CHAPTER 42

GOOSEC OID AND GA STRULATION

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INTRODUCTION

For many years, the transplantation of Spemann’s organizer into a host embryo was the only way of revealing the remarkable biological activity of the dorsal lip of the blastopore. The discovery of goosecoid (gsc) initiated the molecular era of research on Spemann’s organizer (Cho et al. 1991). It became possible to visualize, by following gsc expression, the region of the embryo that corresponds to the inductive activity. The Spemann organizer became a concrete group of cells, rather than an embryological concept. Furthermore, since microinjection of gsc synthetic mRNA is able to execute some of the properties of Spemann’s organizer, these initial studies implicated homeobox genes as key elements in the function of the organizer. Subsequently, many other homeobox genes were found to be expressed in Spemann’s organizer. These transcription factors control the expression of secreted growth factors and growth factor antagonists that mediate the inductive activities of the organizer on neighboring cells. In this chapter, I review studies on goosecoid and what they have taught us about the organizer, how embryonic signaling pathways are integrated at the level of the promoter, and the comparative anatomy of gastrulation.

XENOPUS GOOSEC OID EXPRESSION

goosecoid was isolated from a cDNA library constructed from dissected dorsal lips. The library was screened with degenerate oligonucleotides complementary to a region conserved in most homeobox genes, including Hox genes and bicoid (Blumberg et al. 1991). The gene was named goosecoid to reflect the similarity of its homeodomain region to both Drosophila gooseberry and bicoid, two members of the larger paired-homeodomain family. The gsc homeobox contains a lysine in position 50 of the homeobox (instead of glutamine as in Hox genes). This change is seen in other anterior homeobox genes such as Otx, Siamois, and Xwnt, and correlates with binding to a bicoid-type DNA sequence, rather than an Antennapedia-Hox target sequence (Blumberg et al. 1991).

At early gastrula, goosecoid is expressed in a 60° arc of the dorsal marginal zone, providing an excellent marker for Spemann’s organizer (Fig. 1A). Expression is maximal at stage 10, when the dorsal lip first appears. In hybridizations on histological sections, gsc expression is seen in involuting cells, extending almost to the leading edge (Fig. 1B). The gsc-positive tissue corresponds to the future dorsal mesendoderm, including foregut and pharyngeal endoderm, prechordal plate, and notochord. As involution proceeds, by mid-gastrula, the gsc-expressing region narrows and leaves the blastopore lip (Fig. 2A,B). By the end of gastrulation, gsc expression is seen in the prechordal plate and anterior endoderm, ahead of the Hox gene border provided by Xlabial that separates the head and trunk regions (Fig. 2C). By the late-neurula stage, goosecoid expression is seen in the prechordal plate and in the anterior endomesoderm that will give rise to the pharynx and foregut (Fig. 2D; Fig. 3A). Thus, although gsc initially is expressed in the territory that gives rise to the notochord, it is then switched off in this structure.

As shown in Figure 2, the Xlabial gene, a member of the Hox family, provides a good marker for the formation of the sharp border that demarcates the head and trunk regions of the embryo. This anterior–posterior border originates from the circumblastoporal involution of Xlab-positive cells
Figure 1. Expression of goosecoid in Spemann's organizer in Xenopus and zebrafish. (A) goosecoid in the dorsal lip of an early Xenopus gastrula. (B) Gsc in situ hybridization to a histological section of a Xenopus stage 10 1/2 gastrula. Expression is seen in the inviolated endomesoderm, extending from the dorsal lip (arrowhead) to the leading edge of the endoderm. (C) Expression of Gsc and Xwnt-8 at stage 10 3/4. Gsc is a transcriptional repressor of Xwnt-8, and these two genes acting together pattern the marginal zone. (D) Zebrafish embryos at 50% epiboly hybridized for Gsc (in black) and stained with a Brachyury antibody (in brown). This is the stage of maximal Gsc expression in zebrafish; Brachyury demarcates the trunk mesoderm. We thank Uyen Tran for the embryos shown in A–C. (D, Reprinted, with permission, from Schulte-Merker et al. 1994.)

