Molecular mechanisms of Spemann's organizer formation: conserved growth factor synergy between *Xenopus* and mouse

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Mesoderm induction assays in *Xenopus* have implicated growth factors such as activin, Vg1, Xwnt-8, and noggin as important in directing the formation of dorsal mesoderm (Spemann's organizer). Because these growth factors are structurally very different, they presumably act through distinct cell surface receptors that initiate different intracellular signaling cascades. A consequence of all of these signaling pathways, however, seems to be the induction of goosecoid (gsc) gene expression. To understand how integration of these different signaling pathways results in formation of Spemann's organizer, we sought to identify growth factor-responsive elements within the gsc promoter. Through microinjection of reporter genes we have identified two cis-acting elements, a distal element (DE) and a proximal element (PE), that are required for activin/BVgl and Wnt induction, respectively. We have shown that the DE mediates activin induction in the absence of protein synthesis and therefore constitutes the first activin response element identified to interpret transforming growth factor-β (TGF-β) superfamily member signaling directly. Using a reporter gene construct containing a multimerized DE, we find that an activin/BVgl-type signaling cascade is active throughout the vegetal hemisphere and marginal zone but not in the animal hemisphere. We demonstrate further that both the distal and proximal elements are essential for high-level transcription of the gsc gene, specifically in dorsal mesoderm, strongly suggesting that establishment of Spemann's organizer requires synergistic input from activin/BVgl-like and Wnt signaling pathways. Finally, mechanisms of establishing the organizer are likely to be conserved throughout vertebrate evolution.

[Key Words: Mesoderm; TGF-β; activin; axis specification; Vg1, goosecoid; homeo box; Wnt]

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How the body axis is established during early embryogenesis is one of the fundamental questions in embryology. The most inspiring experiments in the history of the study of embryonic axis formation were those carried out by Spemann and Mangold in 1924. By transplanting a small piece of the dorsal lip of the blastopore (also called the organizer) into the lateral/flank region of another embryo, they showed that the transplanted tissue was able to change the fate of host cells in the area of the transplant, resulting in a twinned embryo [Spemann and Mangold 1924]. Thus, the organizer has the capacity to initiate gastrulation movements, to convert the prospective ventral mesoderm to dorsal mesoderm, and change prospective ectoderm to neural ectoderm. Because of these unique functional capabilities of the organizer, intense efforts have been placed on deciphering its molecular properties.

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ventral endoderm that induces the remainder of the mesoderm. Once the organizer is induced, it is thought to release a third signal within the marginal zone to pattern the mesoderm further. This model of mesoderm induction, known as the three-signal model, has been hypothesized to explain how the D/V patterning of mesoderm is established during early embryogenesis [Dale and Slack 1987b].

Members of the fibroblast growth factor (FGF) and transforming growth factor-β (TGF-β) superfamilies have been implicated as signaling molecules involved in mediating mesoderm induction [Smith 1993]. Particular attention has been focused on members of the TGF-β family, as these are thought to be involved in dorsal mesodermal signaling and, hence, in the establishment of the organizer. One leading candidate is activin, which has been shown to induce different kinds of mesodermal tissues and markers in a concentration-dependent manner in both animal cap explants [Ariizumi et al. 1991; Green et al. 1992] and in the embryo [Gurdon et al. 1994]. Ectopic expression of activin in early cleavage stage Xenopus embryos leads to induction of partial secondary axes [Thomsen et al. 1990]. Additionally, when activin signaling is blocked in the embryo by ectopic expression of a dominant-negative mutant of the activin type-II receptor, mesoderm formation is completely disrupted [Hemmati-Brivanlou and Melton 1992]. However, the role of activin in mesoderm formation has been questioned following recent findings in mice deficient in activin or activin type-II receptor genes, in which the formation of mesoderm was not disrupted [Matzuk et al. 1995a,b]. Vg1, another TGF-β family member, is also a candidate molecule as a dorsal mesoderm inducer. Whereas both Vg1 mRNA and its protein are localized to the vegetal region of oocytes and embryos, the Vg1 protein detected in embryos is biologically inactive as a result of inefficient cleavage processes that fail to release a mature, active peptide [Tannahill and Melton 1989]. However, when Vg1 is processed as a bone morphogenetic protein [BMP–Vg1 chimera [BVg1], the mature Vg1 peptide efficiently induces dorsal mesoderm in animal caps and can rescue UV-ventralized embryos [Thomsen and Melton 1993; Dale et al. 1993].

In addition to molecules such as activin and BVg1 that are capable of directly inducing mesoderm on isolated naive ectoderm, there is another class of molecules with the ability to change the type of the mesoderm induced. These molecules, known as competence modifiers, include nogggin [Smith and Harland 1992] and members of the Wnt family [Christian et al. 1992]. Wnt members are secreted glycoproteins of the wingless/int family. Ectopic expression of synthetic Xwnt-8 RNA in the ventral portion of an early cleavage stage frog embryo leads to the formation of an additional dorsal signaling [Nieuwkoop] center, resulting in a secondary axis [Sokol et al. 1991, Smith and Harland 1991]. Whereas ectopic expression of Xwnt-8 in animal cap ectoderm through injected mRNA is ineffective as a mesoderm inducer, Xwnt-8 appears to alter the response of ectoderm to mesoderm-inducing growth factors such as basic FGF [bFGF] or activin. When these growth factors alone are applied at low concentration, animal caps differentiate into ventral mesoderm. However, with the addition of Xwnt-8, ectodermal cells differentiate into dorsal mesoderm, suggesting that Xwnt-8 modifies the response of cells to growth factors, allowing a shift to more dorsal character [Christian et al. 1992, Sokol and Melton 1992].

The discovery of these competence modifiers has led to an alternative proposal to the three-signal model. This model differs from the traditional three-signal model in that localized competence modifiers [e.g., Xwnt-8] are proposed to regulate the responsiveness of cells to the mesoderm-inducing factors, such as FGF and an activin-like molecules [Kimelman et al. 1992, Moon and Christian 1992]. The competence modifiers are not able to induce mesoderm themselves but are able to work synergistically with mesoderm-inducing factors to establish patterning within the marginal mesoderm and to form mesoderm of dorsal character [Spemann's organizer].

