***pipsqueak* encodes a novel nuclear protein required downstream of *seven-up* for the development of photoreceptors R3 and R4**

Ursula Weber¹, Vivian Siegel^{2,3} and Marek Mlodzik^{1,4}

¹Differentiation Programme, EMBL, Meyerhofstrasse 1, D-69117 Heidelberg, Germany and ²HHMI and Department of Physiology, University of California, San Francisco, CA 94143-0724, USA

³Present address: Cell, 50 Church Street, Cambridge, MA 02138, USA

⁴Corresponding author

Photoreceptor induction in the *Drosophila* eye is mediated by activation of the Ras signal transduction cascade. Although this process is well understood, little is known about how the diversity of photoreceptor subtypes is generated. The *pipsqueak* (*psq*) gene is expressed at high levels in the R3/R4 precursors during eye development and this expression depends on *seven-up* (*svp*) gene function. Moreover, strong *psq* alleles are dominant suppressors of a *svp*-induced cone cell transformation phenotype. Although the gene was previously identified and described as a member of the maternal posterior group of genes, the strong semilethal alleles isolated here demonstrate a specific requirement for *psq* function downstream of *svp* for the development of photoreceptors R3/R4. The gene has three independent 5' ends and codes for several nuclear protein isoforms, some containing the POZ domain which has been implicated in protein–protein interactions. Interestingly, all viable alleles with a maternal posterior group phenotype cluster around one specific 5' exon, while all semilethal alleles have lesions which map to a different alternative 5' exon.

Keywords: *Drosophila*/photoreceptor specification/POZ domain/*seven-up*

Introduction

The development of the *Drosophila* eye recently has been the focus of many studies addressing the determination of specific cell fates in a field of uncommitted precursor cells (Tomlinson, 1988; Ready, 1989; Wolff and Ready, 1993). Activation of the proto-oncogene *ras* is the crucial molecular switch that induces precursor cells to adopt a neuronal photoreceptor (R-cell) fate (reviewed in Dickson and Hafen, 1993; Zipursky and Rubin, 1994). A ligand–receptor interaction activates Ras and its downstream kinase cascade that leads to the phosphorylation of a set of nuclear targets (transcription factors), which are required for R7 development (Bohmann *et al.*, 1994; Brunner *et al.*, 1994; O'Neill *et al.*, 1994). Although this process is best understood for the R7 cell, the Ras signal transduction cascade and its nuclear targets are also required for most (if not all) other photoreceptors (Simon *et al.*, 1991).

The determination of the outer photoreceptors (R1–6) is less well understood, and it is not clear how the

neuronal diversity is generated upon activation of the same molecular switch. Only a few genes are known to be required to specify a subset of outer photoreceptors: *rough* is required for the development of photoreceptors R2 and R5 and encodes a homeodomain protein (Tomlinson *et al.*, 1988), and *seven-up* (*svp*) is required for R1/6 and R3/4 and is an orphan member of the steroid receptor superfamily (Mlodzik *et al.*, 1990). In both cases, mutations in the genes cause a transformation of the respective outer photoreceptors to a different photoreceptor subtype: R2/5 develop as R3/4 in *rough*[−] (Heberlein *et al.*, 1991) and R3/4 and R1/6 are transformed to R7 in *svp*[−] clones. It is not known what mechanism causes the restriction of expression of these genes to specific precursor cells in which their function is required. Nevertheless, this feature is critical to allow normal ommatidial development, since ectopic expression in other precursors causes their transformation to different cell types (Basler *et al.*, 1990; Kimmel *et al.*, 1990; Hiromi *et al.*, 1993). The *Bar* gene has been shown to be required in R1/6, but its function is less well defined (Higashijima *et al.*, 1992). The target genes of these transcription factors are as yet unknown. Other genes implicated in outer photoreceptor development appear less specific in their respective function(s), e.g. *glass* and *tram-track* (*ttk*), both zinc finger-containing transcription factors, are required for all R-cells (Moses *et al.*, 1989; Xiong and Montell, 1993). Interestingly, a hypomorphic *ttk* allele appears to affect only a subset of photoreceptor cells and can sometimes cause an outer photoreceptor to undergo transformation to R7.

Using the enhancer trap technique and screening for specific expression patterns in subsets of cells in ommatidial preclusters, it is possible to isolate lethal genes that are required during ommatidial assembly in specific subsets of photoreceptor precursors (Mlodzik *et al.*, 1990). We have used this approach to identify new genes required for outer photoreceptor development. Here, we show that a mutagenic enhancer detector insertion, specifically expressed in photoreceptor precursors R3 and R4, is allelic to the maternal effect posterior group gene *pipsqueak* (*psq*) (Siegel *et al.*, 1993). We find a new class of *psq* alleles that are semilethal and affect the development of R3 and R4 in the eye and also other adult structures, e.g. wings and legs. Molecular analysis and expression reveal that the gene encodes nuclear proteins containing a POZ domain (Bardwell and Treisman, 1994), which is thought to mediate protein–protein interactions and is also found in *ttk* and other transcription factors required during imaginal disc development.

Results

F112 is expressed in R3/R4 during eye development and is allelic to pipsqueak

The F112 P-element (Mlodzik and Hiromi, 1992) insertion was recovered in an enhancer trap screen for lines with

Fig. 1. F112 *lacZ* expression in imaginal discs. (A–C) Whole eye-antenna, leg and wing disc, respectively, stained for LacZ activity. Note the high level patterned expression in all discs: in eye disc posterior to the morphogenetic furrow (MF, marked by an arrowhead), in leg and antennal discs in the anlagen of distal structures and in wing discs over the wing blade area. Low level expression is uniform as visible in overstained discs (not shown). (D) F112 eye disc stained with anti- β -gal antibody. Expression is elevated in R3/R4 precursors (marked with white numbers in a representative precluster). (E) F112 expression (blue) in an eye disc with the *svp*⁻ clone double stained for *Bar* (brown, expressed in R1/R6). High F112 staining is absent in *svp*⁻ clones (marked by lack of *Bar* staining, indicated by arrow). R3/R4 and R1/R6 are marked with white numbers in a representative cluster. (F) F112 expression in a *ro*⁻ eye disc. Note that 3–4 cells express high levels of LacZ in *ro*⁻ preclusters, reflecting the presence of multiple R3/R4 cells. MF is indicated by an arrowhead, anterior is left.

specific *lacZ* expression in developing ommatidial preclusters in eye imaginal discs. F112 is a semilethal, female-sterile insertion at 47B1-4. All phenotypic aspects (see below) are revertible by precise P-element excision (not shown). The female-sterile *psq* gene has been mapped to the same region (Siegel *et al.*, 1993). Complementation analysis of F112 and different *psq* alleles revealed that F112 is a new allele of *pipsqueak*, and thus it will be referred to as *psq*^{F112}.

