Vertebrate gastrulation

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Our understanding of the mechanisms that control gastrulation is still in its infancy. One problem is that gastrulation is a complex set of coordinated behaviours involving directional cell movements, several types of cell interactions, changes in cell fate and gene expression. Therefore, the successful analysis of its control mechanisms requires simultaneous analysis of more than one of these, or at least some way of separating them. Although progress has been slow, some recent studies have made significant advances in the field and we can probably look forward to some major breakthroughs in the near future.

Introduction

What is gastrulation? The name derives from the Greek root (*gastr-*) for 'stomach'; during this process in early embryonic development, the embryo essentially turns itself 'inside-out'. Some cells that are originally located on the surface of a hollow ball (in amphibians) or a flattened disc (fishes, amniotes) move through an opening to enter the embryo. In some classes of vertebrate embryos (e.g. amphibians), this movement occurs through a discrete hole, or blastopore. In others (most amniotes), cells enter through a slit-like opening, the primitive streak. In both cases, the opening corresponds roughly to the position of the future anus, with the mouth forming from a much more anterior position (derived from the prechordal plate). The movements of gastrulation generate two new layers of cells: the mesoderm, which gives rise to the circulatory system, musculo-skeletal system, dermis, urinary and genital systems and some glands, and the definitive endoderm, which generates the gut. The remainder of the embryo, which does not ingress, is the ectoderm, from which the skin and the entire nervous system are derived.

At the same time as the movements of gastrulation take place, the anteroposterior axis of the embryo can first be recognized by simple observation. But gastrulation is more than just cell movements. It also involves changes in the fates of the participating cells and of those that do not ingress, such that those that enter the embryo become different from those that remain on the outside. At least some of these changes are irreversible: for these cells, transplantation back to their original site does not result in reversal of the original decision. Therefore, these changes must be accompanied by substantive changes in gene expression, probably under the control of transcription factors that, for the most part, remain to be identified.

Thus, gastrulation encompasses three processes: cell movements generating a three-layered embryo; the establishment of the anteroposterior axis of the embryo; and the changes of cell fate that accompany the reorganization of cell populations. Lewis Wolpert's famous dictum is therefore amply justified: "It is not birth, marriage or death, but gastrulation which is truly the most important time in your life", and the current resurgence of interest in this important problem, brought about mainly by a thirst for identifying the genes involved, is hardly surprising.

We know remarkably little about the control of gastrulation at any level; tissue, cellular or molecular. We are not even sure of the lineage relationships between cell types, or about the extent to which cell communication is required for each of the three processes named above, or even whether it is possible to separate the three completely. For a fairly up-to-date review, the reader is referred to the proceedings of a recent meeting on gastrulation that have just appeared [1•]. Here, I will give an overview of the main advances made during the past year, most of which missed being published in the book, classified according to the major processes that appear to play a role.

Cell lineage and fate maps

It seems remarkable that after more than a century of interest in gastrulation, there are few detailed and reliable fate maps of the embryo just before gastrulation. Even in amphibians, where it is relatively easy to map the descendants of single cells because early cell divisions occur without cell growth (cells get smaller at each division), there are almost no studies of the distribution of descendants of single cells in the gastrula, or information

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Abbreviations

FGF—fibroblast growth factor; kFGF—Kaposi sarcoma-FGF; LRD—lysine-rhodamine-dextran; TGF—transforming growth factor.

about cell mixing. During the past year, this has started to change. A major landmark is the publication of the long-awaited detailed study by Lawson *et al.* [2••], in which the descendants of single epiblast cells marked by intra-cellular injection of horseradish peroxidase in the early post-implantation mouse embryo are mapped. The maps obtained agree very well with those published since the 1930s for other vertebrates, but they represent the first direct fate mapping analysis of the embryonic regions of an early mammalian embryo.

