Cell, Vol. 75, 913–926, December 3, 1993, Copyright © 1993 by Cell Press

The TGFβ Homolog *dpp* and the Segment Polarity Gene *hedgehog* Are Required for Propagation of a Morphogenetic Wave in the Drosophila Retina

Ulrike Heberlein,*† Tanya Wolff,† and Gerald M. Rubin†
*Gallo Center
University of California, San Francisco
at San Francisco General Hospital
San Francisco, California 94110
†Howard Hughes Medical Institute
Department of Molecular and Cell Biology
University of California, Berkeley
Berkeley, California 94720

Summary

Development of the Drosophila retina occurs asynchronously; differentiation, its front marked by the morphogenetic furrow, progresses across the eye disc epithelium over a 2 day period. We have investigated the mechanisms by which this front advances, and our results suggest that developing retinal cells drive the progression of morphogenesis utilizing the products of the *hedgehog* (*hh*) and *decapentaplegic* (*dpp*) genes. Analysis of *hh* and *dpp* genetic mosaics indicates that the products of these genes act as diffusible signals in this process. Expression of *dpp* in the morphogenetic furrow is closely correlated with the progression of the furrow under a variety of conditions. We show that *hh*, synthesized by differentiating cells, induces the expression of *dpp*, which appears to be a primary mediator of furrow movement.

Introduction

The developing Drosophila eye is well suited for studying the mechanisms that underlie the formation of a complex structure from a simple unpatterned epithelium, the eye disc. A highly orchestrated series of events, which include regulation of cell division, pattern formation, differentiation, and cell death, transforms the unpatterned epithelium into an ordered array of approximately 800 ommatidia that form the adult retina. The eye disc forms from a few ectodermal cells that invaginate during mid-embryogenesis (Poulson, 1950). Until the third larval instar, the eye disc remains a simple unpatterned epithelium. Retinal pattern formation begins in mid-third instar larvae, in the morphogenetic furrow (MF), which sweeps across the eye disc from posterior to anterior in approximately 2 days (Ready et al., 1976; Tomlinson and Ready, 1987a). The MF is a dorsoventral indentation that results from contraction of the apical–basal dimension of cells. In addition, cells in the MF have greatly reduced apical surfaces (Wolff and Ready, 1991a). Cells located anterior to the MF are actively dividing and appear completely unpatterned; cells posterior to the MF assemble into ommatidia in a stepwise process (for reviews see Tomlinson, 1988; Ready, 1989; Banerjee and Zipursky, 1990). Thus, the furrow is a transient change in the shape of retinal epithelial cells; it follows temporally an inhibition of cell division, it coincides with the first signs of pattern formation, and it precedes ommatidial assembly and differentiation. Owing to the asynchronous nature of eye development, all of these developmental stages are laid out spatially and can be observed simultaneously in a single eye disc. The mechanisms that mediate movement of the furrow across the eye disc are the focus of this paper.

Two different, but nonexclusive, mechanisms have been proposed to explain the advance of the MF. First, cells in the furrow could act as an inductive front that uses the pattern established posterior to the furrow as a template onto which new elements are incorporated. In this model, the information necessary for the anterior progression of morphogenesis is thought to be provided by cells located in or posterior to the MF. This hypothesis, based primarily on the lack of cell lineage restrictions in the retina and the observed pattern of ommatidial assembly just posterior to the furrow, was first suggested by Ready et al. (1976). Second, the furrow and subsequent pattern formation could be the consequence of a developmental program already mapped onto the disc and in progress anterior to the MF. This hypothesis, proposed by Lebovitz and Ready (1986), is supported by a series of transplantation experiments; eye disc fragments from which the MF and the patterned posterior disc region had been removed were able to generate ommatidia after culture in larval hosts.

In this paper we have taken several approaches to studying the mechanisms and the molecules involved in progression of the MF. Our findings suggest that the segment polarity gene *hedgehog* (*hh*; Nüsslein-Volhard and Wieschaus, 1980) and the transforming growth factor β (TGFβ) homolog *decapentaplegic* (*dpp*; Spencer et al., 1982; Padgett et al., 1987) play central roles in mediating cell–cell communication required for MF progression. Analysis of *hh* and *dpp* genetic mosaics revealed that their action is nonautonomous in the eye and that loss of function leads to a failure in morphogenesis. Expression of *dpp* in the MF is abolished in mutants that disrupt the progression of the MF; suppression of this phenotype by the dominant mutation *Rough eye* (*Roi*) coincides with restoration of *dpp* expression. If differentiation of photoreceptor cells is inhibited by loss of Star function, expression of *hh* posterior to the MF and *dpp* in the MF is abolished. We propose that expression of *hh* by differentiating photoreceptor cells posterior to the MF is required for expression of *dpp* in the MF and, consequently, for the progression of the furrow across the eye disc.

Results and Discussion

Mutations That Interfere with the Advance of the MF

To gain insight into the mechanisms of MF movement and its relationship to the closely linked events of pattern formation and ommatidial differentiation, we analyzed a se-

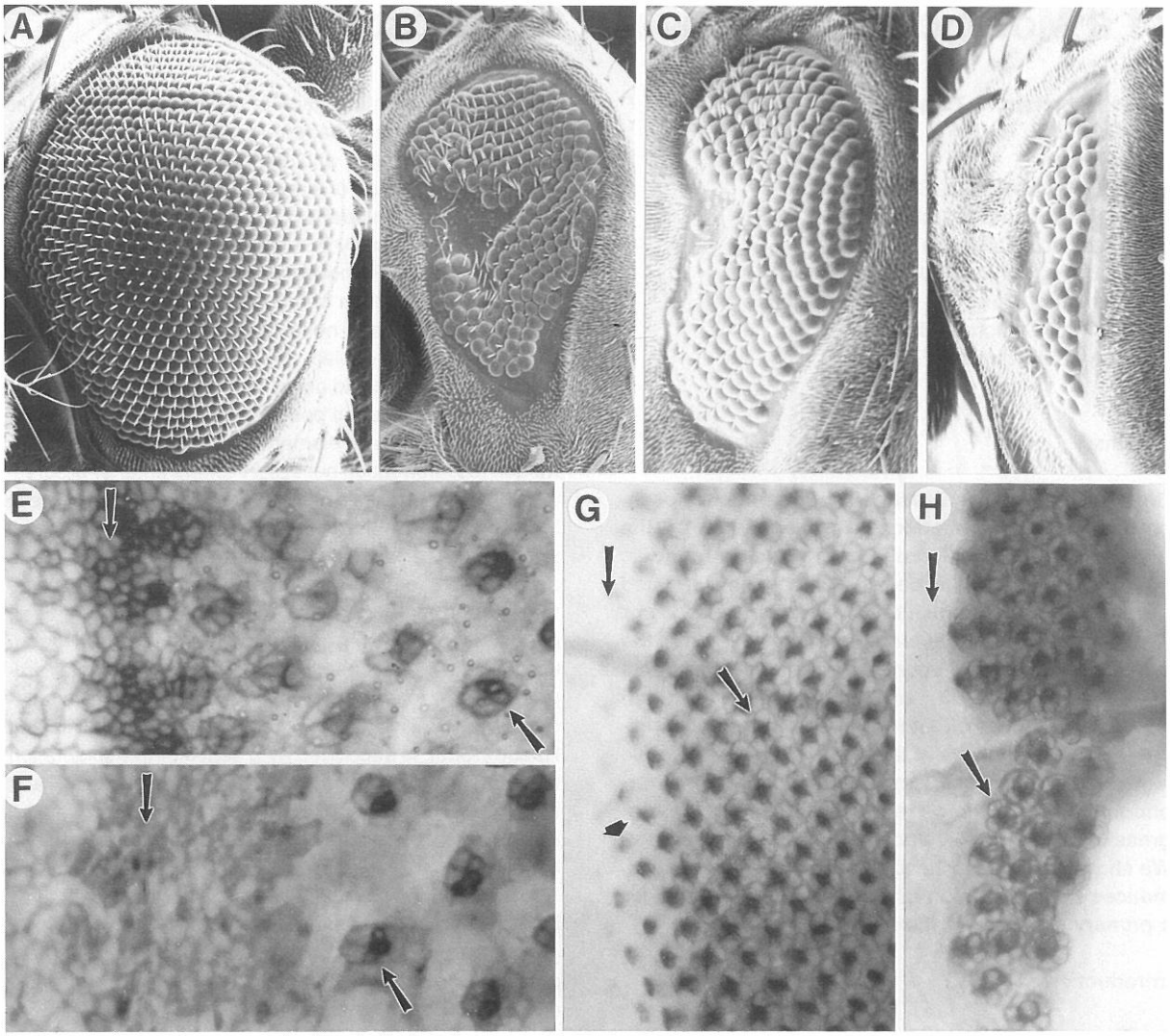


Figure 1. Phenotype of Furrow-Stop Mutants

(A–D) Scanning electron micrographs of adult eyes corresponding to flies of the following genotypes: wild type (A), ro^{DOM}/+ (B), hh^{1}/hh^{1} (C), and *Bar*/*Y* (D).

(E and F) Equatorial regions of eye discs from late third instar larvae stained with cobalt sulfide. Posterior is to the right. (E) Wild type. Cells in the MF (vertical arrow) display reduced apical projections. Anterior to the MF, the epithelium appears unpatterned; posterior to the MF, cells assemble into ommatidial clusters. (F) ro^{DOM}/+. The MF (vertical arrow), which had stopped approximately 12 hr earlier, is observed adjacent to relatively mature ommatidia (arrow). These more mature ommatidia are normally observed several rows posterior to the MF (compare with ommatidium, indicated by an arrow in [E]). In addition, the apical projections of cells in the stopped MF of ro^{DOM}/+ appear to be larger than those observed in the MF of wild-type discs.