(mentioned by a small circle in Fig. 2 A–C), whereas the border of Xlab expression on the sides of the notochord derives from the convergence toward the midline of Xlab-expressing cells flanking the organizer (marked by a small asterisk). Recently, a revision of the dorsal–ventral and anterior–posterior designation of the axes in the Xenopus fate map was proposed (Kumano and Smith 2002; Lane and Sheets 2002) (see Chapter 19). The expression domains of goosecoid and Xlab during Xenopus gastrulation do not support this new proposal, since the anterior–posterior border of Xlab originates from the blastopore circumference (Fig. 2A–C).

EFFECTS OF GOOSECOID OVEREXPRESSION

Microinjection of gsc mRNA into two ventral blastomeres at the 4-cell stage causes the formation of secondary axes lacking head structures (Cho et al. 1991; Yao and Kessler 2001). When gsc is overexpressed in ventral marginal zone explants, twofold increases in gsc mRNA concentration are sufficient to cause dorsalization of mesoderm, and at least three thresholds of dorsal histotopic differentiation can be triggered (Niehrs et al. 1994). Microinjected gsc mRNA has non-cell-autonomous effects, recruiting neighboring uninjected cells into the twinned dorsal axis (Niehrs et al. 1993). These non-cell-autonomous effects of gsc mRNA are mediated by the induction of secreted proteins such as Chordin and Fzrb-1 (Sasai et al. 1994; Leyns et al. 1997).

When overexpressed in dorsal blastomeres, goosecoid promotes cell migration in the dorso-anterior direction (Niehrs et al. 1993). In normal embryos, C1 blastomeres from the 32-cell stage contribute progeny to the leading edge of the head endomesoderm (including pharyngeal endoderm, foregut, and liver), prechordal plate, and the entire length of the notochord (Fig. 3B). The notochord contribution of the C1 blastomere includes its posterior-most end, called the chordonelline hinge, which is homologous to the regressing Hensen's node in amniotes (Gont et al. 1993). In gsc-injected embryos, the C1 progeny undergo a change in cell fate, contributing predominantly to the leading edge involuting cells, while the notochord becomes devoid of labeled cells (Fig. 3C). Since these embryos are phenotypically normal, other cells must have taken the place of C1 progeny in the trunk dorsal axis. Thus, when a dorsal blastomere is dorsalized further, it populates the anterior-most endomesoderm, which occupies the anterior foregut region (Fig. 3). Some of these cells of dorsal origin have recently been found also to contribute to the anterior blood island (Lane and Smith 1999). This is in agreement with earlier observations that dorsal-most endomesodermal cells end up in the liver and foregut region (Fig. 3A) (Niehrs et al. 1993; Bouwmeester et al. 1995).

GOOSECOID AND THE PATTERNING OF THE MARGINAL ZONE

In a loss-of-function situation, Xenopus goosecoid is required for the formation of the head region. Anterior head defects have been obtained by a variety of methods, including antisense gsc mRNA (Steinbeisser et al. 1995), an antimorphic Gsc resulting from the addition of epitope tags (Ferreiro et al. 1998), and fusions of Gsc with the activation domain of VP16 (Latinkic and Smith 1999; Yao and Kessler 2001). Goosecoid functions as a transcriptional repressor, and contains in its amino-terminal domain a conserved heptapeptide (sequence FSIDNIL) that is also present in Drosophila engrailed. This sequence provides a binding site for transcriptional corepressors (Mailhos et al. 1998). Morpholino knockdown experiments of gsc in Xenopus or zebrafish embryos have not been reported yet.

