Genetic analysis of dorsoventral pattern formation in the zebrafish: requirement of a BMP-like ventralizing activity and its dorsal repressor

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According to a model based on embryological studies in amphibia, dorsoventral patterning is regulated by the antagonizing function of ventralizing bone morphogenetic proteins (BMPs) and dorsalizing signals generated by Spemann's organizer. Large-scale mutant screens in the zebrafish, Danio rerio, have led to the isolation of two classes of recessive lethal mutations affecting early dorsoventral pattern formation. dino mutant embryos are ventralized, whereas swirl mutants are dorsalized. We show that at early gastrula stages, dino and swirl mutants display an expanded or reduced Bmp4 expression, respectively. The dino and swirl mutant phenotypes both can be phenocopied and rescued by the modulation of BMP signaling in wild-type and mutant embryos. By suppressing BMP signaling in dino mutants, adult fertile dino-/- fish were generated. These findings, together with results from the analysis of dino-swirl double mutants, indicate that dino fulfills its dorsalizing activity via a suppression of swirl-dependent, BMP-like ventralizing activities. Finally, cell transplantation experiments show that dino is required on the dorsal side of early gastrula embryos and acts in a non-cell-autonomous fashion. Together, these results provide genetic evidence in support of a mechanism of early dorsoventral patterning that is conserved among vertebrate and invertebrate embryos.

[Key Words: Dorsoventral pattern formation; dino; swirl; BMP4; noggin; Spemann's organizer; zebrafish; Danio rerio]
Thus, these factors have opposing activities to, and are counteracted by BMPs [Sasai et al. 1995; Jones et al. 1996]. Follistatin binds activins, which are also members of the TGF-B superfamily, preventing activin signaling [Nakamaru et al. 1989]. Recent evidence indicates that Chordin and Noggin also bind BMPs with high affinity, thereby preventing the interaction of BMPs with their receptors [Piccolo et al. 1996; Zimmermann and Harland 1996]. Thus, the induction of specific dorsoventral positional identities may depend upon the modulation of signaling by BMPs, through the direct blocking action of dorsally localized antagonists.

Interestingly, Chordin shares structural and functional homology with Drosophila Short gastrulation (Sog), which acts as an antagonist of Decapentaplegic (Dpp), the Drosophila BMP2/4 homologue, during patterning of the dorsoventral axis of the Drosophila embryo [Ferguson and Anderson 1992b; Wharton et al. 1993; François et al. 1994]. The opposing distributions and activities of Chordin and BMP4 in Xenopus and Sog and Dpp in Drosophila suggest a common mechanism for dorsoventral patterning in bilateral metazoans [François and Bier 1995; Jones and Smith 1995; DeRobertis and Sasai 1996].

While the relevance of the fly genes for normal dorsoventral pattern formation in vivo has been demonstrated in loss-of-function mutants, similar studies of the vertebrate genes are ambiguous. Gene targeting experiments in the mouse have revealed the general requirement for BMP4 and a type I BMP receptor during early mouse gastrulation and mesoderm formation [Mishina et al. 1995; Winnier et al. 1995]. Homozygous mutant mouse embryos display variable phenotypes. Several affected mutants die during early gastrulation stages and form no or only little mesoderm, making it difficult to address the proposed role of BMP4 signaling during the refinement of mesodermal patterning and neural induction. Those embryos with the least severe phenotype die at early somite stages and have truncations of the posterior axis that might be interpreted as ventral deficiencies.

Recently, screens for randomly introduced mutations in the zebrafish [Haffter et al. 1996] have led to the identification of two distinct classes of mutants that display an altered dorsoventral pattern, either a general ventralization or a general dorsalization. In mutants of both classes, the dorsalmost mesoderm of the shield, which is the fish equivalent of Spemann's organizer in amphibia [Ho 1992; Shih and Fraser 1996], is formed normally. However, the mutants display severe alterations in the patterning of regions outside the organizer. Mutants in dino are ventralized and display an enlargement of ventral lateral mesodermal fates and a reduction of neuroectodermal and dorsolateral mesodermal fates [Hammerschmidt et al. 1996b]. In contrast, swirl mutants are dorsalized and show the opposite phenotype, an enlargement of the neuroectoderm and dorsolateral mesodermal fates [Mullins et al. 1996]. The phenotypes of the mutants suggest that dino and swirl are required for the zygotic control of dorsoventral pattern formation after the initial establishment of a dorsoventral polarity, which appears to be under maternal control. The phenotypes are consistent with the model of counteracting zygotic ventralizing and dorsalizing activities drawn from the aforementioned studies in amphibia.

