Development 122, 869-880 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
DEV1043

A truncated FGF receptor blocks neural induction by endogenous *Xenopus* inducers

Catherine Launay, Valérie Fromentoux, De-Li Shi and Jean-Claude Boucaut*

Groupe de Biologie Expérimentale, Différenciation Moléculaire et Cellulaire du Développement, CNRS URA-1135, Université P. et M. Curie, 9 Quai Saint-Bernard, 75005 Paris, France

*Author for correspondence

SUMMARY

We have examined the role of fibroblast growth factor (FGF) signalling in neural induction. The approach takes advantage of the fact that both *noggin* and the dominant negative mutant activin receptor (Δ*1XAR1*) directly induce neural tissues in the absence of dorsal mesoderm. A truncated FGF receptor (*XFD*) is co-expressed with *noggin* or Δ*1XAR1* in both whole embryos and isolated animal caps. We demonstrate that inhibition of FGF signalling prevents neural induction by both factors. Furthermore, neural induction by organizers (the dorsal lip of blastopore and Hensen's node) is also blocked by inhibiting FGF signalling in ectoderm.

It has been proposed that the specification of anterior neuroectoderm, including the cement gland, occurs in a sequential manner as gastrulation proceeds. We show that the specification of the most anterior neuroectoderm by *noggin* may occur before gastrulation and does not require FGF signalling, since both the cement gland marker *XCG-1* and the anterior neural marker *Otx-2* are normally expressed in ectodermal explants co-injected with *noggin* and *XFD* RNAs, but the cement gland cells are poorly differentiated. In contrast, the expression of both genes induced by *CSKA.noggin*, which is expressed after the mid-blastula transition, is strongly inhibited by the presence of *XFD*. Therefore the *noggin*-mediated neural induction that takes place at gastrula stages is abolished in the absence of FGF signalling. Since inhibition of FGF signalling blocks the neuralizing effect of different neural inducers that function through independent mechanisms, we propose that FGF receptor-related-signalling is required for the response to inducing signals of ectodermal cells from gastrula.

Key words: FGF signalling, *noggin*, organizer, neural induction, truncated FGF receptor, *Xenopus*

INTRODUCTION

The formation of neural tissues in amphibian embryos is a result of interactions between the dorsal mesoderm and the ectoderm. Early embryological work demonstrated that the dorsal mesoderm transplanted to the ventral region could induce a secondary embryonic axis containing a neural tube that was derived from the host (Spemann and Mangold, 1924). In normal development, neural induction takes place in a sequential manner; the anteroposterior axis of the neural tube is patterned during gastrulation by two distinct signals. A strong ectodermal dorsalization leads to neural specification, which is followed by a caudalization signal that functions as a gradient and is responsible for the patterning of the anteroposterior axis (reviewed by Yamada, 1990). A similar interpretation comes from the activation/transformation model (Nieuwkoop, 1952). The activation of ectoderm by anterior mesoderm specifies ectodermal cells to an anterior neural fate, while the transformation process respecifies part of these cells to form posterior neural tissues.

Analysis of the molecular features underlying anteroposterior specification within the neuroectoderm has led to the identification of several molecular markers. For example, cement gland transcript *XCG-1* is expressed in the cement gland anlage, which represents the most anterior neuroectoderm (Sive et al., 1989). The *Xenopus* homeobox gene *Otx-2* is first expressed in the Spemann organizer of early gastrula, and in the presumptive anterior neuroectoderm in subsequent stages (Pannese et al., 1995; Blitz and Cho, 1995). Other homeobox genes such as *Engrailed-2* and *Krox-20* are localized, respectively, to the midbrain-hindbrain junction and to the third and fifth rhombomeres (Hemmati-Brivanlou et al., 1991; Bradley et al., 1993). However, *XlHbox-6* is expressed in the posterior neural tube (Wright et al., 1990). It is of interest to note that not only are these genes neural markers but they may also be involved in anteroposterior specification of the central nervous system. It has been shown that overexpression of *Otx-2* induces ectopic cement gland (Pannese et al., 1995; Blitz and Cho, 1995), while overexpression of *XlHbox-6* produces ectopic tails (Cho et al., 1991a). In addition, *XIF-3* is expressed predominantly in the anterior neural tissues (Sharpe and Gurdon, 1990) and *N-CAM* is a general neural marker (Kintner and Melton, 1987).

Although appreciable numbers of neural markers are

available, little is known about the molecular mechanisms responsible for neural induction. Recently, the *Xenopus* gene *noggin* was shown to be expressed in the Spemann organizer and it has a strong dorsalizing activity in UV-ventralized embryos (Smith and Harland, 1992; Smith et al., 1993). *Noggin* can induce isolated animal caps to form anterior neural tissues in the absence of dorsal mesoderm (Lamb et al., 1993). In addition, the induced anterior neural tissue is dorsoventrally patterned (Knecht et al., 1995). Thus *noggin* can act as an endogenous neural inducer. Another probable candidate for endogenous neural inducer is *follistatin*, which is an antagonist of activin and is expressed in the Spemann organizer of early gastrula. Ectopic expression of *follistatin* in animal caps directly induces neural tissues (Hemmati-Brivanlou et al., 1994). Alternatively, inhibition of activin signalling by a truncated activin receptor (Δ*1XAR1*) also neuralizes ectoderm (Hemmati-Brivanlou and Melton, 1994). Therefore, like *noggin*, *follistatin* is expressed at the right time and in the right place to mediate neural induction. However, it is likely that *noggin* and *follistatin* (or Δ*1XAR1*) neuralize ectoderm through different mechanisms.

There is also evidence that FGF may be involved in neural induction. This mainly comes from the observation that FGF can induce dissociated ectodermal cells from early gastrula to differentiate into neurons and melanophores (Kengaku and Okamoto, 1993, 1995). Nevertheless, whatever the concentrations used, FGF never induces neural tissues in ectodermal explants (Green et al., 1990), and inhibition of FGF signalling by the dominant negative FGF receptor (*XFD*) produces trunk and posterior deficiencies that probably result from gastrulation defects (Amaya et al., 1991, 1993; Isaacs et al., 1994). Although these observations make the involvement of FGF in neural induction unlikely, there are several lines of evidence to suggest a possible role of FGF in the anteroposterior specification of neural tissues. The *Xenopus* gene *int-2* (*FGF-3*) is expressed in the central nervous system and can be activated by mesodermal and neural induction (Tannahill et al., 1992). We have previously found that various FGF receptor mRNAs are localized to defined regions of the brain, except the telencephalon (Launay et al., 1994). Furthermore, we showed that neural induction controls the expression of a spliced FGFR-2 variant that binds specifically aFGF and bFGF (Shi et al., 1994b). These results suggest that FGF may be involved in neural tissue formation or differentiation.

In the present study we investigate the role of FGF signalling in neural induction promoted by neuralizing factors such as *noggin* and Δ*1XAR1*, and by organizers. We show that inhibiting FGF signalling by the dominant negative FGF receptor prevents neural induction in animal cap explants by neuralizing factors, and by *Xenopus* and chick organizers. We also demonstrate that the specification of the most anterior neuroectoderm, including cement gland, may occur before gastrulation and does not require FGF signalling. However, inhibition of neural tissue formation modifies the distribution of cement gland. These observations suggest that FGF receptor-related-signalling is involved in the specification of anteroposterior neuroectoderm.

MATERIALS AND METHODS

Embryos

Xenopus eggs were obtained from females injected with 500 IU of human chorionic gonadotropin (Sigma), and artificially fertilized with minced testis. They were dejellied with 2% cysteine hydrochloride (pH 7.8) and kept in 10% normal amphibian medium (NAM). Embryonic stages were determined according to the table of Nieuwkoop and Faber (1967).

DNA and RNA microinjections

Microinjection of embryos was performed in 10% NAM containing 3% Ficoll 400 (Sigma). After injection the embryos were maintained in this medium for 2 hours and were then cultured in 10% NAM. The plasmid constructs used in this work are the following:

CSKA.noggin

This construct harbours the *noggin* cDNA under the control of cytoskeletal actin (CSKA) promoter (Smith et al., 1993), allowing the expression of noggin protein after mid-blastula transition (MBT). The plasmid was linearized with *Xba*I before microinjection.

Noggin Δ5′

This, and the following plasmids, were used for RNA injection experiments. RNA transcripts from *noggin Δ5′* have a higher dorsalizing activity in the embryos (Smith and Harland, 1992).

Δ1XAR1

This is a dominant negative mutant activin receptor that inhibits axis formation in whole embryos (Hemmati-Brivanlou and Melton, 1992) and promotes neural induction in animal caps (Hemmati-Brivanlou and Melton, 1994).

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 receptors by the formation of non-functional heterodimers.

HAVØ

This is a non-functional form of *XFD* that lacks three consecutive amino acids (His-Ala-Val) involved in homophilic interactions (Amaya et al., 1993). Injection of RNA encoding this protein has no effect on the function of endogenous receptor and thus the RNA was used as a control throughout the experiments.

