

# The evolution of vertebrate gastrulation

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## SUMMARY

The availability of molecular markers now permits the analysis of the common elements of vertebrate gastrulation. While gastrulation appears to be very diverse in the vertebrates, by analyzing a head-organizer marker, *gooseoid*, and a marker common to all forming mesoderm, *Brachyury*, we attempt to identify homologous structures and equivalent stages in *Xenopus*, zebrafish, chick and

mouse gastrulation. Using a tail-organizer marker, *Xnot-2*, we also discuss how the late stages of gastrulation lead to the formation of the postanal tail, a structure characteristic of the chordates.

Key words: Spemann organizer, tailbud, *gooseoid*, *Brachyury*, *Xnot-2*, gastrulation

## INTRODUCTION

Evolutionary and embryological studies have been intertwined throughout their history. The common elements of body plans can be visualized best at mid embryogenesis, the phylotypic stage, as noted initially for the vertebrates by von Baer (1828; Gould, 1977). At an earlier stage, gastrulation, the process by which morphogenetic movements of cell layers produce an embryo consisting of endoderm, mesoderm and ectoderm, appears to be very different in the various vertebrate classes.

In the zebrafish the most striking gastrulation movement is epiboly, in which cells of the embryo proper envelop a spherical yolk mass. In *Xenopus*, which has holoblastic cleavage, the endomesoderm invaginates through a circular blastopore. In the chick the main gastrulation movement is the invagination of the mesoderm and endoderm through a linear primitive streak. In the mouse the gastrula has the shape of a cup, whereas in the human, gastrulation takes place in a flat epithelial epiblast layer and is very similar to that of chick. Mammalian gastrulation is thought to have evolved from ovoviparous ancestors that lost the yolk after nutrients could be obtained from the mother (Kerr, 1919), thus explaining the similarities observed among the amniotes.

Because the outcome of gastrulation in all the animals is a very similar body plan at mid embryogenesis, it is reasonable to assume that the underlying mechanisms are similar in all vertebrates. The availability of molecular markers now permits the identification of the common elements in vertebrate gastrulation.

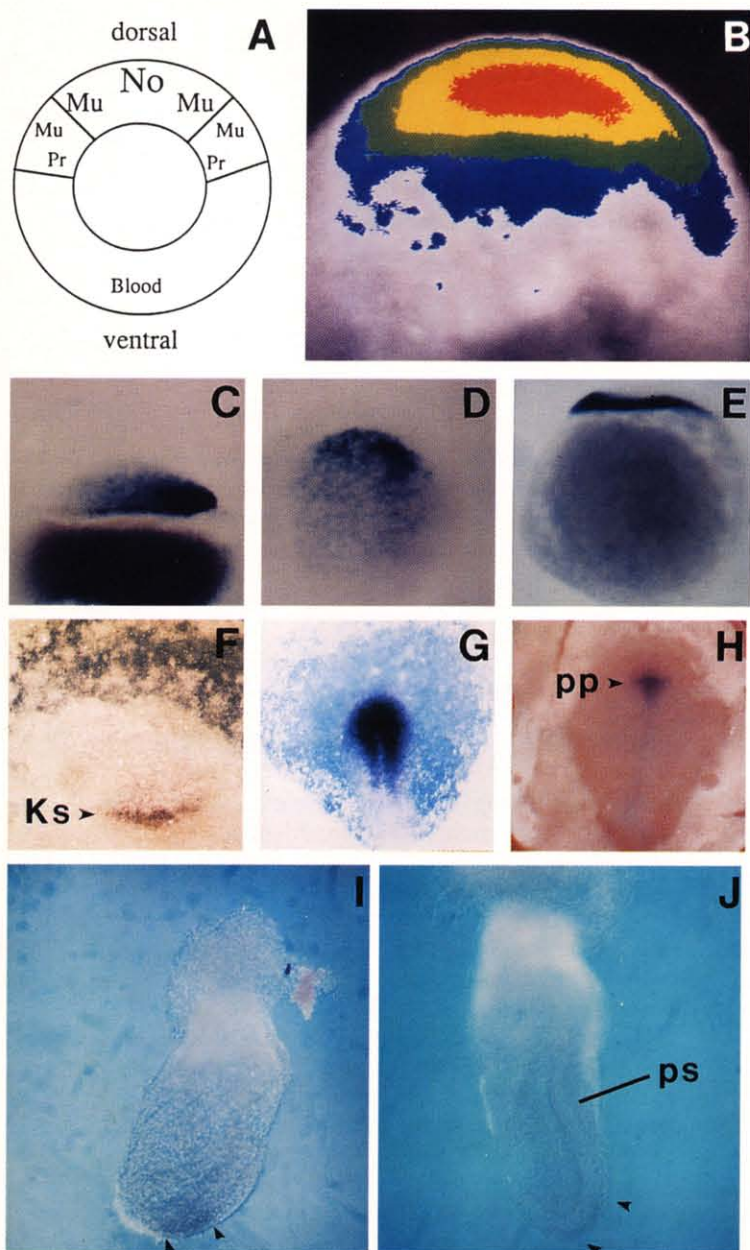
In this paper we will compare the distribution of two gene markers, *gooseoid* and *Brachyury*, in *Xenopus*, zebrafish, chick and mouse embryos, which shed light on the evolution of the organizer and of mesoderm precursor cells. In addition we will examine the mechanism of development of the postanal tail, which arises as a continuation of gastrulation in *Xenopus*, and perhaps in other vertebrates as well.

