Threshold responses to the dorsal regulatory gradient and the subdivision of primary tissue territories in the *Drosophila* embryo

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Dorsoventral patterning in *Drosophila* is initiated by the maternal regulatory factor dorsal (dl), which is a member of the *Rel* family of transcription factors. dl functions as a transcriptional activator and repressor to establish different territories of gene expression in the precellular embryo. Differential regulation of dl target genes may be essential for subdividing each tissue territory (the presumptive mesoderm, neuroectoderm, and dorsal ectoderm) into multiple cell types in older embryos. Different patterns of *snail* (*sna*) and *decapentaplegic* (*dpp*) expression help define the limits of inductive interactions between the mesoderm and dorsal ectoderm after gastrulation. Similarly, the differential regulation of *short gastrulation* (*sog*) and *dpp* may be decisive in the initial subdivision of the dorsal ectoderm, whereas different limits of gene expression within the neuroectoderm might provide the basis for the subsequent subdivision of this tissue into ventral and lateral regions.

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Abbreviations

dl	*dorsal*
dpp	*decapentaplegic*
Mad	*Mothers against dpp*
put	*punt*
rho	*rhomboid*
sax	*saxophone*
scw	*screw*
sim	*single minded*
sna	*snail*
sog	*short gastrulation*
TGF-β	transforming growth factor-β
tin	*tinman*
tld	*tolloid*
tkv	*thick veins*
tsg	*twisted gastrulation*
twi	*twist*
zen	*zerknüllt*

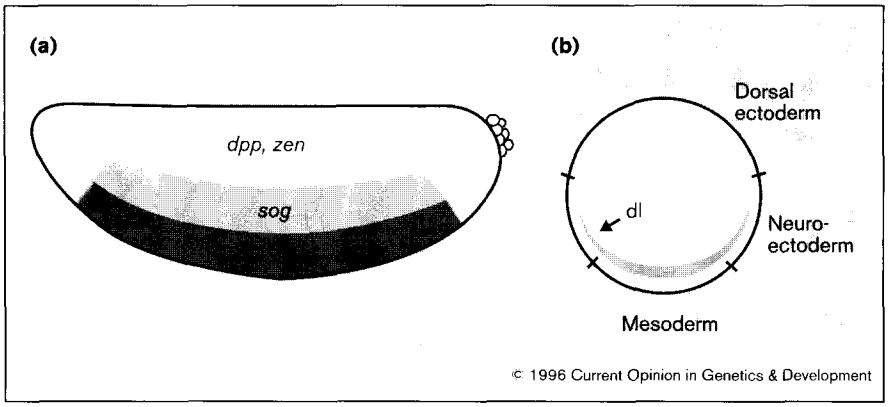
Introduction

Recent studies have provided a detailed overview of dorsoventral patterning in *Drosophila*, beginning with subtle asymmetries in the egg chamber of the ovary and extending to the establishment of primary territories of tissue differentiation in the pregastrula embryo. The purpose of this review is to summarize current information regarding the subdivision of each primary tissue into multiple cell types. For example, the embryonic mesoderm becomes subdivided into two lineages, ventral and lateral, shortly after gastrulation. The lateral mesoderm gives rise to the heart (dorsal vessel) and visceral muscles whereas the ventral mesoderm generates the somatic muscles. Similarly, the dorsal ectoderm is subdivided into amnioserosa and dorsal epidermis and the neuroectoderm gives rise to neuroblasts and the ventral epidermis. In this review, we present evidence that the subdivision of these embryonic tissues is a direct consequence of the primary thresholds of gene expression established by the dorsal regulatory gradient during precellular stages of development.

The dorsal (dl) protein [1] is distributed throughout the cytoplasm of growing oocytes and unfertilized eggs but shortly after fertilization it is subjected to a regulated nuclear transport process [2–4]. At ~90 minutes after fertilization, dl in the ventral regions is released from the cytoplasm and enters nuclei; in contrast, protein in dorsal regions remains in the cytoplasm. This selective nuclear transport creates a broad regulatory gradient, with peak levels of dl in ventral regions, low levels in lateral regions, and little or none in dorsal regions (Fig. 1b). Nuclear transport depends on an elaborate signal transduction pathway that shares many similarities with the interleukin-1 cytokine pathway [5,6,7•], which controls the nuclear transport of NF-κB in several different mammalian tissues, including B lymphocytes and the liver.

