoderm and that *XmyoD* is activated as an immediate early response to eFGF. Moreover, we use morpholino antisense oligonucleotides directed against *eFGF* to show that the specific inhibition of eFGF eliminates early expression of *XmyoD*. We further show that the expression of *eFGF* itself is a direct response to mesoderm induction by activin-like signalling molecules and propose a molecular pathway for *XmyoD* activation in the early mesoderm. We also present evidence that the expression of *XmyoD* may be regulated by an unstable transcriptional repressor.

MATERIALS AND METHODS

Growth factor treatments and explant culture

Xenopus laevis embryos were obtained by artificial fertilisation and cultured in 0.1×normal amphibian medium (NAM) at 23°C. Embryos were de-jellied in a 2.5% cysteine solution and staged according to (Nieuwkoop and Faber, 1967). Animal cap explants were taken at NF stage 7.5-8.5. Manipulations were performed in 0.5×NAM + 0.5% bovine serum albumen (BSA). Caps were cultured in 0.5×NAM + 0.5% BSA, with and without growth factor, (eFGF 20 units/ml or Activin 13 ng/ml). Whole embryos and caps were frozen at the appropriate control stage for later RNAase protection analysis.

Cycloheximide treatment

For experiments involving cycloheximide (CHX) treatment animal cap explants were taken at stage 8-8.5 and cultured for 30 minutes in 0.5×NAM + 0.5% BSA, with and without 7.5 μg/ml CHX. Control explants were left in culture while explants for growth factor treatment were cultured for a further 90 minutes with 20 μ/ml eFGF or 13.5 ng/ml activin in 0.5×NAM + 0.5% BSA, with and without 7.5 μg/ml CHX. After growth factor and CHX treatment, caps were washed twice in 0.5×NAM + 0.5% BSA and cultured in this medium until the appropriate control stage.

Measurement of protein synthesis inhibition

Animal cap culture and CHX treatment was as described above except 15 μCi/ml ^{35}S-methionine was included in the culture medium following the initial 30 minute CHX exposure. Control and CHX treated caps were collected at the appropriate control stage. Caps were homogenised in 10 μl of 0.1 M NaCl, 50 mM Tris (pH 8), 5 mM EDTA and 0.5% SDS (NETS) and 5 μl of homogenate was spotted onto filter paper. Filters were then washed in 10% trichloroacetic acid (TCA) for 30 minutes, twice in water for 10 minutes, once in 95% ethanol for 10 minutes and once in acetone for 10 minutes. Filters were then air dried and activity measured by scintillation counter. Inhibition of radio-methionine incorporation was typically 80-90% over the time course of the experiment.

mRNA and morpholino oligonucleotide injection

The CM control morpholino oligonucleotide is a standard control provided by GeneTools. The eFM oligonucleotide was designed by GeneTools and has the sequence ATGGAACAGTCATCCC-GATCAAC, which is complementary to the nucleotides –10 to +13, which span the initiating codon AUG of *eFGF(i)*. Injections were carried out on four-cell stage embryos cultured in 0.3×NAM + 5% Ficoll 400. Bilaterally injected embryos, for use in RNAase protection analysis, were injected with up to 20 ng into the pigment boundary of each cell. Unilaterally injected embryos, for analysis by in situ hybridisation, were injected with 20 ng into each cell on the left of the embryo. Oligonucleotide-injected embryos for use in animal cap experiments were injected with 40 ng into the animal region of both cells at the two-cell stage. Animal caps for protein treatment taken at stage 8 and were cultured with and without bFGF protein (20 units) until the appropriate control stage. *eFGF* mRNA co-injection experiments used synthetic capped *eFGF* mRNA made from the pSP64T eFGF (un) plasmid which contains the 5′UTR and coding region of *eFGF(i)*. 10 pg *eFGF(i)* mRNA was injected with and without oligonucleotide into both cells at the two-cell stage. Animal caps were excised at stage 8 and cultured until the appropriate control stage. *ssbFGF* mRNA co-injection experiments used synthetic capped ssbFGF mRNA as described in (Thompson and Slack, 1992). *ssbFGF* (100 pg) was injected with and without oligonucleotide into both cells at the two-cell stage embryo. Embryos were cultured until stage 13.

Ribonuclease protection assay

mRNA was extracted in NETS solution followed by phenol/chloroform extraction and ethanol precipitation. RNAase protection analyses were performed using the Ambion RPA III^{TM} kit with a hybridisation temperature of 45°C. *Ornithine Decarboxylase* (*ODC*) mRNA was used as a loading control for all RNAase protections. Templates prepared were *Actin* (Gurdon et al., 1985), *eFGF* (Isaacs et al., 1992), *XmyoDb* (Harvey, 1991), *Xmyf5* linearised with *EcoR1* and transcribed using T7 polymerase, *Xbra* (Smith et al., 1991), *ODC* (Isaacs et al., 1992) and *Xsna* (Sargent and Bennett, 1990).

In situ hybridisation

Embryos were cultured to appropriate stages and then fixed in MEMFA (0.1 M MOPS, 2 mM EDTA, l mM $MgSO_4$, 3.7% formaldehyde) for l hour at room temperature and stored in l00% ethanol at –20º C until further processing. Embryos were rehydrated through a graded series of ethanols and then rinsed in PBS with 0.1% Tween. Proteinase K treatment was carried out for 10 minutes at room temperature with l0 μg/ml of Proteinase K. Hybridisation was carried out overnight at 60°C in 50% formamide, 5×SSC, 1 mg/ml total RNA, 100 μg/ml heparin, 1×Denhardts, 0.1% Tween 0.1% CHAPS, 10 mM EDTA. Extensive washes in 2×SSC and 0.2×SSC at 60°C were followed by washes at room temperature with maleic acid buffer, MAB (0.1 M maleic acid, 0.15 M NaCl, 0.1% Tween, pH 7.8), and

blocking in 2% Roche Blocking Reagent and 20% heat-treated lamb serum for 2 hours at room temperature. Embryos were then incubated with anti-DIG antibody at a dilution of 1/2000 in blocking solution at 4°C overnight. The antibody is detected after extensive washes at room temperature in MAB by a colour reaction using BM purple precipitating alkaline phosphatase detection system (Roche). Probes for in situ hybridisation were transcribed using l×DIG RNA labelling mix (Roche) from linearised plasmids: *XmyoDb* as for RNAase protection (Harvey, 1991); *eFGF* as for RNAase protection (Isaacs et al., 1992); *Xsna* as for RNAase protection (Sargent and Bennett, 1990).

RESULTS

eFGF and XmyoD are co-expressed in the early mesoderm

As discussed above, there is evidence to suggest a role for FGF signalling during myogenesis. We show that the expression domains of *XmyoD* and *eFGF* overlap in the early mesoderm. Both genes are initially co-expressed within a region that encompasses much of the mesoderm (Fig. 1).

