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Relationships between mesoderm induction and the embryonic axes in chick and frog embryos

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Summary

The hypoblast is generally thought to be responsible for inducing the mesoderm in the chick embryo because the primitive streak, and subsequently the embryonic axis, form according to the orientation of the hypoblast. However, some cells become specified as embryonic mesoderm very late in development, towards the end of the gastrulation period and long after the hypoblast has left the embryonic region. We argue that induction of embryonic mesoderm and of the embryonic axis are different and separable events, both in amniotes and in amphibians. We also consider the relationships between the dorsoventral and anteroposterior axes in both groups of vertebrates.

Key words: chick embryo, amphibian embryo, pattern formation, mesoderm induction, embryonic axis, Hensen's node, stem cells.

Introduction

Establishment of the basic body plan in vertebrate embryos depends on two distinct, but interrelated processes. One is gastrulation, whereby the three germ layers and embryonic axes become established. The other is the commitment of cells to mesoderm and the subsequent differentiation of these cells into various mesodermal cell types. In Amphibians, the mesoderm of the early embryo is determined by an inductive interaction between vegetal cells (a transient population of yolky cells; see Hadorn, 1970) and the ectoderm of the marginal zone (Nieuwkoop, 1969, 1985; see Green and Smith, 1991; Slack et al., 1992; Smith and Howard, 1992 for reviews). Certain peptide growth factors are able to replace the vegetal cells in in vitro assays (the 'animal cap assay'; see Green and Smith, 1991): when an isolated piece of animal cap ectoderm is treated with appropriate concentrations of certain members of the FGF or TGFβ families of growth factors, mesodermal cell types differentiate. The higher the concentration of growth factor, the more 'dorsal/axial' the cell type formed. The highest concentrations of activin are able to generate notochord and cells that have organising activity. At these high concentrations, blastopore-specific genes like *Brachyury* (*XBra*) (Smith et al., 1991), *goosecoid* (Cho et al., 1991; Blum et al., 1992; De Robertis et al., 1992), *XFKH1* (Dirksen and Jamrich, 1992) and perhaps *Xlim1* (Taira et al., 1992) are expressed (see Smith and Howard, 1992). Lower concentrations of activin produce muscle and induce α-actin expression. FGF-related growth factors also induce mesoderm in a concentration-dependent manner: low concentrations give blood, mesenchyme and endothelium, higher concentrations give muscle and induce expression of α-actin (see Slack and Tannahill, 1992). These results are generally interpreted to mean that the vegetal cells produce growth factors related to the FGF and TGFβ families, which induce the marginal zone ectoderm cells to become mesodermal. In support of this hypothesis, Asashima et al. (1991) have recently described the presence of maternally derived activin-related activities in the egg and early embryo, and Isaacs et al. (1992) have reported the presence of a member of the FGF family (XeFGF) in the early embryo.

In the chick embryo, mesoderm is thought to arise as a result of a similar interaction, between the hypoblast and the overlying epiblast (Waddington, 1933, repeated and confirmed by Azar and Eyal-Giladi, 1981). When the hypoblast (consisting of yolky entodermal cells that do not contribute to the embryo proper) is rotated with respect to the overlying epiblast, the primitive streak forms according to the orientation of the hypoblast. However, as in the frog (Sokol and Melton, 1991), the epiblast of the chick also has its own polarity: if the hypoblast is dissociated into single cells, and then reaggregated into a sheet before being combined with intact epiblast, a primitive streak forms according to the original orientation of the epiblast (Mitrani and Eyal-Giladi, 1981).

Mitrani and his colleagues have argued that the hypoblast can be replaced by activin, similar to the case in *Xenopus*. If the centre of the embryo is deprived of both hypoblast and marginal zone and incubated in the presence of activin, a normal embryonic axis, containing notochord and somites, develops (Mitrani and Shimoni, 1990; Mitrani et

al., 1990b). Mitrani et al. (1990b) conclude that the hypoblast may be the endogenous source of activin in the embryo and that it may secrete this factor in a graded way, with the highest concentrations being emitted at its posterior midline. However, although it is clear from these experiments and others (e.g. Mitrani et al., 1990a; Cooke and Wong, 1991) that the chick epiblast **can** respond to activin and FGF, there is as yet no direct evidence that these molecules can act as true mesoderm-inducing factors on the chick epiblast (see Slack, 1991). One reason for this is that mesodermal differentiation of chick epiblast cells cannot yet be assessed independently from formation of an embryonic axis.