Gsc overexpression has been shown to repress the expression of genes that control the differentiation of the ventral marginal zone, in particular Xwnt-8 and BMP4, which are antagonistic to organizer function (Christian and Moon 1993; Fainsod et al. 1994; Steinbeisser et al. 1995; Yao and Kessler 2001). Xwnt-8 is a crucial regulator of ventro-
lateral mesoderm, which is expressed in mesoderm in a complementary pattern to that of goosecoid (Fig. 1C). Dorsalization of Xenopus embryos with lithium chloride results in the repression of Xwnt-8 transcription, and this requires the gsc gene product (Steinbeisser et al. 1995). The importance of the repression of Xwnt-8 by Gsc is illustrated by the work of Yao and Kessler (2001), which showed that the anterior truncation caused by VP16-Gsc could be fully rescued by Frzb-1 mRNA, a secreted Wnt inhibitor that can block Xwnt-8 activity. Wild-type Gsc represses Xwnt-8 transcription directly, through four Gsc-binding sites located in the Xwnt-8 promoter (Yao and Kessler 2001). In addition, the gsc promoter has binding sites for Vent-2, which mediate the transcriptional repression of gsc by BMP (Trinidad et al. 1999). These studies suggest that a main function of Gsc is to exclude Xwnt-8 and BMP transcription from Spemann’s organizer, and that this repression is required for normal Xenopus development.

The mutual repressive interactions between gsc in the organizer and BMP and Xwnt-8 in the ventrolateral marginal zone are supported by studies on notochord development. Overexpression of gsc together with a dominant-negative BMP receptor (tBR) causes the formation of notochords at higher frequency than either component alone (Yasuo and Lemaire 2001). This effect of gsc can be mimicked by microinjection of Frzb-1 mRNA and tBR (Yasuo and Lemaire 2001). Since the expression of Frzb-1 and chordin is activated by Gsc (Sasai et al. 1994; Leys et al. 1997), these growth factor antagonists provide a molecular mechanism for the formation of dorsal mesoderm, such as notochord, in ventral mesoderm explants microinjected with gsc mRNA (Niemann et al. 1994). Marginal zone mesoderm is regulated by long-range mutually repressive interactions by which cells in the dorsal or ventral poles of the embryo communicate to ensure that a perfectly proportioned embryo develops each time.

**GOOSECOID TRANSCRIPTIONAL CONTROL**

The gsc promoter has provided a paradigm for understanding how embryonic patterning signals are integrated at the level of individual genes, resulting in spatial patterns of gene expression. Watabe et al. (1995) showed that the goosecoid promoter has two growth-factor-responsive elements. A proximal element mediates activation by β-Catenin/Wnt and a distal element by a TGF-β signal of the
Activin/Vg1/Nodal-related family. As shown in Figure 4, the β-Catenin signal is mediated by binding of the related homeodomain proteins Siamois or Xtwn, which are downstream transcriptional targets of the maternal β-Catenin signal (Laurent et al. 1997). The Activin/Nodal-related signal is transduced by the distal element, which requires a combination of Mixer and Smad2/4 (Germain et al. 2000). Mixer is a paired-homeodomain family transcription factor that is induced in endomesoderm by Activin. Smad2 is phosphorylated by the TGF-β receptors of the Activin family, allowing binding of the cofactor Smad4 and translocation into the nucleus. Mixer has a conserved carboxy-terminal Smad2-binding motif that mediates their mutual interaction on the distal promoter element (Germain et al. 2000). Therefore, Activin/Nodal-related must induce both Mixer transcription and Smad2 phosphorylation to turn on the distal promoter element (Germain et al. 2000). Since both the distal and proximal promoter elements are essential for high-level transcription of the gsc gene, the expression of gsc in the dorsal lip of the gastrula results from the synergistic input of the Activin/Vg1/Nodal-related and β-catenin pathways (Fig. 4).

Many additional transcription factors further modulate gsc transcription, and their binding sites on the Xenopus gsc promoter have been mapped. These include sites for the transcriptional activators Xlim-1 and Otx-2 (Mochizuki et al. 2000) and negative autoregulatory sites for goosecoid itself (Danilov et al. 1998) and for the ventral repressor Vent-2 (Trindade et al. 1999). The negative regulation of gsc by Vent genes plays a fundamental role in the zebrafish embryo. The Vent/vega2 and vox/vega1 genes are expressed in complementary patterns to that of goosecoid (Kawahara et al. 2000). Vent genes are transcriptionally activated by BMP signals. They ventralize the embryo and repress goosecoid when overexpressed. When both zebrafish vent genes (vega1 and vega2) are knocked down with morpholinos, zebrafish embryos become dorsalized, and goosecoid
expression is greatly expanded (Imai et al. 2001). In summary, the goosecoid promoter provides one of the best-studied systems for understanding how multiple signaling pathways are integrated at the level of the DNA during early vertebrate development.