XFR

This is a wild-type *Xenopus* FGF receptor-1 (Amaya et al., 1991), which was used for restoring the effects of *XFD*.

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 synthetic RNA was purified using a Sephadex G-50 column (Pharmacia) and recovered by ethanol precipitation. The amount of RNA was determined both by ethidium bromide staining compared with standard RNA and by incorporation of [^{3}H]UTP in the reaction mixture. Samples of RNA in DEPC-treated water were stored at −80°C.

Embryonic dissections and in vitro neural induction

Dissections of embryonic explants were done in 75% NAM supplemented with gentamycin at 50 μg/ml in an agar-coated culture dish, and dissected explants were maintained in 75% NAM for the desired period.

In vitro neural induction by organizers was performed using both Spemann organizer and Hensen's node. Animal caps derived from stage 9 (late blastula) and stage 10.5 (early gastrula) embryos injected with *XFD* or *HAVØ* RNA were combined with either the dorsal marginal zone from stage 10.5 or with chick Hensen's node, which

was dissected at 16-18 hours of incubation as described (Kintner and Dodd, 1991).

RNase protection assay

The extraction of RNA and synthesis of probes were as previously described (Shi et al., 1992). The EF-1α probe, synthesized at lower specific activity, was used to control for RNA amount. RNase digestion was performed using 250 units/ml RNase T1 (Boehringer Mannheim). Protected fragments were resolved on a 5% polyacrylamide gel and exposed to X-ray film (Kodak) with intensifying screens.

Whole-mount immunocytochemistry and in situ hybridization

Whole-mount immunostaining was performed as described (Hemmati-Brivanlou and Harland, 1989). Both embryos and explants were fixed with DMSO/methanol (1:4) and bleached with H_2O_2. A polyclonal antibody directed against *Xenopus* N-CAM (Levi et al., 1990) was applied, followed by anti-rabbit secondary IgG conjugated with horseradish peroxidase. The presence of N-CAM was visualized by diaminobenzidine substrate.

Whole-mount in situ hybridization was performed as described (Harland, 1991). The *XCG-1* plasmid (Sive et al., 1989) was linearized with *Not*I and antisense RNA was transcribed with T3 RNA polymerase in the presence of digoxigenin-11 UTP (both from Boehringer Mannheim). The chromogenic reaction with alkaline phosphatase was incubated from 10 to 60 minutes at room temperature.

Histology

The explants were fixed in 3.7% formaldehyde and dehydrated in an ethanol series. They were embedded in polyethylene glycol-400 distearate (PEG). Sections were cut at 10 μm thickness and stained with Giemsa stain (Sigma). To cut sections after in situ hybridization, the explants were dehydrated in methanol after chromogenic reaction and embedded in PEG; sections were inspected directly without further staining.

RESULTS

Inhibition of the dorsalizing activity of *noggin* by expression of truncated FGF receptor

It was shown that injection of 50 pg *noggin* RNA into UV-ventralized embryos could rescue nearly normal phenotypes (Smith and Harland, 1992), so this amount was used throughout our experiments. We found that embryos injected with this dose developed exclusively dorsoanterior enhanced structures (Fig. 1A). At late tail-bud stage (stage 35), when compared with uninjected embryos (Fig. 1D), embryos injected with *noggin* RNA lacked the trunk and posterior structures but had externally normal eyes and cement gland.

To see if FGF signalling is required for neural differentiation, we first examined the consequence of inhibiting FGF signalling on the dorsalizing and neuralizing effects of *noggin* in whole embryos. When 2-cell stage embryos were injected with 1 ng *XFD* RNA alone, they had essentially trunk and posterior deficiencies but normal anterior tissues. However, most embryos co-injected with 50 pg *noggin* and 1 ng *XFD* RNAs showed deficiencies in dorsal structures. At stage 35 these embryos were often rounded in shape, without any visible axis. In addition, they had no externally visible anterior tissues such as eyes, as judged by the absence of retinal pigment (Fig. 1C). Surprisingly, although embryos co-injected with *noggin* and *XFD* RNAs had defects in dorsal structures, we noticed that they always had an overdeveloped cement gland. A large area corresponding to cement gland pigment was easily visible during neurulation, and at stage 35, these cement gland pigmentations were frequently dispersed in the presumed head region (Fig. 1C). A total of 90 embryos co-injected with *noggin* and *XFD* RNAs from three independent experiments were scored for external morphology (Table 1). We found that 54 embryos (60%) were dorsal-deficient, with phenotypes as shown in Fig. 1C. 22 embryos (24%) had a short trunk and posterior axis but were also microcephalic; 12 embryos (13%) had a normal head with a short or bent trunk axis. For control experiments, embryos were co-injected with *noggin* and *HAVØ* RNAs. These control embryos had essentially the *noggin* phenotypes (Fig. 1B).

The deficiency of dorsoanterior structures in embryos injected with *noggin* and *XFD* RNAs was further confirmed by whole-mount immunostaining. The anti-N-CAM antibodies labelled specifically the central nervous system and eyes in normal embryos at stage 30 (Fig. 2A). As expected, embryos injected with *noggin* alone developed ectopic neural tissues that were strongly stained by the antibodies (Fig. 2B). In contrast, only small amounts of dispersed neural tissues were present in embryos co-injected with *noggin* and *XFD* RNAs (Fig. 2C). Taken together, these observations indicate that blocking FGF signalling in the whole embryo interferes with the ability of *noggin* in dorsalization and/or neural induction.

To see if *XFD* blocks neural induction and produces anterior defects in whole embryos, we injected 1 ng *XFD* RNA into different blastomeres at the 8-cell stage. When *XFD* RNA was injected into the dorso-animal blastomeres, 87% of the embryos exhibited trunk defects, and significant amounts of the embryos (72%) were also anterior-deficient (Table 2). Injection of *XFD* RNA into dorsovegetal blastomeres resulted essentially in trunk defects (79%), with fewer anterior-deficient embryos (47%). Immunostaining of N-CAM revealed that the anterior-deficient embryos developed small amounts of neural tissues, with small and fused eyes (Fig. 2D). However, in embryos showing only

Table 1. Phenotypes produced by injection of RNAs encoding *noggin* and *XFD*

RNA	Phenotype					
	Normal	Anteriorized	Short/bent axis	Microcephalic	Dorsal-deficient	*n*
noggin	–	82	18	–	–	50
noggin/XFD	3	–	13	24	60	90
noggin/HAVØ	–	74	26	–	–	19

Embryos were injected at the 2-cell stage and allowed to develop until stage 35. Healthy embryos were selected for scoring phenotypes. The results are expressed as percentages except for *n*, which refers to total number of embryos scored.

trunk defects, anterior neural tissues and eyes were normally developed (Fig. 2E). Consistent with the previous observation (Isaacs et al., 1994), the ventro-animal blastomeres were less sensitive to *XFD* injection. These results indicate that inhibiting FGF signalling may interfere with neural differentiation. Nevertheless, the mechanisms underlying these *XFD* phenotypes may be more complex. Firstly, the anterior defects produced by injection of *XFD* RNA into dorso-animal blastomeres may be a consequence of its effect on mesoderm formation and/or on gastrulation. Secondly, the presence of small amounts of neural tissues in these embryos suggests that there may be chimeric (weak) *XFD* expression, or strong and persistent endogenous inducers. Therefore, we turned to explant assay.

Inhibition of neural induction by truncated FGF receptor in animal caps

In order to demonstrate the function of FGF signalling in neural induction directly, we took advantage of the fact that *noggin* induces anterior neural tissues in the absence of dorsal mesoderm (Lamb et al., 1993). As above, 50 pg *noggin* RNA was injected at the 2-cell stage, animal caps were dissected from mid-blastula embryos (stage 8) and cultured to stage 25. We found that animal cap explants derived from embryos injected with *noggin* RNA developed a substantially elongated form, with the cement gland formed frequently in the center as a constricted ring (Fig. 3G). The same morphology was observed when embryos were injected with 50 pg *CSKA.noggin*, which contains *noggin* cDNA under the control of cytoskeletal actin promoter (Smith et al., 1993), but the elongation of explants and cement gland constriction were generally more pronounced (Fig. 3C). This implies that they may have a slightly different neuralizing efficiency. We also extended this analysis by using *Δ1XAR1*, which neuralizes animal caps by inhibiting activin signalling (Hemmati-Brivanlou and Melton, 1994). Injection of 1 ng *Δ1XAR1* RNA resulted in the same morphology as above (Fig. 3I). However, when 1 ng *XFD* RNA was co-injected, nearly all explants became spherical (Fig. 3D,H,J). In addition, animal caps resulting from co-injection with *CSKA.noggin* and *XFD*, as well as with *Δ1XAR1* and *XFD* RNAs, developed like atypical epidermis. They were morphologically indistinguishable from uninjected- or *XFD*-injected animal caps (Fig. 3A,B), and cement gland pigmentations were not visible in these explants. In contrast, diffused cement gland pigmentations were found to be present in animal caps injected with *noggin* and *XFD* RNAs (Fig. 3H; but see also Fig. 8 below). Thus delaying the expression of *noggin* after MBT may have a different effect on cement gland induction.

