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Dynamics of *Drosophila* eye development and temporal requirements of *sevenless* expression

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Summary

The development of the compound eye of *Drosophila* consists of a linear, stereotyped program starting at the posterior end of the eye imaginal disc and progressing towards the anterior border. The determination of the R7 photoreceptor cells is part of this process and is dependent on the *sevenless* gene. In this study, we used a heat-shock-inducible *sevenless* gene as a conditional allele to determine the exact temporal requirements of *sevenless* gene expression and to reveal the stages of ommatidial development during which the presumptive R7 cell can respond to the presence of *sevenless* protein. Our results indicate that *sevenless* gene function is only required during a brief, defined period for the initiation of R7 development; subsequently *sevenless* is dispensable for both differentiation and function of the R7 photoreceptors. Furthermore, using rescue of R7 cells as an internal marker to monitor the progression of eye development we could examine when and at what rate ommatidial columns form.

Key words: *sevenless*, eye development, cell fate determination, conditional allele, phototaxis.

Introduction

Generation of cell type diversity during the development of multicellular organisms involves the successive restriction in the developmental potential of initially totipotent precursor cells. Genetic analysis in *Drosophila* and other organisms have indicated that the individual steps in this process may be controlled by single selector genes. It is of interest to know whether these genes control pathway choices only at a single level in the developmental hierarchy or whether they are used at multiple levels during the development of a specific cell type. To address this question we have studied the temporal and functional requirements for *sevenless*, a gene controlling the development of a particular cell type in the eye of *Drosophila*.

The compound eye of *Drosophila* is an ideal system in which to study the hierarchy of developmental events, since their temporal order is spatially displayed during the differentiation of the imaginal disc that gives rise to the eye. Furthermore, the repetitive, highly ordered array of the ommatidial clusters permits the examination of developmental decisions at the single cell level (for recent review see Ready, 1989). The eye develops from the eye imaginal disc during the third larval instar and pupation. Before this period, the eye disc consists of a single layer epithelium of dividing unpatterned cells. Ommatidial assembly does not occur synchronously throughout the disc, but starts at the posterior margin and progresses anteriorly (Ready *et al.* 1976). Closely associated with the anterior boundary of ommatidial assembly is a morphological depression, called the morphogenetic furrow. Cells anterior to the furrow are unpatterned and randomly organized whereas cells posterior to the furrow become incorporated into the ommatidial clusters in a fixed sequence (Tomlinson and Ready, 1987). This process can be visualized with antibodies against the *sevenless* protein which stain a subpopulation of ommatidial precursor cells in the developing ommatidia (Fig. 1).

Flies homozygous or hemizygous for the *sevenless* mutation lack all R7 photoreceptor cells (Harris *et al.* 1976). The mutant R7 precursor is unable to respond to the inductive signal and develops into a non-neuronal cone cell (Harris *et al.* 1976; Tomlinson and Ready, 1986). The *sevenless* gene has been cloned (Hafen *et al.* 1987; Banerjee *et al.* 1987). It encodes a transmembrane protein with a large extracellular domain and two cytoplasmic domains (Basler and Hafen, 1988; Bowtell *et al.* 1988), one of which is a tyrosine kinase domain. The *sevenless* protein most likely acts on the surface of ommatidial precursors as a receptor for an R7-inducing signal.

The mutant alleles of *sevenless* that have been identified so far are all loss-of-function mutations. The lack of temperature-sensitive alleles for *sevenless* prevented the analysis of the temporal requirements for *sevenless* function. Transient expression of *sevenless* under the control of a heat-shock promoter (*hsp-sev* construct) results in the formation of a narrow stripe of rescued ommatidia in an otherwise mutant background (Basler and Hafen, 1989; Bowtell *et al.* 1989). The position of

the stripe moves anteriorly across the eye with the advancing age of the larva at the time of heat induction, similar to the scarring pattern described for temperature-sensitive alleles of *shibire* and *Notch* (Foster and Suzuki, 1970; Poodry *et al.* 1973). In the present study, we have used the *hsp-sev* construct as a conditional allele to determine the precise temporal requirements of *sevenless* expression for R7 formation and function. Our results demonstrate that *sevenless* product is exclusively required during a brief period of ommatidial assembly to specify an R7 photoreceptor. *Sevenless* expression is not required during later pupal and adult life for R7 photoreceptor function. Furthermore, by using heat-shock-dependent R7 formation to monitor the movement of the morphogenetic furrow across the disc, we determined when and at what rate ommatidial columns are specified.

Materials and methods

Strains

In this analysis we used the *hsp-sev* transformant strain ch21 that carries the $white^{1118}$ and sev^{d2} mutant alleles. This strain is homozygous for a P-insert on the second chromosome (map position 46E) comprising a *white* minigene and a complete *sevenless* cDNA under the control of the *hsp70* promoter (Basler and Hafen, 1989). Wild-type flies are of the Canton-S strain; *sevenless* mutant flies contain the mutant allele sev^{d2}. All experiments were performed on standard *Drosophila* food and at 25°C.

Heat shock conditions

All heat shocks were applied with a programmable heat-shock apparatus. This device consists of an aluminum block with holes to incubate four standard *Drosophila* culture vials. Cooling and heating is achieved by two Peltier electrical elements. Two target temperatures can be set and are selected by a programmable timer. For all experiments the lower temperature was set to 25°C and the inducing temperature to 36°C. All heat shocks were for 30 min.

A potential problem in our analysis is the possible delay in development caused by a heat shock. Indeed, in pilot experiments in which we applied heat shocks every 4 h at 37°C, we observed a substantial retardation of development compared to non-treated flies. However, lowering the temperature to 36°C and inducing only every 6 h, or less frequently, essentially eliminated this delay. Furthermore, for the determination of the temporal requirements for *sevenless*, only single heat shocks at 36°C were used to induce *sevenless* expression and they did not affect the duration of development.

