Drosophila "enhancer-trap" transposants: Gene expression in chemosensory and motor pathways and identification of mutants affected in smell and taste ability

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Abstract.   We have isolated about a thousand *Drosophila* P-element transposants that allow
the *in situ* detection of genomic enhancer elements by a histochemical assay for β-galactosidase
activity. We summarize the β-galactosidase staining patterns of over 200 such transposants
in the adult. Our aim was to identify genes that are likely to be involved in the chemosensory
and motor pathways of *Drosophila*. Based on β-galactosidase expression patterns in the
tissues of our interest, we have chosen some strains for further analysis. Behavioral tests on
a subset of the transposants have, in addition, identified several strains defective in their
chemosensory responses.

Keywords.   Olfaction; taste; muscle; *Drosophila melanogaster*; enhancer-trap; P-element.

1.   Introduction

The enhancer trap method devised by O'Kane and Gehring (1987) has been adapted
and widely applied to identify genes based on their pattern of expression (Bellen *et al.*
1989a; Bier *et al.* 1989; Grossniklaus *et al.* 1989; Wilson *et al.* 1989a). The method
was devised on the assumption that a reporter gene under the control of a weak
constitutive promoter, when brought in proximity to a tissue-specific "enhancer"
element (Serfling *et al.* 1985) would be regulated by the enhancer, resulting in the
expression of the reporter gene in a tissue- and stage-specific pattern similar to that
of the native gene normally controlled by the enhancer. The initial experiments
(O'Kane and Gehring 1987) strongly supported the hypothesis and subsequent studies

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have confirmed that, usually, a cDNA clone can be identified that corresponds to an mRNA expressed in a pattern similar to that of the reporter gene (Bellen *et al.* 1989a, b; Bier *et al.* 1989; Wilson *et al.* 1989a). The expression pattern of the reporter gene, therefore, often closely matches that of a nearby native gene.

One of the advantages of the enhancer-trap approach is that the pattern of expression of the reporter gene can be examined in animals heterozygous for the P-insertion, thereby allowing the analysis of gene expression in recessive lethals at all stages of development (Bellen *et al.* 1989b). Apart from being able to study gene expression *in situ*, the generation of strains of flies bearing single P-inserts allows the direct screening for specific mutant phenotypes and the subsequent rapid molecular analysis of the mutants.

Enhancer-trap expression patterns have been studied extensively, primarily in the embryo (Bellen *et al.* 1989a; Bier *et al.* 1989; Wilson *et al.* 1989a) and in the ovaries (Fasano and Kerridge 1988; Grossniklaus *et al.* 1989). The interests of our groups are in the analysis of the function and development of the chemosensory and motor pathways. We have analyzed over 200 transposants for the histochemical patterns of β-galactosidase expression in the adult brain, thoracic ganglion, antennae, proboscis and muscles. The patterns of expression of transposants of interest to us were then examined at earlier stages of development. In a complementary approach, transposants were tested for behavioral abnormalities and visible phenotypes. The staining patterns in these lines were subsequently analyzed.

Our experiments identify transposants that define loci likely to be involved in the development and function of the chemosensory and motor pathways.

2. Materials and methods

2.1 *Drosophila markers and strains*

All the markers and strains used are described in Lindsley and Grell (1968), in O'Kane and Gehring (1987) and Wilson *et al.* (1989b). The strains bearing the reporter gene and the transposase source were obtained from Cahir O'Kane and Hugh Robertson respectively. Flies were grown in standard corn meal medium (Ashburner 1989) at 22–25°C (room temperature) or at 25°C in an incubator.

2.2 *Isolation of P-element transposants: description of the scheme*

The P-element constructs used in the experiments have the following general properties (O'Kane and Gehring 1987): An in-frame fusion of the P-element transposase gene to the *E. coli lacZ* gene that encodes the enzyme β-galactosidase serves as the reporter gene. This fusion construct is under the control of the P-element transposase promoter which lies close to one P-element terminus and is therefore close to flanking genomic sequences at the site of insertion of the element in the host chromosome. The P-element/reporter constructs also have markers that confer an eye-colour phenotype in the appropriate genetic background and thus allow P-element mobilization to be assayed. More recent versions of the reporter construct (Bellen *et al.* 1989a; Bier *et al.* 1989; Wilson *et al.* 1989a, b) contain a plasmid origin of replication and a gene that confers drug-resistance to bacteria that harbor the plasmid; these features allow rapid
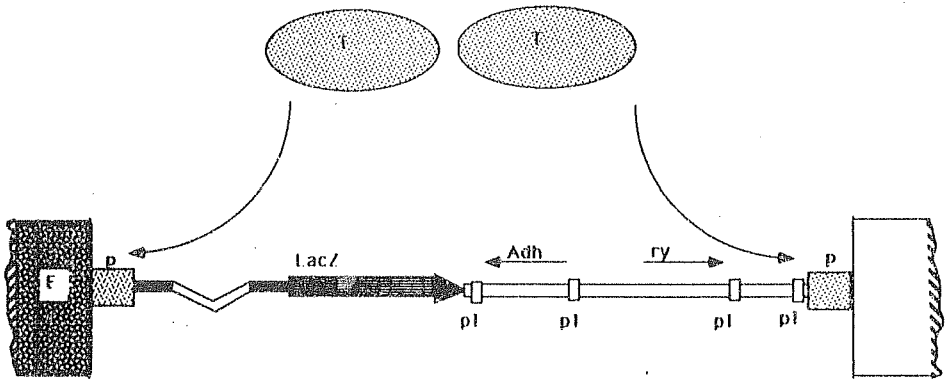
Figure 1. The P-element reporter construct for trapping genomic enhancers. This is a modified schematic representation of the plArB construct described in detail in Wilson *et al.* (1989b). The figure is not to scale and is taken from Vijay Raghavan *et al.* (1990). P = P-element ends. The weak constitutive promoter at the left end drives the reporter gene. LacZ = the transposase gene fused to the *E. coli* lacZ gene. E = genomic enhancer that controls the expression of the reporter gene. Adh = wild-type alcohol dehydrogenase gene from *Drosophila melanogaster* for use in selection schemes. *ry* = structural gene for xanthine dehydrogenase (*rosy*[+]), rescues the eye-color phenotype of the *rosy* hosts in transpositions. T = transposase provided from a genomic source. pl = polylinkers for cloning by plasmid rescue. The bacterial origin of replication and the gene that confers resistance to ampicillin in bacteria are between the two polylinkers on the right of the *ry* gene. These and other details have been omitted from the figure for clarity.