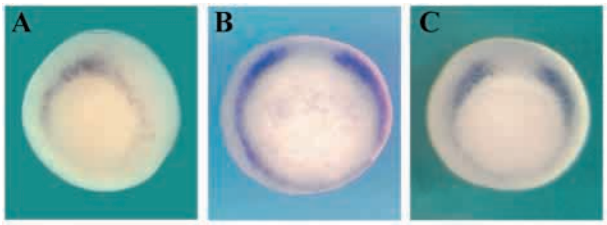

Fig. 1. The normal expression patterns of *eFGF* and *XmyoD* showing co-expression in the early mesoderm. Whole-mount in situ hybridisation showing expression of (A) *eFGF* at stage 10 and (B) *Xmyod* at stage 10 and (C) *Xmyod* at stage 10 (+). Expression of *XmyoD* across the dorsal midline is rapidly excluded as Spemann's organiser signalling is established.

eFGF induces XmyoD expression in ectodermal explants

Using an animal cap assay, we show that eFGF is sufficient to activate the expression of *XmyoD*. Ectodermal explants (or 'animal caps'), which when cultured alone give rise to only ectodermal derivatives, were treated with eFGF protein and assayed for the expression of *XmyoD* and *Xbra* by RNAase protection analysis. Untreated animal caps do not express mesodermal genes, while the expression of the pan-mesodermal gene *Xbra* is a well documented response to induction by FGF signalling (Isaacs et al., 1994; Schulte-Merker and Smith, 1995; Smith et al., 1991). Treatment with 20 units of eFGF protein activates the expression of both *XmyoD* and *Xbra* in ectodermal explants (Fig. 2). This activation is rapid and occurs by early gastrula stage 10, becoming stronger at later gastrula stage 11.5. Another study found that injection of synthetic *bFGF* mRNA (coding for a secreted form of bFGF) was unable to induce *XmyoD* expression in animal caps without the co-injection of *Xwnt8* mRNA (Steinbach et al., 1998). However, we show here that eFGF treatment alone is sufficient to rapidly activate the expression of *XmyoD*.

As previously reported, the TGFβ signalling molecule, activin, which is known to have strong mesoderm inducing activity, also induces the expression of *XmyoD* and *Xbra* in this assay (Harvey, 1991; Smith et al., 1991). However, the expression of these genes over the timescale shown here is much weaker in response to activin than to eFGF. Control animal cap explants show a low level of *XmyoD* expression during early gastrula stages, due to the transient, ubiquitous pulse of *XmyoD* expression that occurs in *Xenopus* embryos at the mid-blastula transition when the zygotic genome becomes active (Harvey, 1990; Rupp and Weintraub, 1991). This low level expression in control caps is no longer apparent by mid to late gastrula stages, so in most of the following experiments we assay for gene expression at late gastrula stage 13.

XmyoD expression is an immediate early response to FGF signalling

We demonstrate here that the expression of *XmyoD* is a direct response to FGF signalling by repeating our induction assay in the presence of the translational inhibitor cycloheximide. This type of analysis has been carried out previously to determine which genes are activated by mesoderm inducing factors in an immediate-early, or cycloheximide-insensitive, manner (Cascio and Gurdon, 1987; Rosa, 1989; Smith et al., 1991). It has been well documented that the expression of *XmyoD* is not an immediate-early response to mesoderm induction by activin-like molecules (Harvey, 1991; Steinbach et al., 1998). However, we demonstrate here that the expression of *XmyoD* in response to FGF signalling does not

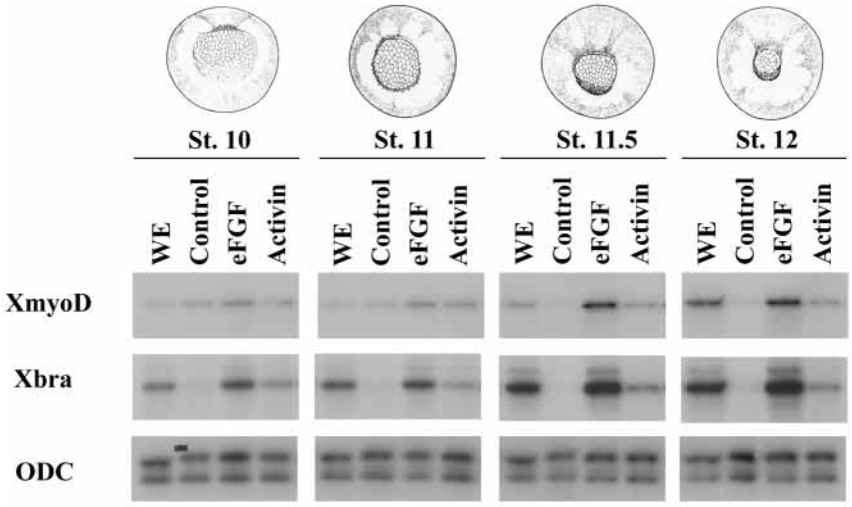

Fig. 2. Gene expression in animal caps treated with activin or eFGF. RNA was extracted at stages 10, 11, 11.5 and 12 from whole embryos, untreated animal cap explants and animal cap explants treated with eFGF or activin. Total RNA (7 μg) from each stage was analysed by RNAase protection for expression of the marker genes *XmyoD*, *Xbra* and the loading control *ODC*.

