Hedgehog directly controls initiation and propagation of retinal differentiation in the *Drosophila* eye

María Domínguez[1,2,3] and Ernst Hafen[1]

[1]Zoological Institute, University of Zürich, CH-8057 Zürich, Switzerland

Patterning of the compound eye begins at the posterior edge of the eye imaginal disc and progresses anteriorly toward the disc margin. The advancing front of ommatidial differentiation is marked by the morphogenetic furrow (MF). Here we show by clonal analysis that Hedgehog (Hh), secreted from two distinct populations of cells has two distinct functions: It was well documented that Hh expression in the differentiating photoreceptor cells drives the morphogenetic furrow. Now we show that, in addition, Hh, secreted from cells at the posterior disc margin, is absolutely required for the initiation of patterning and predisposes ommatidial precursor cells to enter ommatidial assembly later. These two functions of Hh in eye patterning are similar to the biphasic requirement for Sonic Hh in patterning of the ventral neural tube in vertebrates. We show further that Hh induces ommatidial development in the absence of its secondary signals Wingless (Wg) and Dpp and that the primary function of Dpp in MF initiation is the repression of *wg*, which prevents ommatidial differentiation. Our results show that the regulatory relationships between Hh, Dpp, and Wg in the eye are similar to those found in other imaginal discs such as the leg disc despite obvious differences in their modes of development.

[*Key Words: Drosophila* eye; morphogenetic furrow initiation; hedgehog signaling]

Received August 18, 1997; revised version accepted September 25, 1997.

Members of the Hedgehog (Hh) family of proteins have been implicated in patterning of multiple tissues during embryogenesis. These include the neural tube, limbs, bone, sexual organs, and eyes in vertebrates and invertebrates (for review, see Hammerschmidt et al. 1997). The molecular basis of Hh signaling is best understood in its role in patterning the anteroposterior axis of the embryonic segments and the appendages of *Drosophila*. Hh appears to control patterning indirectly by locally inducing the expression of secondary signaling molecules that can act at a distance (for review, see Hammerschmidt et al. 1997). In the embryo and the ventral part of the leg imaginal disc, Hh induces the expression of *wingless* (*wg*) in anterior cells at the anteroposterior compartment boundary (Forbes et al. 1993; Basler and Struhl 1994; Tabata et al. 1995; Ng et al. 1996). In the dorsal part of the leg disc and in the wing disc, Hh induces Dpp (Basler and Struhl 1994; Tabata et al. 1995). Recent experiments have suggested that there is not just a simple linear relationship between Hh and its two secondary signals, Dpp and Wg. On one hand, Dpp and Wg not only control different cell fates in response to Hh signaling, but they also mutually repress each others transcription (Brook and Cohen 1996; Jiang and Struhl 1996; Johnston and Schubiger 1996; Morimura et al. 1996; Peton and Hoffmann 1996; Heslip et al. 1997). It is therefore possible that Dpp specifies cell fate not only by directly acting on target cells in a concentration dependent manner but also indirectly by repressing *wg*. On the other hand, Hh may also control cell fate directly independent of Dpp and Wg. In experiments in which Hh and Dpp are ectopically expressed in the developing wing, Hh but not Dpp is sufficient to induce sensory structures normally found in anterior cells near the compartment boundary (Gómez-Skarmeta and Modolell 1996; Mullor et al. 1997). Furthermore, Hh may also act directly in patterning of the dorsal epidermis (Bokor and DiNardo 1994). Because Dpp is required earlier for the establishment of the dorsoventral axis of the embryo, however, it is unclear whether Hh acts alone or in combination with Dpp to specify cell identities.

Two independent roles for Hh and Dpp have been described in patterning the *Drosophila* compound eye (for review, see Heberlein and Moses 1995). In contrast to the wing and legs, patterning of the developing eye occurs by a lineage-independent mechanism and is closely linked to cellular differentiation (for review, see Wolff and Ready 1993; Bonini and Choi 1995). Pattern formation is initiated at the late second/early third instar larval stage at posterior part of the eye disc and spreads anteriorly by the movement of the morphogenetic furrow (MF). The

[2]Present address: Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 2QH, UK.
[3]Corresponding author.
E-MAIL mdl@mrc-lmb.cam.ac.uk; FAX 44 1223 412142.

sequential induction of the MF is driven by Hh, which is secreted from the differentiating ommatidial cells (for review, see Heberlein and Moses 1995). Hh induces ommatidial assembly in more anterior cells, some of which, in turn, become Hh secreting cells and, thus, the MF advances. Although *dpp* expression is also induced by Hh in the MF (Heberlein et al. 1993), Dpp signaling appears to be dispensable in the wild type for ommatidial assembly and MF propagation (Burke and Basler 1996; Wiersdorff et al. 1996; Chanut and Heberlein 1997; Peton et al. 1997). A reciprocal requirement for Hh and Dpp was observed in the initiation of the MF at the posterior end of the eye disc and in its continuous reinitiation along the lateral margins. MF initiation is blocked in clones of mutant cells lacking the Dpp-receptor Thick veins (Tkv) or the Dpp-downstream protein Mothers against dpp (Mad) that include the posterior and lateral disc margins (Burke and Basler 1996; Wiersdorff et al. 1996). On the basis of three observations, it has been concluded that Hh is not involved in MF initiation from the posterior margin nor in its reinitiation from the lateral margins (Heberlein et al. 1993; Ma et al. 1993; Jarman et al. 1995). Firstly, the MF initiates normally but arrests prematurely in hh^{1} eye discs (Heberlein et al. 1993). hh^{1} is a partial loss-of-function allele that specifically impairs *hh* function in the developing eye (Lindsley and Zimm 1992). Second, when hh^{ts2} larvae were grown at the permissive temperature until early third instar and then shifted to the restrictive temperature, progression in the center of the disc, but not initiation of the MF, was blocked (Ma et al. 1993). Third, the MF still initiates and propagates a short distance before it stops in eye discs mutant for *atonal* (*ato*), where ommatidial development and, hence, *hh* expression in the ommatidial cells is completely abolished (see references in Heberlein et al. 1995).

Here we have reassessed the requirement for Hh in patterning the eye disc and its relation to the function of Dpp and Wg, (1) by examining the effects of complete loss of *hh* function in somatic clones, (2) by examining the expression of *hh* in relation to early cell markers for the MF, and (3) by examining the consequences of constitutive activation of the Hh signal transduction pathway in cells unable to produce Dpp and Wg. In contrast to previous reports, our results show an absolute requirement for Hh in the initiation of patterning in the eye. In addition, we show that Hh and Dpp signaling pathways contribute to eye patterning independently. Hh acts directly in the control of the initiation and propagation of the MF. In contrast, Dpp is required indirectly to prevent marginal cells that receive the Hh signal from expressing *wg*. This repression is important because activation of *wg* in the Hh receiving cells prevents MF induction by Hh.

Results

Hh is required both for initiation and progression of the morphogenetic furrow

We have examined the effects of loss of *hh* function in the eye imaginal disc. To do this, we generated clones of cells mutant for the null allele, hh^{AC} (Ma et al. 1993) by use of the FRT–FLP technique (Xu and Rubin 1993). Two classes of *hh* mutant clones were observed in the eye disc. The first class consists of clones that are situated entirely within the eye field. In these clones, MF propagation and ommatidial differentiation, assessed by the expression of the neuronal marker Elav, is normal (Fig. 1). Only in the center of large clones is the progression of the MF retarded relative to the adjacent tissue (Fig. 1A,B). In addition, the expression level of the proneural gene *ato*, which is required for the initiation of neuronal differentiation in the MF (Jarman et al. 1994), is reduced in the center of the clone (Fig. 1A). These results are consistent with the phenotype described for *hh* clones in the adult eye (Heberlein et al. 1993; Ma et al. 1993), which showed that photoreceptor differentiation occurs normally in the mutant cells and that only in the center of large clones are aberrant ommatidia found. Because neuronal differentiation and MF propagation can proceed normally through and beyond mutant *hh* clones situated entirely within the eye field, it appears that Hh secreted from the neighboring wild-type ommatidia rescues, in a nonautonomous manner, the loss of *hh*. On the basis of the rescue of mutant ommatidial units, we estimate that adequately high levels of Hh protein reach *hh* mutant cells across a distance of about three ommatidial clusters from the boundary with hh^{+} cells.