(G and H) Eye discs stained with the neural-specific MAb BP104. (G) Wild type. Cells posterior to the MF (vertical arrow) become BP104-positive as they are incorporated into ommatidia; near the MF, staining is observed in three presumptive photoreceptor cells (arrowhead), whereas in more posterior regions, staining is observed in all eight developing photoreceptors (arrow). (H) ro^{DOM}/+. Ommatidia located just posterior to the stopped MF (vertical arrow) contain eight BP104-positive cells (arrow), indicating that the furrow in this particular eye disc had stopped at least 12 hr earlier.

ries of mutants in which the furrow fails to complete its progression across the eye disc, resulting in an adult eye that is reduced in size (Figure 1). We described previously the ectopic expression of the homeobox gene *rough* in flies carrying a heat-inducible *rough* gene (*hs-ro* flies) that causes an arrest in morphogenesis, resulting in flies missing portions of the anterior eye (Kimmel et al., 1990). Similarly, a recently isolated dominant allele of *rough* (ro^{DOM}; see Experimental Procedures), in which rough is ectopically expressed anterior to the MF, results in a reduced eye phenotype (Figure 1B). Several existing mutants have analogous phenotypes: hh^{1}, a viable recessive allele of *hh* (Figure 1C; Mohler, 1988), and the dominant mutations *Bar* (Figure 1D) and *Drop* (data not shown).

The development of the eye phenotype of these mutants was analyzed by staining eye discs from third instar larvae with cobalt sulfide (Figures 1E and 1F) to visualize the MF and developing cell clusters that will give rise to the adult

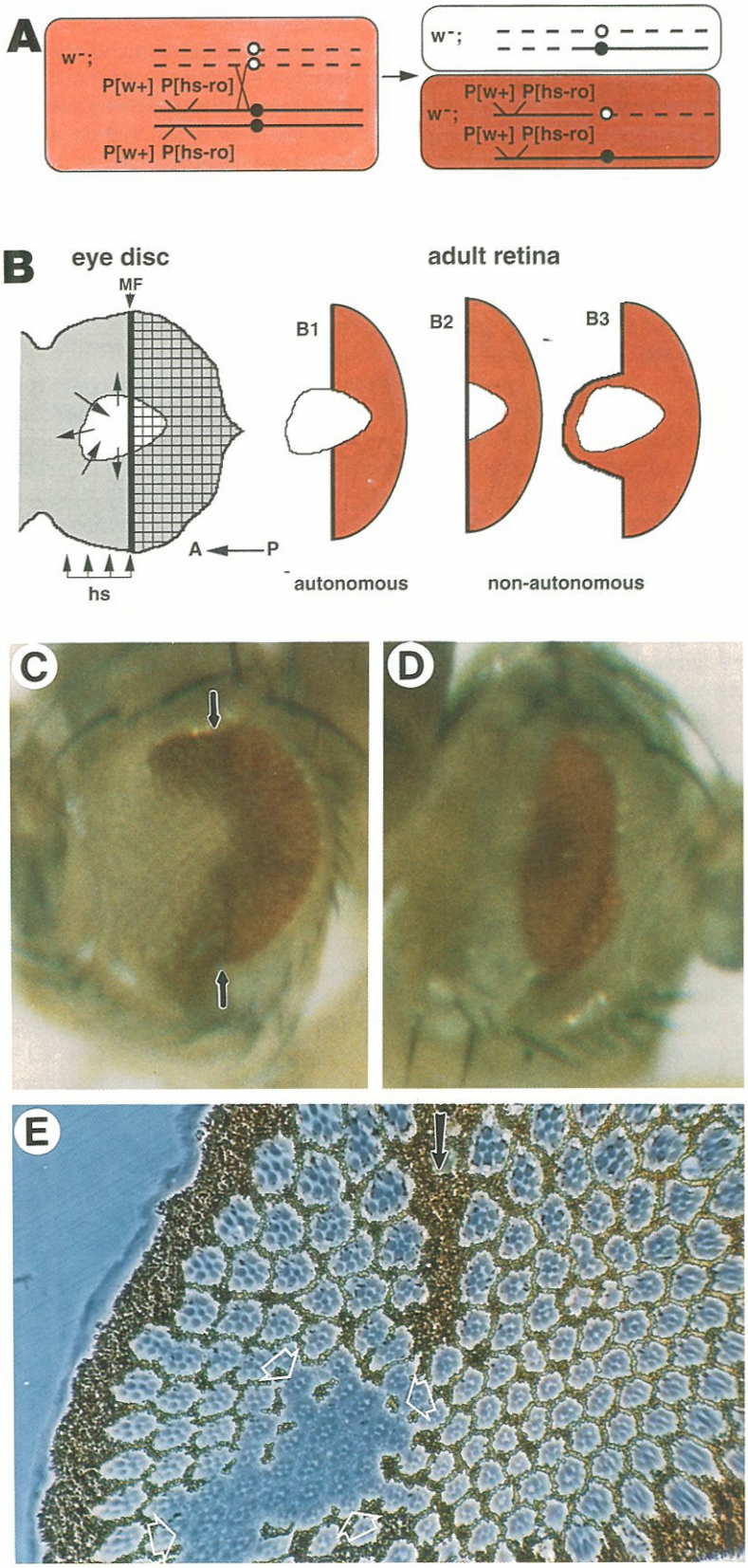


Figure 2. Analysis of *hs-ro* Mosaic Eyes

(A) Schematic representation of the chromosomes used to generate non-*rough*-expressing clones in eye discs from flies carrying an *hs-ro* transgene (P*[hs-ro]*). Heterozygous larvae contain a chromosome carrying the P*[hs-ro]* transgene linked to the cell-autonomous eye pigmentation marker w^+ (P$[w^+]$) and an unmarked wild-type chromosome. X-ray-induced somatic recombination produces a cell homozygous for the unlabeled wild-type chromosome as well as a cell homozygous for P*[hs-ro]* and the P$[w^+]$ marker. These cells divide to generate clones of cells.

(B) Potential phenotypes resulting from mosaic *hs-ro* eyes. Somatic recombination was induced in first instar larvae. Mosaic late third instar larvae are subjected to heat shocks at 12 hr intervals for 2 days, a treatment that causes the MF to stop irreversibly owing to ectopic expression of *rough*. If the MF is crossing a non-*rough*-expressing (unshaded) clone at the time the heat treatments are initiated, one of the following adult phenotypes might be expected: if the effect of *hs-ro* is autonomous, non-*rough*-expressing cells (unshaded area) will continue to develop in the presence of ectopic rough, while the *hs-ro* (shaded) tissue will be prevented from doing so (B1). If the effect of *hs-ro* is nonautonomous, 1 of 2 potential phenotypes are expected: if ectopic rough induces a diffusible molecule that prevents MF progression, this inhibitor may repress the advance of the MF in the non-*rough*-expressing clone (B2); alternatively, if ectopic rough represses the expression of a diffusible molecule normally required for MF progression, cells in the non-*rough*-expressing clone might rescue surrounding *hs-ro* tissue by providing the diffusible molecule (B3).

(C and D) Both eyes of a fly carrying a wild-type clone in a *hs-ro* background and subjected to multiple heat shocks as described above. The left eye (C) contains a large non-*rough*-expressing clone. The position at which eye development would have stopped in the absence of this clone, inferred from the size of the right eye (D), is marked by arrows. Many *hs-ro* ommatidia surrounding the non-*rough*-expressing clone (to the left of the arrows) developed in the presence of ectopic rough, consistent with the nonautonomous model illustrated in B3 of (B).

(E) Tangential section through a mosaic eye similar to that shown in (C). The non-*rough*-expressing clone (outlined by white arrowheads) is recognized by the absence of pigment. The scar (arrow) indicates the position where eye development would have stopped in the absence of the clone of non-*rough*-expressing cells (see Kimmel et al., 1990). Several pigmented *hs-ro* ommatidia developed anterior to the scar adjacent to the clone (to the left of the scar). Posterior is to the right, with the exception of (D) in which posterior is to the left.

ommatidia. The neural-specific monoclonal antibody (MAb) BP104 (Hortsch et al., 1990) (Figures 1G and 1H) was used to observe neuronal differentiation. In ro^{DOM} eye discs, the MF, identified by the reduced apical projection displayed by cells located in it, was observed just anterior to mature ommatidia (Figure 1F), some of which contain a full complement of BP104-positive photoreceptor cells (Figure 1H). Such mature ommatidia are normally located 6–8 rows more posterior (Figures 1E and 1G), indicating that in the mutant the furrow had stopped several hours prior to dissection. Thus, ommatidia that started differentiating prior to the time when the MF stopped continued to develop normally. Curiously, the furrow stopped unevenly; it first stopped near the equator and then gradually in more lateral regions of the eye disc (Figure 1H), resulting in kidney-shaped adult eyes (Figures 1B–1D). Results similar to those shown for ro^{DOM} eye discs were observed with hh^1, *Bar*, and $Drop^{Mio}$ (data not shown). We have collectively called these mutants furrow-stop mutants. A feature that distinguishes them from other mutants with eyeless or reduced eye phenotypes, such as *Lobe* (see Figures 3E and 5E), *eyeless-dominant* (Renfranz and Benzer, 1989), and *eye gone* (data not shown), is that the eye discs appear normal in size at the time when the furrow has stopped. This suggests that the eye phenotypes that we are analyzing are not the primary consequence of a general failure in cell proliferation or excessive cell death, but rather are a specific defect in subsequent morphogenetic events.

