Local inhibition and long-range enhancement of Dpp signal transduction by Sog

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Extracellular gradients of signalling molecules can specify different thresholds of gene activity in development. A gradient of Decapentaplegic (Dpp) activity subdivides the dorsal ectoderm of the *Drosophila* embryo into amnioserosa and dorsal epidermis[1,2]. The proteins Short gastrulation[3] (Sog) and Tolloid[4] (Tld) are required to shape this gradient. Sog has been proposed to form an inhibitory complex with either Dpp[5] or the related ligand Screw[6,7], and is subsequently processed by the protease Tld[5]. Paradoxically, Sog appears to be required for amnioserosa formation[8], which is specified by peak Dpp signalling activity[1,2]. Here we show that the misexpression of *sog* using the *even-skipped* stripe-2 enhancer[9] redistributes Dpp signalling in a mutant background in which *dpp* is expressed throughout the embryo. Dpp activity is diminished near the Sog stripe and peak Dpp signalling is detected far from this stripe. However, a tethered form of Sog suppresses local Dpp activity without augmenting Dpp activity at a distance, indicating that diffusion of Sog may be required for enhanced Dpp activity and consequent amnioserosa formation. The long-distance stimulation of Dpp activity by Sog requires Tld, whereas Sog-mediated inhibition of Dpp does not. The heterologous Dpp inhibitor Noggin[10] inhibits Dpp signalling but fails to augment Dpp activity. These results suggest an unusual strategy for generating a gradient threshold of growth-factor activity, whereby Sog and its protease specify peak Dpp signalling far from a localized source of Sog.

We used the *even-skipped* (*eve*) stripe-2 enhancer and the Flp–FRT system[9] to misexpress *sog* in the *Drosophila* embryo. We visualized *sog* expression using a digoxigenin-labelled antisense RNA probe[11]. In addition to the endogenous ventrolateral stripes[3] (Fig. 1a), we detected an ectopic segmentation stripe of *sog* in transgenic embryos (Fig. 1b). We used *Race* as a marker gene[12] to detect the presumptive amnioserosa and peak Dpp signalling activity[13]. The ectopic *sog* stripe created a gap in the *Race* expression pattern (Fig. 1d; compare with Fig. 1c), consistent with previous evidence that Sog inhibits Dpp signalling by binding either Dpp[5] or another signalling molecule of the transforming growth factor-β (TGF-β) family, Screw[6,7], which is uniformly expressed throughout early embryos[14].

Sog appears to be required for peak Dpp/Screw activity, as *sog* mutants lack amnioserosa[8]. Several amnioserosa marker genes, including *Kruppel*, *rhomboid* and *hindsight*, exhibit broadened patterns of expression that gradually diminish in older embryos[3,5]. In contrast, the *Race* pattern was not transiently expanded in *sog* mutants: instead, expression was nearly lost in central regions by the onset of gastrulation (Fig. 1e). *Race* may represent a more definitive marker for the presumptive amnioserosa than the genes used in previous studies[3,5]. However, the anterior *Race* pattern (Fig. 1e) did not coincide with the amnioserosa, but was associated with head structures such as the optic lobe. The anterior pattern was lost in *dpp* mutants[12] but retained in *sog*$^{-}$ embryos (Fig. 1e), indicating that there may be higher levels of Dpp and/or Screw activity in the presumptive head.

The stripe-2–*sog* transgene created a gap in the *Race* pattern, but did not enhance expression above that directed by the endogenous ventrolateral *sog* stripes (Fig. 1d). We introduced the stripe-2–*sog* transgene into *sog*$^{-}$ mutant embryos to determine whether it could