In 3rd instar larval eye discs, *lacZ* is expressed in *psq*^{F112} at high levels posterior to the morphogenetic furrow (MF) in a subset of photoreceptor precursors (Figure 1A and D), and in other imaginal discs in different patterns (Figure 1A–C). In the antennal and leg discs, highest levels of expression are found in the part of the disc that will give rise to distal structures, and in the wing disc a cross-like pattern is detected over the wing pouch primordia, which is in turn surrounded by higher expression.

To analyze the eye disc expression in detail, third instar discs were stained with an anti- β -galactosidase antibody. Posterior to the MF, ommatidial assembly occurs via a series of inductive events in a well-defined sequence whereby committed cells instruct their undetermined neighbors to adopt specific cell fates (Tomlinson and Ready, 1987; Dickson and Hafen, 1993; Wolff and Ready, 1993). Due to the stereotypic arrangement, all precursor

cells can be identified in developing ommatidial clusters. During this process, *psq*^{F112} is expressed at high levels in photoreceptor precursors R3 and R4 starting in column 3/4 behind the MF throughout the posterior part of the disc (Figure 1D). In strongly stained discs, expression can also be detected at low levels in (all) other cells behind the MF (see also Figure 7).

In *svp*⁻ mutant clones, R3/R4 do not develop as outer photoreceptors but are instead transformed to an R7 cell fate (Mlodzik *et al.*, 1990) and high level *psq*^{F112} expression is not detected (Figure 1E). In the *rough* mutant where R2/R5 are transformed towards the R3/R4 fate in a *svp*-dependent manner (Heberlein *et al.*, 1991), there are often three or four cells of the R3/R4 subtype in each ommatidium, and *psq*^{F112} is expressed in the R2/R5 precursors in addition to its expression in R3/R4, reflecting the transformation to this cell type (Figure 1F). Moreover, *svp* expression in R3/R4 (Mlodzik *et al.*, 1990) precedes *psq*^{F112} by one to two columns. Taken together, these data indicate that high level expression of *psq* is *svp* dependent and thus *psq* might be acting downstream of *svp* for the development of R3/R4 (see below).

Photoreceptors R3 and R4 are affected in *psq* mutants

Homozygous *psq*^{F112} escapers display a variety of adult phenotypes (see below). We have generated additional

alleles by imprecise excision of F112, causing genomic deletions around the site of insertion, and analyzed the phenotypes of *psq*^{F112} and the excision alleles over deficiencies removing the entire locus (for list of alleles and molecular characterization see Table I and below). Transheterozygous escapers (1–5%) of *psq*^{F112} or the excision alleles over the deficiencies Df(2R)17 and Df(2R)27 show similar but stronger phenotypic features as compared with homozygous *psq*^{F112} or excision flies (Figure 2). The eyes of transheterozygous flies are often rough, especially in the posterior part. In such eyes many ommatidia are missing one or two outer photoreceptors (Figure 2A and B). Homozygous mutant escapers as well as homozygous mutant clones of these alleles have the same eye phenotype but with lower penetrance (compare *psq*^{F112} eye clone Figure 2C). To address the identity of the missing outer photoreceptors, we have used a *svp* enhancer trap line, H162, which is expressed in photoreceptors R3 and R4 in adult retinal preparations (Figure 2D; Mlodzik *et al.*, 1990; Y.Hiromi and M.Mlodzik, unpublished). In eyes homozygous for any of these alleles or transheterozygous over the deficiencies, the R3/R4-specific staining is reduced. Often there is only one or sometimes no cell expressing the marker in a single ommatidium (marked by arrowheads and arrows respectively in Figure 2E), indicating that R3 and R4 photoreceptors are missing in such mutant ommatidia. The frequency of affected ommatidia is comparable with the defects seen in sections of eyes of the same genotypes, suggesting that the missing photoreceptors are indeed of R3/R4 identity. Thus, R3/R4 photoreceptors express high levels of *lacZ* and are phenotypically affected in *psq*^{F112} and related alleles. Besides the R3/R4 phenotype, ommatidial orientation can also be affected in these mutants (Figure 2B and E and see Discussion).

To isolate stronger *psq* alleles, we have screened a collection of ethylmethane sulfonate (EMS)-induced mutants from a saturation mutagenesis for lethals over the deficiencies Df(2R)17 and 27 (R.Burgess and T.Schwarz, unpublished). One allele was isolated that failed to complement *psq*^{E39} and *psq*^{F112}, henceforth referred to as *psq*^{D91}. Phenotypic analysis of *psq*^{D91} over the above deficiencies indicates that it is comparable in allelic strength with the other semilethal alleles (e.g. *psq*^{E39}). The same frequency of transheterozygous escapers over Df(2R)17 or 27 is found as with the above alleles and they show similar phenotypic features (Table I).

***psq* alleles are dominant suppressors of *svp*-mediated cone cell transformation**

Expression of either isoform of *Svp* in the cone cells and R7 (*sev-svp*; Hiromi *et al.*, 1993; Begemann *et al.*, 1995) transforms these cells to either R7 or outer photoreceptors, depending on the dosage of the transgene. Due to this dosage sensitivity, modifications of this phenotype can be used as an assay to genetically identify other genes required in the same context (Begemann *et al.*, 1995). Since in *psq*^{F112}, *lacZ* reporter expression in R3/R4 depends on *svp* function (Figure 1E), we tested if ectopic *Svp* expression-mediated cone cell transformation is dependent on *psq* function. Stronger *psq* alleles and Df(2R)17 or 27 are indeed dominant suppressors of the *sev-svp*-mediated phenotype (Figure 3; the weak viable hypomorphic alleles

display no genetic interaction with *sev-svp*). The specificity of this genetic interaction is demonstrated by the failure of the stronger *psq* alleles to suppress other dosage-sensitive phenotypes that also cause a cone cell to R7 transformation, such as activated components of the *Sev*/Ras pathway [e.g. *sev*^{S11} (Basler *et al.*, 1991) and *sE-raf*^{forY9} (Dickson *et al.*, 1992) see Discussion].

