Hedgehog activity, independent of Decapentaplegic, participates in wing disc patterning

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SUMMARY

In the Drosophila wing imaginal disc, the Hedgehog (Hh) signal molecule induces the expression of decapentaplegic (dpp) in a band of cells abutting the anteroposterior (A/P) compartment border. It has been proposed that Dpp organizes the patterning of the entire wing disc. We have tested this proposal by studying the response to distinct levels of ectopic expression of Hh and Dpp, using the sensory organ precursors (SOPs) of the wing and notum and the presumptive wing veins as positional markers. Here, we show that Dpp specifies the position of most SOPs in the notum and of some of them in the wing. Close to the A/P compartment border, however, SOPs are specified by Hh rather than by Dpp alone. We also show that late signaling by Hh, after setting up dpp expression, is responsible for the formation of vein 3 and the scutellar region, and also for the determination of the distance between veins 3 and 4. One of the genes that mediates the Hh signal is the zinc-finger protein Cubitus interruptus (Ci). These results indicate that Hh has a Dpp-independent morphogenetic effect in the region of the wing disc near the A/P border.

Key words: Hedgehog, Decapentaplegic, Patched, Cubitus interruptus, sensory organs, wing vein, Drosophila

INTRODUCTION

The Drosophila wing primordium is first subdivided in two sets of cells that form the anterior (A) and the posterior (P) compartments (García-Bellido et al., 1973). Cell interactions at this compartment boundary induce the expression of certain gene products which have been recently demonstrated to have organizing activities (reviewed by Ingham, 1995; Lawrence and Struhl, 1996). The P compartment cells secrete the protein Hedgehog (Hh), which activates decapentaplegic (dpp), a member of the Transforming Growth Factor β (TGF-β) family of genes (Padgett et al., 1987), in a band of A cells close to the A/P compartment boundary (Masucci et al., 1990). In the remaining of the A compartment, dpp transcription is suppressed by the repressive effect of Patched (Ptc) (Basler and Struhl, 1994; Capdevila et al., 1994; Tabata and Kornberg, 1994). It has been proposed that Dpp mediates the long-range activity of Hh to organize the patterning of the wing disc (Capdevila and Guerrero, 1994; Zecca et al., 1995; Ingham and Fietz, 1995; Lecuit et al., 1996; Nellen et al., 1996), acting as a gradient morphogen (Zecca et al., 1995; Nellen et al., 1996). Also, it has been suggested that Hh may have an organizing activity that is not mediated by Dpp and could act as a short-range morphogen at the A/P compartment border (Jiang and Struhl, 1995; Li et al., 1995; Lawrence and Struhl, 1996).

In the adult, structures derived from the wing disc, like bristles and veins, can be used as references of position. The veins differentiate in precise positions of the wing (Díaz-
MATERIALS AND METHODS

Fly stocks
The UAS-dpp and UAS-hh constructs are described in Capdevila and Guerrero (1994). UAS-ptc and UAS-ci were gifts from M. Scott (Johnson et al., 1995) and K. Basler (Domínguez et al., 1996), respectively. The ptc mutants are ptc\textsuperscript{G20} (Phillips et al., 1990), ptc\textsuperscript{WH109} (Tearle and Nüsslein-Volhard, 1987) and ptc\textsuperscript{moi} (Lindsley and Zimm, 1992), neu-lacz (also called A101 by Bellen et al., 1989; Wilson et al., 1989) is a P-lacZ insertion in the neutralized locus (Boulianne et al., 1991) that marks the nuclei of the sensory mother cells (SMCs) (Huang et al., 1991). Other reporter lines were the dpp-lacZ et al., 1991) that mimics the expression of the dpp RNA (Lee et al., 1992). The temperature-sensitive hh alleles used are hh\textsuperscript{ts1} (also called hh\textsuperscript{ts} Mohler, 1988) and hh\textsuperscript{ts2} (Ma et al., 1993).

