Antagonistic role of *vega1* and *bozozok/dharma* homeobox genes in organizer formation

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During zebrafish development, zygotic gene expression initiated at the midblastula transition converts maternal information on embryo polarity into a transcriptional read-out. Expression of a homeobox gene, *vega1*, is activated at midblastula transition in all blastomeres, but is down-regulated dorsally before gastrulation. Ubiquitous expression of *vega1* is maintained in *bozozok* mutants, in which the dorsal-specific homeobox gene *bozozok*/*dharma (boz*/*dha)* is disrupted and organizer formation is impaired. *Vega1* inhibits expression of *boz*/*dha* and organizer-specific genes, and causes ventralization resulting in a headless phenotype. In contrast, *VP16-vega1*, a fusion including the Vega1 homeodomain and VP16 activation domain, elicits ectopic expression of organizer genes and suppresses several aspects of the *boz* mutant phenotype. We propose that *boz*/*dha*-dependent down-regulation of *vega1* in the dorsal region is an early essential step in organizer formation in zebrafish.

Recent studies in *Xenopus* suggested that transcriptional events between the midblastula transition (MBT) and the beginning of gastrulation establish embryonic polarity and axis formation (1–4). Maternal β-catenin signaling at the dorsal side activates the homeobox genes *siamois* and *twin* immediately after MBT. *Siamois* is a transcriptional activator that positively regulates the expression of Spemann organizer genes and can induce a complete secondary axis (3, 4). In contrast, Vent family homeobox genes, such as *Xvent-1*, function as transcriptional repressors and appear to be activated by bone morphogenetic protein (BMP) signaling only ventrally (5–8). Although Vent genes inhibit organizer formation, these genes do not regulate *siamois* expression (8).

Recently, *bozozok*/*dharma* (*boz*/*dha*; also known as *nieuwkoid*) was identified in zebrafish as a functional mediator of the maternal β-catenin signal on the dorsal side of the blastula (9–11). Zygotic *boz*/*dha* expression arises immediately after MBT in a small group of dorsal blastomeres. By the dome stage, *boz*/*dha* expression becomes progressively restricted to the dorsal yolk syncytial layer (YSL), an extraembryonic structure formed by fusion of marginal blastomeres with the underlying yolk cell. The dorsal YSL appears to possess Nieuwkoop center-like activity (12), contributing to the establishment of the organizer in a non-cell autonomous manner. Misexpression of *boz*/*dha* can induce the organizer gene *goosecoid* (*gsc*) in a non-cell autonomous manner (9, 10). Furthermore, *boz* mutants form an incomplete organizer and exhibit dorso-anterior deficiencies, indicating that *boz*/*dha* is a key component of gastrula organizer formation (11, 13, 14).

Here, we describe a homeobox gene *vega1*, isolated by using a subtracted library enriched in zygotically activated genes. We demonstrate that *vega1* has a mutually antagonistic relationship with *boz*/*dha* and an apparent critical role in early dorso-ventral and antero-posterior patterning of the zebrafish embryo between MBT and organizer formation.

Materials and Methods

Isolation of Vega1. Total RNA of early stage (0–32 cell stage) and dome stage (about 1.5 h after MBT) zebrafish embryos was extracted by using Trizol reagent (GIBCO/BRL), and poly(A)$^+$ RNA was isolated by using the Fast Track Kit (Invitrogen). The early stage cDNA library was subtracted from the dome stage cDNA library, using the PCR-selected cDNA Subtraction Kit (CLONTECH) as described previously (15). cDNAs were selected by further screening using whole-mount *in situ* hybridization. One gene, which we named *vega1*, encodes a homeodomain protein. Full-length *vega1* clones were isolated by rapid amplification of cDNA ends (RACE), using the Marathon cDNA Amplification Kit (CLONTECH). The nucleotide sequence of *vega1* was obtained by sequencing five independent clones. The GenBank accession number of *vega1* is AF193837.

Construction. The coding region of Vega1 (amino acids 1–242) was inserted into pCS2 + vector. Vega1-HA (amino acids 1–242), Vega1-ΔN (amino acids 48–242), ENG-Vega1 (Drosophila Engrailed, amino acids 1–298, plus Vega1, amino acids 100–242), and VP16-Vega1 (Herpes simplex virus I VP16 gene, amino acids 410–490, plus Vega1, amino acids 101–242) were fused to the HA epitope at the C terminus and inserted into the pCS2 + vector. The HA epitope was fused to Vega1-ΔHD (amino acids 1–116) at the N terminus and inserted into the pCS2 + vector. The sequence of inserted cDNAs and their junctions with the backbone were confirmed for all constructs by sequencing.

Dual Luciferase Assay. For luciferase assay experiments, COS-7 cells (0.75×10^5) were plated in a 24-well dish 12 h before transfection. Cells were transfected with total 2 μg of plasmid DNA (0.4 or 1.2 μg of indicated expression vector plus 1.2 or 1.6 μg of pCS2 + vector, 398 ng of p4.0gsclux, and 2 ng of pRL-SV40) and GenePORTER transfection reagent (10 μl) according to manufacturer's directions (Gene Therapy Systems). The dual luciferase assay system (Promega) was used. Twenty-4 h after transfection, cells were harvested and incubated with lysis buffer (100 μl), and aliquots of 20 μl were used for luciferase assays according to the manufacturer's instructions.