The dl transcription factor initiates the differentiation of three embryonic tissues — the mesoderm, neuroectoderm, and dorsal ectoderm — by regulating a number of zygotically active target genes in a concentration-dependent manner (summarized in Fig. 1; reviewed in [8–10]). The promoter regions of five different dl target genes have been characterized in detail (reviewed in [8]). These studies provide strong evidence that different threshold responses to the dl gradient depend on the occupancy of dl protein binding sites in the different target promoters. For example, peak levels of dl in the ventral-most 18–20 nuclei (of the 80 nuclei that are situated in the circumference of the embryo in cross section) determine the boundaries of the presumptive mesoderm by activating the zygotic target genes *twist* (*twi*) and *snail* (*sna*) [11–15]. Both promoters contain low-affinity dl binding sites; however, whereas *twi* can be activated by dl alone, *sna* expression depends on both the dl and twi proteins [15]. The synergistic interactions between the localized dl and twi transcription factors contribute to the sharp 'on'–'off' borders of *sna* expression, which coincide with the boundary between the presumptive mesoderm and neuroectoderm [15].

Figure 1



Gene expression in the primary embryonic tissues. **(a)** Schematic sagittal view of a blastoderm-stage embryo (anterior is to the left and dorsal up), indicating the presumptive mesoderm (dark shading) expressing the *twi* and *sna* genes; the neuroectoderm (light shading) expressing *sog*; and the dorsal ectoderm marked by *dpp* and *zen* expression. **(b)** Cross-section showing the presumptive mesoderm, neuroectoderm, and dorsal ectoderm. Also shown is the graded distribution of nuclear dl protein with peak levels in the ventralmost regions and progressively lower levels in lateral regions.

Low levels of dl activate a different set of target genes in lateral regions of the pre-cellular embryo and these genes initiate the differentiation of the neuroectoderm: in two ventrolateral stripes, 14–16 nuclei wide. At least seven dl target genes appear to be activated in the neuroectoderm, including *rhomboid* (*rho*) [16], *short gastrulation* (*sog*) [17], *lethal of scute* [18], the *m7* and *m8* genes of the *Enhancer of split* complex [19], and *single minded* (*sim*) [20], but only *rho* has been characterized in detail. The *rho* promoter region contains optimal high-affinity dl binding sites. In addition, there are closely linked E box sequences, which bind ubiquitously distributed bHLH activators such as *daughterless* and T4 (*scute*) [21]. *In vitro* binding assays have demonstrated cooperative DNA binding interactions between dl and these bHLH activators, thereby insuring efficient occupancy of the dl operator sites in the *rho* promoter [22,23]. In this way, low levels of dl efficiently activate *rho* in the lateral neuroectoderm. In principle, the high levels of dl in the ventral mesoderm should also activate the *rho* promoter. The gene is kept off in these regions, however, by the *sna* product, which functions as a transcriptional repressor [21,24]. Similar rules might apply to the regulation of other neuroectodermal genes: for example, the *sim* promoter region has been shown to contain high affinity dl binding sites as well as closely linked E boxes and *sna* repressor sites [20].

