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A unique mutation in the *Enhancer of split* gene complex affects the fates of the mystery cells in the developing *Drosophila* eye

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Summary

An unusual recessive allele of the *Drosophila groucho* gene, which encodes a transducin-like protein, affects the fates of specific cells in the eye disc. *groucho* is one of several transcription units in the *Enhancer of split* complex. Most *groucho* mutations are zygotic lethal due to the proliferation of embryonic neural cells at the expense of epidermal cells. In contrast, flies homozygous for the mutant allele described here, gro^{BFP2}, are viable but have abnormal eyes. The *Drosophila* compound eye is composed of several hundred identical facets, or ommatidia, each of which contains eight photoreceptor cells, R1-R8. In gro^{BFP2} mutant retinas, most of the facets contain eight normally determined photoreceptor cells and one or two additional R-cells of the R3/4 subtype. The extra photoreceptors appear to arise from the mystery cells, which are part of the precluster that initiates the ommatidium, but do not normally become neurons. gro^{BFP2} behaves as a partial loss-of-function mutant. Analysis of ommatidia mosaic for wild-type and gro^{BFP2} mutant cells suggests that the focus of action of the gro^{BFP2} mutation is outside of the photoreceptor cells. These results imply that one function of *groucho* is in a pathway whereby neuralization of the mystery cells is inhibited by other non-neural cells in the eye disc. In addition, determination of R3/4 photoreceptors usually requires contact with R2 and R5. Specification of the mystery cells as ectopic R3/4 subtype photoreceptors in gro^{BFP2} mutant eye discs implies that induction by R2 or R5 is not absolutely necessary for R3/4 cell determination.

Key words: *Drosophila*, eye development, *Enhancer of split*, cell communication, neurogenesis.

Introduction

The *Drosophila* compound eye is composed of about eight hundred identical facets, or ommatidia, arranged in a precise hexagonal lattice. Ommatidia assemble stepwise within a monolayer of unpatterned epithelial cells in the eye imaginal disc in the wake of a visible depression called the morphogenetic furrow that moves across the disc from the posterior to the anterior (Ready et al., 1976; Tomlinson, 1985; Tomlinson and Ready, 1987a; Cagan and Ready, 1989a). The eight photoreceptor cells are recruited in the sequence R8, R2/5, R3/4, R1/6 and R7, followed by four cone cells. The pigment cells and bristles are assembled later in the pupal disc. As the morphogenetic furrow advances at the rate of one row of ommatidia every two hours (Campos-Ortega and Hofbauer, 1977), facets at progressive stages of assembly are present behind the furrow in a single eye disc. The cells in a facet are not related by lineage (Ready et al., 1976; Lawrence and Green, 1979; Wolff and Ready, 1991a). Rather, ommatidial assembly is guided by a series of specific cell inductions (reviewed in Tomlinson, 1988; Ready, 1989; Zipursky, 1989; Banerjee and Zipursky, 1990; Moses, 1991; Rubin, 1991). The initial events in the assembly process are less well understood. As the initial stages of eye development involve choosing neurons from a pool of epithelial cells, many genes that mediate the decision between neural and ectodermal cell fates elsewhere in the fly also appear to function during eye development (Dietrich and Campos-Ortega, 1984; Cagan and Ready, 1989b; Baker et al., 1990; Mlodzik et al., 1990a).

The *Enhancer of split* (*E(spl)*) gene complex of *Drosophila* is one of six loci referred to as "neurogenic" genes (recently reviewed in Campos-Ortega, 1991), which were first identified by their role in embryonic neurogenesis (Poulson, 1937; Lehmann et al., 1981, 1983). The *Drosophila* central nervous system arises from neuroectoderm cells which must choose between an ectodermal or neural fate. In embryos mutant for any one of the neurogenic genes, most or all of the neuroectoderm cells become neural and the embryo

dies. Many experiments led to the conclusion that committed neuroblasts inhibit surrounding cells from also acquiring a neural fate by a cell-contact-mediated process (Taghert et al., 1984; Doe and Goodman, 1985; Technau and Campos-Ortega, 1986, 1987; Technau et al., 1988). The structures of the proteins encoded by the neurogenic genes, particularly *Notch* and *Delta*, are consistent with a role in cell communication (Wharton et al., 1985; Kidd et al., 1986; Vassin et al., 1987; Kopczynski et al., 1988). Most of the neurogenic genes also play a role in cell-contact-mediated epidermal/neural commitment decisions in the peripheral nervous system, including the eye (Dietrich and Campos-Ortega, 1984; Cagan and Ready, 1989b, Heitzler and Simpson, 1991; for reviews see Ghysen and Dambly-Chaudiere, 1989; Simpson, 1990). In addition, in the eye, *Notch* also mediates cell interactions involved in other types of cell commitment choices (Cagan and Ready, 1989b).

The *E(spl)* locus, first identified by a dominant mutation, $E(spl)^D$, consists of several closely linked transcription units with complex functional interactions. $E(spl)^D$ enhances the roughened eye phenotype of a unique recessive allele of the *Notch* gene called *split* (Welshons, 1956). In order to ascertain the function of the normal *E(spl)* gene, revertants of the dominant eye phenotype of the $E(spl)^D$ mutation were generated (Lehmann et al., 1983). The revertants, all of which are deficiencies that delete several transcripts, are embryonic lethal and have a typical neurogenic phenotype when homozygous. At least four of the transcripts within the deficiencies participate in neurogenesis and they have been divided into two functional units: the *m5, m7, m8* group and *m9/10* (Delidakis et al., 1991). The *m5, m7* and *m8* transcripts encode proteins containing a helix-loop-helix (HLH) motif (Klambt et al., 1989) characteristic of some transcription factors (Murre et al., 1989). The $E(spl)^D$ mutation results in an altered form of the gene product of *m8* (Klambt et al., 1989). The HLH proteins are at least partly functionally redundant as genetic screens for lethal mutations in trans to *E(spl)* deficiencies that delete the HLH protein transcripts and *m9/10* have identified only mutations in the *m9/10* transcription unit (Preiss et al., 1988). The *m9/10* transcription unit was originally identified by a viable mutant called *groucho* which has specific head bristle duplications (Lindsley and Grell, 1968; Knust et al., 1987; Ziemer et al., 1988). For simplicity, the *m9/10* transcription unit will be referred to as the *groucho* gene. *groucho* encodes a nuclear protein (Delidakis et al., 1991) with a repeated motif present in a G protein subunit, β-transducin (Hartley et al., 1988), in the yeast cell cycle regulatory protein *CDC4* (Yochem and Byers, 1987) and in *PRP4*, a spliceosome component (Dalrymple et al., 1989; Petersen-Bjørn et al., 1989). The functional relationship between *groucho* and the HLH proteins is not well understood.

Here we describe an unusual viable recessive allele of *groucho*, gro^{BFP2}, which has its principal effect on eye development. In homozygous gro^{BFP2} adult eyes, most facets contain one or two extra photoreceptor cells. These cells are likely to originate from the mystery cells, which are part of an undifferentiated ommatidial precluster posterior to the morphogenetic furrow (Tomlinson and Ready, 1987a; Wolff and Ready, 1991*b*). The mystery cells are located between the cells that will become R3 and R4 and are normally excluded from the precluster, but in gro^{BFP2} discs the mystery cells become additional photoreceptors of the R3/4 subtype. Examination of the phenotypes of gro^{BFP2} in trans to lethal *groucho* mutations and observation of clones of the lethal mutants in the eye suggests that gro^{BFP2} is a unique partial loss-of-function mutant. Analysis of individual ommatidia mosaic for wild-type and gro^{BFP2} R-cells suggests that the gro^{BFP2} mutation acts outside of the R-cells. These results imply that cell communication, requiring *groucho* in non-neural cells outside of the developing facets, is necessary to exclude the mystery cells from the ommatidial precluster. In addition, determination of R3/4 subtype cells normally requires inductive signals from the neighboring R2 and R5 cells in the precluster (Tomlinson et al., 1988). Thus, the specification of the mystery cells as ectopic R3/4 subtype photoreceptors in gro^{BFP2} eye discs implies that R3/4 cells can be recruited by an alternative route, not requiring contact with the R2/5 pair.

Materials and methods

Drosophila *genetics*

Fly lines

The *E(spl)* point mutants and deficiencies, and the ry^+E8 transformant were gifts of A Preiss, C. Delidakis and S. Artavanis-Tsakonas, and are described in Preiss et al. (1988) and Delidakis et al. (1991). The *boss* deficiencies, gifts of A. Hart and S. L. Zipursky, are described in Hart et al. (1990). The enhancer trap lines that express β-galactosidase in subsets of photoreceptor cell nuclei were gifts of members of the Rubin laboratory. BG9408 and BGP820 are marked with $rosy^+$, and are on located in polytene chromosome bands 10A and 34A, respectively (M. Mlodzik and G.M.R., unpublished data). BGA2-6, N30, O32, AE127 and X81 are marked with $white^+$. BGA2-6 is inserted into the *scabrous* locus (Mlodzik et al., 1990; Baker et al., 1990), AE127 is inserted into the *seven-up* gene (M. Mlodzik, J.S. Heilig and G.M.R., unpublished data) and X81 is in the *rhomboid* gene (M. Freeman, B.E Kimmel and G.M.R., unpublished data). N30 and O32 are inserted into polytene bands 34A and 65D, respectively (M. Freeman and G.M.R., unpublished data). Other mutant markers are described in Lindsley and Grell (1968). Flies were kept on standard food at 25°C.

