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New perspectives on the role of the fibroblast growth factor family in amphibian development

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Abstract. It has been known for several years that the fibroblast growth factors (FGFs) have potent mesoderm-inducing activity. As a result they have been considered good candidates for one of the endogenous vegetally localized mesoderm-inducing signals in the amphibian *Xenopus laevis*. In this review the properties of the FGFs and their expression patterns in *Xenopus* are described. Recent work is discussed which reveals a close link between FGF signalling and regulation of the *Xenopus brachyury (Xbra)* gene. These data are used to build a model of FGF function which is quite different from what was originally conceived. Present evidence supports the view that during blastula stages the FGFs do not act as vegetally localized inducing signals. Instead, they are required in the animal hemisphere as competence factors, which provide a low level stimulation of the tyrosine kinase signal transduction pathway. FGF activity is necessary for the full range of responses to the vegetal inducing signals, including the activation of *Xbra* transcription in the marginal zone of the late blastula. Xbra is able to activate the zygotic transcription of *eFGF*, which suggests that there is a period of autocatalytic activation of *eFGF* and *Xbra* transcription within the forming mesoderm of the marginal zone. FGF activity continues to be required to maintain the expression of a sub-set of mesodermal genes, including *Xbra*, in the blastopore region and possibly also in the notochord through gastrula and neurula stages. In addition a role for the FGFs in anteroposterior specification and development of the myogenic lineages is discussed.
Key words. *Xenopus*; gastrulation; fibroblast growth factor; dominant negative FGF receptor; brachyury.

Introduction

In amphibians the mesoderm arises as the result of an inductive event in which signals emitted from the unpigmented vegetal hemisphere cause cells within the marginal zone to adopt a mesodermal fate. In recent years there has been a great expansion in our understanding of this inductive process. To a large extent this has resulted from the development of a simple in vitro mesoderm induction assay in *Xenopus laevis*, which has enabled workers to identify a number of purified polypeptide growth factors capable of mimicking the endogenous mesoderm-inducing signals [1–3]. Much work has concentrated on the inducing activities of the fibroblast growth factors (FGF) and members of the transforming growth factor β (TGFβ) family, in particular the activins and bone morphogenetic proteins (BMP) The demonstration that FGFs induce ventral-type mesoderm and the activins induce dorsal-type mesoderm in the mesoderm induction assay quickly established them as candidates for the endogenous inducing signals. This candidacy was further strengthened when members of both families were shown to be present in the early embryo [4–9]. However, as our knowledge of these molecules has increased the views on their functions in development have been modified. Whilst it still seems likely that an activin-like molecule is an important part of the vegetal inducing signal, recent data suggest a rather different role for the FGFs. The objective of this review is to describe the available data on the properties of the FGFs, their activities and distribution within the *Xenopus* embryo. These data are then used to build a model for the role of the FGFs in early development that is quite different from that which was first envisaged.

Mesoderm induction in *Xenopus*

In normal development the animal pole region of a *Xenopus* blastula stage embryo forms ectodermal derivatives. In accordance with this, if it is explanted and cultured in isolation it differentiates as a ball of ciliated epidermis. However, if an 'animal cap' explant is cultured in combination with tissue taken from the vegetal hemisphere it is induced to form mesodermal derivatives, which normally only arise in explants from the marginal zone [10, 11]. Animal/vegetal combinations, also known as 'Nieuwkoop' combinations, provide a powerful tool for investigating the endogenous mesoderm-inducing signals. Experiments of this type show that the vegetal hemisphere can be divided into two regions based upon the dorsoventral nature of inductions elicited in such combinations [12]. A rather large ventrovegetal region is capable of inducing ventral mesodermal tissue types such as blood, mesenchyme and mesothelium. A smaller region of the vegetal hemi-

sphere on the dorsal side, which has been termed the 'Nieuwkoop centre', is capable of inducing dorsal mesoderm, such as muscle and notochord. The relative size of these two regions is reflected in the dorsoventral specification of the mesoderm at the end of the blastula stage. The specification map shows that, when cultured in isolation, most of the marginal zone will differentiate into ventral mesodermal derivatives [12, 13]. At this stage it is only a small region of the dorsal marginal zone that is specified to form dorsal mesoderm possessing the properties of Spemann's organizer [14, 15]. In contrast to the specification map, the fate map shows that in normal development much of the somitic muscle is derived from the ventral blastomeres [12]. This discrepancy between specification and fate map indicates that additional interactions take place within the mesoderm during late blastula and gastrula stages, which further pattern the mesoderm along the dorsoventral axis [13, 16, 17].

The animal cap assay

An important development of 'Nieuwkoop combinations' has been the animal cap serial dilution assay [18]. Using this assay the mesoderm-inducing activity of a given factor can be quantified. Briefly, mid-blastula stage animal cap explants are exposed to serial dilutions of the factor and cultured for up 3 days, after which they are analysed histologically or with molecular markers for mesoderm formation. The animal cap assay has also been extended to test the autoinducing activity of injected mRNAs, where it is assumed that the effective quantities of active proteins produced are proportional to the injected amounts of mRNA [19, 20].

Mesoderm induction by the FGFs

The use of the animal cap assay has greatly assisted in the identification and purification of candidate inducing factors [3, 7, 18]. In this way members of the FGF family were shown to be capable of inducing ventrolateral mesoderm when applied to animal caps at subnanomolar concentrations [1, 21, 22]. Nine members of the FGF family have been identified in mammals [23], and of these acidic FGF (FGF-1), basic FGF (FGF-2), int-2 (FGF-3), kFGF (FGF-4), FGF-5, FGF-6 and FGF-9 have been shown to be active in this assay. Keratinocyte growth factor (FGF-7) is the only member tested so far that is not active (Isaacs and Slack, unpublished observations). At present there are no reports of the mesoderm-inducing activity of FGF-8 [24]. One feature of the FGFs that is of great importance when considering them as candidates for endogenous inducing factors is that some FGFs (acidic FGF, basic FGF and FGF-9) lack recognizable secretory signal peptides. Both secreted and non-secreted forms of FGF are found in the early embryo and this must be taken into consideration in any discussion of the role of these factors in early development.

Animal cap cells are competent to respond to induction by FGFs from early cleavage stages until the start of gastrulation [25], which is very similar to their period of competence for the endogenous inducing signals [26]. Treatment with FGFs over a 100-fold range of concentrations shows that animal caps exhibit an extended dose response to these factors [1, 25]. At low doses of FGF, inductions are of an extreme ventral character. Such animal caps form vesicles consisting of an outer jacket of epidermis surrounding loosely packed mesenchyme and a layer of mesothelium (see fig. 1) At higher doses the inductions are of a more lateral character with increasing quantities of muscle being found. However, even at the highest doses, explants taken from the animal pole region never form notochord in response to FGF treatment. Furthermore, analysis of molecular markers indicates that in contrast to activin-like molecules, the FGFs are unable to induce the expression of *goosecoid*, *noggin* and *LIM* [27–29]. These are genes expressed in the region of Spemann's organizer and considered to be markers of the most dorsal mesoderm. However, mesoderm induction by FGF can be modified to a more dorsal-type in number of ways. For example it has been shown that the injection of *Xwnt-8* mRNA into animal caps results in a more dorsal-type induction following FGF treatment [30]. This has led to the proposal that an FGF could also be a component of the dorsal mesoderm-inducing signal. The ability of one factor to modify the activity of another is an emerging theme and such positive and negative interactions are clearly important in the establishment of the relative sizes of the dorsal and ventral mesodermal territories [31–33].

