A Genetic Screen to Identify Components of the sina Signaling Pathway in Drosophila Eye Development

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ABSTRACT

Specification of the R7 photoreceptor cell in the developing Drosophila eye requires the seven in absentia (sina) gene. We demonstrate that ectopic expression of sina in all cells behind the morphogenetic furrow disrupts normal eye development during pupation, resulting in a severely disorganized adult eye. Earlier events of cell fate specification appear unaffected. A genetic screen for dominant enhancers and suppressors of this phenotype identified mutations in a number of genes required for normal eye development, including UbcD1, which encodes a ubiquitin conjugating enzyme; SR3-4a, a gene previously implicated in signaling downstream of Ras1; and a Drosophila homolog of the Sin3A transcriptional repressor.

During development of multicellular organisms, cell fate determination is often influenced by interactions between neighboring cells. Our understanding of the molecular mechanisms underlying such interactions has advanced greatly in recent years, and has been aided by a number of model systems. Development of the R7 photoreceptor in the Drosophila compound eye has been a particularly profitable system for dissecting the intercellular signaling pathways used to specify cell fate (reviewed by Wasserman et al. 1995; Simon 1994; Zipursky and Rubin 1994). A large number of genetic, molecular, and biochemical studies have led to identification of many of the signaling components required for R7 development, their biochemical functions, and their hierarchy within the R7 signaling pathway.

A primary event of R7 specification is activation at the R7 cell surface of the SEVENLESS (SEV) receptor tyrosine kinase (Tomlinson and Ready 1986). The R7 cell is singled out from a group of five equipotential cells (R7 and four lens-secreting cone cells) by virtue of its contacts with the neighboring R8 photoreceptor, which expresses the ligand for SEV, BRIDE OF SEVENLESS (BOSS; Reinke and Zipursky 1988). Following receptor activation, the signal is passed from the cell surface to the nucleus by a signal transduction pathway common to all eight photoreceptors (Simon et al. 1991). Ligand binding leads to activation of the RAS1 protein, which initiates a series of successive phosphorylation events via the mitogen-activated protein kinase (MAPK) pathway. Activation of this pathway causes activated MAPK to accumulate in the nucleus, where it phosphorylates a number of nuclear proteins that presumably control expression of genes that bring about photoreceptor differentiation. Among such targets of MAPK are the transcription factors JUN and POINTED, whose activities are required for photoreceptor development, and YAN, a negative regulator of photoreceptor fate (Bohmann et al. 1994; O’Neill et al. 1994; Rebay and Rubin 1995).

An additional nuclear protein required for successful transmission of the RAS-MAPK inductive signal is SINA, a ring-finger protein of unknown biochemical function (Carthew and Rubin 1990). Loss of function mutations in seven in absentia (sina) prevent normal specification of the R7 cell, and block transformation of cone cells into photoreceptors caused by experimental manipulation of the RAS-MAPK pathway in these cells (Carthew and Rubin 1990; Fortini et al. 1992). However, the requirement for sina in this pathway is restricted to the R7/cone cell group, as photoreceptors of the R1-6 class are specified correctly in sina mutants. Presumably, neuronal induction by the RAS-MAPK pathway in these cells does not require the biochemical activity provided by SINA, or this activity is provided by an alternative component. Although the precise nature of SINA’s biochemical activity is unknown, we and others have recently shown that SINA is required for the degradation of TRAMTRACK (TTK), a transcriptional repressor of neuronal cell fate (Tang et al. 1997; Li et al. 1997). A potential role for SINA in protein degradation also appears to be conserved in vertebrates (Hu et al. 1997).

