Neural induction by the node and placode induction by head mesoderm share an initial state resembling neural plate border and ES cells

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Around the time of gastrulation in higher vertebrate embryos, inductive interactions direct cells to form central nervous system (neural plate) or sensory placodes. Grafts of different tissues into the periphery of a chicken embryo elicit different responses: Hensen's node induces a neural plate whereas the head mesoderm induces placodes. How different are these processes? Transcriptome analysis in time course reveals that both processes start by induction of a common set of genes, which later diverge. These genes are remarkably similar to those induced by an extraembryonic tissue, the hypoblast, and are normally expressed in the pregastrulation stage epiblast. Explants of this epiblast grown in the absence of further signals develop as neural plate border derivatives and eventually express lens markers. We designate this state as "preborder"; its transcriptome resembles embryonic stem cells. Finally, using sequential transplantation experiments, we show that the node, head mesoderm, and hypoblast are interchangeable to begin any of these inductions while the final outcome depends on the tissue emitting the later signals.

embryonic induction | organizer | gastrulation | embryonic stem cells | pluripotency

During development, cell fate decisions are controlled by inductive interactions: instructive signals from one tissue cause a change of fate in adjacent responding cells. For example, signals from Spemann's organizer (Hensen's node in amniotes) can induce neural tissue from cells that otherwise develop into epidermis (1, 2), and the zone of polarizing activity (ZPA) induces and patterns digits in the limb bud (3). Thus, each inducer elicits a distinct and specific response. However, classical transplantation experiments indicate that some inducers are interchangeable: Hensen's node and the ZPA alike result in digit duplication when grafted into the anterior limb bud (4). These and similar observations have led to the suggestion that, while inducing signals may be universal, it is the responding tissue that confers specificity to inductive events (5–7). Here, we explore two well-characterized inductive events that occur at approximately the same time, but under the influence of signals from different tissues: the induction of the neural plate (NP) by Hensen's node and the induction of placode progenitors by the lateral head mesoderm (lHM).

The neural plate gives rise to the entire central nervous system while sensory placodes contribute to the sense organs and cranial ganglia and arise from the ectoderm surrounding the anterior neural plate [the preplacodal region (PPR)] (8, 9). The neural plate is first defined by the appearance of *Sox2*, and the PPR becomes molecularly distinct at head fold stages by the expression of *Six1*, *Six4*, and *Eya1* or *-2*. At this stage, neural and placode precursors continue to be intermingled with each other as well as with future neural crest and epidermal cells in a territory denominated the "neural plate border" (9, 10), suggesting that they may initially share common properties. Both neural induction and PPR induction require FGF activity, as well as antagonism to BMP and Wnt signaling (2, 11–14), although the relative contribution of each signal still needs to be unraveled.

Significance

It is generally believed that the outcome of many inductive interactions occurring during development is largely dependent on the responding tissue, the source of the signals playing a relatively minor part. Here, we compare induction of the neural plate by the node, and of placodes by the head mesoderm, and show that both inducing tissues elicit a similar initial response but that they later diverge. We characterize the initial common state by a variety of methods and show its similarity to ES cells, suggesting that these inductions may begin with a common "reprogramming" step. This initial state also shares many features in common with the border of the neural plate, suggesting that this region retains features of a "ground state."

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We have previously identified response genes to mesoderm-derived signals (15); we now use transcriptional profiling of node-induced tissue, together with neural and preplacodal tissue from normal embryos, to explore the similarities and differences between the two inductive events. We find that, despite the difference in inducing tissues and in the ultimate outcome, the initial response to the node is largely identical to the response to the lHM; this defines a distinct, common transcriptional state, which is very similar to the early prestreak epiblast and to the neural plate border. When cells from the early epiblast are allowed to develop in vitro without further induction, they differentiate into neural plate border derivatives: lens, neural crest, and neural plate. To demonstrate that this "preborder" state is common to neural and preplacodal induction, we show that induction of this state by short exposure to either the node or the lHM, as well as by the hypoblast (extraembryonic endoderm), can be subsequently directed to each set of fates by short exposure to the appropriate tissue. We propose that inductions by different inducing tissues start by a reprogramming step that converts cells to a common, preborder-like state which is also similar to very early (pregastrulation) epiblast and to embryonic stem cells. Specific lineages diverge under the influence of later, specific signals.

