Copyright © 1996 by the Genetics Society of America

Identification and Characterization of Autosomal Genes That Interact With *glass* in the Developing Drosophila Eye

Chaoyong Ma, Hui Liu, Ying Zhou and Kevin Moses

Department of Biological Sciences, University of Southern California, Los Angeles, California 90089-1340

Manuscript received September 11, 1995
Accepted for publication January 4, 1996

ABSTRACT

The *glass* gene encodes a zinc finger, DNA-binding protein that is required for photoreceptor cell development in *Drosophila melanogaster*. In the developing compound eye, *glass* function is regulated at two points: (1) the protein is expressed in all cells' nuclei posterior to the morphogenetic furrow and (2) the ability of the Glass protein to regulate downstream genes is largely limited to the developing photoreceptor cells. We conducted a series of genetic screens for autosomal dominant second-site modifiers of the weak allele $glass^3$, to discover genes with products that may regulate *glass* function at either of these levels. Seventy-six dominant enhancer mutations were recovered (and no dominant suppressors). Most of these dominant mutations are in essential genes and are associated with recessive lethality. We have assigned these mutations to 23 complementation groups that include multiple alleles of *Star* and *hedgehog* as well as single alleles of *Delta, roughened eye, glass* and *hairy*. Mutations in 18 of the complementation groups are embryonic lethals, and of these, 13 show abnormal adult retinal phenotypes in homozygous clones, usually with altered numbers of photoreceptor cells in some of the ommatidia.

IN Drosophila, the adult compound eye consists of ~800 facets (ommatidia), each of which contains 20 cells: eight photoreceptor cells and 12 accessory cells (WADDINGTON and PERRY 1960). The photoreceptor cells can be subdivided into three subtypes according to their morphology, synaptic specificity, and spectral sensitivity: the six outer photoreceptors, R1–R6, the apical central photoreceptor, R7, and the basal central photoreceptor, R8 (reviewed by TOMLINSON 1988; MEINERTZHAGEN and HANSON 1993). The accessory cells include lens-secreting cone cells, light-insulating pigment cells, and cells comprising a mechano-sensory bristle. The compound eye develops from a monolayer epithelium called the eye imaginal disc, which grows by unpatterned cell divisions during early larval life, without apparent differentiation. Cell-type differentiation begins in the last larval instar, when a transverse groove (the morphogenetic furrow) sweeps across the eye imaginal disc from posterior to anterior (READY *et al.* 1976; HEBERLEIN and MOSES 1995). Anterior to the furrow, cells are dividing and undifferentiated. At the furrow, cell division stops and differentiation begins, and the ommatidial founder cells appear in a regular array. Posterior to the furrow, a final wave of cell division occurs (READY *et al.* 1976; WOLFF and READY 1991) and differentiation proceeds as photoreceptor cells and accessory cells are sequentially specified (TOMLINSON 1988). Many genes have been found to act in photoreceptor cell development. The *eyeless* gene functions in the specification of the nervous system (QUIRING *et al.* 1994; BARINAGA 1995; HALDER *et al.* 1995), and some genes function early to control the growth of the retinal cell precursors, such as *sine oculis* (CHEYETTE *et al.* 1994) and *eyes absent* (BONINI *et al.* 1993). Some genes function in the specification of the regular arrays of ommatidial founder cells, such as *scabrous* (BAKER *et al.* 1990; MLODZIK *et al.* 1990a; ELLIS *et al.* 1994; BAKER and ZITRON 1995), the Drosophila homologue of the epidermal growth factor receptor, *Egfr*, (BAKER and RUBIN 1989; BAKER and RUBIN 1992; ZAK and SHILO 1992), *Notch* (CAGAN and READY 1989; BAKER and ZITRON 1995) and *Delta* (BAKER and ZITRON 1995). Some genes function to specify photoreceptor subtypes, such as *rough* (BASLER *et al.* 1990; KIMMEL *et al.* 1990; HEBERLEIN and RUBIN 1991), *sevenless* (recently reviewed by DICKSON and HAFEN 1994) and *seven up* (MLODZIK *et al.* 1990b; HIROMI *et al.* 1993; BEGEMANN *et al.* 1995; KRAMER *et al.* 1995).

The *glass* (*gl*) gene functions in the terminal differentiation of photoreceptor cells. In *gl* mutants, the presumptive photoreceptor cells begin to develop as neurons but fail to express photoreceptor cell-specific genes and die after about 60 hr of pupal development. Externally, the adult compound eyes are reduced in size, and the regular array of ommatidia is disrupted. Strong *gl* alleles also cause part of the eye to lack pigment, while three independent weak alleles (gl^3, gl^{BX9} and gl^{B24}) produce fully pigmented eyes. *gl* function is required autonomously by all the developing photoreceptor cell types and is not required by the accessory cells (MOSES *et al.* 1989). The *gl* gene encodes a 604-

Corresponding author: Kevin Moses, Department of Biological Sciences, University of Southern California, SHS-172, MC-1340, 825 W. 37th St., Los Angeles, CA 90089-1340.
E-mail: kmoses@mizar.usc.edu

amino acid protein with five zinc finger motifs that can bind to sequences within the enhancer regions of the *Rh1* (rhodopsin) gene and regions within its own promoter (MOSES and RUBIN 1991). At least three of the five zinc finger domains are required for DNA binding, and there is also an activation domain (O'NEILL *et al.* 1995). Sequences resembling Gl-binding sites are found near the promoter of the chicken rhodopsin gene, and they are bound by a retinal-specific, chicken nuclear protein (SHESHBERADARAN and TAKAHASHI 1994). *gl* appears to be the last regulatory gene in its hierarchy, as it acts directly on terminal "realizator" functions (GARCÍA-BELLIDO *et al.* 1975), such as *Rh1*. Thus *gl* may be a master regulatory gene that controls photoreceptor differentiation, and the regulation of *gl* activity may be a critical step in the specification of the photoreceptor cell type.

In Drosophila, both transcription and translation of the *gl* gene begin at the morphogenetic furrow (in all cells) and then persist to the posterior margin of the disc, although at a lower level (MOSES *et al.* 1989; ELLIS *et al.* 1993). Gl protein is detected in the nuclei of all cells posterior to the furrow, but its ability to activate transcription differs between cell types: a Gl-regulated β-galactosidase reporter construct is expressed in the developing photoreceptor cells but not in the accessory cells. In the developing R7 cell *gl* activity is dependent on the *sevenless* tyrosine kinase signal (MOSES and RUBIN 1991). There are at least two classes of model to account for this restriction. (1) There could be photoreceptor cell-specific, positive regulators of *gl* function. Gl protein may be modified in the developing photoreceptor cells: an anti-Gl monoclonal antibody detects multiple forms of the protein on blots (ELLIS *et al.* 1993). (2) Alternatively, there could be negative regulators of *gl* function, which would be present in all cells in the morphogenetic furrow and then later specifically eliminated from the developing photoreceptor cells. This second possibility appears to be more likely, as a sequence adjacent to (and overlapping with) a *gl*-binding site in an *Rh1* enhancer is responsible for the repression of *gl* response in nonphotoreceptor cells (ELLIS *et al.* 1993). However *gl* activity is regulated, genes that act in the *gl* pathway might be identified as dominant, second-site modifiers of *gl*.

Screens for second-site modifiers have been used to identify functionally linked genes in Drosophila (BOTAS *et al.* 1982; BRAND and CAMPOS-ORTEGA 1990; ROGGE *et al.* 1991; SIMON *et al.* 1991; CARTHEW *et al.* 1994; HARDING *et al.* 1995; WEMMER and KLÄMBT 1995). In such a screen, the activity of a gene (in our case *gl*) is reduced, such that an intermediate, or weak, phenotype is seen (Figure 1). In this sensitized condition, a recessive loss-of-function mutation in a functionally related gene may cause a visible phenotype although only one copy of the gene is inactivated. Thus the new mutation may be revealed as dominant, while otherwise it would be recessive. Since the new mutations cause visible phenotypes as heterozygotes, vital genes affecting eye development can be identified. This is important since many genes have pleiotropic effects. It has been estimated that about two-thirds of the vital genes may be essential for eye development (THAKER and KANKEL 1992), however, because most genetic screens for genes with function in eye development have required mutations to be viable (BAKER *et al.* 1992), only a few vital genes affecting eye development have been identified, such as *Notch* (CAGAN and READY 1989), *Delta* (BAKER and ZITRON 1995) and *EgfrE* (BAKER and RUBIN 1989). Some new screening strategies, such as FLP-mediated somatic recombination (XU and RUBIN 1993) and the "enhancer trap" technique (O'KANE and GEHRING 1987), also facilitate the identification of such vital genes, such as *seven-up* (MLODZIK *et al.* 1990b).

We report here the results of a screen for autosomal genes that interact with *gl*, as second-site dominant enhancers or suppressors. All known *gl* alleles (including protein nulls) are completely recessive *in trans* to wild type. This implies that 50% of the normal quantity of Gl protein is sufficient for normal development. Homozygotes of weak *gl* alleles (gl^3, gl^{BX9} and gl^{B24}) have an intermediate, rough eye phenotype (Figure 1B). Unlike null alleles (Figure 1C), their phenotype can be made stronger by placing them *in trans* to a deficiency, and as such they are likely to be hypomorphic alleles (MULLER 1932). Modification of this weak *gl* phenotype may be caused by mutations in genes that are upstream regulators of *gl* and that act in a stoichiometric manner. A loss-of-function mutation in a gene that normally acts to positively regulate *gl* could be recovered as a dominant enhancer of the weak gl^3 phenotype. Similarly, a loss-of-function mutation in a gene that normally acts to negatively regulate *gl* could be recovered as a dominant suppressor of the weak gl^3 phenotype. Modification of this weak *gl* phenotype is less likely to be caused by mutations in genes that are downstream of *gl*. *gl* appears to be the last regulatory gene in its pathway and acts on many terminal "realizators." Mutations in any individual terminal gene are unlikely to have a detectable effect on the phenotype tested in this screen. For instance, mutations in the Rh1 opsin gene (*ninaE* alleles) do not affect external morphology to cause a rough eye and are unlikely to enhance the phenotype of gl^3 as F_1 heterozygotes.

We report here the results from a series of screens for dominant modifiers of gl^3. We used three mutagens (EMS, γ-rays and hybrid dysgenesis) to treat males and examined ~455,000 F_1 progeny. We report the isolation and characterization of 76 dominant enhancer of *gl* mutations in 23 complementation groups.

MATERIALS AND METHODS

Drosophila stocks: Drosophila cultures were carried out at 25° on standard cornmeal-molasses-agar medium. *gl* alleles used were as follows: gl^3 is a weak mutation caused by a spontaneous insertion of ~2.5 kb, gl^{60j} is a null allele caused

by a spontaneous insertion of ~30 kb, gl^{BX9} is a weak mutation induced by X-rays, gl^{B24} is a weak mutation induced by EMS (MOSES *et al.* 1989). The stock used for mutagenesis was isogenic for the second and third chromosomes and is of the genotype w^{1118}; *P[(w,ry)D]3* gl^3 *e*, where *P[(w,ry)D]3* is one cM proximal to *gl* (R. LEVIS and G. M. RUBIN, unpublished results). The *white* gene carried by this *P* element renders the eye red and was used as a reference marker to monitor the efficiency of the mutagenesis by scoring the white-eyed flies in the screens. The following *P*-element insertions were used in mapping experiments and mosaic clone analyses (kindly provided by G. M. RUBIN and T. LAVERTY, see Table 1): *P[(w,ry)A]N22* (in polytene region 30C, 2-34 on the meiotic map), *P[(w,ry)A]1-1* (47A, 2-60), *P[(w,ry)A-R]012* (66E, 3-17), *P[w]21* (89A, 3-58), *P[(w,ry)A]4-4* (100F, 3-108), all from (HAZELRIGG *et al.* 1984); *P[(w,ry)F]4-2* (21D, 2-0), *P[(w,ry)D]1* (25C, 2-15), *P[(w,ry)F]4-1* (57B, 2-95), *P[(w,ry)H]1* (78CD, 3-46), *P[(w,ry)D]3* (90E, 3-62) and *P[(w,ry)E]7* (94D, 3-81), from R. LEVIS and G. M. RUBIN (unpublished results). The marker stocks for mosaic clone analyses were as follows: w^{1118}; *P[(w,ry)A]N22* (for mutations on 2L), w^{1118}; *P[(w,ry)A]1-1* (for mutations on 2R), w^{1118}; *P[gl]-30B; P[(w,ry)H]1* (for mutations on 3L), w^{1118}; *P[gl]30B; P[w]21* (for mutations on 3R). *P[gl]30B* is a gl^+ transgenic insertion in polytene region 30B that fully rescues *gl* mutant's phenotype (MOSES *et al.* 1989). *gl* deletion chromosomes were $Df(3R)gl^{BX3}$, $Df(3R)gl^{BX7}$ and $Df(3R)gl^{BX8}$ (MOSES *et al.* 1989). The balancer chromosomes used were *In(2LR)O*, *TM3 Sb* and *TM6B Tb Hu* (LINDSLEY and ZIMM 1992). A h^{C1} stock was a gift of N. L. BROWN and S. B. CARROLL. A hh^{AC} stock was a gift of J. J. LEE and P. A. BEACHY. A ptc^{IN} stock was a gift of J. E. HOOPER. Stocks containing rn^5 and rn^{20} were gifts of R. GRIFFIN-SHEA. Stocks carrying the following mutations were obtained from the Bloomington and Bowling Green stock centers: arm^{K2}, ci^D, Dl^X, en^{IIB}, eve^{ID19}, ftz^{W20}, fu^{62f3}, *Df(2R)gsb*, gt^{13Z}, h^{5H07}, hb^{14F}, kni^{5F107}, Kr^2, nkd^{7E}, opa_{5H97}, prd^{IB42}, roe^1, roe^3, roe^4, run^{E9}, S^1, slp^{L12}, smo^{Q14}, tll^{L10} and wg^{CX4}.