Inhibition of the MF Is Nonautonomous

To determine whether stopping of the furrow involved cell autonomous or nonautonomous mechanisms, we carried out genetic mosaic studies. We used ectopic *rough* expression, induced by multiple heat shocks of *hs-ro* flies, to inhibit movement of the furrow (Figure 2; see Experimental Procedures). We then asked what effect the presence of a clone of cells that lack the *hs-ro* transgene (marked with *white*$^-$ [w^-]), and therefore could not express *rough* ectopically (referred to as non-*rough* expressing), would have on the differentiation of surrounding *hs-ro* tissue (marked with w^+) (Figures 2A and 2B). If stopping of the furrow is autonomous, we expect that ommatidia in the non-*rough*-expressing clone (w^-) would continue to differentiate, while the surrounding cells (*hs-ro*, w^+) would be inhibited from doing so by ectopic *rough* expression. If the process is nonautonomous, 1 of 2 outcomes would be expected. If ectopic *rough* expression induces a diffusible inhibitor of furrow movement, the non-*rough*-expressing (w^-) clone would be partly or completely prevented from differentiating, resulting in adult eyes in which the w^- clones do not extend significantly beyond the position at which the furrow stopped. Alternatively, if ectopic *rough* expression inhibits a diffusible signal that is necessary for furrow movement, non-*rough*-expressing tissue in the clone might rescue surrounding *hs-ro* (w^+) ommatidia from the inhibition imposed by ectopic rough. Figures 2C and 2D show both eyes of an *hs-ro* fly; the left eye contains a large clone of non-*rough*-expressing cells (Figure 2C) and the right eye (Figure 2D) serves as a control to establish the position of the furrow at the time when heat shock treatments were initiated. The *hs-ro* (w^+) ommatidia that surround w^- clones were rescued from the block in differentiation induced by ectopic rough (Figure 2C). Analysis of sections from mosaic eyes revealed that the *hs-ro* region that developed adjacent to the clone in the presence of ectopic rough was 2–5 ommatidia wide (Figure 2E). These data suggest that normal cells express a diffusible signal required for MF movement and that ectopic rough inhibits the expression of this signal.

dpp Expression in the MF Is Abolished in Furrow-Stop Mutants

A good candidate for mediating the nonautonomous effects described above is the dpp protein, a member of the TGFβ family (Padgett et al., 1987). Dpp is known to act nonautonomously (Bryant, 1988; Posakony et al., 1991), it is expressed in the MF (Masucci et al., 1990; Blackman et al., 1991; Figure 3), and some viable *dpp* alleles display reduced eye phenotypes (Spencer et al., 1982). To ask whether altered expression of *dpp* in furrow-stop mutants might be the cause of the arrest in morphogenesis, we generated flies that carry a disc-specific *dpp* enhancer fused to the bacterial *lacZ* gene (*dpp–lacZ*; Blackman et al., 1991) in the appropriate mutant background. Expression of the *dpp–lacZ* construct, visualized by histochemical detection of β-galactosidase, closely mimics the normal distribution of *dpp* mRNA in imaginal discs (Blackman et al., 1991). The expression of *dpp–lacZ* in the MF was abolished in ro^{DOM} (Figure 3C), hh^1 (Figure 3D), *Bar*, and *Drop* (data not shown) late third instar larvae at a time when the furrow had been stopped for approximately 12–24 hr. Analysis of eye discs from younger ro^{DOM} third instar larvae revealed that β-galactosidase expression was abolished first near the equator (Figure 3B), thus closely paralleling the inhibition of furrow progression both temporally and spatially. As a control, we assayed *dpp–lacZ* expression in the mutant *Lobe* (see Figure 5E) in which the adult reduced eye phenotype is caused by a severe size reduction of the eye disc such that there is little tissue over which the MF can progress. Normal levels of *dpp–lacZ* expression were observed in all third instar *Lobe* eye discs analyzed (Figure 3E), suggesting that loss of *dpp* expression in furrow-stop mutants is not a consequence of the absence of MF movement, but possibly its cause.

A single pulse of heat-induced ectopic *rough* expression administered to middle to late third instar *hs-ro* larvae reversibly stops the MF for 30–32 hr (Kimmel et al., 1990). As expected, this treatment abolished *dpp*-driven β-galactosidase (*dpp–lacZ*) expression in the MF (Figure 3F). To establish further a causal relationship between furrow movement and *dpp* expression, we stained *hs-ro* eye discs at various times after a single heat pulse; expression of β-galactosidase was inhibited after 12 hr (Figure 3F), partially restored after 20 hr (Figure 3G), and completely restored after 26 hr (Figure 3H). The furrow had not obviously resumed its progression across the eye disc at the time when *dpp–lacZ* expression had been restored (24–26 hr after heat shock). Thus, full restoration of *dpp–lacZ* ex-

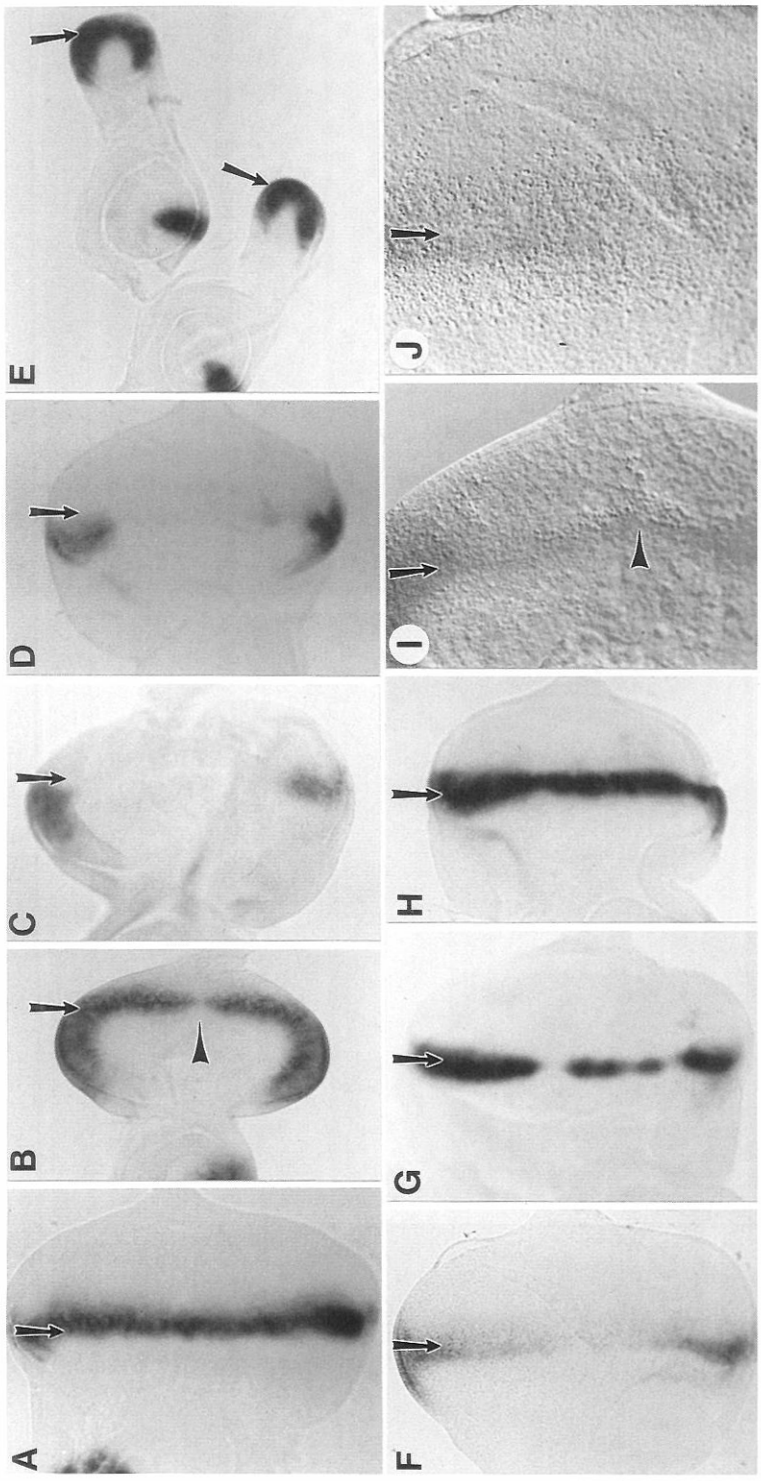


Figure 3. Expression of *dpp–lacZ* in the Eye Discs from Furrow-Stop Mutants

Eye discs from wild-type (A) and mutant (B–J) larvae carrying a *dpp* enhancer fused to *lacZ* (Blackman et al., 1991) were stained for β-galactosidase activity. The position of the MF is indicated by arrows.

(A) Expression of *dpp–lacZ* is restricted to the MF in wild-type late third instar larvae.

(B and C) *dpp–lacZ* expression in ro^{DOM}/+ larvae is still present in eye discs from young mid-third instar larvae (B), but is abolished in older larvae (C). Expression is first inhibited near the equator (arrowhead in [B]) and later in more lateral eye regions.

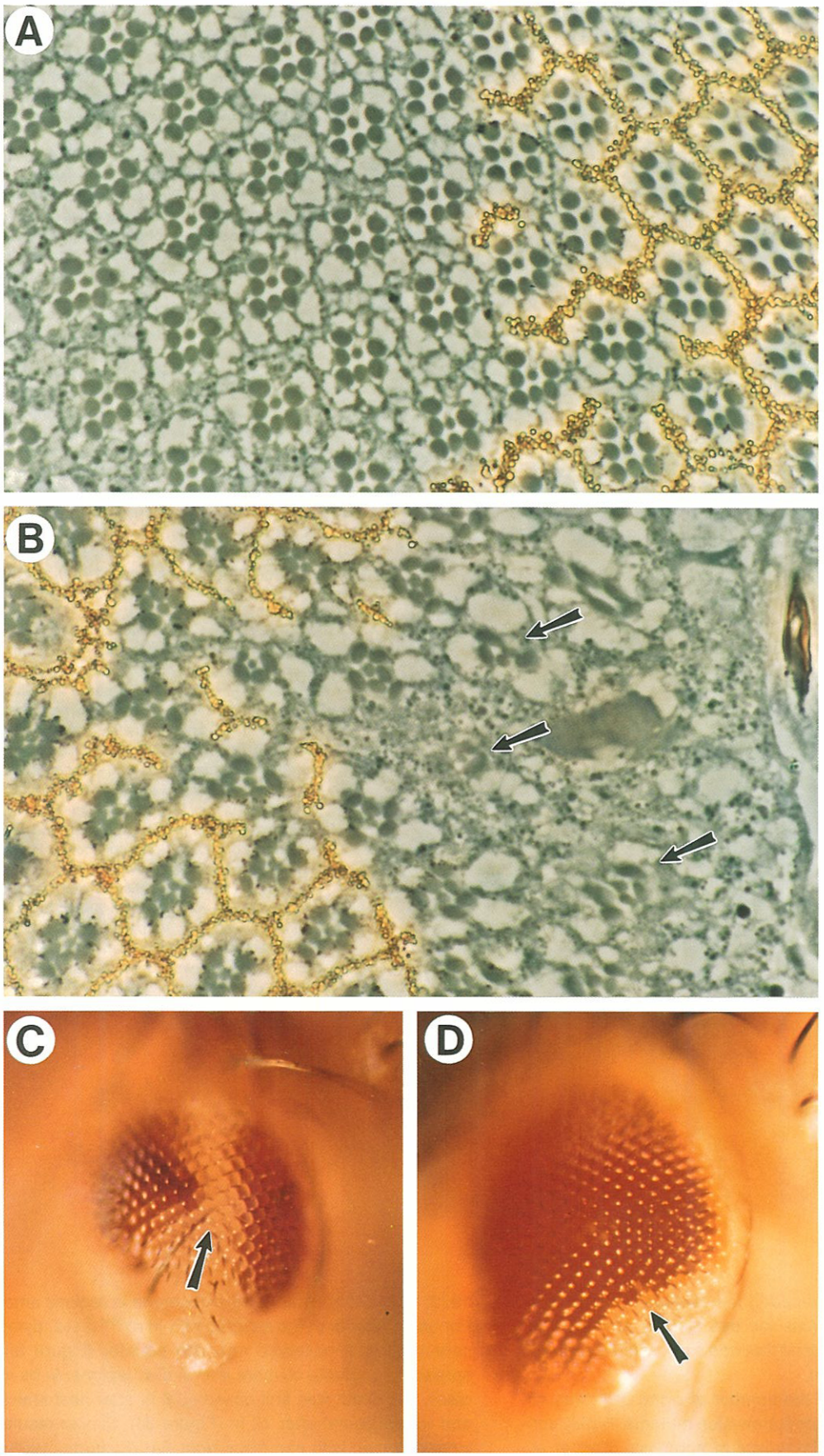
(D) The inhibition of *dpp–lacZ* expression in hh^1/hh^1 larvae follows a similar pattern to that observed in ro^{DOM}/+.

