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wingless inhibits morphogenetic furrow movement in the *Drosophila* eye disc

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SUMMARY

Differentiation of the *Drosophila* eye imaginal disc is an asynchronous, repetitive process which proceeds across the disc from posterior to anterior. Its propagation correlates with the expression of *decapentaplegic* at the front of differentiation, in the morphogenetic furrow. Both differentiation and *decapentaplegic* expression are maintained by Hedgehog protein secreted by the differentiated cells posterior to the furrow. However, their initiation at the posterior margin occurs prior to *hedgehog* expression by an unknown mechanism. We show here that the *wingless* gene contributes to the correct spatial localization of initiation. Initiation of the morphogenetic furrow is restricted to the posterior margin by the presence of *wingless* at the lateral margins; removal of *wingless* allows lateral initiation. Ectopic expression of *wingless* at the posterior margin can also inhibit normal initiation. In addition, the presence of *wingless* in the center of the disc can prevent furrow progression. These effects of *wingless* are achieved without altering the expression of *decapentaplegic.*

Key words: eye development, *Drosophila*, imaginal disc, morphogenetic furrow, *wingless*, *decapentaplegic*

INTRODUCTION

The ordered array of ommatidia in the *Drosophila* compound eye results from a progressive pattern of differentiation in the eye imaginal disc (for review see Thomas and Zipursky, 1994). The eye disc is an epithelial monolayer derived from a primordial group of cells determined during embryogenesis. During the third larval instar, rows of photoreceptor clusters differentiate in succession, starting at the posterior margin and continuing anteriorly. The first morphological marker of differentiation is the morphogenetic furrow, an indentation formed by contraction of the cells in the apical/basal dimension and accompanied by constriction of their apical profiles (Ready et al., 1976). This furrow moves across the disc from posterior to anterior as development proceeds. Immediately anterior to the furrow, cells are arrested in the G_1 phase of the cell cycle (Ready et al., 1976). Within the furrow clusters of cells form in a regularly spaced array (Wolff and Ready, 1991) and, posterior to the furrow, these cells differentiate as photoreceptors in a defined sequence (Tomlinson and Ready, 1987a).

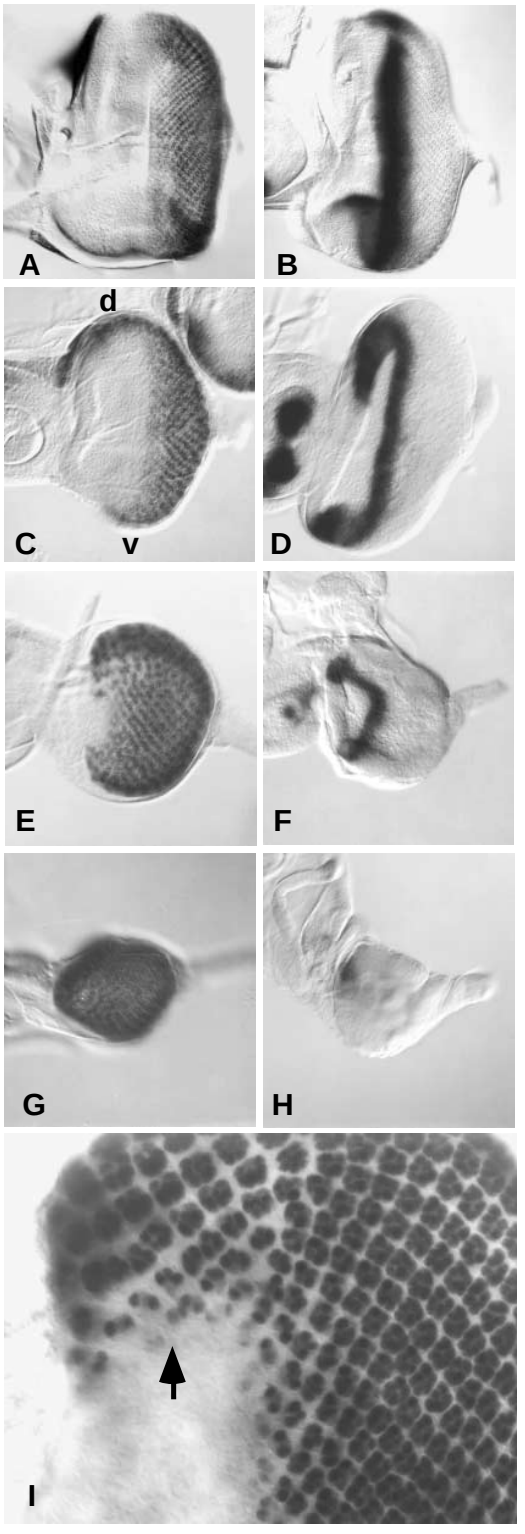
The successive addition of rows of ommatidia has been shown to require expression of *hedgehog (hh)* in the differentiated photoreceptors (Heberlein et al., 1993; Ma et al., 1993); HH is a secreted protein which is thought to constitute an inductive signal (Lee et al., 1992). Its effects may be mediated by *decapentaplegic (dpp)*, a member of the TGF-β family, which shows *hh*-dependent expression within the morphogenetic furrow (Heberlein et al., 1993; Ma et al., 1993). Ectopic expression of *hh* in patches of cells anterior to the furrow is sufficient to induce differentiation which appears to propagate radially (Heberlein et al., 1995). *dpp* is one of the first molecules to be induced by *hh*, and *hh*-independent induction of ectopic *dpp* expression, caused by loss of *protein kinase A* function, has identical effects (Pan and Rubin, 1995; Strutt et al., 1995).

