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9 June 1997

Dr. Philip J. Migliore
Chairman and Research Director
Scientific Advisory Committee of The Moran Foundation
Department of Pathology
Baylor College of Medicine
Texas Medical Center
Houston, TX 77030

Dear Dr. Migliore,

Enclosed please find an updated progress report for funding received during 1996 from the Moran Foundation for a project entitled "An Animal Model System to Study Eye Development" (project number 1-95-0081).

Funding from the Moran Foundation is an important mechanism for initiating new research efforts and is greatly appreciated.

Sincerely,

Graeme Mardon

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Progress Report for Moran Foundation Funded Work

Principal Investigator: **Graeme Mardon, Ph.D.**

Project Title: **An Animal Model System to Study Eye Development**

Project Year: **1996**

Project Number: **1-95-0081**

Summary of Progress

The overall goal of our original proposal was to look for DNA-binding activity of the novel nuclear protein encoded by the *Drosophila* gene *dachshund*. Our approach was two-fold: First, we looked for evidence of DNA-binding activity of the Dachshund protein. Then, we proposed to look for specific DNA sequences that could be bound by Dachshund protein.

We have made excellent progress on the first phase of this project. We expressed Dachshund protein in the larval salivary glands of flies. Chromosomes in the salivary gland of *Drosophila* larvae are polytene; that is, they are present in up to 1000 copies per cell and are all paired together forming large and easily visible chromosomes. Using our monoclonal antibodies prepared against the *Drosophila* Dachshund protein, we were able to visualize specific staining on a subset of chromosomal bands. These results provide preliminary evidence that the Dachshund protein is able to associate with DNA or chromatin in vivo. Moreover, since the protein was detected at specific positions along the chromosomes, it seems likely that Dachshund protein associates with DNA in a site-specific manner. However, whether the Dachshund protein binds directly to DNA or via other DNA-binding proteins is not known.

Now that we have good preliminary evidence that the Dachshund protein may be able to bind DNA, we will move forward with the other experiments outlined in our proposal designed to look for non-specific and specific DNA-binding activity by Dachshund protein in vitro.

Moran Foundation funding has been of great value to us as these results were included as part of an NIH R01 grant application submitted on June 2, 1997.

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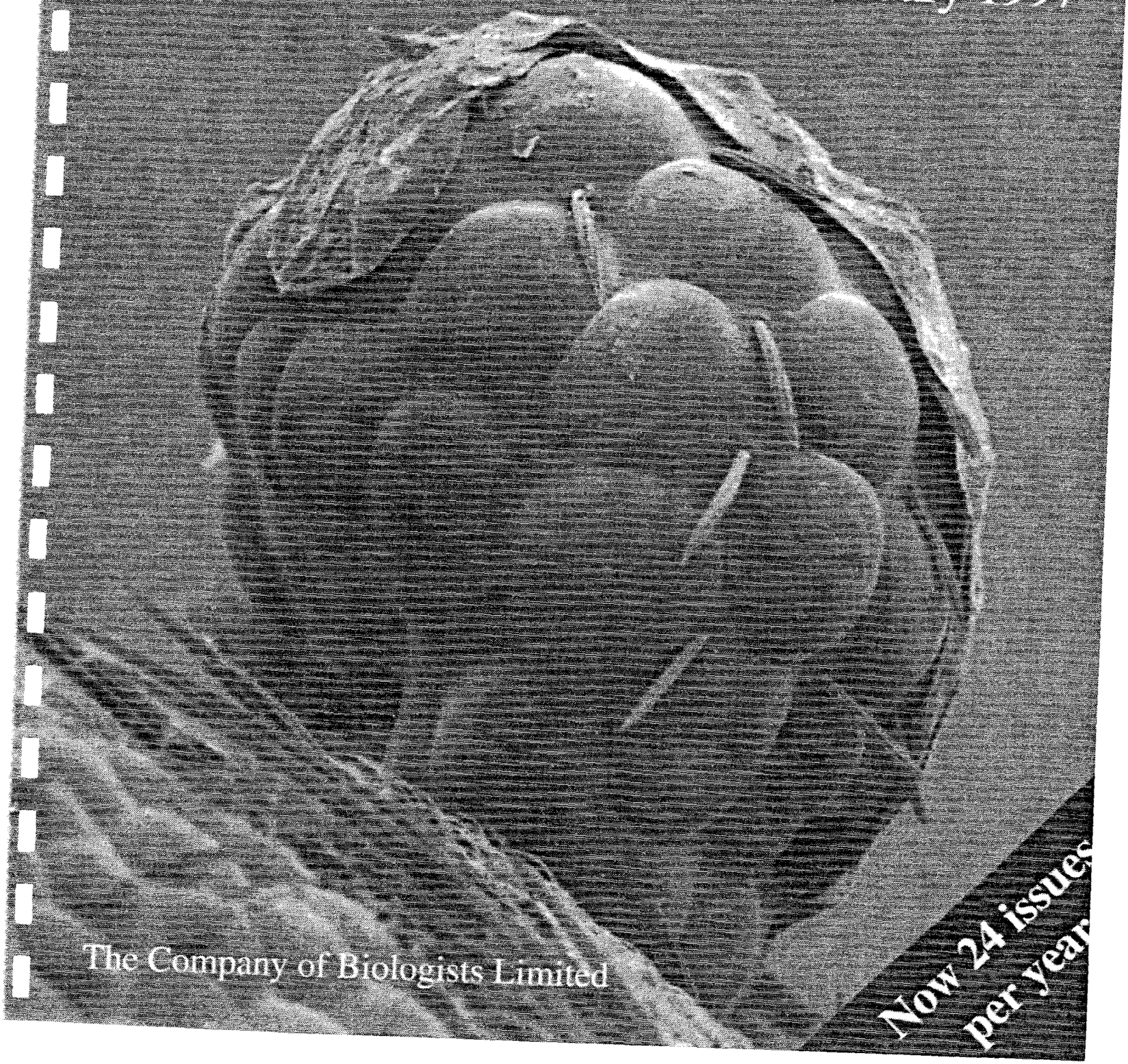