In amphibians, a new technique [3•] combining ^{125}I and a fluorescent tracer, lysine-rhodamine-dextran (LRD), has enabled the mapping of superficial and deep cells of the early gastrulating newt embryo to be studied in detail. One interesting result that has emerged from this study is that there is considerable cell mixing between the two layers, especially in the dorsal part of the embryo. Another study of cell mixing in amphibian ectoderm has been conducted by Wilson and Keller [4] using time-lapse analysis. In the axolotl, using intracellular injection of a *lacZ*-fusion construct, Whiteley and Armstrong [5] have studied the contribution to the mesoderm of the upper two tiers of blastomeres of the 32-cell stage em-bryo. They found, as expected, that the middle tier but not the upper tier contributes to the mesoderm, although some upper tier cells are shed into the blastocoele cavity. Their results, they conclude, argue against an earlier pro-posal suggesting that some mesoderm cells may originate from the blastocoele roof.

In chicks, where intracellular injection of LRD and the carbocyanine dyes DiI and DiO were used to produce a detailed fate map of the 'organizer' region (Hensen's node) during gastrulation [6], there were two main find-ings. The first is that there are sub-regions in the node, each containing cells that normally contribute to only one tissue, although in other regions some cells have mixed progeny. The second finding is that one of these re-gions gives rise to cells restricted to the medial halves of the somites. A study by Ordahl and Le Douarin [7] also reached the conclusion that somites are subdivided along their mediolateral axis by orthotopic and hetero-topic chick/quail transplants. Further transplantation ex-periments have shown that some of the cells are commit-ted to their fates very early in development, whilst others are not [7,8].

Watanabe *et al.* [9] successfully produced quail–chick chimaeras by transplanting quail blastoderm cells into a host chick blastoderm. The stage of the host em-bryo seems to affect the sites at which the transplanted cells are found; interestingly, the fates acquired by at least some cell types appear to be unaffected by the site of the transplant. The technique could be important in the fu-ture both as an assay for the state of commitment of the transplanted cells and as a method for producing trans-genic birds, although other strategies for both have been described by others.

Morphogenetic cell movements, the extracellular matrix and intercellular communication

Most studies in the general area of morphogenetic cell movements are still in the 'embryonic' stage, because few have attempted to link a description of the move-ments of groups of cells to a molecular analysis or even to more detailed cell-lineage studies. Mutants affecting cell movement during vertebrate gastrulation are scarce and at present only available in zebrafish (see [1•]). However, some recent studies do address the important question of how much mixing there is between different pre-sumptive cell types. I have already discussed the study [3•] of double-labelling of deep and superficial newt cells. Wilson and Keller [4] used detailed analysis of time-lapse films and succeeded in analyzing the changing relationships between neighbouring cells during conver-gent extension movements of the ectoderm. In the fish *Fundulus*, Trinkaus and colleagues [10] took advantage of the transparency of these embryos to produce time-lapse films to study in detail the convergent movements of gastrulation. They find that cells most frequently move as clusters rather than as individuals and that cell move-ments do not occur in straight lines; cells appear to mean-der. The rates of movement differ in different regions of the embryo, which they interpret to indicate that factors external to the moving cells control their directionality.

What are these external factors? The extracellular matrix, and in particular fibronectin, has long been suspected to provide guidance cues for cells migrating directionally. In the past, studies of the matrix have been limited mainly to establishing correlations between patterns of immunolocalization and gross patterns of movement, or to disrupting all movements with antibodies or specific peptides (e.g. RGDS [Arg–Gly–Asp–Ser], the cell-binding domain of fibronectin). But neither approach can answer questions about directional control. A recent paper [11•] has attempted to do just that. The authors use a wide variety of techniques and suggest that a combination of cell-intrinsic factors and others extrinsic to the migrating cells guide mesodermal cell movements in the *Xenopus* gastrula. The intrinsic factors appear to increase in impor-tance from the anterior to the posterior part of the gas-trula. Among the extrinsic factors is a fibrillar matrix that can be deposited on an artificial substrate by blastocoele roof explants. They conclude that in the anterior part of the embryo (head mesoderm), the extracellular matrix provides sufficient information for directional migration, but in more posterior regions the intrinsic polarity of the mesoderm dominates. In the chick embryo, agents that interfere with binding to fibronectin appear to disrupt the migration of mesoderm under the epiblast, whilst agents that interfere with binding to laminin (e.g. the peptide YIGSR [Tyr–Ile–Gly–Ser–Arg]) do not affect this migra-tion [12].

