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Search for Drosophila genes based on patterned expression of mini-white reporter gene of a P lacW vector in adult eyes

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Abstract Developmental expression of transduced mini-white (w) gene of Drosophila is sensitive to its flanking genomic enhancers. Taking advantage of this phenomenon, we mobilized a P lacW transposon and screened for new transposant lines which showed patterned expression of the mini-w gene in adult eyes. From a screen of about 1,000 independent P lacW transposant lines on the second chromosome, we identified 7 lines which showed patterned w expression in adult eyes. These P insertions were assigned to engrailed, wingless and teashirt genes based on their chromosomal locations, developmental expression of the lacZ reporter gene, lethal embryonic mutant phenotypes and, finally, their failure to complement the lethal alleles of the respective genetic loci. Our results show that although only a small fraction of the total transposant lines displayed patterned w expression, the genetic loci thus identified are those which play essential roles in pattern formation. Scopes of screens for genetic loci based on w reporter gene expression in adult eyes are discussed.

Key words Drosophila • Mini-white reporter gene • Teashirt • Engrailed • Wingless

Introduction

The white (w) gene of Drosophila melanogaster is frequently used as a marker in transformation vectors (Hazelrigg et al. 1984; Klemenz et al. 1987; Pirrotta 1988). w is also used as a marker in P mutagenesis and enhancer detection studies (Bier et al. 1989; Torok et al. 1993). Its cell autonomous and dose dependent expression causes adult eye pigmentation that is easily detected in a background of bleached white eye colour caused by null mutations of its endogenous alleles. The w marker frequently used in these studies, called the mini-w gene, carries only the essential sequences required for its expression. It lacks part of the intron sequences of the w transcription unit and is under the control of a minimal promoter (Pirrotta 1988). The mini-w marker therefore displays only a basal level of its expression in the transformed flies which can be stimulated by flanking genomic enhancers. Pigmentation due to the mini-w marker in the transformed flies ranges from light yellow, through orange to bright red, i.e. near wild-type eye colour (Hazelrigg et al. 1984; Klemenz et al. 1987; Bier et al. 1989). Intensities of eye pigmentation in these P insertion lines reflect the relative influence of the neighbouring genomic enhancers on w expression in adult eyes (also see Kellum and Schedl 1991).

The first demonstration that the mini-w gene could also be used as a marker for positional information came from the experiments of Hazelrigg et al. (1984). From their collection of transgenic flies they identified two P-w insertions with mosaic w expression. One of these, showed pigmentation of ommatidia only in the anterior part of the adult eyes. The group of ommatidia expressing w therefore appeared to share common positional information (Hazelrigg et al. 1984; Levis et al. 1985). These transduced copies of the w gene thus displayed novel developmental regulation.

Patterned expression of the transduced w gene is distinct from the phenomenon of position-effect variegation (PEV) which results from its translocation or insertion in the proximity of heterochromatic loci (for review, see Spofford 1976; Reuter and Spierer 1992; Henikoff 1990, 1992). PEV is characterized by inactivation of the w gene in random groups of ommatidia in the compound eye. Even though this form of inactivation is clonally stable, the mosaic pattern of eye pigmentation is not heritable. Siblings derived from the same parents, or the two compound eyes of the same individual, do not display identical mosaic patterns. Thus, while PEV results from random inactivation of w gene expression, the patterned w expression (Fig. 1) referred to above is heritable and implies its precise developmental regulation (Hazelrigg et al. 1984; Levis et al. 1985). Expression of the mini-w gene in adult eyes is also modulated when it is placed un-
under the regulatory sequences of genes like en, (en; Kassis et al. 1992) or polyhomeotic, (ph; Fauverque and Dura 1993) in P transformation vectors. Thus, a P transposon carrying a -2.4 to +0.4 kb en regulatory sequence upstream to a mini-w marker displayed patterned pigmentation in adult eyes following its insertion in genetic loci like en or hairy (h; Kassis et al. 1991). Likewise, presence of upstream ph sequences mediated patterned w expression following insertion of the element in several genetic loci (Fauverque and Dura 1993), most of which appeared to be target genes regulated by the Polycomb group (Pe-G) of genes (see Zink and Paro 1989).

