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FGF-8 stimulates neuronal differentiation through FGFR-4a and interferes with mesoderm induction in *Xenopus* embryos

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The role of fibroblast growth factors (FGFs) in neural induction is controversial [1,2]. Although FGF signalling has been implicated in early neural induction [3–5], a late role for FGFs in neural development is not well established. Indeed, it is thought that FGFs induce a precursor cell fate but are not able to induce neuronal differentiation or late neural markers [6–8]. It is also not known whether the same or distinct FGFs and FGF receptors (FGFRs) mediate the effects on mesoderm and neural development. We report that *Xenopus* embryos expressing ectopic FGF-8 develop an abundance of ectopic neurons that extend to the ventral, non-neural, ectoderm, but show no ectopic or enhanced notochord or somitic markers. FGF-8 inhibited the expression of an early mesoderm marker, *Xbra*, in contrast to eFGF, which induced ectopic *Xbra* robustly and neuronal differentiation weakly. The effect of FGF-8 on neurogenesis was blocked by dominant-negative FGFR-4a (ΔXFGFR-4a). Endogenous neurogenesis was also blocked by ΔXFGFR-4a and less efficiently by dominant-negative FGFR-1 (XFD), suggesting that it depends preferentially on signalling through FGFR-4a. The results suggest that FGF-8 and FGFR-4a signalling promotes neurogenesis and, unlike other FGFs, FGF-8 interferes with mesoderm induction. Thus, different FGFs show specificity for mesoderm induction versus neurogenesis and this may be mediated, at least in part, by the use of distinct receptors.

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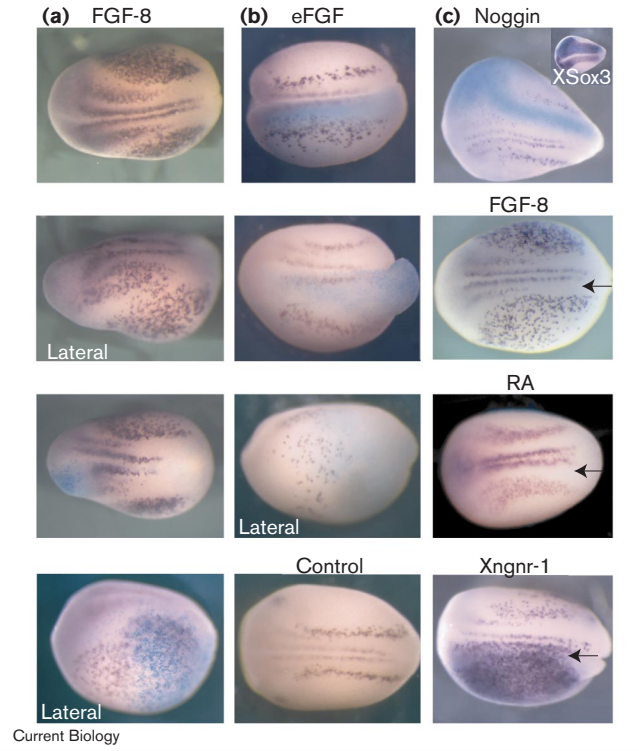
Results and discussion

We injected *FGF-8* RNA (90–270 pg) into one cell of the two-cell stage embryo and assayed for the expression of an early neuronal marker, *N-tubulin* (Figure 1a). Embryos gastrulated normally and the gross morphology of the neurula stage was normal. At the high dose range, there was a narrowing of the anterior neural plate and an enlargement of the proctodeum (Figure 1a), reported previously for *FGF-8* [9] and *eFGF* overexpression [10], respectively. Despite the near normal overall morphology, embryos injected with *FGF-8* RNA showed abundant ectopic *N-tubulin* (Figure 1a; 90%, $n = 89$). The ectopic *N-tubulin* was always more widespread than the coinjected cell-autonomous lineage tracer *lacZ*, as would be predicted if FGF-8 could diffuse from the site of injection (Figure 1a). Indeed, subsequent experiments showed that the cell non-autonomous signal is likely to be FGF-8 itself (see below). In *FGF-8*-injected embryos, ectopic *N-tubulin* spread well into the ventral epidermis but did not enter the anterior or extreme posterior end of the embryo even

