Available online at www.sciencedirect.com

SCIENCE DIRECT

Developmental Biology 279 (2005) 1–19

DEVELOPMENTAL BIOLOGY

www.elsevier.com/locate/ydbio

# Chordin, FGF signaling, and mesodermal factors cooperate in zebrafish neural induction

Eric R. Londin[a,b], Jack Niemiec[a], Howard I. Sirotkin[a,*]

[a]*Department of Neurobiology and Behavior, Stony Brook University, Stony Brook, NY 11794, USA*
[b]*Graduate Program in Genetics, Stony Brook University, Stony Brook, NY 11794, USA*

Received for publication 16 July 2004, revised 4 November 2004, accepted 9 November 2004
Available online 24 December 2004

**Abstract**

The ectoderm gives rise to both neural tissue and epidermis. In vertebrates, specification of the neural plate requires repression of bone morphogenetic protein (BMP) signaling in the dorsal ectoderm. The extracellular BMP antagonist Chordin and other signals from the dorsal mesoderm play important roles in this process. We utilized zebrafish mutant combinations that disrupt Chordin and mesoderm formation to reveal additional signals that contribute to the establishment of the neural domain. We demonstrate that fibroblast growth factor (FGF) signaling accounts for the additional activity in neural specification. Impeding FGF signaling results in a shift of ectodermal markers from neural to epidermal. However, following inhibition of FGF signaling, expression of anterior neural markers recovers in a Nodal-dependent fashion. Simultaneously blocking, Chordin, mesoderm formation, and FGF signaling eliminates neural marker expression during gastrula stages. We observed that FGF signaling is required for *chordin* expression but that it also acts via other mechanisms to repress BMP transcription during late blastula stages. Activation of FGF signaling was also able to repress BMP transcription in the absence of protein synthesis. Our results support a model in which specification of anterior neural tissue requires early FGF-mediated repression of BMP transcript levels and later activities of Chordin and mesodermal factors.
© 2004 Elsevier Inc. All rights reserved.

*Keywords:* Neural induction; FGF; Nodal; Chordin; BMP; Zebrafish

## Introduction

During embryogenesis, the neural plate, which will give rise to the entire central nervous system encompassing the brain and spinal cord, is specified within the ectoderm. The longstanding model for neural induction and initial polarization of the neural plate is the activator-transformer model first proposed by Nieukoop (1952). In this model, an activator signal distinguishes the neural ectoderm from nonneural ectoderm (epidermis). Initially, the neural ectoderm is anterior in character and is subsequently patterned by a transformation step to generate posterior fates. This model predicts that neural and nonneural ectoderms are in equilibrium; therefore, promotion of neural fates should come at the expense of epidermal fates. Conversely, impeding neural induction should expand the epidermal domain.

In classic experiments, Spemann (1924) and Mangold observed that transplantation of the dorsal mesoderm (termed the organizer) to a ventral location induced a complete secondary axis including a well-patterned neural tube. Elegant experiments in *Xenopus laevis* identified molecules expressed in the dorsal mesoderm that have potent neural inductive activity. Prominent among these molecules are *chordin* and *noggin* (Sasai et al., 1995; Smith and Harland, 1992). In vertebrates, neural induction occurs in a dorsal sector of the embryo where bone morphogenetic protein (BMP) signaling has been repressed (Hemmati-Brivanlou and Melton, 1997). Chordin and Noggin inhibit BMP signaling by binding extracellular BMP ligands and

* Corresponding author. Fax: +1 631 632 6661.
*E-mail address:* howard.Sirotkin@sunysb.edu (H.I. Sirotkin).

0012-1606/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2004.11.016

interfering with receptor activation (Piccolo et al., 1996; Zimmerman et al., 1996).

The activities of the organizer and extracellular BMP antagonists are not entirely linked. In zebrafish, dorsal cells with neural inductive activity reside outside the morphological boundaries of the organizer (Grinblat et al., 1998; Saude et al., 2000) and *chordin* expression also stretches beyond the organizer (Miller-Bertoglio et al., 1997). In the chick, the node is able to act as a neural inducer prior to the expression of known BMP antagonists (Streit et al., 1998). While experiments to knockdown Chordin function in *Xenopus* also support the notion that Chordin is an essential component of the neuralizing activity of the organizer (Oelgeschlager et al., 2003), recent experiments also suggest that Chordin is required outside of the organizer for specification of some anterior neural fates (Kuroda et al., 2004).