Isolation of the gro^{BFP2} *allele*

The gro^{BFP2} allele was isolated during an extensive screen for viable recessive mutations on the third chromosome with abnormal eye morphology (J.A F.-V., R. W. Carthew and G M R, unpublished data). More than 20,000 lines of single mutagenized *st* chromosomes were generated as follows. EMS-mutagenized (Lewis and Bacher, 1968) *bw; st* males, isogenic for *st*, were crossed to *bw; TM3/TM6B* virgins. Male progeny (*bw, *st/TM3* or *TM6B*) were singly mated to *bw, TM3/TM6B* virgins. The progeny of this cross were intermated, and the resulting lines were screened for white-eyed (*bw; *st*) flies, indicating viable mutagenized third chromo-

somes. The white-eyed flies in over 8000 viable lines were examined with a dissecting microscope for eye roughness, and then for defects in the reduced corneal pseudopupil, a red trapezoidal image visible in white-eyed flies with incident polarized light (Francheschini and Kirschfeld, 1971; see Banerjee et al , 1987) The *gro^{BFP2}* mutant was recognized on the basis of an abnormal reduced corneal pseudopupil caused by retinal irregularities.

Meiotic mapping of gro^{BFP2}

The *gro^{BFP2}* allele was first approximately mapped by crossing *bw; st gro^{BFP2}/ th st cu sr e ca* virgins with *bw; th st cu sr e ca* males. Many male progeny of all genotypic classes were individually mated with *bw, st gro^{BFP2}/TM6B* virgins to determine whether or not the recombinant chromosome contained *gro^{BFP2}*. By this process, *gro^{BFP2}* was placed distal to *ebony (e)* and various marked *gro^{BFP2}* chromosomes were obtained. The *gro^{BFP2}* mutation was then positioned between *e* and *Serrate (Ser)* by crossing *e gro^{BFP2}/ Ser* virgins to *e gro^{BFP2}* males and scoring all three markers in many progeny Finally, *gro^{BFP2}* was mapped with respect to five P element transformant insertions marked with *white^{+}* (*P[w^{+}]*) in polytene bands 94D, 94E, 95F, 96C, and 96F (Rubin laboratory stock collection), by crossing *w, e gro^{BFP2} /P[w^{+}]* virgins to *w; e gro^{BFP2}* males and scoring many progeny for the *P[w^{+}], e* and *gro^{BFP2}* phenotypes The *gro^{BFP2}* mutation mapped telomeric to all of the *P[w^{+}]* insertions except for the one at 96F. No recombinants between *gro^{BFP2}* and *P[w^{+}]96F* were obtained as compared with 185 recombinants between *e* and *gro^{BFP2}*.

Other fly crosses

Combination of *gro^{BFP2}* with deficiency chromosomes, other *gro* alleles, the *ry^{+}E8* transformant and enhancer trap lines were carried out using standard genetic crosses.

Generation of mosaic eyes

gro^{BFP2} *clones*

Clones of *gro^{BFP2}/gro^{BFP2}* cells marked by the absence of the *white* gene product (no pigment granules associated with photoreceptor cells nor pigment cells) were generated by crossing *w^{1118}; st gro^{BFP2}/TM6B* males with *w^{1118}; P[w^{+}]85D* or *w^{1118}; P[w^{+}]90E* virgins and X-irradiating (1000 rads) their progeny as first instar larvae. Thus, larvae of the genotype *w^{1118}; P[w^{+}]/gro^{BFP2}* have clones of mutant cells of the genotype *w^{1118}; gro^{BFP2}*. *P[w^{+}]85D* and *P[w^{+}]90E* are P element transformant lines marked by expression of the *white* gene with insertions in polytene bands 85D and 90E, respectively (Rubin laboratory stock collection) *w^{-}* clones of cells in the eye were observed in ~1 in 30 flies.

clones of lethal groucho *alleles*

Clones of the *groucho* alleles *E(spl)^{E28}*, *E(spl)^{E48}*, *E(spl)^{E73}*, *E(spl)^{E75}*, *E(spl)^{E77}*, *E(spl)^{E107}*, *l(gro)^{X115}* and the deficiency *E(spl)^{BX22}* were generated as described above using *p[w^{+}]90E*. All of the lethal *groucho* alleles were on *e tx* chromosomes, except *l(gro)^{X115}* and *E(spl)^{BX22}* which were on *ry^{506} tx* chromosomes *E(spl)^{E107}* and *E(spl)^{E28}*clones were obtained at frequencies similar to the *gro^{BFP2}* clones. Clones of all of the other lethal alleles were usually small and were obtained at extremely low frequencies (~1/200 flies)

Sections of eyes

The heads of various mutants and of eyes containing clones were fixed, embedded in plastic and sectioned as previously described (Tomlinson and Ready, 1987b)

Antibody staining eye discs

Third instar larval eye discs were dissected, fixed and stained with mAb22C10 (a gift of S. Benzer) as previously described (Tomlinson and Ready, 1987a). The enhancer trap lines expressing β-galactosidase in cell nuclei in the eye disc were dissected, fixed and stained with a mouse monoclonal antibody raised against β-galactosidase as described previously (Tomlinson and Ready, 1987a; Heberlein et al., 1991).

Results

The gro^{BFP2} *mutant phenotype*

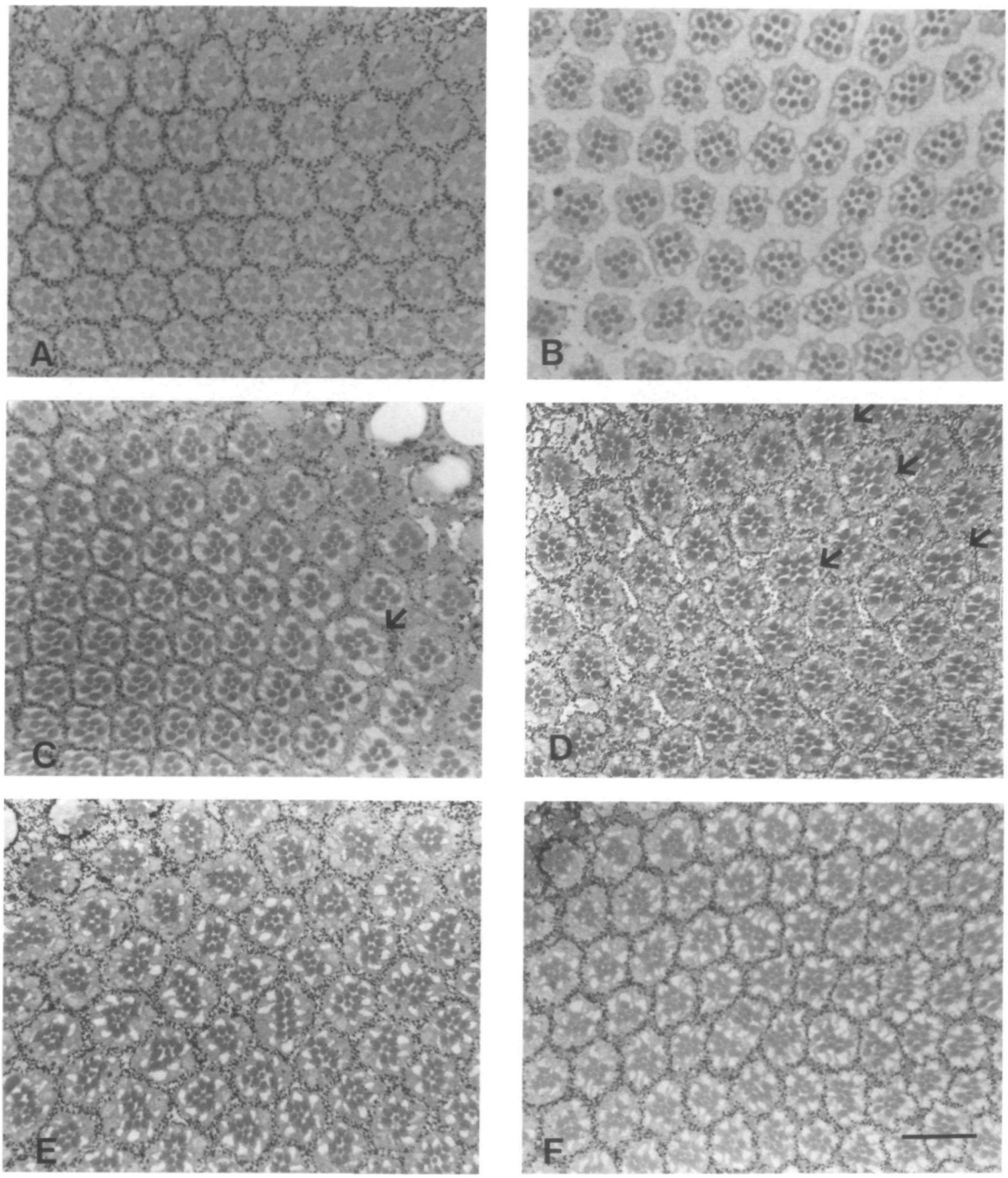
gro^{BFP2} is a recessive mutation that was identified in a screen of the third chromosome for mutants with defects in eye morphology (J.A.F.-V., R.W. Carthew, and G.M.R., unpublished data - see Materials and methods). The external appearance of the eyes of homozygous *gro^{BFP2}* flies is almost normal; they are slightly bulged and have some bristle spacing defects (data not shown). The reduced corneal pseudopupils (see Materials and methods) are blurred indicating some irregularity in the retina (data not shown). In addition to the eye defects, *gro^{BFP2}* mutant males and females are only marginally fertile, and their wings are slightly broader and slightly held out.

