Development 126, 2891-2899 (1999)
Printed in Great Britain © The Company of Biologists Limited 1999
DEV5219

Patterning of *Drosophila* leg sensory organs through combinatorial signaling by Hedgehog, Decapentaplegic and Wingless

Rebecca Hays, Kathryn T. Buchanan, Carrie Neff* and Teresa V. Orenic‡

Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, 2153 Sheridan Road, Evanston, IL 60208, USA

*Present address: Department of Molecular and Cell Biology, University of California at Berkeley, 401 Barker Hall, Berkeley, CA, 94720, USA

‡Author for correspondence (e-mail: tmo773@nwu.edu)

Accepted 14 April; published on WWW 7 June 1999

SUMMARY

During development, global patterning events initiate signal transduction cascades which gradually establish an array of individual cell fates. Many of the genes which pattern *Drosophila* are expressed throughout development and specify diverse cell types by creating unique local environments which establish the expression of locally acting genes. This process is exemplified by the patterning of leg microchaete rows. *hairy* (*h*) is expressed in a spatially restricted manner in the leg imaginal disc and functions to position adult leg bristle rows by negatively regulating the proneural gene *achaete*, which specifies sensory cell fates. While much is known about the events that partition the leg imaginal disc and about sensory cell differentiation, the mechanisms that refine early patterning events to the level of individual cell fate specification are not well understood. We have investigated the regulation of *h* expression along the dorsal/ventral (D/V) axis of the leg adjacent to the anterior/posterior (A/P) compartment boundary and have found that it requires input from both D/V and A/P patterning mechanisms. Expression of the D/V axis *h* stripe (D/V-*h*) is controlled by dorsal- and ventral-specific enhancer elements which are targets of Decapentaplegic (Dpp) and Wingless (Wg) signaling, respectively, but which are also dependent on Hedgehog (Hh) signaling for activation. D/V-*h* expression is lost in *smoothened* mutant clones and is specifically activated by exogenously supplied Cubitus interruptus (Ci). D/V-*h* expression is also lost in clones deficient for Dpp and Wg signaling, but ectopic activation of D/V-*h* by Dpp and Wg is limited to the A/P compartment boundary where endogenous levels of full-length Ci are high. We propose that D/V-*h* expression is regulated in a non-linear pathway in which Ci plays a dual role. In addition to serving as an upstream activator of Dpp and Wg, Ci acts combinatorially with them to activate D/V-*h* expression.

Key words: *hairy*, leg imaginal discs, *hedgehog*, *cubitus interruptus*, *wingless*, *decapentaplegic*, *Drosophila melanogaster*

INTRODUCTION

The patterning of *Drosophila* occurs through a hierarchy of regulatory events which gradually refine spatial organization to the level of individual cell fate specification. Early acting genes establish the position-specific expression of late acting genes which generate diverse cell fates by creating unique local environments. The mechanisms which establish precise, spatially-restricted expression of locally acting genes, however, are not well understood. Here, we address this question using sensory organ development as a model system.

In the *Drosophila* adult, each sensory structure is derived from a single precursor cell, the sensory mother cell (SMC), in a multi-step selection process. First, early patterning events establish expression of the proneural genes *achaete* (*ac*) and *scute* (*sc*), which act locally to confer neural competence to clusters of cells (reviewed by Campuzano and Modolell, 1992; Jan and Jan, 1993). Then, local regulatory events controlled by neurogenic genes select cells from within a proneural cluster to become SMCs and give rise to individual sensory organs (reviewed by Artavanis-Tsakonis and Simpson, 1991; Campos-Ortega, 1993).

Our focus is on the events that organize the microchaete sensory bristles of the *Drosophila melanogaster* leg. It has previously been shown that the longitudinal microchaete rows of the leg are patterned in part through the function of the *hairy* (*h*) gene (Orenic et al., 1993), which encodes a basic helix-loop-helix (bHLH) protein (Rushlow et al., 1989) and is expressed in a spatially restricted manner in the leg imaginal disc (Fig. 1B) (Carroll and Whyte, 1989). In wing imaginal discs, *h* negatively regulates *ac* to pattern the bristle primordia (Cubas et al., 1991; Skeath and Carroll, 1991; Cubas and Modolell, 1992) by binding directly to DNA sequences that govern *ac* expression (Ohsako et al., 1994; Van Doren et al., 194). H also negatively regulates *ac* expression in the leg imaginal disc. In the everted leg, *h* is expressed in four longitudinal stripes (Carroll and Whyte, 1989), each of which is flanked by Ac stripes. In the absence of H function, *ac* expression expands into the domain normally occupied by H, resulting in disorganized bristle rows and ectopic bristle formation (Orenic et al., 1993).

Thus, *h* acts as a link between early global and late local patterning events in the leg imaginal disc. Understanding how *h* expression is spatially regulated in the leg will provide a valuable opportunity to view this developmental process more completely, from the early patterning events that partition the imaginal disc, to those which specify sensory cell fates and direct bristle development and their precise placement.

We have investigated the regulation of *h* expression along the dorsal/ventral (D/V) axis of the leg adjacent to the anterior/posterior (A/P) compartment boundary and find that it requires input from both A/P and D/V patterning mechanisms. Separate dorsal and ventral leg-specific enhancer elements integrate signals from the Hedgehog (Hh), Decapentaplegic (Dpp), and Wingless (Wg) pathways to establish *h* expression along this axis. Cubitus interruptus (Ci) plays a dual role in this pathway. In addition to its requirement as an upstream activator of Dpp and Wg, Ci functions combinatorially with them to activate D/V axis *h* expression.

MATERIALS AND METHODS

Fly strains

Fly lines carrying D/V-*h*-*lacZ* reporters (this work) in combination with 30A-GAL4 (Brand and Perrimon, 1993), *optomotor-blind* (*omb*)-GAL4 (Grimm and Pflugfelder, 1996), UAS-*ci* (Hepker et al., 1997), UAS-*dpp* (Staehling-Hampton and Hoffman, 1994), UAS-*wg* (Hays et al., 1997), and UAS-CiZn/EnRD (Hepker et al., 1997) constructs were prepared by standard genetic methods. *CiZn/EnRD* encodes a functionally dominant-negative Ci. *dpp* hypomorphs were of the genotype dpp^{d6}/dpp^{d12}. dpp^{d6} and dpp^{d12} are regulatory mutants which reduce *dpp* expression in the distal regions of imaginal discs resulting in duplication of *wg* expression and the transformation of dorsal cell fates to ventral fates (Held et al., 1994). *wg* hypomorphs were of the genotype wg^{CX3}/wg^{CX4}. wg^{CX3} is a pupal lethal allele, and wg^{CX4} (Baker, 1988) is null. Wg is present in normal spatial distribution but at reduced levels in these mutants, resulting in ventral duplication of *dpp* expression and transformation of ventral cell fates to dorsal fates. The smo^{IIG26} (van den Heuvel and Ingham, 1996), wg^{CX4}, and $Mad^{1.2}$ (Wiersdorff et al., 1996) alleles were used for clonal analysis. smo^{IIG26} is a null mutation and $Mad^{1.2}$ is strong hypomorph.

Loss-of-function clones

smo mutant clones were made in flies of the genotype *y* hsflp-1/+;smo^{IIG26} FRT40A/hsp-70πmyc36F FRT40A. *Mad wg* doubly mutant clones were made in flies of the genotype *y* hsflp-1/+;$Mad^{1.2}$ wg^{CX4} FRT40A/hsp-70πmyc21C hsp-70πmyc36F FRT40A. Clones were generated by heat shocking larvae for 1 hour at 37˚C. Prior to dissection, animals were heat shocked for 1 hour at 37˚C to induce *myc* expression, and allowed to recover for 1 hour.

Immunohistochemistry and microscopy

Imaginal discs from larvae or pupae were treated according to the methods of Carroll and Whyte (1989) and labeled with antibodies against Ci (Motzny and Holmgren, 1995), β-galactosidase (Zhang et al., 1994), H (Carroll and Whyte, 1989), Dpp (Panganiban et al., 1990), or Myc (Xu and Rubin, 1993). Stainings were visualized on a BioRad MRC Lasersharp Confocal system.