Expression of the mini-w reporter gene in adult flies therefore can be strikingly influenced by the upstream regulatory (Kassis et al. 1991; Whitley et al. 1994) sequences and/or the chromatin organization of the neighboring genomic DNA, as in the case of the targets of Pe-G genes (Fauverque and Dura 1993). We took advantage of this phenomenon to screen for Drosophila genes which could be implicated in pattern formation. In this study, we report the results of a screen for Drosophila genes based on patterned expression of mini-w gene of a P lacW vector (Bier et al. 1989). Seven P lacW insertion lines identified by us on the basis of patterned w expression in adult eyes (from a screen of about 1000 new transposant lines on the second chromosome) were traced to three genetic loci, namely en, wingless (wg) and teashirt (tsh). Our results show that the screen for patterned expression of the mini-w reporter gene allows preferential identification of Drosophila genes involved in pattern formation.

Materials and Methods

Mobilization of a P lacW vector (Bier et al. 1989) from its X chromosomal location was induced by a standard scheme of genetic crosses (for details of these genetic crosses and P lacW vector, see Bier et al. 1989; Torok et al. 1993). Putative single transpositions of the P lacW vector on the second chromosome were recovered in trans over the CyO balancer. Homozygous viability of the individual P insertion lines was subsequently tested (see Bier et al. 1989). Viable stocks were maintained without the CyO balancer chromosome. About 1,000 new transposant lines were screened for patterned expression of the mini-w gene. Lines displaying patterned w expression, characterized by pigmentation of the antennae located in only a certain part of the adult eyes (Fig. 1), were further examined for in situ chromosomal location of the P element, lacZ reporter expression and lethal embryonic phenotype of the P insertion or its excision alleles. A non-radioactive technique of in situ localization of the P insert was carried out (for details, see Torok et al. 1993) and β-galactosidase activity in embryos, imaginal discs and adult cuticle were studied histochemically by staining with the chromogenic compound x-gal (Glysen and O'Kane 1989). Excisions of these P insertions were carried out by inducing their remobilization (see Bier et al. 1989; Torok et al. 1993). Individual P excised lines were tested for viability as homozygotes. Homozygous viability was presumed to suggest precise or nearly precise excisions, whilst lethality implied imprecise excisions of the P elements and, consequently, genetic lesions in and around the sites of their insertions (for further details, see Torok et al. 1993). The first larval cuticular phenotype of embryonic lethal lines was studied as described earlier (see Nusslein-Volhard and Wieschaus 1986).

Results

Patterned expression of the mini-white reporter gene

Transposition of the P element takes place premeiotically in about 2–3% of sperm mother cells (Wilson et al. 1989). One thousand second chromosomal lines were established in our laboratory after screening the progeny of about 11,000 jump starter males. A search for P lacW insertion lines with patterned w expression (see Fig. 1) yielded a total of seven independent transposant lines. The pattern of eye pigmentation in all these lines was heritable and reproducible and, therefore, distinct from PEV observed after translocation or insertion of w gene in the proximity of heterochromatic regions (Fig. 1). General characteristics of these insertion lines are summarized in Tables 1 and 2. Adult flies of these insertion lines displayed eye pigmentation in the anterior half to

Fig. 1 Normal, variegated and patterned white (w) expression in the adult eyes of Drosophila. Schematic comparison of eye pigmentation due to normal white expression in wild type flies (upper left) with that of the variegated expression caused by translocation of w in the proximity of heterochromatic regions (upper right). Lower panel shows patterned w expression in the anterior of posterior region of the compound eye. The mosaic pattern due to variegation of the w gene (upper right) is not heritable whilst those due to patterned w expression (lower panel) are heritable. We screened for P lacW insertions which displayed patterned w expression in the adult eyes. Rectangles in the eye represent ommatidia whilst black dots in the rectangles represent w expression in individual ommatidia

