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Original Article

eFGF is expressed in the dorsal midline of *Xenopus laevis*

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ABSTRACT A detailed study of the expression pattern of *embryonic fibroblast growth factor (eFGF)* during early *Xenopus* development has been undertaken using whole-mount DIG *in situ* hybridization. We show that the zygotic expression of *eFGF* is activated in the mesoderm of the early gastrula and is first visualized as a ring around the blastopore, with significantly higher levels of expression on the dorsal side of the embryo. As gastrulation proceeds, *eFGF* transcripts become increasingly abundant in the dorsal blastopore lip. In the early neurula *eFGF* expression can be detected in the extreme posterior of the embryo around the closed blastopore and in the cells of the notochord. This latter result is significant and represents the first report of a *Xenopus* FGF that is expressed in the notochord. In addition, we show that during gastrula and neurula stages, expression of *eFGF* closely follows the expression of the *Xenopus brachyury (Xbra)* gene. During later development eFGF expression is localized to the tail-bud region and a stripe at the mid-brain/hind-brain junction. These data provide further evidence that FGFs play an important role in regulating the expression of *brachyury* in the developing mesoderm.

KEY WORDS: *brachyury, eFGF, fibroblast growth factor, notochord, Xenopus*

Introduction

In recent years it has become clear that members of the fibroblast growth factor (FGF) family are important for the development of organisms as diverse as *Drosophila* and the mouse (Basilico and Moscatelli, 1992; Shishido *et al.*, 1993). In the vertebrates there is good evidence that the FGFs are involved in a wide range of developmental processes. For example, recent work indicates that the FGFs play an essential role in the specification and subsequent outgrowth of the vertebrate limb (Niswander *et al.*, 1994; Cohn *et al.*, 1995). In addition, work carried out on the amphibian *Xenopus laevis* has demonstrated that the FGFs play important roles during the very earliest stages of development, when the basic vertebrate body plan is established (for review see, Slack, 1994).

In this laboratory we have made an extensive study of the expression patterns and biological activities of the FGFs in *Xenopus* (Isaacs *et al.*, 1992; Tannahill *et al.*, 1992; Song and Slack, 1994; reviewed, Slack, 1994). These data have allowed us to propose that a major role of the FGFs is the regulation of gene expression within the newly formed mesoderm during gastrula and neurula stages (Isaacs *et al.*, 1994). In particular there is good evidence that the FGFs are important for regulating the expression from the *Xenopus* homologue of the *brachyury* gene *(Xbra)* (Smith *et al.*, 1991). A number of pieces of data point to a close link between FGF activity and the regulation of *brachyury* expression. First, it is clear that a functional FGF signal transduction pathway is required for the activation and maintenance of *Xbra* expression in the *Xenopus* embryo (Amaya *et al.*, 1993; Isaacs *et al.*, 1994). Second, there is a striking similarity between the phenotype of *Xenopus* embryos in which FGF function is inhibited and the phenotype of zebrafish and mouse embryos homozygous for mutations in the *brachyury* gene. Both phenotypes are characterized by rather normal anterior development and dramatic reductions in posterior structures (Amaya *et al.*, 1991; Herrmann and Kispert, 1994; Isaacs *et al.*, 1994; Schulte-Merker *et al.*, 1994). Third, there is considerable overlap between the expression domains of FGFs and *Xbra* (Smith *et al.*, 1991; Isaacs *et al.*, 1992; Tannahill *et al.*, 1992). These earlier studies showed that during late gastrula stages both *eFGF* and *Xbra* are expressed in the mesoderm of the blastopore region and in later development are expressed in the developing tail-bud. Finally it has been shown that FGF signalling is not only necessary but is also sufficient to maintain *Xbra* expression in cells of the blastopore region (Isaacs *et al.*, 1994; Schulte-Merker and Smith, 1995).

In addition to being expressed in the mesoderm around the blastopore, *Xbra* transcripts are found in the notochord cells of the dorsal midline during late gastrula and neurula stages. The previous study of *eFGF* expression using radioactive *in situ* hybridization to embryo sections however, did not detect *eFGF* transcripts in the involuted cells of the dorsal midline (Isaacs *et al.*, 1992). This suggested that eFGF was not involved with the regulation and maintenance of *Xbra* expression in the notochord.

Abbreviations used in this paper: FGF, fibroblast growth factor; eFGF, embryonic fibroblast growth factor.