Induction of the mesoderm and its dorso-ventral subdivision still continue throughout gastrulation

In *Xenopus*, it has been suggested that mesodermal cell diversity is generated prior to the establishment of an overt body plan. Different mesoderm types are segregated, such that there is a transition from dorsal mesoderm (e.g. notochord) to ventral (blood, endothelium) across the marginal zone. The 'three signal model' (Slack et al., 1984), proposed to explain both the induction of mesoderm and its subdivision into different dorsoventral cell types, suggests that a ventral vegetal (VV) signal (perhaps FGF) instructs ventral marginal zone cells to become mesodermal. A second, dorsal vegetal (DV) signal, emanating from a small group of cells (the 'Nieuwkoop centre') in the most dorsal part of the vegetal hemisphere, instructs the most dorsal marginal zone cells (the site of the future dorsal lip of the blastopore) to become 'Spemann organizer' cells. These cells would emit an organizing (O) signal, which subdivides the marginal zone mesodermal belt into different dorsoventral cell types, according to their distance from the Spemann organizer. Later in development, the Spemann organizer cells emit neural inducing signal(s), instructing the neighbouring animal cap ectoderm to become neural.

The competence of *Xenopus* animal caps to respond both to vegetal cells and to mesoderm-inducing factors like FGF and activin declines at the beginning of gastrulation (stage 9-10; see Gurdon, 1987; Green and Smith, 1991; Slack and Tannahill, 1992). However, at least some individual cells in the amphibian dorsal lip (Delarue et al., 1992) and chick Hensen's node (Selleck and Stern, 1991) still give rise to progeny that are located in both ectoderm and mesendoderm at the end of gastrulation. Clearly, these cells cannot have been induced to form mesoderm before gastrulation. Therefore, some mechanisms inducing mesoderm must still operate at the end of the gastrula stage, even though the competence of cells to respond to known mesoderm-inducing factors has all but disappeared by this stage.

The mesoderm also retains its ability to be regionalized into different dorsoventral cell types at least until the start of neurulation. Single cells can contribute to notochord and somites at this stage (Selleck and Stern, 1991), and cells located at the posterior end of the paraxial mesoderm can contribute both to somites and to more lateral/ventral mesoderm (mesonephros, endothelium, blood; Stern et al., 1988). Grafts of Hensen's node from a late primitive streak stage quail embryo into the lateral part of a similarly staged chick host embryo can produce paraxial (somite) and lateral mesoderm from the host, although the degree to which somites form depends on distance from the host axis (Hornbruch et al., 1979). Whether these somites form from the lateral plate of the host or from newly induced mesoderm remains to be established, but these experiments show that the competence of the mesoderm to become subdivided into dorsoventral regions has not completely disappeared before the end of gastrulation. Taken together, these conclusions suggest that, at least in the chick, commitment of mesoderm cells is still occurring at the start of neurulation. By this time, the hypoblast has been displaced into extraembryonic regions and is therefore unlikely to be responsible for mesoderm induction or for its regionalisation at these stages.

Thus, mesoderm induction seems to occur over a protracted period of development, in more than one step, and more than one signal must be involved.

Anteroposterior patterning of the mesoderm: evidence for stem cells in Hensen's node

As well as producing a diversity of mesodermal cell types, generation of the basic body plan requires the axes of the embryo to become established. We have seen that in fate maps of Hensen's node, some cells contribute progeny to notochord only, some to somite only and others to both notochord and somite (Selleck and Stern, 1991; see above). But single cell lineage analysis in Hensen's node also revealed an otherwise unsuspected spatial organisation of the mesodermal descendants of the marked cells. Injection of the fluorescent lineage tracer lysine-rhodamine-dextran (LRD) into a single cell in the node generates several clusters of labelled cells, regularly spaced along the length of the notochord or somitic mesoderm (Selleck and Stern, 1991, 1992a, b; Fig. 1). The spacing between clusters differs in the two tissues: in the notochord, clusters are separated by about 1.5-2 somite lengths, whilst in the somitic mesoderm the distance appears to be about 5-7 somites. The results have been interpreted as indicating that the node contains a population of multipotent cells with stem cell properties, which give rise to founder cells with more restricted fates (viz. notochord or somite; Selleck and Stern, 1992b; Fig. 2). The founder cells also have stem cell properties; to account for the differences in spacing between adjacent clusters, the rate of cell division is proposed to be faster in notochord founder cells than in somitic precursors.