cloning of the *Drosophila* DNA flanking the insert by "plasmid rescue" (Pirrotta 1986). The transposase-*lacZ* fusion does not produce active transposase and the P-insert that contains it is therefore immobile in strains lacking the transposase (O'Kane and Gehring 1987). However, presumably because of the nuclear localization signals in the transposase, the fusion protein is almost always found in the nucleus (O'Kane and Gehring 1987; Bellen *et al.* 1989a; Bier *et al.* 1989; Wilson *et al.* 1989a).

Two sets of experiments were done to generate the P-insert bearing lines. In the first set, autosomal lines were generated and the reporter construct used was the original pLacZ construct of O'Kane and Gehring (1987). Subsequent experiments that generated X-chromosome lines used a more recent transposon construct, plArB (Wilson *et al.* 1989a, b), that allows rescue of flanking genomic DNA by plasmid rescue. The crosses to generate the autosomal and X-linked lines are described below. The features of the reporter are shown schematically in figure 1. The transposase source for mobilization of the reporter construct was the P[ry[+], Δ2–3](99B) referred to as Δ2–3(99B) for short in this paper. This source can mobilize, in *trans*, other P-elements but is itself immobile (Robertson *et al.* 1988).

2.3 *Autosomal transposants*

The autosomal transposants were isolated using the pLacZ element described in O'Kane and Gehring (1987). [*Tf*(1)*pLacZ, ry*[+]]/[*Tf*(1)*pLacZ, ry*[+]]; *ry*[506] virgin females were crossed to males of the genotype *Cy/Sp; P*[*ry*[+], Δ2–3](99B), *Sb/TM6, Ubx*. Male progeny carrying the Δ2–3(99B) chromosome can be identified by the *Sb* marker. The transposition events take place in the germline of these flies. These male

progeny were then crossed to ry^{506} flies. Transposant males were identified by their *rosy*$^+$ eye color and crossed to ry^{506} females carrying attached-X chromosomes. The insertion events were mapped to specific chromosomes using the strain $T(2;3)Ata/CyO; TM3, ry^{rk}$.

2.4   *X-linked transposants*

For the isolation of transpositions onto the X-chromosome, a p1ArB P-element containing transposant (Wilson *et al.* 1989b) on the second chromosome was used as the starting strain. Females carrying this element were crossed to $Cy/Sp; P[ry^+, \Delta 2-3](99B)$, $Sb/TM6, Ubx$. males. Male progeny carrying the transposase and the p1ArB element were crossed to females of the genotype $Df(1)Ni10/FM6, B; ry^{506}$ (the deficiency chromosome is used for maintaining the $FM6, B; ry^{506}$ stock and has no relevance otherwise). Females that were $FM6, B$ were screened for the presence of the *rosy*$^+$ marker. These flies could have transposition events on the X-chromosome or on the autosomes. X-insertions were detected in the next generation by crossing candidate transposants to $FM6, B; ry^{506}$ males. If all the bar-eyed female progeny were *rosy*$^+$, then this indicated an X-linked insertion. If half the progeny were *rosy*$^+$ then an autosomal insertion was indicated (note, however, that the *rosy* marker in $FM6, B$ males is not readily identified). The transposition frequency was estimated to be about 25–40%. This approximate figure was arrived at by dividing the number of transpositions on chromosomes other than the starting chromosome by the total number of chromosomes that could have had transpositions. These estimates were done during pilot experiments that generated about 20–100 transposants at a time. Thus, while exact numbers for the whole screen are not provided, the number of new transposition events is operationally very high permitting the ready use of this method even on a small scale. These results are comparable with those obtained in other studies (Bellen *et al.* 1989a; Bier *et al.* 1989; Wilson *et al.* 1989a). We have so far mapped several hundred transposants genetically. These results and our ongoing *in situ* hybridization to the salivary gland polytene chromosomes indicate that most transposants contain a single P-insert at a random chromosomal position.

2.5   *Histochemical analysis*

β-galactosidase activity was assayed histochemically at different stages of development using the chromogenic substrate X-Gal as described in Vijay Raghavan *et al.* (1986). Larvae were dissected in 10 mM phosphate buffer pH 8·0 and immersed in a drop of the staining solution (0·060 ml 5% X-gal; 0·020 ml 100 mM potassium ferrocyanide; 0·020 ml 100 mM potassium ferricyanide; 0·050 ml 1·0 M sodium phosphate pH 8·0 and 0·850 ml 35% Ficoll-400). Preparations were kept in a humid chamber to prevent the solution from drying and were usually analyzed after overnight staining. The tissue was then washed in 50% ethanol, mounted in an aqueous mounting medium (50% glycerol, 50% staining solution without X-gal) and viewed using a compound microscope. Adults were frozen in liquid nitrogen and cut in half with a sharp razor blade. The two halves of the fly were then dipped in a drop of staining solution and incubated for several hours in a moist chamber at room temperature or at 37°C. Cryostat sections of adult parts were also stained directly in the staining solution. Embryos were stained according to the method of Wilson *et al.* (1989b): Timed egg-collections were made on yeast–agar plates, the eggs washed off, dechorionated

in chlorox, washed, fixed and then stained. Most of the methods for handling flies and tissues are described in Ashburner (1989).