(E) Expression of *dpp–lacZ* in eye–antennal disc complexes from a late third instar *Lobe* larva is quantitatively normal, although the size of the eye discs is severely reduced. The MF, visible with Nomarski optics, is still positioned at the posterior edge of the disc and has presumably not advanced because of the lack of tissue anterior to it. The expressivity of *Lobe* is variable; in larvae with larger eye discs, a normal MF and its associated *dpp–lacZ* expression were observed (data not shown), indicating that *Lobe* does not directly affect MF progression.

(F–J) Expression of *dpp–lacZ* in the MF of eye discs from *hs-ro* larvae at different times after a single heat shock has stopped the progression of the MF. Expression is nearly abolished 12 hr after heat shock (F), partially recovered after 20 hr (G), and completely restored after 26 hr (H). The MF does not obviously resume its anterior progression until 30–32 hr after heat shock (Kimmel et al., 1990). The loss of *dpp* expression in the MF of *hs-ro* larvae 12 hr after heat shock was confirmed by in situ hybridization with a *dpp* cDNA (data not shown). Highly refractive dying cells (arrowhead in [I]) are observed with Nomarski optics surrounding the stopped MF in ro^{DOM}/+ larvae (I) and in *hs-ro* larvae 12 hr after the MF had stopped (J). Similar results were obtained when staining with acridine orange was used to visualize cell death (data not shown). Posterior is to the right.

pression in the MF preceded the obvious resumption of furrow movement across the eye disc by at least 4 hr, supporting the hypothesis that *dpp* expression is required for furrow progression.

A large increase in the number of dying cells was observed in eye discs from furrow-stop mutants. Highly condensed refractive cells, typical of apoptosis (Kerr et al., 1972; Wyllie et al., 1980), were observed in regions anterior and posterior to the stopped MF. In ro^{DOM} discs, dying cells were first observed near the equator (Figure 3I), spreading gradually to more lateral disc regions. In *hs-ro* discs, cell death was first observed 8 hr after heat shock and was maximal after 12 hr (Figure 3J). Similar results were obtained with eye discs from *Bar* (Fristrom, 1968),


A
B
C
D

hh^{1}, and *Drop* flies (data not shown). The initial appearance of dying cells in all mutants analyzed followed the inhibition of furrow movement, both spatially and temporally, by several hours, suggesting that cell death is the consequence of the failure in morphogenesis. Extensive cell death is also observed in eye discs from larvae carrying various alleles of *dpp* (Bryant, 1988; Masucci et al., 1990).

In summary, our data show that *dpp* expression (measured as *dpp*-driven β-galactosidase expression) is abolished by all mutations that appear to affect specifically the ability of the MF to progress across the eye disc. In addition, it appears that loss of *dpp* expression is the cause rather than the consequence of the failure in morphogenesis.

dpp Clones Display Nonautonomous Phenotypes Consistent with a Role in Furrow Movement

We have described the loss of *dpp* expression that parallels the inhibition of furrow progression in furrow-stop mutants. To analyze directly the role of *dpp* in eye morphogenesis, we analyzed the eye phenotype of clones of cells homozygous for several embryonic lethal *dpp* alleles (*dpp*^{hr56}, *dpp*^{hr4}, and *dpp*^{hr27}; Irish and Gelbart, 1987; St. Johnston et al., 1990) in genetically mosaic flies. Using the FLP/FRT recombination system (Golic and Lindquist, 1989; Golic, 1991; Xu and Rubin, 1993), we generated clones during the first larval instar and analyzed the phenotype in adult eyes (see Experimental Procedures). Few adult mosaic flies were recovered, particularly with *dpp*^{hr27}, presumably owing to the deleterious effects of homozygous *dpp* clones in other tissues. In addition to the eye phenotypes described below, we recovered many mosaic flies displaying gross deformations of the thorax, wings, and legs. The phenotype of homozygous *dpp*^{hr56} clones in the eye varied depending on the size and position of the clone. Small clones (up to approximately 20 mutant ommatidia) were always phenotypically wild type, presumably because their small size permitted them to be phenotypically rescued by *dpp* secreted by surrounding wild type tissue. Larger phenotypically wild-type clones were also observed in the anterior eye (Figure 4A). Large clones were rarely observed in the posterior eye because cells in the posterior eye disc undergo fewer cell divisions prior to their terminal differentiation. However, at a low frequency (approximately 10 cases in 800 mosaic individuals), we observed mosaic eyes of reduced size (Figure 4C) or in which large parts of the posterior–lateral eye were missing (Figures 4B and 4D); *dpp*^{hr56}/dpp^{hr56} (w^{-}/w^{-}) ommatidia were always observed surrounding this area, suggesting that the missing eye regions corresponded to mutant tissue. Large *dpp* clones in the posterior eye disc may result in an obvious phenotype because there are fewer *dpp*-expressing wild-type cells in the posterior disc to rescue the defect.

Our data show that dpp function is nonautonomous during eye development, as has been described for wing development (Posakony et al., 1991). The phenotypes we observe with homozygous *dpp*^{hr56} clones, together with the known *dpp* expression pattern in the MF (Masucci et al., 1990; Blackman et al., 1991), are consistent with the hypothesis that *dpp* plays a critical role in the morphogenetic events that precede the assembly and differentiation of ommatidia in the eye disc.

The Dominant Mutation *Roi* Suppresses Furrow-Stop Mutations and Restores *dpp* Expression

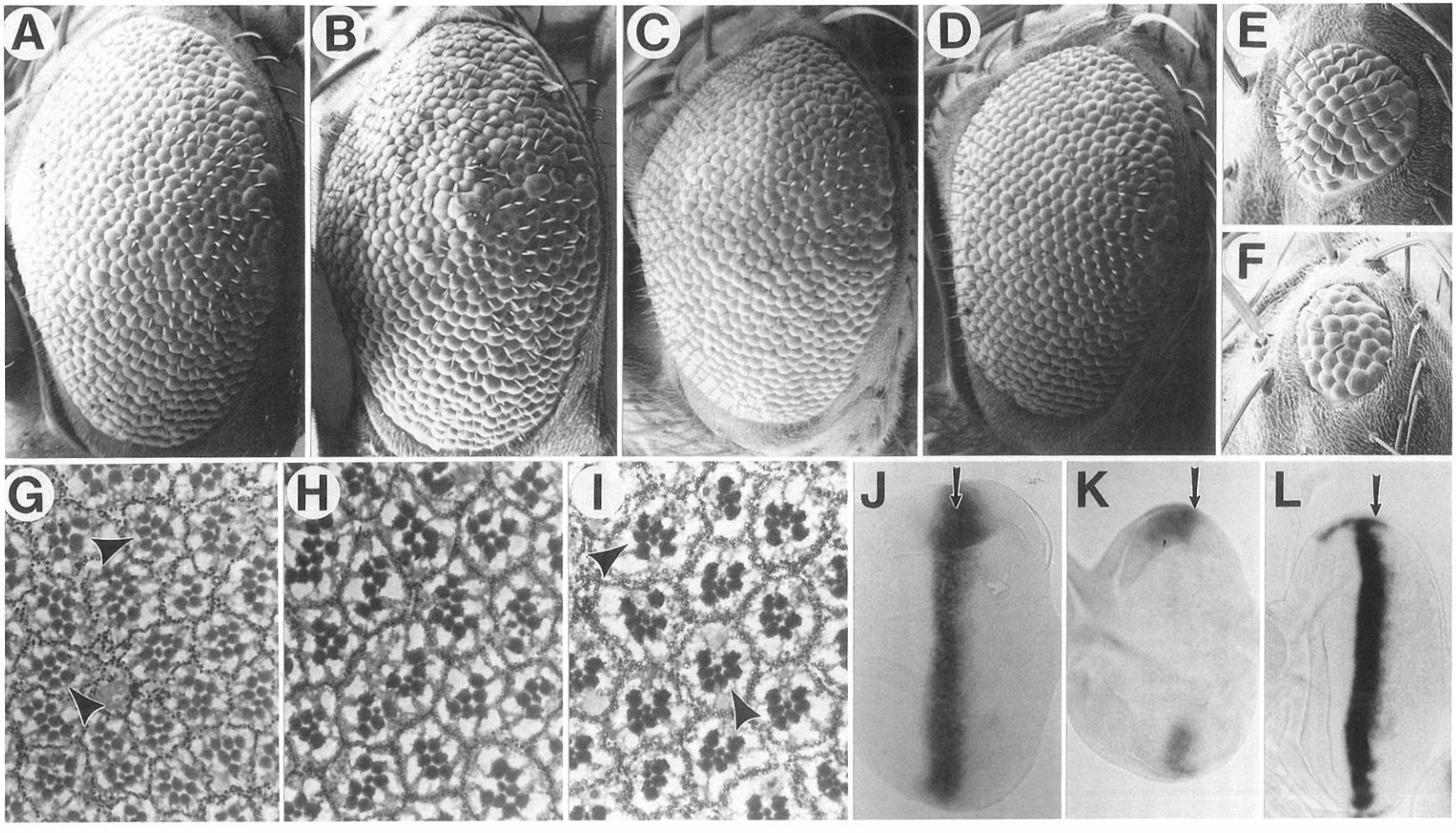
As a first step to finding other genes potentially involved in MF movement, we screened a collection of large chromosomal deletions for their ability to modify the phenotype of one of the furrow-stop mutants, *ro*^{DOM}. Several deletions acted as either enhancers or suppressors of *ro*^{DOM}. Curiously, we observed that chromosomes carrying the *Roi* mutation completely suppressed the reduced eye phenotype of *ro*^{DOM} (Figure 5B). *Roi* is a dominant gain-of-function mutation that causes a strong rough eye phenotype (Figure 5A; Renfranz and Benzer, 1989); the structure of most adult ommatidia is aberrant, containing too few or too many photoreceptor cells (Figure 5G). The phenotype arises early in the ommatidial assembly process; eye discs stained with the neural-specific MAb BP104 (Hortsch et al., 1990) displayed defects in the initial ommatidial spacing near the furrow, as well as numerous defects in later phases of development (data not shown; Renfranz and Benzer, 1989). In addition to *ro*^{DOM}, *Roi* completely suppressed the eye phenotype of *hs-ro* flies raised in the presence of heat shock (Figure 5C) and partially suppressed the eye phenotype of *hh*^{1} (Figure 5D), *Bar* (Lindsley and Zimm, 1992), and *Drop* flies (data not shown). The phenotype of doubly heterozygous flies closely resembled the phenotype of *Roi* (Figures 5B, 5C, and 5H), with the exception of *hh*^{1}, in which a reciprocal suppression was obtained between *hh*^{1} and *Roi* (Figures 5D and 5I; see legend to Figure 5). *Roi* did not suppress other reduced eye mutants such as *Lobe* (Figures 5E and 5F) and *eye gone* (data not shown), suggesting a specific effect on furrow-stop mutants. This observation further strengthens our belief that a common mechanism causes the phenotype of all furrow-stop mutants. The genetic interactions observed were clearly due to the *Roi* mutation, as ethyl methansulfonate–induced revertants of *Roi* failed to suppress furrow-stop mutations (data not shown).