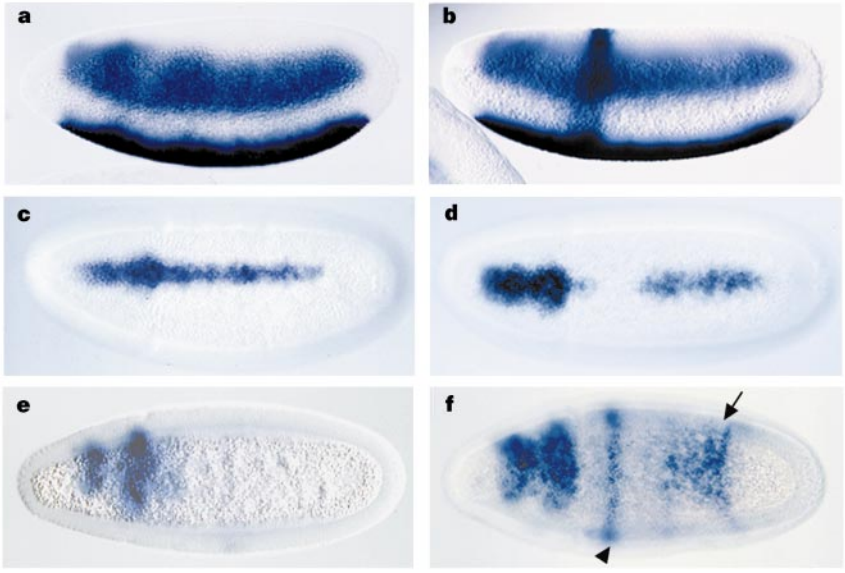

Figure 1 Expression of the stripe-2-*sog* transgene in wild-type and *sog*$^{-}$ embryos. Embryos are orientated with anterior to the left. They were hybridized with digoxigenin-labelled *sog* and/or *Race* antisense RNA probes and histochemically stained with anti-digoxigenin antibodies. **a**, Lateral view of wild-type, late-nuclear-cleavage, cycle-14 embryo stained with a *sog* hybridization probe. *sog* transcripts are detected in two ventrolateral stripes within the presumptive neurogenic ectoderm. **b**, As for **a**, except that the embryo contains the stripe-2-*sog* transgene, which directs an ectopic stripe of *sog* expression. **c**, Dorsal view of a wild-type gastrulating embryo stained with a *Race* hybridization probe. Staining is detected dorsal regions that will form the amnioserosa and head structures. **d**, As for **c**, except that the embryo contains a copy of the stripe-2-*sog* transgene. There is a gap in the *Race* pattern near the ectopic *sog* stripe. A similar gap was obtained with a modified stripe-2-*sog* transgene that contains the CD2 transmembrane protein (data not shown, and Fig. 4). **e**, Dorsal view of a *sog*$^{-}$ embryo stained with the *Race* hybridization probe. Staining is restricted to anterior regions that will form derivatives of head. There is a loss of staining in central regions that form the amnioserosa. Note that the anterior pattern is expanded into dorsolateral regions as compared with wild-type (**c**). **f**, Dorsal view of a *sog*$^{-}$ embryo that contains the stripe-2-*sog* transgene. The embryo was stained with a mixture of *Race* and *sog* RNA probes. There is a gap in the *Race* pattern centred around the *sog* stripe (arrowhead). *Race* expression is upregulated in posterior regions far from the stripe (arrow).