We provide several lines of evidence indicating that the swirl and dino mutant phenotypes result from a modulation of normal BMP signaling. The dorsalizing gene encoded by dino appears to be required on the dorsal side of the pregastrula embryo to suppress a ventralizing swirl-dependent BMP signal. Thus, these genetic studies indicate that zygotic regulation of the vertebrate body plan does have a similar underlying mechanism to that of the Drosophila embryo.

Results

Morphology of dino and swirl mutant embryos

As previously described [Hammerschmidt et al. 1996b], dino mutants appear ventralized, displaying a general shift in the dorsoventral organization of the mesoderm. Dorso lateral cell fates are suppressed, the posterior notochord is lost, and the anterior somites are reduced [Fig. 1B,E], while the blood and pronephros, which derive from more ventral mesoderm, are expanded. In contrast, swirl mutants appear dorsalized. The notochord is broader, the somites extend laterally, and blood and pronephric development is absent [Mullins et al. 1996; Fig. 1C,F]. In addition, dino mutants display a general enlargement of posterior structures at the expense of anterior structures, and an overall reduction of the neuroectoderm, whereas swirl mutants have the opposite phenotype [Figs. 1A–F, 2D,E, 3F,G]. Neither mutant is viable: swirl mutants die within 24 hr after fertilization, and dino mutants at later stages.

Expression of Bmp4 in dino and swirl mutants

To study whether alteration of BMP4 might underlie the dino and swirl phenotypes, we examined the Bmp4 expression in these mutants. At late blastula stages, Bmp4 is uniformly expressed in both wild-type and mutant embryos [not shown]. Starting shortly before gastrulation, Bmp4 expression is progressively lost in dorsolateral regions of wild-type embryos. Expression is maintained in ventrolateral regions and in the anterior dorsal endomesoderm [Fig. 1G,J]. In contrast, in dino mutant embryos Bmp4 expression remains strong in all regions [Fig. 1H,K], while in swirl mutants, Bmp4 expression is lost everywhere except the anterior endomesoderm [Fig. 1L]. This suggests that dino is required for the repression of Bmp4 expression in dorsolateral regions, while swirl is required for the maintenance of ventrolateral Bmp4 expression. Further, the observed alterations in the expression pattern of Bmp4 appear to be consistent with, and might account for, the altered dorsoventral pattern seen in dino and swirl mutant embryos. To test this notion, we studied whether the dino and swirl mutant phenotypes can be phenocopied and rescued by al-
expression of the notochord marker gene \( ntl \) (Schulte-Merker et al. 1992) in the anterior part of the axis (Fig. 2J–L). However, dorsal fates outside the organizer are reduced, as shown by the reduced size of the expression domain of the neuroectodermal marker gene \( jkd3 \) (Fig. 2D–F; cf. Hammerschmidt et al. 1996b) and the lack of \( ntl \) expression in the posterior part of the axis (Fig. 2J–L). In contrast, ventrolateral fates are expanded, as revealed by the broader expression patterns of the ventrolateral marker gene \( eve1 \) at gastrula stages (Fig. 2G–I; Joly et al. 1993) and the hematopoietic marker gene \( gata1 \) at segmentation stages (Fig. 2J–L; Detrich et al. 1995). Therefore, it is likely that the altered dorsoventral organizing BMP signaling in wild-type or mutant embryos, respectively.