Clones of the second class include some of the lateral or posterior margin of the eye disc (Fig. 2). In these clones, no neuronal differentiation is observed (Fig. 2A,C,E–G), except close to the clone boundary, where single *hh* mutant ommatidia can be rescued (see Fig. 2A and arrowheads in 2C). The changes in cell shape that precede and accompany neuronal differentiation are also absent in these marginal clones (Fig. 2E–G). These results show an essential early role of Hh in the initiation of the MF, in addition to its role in MF propagation. Interestingly, there is a significant difference in the requirements for Hh at the disc margins as opposed to the internal eye field, as inferred by the different degree of nonautonomy between marginal (Fig. 2) and internal (Fig. 1) clones.

The analysis of *hh* in clones in the adult eye (Heberlein et al. 1993; Ma et al. 1993) showed that, whereas the majority of clones developed normal ommatidial structures as a result of the nonautonomous rescue by neighboring hh^{+} cells, a small fraction of clones caused gross abnormalities, including the absence of large portions of the eye (Heberlein et al. 1993). This class of clones may correspond to the class of marginal clones.

Blocking the reception of Dpp in *Mad* or *tkv* mutant clones at the margin is often associated with the induction of an ectopic eye field adjacent to the clone (Wiersdorff et al. 1996 and Fig. 2H). In contrast, we have never observed similar reorganizations of eye discs containing *hh* mutant clones (see also Materials and Methods). Moreover, *Mad* mutant cells that fail to form eye structures develop dorsal head structures instead (Wiersdorff et al. 1996). The formation of dorsal head structures can be attributed to the gain of *wg* expression

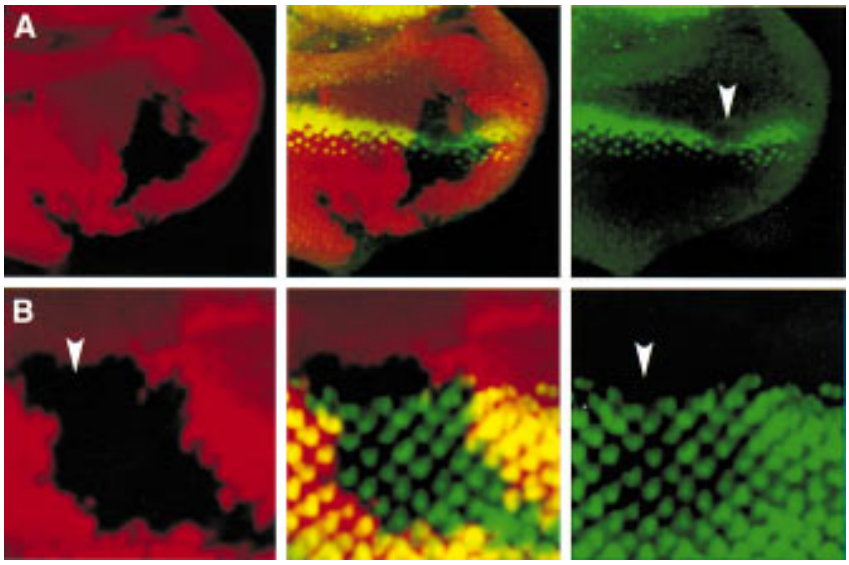

Figure 1. Effects of loss of *hh* function during MF propagation. In all images, eye discs are oriented dorsal to the *right* and anterior *up*. Third instar larval eye discs containing *hh* internal clones double stained with antibodies against β-galactosidase (red) and the proneural protein Ato (green in *A*) or the neuronal marker Elav (green in *B*). Single and superimposed confocal images are shown side by side. The homozygous mutant cells (*hh*/*hh*) are marked by the absence of *lacZ* staining. (*A*) The clone spans the MF and causes a reduction in the levels of Ato protein (arrowhead) in the MF. Note the slightly bent line of *ato* expression, suggesting that the MF has progressed more slowly in the region with limited Hh signal. (*B*) The disc contains two fused *hh* clones. The anterior clone (arrowhead) spans the MF at the time of dissection. The second clone is located posterior to the MF. Examination throughout the depth of the two clones shows that neuronal differentiation, assessed by Elav expression (green), has proceeded normally within the mutant tissue. On the basis of the rescue of mutant ommatidial units, we estimate that adequately high levels of Hh protein reach *hh* mutant cells across a distance of about three ommatidial clusters from the boundary with hh^+ cells.

in the posterior mutant *Mad* cells (Wiersdorff et al. 1996). In contrast, marginal *hh* clones lead to formation of naked cuticle in the adult eye (data not shown; Heberlein et al. 1993). We have not observed ectopic *wg* expression in posterior marginal *hh* clones at third instar larval stage (data not shown).

Early expression of hh, ptc, *and* dpp *in the eye disc*

The results from our clonal analysis of *hh* show an essential role of Hh in the initiation of the MF from the posterior margin of the disc, which is in conflict with a previous observation that *hh* is only expressed in the differentiating photoreceptor cells after the MF has been initiated (Ma et al. 1993). Therefore, we re-examined the expression of *hh* at the time of MF initiation in the late second and early third larval instar stages. The expression of the *patched* (*ptc*) gene, which is activated in response to Hh signaling (Capdevila et al. 1994; Heberlein et al. 1995; Tabata et al. 1995; Strutt and Mlodzik 1996), was also examined at the second and third instar larval stage. To monitor *hh* expression, we used the enhancer-trap line hh^{P30}. This line contains a *P(lacZ)* insertion in the *hh* locus and reproduces the pattern of the endogenous gene (Lee et al. 1992; Ma et al. 1993). *ptc* expression was visualized by use of the ptc^{AT96} enhancer-trap line (see Materials and Methods). The onset of MF initiation was visualized by use of an antibody against the Ato protein (Jarman et al. 1994), which we found is induced shortly before cells enter the MF, assessed by changes in cell shape visualized by the anti-Arm antibody (Fig. 3A). *hh–lacZ* is already expressed in the late second/early third instar larval disc along the posterior and dorso-lateral margins (Fig. 3B–D, G). *hh–lacZ* expression is weak and becomes stronger in cells just posterior to the first Ato positive cells (Fig. 3C). To substantiate that this early expression of β-galactosidase in the hh^{P30} line along the disc margin is related to MF induction, we have examined *hh–lacZ* and *ato* expression in slightly more mature discs, when the MF has moved anteriorly. As shown in Figure 3D, the advance of the MF is closely associated to the anterior expansion of *hh–lacZ*. The *hh* expressing cells lie between discrete Ato-positive cells, which correspond to the presumptive R8 photoreceptor cells (Fig. 3D). It was shown that *hh–lacZ* expression in the developing photoreceptor cells starts several ommatidial rows posterior to the MF as ommatidial cells initiate neuronal differentiation (Heberlein et al. 1993; Ma et al. 1993). At the stage shown in Figure 3D, the neuronal antigen recognized by the Elav antibody is not yet expressed in the differentiating photoreceptor cells (not shown). By this criterion, this early expression of *hh* precedes neuronal differentiation by several hours and, hence, cannot be dependent on it. Furthermore, whereas the later expression of *hh* is restricted to a subpopulation of developing photoreceptors (Ma et al. 1993), the early expression of *hh* is observed in all cells of the posterior margin that themselves will not contribute to the eye field proper, and also in the posterior ommatidial precursors prior to their recruitment. This early expression of *hh* may explain why the MF still initiates in *ato* mutant discs in which neuronal differentiation (Jarman et al. 1994, 1995) and, hence, *hh* expression in differentiating ommatidial cells is prevented. *ptc* expression, which reflects *hh* activity, is also present in the young eye disc (Fig. 3H), supporting the view that the Hh signaling pathway is already active at this stage.

