Development 113, 1245-1256 (1991)
Printed in Great Britain © The Company of Biologists Limited 1991

hairy gene function in the *Drosophila* eye: normal expression is dispensable but ectopic expression alters cell fates

NADEAN L. BROWN[1], CAROL A. SATTLER[2], DONALD R. MARKEY[2] and SEAN B. CARROLL[1,*]

[1]*Howard Hughes Medical Institute and Laboratory of Molecular Biology, 1525 Linden Drive, University of Wisconsin, Madison, WI 53706, USA*
[2]*McArdle Laboratory for Cancer Research, 1400 University Avenue, University of Wisconsin, Madison, WI 53706, USA*

*To whom correspondence should be addressed

Summary

The regulatory gene *hairy* is expressed and required during early embryogenesis to control segmentation gene expression properly and during larval and pupal development to control the pattern of certain adult sensory structures. We have found the *hairy* protein to be expressed transiently during two stages of eye imaginal disc development, including all cells immediately anterior to the morphogenetic furrow that traverses the developing eye disc, and again in the presumptive R7 photoreceptor cells of the developing ommatidia. This pattern is conserved in a significantly diverged *Drosophila* species. We show that, surprisingly, ommatidia formed by homozygous *hairy*$^-$ mutant clones are apparently normal, indicating that *hairy* function in the eye is dispensable. However, we do find that ectopic expression of *hairy* causes numerous structural abnormalities and the alteration of cell fates. Thus, proper regulation of *hairy* is still essential for normal eye development. We suggest that the loss of *hairy* function may be compensated by other regulatory proteins, as has been observed previously for several structurally and functionally related genes involved in sensory organ development. The effects of ectopic *hairy* expression may result from interactions with proneural genes involved in the development of the eye and other sensory organs.

Key words: eye development, *hairy*, *Drosophila melanogaster*, ectopic expression.

Introduction

The *Drosophila* compound eye is made up of approximately 800 repeated units known as ommatidia which are arranged in an invariant hexagonal array. Development of the ommatidia in the eye imaginal disc at the larval third instar stage begins within the morphogenetic furrow as it advances across the disc in a posterior-to-anterior direction (Ready *et al.* 1976). Anterior to this furrow are randomly arranged, undifferentiated cells (Tomlinson, 1985), while posterior to it cellular differentiation and the ordered assembly of cell clusters begins (Tomlinson and Ready, 1987*a*). Development proceeds in a continuous temporal sequence as each ommatidial row posterior to the furrow is approximately 2 h more mature than the row directly anterior to it. Cells of individual ommatidia are not related by lineage (Ready *et al.* 1976; Lawrence and Green, 1979); rather, the development of photoreceptor cell clusters is dependent upon local positional cues. Tomlinson and Ready (1987*a*) have proposed that cell fate is determined by inductive signals sent from differentiated photoreceptor cells to neighboring undifferentiated cells. The identification of genes that participate in the transmission or reception of these inductive signals is a major focus of present research (Rubin, 1989).

At least 20 genes have been shown to act in ommatidial assembly and cellular differentiation (reviewed in Tomlinson, 1988; Ready, 1989; and Banerjee and Zipursky, 1990). The phenotypes of mutations in genes such as *sevenless, bride of sevenless, rough* and *seven-up* demonstrate their essential roles in eye development (Harris *et al.* 1976; Reinke and Zipursky, 1988; Saint *et al.* 1988; Tomlinson *et al.* 1988; Mlodzik *et al.* 1990). All of these genes are expressed within and/or posterior to the morphogenetic furrow. Relatively little is known about the events that precede ommatidial assembly, especially the processes involved in the movement of the morphogenetic furrow or in the regulation of cell behaviors in the 'prefurrow' region.

We have previously shown that the regulatory gene *hairy* is expressed in the prefurrow region of the eye disc (Carroll and Whyte, 1989). The nuclear *hairy* protein is transiently expressed as a dorsal-ventral stripe bordering the anterior edge of the morphogenetic furrow. This stripe moves along with the furrow from the posterior to the anterior of the disc, and cells express *hairy* at their highest levels just prior to entering

the furrow and initiating a defined program of cellular differentiation into various ommatidial cell types. *hairy* is the first regulatory protein that has been found to be expressed in the undifferentiated prefurrow region, although other proteins such as *PS1* and *PS3* (α and β integrin subunits), have been reported to be expressed in all cells anterior to the furrow (Brower *et al.* 1985). Baker *et al.* (1990) have also reported the presence of *scabrous* (an extracellular protein) ahead of the morphogenetic furrow.

The *hairy* gene encodes a helix-loop-helix protein (Murre *et al.* 1989; Rushlow *et al.* 1989) and has multiple developmental functions. It is a primary embryonic pair-rule gene (Ingham *et al.* 1985), and it is also required during larval and pupal development to suppress the formation of certain adult sensory structures (Ingham *et al.* 1985; Ish-Horowicz *et al.* 1985). Null mutations of *hairy* cause a lethal embryonic pair-rule phenotype (Nüsslein-Volhard and Wieschaus, 1980; Ingham *et al.* 1985), while recessive adult viable mutations cause the formation of ectopic mechanosensory bristles on the wing and notum (Moscoso del Prado and Garcia-Bellido, 1984*a*, *b*; Ingham *et al.* 1985). Since the known adult viable alleles do not affect the eye, expression of *hairy* protein in the developing eye disc, particularly in the prefurrow pattern, was unexpected. We were interested in further investigating *hairy* function in the developing eye in order to understand better the wide spectrum of pattern-regulating pathways that utilize the *hairy* gene and to determine whether the analysis of *hairy* function might provide insights into early eye development.

Two approaches were utilized to examine the function of *hairy* in the development of the eye. First, eye development was studied in the absence of *hairy* protein. As no adult viable alleles of *hairy* have been discovered that lack expression in the eye, homozygous null $hairy^-$ mitotic clones were examined. Surprisingly, $hairy^-$ ommatidia are normal, suggesting that proper activation and expression of *hairy* are not essential. Conversely, ectopic expression of this protein was found to interfere with normal photoreceptor and accessory cell development, thus, indicating that the proper regulation of *hairy* expression observed at the onset of ommatidial assembly is necessary for normal eye development.

Materials and methods

Fly strains

The following fly stocks were used: h^{1L79K}, h^{7h94}, h^{C1} (three embryonic null alleles of *hairy*); *hsh 21*, *cn* (heat-shock *hairy*); sc^{10-1} (double mutant of *achaete* and *scute*); sev^{d2} (null allele of *sevenless*); w^{1118};$P(w^+)66E$; and ac^3 (hypomorphic allele of *achaete*). These stocks were obtained respectively from: Eric Wieschaus, David Ish-Horowicz, Seth Blair, F. Michael Hoffmann, Todd Laverty of Gerald Rubin's laboratory and the Bowling Green Stock Center.

Immunohistochemistry

Eye–antennal discs were dissected from larvae as part of a larger imaginal disc complex and fixed and stained with the anti-*hairy* antibody as described previously (Carroll and Whyte, 1989) with the following changes: the affinity-purified rabbit antibody was preincubated with 8–24 h embryos to lower nonspecific background staining and the concentration of H_2O_2 added to the diaminobenzidine (DAB) developing solution was lowered ten-fold. Pupal eye–antennal discs were stained with a monoclonal anti-*achaete* antibody using the methods of Carroll and Whyte (1989). Microscopy was carried out in either bright-field or Nomarski (DIC) optics. Immunofluorescence was carried out as described in Skeath and Carroll (1991).

A modified staining protocol allowed us to obtain better imaginal disc morphology and enabled us to detect cell clusters ahead of the morphogenetic furrow. The fixation and basic staining techniques were those of Carroll and Whyte (1989). Buffers used were those of Tomlinson and Ready (1987*a*), except that BSA was substituted for 10% horse serum in the blocking buffer (5 mg ml^{-1}) and in the washing/antibody incubation buffer (1 mg ml^{-1}). The enhanced disc morphology is most likely due to alteration of the basic buffer composition as this level of morphologic resolution has never been observed with the buffers of Carroll and Whyte (1989). Discs were mounted in 0.1 M phosphate buffer (pH 7.2) containing 10% (v/v) glycerol.

Eye clones

Heterozygotes for mosaic analysis using *w* as a marker were generated by crossing w^-; $h^{allele}/TM3\ Sb$ males to w^{1118}; $P[w+]66E$ females. X-irradiation (1200R) of the progeny was carried out between 24 and 36 h of development on a Phillips X-ray machine. Clones were produced at an average frequency of 1 in 75 flies for all three *hairy* alleles and for wild-type controls. 0.5 μm tangential sections were analyzed using phase-contrast microscopy.

Developmental staging

Larval and pupal age was determined using several criteria. For larvae, HSH and wild-type flies were allowed to lay eggs for 2 h. The midpoint of 1 h was taken to be time zero. The entry into each larval instar was observed grossly as egg hatching (for first instar) or as the shedding of stage-specific mouthhooks (second and third instars). Wandering third instar larvae were defined as those larvae crawling on the side of the bottle ($\geq$118 h old). The beginning of pupal development was noted as the appearance of a white pupal case (0 h APF). Later pupal ages were timed from this point. All staging was done at 25°C.

