A Genetic Method for Generating Drosophila Eyes Composed Exclusively of Mitotic Clones of a Single Genotype

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ABSTRACT

The genetic analysis of a gene at a late developmental stage can be impeded if the gene is required at an earlier developmental stage. The construction of mosaic animals, particularly in Drosophila, has been a means to overcome this obstacle. However, the phenotypic analysis of mitotic clones is often complicated because standard methods for generating mitotic clones render mosaic tissues that are a composite of both mutant and phenotypically normal cells. We describe here a genetic method (called *EGUF/hid*) that uses both the *GAL4/UAS* and *FLP/FRT* systems to overcome this limitation for the Drosophila eye by producing genetically mosaic flies that are otherwise heterozygous but in which the eye is composed exclusively of cells homozygous for one of the five major chromosome arms. These eyes are nearly wild type in size, morphology, and physiology. Applications of this genetic method include phenotypic analysis of existing mutations and F_1 genetic screens to identify as yet unknown genes involved in the biology of the fly eye. We illustrate the utility of the method by applying it to lethal mutations in the synaptic transmission genes *synaptotagmin* and *syntaxin*.

THE compound eye of Drosophila has been an invaluable model system for studying fundamental biological questions in development and physiology. The principal advantages of the Drosophila eye are that phenotypes are recognized with relative ease and that the eye is amenable to molecular genetic analysis. Examples of its utility include the elucidation of both the *sevenless* signaling pathway (reviewed by Simon 1994; Zipursky and Rubin 1994) and the phototransduction cascade (reviewed by Pak 1995; Zuker 1996).

These two aspects of the biology of the fly eye have been suitable for study because key genes in these pathways are not essential for the viability of the organism. Therefore, mutations in nonessential genes involved in these pathways were isolated in screens of adult animals for aberrant phototaxis, electrophysiology, or eye morphology (*e.g.*, Benzer 1967; Pak *et al.* 1969; Heisenberg 1971; Harris *et al.* 1976; Stephenson *et al.* 1983; Reinke and Zipursky 1988; Ondek *et al.* 1992).

To study genes in the fly eye that are essential at earlier developmental stages, several types of F_1 genetic screen have been carried out that attempt to overcome their requirement for adult viability. One form uses a sensitized genotype and relies on recognizing mutants on the basis of suppression or enhancement of a dosage-sensitive eye phenotype (usually a rough eye) created by either a dominant or a homozygous viable allele of a gene in the pathway under study (Simon *et al.* 1991; Dickson *et al.* 1996; Karim *et al.* 1996; Neufeld *et al.* 1998). Though large numbers of flies can be screened easily, the weakness of these screens is that they typically identify only genes that are limiting in a pathway (*i.e.*, where elimination of one copy of the gene product results in a recognizable change in the sensitized phenotype).

Another type of F_1 screen involves identifying genes on the basis of their expression pattern as typically revealed by *lacZ* expression in *P*-element "enhancer trap" lines (Freeman *et al.* 1992). More sophisticated pattern-based screens rely on enhancer trap expression of a protein that produces a phenotype that can be screened for in adult animals (Hay *et al.* 1994; Pignoni *et al.* 1997). These expression pattern-based screens thus overcome the problem of adult viability by recognizing a phenotype in heterozygous animals. However, these screens also have limitations: (1) *lacZ* expression screens are particularly laborious; (2) expression of a gene in a particular tissue by no means ensures that the gene is performing an important function in that tissue; and (3) only a minority of genes can be identified in this way because *P*-element insertion is not random (Engels 1996). Efficiently generating flies that are homozygous mutant only in the eye would make possible function-based screens for uncovering components of both developmental and physiological pathways.

In this article we describe a method to create such a fly by combining the *GAL4/UAS* system (Brand and Perrimon 1993) and the *FLP* recombinase system (Golic and Lindquist 1989; Xu and Rubin 1993) via

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the *UAS-FLP* transgene (Duffy *et al.* 1998). This technique has been made possible by the identification of enhancers active in the developing eye (Hay *et al.* 1994; Hazelett *et al.* 1998) and by progress in the study of apoptosis (Grether *et al.* 1995). Our motivation for developing this method was to determine the electroretinogram (ERG) phenotype of known synaptic transmission mutants as well as to use the method in genetic screens designed to identify novel genes involved in synaptic transmission. However, we believe this method will be a generally applicable and powerful tool for identifying and studying genes involved in nearly all aspects of the biology of the Drosophila eye.