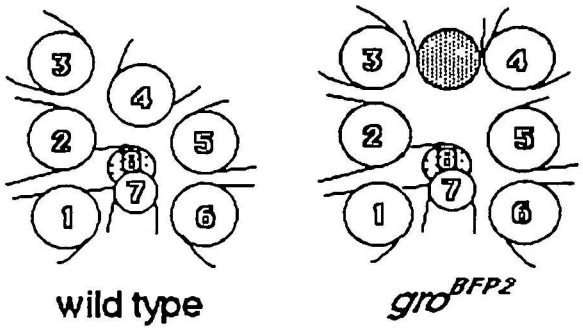
The eyes of *gro^{BFP2}* flies were examined in tangential sections. As shown in Fig. 1A and G, the retina is a hexagonal lattice of identical facets, or ommatidia. Each ommatidium in a wild-type eye has eight photoreceptor cells (R-cells) distinguished by their unique positions in a trapezoid. There are six outer photoreceptor cells, R1-R6, with large rhabdomeres (light-gathering devices), and two inner R-cells, R7 and R8, with small rhabdomeres. The trapezoids are all oriented in the same direction and are symmetrical with respect to a central equator. In the *gro^{BFP2}* retina (Fig. 1B and G), approximately two-thirds of the facets have one or two additional outer photoreceptor cells. The remaining facets have the normal number of photoreceptors. In addition, the orientations of the facets are irregular.

gro^{BFP2} *is an allele of* groucho

By a series of meiotic mapping experiments with several mutant markers, *gro^{BFP2}* was positioned very close to a *white^{+}* transposon inserted in polytene chromosome band 96F (see Materials and methods). Several chromosomes with deficiencies in the 96F region were crossed to *gro^{BFP2}* (Fig. 2). Six of these deficiency chromosomes uncover the *gro^{BFP2}* eye phenotype, and two do not. Thus, *gro^{BFP2}* is located within a chromosomal region including the *m8* and *m9/10 (gro)* transcription units of the *E(spl)* complex.

To test if *gro^{BFP2}* is a mutation in the *groucho* gene, seven lethal *gro* point mutations (Preiss et al., 1988) and the original viable *gro* allele (Knust et al., 1987; Ziemer et al., 1988) were tested for complementation by *gro^{BFP2}* (Table 1). Except for the viable *gro* allele, all of these mutations cause eye defects in trans to *gro^{BFP2}* similar, but not identical to *gro^{BFP2}* homozygotes (see below and Table 1). Moreover, one copy of the P transformant *ry^{+}E8*, which contains the *gro* transcrip-





G

Fig. 1. Adult eye phenotypes of gro^{BFP2} mutant combinations. Shown in A-F are tangential sections of adult eyes (Materials and methods) The sections are apical so that R7 is apparent, rather than R8 (see (G)). The bar in F is 30 μm and applies to all panels. See the text and Table 1 for descriptions of the genotypes and phenotypes. (A) A wild-type eye in the region of the equator. (B) A gro^{BFP2} homozygous eye, in which there are three type of facets. about two-thirds of the facets have 1, 2 or 3 extra outer photoreceptors, approximately one-sixth of the facets are wild-type and one-sixth have the normal number of photoreceptor cells, but R3 and R4 are symmetrical instead of forming a point of the trapezoid. The asymmetry of R3 and R4 is normally initiated in the most mature facets just before pupation (Tomlinson, 1985). In addition, the orientations of the facets are irregular. This particular eye is w^- so that the pigment cells contain no dark staining pigment granules nor are there pigment granules near each rhabdomere. (C) A ry^+E8, gro^{BFP2} eye The arrow indicates one mutant in a field of wild-type facets. In D, $E(spl)^{E107}/gro^{BFP2}$, the arrows also indicate mutant facets. The genotypes in E and F are $E(spl)^{E77}/gro^{BFP2}$ and $E(spl)^{BX22}/gro^{BFP2}$, respectively (G) Wild-type and gro^{BFP2} ommatidia are depicted schematically, the circles denoting rhabdomeres. Note that R8 is located beneath R7. The gro^{BFP2} mutant facet is shown with one ectopic R-cell (shaded) for simplicity. Because the identities of the R-cells are recognized only on the basis of the R-cell positions in a wild-type facet, it is impossible only by observing the gro^{BFP2} mutant eyes shown in B to assign R-cell identities as shown in G. The R-cell labels shown are based on the antibody staining experiments in Figs 4, 5 and 6 Those experiments demonstrate that the ectopic cells are R3/4 subtype cells located adjacent to the normal R3 and R4, and that the other R-cells are normally determined in gro^{BFP2} eye discs. Because gro^{BFP2} facets have three or four cells in the R3/4 position, the labeling of individual R-cells of this group must be somewhat arbitrary. The cells labeled R3 and R4 in the gro^{BFP2} facet in G were so assigned based on their positions next to R2 and R5, and the middle cell (shaded) labeled the ectopic one, primarily because that is where the mystery cells reside in the preclusters (see Fig. 4F).

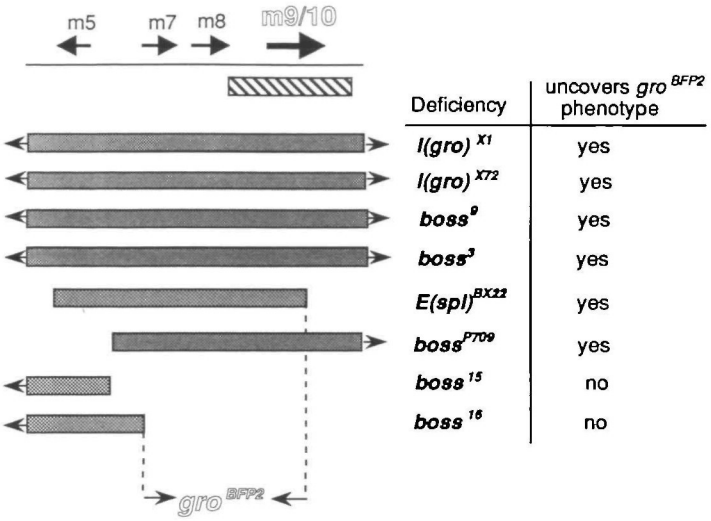


Fig. 2. The gro^{BFP2} mutant phenotype is uncovered by chromosomes deficient for *E(spl)* transcription units *m8* and *m9/10*. At the top is shown an approximately 25 kilobase (kb) portion of the *E(spl)* gene complex (Preiss et al., 1988, Knust et al., 1987). The *m5, m7* and *m8* transcripts are described in the text. The *m9/10* transcripts, which correspond to the *groucho* gene, have different 3′ ends but encode the same protein (Hartley et al., 1988). The arrows indicate the direction of transcription. The striped bar indicates the genomic DNA fragment cloned into the P element within the ry^+E8 transformant line (Preiss et al., 1988). ry^+E8 complements *groucho* point mutations, including gro^{BFP2}. Shaded bars indicate the regions deleted in the deficiency chromosomes indicated at right. Arrows at the ends of the bars indicate that the deletions extend beyond the 25 kb DNA segment depicted. The regions deficient within the chromosomes have been mapped cytologically. $l(gro)^{X1}$ and $l(gro)^{X72}$ delete polytene bands 96F5/7-96F12/14 and 96F5/7-97B1, respectively (Preiss et al , 1988). $boss^9$ and $boss^3$ delete 96E6-97B4/5 and 96F8/11-97B, respectively (Hart et al., 1990). $E(spl)^{BX22}$ is cytologically normal, but has been shown by molecular analysis to contain a deletion of 14 kb (as depicted) and also an inversion of the 14 kb just upstream (to the left) (Preiss et al., 1988; Shepard et al., 1989). $boss^{P709}$ contains a deletion of 96F10/11-97D1/2 (Delidakis et al., 1991). $boss^{15}$ and $boss^{16}$ contain deletions of 96F3/5-11/12 and 96F5/7-12/13, respectively (Hart et al., 1990).

tion unit and complements *gro* mutants completely (Preiss et al., 1988), also rescues the homozygous gro^{BFP2} mutant phenotype (Fig. 1C). All of these data argue that gro^{BFP2} is a viable allele of *groucho*.

gro^{BFP2} *is a partial loss-of-function allele*

The original viable *groucho* allele and gro^{BFP2} each complement the mutant phenotype of the other; gro/gro^{BFP2} flies have normal bristles and normal eyes (data not shown). However, in trans to gro^{BFP2}, all of the lethal mutations and *gro* deficiencies result in viable (or semi-viable) adult flies with mutant eye phenotypes reminiscent of, but not identical to, gro^{BFP2} homozygotes. $E(spl)^{E107}$ and $E(spl)^{E28}$, both pupal lethal, have nearly wild-type eyes in trans to gro^{BFP2} (Fig. 1D and Table 1). The five stronger lethals tested ($E(spl)^{E48}$, $E(spl)^{E73}$, $E(spl)^{E75}$, $E(spl)^{E77}$and $l(gro)^{X115}$) and the embryonic lethal deficiency $E(spl)^{BX22}$ have similar phenotypes in combination with gro^{BFP2} (Fig. 1E, F and Table 1); the retinas look similar to gro^{BFP2} homozygotes, but the defects are more severe. The eyes of trans-heterozygotes have fewer normal looking facets than gro^{BFP2} homozygotes, and the mutant facets do not all have one or two extra outer R-cells neatly added. Instead, many ommatidia have more than two extra R-cells, some facets appear fused, some are missing inner photoreceptor cells, and some rhabdomeres are malformed.

In order to characterize the gro^{BFP2} mutant allele further, it is important to assess the loss-of-function phenotype of *groucho* mutations in the eye. A deficiency that removes only the *groucho* gene is not available and none of the available *groucho* point mutations are known to be complete loss-of-function mutations. Nevertheless, when considered together,

Fig. 3. Clones of cell in the eye homozygous for lethal *E(spl)* alleles. Clones of cells in the eye homozygous for various lethal *E(spl)* alleles were obtained by X-ray-induced somatic recombination (Materials and methods). (A) Tabulation of the number of clones examined for each lethal allele and their characterization into four phenotypic classes. $E(spl)^{E107}$ and $E(spl)^{E28}$ are considered the weakest alleles because homozygotes die as pupae whereas the other alleles cause earlier death (Preiss et al , 1988). See the text, Table 1 and Fig. 2 for further descriptions of the different alleles. (B-D) Tangential sections through representative clones The bar in D is 20 μm, and applies to all panels Clones are marked as w^-, which is seen as the absence of the pigment granules normally associated with each rhabdomere and within pigment cells The pigment granules associated with the R-cells are seen as small black dots near each rhabdomere. (B) A "weak" $E(spl)^{E28}$ clone. The arrow points to the only mutant facet in the clone. (C) A "severe" $E(spl)^{BX22}$ clone The arrow indicates a facet with ectopic R-cells in which every R-cell is $E(spl)^+$ (R8 was not examined). (D) A "moderate" $l(gro)^{X115}$ clone. The arrows indicate mosaic facets with ectopic outer R-cells.

