Patterning of the R7 and R8 photoreceptor cells of Drosophila: evidence for induced and default cell-fate specification

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SUMMARY

Opsin gene expression in the R7 and R8 photoreceptor cells of the Drosophila compound eye is highly coordinated. We have found that the R8 cell specific Rh5 and Rh6 opsins are expressed in non-overlapping sets of R8 cells, in a precise pairwise fashion with Rh3 and Rh4 in the R7 cells of individual ommatidia. Removal of the R7 cells in sevenless, boss or sina mutants, disrupts Rh5 expression and dramatically increases the number of Rh6-expressing R8 cells. This suggests that the expression of Rh5 may be induced by an Rh3-expressing R7 cell, whereas Rh6 expression is most likely a default state of the R8 cell. We found that the paired expression of opsin genes in the R7 and R8 cells occurs in a sevenless and boss independent manner. Furthermore, we found that the generation of both Rh3- and Rh4-expressing R7 cells can occur in the absence of an R8 cell. These results suggest that the specification of opsin expression in the R7 cells may occur autonomously, whereas the R7 photoreceptor cell may be responsible for regulating a binary developmental switch between induced and default cell-fates in the R8 cell.

Key words: Retina, Drosophila melanogaster, Photoreceptor, Cell-fate determination, Rhodopsin, Opsin, Cell patterning

INTRODUCTION

One of the major challenges in developmental biology is to understand how a complex morphological pattern is generated. Pattern formation relies on cell lineage dependent mechanisms as well as signaling between cells. The ommatidial pattern of the Drosophila compound eye is one of the most thoroughly studied systems for elucidating the mechanism of pattern formation. Many aspects of compound eye development in Drosophila are dependent on cell-cell interactions, and mosaic analysis has shown that photoreceptor cell recruitment is independent of lineage (Ready et al., 1976; Lawrence and Green, 1979). One of the best characterized events in ommatidial assembly is R7 photoreceptor cell recruitment, in which a signal from the R8 photoreceptor induces a bipotential precursor to become an R7 cell rather than a non-neuronal cone cell. Here we report our studies of a novel inductive signal from the R7 photoreceptor cell that is responsible for a binary switch between the expression of two different opsins in the R8 cell.

The compound eye of Drosophila contains approx. 750 ommatidia arranged in a repetitive hexagonal array. Each ommatidium is an assembly of 12 accessory cells and eight photoreceptor cells (R cells) (Hardie, 1986; Wolff and Ready, 1993). The photoreceptor cells form rhabdomeres which project into the intraommatidial cavity and are arranged in a trapezoidal pattern (Fig. 1A,B). Six genes encoding the opsins expressed in the adult fly have been isolated and characterized. The sinaE gene (Rhodopsin 1, Rh1) is expressed in the R1-R6 cells which form the peripheral rhabdomeres that surround the central rhabdomeres of the R7 and R8 photoreceptor cells (Scavarda et al., 1983; O’Tousa et al., 1985; Zuker et al., 1985; Feiler et al., 1988). The Rb2 opsins are expressed in the ocelli, simple eyes located on the vertex of the head, and may also be expressed in the male testes (Cowman et al., 1986; Feiler et al., 1988; Pollock and Benzer, 1988; Alvarez et al., 1996). The Rh3 and Rh4 opsins are expressed in non-overlapping sets of R7 cells (Fryxell and Meyerowitz, 1987; Montell et al., 1987; Zuker et al., 1987; Feiler et al., 1992). Rh3 is also expressed in a specialized class of R8 cells along the dorsal margin of the eye (Fortini and Rubin, 1990; Feiler et al., 1992).

We and others have identified Rh5 and Rh6, two novel Drosophila opsins that are expressed in subsets of R8 cells (Chou et al., 1996; Huber et al., 1997; Papatsenko et al., 1997). The expression of Rh5 in the R8 cell of an individual ommatidium is strictly coordinated with the expression of Rh3 in the R7 cell of the same ommatidium, at both the protein and transcript level (Chou et al., 1996; Papatsenko et al., 1997). Likewise it has been proposed that the expression of Rh6 in R8 cells may be paired with the expression of Rh4 in the R7 cells...
of the same ommatidia (Huber et al., 1997). The paired expression of the opsin genes in the R7 and R8 photoreceptor cells of individual ommatidia is likely to result from a specific developmental signal. Because the expression of the Rh5 transcript and protein are disrupted in *sevenless* (*sev*) mutant flies, we propose that the signal responsible for the patterning of R7 and R8 cells may arise in the R7 photoreceptor cell.

