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Functional analysis of the *white*$^+$ gene of *Drosophila* by P-factor-mediated transformation

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A 12-kb DNA segment spanning the *white*$^+$ (w^+) locus of *Drosophila* has been inserted into a P-transposon vector and used for P-factor-mediated germ-line transformation. Several red-eyed transformants were recovered which complement the *white* mutant phenotype. Analysis of the eye pigments and the interaction with the *zeste* mutation indicates that the w^+ gene inserted at several new chromosomal sites is expressed normally. The tissue-specific accumulation of w^+ transcripts, as studied by *in situ* hybridization to tissue sections, is the same in transformant and wild-type larvae. This indicates that all the genetic information specified by the w^+ locus is contained within this 12-kb segment of DNA. By secondary mobilization it was shown that the w^+ sequences have been inserted as a functional $P(w^+)$ transposon which is capable of further transposition.

Key words: Drosophila/white gene/P-factor/transformation

Introduction

In *Drosophila*, molecular cloning methods have been developed to the point where any gene can be isolated even if only its map position is known precisely and chromosomal rearrangements are available which allow the physical mapping of the gene on the cloned segment of DNA. These methods involve 'walking along the chromosome' (Bender *et al.*, 1983), the use of transposons (Gehring and Paro, 1980; Bingham *et al.*, 1981), or microdissection of bands from polytene chromosomes (Scalenghe *et al.*, 1981). In the absence of any biochemical information about the gene products, the *white* (w^+) locus has been cloned by using these methods (Bingham *et al.*, 1981; Goldberg *et al.*, 1982; Pirrotta *et al.*, 1983). To map the w^+ gene on the cloned DNA segment, a variety of mutants, including deletions, inversions, transpositions and insertions, have been used which delimit the locus to a first approximation. In order to define the w^+ locus functionally, we have now used P-factor-mediated transformation. This elegant technique which was developed by Spradling and Rubin (1982; Rubin and Spradling, 1982) allows genetic transformation of the germ line using the P-transposon as a vector. We have inserted a 12-kb DNA segment spanning the w^+ locus into a polylinker of a P-transposon carrying an internal deletion. Upon co-injection of this construct and intact P-transposon DNA into *w* mutant eggs, several red-eyed transformants have been isolated. The analysis of these transformants carrying the $P(w^+)$ transposon indicates that all the information needed for functional expression of the w^+ locus is contained within this 12-kb DNA segment. Similar results have recently been reported by Hazelrigg *et al.* (1984). Using a newly developed method for *in situ* hybridization of cloned DNA to RNA transcripts in tissue sections (Hafen *et al.*, 1983) we have shown that the tissue-specific accumulation of transcripts in the transformants is the same as in the wild-type controls (see accompanying paper by Fjose *et al.*). We also show that an inserted $P(w^+)$-transposon can be mobilized again and transpose to other sites in the genome.

Results

Construction of white$^+$*-P hybrid plasmids*

A 12-kb RI fragment spanning the w^+ locus was isolated from phage M558 (Goldberg *et al.*, 1982) and inserted into the polylinker of p6.3, a newly constructed P-transposon vector (see Materials and methods). The resulting w^+-P hybrid plasmid is designated pWP-2. The restriction map of pWP-2 and its relationship to the map of the *w* locus are shown in Figure 1. The w^+ sequences reach from an artificial RI site at about -5.2 kb to the RI site at $+6.7$ kb (the insertion site of the *copia* element in the *white-apricot* mutation has been defined as the zero coordinate). This DNA segment includes all the *w* mutant sites that have been mapped on the DNA level from w^{sp} at $+4.9$ to w^6 at -2 kb, with the exception of two dominant alleles w^{Bwx} and w^{DZL} which appear to be special cases (Zachar and Bingham, 1982; O'Hare *et al.*, 1983). It also spans the RNA coding regions plus ~3 kb of flanking sequences on either side of the *white*$^+$ locus (O'Hare

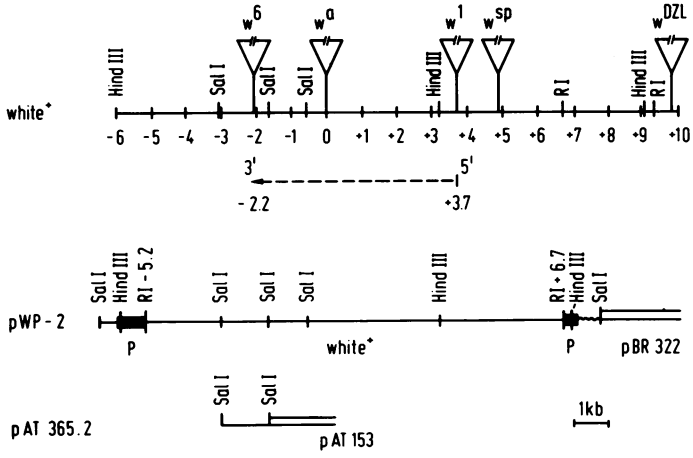

Fig. 1. Restriction map of the w^+ locus and the plasmid pWP-2 used for the transformation experiments. **Top:** restriction map of the w^+ DNA. The site of insertion of transposable elements in five insertion mutants is indicated. The site of insertion of *copia* in the *white-apricot* (w^a) mutant defines the 0 coordinate. The transcribed region is indicated by a dashed line; the direction of transcription is indicated by the arrow. (Based on data from Goldberg *et al.*, 1982; Zachar and Bingham, 1982; O'Hare *et al.*, 1983; Pirrotta and Bröckl, 1984; Fjose *et al.*, 1984.) **Middle:** aligned restriction map pf pWP-2 which was constructed by inserting the w^+ sequences from -5.2 to $+6.7$ into the polylinker of the P-transposon vector p6.3. **Bottom:** restriction map of the subclone pAT365.2 obtained by subcloning the large *Sal*I fragment into pAT153 (A.Fjose, unpublished).