We further examined, on histological sections, the tissues formed in animal caps expressing *XFD* and neuralizing factors. When *noggin*, *CSKA.noggin* or *Δ1XAR1* were injected alone, the explants formed well-differentiated cement glands composed of elongated cells (Fig. 3E). Furthermore, large masses of neural tissues were formed, sometimes with a neural tube present. Co-injection with *XFD* RNA reduced substantially the formation of both cement gland and neural tissues. The analysis of two representative experiments revealed that more than 90% of explants developed as atypical epidermis (Fig. 3F) and fewer than 10% of them had differentiated cement gland cells in the case of co-injection with *noggin/XFD* and *CSKA.noggin/XFD* (Table 3). It should be mentioned that, despite the presence of cement gland pigmentations in explants co-injected with

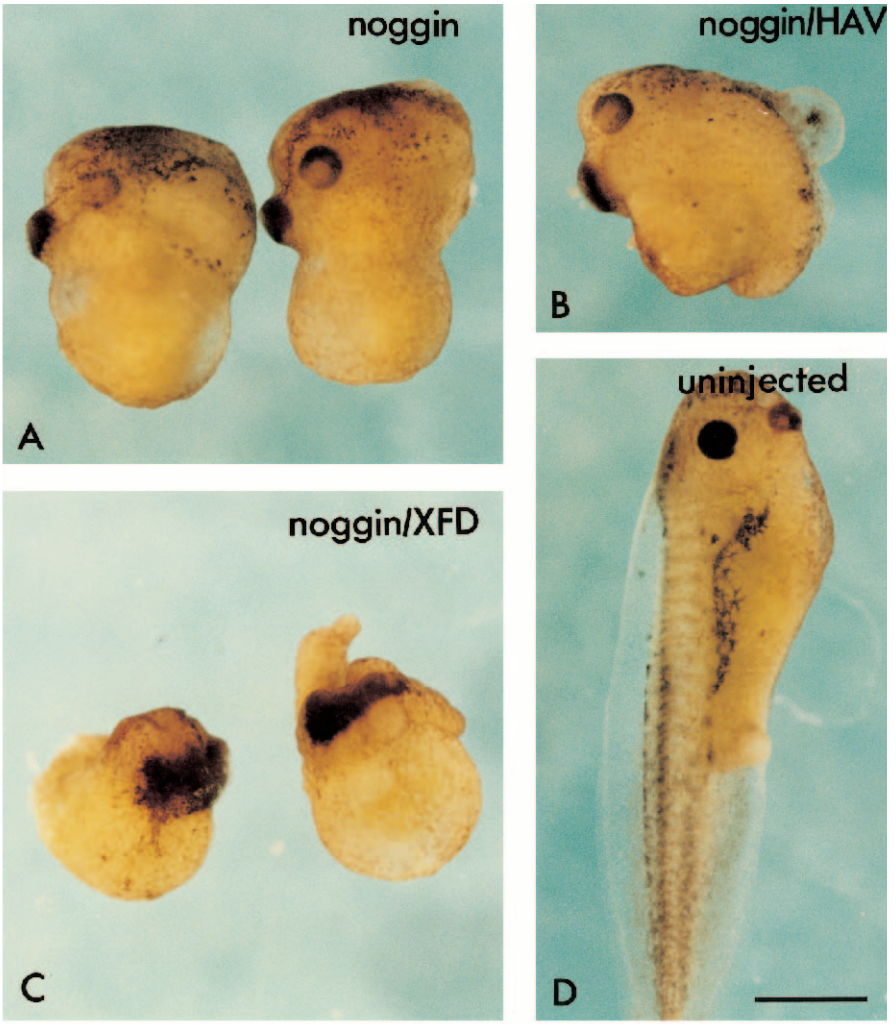

Fig. 1. Overexpression of RNAs encoding *noggin* and *XFD* in whole embryos. (A) Anteriorized embryos at stage 35 resulted from injection of *noggin* RNA. (B) Control embryo injected with RNAs encoding *noggin* and *HAVØ*. The phenotype is essentially similar to that of the *noggin*-injected embryo. (C) Dorsal-deficient embryo resulting from co-injection of *noggin* and *XFD* RNAs. Note that a large area of cement gland pigmentation is visible. (D) An uninjected embryo at stage 35. Scale bar, 500 μm.

noggin and *XFD* RNAs, cement gland cells did not differentiate as elongated cells. Thus in the absence of neural tissues, these explants were considered as atypical epidermis. Although we found that 31% of animal caps derived from embryos co-injected with *Δ1XAR1* and *XFD* RNAs had neural tissues, 67% of the explants developed as atypical epidermis. Therefore, the reduced elongation of explants is well correlated with the inhibition of neural tissue and cement gland formation by *XFD*.

XFD inhibits the expression of neural markers induced by *noggin*, *CSKA.noggin* and *Δ1XAR1*

To further ascertain that *XFD* inhibits neural tissue formation, we performed an RNase protection assay using neuroectodermal markers that are expressed both in anterior (*XCG-1*, *Otx-2* and *XIF-3*) and in all neural tissues (*N-CAM*). In addition, since *noggin*-induced neural tissues were dorsoventrally patterned (Knecht et al., 1995), we assayed the expression of *thrombospondin 1* (*TSP-1*), which is localized to notochord and floor plate cells (D. W. DeSimone and C. A. Whittaker,

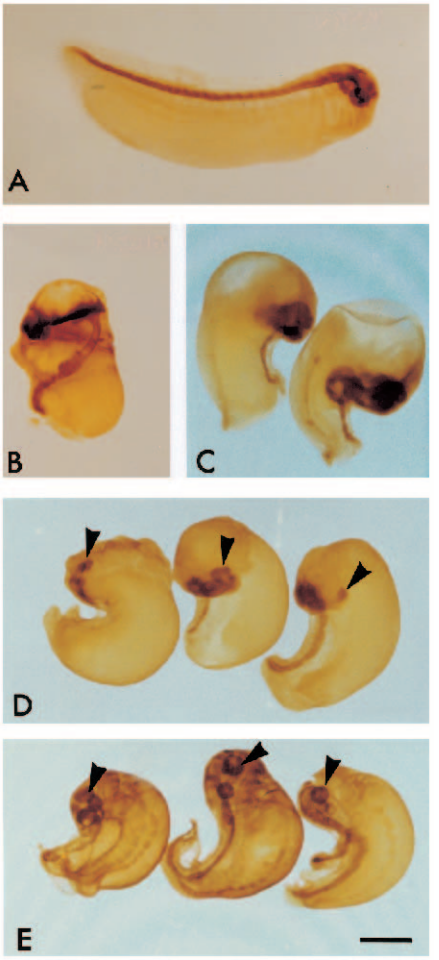

Fig. 2. Whole-mount immunocytochemistry of N-CAM expression in stage-30 embryos. The embryos were labelled by antibodies directed against *Xenopus* N-CAM. (A) Control embryo. N-CAM is detected in the eyes and the entire central nervous system. (B) *Noggin*-injected embryo. Ectopic neural tubes are revealed by anti-N-CAM antibodies. (C) Embryo injected with RNAs encoding *noggin* and *XFD*. Small amounts of N-CAM-positive cells are present. (D) Embryos injected with *XFD* RNA in the dorso-animal blastomeres at the 8-cell stage. Small amounts of neural tissues and fused eyes (arrowheads) are present. (E) Embryos injected with *XFD* RNA in the dorsovegetal blastomeres at the 8-cell stage. Notice the trunk deficiency and unaffected head region with normal eyes (arrowheads). Scale bar, 500 μm.

