An early requirement for FGF signalling in the acquisition of neural cell fate in the chick embryo
Sara I. Wilson*, Enrique Graziano*, Richard Harland*, Thomas M. Jessell† and Thomas Edlund*

Background:
In Xenopus embryos, fibroblast growth factors (FGFs) and bone morphogenetic protein (BMP)-mediated signalling have been implicated in neural induction. The precise roles, if any, that these factors play in neural induction in amniotes remains to be established.

Results:
To monitor the initial steps of neural induction in the chick embryo, we developed an in vitro assay of neural differentiation in epiblast cells. Using this assay, we found evidence that neural cell fate is specified in vitro, before the generation of the primitive streak or Hensen’s node. Early epiblast cells expressed both Bmp4 and Bmp7, but the expression of both genes was downregulated as cells acquired neural fate. During prestreak and gastrula stages, exposure of epiblast cells to BMP4 activity in vitro was sufficient to block the acquisition of neural fate and to promote the generation of epidermal cells. Fgf3 was also found to be expressed in the early epiblast, and ongoing FGF signalling in epiblast cells was required for acquisition of neural fate and for the suppression of Bmp4 and Bmp7 expression.

Conclusions:
The onset of neural differentiation in the chick embryo occurs in utero, before the generation of Hensen’s node. Fgf3, Bmp4 and Bmp7 are each expressed in prospective neural cells, and FGF signalling appears to be required for the repression of Bmp expression and for the acquisition of neural fate. Subsequent exposure of epiblast cells to BMPs, however, can prevent the generation of neural tissue and induce cells of epidermal character.

Background
The induction of neural tissue represents an early and fundamental step in the generation of the vertebrate nervous system. During gastrulation, multipotent ectodermal cells are directed to a neural fate. Much of our understanding of neural induction in vertebrates derives from studies performed in Xenopus embryos. Here, the embryonic ectoderm initially expresses members of the bone morphogenetic protein (BMP)-class of transforming growth factor-β (TGF-β) proteins, and signals mediated by these factors can induce differentiation of epiblast cells into epidermal ectoderm [1,2]. Several BMP inhibitors that are secreted by organism cells have been shown to induce neural differentiation, apparently by blocking BMP signalling activity within the ectoderm or by repressing the expression of genes encoding BMPs [3-8]. Collectively, these studies have led to the prevailing view that BMP inhibitors derived from Spemann’s organiser at gastrula stages have a critical role in neural induction in Xenopus.

In amniotes, however, the role of BMP inhibitors derived from the organiser/node as neural inducers remains ambiguous [9,10]. In mice, neural differentiation proceeds in the absence of activities of the genes encoding the BMP inhibitors noggin, chordin or follistatin, and neural plate cells are also generated in mice lacking both noggin and chordin genes [11-13]. In addition, mouse embryos mutant for the HNF3β gene lack a recognisable node, and both noggin and chordin expression are absent, but still these embryos form neural tissue [14]. In both chick and mouse, cells in the region of the organiser/Hensen’s node can induce ectopic neural cells when transplanted to competent regions of the ectoderm [15,16]. But in avian embryos, chordin alone is unable to mimic the activity of Hensen’s node to induce neural differentiation in prospective extraembryonic tissue [9]. Moreover, in chick, the temporal patterns of expression of noggin, chordin and follistatin do not coincide with the ability of the organiser tissue to induce ectopic neural cells [9,15,17]. These studies leave unresolved the extent to which views of neural induction derived from studies in Xenopus are applicable in amniotes.

As a first step to examine the identity of signals that influence the differentiation of neural tissue in the chick embryo, and to determine the time at which these signals operate, we have developed an in vitro assay of neural cell
differentiation. Explants derived from definitive embryonic tissue in utero, before the initiation of gastrulation. Prospective neural cells expressed genes for fibroblast growth factors (FGFs), and we found that FGF signalling was required for epiblast cells to acquire an overt neural character. Moreover, FGFs acted to repress Bmp expression in epiblast cells, providing a potential link between the signalling activities of FGFs and the extinction of BMP signalling during the induction of neural tissue.