Results

Vega1 is not expressed maternally and is activated immediately after MBT in all blastomeres as seen by *in situ* hybridization (Fig. 1*A*) and Northern blotting (data not shown). About 1 h later (dome stage), *vega1* transcripts are no longer detected in a small dorsal domain including blastomeres and YSL (Fig. 1*A*, ∗). Dorsal exclusion of *vega1* transcripts continues during early gastrulation in the shield, the equivalent of the Spemann organizer, and is maintained throughout gastrulation (Fig. 1*A*). Both

Abbreviations: MBT, midblastula transition; YSL, yolk syncytial layer; VHD, Vent-1 Homology Domain; *boz/dha*, *bozozok/dharma*; BMP, bone morphogenetic protein.

Data deposition: The sequence reported in this paper has been submitted to the GenBank database (accession no. AF193837).

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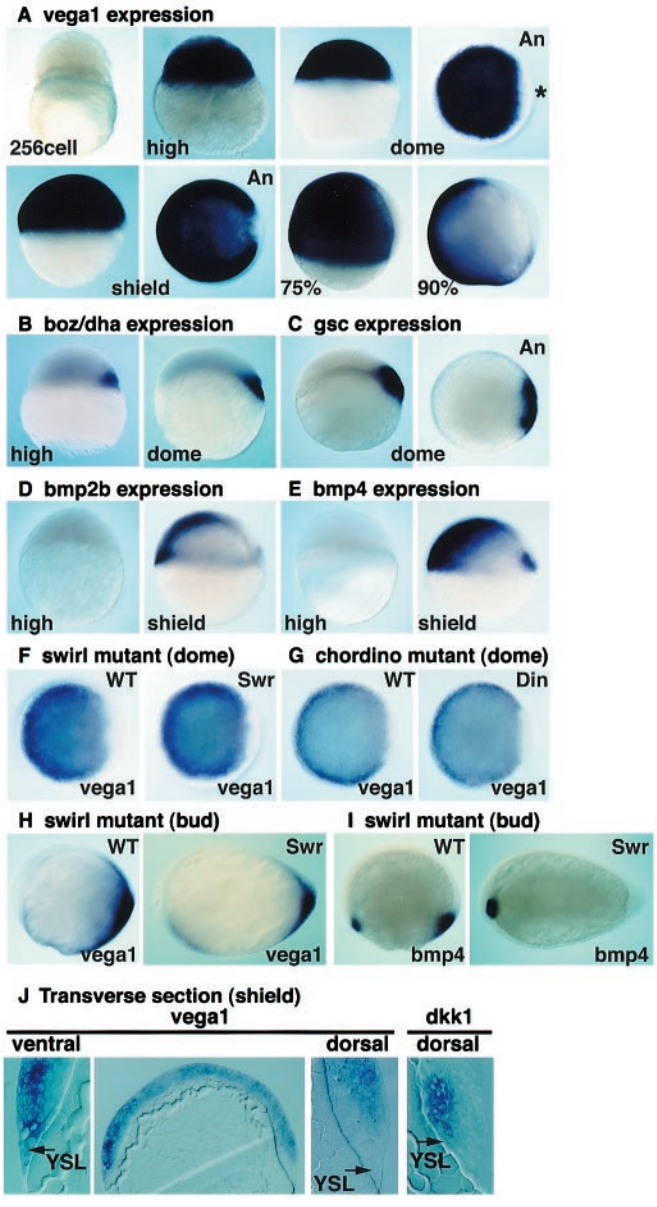

Fig. 1. *Vega1* is activated at the MBT and expressed in a complementary pattern to the dorsal genes, *boz/dha* and *gsc*. Whole-mount *in situ* hybridization of *vega1* (*A*, *F–H*), *boz/dha* (*B*), *gsc* (*C*), *bmp2b* (*D*), and *bmp4* (*E* and *I*). (*A–G*) Dorsal is to the right where known; An, animal view; 75% and 90%, 75% and 90% epiboly stage. Asterisk in dome stage animal view points to *vega1*-depleted region. (*H–I*) Anterior is to the left, dorsal up. (*F* and *G*) Expression of *vega1* is not changed in *swirl*$^{tc300/tc300}$ and *chordino*$^{tt250/tt250}$ mutant embryos at dome stage. Genotyping of mutant embryos was performed by PCR. (*H* and *I*) At bud stage, *vega1* is partly down-regulated in *swirl*$^{tc300/tc300}$ mutant embryos, whereas *bmp4* expression in the posterior region is abolished. (*J*) Transverse section of shield stage embryo stained by *in situ* hybridization for *vega1* or *dkk1*. It is noteworthy that the *dkk1* expression domain is *vega1* negative. Arrows indicate yolk syncytial layer.

dorsal YSL and the adjacent deep blastomeres are *vega1* negative, whereas ventral YSL and blastoderm are strongly stained (Fig. 1J). We compared *vega1* expression to that of dorsal specific homeobox genes *boz/dha* and *gsc*. At the high stage, ubiquitous *vega1* expression overlaps the early *boz/dha* expression in a few dorsal blastomeres (Fig. 1*B*) (9–11). With the exclusion of *vega1* from a dorsal region at dome stage, its expression becomes complementary to that of *boz/dha* and *gsc,* although regions of overlapping expression remain (Fig. 1 *A–C*).

