Dpp signaling thresholds in the dorsal ectoderm of the *Drosophila* embryo

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**SUMMARY**

The dorsal ectoderm of the *Drosophila* embryo is subdivided into different cell types by an activity gradient of two TGFβ signaling molecules, Decapentaplegic (Dpp) and Screw (Scw). Patterning responses to this gradient depend on a secreted inhibitor, Short gastrulation (Sog) and a newly identified transcriptional repressor, Brinker (Brk), which are expressed in neurogenic regions that abut the dorsal ectoderm. Here we examine the expression of a number of Dpp target genes in transgenic embryos that contain ectopic stripes of Dpp, Sog and Brk expression. These studies suggest that the Dpp/Scw activity gradient directly specifies at least three distinct thresholds of gene expression in the dorsal ectoderm of gastrulating embryos. Brk was found to repress two target genes, *tailup* and *pannier*, that exhibit different limits of expression within the dorsal ectoderm. These results suggest that the Sog inhibitor and Brk repressor work in concert to establish sharp dorsolateral limits of gene expression. We also present evidence that the activation of Dpp/Scw target genes depends on the *Drosophila* homolog of the CBP histone acetyltransferase.

Key words: Dpp thresholds, Brinker, CBP, Sog, *Drosophila melanogaster*

**INTRODUCTION**

The maternal Dorsal (Dl) gradient initiates the differentiation of the mesoderm, neurogenic ectoderm and dorsal ectoderm in the precellular *Drosophila* embryo. Dl establishes these tissues through the differential regulation of various target genes, including snail (mesoderm), rho (neurogenic ectoderm) and dpp (dorsal ectoderm) (reviewed by Mannervik et al., 1999). Subsequent signaling interactions subdivide each tissue into multiple cell types during gastrulation. For example, Dpp secreted from the dorsal ectoderm induces the underlying mesoderm to form the heart and gut muscles. In contrast, noninduced mesoderm gives rise to somatic muscles (Staehling-Hampton et al., 1994; Frasch, 1995). The neurogenic ectoderm forms the ventral epidermis and CNS, including three distinct rows of neurons in the ventral nerve cord and a specialized row of mesectodermal cells at the ventral midline (Weiss et al., 1998; McDonald et al., 1998). The dorsal ectoderm is subdivided into at least two tissues, the amnioserosa and dorsal epidermis (reviewed by Rusch and Levine, 1996; Podos and Ferguson, 1999).

This subdivision of the dorsal ectoderm requires a Dpp activity gradient in the dorsal half of the precellular embryo (Ferguson and Anderson, 1992; Wharton et al., 1993). The gradient is dependent on the activity of a second TGFβ signaling molecule, Screw, which potentiates Dpp signaling. Both Scw and Dpp are required to achieve a peak signaling threshold in the presumptive amnioserosa (Arora et al., 1994). Scw activity is modulated by the secreted Sog protein (Neul and Ferguson, 1998; Nguyen et al., 1998), which is related to the Chordin inhibitor of *Xenopus* (Holley et al., 1995). Sog is expressed in broad lateral stripes in the presumptive neurogenic ectoderm (François et al., 1994), possibly in direct response to the Dl gradient. This localized source of Sog is thought to inhibit and enhance Dpp/Scw signaling (reviewed by Podos and Ferguson, 1999). Peak levels of Sog within the lateral stripes completely block Dpp/Scw activity to permit neurogenesis. Intermediate levels of Sog secreted into dorsolateral regions attenuate Dpp/Scw signaling to specify the dorsal epidermis (Biels et al., 1996; Marqués et al., 1997). Finally, low levels of Sog secreted into the dorsalmost regions somehow enhance Dpp/Scw signaling to give a peak threshold response and formation of the amnioserosa (Ashe and Levine, 1999).

