The *Drosophila short gastrulation* gene prevents Dpp from autoactivating and suppressing neurogenesis in the neuroectoderm

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The short gastrulation (*sog*) gene is expressed in broad lateral stripes comprising the neuroectoderm of the *Drosophila* blastoderm embryo. *sog* encodes a predicted secreted protein that functions nonautonomously to antagonize the activity of the TGF-β-like Decapentaplegic (Dpp) signaling pathway in the dorsal region of the embryo. Recently, it has been shown that *sog* and *dpp* are functionally equivalent to their respective *Xenopus* homologs *chordin* and BMP-4. In this report we provide the first direct evidence that *sog* plays a local role in the lateral region of the blastoderm embryo to oppose Dpp activity in the neuroectoderm. In the dorsal region, Dpp signaling both suppresses neurogenesis and maintains expression of genes that promote dorsal cell fates (dorsalization). We show that Dpp also can perform both of these functions in the neuroectoderm. In wild-type embryos, the ability of Dpp to induce expression of dorsal markers including itself (autoactivation) in the neuroectoderm is blocked by *sog*. We propose that Sog protects the neuroectoderm from an invasive positive feedback loop created by Dpp diffusion and autoactivation. We show that the two functions of Dpp signaling, neural suppression and dorsalization, are triggered by distinct thresholds of Dpp activity. Epistasis experiments reveal that all observed *sog* activity can be accounted for by Sog functioning as a dedicated Dpp antagonist. Finally, we provide evidence that Sog functions as a diffusible morphogen in the blastoderm embryo. These data strongly support the view that the primary phylogenetically conserved function of the *Drosophila* *sog* and *dpp* genes and the homologous *Xenopus* *chordin* and BMP-4 genes is to subdivide the primitive embryonic ectoderm into neural versus non-neural domains.

[Key Words: short gastrulation (*sog*); decapentaplegic (*dpp*); TGF-β; morphogen; neuroectoderm; dorsal–ventral axis; evolution; *Drosophila*]

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The vertebrate bone morphogenetic proteins (BMPs) define a subgroup of the transforming growth factor-β (TGF-β) superfamily capable of organizing undifferentiated mesenchymal cells into bone tissue (Rosen and Thies 1992; Kingsley 1994). At least two mouse BMPs are also individually important during development as mutations in these genes (short ear and brachypodism) lead to defects in bone formation and induction between germ layers (Kingsley et al. 1992; Storm et al. 1994). Furthermore, as described below, BMP-4 and its *Drosophila* homolog Dpp play analogous roles during dorsal–ventral (D-V) axis formation and neurogenesis in *Xenopus* and *Drosophila* (Graff et al. 1994; Maeno et al. 1994; Holley et al. 1995; Schmidt et al. 1995a).

Establishment of the D-V axis in *Drosophila* is determined by a cascade of maternally acting genes functioning in both the oocyte and surrounding follicle cells. Ultimately these genes establish a nuclear gradient of the NF-κB-related transcription factor Dorsal (Roth et al. 1989; Rushlow et al. 1989; Steward 1989). The Dorsal nuclear gradient is directly responsible for subdividing the embryo into three primary territories of zygotic gene expression: [1] a ventral zone comprising the presumptive mesoderm, [2] a lateral zone comprising the neuroectoderm, and [3] a dorsal zone giving rise to dorsal ectoderm and amnioserosa. Although Dorsal activates expression of genes in ventral and lateral regions, it specifies the dorsal zone by repressing ventral and lateral expression of key zygotic dorsal group (ZDG) genes, including *decapentaplegic* (*dpp*), *zerknüllt* (*zen*), *tolloid* (*tld*), and *twisted gastrulation* (*tsg*), which are required for the differentiation of dorsal tissue types.

The *dpp* gene, which encodes a protein (Padgett et al. 1987) most similar to vertebrate BMP-2 and BMP-4,
play a key role in patterning the dorsal region. Embryos lacking \textit{dpp} function, which exhibit the most severely ventralized phenotype observed among ZDG mutants [Ray et al. 1991; Arora and Nüsslein-Volhard 1992], fail to make any dorsally derived structures [Irish and Gelbart 1987]. Consistent with its developmental function, \textit{dpp} is expressed throughout the dorsal region of the early precellular blastoderm embryo [St. Johnson and Gelbart 1987]. A variety of evidence suggests that Dpp functions as a morphogen with different levels of Dpp specifying distinct developmental fates in dorsal and lateral regions of the embryo [Ferguson and Anderson 1992a,b; Wharton et al. 1993]. In this model, peak Dpp activity specifies the dorsal-most cell type (amnioserosa), whereas lower levels of activity specify the dorsal ectoderm. It is likely that small amounts of Dpp also diffuse into the lateral zone to influence patterning in the neuroectoderm.

As the level of \textit{dpp} mRNA appears uniform throughout the dorsal zone, it has been speculated that a post-transcriptional mechanism is responsible for establishing graded Dpp activity. The short gastrulation (\textit{sog}) gene may contribute to generating such a Dpp activity gradient. \textit{sog} is expressed in broad lateral stripes abutting \textit{dpp}-expressing dorsal cells [François et al. 1994] and functions nonautonomously to pattern the dorsal region of the embryo [Zusman et al. 1988; Ray et al. 1991; Ferguson and Anderson 1992b; Wharton et al. 1993; François et al. 1994]. The \textit{sog} product is a likely secreted protein [François et al. 1994] that may diffuse into the dorsal region to antagonize Dpp signaling [Zusman et al. 1988; Ferguson and Anderson 1992b; Wharton et al. 1993; François et al. 1994]. It is unclear, however, whether \textit{sog} also functions locally to block \textit{dpp} activity in the neuroectoderm itself. This question is of particular interest as \textit{chordin}, the \textit{Xenopus} homolog of \textit{sog}, prevents the Dpp homolog BMP-4 from suppressing neurogenesis during early frog development [Sasai et al. 1995; Wilson and Hemmati-Breivanelou 1995]. As Chordin has been shown recently to bind BMP-4 with high affinity [Picolla et al. 1996], the mechanism of Chordin/\textit{sog} function may be to bind and sequester BMP-4/Dpp in an inactive form.