FGFs in the early *Xenopus* embryo

Heterochronic combinations of animal and vegetal tissue demonstrate that in *Xenopus* mesoderm induction probably commences in the early blastula [26]. This is before the onset of zygotic transcription, which occurs at the mid-blastula transition (MBT), and indicates that the initial components necessary for mesoderm induction must be deposited maternally in the embryo. At present four members of the FGF family have been identified in *Xenopus*. The first to be identified was basic FGF (FGF-2) [6, 7, 21]. More recently int-2 (FGF-3), embryonic FGF (eFGF) and FGF-9 have been shown to be present in the early embryo [5, 8] (J. Song, personal communication) *bFGF*, *eFGF* and *FGF-9* all show maternal and zygotic expression. However, *int-2* mRNA is not detected until the late blastula stage following the onset of transcription from the zygotic genome. Therefore, using the criterion of temporal expression, bFGF, eFGF and FGF-9 are the best candi-

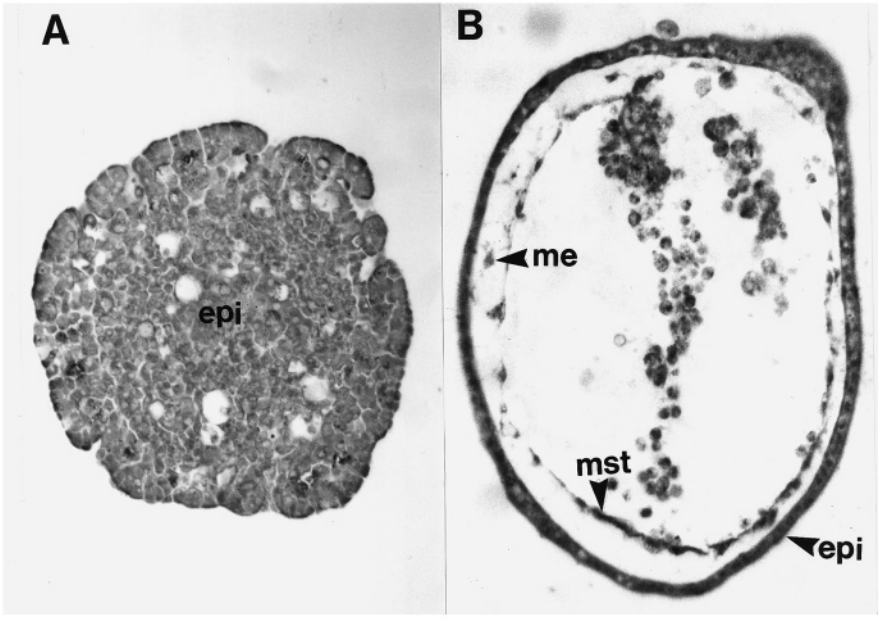

Figure 1. Mesoderm induction by bFGF. (*A*) shows a section through an animal cap cultured in isolation for 3 days. The explant consists of a ball of ciliated epidermis (Epi). All cells within it react to antibodies against cytokeratins. (*B*) shows a section through an animal cap after 3 days of culture following injection with 500 pg of bFGF mRNA. This ventral-type induction is typical of low dose treatment with FGF. The explant forms a transparent vesicle that consists of an epidermal (Epi) jacket surrounding loose mesenchyme (Me) and a layer of mesothelium (Mst).

dates as factors involved in the formation of the mesoderm during blastula stages. However, all four known *Xenopus* FGFs are expressed in the mesoderm after the blastula stage, so it is likely that they have roles to play in its subsequent patterning.

basic FGF

Both *bFGF* mRNA and protein have been shown to be present in the early embryo [6, 7, 21, 34, 35]. The temporal expression of *bFGF* mRNA shows that it is present at relatively low levels in fertilized eggs and that this level falls until early neurula stages when zygotic transcription of *bFGF* is activated. The first report of the spatial expression of bFGF protein in the cleavage and blastula stage embryo indicated that it was primarily localized to the marginal zone and vegetal hemisphere [34]. Such an expression pattern would be very much in keeping with this molecule having a role as an endogenous mesoderm-inducing factor. However, a more recent report shows that the distribution of bFGF protein and mRNA in the blastula does not exhibit a vegetal localization; in fact the converse appears to be the case [35]. On a per unit volume basis bFGF is found predominantly in the animal hemisphere of the blastula. In later development through neurula and tailbud stages *bFGF* has widespread expression in the CNS and somitic tissue [35].

A number of biological experiments cast doubt on a major role for bFGF in mesoderm induction. The first of these experiments provides further evidence that bFGF is not a component of the vegetal inducing signal. Slack [36] shows that a neutralizing antibody specific to *Xenopus* bFGF does not block mesoderm induction by vegetal hemisphere tissue across a fluid-filled gap in a transfilter apparatus. Second, as already discussed, bFGF lacks a signal peptide. Although in some systems FGFs lacking a signal peptide have been shown to be secreted by novel mechanisms [37, 38], there is good evidence that bFGF is not secreted efficiently from cells of the early *Xenopus* embryo [39].

int-2

The *Xenopus* homologue of the mammalian proto-oncogene *int-2* (FGF-3) is only expressed zygotically. int-2 does have weak mesoderm-inducing activity, but given its expression pattern it is unlikely to be involved in the formation of the mesoderm in the blastula [8, 22]. During gastrula stages *int-2* exhibits a distinct posterior domain of expression in the mesoderm of the blastopore region and an anterior ectodermal domain. Subsequently the posterior domain becomes localized to the tailbud and the anterior domain breaks up into a complex pattern of expression in the head [8].

FGF-9

The *Xenopus* homologue of *FGF-9* has recently been cloned (J. Song, personal communication), and shows a remarkably high degree of predicted amino acid identity to the rat and human proteins. *FGF-9* mRNA is present throughout early development. Maternal expression is

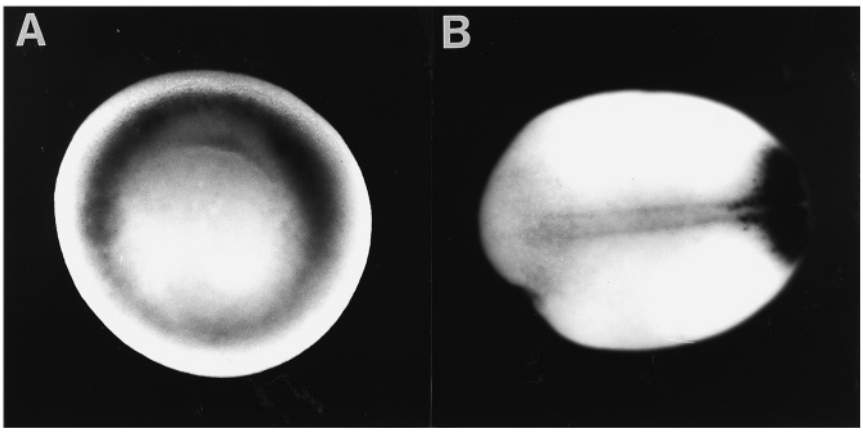

Figure 2. eFGF expression in early development. (*A*) is a vegetal view of a whole-mount in situ hybridization showing eFGF expression in the mesoderm of an early gastrula *Xenopus* embryo (dorsal is towards the top). Note expression is in a complete ring around the closing blastopore but the signal is considerably stronger on the dorsal side of the embryo. (*B*) shows a dorsal view of an early neurula stage *Xenopus* embryo (anterior is to the left). Note strong expression in the extreme posterior of the embryo and in the dorsal midline cells of the notochord.

chiefly in the animal hemisphere and later zygotic expression is throughout the whole of the developing axis. As is the case with bFGF, FGF-9 lacks a recognized secretory signal peptide but unlike bFGF, does appear to enter the secretory pathway and is efficiently secreted from at least some mammalian cell lines. The available data on the biological activities of *Xenopus* FGF-9 protein suggest that it may also reach the cell surface.

eFGF

eFGF is, at the amino acid level, equally related to the mammalian FGF-4 and FGF-6 (about 60%) Both bFGF and eFGF proteins have similar mesoderm-inducing activity when applied exogenously to animal caps. Unlike bFGF, however, eFGF does have a signal peptide, and mRNA overexpression studies indicate that it is much more efficiently secreted from cells in the early embryo [5, 39]. As with *bFGF* and *FGF-9*, the maternal pool of *eFGF* mRNA, on a per unit volume basis, is mainly found in the animal hemisphere. Thus the expression data are not consistent with a role for any of the known maternal FGFs as vegetally localized mesoderm-inducing signals, but rather a role is suggested in the cells that respond to the vegetal signals.

