Development 129, 5421-5436 (2002)

doi:10.1242/dev.00095

Induction and patterning of the telencephalon in *Xenopus laevis*

Giuseppe Lupo[1,2], William A. Harris[2], Giuseppina Barsacchi[1] and Robert Vignali[1,*]

[1]Dipartimento di Fisiologia e Biochimica, Laboratorio di Biologia Cellulare e dello Sviluppo, Università di Pisa, Via G. Carducci 13, 56010 Ghezzano (Pisa), Italy
[2]Department of Anatomy, University of Cambridge, Downing Street, Cambridge CB2 3DY, UK
*Author for correspondence (e-mail: rvignali@dfb.unipi.it)

Accepted 7 August 2002

SUMMARY

We report an analysis of the tissue and molecular interplay involved in the early specification of the forebrain, and in particular telencephalic, regions of the *Xenopus* embryo. In dissection/recombination experiments, different parts of the organizer region were explanted at gastrula stage and tested for their inducing/patterning activities on either naive ectoderm or on midgastrula stage dorsal ectoderm. We show that the anterior dorsal mesendoderm of the organizer region has a weak neural inducing activity compared with the presumptive anterior notochord, but is able to pattern either neuralized stage 10.5 dorsal ectoderm or animal caps injected with BMP inhibitors to a dorsal telencephalic fate. Furthermore, we found that a subset of this tissue, the anterior dorsal endoderm, still retains this patterning activity. At least part of the dorsal telencephalic inducing activities may be reproduced by the anterior endoderm secreted molecule cerberus, but not by simple BMP inhibition, and requires the N-terminal region of cerberus that includes its Wnt-binding domain. Furthermore, we show that FGF action is both necessary and sufficient for ventral forebrain marker expression in neuralized animal caps, and possibly also required for dorsal telencephalic specification. Therefore, integration of organizer secreted molecules and of FGF, may account for patterning of the more rostral part of *Xenopus* CNS.

Key words: Neural induction, Forebrain, Telencephalon, Organizer, Anterior endoderm, Cerberus, Chordin, FGF, *Xenopus*

INTRODUCTION

The vertebrate central nervous system (CNS) is composed of a variety of discrete regions with diverse neuronal morphology and connectivity. Of outstanding interest is the study on how these different areas are generated during development, particularly within the forebrain, which contains the most complex structures in the vertebrate CNS, such as the telencephalon of mammals. Similarly to the Hox genes, which are involved in patterning the trunk CNS, several regulatory genes were proposed to define specific regions within the most rostral brain (Simeone et al., 1992; Bulfone et al., 1993; Shimamura et al., 1995; Shimamura et al., 1996; Rubenstein et al., 1998). Loss-of-function or gain-of-function experiments with these genes, either single or in combination, in fact lead to disruption of proper development within selected areas of the anterior CNS (reviewed by Rubenstein et al., 1998; Wilson and Rubenstein, 2000; Boyl et al., 2001).

Particular interest has been focused on the signals that promote the spatially restricted expression of patterning genes within the developing CNS. Perhaps the best known model that has been proposed to explain neural patterning is the activation/transformation model of Nieuwkoop and co-workers (Nieuwkoop et al., 1952; Nieuwkoop and Nigtevecht, 1954; Foley et al., 2000; Foley and Stern, 2001; Stern, 2001), who suggested that early induction and patterning of the neuroectoderm occurs in two steps. During a first step ('activation'), the dorsal ectoderm is initially induced from the adjacent and underlying mesendoderm to presumptive forebrain neuroectoderm. Subsequently, during the second step ('transformation'), some of this tissue receives caudalizing signals from the posterior dorsal mesoderm. This model has received strong molecular support from studies on *Xenopus*. Several factors that can work as 'activators' have been identified in the secreted molecules noggin (Lamb et al., 1993; Zimmerman et al., 1996), chordin (Sasai et al., 1994; Piccolo et al., 1996), follistatin (Hemmati-Brivanlou et al., 1994), Xnr3 (Smith et al., 1995; Hansen et al., 1997) and cerberus (Bouwmeester et al., 1996; Piccolo et al., 1999). They are all expressed in the dorsal mesendoderm during gastrula/neurula developmental stages and work as extra-cellular antagonists of bone morphogenetic proteins (BMPs). Molecules with characteristics of 'transformers' include retinoic acid, Wnts and FGFs, all of which can activate expression of posterior neural genes in neuroectoderm (Sasai and de Robertis, 1997; Gamse and Sive, 2001).

While the two-signal model may be sufficient to explain how the CNS is subdivided into main regions such as forebrain, midbrain, hindbrain and spinal cord, it does not explicitly account for the complex subregionalization of the forebrain itself. In principle, this could result from either a gradient of a single anterior inducing activity, or from the integration of multiple, qualitatively different, activities. Inhibition of BMP signaling appears to be a crucial step in forebrain induction, as

shown by the double knockout of *chordin* and *noggin* in the mouse (Bachiller et al., 2000). However, several lines of evidence suggest that, within the most anterior region of the neural plate, inhibition of BMP signaling needs to be integrated by other activities that counteract Wnt and Nodal signaling, thereby promoting forebrain development (Glinka et al., 1997; Piccolo et al., 1999). Some of these molecules have been identified as the Wnt-inhibitors Dkk1, Frzb1, crescent and sFRP2 (Leyns et al., 1997; Wang et al., 1997; Glinka et al., 1998; Pera et al., 2000), the Nodal inhibitor Lefty1 (Meno et al., 1999), or cerberus, a triple inhibitor of BMP, Wnt and Nodal (Piccolo et al., 1999), all of which are expressed in anterior mesendodermal tissues. Moreover, IGF signaling also appears to be required for head formation in *Xenopus* (Pera et al., 2001). Finally, patterning of the most anterior parts of the CNS may be integrated by additional signaling molecules, such as FGFs, Nodal, hedgehog proteins, Wnts and BMPs, involved in locally modifying the regional character of the forebrain neuroectoderm after its initial induction (Shimamura and Rubenstein, 1997; Furuta et al., 1997; Ye et al., 1998; Barth et al., 1999; Golden et al., 1999; Gunhaga et al., 2000; Shanmulingam et al., 2000; Heisenberg et al., 2001; Rohr et al., 2001; Wilson and Rubenstein, 2000).

Although all these data have started to clarify the molecular mechanisms that govern induction and patterning of the forebrain region, the fact that experiments were often performed on whole embryos did not allow in many cases the dissection of the activity of single inducing/patterning molecules, and to distinguish their direct actions on the neuroectoderm from indirect actions due to effects on mesendodermal tissues. This can be carried out easily in the frog embryo by means of dissection/recombination and misexpression methods that allow the overexpression of genes in the context of tissue conjugation experiments. In this paper, we report on some of the tissue and molecular signals at work in the induction and patterning of the anterior CNS in *Xenopus*, with particular attention to the telencephalon. We show that dorsoventral patterning of the telencephalon is a complex process that cannot be elicited by simple inhibition of BMP signaling. Moreover, by dissection/recombination experiments, we identify the anterior dorsal endoderm (ADE) of the leading edge of the *Xenopus* gastrula embryo as a source of signals that can regulate dorsoventral patterning of the telencephalon, in possible cooperation with the adjacent prechordal mesendoderm. Finally, in animal cap assays, we have used different combinations of inducing and patterning molecules to show that dorsoventral telencephalic patterning can be reconstructed, at least partially, in naive ectoderm by the combined action of the ADE molecule cerberus and FGF signals.

MATERIALS AND METHODS

Xenopus embryos and in situ hybridization

Embryos were obtained and staged as previously described (Nieuwkoop and Faber, 1967; Newport and Kirschner, 1982). Embryos and explants were processed for whole-mount in situ hybridization as previously described (Harland, 1991), except for proteinase K treatment, which was omitted, and for bleaching of pigment, performed as described by Mayor et al. (Mayor et al., 1995). Fig. 1 shows the expression patterns of the neural markers used in this study at stage 22/23 or stage 30/31.

RNA methods and microinjections

Capped RNAs were synthesized from linearized plasmid templates as described (Krieg and Melton, 1984). Embryos were injected with 10-2000 pg mRNA/embryo at the one- and eight-cell stage as previously described (Vignali et al., 2000). The following template plasmids were used.

cerberus: pcer-HA, pcer-S (Piccolo et al., 1999) and pcer-ΔC1 (Fetka et al., 2000).

chd: pCS2-Chd (Sasai et al., 1994).

ΔXFGFR-4a: ΔXFGFR-4a-pSP64T (Hongo et al., 1999).

Nxfz8: pCS2-Nxfz8 (Deardoff et al., 1998).

Smad7: pCS2-Smad7 (Nakayama et al., 2001).

RT-PCR was performed as described by Henry and Melton (Henry and Melton, 1998). Embryo RNA was extracted with RNA-NucleoSpin kit (Macherey and Nagel) and retro-transcribed with Superscript II (Invitrogen). PCR primers and conditions were drawn from http://www.hhmi.ucla.edu/derobertis/index.html, except for *cpl-1* (see Knecht and Harland, 1997), *XBF-1* and *nrp-1* (see Hongo et al., 1999). For *Xemx1*, 35 cycles were used with primers GCAGAAGCCTTTGTCAGTGG (forward) and CCTCCAGTTT-CTGCCTCTTG (reverse); for *eomes*, 32 cycles were used with primers GCCTACGAAACAGACTACTCCT (forward) and TAATGGAGGGAGGGGTTTCTAC (reverse).

Animal cap and conjugate assays

For animal cap and dissection/recombination assays, RNAs were injected in the animal pole of one-cell stage embryos. Animal caps were dissected from stage 9 or stage 10.5 embryos in 1×MBS; after healing, caps were cultured in 0.5×MBS until early tailbud stage 22/23, or to late tailbud stage 30/31 alongside with sibling embryos.

Dissections and culturing of dorsal ectoderm from gastrula stage embryos were similarly performed.

In conjugate experiments, embryo fragments were similarly dissected, recombined and cultured. Peptide-releasing beads (SIGMA H-5263) were washed in 1×PBS and then incubated overnight at 4°C in 5 μl of 1×PBS, 0.1% BSA containing either human bFGF (100 or 200 ng/μl; ICN) or mouse FGF8b (100, 200 or 400 ng/μl; R&D). Beads were implanted within pairs of animal caps dissected from either injected or uninjected embryos.

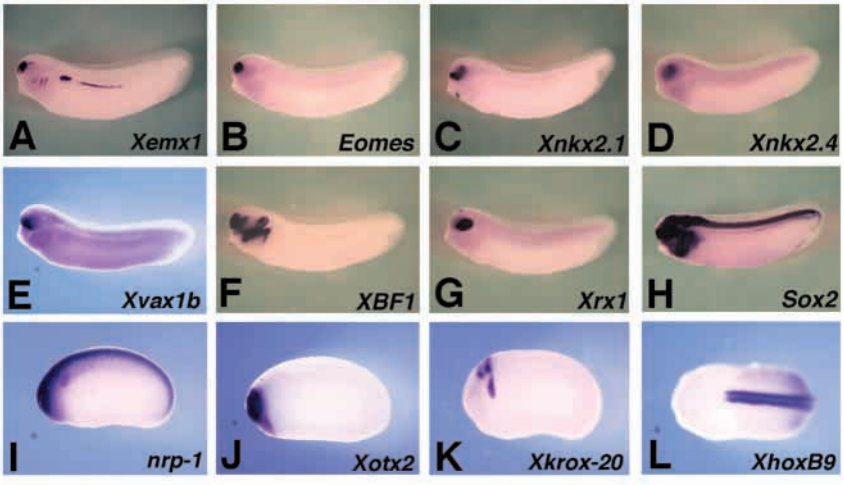

Fig. 1. Expression patterns of the neural markers used in this study, as detected by whole-mount in situ hybridization at stage 30/31 (A-H) or stage 22/23 (I-L). (A-K) lateral views; (L) dorsal view.

