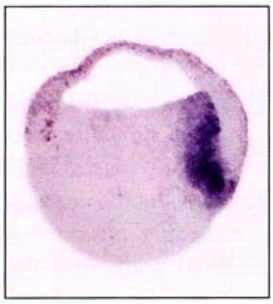

GOOSECOID AND GASTRULATION

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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 GOOSECOID 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 *Xtwn*, 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 mesoderm, 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

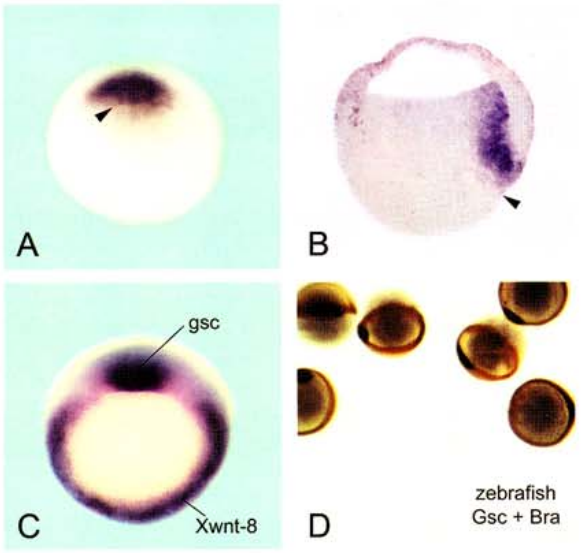

Figure 1. Expression of *goosecoid* in Spemann's organizer in *Xenopus* and zebrafish. (*A*) *goosecoid* in the dorsal lip of an early *Xenopus* gastrula. (*B*) *Gsc* in situ hybridization to a histological section of a *Xenopus* stage 10 1/2 gastrula. Expression is seen in the involuted endomesoderm, 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.)

(marked 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 histotypic 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 Frzb-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 chordoneural 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-