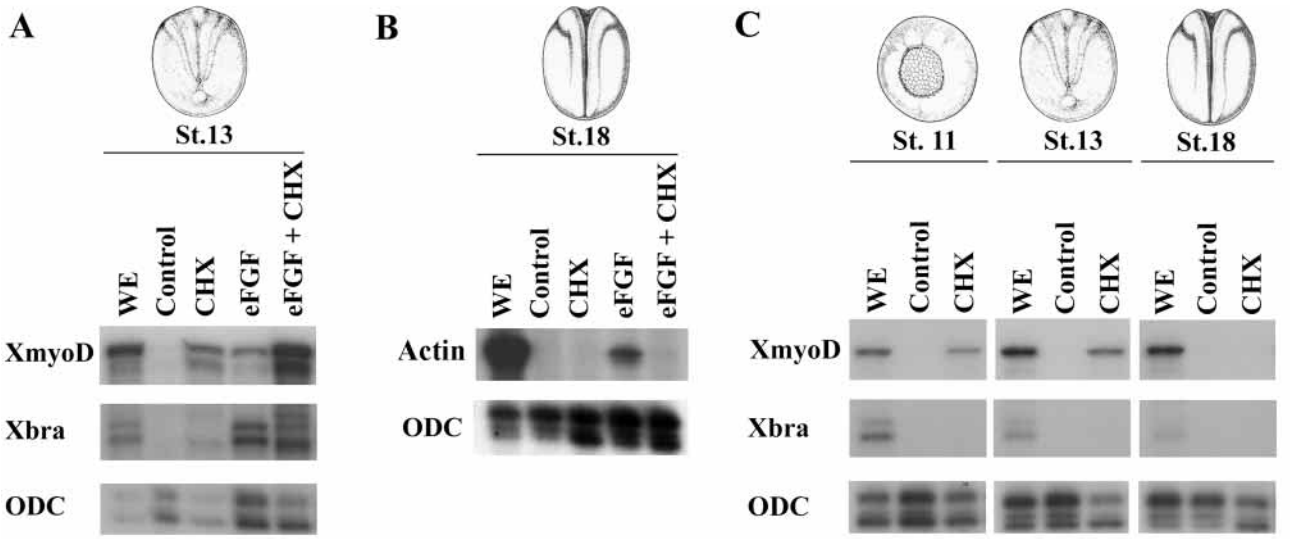

Fig. 3. *XmyoD* induction in eFGF and CHX treated animal caps. (A) RNA was extracted at stage 13 from whole embryos, animal caps in the presence or absence of CHX, and animal caps cultured with eFGF in the presence or absence of CHX. Total RNA (3 μg) was analysed by RNAase protection for the expression of the marker genes *XmyoD, Xbra* and for the loading control *ODC*. (B) RNA was extracted at stage 18 from whole embryos, animal caps in the presence or absence of CHX, and animal caps cultured with eFGF in the presence or absence of CHX. Total RNA (10 μg) was analysed by RNAase protection for the expression of the marker gene *actin* and for the loading control *ODC*. (C) RNA was extracted at stages 11, 13 and 18 from whole embryos and animal caps cultured in the presence or absence of CHX. Total RNA (10 μg) was analysed by RNAase protection for the marker genes *XmyoD, Xbra* and the loading control *ODC*.

require protein synthesis, as the activation of *XmyoD* by eFGF protein is insensitive to cycloheximide (Fig. 3A). This is also true for the expression of *Xbra*, which was shown previously to be an immediate-early response to FGF signalling (Smith et al., 1991). Transcription of *actin* does require protein synthesis and is sensitive to cycloheximide (Fig. 3B).

Interestingly we find that expression of *XmyoD* is super-induced in animal caps treated with eFGF and cycloheximide. We also see that animal caps treated with cycloheximide alone express *XmyoD* (Fig. 3A). Our favoured interpretation of these results is that the regulation of *XmyoD* involves an unstable transcriptional repressor and in the presence of cycloheximide the rapid turnover of this factor is not replenished so that *XmyoD* is no longer repressed. Other studies investigating *XmyoD* expression in animal caps treated with cycloheximide have not described this phenomenon (Harvey, 1991). The timing of our analysis is likely the basis of this difference, as animal caps treated with cycloheximide alone express *XmyoD* during early and late gastrula stages, but no longer express *XmyoD* at neurula stages (Fig. 3B), which is when other studies assayed gene expression (Harvey, 1991).

An antisense morpholino oligo(eFM) directed against eFGF blocks eFGF mRNA induced morphogenetic movements

While we have shown that eFGF is sufficient to activate the expression of *XmyoD*, there are many FGF family members that could be playing this role in vivo. *eFGF*, *Fgf3* and *Fgf8* are expressed in the early mesoderm of *Xenopus laevis* (Christen and Slack, 1997; Isaacs et al., 1995; Lombardo et al., 1998), and all could have some role in regulating *XmyoD* expression. In order to determine if it is eFGF that is required for *XmyoD* expression during normal development, we have adopted an antisense inhibition approach using morpholino oligonucleotides directed against *eFGF*. Injection of antisense morpholino oligos into *Xenopus* embryos has previously been shown to be an effective way of inhibiting translation from specific target mRNAs during early development (Heasman et al., 2000). We designed a 23-mer morpholino oligonucleotide, designated eFM, which is directed against a region spanning the initiating AUG of the *eFGF(i)* mRNA.

Although our experiments seek to analyse the role of eFGF after the period of mesoderm induction, the well characterised mesoderm-inducing activity of eFGF provides an ideal assay to test out the efficacy and specificity of the eFM morpholino oligonucleotide. In order to demonstrate that injection of eFM can inhibit the activity of *eFGF* mRNA, we took animal cap explants that were injected with synthetic mRNA coding for eFGF alone or co-injected with *eFGF* mRNA plus eFM or a control morpholino oligo (CM). Uninjected control animal caps remain as a rounded ball of ectodermal tissue (Fig. 4A, part i). Animal cap explants that are injected with *eFGF* mRNA are induced, and undergo morphogenetic movements and elongation (Fig. 4A, part ii) that are characteristic of the cellular behaviour of mesoderm during gastrula stages. Caps co-injected with *eFGF* mRNA and CM (Fig. 4A, part iii) also undergo elongation movements. However, explants that are co-injected with *eFGF* mRNA and the eFM antisense morpholino oligo are uninduced (Fig. 4A, part iv) and look much the same as the uninjected control explants.

eFM blocks eFGF mRNA induced mesodermal gene expression

Animal cap elongation is an accurate indicator of mesoderm formation, but we further analysed the effect of eFM by looking at the ability of eFM to block mesodermal gene expression induced by injection of *eFGF* mRNA. Fig. 4B shows that co-injection of eFM, but not CM, with *eFGF*

mRNA blocks the activation of expression of both *XmyoD* and *Xbra* in gastrula stage animal caps. We conclude that the eFM oligo, but not the CM control oligo, inhibits the ability of the injected eFGF mRNA to induce mesoderm.

eFM does not block mesodermal gene expression induced by exogenous FGF protein

The above experiments do not rule out the possibility that the eFM oligo inhibits all mesoderm induction in a nonspecific fashion. To test the specificity of eFM we show that eFM does not block the ability of exogenously supplied FGF protein to induce mesoderm. We find that control caps or caps injected with CM or eFM, treated with exogenous FGF protein, show normal induction of mesodermal gene expression (Fig. 4C). We conclude that the eFM oligo does not block mesoderm induction in a nonspecific fashion.

eFGF is specifically required in vivo for XmyoD expression in the early mesoderm

We next showed that eFGF is required for *XmyoD* expression in vivo. Using RNAase protection analysis, we found that although 80 ng of CM has no effect on gene expression, the same amount of eFM completely eliminates *XmyoD* expression during gastrula stages (Fig. 5A, part i). This effect is not a general inhibition of mesodermal gene expression as another pan-mesodermal gene, *Xsna*, continues to be expressed in embryos injected with eFM. At later stages, the expression of *XmyoD* is no longer inhibited by the eFM oligo. In Fig. 5A, part ii we show, as a control for specificity, the rescue of *XmyoD* expression with the co-injection of an mRNA coding for a secreted form of basic FGF (ss-bFGF) (Thompson and Slack, 1992), a protein with similar biological activity to eFGF but with different sequence.