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Ectopic eye development in *Drosophila* induced by directed *dachshund* expression

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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 *Eco*RI site of pUAST (Brand and Perrimon, 1993). Flies were transformed using standard techniques

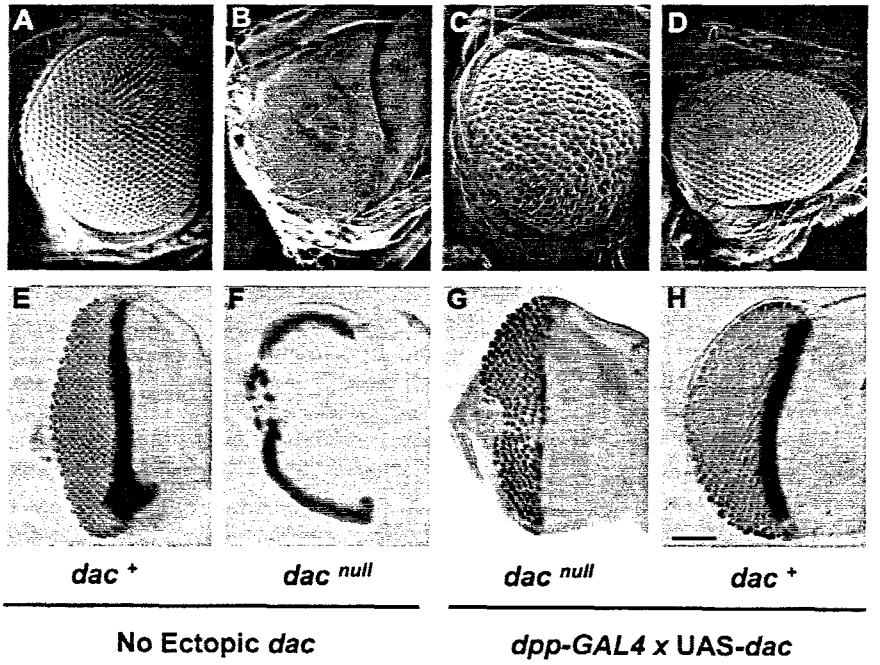

Fig. 1. Rescue of morphogenetic furrow initiation in *dachshund* mutant animals. Scanning electron microscope images of adults eyes (A-D) and light microscope images of late larval eye imaginal discs (E-H) stained to reveal the positions of neurons in brown and *dpp* expression in blue are shown. (A) 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* x 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 μm) 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* (Staehling-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 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.

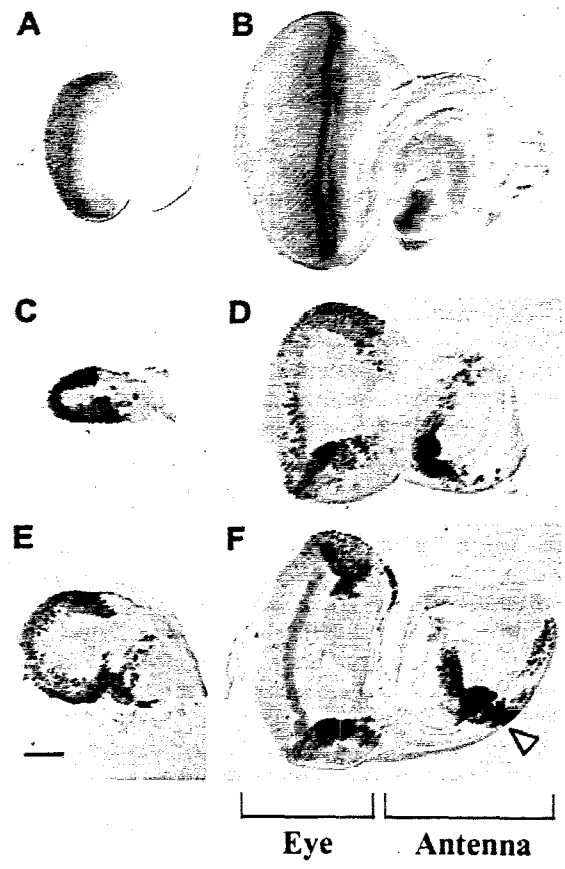

Fig. 2. *dachshund* is required only at the posterior margin for normal furrow movement. Eye-antennal discs were stained to reveal the positions of *dpp* expression in blue (B,D,F) and Dac protein in brown. (A,B) In the wild type, Dac is detected at the posterior margin of the eye disc prior to furrow 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.