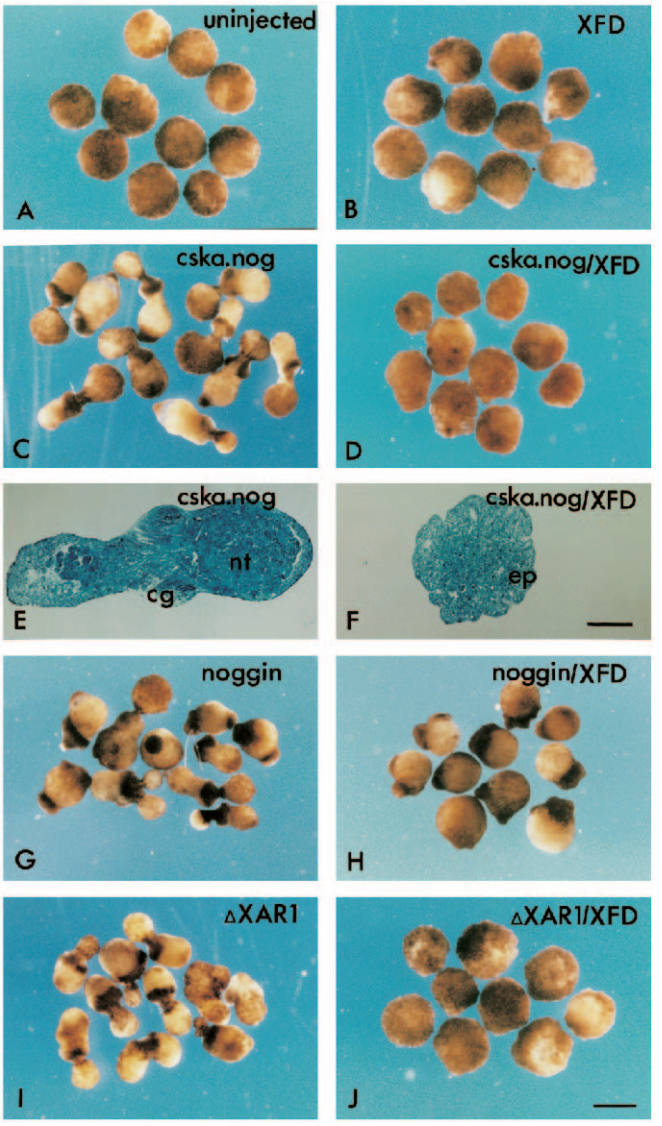

Fig. 3. Expression of neuralizing factors and *XFD* in animal caps cultured to stage 35. (A) Uninjected animal caps. (B) *XFD*-injected animal caps. (C) *CSKA.noggin*-injected animal caps. Cement gland forms in the middle of the elongated explants. (D) Animal caps co-injected with *CSKA.noggin* and *XFD* have a spherical shape and no cement gland pigmentations are visible. (E) Histological section of an explant resulting from injection of *CSKA.noggin* alone. Elongated cement gland cells (cg) and a mass of neural tissue (nt) are present. (F) A section from an explant injected with *CSKA.noggin* and *XFD*. This explant differentiates as atypical epidermis (ep). (G) Animal caps from embryos injected with *noggin* RNA. (H) Animal caps from embryos co-injected with *noggin* and *XFD* RNAs are rounded but cement gland pigmentations are visible. (I) Animal caps from embryos injected with *Δ1XAR1* RNA. (J) Animal caps from embryos co-injected with *Δ1XAR1* and *XFD* RNAs. Scale bars, 500 μm.

Table 2. Phenotypes of embryos resulting from injection with *XFD* into different blastomeres at the 8-cell stage

Site of injection	RNA	Anterior-deficient	Trunk-deficient	Normal	*n*
Dorso-animal	*XFD*	72	87	6	69
	HAVØ	6	7	87	71
Dorso-vegetal	*XFD*	47	79	20	95
	HAVØ	3	3	96	89
Ventro-animal	*XFD*	11	22	74	75
	HAVØ	3	5	92	83

Each blastomere was injected with 0.5 ng *XFD* or *HAVØ* RNA (a total of 1 ng). At stage 35, healthy embryos were selected for scoring phenotypes according to the criteria described in the text and shown in Fig. 2, except that these embryos were not treated by whole-mount immunocytochemistry.

The results are obtained from three different experiments and expressed as percentages except for *n*, which refers to total number of embryos scored.

personal communication). In order to control the specificity of *XFD*, the *HAVØ* RNA was used in every injection experiment.

The results of the RNase protection assay indicate that *CSKA.noggin*, *noggin* and *Δ1XAR1* induced the expression of *Otx-2*, *N-CAM* and *XCG-1* (Fig. 4). The expression of *XIF-3* and *TSP-1* was also significantly enhanced, since both genes were expressed at low levels in uninjected explants. Conversely, the expression of epidermal keratin gene was reduced, especially in explants injected with *CSKA.noggin* or *noggin* RNA. Co-injection of these neuralizing factors with the control RNA (*HAVØ*) did not have any effect on their neural-inducing activities. In contrast, co-injection with *XFD* RNA inhibited completely the expression of *N-CAM* and *TSP-1*, and reduced the expression of *XIF-3* to a background level (Fig. 4). These explants were specified for epidermal differentiation, as judged by the increased expression of epidermal keratin gene, compared with uninjected explants or explants injected with neuralizing factor alone. Interestingly, we observed a differential inhibition of *Otx-2* and *XCG-1*. *XFD* reduced significantly their expression in *CSKA.noggin*- and *Δ1XAR1*-injected explants, while it inhibited *Otx-2* expression in *noggin*-injected explants only to a lesser extent. Consistent with the observation that cement gland was present in explants injected with *noggin* and *XFD* RNAs (see Fig. 3H), our RNase protection analyses reproducibly showed that *XFD* did not affect the amounts of *XCG-1* transcripts in explants co-injected with *noggin* RNA (Fig. 4), although it did modify the distribution and shape of cement gland cells (see Fig. 8H).

In addition, we found that *XFD* inhibits the expression of neural markers in a dose-dependent manner. When 50 pg *CSKA.noggin* or *noggin* RNA were used, a low dose of *XFD* RNA (0.4 ng) reduced significantly the expression of *N-CAM* while that of *Otx-2* was relatively unaffected. However, a high dose (1.5 ng) completely inhibited the expression of *N-CAM* and reduced significantly that of *Otx-2*.

We next examined whether inhibiting FGF signalling affects the early response of ectodermal cells to neural induction. For this purpose, the expression of *XIF-3* and *N-CAM* was analyzed by RNase protection assays at stage 20 and stage 35 respectively. We found that *XFD* inhibited completely the expression of these genes before stage 20, and this inhibition was not reversible at stage 35 (data not shown). Furthermore, our whole-mount immunostaining using anti-N-CAM antibodies showed unequivocally that neural tissues were indeed formed in *noggin*-injected explants cultured to stage 35. Especially, in elongated explants with cement gland in the middle, a mass of neural tissues was generally formed on one side of the explants (Fig. 5A). The nature of the material that is not cement gland or neural tissue is unclear, but it does not contain mesoderm (see Fig. 6A,B). When *noggin* and *XFD* RNAs were co-injected, no neural tissues were formed (Fig. 5B), though these explants still contained poorly differentiated cement gland cells (see Fig. 8). These observations therefore confirm the results obtained by RNase protection analyses and histological observations, and suggest that *XFD* interferes with the initial step of neural induction that leads to the activation of neuroectodermal genes.

The effect of *XFD* is not mediated by inhibition of neural-inducing mesoderm

It was shown that *noggin* acts as a neuralizing factor only in the absence of *XBra*, and in the presence of *XBra* it acts as a dorsalizing factor (Cunliffe and Smith, 1994). This raises the possibility that the inhibition of neural induction by *XFD* would be a consequence of blocking neural-inducing mesoderm. Although this is unlikely in the case of injection using *Δ1XAR1* RNA, it is still important to examine whether mesodermal genes are expressed in explants derived from injection using *noggin* and *CSKA.noggin*. This was done by RNase protection assays using both early and late mesodermal markers.

Animal caps were dissected at stage 8 and cultured to stage 11 for early mesoderm markers *goosecoid* (Cho et al., 1991b) and *XBra* (Smith et al., 1991), and to stage 25 for late mesodermal markers (muscle-specific actin), as well as spinal cord marker *XlHbox-6* (Wright et al., 1990). The size of dissected animal caps was the same in different analyses. At stage 11, the expression of *goosecoid* and *XBra* was not detected in any explants (Fig. 6A). This result was further confirmed at stage 25 by the absence of expression of muscle-specific actin (Fig. 6B) and the posterior mesoderm marker *Xhox-3* (not shown). In addition, *XlHbox-6* was not detected in explants derived

Table 3. Tissues formed in animal caps expressing *XFD* and neuralizing factors

RNA injected	Epidermis	Cement gland	Neural tissues	*n*
Control	100	–	–	19
CSKA.noggin	–	99	100	15
CSKA.noggin/XFD	92*	8	–	13
CSKA.noggin/HAVØ	–	93	99	18
noggin	–	98	99	12
noggin/XFD	91*	9	–	12
noggin/HAVØ	–	100	87	8
Δ1XAR1	–	99	96	21
Δ1XAR1/XFD	67*	25	31	15
Δ1XAR1/HAVØ	–	100	92	13

Embryos were injected at the 2-cell stage. Animal caps were dissected at stage 8 and cultured to stage 35 for histological analysis.

Cement gland refers to histologically recognizable elongated cells.

The results are expressed as percentages except for *n*, which refers to total number of explants scored.

*These explants include undifferentiated (but *XCG-1* expressing) cement gland cells.

Control caps were not injected.