**Phenocopy of dino mutant phenotype by Bmp4 overexpression**

To test the hypothesis that the ventralization of dino mutants is caused by the expanded Bmp4 expression, we studied whether overexpression of Bmp4 in wild-type embryos leads to a dino-like phenotype. While embryos injected with control plasmid (pCSKA–lacZ; Sasai et al. 1995) showed no sign of ventralization (0/63, not shown), embryos injected with a similar construct expressing Bmp4 (pCSKA–BMP4) displayed most phenotypic traits of dino mutant embryos (250/423, 59%). As in dino mutants, the derivatives of Spemann’s organizer appear normal in pCSKA–BMP4-injected wild-type embryos, as revealed by the expression of gsc (Stachel et al. 1993; Schulte-Merker et al. 1994; This et al. 1994) in presumptive prechordal plate cells (Fig. 2G–I) and the expanded gata1 expression domains in the two lateral stripes. The more posterior gata1 expression is delayed in both the mutant and the injected wild-type embryo. At the 15-somite stage, however, this posterior expression domain will also be much broader than in uninjected wild-type embryos (not shown, but see Hammerschmidt et al. 1996b). Also note the increased size of the tissues interposed between the two gata1 stripes posteriorly and the closer apposition anteriorly, which reflects the general enlargement of the posterior region at the expense of the anterior region of the embryo pointed out in Fig. 1.
tion of \textit{dino} mutant embryos results from the observed dorsal expansion of \textit{Bmp4} expression. In the wild-type, \textit{dino} presumably acts as an antagonist of \textit{Bmp4} expression in the dorsolateral region of the gastrulating embryo.

\textbf{Phenocopy of \textit{swirl} mutant phenotype by BMP inactivation}

To determine whether the loss of \textit{Bmp4} expression in \textit{swirl} embryos leads to a dorsalized phenotype, we inactivated \textit{Bmp4} in wild-type embryos (and potentially other related BMPs that might be expressed) by two different means: the injection of synthetic RNA encoding either a truncated, dominant negative form of the \textit{Xenopus} BMP receptor (tBr; Graff et al. 1994; Maeno et al. 1994; Suzuki et al. 1994; Schmidt et al. 1995b) or \textit{Xenopus} Noggin, which binds to BMP2 and BMP4 at picomolar concentrations, preventing the binding of the BMPs to their receptor (Zimmerman and Harland 1996). As in the \textit{Xenopus} embryo, injection of tBr mRNA (50–100 pg per embryo, 117/119, 98%) or noggin mRNA (2–5 pg, 98/98, 100%) into wild-type zebrafish embryos caused a dorsalization. Injected embryos look very similar to dorsalized zebrafish mutants (Mullins et al. 1996), with the body axis wound up in a snailshell-like fashion (Fig. 3B–D). With molecular markers, the dorsalization is already apparent at midgastrula stages. As in \textit{swirl} mutants (Fig. 4G, L), expression of the neuroectodermal marker gene \textit{fkd3} extends into ventralmost regions (Fig. 3H, I), and the expression domain of the notochord marker gene \textit{axial} (\textit{axl}) (Strähle et al. 1993) is much broader. Moreover, expression of \textit{eve1}, a ventrolateral marker, is absent (Fig. 3M, N). This almost perfect phenocopy of the \textit{swirl} mutant phenotype by suppression of ventral BMP signaling in wild-type embryos suggests that the phenotype of the mutant embryos might be caused by the observed loss of endogenous BMP signaling.

\textbf{Partial rescue of \textit{swirl} and \textit{dino} phenotypes by modulation of BMP signaling}

If altered BMP signaling is responsible for the \textit{dino} and \textit{swirl} phenotypes, it should be possible to rescue mutant embryos by modifying BMP signaling. To test this prediction we intercrossed \textit{swirl} heterozygotes and injected their embryos with pCSKA–\textit{Bmp4} to drive ectopic \textit{Bmp4} expression. Significantly less than a quarter of the embryos (11/303, 3.6%) displayed the \textit{swirl}-specific football-like shape (see Fig. 1C) at the end of gastrulation. Moreover, at later stages, some embryos (17/303) showed only a weakly dorsalized phenotype (Fig. 3E, F).
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Figure 4. dino mutants are rescued by the injection of synthetic tBr or nog mRNA. [A–D] Two-day old larvae; [E–H] fkd3 expression, 70% epiboly, [I–L] expression of axl [a] and eve1 [e], 80% epiboly. [A,E,I] Uninjected wild-type; [B,F,J] uninjected dino mutant; [C,C,K] tBr-injected dino mutant; [D,H,L] nog-injected dino mutant. E–L show a dorsal view on the top and an animal view [E–H] or vegetal view [I–L] with dorsal right on the bottom. The arrow in C points to a slight duplication of the ventral tail, the arrow in D to extra cells in the blood islands, characteristics of dino mutants. The embryo in C was raised and shown to be a dino homozygote by segregation analysis. The embryos in G and H show a dino-like fkd3 expression pattern on the left side, and an expanded fkd3 expression on the right side of the embryo. In K and L, sites with some remaining dino-specific expression of axl and eve1 are marked + and −, respectively.