Ovaries and other adult structures are affected in *psq* mutants

Besides the eye (see above) and the ovaries (Siegel *et al.*, 1993) (Table I), other adult structures are malformed in homozygous or transheterozygous *psq* escaper flies. Macrochaetae on the dorsal head capsule and thorax are often kinked, branched or missing. Furthermore, the wing often shows notching along the wing margin and/or vein thickening, and is reduced in size (Table I). Occasionally, the legs of escaper flies are shorter and malformed (not shown).

The maternal phenotypes of *psq* appear to affect several features of oogenesis and embryonic development (Siegel *et al.*, 1993). The weakest *psq* alleles disturb Vasa localization to the posterior pole of developing oocytes and cause a 'grandchildless' posterior group phenotype with missing pole cells in developing embryos and the deletion of from a few up to all abdominal segments. Stronger alleles result in earlier blocks in oogenesis and oocytes rarely enter vitellogenesis. *In trans* to deficiencies, *psq* causes a rudimentary ovary phenotype, suggesting that all previously described *psq* alleles are hypomorphs (Siegel *et al.*, 1993). The female-sterile phenotype of flies homozygous for *psq*^{F112} or the related alleles (or transheterozygous combinations over the deficiencies) appear similar to or even stronger than the strongest *psq* alleles previously reported: such females lay very few eggs and/or have rudimentary ovaries (Table I and not shown).

Transheterozygous combinations of the new *psq* alleles with the previously reported alleles (e.g. *psq*^{P1}, *psq*^{P4}, *psq*^{X1-30}; Siegel *et al.*, 1993) are viable without visible zygotic phenotypes. They are, nevertheless, female-sterile with the typical maternal and oogenesis phenotypes. Furthermore, transheterozygous combinations of most *psq* alleles with Df(2R)12 are viable without zygotic phenotypes, but such flies are again female-sterile, showing the maternal and oogenesis phenotypes. Thus, Df(2R)12 does not complement *psq*, but behaves rather like a weak hypomorph within our allelic series. The molecular analysis of Df(2R)12 supports this conclusion (Table I and Figure 4).

Taken together, while the previously reported female-sterile or grandchildless *psq* alleles affect only the ovaries even as transheterozygotes with stronger alleles or deficiencies, escaper animals of the strong *psq* alleles affect R3/R4 during eye development and also other adult structures (see Discussion).

***psq* has a large and complex transcription unit**

The locus was cloned by virtue of the rescued plasmid from the P-element in *psq*^{F112}. Overlapping genomic fragments flanking the F112 insertion point were used as probes to isolate cDNAs from imaginal disc libraries. The gene spans >100 kb and consists of 11 exons with three alternative 5' exons (Figure 4). The three respective

Table I. *psq* alleles and phenotypes

Table 1. <i>pipsqueak</i> alleles and phenotypes							
<i>psq</i> allele ^a	Viability	Adult phenotypes		Molecular features			
		Eye	Wing				
		Eye	Wing	Leg	Macrochaete on dors. head and thorax	Ovary or maternal phenotype	Molecular features
2905 ^b	Viable	wt	wt	wt	wt	Grandchildless	P-insertion close to exon 1A
6372 ^b	Viable	wt	wt	wt	wt	Grandchildless	Same as 2905
<i>P1</i>	Viable	wt	wt	wt	wt	Grandchildless, <i>vas</i> RNA reduced ^d	P-insertion ^d at exon 1A
<i>P4</i>	Viable	wt	n.d.	n.d.	n.d.	Same as <i>P1</i> ^d	Same as <i>P1</i> ^d
<i>X1–30</i>	Viable	wt	n.d.	n.d.	n.d.	<i>vas</i> RNA more red. than <i>P1</i> ^d	Imprecise excision ^d of <i>psq</i> ^{<i>P1</i>}
<i>13B6</i>	Semilethal	wt	wt	wt	wt	<i>vas</i> undetectable, rudiment. ovary ^d	P-insertion close to 5' end of exon 1B
<i>F112</i>	Semilethal	R3/R4 often missing, irregular omm. array	Shorter, vein thickening/notching along wing margin	Distorted	Kinked, branched or missing	Rudimentary ovaries	P-insertion 224 bp downstream of exon 1B
<i>E34</i>	Semilethal	Same as <i>F112</i>	Same as <i>F112</i>	Same as <i>F112</i>	Same as <i>F112</i>	Same as <i>F112</i>	Excision of <i>psq</i> ^{<i>F112</i>} , 4 kb downstream deletion
<i>E39</i>	Semilethal	Same as <i>F112</i> , increased penetrance	As above, notching almost as severe as <i>ap</i> ^{<i>Xa</i>} (in <i>D91/E39</i>)	Same as <i>F112</i>	Same as <i>F112</i>	Same as <i>F112</i>	Excision of <i>psq</i> ^{<i>F112</i>} , 5 kb deletion removing exon 1B
<i>D91</i> ^c	Semilethal	Same as <i>E39</i>	Same as <i>E39</i>	Same as <i>F112</i>	Same as <i>F112</i>	n.d.	EMS induced ^c
Df(2R)12 ^c	Lethal	n.a.	n.a.	n.a.	n.a.	n.a.	X-ray induced ^c 46E/F–47A/B, deletes exon 1A
Df(2R)17 ^c	Lethal	n.a.	n.a.	n.a.	n.a.	n.a.	X-ray induced ^c 47A–47D, deletes entire locus
Df(2R)27 ^c	Lethal	n.a.	n.a.	n.a.	n.a.	n.a.	Similar to Df(2R)17

The viability of *psq* alleles was tested over the deficiencies Df(2R)17 and 27. All *psq* alleles are viable over Df(2R)12 causing female sterility and maternal phenotypes. All three deficiencies are homozygous lethal. Molecular analysis of the alleles is shown in Figure 4.

^aPhenotypic description is based on the phenotype of the respective allele over deficiencies Df(2R)17 and 27, unless otherwise indicated.

^bIsolated by M.Erdelyi, A.Guichet and A.Ephrussi (unpublished) in a screen for grandchildless mutations.

^cThe D91 allele generated by R.Burgess and T.Schwartz (unpublished), was identified as a lethal EMS mutation over Df(2R)17 and 27.

^dSee Siegel *et al.* (1993) for details.