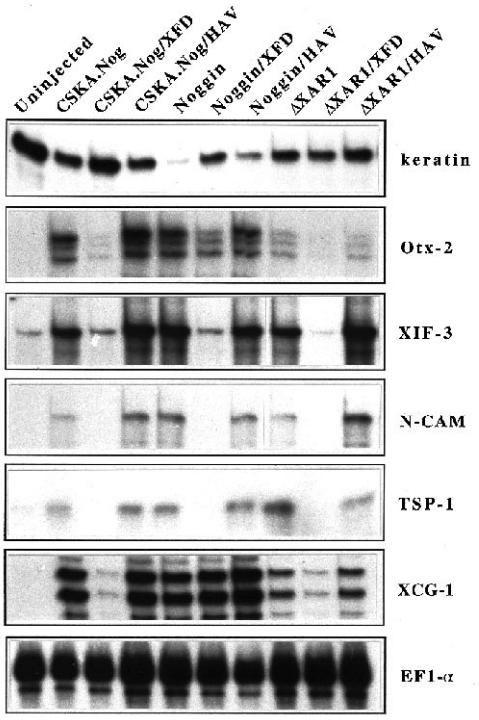

Fig. 4. RNase protection analyses of gene expression in animal caps derived from embryos co-injected with neuralizing factors and *XFD*. Animal caps were dissected at stage 8 and cultured to stage 35. This is a representative RNase protection assay using 60 explants for each condition. An equivalent of one-half or two explants was hybridized with *XCG-1* and epidermal keratin probe, respectively. *N-CAM* and *XIF-3*, and *TSP-1* and *Otx-2* probes, were respectively included in the same hybridization reaction. The type of injection is indicated on the top and the gene analyzed is shown on the right. EF-1α probe is used as a loading control.

from any kinds of injection (Fig. 6B). However, in the same explants, expression of *N-CAM* was induced by *noggin*, *CSKA.noggin* and *Δ1XAR1*, and *XFD* inhibited its expression (Fig. 6B). These observations therefore indicate that the inhibitory effect of *XFD* on neural induction is not mediated by blocking the formation of neural-inducing mesoderm.

The dominant negative effects of *XFD* were further controlled by co-injection of the wild-type *XFR* RNA. In this experiment, we injected 0.5 ng *XFD* RNA, since this amount was sufficient to inhibit *N-CAM* expression induced by *noggin* and *Δ1XAR1*. We found that the inhibitory effects of *XFD* on the morphology of the explants and on *N-CAM* expression were reversed completely by co-injection of twofold (1 ng) and fourfold (2 ng) excess of wild-type *XFR* RNA. The explants derived from embryos co-injected with *noggin* and *XFD* RNAs or *Δ1XAR1* and *XFD* RNAs were rounded, whereas those derived from the triple injection (*noggin/XFD/XFR* or *Δ1XAR1/XFD/XFR*) were elongated (not shown). RNase protection analyses showed clearly that *N-CAM* expression was reversed to the same level as in *noggin*- or *Δ1XAR1*-injected explants, respectively (Fig. 6C). In addition, to see if it is possible to achieve rescue with relatively small amounts of wild-type RNA, we injected 0.25 ng and 0.5 ng *XFR* RNA in this experiment. The result indicated that even an equal relative amount of *XFR* RNA did not rescue efficiently (not shown). This observation suggests that FGF receptor-related-signalling is required for neural induction.

XFD inhibits neural induction by Spemann organizer and Hensen's node

Although we have shown that *XFD* efficiently blocked neural induction by neuralizing factors, the question still remains as to whether FGF signalling is physiologically important for neural induction. To address this, we performed further neural induction using Spemann organizer or Hensen's node. The latter acts as organizer inducing neural tissues in *Xenopus* ectoderm (Kintner and Dodd, 1991). *XFD* or *HAVØ* RNA was injected at the 2-cell stage and animal caps were dissected at stage 9 and stage 10.5 and combined with Hensen's node tissue dissected after 16-18 hours of incubation (Fig. 7A). When the recombinates were cultured to stage 20, we found that neural plates were formed in most *HAVØ*-injected explants (Fig. 7B), whereas nearly all *XFD*-injected explants remained rounded (Fig. 7C). At stage 25, when these explants were analyzed for the expression of *N-CAM*, we observed that *XFD* strongly reduced the expression of *N-CAM* (Fig. 7F). This situation is thus similar to what was observed using neuralizing factors.

Neural induction was also performed using Spemann organizer, which corresponds to the dorsal marginal zone (DMZ) of stage 10.5 gastrula (Fig. 7A). Recombinates were cultured to stage 25 and the result was analyzed by N-CAM immunostaining. Again, strong labelling for N-CAM was observed in *HAVØ*-injected animal caps combined with the DMZ (Fig. 7D). In contrast, only small amounts of N-CAM were present in *XFD*-injected animal caps (Fig. 7E). RNase protection analysis confirmed this observation (Fig. 7F). Therefore, these experiments demonstrate the requirement for FGF signalling in neural induction mediated by different organizers.

XFD differentially modifies the formation of cement gland in animal cap explants injected with *CSKA.noggin*, *noggin* and *Δ1XAR1*

The observation that aberrant cement gland was present in whole embryos and in animal caps co-injected with *noggin* and *XFD* RNAs incited us to examine the formation of cement gland following different injections by whole-mount in situ hybridization. In particular, we were interested to see if differences exist between *noggin* RNA and *CSKA.noggin* in this experimental system. The *XCG-1* probe that specifically recognizes cement gland transcripts was used (Sive et al., 1989).

2-cell stage embryos were injected with neuralizing factor alone or co-injected with *XFD*. Animal caps were isolated at stage 8 and cultured until the equivalent of stage 30. As expected, the expression of *XCG-1* transcripts was induced in all explants derived from embryos injected with neuralizing factors; the labelling corresponded to the constricted region containing cement gland pigment (Fig. 8C,E,I). This region generally represents less than one-third of the explants. When sections were made after the chromogenic reaction, one could see clearly elongated cement gland cells (Fig. 8G). However, to our surprise, a substantial surface area of the explants derived from embryos injected with *noggin* and *XFD* RNAs was labelled by *XCG-1* (Fig. 8F). In particular, *XCG-1* transcripts were also detected in cells that had no visible cement

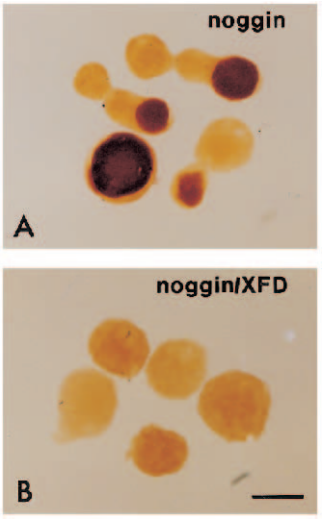

Fig. 5. Whole-mount immunostaining of N-CAM expression in explants cultured to stage 30. (A) Explants derived from embryos injected with *noggin* RNA. A mass of neural tissue is present on one side of the elongated explants. (B) Explants derived from embryos injected with RNAs encoding *noggin* and *XFD* are devoid of N-CAM staining. Scale bar, 500 μm.

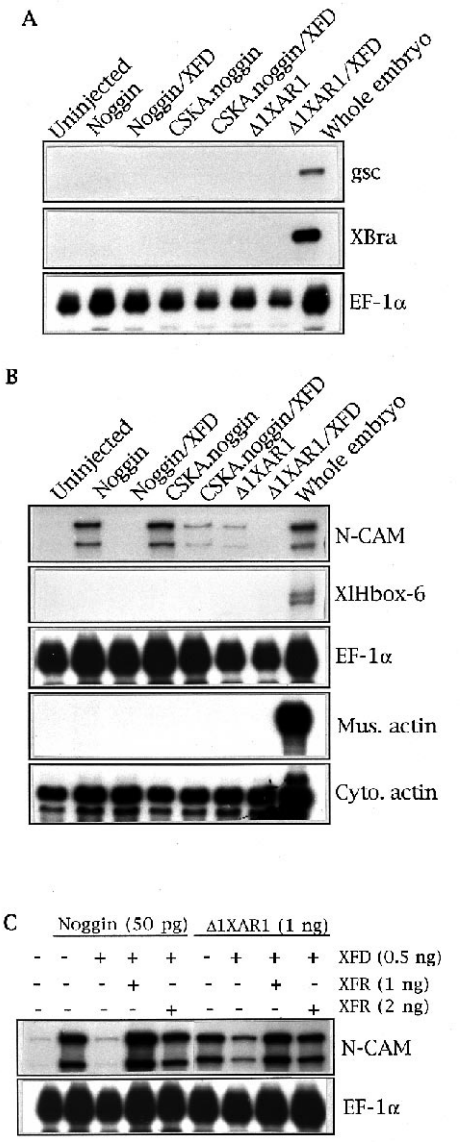

Fig. 6. RNase protection analyses of the expression of mesodermal and neural genes to control for the absence of mesoderm in the explants. (A) At stage 11, the early mesodermal genes *goosecoid* (*gsc*) and *XBra* are not detected in explants derived from all kinds of injection. (B) Expression of neural and late mesodermal genes in explants cultured to stage 25. *N-CAM* is induced by *noggin*, *CSKA.noggin* and *ΔX1AR1*; *XFD* inhibited its expression. *XlHbox-6* and muscle-specific (Mus.) actin are not detectable in these explants. Both EF-1α and cytoskeletal (Cyto.) actin are loading controls. (C) Rescue experiment. Co-injection of the wild-type *XFR* RNA reversed the inhibitory effects of *XFD* on *N-CAM* expression.

gland pigment, such that in most explants nearly the entire surface was *XCG-1*-positive. Histological sections confirmed that *XCG-1*-positive cells were poorly differentiated when compared with the elongated cement gland cells in *noggin*-injected explants (Fig. 8H). Uninjected- and *XFD*-injected explants showed no *XCG-1* expression (Fig. 8A, B). This suggests that the differentiation of cement gland may depend on neural tissue formation.