The signaling molecules Dpp and Wg, which are also

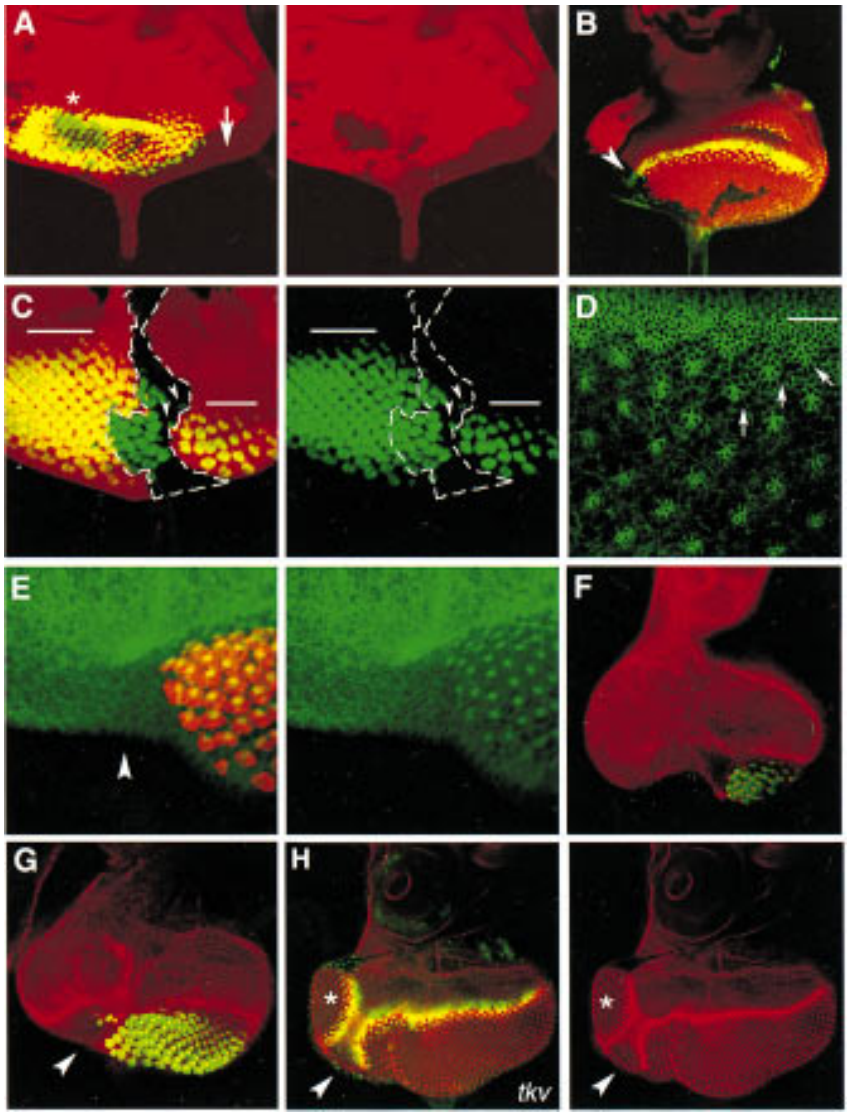

Figure 2. Role of *hh* in the initiation of the MF. (*A–C* and *E–G*) Mosaic eye discs carrying *hh* clones that include the disc margin. (*A–C*) *hh* clones are marked by the absence of *lacZ* (red) staining. (*E–G*) *hh* mutant tissue is marked indirectly by the absence of Elav (green). (*A*) The disc contains several *hh* clones. Single-channel image of the *arm–lacZ* staining to visualize the position of the clones is shown in the *right* panel. Neuronal differentiation, assessed by Elav expression, is normal in the internal *hh* clone (asterisk), whereas it is blocked in the marginal *hh* clone (arrow). The arrow points to the center of the marginal clone and also where marginal ommatidia are missing. (*B*) The expression of *ato* (green) is also affected in marginal *hh* clones. Note that only cells in close proximity to the wild-type border of the clone have detectable Ato protein levels (arrowhead). (*C*) A large *hh* clone (outlined in white) that runs along the center of the disc and spans half of the posterior eye margin. The single channel image of the Elav (green) is shown in the *right* panel. Neuronal differentiation is abolished in the area where the posterior margin is mutant for *hh*. In contrast, the hh^+ margin initiated the MF, which progressed normally through an internal *hh* mutant area (note the presence of green ommatidia). Only a few mutant ommatidia (arrowheads) are rescued adjacent to the borders of the marginal part of the clone. The bars in the image show the approximate position of the MF. (*D*) A portion of a wild-type disc stained with a monoclonal antibody raised against Arm (see also Materials and Methods) to show changes in cell shape associated with cells in the MF (white line) and cells in the ommatidial clusters (some of them are marked with arrows). The image shows an apical focal plane. (*E–G*) Eye discs carrying marginal *hh* clones that span half of the posterior margin. *hh* cells marked by the absence of Elav staining (red in *E*, and green in *F* and *G*) do not constrict apically and fail to assemble into ommatidial clusters, as assessed by the Arm staining (green in *E* and red in *F* and *G*). The disc in *E* was dissected at the mid third instar stage to confirm that failure to initiate ommatidial differentiation is not an indirect effect of loss of *hh* in the ommatidial clusters. The Arm staining is in red, and the Elav in green. The clones in *F* and *G* were induced in a *Minute* background (see Materials and Methods). (*H*) Mosaic eye disc carrying a *tkv* clone also induced in a *Minute* background. The uniform Arm staining (red) and the absence of *ato* expression (green) in the region marked by the arrowhead indicates that this region lacks *tkv*. Adjacent to this area, a supernumerary eye field (asterisk) has formed in the ventral region of the eye disc (see Materials and Methods). The ectopic eye develops an equator as in the endogenous eye, indicating that loss of Dpp reception, but not loss of *hh* (cf. *E–G* with *H*), results in the reprogramming of positional information in the marginal cells to initiate an MF in an ectopic position.

required for patterning of the eye, are already expressed in the late second, early third instar eye disc. *wg–lacZ* is expressed at the disc margin before the onset of *ato* expression (not shown). At these early stages, *wg–lacZ* expression is confined to the most anterior margin of the disc, from which most of the head capsule develops (Fig. 3F). *dpp* is expressed along the posterior and lateral margin of the disc (Masucci et al. 1990; Blackman et al. 1991; and Fig. 3E). We consistently observe a slight temporal difference, however, in the appearance of *hh* and *dpp* expression at the posterior margin of young discs. Whereas *hh* is strongly expressed at the posterior margin of the disc prior to *ato* expression, *dpp* expression in the BS3.0 line appears at the time of *ato* expression (Fig. 3 cf. C and E).