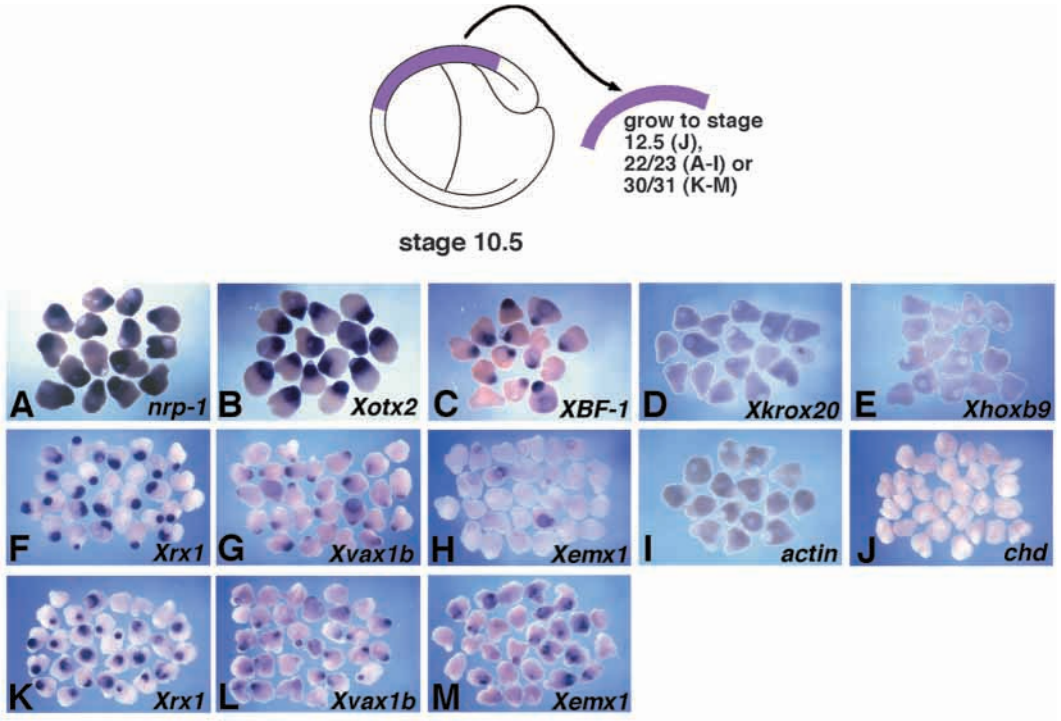

Fig. 2. Specification assays on dorsal ectoderm (DE) of stage 10.5 embryos. DE was explanted as outlined in the scheme, grown to stage 12.5. (J), to stage 22/23 (A-I) or to stage 30/31 (K-M), and finally processed for in situ hybridization with probes for *nrp1, Xotx2, XBF-1, Xrx1, Xvax1b, Xemx1, Xkrox20, XhoxB9*, cardiac actin and *chd* as indicated.

Dissections were performed in the presence of gentamycin (50 μg/ml final concentration).

RESULTS

Specification assays on dorsal ectoderm of stage 10.5 embryos

We have previously shown that BMP antagonists, such as noggin (Lamb et al., 1993) and Xnr3 (Hansen et al., 1997), although able to trigger anterior neural fate in *Xenopus* animal caps, are not sufficient to specify dorsal telencephalon (Pannese et al., 1998). Yet, the dorsal blastopore lip of the early *Xenopus* gastrula can efficiently activate dorsal telencephalic markers in animal cap tissue (Pannese et al., 1998). In order to identify what signals from the dorsal blastopore lip are involved in the induction of dorsal telencephalon, we first aimed to define their timing of action during development. We therefore removed the dorsal ectoderm (DE) from gastrula stage *Xenopus* embryos, cultured the explants up to the corresponding of early tailbud (stage 22/23) or late tailbud (stage 30/31) stage, and assayed their state of specification by in situ hybridization using several neural markers, including dorsal telencephalic markers.

In particular, we dissected, from stage 10.5 midgastrula embryos, fragments of DE of about 500 μm comprised between the animal pole and about half way between the dorsal blastopore lip and the leading edge of the involuting dorsal mesendoderm, as outlined in the scheme in Fig. 2. Data from one to seven independent experiments (Table 1) – depending on the analyzed marker – indicate that this DE region is already specified to develop as anterior neural tissue. In fact, explants cultured up to stage 22/23 showed a strong expression of the pan-neural marker *nrp-1* (Knecht et al., 1995), of the fore/midbrain marker *Xotx2* (Pannese et al., 1995), of the general telencephalic marker *XBF-1* (also expressed in the nasal part of the eye) (Papalopulu and Kintner, 1996), of the eye marker *Xrx1* (Casarosa et al., 1997) or of the ventral forebrain marker *Xvax1b* (Liu et al., 2001) (Fig. 2A-C,F,G; Table 1). However, only few of the explants showed a faint staining for the dorsal telencephalic marker *Xemx1* (Pannese et al., 1998) (Fig. 2H; Table 1). We also found that more posterior markers such as *Xkrox-20* (Bradley et al., 1993) and *XhoxB9* (Wright et al., 1990) were not activated at all, or activated only in few explants (Fig. 2D,E; Table 1). By contrast, when the explants were cultured to stage 30/31, not only did they express *Xrx1, Xvax1b* and the ventral forebrain marker *Xnkx2.1* (Small et al., 2000), but an evident activation of *Xemx1* also occurred (Fig. 2K-M, Table 1; see Fig. 6B and Table 1 for *Xnkx2.1*). To test for possible mesoderm contamination, a proportion of explants were assayed for expression of *chd* (Sasai et al., 1994) (at the equivalent of stage 12.5) or muscle actin (Mohun et al., 1984) (at stage 22/23), and found deprived of expression for either marker (Fig. 2I,J; Table 1). Therefore, although some aspects of forebrain specification have already taken place by midgastrulation, the onset of expression of dorsal telencephalic genes appears to be significantly delayed in stage 10.5 explants. However, when DE was dissected from late gastrula embryos, clear expression of *Xemx1* was already detectable at stage 22/23 (data not shown). These observations suggest that further contact with the dorsal mesendoderm may be required between mid-gastrula and end of gastrulation, to ensure a proper temporal specification of the dorsal telencephalon.

The anterior dorsal mesendoderm plays a role in patterning of the telencephalon

Because signals produced from dorsal mesendoderm may be important for proper induction of dorsal telencephalon

Table 1. Tissue specification and recombination assays

Specification assays: dorsal ectoderm explants dissected at stage 10.5 (Fig. 2)

Experiment	1	2	3	4	5	6	7
Explants cultured to stage 12.5							
chordin					0/28		
Explants cultured to stage 22/23							
nrp1	14/15	16/16					
Xotx2	15/15	15/15				17/17	
Xrx1	13/15	14/16	16/16	12/14	23/28	14/16	13/15
XBF-1	11/13	15/16	16/16				
Xvax1b					22/28		
Xemx1	4/14*	4/15*	2/17*	4/16*	5/28†		
Xkrox20						0/17	2/16
Xhoxb9						0/17	0/15
actin	0/13	0/17					0/15
Explants cultured to stage 30/31							
Xvax1b					22/30		
Xemx1				13/17	17/29		
eomes				14/16			

*Weak signal.
†Three out of five with weak signal.

Molecular identification of ADE and ADME (Fig. 3)

	‘red’ fragment	‘green’ fragment	‘brown’ fragment	‘yellow’ fragment
Explants cultured to stage 12.5				
gsc	19/23	22/22	19/24†	
Xnot2	0/22	4/22*	24/25	
chd	27/27			3/28*
Xhex				25/28

*Weak signal.
†15/19 with weak signal.

Dorsal mesendoderm/animal cap conjugates (Fig. 4)

	‘green’ fragment/an.cap	‘brown’ fragment/an.cap
Explants cultured to stage 22/23		
nrp-1	18/24*	16/16
Xotx2	10/24†	10/18
Xrx1	0/24	2/18
XBF-1	9/23	7/18
Xemx1	6/23‡	2/18

*Seven out of 18 with weak signal.
†Seven out of 10 with weak signal.
‡Five out of six with weak signal.

ADME/DE conjugates (Figs 5, 6)

		‘red’ fragment/ DE stage 10.5	‘green’ fragment/ DE stage 10.5	‘yellow’ fragment/ DE stage 10.5	DE stage 10.5
Explants cultured to stage 22/23 (experiments 1 and 2) or to stage 30/31 (experiment 3)					
Experiment 1	*Xemx1*	24/24	20/20		
Experiment 2	*Xemx1*	21/28		20/23	0/18
	Xrx1				17/17
Experiment 3	*Xnkx2.1*			6/24	17/21
	Xrx1				16/18

Numbers refer to positive explants or conjugates on the total number assayed.

(Pannese et al., 1998), we decided to assay the inducing/patterning abilities of different regions of this tissue.

In order to do this reproducibly, different parts of the involuting mesendoderm were dissected at stage 10.5, cultured to stage 12.5, and assayed with *Xhex, chd, gsc* and *Xnot-2* probes as diagnostic molecular markers. We identified four different pieces, which were used in our recombination experiments. Three of these fragments are contained within one another, and correspond to the yellow, red and green pieces in the schemes of Fig. 3. A first fragment of about 100 μm, corresponding to the anterior dorsal endoderm (ADE; in yellow in Fig. 3), strongly expressed *Xhex* (Jones et al., 1999) (Fig.

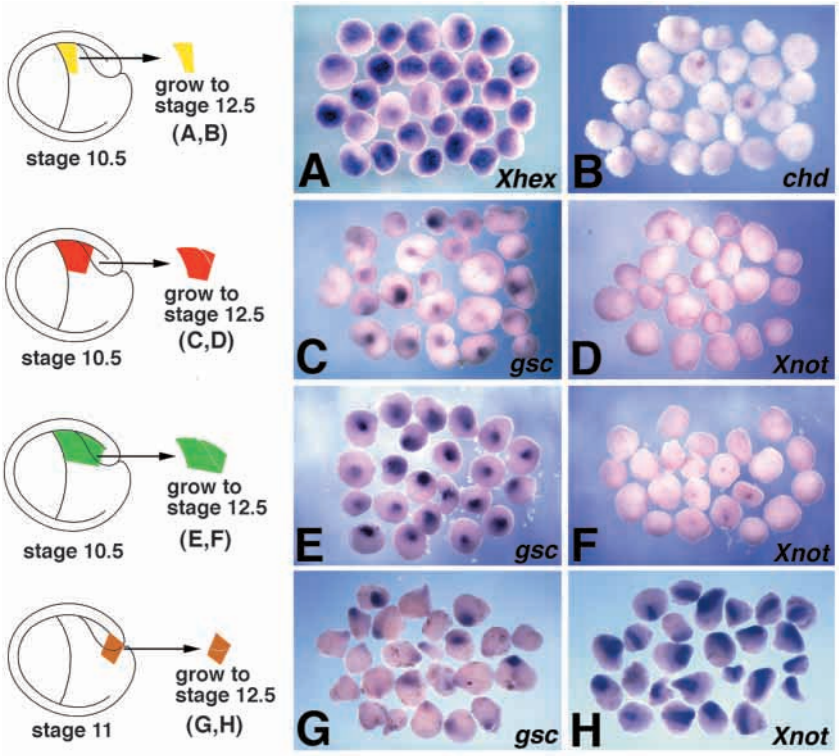

Fig. 3. Characterization of different fragments of dorsal mesendoderm used in recombination experiments. Fragments were reproducibly dissected from stage 10.5 (A-F) or stage 11 (G,H), as shown in the scheme, cultured to stage 12.5, and assayed for expression of the organizer marker genes *Xhex* (A), *chd* (B), *gsc* (C,E,G) and *Xnot* (D,F,H).

3A; Table 1); contamination by prechordal mesoderm was excluded by absence of hybridization to a *chd* probe (Fig. 3B; Table 1), that specifically labels the whole axial mesendoderm, but not the most anterior dorsal endoderm (Sasai et al., 1994). A larger fragment of about 200 μm, corresponding to the anterior half of the involuted anterior dorsal mesendoderm (ADME; in red in Fig. 3), weakly expressed *gsc* (Cho et al., 1991), a marker of prechordal mesoderm, strongly expressed *chd*, but did not express *Xnot-2* (Gont et al., 1993), a marker of presumptive notochord (Fig. 3C,D; Table 1; and data not shown). By contrast, a still larger fragment of about 300 μm, corresponding to the anterior three-quarters of the involuted ADME (in green in Fig. 3), strongly expressed *gsc* while showing a weak spot of *Xnot-2* staining only in a minority of explants (Fig. 3E,F; Table 1). Finally, a fourth fragment of about 120 μm, corresponding to the posterior quarter of the involuted ADME of stage 11 embryos (in brown in Fig. 3), showed a weak *gsc*, but a strong *Xnot-2*, expression (Fig. 3G,H; Table 1). Thus, we conclude that the 'yellow' fragments correspond to the anterior endoderm of the leading edge (ADE), while those in 'red' or 'green' appear to contain exclusively, or almost exclusively, prechordal mesendoderm; finally, the 'brown' fragment is mainly composed of presumptive notochord tissue with little – if any – prechordal mesendoderm.