Table I. Injection of P-transposon (pπ25.1) and *white*$^+$-P plasmid DNA (pWP-2) in fertilized eggs of various M strains

Experiment number	DNA injected (μg/ml)		Recipient M strain	Number of eggs injected	Fertile adults (G0)	Mutable adults (G0) ($sn^w \rightarrow sn^+$ or sn^e)	Adults producing w^+ transformants	w^+ transformants per total progeny (G1)
1	pπ25.1	100	*y sn*^{w};*bw;st*	32	4	3	–	–
2	pπ25.1	50						
			w^1	104	28	–	1	13/185
	pWP-2	200						
3	pπ25.1	50						
			y sn^w;*bw;st*	100	30	5	0	0
	pWP-2	200						
4	pπ25.1	50						
			w^1 *sn*^{w}	137	20	7	1	13/147
	pWP-2	200						

Table II. Mapping of the transduced DNA sequences by *in situ* hybridization to polytene chromosomes

Experiment[a]	Transformant line	Phenotype	w^+ probe (chromosome section)	P probe number of sites[b]	Bacterial vector probe number of sites
1	8.4	sn^+	n.d.[c]	12	n.d.
2	*w47.1 G1*	w^+	82A	6	0
	w47.1 N8	w^+	65A/B	2	0
	w47.3 C3	w^+	65A/B+82A	n.d.	n.d.
	w47.4 L	w^+	82A	5	0
	w47.4 G	w^+	82A	12	0
4	*w 20.1*	w^+	22B	5	n.d.
	w 20.2	w^+	22B	13	n.d.
	w 20.3	w^+	22B	10	n.d.
	w 20.4	w^+	22B	2	0
	w 20.8	w^+	22B	3	0
	w 20.10	w^+	22B+45A	8	0

[a]See Table I.
[b]Including the site(s) of *P* w^+ sequences.
[c]n.d. not determined.

et al., 1983; Pirrotta and Bröckl, 1984; Fjose *et al.*, 1984, accompanying paper).

Isolation of white$^+$ transformants

In a pilot experiment the capacity of the intact P-transposon DNA to insert into germ line chromosomes was tested by injecting pπ25.1 plasmid DNA (Spradling and Rubin, 1982) into fertilized eggs of an M indicator strain with the markers *y sn*^{w}; *bw*; *st* (Engels, 1979, 1981). Mobilization of P-transposons leads to mutations from *singed-weak* (*sn*^{w}) to *singed-extreme* (*sn*^{e}) or *singed*$^+$ at frequencies of 40–60%, which can be diagnosed by the phenotype of the bristles in the progeny of such flies. The results shown in Table I (experiment 1) indicate that the injection of intact P-transposon DNA yields a high frequency of mutable adults: three out of four injected flies (G0 generation) gave rise to *sn*^{e} or *sn*$^+$ progeny suggesting that they were P-transformants. One *sn*$^+$ line resulting from this experiment was analyzed by *in situ* hybridization of pπ25.1 DNA to polytene salivary gland chromosomes (Table II). It carried new P-transposon insertions at 12 additional sites.

Subsequently, the w^+-P hybrid plasmid, pWP-2, was co-injected with intact P-transposon DNA (pπ25.1) into *white* (w^1/w^1) eggs (experiment 2 in Table I). From 104 injected eggs, 28 fertile adults (G0 generation) were obtained, one of which gave rise to 13 red-eyed flies among 185 progeny in G1. These 13 flies turned out to be w^+ transformants as will be shown below. Since the frequency of transformation was lower than expected we used for experiment 3 the same *sn*^{w} recipient strain as the one used in the first experiment, which gave a higher frequency of transformation. Five out of 30 adults in G0 were mutable and gave rise to either *sn*$^+$ and/or *sn*^{e} G1 progeny (Table I). However, the males isolated from such lines, when back-crossed to attached-X *y w f* females, did not produce any red-eyed progeny. Subsequently, a w^1 *sn*^{w} stock was constructed and used as a recipient in experiment 4 in order to detect *white*$^+$ transformants on the basis of both the eye color phenotype and the *sn*^{w} interaction. In this experiment, seven out of 20 fertile adults produced *sn*$^+$ and/or *sn*^{e} progeny, and one of these also produced 13 red-eyed offspring.

Since no silent *P*(w^+) insertions have been discovered (see below), the estimated frequency of transformation in these three experiments is roughly two out of 341 injected eggs (= 0.6%) or 2.6% of the fertile adults. This relatively low frequency is similar to the one obtained by Hazelrigg *et al.* (1984). The 26 red-eyed G1 flies were either back-crossed to the recipient stocks or crossed among themselves in brother-sister crosses, and separate inbred lines were established by isolating single pairs which yielded only red-eyed progeny in subsequent generations. The lines were made homozygous by inbreeding or the use of balancer chromosomes. Similarly *sn*$^+$ and *sn*^{e} homozygous lines were established. Some of the lines proved to be unstable (see below) which presumably is due to the presence of active P-transposons. The possibility that the red-eyed flies carried a non-allelic suppressor mu-

