Role of FGF and Noggin in Neural Crest Induction

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A study of the molecules noggin and fibroblast growth factor (FGF) and its receptor in the induction of the prospective neural crest in Xenopus laevis embryos has been carried out, using the expression of the gene Xslug as a marker for the neural crest. We show that when a truncated FGF receptor (XFD) was expressed ectopically in order to block FGF signaling Xslug expression was inhibited. The effect of XFD on Xslug was specific and could be reversed by the coinjection of the wild-type FGF receptor (FGFR). Inhibition of Xslug expression by XFD is not a consequence of neural plate inhibition, as was shown by analyzing Xsox-2 expression. When ectoderm expressing XFD was transplanted into the prospective neural fold region of embryos Xslug induction was inhibited. The neural crest can also be induced by an interaction between neural plate and epidermis. As this induction is suppressed by the presence of XFD in the neural plate and not in the epidermis, it suggests that the neural crest is induced by FGF from the epidermis. However, treatment of neural plate with FGF was not able to induce Xslug expression, showing that in addition to FGF other non-FGF factors are also required. Previously we have suggested that the ectopic ventral expression of Xslug produced by overexpression of noggin mRNA resulted from an interaction of noggin with a ventral signal. Overexpression of XFD inhibits this effect, suggesting that FGF could be one component involved in this ventral signaling. Overexpression of FGFR produced a remarkable increase in the expression of Xslug in the posterior neural folds and around the blastopore. Injections in different blastomeres of the embryo suggest that the target cells of this effect are the ventral cells. Finally, we proposed a model in which the induction of the neural crests at the border of the neural plate requires functional FGF signaling, which possibly interacts with a neural inducer such as noggin.

INTRODUCTION

The formation of the embryonic nervous system in amphibia is a result of inductive interactions between the dorsal mesoderm and the ectoderm. As a consequence of this interaction, the dorsal ectoderm thickens and becomes a flat sheet of cells called the neural plate. During the process of neurulation the neural folds rise at the border of the neural plate. The neural folds rise and fuse at the dorsal midline to form a cylindrical neural tube that subsequently differentiates into the central nervous system. As the neural tube closes, the neural folds become the neural crest, which differentiates into a variety of neuronal and nonneuronal cell types.

At the closure of the neural tube, in amphibia, the neural crest cells, lying in the ridge on each side, fuse in the midline to form a wedge-shaped cell mass (Schroeder, 1970). After a short period these migrate from the neural tube along defined pathways (Collazo et al., 1993). There are important differences between the cephalic and the trunk neural crest. The cephalic neural crests never come to lie on the top of the neural tube (Sadaghiani and Thiebaud, 1987). In anura the neural crest is derived from two layers of the neuroepithelium (Schroeder, 1970; Essex et al., 1993; Mayor et al., 1993). The discovery of Xenopus genes that are expressed very early in the prospective neural fold cells has facilitated studies of neural crest induction. The genes Xtwi and Xsna (the Xenopus homologues of the Drosophila twist and snail, respectively) are expressed in premigratory and migrating neural crest (Hopwood et al., 1989; Mayor et al., 1993; Essex et al., 1993), but as they are expressed in mesoderm, they are not specific markers for neural fold or neural crest cells at any stage of development. More recently, a Xsna related zinc-finger gene called Xslug (the Xenopus homologue of the chicken Slug, Nieto et al., 1994) has been cloned (Mayor et al., 1995). Xslug is a specific marker for all parts of the cranial and trunk prospective neural crest, from stage 12 until stage 17 (Mayor et al., 1995).

Several hypothesis have been proposed to explain how the prospective neural crest or the neural folds are induced at the border of the neural plate. Raven and Kloos (1945) suggested that a hypothetical neural inducer upregulated
neural crest at the border of the neural plate when the concentration of the inducer was insufficient to induce neural plate but just enough to induce neural folds. On the other hand, Albers (1987) proposed that the size of the neural plate, and therefore its border, is determined by a change in the competence of the ectoderm. Recently, evidence has accumulated in favor of the hypothesis that an interaction between two signals is required for the induction of the neural crest (Moury and Jacobson, 1990; Selleck and Bronner-Fraser, 1995; Mayor et al., 1995; Dickinson et al., 1995; Lien et al., 1995; Mancilla and Mayor, 1996).