**GOOSECOID IN THE ZEBRAFISH**

The identification of goosecoid homologs was instrumental in the visualization of tissues with organizer properties in a number of organisms. Particularly when used in combination with the marginal zone/primitive streak marker Brachyury (Bra), which marks all trunk mesoderm, comparative studies have facilitated a unified view of vertebrate gastrulation. In zebrafish, gsc expression starts at the blastula stage, forming a dorsoventral gradient (Stachel et al. 1993; Schulte-Merker et al. 1994). The moment of maximal gsc expression corresponds to 50% epiboly, a stage that may be considered homologous to early gastrula (stage 10) of Xenopus. Figure 1D shows zebrafish embryos with intense staining of gsc mRNA in the organizer (or embryonic shield), double-stained with a Brachyury antibody. Brachyury protein is seen throughout the ring comprising both dorsal and ventral marginal zone. Initially, individual organizer cells express both gsc and Brachyury, but once the goosecoid-positive cells migrate anteriorly, Brachyury expression is turned off in the prechordal plate (Schulte Merker et al. 1994). This process, by which Brachyury expression is restricted to the notochord, is mediated by repressive Gsc-binding sites in the Xbra promoter (Artinger et al. 1997; Latinik et al. 1997).

In zebrafish, gsc transcription requires Nodal-related signals provided by two genes, cyclops and squint (Feldman et al. 1998; Gritsman et al. 2000; Shimizu et al. 2000). In Xenopus, Agius et al. (2000) used a specific inhibitor of Nodal-related signals, a secreted protein called Cerberus-short, to show that the gsc-inducing signals released by the endodermal "Nieuwkoop center" require Nodal. Thus, both in zebrafish and in Xenopus, Nodal-related signals are required for the induction of the gastrula organizer.

**GOOSECOID IN THE MOUSE**

The mouse gastrula develops from a cup-shaped epiblast. The cloning of mouse Gsc (Blum et al. 1992) helped to identify the location of the mouse organizer. As shown in Figure 5A, at the time of maximal Gsc expression (mid-streak day 6 1/2), Gsc transcripts are found in the anterior primitive streak and, at lower levels, in the anterior visceral endoderm (AVE) (Blum et al. 1992; Belo et al. 1997). The AVE corresponds to the chick hypoblast and also expresses other organizer-specific genes such as Lim-1 and HNF3β (Belo et al. 1997). The discovery that the anterior primitive streak marked the mouse organizer met with some initial skepticism, for mouse embryologists at the time believed that the organizer resided in Hensen’s node, which is formed later. Once the mouse Hensen’s node starts its regression, Gsc expression is down-regulated in the node but remains in the prechordal plate and foregut (Belo et al. 1998). At later stages (day 10 1/2 and later), mouse Gsc has a second phase of expression in the neural crest of pharyngeal arches 1 and 2, floor of the diencephalon, limb buds, and other sites (Gaunt et al. 1993).

Mouse knockouts for Gsc are born alive but die shortly after birth (Rivera-Pérez et al. 1995; Yamada et al. 1995). Homozygous mutants present numerous craniofacial malformations resulting from the late phase of Gsc expression in neural crest. In addition, bone reductions and fusions in the base of the cranium are observed in the midline region.
anterior to the pituitary, which develops in close association with the Gsc-expressing cells of the prechordal plate (Belo et al. 1998). The lack of a gastrulation phenotype was surprising, and contrasted with the results obtained with antimorphic or antisense Gsc constructs in Xenopus (Steinbeisser et al. 1995; Ferreiro et al. 1998; Yao and Kessler 2001). Perhaps the slower developmental pace of the mouse allows for compensation by other redundant repressive mechanisms. One gene that has been demonstrated to compensate in part is HNF3β. Filosa et al. (1997) showed that in Gsc⁺⁺; HNF3β⁻⁻ compound mutants, dorsoventral patterning of the CNS is severely disrupted at an early stage. A second mouse homolog, Gsc-like, may also partly compensate (Funke et al. 1997). A second Gooseoid gene has also been found in chick (Lemaire et al. 1997).