The remaining, nonidentifiable swirl mutant embryos in these experiments were probably in the fraction that were ventralized or that appeared to be wild-type. In embryos that had maintained a swirl-specific marker gene expression pattern in some regions of the embryo, and that were therefore identifiable as swirl homozygotes, we observed a suppression of fkd3 and axl expression and some restoration of eve1 expression [Fig. 3,I,O]. Thus, expression of Bmp4 can partially rescue the swirl mutant phenotype, although we have so far failed to identify viable swirl homozygotes.

In contrast, we were able to recover adult and fertile dino−/− mutants after the inactivation of BMPs by injection of either tBr RNA or noggin RNA. At larval stages, the injected mutants exhibited a dramatic rescue of the mutant phenotype over the entire length of the body axis [Fig. 4,C,D] (tBR: 39/225 identifiable as dino, 30/39 clearly rescued; nog: 3/103 identifiable as dino, 2/3 clearly rescued). Test matings of tBr-rescued adult mutants confirmed their identity as dino homozygotes as the mutant allele was transmitted to all offspring [6/6]. Rescue was dose-dependent. As with wild-type embryos, injection of increasing amounts of either RNA led to a typical snailshell-like dorsalized phenotype in all of the injected embryos derived from dino heterozygous intercrosses [tBr: 200pg, 115/122 dorsalized; nog: 20pg, 246/246 dorsalized]. Thus, the dino mutant phenotype can be rescued or converted into the opposite, dorsalized phenotype, depending on the degree of BMP inactivation.

The response of dino mutants is evident at gastrula stages. In mosaic embryos that can be unambiguously identified as dino homozygotes by the partial retention of a dino-specific gene expression pattern, there is a complementary restoration of fkd3 and axl expression and a suppression of dorsolateral eve1 expression [Fig. 4,G,H,K,L]. Together, these results suggest that the dino mutant phenotype results from the observed lack of BMP4 suppression, and that Dino functions via the repression of BMP4, and/or a related ventralizing signal, on the dorsal side of the embryo.

swirl is epistatic to dino

Genetic data supporting this model come from the analysis of dino–swirl double mutants. According to Mendelian segregation of two unlinked loci, 3/16 (18.75%) of the offspring from a cross of dino/+ × swirl/+ double heterozygous parents should be homozygous for dino, 3/16 homogygous for swirl, and 1/16 (6.25%) homozygous for both dino and swirl. However, we found that, rather than 18.75%, approximately one-quarter of the offspring displayed the dorsalized phenotype typical for swirl single mutants [375/1508, 24.9%, 10 crosses], while dino mutant embryos occurred in the expected proportion [173/930, 18.6%, 8 crosses]. No new doublemutant category was observed. The same distribution of phenotypes was found at gastrula stages when embryos were stained with molecular markers [39/140 were dorsalized], indicating that the dino–swirl double mutant has the same phenotype as the swirl single mutant, and that swirl is epistatic to dino. Clearly, if Dino acts as a repressor of BMPs, there would be no requirement for dino activity in a swirl mutant background in which the ventralizing BMP activity is missing from early gastrula stages [Fig. 11,L].

Interestingly, the partial loss of the swirl-dependent ventralizing activity seems to compensate for the loss of dino. While embryos of the swirl category were uniformly dorsalized, dino mutant embryos displayed significant differences in the strength of their phenotype. Only 8.2% [77/930] embryos showed the strong dino phenotype normally found in a cross of two dino single heterozygotes [Fig. 5B,F,H,J], while 11.4% [106/930] showed a significantly weaker phenotype. Morphologi-
Dino is required on the dorsal side and functions in a non-cell-autonomous fashion