Identification of leg enhancers that direct *h* expression along the D/V axis

Previous genetic studies had placed the *h* longitudinal leg stripe

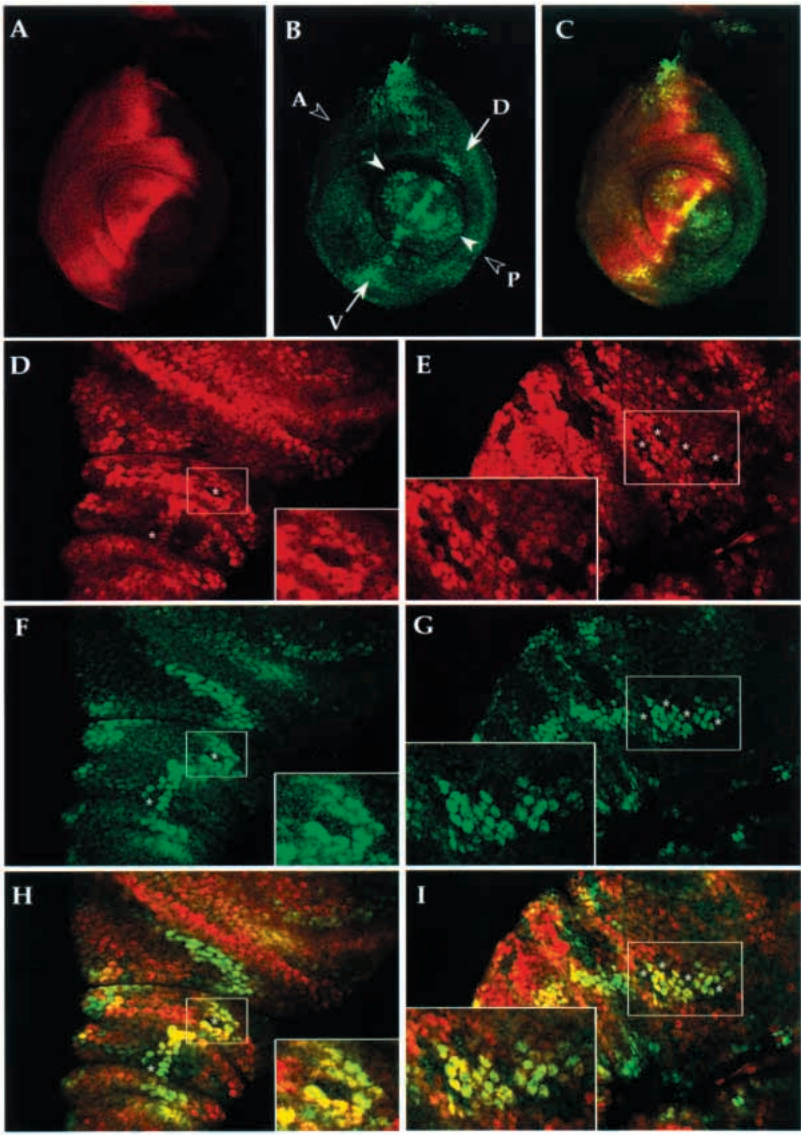

Fig. 1. D/V-*h* is a target of Hh signaling. (A-C) D/V-*h* expression overlaps with high-level, full-length Ci along the A/P compartment boundary. All discs are oriented such that dorsal is up and anterior is to the left. Third instar leg disc doubly labeled for full-length Ci (red) and H (green). At this stage, *h* expression is seen as one stripe along the D/V axis (B, arrows) and two patches along the A/P axis of the distal leg segments (B, arrowheads). After disc eversion, the D/V axis H stripe forms two of the four longitudinal stripes of the leg and the A/P axis patches form five circumferential stripes at the first through fifth tarsal segments. The remaining two longitudinal stripes along the A/P axis do not appear until after disc eversion. Their ultimate position is indicated by open arrowheads in B. (C) Merge of images in A and B. Co-localization of Ci and H appears yellow. (D,F,H) V-*h* stripe in a 2- to 3-hour APF leg disc doubly labeled for Myc (red) and H (green). smo^{IIG26} clones are marked by the loss of Myc. (H) Merge of images in D and F. V-*h* expression is lost in small *smo* clones in a strictly cell autonomous manner (asterisks). (E,G,I) D-*h* stripe in a 2- to 3-hour APF leg disc doubly labeled for Myc (red) and H (green). *smo* clones are marked by the loss of Myc. (I) Merge of images E and G. D-*h* expression is also lost cell-autonomously in small *smo* clones (asterisks).

enhancers a minimum of 14 kb 3′ of the *h* transcription start site (Orenic et al., 1993). Genomic sequences 14-50 kb 3′ of the transcriptional start were cloned from a lambda phage library (Tamkun et al., 1992) and subcloned as 8-9 kb fragments (Fig. 3) into HspCasper, which carries the basal promoter of hsp70 fused to the bacterial *lacZ* gene (Nelson and Laughon, 1993). These constructs were introduced into flies by P-element-mediated germline transformation (Rubin and Spradling, 1982) and tested for the presence of leg-specific enhancers by assessment of their capacity to direct β-gal expression in the *h* leg stripe domains. D/V-*h*-*lacZ* refers to the reporter construct carrying the intact D/V axis stripe enhancer. D-*h*-*lacZ* and V-*h*-*lacZ* refer to reporter constructs carrying isolated dorsal and ventral leg stripe enhancers, respectively.

RESULTS

D/V axis *h* is a target of Ci-mediated Hh signaling

In the third instar leg imaginal disc, *h* is expressed along both the D/V and A/P axes. D/V axis expression appears as a single stripe in the anterior compartment of the disc immediately adjacent to the A/P compartment boundary (Fig. 1B, arrows), while A/P axis expression appears as two wedge-shaped blocks in the distal leg segments on either side of the A/P compartment boundary (Fig. 1B, arrowheads). After disc eversion, the D/V axis stripe forms two of the four longitudinal leg stripes and the A/P axis expression forms five circumferential stripes at the first through fifth tarsal segments. The remaining two longitudinal stripes will be positioned along the A/P axis, intersecting the D/V axis stripes at the distal tip of the leg. These stripes do not appear until 2-3 hours after puparium formation (APF). Their ultimate position is indicated by open arrowheads in Fig. 1B.

The expression pattern of the D/V axis *h* stripe (D/V-*h*) is highly reminiscent of that of known Hh target genes, so we examined constituents of the Hh signal transduction pathway as potential regulators of the stripe. Hh is secreted from the posterior compartment of imaginal discs and influences gene expression in anterior compartment cells (Tabata and Kornberg, 1994). Ci, a zinc finger transcription factor, is expressed throughout the anterior compartment (Eaton and Kornberg, 1990; Motzny and Holmgren, 1995) and is thought to mediate Hh signaling by direct transcriptional regulation of Hh target genes (Alexandre et al., 1996;

