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A role for Siamois in Spemann organizer formation

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SUMMARY

The vertebrate body plan is specified in the early embryo through the inductive influence of the organizer, a special region that forms on the dorsalmost side of the embryo at the beginning of gastrulation. In *Xenopus*, the homeobox gene *Siamois* is activated prior to gastrulation in the area of organizer activity and is capable of inducing a secondary body axis when ectopically expressed. To elucidate the function of endogeneous Siamois in dorsoventral axis formation, we made a dominant repressor construct (SE) in which the Siamois homeodomain was fused to an active repression domain of *Drosophila* engrailed. Overexpression of 1-5 pg of this chimeric mRNA in the early embryo blocks axis development and inhibits activation of dorsal, but not ventrolateral, marginal zone markers. At similar expression levels, SE proteins with altered DNA-binding specificity do not have the same effect. Coexpression of mRNA encoding wild-type *Siamois*, but not a mutated *Siamois*, restores dorsal development to SE embryos. Furthermore, SE strongly blocks axis formation triggered by β-catenin but not by the organizer product noggin. These results suggest that Siamois function is essential for β-catenin-mediated formation of the Spemann organizer, and that Siamois acts prior to noggin in specifying dorsal development.

Key words: *Xenopus laevis*, dorsoventral axis, mesoderm, β-catenin, *noggin*, *goosecoid* promoter, *engrailed*, transcriptional repression, homeodomain

INTRODUCTION

The concept of the organizer as the main signaling center that establishes the vertebrate body plan was initially developed by Hans Spemann (reviewed by Hamburger, 1988). In amphibians, the Spemann organizer is marked at the beginning of gastrulation by formation of the dorsal blastopore lip. Tissue from this region is largely fated to become anterior (head) and dorsal (notochord) mesoderm. When these organizer cells are transplanted to the ventral side of an embryo, they are capable of inducing or 'organizing' a secondary body axis with proper dorsoventral and anteroposterior pattern. Cell populations that are functionally equivalent to the Spemann organizer exist in other vertebrates, i.e. the shield in zebrafish and the node in mice.

The organizer has several distinct morphogenetic activities. It is responsible for induction of the neural plate in the dorsal ectoderm, and it specifies heart and muscle tissue by dorsalizing mesoderm (Sater and Jacobson, 1990; reviewed by Slack, 1991). It may also be involved in endodermal development (Sasai et al., 1996). Additionally, the organizer controls morphogenetic movements of cells during gastrulation and imparts anteroposterior pattern to the body axis (reviewed by Gerhart et al., 1991). The complexity of the organizer has begun to be unraveled in recent years through the identification and study of numerous organizer-specific genes. For example, gene inactivation techniques in the mouse reveal a requirement for *Lim1* (Shawlot and Behringer, 1995) and *Otx2* (Matsuo et al., 1995; Acampora et al., 1995; Ang et al. 1996) in head formation. Mice lacking *nodal* or *HNF-3β* function (Zhou et al., 1993; Ang and Rossant, 1994; Weinstein et al., 1994) and the *floating head* mutants of zebrafish (Talbot et al., 1995) have severe defects primarily in notochord, the dorsalmost mesoderm. In *Xenopus*, several organizer-specific genes, including *noggin* (Smith and Harland, 1992), *chordin* (Sasai et al., 1994), *cerberus* (Bouwmeester, 1996) and the nodal-related genes, *Xnr1*, *Xnr2* and *Xnr3* (Jones et al., 1995; Smith et al., 1995), encode proteins secreted by the organizer. However, despite progress in identifying molecules that are capable of mediating organizer activities, much less is known about the factors that give rise to the organizer itself.

An early signaling molecule implicated in organizer formation is the maternal product of β-catenin. β-catenin acts both in cell adhesion and in the Wnt/wingless signaling pathway (reviewed by Miller and Moon, 1996). Overexpression of different putative members of the Wnt/wingless signal transduction pathway in early *Xenopus* embryos is known to trigger organizer formation, thus mimicking the initial event leading to determination of dorsoventral polarity in the embryo (Pierce and Kimelman, 1995; He et al., 1995; Dominguez et al., 1995; Sokol et al., 1995; Guger and Gumbiner, 1995; Karnovsky and Klymkowsky, 1995). Depletion of maternal β-catenin by antisense oligonucleotides leads to inhibition of dorsal mesoderm formation and results in axis-deficient embryos (Heasman et al., 1994), indicating that maternal β-catenin plays a key role in establishment of the dorsoventral axis and in Spemann organizer formation. Recent evidence

suggests that β-catenin translocates to the nucleus and thus may directly regulate organizer gene expression (Molenaar et al., 1996; Behrens et al., 1996; Huber et al., 1996). However, it is also possible that maternal β-catenin acts in cells that do not give rise to the organizer directly (reviewed by Miller and Moon, 1996; Gerhart et al., 1989).

The paired-class homeobox gene *Siamois* is one of the earliest transcription factors to be expressed after the onset of zygotic transcription at the midblastula stage. Its transcripts accumulate in the dorsovegetal region of the embryo (Lemaire et al., 1995), an area with strong organizer-inducing activity (Gerhart et al., 1991). Interestingly, *Siamois* mRNA has been shown to induce a complete secondary body axis upon overexpression (Lemaire et al., 1995), indicating that it is capable of generating an ectopic organizer. These observations make Siamois an attractive candidate for a zygotic intermediary between early maternal signaling and Spemann organizer formation.

In this study, we investigate the function of Siamois by utilizing a dominant repressor construct in which the Siamois homeodomain is fused to an active repression domain of *Drosophila* engrailed. The N-terminal domain of engrailed is spatially distinct from its homeodomain and has been shown to repress transcription when attached to heterologous DNA-binding domains (Jaynes and O'Farrell, 1991; Han and Manley, 1993). Active transcriptional repression appears to be a more effective method of interfering with gene activation than mere competition for DNA-binding sites. This technique has been successfully used to block the function of several transcription factors such as fushi tarazu (ftz; Jaynes and O'Farrell, 1991; John et al., 1995), c-Myb (Badiani et al., 1994), Xbra (Conlon et al., 1996) and Eomesodermin (Ryan et al., 1996). The Siamois-engrailed recombinant product was therefore expected to interfere with transcription of Siamois target genes.

Embryos overexpressing this dominant transcriptional repressor fail to develop dorsal structures. Analysis of the deficient embryos at the gastrula stage reveals inhibition of dorsal, but not ventrolateral, marginal zone markers. Blocking Siamois function abolishes the axis-inducing ability of β-catenin, but it does not significantly alter similar activity of the Spemann organizer product noggin. Our data suggest that Siamois is essential for organizer formation in the embryo.

MATERIALS AND METHODS

DNA constructs and in vitro transcription

Templates for in vitro RNA synthesis were derived from DNA constructs encoding Siamois (Lemaire et al., 1995), β-catenin (McCrea et al., 1991) and noggin (Smith and Harland, 1992). To generate the dominant repressor construct, we used pTβen, which contains *Drosophila engrailed* cDNA (a gift of J. Jaynes). A *Bgl*II-digested 230 bp DNA fragment corresponding to amino acids 142-219 of Siamois (Lemaire et al., 1995) was cloned in frame into *Bam*HI-digested pTβen to yield Sia-pTβen. Cloning was verified by sequencing on a 373A DNA sequencer (Applied Biosystems, Inc.). The *Hin*dIII/*Eco*RI insert of Sia-pTβen, encoding the first 296 amino acids of engrailed followed by the Siamois homeodomain, was subcloned into pXT7 plasmid (Dominguez et al., 1995) to yield SE-pXT7. For the ΔEn-pXT7 construct, *Bam*HI-digested pTβen was self-ligated and its *Hin*dIII/*Eco*RI insert was subcloned into pXT7 vector.

