Role of Dpp signalling in prepattern formation of the dorsocentral mechanosensory organ in *Drosophila melanogaster*

Yoshinori Tomoyasu¹,², Makoto Nakamura¹,* and Naoto Ueno¹,²

¹Division of Morphogenesis, Department of Developmental Biology, National Institute for Basic Biology, and ²Department of Molecular Biomechanics, School of Life Science, The Graduate University for Advanced Studies, 38 Nishigonaka Myodaijicho Okazaki 444-8585, Japan

*Author for correspondence (e-mail: mack@nibb.ac.jp)

Accepted 25 August; published on WWW 30 September 1998

SUMMARY

A proneural cluster of dorsocentral bristles forms adjacent to the dorsal side of *wg*-expressing cells in the notum region of the wing imaginal disc. It has been shown that 

A proneural cluster of dorsocentral bristles forms adjacent to the dorsal side of *wg*-expressing cells in the notum region of the wing imaginal disc. It has been shown that *wg* activity is required for these structures to form. However, the restriction of this proneural cluster to the dorsal posterior side of the *wg* expression domain in the anterior compartment of the wing imaginal disc has suggested that 

Wg signalling itself is insufficient to establish the dorsocentral proneural cluster. Some factor(s) from the posterior side must participate in this action in cooperation with Wg signalling. We have examined the role of Dpp signalling in dorsocentral bristle formation by either ectopically activating or conditionally reducing Dpp signalling. Ubiquitous activation of Dpp signalling in the notum region of the wing imaginal disc induced additional dorsocentral proneural cluster all along the dorsal side of the *wg* expression domain, and altered *wg* expression. Conditional loss-of-function of Dpp signalling during disc development resulted in the inhibition of dorsocentral proneural cluster formation and expansion of the *wg* expression domain. These results suggest that Dpp signalling has two indispensable roles in dorsocentral bristle formation: induction of the dorsocentral proneural cluster in cooperation with Wg signalling and restriction of the *wg* expression domain in the notum region of the wing imaginal disc.

Key words: Drosophila development, Imaginal disc, decapentaplegic, wingless, Sensory organ, Pattern formation

INTRODUCTION

Two types of sensory organs, large bristles (macrochaetes) and small bristles (microchaetes), develop in fixed numbers at constant positions on the dorsal part of the mesothorax (called notum) of *Drosophila melanogaster*. Prepattern formation of macrochaetes on the notum has provided an ideal model system for studying two-dimensional patterning. The accurate positioning of the macrochaetes is established during the third larval to early pupal stage within the epithelial sheets of the notum region of the wing imaginal discs (Hartenstein and Posakony, 1989; Huang et al., 1991). For convenience, we will refer to this region of the wing disc as the ‘thoracic disc’ to distinguish it from the wing pouch region. Initially, in the thoracic disc, a group of cells called a proneural cluster, characterized by the expression of the proneural genes achaete (*ac*) and scute (*scu*), are formed around the positions where macrochaetes will form (Cubas et al., 1991; Skeath and Carroll, 1991). Next, one or a few sensory mother cells (SMCs) are singled out from the proneural cluster, and each SMC subsequently undergoes two rounds of cell division forming four progeny cells that differentiate into the components of a sensory bristle (Hartenstein and Posakony, 1989; Huang et al., 1991). Thus, precise positioning of the macrochaete on the notum depends on the complex expression pattern of the *ac* and *scu* genes in the thoracic disc. *ac* and *scu* encode transcription factors of the basic helix-loop-helix family that confer upon cells the ability to become SMCs (Cabrera et al., 1987; Gonzalez et al., 1989). The removal of specific proneural clusters by achaete-scute complex (ASC) mutations leads to the absence of the corresponding SMCs and macrochaetes (Cubas et al., 1991; Gomez-Skarmeta et al., 1995).

The complex expression pattern of *ac* and *scu* is controlled through the action of enhancer-like *cis*-regulatory elements (Gomez-Skarmeta et al., 1995; Leyns et al., 1996; Ruiz-Gomez and Ghysen, 1993; Ruiz-Gomez and Modolell, 1987). These elements are presumed to respond to a ‘prepattern’ established by local specific combinations of factors, as first postulated by Stern (1954). The products of prepattern genes would be expected to be distributed asymmetrically in the thoracic disc and they control *ac* and *scu* expression at both transcriptional and post-transcriptional levels (reviewed by Jan and Jan, 1990; Simpson, 1996). The existence of a specific *cis*-regulatory element for individual proneural clusters has suggested that different combinations of prepattern genes promote the complex expression pattern of proneural genes (Gomez-Skarmeta et al., 1995).