Fig. 2. Adult phenotypes of semilethal *psq* alleles. (A–E) Eye phenotypes. (A–C) show sections of *psq*^{F112}/Df(2R)27 (A), *psq*^{E39}/Df(2R)27 (B) and *psq*^{F112} homozygous clone (marked by absence of pigment) in (C). Note that many ommatidia are lacking one or two outer photoreceptors, some are marked by arrowheads in (C). Rarely, R7 can also be missing (see A). (D and E) LacZ activity staining of adult retinas in H162 (enhancer trap insertion in *svp* expressed in R3/R4) of wild-type eye (an example is marked with '3' and '4' accordingly) and *psq*^{E34} eye, respectively. Note in (E) that many ommatidia have only one (examples marked by arrowheads) or sometimes no cell expressing H162 (examples marked by arrows), indicating the absence of R3/R4. Such mutant eyes can also display some orientation defects (compare also with A and B).

Fig. 3. *psq* alleles are dominant suppressors of *sev-svp*. Tangential sections of eyes of the following genotypes are shown. (A) 2×*sev-svp2*/+, (B) *psq*^{D91}/+; 2×*sev-svp2*/+, and (C) *psq*^{E39}/+; 2×*sev-svp2*/+. Note the presence of extra photoreceptors (mostly R7) in many ommatidia in the control (A) and the more wild-type appearance in (B) and (C). The *sev-svp* phenotype is caused by the ectopic expression of the *Svp* isoforms from the *sevenless* enhancer in cone cell precursors. The phenotype is dosage sensitive: increasing copy number causes a more extreme phenotype, which is suppressed by reducing the gene dosage of interacting genes (Hiromi *et al.*, 1993; Begemann *et al.*, 1995). The observed suppression of the D91 and E39 alleles is comparable. The deficiencies Df(2R)17 and 27 removing the entire locus cause a comparable to stronger suppression (not shown).

Fig. 4. Genomic organization of *psq*. Molecular analysis of *psq* alleles is shown in the upper part above the genomic DNA. The positions of the different P-element insertions are indicated by triangles along the genomic DNA. The extents of the excision alleles E34 (~3 kb) and E39 (~5 kb) and the deficiencies Df(2R)12, 17 and 27 are shown by black bars. The centromere is to the left. The bottom part shows the structure of *psq* cDNAs. The gene gives rise to three different transcript classes with alternative 5' ends (to the left) depicted as A, B and C with their unique exons E1A (and E1A'), E1B and E1C. Several independent cDNAs were analyzed for each transcript class. The common exon E3 is split into two exons (E3') by an alternative intron (highlighted by an arrow) spliced out in a 1:2 ratio as judged from the cDNA frequency. Open boxes indicate the extents of cDNAs and black boxes the translated regions in each respective transcript class. In accordance with the A, B and C transcript classes, using a common probe, three major mRNAs of <7, 5.7 and 5 kb are detected on Northern blots.

transcript classes with different 5' ends will be referred to as A, B and C moving from proximal to distal or 5' to 3' along the chromosome. In addition to the differential splicing at the 5' end, there are splice variants within exon 3, where a small intron of 164 bp can be spliced out splitting exon 3 (E3'; arrow in Figure 4). Together these splice variants give rise to at least six different transcripts, which code for three polypeptides with only partially overlapping open reading frames (see below).

The molecular analysis of several *psq* alleles is shown in Figure 4. Interestingly, all alleles which show only the maternal and grandchildless phenotype map to the most 5' of the three alternative 5' exons, 1A (see Table I and Figure 4). However, all the stronger alleles (Table I) cluster around the middle 5' exon, 1B. The alleles *psq*^{E34} and *psq*^{E39} are small deficiencies generated by excising the F112 P-element: they affect or completely delete exon 1B, respectively (Figure 4A, Table I). Cytological analysis of the three non-complementing deficiencies (see above) using exon-specific probes, revealed that while Df(2R)17 and Df(2R)27 delete the entire locus, Df(2R)12 only removes the most 5' of the three alternative 5' exons, 1A. Thus, like the weak hypomorphic alleles (e.g. *psq*^{P1}), which map to this exon, Df(2R)12 deletes only this exon and is viable over stronger *psq* alleles exhibiting only the female-sterile phenotype. Therefore, it apparently does not delete sequences required for the function encoded by transcripts B and C.

***psq* codes for several isoforms and contains a POZ domain**

The three transcripts generated from the different 5' exons have different coding potential (Figure 5). Translation of the open reading frame (ORF) in transcript class A can initiate at an AUG in the alternative 5' exon 1A, giving rise to a polypeptide of 1085 amino acids (ORF A-long). If the alternative intron within exon 3 is not spliced out (arrow in Figure 4), this ORF is truncated after 535 amino

acids (ORF A-short, see Figure 5). Transcript classes B and C do not have an AUG in their respective 5' exons 1B and 1C. Their first AUG is located in the common exon 3 and thus both give rise to a polypeptide of 660 amino acids or 666 residues (if intron 4 is spliced), which is co-linear with the C-terminal half of ORF A-long.

ORFs A-long and A-short (transcript class A) share a homologous domain (underlined in Figure 5) with several *Drosophila* transcription factors [Figure 6; e.g. BR-C (DiBello *et al.*, 1991), Tramtrack (Ttk; Harrison and Travers, 1990)] and a group of poxvirus proteins (Koonin *et al.*, 1992). In all these proteins, this domain of ~110 amino acids is located close to the N-terminus. Bardwell and Treisman (1994) have named the domain POZ domain (also known as Ttk domain or BTB domain; Godt *et al.*, 1993), for its presence in poxviral and zinc finger proteins, and have shown that it mediates protein-protein interactions. All previously reported proteins sharing this domain (except for the poxviral group) are transcription factors containing zinc fingers (reviewed in Bardwell and Treisman, 1994). In *Psq*, however, no classic zinc finger is present. Only in ORF A-short, the truncated ORF from transcript class A, there is a CX₂CX₇HX₄H sequence in its specific C-terminus that could form a novel kind of zinc finger. Nevertheless, this sequence is not translated in ORFs from any other transcript class, A-long and B/C (see also Discussion).

The polypeptides of either class contain several scattered OPA repeats (Wharton *et al.*, 1985) throughout their ORFs (Figure 5). In addition, close to the C-terminus of ORF A-long (or B/C), there is a sequence present that is repeated four times (underlined in Figure 5). These stretches of 46 amino acids are up to 46% identical to each other (Figure 6B). A homologous sequence is found in another *Drosophila* gene, *tkrp* (Haller *et al.*, 1987). However, the biochemical function of these repeats is not known.