Lysine or glutamic acid was substituted for glutamine in position 50 of the Siamois homeodomain by PCR overlap extension (Horton et al., 1990). SE-pXT7 was used as a PCR template with the following sets of oligonucleotide primers (myc tag is underlined, point mutation in bold): (1) A: 5′-GGTTTGGTTT**A**AGAACAGAAG-3′ and B: 5′-GCGAATTCAGATCCTCTTCAGAGATGAGTTTCTGTTC-GAGCTCGCCCGGG-3′ and (2) C: 5′-CTTCTGTTCTT**T**AAAC-CAAACC-3′ and the T7 primer (Promega) for the glutamine-to-lysine substitution; and (1) D: 5′-GGTTTGGTTT**G**AGAACAGAAG-3′ and primer C and (2) E: 5′-CTTCTGTTCT**C**AAACCAAACC-3′ and the T7 primer (Promega) for the glutamine to glutamic acid substitution. The resulting PCR products were digested with *Not*I/*Eco*RI and subcloned into *Not*I/*Eco*RI-digested SE-pXT7 to generate the SEQ>K-pXT7 or SEQ>E-pXT7 constructs encoding single amino acid substitutions. Mutagenesis was verified by sequencing.

The SiaQ>K-pXT7 mutant containing a glutamine-to-lysine substition at position 50 of the homeodomain was generated by PCR overlap extension (Horton et al., 1990) using Siamois-pRN3 (Lemaire et al., 1995) as a template and the following set of primers (point mutation in bold): (1) primers A and F: 5′-GACTCGA-GAATTCAGTTTGGGTAGGGCT-3′ and (2) primers C and G: 5′-TCTAGAGGATCCCATGACCTATGAGGCTG-3′. The final PCR product was digested with *Bam*HI/*Xho*I and ligated together with an *Xho*I/*Pst*I fragment of pXT7 into *Bgl*II/*Pst*I digested pXT7 to yield SiaQ>K-pXT7. A myc-tagged version of SE was generated by PCR with primers G and B, and the product was digested with *Not*I/*Eco*RI and subcloned into *Not*I/*Eco*RI-digested SE-pXT7. Mutagenesis was verified by sequencing.

Capped synthetic mRNAs for microinjections were synthesized in vitro using the Megascript RNA transcription system (Ambion). For template preparation, SE-pXT7, SEQ>K-pXT7, SEQ>E-pXT7 and Sia Q>K-pXT7 plasmids were linearized with *Bam*HI, and ΔEn-pXT7 was linearized with *Xba*I. Amount and quality of synthesized mRNAs were determined by comparison with an mRNA of known concentration on a denaturing 1% agarose gel.

Xenopus embryos and microinjections

Eggs were obtained from female *Xenopus laevis* after injection with 800 units of human chorionic gonadotropin. In vitro fertilization and embryo culture were performed as described (Newport and Kirschner, 1982). Staging was according to Nieuwkoop and Faber (1967).

For microinjection, embryos were transferred to 3% Ficoll, 0.5× MMR (1× MMR: 100 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM Hepes, pH 7.4; Newport and Kirschner, 1982). Normal embryos were injected at the 4- to 8-cell stage with 10 nl of a solution containing approximately 5 pg of mRNA (unless noted otherwise) in the subequatorial region of both dorsal blastomeres. At stage 9, the injected embryos were transferred to 0.1× MMR with 50 μg/ml of gentamicin for long-term culture. Death rate for the injected embryos was usually below 5%. The prospective dorsal and ventral sides were determined by pigmentation differences in the early embryo (Nieuwkoop and Faber, 1967).

In some experiments, embryos were irradiated with short wavelength ultraviolet light (UV) for 63 seconds at 30 minutes postfertilization. The effect of UV irradiation was assessed by the dorsoanterior index (DAI; Kao and Elinson, 1988). The time of irradiation was determined empirically in preliminary experiments to generate DAI scores of 0-1 in more than 80% of treated embryos. UV-treated embryos were injected subequatorially at the 4- to 8-cell stage with 30 pg of β-catenin mRNA, 5 pg of *noggin* mRNA, or with a combination of either mRNA with 5 pg of SE mRNA in a total volume of 10 nl per embryo.

RNA isolation and northern analysis

Total mRNA was extracted from stage 10.5 embryos as described (Itoh et al., 1995) and separated in a 1% formaldehyde-agarose gel using standard techniques (Sambrook et al., 1989). RNA from 2.5 embryos was loaded per lane. RNA was transferred to a GeneScreen

nylon membrane (Dupont) with 20× SSPE and was subsequently hybridized with radiolabeled DNA or RNA probes (Sambrook et al., 1989). ^{32}P-labeled antisense RNA probes were prepared by in vitro transcription of plasmids encoding fibronectin (Krieg and Melton, 1985), Xlim1 (Taira et al., 1992), goosecoid (Blumberg et al., 1991), chordin (Sasai et al., 1994) and Xwnt8 (Christian et al., 1991) with SP6, T3 or T7 RNA polymerases. A ^{32}P-DNA probe for *Xbra* (Smith et al., 1991) and was generated using Klenow enzyme and random hexamer primers (Sambrook et al., 1989). After each hybridization cycle, the membrane was stripped by boiling in distilled water. The same membrane was used for hybridization with all probes.

Western blotting

Embryos were injected in all four blastomeres at the 4-cell stage with myc-tagged versions of SE, SEQ>K, or SEQ>E mRNAs and cultured until stage 11, when they were lysed at 4°C in buffer containing 1% Triton X-100, 1 mM EDTA, 0.05 mM Tris-HCl (pH 7.5), 0.05 M NaCl. One-fifth embryo equivalent per sample was electrophoresed on a 10% SDS-polyacrylamide gel and electroblotted onto polyvinylidene difluoride (PVDF) membrane (Millipore) at 100 V for 1 hour. Membranes were blocked with 5% skim milk for $1\frac{1}{2}$ hours and incubated with 9E10 monoclonal antibody (gift of F. McKeon, Harvard Medical School) for $1\frac{1}{2}$ hours. After washing four times in PBS/0.05% Tween 20, they were incubated with horseradish peroxidase-conjugated goat anti-mouse IgG antibodies/10% goat serum (Jackson ImmunoResearch Labs) for 1 hour. Membranes were then washed four times in PBS/0.05% Tween 20. Peroxidase activity was revealed after a one-minute incubation in a substrate solution (1.25 mM luminol, 0.2 mM paracoumaric acid, 0.03% H_2O_2, 100 mM Tris-HCl, pH 8.25) followed by autoradiography.

Luciferase assays

Embryos were microinjected into each ventral blastomere at the 4-cell stage with 25 pg of –155gsc/Luc plasmid DNA (Watabe et el., 1995), alone or in combination with 5 pg of *Siamois*, 10 pg of SE, or 10 pg of SEQ>E mRNA. At stage 10+, five embryos were homogenized in 100 μl of 50 mM Tris-HCl (pH 7.5) and centrifuged for 5 minutes at 14,000 revs/minute at 4°C. To measure luciferase activity, 50 μl of the supernatant were added to 50 μl of 50 mM Tris-HCl (pH 7.5), and then 300 μl of assay buffer was added per sample, for final concentrations of 33 mM Gly-Gly (pH 7.8), 15 mM potassium phosphate buffer (pH 7.5), 20 mM $MgSO_4$, 5 mM EGTA, 1 mM of DTT, 2.7 mM ATP and 0.25% Triton X-100. Following injection of 100 μl of 1 mM luciferin, luciferase activity was measured in a Monolight 2010 luminometer (Analytical Luminescence Laboratory). Each experiment utilized embryos from the same fertilization batch.