A number of marker genes have been shown to exhibit distinctive patterns of expression in response to the Dpp/Scw activity gradient. For example, *Race* is expressed in the dorsalmost regions in response to peak Dpp/Scw activity (Rusch and Levine, 1997). In contrast, *zerknüllt* (*zen*) (Rushlow et al., 1987) and *rho* (Bier et al., 1990) appear to be expressed in somewhat broader patterns that span the amnioserosa and extend into the presumptive dorsal epidermis in dorsolateral regions. Finally, the GATA-related gene *pnr* (Ramain et al., 1993; Winick et al., 1993) is activated throughout the dorsal ectoderm; the lateral limits of the pattern appear to abut the lateral stripes of *sog* expression within the neurogenic ectoderm (Winick et al., 1993; Heitzler et al., 1996).
A putative transcriptional repressor, Brk, might help establish different Dpp signaling thresholds (Campbell and Tomlinson, 1999; Jazwinska et al., 1999a; Minami et al., 1999). Brk is expressed in lateral stripes in the neurogenic ectoderm in early embryos. These stripes are established by the maternal Dl gradient, and subsequently maintained by Dpp signaling, which suppresses brk transcription in the dorsal ectoderm. Thus far, just one putative Dpp/Scw target gene, pnr, has been shown to be repressed by Brk (Jazwinska et al., 1999b). It is therefore unclear whether Brk is required to yield multiple patterning thresholds in response to Dpp signaling.

In the present study, dpp, brk and sog were misexpressed in transgenic embryos using the eve stripe 2 enhancer (Kosman and Small, 1997). A number of target genes were analyzed, including Race, rho, pnr and members of the ‘ush-group’ of genes required for amnioserosa maintenance (Frank and Rushlow, 1996), such as hindsight (hnt) (Yip et al., 1997), u-shaped (ush) (Cubraba et al., 1997) and tailup (tup) (Thor and Thomas, 1997). The stripe2-brk transgene was found to repress pnr and tup, which are activated by intermediate levels of the Dpp/Scw activity gradient, respectively. The lateral limits of the pnr expression pattern are established by high levels of the Brk repressor, whereas the tup pattern appears to depend on lower levels of Brk. Evidence is also presented that the Drosophila homolog of the CBP histone acetyltransferase is specifically required for the activation of Dpp/Scw target genes at the onset of gastrulation.

MATERIALS AND METHODS

Drosophila stocks

The fly stocks used were as follows: brinker, yw hbr[468]/FM7c ftz-lacZ (Jazwinska et al., 1999a); decapentaplegic, dpp[Hw37] GlaDp(2;2)DTD48; gastrulation defective, gdl/FM3; nejire, nej1 FRT/FM7c (Akimura et al., 1997b). nej1 germine clones were induced using the Flp-DFS technique (Chou and Perrimon, 1996). Briefly, nej1 FRT/FM7c females were mated with ovoD1 FRT; hs-FLP males. Larvae were heat shocked for 3 hours at 37°C on days 3, 4 and 5 after egg laying to induce expression of the FLP recombinase. Females lacking the Bar mutation present on the balancer chromosome were mated with yw males, and embryos were collected and fixed for in situ hybridization.

The misexpression constructs contain a FRT-transcription stop-FRT cassette to circumvent lethality (Kosman and Small, 1997) and were activated using a transgenic line homozygous for a P[ry'β2-tubulin-flp] insertion as described previously (Ashe and Levine, 1999). After removal of the flap-out cassette, it was found that expression of the stripe2-brk, stripe2-sog and stripe2-dpp transgenes is not lethal. To introduce stripe2-brk and stripe2-dpp into the same embryo, females containing the stripe2-dpp transgene were crossed to males carrying the stripe2-brk transgene.

Plasmid construction, P-element transformation and in situ hybridization

A Dral-HindIII (465-2740) fragment of the brk cDNA and a NdeI-SstI (1190-3090) of the dpp cDNA from KSdppNde (Rusch and Levine, 1997) were blunt-end ligated into HindIII-NotI-digested SK+, which has AscI sites engineered into the SacII and SalI sites of the polylinker. The brk and dpp cDNAs were then transferred as AscI fragments into AscI-digested 22FPE (Kosman and Small, 1997). P-element mediated transformation and in situ hybridizations using digoxigenin-labeled RNA probes were performed as described (Jiang et al., 1991).

