

# A green fluorescent protein enhancer trap screen in *Drosophila* photoreceptor cells

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## Abstract

The *Drosophila* ommatidia contain two classes of photoreceptor cells (PR's), the outer and the inner PR's. We performed an enhancer trap screen in order to target genes specifically expressed in PR's. Using the UAS/GAL4 method with enhanced green fluorescent protein (eGFP) as a vital marker, we screened 180 000 flies. Out of 2730 lines exhibiting new eGFP patterns, we focused on 16 lines expressing eGFP in particular subsets of PR's. In particular, we describe three lines inserted near the *spalt major*, *m-spondin* and *furrowed* genes, whose respective expression patterns resemble those genes. These genes had not been reported to be expressed in the adult eye. These examples clearly show the ability of our screen to target genes expressed in the adult *Drosophila* eye. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** *Drosophila* photoreceptors; R1–6 cells; R7 cells; R8 cells; *rhodopsin*; Enhanced green fluorescent protein; Enhancer trap; *GAL4*; *spalt*; *m-spondin*; *furrowed*; Medulla; Lamina; Optic lobe; Antenna; Ocelli; Wing; Leg; Bristle; *labellum*; *scutellum*; *maxillary palpus*

## 1. Introduction

The compound eye of *Drosophila melanogaster* is composed of ~800 ommatidia, each consisting of 20 cells, including eight neuronal photoreceptor cells (PR's), cone cells, pigment cells and bristle cells (Wolf and Ready, 1993). The R1–6 outer PR's are mainly responsible for image formation and project to the lamina part of the optic lobe. They surround the inner PR cells, R7 and R8, located one on top of the other and occupying the apical (R7) or the basal (R8) half of the retinal layer (For review see Hardie, 1985). R7 and R8 are used for the detection of colors and polarized light and both project to the medulla (for a recent review see Pichaud et al., 1999). Many studies have been performed to understand early *Drosophila* eye development using genetic screens based on irradiation, chemical and P-element mutagenesis (Hafen et al., 1993). We are interested in another much less studied aspect, namely genes expressed in differentiated adult PR's which may be involved in specific opsin gene regulation and in phototransduction.

There are six characterized *opsin* genes that are each specifically expressed in subsets of PR's in the adult *Drosophila* eye. *rhodopsin 1* (*rh1/nina E*) is expressed in R1–6; *rh2* is exclusively expressed in ocelli, which represent a distinct visual system. *rh3* and *rh4* genes are expressed in two non-overlapping subsets of R7 (Fortini and Rubin, 1990; Feiler et al., 1992), while *rh5* and *rh6* are expressed in two non-overlapping subsets of R8 (Chou et al., 1996; Papatsenko et al., 1997; Chou et al., 1999). Interestingly, the exclusive expression of *rh3* in a subset of R7 (30%) correlates with the specific expression of *rh5* in the corresponding underlying R8, constituting the *pale* (*p*) ommatidia subset. The remaining 70% of ommatidia express *rh4* in R7 and *rh6* in R8 and constitute the *yellow* (*y*) ommatidia subset (Franceschini et al., 1981; Chou et al., 1996; Papatsenko et al., 1997; Chou et al., 1999). The *y* and *p* ommatidia are distributed stochastically throughout the retina (Franceschini et al., 1981). Furthermore, in the dorsal margin region of the retina, both R7 and R8 contain the same opsin, Rh3 (Wunderer et al., 1989), and the orthogonal orientation of their respective microvilli allows detection of polarized light.

Rhodopsins are composed of a protein opsin and a chromophore, 3-hydroxy-11-cis-retinal (for review see Zuker,

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1996). Under illumination the conformation of the Rhodopsin changes leading to the activation of the phototransduction cascade (for review see Montell, 1999). These reactions generate action potentials transmitted to the brain through the PR axons. Many mutations affecting phototransduction have been identified using an electroretinogram assay (for review see Zuker, 1996). However, this screen only targeted viable recessive genes for which adult flies can be tested.

We used a modification of the GAL4 enhancer trap method (Brand and Perrimon, 1993; Calleja et al., 1996; Timmons et al., 1997) to identify genes specifically expressed in differentiated PR's. Our detection system used an upstream activating sequence (UAS) recognized by GAL4, followed by the cDNA of the enhanced green fluorescence protein (eGFP) gene (substitutions F64L and S65T from Clontech) (Cormack et al., 1996). UAS-eGFP flies were crossed with GAL4 enhancer trap lines obtained by transposition of the enhancerless *GAL4* gene construct pGawB (Brand and Perrimon, 1993). We monitored eGFP expression in the adult eyes of live flies using a fluorescence dissecting microscope (Plautz et al., 1996).

By detecting the eGFP fluorescence in the deep pseudopupil of the *Drosophila* eye, we were able to distinguish outer from inner PR cell expression. The deep pseudopupil is a inner virtual image corresponding to the superimposition of 20–30 ommatidia (Franceschini and Kirschfeld,

1971). The ommatidia in the light beam project to the bottom of the retina, resulting in a larger image of an ommatidium (e.g. Fig. 1a,c). Among 2730 lines showing a new eGFP pattern, we focused on 16 lines with PR-specific expression. Moreover we describe three lines that express *GAL4* in the adult eye: the targeted genes turned out to be the *spalt major* (*salm*) (Reuter et al., 1989; Kuhnlein et al., 1994), *m-spondin* (*mspo*) (Umemiya et al., 1997) and *furrowed* (*fw*) (Leshko-Lindsay and Corces, 1997) genes. These genes had not been previously reported to be expressed in the adult eye. These examples show the ability of our screen to target genuine expression patterns in the eye.