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

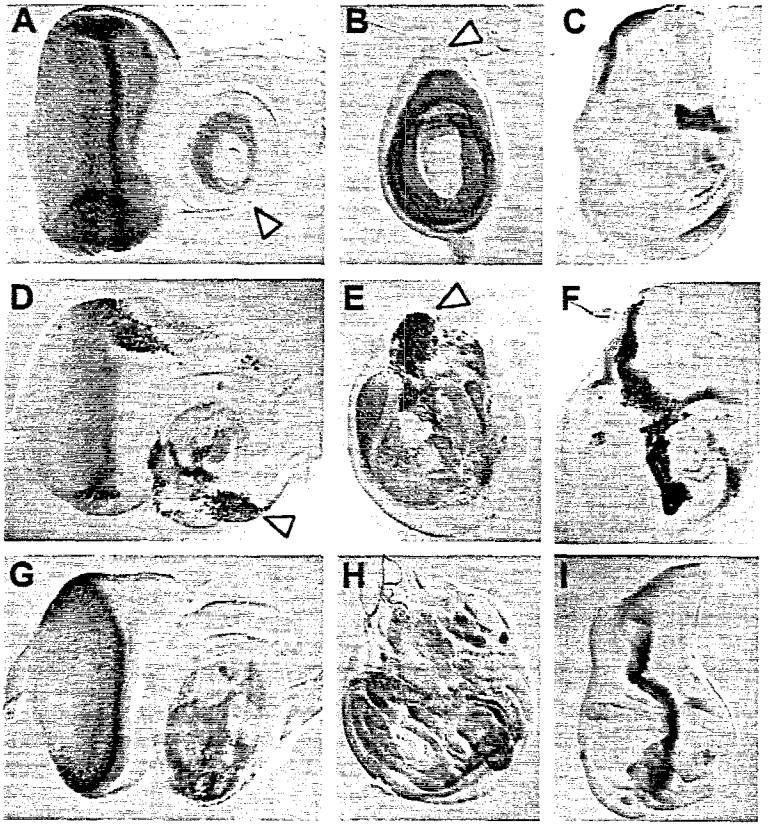

Fig. 3. *eyeless* induces *dachshund* expression. Eye-antennal, leg and wing imaginal discs were stained for Dac protein expression. (A-C) Wild type. (D-F) UAS-*dac* × *dpp-GAL4*. (G-I) UAS-*ey* × *dpp-GAL4*. *dac* is not expressed at the ventral margin of the antennal disc (arrowhead in A), the dorsal 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* x *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.

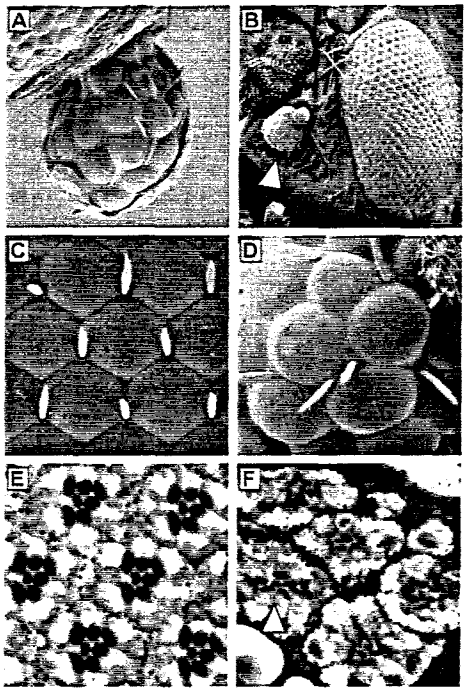

Fig. 4. Targeted *dachshund* expression induces ectopic retinal development. (A,B) Scanning electron microscope images of ectopic eyes (arrowhead in B) induced by *dac* on the adult head. (C) A portion of a wild-type adult eye shows the normal hexagonal array of ommatidia and the regular spacing of interommatidial 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 development as phenotypic males (Koopman et al., 1991). In two other cases, the *LEAFY* (*LFY*) and *APETALA1* (*AP1*) 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

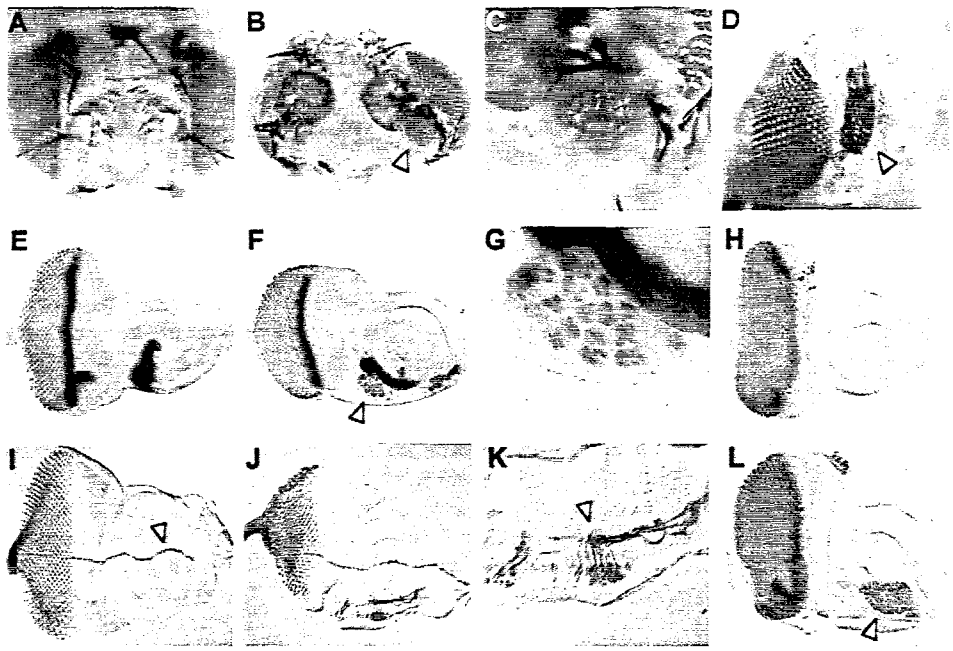

Fig. 5. Photoreceptor development in ectopic eyes. (A) The anterior surface of a wild-type adult head and the antennae. (B-D) *dac*-induced ectopic eyes on the head are located just ventral to the antennae and are normally pigmented (arrowheads). (C) is a higher magnification of the ectopic eye shown 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

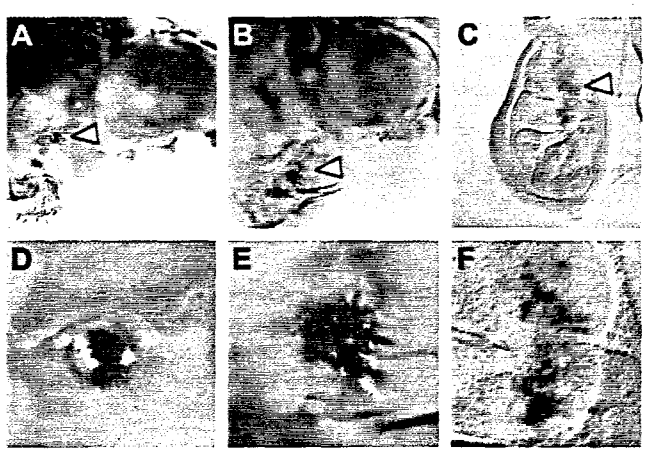

Fig. 6. Ectopic retinal development on the legs and thorax. (A,B) Light microscope images of *dac*-induced ectopic eyes (arrowheads) on the thorax just dorsal to the prothoracic leg. (C) Glass protein is induced by ectopic *dac* expression in leg imaginal discs (arrowhead). *glass* is not expressed in 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.