We wished to understand the molecular mechanisms involved in the establishment of the organizer and its activities in the amphibian to better comprehend the process of vertebrate gastrulation and the formation of primary axes. To initiate such studies, previously we had isolated an organizer-specific homeo box gene, goosecoid [gsc], in Xenopus [Cho et al. 1991]. Ectopic expression of gsc mRNA in the ventral side of the embryo can mimic Spemann's organizer activity by initiating gastrulation movements and inducing a secondary axis [Cho et al. 1991, Niehrs et al. 1993]. Additionally, gsc was shown to be directly inducible by activin in the absence of protein synthesis [Cho et al. 1991], similar to many other genes expressed in the organizer [e.g., Xlim [Taira et al. 1992]; Xbra [Smith et al. 1991]]. Therefore, we reasoned that further analysis of gsc induction by growth factors such as activin/BVg1 and Xwnt-8 should provide insights into organizer formation and the regulation of gastrulation in vertebrates. It should be noted, however, that whereas activin/BVg1 and Xwnt-8 have been implicated to play important roles in organizer formation, these factors may not represent the endogenous factors in the embryo. Instead, they may mimic maternally deposited factors yet to be identified. At present, Xwnt-11 and Xwnt-8b have been shown to be maternally expressed Wnt family members [Ku and Melton 1993; Cui et al. 1995]. Regardless, we decided to take advantage of the properties of activin/BVg1 and Xwnt-8 to study the regulation of gsc in Xenopus because they may aid in elucidating the molecular mechanisms of organizer formation.

This study describes our initial characterization of Xenopus gsc gene regulation. We have identified two promoter elements, which we have named DE [distal element] and PE [proximal element], that are required for the efficient and faithful regulation of gsc expression in vitro as well as in vivo. The DE is the first identified cis-acting element that responds to signaling from members of the TGF-β family. The DE responds to dorsal mesoderm signals mimicked by activin and BVg1, and the PE responds to Wnt signaling. It appears that these two sets of signals synergistically regulate the expression
level of gsc via these elements. These results suggest that formation of the organizer also requires synergistic growth factor signaling via the DE and PE. Furthermore, we show that the DE and PE are conserved structurally and functionally between *Xenopus* and mouse gsc genes and propose that the regulatory mechanisms for the initiation of organizer formation and vertebrate gastrulation have been conserved during evolution at the most basic biochemical level.

**Results**

**Identification of a cis-acting element required for activin responsiveness within the gsc promoter**

A *Xenopus* genomic clone containing the gsc gene was cloned and mapped as shown in Figure 1A. The gene was shown to consist of three exons. Interestingly, the homeo box of the gsc gene is divided into two exons [2 and 3]. The gsc transcription initiation site was mapped, using both primer extension and RNase protection assays (data not shown), to a position 13 nucleotides upstream of the initiator methionine codon (Fig. 1B). A TATA-like sequence was found 30 nucleotides upstream of the transcription initiation site.

Because gsc is directly inducible by activin in the absence of protein synthesis, and activin-like activity has been implicated as important for the establishment of organizer mesoderm, we attempted to identify an activin-responsive element within the promoter of gsc. A 1.5-kb piece of gsc 5′-flanking sequence, including 12 bp of the 5′-untranslated leader, was subcloned into a promoterless luciferase reporter plasmid, pOLuc (de Wet et al. 1987). The resulting plasmid, −1500gsc/Luc, was microinjected into the animal hemisphere of four-cell stage embryos (see Fig. 2A). Animal caps were dissected at the blastula stage, incubated with or without activin for 3 hr, and then prepared as extracts for luciferase assays. The level of activin induction was obtained by calculating the ratio of luciferase activity between activin-treated animal caps and untreated caps. As shown in Figure 2B, activin induces the −1500gsc/Luc reporter gene 40-fold. The induction of the reporter gene by activin is gsc-promoter dependent, because a pOLuc variant containing the reverse orientation of the gsc promoter was not activin inducible (data not shown). Furthermore, microinjection of other luciferase reporter gene constructs containing promoters of cytomegalovirus (CMV) or SV40 did not respond to activin induction (data not shown).

Knowing that −1500gsc/Luc responding to activin signaling, we proceeded to map the 5′ boundary of the region within the gsc promoter required for activin induction, using a series of 5′ deletion constructs. Figure 2B shows that deletion constructs reaching to position −226 relative to the transcription initiation site were still activin-inducible (lines 2,3). Further deletion of the gsc promoter abolished the response to activin (line 4). Because the region between −226 and −198 appeared to be essential for activin induction, point mutations were introduced within the region and tested for activin induction. We found that point mutations between −226 and −198 bp abolished the level of activin induction of the gsc reporter gene (Fig. 2B, line 5). This 29-bp region essential for activin induction is referred to as the DE.

The DE is sufficient for direct activin induction

To test whether the DE was sufficient for activin induction, concatenated DE was subcloned in front of a heterologous promoter (the thymidine kinase [TK] promoter of herpes simplex virus [Luckow et al. 1987]) and the gsc endogenous minimal promoter (104 bp of 5′-flanking region). Animal cap assays using reporter constructs with six copies of the DE [DE[6X]TK/Luc and DE[6X]104gsc/Luc] revealed that the DE was responsive to activin, whereas the TK/Luc vector was not (Fig. 2B, lines 6–8). The increased reporter gene activity of DE[6X]TK/Luc correlated with an increase in the rate of transcription of the reporter gene, as determined by primer extension assay (Fig. 3, lanes 1 and 2). From these analyses, we conclude that the DE is not only necessary but also sufficient to respond to activin.

We next determined whether activin induction
through the DE is protein synthesis independent, reflecting endogenous gsc induction in similar assays. Animal caps injected with DE(6X)TK/Luc were preincubated for 30 min with or without 5 µg/ml of cycloheximide before adding activin and incubated for an additional 90 min before isolating RNA (Rosa 1989). In our hands, these conditions prevented the incorporation of $^{35}$S-methionine into proteins by >95%, as measured by scintillation counting (data not shown). Primer extension analysis of the RNA samples revealed that DE(6X)TK/Luc was transcribed in the presence of cycloheximide after activin challenge (Fig. 3, lane 3), demonstrating that activin directly induces transcription of DE(6X)TK/Luc. To date, DE is the first identified cis-acting element that directly responds to a member of the TGF-β superfamily.

