

# Identification of genes that interact with the *sina* gene in *Drosophila* eye development

(seven in absentia/*Ras1*/*R7* photoreceptor)

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**ABSTRACT** The *sina* gene encodes a nuclear protein that is required for the correct development of R7 photoreceptor cells in the *Drosophila* eye. We conducted a genetic screen for mutations that reduce the activity of *sina* and found mutations that define nine genes whose products may be required for normal *sina* activity. Three of these genes also appear to be essential for signaling by the Sevenless–Ras pathway in R7 cells, of which one gene corresponds to the rolled locus (*rl*). The *rl* gene is known to encode a mitogen-activated protein kinase necessary for signaling by Ras. These results suggest that the products of these three genes may participate in a signaling pathway involving both Ras and Sina, possibly by functionally linking these two proteins.

Cellular differentiation can be influenced by intercellular signals that are received by transmembrane receptors of the protein tyrosine kinase family. Upon binding of their ligands, these receptors initiate a series of events involving Ras (1), exchange factors for Ras (2–4), adaptor proteins (Grb2/Sem5/drk) (5–11), Raf kinase (12–17), and mitogen-activated protein (MAP) kinases (18). The differentiation of R7 photoreceptors during development of the *Drosophila* eye requires the receptor tyrosine kinase Sevenless (19). The Sevenless receptor is activated by a ligand presented by the neighboring R8 cell and encoded by the gene bride of sevenless (*boss*) (20, 21). Several genes appear to be required for transduction of the Sevenless-mediated signal, including ones that encode a Ras protein (*Ras1*; ref. 2), an exchange factor for Ras (*Sos*; refs. 2 and 22), a GTPase activating protein (*Gap1*; refs. 23 and 24), an SH3-SH2-SH3 adaptor protein (*drk*; refs. 5 and 6), a Raf kinase (*DRaf*; ref. 12), and a MAP kinase (*rl*; ref. 25).

Another gene that is required for the decision to develop into an R7 cell is seven in absentia (*sina*) (26). In the absence of this gene, the presumptive R7 cell adopts the fate of a nonneuronal cone cell as it does in sevenless (*sev*) and *boss* mutants. The *sina* gene product contains a C<sub>3</sub>-H-C<sub>4</sub> zinc finger motif characteristic of a number of nuclear regulatory proteins and is concentrated in the cell nucleus, suggesting that it is not likely to be involved in the early steps of signaling through Sevenless. However, *sina* is required for *sev*, *Ras1*, *DRaf*, and *rl* to function in the R7 cell since mutations in *sina* eliminate the ability of constitutively activated alleles of these genes to form R7 cells (refs. 12, 25, and 27; R.W.C., unpublished data). This suggests that Sina functions subsequent to the Sevenless receptor, Ras, Raf, and MAP kinase, possibly in response to signals mediated by those molecules.

We describe in this report a mutation screen for genes whose products may be essential for normal *sina* activity in the R7 cell. This screen has identified nine genes that when heterozygous make the phenotype of a partially defective *sina* allele more severe. Mutations in three of these genes also

attenuate the signaling activity of an activated *Ras1* allele, indicating that products of these genes may be necessary for normal signaling by Ras1. One of these genes is rolled (*rl*), which encodes a MAP kinase necessary for Sevenless signaling.

## MATERIALS AND METHODS

**Genetics.** Fly culture and crosses were performed according to standard procedures. The mutagenesis screen for Enhancer of *sina* [*E(sina)*] mutations was performed as follows. Male *sina*<sup>4</sup> *e tld*<sup>10E95</sup>/*TM1*, *p<sup>P</sup> kni sbd Me* flies that are isogenic for the second and third chromosomes were fed 25 mM ethyl methanesulfonate as described (28) and mated to *st sina*<sup>4</sup> *kni*<sup>IID</sup> *cu/TM3*, *Sb tld e<sup>s</sup>* females. The F<sub>1</sub> progeny (*sina*<sup>4</sup> *e tld*<sup>10E95</sup>/*st sina*<sup>4</sup> *kni*<sup>IID</sup> *cu*) were assayed by the corneal pseudopupil method (29) for the presence of R7 photoreceptors. Approximately 30,000 F<sub>1</sub> progeny were screened. Individuals that displayed abnormal pseudopupils were crossed to *st sina*<sup>4</sup> *kni*<sup>IID</sup> *cu/TM3*, *Sb tld e<sup>s</sup>* flies, and their progeny were examined for abnormal pseudopupils. Mutant individuals were then backcrossed to a *st sina*<sup>4</sup> *kni*<sup>IID</sup> *cu/TM3*, *Sb tld e<sup>s</sup>* strain in order to map the mutation to a chromosome and then balance the mutation. The chromosomes used for segregation analysis and balancing were *FM7c*, *CyO*, and *TM3a*, *st Sb*.