Figure 1



(a) Embryos injected with FGF-8/LacZ RNA show abundant ectopic neurogenesis. **(b)** Compared with FGF-8, eFGF RNA had a very weak effect on neurogenesis. Although some ectopic N-tubulin formed, the percentage of the affected embryos was lower (see text) and the phenotype less severe. The posterior elongation seen in the second eFGF panel was observed in approximately 50% of the high dose eFGF RNA injected embryos ($n = 25$) and was not seen in FGF-8 RNA-injected embryos. **(c)** Comparison of FGF-8 activity to that of noggin, RA, and X-ngnr1 (see text). Black arrows point to the interstripe region. All panels show expression of N-tubulin with the exception of the inset (XSox3). In all panels anterior is to the left and most panels show dorsal views, lateral views are indicated.

when *FGF-8* RNA was localised there (Figure 1a and data not shown).

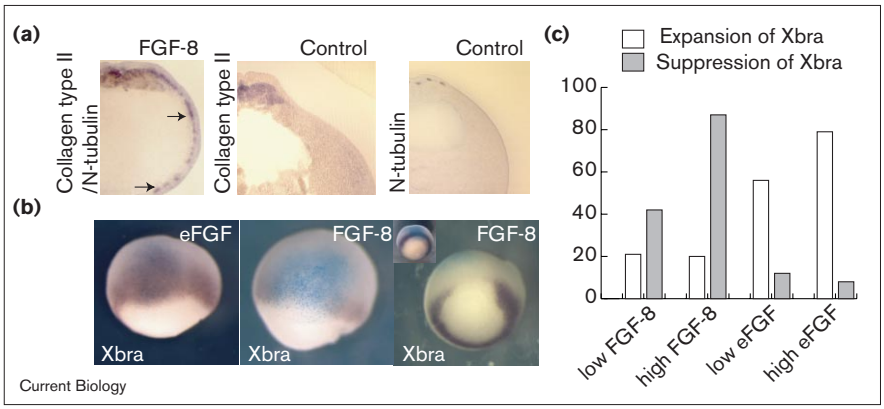
We next determined whether any other early neural inducer would have a similar effect in neurogenesis. Noggin is a bone morphogenetic protein (BMP) antagonist, with dorsalising and neuralising activities in the frog and neural-maintaining properties in the chick (reviewed in [2]). As expected, *noggin* RNA-injected embryos showed ectopic neural tissue formation expressing *XSox3*, a marker of neural precursor cells (Figure 1c, inset). This was either continuous with the host neural tissue or associated with a secondary axis, depending on whether the *noggin* RNA was targeted to the dorsal or ventral side of the embryo, respectively. However, in either case, there was no increased *N-tubulin* associated with the increased neural tissue, in contrast to the *FGF-8*-injected embryos (compare Figure 1c with Figure 1a). We suggest that FGF-8 has an additional role over that of other, BMP-antagonising, neural inducers, which initiate neural induction but do not promote subsequent neuronal differentiation. Our findings do not exclude an early role for FGF-8 in neural induction that may involve BMP inhibition.

FGF-8 shares, with retinoic acid (RA), the ability to posteriorise the embryo and, with neuronal determination factors, such as *neurogenin* (*X-ngnr-1*), the ability to promote ectopic neurogenesis. However, the neuronal-inducing activity of *FGF-8* differs from both of these molecules. In the neural plate stage embryo, *N-tubulin* expression appears in a stereotypical pattern of three stripes on either side of the axial midline of the posterior neural plate and is excluded from the anterior neural plate (Figure 1b, control). Unlike *FGF-8*, RA treatment results in ectopic *N-tubulin* in the anterior end of the embryo but not in the ventral epidermis (Figure 1c and [11]). Unlike *FGF-8*, *X-ngnr-1* induces *N-tubulin* in the region between the normal stripes of *N-tubulin* expression, as well as in the anterior neural plate (Figure 1c and [12]). Thus, the role of FGF-8 signalling may be limited to promoting *N-tubulin* expression to the dorsal, as opposed to the lateral and ventral sides of the embryo. The *FGF-8* phenotype is very similar to that obtained with overexpression of a neuronal inducing and patterning factor, *XBF-1* [13]. However, no ectopic *XBF-1* was induced by *FGF-8* in the posterior neural plate (data not shown).