Histology

Embryos of the desired stage were fixed for 3 hours at room temperature in MEMFA (3.7% formaldehyde, 0.1 M MOPS, 2 mM EGTA, 1 mM $MgSO_4$, pH 7.4; Hemmati-Brivanlou and Harland, 1989). Fixed embryos were dehydrated, embedded in Paraplast and sectioned at 8 μm on a rotary microtome. Sections were stained with hematoxylin/eosin (Sigma) according to the manufacturer's protocol.

RESULTS

The dominant repressor SE inhibits dorsal development

To evaluate the in vivo role of Siamois in dorsoventral axis formation, we made a construct encoding a chimeric protein (SE) in which the Siamois homeodomain is fused to the active repression domain of *Drosophila* engrailed (Fig.1). In the initial set of experiments, we tested whether SE inhibits axis-inducing activity of *Siamois* mRNA. Whereas 5 pg of *Siamois* mRNA induced ectopic secondary axes in 89% of ventrally injected embryos (*n*=27), this activity was completely blocked by coinjection of 5 pg of SE mRNA (*n*=31). This suggests that SE behaves in a dominant negative manner.

To investigate a potential role for endogenous Siamois in early development, SE mRNA was microinjected into different blastomeres of 4- to 8-cell stage embryos. Whereas overexpression of SE in ventral blastomeres does not significantly interfere with embryogenesis, injections into the dorsovegetal region where endogenous *Siamois* is expressed lead to axis deficiencies (Fig. 2). At the beginning of gastrulation, the dorsal blastopore lip is not detectable in embryos that were dorsally injected with 5 pg of SE mRNA (Fig. 2A). A blastopore eventually forms and closes in the majority of SE-injected embryos. At later stages, the injected embryos fail to develop a neural tube as compared with uninjected controls (Fig. 2B,C). By tailbud stages, the majority of embryos injected with SE mRNA exhibit a severely ventralized phenotype with occasional remnants of dorsal fin (Table 1; Fig. 2D). Doses as low as 1-2 pg of SE mRNA are still effective at ventralizing embryos; higher doses result in a complete lack of dorsal structures. This phenotype is similar to that of embryos lacking maternal β-catenin (Heasman et al., 1994) or rendered dorsal-deficient by ultraviolet (UV) irradiation (Gerhart et al., 1989).

Histological sections of SE-injected embryos reveal the presence of three tissue layers, but no dorsal structures such as notochord, muscle or neural tube are apparent (Fig. 2F). Control uninjected embryos and embryos injected dorsally with 5 pg of ΔEn mRNA encoding the engrailed repressor

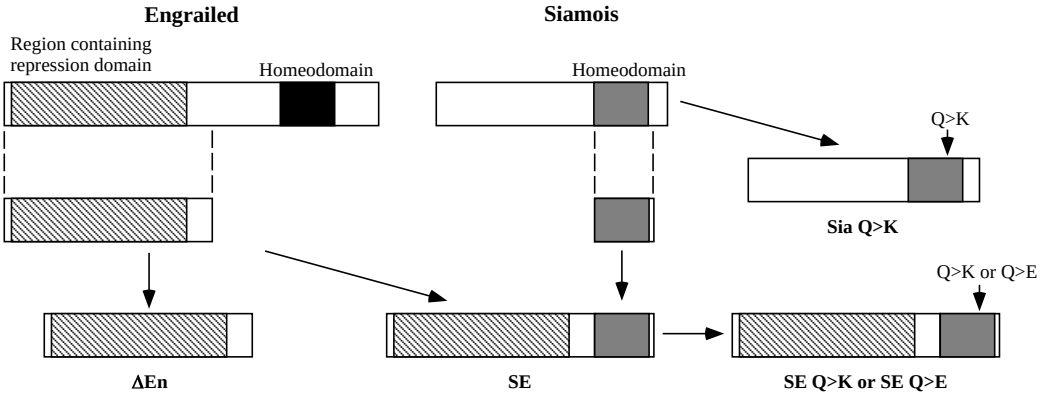

Fig. 1. Constructs used in this study. An engrailed repression domain contained within the first 296 amino acids was fused with the Siamois homeodomain to generate SE. The control construct (ΔEn) contains only the engrailed part of the fusion. The constructs SEQ>K and SEQ>E contain a glutamine-to-lysine (Q>K) or glutamine to glutamic acid (Q>E) mutation at position 50 of the homeodomain. Sia Q>K is identical to wild-type Siamois except for a glutamine-to-lysine substitution at position 50 of the homeodomain.

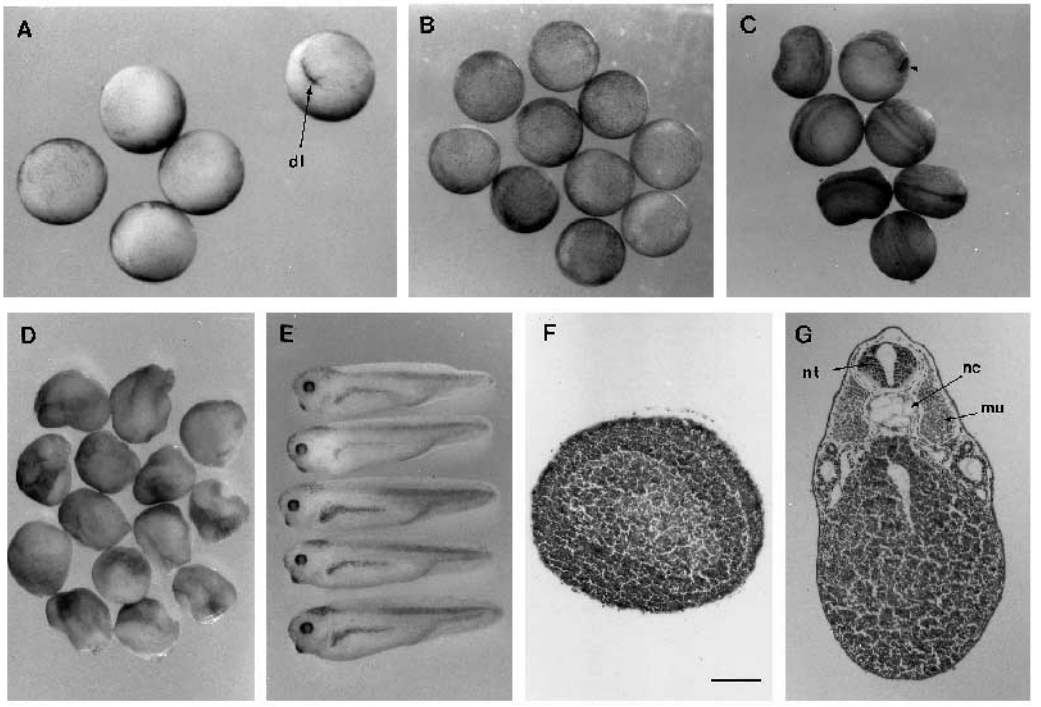

Fig. 2. Siamois function is required for dorsal development. Embryos were injected with 5 pg of SE mRNA (A,B,D,F), 5 pg of SEQ>E mRNA (E) or 5 pg of ΔEn mRNA (G) into both dorsal blastomeres at the 4-cell stage. (A) Early gastrulae (stage 10+). Uninjected control embryo is at top right. (B) Lack of neural tube and head structures in SE embryos at stage 21-22. (C) Uninjected stage 21-22 neurulae. Arrowhead points to the cement gland. (D) Ventralized phenotype of SE embryos at the equivalent of stage 36. (E) Embryos injected with SEQ>E mRNA are indistinguishable from uninjected and ΔEn mRNA-injected controls. (F,G) Cross-sections of injected embryos equivalent to normal embryos at stage 36**.** Bar in F, also refers to G, is 250 μm. Abbreviations: dl, dorsal lip; nt, neural tube; nc, notochord; mu, muscle

domain alone (Fig. 1) do not show any significant developmental abnormalities (Fig. 2C,G). These results demonstrate that overexpression of SE in dorsal blastomeres inhibits axial development and that this inhibition is not likely to be due to nonspecific effects of the engrailed repressor domain.

