Reprogramming cell fate in the developing Drosophila retina: transformation of R7 cells by ectopic expression of rough

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In the developing eye of Drosophila, the homeo box gene rough is required in the developing photoreceptor cells R2 and R5 for the correct development of the neighboring R3 and R4 cells. We have expressed rough ectopically in a limited subset of developing ommatidial cells using the sevenless enhancer. Expression of rough in the presumptive R7 cell transforms this cell into an R1–6 type photoreceptor. This transformation is cell autonomous in contrast to the apparent nonautonomy of the rough mutant phenotype and depends on the presence of the sevenless gene. We propose that in wild type, rough functions autonomously in the specification of R2/5 photoreceptor cell identity but by itself cannot initiate neural development.

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In the developing eye of Drosophila, specification of cell fate can be studied at the single-cell level. This is facilitated by the remarkably precise arrangement of cells within both the developing and the mature retinal epithelia. The compound eye consists of a repetitive array of about 800 identical unit eyes, or ommatidia, each of which is a precise assembly of a few distinct cell types (for review, see Ready 1989). The eight photoreceptor cells (R1–R8) of the ommatidial unit can be grouped into three functional classes on the basis of position, spectral sensitivities, and projection pattern of their axons: R1–6, R7, and R8. During development, they assemble and differentiate in a fixed temporal sequence: R8 is the founder cell in each ommatidium, subsequently R2/R5, R3/R4, and R1/R6 are added pairwise, and finally, R7 is added. Later, the non-neuronal elements, the cone cells, and the pigment cells become incorporated.

It has been proposed that cells are directed to their individual fates by inductive signals communicated by neighboring cells (Tomlinson and Ready 1987). Differentiating cells are thought to express cell-type-specific signals that undetermined cells receive and interpret to choose their differentiation pathways. Mutations in genes whose products are involved in these inductive pathways can be grouped into two classes. Genes encoding products involved in the signaling pathway will show a nonautonomous mutant phenotype. Conversely, genes encoding products involved in the reception of the inductive signals and the subsequent execution of the developmental program will exhibit a cell autonomous mutant phenotype and are required intrinsically for a cell to differentiate correctly.

Three genes have been identified as important components in the communication mechanisms. The sevenless mutation prevents the presumptive R7 cell from differentiating correctly and causes the cell to become a lens-secreting cone cell (Harris et al. 1976; Tomlinson and Ready 1986). The sevenless gene product is a receptor tyrosine kinase required autonomously in the presumptive R7 cell and is thought to receive signals from the adjacent R8 cell (Tomlinson et al. 1987; Basler and Hafen 1988; Bowtell et al. 1988). The boss gene is required only in R8 for the R7 cell to develop appropriately (Reinke and Zipursky 1988). The nonautonomy of boss suggests that it is part of the signaling machinery in R8. Similar to boss, rough is nonautonomously required in cells R2 and R5 for the correct specification of the neighboring R3 and R4 cells (Tomlinson et al. 1988). The rough protein appears to act on the signaling side of the inductive pathway. Surprisingly, molecular analysis of rough revealed that it encodes a homeo domain protein and not a membrane protein or a secreted factor (Saint et al. 1988; Tomlinson et al. 1988). It has been postulated that rough functions in R2 and R5 as a transcriptional regulator for an R3/4 inducing signal (Tomlinson et al. 1988). In contrast, other homeo box genes involved in developmental decisions function as selector genes that control the fate of cells in which they are expressed (Hafen et al. 1983; Gehring 1987).
Results

Ubiquitous expression of rough is lethal during development

Mosaic analysis has indicated that rough has to be expressed in only two cells of the ommatidia, R2 and R5, for correct ommatidial formation (Tomlinson et al. 1988). By use of a specific antiserum, B. Kimmel and G. Rubin have shown that rough is stably expressed not only in R2 and R5 but also in R3 and R4 (pers. comm.; see also accompanying paper; Kimmel et al. 1990).