Neuronal differentiation is promoted by a signal from the axial and/or paraxial mesoderm [14,15]. Several members of the FGF family are potent mesoderm inducers; therefore, any effects on neurogenesis could represent an indirect effect through re-specification of the mesoderm. To test this, we performed animal pole injections of *eFGF* RNA, a potent mesoderm inducer most related to FGF4 and 6 [10,16]. We found that *eFGF* was much less potent than *FGF-8* in inducing ectopic *N-tubulin* (Figure 1b). For example, at 90 pg injected RNA, *FGF-8* expanded and induced ectopic neurogenesis in 90% of the embryos (n = 40) whereas *eFGF* had a similar effect in only 51% of the embryos (n = 54). Importantly, this percentage was not significantly affected by a threefold increase in the quantity of *eFGF* RNA (45%, n = 26). Although more difficult to quantitate, *eFGF* was also less effective when judged by the range of its action as well as by the number of ectopic *N-tubulin* cells formed (for example, compare Figure 1a with Figure 1b).

To exclude further an indirect effect of FGF-8 on neurogenesis through expansion of the inducing mesoderm, we examined the expression of *collagen type II*, a marker of

Figure 2



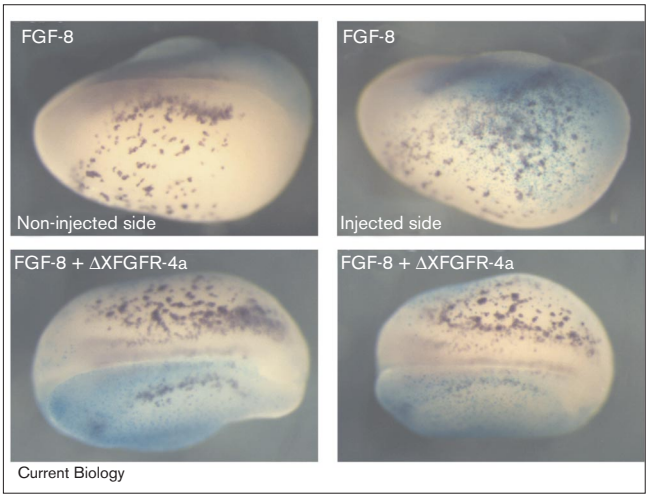
(a) FGF-8 induces ectopic *N-tubulin* without inducing ectopic *collagen type II*. Control and *FGF-8* RNA-injected embryos were hybridised with probes to *collagen type II* and *N-tubulin* (doubly or singly, as indicated) and sectioned. In all embryos, ectopic *N-tubulin* (black arrow) was not underlain by ectopic *collagen type II*. **(b)** Qualitatively different effect of eFGF and FGF-8 on *Xbra* expression. Injection of *eFGF/lacZ* RNA expanded *Xbra* whereas injection of *FGF-8/lacZ* RNA suppressed *Xbra*. Control *lacZ* injections had no effect on *Xbra* (inset). **(c)** Suppression of *Xbra* by FGF-8 and induction of *Xbra* by eFGF is dose dependent. A representative experiment is shown. The concentration difference between the low and high dose was threefold for both FGF-8 and eFGF. Expansion of *Xbra* in response to FGF-8 differed from that obtained with eFGF in that it was weaker in intensity and did not extend to the animal pole. In some *FGF-8* RNA-injected embryos, both suppression and expansion of *Xbra* was observed in the same embryo. Low FGF-8, n = 14; high FGF-8, n = 15; low eFGF, n = 16; high eFGF, n = 24.

notochord and somites, in *FGF-8*-injected embryos. Double staining with *collagen type II* and *N-tubulin* showed that ectopic *N-tubulin* (73%, $n = 11$) did not correlate with ectopic axial or paraxial mesoderm (0%, $n = 11$). Instead, the expression of *collagen type II* was normal both in its spatial distribution and level of expression in the majority of the embryos (74%, $n = 11$; Figure 2a). To exclude an earlier expansion of prospective mesoderm, we examined the expression of the early mesodermal marker *Xbra* in the gastrula. Injection of *FGF-8* RNA caused a very weak expansion of *Xbra* in a minority of cases (see Figure 2c). This is consistent with findings in other assays where FGF-8 has been shown to induce mesoderm very weakly (for example, in animal caps [9]) or not at all (for example, in chick epiblast [8]).