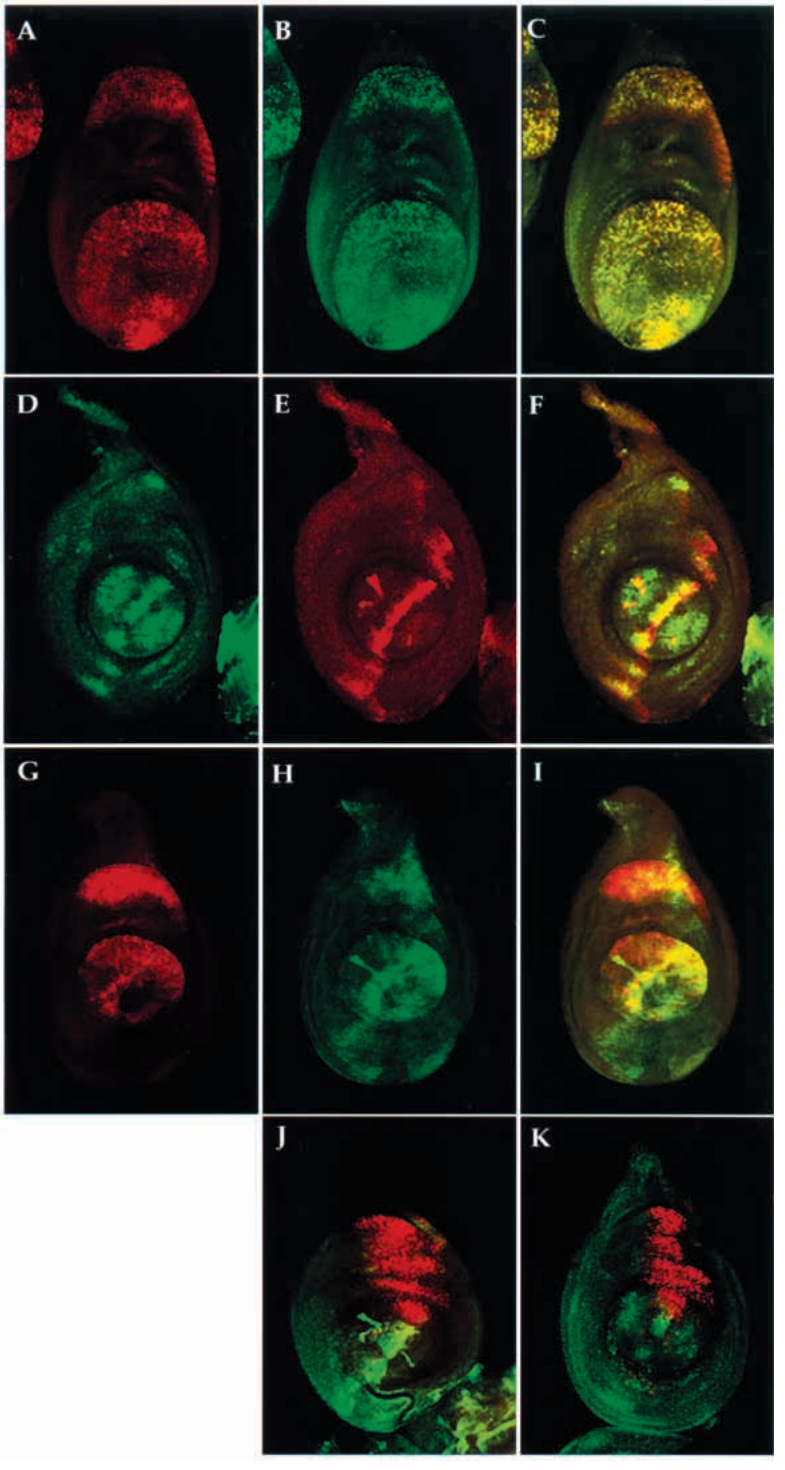

Fig. 2. Ci positively regulates D/V-*h* expression. (A-C) Third instar leg doubly labeled for Ci (red) and H (green). (C) Merge of images in A and B. In the leg disc, the 30A-GAL4 insertion is expressed dorsally in cells of the presumptive femur, in the distal tip of the leg, and in a central ring corresponding to the fifth tarsal segment. Misexpression of a *ci* transgene under the control of 30A-GAL4 results in ectopic expression of endogenous *h* throughout the 30A expression domain. (D-F) A 9 kb genomic fragment 30-40 kb 3′ of the *h* transcription unit contains the D/V-*h* enhancer sequences. Third instar leg carrying D/V-*h*-*lacZ* reporter construct doubly labeled for H (green) and β-gal (red). (F) Merge of images in D and E. The D/V-*h*-*lacZ* stripe superimposes on the endogenous D/V-*h* stripe. (G-I) Ci positively regulates D/V-*h*-*lacZ*. Third instar D-*h*-*lacZ* leg doubly labeled for Ci (red) and β-gal (green). (I) Merge of images in G and H. Misexpression of a *ci* transgene under the control of 30A-GAL4 results in ectopic expression of D/V-*h*-*lacZ* in the posterior compartment of the leg disc and areas of the anterior compartment near the A/P boundary. (J-K) Expression of D/V-*h* and D/V-*h*-*lacZ* are impeded by a dominant-negative Ci. Merged images of third instar legs doubly labeled for CiZn/EnRDmyc (red) and D/V-*h*-*lacZ* (green; J) or endogenous H (green; K). The dorsal portions of D/V-*h*-*lacZ* and D/V-*h* are absent due to the presence of CiZn/EnRDmyc.

Dominguez et al., 1996; Hepker et al., 1997; Von Ohlen et al., 1997). In response to Hh signaling, high levels of full-length Ci accumulate in a stripe along the A/P compartment boundary (Johnson et al., 1995; Aza-Blanc et al., 1997; Hepker et al., 1997) (Fig. 1A) where it is required for activation of the Hh target genes *dpp* and *wg* (Alexandre et al., 1996; Dominguez et al., 1996; Hepker et al., 1997) among others. Away from the A/P boundary the predominant Ci species is a proteolytically processed form which lacks its C terminus but retains its DNA binding domain (Aza-Blanc et al., 1997). This truncated protein has been postulated to function as a repressor of Hh target gene expression in vivo. Hh signaling blocks proteolysis of Ci in cells along the A/P boundary (Aza-Blanc et al., 1997), allowing accumulation of the full-length form. The region of high-level, full-length Ci overlaps with D/V-*h* (Fig. 1A-C), making Ci a strong candidate for regulation of this H stripe.

We investigated the influence of Hh on *h* expression by generating somatic clones which lack functional Smoothened (Smo), a cell surface receptor required for response to Hh signaling. Anterior compartment cells that lack Smo function do not respond to Hh signaling and thus do not express Hh target genes (van den Heuvel and Ingham, 1996). Our data show that within *smo* clones, both dorsally and ventrally, D/V-*h* expression is lost in a cell autonomous manner (Fig. 1D-I), implicating Hh signaling in D/V-*h* regulation.

We extended this analysis to Ci, the only known transcriptional mediator of Hh signaling, by assaying the expression of *h* in legs misexpressing full-length Ci. In the leg disc, the 30A-GAL4 insertion is expressed dorsally in cells of the presumptive femur, in the distal tip of the leg, and in a central ring corresponding to the fifth tarsal segment (Figs 2A,G, 6G). Misexpression of a *ci* transgene under control of the 30A-GAL4 driver results in ectopic expression of *h* throughout the 30A expression domain (Fig. 2A-C), suggesting that Ci can serve as a positive regulator of *h*.

It is impossible to know, however, the identity of ectopic H observed in these experiments. That is, whether or not it is of D/V stripe origin. *h* expression in the leg is quite elaborate and dynamic and is most certainly under the control of multiple *cis*-regulatory elements. Therefore, in order to assay independently the response of D/V-*h* to Ci, we isolated the leg-specific enhancer elements which govern the expression of D/V-*h* and cloned them into a *lacZ* reporter vector. A 9 kb genomic fragment 30-40 kb 3′ of the *h* transcription start site contains sequences that direct β-gal expression in the D/V-*h* stripe domain (Fig. 3). We refer to this reporter construct as D/V-*h*-*lacZ*. The D/V-*h*-*lacZ* stripe lies in the anterior compartment of the disc adjacent to the compartment boundary and superimposes with endogenous H protein (Fig. 2D-F). As with endogenous D/V-*h*, D/V-*h*-*lacZ* expression is lost in *smo* mutant clones (not shown), suggesting that this enhancer element is a target of Hh signaling.

Misexpression of *ci* with the 30A-GAL4 driver results in ectopic expression of D/V-*h*-*lacZ*, throughout the 30A domain (Fig. 2G-I), though the pattern differs somewhat from that seen with endogenous H. D/V-*h*-*lacZ* is consistently expressed at higher levels in the posterior compartment of the leg and in areas of the anterior compartment near the A/P boundary (Fig. 2H). Anterior compartment levels are considerably lower, though not below the limits of detection. This effect is not observable with endogenous H due to the presence of circumferential H stripes in the anterior compartment (Fig. 1B, arrowhead). Nevertheless, this confirms the specific activation of D/V-*h* expression by exogenously supplied Ci.