Analysis of eye phenotypes

The presence or absence of R7 cells was assayed by inspection of the pseudopupils using the optical neutralization technique (Franceschini, 1975). Heads were bisected by a sagittal cut and mounted on a goniometric microscope stage that was illuminated antidromically by a fiber optic light source (kindly provided by R. Wehner). This permitted rotation of the eye under the microscope and thereby allowed assay of essentially the entire retina.

Histological sections through eyes were prepared as described previously (Basler and Hafen, 1988).

Color choice tests

Color choice behavior was determined in a T-maze essentially as described by Ballinger and Benzer (1988). Flies were shortly anaesthetized with CO_2 and distributed in small groups (approximately 20 flies) into clear plastic test tubes (Falcon 2051) that could be inserted into the start cavity of the T-maze apparatus. Green illumination was through a 550 nm narrow-band interference filter with a 150 W fiber-optic source. UV illumination was through a 350 nm narrow-band interference filter (both filters were kindly provided by T. Labhard) with a 18 W UV lamp (long wave, 366 nm). Relative intensities were optimized by varying the distance of the light sources with respect to the T-maze. Flies were given a 20 s period to make a choice and to move to either side. Subsequently, the number of flies that had moved to the UV or green light were counted. For each strain, we assayed four groups of approximately 20 flies. Each group was assayed four times. The numbers given in Table 2 represent the sum of the four values obtained for each group.

Results

Periodic sevenless *induction reveals the dynamics of eye development*

Our previous results with the heat-inducible *sevenless* construct showed that discontinuous supply of *sevenless* product during eye development leads to the formation of alternating vertical stripes of ommatidia containing R7 cells and stripes of ommatidia lacking R7 cells (Basler and Hafen, 1989). The striped pattern reflects the temporal progression of eye development from the posterior to the anterior margin of the disc. Although *sevenless* protein is produced in all cells upon heat induction (Basler and Hafen, 1989; Bowtell *et al.* 1989), only cells in a certain region of the disc and hence in a discrete stage of ommatidial assembly can utilize the *sevenless* protein and become R7 cells. The competence for R7 formation correlates with the movement of the morphogenetic furrow and ommatidial assembly. More posterior ommatidial columns become competent before anterior columns. To investigate the progression of ommatidial assembly, we have used different induction protocols on the *hsp-sev* transformant line ch21 (see Materials and methods).

Induction of the *sevenless* gene by 30 min heat pulses at 36°C every 4 h during late third instar larval and pupal development lead to the formation of R7 cells in all ommatidia. Even exposing larvae only every 6 h to a heat shock still produced essentially wild-type eyes, only in anterior regions some columns exhibited a minor portion of ommatidia lacking R7 cells (Fig. 2A). Under these conditions, the multiple heat shocks did not cause a significant retardation of development (see Materials and methods). When the period of induction was increased to 8 h, narrow regions of only partially rescued columns were detected between stripes with rescued ommatidia (Fig. 2B). Well-defined stripes of wild-type ommatidia were observed with a 12 h period of induction (Fig. 2C). 18 h periods resulted in two stripes separated by a larger mutant zone (Fig. 2D).

In general, the boundaries of the stripes were remarkably sharp, so that only one or two columns