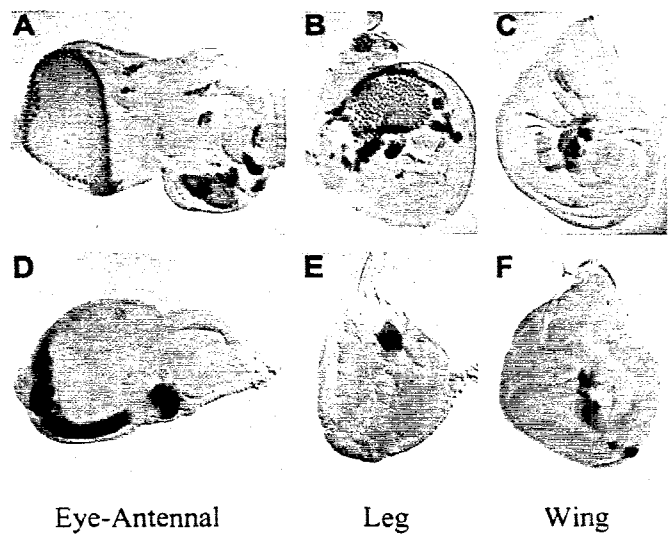

Fig. 7. *dachshund* is required for *eyeless* function. Eye-antennal, leg and wing imaginal discs were stained to reveal neural development in brown and *dpp* expression in blue. (A-C) Discs prepared from UAS-*ey* × *dpp-GAL4* larvae in a wild-type background show ectopic neural development in the antennal (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 downstream 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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It took months of hard work to do this. "It only looked easy after it was finished," says Ketterle. First, he and his colleagues created two condensates by beaming a laser up through the middle of their magnetic trap. The laser light repelled the atoms and split the condensate into two distinct halves. For this test, there was no need to pulse the condensates out of the trap; instead, the group just turned off the trap and let them free fall. As the condensates fell, they expanded into the surrounding vacuum until they overlapped and interfered, demonstrating the atomic version of the bright and dark fringes in an interference pattern.

"The density of the overlapping region is modulated," says Ketterle. "Every 15 microns, we have matter, no matter, matter, no matter. Now, we just shine some light onto the pattern and see this shadow with black-and-white stripes." Says Burnett, "It's not just a little crappy demonstration but a big, juicy interference pattern."

Having proved the condensate is coherent, Ketterle and his colleagues can use the output coupler to extract the condensate in pulses, which makes the setup effectively the first primitive atom laser and raises the question of where they go next. So far, they have been able to get eight pulses out of a condensate before they have to reload, which takes 20 to 30 seconds. One of their first goals is to figure out a way to restock the condensate as they go along to create the atomic version of a continuous wave laser. "Remember, these things are a few weeks old," Ketterle says, "and we need a major improvement in output power, a major reduction in complexity, and also improvement in shaping the pulses."

At that point, any field that relies on beams of atoms might benefit from the brighter and better controlled beams of an atom laser. Atomic clocks, which are based on the vibrations of atoms drifting through a cavity, are one candidate. Another is nanolithography, the technique by which circuit designers lay out minuscule features. It now depends on a mask or stencil to control where atoms or light land on a surface, but an atom laser—which could be focused and directed like a light laser—might provide a way of writing the patterns directly, says University of Texas physicist Dan Heinzen.

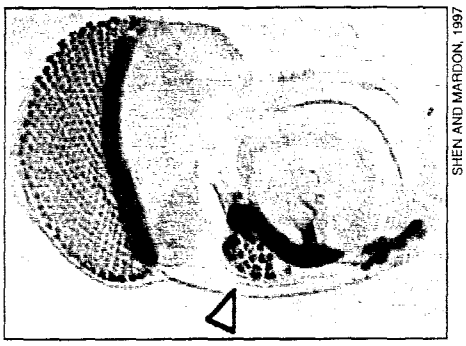
The technology does seem to come with a handicap: Unlike light, an atomic laser beam can't propagate freely through the atmosphere. But Burnett says it's too early to focus on limits. After all, at the birth of the light laser, "people talking about applications really didn't imagine them being in every supermarket check-out counter."

–Gary Taubes

DEVELOPMENTAL BIOLOGY

A "Master Control" Gene for Fly Eyes Shares Its Power

In a startling experiment reported 2 years ago, Swiss biologists caused surplus eyes to sprout on fruit flies' wings, legs, and antennae—all by manipulating a single gene called *eyeless* (*ey*). Grotesque as this spectacle was, researchers hailed it at the time: Besides shedding light on eye development, it also supported the seductive idea of "master control genes" that can single-handedly order up complex organs by turning on other genes. But now it seems that *ey* has a partner—perhaps even two—and the all-powerful master controller may be merely a member of a committee instead.



SHEN AND MARDON, 1997

Eyes up. Expressing the *dac* gene in the wrong place in flies causes eyes to sprout (arrow) where antennae normally grow.