Relationship between Hh, Dpp, and Wg signaling in the initiation of the MF

The results presented here indicate that Hh activity is

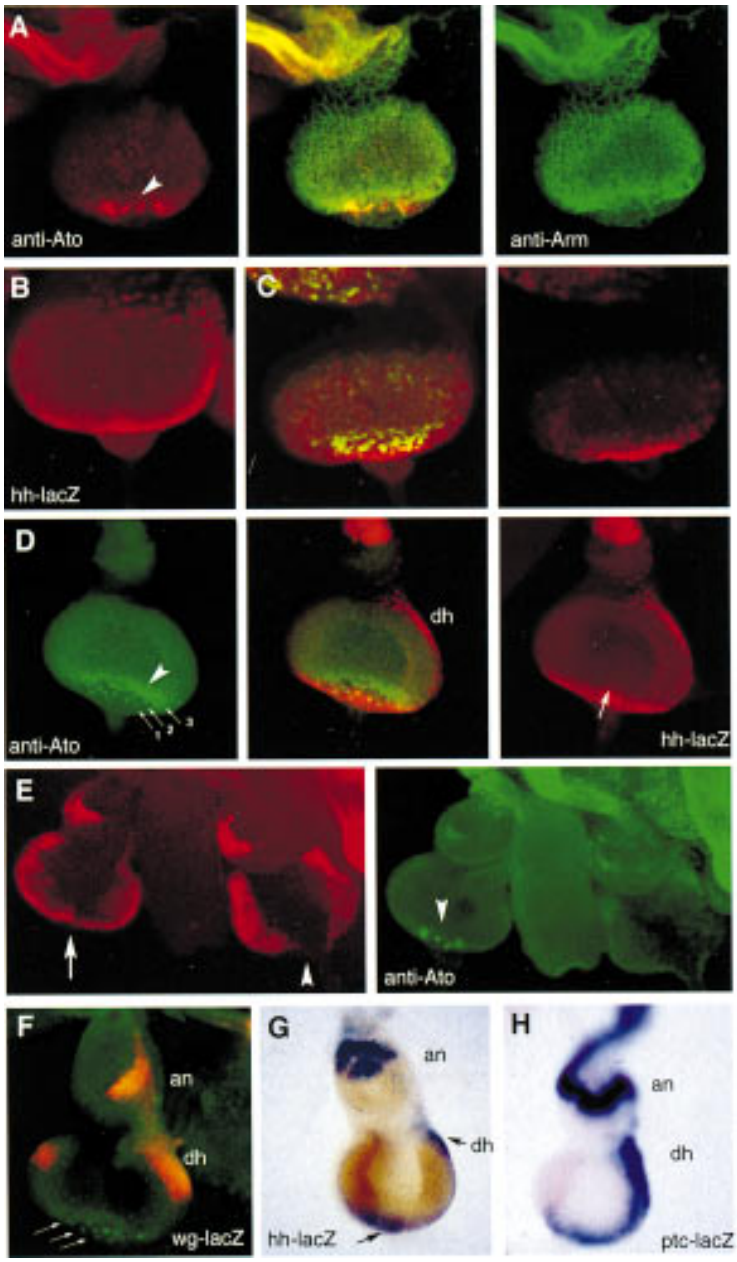

Figure 3. Early pattern of *hh* expression in the second and early third instar eye disc. Anterior is up in all panels. (*A*) The first prominent accumulation of Ato protein (red, *left* panel) in the eye disc marks the onset of the MF, as assessed by Arm staining (green, *right* panel). The middle panel shows the combined image. (*B–F*) Confocal images of increasingly older discs stained with anti-Ato antibody (green) and β-galactosidase (red) to detect *lacZ* expression in the hh^{P30} (*B–D*), in the *dpp-BS3.0* line (*E*) or in a *wg–lacZ* line (*F*). The expression of *hh* occurs in a line of cells along the posterior and dorsal margin of the eye disc prior to the initiation of the MF (*B*). This expression is elevated at the posterior most edge of the disc at the time of MF induction when the first Ato positive cells (green) are detectable (*C*). The *right* panel shows only *hh–lacZ* expression in the same disc. (*D*) An older eye disc, in which the MF has progressed three ommatidial rows (indicated by arrows). The expression of *hh* (arrow) has expanded anteriorly to the region of the presumptive dorsal head (dh) and is located posterior to the band of continuous Ato expression (arrowhead). The *hh–lacZ*-positive cells (red, arrow) are in between the single Ato-positive cells, which correspond to R8 precursor cells. Double staining with anti-Ato and anti-Elav showed no expression of Elav at this early stage (not shown). (*E*) *dpp–lacZ* expression was examined in relation to the early expression of *ato*. Note that the induction of the MF is not synchronous in the two eye discs of the same larva. The disc that shows no detectable accumulation of Ato protein also has weaker expression of *dpp* at the most posterior edge of the disc (arrow). The *dpp–lacZ* expression in this region becomes detectable at the time of *ato* induction, but *dpp* expression is still weaker and discontinuous (arrow) in this region compared with its expression in the lateral margins. (*F*) The disc is of the same age as that shown in *D*. *wg–lacZ* expression is confined to the anterior part of the disc. Note that the prominent *wg–lacZ* expression in the presumptive dorsal head region (dh) overlaps at this stage with *hh–lacZ* expression. (*G–H*) Young eye discs of hh^{P30} (*G*) and ptc^{AT96} (*H*) larvae stained with X-gal (blue). Note that the stripe of *ptc–lacZ* is broader than the stripe of *hh–lacZ* consistent with the notion that secreted Hh can induce gene expression in cells farther from the *hh*-expressing cells. The discrepancy between the time and place of expression of β-galactosidase in the hh^{P30} line shown here and the previous reported *hh* RNA distribution is most likely attributed to the different age of the eye discs used in these two studies. Whereas Ma et al. (1993) analyzed *hh* RNA distribution only in the third instar disc, we see *hh–lacZ* expression at the disc margin already in second instar disc. We used a cell marker (anti-Ato) and a morphological marker (anti-Arm) to assess directly the age of the eye discs. Abbreviations: an, antennal disc; dh, presumptive dorsal head.

required for the initiation of the MF and are consistent with previous reports showing that activation of Hh signaling in cells anterior to the MF is sufficient to induce ectopic MFs (Heberlein et al. 1995; Ma and Moses 1995; Pan and Rubin 1995; Strutt and Mlodzik 1995; Strutt et al. 1995; Wehrli and Tomlinson 1995; Pignoni and Zipursky 1997). Because Wg and Dpp are also implicated in the control of MF initiation—Wg prevents and Dpp promotes initiation—we wondered what the relationship between Hh, Wg, and Dpp in this process was. We tested whether Hh alone is sufficient for this process or whether it acts indirectly via the secondary signals Dpp or Wg. The protein kinase A (Pka) acts downstream in the Hh signaling pathway and loss of *pka* activity mimics the reception of the Hh signal (Jiang and Struhl 1995; Lepage et al. 1995; Li et al. 1995; Pan and Rubin 1995; Strutt et al. 1995). Loss of *pka* function, like ectopic *hh* expression (Heberlein et al. 1995; Pignoni and Zipursky 1997), in anterior cells induces the formation of ectopic MFs (Pan and Rubin 1995; Strutt et al. 1995). Like ectopic *hh* expression (Heberlein et al. 1995; Pignoni and Zipursky 1997), loss of *pka* function activates *dpp* ex-

pression in cells within the eye field (Pan and Rubin 1995; Strutt et al. 1995). We tested whether loss of *pka* also results in ectopic expression of *wg* in any region of the eye disc. As shown in Figure 4A, loss of *pka* function also induces *wg* expression in the anterior part of the eye and in a domain in the antennal disc (Fig. 4A). Next, we tested whether activation of the Hh signaling pathway by loss of *pka* function is sufficient to induce ectopic MFs in the absence of *dpp* and *wg* function. To do this, we generated clones of cells triple mutant for *pka, dpp,* and *wg.* The expression of *ato* was used as an early marker for MF initiation. Clones of cells triple mutant for *dpp, wg,* and *pka* induce autonomously ectopic expression of *ato* (Fig. 4B,C). Triple mutant cells located near to or spanning the endogenous MF cause ectopic MFs and the acceleration of the endogenous MF (Fig. 4B,C), as was described for *pka* single mutant clones (Pan and Rubin 1995; Strutt et al. 1995). In addition, we find that *dpp wg pka* clones located far from the endogenous MF, including the anterior margin of the eye disc, still induce ectopic expression of *ato* at levels comparable with cells in the MF (not shown). The ectopic eyes induced by misexpression of *dpp* are normally formed from the anterior margin of the disc (Heberlein et al. 1995; Pignoni and Zipursky 1997) and induction of ectopic eyes is associated with removal of *wg* expression (Heber-