Co-injection of *XFD* RNA with either *CSKA.noggin* or *Δ1XAR1* RNA resulted in reduced *XCG-1*-positive cells (Fig. 8D,J). In these explants, small patches of *XCG-1*-positive cells were observed. In particular, after double injection with *CSKA.noggin* and *XFD* RNA, these patches were generally smaller or absent. It is unlikely that the differential effect of *XFD* on the induction of cement gland cells in *CSKA.noggin*-versus *noggin*-injected explants was due to the cell-autonomous expression of the plasmid construct, since secreted molecules may diffuse more easily in small explants than in whole embryos. Indeed, like *noggin*, *CSKA.noggin* induces the expression of neuroectodermal genes in isolated animal caps. Thus the similarity between *CSKA.noggin/XFD* and *Δ1XAR1/XFD* explants may result from the delayed effect of both *CSKA.noggin* and *Δ1XAR1*. Our RNase protection assay also indicated that *Δ1XAR1* activated the expression of neural genes later than *noggin* (not shown). These observations suggest that anterior neuroectoderm, including cement gland and *Otx-2*-positive cells, may be specified by *noggin* before gastrulation, independently of FGF signalling.

DISCUSSION

In this report we have analyzed the role of FGF signalling in neural induction. The formation of neural tissues was examined in both whole embryos and cultured animal caps expressing neuralizing factors and truncated FGF receptor (*XFD*). We show that *XFD* inhibited the *noggin*-mediated dorsalization in whole embryos and neural induction in cultured explants. Importantly, *XFD* inhibited neural induction by different organizers. Our results suggest that functional FGF receptor is required for the neuralizing effect of *noggin* and that FGF receptor-related-signalling may represent a graded signal in the determination of anteroposterior neural axis in *Xenopus* embryo.

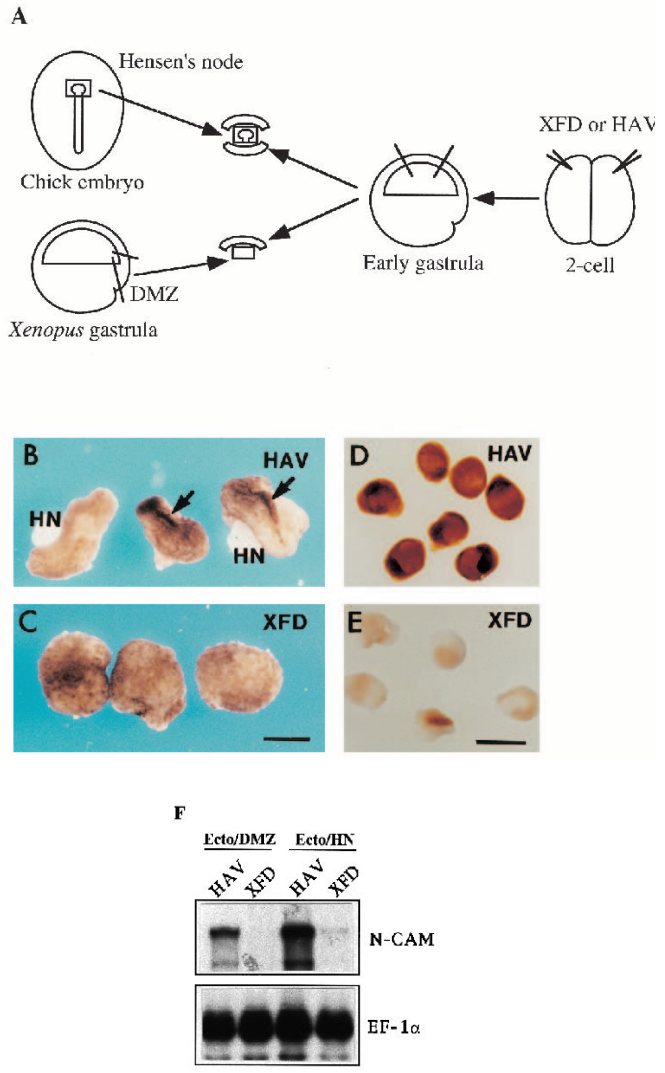

Fig. 7. Effect of *XFD* on neural induction by Hensen's node (HN) and Spemann organizer. (A) Schematic representation of these induction experiments. (B) Explants dissected from stage-9 embryos injected with *HAVØ* RNA. HN induces the appearence of neural plates (arrows). (C) Recombinates between *XFD*-injected ectoderm and HN remain rounded. (D,E) Whole-mount immunostaining of the expression of N-CAM. Recombinates between *HAVØ* caps dissected from stage-10.5 gastrula and DMZ (organizer) are intensely labelled by anti-N-CAM antibodies (D). Low levels of N-CAM are present in recombinates between *XFD* caps and DMZ (E). Scale bars, 500 μm. (F) RNase protection analysis. Both DMZ and HN induce the expression of *N-CAM* in *HAVØ*-injected explants from stage 10.5 gastrula, while low levels of *N-CAM* transcripts are present in recombinates using *XFD* caps.

The function of FGF/FGF receptor in neural tissue formation

We found that blocking the FGF signalling pathway substantially inhibited *noggin*-mediated induction of anterior neural genes. *XFD* also inhibits neural induction promoted by *Δ1XAR1*, which depletes the activin signal. Most importantly, we showed that *XFD* blocked neural induction mediated by Spemann organizer and Hensen's node, indicating that FGF signalling is physiologically important for neural induction.

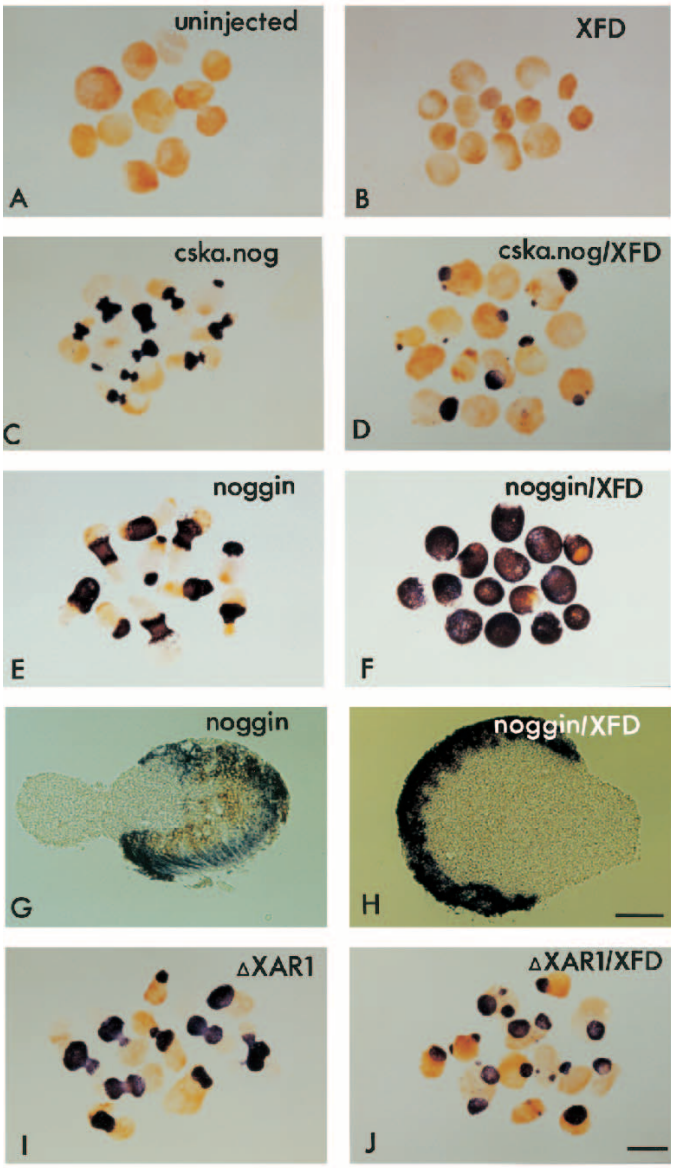

Fig. 8. Whole-mount in situ hybridization of the expression of *XCG-1* transcripts in animal cap explants derived from embryos injected with neuralizing factors and *XFD*. (A,B) Explants from uninjected- and *XFD*-injected embryos. No *XCG-1* labelling is detected. (C) Explants from *CSKA.noggin*-injected embryos. (D) Explants from embryos co-injected with *CSKA.noggin* and *XFD*. Small patches of *XCG-1*-positive cells are present. (E) Explants from embryos injected with *noggin* RNA. (F) Explants from embryos co-injected with *noggin* and *XFD* RNA. Notice that nearly the whole surface of these explants is *XCG-1*-positive. (G) Histological section cut after the chromogenic reaction from a *noggin*-injected explant. Notice the elongated cement gland cells. (H) Section cut from an explant corresponding to (F). The *XCG-1*-positive cells are not elongated. (I) Explants from embryos injected with *Δ1XAR1* RNA. (J) Explants from embryos co-injected with *Δ1XAR1* and *XFD* RNAs. The *XCG-1*-positive areas are reduced to small patches. Scale bars: (G,H), 100 μm; (A-F,I,J), 500 μm.