Initiation of furrow movement must occur by a different mechanism than its propagation, as *hh* is not present prior to differentiation. Since *dpp* is expressed around the edges of the eye disc before initiation (Masucci et al., 1990), it may be involved in this process. The *eyes absent* (*eya*; Bonini et al., 1993), *sine oculis* (*so*; Cheyette et al., 1994) and *dachsund* (*dac*; Mardon et al., 1994) genes are also likely to act in initiation (Thomas and Zipursky, 1994). All of these genes can mutate to eyeless phenotypes, are expressed in a similar early pattern at the margins of the disc and encode nuclear proteins. The *eyeless (ey)* gene is presumably upstream of all these genes, since it is sufficient to initiate eye differentiation in other imaginal discs (Halder et al., 1995).

The *wingless (wg)* gene encodes another secreted protein that is required for segmentation of the embryo and development of the imaginal discs (Rijsewik et al., 1987; Baker, 1988a). It is a member of the *Wnt* gene family (Nusse and Varmus, 1992); vertebrate *Wnt* genes are involved in axis specification (McMahon and Moon, 1989), determination of the midbrain (Thomas and Capecchi, 1990; McMahon and Bradley, 1990) and limb development (Parr and McMahon, 1995). The WNT proteins appear to bind to the extracellular matrix and to act over a short range (Bradley and Brown, 1990; Papkoff and Schryver, 1990; van den Heuvel et al., 1993). Their receptors have not been characterized, although it has been suggested that Notch might act as a WG receptor (Couso and Martinez-Arias, 1994). Genetic methods have been used to identify a number of other molecules acting in the *wg* pathway (reviewed by Klingensmith and Nusse, 1994; Siegfried and Perrimon, 1994); these include

shaggy/zeste-white3 (*sgg/zw3*; Bourois et al., 1990; Siegfried et al., 1990), a protein kinase that is negatively regulated by *wg* (Siegfried et al., 1992).

The functions of *wg* in imaginal disc development have recently been characterized. In the leg imaginal disc, *wg* is expressed in a stripe along the ventral half of the anterior-posterior compartment boundary (Baker, 1988b). At the point where it abuts a stripe of *dpp* along the dorsal half of the A-P boundary, the homeobox genes *distalless* and *aristaless* are induced and direct distal outgrowth of the leg (Campbell et al., 1993; Diaz-Benjumea et al., 1994). Ectopic expression of *wg* promotes ventrolateral cell fates (Struhl and Basler, 1993) and can induce a secondary proximal-distal axis if it is present near the region of *dpp* expression (Struhl and Basler, 1993; Campbell et al., 1993). Even high levels of *wg* expression do not allow cells to adopt the ventralmost fates, although these can be induced by the absence of *sgg/zw3* (Diaz-Benjumea and Cohen, 1994; Wilder and Perrimon, 1995). These results have cast doubt on the theory that WG acts as a graded morphogen and suggest that additional components may contribute to determining ventral cell fates. In the wing disc, *wg* has an early role in specifying the ventral compartment and indirectly determining the entire wing (Morata and Lawrence, 1977; Couso et al., 1993; Williams et al., 1993). Later, it acts through the transcription factors *achaete* and *cut* to form the margin of the wing (Phillips and Whittle, 1993; Couso et al., 1994). In the eye disc, *wg* is expressed at the dorsal and ventral margins in the regions that will form head cuticle (Baker, 1988b). We show here that it acts to prevent *dpp* present in these regions from initiating a wave of photoreceptor development. We also show that ectopic *wg* can inhibit the propagation of normal photoreceptor development. Thus *wg* and *dpp* interact to define the region in which the morphogenetic furrow can initiate.



MATERIALS AND METHODS

Fly strains and transgenic fly lines

The *wg* alleles used were wg^{IL114} (Nusslein-Volhard et al., 1984), with secondary lethals removed (Couso et al., 1994), and wg^{1} (Sharma and Chopra, 1976). Other alleles used were $dpp^{d\text{-}blk}$ (Masucci et al., 1990), hh^{1} (Heberlein et al., 1993) and sgg^{D127} (Bourois et al., 1990). Double mutants were made by standard genetic methods. Reporter genes used were the enhancer traps wg^{P} (Kassis et al., 1992) and P30 (Lee et al., 1992), and the *dpp-lacZ* construct BS3.0 (Blackman et al., 1991). The *Act5c>y*$^{+}$*>wg* lines 411.28 and 411.33 (Struhl and Basler, 1993) were combined with hs-FLP (Xu and Rubin, 1993) to induce *flp*-out clones expressing *wg*. Larvae were heat shocked for 1 hour at 38˚C in both the first and second instar. Clones of *sgg* mutant cells were induced by the same heat-shock regimen, using the 18.2πM stock (Xu and Rubin, 1993). Myc expression was induced by a 1 hour heat shock at 38˚C and larvae were allowed to recover for 30 minutes at 25˚C before dissection. The *dpp*-GAL4 line used was 40.C6 (Staehling-Hampton et al., 1994) and this was crossed to UAS-wg^{ts} M7-2-1 (Wilder and Perrimon, 1995).

Fig. 1. Loss of *wg* function leads to morphogenetic furrow initiation at the lateral margins. (A,C,E,G) *lacZ* expression from the *hh* enhancer trap P30; (B,D,F,H) *lacZ* expression from the *dpp* reporter construct BS3.0. (A,B) Wild-type; (C,D) wg^{IL114} larvae shifted to 25˚C in the early third instar and stained 24 hours later; (E,F) wg^{IL114} larvae shifted to 25˚C in the second instar and stained 48 hours later; (G,H) wg^{IL114} larvae shifted to 25˚C in the first instar and stained 72 hours later. There is a progressive movement inward from the dorsal (d) and ventral (v) edges of *hh* expressed posterior to the morphogenetic furrow and *dpp* expressed in the morphogenetic furrow. (I) wg^{IL114} larva shifted to 25˚C in the second instar and stained 48 hours later with anti-Elav antibody. In the dorsal region of the disc, the immature clusters with few Elav-expressing cells (arrow) are furthest from the dorsal margin. Anterior is to the left and dorsal up in this and all subsequent figures unless otherwise indicated.

