Ras1 signaling and transcriptional competence in the R7 cell of Drosophila

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The receptor tyrosine kinase Sevenless determines R7 cell fate by activation of the Ras1 pathway in a subset of equivalent cells competent to respond in the Drosophila eye. We show that the prospero gene becomes transcriptionally activated at a low level in all Sevenless-competent cells prior to Sevenless signaling, and this requires the activities of Ras1 and two Ras1/MAP kinase-responsive ETS transcription factors. Restriction of high-level prospero expression to the R7 cell appears as a subsequent event, which requires Sevenless activation of the Ras1/MAP kinase pathway. We show that Phyllopod, a nuclear factor whose expression is induced by Sevenless, interacts with another nuclear factor, Sina, to form a complex, and that both factors are involved in upregulating transcription of the prospero gene in the eye. Ultimately, prospero expression is required for proper connectivity of R7 photoreceptor axons to their synaptic targets. Our results suggest that specific transcriptional responses are linked to the mode of activation of the Ras1/MAP kinase signal transduction pathway.

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Receptor tyrosine kinases [RTKs] are involved in signaling between cells to regulate proliferation and differentiation. RTK signaling can be substituted by gain-of-function variants of Ras, MEK, and MAP kinase (MAPK), all of which are components of the same signal transduction pathway [for review, see Zipursky and Rubin 1994; Marshall 1995]. This suggests that Ras-mediated activation of MAPK is a critical event in signal transduction from RTKs. Paradoxically, the Ras pathway is commonly required in many cell types during development, and yet activation of distinct RTKs generates different cellular responses. A central issue in understanding RTK signaling is how a cell-type specific response is generated by use of a common Ras pathway. In rat PC12 cells, the decision to differentiate into a neuron or to proliferate is guided by the duration of MAPK activation [Marshall 1995]. In turn, this is regulated by the specific ligand-RTK interaction triggered in PC12 cells. In the Drosophila eye, specific responses are in part determined by cell-specific factors that function downstream of MAPK [Dickson 1995]. Here we describe a gene, prospero [pros], that is necessary for cell-type specific differentiation in the Drosophila eye and is transcriptionally regulated by multiple signals that activate the same Ras1 pathway.

The Drosophila eye develops by the reiterative patterning of ~800 ommatidia initiated in the larval eye imaginal disc. Differentiation occurs progressively from posterior to anterior across the eye disc, with the leading edge of differentiation marked by an indentation called the morphogenetic furrow. Assembly of each ommatidium behind the furrow occurs by the sequential recruitment of undifferentiated cells into distinct developmental programs [Zipursky and Rubin 1994]. The R7 photoreceptor cell is the last photoreceptor to be recruited into the ommatidial cluster, and is followed by recruitment of four non-neuronal cone cells. Although only one R7 cell develops in each ommatidium, there is a potential for five cells to develop with an R7 cell identity. These cells include the R7 and cone cell precursors and are collectively known as the R7 equivalence group. Determination of R7 cell fate is dependent upon signaling by the Sevenless [Sev] RTK in response to an inductive interaction with the neighboring R8 cell. This is transduced within the presumptive R7 cell by a cascade of proteins including Ras1, Raf, and Rolled/MAPK. All of the cells within the R7 equivalence group can be transformed to an R7 cell fate by activation at any point along the sev/Ras1/rolled signaling pathway [Basler et al. 1991; Fortini et al. 1992; Brunner et al. 1994b]. Conversely, genetic disruption of the pathway results in adoption of a cone cell fate [Tomlinson and Ready 1987; Simon et al. 1991; Biggs et al. 1994].

Biochemical studies of vertebrate MAPK have shown that activated MAPK translocates to the nucleus where it phosphorylates different transcription factors [Marshall 1995]. Nuclear target molecules of activated rolled/MAPK in the R7 equivalence group include the ETS-domain transcription factors, Yan and Pointed [Pnt]. A variety of studies support a model in which the balance between the activities of Pnt and Yan determine whether a cell adopts a cone or R7 cell fate [Lai and Rubin 1992,

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The phyllopod (phyl) gene is a possible target gene of the Ras1 pathway (Chang et al. 1995; Dickson et al. 1995). It is expressed in the R1, R6, and R7 precursor cells where it functions to promote their determination as photoreceptor cells. In the absence of phyl, they are transformed into cone cells. Misexpression of phyl in cone cell precursors transforms their fate to an R7 cell provided the sina gene is present. This is consistent with the demonstrated requirements for sina in normal R7 determination [Carthew and Rubin 1990]. Both phyl and sina encode nuclear factors of unknown biochemical function.

These studies have identified a number of nuclear factors that play important roles in R7 determination. However, target genes of the factors that are specifically expressed in R7 cells and are required for R7 differentiation have not been identified. With the aim of identifying genes that function downstream of sina, we performed a genetic screen for mutations that dominantly enhance a mutant sina phenotype. One of the genes we identified, pros, has been shown previously to encode a putative transcription factor required for differentiation of the embryonic nervous system [Doe et al. 1991; Vaessin et al. 1991]. We show that pros is required in the eye for proper connectivity of R7 photoreceptor axons to their synaptic targets in the brain. We also show that pros transcription is initially activated in all cells of the R7 equivalence group by activation of the Ras1 pathway. Yan and Pnt proteins appear to mediate these effects. Later, R7 cells specifically transcribe pros at higher levels in response to Sev activation of Ras1. Yan and Pnt are also intermediates of the upregulation of transcription. In addition, Phyl is involved in transcriptional upregulation of pros, albeit in a functionally redundant capacity. We show that sina is required for phyl’s effect on pros transcription and that phyl protein forms a specific complex with sina protein both in vivo and in vitro. These results imply that pros transcription is regulated by Ras1 through two different mechanisms, one that initiates transcription in the R7 equivalence group and one that stimulates increased levels of transcription in the R7 cell.