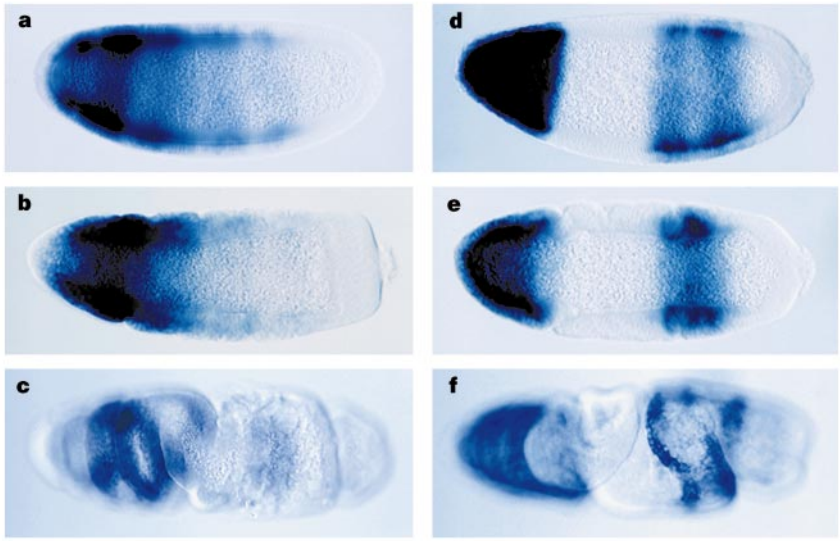

Figure 2 The stripe-2–*sog* transgene induces *Race* expression in *gd*⁻ mutants. Anterior is to the left. Embryos were collected from *gd*⁻ homozygous females and stained with a *Race* hybridization probe. **a**, Cellularized embryo. *Race* transcripts are distributed in a broad anteroposterior gradient, with peak levels in anterior regions. **b**, Gastrulating embryo. *Race* transcripts are gradually lost from posterior regions. **c**, Advanced-stage, elongated embryo. *Race* transcripts are restricted to anterior regions. Weak staining also appears in the anterior and posterior midgut. The mutant embryo has very few amnioserosa cells. **d**, As for **a**, except that the embryo contains the stripe-2–*sog* transgene. There is a loss of *Race* staining in a broad domain on both sides of the *sog* stripe. There is enhanced expression far from the stripe in both anterior and posterior regions. **e**, As for **b**, except that the embryo contains the stripe-2–*sog* transgene. There is a sharp domain of *Race* repression followed by augmented staining in both anterior and posterior regions. This is particularly evident for the posterior pattern. There is virtually no *Race* expression in this region in *gd*⁻ mutants lacking the *sog* transgene (**b**). **f**, As for **c**, except that the embryo contains the stripe-2–*sog* transgene. The *Race* pattern remains off in central regions, but is sustained in posterior regions. The latter staining pattern coincides with amnioserosa tissue, which is largely absent in embryos lacking the stripe-2–*sog* transgene (**c**).

enhance *Race* expression (Fig. 1f). The transgene provided the only source of Sog (arrowhead), and *Race* expression was now detected in posterior regions far from the stripe (Fig. 1f, arrow); this staining was not observed in mutants lacking the transgene (Fig. 1e). We further investigated the ability of Sog both to inhibit and augment Dpp/Screw signalling by studying mutants lacking the Dorsal protein nuclear gradient.

The *gastrulation defective* (*gd*) gene encodes a serine protease required for the activation of the Toll signalling pathway[15], and mutant embryos derived from females homozygous for a null