2.6 *Behavioral tests*

Odor-induced jump assay: The original assay (McKenna *et al.* 1989) as modified by Ayyub *et al.* (1990) was used. Air was blown through a bottle containing the odor solution which was connected by a teflon pipe to a glass tube. 2–4 day old flies were introduced into the glass tube in groups of five. The air flow was turned on when the flies reached two-thirds of the distance up the tube. A jumping event was scored if the fly jumped and hit the bottom of the tube within 5 seconds after the flow had begun. A total of 200 flies were tested for each odorant. Wild type flies were tested in parallel with the experimental sets. In each experimental set, flies were tested against water instead of odorant to test the noise level of jumps in response to air currents or humidity.

Taste behavior was measured by the feeding preference test of Tanimura *et al.* (1982). A 10×6-well microtiter plate was used for the test. Each well was filled with 1% agar solution, alternate wells also contained 2% of the food dye carmoisine red. To assay an attractant response, the stimulus was placed in the uncolored wells. For repellents, the substance was mixed into the wells with the food dye. In a blank plate, containing agar and dye, but no stimulus, flies eat randomly and become colored. Increasing concentrations of stimulus in the uncolored wells result in increasing percentages of uncolored flies. Similarly, an increasing percentage of uncolored flies is observed when the repellent is placed in the colored wells.

Flies grown in uncrowded bottles at 25°C were transferred into fresh medium and allowed to age for 2–4 days. They were then placed in bottles containing moistened filter papers 18 h prior to the test. Approximately 100 flies were introduced into each test plate and left undisturbed in a darkened area for one hour. They were immobilized by cooling and the color of their abdomens scored by inspection under a dissection microscope. Wild-type and mutant flies were tested in parallel under identical conditions. The mean and standard deviation of the response was calculated from a minimum of ten observations. The plates were run on at least three different days using independent batches of flies. Statistical difference between two means was calculated using the Student's *t*-test.

2.7 *In situ hybridization to salivary gland polytene chromosomes*

Chromosome *in situ* hybridization to salivary gland polytene chromosomes followed standard procedures (Langer-Safer *et al.* 1982; Ashburner 1989). A plasmid containing the complete *E.coli lacZ* gene was labelled with bio-16-dUTP (ENZO, New York, USA). Hybridization to salivary gland polytene chromosomes was followed by labelling the biotinylated DNA with streptavidin conjugated with horse-radish peroxidase (ENZO), the complex being detected by a color reaction using diamino-benzidene (Sigma, St. Louis, Mo, USA).

2.8 *Cloning flanking genomic DNA*

"Plasmid-rescue" of flanking genomic DNA followed the method of Pirrotta (1986). Total genomic DNA was extracted from 20–50 flies, digested with Hind III or Sal I,

ligated under conditions that favor mono-molecular ligations and transformed into bacteria that were selected on Ampicillin. DNA from colonies that grew under these conditions were analyzed by restriction digestion. DNA flanking the P-insert, thus cloned, was labeled with ^{32}P (Sambrook *et al.* 1989) and used to screen a wild-type genomic library constructed in the λ-Gem11 vector (Promega, Madison, Wisconsin, USA). Positive plaques were purified and phage DNA isolated.

3. Results and discussion

Over 1000 autosomal transposant lines were isolated. Of these 190 were studied for their pattern of expression of the reporter β-galactosidase gene. The screen for X-chromosomal inserts identified 22 transpositions on the X-chromosome out of 102 insertion containing lines. Thus, a total of 212 transposants were analyzed.

3.1 *Histochemical analysis of patterns of expression*

The histochemical staining pattern of β-galactosidase, using the chromogenic substrate X-gal was first examined in the adults. Our aim in this part of the study was to identify lines in which structures involved in chemosensory and motor responses were histochemically marked. These structures are the antenna, the proboscis, the brain, the thoracic ganglion and the thoracic muscles, all shown in figures 2 and 3a. Animals that stained in the structures of our interest in rapid preliminary screens were examined further in dissected whole-mounts, or cryostat sections. Histochemical assays for β-galactosidase activity at other stages were on dissected tissue (larvae) or whole mounts (embryos) as described in §2.

A summary of the histochemical patterns in the adult is shown in table 1. These results demonstrate that most transposants show β-galactosidase activity. Further, a large number of the transposants stain in the adult nervous system. A similar high frequency of staining in the embryonic nervous system has been found in other screens (O'Kane and Gehring 1987; Bellen *et al.* 1989a; Bier *et al.* 1989). Significantly, the number of inserts that stain in the muscle is small compared to those that stain the nervous system.