To further address the role of Dino as a dorsal repressor of ventralizing activities, we performed cell transplantation experiments. Labeled wild-type cells were transplanted into embryos from a dino heterozygous intercross at both the sphere stage, prior to gastrulation, when the dorsal side cannot be identified in donors and hosts, and at the shield stage, shortly after the onset of gastrulation, when wild-type cells can be taken from and transplanted into defined dorsoventral positions. In all sets of transplantation experiments, the fraction of normal chimeric embryos was close to 75% (110/144), suggesting that a complete rescue of dino mutants did not occur. However, most mutant chimeric embryos with a successful dorsal transplantation of wild-type cells showed a significantly weaker phenotype than nonchimeric mutant siblings, when the cells were transplanted at either the sphere (4/5, Fig. 6C) or shield stage (d → d; 8/11, Fig. 6E,F). In contrast, transplantation of wild-type cells to the ventral side of host embryos had no effect, regardless of whether they were taken from the dorsal or the ventral side at the shield stage (d → v, 0/5; v → v, 0/4; not shown) or at the sphere stage (0/9, Fig. 6D).

The transplantation of dorsal wild-type cells into the
dorsal shield normally led to chimeric embryos with rather few (50–100) wild-type cells restricted to anterior structures of the chimeric larvae (Fig. 6E,F). Nevertheless, the phenotype of these chimeras appeared normalized over the entire length of the axis. The normalizing effect of transplanted wild-type cells was already apparent at gastrula stages. Chimeric mutant embryos, with wild-type cells in the region of the presumptive prechordal plate and the notochord, displayed a significant retraction of the expression of the ventrolateral marker gene *eve1* in regions that are completely devoid of wild-type cells (19/31, Fig. 6H). A weaker, unilateral retraction of *eve1* expression was also observed when wild-type cells were transplanted into a lateral position (9/10, not shown). However, no retraction of *eve1* expression was observed when dorsal *dino* mutant cells were transplanted into either dorsal or lateral positions of *dino* mutants (d → d, 0/17, Fig. 6I; d → l, 0/2, data not shown). Together these data suggest that the *dino* gene product acts in a non-cell-autonomous fashion and is required on the dorsal side of the early embryo to repress ventrolateral fates at early gastrula stages.

**Discussion**

*dino* and *swirl* regulate the dorsoventral patterning of both the mesoderm and the ectoderm

In this paper we describe several experiments carried out with two different zebrafish mutants displaying defects in early dorsoventral pattern formation, the ventralized mutant *dino* and the dorsализed mutant *swirl*. In both mutants, the dorsoventral patterning of both the mesoderm and the ectoderm is affected, and the expansion or reduction of the ventrolateral or dorsolateral mesoderm is coupled with an expansion or reduction of epidermal fates or the neuroectoderm. This indicates that *dino* and *swirl* regulate the dorsoventral patterning of both the marginal and the animal zone of the early gastrula embryo, similar to the recently demonstrated activities of the ventralizing BMPs and the dorsализing signals from Spemann’s organizer in *Xenopus* embryos [e.g., Sasai et al. 1995].

**Requirement of a BMP-like ventralizing signal and a dorsal repressor of BMP signaling**

Although the molecular nature of the *dino* and *swirl* genes is not known as yet, the findings provided in this paper provide compelling genetic evidence that BMP signaling is indeed required for normal ventral development in the vertebrate embryo. In *swirl* mutant embryos, the loss of ventral fates in derivatives of both the marginal and animal zones of the embryo is anticipated by a progressive loss of the ventrolateral expression of *Bmp4*. Further, all aspects of the *swirl* mutant phenotype can be phenocopied and rescued by modulating BMP signaling in wild-type and mutant embryos, respectively.

Interestingly, inactivation of ventral BMP signaling in *Xenopus* animal caps and whole embryos by the injection of mRNA encoding a dominant negative BMP receptor leads not only to a repression of epidermal and ventrolateral mesodermal cell fates, but simultaneously to an activation of neuroectodermal and dorsolateral mesodermal fates [Grafi et al. 1994; Maeno et al. 1994; Suzuki et al. 1994; Sasai et al. 1995; Schmidt et al. 1995b]. Consequently, the establishment of dorsal fates appears to occur by a default pathway, when ventralizing signals are absent or inhibited. During normal development, inhibition of ventralizing signals on the dorsal side of the embryo most likely is regulated by dorsализing signals from Spemann’s organizer. These include *Noggin* [Smith and Harland 1992], *Chordin* [Sasai et al. 1994], and *Follistatin* [Hemmati-Brivanlou et al. 1994], all of which are expressed in the organizer and which counteract BMP signaling [Sasai et al. 1995].