Second chromosome mutations were mapped meiotically with the markers *al*, *dp*, *b*, *pr*, *c*, *px*, and *sp*. Females heterozygous for the *E(sina)* chromosome and an *al*, *dp*, *b*, *pr*, *c*, *px*, and *sp* chromosome and heterozygous for a *st sina*<sup>4</sup> chromosome and *TM6*, *Ubx* were crossed to *al dp b pr c px sp/CyO*; *st sina*<sup>4</sup>/*TM6*, *Ubx* males. Individual males were collected and scored for marker phenotypes, and males carrying crossovers between particular markers were selected. These were mated to *st sina*<sup>4</sup>/*TM3a*, *st Sb* females, and their progeny were scored for the *E(sina)* phenotype. A similar mapping experiment was performed for the third chromosome mutations using *ru h th st cu sr e ca* flies. However, in this case only flies with recombinant chromosomes that also carried *sina*<sup>4</sup> were tested for the *E(sina)* phenotype. In all cases, 25–100 recombinant chromosomes were scored. Mutations were assigned to complementation groups by crossing pairs of lines and scoring for any recessive phenotype.

**Histology.** Fixation and sectioning of adult *Drosophila* eyes was performed as described (26). Scanning electron microscopy was performed as described (30).

**Mosaic Analysis.** Clones of cells homozygous for *E(sina)* mutations were generated by FRT-FLP-mediated mitotic recombination as described (31). The *E(sina)*<sup>+</sup> chromosome was marked with the cell-autonomous white gene (*w*), which was provided on a transposable element (T. Laverty, personal communication). All flies carried one *sina*<sup>+</sup> allele.

which was present in both  $w^-$  and  $w^+$  cells. Eyes that contained  $w^-$  clones were fixed and sectioned as described (26).

## RESULTS AND DISCUSSION

**The Enhancer of *sina* Screen.** To identify genes that interact with *sina*, we screened for extragenic mutations that enhance the phenotype of a weak *sina* mutant. We constructed the screen in such a way that some of these mutations might be detected as loss-of-function mutations, and thus we would find the largest possible number of potential genes that interact with *sina*. Since the level of activity of a gene is proportional to gene copy number in *Drosophila* (32), a loss-of-function mutation in one copy of an interacting gene might cause a reduction in the activity of that gene sufficient to disrupt R7 cell formation. A requirement for detecting loss-of-function mutations in this manner is to reduce *sina* activity to a level in which it is barely able to form R7 cells. Under these conditions, an extragenic loss-of-function mutation would give a dominant *E(sina)* phenotype. Such a strategy for a mutagenesis screen was successful in identifying genes that interact with *sev* in R7 formation (2).

Since it was unclear what level of *sina* activity would be ideal for the screen, we initially tested existing mutant *sina* alleles for their suitability. One allele, *sina*<sup>4</sup>, when homozygous (*sina*<sup>4</sup>/*sina*<sup>4</sup>) gave flies that were predominantly wild type for R7 cell formation, while when heterozygous with a deletion of the *sina* gene (*sina*<sup>4</sup>/-) gave flies that were predominantly missing R7 cells (Fig. 1). The *sina*<sup>4</sup> mutation is a substitution of a tyrosine residue for a histidine (residue 184) in the Sina protein (26), and it does not affect protein abundance or localization as judged by immunohistochemistry of *sina*<sup>4</sup> mutant tissues with antibodies to Sina (data not shown). The screen for *E(sina)* mutations was conducted as outlined in Fig. 1. The assay for the presence of R7 cells was