## ORGANIZER-SPECIFIC GENE MARKERS

An experiment that has had a permanent influence in the way we think about embryos was carried out by Spemann and Mangold in 1924. When the region at which involution starts in the amphibian gastrula, the dorsal lip of the blastopore, was transplanted to the opposite (ventral) side of a host embryo, a twinned embryo resulted. Because the transplanted tissue recruited cells from the host into the secondary axis, this region was called the organizer (Spemann, 1938; Hamburger, 1988). The organizer was able to induce neural tissue and segmented mesoderm such as somites and pronephros, while the graft itself differentiates predominantly into prechordal plate, notochord and part of the tailbud (Hamburger, 1988; Gont et al., 1993).

One of the recent advances has been the isolation of genes that mark the organizer. These include the genes for transcription factors such as *gooseoid* (Blumberg et al., 1991; Cho et al., 1991), *Xlim-1* (Taira et al., 1992), *FKHD-1* (Dirksen and Jamrich, 1992) and *Xnot* (von Dassow et al., 1993; Gont et al., 1993) as well as those for the secreted protein *noggin* (Smith and Harland, 1992) and the membrane bound molecule *integrin  $\alpha 3$*  (Whittaker and DeSimone, 1993).

The homeobox-containing gene *gooseoid* has been studied extensively and its expression pattern in *Xenopus*, zebrafish, chick and mouse is reviewed below. From a functional point of view, ectopic expression of *gooseoid* in ventral blastomeres of *Xenopus* mimics many of the functions of organizer grafts, such as formation of secondary axes (Cho et al., 1991; Steinbeisser et al., 1993), recruitment of neighboring uninjected cells into new cell fates, and triggering of cell migration in the dorsoanterior direction (Niehrs et al., 1993). It also causes dose-dependent formation of dorsal tissues (Niehrs et al., 1994). While *gooseoid* has been studied most intensively, the other dorsal markers are also expected to play roles in the organizer phenomenon. In the case of *noggin* it has been shown



**Fig. 1.** Expression of *goosecoid* mRNA in *Xenopus*, zebrafish, chick and mouse gastrulae. (A) Specification map of the early *Xenopus* gastrula as determined by explantation experiments (Dale and Slack, 1987). No, Notochord; Mu, Muscle; Pr, Pronephros. (B) Graded distribution of *Xenopus goosecoid* in the marginal zone of the stage 10 dorsal marginal zone. Red is maximal staining in this image analysis of a whole-mount in situ hybridized embryo. (C) Zebrafish blastula (3.5 hours) showing graded distribution of *goosecoid* in the cells overlying the yolk mass, side view. (D) Same embryo as C, showing the graded distribution in top view. (E) Zebrafish embryo showing *goosecoid* expression in the prechordal plate at the early tailbud stage. (F) Chick embryo showing *goosecoid* expression in Koller's sickle (Ks) and in cells immediately anterior to it. At this stage (XII) the forming hypoblast has covered 50% of the area pellucida, which remains transparent at the top of the panel. (G) Maximal expression of chick *goosecoid* at stage 3<sup>+</sup>. (H) Expression in cells that have left the node to form the prechordal plate (pp) at stage 4<sup>+</sup>. (I) Mouse gastrula showing *goosecoid* expression (arrowheads) in the anterior region of the early primitive streak. (J) *goosecoid* expression (arrowheads) in a mouse gastrula at the anterior of the primitive streak (ps) at the stage in which *goosecoid* is down-regulated. Panels are reproduced, with permission, from the following publications. (A, B) Niehrs et al. (1994) *Science* **263**, 817-820; (C,D,E) Schulte-Merker et al. (1994) *Development* **120**, 843-852; (F,G,H) Izpisua-Belmonte et al. (1993) *Cell* **74**, 645-659; (I,J) Blum et al. (1992) *Cell* **69**, 1097-1106.

that its ectopic expression produces complete axial rescue of *Xenopus* embryos ventralized by UV light (Smith and Harland, 1992). The analysis of many of the other genes is in progress, particularly by targeted inactivation of their mouse homologues, and one can expect that much information will be forthcoming. In this paper we concentrate on *goosecoid*, which is a marker of the head organizer region.

The *Brachyury* gene has also been intensively studied (reviewed by Beddington and Smith, 1993). Named after its short tailed phenotype in heterozygous mice, it was one of the first embryonic lethal mutations to be identified (Dobrovolskaia-Zavadskaja, 1927; Gluecksohn-Schoenheimer, 1938). The expression pattern of *Brachyury* has been studied in mouse (Herrmann, 1991), *Xenopus* (Smith et al., 1991) and zebrafish (Schulte-Merker et al., 1992). *Brachyury* provides a marker for both ventral and dorsal mesoderm. At mid gastrula *Brachyury*

marks the notochord and uncommitted mesoderm and, together with *goosecoid*, which marks the prechordal plate at this stage, permits one to identify the main territories of the gastrula. At later stages *Brachyury* is expressed in the tailbud and, together with *Xnot-2* (Gont et al., 1993), permits the analysis of the developing tail.

