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goosecoid and the organizer

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Summary

The molecular nature of Spemann's organizer phenomenon has long attracted the attention of embryologists. *goosecoid* is a homeobox gene with a DNA-binding specificity similar to that of *Drosophila bicoid*. *Xenopus goosecoid* is expressed on the dorsal side of the embryo before the dorsal lip is formed. Cells expressing *goosecoid* are fated to become pharyngeal endoderm, head mesoderm and notochord. Transplantation of *goosecoid* mRNA to the ventral side of *Xenopus* embryos by microinjection mimics the properties of Spemann's organizer, leading to the formation of twinned body axes. *goosecoid* is activated by dorsal inducers and not affected by ventral inducers. In the mouse, *goosecoid* is expressed in the anterior tip of the primitive streak. The availability of two early markers, *goosecoid* and *Brachyury*, opens the way for the comparative analysis of the vertebrate gastrula. The results suggest that the *goosecoid* homeodomain protein is an integral component of the biochemical pathway leading to Spemann's organizer phenomenon.

Key words: *goosecoid*, Spemann's organizer, gastrulation, *Drosophila*, *Xenopus*.

Introduction

It is now generally agreed that development results from a series of cell-cell interaction events. The experiment that contributed more than any other to this view was that of Hans Spemann and Hilde Mangold (1924), showing that a small fragment of the gastrula, the dorsal lip, had the ability, after transplantation, to form a twinned body axis in the opposite (ventral) side of a host embryo. The transplanted tissue contributed only a small part of this secondary axis, and thus was able to recruit, or organize, cells into complex anatomical structures. The quest to understand the inductive properties of the organizer still provides great impetus to experimental embryologists (reviewed by Spemann 1938; Nakamura and Toivonen, 1978; Hamburger, 1988; Marx, 1991).

In the present paper, we will discuss how microinjection of purified *goosecoid* homeobox-containing mRNA can mimic most of the properties of Spemann's organizer.

Homeobox genes expressed in the organizer

Homeobox genes encode DNA-binding proteins, which frequently are involved in the specification of positional information in the embryo (reviewed by Gehring 1987; Kessel and Gruss, 1990; De Robertis et al., 1991; McGinnis and Krumlauf, 1992). It is therefore of interest that several such genes are expressed in the dorsal lip region of the *Xenopus* embryo. By screening a cDNA library derived from manually dissected *Xenopus* dorsal lips, four different homeobox genes were isolated by Blumberg et al. (1991). Three were related to genes isolated previously (*X. caudal* 1 and 2, *X. labial*), but the most abundant clone (23 out of 30 isolates) contained a novel type of homeobox. Because its homeobox contained similarities to the *Drosophila* genes *gooseberry* in the initial ⅔ of the homeodomain and to *bicoid* in the DNA recognition helix, this gene was christened *goosecoid*. Additional genes expressed in the dorsal lip have been isolated by other groups from *Xenopus* embryo cDNA libraries by screening with homologous probes. *X-LIM-1* contains a homeobox as well as a conserved cysteine-rich domain (Taira et al., 1992), and while *XFKH-1* lacks a homeobox, it has sequence similarities to the DNA-binding domain of the *Drosophila fork-head* homeotic gene (Dirksen and Jamrich, 1992).

The expression of *goosecoid* in the *Xenopus* gastrula

All the genes mentioned above are surely important in building the body axis, but we will deal here only with *goosecoid*, which is particularly interesting because it has some degree of functional similarity to the *Drosophila* anterior morphogen *bicoid* (Nüsslein-Volhard, 1991). In an *in vitro* assay a *goosecoid* recombinant protein was shown to bind target sequences with a DNA-binding specificity similar to that of *Drosophila bicoid* on target sequences derived from the promoter of the gap gene *hunchback* (Blumberg et al., 1991). In addition, *goosecoid* is expressed very early in *Xenopus* embryos. As can be seen in Fig. 1B, *goosecoid* expression is detectable by whole-mount in situ hybridization as a crescent on the dorsal marginal zone of the