Results
pros is a dominant enhancer of sina
Loss of sina activity in the R7 precursor cell leads to its transformation to a cone cell. In flies homozygous for a weak loss-of-function allele [sina<sup>a</sup>], 20% (<i>n</i> = 660) of the ommatidia are missing the R7 photoreceptor cell (Fig. 1A). To identify genes that interact with sina, we screened a collection of lethal P-element enhancer trap insertions for loss-of-function mutations that dominantly enhance or suppress the sina<sup>a</sup> phenotype. From this screen, we identified an allele of pros, l(3)10419, which enhanced the sina<sup>a</sup> phenotype, increasing the number of R7-less ommatidia to 50% (<i>n</i> = 316; Fig. 1B). A transgenic-null allele of pros, pros<sup>10419</sup> [Vaessin et al. 1991], was tested for its ability to dominantly enhance the sina<sup>a</sup> phenotype. Like the pros allele l(3)10419 [pros<sup>10419</sup>], pros<sup>10419</sup> increased the number of R7-less ommatidia to 50% (<i>n</i> = 1420; Fig. 1C). To demonstrate that a reduction in pros product was responsible for the enhanced phenotype, we increased the amount of Pros by generating transgenic flies in which a pros cDNA was put under the control of the sev enhancer [sev–pros]. This enhancer is active in photoreceptor cells R3, R4, R7, the cone cell precursors, and at low levels in R1 and R6 [Zipursky and Rubin 1994]. The enhancement of the sina<sup>a</sup> phenotype was not observed in flies bearing one copy of the sev–pros transgene in a sina<sup>a</sup>/sina<sup>a</sup>; pros<sup>prosI0419</sup>/+ background (<i>n</i> = 1859; Fig. 1D). Therefore, reducing the dosage of the pros gene enhances the R7 cell defect caused by sina<sup>a</sup>.

pros is required for R7 axon targeting
We wished to determine the homozygous mutant pros phenotype to further understand the function of pros in eye development. Since both pros<sup>10419</sup> and pros<sup>pros</sup> are homozygous lethal, we generated pros<sup>prosI0419</sup> mutant clones in a wild-type eye (Fig. 1E). Within the pros<sup>–</sup> clones, 23% (<i>n</i> = 215) of R7 cells had an abnormal morphology, as evidenced by uncharacteristically small light-sensing organelles or rhabdomeres. We also examined the synaptic connections of pros<sup>–</sup> photoreceptors since pros plays a role in axon guidance within the embryonic nervous system [Doe et al. 1991; Vaessin et al. 1991]. In a wild-type eye, the axons of the outer photoreceptor cells R1–R6 synapse onto a retinotopic unit in the first optic ganglion, the lamina (Fig. 1F). The axons of R7 and R8 bypass the lamina and retinotopically project to the second optic ganglion, the medulla. The R7 and R8 axons terminate at two distinct levels in the medulla. To visualize projections of wild-type R7 axons in the medulla, we filled retinal cells with horseradish peroxidase (HRP). The projections of R1–R6 were densely packed together in the lamina but the individual axons and terminals of R7 were clearly visible in whole mounts of the optic lobe [Fig. 1G]. The axons formed optic columns and terminated at evenly spaced intervals to a unique layer of the medulla. HRP eye fills of sev mutant flies confirmed that these axon projections were from R7 cells [Fig. 1H]. We filled retinal cells within pros<sup>prosI0419</sup> mutant clones and observed a disordered projection pattern (Fig. 1I). R7 axons were irregularly spaced, followed abnormal paths, and terminated at multiple layers in the medulla in a non-retinotopic fashion. The restriction of the phenotype to clones of mutant retinal cells indicates that pros is required in the retina for normal R7 axon connectivity.

pros expression is limited to the R7 equivalence group
The eye phenotype of pros mutants suggests that the gene is involved in determination and differentiation of
R7 photoreceptors. To examine the pattern of pros expression in eye imaginal discs, antibodies specific for the pros protein were generated. Antibody staining of eye discs revealed that pros protein is first detected in presumptive R7 cells of ommatidia ~7–8 columns posterior to the morphogenetic furrow [Fig. 2A,B]. By columns 11–12 its expression is also detected in the four cone cells [Fig. 2C]. Antibody staining is restricted to the nuclei of these five cells. Significantly, Pros is detected in R7 cells several hours prior to their overt differentiation into neurons, as marked by their expression of the neural antigen Elav (Fig. 2D). The timing of pros expression in R7 cells suggests that it is induced early in the R7 developmental program. The specificity of the antibody for pros protein was demonstrated by staining eye discs from sev–pros transgenic larvae. In these eye discs, pros should be detected in the cells which express sev: R3, R4, R7, and the four cone cells. The antibody specifically labeled the nuclei of these cells in sev–pros eye discs [Fig. 2E]. Moreover, the antibody labeled cells in the developing nervous system of wild-type embryos in a pattern indistinguishable from the pattern reported previously for pros [Vaessen et al. 1991], whereas the antibody did not label any cells in homozygous mutant pros<sup>10471</sup> embryos [data not shown].

The specific expression of pros in the R7 and cone cell precursors is primarily achieved through transcriptional regulation. We examined the expression pattern of lacZ in the enhancer-trap line pros<sup>10471</sup>. The P element in pros<sup>10471</sup> is inserted upstream of the transcription start site and expression of the lacZ reporter gene is restricted to the R7 and cone cells [Fig. 2F,G]. Moreover, the onset of lacZ expression closely resembles the timing of pros expression as determined by antibody staining.