P lacW insertions which were traced to three cytogenetic regions, i.e. 40A, 48A and 28A, were subsequently tested for allelism with the mutations of the respective genetic loci, namely tsh, en and wg, by testing their viability in trans over their lethal alleles. The mutant alleles of tsh tested were tshl and tshT (Fasano et al. 1991). Mutant alleles of en tested were enT2 (Kornberg 1981; Eaton and Kornberg 1990), Df(2R)enA (Eberlein and Russell 1983; Gubb 1985), enO (Nusslein-Volhard and Wieschaus 1980) and allelism was also tested against a lethal en allele caused by an insertion of P (en-lacZ) vector ryXho25 within the C en transcription unit (Hama et al. 1990). For insertions in the 28A region, allelism was tested with the lethal allele of wg, namely wgO2 (Baker 1988b; Heuvel et al. 1993).
### Table 1 Allelism of P lacW insertions with known mutant alleles to *teashirt* (tsh), *engrulized* (en) and *wingless* (wg).

<table>
<thead>
<tr>
<th>Insert number</th>
<th>Genetic nomenclature</th>
<th>Cytology</th>
<th>Alleles tested</th>
<th>Mutant lethal alleles of tsh, en and wg tested for lack of complementation</th>
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<tr>
<td>179</td>
<td>tsh179(V)</td>
<td>40A</td>
<td>tsh&lt;sup&gt;17&lt;/sup&gt;(E,L)</td>
<td>tsh&lt;sup&gt;6&lt;/sup&gt;, tsh&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>101</td>
<td>en&lt;sup&gt;nst-101&lt;/sup&gt;(L)</td>
<td>48A</td>
<td>en&lt;sup&gt;nst-101&lt;/sup&gt;(LL)</td>
<td>en&lt;sup&gt;x31&lt;/sup&gt;, en&lt;sup&gt;d34&lt;/sup&gt;, ryXho25, Df(2R)enA</td>
</tr>
<tr>
<td>21</td>
<td>wg&lt;sup&gt;21-P&lt;/sup&gt;(L)</td>
<td>28A</td>
<td>wg&lt;sup&gt;21-P&lt;/sup&gt;(LL)</td>
<td>wg&lt;sup&gt;x2&lt;/sup&gt;</td>
</tr>
<tr>
<td>III-P</td>
<td>wg&lt;sup&gt;III-P&lt;/sup&gt;(V)</td>
<td>28A</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Absence of the Cy<sup>+</sup> progenies implied lack of complementation. At least 500 progenies were scored in each case (Ns not shown, V adult viable, L embryonic lethal, E P excision allele, I P insertion allele).

### Table 2 Summary of patterned (w) expression and β-galactosidase activity of P lacW insertions at tsh, en and wg

<table>
<thead>
<tr>
<th>P lacW insertion</th>
<th>w gene expression in adult eyes</th>
<th>β-galactosidase activity</th>
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<tr>
<td></td>
<td></td>
<td>Embryos</td>
</tr>
<tr>
<td>tsh&lt;sup&gt;179&lt;/sup&gt; (viable)</td>
<td>Anterior three&lt;sup&gt;a&lt;/sup&gt; fourth of the eye</td>
<td>Trunk specific&lt;sup&gt;a&lt;/sup&gt; robust expression pattern</td>
</tr>
<tr>
<td>tsh&lt;sup&gt;75&lt;/sup&gt; (viable)</td>
<td>Anterior three&lt;sup&gt;a&lt;/sup&gt; fourth of the eye</td>
<td>Weak trunk specific&lt;sup&gt;c&lt;/sup&gt; expression</td>
</tr>
<tr>
<td>tsh&lt;sup&gt;253&lt;/sup&gt; (viable)</td>
<td>Anterior three&lt;sup&gt;a&lt;/sup&gt; fourth of the eye</td>
<td>No embryonic&lt;sup&gt;c&lt;/sup&gt; expression</td>
</tr>
<tr>
<td>tsh&lt;sup&gt;55&lt;/sup&gt; (viable)</td>
<td>Anterior three&lt;sup&gt;a&lt;/sup&gt; fourth of the eye</td>
<td>No embryonic&lt;sup&gt;c&lt;/sup&gt; expression</td>
</tr>
<tr>
<td>en&lt;sup&gt;nst-101&lt;/sup&gt; (lethal)</td>
<td>Anterior half&lt;sup&gt;a&lt;/sup&gt; of the adult eye</td>
<td>2–4 cell wide stripes&lt;sup&gt;a&lt;/sup&gt; confined to the posterior compartments of every developing segment,</td>
</tr>
<tr>
<td>wg&lt;sup&gt;III-P&lt;/sup&gt; (viable)</td>
<td>Anterior half&lt;sup&gt;c&lt;/sup&gt; of the adult eye</td>
<td>1–2 cell wide&lt;sup&gt;b&lt;/sup&gt; discontinuous epidermal stripes in the segmental domain of the embryo</td>
</tr>
<tr>
<td>wg&lt;sup&gt;21-P&lt;/sup&gt; (lethal)</td>
<td>Anterior half&lt;sup&gt;c&lt;/sup&gt; of the adult eye</td>
<td>As above&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
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<sup>a</sup> Fig. 2  
<sup>b</sup> Fig. 3  
<sup>c</sup> Not shown here