We first separately analyzed the inducing properties of the prechordal mesendoderm ('green' fragment) compared with those of the presumptive anterior notochord ('brown' fragment). Their different inducing abilities were tested by conjugating either 'green' or 'brown' fragments with stage 9 animal caps, followed by in situ hybridization analysis of the conjugates at the corresponding of stage 22/23. A weak anterior neural induction was detected in the conjugates with the prechordal ('green') fragment, as shown by the occurrence of either weak or no activation of *nrp-1*, *Xotx2*, *Xrx1*, *XBF-1* and *Xemx1* genes after extensive color reaction (Fig. 4A-E; Table 1). By contrast, efficient induction of neural tissue took place in the conjugates made with presumptive anterior notochord ('brown') tissue, as shown by the strong activation of *nrp-1*; localized weak expression of *Xotx2*, *Xrx1*, *XBF-1* and *Xemx1* was detected only in a minority of explants (Fig. 4F-J; Table 2). By RT-PCR assay, very weak or no activation was detected for *nrp-1*, *N-CAM*, *XBF-1*, *Xotx2*, *Xrx1* and *Xemx1* in conjugates with the prechordal mesendoderm (Fig. 4K). Instead *nrp-1*, *N-CAM*, *XBF-1*, *Xotx2* and *Xrx1* were readily detected in conjugates with the anterior chordomesoderm, while *Xemx1* was very weakly expressed in these recombinants (Fig. 4K), possibly owing to the presence of contaminating *gsc*-positive cells in the 'brown' fragment (Fig. 3G). Therefore, the prechordal mesendoderm and the anterior notochord significantly differ in their neural inducing abilities, but neither tissue is able to efficiently induce dorsal telencephalic character in naive ectoderm. Differences between in situ hybridization and RT-PCR results may reflect the different potencies of the two techniques in detecting localized or average levels of expression.

However, when the prechordal mesendoderm ('green' piece of Fig. 3) was conjugated to neuralized stage 10.5 DE (upper scheme of Fig. 5), it was able to restore appropriate expression (both in timing and intensity of signal) of dorsal telencephalic genes (*Xemx1*) within the conjugates cultured up to stage 22/23 (Fig. 5A; Table 1). In fact, a smaller region of this 'green' fragment may be sufficient for this patterning activity: when stage 10.5 DE (as shown in Fig. 2) was removed from embryos together with the underlying fragment of ADME ('red' piece in Fig. 3), as outlined in the lower scheme in Fig. 5, again appropriate strong expression of *Xemx1* was observed at stage 22/23 (Fig. 5B; Table 1).

It has been proposed that the ADE in *Xenopus*, and the corresponding structure known as AVE in the mouse, may play a pivotal role in forebrain development (Bouwmeester et al., 1996; Thomas and Beddington, 1996). We therefore tested whether the ADE alone, without the adjoining prechordal mesendoderm, could elicit *Xemx1* activation in midgastrula DE. We explanted DE fragments from stage 10.5 embryos together with the underlying ADE ('yellow' piece of Fig. 3), as in the lower scheme in Fig. 6, and cultured them up to stage 22/23. Control explants, made of DE alone (upper scheme of Fig. 6), displayed a strong *Xrx1* (as a positive control of neuralization, data not shown), but no *Xemx1* activation (Fig. 6A; Table 1); by contrast, most of the ADE-containing recombinates expressed *Xemx1* (Fig. 6C; Table 1). We also

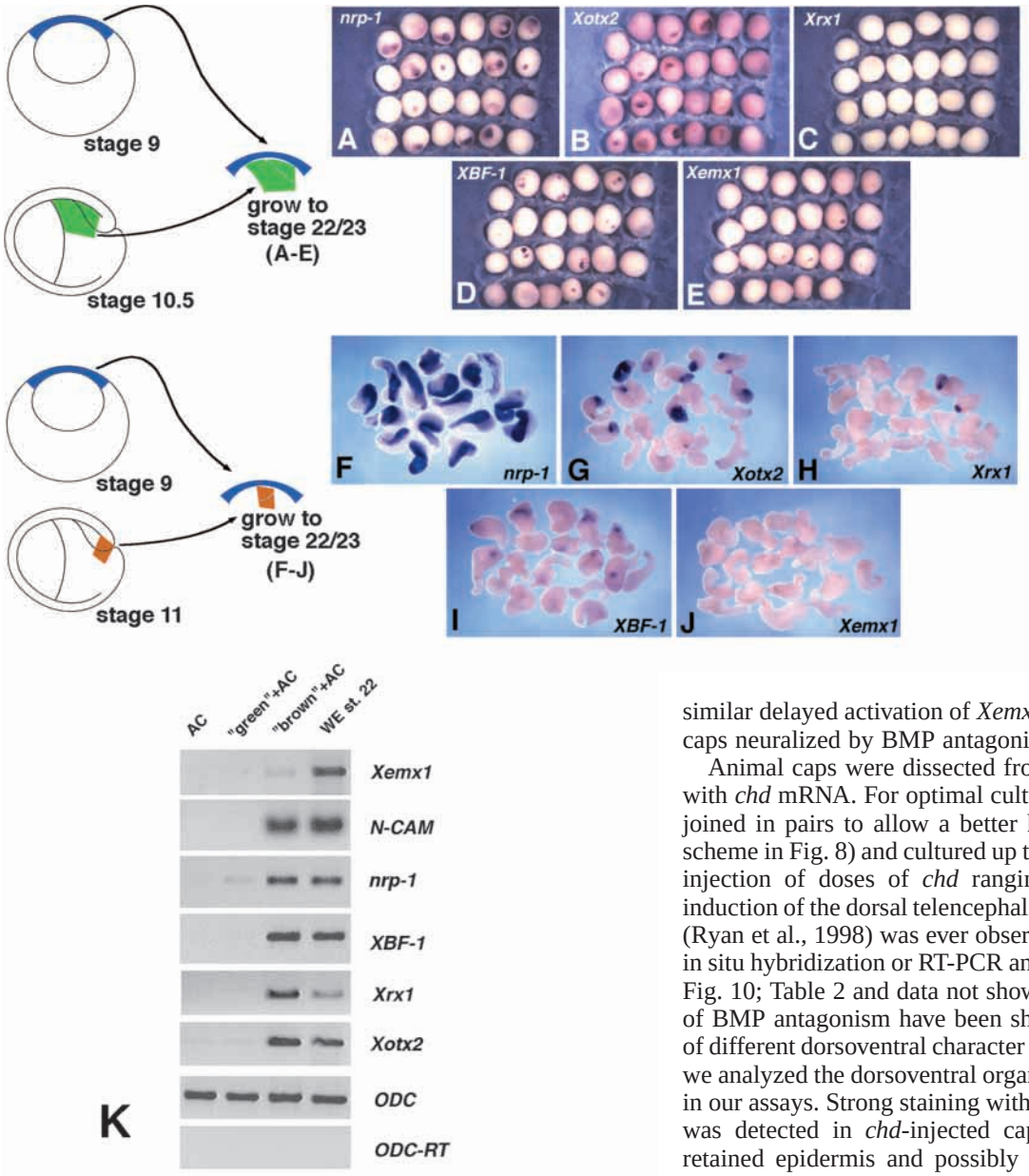

Fig. 4. Tissue recombination induction assays. Conjugates were made by recombining stage 9 animal caps with the involuted anterior dorsal mesendoderm (ADME, green) of stage 10.5 embryo (A-E, upper scheme on the left) or with the presumptive notochordal fragment (brown) of stage 11 embryo (F-J, lower scheme on the left). Conjugates were grown to stage 22/23 and assayed by in situ hybridization for expression of the neural markers *nrp1* (A,F), *Xotx2* (B,G), *Xrx1* (C,H), *XBF-1* (D,I), *Xemx1* (E,J). (K) RT-PCR analysis of the expression of neural markers in similar conjugates: AC, animal caps; WE, whole embryo; 'green' and 'brown' correspond to the colored fragments in the schemes.

tested whether the ADE had any effect on ventral forebrain marker specification, and surprisingly found that while *Xemx1* expression was maintained in stage 30/31 recombinates (data not shown), *Xnkx2.1* expression was suppressed in these recombinates, compared with explants of DE (Fig. 6B,D; Table 1). These results suggest that ADE may be important for specification of dorsal telencephalon, but may have an inhibitory effect on ventral forebrain specification.

Organizer signals and induction of telencephalic markers in animal caps

The organizer-secreted BMP antagonists noggin and Xnr3 are able to activate *Xotx2*, but not *Xemx1* and *Xemx2* expression, in *Xenopus* animal caps grown to stage 22/23 (Pannese et al., 1998). Because DE isolated from midgastrula embryos shows *Xemx1* expression only when cultured up to stage 30/31, and not to stage 22/23 (Fig. 2H,M; Table 1), we asked whether any similar delayed activation of *Xemx1* could take place in animal caps neuralized by BMP antagonists.

Animal caps were dissected from stage 9 embryos injected with *chd* mRNA. For optimal culture to later stages caps were joined in pairs to allow a better healing of the explants (see scheme in Fig. 8) and cultured up to stage 22/23 or 30/31. After injection of doses of *chd* ranging from 10 to 600 pg, no induction of the dorsal telencephalic markers *Xemx1* and *eomes* (Ryan et al., 1998) was ever observed at either stage, either by in situ hybridization or RT-PCR analysis (Fig. 7B,C; Fig. 9E,F; Fig. 10; Table 2 and data not shown). Because different levels of BMP antagonism have been shown to induce neural tissue of different dorsoventral character (Knecht and Harland, 1997), we analyzed the dorsoventral organization of *chd*-injected caps in our assays. Strong staining with the epidermal marker *XK81* was detected in *chd*-injected caps, indicating that explants retained epidermis and possibly a dorsal boundary between neural tissue and epidermis in the conditions used (Fig. 7D; Table 2). Presence of a dorsal neural tube boundary was also addressed by checking the expression of the telencephalic dorsal neural tube boundary marker *cpl-1* (Knecht et al., 1995). *cpl-1* is strongly expressed in caps at low doses of injected *chd*, but still detectable, though at low levels, at high doses (Fig. 10); these results are consistent with earlier observations (Knecht and Harland, 1997) and show that even in conditions that promote *cpl-1* strong expression, *Xemx1* and *eomes* are never induced. To rule out the possibility that these results could be specific to Chd with respect to other BMP antagonists, we also assayed Smad7, a global antagonist of the whole TGF-β pathway (Nakayama et al., 2001), and obtained similar results (Fig. 7E-H; Table 2; Fig. 10).

Because we showed that stage 10.5 ADME had a patterning activity on stage 10.5 neuralized DE, we asked whether it could integrate the action of BMP inhibitors to activate dorsal telencephalic genes. Therefore, stage 9 animal caps were explanted from *chd* injected embryos, conjugated with the

Table 2. Signals involved in forebrain induction and patterning

***chd* and *Smad7* mRNA injection in animal caps (Fig. 7)**

	chd	*Smad7*	Uninjected
Animal caps grown to stage 30/31			
Xemx1	0/82	0/38	0/36
eomes	0/84	0/35	0/33
Xrx1	70/71	39/39	0/35
XK81	39/39	39/39	35/35

***chd*-injected animal caps/ADME conjugates (Fig. 8)**

	chd-injected caps	*chd*-injected caps/ADME	Uninjected caps/ADME	Uninjected caps
Explants grown to stage 30/31				
Xemx1	0/30	20/30	0/32	0/38
eomes	0/30	10/30	0/32	0/37
Xrx1	27/27		0/32	0/37

***cer* and *cer*-Δ*C1* mRNA injections in animal caps (Fig. 9)**

	cer-injected caps	*chd+cer*Δ*C1*-injected caps	*chd*-injected caps	Uninjected caps
Animal caps grown to stage 30/31				
Xemx1	15/50	28/47	0/30	0/28
eomes	25/50	33/49	0/40	0/30
Xrx1	45/45		32/32	

Chd, cerberus and FGFs in forebrain specification (Fig. 11)

	chd+cer-S	*chd+cer-S+ FGF8* (100 ng/ml)	*chd+cer-S+ FGF8* (200 ng/ml)	*chd+cer-S+ bFGF* (100 ng/ml)	*chd+cer-S+ bFGF* (200 ng/ml)
Animal caps grown to stage 30/31					
chd+cer-S+FGFs					
Xemx1	1/24	19/52	2/52	7/40	18/51
eomes	0/23	27/52	24/52	17/38	39/50
Xnkx2.1	0/24	47/47	50/50	24/34	40/42

	cer	*cer+FGF8* (100 ng/ml)	*cer+FGF8* (200 ng/ml)	*cer+FGF8* (400 ng/ml)
cerberus+FGF8				
Xemx1	21/41	22/43	22/46	32/43
eomes	21/39	41/42	35/47	38/43
Xnkx2.1	0/30	31/35	33/37	33/37
Xnkx2.4	0/36	32/39		

Inhibition of FGF signaling on forebrain gene expression (Fig. 12)

	Early dbl+ animal caps	Early dbl+ΔXFGFR-4-injected animal caps
Conjugates were grown to stage 30/31		
Xemx1	15/20	8/24
Xnkx2.1	20/21	7/25
Sox2	19/19	24/24

Numbers refer to positive explants or conjugates on the total number assayed.