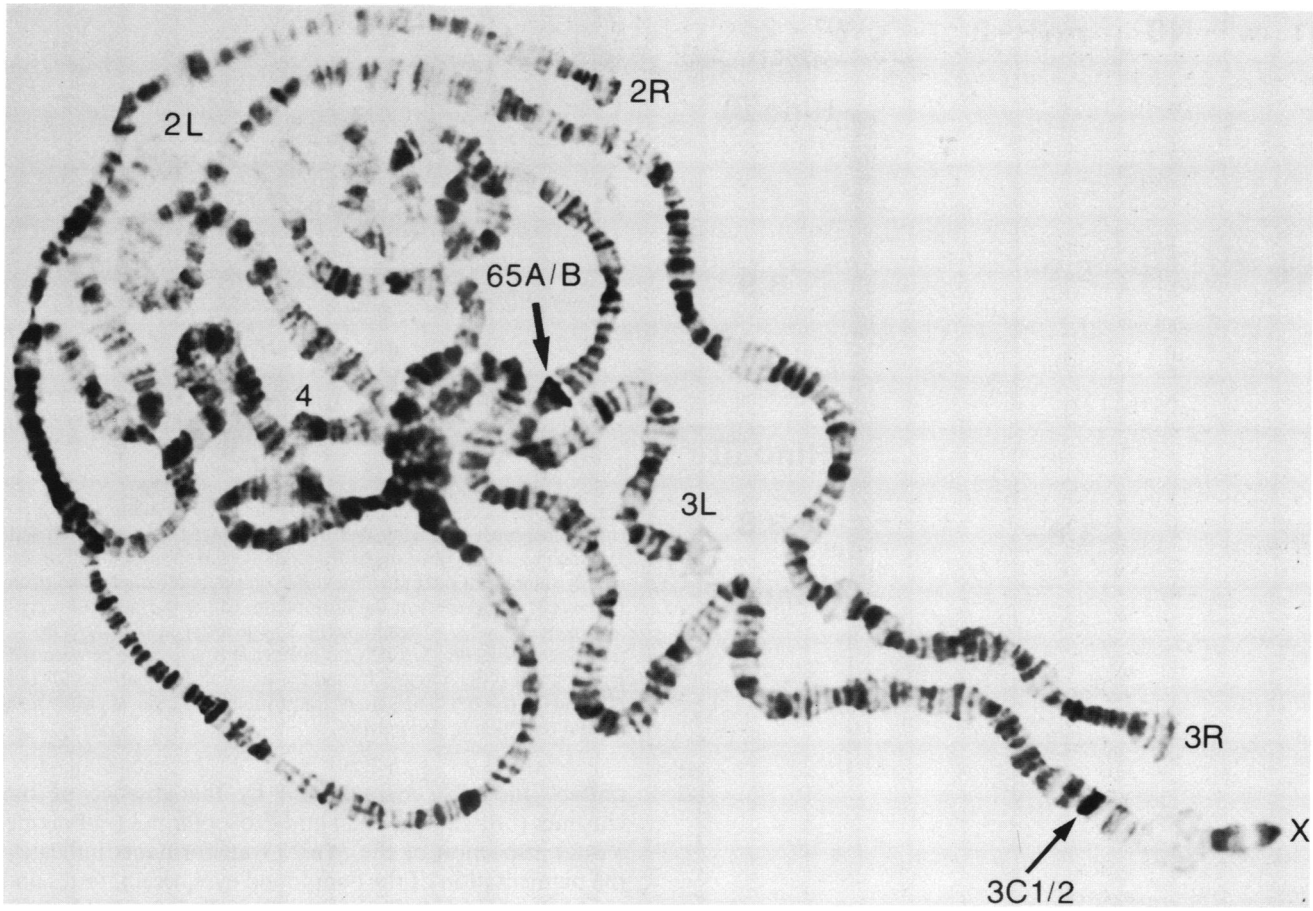

Fig. 2. *In situ* hybridization of a w^+ probe (pAT365.2) to polytene salivary gland chromosomes of the transformant *w47.1 N8*. In addition to the w^1 locus at 3C1/2 one additional site of hybridization at 65A/B on the left arm of chromosome 3 is labeled. The probe was labeled with biotinylated dUTP and the hybridization visualized by an immunoperoxidase reaction.

tation suppressing w^1 was ruled out by establishing a homozygous line in which the original w^1 mutation was replaced by a deletion, *Df(1)w*$^{-55j}$.

Localization and structure of the transduced DNA

Firstly, the w^+ marker was assigned to a given chromosome by genetic mapping using marked balancer chromosomes. Subsequently, the transduced DNA sequences were localized by *in situ* hybridization to polytene chromosomes of homozygous transformant lines. Three different types of probes were used for *in situ* hybridization: a w^+ probe (plasmid pAT-365.2; see Figure 1), or phage M558 carrying sequences from −4.5 to +9.3 of the w^+ locus (Goldberg *et al.*, 1982), a P-transposon probe (pπ25.1) and a probe for bacterial vector sequences (p3013) which carries a single copy sequence of 4.7 kb from the 67B heat-shock locus inserted into pBR322. In the w^1 homozygous recipients the w^+ probe (pAT365.2) hybridizes exclusively to the w^1 locus at 3C1/2 on the X chromosome. M558 also hybridizes to 3C1/2 but in addition, a second, much weaker, site of hybridization was found at 21D on the second chromosome (see Figure 10). This partial cross-homology has been noticed previously by K.Block and G.Ising (personal communication). In the recipient stock, the P probe hybridizes only to the 17C locus on the X chromosome from which it originates (Spradling and Rubin, 1982), and the bacterial vector control hybridizes exclusively to the 67B heat-shock locus because of its single copy insert. The results of the *in situ* hybridizations are summarized in Table II. Among the progeny of the G0 fly *w47* (experiment 2) initially two w^+ insertion sites were detected, one at 82A close to the centromere on the right arm of the third chromosome (3R) and another one at 65A/B (Figure 3) on the left arm of the third chromosome (3L). The transformants recovered from fly *w20* (experiment 4) showed transduced w^+ sequences at 22B on the left arm of the second chromosome (2L) and in section 45 on 2R. The mapping data obtained by *in situ* hybridization are consistent with the genetic mapping. Most lines carried only one copy of w^+, but two lines carried two copies (Table II). Since the *in situ* hybridizations were carried out after several generations of inbreeding, recombination may have occurred and it is difficult to reconstruct the early events following transformation. At a later date, evidence for spontaneous secondary transposition was found in several lines by *in situ* hybridization. The P probe labeled between one and 13 additional chromosomal sites which in many cases were variable from larva to larva, and changed in the course of the generations. No bacterial vector sequences were detected (the limit of detection would be ~1 kb). In experiment 4, eight mutable *sn*$^+$ or *sn*^{e} lines established from three different G0 flies were tested for the presence of 'silent' w^+ insertions by *in situ* hybridization. In all of the eight lines only one site of hybridization of the w^+ probe was detected at the original *w* locus. No 'silent' w^+ copies were detected in this experiment.

In the following, two homozygous transformants, *w47.1 G1* and *w47.1 N8*, which show a single additional site of

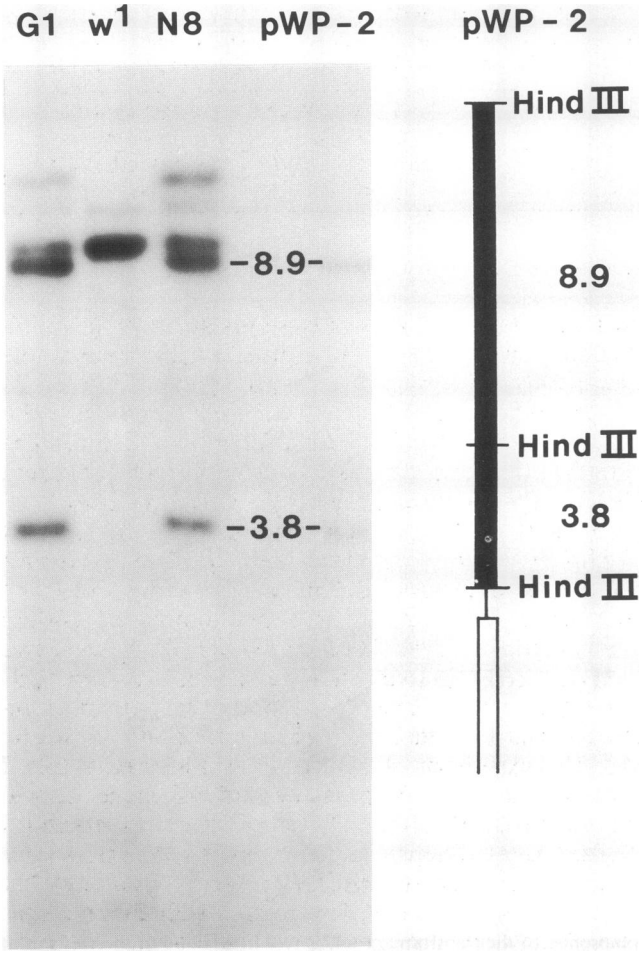

Fig. 3. Analysis of the transduced DNA by Southern blotting. DNA from two transformants *w47.1 G1* and *w47.1 N8*, the w^1 recipient flies, and the plasmid pWP-2 was digested with *Hind*III restriction endonuclease, separated on a 0.5% agarose gel, transferred and probed with the w^+ probe M558. For comparison, a restriction map of pWP-2 is shown at the bottom. The two major restriction fragments of 8.9 and 3.8 kb spanning the entire w^+ insert and most of the flanking P sequences are present in the transformants, in addition to the resident w^1 sequences. The two largest restriction fragments are due to incomplete digestion.

hybridization with the w^+ probe at 82A and 65A/B, respectively, and few sites of P insertions, were chosen for further analysis. The structure of the transduced DNA was analyzed by whole-genome Southern blotting. As shown in Figure 3 the transduced DNA includes the two large *Hind*III fragments that span the entire w^+ insert and most of the flanking P sequences. This is confirmed by the analysis of the *Sal*I digest (data not shown): the two *Sal*I restriction sites flanking the insert in pWP-2 (Figure 1) are not present in the transforming DNA, indicating that the integration must have occurred between the *Hind*III sites in the P sequences and the *Sal*I sites on either side. These results are consistent with the assumption that the donor DNA is transduced as a $P(w^+)$ transposon (proven below), leaving the bacterial vector and sequences outside the transposon behind.