## 2. Results

### 2.1. Detection of eGFP in PR's

In order to assess eGFP expression in the eye, we took advantage of the GAL4/UAS system (Brand and Perrimon, 1993). We first tested our ability to detect eGFP in the eyes of living adult *Drosophila* with constructs under the control of *rh1*, *rh4* and *rh5* promoters. The expression of the *rh1* gene is restricted to R1–6 PR's, while *rh4* and *rh5* are only expressed in the R7y or R8p subsets, respectively. For this

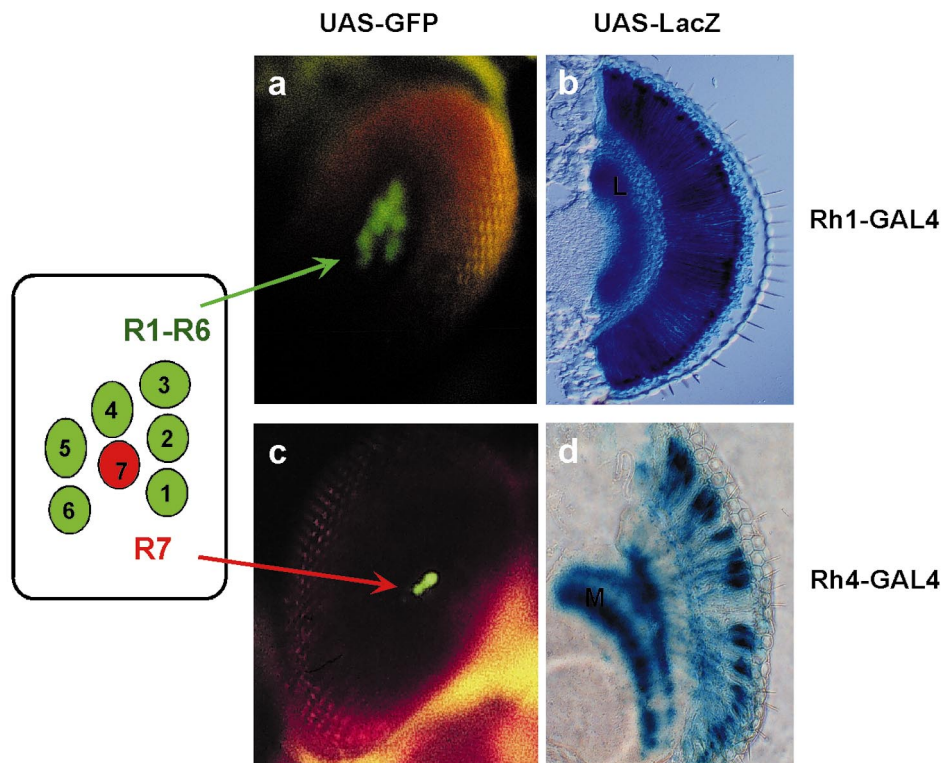


Fig. 1. In vivo detection of eGFP in the adult eye. Visualization of eGFP under the control of the *rh1* (a) or *rh4* (c) promoters in the deep pseudopupil. The fluorescent outer PR's form an enlarged trapezoidal image of an ommatidium (a). The complementary image is formed by R7 cell expressing eGFP (c). Living fly eyes are observed under blue illumination using a stereomicroscope.  $\beta$ -galactosidase activity in horizontal eye cryosection under the control of the *rh1* (b) or *rh4* (d) promoters. R1–6 cell staining spans the whole retina and the lamina (L) (b), while R7 staining is located in the apical part of the retina and in the medulla (M) (d).

purpose UAS-eGFP flies were crossed to transgenic flies carrying the *GAL4* gene under the control of *rh* promoters. Under blue illumination (400–500 nm), we obtained a bright green fluorescence in the deep pseudopupil (Fig. 1a,c). Under *rh1* promoter control (*rh1-GAL4*), eGFP is expressed strongly in the outer PR's resulting in the characteristic trapezoidal pattern of six outer PR's with a hole in the middle corresponding to the inner PR's (Fig. 1a). Alternatively, under the control of the *rh4* promoter (*rh4-GAL4*), we obtained the complementary image, with a single spot, corresponding to the R7 PR cell (Fig. 1c). This system even allows eGFP detection in the deep pseudopupil coming from R8p, in flies carrying the *rh5-GAL4* and *UAS-eGFP* constructs (not shown). Additionally in Fig. 3a, we describe a line selected because of an eGFP signal coming from the R8 cell layer. This proves that our detection system is very sensitive and allows the detection of the deep pseudopupil signal located deep in the retina, under the R7 cell.

As a complementary analysis, the GAL/UAS system allowed us to perform a  $\beta$ -galactosidase activity test on eye cryosections of flies carrying the various *rh-GAL4* and *UAS-lacZ* constructs. With *rh1-GAL4*, we obtained a complete retina staining and the corresponding neuronal projections into the lamina which are characteristic of an outer PR cell expression (Fig. 1b). Alternatively, with *rh4-GAL4*, we clearly distinguished the R7y subset staining pattern in the upper part of the retina, and its corresponding projections into the medulla layer of the optic lobe (Fig. 1d).

Together, these two different *GAL4* expression detection systems allow us to determine specific PR expression patterns in vivo or histologically.