Scanning electron microscopy

Adult fly heads were isolated and immediately fixed in 2% paraformaldehyde (Polysciences), 2.5% glutaraldehyde (Sigma EM grade) in cacodylate wash buffer pH 7.4 (50 ml of 0.2 M sodium cacodylate, 2.7 ml of 0.2 M HCl in 200 ml total) for 4 h at 4°C. Heads were briefly washed and postfixed in cacodylate wash buffer with 2% osmium tetroxide (Sigma) for 2 h at 4°C. Fly heads were washed as before, dehydrated through a graded ethanolic series, critical point dried (Samdri) in CO_2, and platinum-coated. Samples were analyzed on a Hitachi S-900 low voltage scanning electron microscope at 1.5 kV.

Histology

Adult eyes were dissected and fixed immediately after dissection in a 2% glutaraldehyde, 1% osmium tetroxide, 0.1 M cacodylate buffer solution, pH 7.2 for 45 min and postfixed in 2% osmium, 0.1 M cacodylate for 2 h. After a

standard ethanolic dehydration series, eyes were embedded in Eponate resin (Ted Pella, Inc). 0.5 μm tangential sections containing clones were analyzed on a Zeiss Axiophot microscope. Thin sections were cut on a Reichert Ultracut E microtome equipped with a diamond knife. Sections were picked up on Formvar-coated slot grids and stained with uranyl acetate and lead citrate. Micrographs were taken on a Hitachi H-500 transmission electron microscope operated at 75 kV.

Cobalt sulfide staining of cell membranes

Dissected pupal eye discs were stained using the method of Melamed and Trujillo-Cenoz (1975) with several modifications. The ammonium sulfide concentration was lowered from 1.0% to 0.2% (in dH_2O) to slow the reaction and to achieve greater resolution of the stained cell membranes. After staining and destaining in dH_2O, eyes were dissected away from the optic lobes of the brain and the lamina were carefully removed. Discs were then mounted in 50 mM Tris pH 8.8, 10% (v/v) glycerol (BRL Ultra Pure). We noted that the staining faded within 2–4 days.

Results

Biphasic expression of the hairy *protein in developing eye discs*

The eye imaginal disc is a monolayer of epidermal tissue in which all cells extend from the apical to the basal surface except when undergoing cell division. The ordering of cells in this tissue commences early in third instar when a dorsal–ventral morphogenetic furrow begins to traverse the disc from the posterior edge anteriorly (Ready *et al.* 1976). As undifferentiated cells enter the furrow, they are displaced basally. Within the furrow, these cells cluster together to begin forming ommatidia (Tomlinson and Ready, 1987*a*; Cagan and Ready, 1989*a*,*b*). The clustered cells initiate an ordered differentiation pathway to form photoreceptor cells, as reflected by the sequential expression of a neuronal-specific antigen (Tomlinson and Ready, 1987*a*). Based upon these observatons, Tomlinson and Ready (1987*a*) have proposed a model for R cell differentiation in which R8 differentiates first and induces R2 and R5. In subsequent cycles of differentiation, R3 and R4 are induced, followed by R1 and R6 and, lastly R7.

Using antibody staining of third instar eye discs, Carroll and White (1989) have previously demonstrated the presence of *hairy* in the developing eye imaginal disc. The *hairy* protein is spatially expressed in a dorsal-to-ventral stripe located in the undifferentiated prefurrow region of the eye disc adjacent to the morphogenetic furrow (Fig. 1A, D). This stripe of expression traverses the eye disc from posterior to anterior, preceding the morphogenetic furrow. *hairy* protein is found in all cells at least 8–10 cell diameters anterior to the furrow and strongest expression is in those cells that border the edge of the furrow. Protein expression is undetectable in cells within the furrow.

Modified immunohistochemical techniques (see experimental methods) have revealed the presence of spatially ordered cell clusters within the prefurrow stripe of *hairy* expression (Fig. 1B). Cells in physical contact with one another appear to form a rosette-like structure (within one apical to basal focal plane) and have been observed as many as 10–12 cell diameters anterior to the morphogenetic furrow. The cell present within the middle of each rosette is located within a different focal plane and is labeled by the anti-*hairy* antibody as well. In order to verify that the cell clustering events highlighted by the anti-*hairy* antibody are a true morphologic characteristic of the prefurrow region and not an artifact of immunohistochemistry, eye discs were stained with histologic cell membrane stains which also demonstrated the presence of cell clusters (N. Brown, unpublished observations). Similar cell cluster patterns have also been noted in the prefurrow region of eye discs from prefurrow-specific enhancer trap lines stained with anti-*βgal* antibodies (data not shown). These clusters may indicate spatial ordering events prior to R cell differentiation.

Second wave of hairy *expression in the R7 photoreceptor cell*

Further studies with the anti-*hairy* antisera have demonstrated a second wave of expression located posterior to the morphogenetic furrow (Fig. 1D, small arrows and E). This second wave is located in ommatidial rows 8 to 12, counting posteriorly from the morphogenetic furrow in eye discs doubly labeled with anti-*hairy* and the neural-specific monoclonal antibody 22C10 (Zipursky *et al.* 1984; data not shown). Within these four rows, one cell in each ommatidial cluster expresses *hairy* protein (Fig. 1E) but not the 22C10 antigen. We have determined that the *hairy* positive cell is the presumptive R7 photoreceptor cell by its position within the ommatidium and by noting the absence of anti-*hairy* staining within these cells in *sevenless* mutant eye discs (Fig. 1F). Photoreceptor complexes in *sevenless* eyes do not contain photoreceptor cell 7 but instead the cell in R7's position becomes an accessory cone cell (Tomlinson and Ready, 1987*b*). We have also observed the loss of *hairy* expression in the presumptive R7 cell within *boss* mutants (data not shown). The prefurrow expression of *hairy* was normal in both mutant backgrounds as expected. Both *sevenless* and *bride of sevenless* are membrane-associated proteins, which are expressed after the first wave of *hairy* has been turned off at the furrow (Tomlinson *et al.* 1987; Banerjee *et al.* 1987; Hart *et al.* 1990); so it is unlikely that they play any role in the regulation of *hairy* expression in the prefurrow region.

The biphasic expression of hairy *is conserved among* Drosophila *species*

hairy is the first gene to be identified with a biphasic expression pattern in the developing *Drosophila* eye disc. We were interested in determining if both the prefurrow and postfurrow patterns have been conserved during the evolution of *Drosophila*, which would suggest a functional role for the two waves of expression. The divergence between *Drosophila melanogaster* and *Drosophila virilis* is sufficient (60 million years, Beverly and Wilson, 1984) to test this idea.