What is missing from all of these studies, however, is some way of determining not whether particular factors

affect or are required for directional cell migration, but rather what the factors are that initially determine such directionality. To my knowledge, no study has yet addressed this question directly.

An interesting study by Christ *et al.* [13] recently attempted to dissociate the movements of gastrulation from the fates of cells that differentiate from the mesoderm in the absence of such movements. The authors find that in the absence of 'gastrulation', blastoderm cells only differentiate into endothelium. Clearly, more work is required to understand how movements contribute to the production of the remaining mesodermal cell types, but it is obvious that intercellular communication of some sort must play a role, either through diffusible 'morphogens' or through intercellular contact, which may involve specialized junctions. The development of gap junctional connexins has been recently studied in the mouse embryo [14]. During the early stages of implantation, the gap junction proteins are localized mainly to the inner cell mass, but the possible existence of more subtle regional differences in the distribution of connexins during gastrulation was not explored.

Cell type specific gene expression

To elucidate the mechanisms that lead to the diversification of cell types during gastrulation requires the availability of reliable cell type specific markers to identify as early as possible the cell types concerned. The number of attempts that have been made to identify such markers are so numerous that it is impossible to review them all here. However, a few appear particularly interesting and these will be discussed briefly.

In the chick, a homologue of the *Drosophila* gene *caudal*, *CHox-cad*, has been isolated [15]. Its expression appears to be restricted to the definitive (presumptive gut) endoderm of the early embryo, and therefore represents the first marker available for this tissue.

In amphibians, a gene with homology to the *Drosophila* genes *bicoid* and *gooseberry* has been identified, and called *goosecoid* [16]. Its expression is localized to the dorsal lip region of the *Xenopus* embryo even before the blastopore is visible, and thus represents an early marker for the organizer. Another study [17] has dissociated the expression of the 'muscle master gene', *XMyoD*, from muscle differentiation. This gene is currently the earliest muscle-specific marker in *Xenopus*; however, in the early gastrula, it is expressed transiently in a wider subset of mesoderm cells, which includes non-muscle precursors [17,18]. Embryos ventralized with UV make no muscle but still express *XMyoD*. This also argues against what was once thought to be a necessary connection between *MyoD* expression and muscle development. Frank and Harland [17] suggest that stabilization of muscle differentiation requires a dorsalizing signal in addition to mesodermal induction.

Mesoderm induction and peptide growth factors

This field is reviewed elsewhere (C Niehrs and EM De Robertis, this issue, pp 550–555; ME Dickinson and AP McMahon, this issue, pp 562–566; for a recent review see [19]) and therefore a detailed survey of the literature is not provided here. I will, however, single out a few recent studies of importance with direct relevance to the control of gastrulation.

Peptide growth factors are currently the subject of great interest, mainly because of their putative roles as endogenous mesoderm-inducing factors. In particular, members of the fibroblast growth factor (FGF) and transforming growth factor (TGF) families have received much attention. Three recent papers are of interest with respect to the possible roles of FGF-related factors. In the frog, a novel member of the FGF family, XeFGF, which shares homology with FGF-4 (kFGF) and FGF-6, has been identified [20]. Unlike other members of the family, XeFGF is a secreted factor and, most importantly, it is expressed endogenously very early in development; there is even maternal message present in the egg. It is therefore a very good candidate for an endogenous ventral/posterior mesoderm inducer. This conclusion receives strong support from another recent study [21••], in which dominant negative *Xenopus* mutants were made for the FGF receptor. The resulting embryos lack posterior structures and the development of their ventral mesoderm appears to be impaired. Amniotes lag behind *Xenopus* in terms of what we know about mesoderm induction and axis specification. A recent study [22] found that FGF-5 is expressed in the ectoderm of the early preimplantation mouse embryo. The authors suggest, based on the expression pattern observed, that this factor plays some role in mouse gastrulation.