In some *FGF-8*-injected embryos, expression of *collagen type II* was reduced on the injected side (26%, $n = 11$). In addition, at the high end of the dose range, there was a significant incidence of spina bifida (41%, $n = 65$), characteristic of interference with posterior mesoderm development [17]. Consistent with the effect of FGF-8 on *collagen type II*, injection of *FGF-8* suppressed *Xbra* expression in gastrula embryos (Figure 2b) in a dose-dependent manner (48%, $n = 44$ at 90 pg; 93%, $n = 45$ at 270 pg). In contrast, there was no significant suppression of *Xbra* with eFGF at either low or high concentration (a representative experiment is shown in Figure 2c). Instead, *eFGF* was very efficient in inducing enhanced and ectopic *Xbra*, as expected (Figure 2b,c; [16]). Thus, not only is FGF-8 a poor mesoderm inducer but it appears to interfere with the normal process of mesoderm induction. This finding was surprising as all FGFs tested so far have had a positive role on mesoderm induction (reviewed in [18]). Taken together, these results demonstrate that the observed effect of increased and ectopic neurogenesis appears to be independent of an expansion of the inducing mesoderm and, therefore, likely to reflect a direct effect on the ectoderm. Our results also reveal a qualitative difference between the actions of FGF-8 and eFGF, with FGF-8 acting as a potent neuronal inducer but a poor mesodermal inducer whereas eFGF acts as a poor neuronal inducer but a potent mesodermal inducer.

Several types of FGFRs have been identified in vertebrates but the specificity of their biological effects is not well understood (reviewed in [19]). Because these receptors act as dimers, deleting the intracellular signaling domain has been an efficient way to create dominant-negative versions [17]. FGF-8 was found to have a high affinity for FGFR-4 in a cell culture mitogenic assay [20]. Therefore, we determined whether ΔXFGFR-4a would block the effects of FGF-8 on neurogenesis; *ΔXFGFR-4a* RNA was coinjected with *FGF-8*, and *lacZ* RNA as a lineage tracer. The effect of FGF-8 on neurogenesis could be efficiently blocked by ΔXFGFR-4a (75% in a 1:4 FGF-8: ΔX FGFR-4a ratio, $n = 16$; 86% in a 1:6 ratio, $n = 78$). The rescue was cell autonomous as would be predicted because ΔXFGFR-4a contains the membrane-spanning domain of the receptor (Figure 3). Interestingly, areas of the ectoderm that had not inherited *ΔXFGFR-4a* RNA (shown as non-*lacZ*-stained areas) continued to show increased *N-tubulin* expression, demonstrating that they responded to FGF-8 rather than a secondary signal produced by the FGF-8-expressing cells (Figure 3).