The distance of 5-7 somites between adjacent clusters in the somitic mesoderm agrees well with the findings of Primmett and colleagues (Primmett et al., 1988, 1989; Stern et al., 1988). They found that heat shock generates periodic anomalies in the somitic mesoderm, with a spacing of about 6-7 somites, and that this periodicity correlates directly with the rate of cell division of somite precursor cells. Indeed, somite precursors in the segmental plate mesoderm divide about every 10 hours, which is the time taken for about 7 somites to form (Primmett et al., 1989). These experiments suggest that the anteroposterior axis of the mesoderm becomes regionalised in the notochord and somite precur-

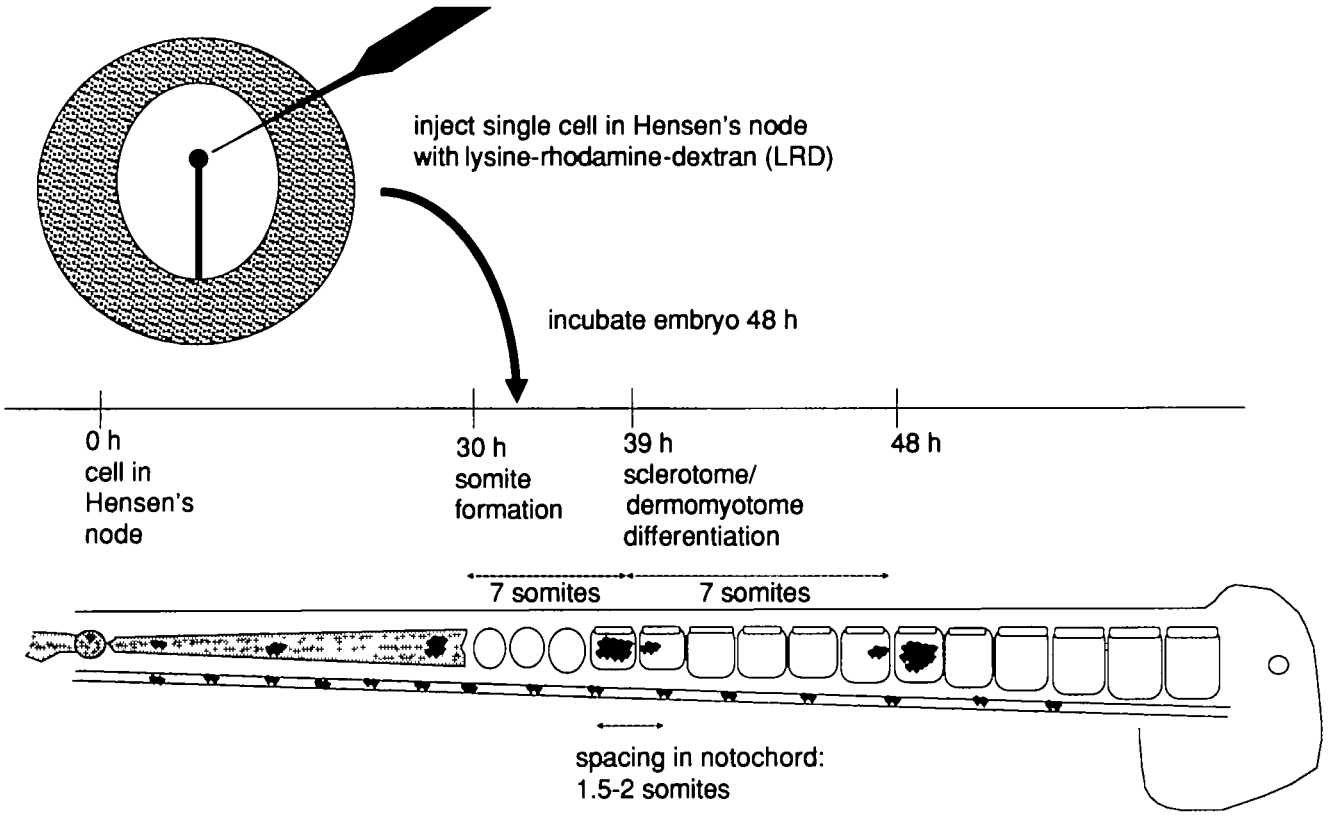

Fig. 1. Diagram summarising periodic clusters of cells revealed by injection of lysine-rhodamine-dextran (LRD) into a single cell in Hensen's node, based on the results of Selleck and Stern (1991, 1992a,b and unpublished observations). The spacing between clusters of labelled cells in the somitic mesoderm is about 6 somites; this is correlated with a time scale following the fate of the somitic descendants of the injected cell over 2 days after leaving Hensen's node. In the notochord, the clusters of descendants of the injected cell are 1.5-2 somite-lengths apart.