Noggin, Follistatin, and Chordin have been proposed as inducers of the neural plate. It has been proposed that they neutralize ectoderm through different mechanisms (Smith and Harland, 1992; Smith et al., 1993; Lamb et al., 1993; Knecht et al., 1995; Hemmati-Brivanlou et al., 1994; Hemmati-Brivanlou and Melton, 1994; Sasai et al., 1994). However, recent evidence shows that noggin, chordin, and possibly follistatin can bind BMPs and inhibit BMP signaling, which is known to inhibit neural development (Zimmerman et al., 1996; Piccolo et al., 1996).

We have shown that overexpression of noggin in animal caps is not able to induce the neural crest marker Xslu. When noggin mRNA is injected at the 1-cell stage a strong ventral expression of Xslu in the ectoderm can be induced (Mayor et al., 1995). This result suggests that while noggin is unable to induce prospective neural crest in caps it can induce Xslu in combination with another signal present in the whole embryo. We have also shown that stage 10 animal caps treated with a concentration below 100 μg/ml of noggin or FGF do not express Xslu, but that a combination of the same concentration of noggin and a FGF is able to induce the expression of the neural crest marker (Mayor et al., 1995). Although these animal caps have greatly reduced competence for mesodermal induction we always detected the mesodermal marker Xbra. Therefore, we could not be certain whether the induction of Xslug in animal caps treated with noggin and FGF was direct or indirect through induction of mesoderm, which in turn induced the neural crest marker. Therefore, we proposed two different explanations for Xslug induction by noggin and FGF: first, FGF could induce (ventral) mesoderm which could be dorsIALIZED by noggin, and this dorsализed mesoderm could induce Xslug expression in the caps or, second, noggin could induce neural plate which could be transformed by FGF into neural crest.

Several lines of evidence suggest that FGF can induce neural plate and neural crest directly (Launay et al., 1994, 1996; Shi et al., 1994; Kengaku and Okamoto, 1993, 1995; Lamb and Harland, 1995). There is also evidence suggesting that induction of the neural crest can occur independently of the induction of the neural plate (Nieuwkoop, 1985; Raven and Kloos, 1945; Holtfreter and Hamburger, 1955; Turner and Weintraub, 1994; Zimmermann et al., 1993). We decided to investigate the importance of FGF in induction of the neural crest and its relationship with noggin. We report that the inhibition of FGF signaling by the dominant negative FGF receptor does not inhibit expression of neural plate markers but prevents Xslu expression in the embryos. We also show, using transplant and conjugate experiments of normal mesoderm and ectoderm expressing the dominant negative FGF receptor, that an intact FGF signaling and signal transduction pathway is required in the ectoderm for Xslu induction. In conjugates of neural plate and ectoderm that result in induction of Xslu (Mancilla and Mayor, 1996), the neural plate must express the FGF signal to induce Xslu. In addition we have shown that the ectopic expression of Xslu induced by overexpression of noggin can be completely blocked by the dominant negative receptor. Finally, we have been able to modify the pattern of expression of Xslu by overexpressing the wild-type FGF receptor into different blastomeres of the embryo. Taken together, these results suggest that FGF is involved in the induction of neural crest by interacting with noggin and that the weaker Xslu expression in the posterior neural folds could be related to the absence of the FGF receptor.

MATERIALS AND METHODS

Embryos and Explants

Xenopus embryos were obtained by artificial fertilization, dejellied in 2% cysteine (Smith and Slack, 1983), reared in 10% normal amphibian medium (NAM) (Slack, 1984), and staged according to Nieuwkoop and Faber (1967). Explants and dissections were made as described in Mancilla and Mayor (1996).

RNA and RLDx Injection

Dejellied 1-, 2-, or 4-cell embryos were placed in 75% NAM with 5% Ficoll, injected with different amounts of RNA as indicated or 10 nl of 25 mg/ml solution of rhodamine dextran (RLDx, Molecular Probes), and subsequently reared at 14°–16°C.