#### ***goosecoid* AND *Brachyury* IN XENOPUS GASTRULATION**

*goosecoid* mRNA is present at low levels in the unfertilized egg (De Robertis et al., 1992; Lemaire and Gurdon, 1994), but shortly after mid blastula the levels of its transcripts increase, reaching a maximum just before the start of gastrulation at stage 10. At this stage *goosecoid* mRNA is localized to the



dorsal side of the marginal zone, forming a gradient over tissue that at this stage is already fated to form dorsal mesoderm (Fig. 1A,B). By culturing fragments of marginal zone (Dale and Slack, 1987) it has been shown that dorsal mesodermal tissues (notochord and muscle) are formed by the organizer (dorsal lip) region, that intermediate regions give rise to pronephros and muscle, and that the rest of the marginal zone produces ventral mesoderm (mesothelium, mesenchyme and blood), as indicated in Fig. 1A. At later stages the more lateral regions of the marginal zone become induced to become muscle under the influence of a 'horizontal' signal emanating from the organizer (reviewed by Slack, 1991), but at the early gastrula only the *gooseoid*-expressing region is specified to form dorsal mesoderm. This early gradient of expression is thought to be involved in mesodermal patterning, because two-fold increments in the amounts of microinjected synthetic *gooseoid* mRNA can trigger the expression of increasingly dorsal molecular markers, forming several sharp thresholds in explanted ventral marginal zones (Niehrs et al., 1994).

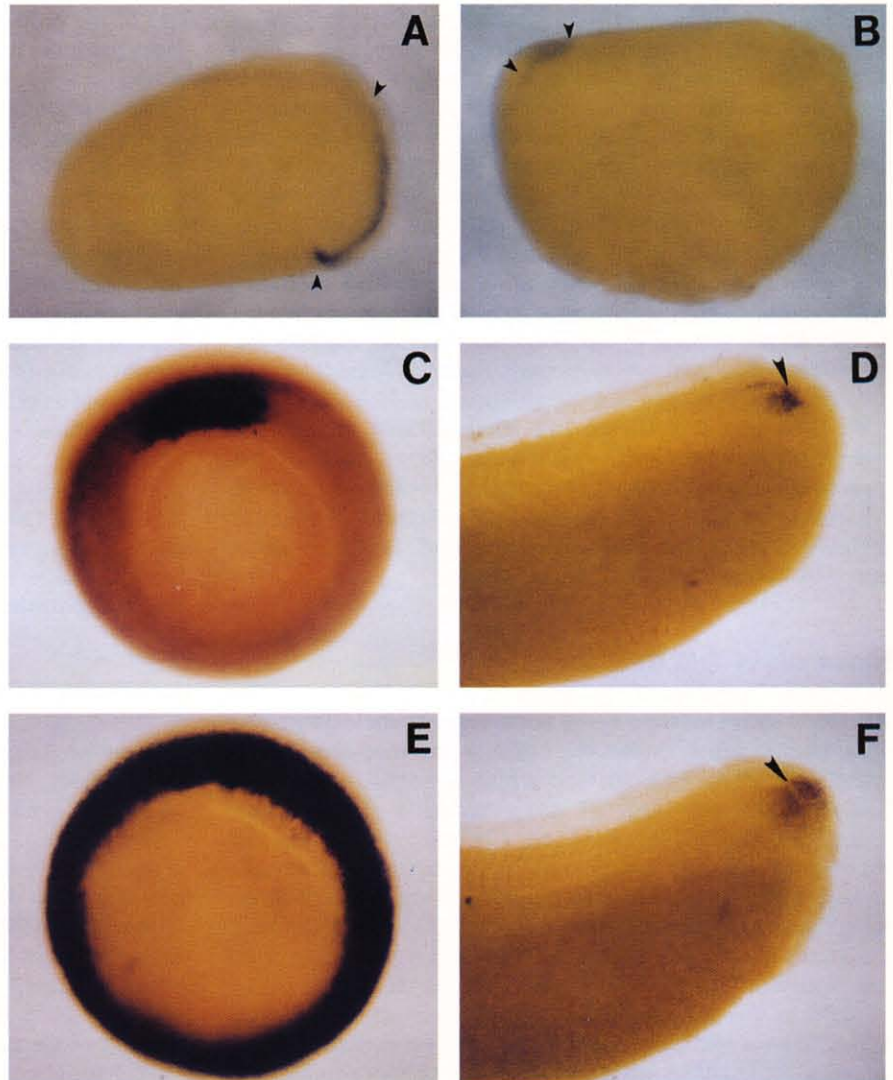
Once gastrulation starts, *gooseoid* is down-regulated, and the cells that continue to express it leave the dorsal lip with the involuting endomesoderm (Fig. 2A), eventually becoming restricted to the small group of cells that form the prechordal plate or head mesoderm (Steinbeisser and De Robertis, 1993), as shown in Fig. 2B. Not all genes expressed in the dorsal lip follow this pattern. For example, the homeobox gene *Xnot-2* is also expressed dorsally, but its expression stays localized in the dorsal lip, and at neurula stages is expressed in the notochord and eventually becomes restricted to the tail of the embryo (Fig. 2C,D).

At the early gastrula stage *Xenopus Brachyury (Xbra)* is expressed in a band or ring encompassing the entire marginal zone (Fig. 2E). In other words, *Xbra* is expressed in the progenitors of both dorsal and ventral mesoderm (Smith et al., 1991). Once cells involute through the circular blastopore, they lose *Xbra* expression (except in the notochord which is transiently positive), so that at later stages the only region expressing *Xbra* is the tailbud, as shown in Fig. 2F. Two regions of the tailbud express *Brachyury*, one of dorsal origin (the chordoneural hinge, which also expresses *Xnot-2*) and the other deriving from the lateral marginal zone (the posterior wall). These two populations are separated by the neurenteric canal (indicated by an arrowhead in Fig. 2F). The role of these two regions in late gastrulation (Gont et al., 1993) will be discussed below.