Mutations affecting different alternative 5' exons display specific phenotypes

Molecular analysis of *psq* alleles (Figure 4) allows their classification into at least two groups. The first group affects only exon 1A, which is the exon encoding the POZ domain. Interestingly, mutations of this class, including deficiency Df(2R)12 (that completely deletes exon 1A) over any *psq* allele, are homozygous viable and display only the maternal *psq* phenotype or are grandchildless (Table I). All alleles that are semilethal (and show the eye phenotype, see Figure 2) have lesions in exon 1B and thus affect isoforms that do not contain the POZ domain. These allele-specific features are further supported by the genetic interactions observed with *sev-svp* (Figure 3). Df(2R)12 that completely deletes exon 1A does not suppress the 2×*sev-svp*2 phenotype. Only alleles affecting exon 1B (or deletions of the entire locus) act as dominant suppressors of the 2×*sev-svp*2 genotype. Taken together, these data suggest that transcripts encoding isoforms containing the POZ domain are required mostly during oogenesis, but show only a weak requirement during eye development (see also Discussion).

***psq* is widely expressed and encodes nuclear proteins**

The expression of *psq* was analyzed throughout embryogenesis and in larval structures. In the embryo, *psq*

Fig. 6. Sequence alignment of Psq with other related proteins. (A) Psq homology to other *Drosophila* genes in the POZ domain. Alignment of Psq with *bric-a-brac* (Bab; Godt *et al.*, 1993), BR-C (DiBello *et al.*, 1991), Ttk (Harrison and Travers, 1990) and Lola (Giniger *et al.*, 1994) is shown. Amino acid numbers of the respective proteins are given on the right side. Within this domain, Psq shares up to 56% identity with the proteins listed. For a complete list of proteins containing this domain see Bardwell and Treisman (1994). (B) Alignment of the four repeats, sharing up to 52% identity, located between amino acids 722 and 926 in ORF A-long and the related sequence in Tkrp (Haller *et al.*, 1987). In both panels, the identical amino acids are boxed in black, similar ones in gray. A minimum of three identical or similar amino acids between all proteins is required for marking. The following amino acids were considered similar: E, D; V, L, I and M; A, G; F, Y and W; S, T; Q, N; K, R.

expressed from this stage on throughout embryonic and larval development (Figure 7 and not shown). Besides a low level uniform expression, high levels of *psq* transcripts are found in the dorso-anterior domain and dorsally in what now can be identified as the amnioserosa (persisting through stages 7/8 as shown in Figure 7C and D; detected with the intronic probe). In later embryogenesis, *psq* mRNA expression is detected in the mandibular, maxillary and labial lobes (not shown).

To confirm the RNA expression patterns and to analyze subcellular protein distribution, antibodies against a peptide corresponding to parts of ORF A-long (including sequences in common with ORFs B/C) were raised (see Materials and methods). This antiserum (see Figure 7F for specificity control) recognizes nuclear proteins and stains nuclei in all tissues analyzed, consistent with the *in situ* hybridization analysis (an example is shown for the eye-antennal disc, Figure 7E, G and H). In eye discs, Psq protein is detected in nuclei of ommatidial precluster cells, with highest levels in R3/R4 (Figure 7H) and, in wing discs, a cross-like pattern in the wing blade primordium can be seen above the uniform low level expression, similar to *lacZ* expression in *psq^{F112}* (Figure 1 and not shown). In summary, the areas of high expression in imaginal discs appear to overlap with high *lacZ* expression in the enhancer detector P-element insertions F112 and 13B6 (Figure 1 and not shown). Since all non-poxviral POZ domain-containing proteins are either *bona fide* transcription factors or at least nuclear proteins (Bardwell and Treisman, 1994), the nuclear localization of Psq is consistent with its homology to this protein family.

Discussion

We have shown that *psq* is required for the development of R3 and R4 in the compound eye and also other adult structures. It codes for nuclear proteins, some of which contain a POZ domain (Bardwell and Treisman, 1994), which has also been called the Tramtrack (Ttk) domain or BTB domain (Godt *et al.*, 1993).

psq and eye development

The *psq^{F112}* allele was isolated as an enhancer detector P-element with expression in photoreceptor precursors R3 and R4 (Figure 1). The phenotypic analysis of this and other similarly strong alleles has revealed that *psq* is required for the differentiation of R3 and R4 (Figure 2). Additional less common eye phenotypes observed in *psq* mutants are orientation defects of some ommatidia and rarely missing R7 cells. The orientation problems are likely to be secondary defects possibly caused by the failure of R3/R4 to differentiate, since these two cells have been shown to be important for ommatidial rotation (Zheng *et al.*, 1995). Similarly, the rare failure of R7 development is observed in ~2–3% of affected ommatidia as compared with the R3/R4 phenotype, and is thus a less specific effect.

Expression of the *lacZ* reporter in *psq^{F112}* in R3/R4 is dependent on *svp* gene activity. In *svp* mutant clones, this expression is not detected, conversely, in *rough* eye discs where more cells depend on *svp* function and develop as R3/R4 (Heberlein *et al.*, 1991), there are more cells expressing *lacZ* in *psq^{F112}*. These observations suggest that high levels of *psq* might be required downstream of *svp* for the development of R3/R4. In addition, we find that stronger loss-of-function *psq* alleles [or also Df(2R)17 and 27 removing the entire locus] are dominant suppressors of the *sev*–*svp*-induced cone cell transformation phenotype. Genetic analysis of other modifiers of *sev*–*svp* has shown that many of these genes are part of the Sev/Ras pathway or interact with this pathway genetically (Begemann *et al.*, 1995). However, we do not detect any genetic interactions between *psq* alleles and *sev^{S11}* (Basler *et al.*, 1991) or *sE-raf^{forY9}* (Dickson *et al.*, 1992), indicating that *psq* probably does not affect activated signaling of the Sev/Ras cascade, and is thus specifically required during *svp*-mediated cell specification. Taken together, our data suggest that *psq* is required downstream of *svp* for the differentiation of outer photoreceptors R3/R4.