In this paper we investigate the role of \textit{sog} in the lateral neuroectoderm of the \textit{Drosophila} blastoderm embryo. We show that \textit{sog} can block two distinct \textit{dpp} functions (i.e., suppression of neural genes and activation of ZDG genes) in both dorsal and lateral regions of the early blastoderm embryo. We show that these two \textit{dpp} functions have different thresholds for Dpp activity as considerably less Dpp activity is required for neural suppression than for dorsalization. Epistasis experiments indicate that \textit{sog} functions primarily or exclusively to block Dpp signaling. We also provide evidence that Sog may act over long distances in a dose-dependent fashion to determine the limits of gene expression domains. These data support the view that \textit{sog} acts locally in the neuroectoderm to block Dpp autoactivation, thereby providing a permissive condition for initiating neurogenesis. In addition, Sog behaves as a long-range diffusible morphogen to define distinct thresholds of Dpp activity in neighboring regions of the blastoderm embryo.

\section*{Results}

\textit{Dpp} suppresses neurogenesis in dorsal cells of early embryos

To assess the role of \textit{dpp} in regulating neurogenesis, we first examined the effect of this signaling pathway on neurogenesis in dorsal cells where \textit{dpp} is known to play a critical role in cell fate determination. The dorsal cuticle of embryos lacking \textit{dpp} activity appears ventralized [Irish and Gelbart 1987], leading to the inference that \textit{dpp} functions normally to suppress lateral cell fates dorsally. This inference is tenuous, however, as the number of differentiated neurons is reduced, not expanded in late \textit{dpp} mutant embryos [B. Biels and E. Bier, unpubl.]. In early gastrulating \textit{dpp} mutants, on the other hand, we observed dorsal expression of neuroectodermal markers such as \textit{thick veins} (\textit{tkv}), which encodes a type I Dpp receptor, and \textit{lethal of scute} (\textit{l'sc}) [Fig. 1, cf. B and E with A and D]. Similarly, neuroblasts visualized by markers such as \textit{scratch} (\textit{scrt}) and \textit{snail} (\textit{sna}) subsequently form ectopically in the dorsal region of gastrulating \textit{dpp} embryos [Wharton et al. 1996; B. Biels, unpubl.]. Consistent with \textit{dpp} acting early to suppress initiation of neurogenesis, ectopic expression of the pronuclear gene \textit{l'sc} is first detectable in dorsal cells of late blastoderm stage \textit{dpp} embryos [see arrow in Fig. 4G]. Paradoxically, the increased number of neuroblasts in \textit{dpp} mutants do not generate a hypertrophied differentiated nervous system. Thus, \textit{dpp} mutants may lack a late positive role for \textit{dpp} in neuronal maturation or may hyperactivate pathways functioning to inhibit subsequent steps in neurogenesis.

\textit{sog} prevents \textit{Dpp} from suppressing neurogenesis laterally in the blastoderm embryo

Superficially, \textit{sog} does not appear to play a major role in early neurogenesis as defects in nervous system formation in \textit{sog} mutant embryos are detectable only during midgastrulation and are relatively mild [François et al. 1994]. Because \textit{sog} behaves genetically as a uniquely potent suppressor of Dpp signaling [Ferguson and Anderson 1992b], we reasoned that it might be possible to exacerbate defects in \textit{sog} embryos by increasing the level of \textit{Dpp}. Thus, we examined expression of various neuroectodermal markers in sensitized \textit{sog} embryos that have twice the normal dose of \textit{dpp} [\textit{sog} \textit{Ddpdp}]; i.e., 0\times \textit{sog} and 4\times \textit{dpp}]. The earliest neuroectodermal marker affected in \textit{sog} embryos is \textit{rhomboid} (\textit{rho}), which is expressed in lateral stripes 8–10 cells wide in wild-type blastoderm embryos [Bier et al. 1990]. Lateral \textit{rho} expression is initiated normally in \textit{sog} embryos \textit{Ddpdp} embryos, but rapidly narrows to stripes 4–6 cells across [Fig. 2, cf. B with A]. Similarly, the lateral extent of \textit{l'sc} expression is reduced in early gastrulating \textit{sog} \textit{Ddpdp} embryos [Fig. 2E] relative to wild type [Fig. 2D], resulting in the subsequent loss of the outer two rows of scrt-
dpp suppresses neurogenesis in the dorsal region of the embryo. Expression of the neuroectodermal markers tkv and l'sc in wild-type (wt) and mutant embryos. Lateral views of embryos are shown with anterior to the left and dorsal at the top in this and subsequent figures unless indicated otherwise. Insets in B, C, E, and F show sagittal sections near the dorsal midline. (A) tkv expression is restricted to the neuroectoderm in an early wild-type germ-band extending embryo. (B) tkv expression in a homozygous dpp null mutant (dpp−/−) embryo occupies the entire dorsal ectoderm as well as the neuroectoderm. Because dpp−/− embryos do not germ-band extend normally, they appear younger than comparably staged wild-type embryos. (C) As in wild type, tkv expression is largely suppressed in the dorsal ectoderm of a homozygous scw null (scw−/−) embryo. scw−/− mutants, like dpp−/− embryos, do not germ-band extend normally. (D) l'sc expression is restricted to the neuroectoderm in an early wild-type germ-band extending embryo. (E) l’sc expression in a homozygous dpp−/− embryo extends into the dorsal ectoderm. Ectopic dorsal expression of l’sc in dpp−/− mutants is first observed during the late blastoderm stage (see Fig. 4G). (F) l’sc expression is largely suppressed as normal in the dorsal ectoderm of a scw−/− embryo.