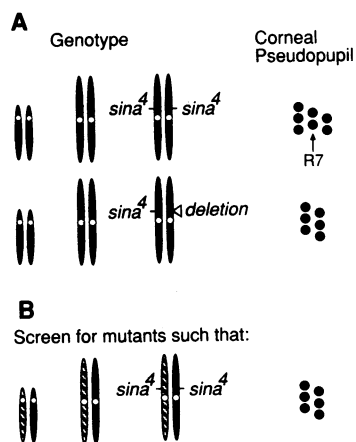


FIG. 1. Scheme for *E(sina)* mutagenesis screen. (A) Summary of the phenotypes of the *sina*<sup>4</sup> allele in different doses. Chromosomal complement of the X and two major autosomes in *Drosophila melanogaster* are represented on the left. The *sina* locus is located on the third chromosome. Solid circles on the right are a representation of the observed corneal pseudopupil in animals with the indicated number of copies of the *sina*<sup>4</sup> allele. The corneal pseudopupil is a virtual image of the photoreceptor endings from a group of neighboring ommatidia. The R7 photoreceptor is represented by the central circle in the image seen in the animal with two copies of the *sina*<sup>4</sup> allele. It is absent in the image seen in the animal with one copy of the *sina*<sup>4</sup> allele, and instead a different pattern is observed. (B) The screen was performed on animals with two copies of the *sina*<sup>4</sup> allele and one or more mutagenized chromosomes. Mutagenized chromosome is hatched; nonmutagenized chromosome is solid. Individuals exhibiting a corneal pseudopupil missing the R7 image were kept as potential *E(sina)* mutants.

the corneal pseudopupil method, which can be carried out on live anesthetized flies (29). Reconstruction experiments established that this assay was sensitive enough to easily distinguish *sina*<sup>4</sup>/- from *sina*<sup>4</sup>/*sina*<sup>4</sup> flies. We screened for ethyl methanesulfonate-induced mutations that resulted in *sina*<sup>4</sup>/*sina*<sup>4</sup> flies with fewer R7 cells. Approximately 30,000 progeny of mutagenized flies were screened, and 14 such *E(sina)* mutations were isolated and mapped to individual chromosomes.

The phenotypes of these mutations were examined in greater detail. Microscopic examination of eye sections from flies carrying *E(sina)* mutations confirmed that the fraction of R7 cells was reduced as initially judged by the corneal pseudopupil assay. Eyes from *sina*<sup>4</sup>/*sina*<sup>4</sup> flies that did not carry an *E(sina)* mutation contained 80% wild-type ommatidia (Fig. 2A). In contrast, eyes from flies with only one copy of *sina*<sup>4</sup> contained 10% wild-type ommatidia (Fig. 2B). Eyes from *sina*<sup>4</sup>/*sina*<sup>4</sup> flies that also carried an *E(sina)* mutation contained 10–54% wild-type ommatidia, depending on the *E(sina)* mutation. The ommatidia that were not wild-type were missing R7 cells. This was the only detectable phenotype for nine mutations (Fig. 2C and D). However, five of the *E(sina)* mutations also led to occasional ommatidia that exhibited other phenotypes. *E(sina)2* and *E(sina)4* occasionally resulted in missing secondary pigment cells (Fig. 2E), and *E(sina)6<sup>M</sup>* resulted in rare ommatidia with an extra outer photoreceptor cell (Fig. 2F). A few ommatidia were missing R7 plus one or two outer photoreceptor cells in flies carrying the *E(sina)2*, *E(sina)4*, *E(sina)5*, or *E(sina)9* mutations (Fig. 2G). The dominant phenotypes of the *E(sina)* mutations were restricted to the eye except for *E(sina)9*, which exhibited a bristle phenotype that resembled the bristle phenotype of strong mutant alleles of *sina*. This mutation appears to enhance both the eye and bristle phenotypes of *sina*. None of these mutations as heterozygotes produced a mutant phenotype in a fly that contained a wild-type *sina* allele.