We conclude that in the early *Xenopus* gastrula *gooseoid* marks most cells specified to become dorsal mesoderm, including notochord and muscle, but that as gastrulation proceeds only those cells forming the prechordal plate continue to express *gooseoid*. *Xbra* is expressed in all mesoderm progenitors at early gastrula, but at later stages becomes restricted to posterior notochord and the tailbud.

### *gooseoid* IN THE ZEBRAFISH

In zebrafish *gooseoid* expression starts at the blastula stage, where it forms a striking dorsoventral gradient (Schulte-



**Fig. 2.** Expression of *gooseoid* (A,B), *Xnot-2* (C,D) and *Brachyury* (E,F) at early and late stages. (A) *gooseoid* at mid-gastrula (stage 11) has left the dorsal lip and involutes with the anterior endomesoderm (arrowheads). (B) At the neurula stage, expression can be seen in the prechordal plate or mesoderm of the head. (C) *Xnot-2* at early gastrula, note expression in the dorsal lip. (D) *Xnot-2* at the tailbud stage, note expression at the chordoneural hinge (arrowhead). The floor plate is seen as a thin trail of expression. (E) *Brachyury* at early gastrula, the entire marginal zone is marked, forming a ring. (F) *Brachyury* at the tailbud stage, two areas of expression can be seen. The arrowhead indicates the neurenteric canal, which separates the chordoneural hinge from the posterior wall area of expression.

Merker et al., 1994), as shown in Fig. 1C,D. These cells then cover the yolk through the movement of epiboly, which is accompanied by convergence towards the dorsal midline (forming the 'embryonic shield' on the dorsal side). Zebrafish *gooseoid* transcripts are maximal at 50% epiboly (Stachel et al., 1993; Schulte-Merker et al., 1994), the stage at which involution of the endomesoderm starts; expression is then down-regulated and follows the involuting cells in the anterior-most regions. The notochord is negative at all times, and by the end of gastrulation expression can be seen in the endomesoderm of the prechordal plate (Schulte-Merker et al., 1994), as shown in Fig. 1E. Patches of expression can be seen in the CNS and branchial arches at later stages (Schulte-Merker et al., 1994).

Zebrafish *Brachyury* (also called *notail* due to the phenotype of its mutant) has been studied in considerable detail, as an excellent antibody is available (Schulte-Merker et al., 1992). Expression starts on the dorsal side and forms a ring along the entire marginal zone, as in *Xenopus*. Interestingly, double staining experiments using *gooseoid* RNA probes and *Brachyury* antibodies have shown that both genes are expressed in the same cells of the dorsal side before involution starts. As gastrulation proceeds, the *gooseoid* signal follows the prechordal plate, which becomes negative for *Brachyury*. *Brachyury* is found in the notochord and tailbud (Schulte-Merker et al., 1992, 1994). Although both genes are expressed initially in the same cells, in *Brachyury* (*notail*) mutants the expression of *gooseoid* is normal (Schulte-Merker et al., 1994).

We conclude that despite the predominance of epiboly movements in zebrafish gastrulation, the expression of *gooseoid* is not unlike that of its *Xenopus* homologue: it starts as a dorso-ventral gradient, has maximal expression at the start of involution of the endomesoderm and is then down-regulated, following the prechordal plate.

### ***gooseoid* IN THE CHICK**

The chick embryo offers many advantages to the experimentalist, one of which is that the embryo develops from a flat epithelial sheet, the epiblast, from which all germ layers derive. This facilitates the study of pre-gastrulation stages. Chick *gooseoid* (Izpisúa-Belmonte et al., 1993) is first detectable in the unincubated egg, which as it is laid by the hen already contains several thousand cells. Expression starts in a thickening of the posterior marginal zone called Koller's sickle, where it is confined to a group of cells located in a middle layer of cells between epiblast and the forming hypoblast. The existence of this cell population had been overlooked, although Koller's sickle had been noted almost 100 years ago, but was revealed by the *gooseoid* marker. It is a very interesting group of cells, for lineage tracing with the hydrophobic dye DiI showed that they migrate anteriorly to the *gooseoid*-expressing region of Hensen's node, indicating that this group of cells constitutes the earliest mesendodermal cells of the chick gastrula (Izpisúa-Belmonte et al., 1993). The Koller sickle region can also induce secondary axes when grafted. The main conclusion from these studies was that in the chick gastrulation starts much earlier than previously thought, in the unincubated egg (Izpisúa-Belmonte et al., 1993). Fig. 1F shows *gooseoid* expression in Koller's sickle in an embryo that has

been incubated for a few hours (stage XII, when the forming sheet of hypoblast covers 50% of the area pellucida).

