**INTRODUCTION**

The adult epidermis in higher dipteran insects such as *Drosophila* is derived from specialized structures in larvae known as imaginal discs. Set aside during mid-embryogenesis as small groups of 10 to 70 cells each, imaginal disc primordia grow and differentiate during larval development as epithelial monolayers. Metamorphosis of imaginal discs during early pupal life generates the adult cuticle and appendages, including the eyes, legs and wings (reviewed in Cohen, 1993). While a morphological description of these events has been well-documented, the genetic and molecular mechanisms controlling pattern formation and morphogenesis of imaginal discs are just now being unraveled.

Eye imaginal disc development creates a highly ordered array of differentiated cells from a pool of equivalent, undetermined cells (Ready et al., 1976). Neural specification and differentiation in the *Drosophila* eye sweep across the unpatterned epithelial monolayer of the eye imaginal disc following a developmental wave termed the morphogenetic furrow. The furrow begins at the posterior margin of the eye imaginal disc and moves anteriorly as a linear front. Progression of the furrow requires the function of *hedgehog*, which encodes a secreted signaling protein. We characterize mutations in *dachshund*, a gene that encodes a novel nuclear protein required for normal cell-fate determination of imaginal disc cells. In the absence of *dachshund* function, cells at the posterior margin of the eye disc fail to follow a retinal differentiation pathway and appear to adopt a cuticle fate instead. These cells are therefore unable to respond to pattern propagation signals such as hedgehog and furrow initiation does not occur. In contrast, cells in more anterior portions of the eye disc are able to differentiate as retinal cells in the absence of *dachshund* activity and respond normally to patterning signals. These results suggest that posterior margin cells are distinct from other cells of the eye imaginal disc by early stages of development. *dachshund* is also necessary for proper differentiation of a subset of segments in the developing leg. Null mutations in *dachshund* result in flies with no eyes and shortened legs.

**SUMMARY**

Neural specification and differentiation in the *Drosophila* eye sweep across the unpatterned epithelial monolayer of the eye imaginal disc following a developmental wave termed the morphogenetic furrow. The furrow begins at the posterior margin of the eye imaginal disc and moves anteriorly as a linear front. Progression of the furrow requires the function of *hedgehog*, which encodes a secreted signaling protein. We characterize mutations in *dachshund*, a gene that encodes a novel nuclear protein required for normal cell-fate determination of imaginal disc cells. In the absence of *dachshund* function, cells at the posterior margin of the eye disc fail to follow a retinal differentiation pathway and appear to adopt a cuticle fate instead. These cells are therefore unable to respond to pattern propagation signals such as hedgehog and furrow initiation does not occur. In contrast, cells in more anterior portions of the eye disc are able to differentiate as retinal cells in the absence of *dachshund* activity and respond normally to patterning signals. These results suggest that posterior margin cells are distinct from other cells of the eye imaginal disc by early stages of development. *dachshund* is also necessary for proper differentiation of a subset of segments in the developing leg. Null mutations in *dachshund* result in flies with no eyes and shortened legs.

Key words: morphogenetic furrow, pattern propagation, cell-fate determination
Anderson, 1992) and encodes a Drosophila homolog of the mammalian transforming growth factor β (TGFβ) family of cytokines that regulate cell proliferation and differentiation (Padgett et al., 1987). The segment polarity gene hh is required for the specification of compartment boundaries in the Drosophila embryo and imaginal discs (Ingham et al., 1991; Tabata and Kornberg, 1994; Basler and Struhl, 1994). Vertebrate homologs of hh exhibit organizing activity during mouse central nervous system and chick limb development (Echelard et al., 1993; Riddle et al., 1993). During eye imaginal disc development, dpp is expressed specifically in the morphogenetic furrow and this expression is correlated with progression of the furrow. Mutations that stop furrow progression also abolish dpp expression. In particular, hh function is required for both dpp expression and furrow progression (Heberlein et al., 1993; Ma et al., 1993). hh is expressed posterior to the furrow in determined photoreceptor cells and it has been proposed that hh protein diffuses anteriorly to positively regulate dpp expression in the furrow. Consistent with this interpretation, ectopic expression of hh in the anterior compartment of the wing imaginal disc is sufficient to induce dpp expression in cells bordering the area of ectopic hh expression (Basler and Struhl, 1994). In the eye disc, dpp and/or hh may diffuse several cell diameters further into the unpatterned epithelium anterior to the furrow to regulate other genes required for undetermined cells to become competent to adopt a neural fate. Indeed, hh function may be required for expression of at least three other genes involved in eye development: glass, scabrous and hairy (Ma et al., 1993); glass and scabrous are normally expressed in and posterior to the furrow while hairy is expressed anterior to the furrow (Moses and Rubin, 1991; Baker et al., 1990; Mlodzik et al., 1990; Brown et al., 1991). Since hh is expressed in differentiated photoreceptors about three columns posterior to the furrow, these data suggest that hh is required for expression of genes that are expressed earlier, in developmental terms, than hh itself. This is possible because all stages of photoreceptor development are in progress simultaneously in a single eye disc and hh is a secreted protein capable of traveling backwards in developmental time (i.e., anteriorly) by means of diffusion (Ma et al., 1993). In this manner, hh is thought to control progression of the morphogenetic furrow.

In contrast to furrow progression, little is known about the mechanisms controlling initiation of the morphogenetic furrow. The furrow begins moving (i.e., initiates) during the late second or early third instar larval stage. However, the signal that causes the furrow to begin moving is not known. It seems likely that a distinct initiation mechanism precedes propagation: while hh is essential for furrow progression, it cannot be involved in furrow initiation because it is not expressed in the eye disc prior to neural differentiation. In addition, not all cells of the eye imaginal disc differentiate as retina: the periphery of the eye disc monolayer gives rise to a portion of the adult head cuticle surrounding the retinal field (Cohen, 1993). Again, the mechanisms controlling the distinction between retinal and cuticle fates in the eye disc are not known. In this paper, we describe mutations in dachshund (dac), which prevent cells at the posterior margin of the eye disc from following a retinal differentiation pathway. dac encodes a novel nuclear protein which is expressed at the posterior margin of the eye imaginal disc prior to furrow initiation and anterior to the furrow during furrow progression. In the absence of dac function, cells at the posterior margin of the eye imaginal disc appear to follow a cuticle differentiation pathway and are unable to respond to the pattern propagation signals required for furrow movement. Furrow initiation fails and little or no photoreceptor cell differentiation is observed. Curiously, the requirement for dac function in retinal cell-fate determination is not observed for cells located in more anterior portions of the eye disc. These results suggest that posterior margin cells possess an identity distinct from that of other cells in the eye imaginal disc from an early stage of development.