The Rh5 opsin has been a useful cell-type-specific-marker for the examination of R7 and R8 photoreceptor cell patterning, and the cloning of the gene encoding Rh6 provides an opportunity to examine other aspects of this system. In this study, we found that the expression of Rh6 in the R8 cell of an individual ommatidium is faithfully coordinated with the expression of Rh4 in the R7 cell. We also found evidence that the regulation of Rh5 versus Rh6 expression in R8 cells is dependent upon the presence of the R7 cell, and that the coordinated expression of visual pigment genes within an individual ommatidium is not dependent upon *sev* or *boss*. We propose that the expression of Rh5 is an induced state, and that in the absence of an Rh3-expressing R7 cell, the R8 cell assumes a default state and expresses Rh6. Our results provide strong evidence for the presence of a novel developmental signal from the R7 photoreceptor cell that is responsible for regulating opsin gene expression in the R8 cell.

**RESULTS**

**Rh6 is expressed in a subset of R8 photoreceptor cells**

To determine the expression pattern of the Rh6 opsin, we generated a polyclonal antiserum against the C-terminus of the Rh6 protein. Immunofluorescence analysis showed that Rh6 is localized to a subset of rhabdomeres that extend from the middle of the retina to the level of the lamina (Fig. 1C). Double labeling with antibodies against both Rh6 and Rh1 also showed that the Rh6 protein is restricted to the proximal retina, and that Rh6 is only expressed in a subset of ommatidia (Fig. 1D). This result was confirmed by preparing dissociated ommatidia from adult fly heads and labeling them with antibodies against Rh6 and Rh1. As Fig. 1E shows, the R8 rhabdomere that contains Rh6 is located basally within the ommatidium and is surrounded by the rhabdomeres of the Rh1-expressing R1-6 cells. We found no evidence of Rh6 expression in other regions of the head, retina, or in the ocelli (data not shown). These findings indicate that Rh6 is expressed specifically in a subset of R8 photoreceptor cells.

**Rh6 expression in R8 cells is coordinated with the expression of Rh4 in adjacent R7 cells of individual ommatidia**

Previous studies have shown that the Rh3 and Rh4 visual pigments of the R7 photoreceptor cells are expressed in a non-overlapping pattern (Montell et al., 1987; Feiler et al., 1992). To determine whether Rh5 and Rh6 are expressed in a similar manner, we performed transmission immunoelectron microscopy on cross sections taken from the basal retina. We found that Rh5 and Rh6 are localized to the R8 rhabdomeres of different ommatidia (Fig. 2). Similarly, in double labeling experiments performed on frozen sections (Fig. 3A) and on individual dissociated ommatidia (Fig. 3D), the Rh5 and Rh6 opsins are expressed in non-overlapping subsets of R8 photoreceptor cells. 29% of ommatidia expressed Rh5 and 71% expressed Rh6 (see Table 1).

To characterize the expression pattern of Rh6 in relation to
other known opsins, we examined frozen sections from the retinas of white-eyed flies using immunohistochemistry with antibodies directed at Rh3, Rh4 and Rh6. As shown in Fig. 3B, double labeling with antibodies against Rh3 and Rh6 illustrates that the R7 and R8 cells labeled by these reagents are not paired in most cases, suggesting that Rh3 and Rh6 are expressed in different ommatidia. Conversely, when labeling with antibodies against Rh4 and Rh6 there is a precise pairing of Rh4 and Rh6 within the R7 and R8 cells of an ommatidium (Fig. 3C). This result suggests that Rh4 and Rh6 are expressed in a paired manner similar to Rh3 and Rh5.

To investigate whether or not the paired expression of Rh4 and Rh6 in the R7 and R8 cells of individual ommatidia is a uniform occurrence throughout the retina and not an artifact of sectioning, we performed double labeling experiments of dissociated ommatidia using antibodies against Rh3 and Rh6. As Fig. 3E shows, Rh3 and Rh6 are expressed in the R7 and R8 cells of different ommatidia in most cases (upper two quadrants of Fig. 3E, see also Table 1). Two exceptional classes of ommatidia were also noted. The lower left quadrant of Fig. 3E shows an ommatidium which expresses Rh3 in both the R7 and R8 rhabdomeres. This is likely to be an ommatidium from the dorsal margin of the eye (Fortini and Rubin, 1990; Feiler et al., 1992). The lower right quadrant of Fig. 3E shows a single ommatidium in which Rh3 and Rh6 are expressed in adjacent R7 and R8 photoreceptor cells. This type of ommatidium was fairly rare, and we found only a small number of ommatidia with this staining pattern (Table 1).