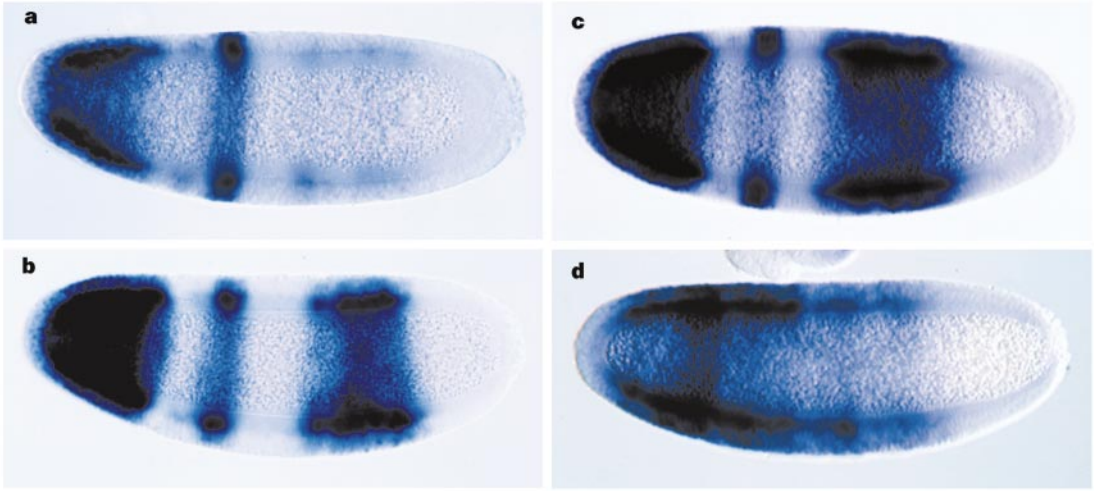

Figure 3 Altering the dose of *dpp* changes the Dpp/Screw signalling pattern. The *gd*⁻ embryos used carried either 1 (**a**), 2 (**b**), or 3 (**c**, **d**) copies of the *dpp*⁺ gene. They were hybridized either with a mixture of *sog* and *Race* RNA probes (**a**–**c**) or with the *Race* probe alone (**d**). **a**, Cellularized *gd*⁻ embryo carrying one copy of *dpp* (*dpp*^{Hin37}/+). *Race* staining is restricted to anterior regions, indicating that the stripe-2–*sog* transgene inhibits Dpp/Screw signalling. However, there is little enhancement in the posterior *Race* pattern (compare with **b**). **b**, Cellularized *gd*⁻ embryo carrying two normal copies of *dpp*. As shown in Fig. 2, there is a gap in the *Race* pattern centred around the *sog* stripe. Enhanced *Race* staining is detected in both anterior and posterior regions. Note that the *sog* stripe is asymmetrically positioned between the two domains of *Race* expression. This might reflect increased activities of Dpp and/or Screw in anterior regions, thereby requiring higher levels of Sog for inhibition. **c**, Cellularized *gd*⁻ embryo carrying three copies of *dpp*. The zone of *Race* inhibition is narrower than that observed in embryos carrying two copies of *dpp*. Moreover, the *sog* stripe is now positioned symmetrically between the two *Race* domains (compare with **b**), indicating that higher levels of Sog may be required to inhibit the increased levels of Dpp. **d**, As for **c**, except that the embryo lacks the stripe-2–*sog* transgene. *Race* staining is generally increased in these embryos as compared with those containing two copies of *dpp* (Fig. 2a).

mutation in *gd* lack a Dorsal nuclear gradient[16]. Consequently, there is no *sog* expression, and *dpp* is expressed ubiquitously in both dorsal and ventral regions[17]. In these mutants, the stripe-2–*sog* transgene provides the only source of Sog products (Fig. 4c; and see later) and these mediate a substantial reorganization in Dpp/Screw signalling activity.

Race was expressed in anterior regions of *gd* mutant embryos (Fig. 2a–c), possibly because of head-specific factors that enhance Dpp signalling. Introduction of the stripe-2–*sog* transgene inhibited *Race* expression within and on either side of the *sog* stripe (Fig. 2d–f). In addition, staining was augmented far from the stripe; this was particularly evident in posterior regions in which *Race* expression is normally lost (Fig. 2e; compare with Fig. 2b). *Race* expression was sustained in advanced-stage embryos (Fig. 2f; compare with Fig. 2c).

The simplest interpretation of these results is that high concentrations of Sog secreted near the stripe inhibit Dpp/Screw signalling, whereas low levels that diffuse far from the stripe augment signalling and activate *Race* expression. However, it is conceivable that the upregulation of *Race* depends on a relay mechanism. The inhibition of Dpp/Screw signalling might trigger formation of the dorsal epidermis in the vicinity of the stripe-2–*sog* pattern. These epidermal cells could then induce neighbouring regions to form amnioserosa through an unknown signal. We investigated this possibility by manipulating the number of copies of the dpp^{+} gene in gd^{-} embryos (Fig. 3). Embryos were stained with a mixture of *sog* and *Race* RNA probes. The stripe-2–*sog* transgene failed to upregulate *Race* expression in embryos carrying just one copy of *dpp* (Fig. 3a; compare with Fig. 3b). There was a general increase in *Race* expression in embryos carrying three copies of *dpp* (Fig. 3d) as compared with two copies (Fig. 2a). Introduction of the stripe-2-*sog* transgene reorganized the *Race* pattern (Fig. 3c), such that a narrow zone of inhibition was formed in the vicinity of the stripe and the posterior domain of enhanced expression was expanded as compared with embryos carrying two copies of *dpp* (Fig. 3b). These results indicate that the specification of cell types is uncoupled by low versus high levels of Dpp signalling. A narrow domain of low Dpp signalling activity was associated with a broadened region of amnioserosa specification (Fig. 3c), whereas a broad domain of low Dpp signalling failed to induce neighbouring cells to express *Race* (Fig. 3a).

More definitive evidence against a relay model was obtained by fusing DNA encoding the rat integral-membrane protein CD2 in-frame at the 3′ terminus of the *sog* coding sequence. The resulting Sog–CD2 fusion protein should be 'tethered' to the plasma membrane and unable to diffuse over long distances. This transgene created a gap in the normal *Race* expression pattern when introduced into wild-type embryos (similar to the pattern in Fig. 1d), indicating that it retains activity (data not shown). The modified *sog* transgene also inhibited the anterior *Race* expression pattern in gd^{-} embryos (Fig. 4b, arrow; compare with Fig. 4a), but did not augment staining in posterior regions (Fig. 4 legend). In contrast, the unmodified stripe-2–*sog* transgene both inhibited the anterior *Race* pattern (Fig. 4d, arrow) and upregulated posterior *Race* expression (Fig. 4d, arrowhead). These results argue against a relay mechanism and indicate that Sog diffusion may be essential in augmenting Dpp/Screw signalling.