Histology and immunohistochemistry

Flies were prepared for scanning electron microscopy as described by Kimmel et al. (1990). Adult eyes were fixed, embedded and sectioned as described by Tomlinson and Ready (1987b). Eye imaginal discs were stained with antibodies as described by Tomlinson and Ready (1987a), except that the detergent used was 0.2% Triton. Rat anti-Elav monoclonal antibody was diluted 1:1; mouse anti-Glass monoclonal antibody (Ellis et al., 1993) was diluted 1:5. Mouse myc ascites was diluted 1:100. For double labeling with antibody and X-gal, the antibody staining was performed first and followed by a wash in PBS and incubation in X-gal staining buffer.

RESULTS

wingless inhibits furrow initiation from the lateral margins of the eye disc

The spatially restricted pattern of expression of the *wingless (wg)* gene in the eye disc suggested that *wg* might function in pattern formation in the eye. *wg* RNA is expressed at the dorsal and ventral edges of the eye disc, in regions fated to become head cuticle (Baker, 1988b; Ma and Moses, 1995; Fig. 3E, 6E).

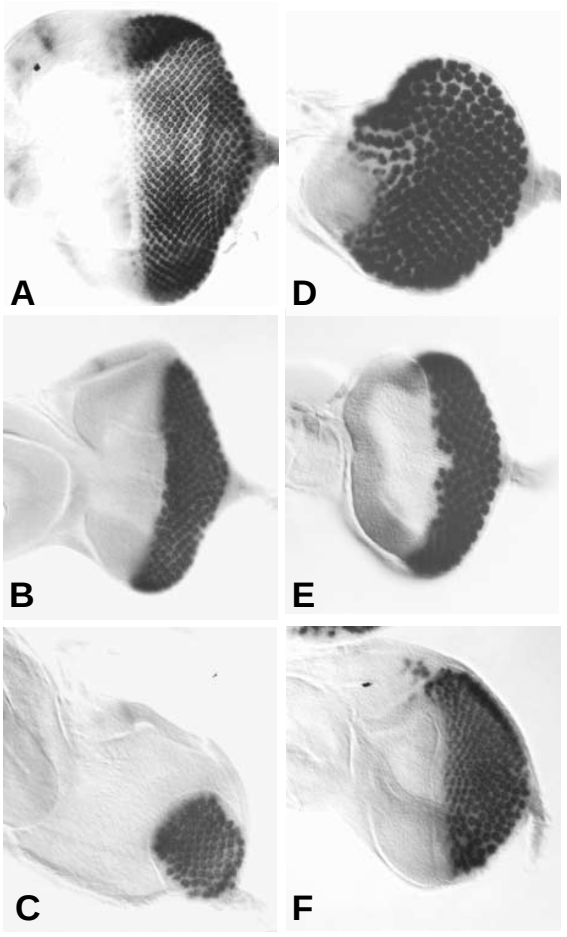


Fig. 2. Furrow movement from the lateral margins requires *hh* and *dpp* function. Eye discs stained with anti-Elav antibody. (A) Wild-type; (B) hh^1; (C) $dpp^{d\text{-}blk}$; (D) wg^{IL114}; (E) wg^{IL114}; hh^1; (F) wg^{IL114}, $dpp^{d\text{-}blk}$. Larvae in (D,E,F) were shifted to 25˚C in the second instar and stained 48 hours later. The inwardly directed furrow movement seen in wg^{IL114} is not present in the double mutants with hh^1 and $dpp^{d\text{-}blk}$.

We have used a temperature-sensitive *wg* mutation, wg^{IL114} (Nusslein-Volhard et al., 1984), to characterize the role of *wg* at this stage of development, and have found that *wg* acts to restrict the initiation of photoreceptor differentiation to the posterior margin of the eye disc.

When *wg* function was inactivated by a shift to the restrictive temperature (25˚C), photoreceptor differentiation progressed medially from the dorsal and ventral margins as well as anteriorly from the posterior margin (Fig. 1). *Hedgehog (hh)* is expressed in differentiated photoreceptors and is thus normally only present posterior to the morphogenetic furrow (Ma et al., 1993; Fig. 1A) but, within 24 hours of a tempera-