Figure 4 shows examples of the staining patterns in the adult. Histochemical activity for β-galactosidase is seen in the second and third antennal segment in the transposant BTJ620 (figure 4c). In this transposant the other tissues of the adult that are positive for β-galactosidase activity (data not shown) are the proboscis, the sub-oesophageal ganglion, the central brain and the thoracic ganglion. The optic lobe is not stained. Another transposant, BTJ409, shows β-galactosidase activity in the antenna and proboscis (figure 4b shows the proboscis). This transposant has been chosen for further analysis and more information on its phenotype is given below. Staining in the thoracic ganglion of BTJ511 is shown in figure 4a. Strong β-galactosidase activity is also seen in the brain in this line, but the staining in the antennae and the proboscis is weak. Amongst the autosomal lines, staining of muscles were clearly seen in two transposants, BTJ629 and BTJ325 (further information on BTJ325 and BTJ629 is given below). Examples of β-galactosidase expression in other developmental stages are shown in figures 5 and 6. Activity in the larval brain of BTJ409 is shown in figure 5a, the cell bodies of several neurons are positive. Figure 5b shows staining in the
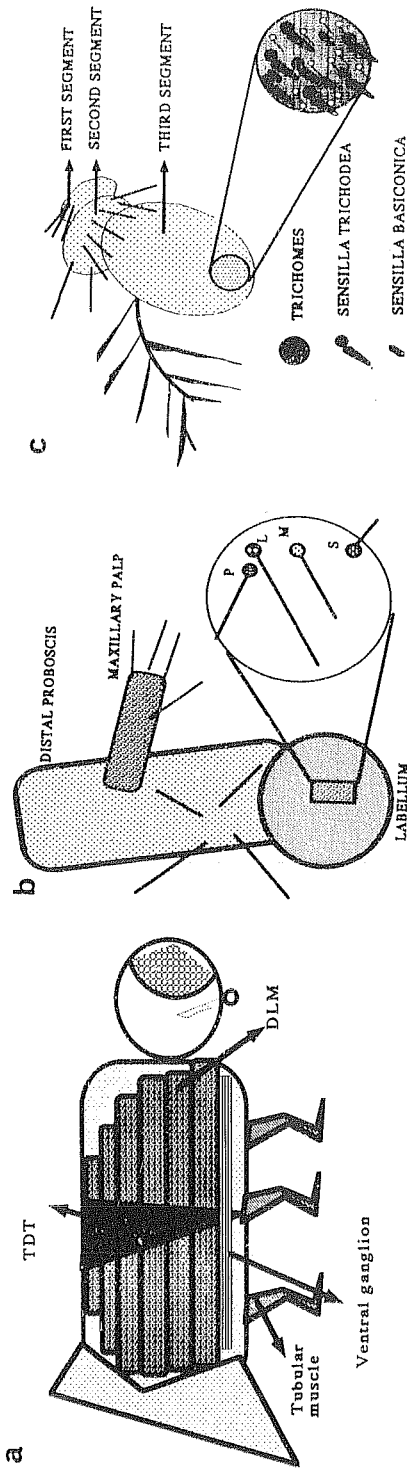
Figure 2. Schematic diagram of the tissues examined by β-galactosidase histochemistry in the adult. The antenna, proboscis, ventral ganglion, the thoracic muscles are shown (parts of the adult brain are shown in figure 3). (a) Saggital view of an adult fly, head to the right and abdomen to the left. The six dorsal longitudinal flight muscles (DLMs) are shown. The jump muscle, the tergal depressor of the trochanter (TDT) is shown in black. The muscles of the leg are called tubular muscles as is the TDT; (b) The proboscis. The taste hairs on the labellum are shown in the inset. P = peripheral, L = large, M = medial and S = small; (c) The antenna. Most of the chemosensory sensillae are on the third antennal segment. These are shown in the inset. (d) Longitudinal view of the adult thorax showing the major muscles. The major muscle mass consists of the indirect flight muscles (also called the fibrillar flight muscles), the DLMs and the dorso-ventral muscles (DVMs). The jump muscle or the TDT is shown. A more ventral view would show the ventral ganglion and additional tubular muscles.
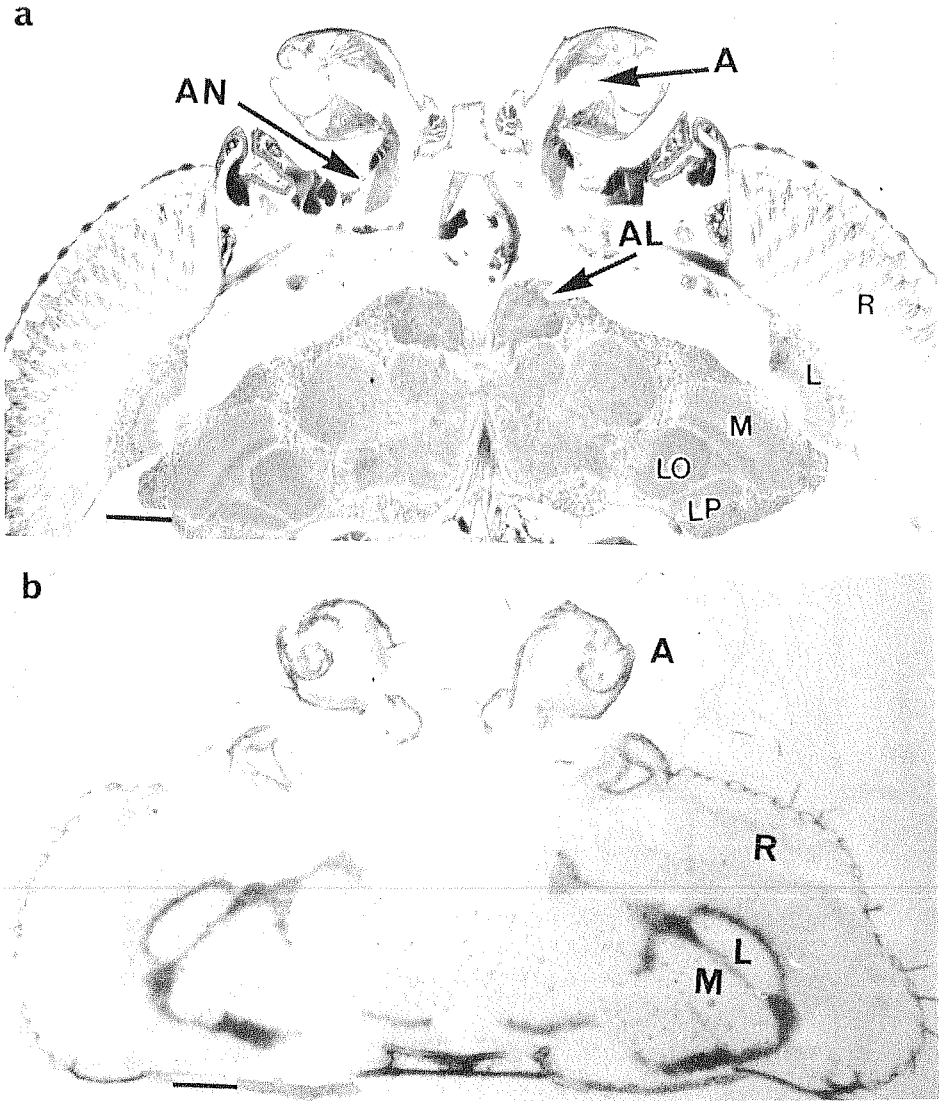
a

