# Conversion of *lacZ* Enhancer Trap Lines to *GAL4* Lines Using Targeted Transposition in *Drosophila melanogaster*

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ABSTRACT

Since the development of the enhancer trap technique, many large libraries of nuclear localized *lacZ* *P*-element stocks have been generated. These lines can lend themselves to the molecular and biological characterization of new genes. However they are not as useful for the study of development of cellular morphologies. With the advent of the *GAL4* expression system, enhancer traps have a far greater potential for utility in biological studies. Yet generation of *GAL4* lines by standard random mobilization has been reported to have a low efficiency. To avoid this problem we have employed targeted transposition to generate glial-specific *GAL4* lines for the study of glial cellular development. Targeted transposition is the precise exchange of one *P* element for another. We report the successful and complete replacement of two glial enhancer trap *P*[*lacZ*, $ry^+$] elements with the *P*[*GAL4*, $w^+$] element. The frequencies of transposition to the target loci were 1.3% and 0.4%. We have thus found it more efficient to generate *GAL4* lines from preexisting *P*-element lines than to obtain tissue-specific expression of *GAL4* by random *P*-element mobilization. It is likely that similar screens can be performed to convert many other *P*-element lines to the *GAL4* system.

THE enhancer trap technique is a widely used method for studying tissue-specific gene expression in *Drosophila melanogaster*. Classically, a *P* element containing the *lacZ* reporter gene under a minimal promoter is mobilized throughout the genome using a transposase source. The *P*-element construct is sensitive to the regulatory elements of the region that it inserts. As a result, detection of β-galactosidase activity by staining reflects the tissue type and timing of the endogenous gene activity (O'Kane and Gehring 1987; Bellen *et al.* 1990). Many libraries of enhancer-trap-staining patterns have been generated in this manner (*e.g.*, Bellen *et al.* 1989; Bier *et al.* 1989; Wilson *et al.* 1989; Klämbt and Goodman 1991). Analyses of such enhancer trap stocks using molecular techniques have resulted in the cloning of many new genes.

A major improvement to the enhancer trap technique employs the *GAL4* transcriptional activator (Brand and Perrimon 1993). In this system, the *GAL4* gene is included in the enhancer trap *P*-element construct and its expression is under control of the local regulatory region just like the expression of the *lacZ* gene in earlier style enhancer traps. The *GAL4* system is far more flexible than earlier *lacZ* reporter systems. *GAL4* enhancer traps can be used to drive the expression of any other gene placed downstream of the upstream activation sequence (*UAS*; Brand and Perrimon 1993).

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The *GAL4* system is becoming a standard tool in Drosophila genetic analyses. However, the availability of *GAL4* enhancer traps is low because the most extensive enhancer trap libraries contain the original nuclear localized *lacZ*. Our own fly stocks are a good example: previously, an enhancer trap screen using the *P*[*lacZ*, $ry^+$] construct generated ~100 lines showing glial-specific nuclear-staining patterns (Klämbt and Goodman 1991), some of which we are currently characterizing in the peripheral nervous system (PNS). Many of these lines allowed the cloning of novel genes, such as gliotactin (Auld *et al.* 1995), glial cells missing (Jones *et al.* 1995), and pointed (Klaes *et al.* 1994). They have also been useful to describe the general stereotypic glial cell patterning in the embryonic nervous system (Klämbt and Goodman 1991). However, we want to characterize the glial morphology more precisely to provide a foundation for further studies in PNS glial developmental roles. To date, no satisfactory cellular marker for peripheral glial cells exists and in many ways the nuclear-staining *lacZ* enhancer trap elements in our glial-specific lines are obsolete for our purposes. Instead, a *GAL4*-containing *P* element at our existing enhancer trap loci would be far more useful in that we would have the ability to express a variety of markers throughout the entire cellular processes enabling morphological analysis. Also, we would have the potential to ectopically express genes in the peripheral glia, which could help us understand their biological roles.