Vega1 encodes a homeodomain protein of 242 amino acids, with 65% sequence identity between its homeodomain and that of *Xenopus* Xvent-1, the most similar protein in the database (Fig. 2 *A* and *B*). A second region of high similarity is named the Vent-1 Homology Domain (VHD) (Fig. 2*C*), whereas the overall sequence identity between Vega1 and any Vent family protein is less than 34%. Because *vega1* functions parallel or upstream of BMP (see below), *vega1* does not appear to be the zebrafish ortholog of *Xvent-1* or of any other reported *Vent* family gene (16).

We hypothesized that *vega1* directly suppresses *gsc* expression ventrally and laterally, and regulates the location and timing of its dorsal expression, because *gsc* expression starts as *vega1* is down-regulated dorsally. To examine this possibility, we used a zebrafish *gsc*/luciferase reporter construct and fusion proteins between the Vega1 DNA binding domain and the previously characterized activation or repressor domains of VP16 and Engrailed, respectively (Fig. 2*D*). Vega1 constructs were co-transfected with p4.0gsclux plasmid (4.0 kb zebrafish *gsc* upstream region linked to luciferase) (17) and internal control plasmid (pRL-SV40) into COS-7 cells. As shown in Fig. 2*E*, *vega1*, truncated constructs, or the Engrailed fusion failed to stimulate the *gsc* promoter; in contrast, *VP16-vega1* strongly activated this promoter. Interestingly, this *VP16-vega1*-induced *gsc* promoter activation was inhibited by cotransfection of either *vega1-HA* or *ENG-vega1*, whereas *vega1-ΔN*, which still carries the homeodomain, did not inhibit the stimulation. Furthermore, analysis of deletion mutants of the *gsc* promoter showed that a 1-kb segment (from −1711 to −738) is required for *VP16-vega1* responsiveness, and wild-type Vega1 protein, but not Vega1-ΔHD, had binding activity for this fragment (data not shown). These results suggest that Vega1 functions as a transcriptional repressor for the *gsc* promoter, and that the N-terminal domain (residues 1–47) of Vega1 is required for repressor activity. Xvent-1 is known to act as a transcriptional repressor for the *XFD-1/XFKH1/pintallavis* promoter by its N-terminal domain (18), which contains a region of high similarity between Vega1 and Xvent-1; this region, named the VHD, may constitute a new type of repressor domain.

We asked how *vega1* expression is suppressed in a small dorsal domain before the dome stage. The dorsal domain exhibits nuclear localization of β-catenin, which leads to the activation of dorsal genes including *boz/dha* and subsequently *gsc*, during blastula stages (11, 19). Injection of *boz/dha* or *gsc* mRNA inhibited *vega1* expression at the dome stage, with *boz/dha* being more effective (Fig. 3 *A–C*). These results suggest that *boz/dha* and to a lesser extent, *gsc,* mediate suppression of *vega1* at the dorsal side of the embryo during blastula stages. To test whether *boz/dha* is required for down-regulation of *vega1* transcripts dorsally, we analyzed expression of *vega1* in *boz*^{m168} mutant embryos (11, 13). We found that ubiquitous *vega1* expression was maintained in *boz* mutants through the shield stage (Fig. 4 *A* and *B*), whereas *gsc* expression was strongly reduced (data not shown) (11). These results indicate that zygotic *boz* function is required for the dorsal repression of *vega1*.

In *Xenopus*, Vent family genes such as *Xvent-1* function as downstream mediators of BMP signaling (5). Therefore, we examined the functional interaction between *vega1* and BMP signaling. When *vega1* is expressed in all blastomeres at the high stage, *bmp2b* expression is just starting and *bmp4* expression is not detectable (Fig. 1 *D* and *E*). At the shield stage, the expression patterns of *vega1* and of *bmp2b/bmp4* which form a ventral-to-dorsal gradient, are quite different (Fig. 1 *A*, *D*, and *E*). Consistently, expression of *vega1* was not affected at the mid-blastula stage in *swirl*^{tc300} (*swr*) and *chordino*^{tt250} (*din*) mutant embryos (Fig. 1 *F* and *G*); *swr* disrupts the *bmp2b* gene, *din* disrupts the BMP antagonist, Chordin (20–23). Furthermore, injection of the constitutively active form of the BMP type