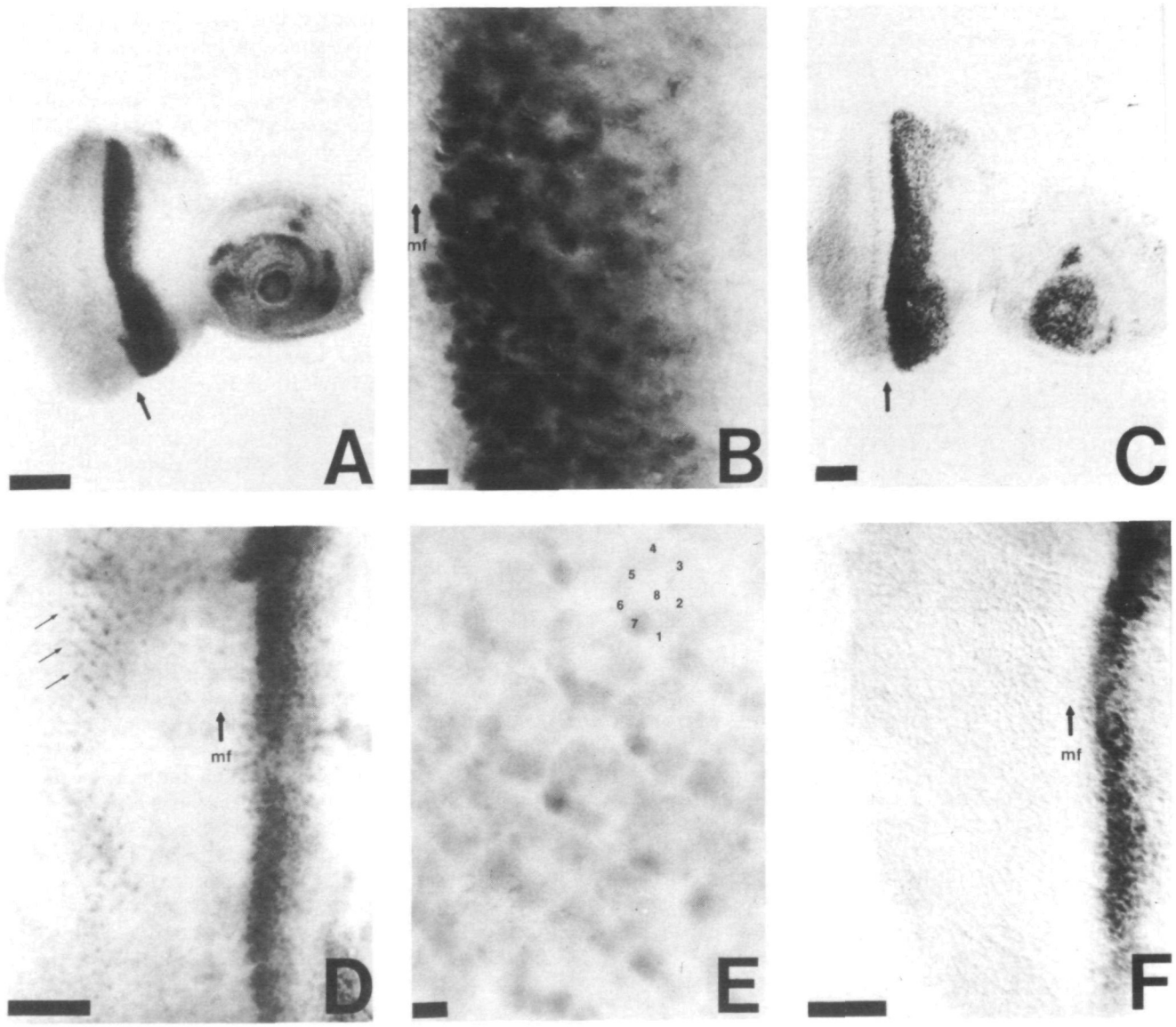

Fig. 1. Biphasic expression of *hairy* protein in the eye and localization of prefurrow cell clusters. Discs in all panels were stained with anti-*hairy* antibody. (A) Whole-mount wild-type eye–antennal disc. The arrow denotes the position of the morphogenetic furrow. (B) High magnification of the prefurrow stripe of *hairy* expression. Note the clustered arrangement of cells within one focal plane in this region. (C) *D. virilis* eye–antennal disc displaying a biphasic *hairy* expression pattern in the eye disc. The arrow marks the position of the morphogenetic furrow. (D) Higher magnification of a wild-type eye disc. The biphasic expression pattern is more prominent at this level. At least four rows of ommatidia can be seen in which the presumptive R7 cell in each ommatidium is positive for *hairy* expression. Small arrows point to several photoreceptor cell complexes whose R7 cell is labeled by the anti-*hairy* antibody. (E) High magnification of photoreceptor cell complexes in which the pre-R7 cell is expressing *hairy* protein. Four rows of ommatidia can be seen in which R7 is stained by the anti-*hairy* antibody. A lower level of background staining in the other R cells is due to the degree of enzymatic development needed to clearly detect the R7 staining. (F) sev^{d2} eye disc. Note the ommatidial expression posterior to the morphogenetic furrow is absent in this mutant disc. The prefurrow expression pattern is unaltered in this mutant background. mf in panels B, D and F denotes the position of the morphogenetic furrow. Posterior is to the left in all panels. Bar, 50 μm (A,C,D,F); 5 μm (B,E).

Drosophila virilis eye discs labeled with the anti-*hairy* antibody demonstrate the presence of the biphasic pattern of *hairy* protein expression (Fig. 1C).

hairy *function is dispensable for eye development*

The biphasic pattern of *hairy* expression in the developing eye imaginal disc and its evolutionary conservation led us to test how this gene is required for normal eye development. As no adult viable alleles of *hairy* have been discovered that lack expression in the eye, it was necessary to generate clones of cells homozygous for embryonic protein null mutations of *hairy*. This was accomplished using somatic recombination techniques (Ready *et al.* 1976; Lawrence and

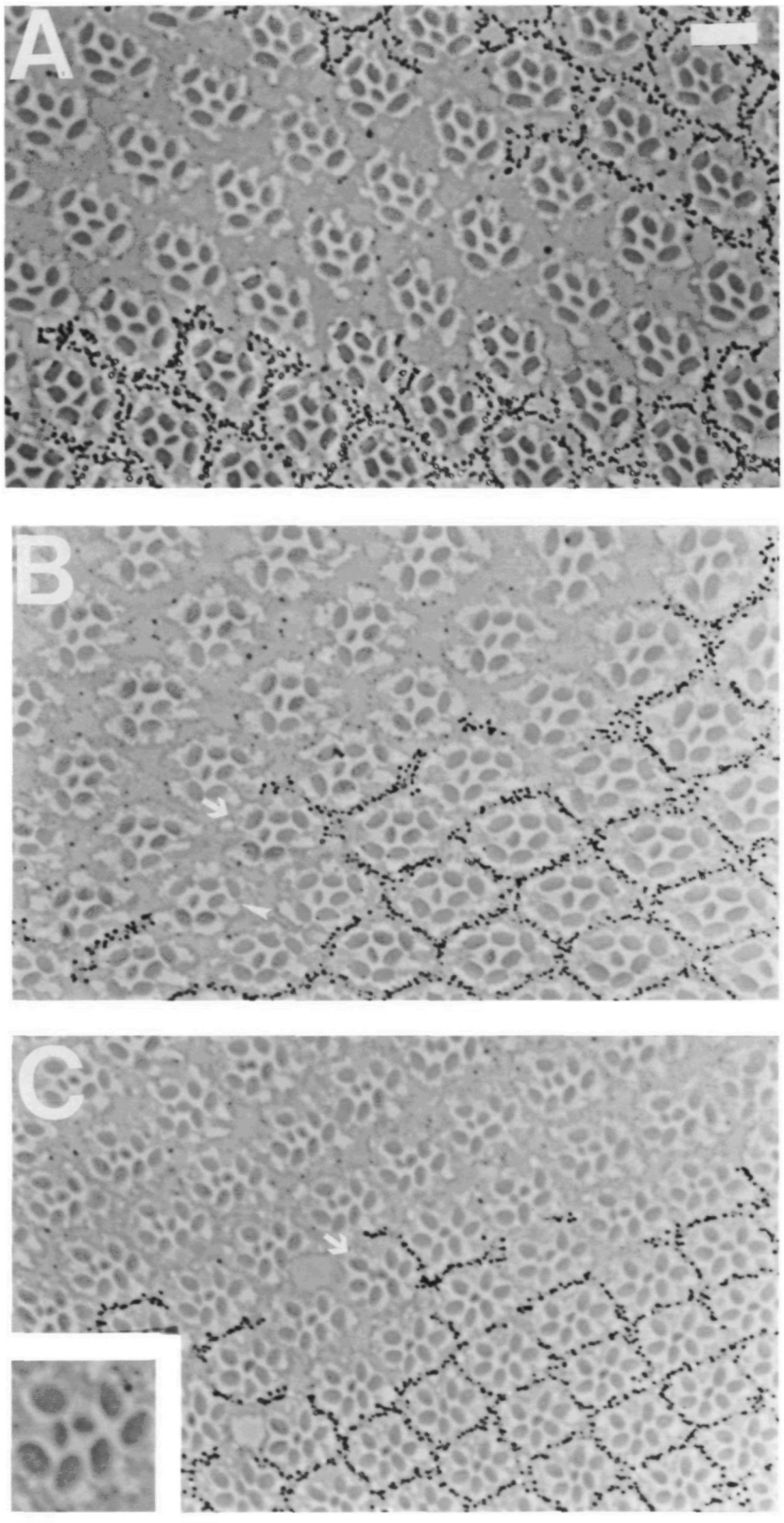

Fig. 2. The ommatidial phenotype of *hairy*$^{-}$ clones. Phase-constrast light micrographs of tangential sections through mutant and mosaic tissue. Wild-type cells are pigmented and homozygous *hairy*$^{-}$ cells are unpigmented. Pigment in photoreceptor cells appears as black granules in the cytoplasm directly adjacent to the base of the rhabdomeres. Pigment in secondary pigment cells can be seen as large yellow granules between ommatidia. (A) Section in the apical retina (at the R7 rhabdomere level) containing both h^{C1} clonal ommatidia and wild-type ommatidia. Note that ommatidia composed partially or entirely of *hairy*$^{-}$ photoreceptor cells have no detectable abnormalities as compared to wild-type ommatidia. (B) Apical retinal section composed of h^{1L79K} clonal and wild-type ommatidia. An extra small rhabdomere was noted in particular sections to be inserted somewhat randomly into the majority of h^{-} ommatidia (arrowhead). This abnormality was never observed in wild-type ommatidia. The extra rhabdomere appears to be distinctly located, as this phenotype is not found in all sections when a particular ommatidium is traced in apical to basal sections. (C) Basal section (at the R8 rhabdomere level) through the identical region as in panel B. The majority of clonal ommatidia contain an extra small (R7 or R8) rhabdomere at this level of the eye. The phenotype of these mutant ommatidia can more easily be seen in the inset. The small arrows in panel B and C denote the same ommatidium shown at two levels within the eye. Anterior is to the left in panel A and up in panels B and C. Bar, 5 μm (A–C); 25 μm (inset in C).