As development continues, the primitive streak forms in the posterior end. As the extension of the streak progresses in the anterior direction, *gooseoid* is expressed at the tip of the primitive streak. Transcripts become more abundant as the streak elongates, and by stage 3<sup>+</sup>, when the streak develops a groove but has not yet reached its maximal extension, *gooseoid* reaches its maximal expression (Fig. 1G). Transcripts are maximal anteriorly and decrease gradually, like the tail of a comet, towards the posterior primitive streak (Izpisúa-Belmonte et al., 1993). This stage may be considered homologous to stage 10 (beginning of dorsal lip formation) in *Xenopus* (Fig. 1B). This is also the stage in which the anterior end of the primitive streak, the young Hensen's node, has its maximal inductive activity. Hensen's node is classically considered to be the equivalent of the amphibian organizer (Waddington, 1933). Once the primitive streak reaches its full extension at stage 4, *gooseoid* is down-regulated, and this correlates with a decrease in inducing activity by the node (Storey et al., 1992). By stage 4<sup>+</sup>, *gooseoid* RNA is found in the cells that fan out from the node ingressing to form the endomesoderm of the head, as shown in Fig. 1H. At later stages (not shown) *gooseoid* expression is found in foregut endoderm, prechordal plate, and ventral diencephalon. The expression pattern of *Brachyury* in the chick has not yet been reported, but from what is known from *Xenopus* and mouse one would expect this gene to be active throughout the length of the primitive streak, where the forming mesoderm is located.

We conclude that while two of the regions in which chick *gooseoid* is found, Hensen's node and prechordal plate, have clear homologues in *Xenopus* and zebrafish, the earliest one, Koller's sickle, is more difficult to fit into the picture of a generalized gastrulation mechanism.

### ***gooseoid* IN THE MOUSE**

The mouse gastrula develops from a cup-shaped epiblast, the egg cylinder. Expression is first detected at the posterior end of the egg cylinder at 6.4 days after fertilization. It is seen as a spot on the side of the egg cylinder on the initial group of cells that leave the epiblast in the initial epithelial-mesenchymal transition that starts formation of the primitive streak (Blum et al., 1992). This early expression may be homologous to that found in Koller's sickle in the chick. Once the primitive streak is formed, *gooseoid* expression is found at its anterior end (Fig. 1I,J). Expression is maximal at day 6.7, also at the anterior of the streak. This early phase of expression is brief, lasting for about 10 hours. Transcripts were not detectable in the prechordal plate or other regions. The whole-mount in situ hybridization signal of mouse *gooseoid* is less intense than in other organisms, perhaps explaining why the signal is lost. After day 10.5 mouse *gooseoid* has a late phase of expression that has been described in some detail (Gaunt et al., 1993). *gooseoid* transcripts are found in limb buds (in the proximal, ventral and anterior region), neural crest of pharyngeal arch 1 (mandibular and maxillary processes) and anterior third of arch 2 (hyoid), the floor of the diencephalon, and other sites (Gaunt et al., 1993). The late expression pattern of *gooseoid* might

be similar in other organisms, but systematic studies have not been carried out.

The studies in the mouse suggest that the anterior primitive streak at day 6.7, when *gooseoid* expression is maximal, should be homologous to the early dorsal lip of the *Xenopus* early gastrula. Mouse embryo fragments containing this region have inducing activity when transplanted into *Xenopus* embryos by the Einsteck procedure (Blum et al., 1992). The mouse node, which at 7.5 days can induce trunk structures when grafted to mouse embryos (Beddington, 1994) would be the equivalent to the late (regressing) Hensen's node of the chick or the late dorsal lip in amphibians, which have trunk-tail organizer activities but do not express *gooseoid*.

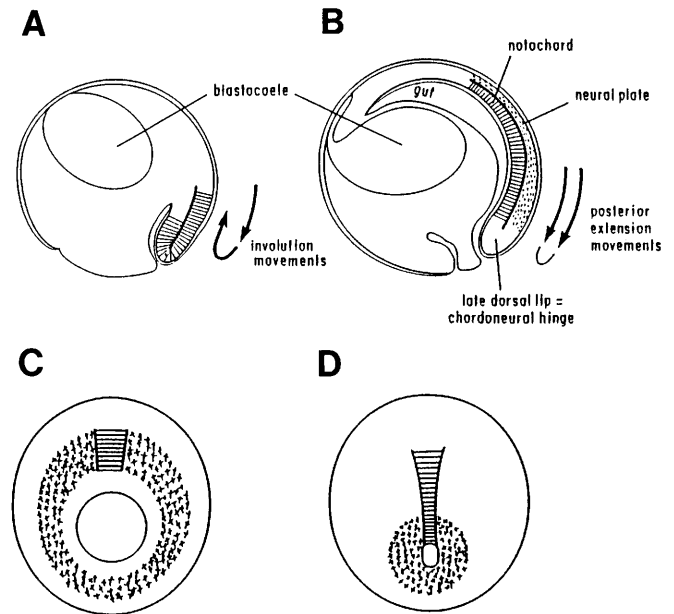
The *Brachyury* gene was initially isolated from the mouse, and it has been studied in detail. It is expressed first in the entire primitive streak and then in the notochord and tailbud (Herrmann, 1991; Beddington and Smith, 1993). In mouse, expression of *Brachyury* in the notochord is more persistent than in *Xenopus*.