The loss of inhibition at later stages is also seen when the eFM oligo is injected unilaterally into two cells at the four-cell stage and gene expression is assayed at gastrula and neurula stages by whole mount in situ hybridisation (Fig. 5B). Expression of *XmyoD* is found to be completely abolished on the eFM injected side of gastrula embryos (Fig. 5B, part i). The expression of *Xsna* is unaffected (Fig. 5B, part iii). The injected side of sibling embryos allowed to develop to neurula stages shows the expression of *XmyoD* is recovering on the injected side (Fig. 5B, part ii). These data support the notion that while the activation and early expression of *XmyoD* in the nascent mesoderm requires eFGF, the later maintenance of *XmyoD* expression in the paraxial mesoderm is independent of eFGF and is regulated by other mechanisms.

eFGF expression is an immediate early response to mesoderm induction

We have shown that *eFGF* is required in vivo for the activation and early expression of *XmyoD*, and that *XmyoD* expression is a direct response to induction by eFGF. To further characterise the molecular pathway leading to *XmyoD* expression we examine if *eFGF* expression is an immediate early response to mesoderm induction by activin-like molecules. Fig. 6 shows the results of an animal cap experiment where we test the ability of activin protein (which is used to mimic the endogenous mesoderm inducing factor) to activate the expression of *eFGF*. We find that activin does activate *eFGF* expression even in the presence of cycloheximide.

DISCUSSION

XmyoD expression in frogs

We have addressed the molecular mechanism for the transcriptional activation of *XmyoD* in cells of the myogenic lineage of the frog *Xenopus laevis*. In *Xenopus*, the earliest expression of *XmyoD* in the skeletal muscle lineage occurs in the nascent mesoderm at the start of gastrulation (Hopwood et al., 1989). This expression is notably earlier than in other vertebrates: in avians *MyoD* expression is associated with somitogenesis (Hirsinger et al., 2001; Pownall and Emerson, 1992) and in mice *MyoD* is expressed in somites sometime after they have formed (Ott et al., 1991). While there is some evidence for low-level *MyoD* transcription in avians prior to somitogenesis (Gerhart et al., 2000), it is clear that both frogs and fish express *MyoD* at high levels in the newly formed mesoderm well before the onset of somitogenesis. (Hopwood et al., 1989; Weinberg et al., 1996).

FGF signalling and mesodermal gene expression

Although *XmyoD* is expressed in the early mesoderm, it has been reported that the expression of *XmyoD* is not a direct response to mesoderm induction (Harvey, 1991; Steinbach et al., 1998). Current thinking is that the endogenous mesoderm inducing signals are members of the TGFβ family of growth factors and may include the *Xenopus* nodal related factors (Jones et al., 1995; Kofron et al., 1999). Historically the FGFs were also considered to play a part in this role; however, as the mesoderm inducing activity of FGF was first described (Slack et al., 1987), the role of FGF signalling during the establishment of the mesoderm has been re-evaluated (Cornell and Kimelman, 1994; Cornell et al., 1995; Isaacs, 1997; Isaacs et al., 1994). Despite its activity in mesoderm induction assays, the bulk of evidence points to a later role for FGFs in regulating mesodermal gene expression during gastrula stages, after the initial phase of mesoderm induction. In this study we examine a possible role for FGF signalling in regulating *XmyoD* expression within the early mesoderm. It has previously been shown that blocking FGF signalling inhibits myogenesis (Amaya et al., 1991; Amaya et al., 1993) and dramatically downregulates *XmyoD* expression (Isaacs et al., 1994) in developing embryos. It has been also been demonstrated that eFGF is able to maintain the expression of *XmyoD* in disaggregated mesoderm cells of gastrula stage embryos (Standley et al., 2001).

eFGF can activate XmyoD in the absence of protein synthesis

This work demonstrates that eFGF is necessary and sufficient to activate *XmyoD* expression in the early mesoderm. Treatment of animal cap ectodermal explants with eFGF rapidly activates the expression of *XmyoD* and can do so even in the presence of the translational inhibitor cycloheximide, indicating that this induction is direct. Interestingly, we have some preliminary data that show that *Xmyf5* is not an immediate early response to eFGF (data not shown) and is therefore regulated by a distinct mechanism.