## 2.2. Mobilization of P-Gal4 to the three chromosomes

The genetic scheme used to mobilize pGawB (Brand and Perrimon, 1993) to the three chromosomes is shown in Fig. 2. The line carrying both the initial P-element and the transposase source was crossed 'en masse' with a fly line that is

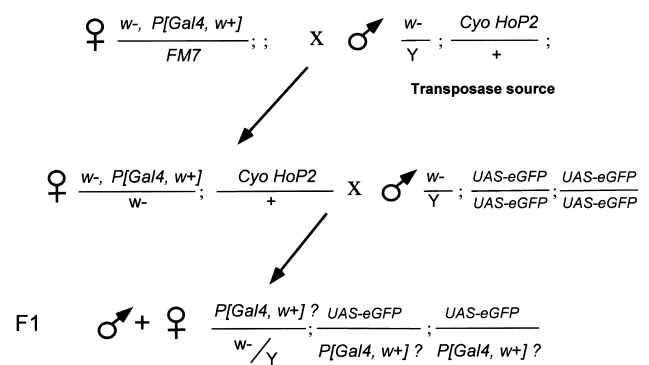


Fig. 2. Genetic scheme for the mobilization of pGawB. The *pGawB* jump start line is crossed with a line carrying a transposase source on the CyO balancer chromosome (named CyOHoP2). In the resulting female progeny carrying *P[GAL4, w+]* and the transposase source, the P-element remobilization occurs in germ cells. These female flies are then crossed with males double homozygous for *UAS-eGFP*, allowing the detection of *GAL4* expression patterns in the progeny with newly inserted *pGawB* P-elements.

double homozygous for two *UAS-eGFP* constructs. We analyzed living adult flies in the F1 for eGFP expression in the deep pseudopupil. We selected lines that exhibit expression in the adult eye and more particularly lines that specifically express eGFP in inner and/or outer PR's only (see Section 2.1). With this method, we could directly identify new insertions in the F1 progeny of the flies in which remobilization occurred, and eventually establish stable lines for those that exhibited interesting patterns.

### 2.2.1. Eye expression patterns

As shown in Table 1A, we screened 180 000 anesthetized F1 flies and 2730 flies exhibited a new eGFP patterns in any part of the body. Among them, 655 lines expressed eGFP in the eye (24% of the targeted genes). We retained 40 lines expressing specifically eGFP in PR's (6% of the lines showing eGFP expression in the eye). As our screen was originally designed to identify genes specifically expressed in

Table 1  
Distribution of expression patterns in flies expressing eGFP in the eye<sup>a</sup>

A	F1	New expresssion	Eye expression	PR expression		
Number of flies	180000	2730	655	40		
<b>B</b>						
Number of lines	5	3	7	7	21	43 Total
PR's	+	—	—	—	—	11.6%
Optic lobes (lamina and/or medulla)	+	—	+	—	+	77%
1st pigment cells	—	+	+	—	—	23%
Other eye cell types	—	—	—	+	—	16%

<sup>a</sup> (A) Among the flies exhibiting a new eGFP pattern in any part of the body, the lines expressing eGFP in the eye were retained. Lines showing PR eGFP expression were kept for further analysis. (B)  $\beta$ -galactosidase activity analysis of flies showing an eGFP signal in the eye. All lines showing PR cell staining exhibited also optic lobe staining. Expression in both primary pigment cells and in optic lobes was found in seven out of ten lines. Lines expressing in other eye cell types (secondary and tertiary pigment cells, cone and bristle cells) showed no costaining in the optic lobes. The percent of the 43 lines expressing in one cell type is shown. Due to coexpression patterns, the total is higher than 100%.