Figure 4. Phenotype of *dpp*^{56} Clones in Mosaic Eyes

dpp^{56}/*dpp*^{56} clones are recognized by the lack of pigment in the adult eye.

(A and B) Phase-contrast photomicrographs of tangential sections through mosaic eyes carrying *dpp*^{56}/*dpp*^{56} clones. (A) A large clone located in the anterior eye is phenotypically wild type. (B) A rare large clone located at the posterior edge of the eye results in missing eye portions and aberrant ommatidial development (arrows).

(C and D) Mosaic eyes reduced in size (C) or missing a posterior/ventral eye region (D) were obtained at low frequency. *dpp*^{56}/*dpp*^{56} cells lacking the P[w^{+}] marker are indicated by arrows. Posterior is to the right.


A
B
C
D
E
F
G
H
I
J
K
L

We have shown that in all furrow-stop mutants, expression of *dpp* in the MF is abolished. To understand the basis of the suppression of these mutants by *Roi*, we analyzed the expression of *dpp*-driven β-galactosidase (*dpp–lacZ*) in some double mutant combinations. Expression of *dpp–lacZ*, which is normal in *Roi* eye discs (Figure 5J), was restored in eye discs from flies doubly heterozygous for *Roi* and either ro^{DOM} (Figure 5L), *Bar*, or hh^1/hh^1 (data not shown). Thus, *Roi* was able to overcome the inhibition of *dpp* expression observed in furrow-stop mutants and restore the progression of morphogenesis.

Inhibition of Photoreceptor Differentiation Posterior to the Furrow Alters Its Normal Progression

The data presented thus far show that *dpp* plays a central role in eye disc morphogenesis. First, expression of *dpp* in the MF closely correlates with the ability of the furrow to traverse the eye disc, and, second, the mutant phenotypes of homozygous *dpp* clones are consistent with their requirement for furrow movement. We next addressed the question of the direction of the driving force that moves the furrow and its associated *dpp* expression across the eye disc. One possibility is that developing ommatidia posterior to the MF induce a signal(s) that pushes the furrow anteriorly across the epithelium. Alternatively, cells anterior to the MF may provide signals for cells located more posteriorly, inducing them to initiate morphogenesis, pattern formation, and differentiation. We addressed these possibilities directly by disrupting differentiation posterior to the MF and determining its effects on the expression of *dpp* and other genes normally expressed in or anterior to the MF.

Inhibition of differentiation was achieved by removing Star function. The *Star* gene is specifically required during the initial phases of ommatidial differentiation posterior to the MF (Heberlein et al., 1993). Loss of Star function, analyzed in mosaic animals owing to the associated embryonic lethality, results in a failure of presumptive photoreceptor cells to differentiate neuronally. Consequently, ommatidial cells die, and prominent scars are observed in the adult eye. Star function, in contrast with dpp, is required autonomously for the differentiation of a subset of photoreceptors just posterior to the MF (Heberlein and Rubin, 1991; Heberlein et al., 1993). Thus, any effect of *Star* on the furrow would have to be indirect. We generated homozygous mutant *Star*⁻ (S^-/S^-) clones in developing eye discs (see Experimental Procedures) and analyzed their effect on the MF and on the expression of *dpp* and *scabrous* (*sca*; Baker et al., 1990; Mlodzik et al., 1990) (Figure 6). To ask whether *dpp* expression was dependent upon differentiation posterior to the MF, we generated S^-/S^- clones in larvae carrying the *dpp–lacZ* construct. Late third instar eye discs were stained for β-galactosidase activity; S^-/S^- clones were identified by the presence of patches of highly refractive dying cells (Heberlein et al., 1993). *dpp–lacZ* expression was clearly inhibited near S^-/S^- clones. In addition, the MF was slightly delayed near all S^-/S^- clones analyzed (Figure 6A).

Similar results were observed in mosaic eye discs doubly stained with antibodies against sca and the neural-specific antigen BP104. S^-/S^- clones were recognized by their aberrant neuronal differentiation (Heberlein et al., 1993; Figure 6B). *sca* is normally expressed in the furrow, including its anterior edge (Baker et al., 1990; Mlodzik et al., 1990), and is required for proper ommatidial spacing but not for furrow progression (Baker et al., 1990). Expression of *sca* was aberrant near S^-/S^- clones; the onset of expression was delayed, and the normal pattern was disrupted (Figure 6B). These data show that *Star*, although expressed just posterior to the furrow (Heberlein et al., 1993), affected the expression of genes in more anterior regions (see Figure 8A for a schematic). More importantly, the progression of the furrow in or near S^-/S^- clones was delayed relative to wild-type regions of the eye disc. This delay on its own is unlikely to cause a disruption in eye development, as *dpp* clones of similar size are phenotypically wild type. We believe that the furrow does not come to a halt in S^-/S^- clones owing to rescue by *dpp* diffusing from adjacent cells. However, the results with *Star* suggest that in the absence of pattern formation and differentiation posterior to the MF, the furrow would not progress across the eye disc (see below).

hh Expression in Differentiating Photoreceptors Is Required for MF Progression

We have shown that *Star*, although autonomously required for the differentiation of a subset of photoreceptor cells posterior to the MF, acts nonautonomously in mediating the normal progression of the MF. *Star* encodes a putative transmembrane protein (Kolodkin et al., submitted) and is unlikely to act as a diffusible factor. Thus, *Star* may act

Figure 5. Suppression of Furrow-Stop Mutants by *Roi*

(A–F) Scanning electron micrographs of adult eyes. (G–I) Tangential sections through adult eyes. (J–L) Late third instar larval eye discs carrying the *dpp–lacZ* construct stained for β-galactosidase activity. The genotypes of flies are as follows: *Roi/+* (A, G, and J), *Roi/+;* $ro^{DOM}/+$ (B, H, and L), *Roi/ hs-ro* (C), *Roi/+;* hh^1/hh^1 (D and I), *Lobe/+* (E), *Roi/L* (F), and ro^{DOM} (K). The reduced eye phenotype of ro^{DOM} ([B]; compare with Figure 1B) and *hs-ro* (C) flies is completely suppressed by *Roi*. The *Roi/hs-ro* larvae and early pupae were raised under heat shock conditions that generate an eyeless phenotype in *+/hs-ro* siblings (Kimmel et al., 1990; data not shown). The *Lobe* mutation was not affected by *Roi* (E and F). The internal eye phenotype of *Roi/+;* $ro^{DOM}/+$ (H) and *Roi/hs-ro* (data not shown) adult eyes is indistinguishable from *Roi* (G). A curious reciprocal suppression was observed between *Roi* and hh^1; *Roi* partially suppresses the reduced eye phenotype of hh^1 ([D]; compare Figure 1C), and hh^1 partially suppresses the rough eye phenotype of *Roi* (compare [G] and [I]). In *Roi/+;* hh^1/hh^1 eyes, approximately 75% of the ommatidia are structured normally (example indicated by arrowheads in [I]), whereas only approximately 15%–20% of the ommatidia in *Roi* eyes are wild type (examples indicated by arrowheads in [G]). Expression of *dpp–lacZ* in the MF of *Roi* larvae is normal (J), whereas expression in ro^{DOM} is inhibited (K). *dpp–lacZ* expression in doubly heterozygous *Roi/+;* $ro^{DOM}/+$ larvae is also normal (L). The MFs in (J)–(L) are indicated by arrows.