Table 1. *Clonal analysis of* hairy *gene function in the developing eye*

hairy allele	Protein truncation[1]	Genetic background	Number of clones analyzed (produced)	Total number of ommatidia examined (avg clone size)	% Defective ommatidia	Comments
h^{C1}	Within basic region	*Ki p^p Aldox*	8 (25)	632 (91)	0.3 %	2 ommatidia with 6 R cells
Wild-type[2]	N/A	*Ki p^p Aldox/ P(w+)66E*	N/A	417	0.5 %	2 ommatidia with 6 R cells
h^{IL79K}	113aa	*Wild-type*	5 (10)	157 (39)	93 %	143 ommatidia with 8 R cells (basal) 3 ommatidia with 6 R cells

[1] Protein truncation denotes where protein translation is terminated.
[2] Ommatidia analyzed are from the wild-type field surrounding the h^{C1} clones

Green, 1979). *hairy*$^-$ cells were genetically marked using the *white* gene as a cell autonomous marker. We analyzed clones for each of three embryonic null alleles, h^{C1}, h^{7h94}, and h^{IL79K} (Ingham *et al.* 1985; Carroll *et al.* 1988; Hooper *et al.* 1989) each of which are capable of producing only truncated forms of the *hairy* protein (D. Ish-Horowicz, personal communication). The results of our analysis are presented in Table 1 and Fig. 2. Clones made with two of the three alleles, h^{C1} and h^{7h94} had very few defective ommatidia (Fig. 2A and data not shown), while the third allele, h^{IL79K}, produced clones with a high frequency of ommatidial defects displaying a novel phenotype (Figs 2B, C).

We observed a very low, insignificant frequency of defective ommatidia within the homozygous h^{C1} clones (Table 1, Fig. 2A). The frequency and nature of defective ommatidia observed in the wild-type zones adjoining the h^{C1}/h^{C1} ommatidia were the same as those found in h^{C1} clones (Table 1). Similar results were obtained with the h^{7h94} allele, although the % of defective ommatidia due to genetic background effects was higher (data not shown).

Possible antimorphic activity of the hairy^{IL79K} *allele*

Unlike the h^{C1} and h^{7h94} clones, the h^{IL79K} clones exhibited a distinct and reproducible phenotype. Serial examination (from the apical to basal level) of histologic sections made from the clones revealed subtle defects at certain levels within the eye. Within the apical R7 level, abnormalities in h^- mutant ommatidia were observed which appeared to involve the insertion of an extra rhabdomere, often at the R8 position, into otherwise normally arranged ommatidia (Fig. 2B arrowhead). Within the basal R8 level the majority of h^- ommatidia also have an extra small rhabdomere photoreceptor cell, this structure always occupies the position of R7 (Table 1 and Fig. 2C). In the most basal sections, this extra photoreceptor cell is absent. The defects observed within h^{IL79K} clones indicate an abnormal spatial arrangement of R cells, particularly an extended region of overlap between the R7 and R8 cells. How this overlap arises as a consequence of the h^{IL79K} product is not clear, and could involve abnormal patterning events in the prefurrow or later defects in ommatidial assembly.

The differences found between h^{IL79K} clones and clones made with the other two alleles (large numbers of mutant h^- ommatidia and a novel phenotype) may be accounted for by the nature of the protein encoded by the h^{IL79K} allele. This protein is truncated C-terminal to the helix-loop-helix (HLH) domain (D. Ish-Horowicz, personal communication); thus the h^{IL79K} protein may retain its ability to interact functionally with other HLH proteins. This interaction could be antimorphic in nature and could effect a phenotypic change within developing ommatidia. Despite this unexpected phenotype for h^{IL79K} clones, we conclude that complete loss of *hairy* protein has little, if any, effect on the development of the eye.

Ectopic expression of hairy *disrupts ommatidial development*

Although we have shown that the *hairy* protein is not required in the eye for its normal development, it is still a formal possibility that the regulation of *hairy* expression can play a role in eye development. For example, the expression of *hairy* protein is strongest in those prefurrow cells bordering the anterior edge of the furrow and yet no protein can be detected in adjacent cells within the furrow. Possible regulatory mechanisms suggested by this observation are the rapid shutoff of *hairy* transcription as cells enter the furrow and/or the rapid degradation of *hairy* protein. To find out whether negative regulation is important, we examined the effects of ectopic induction of *hairy* protein using a fly strain (HSH) homozygous for a P-element containing *hairy* cDNA under the control of the *hsp-70* promoter (Ish-Horowicz and Pinchin, 1987).

Ectopically induced hairy *protein is rapidly degraded*

HSH and wild-type larvae were heat shocked for thirty minutes and eye discs were dissected at various time points following heat shock, immediately fixed and labeled with anti-*hairy* antibody. Virtually every cell from the induced HSH eye discs expressed high levels of *hairy* protein as detected by the antibody (see

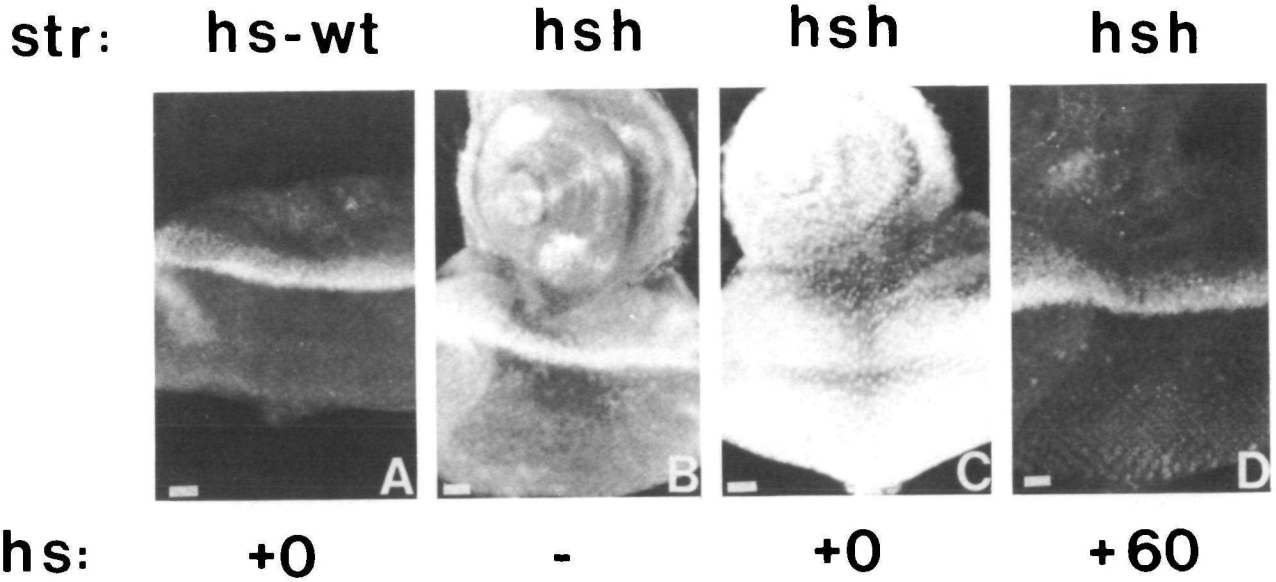

Fig. 3. Rapid turnover of ectopically expressed *hairy* protein. All eye–antennal discs shown (A–D) were taken from wandering third instar larvae and stained with the antibody to the hairy protein. Eye discs in panels A, C and D are from larvae given a 30 min heat shock at 37°C. (A) Wild-type eye disc stained immediately after heat shock. The normal spatial pattern is unaltered although the level of protein expressed is somewhat diminished. (B) HSH eye–antennal disc from unshocked larvae stained to show the normal spatial pattern of *hairy*. The pattern of *hairy* expression in these discs is identical to wild-type. (C) HSH eye–antennal disc stained immediately after heat shock. Every cell in the two disc complex is expressing *hairy* protein. (D) HSH eye–antennal disc heat shocked in tandem with those in panels A and C but allowed to develop for 60 min post shock at 25°C before staining with the anti-*hairy* antibody. Note the return of the wild-type spatial pattern of *hairy* expression with the exception of a few random cells still ectopically expressing *hairy* protein. Anterior is towards the top of all panels. Bar, 25 μm.

Fig. 3C). Identically treated wild-type discs (Fig. 3A) or unshocked HSH discs (Fig. 3B) expressed the normal spatial pattern of *hairy* although the intensity of staining in heat-shocked wild-type discs was somewhat less than unshocked HSH eye discs (see Figs 3A, B). Ectopic expression of *hairy* was sustained for only 60 min (Fig. 3D) and at least half of the induced protein had disappeared by 30–45 min post heat-shock. The rapid turnover of protein in induced eye discs appears to be due to the properties of the *hairy* protein. For example, Kimmel *et al.* (1990) report high levels of ectopic *rough* present in all eye disc cells 2 h post heat-shock and a return to wild-type protein expression was not seen until 6 h post heat-shock. We have also examined the kinetics of ectopic *ftz* expression in the developing eye disc (under identical conditions to ectopic *hairy* induction) and noted no detectable drop in protein intensity until at least 2 h postshock (N. Brown, unpublished observations). The rapid turnover of ectopic *hairy* protein supports our hypothesis that the endogenous *hairy* protein is quickly degraded as cells enter the morphogenetic furrow.