The specificity of DE toward various growth factors was also determined. DE(6X)TK/Luc or -226gsc/Luc was microinjected into embryos, and luciferase activities were measured using animal caps cultured in medium containing activin, TGF-β1, or bFGF. Alternatively, luciferase assays were carried out on animal caps isolated from embryos that were coincubated with reporter genes and synthetic mRNA encoding XBMP-4 (Xenopus bone morphogenetic protein-4), XBMP-7, Xnr3 (Xenopus nodal-related 3, Smith et al. 1995), or BvgI (a processed form of VgI, Thomsen and Melton 1993). As shown in Figure 4, only activin and BvgI induced the expression of both reporter genes, whereas TGF-β1, XBMP-4, XBMP-7, Xnr-3, and bFGF did not. These results suggest that the DE is capable of responding specifically to BvgI and activin, growth factors known to mimic dorsal mesoderm-inducing activities.

Activin/BvgI-type signaling is active throughout the entire vegetal hemisphere

Because DE is able to specifically respond to dorsal signals such as activin and BvgI in vitro, we attempted to determine where these signals would be present in the whole embryo. In Xenopus, the prospective fates of the various blastomeres of the 32-cell stage embryo have been well studied [Fig. 5A; Dale and Slack 1987a]. For example, D1, C1, and C4 blastomeres give rise predominantly to dorsal endoderm (Nieuwkoop center), dorsal mesoderm (Spemann’s organizer), and ventral mesoderm of the embryos, respectively, whereas the blastomeres of the A tier become skin and neural ectoderm. We expected that the activity of DE(6X)104gsc/Luc [Fig. 2, line 8] might be the highest in the C1 and D1 blastomeres of 32-cell stage embryos, where the dorsal mesoderm-inducing activity is thought to reside, according to the three-signal model. Unexpectedly, we found that the reporter gene activity of DE(6X)TK/Luc was uniformly activated [an average of 40-fold] in all blastomeres of the C and D tiers, whereas activities in the blastomeres of the A and B tiers were low [Fig. 5B]. From these results, we conclude that an activin/BvgI-like activity is uniformly

**Figure 2.** Identification of an activin responsive element within the gsc promoter sequence. (A) Schematic representation of the reporter gene assay in animal caps. Embryos are injected into the animal pole of all blastomeres at the four-cell stage with reporter gene constructs. At the blastula stage, animal caps are dissected manually, cultured for 3 hr in the presence or absence of growth factor [e.g., activin], and subjected to luciferase assays. (B) Deleting sequences between -226 and -197 dramatically reduces the ability of the gsc promoter to respond to activin in animal cap assays. Point mutations within this region [M4gsc/Luc] also eliminate the activin response, suggesting that an activin response element [DE] is located within this region. Multimerization of this element [constructs 7 and 8, orientation shown by an arrow] is sufficient for activin responsiveness to the heterologous thymidine kinase [TK] promoter and the endogenous gsc promoter [104gsc/Luc]. The putative responsive elements are shown in the schematic at the top. Induction of reporter genes was calculated as the ratio between growth factor-treated and untreated animal caps.
present, encompassing the entire marginal zone as well as the vegetal hemisphere in the *Xenopus* embryo.

Response of the gsc promoter in whole embryos: in vivo analysis

The uniform localization of activin/BVgl-like activity in *Xenopus* embryos prompted us to test the native gsc promoter in blastomere injections because it appeared that DE alone was not sufficient to faithfully recapitulate organizer-specific expression of gsc. Figure 6 shows the results of microinjection of various gsc promoter reporter genes into blastomeres of A4, C1, or C4. When the −1500gsc/Luc reporter construct was injected into the C1 blastomere, it was activated 297-fold better than in A4 (Fig. 6, line 1). The C4 blastomere was also capable of activating the reporter gene at a moderate level (57-fold), probably because of an activin/BVgl activity that is present throughout the entire marginal zone (see Fig. 5B). The selected activation of −1500gsc/Luc in C1 and C4 is not attributable to nonspecific activation of the reporter because similar injection of the SV40/Luc, Rous sarcoma virus [RSV]/Luc, mouse mammary tumor virus [MMTV]/Luc, and RSV/β-galactosidase failed to exhibit such localized activation (data not shown).

Activation levels of gsc/Luc constructs with 5’ deletions up to position −226 were essentially similar to that of −1500gsc/Luc (Fig. 6, line 2). However, −197gsc/Luc (lacking the DE) was injected, reporter gene activity in C1 was reduced to 40-fold, and activity in C4 was reduced to levels similar to those found in A4 (Fig. 6, line 3). The reduction of reporter gene activity in both C1 and C4 is consistent with a requirement for the DE to respond to an activin/BVgl-like signal present throughout the marginal zone. Moreover, the fact that −197gsc/Luc (lacking the DE) is still inducible up to 40-fold in C1 implies that additional regulatory elements are present downstream of position −197.

Microinjection of other deletion constructs into C1 demonstrated that gsc/Luc constructs extending to position −155 are still capable of being induced (Fig. 6, line 4; data not shown). However, further deletions reduced reporter gene activities to basal levels (Fig. 6, line 5), supporting our hypothesis that an additional element is present within the gsc promoter that responds to a dorsal-specific signal.

The PE specifically responds to Xwnt-8 signal

In addition to mesoderm-inducing molecules, such as activin and BVgl, a competence modifier, Xwnt-8, is capable of inducing gsc when it is ectopically expressed in the ventral region of gastrulating embryos where mesoderm-inducing activities are also present (Christian and Moon 1993). Therefore, we examined whether Xwnt-8 was capable of inducing a reporter gene through the −226 bp of the cloned gsc promoter. Coinjection of Xwnt-8 mRNA into ventral blastomeres (C4) with the −226gsc/Luc reporter construct revealed that Xwnt-8 expression activated the reporter gene 18-fold higher than in control C4 blastomeres injected with reporter alone (Fig. 7, lines 1, 2). When the specificity of gsc for Xwnt-8 signaling was examined using another Wnt-related molecule, Xwnt-5A (Moon et al. 1993), Xwnt-5A was unable to activate the −226 gsc/Luc reporter gene (see Fig. 7, line 3). The result suggests that the response of gsc to Xwnt-8 induction is selective. We also examined the timing of Xwnt-8 action on gsc activation during embryogenesis because ectopic expression of Xwnt-8 mRNA does not distinguish whether the translated protein product acts before or after midblastula transition (MBT). This question is important, because for Xwnt to mimic the Nieuwkoop center activity to induce the organizer the protein must commence its function before (during cleavage stages), but not after MBT (Christian and Moon 1993). We coinjected −226gsc/Luc with a cytoskeletal actin promoter (CSKA)−Xwnt-8 construct that directs the expression of Xwnt-8 only after MBT and
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found that CSKA-Xwnt-8 was unable to activate the reporter gene (Fig. 7, line 4). From this experiment, we infer that ectopic expression of Xwnt-8 mimics Nieuwkoop center activity, which in turn induces a secondary organizer to activate the gsc reporter gene.