Despite the demonstration that both the organizer and the extracellular BMP antagonists are sufficient to induce neural tissue in a variety of assays, genetic evidence suggests that neural induction occurs in the absence of the organizer or extracellular BMP antagonists. Mouse and zebrafish mutants that lack the organizer still undergo neuralization (Ang and Rossant, 1994; Feldman et al., 1998; Gritsman et al., 1999; Klingensmith et al., 1999). Zebrafish mutants lacking the organizer still maintain dorsal expression of *chordin* (Gritsman et al., 1999; Sirotkin et al., 2000). In these mutants, specification of neural tissue may result from extracellular antagonism of BMP signaling by Chordin. The Chordin locus is disrupted in zebrafish *dino* mutants that have reductions in anterior neural tissues. Likewise, mice that are double mutant for *chordin* and *noggin* have anterior neural truncations (Bachiller et al., 2000; Schulte-Merker et al., 1997). Analysis of these mutants suggests that antagonism of BMP signaling by Chordin and related molecules is one key mechanism of neural induction but that additional signaling events play important roles.

One additional class of molecules that has been implicated in neural induction is the fibroblast growth factors (FGFs). Their role in neural induction has been controversial, and experiments in different model systems have indicated that there may be species-specific mechanisms of neural induction. Manipulations of chick embryos suggest a role for FGF in neural induction (Streit et al., 2000; Wilson et al., 2000, 2001) and that FGFs may attenuate BMP signaling by repressing the transcription of BMP4 and BMP7 (Wilson et al., 2000).

However, overexpression of a dominant-negative FGF receptor1 (XFD) or an inhibitory *ras* construct in zebrafish and frog embryos does not prevent formation of anterior neural structures (Amaya et al., 1991; Griffin et al., 1995; Ribisi et al., 2000). These embryos lack all posterior tissue, including spinal cord, but contain hindbrain and anterior neural structures. Furthermore, isolated XFD-expressing cells are capable of becoming spinal cord (Kroll and Amaya, 1996; Ribisi et al., 2000). Together, these results suggest that FGF signaling is not required for neural induction. However, Hongo et al. (1999) demonstrated that in an in vitro culture system blocking FGF signaling with Δ-FGFR-4 or to a lesser extent XFD inhibits neural induction by the organizer and blocks autonomous neuralization of cultured disassociated ectodermal cells. In those experiments, whole embryos injected with Δ-FGFR-4 mRNA still generated anterior neural tissue at late stages. One mechanism by which FGF may act as a neural inducer was suggested by the observation that BMP signaling can be quashed by FGF-mediated phosphorylation of the Smad1 linker region at consensus MAP–ERK kinase phosphorylation sites (Pera et al., 2003).

In this study, we show that in zebrafish, neural tissue is induced as a result of the combined activities of FGF signaling, Chordin and Nodal downstream targets. Inhibition of FGF signaling in wild-type embryos results in early deficits in neural specification and expansion of nonneural ectoderm. However, the anterior neural domain later recovers in a Nodal-dependent fashion following FGF inhibition. Our results demonstrate that FGF acts to diminish BMP transcript levels prior to the start of gastrulation. We show that while FGF induces expression of *chordin* transcripts, it also represses BMP transcript levels by a translation-independent mechanism. Together, these findings suggest that FGF acts at multiple levels to repress BMP signaling and define the neural territory.

## Materials and methods

### *Zebrafish stocks and embryo maintenance*

Adult zebrafish stocks were maintained at 28.5°C. Embryos were produced by natural matings of appropriate adult fish, collected and stored at 28.5°C in embryo medium until desired stage according to Kimmel et al. (1995). The following mutant alleles were used in this study: *dino*$^{tt250}$, *ntl*$^{b160}$, Mz*oep*$^{tz57}$, as well as TL wild-type fish.

### *Pharmacologic treatments*

FGF signaling was pharmacologically inhibited by placing whole embryos of the appropriate stage into 60 μM SU5402 (Calbiochem, La Jolla, CA.). The inducible FGF receptor 1 (iFGFR-1) construct was activated at the appropriate stage with 1.25 μM AP20187 (ARIAD Pharmaceuticals, www.ariad.com/regulationkits). Embryos were left in AP20187 and allowed to develop to the appropriate stage. The iFGFR-1 construct did not have an effect unless embryos were placed into AP20187. Protein synthesis was inhibited by placing embryos into 1 μM cycloheximide (Sigma-Aldrich, St. Louis, MO) for 1 h.

Download English Version:

https://daneshyari.com/en/article/10934291

Download Persian Version:

https://daneshyari.com/article/10934291

Daneshyari.com