To examine the effect of ectopic expression of the rough gene, we designed a rough minigene under the control of a ubiquitous promoter. The rough transcription unit consists of three exons spliced to a 1.3-kb mRNA with an open reading frame of \( \sim 350 \) amino acids (Tomlinson et al. 1988). We chose an inducible promoter to prevent selection against any deleterious effect resulting from inappropriate rough expression. Using in vitro mutagenesis, we assembled a construct—\( hsp-rough \)—as a transcriptional fusion between the Drosophila \( hsp70 \) heat shock promoter/leader and a rough minigene lacking the two introns but comprising the entire open reading frame and the 3'-flanking sequences. This construct was inserted into the pW8 P-element vector (Klementz et al. 1987) to generate germ line transformants. Ten independent lines were obtained and kept as homozygous stocks.

To demonstrate the inducible production of rough protein in the transformed lines, we analyzed protein of third instar larvae at successive time points after a 30-min heat shock at \( 37^\circ \)C. By use of a rough antiserum raised against part of the rough protein synthesized in bacteria, we detected accumulation of rough protein in heat-shocked transformants but not in control larvae (Fig. 1A). The highest level of rough accumulation was detected 1 hr after induction. Repeated heat induction at embryonic, larval and pupal stages resulted in lethality of the \( hsp-rough \) transformants. Under the same conditions, wild-type or \( hsp-sev \) transformants that contain a heat shock-inducible sevenless gene survived. Even single heat shocks at the white prepupal stage caused a substantially lower survival rate. Hatched flies that survived the heat shock have severely reduced eyes bordered anteriorly by a sharp vertical scar (Fig. 1B). The few ommatidia anterior to this scar were highly irregular, whereas ommatidial columns posterior to the scar were normal. In histological sections, however, we occasionally observed an altered rhabdomere pattern in 4–5 adjacent ommatidial columns. This pattern was similar to that caused by the transformation of R7 cells into R1–6 type cells observed in the \( sev-hsp-rough \) transformants (see below). No other changes in the external morphology of these flies were noted.

Ubiquitous expression of rough during development appears to interfere with the normal development of cells. Induction of rough in adult flies, however, has no effect. The greatly reduced eyes resulting from a single heat shock during the third instar stage indicates that ubiquitous expression of rough interferes with the formation of new ommatidial columns in the eye imaginal disc. Heat-shock induction of sevenless carried out in parallel experiments was not lethal, nor did we observe an effect on eye development of wild-type flies. Hence, we assume that this effect is specifically caused by rough. To test whether ubiquitous expression of other homeo domain proteins would produce a similar phenotype, we used a transformed line containing a heat-inducible \( Ubx \) minigene (Gonzalez-Reyes et al. 1990). Ubiquitous expression of \( Ubx \) at the late third instar stage caused almost complete lethality with an even lower survival rate than in the \( hsp-rough \) transformants. The few surviving flies contained rough eyes. In no case did we observe scar formation or a sharp anterior boundary of the eye as in the case of eyes of \( hsp-rough \) flies. Ubiquitous expression of \( Antennapedia \) is also lethal when induced at this stage (Gibson and Gehring 1988). Therefore, the stage around the beginning of pupation is highly sensitive to the ubiquitous expression of different homeo domain proteins. Yet the phenotypes observed in the eyes of surviving flies are clearly different in the case of ubiquitous rough and \( Ubx \) expression.

Localized ectopic expression of rough using the sevenless enhancer

To limit ectopic expression of rough to a defined subset of cells in the developing eye imaginal disc, we used the sevenless enhancer sequences that control the sevenless expression pattern. In contrast to other tissue-specific enhancers that are active only in differentiated cell types
such as the cis-acting sequences that control rhodopsin expression (Mismer et al. 1987), *sevenless* is expressed transiently in a subpopulation of ommatidial precursor cells prior to or at the time of their commitment (Tomlinson et al. 1987). Its expression pattern partially overlaps the rough expression pattern. A comparison of the expression pattern of rough and *sevenless* in the cells of the developing ommatidia is shown in Figure 2. *sevenless* is expressed strongly in cells R3, R4, R7, and the cone cells and weakly in R1 and R6. It has been shown previously that a gene-internal fragment of the *sevenless* gene is responsible and sufficient for the temporally and spatially restricted expression of *sevenless* (Basler et al. 1989; Bowtell et al. 1989b). This regulatory element imposes the *sevenless*-specific expression pattern on heterologous promoters such as the *hsp70* promoter. We inserted an 8-kb restriction fragment containing the *sevenless* enhancer upstream of the *hsp-rough* construct —sev-*hsp-rough*— and generated germ line transformants. Nine independent lines were established with the sev-*hsp-rough* construct. Eye imaginal discs of sev-*hsp-rough* transformants that are stained with the rough antiserum showed a different distribution of rough protein from that observed in wild type (data not shown). The altered rough expression pattern is caused by the expression dictated by the *sevenless* regulatory elements superimposed onto the expression pattern of the endogenous rough gene.