Fig. 7. Analysis of clones of gro^{BFP2} mutant cells in wild-type eyes. Clones of homozygous gro^{BFP2} mutant cells were generated by X-ray-induced somatic recombination and sectioned as described in Materials and methods The mutant cells are marked by the absence of the *white* gene, which results in the absence of the granules normally associated with each photoreceptor and pigment cell. The pigment granules of the R-cells are seen as small black dots near the rhabdomeres. (A) Tangential section through a portion of a clone at the level of R7. The bar is 20 μm. The arrow indicates a mutant facet (it has an ectopic outer R-cell) in which each R-cell has pigment granules associated with it and is thus genotypically wild-type (w^+gro^{BFP2+}). (R8 is not visible in this plane of section). 35 clones were examined for such facets, and 26 examples were found within 15 different clones. 14 of these facets were on the border of the clone, like the example shown in A, and 12 appeared to be outside of the clone, one or two facets away from the border. These facets appear to be separated from the mutant clone probably because the w^- gro^{BFP2-} epithelial cells responsible for the mutant phenotype are no longer adjacent to the mutant facets as they were in the larval disc (see Karpilow et al., 1989). For technical reasons, in the phenotypically mutant facets in which all of the apical R-cells appear to be w^+gro^{BFP2+}, not all of the R8 cells could be scored as w^+or w^- However, all 12 of such facets just outside of the clone border and 6 of the facets at the clone edge could be analyzed definitively and these had w^+ R8 cells. (B) The normally constructed facets in 10 different clones were analyzed cell by cell and the frequency with which each R-cell was w^+gro^{BFP2+} was tabulated. Facets were considered normally constructed if they had 8 R-cells in the appropriate arrangement; the orientation or trapezoidal shape of a facet was not considered. The frequency of individual R-cells being w^+gro^{BFP2+} is nearly random (50%) in all cases The slight deviations upwards from 50% are not surprising. In similar analyses of mosaic ommatidia where strict requirements for gene products were found in specific R-cells, other R-cells related by lineage to the required cells showed upwards deviations from random far greater than those observed here (Tomlinson et al., 1988, Carthew and Rubin, 1990; Mlodzik et al., 1990b; Reinke and Zipursky, 1988). Thus, the deviations observed are likely to reflect the close proximity of the R-cells to the cells within which the gro^{BFP2} mutation acts. If there were a strict requirement for any particular R-cell to be gro^{BFP2+}, taking into account that ~33% of the facets in a gro^{BFP2} mutant eye are normally constructed, it would be expected that 100%-(50%)(~33%)=~84% of those specific R-cells would be w^+gro^{BFP2+} in the mosaic normally constructed facets The number of wild-type and mutant mosaic facets were counted in the same 10 clones. Approximately 50% of the mosaic facets are wild-type (see text).

the eye phenotypes of many strong *gro* point mutations and the smallest deficiency should provide insight into the loss-of-function phenotype of *groucho* in the eye. If, as the genetic data above suggest, gro^{BFP2} is a loss-of-function mutant, the phenotypes of the lethals and the deficiency eye clones should be similar to gro^{BFP2} homozygotes. If the eye phenotype of gro^{BFP2} is completely different from the other *groucho* alleles, then gro^{BFP2} is likely to be performing a novel function in the eye.

Marked clones of cells in the eye, homozygous for seven different lethal *gro* point mutations and the small deficiency $E(spl)^{BX22}$ that removes only transcripts *m5* through *m9/10* (see Fig. 2) were generated by X-ray-induced somatic recombination (Materials and methods). Several clones of each lethal allele were examined in tangential sections and representative results are shown in Fig. 3. None of the clones look exactly like gro^{BFP2} eyes. The clones obtained were grouped into four phenotypic classes (wild-type, weakly mutant, moderate and severe) based on the proportion of mutant facets within the clone, which paralleled the degree of malformation of the facets. Most of the mutant facets have extra photoreceptor cells. However, sometimes facets have too few photoreceptors, and in clones of the stronger alleles, photoreceptor cells are often malformed and there are fusions of facets.

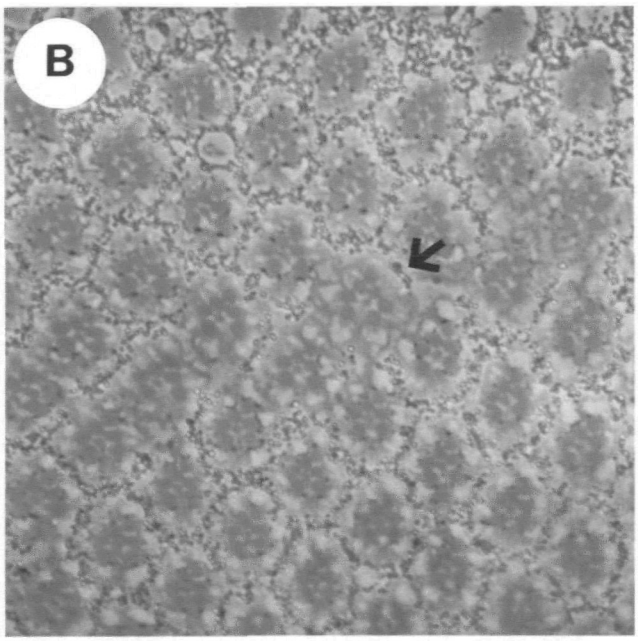
In summary, in trans to strong *gro* mutations or deficiencies, gro^{BFP2} shows a stronger eye phenotype than gro^{BFP2} homozygotes. Also, when homozygous, the strong *groucho* mutations and the deficiency have effects on eye development similar to but more extensive than gro^{BFP2} homozygotes. These observations support the view that gro^{BFP2} is a partial loss-of-function allele.

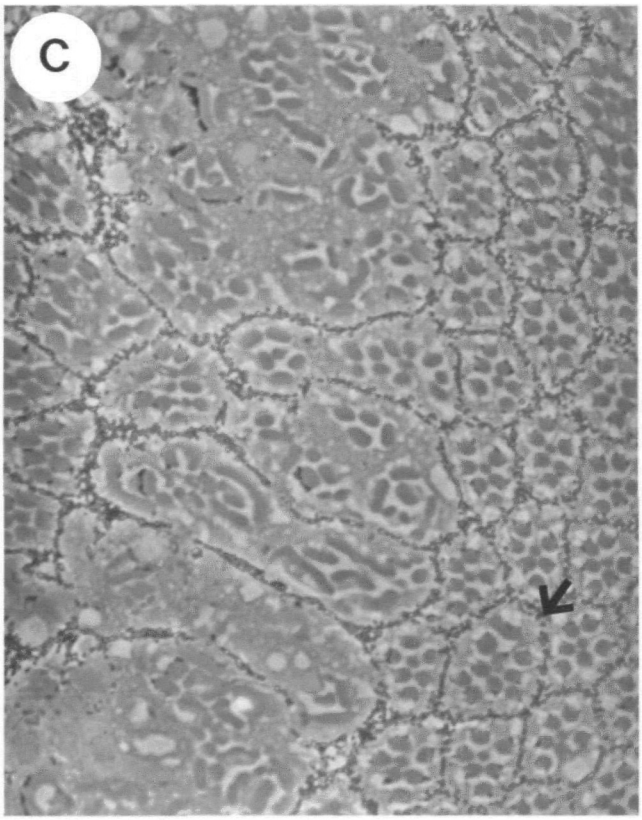
Developmental defects in gro^{BFP2} *larval eye discs*

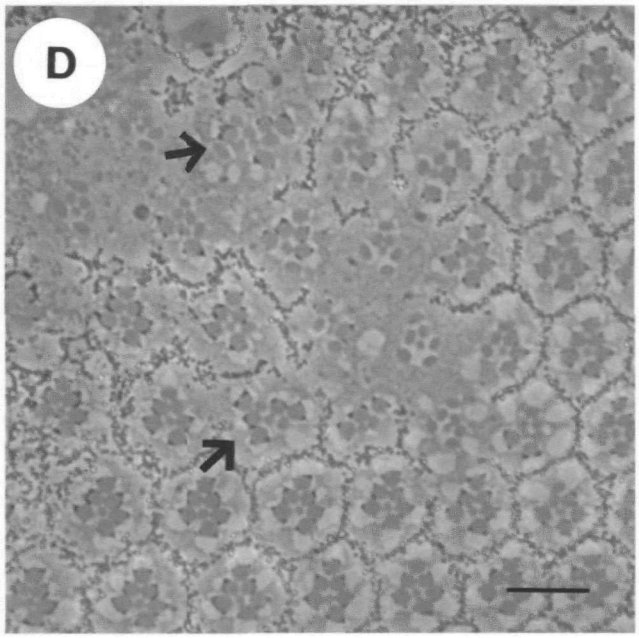
To determine when during ommatidial assembly the extra photoreceptor cells are recruited, the developing eye discs of gro^{BFP2} mutants were stained with the neural specific antibody mAb22C10 (Fujita et al., 1982). mAb22C10 reveals the sequence of photoreceptor cell assembly (R8, R2/5, R3/4, R1/6, R7) as each R-cell begins to express the mAb22C10 antigen when it