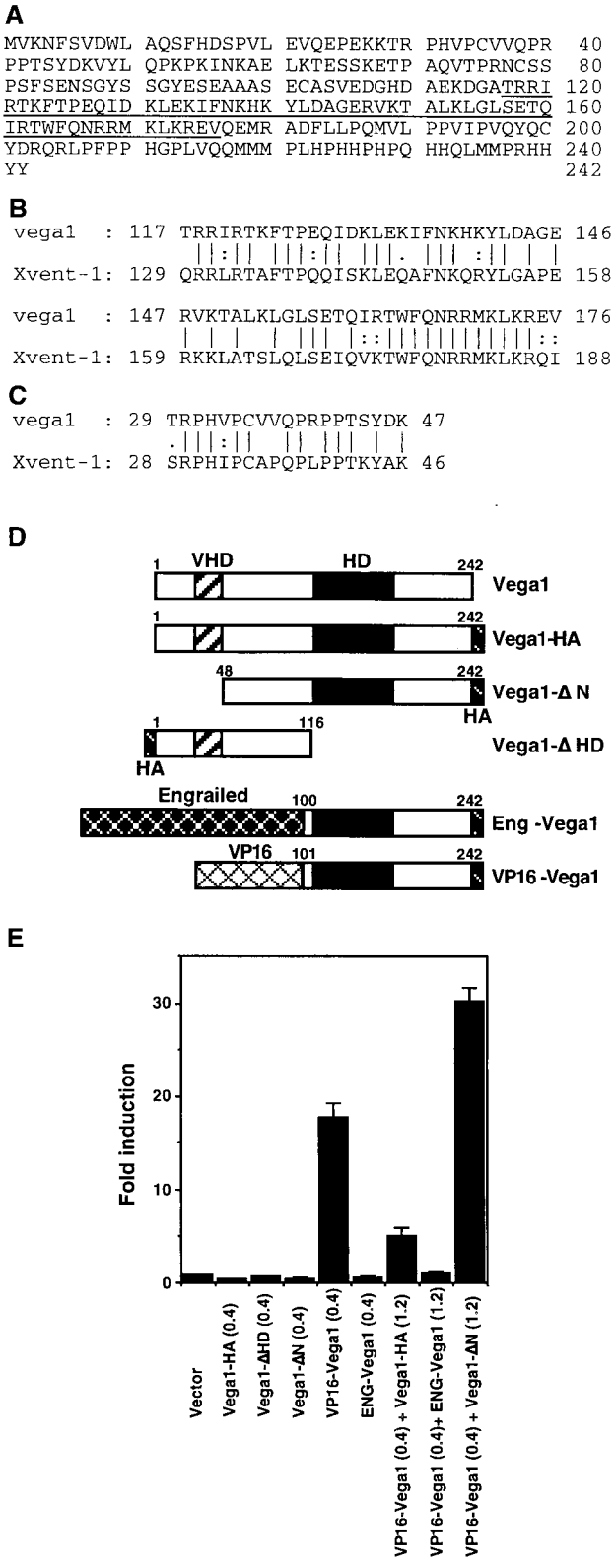

Fig. 2. *Vega1* encodes a homeodomain protein that can regulate the *goosecoid* promoter. (*A*) Predicted amino acid sequence of the Vega1 protein with the homeodomain underlined. (*B*) Sequence alignment of the homeodomains of Vega1 and Xvent-1. Bars and dots mean identical amino acid and similar amino acid, respectively. (*C*) Comparison of N-terminal Vent-1 homology domain (VHD) of Vega1 and Xvent-1. (*D*) Schematic representation of Vega1 mutant constructs. HD and HA indicate homeodomain and influenza hemagglutinin epitope, respectively. (*E*) Regulation of the *gsc* promoter by Vega1 constructs. COS-7 cells were transfected with the indicated cDNA in addition to reporter (p4.0gsclux) and control plasmids (pRL-SV40). Luciferase activity was normalized by the internal control plasmid and expressed relative to the activity of the vector alone. The error bars represent the standard deviation of three experiments. Protein expression levels of Vega1 constructs were comparable as judged by anti-HA Western blotting.

I receptor, BMP-RIA (24), did not cause expansion of the *vega1* domain, whereas *no tail* (*ntl*) expression in the chordamesoderm was strongly suppressed (Fig. 3 D–F). These results suggest that expression of *vega1* is not dependent on BMP signaling during blastula stages. At the end of gastrulation, *vega1* expression was only partly suppressed in the *swirl* mutant, whereas *bmp4* expression was abolished in the posterior domain (Fig. 1 *H* and *I*). Thus, *vega1* expression during gastrulation is regulated by other signaling pathway(s) with only a small contribution from the BMP pathway.

Functional activity of *vega1* and *VP16-vega* was examined by phenotypic changes elicited by injection of synthetic mRNA into zebrafish and *Xenopus* embryos. In zebrafish, both *vega1* and *ENG-vega1* caused ventralization with loss of anterior head structures and tail and axis defects in 53% ($n = 44$) (Fig. 3 *T* and *U*) and 63% ($n = 49$) of embryos, respectively. Likewise, *vega1* mRNA injection into the two dorsal blastomeres of four-cell *Xenopus* embryos led to a head-less phenotype (74%; $n = 66$), whereas ventral injection had no effect (data not shown). In contrast, expression of *VP16-vega1* in zebrafish caused a dorsalized phenotype (62%; $n = 34$) (Fig. 3*V*), similar to the phenotype obtained by ectopic *boz/dha* expression (9). Secondary axis formation was often observed when *VP16-vega1* was injected into the two ventral blastomeres of four-cell *Xenopus* embryos (67%; $n = 55$) (data not shown).

