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The *Drosophila decapentaplegic* and *short gastrulation* genes function antagonistically during adult wing vein development

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SUMMARY

TGF-β-related signaling pathways play diverse roles during vertebrate and invertebrate development. A common mechanism for regulating the activity of TGF-β family members is inhibition by extracellular antagonists. Recently, the *Drosophila short gastrulation* (*sog*) gene was shown to encode a predicted diffusible factor which antagonizes signaling mediated by the TGF-β-like Decapentaplegic (Dpp) pathway in the early blastoderm embryo. *sog* and *dpp*, which are among the earliest zygotic genes to be activated, are expressed in complementary dorsal-ventral domains. The opposing actions of *sog* and *dpp* in the early embryo have been highly conserved during evolution as their vertebrate counterparts, *chordin* and *BMP-4*, function homologously to define neural versus non-neural ectoderm in *Xenopus*. Here we exploit the genetically sensitive adult wing vein pattern to investigate the generality of the antagonistic relationship between *sog* and *dpp*. We show that *dpp* is expressed in vein primordia during pupal wing development and functions to promote vein formation. In contrast, *sog* is expressed in complementary intervein cells and suppresses vein formation. *sog* and *dpp* function during the same phenocritical periods (i.e. 16-28 hours after pupariation) to influence the vein versus intervein cell fate choice. The conflicting activities of *dpp* and *sog* are also revealed by antagonistic dosage-sensitive interactions between these two genes during vein development. Analysis of vein and intervein marker expression in *dpp* and *sog* mutant wings suggests that *dpp* promotes vein fates indirectly by activating the vein gene *rhomboid* (*rho*), and that *sog* functions by blocking an autoactivating Dpp feedback loop. These data support the view that Sog is a dedicated Dpp antagonist.

Key words: *decapentaplegic* (*dpp*), *short gastrulation* (*sog*), antagonism, wing vein development, *Drosophila*

INTRODUCTION

The *decapentaplegic* (*dpp*) gene encodes a member of the TGF-β superfamily (Padgett et al., 1987), which is most related to the vertebrate BMP-4 and BMP-2 proteins (Kingsley, 1994). *dpp* is expressed in the dorsal-most 40% of the early blastoderm embryo (St. Johnston and Gelbart, 1987) where it plays a key role in establishing dorsal cell fates (Irish and Gelbart, 1987; Wharton et al., 1993). Another gene required for patterning the dorsal region of the blastoderm embryo, *short gastrulation* (*sog*), is expressed in broad lateral stripes constituting the neuroectoderm (François et al., 1994). Expression of *sog* in lateral stripes adjacent to the dorsal domain is consistent with genetic evidence that *sog* functions non-autonomously to influence dorsal cell fates (Zusman and Wieschaus, 1988).

dpp and *sog* exert opposing influences in patterning the dorsal region of the blastoderm embryo. For example, expression of the *rhomboid* (*rho*) gene in dorsal-most presumptive amnioserosal cells is abolished in *dpp*$^-$ mutants, but expands ventrally in *sog*$^-$ mutants (François et al., 1994). Genetic evidence further supports the view that *sog* opposes *dpp* activity as reducing the gene dose of *sog* rescues lethality resulting from weak mutations in the Dpp pathway (Ferguson and Anderson, 1992; Wharton et al., 1993; François et al., 1994).

During other stages of embryogenesis (François et al., 1994), *dpp* and *sog* are also expressed in adjacent sets of cells. For example, *dpp* and *sog* are expressed in alternating longitudinal stripes during germ-band extension and retraction, and in a series of parallel non-overlapping rings during gut formation. Such correlated expression patterns are consistent

with the possibility that *dpp* and *sog* interact during later stages of development.

To investigate this possibility in detail, we examined the role of *dpp* and *sog* in the simple context of adult wing vein development. During pupal stages, several forms of cell-cell communication contribute to the ultimate differentiation of continuous and straight veins (García-Bellido, 1977; García-Bellido and de Celis, 1992; Sturtevant and Bier, 1995). These cell-cell interactions include: (1) dorsal-to-ventral (D→V) signal(s) required for maintaining vein fates in cells on the ventral surface of the wing (García-Bellido, 1977; Sturtevant and Bier, 1995), (2) lateral inhibitory signal(s) emanating from vein primordia, which limit the width of veins differentiating within broad vein-competent regions (García-Bellido, 1977; Shellenbarger and Mohler, 1978, Sturtevant et al., 1993; Sturtevant and Bier, 1995), and (3) vein continuity signal(s) promoting vein formation in straight lines along the axis of vein extension (García-Bellido, 1977; Sturtevant and Bier, 1995).

Here, we show that, during early pupal development, *dpp* is expressed in vein primordia and *sog* is expressed in complementary intervein cells. *dpp* and *sog* function contemporaneously to exert opposing influences on the vein versus intervein cell fate choice: *dpp* promotes vein formation while *sog* suppresses vein development. Analysis of vein and intervein marker gene expression in *sog* and *dpp* mutant backgrounds suggests that Sog blocks an autoactivating function of Dpp and thereby channels Dpp activity along the vein axis. These data support models in which *sog* functions as a dedicated antagonist of Dpp signaling. We discuss the possible role of Dpp autoactivation in assuring vein continuity during pupal wing development.

MATERIALS AND METHODS

Fly stocks

All genetic markers and chromosome balancers are described in Lindsley and Grell (1968) and Lindsley and Zimm (1992). Construction of the *8×P{hs-dpp}* stock is described in Twombly et al. (1996). Other stocks were obtained from the Bloomington, Indiana and Bowling Green, Ohio *Drosophila* Stock Centers.

Construction of the HS-*sog* and UAS-*sog* vectors

A *Not*I-*Hin*dIII fragment of a *sog* cDNA (17.12) containing the full predicted Sog protein coding sequence (François et al., 1994) was excised from the pNB40 vector and subcloned into a BlueScript vector. The *Hin*dIII and *Not*I sites were sequentially changed to *Xba*I sites by linker insertion. The resulting *Xba*I *sog* cDNA fragment was then subcloned into the *Xba*I site of the hs-CaSpeR heat-shock P-element vector (Bang and Posakony, 1992) and the *Xba*I site of the pUAST P-element vector (Brand and Perrimon, 1993). Subclones were checked for correct insertional orientation and injected into *white* (*w*) embryos. Transformant flies were identified by screening for the linked *mini-white* (w^+) marker (Rubin and Spradling, 1982).

Enhancer piracy

Enhancer piracy using a HS-*sog* P-element insertion was carried out as described in Noll et al. (1994). Briefly, females homozygous for a w^+ HS-*sog* P-element insertion on the X chromosome (abbreviated EP1-*sog*) were crossed to *yw*; *Δ2-3 Sb* y^+/TM6*Ubx* males. EP1-*sog*; *Δ2-3 Sb* y^+/+ male progeny were collected and crossed to w^- females. Transposition of the w^+ HS-*sog* P-element from the X chromosome to autosomes generates w^+ male progeny. Over 1,000 independent male transposants were induced in individual bottle crosses of this kind, each of which was examined carefully for dominant phenotypes. Males with visible phenotypes were back-crossed to w^- females to determine whether the original phenotype was dominant and heritable. Balanced stocks were made from lines with highly penetrant dominant enhancer piracy phenotypes. 12 enhancer piracy lines (denoted EP2-EP13) were isolated in which various sections of veins were missing (see legend to Table 3). Other than venation defects, we did not recover any other dominant phenotypes in this screen.

Mosaic analysis

Female flies of the genotype *y* f^{36a} sog^6 FRT^{18A}*neo*/FM7c or f^{36a} sog^{P1} FRT^{18A}*neo*/FM7c were crossed to males of the genotype f^+ FRT^{18A}*neo*/Y; MKRS,FLP3/TM6β,Tb and the progeny were heat shocked as first and second instar larvae to generate mosaic adults containing homozygous sog^- clones marked with *y* and *f* (sog^6) or with *f* alone (sog^{P1}).

Heat inductions

Larvae or pupae carrying 8 copies of a HS-*dpp* construct (8×*P{hs-dpp}*, Twombly et al., 1996), which we refer to as 8×HS-*dpp* in this study, 8 copies of the HS-*sog* construct described above (8×HS-*sog*), or 4 copies of the HS-*sog* construct in a dpp^{shv} mutant background (4×HS-*sog*; dpp^{shv}) were heat shocked according to the following regimen, which was repeated for a total of four cycles: 30 minutes of heat shock in a 37-38°C water bath followed by a 30 minute period of recovery at room temperature. Animals were staged at 25°C with respect to formation of white prepupae. Larvae were heat shocked in submerged glass vials, while prepupae and pupae were heat shocked on wet filter paper in a Petri dish. A minimum of 20 flies was scored for each time interval.

Mounting fly wings

Wings from adult flies were dissected in isopropanol and mounted in Canadian Balsam mounting medium (Gary's magic mountant) following the protocol of Lawrence et al. (in Roberts, 1986). Mounted wings were photographed under a compound microscope using Nomarski optics for high magnification exposures (i.e. 20× or 40× lens) and without the condenser or Nomarski optics for low magnification photographs of complete wings (i.e. 4× lens).