**R7 cells are transformed into R1–6-type photoreceptors**

None of the transformed lines carrying one copy of the sev-*hsp-rough* construct exhibits an externally visible phenotype: the arrangement of the ommatidia and the external morphology of the eyes of the transformants is indistinguishable from wild type (see Fig. 3). Histological sections through eyes of heterozygous transformants, however, revealed an irregular rhabdomere pattern in 50–80% of the ommatidia. Wild-type ommatidia contain 8 photoreceptor cells (R1–R8). The rhabdomeres of R1–6 extend through the depth of the retina, and form an asymmetric trapezoid (Fig. 4A, C, E). The rhabdomere of R7 is smaller in diameter than that of the outer photoreceptors and occupies the central position in the distal two thirds of the retina. This position is occupied proximally by the rhabdomere of R8 [Fig. 4E]. Therefore, in each plane of cross section, a highly ordered pattern of...
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Figure 2. Diagram of wild-type and ectopic expression of rough. Precursor cells of the ommatidial 12-cell unit are schematically represented to indicate high levels [grey] or moderate levels [light grey] of expression. Photoreceptors are numbered. (EC) Equatorial cone cell; (AC) anterior cone cell; (PLC) polar cone cell; (PC) posterior cone cell. In the wild type, the four photoreceptor precursor cells R2, R5, R3, and R4 express rough protein [see accompanying paper; Kimmel et al. 1990; diagram at left]. The sevenless enhancer induces the expression pattern illustrated at right [Basler et al. 1989]. Therefore, in sev-hsp-rough transformants [sev-hsp-ro, middle diagram], rough is ectopically expressed in R7, R1, R6, and cone cells. Expression in R2, R5, R3, and R4 has been omitted in the sev-hsp-rough diagram because it is not ectopic.

7 rhabdomeres is visible [Fig. 4A,C,E]. Apical sections of sev-hsp-rough ommatidia also display seven rhabdomeres, but in an abnormal arrangement [Fig. 4B]. The few undisturbed ommatidia can be used as an internal reference for the orientation of the rhabdomere pattern. Comparison with adjacent affected ommatidia indicates that photoreceptors R1–R6 are normal, but that the cell between R1 and R6, corresponding to the R7 cell in wild type, causes the observed irregularity: its rhabdomere is not in the central position, but is instead between that of R1 and R6 [Fig. 4B,E]. Furthermore, its rhabdomere diameter is larger than that of a normal R7 cell, and similar to the size of the R1–6-type rhabdomere. In contrast to wild-type R7 cells, these cells extend through the depth of the retina. Therefore, in more basal sections we observe 8 rhabdomeres instead of 7 in the ommatidia containing transformed R7 cells: in addition to the small R8 rhabdomere surrounded by the rhabdomeres of R1–R6, there is a rhabdomere present between R1 and R6 corresponding to the transformed R7 cell. Another criterion for photoreceptor cell identity is the position of the nucleus. The nuclei of the R1–6 cells are clustered apically, the R7 nucleus is located about 15 μm more proximally, and the R8 nucleus is located basally. The nucleus of the transformed R7 cell is at the same level as the nuclei of the R1–6 cells. Therefore in the sev-hsp-rough transformants, a large fraction of R7 cells assume all of the morphological characteristics of the R1–6-type photoreceptor cells.

