

Interpretation of a BMP Activity Gradient in *Drosophila* Embryos Depends on Synergistic Signaling by Two Type I Receptors, SAX and TKV

Minh Nguyen,^{*||} Sangbin Park,^{*||} Guillermo Marqués,[‡] and Kavita Arora^{*†§}

^{*}Department of Developmental and Cell Biology

[†]Developmental Biology Center

University of California

Irvine, California 92697

[‡]Instituto Cajal

CSIC

Dr. Arce 37

28002 Madrid

Spain

Summary

Graded activity of the DPP signaling pathway is critical for specification of dorsal embryonic cell fates in *Drosophila*. We present evidence that a second BMP ligand, SCW, potentiates DPP activity. Using dominant-negative forms of the type I receptors SAX and TKV, we demonstrate that SAX mediates the SCW signal, while TKV is required for both DPP and SCW activity. We find that while DPP/TKV signaling is obligatorily required, SCW/SAX activity is necessary but not sufficient for dorsal patterning. SAX and TKV act synergistically, suggesting a mechanism for integration of the SCW and DPP signals. Further, we show that the extracellular protein SOG can antagonize SCW, thus limiting its ability to augment DPP signaling in a graded manner.

Introduction

Morphogen gradients play a key role in the establishment of cell identity during embryogenesis in organisms across the phylogenetic spectrum. Several secreted proteins belonging to the transforming growth factor β (TGF β) superfamily are candidate morphogens since they influence growth and patterning in a concentration-dependent manner (reviewed in Hogan, 1996; Lawrence and Struhl, 1996; Neumann and Cohen, 1997). For example, in *Xenopus*, activin and bone morphogenetic protein 4 (BMP4) specify different mesodermal cell fates along the dorsoventral axis in a dose-dependent fashion. In addition, analysis of zebrafish and mice mutants indicates that BMP2 and BMP4 play similar roles in other vertebrates (reviewed in Hogan, 1996; Neumann and Cohen, 1997). Although the signal transduction pathways are fairly well understood, important issues that remain unresolved are how the graded activity of extracellular ligands is generated and how cells interpret this information.

In *Drosophila*, the BMP4 homolog Decapentaplegic (DPP) satisfies the criteria for a classical morphogen in the wing imaginal disc (Padgett et al., 1987; Lecuit et al., 1996; Nellen et al., 1996; Singer et al., 1997). *dpp* is

expressed in a narrow domain of cells at the anterior-posterior compartment boundary and acts as a long-range signal to activate transcription of its target genes, *optomotor blind* and *spalt*, at different concentration thresholds. There is substantial evidence that a gradient of DPP activity is also required for specification of the dorsal region of the embryo (Ferguson and Anderson, 1992a; Wharton et al., 1993). Reduction in the amount of DPP signaling results in progressive loss of dorsal embryonic structures, while increasing concentrations of DPP induce dorsal fates in a dose-dependent manner. However, in contrast to the wing disc, there is no evidence that DPP diffuses extensively from its site of synthesis in the dorsal blastoderm, a prerequisite for an extracellular morphogen. Short gastrulation (SOG), a secreted factor related to *Xenopus* Chordin, is believed to generate an activity gradient of DPP within the dorsal region by preventing DPP from interacting with its receptor (François et al., 1994; Holley et al., 1995, 1996; Schmidt et al., 1995). Similar opposing gradients of Chordin and BMP4 are involved in the establishment of ventral cell fates in vertebrate embryos (Sasai et al., 1995; Piccolo et al., 1997).

We have previously shown that dorsal patterning in the embryo requires a second ligand Screw (SCW) in addition to DPP (Arora et al., 1994). Phenotypic studies suggest an absolute requirement for DPP activity, since all dorsal cell fates are lost in *dpp* mutants (Irish and Gelbart, 1987). In contrast, *scw* function is critical for patterning the dorsal-most cells (Arora and Nüsslein-Volhard, 1992). SCW shares \sim 48% identity with BMP5/6/7 and 40% identity with DPP in the ligand domain (Arora et al., 1994). The requirement for two related ligands in specification of the same region seems paradoxical, given the unconditional requirement for DPP, and raises a number of questions about the mechanism by which multiple signals are integrated in the receiving cell.

According to the current paradigm, BMP ligands signal through a heteromeric complex of type I and type II receptor serine-threonine kinases. Formation of the ligand-receptor complex allows the type II kinase to phosphorylate and activate the type I receptor (reviewed in Derynck and Feng, 1997; Heldin et al., 1997; Kretschmar and Massagué, 1998). In *Drosophila*, a single type II receptor, Punt, and two type I receptors, Thick veins (TKV) and Saxophone (SAX), have been identified (Brummel et al., 1994; Nellen et al., 1994; Penton et al., 1994; Xie et al., 1994; Letsou et al., 1995; Ruberte et al., 1995). While all three receptors have been implicated in the DPP pathway, their *in vivo* specificity has not been determined, leaving open the possibility that they mediate signaling by other ligands. Activation of the type I receptors results in phosphorylation of specific members of the SMAD family of proteins, causing their translocation from the cytoplasm into the nucleus. Genetic screens have led to the identification of Mothers against *dpp* (MAD) and Medea, two SMAD proteins that are involved in DPP signaling (Rafferty et al., 1995; Newfeld et al., 1997). SMADs contain DNA-binding domains and

[§]To whom correspondence should be addressed (e-mail: karora@uci.edu).

^{||}These authors contributed equally to this work.

are thought to regulate the expression of downstream target genes in association with other transcription factors (reviewed in Derynck and Feng, 1997; Heldin et al., 1997; Kretzschmar and Massagué, 1998).

In this paper, we examine the differential requirement for SCW and DPP activity in patterning the dorsal region of the *Drosophila* embryo. We establish that the two ligands display a nonreciprocal relationship in their ability to compensate for one another. SCW is dependent on DPP for its biological activity and functions by potentiating the DPP signal. The interdependence of SCW and DPP signaling is reminiscent of the relationship displayed by SAX and TKV (Nellen et al., 1994; Ruberte et al., 1995). We have used dominant-negative and constitutively activated forms of SAX and TKV to show that SAX transduces the SCW signal, while TKV is required for mediating both SCW and DPP signaling. We also demonstrate a striking synergy between SAX and TKV that may explain how the SCW and DPP signals are integrated in the embryo. Finally, we show that SOG can antagonize SCW activity far more effectively than DPP activity. Thus, diffusion of SOG from the ventral side of the embryo is likely to result in a dorsal gradient of SCW activity that potentiates DPP signaling in a graded manner. The results described in our study provide a novel framework for understanding the relative contribution of multiple BMP signals to cell fate specification and patterning in vertebrate embryos.

Results

SCW Potentiates DPP Signaling in the Embryo

During development, the dorsal-most cells in a wild-type embryo form the amnioserosa, while flanking dorso-lateral cells give rise to the dorsal ectoderm. In a *dpp* null mutant, all dorsal cell fates are missing and the embryos are completely ventralized (Irish and Gelbart, 1987). In contrast, embryos mutant for *scw* are partially ventralized and lack amnioserosa but differentiate a reduced dorsal ectoderm (Arora and Nüsslein-Volhard, 1992). The relative severity of the *dpp* and *scw* mutant phenotypes is not correlated with their expression patterns, since *scw* is transcribed uniformly at syncytial blastoderm stage and *dpp* expression is restricted to the dorsal side of the embryo (St. Johnston and Gelbart, 1987; Arora et al., 1994). An explanation for the different efficacies of the two ligands could be that they differ in abundance or have different affinities for their receptors. Alternatively, the ligands could evoke qualitatively different responses, perhaps by acting through different receptors.

To distinguish between these alternatives, we assayed the ability of SCW mRNA to restore dorsal pattern in *dpp* null embryos. If the difference in the *scw* and *dpp* mutant phenotypes simply reflects their effective concentrations, excess SCW should compensate for the loss of *dpp* function. We first assessed the biological activity of SCW by testing its ability to specify amnioserosa cells in embryos that lack endogenous SCW (Figure 1). Injection of in vitro-transcribed SCW at 0.5 $\mu\text{g}/\mu\text{l}$ rescued amnioserosa in 94% of the embryos (Figures 1A–1C; Table 1A). In contrast, injection of SCW into *dpp*

null embryos did not result in induction of amnioserosa, even at concentrations as high as 5 $\mu\text{g}/\mu\text{l}$ (Figure 1D; data not shown). In the converse experiment, injection of DPP rescued dorsal cell fates in strong loss-of-function (*scw^{S12}*) as well as *scw* null embryos (Figures 1E–1G; Arora et al., 1994). The failure of SCW to restore amnioserosa in embryos that lack *dpp* function suggested that SCW and DPP act in qualitatively distinct ways.

An important inference from these results is that SCW does not signal effectively in the absence of DPP and may be required to enhance a basal level of DPP activity. To test this idea, we examined the dose response to DPP in the presence or absence of SCW. Different concentrations of DPP were injected into embryos derived from flies heterozygous for a deficiency that deletes *scw*, *Df(2L)OD16* (Arora et al., 1994). This allowed us to assay the response of protein null (0 \times SCW) and hemizygous *scw* (1 \times SCW) embryos simultaneously. Control *scw⁻* embryos do not differentiate any amnioserosa cells, while those in the 1 \times SCW class have wild-type amnioserosa. Injection of DPP induced amnioserosa in a dose-dependent manner in both classes of embryos (Figures 1E–1H). In addition, the response to a given concentration of DPP was more robust in 1 \times SCW embryos. For example, at 30 ng/ μl of DPP only 8% of embryos lacking *scw* protein (0 \times SCW) differentiated a circumferential amnioserosa, while a higher proportion (62%) of 1 \times SCW embryos showed the same effect. We conclude that SCW potentiates DPP activity and that the magnitude of the response to DPP is dependent on SCW.