The initial level of pros protein in the presumptive R7 cell is equivalent to the level seen in the cone cells [cf.
Figure 2. Expression of pros in developing eyes. (A–E) Whole mounts of larval eye discs labeled with antibody against Pros. In A and E, discs are presented with posterior up and are aligned to show similar regions of the disc. (A) Wild-type disc, (E) P[sev–pros] disc. No labeling is observed near the morphogenetic furrow (marked with an arrowhead) in A while staining of clusters of cells in E begins immediately adjacent to the furrow. These cells correspond to R3 and R4, as shown in the insert in E. (B–D) are higher magnifications of a wild-type disc labeled with antibody against Pros (shown in red). Anterior is to the right. (B) A basal focal plane showing weak labeling of nuclei from presumptive R7 cells as they are ascending in anterior ommatidia (marked with white arrowheads) and more intense labeling of R7 nuclei in ommatidia beginning three columns posterior. (C) An apical focal plane in a posterior region of the disc showing weak labeling of cone cell nuclei. (D) A disc labeled with antibody against Pros (shown in red) and antibody against Elav (shown in green). Strong staining in both channels appears yellow. The focal plane shows R7 nuclei [red or yellow] and other photoreceptor nuclei [green]. (F,G) Larval eye disc from a pros10417/+ fly stained with X-Gal. High magnification view of staining of R7 cells (marked 7) at one focal plane (F) and cone cells (marked c) at a more apical focal plane (G). (H) A disc labeled with pros antibody coupled to FITC was optically sectioned (1 μm sections) by confocal microscopy. Shown is an apical section of a single ommatidium showing cone cell labeling overlaid with a basal section showing R7 labeling. The signal contribution of the overlying equatorial cone cell was deleted from the composite to keep R7 signal strength constant. Fluorescence intensity was quantitated by NIH Image 6.0 and rendered on a color scale shown to the right. Numbers represent relative intensity values. (I–L) Pupal eye whole mounts (48 hr after pupation) of wild-type pupae labeled with anti-Pros antibody (I–L)] and pros10417/+ pupae stained with X-Gal (K,L)]. Strong labeling of R7 nuclei with antibody and X-Gal observed at one focal plane (I,K) with labeling of underlying bristle neurons evident [arrowheads]. A more-apical focal plane (J,L) shows weak labeling of cone cells. (M–P) Anterior is to the right. (M) Eye disc from a P[sev–Notch] disc labeled with anti-Pros antibody (green) and with the nuclear dye propidium iodide [red]. Staining in both channels appears yellow. The R1, R3, R4, and R6 cells of one ommatidium cluster are labeled. (O,P) X-Gal strongly stains R1, R3, R4, and R6 cells in wild-type flies carrying the H162-lacZ enhancer trap insertion (O), but it stains two cells per ommatidium, R3 and R4, in a P[sev–Notch] background (P).
cell is equivalent to the level seen in the cone cells [cf. Fig. 2B,C]. As the developing ommatidia mature, the level of pros protein in the R7 cell increases, whereas it remains constant in the cone cells. The increase of pros protein in R7 cells begins in column 11 which is coincident with the onset of pros expression in the cone cells. In the most mature ommatidia of the larval eye disc, there is a significant difference in levels of pros protein between R7 and cone cells [Fig. 2H]. The level of pros protein in the R7 cell continues to increase as the ommatidia develop during pupal metamorphosis [Fig. 2L]. pros expression is eventually turned off in all retinal cells between 56–64 hr after pupation [data not shown].

The differential expression of pros protein in the eye was mimicked by a similar differential expression of lacZ in the pros10419 enhancer trap line [Fig. 2K,L]. This suggests that differential levels of pros protein in R7 and cone cells is achieved through differential transcriptional activity of pros in R7 and cone cells.

The restriction of pros expression to the R7 equivalence group is a consequence of the unique time at which these cells are recruited into the ommatidial program. Recruitment of other cells at the same time leads to their misexpression of pros. We overexpressed an activated form of the Notch receptor in defined cells, which transiently blocks their proper recruitment and causes them to adopt different fates [Fortini et al. 1993]. In eye discs from flies carrying the sevNAct gene, we observed two additional cells within each ommatidium that expressed pros at levels comparable to wild-type R7 cells [Fig. 2M]. This result is consistent with the observation that two outer photoreceptor cells are developmentally delayed and adopt an R7 cell fate [Fortini et al. 1993]. It suggests that any cell that is competent to receive a specific cue at this time in development can express pros. Interestingly, this period of competence appears to be fairly extended because developmental delay of the cone cell precursors by sevNAct has no effect on their expression of pros [data not shown].

To determine the identities of the two ectopically stained cells, we used propidium iodide to label all of the nuclei in the eye disc and double-labeled with the Pros antibody. At the eight-cell cluster stage when the nuclei of R3, R4, R1, and R6 are at the apical surface, the Pros antibody labeled the two cells in the posterior position that by position correspond to R1 and R6 [Fig. 2N]. The R1 and R6 precursor cells, developmentally delayed by sevNAct, appear to be recruited into the R7 equivalence group and express pros. To verify that the R1 and R6 precursor cells were recruited into the R7 equivalence group, we examined the effect of sevNAct on seven-up (svp) gene expression. svp is expressed in the outer photoreceptor cells R1, R3, R4, and R6 [Mlodzik et al. 1990, Fig. 2O]. The addition of sevNAct resulted in the loss of svp expression in the R1 and R6 cells [Fig. 2P].

**Regulation of pros expression by the Sev–Ras1 pathway**

The R7 and cone cell precursors have an equivalent developmental potential that is restricted by Sev RTK activity. This suggested the possibility that sev might induce pros expression to higher levels in developing R7 cells. We compared the pros expression pattern in wild-type and sev mutant eye discs [Fig. 3A,B]. Cone cells in null-mutant sev discs expressed pros at a level comparable to wild type. However, no elevated level of pros expression was observed in the transformed R7 cells. Conversely, an average of three cells per ommatidium expressed elevated levels of pros in a constitutively activated sev mutant, SevS11 [Fig. 3C]. The positions of these cells suggest they are the endogenous R7 cell and cone cells transformed to an R7 fate. These results indicate that Sev signaling is not required for normal pros expression in cone cells, nor is it required for low level expression in the R7 cell. However, it is necessary and sufficient for upregulated expression of pros.

Sev signaling is mediated by Ras1. To determine the role of Ras1 in pros expression, a constitutively activated Ras1 mutant, sev–Ras1V12, was examined [Fig. 3D]. Like SevS11, this mutant exhibited elevated pros expression in the endogenous R7 cell and the cone cells that were transformed to an R7 fate. This was observed even in a

![Figure 3](https://example.com/figure3.png)