Ectopic expression of hairy *affects normal eye development*

To determine if ectopic *hairy* expression interferes with normal ommatidial formation, adult eyes were examined using scanning electron microscopy (as the defects noted could not be detected grossly). All adult eyes examined from HSH larvae heat shocked for 30 min during wandering third instar development had only 1 or 2 rows of abnormal ommatidia. The relatively brief period of *hairy* overexpression coupled with the formation of one ommatidial row every two hours suggests that ectopic *hairy* expression phenotypically affects only a small zone of developing cells in the eye.

In order to produce a larger phenotypic effect, we attempted to extend the duration of the heat shock given during larval development. Longer heat shocks of two and four hours were lethal in both HSH and wild-type larvae, therefore, a pulsing heat-shock regime was employed (Basler and Hafen, 1989; Bowtell *et al.* 1988). Identically staged (see experimental methods) HSH and wild-type larvae were heat shocked for 30 min followed by a 90 min rest at 25°C. This scheme was repeated sequentially six times so that the larvae were shocked for thirty minutes every two hours for a total of 12 h (during this time, approximately six rows of ommatidia should develop). Larvae and pupae were heat shocked in 12 h age groups encompassing 0–12 h third instar larvae to 40–52 h pupae. Gross examination of 1150 adult eyes from staged HSH larvae and pupae (in which *hairy* was ectopically induced using the pulsing regime) showed varying areas of eye defects. The nature of the phenotype exhibited by these eyes was directly dependent upon the developmental stage at which the heat shock was administered.

Two distinct phenotypes were revealed by examination of eyes by scanning EM. The first is a change in facet shape and size (Fig. 4F); the second is the

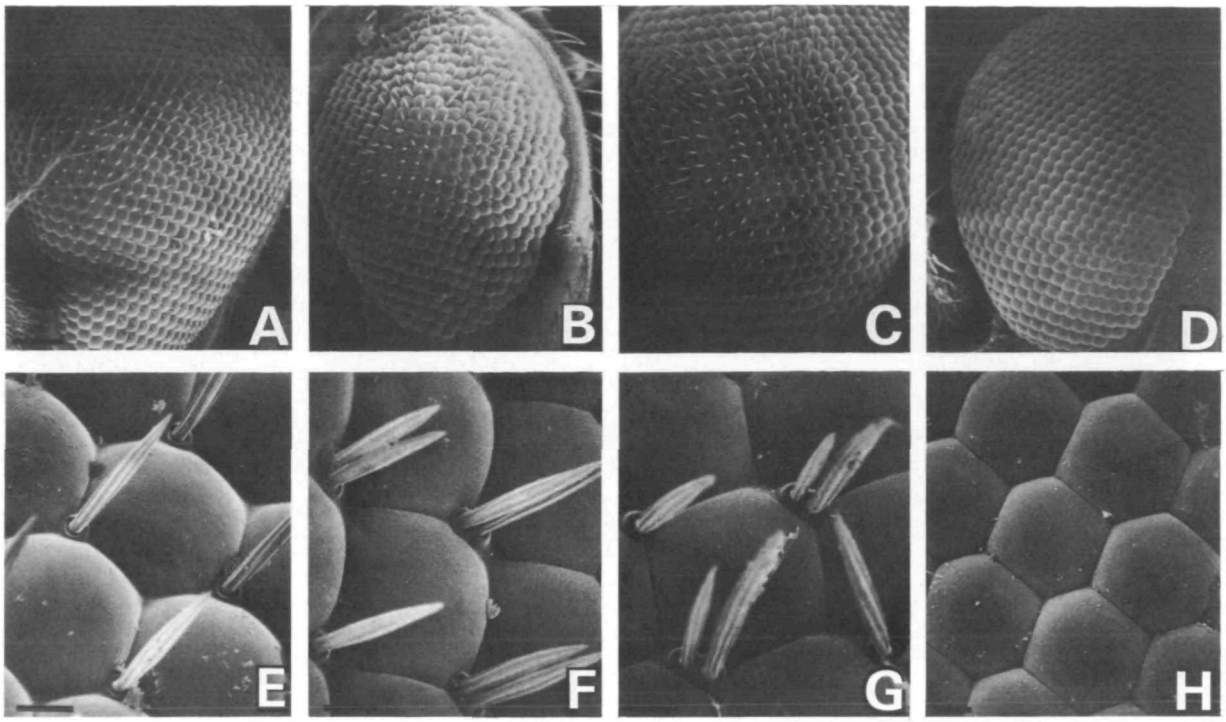

Fig. 4. Ectopic expression of *hairy* phenotypically resembles a strong allele of *achaete* but differs from a double mutant of *achaete–scute*. Scanning electron micrographs of adult eyes from the following genotypes: (A,E) wild-type, (B,F) HSH given 6–30 min heat shocks (separated by 90 min 'rests' at 25°C) during wandering third instar to early pupal development, (C,G) *In(1) ac*^{3}, (D,H) *In (1) sc*$^{10-1}$. Note the strong phenotypic similarity between HSH and *ac*^{3} eyes (F and G). The doubly mutant eye (D,H) is devoid of mechanosensory bristles illustrating the redundant relationship between the *achaete* and *scute* genes. Bar, 50 μm (A–D); 5 μm (E–H).

presence of extra mechanosensory bristles (twins or multiples). Similarly treated wild-type eyes were normal (Fig. 4A, E). Changes in facet size and shape are suggestive of alteration to the normal developmental pathway of accessory pigment cells by ectopically expressed *hairy* protein (Ready *et al.* 1976; see below). This phenotype is often present as a distinct zone (including at least 6 rows of ommatidia). The position of this zone in the eye from posterior to anterior is directly dependent upon the age of the animal at the time of heat shock (N. Brown, unpublished observations). An example of one such eye with a broad area of facet defects located in the middle of the eye is shown in Fig. 4B. Ectopic *hairy* induction administered prior to mid-third instar (94–106 h of age) seemed to have little phenotypic effect. Limitations upon the stringency of staging used prevented us from making a stronger correlation between the time of heat shock and the position of the zone of defects noted.

Ectopic hairy *induction mimics the* achaete *phenotype*

A possible explanation for one of the observed phenotypes in the shocked HSH eyes is that ectopic *hairy* expression is disrupting the function of genes involved in bristle development and facet morphology. One candidate for the former is the *achaete* gene, a known target of *hairy* action in the wing disc (Botas *et al.* 1982; Moscoso del Prado and Garcia-Bellido, 1984*a*,*b*; Skeath and Carroll, 1991). Examination of the eyes from homozygous *In(1) ac*^{3} flies, a strong allele of *achaete* (Figs 4C, G), by scanning electron microscopy reveals a strong resemblance to induced HSH eyes (Figs 4B, F). Both the HSH and *ac* homozygote eyes have ectopic bristles and facet shape changes. Gene dosage and double mutant analyses (Moscoso del Prado and Garcia-Bellido, 1984*a*) have suggested that *hairy* negatively regulates *achaete* in the developing wing disc. The observation here that ectopic *hairy* protein expression in the eye disc produces phenotypic alterations also seen in an *achaete* loss-of-function allele is consistent with this model. Note that bristle pattern is disrupted but not eliminated in these two cases. Clearly, *achaete* is not absolutely required for bristle formation; this is probably due to the redundant function of *achaete* with *scute* during sensory organ formation. *sc*$^{10-1}$, a double mutant for *achaete* and *scute*, lacks all bristles in the eye (Fig. 4D, H).