Table 1. Antibody labeling of dissociated ommatidia

<table>
<thead>
<tr>
<th>Strain</th>
<th>Percentage of ommatidia labeled with antibodies against specific opsins</th>
<th>Total counted</th>
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<tbody>
<tr>
<td>cn1 bw1</td>
<td>Rh5 29%, Rh6 71%</td>
<td>214*</td>
</tr>
<tr>
<td></td>
<td>Rh3 alone 31%, Rh6 alone 53%, Rh3‡,§ Rh3 and Rh6 6%</td>
<td>240</td>
</tr>
<tr>
<td>cn1 bw1</td>
<td>Rh4 and Rh6 96%, Rh6 alone 1.4%, Rh4 alone 0.3%, Rh4‡ 0.3%</td>
<td>274¶</td>
</tr>
<tr>
<td></td>
<td>Rh5 12%**, Rh6 86%, unlabeled 2%</td>
<td>597</td>
</tr>
<tr>
<td>w; sev/4</td>
<td>Rh5 10%**, Rh6 81%, unlabeled 9%</td>
<td>268</td>
</tr>
<tr>
<td>w; boss/cu</td>
<td>Rh3 4%§, Rh6 90%, Rh3 and Rh6 0.4%, unlabeled 6%</td>
<td>242</td>
</tr>
</tbody>
</table>

*Includes only ommatidia labeled with either Rh5 or Rh6 antibody.
‡Labeling in both R7 and R8.
§Rh3-labeled R8 cells are probably from the dorsal margin.
¶Includes only ommatidia labeled with Rh4 and/or Rh6 antibody.
**2/3 of the ommatidia labeled with Rh5 antibody were aberrantly positioned.
**Fig. 2.** Localization of Rh5 and Rh6 to the rhabdomeres of the R8 photoreceptor cells. In tissue sections cut from the basal region of the retina, the rhabdomeres of the R8 cells can be seen projecting between those of R1 and R2. Immunoelectron microscopy of retinas from white eyed flies (w) was performed to determine the subcellular distribution of Rh5 (A, and at higher power in B) and Rh6 (C, and at higher power in D). In both cases there is specific labeling of the rhabdomere of the R8 photoreceptor cell. Anti-Rh5 (mouse monoclonal) labeling was detected with a 1 nm gold-coupled secondary antibody, and anti-Rh6 (rabbit polyclonal) was detected with 10 nm gold-coupled secondary antibody. A control retina incubated without primary antibodies but with both secondary antibodies fails to label the retina (E), whereas anti-Rh1 (rabbit-polyclonal detected by 10 nm gold-coupled secondary) specifically labels the R1-6 rhabdomeres which express the Rh1 visual pigment (F). Scale bars in B, D and F correspond to 0.5, 0.5 and 1.0 μm respectively.

**Fig. 3.** Paired expression of Rh4 and Rh6 in adjacent R7 and R8 cells within the same ommatidia. In longitudinal sections of cn bw retinas, Rh5 and Rh6 are expressed in non-overlapping sets of R8 cells (A). Rh3 and Rh6 are expressed in the R7 and R8 cells of different ommatidia (B), whereas Rh4 and Rh6 appear to be expressed in adjacent R7 and R8 cells in the same ommatidia (C). This observation was confirmed in dissociated ommatidia where we found that Rh5 and Rh6 are expressed in the R8 cells of different ommatidia (D). Likewise, Rh3 and Rh6 are expressed in different ommatidia in most cases (E, two upper quadrants). In the lower left quadrant of E, an ommatidium with labeling against Rh3 in both the R7 and R8 cell is shown. The lower right quadrant of E shows a single ommatidium in which Rh3 and Rh6 are expressed in the R7 and R8 cells, respectively, of the same ommatidium. F shows numerous examples of the normal paired expression of Rh4 and Rh6 in the R7 and R8 cells of individual ommatidia. Scale bar in F, 25 μm.
Dissociated ommatidia double labeled with antibodies against Rh4 and Rh6 are shown in Fig. 3F. In virtually every ommatidium which expresses Rh4 in the R7 cell, Rh6 is expressed in the R8 cell. With the few exceptions noted above and in Table 1, Rh6 is expressed in a non-random manner, in a subset of R8 cells in ommatidia that contain Rh4-expressing R7 cells. This indicates that over most of the compound eye the R7 and R8 cells of individual ommatidia exist as ‘even’ (Rh4/Rh6 expressing) or ‘odd’ (Rh3/Rh5 expressing) pairs. These appear to correspond to the R7 yellow (R7y) and R7 pale (R7p) types of ommatidia, respectively, that have been described in *Calliphora*, *Musca* and *Drosophila* (Kirschfeld et al., 1978; Franceschini et al., 1981; and reviewed by Chou et al., 1996).