Noggin is present maternally in Xenopus embryos (Smith and Harland 1992), and can also function as a competence modifier. Therefore, we examined the possibility that activation of gsc by Xwnt-8 mimics endogenous Noggin signaling. Coinjection of −226gsc/Luc with a high concentration of noggin mRNA (2 ng) results in moderate activation of the reporter in C4 (four-fold) when compared with that of reporter gene injected alone (Fig. 7, line 5). At present, the molecular nature of the weak activation of gsc by noggin is not clear, though our observations are consistent with Lemaire et al. (1995), who demonstrated a stronger axis-inducing activity by Xwnt-8 when compared with noggin.

Identification of a Wnt-responsive element within the promoter of the gsc gene

Because Xwnt-8 was shown to induce −226gsc/Luc reporter gene expression in the ventral side of embryos, we decided to test whether Xwnt-8 induction is dependent on the DE (an activin/BVgl-like signal responsive element). As shown in Figure 8, the −197gsc/Luc construct lacking the DE was still responsive to Xwnt-8 induction in the ventral mesoderm (C4), despite the overall activity being reduced because of the lack of the DE (cf. Fig. 8, lines 1 and 2). The lack of DE involvement in Xwnt-8 induction is consistent with the finding that the DE(6X)TK promoter is capable of responding to activin/BVgl1 fails to respond to the Xwnt-8 signal (data not shown).

Subsequent analysis revealed that reporter genes containing deletions up to position −155 were still capable of responding to the Xwnt-8 dorsalizing signal but further deletions eliminated the Xwnt-8 response (Fig. 8, lines 3,4). Because the region downstream of position −155 appeared to be crucial for the Xwnt-8 response, we generated a series of linker-scanning mutants and tested their responses to Xwnt-8 induction. Mutations in the region between −155 and −105 bp abolished the Xwnt-8 dorsalization response (data not shown). From these experiments we conclude that there are two distinct cis-activating elements in the gsc promoter, a DE and a PE, that respond to different types of growth factor signals, namely an activin/BVgl1 type and an Xwnt-8-type, respectively. It appears that spatiotemporal expression of the gsc gene is controlled via the DE and PE through the combinatorial actions of a general mesoderm inducer and a competence modifier to elicit a dorsal signal. This mechanism of growth factor synergy may broadly reflect how the organizer is established in amphibians.

The DE and PE are conserved between the Xenopus and mouse gsc genes

Isolation and comparison of the genomic counterpart of the Xenopus gsc promoter in the mouse have identified two highly conserved regions (Fig. 9A). The DE between −226 and −197 bp in Xenopus is 87% identical to a sequence located between −582 bp and −554 bp in the mouse gsc promoter, and the Xenopus PE between −151 and −105 bp is 70% identical to a mouse sequence between −524 and −474 bp (Fig. 9A). The conservation of both the DE and PE between Xenopus and mouse suggests that molecular mechanisms supporting gsc regulation may have been preserved during evolution.

Because both the mouse and Xenopus gsc genes are inducible by activin (Cho et al. 1991; Blum et al. 1992)
and the sequences of the DE and PE are conserved between the two species, we studied the extent of functional conservation of the DE and PE by examining the effect of the mouse gsc promoter sequence in the Xenopus in vitro and in vivo systems. Mouse gsc promoter sequence corresponding to the region of the Xenopus gsc promoter between −226 and −105 bp (containing both the DE and PE) was cloned upstream of Xenopus −104gsc/Luc (the minimal gsc promoter/Luc construct). The response of the resulting construct (mDE–PE/104gsc/Luc) to activin was measured in animal caps. As shown in Figure 9B, the mouse promoter sequence responded to activin challenge to the same extent as its Xenopus counterpart, whereas the minimal gsc promoter did not.

We further tested the functional conservation of the mouse gsc promoter sequence by examining the response of mDE–PE/104gsc/Luc to endogenous factors in the Xenopus embryo and to ectopic expression of Xwnt-8 using blastomere co-injection assays. As shown in Figure 9C, the activities of the mouse gsc promoter construct in Xenopus were indistinguishable to those of its Xenopus counterpart (lines 1, 2). Moreover, we found that the mouse gsc construct responded to Xenopus Xwnt-8 induction as revealed by increased reporter gene activity in Xwnt-8-injected C4 blastomeres. These results demonstrate that the DE and PE are structurally and functionally conserved between both the mouse and Xenopus gsc promoter. The interchangeability of these elements between the two species supports the notion that intracellular signaling mechanisms leading to the formation of organizer–equivalent tissues in vertebrates are likely to have been preserved during evolution.

Discussion

Characterization of the gsc promoter has identified important cis-acting elements whose control most likely reflects the regulation involved in establishing the organizer. The DE is the first response element shown to be directly inducible by a TGF-β signaling molecule. Rey
Luciferase activity

1. no mRNA
   - C1
   - C4
2. Xwnt8
   - C1
   - C4
3. Xwnt 5A
   - C1
   - C4
4. CSKA-Xwnt8
   - C1
   - C4
5. noggin
   - C1
   - C4

Figure 7. Ectopic Xwnt-8 specifically activates the gsc promoter. The construct -226gsc/Luc was either injected alone or coinjected with the indicated mRNAs or DNA into either the dorsal [C1] or ventral [C4] blastomere of the 32-cell stage embryos, and the reporter gene activity was measured. Comparison of C1- and C4-injected embryos with the reporter gene activity revealed that the C1-injected embryos induce the reporter gene ~10-fold higher than the C4-injected embryos. Coinjection of Xwnt-8 mRNA caused an 18-fold induction of the reporter gene in the C4 blastomere, which is indicative of dorsoization of the blastomere. This induction was specific for Xwnt-8, as other Wnt molecules and competence modifiers [lines 3,4,5] did not induce the gsc promoter. When Xwnt-8 mRNA was coinjected dorsally, the reporter gene induction was not observed presumably because of the presence and maximal levels of a natural dorsal signal. Data presented are actual luciferase counts. It should be noted that the reporter gene activity in C4 after Xwnt-8 injection was consistently higher than that of C1, whose significance is unknown at present.

porter constructs containing the DE indicate that an activin/BVgl-type signaling cascade is active throughout the vegetal and marginal regions, which is inconsistent with the notion that this activity should be localized to the dorsovegetal region [Nieuwkoop center] of Xenopus embryos [Green et al. 1992]. This suggests that an activin/BVgl-like activity may have a more general role in mesoderm induction than indicated by previous models. Downstream of the DE is a Wnt-responsive element, the PE, which appears to be specific for a dorsal signal mimicked by ectopic expression of Xwnt-8. Together, the DE and PE can synergistically activate the gsc promoter in the dorsal region of the embryo where their respective signal pathways converge.