Expression of the white$^+$ gene in the transformants

Analysis of the eye pigments. In contrast to the wild-type which has red eyes, red ocelli, and yellow testis sheaths and larval Malpighian tubules, the w^- mutant has pure white eyes and colorless ocelli, testes and larval Malpighian tubules (see Phillips and Forrest, 1980). At the biochemical level the w^- mutant flies are characterized by the absence of ommochromes (brown pigments) and drosopterines (red pigments). Visual inspection of the $P(w^+)$ transformants indicates that the pigmentation of the compound eyes, ocelli, testes and larval Malpighian tubules is virtually indistinguishable from the wild-type and clearly differs from the w^1 mutant phenotype. The individual variability in the eye color phenotype is somewhat larger among the transformants than in wild-type stocks. For example, among the progeny of transformant *w47.2*, some individuals with a more brownish-red eye color differing from the Oregon-R wild-type were found. However, attempts to isolate pure brownish-red lines by crossing brownish-red virgins to brownish-red males and continued inbreeding failed, indicating that the variability is phenotypic or unstable.

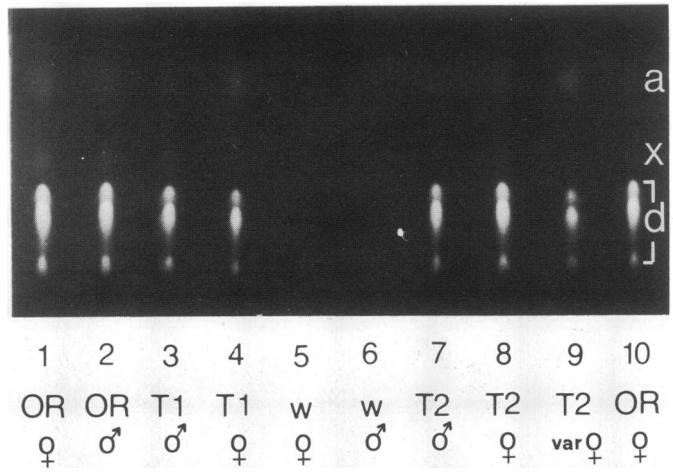

Fig. 4. Analysis of the eye pigments by thin-layer cellulose chromatography. Five adult flies of each genotype were extracted and fractionated as described under Materials and methods. Not all of the weakly fluorescent pteridines are visible on the photograph. The strongly fluorescent spots represent the drosopterins (d); a = 2-amino-4-hydroxypteridine; x = xanthopterin. The *w* recipient flies show no fluorescent pigments. Virtually no difference is detectable between the wild-type controls (OR = Oregon R) and the two transformant lines T1 (*w47.1 N8*) and T2 (*w47.2*). The variant transformants (T2 var ♀) show a slight general reduction in drosopterins.

The eye pigments of several transformed lines were analyzed by t.l.c. Figure 4 shows a one-dimensional chromatogram of transformants *w47.1 N8* and *w47.2* in comparison with the w^1 recipients and w^+ controls. In the case of the *w47.1 N8* transformants, and presumably also in *w47.2*, each fly carries two copies of the w^+ gene, as in the wild-type control females. The wild-type control males carry only one copy of the gene. The problem of dosage compensation will be examined in a separate study. Figure 4 mostly shows the strongly fluorescent red-orange drosopterins, whereas the more weakly fluorescent pteridines are hardly visible on the photograph. No consistent differences were found between transformants and wild-type controls. The transformants clearly differ from the w^1 recipients which virtually lack drosopterins. Only the brownish-red flies isolated from the *w47.2* stock by visual inspection show slightly reduced amounts of drosopterins, but qualitatively all the pigments seem to be present. By two-dimensional t.l.c. the drosopterins can be separated into four components (Wilson and Jacobson, 1977) as shown in Figure 5. The transformants *w47.1 G1* and *w47.1 N8* show the same pattern as the wild-type controls. A minor quantitative difference is found for auro-drosopterin which is

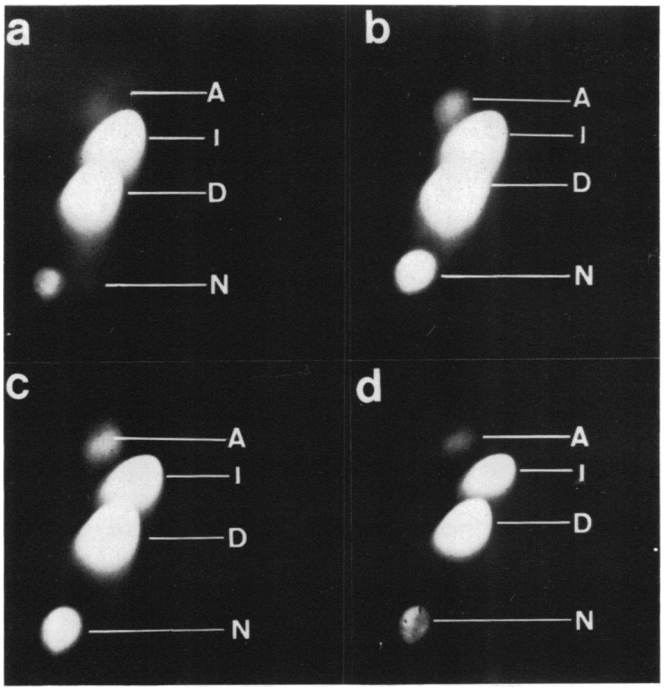

Fig. 5. Analysis of drosopterins in two transformant lines by two-dimensional t.l.c. The fractionation procedure of Wilson and Jacobson (1977) was followed. Five adult flies from each genotype were extracted and the equivalent extract of one fly was loaded per thin-layer plate. First dimension: isopropanol-2% ammonium acetate (1:1); second dimension: 3% ammonium chloride. A = Aurodrosopterin, I = Isodrosopterin, D = Drosopterin, N = Neodrosopterin. **(a)** Wild-type (Oregon-R) ♀; **(b)** *w47.1 N8* ♀; **(c)** *w47.1 G1* ♀; **(d)** *w47.1 G1* ♂.

