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Cell fate control in the *Drosophila* retina by the orphan receptor seven-up: its role in the decisions mediated by the ras signaling pathway

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SUMMARY

***Drosophila* seven-up is an orphan receptor of the steroid receptor family that is required to specify photoreceptor neuron subtypes in the developing compound eye. Expression of seven-up is confined to four of the eight photoreceptor precursors, R3/R4/R1/R6. We show that misexpression of seven-up in any of the other cell types within the developing ommatidium interferes with their differentiation. Each cell type responds differently to seven-up misexpression. For example, ectopic expression in the non-neuronal cone cells using the *sevenless* promoter/enhancer (*sev-svp*) causes the cone cells to take on a neuronal identity. Ectopic expression of seven-up in R2/R5 using the *rough* enhancer (*ro-svp*) causes these neurons to lose aspects of their photoreceptor subtype identity while remaining neuronal. Each cell type appears to have a different developmental time window that is sensitive to misexpressed seven-up. The temporal order of responsiveness of each cell type to misexpressed seven-up is similar but not identical to the order of neuronal differentiation. This suggests that there are processes of specification that are distinct from the specification to become a photoreceptor neuron. We have identified members of the ras signaling pathway as suppressors of the cone cell to R7 neuron transformation caused by *sev-svp*. Suppression of the *sev-svp* phenotype can be achieved by decreasing the gene-dosage of any of the members of the ras-pathway. This suggests that the function of seven-up in the cone cells requires ras signaling. However, a decrease in ras signaling results in enhancement of the phenotype caused by the *ro-svp* transgene. We discuss the relationship between decisions controlled by seven-up and those controlled by ras signaling.**

Key words: steroid receptor, cell fate specification, *sevenless*, *rough*, ommatidium, *Drosophila*

INTRODUCTION

The steroid receptor superfamily represents the largest known family of eukaryotic transcription factors. This family includes ligand-dependent transcription factors, such as glucocorticoid receptor, thyroid hormone receptor and retinoic acid receptor, as well as a large number of so called 'orphan receptors' whose ligands have not been identified (reviewed by Evans, 1988; Green and Chambon, 1988; Tsai and O'Malley, 1994). Ligand-dependent receptors and orphan receptors share a DNA binding domain, which consists of two zinc fingers, and a larger ligand binding domain, which shows more than 20% identity between different members. The activity of steroid receptors is controlled not only by binding of the ligand, but also by other proteins that physically interact with the receptors (reviewed by Smith and Toft, 1993). Furthermore, posttranslational modifications also play a role in the regulation of steroid receptor action (reviewed by Kuiper and Brinkmann, 1994).

A number of orphan receptors have been identified in *Drosophila*, many of which have been shown to play important roles during development (reviewed by Segraves, 1991, 1994). One of the *Drosophila* orphan receptors is encoded by the *seven-up* (*svp*) gene (Mlodzik et al., 1990b). Two types of cDNAs have been isolated from the *svp* locus. *svp* type 1 cDNA encodes a protein with both the DNA binding domain and the ligand binding domain, whereas svp type 2 diverges from type 1 in the middle of the ligand binding domain. While several members of the *Drosophila* steroid receptor family have vertebrate homologs (reviewed by Segraves, 1994), *svp* is unique in that the degree of conservation is extremely high. Homologs of *svp* have been identified from sea urchin (Chan et al., 1992), zebrafish (Fjose et al., 1993), frog (Matharu and Sweeney, 1992), chicken (Lutz et al., 1994), mouse (Qiu et al., 1994) and human (Miyajima et al., 1988; Wang et al., 1989; Ladias and Karathanasis, 1991). The protein encoded by the *svp* type 1 cDNA shares more than 87% identity in both the DNA binding domain and the ligand binding domain to any of these *svp* homologs. The high degree of sequence conservation in the DNA binding domain suggests that these proteins may bind to similar DNA sequences. An equally high degree of conservation in the ligand binding domain implies that this domain interacts with other molecules that are also conserved between various animal species. Such molecules may include the ligand, other molecules that modulate the activity of the receptor and factors that are engaged in the transcription of the target gene. To understand the role these receptors play in development, it is crucial to understand the functional relationship between the receptor and the modulating activities.

Unfortunately, the characterization of the receptor/modulator relationship in vivo has been hindered by the lack of good tools in higher organisms. *Drosophila* offers a system to identify molecules that interact with svp using genetic approaches.

svp is an essential gene; its null mutations are embryonic lethal with defects in CNS and fat body development (Mlodzik et al., 1990b; Hoshizaki et al., 1994; Y. H. unpublished observation). Its function during development is best understood for the compound eye, where it is involved in the specification of photoreceptor subtypes. The compound eye is composed of approximately 800 unit eyes or ommatidia. Each ommatidium is a stereotyped array of 20 cells, eight photoreceptor neurons, R1-R8, four lens-secreting cone cells and eight other accessory cells. The photoreceptor neurons can be classified into three classes (R1-R6, R7, R8) by the size and position of their light-gathering structures, the rhabdomeres, their spectral sensitivity, and their pattern of synaptic connections (reviewed by Hardie, 1986; Wolff and Ready, 1993). Although the six outer photoreceptor cells R1-R6 have the same morphological and physiological properties, molecular differences during eye development suggest that they can be further subdivided into three classes R2/R5, R3/R4, and R1/R6. Cell type specification occurs during the third larval instar in an initially unpatterned epithelium, the eye imaginal disc. An indentation in the epithelium called the morphogenetic furrow moves over the eye imaginal disc in a posterior to anterior direction. As the furrow passes, undifferentiated cells are recruited into the ommatidial cluster in a sequential manner. Specific cell types differentiate from cells located at stereotyped positions within the cluster (Tomlinson and Ready, 1987). This process is thought to be mediated by contact-dependent induction (reviewed by Cagan and Zipursky, 1992; Wolff and Ready, 1993). The best studied example is the induction of the R7 precursor by a signal from R8. Activation of the sevenless receptor tyrosine kinase by its ligand expressed in R8 triggers the ras signaling pathway, resulting in the neuronal determination of R7 (reviewed by Zipursky and Rubin, 1994). R3/R4 and R1/R6 are thought to be induced by R2/R5 (Tomlinson and Ready, 1987; Tomlinson et al., 1988). The molecular nature of the signals involved in the induction of R3/R4/R1/R6 is not known. During ommatidial assembly, svp is expressed in four of the eight future photoreceptor cells, R3/R4/R1/R6. In *svp* mutant ommatidia, these cells differentiate as another type of photoreceptor neuron, the R7 photoreceptor (Mlodzik et al., 1990b). This suggests that *svp* may act as a genetic switch between photoreceptor subtypes. We have previously shown that ubiquitous expression of svp during eye development causes a wide variety of cell fate transformations (Hiromi et al., 1993). Here we show that different cell types within an ommatidium respond differently to misexpression of svp. We further show that the effect of misexpression of svp can be modified by decreasing the ras signaling activity, suggesting an interaction between ras and svp in mediating cell fate decisions in the compound eye.

MATERIALS AND METHODS

Genetics

The mutagenesis screen for suppressors of the *sev-svp2* phenotype was performed as follows. Males that were isogenic for the second chromosome and the third chromosome carrying the markers *st* and *e* were fed ethyl methanesulfonate as described by Lewis and Bacher (1968). Males were then mated to 2x P[*sev-svp2*]/*TM3,ry* virgin females in bulk. The eyes of male F_1 progeny were screened for a less disorganized appearance of the eyes under the dissection microscope. Approximately 40,000 F_1 progeny were screened. F_1 males that had at least one eye with a smoother appearance were backcrossed to 2x P[*sev-svp2*]/*TM3, ry* virgin females and the appearance of the eye surface was examined again. These individuals were used to map suppressor mutations to a chromosome and balance the mutation. Mutations on the second chromosome were mapped meiotically using the *all* chromosome (*al dp b pr c px sp*). Approximately 100 recombinant chromosomes were scored for each case.

The following mutations and deficiencies were tested for effects on phenotypes caused by the *sev-svp* and *ro-svp* transgenes: csw^{e0P} (formerly $E(sev)1A^{e0P}$), Sos^{e4G}, drk^{e0A}, $hsp83^{e1D}$, $cdc37^{e1E}$, $Ras1^{e2F}$, $E(sev)3D^{e0Q}$ (Simon et al., 1991, 1993; Cutforth and Rubin, 1994; M. Simon, personal communication), $Egfr^{1P02}$ (Nüsslein-Volhard et al., 1984; Schejter and Shilo, 1989), *Df(3R)by10* (deficiency for *Ras1*), *Df(3R)by62* (Kemphues et al., 1980; Simon et al., 1991), $D\text{-}raf^{LQ129}$, $Dsor1^{LF133}$ (T. Schüpbach, personal communication), rl^{15M1} (B. Dickson, personal communication). Markers and other mutations used are described by Lindsley and Zimm (1992).