A current working model of organizer formation: growth factor synergism

In this paper we provide in vivo evidence supporting a model whereby dorsal mesoderm and Spemann’s organizer are established by growth factor synergism as shown in Figure 10 [Christian et al. 1992, Kimelman et al. 1992]. According to the model, an interaction between general mesoderm-inducing factors, such as activin/BVgl-like molecules, and competence modifiers, such as Xwnt-8, would be capable of producing dorsal types of mesoderm. Synergistic activation of gsc by Xwnt-8 and activin has been suggested from experiments in vitro using animal cap tissues [Sokol and Melton 1992]. Furthermore, Wnt signaling has been implicated in establishing dorsal mesoderm by two independent observations: Oligonucleotide ablation of β-catenin during Xenopus development eliminates dorsal mesoderm [Heasman et al. 1994], and ectopic expression of Xenopus and Drosophila dishevelled, another component of Wnt signaling, activates the gsc promoter [Rothbächer et al. 1995, U. Rothbächer and K. Cho, unpubl.]. In support of these observations, we have demonstrated that the promoter of gsc contains two independent cis-regulatory elements, one each for activin/BVgl and Xwnt-8 [Fig. 10]. The DE is sufficient to respond to the general mesoderm signal [activin/BVgl-like signal], which is active throughout the vegetal hemisphere of the embryo. When both elements are together, synergistic activation of the
promoter in dorsal mesoderm is found in vivo (Fig. 10). The model predicts that the endogenous gsc gene is expressed at high levels in the dorsal-most tissue of the embryo, and that it should be expressed at low levels throughout the rest of the marginal and vegetal region because of the presence of an activin/BVgl-like activity in these regions. However, the expression of gsc in the ventral/lateral marginal region, for example, may be modified further by the presence of other molecules such as BMP-4 and BMP-7 that are expressed in the ventral/lateral marginal zone and known to suppress the expression of gsc (Fainsod et al. 1994; Gräff et al. 1994; Suzuki et al. 1994; Hawley et al. 1995; Schmidt et al. 1995). Additionally, synergistic and modifying roles for FGF in establishing the mesoderm (Labonne and Whitman 1994; Cornell et al. 1995) may also influence the regulation of gsc in the marginal zone.

The synergistic induction model of mesoderm formation is attractive for several reasons. First, it explains how ectopic expression of Xwnt-8 in animal pole cells (A4 injection) fails to induce the gsc reporter gene, whereas injection into the ventral marginal zone leads to strong expression of gsc on the ventral side (C4 blastomere injection). As we have demonstrated, the A tier does not contain an activin/BVgl-like activity (Fig. 5B) and, therefore, could not synergize with Xwnt-8 as it does in the C4 blastomere. Second, the model provides a molecular basis for the quantitative differences in the levels of gsc reporter gene induction observed when comparing activin-treated animal caps with blastomere injections. That is, we show that −226gsc/Luc is activated only -30- to 50-fold in activin-treated animal caps, whereas in blastomere injection assays, the reporter gene was activated as much as 300-fold (C1/A4 ratio; Fig. 6). Perhaps, the animal cap lacks a Wnt-type activity that appears to be localized in the dorsal region of the em-

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**Figure 9.** The gsc DE and PE are homologous between Xenopus and mouse. (A) Sequence comparison of Xenopus and mouse gsc promoter sequences with optimal alignment of the DE and PE using MacVector's Pustell DNA matrix search (IBI). Identities are shown by vertical bars. Gaps introduced to align the sequences are marked by dots. The Xenopus and mouse DE and PE are 83% and 70% identical, respectively. (X) Xenopus; (M) mouse. The nucleotide positions for the mouse DE and PE are relative to the translational start site. The GenBank accession number for the mouse gsc promoter sequence is U39290. (B) Animal cap assays using the −226gsc/Luc (Xenopus DE-PE) and mDE-PE/104gsc/Luc (mouse DE-PE) reporter genes indicate that the mouse and Xenopus DE-PE have an equivalent response to activin. Activity represents fold induction over untreated animal caps isolated from embryos injected with the same constructs. (C) Similarly, the Xenopus and mouse DE-PE respond equally to endogenous signals and ectopic Xwnt-8 expression in blastomere injection experiments.
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Molecular mechanisms underlying organizer formation are conserved during vertebrate evolution

Gastrulation is possibly the most crucial event of vertebrate development. It is at this stage that the three germ layers (ectoderm, mesoderm, and endoderm) undergo a series of morphogenetic movements that initiate the various cell–cell interactions and inductions that establish the embryonic axis. Each member of the five vertebrate classes undergoes its own distinct morphogenetic movements. However, after the completion of gastrulation, most vertebrate embryos exhibit remarkably similar features [von Baer 1828].

Although gastrulation processes appear different among various species, the fundamental mechanisms regulating gastrulation appear to be conserved across the chordate phylum. Homologs for gsc have been identified in such disparate vertebrate species as mouse, chicken, and zebrafish. In all of these organisms, gsc transcripts appear at the onset of gastrulation precisely in the region where cell ingression is initiated. gsc is expressed in the dorsal lip in Xenopus [Cho et al. 1991], the dorsal blastoderm in zebrafish [Stachel et al. 1993], Koller’s sickle and Hensen’s node in chicken [Izpisua-Belmonte et al. 1993], and the primitive streak in mouse [Blum et al. 1992]. These findings and similar results obtained from comparative studies of Brachyury gene expression [Herrmann 1991, Smith et al. 1991, Schulte-Merker et al. 1992] suggest conservation of the molecular framework governing gastrulation among different species.