ADME ('red' fragment of Fig. 3) of stage 10.5 control embryos and grown to stage 30/31 (see scheme in Fig. 8). Although conjugated pairs of *chd* injected caps did not express *Xemx1* or *eomes* (Fig. 8G,H; Table 2), but expressed the positive control marker *Xrx1* (Fig. 8I; Table 2), *chd* injected caps recombined with the ADME were positive both for *Xemx1* and for *eomes* expression (Fig. 8J,K; Table 2). By contrast, uninjected caps conjugated with the ADME did not show any expression for *Xemx1*, *eomes* and *Xrx1* (Fig. 8D,E,F), confirming the poor, if any, forebrain inducing activity of the ADME. These results demonstrate that the ADME is able to complement the action of BMP antagonists to promote development of dorsal telencephalon.

Head induction has been proposed to result from the triple inhibition of BMP, Wnt and Nodal pathways (Glinka et al., 1997; Glinka et al., 1998; Piccolo et al., 1999) by several secreted proteins. Among them, cerberus has the unique feature of being a triple BMP-Nodal-Wnt-antagonist; moreover, it is

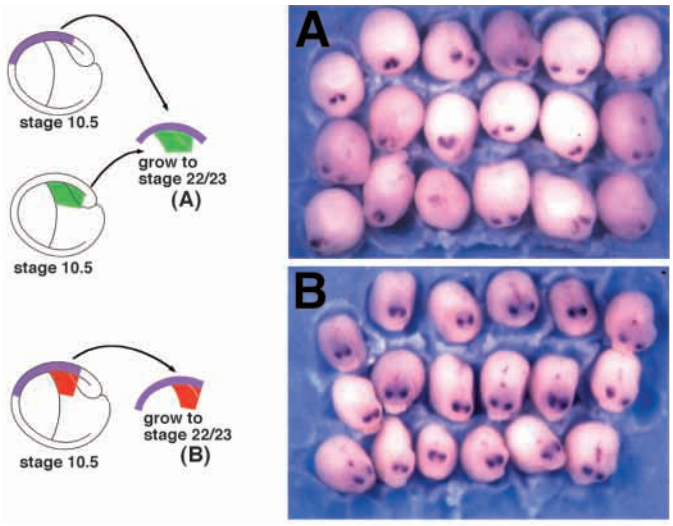

Fig. 5. The involuted ADME of stage 10.5 embryo acts on stage 10.5 DE to elicit proper *Xemx1* expression at stage 22/23. (A) DE (violet) and involuted ADME (green) were explanted and recombined at stage 10.5 (upper scheme), grown to stage 22/23 and assayed for *Xemx1* expression. (B) DE and the underlying involuted ADME (red) were explanted together at stage 10.5 (lower scheme), grown to stage 22/23 and assayed for *Xemx1* expression.

expressed in the ADE, which plays a patterning role on the anterior neuroectoderm (see above). Remarkably, when *cerberus* mRNA was injected into animal caps, besides a strong activation of *Xrx1* in the vast majority of explants, also *Xemx1* and *eomes* expression was found in some of the animal caps (Fig. 9A-C; Table 2).

As cerberus is a triple BMP-Wnt-Nodal-inhibitor, we decided to define which of these inhibitory activities was required for induction of dorsal telencephalic genes. To achieve this, we made use of two previously described constructs, *cer-S* and *cer-ΔC1*, encoding the C-terminal (cer-S) and the N-terminal (cer-ΔC1) regions of cerberus, which have been described as a Nodal-antagonist and as a Wnt-antagonist, respectively (Piccolo et al., 1999; Fetka et al., 2000). When the anti-BMP activity of Chd was coupled to the anti-Nodal activity of cer-S, no activation of either *Xemx1* or *eomes* was detected (Fig. 10), in agreement with the result obtained with the general TGFβ inhibitor Smad7 (Fig. 7F,G; Table 2; Fig. 10). By contrast, the combination of Chd and cer-ΔC1 was clearly able to induce both *Xemx1* and *eomes*, as detected by in situ hybridization and by RT-PCR, while no activation was detectable in *chd* injected caps (Fig. 9E-H; Table 2; Fig. 10). cer-ΔC1 alone was not able to induce any expression of *Xemx1*, *eomes*, *NCAM* and *Xrx1* at doses that were able to induce dorsal telencephalic genes in combination with Chd,

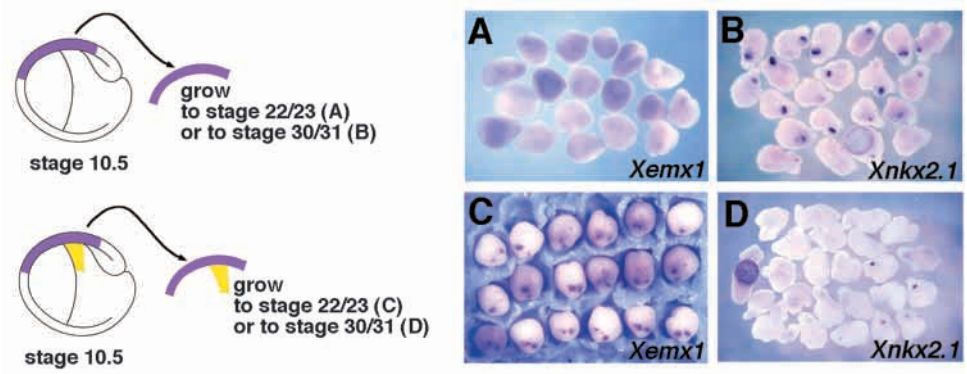

Fig. 6. The ADE promotes *Xemx1* expression and downregulates *Xnkx2.1* expression in explants of DE. (A,B) DE (violet in upper schematic) was explanted from stage 10.5 embryos, cultured to stage 22/23 (A) or 30/31 (B) and assayed for expression of *Xemx1* (A) or *Xnkx2.1* (B). (C,D) DE (violet in lower schematic) was explanted from stage 10.5 embryos together with the ADE (yellow in lower schematic), grown to stage 22/23 (C) or 30/31 (D), and assayed for expression of *Xemx1* (C) or *Xnkx2.1* (D).

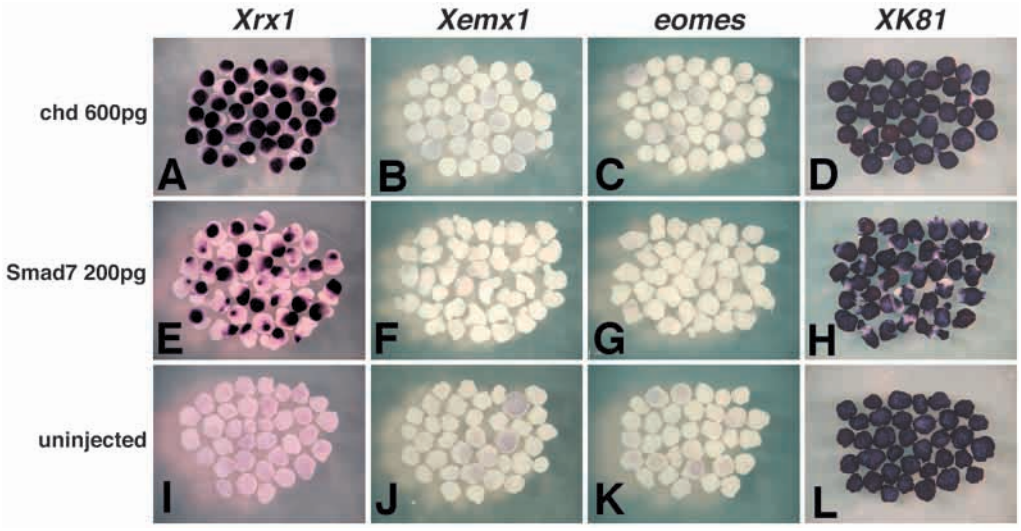

Fig. 7. Injection of *chordin* or of *Smad7* mRNA cannot induce expression of the dorsal telencephalic markers *Xemx1* and *eomes* in animal cap assays. Animal caps from stage 9 embryos injected with 600 pg *chordin* mRNA (A-D), or with 200 pg *Smad7* mRNA (E-H), or from uninjected embryos (I-L) were dissected, grown in pairs to stage 30/31 and assayed for expression of *Xrx1*, *Xemx1*, *eomes* and *XK81* as indicated. (I-L) are uninjected control caps.

suggesting that cer-ΔC1, at least at these doses, lacks neural inducing ability and hence does not retain significant BMP-antagonizing activity (Fig. 10). However, we found that besides the previously described Wnt-blocking activity (Fetka et al., 2000), cer-ΔC1 retains some Nodal-antagonizing activity (data not shown). We also compared the effects of cer-ΔC1 with those of Nxfz8, a potent Wnt-antagonist (Deardoff et al., 1998). In contrast to cer-ΔC1, neither a combination of Chd and Nxfz8, nor a combination of Chd, Nxfz8 and cer-S, was able to induce expression of *Xemx1* or *eomes* (Fig. 10), at doses of Nxfz8 that efficiently induced strong axial defects in whole embryos (Deardoff at al., 1998) (data not shown). Similar results were obtained with the analogous construct ECD8 (Itoh and Sokol, 1999) (data not shown). Because the only qualitative difference between the combinations Chd+cer-ΔC1 and Chd+Nxfz8+cer-S resides in the Wnt-inhibitory activities of cer-ΔC1 and Nxfz8, these results suggest that the dorsal telencephalic inducing activity of cerberus relies on its specific anti-Wnt action; however, we cannot completely rule out the possibility that the residual anti-Nodal activity of cer-ΔC1 may also be required.

In addition, we also tested induction of the ventral forebrain marker *Xnkx2.1* in these same caps. *Xnkx2.1* was not induced by Chd, Smad7, or the combinations of Chd+cer-S and Chd+cer-ΔC1 (Fig. 10), indicating that, though cerberus is able to partially promote dorsal telencephalic fates, a full patterning of the telencephalon may require the integration of different molecular pathways.

Role of FGFs in patterning of the telencephalon

FGFs have been proposed to play important roles both in early neural induction in the frog and the chick (Hongo et al., 1999; Streit et al., 2000), and in later patterning of the anterior neural plate, and particularly the telencephalon, in the mouse (Shimamura and Rubenstein, 1997; Ye et al., 1998; Shanmugalingam et al., 2000) (reviewed by Rubenstein et al., 1998; Wilson and Rubenstein, 2000).