Three-quarters of their compound eyes (Fig. 2a and 3a) and displayed dose dependent expression: the viable or escaper homozygotes exhibited more intense eye pigmentation than the heterozygotes. However, the pigmented area remained the same in both homozygotes and heterozygotes (not shown). Four of these insertions (numbers 179, 75, 55 and 253; Fig. 2a; Table 1 and 2) showed an in situ chromosomal location on 40A, at the base of the left arm of the second chromosome on the polytene chromosome map (Fig. 2b), the cytogenetic locus of the *teashirt* (tsh) homeotic gene (Fasano et al. 1991). These four P insertions were viable as homozygous adults (Table 2). The P insertion line 101 showed embryonic lethality in homozygotes and light yellow pigmentation in the anterior half of the adult eyes of heterozygotes (Fig. 2a). It showed an in situ location on 48A in the right arm of the second chromosome on the polytene chromosome map (Fig. 2c), the cytogenetic position of the segment.
polarity gene engrailed (en) (also see Hama et al. 1990). About 3% of the insertion 101 homozygotes escaped embryonic lethality, survived up to pharate adult stage and showed wing vein defects characteristic of viable mutations of en (not shown here). Adult flies homozygous for the insertion III-P (Fig. 3a) or heterozygous for the lethal insertion 21 (not shown here) showed pigmentation in the anterior half of the adult eyes and an in situ location of the P lacW element on 28A in the polytene chromosome map (not shown), the cytogenetic position of the wingless (wg) segment polarity gene (Sharma and Chopra 1976; Baker 1988a, b, c).

lacZ reporter expression, mutant phenotypes and complementation studies

Insertions at tsh locus

Of the four P lacW insertions which mapped at the chromosomal position 40A, only the line number 179 showed robust lacZ activity (see Table 2). A brief description of lacZ reporter expression of this P lacW insertion is presented here. Embryonic lacZ expression began from early gastrulation (stage 6–7) in parasegments 3–13 (Fig. 2d). Following germ band retraction, β-galactosidase activity was also seen in the internal organs including part of the visceral mesoderm, the central and peripheral nervous systems (Fig. 2e and f). This pattern of activity matched the expression of the tsh homeotic gene reported earlier (Fasano et al. 1991). β-galactosidase activity was also seen in the presumptive ommatidial cells (Fig. 2j) of the eye imaginal discs posterior to the morphogenetic furrow (see Carroll and Whyte 1989), whilst in the wing imaginal discs lacZ expression was seen in a ring surrounding the wing pouch region and nearly covering the entire notal area (Fig. 2k). In the leg discs, too, β-galactosidase activity was seen in a ring encircling the entire disc margin (Fig. 2l); this pattern of expression matched the tsh expression pattern described earlier (see Bryant 1993). β-galactosidase activity was weak or absent in the imaginal discs of the other insertions at 40A (numbers 55, 75 and 253; see Table 2). All four insertions on 40A, however, showed greater uniformity in revealing an adult specific lacZ reporter expression pattern (Table 2), which we interpret as characteristic of the tsh homeotic gene (JB and PS, in preparation). Based on this evidence we have assigned these four insertions to the tsh locus. In a subsequent part of the text, these are referred to as tsh179, tsh75, tsh55, and tsh253 (Table 1). To further test their allelism to the tsh locus, the P lacW vector of the tsh179 insertion line, which displays robust tsh-specific lacZ reporter expression, was excised in genetic crosses (see Bier et al. 1989; Torok et al. 1993). Nearly 40% of the P excisions showed embryonic lethality. First instar larval cuticles of mutant animals showed phenotypes characteristic of lethal alleles of tsh (not shown). Moreover, these lethal P-excision alleles failed to complement the known lethal alleles of the tsh locus (Fasano et al. 1991), namely tsh4 and tsh8 (see Table 1), which further confirmed the insertion of the P lacW vector within or in the vicinity of the tsh locus.