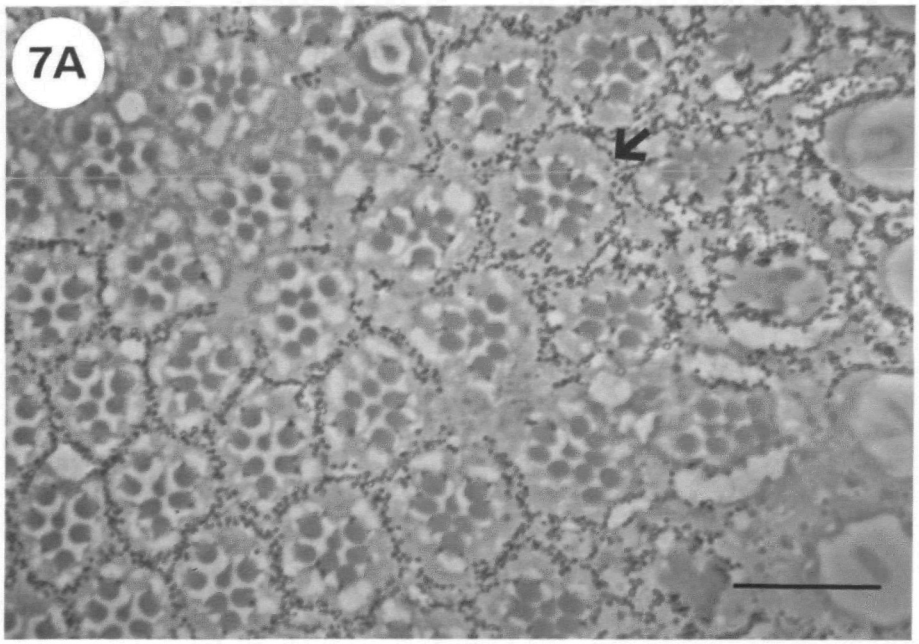
3A

Mutant Allele	Number of Clones			
	wild type	weak	moderate	severe
E(spl)^{E107}	14	2	0	0
E(spl)^{E28}	2	11	2	0
E(spl)^{E48}	0	0	2	2
E(spl)^{E73}	0	0	2	11
E(spl)^{E75}	0	1	2	11
E(spl)^{E77}	0	0	1	0
l(gro)^{X115}	0	0	3	0
E(spl)^{BX22}	0	0	4	10







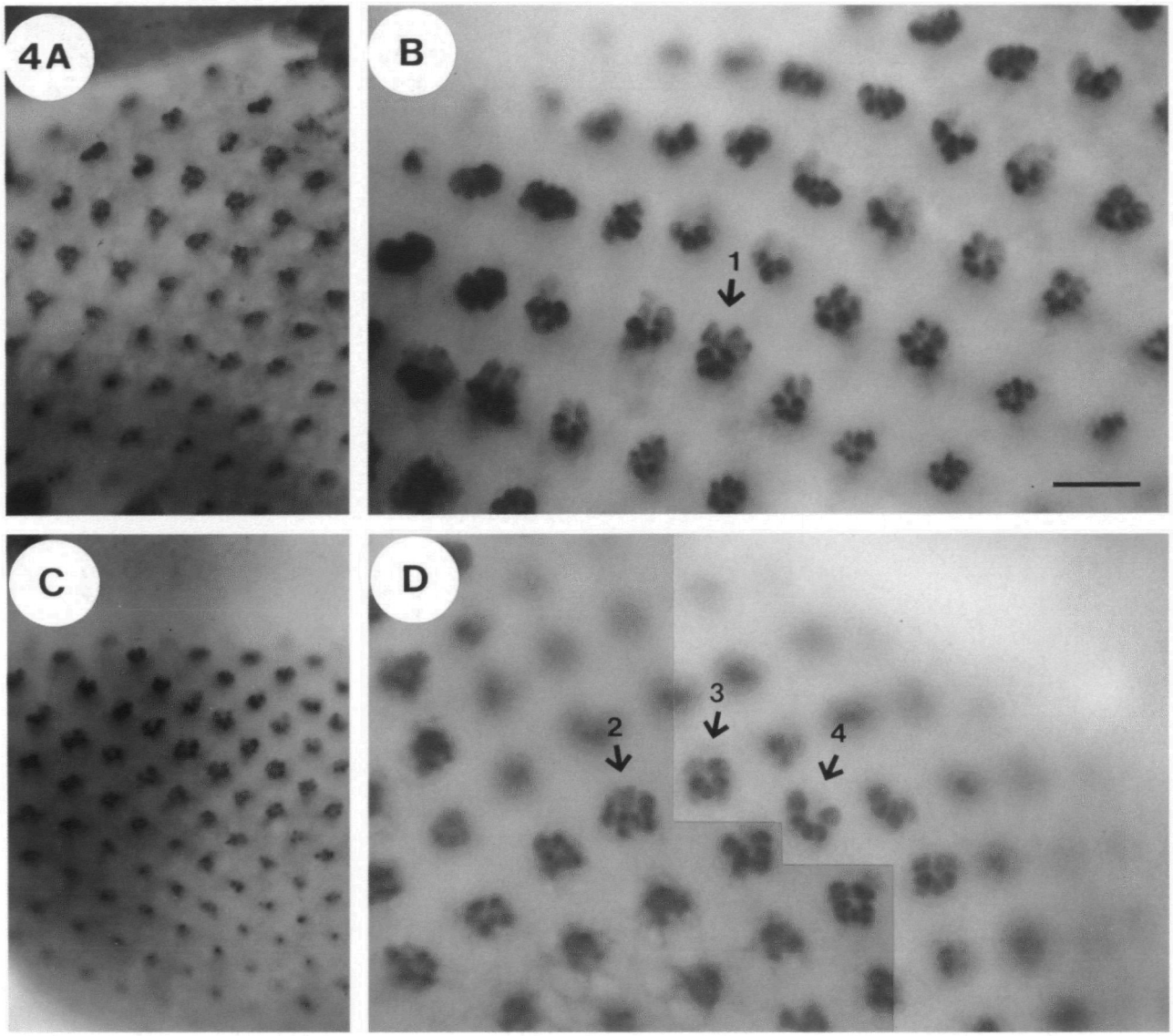


B

Normally Constructed Mosaic Facets

	number w^+gro^{BFP2+} / number counted	% w^+gro^{BFP2+}
R1	84/161	52
R2	85/161	53
R3	87/161	54
R4	99/161	61
R5	95/161	59
R6	93/161	58
R7	92/161	57
R8	66/ 97	68