To explore further the relationship between *hairy* expression and *achaete* regulation, *hairy* was ectopically expressed in HSH 2–4 h pupae and the expression of *achaete* was monitored. The normal pattern of *achaete* expression in an uninduced HSH eye disc is shown in Fig. 5A. Identically stained eye discs from induced (60 min at 37°C) HSH pupae demonstrates that *achaete* protein expression is abolished when *hairy* is inappropriately expressed (Fig. 5B). While this result does not

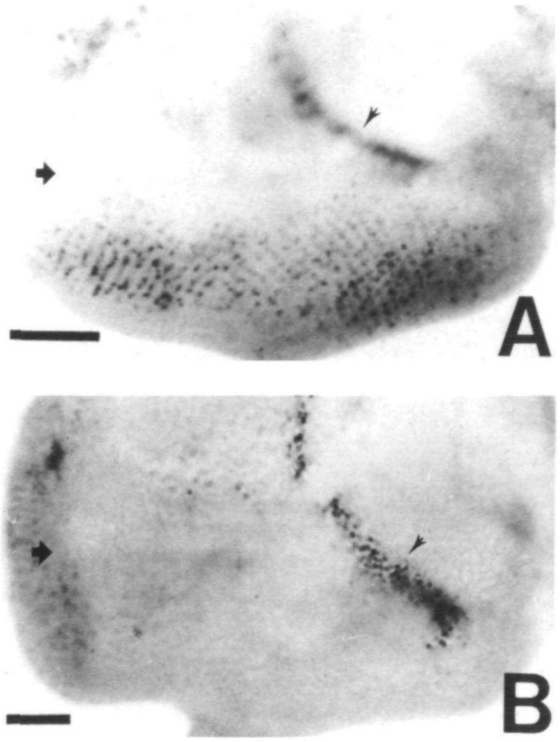

Fig. 5. *achaete* expression is abolished when *hairy* is ectopically expressed. Whole-mount 3–4 h pupal eye discs stained with the anti-*achaete* antibody. The larger arrow in each panel indicates the approximate position of the morphogenetic furrow and the small arrow denotes nonspecfic staining (possibly the peripodial membrane). Because of morphogenetic movements at this stage of development, it is difficult to note the exact position of the furrow. (A) Uninduced HSH pupal eye disc displaying the normal spatial pattern of *achaete* expression. The positively expressing cells are located in a focal plane apical to the photoreceptor complexes and appear to be the mechanosensory bristle precursor sensory mother cells. (B) HSH pupal eye disc induced at 37°C for 60 min and immediately fixed and stained. Note that all *achaete* expression has been abolished from the sensory mother cells but background staining persists (small arrow and low levels throughout disc). Anterior is up in both panels. Bar, 75 μm.

prove that ectopic *hairy* protein expression directly controls *achaete* expression, it does strongly suggest that the similarity of bristle patterns seen in the eye between these two mutants could largely be due to ectopic *hairy* action upon *achaete* expression.

Ectopically expressed hairy *protein affects photoreceptor and accessory cell development*

While examination of induced HSH eyes by scanning EM has demonstrated the presence of several phenotypic defects, it provides no information regarding the proper development of photoreceptor cells when *hairy* is ectopically expressed. The detailed nature of the ommatidial defects induced by shocking the HSH larvae and pupae was further examined by analyzing histologic sections of adult eyes; 17 heat-shocked HSH eyes and 9 heat-shocked wild-type eyes were examined. Sections from induced HSH eyes contained 24% mutant ommatidia (Figs 6B, C) compared with sections from heat-shocked wild-type eyes (Fig. 6A) which had only 0.6% abnormal ommatidia. Although the HSH mutant ommatidia appear to be located in patches within the sections examined, the size of these patches was not restricted to 6 rows of ommatidia. A range of phenotypes was noted for the abnormal HSH ommatidia including: too few photoreceptor cells (Fig. 6B, arrow), too many photoreceptor cells (Fig. 6C, arrow), fused rhabdomeres (Fig. 6C, open arrow), and aberrantly structured ommatidia which could contain either photoreceptor cells with altered cell fates or reduced numbers of R cells resulting in abnormal cluster morphology (Fig. 6B, open arrow).

One aspect of the abnormal ommatidial morphology noted in the scanning electron micrographs of induced HSH eyes is the shape change from hexagonal to squared shape (Figs 4B, F). This defect cannot be fully attributed to either abnormal photoreceptor cell or ectopic bristle development but may in fact reflect abnormal accessory cone or pigment cell development. During late third instar, ommatidial complexes begin to develop accessory cells which are assembled in a fixed pattern. Four cone cells, which during late pupal stage will secrete the corneal lens, differentiate and begin to close over the photoreceptor cells. During early pupal development, primary, secondary and tertiary pigment cells as well as mechanosensory bristles develop and arrange to form a hexagonal array around the cone and photoreceptor cells (Waddington, 1962; Cagan and Ready, 1989*b*). As secondary and tertiary pigment cells form during pupal development, they change the facet shape from a square to a hexagon (Ready *et al.* 1976).

To examine the development of accessory cone and pigment cells in induced HSH eyes, 16 HSH and 16 wild-type pupal eye discs (44 h after puparium formation) were stained with cobalt sulfide to highlight the cell membranes of these cells (Melamed and Trujillo-Cenoz, 1975). The pattern of accessory cells in a normal wild-type eye disc is shown in Fig. 7A. The pupal eyes in Figs 7B–D were heat shocked for 12 h from 24 to 36 h of pupal development (APF). The accessory cell pattern of heat-shocked wild-type pupae (Fig. 7B) is identical to the unshocked pattern (Fig. 7A). The arrangement of accessory cells in heat-shocked HSH eye discs is shown in Figs 7C, D. Note the abnormal number and arrangement of cone cells and the irregular array of the facets. The HSH eyes, although age matched with the heat-shocked wild-type pupae, either appear to be retarded in secondary and tertiary cell development or are totally lacking these pigment cells. Analysis of later stage pupal discs (60 h APF) demonstrated that these pigment cells do develop but appear to lag behind those of heat-shocked wild-type pigment cells and frequently are incorrect in number or position (data not shown). The high incidence of extra bristles noted on the scanning electron micrographs can also be seen in these discs. Thus, ectopic *hairy* expression can

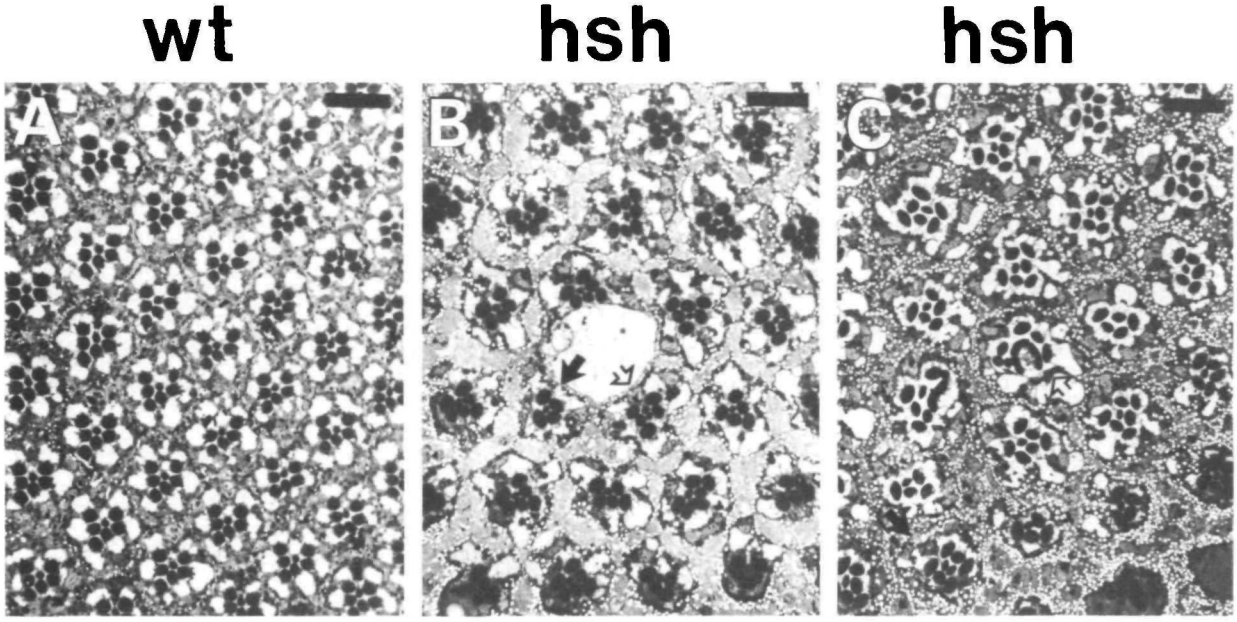

Fig. 6. Ectopic *hairy* expression causes ommatidial defects in photoreceptor cell number and fate. Tangential EM sections from wild-type (A) and HSH (B,C) adult eyes given a 12 h cycled heat shock during wandering third instar/early pupal development. Note the large number of photoreceptor cell defects in panels B and C including missing R cells (arrow in B), extra R cells (arrow in C), and defective rhabdomeres (open arrow in C). Additionally, ommatidia in which incorrect clustering has occurred (open arrow in B) due to either missing R cells or alterations in cell fate were observed. Vacuous spaces, as in panel B, were noted in a number of heat shocked HSH eye sections. Anterior is to the left in panel A. Orientation could not be determined unambiguously in panels B and C. Bar, 10 μm.

interfere with proper photoreceptor cell and all accessory cell (cone, bristle and pigment) fates.