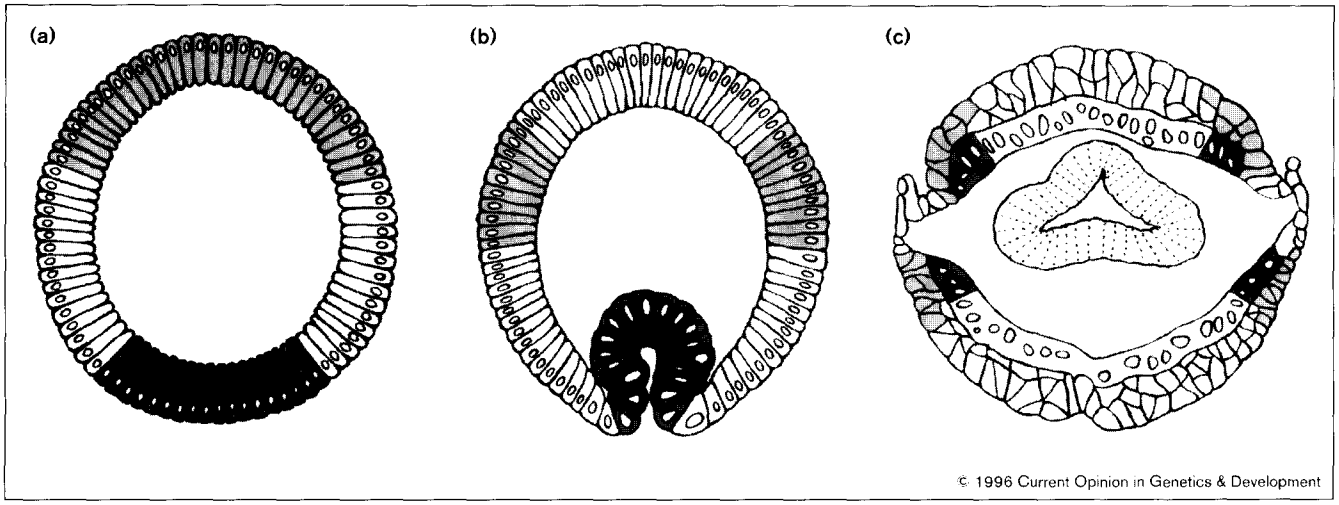
As discussed above, *dl* activates genes in the presumptive mesoderm and neuroectoderm in a concentration-dependent manner. In addition, it works as a repressor. A number of target genes, including *zerknüllt* (*zen*) and *decapentaplegic* (*dpp*), are repressed in ventral and lateral regions by the dl gradient. The expression domains of these genes, in the dorsal half of the embryo spanning ~32–34 nuclei, coincide with the limits of the dorsal ectoderm. In principle, these dorsal-specific genes can be activated throughout the embryo by one or more ubiquitously distributed transcriptional activators, but they are kept off in the ventral mesoderm and lateral neuroectoderm by the dl gradient [25]. The *dpp* and *zen* promoter regions contain dl binding sites which can be occupied efficiently by both high and low levels of dl in ventral and lateral regions [26,27]. There are also closely linked negative response elements which bind 'co-repressors'. Both the dl binding sites and the co-repressor sites are necessary to keep the *zen* and *dpp* promoters off in ventral and lateral regions, thereby restricting the expression patterns to the dorsal ectoderm. The dl protein is intrinsically a transcriptional activator [28] but can function as a repressor by recruiting co-repressors to neighboring negative response elements [29,30•]. This situation appears similar to that of MCM1–α2 interactions in yeast, whereby MCM1 functions as a general activator but mediates repression by recruiting α2 to adjacent binding sites [31]. The exact identities of the dl co-repressors remain controversial. Yeast assays have identified DSP1, a *Drosophila* high mobility group 1 protein, as a potential co-repressor [29]. In contrast, *in vitro* binding assays using crude nuclear extracts taken from *Drosophila* embryos suggest that NTF-1, a previously characterized transcriptional activator encoded by the *grainyhead* gene, may represent a dl co-repressor [30•]. It is conceivable that the co-repressor is composed of several different polypeptides.

Subdivision of the mesoderm

Several recent papers suggest that different threshold responses to the dl regulatory gradient may play a role in the subdivision of each embryonic tissue into multiple cell types. The most information is available for the subdivision of the mesoderm into ventral and lateral lineages. At the onset of gastrulation, the ventral cells that express *twi* and *sna* invaginate into the blastocoel through the ventral furrow (for a description of gastrulation movements, see [32,33]). Mutations in either gene cause a block in ventral furrow formation and a virtual loss of mesoderm derivatives, including somatic muscles, gut muscles, and the heart [34,35].

Evidence has been obtained that invagination and mesoderm differentiation may represent separate processes [36–38]. As discussed below, it would appear that *sna* plays the decisive role in setting the initial limits of

Figure 2



Subdivision of the primary mesoderm. *tin* and *dpp* expression patterns in cross-sections of progressively older embryos. **(a)** Pregastrula embryo. *tin* expression in the presumptive mesoderm is indicated by the dark shading; *dpp* in the dorsal ectoderm is light shaded. **(b)** Gastrulating embryo with the mesoderm invaginated through the ventral furrow. At this time *dpp* expression is restricted to the dorsal ectoderm in two dorsolateral bands. **(c)** Embryo in the germband elongated stage. The mesoderm has spread along the ectoderm to form a monolayer of cells. The dorsalmost mesodermal cells lie underneath *dpp*-expressing cells; these are induced to maintain *tin* expression and form the lateral mesoderm lineages (modified from [69]).