Peak Dpp signalling may depend on the protease Tld[5]. *tld* is expressed in the dorsal ectoderm of wild-type embryos[4], and is expressed ubiquitously in *gd* mutants. The stripe-2–*sog* transgene was expressed in gd^{-} embryos heterozygous for a *tld* mutation. Mutant embryos were stained with a mixture of *sog* and *Race* hybridization probes (Fig. 5). *Race* expression was reduced in posterior regions (Fig. 5b) as compared with gd^{-} embryos containing two wild-type copies of *tld* (Fig. 5a). These results indicate that peak Dpp signalling may depend on the proteolytic degradation of an inhibitory complex containing Sog. In contrast, the stripe-2–*sog* transgene continued to inhibit *Race* expression (Fig. 5b), suggesting that Tld is dispensable for this activity.

To confirm that Sog is specifically required for peak Dpp signalling, we used the *Xenopus* protein Noggin[10] as a heterologous inhibitor to sequester Dpp into an inactive complex. Noggin

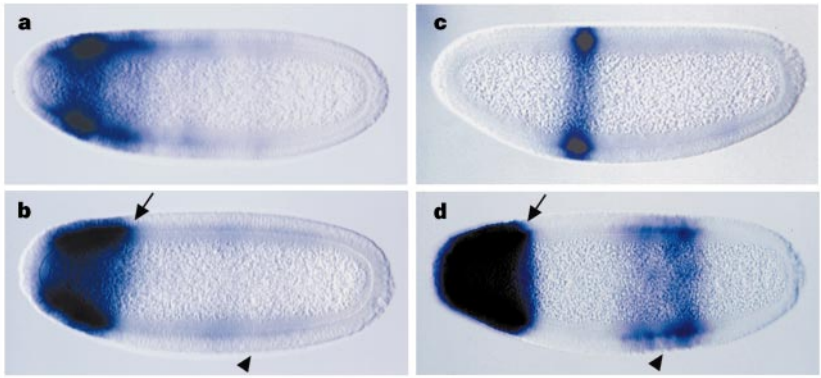

Figure 4 A tethered form of Sog does not mediate long-range enhancement of *Race* expression. The gd^{-} embryos used carried either the wild-type stripe-2-*sog* transgene (**c**, **d**) or a modified transgene encoding the integral-membrane protein CD2 fused in-frame to the 3′ terminus of the *sog* coding region (**b**). Embryos were stained with either the *Race* (**a**, **b**, **d**) or *sog* (**c**) RNA hybridization probe. **a**, Cellularized gd^{-} embryo stained with the *Race* probe. The most intense staining is detected in anterior regions. **b**, As for **a**, except that the embryo contains the modified stripe-2-*sog–CD2* transgene. *Race* expression is restricted to anterior regions (arrow), indicating that the transgene may be able to inhibit Dpp/Screw signalling (compare with **a**). However, there is no increase in the *Race* pattern in posterior regions (arrowhead), in contrast to the situation observed with the unmodified, untethered form of the *sog* transgene (**d**). This result indicates that the Sog–CD2 fusion protein may be unable to diffuse and activate *Race*. However, there appears to be some release of Sog from the modified *sog–CD2* fusion gene, as about half of all older embryos exhibit variable increases in the posterior *Race* pattern. Nonetheless, the wild-type stripe-2-*sog* transgene never produces this pattern in which the anterior domain is repressed and there is no enhancement of expression (**d**). Perhaps the heterologous Sog–CD2 fusion protein is subject to proteolytic cleavage during development. Although unlikely, we cannot completely exclude the possibility that low levels of Sog result in an intermediate threshold of Dpp/Screw which specifies dorsal epidermis and triggers the expression of signalling factors that specify neighbouring cells to form amnioserosa. **c**, A cellularized gd^{-} embryo that contains the unmodified stripe-2-*sog* transgene. The embryo was stained with the *sog* RNA probe to show that the transgene is the sole source of *sog* expression in these mutants. Some embryos exhibit weak and variable staining within the limits of stripe 7 (not shown). In addition, the stripe-2-*sog* expression pattern is broader in younger embryos, but quickly refines into the sharp stripe seen here (data not shown). **d**, *Race* staining pattern in a cellularized gd^{-} embryo that contains the unmodified stripe-2-*sog* transgene. As shown in Fig. 2d, *Race* is inhibited in a broad band in the vicinity of the stripe, but is upregulated in both anterior (arrow) and posterior (arrowhead) regions far from the stripe.