Total number wild-type mosaic facets=161
Total number mutant mosaic facets=170


4A
B
1
C
D
2
3
4

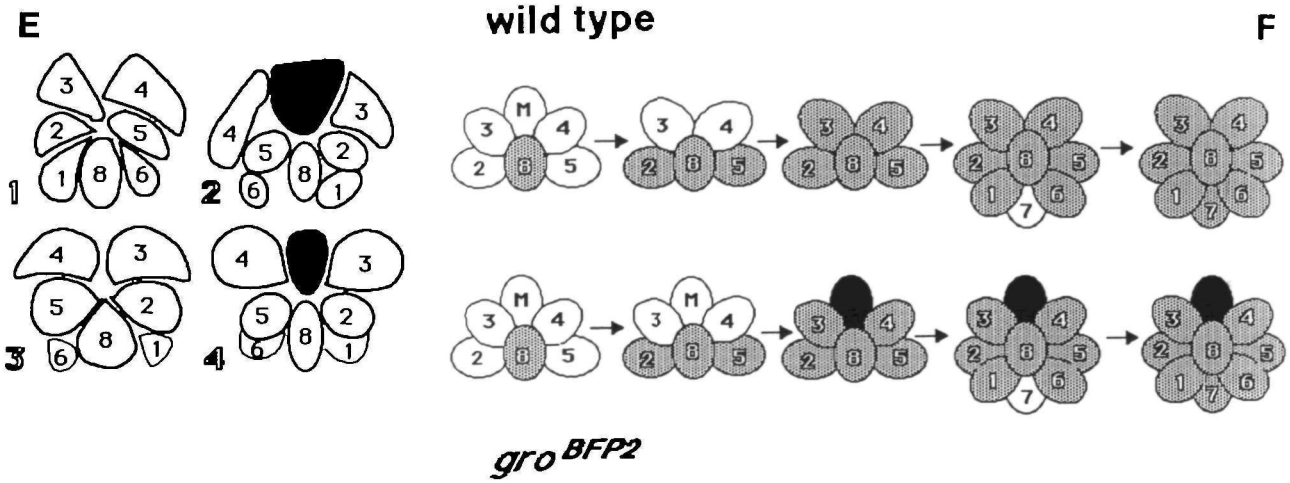

E
wild type
F
1
2
3
4
M
7
gro BFP2

Fig. 4. Ectopic R-cells observed in gro^{BFP2} larval eye discs stained with mAb22C10. Third instar larval eye discs were stained with the neural specific antibody mAb22C10 (Fujita et al., 1982) as described in Materials and methods. mAb22C10 reveals the assembly sequence of R-cells (see F below). The antigen is cytoplasmic, and the stained structures shown are the apical tips of the R-cells The bar is 10 μm in B and D and 20 μm in A and C. In all panels, the morphogenetic furrow is at the top. (A, C) Wild-type and gro^{BFP2} eye discs, respectively, showing that the rows of developing facets are evenly spaced in gro^{BFP2} discs. (B, D) Close-up views of wild-type and gro^{BFP2} discs, respectively. D is a montage so that certain facets are simultaneously in focus. The numbered arrows indicate the facets schematized in E. (E1) A wild-type cluster in ~row 8 of the wild-type disc. The stained apical tips of R8 and R1-R6 are visible. (E2-4) Clusters in ~row 8-9 of the gro^{BFP2} disc. The black cells in E2 and E4 represent the ectopic cells separating R3 and R4 As expected, an additional ectopic cell can sometimes be observed (not shown). E3 appears to be one of the normally assembling facets, which are expected to be present as ~one-third of facets in the gro^{BFP2} adult eye have the normal number of photoreceptors. The extra cells, due to their positions, are likely to be the mystery cells (see F below). Based solely on the mAb22C10 staining pattern observed, we cannot assign the identities to R-cells in the facets shown in E2 and E4. The labels shown in E are based on enhancer trap marker experiments (Figs 5 and 6) which show that the extra cells in the gro^{BFP2} disc are of the R3/4 subtype and that the other R-cells are appropriately determined. R3, R4 and the ectopic cell, as they are all R3/4 subtype cells, were labeled somewhat arbitrarily. R3 and R4 were labeled as such because of their positions next to R2 and R5, and the ectopic cell so labeled because of the position of the "mystery cells" between R3 and R4 in the undifferentiated precluster (see F). (F) A summary of normal R-cell assembly based on the mAb22C10 staining pattern (Tomlinson and Ready, 1987a). The model for the gro^{BFP2} mutant is based on the mAb22C10 staining pattern and also on the enhancer trap experiments (Figs 5 and 6). The cells are shaded in the order that they express the mAb22C10 antigen The 6- to 7-cell preclusters contain R8/2/5/3/4 and one or two mystery cells. Only one mystery cell is shown. In gro^{BFP2} eye discs, the mystery cells do not leave the precluster, but become ectopic R-cells (black) of the R3/4 subtype adjacent to R3/4 (Figs 5 and 6). As explained above, the assignments of R3, R4 and the ectopic cells were somewhat arbitrary. A very small number (~1%) of facets in gro^{BFP2} adult eyes have three ectopic R-cells. The third cell probably originates from an additional mystery cell.

Table 1. *Summary of eye phenotypes of viable* groucho *allele combinations*

genotype	eye phenotype
gro	wild type
gro^{BFP2}	1-2 extra R-cells in 70% of facets and facet orientations disordered
gro^{BFP2}/gro	wild type
$gro^{BFP2}/E(spl)^{E107}$ $gro^{BFP2}/E(spl)^{E28}$	nearly wild type, ~1/100 facets have an extra outer R-cell
$gro^{BFP2}/E(spl)^{E48}$ $gro^{BFP2}/E(spl)^{E73}$ $gro^{BFP2}/E(spl)^{E75}$ $gro^{BFP2}/E(spl)^{E77}$ $gro^{BFP2}/l(gro)^{X115}$ $gro^{BFP2}/E(spl)^{BX22}$	facets have many defects: extra outer R-cells missing outer and inner R-cells disordered orientations malformed rhabdomeres
$ry^{+}E8/+,gro^{BFP2}$	nearly wild type, ~1/100 facets have an extra outer R-cell

Representative examples of each phenotype are shown in Fig 1 The original viable *groucho* allele (*gro*) is described in Lindsley and Grell, 1968, Knust et al , 1987 and Ziemer et al , 1988 All of the *E(spl)* alleles and $l(gro)^{X115}$ are apparent lethal point mutations in the *groucho* gene, except for $E(spl)^{BX22}$ which is a deficiency (Preiss et al , 1988, see Fig 2) $E(spl)^{E107}$ and $E(spl)^{E28}$ are considered the weakest lethals because homozygotes die as pupae, which is later than homozygotes for the other alleles (Preiss et al , 1988) $ry^{+}E8$ is a line transformed with a P element containing a copy of the wild-type *groucho* gene, which rescues the phenotypes of the point mutations (Preiss et al , 1988, see Fig 2)

acquires neural identity (Tomlinson and Ready, 1987a). As ommatidial assembly proceeds in a posterior-to-anterior wave in the eye disc, ommatidia at all stages of photoreceptor cell assembly are observed in one disc (Fig. 4A, B and F). In gro^{BFP2} discs, one or two ectopic R-cells are first observed staining with mAb22C10 in the fifth or sixth column of assembling facets, when the R3/4 pair first stain (Fig. 4C, D, E and F). These extra cells are likely to be the mystery cells, which are normally positioned between R3 and R4 in an undifferentiated 6- to 7-cell precluster (hereafter refered to as the precluster) just posterior to the morphogenetic furrow, but then disappear into the surrounding pool of dividing cells by column 3, without expressing neural antigens (Tomlinson, 1987a; Wolff and Ready, 1991b; Fig. 4F). However, as individual R-cells can be identified only by their positions in a normally assembling facet, other explanations for the unusual mAb22C10-staining structures observed in gro^{BFP2} discs are possible. For example, it is conceivable that the mystery cells are excluded appropriately in the gro^{BFP2} discs and the ectopic cells are recruited from the surrounding epithelial cells into any position in the cluster.

By the fifteenth column, assembling facets have normally gone through 90° rotation with respect to a central equator (Tomlinson and Ready, 1987a). The facets in the gro^{BFP2} disc appear to rotate properly, so the orientation abnormalities apparent in the adult retina must occur in the pupal eye disc.

Eye discs from larvae carrying gro^{BFP2} in trans to several lethal *gro* alleles were also stained with mAb22C10 (data not shown). As expected, $E(spl)^{E107}/gro^{BFP2}$ and $E(spl)^{E28}/gro^{BFP2}$ eye discs appeared normal. The discs of gro^{BFP2} in trans to the stronger lethal alleles or $E(spl)^{BX22}$ looked very similar to gro^{BFP2} homozygous eye discs. Thus, the ectopic R-cells are likely to have the same origin in the trans-heterozygotes as in gro^{BFP2} homozygotes, as they are first observed at the same time during ommatidial assembly. The additional defects apparent in the adult eyes of these genotypes as compared with gro^{BFP2}

homozygotes must occur during pupal eye development.

Photoreceptor cell identities in gro^{BFP2} *eye discs*

To ascertain the subtype and position of the extra photoreceptors in the *gro^{BFP2}* mutant eye disc, and also to investigate whether the other R-cells in the *gro^{BFP2}* disc acquire their normal identities, the *gro^{BFP2}* mutation was combined with seven different enhancer trap lines. Each enhancer trap line expresses β-galactosidase in the nuclei of different subsets of photoreceptor cells, thus allowing the identification of every R-cell in the developing disc by staining with antibodies to β-galactosidase. The results are shown in Figs 5 and 6. In *gro^{BFP2}* mutant eye discs, all seven enhancer trap lines express β-galactosidase in their normal patterns, except that in the four lines that express β-galactosidase in the R3/4 pair, an extra nucleus is often observed next to the R3/4 cells (Figs 5 and 6). No ectopic nuclei stain in lines A2-6, X81 or N30, which express β-galactosidase in R8, R8/2/5 and R1/6/7, respectively (data not shown). In addition, *gro^{BFP2}* discs were stained with an antibody to the *rough* protein, which, behind the morphogenetic furrow, is expressed in the nuclei of R2/5/3/4 (Kimmel et al., 1990). Staining was observed in the four R-cells and also in an ectopic cell between R3 and R4 (data not shown). We conclude, as summarized in Fig. 4F, that the extra R-cells in the *gro^{BFP2}* mutant arise between or next to the normal R3/4 pair, and thus they are very likely to be the mystery cells. In addition, the ectopic cells are of the R3/4 subtype, and the other R-cells in the *gro^{BFP2}* mutant eye disc attain their usual identities.

The neural determination of the mystery cells is independent of their genotype or the genotype of any other photoreceptor cell in gro^{BFP2-}*-wild-type mosaics*

In order to determine which cells in the *gro^{BFP2}* mutant eye disc are responsible for the inappropriate recruitment of the mystery cells as photoreceptors, we generated marked clones of homozygous mutant cells (*w^{-}gro^{BFP2-}*) in wild-type (*w^{+}gro^{BFP2+}*) eyes (Materials and methods and Fig. 7). Within patches of *w^{-}gro^{BFP2-}* cells, the retina looks like that of *gro^{BFP2}* homozygotes and outside of the clones the retina appears wild-type (Fig. 7A and legend). Therefore, the effect of the *gro^{BFP2}* mutation, as is the case for the other *E(spl)* alleles (Fig. 3), is local.

At the clone borders, ommatidia mosaic for *w^{-} gro^{BFP2-}* and *w^{+}gro^{BFP2+}* cells were observed (Fig. 7A). As expected, these genetically mosaic facets were sometimes normally constructed (with 8 R-cells) and sometimes abnormal (with 9, 10 or 11 R-cells.) Approximately 50% of the mosaic ommatidia are normally constructed (Fig. 7B), in contrast with ~33% of the isogenic *gro^{BFP2}* mutant facets (Fig. 1B). Thus, ~17% of the mosaic facets are "rescued" to wild-type (no extra R-cells) by wild-type cells at the borders of the clones.

The genotypes of the different R-cells in 161 normally constructed mosaic ommatidia in 10 different clones were scored to determine if there is a tendency for particular R-cells to be *w^{+}gro^{BFP2+}* The distribution of the R-cell genotypes is nearly random (Fig. 7B). Thus, no particular R-cells in a facet need to be *gro^{BFP2+}* in order to exclude the mystery cells from the ommatidial cluster. Remarkably, we often observed at the clone borders phenotypically mutant facets (9 R-cells; note that only 8 are visible in Fig. 7A) in which all of the R-cells, including the ectopic one, are genotypically wild-type (*w^{+}gro^{BFP2+}*) (Fig. 7A). In 35 clones examined, 26 examples of such facets were observed in 15 different clones (see legend to Fig. 7).