To confirm that the transformed R7 cell has an R1–6 identity, we crossed sev-hsp-rough into an ora [outer rhabdomeres absent] mutant background. ora mutants lack rhodopsin in the R1–6 set of photoreceptors; in addition, this mutation causes the degeneration of the rhabdomeres of the R1–6 photoreceptor cells. Newly emerged flies have small rhabdomeres that shrink as the fly ages until only distal remnants remain [Stark and Sapp 1987]. This phenomenon is strictly specific for R1–6 cells. The rhabdomeres of R7 and R8 are normal in ora flies (Fig. 5C). If the R7 cells in sev-hsp-rough ommatidia have really become R1–6-type cells, they should be susceptible to the ora mutation and should contain degenerated rhabdomeres. As shown in Figure 5D, sev-hsp-rough eyes indeed reveal ommatidia in which all seven rhabdomeres are degenerated. In basal sections of sev-hsp-rough flies, R8 rhabdomeres are present in all ommatidia and are never accompanied by an additional nondegenerated rhabdomere as one would expect if the transformed R7 cell had not become an R1–6-type photoreceptor. This demonstrates clearly that the rough-induced transformation of R7 precursors is complete.

We conclude that ectopic rough expression under the control of the sevenless enhancer results in the transformation of a large fraction of R7 cells into R1–6-type cells. We have never seen R7 cells with morphological characteristics in between those of R7 and R1–6-type photoreceptors. In wild type, rough controls the development of R3 and R4, which belong to the R1–6 class of photoreceptors. Therefore the ectopic role of rough in the transformation of R7 is similar to its normal function.
**Figure 3.** Effect of increased sev-hsp-rough gene dosage on eye morphology. Scanning electron micrographs of two left eyes from transformants carrying one copy (A) versus two copies (B) of the sev-hsp-rough construct. Fivefold enlargements corresponding to the rectangles are shown at right. (A) Heterozygous sev-hsp-rough eyes are indistinguishable from wild-type eyes. (B) In contrast, eyes of transformants homozygous for sev-hsp-rough exhibit a strong blueberry-eye phenotype: The ommatidia are irregularly arranged and have severe lens defects. The short sides of the rectangles correspond to 90 μm. Anterior is to left.

**rough acts cell autonomously in the transformation of R7 cells**

The requirement of the wild-type rough gene in photoreceptors R2 and R5 for normal differentiation of R3 and R4 implies a role for the rough gene product in producing signals required for the specification of other cells (Tomlinson et al. 1988). Because of the apparent nonautonomy of rough function in wild type, it was important to establish whether the transformation of R7 is caused by the ectopic rough expression in R1 and R6 or by the expression in R7.

To test which cells in the ommatidia are responsible for the sev-hsp-rough phenotype, we carried out a mosaic analysis using X-ray-induced mitotic recombination to produce clones of genotypically normal cells in a sev-hsp-rough heterozygous background. To assess the sev-hsp-rough genotype of a retinal cell, we used the white gene included in the sev-hsp-rough construct as a cell-autonomous marker. Because the transformed lines were established in a white- background, only cells containing sev-hsp-rough are pigmented. In this way, the presence of the sev-hsp-rough construct could be unambiguously scored in each photoreceptor cell and in the pigment cells. By induction of somatic recombination in animals heterozygous for the sev-hsp-rough construct, clones of white- cells could be generated that had lost the sev-hsp-rough construct and clones of white+ cells that are homozygous for the construct in an otherwise heterozygous background (Fig. 6A). At the interface between the white- clone and the heterozygous region, cells mix such that some ommatidia contain cells of different genotypes (Fig. 6B). By analyzing a number of these mixed ommatidia, we could determine which cells in an ommatidium always contain the sev-hsp-rough construct when the R7 cell is transformed.