MATERIALS AND METHODS

Drosophila stocks and mutagenesis
The hypermorphic Egfr allele used was Elp^B1 (Baker and Rubin, 1989). The recessive viable Egfr allele top^1 was a gift of Kathryn Anderson. The P-element insertion in the dachshund genomic locus was originally called rK364 and has been renamed dac^P. The suppressor of Elp phenotype did not separate meiotically from this P-element in over 1300 progeny scored. In situ hybridization of a probe representing P-element sequences to salivary gland polytene chromosomes placed the dac^P element at cytological position 36A on the second chromosome (not shown). An additional nine alleles of dac (dac^1-dac^9) were obtained from Iain Dawson, Yale University. The dac locus was originally designated as the l(2)36Ae complementation group (Ashburner et al., 1990) but was later renamed dac. Analysis of polytene chromosomes from dac^2 animals revealed a small deletion that removes the major band at 36A (not shown). This deletion was confirmed by DNA hybridization analysis with genomic probes flanking the site of the P insertion (see below). The dac^4 mutation is, therefore, a complete loss-of-function or null mutation. dac^1, dac^3 and dac^4 are all null mutations for both the eye and leg phenotypes. The other dac mutant alleles are similar to the dac^2 allele with regard to both the eye and leg phenotypes and are assumed to be partial loss-of-function mutations in dac.

Scanning electron microscopy and histology
Adult flies were prepared for electron microscopy as described by Kimmel et al. (1990). Adult eyes were fixed, embedded and sectioned as described by Tomlinson and Ready (1987b). Acidine orange staining of eye and leg imaginal discs was performed as described by Bonini et al. (1993).

Cloning and sequencing
Genomic DNA sequences flanking the P-element insertion in dac were cloned by plasmid rescue (Mlodzik et al., 1990). Beginning with a genomic rescue fragment as a probe, eight independent, overlapping lambda phage clones containing genomic DNA surrounding the P-element were then isolated from a Sau3A partial digestion genomic library cloned in λFix (provided by Kevin Moses). These clones span approximately 30 kb. Ten independent, overlapping cDNA clones derived from this genomic region were isolated from a λgt10 cDNA library prepared from eye imaginal disc RNA (constructed by Alan Cowman). All DNA sequences were obtained using a Pharmacia ALF automated sequencer and analyzed with the Staden software. Two full-length cDNAs (5 kb) were sequenced on both strands using synthetic oligonucleotide primers. The corresponding sequences in genomic DNA were also determined and the putative splice junctions deduced (Mount et al., 1992). Approximately 300 bp from both ends of the ten independent cDNAs were also sequenced, as well as the splice junctions flanking exons two, three and four (see Fig. 2). The site of the P-element insertion was determined by sequencing the ends of the genomic rescue fragment.
Antibody preparation and dac mutant analysis

The P insert in dac is an ‘enhancer trap’ element that contains the bacterial lacZ gene (Mlodzik and Hiromi, 1991). β-galactosidase activity staining of discs prepared from third instar dacP heterozygote larvae reveals high levels of expression in both eye and leg imaginal discs (not shown). Monoclonal antibodies were raised against two non-overlapping, bacterially expressed dac proteins corresponding to amino acids 149-368 and 378-599 (see Fig. 2). The monoclonal preparations are designated mAbdac1-1 and mAbdac2-3, respectively. Both antibody preparations detect the same expression patterns as those seen with β-galactosidase activity staining in the dacP line. Although dac protein staining is still observed in imaginal discs prepared from larvae homozygous for the weak dacP allele, this staining is at significantly lower levels as compared to wild-type (not shown). This is presumed to be full-length, wild-type dac protein, since the P-element is inserted well 5′ of the open reading frame (Fig. 2A). This result is consistent with the partial loss-of-function phenotype of the dacP mutant. As expected, no dac protein is detectable in discs prepared from dacC deletion mutant larvae or in dac null mutant clones (Fig. 5). The two other phenotypic dac null alleles, dacC and dacD, are also protein nulls. In contrast to the dacC deletion mutant, DNA blot analysis indicates that the genomic DNA encompassing the dacC and dacD null alleles is grossly intact (not shown). These results suggest that loss-of-function mutations in the transcription unit defined by the P-element insertion (dacP) are responsible for the mutant phenotypes described. In addition, the null phenotype for this locus is unambiguously defined by these mutants.

Immunohistochemistry

Imaginal discs from second or third instar larvae were dissected in PBS (0.1 M phosphate pH 7.2, 150 mM NaCl), fixed for 15 minutes in 4% formaldehyde in PBS, and washed three times 10 minutes in PBS. Primary antibody incubations were performed in PAXDG (PBS containing 1% BSA, 0.3% Triton X-100, 0.3% sodium deoxycholate, 5% normal goat serum) for 2 hours at room temperature or overnight at 4°C. mAbdac2-3 antibodies were used at a 1:5 dilution, rat monoclonal anti-ELAV antibodies were used at a 1:1 dilution and rabbit anti-β-galactosidase polyclonal antibodies (Organon Teknika) were used at a 1:5000 dilution. Following the primary antibody incubation, discs were washed three times 10 minutes in PAXDG. Discs were then incubated in PAXDG for 2 hours at room temperature with a 1:200 dilution of one of the following horseradish peroxidase-conjugated secondary antibodies: goat anti-rat (for the ELAV primary), goat anti-mouse (for the mAbdac primary), or donkey anti-rabbit (for the β-galactosidase primary). After washing three times 10 minutes in PBS, discs were incubated 10 minutes in PBS with 0.5 mg/ml diaminobenzidine (DAB), 0.003% hydrogen peroxide and 1.5 mM NiCl2 for intensification. The double-label experiment for ELAV and the goat anti-mouse (for the mAbdac primary), or donkey anti-rabbit (for the mAbdac secondary) and then developed using DAB without NiCl2 intensification.

Mosaic analysis

Mitotic clones in eye imaginal discs were generated using the FLP/FRT system essentially as described by Xu and Rubin (1993) with some modifications. dac null alleles, dacC and dacD, were recombined with an FRT sequence at cytological position 40A. In addition, these stocks carried a single copy of either the dpp-lacZ reporter construct (Blackman et al., 1991) or a P-element containing the lacZ gene inserted in hh (line R413 or P30; Ma et al., 1993). Mitotic recombination was induced during the first instar larval stage with a 1 hour heat shock at 38°C to induce expression of the FLP recombinase.

dachshund function in eye and leg morphogenesis

carried on the X chromosome. Eye imaginal discs were dissected from wandering third instar larval animals and fixed as described above. β-galactosidase activity in discs was detected using the X-Gal substrate as described by Simon et al. (1985). The position of homozygous dac null mutant clones was determined by staining discs with anti-dac monoclonal antibodies or with anti-ELAV as described above.