Results

Analysis of Genes Regulated by an Organizer Graft Reveals Similarities Between Neural and Placodal Induction. Grafts of the "organizer," Hensen's node, can induce a complete, patterned nervous system in cells that are not fated to contribute to the neural plate. Previous studies have established that some genes (e.g., *ERNI* and *Sox3*) are induced in responding cells after less than 5 h exposure to the graft (11). The same genes are expressed in a wide territory of the epiblast of the normal embryo before gastrulation begins, which led to the idea that, in the normal embryo, the process of neural induction begins before gastrulation (11, 16). To gain a more comprehensive view of the transcriptional changes occurring at this time point, we performed RNAseq from tissue exposed to organizer signals and compared this to the transcriptome of contralateral control epiblast from the same embryos. Hensen's node from an HH4$^-$ chick donor was grafted onto the area opaca epiblast of a host of the same stage. After 5 h, the node was removed, and the adjacent tissue ("induced epiblast") collected, along with the same region from the contralateral side ("control epiblast") (Fig. 1*A*).

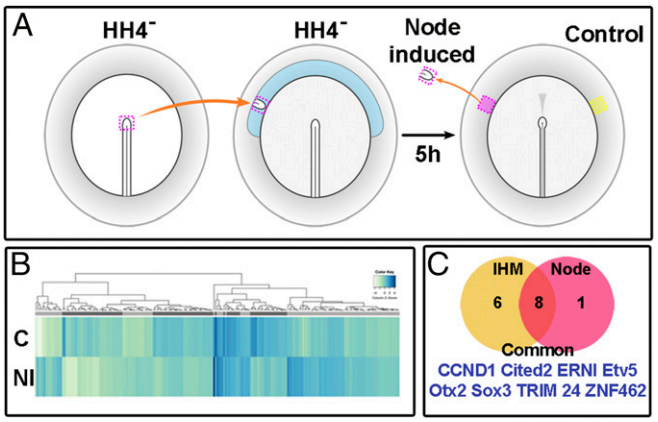

Fig. 1. Comparison of lHM and node grafts at 3 h. (*A*) Schematic of RNAseq tissue collection for node induction. (*B*) Heat map displaying differentially regulated genes between node-induced (NI) and uninduced controls (C) after 5 h. (*C*) Venn diagram showing the overlap between lHM (yellow) and node (red) induced genes at the 3-h time point, common genes in orange.

Fifty control and experimental tissues were collected, processed for RNAseq, and analyzed for differentially expressed genes (1.5-fold change, $P < 0.05$), which revealed a total of 2,477 differentially expressed genes: 1,166 up-regulated and 1,311 down-regulated in the induced epiblast (Fig. 1*B* and Dataset S1).

We noticed that many transcripts up-regulated in response to an organizer graft (252, of which 25 are transcription factors) were also identified in a recent screen for genes involved in sensory placode induction [grafts of lateral head mesoderm (lHM)] (15). This suggests considerable similarity between the initial steps in neural induction by the node and PPR induction by the head mesoderm. To test this directly, we designed a NanoString probe set containing known probes for neural, preplacodal, neural plate border, and nonneural ectoderm cells, as well as other genes identified in our RNAseq data (Dataset S2). Hensen's node from HH4$^-$ or lHM from HH5/6 donors was grafted into the area opaca of HH4$^-$ hosts; after 3 and 5 h, the adjacent epiblast and noninduced epiblast from the contralateral side were collected and processed for NanoString analysis. The node induced 9 genes after 3 h, and the lHM induced 14; 8 of the transcripts were represented in both sets (Fig. S1 and Dataset S3). Five hours after grafting, the responses to the two tissues started to diverge; 33% of the 123 induced genes were shared, and the lHM now induced many genes (51; 41%) that were not induced by the node (Dataset S3).

In summary, many early response genes induced by organizer and lHM grafts are identical. This reveals a striking similarity between the first steps in neural and preplacodal induction and leads us to propose that, before cells acquire their unique identity as central or peripheral nervous system, they transit though a common transcriptional state that primes them for neural development.

The Epiblast Before Gastrulation as a "Preneural State." If the above hypothesis is correct, genes identified in response to node- and lateral head mesoderm-derived signals should be expressed in precursors for both the central and the peripheral nervous system during normal development. To test this further, we analyzed the expression of 27 transcription factors common to both screens by whole-mount in situ hybridization. Twenty transcripts were readily detectable (Fig. S2). At primitive streak stages (HH4$^-$/4), all were broadly expressed in the epiblast encompassing the future neural plate and its border, containing placode and neural crest precursors (15). At head fold stages (HH6-7), when the neural plate is clearly defined and PPR markers begin to be expressed in the adjacent ectoderm, only 2 transcripts were restricted to the neural plate, while 16 continued to be expressed in both tissues. Strikingly, all transcripts were already expressed at preprimitive streak stages (Fig. S2, EGK XII-XIII) similar to *Sox3* and *ERNI* (11), suggesting that the transcriptional profile initially induced in response to neural- and PPR-inducing signals is akin to that of the young epiblast before gastrulation.