We therefore tested whether we could induce telencephalic genes in animal caps by integrating the activities of Chd and cerberus with that of FGF. To do this we conjugated pairs of animal caps, injected with either *chd* or *cerberus* mRNA, around a bead soaked in bFGF or in FGF8 (see Fig. 11). Animal caps were dissected at stage 10.5, when they no longer respond to mesoderm inducing signals, and therefore any effect of FGFs is a direct effect on ectoderm (Lamb and Harland, 1995). Cap competence for mesoderm induction was excluded by failure of either bFGF or FGF8 to induce *Xbra* (Smith et al., 1991) expression, while failure to detect expression of the pan-neural marker *Sox2* excluded any direct neural inducing activity by FGFs (data not shown). However, it proved to be difficult to harvest stage 10.5 caps from *chd*-injected embryos, probably because excess involution of dorsal mesendoderm made it impossible to dissect caps without any underlying mesendoderm. Therefore, it would not be possible to discriminate whether FGF activity, rather than signals from the underlying mesendoderm, was responsible for any effect additional to that of Chd. We therefore co-injected *chd* mRNA with *cer-S*, a *cerberus* construct that, by inhibiting mesoderm formation (Piccolo et

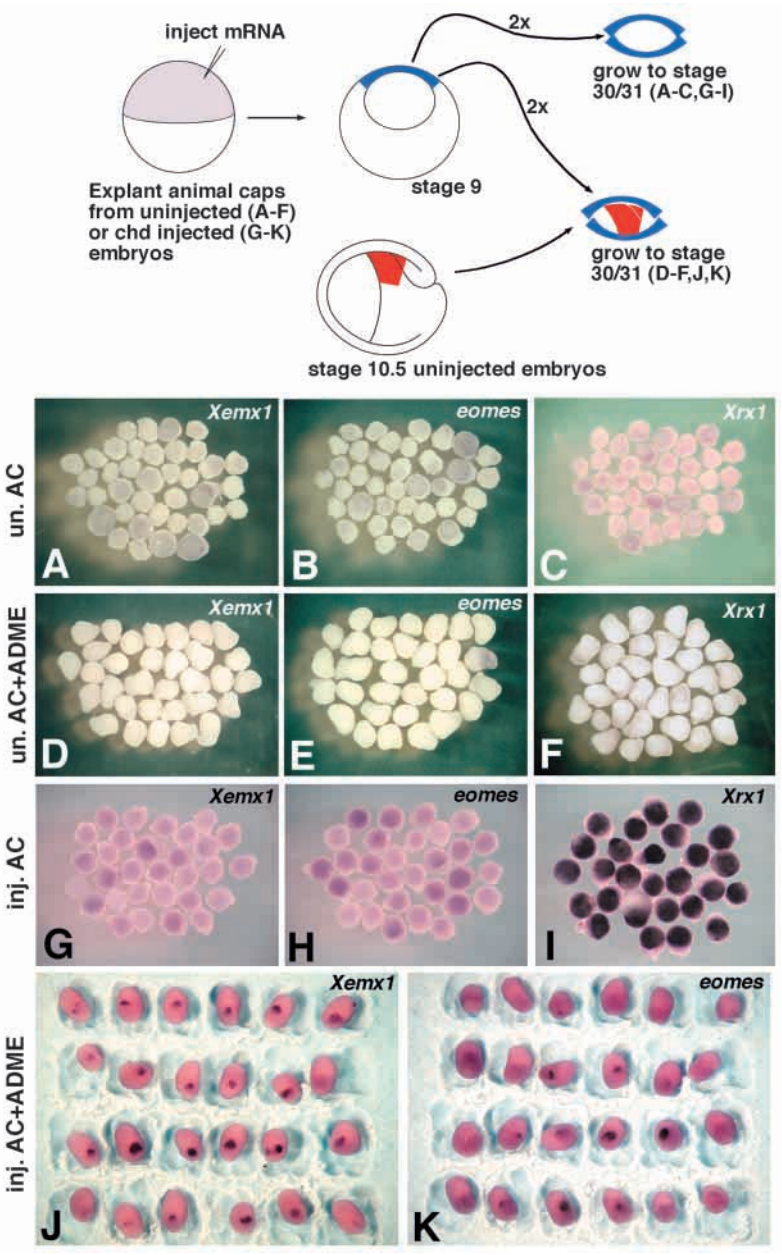

Fig. 8. The involuted ADME of stage 10.5 embryo can trigger expression of the dorsal telencephalic markers *Xemx1* and *eomes* in *chd* injected animal caps. Animal caps (blue in schematic) from stage 9 uninjected (A-F) or injected (G-K) embryos were explanted, conjugated in pairs either without (A-C;G-I) or with (D-F,J,K) the ADME (red) from a stage 10.5 gastrula and grown to stage 30/31. Injected animal caps never express either *Xemx1* (G) or *eomes* (H), but show activation of a control neural marker, *Xrx1* (I). *chd*-injected caps conjugated with the ADME show activation of both *Xemx1* (J) and *eomes* (K), whereas no activation of these genes or of *Xrx1* is detected in conjugates between ADME and uninjected animal caps (D-F). Uninjected caps never show expression of *Xemx1*, *eomes* or *Xrx1* (A-C).

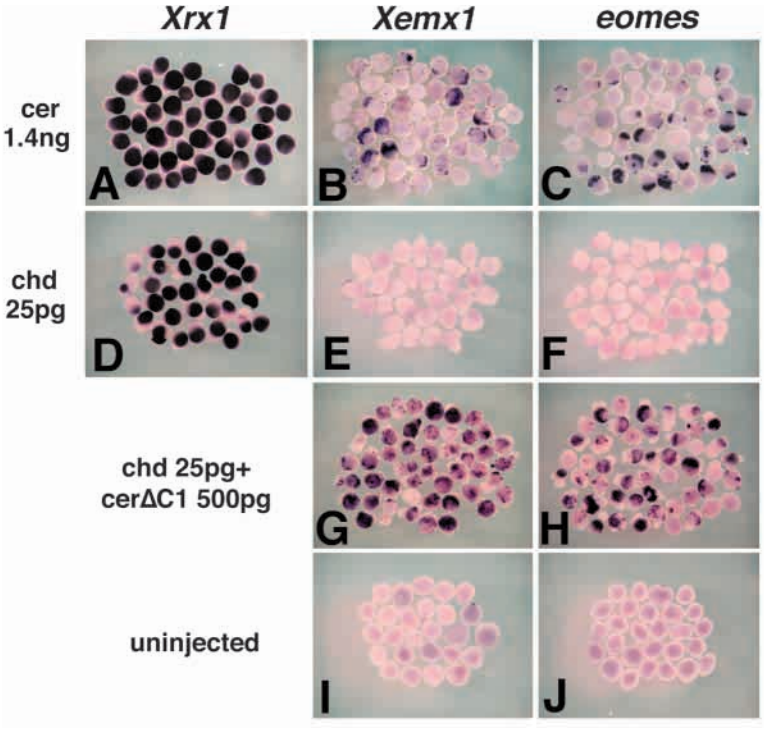

Fig. 9. *cerberus*, but not *chd*, mRNA triggers *Xemx1* and *eomes* expression in injected animal caps. Animal caps were injected with amounts indicated of *cerberus* (A-C) or *chd* (D-F), or a combination of *chd* and *cerΔC1* (G,H) mRNA, or were uninjected (I,J). At stage 30/31 they were assayed for expression of *Xrx1* (A,D), *Xemx1* (B,E,G,I) and *eomes* (C,F,H,J).

al., 1999), was able to prevent any excessive involution of mesendoderm, and afterwards dissected and conjugated the animal caps to FGF beads. The effects of Chd+cer-S on caps dissected at stage 10.5 were not substantially different from those on caps dissected at stage 9, at least for the markers we tested, and resulted in no activation of the ventral gene *Xnkx2.1* (Fig. 11A), essentially no activation of the dorsal genes *eomes* (Fig. 11F) and *Xemx1* (Fig. 11K), and in a strong activation of *Xrx1* (data not shown). However, when FGF8 was added, *Xnkx2.1* expression was strongly activated in animal caps (Fig. 11B,C; Table 2), and activation was also observed for *eomes* (Fig. 11G,H; Table 2); by contrast, slight, if any, activation of *Xemx1* was observed (Fig. 11L,M; Table 2). Similar effects were also observed for bFGF (Fig. 11D,E,I,J,N,O; Table 2).

Different results were obtained when *cerberus*-injected caps were explanted at stage 10.5 and conjugated in pairs either without or with FGF-soaked beads. Again, *Xnkx2.1* and *Xnkx2.4* were not activated by the injected RNA (Fig. 11A′,M′; Table 2); however, clear activation was observed for *eomes* (Fig. 11E′; Table 2) and for *Xemx1* (Fig. 11I′; Table 2); finally, strong activation was observed in all caps for *Xrx1* (data not shown). When FGF was added to *cerberus*-injected caps, *Xnkx2.1* and *Xnkx2.4* were strongly activated in almost all explants (Fig. 11B′-D′,N′; Table 2) and an increase was also observed in the expression of *eomes* (Fig. 11F′-H′; Table 2). Instead, no significant difference was caused by FGFs on *Xemx1* activation compared with *cerberus* alone (Fig. 11J′-L′; Table 2).

These data indicate that FGF signals can promote ventral forebrain fates and may also be important for regulation of dorsal telencephalic fates. To further investigate this, we interfered with the FGF signaling pathway by using a dominant-negative FGF receptor, ΔXFGFR-4a, which blocks the effects of FGF8 on neural tissues (Hongo et al., 1999; Hardcastle et al., 2000). We therefore injected *ΔXFGFR-4a* mRNA in the animal region of *Xenopus* early embryos and subsequently conjugated stage 9 animal caps explanted from these embryos with a full stage 10-10^{+} organizer. Control conjugates were made with uninjected animal caps and the organizer. Experimental and control conjugates were assayed for the ventral marker *Xnkx2.1* and the dorsal marker *Xemx1* at stage 30/31. Although in control explants both genes are strongly activated (Fig. 12A,B; Table 2), in experimental conjugates, expression of both genes was substantially suppressed (Fig. 12D,E; Table 2). By contrast, there was no apparent effect on neural induction, as the expression of the pan-neural marker *Sox2* (Misuzeki et al., 1998) was essentially the same in the two sets of conjugates (Fig. 12C,F; Table 2). These data therefore suggest that FGF signals are required for correct patterning of the forebrain.

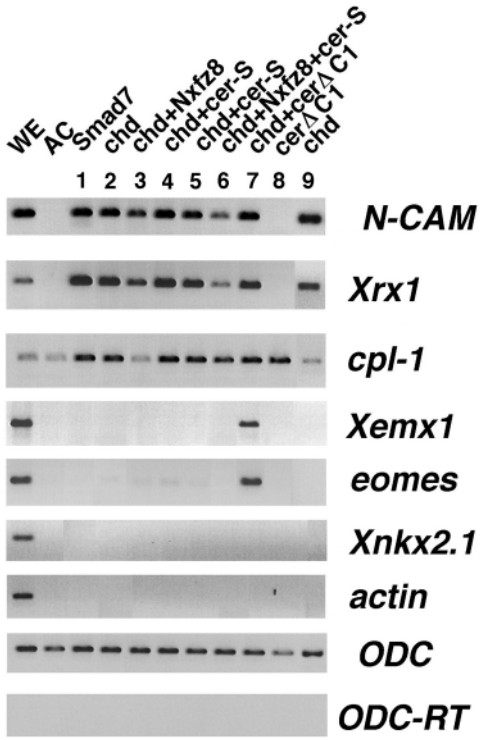

Fig. 10. RT-PCR molecular marker analysis on animal caps injected with various combinations of BMP, Wnt and Nodal inhibitors, as indicated, and grown to stage 30/31. Doses were as follows: (1) 200 pg *Smad7*; (2) 25 pg *chd*; (3) 25 pg *chd*+200 pg *Nxfz8*; (4) 25 pg *chd*+500 pg *cer-S*; (5) 25 pg *chd*+1000 pg *cer-S*; (6) 25 pg *chd*+200 pg *Nxfz8*+500 pg *cer-S*; (7) 25 pg *chd*+500 pg *cerΔC1*; (8) 500 pg *cerΔC1*; (9) 660 pg *chd*.