Table III. Relative xanthommatin content of transformed and control flies

Genotype		Absorption at 492 nm[a]
wild-type (Oregon-R)	positive control	0.13
w47.1 G1	transformant	0.15
w47.1 N8	transformant	0.16
white^1	recipient (negative)	0.01
scarlet	negative control	0.04

[a]The relative xanthommatin content was determined by extraction of 100 adult females, 48 h old and measuring the absorption at 492 nm, as described in Materials and methods.

slightly more abundant in the transformants than in the controls, but the difference may not be significant.

The ommochromes which represent the brown eye pigments were examined by extraction of xanthommatin, the major ommochrome pigment and determining its relative concentration spectrophotometrically (Wiley and Forrest, 1981). The results compiled in Table III indicate that the transformants contain at least as much xanthommatin as the wild-type controls. Whereas *scarlet*, a mutant blocked in ommochrome biosynthesis (Phillips and Forrest, 1980), and *white* lack xanthommatin. Therefore, these biochemical studies of the eye pigments indicate that the expression of the w^+ gene in $P(w^+)$ transformants is virtually normal.

The interaction with zeste. A sensitive genetic test for the proper expression of the *white* gene is based upon the interaction of *white*$^+$ with *zeste*^{1}, which maps further distal on the X chromosome. Flies homozygous for the mutation *zeste*^{1} (z^1)

P	att. X	z^1 w^- *sn*/Y;+/+	♀ x *w*/Y;P w^+ /TM3 ♂
F1	att. X	z^1 w^- *sn*/Y;TM3/+	♀ x *w*/Y;P w^+ /+ ♂
F2	att. X	z^1 w^- *sn*/Y;P w^+ /TM3	♀ x *w*/Y;P w^+ /TM3 ♂
F3	att. X	z^1 w^- *sn*/Y;P w^+ /P w^+ *zeste* ♀	*w*/Y;P w^+ /P w^+ red ♂
	att. X	z^1 w^- *sn*/Y;P w^+ /TM3 red ♀	*w*/Y;P w^+ /TM3 red ♂

Fig. 6. Genetic crosses to test the *zeste-white* interaction.

which carry two w^+ genes in close juxtaposition (z^1 w^+/z^1 w^+) have lemon-yellow eye color, whereas *zeste* homozygotes carrying only one copy of the w^+ gene (z^1 w^+/z^1 w^-) show wild-type red eye color (Gans, 1953; Jack and Judd, 1979). This interaction is highly specific for the w^+ gene and requires the presence of an intact white proximal locus (Green, 1959; Judd, 1961). By crossing a male transformant *w47.1 N8* heterozygous for the balancer chromosome TM3 to an attached-X z^1 w^- *sn* virgin as outlined in Figure 6, females homozygous for z^1 carrying either one or two doses of $P(w^+)$ at the homologous autosomal site were constructed. As indicated in Figure 6, in the F3 generation females heterozygous for $P(w^+)$, ($P(w^+)$/TM3) show red eye color, whereas the homozygotes $P(w^+)/P(w^+)$ are phenotypically *zeste* and show the characteristic lemon-yellow eye color with fine red spots. This experiment shows that $P(w^+)$ inserted at 65A/B in transformant *w47.1 N8* produces a normal interaction with *zeste*, indicating that the w^+ gene is normally expressed. *w47.1 G1* gave a different result. In this case the homozygous females give only a partial *zeste* phenotype: the posterior half of the compound eye is yellow colored with fine red spots (*zeste* phenotype), but the anterior half of the eye has a brownish-red color. A similar phenotype has been described by Hazelrigg *et al.* (1984, Figure 5d) and attributed to a position-effect due to the insertion of the $P(w^+)$ into the β-heterochromatin near the base of chromosome arm 2L. Since *w47.1 G1* has inserted at 82A relatively close to the heterochromatin at the base of 3L, the partial *zeste* phenotype may also be due to a position-effect in this case.

Localization of w^+ transcripts in the transformants by in situ hybridization

In situ hybridization of w^+ cDNA clones to RNA transcripts in tissue sections of wild-type embryos and larvae, described in the accompanying paper (Fjose *et al.*, 1984), indicates that the w^+ gene is expressed specifically in the Malpighian tubules and in the eye imaginal discs. This is in agreement with previous transplantation experiments and observations on position-effect variegation which indicate that w^+ is expressed autonomously in these cells. An additional site of hybridization was detected in the pharyngeal apparatus, in cells which are associated with the larval photoreceptor organ. To investigate whether w^+ is tissue-specifically expressed in transformed larvae, sections of two homozygous transformants, *w47.1 N8* with the genotype w^1/w^1; $P(w^+)$/$P(w^+)$, and *w47.13 1* which is homozygous for both the deletion $Df(1)w^{-55j}$ *spl* and $P(w^+)$, were hybridized with a w^+ cDNA clone (pAT e1, Fjose *et al.*, accompanying paper). Specific hybridization signals were detected over the eye imaginal discs (Figure 7), the cells of the Malpighian tubules (Figure 8a and b), and the pharyngeal cells associated with the larval eye (Figure 8c and d) as in the wild-type (w^+) controls. No hybridization signals were found in $Df(1)w^{-55j}$ and w^1