Molecular function of Psq

Molecular analysis has shown that *psq* encodes novel proteins containing the POZ domain (or Ttk or BTB

Fig. 7. Expression of *psq* transcripts and protein during development. (A–E) Whole mount *in situ* hybridization using digoxigenin-labeled probes of either a cDNA (A and E) or intronic probes (B–D). (F–H) Antibody staining. (A) and (B) A sagittal view of embryos of stage 5 hybridized with a cDNA or intronic probe, respectively. The extent of the dorsal expression domain between 20 and 70% (0% is posterior end) is marked by arrowheads. (C) and (D) Sagittal and dorsal view of stage 7/8 embryos hybridized with an intronic probe. The antero-dorsal domain present in (A–D) is highlighted with a small arrow. Note the nuclear localization of the hybridization signal with the intronic probe in (B–D). Anterior is left and dorsal up (except D: dorsal view). (E) Expression in eye-antennal disc. Note the high signal levels posterior to the MF (marked by arrowhead). Inset: high magnification of the area behind the MF, demonstrating clustered expression. (F–H) Imaginal discs stained with a mouse polyclonal serum raised against a peptide of the C-terminal protein part recognizing ORF A-long and B/C. The specificity of the serum was confirmed by overexpressing the ORFs using the GAL4/UAS_G system (Brand and Perrimon, 1993). (F) *ptc*-GAL4; UAS-*psq* (ORF B/C) driven overexpression in the wing disc. Note the strong staining along the anteroposterior compartment boundary in the *ptc* expression domain confirming the specificity of the antiserum. (G) Eye-antennal disc. (H) High magnification view of the eye disc posterior to MF (marked by an arrowhead in G). Note the high level of staining in R3/R4 precursors (an example is marked accordingly). Staining is nuclear in all tissues analyzed.

domain). This domain is shared among several transcription factors (Bardwell and Treisman, 1994) and is also found in some poxviral proteins and their putative *Drosophila* homolog *kelch* (Koonin *et al.*, 1992; Xue and Cooley, 1993). All the known transcription factor members of this family contain zinc finger DNA binding motives. *Psq*, although also nuclear, does not have a classical zinc finger domain; potentially only one such motif (with an unusual amino acid sequence, Figure 5) is found close to the

C-terminus of ORF A-short. However, neither ORF A-long nor ORFs B/C contain an apparent DNA binding domain. Interestingly, both ORF B and C contain neither the POZ domain nor the zinc finger sequence, but contain the four repeats in the C-terminal part (Figure 5). Such presence of multiple isoforms sharing only parts of the coding potential is also found in several other members of this gene family. *Ttk* combines the N-terminally located POZ domain with two independent C-terminal halves each containing two different zinc fingers (Xiong and Montell, 1993), *BR-C* shares this domain with several independent sets of zinc finger motifs (DiBello *et al.*, 1991), and *lola* codes for two isoforms both sharing this domain spliced to exons either with or without zinc finger motifs (Giniger *et al.*, 1994).

The biochemical function of the POZ domain is to mediate homodimerization or heterodimerization with other proteins containing this domain (Bardwell and Treisman, 1994). Such a mechanism might allow dimer formation between *psq* and other genes sharing the POZ domain. Therefore, it seemed possible that *psq* interacts genetically with some of the other genes containing a POZ domain which are also required during imaginal development: e.g. *bric-a-brac* (*bab*) (Godt *et al.*, 1993), *ttk* (Xiong and Montell, 1993) and *lola* (Giniger *et al.*, 1994) (G.Begemann, C.A.Mayes and M.Mlodzik, unpublished). To test this, we have looked for dosage-sensitive genetic interactions between different alleles of *psq* and null alleles of these other genes (see Materials and methods for details). However, we did not detect any such interaction between *psq* and either *bab*, *ttk* or *lola*. Thus, biochemical analysis of *psq* will be necessary to define its molecular function and possibly identify dimerization partners.

Alleles affecting different alternative 5' exons display different phenotypes

The phenotypic and molecular analysis of existing *psq* alleles puts them into at least two classes. While the previously isolated alleles, such as *psq*^{P1}, *psq*^{P4} and *psq*^{X1-30} (Siegel *et al.*, 1993) and two new P-alleles, *psq*²⁹⁰⁵ and *psq*⁶⁷³² (M.Erdelyi, A.Guichet and A.Ephrussi, unpublished), are homozygous viable but female-sterile or grandchildless, the other new alleles isolated here are semilethal with few homozygous escapers (1–5%) and have zygotic phenotypes affecting the eye and also other adult structures (Table I). Despite significant efforts and many screens, it appears that the strongest *psq* alleles isolated to date are still likely to be hypomorphic alleles, as judged from their molecular nature and genetic behavior: they either affect only one of the three alternative 5' ends of the transcription unit or have stronger phenotypes over large deficiencies removing the entire locus.

It is striking that all viable female-sterile (or grandchildless) alleles cluster around the 5' exon 1A, while the semilethal alleles reported here and *psq*^{13B6} affect the 1B 5' exon. Although, due to the low abundance of the transcripts, it was not possible to detect any differential distribution using specific probes to the alternative 5' exons, the genetic and phenotypic analyses indicate that transcript (and protein) made from exon 1A is mostly required during oogenesis, while exon 1B-derived products would support the zygotic function of the gene after

fertilization. However, only transcripts initiated at exon 1A encode for proteins containing the POZ domain. Therefore, it appears that most of the zygotic requirement (including R3 and R4 development) of *psq* is met by the transcripts containing either exon 1B or 1C and thus missing the POZ domain. This is further supported by the observation that alleles affecting exon 1A do not interact with the *sev-svp* genotype.

Materials and methods

Fly stocks and genetic interactions

psq^{F112} was isolated as an enhancer detector line (Mlodzik and Hiromi, 1992). *psq^{E34}* and *psq^{E39}* are imprecise excision alleles of *psq^{F112}* causing a 4 kb intronic lesion or a complete deletion of exon 1B and 5 kb of upstream sequences, respectively. Escapers of these stocks eclose later than heterozygous animals at a rate of 1–5%. For *psq^{P1}*, *psq^{P4}*, *psq^{X1-30}* and *psq^{13B6}* see Siegel *et al.* (1993). Two grandchildless alleles *psq²⁹⁰⁵* and *psq⁶³⁷²* were isolated in a screen for maternal effect mutants (kindly provided by M.Erdelyi, A.Guichet and A.Ephrussi). X-ray-induced deficiencies Df(2R)12, 17 and 27 uncover either the chromosome region 46E/F–47A/B [Df(2R)12] or 47A–47D (R.Burgess and T.Schwarz, personal communication). EMS allele *psq^{D91}* was isolated as a lethal over Df(2R)17 and 27 (R.Burgess and T.Schwarz, unpublished results).

The following strains were used to test for genetic interactions with *psq* during eye development: *sev-svp* (Hiromi *et al.*, 1993), *sev^{S11}* (Basler *et al.*, 1991) and *sE-ra^{forY9}* (Dickson *et al.*, 1992). Dominant interactions between *psq* alleles [*psq^{F112}*, *psq^{E34}*, *psq^{E39}* and Df(2R)12, 17 and 27] and the following other genes encoding POZ domains were tested, *ttk*: Df(3R)awd^{KRB}, *bab*: Df(3L)emc⁵ and *lola^{mis5D}* (Giniger *et al.*, 1994) [*lola* is uncovered by Df(2R)12, 17 and 27]. Approximately 100 flies for each appropriate genotype and the respective control flies were analyzed. No interactions were detected.