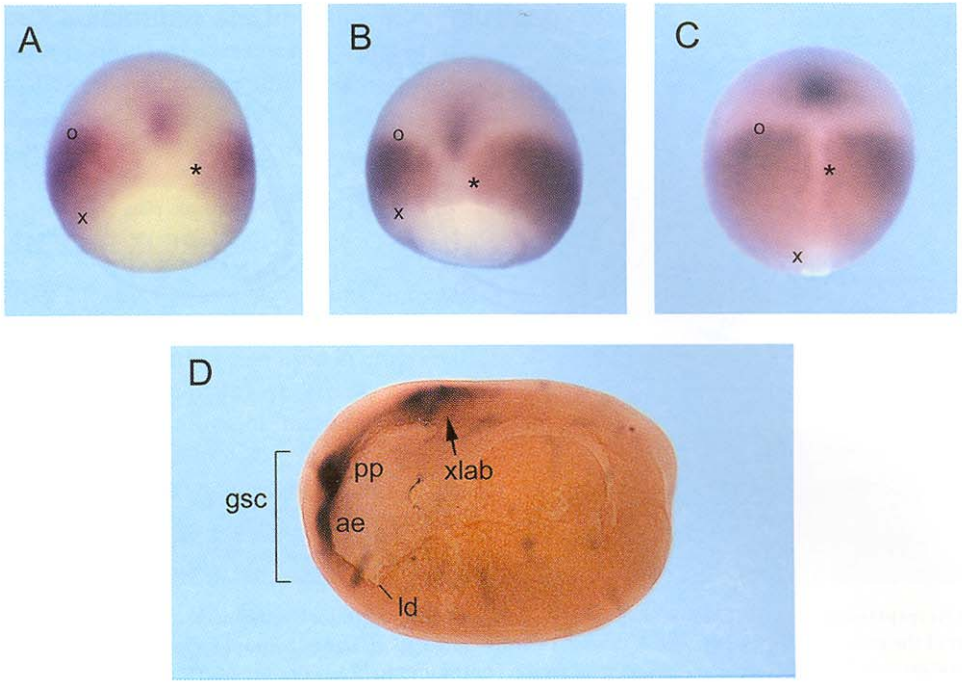

Figure 2. Expression patterns of *goosecoid* and the *Xlab* Hox gene during *Xenopus* gastrulation. (*A–C*) *Gsc*-expressing cells leave the dorsal lip and move into the prechordal plate and anterior endoderm region, while Xlab is observed in involuted mesoderm surrounding the circular blastopore lip. Markings have been placed in the blastopore (*small x*), anterior border of *Xlab* expression (*small o*), and midline border (*small asterisk*) of the notochord to illustrate the relative movements of the two expression domains. Embryos are at stage 11, 11 1/4, and 13. (*D*) Side view of a late-neurula (stage 15) *Xenopus* embryo showing expression of *gsc* in the prechordal plate (pp) just behind the eye anlage and in anterior endoderm (ae). The liver diverticulum (ld) is indicated; expression of *Xlab* in mesoderm, including the first somites, is also indicated. This embryo was cleared after whole-mount in situ hybridization. Photographs courtesy of Sung Kim and Peter Pfeffer.

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 (Trinidade 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; Leyns 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 (Niehrs 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

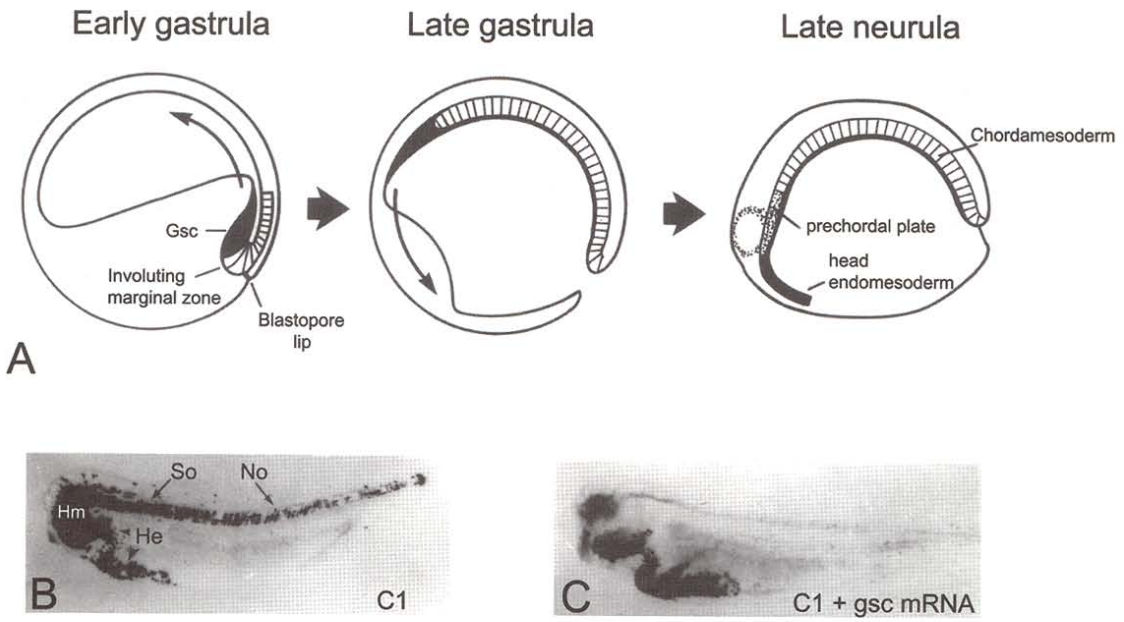

Figure 3. Overexpression of *goosecoid* mRNA promotes dorso-anterior migratory movements. (*A*) Diagram illustrating the movements of the *goosecoid*-expressing region (in *black*) during gastrulation. The notochord region is hatched, and the position of the eye anlage is indicated. Note that the *goosecoid* region contributes to head endomesoderm, including the entire foregut. (*B*) Normal progeny of a C1 blastomere using colloidal gold as lineage tracer. Note staining of the entire length of the notochord (No) including the posterior tip, medial somites (So), prechordal plate (Hm, head mesoderm), and head endomesoderm (He) up to the level of the liver and beyond. (*C*) When *gsc* mRNA is injected into C1 blastomeres, cell progeny is lost from notochord and accumulates in the head endomesoderm. Dorsal blastomeres contribute to the entire head region, including cells that populate the ventral head and trunk. (Modified, with permission, from Niehrs et al. 1993.)