### TAIL FORMATION IS A CONTINUATION OF GASTRULATION

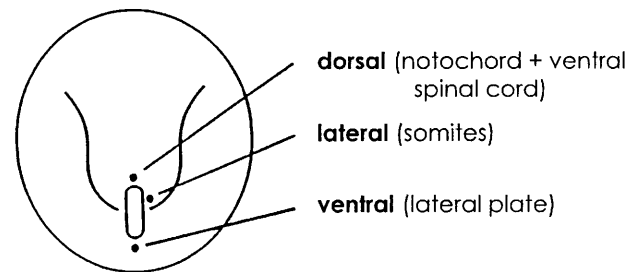
It has been observed recently that the formation of the postanal tail results from a continuation of gastrulation in *Xenopus* and is induced by cells originating from the late dorsal lip (Gont et al., 1993). These findings will be summarized here and then discussed in the context of the evolution of vertebrate gastrulation. Three lines of evidence support the view that tail formation is a continuation of gastrulation: (1) studies with the gene markers *Xnot-2* and *Brachyury*, (2) lineage tracing studies and (3) tail-organizer transplantations (Gont et al., 1993).

The gene marker *Xnot-2* is found first in the dorsal lip (Fig. 2C) and then in a part of the tailbud that forms a hinge between the notochord and floor plate (Fig. 2D). *Brachyury* is expressed as a ring in the marginal zone of the early gastrula (Fig. 2E) but is also found in the tailbud at late stages, although not exclusively in the hinge region, but also in cells located more posteriorly (Fig. 2F). The identification of these two cell populations, called the chordoneural hinge and the posterior wall cells, was our first indication that the tailbud is indeed heterogeneous. Both cell populations are separated by the neurenteric canal. Fifty years earlier Pasteels had reached similar conclusions based on careful examination of histological sections (Pasteels, 1943) but we were not aware of this at the time.

Lineage tracing of the late blastopore lip revealed that the chordoneural hinge is derived from the organizer. As shown in Fig. 3A, after the *gooseoid*-expressing cells that will form the prechordal plate leave the organizer, the predominant movement at mid-gastrula is that of involution (driven by convergence and extension), which leads to the formation of the trunk notochord and somites. At the early neurula (stage 13), the ectoderm and mesoderm attach to each other and the two layers undergo posterior extension movements instead of involution (Fig. 3B). It is these posterior movements of the organizer region (driven by continuing cell intercalations in the neural plate and notochord) that cause tail formation. When marks of DiI were placed in the closing blastopore at the slit stage (Fig. 4) and the embryos were left to develop, it was observed that: (1) the dorsal lip becomes the chordoneural



**Fig. 3.** Diagrams of mid and late gastrulation in *Xenopus*. (A) At the mid gastrula the main movement is involution, which forms the trunk axial structures. (B) At late gastrula/early neurula the main movement is posterior extension, which drives tail formation. Note that the neural plate and notochord are connected at the hinge, which descends from the organizer. (C) Mid-gastrula in vegetal view, the prospective notochord is hatched, the *Xbra*-positive region of the marginal zone is indicated by small crosses. (D) Early neurula, ventral view, the *Xbra*-positive cells from a thick circumblastoporal collar.



**Fig. 4.** Fate map of the late blastopore lip. DiI marks were placed in the slit blastopore of an early neurula (note neural folds) as indicated, and embryos allowed to develop to the tailbud stage. Each mark gives rise to different tissues of the tail. The chordoneural hinge, which gives rise to notochord and ventral spinal cord, derives from the late dorsal lip (from experiments of Gont et al., 1993).

hinge and gives rise to tail notochord and ventral spinal chord, (2) the lateral lip gives rise mostly to posterior wall and the tail somites that derive from it and, (3) the ventral lip gives rise to lateral plate mesoderm as well as tissue spanning the region between the anus and the tailbud. The neurenteric canal, which connects the gut and the spinal cord cavity, is formed by the fusion of the lateral lips and the closure of the neural plate so that the lateral lips become the posterior wall (Fig. 4; Gont et al., 1993). As shown in Fig. 5, at tailbud stages the neurenteric canal separates the chordoneural hinge (which expresses *Xnot*-



2 and *Brachyury*) from the posterior wall (which expresses only *Brachyury*).

Transplantation of the chordoneural hinge into host gastrulae by the Einsteck procedure showed that it has potent organizing activity, inducing tails with structures such as somites, spinal cord and fins, while the graft itself gives rise mostly to chordoneural hinge and notochord (Gont et al., 1993).