Somatic recombination and clonal analysis

svp⁺ clones in imaginal discs were generated with the FLP/FRT system (Xu and Rubin, 1993) using the stock w; P[FRT]82F, *svp^{E22}/TM6B* (kindly provided by Y.Hiromi) crossed into a *psq^{F112}* background. In *svp*⁺ clones, Bar, normally expressed in R1/6, is not detected (Hiromi *et al.*, 1993), and thus Bar was used as a marker for *svp*⁺ ommatidia. Somatic *psq*⁺ clones were induced by X-ray with 1000 rads in 1st instar larvae using a P[white⁺] element at 49D1–4 as autonomous clonal marker.

Histology and immunohistochemistry

β-Galactosidase was detected by either activity or antibody staining as described (Tomlinson and Ready, 1987). Anti-Bar and anti-β-Gal double labelings were done in 0.1 M phosphate buffer (pH 7.2), 0.1% saponin, 0.3% deoxycholate, 0.3% Triton X-100 and 5% normal goat serum. Secondary horseradish peroxidase (HRP)-conjugated antibodies were incubated in 0.1 M phosphate buffer (pH 7.2), 0.1% saponin. The HRP reaction was done in 0.5 mg/ml diaminobenzidine (DAB) for Bar (brown staining). For β-gal detection, the staining solution was supplemented with 0.03% NiCl (black staining).

LacZ activity staining in adult retinas was performed as follows. Flies were fixed in the heptane phase of a mixture of 50% heptane, 25% phosphate-buffered saline (PBS) and 12.5% glutaraldehyde (EM grade) for 20 min, dried briefly on filter paper and collected in PBS. Heads were isolated and fixed for 10 min in PBS, 1% glutaraldehyde, 0.1% Triton X-100. Retinas were dissected with tungsten needles in PBS, post-fixed for 5 min, washed in PBS (2×5 min) and stained for β-galactosidase activity (protocol kindly provided by Y.Hiromi).

Sections of adult retinas were made according to Tomlinson and Ready (1987). Whole mount *in situ* hybridization to detect mRNA was performed using digoxigenin-labeled probes as described (Tautz and Pfeifle, 1989). cDNA or intronic probes were used (see Figure 7).

Generation of anti-Psq antisera

The Psq peptide sequence PTTWPEDLERALEA (amino acids 808–821) was used to generate multiantigenicity peptides which were injected into mice. The G559.1 line, a P[Gal4] insertion in *patched* (kindly provided by A.Brand; Brand and Perrimon, 1993), was used to drive the Gal4 upstream activating sequence (UAS)–ORF A-short or –ORF B/C to test for the specificity of antisera. Following the 4th boost, the serum was used at a 1:1000 dilution in 0.1 M phosphate buffer (pH 7.2), 0.3%

Triton X-100, 0.3% deoxycholate as described above. The DAB staining solution was supplemented with 0.03% NiCl.

Molecular analysis and cytology

Southern and Northern blots and screening hybridizations were done according to Church and Gilbert (1984). The frequency of cDNAs recovered was low, ~1 in 500 000 clones screened with 5' end probes. The first cDNAs or fragments thereof were then used as probes for new rounds of screening for full-length cDNAs and for the genomic clones encompassing them. DNA fragments and exon-specific probes generated by PCR were used as probes. Genomic λ phages encompassing the *psq* exons were mapped on Df(2R)12, 17 and 27 polytene chromosomes with biotinylated probes.

DNA sequences were determined by the chain termination method on single- or double-stranded templates using Sequenase (United States Biochemical Corp.). Sequence assembly and analysis were done with programs from the EMBL implementation of GCG software package 7.1.

Acknowledgements

We are most grateful to R.Burgess and T.Schwarz for providing us with the *psq* deficiencies and allowing us to screen their collection of EMS lethals, and M.Erdelyi, A.Guichet and A.Ephrussi for the grandchildless alleles. We thank Ed Giniger, Hugo Bellen and Kathy Matthews (Bloomington Stock Center) for fly strains, Ann-Mari Voie for help with sequencing and Toby Gibson for help with computer sequence analysis. Thanks to all members of the Mlodzik laboratory for discussions, and Steve Cohen, David Strutt and Volker Wiersdorff for comments on the manuscript.

References

- Bardwell,V.J. and Treisman,R. (1994) The POZ domain: a conserved protein–protein interaction motif. *Genes Dev.*, **8**, 1664–1677.
- Basler,K., Yen,D., Tomlinson,A. and Hafen,E. (1990) Reprogramming cell fate in the developing *Drosophila* retina: transformation of R7 cells by ectopic expression of *rough*. *Genes Dev.*, **4**, 728–739.
- Basler,K., Christen,B. and Hafen,E. (1991) Ligand-independent activation of the sevenless receptor tyrosine kinase changes the fate of cells in the developing *Drosophila* eye. *Cell*, **64**, 1069–1081.
- Begemann,G., Michon,A.M., van der Voorn,L., Wepf,R. and Mlodzik,M. (1995) The *Drosophila* orphan nuclear receptor Seven-up requires the Ras pathway for its function in photoreceptor determination. *Development*, **121**, 225–235.
- Bohmann,D., Ellis,M.C., Staszewski,L.M. and Mlodzik,M. (1994) *Drosophila* Jun mediates Ras-dependent photoreceptor determination. *Cell*, **78**, 973–986.
- Brand,A.H. and Perrimon,N. (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development*, **118**, 401–15.
- Brunner,D., Dückler,K., Oellers,N., Hafen,E., Scholz,H. and Klämbt,C. (1994) The ETS domain protein Pointed-P2 is a target of MAP kinase in the Sevenless signal transduction pathway. *Nature*, **370**, 386–389.
- Church,G.M. and Gilbert,W. (1984) Genomic sequencing. *Proc. Natl Acad. Sci. USA*, **81**, 1991–1995.
- DiBello,P.R., Withers,D.A., Bayer,C.A., Fristrom,J.W. and Guild,G.M. (1991) The *Drosophila* Broad-Complex encodes a family or related proteins containing zinc fingers. *Genetics*, **129**, 385–397.
- Dickson,B., Sprenger,F., Morrison,D. and Hafen,E. (1992) Raf functions downstream of Ras1 in the sevenless signal transduction pathway. *Nature*, **360**, 600–603.
- Dickson,B. and Hafen,E. (1993) Genetic dissection of eye development in *Drosophila*. In Bate,M. and Martinez-Arias,A. (eds), *The Development of Drosophila melanogaster*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 1327–1362.
- Giniger,E., Tietje,K., Jan,L.Y. and Jan,Y.N. (1994) *lola* encodes a putative transcription factor required for axon growth and guidance in *Drosophila*. *Development*, **120**, 1385–1398.
- Godt,D., Couderc,J.L., Cramton,S.E. and Laski,F.A. (1993) Pattern formation in the limbs of *Drosophila*: *bric a brac* is expressed in both a gradient and wave-like pattern and is required for specification and proper segmentation of the tarsus. *Development*, **119**, 799–812.
- Haller,J., Cote,S., Broenner,G. and Jaekle,H. (1987) Dorsal and neural expression of a tyrosine kinase related *Drosophila* gene during embryonic development. *Genes Dev.*, **1**, 862–867.