It would be far more efficient to convert old *lacZ* enhancer trap lines directly to *GAL4* enhancer trap lines

than to repeat an enhancer trap screen for *GAL4* lines. Many of the original *lacZ* enhancer trap lines are viable and isolated on the basis of their expression pattern, which represents a considerable amount of work. Also, the pGawB (*P*[*GAL4, miniwhite*$^+$]) *P*-element mobility is reported to be significantly lower than that of previous *lacZ* constructs (Brand and Perrimon 1993; Gustafson and Boulianne 1996); hence, repeating the earlier type screen would be far more labor intensive. We designed a method based on targeted gene conversion (Engels *et al.* 1990, 1994; Gloor *et al.* 1991; Johnson-Schlitz and Engels 1993; Nassif *et al.* 1994) to isolate direct replacements of original target *P*[*lacZ, rosy*$^+$] elements with a donor *P*[*GAL4, w*$^+$] element.

We present here the results of two independent targeted transposition screens, which successfully converted two glial-specific *lacZ* enhancer traps to the *GAL4* system. The 17.2-kb target *P* elements were on the second and third chromosomes in peripheral and exit glial selective genes. The 11.3-kb donor was the *P*[*GAL4, w*$^+$] located on the X chromosome. The frequency of targeted transposition is reported to be highly dependent on target and donor *P*-element location (Gloor *et al.* 1991). We observed targeted transposition to the peripheral glial and exit glial enhancer traps at 1.3 and 0.4%, respectively, suggesting that the *P*[*GAL4, w*$^+$] donor is at a locus amenable to targeted transposition. Molecular analysis of selected targeted transposition lines revealed that the donor *P* element was copied precisely to the locus of the target enhancer traps and that the entire donor *P*-element sequence had been repaired into these sites. These data suggest that targeted transposition can be an efficient means of converting previously obtained *lacZ* enhancer traps to the *GAL4* system.

## MATERIALS AND METHODS

**Stocks:** Original enhancer trap stocks rL82 and rQ286, used for targeted transposition, arose from a *P*[*lacZ, rosy*$^+$] (pP[PZ]) enhancer trap screen (Klämbt and Goodman 1991). The *P*-element donor stock *P*[*GAL4, miniwhite*$^+$]/*FM7c* (Brand and Perrimon 1993) was provided by G. Boulianne. The *UAS-lacZ* and *UAS-GFP* stocks were obtained from the Bloomington Stock Center. All other marker and balancer chromosomes used for targeted transposition are detailed in Lindsley and Zimm (1992).

**Targeted transposition screen:** All genetic crosses were performed at 25° on standard yeast medium. Preliminary crosses were conducted to generate stocks containing both target and donor *P* elements for the rL82 second chromosome peripheral glial enhancer trap and the rQ286 third chromosome exit glial enhancer traps. These *P*-element stocks were used in the first generations of the targeted transposition screens. The rL82 screen consisted of six generations:

1. *Sp*/*CyO*; *Sb*Δ*2-3*/*TM6* males mated to *P*[*GAL4, w*$^+$]/*FM6*; rL82; + virgin females en masse (females were chosen, as the *P*[*GAL4, w*$^+$] insertion is lethal).
2. Individual female virgins of *P*[*GAL4, w*$^+$]/+; rL82/*CyO*; *Sb*Δ*2-3*/+ were collected and crossed to *w*$^-$; *Gla*/*CyO*.
3. All female virgins carrying the *CyO* balancer but lacking the *Sb*Δ*2-3* chromosome were collected and mated individually to *w*$^-$; *Gla*/*CyO* males: female *P*[*GAL4, w*$^+$]/(*w*$^-$ or +); rL82*/*CyO*; + × male *w*$^-$; *Gla*/*CyO*. rL82* denotes a possible transposed *P*-element chromosome.
4. All males carrying the *CyO* balancer were collected and mated individually to *w*$^-$/*w*$^-$; *Gla*/*CyO* virgin females. Male genotypes for these matings consisted of four possibilities of which the first and second are most desired, but indistinguishable from the others: (i) +; rL82*/*CyO*, (ii) *w*$^-$; rL82*/*CyO*, (iii) *P*[*GAL4, w*$^+$]; rL82/*CyO*, and (iv) *w*$^-$; rL82/*CyO*.
5. Progeny from generation 4 were screened for presence of eye pigmentation. Such progeny were selected for brother-sister matings to maintain a stable stock: *w*$^-$; rL82*/*CyO* males crossed to *w*$^-$/*w*$^-$; rL82*/*CyO* females. Alternate genotypes caused by random *P*-element mobilizations to autosomes were also possible, but indistinguishable from the rL82* chromosome stocks.
6. All resultant stocks were crossed into a *UAS-lacZ* background carried on the third chromosome and embryos were stained for final screening for the rL82* chromosome as represented by staining of peripheral glial processes.