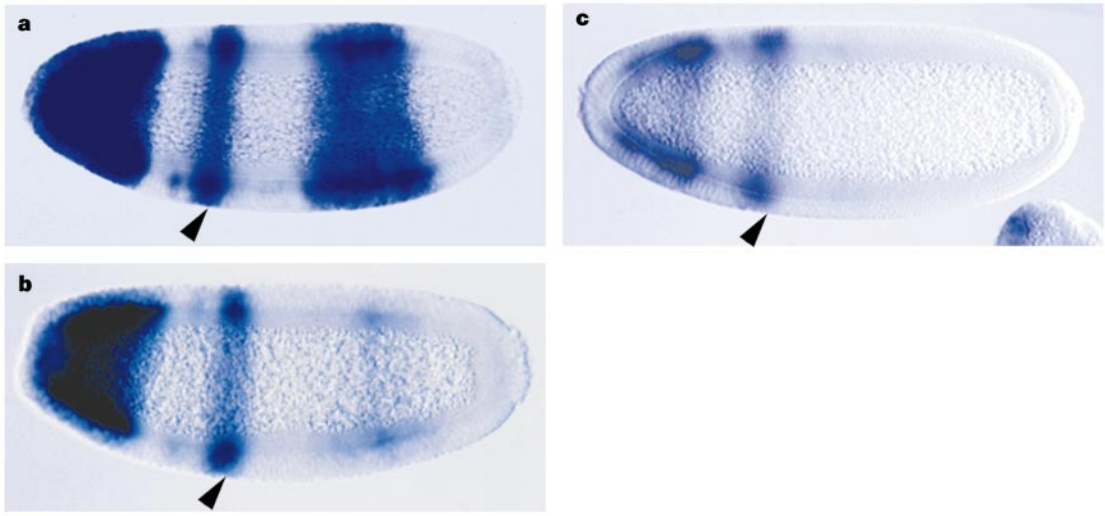

Figure 5 Uncoupling *sog*-mediated enhancement and inhibition of Dpp signalling. The cellularizing gd^{-} embryos used contained either a stripe-2–*sog* or stripe-2–*noggin* transgene, and were stained with a mixture of *Race* and *sog* (**a**, **b**) or *Race* and *noggin* (**c**) hybridization probes. The arrowheads indicate the stripe-2 pattern. **a**, The embryo contains the *sog* transgene. As shown in Fig. 3b, the stripe-2 *sog* pattern is asymmetrically positioned between the anterior and posterior *Race* expression domains. **b**, As for **a**, except that the embryo is heterozygous for a moderate *tld* mutation[25] (tld^{9B}/+). The stripe-2–*sog* transgene continues to inhibit *Race* expression, but there is a substantial loss of staining in posterior regions (compare with **a**). **c**, The embryo contains the stripe-2–*noggin* transgene. *Race* expression is restricted to anterior regions, indicating that Noggin can inhibit, but not enhance, Dpp/Screw signalling. The stripe-2–*noggin* transgene is poorly expressed; however, whenever a *noggin* stripe was observed, there was usually some indication of *Race* inhibition. The embryo shown in this panel represents one of the best examples of robust *noggin* expression.

binds Dpp (and possibly Screw) but is not recognized by Tld, thereby preventing the release of active Dpp[18,19]. Noggin was expressed in the *Drosophila* embryo using the same *eve* stripe-2 Flp–FRT system[9] that we used to misexpress *sog*. *Race* and *noggin* expression were visualized by *in situ* hybridization. Embryos bearing the transgene expressed a stripe of *noggin* that had the same inhibitory effect on *Race* expression as stripe-2–*sog* (Fig. 5c). However, there was no upregulation of *Race* in posterior regions. These results show that Noggin can mimic the inhibitory effect of Sog, but is unable to generate the peak Dpp signalling output.