RESULTS

dachshund is a dominant suppressor of Ellipse

Specification of spacing between ommatidial founder cells in the morphogenetic furrow is crucial to the first step of neural determination and pattern formation in the eye disc. Although this process is not understood, mutations in the Drosophila homolog of the epidermal growth factor receptor, Egfr, prevent normal spacing and differentiation of photoreceptor cells in the developing eye (Clifford and Schüpbach, 1989; Xu and Rubin, 1993). We reasoned that a genetic screen for modifiers of Egfr function might identify other genes involved in this process. Such a screen is facilitated by the dominant Ellipse (Elp) allele of Egfr (Baker and Rubin, 1989; 1992). In contrast to the highly ordered arrangement of ommatidia in wild-type animals, Elp heterozygotes have reduced, rough eyes (Fig. 1A,B). Elp behaves as a hypermorphic allele of Egfr because the Elp phenotype is suppressed by a deletion for this locus. Screens for mutations that dominantly enhance or suppress the Elp rough eye phenotype are likely to identify gene products that affect the activity of Egfr.

A collection of 1170 P-element lines were generated in our laboratory and selected for expression in eye imaginal discs (U. Gaul, L. Higgins and G. M. R., unpublished data). A screen of these lines identified one strong suppressor of the Elp eye phenotype. A single copy of this P-element insert completely suppresses both the external rough eye phenotype of Elp heterozygotes (Fig. 1C) as well as the disorganized ommatidial structure observed in sections of such eyes (not shown). Excision of the P-element reverts both the suppression of Elp and the semi-lethality of the chromosome. Additional alleles that fail to complement the P-element mutant were identified in other genetic screens for modifiers of the Elp eye phenotype (Iain Dawson, personal communication). Due to the recessive leg phenotype associated with these mutants (described below), this locus has been named dachshund (dac). One mutant allele, dacC, is a small deletion encompassing the entire dac locus and is therefore a null mutation (data not shown). These results suggest that loss-of-function mutations in dac reduce the activity of the hyperactive Elp allele of Egfr. Two other dac mutants, dacC and dacD, are also phenotypic and molecular null mutations (see Materials and Methods). All of the experiments described in this paper were performed with the dacC deletion mutant and at least one of the other null alleles. In all cases, identical results were obtained with each of these null mutants.

dachshund is required for eye and leg morphogenesis

Although dac mutants have greatly reduced viability, adult heterozygotes of both the P-element, dacP, and the null alleles are routinely observed. The eyes of dacC homozygotes are reduced and roughened (Fig. 1E), while the eyes of dac null
Fig. 1. The *dac* mutant phenotype. Scanning electron micrographs of adult eyes or prothoracic legs of the following genotypes are shown: wild-type (A,L); *Egfr^Egfr^/+* (B); *dac^P/Egfr^+Xdac^Egfr^+* (C); *dac^P/dac^P* (D); *dac^P/dac^P* (E,M); *dac^P/top^1/dac^P/top^1* (F); *top^1/top^1* (G); *dac^P/dac^P* (H) and (N-P); *dac^P/dac^P*; *hh^hJ413/TM6B* (I); +/-CyO; *hh^hJ413 hh^hJ413* (J); *dac^P/CyO; hh^hJ413 hh^hJ413* (K). All scale bars represent 100 μm. *dac^P/top^1/dac^P/top^1* double homozygote eyes (F) contain approximately half as many ommatidia as *dac^P/dac^P* homozygotes (E). For A-K, scale bar is shown in D; for L-N, scale bar is shown in L; and for O-P, scale bar is in P. C, coxa; Cl, claw; F, femur; Ta, tarsi; Ti, tibia; Tr, trochanter; 4 and 5, fourth and fifth tarsal segments, respectively.
mutant homozygotes are either severely reduced or, in about 50% of adults examined, absent (Fig. 1D, H). Although many of the ommatidia in dacP homozygotes are normally constructed, 50% have either too few or too many photoreceptor cells. The overall array of ommatidia is disrupted, contributing to the rough external eye phenotype of these flies. None of the few ommatidia in dac null mutant eyes has a normal morphology (not shown). In contrast to the compound eye, the external morphology of the adult ocelli appears normal in all dac mutants.

A second phenotype of dac flies inspired its name: dachshund mutants have short, little legs (Fig. 1L-P). The wild-type adult leg is composed of ten discrete segments: the coxa, trochanter, femur, tibia, five tarsal segments and the claw (Fig. 1L). While dacP homozygote legs appear to have a normal proximal and distal morphology, the intermediate segments are fused and condensed (Fig. 1M). The same intermediate segments, the femur, tibia and proximal three tarsi, are severely condensed in null mutant legs. In contrast, the coxa, trochanter, fourth and fifth tarsal segments, and the claw appear to develop normally, even in the null mutant (Fig. 1N-P). Upon eclosion from their pupal cases, these mutants are unable to locomote normally and quickly fall into the food and die. However, if kept away from wet medium, dac homozygotes can live for several days before dying, presumably from dehydration. These helpless homozygotes are able to flail their missshapen legs, albeit to no avail, indicating that at least a portion of the leg neuromusculature develops normally and is functional.

If loss-of-function mutations in dac reduce the activity of the activated Elp allele of Egfr, then the same mutations in dac should enhance the phenotype of partial loss-of-function mutations in Egfr. We looked for such an interaction with the weak top1 allele of Egfr (Clifford and Schüpbach, 1989), which displays a very mild roughening of the adult eye (Fig. 1G). Although we did not observe a dominant effect by loss-of-function mutations in dac on the top1 eye phenotype, we did see what appears to be a recessive synergy between the weak dacP allele and top1. That is, dacP top1 double homozygotes have a reduced, rough eye phenotype that is more severe than either mutant alone or what we would expect from simple additive effects (Fig. 1F). This result suggests that dac and Egfr may function in the same pathway or in parallel pathways during eye development.

dachshund encodes a novel nuclear protein expressed in eye and leg imaginal discs

DNA flanking the P-element insert in dacP was isolated and used to screen cDNA libraries prepared from eye imaginal disc RNA. The longest cDNAs obtained from our library were 5 kilobases (kb) in length and correspond with the major RNA species seen by blot analysis of total imaginal disc RNA (not shown). DNA sequence comparison of genomic and cDNA clones suggests that the locus spans approximately 20 kb and