XmyoD may be regulated by a labile transcriptional repressor

An unexpected finding from some of our experiments is that

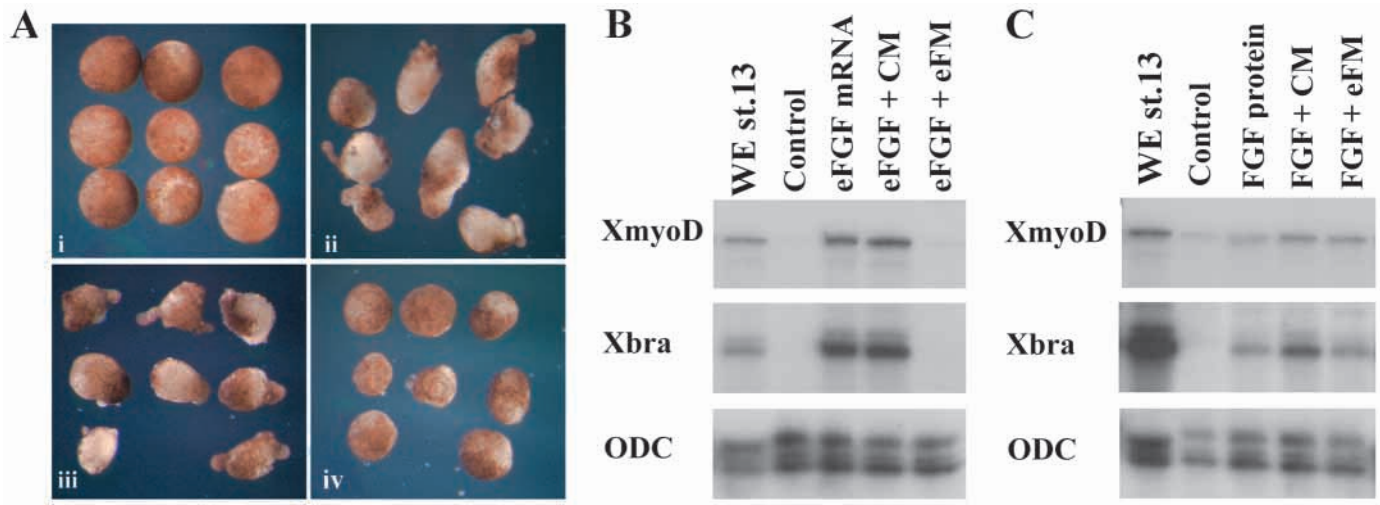

Fig. 4. The effect of an *eFGF*(i) targeted morpholino (eFM) on morphogenic movements and myogenic gene expression. (A) (i) Untreated animal cap controls showing uninduced morphology; (ii) caps from embryos injected with 5 pg of *eFGF* mRNA showing induced morphology; (iii) caps from embryos co-injected with 5 pg of *eFGF* mRNA and 80 ng of control morpholino (CM) showing an induced morphology; and (iv) caps from embryos co-injected with 5 pg of *eFGF* mRNA and 80 ng of eFM showing uninduced morphology. (B) RNA was extracted from whole embryos at stage 13, untreated animal caps, animal caps from embryos injected with 5 pg *eFGF* mRNA and animal caps from embryos co-injected with 5 pg *eFGF* mRNA and 80 ng of either CM or eFM. Total RNA (6 μg) was analysed by RNAase protection for expression of the marker genes *XmyoD, Xbra* and the loading control *ODC.* (C) RNA was extracted from whole embryos at stage 13, untreated animal caps, animal caps treated with eFGF protein, eFGF treated animal caps injected with 80 ng of CM or eFM. Total RNA (6 μg) was analysed by RNAase protection for expression of the marker genes *XmyoD, Xbra* and the loading control *ODC.*

cycloheximide treatment alone activates expression of *XmyoD* during gastrula stages. One interpretation of this finding is that a labile transcriptional repressor inhibits *XmyoD* expression prior to its activation by eFGF. Such unstable inhibitors have been postulated to be involved in the regulation of other genes (Novak et al., 1991). Alternatively, cycloheximide has been shown to activate other signal transduction pathways and could be activating *XmyoD* in this way (Edwards and Mahadevan, 1992). Clearly, however, cycloheximide is not a general activator of all FGF target genes, as expression of *Xcad3* (Isaacs et al., 1998) and *Xbra* (Smith et al., 1991) is not upregulated by cycloheximide treatment alone.

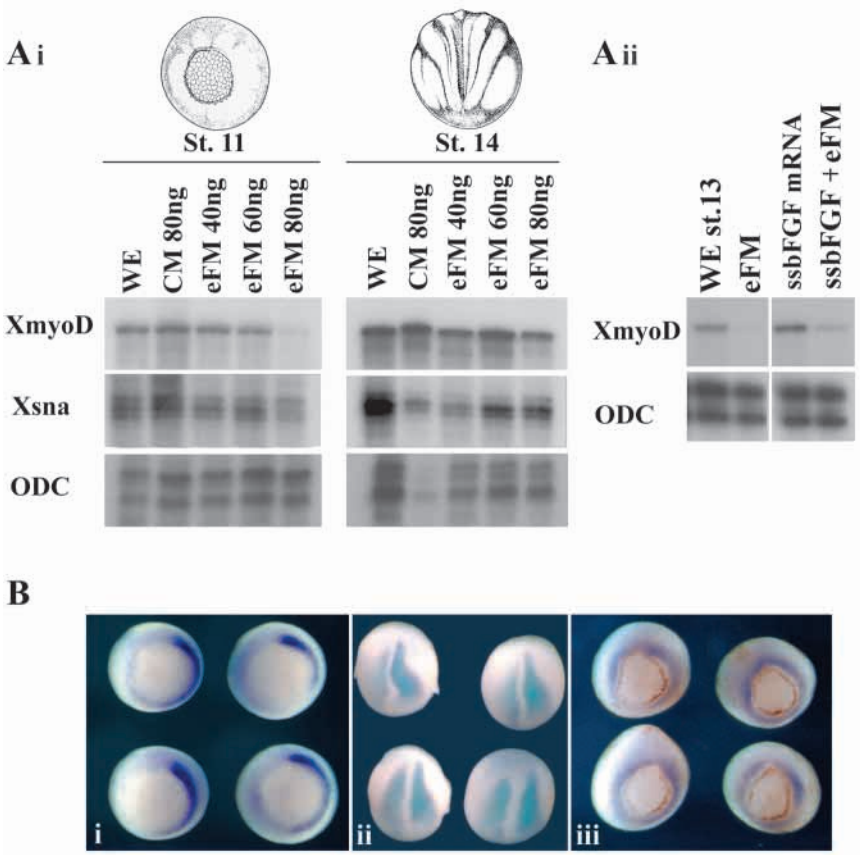

Fig. 5. eFM inhibits *XmyoD* expression in gastrula but not neurula embryos. (A) (i) RNA was extracted at stages 11 and 14 from whole embryos, embryos injected with 80 ng, and embryos injected with 40, 60 or 80 ng of eFM. Total RNA (10 μg) was assayed by RNAase protection for expression of the mesodermal markers *XmyoD* and *Xsna* and for the loading control *ODC*; (ii) RNA was extracted from whole embryos at stage 13, embryos injected with 80 ng of eFM, embryos injected with 200 pg of ssbFGF, and embryos injected with 80 ng of eFM and 200 pg of ssbFGF. Total RNA (10 μg) was analysed by RNAase protection for expression of the marker genes *XmyoD, Xbra* and the loading control *ODC*. (B) Embryos injected unilaterally on the left hand side with 40 ng of eFM were assayed at (i) gastrula stage 10.5 and (ii) neurula stage 14 for *XmyoD* gene expression by in situ hybridisation. (iii) Gastrula stage 10.5 embryos injected unilaterally on the left-hand side with 40 ng of eFM were also assayed by in situ hybridisation for expression of the mesodermal marker *Xsna*.

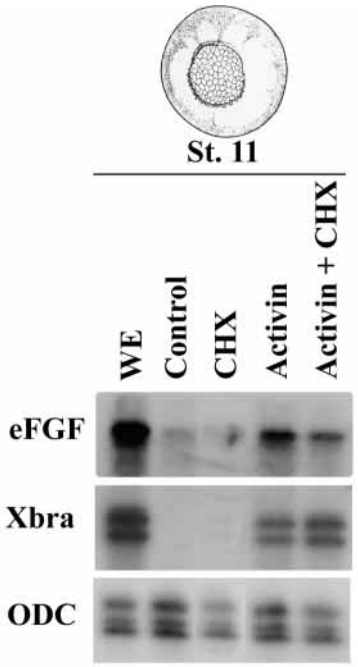

Fig. 6. Activin induces *eFGF* expression in the presence of CHX. RNA was extracted at stage 11 from whole embryos, untreated animal cap explants, CHX treated animal cap explants and caps treated with activin in the presence or absence of CHX. Total RNA (7 μg) was assayed by RNAase protection for expression of *eFGF*, the mesodermal marker *Xbra* and the loading control *ODC*.