sor cells in the node, in association with the cell division cycle of these progenitor cells.

Relationships between the anteroposterior and dorsoventral axes

Several experiments have revealed effects of mesoderm-inducing factors on development of the anteroposterior axis of the early embryo. For example, when dominant-negative mutants are constructed for the FGF receptor in *Xenopus* (Amaya et al., 1991), not only is the ventral mesoderm affected, but also the posterior part of the embryo is deficient or fails to develop. For this reason, as well as the finding that activin-treated explants placed in the blastocoele of a host embryo ('Einsteckung' assay) can generate head structures whilst FGF-treated explants only generate tails (Ruiz i Altaba and Melton, 1989; Sokol and Melton, 1991; Slack and Tannahill, 1992), these results suggest that members of the FGF family are posterior inducers as well as ventral inducers. Microinjection of mRNA encoding goosecoid (Cho et al., 1991; De Robertis et al., 1992) or members of the *wnt* family of proto-oncogenes (Smith and Harland, 1991; Sokol et al., 1991) into ventral blastomeres can also generate an ectopic axis.

Thus, there appears to be a correlation between the ability of a substance to induce dorsal mesoderm with its ability to generate head structures. Therefore, inducing factors are often referred to as 'ventral/posterior' or 'dorsal/anterior' inducers (see Slack and Tannahill, 1992). But if the same factors are responsible for posterior and ventral induction or for anterior and dorsal induction, how do these two axes become separate in development?

Origin of posterior structures

Given the apparent relationship between anterior and dorsal, and posterior and ventral, how do structures such as the notochord (dorsal) of the tail (posterior) become established? In diagrams of the three signal model, the embryo is often shown in mid-sagittal section (e.g. Slack et al., 1984; see also Slack and Tannahill, 1992), with the ventral region being thought of also as posterior. However, fate maps of the early amphibian embryo (e.g. Hadorn, 1970; Nieuwkoop et al., 1985; see also Keller et al., 1992) show the presumptive tail bud region just above the equator, about 90° away from the prospective ventral part of the embryo (Fig. 3).

Classical fate maps of the chick blastoderm before the appearance of the primitive streak (e.g. Rudnick, 1935; Pasteels, 1940; Waddington, 1956; Balinsky, 1975) seem to place the presumptive tail at an equivalent, lateral position close to the marginal zone (Fig. 3). Prior to and during the early stages of primitive streak formation, 'Polonnaise'-like movements of the epiblast make the left and right tail pri-