The MD-638 (M. Calleja and G. Morata, unpublished results) and pannier (pur)-GAL4 lines were generated by M. Calleja (Calleja et al., 1996). The MS-1096 and MS-209 lines (gifts from F. Jiménez and C. Parras) are described in Capdevila and Guerrero (1994). Lines C-765, en-GAL4 and 71B are a gift from A. Brand (Brand and Perrimon, 1993), dpp-GAL4 from M. Hoffman (Morimura et al., 1996), ptc-GAL4 and G455-GAL4 from Campos-Ortega and Hinz (Hinz et al., 1994) and 71ptc from M. Scott (Johnson et al., 1995). The FRT43D ptc\textsuperscript{GD} and y w; FRT43D y\textsuperscript{+} stocks were from T. Kornberg (Tabata et al., 1995). The FRT-y\textsuperscript{+}-FRT-hh transgenic flies (Basler and Struhl, 1994) and the HS-flipase stock (FLP122) (Struhl and Basler, 1993) are from G. Struhl. The FRT-y\textsuperscript{+}-FRT-dpp construct was generated in our laboratory using the tubulin promoter and the yellow gene as a cuticular marker. Two independent transgenic flies were obtained with similar levels of expression. The details of the construction are available upon request.

Ectopic expression of dpp, hh and ptc using the GAL4 system
To modify the levels of expression of the UAS construct, we took advantage of the temperature sensitivity of the GAL4 system and compared the effects of different levels of expression at distinct temperatures, using the same GAL4 line. An extra copy of UAS-dpp was also used to increase the levels of Dpp. For hh and dpp, two different UAS-dpp and UAS-hh lines were used with distinct levels of expression, UAS-dpp\textsuperscript{dpp} (UAS-dpp\textsuperscript{dpp}) and UAS-hh\textsuperscript{hh} (UAS-hh\textsuperscript{hh}).

Generation of mitotic recombinant clones
Clones of ptc mutant cells were generated by FLP-mediated mitotic recombination as described in Golic (1991) and Xu and Rubin (1993). Males of the genotype y w FLP122; FRT43D ptc\textsuperscript{GD}CyO were crossed with y w; FRT43D y\textsuperscript{+} females. FLP-mediated recombination was induced by incubating larvae of 42-60 hours after egg laying (AEL) at 37°C for 60 minutes.

Clones of adult cells expressing either dpp or hh were induced by the ‘flip-out’ system by crossing females of the genotype FLP122 to males of the genotype FRT-y\textsuperscript{+}-FRT-dpp (chromosome 1) or y/+; FRT- y\textsuperscript{+}-FRT-hh. The resultant first instar larvae were heat-shocked at 34°C for 45 minutes and clones were identified in the adult cuticle by the yellow marker.

Whole-mount in situ hybridization and immunostaining of imaginal discs
In situ hybridization was performed according to Tautz and Pfeiffle (1989) modified as in Cubas et al. (1991), using a DIG-labeled probe of the ve/rho cDNA (Sturtevant et al., 1993). Discs were mounted in Epon-Araldite (Fluka). X-Gal staining was performed following standard protocols (Ashburner, 1989). Immunofluorescence staining was performed as described in Sánchez-Herrero et al. (1996). The antibodies used were anti-En (Patel et al., 1989) at a 1/5 dilution, anti-Hh (Capdevila and Guerrero, 1994) at 1/100, anti-β-gal (Cappel) at 1/1000, anti-Ci (Slusarski et al., 1995) at 1/5, anti-Ptc (Capdevila et al., 1994) at 1/100. The discs were mounted in 4% n-propylgallate in 80% glycerol-PBS. Imaginal discs were observed under a Zeiss Laser Scan microscope.

