

Ectopic eye development in *Drosophila* induced by directed *dachshund* expression

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SUMMARY

The *dachshund* gene encodes a nuclear protein that is required for normal eye development in *Drosophila*. In the absence of *dachshund* function, flies develop with severely reduced or no eyes. We show that targeted expression of *dachshund* is sufficient to direct ectopic retinal development in a variety of tissues, including the adult head, thorax and legs. This result is similar to that observed with the highly conserved *Drosophila* gene *eyeless*, which can induce ectopic eye formation on all major appendages.

Here, we show that *dachshund* and *eyeless* induce the expression of each other and that *dachshund* is required for ectopic retinal development driven by *eyeless* misexpression. These results suggest that the control of eye development requires the complex interaction of multiple genes, even at the very highest regulatory levels.

Key words: *Drosophila*, *dachshund*, *eyeless*, organogenesis, ectopic eye

INTRODUCTION

Molecular and genetic studies of development in *Drosophila* have helped to decipher the fundamental mechanisms of cell-fate determination and, due to the highly conserved nature of the proteins involved, have had profound implications for our understanding of vertebrate development. In particular, the *Drosophila* eye has been an extremely informative setting for the study of both cell-cell inductive events and long-range signaling. While our knowledge of the genes and mechanisms controlling morphogenesis and neural differentiation in the *Drosophila* eye has increased dramatically in recent years, relatively little is known about how retinal tissue is specified in the first place. The most important advance in this area was the discovery that the *eyeless* (*ey*) gene is able to initiate an entire cascade of gene activity sufficient to generate complete and properly formed compound eyes in *Drosophila* (Halder et al., 1995). Ectopic expression of *ey* during development directs the formation of retinal tissue on all major appendages, including antennae, legs and wings. *ey* encodes a member of the *Pax-6* family of transcription factors that contain both a paired domain and a homeodomain (Quiring et al., 1994). Loss-of-function mutations in *ey* produce flies that have either reduced eyes or no eyes at all. Moreover, vertebrate homologs of *ey*, including mouse *Small eye* (*Sey*) and human *Aniridia*, are highly conserved both in sequence and in function. The paired and homeodomains encoded by *Sey* and *Aniridia* are more than 90% identical to *Drosophila* *Ey* and all three genes are required for normal eye development in their respective species (Ton et al., 1991; Hill et al., 1991; Jordan et al., 1992; Glaser et al., 1992). Moreover, in spite of the divergence of insects and

mammals more than 500 million years ago, targeted expression of the mouse *Sey* gene also induces the formation of ectopic compound eyes in *Drosophila*. Taken together, these results led to the hypothesis that *ey* is the master control gene for eye morphogenesis (Halder et al., 1995). However, the identity of genes controlling the expression of *ey* and the presumed downstream targets of *ey* function are not known.

Genes known to function early in *Drosophila* eye development, based on both their expression patterns and mutant phenotypes, are candidates for regulators or targets of *ey* activity. One such candidate is the *dachshund* (*dac*) gene, which encodes a novel nuclear protein required for normal eye development in *Drosophila* (Mardon et al., 1994). *dac* is expressed early during eye development and is required for the first steps of eye morphogenesis and photoreceptor determination. The adult *Drosophila* compound eye is composed of approximately 750 unit eyes or ommatidia that are arranged in a precise hexagonal array (Fig. 1A). Each ommatidium comprises 8 photoreceptor cells, as well as lens-secreting cone cells, pigment cells and a mechanosensory bristle. The adult eye is derived from the eye imaginal disc, an epithelial monolayer that is specified in the late embryo and grows throughout larval development (Fig. 1E). Photoreceptor cells first differentiate at the posterior margin of the eye disc and appear progressively in a wave-like fashion over a period of about two days (Wolff and Ready, 1993). Anterior movement of this wave of neural differentiation, termed the morphogenetic furrow, is composed of two genetically separable events: first, the furrow must begin moving (termed initiation) and second, the furrow must propagate across the eye disc (termed progression). Several conserved genes have been identified whose function is required for proper furrow progression,

both as positive and negative regulators, including *hedgehog* (*hh*), *protein kinase A* and *patched* (reviewed in Heberlein and Moses, 1995; Bonini and Choi, 1995). *hh* encodes a secreted signaling molecule that plays crucial roles in pattern formation and cell-fate determination throughout much of the metazoa (reviewed in Ingham, 1995; Johnson et al., 1994; Perrimon, 1995). Ectopic expression of *hh* anterior to the morphogenetic furrow in the undifferentiated epithelium of the eye imaginal disc leads to precocious photoreceptor development (Heberlein et al., 1995; Lee et al., 1994). In contrast, ectopic expression of *hh* in the anterior compartment of other imaginal discs does not induce photoreceptor development; instead, pattern duplications of structures specific to each disc (i.e., antenna, leg or wing) are observed (Basler and Struhl, 1994). Thus, while ectopic *ey* expression is sufficient to redirect differentiation of at least a subset of cells from all imaginal discs to a retinal fate, *hh* acts further downstream during morphogenesis to pattern cells whose primary fate has already been determined.