Our analysis of gsc regulation revealed that in addition to the conserved expression of the gsc homeobox gene during gastrulation, the molecular mechanisms that underlie the induction of gsc are also conserved among vertebrates. The regulatory DNA elements conserved between the mouse and Xenopus gsc genes are functionally interchangeable, suggesting that the signals and the signaling pathways for forming organizing centers are likely to be conserved among vertebrates at the most basic biochemical levels. Further analysis of components of the signal transduction pathways (e.g., DNA-binding proteins for the DE) will give additional insight into this conservation.

Material and methods

Construction of expression plasmids

A Xenopus gsc genomic clone has been described previously [Cho et al. 1991]. The ~1500gsc/Luc reporter was constructed from a blunt-ended EcoRV–PstI 1.5-kb Xenopus gsc gene fragment carrying 3 bp of exon 1 and 5′-promoter sequences inserted into the Smal site of the promoterless luciferase vector pOLuc [de Wet et al. 1987].

Fragments for the deletion constructs were generated by PCR using the same 3′ (downstream) primer [nucleotides −5 to +12 relative to the transcription start site] and various 5′ (upstream) primers representing a series of deletions in the gsc promoter region. The 3′ primer contained a HindIII restriction site at its 5′ terminus and the 5′ primers contained a BamHI restriction site at their 5′ termini for directional cloning into pOLuc. The upstream primer for construction of M4gsc/Luc

Figure 10. A current working model of Spemann’s organizer formation. A map of gsc promoter activity found at gastrulation suggests that during mesoderm formation a general mesoderm-inducing activity, provided by an activin/BVgl-like activity (vertically lined area), would be present throughout the marginal and vegetal region, whereas a dorsalizing activity (horizontally lined area), mimicked by Xwnt-8, would be localized to organize tissue. The map is reminiscent of the synergism model of mesoderm formation described by Moon and Christian (1992), supporting a proposal that growth factor synergy may be a common mode of regulation for genes expressed in the organizer. The two independent signaling cascades synergize to induce gsc gene expression through two independent cis-acting elements within the gsc promoter. This regulation appears to be conserved between mouse and Xenopus and may reflect a general mechanism for establishing organizing centers in vertebrates. At present, the model does not take into account other regulatory factors, such as BMPs, which appear to directly affect transcription of organizer genes as well. (AN) Animal pole, (VG) vegetal pole, (D) dorsal, (V) ventral, (O) presumptive organizer region.
had six single-base mutations in the region between −226 and −210. Sequences of the PCR primers are listed below. Downstream primer: 5’ GGGAAGCTTCAGACTGCAGTCC-TCTT-3’. Upstream primers for −226gsc/Luc, 5’-GGGG-GATCCATTAGATCATACGG-3’; −203gsc/Luc, 5’-GGG-GATCCAAATTGCTCCTGTAAT-3’; −180gsc/Luc, 5’-GGGGGATCCGGTCAGTCCATCCCTGTT-3’; −155gsc/Luc, 5’-GGGGGATCCGGTGATATCT-3’; M4gsc/Luc, 5’-GGGGGATCCAGTGCTGAGGCTGGA-3’ (mutated nucleotides are underlined).

To prepare the multimers of the DE, a set of two oligonucleotides, D.E.L (5’-GATCCTATAGATCCATATAG-3’) and D.E.R (5’-GATCTATAGATCCATATAG-3’) and DE.L (5’-GATCTATAGATCCATATAG-3’) and DE.R (5’-GATCTATAGATCCATATAG-3’) were synthesized. They were phosphorylated, annealed, and concatemered as described (Kadonaga 1986). The multimers were subcloned into the BamHI site of the TK luc-based plasmid and −104pOLuc, and the presence of six copies of the DE was determined by sequencing.

To prepare mDE–PE/104gsc/Luc, the fragment of the mouse counterpart of the Xenopus gsc 5’-flanking sequence between −226 and −105 was generated by PCR using primers that contained a BamHI restriction site at their 5’-termini. The sequences of the PCR primers are upstream, 5’-GGGGGATCCCTATAGATCCATATAG-3’; and downstream, 5’-GGGGGATCCCTATAGATCCATATAG-3’. The fragment was subcloned into the BamHI site of −104gsc/Luc. All subclones were sequenced using Sequenase (U.S. Biochemical).

Preparation of synthetic RNA
The plasmids pSP64T–Bv g-1 (Thomsen and Melton 1993), Xβm–BMP-4, Xβm–BMP-7 (Hawley et al. 1995), Xnt3/pGEM5 (Smith et al. 1995), Xβm–Xwnt-8 (Christian et al. 1992), Xβm–Xwnt-5A (Moon et al. 1993), and previously, pSP64T–noggin (Smith and Harland 1992) were linearized as described previously. Capped mRNAs were synthesized using the Megascript transcription kit (Ambion) and were resuspended in injection buffer (88 mM NaCl, 1 mM KC1, 15 mM Tris- HCl at pH 7.5).

Luciferase assay
Animal caps and embryos were collected and excess medium was removed. Tissues were homogenized in 100 μl of 50 mM Tris (pH 7.6) and cleared by centrifugation. Ten microliters of the supernatant was hydrolyzed at 37°C for 30 min in 1 x MBS containing [35S]methionine (400 μCi/ml), together with cycloheximide and activin, followed by another 30 min incubation in 1 x MBS containing [35S]methionine (400 μCi/ml) and activin. Animal caps were then homogenized in 50 mM Tris (pH 7.6) and cleared by centrifugation. A 2-μl aliquot of the supernatant was hydrolyzed at 37°C for 30 min in 0.25 ml of 1 N NaOH and aminoacyl tRNAs, followed by TCA precipitation and scintillation counting. Other aliquots were used for RNA preparation.

In blastomere injection assays, determination of dorsal versus ventral blastomeres was accomplished as described (Cho et al. 1991). Two nanoliters of DNA was injected into single A1, C1, or C4 blastomeres at the 32-cell stage without or with 200–400 pg of synthetic mRNA of Xwnt-8, Xwnt5-A, or noggin or the DNA expression vector CSKA-Xwnt-8. Embryos were harvested at early gastrula stage (stage 10.5) for luciferase assays.