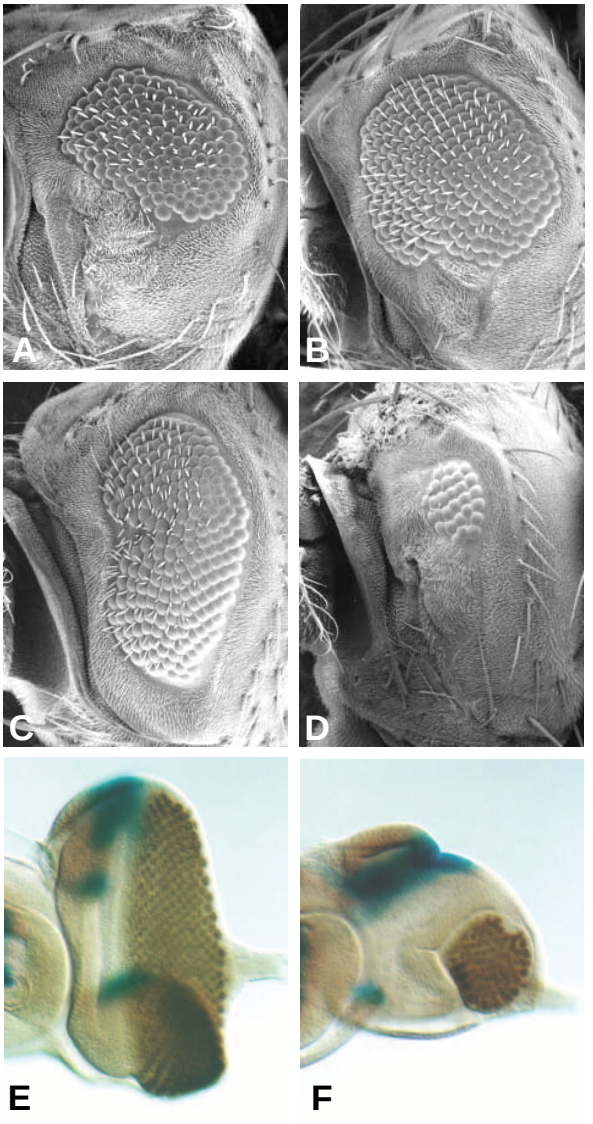


Fig. 3. *wg* suppresses the $dpp^{d\text{-}blk}$ phenotype. (A-D) Scanning electron micrographs of adult eyes. (A) $dpp^{d\text{-}blk}$; (B) $dpp^{d\text{-}blk}$, $wg^{P}/dpp^{d\text{-}blk}$, +; (C) hh^1; (D) hh^1; $dpp^{d\text{-}blk}$. There appears to be some remaining *dpp* in $dpp^{d\text{-}blk}$; its activity is enhanced by the loss of *wg*, making the eye larger and eliminated by the loss of *hh*, making the eye smaller. (E,F) eye discs stained with anti-Elav (brown) and X-gal tp detect *wg-lacZ* (blue). (E) Wild type and (F) $dpp^{d\text{-}blk}$. Neurons only develop in the central region of the $dpp^{d\text{-}blk}$ eye disc; however, *wg-lacZ* expression is not affected.

ture shift, it could also be seen at the dorsal and ventral edges of the eye disc (Fig. 1C). When larvae were shifted at earlier times in development, *hh* expression moved progressively inward, accompanied by a decrease in the size of the third instar disc (Fig. 1E,G). The expression pattern of *decapentaplegic (dpp)* revealed that the morphogenetic furrow curved anteriorly at the edges in the absence of *wg* (Fig. 1D), becoming semicircular 48 hours after a temperature shift (Fig. 1F) and disappearing by 72 hours after a shift (Fig. 1H). Using an antibody to the neuronal protein Elav (Robinow and White, 1991) to reveal the stage of differentiation of the photoreceptor clusters, it was clear that, in the regions of ectopic differentiation, the least differentiated clusters were closest to the center of the disc and to the region of *dpp* expression, confirming that differentiation was progressing in a lateral-to-medial direction (Fig. 1I).

There always appeared to be more ectopic furrow movement on the dorsal side of the disc than the ventral side, correlating with the stronger dorsal than ventral expression of *wg*. However, we have observed ectopic furrow movement initiating predominantly from the ventral margin in the wg^{1} mutant (data not shown), a semiviable allele that causes a deletion of the ventralmost part of the eye (Morata and Lawrence, 1977) in addition to transforming the wing into notal tissue (Sharma and Chopra, 1976; Couso et al., 1993). Thus *wg* does appear to be required at both dorsal and ventral margins.

These results show that in the absence of *wg* the lateral margins are competent to initiate a morphogenetic furrow and *wg* is required to prevent such a furrow from forming. Before furrow initiation, *dpp* is expressed at the dorsal and ventral margins of the eye disc as well as at the posterior margin (Masucci et al., 1990; Fig. 6B). The function of *wg* may be to inhibit its activity at the lateral margins. *wg* itself, as judged by the expression of an enhancer trap inserted in the gene that has been shown to reproduce the pattern of *wg* RNA (Kassis et al., 1992; Couso et al., 1993), is never present at the posterior margin even at early stages (Fig. 6D). To determine whether *hh* and *dpp* were required for the ectopic furrow movement caused by the loss of *wg*, we combined wg^{IL114} with the eye-specific mutations hh^{1} and $dpp^{d\text{-}blk}$. hh^{1} arrests furrow movement after differentiation of 8-10 rows of photoreceptors (Heberlein et al., 1993; Fig. 2B, 3C), while $dpp^{d\text{-}blk}$ allows furrow movement only in the central region of the eye disc (Masucci et al., 1990; Fig. 2C, 3A). The hh^{1} mutation completely abolished the effects of loss of *wg* function (Fig. 2E). In the wg^{IL114}, $dpp^{d\text{-}blk}$ combination, initiation occurred over a wider region than in $dpp^{d\text{-}blk}$ alone, but the extensive inward movement seen in wg^{IL114} alone (Fig. 2D) did not occur (Fig. 2F). Thus the ectopic movement resembles normal furrow movement in its requirement for functional *hh* and *dpp*.

wg suppresses the $dpp^{d\text{-}blk}$ phenotype

In the $dpp^{d\text{-}blk}$ mutant, furrow initiation appears to be inhibited in part of its normal domain (Fig. 2C). This inhibition is alleviated by loss of *wg* function (Fig. 2F), suggesting that *wg* is contributing to the repression. Indeed, even the loss of one copy of *wg* is sufficient to increase the size of the $dpp^{d\text{-}blk}$ eye (compare Fig. 3A and B). However, *wg* expression does not expand into the inhibited regions of the margin (Fig. 3F), so its effect on furrow movement is felt at a distance. This might reflect either diffusion of the WG protein or its indirect action through other factors. It is not clear whether this effect of *wg*, like its effect on the lateral margins of wild-type eye discs, could be achieved by blocking *dpp* function. Although *dpp* RNA is not detectable in third instar $dpp^{d\text{-}blk}$ eye discs (Masucci et al., 1990), several observations lead us to believe that there is some remaining weak or early expression. Firstly, large clones of cells mutant for *dpp* fail to differentiate (Heberlein et al., 1993), and yet there is differentiation in a region of the $dpp^{d\text{-}blk}$ eye. Secondly, if $dpp^{d\text{-}blk}$ mutants did not express *dpp* in the eye disc, we would expect them to be unaffected by the hh^{1} mutation, which causes the loss of *dpp* expression and thus arrests the furrow (Heberlein et al., 1993; Fig. 3C). However, in doubly mutant hh^{1}; $dpp^{d\text{-}blk}$ eye discs, there is also an arrest of furrow movement within the central region (data not shown), reducing the size of the adult eye (Fig. 3D). Finally, the enhancer region deleted in the $dpp^{d\text{-}blk}$ mutant (coordinates 106-110, St. Johnston et al., 1990) does not include all the regulatory sequences active in the eye disc, as it is not sufficient to direct expression in the full *dpp* pattern when fused to a heterologous reporter (see Fig. 5E, F). It is therefore likely that the loss of *wg* suppresses the $dpp^{d\text{-}blk}$ phenotype by allowing a small amount of *dpp* to function more effectively.