SCW Function Is Not Dependent on Dimerization with DPP

Proteins of the TGF β family are synthesized as disulfide-linked dimers that are cleaved to release the mature ligand (Massagué, 1990). We have previously hypothesized that SCW could augment DPP signaling by forming heterodimers with DPP that are more potent than homodimers of either ligand (Arora et al., 1994). To determine whether SCW function in vivo requires the formation of SCW/DPP heterodimers, we used a heterologous *twist* (*twi*) promoter to drive expression of the *scw* cDNA in ventral cells and tested its ability to restore dorsal cell fates in *scw* null embryos (Figure 2A; Jiang and Levine, 1993). Since dimerization occurs intracellularly and *dpp* is transcribed in the dorsal 40% of the embryo at blastoderm stage (St. Johnston and Gelbart, 1987; Ray et al., 1991), the restriction of SCW expression to ventral cells should eliminate production of SCW/DPP heterodimers in embryos lacking endogenous SCW. We observed 62% rescue of the *Df(2L)OD16* homozygotes using two copies of P[*twi*>SCW] and 100% rescue with four copies of the transgene. The rescued embryos showed only slight defects in head involution and differentiated both dorsal epidermis and amnioserosa (Figures 2B–2D). Remarkably, ventrally expressed SCW ligand induces amnioserosa cells in their correct location on the dorsal side of the embryo (Figure 2B; see Discussion). These results strongly argue that formation of SCW/DPP heterodimers is not a prerequisite for the biological activity of SCW in the embryo.

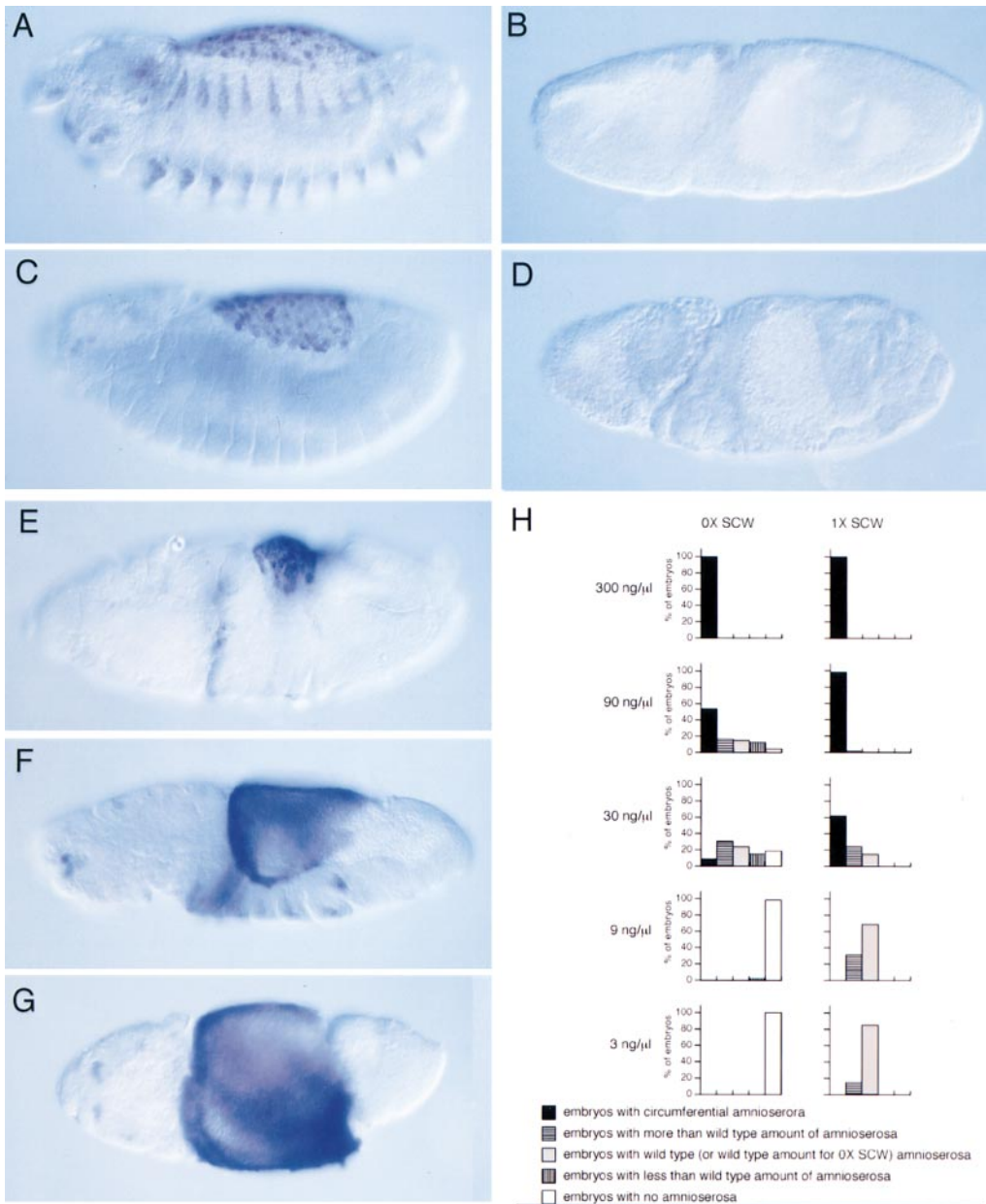


Figure 1. Nonreciprocal Relationship of SCW and DPP Signaling in the Embryo

(A) Lateral view of a wild-type embryo at stage 15 stained to visualize a P[Kr-lacZ] amnioserosa reporter expressed in ~200 cells in the dorsal midbody region. The segmental staining is derived from a P[wg-lacZ] insert in this embryo. In all figures, embryos are oriented anterior to the left and dorsal side up, unless stated otherwise. (B) Amnioserosa is absent in *Df(2L)OD16* embryo lacking the *scw* gene. (C) Injections of SCW (0.5 μg/μl) into *scw*⁻ embryos resulted in induction of amnioserosa and complete rescue of the ventralized morphology. Injection of SCW into *dpp*¹⁴⁸ null embryos at the same (D) or a 10-fold higher concentration (data not shown) did not induce amnioserosa. (E–H) Embryos derived from parents of the genotype *Df(2L)OD16*, P[Kr-lacZ]/*CyO*, P[hb-lacZ], were injected with different concentration of DPP. The P[hb-lacZ] pattern enabled the distinction between *scw*⁻ (0× SCW) and hemizygous (1× SCW) embryos. Injected embryos were classified into five categories based on the magnitude of their response. The data are represented graphically in (H). No amnioserosa, open bar; <wild-type amounts of amnioserosa, vertical stippled bar (see 1E); wild-type amounts of amnioserosa, gray bar; >wild-type amounts of amnioserosa—polar, horizontal stippled bar (see 1F); >wild-type amounts of amnioserosa—circumferential, closed bar (see 1G). Injection of 9 ng/μl DPP induced relatively small patches of amnioserosa in *scw* null embryos (E). Moderate levels of DPP (30 ng/μl) caused an expansion of amnioserosa, but a majority of the embryos retained dorsoventral polarity (F). At higher concentrations, the *scw*⁻ embryos differentiated amnioserosa all around the circumference (G). Injection of DPP resulted in a dose-dependent response in both 0× SCW and 1× SCW embryos (H). However, the magnitude of the response at a given concentration of DPP was distinctly higher in the 1× SCW embryos.