Spemann organizer markers are blocked by SE

To evaluate the effect of SE at the molecular level, the expression of early marginal zone markers was analyzed in SE-injected embryos at stage 10.5. Inhibition of several organizer genes, *Xlim-1*, *chordin* and *goosecoid*, is observed in embryos overexpressing SE dorsally (Fig. 3). In contrast, transcripts for the ventrolateral marker *Xwnt8* may be slightly upregulated, consistent with ventralization of the normal dorsal side. The small decrease in the pan-mesodermal marker *Xbra* most likely reflects a reduction in the dorsal component of its expression (Smith et al., 1991). Marginal zone markers in embryos injected with SEQ>E (see below) or ΔEn are not different from those in uninjected controls.

Morphological and molecular analysis of embryos injected with SE suggests that Siamois may act prior to transcriptional activation of *Xlim-1*, *chordin* and *goosecoid*, and that its function is essential for induction of the Spemann organizer.

The effect of the repressor construct depends on Siamois homeodomain-binding specificity

The third helix and N-terminal arm of the homeodomain are essential for its binding to DNA (reviewed by Mann, 1995). In particular, altering the amino acid in position 50 of the homeodomain can change in vitro binding specificity from one class of homeodomains to another (Hanes and Brent, 1989; Treisman et al., 1989). To address whether the effect of SE is due to nonspecific binding of DNA, we generated two different amino acid substitutions in the Siamois homeodomain (Fig. 1). In the first mutant SEQ>K, glutamine in position 50 is replaced by lysine. Lysine is present in this position in other paired class members such as goosecoid and Otx2 (Wilson et al., 1993). Dorsal injections of the SEQ>K mutant are not as effective at ventralizing embryos, requiring 5- to 10-fold higher levels of protein compared to SE (Table 2 and data not shown). The weaker activity of SEQ>K is consistent with it having lower affinity for SE-recognized target sites in vivo (see Discussion).

Table 1. Effects of SE on dorsal development

mRNA injected	Ventralized DAI 0-1	Partial Axis DAI 2-3	Complete Axis DAI 4-5	Other	Average DAI	Total
SE	109 (82%)	14 (11%)	7 (5%)	2 (2%)	1.2	132
ΔEn	0	0	59 (92%)	5 (8%)	4.9	64
SEQ>E	0	1 (2%)	51 (89%)	5 (9%)	4.9	57
Uninjected	0	1 (1%)	118 (94%)	7 (5%)	5.0	126

Embryos were injected with either 5 pg of SE mRNA, 5 pg of ΔEn mRNA or 5 pg of SEQ>E mRNA in both dorsal blastomeres at the 4- to 8- cell stage. They were scored for axis development at stage 34-35 and categorized as ventralized (no axis or dorsal fin remnant), partial axis (absent or poorly developed head), complete axis (slightly reduced anterior structures or normal), or other (cell division arrest or nonspecific gastrulation defects). Corresponding dorsoanterior index (DAI; Kao and Elinson, 1988) scores are shown. Embryos categorized as 'other' were not assigned a DAI score. Results from six experiments are combined.

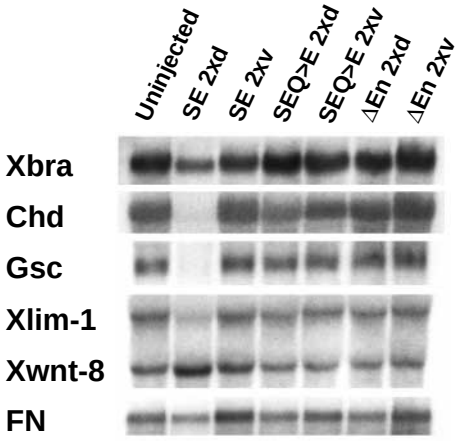

Fig. 3. SE overexpression leads to a loss of dorsal marginal zone markers. Embryos at the 4- to 8-cell stage were injected with a total of 20-30 pg of mRNA as indicated and cultured until stage 10.5 for northern analysis. Total RNA from 2.5 embryos was loaded per lane. Fibronectin is a loading control.

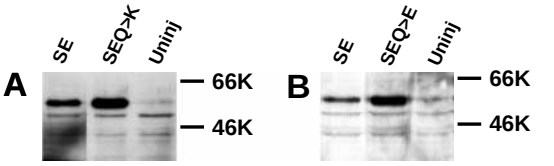

Fig. 4. SE constructs are well-expressed in injected embryos. Embryos were injected equatorially in each blastomere at the 4-cell stage with (A) 5 pg of SE or 5 pg of SEQ>K mRNA, and (B) 10 pg of SE or 5 pg of SEQ>E. Lysates were prepared at stage 11 for western analysis with 9E10 myc-specific monoclonal antibodies.

The second mutant SEQ>E contains a glutamine to glutamic acid substitution at position 50. Glutamic acid is not present in this position in any known homeodomain (Pannese et al., 1995). Dorsal injections of mRNA encoding this mutation yield embryos indistinguishable from normal controls (Fig. 2E; Table 1). The phenotypes of SEQ>E and SEQ>K embryos are not due to low protein levels, as both products are effectively expressed in gastrula stage embryos (Fig. 4). Furthermore, dorsal overexpression of a construct with the Otx2 homeodomain fused to the engrailed repression domain produces head abnormalities distinct from the ventralization phenotype of SE (data not shown). Together, these findings argue that the effect of SE is specific and is not likely to be due to indiscriminate homeodomain binding to DNA.

Wild-type *Siamois* mRNA rescues the SE phenotype

If SE is indeed only interfering with the endogenous target genes of *Siamois*, coexpression of wild-type *Siamois* is expected to correct the developmental abnormalities seen with dorsal injections of SE mRNA. In agreement with this expectation, dorsal coinjection of *Siamois* mRNA with SE mRNA results in complete or partial restoration of the dorsal axis in 51% of injected embryos (n=104, Fig. 5). In contrast, only 6% of embryos (n=98) that are dorsally injected with SE mRNA alone achieve the same degree of axial development. A mutant Siamois with a glutamine-to-lysine substitution (SiaQ>K, Fig. 1), fails to rescue the SE phenotype at equivalent doses (Fig. 5). This indicates that the restoration of dorsal development by wild-type Siamois is specific. The ability of *Siamois* to abrogate the effect of SE argues against SE-mediated repression of genes other than *Siamois* target genes.