The *E(sina)* mutations were mapped by recombination to approximate chromosomal positions. Several mutations mapped to the same regions and were possibly allelic to each other. Flies carrying these mutations were crossed to each other to test if they failed to complement for a detectable recessive phenotype. One group of four mutations failed to complement for a phenotype that resembled a strong *sina* mutant phenotype, and all four mutations mapped to the *sina* locus. These *E(sina)* are likely intragenic mutations of the *sina* gene that exhibit a more severe phenotype. A second group of two mutations failed to complement for a recessive wing vein phenotype. The posterior crossvein was incomplete and sometimes an additional longitudinal vein branched off the crossvein (Fig. 2H). These flies were otherwise wild type in appearance. The results of mapping and complementation experiments are summarized in Table 1.

The chromosomes carrying each *E(sina)* mutation also carried recessive-lethal mutations. The recessive-lethal phenotype was unrelated to the *E(sina)* mutations in *sina*, *E(sina)5*, and *E(sina)6* since multiple mutant alleles of these genes in trans are not recessive lethal. When the other *E(sina)* mutations were mapped by recombination, the recessive-lethal phenotype was not separated from the *E(sina)* phenotypes, suggesting that the same mutation is responsible for both phenotypes.

Analysis of the 14 *E(sina)* mutations identified at most 10 *E(sina)* genes. Most genes are represented by a single allele. The low frequency of alleles could mean that they are either rare gain-of-function alleles or loss-of-function alleles that were difficult to isolate. Five *E(sina)* mutations appear to represent loss-of-function alleles. *E(sina)2* and *E(sina)8* are allelic to chromosomal rearrangements and deletions that also exhibit comparable *E(sina)* phenotypes, and *E(sina)5*, *E(sina)7*, and *E(sina)9* are allelic to loss-of-function muta-

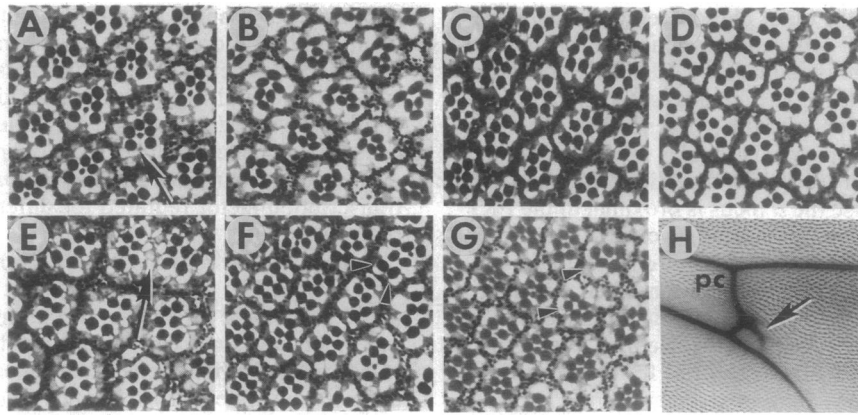


FIG. 2. Phenotypes of the *E(sina)* mutations. (A–G) Light micrographs of 1- $\mu$ m sections of adult eyes. Genotypes are *sina*<sup>4</sup>/*sina*<sup>4</sup> (A), *sina*<sup>4</sup>/*Df(3R)st-k2* (B), *E(sina)2 sina*<sup>4</sup>/*sina*<sup>4</sup> (C), *E(sina)7/+; sina*<sup>4</sup>/*sina*<sup>4</sup> (D), *E(sina)4 sina*<sup>4</sup>/*sina*<sup>4</sup> (E), *E(sina)6<sup>M4</sup>/+; sina*<sup>4</sup>/*sina*<sup>4</sup> (F), *E(sina)5 sina*<sup>4</sup>/*sina*<sup>4</sup> (G). Dark circular structures in each ommatidium are the light-trapping rhabdomeres of the photoreceptors. The rhabdomere of the R7 cell is in the center of each wild-type ommatidium and is highlighted by an arrow in A. Larger outer rhabdomeres belong to photoreceptors R1–R6. Rhabdomere of R8 lies proximally in the retina and is not visible in these sections. In A, the R7 rhabdomere is present in all but two ommatidia. In B–G, many more ommatidia are lacking R7 rhabdomeres and thus R7 photoreceptors. In addition, other defects are occasionally seen. In E, a missing secondary pigment cell is indicated by an arrow; in F, an ommatidium with an extra outer rhabdomere is indicated by arrowheads; in G, missing outer rhabdomeres are indicated by arrowheads. In each panel, the area of the retina shown is in the dorsal/posterior quadrant. The leftmost row of ommatidia shown is five rows from the posterior edge of the retina. This is the area of the retina that was assayed by corneal pseudopupil. Like *sev*, there is an apparent difference in expressivity of the *sina* allele depending on the location of an ommatidium in the retina. Ommatidia in anterior/ventral regions generally lack R7 cells, while ommatidia in posterior/dorsal regions are wild type in a *sina*<sup>4</sup> animal. (H) Part of the wing from an *E(sina)6<sup>M4</sup>/E(sina)6<sup>L3</sup>; sina*<sup>4</sup>/*sina*<sup>4</sup> fly. Although the overall structure of the wing is normal, a short longitudinal vein (arrow) emerges from each posterior crossvein (pc).