Transcription of *eFGF* is activated in animal caps in response to treatment with mesoderm-inducing factors [8, 35, 39]. Similarly, it is likely that the zygotic expression of *eFGF* is activated in the nascent mesoderm of the embryo in response to the vegetal inducing signals. The initial expression of *eFGF* is in a ring around the blastopore with considerably more transcripts present on the dorsal side of the embryo. In the late gastrula and early neurula *eFGF* expression continues to be seen in the blastopore region but can also be seen in the cells of the notochord (see fig. 2) In late neurula and tailbud stage, *eFGF* is found in the extreme posterior of the embryo, becoming localized to the chordoneural hinge and posterior wall of the neuroenteric canal [40]. These structures in the tail-forming region of the embryo are related by lineage to cells of the late blastopore lip [41]. In addition a small domain of high level expression is detected in the brain at the level of the midbrain-hindbrain junction.

The FGF signal transduction pathway

The high affinity FGF receptors are members of the tyrosine kinase receptor family. At present four members of the FGF receptor family have been identified in mammals. This situation is complicated by the fact that each receptor comes in a variety of alternatively spliced isoforms that have different affinities for the various FGF ligands [42]. Two members of the receptor family have been cloned in *Xenopus*. Studies of the distribution of type-1 or *flg*-type receptor mRNA indicate that it is present thoughout the early stages of development [43]. Just as with the FGF ligand mRNA, on a per unit volume basis, the type-1 receptor mRNA is more abundant in the marginal zone and animal hemisphere. Western blot data confirm that the receptor protein also has a predominantly animal localization [44]. In the late gastrula stage immunohistochemical data show that the receptor is most abundant in the blastopore region [11]. The type-2 or *bek*-type receptor is not expressed in the blastula but is first detected in the anterior neural plate during gastrulation [45]. The expression of the type-2 receptor suggests that it is not involved in the establishment of the mesoderm. However, *int-2* has an anterior domain of expression within the ectoderm of the gastrula and it is possible that the type-2 receptor is involved in transducing the int-2 signal. This idea is further supported by recent evidence that shows that *Xenopus* int-2 protein can bind with high affinity to the IIIb and IIIc isoforms of the type-2 receptor [46].

Recently there has been much progress in the understanding of the signal transduction pathway used by this family of growth factor receptors. One important feature of the FGF family is that they all bind to heparin and heparan sulphate [23]. Binding of the ligand to heparan sulphate residues on proteoglycan low affinity receptors in the extracellular matrix greatly increases the affinity of the ligand for the tyrosine kinase signalling receptor. The high affinity receptors also bind heparin, and it has been suggested that a tripartite interaction of this sort may be a prerequisite for the activation of the receptor-ligand complex [47]. Binding of ligand causes dimerization of the high affinity receptor and cross-phosphorylation of its components on tyrosine residues [42]. This in turn leads to activation of the now well characterized *ras*, *raf*, MAP kinase pathway [48]. Although details of this pathway were originally determined in other systems, it is now clear that much of this is directly applicable to *Xenopus* [49].

A number of inhibitory and constitutively active forms of key components in the tyrosine kinase signalling pathway have been produced [49–51]. Use of these mutant components in conjunction with the animal cap assay has demonstrated that the *ras*, *raf*, MAP kinase pathway is of primary importance in transducing the mesoderm-inducing signal provided by FGF stimulation [50]. Experiments of this kind have also revealed an unexpectedly close link between the FGF and activin signal transduction pathways. In animal caps it has been shown that a functioning FGF signalling pathway is necessary for the activation of a number of early mesodermal marker genes in response to activin treatment. Significantly, FGF function is required for the expression of the early pan-mesodermal marker *Xbra* following induction by activin [49, 52]. However, activin does not directly stimulate the FGF signal transduction pathway, which means that in some way FGF activity is required to enable the activation of a subset of mesodermal genes by activin. Furthermore, the activation of *Xbra* transcription in animal caps by mesoderm-inducing factors, including activin, does not require protein synthesis [53]. This indicates that there is sufficient FGF protein present in blastula stage animal caps to allow the initial activation of *Xbra* following treatment with activin and that this must be stored maternally.

These data suggest a role for the maternal FGFs which is in keeping with their expression patterns. There is now good evidence that in the early blastula the FGFs do not act as vegetally localized mesoderm-inducing factors, but rather they provide a sub-threshold stimulation of the tyrosine kinase signal transduction pathway in the cells of the animal hemisphere [54]. This low level of FGF activity in the cells of the animal hemisphere is required for the full repertoire of responses to induction by activin-like molecules, and as such the FGFs can be considered as competence factors necessary for mesoderm induction [44]. The view that the vegetal hemisphere is not a major source of FGF signalling is further supported by recent evidence which demonstrates that, like the cells of the animal hemisphere, vegetal hemisphere cells can express the mesodermal markers *Xbra* and *MyoD* in response to treatment with FGF. However, unlike animal cells, vegetal cells do not express *Xbra* and *MyoD* in response to activin treatment. The fact that *Xbra* and *MyoD* expression is normally excluded from the vegetal hemisphere indicates that an FGF is not a major component of the endogenous vegetal signal [44].

Inhibition of the FGF signal transduction pathway in vivo

The animal cap assay has provided much useful information on the properties of the FGFs as mesoderm-inducing factors. However, definitive proof that the FGFs have an important role in early development must necessarily rely on experiments in which the action of the FGFs is inhibited in vivo. In developmental systems that are amenable to genetic manipulation, such as the mouse and *Drosophila*, this may be achieved by mutational and 'gene knockout' procedures. Unfortunately this approach is not yet feasible in *Xenopus*. However, an increasing range of technologies is being developed which will allow the inhibition of a particular molecule or group of molecules.

One approach to inhibition, which has been used to great effect in *Xenopus*, is the construction of 'dominant negative' forms of growth factor receptors. In the case of the FGF, activin and BMP dominant negative receptors, mutant forms were constructed that lack their intracellular kinase domains. When synthetic mRNAs coding for these mutant forms are injected into the early embryo they are translated, and the resulting mutant proteins are able to form unproductive dimers with the endogenous wild-type receptor. If the mutant receptors are present in excess it can be shown that, in the case of the FGF, activin and BMP mutant receptors, animal caps are refractory to induction by the respective ligands [39, 49, 50, 52, 55–57]. The dominant negative receptor approach has the advantage that, due to the cross-reactivity of ligands and their receptors, it is likely that a particular inhibitory mutant will block the activity of a whole group of ligands [58]. As such it will serve to highlight those processes which require the activity of that group of signalling molecules. Of course the downside of this is that the use of the dominant negative FGF receptor will not give any information as to the identity of the specific FGFs that are important. In the same way, the complete absence of mesoderm in embryos overexpressing the dominant negative activin receptor points towards a crucial role for an activin-like molecule in mesoderm induction, but the identity of this molecule is at present unclear [57, 59].