One approach toward further elucidating SINA’s function is to identify additional signaling components with which it interacts. For example, two-hybrid and im-

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munoprecipitation studies have revealed that SINA can form a complex with TTK and PHYLOPOD, a transcriptional target of the RAS-MAPK pathway (Kaufmann et al. 1996; Tang et al. 1997; Li et al. 1997). Genetic interaction screens can provide a powerful complement to these biochemical studies. We have previously identified a number of mutants that interact with a partial loss of function sina allele (Carta et al. 1994). In this report we describe a complementary screen for extragenic mutations that modify a phenotype caused by overexpression of sina in the developing eye.

MATERIALS AND METHODS

sina misexpression: The sina coding region was amplified by PCR and cloned into the EcoRI site of pGM3 (Hay et al. 1994) to create the construct P[w, GM R-sina]. Transgenic GM R-sina lines were generated by germline transformation. To determine whether functional SINA protein was produced by the P[w, GM R-sina] transgene, weak GM R-sina lines were tested for the ability to rescue a strong loss of function sina mutation. Sections of eyes of the genotype P[w, GM R-sina]/++; sina3 revealed that approximately 50% of the ommatidia contained an R7 cell, whereas no R7 cells were observed in sina3 eyes lacking the P[w, GM R-sina] transgene.

GM R-sina modifier screen: Isolation, balancing, and chromosomal linkage analysis of dominant enhancers and suppressors of the GM R-sina eye phenotype were facilitated by the construction of balancer chromosomes bearing the P[w, GM R-sina] transgene. An X-linked P[w, GM R-sina] insert was mobilized in males carrying the balancers CyO and TM3, and male progeny demonstrating a GM R-sina eye phenotype were scored for the subsequent cosegregation of eye phenotype and dominant balancer markers. Two lines were isolated that carry balancer chromosomes with P[w, GM R-sina] insertions that provide sufficient levels of sina expression to produce a rough eye phenotype suitable for screening. A CyO, P[w, GM R-sina] line was used in the modifier screen. Male w1118 flies isogenic for the second and third chromosomes were fed 25 mM EMS as described (Lewis and Bacher 1968) or treated with 4000 R of X rays (1000 sec at 115 keV, 5 mA), and then mated to w1118; Adv/CyO, P[w, GM R-sina] females. F1 progeny carrying the CyO, P[w, GM R-sina] chromosome and a set of mutagenized chromosomes were assayed for an increase or decrease in the degree of severity of the eye phenotype. Approximately 40,000 (EMS) and 56,000 (X rays) F1 progeny were scored. Putative enhancers and suppressors were backcrossed to the w1118; Adv/CyO, P[w, GM R-sina] stock to test for chromosomal linkage, and balanced over CyO, P[w, GM R-sina] or TM3, P[w, GM R-sina].

Modifier mutants were mapped meiotically by mating w1118; Adv/CyO, P[w, GM R-sina] males with females carrying a representative allele of each complementation group in trans to a multiply marked chromosome. F1 recombinant males with the modifier phenotype were then individually mated to marker females, and the F2 generation was scored for the presence of each marker phenotype. After establishing a meiotic map position for each group, the map positions were further refined by lethal complementation tests using chromosomal deficiencies and P elements from each region, and by cytological examination of polytene chromosomes from X-ray alleles. Non-complementing deficiencies and P elements were as follows: SS2-1: P[w][12][16624 and P[ry][12][623]; SS2-1 and ES3-1: Df(3R)Tk110 and Df(3R)Antp17; SS3-3: P[w][13][1462, P[w][13][1782, P[w][13][1635, P[w][13][128], SS3-4: SR3-4a[192 and SR3-4a[206 (Karim et al. 1996); SS3-5: Df(3L)B119 and Df(3L)stf13; SS3-7: Df(3L)AC1 and P[w][13][2240]; ES2-1: Df(2R)jp1; ES2-2: Df(2L)TRW84, Df(2L)TW161, P[w][12][10523; ES2-3: Df(2R)vg135, Df(2R)vg-C, P[w][12][1740], P[w][12][1869]. The map position of ES1-1 was assigned based on rescue of its lethality by the duplication Dp[12]/v[656].