the ventral furrow and presumptive mesoderm. Just prior to the invagination process, the homeobox gene *tinman* (*tin*, also referred to as *msh-2*) is activated in most cells of the presumptive mesoderm, possibly in response to *twi* [39,40] (Fig. 2a). After invagination, during the rapid phase of germ band elongation, the embryonic mesoderm spreads laterally and forms a monolayer of cells on the inner (basal) side of the ectoderm. During this stage, *tin* expression is shut off in ventral regions of the mesoderm but is maintained in lateral regions (Fig. 2c). The lateral mesoderm gives rise to at least two distinct lineages, including cardiac tissues (the heart/dorsal vessel) and visceral mesoderm (gut muscles) [32,33]. During advanced stages of embryogenesis the *tin* expression pattern is refined progressively to coincide with the cardiac cells [39,41]. Another homeobox gene, *H2.0*, is activated selectively in lateral derivatives that form the visceral mesoderm later in development [42]. Ventral mesodermal cells that lose *tin* expression form somatic derivatives, including body wall muscles.

Considerable efforts have centered on identifying the signaling pathways and regulatory factors responsible for the progressive refinement of the *tin* expression pattern during mesoderm differentiation. A critical clue is provided by classic grafting experiments. Different ectodermal tissues were found to possess distinct inductive activities in lacewing embryos [43]: dorsal ectoderm was found to induce 'naive', multipotent mesoderm to form cardiac and visceral tissues, whereas ventral ectoderm was found to induce somatic mesodermal derivatives. Recent studies suggest that the transforming growth factor-β (TGF-β) homolog *dpp* may be the source of the inductive signal in the dorsal ectoderm [44,45••].

During germ band elongation, the lateral-most regions of the invaginated mesoderm extend beyond the ventral ectoderm (or neuroectoderm) and come into contact with *dpp*-expressing cells in the dorsal ectoderm (Fig. 2c). Recent studies suggest that the mesodermal cells which come into contact with *dpp* are induced to maintain *tin* expression in the lateral mesoderm and ultimately form visceral and cardiac tissues [44,45••]. In contrast, ventral mesodermal cells that stay in contact with the neuroectoderm fail to maintain *tin* expression and form somatic body wall muscles. Ectopic expression of *dpp* in the neuroectoderm causes an expansion of lateral mesoderm derivatives such that cells which would normally form somatic muscles are transformed into visceral and cardiac tissues [44,45••]. Further evidence that *dpp* expression induces lateral mesoderm formation stems from the observation that one of the putative *dpp* receptors, *thick veins* (*tkv*), is expressed selectively in the embryonic mesoderm after invagination [46]. It is unclear currently whether the neuroectoderm plays an instructive role in inducing the underlying ventral mesoderm to form somatic tissues. The neuroectoderm is a localized source of *spitz* expression, a gene which encodes a *Drosophila* homolog of transforming growth factor-α [47].

The dorsal gradient delimits inductive interactions

It would appear that the exact boundaries of the presumptive mesoderm and dorsal ectoderm determine the extent

of *dpp*-mediated induction of lateral mesoderm following gastrulation, as summarized in Figure 2. According to this view, the number of mesodermal cells that come into contact with *dpp*-expressing cells in the dorsal ectoderm depends on the extent of the ventral furrow. Expanding the limits of the ventral furrow — and consequently the amount of invaginated tissue — should lead to a more extensive lateral expansion of mesoderm. This would increase the number of cells in contact with the dorsal ectoderm which would thus be induced to form lateral mesodermal derivatives in response to *dpp* induction. Alternatively, reducing the limits of the mesoderm should diminish the extent of lateral spreading upon invagination, thereby causing fewer cells to come into contact with the dorsal ectoderm. The latter experiment, restricting the extent of mesoderm invagination, was performed recently [38,48•].