Strains carrying a GAL4 enhancer trap construct (Brand and Perrimon, 1993) on the autosomes were generated by inducing secondary jumps from X-linked insertions. Their expression patterns were analyzed by crossing to a strain carrying the UAS_G-*lacZ* transgene (Brand and Perrimon, 1993).

Histology and histochemistry

Antibody staining of imaginal discs was performed as described (Tomlinson and Ready, 1987), except that the peripodial membrane was not removed. Affinity-purified rabbit antibody against BarH1/BarH2 proteins (Higashijima et al., 1992) was a kind gift from K. Saigo. Monoclonal antibody against β-galactosidase was purchased from Promega. Monoclonal antibodies against elav protein and boss protein were gifts from the laboratories of G. M. Rubin and L. Zipursky, respectively. Fixation and sectioning of adult heads was performed as described by Tomlinson and Ready (1987). The following *lacZ* enhancer trap lines were used as cell-type-specific markers: X81, an insertion in the *rhomboid* gene, which expresses β-galactosidase in R2/R5 and R8 (Freeman et al, 1992); P82, which expresses β-galactosidase in R3/R4 and R7 (M. Mlodzik and Y. H. unpublished data; see Carthew and Rubin, 1990; Fischer-Vize et al., 1992); and H214, which express β-galactosidase most strongly in the R7 precursor within the mature ommatidial cluster (Mlodzik et al., 1992).

Plasmid construction and P-element mediated transformation

A 2.8 kb enhancer fragment from the *rough* gene, when fused at position –254 to the *hsp70* promoter, has been shown to direct transcription to R2/R5/R3/R4 (Heberlein et al., 1994). We fused *svp* type 1 and type 2 cDNAs to the *rough* enhancer-*hsp70* promoter fusion to generate *ro-svp1* and *ro-svp2* genes, respectively. First, phsp70CaSpeR (Bell et al., 1991), which contains a *hsp70* promoter in the CaSpeR vector was modified to produce a plasmid with a unique *Xba*I site at position –254 of the *hsp70* promoter. A 1.7 kb *Eag*I fragment of pc162.1 and a 2.4 kb *Eag*I-*Cla*I fragment of pc162.2 (Mlodzik et al., 1990b) was cloned into the polylinker region of the modified phsp70CaSpeR vector to generate pWHS162.1X and pWHS162.2X, respectively. pUH22, containing the 2.8 kb *rough* enhancer fragment in the pHSX vector (Heberlein et al., 1994) was linearized by *Xba*I and cloned into the *Xba*I site of pWHS162.1X and pWHS162.2X to generate P-element vectors containing *ro-svp1* and *ro-svp2* genes, respectively. A P-element vector that carries UAS_G-*svp1* was made by cloning a 1.7 kb *Eag*I fragment of pc162.1 into the

*Not*I site of pUAST (Brand and Perrimon, 1993). Germ-line transformation was performed using w^{1118} as the host strain and pπ25.7wc (Karess and Rubin, 1984) as a helper plasmid. Secondary jumps were made using a strain carrying a genomic source of transposase activity (Robertson et al., 1988).

RESULTS

Ectopic expression of seven-up in any photoreceptor cell causes cell fate transformations

A previous study had shown that ubiquitous expression of *svp* under the control of a heat inducible *hsp70* promoter causes a variety of cell fate changes. While expression of svp anterior to the morphogenetic furrow had little effect on ommatidial assembly, ectopic expression in ommatidial clusters located at progressively posterior positions to the furrow produced specific phenotypes depending on the distance from the furrow. These phenotypes included the loss of outer photoreceptor cells, the loss of R8 and R7, formation of extra R7 cells and the transformation of R7 to an outer photoreceptor cell (Hiromi et al., 1993). Although these experiments reveal the developmental period in which an ommatidial cluster is affected by the ubiquitous expression of svp, they do not provide information about which cell is sensitive to the misexpressed svp. We therefore expressed svp under the control of promoters that have spatial specificity within the ommatidium.

R8

We expressed svp in the R8 precursor using a strain harboring an insertion of the GAL4 enhancer trap construct (Brand and Perrimon, 1993) in the *scabrous* locus. This strain allows