We provide genetic evidence indicating that Dino acts in this way in the zebrafish. Consistent with the activities of *noggin* and *chordin* in ectopic expression studies in *Xenopus*, Dino is required for both the dorsализation of lateral mesoderm in the marginal zone and the induction of neuroectoderm in the dorsal animal zone of the early gastrula zebrafish embryo. Both of these inductions appear to be achieved indirectly via a suppression of BMPs or related ventralizing signals. If Dino acted as an active dorsализer, rather than as a suppressor of ventralizing signals, the dorsal deficiencies in *dino* mutants should not be rescued by the inactivation of ventralizing activities. However, we found that the injection of mRNA encoding a truncated BMP receptor or *Noggin* fully compensates for the loss of *dino* in mutant embryos. Even more important, the generation of *dino–swirl* double mutants does the same, and *swirl* is epistatic to *dino*. This suggests that *dino* encodes not an active dorsализer, but rather a suppressor of a BMP or a similar swirl-dependent ventralizing activity. Moreover, as we were able to raise normal and fertile homozygous *dino* mutant fish after transient expression of RNAs, it would appear that *dino* has no essential function in later developmental processes. In the cell transplantation experiments, the transplantation of wild-type cells into the dorsal shield of early gastrula mutant embryos resulted in a retraction of ventrolateral fates and a rescue of the *dino* mutant phenotype. This indicates that the BMP-repressive function of Dino is required during gastrulation, the same period when BMPs have been shown to be active in *Xenopus* embryos [Jones et al. 1996]. Furthermore, Dino function appears to be restricted to the dorsal side of the embryo, the site of Spemann organizer activity. This finding makes it very unlikely that the *dino* mutant phenotype is caused by a gain-of-function mutation in a component of the ventralizing pathway. In sum, these data provide genetic evidence for the existence and requirement of a dorsal repressor of ventralizing signals, as proposed from embryological studies in *Xenopus*.

**The morphogenetic character of the ventralizing activity**

The antagonizing effects of *dino* and *swirl* in the double-
mutant analysis and of dino and BMP in the injection studies appear to be dose-dependent, as suggested by the observation that the partial inactivation of ventralizing BMP signaling and the partial loss of swirl lead to a rescue of the dino mutant phenotype, while a stronger inactivation of BMP signaling and the complete loss of swirl lead to a conversion of ventralized embryos into the opposite, dorsalized phenotype. This dose-dependence points to a morphogenetic character of the signals involved in the regulation of dorsoventral pattern formation. According to our data, BMP4 or a similar swirl-dependent ventralizing signal functions as the dorsoventral morphogen, while the suppressive function of Dino ensures that this morphogenetic activity is indeed present in the form of a gradient along the dorsoventral axis of the early gastrula embryo, with the highest activity at the ventral and the lowest activity at the dorsal side. According to this model, positional identities along the D-V axis are defined by the action of one, ventralizing morphogen. However, we cannot rule out the existence of an as yet unidentified opposing dorsalizing morphogen.

Dino function and BMP autoregulation

Until the molecular nature of the dino gene is determined, we can only speculate about how Dino might act in repressing BMP activity and Bmp4 expression. The non-cell-autonomous Dino function indicates that it acts as a signaling molecule or upstream of one. It might be a conventional signal that triggers the activation of a transcriptional repressor or the inactivation of a transcriptional activator of the Bmp gene in the dorsolateral target cells. In this case, the absence of dino in the mutant would lead automatically to the observed enhanced dorsolateral Bmp4 transcription. Alternatively, Dino might act similarly to Chordin and Noggin by binding BMP [Piccolo et al. 1996; Zimmerman and Harland, 1996], thereby preventing the binding of BMPs to their receptor. If this were the case, the enhanced dorsolateral Bmp4 transcription in dino mutants could be explained only if the ventralizing BMPs are positive regulators of their own expression. According to this model, the positive autoregulation would be attenuated on the dorsal side of the wild-type embryo by Dino titrating out free BMP proteins. Such a positive feedback has been suggested from ectopic expression studies in Xenopus [Jones et al. 1992] and has been described for the Bmp4 homologue decapentaplegic (dpp) in the visceral mesoderm during Drosophila midgut morphogenesis [Reuter et al. 1990; Panganiban et al. 1990].