**Figure 3. Regulation of pros expression by the Sev–Ras1 pathway.** (A–D) X-Gal staining of pros10419/+ eye discs. All panels are oriented with anterior to the right. (A) Wild-type disc showing R7 cell staining; (B) sev12 eye disc. Note that the transformed R7 cell staining (+) is equivalent to cone cell staining [marked cl.] (C) SevS11 disc; (D) P[sev–Ras1V12] disc. Arrowheads point to cells within one ommatidium that intensely stain with X-Gal. (E,F) Whole mounts of P[sev–Ras1V12] eye discs labeled with anti-Pros antibody. The discs have been overstained to more readily visualize the weak labeling of cone cell nuclei. The region of the disc in E is ~7–10 rows behind the furrow, where Pros-labeled cells normally first appear. An R7 cell [arrowhead] is labeled at the same intensity as the cone cells. A reduced number of R7 precursor cells is labeled in this region of the disc. More posterior in E, a circle outlines a typical ommatidium containing two labeled cells that by position and morphology are indicative of cone cells. There is no evidence of intense R7 cell staining at this stage of development. The region of the disc in F is near the posterior edge where normally four cone cells are labeled. The circle in F highlights a typical ommatidium with two labeled cells that lie in the apical focal plane typical for cone cell nuclei.
sev null background (data not shown). Thus, Ras1 is genetically downstream of sev in regulating pros expression. To examine the effects of removing Ras1 function in retinal cells, a dominant negative form of Ras1 (sev–Ras1N17) was used. The eye phenotype of sev–Ras1N17 is the absence of R7 cells, presumably because of their transformation to a cone cell fate [Allard et al. 1996]. We examined pros expression in sev–Ras1N17 mutant eye discs and found that ~50% of the cone and R7 cells did not express pros, nor did they express elevated levels of pros typical of an R7 cell [Fig. 3E,F]. Therefore Ras1, like sev, appears to be both necessary and sufficient to activate high-level expression. However Ras1, unlike sev, is also required for low-level expression in both R7 and cone cell precursors.

Regulation of pros expression by MAPK-responsive transcription factors

To further define the role of the Ras1 signaling pathway on pros expression, we examined the effects of mutations in two genes encoding ETS-transcription factors: yan and pnt. The activities of both factors are regulated by MAPK phosphorylation and both factors are critical for R7 fate determination [Brunner et al. 1994; O’Neill et al. 1994; Rebay and Rubin 1995]. We tested pros for dominant genetic interactions with partial loss-of-function alleles of yan and pnt. In flies carrying the viable allelic combination pntΔ88/pntT6, 25% (n = 820) of the ommatidia are missing R7 cells [data not shown]. The pntΔ88/pntT6 phenotype was dominantly enhanced by pros01, leading to a roughening of the external eye (Fig. 4A,B). This was accompanied by a reduction in R7 cells from ~0.75 (±0.03 S.D.) cells per ommatidium to ~0.55 (±0.04 S.D.) cells per ommatidium (n = 1690). Conversely, the yan1 rough eye phenotype was dominantly suppressed by pros01 [Fig. 4C,D] and was accompanied by a reduction in superumery R7 cells from 1.8 (±0.2) cells per ommatidium to 1.3 (±0.1) cells per ommatidium (n = 1670). The effect of pros01 on these phenotypes was partial, suggesting that pros is not a major limiting component when yan and pnt activities are reduced.

To examine the effects of yan and pnt on pros expression, we stained mutant eye discs with anti-Pros antibodies. Partial loss-of-function pntΔ88/pntT6 mutants contained a markedly reduced number of pros-expressing cells (Fig. 4E,F). Only two or three pros-positive cone cells per ommatidium were observed, and no elevated expression in R7 cells was observed. Thus, pnt is required for pros expression in both the cone cell and R7 cell precursors. In contrast, the partial loss-of-function yan1 mutant contained a number of cells of unknown identity that ectopically expressed pros in addition to the presumptive R7 and cone cells [Fig. 4G]. The positions of the cells suggest they are recruited from the uncommitted cell population. This result is consistent with the ectopic transformation of uncommitted cells to an R7 fate observed in a yan1 mutant [Lai and Rubin 1992], and suggests that Yan normally represses pros expression.

Yan is hypothesized to be a repressor of gene transcription that is down-regulated when phosphorylated by MAPK [Rebay and Rubin 1995]. If pros transcription is normally activated by Yan phosphorylation, then unphosphorylated Yan should constitutively repress pros transcription. A dominant activated form of yan (yanACT), in which all potential MAPK phosphorylation sites are missing, blocks cell differentiation in the eye and constitutively represses transcription of reporter genes in cell culture [Rebay and Rubin 1995]. We examined pros expression in flies carrying the yanACT gene under control of the sev enhancer (sev–yanACT). Most ommatidia contained substantially fewer cone and R7
cells that expressed pros, and elevated pros expression was not observed in R7 cells [Fig. 4H]. This suggests that activation of pros transcription in all cells within the R7 equivalence group requires the down-regulation of Yan activity through phosphorylation by MAPK in the R7 and cone cell precursors. This is also consistent with the established role for Ras1 in activating pros expression.

The suppression of pros expression by sev–Ras1N17, pntab88/pntab7c, and sev–yanACT mutants was not complete, and indicates that these alleles may contain some residual activity. Although this is very likely for the pntab88/pntab7c mutant, it is not as clear for the other two genes. It is possible that the signal needed to initiate the low level of pros expression in the equivalence group can occur before the sev enhancer becomes active, and, therefore, before yanACT and Ras1N17 are synthesized in these cells.

Activation of pros expression by a parallel pathway

The experiments described above suggest that Yan and Pnt may act on pros transcription in response to Ras1 activity. It was unclear whether their activities are sufficient for Ras1 signaling, because nuclear factors such as Sina and Phyl are also required for R7 determination. Therefore, we examined various sina and phyl mutants to see whether these genes are required for normal pros expression. A sina null mutant expressed levels of pros in the R7 equivalence group comparable to wild type, with elevated levels in transformed R7 cells equivalent to or sometimes greater than that observed in wild-type R7 cells [Fig. 5A,B]. Because sina is absolutely required for R7 fate determination, this result suggests that elevated levels of pros expression are not strictly coupled to R7 fate determination. It also contrasts with the observed absence of elevated expression in the transformed cells of a sev null mutant. We examined pros expression in sev sina double mutant eye discs to determine the relationship between sev and sina in regulating pros transcription. Expression in the double mutant resembled expression in the sev mutant [Fig. 5C], indicating that sev is epistatic to sina. Consistent with this result, the elevated expression of pros in transformed cone cells in a SevN17 mutant was not significantly affected by sina null mutations (data not shown). These epistasis results suggest that sev acts downstream or in parallel to sina.

We also examined the effect of removing phyl function during R7 development. The transformed R7 cells in a phyl mutant expressed pros at a level comparable to wild type [Fig. 5D]. This result suggests that phyl is not absolutely required for elevated pros expression. However, the presence of phyl in cells is sufficient to induce them to express elevated levels of pros. We examined eye discs from flies bearing the phyl gene under control of the sev enhancer [2sev–phyl]. Ectopic expression of phyl in the cone cell precursors resulted in supernumerary cells that express pros at levels comparable to the R7 cell [Fig. 5E]. These cells appeared by position to correspond to transformed cone cells. We conclude that expression of phyl in cone cells is sufficient to induce elevated levels of pros expression. Possibly, one reason that phyl is not absolutely required for pros upregulation in the R7 cell is that its function is redundant, other factors compensate for its absence.