We favour the idea of an unstable repressor of *XmyoD* for several reasons. During blastula stages, there is a transient low-level burst of ubiquitous *XmyoD* expression that is quickly silenced (Harvey, 1990; Rupp and Weintraub, 1991). High levels of mesoderm induction-dependent *XmyoD* expression in the cells of the myogenic lineage could be activated through the inactivation of the transcriptional repressor by modification via FGF signalling. Rupp and colleagues have demonstrated a dominant mechanism restricting *XmyoD* expression in response to known inducers until early gastrula stages, and, although auto-regulation by the XmyoD protein was shown to be direct, its ability to activate *XmyoD* transcription is also restricted until these later stages (Steinbach et al., 1998). The human *MYOD* (*MYOD1* – Human Gene Nomenclature Database) enhancer drives very specific skeletal muscle expression in transgenic mice. However, in tissue culture cells expression driven by the same enhancer is remarkably promiscuous, possibly reflecting the loss of a repressor needed to silence *MyoD* in these derived, non-muscle cell lines (Goldhamer et al., 1992).

eFGF is required for XmyoD expression

Consistent with a role for regulating *XmyoD* in the early mesoderm, several members of the FGF family of signalling molecules (including *eFGF*, *FGF3* and *FGF8*) are co-expressed with *XmyoD* as a ring around the blastopore in the newly formed mesoderm (Christen and Slack, 1997; Isaacs et al., 1995; Lombardo et al., 1998). To determine if eFGF specifically is necessary for *XmyoD* expression in the early mesoderm, we used a morpholino-based antisense oligonucleotide directed against *eFGF(i)*. Our data show that inhibiting eFGF activity results in no *XmyoD* expression in gastrula mesoderm; however, at later stages of development, *XmyoD* expression recovers. This may indicate that later mechanisms for maintaining *XmyoD* expression in the paraxial mesoderm are distinct. Another interpretation is that the antisense oligo is no longer functioning at these later stages; however, work from other laboratories has shown that morpholino oligos are stable and functioning until at least tailbud stages (NF St20+) (Heasman et al., 2000). We favour the interpretation that while *XmyoD* expression during gastrula stages requires eFGF, later maintenance of *XmyoD* in the paraxial mesoderm and somites is independent of eFGF and regulated by another molecular mechanism.

Recently, good evidence has been reported for eFGF-mediated regulation of *XmyoD* during gastrula stages. Standley et al. (Standley et al. 2001) tested members of several different signal transduction pathways for the ability to maintain *XmyoD* expression in disaggregated mesoderm, and found that only eFGF was able to mimic the endogenous community effect (Gurdon et al., 1993; Standley et al., 2001). Their work shows that continued presence of eFGF protein in disaggregated mesoderm cultures is necessary during gastrula stages to maintain high levels of *XmyoD* expression and to substitute for the community effect. Consistent with this, we find that although a pulse of eFGF is able to activate *XmyoD* expression in ectodermal explants (see Fig. 3A), prolonged exposure to eFGF results in higher levels of *XmyoD* expression (see Fig. 2). Our findings further support the work of Standley et al. (Standley et al., 2001) in that we show that eFGF-mediated regulation of *XmyoD* expression is distinct and downstream from mesoderm induction by an activin-like signal. However we extend this consensus by showing that eFGF is required in vivo for *XmyoD* expression and that the expression of *XmyoD* in the myogenic lineage is an immediate-early response to induction by eFGF.

A conserved role for FGF in myogenesis?

Induction and patterning of the early mesoderm has been well studied in frogs and combinations of signalling factors have been shown to act during gastrula stages to pattern the dorsoventral axis. BMP4 and Xwnt8 activity in the ventrolateral mesoderm is modulated by factors such as noggin, chordin and FrzB secreted from the dorsal most mesoderm to promote more dorsolateral fates, such as skeletal muscle (reviewed by Heasman, 1997; Slack, 1994; Smith, 1995). Xwnt8 has been shown to be necessary for *XmyoD* expression during gastrula stages (Hoppler, 1996). However, Xwnt8 signalling is not sufficient for normal myogenesis in vivo. We have previously shown that embryos in which FGF

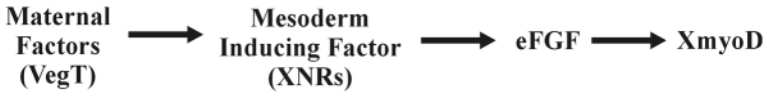

Fig. 7. A pathway for myogenic induction. A molecular pathway depicting the role of eFGF signalling in the induction of *XmyoD* in response to the mesoderm inducing factor. A maternal factor such as VegT induces the expression of a TGFβ family member(s) which act as the endogenous mesoderm inducing factor; this is likely a nodal related factor (Xnr1 and/or Xnr2). We mimic the endogenous mesoderm inducing factor with activin in our experiments and show that it induces the expression of *eFGF* directly. eFGF protein directly induces the expression of *XmyoD*, possibly acting through inhibition of a repressor. XmyoD is crucial in the specification of the myogenic cell lineage.

signalling has been blocked do not express *XmyoD*, despite expressing *Xwnt8* at near normal levels (Isaacs et al., 1994).

Some of these same signals important for myogenesis in frogs have also been shown to play a role in regulating MRF gene expression in somites of higher vertebrates (Munsterberg et al., 1995; Reshef et al., 1998). An intriguing question is whether the molecular mechanisms involving FGF regulation of *XmyoD* in frogs are conserved in higher vertebrates? It is possible that as the early mesodermal expression of *MyoD* has been largely lost, perhaps this mode of *MyoD* activation is also no longer important. However there are some data that support the notion that FGF signalling is involved in regulating myogenesis in chick and mouse. FGFs have long been known to be mitogenic for cultured myoblasts (Linkhart et al., 1981) while inhibiting terminal differentiation. This is also true for muscle-forming cells in the chick limb (Seed and Hauschka, 1988). FGF receptors have been found to be expressed in the segmental plate mesoderm and newly formed somites in the mouse (Yamaguchi et al., 1992). Furthermore, an antibody that can neutralise bFGF has been shown to inhibit dorsal neural tube-derived myogenic signals in a chick explant study (Stern et al., 1997). These data support the notion of some role for FGF signalling during the regulation of myogenesis in chicks and perhaps mice, but the level at which this regulation occurs remains to be determined.