The constituents of prepattern genes are largely unknown,
however, several candidate genes have been reported. Three genes residing at the iroquois (iro) locus, araucan (ara), caupolican (caup) and mirror (mirr), encode a novel family of homeoproteins. These genes are expressed in the lateral half of the thoracic disc and affect proneural cluster formation in this region (Damby-Chaudriere and Leyns, 1992; Gomez-Skarmeta et al., 1996; Gomez-Skarmeta and Modevell, 1996; Kehl et al., 1998; Leyns et al., 1996). Another possible candidate is pannier (pnr) which encodes a protein belonging to the GATA family of transcription factors (Cubadda et al., 1997; Heitzler et al., 1996; Ramain et al., 1993). Loss-of-function mutations of the pnr gene fail to form the proneural clusters in the dorsal side of the thoracic disc (Cubadda et al., 1997; Haenlin et al., 1997). Recently, Haenlin et al. reported that the transcriptional activity of Pnr is regulated negatively by a novel zinc finger protein, U-shaped (Ush). They suggested that the products of pnr and ush cooperate in the regulation of ac and se expression in a specific proneural cluster, the dorsocentral proneural cluster.

Post-transcriptional regulation of proneural gene products also could contribute to prepatterning of the macrochaetae. extramacrochaetae (emc) is genetically described as an ASC repressor and encodes a helix-loop-helix protein devoid of a basic domain (Botas et al., 1982; Ellis et al., 1990; Van Doren et al., 1991). The Emc protein is thought to form a heterodimer with the HLH proteins encoded by the asc and/or daughterless, thereby altering or interfering with their activity (Botas et al., 1982; Ellis et al., 1990; Van Doren et al., 1991).

Besides transcriptional regulators, morphogen gradients generated by secreted proteins could also be involved in macrochaetae prepatterning formation. wingless (wg), hedgehog (hh) and decapentaplegic (dpp) have been shown to generate positional information within imaginal discs (Basler and Struhl, 1994; Ingham and Fietz, 1995; Lecuit et al., 1996; Nellen et al., 1996; Neumann and Cohen, 1997; Tabata and Kornberg, 1994; Zecca et al., 1996). However, there are a few reports regarding the involvement of these secreted factors in prepatterning formation of the macrochaetae. wg is expressed in a stripe of cells along the A/P axis in the thoracic disc (Baker, 1988; Phillips and Whittle, 1993, and also see Fig. 1-B). It has been shown that wg is required for the development of a subset of proneural clusters which appear in or immediately adjacent to the wg-expressing cells (Cousso et al., 1994; Phillips and Whittle, 1993). On the other hand, ectopic activation of either Hh or Dpp signalling in the wing disc results in the induction of SMCs at numerous ectopic positions in the wing disc (Mullor et al., 1997). These results suggest that dpp, hh and wg participate in the prepatterning formation of the macrochaetae on the notum.

Here, we have focused on whether Dpp, as a morphogen gradient, plays a part in the prepatterning of the macrochaetae. Experiments using both gain-of-function and conditional loss-of-function mutants revealed that Dpp signalling participates in this process in two major ways. One is induction of the proneural cluster in cooperation with Wg signalling and the other is restriction of the wg expression domain in the thoracic disc.

**MATERIALS AND METHODS**

**Fly strains**

Flies were raised on standard *Drosophila* medium at 25°C. The mutants and transgenic flies used in this work are as follows. hs-GAL4 and ts-hGAL4 driver lines (Shiga et al., 1996). near-lacZ (A101), wg-lacZ (17en40), emc-lacZ (emc-4218), DC enhancer fragment-3.7x-lacZ (DC-lacZ), ac-lacZ, 3.7x-lacZ (Ghysen and O’Kane, 1989; Gomez-Skarmeta et al., 1995; Huang et al., 1991; Kassis et al., 1992; Van Doren et al., 1992; Wilson et al., 1989). wg\(^{G301}\) is a dominant allele (Neumann and Cohen, 1996), and wg\(^{L144}\) is a temperature sensitive allele of wg (Nusslein-Volhard and Wieschaus, 1980). In flies of genotype wg\(^{G301}\)/wg\(^{L144}\), aDC bristles are constantly missing at 25°C. w; wg\(^{L144}\) tsh-GAL4/SM6a-TM6B flies were crossed with w; UAS-tkv\*/SM6a-TM6B. Pharate adult of genotype w; wg\(^{L144}\) tsh-GAL4/wg\(^{G301}\); UAS-tkv\*/+ flies can be distinguished from their ‘Tubby’ sibs. punt\(^{P1}\)/st punt\(^{155}\) e flies are viable with no phenotypes at 18°C but are lethal at or above 25°C (Letouzou et al., 1995; Simin et al., 1998; Theisen et al., 1996). w; 3.7 sc-lacZ/SM1; st punt\(^{155}\) e/TM6B flies were crossed with w; punt\(^{P1}\)/TM6B and raised at 18°C until late second larval instar. Then the temperature was shifted to 29°C, using a water bath to ensure temperature constancy. Larvae at late wandering to white pupal stage were dissected and wing discs were recovered. Larvae of the w; 3.7 sc-lacZ/+; punt\(^{P1}\)/st punt\(^{155}\) e genotype can be distinguished from their ‘Tubby’ sibs. Tb\* flies, whose genotypes were either w; 3.7 sc-lacZ/+; punt\(^{P1}\)/TM6B or w; 3.7 sc-lacZ/+; st punt\(^{155}\) e/TM6B, were used as wild-type control.