This conclusion is consistent with several lines of evidence showing that both FGF (*FGF-3*) and FGF receptors are activated by neural induction and are also expressed in the brain (Tannahill et al., 1992; Friesel and Brown, 1992; Shi et

al., 1994a,b; Launay et al., 1994). In addition, it has been shown that FGF-2 induces dissociated ectodermal cells from early gastrula to form neural tissues (Kengaku and Okamoto, 1993, 1995). Our results are also consistent with the observation that blocking FGF signalling inhibited activin-mediated neural tissue formation, but not *goosecoid* expression (Cornell and Kimelman, 1994). It is suggested that FGF may be required for the production of neural-inducing signal, or that it is required for neuroectodermal cells to respond to inducing signals. Since *XFD* inhibited neural induction mediated by different neuralizing factors (*noggin* and truncated activin receptor) that function through independent pathways, it is unlikely that these factors are acting through the FGF receptor; rather, *XFD* may affect events downstream from the action of *noggin* or *Δ1XAR1*. In addition, given that FGF is unable to induce neural tissues in animal cap explants (Green et al., 1990), it is likely that FGF signalling is involved in the response of ectodermal cells from gastrula to neural-inducing signals.

The inhibitory effect of *XFD* on neural induction is not mediated by blocking the formation of neural-inducing mesoderm because we have not detected early and late mesodermal markers in explants derived from different kinds of injection. *XFD* did not caudalize neural tissues induced by neuralizing factors either, since no spinal cord marker was expressed in these explants. In fact, blocking mesoderm-inducing signals in ectoderm will promote neuralization, as was illustrated using truncated activin receptor (Hemmati-Brivanlou and Melton, 1994; this work). Therefore, in the absence of mesoderm in our explants, the results demonstrate a direct requirement for FGF signalling in neural induction.

Previous work has shown that *XFD* produced trunk and posterior deficiencies with generally intact anterior neural tissues (Amaya et al., 1991; Isaacs et al., 1994). This result is not necessarily inconsistent with the present observation. Firstly, *XFD* produced dorsoanterior deficiencies (except cement gland) **only** in embryos that were completely dorsalized by *noggin*, probably because the effects of *XFD* are essentially targeted to the anteriorized tissues. Secondly, we can hypothesize that expression of *XFD* alone would preferentially disrupt the formation of trunk and posterior mesoderm, while the unaffected prechordal mesoderm could still induce anterior neural tissues. In this regard, targeting *XFD* RNA in blastomeres fated for neuroepithelium would more efficiently disrupt neural tissue formation. Indeed, we have found that injection of 1 ng *XFD* RNA into dorsovegetal blastomeres at the 8-cell stage resulted in trunk defects. However, if injected into dorso-animal blastomeres, more anterior deficiencies were obtained (Table 2). Thirdly, the inhibitory effect of *XFD* on neural induction by Spemann organizer and Hensen's node provides strong evidence of the involvement of FGF signalling in this aspect of *Xenopus* development.

A possible role of FGF signalling in the specification of anteroposterior neural axis

It has been shown that the future neuroectoderm is first induced to form cement gland at the early gastrula stage, while further induction by mesoderm inhibits its differentiation, except in the most anterior region of the embryo (Sive et al., 1989). Thus the anterior neuroectoderm is progressively induced during gastrulation (Blitz and Cho, 1995). We observed that *XFD* does not inhibit the expression of *XCG-1* in *noggin*-injected animal caps, but leads to poorly differentiated cement gland cells. In contrast, it inhibits strongly the expression of *XCG-1* in *CSKA.noggin*- and *Δ1XAR1*-injected caps. Because the neuralizing activities of *CSKA.noggin* and *noggin* are essentially similar, as judged by the induction of anterior neuroectodermal markers like *XCG-1* and *Otx-2* (see Fig. 4), but *CSKA.noggin* is not expressed before MBT, these observations suggest that the specification of anterior neuroectoderm by *noggin* can occur before gastrulation and does not require FGF signalling. However, the FGF signalling pathway participates in the specification of anterior neuroectoderm that takes place during gastrulation. In addition, a substantial amount of cells may be specified to an anterior neuroectodermal fate in a narrow window after MBT. This explains why the expression of *XCG-1* in *noggin/XFD* explants is unchanged.

In isolated animal caps, the activation of both *XIF-3* and *N-CAM* genes by *noggin* and *Δ1XAR1* was completely inhibited by *XFD*. However, *XFD* differentially inhibited the expression of *XCG-1* and *Otx-2*, depending essentially upon the time at which *noggin* is expressed. When *noggin* was injected as an RNA, *XFD* reduced the expression of *Otx-2* only slightly, while delaying the expression of *noggin* after MBT allowed a more efficient inhibition (see Fig. 4). Furthermore, we observed that *Δ1XAR1* exerts its neuralizing effect later than *noggin*, since it induces lower levels of *N-CAM* expression than *noggin* at neurula stages but maintains this expression more efficiently at late stages. Interestingly, *XFD* also significantly inhibited the expression of *Otx-2* and *XCG-1* in *Δ1XAR1*-injected explants. The cement gland represents the most anterior neuroectodermal tissues of the *Xenopus* embryo. At the early tailbud stage, the anterior border of *Otx-2* expression overlaps the cement gland anlage (Blitz and Cho, 1995). In addition, overexpression of *Otx-2* induces cement gland formation, indicating a connection between these two genes (Pannese et al., 1995; Blitz and Cho, 1995). In the present study we also observed a close correlation between *Otx-2* and *XCG-1*. Reduced expression of *XCG-1* is essentially observed in explants showing a more significant inhibition of *Otx-2* by *XFD* (see Fig. 4). Indeed, in *CSKA.noggin/XFD* and *Δ1XAR1/XFD* explants where the expression of *Otx-2* was significantly reduced, there was a strong inhibition of cement gland formation, as revealed both by RNase protection assays and by whole-mount in situ hybridization.

Furthermore, since FGF signalling is required for ectodermal cells of gastrula to respond to inducing signals, the anterior neuroectoderm will not be induced if the expression of *noggin* is delayed. As a consequence, we observed a more significant inhibition of *XCG-1* and *Otx-2* in *CSKA.noggin/XFD* explants. Delaying the expression of *noggin* also affects its neural-inducing activity in the presence of *XBra* (Cunliffe and Smith, 1994). We can postulate that the requirement for FGF signalling in neural induction is related to the zygotic activation of FGF and their receptors. In this regard, it is noteworthy that both FGF (Tannahill et al., 1992) and FGF receptors (Friesel and Brown, 1992; Shi et al., 1992, 1994b) are shown to be activated at the early gastrula stage. In addition, *XlPOU2* is activated very early (stage 10.5) by *noggin*, and it has a direct neuralizing activity (Witta et al., 1995). Therefore, it will be of interest to test if FGF signalling is required for the activation of this downstream gene.

FGF and the dorsalizing effect of *noggin* in whole embryos

Co-expression of *noggin* and *XFD* results in embryos that have no obvious dorso-anterior neural tissues such as neural tube and eyes. This result is consistent with observations showing that dominant negative FGF receptor inhibits expression of genes that are localized dorsally. For example, *XFD* inhibits the expression of *Xnot*, which is localized in the Spemann organizer (von Dassow et al., 1993), as well as notochord differentiation (Amaya et al., 1993). During mesoderm formation, FGF signalling is required for the expression and function of *XBra*, due to a regulatory loop (Isaacs et al., 1994; Schulte-Merker and Smith, 1995). *Noggin* dorsalizes the response of ectoderm to *XBra* by forming dorsal mesoderm (Cunliffe and Smith, 1994); however, *XFD* blocked the function of *XBra* such that no mesoderm is formed although expression of *N-CAM* was still detected (Schulte-Merker and Smith, 1995). Our result showing that *XFD* inhibited the dorsalizing effect of *noggin* in whole embryos is therefore consistent with this observation. The difference between the *N-CAM* expression level observed in previous work and that in the present report may be due to the amounts of *noggin* or *XFD* RNAs used. We observed that in animal cap explants, *XFD* inhibited *noggin*-induced expression of neural genes in a dose-dependent manner.