Defining the Transcriptional Program for the Preneural State. The above screens were performed in the extraembryonic epiblast of primitive streak stage embryos, which, although competent to respond to neural inducing signals, normally never contributes to the central or peripheral nervous system. We therefore sought to define the transcriptional program for the normal preneural state using RNAseq. We reasoned that this program should comprise genes common to the prestreak epiblast, the neural plate, and the PPR but exclude transcripts specific for the future epidermis, and largely contain *Sox3*-like genes. We performed RNAseq for the medial and anterior prestreak epiblast from EGK XII-XIII embryos, the HH6-7 neural plate, and the nonneural/nonplacodal ectoderm, as well as the anterior and posterior PPR from head fold stage embryos (HH6). Pairwise comparisons (>1.5 fold-change) define genes enriched in each

cell population (Dataset S1; all RNAseq results deposited in GEO under accession number GSE106346), with 505 transcripts being neural plate-specific, 331 genes enriched in the anterior PPR (aPPR), and 266 transcripts in the posterior PPR (pPPR) (Fig. S3 and Dataset S4).

To identify transcripts common to neural plate, anterior and posterior PPR, we compared each tissue to the nonneural/non-placodal ectoderm (NNE) (fold-change cutoff of 1.5) (NNE in Fig. S4). A total of 1,128 transcripts are common to all three tissues but absent from the future epidermis (NNE); these common transcripts ["neural–anterior PPR–posterior PPR" (NAP)] (Fig. S4 *A–E*) include 141 known or putative transcription factors (TFs) (Fig. S4*E*). Anterior prestreak epiblast-specific mRNAs were subtracted from all prestreak genes (Fig. S4*F*); the remaining transcripts were then intersected with genes induced in response to an organizer graft (see above) (Fig. 1). This analysis reveals that the majority of genes induced by the node (1,143/1,167, including 155/158 TFs) were also expressed in the prestreak epiblast (Fig. S4 *H* and *I*) [prestreak–induced (PSI)]. To identify *Sox3*-like transcripts as hallmarks for the preneural state, we intersected NAP and PSI genes (Fig. S4 *J* and *K*). This uncovered 439 transcripts, all of which were also among the genes induced by the node (Fig. 1 and Dataset S1). We refer to this set of genes as "*Sox3*-like." These results support the hypothesis that, in response to neural and placode inducing signals, cells pass through a common, preneural state, which is similar to the epiblast before gastrulation.

Distinct Regulatory Modules Define Preneural, Neural, and PPR States. To uncover whether the common and divergent transcripts from the RNAseq datasets define distinct transcriptional states as cells transit from the preneural to neural plate and PPR fate, we used an unbiased approach, Gene Network Inference with Ensemble of trees (GENIE3) (17). Using a random forest machine-learning algorithm, GENIE3 infers a directed network that predicts functional links between genes. As input, we used fragments per kilobase of transcript per million mapped reads (FPKM) of all known and predicted transcription factors from each RNAseq dataset (FPKM ≥ 10; 805 TFs) and generated a network in Cytoscape based on all interactions with an importance measure ≥0.005 (Fig. S5*A*).

To identify modules within the network, we organized its components according to their connectivity with each other using community clustering (18, 19). This approach generated three major clusters C1–C3 (Figs. S5 *B–D* and S6 and Dataset S5): The majority of preneural (*Sox3*-like; $P = 1.23E{-}03$ as assessed by hypergeometric testing) and PPR (*Six1*-like; $P = 4.59E{-}04$) transcription factors were included in cluster C1 (Fig. S5*B*) while cluster C3 (Fig. S5*D*) was enriched for neural plate factors (*Sox2*-like; $P = 8.01E{-}09$) and contained a few preneural genes. In contrast, cluster C2 did not show significant enrichment of any of the three TF classes (Fig. S5*C* and Dataset S4). Only further subclustering segregated preneural (cluster C1A and C1B; *P* values 5.3E−05 and 2.3E−04, respectively) and PPR genes (cluster C1C; $P = 1.02E{-}07$) (Fig. S5*B*). In conclusion, this analysis reveals that preneural and PPR genes are more interconnected with each other than with neural plate genes.