A molecular pathway for myogenic specification in frogs

Much work has been undertaken to investigate the maternal factors involved in setting off a cascade that specifies the mesoderm in *Xenopus laevis* (Heasman, 1997). Depletion of *VegT*, a maternally deposited T-box transcription factor, results in the extinction of expression of many mesodermal genes, including *XmyoD* and *eFGF*. However, when the TGFβ signalling molecule Xnr2 is introduced into *VegT* depleted frog embryos, the expression of *XmyoD* and *eFGF* is rescued (Kofron et al., 1999). Consistent with this, a mutation in the zebrafish homologue of *VegT*, *spadetail*, results in fish which lack early expression of *MyoD* (Weinberg et al., 1996).

In this study we use activin to mimic the nodal-related endogenous mesoderm inducing factor, and show that *eFGF* expression is a direct response to mesoderm inducing signals. We also show that eFGF can activate *XmyoD* expression even in the presence of protein synthesis inhibitors and that furthermore, *eFGF* is required for the activation of *XmyoD* in vivo.

We are now in a position to propose a molecular pathway from maternally deposited factors to myogenic specification (Fig. 7). Vegetally localised maternal factors such as VegT regulate the expression of TGFβ signalling molecules such as the nodal related factor *Xnr2*. This factor(s) induces mesoderm during blastula stages and activates expression from a subset of genes, including *eFGF*, in the nascent mesoderm. eFGF induces the expression of *XmyoD* in the mesoderm and specifies the cells of the myogenic lineage, possibly via the inhibition of a labile transcriptional repressor of *XmyoD*.

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REFERENCES

Amaya, E., Musci, T. J. and Kirschner, M. W. (1991). Expression of a dominant negative mutant of the FGF receptor disrupts mesoderm formation in Xenopus embryos. *Cell* **66**, 257-270.

Amaya, E., Stein, P. A., Musci, T. J. and Kirschner, M. W. (1993). FGF signalling in the early specification of mesoderm in Xenopus. *Development* **118**, 477-487.

Arnold, H. H. and Winter, B. (1998). Muscle differentiation: more complexity to the network of myogenic regulators. *Curr. Opin. Genet. Dev.* **8**, 539-544.

Cascio, S. and Gurdon, J. B. (1987). The initiation of new gene transcription during Xenopus gastrulation requires immediately preceding protein synthesis. *Development* **100**, 297-305.

Chen, Y., Lee, W., Liu, C. and Tsai, H. (2001). Molecular structure, dynamic expression and promoter analysis of zebrafish (Danio rerio) myf-5 gene. *Genesis* **29**, 22-35.

Christen, B. and Slack, J. M. (1997). FGF-8 is associated with anteroposterior patterning and limb regeneration in Xenopus. *Dev. Biol.* **192**, 455-66.

Cornell, R. A. and Kimelman, D. (1994). Activin-mediated mesoderm induction requires FGF. *Development* **120**, 453-462.

Cornell, R. A., Musci, T. J. and Kimelman, D. (1995). FGF is a prospective competence factor for early activin-type signals in Xenopus mesoderm induction. *Development* **121**, 2429-2437.

Cossu, G., Tajbakhsh, S. and Buckingham, M. (1996). How is myogenesis initiated in the embryo? *Trends Genet.* **12**, 218-23.

Edwards, D. R. and Mahadevan, L. C. (1992). Protein synthesis inhibitors differentially superinduce c-fos and c-jun by three distinct mechanisms: lack of evidence for labile repressors. *EMBO J.* **11**, 2415-2424.

Gerhart, J., Baytion, M., DeLuca, S., Getts, R., Lopez, C., Niewenhuis, R., Nilsen, T., Olex, S., Weintraub, H. and George-Weinstein, M. (2000). DNA dendrimers localize MyoD mRNA in presomitic tissues of the chick embryo. *J. Cell Biol.* **149**, 825-834.

Goldhamer, D. J., Faerman, A., Shani, M. and Emerson, C. P. (1992). Regulatory elements that control the lineage specific expression of myoD. *Science* **256**, 538-542.

Gurdon, J. B., Tiller, B., Roberts, J. and Kato, K. (1993). A community effect in muscle development. *Curr. Biol.* **3**, 1-11.

Gurdon, J. B., Mohun, T. J., Brennan, S. and Cascio, S. (1985). Actin genes in Xenopus and their developmental control. *J. Embryol. Exp. Morphol.* **89**, 125-136.

Harvey, R. P. (1990). The Xenopus MyoD gene: An unlocalised maternal mRNA predates lineage-restricted expression in the early embryo. *Development* **108**, 669-680.

Harvey, R. P. (1991). Widespread expression of MyoD genes in Xenopus embryos is amplified in presumptive muscle as a delayed response to mesoderm induction. *Proc. Natl. Acad. Sci. USA* **88**, 9198-9202.

Heasman, J. (1997). Patterning the Xenopus blastula. *Development* **124**, 4179-4191.

Heasman, J., Kofron, M. and Wylie, C. (2000). Beta-catenin signaling activity dissected in the early Xenopus embryo: a novel antisense approach. *Dev. Biol.* **222**, 124-134.

Hirsinger, E., Malapert, M., Dubrulle, J., Delfini, M. C., Duprez, D. M., Henrique, D., Ish-Horowicz, D. and Pourquié, O. (2001). Notch signalling acts in postmitotic avian myogenic cells to control MyoD activation. *Development* **128**, 107-116.

Hoppler, S., Brown, J. D. and Moon, R. T. (1996). Expression of a dominant-negative Wnt blocks induction of MyoD in Xenopus embryos. *Genes Dev.* **10**, 2805-2817.

Hopwood, N. D., Pluck, A. and Gurdon, J. B. (1989). MyoD expression in the forming somites is an early response to mesoderm induction in Xenopus embryos. *EMBO J.* **8**, 3409-3417.

Hopwood, N. D., Pluck, A. and Gurdon, J. (1991). Xenopus Myf-5 marks early muscle cells and can activate muscle genes ectopically in early embryos. *Development* **111**, 551-560.

Isaacs, H. (1997). New perspectives on the role of the fibroblast growth factor family in amphibian development. *Cell. Mol. Life Sci.* **53**, 350-361.

Isaacs, H. V., Tannahill, D. and Slack, J. M. W. (1992). Expression of a novel FGF in the Xenopus embryo. A new candidate inducing factor for mesoderm formation and anteroposterior specification. *Development* **114**, 711-720.

Isaacs, H. V., Pownall, M. E. and Slack, J. M. W. (1994). eFGF regulates Xbra expression during Xenopus gastrulation. *EMBO J.* **13**, 4469-4481.

Isaacs, H. V., Pownall, M. E. and Slack, J. M. W. (1995). eFGF is expressed in the dorsal midline of Xenopus laevis. *Int. J. Dev. Biol.* **39**, 575-579.

Isaacs, H. V., Pownall, M. E. and Slack, J. M. W. (1998). Regulation of Hox gene expression and posterior development by the *Xenopus* caudal homologue Xcad3. *EMBO J.* **17**, 3413-3427.