Increased expression of pros in transformed 2sev–phyl cells required the sina gene. No supernumerary cells with elevated levels of pros were observed in eye discs from 2sev–phyl sina flies [Fig. 5F]. This result suggests that phyl requires sina activity to stimulate pros expression.

Sina protein forms a complex with Phyl

The close genetic interaction between sina and phyl suggested that the proteins may physically associate and

![Figure 5. Sina and Phyl activate pros expression. X-gal staining of pros1019/+ eye discs (A–C, E, F) or labeling with antibody against Pros (D). All panels are oriented with anterior right. (A) Wild-type disc showing R7 cell staining; (B) sina+ eye disc. Staining of cone cells (marked c) is equivalent to wild type, and staining of transformed R7 cells (one is marked with * ) is somewhat stronger than wild type. (C) sevN17, sina eye disc. Intense staining of transformed R7 cell is abolished and weak cone cell staining appears unaffected. (D) phyl2/phyl2 disc. An average of one cell per ommatidium is intensely stained (*), which by position corresponds to a transformed R7 cell. Posterior in the disc, the ommatidial cell clusters become disorganized and up to five cells display weak staining [data not shown]. Because R1/R6 adopt a cone cell fate in phyl mutants, these ectopically expressing cells may be transformed R1/R6 cells. (E) R7 [2sev–phyl] eye disc. Multiple cells per ommatidium are staining intensely with X-Gal. The position of these cells correspond to transformed cone cells. They all lie on the same focal plane in a position characteristic of R7 cells in this region of the disc. Two strongly staining cells within one ommatidium are marked with arrowheads. The increased number of highly expressing cells was accompanied by a decrease in the number of cells expressing lower levels of pros normally observed in cone cells. The transformation of cone cell identity in 2sev–phyl mutants results in fewer cone cells per ommatidium [Dickson et al. 1995] which likely accounts for the loss of lower-expressing cells. (F) R7 [2sev–phyl] sina2 eye disc. One cell per ommatidium which occupies the position of the transformed R7 stains strongly with X-Gal [+]. The cone cells stain comparably to wild type.](genesdev.cshlp.org)
that this association may play an important role in regulating pros expression. To test this, we employed the yeast two-hybrid system that detects protein interactions [Gyuris et al. 1993]. Full-length Sina protein was fused to a transcriptional transactivation domain (Sina-TA) and Phyl protein was fused to a LexA DNA-binding domain (Phyl-LexA). Interaction was observed by both proteins together transactivating a lacZ reporter gene [Fig. 6A]. In addition, both proteins together transactivated a Leucine reporter gene, resulting in transformant growth on Leu− media (data not shown). Comparable transactivation was observed when the fusion domains were swapped, using Sina-LexA and Phyl-TA. Deletion of amino acids 108–400 of Phyl [A108] abolished the interaction, whereas deletion of amino acids 131–400 [A131] resulted in a strong interaction, as measured by lacZ transactivation. This suggests that amino acids between positions 108 and 130 are critical for interaction. The strength of the A131 interaction is 27-fold greater than the interaction with full-length Phyl. This difference may reflect a peculiarity of one of the constructs or may be of biological relevance. The interaction between Sina and Phyl is specific since bicoid fused to LexA only weakly transactivated lacZ in the presence of Sina–TA. To confirm the apparent in vivo association of Sina and Phyl, in vitro association assays were performed [Fig. 6B]. Phyl protein was translated in vitro and incubated with a Sina–glutathione S-transferase [Sina–GST] fusion protein immobilized to glutathione beads. Phyl remained bound to immobilized Sina–GST following extensive washing of the beads, whereas it was not bound to immobilized Yan–GST or GST alone. Moreover, in control experiments Sina–GST did not associate with heterologous in vitro translated proteins such as TAF60, TAF30α, and TFIIEα [data not shown]. These results establish that Phyl interacts specifically with Sina.

Discussion

The role of pros in photoreceptor development

We have found that pros is required for proper R7 differentiation and axon connectivity in the adult retina. It is not absolutely required for R7 fate determination because loss of pros function causes no significant transformation between different cell types. However, pros may participate in R7 determination because it genetically interacts with sina, pnt, and yan in forming R7 cells. pros appears to control neural connectivity indirectly by regulating gene expression because the protein is nuclear localized and contains a divergent homeo domain. Although pros is expressed in the cone cells at a low level, it is unclear if it is needed for their differentiation.

Regulation of pros expression

The expression of pros is initially activated in the five cells that comprise the R7 equivalence group of the eye disc. Later, R7 cells exhibit elevated levels of pros expression as a response to Sev-mediated activation while transcription remains constant in the cone cells. Several lines of evidence suggest that regulation occurs by a two-step process. First, pros expression is initially detected in the R7 precursor cells prior to the estimated point of time when sev is activated [Mullins and Rubin 1991]. Second, initial detection of pros expression in the R7 precursor cell of sev mutants occurs at the same time as in wild type. Finally, cone cell precursors transformed with activated sev exhibit an initial low level of expression followed by a high level of expression.

It is possible that there is a causal relationship between the initial phase of pros transcription and the subsequent ability to be induced by Sev. Mutations in Ras1 and yan which block the first step also block the second step. It would imply that some genes such as pros are able to respond to induction only by having independently established transcriptional activity. This has been observed with MyoD activation during mesoderm induction in Xenopus [Rupp and Weintraub 1991]. Thus, competence of cells to respond to inductive signals could be regulated by the transcriptional responsiveness of genes. These genes might be required for determination or dif-
ferentiation. Some genes such as phyl may be regulated not by a two-step mechanism but by direct activation. Perhaps a single inductive interaction involves a combination of different modes of transcriptional activation. Our results suggest that after initiation of pros transcription, Sev-mediated induction leads to the accumulation of pros protein above a threshold level in the R7 cell. This level of pros protein might establish a positive feedback loop that stabilizes pros expression into pupal development, long after Sev signaling is complete. In cone cells, the low level of pros protein might lead to stable but low-level expression.