In addition to ectopic activation by Ci, a dominant-negative form of Ci (Hepker et al., 1997) impedes expression of D/V-*h*. *CiZn/EnRD* expressed dorsally along the A/P boundary under the control of *omb*-GAL4 completely eliminates expression of the dorsal component of D/V-*h* (Fig. 2K) and of D/V-*h*-*lacZ* (Fig. 2J). These data suggest that D/V-*h* is a target of Hh signaling and that Ci likely mediates the Hh signal to activate D/V-*h*.

D/V-*h* expression is directed by separate Dpp and Wg responsive elements

The D/V-*h* enhancer sequences are separable into discrete dorsal and ventral components which we refer to as D-*h* and V-*h*, respectively (Figs 3, 4A,D). Both of these elements are responsive to Ci. Leg discs in which UAS-*ci* is driven by 30A-GAL4 show ectopic expression from D-*h*-*lacZ* and V-*h*-*lacZ* identical to that of the full D/V-*h*-*lacZ* (not shown). The separability of these enhancers suggests that D/V-*h* expression is regulated by dorsal- and ventral-specific factors, such as Dpp and Wg, and not by Ci alone. Dpp and Wg specify dorsal and ventral leg fates, respectively (Held, 1995), and have been shown to antagonize each other's function to maintain dorsal and ventral leg territories (Brook and Cohen, 1996; Jiang and Struhl, 1996; Johnston and Schubiger, 1996; Morimura et al., 1996; Penton and Hoffman, 1996; Theisen et al., 1996). In the absence of one signaling molecule, expression of the other and its target genes expands, resulting in the duplication of dorsal or ventral leg structures.

To assess the roles of Dpp and Wg in the regulation of the D-*h* and V-*h* enhancer elements, we assayed the expression of D-*h*-*lacZ* and V-*h*-*lacZ* in legs that were mutant for either *dpp* or *wg*. Expression from D-*h*-*lacZ* is reduced in leg discs that

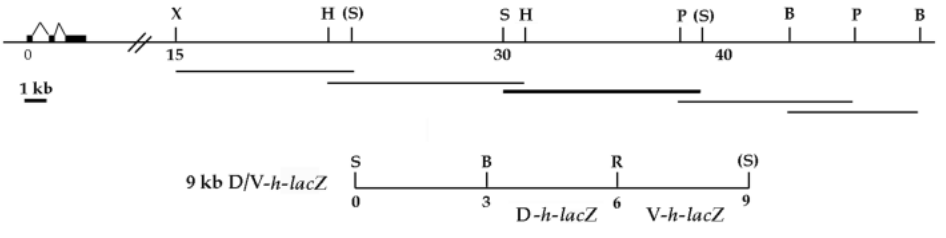

Fig. 3. Map of *h* locus showing the position of the D/V axis *h* leg enhancers. Genomic sequences 3′ of the *h* transcription unit were subcloned into an HspCasper vector as 8-9 kb fragments and introduced into flies by germline transformation. *Sal* (S) sites shown in parenthesis are artificial. The 9 kb *Sal* fragment shown in bold contains D/V axis *h* leg enhancers which direct the expression of a *lacZ* reporter in the D/V axis *h* leg stripe domain. A more detailed map of this fragment is shown below. The D/V-*h* stripe enhancer can be divided into dorsal and ventral elements which map to contiguous 3 kb genomic fragments. The expression pattern of the 6 kb S-R fragment does not differ from that of the 3 kb B-R fragment. X, *Xho*I; H, *Hin*dIII; P, *Pst*I; B, *Bam*HI; R, *Eco*RI.

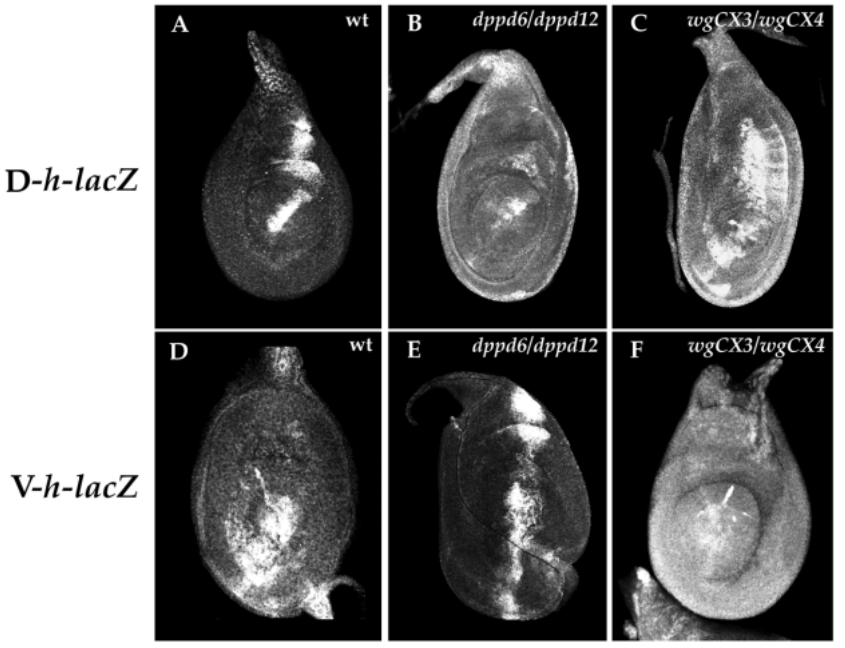

Fig. 4. Separate D/V-*h* enhancer elements are responsive to Dpp and Wg signaling. (A) Wild-type expression of D-*h*-*lacZ*. (B) D-*h*-*lacZ* in a dpp^{d6}/dpp^{d12} leg disc. Decreased Dpp function severely reduces expression of the D-*h*-*lacZ* reporter as compared to the wild-type. (C) D-*h*-*lacZ* expression in a wg^{cx3}/wg^{cx4} leg disc. In response to ventral duplication of Dpp in wg^{cx3}/wg^{cx4} mutant legs, D-*h*-*lacZ* expression is duplicated to reproduce a full-length D/V stripe. (D) Wild-type expression of V-*h*-*lacZ*. (E) V-*h*-*lacZ* expression in a dpp^{d6}/dpp^{d12} leg disc. In response to dorsal duplication of Wg, V-*h*-*lacZ* is duplicated to produce a full-length D/V stripe. (F) V-*h*-*lacZ* expression in a wg^{cx3}/wg^{cx4} leg disc. Reduced Wg activity severely reduces expression of the V-*h*-*lacZ* reporter as compared to the wild-type.

are mutant for *dpp* (Fig. 4B), and is expanded to produce a full D/V axis stripe in discs that are mutant for *wg* (Fig. 4C). Conversely, V-*h*-*lacZ* expression is severely reduced in *wg* mutant discs (Fig. 4F), and duplicated in *dpp* mutant discs (Fig. 4E). Gain-of-function experiments are in agreement with these results. Misexpression of *dpp* or *wg* along the A/P boundary under the control of *dppBlink*-GAL4 (Staehling-Hampton and Hoffman, 1994) expand the dorsal and ventral stripes, respectively, to produce full-length D/V axis stripes (not shown). These findings are in keeping with what has been demonstrated for other Dpp and Wg target genes and suggest that the D-*h* and V-*h* enhancers are targets of Dpp and Wg signaling, respectively. D/V-*h*-*lacZ* was also analyzed in *dpp* and *wg* mutant discs, but no effect was observable in the background of the full D/V axis stripe (not shown).