MATERIALS AND METHODS

Stocks: The balancer chromosomes used in this article are described in Lindsley and Zimm (1992). All FLP recombinase target (*FRT*) chromosomes used in this article are described in Xu and Rubin (1993) and were obtained through the Bloomington Stock Center as was the *CyO* Δ*2-3* stock. *FRT* recombinations were performed as described in Xu and Rubin (1993). The homozygous viable second chromosome insert of the *ey-GAL4* driver was generously provided by Uwe Walldorf (described in Hazelett *et al.* 1998). A homozygous viable third chromosome insert of the *ey-GAL4* driver was generated by transposition of the second chromosome insertion using a *CyO* Δ*2-3* chromosome as a transposase source. Novel *ey-GAL4* insertions were recognized by an increase in *w*+ dosage in the subsequent generation. Third chromosome localization was determined by segregation. Second and third chromosome homozygous viable inserts of *UAS-FLP* are described in Duffy *et al.* (1998). Second and third *EGUF* (*Eyeless-GAL4 UAS-FLP*) chromosomes containing both *ey-GAL4* and *UAS-FLP* were generated by meiotic recombination. The original pGMR-*hid* insertion was obtained from Hermann Steller (described in Grether *et al.* 1995). This second chromosome GMR-*hid* insertion was recombined onto *FRT 40A* and *FRT 42D* and was localized to chromosome arm 2L because only the *FRT40A* GMR-*hid* chromosome showed mitotic recombination-induced suppression of the GMR-*hid* phenotype in the presence of both the corresponding homologous *FRT* chromosome and an *EGUF* chromosome. Insertions of pGMR-*hid* on the X, 3L, and 3R chromosome arms were generated by introducing transposase via a Δ*2-3 CyO* chromosome into the original pGMR-*hid* stock. Progeny flies containing multiple copies of pGMR-*hid* (presumably the original insertion as well as a novel one) were recognized by a change in the single-dose pGMR-*hid* eye phenotype (photoreceptors absent, ~20–30 eye bristles remaining) to the multiple-dose pGMR-*hid* eye phenotype (photoreceptors absent, all eye bristles absent). Localization of new pGMR-*hid* insertions to the X and third chromosomes was determined by segregation. Localization of third chromosome pGMR-*hid* insertions to 3L or 3R was determined analogously as described above for the original second chromosome pGMR-*hid* insertion to produce the *FRT80B* GMR-*hid* and *FRT82B* GMR-*hid* chromosomes. An X chromosome pGMR-*hid* insertion was recombined with *FRT19A* to produce the *FRT19A* GMR-*hid* chromosome. The insertion of pGMR-*hid* on chromosome arm 2R was generated by introducing transposase via a Δ*2-3 CyO* chromosome into males with an X chromosome pGMR-*hid* insertion. Novel autosomal pGMR-*hid* insertions were recognized by the dominant pGMR-*hid* phenotype in males. Second chromosome localization of these pGMR-*hid* insertions was determined by segregation. Chromosome arm 2R localization was determined as described above for the original pGMR-*hid* insertion to produce the *FRT42D* GMR-*hid* chromosome. The cell lethal mutation on chromosome arm 2L was obtained in one of our autosomal ethyl methanesulfonate (EMS) mutagenesis screens for ERG defective mutants (work in progress) and was placed on the *FRT40A* GMR-*hid* chromosome arm by meiotic recombination to give *FRT40A* GMR-*hid CL* (*C*ell *L*ethal). Presumable cell lethal mutations were introduced onto the other four *FRT* GMR-*hid* chromosome arms directly by mutagenesis with 3000 R of gamma rays. *FRT* GMR-*hid CL* chromosomes were recognized by their ability to produce an eye with near wild-type morphology in the presence of both the corresponding *FRT* chromosome and an *EGUF* chromosome. These chromosomes are referred to as *FRT19A* GMR-*hid CL*, *FRT42D* GMR-*hid CL*, *FRT80B* GMR-*hid CL*, and *FRT82B* GMR-*hid CL*. The *synaptotagmin* null allele, syt^{AD4} was described in Di Antonio and Schwarz (1994) and was recombined onto *FRT40A* to give *FRT40A* syt^{AD4}. The *FRT82B* $syntaxin^{L371}$ chromosome was described in Burgess *et al.* (1997).

Electroretinograms: ERGs were performed by placing a reference electrode in the thorax and a recording electrode on the eye and giving 1-sec pulses of light stimuli in a nearly dark room. Both electrodes were filled with 85 mm NaCl. Light stimuli were manually initiated by keystroke with pClamp6 software controlling a shutter (Uniblitz VS35) via a shutter driver (Uniblitz T132).

Scanning electron microscopy: Flies were prepared for scanning electron microscopy (SEM) as described (Simon *et al.* 1991) and were analyzed with a Philips Electron Optics (Eindhoven, The Netherlands) model 505 SEM.

RESULTS

Development of the *EGUF/hid* method: To study essential synaptic transmission genes in photoreceptor neurons, it was necessary to develop a method for generating a fly in which the homozygous mutant phenotype of essential genes could be analyzed by ERG, the standard assay for synaptic transmission in the fly eye. Three features were required of such a fly. First, the fly had to contain homozygous mutant eye clones of sufficient size to produce a signal when analyzed by ERG. Second, the mitotic clones produced had to be highly specific for the eye. Because most genes involved in synaptic transmission are essential, mitotic clones arising in vital cells would prevent survival to adulthood and thus preclude ERG analysis. Third, only photoreceptor neurons homozygous for the mutation of interest could be present in the eye of the fly. Otherwise, the background ERG signal generated by photoreceptor neurons of other genotypes would confuse the interpretation of the ERG.