Parallels in dorsoventral pattern formation among flies and vertebrates

The mechanisms of dorsoventral pattern formation in the Drosophila embryo are well understood. Here, the secreted protein Decapentaplegic [Dpp] acts as a dorsalizing morphogen [Padgett et al. 1987; Ferguson and Anderson 1992a] that is counteracted by the signal Short gastrulation [Sog, François et al. 1994] on the ventrolateral side of the early gastrula embryo. Dpp and Sog are structurally related to the Xenopus proteins BMP4 and Chordin, respectively, and the Drosophila and Xenopus homologues can substitute or mimic each other when ectopically expressed in the other organism [Holley et al. 1995; Smith 1995; DeRobertis and Sasai 1996]. Here, we provide genetic evidence in support of such a mechanistic conservation. Similar to dino and Bmp4 in the zebrafish, sog is required for the refinement of dpp expression in Drosophila [François et al. 1994]. Furthermore, similar to dino and swirl, sog antagonizes dpp genetically in epistasis analyses [Ferguson and Anderson 1992b; Wharton et al. 1993; François et al. 1994].

Materials and methods

For all experiments, the dino allele dinomnd and the swirl allele swtor were used.

In situ hybridization and immunostainings

Whole-mount in situ hybridizations, immunostainings, and double stainings were carried out as described previously [Hammerschmidt et al. 1996b].

RNA and DNA injection

For mRNA injection, the plasmids pSP64T-tBr [Graft et al. 1994] and pSP64Bm-noggin (gift of R.M. Harland, University of California, Berkeley) were linearized with EcoRI, and sense RNA was synthesized using the message machine kit (Ambion) according to the suppliers’ instructions. Approximately 100 pg of tBr mRNA or 5 pg of nog mRNA per embryo were injected into the yolk or into single blastomeres of one- to four-cell-stage embryos as previously described [Hammerschmidt et al. 1996a].

For DNA injection, ~20–40 pg [2 nl] of CsCl gradient-purified pCSKA–BMP4 [Sasai et al. 1995] plasmid DNA was injected into the cytoplasm of one-cell-stage or both blastomeres of two-cell-stage wild-type embryos.

Cell transplantation

The transplantation technique was similar to that described by Ho and Kane [1990]. At the one-cell stage, donor embryos were injected with lysinated rhodamine dextran or biotinylated dextran (MW 10,000; Molecular Probes, Eugene, OR) at a concentration of 0.1 mg/ml. Donor embryos were derived from crosses of two wild-type or two dino heterozygous parents. In the latter case, the donor embryos were kept after the transplantation to determine by their later phenotype if they were wild-type or dino mutant. Host embryos were derived from a din/+ x din/ + cross. Five different transplantations were carried out between donors and hosts of identical stages: (1) transplantation at the sphere stage without a priori knowledge of the position of the transplanted cells in donors and hosts; (2–5) transplantation at shield stage: (2) from the dorsal shield into the dorsal shield [d → dl]; (3) from the dorsal shield into the ventral side [d → v],
from the dorsal shield into a lateral position (d → l), (5) from the ventral side into the ventral side of the hosts (v → v). Chimeric mice that had received rhodamine-labeled cells were analyzed at day 2 of development. Brightfield and fluorescent images were captured with a CCD cooled camera and were processed in Photoshop. Wild-type → din chimeras were judged as rescued when they were more similar to wild-type over the entire body length than their nonchimeric sibling with the mildest mutant phenotype. As a negative control of the transplantsations at the shield stage to rule out that the rescue of the din mutants is caused by the injury of the shield rather than by the introduction of the wild-type cells, shield cells were taken out and implanted back into the shield of the same embryo. In no din mutant embryo treated this way (0/11) was a weakening of the phenotype observed. No formation of a partial or complete secondary axis was observed in → v transplantsations, regardless of whether the recipients were din (0/4) or wild-type (0/11). This indicates a crucial difference between transplantsations to the dorsal and transplantsations to the ventral side. It seems that a few wild-type cells transplanted into the dorsal shield can supplement the din mutant shield to form a normal body axis, while the induction of a secondary axis in the ectopic ventral environment requires an intact shield (Ho 1992, Shih and Fraser 1996).

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