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 3/4 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 (Taia 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.

Blastomeres microinjected with goosecoid are able to recruit neighboring un.injected 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. 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.
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.
or dorsal vegetal blastomeres form different types of mesoderm (ventral mesoderm) or with activin or could be secreted by dorsal and vegetal blastomeres. A dorsal inducer, but a similar substance, as yet uncloned, overlying marginal zone cells.

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 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, Rashash 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 phylotopic 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 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 Grass, 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
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 63/4 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 signals
↓
induction of dorsal marginal zone cells
↓
synthesis of goosecoid DNA-binding protein
↓
Spemann's organizer activity

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