We further examined the roles of Dpp and Wg in D/V-*h* regulation by making somatic clones lacking components of the Dpp and Wg signaling pathways. Given the antagonism between Dpp and Wg in the leg, it was necessary to analyze clones mutant for a component of each pathway. For example, dorsal cells which are deficient in Dpp signaling ectopically express *wg* (Brook and Cohen, 1996; Jiang and Struhl, 1996; Johnston and Schubiger, 1996; Morimura et al., 1996; Penton and Hoffman, 1996; Theisen et al., 1996). Endogenous *h* expression assayed in such cells appears unaffected due to the duplication of V-*h* expression (not shown, see results Fig. 4). Use of the isolated dorsal element in this background eliminates the duplication effect and shows loss of the D-*h*-*lacZ* stripe (not shown), but it is impossible to determine whether this results from the loss of Dpp signaling, or from repression of Dpp targets by ectopic Wg within the clone. The reverse scenario applies to V-*h*. Thus, in order to fairly assess the effects of loss of these patterning signals on D/V-*h* expression, we must examine doubly mutant clones which are deficient for an element of each pathway.

We opted to assay D-*h* expression in clones mutant for *wg* and *Mothers against dpp* (*Mad*). Mad is a downstream effector in the Dpp signaling pathway which has been shown to bind DNA and transcriptionally regulate some Dpp target genes directly (Kim et al., 1997; Szuts et al., 1998). Mad is thought to be essential for all Dpp signaling (Raftery et al., 1995; Newfeld et al., 1996; Wiersdorff et al., 1996; Newfeld et al., 1997), and has been shown to be required for antagonism of Wg (Lecuit and Cohen, 1997). We find that dorsal *Mad wg* clones which intersect the D-*h* stripe show loss of *h* expression (Fig. 5A-C), except for variable low level expression in a single row of cells immediately adjacent to the A/P boundary (Fig. 5B, inset). We can reasonably conclude that loss of D-*h* results from the loss of Mad, since there is no duplicated Wg in these clones. These results not only support the finding that D-*h* is a target of Dpp signaling, it identifies Mad as a potential transcriptional regulator of D-*h*.

We also assayed V-*h* expression in *Mad wg* clones to determine the requirement for Wg signaling without the potential for repression of V-*h* by Dpp. While the non-autonomy of Wg is not an issue in the dorsal region of the leg, it must be considered when assaying ventral *h* expression. We therefore generated very large *Mad wg* clones in order to minimize rescue by Wg, and consistently observed loss of V-*h* expression in the interior of the clones (Fig. 5D-F). This confirms the involvement of Wg signaling in V-*h* regulation. *h* expression is variably rescued 2-3 cell diameters into ventral clones (Fig. 5F, arrows), and cells directly adjacent the A/P boundary show variable low level *h* expression (not shown), similar to that seen with D-*h*. Small *Mad wg* clones show complete rescue of V-*h* expression, as expected (not shown).

Dpp and Wg regulate D/V-*h* in a Ci-dependent fashion

The above findings are consistent with a linear pathway for D/V-*h* regulation in which Ci-mediated Hh signaling activates expression of *dpp* and *wg*, which in turn activate *h*. The response of D/V-*h* and D/V-*h*-*lacZ* to misexpressed *ci* and dominant-negative *ci* could be explained by the effects of Ci on Dpp and Wg alone. By this model, misexpression of *dpp* and *wg* are predicted to elicit the same response from D-*h*-*lacZ*

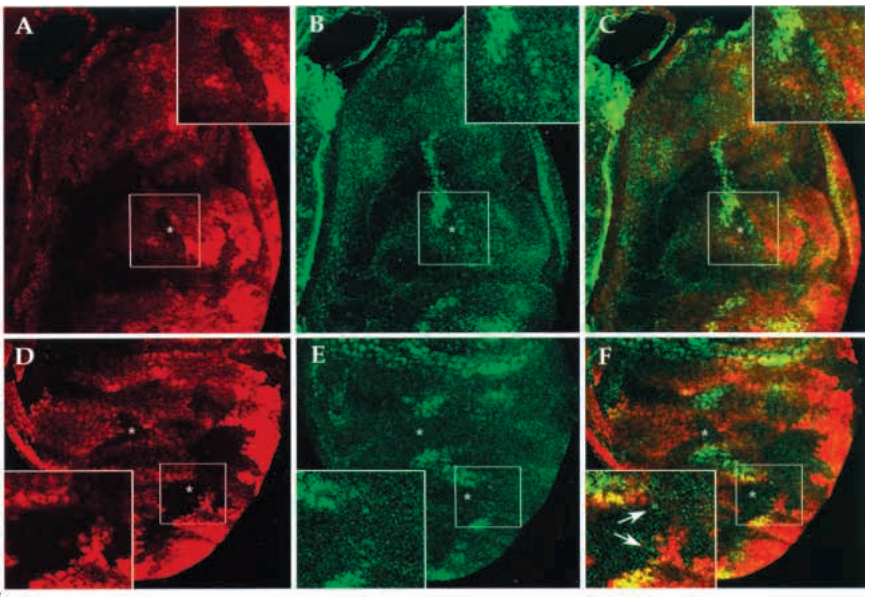

Fig. 5. D/V-*h* expression is lost in *Mad wg* mutant clones. Everting legs doubly labeled for Myc (red) and H (green). *Mad wg* clones are marked by loss of Myc. (A-C) Dorsal H stripe in a leg 3-4 hours APF. (D-F) Ventral H stripe in a leg 1-2 hours APF. D/V-*h* expression is lost both dorsally and ventrally in *Mad wg* clones (asterisks, insets) except for variable low level expression in a single row of cells immediately adjacent to the A/P boundary (B, inset), and non-autonomous rescue by Wg 2-3 cell diameters into ventral clones (F inset, arrows).

and V-*h*-*lacZ*, respectively, as Ci does. We tested this by misexpressing *dpp* and *wg* using the 30A-GAL4 driver and comparing the results to those obtained with Ci. We found that Dpp and Wg do elicit ectopic expression of the reporters, but only in a subset of cells within the 30A-GAL4 domain. Ectopic β-gal is expressed only at the intersection of 30A-GAL4 with the A/P compartment boundary (Fig. 6). In the case of D-*h*-*lacZ*, this appears as a ventral expansion of the stripe (Fig. 6A), and in the case of V-*h*-*lacZ* it appears as a dorsal expansion of the stripe (Fig. 6D). Neither reporter is activated in the posterior compartment of the disc where response to exogenously supplied Ci is the strongest, and neither is activated a significant distance away from the A/P boundary in the anterior compartment where they are responsive to Ci, albeit at low levels. Double labeling of Ci and β-gal in these discs shows clearly that ectopic β-gal is limited to the anterior compartment and lies within the region of elevated full-length Ci (Fig. 6C,F). These results suggest that the response of *h* to Ci is not solely via Dpp and Wg, but that Dpp and Wg induced activation of D/V-*h* is Ci-dependent. When *ci* is misexpressed, ectopic Dpp or Wg results (Basler and Struhl, 1994; Alexandre et al., 1996; Hepker et al., 1997), providing all of the required activators at high levels and activating the D/V stripe elements throughout the 30A-GAL4 expression domain. When *dpp* or *wg* are misexpressed, however, levels of full-length Ci are limiting, and ectopic activation of D-*h* and V-*h* is restricted to areas of the anterior compartment near the A/P boundary where endogenous levels of full-length Ci are high.