Thirty-seven mosaic eyes were serially sectioned to score ommatidia of mixed genotypes. Because the ommatidia of sev-hsp-rough transformants do not all contain transformed R7 cells, our analysis was limited to...
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Figure 4. Transformation of R7 cells into R1–6-type photoreceptor cells. Histological sections through wild-type (A and C) and sev–hsp–rough (B and D) eyes. As schematically illustrated in E, both apical (A and B) and basal sections (C and D) are shown. Each photoreceptor cell has a microvillar stack of membranes containing rhodopsin, called the rhabdomere, that projects toward the center of the ommatidium. In the wild type, the R7 rhabdomere differs morphologically from the rhabdomeres of R1–R6 with respect to position, diameter, and length: it is located in the center of the trapezoid formed by the larger rhabdomeres of R1–R6, it is smaller in diameter, and it extends only two-thirds the depths of the retina. In a high portion of ommatidia of heterozygous sev–hsp–rough transformants, the rhabdomere of the R7 cell has all of the characteristics of an R1–6-type rhabdomere (arrow in B, D, shaded in E, left; marked by asterisk in E, right). In contrast to the seven rhabdomeres observed in basal sections of wild-type eyes (C), eight rhabdomeres are visible in altered ommatidia in corresponding sections of sev–hsp–rough transformants (D). Anterior is down. Magnification, 1000×.
Figure 5. *sev-hsp-rough* in *sevenless* and *ora* mutant background. Sections through eyes of different genotypes are shown: (A) *sev-hsp-rough* in the wild type (for a comparison). (B) *sev-hsp-rough* in a *sevenless* mutant background: In all ommatidia, only six rhabdomeres are visible, indicating that also the formation of the transformed R7 cells is dependent on the *sevenless*+ gene. The identical phenotype was observed in a *boss* mutant background. (C) *ora* without the *sev-hsp-rough* construct: the rhabdomeres of the cells R1–R6 are absent or degenerated, but none of the R7 rhabdomeres is affected. (D) *sev-hsp-rough* in an *ora* homozygous background: note that in contrast to C, a number of R7 cells (e.g., arrow) also have degenerated rhabdomeres, indicating that the transformed R7 cells behave like R1–6-type photoreceptor cells. The section shown in D is from a newly emerged fly and, therefore, shows remnants of the affected rhabdomeres; this allows better orientation within the ommatidia. As the flies age, the rhabdomeres degenerate further until they are completely absent as in C. Anterior is down. Magnification, 1000×.
Figure 6. Mosaic analysis. (A) Generation of wild-type clones in a sev–hsp–rough background: Irradiation of sev–hsp–rough heterozygous larvae can cause somatic recombination between non-sister chromatids, which leads to the simultaneous generation of wild-type and sev–hsp–rough homozygous cells. Mosaic ommatidia occur at the border of wild-type patches. The genotype of the photoreceptor cells can be assessed by the presence or absence of pigment granules (small black spots directly adjacent to the base of the rhabdomeres). (B) Section through an eye containing a typical wild-type clone. The arrow points to the transformed ommatidium that is enlarged in C. In this mosaic ommatidium, the only photoreceptor cell containing pigment is the transformed R7 cell (arrowhead). (D) Another mosaic ommatidium (from a different clone) in which only the transformed R7 cell has pigment (arrowhead). (E) The same ommatidium as in D, but a more basal section in which the R8 cell is also visible. Thus, the only cell in an ommatidium that is required to be of sev–hsp–rough genotype for R7 transformation to occur, is R7 itself. Anterior is down. Magnification, (A) 600×; (B–D) 1400×.

R1–R6. More basally, the R8 cell joined the cluster rendering the wild-type pattern of seven photoreceptors. Therefore, the rough-induced transformation of R7 depends on both boss and sevenless function.

sev–hsp–rough disrupts cone cell development in a dose-dependent manner

Under the control of the sevenless enhancer, rough is also expressed in the cone cells as soon as they become integrated into the ommatidial clusters. The cone cells are non-neuronal cells that lie above the photoreceptor cells and secrete the central part of the corneal lens as well as the liquid-filled pseudocone. In transformed lines heterozygous for the sev–hsp–rough construct, the corneal lenses as well as the regular hexagonal array of the ommatidia is not altered despite the fact that the ma-
The developing eye as an experimental system to study position-dependent cell fate specification has its limits. The deformation of the corneal lens indicates that expression of rough in the developing cone cells disrupts their normal development. Structures derived from the cone cells, such as the central parts of the cornea lens, do not form properly. The effect of rough expression in the cone cells is strikingly dose dependent. When one copy of the sev–hsp–rough construct is present, the lens forms normally. However, with two copies, a large portion of the lenses is affected. The blueberry-eye phenotype is observed in all nine independent sev–hsp–rough lines. Increasing the number of sev–hsp–rough copies to four results in an even more severe blueberry-eye phenotype.