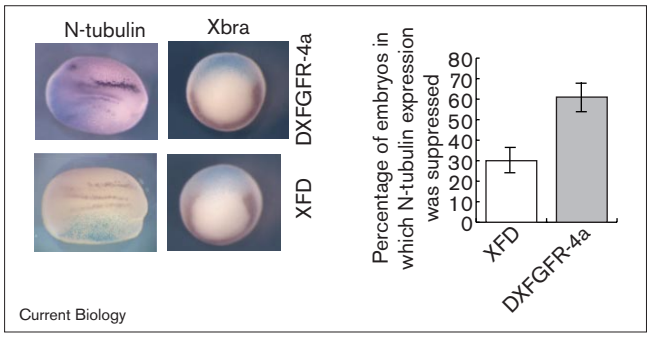
Figure 3



Blocking signalling through FGFR-4a with ΔXFGFR-4a rescues the effect of FGF-8 on neurogenesis. Embryos were injected unilaterally at the two-cell stage with *FGF-8* or *FGF-8* plus *ΔXFGFR-4a* RNA. All were coinjected with *lacZ* and hybridised with *N-tubulin*. Top panels show side views of the same embryo; lower panels show a dorsal view of two embryos. When *FGF-8* was coinjected with *ΔXFGFR-4a*, ectopic *N-tubulin* was abolished and endogenous *N-tubulin* was reduced. Note that abundant ectopic neurogenesis was still observed on the uninjected side, suggesting that it responds directly to FGF-8.

Recently, ΔXFGFR-4a has been shown to work better than dominant-negative FGFR-1 (XFD) in blocking the expression of pan-neural and regionalised neural markers specifically in the anterior neural plate [5]. However, the effect of ΔXFGFR-4a on neuronal differentiation in the posterior neural plate has not been examined. We found that *ΔXFGFR-4a* was very effective in blocking endogenous *N-tubulin* (Figure 4, $n = 136$). *XFD* was, on average, half as effective in blocking the expression of endogenous *N-tubulin* (Figure 4, $n = 193$). This could be explained if FGF-8 signals preferentially through FGFR-4a and to a lesser extent through FGFR-1, consistent with the results in a mitogenic assay [20]. In contrast, *XFD* was very effective in blocking the expression of *Xbra*, as previously reported (100%, $n = 51$) [21]. We found that *ΔXFGFR-4a* was also very efficient in blocking *Xbra* expression (100%, $n = 41$; Figure 4).

Figure 4



Both *ΔXFGFR-4a* (90 pg) and *XFD* (140 pg) were very effective in blocking *Xbra* expression (100%, $n = 51$ for *XFD*; $n = 41$ for *ΔXFGFR-4a*). Using the same concentrations, *ΔXFGFR-4a* was more effective in blocking *N-tubulin* expression. Measurements of *N-tubulin* suppression represent the average percentage of seven experiments. XFD, $n = 193$; ΔXFGFR-4a, $n = 136$.

In conclusion, our results suggest a novel role of FGF-8 in regulating a late step of neural induction in *Xenopus*, such as neuronal differentiation. An indication that FGFs may have a role in neuronal differentiation during development in other species also comes from the recent observation that, in zebrafish FGF-8 mutants (Ace), certain forebrain neuronal clusters are missing [22], and from data in the mouse [23]. Our results show that FGF-8 and eFGF exert a positive inductive effect preferentially on neurogenesis and mesoderm formation, respectively. Our results also suggest that the specificity is due, at least in part, to the use of different types of FGF receptor, with FGFR-4a preferentially mediating the effects of FGF(s) on neuronal differentiation. FGF-8 is expressed in the early neurula in two domains located in the anterior and posterior ends of the embryo [9]. In our experiments, FGF-8 has been able to exert a long-range effect, which our results suggest are mediated by FGF-8 itself. The *Xenopus* FGFR-4a was recently shown to be expressed widely in the early nervous system [5]. Therefore, both receptor and ligand are in the right place at the right time to affect neuronal differentiation in the developing embryo.

Materials and methods

XFD is described in [17] and ΔXFGFR-4a in [5]. *XeFGF* was cloned in pSP64T by E. Amaya. The FGF-8 used here is described in [9] and corresponds to 'variant 1', the same as the FGF-8b isoform of mammalian FGF-8.

Supplementary material

Supplementary material including additional methodological detail is available at http://current-biology.com/supmat/supmatin.htm.