Transcription in the R7 equivalence group

Here, we report evidence implicating Ras1 signaling in the regulation of gene transcription in the R7 equivalence group. This is based on three findings that were made for pros transcription. First, a dominant negative form of Ras1 can block pros expression in any cell of the equivalence group. Second, a constitutively repressive form of yan that is unable to be phosphorylated by MAPK can block pros expression in the equivalence group. Third, in the absence of pnt activity, cells in the equivalence group fail to express pros. The first result establishes that Ras1 activity is required for pros transcription. The second result argues that Ras1 activates pros transcription by downregulating yan activity through a modulation of MAPK activity. The third result argues that pnt activation is critical for pros transcription.

One simple hypothesis to account for the roles of these factors is that the basal level of yan activity is sufficient to repress pros transcription and the basal level of pnt activity is unable to activate pros. Activation of Ras1 in cells of the R7 equivalence group counters the repression of pros by yan (by stimulating MAPK to phosphorylate Yan) and activates pnt to regulate positively pros transcription [Fig. 7A]. This model also explains our observation that the absence of yan causes pros expression in supernumerary cells. Several additional points about this model are worth noting. First, the nature of the signal that activates Ras1 in the R7 equivalence group is unknown. Possibly, the signal is mediated by contacts between uncommitted cells and differentiating photoreceptors in accordance with the sequential inductive mechanism operating at earlier stages of eye development (Zipursky and Rubin 1994). Formation of extra photoreceptor cells leads to formation of extra cone cells as a secondary event (Basler et al. 1991), possibly by increasing the number of cell contacts between differentiated and uncommitted cells. Second, activation of pros transcription depends on developmental timing. Ras1 is activated in all photoreceptor cells and yet only cells in the R7 equivalence group express pros. However, transient delay of R1 and R6 precursor cell fate determination leads to these cells expressing pros. We suggest that a signal timed to occur after R1 and R6 determination confers a specific transcriptional response within cells competent to receive the signal. This competence could be manifested through use of alternative signaling pathways not normally utilized in the other photoreceptors or by inhibition of the signal response in cells already undergoing differentiation. Third, our experiments do not address the possibility that Yan and Pnt act antagonistically toward each other by direct competition for common binding sites in the pros gene. However, both purified Yan and Pnt proteins bind specifically to two 5' - flanking sequences upstream of the pros transcription start site (R.C. Kauffmann and J. Zhang, unpubl.). Possibly, Yan binds to regulatory sequences with higher affinity than Pnt, occupying these elements and occluding Pnt from the gene.

Transcription in the R7 cell

Expression of pros is upregulated in cells of the equivalence group by Sev induction. In Figure 7B, we outline a hypothetical pathway for the relationship between sev and pros. A critical intermediate is Ras1; constitutively activated Ras1 is sufficient to up-regulate pros transcription, while a dominant negative form of Ras1 blocks Sev activation of pros. Likewise, yan and pnt are likely intermediates in sev signal transduction because absence
of *pnt* activity or constitutive *yan* activity blocks sev-mediated upregulation of *pros*.

The placement of *phyl* and *sina* into the pathway is more ambiguous. However, we propose that *sina* and *phyl* function in parallel to another pathway downstream of Ras1. There are two reasons for our proposal. First, only R7 cells that have activated Sev normally up-regulate *pros*. However, when *phyl* is ectopically synthesized in cone cells, *pros* is up-regulated in a *sev*-independent manner. This up-regulation depends on *sina* activity. Second, the absence of *phyl* or *sina* in an R7 cell has no effect on *sev*-dependent upregulation of *pros*. The first result argues that *phyl* activity is sufficient to up-regulate *pros* transcription provided *sina* is also present. The second result suggests that *sina* and *phyl* do not up-regulate *pros* transcription singlehandedly in an R7 cell. A second pathway makes their functions redundant. Possibly, the second pathway utilizes *yan* and *pnt*, which may be modulated to a greater degree in R7 than in other equivalence group members. In support of this hypothesis, it has been observed that Yan stability is lower in R7 than in cone cells (Rebay and Rubin 1995).

A simple hypothesis to account for placement of *sina* and *phyl* in a common pathway is that the two proteins must directly interact to be completely active. In this model, either protein by itself is inactive, but when complexed together they function synergistically to regulate transcription. Complex formation would normally be restricted to the R1/R6 and R7 cell subtypes, ensuring that synergistic activation is limited to these cells. Such a mechanism is reminiscent of muscle-specific gene expression that requires both ubiquitous and myogenic bHLH factors [Lassar et al. 1991]. This model accounts for our finding that Sina and Phyl proteins interact in vivo and in vitro. An alternative model that also fits our data is that interaction between Phyl and Sina is transient, resulting in activation of Sina.

Concluding remarks

Ras1 signaling is required to regulate *pros* expression at low levels in the equivalence group and at high levels in R7. How does signaling through the Ras1 pathway result in two different responses? In PC12 cells, treatment with NGF leads to neural differentiation, whereas treatment with EGF leads to cell proliferation [Marshall 1995]. Both factors signal through Ras and MAPK activation. The addition of NGF results in an elevation of RasGTP levels and prolonged MAPK activation for several hours. In contrast, increased levels of RasGTP and activation of MAPK persist for only a short time following stimulation by EGF. Likewise, the signal that activates Ras1 in the R7 equivalence group may lead only to transient activation of Ras1 and, by inference, MAPK. Transient activation of the signaling pathway would result in modest activation of the *pros* gene. Conversely, activation of the Sev RTK may result in prolonged activation of Ras1 and MAPK to generate higher levels of transcription of *pros* and initiate transcription of other target genes.

Materials and methods

Genetics

The following fly strains were used for genetic interactions: *sev-*N^Ac* (Fortini et al. 1993), *sev-*R^ac12* (Fortini et al. 1992), *sev-*R^ac17* (Allard et al. 1996), *yan*^L^ (Lai and Rubin 1992), *sev-*Y^ah1* (Rebay and Rubin 1995), 2sev-*phyl*, *phyl*, and *phyl*^L^ (Dickson et al. 1995), *sev-*Y^ah1* (Basler et al. 1991), *pnt/^* and *pnt/^* (Brunner et al. 1994a). The recessive lethal P-element insertion line l(3)10419 or *pros^10419* was obtained from the Spradling enhancer trap stock collection. The cytological map position of l(3)10419 was determined to be 86E (Berkeley Drosophila Genome Project). Complementation analysis of l(3)10419 and *pros^10419* indicated they were allelic. The l(3)10419 P-element inserted 5' of the gene in the promoter region as determined by Southern analysis. Clonal analysis in the eye was performed on *pros^10419* using the FLP/FRT system (Xu and Rubin 1993).