In contrast to furrow progression, a largely different set of genes regulate furrow initiation. *wingless* (*wg*) encodes a secreted signaling molecule that is required to prevent premature initiation of the furrow away from the dorsal and ventral margins of the eye disc and thus refines the furrow to a linear (i.e., not curved) wave of development (Ma and Moses, 1995; Treisman and Rubin, 1995). Signaling by *decapentaplegic* (*dpp*), a transforming growth factor β family member, is likely to be required for furrow initiation since loss-of-function mutations in *mothers against dpp* (*mad*) prevent initiation (Wiersdorff et al., 1996). *mad* encodes a novel protein required downstream of *dpp* signaling (Sekelsky et al., 1995). Even though *dpp* is expressed in the furrow throughout progression, *dpp* may not be strictly required for this process because loss of *mad* function does not prevent furrow propagation. Like *dpp*, *dac* is expressed at the posterior margin of the eye disc prior to furrow initiation and neural development (Fig. 2A). In the absence of *dac* function, *dpp* expression remains at the posterior margin of the eye disc, furrow initiation is prevented and adults develop with severely reduced or no eyes (Fig. 1B,F). Although *dac* is highly expressed immediately anterior and posterior to the furrow throughout furrow propagation (Fig. 2B), *dac* is not required for furrow progression or *dpp* expression. However, *dac* is required for proper construction and assembly of ommatidia into the hexagonal array characteristic of the compound eye (Mardon et al., 1994). Since the *dac* and *mad* mutant phenotypes in the eye are very similar, it has been suggested that *dac* may function downstream of *dpp* signaling but, unlike *mad*, is not required for the maintenance of *dpp* expression (Wiersdorff et al., 1996). Based on its early expression pattern at the posterior margin of the eye disc and its essential role in initiation of furrow

movement, *dac* plays a critical role during the earliest stages of morphogenesis and photoreceptor specification in the eye.

In this paper, we show that targeted *dac* expression in the antennal and leg imaginal discs induces ectopic eye development. Ectopic eyes have a nearly normal morphology and contain photoreceptor neurons that project axons in a manner similar to photoreceptors in the eye disc. Moreover, we show that ectopic *dac* expression induces *ey* expression, suggesting that *dac* acts upstream of *ey* during retinal development. Surprisingly, we also found that *ey* induces *dac* expression and that *dac* is required for *ey* function. These results demonstrate that *dac* and *ey* expression are intimately related and that these genes are likely to function together in the control of retinal cell-fate specification at early stages of eye development.

MATERIALS AND METHODS

Fly genetics

All *Drosophila* crosses were carried out at 25°C on standard media. UAS-*dac* constructs were generated using a full-length *dac* cDNA (Mardon et al., 1994) cloned in the *EcoRI* site of pUAST (Brand and Perrimon, 1993). Flies were transformed using standard techniques

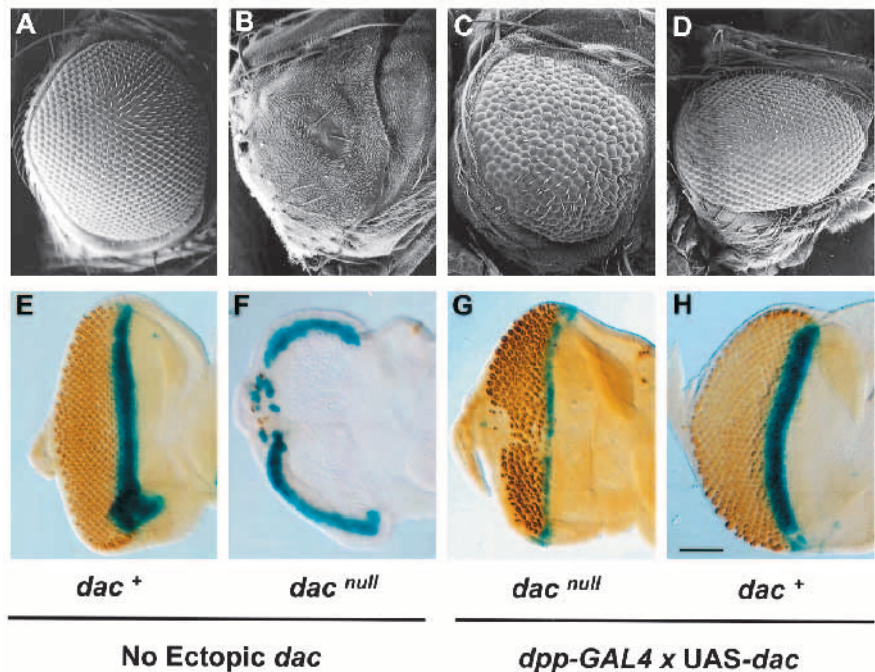


Fig. 1. Rescue of morphogenetic furrow initiation in *dachshund* mutant animals. Scanning electron microscope images of adults eyes (A-D) and light microscope images of late larval eye imaginal discs (E-H) stained to reveal the positions of neurons in brown and *dpp* expression in blue are shown. (A) Wild-type adult eye. (B) *dac* null mutant adults have severely reduced or no eyes. (C) *dpp-GAL4* × UAS-*dac* rescues eye development in *dac* null mutants but the ommatidial array is disorganized. (D) In a wild-type background, *dpp-GAL4* × UAS-*dac* truncates the adult eye in the dorsal-ventral dimension but ommatidial assembly is normal. (E) In wild-type animals, the furrow (blue stripe) is about half-way across the eye disc by the late third instar larval stage. Photoreceptor differentiation (brown dots) occurs immediately posterior to the furrow. (F) In *dac* null mutant eye discs, furrow initiation fails, *dpp* expression remains at the posterior margin and little or no neural development occurs. (G,H) *dpp-GAL4* × UAS-*dac* rescues furrow initiation in *dac* null mutants (G) but has little effect on furrow movement in a wild-type background (H). Posterior is to the left and dorsal is up in all panels. Scale bar (100 μ m) in H is for all panels.