b
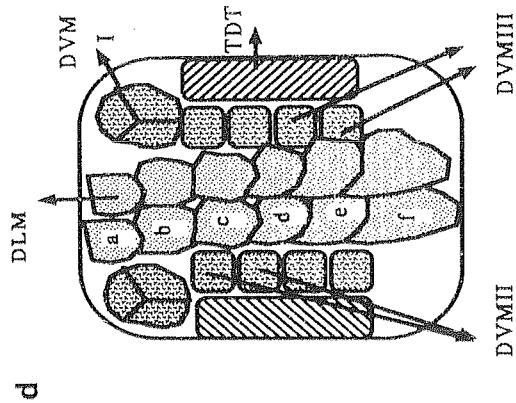
Figure 3. Parts of the adult brain and β-galactosidase expression in the cortex of the brain. (a) 3 μM thick horizontal plastic section through an adult head. Section stained with methylene blue and toluidine blue. A = antenna, AN = antennal nerve, AL = antennal lobe, R = retina, L = lamina, LO = lobula, LP = lobula plate, M = medulla. (b) 10 μM frozen section through the adult head of transposant, BTJ 641. The expression of β-galactosidase in the adult brain was visualized by a histochemical assay using the chromogenic substrate X-gal. Strong staining is seen in the cell bodies of the lamina and medulla in addition to other parts of the brain. Bar for (a) and (b) = 50 μm.

leg imaginal disc. Examples of embryonic staining patterns are shown in figure 6. β-galactosidase activity in BTJ154 is shown in figure 6a, where staining is seen in the precursors of the mouth parts. β-galactosidase activity in the nervous system of the developing embryo is seen in figure 6b. The patterns of expression of the reporter β-galactosidase gene are informative about the way tissue specific genes are regulated.

Table 1. Summary of staining pattern of the autosomal transposants.

Approximately 1000 p-lacZ transposants were isolated. Of these, adults from 190 strains have been examined in detail so far.
75% (143 out of 190) of the lines show activity in the adult and 25% do not. 65% (122 out of 190) of the lines are positive in the nervous system. 1·8% (2 out of 190) were strongly positive in the muscles, though 33% showed ambiguous or diffuse staining in some tubular muscles and 2% were similarly ambiguous in the flight muscles. All the strong muscle positives with the staining clearly localized in the nuclei were in the tubular muscles (BTJ 325 in the jump muscle and BTJ 629 in all the tubular muscles). Two clear fibrillar muscle positives were isolated in the X-chromosome screen out of only 22 transposants analyzed.

Adult tissue positive	Number of lines that stain
1. Antenna	92
2. Proboscis	47
3. Wing blade	8
4. Leg neurons	36
5. Tubular muscle	2
6. Thoracic ganglion	122
7. Brain	107