Table 1. Amnioserosa Induction in Ventralized Embryos in Response to mRNA Injections

mRNA Injected	Embryos with Amnioserosa (%)
(A) SCW mRNA injections into <i>scw</i>⁻ embryos	
SCW (0.2 μg/μl)	38
SCW (0.3 μg/μl)	43
SCW (0.4 μg/μl)	77
SCW (0.5 μg/μl)	94
(B) Ligand alone or ligand plus dominant-negative receptor mRNA injections into <i>scw</i>⁻ embryos	
SCW (0.4 μg/μl)	76
SCW (0.4 μg/μl) + DN SAX (2.0 μg/μl)	35
SCW (0.4 μg/μl) + DN SAX (4.0 μg/μl)	4
SCW (0.4 μg/μl) + DN TKV (2.0 μg/μl)	38
SCW (0.4 μg/μl) + DN TKV (4.0 μg/μl)	19
DPP (20 ng/μl)	71
DPP (20 ng/μl) + DN SAX (2.0 μg/μl)	67
DPP (20 ng/μl) + DN SAX (4.0 μg/μl)	69
DPP (20 ng/μl) + DN TKV (2.0 μg/μl)	36
DPP (20 ng/μl) + DN TKV (4.0 μg/μl)	11
(C) Activated <i>sax</i> (SAX-A) and activated <i>tkv</i> (TKV-A) mRNA injections into <i>dpp</i>⁻ embryos	
SAX-A (1.0 μg/μl)	0
SAX-A (3.0 μg/μl)	0
SAX-A (7.5 μg/μl)	0
SAX-A (15.0 μg/μl)	0
TKV-A (1.0 μg/μl)	4
TKV-A (3.75 μg/μl)	29
TKV-A (7.5 μg/μl)	47
TKV-A (15.0 μg/μl)	73
SAX-A (1.0 μg/μl) + TKV-A (1.0 μg/μl)	27
SAX-A (3.0 μg/μl) + TKV-A (3.75 μg/μl)	92
(D) Ligand alone or ligand plus <i>SOG</i> mRNA injections into <i>scw</i>⁻ embryos	
SOG (0.125 μg/μl)	93
SOG (0.25 μg/μl)	85
SOG (0.5 μg/μl)	50
SOG (1.0 μg/μl)	27
SOG (2.0 μg/μl)	0
SCW (0.4 μg/μl) + SOG (0.5 μg/μl)	0
DPP (20 ng/μl) + SOG (0.5 μg/μl)	72
DPP (20 ng/μl) + SOG (2.0 μg/μl)	75

scw⁻ embryos were derived from *Df(2L)OD16*, P[Kr-LacZ]/*CyO* stocks. A P[Kr-lacZ]; *dpp*¹⁴⁹/*CyO23* stock was used to obtain embryos lacking *dpp* activity. For each set of experiments reported above, greater than 100 embryos of the relevant genotype were injected and scored. Embryos that differentiated more than 2 or 3 cells that expressed Kr-lacZ and were morphologically recognizable as amnioserosa were scored as positive.

SCW and DPP Act Primarily through Independent Receptors

To understand the basis for the differential response of the embryo to SCW and DPP signaling, we examined the interaction of the ligands with the two type I receptors SAX and TKV. The vertebrate DPP ortholog BMP2 has a higher affinity for TKV than for SAX, suggesting that DPP may signal primarily through TKV (Brummel et al., 1994; Penton et al., 1994). Consistent with this view, complete loss of *tkv* in the embryo and in imaginal discs mimics the loss of DPP function (Nellen et al., 1994; Terracol and Lengyel, 1994; Burke and Basler, 1996).

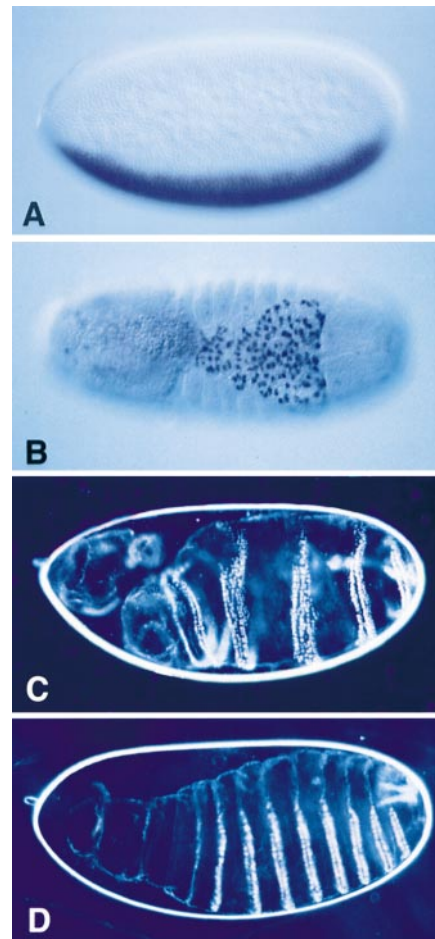


Figure 2. Ventrally Expressed SCW Can Restore Dorsal-Most Cell Fates in *scw* Mutant Embryos

(A) Lateral view of a *scw* null embryo at blastoderm stage, derived from a *Df(2L)OD16/CyO*, P[wg-lacZ] stock carrying a P[twi>SCW] transgene. The embryo was hybridized with an antisense riboprobe for detecting *scw* transcripts. The *twi* promoter restricts *scw* cDNA expression to the ventral-most 12–14 cells. Two copies of the P[twi>SCW] transgene drive expression at levels comparable to wild-type *scw* expression.
 (B) Dorsal view of a stage 15 embryo of the same genotype as in (A) stained with an anti-KR antibody to detect amnioserosa. *scw*⁻ embryos carrying two or more copies of the P[twi>SCW] transgene differentiate amnioserosa cells in their correct dorsal position.
 (C) A moderately ventralized cuticle from a *Df(2L)OD16* homozygous embryo showing extended ventral denticle belts and retracted posterior abdominal segments.
 (D) *scw* null embryos rescued by the P[twi>SCW] transgene differentiated a relatively normal cuticle, including the filzkörper typical of dorsolateral cell fates.

In contrast, *sax* mutant embryos display a weaker ventralized phenotype, similar to the *scw*⁻ phenotype (Brummel et al., 1994; Xie et al., 1994). Defects associated with the loss of *sax* function in imaginal discs are also less severe compared to *tkv* mutant clones, and it has been suggested that SAX may play a purely facilitatory role by augmenting the TKV signal (Ruberte et al., 1995; Singer et al., 1997).

The similarity of the relationship displayed by the receptors SAX/TKV and the ligands SCW/DPP raises the

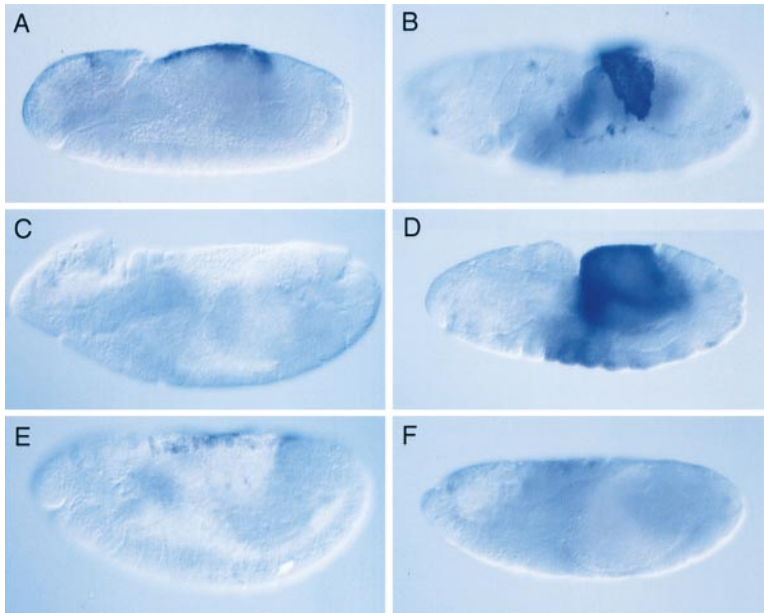


Figure 3. The SAX Receptor Primarily Mediates SCW Activity, while TKV Is Required for Both DPP and SCW Signaling

Lateral views of stage 15 *scw* null embryos stained to visualize a P[Kr-lacZ] amnioserosa reporter. Dorsal injections with 0.4 $\mu\text{g}/\mu\text{l}$ of SCW (A) or 20 $\text{ng}/\mu\text{l}$ of DPP (B) resulted in induction of near wild-type amounts of amnioserosa in 70%–80% of *scw*⁻ embryos. Injection of 2 $\mu\text{g}/\mu\text{l}$ of SAX-DN inhibited the response to SCW (C) but did not prevent induction of dorsal cell fates by DPP (D). Similar injections using TKV-DN (2 $\mu\text{g}/\mu\text{l}$) interfered with induction of amnioserosa fates by both SCW (E) and DPP (F; see Table 1B).

possibility that SAX mediates SCW activity. To address this question, we used dominant-negative forms of SAX and TKV (SAX-DN and TKV-DN) to test whether they specifically blocked signaling by one or both ligands. These receptors retain the ligand-binding domain but lack the intracellular kinase domain and are thought to inhibit signaling by titrating out the ligand (Haerry et al., 1998). We used the ability of SCW and DPP to specify amnioserosa in *scw*⁻ embryos as an assay for ligand activity. Concentrations of ligand mRNA that elicit biologically equivalent responses (amnioserosa induction in 70%–80% of *scw*⁻ embryos) were used as a baseline (Table 1B; Figures 3A and 3B). We found that the response to SCW mRNA was significantly reduced by coinjection with 2 $\mu\text{g}/\mu\text{l}$ of SAX-DN and abolished at higher concentrations (Table 1B; Figure 3C). In marked contrast, SAX-DN was ineffective in attenuating the response to DPP (Table 1B; Figure 3D). Unlike SAX-DN, the TKV-DN receptor was able to inhibit the response to both SCW and DPP. Coinjection of 4 $\mu\text{g}/\mu\text{l}$ of TKV-DN mRNA reduced the peak response to SCW and DPP by 4- and 6-fold, respectively, but did not abolish the response to either ligand (Figures 3E and 3F; Table 1B). Thus, TKV function is required for the response to both ligands, while the ability of SAX-DN to interfere specifically with SCW and not DPP signaling strongly argues that SAX preferentially mediates the response to SCW.