In situ hybridization to whole-mount embryos or discs

In situ hybridization to whole-mount wings was performed using digoxigenin (Boehringer-Mannheim, 1093 657) or biotin-labeled RNA probes (O'Neill and Bier, 1994) as previously described (e.g. Sturtevant et al., 1993; O'Neill and Bier, 1994).

RESULTS

dpp and *sog* are expressed in complementary patterns during pupal wing development

To determine whether the theme of correlated *sog* and *dpp* expression observed during embryogenesis (François et al., 1994) continues into adult development, we examined the pattern of *sog* expression in imaginal discs and pupal wings. In wing and leg imaginal discs, *sog* is expressed in stripes running parallel to the *dpp* stripe along the compartment border (data not shown). In early prepupae, *dpp* expression along the A/P boundary disappears. In late prepupae, 6-9 hours after pupariation (AP), *dpp* expression is initiated in a series of stripes corresponding to vein primordia (data not shown). Following a cycle of separation and reapposition of the two wing surfaces (Fristrom et al., 1994), expression of *dpp* in vein primordia reemerges in early P1 stage pupae (18-20 hours AP; Fig. 1A). *dpp*

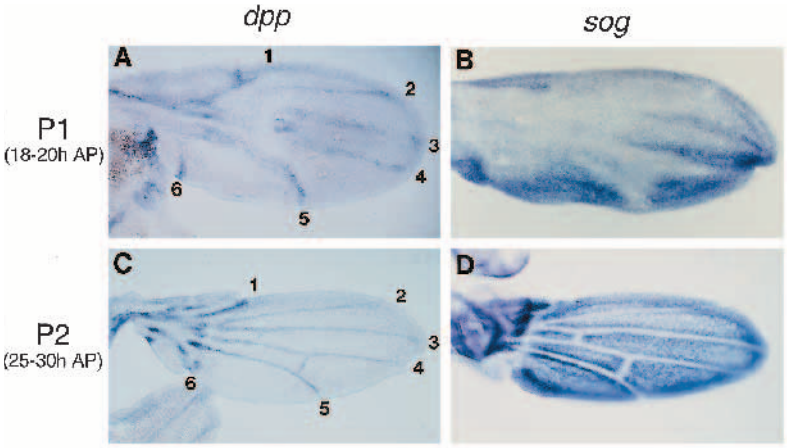


Fig. 1. *dpp* and *sog* are expressed in complementary patterns during pupal wing vein development. *dpp* and *sog* transcription patterns in developing pupal wings were visualized by in situ hybridization with digoxigenin-labeled single-stranded antisense RNA probes (O'Neill and Bier, 1994). Pupal wings shown in this and subsequent figures have been categorized into one of two different stages (P1 or P2) for convenience. Stage P1, the earliest pupal wings that can be stained histologically following apolysis (approximately 18-20 hours AP) have clearly visible longitudinal veins L1-L6, but no cross veins and a poorly developed alula. Stage P2, pupal wings in the terminal phase of vein versus intervein differentiation (25-30 hours AP), have both anterior and posterior cross veins and a well-defined alula. Times AP are with respect to incubation at 25°C, but are not strictly correlated with developmental stage due to significant variability in developmental rates between individual pupae (see Fristrom et al., 1993). (A) *dpp* expression in a P1 pupal wing. *dpp* expression is restricted to the primordia of longitudinal veins. Longitudinal veins L1-L6 are labeled 1-6. (B) *sog* expression in a P1 pupal wing is strongest in the center of intervein regions. *sog* is also expressed along the margin. (C) *dpp* expression in P2 pupal wings is limited to the primordia of longitudinal veins and cross veins within the wing blade, but is not expressed in the marginal vein. Longitudinal veins L1-L6 are labeled 1-6. (D) *sog* expression in a P2 pupal wing is confined to intervein regions and the margin.

expression remains restricted to vein primordia in later stage P2 pupae (25-30 hours AP; Fig. 1C). In P1 pupae, *sog* expression is initiated in the center of intervein domains (Fig. 1B) and then expands in P2 pupae to include nearly all intervein cells (Fig. 1D). Double-label in situ hybridization with a digoxigenin-labeled *dpp* probe and a biotin-labeled *sog* probe reveals that the *dpp* and *sog* expression domains are strictly complementary throughout most of the wing except in the proximal region of the L5 vein, where there is a gap approximately one cell wide between *dpp* and *sog* expressing cells (data not shown). No wing cells express both *dpp* and *sog*, however. Thus, as in the early embryo, *sog* and *dpp* are expressed in abutting domains during pupal wing development.

dpp promotes vein development during pupariation

A class of loss-of-function *dpp* alleles (Fig. 2B; Segal and Gelbart, 1985; St Johnston et al., 1990; Posakony et al., 1990), certain combinations of Dpp receptor mutants (Nellen et al., 1994; Penton et al., 1994; Brummel et al., 1994; Terracol and Lengyel, 1994; Ruberte et al., 1995; Letsou et al., 1995) and loss-of-function clones of *schnurri*, which encodes a transcription factor likely to propagate a portion of the *dpp* signal (Arora et al., 1995; Grieder et al., 1995; Staehling-Hampton et al., 1995), lead to vein-loss phenotypes. Other combinations of Dpp receptor alleles, however, generate thick-vein phenotypes (Penton et al., 1994; Terracol and Lengyel, 1994). In addition, small clones of cells mis-expressing *dpp* have been observed to form localized patches of ectopic vein material (Zecca et al., 1995). These observations implicate *dpp* signaling in vein development. That *dpp* plays a role in vein formation per se remains unclear, however, since wing vein phenotypes associated with Dpp pathway mutants include thick veins as well as loss of veins. An additional complication is that *dpp* also functions earlier during larval development to supply anterior-posterior positional information and to direct imaginal disc outgrowth. The early function of *dpp* in anterior-posterior patterning indirectly affects vein formation (Segal and Gelbart, 1985; Sturtevant and Bier, 1995; Zecca et al., 1995; M. A. Sturtevant and E. Bier, unpublished observations).

To assess whether Dpp signaling plays a specific role in vein formation, we mis-expressed *dpp* during larval and pupal development in short pulses using the 8×HS-*dpp* stock (Twombly et al., 1996), which contains eight copies of a HS-*dpp* transgene (Table 1). Heat induction of 8×HS-*dpp* flies during larval or prepupal stages, when endogenous *dpp* is expressed in a stripe along the A/P compartment boundary, had

Table 1. Temporal profile of HS-*dpp* ectopic vein phenotypes

Stage[a]	Phenotype
3rd Instar[b]	high mortality[e], escapers and dissected pharate adults: < 50% mild broad wing, >75% extra dorso-central macrochaetae[f]
0h AP[c]	>75%: mild broad wing
0-4h AP[d]	> 50%: mild broad wing, >75%: twigged posterior cross vein
4-8h AP[d]	>75%: twigged posterior cross vein and distal tips of L4, L5
8-12h AP[d]	>75%: twigged posterior cross vein and distal tips of L4, L5
12-16h AP[d]	>75%: twigged veins and short ectopic veins (most branching anteriorly from L3)
16-20h AP[d]	**> 90%: thick and long ectopic veins**
20-24h AP[d]	**> 90%: thick and long ectopic veins**
24-28h AP[d]	**> 90%: thick and long ectopic veins**
28-32h AP[d]	> 90%: massive central blister, occasional ectopic veins
32-36h AP[d]	> 90%: thick veins, 50%: mild blisters at post. cross vein and/or short ectopic veins
36-40h AP[d]	> 75%: thick posterior cross vein, <50%: mild blisters and/or short ectopic veins
40-44h AP[d]	> 90%: wild type

(a) Stage when heat induction was begun. The heat-shock protocol consisted of four cycles of 30 minute heat shocks at 38°C separated by 30 minutes of recovery (see Materials and Methods for details), (b) feeding and wandering third instar larvae, (c) white prepupae were collected over a brief period of time and then heat shocked, (d) white prepupae were collected during 4 hour time intervals and then subjected to the heat-shock regimen, (e) 29/44 wandering third instar larvae and 20/22 feeding third instars failed to eclose, and (f) extra dorsocentral macrochaetae were found spaced between the normal dorsocentrals and were usually shorter than the normal dorsocentrals (ectopic spaced scutellar macrochaetae were also occasionally observed, but the pattern of macrochaetae in other positions was normal). The only phenotype observed in flies heat shocked in 4 hour intervals between 44 and 60 hours AP was a short ectopic vein extending from the posterior cross vein. This latter phenotype is likely to be a heat-shock artifact, however, as it was also observed in heat-shocked wild-type flies.