RESULTS

Hh, but not Dpp, restores the structures of the wing lost by an excess of Ptc
Ectopic expression of either dpp or hh, gave similar but not identical transformations of the wing (Capdevila and Guerrero, 1994). In order to explore this difference further, we blocked Hh signaling with an excess of Ptc (Sampedro and Guerrero, 1991; Ingham et al., 1991; Capdevila et al., 1994) causing the loss of pattern elements (Johnson et al., 1995). By adding ectopic Hh or Dpp, we can see which structures can be recovered by each signal when Hh signaling is prevented.

In the wing disc, ectopic Ptc induced by the GAL4 system (Brand and Perrimon, 1993) has maximal effects at the A/P compartment border when driven by the 71B-GAL4 line at 29°C (Johnson et al., 1995). In this combination, the presumptive veins 3 and 4 are deleted, along with the region between them, as revealed by the absence of SOPs in L3 (Fig. 1B, compare to wild type in A) and of ve/rho expression (Fig. 1F, compare to wild type in E). We attempted to restore the pattern elements missing in 71B/UAS-ptc wing discs by adding either Dpp or Hh (see Materials and Methods). In 71B/UAS-ptc/UAS-hh\textsuperscript{hh}, ectopic Hh completely restores the A/P compartment border SMCs (Fig. 1C) and veins 3 and 4, and these discs are indistinguishable from wild type (Fig. 1E).

Using UAS-hh\textsuperscript{hh}, the higher levels of Hh inhibit the formation of vein 3 (Fig. 1G) and of the SOPs (data not shown) while restoring vein 4. By contrast, in 71B/UAS-ptc/UAS-dpp discs, ectopic Dpp does not restore either the Sc25, L3 or ACV sensilla precursors (Fig. 1D), or the presumptive veins 3 and 4 (Fig. 1H). Moreover, in 71B/UAS-dpp discs, ectopic Dpp does not affect vein 3, its associated sensilla and vein 4 (data not shown).

The effects just described in the disc cannot be studied in wings because 71B/UAS-dpp animals die as early pupae. For this purpose, we used the MD-638 line (expression pattern shown in Fig. 2B) which is viable with all the UAS constructs used. MD-638/UAS-ptc flies lack most of the wing, with the exception of proximomarginal structures (Fig. 2C compare with wild type in Fig. 2A), presumably because all genetic interactions induced by Hh are prevented by ectopic Ptc in this condition. In UAS-dpp/MD-638 flies, the uniform high levels of Dpp provoke the loss of many pattern elements like veins 2 and 5, costa and allula; however, veins 3 and 4 and the distance between them are unaffected (Fig. 2D). In UAS-dpp/UAS-ptc/MD-638 flies, most of the wing tissue lost by an excess of Ptc is recovered by Dpp (Fig. 2E). The wing obtained is similar to the UAS-dpp/MD-638 wing, except that vein 3 and its associated sensilla and vein 4 are not rescued.


Independent activities of Hh and Dpp signals organize the wing disc

By contrast, when UAS-\textit{hh} is introduced in MD-638/UAS-\textit{ptc} flies, we find recovery of the P compartment veins, ectopic induction of vein 3 and L3 sensilla and an enlargement of the region between veins 3 and 4 (Fig. 2F). Taking together the results in wings and wing discs, we conclude that Hh, but not Dpp, is able to restore the pattern elements lost at the A/P border when Hh signaling is prevented.

**Independent effects of Hh and Dpp on the induction of the SOs located close to the A/P compartment border**

To study in detail the effects of Dpp and Hh expression over the induction of SOs in different regions of the wing disc, we induced clones of cells expressing ectopically these two products (Struhl and Basler, 1993). In agreement with previous reports (Capdevila and Guerrero, 1994; Zecca \textit{et al}., 1995; Ingham and Fietz, 1995), ectopic Dpp clones duplicate most structures of the wing, but vein 3 and the distance between veins 3 and 4 are normal (Zecca \textit{et al}., 1995; Morimura \textit{et al}., 1996; this work). In the notum, the clones always give rise to extra bristles (Fig. 3A,B), except in the scutellum (Fig. 3B). Thus, the structures located at the maximum of \textit{dpp} expression are not affected by adding more Dpp.