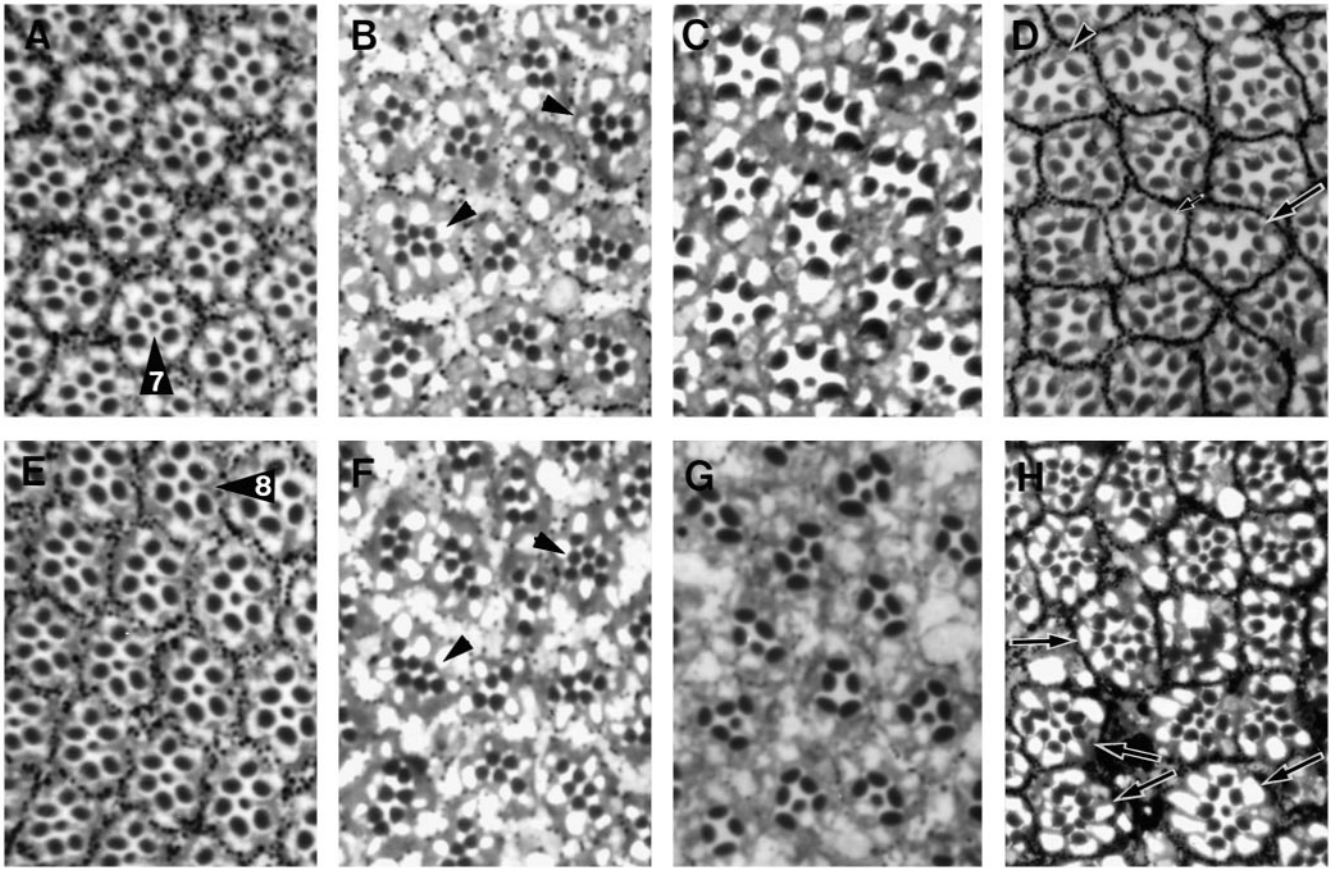

Fig. 1. Ectopic expression of *svp* in all cells of the ommatidium causes cell fate transformations. (A,E) Tangential retinal sections of wild-type animals at the apical (A) and basal level (E). Rhabdomeres of central photoreceptor cells R7 and R8 are indicated. (B,F) Ectopic expression in R8 caused by *sca-GAL4*/UAS_G*-svp1*. The same region in shown for the apical (B) and the basal level (F). All ommatidia lack both R7 and R8. Some ommatidia have an extra outer photoreceptor cell. Two such ommatidia are indicated by arrowheads in the apical (B) and the corresponding basal (F) sections. (C,G) Ectopic expression in R2/R5 caused by one copy of *ro-svp1* (C), and one copy of the *ro-svp2* (G) transgenes. A majority of the ommatidia lack one outer photoreceptor cell. Note that *ro-svp1* and *ro-svp2* eyes contain approximately 5-10% ommatidia that exhibit an extra R7 cell and approximately 5% ommatidia that have no R7 cell. The section of the eye of the *ro-svp2* animal overrepresents the latter class. The R8 cell is present in *ro-svp1* and *ro-svp2* ommatidia (not shown). (D,H) Ectopic expression in the R7 cell and the cone cells caused by the *sev-svp2* transgene. (D) Two copies of *sev-svp2*. Note ommatidia with an extra R7 cell (small arrow), with an extra outer photoreceptor cell (arrowhead) and an ommatidium that has lost the R7 neuron and gained two extra outer photoreceptor neurons (large arrow). (H) Three copies of *sev-svp2*. Ommatidia with eight outer photoreceptor cells are indicated by arrows. These outer photoreceptor neurons extend throughout the depth of the retina and R8 is present at the basal level (not shown). For one copy of *sev-svp2*, see Figure 7A of Hiromi et al. (1993). Sections are at the apical level where R7 is visible unless noted otherwise. Animals shown in B,C,F and G carry P[$white^+$] insertions in the presence of the *white* mutation and have reduced amounts of pigment granules.

expression of a UAS_G linked reporter gene in the R8 cell starting from the morphogenetic furrow (Mlodzik et al., 1990a; Baker et al., 1990). Expression of *svp* type 1 cDNA in the *sca* pattern was achieved by crossing the *sca-GAL4* strain to a strain carrying a *UAS_G-svp1* fusion gene. The majority of *sca-GAL4*/*UAS_G-svp1* individuals died as embryos or larvae, presumably due to the misexpression of svp in the neuronal precursor cells in the embryo (data not shown). A small number of animals that survived to adulthood showed a mild roughening of the eye surface. Sections of such eyes revealed that all ommatidia lacked central photoreceptor cells R7 and R8 (Fig. 1B, F). In addition, some ommatidia lacked one to two outer photoreceptor cells, in extents varying from individual to individual. This phenotype is similar to one of the phenotypes produced by ubiquitous expression of svp using the *hsp70* promoter (Hiromi et al., 1993). We have previously shown that the loss of R7 caused by ubiquitous expression of svp can be explained by a perturbation of R8 development that occurs after the initial specification of R8. To test whether the loss of R8 and R7 in *sca-GAL4*/*UAS_G-svp1* individuals is caused by the same mechanism as the phenotype seen with ubiquitous expression of svp, we examined the expression of the boss protein, the ligand for the sevenless receptor tyrosine kinase, in the eye imaginal disc. In wild-type eye imaginal discs, the boss protein is expressed in R8 from three rows posterior to the morphogenetic furrow to the posterior edge of the disc (Krämer et al., 1991; Van Vactor et al., 1991; Fig. 2A). In *sca-GAL4/UAS_G-svp1* discs, expression of boss was severely affected. The region close to the equator, the dorsoventral midline of the eye, usually entirely lacked boss expression. In the more lateral region, a single row of boss-expressing cells was present near the morphogenetic furrow, indicating that boss expression was initiated, but failed to be maintained (Fig. 2C). In wild-type imaginal discs, the boss protein is internalized into the R7 cell starting in row 6 (Krämer et al., 1991; Cagan et al., 1992), before the sevenless tyrosine kinase activity is required to specify the neuronal fate of R7 (Basler and Hafen, 1989; Mullins and Rubin, 1991). Since boss is necessary as a signal to induce R7, the failure to initiate or maintain boss expression in early stages of ommatidial assembly accounts for the loss of R7. The similarity of the cellular phenotypes observed in *sca-GAL4*/*UAS_G-svp1* animals and with ubiquitous expression of svp strongly suggests that the loss of R8 and R7 observed upon ubiquitous expression of svp is due to misexpression of svp in the R8 cell.

The fate of the R8 cell was studied by examining the expression of the neuron-specific antigen elav (Robinow and White, 1991). Expression of the elav protein in the wild-type disc starts in row 1 in R2/R5, followed by expression in R8, R3/R4, R1/R6, and finally in R7 (Fig. 2B). In *sca-GAL4*/*UAS_G-svp1* discs, elav expression in the R8 cell was indistinguishable from wild-type discs in row 5, and was still detectable in row 7 (Fig. 2D). However, the behavior of the R8 cell was not normal, because its nucleus failed to move basally as it does in normal discs. Since no R8 neuron is present in the adult retina, we assume that the R8 precursor undergoes cell death later in eye development. We cannot, however, exclude the possibility that some R8 precursors differentiate as outer

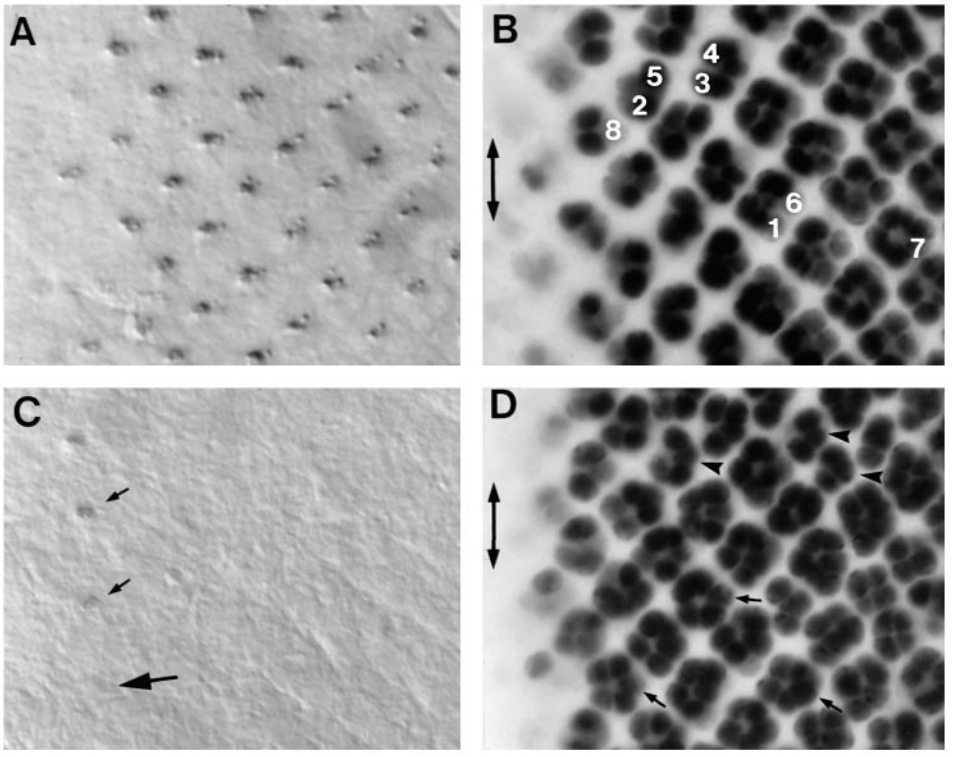

Fig. 2. Effects of misexpression of svp in R8. (A,C) Imaginal discs labeled with anti-boss antibody. (A) A wild-type disc. The morphogenetic furrow is at the left edge of the field of view. Expression of boss is first detected in the apical tip of the R8 cell in row 3 and persists to the posterior edge of the disc. (C) A *sca-GAL4*/*UAS_G-svp1* disc. Expression of boss initiates in row 3 in many of the ommatidial clusters, however, no boss expression is detectable in more posterior rows. A small percentage of R8 cells fail to initiate any detectable boss expression. These cells are usually located in clusters in the central part of the disc. Several boss-positive cells are indicated by small arrows. The large arrow indicates a boss-negative region in row 3. (B,D) Imaginal discs stained with anti-elav antibodies. (B) A wild-type imaginal disc. elav expression begins at the morphogenetic furrow (double headed arrow) in R2 and R5, followed shortly by staining in R8. Staining is then detected in R3/R4, R1/R6 and finally the R7 cell. The identity of each photoreceptor cell is indicated. (D) A *sca-GAL4*/*UAS_G-svp1* disc. The initiation of elav staining in R8 (small arrows) appears normal near the morphogenetic furrow and can be detected posterior to approximately row 7. The nuclei of R8, however, often do not move basally as they do in wild-type discs (compare row 5 in B and D). We could not unambiguously identify the R8 nuclei in ommatidia posterior to row 7. Ommatidia containing outer photoreceptor cell precursors that fail to express the elav antigen are indicated by arrowheads. Posterior is to the right in all panels.

photoreceptor neurons upon misexpression of svp. Indeed, we observed a small fraction of ommatidia that contained seven outer photoreceptor neurons, and lacked both central photoreceptor neurons (Fig. 1B,F). Since we did not observe any extra elav-positive nuclei in the eye imaginal discs, the extra outer photoreceptor does not originate from the cone cells or mystery cells, cells that are present in the early stages of ommatidial assembly but do not differentiate as photoreceptor neurons (Wolff and Ready, 1993).