In light of the strict dependence of *sna* expression on twi activity, *twi* mutants effectively are double mutants that lack both *twi* and *sna* products. It is thus difficult to dissociate the regulatory activities of these genes in ventral furrow formation, invagination, and mesoderm differentiation. The activity of *sna* was uncoupled from *twi* regulation by attaching the *sna* coding sequence to a heterologous promoter (a modified *twi* promoter element) that is activated solely by dl. The *sna* transgene is expressed in ventral regions of pre-cellular embryos, even in the absence of *twi* activity. Expression of *sna* is sufficient for the formation of the ventral furrow. The invaginated cells, however, fail to differentiate mesodermal derivatives in *twi*⁻ embryos. In some instances, the invaginated cells form mesectodermal tissues, suggesting that furrow formation and mesoderm differentiation can be uncoupled. These results were taken as evidence that *sna* controls invagination, whereas *twi* functions as a mesoderm determinant to activate target genes which are essential for mesoderm differentiation [38].

The *sna* transgene analyzed in these studies does not direct a normal pattern of expression. The ventrolateral limits are narrower than the endogenous pattern and, consequently, fewer cells invaginate through the ventral furrow. After lateral spreading of the invaginated cells, very few come into contact with *dpp*-expressing cells in the dorsal ectoderm [48•]. Consequently, there is a reduction in lateral mesoderm derivatives, including gut muscles and the heart. In contrast, ventral mesoderm derivatives — such as somatic body wall muscles — are less disrupted and appear to be formed at normal levels. These results suggest that different threshold responses to the *dl* regulatory gradient are directly responsible for setting the limits of inductive interactions between germ layers after gastrulation.

Subdivision of the dorsal ectoderm

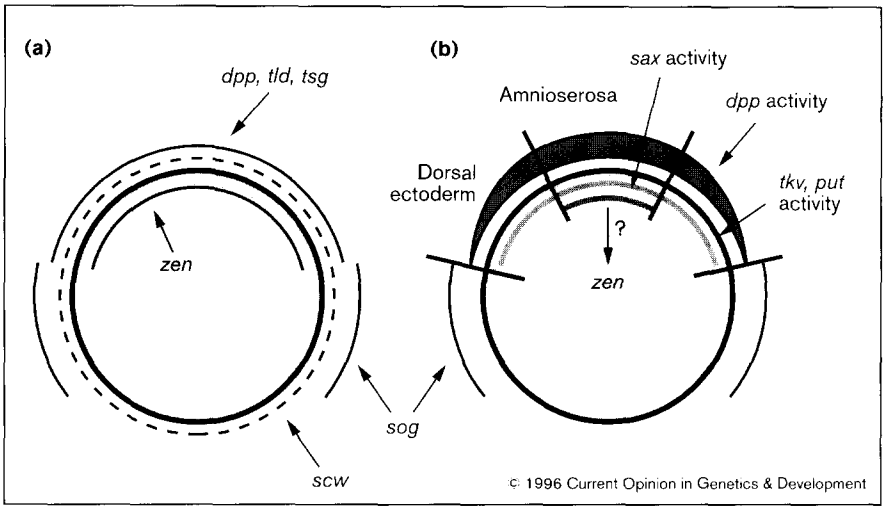
Dorsal and dorsolateral regions of the pre-cellular embryo give rise to the dorsal ectoderm (Fig. 1). Shortly after the onset of gastrulation, the dorsal ectoderm is subdivided into two distinct lineages: the amnioserosa, which arises from the dorsal-most regions of the fate map, and the dorsal epidermis. The dorsal epidermis derives from dorsolateral regions of the embryo and gives rise both to epidermal derivatives and sensory organs of the peripheral nervous system [33]. Several target genes that are repressed directly by the dl gradient appear to be expressed within the initial limits of the presumptive dorsal ectoderm. These include *dpp* and *zen*, as discussed above, plus *tolloid* (*tld*) [49] and *twisted gastrulation* (*tsg*) [50]. Each gene is essential for the subsequent subdivision of the dorsal ectoderm after gastrulation. For example, *tld*, *tsg*, and *zen* mutants lack amnioserosa, as do hypomorphic mutations in *dpp*. The complete loss of *dpp* activity results in a total transformation of the dorsal ectoderm into neuroectoderm; both the amnioserosa and dorsal epidermis are lost in these mutants.