tions in previously identified genes (Table 1; see ref. 33). The most likely reason for the low frequency in isolating more alleles of these genes is that difficulties were encountered when isolating and mapping many of the *E(sina)* mutations that were initially detected. In addition to their effects on photoreceptor development, *sina* mutants also result in a reduction in adult vitality and fertility, and it is possible that many potential *E(sina)* mutants increased the severity of these phenotypes.

Table 1. Summary of *E(sina)* genes

Gene	Mutant allele(s)	Enhancer strength*	Recombination map position
<i>sina</i>	C2, F1, I1, N6	3.9	3–46
<i>E(sina)1</i>	D1	1.9	3–20
<i>E(sina)2</i> , <i>E(sev)3E</i> <sup>†</sup>	H3	3.2	3–55; 88A <sup>‡</sup>
<i>E(sina)3</i>	K1	1.9	2–8
<i>E(sina)4</i>	K5	2.8	3–44
<i>E(sina)5</i> , <i>gl</i>	L1	2.4	3–62
<i>E(sina)6</i>	L3, M4	2.6	2–64
<i>E(sina)7</i> , <i>rl</i>	M1 <sup>¶</sup>	2.6	2–55
<i>E(sina)8</i> , <i>pnut</i>	H2	3.9	2–60
<i>E(sina)9</i>	12.26	3.8	3–107

\*Calculated as ratio of percentage of R7<sup>-</sup> ommatidia in *E(sina)/+; sina*<sup>4</sup> eyes over percentage of R7<sup>-</sup> ommatidia in *sina*<sup>4</sup> eyes. For *sina*<sup>4</sup>/*Df(3R)st-k2* flies, this ratio is 4.0.

<sup>†</sup>Isolated by T. Cutforth and G.M.R. and tested positive for non-complementation.

<sup>‡</sup>Based on breakpoint of an isolated translocation (T. Cutforth and G.M.R., unpublished data).

<sup>§</sup>Repeated recombination mapping has failed to localize mutation. Since polytene chromosomes appear cytologically normal, the mutation may represent a multigenic alteration.

<sup>¶</sup>The M1 chromosome contained two recessive-lethal mutations, one in the *rl* locus and another that mapped to 46C1-2. When these mutations were separated by recombination, each had an *E(sina)* phenotype. The mutation that mapped to the *rl* locus had the indicated phenotypic strength, and the mutation that mapped to 46C1-2 had a phenotypic strength of 1.4. It is not included in the table.

*E(sina)5* mapped near 62 map units on the third chromosome and failed to complement several alleles of the *glass (gl)* gene. *gl* is required for proper differentiation of all photoreceptors and encodes a zinc finger transcription factor (34). The *E(sina)5* mutation was similar in its phenotype to loss-of-function *gl* alleles. We henceforth refer to *E(sina)5* as *gl<sup>ES5</sup>*.