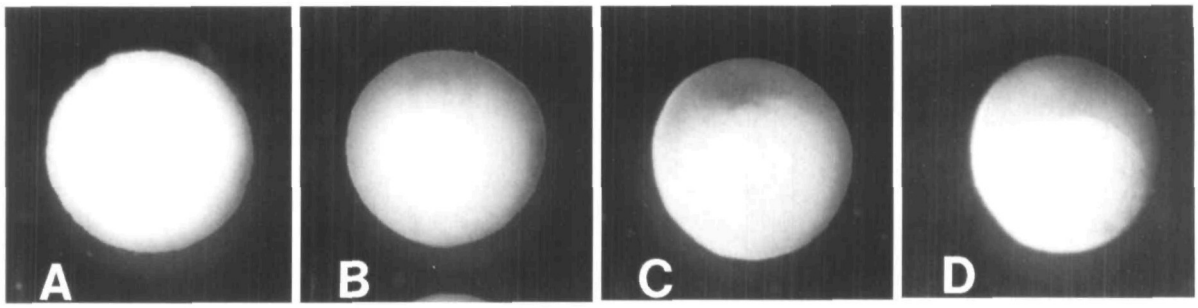

Fig. 1. Time course of *goosecoid* expression in *Xenopus* embryos. Whole-mount *in situ* hybridization (Harland, 1991) at (A) stage 8, mid blastula; (B) stage 9, late blastula one hour before the onset of gastrulation; (C) stage 10, early gastrula in which the dorsal lip is formed; (D) stage 11, mid gastrula with a circular blastopore. Note that the patch of goosecoid expression in the marginal zone is present before gastrulation starts (B), and that it invaginates into the interior of the embryo during gastrulation (D). Unfortunately the black and white reproduction of this figure, which was originally in color, does not have enough contrast.

embryo at least one hour before the start of gastrulation. At the start of gastrulation (Fig. 1C) *goosecoid* is most intense in an arc of about 60° just above the dorsal lip (Cho et al., 1991). This corresponds to the region where Spemann's organizer, as defined by transplantation experiments, is located (Cooke, 1972; Gerhart et al., 1989).

In sagittal sections, *goosecoid* is seen to be expressed in the internal layer of the dorsal lip region. As shown in Fig. 2, cells close to the incipient indentation of the dorsal lip (indicated by an arrow) contain mostly nuclear transcripts, while cells located further away from the dorsal lip contain increasing amounts of cytoplasmic *goosecoid* RNA. The cells that express *goosecoid* correspond to the dorsal-most invaginating cells. Their normal fate is to become pharyngeal endoderm, head endoderm and notochord (Keller, 1976, 1991).

Microinjection of *goosecoid* mRNA gives rise to a twinned body axis

When full-length *goosecoid* mRNA is microinjected into the ventral side of the four-cell embryo (Cho et al., 1991), the formation of a second dorsal lip, and subsequently of a twinned body axis, ensues (Fig. 3). Some of these axes are complete, containing head structures such as eyes, cement gland and hatching glands. More frequently, only trunk structures containing massive amounts of notochord are formed (Cho et al., 1991). These structures are made at the expense of the tail, so that the resulting tadpoles are considerably shortened.

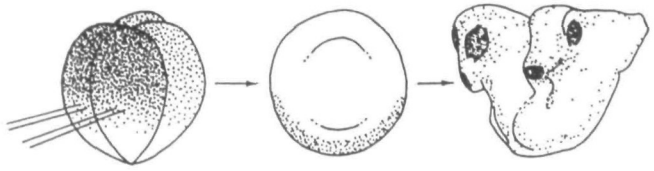

Fig. 3. Diagram depicting the effects of injecting *goosecoid* mRNA into the two ventral blastomeres of a 4-cell *Xenopus* embryo (i.e., opposite to the site where this gene will normally be expressed). A second dorsal lip is formed, which leads to the formation of a twinned body axis. After experiments of Cho et al. (1991), redrawn by Koichiro Shiokawa.

Blastomeres microinjected with *goosecoid* are able to recruit neighboring uninjected cells into the secondary axes (C. Niehrs, K.W.Y. Cho and E. De Robertis, unpublished results), as occurs in Spemann's organizer phenomenon. Thus, the *goosecoid* homeobox protein has non-cell autonomous effects, a property that has been noted for some *Drosophila* homeobox genes during midgut development (Immerglück et al., 1990; Reuter and Scott, 1990).

The conclusion from these microinjection experiments is that transplantation of a single gene product, *goosecoid* mRNA, can mimic Spemann's organizer phenomenon.