In embryos co-injected with *noggin* and *XFD* RNAs, no anterior neural tissues were formed. However, as in isolated animal caps, cement gland was always present. This further suggests that the induction of cement gland that takes place before gastrulation does not require FGF signalling. The aberrant distribution of cement gland may result from the inhibition of neural tissue formation. During development the induction of cement gland precedes that of anterior neural tissue. The interaction with mesoderm converts part of the cement gland cells to the neural state and restricts the formation of cement gland to the most anterior region of the embryo (Sive et al., 1989). Therefore, disturbing anterior neural tissue formation by *XFD* modifies the location of cement gland. Although further study is necessary to determine precisely the mechanism by which *XFD* inhibits neural induction, the present work provides direct evidence that FGF signalling plays a role in the patterning of the anteroposterior axis of the neuroectoderm.

We are grateful to Drs Richard Harland, Douglas Melton, Marc Kirschner, Colin Sharpe, Hazel Sive, Douglas W. DeSimone, Florence Broders, Jim C. Smith, Eddy DeRobertis and Giovanni Levi for providing us with the cDNAs and antibodies used in this work. We also acknowledge Dr Huguette Lovtrop for her advice on whole-mount in situ hybridization, and Drs Jim C. Smith, Douglas W. DeSimone and Claudio Stern for helpful discussions during this work. We wish to thank Drs Michel Delarue and Jean-Loup Duband for teaching us the dissection of Hensen's node, and our colleagues for their encouragement throughout this work. We thank Drs Jim C. Smith and Muriel Umbhauer for reading the manuscript and for suggestions. This work was supported by grants from CNRS, AFM and ARC.

REFERENCES

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

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

Blitz, I. L. and Cho, K. W. Y. (1995). Anterior neuroectoderm is progressively induced during gastrulation: the role of the *Xenopus* homeobox gene *orthodenticle*. *Development* **121**, 993-1004.

Bradley, L., Snape, A., Bhatt, S. and Wilkinson, D. (1993). The structure and expression of the *Xenopus Krox-20* gene: conserved and divergent patterns of expression in rhombomeres and neural crest. *Mech. Dev.* **40**, 73-84.

Cho, K. W. Y., Morita, E. A., Wright, C. V. E. and De Robertis, E. M. (1991a). Overexpression of a homeodomain protein confers axis-forming activity to uncommitted *Xenopus* embryonic cells. *Cell* **65**, 55-64.

Cho, K. W. Y., Morita, E. A., Wright, C. V. E. and De Robertis, E. M. (1991b). Molecular nature of Spemann's organizer: the role of the *Xenopus* homeobox gene *goosecoid*. *Cell* **67**, 1111-1120

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

Cunliffe, V. and Smith, J. C. (1994). Specification of mesodermal pattern in *Xenopus laevis* by interactions between *Brachyury*, *noggin* and *Xwnt-8. EMBO J.* **13**, 349-359.

Friesel, R. and Brown, S. (1992). Spatially restricted expression of fibroblast growth factor receptor-2 during *Xenopus* development. *Development* **116**, 1051-1058.

Green, J. B. A., Howes, G., Symes, K., Cook, J. and Smith, J. C. (1990). The biological effects of XTC-MIF: quantitative comparison with *Xenopus* bFGF. *Development* **108**, 173-183.

Harland, R. M. (1991). In situ hybridization: an improved whole mount method for *Xenopus* embryos. *Meth. Cell Biol.* **36**, 685-695.

Hemmati-Brivanlou, A. and Harland, R. M. (1989). Expression of an *engrailed*-related protein is induced in the anterior neural ectoderm of early *Xenopus* embryos. *Development* **106**, 611-617.

Hemmati-Brivanlou, A., de la Torre, J. R., Holt, C., and Harland, R. M. (1991). Cephalic expression and molecular characterization of *Xenopus En-2. Development* **111**, 715-724.

Hemmati-Brivanlou, A. and Melton, D. A. (1992). A truncated activin receptor dominantly inhibits mesoderm induction and formation of axial structures in *Xenopus* embryos. *Nature* **359**, 609-614.

Hemmati-Brivanlou, A. and Melton, D. A. (1994). Inhibition of activin receptor signaling promotes neuralization in *Xenopus. Cell* **77**, 273-281.

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.

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

Kengaku, M. and Okamoto, H. (1993). Basic fibroblast growth factor induces differentiation of neural tube and neural crest lineages of cultured ectoderm cells from *Xenopus* gastrula. *Development* **119**, 1067-1078.

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.

Kintner, C. R. and Melton, D. A. (1987). Expression of *Xenopus* N-CAM RNA in ectoderm is an early response to neural induction. *Development* **99**, 311-325.

Kintner, C. R. and Dodd, J. (1991). Hensen's node induces neural tissue in *Xenopus* ectoderm. Implications for the action of the organizer in neural induction. *Development* **113**, 1495-1505.

Knecht, A. K., Good, P. J., Dawid, I. B. and Harland, R. M. (1995). Dorsal-ventral patterning and differentiation of noggin-induced neural tissue in the absence of mesoderm. *Development* **121**, 1927-1936.

Lamb, T. M., Knecht, A. K., Smith, W. C., Stachel, S. E., Economides, A. N., Stahl, N., Yancopolous, G. D. and Harland, R. M. (1993). Neural induction by the secreted polypeptide noggin. *Science* **262**, 713-718.

Launay, C., Fromentoux, V., Thery, C., Shi, D. L. and Boucaut, J. C. (1994). Comparative analysis of the tissue distribution of three fibroblast growth factor receptor mRNAs during amphibian morphogenesis. *Differentiation* **58**, 101-111.

Levi, G., Broders, F., Dunon, D., Edelman, G. and Thiery, J. (1990). Thyroxine-dependent modulations of the expression of the neural cell adhesion molecule N-CAM during *Xenopus laevis* metamorphosis. *Development* **109**, 681-692.

Nieuwkoop, P. D. (1952). Activation and organization of the central nervous

system in amphibians. Part III. Synthesis of a new working hypothesis. *J. Exp. Zool.* **120**, 83-108.

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

Pannese, M., Polo, C., Andreazzoli, M., Vignali, R., Kablar, B., Barsacchi, G. and Boncinelli, E. (1995). The *Xenopus* homologue of *Otx-2* is a maternal homeobox gene that demarcates and specifies anterior body regions. *Development* **121**, 707-720.

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

Sharpe, C. R. and Gurdon, J. B. (1990). The induction of anterior and posterior neural genes in *Xenopus laevis*. *Development* **109**, 765-774.

Shi, D. L., Feige, J. J., Riou, J. F., Desimone, D. W. and Boucaut, J. C. (1992). Differential expression and regulation of two distinct fibroblast growth factor receptors during early development of the urodele amphibian *Pleurodeles waltl*. *Development* **116**, 261-273.

Shi, D. L., Fromentoux, V., Launay, C., Umbhauer, M. and Boucaut, J. C. (1994a). Isolation and developmental expression of the amphibian homolog of the fibroblast growth factor receptor-3. *J. Cell Sci.* **107**, 417-425.

Shi, D. L., Launay, C., Fromentoux, V., Feige, J. J. and Boucaut, J. C. (1994b). Expression of fibroblast growth factor receptor-2 splice variants is developmentally and tissue-specifically regulated in the amphibian embryo. *Dev. Biol.* **164**, 173-182.

Sive, H., Hattori, K. and Weintraub, H. (1989). Progressive determination during formation of the anteroposterior axis in *Xenopus laevis*. *Cell* **58**, 171-180.

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

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.

Smith, W. C., Knecht, A. K., Wu, M. and Harland, R. M. (1993). Secreted *noggin* protein mimics the Spemann organizer in dorsalizing mesoderm. *Nature* **361**, 547-549.

Spemann, H. and Mangold, H. (1924). Über Induction von Embryonalanlagen durch Implantation Artfremder Organisatoren. *Roux' Arch. Entw. Mech. Org.* **100**, 599-638.

Tannahill, D. Isaacs, H. V. 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.

von Dassow, G., Schmidt, J. E. and Kimelman, D. (1993). Induction of the *Xenopus* organizer: expression and regulation of *Xnot*, a novel FGF and activin-regulated homeo box gene. *Genes Dev.* **7**, 355-366.

Witta, S. E., Agarwal, V. R. and Sato, S. M. (1995). *XlPOU2*, a noggin-inducible gene, has direct neuralizing activity. *Development* **121**, 721-730.

Wright, C. V., Morita, E. A., Wilkin, D. J. and De Robertis, E. M. (1990). The *Xenopus* XlHbox 6 homeo protein, a marker of posterior neural induction, is expressed in proliferating neurons. *Development* **109**, 225-223.

Yamada, T. (1990). Regulations in the induction of the organized neural system in amphibian embryos. *Development* **110**, 653-659.

(Accepted 30 November 1995)