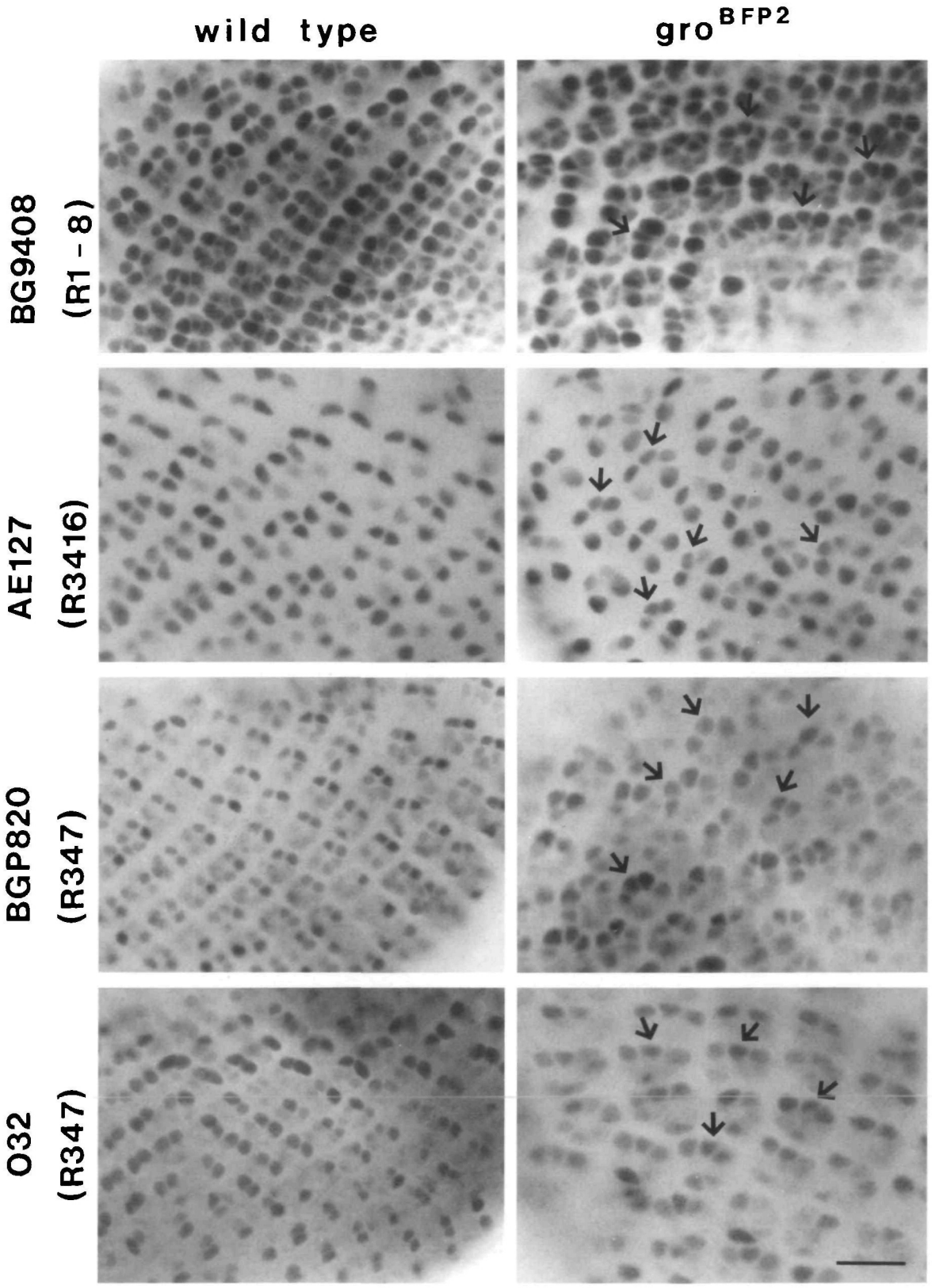
A similar detailed analysis of mosaic facets in the eye clones of lethal *groucho* mutations was not attempted because the eye phenotypes of these alleles were either too weak or too complex (Fig. 3). However, several examples of facets with ectopic R-cells were observed at the clone borders that appeared to be composed of genotypically wild-type R-cells (Fig. 3 and legend).

In summary, these observations imply that defects in cells outside of the photoreceptors and mystery cells result in the recruitment of ectopic R-cells in *gro^{BFP2}* mutant eye discs. As *gro^{BFP2}* is completely recessive and behaves as a partial loss-of-function mutation, the focus of action of the mutant protein is likely to be in at least a subset of the cells in which the wild-type *groucho* protein functions. Thus, we conclude that cells outside of the photoreceptors or mystery cells require *groucho* to inhibit the neuralization of the mystery cells.

Fig. 5. Photoreceptor cell identities in *gro^{BFP2}* larval eye discs. Seven different enhancer trap lines, identified at the left, that express β-galactosidase in the subsets of R-cell nuclei indicated in parentheses, were stained with anti-β-galactosidase antibodies (Materials and methods) in wild-type and *gro^{BFP2}* backgrounds. The enhancer trap lines are described in detail in Materials and methods. The morphogenetic furrow is at the top in all panels. Shown are the four enhancer trap lines that normally express β-galactosidase in the R3/4 pair and also in adjacent ectopic cells in *gro^{BFP2}* discs. The staining patterns are identical in wild-type and *gro^{BFP2}* discs except for the appearance of the extra R-cells in the mutant discs. The arrows indicate some of the ectopic cells (see Fig. 6). The bar in the lower right-hand panel is 10 μm and 15 μm in all of the other panels except for the two at the lower left in which it is 20 μm See Fig. 6 for enlargements of individual assembling facets

Discussion

We have described an unusual viable mutation in the *Drosophila groucho* gene, *gro^{BFP2}*, that results in ectopic photoreceptors in adult eyes. We show that *gro^{BFP2}* is likely to be a partial loss-of-function mutation by its genetic behavior and also by comparing *gro^{BFP2}* eyes to eye clones homozygous for lethal *groucho* alleles. There are two main conclusions from the analysis of *gro^{BFP2}* eyes. First, by examining the early development of *gro^{BFP2}* eye discs with neural and R-cell-specific markers, we show that many facets in *gro^{BFP2}* retinas contain extra R3/4 subtype photorecep-



tors adjacent to the normal R3 and R4. Second, by observing the genotypes of R-cells in facets mosaic for gro^{BFP2-} and wild-type cells, we find that the focus of action of the gro^{BFP2} mutation appears to be outside of the photoreceptors, including the ectopic ones, within a particular facet.

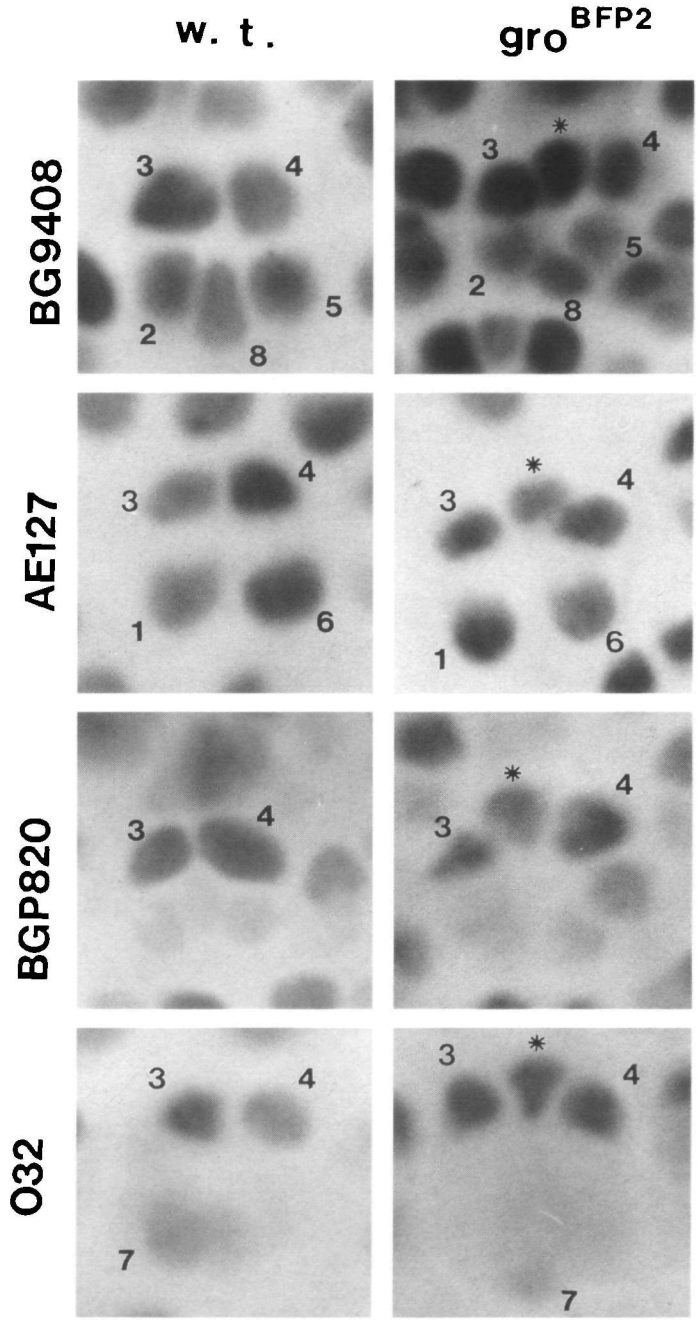


Fig. 6. Ectopic R3/4 subtype photoreceptor cells in *gro*^{BFP2} eye discs. Shown are enlarged images of individual facets from Fig. 5A. The R3/4 pair are the only R-cells in which all four enhancer trap lines, indicated at the left, express β-galactosidase (see Fig. 5A). β-galactosidase is also expressed in the ectopic R-cell nuclei of *gro*^{BFP2} mutant eye discs. The identities of the nuclei are as shown and asterisks (*) indicate the ectopic nuclei seen in *gro*^{BFP2} discs. As there are three R3/4 subtype nuclei shown in each panel of facets from *gro*^{BFP2} discs, the labeling of cells as R3, R4 or * is somewhat arbitrary. R3 and R4 were labeled according to their positions adjacent to R2 and R5, and the ectopicnucleus was labeled between R3 and R4 as that is the normal position of the mystery cells in the undifferentiated precluster (see Fig. 4F).

groucho *function in the eye*

The extensive eye defects in strong *groucho* mutant clones suggests that *groucho* may be involved in many different aspects of eye development. Indeed, the *groucho* protein is found in all cell nuclei in the eye disc, both anterior and posterior to the morphogenetic furrow (Delidakis et al., 1991; our observations). Using a temperature-sensitive allele, it has been demonstrated that another "neurogenic" gene, *Notch*, mediates cell interactions in all types of commitment decisions in the developing eye disc (Cagan and Ready, 1989b). Perhaps pleiotropic function will prove to be characteristic of many neurogenic genes.