How is *goosecoid* activated?

Our understanding of mesoderm induction has progressed greatly in the past few years. The pioneering work of Peter Nieuwkoop showed that mesoderm induction results from signals emanating from the vegetal cells (reviewed by Nieuwkoop, 1973). Furthermore, ventral and dorsal vegetal cells differ in their inductive power when placed in contact with pluripotent animal cap cells. As indicated in Fig. 4, ventral induction leads to the formation of tissues such as blood, mesothelium (coelom) and lateral plate mesoderm. Addition of FGF to animal cap fragments mimics this ventral induction (Slack et al., 1987; Slack, this volume). Dorsal and vegetal blastomeres induce notochord and muscle when conjugated with animal cap cells (Boterenbrood and Nieuwkoop, 1973). Growth factors of the activin type (Smith et al., 1989; Green and Smith, 1990) and of the *Wnt* type (McMahon and Moon, 1989a,b) can mimic this dorsal induction.

The nature of the molecules that cause mesoderm induction in vivo is not known (Slack, 1991), but this is a very active area of research at present. Interesting recent developments suggest that (1) the microinjection of activin mRNA into single blastomeres can produce secondary axes (Thomsen et al., 1990) and (2) that injection of *Wnt*-8 mRNA into vegetal blastomeres can produce extensive secondary axes, including complete rescue of UV-treated embryos, presumably via induction of Spemann organizer function (Sokol et al., 1991; Smith and Harland, 1991). Because *Wnt*-8 is normally localized in the ventral side of the embryo, it is considered unlikely to be the endogenous