Mutant screens: For chemical and radiation mutagenesis, males of the genotype w^{1118}; *P[(w,ry)D]3* gl^3 *e* were treated with 25 mM EMS (Sigma #M0880) as described in ASHBURNER (1989) or γ-rays (4000 rads from a ^{137}Cs source) and crossed to w^{1118}; gl^3 virgin females. About 395,000 F_1 progeny were inspected under dissecting microscope for modified eye phenotypes. Mutations of the *white* gene on the *P[(w,ry)D]3* element were also scored as a monitor of the efficiency of mutagenesis. As a second method to monitor the mutagenesis efficiency, for each mutagenesis run we measured the frequency of induced *X*-linked lethals, by crossing mutagenized males *en masse* to *FM6K y w B* virgin females. The F_1 progeny were allowed to mate *inter se*, and then 100 single females were bred in individual vials. We scored for the presence or absence of B^+ males in the F_2 progeny of each female. Each case of the absence of such males was scored as one *X*-linked lethal. For *P*-element transposon mutagenesis, *Birm-2/+; Ki* Δ*2-3 ry 506*/gl^3 males were crossed to w^{1118}; gl^3 virgin females. The Δ2-3 element is a stably integrated source of *P*-element transposase (at polytene band 99B) and *Birm-2* is a second chromosome isolated from the *Birmingham* (*Birm*) stock of *D. melanogaster*, which has a large number of defective *P* elements and no complete ones. The *Δ2-3; Birm-2*-combined genotype results in a relatively high level of *P*-element transposition, which can then be stabilized by selecting against *Ki* (and thus *Δ2-3*) in the next generation (LASKI *et al.* 1986; ROBERTSON *et al.* 1988). We scored ~60,000 F_1 Ki^+ progeny for modified eye phenotypes. The mutants obtained were crossed to w^{1118}; gl^3 flies. If mutations from any of the screens bred true, they were crossed to the following balancer stocks, w^{1118}; *In(2LR)O/Sco*, gl^3 and w^{1118}; *TM3 Sb e* gl^{B2}/*TM6B Tb Hu e* gl^{BS1}, and maintained as balanced stocks. We also tested 25 known autosomal genes for their ability to act as dominant enhancers of gl^3. The genes tested were *armadillo, cubitus interuptus, engrailed, even skipped, fused, fushi tarazu, giant, glass, gooseberry, hairy, hunchback, knirps, Krüppel, naked, odd paired, paired, patched, roughened eye, rotund, runt, sloppy paired, smooth, Star, tailless* and *wingless* (see alleles listed above and RESULTS section below).

Linkage and complementation tests: The mutations were assigned to linkage groups by their patterns of inheritance relative to the dominant markers on the balancer chromosomes, and on whether they were linked to the third chromosome *P[(w,ry)D]3* element. The *E(gl)* mutations that are associated with recessive lethality were assigned to lethal complementation groups by complementation tests (within each linkage group). In those cases where map position and/or mutant phenotype suggested that a particular enhancer of *gl* might be an allele of a known gene, we tested this by complementation.

Mapping: We determined the approximate meiotic map position of one allele of each previously unidentified complementation group obtained in the screens. We determined the position of both the dominant *E(gl)* phenotype and the recessive lethality associated with it (when applicable) in independent experiments. We mapped the positions of the dominant enhancers of *gl* by scoring the enhancer and eye color phenotypes, of the progeny of the outcross of heterozygous females (genotype: w^{1118}; $E(gl)/E(gl)^+$ $P[w^+]$, in a gl^3 homozygous background) crossed to males of the w^{1118}; gl^3 stock. We mapped the positions of the recessive lethality associated with most of the dominant enhancers of *gl* by back-crossing F_1 heterozygous females (genotype: w^{1118}; $E(gl)/E(gl)^+$ $P[w^+]$, in a gl^3 homozygous background) to males carrying a mutation in the same lethal *E(gl)* complementation group (using a different allele, when available) of the genotype w^{1118}; *E(gl)/balancer* and then scoring the eye color phenotypes of the nonbalancer progeny. The scale of our mapping experiments and the distribution of the *white*$^+$ markers were such that the resulting positions are accurate to only ±5 cM.

Mosaic clone analysis: Mutants were crossed to the marker stocks described above, as appropriate to their map positions. To induce mitotic recombination, heterozygous F_1 progeny in the early first instar were treated with γ-rays from a ^{137}Cs source (1000 Rad) or X-rays (1000 Rad at 120 kv, 5 mA). Adults were then screened for mosaic clones in the eye, marked by a lack of retinal pigment. Heads of such flies were fixed and embedded in resin, and 3-μm retinal sections were cut as described by TOMLINSON and READY (1987a). The sections were examined and photographed using phase contrast lenses.

Electron microscopy, cuticle preparation, and histochemistry: Scanning electron microscopy was performed as described (MOSES *et al.* 1989). Embryonic cuticles were prepared as described in WIESCHAUS and NÜSSLEIN-VOLHARD (1986). Embryos were stained as described by DEQUIN *et al.* (1984). The antibody used for embryonic central nervous system staining was mAb BP102, gift of C. GOODMAN (HORTSCH *et al.* 1990). The antibody used for embryonic peripheral nervous system staining was mAb 22C10, gift of S. L. ZIPURSKY (FUJITA *et al.* 1982). Eye imaginal discs were stained as described by TOMLINSON and READY (1987b) as modified by MA *et al.* (1993). Antibodies used were anti-Elav, from the University of Iowa developmental biology hybridoma bank (BIER *et al.* 1988), anti-Hairy, gift of S. CARROLL (CARROLL and WHYTE 1989), anti-Scabrous, gift of N. E. BAKER (MLODZIK *et al.* 1990a), and anti-Dpp, gift of F. M. HOFFMANN (PANGANIBAN *et al.* 1990).

Genomic DNA blots: DNA was isolated from adults as described (MOSES *et al.* 1989). The DNA was digested with six restriction endonucleases, *Bam*HI, *Eco*RI, *Hin*dIII, *Pst*I, *Sac*I

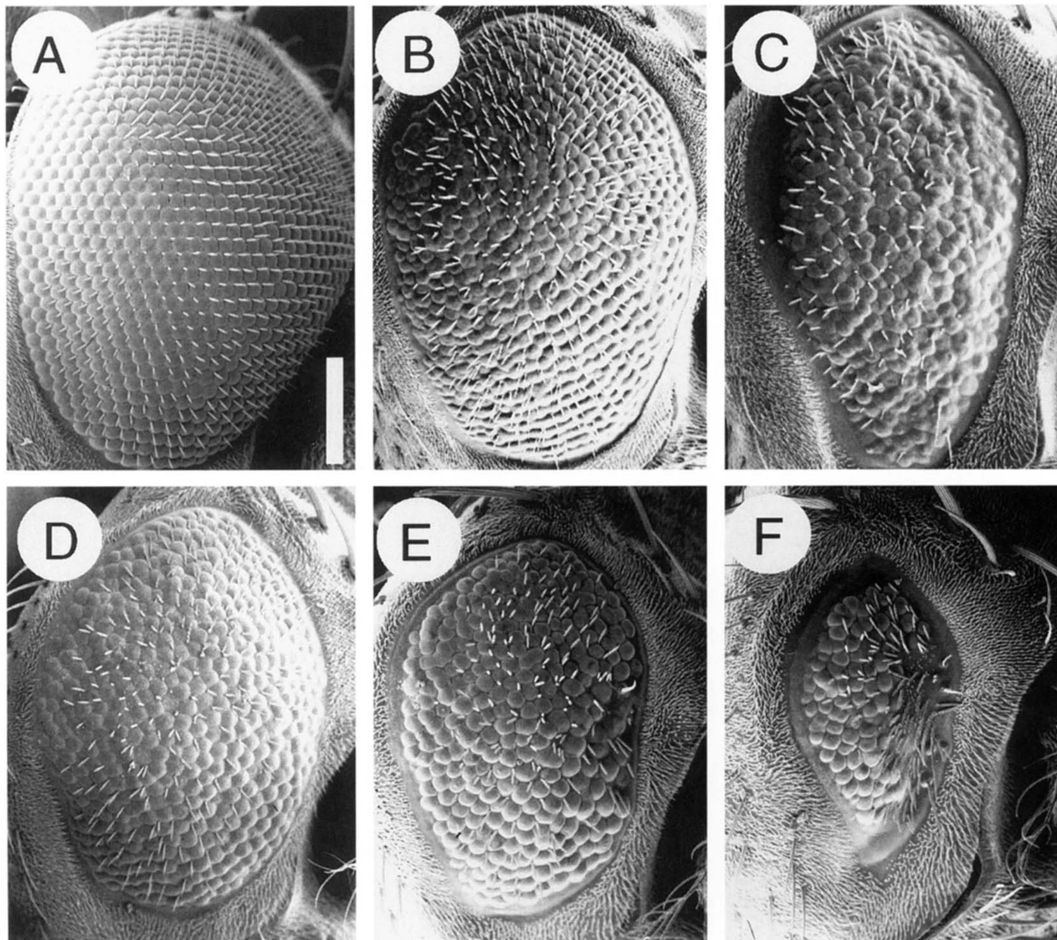

FIGURE 1.—External retinal morphologies of *E(gl)* mutants. All panels are SEMs of adult retinas. Anterior is to the right and dorsal is up. All panels are to the same scale, bar in A, 100 μm. (A) Wild type. (B) gl^3 homozygote. (C) gl^{60j} homozygote, a null allele. D–F are examples of *E(gl)* heterozygotes in a gl^3 homozygous background. (D) *E(gl)2D,* a weak enhancer, (E) h^{SCG1}, an intermediate strength enhancer, (F) *E(gl)3C,* a strong enhancer.

and *Xho*I, then run on agarose gels and transferred to nitrocellulose. Blots were hybridized to ^{32}P nick-translated probes covering the genomic region of the *hh* gene from a *Hin*dIII site at −5.5 kb to the *Eco*RI site at +16.4 kb, position numbers as LEE *et al.* (1992).

RESULTS

Mutant screens: The compound eyes of gl^3 homozygotes have a phenotype that is intermediate between that of wild type and that of a *gl* null (compare Figure 1B with 1A and 1C). We recovered 76 dominant enhancers of gl^3 in a total of ~455,000 F_1 flies: 49 EMS-induced mutants (in ~282,000), 24 γ-ray-induced mutants (in ~113,000), and three dysgenic mutants (in ~60,000) (see MATERIALS AND METHODS). No dominant suppressors were found. Seventy of the mutations are homozygous lethal when cultured at 25°. One mutation was found to be temperature sensitive, and the homozygotes are viable at 18°. Pair-wise complementation tests were used to assign the *E(gl)* mutations to 23 complementation groups. Figure 1 shows external views of the dominant modified-eye phenotypes of one weak, one intermediate and one strong *E(gl)* complementation group (shown in a gl^3 homozygous background). The meiotic map position of each *E(gl)* complementation group was determined relative to several transgenic *P* elements carrying a *white*$^+$ gene (see Tables 1 and 2, Figure 2 and MATERIALS AND METHODS).