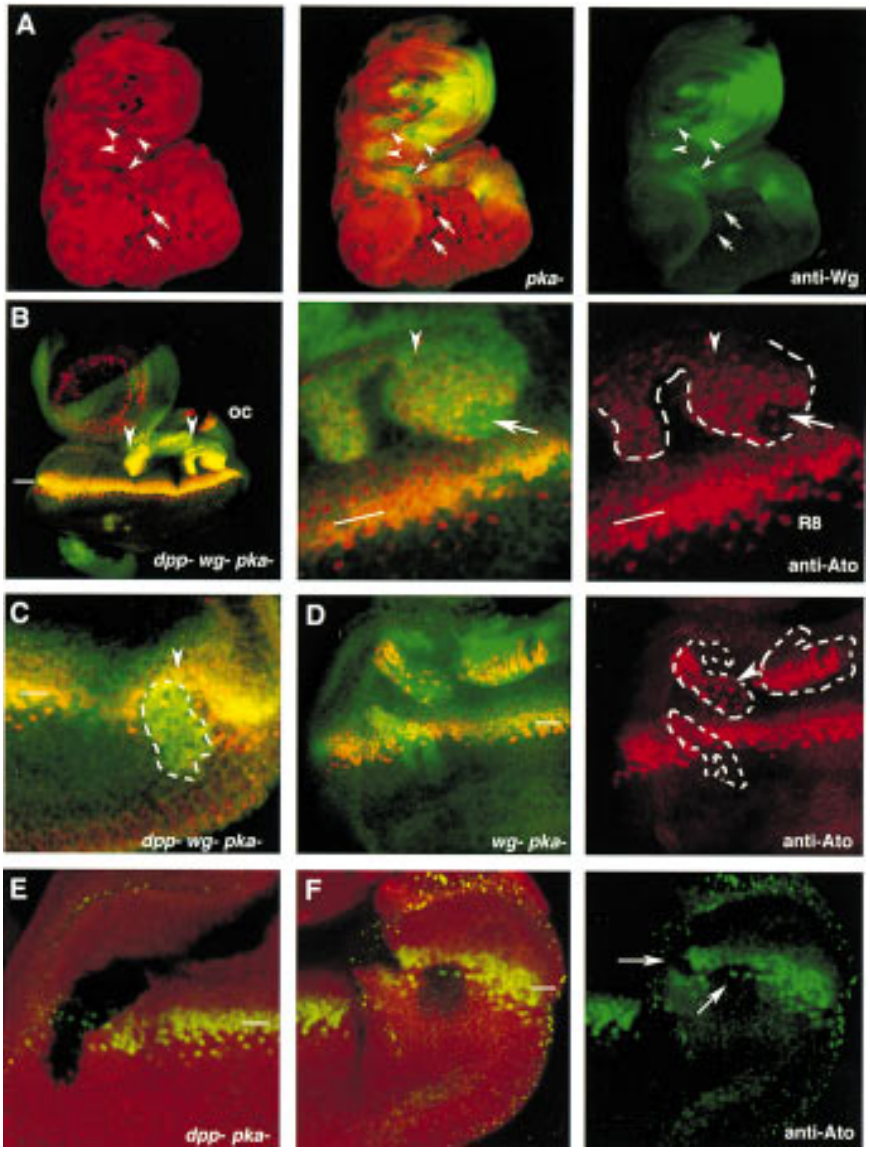

Figure 4. Constitutive activation of the Hh signaling pathway, in the absence of *wg* and *dpp* activities, activates the expression of the proneural gene *ato* and causes ectopic MFs. (*A*) A third instar larval eye disc stained with anti-wg (green) and carrying multiple pka^{DCO-B3} clones marked by the absence of *arm–lacZ* (red). The arrows point to eye internal single pka^{DCO-B3} clones that do not activate *wg* expression. The arrowheads point to clones where *wg* expression is activated in response to removal of *pka*. (*B,C*) Eye discs carrying dpp^{H61} $wg^{CX4}pka^{DCO-B3}$, which are marked by the increased accumulation of Ci occurring in the *pka* mutant cells (Johnson et al. 1995; M. Domínguez and E. Hafen unpubl.). The dpp^{d12} pka^{DCO-B3} clones are marked by the absence of *arm–lacZ* staining. (*B*) A large dpp^{H61} $wg^{CX4}pka^{DCO-B3}$ triple mutant clone (arrowheads) located in the anterior part of the eye disc. *ato* expression is induced autonomously in the mutant cells. A detail of the posterior part of the clone is shown in the *middle* panel. The *right* panel shows a single image of *ato* expression in the posterior part of the clone (outlined in white) and *ato* expression in the endogenous MF (indicated with the bar). In the MF (white line), the uniform levels of Ato protein evolve into discrete single Ato-positive cells, the R8 photoreceptor cells. Note that most cells in the clone have uniform levels of Ato (arrowhead) as those found in the endogenous MF. Some of the mutant cells (arrow) have initiated ommatidial development, as inferred by the presence of regularly spaced Ato-positive cells. (*C*) A dpp^{H61} $wg^{CX4}pka^{DCO-B3}$ clone located posterior to the MF at the time of dissection. The clone caused an acceleration of the endogenous MF (arrowhead). (*D*) Aneye disc carrying three $wg^{CX4}pka^{DCO-B3}$ mutant clones. Note the ectopic activation of *ato* expression even in the clones located far from the endogenous MF. The arrowhead points to some mutant cells where ectopic expression of *ato* is in separated singular cells. (*E,F*) Two eye discs carrying dpp^{d12} pka^{DCO-B3} mutant clones. Note that *ato* expression (green) is not induced in these clones, which are marked by the absence of *arm–lacZ* staining (red). The clones in *F* span the endogenous MF and have caused a reduction in the levels of expression of *ato* (arrowheads). (oc) Occellar region. The position of the endogenous MF is indicated by white bars.

lein et al. 1995; Pignoni and Zipursky 1997). Our results of the triple mutant clones suggest a dispensable role for Dpp in the initiation of MFs and, thus, are in contrast to the report of Wiersdorff et al. (1996) that Dpp signaling via the Mad protein is required to initiate MFs from *pka* cells. One explanation for the difference between *pka Mad* and *dpp wg pka* clones could be the failure to activate *wg* expression in the latter. To test whether the loss of *wg,* rather than the gain of *dpp,* is necessary for the formation of ectopic MFs, we compared the triple mutant clones with clones double mutant for *wg pka* (Fig. 4D) or *dpp pka* (Fig. 4E–F). Whereas *wg pka* clones located in the anterior part of the disc behave as triple clones, *dpp pka* mutant cells, like *pka Mad* mutant cells (Wiersdorff et al. 1996) are unable to activate *ato* (Fig. 4E). These results indicate that, at least in the lateral and anterior region of the disc, in the absence of Wg, Dpp is dispensable for the initiation of a MF by the Hh signaling pathway. Posterior triple mutant clones, as single mutant *pka* clones (Strutt et al. 1995; Pan and Rubin 1995), showed normal retinal differentiation (not shown). Although we cannot exclude the possibility that neighboring dpp^{+} tissue rescues the loss of *dpp* in the posterior marginal clones, it is important to bear in mind that in dpp^{blink} mutants, where no *dpp* expression is detected in the eye disc, the MF still initiates in the center of the posterior margin (Treisman and Rubin 1995). Our results also show that activation of Wg in the Hh-receiving cells alters the competence of cells to respond to Hh signals.

Discussion

Hh acts directly on cells in the eye imaginal disk to induce ommatidial differentiation

Our results show an essential and direct function of Hh in the initiation and propagation of the MF. Loss of *hh* from the disc margin, where it is expressed prior to the onset of eye patterning, impedes growth of the disc and prevents all aspects of MF initiation. Furthermore, we show that the activation of the Hh signaling pathway is sufficient to induce ommatidial development in the absence of *wg* and *dpp* function.