Several of the inserts show nervous system-specific staining. Amongst these almost all are expressed in a subset of cells in the nervous system, though there are a few that are expressed in most, if not all, cells of the nervous system. However, the pattern of expression varies considerably during development. For example, BTJ540 is expressed in very few cells in the nervous system of the embryo. In the third instar larva, however, staining in the ventral nerve cord is extensive. Amongst the transposants that express the β-galactosidase reporter gene in the adult brain the pattern of expression is different in each transposant. All the transposants that express β-galactosidase in the muscles do so in parts of the nervous system as well. What does the high percentage of reporter gene expression in the nervous system imply? One viewpoint, which we favor, is that this reflects the large number of genes that are expressed in the nervous system (Palazzolo *et al.* 1989). Another reason for the high frequency of staining in the nervous system could be due to better access of enhancer regions of this tissue to transposition in the male germline where the transpositions have taken place. We have not come across any evidence that suggests that this is the case. Product(s) of the same gene can be used in several tissues at different times during development. This is consistent with the complex patterns of expression that are observed. These conclusions are independently supported by analysis of the developmental pattern of expression of cDNA clones that correspond to low-abundance mRNA species (Palazzolo *et al.* 1989). cDNA clones corresponding to mRNAs that are expressed in the adult head, but not the 0–1 hour embryos, were labelled and used on blots of
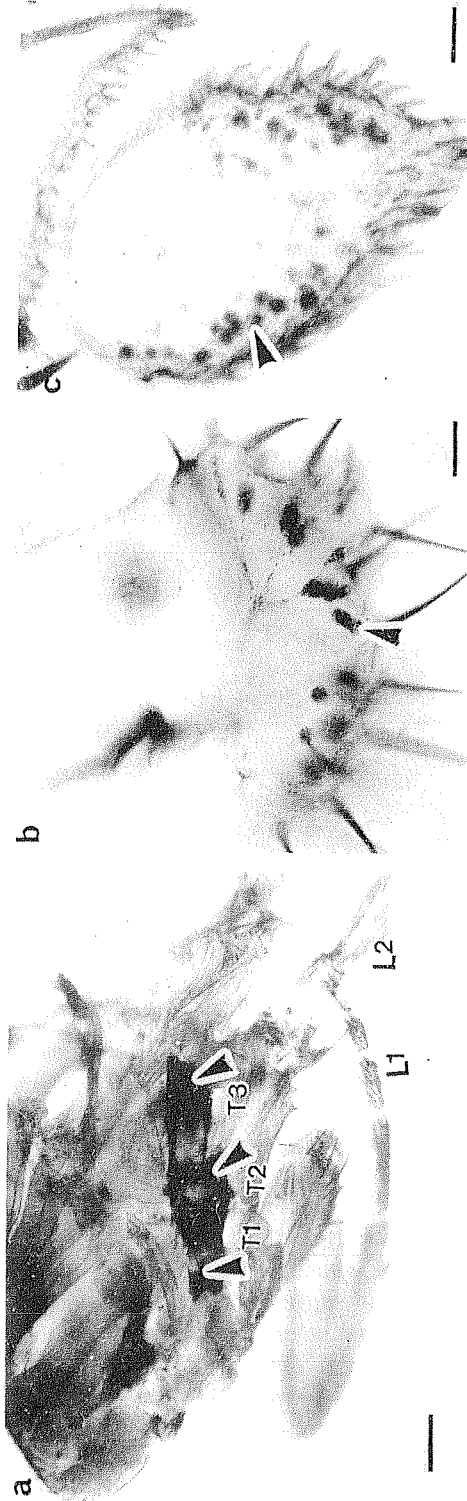
Figure 4. Gene expression in the antenna, proboscis and the thoracic ganglion, arrows point to the staining. (a) Thoracic ganglion staining. T2 = mesothoracic part of the ventral ganglion, T3 = metathoracic. L2 = second leg. L3 = third leg. Intense staining is seen in most cells in the ventral ganglion. Bar = 50 µm. (b) Proboscis staining in BTJ 409. Arrow points to staining in the cell bodies of the neurons innervating the taste sensilla. (c) Antennal staining in BTJ 620. Arrow points to labelled neurons in the third antennal segment. Bar in (b) and (c) = 20 µm.
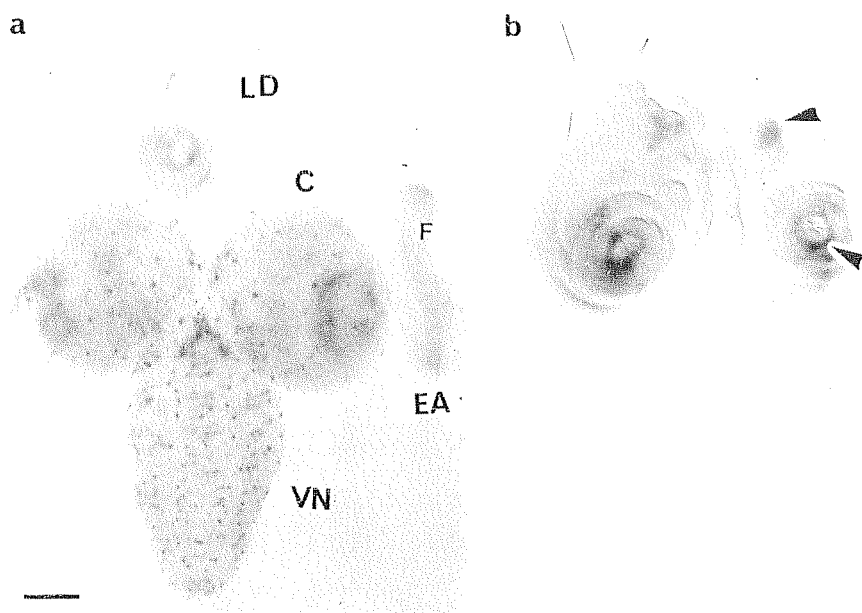
Figure 5. Staining in the larval brain and imaginal discs of BTJ 409. (**a**) β-galactosidase staining in the brain of the third instar larva. Dark staining is seen in the proliferation centers of the optic lobes and spots of staining show that a small number of cell-bodies are positive. Staining in the imaginal discs is confined to specific regions. VN = ventral nerve cord. C = cerebral cortex. LD = leg disc. EA = eye-antennal disc. F = morphogenetic furrow in the eye disc. Bar = 100 μm. (**b**) Staining in paired imaginal discs from a third instar larva. Arrow points to staining.

RNA from different tissues and developmental stages. Analysis of the blots showed a complex pattern of expression of the mRNAs. RNAs corresponding to some cDNA clones were expressed only in the head, others in the head and body, yet others in the head and 24-hour embryo, etc. The chromosomal sites of insertion of the transposants could therefore be in or near genes that are expressed in the male germline in addition to other tissues, since it has been suggested that P-element insertion is biased towards actively transcribed regions (Engels 1988). This can be tested by examining the staining patterns of the inserts in the male germline.

Of the transposants examined we have chosen a small number for detailed investigation based on the expression pattern of the reporter gene. The patterns of expression of some these in the adult are summarized briefly.

3.2 *Examples of transposants that express β-galactosidase in the chemosensory pathways*

The organization of the adult brain is shown in figure 3a. The sense organs that detect chemical stimuli are the multi-innervated sensillae which are located on the antenna, maxillary palps, proboscis (figure 2b, c), tarsi and wings. The olfactory neurons project from the antenna to olfactory centers in the antennal lobe (Pinto *et al.* 1988). The taste neuropil is in the sub-oesophageal ganglion (Nayak and Singh
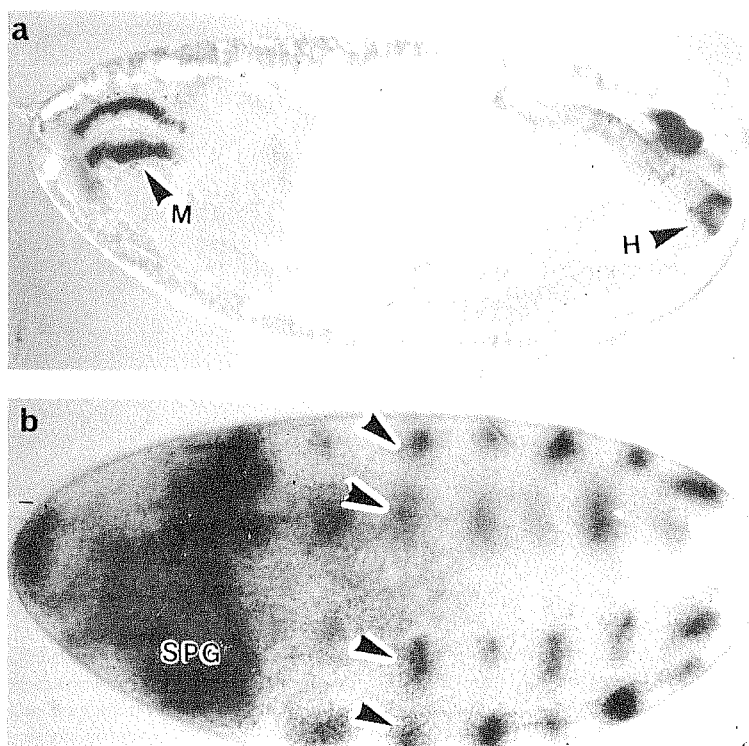
Figure 6. β-galactosidase staining in the embryo. Anterior is to the left. (a) Dorsal view of a BTJ 154 embryo showing staining of the cells that give rise to the mouth hook (M) of the larva and the region that gives rise to the hind gut (H). This is a late embryo where germ band retraction has taken place (Campos-Ortega and Hartenstein 1987). (b) Dorsal view (slightly rotated of an ETX 86 embryo. There is strong β-galactosidase activity in the head (SPG = supraesophageal ganglion). The cells that stain will form the brain of the first instar larva. Arrows point to staining on the surface of the embryo.