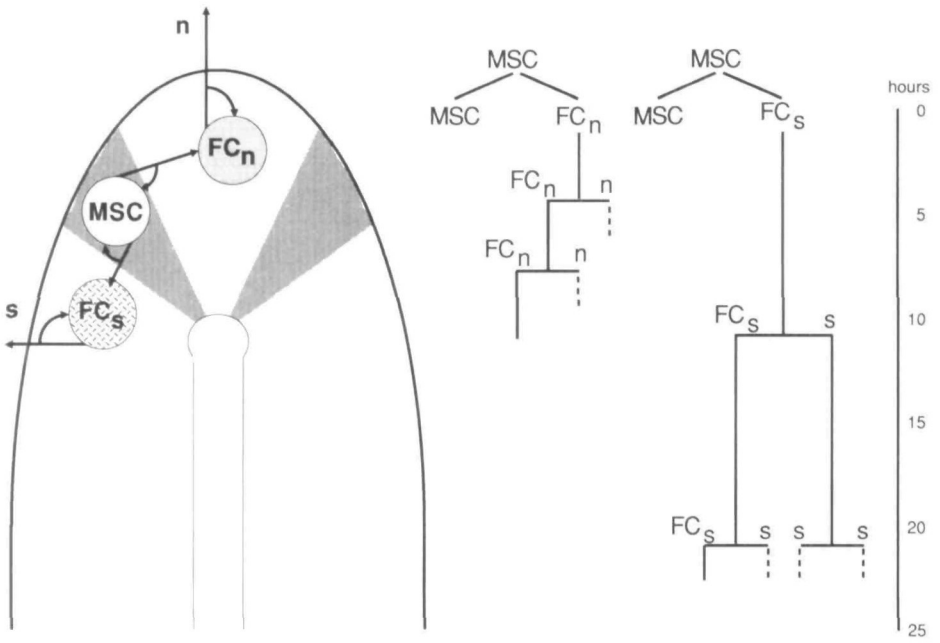

Fig. 2. A model of the organization of stem cells and founder cells in Hensen's node of the chick embryo at stage 3+. The median, anterior quadrant of the node contributes cells to the notochord but not to the somites, the regions lateral to the primitive pit populate the medial halves of the somites, and the region between the previous two (shaded) contributes to both notochord and somites (Selleck and Stern, 1991). Based on these results and those summarised in Fig. 2, the following scheme is proposed (see Selleck and Stern, 1992b): the region that contributes cells to both notochord and somites (shaded) contains multipotent stem cells (MSC) which renew themselves and give rise to founder cells that contribute progeny to either the notochord (FC_n) or the somite (FC_s). Each of these founder cells also have stem cell properties, since they can also renew themselves. The same model is displayed as a lineage tree on the right. n, notochord cells; s, medial somite cells. In the lineage tree, the length of the vertical lines is proportional to the length of the cell division cycle of the cell shown at the top of the line.

mordia converge towards the posterior margin, whilst the cells that originally lay posteriorly in the marginal zone shift forwards (see Stern, 1990). Some of these cells end up in Hensen's node and subsequently contribute to the prechordal plate, definitive (gut) endoderm and chordamesoderm (see Selleck and Stern, 1991). Although the classical fate maps mentioned above were produced mostly without good lineage markers and before a good staging system was available for pre-primitive streak stages of chick development (Eyal-Giladi and Kochav, 1976), they appear to be remarkably accurate; recent studies in our laboratory (Selleck and Stern, 1991, 1992a; YH and CDS, in preparation) confirm their conclusions.

Such fate maps of both chick and amphibian embryos, indicate that: (a) presumptive head structures and prospective dorsal mesoderm are located close to each other, in the region of Hensen's node or the dorsal lip of the blastopore of the gastrula-stage embryo; (b) ventral mesoderm originates from a region which in the amphibian appears to be located ventrally in the blastula; in the chick, the ventral mesoderm seems to come from cells situated in the more central epiblast, away from the marginal zone (see Stern and Canning, 1990; Stern, 1992); (c) posterior ventral structures come from a region about 90° away from both the prospective dorsal lip and ventral marginal zone in the amphibian blastula, and from a marginal region about 90° away from the posterior margin of the chick blastoderm. But this still leaves us with the question: where are the progenitors of the dorsal structures of the trunk and tail?

If, as we have discussed above, the node contains notochord and somite precursor cells with stem cell properties, then the posterior notochord and somites will be derived from progenitors common to more anterior notochord and somites at the late primitive streak stage. Somehow, the descendants of these progenitors must acquire their anteroposterior positional information **after** this stage. One possibility is that such positional information is dependent on the number of cell divisions undergone by stem cells before each of their descendants leaves the node region. For example, the progenitor cells might become posteriorised by exposure to some substance, like retinoic acid, present locally within the node such that, the longer the time spent in the node, the more posterior the character of their descendants.

One further piece of evidence supports this conclusion. When Hensen's nodes of increasing age are grafted into the area opaca of a competent host embryo, the anteroposterior extent of the structures formed from the host depends on the age of the node (see Storey et al., 1992; Kintner and Dodd, 1991; for amphibians, see Spemann and Mangold, 1924; Nieuwkoop et al., 1985; Hemmati Brivanlou et al., 1990; Sharpe, 1990). The older the node, the more posterior the structures that develop. However, host-derived structures only form if the transplanted node comes from a donor embryo younger than the definitive streak stage. If the node is older than this, the structures formed are derived from self-differentiation of the grafted node; nevertheless, they express the most posterior markers as if the grafted node were able to pattern itself as far as the tail. This conclusion is consistent with the idea that patterning posterior to the otic vesicle (the level at which Hensen's node appears to be located at the definitive streak stage; see Rudnick, 1935; Balinsky, 1975) is related to the length of time spent by progenitor cells within the node region.