RESULTS

Different dorsal ectoderm genes were examined in a variety of mutant and transgenic embryos using digoxigenin-labeled RNA probes and in situ hybridization (Jiang et al., 1991). The normal expression patterns suggest the occurrence of at least three thresholds of gene activity in response to the Dpp/Scw activity gradient (Fig. 1). The Race and hnt genes are expressed in narrow strips in the dorsalmost regions of the embryo (Fig. 1A,B, respectively), although the anteroposterior limits of the two patterns are distinct. It is conceivable that early-acting segmentation genes are responsible for repressing Race in posterior regions and hnt in anterior regions. Somewhat broader expression patterns are observed for tup and ush (Fig. 1C,D). These patterns encompass the presumptive amnioserosa and dorsal regions of the dorsal epidermis.

Broad staining patterns are observed for two genes encoding GATA transcription factors, dGATAc (Lin et al., 1995) and pnr (Fig. 1E,F, respectively). pnr is expressed throughout the dorsal ectoderm in the presumptive thorax and abdomen (Fig. 1F). dGATAc exhibits a nearly reciprocal pattern in anterior and posterior regions; staining is mainly excluded from regions expressing pnr, although a weak patch of staining is detected in a portion of the presumptive amnioserosa. Most of the subsequent analyses on gradient thresholds focus on the regulation of hnt (Fig. 1B), tup (Fig. 1C) and pnr (Fig. 1F).

Manipulating dpp dose

All of the aforementioned genes are virtually silent in the dorsal ectoderm of dpp/−dpp− embryos (data not shown), while changes in dpp+ gene dose cause altered patterns of expression (Fig. 2). For example, increasing the number of dpp+ copies from two (Fig. 2A) to three (Fig. 2B) to four (Fig. 2C) results in a sequential expansion of the hnt expression pattern, whereas expression is lost in dpp+/+ heterozygotes (data not shown). In contrast, ush is expressed in dpp/+ heterozygotes, although there is a marked narrowing in the expression pattern as compared with wild-type embryos (Fig. 2G, compare with H). Three copies of dpp+ cause an expansion of the ush pattern (Fig. 2I). Similarly, the tup expression pattern is narrower in dpp/+ heterozygotes and expanded in embryos with three copies of dpp (data not shown).

Further evidence that hnt and ush are early targets of the Dpp signaling pathway was obtained by analyzing transgenic embryos that contain the dpp-coding sequence attached to the eve stripe 2 enhancer (Fig. 2E). These embryos exhibit both the endogenous dpp pattern in the dorsal ectoderm (St Johnston and Gelbart, 1987) as well as an ectopic stripe of expression. The dpp stripe results in an expansion in both the hnt (Fig. 2D) and ush (Fig. 2F) expression patterns. The broadening of these patterns is particularly evident in anterior regions in the vicinity of the eve stripe. Increases in dpp+ gene dose do not expand the pnr expression pattern (data not shown). For example, four copies of dpp+ result in augmented levels of pnr expression, but the dorsoventral limits of expression are essentially normal. The stripe2-dpp transgene has no obvious effect on the early sog and brk expression patterns (data not shown).

Brinker represses pnr and tup

Previous studies have shown that the pnr expression pattern expands into neurogenic regions in brk− mutant embryos. No
contain different doses of cellularization. The embryos gastrulate. A lateral view of the embryos at the initial phase of dose. Dorsal views (except E) of containing two copies of midline in wild-type embryos left. (A–C)

hnt

and are oriented with anterior to the digoxigenin-labeled RNA probes and are oriented with anterior to the left. (A) Race hybridization probe. Staining is detected in a strip of cells along the dorsal midline. Staining in anterior regions corresponds to the presumptive brain and optic lobe, while the narrower strip in posterior regions corresponds to the developing amnioserosa. (B) hnt hybridization probe. Staining is restricted to a narrow strip at the dorsal midline. The dorsolateral limits of expression are similar to those observed for Race, but staining is excluded from anterior regions and extends to the posterior pole. (C) Tup hybridization probe. Staining is detected in the developing amnioserosa and also extends into dorsal regions of the presumptive dorsal epidermis. (D) Usb hybridization probe. Staining is detected in a broad band that encompasses the developing amnioserosa and extends into dorsolateral regions of the dorsal ectoderm. This pattern is similar to that observed for Tup (C) except that the Tup pattern extends into more anterior regions. (E) dGata9 hybridization probe. A broad band of staining is detected in anterior regions. There is a stripe near the posterior transverse furrow, and a patch of staining that includes an anterior portion of the amnioserosa. (F) Pnr hybridization probe. A series of broad stripes are detected throughout the dorsal ectoderm in the posterior half of the embryo.