Insertion at en locus

lacZ expression of insertion 101 heterozygous animals was robust (Fig. 2g–i), appeared early during embryogenesis — beginning around the cellular blastoderm stage — and revealed β-galactosidase activity in 2–4 cell wide stripes in the posterior compartments of the developing segments (Fig. 2g–i). Domains of this expression and its developmental pattern matched the en-specific lacZ reporter activity described earlier (Hama et al. 1990). About 5% of the 101 insert homozygotes which escaped embryonic lethality showed posterior compartment specific expression in the imaginal discs of the third instar larvae (Fig. 2m), whereas imaginal discs of insert 101 heterozygotes did not show strong β-galactosidase activity (not shown). Homozygous adult escapers also showed posterior compartment specific β-galactosidase activity in the cuticle derived from different imaginal discs and the abdominal histoblast nests (not shown here; also see Hama et al. 1990).

The insertion line 101 showed embryonic lethality whilst its excision alleles showed an embryonic mutant phenotype characteristic of en (not shown here, see Kornberg 1981). Both P insertion and its excision alleles displayed lethality over chromosomes deficient for the en-inv region, i.e. Df(2R)enA (Eberlein and Russell 1983; Gubb 1985) and enK31 (Kornberg 1981; Kuner et al. 1985) lethal alleles (see Table 1). Moreover, these P-excision alleles showed lethality over the en1034 mutation (Nusslein-Volhard and Wieschaus 1980) and the ryXho25 insertion which, respectively, represent a lesion (see Eaton and Kornberg 1990) and a lethal insertion of a P (en-lacZ) transposon in the en transcription unit (Hama et al. 1990). Taken together with the pattern of lacZ reporter expression (see above), these results suggest that the P lacW insertion of line number 101 is located in the en-inv region, presumably within the en region. Moreover, 101 insertion homozygotes and its P-excision alleles showed, respectively, weak and strong en mutant phenotypes (AS and PS, unpublished). Based on this evidence the insertion 101 is assigned to the en locus and designated as enB-101.

Insertions at wg locus

The insertion lines III-P (Fig. 3b) and 21-P (not shown) revealed lacZ reporter activity in stripes in the developing embryo, characteristic of the segment polarity gene wg (see Baker 1988a, c; Heuvel et al. 1989; Kassis et al. 1992). lacZ reporter expression in the wing imaginal discs of both these insertions was identical to the pattern displayed by a wg-specific lacZ reporter gene (Kassis et al. 1992; for review see Cohen 1993). This was characterized by a stripe along the dorsoventral
Fig. 3a–d Characteristics of P lacW insertions in the wingless (wg) locus. a Patterned w expression of homozygous adult flies in insertion III-P(wg). b lacZ activity during embryonic development in a stage 16 embryo. Note the striped pattern in every segment of the embryo and a very prominent foregut (fg) staining. c lacZ reporter activity in wing imaginal discs was characterized by two distinct stripes, one on the wing pouch along the primordial dorsal-ventral boundary (arrow) and the other on the notal region (arrowhead). In addition, lacZ expression was also seen in a ring over the entire presumptive wing pouch region. d In the leg disc, reporter activity of this insert was restricted to the anterior ventral sector.
Discussion