In the absence of R7, the R8 cells assume a default state and express Rh6.

If an inductive event between the R7 and R8 cells is responsible for the coordinated expression of the opsin genes in these two cells, then we would predict that removal of the R7 cell might have an effect on the expression of opsin genes in the R8 cell. To test this hypothesis, we previously examined the expression of Rh5 in *sev* mutant flies, that lack R7 photoreceptor cells, and found that Rh5 expression is disrupted (Chou et al., 1996). This result suggested that the expression of Rh5 in a subset of R8 cells is likely to be an induced event that is dependent upon a signal from an Rh3-expressing R7 cell.

To determine whether the expression of Rh6 within the R8 cell is also an induced event, or whether there is a default pathway of opsin specification in the R8 cell, we examined the expression of Rh6 in *sev* mutant flies. Interestingly, while the R8 cells of normal flies express either Rh5 or Rh6 (Fig. 4A), almost all R8 cells express Rh6 in *sev* flies (Fig. 4B, Table 1). Our finding that the proportion of R8 cells expressing Rh6 increases dramatically in *sev* flies suggests that in the absence of an R7 cell, or an Rh3-expressing R7 cell, the R8 cell assumes a ‘default’ fate to become an Rh6-expressing R8 cell. Curiously, we found that Rh5 expression was not completely eliminated in *sev* flies. We found rare examples of Rh5-containing cells in some sections (indicated in Fig. 4B), and many of these rhabdomeres were either longer than normal or aberrantly localized to the apical region of the retina. Although there are no Rh3-expressing R7 cells in the ommatidia in which these rare Rh5-expressing cells are found, there must be some mechanism responsible for their induction.

To determine whether the changes in the expression of the Rh5 and Rh6 proteins in *sev* mutant flies is reflected in the steady-state transcript levels of the genes, we compared the level of mRNAs in wild-type and *sev* mutant flies. As shown in the left three lanes of Fig. 5, the transcripts for both Rh5 (upper panel) and Rh6 (middle panel) were detectable in mRNAs prepared from the heads of wild-type (*cn bw*) flies. No transcript for either gene detectable in the heads of *eya* flies, which lack retinal structures (Sved, 1986), consistent with both Rh5 and Rh6 being expressed specifically in the retina in adult flies. The blots also show that the Rh5 transcript is dramatically reduced in *w; sev* mutant flies that lack R7 cells, whereas the Rh6 gene is still expressed in this mutant.

Fig. 4. Loss of R7 photoreceptor cells disrupts Rh5 and Rh6 expression. Compared to the normal pattern of Rh5 and Rh6 expression (A), mutants which lack R7 cells show a dramatic reduction in the number of cells expressing Rh5, whereas Rh6 is expressed in virtually every R8 cell. This occurs in *sev* (B), *boss* (C) and *sina*/sina* mutants (D). In each of these mutant backgrounds, the expression of Rh5 is not entirely eliminated. There are rare examples of Rh5 expression in cells that have abnormally long rhabdomeres (B, arrow), as well as those that have rhabdomeres that are displaced into an apical position (B, arrowhead). The morphology of the Rh6-expressing cells is similarly affected (readily apparent in B, C and D).