New work reported in the January issue of the journal *Development* shows that a fly gene called *dachshund* (*dac*) can, like *ey*, give rise to ersatz eyes when turned on in out-of-the-way places such as a developing leg or antenna. And the researchers also discovered that *ey* can't build these so-called ectopic eyes in flies missing *dac*—an indication that the two genes normally work together. "It's really an oversimplification to say that any one gene is the master-control gene for eye development," concludes developmental geneticist Graeme Mardon of Baylor College of Medicine in Houston, who authored the study with technician Weiping Shen.

The Baylor team's result "is a very interesting discovery," agrees Nancy Bonini, a *Drosophila* geneticist at the University of Pennsylvania. "If *dac* had been found before *ey*, you might say that *dac* is 'the' master regulatory gene in eye development. So, maybe we should think differently about these terms." But Walter Gehring, the Swiss geneticist who led the original dramatic *ey* study—and whose lab recently discovered yet another eye-forming fly gene, christened *twin-of-eyeless* (*toy*)—says *eyeless* is still the master switch. "I don't think this [label] has to be revised," he says.

The eye-popping powers of *dac* were discovered by accident. The gene got its name several years ago, when Yale University biologist Iain Dawson came across a mutation in the fruit fly *Drosophila melanogaster* that resulted in short, stubby legs—and also affected the arrangement of the 800-some individual eyes (called ommatidia) in each of the flies' compound eyes. Mardon, then a postdoc in the lab of geneticist Gerald Rubin at the University of California, Berkeley, found the gene independently. He went on to clone it and discovered that the protein it encodes resides in the cell nucleus, suggesting that *dac* helps regulate the expression of other genes.

But Mardon couldn't find which genes those might be. Then, in 1995, Gehring and colleagues Georg Halder and Patrick Callaerts at the University of Basel in Switzerland published their study on *ey*. To trick the gene into becoming active where it should be dormant, they used genetically engineered fly larvae that produced a gene-activating protein called GAL4 in many different body parts, such as wings, legs, and antennae. Then, they mated these flies to others in which *ey* was connected to a control switch activated by GAL4. The result was a brood of flies with eyes in unorthodox places (*Science*, 24 March 1995, pp. 1766 and 1788).

Mardon, eager "to see what *dac* might be doing," borrowed the technique, linking not *ey* but *dac* to the GAL4-activated control switch. He and Shen found that 20% of the resulting flies developed clusters of fully formed ommatidia in odd locations. That's a much lower fraction than the Gehring team's 100%—perhaps, Mardon speculates, because making eyes in certain places in the body would require genes that *dac* does not activate, but *ey* does. Intriguingly, the Baylor team also found that ectopic expression of *ey* induces *dac* expression in the same places, and that *dac* can also turn on *ey* in a subset of these cells. And when Gehring's experiment is repeated in flies lacking *dac*, no ectopic eyes form. All this suggests to Mardon that the two genes evolved as partners, reinforcing each other's eye-building signals in a positive feedback loop.

So, which is the true master-control gene for the fly eye? Neither, says Mardon. Both, suggests Ulrike Heberlein, a *Drosophila* geneticist at the University of California, San Francisco. "Maybe we need to talk about a hierarchy of master regulators," she says. But Gehring maintains that between *ey* and *dac*, *ey* is still the

master. In his view, the term means that "if you make a gain-of-function mutation or switch the gene on ectopically, you get a complete wing or leg or eye or body segment," he says. *dac* fits this definition, but he thinks it doesn't quite qualify as a master gene, because *ey* always induces *dac*, but *dac* can't always induce *ey*. To him, this suggests that *ey* is higher in the regulatory hierarchy. Or perhaps, suggests University of Southern California geneticist Kevin Moses, it all boils down to semantics: "Any genetic element that can become critical can be seen as a 'master regulator.' "

The "big game" now, says Gehring, is to map out the eye-development pathway in detail, assigning places to *ey*, *dac*, and a handful of other genes known to be involved—including the recently discovered *toy*, a possible "co-master" regulator that seems to help activate *ey*, as Gehring reported at a conference in Tennessee last June. The gene *toy* is even more closely related than *ey* to *pax-6*, a mammalian gene involved in eye development, and may be the ancestral fly-eye gene, with *ey* an accidental duplicate that later took over most of *toy*'s job, Gehring speculates. Whichever gene comes out on top, there will be plenty of depth left to plumb below.

–Wade Roush

ASTRONOMY

Gas Clouds May Be Relics of Creation

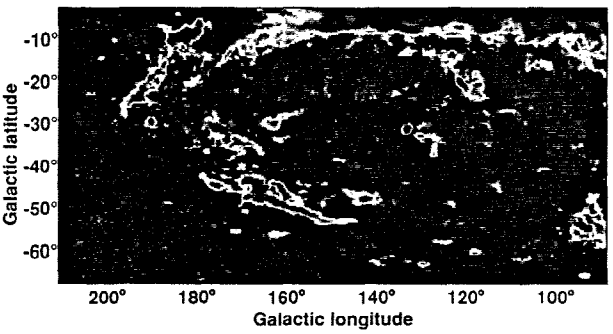
TORONTO—Astronomers who study a mysterious set of gas clouds speeding through the Milky Way can appreciate the plight of the very nearsighted tourist in Africa. A gnat seems to be crawling across her glasses, but when she removes them, the gnat is still there; finally she realizes that a rhino is charging over a ridge. Astronomers haven't had a similar flash of recognition yet. But some have proposed that what they thought were gnats—the spindrift of supernovas exploding in our galaxy—might be something much grander: huge, distant remnants of the galaxy's formation that extend well beyond the Milky Way and could fuel the formation of new stars for billions of years into the future.