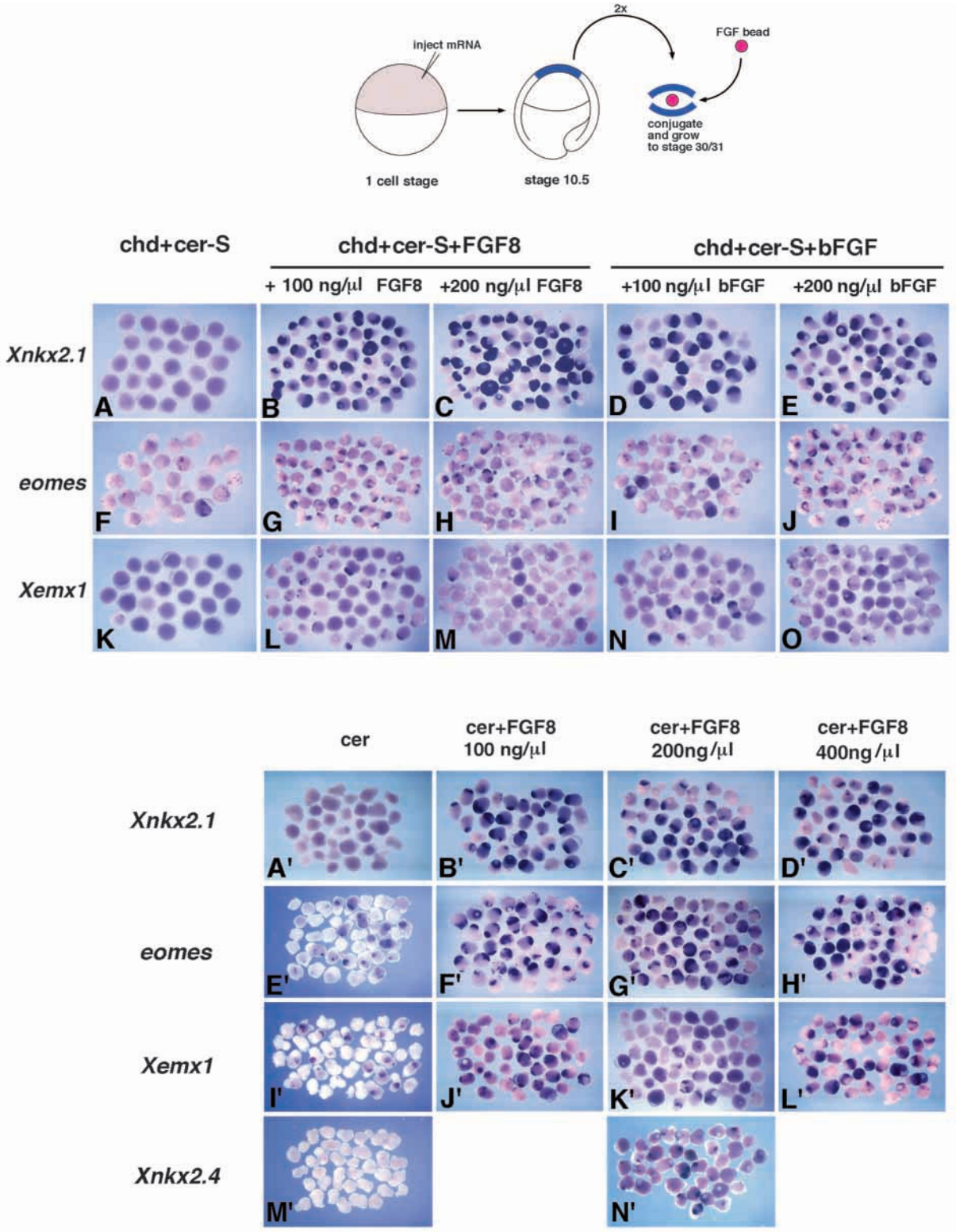

Fig. 11. FGF effect on neuralized animal caps. As shown in the scheme, animal caps were dissected from stage 10.5 embryos injected either with chordin (660 pg)+cer-S (2 ng) mRNA (A-O), or with cerberus mRNA (2 ng) (A′-N′), and recombined in pairs either without addition of FGF-soaked beads (A,F,K,A′,E′,I′,M′) or with FGF-soaked beads (B-E,G-J,L-O,B′-D′,F′-H′,J′-L′,N′). After reaching stage 30/31, they were processed by in situ hybridization for the expression of *Xnkx2.1* (A-E,A′-D′), *eomes* (F-J,E′-H′), *Xemx1* (K-O,I′-L′) or *Xnkx2.4* (M′,N′). Concentrations used for FGF8 were 100 ng/µl (B,G,L,B′,F′,J′), 200 ng/µl (C,H,M,C′,G′,K′,N′) or 400 ng/µl (D′,H′,L′); concentrations used for bFGF were 100 ng/µl (D,I,N) or 200 ng/µl (E′,J′,O′).

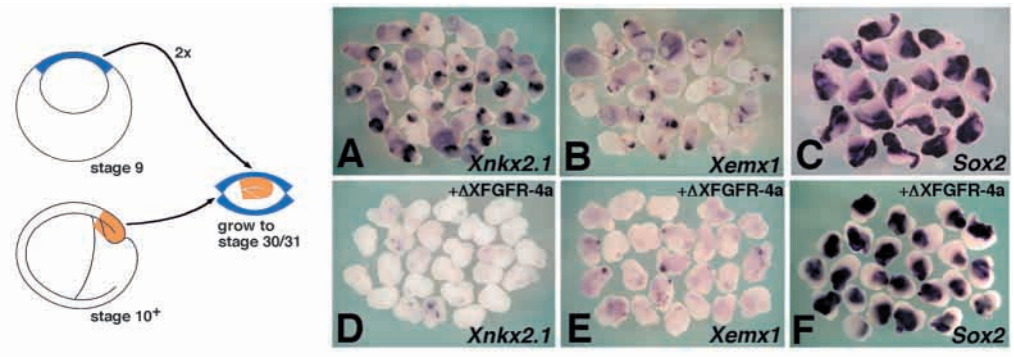

Fig. 12. FGF signaling is required for telencephalic gene expression. As shown in the schematic, the early dorsal blastopore lip (brown) of a stage 10-10$^+$ gastrula was sandwiched either between two uninjected stage 9 animal caps (A-C) or animal caps injected with ΔXFGFR-4a (320 pg/blastomere) (blue) in all four animal blastomeres of eight-cell stage embryos (D-F). Conjugates were grown to stage 30/31 and assayed for expression of *Xnkx2.1* (A,D), *Xemx1* (B,E) or *Sox2* (C,F).

DISCUSSION

Dorsoventral patterning of the telencephalon requires complex signaling

Classical models suggest that neural induction and patterning result from the combined action of two different signaling steps acting on the DE: a first step (activation) that is due to a uniform forebrain-inducing signal, and a second step in which forebrain-induced tissue is posteriorized to presumptive hindbrain and spinal cord (reviewed by Gamse and Sive, 2001; Foley and Stern, 2001; Stern, 2001). Our results on injected animal caps show that molecular signaling proposed to mediate the activation step (namely BMP inhibition) is not sufficient to induce dorsal and ventral telencephalic fates, suggesting that full patterning of the forebrain requires the integration of complex signaling. In fact, although some aspects of forebrain specification may be triggered by BMP inhibitors, as shown by the activation of *Xrx1* (and *XBF-1*, data not shown) (Andreazzoli et al., 1999) in *chd*-injected animal caps, expression of the dorsal telencephalic markers *Xemx1* or *eomes*, or of the ventral forebrain marker *Xnkx2.1* was never observed. Therefore, signals are required to integrate the action of BMP inhibitors in order to specify both dorsal telencephalic values and ventral forebrain values. Because dorsal blastopore lip of stage 10-10$^+$ *Xenopus* embryo is able to induce the dorsal telencephalic markers *Xemx1* and *Xemx2* in naive ectoderm (Pannese et al., 1998), additional signaling may reside in the tissues of the organizer region, namely the ADME. In fact, our recombination experiments with explanted stage 10.5 DE and different region of ADME show that, in spite of its poor neural inducing activity, the ADME can play a patterning role on neuralized DE.

The ADE may be involved in controlling the dorsoventral patterning of the telencephalon

In the last few years, work on several vertebrate models has unravelled a crucial role of anterior endodermal tissues in forebrain development. In particular, the mouse anterior visceral endoderm (AVE) is essential for forebrain induction and patterning, as shown by both embryological and genetic manipulations. Indeed, removal of the AVE at the earliest stages of gastrulation impairs activation of rostral CNS markers in the epiblast (Thomas and Beddington, 1996). Moreover, before their activation in the axial mesendoderm, several genes required for forebrain development, such as *Lim1*, *Otx2*, *HNF3β* and *nodal*, are expressed in the pregastrula stage AVE, where their activities are specifically required for proper forebrain formation (reviewed by Beddington and Robertson, 1998). Recently, the chick hypoblast has been proposed as the embryological and functional equivalent of the mouse AVE. In fact, genetic activities characteristic of mouse AVE are also detectable in chick hypoblast at pre-streak stages; moreover, the hypoblast induces pre-forebrain markers in the epiblast before streak formation and protects the forebrain territory from caudalizing signals by directing cell movements that distance the anterior epiblast from the organizer (Foley et al., 2000). During gastrulation, both mouse AVE and chick hypoblast are displaced by the involuting foregut endoderm; also this tissue has important functions for proper forebrain formation: in chick, removal of the foregut endoderm during gastrulation results in severely compromised forebrain patterning (Withington et al., 2001). In addition, the foregut endoderm shares some of the genetic activities of the mouse AVE or chick hypoblast, such as *cerberus* and *Hex*. Knock-out of the *Hex* gene in the mouse and analysis of chimeric embryos showed that *Hex* function is specifically required in the foregut endoderm for normal forebrain development (Martinez Barbera et al., 2000). Therefore it is likely that the AVE/hypoblast and the foregut endoderm may play similar roles and that the anti-caudalizing activity of the AVE/hypoblast is taken over at later stages by the foregut endoderm and/or prechordal mesendoderm (Foley et al., 2000; Foley and Stern, 2001; Stern, 2001). Although in chick and mouse this activity occurs in two separate tissues (the AVE/hypoblast and the foregut endoderm), in *Xenopus*, the anterior dorsal endoderm (ADE) that constitutes the leading edge of the involuting dorsal mesendoderm may possess the signaling properties of both amniote tissues. Like them, the ADE is the only frog tissue that expresses *cerberus* and *Hex*. Moreover, it displays cell movements reminescent of the mouse AVE (Jones et al., 1999; Foley and Stern, 2001). Finally, the ADE will contribute to the foregut, and, similarly to the foregut endoderm of chick and mouse, it may be important to confer anterior character to the overlaying ectoderm, as judged by the ability to trigger cement gland markers in gastrula ectodermal explants (Bradley et al., 1996; Jones et al., 1999).

Our data suggest a new potential role for the *Xenopus* ADE in the dorsoventral patterning of the forebrain, possibly in synergism with the adjacent prechordal mesendoderm. In fact, the ADE was able to activate the dorsal telencephalic marker *Xemx1* in midgastrula DE explants that, although already specified to forebrain fates, would not express *Xemx1* at the

early tailbud stages. Moreover, expression of the ventral forebrain marker *Xnkx2.1* was suppressed in stage 10.5 DE explants conjugated to the ADE. These data suggest that the ADE may be involved in inducing dorsal telencephalic fates and repressing ventral fates within the prospective forebrain region. This patterning role was further supported by the striking observation that a fragment of ADME, including the ADE together with the anteriormost prechordal mesendoderm, was able to elicit *Xemx1* and *eomes* expression in *chd*-injected caps, where expression of these dorsal telencephalic markers was otherwise never detected. Notably, removal of the anterior definitive endoderm in chick embryos seems to impair proper regionalization of dorsal, but not ventral, forebrain territories, although a more specific molecular marker analysis was not performed (Whitington et al., 2001).

Previous work in *Xenopus* has shown that planar signals spreading from the dorsal mesendoderm are sufficient to induce, in the adjacent ectoderm, neural tissue with a remarkable degree of anteroposterior patterning, including forebrain characters (Doniach at al., 1992; Papalopulu and Kintner, 1993). However, additional vertical signaling is required from the involuting mesendoderm for proper differentiation, morphogenesis and patterning of the nervous system (Dixon and Kintner, 1989; Ruiz i Altaba, 1992). In line with these observations, our results suggest that vertical signals from the ADE and possibly the adjacent ADME may be specifically responsible for proper dorsoventral patterning of the telencephalon during gastrulation.

Molecular signaling specifying dorsal and ventral telencephalic fates

A crucial question concerns the identity of molecules mediating the patterning activity of the ADE. The secreted molecule cerberus was a likely candidate to mediate part of this activity: its expression is restricted to the ADE throughout gastrulation (Bouwmeester et al., 1996), and besides providing a BMP antagonistic effect, cerberus is also endowed with anti-Wnt and anti-Nodal activities (Piccolo et al., 1999), which could account for the patterning effects of the ADE. Remarkably, we found that cerberus was not only able to trigger anterior neural induction and early forebrain markers (such as *Xrx1* and *XBF-1*) (see Results; data not shown) in animal caps, as do other BMP inhibitors, but also to induce the expression of the dorsal telencephalic markers *Xemx1* or *eomes*. We then attempted to define which of the three inhibitory activities of cerberus are required for the induction of these genes. When the anti-Nodal activity of cer-S (Piccolo et al., 1999) and the anti-BMP activity of Chd were combined together, they were not able to induce *Xemx1* and *eomes*. Instead, their efficient induction was obtained by the combination of cer-ΔC1, containing the Wnt-inhibitory activity of cerberus (Fetka et al., 2000), and Chd, while cer-ΔC1 alone did not show any telencephalic or neural inducing activity, at least in the conditions we used. Taken together, with respect to the induction of dorsal telencephalic genes, these results suggest that: (1) the anti-BMP and the anti-Wnt activities of cerberus are both required; and (2) neither of them alone is sufficient, but they might be possibly sufficient in combination. However, in our hands, cer-ΔC1 seemed to retain a partial anti-Nodal activity that has not been previously described (Fetka et al., 2000); thus, at present, a requirement for the anti-Nodal activity of cerberus in the activation of dorsal telencephalic genes cannot be completely excluded. When a different Wnt-antagonist, Nxfz8 (Deardoff et al., 1998), was tried, it did not trigger *Xemx1* or *eomes*, either in combination with Chd or with the further addition of the anti-Nodal activity of cer-S. Because in all the different combinations that we assayed, dorsal telencephalic genes were only induced when the Wnt-inhibitory action of cerberus was included, these results would suggest that dorsal telencephalic induction may require a particular specificity of Wnt inhibition. Besides cerberus, several other inhibitors of Wnt signaling are secreted from the ADE and/or the adjacent prechordal mesendoderm, such as Dkk1 (Glinka et al., 1998), Frzb1 (Leyns et al., 1997; Wang et al., 1997), crescent and Sfrp2 (Pera and De Robertis, 2000). They have different anti-Wnt specificities and different biological activities (Kazanskaya et al., 2000; Pera and De Robertis, 2000); some of them may cooperate with cerberus in inducing the dorsal telencephalon. The requirement of the anti-Wnt activity of cerberus for the induction of dorsal telencephalic genes in animal caps raises the question of which Wnts need to be inhibited. In *Xenopus*, *Xwnt7B* (Chang and Hemmati-Brivanlou, 1998) and *Xwnt8b* (Cui et al., 1995) are widely expressed in the ectodermal region of the embryo during gastrula and neurula developmental stages; furthermore, *Xwnt7B* expression is maintained in animal caps dissected from blastula stage embryos (Chang and Hemmati-Brivanlou, 1998). Therefore, *Xwnt7B* and *Xwnt8b* potentially represent two Wnt activities whose inhibition may be necessary for patterning of the telencephalon in *Xenopus*. This hypothesis is strongly supported by recent work in zebrafish, showing requirement of local Wnt antagonism for telencephalic gene expression within the anterior neuroectoderm, and identifying Wnt8b as a likely target for this antagonism (Houart et al., 2002).