Capped RNAs were synthesized from linearized plasmids using an appropriate RNA polymerase (Boehringer Mannheim) in the presence of 500 μM 5'-mGpppG-3' cap analog, rUTP, rATP, rCTP, and 50 μM rGTP.

The RNAs, injected at the animal pole, were as follows. XFD: This is a dominant negative mutant Xenopus FGF receptor-1 lacking the tyrosine kinase domain (Amaya et al., 1991). It interferes with the activity of endogenous FGF receptor by the formation of nonfunctional heterodimers. Controls were made in which the XFD mRNA was injected at the 1-cell stage or in 1 cell of the 2-cell stage and the expression of the mesodermal marker Xbra was analyzed by in situ hybridization at stage 10.5. N o Xbra expression was detected in the injected cells. Noggin: This is a neural inducer and a dor- salizer (Smith and Harland, 1992). FGFR: This is a wild-type Xenopus FGF receptor-1 (Amaya et al., 1991).

Transplantation of Tissues and Conjugates

Tissues were transplanted from injected donor to the prospective neural fold region of uninjected hosts, as described under Results. During healing, transplants were held in place with small curved glass bridges (Henry and Grainger, 1987). Conjugates of ectoderm with mesoderm were prepared as described by Nieuwkoop (1969). Conjugates of neural plate and epidermis were made as described in Mancilla and Mayor (1996). Only anterior neural plates were used in these conjugates, for two reasons: first, the posterior neural
Inhibition of Xslu Expression by XFD Is Not a Consequence of Neural Plate Inhibition

It has been reported that FGF can induce neural plate markers and that the neural inducer activity of Noggin can be inhibited by overexpression of XFD, which suggest that XFD can inhibit neural plate formation in the embryos (Lau-nay et al., 1996; Shi et al., 1994; Kengaku and Okamoto, 1993, 1995; Lamb and Harland, 1995). However, a recent report, using transgenic animals shows that the expression of XFD is not able to block the induction of the neural plate in the embryo (Kroll and Amaya, 1996).

As it has been shown that the neural crest can be induced by an interaction between neural plate and epidermis (Moury and Jacobson, 1990; Selleck and Bronner-Fraser, 1995; Mayor et al., 1995; Dickinson et al., 1995; Liem et al., 1995; Mancilla and Mayor, 1996), a possible explanation for the inhibition of Xslu expression by XFD is that these embryos do not have a neural plate to interact with the epidermis to induce the neural crest marker Xslu. In order to analyze this possibility, and as different reports describes opposite results in the ability to block neural plate induction by XFD, we tested if the conditions that we used to inhibit Xslug expression were able to inhibit neural plate formation as well.

Embryos were injected in one blastomere at the 2-cell stage with 0.2 ng of XFD mRNA (n = 56). They were cultured until stage 17, fixed, and analyzed by whole mount in situ hybridization for the expression of the neural plate marker Xsox-2 (Fig. 2A). Embryos injected with XFD failed to close the blastopore, presumably because mesodermal induction was affected, as Xbra was not expressed in these embryos (not shown, see Materials and Methods). However, the expression of Xsox-2 was not affected (Fig. 2B, 89% of the embryos expressed Xsox-2, 11% of the embryos showed a partial inhibition in the posterior neural plate), suggesting that under these conditions the induction of the neural plate in the embryo was not inhibited by XFD overexpression. Although only the expression of one gene was analyzed, this is a pan-neural marker expressed in the whole neural plate and, in addition, our results confirm a previous report where several neural plate markers were expressed in XFD transgenic embryos (Kroll and Amaya, 1996).

These results show that the inhibition of Xslu expression by XFD cannot be explained by the inhibition of the neural plate.

Inhibition of Xslu Expression by XFD Is Not a Consequence of Mesodermal Inhibition

It has been shown that the neural crest can be induced in animal caps by conjugating it with mesoderm (Hopwood et al., 1989; Mayor et al., 1995; Mancilla and Mayor, 1996); on the other hand, XFD inhibits mesodermal induction (Amaya et al., 1991); therefore, a possible explanation of the inhibition of the neural crest marker Xslu by XFD is that XFD inhibits the development of some mesodermal tissue required to induce neural crest in the ectoderm. In order to rule out this possibility we decided to have embryos that...
express XFD only in the ectoderm while the mesoderm was normal and analyze the expression of Xslug.