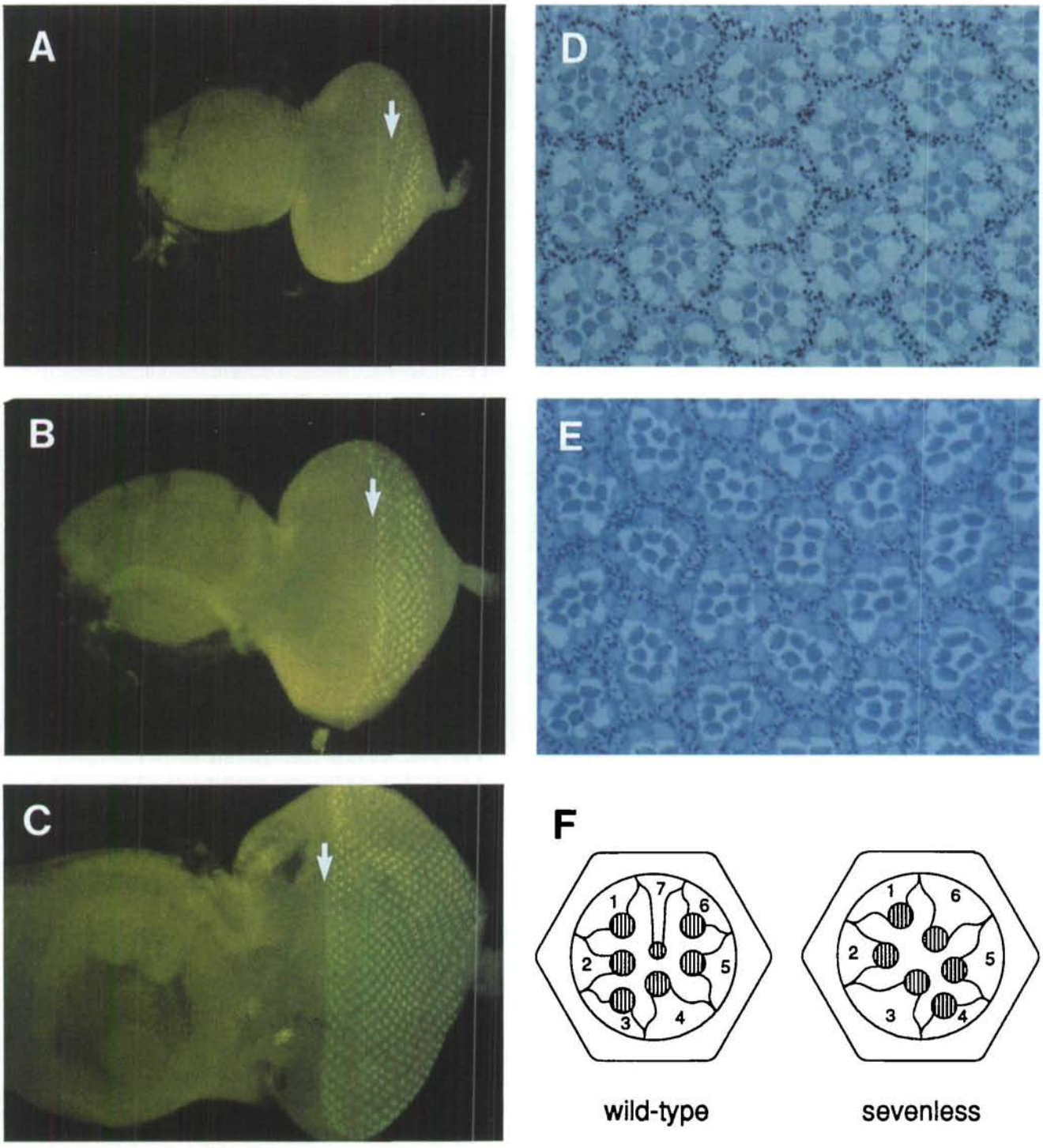


Fig. 1. Development and structure of the compound eye. Anterior is always to the left. (A–C) The progression of eye development is visualized by staining eye imaginal discs of increasingly older third instar larvae with an antibody against the *sevenless* protein. As the morphogenetic furrow (arrow) moves anteriorly across the disc, a smoothly graded series of ommatidia at different developmental stages is laid out along the anterior–posterior axis of the disc. Magnification, ×150. (D, E) Tangential sections through the retina of a wild-type (D) and a *sevenless* mutant (E) strain. The arrangement of the rhabdomeres is very regular in wild-type (D). The rhabdomeres of the outer photoreceptors (R1–R6) are arranged in a trapezoidal pattern surrounding the central rhabdomere of photoreceptor R7 (D). In the *sevenless* mutant ommatidia, the R7 cell and its rhabdomere are missing (E). Magnification, ×100. (F) A schematic representation of the tangential sections through a wild-type and a *sevenless* mutant ommatidium, illustrating the positions and numbers of the photoreceptor cells.

flanking either side of the stripe contained mixed wild-type and *sevenless* ommatidia. The percentage of wild-type ommatidia in adjacent columns after *sevenless* induction in 12 h intervals is shown as a bar diagram in Fig. 3. In our further analyses, we designated a column as wild-type if in more than 50 percent of the ommatidia that could be scored the R7 cell was present.

The increasing distance between wild-type stripes correlates with the progressively longer intervals between heat-shocks and reflects the dynamic process of the eye development. The number of ommatidial columns between the beginnings or the ends of two stripes corresponds to the migration of the morphogenetic furrow over the undifferentiated eye disc epithelium between the two heat pulses. Therefore, this distance is a measure for the speed of the advancing furrow. We have analyzed sections of eyes containing three wild-type stripes that resulted from repeated heat pulses applied in 12 h intervals. Due to the curvature of the eye, not all the ommatidial columns could be assayed in serial sections. Of the approximately 30 ommatidial columns per eye; 20 could be scored in these sections. In 11 of the 13 eyes analyzed, three wild-type stripes were at least partially visible. The number of columns either between the beginnings or the ends of two stripes was recorded (Table 1). In the 11 eyes analyzed, the average distance between the posterior and the middle stripe was 6.9 columns whereas this distance was 10.6 columns between the middle and the anterior stripe. Since the heat shocks were applied at constant 12 h intervals, the morphogenetic furrow must have passed over more ommatidial columns per time interval in the anterior than in the posterior part of the disc. We conclude that eye development does not proceed uniformly over the eye disc but accelerates towards the anterior end of the disc.

Table 1. *Progression of the morphogenetic furrow in a 12 h interval reflected by the spacing of wild-type stripes upon* sevenless *induction every 12 h*

Posterior period:	Anterior period:
7	11
7	10
7	10
6	11
8	11
7	12
6	11
8	12
6	10
7	9
7	10
mean: 6.9±0.7	mean: 10.6±0.9