To determine whether the interactions observed with SAX-DN and TKV-DN extend to other tissues in which DPP signaling is required, we used the UAS-GAL4 system to coexpress these proteins in the wing imaginal discs (Brand and Perrimon, 1993; Figures 4A–4C). *dpp* is expressed extensively during larval and pupal development and has a well-characterized role in growth and patterning of imaginal disc derivatives (reviewed in Gelbart, 1989; Lawrence and Struhl, 1996; Neumann and Cohen, 1997). In contrast, expression of the *scw* gene is confined to early blastoderm embryos and cannot be detected later in development (Arora et al., 1994).

Ubiquitous expression of a single copy of UAS:SCW in the wing disc, using the A9 driver, produces adult wings with ectopic venation and thickening of the normal veins (Figure 4A). The posterior compartment is enlarged, and the distal region often contains a blister. These phenotypes are comparable to the effect of ectopic expression of DPP at low levels (data not shown). Expression of higher levels of DPP results in a stronger phenotype characterized by small blistered wings that have pigmentation and hairs typical of vein tissue (Figure 4A).

SAX and TKV display a ligand specificity in imaginal discs that is similar to their specificity during embryogenesis. Flies expressing two copies of UAS:SAX-DN have wings that are narrower than wild type and lack longitudinal vein L5 and the posterior crossvein (Figure 4B). This phenotype may result from inhibition of endogenous signaling by DPP or a related ligand required for growth of the wing disc and vein differentiation (Singer et al., 1997). Expression of a single copy of UAS:SAX-DN in the wing caused no patterning defects (data not shown) but suppressed the ectopic SCW phenotype to almost wild type (Figure 4B). SAX-DN has no inhibitory effect on UAS:DPP signaling in the wing. In contrast, coexpression of TKV-DN suppressed the phenotypes resulting from both DPP and SCW ectopic expression. As seen in the embryo, suppression by TKV-DN was partial. Expression of TKV-DN alone resulted in defects similar to those caused by expression of SAX-DN but that affect different regions of the wing. The L2 and L4 veins were partially lost (Figure 4C). Wings from animals coexpressing SCW and TKV-DN no longer developed blisters, but ectopic venation and overgrowth of the region posterior to L5 persisted. Suppression of the ectopic DPP phenotype by TKV-DN resulted in wings that were closer to wild type in size, and the longitudinal veins L1–L3 were restored.

Our results provide evidence that TKV mediates signaling by both SCW and DPP, while SAX is dedicated to transducing the SCW signal. This view is supported by

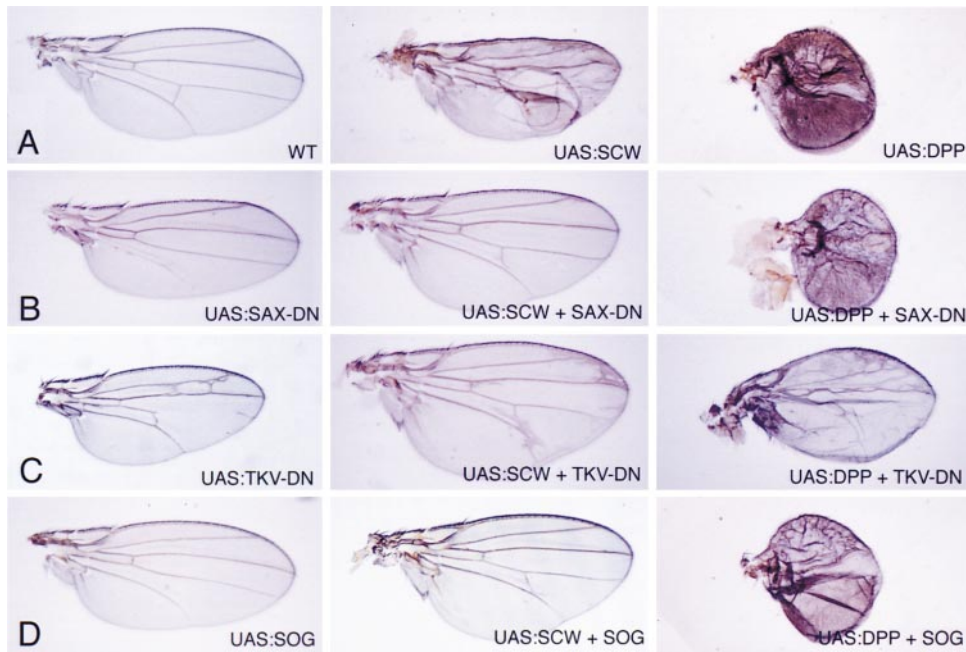


Figure 4. SCW and DPP Activity in the Wing Is Differentially Sensitive to Dominant-Negative Receptors and SOG

(A) Phenotypes resulting from ectopic expression of UAS:SCW and UAS:DPP are compared with a wild-type wing. These wings serve as controls for (B–D). The A9:GAL4 line used in these experiments drives ubiquitous expression at high levels in wing imaginal discs. At late third instar stages, expression is enhanced in the dorsal wing pouch. All wings are from female flies reared at 25°C. Overexpression of SCW resulted in blistering and ectopic venation reminiscent of low levels of ectopic DPP. Strong expression of DPP caused overgrowth of discs but resulted in small and blistered adult wings characterized by pigmentation and hairs typical of vein tissue.

(B) Expression of two copies of UAS:SAX-DN in a wild-type background resulted in narrow wings, partial loss of the longitudinal vein L5, and the posterior crossvein. Coexpression of a single copy of UAS:SAX-DN strongly suppressed the UAS:SCW phenotype but had no effect on the UAS:DPP phenotype.

(C) Expression of two copies of UAS:TKV-DN resulted in partial loss of vein L4, while L2 began to merge with L3. Coexpression of a single copy of UAS:TKV-DN partially suppressed the ectopic SCW phenotype. However, signs of ectopic venation persisted, and the characteristic overgrowth of the posterior compartment was apparent. Suppression of the UAS:DPP phenotype by TKV-DN resulted in a wing morphology that is closer to wild type, both in size and appearance.

(D) Expression of two copies of UAS:SOG resulted in loss of the posterior crossvein, a relatively mild phenotype with poor penetrance. Coexpression of a single copy of UAS:SOG resulted in strong suppression of the ectopic SCW phenotype to near wild type. Simultaneous expression of SOG was strikingly ineffective in suppressing the effects of UAS:DPP, even when four copies of UAS:SOG were used (data not shown).

analysis of double mutants lacking receptor and ligand function. Zygotic loss of both *scw* and *tkv* results in a completely ventralized phenotype that is much stronger than the partial ventralization observed in *scw* null embryos, suggesting that TKV mediates signaling by another ligand in addition to SCW (Terracol and Lengyel, 1994). We reasoned that if SAX functions as a receptor for SCW, the *sax*, *scw* double mutant phenotype should be no stronger than either mutant alone. We examined the phenotype of double mutant embryos laid by *sax*², *Df(2L)OD16/sax*¹ mutant females mated with *scw*^{S12} males. These embryos appear similar to *scw*⁻ embryos, as judged by cuticular and amnioserosa phenotypes (data not shown), reinforcing the idea that the primary role of SAX during early embryogenesis is to convey the SCW signal.

SAX and TKV Have a Synergistic Effect on Embryonic Patterning

The distinct roles that SCW and DPP play in embryogenesis may result from inherent differences in the way SAX and TKV function. Recent studies have shown that type

I receptors can be constitutively activated by a change in the GS box that mimics endogenous signaling (Derynck and Feng, 1997; Heldin et al., 1997; Kretzschmar and Massagué, 1998). An activated form of TKV can rescue dorsal cell fates in a *dpp*⁻ embryo and drives nuclear translocation of MAD in COS cells (Holley et al., 1996; Hoodless et al., 1996). We decided to test the potency of constitutively activated forms of SAX (SAX-A) and TKV (TKV-A) to gain an insight into whether these receptors differ in their signaling capacity.

We wished to determine whether SAX-A could override the requirement for DPP in the embryo. Injection of *TKV-A* into *dpp*⁻ embryos resulted in differentiation of amnioserosa in a concentration-dependent manner (Table 1C; Figure 5A). In striking contrast, injection of *SAX-A* over the same 15-fold concentration range (1–15 µg/µl) was ineffective in promoting amnioserosa cell fates (Figure 5B; Table 1C). The observation that *SAX-A* has very limited potency compared to *TKV-A* argues that SAX and TKV may have qualitatively distinct activities. Based on genetic considerations (Brummel et al., 1994; Nellen et al., 1994; Ruberte et al., 1995; Singer et al.,

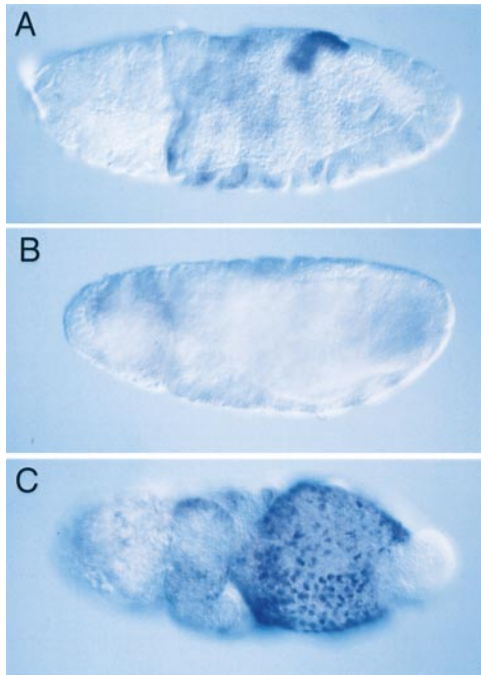


Figure 5. The SAX and TKV Receptors Synergize to Induce Dorsal Cell Fates in the Embryo

Lateral views of stage 15 *dpp*⁴⁴⁸ embryos stained to visualize a P[Kr-lacZ] amnioserosa reporter. While a majority of embryos injected with 1 μg/μl of *TKV-A* were below threshold (see Table 1C), injections with 15 μg/μl of *TKV-A* were sufficient to induce a small but significant amount of amnioserosa in 73% of the *dpp*⁻ embryos (A). Injections of *SAX-A* in the same concentration range did not promote induction of amnioserosa in *dpp*⁻ embryos (B). Coinjection with 3.75 μg/μl of *TKV-A* and 3.0 μg/μl *SAX-A* resulted in synergistic induction of amnioserosa in 92% of embryos lacking endogenous DPP activity, often in a circumferential manner (C; see Table 1C).