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**Fig. 2.** dac encodes a novel protein. (A) A map of the genomic dac locus is shown with 5′ and 3′ non-coding exons depicted as open boxes and coding exons as black boxes. The site of the P insertion in dac is shown by an arrow. Comparison of partial sequences from ten independent cDNA clones indicate that exon 3, only 27 base pairs (bp) in length, is alternately spliced and appears in six out of ten clones analyzed. In addition, exon 4 has three alternate splice acceptor sites, separated by 21 and 6 bp. Each of these alternate acceptor sites appears to be used with approximately equal frequency. All of the predicted splice acceptor and donor sites in the cDNA are in good agreement with the consensus splice sequences (Mount et al., 1992; not shown). (B) The amino acid sequence of the predicted dac protein is shown beginning with the first ATG in the longest open reading frame. Splice sites based on comparison of the genomic and cDNA sequences are indicated with solid arrows. This sequence is characterized by multiple polyalanine, polyasparagine, polyglycine and polyglutamine runs throughout the protein. In addition, there are unusually high percentages of serine (11%) and proline (6%) residues present in the predicted sequence. The entire cDNA sequence and splice junction sequences have been deposited in GenBank.
comprises 12 exons, some of which can be alternately spliced (Fig. 2). The dac P-element is inserted after a position corresponding to base pair 11 of our longest cDNA clone (Fig. 2A). DNA sequence analysis reveals a single long open reading frame encoding a predicted protein of 1081 amino acids, which shares no significant similarity to any sequence in the databases (Fig. 2B).

Expression of dac is readily apparent prior to imaginal disc morphogenesis. In the eye disc, dac is expressed at the posterior margin prior to initiation of the morphogenetic furrow (Fig. 3A). This expression pattern is similar to that of dpp at the same early stage of development (Fig. 3B). Strong dac expression is also detected in the unpatterned epithelium preceding the morphogenetic furrow as it moves anteriorly across the eye disc (Fig. 3C,D). Posterior to the furrow, dac expression is detected primarily in photoreceptors R1, R6 and R7 as well as the cone cells (not shown). dac is expressed only in that part of the eye disc fated to become retina; the periphery of the disc destined to become head cuticle does not express dac (Fig. 3D). The ring pattern of dac expression in the leg disc is established at an early stage of leg disc development (Fig. 3E), well before the characteristic epithelial folds of a mature leg disc are seen (Fig. 3F,G). In all cases, dac protein appears to be localized to the nucleus.

In addition to strong expression in eye and leg discs, dac protein is found in the third antennal disc segment and the wing.

Fig. 3. dac is a nuclear protein expressed in the embryo and imaginal discs. All panels except B show expression of dac protein in wild-type imaginal discs and embryos as detected by anti-dac monoclonal antibody mAbdac2-3. β-galactosidase activity staining of an eye-antennal disc prepared from a second instar larva carrying the dpp-lacZ reporter is shown in B. Second (A,B,E) and third (C,D,F-H) instar larval discs of the following types are shown: eye-antennal (A-D), with eye discs to the left; prothoracic leg (E-G) and wing (H). Expression of dac protein is found in most or all nuclei anterior to the furrow and remains detectable primarily in photoreceptors R1, R6 and R7 posterior to the furrow (not shown). MF, morphogenetic furrow. A side view of a leg disc similar to that in F is shown in G. The arrow in G indicates the portion of the disc fated to give rise to the distal tarsal segments and claw of the adult leg. Ventral views of stage 13 (I) and stage 16 (J) embryos are shown. Posterior is to the left for A-D; on top for E, F and H; and to the right for I, J. Scale bars for A-C and E-J are 50 μm. Scale bar in D is 10 μm.
imaginal disc (Fig. 3C,H). dac expression is also detected in the central nervous system of embryos (Fig. 3I,J) and in the optic lobe of the larval brain (see cover picture). In spite of these distinct patterns of expression, no obvious morphological phenotype is observable in the embryonic CNS or the adult antennae and wings in dac null mutant animals. The function of dac in these structures remains to be determined.

**dachshund is essential for the development of intermediate leg segments**

The expression pattern of dac during larval leg disc development is consistent with the mutant phenotype of the adult leg. The leg imaginal disc is composed of concentric folds of epithelia such that the outermost portions of the disc give rise to the most proximal segments (i.e. the coxa and claw) and while the most distal structures (i.e. the tarsal segments) are derived from the central portion of the disc. As shown by antibody staining, dac is strongly expressed specifically in the presumptive epithelium that is fated to give rise to the femur, tibia and proximal tarsal segments (Fig. 3E-G), the same structures most severely affected in dac mutant legs (Fig. 1M,N). There is no dac expression detected in either the center or the periphery of the leg imaginal disc, and the structures derived from these portions of the disc appear to develop normally in dac mutant flies (Fig. 1O,P).

Although the adult legs of dac mutants are greatly shortened, leg imaginal discs prepared from dac mutant third instar larvae appear morphologically normal, both in size and in their concentric segmental structure (not shown). However, a significant increase in cell death in dac mutant leg discs is revealed by staining with acridine orange (Spreij, 1971), a dye that is actively excluded from living cells (Fig. 4G,H). This cell death is limited primarily to the region of the disc where dac is expressed and most likely accounts for the failure of disc eversion during early pupal stages. By 6 hours into pupal development, wild-type leg discs have undergone rapid elongation, primarily as a result of cell-shape change (Condic et al., 1991). Such elongation does not occur in dac mutant pupal legs, consistent with the adult leg phenotype (not shown). Thus, there is a failure of morphogenesis in dac mutant leg discs during late larval and early pupal development.

**Photoreceptor development is prevented in dachshund mutant eye discs**

The near or total absence of ommatidia in complete loss-of-function dac mutant adult eyes (Fig. 1D,H) could be the result of either an initial failure of photoreceptor determination during larval development or from a degenerative event following neural determination and differentiation, such as in the glass mutation (Moses et al., 1989). We examined the state of neural development in dac mutant eye discs using a monoclonal antibody that recognizes the nuclear ELAV antigen (Robinow and White, 1991). ELAV is expressed in all neurons in Drosophila and is apparent immediately posterior to the morphogenetic furrow in the eye imaginal disc. In contrast to the highly ordered array of photoreceptors seen in wild-type eye discs, fewer neurons differentiate in dac\(^p\) imaginal discs (Fig. 4A,B). Close examination of these discs reveals that highly variable numbers of photoreceptors per ommatidial cluster are already present at this stage (not shown) and can account for the adult phenotype of dac\(^p\) homozygote eyes. Moreover, null mutant eye discs have just a few ELAV-positive clusters or none at all (Fig. 4C). When present, these presumptive photoreceptors are always located near the posterior margin of the disc, where neural development normally begins (Fig. 4D). These results demonstrate that normal photoreceptor development is blocked in dac null mutants.