(Rubin and Spradling, 1982). 14 transformants were analyzed in detail. *dpp-GAL4* flies express *GAL4* in the antennal disc and at the A-P compartment boundaries of the leg and wing discs in a pattern that approximates that of *dpp* (Stahling-Hampton et al., 1994). For an unknown reason, the *dpp-GAL4* line does not express *GAL4* in the morphogenetic furrow during furrow progression. Instead, *GAL4* is expressed specifically at the posterior margin of the early eye disc and then fades away from the central portion of the disc rapidly following furrow initiation (see Fig. 2). Rescue of *dac* null mutant animals were assayed as follows: *dac[3], dpp-lacZ*; *dpp-GAL4 / SM6-TM6B* flies were crossed to *dac[1]*; *UAS-dac / SM6-TM6B* flies and non-*Tb* larvae or non-*Hu* adults were selected as *dac[3], dpp-lacZ / dac[1]*; *dpp-GAL4 / UAS-dac*. Targeted expression of *ey* in a *dac* null mutant background was carried out as follows: *dac[3], dpp-lacZ*; *dpp-GAL4 / SM6-TM6B* flies were crossed to *dac[1]*; *UAS-ey / SM6-TM6B* flies and non-*Tb* larvae were selected as *dac[3], dpp-lacZ / dac[1]*; *dpp-GAL4 / UAS-ey*.

Scanning electron microscopy and histology

Due to severe truncation of the legs and wings, animals carrying both *UAS-dac* and *dpp-GAL4* constructs fail to emerge from their pupal cases. Thus, all adult images were prepared from animals dissected from late pupae. Samples were prepared for scanning electron microscopy and histological sections as previously described (Kimmel et al., 1990; Tomlinson and Ready, 1987).

Immunohistochemistry

Imaginal discs were dissected and stained as previously described (Mardon et al., 1994). All ectopic *dac* expression studies in this paper were performed using *dpp-GAL4* × *UAS-dac* animals. *dpp* expression was shown using a β-galactosidase reporter construct specific for imaginal discs (Blackman et al., 1991). Anti-Elav (Robinow and White, 1991), anti-Dac (Mardon et al., 1994), anti-Glass (Moses and Rubin, 1991), anti-Neuroglian (Hortsch et al., 1990) and anti-Ey (Halder et al., 1995) stainings were all performed using the same protocol with the following exceptions. For anti-Glass staining, imaginal discs were dissected in PBS and fixed in PLP (2% paraformaldehyde, 10 mM sodium periodate, 75 mM lysine, 37 mM sodium phosphate pH 7.2) for 25 minutes on ice. For anti-Ey staining, imaginal discs were dissected in PBS and fixed in PEM (0.1 M Pipes pH 7.0, 0.2 mM EGTA, 4% paraformaldehyde) for 25 minutes on ice. All discs were mounted in 80% glycerol in PBS.

RESULTS

Rescue of the *dachshund* mutant eye phenotype

We sought to further our understanding of *dac* gene function during development through ectopic expression studies using the *GAL4* system (Brand and Perrimon, 1993; Brand and Dormand, 1995). Our first step toward this goal was to establish that our *dac* cDNA clone encodes a functional protein using rescue of the *dac* mutant eye phenotype as an assay. Confirming predictions based on our previous analyses, expression of *dac* specifically at the posterior margin of the eye disc early in development (Fig. 2C,E) is able to fully rescue morphogenetic furrow initiation in a *dac* null mutant background (Fig. 1C,G). In this setting, even though *dac* is not expressed anterior or posterior to the furrow during most of progression, the furrow still moves normally across the entire eye imaginal disc (Fig. 2D,F). However, the requirement for *dac* function during furrow progression for normal ommatidial assembly is readily apparent in these preparations: rescued eyes are disorganized, both during larval stages and in the adult (Fig. 1C,G). Although ectopic *dac* expression at the posterior margin of the eye disc

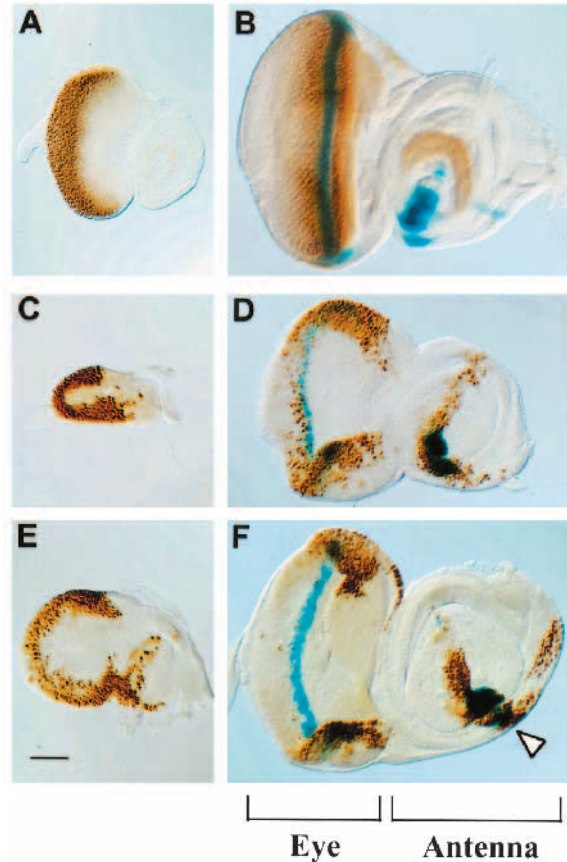


Fig. 2. *dachshund* is required only at the posterior margin for normal furrow movement. Eye-antennal discs were stained to reveal the positions of *dpp* expression in blue (B,D,F) and Dac protein in brown. (A,B) In the wild type, Dac is detected at the posterior margin of the eye disc prior to furrow initiation (A) and both anterior and posterior to the furrow (blue stripe) throughout progression (B). (C-F) *dpp-GAL4* × *UAS-dac* in a *dac* null mutant background strongly induces Dac protein at the posterior margin of early larval eye discs (C,E) and is sufficient to initiate furrow movement. By mid-third instar larval development, Dac protein is only weakly detectable in the center of the eye disc (D) and is gone by late larval stages (F). At no stage is Dac protein detectable anterior to the furrow. Nevertheless, the morphogenetic furrow (blue stripe) progresses normally across the eye disc. In contrast, Dac protein is strongly induced at the dorsal and ventral margins of the eye disc throughout the second and third instar larval stages (C-F). In the antennal disc, *dpp-GAL4* × *UAS-dac* induces Dac protein at the ventral margin of the disc and along the A-P midline of the disc during larval development (C-F, arrowhead in F). Posterior is to the left and dorsal is up in all panels. Scale bar (100 μm) in E is for all panels.