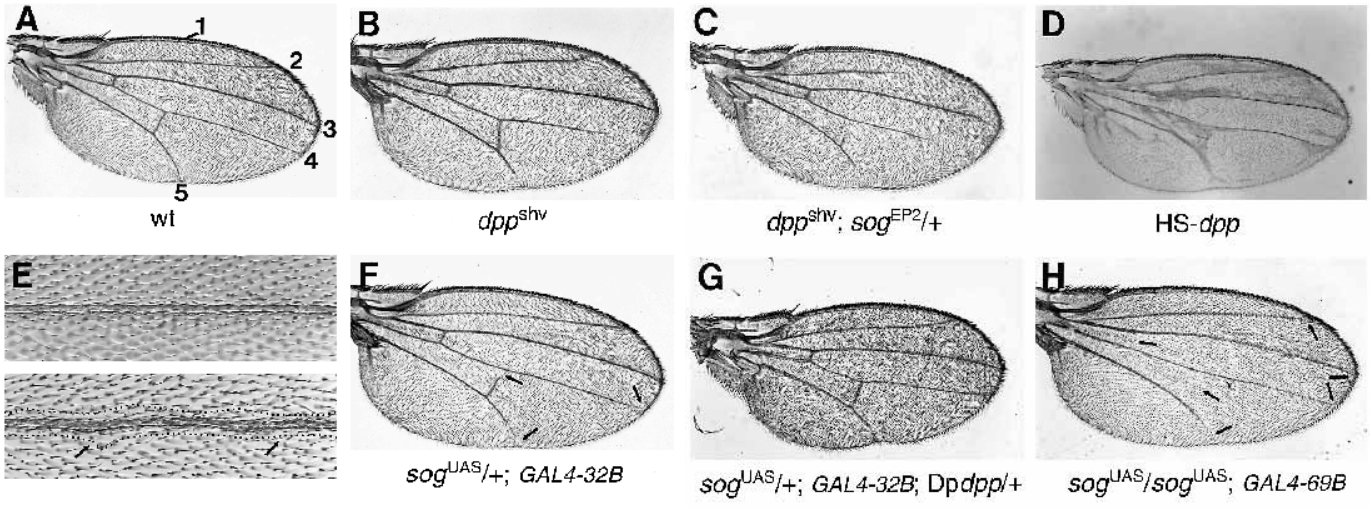


Fig. 2. *dpp* and *sog* play opposing roles during vein development. (A) A wild-type adult wing. Longitudinal veins L1-L5 are labeled 1-5. (B) A dpp^{shv}/dpp^{shv} wing. The distal portion of L4 is missing. (C) A dpp^{shv}/dpp^{shv}; sog^{EP2}/+ wing. All longitudinal veins are significantly truncated. In a wild-type background, sog^{EP2}/+ flies lack only the anterior half of the posterior cross vein. (D) A wing derived from a cohort of 8×HS-*dpp* flies heat shocked between 28 and 32 hours AP. Most of the flies in this cohort have blisters, but those without blisters have strong ectopic vein phenotypes similar to those commonly observed in the 24-28 hours AP cohort. (E) Top panel: a mid-section of a wild-type L2 vein. Note that it is straight and uniform in diameter. Bottom panel: a mid-section of an L2 vein from a fly of the genotype $f\ sog^{P1}\ FRT/\ f^{+}\ sog^{+}\ FRT$; HS-*FLP*/+ which was heat shocked during the first or second larval instar to generate a $f\ sog^{P1}\ FRT/f\ sog^{P1}\ FRT$ homozygous clone running along the vein the ventral surface (within dotted lines). On the dorsal surface, there are also two smaller clones 1-2 cells wide, which cross L2 diagonally in regions where the vein is kinked (arrows). Longitudinal veins in such clones often meander over a domain 5-7 cells wide, which most likely corresponds to the vein-competent pro-vein region morphologically visible 25-30 hours AP (Sturtevant et al., 1993; Sturtevant and Bier, 1995). sog^{-} mutant veins also vary in thickness (i.e. 1-6 cells wide versus the normal 2-3 cells wide). Similar phenotypes are observed in wings with larger sog^{-} clones and in wings with sog^{-} clones covering both the dorsal and ventral components of the vein. Even in wings, which were composed almost entirely of sog^{-} cells, we did not observe any global defects in wing patterning indicative of an earlier role for *sog* in A/P patterning. (F) A wing from a fly of the genotype sog^{UAS2}/+; *GAL4-32B*/+. Flies of this genotype consistently lack the anterior half of the posterior cross vein and the distal tips of L4 and L5. Occasionally, the distal tip of L3 is also missing. Arrows point to missing vein segments. (G) A fly of the genotype sog^{UAS2}/+; Dp *dpp*/+; *GAL4-32B*/+. Flies of this genotype always have a fully restored wild-type vein pattern. (H) A wing from a fly of the genotype sog^{UAS2}/sog^{UAS2}; *GAL4-69B*/+. These flies have the most severe vein-loss phenotype resulting from our various forms of *sog* mis-expression in a wild-type background (i.e. loss of the distal tips of L2-L5 and loss of both cross veins – see arrows). This phenotype is very similar to the cumulative vein-loss pattern observed for combinations of loss-of-function *dpp* signaling pathway mutations.

little effect on venation. In contrast, heat shocks delivered between 16 and 40 hours after pupariation (AP) caused severe ectopic vein and blister phenotypes. Strong penetrant production of ectopic veins dominated in flies heat shocked 16-28 hours AP (Fig. 2D). Blistering, another phenotype commonly associated with widespread ectopic venation (Sturtevant et al., 1993; Noll et al., 1994; Fristrom et al., 1994; Sturtevant and Bier, 1995), was the most frequent phenotype at later times (28-40 hours AP). Heat shocks administered after 44 hours AP had no effect (Table 1). As the critical period during which *dpp* mis-expression generates ectopic veins coincides with restricted *dpp* expression in vein primordia (Fig. 1A,B), it is likely that Dpp acts locally to promote vein development. Genes such as *rho* (Sturtevant and Bier, 1995) and *Notch* (Shellenbarger and Mohler, 1978) also function during this period to influence the vein-versus-intervein cell-fate choice.

sog suppresses vein development during pupariation

To determine whether *sog* plays a role in vein formation, we first generated homozygous loss-of-function *sog* mutant clones in the wing using the FLP-FRT somatic recombination system (Golic, 1991). While the vein pattern is grossly normal in mosaic wings containing sog^{-} clones, we consistently observed

Table 2. Temporal profile of HS-*sog* vein-loss phenotypes

Stage[a]	Phenotype
3rd Instar[b]	>90% mortality[e], wild type escapers
0h AP[c,f]	0% vein loss
0-4h AP[d]	>25%: truncated L4
4-8h AP[d]	<10%: truncated L4
8-12h AP[d]	<10%: truncated L4
12-16h AP[d]	<10%: truncated L4
16-20h AP[d]	**>40%: truncated L4 and/or posterior cross vein**
20-24h AP[d]	**>60%: truncated L4, L5, and/or posterior cross vein**
24-28h AP[d]	**>40%: truncated L4, L5, and/or posterior cross vein**
28-32h AP[d]	<10%: truncated L4
32-36h AP[d,f]	<10%: truncated L4
36-40h AP[d,f]	<10%: truncated L4
40-44h AP[d]	>90% wild type

(a) Stage when heat induction protocol was started, (b) wandering third instar larvae, (c) white prepupae were collected over a brief period of time and then heat shocked, (d) white prepupae were collected during 4 hour time intervals and then subjected to the heat-shock regimen, (e) 20/22 wandering third instar larvae failed to eclose and (f) short perpendicular vein segments (<20%) branching from L2 or L3 (0 hours and 32-40 hours AP) or long perpendicular vein segments (<30%) which occasionally formed cross veins (44-52 hours AP) were observed in heat-shocked 8×HS-*sog* wings. Similar ectopic vein phenotypes were observed sporadically in various combinations of UAS-sog × GAL 4 lines. The basis for this poorly penetrant phenotype, which occurs outside of the peak period for vein-loss phenotypes, remains to be determined. p. cross vein = posterior cross vein.