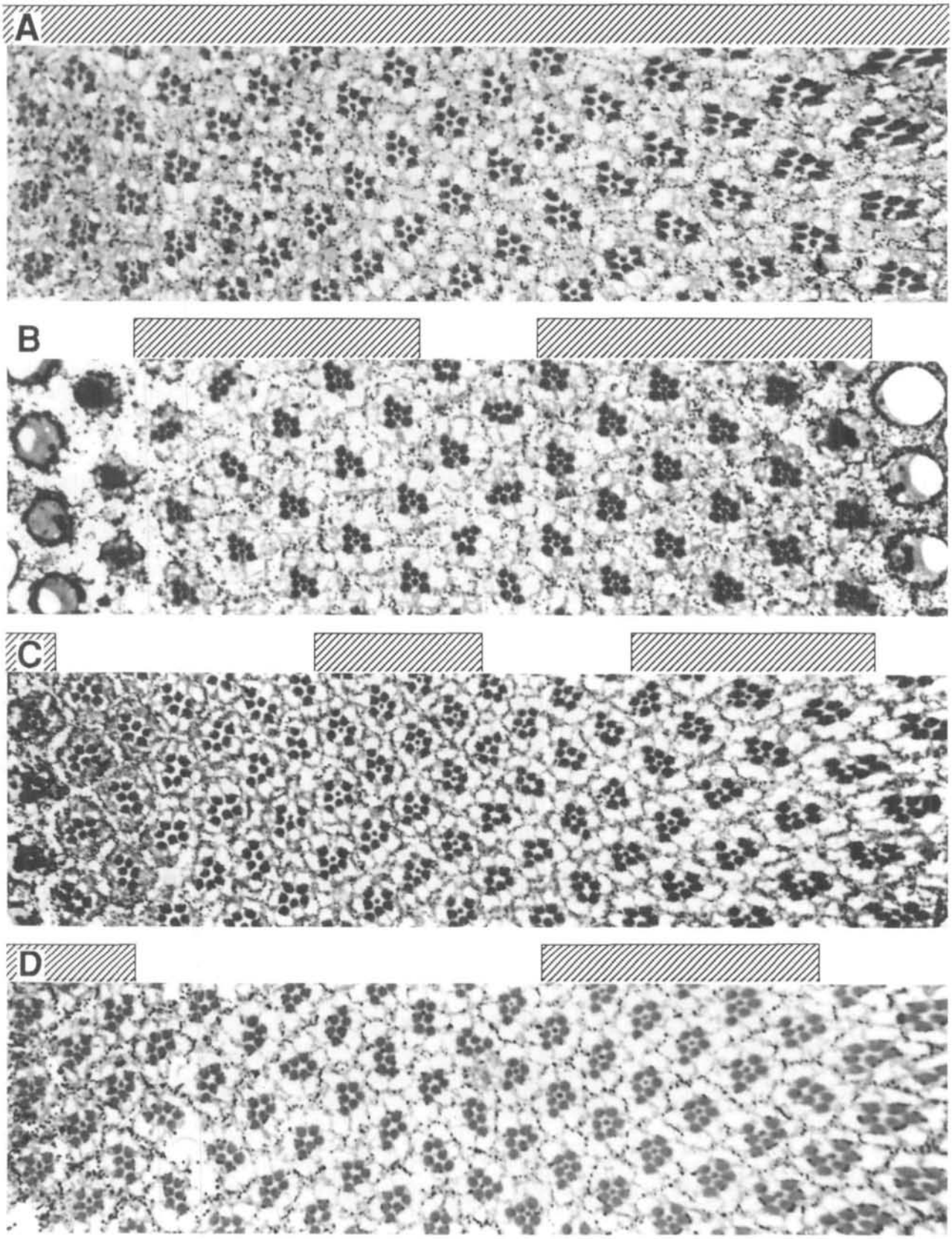
Upon induction of *sevenless* every 12 h during third instar and pupal development, the eyes of the eclosing flies contain 3 dorsoventral stripes of wild-type ommatidia each 4 to 5 columns wide. The distance between the beginnings or the end of two adjacent stripes reflects the movement of the morphogenetic furrow over the disc in the 12 h interval. Hence this distance is a measure for the speed of movement of the furrow. The distance measured in ommatidial columns between the posterior and the central stripe (posterior period) and between the central and the anterior stripe (anterior period) was recorded from sections through 11 eyes. The bottom row contains the means and standard deviations.

hsp-sev *as a conditional allele to determine the temporal requirements of* sevenless *expression during development*

For the exact determination of the end point of required *sevenless* expression, we analyzed at what time during pupal development we had to induce *sevenless* expression to rescue the anterior-most ommatidial columns. To reduce the heterogeneity in age in the larval populations that occurred after 5 days of development and to link our analysis to a precise developmental stage, we used the white prepupae formation as a marker of development. This stage, when the larval cuticle is still white, lasts only a few minutes, and thus is an accurate time mark between larval and pupal development. Groups of white prepupae were collected during 15 min and subjected to a single heat shock either 0, 6, 9, 12, 15 or 18 h later. After hatching, we localized the vertical stripe of rescued ommatidia by inspecting their pseudopupils. The stripe was always 4 to 5 columns wide. The result of this analysis is illustrated in Fig. 4; each arrow represents the position of the heat-shock-induced stripe of rescued ommatidia in an individual fly. Sixteen hours after the white prepupal stage, induction of *sevenless* expression could no longer affect R7 formation in the anterior-most rows. Induction of *sevenless* 12 h after white prepupae formation was the last time point that rescued the anterior-most 4 columns.

To characterize the onset of *sevenless* requirement in an equally precise manner, we also used white prepupae formation as a reference. Several vials containing third instar larvae were given single heat shocks at different time points. White prepupae were collected from these vials over a 15 min period 16, 19, 22, or 25 h later and set aside for the rest of their development. The position of the stripe of rescued ommatidia was again determined by pseudopupil inspection (Fig. 4). For the correct specification of R7 cells in the posterior-most 4 to 5 rows, induction of *sevenless* expression is required 20 h prior to white prepupae formation. Therefore, the total period of *sevenless* expression required for full rescue of the entire eye is 36 h, starting 24 h before and lasting until 12 h after the white prepupal stage.

To correlate the time of R7 determination with the initiation of ommatidial assembly in the morphogenetic furrow, we determined which ommatidial columns were rescued when *sevenless* was induced at the white prepupal stage. At this stage the morphogenetic furrow has reached column 25, as determined by counting columns in six eye discs that had been stained with an antiserum against the *sevenless* protein (Fig. 1C and data not shown). Induction of *sevenless* expression at this stage rescues ommatidial columns 17 to 20, counted from the posterior margin of the eye (Fig. 4). We assume that there is a 2 to 3 h delay between the heat shock and the appearance of functional *sevenless* protein on the surface of all cells based on time-course studies using Western blots (data not shown). During this lag period,



the furrow moves over two more columns. The ommatidial columns where the presence of *sevenless* protein first results in the specification of R7 cells correspond to the most posterior column (column 17) within the stripe of wild-type ommatidia. Since the furrow has reached column 27 by the time functional *sevenless* protein is present, the first cells that can utilize the *sevenless* protein are the ommatidial precursors in the tenth

Fig. 2. Tangential sections through eyes derived from *hsp-sev* transformants that were repeatedly heat-shocked during development. In each section approximately two thirds of all ommatidial columns are visible. An increasing period of *sevenless* induction results in the formation of dorsoventral stripes of rescued ommatidia that are separated by increasingly broader regions of *sevenless* mutant ommatidia. The approximate extent and location of the wild-type regions are indicated above each photomicrograph (hatched area). (A) Induction of the *sevenless* gene every 6 h during late third instar larval and pupal development leads to the formation of R7 cells in virtually all ommatidia. (B) When the period of induction is increased to 8 h narrow regions of non-rescued ommatidia can be detected between stripes of rescued columns. (C) Three distinct stripes are observed with a 12 h induction, usually about 4 columns wide. (D) With an 18 h period of induction only two stripes are formed. Magnification, ×800.