The molecular pathways influenced by *vega1* or *VP16-vega1* injection were analyzed by the use of marker genes. Injection of *vega1* mRNA into zebrafish embryos inhibited *gsc*, *boz/dha*, and *chordin* (*din*) expression (Fig. 3 *H*, *K*, and *N*). Conversely, ectopic expression of these dorsal genes was detected in *VP16-vega1* injected embryos (Fig. 3 *I*, *L*, and *O*), whereas *bmp2b* expression was inhibited (Fig. 3*R*). Furthermore, *VP16-vega1* injections resulted in expansion of the expression domains of the dorsal genes *dkk1*, *floating head* (*flh*), *squint* (*sqt*) and *cyclops* (*cyc*), whereas *gata2* expression was inhibited (data not shown). Conversely, the expression of these genes except for *gata2* was strongly suppressed by *vega1* injection. Thus, *vega1* acts as a ventralizing factor suppressing dorsal genes whereas *VP16-vega1,* an apparent antimorphic form, acts as a dorsalizing factor. Because BMP signaling is critical for maintaining ventral identity in the embryo, we examined the effects of *vega1* and *VP16-vega1* on *swr* (dorsalized) and *din* (ventralized) mutants. Injection of *vega1* could not suppress the expansion of neuroectoderm in the *swr* mutant, but resulted in a loss of notochord and an anterior shift of *pax2.1* expression domain ($n = 129$) (Fig. 3 *Y* and *Z*). This phenotype is similar to that obtained by overexpression of Noggin or Chordin in *boz* mutants (25), or exhibited by *boz;swr* double mutants (L.S.K., unpublished observation). Likewise, injection of *VP16-vega1,* which consistently dorsalized wild-type embryos, could not rescue the ventralized phenotye of *din* mutant embryos as judged by the shape of the ventral tail (Fig. 3 *W* and *X*, arrowhead). These results indicate that *vega1* functions parallel or upstream of BMP signaling in axis formation in zebrafish.

Because *boz/dha* and *vega1* are the earliest-expressed zygotic genes implicated in axis formation, we examined their functional interactions in the embryo. *Boz/dha* mRNA injection into the ventral blastomeres of four-cell *Xenopus* embryos induced secondary axes (86%, $n = 57$), which was inhibited by