The results presented here conflict with previous reports suggesting that Hh is not required for the MF initiation (Heberlein et al. 1993; Ma et al. 1993; Jarman et al. 1995). This notion was based on the following evidence. MF initiation is normal in the hypomorphic hh^{1} allele (Heberlein et al. 1993) and in *ato* mutants (Jarman et al. 1995) where *hh* is not expressed in the developing photoreceptors. We show that *hh* is already present along the margin of the late second/early third instar disc prior to *ato* expression and that MF initiation is dependent on Hh function in the disc margin. Furthermore, we show that *ato* expression is strictly dependent on Hh input. Therefore, by genetic criteria, hh^{1} is a partial loss-of-function allele. It is likely that MF initiation in hh^{1} mutants initiates normally because the early expression of *hh* in the margin, as *hh* expression in other imaginal discs, is normal in the hh^{1} mutant. Temperature-shift experiments performed with the hh^{ts2} allele at the early third instar stage did not prevent initiation of the MF, presumably because the requirement for Hh begins already at an earlier stage. Because of the perdurance of the β-galactosidase protein used to analyze *hh* expression in the second instar, we cannot accurately determine the onset of *hh* expression in the disc margin, and hence, its time of action. Although we cannot exclude the possibility that Hh function begins at an earlier stage than the onset of *ato* expression, we believe that Hh's function is also involved later in the initiation of the MF. Clones of *pka* induced at the second instar larval stage still express *ato* and generate ectopic MFs (Strutt et al. 1995; Pan and Rubin 1995; M. Domínguez and E. Hafen unpubl.). Similarly, late-induced *pka wg dpp* clones still induce ectopic *ato* expression, supporting the view that cells must receive a direct input from Hh to initiate the MF. In vertebrates, inhibition of protein kinase A phenocopies the effects of ectopic expression of vertebrate Hh proteins in the developing eye (Hammerschmidt et al. 1996) suggesting that the mechanisms of the Hh signaling is conserved.

The early function of Hh in *Drosophila* eye development described here may have parallels during vertebrate eye development. In the Zebrafish embryo, two *hh* genes are expressed in the floor of the diencephalon adjacent to the developing optic vesicles that express *Pax2* adjacent to the Hh domain and *Pax6* in the presumptive distal portion far from the source of Hh (Ekker et al. 1995). High levels of Sonic hedgehog (SHh) reduce *Pax6* expression and result in the expansion of *Pax2* expression. A similar regulatory relationship between Shh and *Pax6* has recently been shown in the developing neural tube (Ericson et al. 1997). Hence, it is possible that one of the early functions of Hh during *Drosophila* eye development is the regulation of *eyeless,* the *Pax6* homolog in *Drosophila.* Consistent with this notion is the fact that the early expression we detect in second instar eye imaginal discs is confined to the region surrounding the eye field.

In contrast to the direct role of Hh in MF initiation, it appears that the control of MF initiation by Dpp is indirect; it acts by repressing *wg.* The negative regulation of *wg* by Dpp at the disc margins (Wiersdorff et al. 1996; Chanut and Heberlein 1997; Pignoni and Zipursky 1997) provides an explanation for why ectopic *dpp*-expressing cells can only initiate an ectopic MF from the anterior margin (Chanut and Heberlein 1997; Pignoni and Zipursky 1997), where *wg* is normally expressed (Ma and Moses 1995; Heslip et al. 1997). The ectopic MFs induced by Dpp at the anterior margin are likely initiated by Hh, which is also expressed at the dorsal margin in the young eye disc (Fig. 3B,C,G). A role for Hh in MF initiation at the anterior disc margin caused by temporally removing *wg* function is supported by the observation that this phenotype is suppressed by *hh* (Treisman and Rubin 1995).

A striking phenotype is the formation of ectopic eye fields in the vicinity of marginal *Mad* (Wiersdorff et al.

1996) and tkv^{strII} (Fig. 2H) clones. This dramatic reorganization of the eye disc resembles the production of ectopic appendages as a consequence of local loss of Dpp reception at the compartment boundary in the leg discs (Brook and Cohen 1996; Jiang and Struhl 1996; Johnston and Schubiger 1996; Morimura et al. 1996; Peton and Hoffmann 1996; Theisen et al. 1996). These ectopic appendages appear to result from the novel juxtaposition of *dpp*-expressing cells and cells expressing *wg* because of their failure to respond to Dpp. A similar mechanism could account for the formation of ectopic eye fields in mosaic *Mad* and *tkv* eye discs because it has been shown that *wg* expression is activated in *Mad* mutant clones and in partial loss-of-function mutants of *dpp* in the eye (Wiersdorff et al. 1996; Chanut and Herberlein 1997).

Like in the leg disc, the early expression of *dpp* and *wg* in the disc margins may be induced by Hh. Several lines of evidence suggest that this is the case. First, *hh* is expressed at the disc margin of the second instar disc and its expression overlaps with the domain of *wg* and *dpp* at this stage. Second, removal of *pka,* which mimics the effects of ectopic Hh expression, results in ectopic expression of *dpp* or of *wg.* Like in the antenna and leg disc, the response to the removal of *pka* is limited to cells anterior to the *hh*-expressing cells, and differs in different regions of the eye disc. In the anterior margin of the eye disc, loss of *pka* induces expression of *wg* like in the ventral domain of the leg and the antenna. In the internal part of the eye disc, loss of *pka* induces *dpp* expression like the dorsal region of the leg and antenna. Furthermore, the repression of *wg* by Dpp occurs not only in the margin but also in eye internal cells lacking *pka.* It is, therefore, likely that Hh directly induces early expression of *wg* and *dpp* by antagonizing *pka* activity at the eye disc margin. Thus, the regulatory relationship between Hh, Dpp, and Wg may be similar in the eye disc and the leg disc despite obvious differences in the way the discs develop. Patterning of the leg is controlled by the juxtaposition of two clonally unrelated cell populations, anterior and posterior cells, and is not linked to differentiation (Basler and Struhl 1994; Brook and Cohen 1996; Jiang and Struhl 1996). The eye disc, however, develops by a lineage-independent mechanism and differentiation is a prerequisite for progression of the patterning process.

Different thresholds for Hh activity to induce ommatidial assembly

Our analysis of *hh* in the eye disc has shown that there are different requirements for Hh activity at the disc margins and in the internal part of the eye disc, as observed by the different degree on nonautonomy between *hh* internal and *hh* marginal clones. Loss of *hh* activity in the internal region of the disc has very little consequences, presumably because the presence of hh^{+} tissue is sufficient to rescue the lack of *hh* function over a relatively long distance (about three ommatidial units). In contrast, the loss of *hh* expression at the margin, even in small clones, is not completely rescued by the adjacent Hh-producing ommatidia. As the MF progresses in a mosaic disc carrying a marginal *hh* clone, only a single ommatidial unit adjacent to Hh-secreting ommatidia is rescued. Thus, there appears to be an essential requirement for *hh* function at the disc margin to induce ommatidial differentiation in the internal cells. There is no detectable *hh* expression in the disc margins at the time of MF progression in the late third instar larval disc (Ma et al. 1993; M. Domínguez and E. Hafen unpubl.). Thus, this requirement for Hh may be related to its early expression in the disc margin. In the absence of early marginal expression of *hh,* the initiation of ommatidial development appears to require a higher concentration of Hh so that only the mutant cells at the boundary to the Hh-secreting cells receive sufficient Hh to be rescued. The early expression of *hh* at the margin would result in a gradient of Hh activity toward the center of the eye disc, predisposing cells in the presumptive eye field to become ommatidial cells later. In support of this, we observed that expression of *dpp–lacZ* and of *ptc–lacZ* are graded from the margin toward the center of the disc. The graded expression of *dpp* becomes uniform and expands throughout the posterior part of the disc upon ectopic expression of *hh* in the second instar larval stage (Pignoni and Zipursky 1997). It is interesting to note that in hh^{1} eye discs, where no Hh protein is detected during the mid to late third instar stage (Huang and Kunes 1996) the MF progression stops first in the center of the disc and only later near the disc margin (Heberlein et al. 1993). This observation is consistent with the notion that in the wild type, central cells farthest away from the disc margin require higher Hh levels to initiate neural development than cells located near the margins.

A similar early and late requirement of Hh signaling as proposed here for eye patterning has recently been shown for the differentiation of motor neurons in the vertebrate neural tube (Ericson et al. 1996). In this system, early expression of *SHh* in the notochord is important to induce a ventralized state in the cells of the neural tube. This ventralized state is marked by the repression of *pax7* and *pax3* expression in the ventral neural tube and is required for later specification of motor neurons by Hh secreted from floor-plate cells. In each case, the same cells, ommatidial precursor cells or motor neuron precursors, require for their differentiation distinct phases of SHh signaling originating from different cell populations: marginal cells and differentiating photoreceptor cells in the eye disc and notochord cells and floor plate cells in the neural tube. We note that the reiterative use of the Hh signaling pathway during patterning of the *Drosophila* eye and the differentiation within the vertebrate neural tube is similar to the reiterative use of the Ras/MAP kinase pathway during the specification of the different cell types in the developing eye (Freeman 1996). Hh signaling in the *Drosophila* eye may thus be another example of how the same signaling pathway is used repeatedly to advance the developmental state of cells and tissues in a ratchet-like manner.