Preferential identification of Drosophila genes based on patterned expression of mini-w reporter gene

Insertions of Drosophila P elements are generally random (O'Hare and Rubin 1983). Therefore, various enhancer detection screens (Bier et al. 1989; Wilson et al. 1989) demand the examination of lacZ reporter gene activity in different cell and tissue types from a large collection of enhancer trap lines. An interesting modification of such genetic screens would be site-specific P insertions so as to study a desired set of genetic loci. The possibility of site specific P transposition was realized for the first time by the observation of Hama et al. (1990). They showed that the presence of a large part of en regulatory sequence in the transposon resulted in its preferential insertion in and around the en genetic region. Using similar strategies, Kassis et al. (1992) showed that transposons carrying en sequences (referred to as P[en]) insert preferentially on genetic loci which are generally expressed in striped patterns in embryos. Somewhat comparable results were obtained using a transposon carrying sequences of the polyhomeotic (ph) gene which also showed preferential insertions in the chromosomal regions which are prefered sites of binding of proteins of the Pc-G genes (Fauvarque and Dura 1993). P insertions therefore become site specific when they carry the regulatory sequences of genes like en (Kassis et al. 1992) and ph (Fauvarque and Dura 1993; for review see Paro 1993). Our results show that, in a screen based on patterned expression of the mini-w reporter gene, one can preferentially search for a variety of Drosophila genetic loci essential for pattern formation in the fruitfly. Although such a screen does not imply a site-specific transposition in the sense that the P[en] and P[ph] vector does, the screen permits preferential selection of a category of patterning genes. The ease of the preferential detection of these genes based on patterned w expression can be further appreciated from the fact that out of the seven P lacW inserts from the present screen, one was localized on en, two on wg and four on tsh. In contrast, screens primarily based on lacZ reporter gene expression, as in the case of the study of Bier et al. (1989) required examination of 3678 lines which yielded one insertion on en. In a separate attempt in this laboratory, screening for P lacW insertions in the third chromosome displaying patterned mini-w expression led to the identification of a novel locus at polytene band 86C, which is apparently required for patterning of the Drosophila appendages in the proximal-distal axis (LM, AM and PS, in preparation). In an independent investigation, 21 genetic loci (including those of tsh and en) have been identified by Sun et al. (1995) based on the patterned expression of the mini-w reporter gene of a P lacW vector (Y. H. Sun, pers. comm.). Genetic screens based on patterned expression of the mini-w reporter gene thus provide the prospect of preferentially searching for the Drosophila genes required for pattern formation.
that chromosomal loci where $P$ lacW insertions displayed patterned mini-$w$ expression, such as 40A ($tsh$), 28A ($wg$) and 48A ($en$), are all targets of in vivo binding by the proteins of Pc-G genes (see Zink and Paro 1989; DeCamillis et al. 1992; Martin and Adler 1993; Rastelli et al. 1993). These genes are presumed to be developmentally regulated by compaction of their chromatin structure resulting in a heritable silencing of their expression in specific cell types (for review, see Paro 1993). We believe that a genetic screen based on patterned mini-$w$ expression would preferentially detect these genetic loci. In agreement with this proposition, we find that over half of the genetic loci which displayed patterned $w$ expression following insertion of various $P(w^+)\text{ vectors }$ (see Hazelrigg et al. 1984; Levis et al. 1985; Kassis et al. 1991; Kassis 1994; Fauvarque and Dura 1993; Y. Henry Sun, personal communication) are targets of Pc-G protein binding (see Zink and Paro 1989; Martin and Adler 1993; DeCamillis et al. 1992; Rastelli et al. 1993). The screen described here therefore would imply detection of genetic loci based on the "silencing" (see Paro 1993) of mini-$w$ expression rather than its selective expression under the influence of neighbouring enhancers. These speculations, however, need further experimental tests.

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References