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 (Trinidade 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*

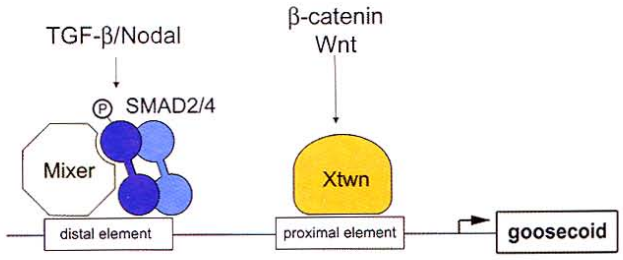

Figure 4. Diagram of the *goosecoid* promoter region, showing how signals from the β-catenin and the TGF-β pathway are integrated at the level of the DNA. Diagram courtesy of Oliver Wessely and Uyen Tran.

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

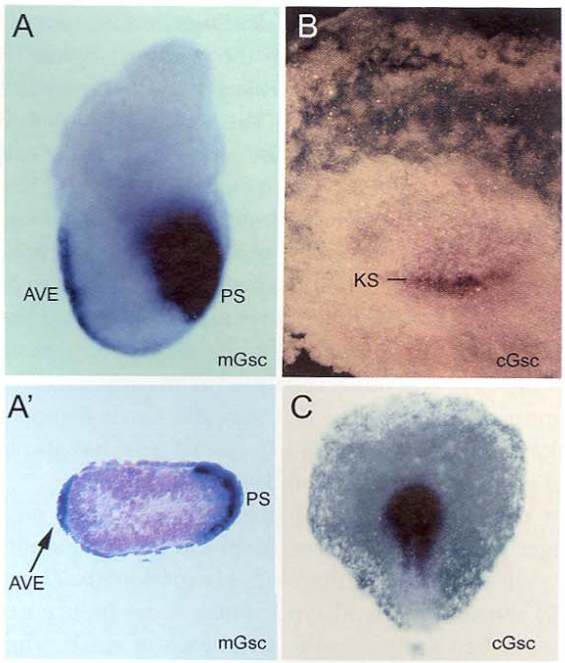

Figure 5. Expression of *GSC* in mouse and chick embryos. (*A*) Mouse mid-streak gastrula at the time of maximal *Gsc* expression. Note expression in anterior primitive streak (PS) and in anterior visceral endoderm (AVE). (*A'*) Transverse section through the same egg cylinder at the level of the anterior primitive streak. (*B*) Stage XII chick embryo in which the hypoblast has covered the posterior half of the area pellucida. Note *Goosecoid* expression in Koller's sickle (KS) and more weakly in the hypoblast anterior to it. (*C*) Expression of chick *Goosecoid* in the anterior third of the primitive streak, at stage 3+, when *Gsc* expression reaches its maximum. (*A, A'*, courtesy of J.A. Belo; *B,C*, reproduced, with permission, from Izpisúa-Belmonte et al. 1993.)

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 $Gsc^{-/-}$; $HNF3\beta^{+/-}$ 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 *Goosecoid* 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 nodes induce neural markers when grafted to the area opaca of chick, whereas $Gsc^{-/-}$ nodes are severely impaired in their neural inducing activity. Even $Gsc^{+/-}$ nodes 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.

GOOSECOID 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 *Goosecoid* 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 *Goosecoid* marker. Figure 5B shows *Goosecoid* 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, *Goosecoid* 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, *Goosecoid* 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 *Goosecoid* gene, *GSX*, is initially expressed together with *Goosecoid* 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 *Goosecoid* 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.

GOOSECOID AND THE MOLECULAR ANATOMY OF VERTEBRATE GASTRULATION

By analyzing the expression of *Goosecoid* 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 yolky 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; García-Martínez 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 lineages 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 downstream 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 all 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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