R2/R5

Among the outer photoreceptor cells all of which have the same physiological properties, R2 and R5 do not express svp. We induced ectopic expression of *svp* type 1 and type 2 cDNAs in R2/R5 using the enhancer element from the *rough* gene. The *rough* enhancer element/*hsp70* promoter fusion drives expression in R2/R5 and in R3/R4 (Heberlein et al., 1994), thus achieving ectopic expression of svp in R2/R5. Several transgenic strains that carry *ro-svp1* or *ro-svp2* transgenes were established. These transgenic strains exhibit a roughening of the eye surface in a gene dosage-dependent manner. Sections of adult eyes of either *ro-svp1* or *ro-svp2* animals showed the loss of one or more outer photoreceptor neurons from a majority of the ommatidia (Fig. 1C,G). As the copy number of the transgenes was increased, there was a decrease in the average number of outer photoreceptor cells per ommatidium, concomitant with an increase in the percentage of ommatidia with a reduced number of outer photoreceptor cells. In addition, a small number of ommatidia contained an extra R7 cell or had lost the R7 cell. Both *ro-svp1* and *ro-svp2* transgenes caused qualitatively similar phenotypes. A higher copy-number of the *ro-svp1* transgene was required, however, to achieve a phenotype that is similar in strength to the phenotype caused by the *ro-svp2* gene.

We examined neuronal differentiation in the *ro-svp* eye imaginal discs using an antibody against elav. The expression pattern of elav in both *ro-svp1* and *ro-svp2* discs was indistinguishable from wild-type discs up to row 4. In the five cell precluster, however, a small percentage of the clusters contained only four elav-positive cells, instead of the normal five. This suggested that one cell of the R3/R4 pair failed to initiate neuronal differentiation. In subsequent stages, a

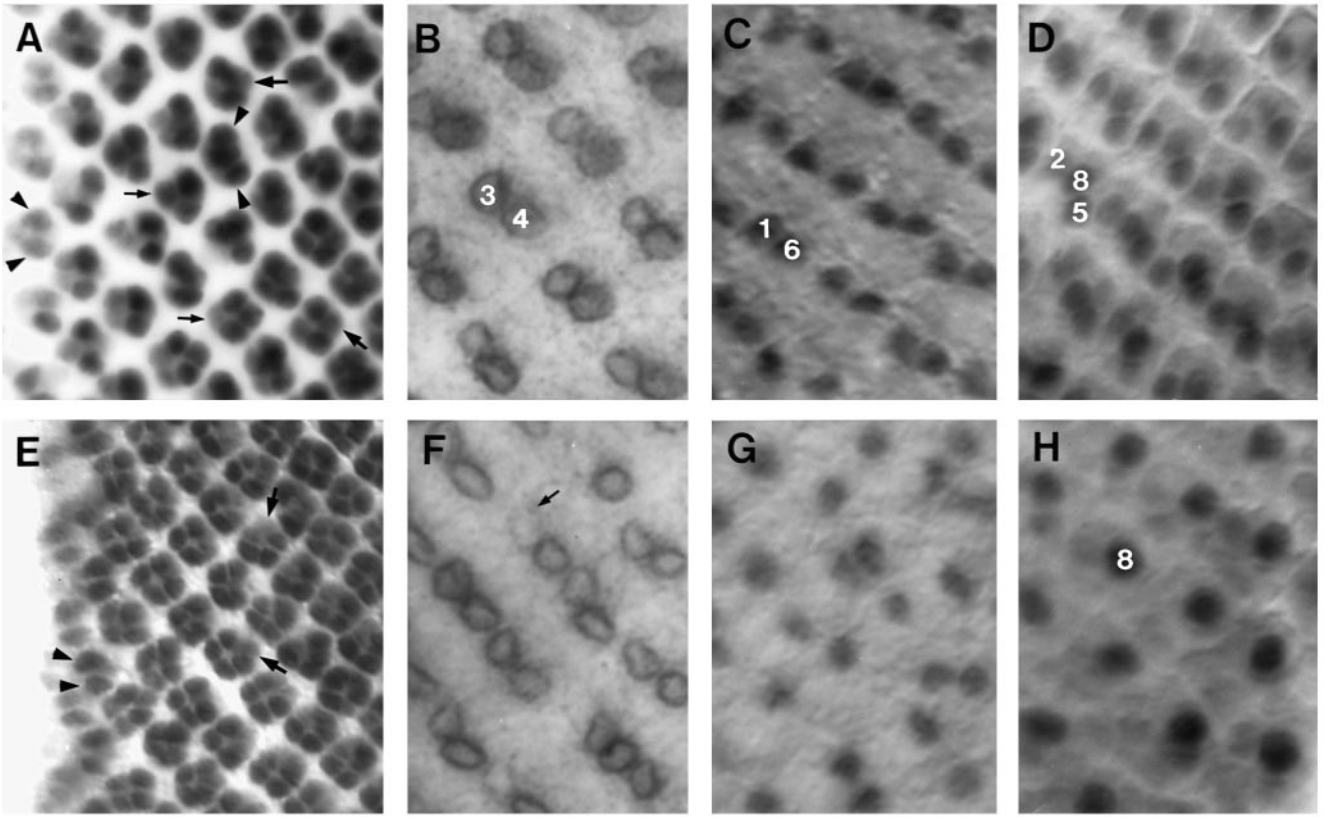

Fig. 3. The fates of the outer photoreceptor cells in *ro-svp* animals. Expression of molecular markers for eye imaginal discs of third instar larvae of wild-type animals (B-D), animals expressing one copy of *ro-svp2* (A) or two copies of *ro-svp1* (E-H). Anti-elav staining of *ro-svp2* (A) and *ro-svp1* (E) eye imaginal discs. Refer to Fig. 2B for a wild-type control. Expression of elav initiates normally in R2/R5 (arrowheads) in *ro-svp1* (E) as well as *ro-svp2* (A) eye discs. The nuclei of R2/R5 fail to descend to the basal level in the five-cell precluster (arrowheads in A). In *ro-svp1* eye discs (E) one cell of the R1/R6 cell pair frequently fails to express elav (large arrow). In *ro-svp2* eye discs (A) one cell of R3/R4 cell pair (small arrow) and/or one cell of the R1/R6 cell pair (large arrow) frequently fail to express elav. (B,F) Expression of the R3/R4-specific enhancer trap insertion P82 in wild type (B) and *ro-svp1* eye discs (F). Note the absence of β-galactosidase expression in one cell of the R3/R4 cell pair in some clusters (arrow). (C,G) Expression of the R1/R6-specific BarH1/H2 antigen in wild-type (C) and *ro-svp1* eye discs (G). A majority of clusters contain only one cell that expresses the BarH1/H2 antigen. (D,H) Anti-β-galactosidase staining of eye imaginal discs carrying enhancer trap insertion X81. β-galactosidase is expressed in R8, R2 and R5 in wild-type eye discs (D). β-galactosidase expression in R2 and R5 is absent in *ro-svp1* eye discs (H). Posterior is to the right in all panels.

majority of the ommatidial clusters showed only one elav-positive cell in the position of R1/R6 (Fig. 3A,E). Thus, the reduction in the number of outer photoreceptor cells seen in the adult retina is likely to be due to a failure to initiate neuronal differentiation, rather than cell death or degeneration occurring later in development.