To fulfill the first two requirements, we combined the advantages of both the *GAL4/UAS* and *FLP/FRT* systems to generate mitotic clones in the eye. Specifically, we used the eye-specific *GAL4* driver *ey-GAL4* (Hazelett *et al.* 1998) in combination with a *UAS-FLP* transgene (Duffy *et al.* 1998) to express the site-specific recombinase *FLP* in mitotically active eye precursor cells. When appropriately matched homologous chromosomes containing *FRT*s are present in these cells, *FLP*-mediated site-specific mitotic recombination results, thus creating

the possibility for homozygous mitotic clones to be produced at cell division. To satisfy the third requirement of eliminating all photoreceptor cells not homozygous for the mutation of interest, we generated five chromosomes, one for each of the five major Drosophila chromosome arms, that each contains both an *FRT* at the base as well as a more distally located insertion of the dominant photoreceptor cell lethal transgene GMR-*hid* (Gretherer *et al.* 1995). This transgene kills photoreceptor cells because of eye-specific expression of the cell death gene *hid* during metamorphosis. By using the appropriate GMR-*hid* chromosome as homolog to the chromosome arm of interest, our method insures that only cells homozygous for the chromosome arm of interest are present in the adult fly. Note that mitotic clones are induced by the eyeless enhancer early in the development of the visual system, well before the GMR enhancer is activated and photoreceptor degeneration begins.

The diagram shown in Figure 1 depicts the *FLP*-mediated mitotic recombination events that occur in premitotic photoreceptor cells with this method (hereafter, the *EGUF/hid* method). At the far left of the diagram a photoreceptor cell is depicted that has completed S phase but has not undergone cell division. Mitotically active eye precursor cells at this stage that undergo an even number of recombination events between nonidentical (homologous) chromosome arms follow the pathway indicated by the downward arrow and generate identical heterozygous daughter cells of the same genotype as the parental cell. Precursor cells that undergo an odd number of such recombination events follow the pathway indicated by the upward arrow and have a 50% chance of giving rise to either heterozygous or homozygous daughter cells depending on the chromosome segregation pattern at cell division. Once a homozygous cell is generated, it is fixed in genotype. Consequently, during subsequent rounds of cell divisions, all its progeny will necessarily be identical, irrespective of additional mitotic recombination events. In contrast, heterozygous cells can give rise to homozygous progeny during subsequent rounds of cell division because additional mitotic recombination events will continue to occur as a result of the sustained expression of *FLP*ase via the eyeless promoter.

To calculate the theoretical maximum number of homozygous cells that could be produced in the adult eye by the *EGUF/hid* method, we developed the following model describing the generation of mitotic clones in the developing eye. This model makes two assumptions: (1) the eyeless enhancer used in the *EGUF/hid* method mimics the known expression of the eyeless gene in that it becomes active at the end of embryogenesis and remains active throughout larval development (Quiring *et al.* 1994) and (2) the level of *FLP*ase expression generated by the eyeless driver results in a sufficiently high rate of mitotic recombination in every cell during each round of cell division that the probabilities

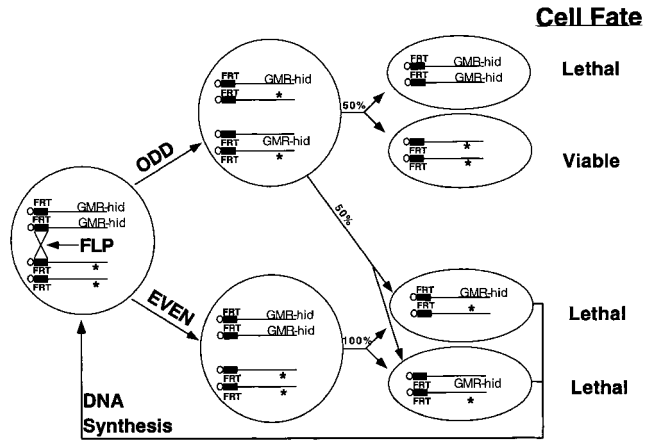

Figure 1.—Schematic diagram of mitotic recombination occurring in eye precursor cells with the *ey-GAL4/UAS-FLP/* GMR-*hid* (*EGUF/hid*) method. At the far left, a G2 premitotic photoreceptor precursor cell is depicted that is undergoing *FLP*-mediated mitotic recombination between nonidentical (homologous) chromosome arms. Premitotic photoreceptor precursors that undergo an even number of this type of recombination event follow the pathway indicated by the downward arrow and do not give rise to homozygous progeny cells. Premitotic photoreceptor precursors that undergo an odd number of these recombination events follow the pathway indicated by the upward arrow and have a 50% chance of giving rise to either heterozygous or homozygous progeny cells depending on the chromosome segregation pattern at cell division. "Silent" G2 recombination events between identical (sister) chromosome arms as well as G1 recombination events between homologous chromosomes will also be occurring, but are inconsequential with regard to generating mitotic clones. Heterozygous progeny cells from a given round of cell division can give rise to homozygous progeny cells during subsequent rounds of cell division. The adult photoreceptor cell phenotype of each photoreceptor cell genotype is indicated in the column at the far right. Only photoreceptor cells that are homozygous for the *FRT* * chromosome survive to adulthood because of the dominant photoreceptor lethality of the GMR-*hid* transgene.

that a dividing cell will take either the even or odd pathways (Figure 1) are equal. The model also takes into account that 6 to 23 late-embryonic precursor cells give rise to the adult eye (Wolff and Ready 1993) and thus that 10 to 12 rounds of postembryonic cell division are necessary to generate the ~16,000 cells of the adult eye. According to this model, during each round of cell division heterozygous cells will increase at a rate of 1.5× the initial heterozygous cell population and homozygous cells will increase at a rate of 2× the initial homozygous cell population plus 0.25× the initial heterozygous cell population. Over many rounds of cell division, because the homozygous population grows at a faster rate, they will overtake the heterozygous cell population. An alternative way of describing what occurs during each round of cell division is that the percentage of heterozygous cells decreases 0.75-fold. Because 10 to 12 rounds of postembryonic cell division occur to produce the number of cells present in the adult eye, heterozygous