expressing neuroblasts [Fig. 2, cf. sog− in H with G]. As these phenotypes are only observed in sog−; Dpdpp double mutant embryos and not in either single mutant, other genes must normally function in parallel with sog to block the action of Dpp (see below). It is also possible to overcome the protective function of sog in the neuroectoderm by forcing strong ubiquitous expression of dpp in a sog+ background. Thus, heat induction of blastoderm embryos carrying eight copies of a HS-dpp construct (8× HS-dpp) reduces lateral expression of rho to stripes 5–7 cells wide [Fig. 2C]. Such ubiquitous dpp expression also narrows the subsequent expression of l’sc [data not shown], tkv [data not shown], and scrt [Fig. 2I] during germ-band extension. The neuronal loss phenotype of HS-dpp embryos is enhanced in sog− embryos that lack all but the ventral-most neural progenitors [Fig. 2F]. These early neuronal loss phenotypes are consistent with the moderate loss of ventral denticles observed in sog− mutant cuticles [Wieschaus et al. 1984; Zusman et al. 1988] and with the exacerbation of this cuticular phenotype by extra copies of dpp [Ferguson and Anderson 1992b].

dpp continues to exert a neural suppressive function during early gastrulation as HS-dpp induction during germ-band extension causes a significant reduction in neuroblast number [data not shown]. As the D-V axis is well established by germ-band extension, the continued neural suppressive activity of dpp during this stage suggests that Dpp signaling functions by suppressing neurogenesis per se rather than acting indirectly by suppressing the establishment of ventral positional values. Cumulatively, the above results show that sog acts in concert with other genes during the blastoderm stage to prevent Dpp signaling from suppressing neurogenesis in lateral cells.

Dpp can activate expression of dorsal domain genes in the neuroectoderm

In addition to suppressing expression of neuroectodermal genes in the dorsal region, Dpp also plays an active dorsalizing role by maintaining expression of ZDG genes such as zen. In dpp− or sog− embryos, expression of zen is initiated correctly but then disappears rapidly [Ray et al. 1991]. Therefore, we asked whether dpp could activate expression of dorsal markers in the early neuroectoderm of sog−; Dpdpp mutants. Surprisingly, we observed that expression of dpp itself was induced throughout the neuroectoderm in sog−; Dpdpp embryos [Fig 3, cf. B with A]. This provides the first evidence that dpp is capable of autoactivating during early embryogenesis. The ventral limit of dpp expression (blue) is sharp and lies one cell diameter within the sna [brown]-expressing mesoderm [Fig 3B; inset]. Double label experiments with a combination of rho and dpp probes revealed that dpp autoac-
Figure 2. Dpp can suppress neurogenesis in the lateral region of the embryo. Expression of neuroectodermal markers during the early precellular blastoderm stage (rho; A–C), early gastrulation before neuroblast delamination (l'sc; D–F), and late germ-band extension (scrt; G–I). Embryos in D–I are viewed from a ventral perspective with anterior to the left. In D–F surface views are shown and in G–I the focal plane is subectodermal. The ventral midline in D–I is denoted by an arrowhead. (A) rho expression in lateral stripes is 8–10 cells wide in a wild-type precellular blastoderm embryo. (B) The dorsal extent of the lateral rho stripes is reduced by about four cells in sog^U2/Y; Dpdpp/Dpdpp (sog; Dpdpp) embryos. (C) Lateral rho stripes are similarly narrowed in sog^- embryos carrying eight copies of the heat shock dpp construct (8x HS-dpp) after a 20-min heat shock and 30-min of recovery. (D) Wild-type expression of l'sc in lateral stripes in an early gastrulating embryo. (E) The dorsal extent of l'sc expression is significantly reduced in sog^-; Dpdpp embryos. (F) l'sc expression in a sog^U2/Y embryo carrying four copies of the HS-dpp construct (sog^-; 4x HS-dpp) that was heat shocked for 30 min during the blastoderm stage and allowed to develop until midgastrulation. Embryos of this kind have the most pronounced reduction of l'sc expression we observed. (G) scrt expression in S_1 and early S_2 neuroblasts in a sog^-/Y hemizygous embryo. The number of neuroblasts expressing scrt is mildly reduced. Typically, the thoracic and most anterior abdominal segments have the full three rows (r_1-r_3) of S_1 neuroblasts, the middle abdominal segments have the two inner rows (r_1, r_2), and the posterior segments have only the inner r_1 row of neuroblasts [not visible in this local plane, see Fig. 1H in François et al. (1994)]. (H) scrt expression in neuroblasts is reduced to the inner r_1 row along the entire length of a sog^-; Dpdpp embryo. (I) A similar strong reduction in neuroblasts is observed in sog^+; 8x HS-dpp embryos that were heat shocked only 13 min and then allowed to develop until germ-band extension. With this minimal heat induction there is no detectable ectopic expression of dorsal markers in the neuroectoderm (data not shown).

activation precedes or is contemporaneous with the narrowing of lateral rho expression in sog^-; Dpdpp embryos [Fig. 2B]. We also observed ectopic expression of zen [Fig. 3, cf. D with C] and tld [data not shown] in the neuroectoderm of sog^-; Dpdpp embryos. As in the case of neural suppression, dpp autoactivation and misexpression of zen and tld were not observed in either sog^- or Dpdpp single mutant embryos [data not shown]. These data reveal that Dpp signaling, if unopposed by sog, can invade the neuroectoderm by a chain of diffusion followed by autoactivation. Ubiquitous expression of dpp in a sog^- background via HS-dpp also leads to ectopic activation of zen expression in parts of the neuroectoderm [Fig. 3E] and mesoderm [Fig. 3E, inset; see also Fig. 5D,E below]. The effect of ubiquitous dpp expression is enhanced greatly in a sog^- background [Fig. 3F], resulting in zen expression throughout the entire trunk neuroectoderm and mesoderm [see also Fig. 5F below].