Table 3. Vein-loss phenotypes resulting from *sog* mis-expression

Genotype		Vein loss phenotype		
sog/dpp	*GAL4*	Ant. cross vein	Post. cross vein	Longitudinal veins
UAS1/+	32B/+	0	+	+ (L5)
*UAS2/+	32B/+	0	+	++ (L4,L5)
UAS2 EP1/+	32B/+	0	**++**	**+++** (L3,L4,L5)
UAS2 +/+ *sog*$^{-}$	32B/+	0	**0**	**0**
*UAS2/+; Dp*dpp*/+	32B/+	0	**0**	**0**
UAS3/+	32B/+	0	+	++ (L4,L5)
UAS1/+	69B/+	0	++	0
UAS2/+	69B/+	0	+++[a]	0
*UAS2/UAS2	69B/+	+++[a]	+++[a]	+++ (L2,L3,L4,L5)
UAS2 EP1/+	69B/+	0	+++[a]	++ (L4,L5)
UAS2/Dp(1;Y)*sog*†	69B/+	0	+++[a]	+++ (L3,L4,L5)
UAS2 +/+ *sog*$^{-}$	69B/+	0	**0**	0
UAS2/+; Dp*dpp*/+	69B/+	0	**0**	0
UAS3/+	69B/+	0	++	++ (L4,L5)
UAS1/+	ptc/+	+	0	0
UAS2/+	ptc/+	++	0	0
EP1	-	0	0	0
EP2,3,4,5,6/+	-	0	++	0
EP2,3,4,5,6 homozygous	-	0	+++[a]	0
EP7,8,9,10,11/+	-	0	0	++ (L4,L5)
EP7 homozygous	-	0	0	++ (L3,L4,L5)
EP12,13/+	-	0	0	++ (L5)
EP12 homozygous	-	0	0	++ (L4,L5)
EP7/Dp*dpp*	-	0	0	**0**
EP7/*dpp*$^{-}$	-	0	0	++ (L3,L4,L5)
**dpp*^{shv}*/dpp*^{shv}	-	0	0	++ (L4)
**dpp*^{shv}*/dpp*^{shv}; EP2,3/+	-	0	++	+++ (L2,L3,L4,L5)
dpp^{shv} EP7/*dpp*^{shV} +	-	0	0	+++ (L2,L3,L4,L5)
tkv/tkv	-	0	0	0
tkv/tkv; EP2/+	-	0	0	++ (L4)
tkv EP7/*tkv* +	-	0	0	+++ (L4,L5)
det/det	-	0	++	0
dpp^{shv}*/dpp*^{shv}*; det/det*	-	0	+++	+++ (L4)

Symbol key: Ant. cross vein, anterior cross vein; Post. cross vein, posterior cross vein; 0, wild-type vein pattern; +, slight and partially penetrant (<50% of wings) vein truncation; ++, moderate and frequent (>50% of wings) vein truncation; +++, strong and highly penetrant (>80% of wings) vein truncation; a, entire cross vein missing; parentheses indicate which longitudinal veins are affected; UAS, UAS-*sog* lines (UAS1 is a weaker responder than UAS2 or UAS3); EP, *sog* enhancer piracy lines recovered in an enhancer piracy screen (Noll et al., 1994) of more than 1,000 independent jumps of a HS-*sog* p-element vector (EP1) from the X-chromosome to the autosomes (see Materials and Methods), EP1 has no phenotype in a wild-type background, while EP2, EP3, EP4, EP5 and EP6 lack the anterior half of the posterior cross vein with high penetrance (>70% of wings), EP7, EP8, EP9, EP10 and EP11 lack the distal tip of L4 and L5 (>70% of wings), and EP12 and EP13 lack the distal tip of L5 (>70% of wings). EP2 and EP3 express the *sog* transgene in the intervein regions of third instar larval discs and pupal wings at high levels, suggesting their vein-loss phenotypes result from a non-autonomous action of the overexpressed *sog* transgene; GAL4-32B and GAL4-69B express GAL-4 throughout most of the wing disc (Brand and Perrimon 1993), and GAL4-*ptc* expresses GAL4 in a narrow stripe running anterior to the anterior-posterior compartment boundary that intersects the future anterior cross vein; *, data shown in Fig. 2; †, construction of Dp(1;Y)y^{+} Bs *sog*$^{+}$ by L. Deutsch and D. L. Lindsley will be reported in the *Drosophila* Information Service; bold entries indicate genetic interactions.

that veins meander about their normal trajectories and vary in thickness in clones covering veins (Fig. 2E, bottom panel). These irregularities contrast with the straight and uniform diameter veins typical of wild-type wings (Fig. 2E, top panel).

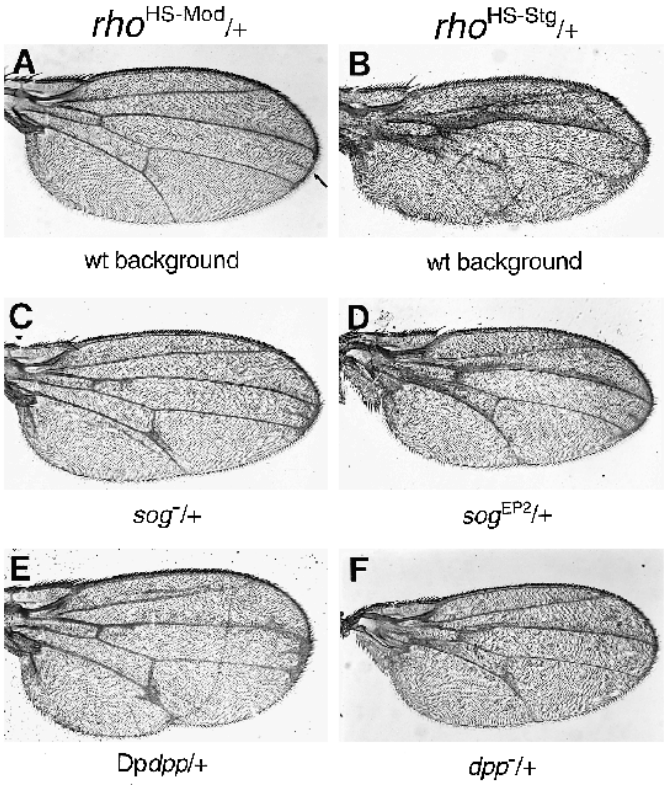
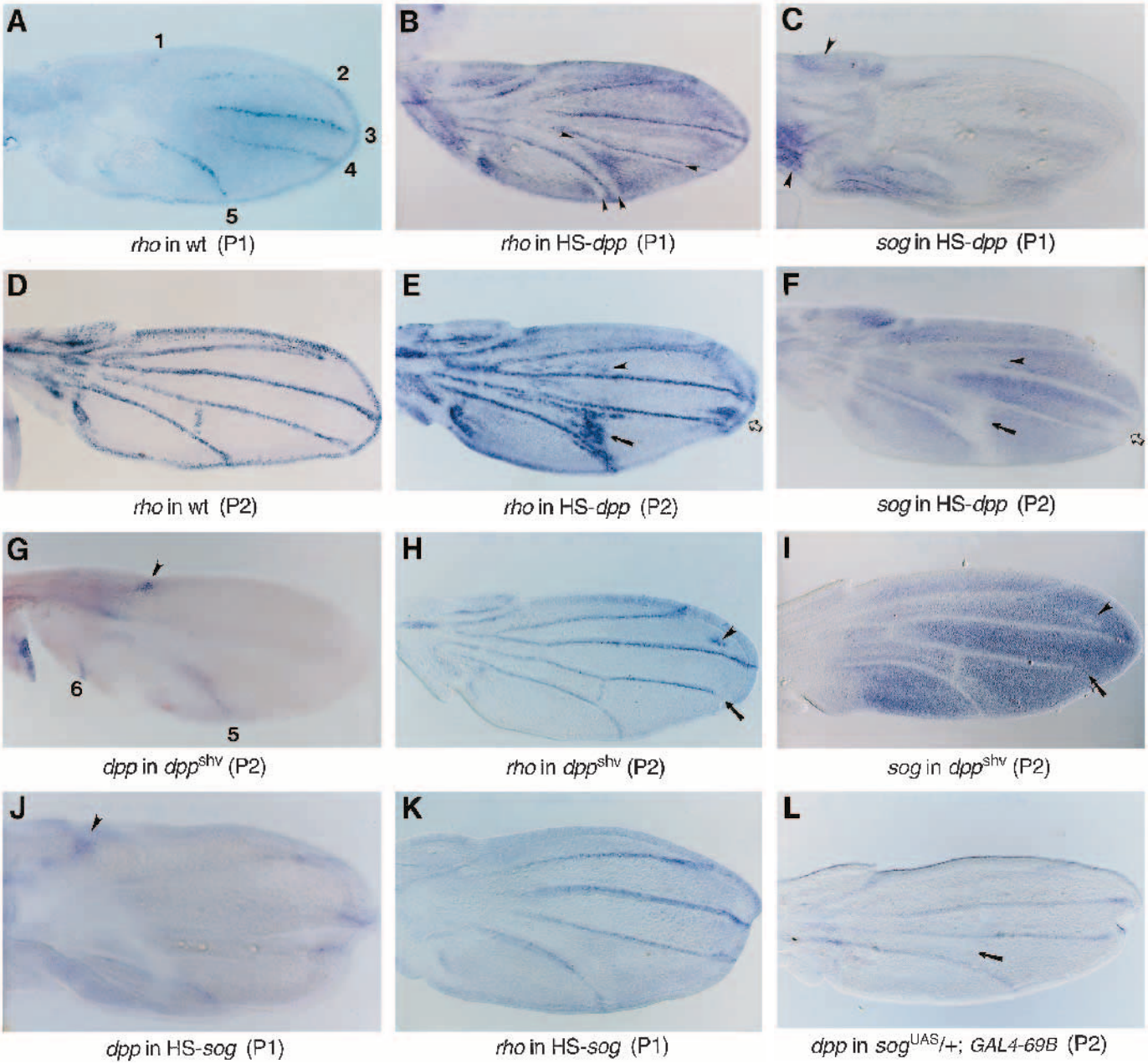