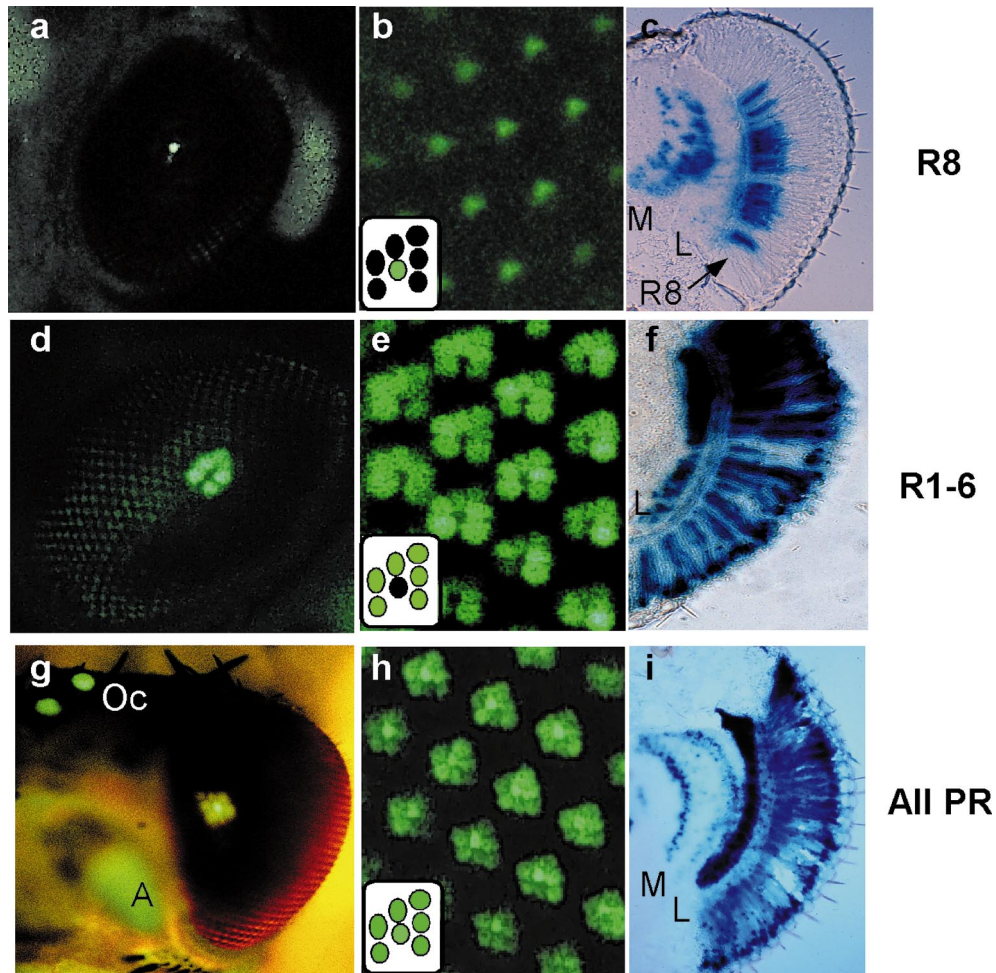


Fig. 3. Enhancer trap lines expressing GAL4 in PR's. The eGFP signal, detected in living flies, is visualized in the deep pseudopupil (a,d,g) or with the cornea neutralization technique (b,e,h) under blue illumination.  $\beta$ -galactosidase activity is assessed in horizontal eye sections (c,f,i). The R8.2.3 line specifically expresses eGFP in the R8 cells: the signal forms a unique spot in the center of the pseudopupil (a), while the R8 cells layer and the medulla exhibit X-Gal staining (c). In the R1–6M12.16 line, the deep pseudopupil shows the trapezoidal shaped R1–R6, which surround a blank spot corresponding to the inner PR's (d); the whole retina and lamina show X-Gal staining (f). The P9.2 line expresses eGFP in all PR's and deep pseudopupil shows inner and outer PR's (g); the whole retina, the lamina and the medulla exhibit X-Gal staining (i). Cornea neutralization shows that individual ommatidia express eGFP in inner (b), outer (e) or both inner and outer PR's (h).

PR's, all the lines expressing eGFP elsewhere in the eye were not further analyzed. However, using immersion epifluorescence and the *lacZ* staining, we classified the first 43 lines found to express eGFP in the eye in four major groups of expression (Table 1B). This classification gives a rough approximation of how genes are expressed in the adult *Drosophila* eye. A large proportion of the lines detected showed an expression in the optic lobe (77%) and 12 of them expressed only in lamina and not in PR's (not shown). These lines were detected because a strong eGFP signal from the lamina crosses the retina through the R7/R8 rhabdomeres and is detectable in the deep pseudopupil. However, we were still able to distinguish a true R7/R8 eGFP signal, which was larger and sharper. In two lines, only medulla staining but not PR expression was specifically detected (not shown). This lower proportion may be explained because the eGFP signal comes from

deeper in the brain and might be more difficult to detect. Such a detection system gives an efficient way to screen for genes expressed in the optic lobes. A high percentage of flies (23%) were isolated with a primary pigment cell expression. These flies are easily recognizable because the pigment cells are the only cells localized at the top of the retina which do not completely cross the retina and give a clear signal with the X-Gal assay. This pattern of expression is shown in Fig. 4g–i with the insertion in the *furrowed* gene. The 'other eye cell types' reported in Table 1B, include, secondary, tertiary pigment, cone, and bristle cells; they all exhibited a complete retina staining by X-Gal but showed no costaining in the optic lobes. At this point, we concluded that we could rely on eGFP detection in the pseudopupil to look for specific PR expression and were not constrained to perform a systematic histological X-Gal analysis on all lines showing eGFP expression in the eye.