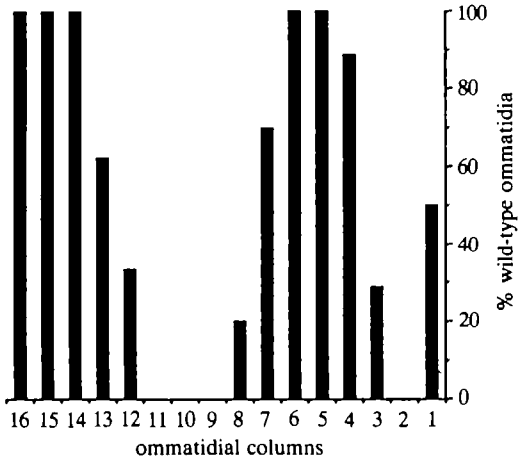


Fig. 3. Percentage of wild-type ommatidia in ommatidial columns after *sevenless* induction in 12 h intervals. Heads of flies that were heat-shocked every 12 h during the larval and pupal period were embedded and sectioned. The percentage of wild-type ommatidia in each column visible in the sections was recorded. The analysis for a single eye is shown. Columns are numbered starting with the posterior-most column that could be scored in the section.

column behind the morphogenetic furrow. Cells in more posterior columns can no longer utilize the *sevenless* protein.

Sevenless *expression in the adult is not required for R7 cell function*

Knowing the exact temporal window during which *sevenless* expression is necessary for R7 cell determination, we can address the question of whether *sevenless* is also required for R7 function later in development. ch21 flies were raised under conditions supplying *sevenless* only during the 36 h window in which we showed *sevenless* to be required for R7 formation. The *sevenless* gene was not induced during the last 60 h of development and in the adult stage. These induction conditions are illustrated in Fig. 5 (+hs[A]). A control group was raised under complementary conditions (+hs[B]). The function of R7 cells was then tested in a behavioral assay for color choice. When wild-type flies are given a choice between ultraviolet and green light, they choose UV over green with a high probability. Sevenless mutant flies exhibit a reversed phototactic behavior due to the lack of the R7 photoreceptors that constitute the primary UV receptors (Harris *et al.* 1976). The color preference of groups of flies raised under conditions A, B, or non-heat-shocked was compared with wild-type and *sevenless* mutant flies. The results of this analysis are given in Table 2 and are graphically represented in Fig. 6. sev^{d2} mutant flies, untreated ch21 flies and the flies from group B that were not heat-shocked during R7 specification preferred the green light source. In contrast, group A flies were equally attracted to UV light as wild-type or the sev^{d2} strain transformed with a genomic wild-type *sevenless* gene (Basler and Hafen, 1988). These results indicate that *sevenless* protein is not required in the adult for R7 cell function.

Discussion

Dynamics of the eye development

Examination of eyes containing multiple stripes of wild-type ommatidia upon repeated thermal *sevenless* induc-

Table 2. *Color choice test of normal, mutant, transformed and heat-shocked flies*

	UV	Green		UV	Green
Canton-S	59	10	*hsp-sev* −hs	9	47
	60	11		2	55
	49	10		4	58
	51	7		3	61
sev^{d2}	4	34	*hsp-sev* +hs[A]	54	1
	4	45		54	4
	4	67		65	3
	6	54		62	5
$Tsev^{Lys2242}$	62	1	*hsp-sev* +hs[B]	11	72
	42	2		6	85
	55	5		4	41
	53	6		4	71

Flies were tested in a T-maze for preference between UV and green light. Each number is the sum of four successive assays with the same group of flies.