The proposal, presented during an American Astronomical Society meeting held here from 12 to 16 January, relies on computer simulations of gas left over from the formation of the first galaxies and clusters of galaxies. The simulations show that these leftovers could survive until the present as clouds of gas roiling in the gravity of our Local Group of galaxies. "We start these [models] at about 1 billion years after the big bang and just let them evolve," says Leo Blitz of the University of California, Berkeley. Fast-forwarding to the present, "we get what we see"—assuming the observed high-velocity clouds are lumbering masses in deep intergalactic space.

Few astronomers believe that the evidence is strong enough yet to prove these cosmic claims. But by pointing to the kinds of observations that could finally pin down the nature of the clouds, says Joel Bregman of the University of Michigan, "this breathes new life into the problem."

The mystery dates back to the 1960s, when observations of radio emissions from hydrogen atoms in interstellar space showed that some of them belonged to clouds stampeding in all directions at hundreds of kilometers per second relative to Earth. The most complete catalog to date, compiled by Bart Wakker of the University of Wisconsin, lists about 550 of these rogue clouds. The biggest obstacle to understanding them is astronomers' ignorance of their distance and thus their actual size. "Distance is the most critical, but the most difficult," says Wakker.

Still, Wakker thinks the clouds are closely associated with our galaxy. One possibility is that they are the handiwork of supernovas. By driving gas out of the plane of the galaxy in "fountains" that would tumble back, supernovas could stir up clumps of interstellar gas. "There are supernovas in the [galactic] plane; they do explode, so where does the gas go?" asks Wakker. "The galactic fountain seems reasonable." Wakker has shown that this picture can account for most of the observations, although he and Bregman concede that it has a hard time explaining the very fastest clouds.



All in tatters. Radio-emitting clouds of hydrogen (red and yellow) could be left over from the galaxy's formation.

DAP HARTMANN/HARVARD-SMITHSONIAN CENTER FOR ASTROPHYSICS

Blitz, along with David Spergel of Princeton University, Dap Hartmann of the Harvard-Smithsonian Center for Astrophysics in Cambridge, Massachusetts, W. Butler Burton of the University of Leiden in the Netherlands, and Peter Teuben of the University of Maryland, College Park, decided to try out a grander picture. They suggest that, rather than lying 10,000 or 15,000 light-years away, the high-velocity clouds are scattered on scales of more than a million light-years, stretching well beyond our galaxy toward its neighbors. And instead of being run-of-the-mill interstellar gas, they are relics of the great filament of primordial gas that coalesced to form the entire Local Group of galaxies. If so, the gravity of the Andromeda galaxy and the Milky Way could be accelerating the clouds to the high velocities that have puzzled observers.

To test this idea, says Blitz, the team used "an extremely simplified model of our local region of the universe." In the model, the newborn Milky Way and Andromeda galaxies first draw apart with the general expansion of the universe, then move closer again because of their mutual attraction. Based on the changing gravitational field created by the two galaxies, the model calculates how nearby gas clouds should move, how much of the gas should get swallowed up by the galaxies, and how much should survive to the present epoch.

Hartmann says the model's predictions of where the clouds should tend to congregate in the sky and how fast they should move just about match his own detailed radio observations. The model predicts, for example, that clouds should be concentrated along a line connecting the Milky Way and Andromeda—the orientation of the original gas filament that formed the Local Group. The clouds do seem to cluster along that line, which impresses David Weinberg, a specialist in cosmic structure formation at Ohio State University. Throughout space, galaxies form patterns "like beads on a string," says Weinberg. "If this is correct, then in addition to seeing the beads, you can still see the string."

These leftovers, if that's what they are, should amount to roughly 100 billion solar masses of material—enough to nourish star formation in the Milky Way for billions of years. That would brighten the galaxy's future, says Blitz, who notes that observers have had a hard time finding enough fresh gas to sustain the present rate of star formation for much longer. Before astronomers draw too many conclusions, though, they want definitive evidence for or against the new cloud theory. So far, one observation has given it a boost by showing that the composition of one high-speed cloud could be extragalactic. But another has raised doubts by showing that a different cloud lies relatively nearby—too close for comfort in the new scenario. The cloud watchers are still waiting for that shock of recognition.

–James Glanz

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Focusing on Eye Evolution

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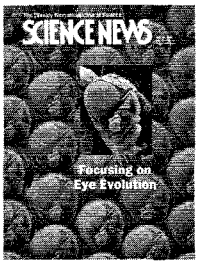
This Week

Research Notes

Articles

Cover: In fruit flies, a vertebrate gene governing eye development can induce extra eyes, such as this small, misshapen eye below the normal one (inset). Such findings challenge the traditional view that the remarkably different eyes of insects and vertebrates evolved independently. (Photo: A. Hefti, University of Basel, Switzerland; background illustration: Mark Gilvey, Design Imaging)



Departments

Letters

A chilly prospect

"New station recommended for the South Pole" (SN: 3/22/97, p. 175) includes a sketch of a proposed science station for the South Pole. The photo of the original shows a building (dome) with a small surface area relative to its interior volume. The proposed building is the very opposite, with a large amount of surface area for the interior volume. There also appear to be windows all around. Given the low temperatures, I would want to spend as little of my budget on heating as possible!

Are you sure that the sketch is not for a tropical office building?

Mike Sitz
Park Rapids, Minn.

Accounting for urban hot spots

"Global Temperatures Spark Hot Debate" (SN: 3/15/97, p. 156) does not take into account the fact that ground-based thermometers are located in cities. Cities are local hot spots that get warmer as their populations increase, a well-known phenomenon.

Harvey Morgan
Deming, N.M.

The records of ground-based thermometers take into account the well-known "heat-island effect" of nearby cities. Moreover, not all stations are located near cities. — R. Monastersky

Defending the defenseless

Aren't most of us descendents of survivors of the 1918 flu pandemic ("A Doughboy's Lungs Yield 1918 Flu Virus," SN: 3/22/97, p. 172)? Doesn't our DNA harbor natural genetic resistance to the 1918 strain?