Mutations in genes that are not functionally related to *gl* may be recovered in this screen, and secondary tests were required to eliminate such false positives. Such extraneous genes can be recovered through the neomorphic or antimorphic mutation of genes with products that do not normally act in the *gl* pathway. These mutations are inherently rare and are usually recovered as the sole allele in a complementation group. Indeed, the single *hairy* allele appears to be antimorphic (see below). All *E(gl)* mutations were compared to known loss-of-function alleles of the same gene, whenever possible. Also haplo-insufficient, dominant rough eye mutations (such as *Star*) may have a simple additive effect with the rough eye mutation gl^3 and thus appear in our screen. To test for such nonspecific interactions, members of each complementation group were crossed to wild type (Canton-S), and their progeny were examined for dominant eye phenotypes in this gl^+/gl^3 background. Mutations in seven of the *E(gl)* loci show this phenotype and thus are not consid-

TABLE 1

***P*-element insertion markers**

Name	Abbreviation	Polytene	Map	Reference[a]
P[(*w*,*ry*)*F*] *4-2*	2-I	21D	2-0	1
P[(*w*,*ry*)*D*] *1*	2-II	25C	2-15	1
P[(*w*,*ry*)*A*] *N22*	2-III	30C	2-34	2
P[(*w*,*ry*)*A*] *1-1*	2-IV	47A	2-60	2
P[(*w*,*ry*)*F*] *4-1*	2-V	57B	2-95	1
P[(*w*,*ry*)*A-*	3-I	66E	3-17	2
P[(*w*,*ry*)*H*] *1*	3-II	78CD	3-46	1
P[*w*] *21*	3-III	89A	3-58	2
P[(*w*,*ry*)*D*] *3*	3-IV	90E	3-62	1
P[(*w*,*ry*)*E*] *7*	3-V	94D	3-81	1
P[(*w*,*ry*)*A*] *4-4*	3-VI	100F	3-108	2

[a] 1, R. LEVIS and G. M. RUBIN, unpublished results; 2, HAZELRIGG *et al.* (1984).

ered to be specific enhancers of *gl* (see "Specific Enhancer" column in Table 2).

The gl^3 mutation was spontaneously induced (CSIK 1929) and is associated with an insertion of 2.5 kb near the 5′ end of the gene (which may be a transposable element) (MOSES *et al.* 1989). It may be possible for a second-site enhancer mutation to identify a gene that interacts with gl^3 through regulatory effects on the insertion, and that itself does not have any normal function in eye development. An example of such an interaction is that between a *gypsy*-induced allele of *Hairy wing* and mutations at the *suppressor of Hairy wing* [*su(Hw)*] locus (PARKHURST and CORCES 1986; PARKHURST *et al.* 1988; HOOVER *et al.* 1992; HARRISON *et al.* 1993; SHEN *et al.* 1994; SMITH and CORCES 1995). To rule out such allele-specific interactions, members of each complementation group were crossed to two other weak *gl* alleles: gl^{BX9} (induced by X-rays) and gl^{B24} (induced by EMS) and that are unlikely to harbor the same transposon as gl^3. By this test we found that none of the mutations discovered in our screen interacts specifically with the gl^3 allele alone. As the homozygous hypomorphic phenotype of gl^3 is stronger when placed *in trans* to a null allele, we could (and did) recover a new *gl* mutation in this screen (see below).

The most direct test of the function of a gene in eye development is to examine the homozygous phenotype of null mutations of that gene alone, in an otherwise wild-type genetic background. For homozygous lethal mutations, this can only be done through the examination of homozygous somatic mosaic clones. To examine the retinal phenotypes of the recessive lethal mutations (in a gl^+ genetic background), we made retinal mosaic clones of members of each complementation group that were marked with *white* and examined them in adults by sectioning and microscopy (see MATERIALS AND METHODS). Of the 18 genes that can mutate to recessive lethality, 13 showed retinal phenotypes in this test (see below and Table 2). We also examined the embryonic phenotypes of members of each recessive lethal complementation group by embryo cuticle preparation and by immunohistochemical staining of the nervous system (see below).

Characterization of identified loci: Six complementation groups were identified based on their mutant phenotypes, map positions, and complementation tests with mutations of known genes. They are *Delta, glass, hairy, hedgehog, roughened eye* and *Star* (Table 2).

***Delta* (*Dl*):** *Dl* encodes a protein with transmembrane domain and EGF-like repeats (KOPCZYNSKI *et al.* 1988; ALTON *et al.* 1989). In the eye, ommatidia formed by cells homozygous for weak *Dl* loss-of-function alleles contain an increased number of rhabdomere-bearing cells (which are likely to be photoreceptor cells) (DIETRICH and CAMPOS-ORTEGA 1984), and *Dl* plays a role (with *Notch* and *scabrous*) in spacing the array of ommatidial preclusters in the morphogenetic furrow (BAKER and ZITRON 1995).

***glass* (*gl*):** gl^{SCG1} is associated with a lethal deletion between polytene bands 90F and 91F (data not shown). *gl* is located in 91A1-2, and gl^{SCG1} fails to complement the lethality associated with three extant lethal *gl* deletions: $Df(3R)gl^{BX3}$, $Df(3R)gl^{BX7}$, and $Df(3R)gl^{BX8}$ (MOSES *et al.* 1989).

***hairy* (*h*):** Homozygotes of h^{SCG1} show a typical *h* cuticular pair-rule phenotype (NÜSSLEIN-VOLHARD and WIESCHAUS 1980; INGHAM *et al.* 1985) and fail to complement the lethality associated with two known *h* alleles (h^{5H07} and h^{C1}). Ommatidia that are homozygous for h^{SCG1} (in retinal mosaic clones, Figure 3G) are often missing rhabdomere-bearing cells (20% of such ommatidia). *h* encodes a basic helix-loop-helix (bHLH) family transcription factor (MURRE *et al.* 1989; RUSHLOW *et al.* 1989). H protein is normally expressed anterior to the morphogenetic furrow in the developing eye, and ectopic expression of *h* can alter retinal cell fates (CARROLL and WHYTE 1989). However, homozygous *h* retinal mosaic clones show no mutant phenotype (BROWN *et al.* 1991), and it is likely that H acts redundantly in the developing eye to regulate other bHLH proteins (BROWN *et al.* 1995; HEBERLEIN and MOSES 1995). We showed that a *h* null mutation is not a dominant en-

TABLE 2

Summary of the *Enhancer-of-glass* mutations

Gene	Allele(s)[a]	Map position[b]	Distance from left flanking w^+ marker[c]	Distance from right flanking w^+ marker[c]	Specific enhancer	Lethal	Note
Dl	*SC1*	3-66			No	Yes	
gl	*SCG1*	3-63			Yes	Yes	
h	*SCG1*	3-26			Yes	Yes	Antimorph
hh	*SC1-SC21* *SCG1-SCG5*	3-81			Yes	Yes	One viable allele, one temperature-sensitive
roe	*SC1*	3-47			Yes	No	
S	*SC1-SC12* *SCG1-SCG12* *SCP1-SCP3*	2-1.3			No	Yes	
E(gl) 2A	*SC1*	2-24	2-II, 6 cM (16/271)	2-III, 10 cM (22/212)	Yes	Yes	No obvious retinal[d] or embryonic[e] defects.
Opl	*SC1*	2-50	2-III, 21 cM (18/87)	2-IV, 10 cM (9/93)	No	Yes	No obvious embryonic[e] defects. Homeotic phenotypes in some retinal[d] clones (Figure 7). Other retinal clones contain ommatidia with both increased and decreased numbers of rhabdomeres (average number 6.8, Figure 3B)
E(gl) 2B	*SC1*	2-65	2-IV, 4 cM (6/142)	2-V, 25 cM (75/305)	Yes	Yes	No obvious retinal[d] or embryonic[e] defects.
E(gl) 2C	*SC1*	2-65	2-IV, 5 cM (3/63)	2-V, 33 cM (15/45)	Yes	Yes	No obvious embryonic[e] defects. Retinal clones[d] contain ommatidia with both increased and decreased numbers of rhabdomeres (average number 8.1, Figure 3C)
E(gl) 2D	*SC1*	2-70	2-IV, 11 cM (46/408)	2-V, 38 cM (76/200)	Yes	Yes	No obvious embryonic[e] defects. Retinal clones[d] contain ommatidia with both increased and decreased numbers of rhabdomeres (average number 7.9). Also 67% of the ommatidia are miss-oriented Figure 3D)
E(gl) 2E	*SC1*	2-70	2-IV, 10 cM (18/174)	2-V, 23 cM (44/190)	Yes	Yes	No obvious retinal[d] or embryonic[e] defects.
E(gl) 2F	*SC1*	2-70	2-IV, 11 cM (25/227)	2-V, 30 cM (80/268)	Yes	Yes	No obvious embryonic[e] defects. Retinal clone[d] contain rare ommatidia with decreased numbers of rhabdomeres (average number 7.9, Figure 3E)
E(gl) 2G	*SC1*	2-70	2-IV, 11 cM (15/141)	2-V, 31 cM (80/255)	No	Yes	No obvious retinal[d] or embryonic[e] defects.
E(gl) 2H	*SC1*	2-97[f]	2-IV, 40 cM (72/182)	2-V, 2 cM (4/189)	Yes	No	No obvious retinal[d] defects.
Pas	*SC1*	3-0[f]	3-I, 26 cM (62/239)	3-II, 44 cM (131/298)	No	Yes	Embryonic nervous system defects, dominant retinal[d] defects (see text).
dog	*SC1, SC2*	3-42	3-I, 15 cM (44/258)	3-II, 4 cM (14/320)	Yes	Yes	No obvious embryonic[e] defects, required by all retinal[d] cells (see text).
E(gl) 3A	*SC1*	3-43	3-II, 43 cM (74/173)	3-II, 3 cM (10/333)	Yes	No	No obvious retinal[d] defects.
Roa	*SCG1*	3-45	3-II, 1 cM (1/166)	3-III, 14 cM (32/229)	No	Yes	No obvious embryonic[e] defects, dominant retinal[d] defects (see text).
Oab	*SCG1*	3-46	3-II, 0 cM (0/77)	3-III, 11 cM (4/38)	No	Yes	No obvious embryonic[e] defects, dominant retinal[d] defects (see text).
E(gl) 3B	*SCG1*	3-81	3-V, 0 cM (0/69)	3-VI, 2 cM (3/185)	Yes	Yes	No obvious embryonic[e] defects. Retinal clones[d] contain rare ommatidia with decreased numbers of rhabdomeres (average number 7.9, Figure 3L)
E(gl) 3C	*SCG1*	3-99	3-V, 19 cM (50/267)	3-VI, 24 cM (52/213)	Yes	No	Homozygous adult retinas lack 2% of the ommatidia, and the surviving ommatidia occasionally have both increased and decreased numbers of rhabdomeres (average number 6.5, Figure 3O).
E(gl) 3D	*SC1, SCG1*	3-108	3-V, 26 cM (25/98)	3-VI, 0 cM (0/47)	Yes	Yes	Embryonic nervous system defects (Figure 8). No obvious retinal[d] defects.

[a] EMS alleles are listed as SC numbers. γ-ray induced alleles are listed as SCG numbers. Hybrid-dysgenesis induced alleles are listed as SCP numbers.

[b] The map positions of known genes are taken as published in LINDSLEY and ZIMM (1992).

[c] In each case, the abbreviated name of the marker is given first, then the estimated map distance to it in cM, and then the number of recombinants per total number in parentheses.

[d] The retinal phenotypes of lethal genes were examined in adults in negatively marked mosaic clones, see MATERIALS AND METHODS.

[e] The embryonic cuticular and nervous system phenotypes of homozygous embryos were examined, see MATERIALS AND METHODS.

[f] Gene is distal to the outer marker, thus the two closest proximal markers were used.