Discussion

The developing eye as an experimental system to study position-dependent cell fate specification has its limitation in that experimental manipulations such as laser ablation of individual cells and single-cell transplantations are not possible. These techniques have been extremely useful in other systems such as Caenorhabditis elegans (Greenwald 1989) and the grasshopper (Doe and Goodman 1985) to assay the developmental potential of individual cells in an altered environment. This shortcoming can be overcome by molecular genetic manipulation of the system. Genes controlling developmental decisions or specifying positional information can be fused to heterologous promoters and reintroduced into the germ line. In this way, positional values in the developing field can be changed without the inherent side effects of experimental manipulation. The specificity of the molecular genetic manipulation depends largely on the genes used, and just as importantly, on the specificity of the control elements that confer ectopic expression. Ectopic expression may be achieved by fusing the structural gene to an inducible promoter such as the hsp70 promoter. Indiscriminate expression of genes, however, is often lethal, and this lethal phenotype is then difficult to relate to the normal function of the gene. Similarly, the results reported here on the effect of ubiquitous expression of rough are difficult to compare with the normal function of rough in specifying photoreceptor cell identity in postmitotic cells. In contrast, by use of the sevenless enhancer to drive rough expression, we expressed rough ectopically in only a small number of related cells and indeed obtained specific information on the function of rough. Although the promoters of other genes have been characterized that are specifically expressed in subsets of ommatidial cells (i.e., rhodopsin; Mismer et al. 1987), the sevenless enhancer is unique in that it is active early, during the determination process of retinal precursor cells. This approach will become more widely applicable as more stage- and tissue-specific enhancers are identified and characterized by the enhancer trap method (O’Kane and Gehring 1987; Bier et al. 1989; Wilson et al. 1989).

Expression of rough in the presumptive R7 cell causes this cell to differentiate as an R1–6 photoreceptor cell type. This transformation is a cell-autonomous phenomenon and contrasts the apparent nonautonomy of the rough mutant phenotype: In the developing ommatidia, the rough protein is required in R2 and R5 for the ommatidia to develop correctly. In its absence from these two cells, R3 and R4 develop aberrantly, but R2 and R5, as judged from morphological criteria, develop normally. Given the role of the rough homeo domain protein as a putative transcription factor, it has been proposed that rough controls the expression of a gene encoding the R3/4 inducing signal in R2 and R5 (Tomlinson et al. 1988). One way to reconcile this contradiction is to suggest that in the presumptive R7 cell, the rough protein transcriptionally activates the R3/4 inducing signals which, in an autocrine way, triggers the R1–6 developmental pathway in R7. We think, however, that this is unlikely and propose instead that rough serves an autonomous role in controlling the identity of the R2 and R5 cells. Because markers that distinguish between the identities of the outer photoreceptor cells are not available, the failure to obtain R2/5 identity in rough mutants could not be detected. According to this model, the incorrect development of R3 and R4 in rough mutants is a mere consequence of the incomplete differentiation of R2 and R5. It appears, therefore, that rough, similar to other homeotic genes, is a selector gene that distinguishes between alternative fates of the cells where it is expressed. Although the progenitors of R2 and R5 enter the photoreceptor cell pathway in rough mutants, they fail to assume an R2/5 identity, which is manifested in their failure to induce R3 and R4. In wild type, rough is also expressed in R3 and R4 (see accompanying paper; Kimmel et al. 1990). According to our model, the presence of rough protein in R3 and R4 also causes these cells to assume an R2/5 identity and thereby distinguishes them from R1 and R6. The failure to assume this identity in rough mutant clones, however, cannot be detected because R3 and R4 do not recruit other photoreceptor cells. Similarly, a potential, incorrect signal sent by the transformed R7 cells does not induce a change in the cell fate of neighboring cone and pigment cells, pre-
sumably because they are not competent to respond to such a signal.