The *E(sina)7* mutation was mapped very close to *rl* on the second chromosome (Table 1). It has been shown that the *rl* gene encodes a homolog of MAP kinase that is required for Sevenless-mediated signaling (25). Two lines of evidence suggest that *E(sina)7* is a loss-of-function mutation of *rl*. First, the *E(sina)7* mutation failed to complement the lethality of *rl* loss-of-function alleles *rl<sup>10a</sup>* and *rl<sup>10b</sup>*. Second, the *rl<sup>10a</sup>* and *rl<sup>10b</sup>* mutations exhibit an *E(sina)* phenotype similar in strength to the *E(sina)7* mutation (data not shown). Therefore, we henceforth refer to *E(sina)7* as *rl<sup>ES7</sup>*.

*E(sina)8*, which we have renamed peanut (*pnut*), was cloned by transposon tagging and is related in sequence to the CDC3, CDC10, CDC11, and CDC12 genes of *Saccharomyces cerevisiae* (33). Animals homozygous for null alleles of *pnut* show defects in cytokinesis and die at the larval–pupal transition.

*E(sina)9* mapped near the tip of the right arm of the third chromosome and failed to complement several mutations and deficiencies in this region. Cytological analysis of the *E(sina)9* chromosome revealed a small deficiency in the 100D region (data not shown). One gene that maps to this region, fat facets (*faf*), is required for cell interactions in the developing eye (35). Mutations in *faf* failed to complement the *E(sina)9* mutation and act as enhancers of *sina*. However, the strength of enhancement by null *faf* alleles is considerably less than that of *E(sina)9* (data not shown), indicating that the enhancement effect of *E(sina)9* may be due to the loss of multiple genes in this region.

**Function of *E(sina)* Genes in Eye Development.** Mutations in both copies of the *E(sina)6* gene resulted in animals with completely wild-type eyes (data not shown). However, since the other *E(sina)* mutations reside on recessive-lethal chromosomes, it was not clear what the consequence of mutating both copies of the other *E(sina)* genes would be on develop-

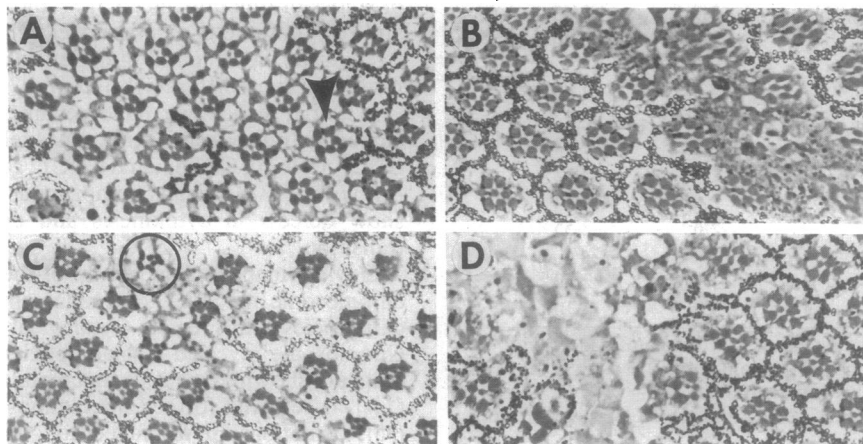
ment of the R7 cell and the other photoreceptors. Therefore, we generated clones of cells homozygous for the *E(sina)* mutations in heterozygous animals by FRT-FLP-mediated mitotic recombination (31). The homozygous *E(sina)* cells were marked by the absence of the *w*<sup>+</sup> gene. The *w* gene is required for formation of pigment granules in photoreceptors and pigment cells, which are visible in sections through adult retinas (Fig. 3A). Because the *rl* locus is proximal to the FRT element on the second chromosome, we were unable to generate mitotic clones of the *rl*<sup>ES7</sup> mutation. The homozygous phenotypes of the other *E(sina)* genes fell into three different classes. First, the homozygous *E(sina)2* clones are wild type, suggesting that this mutant allele may retain some partial activity or is rescued by activities from other genes. Second, no mitotic clones were observed with the *E(sina)9* mutation, as expected for a chromosomal deficiency. Third, mutations in *E(sina)1*, *E(sina)4*, *gl*<sup>ES5</sup>, and *E(sina)8* appear to affect photoreceptor development. Analysis of a number of mosaic ommatidia, as judged by the presence of at least one wild-type and one mutant pigment cell, indicates that cells homozygous for the *E(sina)1* and *gl*<sup>ES5</sup> mutations are greatly underrepresented as photoreceptors. For *gl*<sup>ES5</sup>, no homozygous mutant photoreceptors were observed (Fig. 3D) as was previously observed with other *gl* alleles (34). Clones of *E(sina)1* cells exhibited abnormal mutant photoreceptors (Fig. 3B). Most *E(sina)4* mutant ommatidia had the normal complement of photoreceptors, but their rhabdomeres were greatly reduced in size (Fig. 3C). These results suggest that these genes play a role in processes common to the development of all photoreceptors. A similar observation has been made with several of the Enhancer of sevenless [*E(sev)*] genes that are also required for development of all photoreceptors (2). Clones of the *E(sina)8* mutation were much smaller than their wild-type twin spots, suggesting that this mutation affects cell proliferation or survival. Most ommatidia in these clones were wild type, but occasional extra or missing photoreceptor cells were observed.