We tested how ectopic Hh clones affected the induction of the SOs at the A/P compartment border. Since the effects of clones of ectopic expression of \textit{hh} in wing and notum are similar to those produced by the lack of \textit{ptc} function, we also studied the role of Ptc in the induction of SOs. In the wing, both types of clones produce, in the A compartment, a mirror-image duplication of wing structures (Phillips \textit{et al}., 1990; Tabata \textit{et al}., 1995; Basler and Struhl, 1994; our unpublished observations). Additionally, in ectopic \textit{hh}-expressing clones, there is always vein 3 tissue surrounding the clone (Basler and Struhl, 1994; our unpublished observations). In the scutum and prescutum, these clones do not survive, although, before they die, they cause growth and pattern alterations (Fig. 3C), probably due to the Dpp induction by Hh. By contrast, in the scutellum both \textit{ptc} clones and ectopic \textit{hh}-expressing clones survive and form extra bristles, most of them marked with \textit{yellow} (Fig. 3D,E). These results indicate that the scutellum is competent to respond to ectopic high levels of Hh without cell death, unlike the rest of the notum. This response of the scutellum and the veins contrasts with the lack of effect of ectopic Dpp clones in these structures (Fig. 3B).

It has been suggested that the lack of ectopic vein 3 in \textit{dpp}-expressing clones could be due to insufficient Dpp levels induced by the flp-out system since, in wild-type wing discs,
vein 3 is located where the Dpp levels are the highest (Zecca et al., 1995; Lawrence and Struhl, 1996). To circumvent this problem, we have used the GAL4 system to express Dpp in known areas of the wing disc at different levels (see Materials and Methods). The levels obtained of ectopic Dpp are comparable to those of the wild type because high levels of Dpp in dppd12/dppd5 mutants, which have vestigial wings, partially rescue the wing, including vein 3 (E. Sánchez-Herrero and I. G., unpublished observations).

We used lines that drive ubiquitous (like C-765; Guillén et al., 1995) or local (like MS-1096, MS-209; Capdevila and Guerrero, 1994) expression in the wing disc. The SOPs located at the A/P border are not altered by ectopic Dpp expression, except for some SOPs that correspond to the Sc12 cluster of the DR and some clusters of the VR (Fig. 4A). Thus, levels of Dpp sufficient to induce ectopic Sc12 sensilla do not affect most sensilla located close to the A/P compartment border region. The rest of the precursors far from the A/P compartment boundary of the wing pouch show a variable response depending on the ectopic Dpp levels induced, being duplicated at low levels and deleted at high levels (data not shown). In the case of the ectopic Dpp clones, we always obtain extra bristles due to the low levels of Dpp induced.

High levels of Dpp induced in its own domain with the dpp-GAL4 line do not have any effect on wing pattern (Morimura et al., 1996; our unpublished observations). By contrast, ectopic Hh induced in the same domain with either the dpp-GAL4 or the C-734 GAL4 lines gives a repetitive distribution of all the wing SOPs normally located close to the A/P compartment border (Fig. 4B), similar to that observed in some ptc mutant combinations (Phillips et al., 1990; Capdevila et al., 1994). This indicates that the effects produced by Hh at the A/P compartment border are not mediated by Dpp, but by another factor induced by Hh.

In the notum (wild type in Fig. 4G), as with flp-out clones, low levels of ectopic Dpp (induced by the C-765 GAL4 line at 17°C) give a general excess of bristles, except in the scutellum, which is located at the A/P compartment border (Fig. 4H), although very rarely we observe a duplication of the anterior SC bristles. Higher levels of ectopic Dpp in the notum (induced with the same C-765 line at 25°C (data not shown) or with pnr-GAL4 (Calleja et al., 1996)) cause disappearance of most of...
the scutum and prescutum bristles, but cannot give extra bristles in the scutellum, which is even reduced (Fig. 4I) (Morimura et al., 1996).