1997) and the data presented above, it is likely that SAX is dependent on a basal level of DPP/TKV signaling for its activity.

To test the interdependence of TKV and SAX signaling in embryonic patterning, we coinjected low levels of *TKV-A* along with *SAX-A* (Table 1C; Figure 5). When *dpp*⁻ embryos are injected with *TKV-A* at 1.0 μg/μl, a majority of the embryos are below the threshold for amnioserosa induction (Table 1C). However, coinjection of *TKV-A* and *SAX-A* (each at 1.0 μg/μl) resulted in induction of amnioserosa in 27% of the embryos, a response that was 6-fold greater than with *TKV-A* alone (Table 1C). When higher concentrations of *TKV-A* (3.75 μg/μl) and *SAX-A* (3.0 μg/μl) were tested, 92% of the embryos differentiated an amnioserosa, in contrast to 29% with *TKV-A* alone (Table 1C; Figure 5C). Coinjections of the same concentrations of *TKV-A* with wild-type *SAX*, or *SAX-A* with wild-type *TKV*, did not result in induction of amnioserosa (data not shown). These results demonstrate that coexpression of *SAX-A* and *TKV-A* has a synergistic effect that is sufficient to promote specification of the dorsal-most pattern element. This synergy between *SAX* and *TKV* could provide the basis by which *SCW* potentiates DPP signaling in the embryo (see Discussion).

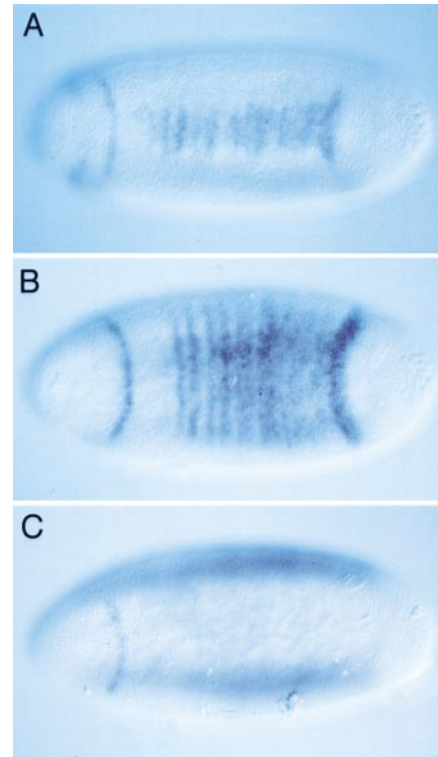


Figure 6. *scw* Is Epistatic to *sog*

The dorsal expression of *rho* at blastoderm stage was used as a marker for examining the epistatic relationship between *sog* and *scw*. Embryos were hybridized with digoxigenin-labeled *rho* and *lacZ* antisense riboprobes. (A) Dorsal view of *rho* expression in a wild-type embryo. (B) *sog*^{Y26} embryos showed expansion of *rho* laterally. Dorsal expression of *rho* was lost in a *scw*⁻ embryo (data not shown), as well as in a *sog*^{Y26}; *Df(2L)OD16* double mutant (C). Mutant embryos were unambiguously identified by the absence of *lacZ* inserts on the *FM7* and *CyO* balancers.

SOG Antagonizes SCW Activity in the Embryo

The *sog* gene is expressed in two ventrolateral domains that about the dorsal domain of *dpp* expression at cellular blastoderm (François et al., 1994). It has been proposed that SOG diffuses dorsally and limits DPP activity by preventing the ligand from binding its receptors (Holley et al., 1996). The dorsally expressed metalloprotease TLD negates this effect by mediating SOG cleavage, thus promoting the release of active ligand (Shimell et al., 1991; Marqués et al., 1997). While these data strongly imply that DPP and SOG can interact, they do not address the question of whether SOG exclusively targets DPP activity in the embryo.

To determine whether *sog* antagonizes *scw* function, we examined the phenotype of *sog*; *scw* double mutant embryos, using the dorsal domain of *rho* expression as a marker for cell fate (Figure 6A; Biehs et al., 1996). This aspect of *rho* expression is abolished in *scw* null embryos (Biehs et al., 1996). In contrast, loss of *sog* function results in an increase in dorsolateral pattern elements and concomitant expansion of dorsal *rho* expression (Figure 6B). As shown in Figure 6C, the phenotype of *sog*; *scw* double mutants is indistinguishable from that of *scw* null embryos. This result demonstrates

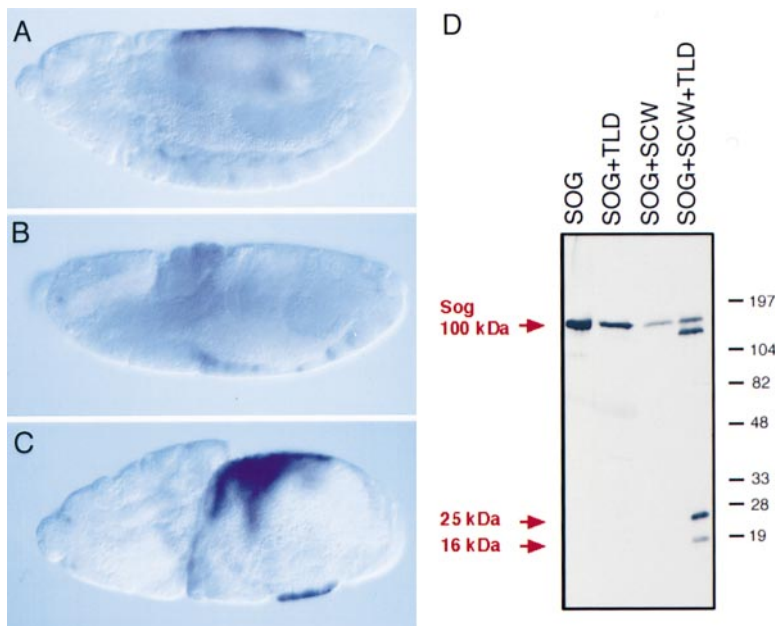


Figure 7. SOG Inhibits the Induction of Dorsal Cell Fates by SCW but Not DPP

(A–C) Lateral views of stage 15 *scw* null embryos stained to visualize a P[Kr-lacZ] amnioserosa reporter. Injection of 0.4 $\mu\text{g}/\mu\text{l}$ SCW resulted in induction of amnioserosa (A) that was effectively blocked by coinjection with 0.5 $\mu\text{g}/\mu\text{l}$ SOG (B). In contrast, coinjection of SOG did not interfere with the ability of DPP (20 $\text{ng}/\mu\text{l}$) to induce amnioserosa (C).

(D) *Drosophila* S2 cells were transfected with the indicated constructs to assay TLD-mediated cleavage of SOG in the presence of SCW ligand. The conditioned media were analyzed on an SDS-PAGE gel and the Western blot probed with an anti-FLAG polyclonal antibody (D-8) that recognizes the C-terminal tag in SOG. The level of expression of the individual proteins was verified independently (data not shown). Expression of SOG and TLD in the presence of the SCW ligand resulted in the generation of three distinct cleavage products of SOG (100 kDa, 25 kDa, and 16 kDa), and an occasional 45 kDa band at much lower intensity.

that *scw* is epistatic to *sog*, consistent with a role for SOG as an inhibitor of SCW function.

Although the epistasis experiment illustrates the relationship between SOG and SCW, it does not reveal whether antagonism of SCW is mediated through inhibition of DPP activity. In order to evaluate whether SOG activity was ligand specific, we coinjected SOG along with SCW or DPP into *scw*⁻ embryos. Concentrations of ligand mRNA that evoke biologically equivalent responses were chosen as a baseline (see Table 1B). Coinjection of 0.5 $\mu\text{g}/\mu\text{l}$ of SOG completely inhibited the ability of SCW to induce amnioserosa (Figures 7A and 7B; Table 1D). Remarkably, SOG at concentrations as high as 2 $\mu\text{g}/\mu\text{l}$ did not block induction of amnioserosa by DPP, although it was sufficient to abolish endogenous signaling in a wild-type embryo (Figure 7C; Table 1D). These results indicate that SOG is an effective antagonist of SCW activity at concentrations that have no effect on DPP signaling.