Neural suppression is induced by a lower threshold of Dpp activity than dorsalization

A striking feature of the data presented above is that Dpp-mediated neural suppression and dorsalization appear to have markedly different thresholds for Dpp ac-
Figure 3. **sog** opposes Dpp autoactivation in the neuroectoderm. Expression of the early dorsal markers **dpp** (A, B, G, H) and **zen** (C–F) in the neuroectoderm is opposed by **sog**. Double label in situ hybridizations (O’Neill and Bier 1994) were performed with digoxigenin-labeled probes (visualized by a blue alkaline phosphatase reaction product) and biotin-labeled probes (visualized by a brown peroxidase reaction product). (A) **dpp** (blue) and **sna** (brown) expression in a wild-type (wt) blastoderm stage embryo. **sna** expression marks the prospective mesoderm. (B) **dpp** and **sna** expression in a **sog**; **Dpdpp** embryo. **dpp** is expressed ectopically throughout the neuroectoderm and descends into the dorsal-most row of mesoderm cells [small arrow; arrowhead in inset]. The large arrow indicates a stripe of elevated ectopic **dpp** expression. (C) **zen** (blue) and **sna** (brown) expression in a wild-type embryo. (D) **zen** and **sna** expression in a **sog**; **Dpdpp** embryo. **zen** is expressed ectopically in a large portion of the neuroectoderm and extends ventrally into the most dorsal row of mesoderm cells [arrow; arrowhead in inset]. (E) **zen** and **sna** expression in a **sog**; 8× HS-**dpp** embryo after 30 min of heat shock and 30 min of recovery. **zen** expression extends ventrally four to six cells in the central region of the neuroectoderm and encircles the embryo in vertical stripes at the head and tail [inset shows a cross-sectional focal plane in the mesoderm]. The ventral and lateral portions of the neuroectoderm do not express **zen**. Identical phenotypes are observed in **sog**; 4× HS-**dpp** embryos after 30 min of heat shock and 30 min of recovery. (F) **zen** and **sna** expression in a **sog**; 4× HS-**dpp** embryo after 30 min of heat shock and 30 min of recovery. **zen** expression occupies the entire trunk neuroectoderm and mesoderm in these embryos. In sibling **sog**; +, 4× HS-**dpp** embryos the pattern of **zen** expression in the neuroectoderm is similar to that shown in E. (G) **dpp** (blue) and **sna** (brown) expression in a wild-type germ-band extended embryo. Note that the ventral limit of the **dpp** domain precisely abuts the central nervous system neuroectoderm at this stage [arrow]. (H) **dpp** and **sna** in a **sog**; Y embryo derived from mothers heterozygous for a loss of function **dorsal** allele (**dl- */+**). There is significant ectopic **dpp** expression in the central nervous system. Ectopic **dpp** expression also can be observed during the cellular blastoderm stage when the limit of **dpp** expression is shifted ventrally by four to six cells and clusters of six to eight **dpp**-expressing cells are found near the mesoderm in the region of the cephalic furrow [this area is indicated by the large arrow in B].

Activity. In all cases examined, much less Dpp activity was required to suppress expression of neuroectodermal genes than was required to activate dorsal markers. For example, brief submaximal heat induction of **sog**; HS-**dpp** blastoderm embryos led to nearly maximal suppression of **l’sc**, **scrt** [Fig. 2I], or **sna** expression during germ-band extension. Under these conditions, however, there is no detectable ectopic expression of **zen** in the neuroectoderm [data not shown]. In contrast, maximal HS-**dpp** induction is required to induce neuroectodermal expression of **zen** [the most sensitive dorsal marker] in a **sog**; background [Fig. 3F]. Similarly, in dorsolateral cells of strongly induced **sog**; HS-**dpp** blastoderm embryos, lateral **rho** expression is suppressed [Fig. 2C] without inducing ectopic **zen** expression [Fig. 3E]. More Dpp activity is also required to activate **zen** than to suppress neural markers in dorsal cells. Thus, as in **dpp** embryos, **zen** expression is not maintained in **scw** mutants [Ray et al. 1991] or in heat-shocked HS-**sog** embryos [B. Biels, unpubl.; see below for description of the HS-**sog** phenotype]. In contrast, neural suppression remains largely intact in the dorsal region of **scw** embryos [Fig. 1; cf. C and F with B and E] or HS-**sog** embryos [data not shown]. These observations are also consistent with weak neural suppression but lack of ectopic dorsal gene expression in the neuroectoderm of **sog** single mutants.
Dorsal acts in parallel with sog to repress dpp expression in the neuroectoderm

During establishment of primary D-V domains of zygotic gene expression, maternally supplied Dorsal actively represses expression of dpp and other ZDG genes in lateral and ventral regions of the blastoderm embryo [Huang et al. 1993, 1995; Jiang et al. 1993]. Thus, dorsal seemed a likely candidate for one of the genes acting in concert with sog to block dpp autoactivation in the neuroectoderm. Consistent with this expectation, dpp is expressed ectopically throughout the central nervous system of sog" germ-band extended embryos, which are derived from mothers with only one functional copy of dorsal (dl-+/; Fig. 3H). There are also earlier defects in blastoderm stage embryos of this genotype including a shift in the ventral limit of dpp expression by approximately four cells and small patches of ectopic dpp expression where the neuroectoderm/mesoderm boundary intersects the cephalic furrow [data not shown]. Similar blastoderm stage phenotypes are observed in embryos with four copies of dpp derived from dl-+/+ mothers. Thus, maternal Dorsal is likely to be one of the genetic elements acting in parallel with sog to prevent Dpp from autoactivating in the neuroectoderm.

sog functions upstream of dpp

The observation that strong ubiquitous dpp expression in a sog" background leads to phenotypes similar to those found in sog"; Dpp embryos is consistent with models in which sog functions primarily or exclusively to block Dpp signaling. To determine the epistatic relationship between sog and dpp in greater detail, we examined expression of several marker genes in sog"; dpp" double mutant embryos [Fig. 4]. The most informative markers for this purpose are misexpressed in opposite ways in sog" versus dpp" mutants. For example, the dorsal stripe of rho expression, which gives rise to the amnioserosa in wild-type embryos [Figs. 4A and 5A], is expanded in sog" mutants to include nearly the full dorsal region [Figs. 4B and 5C], but is entirely lacking in dpp" mutants [Fig. 4D; François et al. 1994]. In sog"; dpp" double mutant embryos, dorsal rho expression is lost [Fig. 4E] as it is in dpp" single mutants [Fig. 4D]. Similarly, expression of race, another early amnioserosa marker [Tartari et al. 1995], is expanded in sog" embryos, but is absent in both dpp" single mutants and sog"; dpp" double mutants [data not shown]. Conversely, expression of neural markers such as l'sc, scrt, sna, or tkv expands into the dorsal region of gastrulating dpp" mutant embryos (e.g., Fig. 1B,E), but is either narrowed (e.g., scrt and sna, see Fig. 2G) or unaffected (e.g., l'sc and tkv) in sog" mutants. In sog"; dpp" double mutant embryos, expression of l'sc [Fig. 4H] and other neural marker genes [data not shown] is indistinguishable from that in dpp" single mutants. Furthermore, we found no differences between the expression of the dorsal markers dpp, zen, and tld and the ventral ectodermal marker otd in sog"; dpp" double mutant embryos versus dpp" single mutants [data not shown].