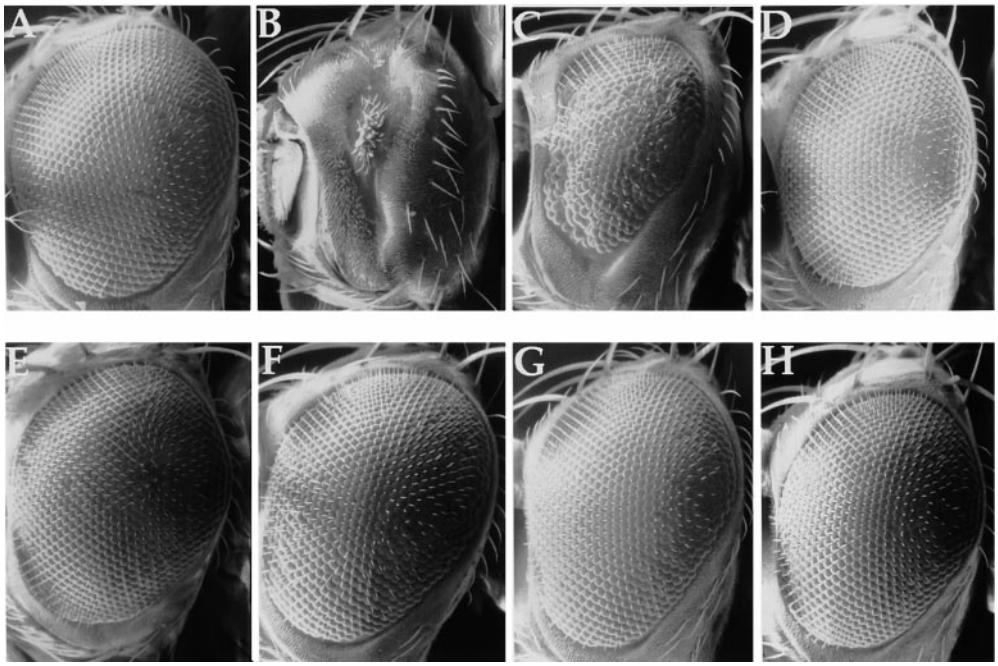

Figure 2.—Application of the *EGUF/hid* method. Scanning electron microscopy of adult Drosophila eyes representative of the following genotypes: (A) Canton-S, (B) *yw*; *FRT40A* GMR-*hid2L*/+; *EGUF*/+, (C) *yw*; *FRT40A* GMR-*hid2L*/*FRT40A*; *EGUF*/+, (D) *yw*; *FRT40A* GMR-*hid2L CL2L*/*FRT40A*; *EGUF*/+, (E) *yw*; *FRT42D* GMR-*hid2R CL2R*, *y*+/*FRT42D*; *EGUF*/+, (F) *yw*; *EGUF*/+; *FRT80B* GMR-*hid3L CL3L*, *y*+/*FRT80B*, (G) *yw*; *EGUF*/+; *FRT82B* GMR-*hid3R CL3R*/*FRT82B*, and (H) *yw FRT19A* GMR-*hidX CLX*/*yw FRT19A*; *EGUF*/+. (C–H) Mitotic recombination in the eye precursor cells largely suppresses the dominant photoreceptor lethality of the GMR-*hid* transgene. (D–H) The presence of a recessive *CL* mutation on the GMR-*hid* chromosome improves the morphology of the recombinant eye. When not suppressed by recombination, the GMR-*hid* inserts on each of the five major chromosome arms showed a lack of photoreceptor phenotype similar to that shown in B.

cells could account for as few as $(0.75)^{10}$ to $(0.75)^{12}$ (5.6 to 3.1%) of the adult eye. The remainder will be divided equally among homozygous clones of the two original chromosome arms. Thus, even though only heterozygous eye precursor cells are present prior to the initial expression of *FLP*ase, this model explains how cells of homozygous genotype can become predominant.

The fate of each of the three possible genotypes of the progeny cells is shown in the far right column of Figure 1. As indicated, any photoreceptor cell possessing even a single copy of GMR-*hid* will die, thus leaving in the adult fly only photoreceptor cells homozygous for the chromosome arm containing the mutation of interest. This method thus accomplishes suppression of the dominant phenotype of GMR-*hid* through mitotic recombination.

The *in vivo* results of this method of generating mitotic eye clones are shown in Figure 2. In Figure 2, B and C, animals are shown that are heterozygous for the same GMR-*hid* insertion except that in Figure 2C we have applied the *EGUF*/*hid* method to induce mitotic recombination in the eye. As can be seen in Figure 2B, GMR-*hid* heterozygous animals lack photoreceptors. In contrast, in the GMR-*hid* heterozygote shown in Figure 2C in which eyeless driven *FLP* has induced mitotic recombination, this phenotype has been significantly suppressed. The size and morphology of eyes engineered in this fashion were remarkably consistent. In contrast to the variable degree of mosaicism encountered with heat-shock-driven recombination, the chromosomes described here use endogenous, developmentally driven enhancers that appear to cause sustained and consistent activation of the recombinase and the dominant cell lethal, thus yielding uniform results.