We injected 1-cell stage embryos with a lineage-labeling dye (RLDx), and the same embryos were injected at the 2-cell stage with 0.2 ng of XFD per blastomere, to inhibit the FGF signaling as we had previously shown (Fig. 1D). The embryos were cultured until stage 10.5, at which point a piece of ventral ectoderm, which is no longer competent to respond to mesodermal induction but is competent to respond to neural fold induction (Mancilla and Mayor, 1996), was grafted into the prospective neural fold region of a normal embryo (Fig. 3A) to create an otherwise normal embryo with one prospective neural fold expressing XFD. The embryos were cultured until stage 18 and Xslug expression was analyzed. Only 1 of the 12 transplanted grafts containing XFD expressed the Xslug gene, by contrast with 13 of the 18 grafts when the ectoderm was taken from a normal embryo (Fig. 3B). The fluorescent transplant (Fig. 3C) did not express the marker, although the control neural fold in the same embryo exhibited normal Xslug expression (Fig. 3D). In order to confirm this result we analyzed the ability of dorsal mesoderm taken from a XFD-injected embryo to induce Xslug expression in normal ectoderm. Embryos were injected at the 2-cell stage with 0.2 ng of XFD mRNA per blastomere; at stage 10.5 dorsal mesoderm was dissected and conjugated with animal caps taken from normal stage 10.5 embryos, cultured until the equivalent of stage 17, and analyzed for Xslug expression. Control ectoderm alone never expressed the marker (Fig. 3E, n = 35); however, a strong Xslug induction was observed when the ectoderm was conjugated with normal mesoderm (95%, n = 30) or with mesoderm taken from a XFD injected embryo (87%, n = 35; Fig. 3F). These results suggest that ectoderm, and not mesoderm, requires a functional FGF signaling pathway for induction of the neural crest.
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FIG. 2. Neural plate induction in XFD-injected embryos. (A) Control embryo showing Xsox-2 expression in the neural tube. (B) Embryo injected in one blastomere at the 2-cell stage with 0.2 ng of XFD mRNA. Notice the abnormal closure of the blastopore but the normal Xsox-2 expression at both sides of the embryo, n = 56.

**Xslu Induction in Neural Plate/Epidermis Conjugates Requires a Normal FGF Receptor in the Neural Plate**

Because we had previously shown that Xslu can be induced by an interaction between neural plate and epidermis (Mancilla and Mayor, 1996), we decided to test if the interaction between neural plate and epidermis was also inhibited by XFD expression and in which of these tissues the FGF receptor was required. Conjugates were prepared of anterior neural plate and ventral epidermis taken from stage 12.5 embryos. Xslu expression was induced in conjugates of neural plate and epidermis taken from normal embryos (Fig. 4A, 55% of the conjugates express Xslu) or when control neural plate was conjugated with XFD injected epidermis (Fig. 4C, 45% of the conjugates express Xslu). However, a strong inhibition was observed when the conjugate was made of normal epidermis and XFD expressing neural plate (Fig. 4B, 0% of the conjugates express Xslu) or when both tissues expressed XFD (Fig. 4D, 6% of the conjugates expressed Xslu). This result shows that the induction of neural crest by the interaction between neural plate and epidermis depends on an FGF signal and that probably FGF is produced by the epidermis to induce Xslu in the neural plate. To analyze if FGF was enough to induce neural crest, we treated neural plate taken from a stage 13 embryo with 80 units/ml of FGF, the explants were cultured until the equivalent of stage 17, and Xslug expression was analyzed. No Xslug expression was detected in the explants (n = 28), suggesting that FGF requires an additional factor to induce Xslug in the ectoderm (see Discussion).