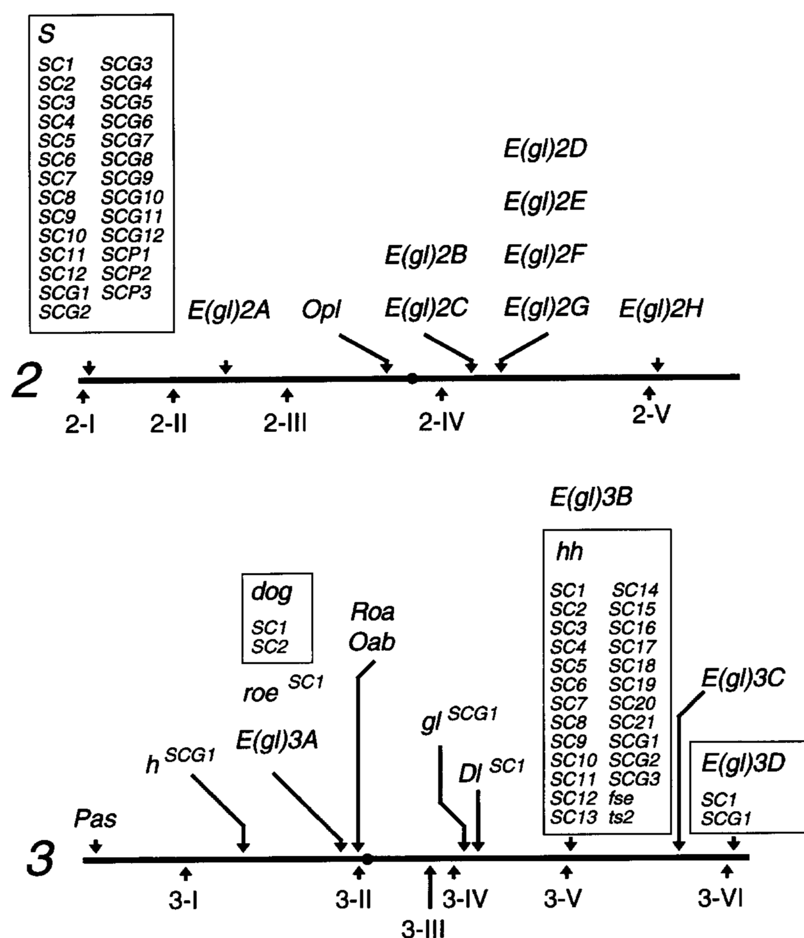

FIGURE 2.—Genetic map of *E(gl)* mutations and mapping markers. The two heavy lines marked 2 and 3 represent the second and third chromosomes of *D. melanogaster*. Below each chromosome are the positions of the transgenic w^+ mapping insertions used (see Table 1 and MATERIALS AND METHODS). Above each chromosome are shown the positions and names of all autosomal *E(gl)* mutations recovered (see Table 2). Genes with multiple alleles are boxed.

hancer of gl^3 (by examining the genotype h^{C1}/h^+; gl^3/gl^3), and it appears that h^{SCG1} is a rare antimorphic allele (h^{SCG1} homozygous embryos have a more extreme phenotype than that of a null mutation) (NADEAN BROWN and SEAN CARROLL, personal communication).

***hedgehog* (*hh*):** We recovered 26 alleles of *hh* as dominant enhancers of gl^3 (21 induced by EMS, and five induced by γ-rays, see Table 2), as confirmed by their embryonic segment polarity phenotype, map position and failure to complement the lethality associated with a known *hh* null allele, hh^{AC} (LEE *et al.* 1992). *hh* is a zygotic segment polarity gene (NÜSSLEIN-VOLHARD and WIESCHAUS 1980), which has nonautonomous effects on neighboring cells in the developing embryo and elsewhere (MOHLER 1988). *hh* encodes a secreted and cleaved protein, which comprises an extracellular signal (LEE *et al.* 1992; MOHLER and VANI 1992; TABATA *et al.* 1992; TASHIRO *et al.* 1993; LEE *et al.* 1994; BUMCROT *et al.* 1995; MARTÍ *et al.* 1995; PORTER *et al.* 1995). *hh* homologues have been found to act in several aspects of vertebrate development, including the eye (SMITH 1994; CONCORDET and INGHAM 1995; EKKER *et al.* 1995).

One of the γ-induced *hh* alleles (hh^{ts2}) is temperature sensitive. The coding sequence of hh^{ts2} has been determined, and it shows a replacement of seven amino acid residues by five others (PORTER *et al.* 1995). We have found that *hh* is expressed posterior to the morphogenetic furrow, and that it is required for the progression of the furrow (MA *et al.* 1993), where it appears to act through the TGFβ homologue *decapentaplegic* (*dpp*). These results are consistent with the effects of *hh* in retinal mosaic clones (HEBERLEIN *et al.* 1993b; MA *et al.* 1993), and ectopic Hh expression anterior to the furrow is sufficient for furrow initiation (HEBERLEIN and MOSES 1995; HEBERLEIN *et al.* 1995).

Another of the γ-induced *hh* alleles ($hh^{furrow\ stops\ early}$ or hh^{fse}) is homozygous viable with no embryonic defects. Homozygous hh^{fse} animals have a kidney-shaped eye that is very similar to the first *hh* mutation discovered, *bar-3* (now known as hh^1). We examined developing eye imaginal discs from hh^{fse} homozygous (Figure 4) and found that the phenotype is indistinguishable from that of *bar-3*: the furrow stops early (HEBERLEIN *et al.* 1993b). The *bar-3* mutation is associated with a 1.7-kb deletion in the first intron of the *hh* transcription unit (LEE *et al.* 1992). We examined genomic DNA from hh^{fse} and compared it to its progenitor chromosome and to *bar-3* by genomic DNA gel blot. Four of six restriction enzymes used reveal the presence of a new 0.8- to 1-kb deletion associated with hh^{fse} (data not shown). This new deletion overlaps with that of *bar-3* (Figure 5).

***roughened eye* (*roe*):** roe^{SC1} homozygotes are viable and show defects in the eyes, wings, and legs. The eyes are rough and reduced in size (Figure 6A) with variable and

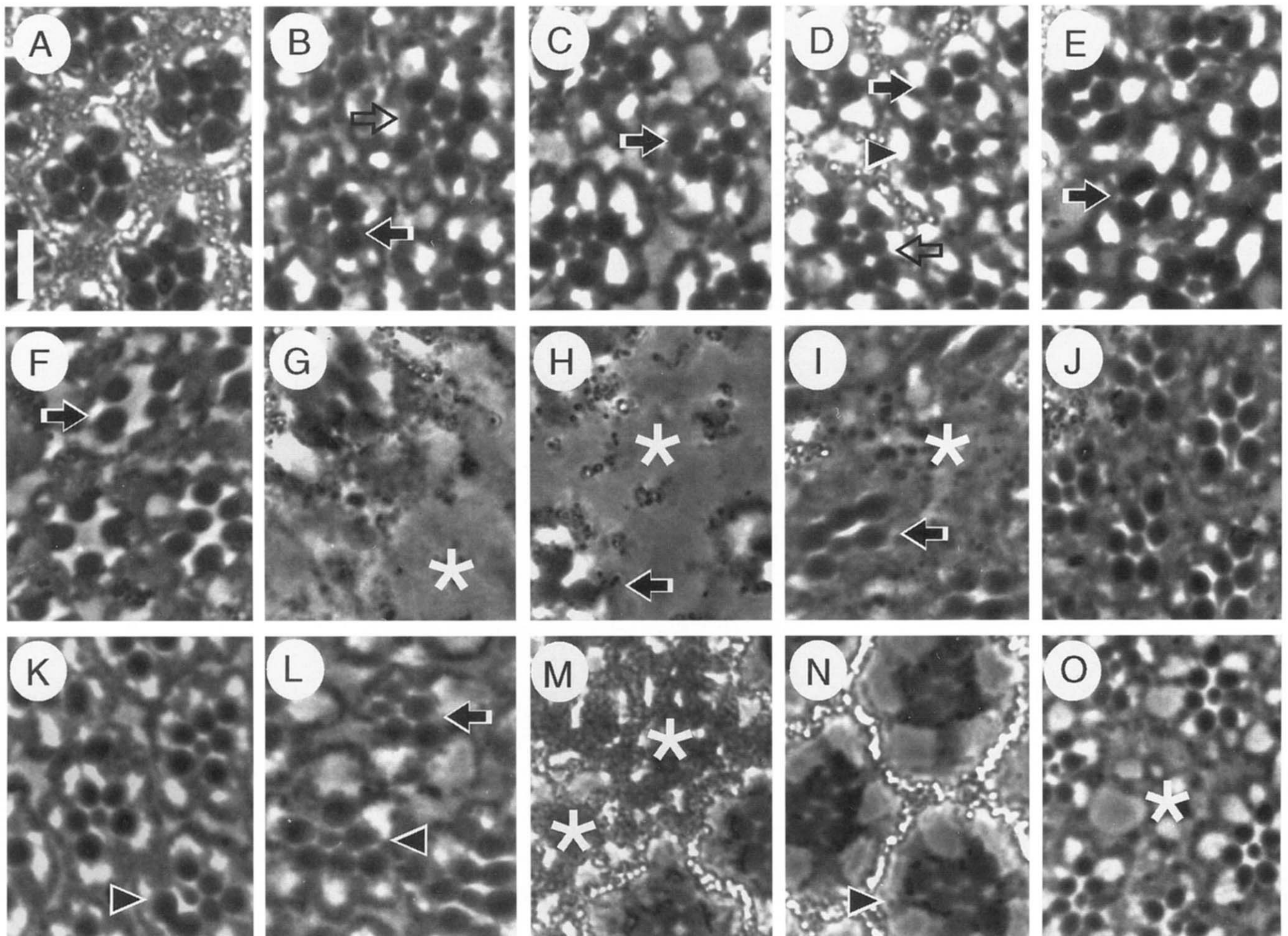

FIGURE 3.—Retinal sections of *E(gl)* mutants in a gl^3/gl^+ genetic background. All panels are shown as the dorsal half of the right eye. Anterior is to the right and dorsal is up. All panels are to the same scale, bar in A, 5 μm. (A) Wild type. B–L are mosaic clones that are homozygous for *E(gl)* mutations and are marked with w^-. (B) *Opl,* (C) *E(gl)2C,* (D) *E(gl)2D,* (E) *E(gl)2F,* (F) *Pas,* (G) h^{SCG1}, (H) dog^{SC1}, (I) *Roa,* anterior clone, (J) *Roa,* posterior clone, (K) *Oab,* (L) *E(gl)3B.* (M) *Roa* heterozygote (anterior region of the eye), (N) *Oab* heterozygote, (O) *E(gl)3C* homozygote. Arrowheads show abnormally oriented ommatidia, open arrows show ommatidia with extra rhabdomere-bearing cells, arrows show ommatidia with reduced numbers of rhabdomere-bearing cells, white asterisks show the positions of missing ommatidia.

reduced numbers of photoreceptors in almost all ommatidia (Figure 6B). The wings are short, with the distal part deleted (compare Figure 6, C and D), and the legs are also short due to the fusion of tarsal segments (compare Figure 6, E and F). These phenotypes are similar to those of some mutations in *dpp* (SPENCER *et al.* 1982) and *dachshund* (*dac*) (MARDON *et al.* 1994), which are known to affect the morphogenetic furrow (HEBERLEIN and MOSES 1995). *roe* mutations have also been recovered as dominant enhancers of N^{spl} (BRAND and CAMPOS-ORTEGA 1990). roe^{SC1} maps close to the *rotund* (*rn*) and *roe* loci (KERRIDGE and THOMAS-CAVALLIN 1988; AGNEL *et al.* 1989, 1992). Complementation tests were carried out between roe^{SC1} and rn^5 (a strong *rotund* mutation), and rn^{20} (a deletion in the *rotund* region that also includes *roe*) (R. GRIFFIN-SHEA, personal communication). roe^{SC1}/rn^5 flies show the same wing and leg defect as described above. roe^{SC1}/rn^{20} flies show small and rough eye phenotype in addition to the wing and leg defect. This eye phenotype is the same as that caused by *roe* mutations, which are adjacent to *rn* (AGNEL *et al.* 1989). We found that roe^1, roe^3, and roe^4 fail to complement the small and rough eye phenotype of roe^{SC1}. Therefore, roe^{SC1} lesion inactivates both *rn* and *roe.* The *roe* mutation is responsible for the enhancement of *gl* phenotype, since while roe^1, roe^3, roe^4 and the deletion of the region (rn^{20}) are all dominant enhancers of the phenotype of gl^3, the strong *rn* allele (rn^5) is not. Thus *roe* and *rn* show complex complementation, and it is possible that they are two classes of mutation of the same gene, each of them disrupting a subfunction: class I represented by roe^1, roe^3 and roe^4 and class II represented by rn^5, and several more alleles in these classes exist (LINDSLEY and ZIMM 1992). roe^{SC1} and the rn^{20} deletion disrupt both classes. There are several transcripts from this genomic location (AGNEL *et al.* 1992). However, some *roe* and *rn* alleles are separable by meiotic recombination, and we found 0.023% recombinants between roe^1 and rn^5 (1/4267 recombinant/total progeny).