A complex signaling pathway is responsible for the subdivision of the dorsal ectoderm. At least seven zygotically active genes participate in this process (*dpp*, *tld*, *tsg*, *screw* [*scw*], *sog*, *zen*, and *shrew* [*srw*]; summarized in Fig. 3a), plus three maternally encoded receptor serine-threonine kinases (*punt* [*put*]), *saxophone* [*sax*], and *tkv*; see Fig. 3b). The linch-pin of this group of interacting genes is *dpp*. Microinjection of *dpp* RNA into wild-type and mutant embryos suggests that high concentrations of *dpp* specify formation of the amnioserosa whereas low levels trigger the differentiation of the dorsal epidermis [51].

It has been proposed that dorsal regions of the pre-cellular embryo possess an extracellular *dpp* activity gradient with peak concentrations in the dorsal-most regions and lower levels in progressively more lateral regions [51]. This putative gradient has not been visualized and, consequently, the concept remains in the realm of theory. Nonetheless, a prevailing view is that several of the zygotically active patterning genes which control the subdivision of the dorsal ectoderm are responsible for generating a *dpp* gradient [50,52–54]. This, in turn, functions as a morphogen to specify the amnioserosa and dorsal epidermis as discussed above. There is some controversy surrounding the instructive capacity of this putative *dpp* gradient. Does it work in a continuous fashion to specify multiple cells within each basic subdivision of the dorsal ectoderm or are there just two thresholds of *dpp* activity, one specifying amnioserosa and the other directing formation of dorsal epidermis?

A critical component of the *dpp* signaling pathway is *sog*, which is expressed in two broad ventrolateral stripes in pre-cellular embryos [17,55•] (Fig. 3). These stripes probably coincide with the initial limits of the embryonic neuroectoderm. The putative secreted protein encoded by *sog* is related to CHORDIN, a protein identified in *Xenopus* which plays an essential role in the subdivision of the embryonic mesoderm into ventral and dorsal lineages

Figure 3



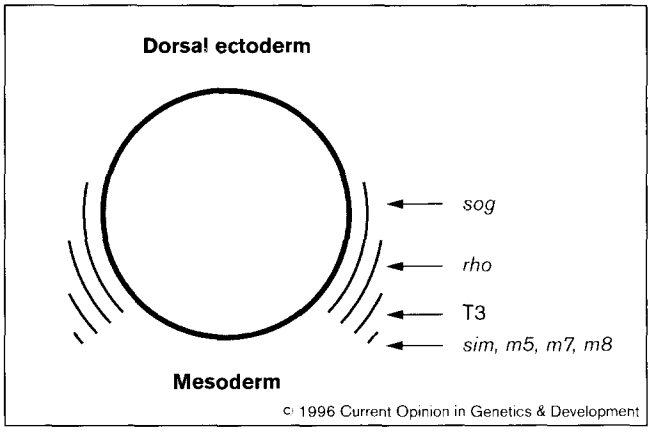
Genes involved in the subdivision of the dorsal ectoderm. **(a)** Schematic cross section of a blastoderm stage embryo, indicating the expression patterns of zygotically active genes involved in the formation of the amnioserosa and dorsal epidermis. **(b)** Cross-section indicating the components of the *dpp* and *scw* signaling pathways. Peak levels of DPP and low levels of scw lead to the activation of the *tkv* and *sax* type I receptors, and ultimately the induction of the *put* type II receptor. Activation of both type I receptors leads to the maintenance of the *zen* expression pattern in the dorsal-most regions of the embryo that will form the amnioserosa.

[55•,56,57]. Overexpression of *chordin* in *Xenopus* embryos results in a transformation of ventral mesoderm into dorsal mesoderm and a corresponding expansion of the notochord and paraxial mesodermal derivatives [55•,56]. Expression of *sog* is thought to inhibit *dpp* activity so that there are only low effective levels in dorsolateral regions that generate the dorsal epidermis. In contrast, the dorsal-most regions of the embryo are farthest from the localized *sog* inhibitor and, consequently, there are peak levels of *dpp* activity in those regions that will form the amnioserosa. As summarized in Figure 3, localized *sog* expression provides a key asymmetry in the pre-cellular embryo that leads to the subdivision of the dorsal ectoderm after gastrulation.