In contrast to the mild phenotype in intact mice, a stronger requirement is seen when the inducing ability of Gsc⁺⁺ cells is tested in embryological experiments. Zhu et al. (1999) showed that wild-type mouse nodose induce neural markers when grafted to the area opaca of chick, whereas Gsc⁻⁻ nodose are severely impaired in their neural inducing activity. Even Gsc⁻⁻ nodose showed decreased inducing activity. Similarly, the expression of Nkx2.1 in ventral forebrain in response to anterior mesoderm ablation is altered in Gsc⁻⁻ (Camus et al. 2000). Thus, a requirement for Gsc in the mouse organizer can be uncovered by embryological manipulations even when it is not apparent in the intact mouse embryo.

**GOOSEOID IN THE CHICK**

Because the chick blastoderm is flat and translucent, transcript detection can be achieved with a much better resolution than in Xenopus or mouse. Chick Gooseoid expression has been studied in considerable detail (Izpisúa-Belmonte et al. 1993; Chapman et al. 2002; Skromme and Stern 2002) and is first detectable in the unincubated egg, which already contains several thousand cells. Expression starts in a thickening of the posterior edge called Koller's sickle, where it is confined to a group of cells located in a middle layer between the epiblast and the forming hypoblast. The existence of this cell population had been overlooked by embryologists, even though Koller's sickle had been described almost 100 years earlier. Its importance for the gastrulation process was revealed by the Gooseoid marker. Figure 5B shows Gooseoid expression in Koller's sickle in an embryo incubated for a few hours (stage XII, when the forming sheet of hypoblast covers 50% of the area pellucida). At slightly later stages (and only in the White Leghorn strain), Gsc becomes stronger in the hypoblast (Hume and Dodd 1993; Foley et al. 2000; Chapman et al. 2002), a tissue that is homologous to the mouse AVE (see Chapter 15).

As development continues, the primitive streak forms, and as it progresses in the anterior direction, Gooseoid is expressed in its anterior region. Transcripts become more abundant as the streak elongates, and by stage 3', before the streak reaches its maximal extension, Gooseoid attains its maximal expression in the anterior third of the streak (Fig. 5C). This is also the stage at which the anterior end of the primitive streak, the young Hensen's node, presents its maximal neural inducing activity. Once the primitive streak reaches its full extension at stage 4, Gsc expression becomes confined to the morphological node, and immediately afterward (stage 4') is down-regulated except in cells that move anteriorly out of the node, ingressing to form the endomesoderm of the head process and the prechordal plate that underlies the future forebrain.

The second chick Gooseoid gene, GSX, is initially expressed together with Gooseoid in Koller's sickle and early primitive streak. Later in development, the two expression domains separate. GSX is expressed in the primitive streak, excluding Hensen's node, and in the neural plate, in which it remains (Lemaire et al. 1997).

The inducing potency of the chick organizer correlates with cells having expressed Gooseoid during their development. Grafts of Koller's sickle cells are able to induce the formation of partial ectopic axes and can induce neighboring cells to express Gsc (Izpisúa-Belmonte et al. 1993; Streit et al. 2000). At stage 3', grafts from anterior or posterior primitive streak induce mesodermal structures, but only the anterior third of the streak has the ability to induce neural tissues and Gsc-positive cells. At stage 4', when Gsc-positive cells leave the node, the neural inducing activity of the node itself drastically decreases. The neural inducing activity is found in the head process, both in cells that continue to express Gsc and in cells that expressed Gsc at earlier stages (Storey et al. 1992; Izpisúa-Belmonte et al. 1993).

The chick embryo, due to its flat anatomy, has provided some of the best information on the sequence of events that lead to organizer formation. Studies on chick Gsc expression allowed a precise analysis of the inductive powers of tissues with organizer activity. Unexpectedly, these studies led to the conclusion that the organizer starts much earlier than previously thought, in Koller's sickle of the unincubated egg. Perhaps the most important contribution of these studies was to our understanding of the general architecture of gastrulation in vertebrates, as discussed below.