SE blocks Siamois-mediated activation of the *goosecoid* promoter

goosecoid is an organizer-specific gene (Blumberg et al., 1990) which is thought to be activated synergistically by mesoderm-inducing factors and Wnt-like competence modifiers (Sokol and Melton, 1992; Watabe et al., 1995). It has previously been demonstrated that the −155 to −105 region of the *goosecoid* promoter corresponds to an element (named PE, proximal element) which is sufficient to mediate Wnt responses (Watabe et al., 1995). To examine whether Siamois can activate transcription of this organizer-specific gene, we utilized the −155gsc/Luc construct in which a portion of the *goosecoid* promoter containing PE is linked to the luciferase reporter gene (Watabe et al., 1995). Ventral injection of *Siamois* mRNA and the −155gsc/Luc plasmid results in 15-fold increase in luciferase activity (Fig. 6), indicating that Siamois can lead to transcriptional activation from the *goosecoid* PE-containing promoter. Coinjection of SE mRNA leads to a sharp reduction in the level of Siamois-induced luciferase activity (Fig. 6). At higher ratios of SE to *Siamois* mRNA, this reduction more closely approaches basal levels (data not shown). Siamois-induced luciferase activity is not affected by the mutant SEQ>E (Fig. 6). These results are consistent with specific inhibition of Siamois target genes by SE and further support the idea that Siamois is required for induction of organizer genes in vivo.

Differential effects of SE on the rescue of dorsoventral axis formation in ventralized embryos

These experiments indicate that Siamois is required for dorsoventral axis formation in the embryo. To investigate the possible relationship between Siamois and other gene products implicated in dorsoventral axis determination, we tested the ability of SE to interfere with the axis-rescuing ability of β-

Table 2. Comparison of the effects of SE and SEQ>K

mRNA injected	Ventralized DAI 0-1	Partial Axis DAI 2-3	Complete Axis DAI 4-5	Average DAI	Total
SE	17 (85%)	3 (15%)	0	1.1	20
SEQ>K	3 (14%)	6 (27%)	13 (59%)	3.5	22
Uninjected	0	0	14 (100%)	5.0	14

Embryos were injected in both dorsal blastomeres at the 4- to 8-cell stage and scored for axis development at stage 34-35 (see Table 1 for criteria). The injected embryos expressed similar levels of SE and SEQ>K proteins (data not shown and Fig. 4A).

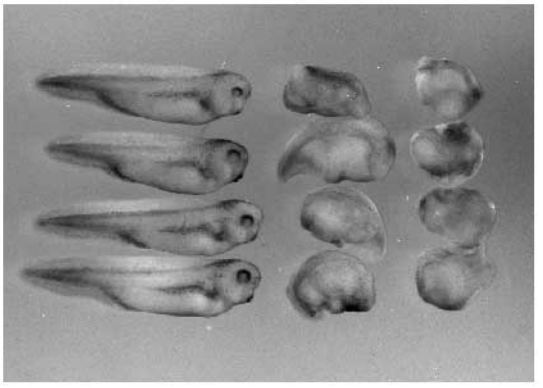

Fig. 5. Wild-type *Siamois* mRNA corrects dorsal deficiences of SE embryos. Embryos were injected in both dorsal blastomeres at the 4-cell stage with either (center) 5 pg of SE mRNA alone or with a mixture of (left) 5 pg of SE and 10 pg *Siamois* mRNAs, or (right) 5 pg of SE and 10 pg of Sia Q>K mRNAs. Injections of SE mRNA alone resulted in ventralized embryos indistinguishable from those in Fig. 2D.

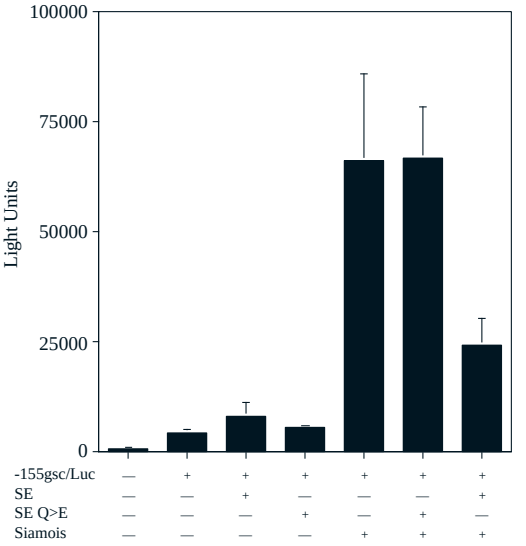

Fig. 6. SE inhibits Siamois-mediated activation of a *goosecoid* promoter element. A representative experiment is shown. Embryos were microinjected in both ventral blastomeres at the 4-cell stage with −155gsc/Luc plasmid DNA (Watabe et al., 1995), either alone or in combination with indicated mRNAs. For luciferase activity measurements, lysates were prepared from embryos at stage 10+. Data are expressed as the means from triplicate samples ± the standard deviations.

catenin and *noggin* mRNAs in embryos rendered axis-deficient by UV irradiation (Smith and Harland, 1992; Guger and Gumbiner, 1995). If Siamois functions downstream of β-catenin during normal development, then SE should block the ability of β-catenin to induce an axis in UV-treated embryos. Additionally, as Siamois appears to be necessary for organizer formation, SE is not expected to interfere with activity of noggin, a zygotic product of the organizer.

Injection of 30 pg of β-catenin mRNA restores body axes in 87% of UV-treated embryos (*n*=69), whereas coinjection with SE leads to a significant reduction of this number (Table 3; Fig. 7A-C). Induction of secondary axes by β-catenin upon ventral injection of normal embryos is also inhibited by SE (data not shown). Since interference with Siamois function blocks β-catenin's ability to induce an axis, Siamois appears to be necessary for β-catenin-mediated induction of the organizer.

The ability of the dominant repressor to interfere with noggin-induced axis formation was also evaluated. Under our experimental conditions, noggin seems to be less effective in axis induction than β-catenin, with the average DAI rarely exceeding 3-3.5 (Table 3; Fig. 7D). Injection of 5 pg SE mRNA does not affect the ability of 5 pg *noggin* mRNA to rescue UV embryos (Table 3; Fig. 7D,E). Similarly, ventral injections of normal embryos with *noggin* mRNA result in ectopic partial secondary axes (up to the hindbrain level) which are not significantly affected by SE mRNA (data not shown). Thus our observations suggest that Siamois is not required for the axis-inducing activity of noggin. Secondary axis induction by another organizer product, chordin, also does not appear to be affected by SE (data not shown).

Axes induced by *noggin* mRNA in these experiments infrequently include the most anterior derivatives such as eyes and cement gland, and coinjection of SE mRNA appears to suppress these anterior structures. Importantly, axial structures posterior to the hindbrain level are not inhibited. Induction of anterior structures by noggin may reflect its properties as a neural inducer (Lamb et al., 1993) rather than its role in dorsoventral axis formation. These observations raise the possiblity that Siamois may participate in neural development in addition to axis induction.

Together these findings support a model for organizer formation in which Siamois acts downstream of β-catenin-mediated signaling and prior to the function of organizer-specific factors such as noggin.

Table 3. SE blocks axis rescue by β-catenin but not noggin in UV embryos

mRNA injected	Ventralized DAI 0-1	Partial or Complete Axis DAI 2-5	Other	Average DAI	Total
β-catenin	1 (1%)	60 (87%)	8 (12%)	2.6	69
β-catenin+SE	54 (78%)	14 (20%)	1 (2%)	0.7	69
noggin	6 (6%)	76 (82%)	11 (12%)	2.4	93
noggin+SE	5 (5%)	67 (72%)	21 (23%)	2.7	93
None	121 (83%)	24 (17%)	0	0.6	145

Ultraviolet (UV)-irradiated embryos were injected with either 30 pg of β-catenin mRNA or 5 pg of *noggin* mRNA, with or without coinjection of 5 pg of SE mRNA, in one ventral blastomere at the 4- to 8-cell stage. Embryos were scored at stage 34-35 for axis development (see Table 1 for criteria). Normal control embryos had an average DAI of 5.0. Results from five experiments are combined.