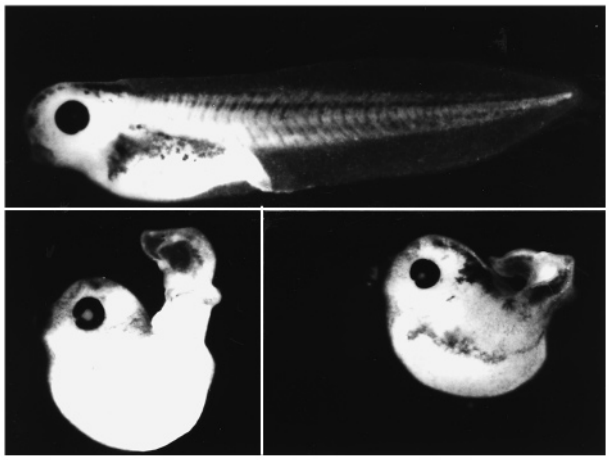

Figure 3. Inhibition of FGF activity in vivo. The top embryo is a swimming larval stage control *Xenopus* embryo after 3 days of development. The bottom embryos are also 3 days old but were injected with 125 pg of dominant negative FGF receptor mRNA into both dorsal blastomeres at the 4-cell stage. Note the characteristic reduction in trunk and tail development that results from inhibition of the FGF signal transduction in the embryo.

Dominant negative FGF receptor embryos

Injection of mRNA coding for the dominant negative FGF receptor or dominant inhibitory *raf* kinase into the early *Xenopus* embryo leads to a perturbation in normal development, which is characterized by normal development of head structures and a dramatic reduction in trunk and tail structures (see fig. 3) [39, 50, 51, 55]. Histological examination shows that in dominant negative FGF embryos the dorsoventral pattern of the mesoderm is disrupted and the total amount of mesoderm present is greatly reduced. There is a considerable reduction in the amount of blood, somitic muscle and notochord which indicates that the differentiation of both ventral and dorsal tissues is affected [50]. However, there are likely to be many cell-cell interactions subsequent to mesoderm induction which are necessary for the formation of specific tissue types [60]. Therefore it is perhaps unwise to use these effects of the dominant negative FGF receptor on terminal differentiation as evidence to suggest that the FGFs are needed for the very earliest stages of mesoderm formation.

FGF and the regulation of mesodermal gene expression

The best evidence that FGFs have a role in the earliest stages of mesoderm formation comes from looking at the effects of blocking the FGF signal tranduction pathway on the expression of early mesodermal markers in the embryo at the start of gastrulation [31, 55, 61]. Any effects on gene expression at this time must result from interference with FGF signalling during mesoderm induction in the blastula. The expression of a wide range of these early markers of mesoderm has now been examined in embryos overexpressing the dominant negative FGF receptor. In such embryos the expression of the dorsal regional marker *goosecoid* is not significantly affected, neither is the lateroventral marker *Xwnt-8*, nor is *Xsna*, which is normally expressed in the whole of the marginal zone at the start of gastrulation. However, the inhibition of the FGF signalling pathway does result in a dramatic down-regulation in the expression of *Xcad3* and *Xbra*, which like *Xsna* are expressed in the whole of the marginal zone at the start of gastrulation (see fig. 4). The expression of the dorsal mesoderm marker gene *noggin* is also down-regulated, but to a lesser degree. The initial expression of the myogenic basic helix-loop-helix gene *XmyoD* at the start of gastrulation is not affected but its expression in the late gastrula and neurula is greatly reduced. Interestingly, the onset of transcription from *Xhox36* and *Xlhbox1*, which are members of the *Xenopus* HOX complex, is delayed. The HOX genes are expressed in both mesoderm and ectoderm lineages, indicating an additional requirement for FGF activity in patterning of the ectoderm.

The present evidence suggests that FGF function is required for the correct regulation of a subset of genes that are expressed in the newly formed mesoderm and this requirement is not limited to the dorsal or ventral side of the embryo. This is in contrast to the data obtained using the dominant negative activin receptor which suggest that this signalling pathway is required for the expression of all mesodermal genes [44]. Hence the blocking of the activin signalling pathway in the embryo results in a complete absence of all mesodermal structures [57]. These data indicate that the FGFs are likely to have multiple and possibly independent roles in regulating gene expression within the nascent mesoderm. However, at present the best characterized role of FGF is in regulating the expression of the gene *Xbra*.

FGF and the regulation of Xbra expression

Xbra is the *Xenopus* homologue of the *brachyury* gene; a putative transcription factor that has been shown to have an important role in the formation of the mesoderm in a number of vertebrates (reviewed in ref. 62). Overexpression of *Xbra* in animal caps leads to the activation of a number of mesodermal marker genes and induces the formation of ventral-type mesoderm, indicating that it plays a similar role in *Xenopus* [63]. As already discussed, the activation of expression of *Xbra* in animal caps is an immediate early response to treatment by mesoderm-inducing factors [53]. This response requires a functional FGF signalling pathway [49, 52] and this is also the case for the activation of *Xbra* expression in the embryo by the endogenous mesoderm-inducing signals [39, 55]. Additional complexity to the relationship of Xbra with FGF is indicated by the observation that overexpression of *Xbra* in animal caps

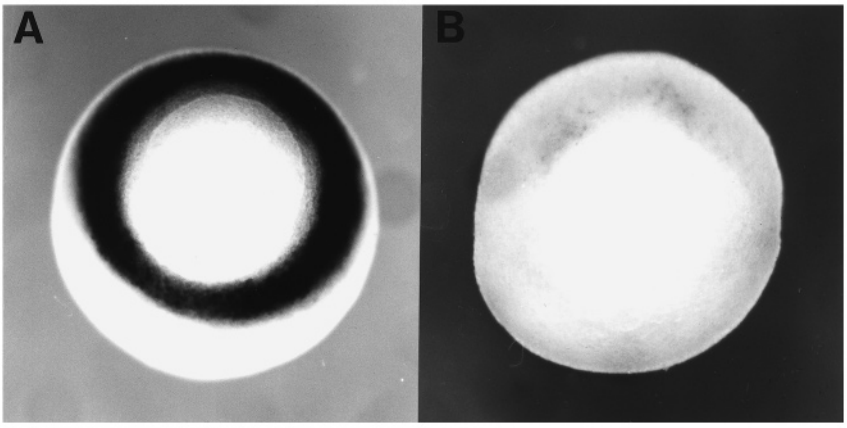

Figure 4. FGF function is required for the normal expression of Xbra. (*A*) is a vegetal view of a whole-mount in situ hybridization showing Xbra expression in the mesoderm of an early gastrula *Xenopus* embryo (dorsal is towards the top). Note that the domain of expression is very similar to that of eFGF in fig. 2A. (*B*) is a vegetal view of a whole-mount in situ hybridization showing the almost complete absence of Xbra expression in an early gastrula *Xenopus* embryo that was injected with 0.5 ng of dominant negative FGF receptor mRNA into each blastomere at the 4-cell stage.

activates the expression of *eFGF*. The ability of Xbra and eFGF to activate the expression of each other suggests that they may be components of an autocatalytic loop involved in the formation of the mesoderm in the late blastula.