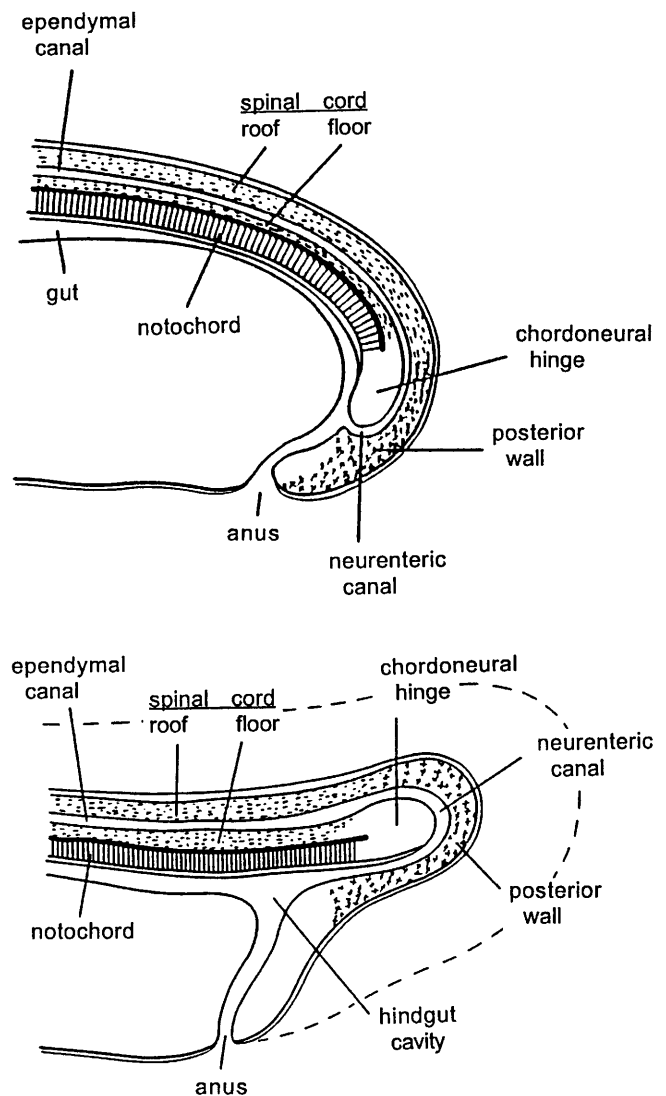
The main conclusion is that the organizer region retains its activity at tailbud stages and occupies a distinct region of the tailbud. This has implications on the way we think about *Xenopus* gastrulation. For example, the 'horizontal' signal emanating from the organizer that induces somite formation (Slack, 1991) might continue well into tailbud stages. The source of inductive signals would be the chordoneural hinge, which as it develops could recruit uncommitted cells from the *Brachyury*-positive posterior wall into the myotome pathway. In addition, the experiments in *Xenopus* tell us that the gastrulation process continues much longer than previously thought in the tail region (Gont et al., 1993). We will discuss below whether other vertebrates might share a similar mechanism of late gastrulation.

## COMPARATIVE MOLECULAR ANATOMY OF VERTEBRATE GASTRULATION

Despite their considerable anatomical differences, the common elements of gastrulation in the various vertebrate classes are highlighted by analyzing the expression of two markers, *gooseoid* and *Brachyury*. The organizer is marked by *gooseoid*. The period of highest *gooseoid* expression corresponds to that of maximal organizer activity in *Xenopus* and chick, the two organisms in which detailed transplantation analyses have been possible.

The future mesoderm, both dorsal and ventral, is marked by *Brachyury*. In *Xenopus* and zebrafish, this can be seen as a ring encircling the spherical embryo, the marginal zone. In the mouse embryo, it is expressed in the entire primitive streak. Thus, the marginal zone ring has become flattened, forming two parallel accumulations of cells in the primitive streak in the amniote embryo. At the anterior end of the early primitive streak lies the organizer, which expresses *gooseoid*. We conclude that the primitive streak is homologous to the circumblastoporal marginal zone present in *Xenopus* and zebrafish.

It might be worthwhile to attempt to identify the equivalent stages of gastrulation from the expression patterns. The maximal expression of *gooseoid* correlates with maximal inducing activity, tested by grafting, in *Xenopus* and chick. These stages may therefore be considered truly homologous. Furthermore, maximal expression of *gooseoid* is followed by the migration of cells that form the endomesoderm of the head in *Xenopus*, zebrafish, chick and mouse. These anterior cells continue to express *gooseoid*, while the gene is down-regulated in other cells that will become a number of other dorsal tissues. The region of maximal expression corresponds to the dorsal marginal zone in *Xenopus*, the embryonic shield in zebrafish, the early Hensen's node in chick and the anterior primitive streak in the mouse. Maximal expression of *gooseoid* is achieved just before dorsal lip formation in *Xenopus* (stage 10, Fig. 1B), before the primitive streak is fully



**Fig. 5.** Anatomy of the *Xenopus* tailbud. (A) Stage 23. (B) Stage 28. The neurenteric canal connects the ependymal and gut cavities. The chordoneural hinge gives rise to notochord, floor plate and roof of the postanal gut. The notochord is hatched, the *Xbra*-positive cells of the posterior wall are indicated by small crosses, and the spinal cord is stippled. All these structures form as a consequence of late gastrulation movements.

extended in the chick (stage 3<sup>+</sup>, Fig. 1G), at 50% epiboly in zebrafish (Stachel et al., 1993; Schulte-Merker et al., 1994), and at day 6.7 in the mouse (Blum et al., 1992). We propose that these stages are equivalent in the vertebrate gastrula.

*Xenopus* embryologists usually consider the beginning of gastrulation as the start of involution of cells through the dorsal lip at stage 10. However, if we consider this stage to be homologous to the chick embryo as it reaches full extension of the primitive streak (stage 3<sup>+</sup> to 4), it is evident that in the chick gastrulation starts much earlier. The extensive morphogenesis involved in the formation of the primitive streak, starting from Koller's sickle, is being ignored in most current *Xenopus* work. However, there are indications that also in *Xenopus*, pregastrulation movements are much more extensive than commonly

thought (Hausen and Riebsell, 1991; Bauer et al., 1994). The challenge for the future lies in discovering whether homologous events exist at the very early stages of vertebrate gastrulation.

## THE EARLIEST ORGANIZER CELLS

In *Xenopus* inducing activity is first found in dorsal-vegetal blastomeres, in a region designated the Nieuwkoop center (Gerhart et al., 1989). As early as the 32- to 64-cell stage the vegetal cells are thought to emit a signal that induces organizer activity in the overlying marginal zone. Nieuwkoop center cells do not themselves participate in the axis, but remain as yolk endodermal cells (Smith and Harland, 1991). The next step for those working in zebrafish, chick or mouse would be to identify the source of the equivalent Nieuwkoop center signal in these animals, if one exists. In zebrafish we may assume that the early gradient of *gooseoid* transcripts that is observed at blastoderm (Fig. 1C,D) reflects the intensity of the inductive signal from the Nieuwkoop center equivalent. This signal could emanate for example from the yolk region.