Results

In previous studies, we found that, at Hamburger and Hamilton (HH) stage 3, the definitive primitive streak stage, prospective neural cells isolated from the chick epiblast and grown in vitro acquire forebrain character, as assessed by a series of molecular markers [18]. To extend these studies, we examined whether cells in younger pre-streak-stage chick embryos also acquired neural character when grown in vitro. We monitored the expression of several transcription factors to assess the early neural or epithelial character of epiblast cells. Sox3 is initially expressed widely by epiblast cells but its expression gradually becomes restricted to neural plate cells [19], whereas Sox2 expression is initially restricted to neural plate cells [19]. The expression of Otx2 defines neural cells normally located at levels rostral to the hindbrain [20], whereas the expression of Pax6 defines cells at forebrain, hindbrain and spinal cord levels of the neural tube [21]. Although not all of these markers are initially restricted exclusively to neural cells, the coincidence in their expression serves to define neural cells in an unambiguous manner. The expression of Mx1/2 by early chick embryos is found in cells located at the border between the epidermis and the neural plate [22], and Mx1 activity can induce epidermal cells and block the generation of neural tissue, at least in Xenopus [23]. GATA2 is expressed in the lateral regions of pre-streak embryos in an anterior to posterior pattern and, from late gastrula stages, its expression defines the entire non-neural ectoderm and is excluded from the prospective neural plate [24]. Thus, the coincidence in expression of Mx1/2 and GATA2 defines non-neural cells of epithelial character.

Specification of neural cells in chick is initiated in utero

We first attempted to define the time at which epiblast cells acquire neural character when isolated and grown in vitro. Epiblast tissue was isolated from medial and lateral regions of the area pellucida of Elyal-Giladi and Kochav stage VIII–IX chick embryos [25] (Figure 1a,n). Explants were grown in vitro for 40 hours and then assayed for the generation of epidermal and neural marker expression. Stage VIII medial explants generated Msx1/2 cells, whereas no expression of Sox2, Sox3, Otx2, Pax6 or GATA2 was detected. Stage VIII lateral explants generated Msx1/2 cells and GATA2 cells (Figure 1b–m). Stage IX medial explants generated Sox2+ cells, Sox3+ cells, Otx2+ cells, Pax6+ cells and also generated Mx1/2+ cells in non-overlapping regions of individual explants, but no GATA2+ cells were detected (Figure 1n–o). Stage IX medial explants generated Sox2+ cells, Sox3+ cells, Otx2+ cells, Pax6+ cells and also generated Mx1/2+ cells in non-overlapping regions of individual explants, but no GATA2+ cells were detected (Figure 1n–o).

Figure 1

Specification of cells in stage VIII and IX chick embryos. (a) Schematic representation of an Elyal-Giladi and Kochav stage VII embryo. The area pellucida (white) is indicated. The boxed areas indicate where the medial (M, blue) and lateral (L, yellow) area pellucida explants were isolated for in vitro studies. (b–g) Stage VIII medial explants cultured for 40 h (n = 6) did not generate (b) Sox2+ cells, (c) Sox3+ cells, (d) Pax6+ cells, (e) Otx2+ cells or (f) GATA2+ cells, but did generate (g) Mx1/2+ cells (95 ± 5% cells per section). (h–n) Stage VIII lateral explants cultured for 40 h (n = 6) did not generate (h) Sox2+ cells, (i) Sox3+ cells, (j) Pax6+ cells or (k) Otx2+ cells, but did generate (l) Mx1/2+ cells (96 ± 5% cells per section) and (m) GATA2+ cells. (n) Schematic representation of a stage IX embryo. The area pellucida and the regions where medial and lateral explants were derived are indicated. (o–z) Stage IX medial explants cultured for 40 h (n = 6) generated (o) Sox2+ cells, (p) Sox3+ cells, (q) Pax6+ cells, (r) Otx2+ cells (95 ± 5% cells per section) and (s) Mx1/2+ cells (95 ± 10% cells per section), but no (t) GATA2+ cells. (u–z) Stage IX lateral explants cultured for 40 h (n = 6) did not generate (u) Sox2+ cells, (v) Sox3+ cells, (w) Pax6+ cells or (x) Otx2+ cells, but did generate (y) Mx1/2+ cells (96 ± 5% cells per section) and (z) GATA2+ cells. The scale bar in (z) represents 100 μM and applies to (b–m, o–z).
lateral explants, in contrast, generated only Msx1/2+ cells and GATA2+ cells (Figure 2a–g). These results provide evidence that medial epiblast cells acquire the capacity to express neural markers in vitro, soon after stage VIII.

We next isolated medial and lateral explants from stage XII embryos (Figure 2a). At this stage, medial explants generated Sox2+ cells, Sox3+ cells, Otx2+ cells and Pax6+ cells, but no expression of Msx1/2 or GATA2 was detected (Figure 2b–g). Lateral explants generated Msx1/2+ cells and GATA2+ cells (Figure 2h–m).