Origin of the definitive (gut) endoderm, neural induction and regionalisation

During the early stages of chick gastrulation, cells destined

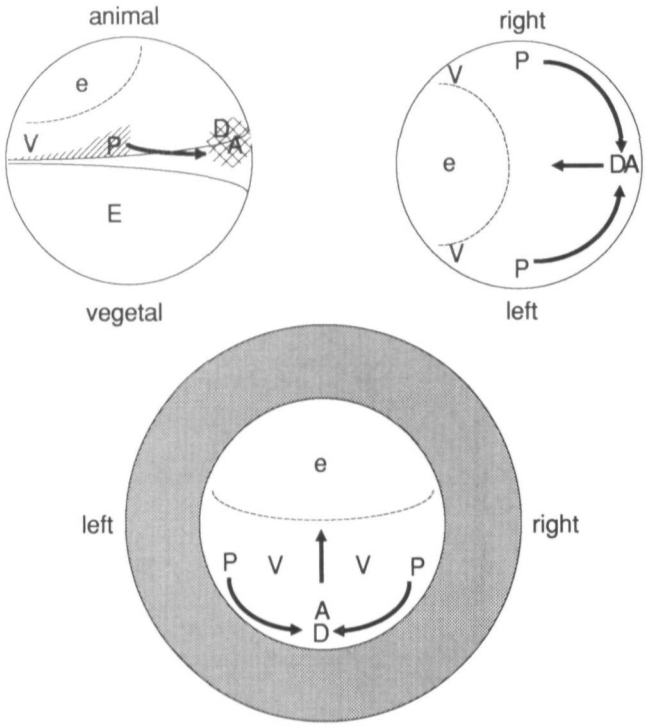

Fig. 3. Presumptive dorsoventral and anteroposterior axes in the fate maps of an amphibian late blastula (upper diagrams) and chick blastoderm at about stage XI (lower diagram). The amphibian map on the left shows the surface of the blastula viewed from its left side, and the map on the right shows a similar blastula observed from the animal pole. The chick blastoderm is viewed from the epiblast side. D, dorsal; V, ventral; A, anterior; P, posterior; E, yolky vegetal 'entoderm' (amphibian); e, surface ectoderm. The arrows show the direction of some of the main cell movements. In the upper left diagram, the presumptive tail region is shown as a shaded area and the presumptive head is shown by cross-hatching. In the chick diagram, the area opaca is shaded.

to form the tail bud in amphibians and birds converge from the lateral margins to the future posterior margin, as we have discussed above. At this time, presumptive definitive endoderm cells become located close to precursors of the notochord (Rudnick, 1935; Pasteels, 1940; Waddington, 1956; Rosenquist, 1966, 1971, 1983; Nicolet, 1970, 1971; Balinsky, 1975; Selleck and Stern, 1991; YH and CDS, in preparation). The regions giving rise to each of these tissues all seem to meet at Hensen's node. The endoderm cells have been suggested to be responsible for neuralization of the overlying ectoderm (see Dias and Schoenwolf, 1990; Storey et al., 1992), whilst the notochord and/or somitic precursors may be responsible for its regionalization (Storey et al., 1992).

Conclusions

The above discussion suggests that in the tail and posterior trunk regions, the dorsal and paraxial mesoderm components are derived from late descendants of chordal and somitic founder cells in the node region, and which were located in the midline of the mid-blastula stage embryo. Intermediate and lateral mesodermal components of the tail, on the other hand, are recruited by the regressing primitive streak from cells that have migrated towards the midline during the early stages of gastrulation.

This brings us back to the original question: what is the relationship between induction of the mesoderm and specification of the embryonic axes? Examination of fate maps and analysis of other experimental findings can help us to separate dorsal from anterior, ventral from posterior, and all of the above from mesoderm induction. But we still have to address the questions of when each of these axes is specified, and whether the role of the hypoblast in the chick and of the vegetal tissue of the frog is mainly to induce mesoderm, to set up dorsoventral pattern or to specify the axes of the embryo. Acknowledgement of the identity and location of these three embryonic dimensions could help us to understand better the role of the so-called mesoderm-inducing factors in early vertebrate development.

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