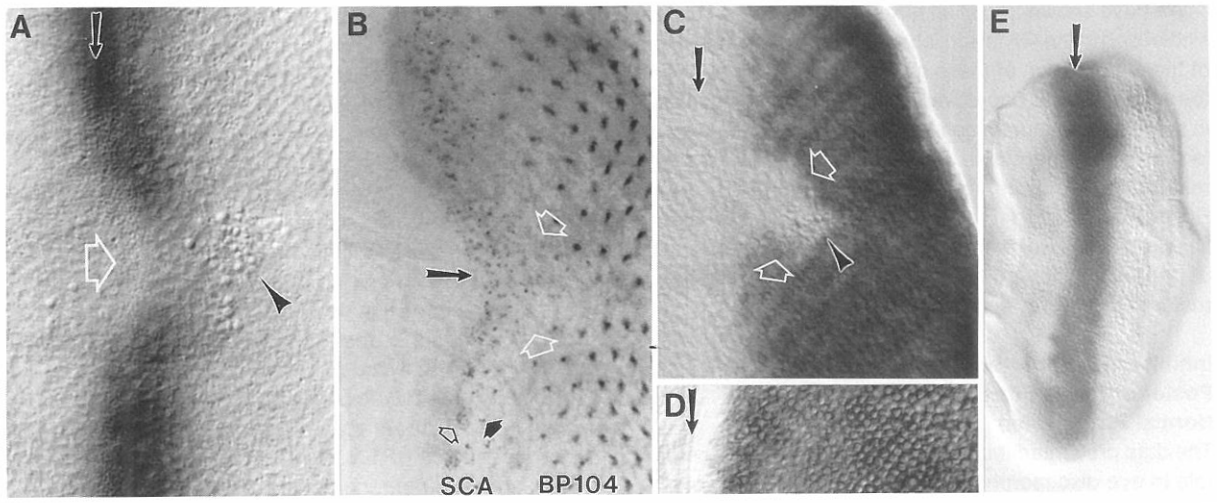


Figure 6. Loss of Star Function Affects Furrow Progression and Expression of *dpp* and *hh*

(A) A S^-/S^- clone was induced in a larva carrying the *dpp–lacZ* construct (see Experimental Procedures). Expression of *dpp–lacZ* is inhibited in or near a S^-/S^- clone, which is recognized by a patch of refractive dying cells posterior to the MF (arrowhead). The progression of the MF (vertical arrow) is delayed in the S^-/S^- clone (open white arrow).

(B) Eye disc from a larva carrying a S^-/S^- clone doubly stained with MAb BP104 and a polyclonal antibody against sca; the clone was recognized by aberrant neuronal differentiation (open white arrows). Sca is normally expressed in groups of cells at the anterior edge of the MF (open small arrowhead), followed by expression in single R8 cell precursors in the MF (closed arrowhead) (Baker et al., 1990). In or near the S^-/S^- clone, the onset of sca expression is delayed (horizontal arrow), and the normal pattern of expression is disrupted. The domains of BP104 and sca expression are indicated.

(C) A S^-/S^- clone (indicated by the open white arrows), recognized by a patch of dying cells (closed arrowhead), was induced in a larva carrying an enhancer trap insertion in the *hh* gene (see Experimental Procedures). *hh*-driven β-galactosidase in the S^-/S^- clone was abolished. The position of the MF is indicated by a vertical arrow.

(D) Expression of β-galactosidase in an eye disc from a larva carrying the *hh* enhancer trap starts posterior to the MF (arrow); the plane of focus shows the MF more clearly here than in (C).

(E) Expression of *dpp–lacZ* in the MF (arrow) of an $Egfr^{Elp}/Egfr^{Elp}$ larva is normal. Nomarski optics were used for photomicroscopy. Posterior is to the right.

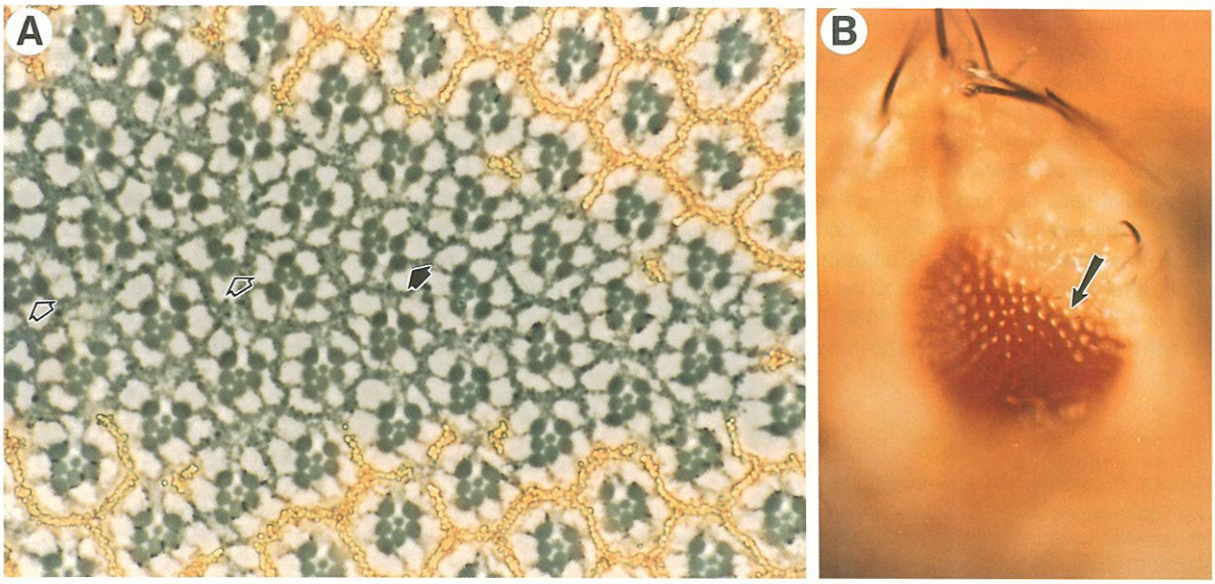


Figure 7. Phenotype of *hh* Clones in Mosaic Eyes

(A) Phase-contrast photomicrograph of a section through a large hh^-/hh^- clone located in the anterior eye. The clone is recognized by the absence of pigment. With a few exceptions (open arrowheads), most ommatidia are phenotypically wild type (an example is indicated by the closed arrowhead). The organization of ommatidial rows is slightly disrupted, particularly in the more anterior areas of clones.

(B) A large *hh* clone located in the posterior–dorsal eye results in a reduced eye phenotype; hh^-/hh^- (w^-) ommatidia (arrow) surround the missing eye region. Posterior is to the right.

indirectly by permitting differentiation of cells that express a diffusible signal, which in turn induces expression of *dpp* and furrow progression. A good candidate for this signal is *hh*. We have shown that *dpp* expression was abolished in eye discs from hh^1 flies (see Figure 3D), suggesting that *hh* is required for normal expression of *dpp* in the MF. In addition, expression of *dpp* and MF movement are impaired in or near S^-/S^- clones. Thus, if *hh* mediates the posterior to anterior information flow between differentiating photoreceptors and *dpp*, *hh* expression should be dependent upon Star function. To test this directly, we generated S^-/S^- clones in larvae carrying an enhancer trap insertion in the *hh* gene (see Experimental Procedures). We found that *hh* expression was abolished in or near S^-/S^- clones, which were identified by the presence of dying cells (Figure 6C).

hh expression, judged by the expression of β-galactosidase in several enhancer trap insertions, starts several rows posterior to the MF (Figures 6C and 6D; Lee et al., 1992; Ma et al., 1993 [this issue of *Cell*]). Thus, *hh* must act on cells located anterior to its expression domain, implying that its function may be nonautonomous in the eye. To test this directly, we analyzed the effect of loss of hh function in mosaic eyes (Figure 7). Although the viable partial loss-of-function hh^1 allele affects furrow progression, most hh^-/hh^- clones analyzed were phenotypically wild type. A subtle phenotype was observed in large hh^-/hh^- clones located in the anterior of the eye; although the great majority of ommatidia were constructed properly, their general organization was slightly disrupted (Figure 7A). This phenotype, which is more pronounced near the center of clones, could be the consequence of aberrant ommatidial spacing caused by a delay in MF progression. At a low frequency (approximately 1%), we observed mosaic eyes with obvious defects, such as missing eye regions (Figure 7B; also see Ma et al., 1993). We believe that these defects are caused by large hh^-/hh^- clones that fail to be rescued by surrounding wild-type tissue. Thus, function of hh is nonautonomous in the eye, an observation that is consistent with its previously reported nonautonomy during development of other imaginal disc-derived structures (Mohler, 1988). In addition, the phenotypes observed with *dpp* and *hh* clones are very similar, arguing that both genes may be involved in the same process.

Our data suggest a simple model in which differentiating ommatidial cells posterior to the MF produce hh protein that diffuses anteriorly and mediates the induction of *dpp* expression in the MF and subsequent progression of the furrow across the eye disc. In this model, any block in the differentiation of ommatidial cells, such as that caused by the lack of Star function, might be expected to prevent hh production and thus stop MF progression. An apparent contradiction to this model is presented by the *Ellipse* ($Egfr^{Elp}$) mutation, a dominant mutation in the Drosophila epidermal growth factor receptor homolog (Baker and Rubin, 1989). Ommatidial assembly and differentiation are severely inhibited in eye discs from larvae homozygous for *Ellipse* ($Egfr^{Elp}/Egfr^{Elp}$). Nevertheless, the MF does progress across the eye disc (Baker and Rubin, 1992), and expression of *dpp–lacZ* in the MF is relatively normal in $Egfr^{Elp}/Egfr^{Elp}$ eye discs (see Figure 6E). Thus, the $Egfr^{Elp}$ mutation appears to uncouple the requirement of differentiation posterior to the MF from the expression of *dpp* in the MF and its ability to progress across the disc. However, movement of the furrow in $Egfr^{Elp}/Egfr^{Elp}$ eye discs still appears to depend on hh function: $Egfr^{Elp}/+$; *hh*/+ flies have very reduced eyes, while the eyes of flies heterozygous for either gene alone are normal in size (data not shown).