***Star* (*S*):** *S* mutations are haplo-insufficient dominants with a rough eye phenotype (RENFRANZ and BE-

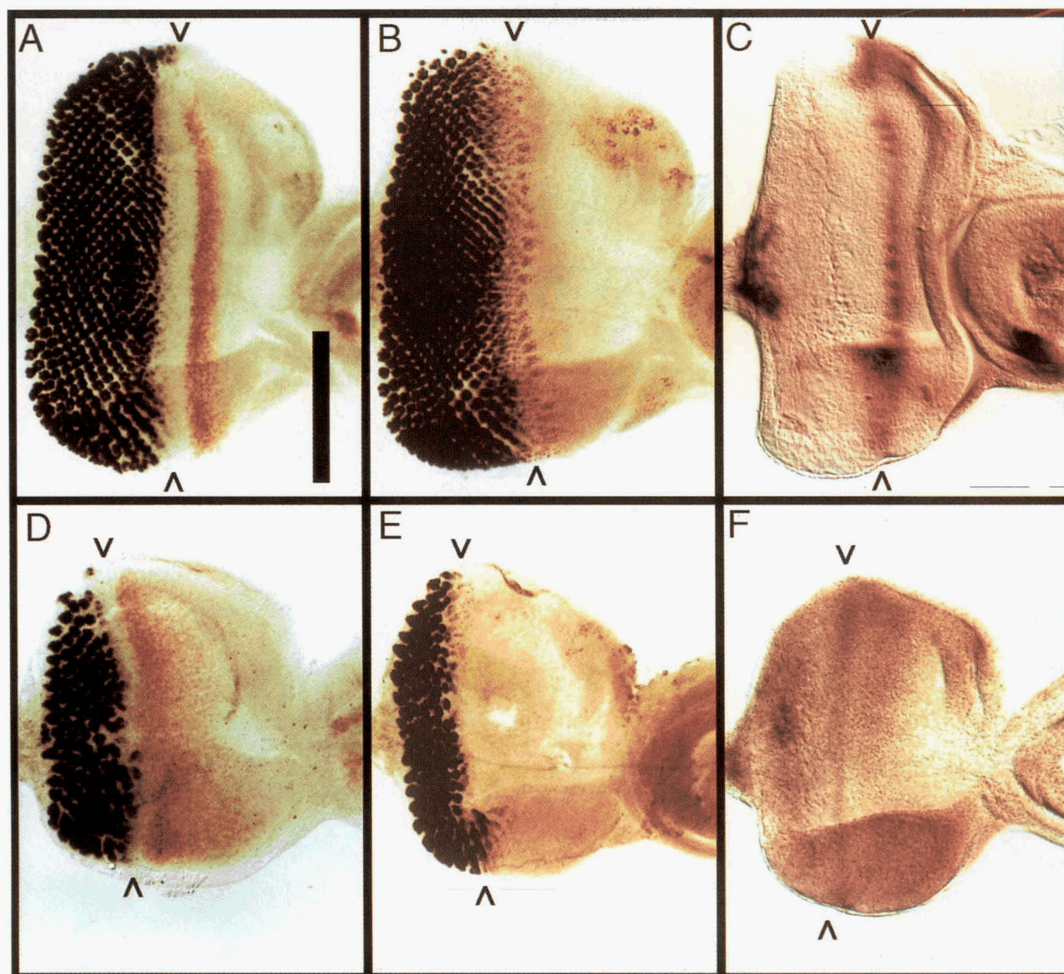

FIGURE 4.—hh^{fse} mutant phenotypes. All panels are third instar eye-imaginal discs. Anterior is to the right and dorsal is up. All panels are to the same scale, bar in A, 100 μm. V's show the position of the furrow. A–C are wild type. D–F are hh^{fse} homozygotes. A and D are stained to show the expression of Elav (black) and Hairy (brown) (see MATERIALS AND METHODS). B and E are stained to show Elav (black) and Scabrous (brown) (see MATERIALS AND METHODS). C and F are stained to show Dpp (see MATERIALS AND METHODS). Note, in hh^{fse} mutants, overly mature clusters are seen at the furrow, and Scabrous and Dpp expression are repressed.

NZER 1989) and are recessive lethal, due to an essential embryonic function in ventral midline development. *S* is one of six genes in the "*spitz*" group (NÜSSLEIN-VOLHARD *et al.* 1984; MAYER and NÜSSLEIN-VOLHARD 1988), and *S* encodes a possible transmembrane protein, required for the prospective photoreceptor cells to differentiate as neurons (HEBERLEIN and RUBIN 1991; HEBERLEIN *et al.* 1993a; KOLODKIN *et al.* 1994).

Characterization of unidentified loci: We were unable to assign mutations at 17 loci to known complementation groups, and of these, 15 are represented by only one allele (Table 2 and Figure 2). Nine of them are on the second chromosome, and eight are on the third (none lie on the fourth). We have named some of them according to their mutant phenotype and the rest are identified only by the generic *E(gl)* with the chromosome number and a letter as a specific suffix (*e.g., E(gl)2A*) (see Table 2).

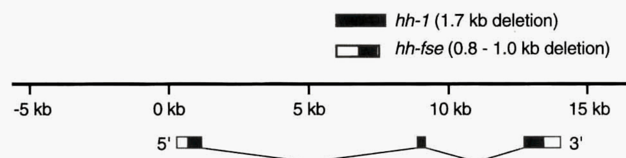

FIGURE 5.—Map of the *hh* genomic locus. The heavy line represents genomic DNA, coordinates in kb, and the structure of the transcript are after LEE *et al.* (1992). The deletions associated with *bar-3* and hh^{fse} are shown. End-point uncertainty is shown by the open box for hh^{fse}.

***Opthalmopedia-like* (*Opl*):** We examined homozygous *Opl* tissue in negatively marked (w^-) retinal mosaics, and in some cases (3/15 clones in one experiment) the mutant retinal tissue was transformed into an appendage-like extrusion (Figure 7). We cut thin sections of five mosaic retinal clones, in which no such transformation was seen. Of these, two show no obvious defects, and in three ~70% of *Opl* homozygous ommatidia (32/46 examined) show reduced numbers of photoreceptor cell rhabdomeres, and one ommatidium (in 46) had an extra cell (an overall average of 6.8 rhabdomeres, Figure 3B).

***Partial sevenless* (*Pas*):** *Pas* homozygotes die as late

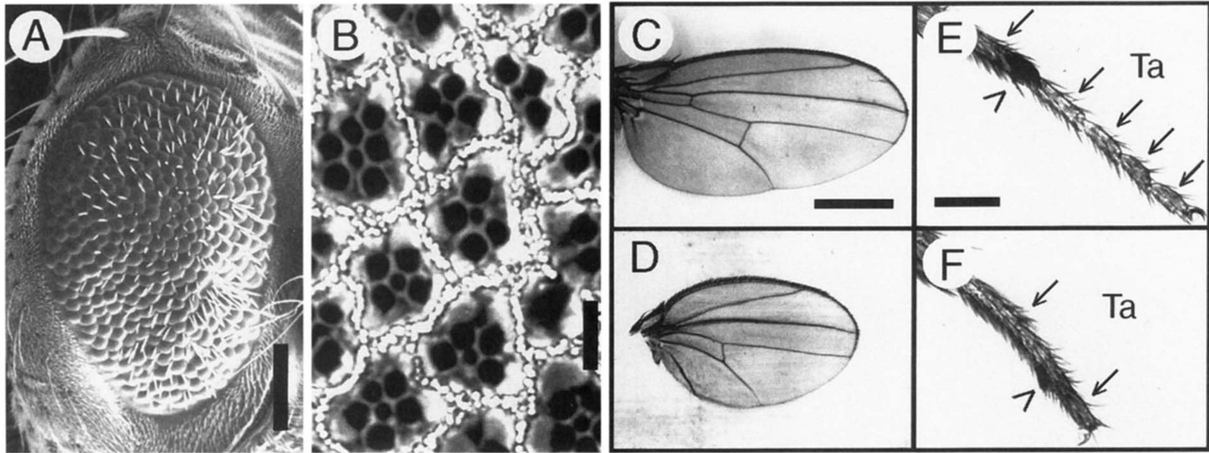

FIGURE 6.—*roe* phenotypes. A is an SEM of the adult retina of roe^{SC1}/rn^{20} (rn^{20} is a deletion of the region). Anterior is to the right, dorsal is up. Bar, 100 μm. B is a section of an eye of the same genotype as A. Anterior is to the right, dorsal is up. Bar, 5 μm. Note the reduced numbers of rhabdomere-bearing cells. C and D are whole mounts of wings. Bar, 500 μm. E and F are whole mounts of first male legs. Bar, 100 μm. Ta, tarsal segments; V, sex combs. C and E are wild type, and D and F are roe^{SC1} homozygotes. Note the shortened wing and fused tarsi.

embryos, with defects in their nervous systems (Figure 8, E and F). In a gl^+/gl^3 genetic background, *Pas* shows a dominant effect: ~40% (123/321) of the ommatidia in a *Pas* heterozygote lack the apical central photoreceptor rhabdomere, appearing to be "sevenless", and often also one or more outer cells (average is 6.1 rhabdomere-bearing cells per ommatidium). We examined homozygous *Pas* tissue in three negatively marked (w^-) retinal mosaics, and we found that the average number of rhabdomere-bearing cells per ommatidium is also 6.1 (Figure 3F).

***dog of glass* (*dog*):** *dog* heterozygous flies are sick (*sic.*), and heterozygotes have much shorter life span than that of wild-type flies. We examined homozygous, negatively marked (w^-) retinal mosaic clones for both dog^{SC1} and dog^{SC2} (six and two clones, respectively), and we found that *dog* is absolutely required for the development of all the retinal cells, as no w^- photoreceptor or pigment cells remain (Figure 3H). It is thus likely that

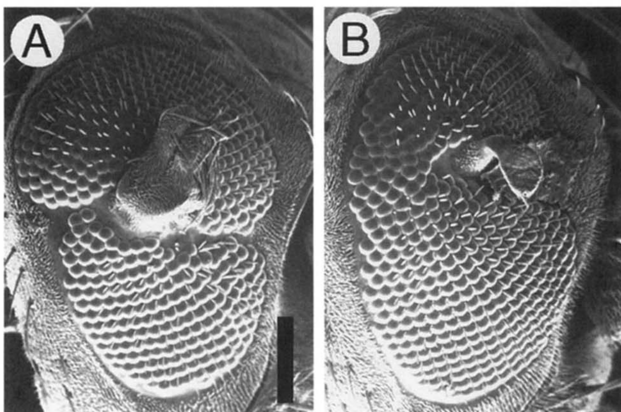

FIGURE 7.—*Opl* mosaic clones transform retinal tissue to another fate. A and B are SEMs of two examples of homozygous mosaic clones of *Opl* in a heterozygous background. Anterior is to the right and dorsal is up. Bar, 100 μm. Note bizarre objects growing from the clones.

dog is recessive cell-lethal. In the center of the mosaic tissue, there are no ommatidia and along the periphery there are abnormal ommatidia with reduced numbers of rhabdomere-bearing cells. We scored 189 rhabdomere-bearing cells in 50 such ommatidia and found that all the rhabdomere-bearing cells carry pigment granules, marking them as dog^+. Homozygous *dog* retinal clones are associated with vacuoles in the underlying lamina region of the optic lobe of the brain (data not shown).

***Rough anteriorly* (*Roa*):** *Roa* heterozygotes show a dominant retinal mutant phenotype (in a gl^+/gl^3 background): the anterior half of the eye is rough, flattened and devoid of bristles. Sections of the mutant region show that there are only sparse ommatidia isolated by massive pigment cells (Figure 3M). Forty percent of the remaining ommatidia contain reduced numbers of rhabdomere-bearing cells (average number is 4.3). Because the anterior part of the eye in a *Roa* heterozygote is mutant, we analyzed *Roa* homozygous clones in the anterior and posterior parts of the eye separately. Anterior *Roa* homozygous clones have a phenotype that is very similar to that of the heterozygous tissue (Figure 3I). Posterior homozygous *Roa* clones show no mutant phenotype (Figure 3J).