Materials and methods

Fly stocks

The *hh* allele used, hh^{AC}, is a null allele that is a small deletion of sequences of the *hh* promoter and part of the coding region (Ma et al. 1993). In genetic clones, hh^{AC} behaves as other null alleles (Ma et al. 1993; Basler and Struhl 1994). dpp^{H61} and wg^{CX4} are null alleles. dpp^{d12} and pka^{DCO-B3} are strong loss-of-function alleles (described in Jiang and Struhl 1995; Li et al. 1995). The enhancer-trap strain hh^{P30} (Ma et al. 1993) was used to monitor expression of the *hh* gene. *ptc* expression was monitored by use of the ptc^{AT96} enhancer-trap line (kindly provided by G. Struhl). In the eye disc, Ptc protein is detected at low levels in all anterior cells and at higher levels in cells in the MF and in some cells posterior to the MF (Strutt and Mlodzik 1996). *ptc–lacZ* expression in the ptc^{AT96} enhancer-trap line is high in cells posterior to the MF. *dpp* expression was monitored with a BS3.0 reporter construct (Blackman et al. 1991) inserted on the second chromosome. This reporter line reproduces the expression of the endogenous *dpp* gene (Masucci et al. 1990).

Somatic clones

Mitotic recombination clones were generated by use of the FRT–FLP technique (Xu and Rubin 1993) alone or in combination with the *Minute* technique (Morata and Ripoll 1975) to give the mutant clone a growth advantage. The genotype of the larvae in Figure 1 and 2A–C are *y w hs–FLP122;* FRT^{82B} hh^{AC}/ FRT^{82B} *arm–lacZ*. In these experiments, the hh^{AC}/hh^{AC} homozygous mutant tissue and the twin spot (hh^{+} *arm–lacZ*/hh^{+} *arm–lacZ*) are marked, respectively, by the absence or the increased levels of *lacZ* staining in relation to the heterozygous tissue. The genotype of discs shown in Figure 2E–G is *y w hs–FLP122;* FRT^{82B} hh^{AC}/FRT^{82B} $M(3R)^{67C}$. The genotype of the disc shown in Figure 2H is *y w hs–FLP122;* tkv^{strII} FRT^{40}/ $M(2)^{25A}$ FRT^{40}. The tkv^{strII} allele (provided by K. Basler) is an amorphic allele, and at the margin, but not in the internal region, of the eye disc only very small clones are recovered. To generate larger clones of tkv^{strII} at the margin, the somatic clones were induced in a *Minute* background. To compare the phenotype of loss of *hh* and loss of Dpp-reception, clones of cells mutant for hh^{AC} or tkv^{strII} induced in a *Minute* background were generated. Eye–antennal discs with supernumerary eye field and duplicated antenna were obtained only in discs of the genotype tkv^{strII} M^{+}. In addition, we found that mosaic discs of the genotype hh^{AC} M^{+} showed signs of cessation of the MF, such as mature ommatidia in the leading front of Elav expression, and absence of *dpp–lacZ* expression. These phenotypes presumably result from the loss of *hh* in a large internal area.

The genotype of the discs carrying triple mutant clones shown in Figure 4A is pka^{DCO-B3} *stc* FRT^{40}/*arm–lacZ*, FRT^{40} in Figure 4, B and C is dpp^{H61} wg^{CX4} pka^{DCO-B3} *stc* FRT^{39E}/ *Dp(2;2)VT1* (dpp^{+}) *Dp(1;2)sc*^{19} (y^{+}) FRT^{39E} *hs–Flp,* in Figure 4D is *y hs–Flp122*/+; wg^{CX4} pka^{DCO-B3} FRT^{40}/*arm–lacZ* FRT^{40}, and in Figure 4, E and F, is *y hs–Flp122*/+; dpp^{d12} pka^{DCO-B3} FRT^{40}/ *arm–lacZ* FRT^{40}. In all cases, the *FLP* gene was activated in first instar larvae by heat shocking the larvae for 1 hr at 38°C. Wandering third instar eye discs were dissected for histochemistry.

Immunofluorescence staining

Eye imaginal discs from second to third instar larvae were dissected, stained, and analyzed as described by Gaul et al. (1992). A rat monoclonal anti-Elav antibody diluted 1:50, a rabbit polyclonal anti-Ato antibody diluted 1:2000, a rabbit polyclonal anti-β-galactosidase antibody (Cappel) diluted 1:400 or a mouse monoclonal anti-β-galactosidase antibody (Cappel) diluted 1 : 2000 were used. In the experiments in which loss of Elav staining was used to mark the clones indirectly, discs were double-stained with a mouse anti-Arm antibody (N2 7A1 Armadillo) from the Hybridoma Bank. Arm protein accumulates in the adherens junctions and becomes concentrated around the apical tips of cells in the MF and in photoreceptor cells as they gather into clusters. Modulation of Arm protein was used as morphological criterion for the differentiation stage of cells in the eye. Secondary antibodies, either a FITC- or a Texas-Red-conjugated, were from Jackson Inc. Eye discs were incubated overnight with a mixture of the two primary antibodies, washed several times and then incubated for 2 hr in a mixture of the two secondary antibodies.

Acknowledgments

We thank K. Dücker and J. Riesgo for discussions and D. Gubb, J.F. de Celis, M. Freeman, P. Lawrence, and K. Basler for critical comments of the manuscript. We thank K. Moses, K. Basler, and G. Struhl for fly stocks, Y. Jan for the Ato antibody. Part of this work was carried out in Dr. P. Lawrence's laboratory. M.D. was supported by a postdoctoral fellowship from Human Frontiers Science Program and is presently supported by a postdoctoral fellowship from European Molecular Biology Organization (EMBO). E.H. acknowledges the support from the Swiss National Science Foundation.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked ''advertisement'' in accordance with 18 USC section 1734 solely to indicate this fact.

References

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

Blackman, R.K., M. Sanicola, L.A. Raftery, T. Gillevet, and W.M. Gelbart. 1991. An extensive 3′ cis-regulatory region directs the imaginal disk expression of decapentaplegic, a member of the TGF-beta family in Drosophila. *Development* **111:** 657–666.

Bonini, N.M. and K.W. Choi. 1995. Early decisions in Drosophila eye morphogenesis. *Curr. Opin. Genet. Dev.* **5:** 507–515.

Bokor, P. and S. DiNardo. 1996. The roles of *hedgehog, wingless,* and *lines* in patterning of the dorsal epidermis in *Drosophila. Development* **122:** 1083–1092.

Brook, W.J. and S.M. Cohen. 1996. Antagonistic interactions between Wingless and Decapentaplegic responsible for dorsal-ventral pattern in the *Drosophila* leg. *Science* **273:** 1373–1377.

Burke, R. and K. Basler. 1996. Hedgehog dependent patterning in the *Drosophila* eye can occur in the absence of Dpp signaling. *Dev. Biol.* **179:** 360–368.

Capdevila, J., M.P. Estrada, H.E. Sánchez, and I. Guerrero. 1994. The Drosophila segment polarity gene patched interacts with decapentaplegic in wing development. *EMBO J.* **13:** 71–82.

Chanut, F. and U. Heberlein. 1997. Role of *decapentaplegic* in initiation and progression of the morphogenetic furrow in the developing *Drosophila* retina. *Development* **124:** 559–567.

Ekker, S.C., A.R. Ungar, P. Greenstein, D.P. von Kessler, J.A. Porter, R.T. Moon, and P.A. Beachy. 1995. Patterning activi-

ties of vertebrate hedgehog proteins in the developing eye and brain. *Curr. Biol.* **5:** 947–955.