The *P(sev-pros)* transformation vector was made by cloning a 5.3-kb *pros* cDNA [Vaessin et al. 1991] into a *sev* expression plasmid [Chang et al. 1995]. Transgenic lines were generated by P-element-mediated germ-line transformation.

Prospéro antibodies

The start codon of *pros* cDNA was replaced with a DNA sequence encoding MetHisGly by PCR mediated site-directed mutagenesis. An Ndel–BanHI fragment from the engineered DNA corresponding to Met-1 to Ser-408 [Vaessin et al. 1991; GenBank accession number M61389] of the Pros protein was inserted into pET3c to generate pPros1. The Pros1 protein was purified from bacterial lysate by Ni^2+^-NTAagarose (Qiagen) affinity column and subsequently preparative SDS-PAGE for the immunization of mice [Harlow and Lane 1988]. Affinity purified antibodies were prepared by passing serum over a Pros1 affinity column and eluting with 0.1 M glycine-HCl (pH 2.5).

Histology and immunohistochemistry

Eye fills were done as previously described (Buckles et al. 1992). The adult heads were fixed and embedded, and sections were cut as described by Carthew and Rubin (1990). Samples were prepared for scanning electron microscopy as described by Kimmel et al. (1990) with the exception that hexamethyldisilazane [Ted Pella] was substituted for freon. The β-galactosidase activity of *pros*^10419* discs was assayed with X-gal as described by Moses and Rubin (1991).

Immunolabeling of eye discs and pupal retinas was by done as described by Zheng et al. (1995). For immunofluorescence labeling, Cy3- and FITC-conjugated IgGs were used [Chemicon and Cappel, respectively]. Staining of nuclei with propidium iodide was done as described in Neufield and Rubin (1994).

Expression plasmids

Plasmids expressing Yan119-GST (amino-terminal region of Yan with amino acids 1–389) and Yan121-GST (carboxy-terminal region of Yan with amino acids 388–732) were provided by Z.C. Lai [Pennsylvania State University, State College]. pP^ntC/GST was generated by ligating a 0.6-kb SacI–SalI fragment from pAcptn2 [O’Neill et al. 1994] into pGex-3X to generate pPntC/GST. The carboxy-terminal region of PntP2 from Ser-479 to Asp-718 is linked to GST through a single serine linker. pSina/GST was constructed by PCR amplification of Sina sequences from position 903 to 1851 [Carthew and Rubin 1990] and insertion into pGex-2T to create pSina/GST. *phyl* cDNA sequence was amplified by PCR from nucleotides 234–
The Phyl two-hybrid expression plasmids were derived from the vectors pEG202 and pG4-5 (Gyuris et al. 1993). Phyl PCR product (234–1446) was inserted into pG4-5 and pEG202 to generate pG4-5/Phyl and pEG202/Phyl, respectively. Deletion mutants PhylA130–400 and PhylA107–400 were constructed by a partial PstI digestion of pG4-5/Phyl and self-ligation to the XhoI site, resulting in removal of 3' coding sequences. The Sina two-hybrid expression plasmids were derived from pSina/GST by insertion of a fragment containing the entire sina coding sequence into pEG202 and pG4-5 to make pEG202/Sina and pG4-5/Sina, respectively. Met-1 of Sina is linked to the two-hybrid expression plasmids were derived from pSina/GST by insertion of a fragment containing the entire sina coding sequence into pEG202 and pJG4-5 to make pEG202/Sina and pJG4-5/Sina, respectively. Met-1 of Sina is linked to the two-hybrid domains through a nine amino acid linker sequence (FELGTRGSP). All ligation junction sequences and complete sequences of PCR products were confirmed by automated DNA sequencing (ABI).

In vitro binding
GST fusion proteins were produced in strain HMS174(DE3)-pLysS induced with 1 mM IPTG and grown at 37°C for 3 hr in LB medium supplemented with 1 mM ZnSO₄. Proteins were purified from whole cell extracts as described [Ausubel et al. 1993] with the addition of a protease inhibitor cocktail (1 µg/ml leupeptin, 1 µM benzamidine, 1 µg/ml pepstatin, 0.2 mM PMSF) to the lysis buffer. Phyl protein was produced by in vitro translation using pET/Phyl with the TNT Coupled Reticulocyte Lysate System [Promega]. GST fusion proteins were prebound to glutathione-Sepharose (Pharmacia) such that the concentration of protein on beads was 0.2 mg/ml. The protein–bead complexes were equilibrated in Buffer B [50 mM HEPES (pH 7.4), 120 mM NaCl, 1 µM ZnSO₄, 0.1% NP-40]. For in vitro binding, 12 µl of equilibrated protein–bead complex was mixed with 150 µl Buffer B, 7.5 µl 10 mg/ml BSA, and 12 µl of reticulocyte translate. The reaction was incubated at 4°C for 3 hr. Matrices were washed three times with 1 ml of Buffer B prior to electrophoresis and autoradiography.

Yeast two-hybrid association
Two-hybrid plasmids were transformed into the yeast strain EGY48 and selected as described [Gyuris et al. 1993]. For one two-hybrid test, strains were tested for galactose-dependent growth on Lcu– plates. For a second test, colonies were grown in liquid glucose/CM Ura– His– Trp– overnight and diluted into galactose/CM Ura– His– Trp– or glucose/CM Ura– His– Trp– to achieve log phase growth. β-Galactosidase activity was quantitatively assayed with ONPG as described [Ausubel et al. 1993].

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References
Allard, J.D., H.C. Chang, R. Herbst, H. McNeill, and M.A. Simon. 1996. The SH2-containing tyrosine phosphatase corkscrew is required during signaling by sevenless, Ras1 and Raf. Development 122: 1137–1146.

Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl. 1993. Current Protocols in Molecular Biology. Greene Publishing and John Wiley & Sons, Inc., New York, NY.

Basler, K., B. Christen, and E. Hafen. 1991. Ligand-independent activation of the sevenless receptor tyrosine kinase changes the fate of cells in the developing Drosophila eye. Cell 64: 1069–1081.