Fig. 5. Regulation of Rh5 and Rh6 mRNA expression. Northern analysis revealed that the Rh5 (upper panel) and Rh6 (middle panel) genes are transcribed as 1.4 and 1.35 kb mRNAs, respectively. The lower panel shows the 0.6 kb transcript of RP 49, which was used as a loading control (O’Connell and Rosbash, 1984). Both the Rh5 and Rh6 transcripts are present in the heads (H) but not the bodies of white eyed *cn bw* flies. There is no transcript for either gene detectable in the heads of *eya* flies, which lack retinal structures (Sved, 1986), consistent with both Rh5 and Rh6 being expressed specifically in the retina in adult flies. The blots also show that the Rh5 transcript is dramatically reduced in *w; sev* mutant flies that lack R7 cells, whereas the Rh6 gene is still expressed in this mutant.
transcripts were observed in mRNAs prepared from the bodies of these animals, or from the heads of *eyes absent* (*eya*) mutant flies, which lack retinal tissues (Sved, 1986). This retinal specific expression is consistent with both Rh5 and Rh6 encoding visual pigments of the compound eye. In mRNAs prepared from the heads of sev*14* mutant flies, the level of Rh5 transcript, but not the Rh6 transcript, was dramatically reduced. These results suggest that the alterations in the expression of the Rh5 opsin in sev*14* mutants are likely to be regulated at the level of transcription.

The normal expression pattern of Rh5 and Rh6 is dependent on the presence of the R7 cell, not the sev or boss proteins

To determine whether the change in R8 cell fate is due to the lack of the R7 cell or to the lack of the sev protein itself, we first examined frozen sections taken from boss and seven in absentia (*sina*) mutant flies (Reinke and Zipursky, 1988; Carthew and Rubin, 1990). We found that in both boss*1* (Fig. 4C) and sina*2*/sina*3* (Fig. 4D) mutants Rh5 expression is disrupted, whereas the proportion of R8 cells expressing Rh6 is substantially increased in a manner identical to that seen in sev*14* mutants. This result was confirmed by examining dissociated ommatidia of boss*1* mutant flies (Table 1). These results are consistent with our observations in sev mutant flies, and provide additional support for the presence of a default state. In addition, these experiments indicate that in the absence of a fully differentiated R7 cell, the presence of sev (in boss mutants), boss (in sev mutants) or both sev and boss (in sina mutants) is insufficient to rescue normal Rh5 expression.

To determine whether sev is required for the expression of Rh5, we examined mutants in which the R7 cells were rescued in a sev-independent manner. Ras1 is an essential component of the sev signaling cascade, and it has been shown that the expression of an activated form of this molecule (Ras1V12) in R7 cell precursors is sufficient to rescue R7 cell formation in sev mutant flies (Fortini et al., 1992). As Fig. 6A shows, we found that when Ras1V12 expression is driven by the sev enhancer in a sev*14* mutant background, Rh5 expression was restored. Rh5 expression in this mutant background occurred predominantly in a normal paired manner beneath the rhabdomeres of Rh3-expressing R7 cells. Similar experiments performed in a boss*1* mutant background yielded identical results (data not shown). These findings indicate that there is no specific requirement for the sev or boss proteins in inducing the expression of the Rh5 opsin in R8 cells. To determine whether the pairing of opsin expression in the R7 and R8 cells is precisely maintained in sev*14* mutants when the R7 cells are rescued by the expression of Ras1V12, we examined opsin expression at the level of dissociated ommatidia. We found that in most cases, Rh3-expressing R7 cells pair with Rh5-expressing R8 cells (Fig. 6B), and likewise for cells expressing Rh4 and Rh6 (data not shown). However, there are some examples where Rh3 and Rh6 are found in the same ommatidium (Fig. 6C), and rare instances where Rh4 and Rh5 are also expressed in the same ommatidium (Fig. 6D). In the later case, although Rh4 and Rh5 are expressed in the same ommatidium, the rhabdomeres containing Rh4 and Rh5 are not arranged in tandem as we have observed in the normal flies.

This abnormal patterning may be explained by the formation of multiple R7 cells in individual ommatidia, which has been observed in sev mutants expressing the Ras1V12 transgene (Fortini et al., 1992). Flies ectopically expressing seven-up (*svp*) or boss have also been shown to generate multiple R7 cells within individual ommatidia. These ommatidia appear to contain both Rh3 and Rh4-expressing R7 cells as indicated by Rh4-lacZ transgene expression (Van Vactor et al., 1991; Hiromi et al., 1993). To determine whether the 'unpaired' expression of Rh3 and Rh6 or Rh4 and Rh5 in some ommatidia was due to the presence of multiple R7 cells expressing Rh3 and Rh4...
in different cells within the same ommatidium, we performed additional double and triple labeling experiments on dissociated ommatidia from this genetic background. We found that most ommatidia contain single R7 cells that produce either Rh3 or Rh4 (Fig. 6E left of panel). In ommatidia containing multiple R7 cells, we found examples where the R7 cells either have the same or different opsins (Fig. 6E, middle and right of panel respectively). In triple labeling experiments, we found ommatidia expressing Rh4 in one or both R7 cells that are paired with Rh6 expression in the R8 cell (Fig. 6F, two ommatidia on left). In ommatidia containing both Rh3- and Rh4-expressing R7 cells, the R8 cells express either Rh6 (Fig. 6F, three right ommatidia) or Rh5 (Fig. 6G). These results suggest that the geometry of the R7 and R8 cells with respect to each other may play a role in determining whether the R8 cell will express Rh5 or Rh6. This result is completely consistent with the idea that an intercellular signal from the R7 cell is involved in regulating opsin gene expression in the R8 cell.