in a wild-type (*dac*⁺) background had little or no effect on furrow initiation or progression, the adult eyes of such animals are truncated in the dorsal-ventral dimension (Fig. 1D,H). This may be attributable to the strong ectopic expression of *dac* at the dorsal and ventral margins of the eye disc (Fig. 3D). These experiments confirmed that our *dac* cDNA encodes a functional protein capable of rescuing furrow initiation in *dac* mutant animals and that little or no Dac protein is required for furrow progression.

dachshund induces ectopic eyes

In addition to the eye disc, *dac* is normally expressed only in specific domains of the antennal, leg and wing discs (Fig. 3A-C) and the embryo (data not shown). These restricted patterns of *dac* expression are important for normal development: a single 30 minute heat shock (hs) of animals carrying both *hs-GAL4* and *UAS-dac* transgenes at any time during development causes complete lethality (data not shown). More subtle phenotypes were produced using a *dpp-GAL4* construct to drive *dac* expression in the antennal disc and at the anterior-posterior (A-P) compartment boundary of the leg and wing discs (Cohen, 1993). In each case, ectopic expression of Dac protein caused strong disruption of the normal *dac* pattern of expression (Fig. 3D-F), increased cell death and truncation of the resulting appendage (data not shown). Strikingly, about 20% of these animals develop ectopic eyes just ventral to the antenna on the anterior surface of the head (Fig. 4A,B,D). This position in the adult corresponds to the site of strongest ectopic *dac* expression at the ventral margin of the antennal disc (arrowhead in Fig. 3D). Ectopic eyes derived from the ventral antennal disc often comprise up to 40 or 50 ommatidia (Fig. 4A). Ectopic retinal development can occur in a *dac* null mutant background where the *UAS-dac* transgene is the only source of Dac protein (data not shown).

The external morphology of *dac*-induced ectopic eyes closely resembles that of normal adult eyes, including lens and interommatidial bristle formation (Fig. 4C,D). Sections of ectopic eyes reveal a typical ommatidial structure that includes the densely staining rhabdomeres (the light-sensing organelles of the retina) and pigment granules that serve to optically insulate each ommatidium (Fig. 4E,F). However, the normally precise hexagonal shape of most ommatidia is absent (Fig. 4C,D) and a minority of ommatidia display the characteristic trapezoidal arrangement or number of rhabdomeres (Fig. 4E,F). Ectopic eyes derived from *dac* misexpression are normally pigmented (Fig. 5B-D).

Ectopic *dac* expression also induces retinal development on a portion of the body wall (thorax) that is derived from leg imaginal discs. While 95% of animals display at least some red pigment on the thorax just dorsal to the articulation of the leg and body, only 5% present obvious ommatidial structures (Fig. 6). This position again corresponds to the site of strongest ectopic *dac* expression in the leg imaginal disc (arrowheads in Fig. 3B,E). In about one percent of cases, pigment is also clearly visible on the leg per se (data not shown). Patches of red pigment are only rarely associated with wing structures and no well-formed ommatidia have been observed. Truncation of the adult appendages demonstrates that ectopic *dac* expression along the A-P compartment boundary significantly alters the fate of cells in each of the imaginal discs, indicating that *dac* plays an instructive role during development. Moreover, because ectopic *dac* expression in the antennal and leg imaginal discs is sufficient to direct some cells to adopt a retinal fate, *dac* must function at or near the very highest levels of the regulatory hierarchy controlling eye development.

Developmental analysis of ectopic photoreceptors

Ectopic neural development resulting from targeted *dac* expression is readily apparent in antennal and leg discs during late larval stages. The nuclear protein Elav is detected in all neurons in *Drosophila* (Robinow and White, 1991) and is not normally expressed in the antennal disc (Fig. 5E). Ectopic *dac* expression induced formation of Elav-positive cells in the ventral antennal disc in about 20% of samples analyzed, in good agreement with the frequency of ectopic eyes observed in adults (Fig. 5F,G). These cells are likely to be ectopic photoreceptor neurons. Indeed, we found evidence for retina-specific development in the antennal disc by the ectopic appearance of Glass-expressing cells (Fig. 5H,L). *glass* encodes a zinc-finger protein that is specific for visual system development in *Drosophila* and is not normally expressed in antennal or leg imaginal discs (Moses and Rubin, 1991; Moses et al., 1989). Ectopic *glass* expression is also observed in the dorsal leg disc in response to