Meanwhile, in the scutum and prescutum, ectopic Hh produces extra bristles (data not shown), similar to the ectopic dpp expression. This is expected since dpp is activated by Hh. However, using the C-734 line, ectopic Hh also gives an overgrowth of the scutellum which is covered with bristles (Fig. 4J and B for extension of the scutellar SOPs). Raising the temperature of this combination (data not shown) or with the pnr-GAL4 line at 25°C (Fig. 4L), high levels of Hh repress almost completely the formation of the scutum and prescutum, but not that of the scutellum, which is enlarged, contrasting with the effect of Dpp (compare Fig. 4I and L). This phenotype of the scutellum is similar to that observed in hh-expressing clones, in null clones of ptc and in the ptc<sup>md1</sup>/ptc<sup>W1109</sup> mutant combination (Fig. 4K) (Phillips et al., 1990; Capdevila et al., 1994).

All these results support the previous conclusion that Hh induces elements close to the A/P border and this effect is not mediated by Dpp, although its presence may also be needed.

Effects of ectopic Ci on induction of SOs and wing growth

One of the targets of the Hh-Ptc signal transduction cascade is the gene <i>cubitus interruptus</i> (ci), which encodes a zinc finger protein (Orenic et al., 1990). Ci renders A cells competent to respond to Hh and mediates the transduction of this signal (Domínguez et al., 1996; Alexandre et al., 1996). Although ci is uniformly transcribed in the A compartment, the blocking of Ptc by Hh in cells near the compartment boundary results in the accumulation of high levels of Ci in a band of cells abutting the A/P compartment border (Motzny and Holmgren, 1995; Slusarski et al., 1995; Sánchez-Herrero et al., 1996; Domínguez et al., 1996; Alexandre et al., 1996). These high
levels coincide with the position of those SOPs that are specifically activated by Hh. Thus, Cî is a candidate to be an effector gene in promoting development of vein 3 SOs by Hh.

We used an UAS-cî construct (Domínguez et al., 1996) to study the effect of ectopic expression of Cî and found an effect on SOPs distribution similar to that observed by induction of Hh expression. Thus, when using the MD-638 line (Fig. 2B), the L3 SOPs extend into the A compartment (Fig. 4D). We also observed extra growth of the region between veins 3 and 4, slightly displacing vein 3 anteriorly and inhibiting the
formation of vein 4 (data not shown). This inhibition of vein 4 formation is like that observed in ci mutants that show ectopic expression of Ci in the P compartment (Slusarski et al., 1995).

**The specific role of Hh in positioning the vein 3 coincides with the late activation of en in anterior compartment cells**

In wild-type discs, ci and en are expressed in cells of the A and P compartments, respectively, although, in mature third instar discs, their expression domains overlap in a region between the presumptive vein 3 and the A/P compartment border (Blair, 1992). This late en expression in the A compartment is supposed to be specifically induced by Hh (Guillén et al., 1995; de Celis and Ruiz-Gómez 1995). When blocking the Hh signal by an excess of Ptc, the late en expression in the A compartment (Fig. 5A) does not occur (Fig. 5B) and it is not recovered by adding Dpp (Fig. 5C). Thus, this en activation is a good example of a Dpp-independent Hh signaling.

We took advantage of this late en expression to study the effect of Hh on cells close to the A/P compartment border by using the en-GAL4 line. In en-GAL4/UAS- hhts the ‘anterior en-expressing region’ is expanded (Fig. 5D). This anterior en expression does not induce endogenous hh transcription in wild-type or mutant discs (Fig. 5A,D), probably due to a direct repression of hh by Ci in the A compartment (Domínguez et al., 1996). Ci expression overlaps with En in this region (Fig. 5E), but the maximum levels of Ci protein are moved more anteriorly, abutting the region of En expression (Fig. 5E,G,I). By contrast, maximum levels of Ptc largely coincide with those cells expressing En (Fig. 5G,H). We think that high levels of Hh would induce strong expression of ptc, whereas low levels would induce high levels of Ci. At the end of the third larval instar, the band of dpp expression is severely reduced in the wild type (data not shown). In UAS-hh-lacZ discs, dpp is broadly expressed in early stages, but disappears in the en-expressing region at prepupal stages (Fig. 5F). Thus, in the wild type, the late en expression in the A compartment could be of functional significance in the repression of dpp expression at the A/P compartment border.