**FGF Interacts with Noggin to Induce Xslu**

Xslu expression is very strong in the cephalic region and becomes progressively weaker toward the posterior region of the spinal chord. When noggin mRNA was injected at the 1-cell stage the dorsal Xslu expression was transformed into a ring of Xslu expressing cells extended to the ventral ectoderm (Figs. 5A and 5B). We had previously interpreted this result to mean that noggin, normally present only in the dorsal side of the embryo, interacted with a ventral signal to induce the neural crest marker Xslu (Mayor et al., 1995). To test if FGF was involved in this ventral signaling we injected 1-cell embryos with noggin in order to produce the ventral extension of the Xslu expression (Fig. 5B), and at the 2-cell stage the same embryos were injected in one blastomere with XFD. Overexpression of XFD was enough to inhibit partially or totally the effect of noggin on Xslu expression, suggesting that FGF interacts with Noggin to induce the neural crest marker Xslu (Figs. 5C and 5D). Interestingly, about 50% of the embryos exhibited a remain of Xslug expression in the dorsal side of the embryo, similar to the expression of a normal embryo, suggesting that XFD was inhibiting the effect produced by noggin overexpression.

**The Xslu Expression Can Be Modified by Overexpression of the FGF Wild-Type Receptor**

As our results suggest that FGF is involved in the induction of the neural crest, we analyzed the effect of overexpressing the Xenopus wild-type FGF receptor on Xslu expression. We injected different concentrations of the FGFR
FIG. 3. Inhibition of Xslu by overexpression of XFD in the ectoderm. (A) Embryos were injected with RLDx at the 1-cell stage and with 0.2 ng of XFD mRNA at the 2-cell stage. The injected embryos were cultured until stage 10.5 and a piece of ectoderm was dissected and grafted into the anterior neural fold of a normal stage 13 embryo. As controls ectoderm was dissected from normal stage 10.5 embryos and grafted as described. The grafted embryos were cultured until stage 17 and Xslu expression was analyzed. (B) The grafts of normal (N) or XFD-injected (XFD) ectoderm were recognized by fluorescence and the Xslu expression in the graft was scored. For N, n = 18; for XFD, n = 12. (C, D) The embryos were sectioned and photographed under white light (D) for comparison with fluorescent images (C). Arrow, normal neural fold; arrowhead, grafted neural fold. (E) Animal caps taken at stage 10.5 and cultured until the equivalent of stage 17, when Xslug expression was analyzed. (F) Conjugates of animal caps taken from a normal stage 10.5 embryo and dorsal mesoderm taken from an embryo injected at the 2-cell stage with 0.2 ng of XFD mRNA per blastomere. The conjugates were cultured until the equivalent of stage 17, when Xslug expression was analyzed. Arrows, Xslug expression induced in the conjugate.

mRNA into 1-cell stage embryos. Low concentrations (0.4 ng) of FGFR mRNA did not produce any effect on Xslu expression (Fig. 1B); however, higher concentrations of the same mRNA produced a remarkable and reproducible effect on the expression of the neural crest marker. In a normal embryo Xslu is expressed very strongly in the prospective cephalic crest, its expression decreases in the prospective trunk neural crest and disappears completely near the blastopore (Figs. 6A and 6C). However, when 1.2 ng of FGFR mRNA was injected at the 1-cell stage the expression in the posterior prospective trunk neural crest increased and a strong expression around the blastopore appeared, including the ventral region of the blastopore (Figs. 6B and 6D). In order to analyze the target cells that produced this phenotype, we overexpressed FGFR in different blastomeres. We injected FGFR into the dorsal or ventral blastomeres of a 4-cell stage embryo. When the injection was made into the dorsal blastomeres there was no difference in Xslu expression relative to the controls (Fig. 6E). On the other hand, when the injection was made into the ventral blastomeres...
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Synergistic Interaction between FGF Signaling and Noggin

Both Noggin and FGFR overexpression induce ectopic expression of the neural crest marker, but the location of ectopic Xslu expression is different for each inducer. Noggin induces the ectopic expression of Xslu in the anterior ventral ectoderm, while FGFR induces it in the posterior ventral ectoderm (Figs. 5 and 6). To compare these effects we injected both mRNAs at the 1-cell stage and the embryos were cultured to stage 17 for analysis of Xslu expression.