Early Epiblast and Newly Induced Ectoderm Have Properties of the Neural Plate Border. The above results uncovered a set of *Sox3*-like, or preneural, genes that are induced in peripheral epiblast within 3–5 h in response to a graft of either Hensen's node (neural inducing) or the lHM (placode inducing). In normal embryos, these genes are initially expressed in a broad region of the preprimitive-streak stage epiblast (Fig. S2). Could the prestreak epiblast be specified as preneural? It was previously reported that, when cultured in isolation, epiblast explants express neural plate border (20) or neural markers (21) (although the combination of *Sox2*, *Otx2*, and *Pax6* expression reported in the latter study could also be interpreted as lens tissue).

To address the specification state of the early epiblast, we cultured epiblast explants from prestreak stage embryos and assessed the expression of markers for various ectodermal derivatives. We distinguished explants from the medial and lateral prestreak epiblast since they had previously been reported to

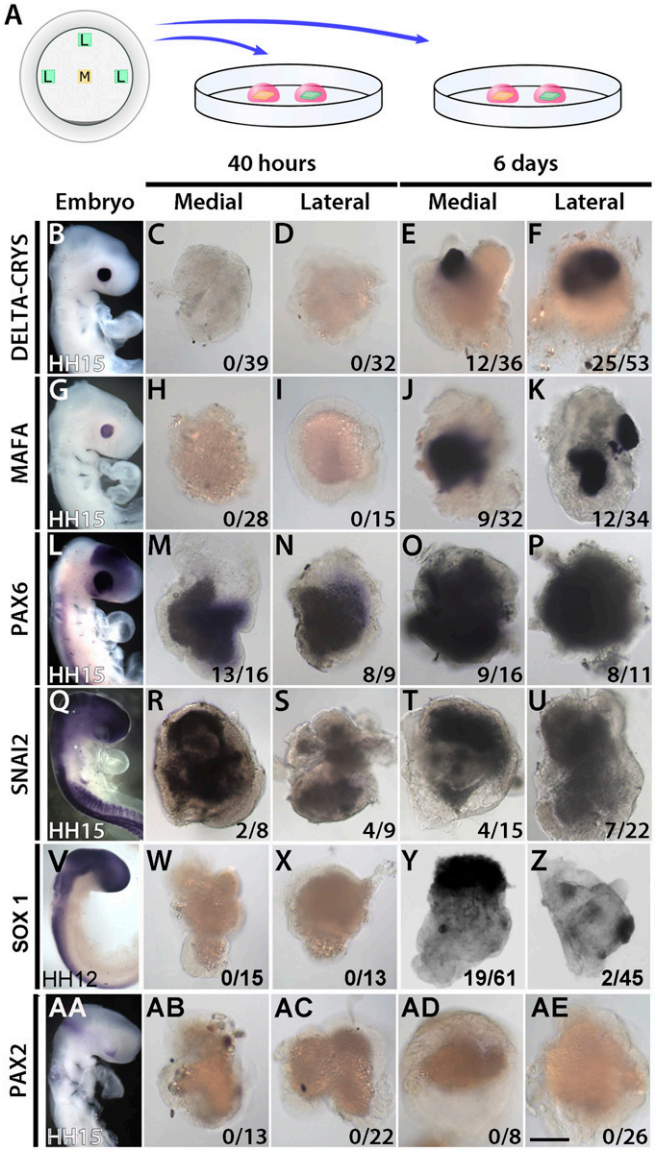

Fig. 2. Specification assay for the early epiblast. (*A*) Explants were obtained from medial (M) and lateral (L) regions of preprimitive streak epiblast and placed in culture. Expression of various markers (*B*, *G*, *L*, *Q*, *V*, and *AA* show the expression of each of these in normal embryos. The remaining panels *C–F*, *H–K*, *M–P*, *R–U*, *W–Z*, and *AB–AE* show explants cultured for the periods indicated above: 40 h and 6 days) was assessed after either 40 h or 6 d. *MAFA* and *δ-crystallin* are lens markers (*B–K*), *Pax6* marks anterior neural and placodal regions as well as the eye (*L–P*), *Snail-2* is a marker for neural crest (*Q–U*), *Sox1* is a neural marker (*V–Z*), and *Pax2* marks the otic vesicle (*AA–AE*). After 40 h (see also Fig. S6) the explants express neural plate border markers. By 6 d, about one-third of explants have differentiated into lens tissue (*E*, *F*, *J*, and *K*), about a third as neural (*O*, *P*, *Y*, and *Z*), and about a third as neural crest (*T* and *U*). No differentiation into otic placodes is detected (*AD* and *AE*). The number in each panel indicates the proportion of explants expressing the marker. Scale bar, 100 μm (applies to all panels with explants: *C–F*, *H–K*, *M–P*, *R–U*, *W–Z*, and *AB–AE*).