sina interaction test: A representative allele of each group was crossed into a sina genetic background to generate flies heterozygous for the modifier mutation, and homozygous for sina. Ten eyes were sectioned for each group, and scored for percentage ommatidia lacking R7 cells. Tester alleles and percentage R7– ommatidia were as follows: SS2-1[LS], 22%; SS3-3[20min], 17%; SS3-4[65], 23%; SS3-6[97], 39%; SS3-7[98], 39%; ES1-1[64], 29%; ES1-4, 15%; ES2-2, 19%; ES2-3, 62%; ES3-4[20], 67%; ES3-5[20], 11.5%; sina2 homozygotes without a modifier mutation lack R7 cells in 20% of ommatidia. No recombinants were obtained between sina and the SS3-5 locus.

FRT-P-element transgenesis: One or more representative alleles of each group was recombined onto a centromere-proximal FRT chromosome, and mitotic clones were generated as described (Xu and Rubin 1993). No recombinants were obtained between the ES3-1 and SS3-5 groups and their centromere-proximal FRT sites, so we were unable to examine the clonal phenotypes of these groups. In addition, Star and glass homozygous phenotypes have been described previously (Hberlein and Rubin 1991; Moses et al. 1989) and were not analyzed in this study.

Molecular analysis: Plasmid rescue (Pirotta 1986) was used to isolate genomic DNA adjacent to the P element insertions P[w][12][1740] and P[w][12][1869] (ES2-3); and P[w][13][1462] and P[w][12][1328] (SS3-3). These genomic fragments were then used as probes to screen cDNA libraries prepared from eye-antennal imaginal disc (A. Cowman, unpublished results) and embryonic (L. Hong and G. M. Rubin, unpublished results) transcripts. cDNAs that hybridized to genomic DNA on both sides of the P insertion sites were further characterized by sequence analysis. P[w][12][1328] was found to be inserted in the 5′ untranslated region of the Ubx-D1 gene, 333 nucleotides upstream of the translation start site, between the t1 and t2 trinucleotide insertions (Cenci et al. 1997). Several related cDNAs that span the ES2-3 insertions P[w][12][1740] and P[w][12][1869] were identified and characterized; the P insertion sites were localized to a common 2 kilobase EcoRI fragment at the 5′ end of these transcripts by hybridization to genomic sequences on both sides of the P insertions. Sequence analysis of approximately 1 kilobase of genomic DNA surrounding the insertion sites revealed no sequence in common with that of the cDNAs, indicating the P[w][12][1740] and P[w][12][1869] elements are inserted within an intron of this transcription unit. BLAST and PILEUP algorithms (Genetics Computer Group, University of Wisconsin, Madison) were used for protein database searches and amino acid sequence alignments.

RESULTS

sina misexpression phenotypes: SINA protein is normally expressed throughout the developing eye disc, with increased levels in cells that have joined an ommatidial group (Carta et al. 1990). To ask whether this wild-type pattern of sina expression is required for normal eye development, we generated transgenic lines bearing a wild-type sina gene in the expression vector pGM3, which contains a multimerized segment of the Rh1 promoter that acts as a binding site for the Zn-finger protein GLASS (Hay et al. 1994). This
vector provides high levels of glass-dependent expression in all eye disc cells posterior to the morphogenetic furrow during larval development, and in all cells except cone cells in the pupal eye (Hay et al. 1994).