**Plasmid constructions and fly transformations**

A point mutation in the tkv cDNA (Okano et al., 1994), changing a glutamine residue (position 199) to aspartic acid, was generated by PCR using mutagenic primers. It has been reported that the same amino acid substitution in Tkv results in the constitutive activation of this receptor (Hoodless et al., 1996; Nellen et al., 1996). A Nof-Xho fragment containing the constitutively active version of tkv (tkv\*) cDNA was subcloned from pluescript II KS- into pUAST (Brand and Perrimon, 1993). The cDNA containing the entire punt ORF (Ruberte et al., 1995) was also subcloned into pUAST at the appropriate restriction sites. P-element-mediated transformation was performed using standard procedures (Rubin and Spradling, 1982; Spradling and Rubin, 1982).

**Mosaic expression and conditional overexpression of tkv\* and punt**

In the AyGAL4 construct, a FLP-out cassette containing the hsp70 termination signals flanking the yellow\* gene, flanked in turn by two FRT sites, is inserted between the Act5C promoter and the GAL4 gene (Ito et al., 1997), w; AyGAL4 UAS-GFP\** were crossed to flies of the genotype y w hs-fjun; UAS-tkv\*. The resulting progeny were subjected to a heat shock (20 minutes at 37°C) during the first larval instar. tkv\* expression mosaics were monitored by GFP fluorescence. Flies of the genotype hs-GAL4; UAS-tkv\* (or UAS-punt) were subjected to two heat shocks at 37°C for 30 minutes separated by a 1 hour recovery at 25°C during the second to third instar larval stage, and then aged at 25°C. White pupae were collected every two hours. The heat shock time indicates the period from the beginning of the first heat shock to the pupal collection, referred to as hours ‘before puparium formation’ (BPF). Each dot in Fig. 1G represents an average of more than 20 animals.

**Imaginal discs staining**

The discs were fixed with 3.7% formaldehyde in PBS for 30 minutes at room temperature. After several washes, the discs were incubated with primary antibodies diluted in PBS containing 0.3% Triton X-100 and 10% normal goat serum (blocking solution) at 4°C overnight. After washing several times in PBS containing 0.3% Triton X-100 (PBT), discs were incubated for 2 hours at room temperature with secondary antibodies diluted in the blocking solution. After several washes in PBT, discs were mounted on the slide glass with GEL MOUNT™ (Biomeda). Confocal fluorescent images were obtained...
using Zeiss LSM410 or LSM510 microscopes. Antibodies were diluted as follows: anti-Wg (1:5; gift from S. Cohen); anti-β-galactosidase rabbit polyclonal antibody (1:500; Cappel); anti-rabbit IgG LRSC-conjugated (1:100; Jackson); anti-mouse IgG FITC-conjugated (1:100; Jackson).

RESULTS

Ectopic activation of Dpp signalling induces extra macrochaete formation on the notum

Fig. 1A shows the wild-type macrochaete pattern of the notum. An anterior-dorsocentral bristle (aDC) and a posterior-dorsocentral bristle (pDC) are formed along the anterior/posterior (A/P) axis on the notum. It has been shown that wg activity is necessary for the formation of both aDC and pDC (Couso et al., 1994; Phillips and Whittle, 1993). However, these SMCs are not induced all along the wg expression domain, but induced only adjacent to the dorsal posterior side of the wg expression domain in the anterior compartment of the thoracic disc (Fig. 1B, we will refer to the two sides of the wg expression domain as the ‘dorsal side’, where the dorsocentral SMCs are formed, and the ‘lateral side’, for the opposite side). This suggests that Wg signalling alone is insufficient to induce SMCs of aDC and pDC, and that another factor(s) which resides on the dorsal posterior side of the thoracic disc is also required for inducing these SMCs. One candidate factor is Dpp. In the thoracic disc, dpp is induced in a stripe of cells located posterior to the dorsocentral SMCs (Fig. 1B). This expression pattern and the property of Dpp as a morphogen suggests that Dpp signalling may also participate in prepattern formation of the macrochaetes on the notum.