Fig. 1. dpp target genes. Dorsal views of wild-type embryos at the initial phase of gastrulation. Embryos were hybridized with digoxigenin-labeled antisense RNA probes and are oriented with anterior to the left. (A) Race hybridization probe. Staining is detected in a strip of cells along the dorsal midline. Staining in anterior regions corresponds to the presumptive brain and optic lobe, while the narrower strip in posterior regions corresponds to the developing amnioserosa. (B) hnt hybridization probe. Staining is restricted to a narrow strip at the dorsal midline. The dorsolateral limits of expression are similar to those observed for Race, but staining is excluded from anterior regions and extends to the posterior pole. (C) Tup hybridization probe. Staining is detected in a broad band that encompasses the developing amnioserosa and extends into dorsolateral regions of the dorsal ectoderm. This pattern is similar to that observed for Tup (C) except that the Tup pattern extends into more anterior regions. (E) dGata9 hybridization probe. A broad band of staining is detected in anterior regions. There is a stripe near the posterior transverse furrow, and a patch of staining that includes an anterior portion of the amnioserosa. (F) Pnr hybridization probe. A series of broad stripes are detected throughout the dorsal ectoderm in the posterior half of the embryo.

Fig. 2. Altered expression patterns in response to altered dpp+ gene dose. Dorsal views (except E) of embryos at the initial phase of gastrulation. A lateral view of the embryo in E undergoing cellularization. The embryos contain different doses of dpp+ or express a stripe2-dpp transgene. They were hybridized with different digoxigenin-labeled RNA probes and are oriented with anterior to the left. (A–C) hnt hybridization probe. Staining is restricted to the dorsal midline in wild-type embryos containing two copies of dpp+ (A; see Fig. 1B). The pattern is broader in embryos carrying three copies of dpp+ (B) and perhaps even slightly broader in embryos carrying four copies of dpp+ (C). (D–F) Transgenic embryos carrying the stripe2-dpp fusion gene. hnt (D), dpp (E) and ush (F) expression patterns. The hnt and ush patterns are substantially broader than those seen in normal embryos, particularly in anterior regions in the vicinity of the stripe2-dpp expression pattern. These transgenic embryos contain both the endogenous dpp pattern in the dorsal ectoderm and the ectopic stripe 2 pattern (E). (G–I) Usb hybridization probe. The embryos contain different copies of the dpp+ gene. (G) a dpp+/+ heterozygote; (H) wild-type (2 copies of dpp+); (I) embryo contains three copies of dpp+. There is a progressive expansion of the ush expression pattern. The dpp+/ heterozygote (G) exhibits an abnormally narrow expression pattern. Genes requiring higher levels of dpp+ activity, such as hnt and Race, are not expressed in these embryos (data not shown).

such expansion was observed for other Dpp/Scw target genes that were previously examined, including ush (Jazwinska et al., 1999b). To test the role of the Brk repressor in establishing the different responses of Dpp target genes, the brk-coding sequence was attached to the eve stripe 2 enhancer.

Transgenic embryos carrying the stripe2-brk transgene exhibit both the normal pattern (lateral stripes) in the neurogenic ectoderm (Jazwinska et al., 1999b) as well as an ectopic stripe of expression (Fig. 3B, compare with A). Pnr is normally expressed in a series of 5 stripes in the dorsal ectoderm (Fig. 3C; Winick et al., 1993). The anteriormost stripe is lost in transgenic embryos carrying the stripe2-brk fusion gene (Fig. 3D, compare with C). This result suggests that Brk is sufficient to repress pnr in an ectopic location in the embryo.

Additional Dpp/Scw target genes were examined for repression by the stripe2-brk transgene. Those showing altered patterns of expression include tup, rho, hnt and Race (Fig. 3F, data not shown). The normal tup expression pattern encompasses both the presumptive amnioserosa and dorsal regions of the dorsal epidermis (Figs 1C, 3E). In transgenic embryos, there is a gap in the pattern in regions where the stripe2-brk fusion gene is expressed (Fig. 3F). These results suggest that Brk represses tup, even though it appears to respond to a different threshold of Dpp/Scw signaling than pnr. Additional experiments were done to determine whether Brk directly represses tup expression, or works indirectly by inhibiting Dpp signaling.