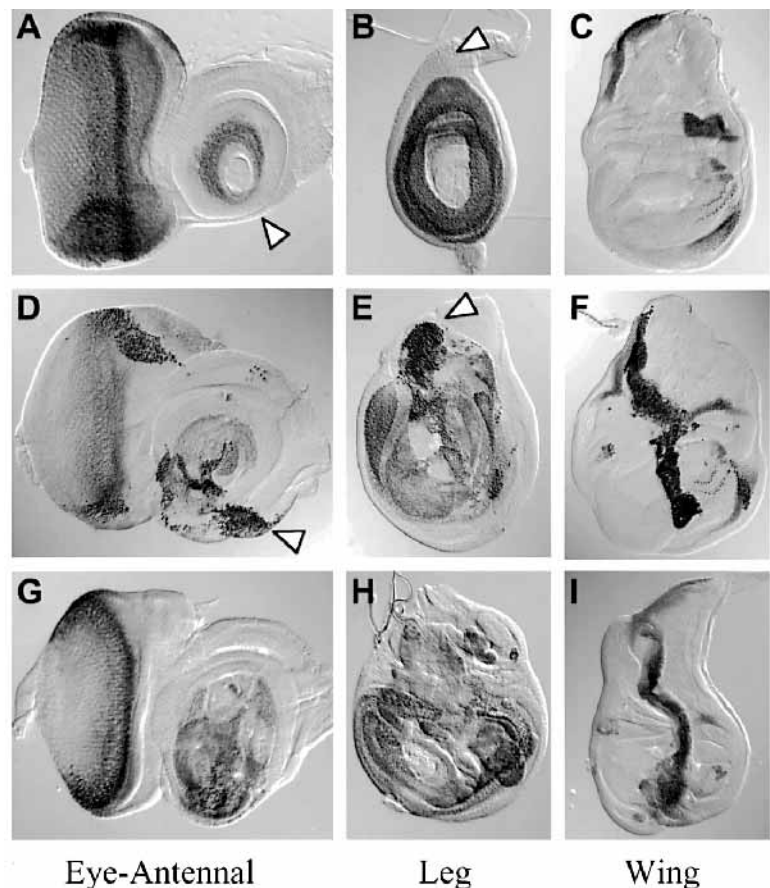


Fig. 3. *eyeless* induces *dachshund* expression. Eye-antennal, leg and wing imaginal discs were stained for Dac protein expression. (A-C) Wild type. (D-F) *UAS-dac* × *dpp-GAL4*. (G-I) *UAS-ey* × *dpp-GAL4*. *dac* is not expressed at the ventral margin of the antennal disc (arrowhead in A), the dorsal margin of the leg disc (arrowhead in B) or along most of the A-P compartment boundary in the wing disc (C) of wild-type larvae. Ectopic expression of *dac* in *UAS-dac* × *dpp-GAL4* larvae disrupts the normal pattern of *dac* expression in all discs (D-F). The arrowheads in D, E show the positions of ectopic retinal development. Ectopic expression of *ey* in *UAS-ey* × *dpp-GAL4* larvae also disrupts the normal pattern of *dac* expression in all discs (G-I) and is likely to be the result of induction of *dac*. *ey* induction of *dac* is most clearly seen in the antennal and wing discs (G,I). Posterior is to the left and dorsal is up in all panels.

dac misexpression (Fig. 6C,F). Remarkably, ectopic photoreceptors in the antennal disc send out axonal projections that first extend medially and then turn sharply posteriorly (Fig. 5J,K). This is very similar to the pattern of normal axonal projection in the wild-type eye disc where photoreceptor axons exit posteriorly through the optic stalk to synapse in the optic lobe of the larval brain (Fig. 5I). Whether the axonal projections of ectopic photoreceptor cells in the antennal disc are able to find their way to targets in the brain (or elsewhere) is not known.

We looked for evidence of morphogenetic furrow movement by examining the pattern of *dpp* expression in antennal discs positive for photoreceptor cell development. *dpp* expression marks the position of the furrow as it progresses across the eye imaginal disc. In the antennal disc, *dpp* is normally expressed in a sector abutting the ventral margin of the antennal disc (Fig. 5E). In antennal discs that show ectopic neural development, *dpp* expression appears to have moved away from the ventral margin toward the center of the disc as an elongated stripe (Fig. 5F,G). These results suggest that ectopic *dac* expression is sufficient to initiate movement of an ectopic furrow at the ventral margin of the antennal disc and induce photoreceptor development.

Relationship between *dachshund* and *eyeless*

Ectopic eye development caused by targeted expression of the

dac gene is remarkably similar to results obtained with *ey* (Halder et al., 1995). We therefore sought to examine the relationship between *dac* and *ey* using both molecular and genetic approaches. Three independent results suggest that *dac* functions downstream of *ey*. First, misexpression of *ey* in the antennal, leg and wing imaginal discs is sufficient to induce ectopic *dac* expression and disrupt the normal pattern of *dac* expression in all discs (Fig. 3G-I). These results suggest that *ey* activity positively regulates *dac* transcription, either directly or indirectly, and raised the possibility that *dac* function may be required downstream of *ey*. Indeed, we found that targeted *ey* expression was unable to induce ectopic eye formation in a *dac* null mutant background (Fig. 7). Finally, if *dac* functions downstream of *ey* in a simple linear pathway, then *dac* function should not be required for *ey* expression. *ey* is normally expressed anterior to the morphogenetic furrow in the eye imaginal disc (Fig. 8A) and is not expressed in other imaginal discs (Quiring et al., 1994). We found that *ey* is still expressed in *dac* null mutant eye discs, demonstrating that *dac* is not essential for *ey* expression (Fig. 8B). However, *ey* expression is restricted to the posterior margin of the disc, presumably due to the failure of furrow initiation in *dac* mutants. These results suggest that *dac* functions downstream of *ey* and, considering that *dac* can induce ectopic retinal development, are consistent with the idea that *dac* may be a direct target of *ey* function.