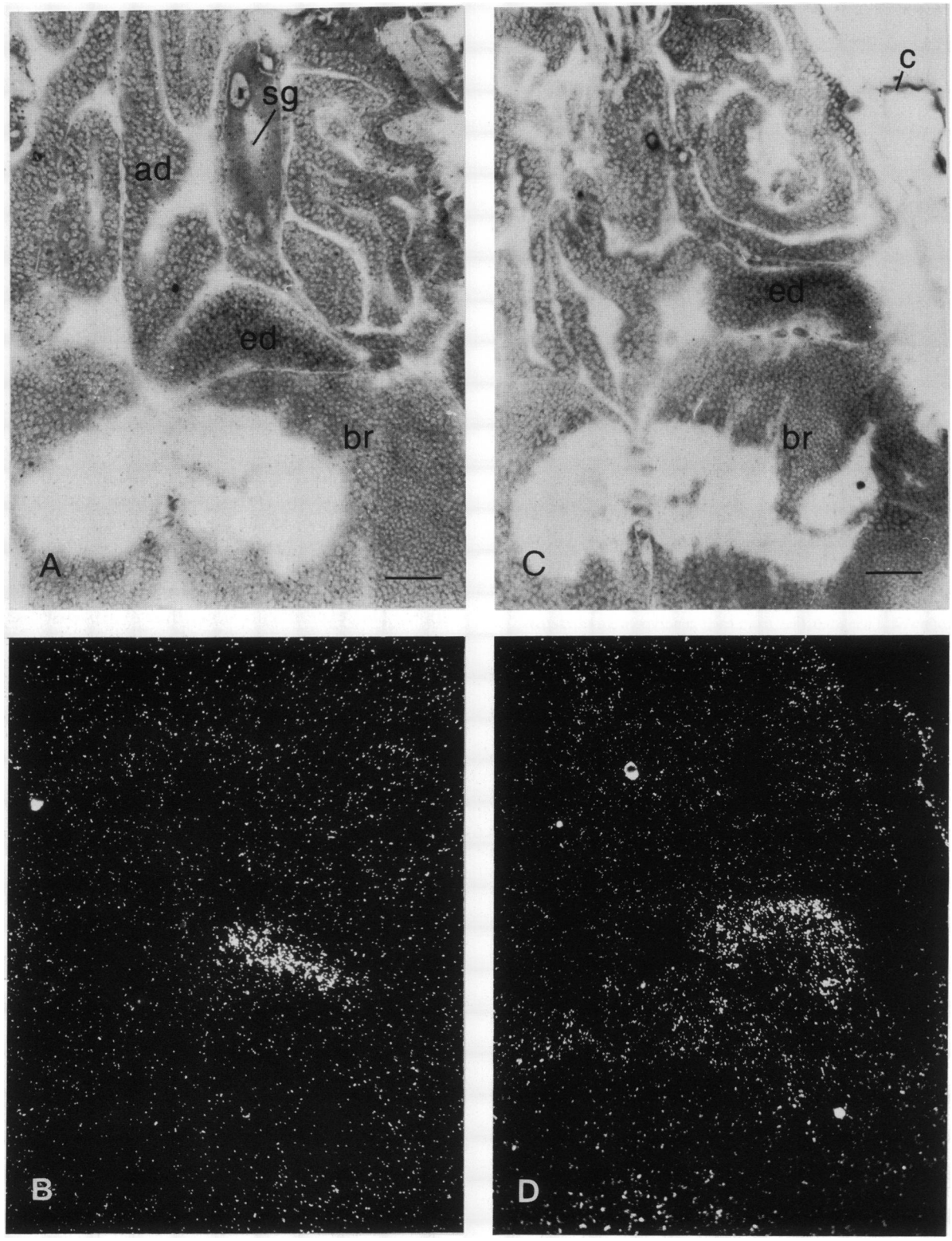

Fig. 7. *In situ* hybridization of cloned w^+ cDNA to tissue sections of third instar larvae. **(A)** Section through the head region of a wild-type (Oregon-R) larva; **(B)** corresponding dark-field photograph; **(C)** section through the head region of a transformant larva (*w47.1 N8*); **(D)** corresponding dark-field photograph. ad = antennal disc, br = brain hemisphere, c = cuticle, ed = eye imaginal disc, sg = salivary gland duct. Specific labeling is observed over the eye imaginal disc. Probe: pAT e1 (Fjose *et al.*, 1984). Time of exposure: 7 weeks. The horizontal bar represents 50 μm.

larvae. Therefore, the w^+ gene appears to be expressed normally in the transformants with respect to tissue specificity. Subtle quantitative differences in gene expression would not be detected by our method, but at least qualitatively the pattern of expression is the same as in the wild-type controls.

Secondary mobilization of the white$^+$-P-transposon

If the w^+-P hybrid plasmid has been integrated in the transformants as a transposon, flanked by the inverted repeats of P, it should be possible to mobilize it by a P-M interaction.