Members of the TGF-β family are also reported to be present in early mouse [23] and chick [24] embryos. In the former, expression of TGF-β_2 appears localized in the extra-embryonic regions and in the visceral endoderm at early post-implantation stages. In chick, expression of TGF-β_1 (which does not have mesoderm-inducing activity but which can act synergistically with inducing factors) has only been studied at the end of the primitive streak period. However, addition of this factor to cultured epiblast or mesoderm cells obtained from such embryos alters various aspects of their behaviour *in vitro*. A more remote member of the family is activin, which does have strong mesoderm-inducing activity. A recent study has reported the endogenous presence of a number of activin-related molecules even as maternally inherited components in the *Xenopus* oocyte and early embryo [25].

Establishment of the embryonic axis

Mesoderm induction, and indeed gastrulation, are intimately connected with the establishment of the anteroposterior axis of the embryo (for a recent re-

view see [26]). In frogs, it is widely believed that the dorsoventral character (where dorsal = notochord, and ventral = blood and endothelium) of the mesoderm is equivalent to the later anteroposterior axis; many workers assume that the same inducing factors are responsible for the simultaneous generation of both axes, although this does not appear to make much geometric sense. Nevertheless, it is clear that some connection exists, for example FGFs, which induce only ventral mesoderm, appear to be required for the normal development of posterior structures, as demonstrated by the important experiments of Amaya *et al.* [21••]. In contrast, another recent study [27] succeeded in separating anterior induction from dorsal axial mesoderm development in the frog, *Rana*, by using chimaeric embryos engineered by hybridizing macrocephalic and normal blastulae; some of the chimaeras were UV-treated to ventralize them. This treatment eliminated the dorsal axial structures but did not eliminate the head. Clearly, much remains to be learnt about the relationship between the axes of the embryo, for which cellular, as well as molecular studies are required.

The organizer and neural induction

One of the products of mesodermal induction, generally thought to be the most dorsal, is organizer tissue. This tissue is composed of cells that have the ability to dorsalize other mesoderm, or to induce ectoderm to become neural plate. Three recent and important studies have been conducted on two genes, *goosecoid* [16,28•] and *Brachyury* (the *T* gene) [29•], which are expressed specifically in cells at or near the organizer region of the frog embryo. In both cases, not only is expression localized to the cells in this important region of the *Xenopus* embryo, but expression of both genes responds rapidly and strongly to high concentrations of activin, which is known to induce the organizer property in responding cells (reviewed in [19]). Expression of *Brachyury* but not of *goosecoid* can be induced by FGF; this is perhaps unexpected, as FGF does not induce notochord. Injection of *goosecoid* mRNA into two vegetal blastomeres of the early embryo results in complete duplications of the axis, suggesting that expression of *goosecoid* is sufficient to cause axial duplication. However, other genes also appear to be sufficient to elicit a similar response in frogs. For example, when mRNA encoding factors related to the *Wnt* family [30•,31•] is injected into vegetal blastomeres, the embryo develops a supernumerary axis, and this axis includes the head of the embryo.

In chick embryos, it was recently shown [32] that neural induction by the chick equivalent of the amphibian dorsal lip, Hensen's node, occurs before the end of the gastrula stage in heterochronic and heterotopic quail–chick chimaeras. Specification of the anteroposterior character of the axis (revealed by the expression of four region-specific markers) can, however, continue over a much longer period, and can even be respecified after formation of the early neural plate [33••,34•]. Another study [35••] imagi-

natively investigates the same question by sandwiching a chick Hensen's node between two pieces of amphibian animal cap. Remarkably, although the chick node does not develop visible structures under these conditions, it induces neural differentiation in the amphibian ectoderm, and this ectoderm also expresses regional markers.

Conclusions

The literature surveyed in this brief review, which is by no means exhaustive, should give a glimpse of the degree of current interest in vertebrate gastrulation. During the past summer, a NATO meeting on the development of embryonic mesoderm took place at Banff, Canada (the proceedings are in press). A few months earlier, another meeting on gastrulation took place at Bodega Bay, California [1•]. Furthermore, a major symposium on gastrulation has just taken place in Brighton, with the auspices of the British Society for Developmental Biology and the Company of Biologists and with an attendance of 700. The proceedings of this meeting will appear as the 1992 supplement of the journal *Development*.