Improving the *EGUF/hid* method: Although this initial trial was successful, we attempted to improve it by making recombinant eyes whose size and organization would more closely resemble those of wild-type animals. To do so we constructed a chromosome arm that contains, in addition to a dominant cell lethal GMR-*hid* insert, a recessive cell lethal (CL) mutation. The rationale for this modification is as follows. Because GMR-*hid* does not induce cell death until the beginning of pupal development (Hay *et al.* 1994), or after cell division in the developing eye disc has nearly ceased, there is relatively little developmental time left before adulthood for the eye disc to compensate for the injury. Consequently, we reasoned that cell death induced at an earlier developmental stage in homozygous GMR-*hid* cells, by the addition of a recessive cell lethal mutation to the GMR-*hid* chromosome arm, would give the eye disc more time to make compensations and thus produce a recombinant adult eye that more closely resembles wild type. The improvement in the recombinant eye that results from implementing this modification can be seen by comparing Figure 2D (GMR-*hid* with recessive cell lethal mutation) to Figure 2C (GMR-*hid* without recessive cell lethal mutation). In fact, comparison of the recombinant eye shown in Figure 2D with the wild-type eye shown in Figure 2A reveals that this modification of the method results in the production of recombinant eyes that approach wild type in both size and morphology. Figure 2, E–H shows recombinant eyes generated with modified versions of the other four

GMR-*hid* chromosome arms. All eyes shown in Figure 2 are entirely representative of the populations examined; there was little or no morphological variation among the individuals of these genotypes. A minor limitation of the *EGUF/hid* method should, however, be noted. The presumed cell lethal mutations on these *FRT* GMR-*hid* CL chromosome arms have not been characterized. The *EGUF/hid* method could not be applied to these cell lethal genes or any other recessive lethals that these chromosomes carry because those mutations would be lethal in combination with the *FRT* GMR-*hid*CL chromosomes. A list of each of these stocks is shown in Table 1.

ERG analysis of *EGUF/hid* recombinant eyes: To determine whether the photoreceptors in the recombinant eyes were capable of phototransduction and synaptic transmission, we performed ERG analysis. We compared eyes from wild-type flies and eyes composed of recombinant clones, made as described above, in which an *FRT* chromosome that was otherwise wild type had been made homozygous (Figure 3, A and B). The ERG waveform reflects phototransduction in the rhabdomeres and synaptic activation of the optic ganglia (Pak *et al.* 1969). The sustained downward deflection in the ERG arises from the activity of the light-dependent current in the photoreceptor cells while the "on/off" transients present at the initiation and cessation of the light represent downstream events that occur in the lamina. The latter depend on the competence of the photoreceptors to release neurotransmitter. The recombinant and wild-type eyes are very similar in their waveforms; thus the extensive cell death and reorganization that occurred as a consequence of the transgene expression during the development of the recombinant eyes did not prevent their photoreceptors from assembling the appropriate machinery for detecting light, responding electrically, and communicating the signal to downstream cells in the optic ganglia.

Testing the *EGUF/hid* method on mutants of *synaptotagmin* and *syntaxin*: We next applied the method to two lethal mutations and determined the ERG phenotypes of these essential genes. These experiments tested the specificity of the mitotic recombination for the eye; the generation of vital cells lacking an essential gene would cause lethality to the organism. *Synaptotagmin* (*syt*) mutants are defective in synaptic transmission and null mutations die as embryos or paralyzed first instar larvae (DiAntonio and Schwarz 1994). *Syntaxin* (*syx*) is re-

TABLE 1

Stocks

yw FRT 19A GMR-*hid* X CLX; EGUF/EGUF
yw; FRT 40A CMR-*hid* 2L CL2L/Cy0; EGUF/EGUF
yw; FRT 42D GMR-*hid* 2R CL2R, *y* +; EGUF/EGUF
yw; EGUF/EGUF; FRT 80B GMR-*hid* 3L CL3L, *y* +
yw; EGUF/EGUF; FRT 82B GMR-*hid* 3R CL3R