Ectopic *wg* inhibits normal furrow initiation and progression

The effects of loss of normal *wg* function showed that *wg* is necessary to inhibit abnormal furrow movement. We next wished to determine whether *wg* was sufficient to inhibit normal furrow movement. We did this by ectopically expressing *wg*, using both the *flp*-out (Struhl and Basler, 1993) and the GAL4 (Brand and Perrimon, 1993) systems. In the adult eye, induction of *wg* expression in random clones of cells using the *flp*-out system led to scar formation; scars always ran in the anterior-posterior direction and usually extended to the anterior margin of the eye (Fig. 4A). These scars were associated with bristle loss in the surrounding ommatidia, an effect that has been shown to be produced by expression of *wg* under the control of the *sevenless* promoter (R. Nusse, personal communication); it is therefore likely that the scars do result from the presence of *wg*-expressing cells. Sections revealed that the scars were devoid of ommatidia but appeared to contain pigment cells. In addition, the dorsal-ventral polarity was reversed in the row of ommatidia next to the scar on the side further from the equator (Fig. 4B). These scars appeared to result from regions in the eye disc where development was delayed or inhibited. In these regions, expression of neuronal markers and of *dpp* and *hh* was delayed relative to the surrounding tissue and there appeared to be overproliferation (Fig. 4C-E and data not shown). The extent of the adult scars suggests that once blocked, the furrow does not reinitiate beyond the position of the block. We assume that these blocked regions result from the presence of *wg*-expressing cells, as they are never observed in wild-type eye discs. However, since the clones of *wg*-expressing cells are not marked, we cannot evaluate the range of *wg* action. No activation of the *wg* enhancer trap was associated with the blocks, suggesting that the endogenous *wg* gene is not required (Fig. 4E).

Similar blocks to furrow progression were caused by loss

of *shaggy (sgg)* function in clones of cells (Fig. 4F). Since *sgg* is inhibited by *wg* (Siegfried et al., 1992), *sgg*$^{-}$ cells behave as though they have received the *wg* signal. Almost all such mutant cells failed to differentiate (Fig. 4G,H) and were not present in the adult eye (data not shown). In addition, wild-type cells anterior to the *sgg*$^{-}$ cells did not express neuronal markers (Fig. 4H), confirming that the furrow is unable to reinitiate beyond a block caused by the *wg* pathway.

Occasionally, regions in the disc where the furrow had failed to initiate were observed after induction of clones of *wg*-expressing cells or *sgg* mutant cells (Fig. 5A,B). This resulted in lack of expression of the Elav and Glass proteins. However, *dpp-lacZ* continued to be expressed at a high level (Fig. 5A). We hypothesized that such blocks resulted from the presence of *wg* pathway function at the posterior margin, which would interfere with initiation. To confirm this, we also misexpressed *wg* specifically at the posterior margin of the eye disc, using a disc-specific enhancer from the *dpp* gene to drive expression of GAL4 at the margins of the disc (Staehling-Hampton et al., 1994) and a GAL4-responsive UAS sequence to drive a temperature-sensitive form of *wg* (Wilder and Perrimon, 1995). When shifted to the permissive temperature (18˚C), ectopic *wg* appears to delay normal furrow initiation. The central region of the disc is least affected, so that initiation occurs in the center ahead of more lateral regions, resembling the phenotype of *dpp*$^{d\text{-}blk}$. However, the expression of *dpp-lacZ* is not affected (Fig. 5D), suggesting that *wg* interferes with the function of *dpp* rather than with its expression.

The eye does eventually develop almost normally in flies carrying the *dpp*-GAL4/UAS-wg^{ts} combination (data not shown), so the block imposed by *wg* is not permanent in this case. The temperature-sensitive form of WG may be less active, even at the permissive temperature, than wild-type WG present at the lateral margins. It has been shown to be less efficiently secreted than the wild-type protein (van den Heuvel et al., 1993), although it is sufficiently active to direct normal embryonic development (Baker, 1988a). There is also a difference between the expression pattern of the *dpp*-GAL4 construct, which contains the enhancer region deleted in the *dpp*$^{d\text{-}blk}$ mutant, and that of *dpp* itself. When crossed to UAS-lacZ, this *dpp*-GAL4 construct did induce early *lacZ* expression at the posterior margin as well as at the lateral margins, reflecting the normal pattern of *dpp* expression at this stage, but this expression remained at the posterior margin rather than progressing anteriorly with the furrow (Fig. 5E,F). However, when used in conjunction with UAS-dpp, the same construct is sufficient to rescue the *dpp*$^{d\text{-}blk}$ phenotype (Staehling-Hampton et al., 1994). Despite these caveats, we can conclude that the presence of *wg* at the posterior margin has at least a mild inhibitory effect on furrow initiation.