**dachshund is required for normal movement of the morphogenetic furrow**

We examined furrow movement in wild-type and dac mutant imaginal discs using a lac\(Z\) reporter construct for dpp (Blackman et al., 1991). dpp is expressed specifically in the morphogenetic furrow and can serve as a marker for the position of the furrow in the eye disc (Heberlein et al., 1993). While the morphogenetic furrow has normally progressed about half-way across the eye disc by the late third instar larval stage, dpp-lac\(Z\) expression remains at the posterior margin of dac null mutant discs (Fig. 4A,C). In about half of the dac null mutant eye discs examined, there is a small amount of furrow movement observed at the posterior midline of the disc, near the optic stalk. This movement is usually associated with the appearance of a few ELAV-positive cells (Fig. 4C,D). In all other cases studied, there is neither furrow movement nor neural differentiation (not shown). Thus, dac does not appear to be required specifically for neural differentiation. Instead, dac function is required for normal movement of the morphogenetic furrow.

In the weak dac\(^p\) mutant, furrow movement is uneven. Normally, the furrow moves across the eye disc as a linear front with neural development beginning immediately in its wake (Fig. 4A). In contrast, the partial loss-of-function dac\(^p\) mutation prevents the furrow from moving normally in lateral regions of the eye disc. This results in a curved furrow that has advanced further at the midline than at the periphery of the disc (Fig. 4B). Nevertheless, neural development closely follows the furrow in all cases examined.

Eye discs of third instar dac null mutant larvae are approximately normal in size, suggesting that the failure of furrow movement is not the result of inadequate cell proliferation (Fig. 4C). Nevertheless, in the absence of furrow movement, cells in dac mutant eye discs fail to adopt a neural fate and are likely to remain in an undifferentiated state. The fate of these undifferentiated cells is death. As detected by acridine orange staining, a large increase in cell death is seen in dac mutant eye discs (Fig. 4E,F).

To analyze further the role that dac plays in furrow movement, we made homozygous dac null mutant clones of cells in a heterozygous dac background and examined the phenotype of such clones in both larvae and adults. Dramatically different results were obtained depending on the position of these clones in the developing eye. dac clones occurring anywhere in the eye but not including the posterior margin (‘interior’ clones) always give rise to ommatidia (Fig. 5A). This suggests that the morphogenetic furrow is able to progress through patches of dac mutant cells given the chance to first initiate movement in wild-type tissue. In contrast to interior clones, posterior margin dac mutant clones usually fail to develop into retinal tissue; head cuticle is found in its place (Fig. 5B,C). In about one third of cases examined a few mutant
(white-minus) ommatidia are found at the borders of dac clones (Fig. 5L) and about 5% of posterior margin clones will give rise to a substantial patch of mutant ommatidia (Table 1). The reason for this variability is not known.

dac mutant clones in larval discs are consistent with the observed adult phenotypes. While neural differentiation is detected in interior dac clones (Fig. 5D), few or no ELAV-positive cells are found in posterior margin dac clones (Fig. 5E,F). Using the dpp-lacZ reporter as an assay, we found that the furrow indeed progresses through dac null mutant clones, as long as they do not include the posterior margin of the eye disc (Fig. 5G). However, the rate of furrow movement seems to be slowed in such clones as compared to the surrounding wild-type tissue. Interestingly, this effect on the rate of furrow progression exhibits local dominating non-autonomy: the furrow is also slowed in the genotypically wild-type epithelium immediately adjacent to dac mutant clones (not shown). This effect on furrow movement correlates with the appearance of additional photoreceptors in genotypically wild-type ommatidia located next to dac mutant clones in adult eyes (Fig. 5J). Consistent with the phenotype of whole dac null mutant eye discs (Fig. 4C), the furrow does not initiate movement in clones that include the posterior margin of the eye disc. Once the furrow has moved past such a clone in the surrounding wild-type tissue, normal furrow progression anterior to the clone resumes (Fig. 5H,I,M).

Thus, our clonal analyses indicate that loss of dac function prevents furrow initiation at the posterior margin of the eye disc and slows but does not prevent movement of the furrow during progression.

Although the morphogenetic furrow is able to move through patches of dac mutant cells, sections of dac mutant clones demonstrate that dac is required for normal ommatidial assembly: null mutant clones are clearly disrupted with most ommatidia containing an abnormal number and arrangement of photoreceptors (not shown). Nonetheless, complete and properly formed ommatidia composed entirely of dac mutant cells are occasionally observed (Fig. 5K), suggesting that there is no absolute requirement for dac in any given photoreceptor. In addition, mosaic ommatidia at the border of clones – those comprising both wild-type and dac mutant cells – are most often abnormally constructed, suggesting that dac is required cell autonomously for ommatidial assembly (not shown). That is, dac-dependent activity from surrounding wild-type cells is unable to rescue the aberrant ommatidial structure of dac mutant clones.

**hedgehog and dachshund**

Additional support for a role for dac during furrow progression comes from genetic analyses of interactions between dac and hedgehog (hh). hh is expressed in differentiating photoreceptors and is required for progress of the morphogenetic furrow (Ma et al., 1993; Heberlein et al., 1993). A lethal loss-of-function P-element insert in hh (rJ413) dominantly enhances the recessive eye phenotype of the weak dac<sup>2</sup> allele (Fig. 1E,I). Similarly, loss of one copy of dac acts as a dominant enhancer of the recessive eye phenotype of the viable hh<sup>1</sup> mutation (Fig. 1J,K).

**Fig. 4.** dac is required for movement of the morphogenetic furrow. Eye imaginal discs were double-labeled to reveal the position of the morphogenetic furrow and differentiating neurons. Wild-type (A,E,G), dac<sup>−/−</sup> (B) or dac<sup>−/−</sup> (C,D,F,H) imaginal discs were prepared from late third instar larvae also carrying the dpp-lacZ reporter and stained with anti-ELAV (black) and anti-β-galactosidase (brown). Third instar eye (E,F) and leg (G,H) imaginal discs were also stained with acridine orange to detect cell death. Dying cells are identified as brightly staining spots. Scale bar for A-C is 50 μm. Scale bar in D is 10 μm. Scale bars for E,F and G,H are 100 μm.
Fig. 5. Mosaic analysis of *dac* function. Light microscope photographs of adult eyes with homozygous *dac<sup>4</sup>* mutant clones (A-C,L) are shown. Interior clones – those that do not include the posterior margin – always give rise to retinal tissue (A). In contrast, posterior margin-containing clones (B,C,L) usually give rise to head cuticle and fail to respond to pattern propagation signals. Homozygous *dac<sup>4</sup>* mutant clones in eye discs were detected by staining with anti-*dac* monoclonal antibody (D-G), shown in brown (D-F) or black (G). Clones are located in areas that do not stain. Discs were also stained for the neuronal ELAV antigen, shown in black (D-F) or brown (H,I,M). *dpp-lacZ* expression was detected by β-galactosidase activity staining, shown in blue (G-I,M). In most eye discs examined, the level of *dpp* expression is not affected by loss of *dac* function. However, the position of expression of *dpp* is either slowed in interior *dac* clones (G) or fails to move at all in posterior margin clones (H,I,M). In a minority of cases (less than 10%), *dpp* expression is reduced or absent in both classes of clones (not shown). The basis for this variability of *dpp* expression is not known. The arrows in (L) indicate the morphological boundary that is usually apparent at the border of posterior margin *dac* clones. This boundary is already apparent in larval eye disc clones such as that shown in (F). Also visible in (L) are several white-minus *dac* mutant ommatidia bordering the clone. (J) A section of an abnormal ommatidium containing one additional outer photoreceptor but composed entirely of wild-type cells is located near a *dac* mutant clone. (K) A section of a normally constructed ommatidium composed entirely of *dac* mutant cells lacking pigment granules is shown. Posterior is at the top for all panels. Scale bars represent 50 μm (A,B), 25 μm (C,F,I,L,M), 50 μm (D,E,G,H) or 2 μm (J,K).
which presents a significant reduction of the anterior portion of the eye (Mohler, 1988). These genetic results suggest that \textit{dac} and \textit{hh} act in the same or in parallel pathways during eye development.