have different properties (12, 21). After 40 h culture (Fig. 2 and Fig. S7), medial and lateral explants expressed the neural plate border markers *Dlx5*, *Gata3*, and *ERNI* and the neural crest specifiers *Msx1* and *Pax7*, as well as the definitive neural crest marker *Snail2*, the preplacodal markers *Six4* and *Eya2*, *Pax6*, which marks lens and olfactory progenitors, *Sox3*, expressed in both the neural plate and PPR, and the neural plate marker *Sox2* (Fig. 2 and Fig. S7). In contrast, the "definitive" neural plate marker *Sox1*, mesodermal (*Tbx6*), and nonneural ectoderm transcripts (*Gata2*) are absent (Fig. 2 and Fig. S7). No differences were seen between medial and lateral epiblast explants. In the developing embryo, this combination of gene expression uniquely identifies the neural plate border, where precursors for the neural plate, neural crest, and placodes overlap (22). These observations suggest that cells in the preneural epiblast at the preprimitive streak stage are specified as neural plate border.

If this is indeed the case, after prolonged culture, these explants should develop into lenses, the default fate of placode progenitors (23), as well as into neural crest and central nervous system. Indeed, after 6 d, a subset of prestreak explants differentiated into lens-like structures expressing *Pax6*, *Mafa*, and the lens differentiation marker *δ-crystallin* (Fig. 2 *E*, *F*, *J*, and *K*) while another subset continued to express the neural crest cell marker *Snail2* (Fig. 2 *T* and *U*) and a third set of explants expressed the definitive neural marker *Sox1* (Fig. 2 *Y* and *Z*). In contrast, markers of other placodes like *Pax2* (otic) were never expressed (Fig. 2 *AB–AE*). Thus, like the PPR, the prestreak epiblast has an autonomous tendency to develop into lens-like structures, while also generating other border derivatives like neural crest and neural plate cells. The presence of neural crest cells could account for why only a proportion of explants (about one-third) express lens markers and also why this expression tends to be confined to a patch within the explants, since neural crest cells have been shown to inhibit lens development (23). Together, these data suggest that, before gastrulation, epiblast cells are specified as neural plate border. It is therefore more appropriate to refer to the preneural state as a preborder state, and we will use this designation henceforth.

Signals from the Hypoblast Induce the Preborder State. At preprimitive-streak stages, before gastrulation, a layer of extraembryonic hypoblast cells underlies the epiblast (which forms the embryo proper). We have previously shown that grafts of the hypoblast transiently induce four *Sox3*-like genes: *ERNI*, *Sox3*, *Otx2*, and *Cyp26A1* (24). Is the hypoblast sufficient to induce the full transcriptional profile of the preborder state? To assess this, we grafted hypoblast from preprimitive streak stage donors into the peripheral area opaca of primitive streak stage hosts (HH4$^-$). After 5 h, the grafts were removed and the adjacent epiblast collected, along with the same tissue from the contralateral side. Transcriptional changes were assessed using NanoString. Many transcripts that were rapidly induced by the organizer and lateral head mesoderm were also significantly induced by the hypoblast (Fig. S8 and Dataset S3). These results suggest that the hypoblast may be responsible for priming the overlying epiblast by inducing a set of transcripts that characterize a common preborder state.

Testing the Hypothesis of a "Common State." If the preborder state truly represents a common state that initiates the responses of epiblast to signals from different inducing tissues, it should be possible to replace any of these tissues by another for the early steps, the outcome of the induction being determined by the final inducing tissue. To test this, we first determined the minimum time required to induce placodal (*Six1*) and neural (*Sox2*) markers by the normal inducing tissue. We found that *Six1* induction by the lHM requires 8 h (15), and induction of *Sox2* by Hensen's node requires 7 h. Next, we grafted each tissue into the area opaca, replaced it after 3 h by the other tissue, and cultured for a further 5 h (for *Six1*) or 4 h (for *Sox2*). We found that either tissue (lHM or node) can provide the initial signals, but it is the subsequent graft that determines whether PPR (*Six1*, induced by lHM) or the neural plate (*Sox2*, induced by the node) is induced (Fig. 3 *A–I*).