Despite all this excellent literature, we still know very little about gastrulation. The basic questions posed by the pioneer experimental embryologists at the turn of the century still remain unanswered. Good maps of cell fate and specification, and of patterns of gene expression will not be sufficient for complete understanding of the control of the events involved, and mutations affecting gastrulation are not yet available in vertebrates. I believe that an understanding of vertebrate gastrulation will only be accomplished when experimental embryology can be combined successfully with a molecular analysis. The papers published over the past year show that this approach is only just starting to be exploited.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
- •   of special interest
- ••   of outstanding interest

1.   KELLER RA, CLARK WH JR, GRIFFIN F (EDS.): *Gastrulation: Move-*
•   *ments, Patterns and Molecules.* New York: Plenum Press; 1991.
An interesting, important and up-to-date survey of the field, arising from a conference on gastrulation that took place last year at Bodega Bay, California.

2.   LAWSON KA, MENESES JJ, PEDERSEN RA: Clonal Analysis of Epi-
••   blast Fate during Germ Layer Formation in the Mouse Embryo. *Development* 1991, 113:891–912.
A landmark for mouse embryology. For the first time, the lineage of single cells in the early post-implantation mouse embryo is studied. Fate maps are presented for these stages of early mouse development and are shown to be remarkably consistent with those produced for amphibian and chick embryos.

3.   DELARUE M, SANCHEZ S, JOHNSON KE, DARRIBÈRE T, BOUCAUT
•   J-C: A Fate Map of Superficial and Deep Circumblastoporal Cells in the Early Gastrula of *Pleurodeles waltl. Development* 1992, 114:135–146.

Using orthotopic grafts of small groups of cells from a donor newt in which the superficial cells were labelled by ^{125}I and the deep cells by intracellular injection of LRD into unlabelled hosts, a fate map of the dorsal (blastopore) part of the newt embryo is constructed. The maps reveal a high degree of cell mixing between superficial and deep cells, particularly at the dorsal side of the embryo.

WILSON P, KELLER R: Cell Rearrangement during Gastrulation in *Xenopus*. Direct Observation of Cultured Explants. *Development* 1991, 112:289–300.

5. WHITELEY M, ARMSTRONG JB: On the Origin of the Mesoderm in the Mexican Axolotl, *Ambystoma mexicanum. Can J Zool* 1991, 69:1221–1225.

6. SELLECK MAJ, STERN CD: Fate Mapping and Cell Lineage Analysis of Hensen's Node in the Chick Embryo. *Development* 1991, 112:615–626.

7. ORDAHL CP, LE DOUARIN NM: Two Myogenic Lineages in the Developing Somite. *Development* 1992, 114:339–353.

8. SELLECK MAJ, STERN CD: Commitment of Mesoderm Cells in Hensen's Node of the Chick Embryo to Notochord and Somite. *Development* 1992, 114:403–415.

9. WATANABE M, KINUTANI M, NAITO M, OCHI O, TAKASHIMA Y: Distribution Analysis of Transferred Donor Cells in Avian Blastodermal Chimeras. *Development* 1992, 114:331–338.

10. TRINKAUS JP, TRINKAUS M, FINK RD: On the Convergent Cell Movements of Gastrulation in *Fundulus. J Exp Zool* 1992, 261:40–61.

11. WINKLBAUER R, NAGEL M: Directional Mesoderm Cell Migra-
• tion in the *Xenopus* Gastrula. *Dev Biol* 1991, 148:573–589.
An extensive array of techniques is used to demonstrate that the direction of migration of mesoderm cells is controlled both by factors intrinsic to the migrating cells and by others associated with the extracellular matrix deposited by the blastocoele roof. The relative importance of the two factors varies along the anteroposterior axis.

12. BROWN AJ, SANDERS EJ: Interactions between Mesoderm Cells and the Extracellular Matrix Following Gastrulation in the Chick Embryo. *J Cell Sci* 1991, 99:431–441.

13. CHRIST B, GRIM M, WILTING J, VON KIRSCHHOFER K, WACHTLER F: Differentiation of Endothelial Cells in Avian Embryos does not Depend on Gastrulation. *Acta Histochem* 1991, 91:193–199.