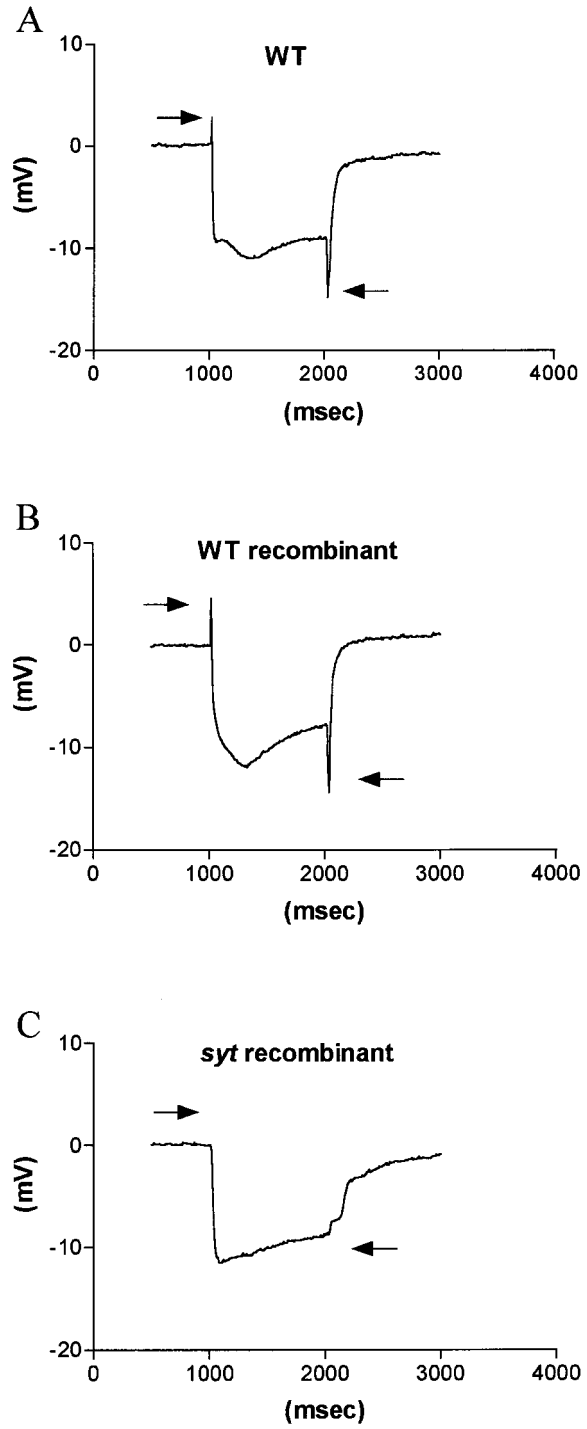

Figure 3.—Electroretinograms from *EGUF-hid* recombinant eye flies. ERGs were recorded from the eyes of (A) *yw*; *EGUF/+*, (B) *yw*; *FRT40A* GMR-*hid2L CL2L/FRT40A*; *EGUF/+*, and (C) *yw*; *FRT40A* GMR-*hid2L CL2L/FRT40A syt*^{AD4}; *EGUF/+* flies. The period of light stimulation was from 1000 to 2000 msec. Arrows indicate the on/off transients (when present). ERGs of (B) recombinant eye flies are essentially identical to similarly pigmented nonrecombinant eye flies. ERGs from (C) *syt* recombinant eyes are missing the on/off transients, indicating that photoreceptor synaptic transmission is not occurring normally in *syt* mutant photoreceptors.

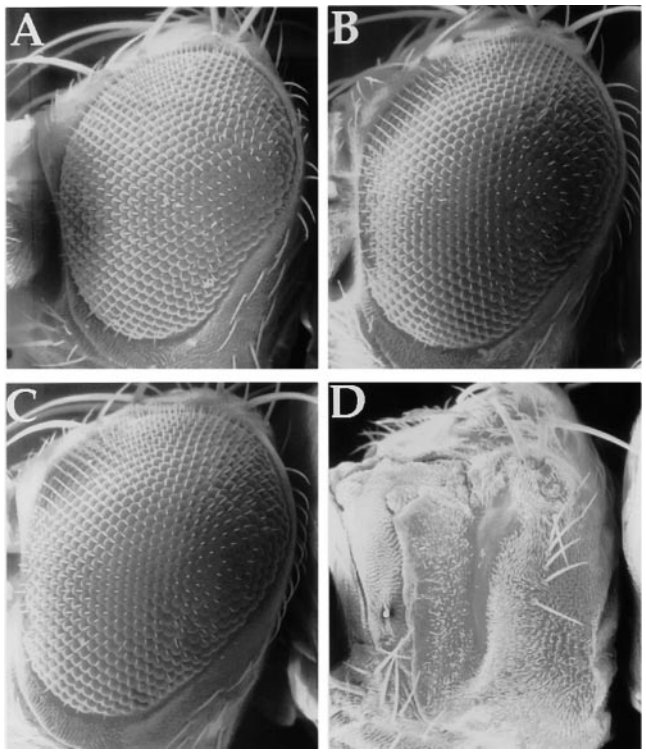

Figure 4.—Demonstrating the *EGUF/hid* method on lethal mutations in *synaptotagmin* and *syntaxin*. Shown are scanning electron micrographs of (A) *yw*; *FRT40A* GMR-*hid2L CL2L*/*FRT40A*; *EGUF*/+, (B) yw; *FRT40A* GMR-*hid2L CL2L*/*FRT40A* syt^{AD4}; *EGUF*/+, (C) yw; *EGUF*/+; *FRT82B* GMR-*hid3R CL3R*/*FRT82B*, and (D) yw; *EGUF*/+; *FRT82B* GMR-*hid3R CL3R*/*FRT82B* syx^{L371} flies. (B) *FRT40A syt* eyes are morphologically indistinguishable from (A) *FRT40A* eyes. In contrast, in D, *FRT82B* syx^{L371} eyes photoreceptors are absent (compare to C), indicating that syx^{L371} is required for the viability of photoreceptor cells. Successful application of the *EGUF-hid* method to these test cases suggests that the method will be applicable to other lethal genes.

quired for membrane trafficking in several cell types and is essential for synaptic vesicle fusion. Clonal analysis has shown that *syx* is required for cell viability (Schulze and Bellen 1996; Burgess *et al.* 1997). Mutations in *syt* and *syx* thus provide stringent tests of the method.