To define in more detail the location of prospective neural cells in stage XII embryos, we dissected the epiblast into 100 explants and monitored the generation of neural and epidermal cells after 40 hours of culture. Explants isolated from the central region of the epiblast generated Sox2+ cells, Sox3+ cells, Otx2+ cells and Pax6+ cells and no Msx1/2+ cells (Figure 3a). Explants isolated from the peripheral region of the epiblast generated Msx1/2+ cells, but no expression of Sox2, Sox3, Otx2 or Pax6 was detected (Figure 3b). Most or all individual explants isolated from the region between the central and peripheral epiblast generated both Msx1/2+ cells and Sox2+ cells, Sox3+ cells, Otx2+ cells and Pax6+ cells in non-overlapping domains of the explants (Figure 3a,b). Explants that generated Sox2+ cells, Sox3+ cells, Otx2+ cells and Pax6+ cells covered approximately two-thirds of the area pellucida. This central region was oval in shape and extended posteriorly towards the centre of the Koller’s sickle (Figure 2n). Cells that acquired Msx1/2 expression were located peripheral to prospective neural cells at all anteroposterior positions of the area pellucida (Figure 2n). Cells that acquired GATA2 expression were located in the most peripheral regions of the area pellucida except, in the most posterior region of the embryo, where epiblast cells failed to express GATA2. Thus, at stage XII, prospective neural cells are located in the central region of the embryo, and prospective epidermal cells are found in the peripheral region of the embryo.

Fate maps and lineage analyses have provided evidence that the epiblast of stage XII embryos consists of a mosaic of presumptive ectodermal, mesodermal and neuroectodermal cells [26]. To examine whether mesoderm, a potential source of neural-inducing signals, is generated during culture of medial explants from stage XII, we monitored the expression of three mesodermal markers Brachyury, chordin and Raldh2, by reverse transcription (RT)-PCR. Brachyury is expressed in mesoderm precursor cells located in the primitive streak, in Hensen’s node and in axial mesoderm [27]. Chordin is expressed in Hensen’s node and subsequently in axial mesoderm [9], and Raldh2 is selectively expressed in paraxial mesoderm [28]. No expression of Brachyury, chordin or Raldh2 was detected in stage XII medial explants after 40 hours of culture (Figure 2o, lane 2). Thus, in stage XII medial explants, neural markers are expressed in the absence of the generation of mesoderm.

Downregulation of Bmp4 and Bmp7 expression in prospective neural cells in Xenopus, the exposure of ectodermal cells to BMPs has been shown to block the generation of neural tissue and
to induce epidermal character [1]. The expression of Bmp4 is downregulated in the prospective neural plate cells during neural induction [1,2], and there is evidence implicating Msx1 in the BMP-mediated induction of epi-
dermal tissue [23]. Similarly, in the embryonic region of prestreak chick embryos, Bmp4 and Bmp7 are expressed at low levels and are downregulated before the formation of the primitive streak [9].

To determine whether the extinction of Bmp expression correlates with the generation of neural cells in chick epi-
blast explants, we examined whether Bmp4 and Bmp7 expression is downregulated in stage XII medial and lateral explants grown in culture. Bmp4 and Bmp7 were initially expressed in both stage XII medial and lateral epiblast tissue prior to growth in vitro (Figure 4a, lanes 1,5). The expression of both Bmps was downregulated in medial explants (Figure 4a, lane 2), but was maintained in lateral explants (Figure 4a, lane 6) after 40 hours in vitro. Thus, the extinction of Bmp4 and Bmp7 expression by prospective neural cells correlates with the acquisition of neural character.

**BMP4 induces epidermal character in epiblast cells**

In stage XII embryos, Bmp7 is expressed at high levels in the area opaca [9], a region located adjacent to the lateral area pellucida cells which acquire epidermal character. Moreover, Msx1/2 can be induced by BMP signalling in chick embryos [29,30]. We therefore determined whether the exposure of prospective neural epiblast cells to BMPs induces epidermal character and blocks the acquisition of neural character. Stage XII medial epiblast explants grown in the presence of BMP4 (1.3 nM) for 40 hours generated Sox2+ cells, Sox3+ cells and Otx2+ cells, but no Pax6+, Msx1/2+ or GATA2+ cells were detected (Figure 5a-f). Thus, BMP4 induces the generation of epidermal cells in prestreak and early gas-
tula stage epiblast explants. By late gastrula stages, however, prospective neural cells appear to be refractory to the actions of BMP4.