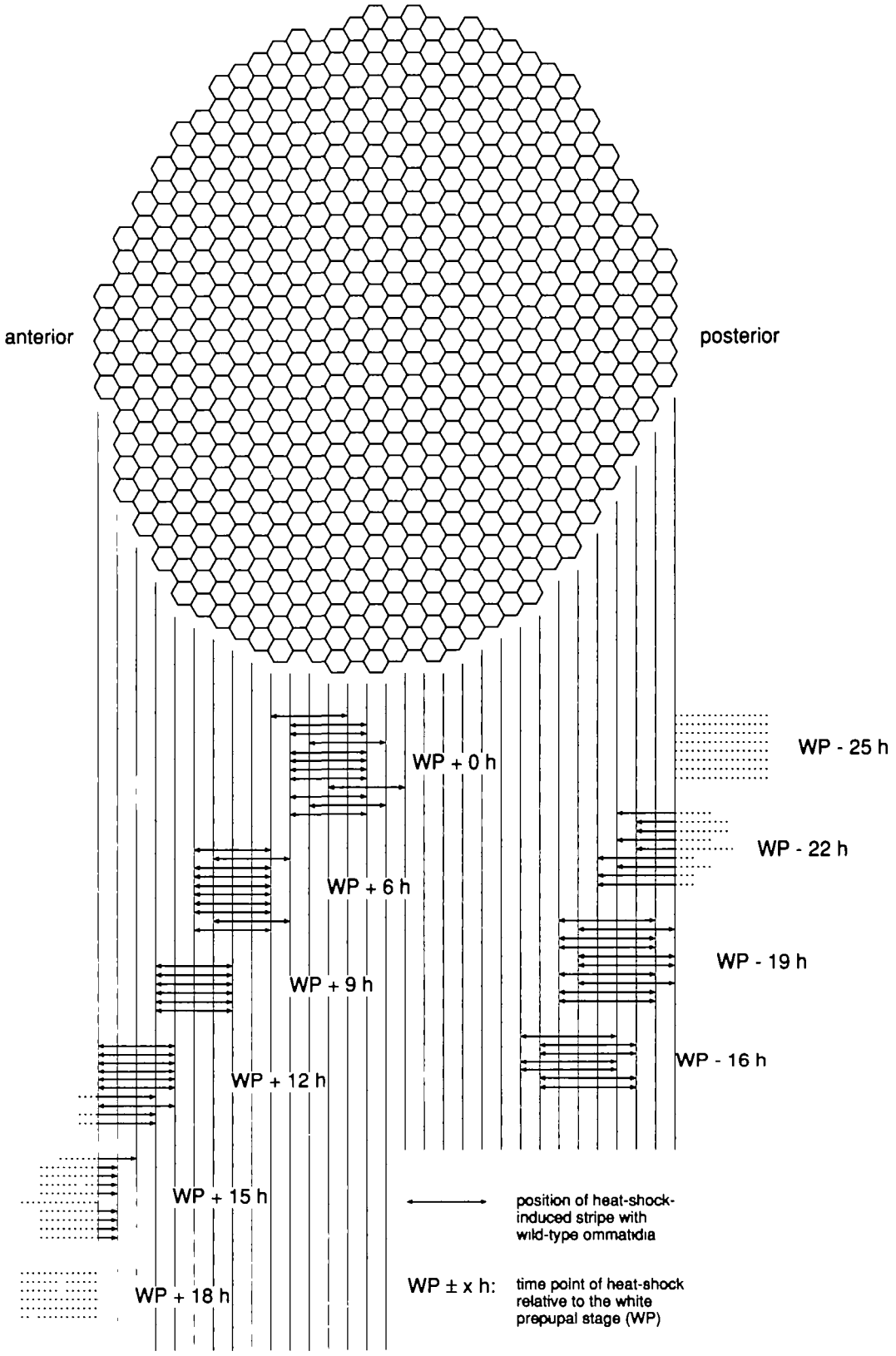


Fig. 4. Summarized results of the single stripe analysis examining the temporal requirements of *sevenless* expression for eye development. An entire eye is shown schematically to indicate the vertical ommatidial columns. Each arrow represents the position of rescued columns in an individual eye derived from an *hsp-sev* transformant that was heat-shocked at the time point indicated relative to the white prepupal stage (WP). For example, the induction of *sevenless* expression by applying a heat shock 9 h after white prepupae formation resulted in a vertical stripe with wild-type ommatidia extending from the fourth to the seventh anterior-most columns of the adult eye.

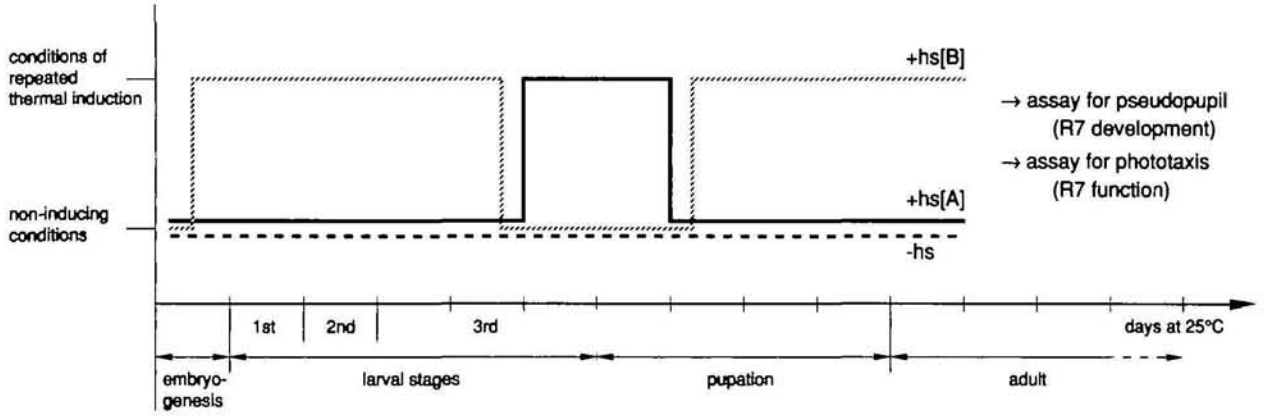


Fig. 5. Induction conditions used for phototaxis assay. *hsp-sev* transformants were raised under the illustrated conditions. Group *+hs[A]* flies were supplied with *sevenless* protein only during the period of R7 cell formation; before and after this period no *sevenless* product was produced. Complementary conditions were chosen for group *+hs[B]*. Conditions of repeated thermal induction were achieved by repeated heat shocks every 6 h for 30 min at 36 °C.

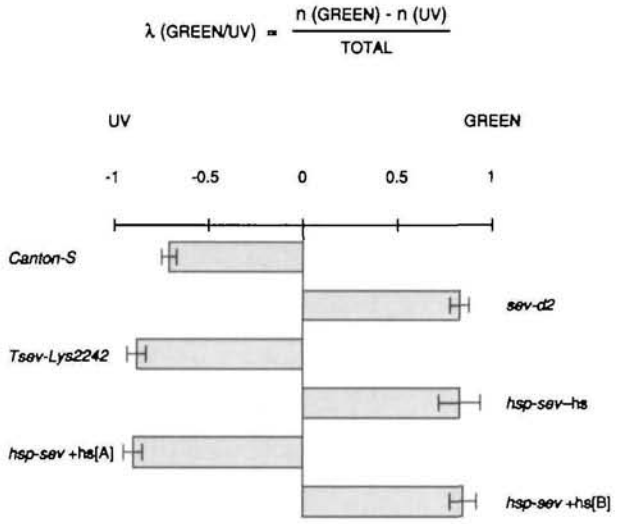


Fig. 6. Color choice preference of wild-type and mutant flies in a T-maze. Flies were tested for their color choice preference between 350 nm UV light and 550 nm green light. The phototactic value λ that is graphically represented indicates the mean of four independent measurements±standard deviation (see data in Table 2). *hsp-sev* transformants raised under conditions +hs[A] (see Fig. 5) behave identical to wild-type flies (*Canton-S*) or *sevenless* mutants that were transformed with the wild-type *sevenless* gene ($Tsev^{Lys2242}$, Basler and Hafen, 1988), even though they were lacking *sevenless* gene function during later stages of development. In contrast, *hsp-sev* transformants without thermal induction or under conditions +hs[B] were not attracted by UV light, just as *sevenless* mutant flies (sev^{d2}).