Thirteen independent GMR-sina transformant lines were isolated, each of which displayed eyes with abnormal exteriors, ranging from a slight roughening of the ommatidial lattice to a gross disruption of normal eye morphology (Figure 1, C and E). This range in phenotype is most likely due to variances in expression among the lines, since homozygosing each line resulted in a stronger phenotype. Eyes from strong GMR-sina lines were notably smaller and less pigmented than wild-type, and a fusion of ommatidial surfaces resulted in a glazed cuticle covering the eye (Figure 1E). Microscopic examination of sections through such eyes revealed corresponding abnormalities in the underlying retinal cells (Figure 1F). In these lines retinal patterning appeared severely disrupted, and no normal ommatidia were identified. However, no cell types appeared to be lacking, as judged by the presence of pigment granules (pigment cells), lens structures (cone cells), rhabdomeres (photoreceptor cells) and bristles. Sections through eyes from the weaker GMR-sina lines with mild exterior phenotypes displayed more subtle defects, including defective ommatidial rotation, lattice disorganization, and occasional missing photoreceptors (Figure 1D). In no case were extra R7 photoreceptor cells observed, indicating that misexpression of sina in uncommitted cells in the eye disc is insufficient to direct them into a neuronal program of development.

To determine the developmental stage at which sina misexpression results in retinal disruption, we examined the program of ommatidial assembly in larval and pupal eye discs of strong GMR-sina lines. In wild-type eye discs the neuronal antigen ELAV is expressed in a characteristic sequence as photoreceptor cells assume their neuronal identities, and expression continues during pupal development in a pattern that anticipates the highly ordered appearance of the adult eye (Robinson and White 1991). Eye discs from third instar larvae of strong GMR-sina lines displayed a normal pattern of ELAV expression (data not shown), indicating that sina misexpression during the period of photoreceptor recruitment does not disrupt ommatidial assembly, and in particular does not cause formation of extra photoreceptor cells. In contrast, by 40 hr after pupation, signs of ommatidial disorganization were readily apparent (Figure 1, G and H). ELAV-positive cells were no longer arranged in ommatidial clusters as in wild-type.

Figure 1.—sina misexpression phenotypes. Light photomicrographs of retinae from wild-type (A, B, G), and medium (C, D) and strong (E, F, H) GMR-sina lines are shown. (A, C, E) Adult eye surfaces. Note the disorganization of the lattice and loss of pigment in the transgenic eyes. (B, D, F) Tangential sections through adult eyes. Sina misexpression disrupts the integrity of each ommatidium, but all cell types are present. (G, H) Photoreceptor nuclei in 40 hr pupal discs, marked by ELAV expression.
but instead appeared scrambled about the pupal disc. Although this disorganization prevented an accurate determination of the number of photoreceptors in each ommatidium, the overall density of ELAV-positive cells in GMR-sina pupal discs appeared similar to that of wild type. A similar effect was observed in eye discs stained with an antibody against the CUT protein, which specifically labels cone cell nuclei: the patterning of cone cells appeared normal in GMR-sina third instar eye discs, but was clearly disorganized by the 40 hr pupal stage (data not shown).

**Enhancers and suppressors of GMR-sina:** The sensitivity of the GMR-sina eye phenotype to dosage of the transgene (see above) suggested that sina activity was at a threshold level, and might be sensitive to perturbations in genes encoding potential regulators or effectors of sina. To test this idea, we asked whether mutations in genes implicated in sina function were capable

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**Figure 2.—Suppressors and enhancers of GMR-sina.** Light photomicrographs of adult eyes of the following genotypes: (A) GMR-sina/+. (B) GMR-sina/SS2-1s35 (C) GMR-sina/ES2-1el.

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**TABLE 1**

Enhancers and suppressors of GMR-sina

<table>
<thead>
<tr>
<th>Enhancer groups</th>
<th>No. of alleles</th>
<th>Map position</th>
<th>sina^4 interaction</th>
<th>Clonal phenotype</th>
</tr>
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<tbody>
<tr>
<td>ES1-1</td>
<td>11</td>
<td>10F7-11A7</td>
<td>1.5</td>
<td>Occasional missing R cells</td>
</tr>
<tr>
<td>ES2-1</td>
<td>26</td>
<td>51C-52F</td>
<td>0.8</td>
<td>Wild-type</td>
</tr>
<tr>
<td>ES2-2</td>
<td>15</td>
<td>39B-12</td>
<td>1.0</td>
<td>Wild-type</td>
</tr>
<tr>
<td>ES2-3 Sln3A</td>
<td>2</td>
<td>49B3-6</td>
<td>3.1</td>
<td>Very small clones or scars</td>
</tr>
<tr>
<td>ES2-4 Star</td>
<td>6</td>
<td>21E1-2</td>
<td>3.4</td>
<td>n.d.</td>
</tr>
<tr>
<td>ES3-1</td>
<td>5</td>
<td>84B1-2</td>
<td>0.6</td>
<td>n.d.</td>
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</table>