DISCUSSION

We have shown that *wg* acts at the dorsal and ventral edges of the eye disc to prevent these marginal regions from initiating neuronal differentiation. This ensures that differentiation initiates only at the posterior margin, in spite of the early expression of *dpp* around the entire margin. Ectopic *wg* can also inhibit both initiation from the posterior margin and progression in the center of the disc. In these instances, the expression of *dpp* is not reduced, although it fails to advance normally.

The requirements for initation are different in three marginal zones

The results in combination suggest a model dividing the margin of the eye disc into three zones (Fig. 6). In the posterior central zone, surrounding the optic stalk, initiation is robustly driven by an unidentified signal, which does not require *hh*, can overcome the presence of *wg* and functions even with greatly reduced levels of *dpp*. This mechanism accounts for the differentiation seen in *dpp*$^{d\text{-}blk}$ and in discs in which *wg* is ectopically expressed at the posterior margin. Initiation only from this zone is also seen in weak alleles of *dac* (Mardon et al., 1994) or *eya* (Bonini et al., 1993); these genes might control the expression of *wg* or act downstream to block its function. There does appear to be some ectopic *wg* expression at the margin of *dac* mutant discs (data not shown). In the posterior zones lateral to this central zone, the furrow initiates at a time when very little *hh* is present, and initiation still occurs in the *hh*^{1} mutant but not in *dpp*$^{d\text{-}blk}$. Thus initiation in this region requires *dpp* but probably does not require *hh*. Ectopic *wg* has an inhibitory effect on initiation from this zone. There may not be a sharp boundary between the posterior central and posterior lateral zones; an alternative would be a gradient of a molecule conferring initiation capability (X in Fig. 6), perhaps derived from a source at the optic stalk. Finally, in the lateral zones, which normally express *wg*, initiation requires the presence of both *dpp* and *hh* and the absence of *wg*. The lateral zone is distinguished from the posterior-lateral zone by its lack of any initiation in the absence of both *wg* and *hh*, as seen in the *wg*^{ts}*; hh*^{1} double mutant.

Although *wg* prevents initiation from the lateral margins (Fig. 1), and *wg* expressed in the central region can block furrow progression (Fig. 4), the furrow is able to progress adjacent to these *wg*-expressing regions. The most likely explanation of this is that *hh* allows the furrow to move past *wg*-expressing cells but not through them. As WG protein is thought to act over a short range, it may produce only a narrow zone of cells unable to respond to *hh*. Another possibility is that *hh* may act indirectly on the transcription of *wg* to inhibit its expression at the edges. The *wg*-expressing clones that we generated using the *flp*-out system express *wg* under the control of the *Actin5C* promoter (Struhl and Basler, 1993), which would presumably not respond to the *hh* signal and would therefore result in a more permanent block to differentiation.

What is the primary effect of *wg*?

The effect of *wg* on furrow initiation could be described as a decision between head cuticle fate and eye fate; regions determined by *wg* to become cuticle would be unable to respond to signals directing eye morphogenesis. Alternatively, the primary function of *wg* could be to prevent cells from responding to the *dpp* signal, with cuticle formation being the default fate. The effect of clones of ectopic *wg* seems to support the latter model. Centrally located cells expressing *wg* fail to respond to *dpp* and the furrow is unable to progress through them. However, their later fate appears to be the formation of

pigment cells rather than cuticle; pigment cells may be the default state of cells in the central region of the eye disc that do not become photoreceptors or cone cells, since these cells are determined synchronously at a stage after passage of the morphogenetic furrow is complete (Cagan and Ready, 1989).

wg may influence eye development in ways other than directly affecting differentiation. Discs in which *wg* has been inactivated reach a smaller final size than wild-type discs. This could be due to stimulation of cell division by *wg*, an effect that has been observed in other tissues (Skaer and Martinez-Arias, 1992; Wilder and Perrimon, 1995). Induction of ectopic *wg* in clones produces outgrowths of disc tissue (Fig. 4C), supporting this hypothesis. Alternatively, the earlier differentiation of cells anterior to the furrow in wg^{ts} discs may simply leave insufficient time for the normal number of cell divisions. However, when differentiation anterior to the furrow is induced by ectopic *hh* expression, it is accompanied by disc overgrowth (Heberlein et al., 1995). The reverse effect seen when such differentiation is induced by loss of *wg* suggests that *wg* may have an active role in directing proliferation.

wg may also influence dorsoventral polarity, which is manifested in the eye as the rotation of ommatidia in opposite directions on the dorsal and ventral sides of the equator (Ready et al., 1976). The direction of ommatidial rotation is reversed in ommatidia adjacent to a scar caused by ectopic *wg* expression (Fig. 4B). Possibly *wg* expressed at the edges of the disc is one of the signals directing ommatidial rotation. This might explain the polarity defects seen in *dishevelled (dsh)* mutant eyes (Theisen et al., 1994), since *dsh* has been shown to act downstream of *wg* (Noordemeer et al., 1994; Siegfried et al., 1994).

The interactions between *dpp* and *wg* vary in different tissues

Although *dpp* and *wg* both affect the development of many tissues in the fly, the relationship between them is not invariant. During embryogenesis, *wg* acts as a segment polarity gene, determining cell identity along the anterior-posterior axis of each segment (Baker, 1988a); *dpp* acts dorsally to determine the perpendicular dorsoventral axis (Ferguson and Anderson, 1992). In wing development, *wg* and *dpp* again direct development along mutually perpendicular axes, but *wg* establishes the dorsoventral and *dpp* the anterior-posterior axis (Williams et al., 1993; Couso et al., 1993, 1994; Basler and Struhl, 1994).