The rQ286 enhancer-trap-targeted transposition screen consisted of six generations:

1. *Sp*/*CyO*; *Sb*Δ*2-3*/*TM6* males were crossed to *P*[*GAL4, w*$^+$]; rQ286 virgin females in a bottle.
2. *P*[*GAL4, w*$^+$]/+ ; *Sb*Δ*2-3*/rQ286 virgin females were collected and mated to *w*$^-$; *Gl*/*TM3* males en masse.
3. All males were collected, except for *Sb*Δ*2-3*/*Gl* males, and mated to *w*$^-$/*w*$^-$; *Gl*/*TM3* virgin females as single-pair matings.
4. Single-pair matings were screened for male flies with pigmented eyes, indicating a mobilization of the *P*[*GAL4, w*$^+$] transposon to an autosome. These males were mated to virgin female sisters with similar eye pigment for a stable stock: *w*$^-$; rQ286*/*TM3* males × *w*$^-$/*w*$^-$; rQ286*/*TM3* females.
5. All such stocks were crossed into a *UAS-lacZ* background and screened for staining of exit glial processes.

**Embryo staining:** Expression of β-galactosidase in embryos was detected with X-Gal staining (Klämbt *et al.* 1991) and HRP immunohistochemistry (Patel *et al.* 1987). PNS glia were labeled with polyclonal mouse anti-β-galactosidase antibody 1:250 (Sigma, St. Louis) and motor axons were labeled with mouse monoclonal antibody 1D4 (anti-Fasciclin II) 1:10 (Van Vactor *et al.* 1993). Sensory axons were labeled with mAb 22C10. Embryos were cleared in 90% glycerol with PBS, dissected, and photographed. For GFP visualization, embryos were dechorionated for 4 min in 50% household bleach, and then rinsed and mounted in PBS for photography.

**Southern analysis:** Genomic DNA from adult flies was prepared from two control stocks, (i)*white*$^{1118}$ and (ii)*P*[*GAL4, w*$^+$]/*FM7c*, two parental stocks, (iii) rL82 and (iv) rQ286, and selected targeted transposition lines, (v) *w*$^-$; rL82#4, (vi) *w*$^-$; rL82#8, (vii) *w*$^-$; rL82#29, (viii) *w*$^-$; rQ286#2, and (ix) *w*$^-$; rQ286#5. A total of 10 μg of genomic DNA was digested with *Pst*I and blotted using standard techniques. The *GAL4* 3-kb coding sequence and the pUAST vector (containing the *miniwhite*$^+$ gene and plasmid sequences) were used as probes.

**PCR analysis:** Genomic DNA obtained for Southern analysis was also used for PCR analysis. Oligonucleotide primers specific for the gliotactin gene surrounding the *P*-insertion site were used: rL82 primer (5′-CGG GAT CCT GTC TCG CCG AGA GAA GGC GC-3′) and RNA II primer (5′-CCT GGC CAA CAA TTC TTT CGT TTG TAT TGA GCG-3′). Primers internal to the *GAL4* enhancer trap construct were used as well: *GAL4* primer (5′-GTC AAT CGA TAC ACT CAA CTG TCT TTG