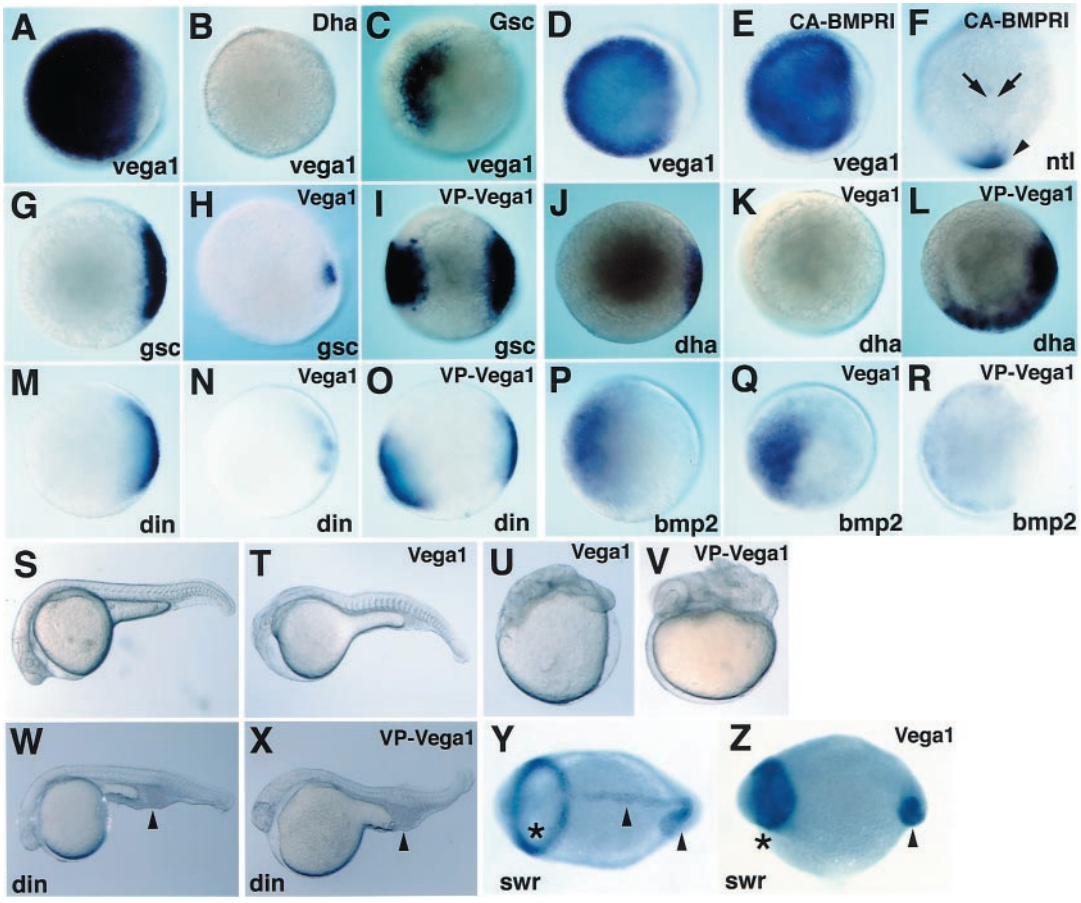

Fig. 3. Regulation of *vega1* expression and analysis of *vega1* function. Whole-mount *in situ* hybridization for *vega1* (*A–E*), *ntl* (*F*), *gsc* (*G–I*), *boz/dha* (*J–L*), *chordin* (*M–O*), and *bmp2b* (*P–R*); animal views (*A–E*, *G–R*), dorsal view (*F*), dome stage (*A–E*, *G–I*, *M–O*), 30% epiboly stage (*P–R*), shield stage (*J–L*), bud stage (*F*). (*A–C*) *boz/dha* mRNA (*B*; 25pg), *gsc* mRNA (*C*; 50pg) were injected into one to two cell stage embryos; (*A*) uninjected control. (*D–F*) RNA injections; *CA-BMPRI* mRNA (*E* and *F*; 200 pg) was injected into one to two cell stage embryos; (*D*) uninjected control. (*E*) *Vega1* expression was unchanged. (*F*) Expression of *ntl* in the chordamesoderm region was suppressed (arrows), but not affected in the tailbud domain (arrowhead). (*G–R*) *vega1* mRNA (*H*; 250 pg; and *K*, *N*, and *Q*, 100 pg) and *VP16-vega1* mRNA (*I*, *L*, *O*, and *R*, 50 pg) were injected into one to two cell and two to eight cell stage embryos, respectively. (*G*, *J*, *M*, and *P*) Uninjected controls. (*S–V*) Phenotypic effects; lateral views, prim-6 (25 hpf) stage. Embryos were injected with *vega1* mRNA (*T* and *U*; 100pg) or *VP16-vega1* mRNA (*V*; 50pg) at the one to two cell stage. (*S*) Uninjected control. (*W* and *X*) Embryos from a $din^{tt250/+}$ X $din^{tt250/+}$ cross were injected with *VP16-vega1* mRNA (*X*; 100pg) at one to two cell stage. *VP16-vega1* could not restore the ventral tail phenotype (arrowhead), whereas wild-type embryos injected with *VP16-vega1* were dorsalized (*V*). (*W*) Uninjected $din^{tt250/tt250}$ mutant. (*Y* and *Z*) Whole-mount *in situ* hybridization for *pax2.1* plus *ntl*; 5-somite stage, dorsal view. Asterisk and arrowhead show *pax2.1* and *ntl* staining domain, respectively. (*Z*) Embryos from a $swirl^{tc300/+}$ X $swirl^{tc300/+}$ cross were injected with *vega1* mRNA (150pg) at one to two cell stage. (*Y*) Uninjected $swirl^{tc300/tc300}$ mutant.

coinjection of *vega1* (7%, $n = 58$) (data not shown). Furthermore, we tested whether *VP16-vega1* can restore *gsc* expression and rescue other aspects of the *boz* mutant phenotype. *VP16-vega1* mRNA injection into *boz* mutant embryos at the one-cell stage strongly induced *gsc* expression (100%, $n = 39$, Fig. 4*E*). Forebrain and notochord deficiencies of *boz* mutants were largely suppressed by injection of *VP16-vega1* mRNA as evidenced by morphological analysis (Fig. 4*F*), and by the restoration of *six3* (forebrain) and *ntl* (notochord) expression at the 8 somite stage (data not shown). These results suggest that *vega1* and *boz/dha* functionally antagonize each other, and that repression of *vega1* is an important aspect of *boz/dha* function in axis formation.

Discussion

The zebrafish *boz/dha* and the *Xenopus siamois* and *twin* genes are functional mediators of maternal β-catenin signaling in the establishment of the gastrula organizer. In *Xenopus*, *siamois* contributes to organizer formation acting as transcriptional activator of other dorsal genes (26–28). In contrast, the N-terminal domain of Boz/Dha shares sequence similarity with a characteristic domain of the transcriptional repressors Gsc and Engrailed, the so-called Gsc-Engrailed homology (GEH) domain (10). Furthermore, a Boz/Dha homeodomain-Engrailed repressor domain fusion protein has similar dorsalizing activity as wild type Boz/Dha, suggesting that this factor functions as a transcriptional repressor (M. Hibi, Y. Yamanaka, T. Hirano, personal communication). Therefore, the mechanism of *boz/dha* action appears distinct from that of *siamois*.

In this paper, we have demonstrated an antagonistic interaction between *vega1* and *boz/dha* that is essential for organizer formation, providing a plausible mechanism through which the repressor Boz/Dha may exercise its dorsalizing role. The rapid clearing of *vega1* mRNA from the future dorsal side of the embryo immediately follows the initiation of *boz/dha* expression and depends on zygotic *boz/dha* function. This dependence on *boz* is specific, as no change in *vega1* expression was observed in the BMP signaling mutants *swr* and *din*. Interestingly, *boz/dha* is also required for down-regulation of the *bmp2b* (14, 25), and Boz/Dha protein directly binds to the promoter region of *bmp2b*