No null mutant allele of *ey* is available (Quiring et al., 1994) and all existing *ey* alleles cause highly variable phenotypes in the eye, ranging from wild type to total absence in the same animal (data not shown). Thus, we are unable to test whether *ey* is required for *dac* expression or function. Surprisingly, however, we found that ectopic *dac* expression in the antennal disc is sufficient to induce *ey* expression (Fig. 8C). In addition, the normal pattern of *ey* expression in the eye disc is disrupted as a result of ectopic *dac* expression. Extremely weak or no *ey* expression was observed in the leg or wing discs in response to directed *dac* expression (data not shown). Thus, *dac* is unable to drive *ey* expression at detectable levels in all cells in which it is present. Nevertheless, *dac*-mediated induction of *ey* expression in the antennal disc, in addition to the results presented above, suggests that *dac* may function both upstream and downstream of *ey* during retinal development.

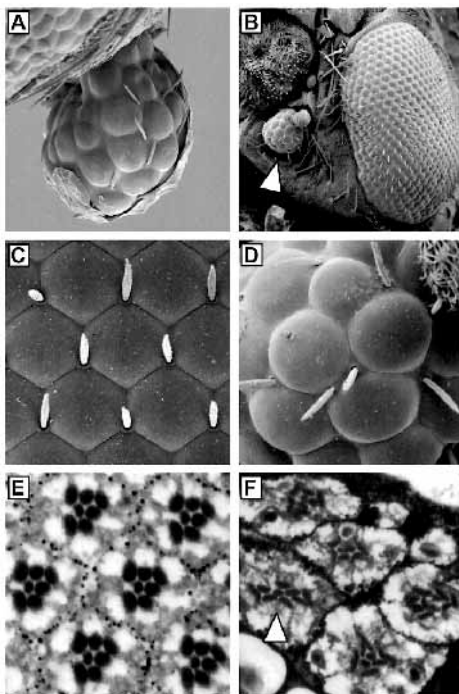
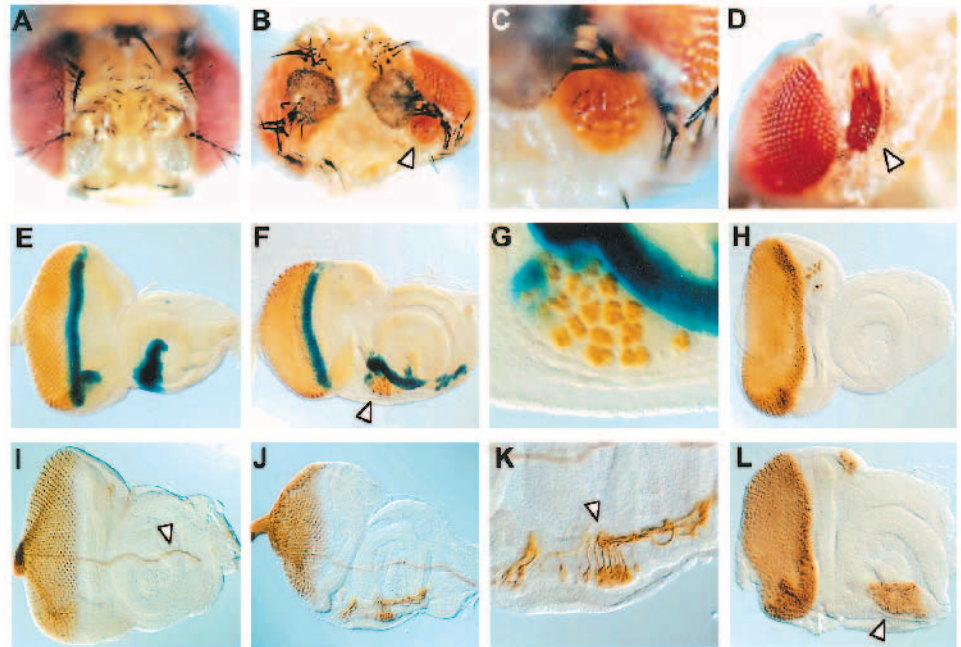


Fig. 4. Targeted *dachshund* expression induces ectopic retinal development. (A,B) Scanning electron microscope images of ectopic eyes (arrowhead in B) induced by *dac* on the adult head. (C) A portion of a wild-type adult eye shows the normal hexagonal array of ommatidia and the regular spacing of interommatidial bristles. (D) A higher magnification of (B) reveals the abnormal ommatidial shape and irregular bristle arrangement of a *dac*-induced ectopic eye. (E,F) Sections of wild-type (E) and *dac*-induced ectopic (F) eyes. Rhabdomeres are large, darkly staining organelles that contain the light-sensitive pigment rhodopsin (E). Compared to wild type, the rhabdomeres of ectopic eyes are usually small and disorganized (arrowhead in F) but can often appear wild type (data not shown).

DISCUSSION

Proper construction of tissues and organs during development requires the precise regulation of complex genetic hierarchies. Only a few cases have been described where expression of a single gene is sufficient to direct the development of complete and properly formed organs or systems. For example, expression of the *Sry* gene is able to cause genotypically XX mice to develop as phenotypic males (Koopman et al., 1991). In two other cases, the *LEAFY (LFY)* and *APETALA1 (API)* genes each direct precocious flower development in *Arabidopsis* (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995). Most recently, targeted expression of the *Drosophila* gene *vestigial (vg)* was shown to generate wing-like outgrowths from the eyes, legs and antennae of adult flies (Kim et al., 1996). Finally, the highly conserved *Drosophila* gene *eyeless (ey)* is sufficient to direct the formation of compound eyes on all the major appendages of flies when ectopically