In chick, the *gooseoid*-positive cells of Koller's sickle have been shown by fate mapping to contribute to the main body axis, in particular Hensen's node and its derivatives (Izpisua-Belmonte et al., 1993). Therefore these cells are not homologous to the Nieuwkoop center, whose cells can induce but do not themselves form part of the axis. If a Nieuwkoop center exists in the chick, one would venture that it should signal before the chick egg is laid and that it would be located in the posterior marginal zone, in the vicinity of the region where Koller's sickle is formed. Another possibility is that Koller's sickle cells may not require a separate localized inducing center.

In *Xenopus* there is recent evidence suggesting that the redistribution of egg cytoplasmic determinants may be all that is required for the activation of *gooseoid*. Lemaire and Gurdon (1994) marked eggs on the dorsal or ventral side with vital dyes and then cultured dissociated blastomeres. Even in the absence of any cell-cell interactions, *gooseoid* was turned on in dorsal blastomeres and *Xwnt-8*, a ventral marker, was activated in ventral blastomeres. One possibility worth exploring is that perhaps the inductive activity found in vegetal-dorsal blastomeres (Nieuwkoop center) is just a manifestation of a diffuse organizer present from the earliest stages in the dorsal side of the *Xenopus* embryo. Perhaps the vegetal cells do not participate in the axis proper because they are too bulky to migrate, while smaller more dorsal cells might be able to migrate and still have similar inductive properties. While the possibilities mentioned in the last two paragraphs are entirely hypothetical, they were raised here to illustrate how a comparative approach to the Nieuwkoop center/Koller's sickle problem helps us think about how the vertebrate embryo is formed.

## COMMON MECHANISMS IN LATE GASTRULATION?

Unlike the studies on *gooseoid* and *Brachyury*, the experiments reviewed here on the development of the tail apply only to *Xenopus*. Given the conservation of developmental mechanisms, we would propose that the mechanism of tail formation will turn out to be very similar in all vertebrates. However, it could be argued that what happens in *Xenopus* might be the

exception, because the tadpole is under strong selective pressure to develop a tail rapidly in order to swim for survival. The currently prevailing view states that the tailbud of a chick or a mouse is a proliferation blastema of undifferentiated cells, from which the various tissues of the tail arise (Griffith et al., 1992).

The comparative approach could be very productive in advancing knowledge in this case. If the *Xenopus* tail formation mechanism (Gont et al., 1993) operates in amniotes, several strong predictions can be made. For example, a population of cells derived from the organizer should regress through the primitive streak (the region equivalent to the lateral blastopore lip and the posterior wall of *Xenopus*). This population of cells would be homologous to the chordoneural hinge of *Xenopus* and should be endowed with potent organizing properties, inducing somites and other tail structures as it comes in contact with pluripotent primitive streak cells. Some observations on mouse somite formation (Tam and Tan, 1992) may be considered to be at least consistent with the mechanism proposed here. Gene markers will be useful, but ultimately definitive answers will require the traditional transplantation and lineage tracing tools available to the embryologist.

In the case of chick and mouse, the tail formation mechanism might be masked by the extensive cell proliferation required for the growth of the embryo which, unlike *Xenopus*, greatly increases in size at late gastrulation. Perhaps the largest obstacle is the lack of a visible neurenteric canal, which serves as a useful landmark, in chick and mouse. However, this canal must be an ancestral chordate characteristic, for it is present in *Amphioxus*, shark, turtle and human embryos (see Gont et al., 1993).

Thus in the immediate future the task will be to establish whether a common mechanism exists in the development of the postanal tail, which is a structure found only in the chordates. By focusing attention on this problem it is hoped that more will be learnt about the mechanisms that control late gastrulation in the vertebrates.

## CONSERVATION VERSUS VARIATION

We have emphasized throughout this paper how developmental mechanisms that control gastrulation in the vertebrates appear to be conserved when examined through the narrow prism of gene markers. One might argue that a productive area of research might be to extend these comparisons by studying homologues of organizer-specific genes in invertebrates, e.g., hemichordates, echinoderms and even in protostomes. Such studies might help answer the question of whether the organizer phenomenon is restricted only to the vertebrates. On the other hand, one could also argue that understanding how differences in body shape arise will be of greater importance in evolutionary studies. After all, mutations in the genes that regulate development may provide one of the main sources of the variation upon which natural selection acts in metazoans (reviewed by De Robertis, 1994).

The choice of emphasizing either similarities or differences can be seen in articles throughout this volume. But this is not something new in evolutionary biology. In 1830 a famous debate took place in the French Academy between Cuvier and Geoffroy Saint-Hilaire (see Appel, 1987). The latter defended the principle of unity of composition among animals. Those

who today choose to emphasize homologies marvel at the unity of composition of the mechanisms of development. But others marveled before. One of the observers of the 1830 debate summarized his views in the following way:

*“Il n’y a qu’un animal. Le créateur ne s’est servi que d’un seul et même patron pour tous les êtres organisés.”*

*“There is but one animal. The Creator used only a single pattern for all organisms.”*

Honoré de Balzac  
in “La Comédie Humaine”, 1842

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