<table>
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<tr>
<th>Suppressor groups</th>
<th>No. of alleles</th>
<th>Map position</th>
<th>sina^4 interaction</th>
<th>Clonal phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS2-1</td>
<td>40</td>
<td>23C1-2</td>
<td>1.1</td>
<td>Small rhabdomeres</td>
</tr>
<tr>
<td>SS3-1</td>
<td>2</td>
<td>84B1-2</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>SS3-2 glass</td>
<td>11</td>
<td>91A1-2</td>
<td>2.4</td>
<td>n.d.</td>
</tr>
<tr>
<td>SS3-3 Ubcd1</td>
<td>14</td>
<td>88D5-6</td>
<td>0.9</td>
<td>Missing R cells; scars</td>
</tr>
<tr>
<td>SS3-4 Srib3-4a</td>
<td>24</td>
<td>76D3-E</td>
<td>1.2</td>
<td>Very small clones or scars</td>
</tr>
<tr>
<td>SS3-5</td>
<td>2</td>
<td>72C1-73A3</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>SS3-6</td>
<td>8</td>
<td>64C5-E1</td>
<td>2.0</td>
<td>Fallen rhabdomeres</td>
</tr>
<tr>
<td>SS3-7</td>
<td>2</td>
<td>67C4-5</td>
<td>2.0</td>
<td>Fallen rhabdomeres</td>
</tr>
</tbody>
</table>

n.d., not determined.

Six enhancer and eight suppressor groups were identified by lethal complementation. Not shown in the table are 73 mutants that complement all groups. Map positions were initially defined by meiotic recombination analysis of the modifier phenotype, and subsequently refined by lethal complementation tests using deficiencies and P element lines. Alleles of ES3-1 and SS3-1 mapped to the same location and failed to complement for recessive lethality, and thus are likely opposing alleles of the same locus. Modification of the sina^4 phenotype was scored in eye sections from flies heterozygous for a given modifier mutant, and homozygous for sina^4 (see materials and methods for details). Interaction strength was calculated as the ratio of percent R^7^- ommatidia in Modifier/-; sina^4 eyes over percent R^7^- ommatidia in sina^4 eyes. Values between 0.8 and 1.2 represent insignificant interaction. Glass-sina^4 data is from Carthew et al. 1994.
of enhancing or suppressing the GMR-sina eye phenotype. Of nine Enhancers of sina previously identified in a screen for dominant modifiers of a weak sina allele (Carthew et al. 1994), three were found to modify the severity of the GMR-sina phenotype: E(sina)5 and E(sina)6 suppressed the phenotype, whereas E(sina)2 behaved as an enhancer in this assay (data not shown). E(sina)5 is allelic to glass, and probably alleviates the eye phenotype by decreasing expression of the glass-dependent pGMR transgene. Interestingly, E(sina)2 enhances the eye phenotypes caused by both overexpression and loss of sina function. This locus was also isolated as a dominant enhancer of a temperature-sensitive allele of sevenless (E[sev]3E; Cutforth 1994). In contrast to these results, the GMR-sina phenotype was not modified by the Ras signaling pathway mutants Ras1eb, SosAG, GAP113P, nor by strong mutations in sina itself (alleles C2, F1, I1, and N6; data not shown). Although limited in scope, these results suggested that the GMR-sina phenotype is sensitive to the dosage of at least some genes implicated in sina function, and that this phenotype could be used to identify mutations in additional components. We therefore decided to conduct a screen for such mutants.