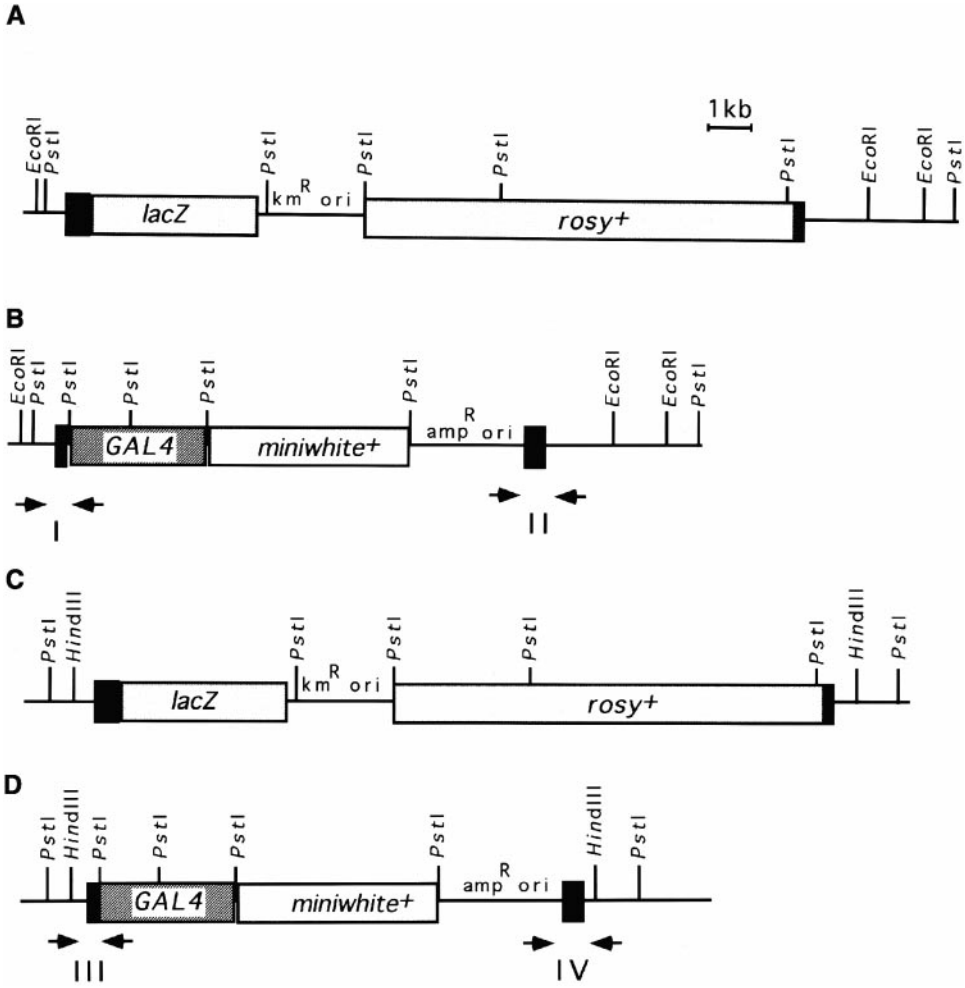


Figure 1.—Schematic diagram of the enhancer trap *P* elements involved in targeted transposition and their surrounding genomic regions. *P*-element termini are indicated as black boxes. (A) rL82 (*P*[*lacZ*, $ry^+$]) insertion into the 5′ region of the gliotactin gene. (B) The *P*[*GAL4*, $w^+$] element in the original rL82 insertion site. The size and position of PCR products generated using primer pairs I and II are shown. (C) rQ286 (*P*[*lacZ*, $ry^+$]) insertion site at 63F on the third chromosome. (D) The *P*[*GAL4*, $w^+$] element that has replaced the original rQ286 element. The size and position of the PCR products generated by primer pairs III and IV are indicated.

ACC-3′) for the *GAL4* gene and the reverse primer (5′-TTC ACA CAG GAA ACA G-3′) for the pBluescript plasmid. For analysis of exit glial enhancer-trap-targeted transpositions the genomic region primers were rQ286 5′ primer (5′-GCC GCA GCT GGG AAA TGC TGA TGG CGC CCG-3′) and rQ286 3′ primer (5′-CAT GTA TGT TTG AAC TTA CC-3′). For rL82 PCR analysis, amplification conditions were 30 sec at 94°; 1 min at 55°; 2 min, 30 sec at 72°; 30 sec at 94°; 1 min at 55°; 2 min, 30 sec at 72°; touch down from 55° by 2° going to first step, 30 sec at 94°, for 3 cycles (steps 6–9); 1 min at 49°; 2 min, 30 sec at 72°; cycle 26 times to step 9; 5 min at 72°. For rQ286 PCR analysis, analogous touchdown programs were used, but final annealing temperatures of 51° for pairs with rQ286 3′ primer and 60° for pairs with rQ286 5′ primer were substituted. All reactions contained standard PCR solutions (Pharmacia, Piscataway, NJ) and included 2.5 mm $Mg^{2+}$.

***In situ* hybridization to polytene chromosomes:** Salivary gland polytene chromosomes were prepared using standard techniques. Preparations were probed with a digoxygenin-11-dUTP-labeled 3-kb *GAL4* fragment and detected with an alkaline phosphatase reaction according to manufacturer's instructions (Boehringer Mannheim, Indianapolis).