We confirmed the specificity of the SCW–SOG interaction by examining the effect of SOG on ectopic expression of SCW and DPP in imaginal discs (see Figure 4). Expression of UAS:SOG had no effect on wing morphology, barring the occasional loss of the posterior crossveins (Figure 4D). Consistent with our observations in the embryo, coexpression of SOG resulted in a remarkable suppression of the ectopic SCW phenotype but was unable to suppress the patterning defects caused by ectopic DPP expression in the wing (Figure 4D).

To obtain biochemical evidence for an interaction between SOG and SCW, we examined the ability of TLD to cleave SOG in the presence of the SCW ligand. Expression of TLD in S2 cells has been shown to result in a low level of SOG cleavage that is significantly stimulated by the presence of ligands such as DPP and BMP2 (Marqués et al., 1997). To test whether SOG cleavage is similarly enhanced by SCW, S2 cells were transfected with SOG-FLAG and TLD-Myc in the presence or absence of SCW. Full-length SOG and all processed forms

retaining the C-terminal tag were visualized by probing Western blots with an anti-FLAG antibody. As shown in Figure 7D, SOG is efficiently cleaved in the presence of SCW. Taken together, the genetic epistasis studies, coexpression experiments, and biochemical data argue that SOG is a potent antagonist of SCW activity *in vivo*.

Discussion

SCW Is Required for Maximal Levels of DPP Signaling

The requirement for two BMP ligands, SCW and DPP, in patterning the same subset of dorsal structures seems counterintuitive, given that exogenous DPP is capable of specifying the full range of dorsal pattern (this work; Ferguson and Anderson, 1992a; Wharton et al., 1993). A resolution to this paradox may lie in the fact that the endogenous levels of DPP present in the embryo have a limited patterning capacity. This is clearly illustrated by the phenotype of *scw* mutant embryos, in which *dpp* is expressed normally but is insufficient to specify amnioserosa cells (Arora et al., 1994). In this study, we demonstrate that SCW functions by potentiating DPP activity in the embryo in order to achieve peak levels of signaling in the dorsal-most blastoderm cells. Similar results have been reported by Neul and Ferguson (1998 [this issue of *Cell*]). Data from embryo injection assays do not support a simple model in which SCW augments DPP by binding to the same receptors and eliciting a qualitatively similar signal. SCW injections are ineffective in restoring dorsal pattern in *dpp*⁻ embryos, suggesting that SCW and DPP have distinct roles in embryonic patterning (Figure 1). Since SCW does not signal effectively in the absence of DPP, an important corollary is that the *dpp* null phenotype represents the loss of both DPP and SCW signaling and emphasizes the absolute requirement for DPP in dorsal patterning.

A possible explanation for the dependence of SCW activity on DPP could be that SCW functions as an

obligate heterodimer with DPP (Arora et al., 1994). Indeed, studies using vertebrate embryos and cell culture assays have reported that BMP4/7 heterodimers are more effective at signaling than BMP4 or BMP7 (Aono et al., 1995; Suzuki et al., 1997). However, our data show that expression of SCW under conditions that preclude heterodimer formation with DPP is sufficient to rescue amnioserosa in a *scw*⁻ embryo. Therefore, it seems unlikely that SCW boosts DPP activity through heterodimer formation. Significantly, the formation of amnioserosa at the correct location suggests that SCW can diffuse over several cell diameters (Figure 2). These results also indicate that *scw*⁻ embryos have an inherent polarity that may result from the restriction of DPP and/or TLD to dorsal cells.

SAX and TKV Mediate Signaling by Independent Ligands

Mutations in *tkv* and *sax* result in phenotypes that differ in severity. In addition, the ligand-binding domains of SAX and TKV are relatively nonconserved, suggesting that they could have different ligand specificities (Brummel et al., 1994; Nellen et al., 1994; Penton et al., 1994; Xie et al., 1994). Consistent with this idea, a dominant-negative form of SAX can block the response to exogenous SCW but does little to alter the response to DPP (Figures 3 and 4). We conclude from these results that SAX mediates SCW but not DPP signaling. In similar assays, TKV-DN inhibited peak levels of signaling by both DPP and SCW. These data imply that TKV is the primary receptor for DPP in the embryo, since DPP signaling is only affected by TKV-DN.

While an obvious explanation for the inhibitory effect of TKV-DN on SCW activity is that TKV binds SCW directly, this results in an apparent contradiction. If SCW can bind TKV, why is excess SCW unable to specify pattern in the absence of DPP? One can argue that SCW/TKV binding results in a qualitatively different output from that of DPP/TKV signaling. Alternatively, inhibition of SCW signaling by TKV-DN may not require a direct interaction. Coimmunoprecipitation and functional complementation data reveal homomeric interactions between TGF β type I receptors, suggesting that the ligand-receptor complex may consist of multiple type I and type II forms (reviewed in Derynck and Feng, 1997; Heldin et al., 1997). If TKV and SAX participate in a multimeric complex, it could explain how TKV-DN interferes with SCW signaling without invoking direct binding. By the same logic, it might be expected that the SAX-DN receptor would inhibit DPP signaling. However, since our assays were carried out in the absence of endogenous SCW, the contribution of SAX to such a complex would not be detected (Figures 3 and 4). An obligatory requirement for SAX to form a higher order complex with TKV may provide an explanation for the dependence of SCW signaling on DPP. In contrast, the ability of TKV to signal in the absence of SAX (this work; Brummel et al., 1994) argues that a DPP/TKV complex may be functional on its own. The involvement of higher order complexes may also provide the basis for the genetic interactions observed between dominant-negative alleles of *sax* and *scw* with partial loss-of-function alleles of *dpp* (Xie et al., 1994; Raftery et al., 1995).

While we propose a primary role for SAX in transducing SCW activity in the embryo, it is clear that SAX can mediate the response to other ligands. *scw* is not expressed later in development when *sax* is required for the growth and patterning of imaginal discs (Arora et al., 1994). Recent work by Haerry et al. (1998) demonstrates that the BMP-related *gbb-60A* gene acts through SAX to augment DPP signaling in imaginal discs.

SOG May Be Involved in Generating a Gradient of SCW Activity

Genetic and phenotypic studies have established that *sog* and *dpp* exert opposing influences on dorsal patterning, leading to the suggestion that SOG functions as an antagonist of DPP activity (Ferguson and Anderson, 1992b; Holley et al., 1995; Schmidt et al., 1995; Biehs et al., 1996). We have demonstrated that levels of SOG that do not affect DPP signaling can block the ability of SCW to promote dorsal cell fates (Figure 7). The ability of SOG to specifically interfere with SCW does not conflict with previous studies showing a genetic antagonism of *dpp* activity by *sog*. Since SCW augments DPP signaling, the inhibition of SCW activity by SOG is equivalent to antagonism of DPP. In fact, results from earlier studies support our assertion that SOG preferentially targets SCW activity in the embryo. Holley et al. (1996) reported that injections of SOG mRNA into wild-type embryos resulted in a partially ventralized phenotype, suggesting that SOG can inhibit only a subset of the endogenous "dorsalizing activity." In an independent study, ubiquitous expression of the *sog* cDNA at high levels did not phenocopy *dpp* mutants, but was comparable to loss of *scw* function (Biehs et al., 1996). Thus, we propose that one way by which SOG mediates its negative effect on dorsal patterning is by antagonizing SCW function.

Our data are also inconsistent with a central role for *sog* in modulating DPP activity in late development. Ectopic expression of SOG in the wing disc using a variety of GAL4 drivers caused no significant phenotypic defects (this work; Yu et al., 1996). This is quite striking given the prominent role of DPP in organizing pattern along the anterior-posterior axis in the wing disc. It is worth noting that the loss of posterior crossveins caused by expression of SOG is similar to the defect caused by SAX-DN, rather than TKV-DN (see Figure 4). Somatic clones lacking *sog* function also cause relatively mild phenotypic effects restricted to wing venation (Yu et al., 1996). An explanation for the failure of SOG to target DPP could be that DPP is bound to extracellular matrix components or forms a high-affinity complex with its receptor. Alternatively, the observation that *Xenopus* Noggin can severely ventralize *Drosophila* embryos (Holley et al., 1996) raises the possibility that a Noggin-like factor may be the functionally relevant DPP antagonist.

If SOG primarily blocks SCW activity during embryogenesis, the role of TLD may be to potentiate SCW signaling by releasing it from an inhibitory complex. We have shown that SCW can promote TLD-dependent cleavage of SOG (Figure 7). This may explain why the loss of *tld* function results in a partially ventralized phenotype similar to that of *scw*⁻ mutants, rather than the complete ventralization typical of *dpp* null embryos (Shimell et al., 1991). The observation that embryos lacking

both *scw* and *tld* function do not display a more severe phenotype is also compatible with this view (K. A., unpublished data).

While we favor the idea that SOG preferentially inhibits SCW activity, there could be other explanations for the inability of SOG to antagonize DPP, specific to our assays (Figures 4 and 7). It is possible that SOG is dependent on another component to block DPP activity, and this factor is not expressed, or does not function, in a *scw*⁻ background. Arguably, this factor could be present in the heterologous *Xenopus* system where SOG can effectively inhibit DPP signaling, as well as in *Drosophila* S2 cells where DPP enhances cleavage of SOG (Holley et al., 1995; Marqués et al., 1997).