We have shown that a localized stripe of *sog* expression is sufficient to reorganize the dorsoventral pattern of mutant embryos lacking a Dorsal nuclear gradient. Cells located near the Sog source exhibit repression of dorsal-ectoderm marker genes, whereas those located far from the source (>10 cell diameters) exhibit peak Dpp signalling, including upregulation of *Race* expression and the restoration of amnioserosa in advanced-stage embryos. Different models have been proposed to explain the requirement for Sog in generating peak Dpp activity. One invokes the diffusion of Sog–Dpp or Sog–Screw complexes away from the ventrolateral Sog stripes, thereby focusing Dpp and/or Screw at the dorsal midline[6,7,19]. An alternative model is that a product resulting from the cleavage of Sog directly signals formation of the amnioserosa[5], possibly by augmenting the binding of Dpp or Screw to the receptors Thick veins and Saxophone.

Results from studies of *Xenopus* and *Drosophila* indicate that extracellular signalling molecules of the TGF-β family can generate different thresholds of gene activity through a classical 'French flag' mechanism of positional information[20]. Cells located near the source of signal exhibit a peak threshold of gene activity, whereas those located progressively farther from the source express target genes that can be activated only by low levels of signal. A localized source of activin leads to different patterns of *goosecoid* and *Xbra* expression in *Xenopus* animal caps[21,22], and a localized source of Dpp at the boundary between the anterior and posterior compartments of *Drosophila* wing imaginal discs leads to differential patterns of *spalt* and *optomotor-blind* expression[23,24]. Our results indicate that the patterning of the *Drosophila* dorsal ectoderm may depend on a different mechanism[5,19]. In the absence of Sog, Dpp signalling is just below the critical threshold required for the specification of the amnioserosa. The activation of amnioserosa-specific genes, such as *Race*, depends on enhancing Dpp/Screw signalling. This can be achieved by overexpressing Dpp[25] (Fig. 3d), but is normally achieved with a localized source of Sog. Given the evolutionary conservation of Sog and Dpp (chordin and bone morphogenetic protein in *Xenopus*)[26], it is conceivable that this mechanism is used generally in metazoans. □

Methods

Plasmid construction, P-element transformation and *in situ* hybridization. A *Hin*dIII–*Not*I fragment of the *sog* cDNA (nucleotides 0–4,541) from pBS*sog* (a gift from E. Bier) and an *Eco*RI–*Eco*RV *noggin* cDNA fragment from pNogginΔ5 (ref. 10) were blunt-end-ligated into 22FPE (ref. 9) (provided by S. Small). Rat *CD2* cDNA was fused to *sog* in pBS*sog* by first mutagenizing the *sog* stop codon by the polymerase chain reaction (PCR), creating a unique *Asp*718 site. A *Pst*I fragment of rat *CD2* cDNA (from FC15; a gift from G. Struhl) lacking the signal sequence was inserted in-frame into the *Asp*718 site in a blunt-ended ligation. The *sog*–*CD2* cDNA was then transferred into 22FPE. P-element-mediated transformation by injection into yw^{67c23} embryos was as described[27]. Multiple lines were tested for all constructs. *In situ* hybridizations using digoxigenin-labelled antisense RNA probes and alkaline phosphatase substrate were performed as described[11,28].

***Drosophila* stocks and genetic crosses.** The fly stocks used were as follows: a transgenic line homozygous for a P[ry^{+} β_2-*tubulin*–*flp*] insertion provided by G. Struhl); *gastrulation defective*, gd^{7}/FM3; *decapentaplegic*, dpp^{Hin37}/GlaDp(2;2)DTD48; *short gastrulation*, sog^{S6}/FM7c; and *tolloid*, tld^{9B}/TM3. Females containing the misexpression constructs were crossed to males carrying the β_2-*tubulin*–*flp* gene to obtain males containing both transgenes; in these males the misexpression constructs are activated by the spermatocyte-specific removal of a flp-out cassette catalysed by β_2-*tubulin*–*flp*. These males were crossed to yw^{67c23}, gd^{7}/gd^{7}, sog^{S6}/FM7c (Fig. 1f), gd^{7}/gd^{7}; GlaDp(2;2)DTD48/+ (Fig. 3c) or gd^{7}/gd^{7}; dpp^{Hin37}/GlaDp(2;2)DTD48 (Fig. 3a) females and embryos were collected and analysed by *in situ* hybridization. gd^{7}/gd^{7}; GlaDp(2;2)DTD48/+ females, which contain an extra copy of *dpp*, were obtained by first crossing dpp^{Hin37}/GlaDp(2;2)DTD48 males with gd^{7}/FM3 females. gd^{7}; GlaDp(2;2)DTD48/+ males from this cross were