In principle, the regulation of the *sog* pattern might be similar to the situation described for *rho*; both genes are expressed in ventrolateral stripes along the length of the embryo in the presumptive neuroectoderm. The primary difference between the *sog* and *rho* expression patterns is that the dorsal limit of *sog* extends beyond *rho*. Each *sog* lateral stripe is ~14–16 cells in width, whereas the *rho* stripes are 8–10 cells. The *sog* promoter region has not been characterized but it is reasonable to anticipate that it contains an overall organization similar to the *rho* promoter, including a series of high affinity dl binding sites and closely linked E boxes. The expanded *sog* pattern may result from a more sensitive threshold response to vanishing levels of dl.

The putative *dpp* activity gradient is interpreted by the cells in the dorsal half of the embryo via three maternally expressed TGF-β receptors, *put*, *sax*, and *tkv* [46,58–60,61•]. All three receptors show ubiquitous localization of transcripts initially but whereas *put* and *tkv* are required for the patterning of the entire dorsal half of the embryo, *sax* appears to be necessary only for the formation of the amnioserosa, indicating that there are differences in the range of activity between the different receptors [58,60]. It is conceivable that this phenomenon is caused by different affinities of the receptors to their ligands, for instance one could imagine that the *sax* receptor is activated only by peak levels of *dpp* and/or *dpp/scw* multimers whereas *tkv* and *put* could be activated both by high and low levels of *dpp*. The presumed activity gradients of *dpp*, *sax*, *tkv* and *put* are summarized in Figure 3b.

Figure 4



Expression patterns in the neuroectoderm. Schematic cross-section indicating the overlapping but distinct patterns of different neuroectodermal genes. *sog* is expressed in the entire neuroectoderm, in lateral stripes 14–16 cells wide [17]. The *rho* stripes are 8–10 cells in width [70] whereas *lethal of scute* (T3) is expressed in clusters spanning up to 4 cells. *sim*, *m5*, *m7*, and *m8* are expressed in just 1–2 cells beyond the presumptive mesoderm.

Recent studies have identified potential 'downstream' genes in the DPP signaling pathway. These include *Mothers against dpp* (*Mad*) and *Medea*; *Mad* appears to encode a novel, cytoplasmic protein which may function

in the pathway leading to the activation of downstream targets such as *zen* [62,63•] (Fig. 3b). A putative zinc finger transcription factor, *schnurri*, might represent a nuclear target of the pathway [64•–66•].

The simplest interpretation of the information that is currently available is that the differential activation of *sog* and repression of *dpp* by the dl gradient initiates the formation of a *dpp* activity gradient and the subsequent subdivision of the dorsal ectoderm.

Subdivision of the neuroectoderm

As discussed above, the initial pattern of *sog* expression appears to coincide with the limits of the presumptive neuroectoderm. After gastrulation and germ band elongation, this embryonic tissue gives rise to neurons of the CNS and ventral epidermis. It is conceivable — but currently a matter of speculation — that differential threshold responses to the dl regulatory gradient provide the basis for an initial subdivision of the neuroectoderm into ventral and dorsal components.

As mentioned earlier, several dl target genes are expressed in the neuroectoderm during the blastoderm stage, including *rho*, *sog*, *sim*, and genes of the *achaete-scute* and *Enhancer of Split* complexes [19,67,68]. Of these, only *sog* is expressed in the entire 14–16 cell wide domain, the others being found in smaller territories. For example, the *rho* domain is only 8–10 cells wide, whereas *m7*, *m8*, and *sim* are expressed in just one or two cells beyond the *sna* border (summarized in Fig. 4). Progressively more ventral regions of the presumptive neuroectoderm thus express an increasing number of regulatory genes. Fate map studies suggest that the two innermost rows of neurons which form the ventral nerve cord, rI and rII, arise from the ventral part of the neuroectoderm expressing *rho* in addition to *sog*, whereas the outer row, rIII, arises from the dorsal part expressing only *sog* [33]. Perhaps the different combinations of early neuroectodermal genes help subdivide this territory into distinct derivatives of the CNS.

Conclusions

In conclusion, we have reviewed evidence that differential threshold responses to the dl regulatory gradient initiate the subdivision of each primary embryonic tissue during gastrulation. Future studies could provide critical tests of these proposals by manipulating the expression patterns of specific dl target genes. For example, expansion of the *rho* or *Lethal of scute* patterns in the presumptive neuroectoderm might alter the subdivision of tissue into ventral and lateral regions.

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