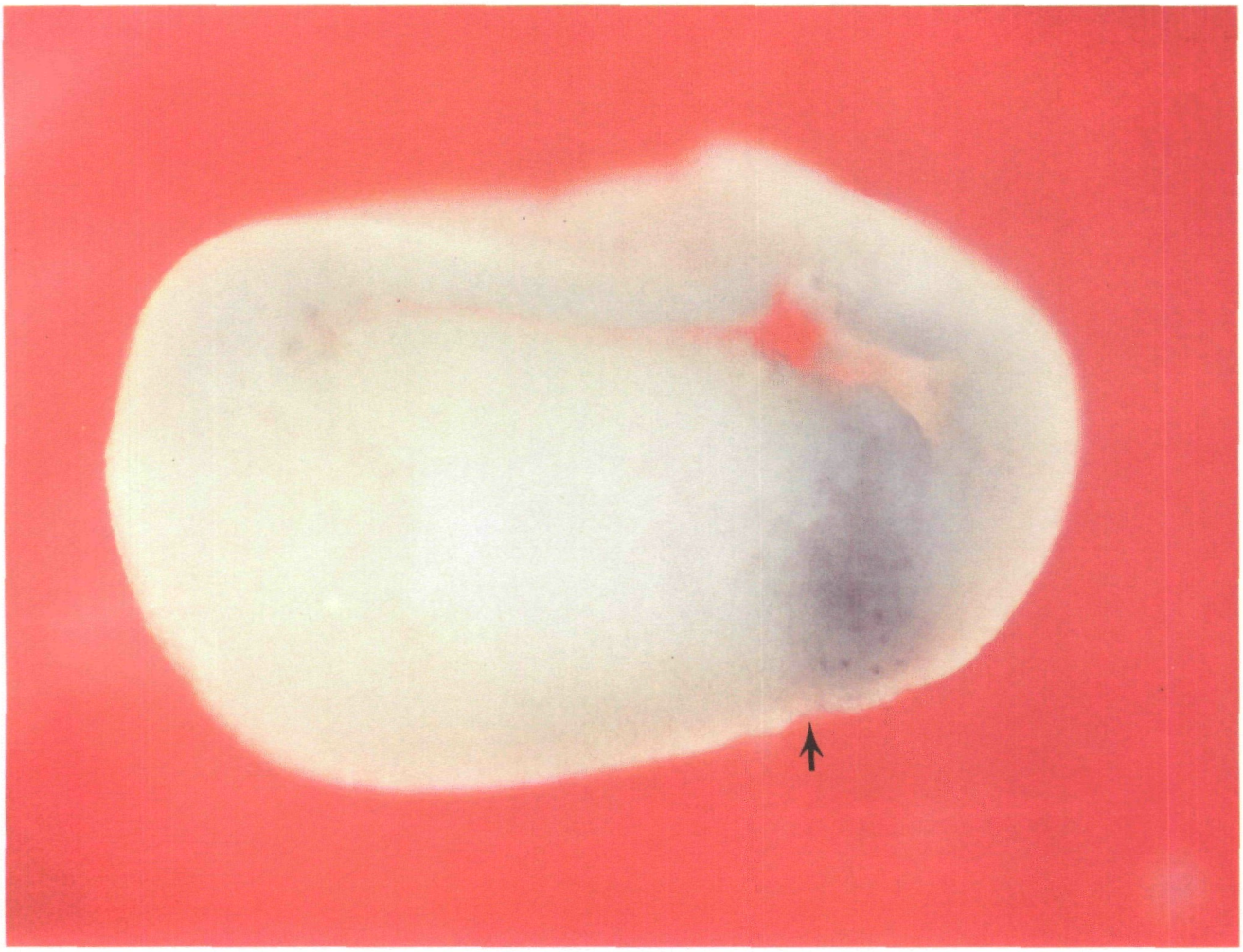

Fig. 2. *goosecoid* expression at the start of gastrulation, sagittal section through the organizer region of a *Xenopus* embryo. The arrow indicates the incipient dorsal blastopore lip. Note that *goosecoid* RNA is nuclear in the proximity of the dorsal lip (suggesting that the gene is first transcribed at this site) and cytoplasmic as cells move into deeper layers of the marginal zone. The fate of *goosecoid*-expressing cells is to become pharyngeal endoderm, head mesoderm and notochord.

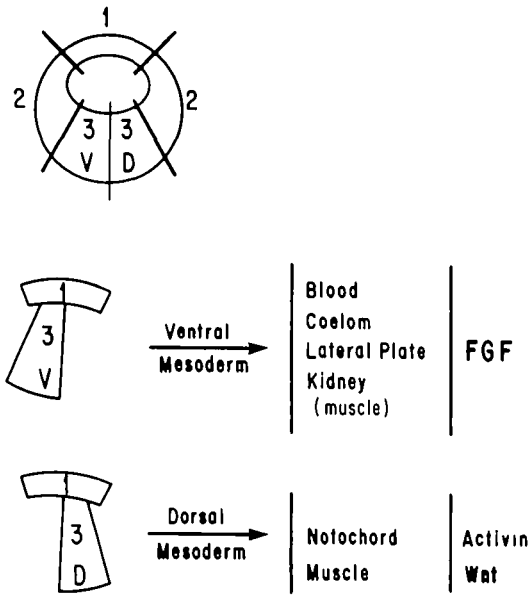

Fig. 4. Mesoderm induction can be of a ventral or dorsal nature. Explanted animal caps conjugated and cultured with either ventral or dorsal vegetal blastomeres form different types of mesoderm (after experiments by Boterenbrood and Nieuwkoop, 1973). These inductions can be mimicked by treating animal cap cells with FGF (ventral mesoderm) or with activin or *Wnt* (dorsal signals).

dorsal inducer, but a similar substance, as yet uncloned, could be secreted by dorsal and vegetal blastomeres.

The current model, summarized in Fig. 5, is therefore that there exists a radial signal (or signals) that induces a ring of cells in the overlying marginal zone to become ventral mesoderm. On the dorsal side, an additional signal (or signals) is released from the vegetal cells (Boterenbrood and Nieuwkoop, 1973; Gimlich and Gerhart, 1984), originating in a region that has been designated the Nieuwkoop center (Gerhart et al., 1989). The signal from the Nieuwkoop center acts upon the overlying marginal zone cells and induces Spemann's organizer tissue. *goosecoid* is expressed in the latter region.