Synergistic Signaling by SAX and TKV Is Involved in Patterning the Dorsal Side of the Embryo

Both *DPP* and activated *TKV* can induce amnioserosa cells in the absence of endogenous SCW in a dose-dependent manner (Figure 1; data not shown). In contrast, neither excess *SCW* nor constitutively activated *SAX* can specify extreme dorsal cell fates in the absence of *DPP*. However, levels of *SAX-A* that are ineffective alone can achieve the threshold required for differentiation of amnioserosa in combination with suboptimal levels of *TKV-A* receptor (Table 1; Figure 5). The mechanistic basis for this synergy could lie at several levels. One possibility is that direct interactions between *SAX* and *TKV* (perhaps cross-phosphorylation events) increase their combined output. An alternative is that a shared downstream component such as *MAD* could be hyperphosphorylated when both type I receptors are activated. A third possibility is that *SAX* and *TKV* activate different *SMADs* that synergize in the nucleus.

We propose that in the embryo, *SOG* diffusion results in a ventral-to-dorsal gradient of inhibitor that acts on ubiquitously distributed *SCW* to generate a ligand activity gradient of the opposite polarity. Thus, although *SAX* is expressed ubiquitously, its activation would occur in a graded manner. The restricted expression of *DPP* and *TKV* is likely to result in a basal level of *TKV* signaling in all dorsal cells. The ability of *SAX* and *TKV* to synergize would allow the dorsal-most cells to achieve the highest threshold of signaling required for specification of amnioserosa. Signaling by *SCW/SAX* impacts the specification of at least two thresholds, since in *scw*⁻ embryos, in addition to loss of amnioserosa, the boundary between the dorsal ectoderm and the ventral ectoderm is also shifted dorsally (Arora et al., 1994). The nonlinear enhancement of *TKV* signaling by *SCW/SAX* is likely to be critical in achieving distinct thresholds by accentuating small differences in the effective concentration of the two ligands. The apparent lack of *scw* signaling in ventral cells could result from inhibition by *sog* in ventrolateral cells or the dependence of *SCW/SAX* activity on *TKV* signaling.

Based on our model, mutations in *sog* should result in peak levels of *DPP* and *SCW* signaling in all dorsal cells and production of excess amnioserosa. However, in *sog*⁻ embryos the amnioserosa is reduced, while the dorsal ectoderm is expanded (Ferguson and Anderson, 1992b; François et al., 1994). Holley et al. (1996) proposed that in addition to antagonizing ligand activity in

ventral cells, *SOG* may also enhance signaling in dorsal cells by facilitating diffusion of the ligand. Thus, it is possible that mutations in *sog* result in a broader but flatter gradient of *SCW* activity. This aspect of *SOG* function may also explain why *dorsal* (*dl*) mutant embryos differentiate a mosaic of dorsal ectoderm and amnioserosa cell fates, although they express *scw* and *dpp* ubiquitously (Ray et al., 1991). Alternatively, the commitment to dorsal ectoderm and amnioserosa cell fates may require secondary interactions between these tissues that involve other genes.

In the wing imaginal disc, diffusion of *DPP* can account for the formation of a morphogen gradient (Lecuit et al., 1996; Nellen et al., 1996). In contrast, we have delineated how modulation of ligand activity at multiple levels contributes to the establishment of a *BMP* activity gradient in the *Drosophila* embryo. The role of *SOG* in inhibition of *BMP* signaling by diffusion into the dorsal region underscores the fact that a gradient of *SOG* activity is critical for patterning dorsal embryonic cells. Similar mechanisms may be involved in other embryonic systems where cells integrate information from multiple ligands that are expressed in overlapping patterns.

Experimental Procedures

Drosophila Stocks

Wild-type embryos used for injection assays carried a P[Kr-lacZ] insert on chromosome III. *Df(2L)OD16*, P[Kr-lacZ]/*CyO* stocks were used to obtain *scw* null embryos (Arora et al., 1994). *scw*⁻ embryos were identified by the absence of P[hb-lacZ] or P[wg-lacZ] inserts on the *CyO* chromosome. A P[Kr-lacZ]; *dpp*⁴⁴⁸/*CyO*23 stock was used to obtain embryos lacking *dpp* activity. *dpp*⁻ embryos were identified by their characteristic ventralized morphology and distinctive dorsal cleft in the cephalic region even in injected embryos that differentiated an amnioserosa. *sog*^{YL26}, *sax*¹, *sax*², and *scw*^{S12} have been described (Ferguson and Anderson 1992b; Arora et al., 1994; Brummel et al., 1994). The *dpp* and *sax* stocks were obtained from V. Twombly and W. M. Gelbart. The GAL4:A9 stock and UAS lines of *TKV-DN*, *SAX-DN*, and *DPP* were kindly provided by T. Haerry and M. O'Connor (Haerry et al., 1998). The UAS:*SOG* line was a gift from E. Bier.

Plasmid Constructs

pBluescript clones of *TKV-DN*, *SAX-DN*, *TKV-A*, and *SAX-A* were a generous gift from T. Haerry and M. O'Connor (Haerry et al., 1998). *TKV-DN* was derived from the *tkv-1* splice variant (Brummel et al., 1994). cDNAs used for mRNA injections were subcloned into a pSP64 vector that carries a 5' globin leader and 3' flanking poly A sequences. The *scw*, *sog*, and *dpp* cDNAs have been described (Padgett et al., 1987; Arora et al., 1994; François et al., 1994).

P[*twi*>*SCW*] was constructed by replacing the β -*gal* fragment in pCaSpeR-4XPE *twi*-lacZ fusion construct (Jiang and Levine, 1993) with the *scw* coding region. pUAST-*SCW* and pRmHa1-*SCW* contain the full-length *scw* coding sequence downstream of the respective promoters. pRmHa1-*SOG*-FLAG and pRmHa1-TLD-Myc have been described in Marqués et al. (1997).

Embryo Injections

Germline transformations to generate the UAS:*SCW* lines were carried out as described in Arora et al. (1994). mRNAs for microinjection were generated using the Message Machine kit (Ambion). Embryos were prepared as described in Park and Lim (1995) and injected according to Ferguson and Anderson (1992a). Syncytial blastoderm stage embryos were injected by inserting the needle laterally, and ~4 nl of mRNA was placed beneath the dorsal surface of the embryo. Embryos were aged until stage 14–15 to assay β -*gal* expression.

Detection of Gene Expression

In situ hybridization to detect *rho* and *scw* expression was carried out using digoxigenin-labeled antisense riboprobes (Arora et al., 1994). Expression of *Kr* and β -*gal* was detected using antibodies directed against these proteins (Arora et al., 1994).

Transfection and Western Blot Analysis

Drosophila S2 cells were used for transfection and SOG cleavage assays as described in Marqués et al. (1997). Western blots were probed with an anti-FLAG D-8 antibody (Santacruz Biotech) to detect SOG; 9E10 anti-Myc antibody (Santacruz Biotech) to detect TLD; and a polyclonal rat antibody to detect SCW.

Acknowledgments

The insightful comments and suggestions of an anonymous reviewer substantially improved this manuscript. We are grateful to M. B. O'Connor and T. Haerry for their generous gift of plasmids and stocks used in this study. We thank M. B. O'Connor and E. Ferguson for communication of data prior to publication. This work has benefited immensely from discussions with Rahul Warrior. We thank Rahul Warrior, Mark Stapleton, Jesus Torres-Vásquez, and Tim Hesp for comments on the manuscript, and Michelle Reyes for support. K. A. is a Searle Scholar funded by the Kinship Foundation. This work was supported by grants from the National Institutes of Health (GM55442), the March of Dimes Foundation, and the Cancer Research Coordination Committee (K. A.).

Received May 18, 1998; revised September 21, 1998.

References

Aono, A., Hazama, M., Notoya, K., Taketomi, S., Yamasaki, H., Tsukuda, R., Sasaki, S., and Fujisawa, Y. (1995). Potent ectopic bone-inducing activity of bone morphogenetic protein-4/7 heterodimer. *Biochem. Biophys. Res. Commun.* **210**, 670–677.

Arora, K., and Nüsslein-Volhard, C. (1992). Altered mitotic domains reveal fate map changes in Drosophila embryos mutant for zygotic dorsoventral patterning genes. *Development* **114**, 1003–1024.

Arora, K., Levine, M., and O'Connor, M. (1994). The *screw* gene encodes a ubiquitously expressed member of the TGF β family required for specification of dorsal cell fates in the *Drosophila* embryo. *Genes Dev.* **8**, 2588–2601.

Biehs, B., François, V., and Bier, E. (1996). The *Drosophila short gastrulation* gene prevents Dpp from autoactivating and suppressing neurogenesis in the neuroectoderm. *Genes Dev.* **10**, 2922–2934.

Brand, A.H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401–415.

Brummel, T.J., Twombly, V., Marqués, G., Wrana, J.L., Newfeld, S.J., Attisano, L., Massagué, J., O'Connor, M.B., and Gelbart, W.M. (1994). Characterization and relationship of Dpp receptors encoded by the *saxophone* and *thick veins* genes in Drosophila. *Cell* **78**, 251–261.

Burke, R., and Basler, K. (1996). Dpp receptors are autonomously required for cell proliferation in the entire developing *Drosophila* wing. *Development* **122**, 2261–2269.