Since the hypoblast also induces the preborder genes, we tested whether 3 h of hypoblast signaling is sufficient to prime cells to respond to neural and placode precursor inducing signals using the same experimental paradigm. The hypoblast alone did not induce neural or preplacodal markers after any length of time. However, when 3 h hypoblast signaling was followed by either a node (a further 4 h) or the lHM (a further 5 h), *Sox2* and *Six1* were induced, respectively. In the same experiment, replacing the hypoblast with a node (5 h) did not induce *Six1* and with the lHM (4 h) did not induce *Sox2* (Fig. 3 *A–I*).

Together, our experiments indicate that the response of epiblast to induction by different signaling tissues first elicits a common set of responses irrespective of the inducing tissue and that the final direction of differentiation is determined by the signals received in the following few hours. During normal development, the initial state characterizes the early (preprimitive streak stage) epiblast and may be induced by signals from the underlying hypoblast. These findings are summarized diagrammatically in Fig. 3*J*.

Discussion

Our results lead us to propose that tissues (like Hensen's node and the lateral head mesoderm lHM) that induce diverse fates, such as the neural plate (central nervous system, CNS), the neural crest, and placodes (peripheral nervous system, PNS) at the gastrula (primitive streak) stage, initiate their action (around 3 h) by eliciting a common, preborder state, which only then diverges to follow distinct pathways depending on the inducing tissue. During normal development, this initial common state is displayed by the epiblast of the preprimitive streak stage embryo and is also induced by the hypoblast (equivalent to the mouse anterior visceral endoderm) (25, 26). The specification state of this early epiblast is so similar to border/placodal precursors that, when cultured in the absence of added signals, it will develop into a lens, the "default" state of preplacodal cells (23).

The epiblast of early mouse embryos can generate stem cells ["embryonic stem cells" (ESCs)] when cultured: the cells acquire the ability to self-renew in vitro and, when allowed to differentiate, can give rise to all cell types (pluripotency). Could the preborder state of preprimitive streak chick epiblast and peripheral epiblast exposed to an inducing tissue for a short time be equivalent to pluripotency? To explore this, we compared the genes in our PSI, *Sox3*-like, and NP- and PPR-enriched TF lists to Embryonic Stem Cell Atlas from Pluripotency Evidence (ESCAPE) databases (27). This unbiased comparison shows that PSI and *Sox3*-like TFs are enriched for genes characteristic of mouse ESCs (embryonic stem line Bruce4 p13 and embryonic_stem_line_V26_2_p16) whereas TFs enriched in NP, PPR, and NNE transcriptomes are not (Fig. S9). This suggests that the preborder state shared by preprimitive streak stage epiblast and later peripheral epiblast starting to respond to signals from different inducing tissues may resemble ESCs.

These conclusions support recent findings (28) that the animal pole of *Xenopus* embryos at the blastula stage is made up of cells that are "pluripotent," in that they not only contribute to neural crest and other neural border derivatives (see ref. 20) but can also give rise to the mesoderm. Accordingly, the transcriptome of these animal pole cells includes genes normally associated with pluripotency, such as *PouV*, *Vent3*, *Sox3*, *Id3*, and others (28). These findings are particularly interesting because, to date, it has not been possible to generate truly self-renewing pluripotent cells from any early amphibian or fish embryo. This is probably