Because FGF8, as other FGFs (Shinya et al., 2001), is expressed in the anterior neural ridge (Crossley and Martin, 1995), and seems to mediate the ability of the latter to promote expression of the telencephalic marker *XBF1* (Shimamura and Rubenstein, 1997; Ye et al., 1998) and also later aspects of telencephalic patterning (Fukuchi-Shigomori and Grove, 2001), we tested whether FGF could have a role in the regulation of dorsal and ventral telencephalic genes. We here show that FGF8 is able to potentiate *eomes* expression in Chd+cer-S or cerberus injected caps. Moreover, *Xemx1* activation in animal caps by the head organizer was severely compromised by overexpression of the dominant-negative ΔXFGFR-4a receptor. Together, these results suggest that cerberus and FGF8 may interact in the specification of the dorsal telencephalon.

We have also found that FGF signals (FGF8 or bFGF) are able to promote strong *Xnkx2.1* expression in animal caps neuralized by cerberus or by the combination of Chd+cer-S; conversely, the dominant negative ΔXFGFR-4a receptor almost completely prevents activation of *Xnkx2.1* in animal caps conjugated to early organizer tissue, without preventing neural induction. These results strongly suggest that FGF signals may be essential for specification of the ventral forebrain. Similar conclusions have been recently reached by Shinya et al. (Shinya et al., 2001), who showed that inhibition of FGF signaling, particularly from FGF3 and FGF8, suppressed development of the ventral telencephalon in zebrafish embryos.

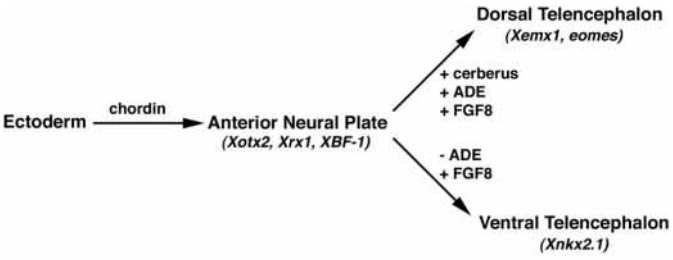

Fig. 13. A proposed model for signaling events occurring during induction and patterning of the telencephalon within the anterior neural plate. Anterior neural plate fate is induced in the ectoderm by secreted BMP inhibitors (such as chordin), which start the expression of region-specific forebrain markers, like *XBF-1* (telencephalon) and *Xrx1* (retina). On this early anterior neural plate, ventral forebrain fates are induced by FGF8 and inhibited by the ADE, while the combined action of cerberus (and possibly other ADE-secreted signals) and FGF8 promotes dorsal telencephalic fates.

In conclusion, our work provides evidence that inductive signals leading to specification of early dorsal and ventral telencephalic territories can be reconstructed, at least in part, on naive animal caps, by specific combinations of signaling molecules. BMP inhibition, though able to possibly provide a general telencephalic fate, is not sufficient for dorsal and ventral telencephalic specification, as it does not activate the dorsal telencephalic markers *Xemx1* and *eomes* or the ventral forebrain marker *Xnkx2.1*. Strong *Xnkx2.1* activation instead occurred when either FGF8 or bFGF were administered to neuralized caps. By contrast, activation of both *Xemx1* and *eomes* expression was detected in animal caps injected with cerberus or the combination of Chd and N-terminal fragment of cerberus, cer-ΔC1, and *eomes* induction was reinforced by the further addition of FGF8 to the explants. A model that summarizes a possible interaction between the molecules and tissues we have studied for dorsoventral patterning of the telencephalon is shown in Fig. 13. According to this, the anterior neural plate is induced in dorsal ectoderm by the action of BMP inhibitors, such as Chd; this initial forebrain-presumptive region may already express region-specific genes such as *Xotx2, Xrx1* and *XBF-1*. Upon this ground, ventral forebrain fates would be induced by FGF signals, possibly secreted from the anterior neural ridge, and inhibited by the ADE. On the same ground, cerberus, possibly through its Wnt-inhibitory activity, and FGF signaling may cooperate in the activation of *Xemx1* and *eomes* and the specification of dorsal telencephalon.

This paper is dedicated to the memory of Rosa Beddington, who shed light onto the role of the anterior endoderm in rostral brain development. Special thanks to Gaia Gestri and Irene Appolloni for help with in situ hybridization. We thank Richard Harland and Steve Wilson for comments on the manuscript, and Hazel Sive for suggestions in starting this work. We thank Tewis Bouwmeester, Jan Christian, Eddy De Robertis, Richard Harland, Peter Klein, Paul Krieg, Harumasa Okamoto, Nancy Papalopulu, Stefano Piccolo, Kenneth Ryan, Yoshiki Sasai, Hazel Sive, Jim Smith, David Wilkinson for plasmids; Donatella De Matienzo, Marzia Fabbri and Dean Pask for technical assistance; and Salvatore Di Maria and Denise O'Connor for frog care. G. L. is grateful to E. M. for support and encouragement. This work was supported by Cofinanziamento MURST-Università di Pisa and the Wellcome Trust. G. L. was supported by an EMBO long-term post-doctoral fellowship.

REFERENCES

Andreazzoli, M., Gestri, G., Angeloni, D., Menna, E. and Barsacchi, G. (1999). Role of *Xrx1* in *Xenopus* eye and anterior brain development. *Development* **126**, 2451-2460.

Bachiller, D., Klingensmith, J., Kemp, C., Belo, J. A., Anderson, R. M., May, S. R., McMahon, J. A., McMahon, A. P., Harland, R. M., Rossant, J. and de Robertis, E. M. (2000). The organizer factors Chordin and Noggin are required for mouse forebrain development. *Nature* **403**, 658-661.

Barth, K. A., Kishimoto, Y., Rohr, K., Seydler, C., Schulte-Merker, S. and Wilson, S. W (1999). Bmp activity establishes a gradient of positional information throughout the entire neural plate. *Development* **126**, 4977-4987.

Beddington, R. S. and Robertson, E. J. (1998). Anterior patterning in mouse. *Trends Genet.* **14**, 277-284.

Bouwmeester, T., Kim, S., Sasai, Y., Lu, B. and de Robertis, E. M. (1996). Cerberus is a head-inducing secreted factor expressed in the anterior endoderm of Spemann's organizer. *Nature* **382**, 595-601.

Boyl, P. P., Signore, M., Annino, A., Barbera, J. P., Acampora, D. and Simeone, A. (2001). *Otx* genes in the development and evolution of the vertebrate brain. *Int. J. Dev. Neurosci.* **19**, 353-363.

Bradley, L. C., Snape, A., Bhatt, S. and Wilkinson, D. G. (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.

Bradley, L., Wainstock, D. and Sive. H. (1996). Positive and negative signals modulate formation of the *Xenopus* cement gland. *Development* **122**, 2739-2750.

Bulfone, A., Puelles, L., Porteus, M. H., Frohman, M. A., Martin, G. R. and Rubenstein, J. L. R. (1993). Spatially restricted expression of Dlx-1, Dlx-2 (Tes-1), Gbx-2, and Wnt-3 in the embryonic day 12.5 mouse forebrain defines potential transveres and longitudinal segmental boundaries. *J. Neurosci.* **13**, 3155-3172.

Casarosa, S., Andreazzoli, M., Simeone, A. and Barsacchi, G. (1997). *Xrx1*, a novel *Xenopus* homeobox gene expressed during eye and pineal gland development. *Mech. Dev.* **61**, 187-198.

Chang, C. and Hemmati-Brivanlou, A. (1998). Neural crest induction by *Xwnt7B* in *Xenopus*. *Dev. Biol.* **194**, 129-134.

Cho, K. W. Y., Blumberg, B., Steinbeisser, H. and de Robertis, E. M. (1991). Molecular nature of Spemann's organizer: the role of the *Xenopus* homeobox gene *goosecoid. Cell* **67**, 1111-1120.

Crossley, P. H. and Martin, G. R. (1995). The mouse Fgf8 gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo. *Development* **121**, 439-451.

Cui, Y., Brown, J. D., Moon, R. T. and Christian, J. L. (1995). *Xwnt-8b*: a maternally expressed *Xenopus Wnt* gene with a potential role in establishing the dorsoventral axis. *Development* **121**, 2177-2196.

Deardoff, M., Tan, C., Conrad, L. J. and Klein, P. S. (1998). Frizzled-8 is expressed in the Spemann organizer and plays a role in early morphogenesis. *Development* **125**, 2687-2700.

Dixon, J. E. and Kintner, C. R. (1989). Cellular contacts required for neural induction in *Xenopus* embryos: evidence for two signals. *Development* **106**, 749-757.

Doniach, T., Phillips, C. R. and Gerhart, J. C. (1992). Planar induction of anteroposterior patterning in the developing central nervous system of *Xenopus laevis*. *Science* **257**, 542-545.

Fetka, I., Doederlein, G. and Bouwmeester, T. (2000). Neuroectodermal specification and regionalization of the Spemann organizer in *Xenopus*. *Mech. Dev.* **93**, 49-58.

Foley, A. C., Skromne, I. and Stern, C. D. (2000). Reconciling different models of forebrain induction and patterning: a dual role for the hypoblast. *Development* **127**, 3839-3854.

Foley, A. C. and Stern, C. D. (2001). Evolution of vertebrate forebrain development: how many different mechanisms? *J. Anat.* **199**, 35-52.

Fukuchi-Shigomori, T. and Grove, E. A. (2001). Neocortex patterning by the secreted signaling molecule FGF8. *Science* **294**, 1071-1074.

Furuta, Y., Piston, D. W. and Hogan, B. L. M. (1997). Bone morphogenetic proteins (BMPs) as regulators of dorsal forebrain develoment. *Development* **124**, 2203-2212.

Gamse, J. T. and Sive, H. (2001). Early anteroposterior division of the presumptive neurectoderm in *Xenopus*. *Mech. Dev.* **104**, 21-36.

Glinka, A., Wu, W., Onichtchouk, D., Blumenstock, C. and Niehrs, C. (1997). Head induction by simultaneous repression of Bmp and Wnt signalling in *Xenopus*. *Nature* **389**, 517-519.

Glinka, A., Wu, W., Onichtchouk, D., Blumenstock, C. and Niehrs, C.

(1998). Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* **3**, 357-362.

Golden, J. A., Bracilovic, A., McFadden, K. A., Beesely, J. S., Rubenstein, J. L. R. and Grinspan, J. B. (1999). Ectopic BMP5 and BMP4 in the chick forebrain leads to cyclopia and holoprosencephaly. *Proc. Natl. Acad. Sci. USA* **96**, 2439-2444.

Gont, L. K., Steinbeisser, H., Blumberg, B. and de Robertis, E. M. (1993). Tail formation as a continuation of gastrulation: the multiple cell populations of the *Xenopus* tailbud derive from the late blastopore lip. *Development* **119**, 991-1004.

Gunhaga, L., Jessel, T. M. and Edlund, T. (2000). Sonic hedgehog signaling at gastrula stages specifies ventral telencephalic cells in the chick embryos. *Development* **127**, 3283-3293.