**GOOSEOID AND THE MOLECULAR ANATOMY OF VERTEBRATE GASTRULATION**

By analyzing the expression of Gooseoid and of the primitive-streak marker Brachyury, it is possible to discern the homologous elements of vertebrate gastrulation. This is
best illustrated by analyzing the chick embryo. At stage 3+, expression of Gsc is maximal and located in the anterior streak (Fig. 5C). Brachyury is expressed throughout the length of the streak, including its posterior end (Kispert et al. 1995). Shortly afterward, Gsc-positive cells move out of the streak and into the head process endomesoderm, while Brachyury remains in the streak. The equivalent stage of maximal expression of Gsc, before it exits into the head process, corresponds to stage 10 in Xenopus, 50% epiboly in zebrafish, and mid-streak in mouse (Figs. 1A,D and 5A). In the mouse, Brachyury is also expressed throughout the length of the primitive streak mesoderm. In zebrafish and Xenopus, however, Brachyury is expressed as a ring around yolk-laden cells, in what is called the marginal zone (Fig. 1D) (Smith et al. 1991; Schulte-Merker et al. 1994). The main difference between the ring-shaped marginal zone of Xenopus and the primitive streak of the chick or mouse is the interposition of yolk cells in the midline. Both sides of the marginal zone are joined in the midline of the primitive streak of amniotes (De Robertis et al. 1994).

**GOOSECOID IS A DORSAL MARKER**

As mentioned above, a revision in the nomenclature of the Xenopus marginal zone has been proposed recently, by which the vertical short axis across the mesoderm ring would be renamed dorsal-ventral (see Fig. 1B in Gerhart 2002). If one accepts the view of the primitive streak of amniotes being homologous to the Brachyury-expressing marginal zone of Xenopus, then the revised Xenopus proposal (Kumano and Smith 2002; Lane and Sheets 2002) could not apply to chick or mouse gastrulation. This is because extensive lineage analyses have shown that the primitive streak has a very different organization. Mesodermal cells in the chick primitive streak are arranged in a rostro-caudal sequence so that the more rostral parts of the streak contribute to dorsal elements such as somites, intermediate ones to kidney, and the more caudal primitive streak to ventral elements such as lateral plate mesoderm (Rosenquist 1966; Nicolet 1970; Schoenwolf et al. 1992; Garcia-Martinez et al. 1993; Catala et al. 1996: Psychoyos and Stern 1996). In the mouse, a similar movement of ventral divergence is seen in the mesoderm emerging from the primitive streak (Tam and Beddington 1987; Lawson et al. 1991; Smith et al. 1994; Wilson and Beddington 1996). In Xenopus, at the end of gastrulation (stage 13), the fate map of the mesoderm of the slit blastopore has the same arrangement as in chick or mouse (Gont et al. 1993). At earlier stages of gastrulation in Xenopus, this issue will have to be resolved by a detailed lineage analysis of the marginal zone at mid gastrula, after gsc-positive cells move into the head process (Fig. 2A) (the use of 32-cell blastomere lineage-may confuse the issue for the lineages of the trunk, because the entire head region arises from dorsal blastomeres in Xenopus; Fig. 3). In the meantime, the common elements of vertebrate gastrulation indicate that gsc is a dorsal marker.

**CONCLUSIONS AND PERSPECTIVES**

Twelve years after the isolation of Goosecoid, much has been learned about the molecular nature of the vertebrate organizer. Gsc is part of a gene hierarchy that executes organizer activity, and is downstrm of β-Catenin and Nodal-related signals. We have learned that many other genes participate in organizer formation, resulting in convergent redundant mechanisms. It is noteworthy that goosecoid is found in many metazoans examined, from Hydra up (e.g., Broun et al. 1999). In general, in the invertebrates, gsc is also expressed during gastrulation and marks the foregut (see, e.g., Arendt et al. 2001). In the vertebrates, gsc provides an excellent marker first for Spemann's organizer, and later for prechordal plate and foregut. Comparative analyses of gsc expression patterns during gastrulation in Xenopus, zebrafish, mouse, and chick have helped uncover the homologous components and mechanisms of vertebrate gastrulation. Despite progress in the molecular exploration of gastrulation, much remains to be understood, as explained in this volume.

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