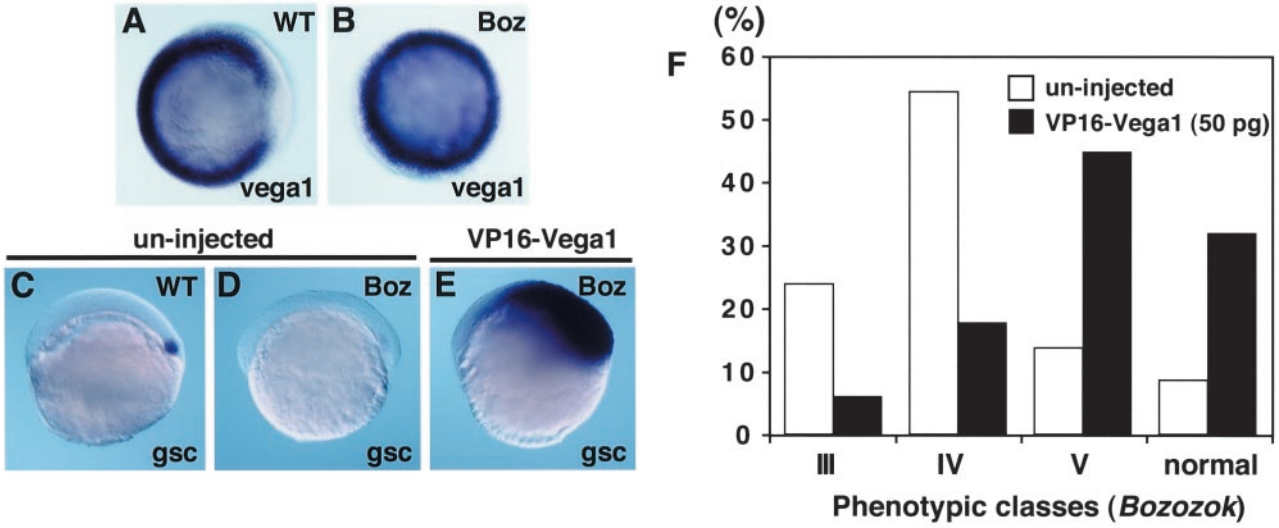

Fig. 4. *VP16-vega1* can rescue both *gsc* expression and *boz* phenotype. Whole-mount *in situ* hybridization for *vega1* (*A* and *B*, shield stage, animal view) and *gsc* (*C–E*, 40% epiboly stage, lateral view). Genotyping of *boz* mutants was carried out by restriction fragment polymorphism (11). (*A*) $boz^{m168/+}$ phenotypically wild type. (*B*) $boz^{m168/m168}$ mutant. (*C*) Uninjected wild-type embryo. (*D*) Uninjected $boz^{m168/m168}$ embryo. Expression of *gsc* was suppressed in all uninjected *boz* embryos ($n = 15$). (*E*) *VP16-vega1* mRNA (100pg) was injected into one-cell stage of embryos obtained from a $boz^{m168/m168} \times boz^{m168/m168}$ cross. Expansion of *gsc* was observed in all *VP16-vega1*-injected *boz* embryos (100%, $n = 39$). (*F*) Suppression of *boz* phenotype by *VP16-vega1* injection. *VP16-vega1* mRNA (50 pg) was injected into one-cell stage of embryos obtained from $boz^{m168/m168} \times boz^{m168/m168}$ crosses. The *boz* phenotype was classified by morphological criteria at 30 hpf as described previously (11). The number of uninjected and *VP16-vega1*-injected embryos was 59 and 85, respectively. Class V has a small break in trunk notochord with normal head structure; there is a two-to-several somite wide gap in the trunk notochord of class IV; class III shows partial cyclopia with anterior head deficiency and usually a large gap in the trunk notochord. Essentially identical results were obtained in several independent experiments.

gene (T.C. Leung, W. Driever, personal communication), indicating that *boz/dha* contributes the dorsal suppression of both *vega1* and *bmp2b* genes. We have shown that *vega1* and *boz/dha* as well as *vega1* and *gsc* reciprocally repress each other's expression in the blastula embryo. Therefore, the initial stage of axis formation by zygotically activated genes may be based on the balance of expression levels of these homeobox genes and involves progressive refinement of their expression domains. One attractive possibility is that both *boz/dha* and subsequently *gsc* directly bind to the *vega1* promoter and repress its activity. Functional antagonism is also indicated by the ability of *vega1* to inhibit the axis-inducing activity of *boz/dha*, whereas *VP16-vega1* can largely restore marker expression and morphology in *boz* mutant embryos. Furthermore, analysis of zebrafish mutants with elevated BMP signaling demonstrates that *vega1* functions in parallel or upstream of BMP signaling in axis specification in the zebrafish.

We also were able to show that *vega1* can affect the expression of several dorsal genes. The effect of *vega1* constructs on *gsc* reporter activity and the restoration of *gsc* expression in *boz* mutants by *VP16-vega1* indicate that *gsc* is one of the direct targets of the Vega1 repressor. Furthermore, *VP16-vega1* strongly induced the ectopic expansion of *dkk1*, *sqt*, and *cyc* genes that encode secreted factors involved in axis formation (29–32). Because early expression of *nodal* genes is not dependent on *boz/dha* (33), these observations suggest that *vega1* may inhibit the expression of *nodal* genes in addition to *boz/dha*. In summary, we suggest that elimination of *vega1* transcripts from a small dorsal domain by *boz/dha* is the first post-MBT step in axis formation, generating a permissive region for the expression of dorsal genes such as *gsc*, leading to the development of the gastrula organizer.