The shift of maximal levels of Ci to a more anterior region observed in en-GAL4/UAS- hhts discs (Fig. 5E,G,I) seems to be correlated with the displacement of vein 3 to a more anterior position in the wing (Fig. 6A-C). Accordingly, high levels of Ci coincide with vein 3 localization in the wild type (see scheme in Fig. 6E,F). If we express lower levels of Hh by changing the temperature or using a weaker UAS-hh line, we observe that the sensilla campaniformia of vein 3 are also lacking (unpublished observations). Thus, the Hh-Ptc interaction in the A/P border that occurs late in larval development is responsible for the location of vein 3 and the formation of the scutellar region through a Dpp-independent signal.

**DISCUSSION**

Dpp signal is not the only organizer of the A/P axis in the wing disc

It has been proposed that Dpp may be a long-range morphogen that patterns the wing. In this model, the pattern is established by a gradient of Dpp with its source in the band of cells transcribing dpp close to the A/P compartment border in the A compartment. The graded concentration of Dpp would specify the type, sequence and spacing of the veins and SO in both compartments (Zecca et al., 1996; Nellen et al., 1996; reviewed in Lawrence and Struhl, 1996). However, in all the experiments that we and others have made (Capdevila and Guerrero, 1994; Zecca et al 1995; Ingham and Fietz, 1995; this work), the ectopic expression of Dpp can duplicate all veins except vein 3 and its associated sensilla.

To test the hypothesis that Dpp is the sole organizer of the A/P coordinates, we have used the local induction of SOPs and vein precursors as markers, when Dpp is expressed. As demonstrated in this work, the levels of ectopic Dpp induced in our GAL4 experiments were comparable to or higher than those at the A/P compartment border in wild type. However, the specification of SOs at the A/P compartment border, the spacing between veins 3 and 4 and the scutellar region are not much affected by varying levels of Dpp. Our results also suggest that the SOPs that are far away from the A/P compartment border depend on Dpp levels for proper induction or repression. This induction is an early effect that promotes formation of proneural clusters and not lateral inhibition, because the expression of ace/sc is like that of the neu-lacZ line (data not shown). Thus, Dpp would establish the A/P coordinates at which some proneural clusters will arise. These findings support the hypothesis of Dpp acting as a long-range morphogen, except in cells close to the A/P compartment boundary, precisely those located at the highest point of the Dpp gradient.