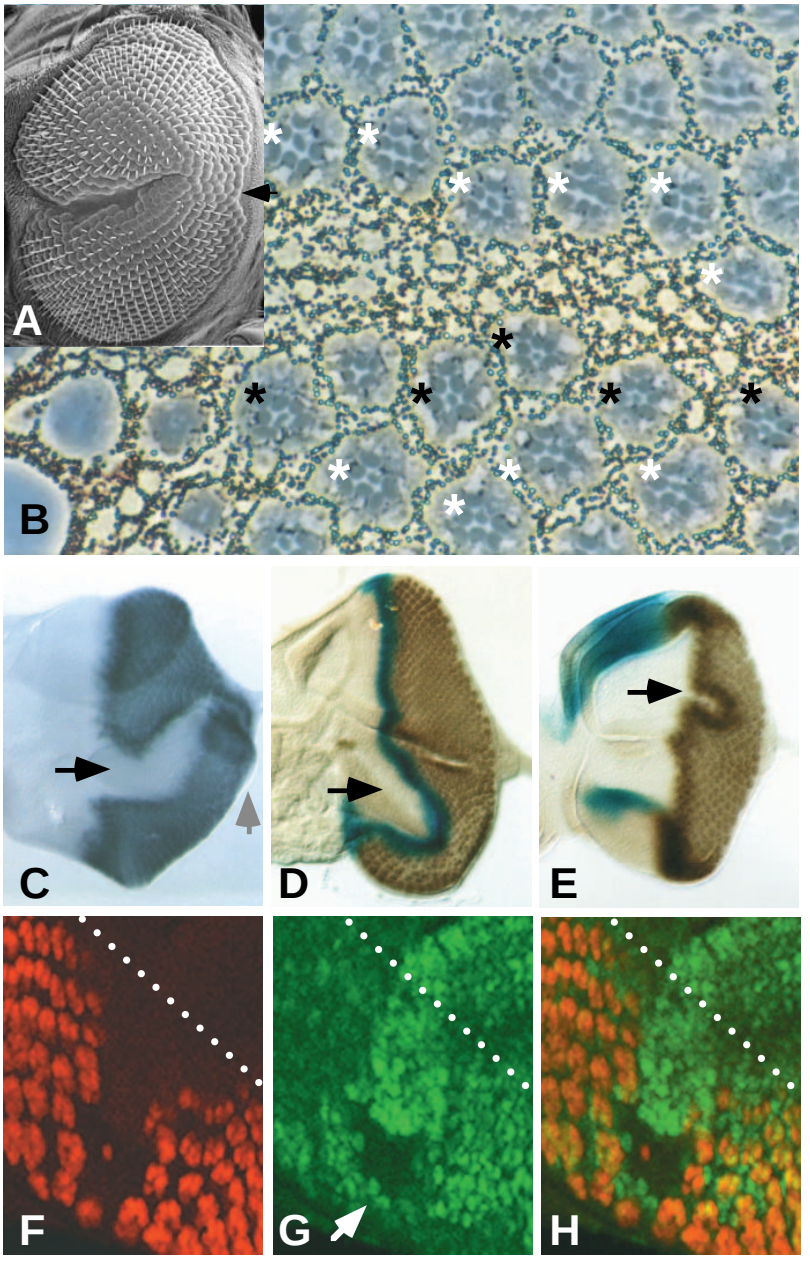


Fig. 4. Clones of cells expressing *wg* or mutant for *sgg* prevent furrow progression. (A) Scanning electron micrograph of an adult eye in which a clone expressing *wg* from the *Actin5c* promoter was induced at first or second instar using the *flp*-out system. A scar extends to the anterior margin of the eye and bristles are missing from the surrounding ommatidia (arrow). (B) Section through an adult eye similar to that shown in A. A scar across the eye contains no ommatidia, but appears to contain pigment cells. One column of ommatidia adjacent to the scar shows reversed dorsoventral polarity (black asterisks; white asterisks identify ommatidia with normal polarity on both sides of the scar. The polarity of mutant ommatidia cannot be scored, so these are unmarked). The equator is above the top of the region shown. (C-E) Eye discs in which clones of cells expressing *wg* have been induced as in A. (C) Anti-Glass staining shows a large delay in differentiation (black arrow) accompanied by an overgrowth of disc tissue (grey arrow). (D) Anti-Elav (brown) and X-gal staining to detect *dpp-lacZ* (blue) shows that *dpp* expression remains posterior to the block (arrow) but does not progress through it. (E) Anti-Glass (brown) and *wg-lacZ* (blue). A region of delayed differentiation (arrow) is seen; note that ectopic *wg-lacZ* is not induced. (F-H) Confocal images of a clone of sgg^- cells (arrow in G) identified by lack of expression of the π-myc marker (green in G and H). The neuronal protein Elav (red in F and H) is not expressed in either the sgg^- cells or the wild-type cells anterior to them. Anterior is up and to the right; the morphogenetic furrow is marked by dotted lines.