**FGF signalling is required for the specification of neural cells**

We next examined the pattern of expression of genes encoding BMP inhibitors in the early epiblast. No expres-
sion of the genes encoding the secreted BMP inhibitors noggin, chordin, follistatin or Caronte was detected in stage XII medial or lateral epiblast explants at the time of isola-
tion or after 40 hours in culture (Figure 2o and data not shown). Thus, the generation of neural cells in stage XII medial explants is not likely to require the activity of these BMP inhibitors.

FGFs have been implicated in neural induction in the chick embryo [31,32], and Fgf3 is expressed in early gas-
tula stage embryos [33]. We therefore tested whether Fgf3 was expressed by epiblast cells in the area pellucida of prestreak stage XII embryos. Fgf3 expression was detected in stage XII medial and lateral epiblast explants at the time of isola-
tion and after 40 hours of culture (Figure 4a, lanes 1,2 and 5,6).

FGF3 signalling is mediated mainly through activation of the FGF receptors (FGFRs) 1b and 2b [34,35]. The Fgfr2b gene was expressed in stage XII medial explants at the time of isolation and after 40 hours of culture (Figure 4b, lanes 1,2). To test whether ongoing FGF sig-
nalling was required for the acquisition of neural charac-
ter by epiblast cells, we cultured stage XII medial
explanations in the presence of different concentrations of the FGF-receptor tyrosine kinase inhibitor SU5402 [18,36]. SU5402 has been shown to attenuate FGF signalling in chick neural plate explants and in chick embryos [37] without affecting the signalling pathways mediated by BMP and Hedgehog factors [18]. In the presence of SU5402 (2 µM), stage XII medial explants grown for 40 hours in vitro generated Msx2+ cells and GATA2+ cells. In contrast, no Sox2+, Sox3+, Otx2+ or Pax6+ cells were generated (Figure 6a–l). In addition, Fgf3 expression was not detected (Figure 4a, lane 3). Addition of FGF1 (33 nM) in the presence of SU5402 (2 µM) to stage XII medial explants was able to overcome the block in neural differentiation. Sox2+, Sox3+, Otx2+ and Pax6+ cells, but few Msx2+ cells and no GATA2- cells were detected (data not shown). These findings support the idea that the action of SU5402 is mediated by blockade of FGF signalling rather than by inhibition of other signalling pathways. They also suggest that ongoing FGF signalling is required to maintain the potential for neural differentiation in cells in stage XII medial epiblast explants, and also to maintain Fgf3 expression.

**FGF signalling downregulates Bmp expression in prospective neural cells**

The results described above indicate that attenuation of FGF signalling and exposure to BMP4 can each suppress the acquisition of neural character in stage XII medial explants. In view of these findings, we considered whether FGF signalling might have a role in the repression of Bmp expression in epithelial cells of prospective neural character. To test this, stage XII medial explants were exposed to SU5402 (2µM) and the expression of Bmp4 and Bmp7 analysed. In marked contrast to the normal loss of Bmp4 and Bmp7 expression, the expression of both genes was maintained in the presence of SU5402 (Figure 4a, lane 3).

To examine whether blockade of BMP signalling could result in the generation of neural cells under these conditions, we cultured stage XII medial explants in the presence of both SU5402 (2 µM) and Xenopus noggin (64 nM). Under these conditions, stage XII medial explants generated Sox2+ cells, Sox3+ cells, Otx2+ cells and Pax6+ cells (Figure 6g–j). In addition, Fgf3 expression was maintained, although Msx2+ cells or GATA2+ cells were detected after 40 hours in culture (Figure 4a, lane 4; Figure 6k–l). Under these conditions, Bmp4 and Bmp7 were still expressed (Figure 4a, lane 4). Similar results were obtained when stage XII medial explants were cultured with either medium containing chick chordin (data not shown) or soluble isoforms of the dominant negative BMP receptor, 1A-Fc and 1B-Fcs (0.5 µM each) (Figure 6m–r). Thus, the attenuation of FGF signalling appears to prevent the downregulation of Bmp expression in epithelial explants. Nevertheless, the blockade of BMP signalling activity under these conditions is sufficient to promote the acquisition of neural character and to maintain Fgf3 expression. Together, these results suggest that ongoing FGF signalling during early phase of neural cell differentiation functions to attenuate BMP signalling, at least in part by repressing Bmp expression in prospective neural epithelial cells.