Many of the genes that interact with *sev* and *sina* appear to function in aspects of development other than R7 specification, including development of other cells in the eye. This is borne out by the number of *E(sev)* and *E(sina)* genes that

appear to be essential for both viability and proper development of all photoreceptor cells. Some of the *E(sev)* genes encode components of a second messenger system downstream of several receptor tyrosine kinases and include the *Ras1*, *Sos*, and *drk* genes (2, 5). At least one of the *E(sina)* genes, *rl*, encodes another component of the same second messenger system (25). Another *E(sina)* gene, *gl*, encodes a transcription factor required for differentiation of all photoreceptors (34).

**Genetic Interactions Between *E(sina)* and *Ras1*.** This screen was designed to identify genes that encode proteins that might participate directly in mediating regulation of R7 development by *Sina*. One of the identified genes encodes a MAP kinase that functions in the second messenger pathway downstream of Sevenless. It is possible that some of the other genes also encode elements of this pathway, although it is unlikely that they encode earlier-acting elements since none appears to coincide with any of the previously characterized *E(sev)* genes. Moreover, null alleles of *sev*, *Ras1*, *DRaf*, and *DSor* do not behave as *E(sina)* mutations in our genetic assay (R.W.C. and M. Simon, unpublished data). To test whether the *E(sina)* genes encode elements downstream of *Ras1*, we examined the abilities of the *E(sina)* mutations to attenuate *Ras1* activity in forming R7 cells. We tested each *E(sina)* mutation for its ability to suppress the phenotype of a constitutively activated form of the *Ras1* gene. This activated *Ras1* allele results in heterozygous animals containing supernumerary R7 cells in the eye (27). This phenotype was dependent on the dosage of the activated *Ras1* transgene (data not shown), suggesting that the phenotype was sensitive to different levels of *Ras1* activity. If the *E(sina)* mutations attenuate signaling by activated *Ras1*, the effect would be seen as a suppression of the activated *Ras1* phenotype.

Flies carrying each *E(sina)* mutation were crossed to flies carrying the activated *Ras1* gene, and flies heterozygous for both genes were examined. While 11 of the *E(sina)* mutations had no effect on the *Ras1* phenotype, three mutations suppressed the *Ras1* phenotype and restored the eye to a nearly wild-type appearance (Fig. 4). Sections of eyes from these flies confirmed that the suppression was due to a dramatic reduction in the number of supernumerary R7 cells seen in