Concluding Remarks

We are interested in investigating the mechanisms by which positional information is generated and propagated across the developing eye epithelium. The MF marks the front edge of morphogenesis and coincides with the first obvious manifestations of pattern formation. We show that progression of the MF is mediated by genes, such as *dpp* and *hh*, that function in highly nonautonomous ways. This observation is consistent with our finding that progression of the MF itself is mediated by a nonautonomous mechanism. In addition, we show that inhibition of ommatidial differentiation posterior to the MF interferes with its anterior progression. We propose that proper differentiation and normal *hh* expression posterior to the MF are required for the expression of *dpp* in the MF and, consequently, the progression of the morphogenetic wave.

The dpp protein, a member of the TGFβ superfamily, plays multiple roles during development (for reviews see Gelbart, 1989; Hoffman, 1992). Our data suggest that dpp plays a central role in propagating the morphogenetic wave across the eye disc. First, we find that expression of *dpp*, normally restricted to the MF (Masucci et al., 1990; Blackman et al., 1991), is abolished in mutants in which the anterior progression of the MF is disrupted. Second, restoration of *dpp* expression correlates with restoration of morphogenesis in eye discs from *hs-ro* larvae subjected to a single heat shock and in eye discs from furrow-stop mutants suppressed by *Roi*. Third, the phenotype of homozygous mutant *dpp* tissue in mosaic eyes is consistent with its proposed role in positional signaling. In the embryo, dpp protein has been shown to be secreted and to induce specific patterns of gene expression in adjacent tissues (Panganiban et al., 1990; Immerglück et al., 1990; Reuter et al., 1990). Similarly, dpp may diffuse from the MF, assuring its forward propagation.

Our analysis of hh function in eye development, together with the observations of others, reveals its essential signaling role in eye disc morphogenesis. First, the viable hypomorphic allele hh^1 causes a reduced eye phenotype owing to a premature halt in the progression of the MF; temperature-sensitive alleles of *hh* display a similar phenotype when shifted to the restrictive temperature during the late third instar larval period (Mohler, 1988; Ma et al., 1993). Second, the phenotype of homozygous mutant *hh* clones is nonautonomous; only large clones display a phenotype that is consistent with a role for *hh* in eye morphogenesis. *hh* expression, inferred from the expression of several independent enhancer trap insertions (Lee et al., 1992; see Figure 6D), does not start until several rows posterior to the MF. Thus, *hh* mediates events that occur anterior to its expression domain. The sequence of the hh protein

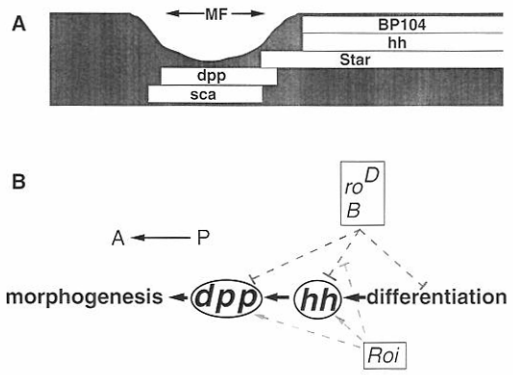


Figure 8. Model for the Information Flow That Drives the MF across the Eye Disc

(A) The domains of expression of several genes discussed in this paper are diagrammed.

(B) *hh*, synthesized by differentiating cells posterior to the MF, induces the expression of *dpp* in the MF and, consequently, the progression of the furrow across the eye disc. See text for details.

suggests that it is secreted (Lee et al., 1992; Mohler and Vani, 1992; Tabata et al., 1992; Tashiro et al., 1993), which is consistent with its nonautonomous genetic behavior.

The evidence discussed above suggests a mechanism that involves the transmission of positional information from the more mature posterior areas of the eye disc to the undifferentiated anterior regions. A model that includes all of our observations is diagrammed in Figure 8B. In brief, differentiating cells posterior to the MF express *hh*; *hh* mediates a diffusible signal that acts on cells located more anteriorly, resulting in expression of *dpp* in the MF; *dpp* mediates, directly or indirectly, progression of the MF across the eye disc. Mutations that stop progression of the MF (*hs-ro*, *ro^{DOM}*, *Bar*) could block the signaling cascade at several points; interference with differentiation, hh function, or *dpp* expression would all result in the same final outcome, an inhibition of morphogenesis. Specific suppression of this phenotype by *Roi* could be achieved either by interference with the inhibitory effect of furrow-stop mutations or by a direct activation of hh function or *dpp* expression. The model presented is only meant to summarize our findings regarding the directionality of informational flow during eye disc development; it is clearly an oversimplification of a very complex process. As in the eye disc, the domains of expression of *hh* and *dpp* in developing wing and leg discs are adjacent; *hh* is expressed in the posterior compartment (Lee et al., 1992; Tashiro et al., 1993), whereas *dpp* is expressed in a stripe just anterior to the compartment boundary (Masucci et al., 1990; Blackman et al., 1991; Posakony et al., 1991). Thus, although the development of the eye is quite different from that of the other imaginal discs (Bryant, 1978; Whittle, 1990; Wilkins and Gubb, 1991; Couso et al., 1993), the molecular mechanisms underlying positional signaling may be similar.

The eye disc fragment transplantation experiment of Lebovitz and Ready (1986) clearly showed that cells anterior to the MF are committed to develop as retina and that the anterior–posterior axis of the eye disc may already have been established at a considerable distance anterior to the MF. In agreement with these findings is the recent observation that several genes (*hairy* [Brown et al., 1991]; *string* [Alphey et al., 1992]; and *eyes absent* [Bonini et al., 1993]) are expressed in spatially restricted regions anterior to the MF. The question we addressed in this paper is not whether cells anterior to the furrow have already acquired positional information, but rather where the information came from. Based on the data presented, we conclude that cells posterior to the MF provide information that is transmitted nonautonomously to cells located anterior to the MF by a mechanism(s) utilizing the products of the *hh* and *dpp* genes.

Experimental Procedures

Fly Stocks

ro^{DOM} was isolated in an X-ray screen designed for a different purpose and was kept because of its reduced eye phenotype. Based on cytological and molecular criteria, *ro^{DOM}* flies carry a T(2; 3) that breaks near the *rough* gene. Ethyl methansulfonate–induced revertant of *ro^{DOM}* results in a recessive rough phenotype. Immunocytochemistry with a MAb against rough (Kimmel et al., 1990) revealed weak ectopic *rough* expression anterior to the MF and in the brain (data not shown). *Bar*, *Drop^{Mlo}*, *Lobe*, *eye gone*, *bar^{3}* (now called *hh^{1}*), and *In(2L)t*, *In(2R)Cy*, and *Roi* were obtained from the Drosophila Stock Centers at Bloomington, Indiana, and Bowling Green, Ohio. Fly stocks carrying a construct in which the *dpp* enhancer is fused to *lacZ* (line BS3.0) were obtained from R. Blackman and W. Gelbart; insertions on either the second and third chromosome were used. The enhancer trap insertion in *hh* (*hh^{ry413}*) was generated by U. Gaul, L. Higgins, and G. M. R. Flies were reared at 25°C.

Generation of Somatic Mosaics

hs-ro *Mosaics*

A second chromosome carrying a *P[w^{+}]* element located at 30A and a *P[hs-ro, ry^{+}]* element located at 28B was constructed using standard recombination schemes. Virgin females of genotype *w^{-}*; *P[hs-ro, ry^{+}], P[w^{+}]/CyO* were mated to *w^{-}* males. First instar larvae (24–48 hr old) were exposed X-rays (1000 rad). Wandering third instar larvae were subjected to 45 min heat shock treatments at 12 hr intervals for 2 days. Adult flies were screened for mosaic eyes.

dpp *Mosaics*

Second chromosomes carrying either *dpp^{hr56}*, *dpp^{hr27}*, or *dpp^{hr4}* and an FRT element at 40A were generated. Virgin females of genotype *40-w^{+} F* were mated with males of genotype *w^{-}*, *40-dpp^{hr}/CyO* males (for nomenclature of FLP/FRT chromosomes, see Xu and Rubin, 1993). First instar larvae were subjected to a 60 min heat shock to induce the FLP recombinase. Adult eyes were screened for regions of *w^{-}/w^{-}* (*dpp^{hr}/dpp^{hr}*) tissue. X-ray-induced clones were generated with alleles *dpp^{H47}*, *dpp^{H37}*, *dpp^{14}*, and *dpp^{dblk}*; clones were recovered at very low frequencies (~0.1%). All clones were relatively small and phenotypically wild type (data not shown).

hh *Mosaics*

Third chromosomes carrying either *hh^{13C}* (Jürgens et al., 1984) or *hh^{E23}* and an FRT element at 82B were constructed. Virgin females of genotype *82-w F* were mated with males of genotype *w^{-}*; *82-hh/TM3*. Clones were induced in first instar larvae and recovered in adult eyes.

Star *Mosaics*

The chromosomes used to generate *S^{-}/S^{-}* clones have been described (Heberlein et al., 1993). Alleles *S^{218}* and *S^{x155}* were used with indistinguishable results. To analyze the expression of *dpp–lacZ* in *S^{-}/S^{-}* clones, virgin females of genotype *40-1 F* were crossed to males of genotype *w^{-}*; *40-S^{-}*; *P[dpp–lacZ]/+*. Clones were induced in first instar larvae and analyzed in wandering late third instar larvae. To analyze the expression of *hh* in *S^{-}/S^{-}* clones, virgin females of genotype *40-1 F* were crossed to males of genotype *w^{-}*; *40-S^{-}*; *hh^{ry413}/+*. Clones were induced in first instar larvae and analyzed in wandering late third instar larvae.

Histochemistry and Immunohistochemistry

The expression of β-galactosidase in enhancer traps and *lacZ* constructs in imaginal discs was assayed by an activity stain using the substrate X-Gal (Simon et al., 1985). Eye imaginal discs were immunostained with antibodies against sca and BP104 as described in Tomlinson and Ready (1987a). The polyclonal antibody against sca was a gift from N. Baker. Cobalt sulfide–stained eye discs were obtained as described by Wolff and Ready (1991b).