**Discussion**

In this study, we have provided evidence that the initial steps in the specification of neural cell fate in the chick embryo are initiated in utero, and that early epithelial cells require ongoing FGF signalling to promote neural character and to repress Bmp expression. We discuss these
findings in the context of current views of neural induction in vertebrate embryos.

**Specification of neural cells begins in utero**

In the chick embryo, signals from Hensen’s node are sufficient to induce the formation of neural tissue in extraembryonic regions [15,38,39]. Our results provide evidence, first, that the specification of neural cells in chick begins much earlier than the time of Hensen’s node formation, shortly after the formation of the area pellucida in utero; second, that the neural fate of epiblast cells has been consolidated by the late gastrula stage; and, third, that in early prestreak embryos cells in the medial region of the area pellucida are specified to a neural fate and that cells in the lateral region are specified to an epidermal fate.

The differential patterns of expression of Dlx5 [40] and GATA2 [24] by cells in the medial and lateral regions of prestreak embryos provide independent evidence for a distinction in the fates of cells in these regions. In addition, the pattern of expression of GATA2 at early stages indicates that the prospective epidermis is patterned along its anteroposterior axis [24]. Collectively, these results support the idea that the specification of neural and epidermal cells in chick is initiated in utero, before the generation of the primitive streak and Hensen’s node.

In *Xenopus*, Wnt signalling at blastula stages has recently been implicated in the downregulation of *Bmp4* expression in prospective neural plate cells and in the induction of neural cells [41]. In the chick, however, a nuclear-localised form of β-catenin, a marker of active Wnt signalling, is detected in cells in the lateral but not in the medial region of stage VIII–X embryos [42]. The involvement of Wnt signalling in the generation of neural and epidermal cells may therefore differ in *Xenopus* and avian embryos.

**BMP signalling promotes the generation of epidermal cells at the expense of neural tissue**

In *Xenopus* embryos, BMP inhibitors derived from the Spemann’s organiser region are believed to contribute to neural induction by sequestering BMP protein [2]. In chick, noggin and chordin appear unable to induce neural cells in extraembryonic tissue [9,10]. Moreover, neither noggin, chordin or follistatin exhibit temporal patterns of expression that correlate with the ability of Hensen’s node to induce ectopic neural tissue [9,15,17]. Therefore, there remains little evidence that the generation of neural ectoderm in amniotes results from the blockade of BMP signalling by secreted inhibitors.

Nevertheless, in embryonic regions of prestreak stage chick embryos, *Bmp4* and *Bmp7* are expressed at low levels and the expression of both genes is extinguished in prospective neural cells before the formation of the primitive streak. Thus, the extinction of BMP expression from the prospective neural plate may represent a common link in neural induction in different vertebrate embryos. In different vertebrate organisms, *Bmp7* is expressed at high levels in cells of the extra-embryonic region of pre-streak embryos, that is, adjacent to the lateral region of area pellucida that generates epidermal cells when cultured *in vitro*. Consistent with these observations, application of BMP4 to the area pellucida at prestreak stages promotes the generation of cells of epidermal character and prevents the generation of neural tissue. Thus, it is possible that, in
the chick embryo, the repression of *Bmp4* and *Bmp7* expression may be causally linked with the acquisition of neural character and that cells that acquire epidermal character maintain *Bmp4* and *Bmp7* expression.

**FGF signalling in epiblast cells is required for the acquisition of neural character**

In both *Xenopus* and chick embryos, FGF has been suggested to play a role in neural induction [31,32,43,44]. Our results provide evidence that Fgf3 is expressed in early epiblast cells before the formation of the primitive streak and that ongoing FGF signalling is required for effective repression of *Bmp4* and *Bmp7* expression in epiblast cells and for the acquisition of neural character. Signalling by Fgf3 is mediated primarily through the activation of FGFRs 1b and 2b [34,35]. Genetic evidence in mouse suggests that the FGFR2 is required for pregastrulation development [45] and, thus, it remains possible that in mouse, as it has been in mouse, as it has been in chick, an early phase of FGF signalling is required to promote neural character.

Our results show also that exposure of epiblast cells to noggin, chordin or soluble dominant-negative BMP receptors is sufficient to promote the generation of neural cells under conditions when FGF signalling is attenuated and *Bmp* expression is maintained in area pellucida cells. These results raise the possibility that an important role of FGF signalling during early neural differentiation is to attenuate BMP signalling by repressing the expression of *Bmp* in epiblast cells of prospective neural character.