Further evidence for the dual requirement of Ci in this pathway comes from the observation that loss of D/V-*h* expression in *smo* clones is strictly cell autonomous (Fig. 1D-

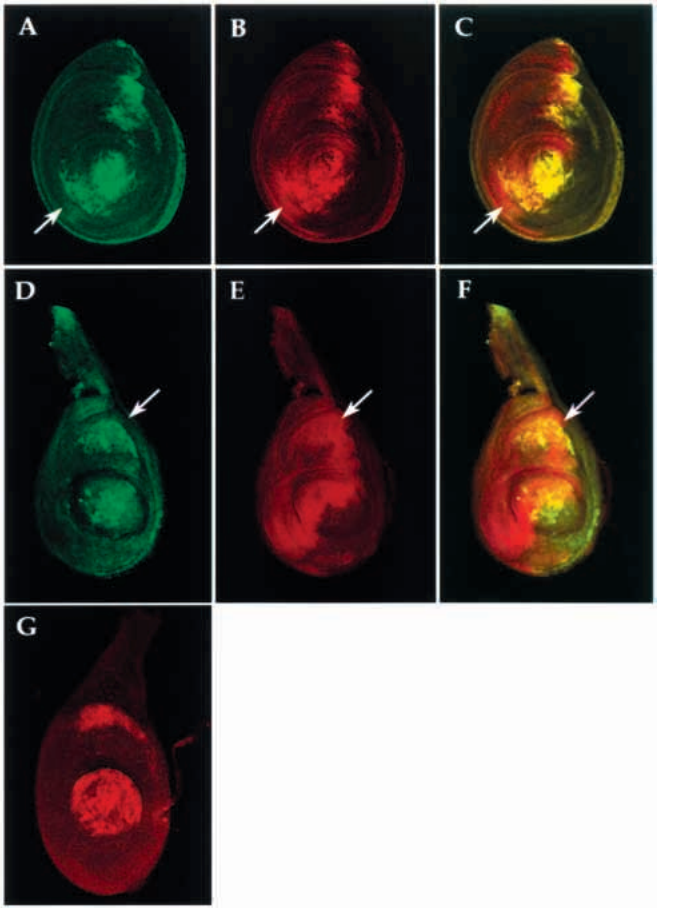

Fig. 6. Ci is acts combinatorially with Dpp and Wg to activate D/V axis *h* expression. (A-C) D-*h*-*lacZ*;30A-GAL4;UAS-*dpp* leg disc doubly labeled for β-gal (A) and Ci (B). (C) Merge of images in A and B. Co-localization of Ci and β-gal appears yellow. D-*h*-*lacZ* is ectopically expressed in response to *dpp* (arrow), but is limited to an area near the A/P boundary within the full-length Ci stripe. *dpp* does not activate D/V-*h*-*lacZ* in the posterior compartment, where induction by exogenously supplied Ci is maximal. (D-F) V-*h*-*lacZ*;30A-GAL4;UAS-*wg* leg disc doubly labeled for β-gal (D) and Ci (E). (F) Merge of images in D and E. V-*h*-*lacZ* is ectopically expressed dorsally along the A/P boundary within the full-length Ci stripe (arrow). The most ventral portion of the endogenous V-*h*-*lacZ* stripe is not visible due to overwhelmingly high levels of ectopic β-gal expression. (G) 30A-GAL4;UAS-*dpp* leg labeled for Dpp demonstrating the full compliment of 30A-GAL4 expression domain.

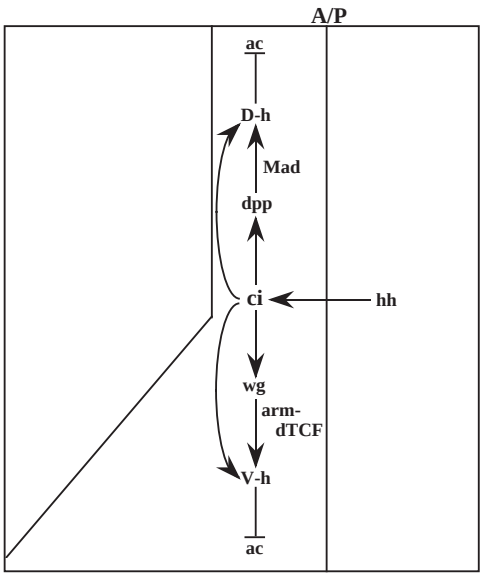

Fig. 7. Model of D/V-*h* regulation. Ci acts combinatorially with Dpp and Wg to regulate D/V-*h*. Hh signaling from the posterior compartment establishes a stripe of full-length Ci along the A/P compartment boundary which is required for the expression of *dpp* and *wg*. Ci acts together with Dpp and Wg to regulate D-*h* and V-*h* expression, respectively. Repression of *ac* expression by H positions the D/V axis sensory microchaete rows.

I). If Ci were only required upstream of Dpp and Wg in this pathway, we would expect *h* expression within small *smo* clones to be non-autonomously rescued by Dpp and Wg. In fact, we see no rescue at all within the clones. This differs from results we obtained in the eye imaginal disc. In the developing eye, *h* is expressed in a stripe anterior to the morphogenetic furrow where it functions to regulate the rate of furrow progression and neuronal differentiation (Brown et al., 1995). It has been shown that expression of the *h* eye stripe is dependent on Dpp signaling from within the morphogenetic furrow. *h* expression is activated anterior to the furrow by long range Dpp signaling, and repressed within the furrow by high level Dpp signaling (Heberlein et al., 1995). As in the leg, Hh signaling in the eye disc activates the expression of *dpp* (Heberlein et al., 1993; Ma et al., 1993; Royet and Finkelstein, 1997). We have observed that small *smo* clones in the eye show no loss or attenuation of *h* expression (not shown), consistent with non-autonomous rescue of *h* expression by Dpp. The absence of D/V-*h* rescue in small *smo* clones in the leg reveals a dependence on Hh signaling separate from its role as an upstream activator of *dpp* and *wg*.

We have also examined activation of V-*h*-*lacZ* by Armadillo (Arm)-dTCF, the only known transactivator of Wg target genes (Brunner et al., 1997; van de Wetering et al., 1997). Misexpression of a constitutively active Arm driven by 30A-GAL4 shows the same restricted ectopic β-gal observed in response to misexpression of *wg*. Dorsal expression of V-*h*-*lacZ* is limited to areas within the full-length Ci stripe along the A/P compartment boundary (not shown). This corroborates the finding that V-*h* activation is responsive to Wg signaling and that its activation by Wg is Ci-dependent.

DISCUSSION

D/V-*h* expression is regulated by the integrated signals of Hh, Dpp, and Wg

Our results show that the positioning of a longitudinal H stripe along the D/V axis of the *Drosophila* leg requires input from both A/P and D/V patterning mechanisms (Fig. 7). Independent dorsal and ventral *cis*-regulatory elements at the *h* locus integrate Hh, Dpp and Wg signals to establish D/V-*h* expression and allow for the precise placement of sensory bristles in the adult leg. Through loss- and gain-of-function experiments we show that the D-*h* and V-*h* enhancer elements require multiple signals for the activation of *h* expression. Wg or Dpp signaling alone is insufficient for activation of D/V-*h* and D/V-*h*-*lacZ* as demonstrated by the loss of *h* and *lacZ* expression in small *smo* clones at the A/P boundary which fail to be rescued by Dpp or Wg (Fig. 1, not shown). Neither is Hh signaling alone sufficient for wild-type activation of D/V-*h* as seen by the loss of *h* expression in *Mad wg* clones (Fig. 5). D/V-*h* can respond solely to Hh signal if the level of signaling is very high, as both dorsal and ventral *Mad wg* clones occasionally show *h* expression in a single row of cells at the A/P boundary. This expression is variable and low level, however, and is not representative of wild-type D/V-*h* expression.

D/V-*h* is ectopically expressed throughout the 30A-GAL4 domain in response to misexpressed *ci* (Fig. 2), but ectopic activation by Dpp and Wg is limited to areas of the disc near the A/P boundary, where levels of full-length Ci are high (Fig. 6). Again, this demonstrates a requirement for multiple signals at the D/V-*h* enhancers for *h* expression along this axis of the leg. The low level response of D/V-*h*-*lacZ* to exogenously supplied Ci in the anterior compartment of the leg could be due to antagonism by truncated Ci present in this compartment or to the fact that it is beyond the range of high level Hh signaling. The same phenomenon has been observed with other Hh target genes (Hepker et al., 1997). Recall that it is not possible to observe this effect on endogenous *h* expression due to the presence of the circumferential H stripes in the anterior compartment of the disc (See Results, compare Figs. 1B, 2B, 2H).

Our data suggest that Ci, Mad and Arm-dTCF may be the mediators of the Hh, Dpp, and Wg signals at the D-*h* and V-*h* cis-regulatory elements. However, the conclusions we draw from analysis at this level are strictly genetic. It is entirely possible that the effects we observe are indirect, and that D/V-*h* is downstream of other Ci, Dpp and Wg target genes. Molecular analysis of the D/V-*h* enhancer elements is necessary to identify the transactivators of D-*h* and V-*h* expression. Nonetheless, our current findings clearly demonstrate a convergence of the Hh, Dpp and Wg pathways with respect to D/V-*h* regulation.