Sog inhibition versus Brk repression

To examine the relative contributions of the Sog inhibitor and the Brk repressor in establishing different thresholds of Dpp/Scw signaling activity, target genes were analyzed in gastrulation defective (gd) mutants that express either a stripe2-sog (Ashe and Levine, 1999) or stripe2-brk transgene (Fig. 4). Mutant embryos collected from gd+/gd+ females lack
all stripe2 pattern detected in the lateral stripes within the neurogenic ectoderm. In both cases, staining is observed in lateral stripes within the neurogenic ectoderm. In addition, there is an ectopic stripe2 pattern detected in the transgenic embryo (B). (C,D) pnr expression in a wild-type (C) and transgenic embryo (D) carrying the stripe2-brk transgene. The anteriormost pnr stripe is lost in the transgenic embryo (D, compare with C), indicating repression by the stripe2-brk fusion gene. (E,F) tup expression in a wild-type (E) and transgenic embryo (F) carrying the stripe2-brk transgene. The transgene creates a gap in the normal tup pattern (F, compare with E).

Dl nuclear gradient and therefore lack ventral tissues such as the mesoderm and neurogenic ectoderm. All tissues along the dorsoventral axis form derivatives of the dorsal ectoderm, mainly dorsal epidermis (e.g., Roth et al., 1989). Hereafter, we shall refer to such embryos as gd−. These mutants lack endogenous sog and brk products, so that the stripe2 transgenes represent the only source of expression. Although the stripe2-sog transgene inhibits Dpp signaling, it does not cause activation of brk (data not shown).

The pnr and tup expression patterns are derepressed in gd− mutants, and exhibit uniform staining in both dorsal and ventral regions (Fig. 4A,D). These expanded patterns correlate with the expanded expression of dpp, which is normally repressed in ventral and lateral regions by the Dl gradient (e.g., Huang et al., 1993). As seen in wild-type embryos (Fig. 3), the stripe2-brk transgene represses the anterior portion of the pnr expression pattern (Fig. 4B, compare with A). In contrast, the stripe2-sog transgene has virtually no effect on the pattern (Fig. 4C). These observations suggest that Brk is the key determinant in establishing the lateral limits of the pnr pattern at the boundary between the dorsal ectoderm and neurogenic ectoderm. The failure of stripe2-sog to inhibit pnr expression might be due to redundancy in the action of the Dpp and Scw ligands. Perhaps either Scw alone or just one copy of dpp is sufficient to activate pnr. This would be consistent with the observation that the initial pnr expression pattern is essentially normal in dpp−/dpp− and scw−/scw− mutant embryos (data not shown).

The limits of the tup expression pattern seem to depend on both Sog and Brk (Fig. 4D-F). The introduction of the stripe2-brk transgene leads to a clear gap in the tup expression pattern (Fig. 4E), although there is a narrow stripe of repression in gd− mutants lacking the transgene (Fig. 4D). The stripe2-sog transgene causes a more extensive gap in the tup pattern (Fig. 4F). The stripe2-brk transgene was also found to repress Race, hnt and rho in this assay (data not shown).

In principle, the gap in the tup pattern caused by the stripe2-brk transgene (Figs 3F, 4E) could be indirect, and caused by the repression of dpp. Previous studies have shown that the early dpp expression pattern expands into the ventral ectoderm in brk− mutant embryos (Jazwinska et al., 1999b). To investigate this possibility, tup expression was monitored in brk− embryos, and in wild-type embryos carrying both the stripe2-brk and stripe2-dpp transgenes (Fig. 5).