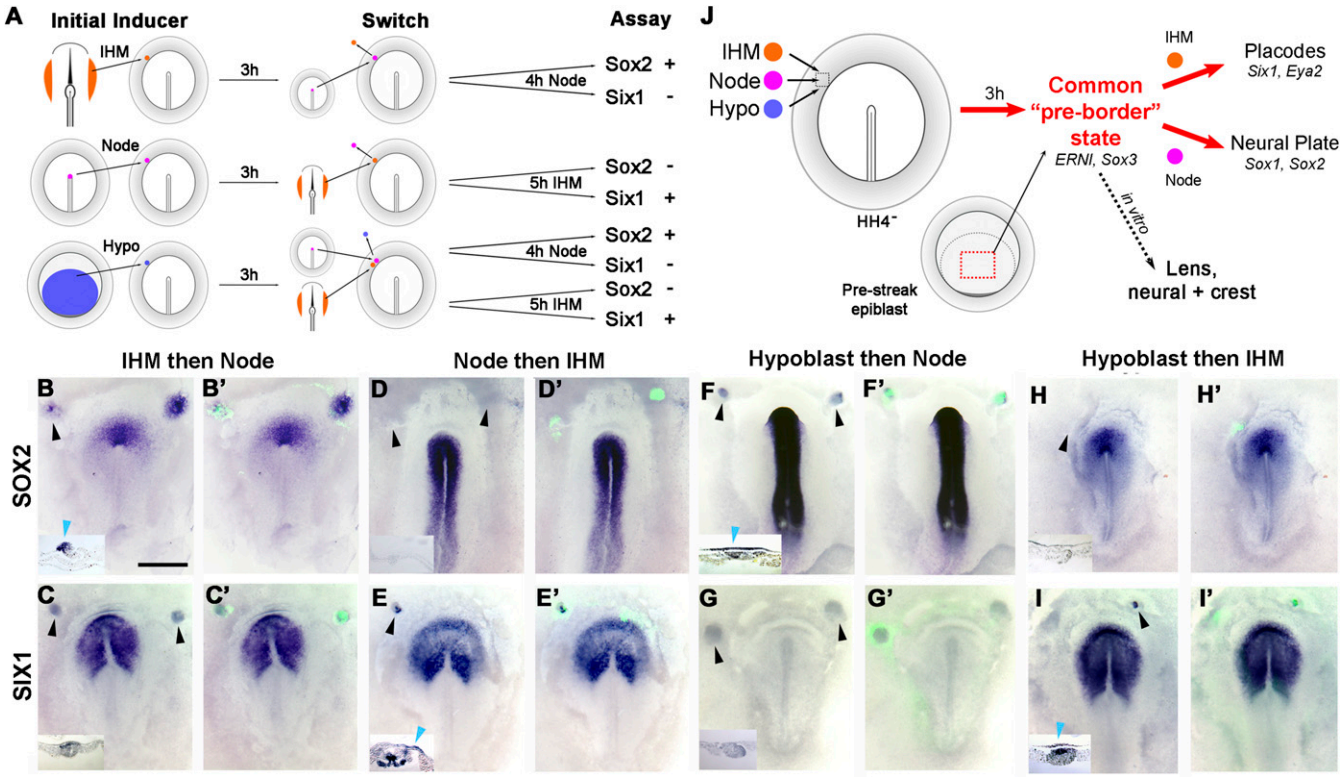

Fig. 3. Sequential transplantation demonstrates common state. (*A*) Diagram of the experimental design. After a period of 3 h following a graft of lHM, Hensen's node, or hypoblast (Hypo), the initial graft was removed and replaced by another inducing tissue (node or lHM); markers of neural plate (*Sox2*) or placode (*Six1*) were assessed after a further 4–5 h incubation. (*B* and *C*) lHM was grafted to an HH4$^-$ host for 3 h and exchanged by a node for 4 h (total time 7 h); *Sox2* was induced in 4/6 cases (compared with 0/5 for 4 h node-only grafts; node 7 h: 4/5). *Six1* was not induced (0/8). (*D* and *E*) Node was grafted to an HH4$^-$ host for 3 h and exchanged for lHM for 5 h (total 8 h): *Six1* was induced (5/8; lHM-only grafts: 0/5 in 5 h, 4/4 in 8 h) (*G*), but *Sox2* was not (0/11). (*F* and *G*) Hypoblast was grafted to an HH4$^-$ host for 3 h and then replaced by a node for a further 4 h (total time 7 h); *Sox2* was induced in 7/12 (c.f.: 0/5 in 4-h node grafts). (*H* and *I*) Hypoblast was grafted to HH4$^-$ for 3 h and then exchanged for lHM for a further 5 h (total time 8 h); *Six1* was induced (10/14; 5 h lHM alone: 0/4), but *Sox2* was not (0/7). (*Insets*) sections at the level of the graft; cyan arrows indicate expression in host ectoderm. *B–I* show in situ hybridizations of the embryos in whole mount. The inset in *B–I* shows a section through the graft and reveals expression in the epiblast in *B*, *E*, *F*, and *I* (blue arrows) and lack of expression in *C*, *D*, *G*, and *H*. Panels designated with a prime (*B′–I′*) show the fluorescence emitted by the graft-derived GFP$^+$ cells. *Sox2* was not induced in host embryos when the lHM was removed after 3 h and cultured for 4 h (0/6); *Six1* was not induced in host embryos when the lHM was removed after 3 h and cultured for 5 h (0/5). Black arrows indicate switch grafts. (*J*) Summary of the main findings. Grafts of either the node, lHM, or hypoblast induce a common state (or preborder state) in host epiblast in 3–4 h. When this tissue is isolated in culture for 6 d, it differentiates into lens, neural, and neural crest. Among the characteristic genes for the common/preborder state are *Sox3* and *ERNI*. Epiblast isolated from preprimitive streak stage embryos shares a genetic signature with this state. When further exposed to node or to lHM, cells in the preborder state can be directed to differentiate into neural plate (by the node) and express *Sox2*/*Sox1*, or into placodes (by lHM), expressing *Six1/Eya2*. The scale bar shown in *B* represents 300 μm in all main panels and 200 μm in the insets.