Histology and Scanning Electron Microscopy of Adult Heads

Adult Drosophila heads were fixed and embedded in Durcapan resin essentially as described by Tomlinson and Ready (1987b). Sections (2 μm) were mounted in DPX medium (Fluka) and viewed under phase-contrast optics. Samples for scanning electron microscopy were prepared as described by Kimmel et al. (1990).

Acknowledgments

We thank C. Ferguson and J. Treisman for many enlightening discussions; A. Penton for isolating ro^{DOM}; R. Blackman, W. Gelbart, J. Mohler, and T. Xu for fly stocks; D. Pardoe for scanning electron microscopy; T. Laverty for chromosome cytology; and K. Moses for sharing unpublished data. We are grateful to C. Ferguson, J. Fischer-Vize, J. Treisman, G. Mardon, and D. Wassarman for thoughtful comments on the manuscript.

Received August 10, 1993; revised September 15, 1993.

References

Alphey, L., Jimenez, J., White-Cooper, H., Dawson, I., Nurse, P., and Glover, D. M. (1992). *twine*, a *cdc25* homolog that functions in the male and female germline of Drosophila. Cell *69*, 977–988.

Baker, N. E., and Rubin, G. M. (1989). Effect on eye development of dominant mutations in *Drosophila* homologue of the EGF receptor. Nature *340*, 150–153.

Baker, N. E., and Rubin, G. M. (1992). *Ellipse* mutations in the *Drosophila* homologue of the EGF receptor affect pattern formation, cell division, and cell death in eye imaginal discs. Dev. Biol. *150*, 381–396.

Baker, N. E., Mlodzik, M., and Rubin, G. M. (1990). Spacing differentiation in the developing *Drosophila* eye: a fibrinogen-related lateral inhibitor encoded by *scabrous*. Science *250*, 1370–1377.

Banerjee, U., and Zipursky, S. L. (1990). The role of cell–cell interaction in the development of the Drosophila visual system. Neuron *4*, 177–187.

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

Bonini, N. M., Leiserson, W. M., and Benzer, S. (1993). The *eyes absent* gene: genetic control of cell survival and differentiation in the developing Drosophila eye. Cell *72*, 379–395.

Brown, N. L., Sattler, C. A., Markey, D. R., and Carroll, S. B. (1991). *Hairy* gene function in the *Drosophila* eye: normal expression is dispensable but ectopic expression alters cell fates. Development *113*, 1245–1256.

Bryant, P. J. (1978). Pattern formation in imaginal discs. In Genetics and Biology of *Drosophila*, Volume 2C, M. Ashburner and T. R. F. Wright, eds. (New York: Academic Press), pp. 229–335.

Bryant, P. J. (1988). Localized cell death caused by mutations in a *Drosophila* gene coding for a transforming growth factor-beta homolog. Dev. Biol. *128*, 386–395.

Couso, J. P., Bate, M., and Martinez-Arias, A. (1993). A *wing-less* dependent polar coordinate system in *Drosophila* imaginal discs. Science *259*, 484–489.

Fristrom, D. (1968). Cellular degeneration in wing development of the mutant vestigial of *Drosophila melanogaster*. J. Cell Biol. *39*, 488–491.

Gelbart, W. M. (1989). The *decapentaplegic* gene: a TGF-β homologue controlling pattern formation in *Drosophila*. Development (Suppl.) *107*, 65–74.

Golic, K. G. (1991). Site-specific recombination between homologous chromosomes in *Drosophila*. Science *252*, 958–961.

Golic, K. G., and Lindquist, S. (1989). The FLP recombinase of yeast catalyzes site-specific recombination in the Drosophila genome. Cell *59*, 499–509.

Heberlein, U., and Rubin, G. M. (1991). *Star* is required in a subset of photoreceptor cells in the developing *Drosophila* retina and displays dosage sensitive interactions with *rough*. Dev. Biol. *144*, 353–361.

Heberlein, U., Hariharan, I. K., and Rubin, G. M. (1993). *Star* is required for neuronal differentiation in the *Drosophila* retina and displays dosage sensitive interactions with *Ras1*. Dev. Biol., in press.

Hoffman, F. M. (1992). TGF-β family factors in *Drosophila* morphogenesis. Mol. Repr. Dev. *32*, 173–178.

Hortsch, M., Bieber, A. J., Patel, N. H., and Goodman, C. S. (1990). Differential splicing generates a nervous system-specific form of Drosophila neuroglian. Neuron *4*, 697–709.

Immerglück, K., Lawrence, P. A., and Bienz, M. (1990). Induction across germ layers in Drosophila mediated by a genetic cascade. Cell *62*, 261–268.

Irish, V. F., and Gelbart, W. M. (1987). The *decapentaplegic* gene is required for dorsal–ventral patterning of the *Drosophila* embryo. Genes Dev. *1*, 868–879.

Jürgens, G., Wieschaus, E., Nüsslein-Volhard, C., and Kludig, H. (1984). Mutations affecting the pattern of the larval cutical in *Drosophila melanogaster*. II. Zygotic loci on the third chromosome. Roux's Arch. Dev. Biol. *193*, 283–295.

Kerr, J. F., Wyllie, A. H., and Currie, A. R. (1972). Apoptosis: a basic biological phenomenon with wide ranging implications in tissue kinetics. Br. J. Cancer *26*, 239–257.

Kimmel, B. E., Heberlein, U., and Rubin, G. M. (1990). The homeo domain protein *rough* is expressed in a subset of cells in the developing *Drosophila* eye where it can specify photoreceptor cell subtype. Genes Dev. *4*, 712–727.

Lebovitz, R. M., and Ready, D. F. (1986). Ommatidial development in *Drosophila* eye disc fragments. Dev. Biol. *117*, 663–671.

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

Lindsley, D. L., and Zimm, G. G. (1992). The Genome of *Drosophila melanogaster* (New York: Academic Press).

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

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

Mlodzik, M., Baker, N. E., and Rubin, G. M. (1990). Isolation and expression of *scabrous*, a gene regulating neurogenesis in *Drosophila*. Genes Dev. *4*, 1848–1861.

Mohler, J. (1988). Requirements for *hedgehog*, a segmental polarity gene, in patterning larval and adult cuticle of *Drosophila*. Genetics *120*, 1061–1072.

Mohler, J., and Vani, K. (1992). Molecular organization and embryonic expression of the *hedgehog* gene involved in cell–cell communication in segmental patterning of *Drosophila*. Development *115*, 957–971.

Nüsslein-Volhard, C., and Wieschaus, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. Nature *287*, 795–801.

Padgett, R. W., St. Johnston, R. D., and Gelbart, W. M. (1987). A transcript from a *Drosophila* pattern gene predicts a protein homologous to the transforming growth factor-beta family. Nature *325*, 81–84.

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

Posakony, L. G., Raftery, L. A., and Gelbart, W. M. (1991). Wing formation in *Drosophila melanogaster* requires *decapentaplegic* gene func-

tion along the anterior–posterior compartment boundary. Mech. Dev. *33*, 69–82.

Poulson, D. F. (1950). Histogenesis, organogenesis, and differentiation in the embryo of *Drosophila melanogaster meigen*. In Biology of *Drosophila*, M. Demerec, ed. (New York: Wiley), pp. 168–274.

Ready, D. (1989). A multifaceted approach to neural development. Trends Neurosci. *12*, 102–110.

Ready, D. F., Hanson, T. E., and Benzer, S. (1976). Development of the *Drosophila* retina, a neurocrystalline lattice. Dev. Biol. *53*, 217–240.

Renfranz, P. J., and Benzer, S. (1989). Monoclonal antibody probes discriminate early and late mutant defects in development of the *Drosophila* retina. Dev. Biol. *136*, 411–429.

Reuter, R., Panganiban, G. E. F., Hoffmann, F. M., and Scott, M. P. (1990). Homeotic genes regulate the spatial expression of putative growth factors in the visceral mesoderm of *Drosophila* embryos. Development *110*, 1031–1040.

Simon, J. A., Sutton, R. B., Lobell, R. L., Glazer, R. L., and Lis, J. T. (1985). Determinants of heat shock-induced chromosome puffing. Cell *40*, 805–817.

Spencer, F. A., Hoffmann, F. M., and Gelbart, W. M. (1982). Decapentaplegic: a gene complex affecting morphogenesis in Drosophila melanogaster. Cell *28*, 451–461.

St. Johnston, R. D., Hoffmann, F. M., Blackman, R. K., Segal, D., Grimaila, R., Padgett, R. W., Irick, H. A., and Gelbart, W. M. (1990). Molecular organization of the *decapentaplegic* gene in *Drosophila melanogaster*. Genes Dev. *4*, 1114–1127.

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

Tashiro, S., Michiue, T., Higashijima, S., Zenno, S., Ishimaru, S., Takahashi, F., Orihara, M., Kojima, T., and Saigo, K. (1993). Structure and expression of *hedgehog*, a *Drosophila* segment-polarity gene required for cell–cell communication. Gene *124*, 183–189.

Tomlinson, A. (1988). Cellular interactions in the developing *Drosophila* eye. Development *104*, 183–193.

Tomlinson, A., and Ready, D. F. (1987a). Neuronal differentiation in the *Drosophila* ommatidium. Dev. Biol. *120*, 366–376.

Tomlinson, A., and Ready, D. F. (1987b). Cell fate in the *Drosophila* ommatidium. Dev. Biol. *123*, 264–275.

Whittle, J. R. S. (1990). Pattern formation in imaginal discs. Semin. Cell Biol. *1*, 241–252.

Wilkins, A. S., and Gubb, D. (1991). Pattern formation in the embryo and imaginal discs of *Drosophila*: what are the links? Dev. Biol. *145*, 1–12.

Wolff, T., and Ready, D. F. (1991a). The beginning of pattern formation in the *Drosophila* compound eye: the morphogenetic furrow and the second mitotic wave. Development *113*, 841–850.

Wolff, T., and Ready, D. F. (1991b). Cell death in normal and rough eye mutants of *Drosophila*. Development *113*, 825–839.

Wyllie, A. H., Kerr, J. F., and Currie, A. R. (1980). Cell death: the significance of apoptosis. Int. Rev. Cytol. *68*, 251–306.

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