Fig. 5. Photoreceptor development in ectopic eyes. (A) The anterior surface of a wild-type adult head and the antennae. (B-D) *dac*-induced ectopic eyes on the head are located just ventral to the antennae and are normally pigmented (arrowheads). (C) is a higher magnification of the ectopic eye shown in (B). (E-G) Eye-antennal discs were stained for the neuron-specific Elav protein in brown and *dpp* expression in blue. No Elav-staining cells are found in the wild-type antennal disc (E). Ectopic *dac* expression in the antennal disc induces clusters of Elav-positive cells just ventral to the *dpp* stripe which has moved away from the disc margin (arrowhead in F). A higher magnification of (F) reveals that ectopic groups of neurons induced by *dac* resemble wild-type ommatidial clusters (G). (H and L) The visual system specific Glass protein is not expressed in the wild-type antennal disc (H). Induction of *glass* by targeted *dac* expression (arrowhead in L) demonstrates that ectopic neurons are presumptive photoreceptor cells. (I-K) The *Drosophila* protein Neuroglian is present in all neurons and their axons and was detected using monoclonal antibody BP104. In the wild type, the only staining seen in the antennal disc is the larval Bolwig's nerve (arrowhead in I). *dac*-induced ectopic neurons in the antennal disc (J) project axons to towards the midline (arrowhead in K) and then posteriorly. Posterior is to the left for eye discs and to the right for antennal discs and dorsal is up in all panels.



expressed during development (Halder et al., 1995). Most of these genes encode nuclear proteins (Sry, AP1, Vg and Ey) that may control elaborate gene networks governing organ formation. In particular, *ey* encodes a member of the *Pax-6* family of DNA-binding transcription factors that includes mouse *Small eye* (*Sey*) and human *Aniridia* (Quiring et al., 1994). Targeted expression of *ey* or *Sey* causes ectopic eye development in *Drosophila*, suggesting that genetic mechanisms of retinal development may be more highly conserved

than previously anticipated (Halder et al., 1995). Since *ey*, *Sey* and *Aniridia* are each required for normal eye development and *ey* and *Sey* are functionally conserved, these genes are likely to represent descendants of a common ancestral gene that existed prior to the divergence of insects and mammals more than 500 million years ago. Moreover, these results have forced

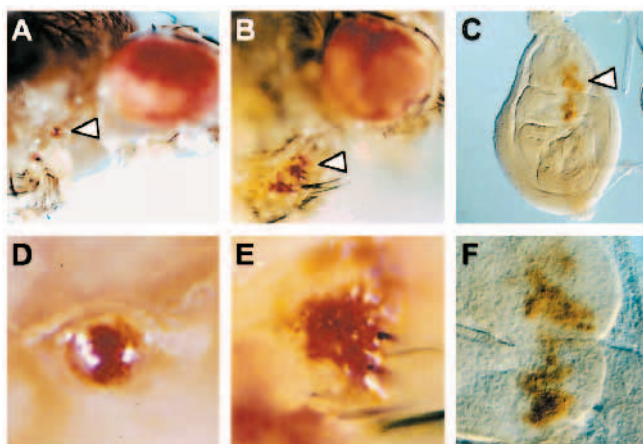


Fig. 6. Ectopic retinal development on the legs and thorax. (A,B) Light microscope images of *dac*-induced ectopic eyes (arrowheads) on the thorax just dorsal to the prothoracic leg. (C) Glass protein is induced by ectopic *dac* expression in leg imaginal discs (arrowhead). *glass* is not expressed in wild-type leg discs (data not shown). (D-F) Higher magnification views of A-C, respectively. A group of about 8 ommatidia are visible in E. Posterior is to the left and dorsal is up in all panels.

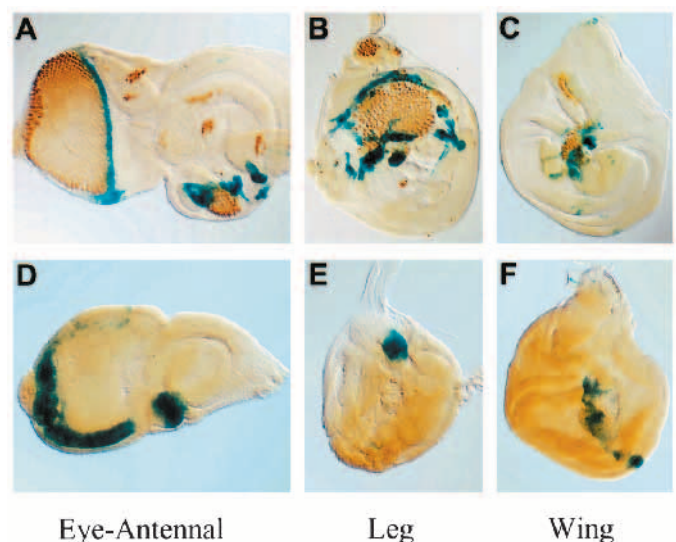


Fig. 7. *dachshund* is required for *eyeless* function. Eye-antennal, leg and wing imaginal discs were stained to reveal neural development in brown and *dpp* expression in blue. (A-C) Discs prepared from *UAS-ey* × *dpp-GAL4* larvae in a wild-type background show ectopic neural development in the antennal (A), leg (B) and wing discs (C). (D-F) Targeted *ey* expression is unable to induce ectopic neural development in any disc in a *dac* null mutant background. Posterior is to the left and dorsal is up for all panels.



Fig. 8. *dachshund* induces *eyeless* expression. Eye-antennal discs were stained for Ey protein expression. (A) In wild-type larvae, *ey* is expressed anterior to the furrow in the eye disc and is not expressed anywhere in the antennal disc. (B) *dac* is not required for *ey* expression. In *dac* null mutant larvae, *ey* expression remains at the posterior margin of the eye disc. (C) Ectopic *dac* expression in UAS-*dac* x *dpp-GAL4* larvae induces *ey* at the ventral margin of the antennal disc (arrowhead) and disrupts the normal pattern of *ey* expression in the eye disc. Posterior is to the left and dorsal is up for all panels.

a reevaluation of the traditional view that insect and vertebrate visual systems evolved independently (Quiring et al., 1994).