We also addressed the epistasis between sog and dpp by comparing the effects of ectopically expressing sog in wild-type versus dpp" mutant backgrounds. The motivation for these experiments is that if sog acted on a pathway independent of the Dpp pathway, overexpressing sog might aggravate the dpp" mutant phenotype.

We first characterized the effect of ubiquitous sog misexpression in a wild-type background using a line of flies carrying eight copies of a HS-sog construct (8x HS-sog). When 8x HS-sog blastoderm embryos were heat shocked, we observed phenotypes typical of moderate loss of function ZDG mutants. For example, in 8x HS-sog embryos, rho expression is eliminated in the dorsal stripe [Fig. 4F], but is unaffected in the head stripe and lateral stripes, which are independent of dpp activity [see Fig. 4D]. This HS-sog phenotype is opposite to that of HS-dpp embryos in which the dorsal rho stripe expands beyond the limits of the normal dorsal domain [Fig. 4C]. In 8x HS-sog embryos, as in dpp" mutants, we observed that tld expression was normal and that zen expression was initiated normally but not maintained [data not shown]. Ectopic sog expression also relieves neural suppression dorsally resulting in ectopic dorsal stripes of l'sc expression [Fig. 4I]. Such early 8x HS-sog embryos subsequently give rise to ectopic scrt-expressing neuroblasts [data not shown]. The extent of ectopic l'sc and scrt expression in 8x HS-sog embryos is similar to that in scw" mutants, which is less extreme than in dpp" null mutants.

To test whether ectopic sog expression enhanced the dpp" phenotype, we conducted parallel HS-sog inductions in dpp" embryos carrying four copies of the HS-sog transgene [4x HS-sog]. We compared expression of dorsal markers [rho, zen, and tld] and neural markers [l'sc and scrt] in 4x HS-sog; dpp" embryos to that in 4x HS-sog; dpp" embryos. As in the case of the 8x HS-sog line, we observed highly penetrant loss of the dorsal rho stripe and ectopic dorsal expression of l'sc and scrt in 4x HS-sog; dpp" embryos. On the other hand, sibling 4x HS-sog; dpp" embryos had a phenotype indistinguishable from dpp" single mutants [data not shown]. Similarly, dorsal expression of tld is normal in 4x HS-sog; dpp" double mutants and expression of zen is initiated normally and then fades with the same time course as observed in dpp" single mutants. Thus, although ectopic sog expression in a wild-type background generates a phenotype comparable to that of scw" mutants, identical sog misexpression in a dpp" background has no detectable effect.

Sog acts over a long distance in a dose-dependent manner

Previous studies indicated that sog functions nonautonomously to influence cell fates over significant distances [Zusman et al. 1988; François et al. 1994]. It was also observed that reducing the gene dose of sog could rescue the lethality of weak ZDG mutants [Ferguson and Anderson 1992b; Wharton et al. 1993; François et al. 1994]. To determine whether the dose of sog can influ-
dpp is epistatic over sog. Expression of rho [A–F] and l'sc [G–I] in sog and dpp mutant embryos is consistent with Sog functioning primarily or exclusively to block Dpp signaling. Surface views of embryos are shown in A–F and cross-sectional views are shown in G–I. [A] rho expression in a wild-type embryo. The arrow indicates the head stripe that serves as a reference point. Note that the dorsal rho stripe does not extend as far ventrally as the head stripe. [B] rho expression in a sog122/Y RNA null mutant embryo. This embryo was double labeled with a biotin-sog probe to score for the sog genotype. Note that the dorsal rho stripe is expanded such that the ventral border of this domain coincides with the ventral extent of the head stripe [cf. wild-type embryo in A; see also Figs. 5A, C for dorsal views of the same genotypes]. [C] rho expression in a heat-shocked 8× HS-dpp embryo. Embryos were heat shocked for 30 min at 38°C, allowed to recover at room temperature for another 30 min, and then fixed for analysis. The extreme ventral expansion of the dorsal rho stripe can be gauged by the small gap [four to six cells] separating dorsal and lateral rho stripes. [D] rho expression in a Dfdpp embryo. This embryo was double hybridized with a digoxigenin labeled dpp probe to identify unambiguously mutant embryos [i.e., those embryos not expressing dpp]. Identical phenotypes were observed in embryos homozygous for two other dpp null point alleles. The dorsal rho stripe is entirely lacking at all developmental stages in dpp null embryos. rho expression in the head stripe, which is independent of Dpp activity, and in lateral stripes [out of focus] is normal, however. [E] rho expression in a sog122/Y, dpp1B/dpp1B double mutant embryo [sog−, dpp−]. This embryo was double hybridized with a biotin-sog probe. The phenotype of sog−; dpp− embryos is indistinguishable from that of dpp− single mutants [e.g., cf. D]. [F] rho expression in 8× HS-sog embryos is very similar to that observed in dpp− mutants. Embryos were heat shocked for 30 min at 38°C, allowed to recover at room temperature for another 30 min, and then fixed for analysis. Similar, although weaker, phenotypes were also observed by expressing sog ectopically in dorsal blastoderm cells by means of a UAS-sog transgene activated by zen-GAL4 or dpp-GAL4 driver constructs [data not shown]. [G] l'sc expression in a sog−; dpp− sibling of the sog−; dpp− double mutant embryo shown in H. The arrow points to abnormal dorsal expression of l'sc. In wild-type or sog− embryos this region is devoid of staining. [H] l'sc expression in a sog−; dpp− double mutant embryo shown in I. Ectopic l'sc expression in the dorsal region of this embryo is similar to that observed in dpp−, in 4× HS-sog, dpp−, and in 4× HS-sog, dpp− embryos [data not shown]. The arrow indicates abnormal dorsal expression of l'sc.
sog protects the neuroectoderm from Dpp activity