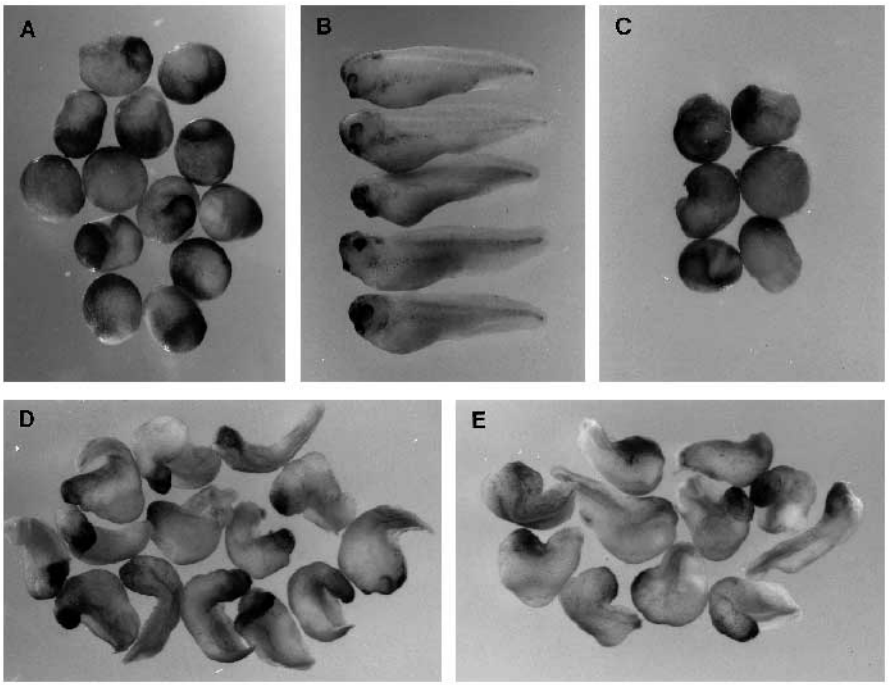

Fig. 7. SE blocks the axis rescuing activity of β-catenin, but not noggin. UV-treated embryos were microinjected subequatorially at the 4- to 8-cell stage with 30 pg of β-catenin mRNA (B,C) or 5 pg of *noggin* mRNA (D,E), alone or in combination with 5 pg of SE mRNA (C,E). (A) Uninjected UV-treated embryos.

DISCUSSION

In this study, we show that inhibition of endogenous Siamois function by the repressor construct SE results in severe deficiencies of dorsal development and in a loss of organizer-specific gene expression, indicating that Siamois is required for Spemann organizer formation in vivo. Siamois activity is also essential for the axis-inducing activity of β-catenin, but not that of noggin. The formation of the organizer is a gradual process that involves both maternal and zygotic gene products. Siamois appears to function as an intermediary between maternal factors such as β-catenin and zygotic signals produced by the organizer.

Specificity of target gene repression by SE is indicated by several lines of evidence. The engrailed repressor alone and the SEQ>E mutant do not alter normal development. A similarly constructed Otx2-engrailed chimeric repressor leads to a phenotype distinct from that of SE (data not shown). Together with the ability of wild-type Siamois to rescue the effect of SE, these experiments argue that the SE repressor has specific DNA-binding activity in vivo. SE is effective at extremely low levels, making it unlikely that it interferes with non-Siamois target genes.

The SEQ>K mutant apparently retains low affinity binding to SE target genes, as is evidenced by its ventralizing activity at high protein levels. The weak effect of SEQ>K is consistent with results from ventral injections of the SiaQ>K mutant, which induces only rudimentary axial derivatives even at high doses (data not shown). In contrast, comparable doses of wild-type Siamois generate well-developed secondary axes in almost all embryos injected ventrally (data not shown). Moreover, a similar glutamine-to-lysine (Q>K) mutation in position 50 of the ftz homeodomain results in a protein that still recognizes wild-type ftz-binding sites in vivo, albeit at a lower affinity, and does not activate bicoid-binding sites that are recognized by the Q>K mutant in vitro (Schier and Gehring, 1993). Thus, based on our work and that of others (Schier and Gehring, 1993), residue 50 cannot fully account for the functional specificity of homeoproteins in vivo.

The SE construct may repress gene transcription by interfering with the function or formation of the basal transcriptional machinery, by associating with the basal machinery and preventing productive interactions with all activator proteins, or by inhibiting activation from other specific promoter-bound factors (reviewed in Hanna-Rose and Hansen, 1996). As the mechanism of engrailed repression is not fully understood, alternative approaches such as genetic knockout experiments are necessary to confirm and extend our conclusions based on the activity of the SE repressor.

To date, it has not been demonstrated whether Siamois is a transcriptional activator or repressor. Our findings that SE inhibits the ability of Siamois to induce an ectopic axis and to activate the *goosecoid* promoter suggest that SE and Siamois have counteracting effects. This observation argues that the activity of SE on dorsal development is unlikely to be due to further inhibition of genes already repressed by endogenous Siamois. A higher dose of Siamois corrects dorsal deficiencies in SE-repressed embryos, which is also consistent with Siamois functioning as a transcriptional activator. Formal proof that Siamois is a transcriptional activator will require direct binding and gene activation studies.

It is possible that Siamois directly activates the *goosecoid* promoter, as *Siamois* and *goosecoid* are expressed in the same region of the embryo at gastrula stages (Lemaire and Kodjabachian, 1996). Alternatively, Siamois could modulate the transcription of other genes, which may then affect *goosecoid* expression. We do not see much effect of the SE construct on basal transcription levels of the *goosecoid* promoter (Fig. 6), which is consistent with an indirect effect of Siamois on *goosecoid* transcription.

Evidence is mounting that β-catenin-mediated signaling may directly affect gene expression. Studies have shown that β-catenin can associate with the high mobility group (HMG) box transcription factors XTcf-3 and LEF-1 (Molenaar et al., 1996; Behrens et al., 1996; Huber et al., 1996). These interactions appear to be important for dorsoventral axis formation, since dominant negative forms of these transcription factors cause ventralization (Molenaar et al., 1996; Behrens et al., 1996). Furthermore, LEF-1 and XTcf-3 are capable of translocating cytosolic β-catenin into the nucleus. In fact, endogenous β-catenin is preferentially accumulated in the nuclei on the dorsal side of the embryo by the onset of zygotic transcription (Schneider et al.,1996). Together these findings suggest that β-catenin may directly regulate transcription of the earliest genes expressed after the midblastula transition. *Siamois* mRNA accumulates rapidly by stage 8.5 (Lemaire et al., 1995), raising the possibility that Siamois is a direct downstream target of β-catenin-mediated signal transduction. This idea is supported by the fact that β-catenin activates *Siamois* expression in dissociated animal blastomeres (Brannon and Kimelman, 1996). Additionally, many putative members of the Wnt pathway (Xwnt-8, rat frizzled 1, dominant negative GSK-3β, APC and β-catenin) can induce *Siamois* in animal cap assays (Yang-Snyder et al., 1996; Brannon and Kimelman, 1996; Vleminckx et al., 1997; Fagotto et al., 1997).

Consistent with the idea that Siamois functions downstream of β-catenin, Siamois can induce an axis in embryos in which β-catenin signaling has been inhibited (Wylie et al., 1996; Fagotto et al., 1997). Carnac et al. (1996) have shown that Siamois is able to activate transcription of *noggin*, *chordin*, *goosecoid* and *Xnr3* in animal caps, consistent with a model whereby Siamois functions to establish an organizer. Interestingly, *Xlim-1* and *Xbra* are not induced by Siamois (Carnac et al., 1996), suggesting that it is not solely responsible for activation of all organizer genes. Our data demonstrate that Siamois is required for expression of all tested dorsal marginal zone markers including Xlim-1 and, to a lesser extent, Xbra. Together, these studies indicate that Siamois is necessary but not sufficient for dorsoventral axis formation. Other factors present in the marginal zone are likely to cooperate with Siamois to establish full spatial and functional complexity of the organizer. Further studies are needed to evaluate the requirement for Siamois in signaling pathways other than the Wnt pathway. It will also be important to determine if Siamois participates in later developmental processes such as neural patterning, and whether its function is conserved in other vertebrates.