In *sevenless* mutants, the R7 precursor appears never to enter the photoreceptor cell pathway. *rough* expression in the absence of either *sevenless* or *boss* is not sufficient to initiate the photoreceptor cell pathway. This suggests that specification of photoreceptor cell fate requires more than one function. *boss* and *sevenless* appear to be involved in the initiation of this pathway in R7, whereas *rough* functions in the specification of photoreceptor cell identity. *rough* expression in R7 can compete with or even override the step that leads to the specification of the R7 cell fate. The dependence of *rough* function on a prior commitment of the *rough*-expressing cell to photoreceptor development is consistent with its normal function in R2 and R5, because these cells apparently initiate photoreceptor cell development in the absence of *rough*. Although *rough* controls photoreceptor cell identity, it is not sufficient to confer neural fate to undetermined cells. This contrasts the effect of MyoD expression that induces muscle differentiation in a variety of different cell types (Davis et al. 1987; Weintraub et al. 1989). Whether a gene exists in *Drosophila* for photoreceptor cell development with properties similar to those of MyoD for muscle differentiation is not known.

*rough* expression in ommatidial precursor cells can elicit distinct developmental programs depending on the activity of other proteins in the cells. In wild-type R2 and R5 cells, it specifies R2/5 identity. When ectopically expressed in the R7 precursor, it also specifies the developmental pathway of R1–6 photoreceptor cells, if *sevenless* is activated. In high doses in cone cells, *rough* disrupts cone cell development. It is tempting to speculate that the disruption of ommatidial row formation observed in *hsp-rough* transformants may be caused by the transformation of R8 into R2/5-type cells, thereby preventing the normal recruitment of other ommatidial cells by R8.

Our results demonstrate that the restricted *rough* expression observed in wild type is critical for proper differentiation of several cell types in the compound eye. In contrast, the restricted expression pattern of *sevenless* —a putative receptor for an inductive signal—is not important for the correct determination of retinal precursor cells (Basler and Hafen 1989; Bowtell et al. 1989a). This reflects the different roles of *rough* and *sevenless* in cell fate specification. Whereas *rough* has an instructive role, *sevenless* acts as a sensory protein in cell fate specification.

The results presented here indicate that the R7 precursor is multipotent. It can assume four distinct fates dependent on the state of activity of the *sevenless* and *rough* gene products (Table 1): In wild type, *sevenless* protein is activated—probably by *boss*—which results in an R7 fate. If *rough* is also present in the cell, it can become an R1–6-type photoreceptor cell. If *sevenless* is not activated, the cell becomes a normal cone cell. We assume that, as in the case of normal cone cells, high levels of *rough* protein abort development of this cell.

The observed multipotency indicates that the precision with which cell fate is determined in the eye depends in part on the spatially and temporally restricted expression of positional cues and additionally on the combination of different intracellular signal transducers that respond to the inductive stimulus.

The dose-dependent blueberry-eye phenotype caused by expression of *rough* in the cone cells can be used to genetically identify revertants of this phenotype. Second-site suppressors of the blueberry-eye phenotype should identify genes that act downstream of *rough* and also genes that control the *sevenless* expression pattern. Because two doses of *sev*–*hsp-rough* are required to see the blueberry-eye phenotype, it is expected that the inactivation of just one gene copy of a potential downstream gene could cause reversion.

**Experimental procedures**

**Plasmid constructions**

The strategy to construct the two plasmids pW8[*hsp-rough*] and pW8[sev–*hsp-rough*] was as follows: A unique cloning site was introduced into the untranslated leader sequence of the genomic *rough* gene by site-directed mutagenesis; subsequently, the two introns were removed by replacing a genomic restriction fragment with the corresponding cDNA sequences. The resulting *rough* minigene containing the endogenous termination-processing sequences was inserted into both a derivative of pW8 (Klemenz et al. 1987) that contained the *hsp70* promoter and into a plasmid that constructed the *sevenless* regulatory sequences upstream of the *hsp70* promoter.