14. NISHI M, KUMAR NM, GILULA NB: Developmental Regulation of Gap Junction Gene Expression during Mouse Embryonic Development. *Dev Biol* 1991, 146:117–130.

15. FRUMKIN A, RANGINI Z, BEN-YEHUDA A, GRUENBAUM Y, FAINSOD A: A Chicken *caudal* Homologue, *CHox-cad*, is Expressed in the Epiblast with Posterior Localization and in the Early Endodermal Lineage. *Development* 1991, 112:207–219.

16. BLUMBERG B, WRIGHT CVE, DE ROBERTIS EM, CHO KWY: Organizer-specific Homeobox Genes in *Xenopus laevis* Embryos. *Science* 1991, 253:194–196.

17. FRANK D, HARLAND RM: Transient Expression of XMyoD in Non-somitic Mesoderm of *Xenopus* Gastrulae. *Development* 1991, 113:1387–1394.

18. HARVEY RP: Widespread Expression of MyoD Genes in *Xenopus* Embryos is Amplified in Presumptive Muscle as a Delayed Response to Mesoderm Induction. *Proc Natl Acad Sci USA* 1991, 88:9198–9202.

19. GREEN JBA, SMITH JC: Growth Factors as Morphogens: Do Gradients and Thresholds Establish Body Plan? *Trends Genet* 1991, 7:245–250.

20. ISAACS HV, TANNAHILL D, SLACK JMW: Expression of a Novel FGF in the *Xenopus* Embryo. A New Candidate Inducing Factor for Mesoderm Formation and Anteroposterior Specification. *Development* 1992, 114:711–720.

21. AMAYA E, MUSCI TJ, KIRSCHNER MW: Expression of a Dominant
•• Negative Mutant of the FGF Receptor Disrupts Mesoderm Formation in *Xenopus* Embryos. *Cell* 1991, 66:256–270.

A landmark paper, utilizing a new approach for understanding the role played by peptide growth factors during gastrulation and mesoderm induction. Dominant negative mutants for the FGF receptor are produced. The resulting embryos lack posterior structures, although their head parts develop in a remarkably normal manner.

22. HÉBERT JM, BOYLE M, MARTIN GR: mRNA Localization Studies Suggest that Murine FGF-5 Plays a Role in Gastrulation. *Development* 1991, 112:407–415.

23. SLAGER HG, LAWSON KA, VAN DEN EIJNDEN-VAN RAAIJ AJM, DE LAAT SW, MUMMERY CL: Differential Localization of TGF-β_2 in Mouse Preimplantation and Early Postimplantation Development. *Dev Biol* 1991, 145:205–218.

24. SANDERS EJ, PRASAD S: Possible Roles for TGF-β_1 in the Gastrulating Chick Embryo. *J Cell Sci* 1991, 99:617–626.

25. ASASHIMA M, NAKANO H, UCHIYAMA H, SUGINO H, NAKAMURA T, ETO Y, EJIMA D, NISHIMATSU S-I, UENO N, KINOSHITA K: Presence of Activin (Erythroid Differentiation Factor) in Unfertilized Eggs and Blastulae of *Xenopus laevis. Proc Natl Acad Sci USA* 1991, 88:6511–6514.

26. SLACK JMW, TANNAHILL D: Mechanism of Anteroposterior Axis Specification in Vertebrates: Lessons from the Amphibians. *Development* 1992, 114:285–302.

27. ELINSON RP: Separation of an Anterior Inducing Activity from Development of Dorsal Axial Mesoderm in Large-headed Frog Embryos. *Dev Biol* 1991, 145:91–98.

28. CHO KWY, BLUMBERG B, STEINBESSER H, DE ROBERTIS EM:
• Molecular Nature of Spemann's Organizer: the Role of the *Xenopus* Homeobox Gene *goosecoid* in Gastrulation. *Cell* 1991, 67:1111–1120.
The frog gene *goosecoid*, which shares homology with the fly genes *gooseberry* and *bicoid*, is expressed in the dorsal lip region of the blastopore. LiCl-dorsalization markedly extends the *goosecoid* expression domain, whilst UV-induced ventralization inhibits expression. Activin, but not FGF, induces expression of the gene. Most dramatically, injection of *goosecoid* mRNA into two ventral blastomeres of an early frog embryo results in the formation of an extra blastopore, and later in complete duplication of the embryonic axis.