Despite the apparent complexity of the role of *groucho* in eye development, using the *gro*^{BFP2} allele, it is possible to ask where *groucho* is required to perform the function of preventing some mystery cells from becoming photoreceptors. The simplest interpretation of the mosaic analysis is that *groucho* is required outside of both the mystery cells and the photoreceptor cell precursors. First, the genotypes of the R-cells in the wild-type mosaic facets are random, consistent with the *gro*^{BFP2} mutation having no effect in photoreceptor cells. Second, the observation that the R1-R8 appear to be correctly determined in *gro*^{BFP2} mutant discs is consistent with the *gro*^{BFP2} mutant affecting cells other than photoreceptors. Finally, the frequent appearance at the *gro*$^{BFP2-}$ clone borders of facets containing an ectopic R-cell, in which every R-cell is *gro*$^{BFP2+}$, is particularly compelling evidence in support of this interpretation. We performed a similar mosaic analysis with a mutant of a different gene with a null phenotype in the eye similar to *gro*^{BFP2}, that is, another mutant in which the mystery cells become photoreceptors. In 30 clones examined, not one example of a facet containing an extra R-cell in which all of the R-cells were genotypically wild-type was ever observed either at the clone border or outside of the clone (J.A.F.-V. and G.M.R., unpublished data). Moreover, genotypically wild-type, phenotypically mutant (with extra R-cells) facets were also observed at the borders of clones of lethal *groucho* mutations.

We cannot rule out more complicated models to explain the results of the mosaic experiments. For example, the phenotypically mutant (containing an extra R-cell) facets in which every R-cell is *gro*$^{BFP2+}$ observed at clone borders could be explained by proposing that *gro*$^{BFP2-}$ R3 or R4 fail to signal the mystery cells to leave the facet, and are then sometimes competed out of the facet when surrounded by wild-type cells. "Sometimes" is emphasized as we often observed facets with ectopic photoreceptors in which *gro*$^{BFP2-}$ cells in the positions of R3 or R4 were surrounded by wild-type cells. This model would require that a signal from R3 or R4 be propagated across more than one cell. In addition, the failure to observe a bias towards *gro*$^{BFP2+}$ R3 and R4 cells in normally constructed mosaic facets is not easily explained by this model. Another possibility is that the genotypes of cells from neighboring facets could influence the fates of the mystery cells. This interpret-

ation is unlikely because the phenotype of *Ellipse* mutations shows that facets develop autonomously (Baker and Rubin, 1989).

Previous observations suggest that mystery cell fate is controlled by cells within the developing facet. The *seven-up (svp)* gene product is required in R3/4/1/6 cells to repress the R7 developmental pathway (Mlodzik et al., 1990b). In *seven-up* mutant clones, an extra outer photoreceptor cell of unknown subtype sometimes appears adjacent to svp^- R3 cells, and always between svp^- R3 and R4, independent of its own genotype (Mlodzik et al., 1990b). Thus, R3 and to some extent R4 influence the fate of the mystery cells. Our results with gro^{BFP2} suggest that mystery cell fate is also controlled by cells outside of the precluster. Wolff and Ready (1991b) have shown that the first structure to emerge from the morphogenetic furrow is a rosette in which 10-15 cells, including the R8, R2/5, R3/4 precursors and the mystery cells form a ring around 4-5 core cells. The ring then opens and the 6- to 7-cell preclusters, containing the precursors to R8/2/5/3/4 and the mystery cells, are formed. Precisely when the mystery cells are determined to leave the precluster is unknown. Our results suggest that *groucho* mediates this process, presumably through cell contact. Thus, the cells that require *groucho* to prevent neurogenesis of the mystery cells could be the core cells or cells next to the mystery cells within the ring. Alternatively, if the cell communication process interrupted by the gro^{BFP2} mutation occurs later, during the 6- to 7-cell precluster stage, epithelial cells surrounding the precluster could be involved. Unfortunately, these cells cannot be identified in the adult eye.

These results suggest that the cells requiring *groucho* to signal the mystery cells are uncommitted cells. All of the "neurogenic" genes, including *E(spl)*, appear to play key roles in cell-contact-mediated neural inhibition in the embryonic neurectoderm that forms the CNS and probably also in the proneural regions of imaginal discs from which bristles arise. In these processes, cells compete for neural determination and the victor then inhibits its neighbors from also becoming neural cells. The particular role of *groucho* described here is different in that the cells sending the inhibitory signals appear to be uncommitted epithelial cells.

In cell transplantation experiments, cells containing *E(spl)* deletions behave autonomously, that is they always become neural when surrounded by wild-type cells (Technau and Campos-Ortega, 1987). This observation implies that *E(spl)* is required for the reception of a neural inhibition signal in embryonic cells. Our mosaic results suggest a non-autonomous role for *groucho*, in that cells outside of the mystery cells require *groucho* to influence mystery cell fate. However, our results do not necessarily contradict the previous findings, as the apparently non-autonomous role we find for *groucho* could be indirect. In other words, it is possible that *groucho* is autonomously required by the cells that direct the mystery cells to leave the precluster.

Specification of photoreceptor cell subtype

Developing ommatidia display a particular sequence of determination of specific cell types and assembling clusters have characteristic structures and cell contacts (Tomlinson, 1985; Tomlinson and Ready, 1987). These observations led Tomlinson and Ready to hypothesize that local cell contacts instruct cells to acquire particular fates. An extreme version of this model would predict that in the precluster, R8 would cue R2 and R5, and those three cells would then instruct the specification of R3 and R4. In gro^{BFP2} eye discs, the specification of the mystery cells as R3/4 subtype photoreceptors appears to break the rules for cell specification in the precluster. How much evidence is there that cells within the initial 6- to 7-cell precluster normally cue each others determination? The best evidence comes from studies of the *rough* gene, which encodes a homeobox protein required only in cells R2 and R5 for their appropriate differentiation (Tomlinson et al., 1988; Saint et al., 1988; Heberlein et al., 1991). In *rough* mutant eye discs, although the appropriate R2/5 precluster cells become outer photoreceptors, they are not properly specified as the R2/5 subtype, and presumably do not send the necessary signals to R3/4 so that these cells often fail to join the developing precluster. It is unknown whether or not cues from R8 are also necessary for R3/4 determination (see Banerjee and Zipursky, 1990). Also, mutations disrupting communication between R8 and R2/5 have not yet been identified.

How can the mystery cells become R3/4 subtype photoreceptors? Our interpretation of the analysis of gro^{BFP2+}/gro^{BFP2-} mosaic ommatidia is that the cells responsible for the extra R3/4 cells in gro^{BFP2} mutants are outside of the R-cells in the facet, including the extra R-cells. Moreover, the appropriate expression of many markers implies that the R-cells are properly determined in gro^{BFP2} eye discs. Therefore, it cannot be argued that, in gro^{BFP2} eye discs, the R-cells in contact with the mystery cells (R8 and R3/4), because they are mutant cells, send inappropriate signals to the mystery cells thus recruiting them as R3/4 cells. Likewise, it is inconsistent with our data to suppose that the mystery cells, because they are gro^{BFP2} mutant cells, inappropriately receive positional cues from R3/4 and/or R8.

More likely explanations for the determination of the mystery cells as R3/4 subtype photoreceptors in gro^{BFP2} eye discs allow that R8 and R3/4 act normally, but the mystery cells are in an unusual environment because they remain in contact with the developing ommatidial precluster longer than they would normally. For example, the R3/4 cells may normally send positional cues similar to those of R2/5 cells. These cues from R3/4 usually would be inconsequential because there are no cells between R3 and R4 after the mystery cells leave. The R3/4 pair express *rough* (Kimmel et al., 1990) although no requirement for *rough* in cells other than R2/5 is apparent, suggesting that these subtypes may share some signalling pathways. Alternatively, the mystery cells could acquire R3/4 fate by receiving a signal solely from R8, and thus by a pathway at least

partially different from that of the normal pre-R3/4 cells. The combination of gro^{BFP2} with mutations that disrupt R8 or R3/4 differentiation may help to distinguish among these alternatives.

groucho *is involved in several different neural repression pathways*

The level and nuclear distribution of *groucho* antigen appears, at the light microscope level, to be normal in gro^{BFP2} eye discs (data not shown). The mutation may therefore affect the structure of the protein in a manner that is critical to one of its functions in the eye. The ability to obtain *groucho* mutants that specifically affect a subset of its many functions reveals that *groucho* is likely to be involved in several different cell signaling pathways that prevent neural cell determination. The original *groucho* allele very specifically affects the ability of the *groucho* gene product to repress the formation of specific head bristles. Similarly, although $E(spl)^{E107}$ is pupal lethal due to weak neural overgrowth, it has little effect on eye development. Likewise, gro^{BFP2} perturbs a small subset of the many roles of the normal *groucho* protein in the eye.

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