Independent stripe elements may allow for stripe refinement and differential spatial regulation of D/V-*h*

These findings address two important aspects of D/V-*h* regulation: the definition of a stripe at the A/P boundary, and differential spatial regulation of the dorsal and ventral components of the stripe. The dual requirement for Ci in this pathway may provide a mechanism for refinement of the D/V-

h stripe, which is narrower than the stripes of full-length Ci, Dpp and Wg. The truncated form of Ci present in areas away from the A/P boundary has been postulated to function as a transcriptional repressor (Aza-Blanc et al., 1997), and may compete with full-length Ci for DNA binding sites within target gene promoters. Changes in the relative levels of full-length and truncated Ci, then, may be an important determinant for the positioning of this H stripe.

Separation of the D/V-*h* enhancer sequences into two elements may allow for differences in spatial regulation of *h* in the dorsal and ventral regions of the leg. Late in development, the dorsal and ventral components of D/V-*h* are shifted relative to each other and to the compartment boundary. In the third instar leg disc, the entire D/V-*h* stripe directly abuts the A/P compartment boundary. In the pupal leg, however, the dorsal component of this stripe lies far enough away from the boundary to accommodate an *ac* stripe in the anterior compartment, while the ventral portion of the stripe remains directly adjacent the A/P boundary (Orenic et al., 1993).

Regulation of D/V-*h*: establishing missing links

The precise spatial alignment of sensory organs in the leg of the *Drosophila* adult is a consequence of the positioning of *ac* stripes during pupal leg development. A key regulator of *ac* is *h*, which functions to establish one boundary of each *ac* stripe. We show that D/V axis *h* is a target of the global patterning regulators Hh, Dpp and Wg. Thus H is a critical link between global and local patterning events, and an understanding of its regulation will reveal much about this aspect of sensory organ development.

These results allow us to begin to dissect the hierarchy of interactions that control the positioning of leg sensory organs. Our findings are remarkably consistent with those of Gomez-Skarmeta and Modolell (Gomez-Skarmeta and Modolell, 1996) who have identified Araucan (Ara) and Caupolican (Caup) as positive regulators of the proneural genes *ac* and *sc*. *ara-caup* is expressed on either side of the D/V boundary in the *Drosophila* wing near the A/P compartment boundary and is required for the development of the prospective vein L3 and associated sensory organs through the activation of *rhomboid*/*veinlet* and *ac*/*sc*. *ara-caup* expression is activated by combinatorial signaling by Hh and Dpp signaling. Thus, like H, Ara-Caup acts as a link between global and local patterning events.

Several questions remain open regarding the placement of leg sensory organs. There is much to be learned about the regulation of the eight Ac leg stripes. In the absence of H function, *ac* is expressed in four broad stripes which may represent four zones that are competent to express *ac*. The expression of *ac* in these zones could be under the control of independent *cis*-regulatory elements as are D-*h* and V-*h*. The D/V axis stripes would likely be regulated by high level Dpp and Wg signaling. The A/P axis stripes could be sensitive to low levels of Dpp and Wg signaling. This may also be the case for the A/P-*h* stripes, whose regulation is also not yet understood. Alternatively, the whole leg may be competent to express *ac* with the interstripes established by H and an unknown factor that represses the expression of *ac* in the four non-H-expressing interstripe regions.

We are very grateful to R. Holmgren for many fruitful discussions and insights. We also thank S. Carroll, in whose lab this work was initiated, for discussion and suggestions during the early stages of this project. Many thanks to S. Blair and A. Laughon for fly stocks. We thank R. Holmgren, J. Skeath, J. Hepker, and Q. Wang and H. Dierick for critical reading of this manuscript. This work was supported by NSF, grant IBN-9404878. R. Hays was supported in part by a Gramm Fellowship award from the Lurie Cancer Center of Northwestern University.

REFERENCES

Alexandre, C., Jacinto, A. and Ingham, P. W. (1996). Transcriptional activation of *hedgehog* target genes in Drosophila is mediated directly by the *Cubitus interruptus* protein, a member of the the GLI family of zinc finger DNA-binding proteins. *Genes Dev.* **10**, 2003-2013.

Artavanis-Tsakonis, S. and Simpson, P. (1991). Choosing a cell fate: A view from the Notch locus. *Trends Genet.* **7**, 403-408.

Aza-Blanc, P., Ramirez-Weber, F. A., L., M. P., Schwartz, C. and Kornberg, T. B. (1997). Proteolysis that is inhibited by *hedgehog* targets cubitus interruptus protein to the nucleus and converts it to a repressor. *Cell* **89**, 1043-1053.

Baker, N. (1988). Transcription of the segment-polarity gene wingless in the imaginal discs of *Drosophila*, and the phenotype of a pupal-lethal *wg* mutation. *Development* **102**, 489-497.

Basler, K. and Struhl, G. (1994). Compartment boundaries and the control of *Drosophila* limb pattern by *hedgehog* protein. *Nature* **368**, 208-214.

Brand, A. H. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415.

Brook, W. J. and Cohen, S. M. (1996). Antagonistic interactions between wingless and decapentaplegic responsible for dorsal-ventral pattern in the *Drosophila* leg. *Science* **273**, 1373-1377.

Brown, N., Sattler, C., Paddock, S. and Carroll, S. (1995). Hairy and emc negatively regulate morphogenetic furrow progression in the Drosophila eye. *Cell* **80**, 879-887.

Brunner, E., Peter, O., Schweizer, L. and Basler, K. (1997). pangolin encodes a Lef-1 homologue that acts downstream of Armadillo to transduce Wingless signal in Drosophila. *Nature* **385**, 829-833.

Campos-Ortega, J. (1993). Early neurogenesis in *Drosophila melanogaster*. In The development of *Drosophila melanogaster*. (ed. C. Bate and A. Martinez-Arias), pp. 109-1129. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Campuzano, S. and Modolell, J. (1992). Patterning of the *Drosophila* nervous system: the *achaete-scute* gene complex. *Trends Genet.* **8**, 202-208.

Carroll, S. B. and Whyte, J. S. (1989). The role of the *hairy* gene during *Drosophila* morphogenesis: stripes in imaginal discs. *Genes Dev.* **3**, 905-916.

Cubas, P., de Celis, J.-F., Campuzano S. and Modolell, J. (1991). Proneural clusters of *achaete scute* expression and the generation sensory organs in the *Drosophila* imaginal wing discs. *Genes Dev.* **5**, 996-1008.

Cubas, P. and Modolell, J. (1992). The extramacrochaetae gene provides information for sensory organ patterning. *EMBO J.* **11**, 3385-3393.

Dominguez, M., Brunner, M., Hafen, E. and Basler, K. (1996). Sending and receiving the Hedgehog signal: control by the *Drosophila* Gli protein Cubitus interruptus. *Science* **272**, 1621-1625.

Eaton, S. and Kornberg, T. (1990). Repression of ci^D in posterior compartments of *Drosophila* by *engrailed*. *Genes Dev.* **4**, 1068-1077.

Gomez-Skarmeta, J. L. and Modolell, J. (1996). *araucan* and *caupolican* provide a link between compartment subdivisions and patterning of sensory organs and veins in the Drosophila wing. *Genes Dev.* **10**, 2935-2945.

Grimm, S. and Pflugfelder, G. O. (1996). Control of the gene *optomotor-blind* in *Drosophila* wing development by *decapentaplegic* and *wingless*. *Science* **271**, 1601-1604.

Hays, R., Gibori, G. and Bejsovec, A. (1997). *wingless* signaling generates epidermal pattern through two distinct mechanisms. *Development* **124**, 3727-3736.

Heberlein, U., Singh, C., Luk, A. and Donohoe, T. (1995). Growth and differentiation in the *Drosophila* eye coordinated by *hedgehog*. *Nature* **373**, 709-711.

Heberlein, U., Wolff, T. and Rubin, G. (1993). The TGFβ homolog *dpp* and

the segment polarity gene *hedgehog* are required for propagation of a morphogenetic wave in the *Drosophila* retina. *Cell* **75**, 913-926.