John P. MacLean
Stafford, Texas

Survivors of the epidemic do harbor antibodies to the 1918 strain. Unfortunately, disease-specific antibodies are not passed from generation to generation, so the rest of us remain essentially defenseless. — S. Sternberg

Essential difference

How apt that Ivars Peterson invoked Paul Erdős' notion of God's book of exquisite mathematical proofs in his article "Computers and Proof" (SN: 3/22/97, p. 176)! While the recent computer proof of the Robbins conjecture may not be in "the book," the real difference between a computer and a mathematician may be that the computer can't decide whether it's in "the book" or not.

Mike Meadows
Nederland, Colo.

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All letters subject to editing.

Eye-Opening Gene

How many times did eyes arise?

By JOHN TRAVIS

Several years ago, Walter J. Gehring of the University of Basel in Switzerland was working on a zoology textbook. When it came time to write a section that dealt with the evolution of eyes, Gehring unhesitatingly recited the traditional view that eyes had evolved independently dozens of times.

For the next edition, he'll pen a different scenario.

The discovery of a gene shared by fruit flies, mice, squid, and humans and the creation of unusual fruit flies that sprout eyelike structures in places such as wings, legs, and antennae have persuaded Gehring that all modern animals with eyes evolved from a common ancestor that possessed a primitive image-forming organ.

In essence, he contends that the eye probably evolved just once in life's evolutionary history—an assertion not everyone is willing to accept.

The eye has always been a thought-provoking organ in discussions of evolution. Creationists have regularly pointed to it as something so complex and specialized that it could not have developed on its own.

Charles Darwin also considered eyes a formidable challenge to his theory of natural selection. "To suppose that the eye, with all its inimitable contrivances for adjusting the focus to different distances, for admitting different amounts of light, and for the correction of spherical and chromatic aberration, could have been formed by natural selection, seems, I freely confess, absurd in the highest possible degree," he wrote in *On the Origin of Species*.

Yet Darwin quickly dismissed this concern, arguing that the complex eyes of modern animals could have evolved slowly from light-sensitive nerve cells and not much else.

In more recent years, evolutionary biologists have been asking a different question: How often can such an organ develop from scratch? While many creatures have an ability to sense light, a survey of the animal world shows that a minority of the major animal groups, or phyla, have true eyes.

"Only 6 out of 30 phyla have complex eyes, which are able to give you images, but because [such eyes] give them so many evolutionary advantages, these phyla dominate," says Stanislav I. Tomarev of the National Eye Institute (NEI) in Bethesda, Md. Researchers estimate that species with complex eyes comprise 95 percent of the animals on the planet, notes Tomarev.

While image-forming eyes are commonplace, no one design for eyes dominates. Scientists have described almost a dozen distinct blueprints, from the alien-seeming compound eyes of insects and many other species to the cameralike single eyes of vertebrates like us.

Gehring, P. Callaerts, and G. Halder

Abnormal activity of the eyeless *gene has generated an eye on the leg of a fly.*

The exotic appearance of the compound insect eye, with its hundreds of miniature eyes called ommatidia, helps explain why scientists have assumed that it evolved independently of the vertebrate eye.

Even superficially similar eyes provide evidence of independent evolution. At first glance, the eyes of cephalopods such as squid and octopuses closely resemble those of vertebrates. A closer examination reveals that the organs emerge from different embryonic tissues and differ considerably in the fine details of their construction. Consequently, the two groups of eyes have been thought a classic example of convergent evolution.

"They appeared independently and somehow evolved to form the very similar structures we observe now," says Tomarev.

Indeed, the majority of scientists studying eye evolution ultimately decided that the wide variety of eyes spread across the animal kingdom is evidence that the organ could not have developed just once. In 1977, L. Von Salvini-Plawen and Ernst Mayr, both of Harvard University, placed this conventional wisdom solidly on the record when they published a landmark paper concluding that eyes had arisen independently at least several dozen times.

That's where the story of eye evolution stood until 1993. That year, Gehring and Rebecca Quiring, also of the University of Basel, were studying fruit flies and looking for transcription factors—proteins that regulate the activity of genes.

Quiring finally identified a protein that binds to DNA, a common feature of transcription factors. Although it wasn't the kind of transcription factor Gehring was interested in, the researchers sent information on the discovery of this protein and its gene to a worldwide computer database to see if any similar genes, or homologues, had already been reported.

The database search highlighted two genes, one from mice that is called *Pax-6* (or *small eye*) and one from people that is called *Aniridia*. Both genes, which are nearly identical to the fruit fly gene, encode proteins crucial to eye development. If mutations exist in both copies of the mouse gene, embryos don't form eyes at all. In people, a mutation in one of *Aniridia*'s two copies usually produces defects in the eyes.

Gehring was surprised that the fly gene was so similar to the two vertebrate genes, but the real astonishment came when he realized that the insect gene also plays a role in eye development.

That finding, reported in the Aug. 5, 1994 SCIENCE, emerged after Gehring and his colleagues had mapped the location of the new gene. They found it at a chromosomal site harboring mutations in flies with developmental eye defects ranging from too-small compound eyes to a complete absence of the organs.

The new gene, named *eyeless*, turned out to be more than just a cog in the

genetic machinery that makes a compound eye. When *eyeless* was turned on in parts of the developing fruit fly where it is normally inactive, it could sometimes initiate the development of additional eyes in odd places.