As expected, *goosecoid* is induced by activin but not by

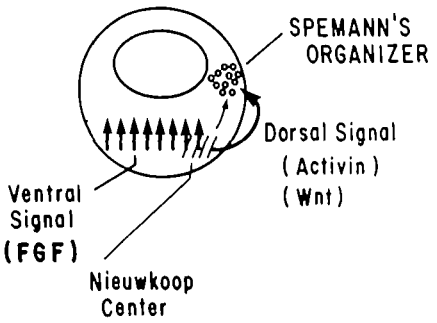

Fig. 5. A current view of mesoderm induction in *Xenopus* (see Gerhart et al., 1989). A ventral signal is released radially by the vegetal cells (e.g., FGF), inducing the entire marginal zone to become mesoderm. On the dorsal side an additional signal (perhaps activin- or *wnt*-like) is released by vegetal cells of the Nieuwkoop center, inducing Spemann organizer tissue in the overlying marginal zone cells.

the ventral inducer FGF (Cho et al., 1991). The effect of *Wnt*-type factors is presently under investigation. *goosecoid* is a primary response gene to activin induction, i.e., it's transcripts will accumulate even in the absence of protein synthesis. We have previously argued that this result would place *goosecoid* high up in the hierarchy of genetic events leading to axis formation (Cho et al., 1991). This interpretation should now be reassessed in view of recent findings that a great many genes are primary targets for activin in *Xenopus* animal cap explants. These include *Mix-1* (an endoderm-specific homeobox gene isolated by screening for activin-induced transcripts; Rosa, 1989), *Myo D* (Rupp and Weintraub, 1991), *Brachyury* (a gene expressed as a ring in the marginal zone of the *Xenopus* embryo as well as in the organizer region; Smith et al. (1991) and known to be required for notochord and posterior axis formation in the mouse, Rasbash et al., 1991), *X-LIM-1* (Taira et al., 1992) and *XFKH1* (Dirksen and Jamrich, 1992). In fact, transcripts for many of these genes are present at low levels already at the time when the animal cap are excised at the mid blastula stage. This applies to *Myo D* (Rupp and Weintraub, 1991), *X-LIM-1* (Taira et al., 1992), *goosecoid* (Cho et al., 1991) and presumably other inducible genes. Low levels of *goosecoid* mRNA are present in the *Xenopus* unfertilized egg and early cleavage stages (H. Steinbeisser, unpublished observations) but went unnoticed in our initial study (Blumberg et al., 1991). These maternal transcripts are not detectable by whole-mount in situ hybridization (Fig. 1A).

In order to dissect the hierarchy of these genes in axis formation, if indeed one exists, it will be necessary to carry out loss-of-function studies. For example, one would like to know whether *goosecoid* is expressed in a *Brachyury* mutant, and vice versa. Such studies should be possible in the mouse embryo.

goosecoid in mouse gastrulation

As is well known, all vertebrate embryos appear very similar to each other at mid-embryogenesis (the so-called phylotypic stage of the vertebrate embryo, Wolpert, 1991; Feduccia and McCrady, 1991). On the other hand, embryos from the various vertebrate classes appear to differ greatly at the gastrula stage. For example, in teleosts the main gastrulation movement is epiboly, in which the embryo proper envelops the yolk mass; in amphibians, which in general have holoblastic cleavage, the main morphogenetic movement is the invagination of the endomesoderm through the circular blastopore; while in birds and mammals (anmiotes) the main morphogenetic movement is the delamination of the future endodermal and mesodermal cells through the linear primitive streak. One of the main lessons that we have learned in the past few years, in particular from the extraordinary conservation of the workings of *Hox* genes (reviewed by De Robertis et al., 1990; Kessel and Gruss, 1990; McGinnis and Krumlauf, 1992), is that while embryogenesis may appear to differ greatly, the molecular mechanisms involved are universal. Southern blot analysis indicates that all classes of vertebrates contain a *goosecoid* homologue (Blum et al., 1992). Thus the *goosecoid* marker