First, we attempted to ectopically activate Dpp signalling in the thoracic disc during larval development using the GAL4-UAS system (Brand and Perrimon, 1993). It has been shown that ectopic expression of either Dpp type-II receptor Punt, or type-I receptor Thick veins (Tkv) in which glutamine residue 199 is replaced with aspartic acid (Tkv*), activates Dpp signalling in a ligand independent manner (Hoodless et al.,...
We have tested several GAL4 drivers which promote GAL4 expression in the thoracic disc. Overexpression of either tkv* or punt (data not shown) using tsh-GAL4 driver (Shiga et al., 1996; the GAL4 expression pattern in the wing disc is also shown in Fig. 1H) alters the macrochaete pattern on the notum (Fig. 1C). More than seven macrochaetes (per heminotum) are ectopically induced in the dorsolateral region (but not in the most dorsal region) of the notum. Ectopic macrochaetes seem to be induced cell-autonomously, only within the tkv* expressing mosaic clones (Fig. 1D). To look for a correlation between the timing of ectopic Dpp signalling and macrochaete induction, tkv* was induced for a short time period at different stages during larval development using hs-GAL4 driver. Ectopic expression of either tkv* (Fig. 1E) or punt (Fig. 1F) induces extra macrochaetes without significant notum morphology change. Fig. 1G shows the number of additional macrochaetes induced by tkv* near the endogenous dorsocentral bristles (dorsocentral region) at different heat shock timings. A heat shock treatment around 45 hours before puparium formation (BPF) significantly induces additional macrochaetes, about four extra macrochaetes on average per dorsocentral region of the notum. A time lag of several hours between heat shock initiation and Tkv* protein expression should exist due to the indirect induction of the transgene via heat induced GAL4 proteins. Considering this, the effective period of ectopic Dpp signalling seems to be near the beginning of endogenous proneural gene expression in the thoracic disc (Cubas et al., 1991). These results suggest that Dpp signalling participates in the prepatterning of the macrochaetes, presumably in the transcriptional activation of proneural genes in the thoracic disc.

**Ectopic Dpp signalling induces additional SMCs and suppresses wg expression in the thoracic disc**

To investigate more precisely the positioning of ectopically formed macrochaetes, we observed the locations of SMCs in the thoracic discs. Ubiquitous tkv* expression in the thoracic disc using tsh-GAL4 induces numerous ectopic SMCs (Fig. 2B). Ten to fifteen ectopic SMCs formed along the dorsal side of the wg expression domain and also several SMCs formed lateral to the wg expression domain. This asymmetric induction suggests that Wg signalling is necessary for the induction of extra SMCs by ectopic Dpp signalling. Thoracic discs of UAS-tkv*: tsh-GAL4 are expanded along the A/P axis, presumably due to over proliferation of the cells in the wing disc. Ectopic activation of Dpp signalling also alters the wg expression. In the wild-type thoracic disc, wg is expressed in a stripe of cells with a smooth boundary (Fig. 2A). In the UAS-tkv*: tsh-GAL4 disc, wg-expressing cells exist within a narrow stripe and occasionally appear as small patches (Fig. 2B). Weak expression of tkv* using hs-GAL4 driver also induces additional SMCs and repression of the wg expression (Fig. 2C). This level of ectopic Dpp signalling induces additional SMCs only on the posterior side of the anterior compartment near the endogenous Dpp source (Fig. 2C). Relatively higher levels of tkv* expression in the 2×UAS-tkv*: hs-GAL4 disc, induces ectopic SMCs more anteriorly (Fig. 2D). These results indicate that high levels of Dpp signalling activity are necessary to induce SMCs. Low levels of ectopic Dpp signalling could recruit cells, which have already received sub-threshold levels of endogenous Dpp signalling, to form additional SMCs. On the other hand, even in the 1×UAS-tkv*: hs-GAL4 discs, reduction of wg expression was observed not only near the endogenous Dpp source but also around the most anterior region of the wg expression domain (Fig. 2C). Thus, low levels of Dpp signalling appear to be sufficient to repress wg expression.

Together, these results suggest that Dpp signalling has two important roles for macrochaete formation, one is induction of SMCs in cooperation with Wg signalling and the other is restriction of wg expression. A difference should exist between the threshold level of Dpp signalling required for SMC induction and that required for repression of wg expression.