The close relationship between *Xbra* expression and FGF activity continues through later development. At the start of gastrulation *eFGF* and *Xbra* are expressed in the mesoderm of the blastopore region [5, 40, 53]. When explants are taken from this region, and the cells are dissociated in divalent cation-free medium, the expression of *Xbra* is rapidly down-regulated. This indicates a requirement for cell-cell signalling in the maintenance of *Xbra* expression in the blastopore region. It has been shown however, that the addition of eFGF to the culture medium maintains the expression of *Xbra* in the these dissociated cell cultures [39, 64]. Given that *Xbra* and *eFGF* are coexpressed in the blastopore region, it is likely that eFGF contributes to this maintenance activity in the embryo and that interference with this function in the gastrula underlies at least some aspects of the dominant negative FGF receptor phenotype. Recent data show that *eFGF* is also coexpressed with *Xbra* in the notochord in the late gastrula and neurula stages, suggesting that eFGF may also regulate *Xbra* expression in the dorsal midline [40].

Brachyury mutants in other organisms

A number of naturally occurring mutations in the homologues of the *Xbra* gene have been identified in other organisms. The phenotypes of *T* mutant mice and *no tail* mutant zebrafish exhibit a deficiency in trunk and posterior structures (reviewed in ref. 62) This has similarities to the phenotype of *Xenopus* embryos in which the activity of the FGFs has been inhibited [39, 50, 51]. At present it is not clear if the main function of *brachyury* is associated with the control of cell movements or as a 'genetic switch' which specifies posterior mesoderm. Certainly its function is not required for the formation of the mesoderm per se because in mouse and zebrafish *brachyury* mutants the development of anterior mesodermal structures, including anterior somites, is quite normal. Brachyury is not required for the initial specification of the notochord in zebrafish *no tail* mutants, but is required for its terminal differentiation. It is likely that the absence of differentiated notochord in *Xenopus* embryos injected with the dominant negative FGF receptor is due to an interference with the regulation of *Xbra* function in the dorsal midline.

The formation of ventral mesoderm in animal caps injected with *Xbra* mRNA is suggestive of a role in ventroposterior specification. However, this observation may be misleading as to the in vivo function of *Xbra*. A low dose injection of the dominant negative FGF receptor into the ventral side of a *Xenopus* embryo down-regulates *Xbra* expression but has very little effect on development, while a similar dose injected into the dorsal side recapitulates the full posterior truncated phenotype [39]. This indicates that the most sensitive requirement for FGF and Xbra activity is on the dorsal side of the embryo. It has been suggested that in *T* mutant mice abnormal cell movements within the primitive streak lead to the characteristic posterior truncation [65]. Gastrulation movements are also disturbed in *Xenopus* embryos injected dorsally with the dominant negative FGF receptor. Instead of involuting and extending along the anteroposterior axis, as in normal development, the dorsal mesoderm spreads laterally and ventrally around the open blastopore [39]. Given the close link between FGF and Xbra activity and the intimate relationship between morphogenesis and patterning during gastrulation, it is tempting to suggest that the reduction of posterior structures in both

brachyury mutant organisms and FGF dominant negative receptor embryos results primarily from abnormal cell movements in the dorsal mesoderm. Certainly there is evidence from studies in the mouse which indicates that the extracellular matrix of *brachyury* mutant-derived cells is very much reduced in quantity from that of wild-type cells [66, 77]. These changes may underlie the aberrant cell movements in both dominant negative FGF receptor embryos and *brachyury* mutants.

Although the phenotype of embryos lacking a functional FGF signalling pathway is similar to *brachyury* mutant embryos, there are differences, and these differences serve to highlight additional processes for which FGF function is required. Most notably a number of anterior somites are present in both mouse and zebrafish *brachyury* mutants whereas there is a complete absence of somites in *Xenopus* embryos in which FGF function is inhibited. This indicates that the FGFs are required for the development of the somites and in particular the muscle lineages in *Xenopus*, and that this function is independent of the FGF role in regulating *brachyury* expression. Such a role is certainly in keeping with the expression of both *eFGF* and *XmyoD* in the marginal zone at the start of gastrulation. Recent work in avians suggests that signals from the dorsal midline are required for the stabilization and maintenance of the myogenic lineages; given that *eFGF* is also expressed in the notochord it is possible that it may contribute to this midline signal.

Overexpression of the FGFs in vivo

The inhibition of the FGF signal transduction pathway in vivo has provided much information concerning the role of these factors in early development. Another approach that has proved useful in the analysis of FGF function has been the overexpression of the FGF ligands in the embryo. Injection of synthetic mRNA coding for secreted FGFs, such as eFGF, into the zygote or early cleavage stage embryo demonstrates the potent mesoderm-inducing activity of these factors [20, 39]. However, the formation of large quantities of ectopic mesoderm in the animal hemisphere of such embryos blocks normal gastrulation movements, making this an unsatisfactory approach for the analysis of FGF function during later development [20]. These problems associated with mRNA injection can be overcome by the use of DNA constructs that drive expression after the MBT. Several studies show that large quantities of mRNA transcribed from injected DNA plasmids of this type do not accumulate until the late blastula or early gastrula stage, by which time the competence of the animal hemisphere to respond to mesoderm inducing factors is fading [31, 68]. When such a construct, which drives *eFGF* expression under the control of a cytoskeletal actin promoter, is injected into the two-cell embryo

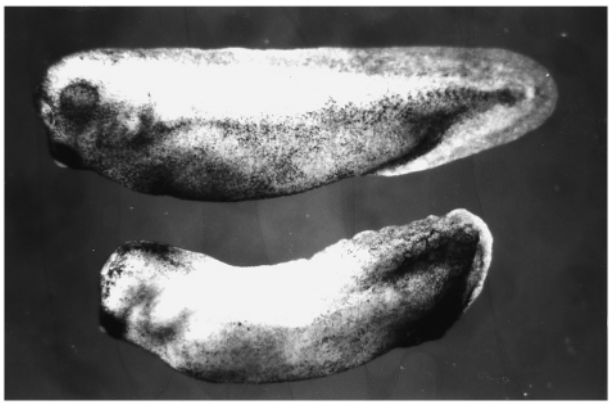

Figure 5. Overexpression of *eFGF* during gastrulation. The top embryo is a tailbud stage control *Xenopus* embryo. The bottom embryo was injected with 10 pg of a DNA construct that drives expression of *eFGF* after the mid-blastula transition. Note the reduction in anterior structures, in particular the loss of eyes (picture courtesy of Betsy Pownall).

the majority of embryos gastrulate normally. During later development, however, these embryos exhibit reductions in the development of anterior structures and a gross enlargement of the posterior, particularly the proctodeum (see fig. 5). This phenotype appears to result from a coordinate posteriorization of the embryo and this view is supported by molecular data which show that in such embryos there is ectopic expression of posterior markers in anterior regions [39].