***Orientation abnormal* (*Oab*):** *Oab* heterozygotes show a dominant retinal mutant phenotype (in a gl^+/gl^3 background): a slightly rough eye. We cut sections of these eyes, and we found that the orientations of ~23% (56/245) of the mutant ommatidia are randomized and the normally regular array of ommatidia is disrupted (Figure 3N). We also examined two negatively marked (*w*) retinal mosaics, and we found similar defects: ~30% (15/49) of the mutant ommatidia showed an orientation defect (Figure 3K).

Tests of mutations in candidate genes as dominant modifiers of *gl*: As mutations of two segmentation genes (*hedgehog* and *hairy*) were recovered as dominant

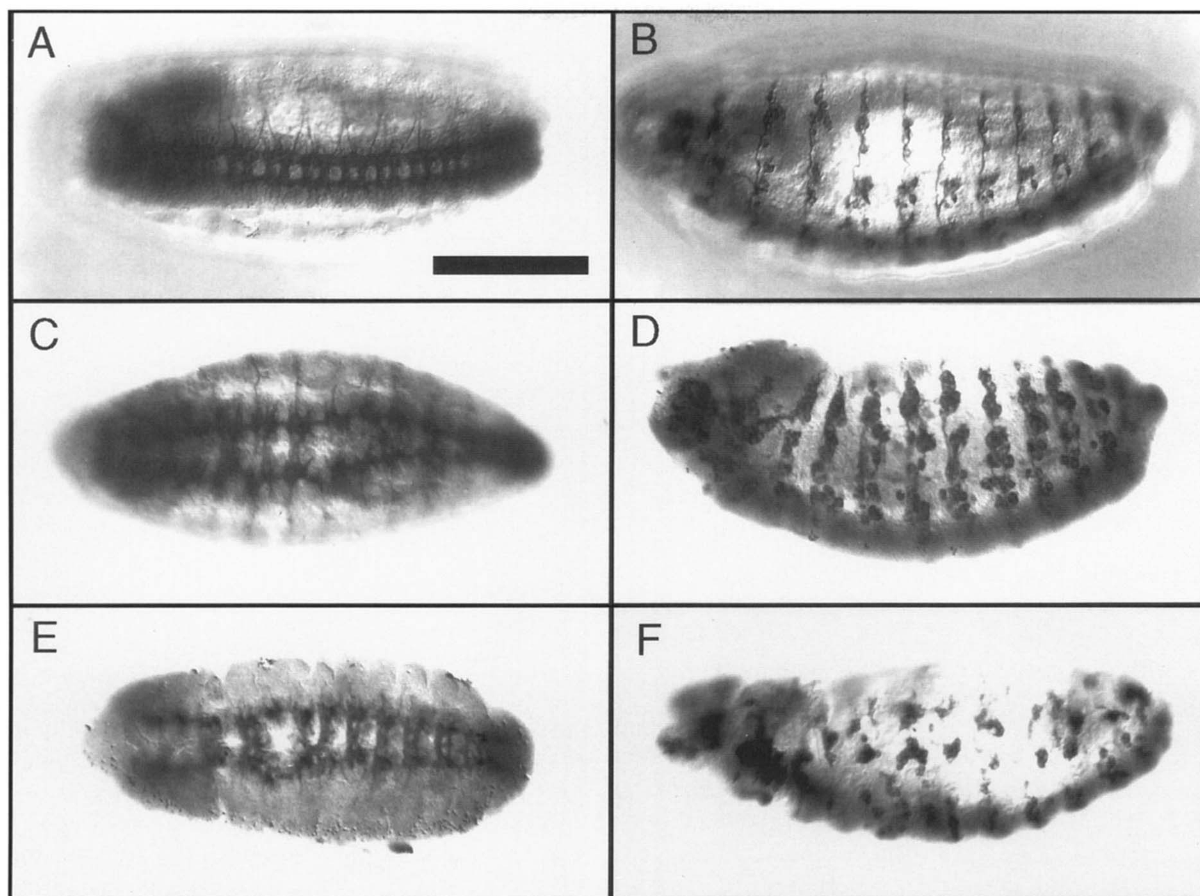

FIGURE 8.—Embryonic phenotypes of two *E(gl)* mutations. Late embryos are shown; all panels are to the same scale. Bar in A, 100 μm. Anterior is to the left. A, C and E are ventral views of embryos stained with mAb BP102 to show the central nervous system (CNS) (see MATERIALS AND METHODS). B, D and F are lateral views, ventral down, of embryos stained with mAb 22C10 to show the peripheral nervous system (PNS). A and B are wild type. C and D are *E(gl)3D* homozygotes. Note the commisural failures in the CNS and the neural hypertrophy in the PNS. E and F are *Pas* homozygotes. Note the commisural failures in the CNS and the neural disruption in the PNS.

enhancers of *gl,* we tested 25 known autosomal genes (see MATERIALS AND METHODS) for this genetic interaction. We constructed genotypes that are heterozygous for each of the 25 individual segmentation mutations and homozygous for gl^3. None of them was a dominant modifier of gl^3. However, we found that although ptc^{IN} mutation does not modify the gl^3 homozygous phenotype, it does suppress the rough eye phenotype caused by the hh^{ts2} mutation, which is a dominant enhancer of *gl* (MA *et al.* 1993; MA and MOSES 1995). In the course of the EMS screen, we recovered nine alleles of *Notch* (*N*), which were not retained. We constructed the genotypes N^{spl}/N^+; gl^3/gl^3 and N^{ts}/N^+; gl^3/gl^3 and found that loss-of-function *N* alleles are dominant enhancers of *gl.* While we do not know if null alleles of *Dl* are enhancers of *gl,* this appears unlikely, as we only recovered one *Dl* allele in this screen.

DISCUSSION

In this screen we set out to isolate autosomal second-site dominant modifiers of the phenotype of a weak *gl* allele. Such a screen might have been expected to detect genes that act to regulate *gl* activity. We recovered 76 mutations in 23 genes, as well as nine alleles of *Notch* (*N,* which were not retained), and these fall broadly into two groups: genes for which a large number of alleles were recovered (*N, S* and *hh*) and genes at which only one or two were found (all the rest). Enough is known of the molecular biology of *N, S* and *hh* to state that while these genes clearly play crucial roles in eye development, they do not interact with *gl* in a direct way. *N, S* and *hh* are all involved in the function of the morphogenetic furrow. *N* is required for the correct spacing of neurons in the furrow and later functions (CAGAN and READY 1989; BAKER and ZITRON 1995). *S* is required for the correct recruitment of those photoreceptor cells that immediately follow the founding R8 cell (HEBERLEIN and RUBIN 1991) and is likely to be involved in the EGF receptor pathway (KOLODKIN *et al.* 1994). *hh* encodes a diffusible signal that is produced in differentiating photoreceptor cells behind the furrow and acts to induce more anterior cells to enter the furrow (HEBERLEIN *et al.* 1993b, 1995; MA *et al.* 1993; HEBERLEIN and MOSES 1995). Thus all three of these genes are required for the normal specification and

differentiation of photoreceptor cells, and in that (indirect) sense, they act upstream of *gl*. It is worth noting that a screen for genes that interact with *N* in the eye recovered alleles of *gl* as well as other loci identified in this screen (*roe* and *Dl*) (BRAND and CAMPOS-ORTEGA 1990). For the most other loci detected in this screen, we do not yet have sufficient information to determine their biochemical relationship to *gl*.

It is thought that the photoreceptor cell specific activity of *gl* is due to a negative factor that binds to a DNA sequence adjacent to that of the Gl protein-binding site (ELLIS *et al.* 1993). If this is the case, the negative factor itself must be either be expressed only in the nonphotoreceptor cells, or it must be downregulated in the photoreceptor cells as they differentiate. Because that factor has not been identified yet, these possibilities remain open. One would expect loss-of-function mutations in this gene to be dominant suppressor of *gl*. However, we did not recover any dominant suppressor mutations. It is possible that one of the single-hit *E(gl)* mutations is a gain-of-function mutation of such a gene.

Why were mutations at 21 loci detected at a 10-fold lower frequency than *N, S* and *hh*? It could simply be that the level of saturation in this screen was insufficient to detect many genes. This is unlikely, based on three criteria: the number of control mutations recovered in the *white* gene, the number of recessive lethals induced on the *X* chromosome in control experiments, and the curves seen when the number of loci and the number of mutations are plotted against the number of flies screened. In this screen the mutagenized males' third chromosomes carried a transgenic copy of *white,* and as they were crossed to *white* mutant females, new *white* alleles could easily be detected in the F_1 (and tested for true-breeding in the F_1 males). In the course of these screens, we recovered 70 true-breeding *white* alleles. The *white* gene is not known for hypermutability with EMS or radiation, and thus this large number argues strongly for a high degree of saturation. The average frequency of *X*-linked lethal mutations in the EMS screens was 14%, and in the γ-ray screens it was 5%. The total number of flies inspected in those screens were 282,000 and 113,000, respectively. Assuming that there are ~1000 vital genes on the *X* chromosome, the average number of mutations per locus was 39 in the EMS screens and six in the γ-ray screens. Plotting the number of mutations recovered in the screens against the number of F_1 flies screened (open circles and solid line in Figure 9) yields a constantly increasing linear relationship. When we plot the number of genes against the number of flies screened (filled circles and dotted line in Figure 9), a much lower slope is seen and the lines cross. Perhaps the simplest explanation of these data are that there are two classes of potential targets in this (and perhaps any) genetic screen: those loci at which a simple loss-of-function mutation will produce the required phenotype (in this case *N, S* and *hh*), and those loci at which only a rare more complex mutation

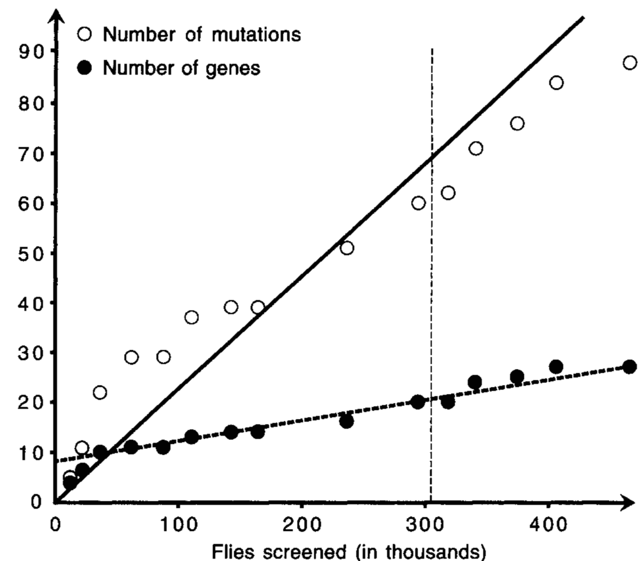

FIGURE 9.—Graph showing screen saturation. Abscissa is the number of F_1 progeny screened. Ordinate is the number of genes (●) and mutations (○). Left of the vertical dashed line, the mutagen was EMS. Right of the vertical dashed line, the mutagen was γ-rays. Hybrid-dysgenic screen data are not shown. Two lines were fitted by eye: the solid line through the open circles and the dotted line through the filled circles. Note that these two lines intersect (see text).

can yield the required phenotypic effect (this class could include a vast number of genes). This may be true in many cases for the 22 one- or two-hit loci we recovered. Indeed, the one allele of *h* appears to behave as an antimorph.

In some cases three other factors may produce a paucity of mutations. Mutations at some loci are subviable, even as heterozygotes. In this screen both *dog* mutations are subviable as heterozygotes. Thus many potential *dog* mutant F_1 flies may simply not have survived to be detected in the screens. Mutations at some loci have only very weak effects (low expressivity), for example *E(gl)2D* (Figure 1D). Such mutations are difficult to detect (by definition) and may often be missed. Similarly, some loci may produce variable phenotypes (low penetrance) and thus can also be missed in the F_1.