**Ectopic Dpp signalling induces dorsocentral proneural cluster formation all along the dorsal side of the wg-expressing domain**

In the wild-type thoracic disc, ac expression associated with
the dorsal central proneural cluster appears only adjacent to the dorsal posterior side of the wg-expressing cells (Fig. 3A). Ectopic Dpp signalling using tsh-GAL4 driver abnormally extends ac expression to the anterior end of the thoracic disc (Fig. 3B). Dorsocentral specific proneural gene expression was also monitored using DC-lacZ reporter (Gomez-Skarmeta et al., 1995). This reporter line selectively expresses β-galactosidase in the dorsal central proneural cluster in the thoracic disc (Fig. 3C and Gomez-Skarmeta et al., 1995). As this reporter contains an SMC enhancer, it also expresses β-galactosidase in all the SMCs in the wing disc (Culi and Modolell, 1998). β-Galactosidase expression in the proneural cluster can easily be distinguished from that in SMCs based on the shape and intensity of expression. In the UAS-tkv*: tsh-GAL4 discs, DC-lacZ proneural expression extends to the anterior edge of the thoracic disc, however, it appears only on the dorsal side of the wg expression domain (Fig. 3D). This result indicates that ectopically induced SMCs on the dorsal side of the wg expression domain are SMCs of the dorsal central proneural bristles. Ectopic DC-lacZ expression was observed only near the wg expression domain. This expression is likely to complement the wg expression domain (Fig. 3D). These results seem to indicate that Wg signalling is required for dorsocentral proneural cluster formation, but that only those cells which do not express wg have the potential to become dorsocentral proneural cells.

On the other hand, ac-lacZ expression lateral to the wg expression domain corresponds to the expression of another reporter line, 6.0-0.0 kb enhancer fragment-3.7 sc-lacZ (Gomez-Skarmeta et al., 1995) (data not shown). The latter reporter expression reflects the locations of several proneural clusters in the thoracic disc (aNP, aPA, tr1 and tr2). It has been shown that wg activity is not required for the formation of these proneural clusters (Phillips and Whittle, 1993). Dpp signalling appears to cooperate with other factor(s) to induce several wg independent proneural clusters.

**Ectopic expression of tkv* does not affect emc expression**

Anterior expansion of the dorsocentral proneural cluster in the presence of ectopic Dpp signalling could result from alteration of the ASC modulator(s). Emc is a helix-loop-helix protein that lacks a transcriptional activator domain (Botas et al., 1982; Ellis et al., 1990; Van Doren et al., 1991). Emc protein appears to suppress the formation of Ac-Da and/or Sc-Da complexes and inhibit their transcriptional activities. The loss-of-function emc mutation results in the appearance of some additional bristles near the endogenous ones. This phenotype is similar to that of the flies expressing low levels of tkv* as shown in Fig. 1E. We observed emc expression using an emc-lacZ reporter in UAS-tkv*: tsh-GAL4 discs. Emc expression is retained even in the presence of ectopic Dpp signalling (Fig. 4B). This result indicates that emc expression is independent of Dpp signalling. Importantly, ectopic proneural clusters and SMCs are induced even in the emc
expressing region (Fig. 4B, compare with Figs 2B and 3B). Therefore it is possible that ectopically activated Dpp signalling causes the induction of proneural genes at high levels and that these activities could overcome the inhibitory effects of the Emc protein.

Endogenous Dpp signalling is required for dorsocentral proneural cluster induction and repression of wg expression

Dpp signalling is important for induction and proliferation of imaginal discs (Lecuit et al., 1996; Nellen et al., 1996). To minimize the activity of dpp in early morphogenesis of imaginal discs (and to focus on the induction of the proneural clusters), we used conditional loss-of-function Dpp signalling mutants. Some allelic combinations of the punt mutations exhibit temperature sensitivity for Dpp signalling (Letou et al., 1995; Simin et al., 1998; Theisen et al., 1996). punt^6/punt^15 (termed punt-ts) is permissive at 18°C and non-permissive at 29°C. punt-ts flies were cultured at 18°C and transferred to 29°C at the second to early third larval instar stage. We monitored the position of SMCs and wg expression in the punt-ts discs. In this condition, wg expression was expanded to the dorsal edge of the thoracic disc (Fig. 5B). Expansion of the wg expression domain in this mutant disc was also confirmed by using wg-lacZ reporter (data not shown). High levels of lacZ protein were observed in dorsocentral SMCs in wild-type discs (Fig. 5A) but not in punt-ts discs (Fig. 5B). This result indicates that both dorsocentral proneural cluster induction and repression of wg expression are promoted by endogenous Dpp signalling.