Hansen, C., Marion, C. D., Steele, K., George, S. and Smith, W. C. (1997). Direct neural induction and selective inhibition of mesoderm and epidermis inducers by Xnr3. *Development* **124**, 483-492.

Hardcastle, Z., Chalmers, A. D. and Papalopulu, N. (2000). FGF-8 stimulates neuronal differentiation through FGFR-4a and interferes with mesoderm induction in *Xenopus* embryos. *Curr. Biol.* **10**, 1511-1514.

Harland, R. M. (1991). In situ hybridization: an improved wholemount method for *Xenopus* embryos. *Methods Cell Biol.* **36**, 675-685.

Heisenberg, C., Houart, C., Take-Uchi, M., Rauch, G., Young, N., Coutinho, P., Masai, I., Caneparo, L., Concha, M., Geisler, R. et al. (2001). A mutation in the Gsk3-binding domain of zebrafish Masterblind/Axin1 leads to a fate transformation of telencephalon and eyes to diencephalons. *Genes Dev.* **15**, 1427-1434.

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.

Henry, G. L. and Melton, D. A. (1998). *Mixer,* a homeobox gene required for endoderm development. *Science* **281**, 91-96.

Hongo, I., Kengaku, M. and Okamoto, H. (1999). FGF signaling and the anterior neural induction in *Xenopus*. *Dev. Biol.* **216**, 561-581

Houart, C., Caneparo, L., Heisenberg, C. P., Barth, K. A., Take-Uchi, M. and Wilson, S. W. (2002). Establishment of the telencephalon during gastrulation by local antagonism of Wnt signalling. *Neuron* **35**, 255-265.

Itoh, K. and Sokol, S. Y. (1999). Axis determination by inhibition of Wnt signaling in Xenopus. *Genes Dev.* **13**, 2328-2336.

Jones, C. M., Broadbent, J., Thomas, P. Q., Smith, J. C. and Beddington, R. S. (1999). An anterior signalling centre in *Xenopus* revealed by the homeobox gene *XHex*. *Curr. Biol.* **9**, 946-954.

Kazanskaya, O., Glinka, A. and Niehrs, C. (2000). The role of *Xenopus dickkopf1* in prechordal plate specifcation and neural patterning. *Development* **127**, 4981-4992.

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.

Knecht, A. K. and Harland, R. M. (1997). Mechanisms of dorsal-ventral patterning in noggin-induced neural tissue. *Development* **124**, 2477-2488.

Krieg, P. A. and Melton, D. A. (1984). Functional messenger RNAs are produced by SP6 in vitro trasncription of cloned cDNAs. *Nucleic Acids Res.* **12**, 7057-7070.

Lamb, T. M. and Harland, R. M. (1995). Fibroblast growth factor is a direct neural inducer, which combined with noggin generates anterior-posterior neural pattern. *Development* **121**, 3627-3636.

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.

Leyns, L., Bouwmeester, T., Kim, S.-H., Piccolo, S. and de Robertis, E. M. (1997). Frzb-1 is a secreted antagonist of Wnt signaling expressed in the Spemann Organizer. *Cell* **88**, 747-756.

Liu, Y., Lupo, G., Marchitiello, A., Gestri, G., He, R.-Q., Banfi, S. and Barsacchi, G. (2001). Expression of the *Xvax2* gene demarcates presumptive ventral telencephalon and specific visual structures in *Xenopus laevis*. *Mech. Dev.* **100**, 115-118.

Martinez Barbera, J. P., Clements, M., Thomas, P., Rodriguez, T., Meloy, D., Kioussis, D. and Beddington, R. S. (2000). The homeobox gene *Hex* is required in definitive endodermal tissues for normal forebrain, liver and thyroid formation. *Development* **127**, 2433-2445.

Mayor, R., Morgan, R. and Sargent, M. (1995). Induction of the prospective neural crest of *Xenopus*. *Development* **121**, 767-777.

Meno, C., Gritsman, K., Ohishi, S., Ohfuji, Y., Heckscher, E., Mochida, K., Shimono, A., Kondoh, H., Talbot, W. S., Robertson, E. J., Schier, A. F. and Hamada, H. (1999). Mouse Lefty2 and zebrafish antivin are feedback inhibitors of nodal signaling during vertebrate gastrulation. *Mol. Cell* **4**, 287-298.

Misuzeki, K., Kishi, M., Matsui, M., Nakanishi, S. and Sasai, Y. (1998). *Xenopus* Zic-related-1 and Sox-2, two factors induced by chordin, have distinct activities in the initiation of neural induction. *Development* **125**, 579-587.

Mohun, T. J., Brennan, S., Dathan, N., Fairman, S. and Gurdon, J. B. (1984). Cell type-specific activation of actin genes in the early amphibian embryo. *Nature* **311**, 716-721.

Nakayama, T., Berg, L. K. and Christian, J. (2001). Dissection of inhibitory Smad proteins: both N- and C-terminal domains are necessary for full activities of Xenopus Smad6 and Smad7. *Mech. Dev.* **100**, 251-262.

Newport, J. and Kirschner, M. (1982). A major developmental transition in early *Xenopus* embryos. II. Control of the onset of transcription. *Cell* **30**, 687-696.

Nieuwkoop, P. D. and Nigtevecht, G. V. (1954). Neural activation and transformation in explants of competent ectoderm under the influence of fragments of anterior notochord in urodeles. *J. Embryol. Exp. Morphol.* **2**, 175-193.

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

Nieuwkoop, P. D., Botterenbrood, E. C., Kremer, A., Bloesma, F. F. S. N., Hoessels, E. L. M. J., Meyer, G. and Verheyen, F. J. (1952). Activation and organization of the central nervous system in Amphibians. *J. Exp. Zool.* **120**, 1-108.

Pannese, M., Polo, C., Andreazzoli, M., Vignali, R., Kablar, B., Barsacchi, G. and Boncinelli, E. (1995). The *Xenopus* homologue of *Otx2* is a maternal homeobox gene that demarcates and specifies anterior body regions. *Development* **121**, 707-720.

Pannese, M., Lupo, G., Kablar, B., Boncinelli, E., Barsacchi, G. and Vignali, R. (1998). The *Xenopus Emx* genes identify presumptive dorsal telencephalon and are induced by head orgnaizer signals. *Mech. Dev.* **73**, 73-83.

Papalopulu, N. and Kintner, C. (1993). *Xenopus Distal-less* related homeobox genes are expressed in the developing forebrain and are induced by planar signals. *Development* **117**, 961-975.

Papalopulu, N. and Kintner, C. (1996). A posteriorizing factor, retinoic acid, reveals that anteroposterior patterning controls the timing of neuronal differentiation in *Xenopus* neuroectoderm. *Development* **122**, 3409-3418.

Pera, E. M. and de Robertis, E. M. (2000). A direct screen for secreted proteins in *Xenopus* embryos identifies distinct activities for the Wnt antagonists Crescent and Frzb-1. *Mech. Dev.* **96**, 183-195.

Pera, E. M., Wessely, O., Li, S.-Y. and de Robertis, E. M. (2001). Neural and head induction by insulin-like growth factor signals. *Dev. Cell* **1**, 655-665.

Piccolo, S., Sasai, Y., Lu, B. and de Robertis, E. M. (1996). Dorsoventral patterning in *Xenopus*. Inhibition of ventral signals by direct binding of Chordin to BMP-4. *Cell* **86**, 589-598.

Piccolo, S., Agius, E., Leyns, L., Bhattacharyya, S., Grunz, H., Bouwmeester, T. and de Robertis, E. M. (1999). The head inducer Cerberus is a multifunctinal antagonist of Nodal, BMP and Wnt signals. *Nature* **397**, 707-710.

Rohr, K. B., Barth, K. A., Varga, Z. M. and Wilson, S. W. (2001). The nodal pathway acts upstream of hedgehog signaling to specify ventral telencephalic identity. *Neuron* **29**, 341-351.

Rubenstein, J. L., Shimamura, K., Martinez, S. and Puelles, L. (1998). Regionalization of the prosencephalic neural plate. *Annu. Rev. Neurosci.* **21**, 445-477.

Ruiz i Altaba, A. (1992). Planar and vertical signals in the induction and patterning of the *Xenopus* nervous system. *Development* **116**, 67-80.

Ryan, K., Butler, K., Bellefroid, E. and Gurdon, J. B. (1998). *Xenopus eomesodermin* is expressed in neural differentiation. *Mech. Dev.* **75**, 155-158.

Sasai, Y. and de Robertis, E. M. (1997). Ectodermal patterning in vertebrate embryos. *Dev. Biol.* **182**, 5-20.

Sasai, Y., Lu, B., Steinbeisser, H., Geissert, D., Gont, L. K. and de Robertis, E. M. (1994). *Xenopus* chordin: a novel dorsalizing factor activated by Organizer-specific homeobox genes. *Cell* **79**, 779-790.

Shanmugalingam, S., Houart, C., Picker, A., Reifers, F. MacDonald, R., Barth, K. A., Griffin, K., Brand, M. and Wilson, S. W. (2000). Ace/fgf8 is required for forebrain commissure formation and patterning of the telencephalon. *Development* **127**, 2549-2561.

Shimamura, K. and Rubenstein, J. L. R. (1997). Inductive interactions

direct early regionalization of the mouse forebrain. *Development* **124**, 2709-2718.

Shimamura, K., Hartigan, D. J., Martinez, S., Puelles, L. and Rubenstein, J. L. R. (1995). Longitudinal organization of the anterior neural plate and neural tube. *Development* **121**, 3923-3933.

Shimamura, K., Martinez, S., Puelles, L. and Rubenstein, J. L. R. (1996). Patterns of gene expression in the neural plate and neural tube subdivide the embryonic forebrain into transverse and longitudinal domains. *Dev. Neurosci.* **19**, 88-96.

Shinya, M., Koshida, S., Sawada, A., Kuroiwa, A. and Takeda, H. (2001). Fgf signalling through MAPK cascade is required for development of the subpallial telencephalon in zebrafish embryos. *Development* **128**, 4153-4164.

Simeone, A., Acampora, D., Gulisano, M., Stornaiuolo, A. and Boncinelli, E. (1992). Nested expression domains of four homeobox genes in developing rostral brain. *Nature* **358**, 987-690.

Small, E. M., Vokes, S. A., Garriock, R. J., Li, D. and Krieg, P. A. (2000). Developmental expression of the *Xenopus Nkx2-1* and *Nkx2-4* genes. *Mech. Dev.* **96**, 259-262.

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

Smith, W. C., McKendry, R., Ribisi, S. and Harland, R. M. (1995). A nodal-related gene defines a physical and functional domain within the Spemann organizer. *Cell* **82**, 37-46.

Stern, C. D. (2001). Initial patterning of the central nervous system: how many organizers? *Nat. Rev. Neurosci.* **2**, 92-98.

Streit, A., Berliner, A. J., Papanoyotou, C., Sirulnik, A. and Stern, C. D. (2000). Initiation of neural induction by FGF signalling before gastrulation. *Nature* **406**, 74-78.

Thomas, P. and Beddington, R. (1996). Anterior primitive endoderm may be responsible for patterning the anterior neural plate in the mouse embryo. *Curr. Biol.* **6**, 1487-1496.

Vignali, R., Poggi, L., Madeddu, F. and Barsacchi, G. (2000). *HNF1β* is required for mesoderm induction in the *Xenopus* embryo. *Development* **127**, 1455-1465.

Wang, S., Krinks, M., Lin, K., Luyten, F. P. and Moos, M., Jr (1997). Frzb, a secreted protein expressed in the Spemann organizer, binds and inhibits Wnt-8. *Cell* **88**, 757-766.

Wilson, S. W. and Rubenstein, J. L. R. (2000). Induction and dorsoventral patterning of the telencephalon. *Neuron* **28**, 641-651.

Withington, S., Beddington, R. and Cooke, J. (2001). Foregut endoderm is required at head process stages for anteriormost neural patterning in chick. *Development* **128**, 302-320.

Wright, C. V. E., Morita, E. A., Wilkin, D. J. and de Robertis, E. M. (1990). The *Xenopus* XlHbox6 homeo protein, a marker of posterior neural induction, is expressed in proliferating neurons. *Development* **109**, 225-234.

Ye, W., Shimamura, K., Rubenstein, J. L. R., Hynes, M. A. and Rosenthal, A. (1998). FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. *Cell* **93**, 755-766.

Zimmermann, L. B., de Jesùs-Escobar, J. M. and Harland, R. M. (1996). The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein-4. *Cell* **86**, 599-606.