tion indicates that ommatidial columns are not specified at a uniform rate throughout the disc. The spacing of two wild-type stripes, caused by heat shocks in 12 h intervals, is 6.9 columns in the posterior and 10.6 in the anterior part of the eye (Table 1). Therefore, a new ommatidial column is specified about every 100 min in the posterior portion of the eye disc and even every 60 min in the anterior part of the disc. Previous studies assumed a uniform movement of the morphogenetic furrow during which a new ommatidial column is formed every 2 h (Campos-Ortega and Hofbauer, 1977). If eye development proceeded at this speed it would take 60 h to specify all 30 ommatidial columns of a wild-type eye. Our analysis using single heat shocks indicates that R7 cells in the anterior-most ommatidial column are specified 36 h after R7 cells in the posterior-most column. This corresponds to an average speed of the furrow of 70 min per column which is in good agreement with our analysis of eyes containing three wild-type stripes. We do not know the reason for the non-uniform movement of the morphogenetic furrow across the disc. It is possible that changes in ectysone levels at pupariation influence the rate of ommatidial development since ectysones have been reported to alter both the division rate and the differentiation of imaginal disc cells in culture (Siegel and Fristrom, 1978).

We have calculated that only ommatidial precursor cells in columns 9 to 10 behind the furrow can utilize the ubiquitously produced *sevenless* protein. Previous studies have shown that the different photoreceptor cells express neural antigens in a defined temporal sequence which is spatially displayed on the disc with respect to the morphogenetic furrow (Tomlinson and Ready, 1987). A compilation of all the available data is shown in Fig. 7. Our estimate of the functional requirement of *sevenless* protein coincides with the time when *sevenless* protein is first detected in the R7 precursors in wild-type eyes (Tomlinson *et al.* 1987). According to the Tomlinson and Ready model, R7 determination depends on the signals from its differentiating neighbors R1/6 and R8 (Tomlinson and Ready, 1987). Hence it is possible that ommatidial precursors in regions that are anterior to columns 9 to 10 are unable to utilize the heat-shock-induced *sevenless* protein because the putative ligand for *sevenless* is not yet available. Conversely, cells posterior to column 11 are refractory to the presence of *sevenless* protein possibly because their

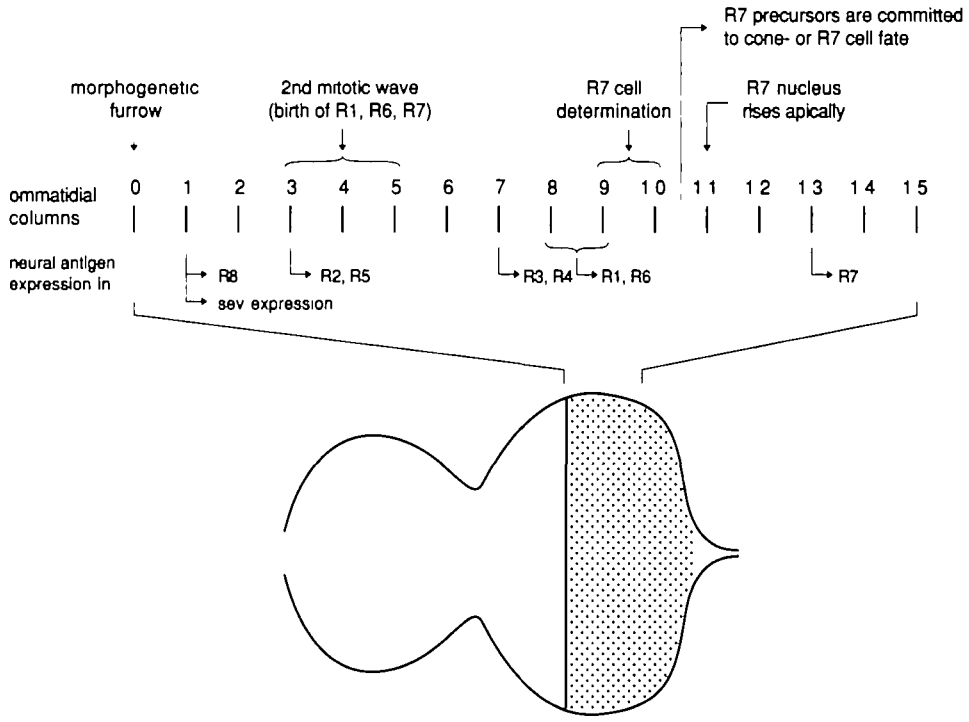


Fig. 7. Compilation of events in the ommatidial assembly sequence. Depicted is an idealized eye imaginal disc at the white prepupal stage. Columns are numbered relative to the center of the morphogenetic furrow. Anterior is to the left. The start positions of neural antigen expression in the various precursor cell types are derived from a study by Tomlinson and Ready (1987) in which they examined neuronal differentiation using the monoclonal antibody 22C10. The estimation of when R7 cells become determined is based on our thermal induction experiments at the white prepupal stage and is corrected for a predicted 3 h lag between induction time and presence of functional *sevenless* protein (see Results).

commitment to either photoreceptor or cone cell fate has already occurred at this stage.