Fig. 3. Opposite effects of *dpp* and *sog* gene dosage on ectopic vein phenotypes. (A) A wing from a fly carrying one copy of the *rho*$^{HS\text{-}Mod}$ enhancer piracy insertion, which has a moderate ectopic vein phenotype typified by a short ectopic segment of vein between L3 and L4 near the margin (arrow) and slight L3 and L4 deltas. This phenotype results from low ubiquitous levels of *rho* expression. (B) A wing from a fly carrying one copy of the *rho*$^{HS\text{-}Stg}$ enhancer piracy insertion which has a strong ectopic vein phenotype. This phenotype results from strong ubiquitous *rho* expression (Sturtevant et al., 1993). (C) A wing from a fly of the genotype *sog*^{6}/+; *rho*$^{HS\text{-}Mod}$/+. Note the strong enhancement of the *rho*$^{HS\text{-}Mod}$/+ extra vein phenotype including additional ectopic veins running parallel to L2 and L5. (D) A wing from a fly of the genotype *sog*^{EP2}/+; *rho*$^{HS\text{-}Stg}$/+. The severe ectopic vein phenotype is strongly suppressed. *sog*^{EP2}/+ flies lack only the anterior half of the posterior cross vein. Doubling the dose of genomic *sog* (i.e. Dp(1;Y)*sog*, see Table 1), which on its own has no vein phenotype, also strongly suppresses the *rho*$^{HS\text{-}Stg}$/+ phenotype (data not shown). (E) A wing from a fly of the genotype Dp*dpp*/+; *rho*$^{HS\text{-}Mod}$/+. The degree of enhancement of the *rho*$^{HS\text{-}Mod}$/+ phenotype is similar to that observed by reducing the dose of *sog* by a factor of two (C). (F) A wing from a fly of the genotype *dpp*$^{-}$/+; *rho*$^{HS\text{-}Stg}$/+. This dramatic suppression is as strong as the most potent suppression of the *rho*$^{HS\text{-}Stg}$/+ phenotype that we have observed by mutants in the EGF-R pathway such as *Star* (Sturtevant et al., 1993) or by the wide variety of existing wing vein mutants that we have surveyed such as *vvl* or *Serrate* (Sturtevant and Bier, 1995).

The intervein pattern of *sog* expression and the irregular course of veins in *sog*$^{-}$ clones suggest that *sog* normally plays a role in restricting vein formation to the center of broad vein-competent regions.

Another way that we examined the function of *sog* during vein development is by mis-expressing *sog* ubiquitously at various developmental stages (Table 2) in 8×HS-*sog* flies, which carry eight copies of a HS-*sog* transgene. We also



ectopically expressed *sog* using the GAL4-UAS system (Brand and Perrimon, 1993) and enhancer piracy (Noll et al., 1994) (Table 3). Mis-expression of *sog* by each of these methods leads to various vein-loss phenotypes (Table 3; Fig. 2F,H), the most extreme of which (Fig. 2H) is very similar to the cumulative pattern of vein-loss resulting from various combinations of Dpp signaling mutants (Nellen et al., 1994; Penton et al., 1994; Brummel et al., 1994; Terracol and Lengyel, 1994; Ruberte et al., 1995; Letsou et al., 1995). The gain-of-function *sog* phenotype observed in UAS2-*sog*; GAL4-69B flies is due to a combination of endogenous and transgene encoded *sog*, since reducing the dose of endogenous *sog* normalized the vein pattern, whereas increasing the dose of endogenous *sog* aggravated the vein-loss phenotype (Table 3).

We determined the phenocritical period for HS-*sog*-induced vein-loss from a series of staged 8×HS-*sog* heat inductions (16-28 hours AP, Table 2) and found that it coincides with the period for generating ectopic veins in 8×HS-*dpp* pupae (Table 1). Thus, *sog* and *dpp* function during the same developmental interval to influence the vein-versus-intervein cell-fate choice.

dpp and *sog* mutations interact antagonistically during vein development

Consistent with the opposite vein phenotypes resulting from *dpp* versus *sog* mis-expression, we observed strong antagonistic genetic interactions between these two genes during vein development. For example, gain-of-function EP-*sog* alleles, which on their own generate only a partial truncation of the posterior cross vein (Table 3), greatly enhance the loss of longitudinal veins associated with the dpp^{shv} allele (Fig. 2C). Enhancement of the dpp^{shv} vein-loss phenotype, which is also observed as a result of heat shocking 4×HS-*sog*; dpp^{shv} pupae,

Fig. 4. Regulatory relationships between *dpp*, *sog* and *rho* in pupal wings. (A) *rho* expression in a wild-type P1 (18-20 hours AP) pupal wing is restricted to longitudinal veins. No cross veins have formed at this stage. L1-L5 veins are labeled 1-5. (B) *rho* expression in a 8×HS-*dpp* P1 pupal wing derived from an individual that was heat shocked for 30 minutes and allowed 30 minutes of recovery prior to fixation for in situ hybridization. *rho* expression is ectopically induced throughout intervein regions, except in narrow strips of cells running along veins (arrowheads). There is a wedge of elevated staining in the region between L4 and L5 in which the posterior cross vein will ultimately form (see E). *rho* expression is normal in a parallel cohort of heat-shocked wild-type wings (data not shown). (C) *sog* expression in a 8×HS-*dpp* P1 pupal wing derived from an individual that was heat shocked for 30 minutes and allowed 30 minutes of recovery prior to fixation for in situ hybridization. *sog* expression is globally reduced in distal regions of the wing (compare with Fig. 1B). Arrowheads indicate proximal regions of the wing in which *sog* expression is nearly normal. This labeling serves as an internal positive control for staining efficiency. *sog* expression is normal in a parallel cohort of heat-shocked wild-type wings (data not shown). (D) *rho* expression in a wild-type P2 pupal wing (25-30 hours AP) is observed in both longitudinal veins and cross veins. (E) *rho* expression in an 8×HS-*dpp* P2 pupal wing derived from an individual that was heat shocked for 30 minutes and aged 5 hours prior to fixation for in situ hybridization. Note the prominent restricted domains of ectopic *rho* expression near the posterior cross vein (solid arrow), anterior to L2 (arrowhead) and at the distal tip of the wing near L3 (open arrow). The P1 pupal wing shown in B would develop into a wing such as this. (F) *sog* expression in a 8×HS-*dpp* P2 pupal wing derived from an individual that was heat shocked for 30 minutes and aged 5 hours prior to fixation for in situ hybridization. The regions of strong down-regulation (compare with Fig. 1D) coincide with those ectopically expressing *rho* (indicated as in E of this figure). The P1 pupal wing shown in C would develop into a wing such as this. (G) *dpp* expression in longitudinal veins in a dpp^{shv}/dpp^{shv} P2 pupal wing is virtually abolished (compare with Fig. 1C). Staining in the proximal (arrowhead points to L1 and L6 is indicated by 6) and hinge (out-of-focus) regions of the wing is relatively normal and there is low-level residual *dpp* expression in the L5 vein of this wing (indicated by 5). (H) *rho* expression in longitudinal veins is normal in a dpp^{shv}/dpp^{shv} P2 pupal wing except in the distal region of L4 (arrow), which is missing in dpp^{shv}/dpp^{shv} mutant wings (see Fig. 2B). Another anomaly typical of the dpp^{shv}/dpp^{shv} mutation in certain genetic backgrounds (Díaz-Benjumea and García-Bellido, 1990) is a small ectopic vein segment between L2 and L3 (arrowhead). In our dpp^{shv} stock, however, this ectopic vein is found only occasionally in adult wings, although it is present in most or all pupal wings. (I) *sog* expression in longitudinal veins is normal in a dpp^{shv}/dpp^{shv} P2 pupal wing (compare with Fig. 1D) except in the distal region of L4 (arrow), where *rho* expression is lost (see H). Also note that the small hole in *sog* expression between L2 and L3 (arrowhead) is in the same location as the ectopic island of *rho* expression shown in panel H. (J) *dpp* expression in a 8×HS-*sog* P1 pupal wing is strongly suppressed. Some residual labeling is observed in proximal (arrowhead) and extreme distal regions of the wing (compare with Fig. 1A). Similar heat-shock treatments of wild-type pupae also generate sporadic loss of *dpp* expression, particularly in the L2 primordium. This effect of heat shocking wild-type wings is significantly less severe than that observed in heat-shocked HS-*sog* wings such as the one shown in this panel, and may reflect interruption of the Dpp autoregulatory loop during the heat-shock treatment. (K) *rho* expression in a 8×HS-*sog* P1 pupal wing is indistinguishable from wild-type (e.g. A of this figure). (L) *dpp* expression in a UAS-*sog*; *GAL4-69B* P2 pupal wing is strongly suppressed (compare with Fig. 1D). Some residual intermittent labeling is observed. Typically, expression in L2 and the dorsal surface of L3 is most strongly affected. The arrow points to the absence of a posterior cross vein, which would be present in a corresponding wild-type P2 wing, but is missing with high penetrance in UAS-*sog*; GAL4 69B wings. In contrast, *rho* expression is normal in UAS-*sog*; GAL4 69B pupal wings except in the regions where veins are missing from adult wings (e.g. the posterior cross vein).