We examined \textit{hh} expression in wild-type and \textit{dac} mutant imaginal discs using a \textit{lacZ} enhancer trap insert in \textit{hh} that faithfully reproduces the normal expression pattern of \textit{hh} (Ma et al., 1993). Since little or no neural development takes place in \textit{dac} null mutant eye discs and \textit{hh} is expressed in the eye specifically in determined photoreceptors, it is not surprising that \textit{hh} is not expressed in \textit{dac} mutant eye discs (Fig. 6A,B). However, \textit{hh} expression in other imaginal discs, including the antennal, leg and wing discs, is not affected by loss of \textit{dac} function (for example, compare the antennal discs shown in Fig. 6A,B).

Expression of \textit{hh} in differentiating photoreceptors lacking \textit{dac} function was examined in homozygous \textit{dac} null mutant clones in eye imaginal discs. We found that \textit{hh} expression is normal in the absence of \textit{dac} activity using a single copy of the viable \textit{hh} enhancer-trap insertion P30 as an assay (Fig. 6C). Thus, \textit{dac} function is not cell autonomously required for \textit{hh} expression. The dominant enhancement of the adult \textit{dac} mutant eye phenotype by a single copy of the lethal \textit{hh} mutation rJ413 (Fig. 1I) is also apparent during larval development. As judged by the near absence of ELAV or \textit{hh} expression in \textit{dac} null mutant clones, loss-of-function of one copy of \textit{hh} by the rJ413 lethal insertion results in delayed or reduced neural differentiation in such clones (Fig. 6D).

### DISCUSSION

Substantial progress has been made toward elucidating the molecular mechanisms controlling pattern formation and morphogenesis during development of both vertebrate and invertebrate species. Genetic and molecular studies of embryonic development in \textit{Drosophila} have been particularly fruitful in identifying and understanding the function of phylogenetically conserved genes that are essential for organizing tissue polarity, specifying segment boundaries and controlling cell-fate determination. In many cases, intercellular signaling is achieved by diffusion of a secreted protein acting over several cell diameters (Bryant, 1993). For example, the product of the segment polarity gene \textit{hh} acts as a true morphogen in \textit{Drosophila}, specifying cell fates in the embryo in a concentration-dependent manner (Heemskerk and DiNardo, 1994), vertebrate homologs of \textit{hh} have recently been shown to exhibit organizing activity in the chick limb and in central nervous system development of mice (Riddle et al., 1993; Echelard et al., 1993). Similarly, \textit{nodal}, a member of the TGF\textbeta superfamily of secreted proteins, is expressed in the mouse node at the anterior of the primitive streak and is required for mesoderm formation (Zhou et al., 1993). A \textit{Drosophila} homolog of TGF\textbeta, \textit{dpp}, acts as a morphogen controlling dorsal-ventral pattern formation in the embryo (Ferguson and Anderson, 1992).

Eye imaginal disc development in \textit{Drosophila} provides an excellent system for deciphering mechanisms of pattern formation and morphogenesis. Organization of the unpatterned epithelium of the eye disc into a highly ordered array of differentiated cells follows movement of an indentation across the epithelial monolayer termed the morphogenetic furrow. Furrow progression is not associated with cell migration; instead, furrow movement is detected as a wave of changes in cell shape, cell cycle, gene expression and neural differentiation. Progression of the furrow requires the function of \textit{hh} and is likely to involve \textit{dpp}. The developmental events surrounding morphogenetic furrow movement in \textit{Drosophila} may be analogous to those associated with the Spemann organizer in \textit{Xenopus} and Hensen’s node and the zone of polarizing activity in chick: organizing centers determine cell fates of adjacent tissues through the action of secreted morphogens (reviewed in Slack, 1991; Riddle et al., 1993).

\textit{hh} plays a central role in progression of the morphogenetic furrow during eye disc development (Ma et al., 1993; Heberlein et al., 1993). In this case, \textit{hh} protein is expressed by determined neural cells posterior to the furrow and is thought to diffuse anteriorly, thereupon controlling expression of other genes required for furrow movement. One such putative target is \textit{dpp} (Heberlein et al., 1993). Removal of \textit{hh} function during furrow progression abolishes both the expression of \textit{dpp} in the furrow as well as furrow propagation. \textit{dpp} or \textit{hh}, in turn, are likely to regulate the expression of other genes in the unpatterned epithelium anterior to the furrow. Transplantation experiments have suggested that positional information sufficient to direct neural development is present in the morphologically undifferentiated epithelium immediately anterior to the furrow in the eye disc (Lebovitz and Ready, 1986). More recently, molecular analyses have shown that several genes are indeed expressed just anterior to the furrow, including \textit{string}, \textit{eyes absent}, \textit{hairy} and \textit{atonal} (Alphay et al., 1992; Bonini et al., 1993; Brown et al., 1991; Jarman et al., 1994). However, none of these genes expressed anterior to the furrow has been shown to participate directly in either initiation or propagation of the morphogenetic furrow.

While some of the genes involved in furrow progression have now been identified, little is known about the events surrounding initiation of the morphogenetic furrow. In this paper, we present evidence that \textit{dachshund} (\textit{dac}) is expressed at the posterior margin of the eye imaginal disc prior to movement of the morphogenetic furrow and is required for cells at the posterior margin to respond to pattern propagation signals such as those encoded by \textit{dpp} and \textit{hh}. This may reflect a requirement for \textit{dac} to control a cell-fate choice between retina and cuticle. Similar to other genes controlling pattern formation in multiple tissues, such as \textit{hh} and \textit{dpp}, \textit{dac} is also required for normal leg morphogenesis.