Several of the *E(gl)* mutations have intriguing phenotypes. The subviability of *dog* heterozygotes may suggest that the gene encodes a dose-sensitive function required for cell viability. The specificity of its interaction with *gl* might suggest that this function is associated with zinc finger transcription factors. The homeotic transformation seen in the homozygous *Opl* clones may be toward wing fates. This may correlate to the observation from disc transplantation experiments, that eye imaginal disc tissue can most easily transform to wing (HADORN 1968). Further studies of these, and other loci from these screens, may produce new insights into Drosophila retinal development in the future.

We thank G. LANDIS for help with the screen and N. E. BAKER, N. L. BROWN, S. CARROLL, C. GOODMAN, R. GRIFFIN-SHEA, J. E. HOOPER, F. M. HOFFMANN, T. LAVERTY, G. M. RUBIN and S. L. ZIPUR-

SKY for materials and reagents. This research was supported by National Eye Institute/National Institutes of Health grant 1RO1-EYO-9299.

LITERATURE CITED

AGNEL, M., S. KERRIDGE, C. VOLA and R. GRIFFIN-SHEA, 1989 Two transcripts from the rotund region of *Drosophila* show positional specificities in imaginal disc tissues. Genes Dev. **3:** 85–95.

AGNEL, M., L. RÖDER, R. GRIFFIN-SHEA and C. VOLA, 1992 The spatial expression of *Drosophila rotund* gene reveals that the imaginal discs are organized in domains along the proximal-distal axis. Roux's Arch. Dev. Biol. **201:** 284–295.

ALTON, A. K., K. FECHTEL, C. C. KOPCZYNSKI, S. B. SHEPARD, P. J. KOOH *et al.*, 1989 Molecular genetics of *Delta*, a locus required for ectodermal differentiation in *Drosophila*. Dev. Genet. **10:** 261–272.

ASHBURNER, A., 1989 *Drosophila. A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

BAKER, N. E., and G. M. RUBIN, 1989 Effect on eye development of dominant mutations in *Drosophila* homologue of the EGF receptor. Nature **340:** 150–153.

BAKER, N. E., and G. M. RUBIN, 1992 *Ellipse* mutations in the *Drosophila* homologue of the EGF receptor affect pattern formation, cell division, and cell death in eye imaginal discs. Dev. Biol. **150:** 381–396.

BAKER, N. E., and A. E. ZITRON, 1995 *Drosophila* eye development: *Notch* and *Delta* amplify a neurogenic pattern conferred on the morphogenetic furrow by *scabrous*. Mech. Dev. **49:** 173–189.

BAKER, N. E., M. MLODZIK and G. M. RUBIN, 1990 Spacing differentiation in the developing *Drosophila* eye: a fibrinogen-related lateral inhibitor encoded by *scabrous*. Science **250:** 1370–1377.

BAKER, N. E., K. MOSES, D. NAKAHARA, M. C. ELLIS, R. W. CARTHEW *et al.*, 1992 Mutations on the second chromosome affecting the *Drosophila* eye. J. Neurogenet. **8:** 85–100.

BARINAGA, M., 1995 Focusing on the *eyeless* gene. Science **267:** 1766–1767.

BASLER, K., D. YEN, A. TOMLINSON and E. HAFEN, 1990 Reprogramming cell fate in the developing *Drosophila* retina: transformation of R7 cells by ectopic expression of *rough*. Genes Dev. **4:** 728–739.

BEGEMANN, G., A.-M. MICHON, L. VAN DE VOORN, R. WEPF and M. MLODZIK, 1995 The *Drosophila* orphan nuclear receptor Seven-up requires the Ras pathway for its function in photoreceptor determination. Development **121:** 225–235.

BIER, E., L. ACKERMAN, S. BARBEL, L. JAN and Y. N. JAN, 1988 Identification and characterization of a neuron-specific nuclear antigen in *Drosophila*. Science **240:** 913–916.

BLAIR, S. S., 1995 Compartments and appendage development in *Drosophila*. BioEssays **17:** 299–309.

BONINI, N. M., W. M. LEISERSON and S. BENZER, 1993 The *eyes absent* gene: genetic control of cell survival and differentiation in the developing *Drosophila* eye. Cell **72:** 379–395.

BOTAS, J., J. MOSCOSO DEL PRADO and A. GARCIA-BELLIDO, 1982 Gene-dose titration analysis in the search of trans-regulatory genes. EMBO J. **1:** 307–310.

BRAND, M., and J. A. CAMPOS-ORTEGA, 1990 Second-site modifiers of the *split* mutation of *Notch* define genes involved in neurogenesis in *Drosophila melanogaster*. Roux's Arch. Dev. Biol. **198:** 275–285.

BROWN, N. L., C. A. SATTLER, D. R. MARKEY and S. B. CARROLL, 1991 *hairy* gene function in the *Drosophila* eye: normal expression is dispensable but ectopic expression alters cell fates. Development **113:** 1245–1256.

BROWN, N. L., C. A. SATTLER, S. W. PADDOCK and S. B. CARROLL, 1995 Hairy and Emc negatively regulate morphogenetic furrow progression in the Drosophila eye. Cell **80:** 879–887.

BUMCROT, D. A., R. TAKADA and A. P. MCMAHON, 1995 Proteolytic processing yields two secreted forms of sonic hedgehog. Mol. Cell. Biol. **15:** 2294–2303.

CAGAN, R. L., and D. F. READY, 1989 *Notch* is required for successive cell decisions in the developing *Drosophila* retina. Genes Dev. **3:** 1099–1112.

CARROLL, S. B., and J. S. WHYTE, 1989 The role of the *hairy* gene during *Drosophila* morphogenesis: stripes in imaginal discs. Genes Dev. **3:** 905–916.

CARTHEW, R. W., T. P. NEUFELD and G. M. RUBIN, 1994 Identification of genes that interact with the *sina* gene in *Drosophila* eye development. Proc. Natl. Acad. Sci. USA **91:** 11689–11693.

CHANG, D. T., A. LÓPEZ, D. P. VON KESSLER, C. CHIANG, B. K. SIMANDL *et al.*, 1994 Products, genetic linkage and limb patterning activity of a murine *hedgehog* gene. Development **120:** 3339–3353.

CHEYETTE, B. N. R., P. J. GREEN, K. MARTIN, H. GARREN, V. HARTENSTEIN *et al.*, 1994 The *Drosophila sine oculis* locus encodes a homeodomain-containing protein required for the development of the entire visual system. Neuron **12:** 1–20.

CONCORDET, J.-P., and P. INGHAM, 1995 Patterning goes sonic. Nature **375:** 279–280.

CSIK, L., 1929 Ein neuer Erbfaktor bei *Drosophila melanogaster*. Biol. Zentbl. **49:** 419–421.

DEQUIN, R., H. SAUMWEBER and J. W. SEDAT, 1984 Proteins shifting from the cytoplasm into the nuclei during early embryogenesis of *Drosophila melanogaster*. Dev. Biol. **104:** 37–48.

DICKSON, B., and E. HAFEN, 1994 Genetics of signal transduction in invertebrates. Curr. Opin. Genet. Dev. **4:** 64–70.

DIETRICH, U., and J. A. CAMPOS-ORTEGA, 1984 The expression of neurogenic loci in imaginal epidermal cells of *Drosophila melanogaster*. J. Neurogenet. **1:** 315–332.

DIETRICH, W., 1909 Die facettenaugen der Dipteren. Z. Wiss. Zool. **92:** 465–539.

ECHELARD, Y., D. J. EPSTEIN, B. ST. JACQUES, L. SHEN, J. MOHLER *et al.*, 1993 Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. Cell **75:** 1417–1430.

EKKER, S. C., A. R. UNGAR, P. GREENSTEIN, D. P. VON KESSLER, J. A. PORTER *et al.*, 1995 Patterning activities of vertebrate hedgehog proteins in the developing eye and brain. Curr. Biol. **5:** 944–955.

ELLIS, M. C., E. M. O'NEILL and G. M. RUBIN, 1993 Expression of *Drosophila glass* protein and evidence for negative regulation of its activity in non-neuronal cells by another DNA-binding protein. Development **119:** 855–865.

ELLIS, M. C., U. WEBER, V. WIERSDORFF and M. MLODZIK, 1994 Confrontation of *scabrous* expressing and non-expressing cells is essential for normal ommatidial spacing in the *Drosophila* eye. Development **120:** 1959–1969.

FUJITA, S. C., S. L. ZIPURSKY, S. BENZER, A. FERRÚS and S. L. SHOTWELL, 1982 Monoclonal antibodies against the *Drosophila* nervous system. Proc. Natl. Acad. Sci. USA **79:** 7929–7933.

GARCÍA-BELLIDO, A., P. A. LAWRENCE and G. MORATA, 1979 Compartments in animal development. Sci. Am. **241:** 102–110.

HADORN, E., 1968 Transdetermination in cells. Sci. Am. **219:** 11–120.

HALDER, G., P. CALLAERTS and W. J. GEHRING, 1995 Induction of ectopic eyes by targeted expression of the *eyeless* gene in *Drosophila*. Science **267:** 1788–1792.

HARDING, K. W., G. G. N. MCGINNIS and W. MCGINNIS, 1995 A screen for modifiers of *Deformed* function in *Drosophila*. Genetics **140:** 1339–1352.

HARRISON, D. A., D. A. GDULA, R. S. COYNE and V. G. CORCES, 1993 A leucine zipper domain of the suppressor of Hairy-wing protein mediates its repressive effect on enhancer function. Genes Dev. **7:** 1966–1978.

HAZELRIGG, T., R. LEVIS and G. M. RUBIN, 1984 Transformation of *white* locus DNA in *Drosophila:* dosage compensation, *zeste* interaction, and position effects. Cell **36:** 469–481.

HEBERLEIN, U., and K. MOSES, 1995 Mechanisms of *Drosophila* retinal morphogenesis: the virtues of being progressive. Cell **81:** 987–990.

HEBERLEIN, U., and G. M. RUBIN, 1991 *Star* is required in a subset of photoreceptor cells in the developing *Drosophila* retina and displays dosage sensitive interactions with *rough*. Dev. Biol. **144:** 353–361.

HEBERLEIN, U., I. K. HARIHARAN and G. M. RUBIN, 1993a *Star* is required for neuronal differentiation in the *Drosophila* retina and displays dosage-sensitive interactions with *Ras 1*. Dev. Biol. **160:** 51–63.

HEBERLEIN, U., T. WOLFF and G. M. RUBIN, 1993b The TGFβ homolog dpp and the segment polarity gene *hedgehog* are required for propagation of a morphogenetic wave in the *Drosophila* retina. Cell **75:** 913–926.

HEBERLEIN, U., C. M. SINGH, A. Y. LUK and T. J. DONOHOE, 1995

Growth and differentiation in the *Drosophila* eye coordinated by *hedgehog*. Nature **373:** 709–711.

HIROMI, Y., M. MLODZIK, S. R. WEST, G. M. RUBIN and C. S. GOODMAN, 1993 Ectopic expression of seven-up causes cell fate changes during ommatidial assembly. Development **118:** 1123–1135.

HOOVER, K.-K., T. I. GERASIMOVA, A. J. CHIEN and V. G. CORCES, 1992 Dominant effects of *suppressor of Hairy-wing* mutations on gypsy-induced alleles of *forked* and *cut* in *Drosophila melanogaster*. Genetics **132:** 691–697.

HORTSCH, M., A. J. BIEBER, N. H. PATEL and C. S. GOODMAN, 1990 Differential splicing generates a nervous system-specific form of *Drosophila* neuroglian. Neuron **4:** 697–709.

INGHAM, P. W., 1994 *Hedgehog* points the way. Curr. Biol. **4:** 347–350.

INGHAM, P. W., S. M. PINCHIN, K. R. HOWARD and D. ISH-HOROWICZ, 1985 Genetic analysis of the *hairy* locus in *Drosophila melanogaster*. Genetics **111:** 463–486.

KERRIDGE, S., and M. THOMAS-CAVALLIN, 1988 Appendage morphogenesis in *Drosophila:* a development study of the rotund (rn) gene. Roux's Arch. Dev. Biol. **197:** 19–26.

KIMMEL, B. E., U. HEBERLEIN and G. M. RUBIN, 1990 The homeo domain 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.

KOLODKIN, A. L., A. T. PICKUP, D. M. LIN, C. S. GODDMAN and U. BANERJEE, 1994 Characterization of *Star* and its interactions with *sevenless* and EGF receptor during photoreceptor cell development in *Drosophila*. Development **120:** 1731–1745.