From a syt^{AD4}-bearing *FRT* chromosome, viable adult flies with homozygous syt^{AD4} eyes were successfully recovered. This result indicates that the *EGUF/hid* method does not induce mitotic clones in essential neurons to an extent that they prove lethal to the organism. Scanning electron micrographs of eyes homozygous for syt^{AD4} do not reveal any effect of the mutation on eye morphology (Figure 4B, compare with Figure 4A); as expected, *syt* is not required for cell viability or for the external morphology of the photoreceptors. Electroretinograms of these flies indicated that phototransduction in these eyes is normal but that synaptic transmission is defective as indicated by the 100% penetrant phenotype of no on/off transients in the ERG ($n = 10$) of flies with homozygous *syt* eyes (Figure 3C; compare with Figure 3B).

From the syx^{L371}-bearing *FRT* chromosome, viable adult flies could also be generated despite the *EGUF/hid*-induced mitotic recombination. Because *syx* is believed to be required for cell viability in all cells, this result suggests that mitotic recombination is not induced to an appreciable degree in any essential cells. Scanning electron micrographs of a representative homozygous syx^{L371} eye (Figure 4D, compare with Figure 4C) confirm the cell lethality of this mutation; no photoreceptors are seen in homozygous syx^{L371} eyes. This phenotype was also 100% penetrant ($n > 50$). Recombinant *syx* eyes also demonstrate that the *EGUF/hid* method is completely effective in suppressing the development of any clones in which the syx^{L371} chromosome had not been made homozygous. Consistent with the absence of photoreceptors in these flies, the electroretinogram found no light-dependent changes in electrical activity (data not shown).

Success in applying the *EGUF/hid* method to lethal mutations in *syt* and *syx* strongly suggests that it will be possible to use it to study the vast majority of genes expressed in the fly eye. In particular, its application to a cell lethal gene may be the most stringent possible test of the completeness and specificity of the method. Indeed, the only limitation to the *EGUF/hid* method may be that the mutations must be located distally to the basally located *FRT* sites on one of the five major chromosome arms—a limitation inherent to all *FRT*-based methods.

DISCUSSION

It has been estimated that two-thirds of the essential genes in the Drosophila genome are required for the proper development of the fly eye (Thaker and Kankel 1992). We have described in this article a genetic method that greatly facilitates the determination of the eye phenotype of mutations in the vast majority of these essential genes. The *EGUF/hid* method makes this analysis possible because of its ability to generate flies with eyes composed exclusively of mitotic clones of a single genotype. The method does this by using a highly specific endogenous developmental promoter to drive expression of the site-specific recombinase *FLP*ase in eye precursor cells. This results in the consistent and reliable production of flies with mitotic eye clones. Compared to the standard method of inducing mitotic clones with a heat-shock-controlled *FLP*ase, the *EGUF/hid* method has several advantages: (1) eye clones are produced more frequently and consistently; (2) deleterious clones outside the eye do not appear to be produced at all; and (3) the entire eye is made homozygous for the mutation (*i.e.*, the phenotype need not be analyzed only in small clonal patches that are often difficult to identify).

While this method will be of immediate use in determining in a single generation cross the homozygous eye phenotype of any mutation located distally on an appropriate *FRT* chromosome, we believe it will be more

valuable in the long term because of the understanding of the biology of the fly eye that will result from the genes identified in the F_1 genetic screens that it makes possible. We discuss below several possibilities for such screens.

Potential F_1 genetic screens using the *EGUF/hid* method: For screening purposes, the most straightforward application of our method will be F_1 genetic screens for mutations that produce anomalies in specific pathways or processes of interest. Such F_1 screens could involve selection on the basis of behavioral, morphological, or physiological phenotypes. The most appropriate primary screening criteria will vary depending on the particulars of the process under investigation. One potentially productive approach might be to repeat selections that have been carried out in the past. The difference, of course, is that with the *EGUF/hid* method such screens will not be limited to genes required for adult viability. For instance, early investigations of vision in Drosophila attempted to identify vision defective mutants on the basis of aberrant phototactic behavior (Benzer 1967; Pak *et al.* 1969; Heisenberg 1971) or aberrant ERGs (Pak 1975; reviewed in Pak 1991). Repeating these screens with the *EGUF/hid* method might result in the identification of a significantly different collection of mutants than those identified in previous screens. A schematic diagram of the most straightforward *EGUF/hid*-based phenotypic screen is shown in Figure 5A. This screen could, for example, select for defects in UV-phototaxis, the phenotype that originally identified the *sevenless* gene. The ability of such a screen to uncover the genes involved in such a behavior would be limited only in its omission of genes located very close to centromeres. Also, the phenotype of mutations that are cell lethal could not be examined in a more detailed fashion unless hypomorphic alleles are recovered. Using a similar scheme, it should be possible to genetically dissect nearly any other aspect of the biology of the fly eye.

Another potential F_1 screen using the *EGUF/hid* method is shown in Figure 5B. In this type of screen, mutations in a gene of interest are distinguished in the F_1 generation by modifying the *EGUF/hid* method to include a gene-specific transgenic rescue construct (a requirement of this particular type of screen). From mutagenized parents, the *EGUF/hid* technique generates F_1 progeny that are heterozygous for a rescue construct everywhere except for the eye where the rescue construct has been eliminated. This is accomplished by recombining the rescue construct onto an *FRT* GMR-*hid* chromosome arm and thus coupling it to photoreceptor cells fated to die. Mutations in the gene of interest are rescued everywhere except in the eye, where the effect of the mutation is revealed by the use of a deletion or other mutation in the gene of interest on the homologous chromosome. For nearly any gene that mutates to give a morphologically recognizable eye phenotype, this type of F_1 screen could be used to generate a much larger number of alleles for the same effort as compared to widely used F_2 lethal screens, regardless of whether the eye is the tissue of ultimate phenotypic interest. This type of screen may make it possible to extract from a genetic screen detailed structure/function information.