Is sevenless *required for functions other than R7 specification?*

Since the isolation of the *sevenless* mutation and the characterization of the gene, it has been puzzling that an organism would expend a gene exclusively for the determination of the R7 cell. *Sevenless* might additionally be involved in other pathways, e.g. during embryogenesis or in the adult brain. No difference however could be detected between wild-type and mutant flies other than the absence of R7 cells. We are aware that subtle changes in non-retinal tissues could have escaped detection. The altered phototactic behavior of *sevenless* mutant flies is thought to be a consequence of the absence of R7 photoreceptor cells. Since *sevenless* mutants have no R7 cells, it could not be excluded, however, that the *sevenless* protein is also required for the correct function of R7 cells or in the processing of visual information. In fact, high levels of *sevenless* transcripts are detected in adult heads and to a lesser extent also in adult bodies. The expression of *sevenless* in the adult head would be consistent with an additional role for *sevenless* in R7 function. Ballinger and Benzer (1988) have recently reported the isolation of a behavioral mutation, called *Photophobe*. This mutation was isolated in a screen for suppressor mutations that revert the abnormal color preference of *sevenless* mutant flies. *Photophobe* is a dominant mutation and suppresses the behavioral phenotype of *sevenless* mutant flies without causing the return of R7 cells. Since the mutation exhibits an allele-specific interaction with *sevenless*, it has been proposed that the *sevenless* gene plays a role along with *Photophobe* in a common visual information-processing pathway (Ballinger and Benzer, 1988).

The availability of a conditional *sevenless* allele enabled us to probe R7-dependent phototactic behavior in the absence of *sevenless* gene function. By inducing *sevenless* protein only during the time of R7 specification, we generated flies with R7 cells but lacking the *sevenless* protein during the last 60 h of pupal development and during adult life. The phototactic behavior of these flies was indistinguishable from that of wild-type flies containing *sevenless* protein. This indicates that *sevenless* is not required for R7 function and wild-type phototactic behavior. The *sevenless* transcripts present in adult wild-type flies might not serve a function at all, since there seems to be only little selection pressure for a more precise regulation of wild-type *sevenless* expression (Basler *et al.* 1989; Bowtell *et al.* 1989). We cannot formally exclude that under our experimental conditions some *sevenless* protein is still present in the *hsp-sev* transformants after it has been induced several days earlier; however, we think that this is unlikely. The fact that stripes of rescued ommatidia are produced by inducing *sevenless* every 8 h suggests that in less than 8 h the concentration of *sevenless* protein drops below functional levels. Therefore, *sevenless* appears to function exclusively at a single level in the R7 developmental pathway. It is only required for the initiation of R7 development; both differentiation and function of R7 photoreceptors can occur in the absence of *sevenless* protein.

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References

BALLINGER, D. G. AND BENZER, S. (1988). Photophobe (Ppb), a *Drosophila* mutant with a reversed sign of phototaxis; the mutation shows an allele-specific interaction with sevenless. *Proc. natn. Acad. Sci. U.S.A.* **85**, 3960–3964.

BANERJEE, U., RENFRANZ, P. J., HINTON, D. R., RABIN, B. A. AND BENZER, S. (1987). The sevenless protein is expressed apically in cell membranes of developing *Drosophila* retina; it is not restricted to cell R7. *Cell* **51**, 151–158.

BASLER, K. AND HAFEN, E. (1988). Control of photoreceptor cell fate by the sevenless protein requires a functional tyrosine kinase domain. *Cell* **54**, 299–311.

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

BASLER, K., SIEGRIST, P. AND HAFEN, E. (1989). The spatial and temporal expression pattern of sevenless is exclusively controlled by gene-internal elements. *EMBO J.* **8**, 2381–2386.

BOWTELL, D. D. L., SIMON, M. A. AND RUBIN, G. M. (1988). Nucleotide sequence and structure of the sevenless gene of *Drosophila melanogaster*. *Genes and Dev.* **2**, 620–634.

BOWTELL, D. D. L., SIMON, M. A. AND RUBIN, G. M. (1989). Ommatidia in the developing *Drosophila* eye require and can respond to sevenless for only a restricted period. *Cell* **56**, 931–936.

CAMPOS-ORTEGA, J. A. AND HOFBAUER, A. (1977). Cell clones and pattern formation: on the lineage of photoreceptor cells in the compound eye of *Drosophila*. *Wilhelm Roux' Arch. devl Biol.* **181**, 227–245.

FOSTER, G. G. AND SUZUKI, D. T. (1970). Temperature-sensitive mutations in *Drosophila melanogaster*, IV. A mutation affecting eye facet arrangement in a polarized manner. *Proc. natn. Acad. Sci. U.S.A.* **67**, 738–745.

HAFEN, E., BASLER, K., EDSTROEM, J. E. AND RUBIN, G. M. (1987). Sevenless, a cell-specific homeotic gene of *Drosophila*, encodes a putative trans-membrane receptor with a tyrosine kinase domain. *Science* **236**, 55–63.

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.

POODRY, C. A., HALL, L. AND SUZUKI, D. T. (1973). Developmental properties of shibire^{ts1}: a pleiotropic mutation affecting larval and adult locomotion and development. *Devl Biol.* **32**, 373–386.

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

READY, D. F., HANSON, T. E. AND BENZER, S. (1976). Development of the *Drosophila* retina, a neurocrystalline lattice. *Devl Biol.* **53**, 217–240.

SIEGEL, J. G. AND FRISTROM, J. W. (1978). The biochemistry of imaginal disc development. In *The Genetics and Biology of Drosophila* (ed. M. Ashburner and T. R. F. Wright), pp. 317–394. New York: Academic press.

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. AND READY, D. F. (1986). *Sevenless*: a cell-specific homeotic mutation of the *Drosophila* eye. *Science* **231**, 400–402.

TOMLINSON, A. AND READY, D. F. (1987). Neuronal differentiation in the *Drosophila* ommatidium. *Devl Biol.* **120**, 366–376.

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