### Table 1. Adult posterior margin \textit{dac} clones

<table>
<thead>
<tr>
<th>\textit{dac} allele</th>
<th>Cuticle only</th>
<th>1-5 w\textsuperscript{−} ommatidia</th>
<th>&gt;5 w\textsuperscript{−} ommatidia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{dac}\textsuperscript{−}</td>
<td>82 (74%)</td>
<td>24 (22%)</td>
<td>5 (4%)</td>
<td>111</td>
</tr>
<tr>
<td>\textit{dac}\textsuperscript{−}</td>
<td>65 (67%)</td>
<td>25 (26%)</td>
<td>7 (7%)</td>
<td>97</td>
</tr>
<tr>
<td>\textit{dac}\textsuperscript{−}</td>
<td>67 (68%)</td>
<td>29 (30%)</td>
<td>2 (2%)</td>
<td>98</td>
</tr>
</tbody>
</table>

Homozygous \textit{dac} mutant clones for all three \textit{dac} null alleles were generated using the FLP-FRT system and were examined using a light dissecting microscope. Approximately three-quarters of the clones scored did not include the posterior margin of the eye (n=937) and always developed as \textit{dac} mutant (white) ommatidia. Clones including a portion of the posterior margin were scored and categorized based on the number of white minus ommatidia found bordering each clone.
**dachshund and leg development**

In the absence of *dac* function, flies develop with severely shortened legs. *dac* is expressed in early larval leg imaginal discs and this expression continues into pupal development. *dac* activity is necessary to specify the identity of a subset of segments in the leg: the femur, tibia and proximal three tarsi. Loss-of-function mutations in *dac* result in legs with these intermediate structures fused and condensed. Thus, *dac* is required in those leg segment primordia in which it is expressed for cells to adopt a fate appropriate to their position. Greatly increased cell death is apparent in third instar *dac* mutant leg discs, prior to the elongation that occurs during early pupal development. Thus, *dac* function is required prior to leg disc eversion. *dac* is likely to act downstream of a proximal-distal patterning signal, such as Distal-less, to establish segment boundaries or identities (Cohen and Jürgens, 1998). The *dac* mutant leg phenotype is consistent with a model in which establishment of proximal and distal structures during limb development precedes elaboration of intermediate positional information (Cohen, 1993).

**Morphogenetic furrow movement and dachshund function**

In the absence of *dac* activity, the morphogenetic furrow remains at the posterior margin of the eye imaginal disc. However, furrow movement is not prevented in *dac* mutants by a lack of sufficient epithelial tissue. Eye discs of *dac* null mutants are approximately normal in size, indicating that cell proliferation in the eye disc is not inhibited in the absence of *dac* function or furrow progression. This stands in contrast to other eyeless or reduced-eye mutants, such as *eyes absent* and *Lobe*, where a failure of retinal development is associated with larval eye discs that are significantly reduced in size (Bonini et al., 1993; Heberlein et al., 1993). While proliferation is normal, cells of *dac* mutant eye discs either fail to receive or perhaps respond to the normal cues specifying a neural identity. These cells eventually die, possibly as a result of inappropriate cell-fate specification. Alternatively, these dying cells may reflect the action of a programmed cell-death mechanism designed to remove undifferentiated cells during development, thereby preventing disruption of normal tissue organization (Ma et al., 1993).

Although loss of *dac* function prevents all movement of the morphogenetic furrow in posterior lateral regions of the eye disc, some furrow movement at the midline of the disc is observed. Here, the furrow is often able to move a small distance and this is sufficient to allow differentiation of a few photoreceptor cells. These cells develop as apparently normal photoreceptors, able to support the determination of the cone, pigment and bristle accessory cells found in wild-type ommatidia. Thus, *dac* is not required specifically for photoreceptor differentiation. In support of this view, photoreceptor development also occurs in interior *dac* mutant clones in the eye, indicating that there is no cell-autonomous requirement for *dac* in photoreceptor determination.

The eye phenotype of weak *dac* mutants is different from other mutants that affect furrow movement. For example, the partial loss-of-function *hh* allele eliminates *dpp* expression and furrow progression first in the middle of the eye disc, giving the adult eye a kidney-shaped appearance (Fig. 1J and Heberlein et al., 1993). In contrast, in weak *dac* mutants, *dpp* expression is unaffected and the furrow progresses nearly normally at the center of the eye disc but is delayed at the periphery (Fig. 4B). This difference in furrow progression may reflect a requirement for *dac*, but not *hh*, during furrow initiation.

Comparison of the phenotype of *dac* and *hh* mutant clones at the posterior margin of the eye disc suggests that *dac* plays an essential role in either furrow initiation or retinal cell-fate determination: while a few ommatidial columns are observed in *hh* mutant clones located at the posterior margin of the eye (Ma et al., 1993), *dac* mutant clones that include the posterior margin usually fail to give rise to retinal tissue (Fig. 5B,C,L). Consistent with this adult phenotype, the *dpp-lacZ* reporter expression remains at the posterior margin in *dac* mutant clones in the eye disc (Fig. 5H,I,M). Thus, our clonal analysis indicates that *dac* is cell autonomously required for initiation of movement of the morphogenetic furrow away from the posterior margin of the eye imaginal disc. Moreover, the failure of furrow initiation in *dac* mutant clones is unlikely to result from either a failure of photoreceptor differentiation or the absence of *hh* expression, since diffusion of *hh* protein from nearby wild-type photoreceptor cells is unable to rescue furrow initiation in *dac* mutant clones. Thus, *dac* is clonally required for cells at the posterior margin of the eye disc to respond to pattern propagation signals normally sufficient to cause furrow movement. This may reflect a direct requirement for *dac* in furrow initiation. Alternatively, *dac* may be required for an earlier step of cell-fate determination such that clones of *dac* mutant cells at the posterior margin of the eye disc are unable to differentiate as retinal tissue.

In addition to an essential role in early furrow movement, *dac* is also required for some aspect of ommatidial assembly. *dac* mutant clones in the adult eye are comprised of mostly abnormal ommatidia, even at the border of such clones. This is in sharp contrast to *hh* mosaic studies, where even large *hh* mutant clones are normally constructed (Ma et al., 1993; Heberlein et al., 1993). That is, unlike furrow progression, *dac* function is required cell autonomously for ommatidial assembly. Since *dac* is a nuclear protein, it may be involved in regulating other genes functioning in this process. One candidate is the *Egfr* gene. *dac* mutants were isolated by their ability to dominantly suppress the rough-eye phenotype of the activated *Ellipse* allele of *Egfr*. We have also shown that loss-of-function mutations in *dac* and *Egfr* display an apparent recessive synergy during eye development. Both results suggest that *dac* may positively regulate *Egfr*. Consistent with the possibility that *dac* controls *Egfr* expression and that this effects part of the *dac* mutant phenotype, *Egfr* is expressed anterior to the morphogenetic furrow (Zak and Shilo, 1992) and is required for normal photoreceptor determination (Clifford and Schüpbach, 1989; Xu and Rubin, 1993).