Figure 5. sog exerts a long range dose-dependent influence on gene expression. Wild-type embryos carrying two functional copies of sog (2x sog), embryos heterozygous for a sog null allele (1x sog), and embryos lacking all sog function (0x sog) express marker genes in a dose-dependent fashion. Embryos in A–C were hybridized with a digoxigenin-labeled rho antisense RNA probe and (B, C) with a biotin-labeled sog probe. Embryos are viewed ventral side up with anterior facing to the left. The arrowhead in A–C denotes the dorsal midline. Embryos in D–F were double hybridized with a digoxigenin-labeled zen probe and a biotin-labeled sna probe and are viewed ventral side up with anterior facing to the left. The arrowhead in D–F denotes the ventral midline. (A) Dorsal rho expression in a wild-type embryo (2x sog) is ~10–13 cells in maximum width. (B) Dorsal rho expression in a sog (2x sog) embryo increases to 17–20 cells across. This embryo can be scored as sog +/+ as it expresses sog weakly (see inset), and because in parallel hybridizations of wild-type embryos such an expansion of the dorsal rho stripe is not observed. The dorsal rho stripe is expanded comparably in each of the three sog alleles we tested (i.e., sog 0/sog 0, sog 0/sog 1, and sog 1/sog 1). A similar expansion of the dorsal rho stripe is observed in embryos with two copies of sog and four copies of dpp (i.e., sog 0/+, Dpdp). (C) The dorsal rho domain in a sog +/Y (0x sog) embryo expands to ~100–130 cells across. The genotype of this embryo can be scored as sog 0/Y as it is not labeled by the biotin-sog probe (see inset). (D) Ectopic expression of zen in the mesoderm of a heat-shocked 4x HS-dpp embryo in a wild-type sog background (i.e., 2x sog), zen expression in the mesoderm is confined to a narrow stripe (zero to three cells wide) running along the ventral midline and to two wedge-shaped patches at the anterior and posterior ends of the mesoderm. (E) Ectopic zen expression in the mesoderm of a heat-shocked sog +/+; 4x HS-dpp embryo (i.e., 1x sog) is significantly broader (10–12 cells wide) than in similarly treated sog 0/sog 0; 4x HS-dpp embryos (D). This embryo derives from a cross of 8x HS-dpp males to sog 0/sog 1 FM7c females. The genotype of this embryo can be determined by virtue of the fact that there is a much more severe ectopic zen phenotype in the neuroectoderm of sog 0/Y; 4x HS-dpp embryos (see F and also Fig. 3F) and because there is no such intermediate class of embryos observed in a parallel cross of 8x HS-dpp males to wild-type females (D). (F) zen expression occupies the entire trunk mesoderm (18 cells wide) and neuroectoderm of a heat-shocked sog 0/Y; 4x HS-dpp embryo (i.e., 0x sog). Ectopic zen expression is never observed in the terminal regions of the embryo, although pole cells are frequently labeled. The genotype of these embryos was determined by parallel double label experiments using a combination of zen and sog probes.
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embryo. Because Sog seems to function primarily or exclusively by antagonizing Dpp signaling (see above), it may create a reciprocal gradient of Dpp activity in the dorsal region of the embryo. In this respect, Sog displays many features of a classic morphogen.

Discussion
dpp promotes dorsal gene expression and suppresses neural gene expression dorsally

Previous experiments [Irish and Gelbart 1987; Ray et al. 1991; François et al. 1994] and data presented in this paper reveal two functions of Dpp signaling in the dorsal region of the blastoderm embryo. The first is a positive action of Dpp to maintain expression of dorsally expressed genes such as zen [Ray et al. 1991], and at peak levels, to trigger expression of amnioserosal markers such as the dorsal rho stripe [François et al. 1994; Fig. 4C,D]. The second role of Dpp signaling is to suppress expression of neuroectodermal genes such as l'sc [Fig. 4G]. This latter role of Dpp activity dramatically influences the extent of neuroblast formation during gastrulation [Fig. 2, Wharton et al. 1996]. Although dpp mutants generate large numbers of ectopic neuroblasts dorsally, the final result is a hypertrophied rather than an enlarged nervous system. The basis for the ultimate nervous system defect remains to be determined.

sog prevents Dpp from autoactivating in the neuroectoderm

Our results reveal a very early role of sog in opposing dpp signaling within the neuroectoderm well before gastrulation begins. sog opposes both the autoactivating and neural suppressive functions of Dpp in the neuroectoderm [Fig. 6]. In sog−; Dpdppe embryos, Dpp, which presumably diffuses laterally from the dorsal region, autoactivates dpp expression in the neuroectoderm. Such unopposed Dpp activity in the neuroectoderm induces expression of the dorsal markers zen and tld. Similarly, ectopic dpp produced in sog−; HS-dpp embryos induces zen expression throughout the neuroectoderm and causes the dorsal rho stripe to expand partway into the neuroectoderm. The fact that the dorsal rho stripe extends only into the dorsal portion of the neuroectoderm in sog−; Dpdppe or sog−; HS-dpp embryos indicates that these embryos retain sufficient D-V polarity to subdivide the enlarged dorsal ectoderm into dorsal-most (rho + zen) and ventral (zen only) domains.

sog also prevents Dpp signaling from suppressing neurogenesis in lateral cells. In sog−; Dpdppe embryos, expression of l'sc is confined to a narrow ventral strip of neuroectoderm. These embryos give rise to only the inner row of scrt-expressing neuroblasts. These inner-row neuroblasts appear to be specified by a Dpp-insensitive mechanism as they are largely immune to even the most extreme conditions of ectopic Dpp expression [i.e., sog−; HS-dpp embryos].