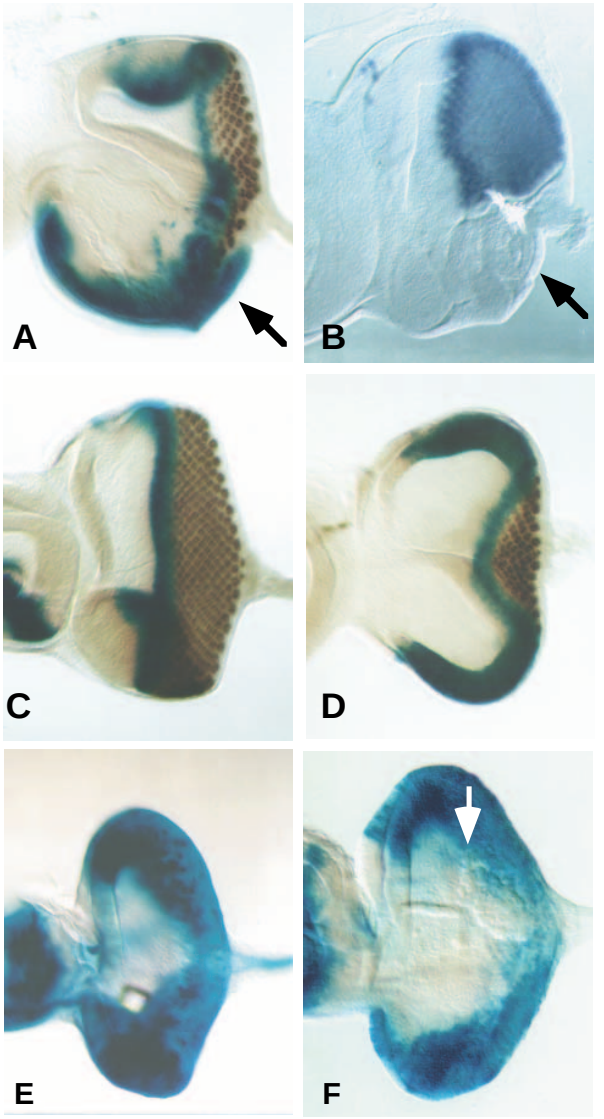


Fig. 5. *wg* function at the posterior margin inhibits initiation. (A,C,D) Eye discs stained with anti-Elav (brown) and X-gal to detect *dpp-lacZ* (blue). (B) Eye disc stained with anti-Glass. (A,B) Flies in which clones of *wg*-expressing cells (A) or *sgg* mutant cells (B) were induced at first or second instar. Initiation from a region of the margin is inhibited (arrows). (C,D) Flies carrying *dpp*-GAL4 alone (C) or crossed to UAS-wg^{ts} (D). Eggs were collected for 24 hours at 25˚C, aged at 25˚C for 2 days and then shifted to 18˚C and grown for 6 days; larvae were dissected at the wandering third instar stage. The presence of *wg* at the posterior margin delays furrow initiation and has a stronger effect in posterior-lateral than central regions. (E,F) Eye discs from flies carrying *dpp*-GAL4 and UAS-*lacZ*, stained with X-gal. (E) Early third instar; (F) late third instar. The *dpp* enhancer directs expression at the lateral and posterior margins at both developmental stages and not in the morphogenetic furrow (arrow in F).

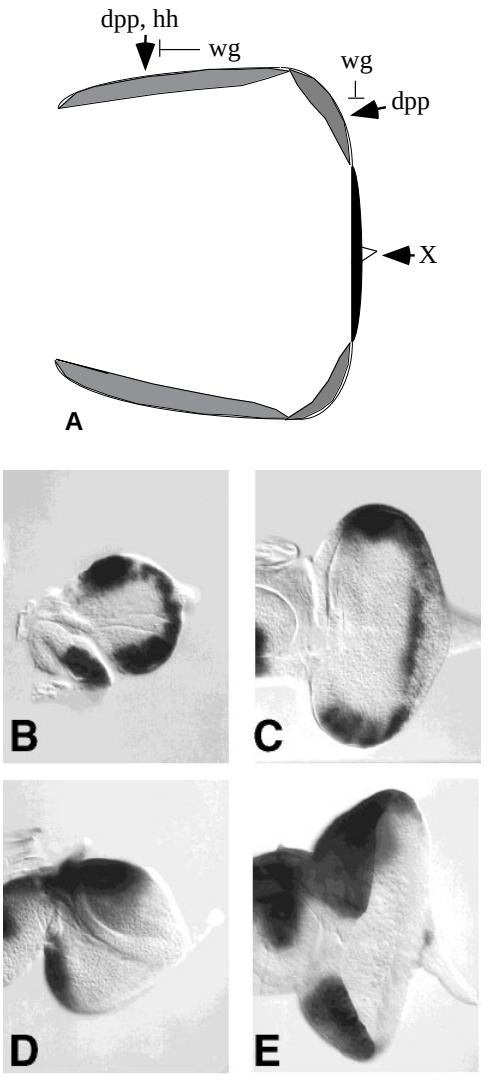


Fig. 6. Three zones can be distinguished at the margin of the eye disc. (A) A diagram showing the requirements for *hh*, *dpp* and *wg* in all three zones; only those molecules affecting initiation in each zone are shown. In the posterior central zone (black), initiation is driven by an unknown signal, X, which requires neither *hh* nor high levels of *dpp* and is not inhibited by *wg*. In the posterior lateral zone (striped) initiation requires *dpp* but not *hh* and can be inhibited by *wg*. In the lateral zone (shaded), where *wg* is normally expressed, initiation requires both *dpp* and *hh* and is inhibited by *wg*. (B,C) The expression pattern of a *dpp-lacZ* reporter construct and (D,E) the expression pattern of a *wg* enhancer trap. (B,D) Early third instar, prior to furrow initiation; (C, E) mid third instar, just after initiation. *dpp* is initially present at all the margins and *wg* at the lateral margins; the furrow initiates only at the posterior margin, where *dpp* but not *wg* is present. The *dac*, *so* and *eya* genes are also expressed at the posterior and lateral margins, but the nature of their interaction with *hh*, *dpp* and *wg* is not known.

Gut development requires *dpp* and *wg* to act on the common target *labial* (Immergluck et al., 1990). During leg development, specification and outgrowth are both induced by the presence of cells expressing *wg* adjacent to cells expressing *dpp* (Cohen, 1990; Campbell et al., 1993; Basler and Struhl, 1994). In the optic lobes of the brain, *wg* activates expression of *dpp* in an adjacent domain (Kaphingst and Kunes, 1994). Finally, we show here that, in the eye disc, *wg* interferes with the function of *dpp* but not with its expression. In vertebrates, preliminary indications also favor a variety of relationships between members of the *Wnt* and TGF-β gene families (Nusse and Varmus, 1992; Kingsley, 1994). These signaling molecules may have been co-opted for many different uses, with their relationship to each other remaining flexible.

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