The expression of Rh3 and Rh4 in the R7 photoreceptor cells is not dependent on the presence of the R8 photoreceptor cells

From the evidence presented above we propose that a novel, and as yet unknown, developmental signal from the R7 cell regulates a binary switch between the expression of Rh5 and Rh6 in the R8 cells, and that in the absence of such a signal the R8 cell adopts a default fate and expresses Rh6. An immediate question raised by this model concerns the mechanism by which opsin expression in the R7 cells is specified. The finding that both Rh3 and Rh4-expressing R7 cells can be generated within a single ommatidium in sev-RasVAl12 flies suggests that the specification of opsin expression in the R7 photoreceptor cell may occur autonomously (Fig. 6E-G). If the R7 cell’s opsin expression is indeed autonomous and independent of the R8 cell, we would predict that removal of the R8 cell would not affect the ability of R7 to assume either an Rh3- or Rh4-expressing state. However, because the R8 photoreceptor cell is essentially the founder cell of the developing compound eye, and is the source of an inductive signal (boss) that is required for R7 photoreceptor cell recruitment, this approach is problematic. Nonetheless, previous studies have shown that ectopic expression of svp in the R8 cells, at a time following the induction of the R1-6 cells but prior to R7 cell recruitment, leads to the generation of ommatidia that lack both central photoreceptor cells (R7 and R8) (Hiromi et al., 1993; Kramer et al., 1995). These studies have suggested an alternative means to generate flies having ommatidia that lack only the R8 cells, by introducing the sev-RasVAl12 transgene to rescue the R7 cells in the background of flies expressing svp1 under the control of scabrous (sca).

The R7 and R8 cells are distinguishable within the ommatidium based upon their position and the visual pigments they express. In the apical region of the retina in wild-type flies, the rhabdomeres of the R7 cells project from the photoreceptor cell body between the rhabdomeres of the R1 and R6 cells (Figs 7A, 1B). In the basal region of the retina, the rhabdomeres of the R8 cells project between the rhabdomeres of the R1 and R6 cells (Figs 7B, 1B). The R7 and R8 cells are distinguishable within the ommatidium based upon their position and the visual pigments they express. In the apical region of the retina in wild-type flies, the rhabdomeres of the R7 cells project from the photoreceptor cell body between the rhabdomeres of the R1 and R6 cells (Figs 7A, 1B). In the basal region of the retina, the rhabdomeres of the R8 cells project between the rhabdomeres of the R1 and R6 cells (Figs 7B, 1B). The R7 and R8 cells are distinguishable within the ommatidium based upon their position and the visual pigments they express. In the apical region of the retina in wild-type flies, the rhabdomeres of the R7 cells project from the photoreceptor cell body between the rhabdomeres of the R1 and R6 cells (Figs 7A, 1B). In the basal region of the retina, the rhabdomeres of the R8 cells project between the rhabdomeres of the R1 and R6 cells (Figs 7B, 1B). Flies lacking both central photoreceptor cells were generated using the GAL4/UAS system, by crossing flies carrying the sca-GAL4 transgene to those carrying the UASG-svp1 fusion gene (Brand et al., 1994; Kramer et al., 1995). As shown in both...
apical and basal sections (Fig. 7C,D respectively) all of the ommatidia in *sca-GAL4/UAsg-svp1* flies lack both central R7 and R8 photoreceptor cells. Some ommatidia also lack one or two outer photoreceptor cells, and some have an extra outer photoreceptor cell. As would be expected in animals that lack all of the central R7 and R8 photoreceptors, Rh3 and Rh4 expression are absent (Fig. 7G), and Rh5 and Rh6 expression are absent as well (Fig. 7H).