Discussion

We have examined the function of the *hairy* gene in developing *Drosophila* eye discs. Analysis of two of three different embryonic functional null alleles (h^{C1} and h^{7h94}) in *hairy*$^-$ eye clones has revealed no significant alterations in photoreceptor cell development in those ommatidia lacking *hairy* gene product. The low frequency of defects observed in these clones can be attributed entirely to the genotype of the chromosomes that carry these *hairy* alleles. Because *hairy* protein is localized to the nucleus (Carroll *et al.* 1988), it is unlikely that *hairy* from nearby wild-type ommatidia could rescue the clonal ommatidia, further strengthening our finding that loss of *hairy* in the developing ommatidia has little phenotypic effect. This is consistent with previous data showing *hairy* to be cell autonomous in the developing wing blade (Ingham *et al.* 1985). Alternatively, a downstream extracellular protein could rescue the ommatidia in the h^- clone thus masking our ability to detect defects due to loss of *hairy* alone. We conclude from these experiments in either case that the loss of functional *hairy* protein alone in clonal ommatidia is not sufficient to alter normal eye development detectably.

A third allele, h^{IL79K}, produces clones with a high frequency of mutant ommatidia with disrupted R cell arrangement. Because the h^{IL79K} protein retains its helix-loop-helix domain, it can potentially interact with other HLH proteins in an antimorphic manner to manifest the ommatidial defects observed. Careful analysis of these mutant ommatidia suggests to us that the nature of the defects seen are most likely due to alterations in R cell positioning and not the presence of extra photoreceptor cells. The h^{IL79K} protein could potentially interfere with correct cell positioning either within the prefurrow or at the time when presumptive R7 is positioning itself. It is interesting to note that the potentially antimorphic activity observed here for the h^{IL79K} protein in the eye has not been observed in the embryo (Ingham *et al.* 1985). Perhaps the potential interacting protein(s) for such an activity is (are) not expressed during embryogenesis.

A redundant role for hairy?

We propose that the function of the *hairy* protein is redundant during eye development. The loss of one or more gene products in addition to *hairy* may be required to produce a significant phenotypic change in the developing eye disc and to uncover any potential role of this gene in eye development. There are two main arguments supporting a redundant role for *hairy* in eye development. First, the biphasic expression of *hairy* protein in the developing third instar and pupal eye discs has been conserved over a reasonable period of *Drosophila* evolution, since *Drosophila virilis* also exhibits the biphasic *hairy* pattern (Fig. 1C). This finding would argue in favor of the conservation of a function for the *hairy* protein in eye development.

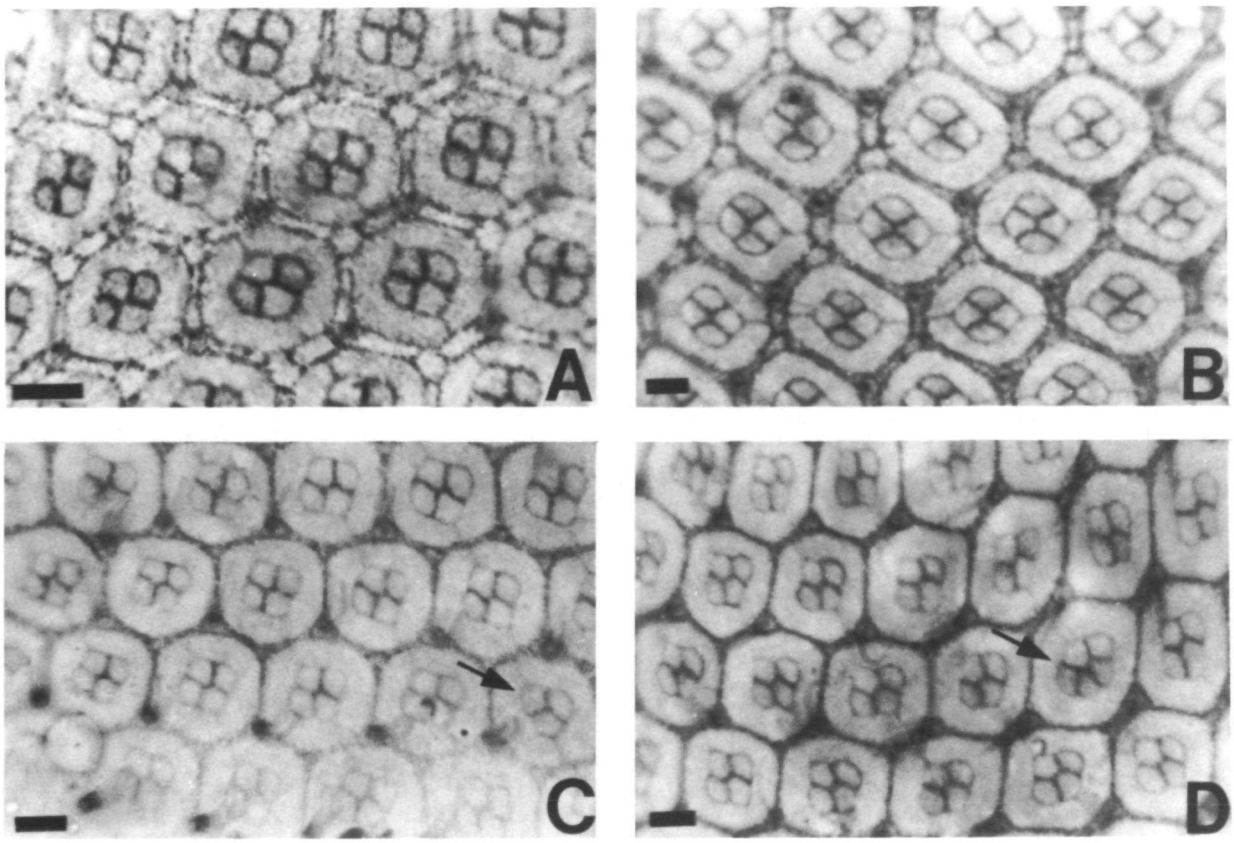

Fig. 7. Ectopic *hairy* expression alters accessory cell fate. Cobalt sulfate staining of wild-type (A,B) and HSH (C,D) pupal eye discs. Discs in panels B, C, D were taken from 44 h pupae heat shocked at 24 h APF 6 times for 30 min at 37°C (with 90 min rests at 25°C inbetween each shock). Heat-shocked wild-type (B) ommatidial accessory cell arrangement is identical to wildtype (A). Arrows indicate abnormal cone cell arrangements in the HSH pupal eyes (C,D). Anterior is to the upper right in panels A, C, D and up in panel B. Bar, 10 μm.

Secondly, there is a precedent for other HLH domain proteins to act redundantly in development, including *achaete* and *scute* in certain aspects of central and peripheral nervous system development (Dambly-Chaudiere *et al.* 1987). Perhaps another HLH protein can substitute for *hairy* function in eye development. A new member of the *Drosophila* helix-loop-helix protein family, the *deadpan* gene product, has been identified and shown to possess strong sequence similarity to *hairy* (including a proline residue disrupting the basic region of the protein; E. Bier, personal communication). Although anti-*deadpan* labeling of third instar eye discs demonstrates that *deadpan* expression does not coincide with *hairy*, perhaps these two genes have overlapping redundant functions in tissues where they are co-expressed (H. Vaessin and Y.H. Jan, personal communication).

Why is hairy *tightly regulated?*

Our studies of ectopic induction of *hairy* expression during eye development indicate that, while proper activation of *hairy* is not required, proper shutoff of this gene is essential to normal eye development. Improper expression of *hairy* alters both photoreceptor cell and accessory cell fates and arrangement. Interestingly, ectopic *hairy* expression induces a phenotype, as shown by scanning EM, resembling mutants for ac^3, a strong allele of the *achaete* gene (and another HLH protein), which could be due to a direct effect of *hairy* upon *achaete* expression. This interaction is consistent with previous genetic experiments that demonstrated a negative interaction between *hairy* and *achaete* in the developing wing disc (Moscoso del Prado and Garcia-Bellido, 1984*a*,*b*). Results presented here are consistent with this model although the nature of the interaction (protein–DNA or protein–protein) is still unknown. Not all of the cell fate changes noted in HSH eye discs are necessarily caused by the misregulation of *achaete* by *hairy*. As *achaete* expression in the eye disc cannot be detected until 2 h after puparium formation (Fig. 5A and J. Skeath, personal communication), it is not likely that ectopic expression of *hairy* mediates its effect on photoreceptor cell development *via achaete*. The abnormally structured ommatidia in heat-shocked HSH eyes have phenotypes similar to those of various eye mutations that affect proper photoreceptor cell differentiation and development. As the staining of heat-shocked HSH eye discs with 22C10 at various times post-heat shock has not revealed to us any obvious R cell defects, it is difficult to determine the exact nature and cause of the photoreceptor cell abnormalities

observed within the sections of adult HSH eyes (N. Brown, unpublished observations).

Cell clustering in the prefurrow and other prefurrow genes

The observation that 'undifferentiated' cells within the prefurrow (and especially, within the *hairy* expressing region) contact each other in a patterned manner (Fig. 1B) leads us to hypothesize that cellular and genetic regulatory events may occur earlier in the development of the eye disc than has been previously appreciated. *hairy* is the first regulatory gene shown to be expressed anterior to the morphogenetic furrow (as well as in a biphasic manner). We expect that other genes are exclusively expressed within this region of the eye disc. It is conceivable that gene activity within the prefurrow may regulate morphogenetic events such as the formation and movement of the morphogenetic furrow or cellular inductive events which may occur prior to those involving photoreceptor cell R8 within the furrow. Additional gene activity may be needed to carry out cell movements or to participate in cell–cell interactions that may occur when the prefurrow cells cluster together. In order to identify some of these gene products, we are attempting to characterize prefurrow specific enhancer trap lines as well as to investigate more fully the nature of the cell patterning process observed within the prefurrow of the *Drosophila* eye imaginal disc.