Ericson, J., S. Morton, A. Kawakami, H. Roelink, and T.M. Jessell. 1996. Two critical periods of sonic hedgehog signaling required for the specification of motor neuron identity. *Cell* **87:** 661–674.

Ericson, J., P. Rashbass, A. Schedl, S. Brennermorton, A. Kawakami, V. Vanheyningen, T.M. Jessell, and J. Briscoe. 1997. Pax6 controls progenitor cell identity and neuronal fate in response to graded shh signaling. *Cell* **90:** 169–180.

Forbes, A.J., Y. Nakano, A.M. Taylor, and P.W. Ingham. 1993. Genetic analysis of hedgehog signalling in the Drosophila embryo. *Development* **119:** 115–124.

Freeman, M. 1996. Reiterative use of the EGF receptor triggers differentiation of all cell types in the Drosophila eye. *Cell* **87:** 651–660.

Gaul, U., G. Mardon, and G.M. Rubin. 1992. A putative Ras GTPase activating protein acts as a negative regulator of signaling by the sevenless receptor tyrosine kinase. *Cell* **68:** 1007–1019.

Gómez-Skarmeta, J.L. and J. Modolell. 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.

Hammerschmidt, M., M.J. Bitgood, and A.P. McMahon. 1996. Protein kinase A is a common negative regulator of hedgehog signaling in the vertebrate embryo. *Genes* & *Dev.* **10:** 647–658.

Hammerschmidt, M., A. Brook, and A.P. McMahon. 1997. The world according to hedgehog. *Trends Genet.* **13:** 14–21.

Heberlein, U. and K. Moses. 1995. Mechanisms of Drosophila retinal morphogenesis: The virtues of being progressive. *Cell* **81:** 987–90.

Heberlein, U., T. Wolff, and G.M. Rubin. 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.

Heberlein, U., C.M. Singh, A.Y. Luk, and T.J. Donohoe. 1995. Growth and differentiation in the Drosophila eye coordinated by hedgehog. *Nature* **373:** 709–711.

Heslip, T.R., H. Theisen, H. Walker, and L. Marsh. 1997. Shaggy and Dishevelled exert opposite effects on *wingless* and *decapentaplegic* expression and on positional identity in imaginal discs. *Development* **124:** 1069–1078.

Huang, Z. and S. Kunes. 1996. Hedgehog, transmitted along retinal axons, triggers neurogenesis in the developing visual centers of the Drosophila brain. *Cell* **86:** 411–422.

Jarman, A.P., E.H. Grell, L. Ackerman, L.Y. Jan, and Y.N. Jan. 1994. *atonal* is the proneural gene for Drosophila photoreceptors. *Nature* **369:** 398–400.

Jarman, A.P., Y. Sun, L.Y. Jan, and Y.N. Jan. 1995. Role of the proneural gene, *atonal,* in formation of *Drosophila* chordotonal organs and photoreceptors. *Development* **121:** 2019–2030.

Jiang, J. and G. Struhl. 1995. Protein kinase A and hedgehog signaling in Drosophila limb development. *Cell* **80:** 563–572.

———. 1996. Complementary and mutually exclusive activities of *decapentaplegic* and *wingless* organise axial patterning during *Drosophila* leg development. *Cell* **86:** 401–409.

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

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

Lee, J.J., K.D. Von, S. Parks, and P.A. Beachy. 1992. Secretion and localized transcription suggest a role in positional signaling for products of the segmentation gene hedgehog. *Cell* **71:** 33–50.

Lepage, T., S.M. Cohen, F.J. Díaz-Benjumea, and S.M. Parkhurst. 1995. Signal transduction by cAMP-dependent protein kinase A in Drosophila limb patterning. *Nature* **373:** 711–715.

Li, W., J.T. Ohlmeyer, M.E. Lane, and D. Kalderon. 1995. Function of protein kinase A in hedgehog signal transduction and Drosophila imaginal disc development. *Cell* **80:** 553–562.

Lindsley, D.L. and G.G. Zimm. 1992. The genome of *Drosophila melanogaster.* Academic Press, San Diego, CA.

Ma, C. and K. Moses. 1995. *wingless* and *patched* are negative regulators of the morphogenetic furrow and can affect tissue polarity in the developing *Drosophila* compound eye. *Development* **121:** 2279–2289.

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

Masucci, J.D., R.J. Miltenberger, and F.M. Hoffmann. 1990. Pattern-specific expression of the *Drosophila* decapentaplegic gene in imaginal disks is regulated by 3′ cis-regulatory elements. *Genes* & *Dev.* **4:** 2011–2023.

Morata, G. and P. Ripoll. 1975. Minutes: Mutants of Drosophila autonomously affecting cell division rate. *Dev. Biol.* **42:** 211–221.

Morimura, S., L. Maves, Y. Chen, and F.M. Hoffmann. 1996. *decapentaplegic* overexpression affects *Drosophila* wing and leg imaginal disc development and *wingless* expression. *Dev. Biol.* **177:** 136–151.

Mullor, J.L., M. Calleja, J. Capdevilla, and I. Guerrero. 1997. Hedgehog activity, independent of Decapentaplegic, participates in wing disc patterning. *Development* **124:** 1227–1237.

Ng, M., F.J. Diaz-Benjumea, J. P. Vincent, J. Wu, and S.M. Cohen. 1996. Specification of the wing by localized expression of *wingless* protein. *Nature* **381:** 316–318.

Pan, D. and G.M. Rubin. 1995. cAMP-dependent protein kinase and hedgehog act antagonistically in regulating decapentaplegic transcription in Drosophila imaginal discs. *Cell* **80:** 543–552.

Peton, A. and M. Hoffmann. 1996. Decapentaplegic restricts the domain of *wingless* during *Drosophila* limb patterning. *Nature* **382:** 162–165.

Peton, A., S.B. Selleck, and M. Hoffmann. 1997. Regulation of cell cycle synchronization by *decapentaplegic* during *Drosophila* eye development. *Science* **275:** 203–206.

Pignoni, F. and L. Zipursky. 1997. Induction of *Drosophila* eye development by Decapentaplegic. *Development* **124:** 271–278.

Strutt, D.I. and M. Mlodzik. 1995. Ommatidial polarity in the *Drosophila* eye is determined by the direction of furrow progression and local interactions. *Development* **121:** 4247–4256.

———. 1996. The regulation of *hedgehog* and *decapentaplegic* during *Drosophila* eye imaginal disc development. *Mech. Dev.* **58:** 39–50.

Strutt, D.I., V. Wiersdorff, and M. Mlodzik. 1995. Regulation of furrow progression in the Drosophila eye by cAMP-dependent protein kinase A. *Nature* **373:** 705–709.

Tabata, T., S. Eaton, and T.B. Kornberg. 1992. The Drosophila *hedgehog* gene is expressed specifically in posterior compartment cells and is a target of engrailed regulation. *Genes* & *Dev.* **6:** 2635–2645.

Tabata, T., C. Schwartz, E. Gustavson, Z. Ali, and T.B. Kornberg. 1995. Creating a Drosophila wing de novo, the role of engrailed, and the compartment border hypothesis. *Development* **121:** 3359–3369.

Theisen, H., J. Purcell, M. Bennett, D. Kansagara, A. Syed, and J.L. Marsh. 1996. Developmental territories created by mutual antagonism between WG and DPP. *Development* **122:** 3939–3948.

Treisman, J. and G.M. Rubin. 1995. *wingless* inhibits morphogenetic furrow movement in the *Drosophila* eye disc. *Development* **121:** 3519–3527.

Wehrli, M. and A. Tomlinson. 1995. Epithelial planar polarity in the developing *Drosophila* eye. *Development* **121:** 2451–2459.

Wiersdorff, V., T. Lecuit, S.M. Cohen, and M. Mlodzik. 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.

Wolff, T. and D.F. Ready. 1993. Pattern formation in the *Drosophila* retina. In *The development of Drosophila melanogaster* (ed. M. Bate and A. Martinez-Arias), pp. 1277–1326. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Xu, T. and G.M. Rubin. 1993. Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development* **117:** 1223–1237.