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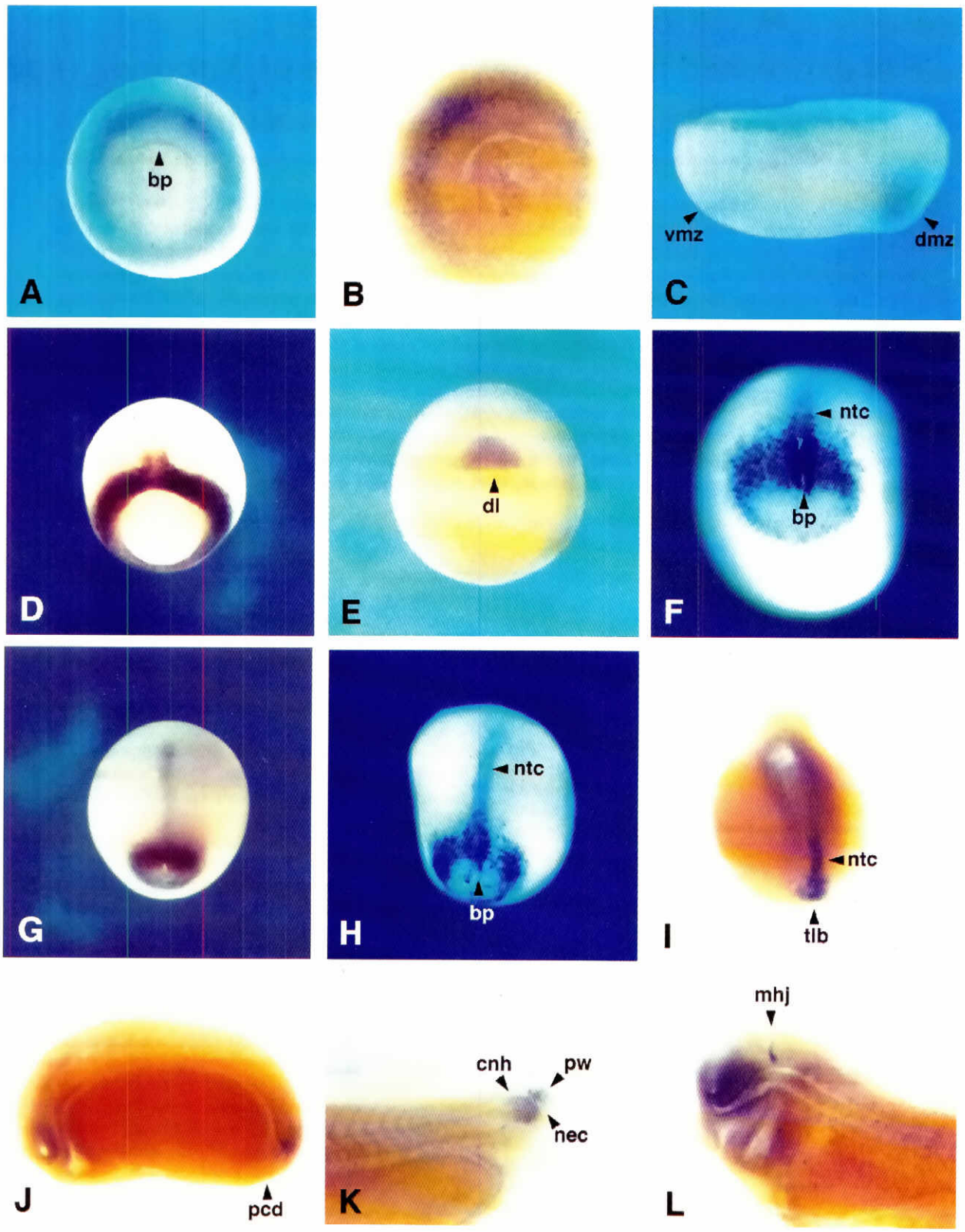
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We have now extended our earlier characterization of the *eFGF* expression pattern using the sensitive technique of whole-mount DIG *in situ* hybridization and confirm that in the early gastrula *eFGF* and *Xbra* are coexpressed in the blastopore region. Later expression of *eFGF* is also detected in specific regions of the developing central nervous system and tailbud. However, the whole-mount technique unexpectedly reveals that *eFGF* expression becomes increasingly prominent within the dorsal mesoderm during mid and late gastrula stages and continues to be expressed in the notochord through neurula stages. These data raise the possibility that *eFGF* not only regulates *Xbra* expression in the blastopore region but also in the notochord and suggests that eFGF is likely to have a role in the specification and patterning of dorsal midline structures.