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1. Wylie, C., Kofron, M., Payne, C., Anderson, R., Hosobuchi, M., Joseph, E. & Heasman, J. (1996) *Development (Cambridge, U.K.)* **122,** 2987–2996.
2. Fagotto, F., Guger, K. & Gumbiner, B. M. (1997) *Development (Cambridge, U.K.)* **124,** 453–460.
3. Lemaire, P., Garrett, N. & Gurdon, J. B. (1995) *Cell* **81,** 85–94.
4. Kessler, D. S. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 13017–22.
5. Gawantka, V., Delius, H., Hirschfeld, K., Blumenstock, C. & Niehrs, C. (1995) *EMBO J.* **14,** 6268–6279.
6. Ladher, R., Mohun, T. J., Smith, J. C. & Snape, A. M. (1996) *Development (Cambridge, U.K.)* **122,** 2385–2394.
7. Schmidt, J. E., von Dassow, G. & Kimelman, D. (1996) *Development (Cambridge, U.K.)* **122,** 1711–1721.
8. Shapira, E., Marom, K., Levy, V., Yelin, R. & Fainsod, A. (2000) *Mech. Dev.* **90,** 77–87.
9. Yamanaka, Y., Mizuno, T., Sasai, Y., Kishi, M., Takeda, H., Kim, C. H., Hibi, M. & Hirano, T. (1998) *Genes Dev.* **12,** 2345–2353.
10. Koos, D. S. & Ho, R. K. (1998) *Curr. Biol.* **8,** 1199–1206.
11. Fekany, K., Yamanaka, Y., Leung, T., Sirotkin, H. I., Topczewski, J., Gates, M. A., Hibi, M., Renucci, A., Stemple, D., Radbill, A., *et al.* (1999) *Development (Cambridge, U.K.)* **126,** 1427–1438.
12. Kodjabachian, L., Dawid, I. B. & Toyama, R. (1999) *Dev. Biol.* **213,** 231–245.
13. Solnica-Krezel, L., Stemple, D. L., Mountcastle-Shah, E., Rangini, Z., Neuhauss, S. C., Malicki, J., Schier, A. F., Stainier, D. Y., Zwartkruis, F., Abdelilah, S. & Driever, W. (1996) *Development (Cambridge, U.K.)* **123,** 67–80.
14. Koos, D. S. & Ho, R. K. (1999) *Dev. Biol.* **215,** 190–207.
15. Kawahara, A. & Dawid, I. B. (2000) *Mech. Dev.*, in press.
16. Rastegar, S., Friedle, H., Frommer, G. & Knochel, W. (1999) *Mech. Dev.* **81,** 139–149.
17. McKendry, R., Harland, R. M. & Stachel, S. E. (1998) *Dev. Biol.* **204,** 172–186.
18. Friedle, H., Rastegar, S., Paul, H., Kaufmann, E. & Knochel, W. (1998) *EMBO J.* **17,** 2298–2307.
19. Stachel, S. E., Grunwald, D. J. & Myers, P. Z. (1993) *Development (Cambridge, U.K.)* **117,** 1261–1274.

20. Kishimoto, Y., Lee, K. H., Zon, L., Hammerschmidt, M. & Schulte-Merker, S. (1997) *Development (Cambridge, U.K.)* **124,** 4457–4466.
21. Nguyen, V. H., Schmid, B., Trout, J., Connors, S. A., Ekker, M. & Mullins, M. C. (1998) *Dev. Biol.* **199,** 93–110.
22. Schulte-Merker, S., Lee, K. J., McMahon, A. P. & Hammerschmidt, M. (1997) *Nature (London)* **387,** 862–863.
23. Piccolo, S., Sasai, Y., Lu, B. & De Robertis, E. M. (1996) *Cell* **86,** 589–598.
24. Nikaido, M., Tada, M., Takeda, H., Kuroiwa, A. & Ueno, N. (1999) *Development (Cambridge, U.K.)* **126,** 181–190.
25. Fekany-Lee, K., Gonzalez, E., Miller-Bertoglio, V. & Solnica-Krezel, L. (2000) *Development (Cambridge, U.K.)* **127,** 2333–2345.
26. Lemaire, P. & Kodjabachian, L. (1996) *Trends Genet.* **12,** 525–531.
27. Harland, R. & Gerhart, J. (1997) *Annu. Rev. Cell Dev. Biol.* **13,** 611–667.
28. Moon, R. T. & Kimelman, D. (1998) *BioEssays* **20,** 536–545.
29. Rebagliati, M. R., Toyama, R., Haffter, P. & Dawid, I. B. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 9932–9937.
30. Feldman, B., Gates, M. A., Egan, E. S., Dougan, S. T., Rennebeck, G., Sirotkin, H. I., Schier, A. F. & Talbot, W. S. (1998) *Nature (London)* **395,** 181–185.
31. Sampath, K., Rubinstein, A. L., Cheng, A. M., Liang, J. O., Fekany, K., Solnica-Krezel, L., Korzh, V., Halpern, M. E. & Wright, C. V. (1998) *Nature (London)* **395,** 185–189.
32. Hashimoto, H., Itoh, M., Yamanaka, Y., Yamashita, S., Shimizu, T., Solnica-Krezel, L., Hibi, M. & Hirano, T. (2000) *Dev. Biol.* **217,** 138–152.
33. Shimizu, T., Yamanaka, Y., Ryu, S., Hashimoto, H., Yabe, T., Hirata, T., Bae, Y., Hibi, M. & Hirano, T. (2000) *Mech. Dev.* **91,** 293–303.