Derynck, R., and Feng, X.-H. (1997). TGF β receptor signaling. *Biochim. Biophys. Acta* **1333**, F105–F150.

Ferguson, E.L., and Anderson, K.V. (1992a). *decapentaplegic* acts as a morphogen to organize dorsal-ventral pattern in the Drosophila embryo. *Cell* **71**, 451–461.

Ferguson, E.L., and Anderson, K.V. (1992b). Localized enhancement and repression of the activity of the TGF β family member, *decapentaplegic*, is necessary for dorsal-ventral pattern formation in the Drosophila embryo. *Development* **114**, 583–597.

François, V., Solloway, M., O'Neill, J.W., Emery, J., and Bier, E. (1994). Dorsal-ventral patterning of the *Drosophila* embryo depends on a putative negative growth factor encoded by the *short gastrulation* gene. *Genes Dev.* **8**, 2602–2616.

Gelbart, W.M. (1989). The *decapentaplegic* gene: a TGF β homolog controlling pattern formation in Drosophila. *Development* **107** (Suppl.), 65–74.

Haerry, T.E., Khalsa, O., O'Connor, M.B., and Wharton, K.A. (1998). Synergistic signaling by two BMP ligands through the SAX and TKV receptors controls wing growth and patterning in Drosophila. *Development* **125**, 3977–3987.

Heldin, C.-H., Miyazono, K., and ten Dijke, P. (1997). TGF β signaling from cell membrane to nucleus through SMAD proteins. *Nature* **390**, 465–471.

Hogan, B.L. (1996). Bone morphogenetic proteins: multifunctional regulators of vertebrate development. *Genes Dev.* **10**, 1580–1594.

Holley, S.A., Jackson, P.D., Sasai, Y., Lu, B., DeRobertis, E.M., Hoffman, F.M., and Ferguson, E.L. (1995). A conserved system for dorsal-ventral patterning in insects and vertebrates involving sog and chordin. *Nature* **376**, 249–253.

Holley, S.A., Neul, J.L., Attisano, L., Wrana, J.L., Sasai, Y., O'Connor, M.B., De Robertis, E.M., and Ferguson, E.L. (1996). The Xenopus dorsalizing factor noggin ventralizes Drosophila embryos by preventing DPP from activating its receptor. *Cell* **86**, 607–617.

Hoodless, P.A., Haerry, T., Abdollah, S., Stapleton, M., O'Connor, M.B., Attisano, L., and Wrana, J.L. (1996). MADR1, a MAD-related protein that functions in BMP2 signaling pathways. *Cell* **85**, 489–500.

Irish, V.F., and Gelbart, W.M. (1987). The *decapentaplegic* gene is required for dorsal-ventral patterning of the Drosophila embryo. *Genes Dev.* **1**, 868–879.

Jiang, J., and Levine, M. (1993). Binding affinities and cooperative interactions with bHLH activators delimit threshold responses to the dorsal gradient morphogen. *Cell* **72**, 741–752.

Kretzschmar, M., and Massagué, J. (1998). SMADs: mediators and regulators of TGF β signaling. *Curr. Opin. Genet. Dev.* **8**, 103–111.

Lawrence, P.A., and Struhl, G. (1996). Morphogens, compartments, and pattern: lessons from Drosophila? *Cell* **85**, 951–961.

Lecuit, T., Brook, W.J., Ng, M., Calleja, M., Sun, H., and Cohen, S.M. (1996). Two distinct mechanisms for long-range patterning by Decapentaplegic in the Drosophila wing. *Nature* **381**, 1387–1393.

Letsou, A., Arora, K., Wrana, J.L., Simin, K., Twombly, V., Jamal, J., Staehling-Hampton, K., Hoffman, F.M., Gelbart, W.M., Massagué, J., and O'Connor, M.B. (1995). Drosophila Dpp signaling is mediated by the *punt* gene product: a dual ligand binding type II receptor of the TGF β receptor family. *Cell* **80**, 899–908.

Marqués, G., Musacchio, M., Shimell, M.J., Wünnenberg-Stapleton, K., Cho, K.W.Y., and O'Connor, M.B. (1997). Production of a DPP activity gradient in the early Drosophila embryo through the opposing actions of the SOG and TLD proteins. *Cell* **91**, 417–426.

Massagué, J. (1990). The transforming growth factor- β family. *Annu. Rev. Cell Biol.* **6**, 597–641.

Nellen, D., Affolter, M., and Basler, K. (1994). Receptor serine/threonine kinases implicated in the control of Drosophila body pattern by *decapentaplegic*. *Cell* **78**, 225–237.

Nellen, D., Burke, R., Struhl, G., and Basler, K. (1996). Direct and long-range action of a DPP morphogen gradient. *Cell* **85**, 357–368.

Neul, J.L., and Ferguson, E.L. (1998). Spatially restricted activation of the SAX receptor by SCW modulates DPP/TKV signaling in *Drosophila* dorsal-ventral patterning. *Cell* **95**, this issue, 483–494.

Neumann, C., and Cohen, S. (1997). Morphogens and pattern formation. *Bioessays* **19**, 721–729.

Newfeld, S.J., Mehra, A., Singer, M.A., Wrana, J.L., Attisano, L., and Gelbart, W.M. (1997). *Mothers against dpp* participates in a DPP/TGF-beta responsive serine-threonine kinase signal transduction cascade. *Development* **124**, 3167–3176.

Padgett, R.W., Johnston, R.D.S., and Gelbart, W.M. (1987). A transcript from a Drosophila pattern gene predicts a protein homologous to the transforming growth factor- β family. *Nature* **325**, 81–84.

Park, S., and Lim, J.K. (1995). A microinjection technique for ethanol-treated eggs and a mating scheme for detection of germ line transformants. *Dros. Info. Serv.* **76**, 197–199.

Penton, A., Chen, Y., Staehling-Hampton, K., Wrana, J.L., Attisano, L., Szidonya, J., Cassill, J.A., Massagué, J., and Hoffmann, F.M.

(1994). Identification of two bone morphogenetic protein type I receptors in *Drosophila* and evidence that Brk25D is a *decapentaplegic* receptor. *Cell* **78**, 239–250.

Piccolo, S., Agius, E., Lu, B., Goodman, S., Dale, L., and De Robertis, E.M. (1997). Cleavage of Chordin by Xolloid metalloprotease suggests a role for proteolytic processing in the regulation of Spemann organizer activity. *Cell* **91**, 407–416.

Rafferty, L.A., Twombly, V., Wharton, K., and Gelbart, W.M. (1995). Genetic screens to identify elements of the *decapentaplegic* signaling pathway in *Drosophila*. *Genetics* **139**, 241–254.

Ray, R.P., Arora, K., Nüsslein, V.C., and Gelbart, W.M. (1991). The control of cell fate along the dorsal-ventral axis of the *Drosophila* embryo. *Development* **113**, 35–54.

Ruberte, E., Marty, T., Nellen, D., Affolter, M., and Basler, K. (1995). An absolute requirement for both the type II and type I receptors, punt and thick veins, for Dpp signaling in vivo. *Cell* **80**, 889–897.

Sasai, Y., Lu, B., Steinbeisser, H., and De Robertis, E.M. (1995). Regulation of neural induction by the Chd and BMP4 antagonistic patterning signals in *Xenopus*. *Nature* **376**, 333–336.

Schmidt, J., François, V., Bier, E., and Kimelman, D. (1995). *Drosophila short gastrulation* induces an ectopic axis in *Xenopus*: evidence for conserved mechanisms of dorsal-ventral patterning. *Development* **121**, 4319–4328.

Shimell, M.J., Ferguson, E.L., Childs, S.R., and O'Connor, M.B. (1991). The *Drosophila* dorsal-ventral patterning gene *tolloid* is related to human bone morphogenetic protein 1. *Cell* **67**, 469–481.

Singer, M.A., Penton, A., Twombly, V., Hoffmann, F.M., and Gelbart, W.M. (1997). Signaling through both type I Dpp receptors is required for anterior-posterior patterning of the entire *Drosophila* wing. *Development* **124**, 79–89.

St. Johnston, R.D., and Gelbart, W.M. (1987). *decapentaplegic* transcripts are localized along the dorsal-ventral axis of the *Drosophila* embryo. *EMBO J.* **6**, 2785–2791.

Suzuki, A., Kaneko, E., Maeda, J., and Ueno, N. (1997). Mesoderm induction by BMP4 and -7 heterodimers. *Biochem. Biophys. Res. Commun.* **232**, 153–156.

Terracol, R., and Lengyel, J.A. (1994). The *thick veins* gene of *Drosophila* is required for dorsoventral polarity of the embryo. *Genetics* **138**, 165–178.

Wharton, K.A., Ray, R.P., and Gelbart, W.M. (1993). An activity gradient of *decapentaplegic* is necessary for the specification of dorsal pattern elements in the *Drosophila* embryo. *Development* **117**, 807–822.

Xie, T., Finelli, A.L., and Padgett, R.W. (1994). The *Drosophila saxophone* gene: a serine-threonine kinase receptor of the TGF β superfamily. *Science* **263**, 1756–1759.

Yu, K., Sturtevant, M.A., Biehs, B., François, V., Padgett, R.W., Blackman, R.K., and Bier, E. (1996). The *Drosophila decapentaplegic* and *short gastrulation* genes function antagonistically during adult wing vein development. *Development* **122**, 4033–4044.