Held, L. I. (1995). Axes, boundries, and coordinates: the ABCs of fly leg development. *BioEssays* **17**, 721-732.

Held, L. I., Heup, M. A., Sappington, M. and Peters, S. (1994). Interactions of *decapentaplegic*, *wingless*, and *Distal-less* in the *Drosophila* leg. *Roux's Arch. Dev.* **203**, 310-319.

Hepker, J., Wang, Q. T., Motzny, C. K., Holmgren, R. A. and Orenic, T. (1997). *Drosophila cubitus interruptus* forms a negative feedback loop with *patched* and regulates expression of Hedgehog target genes. *Development* **124**, 549-558.

Jan, Y. and Jan, L. (1993). HLH proteins, fly neurogenesis, and vertebrate myogenesis. *Cell* **75**, 827-830.

Jiang, J. and Struhl, G. (1996). Complementary and mutually exclusive activities of decapentaplegic and wingless organize axial patterning during *Drosophila* leg development. *Cell* **86**, 401-409.

Johnson, R. L., Grenier, J. K. and Scott, M. (1995). *patched* overexpression alters wing disc size and pattern: transcriptional and post-transcriptional efects on *hedgehog* targets. *Development* **121**, 4161-4170.

Johnston, L. A. and Schubiger, G. (1996). Ectopic expression of *wingless* in imaginal discs interferes with *decapentaplegic* expression and alters cell determination. *Development* **122**, 3519-3529.

Kim, J., Johnson, K., Chen, H., Carroll, S. and Laughon, A. (1997). *Drosophila* Mad binds to DNA and directly mediates activation of *vestigial* by Decapentaplegic. *Nature* **388**, 304-308.

Lecuit, T. and Cohen, S. (1997). Proximal-distal axis formation in the Drosophila leg. *Nature* **388**, 139-145.

Ma, C., Zhou, Y., Beachy, P. and Moses, K. (1993). The segment polarity gene *hedgehog* is required for progression of the morphogenetic furrow in the developing Drosophila eye. *Cell* **75**, 927-938.

Morimura, S., Maves, L., Chen, Y. and Hoffman, M. (1996). *decapentaplegic* overexpression affects *Drosophila* wing and leg imaginal disc development and *wingless* expression. *Dev. Biol.* **177**, 136-151.

Motzny, C. K. and Holmgren, R. (1995). The Drosophila cubitus interruptus protein and its role in the *wingless* and *hedgehog* signal transduction pathways. *Mech. Dev.* **52**, 137-150.

Nelson, H. B. and Laughon, A. (1993). Glial architecture and development in analysis using a collection of new cell-specific markers. *Drosophila* embryo. *Roux's Arch. Dev.* **202**, 341-354.

Newfeld, S., Chartoff, E., Graff, J., Melton, D. and Gelbart, W. (1996). Mothers against dpp encodes a conserved cytoplasmic protein required in DPP/TGF-beta responsive cells. *Development* **122**, 2099-2108.

Newfeld, S., Mehra, A., Singer, M., Wrana, J., Attisano, L. and Gelbart, W. (1997). Mothers against dpp participates in a DDP/TGF-beta responsive serine-threonine kinase signal transduction cascade. *Development* **124**, 3167-76.

Ohsako, S., Hyer, J., Panganiban, G., Oliver, I. and Caudy, M. (1994). Hairy function as a DNA-binding helix-loop-helix repressor of *Drosophila* sensory organ formation. *Genes Dev.* **8**, 2743-2755.

Orenic, T. V., Held, L. I., Jr., Paddock, S. W. and Carroll, S. B. (1993). The spatial organization of epidermal structures: *hairy* establishes the geometrical pattern of *Drosophila* leg bristles by delimiting the domains of *achaete* expression. *Development* **118**, 9-20.

Panganiban, G., Reuter, R., Scott, M. P. and Hoffman, F. M. (1990). A Drosophila growth factor homolog, *decapentaplegic*, regulates homeotic gene expression within and across germ layers during midgut morphogenesis. *Development* **110**, 1041-1050.

Penton, A. and Hoffman, M. (1996). Decapentaplegic restricts the domain of *wingless* during *Drosophila* limb patterning. *Nature* **382**, 162-165.

Raftery, L., Twombly, V., Wharton, K. and Gelbart, W. (1995). Genetic screens to identify elements of the decapentaplegic signaling pathway in Drosophila. *Genetics* **139**, 241-54.

Royet, J. and Finkelstein, R. (1997). Establishing primordia in the *Drosophila* eye-antennal imaginal disc: the roles of *decapentaplegic*, *wingless* and *hedgehog*. *Development* **124**, 4793-4800.

Rubin, G. and Spradling, A. (1982). Genetic transformation of Drosophila with transposable element vectors. *Science* **218**, 348-353.

Rushlow, C. A., Hogan, A., Pinchin, S. M., Howe, K. M., Lardelli, M. and Ish-Horowicz, D. (1989). The *Drosophila* hairy protein acts in both segmentation and bristle patterning and shows homology to N-myc. *EMBO J.* **8**, 3095-3103.

Skeath, J. B. and Carroll, S. B. (1991). Regulation of *achaete-scute* gene expression and sensory organ pattern formation in the *Drosophila* wing. *Genes Dev.* **5**, 984-995.

Staehling-Hampton, K. and Hoffman, F. M. (1994). Ectopic decapentaplegic in the Drosophila midgut alters the expression of five homeotic genes, *dpp*, and *wg*, causing specific morphological defects. *Dev. Biol.* **164**, 502-512.

Szuts, D., Eresh, S. and Bienz, M. (1998). Functional intertwining of Dpp and EGFR signaling during *Drosophila* endoderm induction. *Genes Dev.* **12**, 2022-2035.

Tabata, T. and Kornberg, T. B. (1994). Hedgehog is a signaling protein with a key role in patterning Drosophila imaginal discs. *Cell* **76**, 89-102.

Tamkun, J., Deuring, R., Scott, M., Kissinger, M., Pattatucci, A., Kaufman, T. and Kennison, J. (1992). brahna: A regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell* **68**, 561-572.

Theisen, H., Haerry, T. E., O'Conner, M. B. and Marsh, L. (1996). Developmental territories created by mutual antagonism between wingless and decapentaplegic. *Development* **122**, 3939-3948.

van de Wetering, M., Cavallo, R., Dooijes, D., van Beest, M., van Es, J., Loureiro, J., Ypma, A., Hursh, D., Jones, T., Bejsovec, A., Peifer, M., Mortin, M. and Clevers, H. (1997). Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene dTCF. *Cell* **88**, 789-799.

van den Heuvel, M. and Ingham, P. W. (1996). *smoothened* encodes a receptor-like serpentine protein required for *hedgehog* signalling. *Nature* **382**, 547-551.

Van Doren, M., Baily, A. M., Esnayra, J., Ede, K. and Posakony, W. (1994). Negative regulation of proneural gene activity: *hairy* is a direct transcriptional repressor of *achaete*. *Genes Dev.* **8**, 2729-2742.

Von Ohlen, T., Lessing, D., Nusse, R. and Hooper, J. (1997). Hedgehog signaling regulates transcription through *cubitus interruptus*, a sequence-specific DNA binding protein. *Proc. Natl. Acad. Sci. USA* **94**, 2404-2409.

Wiersdorff, V., Lecuit, T., Cohen, S. and Mlodzik, M. (1996). Mad acts downstream of Dpp receptors, revealing a differential requirement for dpp signaling in initiation and propagation of morphogenesis in the Drosophila eye. *Development* **122**, 2153-2162.

Xu, T. and Rubin, G. M. (1993). Analysis of genetic mosaics in developing and adult *Drosphila* tissues. *Development* **117**, 1223-1237.

Zhang, Y., Ungar, A., Fresquez, C. and Holmgren, R. (1994). Ectopic expression of either the *Drosophila gooseberry-distal* or *proximal* gene causes alterations of cell fate in the epidermis and central nervous system. *Development* **120**, 1151-1161.