FIG. 4. Interaction between neural plate and epidermis requires FGF signaling. Anterior neural plate and ventral epidermis were dissected from a stage 12.5 embryo, the tissues were conjugated and cultured until stage 17, and then Xslu was analyzed. Some tissues were dissected from embryos injected at the 1-cell stage with 0.4 ng of XFD mRNA. (A) Control neural plate conjugated with control epidermis showing Xslu induction (arrows), n = 15. (B) Conjugates of normal epidermis and XFD-injected neural plate, n = 12. (C) Conjugates of normal neural plate and XFD-injected epidermis, n = 10. Photograph in C was taken with higher magnification than A, B, and D. (D) Conjugates of XFD-injected neural plate and XFD-injected epidermis, n = 14.

a ventroposterior extension was detected similar to that observed in the 1-cell stage-injected embryo (Fig. 6F). This result shows that the effect produced by the overexpression of FGFR on the Xslu pattern is due to its action on the ventral region of the embryo.

Our results suggest that the signals that induce neural crest are present in the anterior and posterior regions of the embryo; however, the posterior regions are not able to respond to these. A possible explanation of this observation could be that the competence of ectoderm to respond to neural crest induction is regulated by the presence of the receptor. In order to analyze this possibility we decided to study if the loss of neural crest competence during gastrulation (Mancilla and Mayor, 1995) could be affected by overexpression of the FGFR. Ectoderm was taken from embryos at different stages during gastrulation and conjugated with dorsal marginal zone taken from a stage 10.5 embryos (Mayor et al., 1995; Mancilla and Mayor, 1996). The ectoderm was taken from embryos injected with FGFR mRNA or from control embryos. After sibling embryos reached stage 17, the conjugates were fixed and Xslu expression was analyzed. A loss in the competence from stage 10 to stage 12 was observed, as described previously (Mancilla and Mayor, 1996); however, no difference was detected between controls and FGFR-injected ectoderm (Fig. 7). This result indicates that the overexpression of the FGFR is not enough to extend the period of neural crest competence.

FIG. 5. An interaction of FGF and Noggin induces Xslu. (A) Embryo injected with noggin. (Top) Lateral view; (bottom) posterior view; D, dorsal; V, ventral; A, anterior; P, posterior; red band represents Xslu expression. (B) Embryo injected with Noggin mRNA as in bottom in A. (B) Noggin mRNA was injected at the 1-cell stage, the embryos were cultured until stage 17, and Xslu expression was analyzed. Notice a ring of expression around the embryo, d, dorsal side of the embryo; n = 35. (C, D) Noggin mRNA was injected at the 1-cell stage and at the 2-cell stage embryos were injected with 0.2 ng of XFD per blastomere. Notice the partial (C, 47% of inhibition) or total inhibition (50% of inhibition, D) of Xslu expression in the injected side of the embryo (arrowhead). The remaining expression in the partially inhibited embryos was always in the dorsal side of the embryo (arrow). d, dorsal side of the embryo; n = 38.
separately. The combination of factors induced a more posterior expression of Xslu than that with noggin alone but not as posterior as that with FGFR alone. However, the ectopic Xslu expression produced by overexpression of both factors simultaneously could not be described as the simple addition of the ectopic Xslu expression induced by each factor independently. Taken together these results suggest that Noggin and FGF probably act on different cell populations.

**DISCUSSION**

Several lines of evidence suggest that the neural crest can be induced by an interaction between dorsal or neural signals and ventral or epidermal signals (Moury and Jacobson, 1990; Selleck and Bronner-Fraser, 1995; Mayor et al., 1995; Dickinson et al., 1995; Liem et al., 1995; Mancilla and Mayor, 1996). It has been proposed that the epidermal signal could be a member of the BMP family (Liem et al., 1995). It has been shown in Xenopus that BMP-4 is an inhibitor of neural development (Dale et al., 1994; Graff et al., 1994; Maeno et al., 1994; Schmidt et al., 1995), which could be the default pathway of ectodermal differentiation. On the other hand, it has been shown that the neural inducers noggin and chordin bind to BMP-4 and inhibit its action (Piccolo et al., 1996; Zimmerman et al., 1996). In this model, the neural plate is induced by reducing the concentration of BMP-4 and the neural crest could be induced by an intermediate concentration, which could be reached at the border of the neural plate by the regulatory activity of neural inducer on BMP-4. However, it is possible that other molecules can be involved in the induction of the neural crest independently of BMPs or by regulating its action.