Since svp is expressed ectopically in R2/R5 in *ro-svp* animals, it was rather surprising that the effect on neuronal differentiation appeared to take place in R1/R6 and R3/R4, rather than R2/R5. To confirm the identities of the affected photoreceptor cells, we examined the expression patterns of molecular markers that are specifically expressed in the R1/R6 and in the R3/R4 cell pair, respectively. The fate of the presumptive R3/R4 cell pair was examined using enhancer trap line P82 which expresses β-galactosidase in R3/R4 and R7. A small proportion of the ommatidial clusters in *ro-svp1* or *ro-svp2* discs contained only one β-galactosidase-positive cell in the position of the R3/R4 precursors (Fig. 3B,F). The frequency of such clusters, however, was far too small to account for the missing outer photoreceptor cells observed in adult retinae. The differentiation of R1/R6 was monitored using the BarH1/H2 antigen as a marker, which is expressed in the R1/R6 cell pair but not in other photoreceptor cells (Higashijima et al., 1992). A majority of the ommatidial clusters in the eye discs of *ro-svp* larvae contain only one cell that expresses BarH1/H2 (Fig. 3C,G). For example, in an eye disc from an animal with two copies of the *ro-svp1* transgene, approximately 90% of the ommatidial clusters contained one BarH1/H2-positive cell while the remaining 10% contained two BarH1/H2-positive cells. The frequency of ommatidia that contain only one BarH1/H2-positive cell correlates well with the percentage of the ommatidia that show a reduction in the number of outer photoreceptor cells in adult retinae. The frequency of photoreceptor precursors that fail to express the P82 marker correlates with the percentage of the ommatidia that lack two outer photoreceptor neurons in the retina. We conclude that the majority of the photoreceptor cells that are missing in *ro-svp* retinae are of the R1/R6 subtype, with a smaller percentage of the R3/R4 subtype also failing to differentiate. This indicates that *ro-svp* expression produces an indirect effect on the differentiation of R1/R6 through misexpression in R2/R5. Loss of R3/R4 could be due to either a nonautonomous effect through R2/R5 or to overproduction of svp in R3/R4.

To examine the effect of *ro-svp* transgenes on the differentiation of R2/R5, we used an enhancer trap line, X81, that shows specific expression of β-galactosidase in R2, R5 and R8 (Freeman et al., 1992). In both *ro-svp1* and *ro-svp2* discs, the presumptive R2 and R5 cells fail to express β-galactosidase, whereas β-galactosidase expression is unaffected in the R8 cell (Fig. 3). These data indicate that ectopic expression of svp in R2 and R5 causes these two cells to lose some aspects of their identity while remaining neuronal. Since we found no evidence for misexpression of either the BarH1/H2 antigen or the P82 marker in R2/R5, the neuronal subtype of the affected R2/R5 cells is not known. The ability to affect neuronal differentiation of R3/R4/R1/R6 by altering R2/R5 development supports the idea that R3/R4/R1/R6 are induced by R2/R5 (Tomlinson and Ready, 1987). The preferential loss of only one cell of the R1/R6 pair may mean that there is a competition for the signal from R2/R5.

In retinae from animals in which svp was ubiquitously expressed, ommatidia that lack one to two outer photoreceptor cells were present in the anteriormost portion of the affected region (Hiromi et al., 1993). In the imaginal disc, a stripe of ommatidia that lack X81 marker expression was present immediately posterior to the morphogenetic furrow (data not shown). The similarity of this phenotype to the one caused by *ro-svp* transgenes suggests that ubiquitous expression of svp affects R2/R5 close to the morphogenetic furrow.

R7

In order to test the effect of ectopic expression of svp in R7, we had previously generated transgenic strains that express *svp* type 1 and type 2 cDNAs under the control of the *sevenless* enhancer (*sev-svp1* and *sev-svp2*). These fusion genes direct misexpression of svp to the R7 precursor, as well as to the cone cells. The major phenotype caused by these transgenes was the transformation of cone cells towards R7 neurons (Hiromi et al., 1993). Retinae of these transgenic animals nevertheless contained ommatidia that had seven or more outer photoreceptor cells, in addition to one or more R7 cells (Fig. 1D,H). This phenotype appeared to require more svp product than the cone cell to R7 transformation, because it was only seen when the gene dosage of *sev-svp2* was two or more. We assume that this represents a transformation of R7 to an outer photoreceptor cell. This phenotype was most readily visible when the cone cell to R7 transformation was suppressed as R7 precursors that do not express an R7-specific marker (see below). Although the specificity of the *sevenless* enhancer does not allow us to pinpoint the cell in which svp has to be expressed in order to generate this phenotype, we think it is likely that expression of svp in R7 causes transformation of R7 to an outer photoreceptor cell.

In summary, we have shown that misexpression of svp in any cell within a mature ommatidial cluster that normally does not express svp (R8, R2/R5, R7 and cone cells) causes cell fate transformations. Each of these phenotypes corresponds to one of the phenotypes caused by transient but ubiquitous

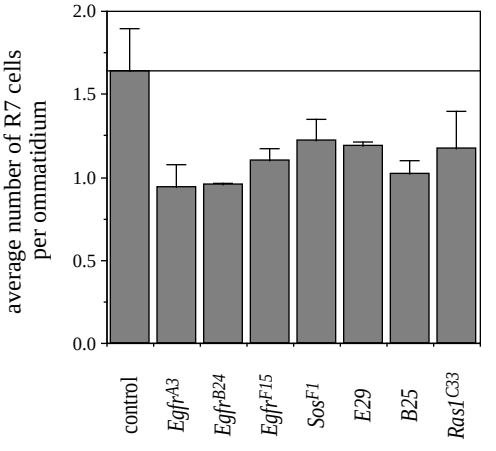

Fig. 4. Effect of *Su(sev-svp)* on the *sev-svp2* phenotype. The average number of R7 cells per ommatidium was determined in apical tangential plastic sections of eyes of animals that carry two copies of the *sev-svp* transgene without a suppressor mutation (control) or *sev-svp* animals that are heterozygous for the indicated suppressor mutation.

expression of svp, each of which has a specific sensitive period. Thus, different cells enter the sensitive period at different stages during ommatidial assembly and respond differently to misexpressed svp.

Identification of genes that are required for svp activity

Since *svp* encodes a steroid receptor-like molecule, phenotypes caused by misexpressing svp may be caused by misregulation of the transcriptional target genes of svp. The activity of svp as a transcriptional regulator may be influenced by other molecules such as its ligand or a heterodimeric partner. The restriction of the sensitivity to svp to a certain developmental period may mean that there are modulating factors that are necessary for svp activity. We chose to identify molecules that are required for svp activity using a genetic screen for dominant suppressors of the ectopic expression phenotypes. The phenotype caused by misexpression of svp is strongly dependent on the dosage of the transgenes, thus on the amount of the misexpressed svp protein. It is therefore possible that a decrease in the activity of another molecule that is required for svp function would sufficiently reduce svp activity so as to suppress the phenotypes caused by misexpression of svp.

We carried out a genetic screen for suppressors of the rough eye phenotype caused by the *sev-svp2* transgene. Seven extragenic suppressors of the *sev-svp2* phenotype were isolated in an F_1 screen. All suppressor mutations except one were homozygous lethal. Complementation tests with other mutations that are known to affect eye development (reviewed by Dickson and Hafen, 1993) showed that three suppressors were alleles of the *Egfr* locus (also known as *torpedo*, *faint little ball* or *DER*), one was an allele of the *Ras1* gene, and another was an allele of *Son of sevenless* (*Sos*). These mutations will be referred to as $Egfr^{A3}$, $Egfr^{B24}$, $Egfr^{F15}$, $Ras1^{C33}$ and Sos^{F1}.