**FIG. 3.** Requirement for *E(sina)* genes in eye development. Clones of cells homozygous for an *E(sina)* mutation and for a null allele of the marker *w* gene were generated by mitotic recombination. The *w* gene is required for formation of pigment granules, which are seen in photoreceptors as black specks at the base of each rhabdomere and are seen in pigment cells as a lattice of granules. (A) Clone of *w*<sup>-</sup> cells without a linked *E(sina)* mutation. Arrowhead marks a *w*<sup>+</sup> R5 cell in an otherwise *w*<sup>-</sup> ommatidium. (B–D) Clones of *w*<sup>-</sup> cells that are also homozygous for the *E(sina)1* (B), *E(sina)4* (C), and *gl*<sup>ES5</sup> (D) mutations. Ommatidia in the *E(sina)1* clone are spaced incorrectly and contain far fewer numbers of photoreceptors than normal. Cells homozygous for *E(sina)1* were represented as photoreceptors in mosaic ommatidia by a 1:2 ratio with wild-type cells rather than the predicted 1:1 ratio observed in A. Analysis of normally constructed mosaic ommatidia revealed no absolute requirement for *E(sina)1* in any subset of photoreceptors. However, there was a significant bias for *w*<sup>+</sup> R1, R6, and R7 cells in such ommatidia. Photoreceptors homozygous for *E(sina)4* contain rhabdomeres that are much smaller than wild type (circle outlines a mutant ommatidium) and may possibly be R7-like (C). Cells homozygous for *gl*<sup>ES5</sup> did not form photoreceptors in any of the clones that were analyzed (D). All of the photoreceptors at the borders of clones are *w*<sup>+</sup>, and many ommatidia at the clone boundaries contain fewer photoreceptors than normal.

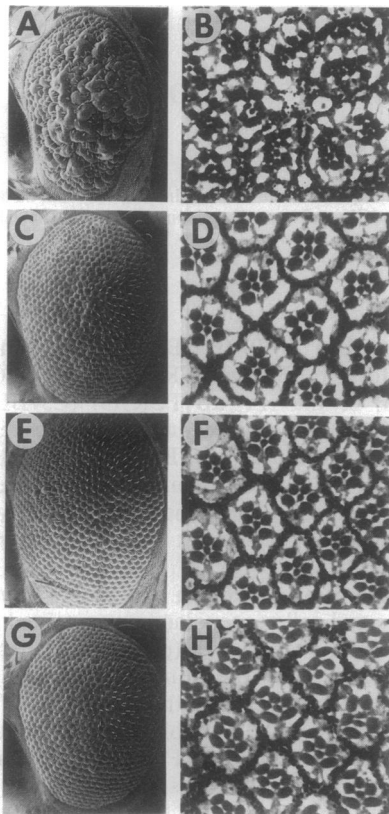


FIG. 4. Effect of *E(sina)* mutations on the phenotype of activated *Ras1*. (A, C, E, and G) Scanning electron micrographs of adult eyes from flies carrying one copy of *P[ry, sevRas1<sup>Val12</sup>]5\*3*. (B, D, F, and H) Plastic sections through adult eyes of flies carrying one copy of *P[ry, sevRas1<sup>Val12</sup>]5\*1*. The *5\*3* P-element insert results in a strongly activated *Ras1* phenotype, whereas the *5\*1* P-element insert results in a weaker phenotype. A and B correspond to *sevRas1<sup>Val12</sup>; +/+* flies. The eye appears severely deformed because of the large number of supernumerary R7 cells in each ommatidium. C and D correspond to *sevRas1<sup>Val12</sup>; E(sina)3/+* flies. The eye appears more wild type, and the number of supernumerary R7 cells in each ommatidium is greatly reduced. Similar results are seen in *sevRas1<sup>Val12</sup>; gl<sup>ES5</sup>/+* (E and F) and in *sevRas1<sup>Val12</sup>; rl<sup>ES7</sup>/+* (G and H) flies.

*Ras1* flies (Fig. 4). The interaction between the *E(sina)* genes and *Ras1* was observed with three different activated *Ras1* alleles. One of the suppressing mutations is *rl<sup>ES7</sup>*. Indeed, other alleles of *rl* exhibit a similar interaction (25) consistent with a mechanism by which Ras proteins transduce their signals through MAP kinase activation (18). The complex genetic interactions suggest that *rl* and some *E(sina)* genes participate in both *Ras1*- and *sina*-mediated activities, possibly by functionally linking the two elements.

**Concluding Remarks.** We have isolated mutations that decrease the effectiveness of *sina* to function in R7 cell development. These mutations define nine genes whose normal products may be required for *sina* activity. At least four of these genes function in normal eye development since mutant alleles prevent photoreceptor development. Three of the genes also appear to be essential for signaling by *Ras1* during R7 cell development, and one corresponds to the gene *rl*, which encodes a MAP kinase required for Sevenless signaling.

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