The role of FGF in the induction of the prospective neural crest has been examined using the specific molecular marker Xslu to study the induction of the neural crest. We

**FIG. 6.** Effect of the overexpression of FGFR on Xslu pattern. (A) Lateral and (C) posterior views of a stage 17 normal embryo showing Xslu expression very strongly in the anterior neural folds, weaker in the posterior neural folds, and absent around the blastopore. (B) Lateral and (D) posterior views of a stage 17 normal embryo, injected at the 1-cell stage with 1.2 ng of FGFR mRNA and hybridized for Xslu expression. Ectopic expression was observed in the posterior neural folds and around the blastopore (arrow); n = 22. (E, F) Embryos were injected at the 4-cell stage with 0.4 ng of FGFR mRNA in the dorsal (E, n = 17) or ventral (F, n = 23) blastomeres. Notice the ectopic expression of Xslu in F. b, blastopore; arrowhead, normal Xslu expression.

An ectopic Xslu expression was observed in the anterior-middle ventral ectoderm (Fig. 8), which was much stronger than the effect induced by Noggin or FGFR mRNAs injected separately. The combination of factors induced a more posterior expression of Xslu than that with noggin alone but not as posterior as that with FGFR alone. However, the ectopic Xslu expression produced by overexpression of both factors simultaneously could not be described as the simple addition of the ectopic Xslu expression induced by each factor independently. Taken together these results suggest that Noggin and FGF probably act on different cell populations.

**FIG. 7.** Competence of the neural crest induction. Ventral ectoderm was dissected from embryo at different stages and conjugated with dorsal mesoderm taken from a stage 10.5 embryo. The conjugates were cultured in vitro until the equivalent of stage 17 and Xslu expression was analyzed. The percentage of conjugated expressing Xslu was scored. The ectoderm was taken from normal embryos (white circles) or from embryos injected with 0.4 ng of XFD at the 1-cell stage (black circles); n = 10–15 for each point.

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Our results suggest a difference between the requirements of FGF in the induction of the neural plate and the induction of the neural crest, confirming previous reports that indicated that the induction of the neural crest can be independent of the induction of the neural plate. It has been proposed that the induction of the neural plate could be an earlier event than the induction of the neural plate border, including the neural crest and the placode tissue (Nieuwkoop et al., 1985). Raven and Kloos (1945) have shown that the mesoderm underlying the neural folds from an early neurula is able to induce neural crest derivatives without neural plate. Holtfreter and Hamburger (1955) have also claimed that in many induction experiments neural crest is a more frequent outcome than neural plate induction, suggesting again that these two inductive processes could be independent. Recent experiments have shown that the Xenopus homologues of Drosophila proneural and neurogenic genes, called Xash and Xotch, can increase the size of the neural plate at the expense of neural crest cells and epidermal cells (Turner and Weintraub, 1994; Zimmerman et al., 1993). These results suggest that the factors that induce the neural plate are not enough to induce the neural crest cells.

We have also analyzed the role of FGF in the interaction between neural plate and epithelium that causes induction of the neural crest cells (Mancilla and Mayor, 1996). In conjugates of neural plate and epithelium in which one or both tissues were injected with XFD, XSlu induction was blocked only when the neural plate contained XFD. As the neural plate never expresses XSlu when cultured in isolation it appears that FGF is being produced by the epidermis. However, in experiments where the neural plate was treated with FGF, no XSlu induction was detected. It is possible that treating neural plate with FGF is not equivalent to conjugating it with epidermis, even if FGF is the molecule that induces neural crest. A possible explanation of this result is that although FGF is indeed the molecule that induces neural crest, it is not sufficient to mediate the effect of epidermis on neural plate and other non-FGF factors are also required. Alternatively, FGF may not be part of the epidermal signal at all; instead, FGF signaling may be required within the prospective epidermal, as a part of the response to non-FGF signals emanating from the epidermis and/or to render cells competent to respond to such signals from the epidermis.