The tup expression pattern exhibits a transient expansion in brk− mutant embryos (Fig. 5C). However, this expansion is only seen in early embryos, prior to the completion of cellularization. By the onset of gastrulation, the pattern is essentially normal (Fig. 5B, compare with A). The stripe2-brk transgene creates an early gap in the normal dpp expression pattern in wild-type embryos (Fig. 5D). This observation raises the possibility that the repression of tup (Figs 3F, 4E) and rho (data not shown) is indirectly mediated by the inhibition of Dpp...
different mechanisms of Dpp signaling since threshold of gene activity. The similar patterns might involve and at the dorsal midline. The levels of Dpp and Scw activity lead to the activation of different thresholds of gene activity in the dorsal ectoderm. Peak that the Dpp/Scw activity gradient might generate as many as four an opposing Brk repressor gradient through the limited diffusion of that the early lateral stripes of tup/ush is not (data not shown). Finally, the broad expression pattern in regions where the transgenes are co-expressed. The gap is more pronounced than the one obtained tup pattern is expanded in anterior regions where the transgene is active (compare with Fig. 2D,F). tup expression pattern in an early gastrulating embryo that contains both the stripe2-dpp and stripe2-brk transgenes. There is a broad gap in the expanded tup expression pattern in regions where the transgenes are co-expressed. The gap is more pronounced than the one obtained with the stripe2-brk transgene alone (Fig. 3F) since the stripe2-dpp transgene directs a broad domain of enhanced Dpp/Scw signaling. can repress tup even in regions containing high levels of Dpp signaling. Similar assays suggest that Race, hnt and rho are not directly repressed by Brk (data not shown).

The CBP coactivator is required for Dpp signaling thresholds

Previous studies have identified mutations in the Drosophila homolog of the mammalian CBP histone acetyltransferase gene, nej (Akimaru et al., 1997a). nej is maternally expressed so that the detection of early patterning defects depends on the analysis of embryos derived from females containing nej germline clones. The complete loss of nej activity results in a failure to make mature eggs. However, it is possible to obtain embryos from a strong hypomorphic allele, nej. These embryos exhibit dorsoventral patterning defects (Akimaru et al., 1997b). Recent studies have shown that CBP interacts with Smad proteins (Feng et al., 1998; Janknecht et al., 1998; Topper et al., 1998) including the Drosophila protein Mad (Waltzer and Bienz, 1999), a transcription factor downstream of Dpp signaling (reviewed by Raftery and
DISCUSSION

We have presented evidence that the Dpp/Scw activity gradient specifies at least three distinct thresholds of gene activity in the dorsal ectoderm of the early Drosophila embryo. These thresholds depend on the interplay between broadly distributed Mad/Medea transcriptional activators and the spatially localized Brk repressor (Fig. 7). Different levels of the Brk repressor help establish distinct limits of pnr and tup expression. We have also presented evidence that the general CBP coactivator protein plays a surprisingly specific role in the patterning of the dorsal ectoderm, and is particularly important for the regulation of target genes that depend on high and intermediate levels of Dpp/Scw signaling. Despite the fact that CBP has been implicated as a general transcriptional coactivator (e.g., Mannervik et al., 1999), specification of the mesoderm and neurogenic ectoderm appears normal in mutant embryos derived from nej1 germline clones.

How many thresholds?
The distinct patterns of hnt, tup and pnr expression (Fig. 1) suggest that the Dpp/Scw activity gradient specifies at least three thresholds of gene activity. These genes exhibit differential responses to Dpp dose (Fig. 2) and to the Sog inhibitor (Fig. 4) and Brk repressor (Figs 3–5). It has been previously shown that Brk represses the early patterns of zen, tld and dpp expression (Jazwinska et al., 1999b). However, the initial activation of these genes depends on the maternal DI gradient and, consequently, they probably do not represent a distinct readout of the Dpp/Scw activity gradient.

The preceding arguments suggest that Dpp and Scw specify three thresholds of gene activity (summarized in Fig. 7). However, only two patterning thresholds, dorsal epidermis and amnioserosa, can be discerned on the basis of analyzing cuticle preparations. It is possible that staggered patterns of pnr and ush expression define a domain in dorsolateral regions where sensory organs can form. This would be analogous to the specification of the Do sensory bristle in the adult notum. Pnr can activate the pro-neural genes, achaete and scute, in a broad region of the notum, but expression is restricted to the Do primordium by ush, which inhibits Pnr through direct protein-protein interactions (Cubbada et al., 1997; Haenlin et al., 1997).