Figure 6. A model for how Sog and Chordin block Dpp and BMP-4 from autoactivating and suppressing neurogenesis in the neuroectoderm. It is inherently difficult to limit expression of genes such as dpp or BMP-4 that encode diffusible autoactivating molecules. We propose that the key phylogenetically conserved function of Sog and Chordin is to protect the neuroectoderm from the invasive positive feedback loop resulting from Dpp/BMP-4 diffusion and autoactivation. The maternal mechanisms by which sog and dpp are activated in abutting territories in fly embryos [i.e., direct transcriptional threshold responses to the Dorsal morphogen gradient] appear to differ fundamentally from those leading to the complementary activation of chordin and BMP-4 in frog embryos [i.e., Wnt and Activin-like induction of goosecoid expression in the Spemann organizer leading to chordin expression dorsally, and FGF plus a low Activin-like signal inducing BMP-4 expression ventrally]. In contrast, once the primary zygotic response genes sog/chordin and dpp/BMP-4 are expressed in abutting territories, they specify neural vs. non-neural ectoderm through a highly conserved mechanism. The Dpp/BMP-4 signaling pathway plays two major roles in establishing non-neural cell fates: (1) potent suppression of neurogenesis and (2) autoactivation to maintain the expression of dpp (BMP-4) and associated genes [e.g., zen and tld in flies]. Sog or Chordin prevent Dpp or BMP-4, which diffuse into the neuroectoderm from neighboring cells, from reaching the threshold level necessary to trigger autoactivation. Because neural suppression has a lower threshold for Dpp signaling than does dorsalization, Dpp/BMP-4 signaling will suppress neurogenesis once the threshold required for autoactivation is achieved [e.g., as occurs in sog; Dpdppe fly embryos].

The hypothesis that Sog functions in the neuroectoderm to block a chain of Dpp diffusion followed by autoactivation provides a potential explanation for the high degree of evolutionary conservation between sog and dpp and their vertebrate counterparts [see below]. Dpp diffusion coupled to autoactivation constitutes an intrinsically invasive positive feedback loop. If unopposed, this action of Dpp would force all ectodermal cells to express dpp, even though dpp expression is initiated in a localized pattern [Fig. 6].

Distinct thresholds of Dpp signaling trigger neural suppression versus dorsalization

We have observed consistently that significantly less dpp activity is required to suppress expression of neural markers such as l'sc, scrt, and sna than to activate or maintain expression of dorsal target genes such as zen or tld. This relation appears to hold in both the lateral and dorsal regions of the embryo. For example, potent neural
suppression is observed in the neuroectoderm under mild conditions that do not induce ectopic expression of *zen*. Similarly, in the dorsal region of the blastoderm embryo, we have identified a threshold of Dpp signaling (e.g., in *scw*− or HS-*sog* embryos) that is largely adequate for suppressing *I sc* expression dorsally but is insufficient for maintaining *zen* expression.

**Dpp and Sog consolidate pattern initiated by the Dorsal morphogen gradient**

Because the protective action of *sog* in the neuroectoderm is only strictly required in embryos having elevated levels of Dpp, parallel pathways are likely to act together with *sog* to block Dpp from autoactivating in the neuroectoderm. The maternal morphogen Dorsal is one likely candidate for such a parallel pathway as ectopic *dpp* expression is observed in the neuroectoderm of *sog*− [Fig. 3H] or Dpdpp embryos derived from *dl*−/+ mothers. Dorsal, an NF-κB-related transcription factor, activates expression of key patterning genes in ventral and lateral regions of the embryo but represses expression of several ZDG genes including *dpp* in these same cells [Huang et al. 1993, 1995; Jiang et al. 1993]. Data presented here suggest that *sog* stabilizes the primary subdivision of the ectoderm into neural versus non-neural components by Dorsal. Dpp activity in dorsal cells exerts a similar stabilizing influence on primary maternal patterning as Dpp maintains expression of several ZDG genes while suppressing expression of genes promoting the alternative neuroectodermal fate.

Another example of parallel processing is that *dpp* is not required dorsally to maintain expression of ZDG genes such as *tld* or *dpp* itself, although ectopic expression of *dpp* in the neuroectoderm is sufficient for inducing expression of these genes. Similarly, although *dpp* function is not required to set the limits of the lateral rho stripe, ectopic *dpp* expression reduces the width of this domain. This suggests that Dpp diffusing laterally from the dorsal domain works in parallel with Dorsal (Ip et al. 1993; François et al. 1994; Yu et al. 1996). Tentatively, we favor models in which an active form of Dpp is insensitive to inhibition by *sog*, or in which *sog* interferes with the action of molecules potentiating Dpp activity or propagating the Dpp signal. Consistent with this view, we have obtained recent evidence that *sog* may block only one of two effects of Dpp signaling during wing vein development [Yu et al. 1996].

**Sog may be a dedicated Dpp antagonist**

The observation that the *sog*−; *dpp*− double mutant phenotype is indistinguishable from that of *dpp*− single mutants is consistent with *sog* functioning as a dedicated antagonist of Dpp signaling. The absence of any aggravating effect of ectopic *sog* expression on the *dpp*− phenotype also supports models in which *sog* functions specifically to block Dpp action. Although it is possible that there are markers that would reveal a stronger phenotype in HS-*sog*; *dpp*− embryos than in *dpp*− single mutants, *I sc*, *tld*, and *zen* should be sensitive indicators of such a difference as they are either expressed normally or misexpressed to only a limited extent in *dpp*− single mutants.

Recently it has been shown that Chordin, the vertebrate homolog of *Sog*, binds to BMP-4 (the vertebrate homolog of Dpp) [Picolla et al. 1996]. If *Sog* functioned by a similar mechanism, one might expect that ectopic expression of *sog* could phenocopy *dpp*− mutants. The most extreme phenotype resulting from ubiquitous *sog* expression we observed, however, is substantially weaker [i.e., comparable to that of *scw*− mutants]. Similarly, analysis of cuticle patterns suggested that dorsal injection of maximal quantities of *sog* mRNA generates ventralized phenotypes significantly less extreme than observed in *dpp*− embryos [Holley et al. 1995]. These data do not support simple models in which *Sog* binds and sequesters all forms of Dpp in inactive complexes. Although it is possible that the amounts of ectopic *sog* produced by 8× HS-*sog* and by *sog* mRNA injection are insufficient to bind all available Dpp, we note that maximal 8× HS-*sog* phenotypes can also be obtained at similar frequencies and with similar penetrance in 4× HS-*sog* embryos, and that increasing the amount of *sog* mRNA injected into embryos does not increase the severity of the cuticular phenotype [Holley et al. 1995]. This is in contrast to the extreme dosage-sensitive interactions observed between *dpp* and *sog* mutants [Ferguson and Anderson 1992b; Wharton et al. 1993; François et al. 1994, Yu et al. 1996]. Tentatively, we favor models in which an active form of Dpp is insensitive to inhibition by *Sog*, or in which *Sog* interferes with the action of molecules potentiating Dpp activity or propagating the Dpp signal. Consistent with this view, we have obtained recent evidence that *sog* may block only one of two effects of Dpp signaling during wing vein development [Yu et al. 1996].