then mated with gd^7/FM3 females to obtain gd^7/gd^7; GlaDp(2;2)DTD48/+ females. This cross also generated gd^7/FM3; GlaDp(2;2)DTD48/+ females, which were first crossed to dpp^{Hin37}/GlaDp(2;2)DTD48 males. The resulting males (including gd^7; dpp^{Hin37}/GlaDp(2;2)DTD48 males) were backcrossed to gd7/FM3; GlaDp(2;2)DTD48/+ females. From this cross, females that lacked the FM3 balancer (including gd^7/gd^7; dpp^{Hin37}/GlaDp(2;2)DTD48 females) were crossed to P[*st2-sog*]; P[$ry^+\beta_2$-*tubulin–flp*] males to obtain st2-*sog*, gd^-, *dpp*/+ embryos.

The dose of *tld* was lowered by first crossing P[*st2-sog*] females to tld^{9B}/TM3 males. P[*st2-sog*]; tld^{9B} females were then crossed to P[$ry^+\beta_2$-*tubulin–flp*] males generate P[*st2-sog*]; tld^{9B}/P[$ry^+\beta$-*tubulin–flp*] males, which can activate the transgene. Embryos were collected from crosses between these males and gd^7/gd^7; tld^{9B} females, obtained by crossing gd^7/FM3 females with tld^{9B}/TM3 males then backcrossing gd^7; tld^{9B} males with gd^7/FM3 females. The embryos shown in Figs 3, 5 were represented at the predicted frequency based on the crosses.

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A new secreted protein that binds to Wnt proteins and inhibits their activites

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The Wnt proteins constitute a large family of extracellular signalling molecules that are found throughout the animal kingdom and are important for a wide variety of normal and pathological developmental processes[1,2]. Here we describe Wnt-inhibitory factor-1 (WIF-1), a secreted protein that binds to Wnt proteins and inhibits their activities. WIF-1 is present in fish, amphibia and mammals, and is expressed during *Xenopus* and zebrafish development in a complex pattern that includes paraxial presomitic mesoderm, notochord, branchial arches and neural crest derivatives. We use *Xenopus* embryos to show that WIF-1 overexpression affects somitogenesis (the generation of trunk mesoderm segments), in agreement with its normal expression in paraxial mesoderm. *In vitro*, WIF-1 binds to *Drosophila* Wingless and *Xenopus* Wnt8 produced by *Drosophila* S2 cells. Together with earlier results obtained with the secreted Frizzled-related proteins[1,2], our results indicate that Wnt proteins interact with structurally diverse extracellular inhibitors, presumably to fine-tune the spatial and temporal patterns of Wnt activity.

There are two families of secreted molecules known to inhibit Wnt signalling activity: the secreted Frizzled-related protein (sFRP) family, whose members all have an amino-terminal cysteine-rich domain (CRD) that is highly homologous to the ligand-binding domain of Frizzled proteins, which are transmembrane Wnt receptors[1,2]; and the protein family Dickkopf (Dkk), whose mechanism of action is at present unknown[3].

WIF-1 was first identified as an expressed sequence tag from the human retina (J. P. Macke, P.M.S. and J.N., unpublished results), and highly conserved orthologues have been isolated from mouse, *Xenopus* and zebrafish (Fig. 1). The deduced amino-acid sequence of WIF-1 has an N-terminal signal sequence, a domain of ~150 amino acids (the WIF domain), five epidermal growth factor (EGF)-like repeats that are most similar to those of the extracellular matrix protein tenascin, and a hydrophilic domain of ~45 amino acids at the carboxy terminus.

In the adult mouse, WIF-1 expression is highest in the heart and lung, and lower in the brain and eye (Fig. 2a). Northern-blot hybridization with *Xenopus* total RNA revealed the presence of a single transcript expressed first at neurula stages (not shown), and *in situ* hybridization to *Xenopus* or zebrafish embryos confirmed that no messenger RNA is detectable at the gastrula stage. In *Xenopus*, WIF-1 is expressed during somitogenesis predominantly in the unsegmented paraxial presomitic mesoderm and to a much lesser extent in newly segmented somites (Fig. 2b–e). In zebrafish, WIF-1 is highly expressed in unsegmented paraxial mesoderm and is virtually undetectable in mature somites (Fig. 2g–i). WIF-1 expression is visible in both species in the notochord in register