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REFERENCES

Acampora, D., Mazan, S., Lallemand, Y., Avantaggiato, V., Maury, M., Simeone, A. and Brulet, P. (1995). Forebrain and midbrain regions are deleted in Otx2-/-mutants due to a defective anterior neuroectoderm specification during gastrulation. *Development* **121**, 3279-3290.

Ang, S.-L. and Rossant, J. (1994). *HNF-3β* is essential for node and notochord formation in mouse development. *Cell* **78**, 561-574.

Ang, S.-L., Jin, O., Rhinn, M., Daigle, N., Stevenson, L. and Rossant, J. (1996). A targeted mouse *Otx2* mutation leads to severe defects in gastrulation and formation of axial mesoderm and to deletion of rostral brain. *Development* **122**, 243-252.

Badiani, P., Corbella, P., Kioussis, D., Marvel, J. and Weston, K. (1994). Dominant interfering alleles define a role for c-Myb in T-cell development. *Genes Dev.* **8**, 770-782.

Behrens, J., von Kries, J. P., Kühl, M., Bruhn, L., Wedlich, D., Grosschedl, R. and Birchmeier, W. (1996). Functional interaction of β-catenin with the transcription factor LEF-1. *Nature* **382**, 638-642.

Blumberg, B., Wright, C. V. E., De Robertis, E. M. and Cho, K. W. Y. (1991). Organizer-specific homeobox genes in *Xenopus laevis* embryos. *Science* **253,** 194-196.

Bouwmeester, T., Kim, S.-H., Sasai, Y., Lu, B. and De Robertis, E. M. (1996). Cerberus is a head-inducing secreted factor expressed in the anterior ectoderm of Spemann's organizer. *Nature* **382**, 595-601.

Brannon, M. and Kimelman, D. (1996). Activation of *Siamois* by the *Wnt* pathway. *Dev. Biol.* **180**, 344-347.

Carnac, G., Kodjabachian, L., Gurdon, J. B. and Lemaire, P. (1996). The homeobox gene *Siamois* is a target of the Wnt dorsalisation pathway and triggers organiser activity in the absence of mesoderm. *Development* **122**, 3055-3065.

Christian, J. L., McMahon, J. A., McMahon, A. P. and Moon, R. T. (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.

Conlon, F. L., Sedgwick, S. G., Weston, K. M. and Smith, J. C. (1996). Inhibition of Xbra transcription activation causes defects in mesodermal patterning and reveals autoregulation of Xbra in dorsal mesoderm. *Development* **122**, 2427-2435.

Dominguez, I., Itoh, K. and Sokol, S. Y. (1995). Role of glycogen synthase kinase 3ß as a negative regulator of dorsoventral axis formation in *Xenopus* embryos. *Proc. Natl. Acad. Sci. USA* **92,** 8498-8502.

Fagotto, F., Guger, K. and Gumbiner, B. M. (1997). Induction of the primary dorsalizing center in *Xenopus* by the Wnt/GSK/β-catenin signaling pathway, but not by Vg1, Activin or Noggin. *Development* **124**, 453-460.

Gerhart, J., Danilchik, M., Doniach, T., Roberts, S., Rowning, B. and Stewart, R. (1989). Cortical rotation of the *Xenopus* egg: consequences for the anterioposterior pattern of embryonic dorsal development. *Development* **107 Supplement**, 31-51.

Gerhart, J., Doniach, T. and Stewart, R. (1991). Organizing the *Xenopus* organizer. In *Gastrulation* (ed. R. Keller, W. H. Clark, Jr., and F. Griffin), pp. 57-77. New York: Plenum Press.

Guger, K. and Gumbiner, B. M. (1995). β-catenin has Wnt-like activity and mimics the Nieuwkoop signaling center in *Xenopus* dorsal-ventral patterning. *Dev. Biol.* **172**, 115-125.

Hamburger, V. (1988). *The Heritage of Experimental Embryology: Hans Spemann and the organizer*. New York: Oxford University Press.

Han, K. and Manley, J. L. (1993). Functional domains of the *Drosophila* Engrailed protein. *EMBO J.* **12**, 2723-2733.

Hanna-Rose, W. and Hansen, U. (1996). Active repression mechanisms of eukaryotic transcription repressors. *Trends Genet.* **12**, 229-234.

Hanes, S. D. and Brent, R. (1989). DNA specificity of the Bicoid activator protein is determined by homeodomain recognition helix residue 9. *Cell* **57**, 1274-1283.

He, X., Saint-Jeannet, J.-P., Woodgett, J. R., Varmus, H. E. and Dawid, I. B. (1995). Glycogen synthase kinase-3 and dorsoventral patterning in *Xenopus* embryos. *Nature* **374**, 617-622.

Heasman, J., Crawford, A., Goldstone, K., Garner-Hamrick, P., Gumbiner, B., McCrea, P., Kintner, C., Noro, C. Y. and Wylie, C. (1994). Overepxression of cadherins and underexpression of β-catenin inhibit dorsal mesoderm induction in early Xenopus embryos. *Cell* **79**, 791-803.

Hemmati-Brivanlou, A. and Harland, R. M. (1989). Expression of an *engrailed*-related protein is induced in the anterior neural ectoderm of early *Xenopus* embryos. *Development* **106**, 611-617.

Horton, R., Cai, Z., Ho, S. and Pease, L. (1990). Gene splicing by overlap extension: tailor made genes using the polymerase chain reaction. *BioTechniques* **8**, 528-535.

Huber, O., Korn, R., McLaughlin, J., Ohsugi, M., Herrmann, B. G. and

Kemler, R. (1996). Nuclear localization of β-catenin by interaction with transcription factor LEF-1. *Mech. Dev.* **59**, 3-10.

Itoh, K., Tang, T. L., Neel, B. G. and Sokol, S. Y. (1995). Specific modulation of ectodermal cell fates in *Xenopus* embryos by glycogen synthase kinase. *Development* **121**, 3979-3988.

Jaynes, J. B. and O'Farrell, P. H. (1991). Active repression of transcription by the Engrailed homeodomain protein. *EMBO J.* **10**, 1427-1433.

John, A., Smith, S. T. and Jaynes, J. B. (1995). Inserting the Ftz homeodomain into Engrailed creates a dominant transcriptional repressor that specifically turns off Ftz target genes in vivo. *Development* **121**, 1801-1813.

Jones, C. M., Kuehn, M. R., Hogan, B. L. M., Smith, J. C. and Wright, C. V. E. (1995). Nodal-related signals induce axial mesoderm and dorsalize mesoderm during gastrualtion. *Devleopment* **121**, 3651-3662.

Kao, K. R. and Elinson, R. P. (1988). The entire mesodermal mantle behaves as Spemann's organizer in dorsoanterior enhanced *Xenopus laevis* embryos. *Dev. Biol.* **127**, 64-77.

Karnovsky, A. and Klymkowsky, M. W. (1995) Anterior axis duplication in *Xenopus* induced by the over-expression of the cadherin-binding protein plakoglobin. *Proc. Natl. Acad. Sci. USA* **92**, 4522-4526.

Krieg, P. A. and Melton, D. A. (1985). Developmental regulation of a gastrula specific gene injected into fertilized *Xenopus* eggs. *EMBO J.* **4**, 3463-3471.