Results

eFGF is expressed in the mesoderm of the early gastrula

Low levels of maternal *eFGF* mRNA can be detected by RNAase protection in fertilized eggs (Isaacs *et al.*, 1992). However, using the techniques of radioactive and DIG *in situ* hybridization, we have not been able to visualize the spatial distribution of maternal *eFGF* mRNA during early cleavage or blastula stages. We are first able to detect *eFGF* expression using whole-mount DIG *in situ* hybridization, following the onset of zygotic transcription in the early gastrula. At stage 10.5 *eFGF* mRNA is present in a complete ring around the blastopore, although the signal is considerably stronger on the dorsal side of the embryo (Fig. 1A,B and C). This pattern is very similar to the gradient of *Xbra* expression in the marginal zone of the early gastrula reported by Ruiz i Altaba and Jessell (1992) and supports the view that there is a close link between the regulation *eFGF* and *Xbra* expression.

eFGF is expressed in the blastopore region and the notochord of the late gastrula and neurula

Figure 1D shows that by stage 11.5 *Xbra* is expressed in a complete ring around the closing blastopore and in addition is expressed in notochord cells of the dorsal midline. Our previous study of *eFGF* expression failed to detect any *eFGF* expression in the notochord suggesting that other factors are involved in the regulation of *Xbra* expression in the dorsal midline. However, Figure 1E shows that *eFGF* is expressed at high levels in the involuting dorsal mesoderm of the stage 11.5 gastrula. This raises the possibility that *eFGF* is also required for the activation and maintenance of *Xbra* expression in the notochord. At this stage the expression of *eFGF* is very faint in the lateral and ventral aspects of the blastopore lip. By the end of gastrulation *eFGF* transcripts are clearly detected in the cells of the notochord and in wings of tissue that extend laterally and ventrally around the blastopore (Fig. 1F). In the open neural plate stage 13 it can be seen that *Xbra* (Fig. 1G) and *eFGF* (Fig. 1H) are both expressed in very similar domains in the posterior of the embryo around the closed blastopore and in the notochord.

eFGF is expressed in the posterior notochord of the early tailbud stage

During later neurula stages and in the early tailbud the expression of *eFGF* becomes increasingly restricted to the extreme posterior of the embryo. The dorsal posterior view of a cleared stage 23 embryo in Figure 1I shows *eFGF* mRNA is present in the posterior notochord and tailbud region. The side view of an embryo from the same stage shows that *eFGF* expression is absent from all but the most posterior part of the dorsal axis (Fig. 1J).

eFGF is expressed in the CNS and tail forming region during tailbud extension

Figure 1K shows that during tailbud stages *eFGF* expression is not detected in the mature notochord but is present in both the chordoneural hinge and the posterior wall of the neuroenteric canal in the tail forming region (Gont *et al.*, 1993; Tucker and Slack, 1995). The expression of *eFGF* within these structures is very much in keeping with lineage studies which show that the chordoneural hinge derives from cells of the late dorsal lip and that the posterior wall of the neuroenteric canal arises from the lateral lips of the late blastopore (Gont *et al.*, 1993). In addition to this posterior domain, a prominent stripe of *eFGF* expression is seen at the midbrain/hind brain junction (Fig. 1L). It is likely that the staining in the forebrain cavity and branchial arches at this stage represents background staining.

Discussion

Previous studies have shown that a functional FGF signal transduction pathway is necessary for the activation of expression from the *Xenopus* homologue of the *brachyury* gene *(Xbra)* in normal development (Amaya *et al.*, 1993; Isaacs *et al.*, 1994)

Fig. 1. *eFGF* and *Xbra* expression during development. (A,B,C,E,F,H,I,J,K and L) *Embryos hybridized to eFGF antisense RNA.* **(D and G)** *Embryos hybridized to Xbra antisense RNA.* **(A and B)** *Vegetal views (dorsal is to the top) of stage 10.5 embryos showing eFGF expression in the mesoderm of the marginal zone around the blastopore. The arrow indicates the position of the dorsal lip (bp) The specimen in* **(B)** *has been cleared.* **(C)** *shows the cut surface of a stage 10.5 embryo that has been manually dissected along the dorsoventral midline to reveal staining in the deep layers of the marginal zone (dorsal is to the right and animal to the top) showing that there is a higher level of eFGF expression in the dorsal marginal zone (dmz) than in the ventral marginal zone (vmz) (note the animal hemisphere has collapsed onto the vegetal yolk mass during processing).* **(D)** *Dorsovegetal view of Xbra staining in the blastopore region and involuting dorsal mesoderm of a stage 11.5 embryo.* **(E)** *Dorsovegetal view showing high levels of eFGF expression in the involuting dorsal mesoderm of a stage 11.5 embryo above the dorsal lip (dl) of the blastopore.* **(F)** *Posterior view of a stage 13 embryo (dorsal to the top) showing eFGF expression in the notochord (ntc) and around the blastopore (bp).* **(G and H)** *Dorsal views of stage 13 embryos (anterior to the top) showing expression of Xbra and eFGF respectively, in the notochord and posterior around the closed blastopore (bp).* **(I)** *Posterior dorsal view of a cleared stage 23 embryo (anterior is into the page) showing eFGF expression in tail-bud (tlb) and in the posterior notochord (ntc).* **(J)** *Side view of a cleared stage 23 embryo (anterior to the right) showing that eFGF expression is restricted to the extreme posterior of the developing axis. The arrow shows the position of the proctodeum (pcd).* **(K)** *Side view of the tail forming region from a cleared stage 31 embryo showing eFGF expression in the chordoneural hinge (cnh) and posterior wall (pw) of the neuroenteric canal (nec).* **(L)** *Side view of a cleared stage 31 embryo showing a prominent stripe of eFGF expression at the midbrain/hindbrain junction (mhj) (note the staining in the brain and branchial arch cavities is background).*