1983, 1985). Several of the transposants stain in the sensillae on the antenna and proboscis (examples shown in figures 4b and c). We have selected some lines, whose patterns of staining suggest that the trapped genes have roles in the development or function of the chemosensory pathways, for more detailed analysis and these are described below.

3.2a   BTJ409: Adults of this transposant stain for β-galactosidase activity in the antenna, proboscis, central brain and thoracic ganglion. In the antenna, a small subset of olfactory sensillae on the third antennal segment are stained. Mechanosensory sensillae on the second antennal segment are also stained. The staining in the sensillae of the proboscis and antenna is restricted to the bases of the hairs (e.g. figure 4b). The staining pattern observed in the antennae of BTJ409 is very similar to that shown for BTJ620 shown in figure 4b. Staining of cryostat sections with the neuron-specific antibody 22C10 suggests that the neurons innervating these sensillae are also stained (data not shown). In the brain β-galactosidase activity is observed in a small number

of cell bodies around the antennal lobes. In the larva, staining is seen in several cell bodies of the larval brain and in a small number of cells in the imaginal discs (figures 5a and 5b). The staining in the imaginal discs could represent activity in the precursors of the cells that are seen to be stained in the adult. If this is indeed so, BTJ409 will be a useful marker for the study of the development of the adult olfactory and taste neurons.

3.2b   BTJ540: A subset of antennal sensillae distinct from those staining in BTJ409 are stained. Neurons in the proboscis are also strongly stained. Staining in the brain is limited to the cell cortex of the mid-brain and excludes the optic ganglia. Staining is prominent in the dorsal brain near the calyx of the mushroom bodies. This region of the brain is likely to be involved in processing of olfactory information.

3.3   *Examples of transposants that express in the muscles*

The principal muscles of the fly are outlined schematically in figures 2a and d. The major muscles of the thorax are the fibrillar indirect flight muscles. The tergal depressor of the trochanter (TDT, or jump muscle) and the muscles of the leg are called tubular muscles. There are other muscles of the dorsal thorax, called the direct flight muscles and these are similar to the tubular muscles in their physiology.

3.3a   BTJ325: β-galactosidase activity is seen in the jump muscles, the muscles connecting the head to the thorax. The fibrillar flight muscles and the other muscles of the thorax do not stain. Light activity for β-galactosidase is detected in the brain and in the thoracic ganglion. Strong staining is seen in the lamina (figure 3a)

3.3b   BTJ629: A subset of tubular muscles of the adult stain, the fibrillar flight muscles being negative. The staining is intense and obscures the few tubular muscles that do not stain. These can however be observed in polarized light under a compound microscope. In the nervous system, few cells show β-galactosidase activity in the protocereberum and thoracic ganglion.

3.3c   ETX28: All the fibrillar muscles stain for β-galactosidase activity, the tubular muscles being negative. The staining pattern in this complements that of BTJ629 where no staining is observed in the fibrillar muscles.

3.4   *Behavioral assays for abnormal chemosensory responses*

Preliminary tests were undertaken to identify transposants that had abnormal chemosensory responses. The X-chromosome insert lines and a subset of the autosomal lines were tested in behavioral paradigms for their responses to smell and taste stimuli. The olfactory responses were measured by a stimulus-induced jump test (McKenna *et al.* 1989; Ayyub *et al.* 1990). This test measures the ability of flies to jump in response to an air stream bearing an odorant. Approximately 90% of wild type flies jump in response to undiluted benzaldehyde or isoamyl acetate. Any line in which less than 50% of flies responded positively was re-tested. ETX3 and ETX20 showed olfactory defects. The gustatory response was measured in a feeding preference test (Tanimura *et al.* 1982; Arora *et al.* 1987). Normal flies accept sucrose solutions and low

concentrations of sodium chloride, but are repelled by high NaCl and quinine. Three X-chromosome transposant lines-ETX3, ETX4, and ETX48 and an autosomal line ET6, were identified in the taste screens. The phenotypes of ETX3 and ETX4 are distinguishable. ETX4 shows a general taste defect while ETX3 is affected only in its response to salts. These tests identify candidate mutants for further genetic and molecular analysis.

With the exception of the olfactory phenotype of ETX3 which was dominant, all phenotypes were recessive. Behavioral experiments on strains in which the P-element insertions have been precisely excised are required to prove the association of the phenotype with the P-insert. Such experiments are in progress.