To test whether the Rh3- and Rh4-expressing states of the R7 cell can be acquired in the absence of the R8 cell, we introduced the *sev-Ras1Val12* transgene into the *sca-GAL4/UAsg-svp1* background. As expected, in *Ras1Val12/sca-GAL4/UAsg-svp1* flies the formation of the R7 cells was rescued (Fig. 7E). In cross sections through the apical retina, we observed numerous examples of central photoreceptor cells having small rhabdomeres, several examples of ommatidia with multiple central photoreceptor cells, and some ommatidia in which the R7 cell had not been rescued. Although the ommatidial morphology is somewhat irregular in this genotype due to the presence of extra R7 cells, as well as the gain or loss of some outer photoreceptor cells, we found numerous examples of ommatidia having normal morphology in which the R7 rhabdomeres projected between those of R1 and R6. Interestingly, in the basal region of the retina we also found numerous examples of central photoreceptor cells (Fig. 7F). In morphologically normal ommatidia, the rhabdomeres of the central photoreceptor cells were also projected between those of R1 and R6 in many cases, suggesting that the rhabdomeres of the R7 cell may extend into the basal region of the retina in the absence of R8, in a manner similar to that observed above (Fig. 6D,G). Consistent with this assumption, we found that sections of the retinas of these animals failed to label with antibodies against Rh5 and Rh6 (Fig. 7I). Furthermore, we found that both Rh3- and Rh4-expressing R7 cells are present in these animals, and that many of their rhabdomeres do indeed extend into the basal region of the retina (Fig. 7I). These results provide very strong evidence that at the time of R7 cell induction, the presence of the R8 cell is not required for the generation of the two types of R7 cells. These findings are consistent with the idea that while opsin specification in the R8 cell expresses Rh6. An immediate question raised by these results is the nature of this novel signaling pathway and the identity of the molecules that mediate it. As we have described in this paper, it is quite clear that neither sev nor boss are required for the establishment of the paired expression of visual pigments in the R7 and R8 cells, and that the presence of either boss or sev or both is insufficient to restore Rh5 expression in the absence of an R7 cell. These results indicate that there must be other novel signaling molecules that are responsible for patterning of the R7 and R8 cells.

In the course of these experiments, we have observed a number of apparent exceptions to the predominant even or odd pairing of opsins expressed in the R7 and R8 photoreceptor cells. In particular, we found instances of expression of Rh3 and Rh6 in the R7 and R8 cells of individual ommatidia (Fig. 3E and Table 1), occurring in 6% of cases. Because Rh5 is not expressed in these R8 cells, and because Rh5 expression appears to be an induced state, it may be that the geometry of the R7 and R8 cells in these exceptional ommatidia was somehow abnormal at the time when this signal is required. Thus, these R8 cells may have failed to receive the inductive signal, and then assumed the Rh6-expressing default state. Consistent with this, studies of ommatidia having multiple R7 cells (Figs 6D-G) seem to support the idea that the signal to induce Rh5 expression must be highly spatially restricted. Additionally, we have never observed ommatidia from wild type strains that showed paired expression of Rh4 and Rh5. This suggests that while the inductive signal may fail in some rare cases, Rh5 can never be induced in a normal eye by an Rh4-expressing R7 cell because it does not transmit the inductive signal. Curiously, in mutant strains that lack R7 cells, Rh5 expression is not completely abolished (Fig. 4B). Many of these Rh5-expressing cells are morphologically abnormal, in that they have long rhabdomeres or rhabdomeres in an apical position. Such photoreceptor cells have been observed previously in *sev* mutants and shown to be R8 cells, based upon their axonal projections and the timing of their terminal mitoses (Campos-Ortega et al., 1979). Perhaps these cells are receiving or improperly processing an inductive signal, based on their abnormal position within the eye. This result does show however, that Rh5 expression is not absolutely dependent upon the presence of the R7 cell. The signal used for the induction of Rh5 could be used elsewhere within the eye for other purposes, or the signal from the R7 cell may not be direct. Identification of the genes required to establish R7 and R8 cell patterning should allow us to resolve some of these questions.