To our surprise, in thoracic discs from punt-ts mutants shifted to milder conditions (25°C) at the same stage (we will refer to this mutant as ‘mild punt-ts’) one or a few extra SMCs are formed (Fig. 5C). It is also worth noting that extra SMCs appear to be formed in a more posterior region compared to the endogenous dorsocentral SMCs (Fig. 5C compare with Fig. 5A). In the mild punt-ts disc, the wg expression domain is slightly expanded dorsally and posteriorly (Fig. 5C). One possible explanation for this phenotype is that the region receiving sufficient levels of both Dpp and Wg signals to induce dorsocentral proneural cluster has expanded in mild punt-ts mutants. We will discuss more about this controversial issue later.

wg activity is necessary for induction of ectopic dorsocentral bristle formation by ectopic Dpp signalling

Finally, we examined whether wg activity is required for tkv* induced ectopic dorsocentral bristle formation. Fig. 6A shows a bristle pattern of the allelic combination of the wg mutants (wg^IL14/wg^Sp1). aDC is constantly missing in flies of this genotype. Ectopic tkv* expression by tsh-GAL4 fails to induce any ectopic dorsocentral bristles (Fig. 6B and compare to Fig. 1C). In contrast to the dorsocentral bristles, wg independent macrochaetes, such as aPA and pSA, are ectopically induced by tkv* even in the wg mutant background (Fig. 6B). These results confirm that Wg signalling is absolutely required for ectopic dorsocentral bristle formation.

Fig. 5. Reduction of Dpp signalling activity leads to ectopic wg expression and inhibition of dorsocentral SMC formation. (A-C) Thoracic discs in the late third larval stage labeled with anti-Wg antibody (green) and 3.7 sc-lacZ expression (red) are shown. High levels of sc-lacZ expression localize in SMCs at this stage. (A) Positions of the dorsocentral SMCs in the wild-type disc are indicated with arrows. (B) A punt^6/punt^15 (punt-ts) thoracic disc from larvae which were transferred from 18°C to the nonpermissive temperature (29°C) about 48 hours BPF; we referred to this mutant as ‘severe punt-ts’. In the severe punt-ts mutant disc, wg expression expands to the dorsal edge of the disc. SMCs lateral to the wg expression domain still exist in the mutant discs (indicated with arrowheads in B and C). However, the dorsocentral SMCs are no longer observed within the expanded wg expression domain. The severe punt-ts mutant disc is smaller than that of wild type. (C) A thoracic disc of a punt-ts mutant which was shifted to a mild heat condition (at 25°C); we referred to this as ‘mild punt-ts’. In the mild punt-ts disc, the wg expression domain is slightly expanded toward the dorsal edge and additional SMCs are formed (arrows). The discs are shown with anterior left and dorsal down.

Fig. 6. Effect of tkv* overexpression on bristle development in a wg mutant background. (A) Bristle pattern of the wg^IL14 tsh-GAL4/wg^Sp1 notum. aDC is constantly missing in the flies of this genotype (position where it should be formed is indicated by an arrow). (B) Pharate adult notum of wg^IL14 tsh-GAL4/wg^Sp1; UAS-tkv^+/+. Ectopic tkv* expression by tsh-GAL4 in this wg mutant no longer induces ectopic bristles in the dorsocentral region of the notum. Duplication of the wg-independent bristles (aPA and pSA) is frequently observed (indicated by blue arrowheads).
DISCUSSION

Dpp signalling participates in dorsocentral bristle development

We have shown that ectopic Dpp signalling induces additional dorsocentral proneural clusters and SMCs all along the dorsal side of the wg expression domain in the thoracic discs. Mosaic expression of the tkv* indicated that Dpp signalling is required cell autonomously to induce ectopic proneural clusters. Loss-of-function experiments using punt-ts flies also indicated that endogenous Dpp signalling is necessary for the formation of the dorsocentral SMCs. Moreover, in the wg mutant flies (wg3pl/wgIII14), ectopic Dpp signalling did not induce any additional dorsocentral bristles. These results indicate that the dorsocentral proneural cluster is formed through the activities of both Dpp and Wg signalling.

There are many genes known to be regulated by both Dpp and Wg signalling. For instance, a midgut enhancer of the Ultrabithorax gene has been shown to be regulated directly by both Wg and Dpp signal transducers (Ereshe et al., 1997; Riese et al., 1997), vestigial (vg) quadrant enhancer has been shown to be activated by Dpp signalling (Kim et al., 1996, 1997). vg expression is also regulated by Wg signalling in the wing pouch (Kim et al., 1996, 1997). The regulatory mechanism for the cis-element(s) of the DC-enhancer is totally unknown. Cell autonomous effects of both Dpp and Wg signals (Fig. 1D, and R. G. Phillips et al., personal communications) suggest the possibility that a Dpp and Wg signal transducer directly effects the DC-enhancer to induce proneural genes. However, we cannot rule out another possibility that Dpp and/or Wg signalling control the expression (or activity) of other prepatterning genes that directly activate the DC-enhancer. Analysis of the DC-enhancer element is necessary to address how Dpp and Wg signals cooperate in the induction of the proneural genes at the dorsocentral region.