KOPCZYNSKI, C. C., A. K. ALTON, K. FECHTEL, P. J. KOOH and M. A. MUSKAVITCH, 1988 *Delta*, a *Drosophila* neurogenic gene, is transcriptionally complex and encodes a protein related to blood coagulation factors and epidermal growth factor of vertebrates. Genes Dev. **2:** 1723–1735.

KRAMER, S., S. R. WEST and Y. HIROMI, 1995 Cell fate control in the *Drosophila* retina by the orphan receptor seven-up: its role in the decisions mediated by the ras signaling pathway. Development **121:** 1361–1372.

KRAUSS, S., J. P. CONCORDET and P. W. INGHAM, 1993 A functionally conserved homolog of the *Drosophila* segment polarity gene *hh* is expressed in tissues with polarizing activity in zebrafish embryos. Cell **75:** 1431–1444.

LASKI, F. A., D. C. RIO and G. M. RUBIN, 1986 Tissue specificity of *Drosophila* P element transposition is regulated at the level of mRNA splicing. Cell **44:** 7–19.

LAUFER, E., C. E. NELSON, R. L. JOHNSON, B. A. MORGAN and C. TABIN, 1994 *Sonic hedgehog* and *Fgf-4* act through a signaling cascade and feedback loop to integrate growth and patterning of the developing limb bud. Cell **79:** 993–1003.

LEE, J. J., D. P. VON KESSLER, S. PARKS and P. A. BEACHY, 1992 Secretion and localized transcription suggest a role in positional signaling for products of the segmentation gene *hedgehog*. Cell **71:** 33–50.

LEE, J. J., S. C. EKKER, D. P. VON KESSLER, J. A. PORTER, B. I. SUN *et al.*, 1994 Autoproteolysis in hedgehog protein biogenesis. Science **266:** 1528–1537.

LINDSLEY, D. L., and G. G. ZIMM, 1992 *The Genome of Drosophila melanogaster*. Academic Press, San Diego.

MA, C., and K. MOSES, 1995 *wingless* and *patched* are negative regulators of the morphogenetic furrow and can affect tissue polarity in the developing *Drosophila* compound eye. Development **121:** 2279–2289.

MA, C., Y. ZHOU, P. A. BEACHY and K. MOSES, 1993 The segment polarity gene *hedgehog* is required for progression of the morphogenetic furrow in the developing *Drosophila* eye. Cell **75:** 927–938.

MADEN, M., 1994 The limb bud—part two. Nature **371:** 560–561.

MARDON, G., N. M. SOLOMON and G. M. RUBIN, 1994 *dachshund* encodes a nuclear protein required for normal eye and leg development in *Drosophila*. Development **120:** 3473–3486.

MARTÍ, E., D. A. BUMCROT, R. TAKADA and A. P. MCMAHON, 1995 Requirement of 19K form of sonic hedgehog for induction of distinct ventral cell types in CNS explants. Nature **375:** 322–325.

MAYER, U., and C. NÜSSLEIN-VOLHARD, 1988 A group of genes required for pattern formation in the ventral ectoderm of the *Drosophila* embryo. Genes Dev. **2:** 1496–1511.

MEINERTZHAGEN, I. A., and T. E. HANSON, 1993 The development of the optic lobe, pp. 1363–1491 in *The Development of Drosophila melanogaster*, edited by M. BATE and A. MARTINEZ-ARIAS. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

MELAMED, J., and O. TRUJILLO-CENÓZ, 1975 The fine structure of the eye imaginal disks in Muscoid flies. J. Ultrastr. Res. **51:** 79–93.

MLODZIK, M., N. E. BAKER and G. M. RUBIN, 1990a Isolation and expression of *scabrous*, a gene regulating neurogenesis in *Drosophila*. Genes Dev. **4:** 1848–1861.

MLODZIK, M., Y. HIROMI, U. WEBER, C. S. GOODMAN and G. M. RUBIN, 1990b The *Drosophila seven-up* gene, a member of the steriod receptor gene superfamily, controls photreceptor cell fates. Cell **60:** 211–224.

MOHLER, J., 1988 Requirements for *hedgehog*, a segmental polarity gene, in patterning larval and adult cuticle of *Drosophila*. Genetics **120:** 1061–1072.

MOHLER, J., and K. VANI, 1992 Molecular organization and embryonic expression of the *hedgehog* gene involved in cell-cell communication in segmental patterning of *Drosophila*. Development **115:** 957–971.

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.

MOSES, K., M. C. ELLIS and G. M. RUBIN, 1989 The *glass* gene encodes a zinc-finger protein required by *Drosophila* photoreceptor cells. Nature **340:** 531–536.

MULLER, H. J., 1932 Further studies on the nature and causes of gene mutations, pp. 213–255 in *International Congress of Genetics*, edited by D. F. JONES. Brooklyn Botanic Garden, Brooklyn, NY.

MURRE, C., P. S. MCCAW and D. BALTIMORE, 1989 A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD, and myc proteins. Cell **56:** 777–783.

NISWANDER, L., S. JEFFREY, G. R. MARTIN and C. TICKLE, 1994 A positive feedback loop coordinates growth and patterning in the vertebrate limb. Nature **371:** 609–612.

NOHNO, T., Y. KAWAKAMI, H. OHUCHI, A. FUJIWARA, H. YOSHIOKA *et al.*, 1995 Involvement of the *Sonic hedgehog* gene in chick feather formation. Biochem. Biophys. Res. Com. **206:** 33–39.

NÜSSLEIN-VOLHARD, C., and E. WIESCHAUS, 1980 Mutations affecting segment number and polarity in *Drosophila*. Nature **287:** 795–801.

NÜSSLEIN-VOLHARD, C., E. WIESCHAUS and H. KLUDING, 1984 Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. I. Zygotic loci on the second chromosome. Roux's Arch. Dev. Biol. **183:** 267–282.

O'KANE, C. J., and W. J. GEHRING, 1987 Detection *in situ* of genomic regulatory elements in *Drosophila*. Proc. Natl. Acad. Sci. USA **84:** 9123–9127.

O'NEILL, E. M., M. C. ELLIS, G. M. RUBIN and R. TJIAN, 1995 Functional domain analysis of Glass, a zinc-finger-containing transcription factor in *Drosophila*. Proc. Natl. Acad. Sci. USA **92:** 6557–6561.

PANGANIBAN, G., K. E. RASHKA, M. D. NEITZEL and F. M. HOFFMANN, 1990 Biochemical characterization of the *Drosophila dpp* protein, a memeber of the Transforming Growth Factor b family of growth factrors. Mol. Cell. Biol. **10:** 2669–2677.

PARKHURST, S. M., and V. G. CORCES, 1986 Retroviral elements and suppressor genes in *Drosophila*. BioEssays **5:** 52–57.

PARKHURST, S. M., D. A. HARRISON, M. P. REMINGTON, C. SPAN, R. L. KELLEY *et al.*, 1988 The *Drosophila su(Hw)* gene, which controls the phenotypic effect of the *gypsy* transposable element, encodes a putative DNA-binding protein. Genes Dev. **2:** 1205–1215.

PEIFER, M., and A. BEJSOVEC, 1992 Knowing your neighbors: cell interactions determine intrasegmental patterning in *Drosophila*. Trends Genet. **8:** 243–249.

PORTER, J. A., D. P. VON KESSLER, S. C. EKKER, K. E. YOUNG, J. J. LEE *et al.*, 1995 The product of Hedgehog autoproteolytic cleavage active in local and long-range signaling. Nature **374:** 363–366.

QUIRING, R., U. WALLDORF, U. KLOTER and W. J. GEHRING, 1994 Homology of the *eyeless* gene of *Drosophila* to the *small eye* gene in mice and *Aniridia* in humans. Science **265:** 785–789.

READY, D. F., T. E. HANSON and S. BENZER, 1976 Development of the *Drosophila* retina, a neurocrystalline lattice. Dev. Biol. **53:** 217–240.

RENFRANZ, P. J., and S. BENZER, 1989 Monoclonal antibody probes discriminate early and late mutant defects in development of the *Drosophila* retina. Dev. Biol. **136:** 411–429.

ROBERTSON, H. M., C. R. PRESTON, R. W. PHILLIS, D. M. JOHNSON-

SCHLITZ, W. K. BENZ *et al.*, 1988 A stable genomic source of P element transposase in *Drosophila melanogaster*. Genetics **118:** 461–470.

ROELINK, H., A. AUGSBURGER, J. HEEMSKERK, V. KORZH, S. NORLIN *et al.*, 1994 Floor plate and motor neuron induction by *vhh-1*, a vertebrate homolog of *hedgehog* expressed by the notochord. Cell **76:** 761–775.

ROGGE, R. D., C. A. KARLOVICH and U. BANERJEE, 1991 Genetic dissection of a neurodevelopmental pathway: son of sevenless functions downstream of the sevenless and EGF receptor tyrosine kinases. Cell **64:** 39–48.

RUSHLOW, C. A., A. HOGAN, S. M. PINCHIN, K. M. HOWE, M. LARDELLI *et al.*, 1989 The *Drosophila hairy* protein acts in both segmentation and bristle patterning and shows homology to *N-myc*. EMBO J. **8:** 3095–3103.

SHEN, B., J. KIM and D. DORSETT, 1994 The enhancer-blocking suppressor of Hairy-wing zinc finger protein of *Drosophila melanogaster* alters DNA structure. Mol. Cell. Biol. **14:** 5645–5652.

SHESHBERADARAN, H., and J. S. TAKAHASHI, 1994 Characterization of the chicken rhodopsin promoter: identification of retina-specific and *glass*-like protein binding domains. Mol. Cell. Neurosci. **5:** 309–318.

SIMON, M. A., D. D. L. 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.

SMITH, J. C., 1994 Hedgehog, the floor plate, and the zone of polarizing activity. Cell **76:** 193–196.

SMITH, P. A., and V. G. CORCES, 1995 The suppressor of Hairy-wing protein regulates the tissue-specific expression of the *Drosophila* gypsy retrotransposon. Genetics **139:** 215–228.

SPENCER, F. A., F. M. HOFFMANN and W. M. GELBART, 1982 *Decapentaplegic:* a gene complex affecting morphogenesis in *Drosophila melanogaster*. Cell **28:** 451–461.

TABATA, T., and T. B. KORNBERG, 1994 Hedgehog is a signaling protein with a key role in patterning *Drosophila* imaginal discs. Cell **76:** 89–102.

TABATA, T., S. EATON and T. B. KORNBERG, 1992 The *Drosophila hedgehog* gene is expressed specifically in posterior compartment cells and is a target of *engrailed* regulation. Genes Dev. **6:** 2635–2645.

TASHIRO, S., T. MICHIUE, S.-I. HIGASHIJIMA, S. ZENNO, S. ISHIMARU *et al.*, 1993 Structure and expression of *hedgehog*, a *Drosophila* segment-polarity gene required for cell-cell communication. Gene **124:** 183–189.

THAKER, H. M., and D. R. KANKEL, 1992 Mosaic analysis gives an estimate of the extent of genomic involvement in the development of the visual system in *Drosophila melanogaster*. Genetics **13:** 883–894.

TOMLINSON, A., 1988 Cellular interactions in the developing *Drosophila* eye. Development **104:** 183–193.

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

TOMLINSON, A., and D. F. READY, 1987b Neuronal differentiation in the *Drosophila* ommatidium. Dev. Biol. **120:** 366–376.

WADDINGTON, C. H., and M. M. PERRY, 1960 The ultra-structure of the developing eye of *Drosophila*. Proc. Roy. Soc. Lond. B **153:** 155–178.

WEMMER, T., and C. KLÄMBT, 1995 A genetic analysis of the *Drosophila* closely linked interacting genes *bulge, argos,* and *soba*. Genetics **140:** 629–641.

WIESCHAUS, E., and C. NÜSSLEIN-VOLHARD, 1986 Looking at embryos, pp. 199–227 in *Drosophila: A Practical Approach*, edited by D. B. ROBERTS. IRL Press, Oxford.

WOLFF, T., and D. F. READY, 1991 The beginning of pattern formation in the *Drosophila* compound eye: the morphogenetic furrow and the second mitotic wave. Development **113:** 841–850.

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

ZAK, N. B., and B.-Z. SHILO, 1992 Localization of DER and the pattern of cell divisions in wild-type and *Ellipse* eye imaginal discs. Dev. Biol. **149:** 448–456.

Communicating editor: T. SCHÜPBACH