All of the suppressor mutations that were isolated by virtue of suppressing the rough eye phenotype suppressed the formation of extra R7 cells in *sev-svp2* retinae. In the presence of a suppressor mutation, the average number of R7 cells per ommatidium was significantly decreased (Fig. 4). An example of the effect of a suppressor mutation, the $Ras1^{C33}$ mutation, on the *sev-svp2* phenotype is shown in Fig. 5A. To examine the way in which the reduction in the number of R7 cells is achieved, we

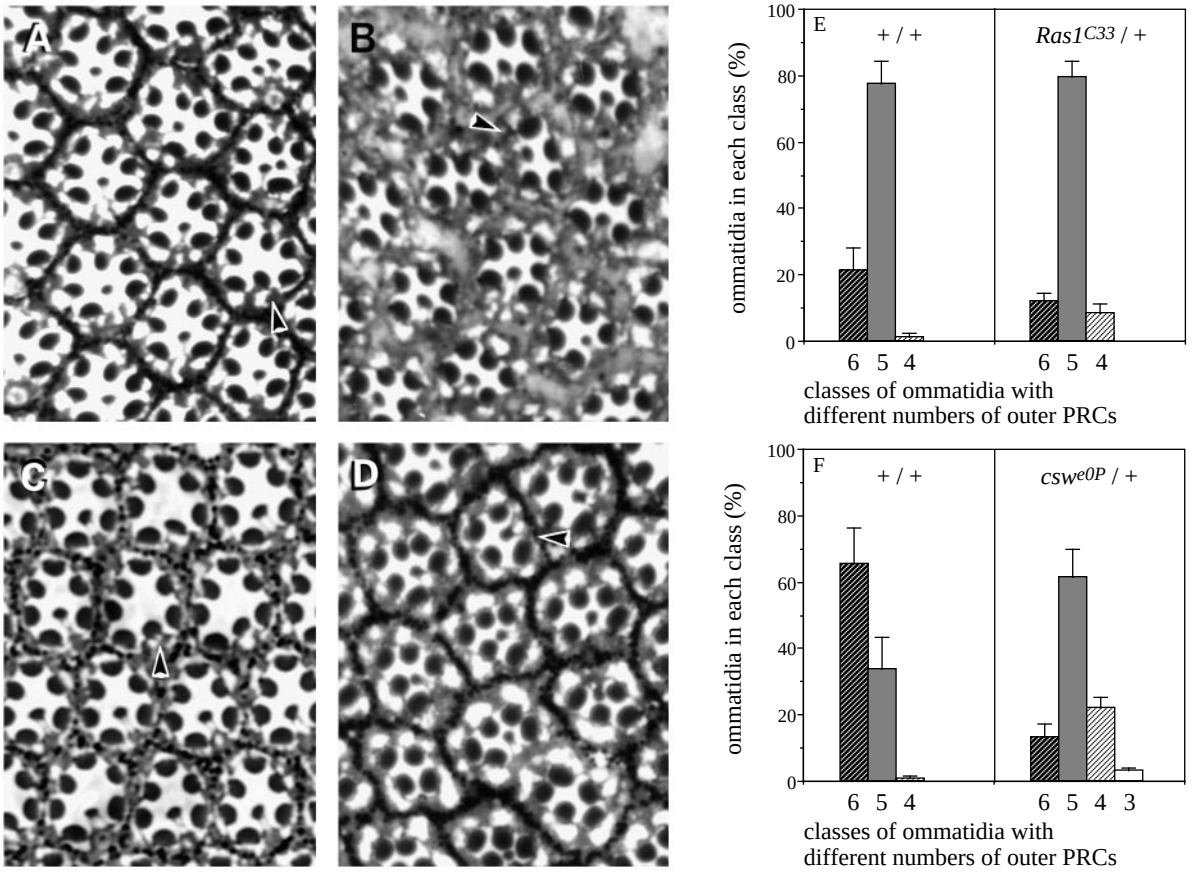

Fig. 5. Effect of *Su(sev-svp)* on the *sev-svp* and the *ro-svp* phenotype. (A,C) Tangential retinal sections of *sev-svp2* animals that are heterozygous for $Ras1^{C33}$ (A) and csw^{e0P} (C). Refer to Fig. 1D for a *sev-svp2* control. (B,D) Retinal sections of *ro-svp1* animals that are heterozygous for $Ras1^{C33}$ (B) and csw^{e0P} (D) mutations. See Fig. 1C for a *ro-svp1* control. The *ro-svp1* control for csw^{e0P} has a weaker phenotype than the control shown in Fig. 1C and is not shown (compare E and F). (E,F) The number of normal ommatidia and of ommatidia that were lacking one, two or three outer photoreceptor neurons were determined in tangential retinal sections of *ro-svp1*; + / *st e* and *ro-svp1*; + / $Ras1^{C33}$ *st e* animals (E) and for sev^{d2} / + ; *ro-svp1* and csw^{e0P}, sev^{d2} / + ; *ro-svp1* animals (F). Eight sections each were scored for *ro-svp1*; + / *st e* and *ro-svp1*; + / $Ras1^{C33}$ *st e* animals and the highest and lowest data points were omitted in the calculation of the mean number of outer PRCs and the standard deviation shown in E. The mean number of outer photoreceptor cells for *ro-svp1*; + / $Ras1^{C33}$ *st e* and *ro-svp1*; + / *st e* are distinct with $P< 0.01$ as determined from a *t*-test for eight samples each of $Ras1^{C33}$ and control.

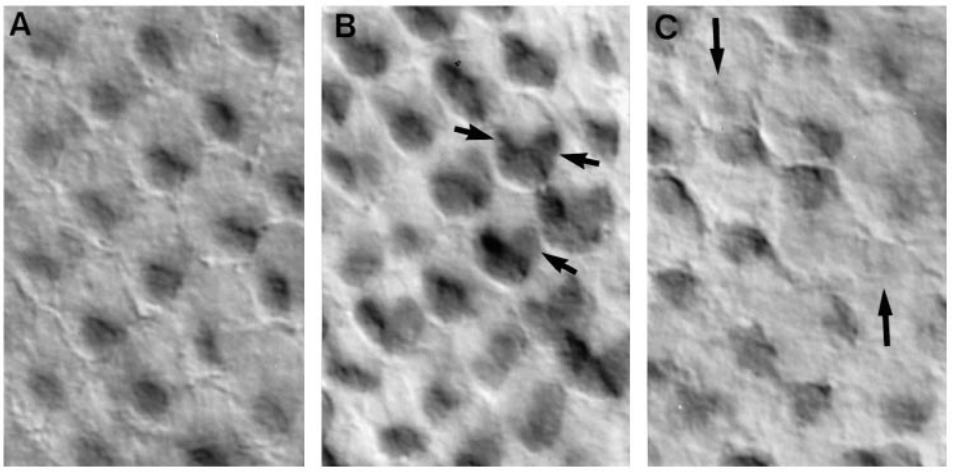

Fig. 6. Suppression of cone cell to R7 transformation by loss of Ras1 activity. (A-C) Anti-β-galactosidase staining of eye imaginal discs with the H214 enhancer trap insertion. Posterior is to the right. The anterior-most row is row 10 in all panels. The H214 line has weak expression of β-galactosidase in the outer photoreceptor precursors early in cluster formation; more posterior expression is restricted to the developing R7 precursor. (A) Wild type. (B) Three copies of *sev-svp2*. Ectopic expression of svp in the cone cells driven by the *sevenless* promoter/enhancer elements causes them to develop as R7 cells. This transformation results in ectopic expression of β-galactosidase in the cone cell precursors. Examples are indicated by arrows. (C) Introduction of the $Ras1^{e2F}$ loss of function allele into the three copy *sev-svp2* background results in almost total suppression of the ectopic cone cell staining. We also observe a small percentage of ommatidia that lack R7 cell staining (large arrows). These clusters most likely represent the transformation of the R7 cell precursor to an outer photoreceptor cell.

examined the expression of an enhancer trap marker for R7 in animals carrying the *Ras1* mutation. In the presence of the *Ras1* mutation, expression of β-galactosidase in the cone cells caused by the *sev-svp2* transgene is clearly suppressed (Fig. 6). Thus suppressors of *sev-svp2* act by suppressing the cone cell to R7 transformation elicited by the *sev-svp2* transgene.

A significant fraction (10-15%) of ommatidia from individuals that carry a suppressor mutation and a *sev-svp2* transgene contain seven outer photoreceptor cells but no R7 cell (Fig. 5A, C). A similar fraction of ommatidial clusters in the imaginal disc lacks expression of an R7-specific enhancer trap marker in the R7 precursor (Fig. 6C). This suggests that suppressors suppress the cone cell to R7 cell transformation, but not the R7 to outer photoreceptor cell transformation. Since the latter transformation requires a higher dosage of the transgene, failure of the suppressor mutations to suppress this phenotype may mean that the suppressor genes are differentially expressed within an ommatidium, or that misexpressed svp has different activities in the cone cells and in the R7 cell.

To determine if any of the suppressors of the *sev-svp2* phenotype are specific for the *svp* type 2 isoform, we tested whether the suppressors of the *sev-svp2* phenotype suppressed phenotypes caused by *sev-svp1*. All mutations isolated as suppressor of *sev-svp2* also suppressed the *sev-svp1* phenotype, indicating that they affect functions that are common to both type 1 and type 2 isoforms (Table 1).