The FGFs and anteroposterior development

The anterior reductions and overdevelopment of posterior structures caused by the late overexpression of *eFGF* is in many ways the converse of the dominant negative FGF receptor phenotype and suggests that the FGF system has an important role in establishing the anteroposterior pattern of the embryo during gastrula and later stages. There is a body of embryological data that suggests that during A-P specification of the nervous system the default state is anterior and that the acquirement of posterior fate requires the action of a putative 'posteriorizing' influence (reviewed in ref. 69). The presence of FGFs in the posterior of the embryo and the posteriorized phenotype of embryos overexpressing eFGF make the FGFs good candidates for such a posteriorizing agent. This view is further supported by the observation that gastrula ectoderm cells can be induced to form neural tissue of a posterior character following brief dissociation and treatment with bFGF [70]. This suggests that the FGF may be able to induce posterior neural structures directly in competent ectoderm. Interestingly, the neural tissue induced by the noggin and follistatin proteins is of an anterior character [71, 72]. However, noggin-type neural inductions can be made more posterior in character by treatment with eFGF during gastrula stages [73] (Betsy

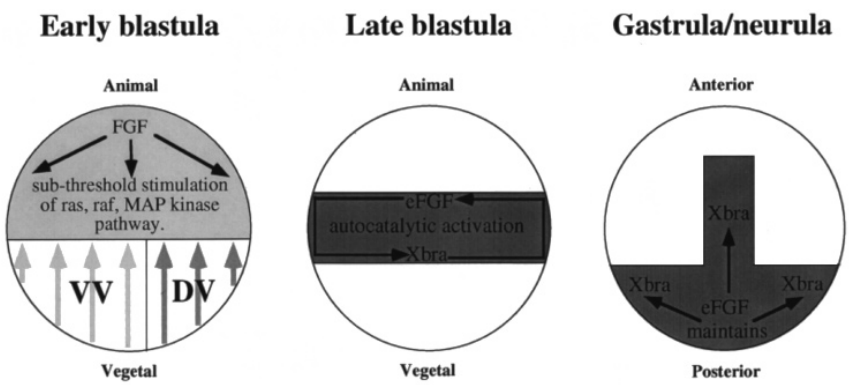

Figure 6. A model for FGF regulation of Xbra in early amphibian development. In the early blastula maternally deposited FGF is required in the animal hemisphere to provide sub-threshold stimulation of the tyrosine kinase signal transduction pathway. This is necessary to allow activation of *Xbra* transcription in the late blastula. Xbra activates the expression of eFGF in the newly formed mesoderm. This leads to a period of autocatalytic activation of eFGF and Xbra. During gastrula and neurula stages eFGF continues to regulate the expression of Xbra in the blastopore region and in the notochord. The FGF-Xbra regulatory pathway plays an important role in the establishment of the mesoderm in the blastula. During gastrulation this pathway continues to be important for maintaining the properties of the mesoderm necessary for gastrulation.

Pownall, personal communication) Thus noggin may be likened to the activating principle and eFGF to the transforming principle in Nieuwkoop's 'activation-transformation' model of neural development [74].

In recent years there has been much discussion as to the relative importance of 'planar' versus 'appositional' signals involved in the induction and patterning of the nervous system. Further work will be required to determine if the posteriorizing influence of the FGFs in vivo is transmitted through the plane of the neural plate or through vertical signals from the underlying axial mesoderm. The expression pattern of *eFGF* is consistent with both possibilties.

A model for the role of the FGFs in amphibian development

It is now possible to outline a model for the role of FGFs during amphibian development (see fig. 6). The scheme below relies heavily on data that highlight those processes in the embryo for which the activity of the FGF signalling pathway is necessary; clearly the involvement of other factors in these processes cannot be excluded and indeed is to be expected. In particular, the recent demonstration that cell adhesion molecules can interact specifically with FGF receptors suggests that direct cell-cell contacts are likely to be important in regulating the activity of the pathway FGF [75]. Furthermore, as discussed earlier, the activities of the FGFs can be modified by other growth factor-like molecules, which are known to be present in the developing embryo. So it is to be expected that the FGF signal transduction pathway interacts in a complex fashion with that of members of the TGFβ, Wnt and noggin families of secreted factors.

Early models of FGF function during development suggested that they may act as vegetally localized mesoderm-inducing molecules. This now seems unlikely because the known FGFs in *Xenopus* are more abundant in the animal hemisphere. However, experiments with the dominant negative FGF receptor indicate that FGF activity is required during blastula stages for certain aspects of mesoderm formation. Current data support the notion that the maternal pool of FGFs is required to provide sub-threshold stimulation of the tyrosine kinase signal transduction pathway in the animal hemisphere. The maternal FGFs may be viewed as competence factors which need to be present for the full range of responses to the vegetally localized inducing molecules. It is likely that a major component of the vegetal signal is an activin-like molecule such as vg1 [9, 57], perhaps acting in combination with a member of the Wnt family. Cornell and Kimelman [52] have proposed that the mesoderm forms in the marginal zone because it is only here that cells are exposed to both an FGF and an activin-like signal. Absence of FGF 'competence' activity from the vegetal hemisphere explains why the whole of the vegetal hemisphere does not mesodermalize in response to the endogenous inducing signals and at the same time explains why inhibition of the activin signalling pathway blocks the formation of all mesoderm.

Of particular importance is that a low level of maternal FGF activity is required for the transcription of *Xbra* in the marginal zone of the late blastula. *Xbra* in turn activates the zygotic expression of *eFGF* in the early mesoderm. This leads to a period of autocatalytic activation of eFGF and Xbra in the nascent mesoderm of the marginal zone. During this phase eFGF may function as a secondary mesoderm-inducing factor which amplifies and spreads the effect of the primary vegetal inducers, resulting in activation and maintenance of gene expression within the marginal zone, and in this way contributing to the stability of the mesoderm. The

spread of this region of autocatalytic activity is likely to be limited by the loss of competence within the animal hemisphere which occurs at the start of gastrulation. At present it is not known if Xbra and eFGF are components of a bistable circuit. However, we know that the expression from both of these genes is activated in response to activin. The formation of such a bistable circuit would provide one mechanism for the generation of the sharp thresholds of gene activation, including *Xbra*, seen in animal cap cells treated with activin [76].

In gastrula and neurula stages, eFGF activity continues to regulate the expression of *Xbra* in the blastopore region and possibly also in the notochord. The activity of the FGFs is also required for the correct regulation of other genes expressed in the mesoderm, most likely on parallel pathways to that of *Xbra*. FGF function is necessary for the correct development of *XmyoD* expression, which indicates a role in the development of the myogenic lineages. In addition to activities within the early mesoderm there is evidence that the FGFs function as posteriorizing agents involved in the anteroposterior specification of the neuroectoderm.

The recent demonstration that *eFGF* is expressed in the notochord raises the possiblity that FGF signalling constributes to other midline patterning processes. The signalling molecule sonic hedgehog is also expressed in the notochord and has been implicated in dorsoventral specification in the neural tube [77–79]. A close interaction between sonic hedgehog and FGF signalling has been demonstrated in the developing limb-bud [80]. It is intriguing to speculate that a similar close interaction of these two signalling molecules may also be involved in the patterning of dorsal midline structures.

After neurula stages, *eFGF* continues to be expressed in the tailbud of the embryo. Some workers have suggested that the extension of the tailbud to form the posterior of the embryo represents a continuation of the processes involved in gastrulation [41]. It is tempting to speculate that during tail formation the FGFs continue to be involved in similar processes to those that are indicated for them during gastrulation.

Conclusions

Close homologues of the molecules discussed in this account are present in other organisms and, given the high conservation of developmental mechanisms between vertebrate species, it is likely that much of what has been learnt concerning the targets and regulation of FGF activity in *Xenopus* will be generally applicable. However, additional roles for the FGFs are also suggested in higher vertebrates. During the early development of the amphibia there is no growth in size but early tissue specification and patterning in the higher vertebrates is accompanied by substantial growth. Recent data from the knockout of murine *FGF-4* gene function strongly suggest that there is a requirement for FGF activity to stimulate growth of the inner cell mass before the onset of gastrulation and the homologous patterning events described in this review [81]. Such early growth requirements will necessarily complicate the analysis of FGF function in the amniotes and makes it likely that the amphibian model will continue to provide useful insights into the role of the FGFs in vertebrate development.