Recently, it has been reported that some genes are activated in large central domains of the wing disc in response to Dpp, subdividing the wing into different regions (de Celis et al., 1996; Grimm and Pflugfelder, 1996; Lecuyt et al., 1996; Nellen et al., 1996). These downstream genes, spalt (sal), spalt-related (sal-r) and optomotor-blind (omb) have expression patterns that extend to both sides of the A/P compartment border. However, sal and sal-r mutant clones affect veins 2 and 5, but do not affect veins 3 and 4 (de Celis et al., 1996), and cells lacking omb still differentiate vein 3 (Grimm and Pflugfelder, 1996). These results are in accordance with our data, since vein 3, its associated sensilla and vein 4 are not affected by ectopic Dpp. Although, Dpp organizes the pattern and growth of the disc during larval development (Burke and Basler, 1996a), it probably does not have a function in defining, later in third instar larvae, the pattern of the structures near the A/P compartment border. In spite of these results, we cannot rule out a requirement for Dpp, in cooperation with another factor, to
Fig. 5. Hh modulates the response of effector genes at the A/P border. (A-D) En expression is in red and hh expression in green. (A) Wild-type wing pouch. Bracket indicates the ‘anterior en-expressing region’. (B) 71B/UAS-pter wing pouch. Arrowhead shows the loss of en anterior expression. (C) 71B/UAS-pter/UAS-dpp wing pouch. Arrowhead shows that the en anterior expression is not recovered by ectopic Dpp. Note the enlargement of the wing pouch by the effect of Dpp. (D-I) en-GAL4/UAS-hh wing pouch. Arrowhead shows the ‘anterior en-expressing region’ is enlarged (bracket) compared to wild type in A. Endogenous hh expression is not induced in the A compartment by the late en expression, either in wild type (A) or in mutant discs (D); Ci is probably repressing activation of hh. (E) Overlapping of En (green) and Ci (red) expression domains in the A compartment. (F) dpp and En expression patterns. dpp expression (green) is displaced anteriorly and outside the en-expressing region. Also, the early induction of dpp next to the A/P compartment border (asterisk) may be repressed by En (red). (G,H) Ci (red) and (G,I) Ptc (green) overlapping patterns. The highest levels of Ptc are in the en-expressing region (H), whereas the peak of Ci abuts this region anteriorly (arrow in I shows how Ci peak has been displaced anteriorly).

Fig. 6. Ectopic Hh in the anterior en-expressing domain alters the scutellum and the location of vein 3. (A,B) en-GAL4/UAS-hh flies incubated at 17°C. (A) Displacement of vein 3 anteriorly with an increase of the distance between veins 3 and 4. (B) Detail of the wing in which the arrows show where the L3 sensillae are located, having been pushed more anteriorly, but not as far as the vein 3. (C,D) en-GAL4/UAS-hh flies incubated at 25°C. (C) Wing with vein 3 displaced even further (thick arrow). This vein almost disappears and the L3 sensillae are also absent. The empty arrow shows the duplication of the costa produced by the ectopic Dpp induced by Hh. (D) Notum with duplicated SC bristles (thick arrows). The wing and notum phenotypes shown in this figure are similar to the ptc+/pter/W1109 phenotypes (Fig. 4K). (E,F) Schematic drawing of the location of vein 3 and 4 related to Ci (green) and En (red) expressions in wild-type wing (E) and in en-GAL4/UAS-hh wing (F). The region of overlapping between Ci and En (‘anterior en-expressing region’) is shown by coloured stripes indicating that the protein levels are lower than in their respective domains. The ‘anterior en-expressing region’ is enlarged in en-GAL4/UAS-hh flies because of the higher concentrations of Hh there. The position of vein 3 is displaced along with the peak of Ci concentration.
Hh acts as a short-range organizer

We demonstrate in this work some morphogenetic effects of Hh that do not depend on Dpp. The structures affected by this function of Hh, namely vein 3, its associated sensilla and the scutellum, are very close to the source of Hh (the P compartment) indicating a short-range activity of Hh. This Hh activity is also seen in experiments with protein kinase A (pkA) dpp null mutant clones. In pkA− clones, dpp and ci, but not hh, are derepressed autonomously anywhere in the A compartment of the wing disc, giving the cells of the clone an identity similar to that of the cells close to the A/P compartment border (Jiang and Struhl, 1995; Li et al., 1995; Lepage et al., 1995; Pan and Rubin, 1995). dpp− pkA− clones suppress most of the pkA− clonal phenotype, but they still show an increase in SOs and cause local bifurcation of veins (Jiang and Struhl, 1995; Li et al., 1995). These results suggest a signaling mechanism for patterning of the wing that does not depend on Dpp. Furthermore, since vein 3 is induced non-autonomously in pkA− clones, another secreted signal, which is neither Dpp (this work) nor Hh (Jiang and Struhl, 1995; Li et al., 1995), must be responsible for this phenotype. We called this putative signal factor X in the scheme of Fig. 7.