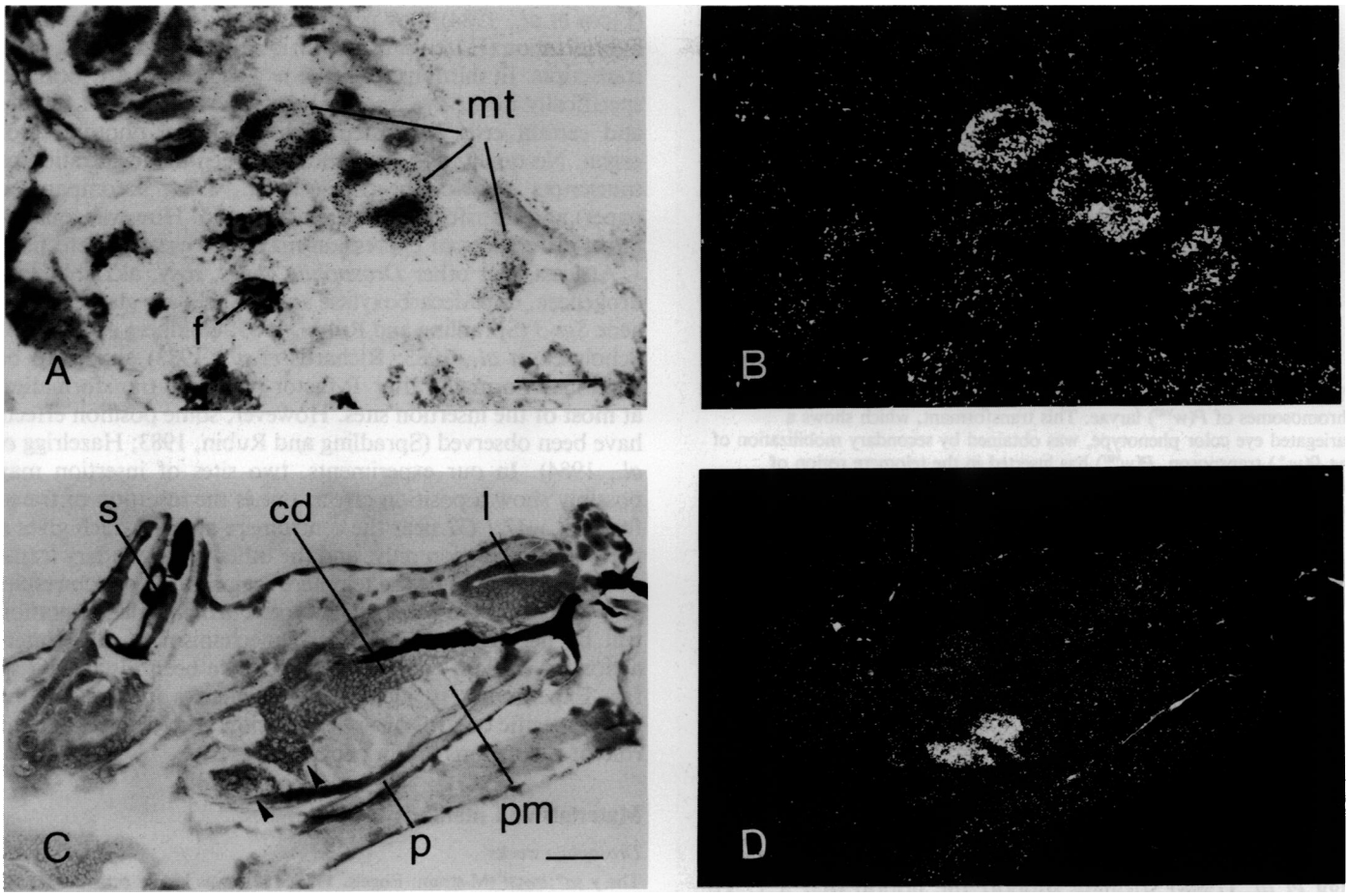

Fig. 8. *In situ* hybridization of cloned w^+ cDNA to sections across the Malpighian tubules and the pharynx of transformed third instar larvae (*w47.1 N8*). **(A)** Malpighian tubules. **(B)** Corresponding dark-field photograph. Only the cells of the Malpighian tubules (mt) are specifically labeled. Little or no labeling is observed over the fat-body cells (f). **(C)** Oblique longitudinal section through the pharynx region. **(D)** Corresponding dark-field photograph. cd = cibarial disc, l = labial disc, p = pharynx, pm = pharyngeal muscles, s = spiracle. Arrows: labeled cells associated with larval photoreceptor organ. Probe: pAT e1. Exposure time: 4 weeks. The horizontal bar represents 50 μm.

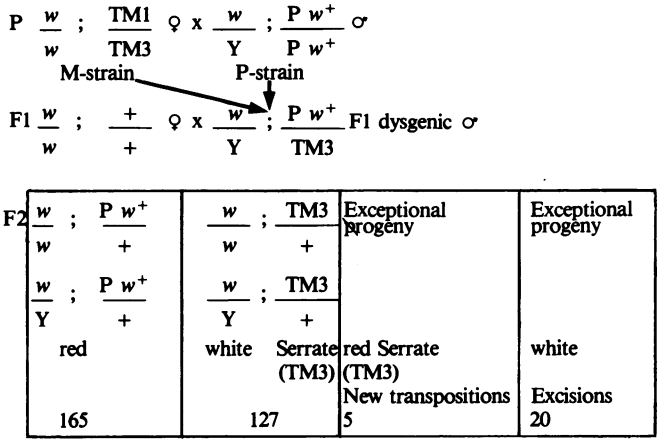

Fig. 9. Test cross for secondary transpositions.

For these experiments we used the *w47.4 L* line which originally contained five P-transposons. After approximately one year of culturing, this line had accumulated up to 15 P-transposons in addition to $P(w^+)$, which maps on the third chromosome. If this line behaves as a P strain when crossed to M females one would expect that mobilization of $P(w^+)$ can occur. The test cross is outlined in Figure 9. *w47.4 L* males (P strain) are crossed to *w* females (M strain) heterozygous for two balancer third chromosomes. The F1 hybrid dygenic males (*w*/Y; $P(w^+)$/TM3) are back-crossed to *w* females. In this experiment, $P(w^+)$ behaves as a dominant third chromosome marker and should segregate from the balancer chromosome TM3, which carries the dominant *Serrate* marker. In the F2 most of the progeny are phenotypically either red or white Serrate as expected. However, some exceptional progeny carrying either both or none of the dominant markers were found. The exceptional *white* progeny, lacking both dominant markers, breed true and are interpreted as excisions of the $P(w^+)$ transposon. For at least three of the five red Serrate flies it was shown that they are new transpositions of $P(w^+)$ to other chromosomes. One of the new transpositions is X-linked, the second inserted into the TM3 chromosome, and the third differs from all other transformants in that it shows variegated red-orange eye color. *In situ* hybridization of a w^+ probe to polytene chromosomes of the variegated line show that the $P(w^{var})$ has inserted in the telomere region of the left arm of the second chromosome (Figure 10). It remains to be shown whether the variegated eye color is due to a position effect since the transposon has inserted near the repetitive sequences of the telomere, or whether there is a mutational change in the transposon itself. These mobilization experiments indicate

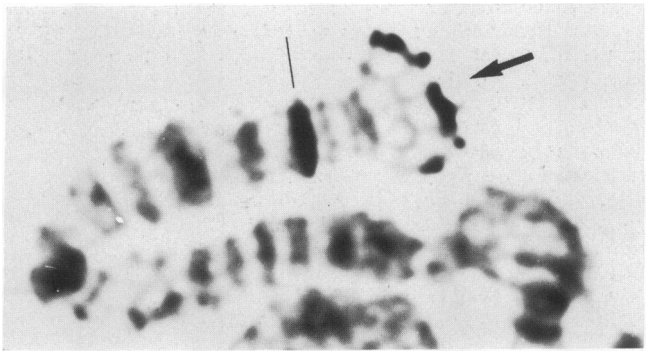

Fig.10. *In situ* hybridization of w^+ DNA to polytene salivary gland chromosomes of $P(w^{var})$ larvae. This transformant, which shows a variegated eye color phenotype, was obtained by secondary mobilization of the $P(w^+)$ transposon. $P(w^{var})$ has inserted in the telomere region of chromosome 2L (large arrow). The w^+ probe M558 cross-hybridizes weakly with some DNA sequences at 21D.

that the transduced DNA is inserted as a $P(w^+)$ transposon which can undergo further transposition events. In the same test cross two other transformant lines, *w47.1 N8* and *w47.1 G1*, did not produce any exceptional progeny.

Discussion

P-factor-mediated germ-line transformation provides a powerful functional assay for cloned genes. On the basis of chromosomal rearrangements, the w^+ gene has previously been assigned to a cloned DNA region from the X chromosome. The experiments reported above and those of Hazelrigg *et al.* (1984) strongly support the notion that a 12-kb DNA segment spanning the w^+ locus contains all the genetic information needed for the complementation of *w* mutants and the proper expression of the w^+ gene. This DNA segment contains the RNA coding region (O'Hare *et al.*, 1983; Pirrotta and Bröckl, 1984; Fjose *et al.*, 1984) and ~3 kb of flanking DNA on either side of the RNA coding sequences. It also accommodates all the *w* mutants that have been mapped physically, with the exception of w^{DZL} and possibly w^{Bwx} (Zachar and Bingham, 1982; O'Hare *et al.*, 1983). However, the dominant phenotype of w^{DZL} is most likely due to a position effect of transposed foreign DNA sequences on the w^+ locus (Levis and Rubin, 1982; Zachar and Bingham, 1982) and the physical localization of w^{Bwx} is uncertain (Hazelrigg *et al.*, 1984). P-factor-mediated transformation allows the insertion of a single defined piece of DNA flanked by short P sequences at numerous chromosomal sites with a relatively high frequency, of the order of a few percent. Our secondary mobilization experiments indicate that the transduced sequences are integrated as a functional $P(w^+)$ transposon, which is susceptible to a secondary P-M interaction.