In addition to the genetic screens just described, it should also be possible to use the *EGUF/hid* method to perform F_1 suppressor/enhancer screens. The principal

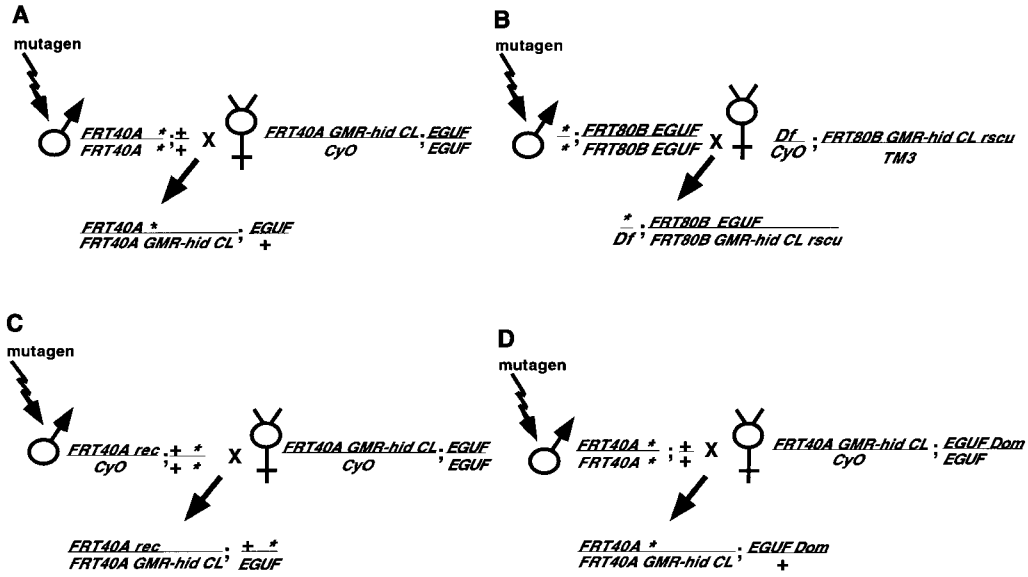

Figure 5.—Possible F_1 genetic screens using the *ey-GAL4/UAS-FLP/*GMR-*hid* method. The chromosomes on which the mutations are to be recovered are marked with asterisks. (A) Screen of chromosome arm 2L in which F_1 progeny can be screened for behavioral, morphological, or physiological phenotypes. F_1 progeny have recombinant eyes composed exclusively of cells homozygous for the mutagenized chromosome of the parental male. (B) Screen for alleles of a specific gene located on chromosome 2 and uncovered by a local deficiency (Df). A rescue construct for this gene (rscu) is located on a *FRT80B* GMR-*hid* chromosome arm to prevent lethality because of phenotypes outside the eye. Within the eye, however, cells expressing the rescue construct are selectively removed by the linkage to GMR-*hid*. In the remaining cells of the eye, a phenotype can be assayed. (C) Screen for third chromosome dominant modifiers of a recessive phenotype located on chromosome arm 2L (rec, recessive mutation). (D) Screen for chromosome arm 2L recessive modifiers of a third chromosome dominant phenotype (Dom, dominant mutation). A screen for recessive modifiers of recessive phenotypes is not shown but should be possible.

difference between this type of screen and the straightforward type of screen described above is that the suppressor/enhancer screen starts with a fly that already possesses a mutant eye phenotype. Thereafter, suppressor/enhancer screens use the same strategy of screening for mutations that produce a fly eye that is phenotypically different from the parental fly eye, be it a behavioral, morphological, or a physiological phenotype. As mentioned in the Introduction, significant effort has gone into carrying out modifier screens for genes involved in eye development. While those screens typically relied on identifying dominant modifiers of dominant phenotypes, the *EGUF/hid* method allows suppressor/enhancer screens to be extended to include dominant modifiers of recessive phenotypes (Figure 5C), recessive modifiers of dominant phenotypes (Figure 5D), and even recessive modifiers of recessive phenotypes (not shown).

Last, we point out that the *EGUF/hid* method may facilitate biochemical studies by providing a tissue source enriched in mutant forms of essential proteins that are expressed preferentially in the eye. Because large quantities of Drosophila heads can be isolated easily (Gitschier *et al.* 1980) and because the eye constitutes a substantial portion of the head, the mutant isoforms should predominate in extracts of heads from heterozygotes in which the eyes were made homozygous for the mutation.

In summary, we envision five uses of the *EGUF/hid* technique described above: the analysis in the fly eye of known mutations (as shown for *syt* and *syx*); phenotypic F_1 screens for new loci; F_1 screens for identified genes that exhibit morphological phenotypes; F_1 screens for novel enhancers and suppressors; and biochemical studies of (or screens for) mutant proteins. In each case the advantages of the method stem from the generation of an eye that is uniformly homozygous for a given mutation within an animal that is otherwise uniformly heterozygous. Extension of the method to other adult structures that are not essential for viability (*e.g.*, wings, antennae, reproductive organs, or even nonessential subsets of neurons) should be possible and is limited only by the availability of appropriate tissue-specific enhancers to drive *FLP*ase and a dominant cell lethal like *hid*.

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