Primer extension analysis
The transcriptional start site of the luciferase mRNA from the reporter constructs was determined by primer extension (Maniatis et al. 1982) using a polynucleotide kinase end-labeled oligonucleotide that was complementary to nucleotides 22–39 of the luciferase-coding region (de Wet et al. 1987). Template RNA was isolated from animal caps using TRIzol according to the manufacturer’s recommendations (GIBCO BRL).

Isolation of genomic clones containing mouse gsc sequences
An ~250-bp PCR fragment of mouse gsc gene exon 1 sequence was amplified from mouse genomic DNA (Clonetech) using two primers containing BamHI linkers. The sequence of the upstream primer was 5’-GGGGGATCCATATAGATCATAGGACAAAC-3’. The sequence of the downstream primer was 5’-GGGGGATCCTAGAATGCTGTTGTA-3’. Three PCR fragments were used as a probe to screen ~105 plaques of a genomic mouse library (Stratenege), which resulted in the isolation of a 3.3-kb gsc genomic fragment. Hybridizations were carried out in buffer containing 6 x SSC, 5 x Denhardt’s solution, 45% formamide, 0.1% SDS, 50 mM phosphate buffer (pH 7.6), 100 μg/ml of yeast torula RNA, and 5% dextran at 37°C. The final washes were performed in 0.5 x SSC at 50°C for 30 min.

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References

Arizumi, T., K. Sawamura, H. Uchiyama, and M. Asashima. 1991. Dose and time-dependent mesoderm induction and outgrowth formation by activin A in Xenopus laevis. Int. J. Dev. Biol. 35: 407–414.

Blum, M., S.J. Gaunt, K.W.Y. Cho, H. Steinbeisser, B. Blumberg, D. Bittner, and E.M. DeRobertis. 1992. Gastrulation in the mouse: The role of the homeobox gene goosecoid. Cell 69: 1097–1106.

Cho, K.W.Y., B. Blumberg, H. Steinbeisser, and E.M. DeRobertis. 1991. Molecular nature of Spemann’s organizer: The role of the Xenopus homeobox gene goosecoid. Cell 67: 1111–1120.

Christian, J.L., and R.T. Moon. 1993. Interactions between Xwnt-8 and Spemann organizer signaling pathways generate dorsoventral pattern in the embryonic mesoderm of Xenopus. Genes & Dev. 7: 13–28.

Christian, J.L., J.A. McMahon, A.P. McMahon, and R.T. Moon. 1991. Xwnt-8, a Xenopus wnt-1/int-1 related gene responsive to mesoderm-inducing growth factors, may play a role in ventral mesodermal patterning during embryogenesis. Development 111: 1045–1055.

Christian, J.L., D.J. Olson, and R.T. Moon. 1992. Xwnt-8 modifies the character of mesoderm induced by bFGF in isolated Xenopus ectoderm. EMBO J. 11: 33–41.

Cornell, R.A., T.J. Musci, and D. Kimelman. 1995. FGF is a prospective competence factor for early activin-type signals in Xenopus mesoderm induction. Development 121: 2429–2437.

Cui, Y., J.D. Brown, R.T. Moon, and J.L. Christian. 1995. Xwnt-8b: a maternally expressed Xenopus Wnt gene with a potential role in establishing the dorsoventral axis. Development 121: 2177–2186.

Dale, L. and J.M. Slack. 1987a. Fate map for the 32-cell stage of Xenopus laevis. Development 99: 527–531.

———. 1987b. Regional specification within the mesoderm of early embryos of Xenopus laevis. Development 100: 279–295.

Dale, L., G. Matthews, and A. Colman. 1993. Secretion and mesoderm inducing activity of the TGFβ related domain of Xenopus Vg1. EMBO J. 12: 4471–4480.

de Wet, J.R., K.V. Wood, M. DeLuca, D.R. Helinski, and S. Subramani. 1987. Firefly, luciferase gene: Structure and expression in mammalian cells. Mol. Cell. Biol. 7: 725–737.

Fainsod, A., H. Steinbeisser, and E.M. DeRobertis. 1994. On the function of BMP-4 in patterning the marginal zone of the Xenopus embryo. EMBO J. 13: 5015–5025.

Graff, J.m., R.S. Thies, J.J. Song, A.J. Celeste, and D.A. Melton. 1994. Studies with a Xenopus BMP receptor suggest that ventral mesoderm-inducing signals override dorsal signals in vivo. Cell 79: 169–179.

Green, J.B.A., H.V. New, and J.C. Smith. 1992. Responses of embryonic Xenopus cells to activin and FGF are separated by multiple dose thresholds and correspond to distinct axes of the mesoderm. Cell 71: 731–739.

Gurdon, J.B., P. Harger, A. Mitchell, and P. Lemaire. 1994. Activin signalling and response to a morphogen gradient. Nature 371: 487–492.

Hawley, S.H.B., K. Wünnenberg-Stampleton, C. Hashimoto, M.N. Laurent, T. Watabe, B. Blumberg, and K.W.Y. Cho. 1995. Disruption of BMP signals in embryonic Xenopus ectoderm leads to direct neural induction. Genes & Dev. 9: 2923–2935.

Heasman, J., A. Crawford, K. Goldstone, P. Garner-Hamrick, B. Gumbiner, P. McCrea, C. Kinner, C. Yoshida Noro, and C. Wylie. 1994. Overexpression of cadherins and underexpression of β-Catenin inhibit dorsal mesoderm induction in early Xenopus embryos. Cell 79: 791–803.

Hemmati-Brivanlou, A. and D.A. Melton. 1992. A truncated activin receptor inhibits mesoderm induction and formation of axial structures in Xenopus embryos. Nature 359: 609–614.

Herrmann, B.G. 1991. Expression pattern of the Brachyury gene in the whole-mount T(Tw)/T(Tw) mutant embryos. Development 113: 913–917.

Izpisua-Belmonte, J.C., E.M. DeRobertis, K.G. Storey, and C.D. Stern. 1993. The homeobox gene goosecoid and the origin of the organizer cells in the early chick blastoderm. Cell 74: 645–659.

Kadonaga, J.T. and R. Tjian. 1986. Affinity purification of sequence-specific DNA binding proteins. Proc. Natl. Acad. Sci. 83: 5889–5893.

Kimelman, D., J.L. Christian, and R.T. Moon. 1992. Synergistic principles of development: Overlapping patterning systems in Xenopus mesoderm induction. Development 116: 1–9.