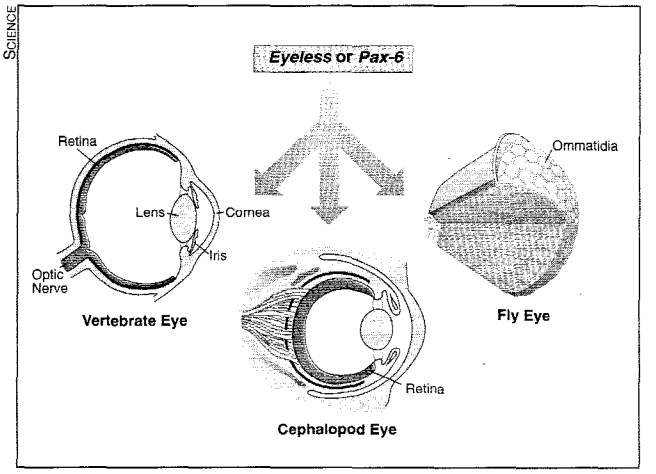
Even more remarkable, *Pax-6* and *Aniridia* did the same. Gehring's group added to fruit flies copies of the vertebrate genes that had been engineered to become active in imaginal disks, embryonic tissues that give rise to adult insect structures like wings and legs.

This unusual experiment, described in the March 24, 1995 SCIENCE, generated flies that had extra eyes growing out of their legs, wings, and other body parts. While the eyes were not wired to the brain, they were light-sensitive and looked superficially much like normal compound insect eyes.

Even before the startling pictures of these mutant insects hit the newspapers, Gehring and other researchers set off to find *eyeless* counterparts in more species, especially ones that might offer further insight into eye evolution.

The group began to collaborate with Tomarev and Joram Piatigorsky, also of NEI, to study the squid *Loligo opalescens*, for example. This animal indeed has its own version of *eyeless*, the researchers reported in the March 18 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES.

Moreover, the squid gene, like the vertebrate genes, initiated formation of extra eyes when activated in developing fruit flies.



The insect gene eyeless *and its counterparts in vertebrates and cephalopods appear to control the formation of distinctly different eyes.*

Although the ability to create eyes where none should exist is an impressive feat, researchers are still struggling to understand what *eyeless* and its noninsect relatives do during development.

Gehring likes to call *eyeless* a "master control gene" for eye development, one that sits at the top of the network of genes, estimated at more than 2,000, used to form eyes. "It's like the main electrical switch in a building. You turn on the main switch and all the lights can go on," explains Gehring.

This interpretation of *eyeless*' role rubs some researchers the wrong way.

"It greatly oversimplifies the way you build organs," argues Graeme Mardon of Baylor College of Medicine in Houston. "The idea that one gene can do the whole job is wrong."

Mardon emphasizes that there are many genes before and after *eyeless* in the genetic hierarchy driving eye development, making it impossible to pick *eyeless* or any other single gene as a master control gene. For example, the fruit fly gene *dachshund*, which he studies, can also trigger the formation of extra eyes, though Mardon acknowledges that it may work by turning on *eyeless*. (In addition to eye defects, flies with mutant *dachshund* genes have short legs.)

Mardon further points out that *eyeless* clearly needs the proper environment to make an eye. "There are many cells in the developing [fruit fly] larva that will not respond to *eyeless*. You turn on *eyeless* and they go 'Ha, we're not making an eye here,'" he says.

Adding to the confusion about *eyeless* are observations indicating that the gene does much more than guide eye development. The fruit fly gene is naturally active in embryonic regions other than those destined to give rise to eyes, as are the gene's counterparts in other animals.

In mice, *Pax-6* plays a role in the formation of the nose. If both copies of *Pax-6* have mutations, a mouse embryo will die because it has no nose and cannot breathe properly. The squid version of *Pax-6* is active in the animal's tentacle development, adding to the idea that the gene plays a role in the formation of the many organs that process sensory information.

Even more controversial than Gehring's calling *eyeless* the master control gene for eye development is his belief that its discovery in several disparate species shatters the dogma that eyes evolved independently on many occasions.

"We now think that this event happened only once," asserts Gehring.

Mayr, however, is emphatic that the new research does not conflict with his proposal of 2 decades ago. He notes that species of worms that do not have eyes also employ *eyeless*-like genes during development.

"What we claimed, and it's as correct as ever, is that the eyes themselves, the photoreceptive organs, developed independently at least 40 times. This is not in the slightest touched by finding that there are genes used in making eyes that existed long before eyes," says Mayr. "You should go to the species that have no eyes but have this gene and find out what it's doing."

Mayr and other researchers suspect that *eyeless* was originally part of a group of genes shaping the developing nervous system. As eyes evolved in various organisms, this genetic cascade was adapted to the specific task of eye development.

"These genes may have been involved in some sort of primitive genetic network that then got co-opted into eye development, either once or multiple times," says Nancy Bonini of the University of Pennsylvania in Philadelphia. "I'm not sure how easy it will be to resolve those two choices."

Even Mardon, who says that he favors Gehring's hypothesis that eyes evolved just once, agrees that the jury is still out. "It's not a foregone conclusion that because *eyeless* and *Aniridia* are both involved in eye development, it must be true that there was a common eye precursor in a species prior to the divergence of insects and vertebrates," he says.

The challenge facing Gehring and other scientists is to identify many more genes involved in the early stages of eye formation, understand how those genes function as a group, and then determine whether all animals share this developmental genetic network.

There has already been significant progress in that respect. Scientists have identified other fruit fly genes—*eyes absent* and *sine oculis*, as well as *dachshund*—that play some role in the early stages of insect eye formation and have vertebrate counterparts.

Ultimately, they hope to chart how these and other genes are linked to *eyeless* and together form the compound insect eye. If large parts of this genetic network are in fact shared by widely divergent animals, then it may become more and more difficult for Mayr and other evolutionary biologists to argue that eyes evolved independently dozens of times.

"When you have a pathway that's conserved, the argument that [eyes] evolved independently is much more tenuous," says Mardon.

As for Gehring, he's already confident enough in his interpretation that eyes probably developed just once that he has begun to plan how he should revise his textbook's section on eye evolution.

"It's nice to disprove your own text, as long as it doesn't happen too many times," he laughs. □