Specifically, we cloned the genomic 1.2-kb *Salt–BamHI* fragment of *P[rry,ro]* (positions 57–1169; Tomlinson et al. 1988) into M13mp18 and used the oligonucleotide 5′-GTCGCCGCCC-CCCGTACCCAAATGA-3′ according to the method of Taylor et al. (1985) to introduce a *KpnI* site at position 14. The double-stranded M13 insert was cloned as a *Salt–EcoRI* fragment into pKB216, which was obtained by inserting the 2-kb genomic *EcoRI–Xhol* fragment (positions 3024–4945) as an *EcoRI– HindIII* × *T4 polymerase* fragment into pBluescript digested with *EcoRI* and *BamHI* × *T4 polymerase*. The resulting plasmid pKB219 was digested partially with *KpnI* and religated to get rid of the upstream 80-bp *KpnI* fragment that contains an *Aatt* site interfering with the subsequent cloning step. The 2.1-kb *Aatt* fragment of the resulting pKB227 was replaced with the corresponding 829-bp *Aatt* fragment (genomic positions 251–4215) of cDNA proc4-2 (Tomlinson et al. 1988), yielding the intronless *rough* minigene pKB228. Its 1.8-kb insert was released by digestion with *KpnI* and *PstI* and inserted into pW8hsp (Basler and Hafen 1989) to yield the P-element transformation plasmid pW8[*hsp-rough*]. The plasmid pW8[sev–*hsp-rough*] was obtained by cloning the same insert into a derivative of pW8hsp that contains a genomic 8-kb *sevenless* fragment (positions 86–8332, Basler and Hafen 1988) inserted in reversed orientation into the *EcoRI* site upstream of the *hsp70* promoter (analogous to construct 202 in Basler et al. 1989).

**Generation of antisera**

To obtain bacterially produced protein corresponding to amino acids 24–236 of the *rough* protein, we used the T7 polymerase expression system (Studier and Moffatt 1986). The 640-bp *Xhol* fragment of pKB228 (genomic positions 133–3543; Tomlinson et al. 1988) was inserted at the *AatII* site of pW8hsp (Basler and Hafen 1988) to yield the pW8[sev–*hsp-rough*] plasmid. The resulting plasmid was co-transfected into *Escherichia coli* strain DH5α, in which the minigene was expressed as a polyhistidine tag fusion protein. Purified protein was used to generate antiserum against the amino acids 24–236 of *rough*.
Thirty-seven eyes were serially sectioned. All sections (2 ~m) were identified as white patches in otherwise red eyes. We found 382 mosaic eyes among 10'720 flies, the frequency of mosaic integrations were mapped on the basis of the segregated sevenless (sev) gene product in the neighboring R8 cell. The expression of rough in the R7 precursor (ro+) by sev-hsp-rough causes the cell to become an R1−6 type cell. If sevenless protein is not activated in boss mutants or is not functional in sevenless mutants (sev−), then the R7 precursor assumes the cone cell fate.

*The strong rough expression in cone cells (ro+) of sev-hsp-rough homozygotes causes abortive development.

Table 1. Potential fates of the R7 precursor cell depending on different combinations of sevenless and rough activity

| Activity       | R7 precursor cell fate |
|---------------|------------------------|
| sev+/ro−      | R7                     |
| sev+/ro+      | R1−6                   |
| sev−/ro−      | cone cell              |
| sev−/ro+ *    | —                      |

Activation of the sevenless protein (sev+) in the wild-type R7 precursor leads to normal development and is most likely dependent on the boss gene product in the neighboring R8 cell. As expected from previous work [Basler and Hafen 1988; Smelth, 1989; et al. 1989], the sevenless gene product is not functional in boss (bo) mutants or is not functional in sevenless (sev−) homozygotes. The R7 precursor assumes the cone cell fate.

Adult flies used for scanning electron microscopy were stored in 70% acetone before they were critical-point dried and coated with gold to examine in a Hitachi S-4000. Heads to be sectioned were fixed and embedded in Spurr’s medium as described previously [Basler and Hafen 1988]. Semithin sections (1 μm) were prepared for light microscopy and examined on a Zeiss Axiophot.

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