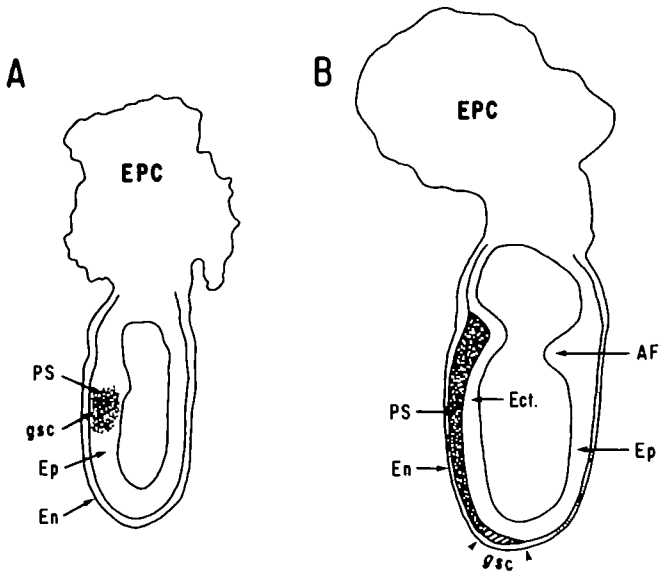

Fig. 6. Expression of *goosecoid* in the mouse gastrula. (A) At 6.4 days *goosecoid* (gsc) mRNA is expressed as a patch on the side of the epiblast, coinciding with the site at which the epithelial-mesenchymal transition that initiates primitive streak formation takes place. (B) At $6^3/_4$ days the region of *goosecoid* expression is located in the mesoderm at the anterior end of the primitive streak, located at the tip of the egg cylinder (gsc). This region corresponds to the future head process from which the definitive endoderm, head mesoderm, and notochord are derived. The *goosecoid* signal is represented by hatched bars; the primitive streak is stippled. The epiblast (Ep), embryonic endoderm (En), primitive streak mesoderm (PS), ectoderm (Ect), amniotic fold (AF) and ectoplacental cone (EPC) are indicated. After studies by Blum et al., 1992.

provides an opportunity for a comparative analysis of the organizer region in gastrulation.

In mammals, gastrulation occurs at the time in which the embryo, due to its small size and uterine implantation, is most inaccessible to study. Mouse *goosecoid* has been cloned, and analysis of its expression provides a useful marker for dorsal development. The mouse epiblast has the shape of a cup, called the egg cylinder (see Lawson, 1991). At 6.4 days post-coitum mouse *goosecoid* mRNA can be seen on the side of the egg cylinder, in the cells of the epiblast that begin the epithelial-mesenchymal transition that marks the start of primitive streak formation (Fig. 6A). As the primitive streak elongates, the *goosecoid*-expressing cells are seen to be located at the anterior end of the advancing mesoderm (Blum et al., 1992). The direction of these movements is in agreement with the mouse gastrula fate map (Lawson, 1991). By $6^3/_4$ days the *goosecoid*-expressing cells are located at the anterior-most tip of the primitive streak, as indicated in Fig. 6B. This region is the precursor of the head process, which gives rise to notochord, head mesoderm and the definitive endoderm. Shortly thereafter *goosecoid* mRNA becomes undetectable (Blum et al., 1992), and does not reappear until $3^1/_2$ days later (Gaunt et al., 1992).

The distribution of *goosecoid* mRNA is in agreement with transplantation experiments of mouse gastrula fragments into *Xenopus* embryos. Head organizer activity was found at the tip of the egg cylinder at about $6^3/_4$ days, while the base of the egg cylinder, which contains most of the primitive streak, induces tail or no structures at all (Blum et al., 1992).

The availability of two markers, *goosecoid*, which marks the organizer and invaginating prechordal plate (Cho et al., 1991; Blum et al., 1992), and *Brachyury*, which marks the notochord as well as the marginal zone in *Xenopus* and the primitive streak in the mouse (Smith et al., 1991; Herrmann, 1991), now permits the identification of homologous regions in these two gastrulae. Together with the transplantation experiments described above, the molecular studies are consistent with the anterior end of the mouse primitive streak (Fig. 6B) corresponding to the *Xenopus* dorsal lip and the more posterior primitive streak to the lateral and ventral blastopore lip regions.

Conclusions

The isolation of *goosecoid* provides a molecular marker that should permit identification of the equivalent of the organizer region in a number of species in which manipulative experiments are not possible. Microinjection of a purified molecule, *goosecoid* mRNA, into ventral blastomeres mimics Spemann's organizer phenomenon. This gene is induced in the marginal zone by dorsal inducers which in turn mimic the signal released by the Nieuwkoop center. While surely many more components remain to be discovered, at present the results seem to suggest that the *goosecoid* DNA-binding protein is part of the following pathway:

Nieuwkoop center cells
↓
release of dorsal intercellular signal/s
↓
induction of dorsal marginal zone cells
↓
synthesis of *goosecoid* DNA-binding protein
↓
Spemann's organizer activity

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