In general, the transduced sequences seem to be expressed normally. Our analysis of the ommochrome and pteridine eye pigments shows no significant differences between transformants and wild-type flies, and the $P(w^+)$ can also interact properly with the *zeste*^{1} mutation (Gans, 1953; Jack and Judd, 1979) which is perhaps the most sensitive test for w^+ expression. Since pigmentation is relatively far removed from the primary gene action, we have also examined the tissue-specific localization of w^+ transcripts. Although w^+ transcripts are rare and constitute only between 0.0003% of the total poly(A)$^+$ RNA in embryos and 0.003% in larvae (Fjose *et al.*, 1984), our newly developed method for *in situ* hybridization (Hafen *et al.*, 1983) is capable of detecting these transcripts. In third instar larvae w^+ transcripts are localized specifically in the eye imaginal disc, the Malpighian tubules and certain cells associated with the larval photoreceptor organ. No qualitative differences between the localization of transcripts in wild-type (Fjose *et al.*, 1984, accompanying paper) and transformed larvae was found. However, quantitative differences of expression may have escaped detection.

At least four other *Drosophila* genes, rosy, alcohol dehydrogenase, dopadecarboxylase and the salivary gland protein gene *Sgs-3* (Spradling and Rubin, 1983; Goldberg *et al.*, 1983; Scholnick *et al.*, 1983; Richards *et al.*, 1983) appear to be expressed normally after P-factor-mediated transformation at most of the insertion sites. However, some position effects have been observed (Spradling and Rubin, 1983; Hazelrigg *et al.*, 1984). In our experiments, two sites of insertion may possibly show a position effect; one is the insertion of transformant *w47.1 G1* near the centromere at 82A which gives a partial *zeste* reaction only, and the other is a secondary transposition of $P(w^+)$ to the telomere region of 2L which results in a variegated eye color phenotype. Further experimentation will be needed to elucidate the mechanism of such interactions. No silent copies of $P(w^+)$ have been discovered so far. Experiments to identify the sequences involved in the tissue-specific control of w^+ expression and to determine the role of the introns are in progress.

Materials and methods

Drosophila stocks

The *y sn*^{w};*bw*;*st* (M-strain; Engels, 1979. 1981) was kindly provided by W. Engels. *Df(1)w*$^{-55j}$ is a partial deletion of the *w* locus (Goldberg *et al.*, 1982) and was obtained from M.M.Green. The attached-X z^1 w^- *sn* chromosome was constructed and kindly provided by G.Ising. The remaining stocks used are described by Lindsley and Grell (1967).

Construction of recombinant plasmids

The plasmid p6.1 which contains a defective P-transposon and which was generously provided by A.Spradling and G.Rubin was modified in such a way as to be more versatile for subcloning DNA fragments. p6.1 was cut with *Bam*HI and *Eco*RI, treated with S1 nuclease and religated in order to delete the 375-bp *Bam*HI-*Eco*RI fragment and to destroy the *Bam*HI and *Eco*RI sites. The 40-bp *Hae*III-*Hind*III polylinker fragment, isolated from pUC9, and p6.1 opened at the unique *Xho* site were made blunt ended by treatment with the Klenow fragment of DNA polymerase I and fused together with T4 DNA ligase. The resulting plasmid p6.3 contains, within the P-element sequence, unique sites for the restriction enzymes *Eco*RI, *Sma*I and *Bam*HI. The 12-kb *Eco*RI fragment spanning the region from −5.2 to +6.7 in the *white* locus was isolated from the recombinant phage M558 (Goldberg *et al.*, 1982) and inserted into the RI site of p6.3. This plasmid was designated pWP-2 and used in the transformation experiments.

Plasmid DNA was purified by preparing a cleared supernatant as described by Clewell and Helinski (1970) followed by banding in CsCl-ethidium bromide gradients.

P-factor-mediated germ-line transformation

Essentially the procedure described by Spradling and Rubin (1982; Rubin and Spradling, 1982) has been followed. The egg injections were carried out according to the method of Van Deusen (1976) developed in our laboratory, except that micropipettes of a diameter of 4−5 μm were used, which were filled from the back.

In situ hybridization to polytene chromosomes

The probes were labelled by nick-translation with biotinylated dUTP which was kindly synthesized by M.Mlodzik, using the 11-carbon linker arm, according to Langer-Safer *et al.* (1981). The hybridizations were carried out according to the procedure described by Langer-Safer *et al.* (1982) and the hybridized DNA sequences were detected by an immunoperoxidase method.

Fly DNA preparation and Southern hybridization

Fly DNA was prepared following a slightly modified protocol from W.

McGinnis (W.McGinnis, Ph.D. Thesis, University of California, Berkeley). Ten flies were homogenized in an Eppendorf tube with a glass pestle in 200 μl 0.1 M NaCl, 30 mM Tris-HCl, pH 8.0, 10 mM EDTA, 10 mM β-mercaptoethanol, 0.5% Triton X-100. After a 1 m in centrifugation at 10 000 *g* the supernatant was discarded and the pellet resuspended in buffer A (100 mM NaCl, 100 mM Tris-HCl, pH 8.5, 20 mM EDTA). After brief centrifugation the pellet was resuspended in 30 μl buffer A containing 1% sarcoysl and 100 μg/ml proteinase K and incubated at 50°C for 1 h. Following two extractions with phenol-chloroform and one with chloroform, RNA was digested with 100 μg/ml RNase A for 1 h at 42°C. After two phenol-chloroform and two chloroform extractions the DNA was precipitated with ethanol. 10 μg DNA was digested with restriction enzyme and the fragments separated on a 0.5% agarose gel. Transfer of DNA to nitrocellulose and hybridization with ^{32}P-labelled DNA was done according to Maniatis *et al.*, (1982).

Analysis of eye pigments

For one-dimensional chromatography of pteridine pigments, five adult flies of each genotype, aged for 3 days, were anesthetized, sonicated in 100 μl of a 2:1 mixture of n-propanol and 3.5% ammonia (v/v) in an Eppendorf tube, and centrifuged for 5 min. 10 μl of each extract were spotted on thin-layer cellulose plates (0.1 mm thick, Merck) and fractionated by chromatography using a 1:1 mixture of n-propanol and 2 M Na-acetate as a solvent. The dried chromatogram was examined under u.v. illumination at 366 nm and photographed with a u.v. filter (Vivitar).

Two-dimensional chromatography of drosopterins was carried out according to Wilson and Jacobson (1977).

Xanthommanin determinations were carried out according to Butenandt *et al.* (1960) and Wiley and Forrest (1981). 100 adult females of the different genotypes were anesthetized and homogenized in 3 ml 2 M HCl on ice. 4 ml of n-butanol and 5 mg of sodium metabisulfite ($Na_2S_2O_5$) were added and xanthommatin extracted by vigorous shaking in the cold. The phases were separated by centrifugation and the absorbance was measured at 492 nm.

In situ hybridization of cloned cDNAs to tissue sections

The procedure of Hafen *et al.* (1983) was used with minor modifications.

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