29. SMITH JC, PRICE BMJ, GREEN JBA, WEIGEL D, HERRMANN BG:
• Expression of a *Xenopus* Homolog of Brachyury (*T*) is an Immediate-early Response to Mesoderm Induction. *Cell* 1991, 67:79–87.
This paper reports the cloning of *XBra*, a *Xenopus* homologue of the mouse *T* gene, which is responsible for the murine *Brachyury* mutation. *XBra* is first expressed in cells around the frog blastopore and later in the notochord. Its expression is rapidly induced by activin-A in the absence of protein synthesis. Interestingly, FGF, which does not induce notochord, also induces expression of *XBra*.

30. SOKOL S, CHRISTIAN JL, MOON RT, MELTON DA: Injected Wnt
• RNA Induces a Complete Body Axis in *Xenopus* Embryos. *Cell* 1991, 67:741–752.
Injection of mRNA encoding either of two *Wnt* proto-oncogenes (*Wnt-1* and *XWnt-8*) into the ventral region of an early frog embryo can induce an ectopic axis that includes head structures. Such injections can rescue the phenotype of ventralized embryos resulting from UV irradiation.

31. SMITH WC, HARLAND RM: Injected XWnt-8 RNA Acts Early
• in *Xenopus* Embryos to Promote Formation of a Vegetal Dorsalizing Center. *Cell* 1991, 67:753–765.
Here, the cloning of *XWnt-8* is reported. Injection of mRNA encoding this proto-oncogene (see [30•]) can generate an ectopic, complete axis and can rescue UV-irradiated embryos. Curiously, the authors found that *XWnt-8* mRNA is most abundant in UV-treated (ventralized) embryos and least abundant in LiCl-treated (dorsalized) embryos, and no dorsalizing activity could be detected in RNA from UV-treated or normal embryos.

32. STOREY KG, CROSSLEY JM, DE ROBERTIS EM, NORRIS WE, STERN CD: Neural Induction and Regionalisation in the Chick Embryo. *Development* 1992, 114:729–741.

33. MARTÍNEZ S, WASSEF M, ALVARADO-MALLART RM: Induction of
•• a Mesencephalic Phenotype in the 2-Day-Old Chick Pros-

encephalon is Preceded by the Early Expression of the Homeobox Gene *en. Neuron* 1991, 6:971–981.

A series of beautifully designed experiments, where the authors exploit the fact that the *engrailed* proteins of mouse and rat embryos are not recognised by monoclonal antibody 4D9, unlike their chick counterparts. They performed interspecific grafts and show that when a piece of *engrailed*-expressing neuroepithelium (presumptive cerebellum) is grafted anteriorly into the diencephalon, the adjacent diencephalic tissue expresses *engrailed*. Moreover, they present evidence that the diencephalic tissue develops cerebellar traits.

34.   GARDNER CA, BARALD KF: The Cellular Environment Con-
•   trols the Expression of *engrailed*-like Protein in the Cranial Neuroepithelium of Quail–Chick Chimeric Embryos. *Development* 1991, 113:1037-1048.

Using quail-chick chimaeras, the authors show that *engrailed*-expressing neuroepithelium can induce the expression of *engrailed* when grafted into the diencephalon of a host embryo (see also [33••]).

35.   KINTNER CR, DODD J: Hensen's Node Induces Neural Tis-
••   sue in *Xenopus* Ectoderm. Implications for the Action of the Organizer in Neural Induction. *Development* 1991, 113:1495–1505.

A stunning demonstration of the conservation of developmental mechanisms. Chick Hensen's node is sandwiched between two pieces of *Xenopus* animal cap. Remarkably, despite the failure of the donor chick node to develop under these conditions, it is able to induce a neural plate from the frog explants, as well as expression of neural- and region-specific markers.

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