In this paper, we have shown that Rh6 is expressed in a subset of R8 photoreceptor cells, and that its expression is coordinated with the expression of Rh4 in the R7 cell of the same ommatidium. This patterning of opsin expression in the R7 and R8 cells is similar to what we and others have observed for the expression of Rh3 and Rh5 (Chou et al., 1996; Papatsenko et al., 1997) As we have demonstrated, this unique patterning is dramatically altered in mutants that lack R7 cells, resulting in the disruption of Rh5 expression and the expression of Rh6 in a dramatically increased proportion of R8 cells. These results are consistent with a model in which a developmental signal from the Rh3-expressing R7 cell induces the expression of Rh5 in the R8 cell, and represses the expression of Rh6. In the absence of this signal the R8 cell assumes a default state and expresses Rh6. An immediate question raised by these results is the nature of this novel signaling pathway and the identity of the molecules that mediate it. As we have described in this paper, it is quite clear that neither sev nor boss are required for the establishment of the paired expression of visual pigments in the R7 and R8 cells, and that the presence of either boss or sev or both is insufficient to restore Rh5 expression in the absence of an R7 cell. These results indicate that there must be other novel signaling molecules that are responsible for patterning of the R7 and R8 cells.

**DISCUSSION**

In this paper, we have shown that Rh6 is expressed in a subset of R8 photoreceptor cells, and that its expression is coordinated with the expression of Rh4 in the R7 cell of the same ommatidium. This patterning of opsin expression in the R7 and R8 cells is similar to what we and others have observed for the expression of Rh3 and Rh5 (Chou et al., 1996; Papatsenko et al., 1997) As we have demonstrated, this unique patterning is dramatically altered in mutants that lack R7 cells, resulting in the disruption of Rh5 expression and the expression of Rh6 in a dramatically increased proportion of R8 cells. These results are consistent with a model in which a developmental signal from the Rh3-expressing R7 cell induces the expression of Rh5 in the R8 cell, and represses the expression of Rh6. In the absence of this signal the R8 cell assumes a default state and expresses Rh6. An immediate question raised by these results is the nature of this novel signaling pathway and the identity of the molecules that mediate it. As we have described in this paper, it is quite clear that neither sev nor boss are required for the establishment of the paired expression of visual pigments in the R7 and R8 cells, and that the presence of either boss or sev or both is insufficient to restore Rh5 expression in the absence of an R7 cell. These results indicate that there must be other novel signaling molecules that are responsible for patterning of the R7 and R8 cells.

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photoreceptor cell-fate has been specified, this even or odd identity is then communicated to the R8 cell. This putative signal would regulate a binary cell-fate decision in R8 cells, between the proposed induced (Rh5 expressing) and default (Rh6 expressing) states.

Binary cell-fate decisions have been characterized in a number of different systems. In Drosophila, the glial cells missing gene regulates the binary decision between neuronal and glial cell fates (Hosoya et al., 1995; Jones et al., 1995). Similarly, lineage based cell-fate determination may also occur in a binary manner (Jan and Jan, 1998). Likewise, R7 photoreceptor cell recruitment via the boss/sev pathway also serves as a potential example of a binary cell-fate decision (Banerjee and Zipursky, 1990; Zipursky and Rubin, 1994). The main difference between these examples and R8 photoreceptor cell opsin specification is that, in the later case, the two alternative cell-fates are only subtly different from one another. Nonetheless, the regulated expression of different visual pigments in different types of photoreceptor cells has obvious biological importance because this is the basis for color vision in many organisms. As we have discussed previously, physiological studies of Musca and Calliphora suggest that the pairing of different types of R7 and R8 cells may play an important role in tuning photoreceptor spectral sensitivity, by coordinating the expression of a screening pigment in Rh4-expressing R7 cells that serves as an optical filter for Rh6-expressing R8 cells (Kirschfeld et al., 1978; Hardie, 1979; Chou et al., 1996).

The current work has special relevance within the field of photoreceptor cell differentiation, in which there is growing evidence from other experimental systems that supports the idea that local interactions are involved in regulating the expression of visual pigments in specific cells. In studies of both the chicken and goldfish retinas, it has been shown that the expression of a specific opsin in a cell is more closely related to the cell's position than to the cell's birthdate (Bruhn and Cepko, 1996; Stenkamp et al., 1997). These results suggest that extrinsic signals are intimately involved in the determination of photoreceptor cell fate and the specification of photoreceptor cell opsin phenotype. Thus, our finding of a likely inductive signaling mechanism between the Drosophila R7 and R8 photoreceptors, that is involved in the specification of the opsin phenotype, may ultimately provide a means to identify signaling molecules mediating similar signals in other organisms and organ systems.

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