Our data from both gain-of-function and loss-of-function experiments suggest that Dpp signalling also has an important role in the specification of the wg expression domain in the thoracic disc. A mutual inhibitory interaction between dpp and wg in the leg disc has been extensively analyzed (Brook and Cohen, 1996; Jiang and Struhl, 1996; Penton and Hoffmann, 1996; Theisen et al., 1996). Wg is expressed ventrally and Dpp expression is at high levels dorsally along the A/P boundary in the leg discs and both expressions are controlled by Hh (Basler and Struhl, 1994). By contrast, in the wing disc, Hh controls expression of dpp but not wg. The involvement of inhibitory interactions between wg and dpp in transcriptional regulation in the wing disc has been controversial. Penton and Hoffmann (1996) reported that the punt mutant clone, puntP62, ectopically expressed wg only in a restricted portion of the wing pouch. However, Theisen et al. (1996) reported that the pattern of wg expression in the wing disc of the punt-ts mutants is normal even if the animals are upshifted to the nonpermissive temperature, 25°C, for 70 hours BPF, while the maximal ectopic wg expression in the leg disc is seen after 40 hours at the restrictive temperature. We have shown that wg expression is expanded dorsally in the thoracic discs of puntP1/punt135, the same allelic combination as Theisen et al. used, however, we set much more severe conditions, with a temperature shift from 18 to 29°C. We suggest that transducing activity associated with Dpp signalling is reduced in the punt-ts mutant at 25°C, but it still retains partial activity to restrict the wg expression domain in the thoracic disc (Fig. 5C). At 29°C, Dpp signalling activity is reduced below a threshold level and the wg expression domain expands (Fig. 5B). dpp expression in the wing disc seems not to be regulated by Wg signalling, since the dpp expression pattern is normal in the wg-ts mutants under nonpermissive conditions (Theisen et al., 1996; Y. Tomoyasu et al., unpublished data).

Interestingly, even when high levels of tkv* were expressed in the whole thoracic disc, complete repression of wg expression was not observed. A narrow band of wg expression always remained, presumably corresponding to the lateral side of the endogenous wg expression domain (Fig. 2B). It is also worth noting that wg expression never expanded toward the lateral side in the punt-ts mutant discs (Fig. 5B and C). These results suggest that the mechanism which establishes the lateral side of the wg expression boundary may be different from that which defines the dorsal side of its expression domain. The lateral side of the wg expression domain may be regulated by a Dpp-independent mechanism.

Models for formation of the dorsocentral bristles

It appears that different thresholds of Dpp signalling are required for proneural gene induction and repression of wg expression. Dpp is secreted from the A/P border and generates a concentration gradient along the A/P axis within the thoracic disc. The schematic model shown in Fig. 7A illustrates a possible relationship between Dpp signal activity and position in the thoracic disc, along the A/P axis. This model assumes that Dpp molecules simply diffuse and generate a gradient of Dpp signal activity along the A/P axis. Presumably, low levels of Dpp still reach the most anterior region of the thoracic disc and this level of Dpp signal activity is sufficient for wg repression (Fig. 7C). The threshold levels required for proneural gene induction, on the other hand, appear to be higher. Only those cells which are located in the vicinity of both dpp and wg expression domains receive sufficient levels of both signals for proneural induction (Fig. 7C).

It is possible that alterations in Dpp signal activity cause a shift in the activities slope, with an upshift in gain of function mutants and a downshift in loss of function mutants (Fig. 7A). This model is consistent with our experimental results except for the results that were observed in the mild punt-ts mutants. According to this model, weak reduction of Dpp signal transduction activity should result in a downshift in gain of function mutants and a downshift in loss of function mutants (Fig. 7A). This model is consistent with our experimental results except for the results that were observed in the mild punt-ts mutants. According to this model, weak reduction of Dpp signal transduction activity should result in a downshift in gain of function mutants and a downshift in loss of function mutants (Fig. 7A). This model is consistent with our experimental results except for the results that were observed in the mild punt-ts mutants. According to this model, weak reduction of Dpp signal transduction activity should result in a downshift in gain of function mutants and a downshift in loss of function mutants (Fig. 7A).
in weak reduction of the wg repression area, while the proneural induction area would not be reduced. Thus, the region receiving sufficient levels of both Dpp and Wg signalling to induce proneural genes seems to have expanded, resulting in the formation of additional SMCs.