Approximately 96,000 progeny of mutagenized flies were examined for their ability to dominantly modify the external eye phenotype of an intermediate strength GMR-sina line (see materials and methods). This screen yielded 242 dominant modifier mutants, 87 of which enhanced and 155 suppressed the eye phenotype. The strongest suppressor mutants were able to rescue GMR-sina eyes to a nearly wild-type appearance (Figure 2B), whereas strong enhancers increased the severity of the intermediate GMR-sina tester to that resembling a strong GMR-sina line (Figure 2C). Mutants of low to medium strength produced more subtle modifications of the GMR-sina phenotype (not shown).
ing 73 mutants complement all groups and each other, and thus may represent single alleles of loci that were disrupted only once in this screen, or multiple alleles of one or more complementation groups with no apparent homozygous phenotype, which would not have been detected in our complementation analysis. In addition, some of these mutants may represent viable alleles of one or more of the 14 defined complementation groups.

Three of the groups represent new alleles of genes with previously described roles in eye development. As expected from our pilot screen, several new alleles of glass (SS3-2, for Suppressor of GMR-sina, chromosome 3, group 2) were identified as strong suppressors of GMR-sina (Table 1). ES2-4 (Enhancer of GMR-sina, chromosome 2, group 4) represents six new alleles of Star, a member of the spitz group of mutants which acts in the EGF signaling pathway (Kolodkin et al. 1994). The 24 alleles of SS3-4 were found to be allelic to SR3-4a, a gene of unknown function identified in a screen for genes that act downstream of Ras1 during eye development (Karim et al. 1996). The remaining 11 groups represent genes not previously implicated in eye development.

**Interactions with a hypomorphic sina allele:** To begin to characterize these new mutations, we tested whether they interact genetically with a partial loss of function sina allele, sina^{4} (Carthew and Rubin 1990). This allele provides a reduced amount of sina activity, and has been useful in detecting genetic interactions between sina and putative regulators and effectors of sina (Carthew et al. 1994). We expected that some of the modifier mutants might act in an opposite direction in this assay; that is, that suppressors of GMR-sina would enhance the loss of function sina^{4} phenotype, and GMR-sina enhancers would suppress sina^{4}.

Of twelve groups tested, seven acted as dominant modifiers of sina^{4} (Table 1). Four of these groups acted in the expected direction: SS3-2, SS3-6, and SS3-7 enhanced sina^{4}, and ES3-1 behaved as a suppressor. In addition, three groups, ES1-1, ES2-3, and ES2-4 enhanced the severity of both GMR-sina and the hypomorphic
Screen for Modifiers of sina 

The screen for modifiers of sina had little or no effect on the severity of sina, suggesting the dosage of these genes is limiting when an excess of SINA protein is present, but apparently not when sina activity is reduced.