Table 4. Temporal profile for the dpp^{shv} ↔ HS-*sog* interaction

	Vein loss phenotype		
Stage[a]	Unmodified[d] dpp^{shv}	Moderately enhanced[e] dpp^{shv}	Strongly enhanced[f] dpp^{shv}
no heat shock	50%	50%	0%
3rd Instar[b]	50%	50%	0%
4-16h AP[c]	30%	70%	0%
16-28h AP[c]	**0%**	**50%**	**50%**
28-40h AP[c]	50%	50%	0%

(a) Stage when heat induction protocol was started, (b) wandering third instar larvae, (c) white prepupae were collected over a brief period of time and then heat shocked, (d) unmodified dpp^{shv} phenotype (i.e. truncated L4 only), (e) moderately enhanced dpp^{shv} phenotype (i.e. truncated L2 and L4), and (f) strongly enhanced dpp^{shv} phenotype (i.e. truncated L2, L3 and L4).

Table 5. Opposite effects of *sog* versus *dpp* gene dosage on ectopic vein phenotypes

	Effect on gain-of-function *rho* phenotypes		
Test Genotype	$rho^{HS\text{-}Wk}$/+	$rho^{HS\text{-}Mod}$/+	$rho^{HS\text{-}Stg}$/+
sog^{-}/+	↑	↑↑*	↑
Dp(1;Y)*sog*/+	-	↓	↓
$sog^{EP2,3}$/+	-	↓↓	↓*
dpp^{-}/+	-	↓↓	↓↓*
Dp*dpp*/+	±↑	↑↑*	↑

Symbol key: ↑↑, strongly enhanced rho^{HS} phenotype; ↑, enhanced rho^{HS} phenotype; ↓↓, strongly suppressed rho^{HS} phenotype; ↓, suppressed rho^{HS} phenotype; ±, weak incompletely penetrant modification of rho^{HS} phenotype; -, $rho^{HS\text{-}Wk}$/+ wings have slight ectopic vein phenotypes that are too subtle to score reliably for suppression, $rho^{HS\text{-}Mod}$/+ wings (Fig. 3A) have a mild ectopic vein phenotype typified by a short vein segment between L3 and L4 near the margin, $rho^{HS\text{-}Stg}$/+ wings have a large amount of ectopic vein material, which often results in separation of the dorsal and ventral surfaces in blisters (75% of wings have blisters, *n*=28; Fig. 3B). The frequency of $rho^{HS\text{-}Stg}$/+ blistering was strongly suppressed by sog^{EP}/+ (10% of wings have blisters, *n*=40) and by Dp*sog* (10% of wings have blisters, *n*=80). dpp^{-}/+; $rho^{HS\text{-}Stg}$ escapers were only rarely recovered due to *dpp* haplolethality, but all (*n*=4) showed the same extreme suppression of the $rho^{HS\text{-}Stg}$ phenotype (Fig. 3F). See Sturtevant et al. (1993) and Sturtevant and Bier (1995) for examples of genetic interactions between other mutants and these three rho^{HS} lines. *, data shown in Fig. 3C-F. In addition to suppressing rho^{HS} ectopic vein phenotypes, sog^{EP}/+ also enhances the vein-loss phenotype of rho^{ve}.

derives from *sog* mis-expression during the same phenocritical period (16-28 hours AP) as that determined for the HS-*dpp* (Table 1) and HS-*sog* (Table 2) vein phenotypes (Table 4). Reciprocally, vein-loss phenotypes generated by *sog* mis-expression can be suppressed by only a 50% increase in *dpp* levels (i.e. three versus two copies of *dpp*, Fig. 2G).

Since we previously characterized the role of the *rho* gene (Bier et al., 1990) in promoting wing vein development (Sturtevant et al., 1993; Noll et al., 1994; Sturtevant and Bier 1995), we also tested for genetic interactions between loss- or gain-of-function *sog* and *dpp* mutations and gain-of-function rho^{HS} enhancer piracy alleles (Table 5). Different rho^{HS} lines (Fig. 3A,B) have varying degrees of ectopic vein formation resulting from different levels and patterns of *rho* mis-expression in intervein regions (Sturtevant et al., 1993; Noll et al., 1994). Decreasing the amount of Dpp by a factor of two dramatically suppresses even strong rho^{HS} ectopic vein phenotypes (Fig. 3F). Reciprocally, rho^{HS} flies with three copies of Dpp have exaggerated extra vein phenotypes (Fig. 3E). On the contrary,

reducing the level of *sog* by 50% strongly enhances rho^{HS} ectopic vein phenotypes (Fig. 3C), while elevated *sog* expression markedly suppresses the formation of rho^{HS} veins (Fig. 3D; Table 5).

Ectopic *dpp* activates *rho* expression and suppresses *sog* expression

To further analyze the role of *dpp* in promoting vein formation, we examined expression of several vein and intervein markers in 8×HS-*dpp* pupal wings. Shortly after a pulse of ubiquitous 8×HS-*dpp* expression, we observed widespread ectopic expression of the vein gene *rho* (Fig. 4B) and generalized suppression of *sog* expression in intervein regions (Fig. 4C). Narrow strips of cells running along veins (near-vein cells) appear to be refractory to the vein promoting activity of Dpp, however (Fig. 4B, arrows). When 8×HS-*dpp* pupal wings were allowed to develop for an additional 5 hours following the heat induction, a more restricted pattern of ectopic *rho* expression was observed (Fig. 4E). This patterned ectopic *rho* expression was most prominent in the vicinity of the posterior cross vein, in a strip of cells anterior to L2, and at the distal tips of L2 and L3, prefiguring the final ectopic vein phenotype of HS-*dpp* flies. When pupal wings from the same 8×HS-*dpp* batch were hybridized with a *sog* probe, we observed a pattern of suppressed *sog* expression (Fig. 4F) strikingly similar to that of ectopic *rho* expression (compare with Fig. 4E). These data suggest that Dpp acts indirectly to suppress *sog* expression, possibly by activating *rho*, which in turn suppresses *sog* expression.

Induction of *rho* expression in intervein cells in HS-*dpp* wings most likely results from a Dpp activity normally functioning to maintain *rho* expression in vein primordia. Other mechanisms must be working in parallel with *dpp*, however, since *rho* expression is largely normal in situations where *dpp* expression is severely reduced or eliminated (see below). Further support for a primary role of *rho* in establishing the vein fate is that ubiquitous expression of *rho* potently induces *dpp* expression and suppresses *sog* expression (data not shown – see legend to Fig. 5).

dpp^{shv} is likely to be an enhancer mutation

Previous genetic and molecular analyses suggested that the dpp^{shv} allele is likely to disrupt the function of a specific *dpp* enhancer element (Segal and Gelbart, 1985; St. Johnston et al., 1990). Consistent with this possibility, we observed a strong reduction of *dpp* expression along the length of all vein primordia in homozygous dpp^{shv} pupal wings (Fig. 4G). This widespread loss of *dpp* expression is far more severe than the final adult dpp^{shv} vein phenotype (Fig. 2B). In contrast to the residual and spotty expression in veins, *dpp* is expressed at normal levels in the wing hinge. *dpp* expression also is normal in dpp^{shv} third instar discs and early prepupal wings. The specific loss of *dpp* expression in vein primordia is consistent with the causative defect in dpp^{shv} mutants being a deletion of a vein-specific enhancer element (St. Johnston et al., 1990). The loss of *dpp* expression in dpp^{shv} mutants does not result from an indirect effect on vein formation per se since *rho* is expressed normally in dpp^{shv} pupal wing veins except at the tip of L4 (Fig. 4H), which is missing in adult dpp^{shv} wings (Fig. 2B). *sog* expression in dpp^{shv} pupal wings is excluded from vein cells expressing *rho* (Fig. 4I). As *dpp* expression is globally compromised in dpp^{shv} wings, it seems unlikely that *sog* is a direct target of the Dpp pathway. On the other hand, the correlated expression of *rho* and suppression of *sog* in HS-*dpp* and dpp^{shv} wings suggests that *rho* may be more directly involved in excluding *sog* from vein primordia than is Dpp. In addition, these experiments reveal that *rho* expression is not dependent on high levels of *dpp* activity throughout most of the wing.

sog may function by blocking Dpp autoactivation

One important function of Dpp signaling during embryogenesis is to activate expression of *dpp* itself (autoactivation). To determine whether Dpp might play a similar autoactivating role during vein development, we examined *dpp* expression in HS-*sog* wings since the only known function of *sog* is to block Dpp signaling (B. Biehs et al., unpublished data). A pulse of ectopic *sog* expression in 8×HS-*sog* pupal wings mimics the dpp^{shv} phenotype in that *dpp* expression is rapidly lost (Fig. 4J) without affecting the pattern of *rho* expression (Fig. 4K). A similar reduction in *dpp*, but not *rho*, expression was observed in pupal wings mis-expressing *sog* via the GAL4-UAS system (Fig. 4L).