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1 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
2 Slack J. M. W. (1994) Inducing factors in *Xenopus* early embryos. Curr. Biol. **4:** 116–126
3 Smith J. C. (1989) Mesoderm induction and mesoderm-inducing factors in early amphibian development. Development **105:** 665–677
4 Dale L., Howes G., Price B. M. J. and Smith J. C. (1992) Bone morphogenetic protein 4: a ventralizing factor in early *Xenopus* development. Development **115:** 573–585
5 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
6 Kimelman D., Abraham J. A., Haaparanta T., Palisi T. M. and Kirschner M. W. (1988) The presence of fibroblast growth factor in the frog egg: its role as a natural mesoderm inducer. Science **242:** 1053–1056
7 Slack J. M. W. and Isaacs H. V. (1989) Presence of basic fibroblast growth factor in the early *Xenopus* embryo. Development **105:** 147–154
8 Tannahill D., Isaacs H. V., Close M. J., Peters G. and Slack J. M. W. (1992) Developmental expression of the *Xenopus* int-2 (FGF-3) gene: activation by mesodermal and neural induction. Development **115:** 695–702
9 Thomsen G. and Melton D. A. (1993) Processed vg1 protein is axial mesoderm inducer in *Xenopus*. Cell **74:** 433–441
10 Boterenbrood E. C. and Nieuwkoop P. D. (1973) The formation of the mesoderm in urodelan amphibians. V. Its regional induction by the endoderm. W. Roux's Arch. Dev. Biol. **173:** 319–332
11 Ding X. Y., McKeehan W. L., Xu J. M. and Grunz H. (1992) Spatial and temporal localization of FGF receptors in *Xenopus laevis*. W. Roux's Arch. Dev. Biol. **201:** 334–339
12 Dale L. and Slack J. M. W. (1987) Regional specification within the mesoderm of early embryos of *Xenopus laevis*. Development **100:** 279–295
13 Lettice L. A. and Slack J. M. W. (1993) Properties of the dorsalizing signal in gastrulae of *Xenopus laevis*. Development **117:** 263–272
14 Smith J. C. and Slack J. M. W. (1983) Dorsalization and neural induction: properties of the organizer in *Xenopus laevis*. J. Embryol. Exp. Morph. **78:** 299–317
15 Spemann H. and Mangold H. (1924) Über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren. Arch. f. mikr. Anat. u. Entw. Mech. **100:** 599–638
16 Sive H. L. (1993) The frog prince-ss: A molecular formula for dorsoventral patterning in *Xenopus*. Genes Dev. **7:** 1–12
17 Slack J. M. W. and Forman D. (1980) An interaction between dorsal and ventral regions of the marginal zone in early amphibian embryos. J. Embryol. Exp. Morph. **56:** 283–299
18 Godsave S. F., Isaacs H. V. and Slack J. M. W. (1988) Mesoderm inducing factors: a small class of molecules. Development **102:** 555–566

19 Kimelman D. and Maas A. (1992) Induction of dorsal and ventral mesoderm by ectopically expressed *Xenopus* basic fibroblast growth factor. Development **114:** 261–269
20 Thompson J. and Slack J. M. W. (1992) Overexpression of fibroblast growth factors in *Xenopus* embryos. Mech. Dev. **38:** 175–182
21 Kimelman D. and Kirschner M. (1987) Synergistic induction of mesoderm by FGF and TGF-β and the identification of an mRNA coding for FGF in the early *Xenopus* embryo. Cell **51:** 869–877
22 Paterno G. D., Gillespie L. L., Dixon M. S., Slack J. M. W. and Heath J. K. (1989) Mesoderm inducing properties of int-2 and kFGF: two oncogene encoded growth factors related to FGF. Development **106:** 79–83
23 Basilico C. and Moscatelli D. (1992) The FGF family of growth-factors and oncogenes. Adv. Cancer. Res. **59:** 115–165
24 Tanaka A., Miyamoto K., Minamino N., Takeda M, Sato B., Matsuo H. et al. (1993) Cloning and characterization of an androgen-induced growth-factor essential for the androgen-dependent growth of mouse mammary-carcinoma cells. Proc. Natl. Acad. Sci. USA **89:** 8928–8932
25 Slack J. M. W., Isaacs H. V. and Darlington B. G. (1988) Inductive effects of fibroblast growth factor and lithium ion on *Xenopus* blastula ectoderm. Development **103:** 581–590
26 Jones E. A. and Woodland H. R. (1987) The development of animal cap cells in *Xenopus*: a measure of the start of animal cap competence to form mesoderm. Development **101:** 557–563
27 Cho K. W. Y., Blumberg B., Steinbeisser H. and De Robertis E. M. (1991) Molecular nature of Spemann's organizer: the role of the *Xenopus* homeobox gene goosecoid. Cell **67:** 1111–1120
28 Smith W. C. and Harland R. M. (1992) Expression cloning of noggin, a new dorsalizing factor localized to the Spemann organizer in *Xenopus* embryos. Cell **70:** 829–840
29 Taira M., Jamrich M., Good P. J. and Dawid I. B. (1992) The lim domain containing homeobox lim1 is expressed specifically in the organizer region of *Xenopus* gastrula embryos. Genes Dev. **6:** 356–366
30 Christian J. L., Olson D. J. and Moon R. T. (1992) Xwnt8 modifies the character of mesoderm induced by bFGF in isolated *Xenopus* ectoderm. EMBO J. **11:** 33–41
31 Christian J. L. and Moon R. T. (1993) Interactions between *Xwnt8* and Spemann organizer signaling pathways generate dorsoventral pattern in the embryonic mesoderm of *Xenopus*. Genes Dev. **7:** 13–28
32 Fainsod A., Steinbeisser H. and De Robertis E. M. (1994) On the function of bmp-4 in patterning the marginal zone of the *Xenopus* embryo. EMBO J. **13:** 5015–5025
33 Kimelman D., Christian J. L. and Moon R. T. (1992) Synergistic principles of development: overlapping patterning systems in *Xenopus* mesoderm induction. Development **116:** 1–9
34 Shiurba R. A., Jing N., Sakakura T. and Godsave S. F. (1991) Nuclear translocation of fibroblast growth factor during *Xenopus* mesoderm induction. Development **113:** 487–494
35 Song J. and Slack J. M. W. (1994) Spatial and temporal expression of basic fibroblast growth factor (FGF-2) mRNA and protein in early *Xenopus* development. Mech. Dev. **48:** 141–151
36 Slack J. M. W. (1991) The nature of the mesoderm inducing signal in *Xenopus*: a transfilter induction study. Development **113:** 661–671
37 Jackson A., Freidman S. Zhan X., Engleka K. A., Forough R. and Maciag T. (1992) Heat shock induces the release of fibroblast growth factor-1 from NIH-3T3 cells. Proc. Natl. Acad. Sci. USA. **89:** 10691–10695
38 Mignatti P., Morimoto T. and Rifkin D. B. (1992) Basic fibroblast growth factor, a protein devoid of secretory signal sequence, is released by cells via a pathway independent of the endoplasmic reticulum-Golgi complex. J. Cell. Physiol. **151:** 81–93
39 Isaacs H. V., Pownall M. E. and Slack J. M. W. (1994) eFGF regulates Xbra expression during *Xenopus* gastrulation. EMBO J. **19:** 4469–4481
40 Isaacs H. V., Pownall M. E. and Slack J. M. W. (1995) eFGF is expressed in the dorsal mid-line of *Xenopus* laevis. Int. J. Dev. Biol. (in press)
41 Gont L. K., Steinbeisser H., Blumberg B. and De Robertis E. (1993) Tail formation as a continuation of gastrulation: the multiple cell populations of the *Xenopus* tailbud derive from the late blastopore lip. Development **119:** 991–1004
42 Johnson D. E. and William L. T. (1993) Structural and functional diversity in the FGF receptor multigene family. Adv. Cancer. Res. **60:** 1–41
43 Musci T. J., Amaya E. and Kirschner M. W. (1990) Regulation of the fibroblast growth factor receptor in early *Xenopus* embryos. Proc. Natl. Acad. Sci. USA **87:** 8365–8369
44 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
45 Freisel R. and Brown S. (1992) Spatially restricted expression of fibroblast growth factor receptor 2 during *Xenopus* development. Development **116:** 1051–1058
46 Mathieu M., Kiefer P., Mason I. and Dickson C. (1995) Fibroblast growth factor (FGF) 3 from *Xenopus laevis* (XFGF3) binds with high affinity to FGF receptor 2. J. Biol. Chem. **270:** 6779–6787
47 Kejii I. and Sokol S. Y. (1994) Heparan sulfate proteoglycans are required for mesoderm formation in *Xenopus* embryos. Development **120:** 2703–2711
48 Egan S. E. and Weinberg R. A. (1993) The pathway to signal achievement. Nature **365:** 781–783
49 LaBonne C. and Whitman M. (1994) Mesoderm induction by activin requires FGF-mediated intracellular signals. Development **120:** 463–472
50 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
51 MacNichol A. M., Muslin A. J. and Williams L. T. (1993) Raf-1 kinase is essential for early *Xenopus* development and mediates the induction of mesoderm by FGF. Cell. **73:** 571–583
52 Cornell R. A. and Kimelman D. (1994) Activin-mediated mesoderm induction requires FGF. Development **120:** 453–462
53 Smith J. C., Price B. M. J., Green J. B. A., Weigel D. and Herrman B. G. (1991) Expression of a *Xenopus* homolog of Brachyury (T) is an immediate early response to mesoderm induction. Cell **67:** 79–87
54 LaBonne C. Burke B. and Whitman M. (1995) Role of map kinase in mesoderm induction and axial patterning during *Xenopus* development. **121:** 1475–1486
55 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
56 Graff J. M., Thies R. S., Song J. J., Celeste A. J. and Melton D. A. (1994) Studies with a *Xenopus* bmp receptor suggest that ventral mesoderm-inducing signals override dorsal signals in-vivo. Cell. **79:** 169-179
57 Hemmati-Brivanlou A. and Melton D. A. (1992) A truncated activin receptor inhibits mesoderm induction and formation of axial structures in *Xenopus* embryos. Nature **359:** 609–614
58 Ueno H., Gunn M., Dell K., Tseng A. and Williams L. T. (1992) A truncated form of fibroblast growth factor receptor 1 inhibits signal transduction by multiple types of fibroblast growth factor receptor. J. Biol. Chem. **267:** 1470–1476
59 Schulte-Merker S., Smith J. C. and Dale L. (1993) Effects of truncated activin and FGF receptors and of follistatin on the inducing activities of BVg1 and activin: does activin play a role in mesoderm induction? EMBO J. **13:** 3533–3541
60 Gurdon J. B. (1988) A community effect in animal development. Nature **336:** 772-774
61 Northrop J. L. and Kimelman D. (1994) Dorsal-ventral differences in xcad-3 expression in response to FGF-mediated induction in *Xenopus*. Dev. Biol. **161:** 490–503
62 Herrmann B. G. and Kispert A. (1994) The T genes in embryogenesis. Trends. Genet. **10:** 280–286