Homozygous mutant phenotypes: To test more directly the roles of the modifier groups in eye development, we examined eye phenotypes caused by homozygous loss of these loci. Since all of the groups except SS3-2 (glass) reside on recessive lethal chromosomes, clones of cells homozygous for these mutations were generated by mitotic recombination using the FLP-FRT technique (Xu and Rubin 1993). Five classes of adult eye phenotypes were observed (Table 1). Two groups, ES2-1 and ES2-2, gave rise to large clones of wild-type appearance, and thus appear to be dispensible for normal eye development (Figure 3A). A second class (ES2-3, SS3-4, and most SS3-3 alleles) did not give rise to clones, but instead generated scars across the eye. Sections through such scars revealed missing photorecept-
tor and pigment cells, and the presence of large vacuoles (Figure 3B). These groups most likely affect cell survival or proliferation. ES1-1 and three weak alleles of SS3-3 comprise a third phenotypic class (Figure 3C), producing clones of nearly normal size, with occasional reductions in the number of photoreceptors per ommatidia. Two final classes were assigned based on defects in the morphology of cells within clones. Photoreceptors within SS2-1 clones (class 4) were found to bear extremely small rhabdomeres (Figure 3D). The mutant rhabdomeres were smaller than wild-type R7 rhabdomeres, indicating that they probably are not the result of a transformation to the R7 fate. The overall smaller cell size resulted in a distortion of the regular lattice across the clone. A similar phenotype has been observed in Esina4 homozygous mutant clones (Carthew et al. 1994). Finally, photoreceptor cells within clones of SS3-6 and SS3-7 mutants (class 5) also displayed rhabdomeres with abnormal morphology. In such clones, rhabdomeres were often projected tangentially in the retinal plane, rather than radially as in wild-type (Figure 3E). This collapsed rhabdomere phenotype is similar to that described for the marbles mutant (Fischer-Vize and Mosley 1994). A marbles-like phenotype was also observed in developing eye discs of SS3-7 homozygotes (which survive until pupation). Photoreceptor nuclei in SS3-7 mutants often failed to migrate to the apical surface of the disc (Figure 3, F and G), as has been described for marbles (Fischer-Vize and Mosley 1994).

**Molecular analysis:** Mapping by meiotic recombination and deficiency analysis revealed that several of the GMR-sina modifier groups are located near the sites of lethal P-element insertions maintained as part of the Berkeley Drosophila Genome Project (Spradling et al. 1995). Complementation tests between these lines and the modifier groups identified five groups allelic to one or more lethal P lines, providing a starting point for the molecular cloning of these loci. We report here the molecular characterization of SS3-3 and ES2-3.

The SS3-3 group failed to complement four lethal P element lines in the 88D5-6 region. Sequence analysis of cDNAs spanning the insertion sites of two such lines (see materials and methods) identified this locus as the UbcD1 gene, which encodes a ubiquitin conjugating enzyme involved in selective protein degradation (Treier et al. 1992). Male-sterile alleles of this locus have recently been identified and are known as efete (Cenci et al. 1997).

Two P lines at 49B3-6 were found to be allelic to the ES2-3 group. The transcription unit disrupted by these P insertions (see materials and methods) is predicted to encode a polypeptide sequence with high similarity to members of the Sin3 family of transcriptional repressors (Wang et al. 1990; Ayer et al. 1995). The protein sequence in the database most closely related to the deduced ES2-3 sequence is that of mouse mSin3A, and we have therefore renamed the ES2-3 locus Sin3A. Figure 4 shows an alignment of the fly and mouse SIN3A sequences. The two proteins share 33% amino acid identity overall, with the highest level of sequence conservation centered on the four paired amphipathic helix (PAH) domains and the region between PAH3 and PAH4, which is required for association of mSin3A with histone deacetylase enzymes (Laherty et al. 1997). In addition, the Drosophila sequence is longer by 529 amino acids than the mouse, due to a longer amino-terminal tail and insertions between the PAH domains.

**DISCUSSION**

Expression of sina at high levels in all cells of the eye disc causes a severe disruption of the structure and organization of the adult eye. This disruption is a relatively late event, occurring after the initial period of ommatidial assembly and photoreceptor cell fate specification. Given the requirement for sina in R7 photoreceptor development, a reasonable expectation was that ectopic sina expression might cause transformation of cone cell precursors into R7 photoreceptors, as observed for ectopic expression of boss (Van Vactor et al. 1991), phyllopod (Chang et al. 1994), and activated Ras1 (Fortini et al. 1992). However, this appears not to be the case for sina, as no evidence of supernumerary photoreceptor cell formation was observed in GMR-sina flies. Since defects in retinal structure were first observed during the pupal stage, a time at which SINA is normally expressed in all cells in the eye disc (Carthew and Rubin 1990), we conclude that the defects observed in GMR-sina flies are most likely caused by higher than normal levels of SINA protein in these cells, rather than by inappropriate timing or patterning of SINA expression.