Cells in the lateral region of stage XII epiblast cells express Fgf3, but these cells do not downregulate *Bmp* expression in *vitro*. Moreover, in these lateral epiblast regions, noggin, alone or in combination with FGF1, an activator of all FGFRs, was not sufficient to induce cells of neural character (data not shown). Thus, FGF signalling is not sufficient to repress BMP expression in prospective epidermal cells, nor does the blockade of BMP signalling at this stage appear to be sufficient to induce neural character. This distinction could be explained if medial but not lateral epiblast cells are exposed to additional signals that are normally required for the induction of neural character. Alternatively, lateral cells may become refractory to the actions of FGF at early stages. Despite these uncertainties, the present explant assay system may provide a means of characterising in more detail the mechanism through which epiblast cells acquire neural fates at early stages of chick embryogenesis.

**Materials and methods**

**Preparation of inducing factors**

COS-7 cells were transfected with chick chordin (in pMT23) [46] using Lipofectamine (Gibco BRL). The chordin-conditioned medium was prepared by incubating transfected COS cells for 48 h in OPTI-MEM medium, and was used undiluted. Human recombinant BMP4 and FGF1 (R & D systems) were used at concentrations of 1.3 nM and 33 nM, respectively. Recombinant mouse dominant-negative BMP receptors IAFc and IIBFc (R & D systems) were used at a final concentration of 0.5 µM. Purified recombinant *Xenopus* noggin was used at a concentration of 64 nM. The FGF inhibitor SU5402 (Calbiochem) was used at a concentration of 2 µM.

**Isolation and growth of explants**

Explants were isolated from stage VII–XII [25] chick embryos, as described in the text (Figures 1 and 2). Epiblast ectoderm located rostral to the tip of the primitive streak was isolated from stage 2 and stage 4 (47) chick embryos. Explants were cultured for 40 h at 37°C on a collagen bed in OPTI-MEM medium supplemented with N-2 (Gibco BRL) in the presence or absence of factors as described in the text.

**Immunohistochemistry**

Immunohistochemical localisation of proteins was performed as previously described [48]. Neural tissue was defined with rabbit anti-Sox2 antibody provided by R. Lovell-Badge [49], rabbit anti-Sox3 antibody, mouse
anti-Parf and rabbit anti-65k antibodies [30], provided by E. Boncinelli. Mac1/2 were detected with monoclonal antibody 451. GATA2 was detected with mouse anti-GATA2 IgG (Santa Cruz Biotechnologies).

RT–PCR analysis

RT–PCR was performed on pooled stage XI medial and lateral explants. Total RNA was prepared from the following: either 10 pooled uncultured explants or six pooled explants cultured for 40 h with or without factors as described in the text. Control RNA was prepared from the embryonic region of a HH stage 4 embryo. Total RNA was reverse transcribed under standard conditions. The decamer primer 5′-[alpha-<sup>32</sup>P]GTP]-CGTGTGGTACTGGTCCGAC-3′ was used as a semiquantitative control to ensure that equivalent concentrations of RNA were transcribed. Olignucleotide primer sets included: 5′-CGGCTCAGTTGCTGGAATGTGGAAC-3′, 5′-SETTCCCAGGAATTGTCATCCTGC-3′, 5′-AGCTGAGATG-3′. RT-PCR analysis was performed at an annealing temperature of 55°C (Bmp7, Bmp4, and Bmp7), or 55°C (Fgf8, Fgf8r2, and Raldh2) or 60°C (chordin). Fgf8, Bmp4, and Bmp7 were amplified for 25 cycles and subjected to Southern blot analysis. Fgf8R2 was amplified for 35 cycles. chordin, chordin, and Raldh2 were subjected to 35 cycles of amplification from RNA prepared from stage XI medial explants after 40 h of culture, while RNA prepared from the control HH stage 4 embryo was amplified for 30 cycles under parallel conditions.

Acknowledgements

We are grateful to members of the Edlund lab for helpful discussions and to C. Stem for discussion of unpublished results. T.E. is supported by the Swedish Medical Research Council and by the Foundation for Strategic Research. R.H. is supported by the NIH. T.M.J. is supported in an Investigator of the Howard Hughes Medical Institute.

References


Because Current Biology operates a ‘Continuous Publication System’ for Research Papers, this paper has been published on the internet before being printed. The paper can be accessed from http://biomednet.com/cbiology/cub – for further information, see the explanation on the contents page.