Biggs, W.H., K.H. Zavitz, B. Dickson, A. VanderStraten, D. Brunner, E. Hafen, and S.L. Zipursky. 1994. The Drosophila rolled locus encodes a MAP kinase required in the sevenless signal transduction pathway. EMBO J. 13: 1628–1635.

Brunner, D., K. Ducker, N. Oellers, E. Hafen, H. Scholz, and C. Klamt. 1994a. The ETS domain protein Pointed-P2 is a target of MAP kinase in the Sevenless signal transduction pathway. Nature 370: 386–389.

Brunner, D., N. Oellers, J. Szabad, W.H. Biggs, S.L. Zipursky, and E. Hafen. 1994b. A gain-of-function mutation in Drosophila MAP kinase activates multiple receptor tyrosine kinase signaling pathways. Cell 76: 875–888.

Buckles, G.R., D.J. Smith, and F.N. Katz. 1992. mip causes hyperinnervation of a retinotopic map in Drosophila by excessive recruitment of R7 photoreceptor cells. Neuron 8: 1015–1029.

Carthew, R.W. and G.M. Rubin. 1990. seven in abstentia, a gene required for specification of R7 cell fate in the Drosophila eye. Cell 63: 561–577.

Chang, H.C., N.M. Solomon, D.A. Wassarman, F.D. Karim, M. Thirrien, G.M. Rubin, and T. Wolff. 1995. phyllodip functions in the fate determination of a subset of photoreceptors in Drosophila. Cell 80: 463–472.

Dickson, B. 1995. Nuclear factors in sevenless signaling. Trends Genet. 11: 106–111.

Dickson, B.J., M. Dominquez, A. van der Straten, and E. Hafen. 1995. Control of Drosophila photoreceptor cell fates by Phyllodip, a novel nuclear protein acting downstream of the Raf kinase. Cell 80: 453–462.

Doc, C.O., Q. Chu-LaGriff, D.M. Wright, and M.P. Scott. 1991. The prospero gene specifies cell fates in the Drosophila central nervous system. Cell 65: 451–464.

Fortini, M.E., M.A. Simon, and G.M. Rubin. 1992. Signalling by the sevenless protein tyrosine kinase is mimicked by Ras1 activation. Nature 355: 559–561.

Fortini, M.E., I. Rebay, L.A. Caron, and S. Artavanis-Tsakonis. 1993. An activated Notch receptor blocks cell-fate commitment in the developing Drosophila eye. Nature 365: 555–557.

Gyuris, J., E. Golemis, H. Chertkov, and R. Brent. 1993. Cdil, a gene with Cdk2. Nature 365: 559–561.

Antibodies: A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Kimmel, B.E., U. Heberlein, and G.M. Rubin. 1990. The home-
Kauffmann et al.

odomain protein rough is expressed in a subset of cells in the developing *Drosophila* eye where it can specify photoreceptor cell subtype. *Genes & Dev.* 4: 712–727.

Lai, Z.C. and G.M. Rubin. 1992. Negative control of photoreceptor development in *Drosophila* by the product of the yan gene, an ETS domain protein. *Cell* 70: 669–620.

Lassar, A.B., R.L. Davis, W.E. Wright, T. Kadesch, C. Murre, A. Voronova, D. Baltimore, and H. Weintraub. 1991. Functional activity of myogenic HLH proteins requires hetero-oligomerization with E12/E47-like proteins in vivo. *Cell* 66: 305–315.

Marshall, C.J. 1995. Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-related kinase activation. *Cell* 80: 179–185.

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

Moses, K. and G.M. Rubin. 1991. *glass* encodes a site-specific DNA-binding protein that is regulated in response to positional signals in the developing *Drosophila* eye. *Genes & Dev.* 5: 583–593.

Mullins, M.C. and G.M. Rubin. 1991. Isolation of temperature-sensitive mutations of the tyrosine kinase receptor sevenless (sev) in *Drosophila* and their use in determining its time of action. *Proc. Natl. Acad. Sci.* 88: 9387–9391.

Neufeld, T.P. and G.M. Rubin. 1994. The *Drosophila* peanut gene is required for cytokinesis and encodes a protein similar to yeast putative bud neck filament proteins. *Cell* 77: 371–379.

O’Neill, E.M., I. Rebay, R. Tjian, and G.M. Rubin. 1994. The activities of two Ets-related transcription factors required for *Drosophila* eye development are modulated by the Ras/MAPK pathway. *Cell* 78: 137–147.

Rebay, I. and G.M. Rubin. 1995. Yan functions as a general inhibitor of differentiation and is negatively regulated by activation of the Ras1/MAPK pathway. *Cell* 81: 857–866.

Rupp, R.A. and H. Weintraub. 1991. Ubiquitous MyoD transcription at the midblastula transition precedes induction-dependent MyoD expression in presumptive mesoderm of X. laevis. *Cell* 65: 927–937.

Simon, M.A., D.D. Bowtell, G.S. Dodson, T.R. Laverty, and G.M. Rubin. 1991. Ras1 and a putative guanine nucleotide exchange factor perform crucial steps in signaling by the sevenless protein tyrosine kinase. *Cell* 67: 701–716.

Tomlinson, A. and D.F. Ready. 1987. Cell fate in the *Drosophila* ommatidium. *Dev. Biol.* 123: 264–275.

Vaessin, H., E. Grell, E. Wolff, E. Bier, L.Y. Jan, and Y.N. Jan. 1991. *prospero* is expressed in neuronal precursors and encodes a nuclear protein that is involved in the control of axonal outgrowth in *Drosophila*. *Cell* 67: 941–953.

Xu, T. and G.M. Rubin. 1993. Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development* 117: 1223–1237.

Zheng, L., J. Zhang, and R.W. Carthew. 1995. *frizzled* regulates mirror-symmetric pattern formation in the *Drosophila* eye. *Development* 121: 3045–3055.

Zipursky, S.L. and G.M. Rubin. 1994. Determination of neuronal cell fate: lessons from the R7 neuron of *Drosophila*. *Annu. Rev. Neurosci.* 17: 375–397.
Ras1 signaling and transcriptional competence in the R7 cell of Drosophila.

R C Kauffmann, S Li, P A Gallagher, et al.

*Genes Dev.* 1996, 10:
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