and in response to mesoderm induction by activin (Cornell and Kimelman, 1994; Labonne and Whitman, 1994). Data from experiments, which involve disaggregated cell culture of explants from the blastopore region of gastrulae, indicate that the maintenance of *Xbra* expression in the nascent mesoderm is dependent upon intercellular signals (Isaacs *et al.*, 1994; Schulte-Merker and Smith, 1995). These experiments also showed that the addition of eFGF protein to such disaggregated cell cultures can mimic the endogenous *Xbra* maintenance signal. Furthermore, *eFGF* shares overlapping domains of expression with *Xbra* in the blastopore and tail forming regions of the embryo. These data provide good evidence that *eFGF* functions as an autocrine factor involved in the regulation of *Xbra* expression within the mesoderm of the blastopore region. However, *Xbra* is also expressed in the notochord, which is the most dorsal mesodermal tissue-type. Using radioactive *in situ* hybridization it was not possible to detect *eFGF* expression within the notochord, suggesting that the regulation of *Xbra* within the cells of the notochord was independent of *eFGF*.

In this study we use the technique of whole-mount *in situ* hybridization to confirm that during early gastrula stages *eFGF* is coexpressed with *Xbra* in the blastopore region. However, the new data show that *eFGF* is also expressed in the notochord cells of the late gastrula and early neurula. This suggests that *eFGF* may be involved in regulating *Xbra* expression in the mesoderm of the dorsal midline. Later in development the expression of *eFGF* is lost from the mature notochord but continues to be coexpressed with *Xbra* in the chordoneural hinge and posterior wall of the tailbud. These data show that throughout tailbud stages *eFGF* is expressed in populations of cells that are related by lineage to cells derived from the blastopore lip at the end of gastrulation. It is likely that eFGF plays a role in regulating the properties of these cells necessary for the complex morphogenetic activity of gastrulation and tailbud extension.

In this paper we report the first example of a *Xenopus* FGF family member being expressed in the cells of the notochord. These data add considerable weight to the view that the FGFs, and in particular *eFGF*, are involved in the regulation of *Xbra* expression, not only within the mesoderm of the blastopore region, but also in the notochord. The presence of *eFGF* within the notochord also has implications for the signalling pathways involved in the development of dorsal midline structures. The signalling molecule *sonic hedgehog (shh)* has also been shown to be expressed in the notochord of *Xenopus* (Ekker *et al.*, 1995). In this context it is interesting to note that *FGF-4*, to which *eFGF* is most closely related, and *shh* have been implicated in the growth and patterning of limb and feather buds (Niswander *et al.*, 1994; Nohno *et al.*, 1995). Further work will be required to determine if the *eFGF* and *shh* signalling pathways interact in a similar way during the induction and patterning of dorsal midline structures.

Materials and Methods

Embryos

Embryos were cultured as per the methods of Godsave *et al.* (1988) and staged according to Nieuwkoop and Faber (1967).

In situ hybridization

Whole-mount *in situ* hybridization procedures were carried out on albino embryos according to Harland (1991). Embryos were treated with proteinase K (10 μg/ml) for 10 to 20 min. Hybridization was carried out overnight at 60°C. In a number of cases the RNAsing step was omitted as this was found to considerably increase the sensitivity of the technique without a significant increase in background. Color reactions for *Xbra* were carried out for 1 h and overnight for *eFGF*. Embryos were cleared in 100% 1,2,3,4-tetrahydronaphthalene (Aldrich).The antisense *eFGF* DIG labeled probe was transcribed from *Eco RI* linearized *XeFGF*(i) GS (Isaacs *et al.*, 1992). The *Xbra* probe was transcribed from *ClaI* linearized *Xbra* pSp73 (Smith *et al.*, 1991).

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