We are grateful to David Ish-Horowicz for providing us with unpublished sequence data for the three *hairy* alleles used in the eye clone analysis, his helpful advice and fly stocks. We would like to thank Ethen Bier, Harrold Vaessin, and Yuh Nan Jan for sharing information with us prior to publication. We are also indebted to Nick Baker for thoughtful discussions regarding the analysis of the clonal data and to Don Ready for initially helping us to identify the *hairy* positive photoreceptor cell as R7. Our appreciation is extended to Mark Tengowski at the UW-Madison Integrated Microscope Resource (NIH Biomedical Research Technical Resource RR570) for his help with scanning electron microscopy, and to Peisu Zhang for her work on the electron micrographs. A special thanks goes to Jim Skeath for his help in developing dissection and antibody staining techniques as well as for providing the *achaete* antibody. We acknowledge technical assistance from Jill Whyte and Kathy Vorwerk. We thank Teresa Orenic, Allen Laughon, and F. Michael Hoffman for critical reading of the manuscript; Leanne Olds for her help with the figures; and Jamie Wilson for help with the manuscript. This work was supported by an NIH predoctoral traineeship (HDO7118) to N.L.B., a NSF Presidential Young Investigators Award, the Shaw Scholar's Program of the Milwaukee Foundation, and the Howard Hughes Medical Institute. C.A.S. and D.R.M. are supported by a National Cancer Institute grant (CA07175).

References

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

Banerjee, U., Renfranz, P. J., Hinton, D. R., Rabin, D. A. and Benzer, S. (1987). The *sevenless*$^{+}$ protein is expressed apically in cell membranes of the developing *Drosophila* retina; it is not restricted to cell R7. *Cell* **51**, 151–158.

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

Basler, K. and Hafen, E. (1989). Ubiquitous expression of *sevenless*: position-dependent specification of cell fate. *Science* **243**, 931–934.

Beverly, S. M. and Wilson, A. C. (1984). Molecular evolution in *Drosophila* and higher *Diptera* II. A time scale for fly evolution. *J. molec. Evol.* **21**, 1–13.

Botas, J., Moscoso del Prado, J. and Garcia-Bellido, A. (1982). Gene-dose titration analysis in the search of trans-regulating genes in *Drosophila*. *EMBO J.* **1**, 307–310.

Bowtell, D. D. L., Simon, M. A. and Rubin, G. M. (1988). Nucleotide sequence and structure of the *sevenless* gene of *Drosophila melanogaster*. *Genes Dev.* **2**, 620–634.

Brower, D. L., Piovant, M. and Reger, L. A. (1985). Developmental analysis of *Drosophila* position-specific antigens. *Devl Biol.* **108**, 120–130.

Cagan, R. L. and Ready, D. F. (1989*a*). *Notch* is required for successive cell divisions in the developing *Drosophila* retina. *Genes Dev.* **3**, 1099–1112.

Cagan, R. L. and Ready, D. F. (1989*b*). The emergence of order in the *Drosophila* pupal retina. *Devl Biol.* **136**, 346–362.

Carroll, S. B., Laughon, A. and Thalley, B. S. (1988). Expression, function, and regulation of the *hairy* segmentation protein in the *Drosophila* embryo. *Genes Dev.* **2**, 883–890.

Carroll, S. B. and Whyte, J. S. (1989). The role of the *hairy* gene during *Drosophila* morphogenesis: stripes in imaginal discs. *Genes Dev.* **3**, 905–916.

Dambly-Chaudiere, C. and Ghysen, A. (1987). Independent subpatterns of sense organs regulate independent genes of the *achaete-scute* complex in *Drosophila* larvae. *Genes Dev.* **1**, 297–306.

Harris, W. A., Stark, W. S. and Walker, J. A. (1976). Genetic dissection of the photoreceptor system in the compound eye of *Drosophila melanogaster*. *J. Physiol.* **256**, 415–439.

Hart, A. C., Kramer, H., Van Vector, D. L., Jr, Paidhungat, M. and Zipursky, S. L. (1990). Induction of cell fate in the *Drosophila* retina: the *bride of sevenless* protein is predicted to contain a large extracellular domain and seven transmembrane segments. *Genes Dev.* **4**, 1835–1847.

Hooper, K. L., Parkhurst, S. M. and Ish-Horowicz, D. (1989). Spatial control of *hairy* protein expression during embryogenesis. *Development* **107**, 489–504.

Ingham, P. W., Pinchin, S. M., Howard, K. R. and Ish-Horowicz, D. (1985). Genetic analysis of the *hairy* gene in *Drosophila*. *Genetics* **111**, 463–486.

Ish-Horowicz, D., Howard, K. R., Pinchin, S. M. and Ingham, P. W. (1985). Molecular and genetic analysis of the *hairy* locus in *Drosophila*. *Cold Spring Harbor Symp. quant. Biol.* **50**, 135–144.

Ish-Horowicz, D. and Pinchin, S. M. (1987). Pattern abnormalities induced by ectopic expression of the *Drosophila* gene *hairy* are associated with repression of *ftz* transcription. *Cell* **51**, 405–415.

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

Lawrence, P. A. and Green, S. M. (1979). Cell lineage in the developing retina of *Drosophila*. *Devl Biol.* **71**, 142–152.

Melamed, J. and Trujillo-Cenoz, O. (1975). The fine structure of the eye imaginal disks in muscoid flies. *J. Ultrastruct. Res.* **51**, 79–93.

Mlodzik, M., Hiromi, Y., Weber, U., Goodman, C. S. and Rubin, G. M. (1990). The *Drosophila seven-up* gene, a member of the steroid receptor gene superfamily, controls photoreceptor cell fates. *Cell* **60**, 211–224.

Moscoso del Prado, J. and Garcia-Bellido, A. (1984*a*). Genetic regulation of the *achaete-scute* complex of *Drosophila melanogaster*. *Wilhelm Roux's Arch. devl. Biol.* **193**, 242–245.

Moscoso del Prado, J. and Garcia-Bellido, A. (1984*b*). Cell interactions in the generation of chaete pattern in *Drosophila*. *Wilhelm Roux's Arch. devl. Biol.* **193**, 246–251.

Murre, C., McCaw, P. S. and Baltimore, D. (1989). A new DNA binding and dimerization motif in immunoglobulin enhancer binding, *daughterless*, *MyoD*, and *myc* proteins. *Cell* **56**, 777–783.

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

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

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

Reinke, R. and Zipursky, S. L. (1988). Cell–cell interaction in the *Drosophila* retina: the *bride of sevenless* gene is required in photoreceptor cell R8 for R7 cell development. *Cell* **55**, 321–330.

Rubin, G. M. (1989). Development of the *Drosophila* retina: inductive events studied at single cell resolution. *Cell* **57**, 519–520.

Rushlow, C. A., Hogan, A., Pinchin, S. M., Howe, K. M., Lardelli, M. and Ish-Horowicz, D. (1989). The *Drosophila hairy* protein acts in both segmentation and bristle patterning and shows homology to N-*myc*. *EMBO J.* **8**, 3095–3103.

Saint, R., Kalonis, B., Lockett, T. J. and Eliziur, A. (1988). Pattern formation in the developing eye of *Drosophila melanogaster* is regulated by the homeobox gene *rough*. *Nature* **334**, 151–154.

Skeath, J. B. and Carroll, S. B. (1991). Regulation of *achaete-scute* gene expression and sensory organ pattern formation in the *Drosophila* wing. *Genes Dev.* **5**, 984–995.

Tomlinson, A. (1985). The cellular dynamics of pattern formation in the eye of *Drosophila*. *J. Embyol. exp. Morph.* **89**, 313–331.

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

Tomlinson, A., Bowtell, D. D. L., Hafen, E. and Rubin, G. M. (1987). Localization of the *sevenless* protein, a putative receptor for positional information in the eye imaginal disc of *Drosophila*. *Cell* **51**, 143–150.

Tomlinson, A., Kimmel, B. E. and Rubin, G. M. (1988). *rough*, a *Drosophila* homeobox gene required in photoreceptors R2 and R5 for inductive interactions in the developing eye. *Cell* **55**, 771–784.

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

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

Waddington, C. H. (1962). New Patterns in Genetics and Development. New York: Columbia Univ. Press.

Zipursky, S. L., Venkatesh, T. R., Teplow, D. B. and Benzer, S. (1984). Neuronal development in the *Drosophila* retina: Monoclonal antibodies as molecular probes. *Cell* **36**, 15–26.

(*Accepted 3 September 1991*)