Lamb, T. M., Knecht, A. K., Smith, W. C., Stachel, S. E., Economides, A. N., Stahl, N., Yancopolous, G. D. and Harland, R. M. (1993). Neural induction by the secreted polypeptide noggin. *Science* **262**, 713-718.

Lemaire, P., Garrett, N. and Gurdon, J. B. (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.

Lemaire, P. and Kodjabachian, L. (1996). The vertebrate organiser: structure and molecules. *Trends Genet.* **12**, 525-531.

Mann, R. S. (1995). The specificity of homeotic gene function. *BioEssays* **17**, 855-863.

Matsuo, I., Kuratani, S., Kimura, C., Takeda, N. and Aizawa, S. (1995). Mouse *Otx2* functions in the formation and patterning of rostral head. *Genes Dev.* **9**, 2646-2658.

McCrea, P., Turck, C. W. and Gumbiner, B. (1991). A homolog of the armadillo protein in Drosophila (plakoglobin) associated with E-cadherin. *Science* **254**, 1359-1361**.**

Miller, J. R. and Moon, R. T. (1996). Signal transduction through β-catenin and specification of cell fate during embryogenesis. *Genes Dev.* **10**, 2527-2539.

Molenaar, M., van de Wetering, M., Oosterwegel, M., Peterson-Maduro, J., Godsave, S., Korinek, V., Roose, J., Destrée, O. and Clevers, H. (1996). XTcf-3 transcription factor mediates β-catenin-induced axis formation in *Xenopus* embryos. *Cell* **86**, 391-399.

Newport, J. and Kirschner, M. (1982). A major developmental transition in early*Xenopus* embryos: I. Characterization and timing of cellular changes at the midblastula stage. *Cell* **30**, 675-686.

Nieuwkoop, P. D. and Faber, J. (1967). *Normal Table of Xenopus laevis (Daudin)*. Amsterdam, Holland: North Holland Publ.

Pannese, M., Polo, C., Andreazzoli, M., Vignali, R., Kablar, B., Barsacchi, G. and Boncinelli, E. (1995). The *Xenopus* homologue of *Otx2* is a maternal homeobox gene that demarcates and specifies anterior body regions. *Development* **121**, 707-720.

Pierce, S. B. and Kimelman, D. (1996). Overexpression of Xgsk-3 disrupts anterior ectodermal patterning in *Xenopus*. *Dev. Biol.* **175**, 256-264.

Ryan, K., Garrett, N., Mitchell, A. and Gurdon, J. B. (1996). *Eomesodermin*, a key early gene in Xenopus mesoderm differentiation. *Cell* **87**, 989-1000.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular cloning: A Laboratory Manual*. New York: Cold Spring Harbor Laboratory Press.

Sasai, Y., Lu, B., Steinbeisser, H., Geissert, D., Gont, L. K. and De Robertis, E. M. (1994). Xenopus *chordin*, a novel dorsalizing factor activated by organizer-specific homeobox genes. *Cell* **79**, 779-790.

Sasai, Y., Lu, B., Piccolo, S. and De Robertis, E. M. (1996). Endoderm induction by the organizer-secreted factors chordin and noggin in Xenopus animal caps. *EMBO J.* **15**, 4547-4555.

Sater, A. K. and Jacobson, A. G. (1990). The role of the dorsal lip in the induction of heart mesoderm in Xenopus laevis. *Development* **108,** 461-470.

Schier, A. F. and Gehring, W. J. (1993). Functional specificity of the homeodomain protein fushi tarazu: The role of DNA-binding specificity *in vivo*. *Proc. Natl. Acad. Sci. USA* **90**, 1450-1454.

Schneider, S., Steinbeisser, H., Warga, R. M. and Hausen, P. (1996). β-catenin translocation into nuclei demarcates the dorsalizing centers of frog and fish embryos. *Mech. Dev.* **57**, 191-198.

Shawlot, W. and Behringer, R. R. (1995). Requirement for *Lim1* in head-organizer function. *Nature* **374**, 425-430.

Slack, J. M. W. (1991). *From Egg to Embryo: Regional Specification in Early Development.*, Cambridge, UK: Cambridge Univ. Press.

Smith, J. C., Price, B. M. J., Green, J. B. A., Weigel, D. and Herrmann, B. G. (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 Harland, R. M. (1992). Expression cloning of noggin, a new dorsalizing factor localized to the Spemann organizer in *Xenopus* embryos. *Cell* **70**, 829-840.

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

Sokol, S. Y. and Melton, D. A. (1992). Interaction of Wnt and activin in dorsal mesoderm induction in *Xenopus*. *Dev. Biol.* **154**, 348-355.

Sokol, S. Y., Klingensmith, J., Perrimon, N. and Itoh, K. (1995). Dorsalizing and neuralizing properties of Xdsh, a maternally expressed *Xenopus* homolog of *dishevelled*. *Development* **121**, 1637-1647.

Taira, M., Jamrich, M., Good, P. J. and Dawid, I. B. (1992). The LIM domain-containing homeo box gene *Xlim-1* is expressed specifically in the organizer region of *Xenopus* gastrula embryos. *Genes Dev.* **6**, 365-366.

Talbot, W. S., Trevarrow, B., Halpern, M. E., Melby, A. E., Farr, G., Postlethwait, J. H., Jowett, T., Kimmel, C. B. and Kimelman, D. (1995). A homeobox gene essential for zebrafish notochord development. *Nature* **378**, 150-157.

Treisman, J., Gönczy, P., Vashishtha, M., Harris, E. and Desplan, C. (1989). A single amino acid can determine the DNA binding specificity of homeodomain proteins. *Cell* **59**, 553-562

Vleminckx, K., Wong, E., Guger, K., Rubinfeld, B., Polakis, P. and Gumbiner, B. M. (1997). Adenomatous polyposis coli tumor suppressor protein has signaling activity in *Xenopus laevis* embryos resulting in the induction of an ectopic dorsoanterior axis. *J. Cell Biol.* **136**, 411-420.

Watabe, T., Kim, S., Candia, A., Rothbächer, U., Hashimoto, C., Inoue, K. and Cho, K. W. Y. (1995). Molecular mechanisms of Spemann's organizer formation: conserved growth factor synergy between *Xenopus* and mouse. *Genes Dev.* **9**, 3038-3050.

Weinstein, D. C., Ruiz i Altaba, A., Chen, W. S., Hoodless, P., Prezioso, V. R., Jessell, T. M. and Darnell, Jr., J. E. (1994). The winged-helix transcription factor *HNF-3β* is required for notochord development in the mouse embryo. *Cell* **78**, 575-588.

Wilson, D., Sheng, G., Lecuit, T., Dostatni, N. and Desplan, C. (1993). Cooperative dimerization of Paired class homeodomains on DNA. *Genes Dev.* **7**, 2120-2134.

Wylie, C., Kofron, M., Payne, C., Anderson, R., Hosobuchi, M., Joseph, E. and Heasman, J. (1996). Maternal β-catenin establishes a 'dorsal signal' in early Xenopus embryos. *Development* **122**, 2987-2996.

Yang-Snyder, J., Miller, J. R., Brown, J. D., Lai, C.-J. and Moon, R. T. (1996). A *frizzled* homolog functions in a vertebrate *Wnt* signaling pathway. *Curr. Biol.* **6**, 1302-1306.

Zhou, X., Sasaki, H., Lowe, L., Hogan, B. L. M. and Kuehn, M. R. (1993). Nodal is a novel TGF-β like gene expressed in the mouse node during gastrulation. *Nature* **361**, 543-547.

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