Jones, C. M., Kuehn, M. R., Hogan, B. L. M., Smith, J. C. and Wright, C. V. E. (1995). Nodal-related signals induce axial mesoderm and dorsalize mesoderm during gastrulation. *Development* **121**, 3651-3662.

Kiefer, J. C. and Hauschka, S. D. (2001). Myf-5 is transiently expressed in nonmuscle mesoderm and exhibits dynamic regional changes within the presegmented mesoderm and somites I-IV. *Dev. Biol.* **232**, 77-90.

Kofron, M., Demel, T., Xanthos, J., Lohr, J., Sun, B., Sive, H., Osada, S., Wright, C., Wylie, C. and Heasman, J. (1999). Mesoderm induction in Xenopus is a zygotic event regulated by maternal VegT via TGFbeta growth factors. *Development* **126**, 5759-5770.

Lin-Jones, J. and Hauschka, S. D. (1996). Myogenic determination factor expression in the developing avian limb bud: an RT-PCR analysis. *Dev. Biol.* **174**, 407-422.

Linkhart, T. A., Clegg, C. H. and Hauschka, S. D. (1981). Myogenic differentiation in permanent clonal myoblast cell lines: regulation by macromolecular growth factors. *Dev. Biol.* **86**, 19-30.

Lombardo, A., Isaacs, H. V. and Slack, J. M. (1998). Expression and functions of FGF-3 in Xenopus development. *Int. J. Dev. Biol.* **42**, 1101-1107.

Munsterberg, A., Kitajewski, J., Bumcrot, D. A., McMahon, A. P. and Lassar, A. B. (1995). Combinatorial signaling by sonic hedgehog and wnt family members induce myogenic bHLH gene expression in the somite. *Genes Dev.* **9**, 2911-2922.

Nieuwkoop, P. D. and Faber, J. (1967). *Normal Table of* Xenopus laevis *(Daudin).* Amsterdam: North-Holland.

Novak, U., Cocks, B. G. and Hamilton, J. A. (1991). A labile transcriptional repressor acts through the NFKB-like binding-sites of the human urokinase gene. *Nucleic Acids Res.* **19**, 3389-3393.

Ott, M. O., Bober, E., Lyons, G., Arnold, H. H. and Buckingham, M. (1991). Early expression of the myogenic regulatory gene myf5 in precursor cells of skeletal muscle in the mouse embryo. *Development* **111**, 1097-1107.

Pownall, M. E. and Emerson, C. P. (1992). Sequential activation of three myogenic regulatory genes during somite morphogenesis in quail embryos. *Dev. Biol.* **151**, 67-79.

Pownall, M. E., Tucker, A. S., Slack, J. M. W. and Isaacs, H. V. (1996). eFGF, Xcad3 and Hox genes form a molecular pathway that establishes the anteroposterior axis in Xenopus. *Development* **122**, 3881-3892.

Reshef, R., Marato, M. and Lassar, A. B. (1998). Regulation of dorsal somitic cell fates: BMPs and Noggin control the timing and pattern of myogenic regulator expression. *Genes Dev.* **12**, 290-303.

Rosa, F. (1989). Mix.1, a homeobox mRNA inducible by mesoderm inducers, is expressed mostly in the presumptive endodermal cells of Xenopus embryos. *Cell* **57**, 965-974.

Rudnicki, M. A., Schnegelsberg, P. N. J., Stead, R. H., Braun, T., Arnold, H. H. and Jaenisch, R. (1993). Myod or myf-5 is required for the formation of skeletal-muscle. *Cell* **75**, 1351-1359.

Rupp, R. A. W. and Weintraub, H. (1991). Ubiquitous MyoD transcription at the midblastula transition precedes induction-dependent MyoD expression in presumptive mesoderm of Xenopus Laevis. *Cell* **65**, 927-937.

Sargent, M. G. and Bennett, M. F. (1990). Identification in Xenopus of a structural homologue of the Drosophila gene snail. *Development* **109**, 967-973.

Schulte-Merker, S. and Smith, J. C. (1995). Mesoderm formation in response to brachyury requires FGF signalling. *Curr. Biol.* **5**, 62-67.

Seed, J. and Hauschka, S. D. (1988). Clonal analysis of vertebrate myogenesis. VIII. Fibroblasts growth factor (FGF)-dependent and FGF-independent muscle colony types during chick wing development. *Dev. Biol.* **128**, 40-49.

Slack, J. M. W. (1994). Inducing factors in Xenopus early development. *Curr. Biol.* **4**, 116-126.

Slack, J. M. W., Darlington, B. G., Heath, J. K. and Godsave, S. F. (1987). Mesoderm induction in early Xenopus embryos by heparin-binding growth factors. *Nature* **326**, 197-200.

Smith, J. C. (1995). Mesoderm-inducing factors and mesodermal patterning. *Curr. Opin. Cell Biol.* **7**, 856-861.

Smith, J. C., Price, B. M. J., Green, J. B. A., Weigel, D. and Herrmann, B. G. (1991). Expression of a Xenopus homolog of Brachyury (T) is an immediate-early response to mesoderm induction. *Cell* **67**, 79-87.

Standley, H. J., Zorn, A. M. and Gurdon, J. B. (2001). eFGF and its mode of action in the community effect during Xenopus myogenesis. *Development* **128**, 1347-1357.

Steinbach, O. C., Ulshöfer, A., Authaler, A. and Rupp, R. A. (1998). Temporal restriction of MyoD induction and autocatalysis during Xenopus mesoderm formation. *Dev. Biol.* **202**, 280-292.

Stern, H. M., Lin-Jones, J. and Hauschka, S. D. (1997). Synergistic interactions between bFGF and a TGF-b family member may mediate myogenic signals from the neural tube. *Development* **124**, 3511-3523.

Thompson, J. and Slack, J. M. W. (1992). Over-expression of fibroblast growth factors in Xenopus embryos. *Mech. Dev.* **38**, 175-182.

Weinberg, E. S., Allende, M. L., Kelly, C. S., Abdelhamid, A., Murakami, T., Andermann, P., Doerre, O. G., Grunwald, D. J. and Riggleman, B. (1996). Developmental regulation of zebrafish myod in wild-type, no tail and spadetail embryos. *Development* **122**, 271-280.**Weintraub, H.** (1993). The myoD family and myogenesis redundancy, networks and thresholds. *Cell* **75**, 1241-1244.

Yamaguchi, T. P., Conlon, R. A. and Rossant, J. (1992). Expression of the fibroblast growth factor receptor FGFR-1/flg during gastrulation and segmentation in the mouse embryo. *Dev. Biol.* **152**, 75-88.