There is a substantial distance between dorsocentral SMCs and the dpp expression domain in wild-type discs (Fig. 1B). One explanation for the existence of this gap is that the highest level of Dpp signalling inhibits the formation of proneural clusters. This hypothetical Dpp signal activity is useful to explain the observation that additional dorsocentral SMCs were formed more posteriorly in the mild punt-ts discs (Fig. 5C). A down shift of the Dpp activities slope would release the area in which proneural induction is inhibited by the highest levels of Dpp signalling from such inhibition. However, there is one more paradox to the adoption of the inhibitory action of Dpp signalling. Considering this inhibitory effect in terms of the model shown in Fig. 7A, ectopic activation of Dpp signalling should have expanded the area in which proneural induction is inhibited. This was not the case for UAS-tkv*: tsh-GAL4 discs (Fig. 2B). One solution to this paradox is to alter the linear activity slope, as illustrated in Fig. 7A, to the nonlinear slope, as illustrated in Fig. 7B. The alternative model is able to simulate both phenotypes of UAS-tkv*: tsh-GAL4 and mild punt-ts without contradiction. However, it is clear that this model still includes several assumptions which may be addressed by future experiments. For instance, a concentration gradient of Dpp protein within the thoracic disc should be directly visualized. The mechanism by which the highest levels of...
Dpp signalling inhibits proneural induction is unclear and should be studied at the molecular level.

It is worth noting that the effective range of wg from its source for proneural cluster induction seem to be different to that of dpp. The dorsocentral proneural cluster is formed within approximately five cell diameters from the wg expression domain, whereas it can be formed more than ten cell diameters from the dpp source (Fig. 3A). This difference must contribute to the oval shape of the proneural cluster, which is longest along the A/P axis. wg expression is not uniform in the notal stripe, it is lower at the A/P compartment border (Fig. 1B). It is possible that the difference in wg expression levels along the A/P axis also affects the precise positioning of the dorsocentral proneural cluster.

Interaction to other genes in the macrochaete prepatternning on the notum

Recently, it has been reported that pnr, which encodes a GATA family transcription factor, and ush, which encodes a novel zinc finger protein, have a regulatory role in dorsocentral proneural cluster formation, presumably at the level of ASC gene expression (Cubadda et al., 1997; Haenlin et al., 1997). It has shown that Pnr transactivates the α-globin promoter in a cultured cell system and that Ush negatively regulates the activity of Pnr (Cubadda et al., 1997). They have not described whether or not Pnr proteins act as a transcriptional activator against DC-enhancer of ASC genes. The relation between Dpp signalling and these transcriptional regulators is largely unknown. Dpp signalling may regulate the expression or activity of these proteins. Some interesting results are also reported by Calleja et al. (1996), who have shown that wg expression is affected in pnr mutants. In pnr+/pnr+/2 discs, both are loss-of-function alleles of the pnr gene, resulting in no wg-lacZ expression in the thoracic disc. On the other hand, wg expression extends dorsally in the disc of another heteroallelic combination, pnr+/pnr-md237 (pnr-D1 seems to be a gain-of-function allele). These results suggest that pnr and ush will regulate wg expression in the thoracic disc.

Ectopic Dpp signalling also induces wg independent proneural cluster formation (Fig. 3B). It has been shown that formation of these proneural clusters depends on the activity of the iro locus. This suggests that Dpp signalling positively interacts with the products of the iro locus (Dambly-Chaudiere and Leyns, 1992; Gomez-Skarmeta et al., 1996; Gomez-Skarmeta and Modolell, 1996; Kehl et al., 1998; Leyns et al., 1996). Whether an epistatic relationship exists between dpp and iro in wg independent proneural cluster is unknown. Further studies are necessary to determine how dpp and other prepattern genes cooperate in the regulation of ac and sc in the thoracic disc.

We are grateful to K. Iizuka for help in many experiments. We thank R. Phillips, N. Warner and J. Whittle for communication of results prior to publication. We also thank J. Coleman, A. Ghysen, M. Haenlin, K. Neal and P. Simpson for comments on the manuscript, S. Cohen for Wg-antibody, H. Okano and K. Basler for tkv and punt cDNAs, respectively, S. Goto, S. Hayashi, Y. Hiromi, K. Itoh, K. Kimura, J. Modolell, R. Ueda, T. Uemura and the Bloomington Stock Center for fly stocks. This work was supported by Grants-in-Aids for scientific research from the Ministry of Education, Science, and Culture of Japan and the ‘Research for the Future’ program of the Japanese Society for the Promotion of Science.

REFERENCES


Role of dpp in macrochaete prepatterning 4223