**A model for movement of the morphogenetic furrow**

Three lines of evidence suggest that a primary initiation signal acts at the posterior midline of the eye disc. First, as judged by *dpp* expression, the furrow normally initiates movement at the midline of the eye disc. Second, neural-specific markers, such as ELAV and hh, first appear at the posterior midline. Third, in the absence of *dac* function, the only movement of the furrow occurs at the posterior midline. Since this movement is
were generated in larvae carrying a single copy of either of two P-element insertions in disc in D was stained for the neuronal ELAV antigen, showing the delay in neural differentiation caused by the lethal antibody, shown in brown. Clones are located in areas that do not stain brown, seen as a white background behind the blue eye disc to respond to pattern propagation signals from sur-
amount. Clones in (C) are revealed by staining with anti-dac monoclonal antibody, shown in brown. Clones are located in areas that do not stain brown, seen as a white background behind the blue hh expression. The disc in D was stained for the neuronal ELAV antigen, showing the delay in neural differentiation caused by the lethal hedgehog mutation rJ413. Posterior is to the left for all panels.

Fig. 6. hedgehog enhances the dac clonal phenotype in eye discs. Third instar eye-antennal imaginal discs prepared from wild-type (A) and dac<sup>+</sup> mutant (B) larvae carrying a P-element insertion in hedgehog (line rJ413) were fixed and stained for β-galactosidase activity (blue). Eye discs are to the left and antennal discs are to the right. hedgehog is not expressed in dac null mutant eye discs. Homozygous dac<sup>+</sup> mutant clones were generated in larvae carrying a single copy of either of two P-element insertions in hedgehog: viable insert P30 (C) or lethal insert rJ413 (D). Both eye discs were fixed and stained for β-galactosidase activity (blue) to show the expression pattern for hedgehog. Expression of the hedgehog insert P30 is not affected by loss of dac function. The position of dac clones in (C) are revealed by staining with anti-dac monoclonal antibody, shown in brown. Clones are located in areas that do not stain brown, seen as a white background behind the blue hh expression. The disc in D was stained for the neuronal ELAV antigen, showing the delay in neural differentiation caused by the lethal hedgehog mutation rJ413. Posterior is to the left for all panels.

observed in about half of the discs examined, dac may serve some role in receiving this primary initiation signal but is not absolutely required for this step. This initial step cannot require hh function because hh is not expressed until several rows of ommatidia posterior to the furrow. The initial signal that triggers furrow movement may be transmitted directly via the optic stalk, thereby localizing initiation to the posterior midline. Alternatively, there may be a diffusable primary initiator, such as an ecdysone pulse (Richards, 1981), that is received only by specialized cells at this position.

The expression patterns of dpp and ELAV indicate that the furrow does not begin moving away from the entire posterior margin of the eye disc at once. Instead, the furrow advances as a linear front such that movement away from the curved posterior margin occurs over time as a function of the distance from the midline of the eye disc. The simplest mechanism to control propagation of pattern formation and neural differentiation in this manner would be by diffusion of a secreted signaling molecule such as hh. Transmission of this signal does not require dac activity since normal furrow movement resumes lateral to dac mutant clonal clones located at the posterior margin of the eye (Fig. 5). That is, lateral propagation of signals for furrow movement away from the posterior margin of the eye disc is not prevented by intervening clones of dac mutant cells.

Our clonal analysis demonstrates that dac function is cell autonomously required for cells at the posterior margin of the eye disc to respond to pattern propagation signals from surrounding wild-type tissue. This requirement is not found in cells located in interior portions of the eye disc. In most posterior margin dac clones, both in larvae and in adults, there is a morphological boundary that forms between dac mutant clones and the adjacent retinal field (Fig. 5F,L), suggesting that dac mutant cells that fail to develop as retina possess different adhesive properties than those of the surrounding cells. The eye imaginal disc is fated to give rise not only to the retina but to a portion of the surrounding head cuticle as well (Cohen, 1993). A boundary similar to that observed in posterior margin dac clones is normally seen at the periphery of wild-type eye discs (Fig. 3D), presumably representing a physical distinction between cuticle and retinal fields. Such boundaries are not observed in interior dac clones. These observations suggest the possibility that dac may be involved in controlling a choice between a cuticular versus a retinal fate. Specifically, dac may be required to suppress a cuticle fate in cells derived from the posterior margin. In the absence of dac function, clones that originate from a posterior margin cell follow a cuticle pathway and are usually unable to respond to the pattern propagation signals, such as dpp and hh, that are present but apparently ineffective. Consistent with this model, dac is not expressed in the peripheral margin of the eye disc destined to become cuticle (Fig. 3D). Thus, dac is at least indirectly required for initiation of the morphogenetic furrow and the onset of neural differentiation. However, this requirement is not absolute since mutant ommatidia bordering posterior margin clones, although usually few in number, are frequently observed. Moreover, if a dac clone is derived from an ‘interior’ cell of the eye disc, these cells appear to respond relatively normally to differentiation signals. This difference in responsiveness to pattern propagation signals suggests that posterior margin cells may
be different from other cells in the eye disc from an early stage of development. Consistent with this view, both dpp and dac are expressed specifically at the posterior margin of the eye disc prior to furrow initiation or neural differentiation.

In addition to dac, the product of the dpp gene may also be involved in some aspect of furrow initiation. This seems likely for three reasons. First, like dac, dpp is expressed along the posterior margin of the eye disc, prior to furrow initiation (Masucci et al., 1990; Ma et al., 1993). Second, mutant dpp clones located at the posterior margin of the eye disc result in a reduced retinal field and fail to give rise to ommatidia at the posterior border of such clones (Heberlein et al., 1993). Finally, dpp expression is unaffected in a dac mutant background, indicating that dpp is likely to either be genetically upstream of dac or act in a parallel pathway. Since dac plays at least an indirect role in furrow initiation, these observations suggest that dpp may also be involved. However, a direct role for dpp in this process remains to be demonstrated.

Consistent with the proposal that there are distinct mechanisms for furrow initiation and progression, dpp expression is hh-independent prior to initiation of the morphogenetic furrow. dpp is expressed at the posterior margin of the eye disc prior to neural determination and this expression persists in a dac mutant background. Since hh is expressed in the eye only in cells posterior to the morphogenetic furrow and there is little or no furrow movement in the absence of dac function, dpp expression must not require hh activity prior to furrow initiation. However, once the furrow has begun moving across the eye disc, the product of the hh gene is required in differentiating photoreceptors for continued expression of dpp in the furrow and for furrow propagation.

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