DISCUSSION

dpp and *sog* exert opposite influences on vein development

The data presented above demonstrate that *dpp* promotes vein development during pupal stages and that *sog* antagonizes Dpp signaling in intervein regions. The vein-promoting activity of *dpp* appears to be entirely separate from its earlier role in establishing anterior-posterior polarity during larval development of the wing imaginal disc, although defects in this earlier function may also lead to venation defects (Segal and Gelbart, 1985; St. Johnston et al., 1990; Posakony et al., 1990; Sturtevant and Bier, 1995). As *sog* antagonizes *dpp* function in dorsal (Ferguson and Anderson, 1992; Wharton et al. 1993; François et al., 1994) and lateral (Biehs et al., 1996) regions of the blastoderm embryo, it may be a general rule that the Sog product interferes with Dpp signaling. Preliminary data indicate that *sog* also blocks *dpp* activity during later stages of embryogenesis (B. Biehs and E. Bier, unpublished observations). Further analysis of *sog* versus *dpp* function in other developmental contexts will be required to address the generality of *dpp* and *sog* constituting a signal-and-inhibitor genetic cassette.

A network of gene interactions promotes vein fates

The diagram in Fig. 5A summarizes the two basic results of our analysis of vein and intervein gene expression in various mutant backgrounds. The first of the proposed gene interactions is that *dpp* promotes vein development indirectly, possibly by maintaining *rho* expression in veins, which plays a key role in defining the vein fate by hyperactivating EGF-R signaling (Sturtevant et al., 1993; Noll et al., 1994; Sturtevant and Bier, 1995). Thus, when *dpp* is ectopically expressed, *rho* expression is rapidly induced in intervein regions, except in narrow strips of cells flanking veins (i.e. near-vein regions), which appear to be refractory to the effects of Dpp. Ectopic *rho* expression in intervein cells presumably reflects a Dpp activity which

normally functions to maintain *rho* expression in veins. The basis for the refractory behavior of near-vein cells is not known; however, we note that these cells express enhanced levels of various intervein markers including *tkv* (B. Biehs and E. Bier, unpublished data) which encodes a type I Dpp receptor (Nellen et al., 1994; Penton et al., 1994; Brummel et al., 1994). When heat-induced HS-*dpp* wings are allowed to develop, ectopic *rho* expression becomes restricted to a specific pattern. While the basis for this spatial bias is unknown, it provides an insight into the mechanism by which Dpp suppresses *sog* expression since the pattern of *sog* down-regulation in these aged wings correlates with that of ectopic *rho* expression. Another indication that *rho* is likely to play a more direct role than *dpp* in regulating *sog* is that *sog* expression is excluded from veins in dpp^{shv} mutant wings. These vein primordia express normal levels of *rho* but do not accumulate appreciable levels of *dpp*. Thus, the pattern of *sog* expression in dpp^{shv} mutant wings is complementary to that of *rho,* not *dpp*, which is nearly absent in this regulatory mutant. Finally, ectopic *rho* suppresses *sog* expression in a similar pattern to that of induced endogenous *rho* expression (data not shown).

The uncoupling of *dpp* and *rho* expression in HS-*sog* and dpp^{shv} mutant wings suggests that there are other pathways maintaining *rho* expression, which function in parallel with Dpp. The EGF-R pathway is a candidate for such a parallel genetic function since ectopic expression of a *rho* transgene induces endogenous *rho* expression. Furthermore, *rho* and *Egf-r* interact throughout the course of vein development to promote vein development (Sturtevant et al., 1993; Noll et al., 1994; Sturtevant and Bier, 1995).

The second major feature of the model depicted in Fig. 5A is that Sog antagonizes Dpp signaling by blocking Dpp autoactivation. Thus, ectopic *sog*, produced by either heat shock or the GAL4-UAS system, leads to dramatic reduction in *dpp* expression without affecting *rho* expression. Since available data support the view that Sog functions as a dedicated Dpp antagonist (this study and Biehs et al., 1996), the most plausible explanation for the loss of *dpp* expression in HS-*sog* wings is that maintenance of Dpp expression requires an autoactivation loop, which can be broken by Sog. It remains possible, however, that Sog acts through some unknown independent pathway to antagonize *dpp* expression.

Potential functions for *dpp* and *sog* during vein development

Several types of cell-cell communication have been described during the latter stages of pupal wing vein development including: (1) lateral inhibitory signal(s) elaborated by presumptive vein cells restricting vein formation to the center of broad vein-competent domains, (2) dorsal-to-ventral signal(s) required by ventral vein cells to maintain their vein identity, and (3) vein continuity signals promoting vein formation along the axis of vein extension (García-Bellido, 1977; Díaz-Benjumea and García-Bellido, 1990; García-Bellido and De Celis, 1992; Sturtevant and Bier, 1995). These various signals presumably collaborate to insure that the dorsal and ventral components of veins are strictly aligned and uninterrupted.

It is not certain whether the *dpp* and *sog* activities described in this study correspond to any of these known signaling functions or represent novel genetic functions for channeling veins along straight trajectories. We briefly consider whether the interaction between *dpp* and *sog* could be involved in each of the three types of cell-cell interaction mediating vein development. As discussed below, we favor the possibility that Dpp functions as a vein continuity signal.

A potential role for Dpp signaling in lateral inhibition is suggested by the thick-veins phenotype that is associated with various loss-of-function alleles of genes encoding components Dpp pathway (Penton et al., 1994; Terracol and Lengyel, 1994). Also, as described in this study, we observed irregularly thickened veins in HS-*dpp* and sog^{-} mosaic wings. However, we believe that the vein phenotypes that we observed are unlikely to result from defects in lateral inhibition. The thickened veins typical of lateral inhibition mutants such as *Notch* and *Delta* are more solid and uniformly broad than those that we observed. In addition, we would expect that Dpp misexpression would generate a vein-loss phenotype not an ectopic vein phenotype if Dpp were functioning as a lateral inhibitory signal. It is possible, however, that Dpp plays a lateral inhibitory role during another distinct developmental stage. We tested for potential HS-*dpp*-induced vein-loss phenotypes over a broad window of pupal development and found no evidence for such an activity (Table 1). Furthermore, certain specific regions of veins are more affected than others in lateral inhibition mutants (e.g. the entire length of L3), while the vein thickening that we observe in HS-*dpp* or sog^{-} mosaic wings is chaotic. Finally, we attempted to reveal a role for *sog* in regulating lateral inhibition by mis-expressing *sog* in a *tkv* mutant background. We expected that the EP-*sog*; *tkv* combination (Table 3) might aggravate the thick vein phenotype if *sog* further reduced the level of Dpp signaling mediated by the *tkv* receptor. Such enhancement of the *tkv* phenotype has been observed by lowering the level of *schnurri* (Staehling-Hampton et al., 1995), which propagates part of the Dpp signal. Surprisingly, however, we observed a loss-of-vein phenotype in EP-*sog*; *tkv* wings, which was much more severe than the mild EP-*sog* phenotype alone (Table 3). Thus, while *tkv* may mediate both lateral inhibitory and vein-promoting signals, it appears that *sog* can oppose only the vein-promoting activity of Dpp. It is possible that *dpp* functions through a *sog*-independent pathway to influence lateral inhibition or that another TGF-β family member serves as the lateral inhibitory ligand.

A role for Dpp as a dorsal-to-ventral signal is an intriguing possibility. If this were the case, one would expect to observe strong asymmetric effects resulting from eliminating or ectopically inducing *dpp* activity on either the dorsal or ventral surfaces. For example, ventral clones of loss-of-function Dpp receptor mutants should have stronger phenotypes than similar dorsal clones. One would also expect that ectopic expression of *dpp* dorsally should induce ectopic veins with dorsal and ventral components while ventral mis-expression either should have no effect or only generate a ventral vein component. These types of asymmetries have not been noted to date (Posakony et al., 1990; Grieder et al., 1995; Zecca et al., 1995; E. Bier, unpublished observations), but more in depth analyses of such experiments with these predictions in mind are necessary to resolve this point.