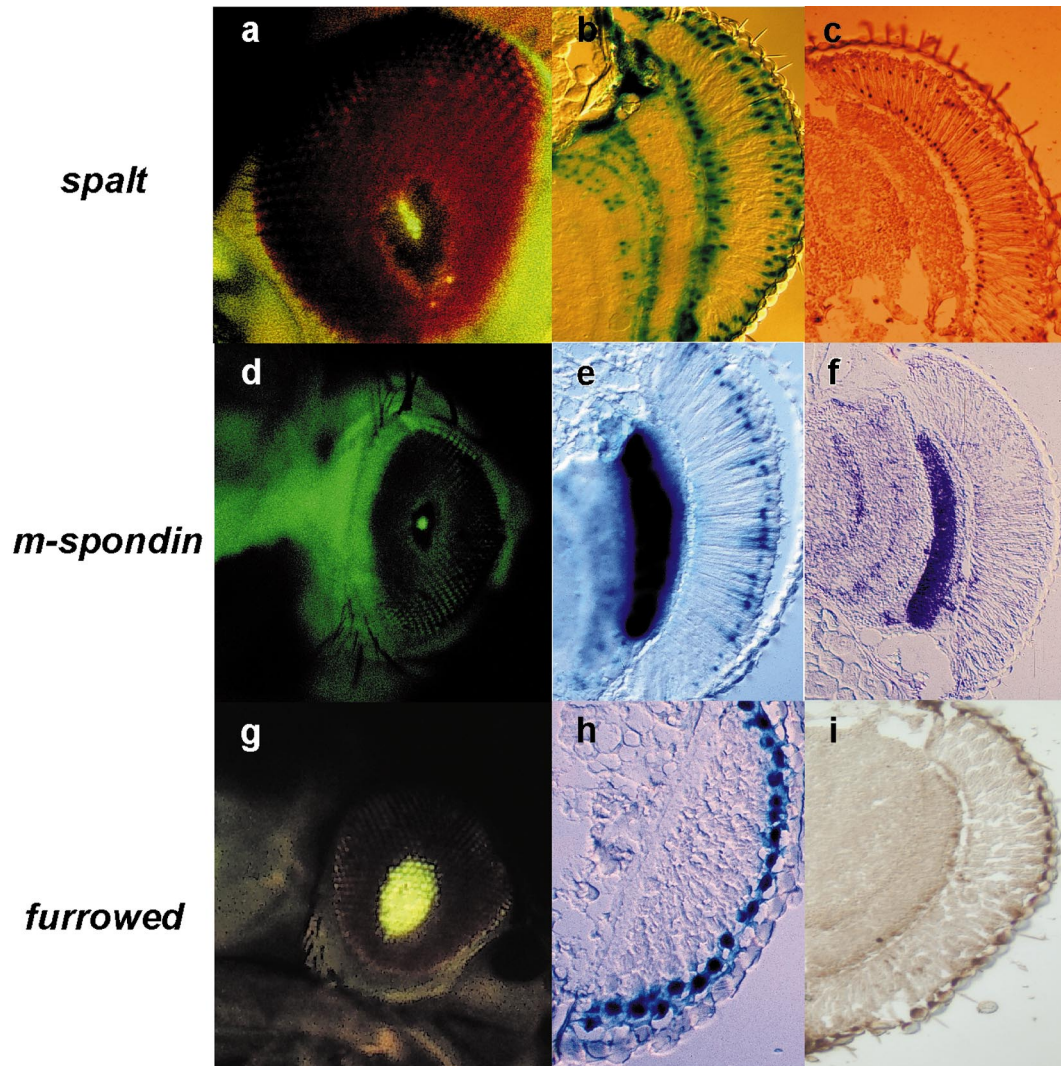


Fig. 4. Patterns of expression of the *salm*, *mspto* and *fw* genes. The R7T3.8 enhancer trap line inserted in *salm* shows an eGFP signal in the deep pseudopupil corresponding to inner PR expression (a). The corresponding X-Gal staining exhibits R7, R8 and medulla staining. Antibody staining performed against Salm also reveals R7 and R8 staining (c). The R7Ts12.7 enhancer trap line inserted in *mspo* shows an eGFP signal in inner PR's (d). X-Gal analysis exhibits R7 cell, lamina and medulla staining (e). Antibody staining against Mspo reveals lamina and medulla staining (f). The POC2.1 enhancer trap line inserted in *furrowed* gives a strong eGFP spot in the eye (g). X-Gal staining is located at the top of the retina, suggesting expression in primary pigment cell (h). Antibodies staining against Fw also reveal primary pigment cell staining.

### 2.2.2. Inner PR expression patterns

By performing plasmid rescue analysis on most of the 40 lines expressing eGFP in PR's (6% of the flies expressing eGFP in the eye) (Table 1A) and by comparing GAL4 expression pattern in the retina and in the rest of the body, we can estimate that at least 16 lines are independent insertions (Table 2; Figs. 3 and 4). The lines for which the plasmid rescue was not successful but presenting similar pattern in terms of photoreceptor expression were deemed distinct insertion events if they also exhibited distinct expression in other parts of the body. In Figs. 3a and 4a,d, the GAL4 system allows us to clearly distinguish the eGFP signal coming from the inner PR's and can be visualized in the center of the deep pseudopupil: after crossing the F1 with transgenic flies carrying the

UAS-*lacZ* constructs, X-Gal staining on horizontal eye cryosections showed localization of GAL4 in R7 (e.g. lines R7T3.8 and R7Ts12.7 in Fig. 4b,e) or in R8 (line R8.2.3, Fig. 3b). R7 cells are stained in the apical half of the retina, while R8 cells are stained in the basal half, and both their axons project to the medulla. Among the lines showing expression in inner PR's, we focused on one R7, one R8 and two R7+R8 specific lines (see 'R7+R8' in Table 2). As a complementary analysis, we used the cornea neutralization technique (Franceschini et al., 1981) which allows visualization of individual ommatidium expressing eGFP. In this way, we could see that most of these lines expressed eGFP in all R7 or R8 cells, and not only in *y* or *p* subtypes (shown only for the R8.2.3 line in Fig. 3b).

Table 2  
Enhancer trap lines with GAL4 expression in PR's<sup>a</sup>

Lines	Chromosome location	Targeted sequence	Eye pattern	Body pattern
<b>Inner PR</b>				
R7T3.8	II	<i>spalt</i>	R7 + R8	Thorax
R7TS12.7	II	<i>m-spondin</i>	R7	Thorax, scutellum
PTA	X	<i>waclaw</i>	Weak R7 + R8	Thorax, abdomen and scutellum
R8.2.3	II	ND	R8	None
<b>Outer PR</b>				
R1–6AM12.16	X	BACR20N11	R1–6	Antenna, maxillary palp
PB7.30	X	ND	R1–6	Thorax, abdomen
OmBrA6.9	ND	ND	R1–6	Bristle base, antenna
R1–6H12.6	ND	ND	R1–6	Head
R1–6T12.22	ND	ND	R1–6	Thorax
R1–6M12.17	ND	ND	R1–6	Maxillary palp
<b>All PR</b>				
P9.2	X	BACR48E01	R1–8	None
PRAb12.7	X	ND	R1–8	Abdomen
<b>Other PR pattern</b>				
R1–6OcB1.20	III	BACN11C06	R1–6 + some inner	Ocelli, thorax, abdomen
B8.6.4	X	ND	Some outer	Thorax, abdomen
PR2.27	III	ND	Some outer and inner	None

<sup>a</sup> Lines showing eGFP expression in the deep pseudopupil were further analyzed using immersion epifluorescence (cornea neutralization) and X-Gal staining, in order to determine those expressing in PR's. With the exception of the *spalt* and *m-spondin* insertions for which the GAL4 patterns correspond to the antibody staining (Fig. 4), the profiles of expression of the reported targeted sequences have not been compared to the GAL4 expression pattern. All lines reported in this table that exhibit similar PR expression differ by their expression pattern in other parts of the body.

### 2.2.3. Other PR expressions

We also isolated several lines expressing *GAL4* only in the outer, or in all the PR's, as shown in Fig. 3d–i. All outer PR expressing lines showed the characteristic trapezoidal eGFP expression pattern in the deep pseudopupil formed by the R1–6 cells (Fig. 3d). X-Gal staining confirmed outer PR expression with projections to the lamina region of the optic lobe (Fig. 3f). A line expressing *GAL4* in both outer and inner PR's is shown in Fig. 3g–i. X-Gal staining shows axonal projections to both the lamina and the medulla (Fig. 3i). Additionally we confirmed the specificity of the photoreceptor profile by performing the cornea neutralization technique (Fig. 3e,h). These images demonstrate that the deep pseudopupil image shown correspond to the superimposition of individual photoreceptor cell expression. We also found three lines showing atypical expression patterns in PR's (classified as 'other PR patterns' in Table 2). Some of the outer and inner PR's expressed eGFP in an apparent random manner that seemed to vary from one ommatidium to another (not shown). We could not conclude whether these patterns reflected true expression pattern or were the consequence of position effect variegation.