We have shown that overexpression of noggin mRNA is not able to induce XSlu directly in animal caps, but needs to interact with another signal present presumably in the ventral side of the embryo (Mayor et al., 1995). The inhibitory effect of noggin on XSlu expression could be explained...
by an induction of neural plate which later interacts with the ventral signal to induce Xslug. The extension of Xslug expression to the ventral side of the embryo produced by noggin mRNA can be inhibited by XFD, suggesting that the ventral signal that interacts with noggin could be FGF. Ectopic expression induced by noggin and the FGF receptor is qualitatively different, and the combination of both produces a different pattern of ectopic expression. These synergistic effects of FGF and noggin suggest that they activate different signaling pathways and/or that a third component is involved in the induction of the neural crest. The idea that the position of the border of the neural plate is established by a balance between dorsal and ventral signals is not new (Zhang and Jacobson, 1993; Mayor et al., 1995; Liem et al., 1995). As we do not know which member of the FGF family or its receptors participate in this process, we cannot analyze its pattern of expression to interpret our results. However, it is very well established that noggin is expressed in the dorsal mesoderm, which is not immediately adjacent to the neural crest region. Two alternatives can explain this difference in the expression of noggin and its proposed function. First, as noggin is a secreted molecule it can diffuse from the dorsal mesoderm to the borders of the neural plate where it participates in the induction of the neural crest. Second, it could participate indirectly in the induction of the neural crest by, for example, inducing neural plate which in turn induces neural crest by interacting with epidermis. Finally, although we have used noggin overexpression to generate neural signal, such a signal is required endogenously during neural crest induction it could also be generated by chordin, follistatin, or other molecules which inhibit BMP signaling.

Xenopus embryos in which FGFR mRNA is overexpressed exhibited ectopic Xslug expression in the ventral ectoderm around the blastopore and an increase in Xslug expression in the most posterior neural folds. The number of cells in the cephalic neural crest is always bigger than that in the posterior crest; on the other hand, some of the FGF receptors that are expressed in the nervous system are more strongly or exclusively expressed in the anterior neural tissue (Tannahill et al., 1992; Launay et al., 1994). These facts suggest that the posterior endodermal cells of normal embryos contain normal levels of FGF, but are not strongly induced because the FGF receptor or other components of the signal transduction is not sufficient. When the level of the FGF receptor is increased by expressing a high level of FGFR (1.2 ng/embryo), posterior cells are induced as strongly as anterior cells. However, when only moderate levels of FGFR are expressed (0.4 ng/embryo), no effect on neural crest was detected, suggesting that the limiting factor is not the FGF receptor, but possibly other elements of the FGF signal transduction pathway. This effect on posterior tissues is confirmed by the observation that the only cells that responded to overexpression of FGFR were the ventral blastomeres which produce part of the posterior tissues. No modification of the Xslug pattern was observed when the FGFR mRNA was injected into the dorsal blastomeres that produce more anterior structures. Furthermore we have shown previously that only anterior neural plate can interact with the epidermis to induce Xslug (Mancilla and Mayor, 1996). Interestingly, the overexpression of XFD modified only the posterior domain of Xslug expression and the overexpression of FGFR modified only the posterior domain of Xslug expression. This observation suggests that the posterior ectoderm is more labile to the changes in FGF activity, which is consistent with a posteriorizing role proposed for FGF (Lamb and Harland, 1995; Cox and Hemmati-Brivanlou, 1995).

Neural plate competence is lost at the end of gastrulation (Sharpe et al., 1987; Servetnick and Grainger, 1991) and the neural crest competence is lost earlier, during gastrulation (Mancilla and Mayor, 1996). We have shown in this report that this loss in competence for induction of the neural crest can not be modified by the overexpression of the FGFR, suggesting that the explanation of the temporal changes in the competence have to be explained by changes downstream of the FGFR or changes in other receptors.

**ACKNOWLEDGMENTS**

We thank Dr. Michael Sargent and Dr. Catherine Allende for comments on the manuscript and Dr. Enrique Amaya, Dr. Alison Snape, and Dr. Robert Grainger for providing us the XFD, the FGFR, and the Xsox-2 clones, respectively. We acknowledge the technical assistance of Florencio Espinoza. This investigation was supported by Fondecyt (Grant 1960910) and the University of Chile.

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Received for publication February 26, 1997
Accepted May 20, 1997