In vertebrates, one of the homologues of Hh, Sonic hedgehog (Shh), can also act as a morphogen independently of the Dpp homologue BMP-2, specifying either floor plate or motor neuron fate (reviewed in Johnson and Tabin, 1995). During limb patterning, Shh can mimic the Zone of Polarizing Activity and induces the expression of BMP-2, in adjacent regions. However, BMP-2 does not seem to mediate the role of Shh (Francis et al., 1994) and Shh could also be acting as a morphogen signal in this system. This function of Hh as a morphogen has been already demonstrated in the Drosophila embryo in patterning of the dorsal epidermis (Heemskerk and DiNardo, 1994). Also, in the morphogenesis of the adult structures, a Dpp-independent role of Hh has been found. Thus, Hh, but not Dpp, plays a major role in furrow progression, in ommatidial development (Burke and Basler, 1996b) and in early oogenesis (Forbes et al., 1996). These parallel results support our proposal of a Dpp-independent morphogenetic role of Hh in the wing.

Interactions at the A/P compartment border

The interactions at the A/P compartment border are mediated by Ptc and directed by the concentration of secreted Hh diffusing into the A compartment (Capdevila et al., 1994; Tabata and Kornberg, 1994). The segment polarity genes that mediate the Hh signal transduction pathway demarcate the A/P coordinates at which the proneural clusters and the position of veins will arise. Here, we have shown that the lack of Ptc mimics the effects of ectopic expression of hh in the induction of SOs. We have also demonstrated that by blocking the interactions at the A/P compartment border with an excess of Ptc protein, the wing is not formed, since the signals responsible for growth and pattern are not induced. By adding ectopic Dpp, which acts as one of these signals, most of the wing tissue is restored, but not all the wing structures. Similarly, in the notal region, the scutellum (located at the A/P compartment border of the notum) cannot be restored by adding Dpp (data not shown). In both cases, however, Hh protein fully restores the missing structures. These results support the hypothesis that the specification of the scutellar region, vein 3, its associated sensilla and the space between veins 3 and 4 are a consequence of Hh-Ptc interactions mediated by Dpp-independent signals. This conclusion is supported by the results obtained with temperature-sensitive alleles of hh. When hhts alleles are shifted to the restrictive temperature at the end of the third instar, dpp expression is not affected, but the scutellum and the distance between veins 3 and 4 are reduced. This late gene interactions at the A/P border are revealed by the late expression of en in some cells of the A compartment close to the A/P compartment border (Blair, 1992) induced by high levels of Hh. This En invasion of the A compartment was also blocked by an excess of Ptc and can not be re-established by Dpp.

The zinc finger protein encoded by the segment polarity gene ci is a good candidate to be the transcriptional activator of this pathway in the A compartment, since the levels of Ci protein are positively modulated at the A/P compartment border by the Hh signal (Metz and Holmgren, 1995; Johnsson et al., 1995; Alexandre et al., 1996; Domínguez et al., 1996; Sánchez-Herrero et al., 1996). Ci will in turn inhibit hh expression in the A compartment (Domínguez et al., 1996). The late induction of en expression in the A compartment seems to regulate the levels of Ci. This is shown by the lower levels of Ci in the cells co-expressing en, which give rise to the region between vein 3 and the A/P compartment border. The higher levels of Ci would locate vein 3 and SOs close to the A/P compartment border. Therefore, Ci is one of the mediators of the described morphogenetic effect of Hh.

We propose (Fig. 7) that late in development, the Hh signal, acting as a short-range organizer, is responsible for both growth and the specification of fine pattern elements in the region close to the A/P compartment border of the wing disc. This late morphogenetic effect of Hh does not depend on Dpp alone,
although Dpp could also be required somehow. Thus, the Hh signal results in long-range effects through the activity of Dpp and short-range effects through another signal (called factor X in Fig. 7), both activated by Ci.

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