### 2.2.4. Body expression patterns

We found many specific body patterns. An example is shown in Fig. 5 to emphasize the potential of this screen. The PAMBr5.21 insertion lines shows an expression in the antenna, the bristles cells, but also in other external structures such as the maxillary palpus, the labellum, the legs and

the wings. Thus, this screen is a useful tool to isolate genes expressed in many adult structures.

### 2.3. What are the genes leading to the eGFP/lacZ patterns?

Although a complete description of the genes targeted by the P-element is out of the scope of this article, we describe a few examples of genes that have been previously characterized. Using the plasmid rescue technique, three lines were found to be insertions in the promoter sequences of the *spalt major (salm)* (552 bp upstream of the start codon) (Reuter et al., 1989; Kuhnlein et al., 1994), *m-spondin (mspo)* (179 bp upstream of the transcription start) (Umekiya et al., 1997) and *furrowed (fw)* (7296 bp upstream of the start codon) (Leshko-Lindsay and Corces, 1997) genes. These genes encode molecules that had not been reported to be expressed in the adult eye. These three examples clearly show the ability of our screen to target genes expressed in the adult *Drosophila* eye.

The *salm* line exhibited an eGFP signal coming from the inner PR cells (Fig. 4a) and a X-Gal staining in R7 and R8 cells with the corresponding projection to the medulla (Fig. 4b). An additional row of cells at the top of the retina was stained corresponding to primary pigment cell staining. Antibody staining performed against the Salm protein (Kuhnlein et al., 1994) revealed R7 and R8 cells staining (Fig. 4c), thus confirming that *salm* and *GAL4* expression are under the control of similar enhancers. The absence of the primary pigment cell staining with the antibody against Salm suggests that the P-element line may be under the control of an additional enhancer. The gene *salm* encodes

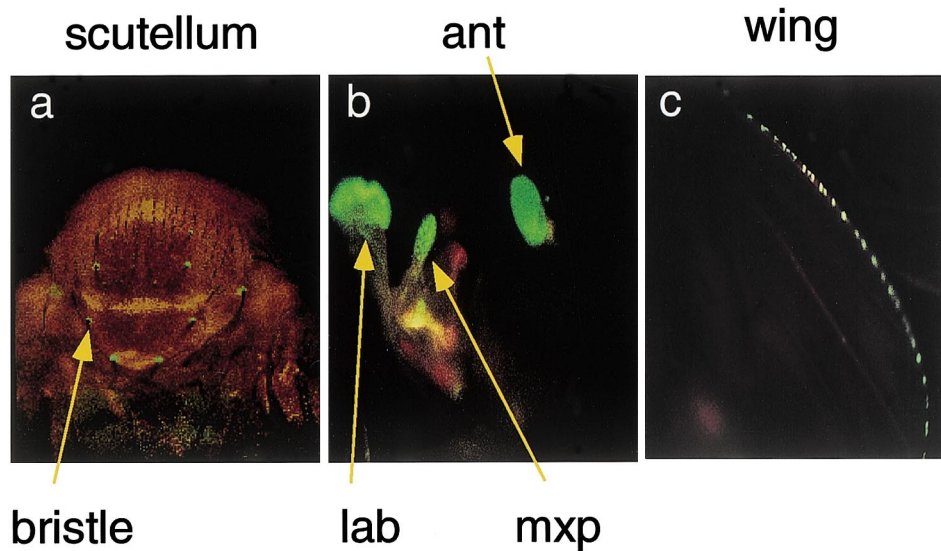


Fig. 5. Enhancer trap line with specific eGFP expression in the body. The PAMLBr5.21 line gives eGFP expression in different external structures: (a) base of bristles. (b) Olfactory organs; (c) wing margin; lab, labellum; mxp, maxillary palpus; ant, antenna.

a transcription factor with double zinc-finger domains (for review see Burke and Basler, 1997). In the embryo, *salm* promotes the specification of terminal pattern elements as opposed to trunk (Kuhnlein et al., 1994). In the wing imaginal disc, it activates the transcription of the *dpp* targets (Lecuit et al., 1996; Sturtevant et al., 1997). Interestingly in the eye imaginal disc, *salm* has been shown to be expressed in R3 and R4 and in the cone cells (Barrio et al., 1999; Fanto and Mlodzik, 1999) but no function has yet been reported in the eye. Indeed no visible phenotype was observed in homozygous clones for *salm* null mutant in the eye (Marek Mlodzik, personal communication).

The *mspo* insertion exhibited strong eGFP expression in the inner PR cells (Fig. 4d) while X-Gal revealed staining in R7 cells, in lamina, and in medulla (Fig. 4e). The presence of stained R7 cells was confirmed by double staining of eye sections with X-Gal and Hoechst (bis benzimide) (not shown). The Hoechst dye, which labels all nuclei, allows us to distinguish the R7 nuclei, which are located beneath the R1–6 nuclei layer in the apical part of the retina. Antibody staining performed against M-spondin (antibodies are a gift of A. Nose) revealed strong lamina staining as was also observed with the enhancer trap line (Fig. 4e–f). Although the Fig. 4f does not show R7 staining due to a low affinity of the antibody, we could detect the R7 cells by performing longer incubations (not shown). *Mspo* is a component of the extracellular matrix, reported to be localized to the muscle attachment sites in the *Drosophila* embryo (Umekiya et al., 1997). *Mspo* is highly homologous to vertebrate Mindin (Higashijima et al., 1997), a secreted protein that has been shown to promote neurite outgrowth (Feinstein et al., 1999). The PR and lamina expression observed in the *mspo* insertion line is reminiscent of the neural vertebrate homologue *mindin*, which is also expressed in neurons, suggesting a possible role of *mspo*

in PR's outgrowth. However, we analyzed the morphology of the adult PR projections in *mspo* null mutant flies (gift of A. Nose) using the 24B10 anti neuronal antibody, but no defect was observed (not shown).