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References

1. Mason I: **Neural induction: do fibroblast growth factors strike a cord?** *Curr Biol* 1996, **6:**672-675.
2. Streit A, Stern CD: **Neural induction. A bird's eye view.** *Trends Genet* 1999, **15:**20-24.
3. Streit A, Berliner AJ, Papanayotou Sirulnik A, Stern CD: **Initiation of neural induction by FGF signalling before gastrulation.** *Nature* 2000, **406:**74-77.
4. Wilson SI, Graziano E, Harland R, Jessell T, Edlund T: **An early requirement for FGF signalling in the acquisition of neural cell fate in the chick embryo.** *Curr Biol* 2000, **10:**421-429.
5. Hongo I, Kengaku M, Okamoto H: **FGF signaling and the anterior neural induction in *Xenopus*.** *Dev Biol* 1999, **216:**561-581.
6. Storey KG, Goriely A, Sargent CM, Brown JM, Burns HD, Abud HM, Heath JK: **Early posterior neural tissue is induced by FGF in the chick embryo.** *Development* 1998, **125:**473-484.
7. Henrique D, Tyler D, Kintner C, Heath JK, Lewis JH, Ish-Horowicz D, Storey KG: ***cash4*, a novel *achaete-scute* homolog induced by Hensen's node during generation of the posterior nervous system.** *Genes Dev* 1997, **11:**603-615.
8. Streit A, Stern CD: **Establishment and maintenance of the border of the neural plate in the chick: involvement of FGF and BMP activity.** *Mech Dev* 1999, **82:**51-66.
9. Christen B, Slack JMW: **FGF-8 is associated with anteroposterior patterning and limb regeneration in *Xenopus*.** *Dev Biol* 1997, **192:**455-466.
10. Isaacs HV, Tannahill D, Slack JMW: **Expression of a novel FGF in the *Xenopus* embryo: a new candidate inducing factor for mesoderm formation and anteroposterior specification.** *Development* 1992, **114:**711-720.
11. Papalopulu N, Kintner C: **A posteriorising factor, retinoic acid, reveals that the anteroposterior patterning controls the timing of neuronal differentiation in *Xenopus* neuroectoderm.** *Development* 1996, **122:**3409-3418.
12. Ma Q, Kintner C, Anderson DJ: **Identification of neurogenin, a vertebrate neuronal differentiation gene.** *Cell* 1996, **87:**43-52.
13. Bourguignon C, Li J, Papalopulu N: ***XBF-1*, a winged helix transcription factor with dual activity, has a role in positioning neurogenesis in *Xenopus* competent ectoderm.** *Development* 1998, **125:**4889-4900.
14. Lamb TM, Knecht AK, Smith WC, Stachel SE, Economides AN, Stahl N, *et al.*: **Neural induction by the secreted polypeptide noggin.** *Science* 1993, **262:**713-718.
15. Bang AG, Papalopulu N, Goulding MD, Kintner C: **Expression of Pax-3 in the lateral neural plate is dependent on a Wnt-mediated signal from posterior nonaxial mesoderm.** *Dev Biol* 1999, **212:**366-380.
16. Isaacs HV, Pownall ME, Slack JMW: **eFGF regulates *Xbra* expression during *Xenopus* gastrulation.** *EMBO J* 1994, **13:**4469-4481.
17. Amaya E, Musci T, Kirschner MW: **Expression of a dominant negative mutant of the FGF receptor disrupts mesoderm formation in *Xenopus* embryos.** *Cell* 1991, **66:**257-270.
18. Isaacs HV: **New perspectives on the role of the fibroblast growth factor family in amphibian development.** *Cell Mol Life Sci* 1997, **53:**350-361.
19. Johnson DE, Williams LT: **Structural and functional diversity in the FGF receptor multigene family.** *Adv Cancer Res* 1993, **60:**1-41
20. Ornitz DM, Xu J, Colvin JS, McEwen DG, MacArthur CA, Coulier F, *et al.*: **Receptor specificity of the fibroblast growth factor family.** *J Biol Chem* 1996, **271:**15292-15297.
21. Amaya E, Stein PA, Musci TJ, Kirschner MW: **FGF signalling in the early specification of mesoderm in *Xenopus*.** *Development* 1993, **118:**477-487
22. Shanmugalingam S, Houart C, Picker A, Reifers F, Macdonald R, Barth A, *et al.*: **Ace/FGF8 is required for forebrain commisure formation and patterning of the telencephalon.** *Development* 2000, **127:**2549-2561.
23. Ye W, Shimamura K, Rubenstein JLR, Hynes MA, Rosenthal A: **FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate.** *Cell* 1998, **93:**755-766.