- Harrison,S.D. and Travers,A.A. (1990) The *tramtrack* gene encodes a *Drosophila* finger protein that interacts with the *ftz* transcriptional regulatory region and shows a novel embryonic expression pattern. *EMBO J.*, **9**, 207–216.
- Heberlein,U., Mlodzik,M. and Rubin,G.M. (1991) Cell fate determination in the developing *Drosophila* eye: role of the rough gene. *Development*, **112**, 1123–1135.
- Higashijima,S., Kojima,T., Michiue,T., Ishimaru,S., Emorei,Y. and Saigo,K. (1992) Dual *Bar* homeo box genes of *Drosophila* required in two photoreceptor cells, R1 and R6, and primary pigment cells for normal eye development. *Genes Dev.*, **4**, 1835–1847.
- Hiroimi,Y., Mlodzik,M., West,S.R., Rubin,G.M. and Goodman,C.S. (1993) Ectopic expression of *seven-up* causes cell fate changes during ommatidial assembly. *Development*, **118**, 1123–1135.
- Kimmel,B.E., Heberlein,U. and Rubin,G.M. (1990) The homeo domain protein *rough* is expressed in a subset of cells in the developing *Drosophila* eye where it can specify photoreceptor cell subtype. *Genes Dev.*, **4**, 712–727.
- Koonin,E.V., Senkevitch,T.G. and Chernos,V.I. (1992) A family of DNA virus genes that consists of fused portions of unrelated cellular genes. *Trends Biochem. Sci.*, **17**, 213–214.
- Mlodzik,M. and Hiroimi,Y. (1992) Enhancer trap in *Drosophila*: its application to neurobiology. *Methods Neurosci.*, **9**, 397–414.
- Mlodzik,M., Hiroimi,Y., Weber,U., Goodman,C.S. and Rubin,G.M. (1990) The *Drosophila* *seven-up* gene, a member of the steroid receptor gene superfamily, controls photoreceptor cell fates. *Cell*, **60**, 211–224.
- Moses,K., Ellis,M.C. and Rubin,G.M. (1989) The *glass* gene encodes a zinc-finger protein required by *Drosophila* photoreceptor cell. *Nature*, **340**, 531–536.
- O'Neill,E.M., Rebay,I., Tjian,R. and Rubin,G.M. (1994) The activities of two Ets-related transcription factors required for *Drosophila* eye development are modulated by the Ras/MAPK pathway. *Cell*, **78**, 137–147.
- Ready,D.F. (1989) A multifaceted approach to neural development. *Trends Neurosci.*, **12**, 102–110.
- Siegel,V., Jongens,T.A., Jan,L.Y. and Jan,Y.N. (1993) *pipsqueak*, an early acting member of the posterior group of genes, affects *vasa* level and germ cell–somatic cell interaction in the developing egg chamber. *Development*, **119**, 1187–1202.
- Simon,M.A., Bowtell,D.D.L., Dodson,G.S., Lavery,T.R. and Rubin,G.M. (1991) Ras1 and a putative guanine nucleotide exchange factor perform crucial steps in the signaling by the sevenless protein tyrosine kinase. *Cell*, **67**, 701–716.
- Tautz,D. and Pfeifle,C. (1989) A non-radioactive *in situ* hybridization method for localization of specific mRNAs in *Drosophila* embryos reveals a translational control of the segmentation gene *hunchback*. *Chromosoma*, **98**, 81–85.
- Tomlinson,A. (1988) Cellular interactions in the developing *Drosophila* eye. *Development*, **104**, 183–193.
- Tomlinson,A. and Ready,D.F. (1987) Neuronal differentiation in the *Drosophila* ommatidium. *Devel. Biol.*, **120**, 366–376.
- Tomlinson,A., Kimmel,B.E. and Rubin,G.M. (1988) *rough*, a *Drosophila* homeobox gene required in photoreceptors R2 and R5 for inductive interactions in the developing eye. *Cell*, **55**, 771–784.
- Wharton,K.A., Yedvobnick,B., Finnerty,V.G. and Artavanis-Tsakonas,S. (1985) *opa*: a novel family of transcribed repeats shared by the *Notch* locus and other developmentally regulated loci in *Drosophila* in *D.melanogaster*. *Cell*, **40**, 55–62.
- Wolff,T. and Ready,D.F. (1993) Pattern formation in the *Drosophila* retina. In Bate,M. and Martinez-Arias,A. (eds), *The Development of Drosophila melanogaster*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 1277–1325.
- Xiong,W.C. and Montell,C. (1993) *tramtrack* is a transcriptional repressor required for cell fate determination in the *Drosophila* eye. *Genes Dev.*, **7**, 1085–1096.
- Xu,T. and Rubin,G.M. (1993) Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development*, **117**, 1223–1237.
- Xue,F. and Cooley,L. (1993) *kelch* encodes a component of intercellular bridges in *Drosophila* egg chambers. *Cell*, **72**, 681–693.
- Zheng,L., Zhang,J. and Carthew,R.W. (1995) *frizzled* regulates mirror-symmetric pattern formation in the *Drosophila* eye. *Development*, **121**, 3045–3055.
- Zipursky,S.L. and Rubin,G.M. (1994) Determination of neuronal cell fate: lessons from the R7 neuron of *Drosophila*. *Annu. Rev. Neurosci.*, **17**, 373–397.

Received on May 25, 1995; revised on September 14, 1995