Ku, M. and D.A. Melton. 1993. Xwnt-11: A maternally expressed Xenopus Wnt gene. Development 119: 1161–1173.

Labonne, C. and M. Whitman. 1994. Mesoderm induction by activin requires FGF-mediated intracellular signals. Development 120: 463–472.

Lemaire, P., N. Garrett, and J.B. Gurdon. 1995. Expression cloning of Siamois, a Xenopus homeobox gene expressed in dorsal vegetal cells of blastulae and able to induce a complete secondary axis. Cell 81: 85–94.

Luckow, B. and G. Schutz. 1987. CAT constructions with multiple unique restriction sites for the functional analysis of eukaryotic promoters and regulatory elements. Nucleic Acids Res. 15: 5490.

Mamatis, T., E.F. Frisch, and J. Sambrook. 1982. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Matzuk, M.M., T.R. Kumar, A. Vassalli, J.R. Bickenbach, D.R. Roop, R. Jaenisch, and A. Bradley. 1995a. Functional analysis of activins during mammalian development. Nature 374: 354–356.

Matzuk, M.M., T.R. Kumar, and A. Bradley. 1995b. Different phenotypes for mice deficient in either activins or activin receptor type-II. Nature 374: 356–360.

Moon, R.T. and J.L. Christian. 1992. Competence modifiers synergize with growth factors during mesoderm induction and patterning in Xenopus. Cell 71: 709–712.

Moon, R.T., R.M. Campbell, J.L. Christian, L.L. McGrew, J. Shih, and S. Fraser. 1993. Xwnt-5A: A maternal Wnt that affects morphogenetic movements after overexpression in embryos of Xenopus laevis. Development 119: 97–111.

Niehrs, C., R. Keller, K.W.Y. Cho, and E.M. DeRobertis. 1993. The homeobox gene goosecoid controls cell migration in Xenopus embryo. Cell 72: 491–503.

Nieuwkoop, P.D. 1969. The formation of the mesoderm in urodele amphibians. I. Induction by the endoderm. Wilhelm Roux’s Arch. Entwicklungsmech. 162: 341–373.

———. 1973. The organization center of the amphibian embryo:

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Its origin, spatial organization, and morphogenetic action. 
Adv. Morphol. 10: 1–39.

Nieuwkoop, P.D. and J. Faber. 1967. A normal table of Xenopus laevis (Daudin). North Holland Publishing Co., Amsterdam, The Netherlands.

Rosa, F.M. 1989. Mix.I, a homeo box mRNA inducible by mesoderm inducers, is expressed mostly in the presumptive endodermal cells of Xenopus embryos. Cell 57: 965–974.

Rothbacher, U., M.N. Laurent, I.L. Blitz, T. Watabe, J.L. Marsh, and K.W.Y. Cho. 1995. Functional conservation of the Wnt signaling pathway revealed by ectopic expression of Drosophila dishevelled in Xenopus. Dev. Biol. 170: 717–721.

Schmidt, J.E., A. Suzuki, N. Ueno, and D. Kimelman. 1995. Localized BMP-4 mediates dorsal/ventral patterning in the early Xenopus embryo. Dev. Biol. 169: 37–50.

Schulte-Merker, S., R.K. Ho, B.G. Herrmann, and C. Nüsslein-Volhard. 1992. The protein product of the zebrafish homologue of the mouse T gene is expressed in nuclei of the germ ring and the notochord of the early embryo. Development 116: 1021–1032.

Smith, J.C. 1993. Mesoderm-inducing factors in early vertebrate development. EMBO J. 12: 4463–4470.

Smith, J.C., B.M. Price, J.B. Green, D. Weigel, and B.G. Herrmann. 1991. Expression of a Xenopus homolog of Brachyury [T] is an immediate-early response to mesoderm induction. Cell 67: 79–87.

Smith, W.C. and R.M. Harland. 1991. Injected Xwnt-8 RNA acts early in Xenopus embryos to promote formation of a vegetal dorsalizing factor. Cell 67: 829–840.

Smith, W.C. and R.M. Harland. 1992. Expression cloning of noggin, a new dorsalizing factor localized to the Spemann organizer in Xenopus embryos. Cell 70: 829–840.

Smith, W.C., R. McKendry, S. Ribisi Jr., and R.M. Harland. 1995. A nodal-related gene defines a physical and functional domain within the Spemann organizer. Cell 82: 37–46.

Sokol, S. and D.A. Melton. 1992. Interaction of Wnt and activin in dorsal mesoderm induction in Xenopus. Dev. Biol. 154: 348–355.

Sokol, S., J.L. Christian, R.T. Moon, and D.A. Melton. 1991. Injected Wnt-8 RNA induces a completed body axis in Xenopus embryos. Cell 67: 741–752.

Spemann, H. and H. Mangold. 1924. Über induktion von embryonalanlagen durch implantation artfremder organisatoren. Wilhelm Roux's Arch. Dev. Biol. 100: 599–638.

Stachel, S.E., D.J. Grunwald, and P.J. Myers. 1993. Lithium perturbation and goosecoid expression identify a dorsal specification pathway in the pregastula zebrafish. Development 117: 1261–1274.

Suzuki, A., R.S. Thies, Y. Yamaji, J.J. Song, I.M. Wozney, K. Murakami, and N. Ueno. 1994. A truncated bone morphogenetic protein receptor affects dorsal-ventral patterning in the early Xenopus embryo. Proc. Natl. Acad. Sci 91: 10255–10259.

Taira, M., M. Jamrich, P.I. Good, and I.B. Dawid. 1992. The LIM domain-containing homeo box gene Xlim-1 is expressed specifically in the organizer region of Xenopus gastrula embryos. Genes & Dev. 6: 356–366.

Tannahill, D. and D.A. Melton. 1989. Localized synthesis of the Vg1 protein during early Xenopus development. Development 106: 775–785.

Thomsen, G. and D.A. Melton. 1993. Processed Vg-1 protein is an axial mesoderm inducer in Xenopus. Cell 74: 433–441.

Thomsen, G., T. Woolf, M. Whitman, S. Sokol, J. Vaughan, W. Vale, and D.A. Melton. 1990. Activins are expressed early in Xenopus embryogenesis and can induce axial mesoderm and anterior structures. Cell 63: 485–493.
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