In the *fw* insertion line, we found a strong eGFP spot in the center of the eye (Fig. 4g). The corresponding *lacZ* staining gave a strong signal at the top of the retina, characteristic of primary pigment cells (Fig. 5h). The pattern observed was confirmed by antibody staining against Fw (gift of V. Corces) (Fig. 5i). Fw is an adhesion molecule highly homologous to vertebrate selectins, which allow carbohydrate-protein interactions. Strong mutant alleles of *fw* show defects in the development of the eye and mechanosensory bristles. The adult eye morphology is strongly affected and exhibits deep furrows or crevices suggestive of an effect on the structural integrity of the retina (Leshko-Lindsay and Corces, 1997). In addition, ommatidia have an altered morphology and often contain an abnormal number of cells (Leshko-Lindsay and Corces, 1997). The role of *fw* adult expression in primary pigment cells still remains to be investigated.

### 3. Discussion

#### 3.1. eGFP expression patterns

We used the deep pseudopupil image as a useful method allowing visualization of eGFP expression in PR cells. The interest of the deep pseudopupil image, which represents the average of 20–30 ommatidia, is that it allows a quick and reliable assessment of eGFP expression in the PR cells of living adult *Drosophila* without performing any histological analysis. By adapting the enhancer-trap method using eGFP as a vital marker, we performed an F1 screen to analyze a

large number of flies. We generated and analyzed 2730 lines exhibiting new eGFP patterns. This represents an underestimation of the rate of new insertions since it only accounts for patterns that can be detected in the adult. All these strains show a great variety of eGFP expression patterns, reflecting the influence of many different genomic regulatory elements on the *GAL4* reporter gene.

A number of previous screens have shown that enhancer detectors can be used to identify genes on the basis of their expression patterns (Bellen et al., 1989; Torok et al., 1993; Calleja et al., 1996; Timmons et al., 1997). eGFP detection in the deep pseudopupil is a highly reliable way to isolate specific lines with a PR cell expression. We were even able to isolate a specific R8 expressing line with a eGFP signal emanating from the basal part of the retina (Fig. 3a), in addition to several lines expressing in the lamina only and two lines from the medulla only (not shown).

We found several lines specifically expressing *GAL4* in R7 and/or R8 (Table 2). These lines are of great interest because they might have targeted *rh* regulators specific to R7 and/or R8 PR's. Except for the PTA line, none of these lines gave a specific expression in a subset of R7 or R8 cells, like the *rh* genes themselves, suggesting that we did not yet pick up genes which might be involved in the specific regulation of *rh* in *y* or *p* subsets of R7 or R8.

Surprisingly we found six independent lines expressed solely in outer PR's (Table 2). These are large PR's that all express *rh1*, and whose rhabdomeres span the complete retina and project to the lamina. Genes specifically expressed in these cells could be involved in *rh1* regulation, in the projection to the lamina or could be some specific architectural components. By contrast, we isolated only three lines expressing *GAL4* in all PR's (Table 2), although we would have expected to find many genes expressed in all PR's, e.g. involved in the phototransduction cascade (for review see Zuker, 1996; Montell, 1999). Because many genes are involved in this specific cascade, this indicates that we did not yet reach saturation in our screen.

Another *GAL4* enhancer trap screen in living flies performed by Calleja et al. (1996) has already been very successful. Using the *yellow* cuticle pigmentation marker, the authors isolated genes specifically expressed in third instar larvae and the adult body. Whereas the *yellow* marker is restricted to the cuticle, the eGFP fluorescent emission is able to cross through the cuticle or the cornea and allows its detection in internal structures. Indeed, in addition to the selected PR lines, we have isolated several lines showing specific body patterns (e.g. see Fig. 5). This demonstrates that the eGFP enhancer trap screen can be used to efficiently screen for genes expressed in other adult structures.

### 3.2. P-*GAL4* lines

The advantage of the *GAL4* P-element lines compared to classical *lacZ* lines, is that they can be used for misexpression experiments. An interesting misexpression experiment

has been performed in *Drosophila* outer PR's by expressing the diphtheria toxin (DT) under the control of the *rh1* promoter (Bellen et al., 1992). The authors obtained an efficient R1–6 cell killing without altering R7 and R8 shapes, as assessed by electron microscopy. In a similar manner, the specific killing of R7 or R8 cells could be an interesting tool for the understanding of the communication between those cells.

We found three known genes *salm*, *mspo* and *fw* to be expressed in the adult eye.

*salm* has a very dynamic expression pattern in several organs and has been implicated in multiple developmental processes (Kuhnlein et al., 1994; Barrio et al., 1999). Interestingly it has been shown to be expressed in R3, R4 and cone cells in the eye imaginal disc (Barrio et al., 1999; Fanto and Mlodzik, 1999). We found it expressed in R7 and R8 PR's in the adult eye, suggesting multiple roles at different stages of eye development. However, no morphological defects were observed in eye mosaic clones for *salm* (Marek Mlodzik, personal communication) suggesting a very subtle phenotype or possible redundancy with the related gene *salr*. Indeed *salm* and *salr* have very similar expression patterns due to the organization of their regulatory region in a common DNA fragment located 3' of both transcription units (Barrio et al., 1999). The detection of a primary pigment cell staining by X-Gal in the enhancer trap line R7T3.8 but not in the *salm* antibody staining (Fig. 4b,c), suggests that *GAL4* may be under the control of *salr* enhancer.