63 Cunliffe V. and Smith J. C. (1992) Ectopic mesoderm formation in *Xenopus* embryos caused by widespread expression of a *Brachyury* homologue. Nature **358:** 427–430
64 Schulte-Merker S. and Smith J C. (1995) Mesoderm formation in response to brachyury requires FGF signalling. Curr. Biol. **5:** 62–67
65 Beddington R. S. P., Rashbass P. and Wilson V. (1992) *Brachyury* – a gene affecting mouse gastrulation and organogenesis. Development Suppl. 157–171
66 Hashimoto K., Fujimoto H. and Nakatsuji N. (1987) An ECM substratum allows mouse mesodermal cells isolated from the primitive streak to exhibit motility similar to that inside the embryo and reveals a deficiency in the T/T mutant cells. Development **100:** 587-598
67 Jacobs-Cohen R. J., Spiegelman M. and Bennett D. (1983) Abnormalities of cells and extracellular matrix T/T embryos. Differentiation **25:** 48–55
68 Smith W. C., Knecht A. K., Wu M. and Harland R. M. (1993) Secreted noggin mimics the Spemann organizer in dorsalizing *Xenopus* mesoderm. Nature **361:** 547–549
69 Slack J. M. W. and Tannahill D. (1992) Mechanism of anteroposterior axis specification in vertebrates – lessons from the amphibians. Development **114:** 285–302
70 Kengaku M. and Okamoto H. (1995) bFGF as a possible morphogen for the anteroposterior axis of the central nervous system in *Xenopus*. Development **121:** 3121–3130
71 Hemmati-Brivanlou A., Kelly O. G., and Melton D. A. (1994) Follistatin, an antagonist of activin, is expressed in the Spemann organizer and displays direct neuralizing activity. Cell. **77:** 283–295
72 Lamb T. M., Knecht A. K., Smith W. C., Stachel S. E., Economides A. N., Stahl N. et al. (1993) Neural induction by the secreted polypeptide noggin. Science **262:** 713–718
73 Lamb T. M. and Harland R. M. (1995) Fibroblast growth factor is a direct neural inducer, which combined with noggin generates anterior-posterior neural pattern. Development **121:** 3627–3636
74 Nieuwkoop P. D., Johnen A. G. and Albers B. (1985) The epigenetic nature of early chordate development. Cambridge University Press, Cambridge
75 Williams E. J., Furness J., Walsh F. S. and Doherty P. (1994) Activation of the FGF receptor underlies outgrowth stimulated by L1, N-CAM, and N-cadherin. Neuron **13:** 583–594
76 Green J. B. A., New H. V. and Smith J. C. (1992) Responses of embryonic *Xenopus* cells to activin and FGF are separated by multiple dose thresholds and correspond to distinct axes of the mesoderm. Cell **71:** 731-739
77 Ekker S. C., Mcgrew L. L., Lai C.-J., Lee J. J., von Kessler D. P., Moon et al. (1995) Distinct expression and shared activities of memebers of the hedgehog gene family in *Xenopus laevis*. Development **121:** 2337–2347
78 Pownall M. E. 1994. More to patterning than sonic hedgehog. Bioessays **16:** 381–383
79 Smith J. C. (1994) Hedgehog, the floor plate, and the zone of polarizing activity. Cell **76:** 193–196.
80 Niswander L., Jeffrey S., Martin G. R. and Tickle C. (1994) A positive feedback loop coordinates growth and patterning in the vertebrate limb. Nature **371:** 609–612
81 Feldman B., Poueymirou W., Papaioannou V. E., Dechiara, T. M. and Goldfarb M. (1995) Requirement of FGF-4 for postimplantation mouse development. Science **267:** 246–249

.