Decrease in ras-activity suppresses the *sev-svp* phenotype

The *sev-svp* phenotype is similar to the phenotype that is observed with constitutive activation of the ras pathway in the cone cells. Activation of the ras pathway in cone cells can be achieved using a ligand-independent allele of *sevenless*, Sev^{S11} (Basler et al., 1991), or a constitutively active allele of *Ras1*, $Ras1^{V12}$, under the control of the *sevenless* promoter (Fortini et al., 1992). The extra R7 cells that are formed in *sev-svp*, Sev^{S11} or *sev-*$Ras1^{V12}$ ommatidia are dependent on *sina* gene function, but do not require *sevenless* or *boss* (Basler et al., 1991; Fortini et al., 1992; Hiromi et al., 1993). The similarity of the phenotypes that are elicited by expression of Sev^{S11}, *sev-*$Ras1^{V12}$ or *sev-svp* in the cone cells suggests that these phenotypes might be mediated by similar mechanisms. The identification of mutations in two genes that act by decreasing ras activity (*Ras1* and *Sos*) as suppressors of *sev-svp* is consistent with this view.

Activated ras is known to act through a kinase cascade in the R7 cell and the cone cells (reviewed by Zipursky and Rubin, 1994). We determined if mutations in other genes of the ras pathway suppressed the *sev-svp* phenotype. In addition to mutations in *Ras1* and *Sos*, we tested loss of function alleles

Table 1. Effect of decreased ras activity on the *sev-svp* and the *ro-svp* phenotype

	Dominant effect on		
Mutation	*sev-svp2*	*sev-svp1*	*ro-svp1*
E29	S	S	NE
Sos^{F1}	S	S	S
$Egfr^{A3}$	S	S	E
$Egfr^{B24}$	S	S	NE
$Egfr^{F15}$	S	n.d.	n.d.
$Ras1^{C33}$	S	S	E
B25	S	S	n.d.
$Egfr^{1PO2}$	S	n.d.	E
csw^{e0P}	S	n.d.	E
drk^{e04}	S	n.d.	E
Sos^{e4G}	S	n.d.	E
$Ras1^{e2F}$	S	n.d.	NE
Df(Ras1)	S	n.d.	E
$D\text{-}raf^{LQ129}$	S	n.d.	E
rl^{15M1}	S	n.d.	n.d.
$Dsor1^{LF133}$	S	n.d.	NE
$hsp83^{e1D}$	NE	n.d.	NE
$cdc37^{e1E}$	NE	n.d.	NE
$E(sev)3D^{e0Q}$	NE	n.d.	NE

The dominant effect of the mutations indicated on the phenotypes caused by *sev-svp2*, *sev-svp1* and *ro-svp1* were scored in apical tangential 1 μm sections of adult eyes and compared to the effect of an appropriate control chromosome. *Df(Ras1)* is *Df(3R)by10* which includes *Ras1*. S, suppression; E, enhancement; NE, no effect; n.d., not determined.

of *csw*, *drk*, *D-raf* (*l(1)polehole*/MAPKKK), *Dsor1* (MAPKK) and *rolled* (MAPK). Mutations in all of these genes acted as dominant suppressors of the *sev-svp2* phenotype (Table 1). Suppression was visible as a less disorganized appearance of the exterior of *sev-svp2* eyes as well as in a decrease in the number of R7 cells in apical sections. We conclude that a reduction in ras signaling activity suppresses the cone cell to R7 cell transformation caused by *sev-svp*. Of the genes that are required for efficient signaling of the sevenless receptor tyrosine kinase, mutations in *hsp83, cdc37* and *E(sev)3D* do not suppress the *sev-svp2* phenotype (Table 1). Mutations in these three genes also do not suppress the phenotype of a hyperactive Egfr receptor tyrosine kinase, suggesting that they may act in a different pathway (Simon et al., 1991).

The largest class of *sev-svp2* suppressors that we isolated are mutations in the *Egfr* gene. Although the Egfr receptor tyrosine kinase was known to be required for neuronal specification of photoreceptor precursors (Baker and Rubin, 1992; Xu and Rubin, 1993), it was not identified in a screen for enhancers of a hypomorphic allele of *sevenless* (Simon et al., 1991). The strong loss of function allele $Egfr^{1P02}$ also suppressed the cone cell to R7 transformation caused by *sev-svp2* (Table 1), but not the same phenotype caused by Sev^{S11} (data not shown). Since the Egfr receptor tyrosine kinase is thought to act through the ras pathway (Simon et al., 1991; Diaz-Benjumea and Hafen, 1994) the mechanism of suppression of the *sev-svp* phenotype by the *Egfr* mutations could also be due to a reduction in ras signaling.

Effect of suppressors of *sev-svp* on the *ro-svp* phenotype

If suppressors of *sev-svp* affect molecules that are required for svp activity, they may suppress dominant phenotypes caused by the misexpression of svp in cells other than the cone cells. We determined the effect of the suppressors of *sev-svp2* on the *ro-svp1* phenotype. Of the five mutations isolated as suppressors of *sev-svp2* that were tested, two, $Ras1^{C33}$ and $Egfr^{A3}$ enhanced, rather than suppressed, the *ro-svp1* phenotype (Table 1, Fig. 5B, D-F). The average number of outer photoreceptor cells per ommatidium was 5.03±0.03 in the presence of the $Ras1^{C33}$ mutation, and 5.05±0.07 in the presence of $Egfr^{A3}$, as compared to the control value of 5.2±0.07. In addition, the *ro-svp1* phenotype was also enhanced by most of the other mutations in the ras pathway (Table 1). Two alleles of *Sos* exhibited opposite effects on the *ro-svp* phenotype. While Sos^{e4G} enhanced the *ro-svp1* phenotype, Sos^{F1} suppressed it; the average number of outer photoreceptor cells increased to 5.41±0.12 in the presence of the Sos^{F1} mutation. As Sos^{e4G} is likely to be an amorph of *Sos* (Simon et al., 1991), the effect of Sos^{F1} is likely to be allele specific. Moreover, we have identified two alleles of *Ras1* in a screen for enhancers of the *ro-svp1* phenotype (S. K., Q.-L. Ch'ng and Y. H., unpublished observation). We conclude that a decrease in ras signaling results in the enhancement of the *ro-svp1* phenotype.

DISCUSSION

Each cell type within an ommatidium has a specific period that is sensitive to svp

The initial characterization of the loss of function phenotype of *svp* suggested that *svp* might act as a developmental switch by implementing a particular developmental program or a cell fate, such as the R3/R4/R1/R6 fate (Mlodzik et al., 1990b). Our analysis of the gain of function phenotypes caused by the misexpression of svp offers an alternative view. We find that the misexpression of svp in any cell type within an ommatidium that normally does not express svp causes a specific response that is unique to each cell type. For example, misexpression in R2/R5 causes these cells to lose R2/R5 characteristics while remaining neuronal, and misexpression in the cone cells causes their transformation towards R7 neurons. This suggests that the function of svp may not be to specify a particular cell type (R3/R4/R1/R6), but to act as a component of a widely used cell fate switch. Each of the phenotypes caused by the misexpression of svp in a specific subset of cells within an ommatidium resembles one of the phenotypes caused by expressing svp ubiquitously under the *hsp70* promoter (Hiromi et al., 1993). This spatially ubiquitous but temporally restricted expression of svp results in specific phenotypes depending on the developmental stage of the ommatidium at the time of svp expression. Assuming that the similar phenotypes caused by cell type specific misexpression and by transient ubiquitous expression are due to the same cellular response, we can assign a sensitive cell type to each developmental stage (Fig. 7). In this model, each cell enters the period that is sensitive to the misexpression of svp at a different developmental stage during ommatidial assembly. One possible explanation for the sensitive period is that the activity of svp is modulated in each cell so that it is activated only at the sensitive stage. It is also possible that this sensitive stage represents a time at which an intrinsic cell fate decision is taking place, and that the expression of svp somehow interferes with the decision, causing the cell to assume an alternative or default cell fate. These two possibilities are not mutually exclusive and both may contribute to the occurrence of a narrow time window in which svp can exert its effect.