**Sog has properties of a morphogen**

In this paper we extended previous observations suggesting that *Sog* not only functions locally to block Dpp signaling in the neuroectoderm, but that it also diffuses long distances to influence gene expression in neighboring cells [Zusman et al. 1988, François et al. 1994]. We observed that the width of the dorsal rho stripe depends on the dose of *sog*, and similarly, that the extent of the ventral stripe of ectopic *zen* expression in HS-*dpp* embryos is sensitive to *sog* dosage. These data are compatible with *Sog* diffusing 12- to 15-cell diameters dorsally to establish a gradient of Dpp activity. In this sense *Sog* could be considered a morphogen. It is also possible, however, that other genes function in parallel with *sog* to determine the limits of target gene expression. If this were true, one could imagine that *sog* functions only as an on–off switch to reveal existing prepatterns in responding dorsal or ventral cells. Although we have no direct evidence to contradict this possibility, there are two reasons why it seems unlikely. First, the dorsal rho
stripe and the ventral zen stripe (in HS-dpp embryos) assume three different diameters in embryos carrying two, one, or zero doses of sog. In the simple case that sog were functioning as a binary on–off switch to trigger gene expression according to a prepattern of sensitive cells [e.g., dorsal ectoderm] versus insensitive cells (e.g., amnioserosa), one would expect two, not three, potential boundaries. Second, there is no known basis for suspecting the existence of prepatterns in either the dorsal or ventral regions of the blastoderm embryo. In fact, current data suggest that all cells in the dorsal region are equivalent and specified as such by the absence of nuclear Dorsal. Direct visualization of the pattern of Sog proteins will help resolve this question.

In summary, Sog and Dpp act locally to consolidate the primary subdivision of the embryo into neural versus non-neural ectoderm. These genes also function nonautonomously to trigger the next round of subdivision within each of these domains.

**Mechanisms for subdividing the ectoderm into neural versus nonneural components have been conserved during evolution**

From an evolutionary point of view, it is striking that the *Xenopus* BMP-4 and *chordin* genes, like *dpp* and *sog*, are expressed in abutting D-V domains [François et al., 1994; Graff et al., 1994; Maeno et al., 1994; Sasai et al., 1994; Schmidt et al., 1995b] and encode molecules highly related to *dpp* and *sog* [François and Bier 1995], respectively. These vertebrate and invertebrate genes play homologous roles during *Xenopus* and *Drosophila* embryogenesis [Padgett et al., 1993; Holley et al., 1995; Schmidt et al., 1995a]. There are also strong parallels between the biological actions of these homologous gene sets. Thus, BMP-4 has been shown to function as a potent neural suppressor during *Xenopus* development [Sasai et al., 1995; Schmidt et al., 1995b; Wilson and Hemmati-Brivanlou 1995] and is capable of autoactivating in *Xenopus* [Jones et al., 1992; Schmidt et al., 1996]. The data presented here reinforce the view that mechanisms for subdividing the ectoderm into neural versus non-neural territories have been conserved in detail during the course of evolution [Padgett et al., 1993; Wilson and Hemmati-Brivanlou 1995; François and Bier 1995; Holley et al., 1995; Sasai et al., 1995; Schmidt et al., 1995a; Fig. 6].

**Materials and methods**

**Fly stocks**

The *sog*°° and *sog*° alleles are described in François et al. (1994). The *sog*° allele, [Wieschaus et al. 1984], was obtained from the Bloomington Indiana Stock Center. *sog*°° and *sog*° are RNA null alleles [François et al. 1994]. The CyODpdp transgene (CyO23, kindly supplied by W. Gelbart, Harvard University) carries a genomic dpp transgene expressed as the wild-type gene during early embryogenesis in addition to a wild-type copy of the *dpp* locus. The 8× HS-dpp stock was kindly provided by Dr. Ronald K. Blackman (University of Illinois, Urbana). Construction of the 8× HS-sog line is described in Yu et al. [1996]. Other balancers and chromosomal markers (Lindsley and Grell 1968, Lindsley and Zimm 1992) were obtained from either the Bloomington Indiana Stock Center or the Bowling Green Stock Center.

**Scoring the genotype of sog−; Ddpdp and sog−; dpp− embryos**

To identify embryos of the genotype sog−/Y, Ddpdp/Ddpdp in collections from sog°°/FM7c, CyODpdp parents, embryos were either triple hybridized with probes for rho (digoxigenin labeled), dpp (digoxigenin labeled), and sog (biotin labeled), or were double stained for lsc or scr (digoxigenin labeled) and sog (biotin labeled) and scored for dpp function by the severe gastrulation phenotype typical of dpp− mutants. These experiments revealed premature ectopic dorsal expression of rho in sog−; Ddpdp embryos [i.e., such embryos did not label with a sog probe but did label with a dpp probe]. Similar experiments without addition of the dpp probe were also performed on stocks carrying a null dpp point mutant allele (sog°°/FM7c, dpp°°/CyODpdp). The sog−; Ddpdp embryos shown in Figure 2 were obtained from parallel single label experiments with lower background signals as initial experiments revealed that sog−; Ddpdp embryos could be identified unambiguously by expression of the marker genes alone. In the multilabel experiments described above, it was also possible to identify unambiguously sog−; dpp− double mutant embryos as shown in Figure 4.

**Double label in situ hybridization**

Double in situ hybridization with digoxigenin- and biotin-labeled RNA probes is described in detail in O’Neill and Bier [1994].

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