We have identified the *Drosophila* gene *dachshund* (*dac*) as another member of this small group of genes known to occupy positions high in the regulatory hierarchies directing organ development. *dac* is expressed in nearly all cells of the eye imaginal disc throughout larval development and in the lamina of the optic lobe where most photoreceptor axons first synapse in the brain. *dac* encodes a novel nuclear protein that is necessary for the initiation of eye morphogenesis and proper assembly of the retinal field in *Drosophila* (Mardon et al., 1994). Here, we have shown that *dac* is also sufficient to induce properly formed ectopic eye structures in a variety of tissues in *Drosophila*. Taken together, these results suggest that *dac* functions at many levels of the genetic hierarchy controlling eye development and that *dac* can switch on a pathway that is likely to involve thousands of genes (Halder et al., 1995; Thaker and Kankel, 1992).

Targeted expression of *dac* is sufficient to induce ectopic *ey* expression in one or more imaginal tissues during larval development. Similarly, *ey* can induce *dac* expression in most imaginal cells. Whether a similar regulatory relationship normally exists between *dac* and *ey* in the eye disc is not known. In wild-type eye discs, *dac* and *ey* expression anterior to the morphogenetic furrow overlap significantly (Figs 2B, 8A). In addition, ectopic expression of either gene at the posterior and lateral margins of the eye disc disrupts the normal pattern of expression of the other (Figs 3G, 8C). Thus, it seems likely that *dac* and *ey* contribute to the regulation of each other during normal eye development.

We have shown that *dac* is required for induction of ectopic eye development by *ey* misexpression. In contrast, we do not know whether *ey* is required for *dac* function because no strong or highly penetrant mutant alleles of *ey* exist. Although *dac* does not induce strong *ey* expression in the leg imaginal disc, *dac* can efficiently induce pigment development and, in some cases, ommatidial formation in structures derived from the leg disc. Thus, if *dac* induction of ectopic retinal development is mediated by and requires *ey* function, then only low levels of *ey* are required for this process. Alternatively, *dac* may act either downstream of *ey* or in a parallel pathway and not require *ey* function in this regard.

Since *dac* is able to induce ectopic *ey* expression in the antennal disc, *dac* can function as a positive regulator of *ey*. Three lines of evidence suggest that *dac* also functions down-

stream of *ey*. First, *ey* induces *dac*. Second, *dac* is not required for *ey* expression and third, *dac* is required for ectopic eye induction by *ey*. Thus, it is possible that *dac* and *ey* participate in a positive regulatory feedback loop during eye development. This is reminiscent of the regulatory relationships proposed for *wingless*, *hedgehog* and *engrailed* in the establishment of parasegmental boundaries in the *Drosophila* embryo (Hooper, 1994; Manoukian et al., 1995) and *Sonic hedgehog* and *Fgf-4* in specifying growth and patterning of the vertebrate limb (Laufer et al., 1994; Niswander et al., 1994). Whether *Dac* and *Ey* each act directly or indirectly to control transcription of the other remains to be determined. Since *dac* is expressed in a wide variety of tissues and locations where *ey* is not, including the antennal, leg and wing discs, it is clear that neither gene is solely necessary or sufficient for expression of the other. Moreover, the nature of the regulatory relationship between *dac* and *ey* must depend upon the local cellular environment.

Targeted expression of *dac* using *dpp-GAL4* induces ectopic retinal development in about 20% of antennal discs and, to a lesser extent, in leg and wing discs. In contrast, targeted expression of *ey* in the same manner is able to direct retinal development in all imaginal discs with complete penetrance. This suggests that *ey* may induce the expression of one or a few other genes that facilitate eye development and that are not efficiently induced by *dac*. However, neither gene is able to induce photoreceptor development in all cells in which it is expressed. For example, ectopic *dac* expression is unable to efficiently induce retinal development along any part of the A-P compartment boundary of the wing imaginal disc. Similarly, targeted *ey* expression fails to induce photoreceptor development along the ventral portion of the A-P compartment boundary of the wing disc (data not shown). These results suggest that *dac* and *ey* do not act alone in the control of gene expression or retinal cell-fate specification. Instead, these genes are likely to require the cooperation of, or be inhibited by, other factors that are expressed in a spatially or temporally restricted pattern during development. Genes acting early in retinal development are potential candidates for such factors, and include *dpp*, *sine oculis* (*so*) and *eyes absent* (*eya*). Both *so* and *eya* encode nuclear proteins that are expressed early in the eye imaginal disc and are required for normal eye development (Bonini et al., 1993; Cheyette et al., 1994). Further experiments examining the relationships among these and other genes will be required to decipher the molecular and genetic mechanisms controlling retinal cell-fate specification.

Although a vertebrate homolog of *dac* has not been identified, highly conserved *dac* homologs have been isolated from several invertebrate species, including *Drosophila virilis*, the flour beetle *Tribolium* and the butterfly *Precis coenia*. The amino acid sequences predicted from these *dac* homologs are 60 to 85% identical to the *Drosophila melanogaster* *Dac* protein (W. Shen, G. Mardon, unpublished data and T. Heanue and C. Tabin, personal communication). Since these species are 60 to 250 million years diverged from *Drosophila melanogaster* and most of the known genes required for eye development in *Drosophila* are highly conserved in vertebrates, it is likely that one or more vertebrate homologs of *dac* exists (Beverley and Wilson, 1984).

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