Transcriptional repression and Dpp signaling thresholds
At least two of the transcription thresholds that are specified by the Dpp/Scw activity gradient depend on the Brk repressor (Fig. 7). In principle, pnr can be activated throughout the dorsal half of the embryo but expression is restricted to the presumptive dorsal ectoderm by high levels of the Brk repressor (Jazwinska et al., 1999b; Figs 3, 4 of the present study). In contrast, lower levels of Brk might work together with the Sog inhibitor to specify sharp lateral limits of the tup expression pattern (Figs 4, 5). Perhaps the early lateral stripes of brk expression permit limited diffusion of the Brk protein in the syncitial nuclei of precellular embryos. Such diffusion might produce a Brk repressor gradient that is complementary to the Dpp/Scw signaling gradient. Presumably, Sog-Dpp/Scw interactions create a gradient of activated Mad/Meda complexes in the nuclei of the dorsal ectoderm (reviewed by Raftery and Sutherland, 1999; Fig. 7). Perhaps the lowest levels of Brk that can repress tup correspond to the lowest levels of the Mad/Meda gradient that can activate it. According to this view, the Brk gradient functions redundantly with the Mad/Meda gradient to specify the lateral limits of the tup expression pattern. This would be similar to the specification of the posterior border of eve stripe 2. This border depends on both limiting amounts of the anteroposterior gradient of Bicoid (Bcd) activator and on the Krüppel (Kr) repressor gradient emanating from central regions of the embryo. Stripe2-lacZ fusion genes lacking critical Kr repressor sites exhibit only a slight posterior expansion of the stripe border due to limiting amounts of Bcd. The interplay between Bcd and Kr helps produce a sharp stripe border (Stanojevic et al., 1991; Small et al., 1992). Similarly, it is conceivable that Mad/Meda and Brk produce sharp borders of tup expression (see Fig. 3E).

Thus, the situation we observe in the dorsal ectoderm of early embryos is quite similar to the patterning of wing imaginal disks. A Dpp activity gradient at the A/P compartment boundary of the wing disk establishes different limits of spalt (sal) and optomotor blind (omb) expression. High levels of the Brk repressor specify the lateral limits of the broad omb pattern, whereas the combination of low levels of Brk and intermediate levels of Dpp signaling regulate the narrower sal pattern (Campbell and Tomlinson, 1999; Jazwinska et al., 1999a). A difference between the wing disk and embryo is that there are target genes in the embryo which are activated by peak levels of Dpp signaling (e.g., Race and hnt) but not repressed by Brk. Such genes have not yet been described in the wing disk.

The role of CBP in establishing Dpp/Screw signaling thresholds
A remarkable number of genes are required for producing and
receiving the Dpp/Scw gradient in the dorsal ectoderm (reviewed by Podos and Ferguson, 1999). It would appear that an increasing number of nuclear factors are also required for generating different thresholds of gene activity in response to this gradient. In addition to Mad/Medea and Brinker, we have obtained evidence that the general transcriptional coactivator, CBP, is essential in this process. Previous studies have shown that the expression of a Race-lacZ fusion gene is lost in the presumptive amnioserosa of mutant embryos derived from nej1 germline clones (Waltzer and Bienz, 1999). Here we have shown that there is an extensive, but specific, loss of dorsal ectoderm derivatives in these mutants. There is a loss of high Dpp/Scw signaling thresholds including Race, hnt and rho. Moreover, target genes that are activated by intermediate levels of Dpp/Scw signalling, such as ush, exhibit abnormally narrow patterns of expression. However, unlike dpp/+ heterozygotes or scw+/scw− homozygotes there is also a narrowing of the pnr expression pattern in nej mutants.

These disruptions in Dpp patterning thresholds may reflect relatively specific CBP-Mad interactions in the early embryo. It is possible that Mad recruits CBP to the promoter regions of Dpp target genes (Waltzer and Bienz, 1999). Alternatively, CBP-mediated chromatin decondensation may be essential for the binding of Mad/Medea to chromatin templates in vivo. The promoter regions of hnt and rho might contain low-affinity Mad/Medea operator sites and thereby mediate activation only by high levels of Dpp signaling. Such genes would be expected to exhibit particular sensitivity to reductions in CBP activity. The pnr promoter region might contain optimal, high-affinity Mad/Medea operator sites. The narrowing of the pnr pattern in nej mutants might indicate that CBP is required for the efficient occupancy of even optimal sites in dorsolateral regions where there are diminishing levels of Dpp/Scw signaling.

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