The relatively late manifestation of the GMR-sina phenotype suggests that cells in the eye disc remain responsive to sina activity beyond the time at which the R7 cell is normally recruited. A requirement for sina during these later stages of eye development has been described previously for a subset of R1-6 photoreceptors, which in strong sina mutants initially appear to be properly specified, but subsequently can lose their normal photoreceptor morphology during pupation (Carthew and Rubin 1990). Moreover, loss of function mutations in the ttk gene result in formation of supernumerary R7 cells during the pupal stage (Lai et al. 1996). We and others have recently demonstrated that ttk interacts physically and genetically with sina, and that sina is required for degradation of TTK in response to activation of the RAS-MAPK pathway (Tang et al. 1997; Li et al. 1997; and see below). Taken together, these results suggest that some cell types in the developing eye may require continuous inductive signals to maintain their particular developmental program, and...
that these signals are dependent upon proper levels of SINA protein. Alternatively, sina may have other functions during the later stages of eye development.

A major goal of this study was to identify genes involved in regulating and effecting sina activity. Among our collection of 14 GM R-sina enhancer and suppressor groups are such candidate genes. Of the three groups with known roles in eye development, SS2-2 (glass), ES2-4 (Star), and SS3-4 (SR3-4), two appear to interact nonspecifically with GM R-sina. Although genetic interaction between sina and glass was described in a previous report (Carter et al. 1994), the primary suppressive effect of glass mutants on the GM R-sina phenotype is most likely due to reduced expression of the glass-dependent transgene. Additionally, like previously existing Star alleles, ES2-4 mutants have a dominant rough eye phenotype independent of GM R-sina, and thus the interaction observed between Star and GM R-sina appears to reflect independent additive effects of each mutation.

Mutations allelic to SS3-4 have been isolated previously as dominant suppressors of a rough eye phenotype caused by an activated Ras1 transgene (Karim et al. 1996). sina has been shown to be required for the transformation of cone cells to photoreceptors caused by activated Ras1 (Fortini et al. 1992), a requirement we attribute to SINA’s role in degrading TTK. Characterization of mutants that interact with both sina and Ras1 may lead to a further understanding of the relationship of these genes. Two additional suppressor groups (SS2-1 and SS3-3; data not shown) also tested positive for interactions with activated Ras1, as did several mutants isolated in a previous screen for enhancers of sina (Carter et al. 1994).

Molecular cloning of the remaining genes identified in this screen has begun to provide additional insight into the mechanisms of SINA function. The finding that SS3-3 encodes the ubiquitin conjugating enzyme UBCD1 is consistent with a role for SINA in ubiquitin-dependent processes, a possibility initially suggested by interactions between sina and fat facets, which encodes a deubiquitinating enzyme (Carter et al. 1994; Huang et al. 1995). Recent experiments have found that sina-dependent degradation of TTK in cell culture probably occurs via the ubiquitin-dependent proteolysis pathway (Li et al. 1997). The genetic interaction between sina and UbcD1 presented here, as well as our demonstration of physical interaction between SINA and UBCD1 (Tang et al. 1997), provides a molecular framework for beginning to understand how SINA may regulate the stability of proteins such as TTK. Members of the Sin3 class of transcriptional corepressors serve as requisite components of the Mad-Max repressor complex (Ayer et al. 1995). In this report, we have demonstrated that a Drosophila member of this family, Sin3A, interacts genetically with sina. At present, the role played by Sin3A during development is unclear. We were unable to recover clones of cells homozygous for Sin3A mutations, suggesting that this gene is required for cell proliferation or survival. Identification of mutations in Drosophila Sin3A should contribute to our understanding of this important class of transcriptional regulators. Elucidation of the roles played by the products of the remaining genes identified in this screen awaits their further characterization.

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LITERATURE CITED


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