We believe that Dpp signaling is most likely to be to provide a vein continuity function (Fig. 5B). The meandering vein phenotype observed in large sog^{-} patches is consistent with disruption of a mechanism constraining vein formation to the center of vein-competent domains (Fig. 5C). Expression of *sog*

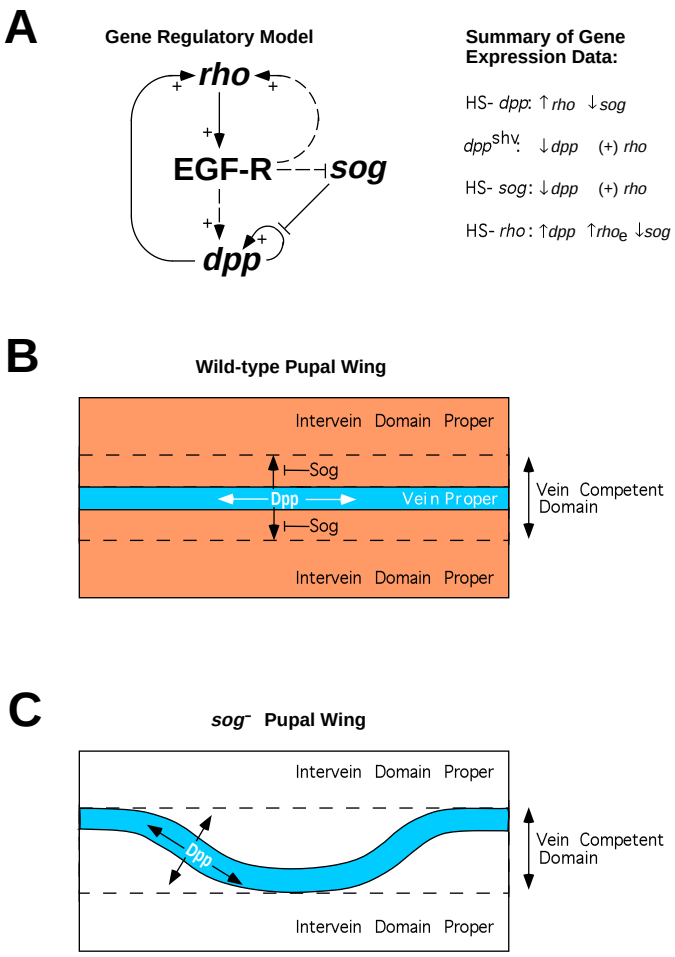


Fig. 5. Model for *sog* and *dpp* function in vein formation. (A) A model of gene interactions to explain the experimental observations reported in this study (left panel) and a summary of the data from which this model is constructed (right panel). We propose that Dpp signaling represents a sufficient, but not necessary, condition for maintaining *rho* expression in veins. Thus, *rho* expression can be triggered by ectopic *dpp* expression, but does not require Dpp function (i.e. in dpp^{shv} *or* HS-*sog* wings, *dpp* expression is severely reduced or eliminated, but *rho* expression is normal). As available data indicate that the sole function of Sog is to block Dpp signaling (Biehs et al., 1996), the observation that HS-*sog* suppresses *dpp* expression suggests that Sog antagonizes Dpp signaling by blocking a Dpp autoregulatory loop necessary for maintaining *dpp* expression. Other possible interactions are indicated with dotted lines since additional data will be required to rigorously establish these potential direct connections. The reason for suspecting that EGF-R signaling may directly activate *dpp* expression is that ectopic *rho* expression potently induces *dpp* expression throughout intervein regions immediately following HS-*rho* induction and along veins following a 5 hour rest period. It is notable that HS-*rho*-induced ectopic *dpp* expression is considerably stronger and more widespread than ectopic activation of endogenous *rho*. If EGF-R signaling directly activates *dpp* expression, it is not sufficient to support *dpp* expression (i.e. *rho* is expressed as normal in HS-*sog* wings, yet *dpp* expression is strongly compromised). It is possible that the effect of ectopic *rho* on *dpp* expression is mediated indirectly by down-regulation of *sog*. This seems unlikely for two reasons, however. First, the pattern of *sog* down-regulation in HS-*rho* wings is similar to the relatively mild ectopic expression of *rho* rather than to the widespread pattern of ectopic *dpp* expression. Second, the lack of ectopic veins in *sog* mosaic clones would be puzzling since *dpp* mis-expression does induce ectopic vein formation. The connection between EGF-R signaling and *rho* expression derives from the observation that ectopic *rho* induces endogenous *rho* expression, and by genetic data indicating that *rho* hyperactivates *Egf-r* function throughout vein development to promote the vein fate (Sturtevant et al. 1993; Noll et al., 1994; Sturtevant and Bier, 1995). Since HS-*rho* induces stronger ectopic *dpp* expression than ectopic *rho* expression, however, it is possible that the HS-*rho*-induced endogenous *rho* is mediated indirectly by Dpp signaling. Consistent with this latter possibility, the degree of ectopic endogenous *rho* expression is greater after 5 hours of intervening development than immediately following heat induction. In the summary of gene expression data the following symbols are used: ↑, ectopic gene expression, (+), no effect on gene expression, ↓, reduction or loss of gene expression. rho_e, endogenous *rho* expression, which was detected using a 3′ *rho* probe hybridizing to sequences present in the endogenous *rho* transcription unit, but missing from the HS-*rho* transgene. We observed mild ectopic endogenous *rho* expression immediately following HS-*rho* expression which became stronger and more defined following 5 hours of aging. The pattern of late rho_e expression was similar to, although less extensive than, that observed following ectopic *dpp* expression (Fig. 4E). As in the case of HS-*dpp*, *sog* expression in HS-*rho* wings was down-regulated in a pattern mirroring that of ectopic rho_e (data not shown). As mentioned above, HS-*rho* induced stronger and more general ectopic expression of *dpp* than rho_e (data not shown). (B) Diagram of a wild-type wing in which Dpp expressed in veins diffuses along the axis of the developing vein and into the intervein region. In intervein regions, however, Sog prevents Dpp from functioning (i.e. by blocking Dpp autoactivation) and thereby restricts Dpp activity to the developing vein. According to this model, Sog is essential for channeling Dpp activity down the center of the broader vein-competent domain. (C) Diagram of a wing containing a homozygous loss-of-function sog^{-} clone. Dpp signaling is no longer limited to the center of the vein-competent region by the sharp intervein expression of *sog*. Thus, a continuous vein forms (because Dpp signaling is intact) that meanders about the vein-competent region in an irregular path (because *sog* is not present to confine Dpp signaling to a straight channel down the center of the vein-competent domain).

in intervein regions could contribute to establishing the directionality of vein extension by providing straight narrow channels of Dpp-responsive cells (Fig. 5B). A role for Dpp in promoting vein continuity is also appealing in light of the autoactivating function of Dpp in veins (see above). Thus, *sog* may provide a conduit within which Dpp can diffuse and autoactivate, thereby spreading a signal to all cells along the vein primordium to retain the vein fate. This mechanism for achieving a uniform cell fate along the axis of the vein is ideally suited for preventing any interruption of vein continuity. Another indication that *dpp* and *sog* may contribute to vein extension is that mis-expression of *sog* leads to detached cross veins in weakly affected individuals of various genotypes (more strongly affected individuals of these same genotypes typically lack the affected cross veins altogether, see Table 3). A similar floating cross vein phenotype is observed in *detached* (*det*) mutants and ectopic floating veins are common in *net*; *det* double mutants (Sturtevant and Bier, 1995). These phenotypes have been interpreted tentatively as disruption of a vein continuity function (Sturtevant and Bier, 1995). Consistent with this view, *det* is a potent enhancer of dpp^{shv} (Table 3). Further genetic and mosaic analysis will be required to distinguish between the various possible roles for Dpp and Sog during vein development.

Generality of *dpp* and *sog* antagonism

Antagonism of *dpp* activity by *sog* appears to be a phylogenetically conserved mechanism for subdividing the ectoderm into neural versus non-neural domains. In *Xenopus*, *BMP-4* (a *dpp* homologue) and *chordin* (a *sog* homologue) are expressed in complementary dorsal-ventral domains (Fainsod et al., 1994; Sasai et al., 1994; Schmidt et al., 1995a). While the relative dorsal-ventral order of abutting *BMP-4* and *chordin* expression domains is reversed in *Xenopus* relative to that of *dpp* and *sog* in *Drosophila* (Arendt and Nüblerjung, 1994; François and Bier, 1995), the developmental potential of cells arising from these juxtaposed domains are similar (i.e. neuroectoderm forms from *sog* or *chordin*-expressing cells while non-neural ectoderm derives from *dpp* or *BMP-4*-expressing regions). In addition, *BMP-4* and *dpp* are functionally equivalent in both *Drosophila* and *Xenopus* embryos (Padgett et al. 1993; Holley et al., 1995) and *chordin* and *sog* have similar functions in both organisms (Holley et al., 1995; Schmidt et al., 1995b). The mechanism of action of this pair of genes may even be conserved. In *Xenopus*, BMP-4 suppresses the default program of neurogenesis and Chordin alleviates this repression to define a BMP-4-free zone in which neurogenesis is permitted (Wilson and Hemmati-Brevanlou; Sasai et al., 1995). We have recently obtained evidence that *dpp* plays an analogous role in suppressing neurogenesis at a very early stage of *Drosophila* embryogenesis and that *sog* blocks this action of *dpp* in the neuroectoderm (Biehs et al., 1996). As *BMP-4* and *chordin* are both expressed during subsequent stages of vertebrate development, such as formation of the neural tube, it will be interesting to determine whether they also function antagonistically in these developmental settings.

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