As a transcription factor expressed in R7 and R8, *Salm* could play a role in the transcription of *rh3-6* in R7 and R8; alternatively *Salm* could mediate repression of *rh1* in inner PR cells. We are currently testing this hypothesis in *salm* mosaic clones generated in the eye.

The *mspo* insertion line shows X-Gal staining in R7 cells, together with strong lamina and medulla staining (Fig. 4e). Umemiya et al. observed no phenotype at the embryo muscle attachment sites in *mspo* null mutants (Umemiya et al., 1997). Similarly we did not find any morphological defects in the adult eye of the *mspo* null mutant. This could be due to a possible redundancy between *Mspo* and two other related genes found in *Drosophila* (A. Nose, unpublished).

*fw* mutants have been reported to exhibit a strong morphological phenotype in the adult eye as well as defects at the level of the mechanosensory bristles (Leshko-Lindsay and Corces, 1997). The authors offered a possible explanation to the eye phenotype by showing *fw* expression in the eye imaginal disc, suggesting a role of *fw* in early eye development. However, it is possible that the mutant phenotype is due to the loss of the strong adult expression that we observed in the primary pigment cells (Fig. 5i).

Taken together, the results of our screen have demonstrated the ability to target different PR cell subtypes, as well as known genes expressed in the adult eye. Further analysis should help to improve our understanding of *opsins* regulation and the phototransduction cascade.



## 4. Experimental procedures

### 4.1. GAL UAS fusion genes

We used the pUAST vector from Brand and Perrimon (Brand and Perrimon, 1993) to subclone eGFP (from Clontech (Cormack et al., 1996)) behind the GAL4 UAS sequence.

UAS-eGFP: the mut-1 modified eGFP (substitutions F64L and S65T) (Cormack et al., 1996), was removed from a pBlueScript-eGFP vector by digestion with *Bam*HI and *Xba*I and was subcloned into the *Bgl*III and *Xba*I sites of pUAST.

Brand and Perrimon kindly provided UAS-*lacZ* lines on three chromosomes (Brand and Perrimon, 1993).

*rh1-GAL4* (3 kb promoter) and *rh4-GAL4* (480 bp promoter) lines on the three chromosomes were kindly provided by J. Treisman.

The *rh5-GAL4* construct was obtained through a tripartite ligation between an *Eco*RI/*Bam*HI *rh5* promoter fragment (−680, +49), a *Bam*HI/*Not*I *GAL4* gene fragment (*pGaTB* vector from A. Brand and N. Perrimon), and *Eco*RI/*Not*I *pCaSpEr* (*CHABΔXba* vector (Wimmer et al., 1997)), transgenic lines were obtained through standard procedures (Papatsenko et al., 1997).

### 4.2. Enhancer detection screen

We generated new insertions by mobilizing a single X-linked enhancer detection GAL4 vector, *pGawB* (gift from Brand and Perrimon (Brand and Perrimon, 1993)). The original insertion line we used is homozygous lethal and is carried over an *FM7* balancer chromosome (*GAL4-lethal/FM7*). The P-element transposon was mobilized using a transposase source carried on the *CyO* balancer chromosome by a hobo element (*CyOHO2*, gift from W. Gelbart). Insertions segregating with the X chromosome were detected by examining the segregation of the eGFP fluorescence in males and females. Autosomal insertions were mapped by standard genetic methods using *w*−/*y*; UAS-eGFP/*CyO*; UAS-eGFP/MKRS and *w*−/*w*−; UAS-eGFP/*CyO*; UAS-eGFP/TM2 flies. Each of the lines showing a eGFP PR cell pattern was crossed to a line carrying the UAS-*lacZ* reporter.

### 4.3. eGFP detection through deep pseudopupil and cornea neutralization

Flies were screened under a fluorescent dissecting microscope using a GFP cube filter (Kramer Scientific Corporation). Further characterization was performed by using the cornea neutralization technique (Franceschini et al., 1981), adapted to eGFP visualization in living flies by F. Pichaud (FP and CD, in preparation).

### 4.4. X-Gal and antibody stainings

Horizontal eye sections were performed using a cryostat microtome (Zeiss) and deposited on superfrost Plus slides (Fisher). Slides were fixed 5–10 min in PBS 0.25% glutaraldehyde. They were stained in a solution of 7.2 mM Na<sub>2</sub>HPO<sub>4</sub>, 2.8 mM NaH<sub>2</sub>PO<sub>4</sub>, 150 mM NaCl, 1 mM MgCl<sub>2</sub>, 3 mM K<sub>3</sub>[Fe(CN)<sub>6</sub>], 3 mM K<sub>4</sub>[Fe(CN)<sub>6</sub>], containing a 1/30 dilution of X-Gal (30 mg/ml in dimethyl formamide). After washing in PBS, slides were mounted in aquamount (Lerner Laboratories, Fisher).

Rat serum against Mspo (Umekiya et al., 1997) and rabbit serum against Fw (Leshko-Lindsay and Corces, 1997) were kindly provided by A. Nose and V. Corces, respectively. Anti-Mspo (diluted at 1/300 in BBT: PPS 1×, 0.1% BSA, 0.1% Tween 20) or anti-FW (diluted at 1/200 in BBT) antibodies were incubated overnight at 4°C on horizontal eye section, and revealed using a Horse Radish Peroxidase kit (Renaissance kit, NEN).

Rabbit antibody against Salm (Kuhnlein et al., 1994) (diluted at 1/25) was incubated overnight at 4°C on cryostat eye section. After washes, a secondary Goat anti-rabbit antibody coupled to the alkaline phosphatase enzyme (Jackson) (diluted at 1/500) was incubated for two hours at room temperature. The reaction was revealed using NBT and BCIP reagents (Gibco BRL).

### 4.5. Plasmid rescue

Plasmid rescue analysis were performed according to (Ashburner, 1989). Ligated plasmids were then transformed in Epicurean XL10-Gold™ ultracompetent cells (Stratagene).

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