because the first 10 cell divisions of fish and amphibian blastomeres do not have G_1 or G_2 phases, there is no (or negligible) zygotic gene expression, and these cell divisions are not accompanied by cell growth (cells become smaller at each division): these properties do not allow true self-renewal (see ref. 26 for discussion). Therefore, the common program shared by neural, neural crest, and placodal lineages, manifested by the pregastrula stage ectoderm, precedes the evolutionary divergence of amniotes and anamniotes.

Experiments mainly in *Xenopus* suggested that the default state of cells in the animal pole of the blastula stage embryo is neural and that this is inhibited by endogenous BMP signals (29–32). However, BMP inhibition alone is not sufficient to induce a neural fate either in chick epiblast or *Xenopus* ectodermal cells that lie remote from the neural plate (14, 20, 33). It can only expand the neural plate territory when BMP inhibited cells are in contact with the neural plate border. Our results, together with those of others (28), suggest that the default state of the early epiblast/ectoderm of both *Xenopus* and chick is in fact a "border-like state" and that explants of this tissue have a strong tendency to differentiate into neural crest and placode (lens) derivatives.

As ectodermal cells acquire neural identity, they transit through successive states, characterized by specific sets of genes. The order in which these genes are induced by an organizer graft closely mimics the chronology of their expression during normal neural development (11, 16, 34–37). Likewise, a cascade of states has been suggested to accompany placodal induction (15), reminiscent of the successive states originally proposed by Jacobson to lead to lens and other inductions (38). Are the same signals responsible during normal development? When do these pathways diverge, and what determines the outcome of inductive interactions? It was believed long ago that the specificity of inductive interactions depends largely on the responding tissue (6, 7), a view also supported by some grafting experiments: for example, the finding that a graft of Hensen's node (Spemann organizer) or of the floor plate of the neural tube to the anterior limb bud can induce digit duplication (4, 39). Our results suggest that at least for early-occurring inductive interactions, the specificity of the induction caused in one particular tissue is determined at least in part by the inducing tissue: although the initial response is the same, the inducing tissue then directs the subsequent and final outcome. A likely initial signal is a member of the FGF family or

other ligands that activate this pathway (2, 9, 24, 40), but the appropriate signaling tissues must also produce specific signals to account for the divergence into distinct pathways and fates that follow the initial, common state. A challenge will be to determine what these signals are and how they are interpreted by responding cells.

Materials and Methods

Detailed materials and methods are described in *SI Materials and Methods*.

Hens' eggs were incubated and staged according to Hamburger and Hamilton (HH) (41) or Eyal-Giladi and Kochav (EGK) (42) for preprimitive streak (prestreak) stages and cultured using a modified New culture method (43, 44). Induction assays were conducted as previously described (45–47). Hensen's node was isolated from HH4^{-} chicken embryos (45) and hypoblast from EGK XII-XIII embryos (24). The lateral head mesoderm (lHM) at this stage corresponds to the lateral-most part of the head mesoderm, whose fate is to contribute to the heart—it does not include the paraxial head mesoderm (see refs. 13 and 15 for details). Grafts were placed into the inner lateral area opaca of HH4- stage hosts (46, 47). In situ hybridization was performed as previously described (48, 49). Explant cultures were set up as described (20, 23).

Transcriptional responses to induction were assessed by RNA sequencing and using the NanoString nCounter analysis system using a custom made probe set of 386 genes (Dataset S2). Experiments were performed in triplicate. All transcription factors from RNAseq with FPKM of ≥10 were used for predicting gene regulatory interactions using Genie3 (17). Interactions with importance measure (IM) of ≥0.005 were extracted and visualized with Cytoscape 3.2.0.

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