3.5 *Other phenotypes*

One of the transposants, ETX52 is located at chromosomal position 4C1 and is a recessive lethal. X-gal staining of lethal embryos shows a strong hypertrophy of the nervous system. This phenotype and its chromosomal location strongly suggest that this transposant is allelic to the neurogenic gene *Notch*. This conclusion is supported by the fact that a chromosomal duplication for *Notch* region rescues the lethal phenotype. We therefore tested ETX52 for complementation with a known mutant in the *Notch* locus. ETX52 fails to complement Notch[ts] (N[ts]).

ETX4, located at 13E-F on the salivary chromosome map, has a wing blade phenotype similar to the gene *scalloped* that maps in the same region. The phenotype and detailed complementation analysis with a deficiency and an independently isolated

Table 2. Chromosomal positions of some transposants.

Transposant	Chromosomal position
BTJ 36	4th chromosome
BTJ 154	77 F
BTJ 325[†]	100 D/E
BTJ 409*	70 A
BTJ 540*	99 C/D
BTJ 629[†]	102
BTJ 641	28 A
ETX3 (1, 2)	2B1
ETX4 (1, 2, 3)	13 EF
ETX6	74 E
ETX13 (L)	14 EF
ETX20 (1, 2)	1B1
ETX52 (4, L)	3 CD
ETX81 (L)	13 EF

* – β-galactosidase staining pattern of interest for chemosensory analysis.[†] – β-galactosidase staining pattern of interest for muscle development.
1 – Chemosensory mutant phenotype. 2 – Flanking genomic DNA cloned. 3 – Insertion in the *scalloped* locus. 4 – Insertion in the *Notch* locus. L = recessive lethal.

P-insert in the region (Manisha Bhave, personal communication) suggest that ETX4 is indeed a *scalloped* mutant.

ETX100 on the X-chromosome has wing position abnormality and β-galactosidase staining of the adults mark the flight muscles. These results suggest that the upheld wing phenotype is due to a muscle abnormality.

3.6 *In situ hybridization to the salivary gland polytene chromosomes and cloning flanking genomic DNA*

The chromosomal localization of some of the transposants was done using biotinylated lacZ DNA as a probe on the salivary gland polytene chromosomes. These chromosomal positions are shown in table 2. The genomic DNA flanking the P-insert has been cloned in several cases and genomic clones isolated from a wild-type genomic library. These results are also listed in table 2.

4. Conclusion

Understanding the molecular basis of the functional complexity of the nervous system requires the study of a large number of molecules (Palazzolo *et al.* 1989). The enhancer trap approach is one way to identify molecules that are expressed in the nervous system. The expression of the reporter gene in the nervous system suggests the presence of a nearby gene whose expression pattern matches the β-galactosidase activity observed histochemically. In several cases analyzed, this indeed seems to be the case (e.g. Bellen *et al.* 1989; Bier *et al.* 1989; Wilson *et al.* 1989a). The analysis of transposants identified by this method therefore requires rapid isolation of cDNA clones from the region of insertion. The design of the new P-element vectors (Wilson *et al.* 1989b) and other advances in cDNA cloning systems (Palazzolo *et al.* 1990) readily allow the isolation of cDNA clones, the study of patterns of RNA expression by *in situ* hybridization to tissue, the generation of antibodies to proteins encoded by the RNA and the analysis of the function of the gene product *in vivo* by the study of mutants in the gene of interest.

The *in situ* pattern of reporter gene expression is not an all-encompassing way to identify important loci. In our experiments, this is best illustrated by the insertion that we obtained in the *Notch* locus. In this transposant (ETX52) the pattern of expression of β-galactosidase in animals heterozygous for the P-insertion would not have identified the gene as important for neurogenesis since the gene product is expressed in many tissues. However, the insertion results in a recessive lethal phenotype that shows a strong hypertrophy of the embryonic nervous system. Thus, it is important to be aware that the "enhancer-trap" method is one way to identify genes of interest but is not a method for identifying all genes. Other recent methods that complement this approach are the isolation of rare cDNA clones (Palazzolo *et al.* 1989) combined with reverse genetics (Ballinger and Benzer 1989; Hamilton *et al.* 1990; Kaiser and Goodwin 1990) and the use of single P-insert lines to isolate genes on the basis of their mutant phenotype (Cooley *et al.* 1988, this study).

There are several aspects of the study of chemosensory information processing that is facilitated by the identification of gene bases on the spatial localization of their products. The adult brain (figure 3a) can be divided into functional regions. In the

olfactory system, evidence exists for the coding of information regarding odor quality as spatial maps in the antennal lobe (Rodrigues 1988). The expression of β-galactosidase in specific subsets of antennal sensillae as well as discrete cell bodies around the antennal lobe in some of the transposants identifies loci that may be important for the function of these cells and the facile genetics that is possible with a marked P-insert allows such roles to be tested. The genetic approach to the study of the chemosensory pathway has identified several genes based on mutant phenotype (Rodrigues and Siddiqi 1978; Siddiqi 1987; McKenna *et al.* 1989; Ayyub *et al.* 1990). Similar questions can be asked in the development of precise neuromuscular connections and muscle pattern (Crossley 1978; Lawrence 1982). Here too, the genetic approach has identified genes that affect the development of the adult flight muscles (Deak *et al.* 1982; Costello and Wyman 1986). P-element insertional mutagenesis and enhancer detection schemes serve to identify and rapidly analyze genes involved in such complex pathways.

We have described the initial survey of over 200 enhancer trap lines on the autosomes and X-chromosomes of *Drosophila melanogaster*. Our screen was aimed at identifying genes that are of interest in our study of the chemosensory and neuromuscular development. Based on β-galactosidase patterns we have chosen BTJ409, and 540 for the study of the chemosensory pathway and BTJ 325, 629 and ETX28 for the study of muscle development. We have also studied genes on the basis of phenotypes most likely caused by the P-element insertion. From behavioral tests we have identified several transposants that show chemosensory abnormalities and are currently analyzing ETX3, ETX4 and ETX20 in detail.

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