Misexpression of svp in R2/R5 mimics the *rough* mutant phenotype

The ability of svp to affect all cell types within the ommatidial cluster that do not express svp could mean that the misexpressed svp protein interferes with the expression or the function of a gene product that is utilized for various cell fate decisions within the ommatidium. Alternatively, the misexpressed svp might interact with different target genes or molecules depending on the cell type. One of the phenotypes caused by the misexpression of svp can be explained by an interference with the function of a gene that is known to be involved in photoreceptor subtype specification. The phenotype caused by the misexpression of svp in R2/R5 is similar to the *rough* mutant phenotype. In *rough* mutant ommatidia, R2/R5 differentiate as photoreceptor neurons, but the subsequent recruitment of R3/R4 and R1/R6 fail (Tomlinson et al., 1988; Higashijima et al., 1992). The *rough* gene is expressed not only in R2/R5, where its activity is required, but also in R3/R4. Thus the expression of the *ro-svp* transgene generates the same situation (*rough*-ON, *svp*-ON) in R2/R5 as in the normal R3/R4. In *ro-svp* ommatidia *rhomboid* gene expression, as visualized by the enhancer trap marker X81, is absent in R2/R5. As *rhomboid*

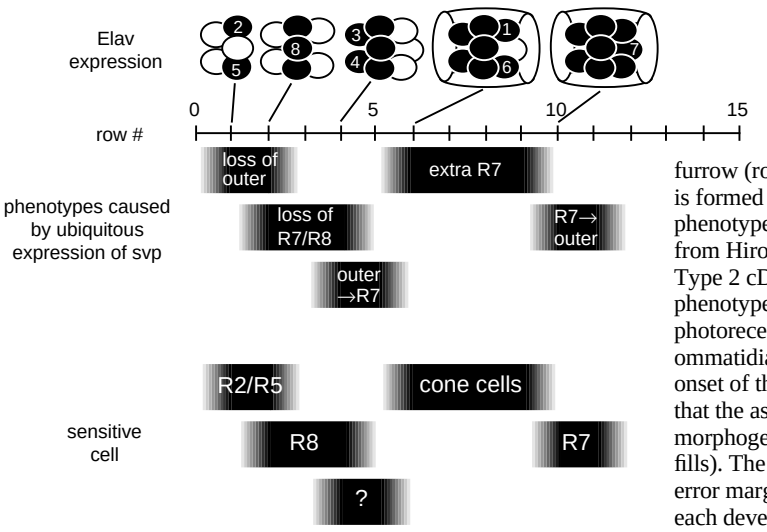

Fig. 7. A comparison of the timing of ommatidial differentiation and sensitivity to misexpressed svp. Top: the temporal expression pattern of the neuron-specific elav protein, indicated by shaded nuclei. The cone cells begin differentiation around row 7. The developmental position at which each event occurs with respect to the morphogenetic furrow (row 0) is indicated below on the row number timeline. A new row is formed every 1.5-2 hours (Basler and Hafen, 1989). Middle: specific phenotypes caused by ubiquitous expression of svp (redrawn using the data from Hiromi et al., 1993). This represents the effects of the type 1 cDNA. Type 2 cDNA produces similar, but not identical results. 'outer→R7' phenotype indicates ommatidia that have a reduced number of outer photoreceptor cells and extra R7 cells. 'extra R7' phenotype refers to the ommatidia with six outer photoreceptor cells and two or more R7 cells. The onset of the 'loss of R7/R8' phenotype was taken to be row 2. We estimate that the assignment of the phenotypes to the position with respect to the morphogenetic furrow could be off by one row (indicated by the graded fills). The relative positions among different phenotypes are more accurate, error margin of approximately half a row. Bottom: cells that are sensitive at each developmental stage, as inferred from comparisons of phenotypes caused by transient ubiquitous expression and cell-specific misexpression

gene expression in the same cells is dependent on *rough*$^{+}$ function (Freeman et al., 1992), it is possible that svp causes an effect in R2/R5 by interfering with rough function. Since svp is ectopically expressed in R2/R5 in *rough* mutant ommatidia (Heberlein et al., 1991), it is also possible that the dependence of *rhomboid* expression on *rough* is mediated by a double negative regulation via *svp*. In this case, svp could directly repress *rhomboid* expression. Since rough is not expressed in R7 or in the cone cells, the effect of svp in these cells cannot be explained by the interference with rough function.

Possible relationships between ras signaling and svp function

One of the known pathways used for cell fate decisions in the eye is the ras signaling pathway. Extensive genetic and molecular analyses have shown that the ras signaling pathway is activated in the R7 precursor through the sevenless receptor tyrosine kinase to specify a neuronal fate (reviewed by Rubin, 1991; Zipursky and Rubin, 1994). The other photoreceptor precursors also require the ras signaling pathway to become neuronal (Simon et al., 1991; Rogge et al., 1991; Dickson et al., 1992; Biggs et al., 1994; Brunner et al., 1994; O'Neill et al., 1994; Bohmann et al., 1994). In these cells ras is presumably activated by the Egfr receptor tyrosine kinase (Baker and Rubin, 1992; Xu and Rubin, 1993). Of the various phenotypes caused by svp misexpression, the phenotype caused by expression in the cone cells is similar to the effects of the ectopic activation of ras signaling. Expression of a ligand independent sevenless kinase or an activated form of the *Ras1* gene in the cone cells results in their differentiation as R7 neurons (Basler et al., 1991; Fortini et al., 1992). It is thus possible that ras signaling is activated by the misexpression of svp or that svp is activated by ras signaling. In either case, phenotypes caused by misexpression of svp could be suppressed by decreasing ras signaling. We found, however, that the phenotype caused by misexpression of svp in the cone cells is suppressed by a reduction in Egfr activity, a putative upstream component. This result renders the possibility that misexpressed svp acts upstream of ras less likely.

The model we find most attractive is that ras signaling is required in some way for svp activity. In one extreme view, svp or its heterodimeric partner could be phosphorylated by one of the kinases of the ras signaling pathway, and such a phosphorylation may be required for svp to be active. At another extreme, ras signaling may be required to make the cell competent to respond to svp. This model for svp function predicts that the svp sensitivity occurs after ras activation. For the cone cells, it is not known whether ras signaling is activated at a specific developmental stage. Photoreceptor cells, however, require activities of *Ras1* and other genes in the ras signaling pathway for neuronal specification. It is thus possible that ras signaling is activated in a sequential manner in different photoreceptor subtypes making them responsive to svp. The activation of ras signaling in R7 is triggered by the interaction of sevenless with its ligand boss. The internalization of boss by R7 starts in row 6 (Krämer et al., 1991), whereas the svp sensitive period for R7 maps to rows 10-11 (Fig. 7), prior to the initiation of neuronal differentiation at row 12 (Tomlinson and Ready, 1987). This timing of svp sensitivity is consistent with the idea that R7 becomes sensitive to svp upon ras activation.

The order of neuronal differentiation does not appear to strictly follow the order of the sensitive periods for svp. R8, for instance, initiates neuronal differentiation before R2/R5 (Tomlinson and Ready, 1987). However, the svp-sensitive period of R2/R5 is immediately posterior to the morphogenetic furrow; one row anterior to the sensitive period for R8 (Fig. 7). This may be due to different mechanisms that control neuronal specification in these two cell types. Neuronal specification of R8 is dependent on the proneural gene *atonal* (Jarman et al., 1994), whereas the other photoreceptors are thought to be recruited by induction. Perhaps ras signaling is activated by the induction signal and does not rise immediately during neuronal differentiation of R8. In contrast to mAb22C10 and anti-HRP used in the studies of Tomlinson and Ready (1987), the neuron-specific elav protein (Robinow and White, 1991) is expressed in R2/R5 slightly earlier than in R8, following the same order as the sensitive periods for svp misexpression (Fig. 7).

The observation that a decrease in ras signaling enhances the *ro-svp* phenotype appears to contradict our model of ras signaling being required for svp function. This is likely to be due to the difference in the cells in which svp is misexpressed and those in which a decrease in ras signaling exerts its effect. In *ro-svp* ommatidia, the misexpression of svp is directed to R2/R5, whereas the outer photoreceptor cells that fail to become neuronal are of the R3/R4/R1/R6 subtype. The latter cell types may have a limiting amount of ras activity, due to inefficient induction by R2/R5. In this case, a decrease in ras signaling may exert a prominent effect only in R1/R6/R3/R4. Thus the enhancement of the *ro-svp* phenotype by a reduction in the gene dosage of the components of the ras signaling pathway does not necessarily imply a direct relationship between ras signaling and svp function. The failure of a decrease in ras signaling to suppress the transformation of R7 to an outer cell suggests that ras activity is not limiting in the R7 precursor, where ras signaling is activated through the boss-sevenless interaction.

In conclusion, we suggest that ras signaling is required for svp function. This would provide a molecular basis to explain how induction triggers competence to respond to other signals, such as svp, that control neuronal diversity.

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