## RESULTS

**Generation and detection of targeted transposition lines:** A previous enhancer trap screen generated a number of lines that selectively label glial cells (Klämbt and Goodman 1991). One such line, rL82, contains the *lacZ* enhancer trap insert at 35F1-2 in the gliotactin gene on the second chromosome and shows selective labeling of the peripheral glia (Figures 1A and 3A; Auld *et al.* 1995). Another line, rQ286, contains the same enhancer trap *P* element inserted to 63F1-2 on the third chromosome and results in the staining of the exit glia (Figures 1C and 3D). The exit glia are a subset of the peripheral glia that wrap peripheral nerve roots just distal to the CNS. A targeted transposition screen was carried out with the goal of efficiently replacing the original ("target") rL82 and rQ286 enhancer trap *P* elements with a new ("donor") enhancer trap *P* element containing the *GAL4* gene located at position 12 on the X chromosome.

The basis of our screen is outlined in Figure 2A. First, the target *P* element of the original enhancer trap line is excised in the presence of a transposase source. This will leave behind a 10 to 20-bp footprint from each inverted repeat terminus (Searles *et al.* 1982; Takasu-Ishikawa *et al.* 1992; Johnson-Schlitz and Engels 1993) with a double-stranded gap (Gloor *et al.* 1991). Gap repair machinery compares homology of the broken ends to other regions of the genome. The DNA repair machinery bypasses the homologous chromosome as a template, because in our screen it is represented by a multiply inverted balancer chromosome,

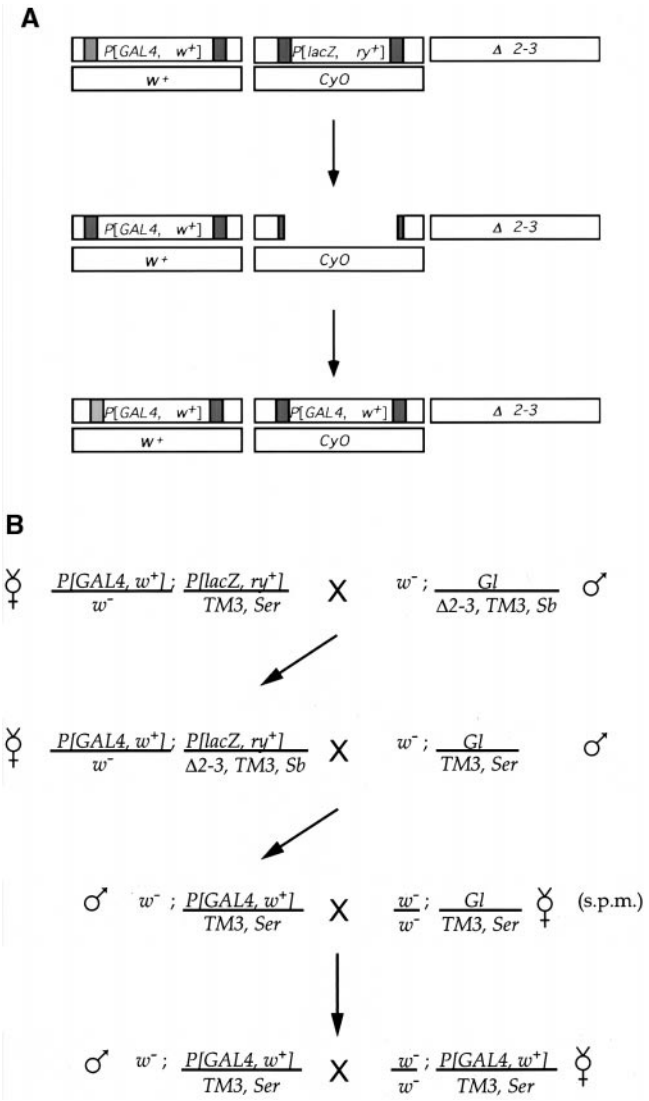


Figure 2.—Schematic diagrams of *P*-element replacement and a crossing scheme for targeted transposition. (A) Target *lacZ P*-element excision on the second chromosome is induced by a source of active transposase (Δ*2-3* chromosome). This results in a double-stranded DNA gap with remnants of *P*-element termini on either side. Repair of the resultant double-stranded gap using the sister chromosome is prevented by use of a balancer chromosome, *CyO*. Rather, recognition of homologous sequence at the *GAL4* donor *P*-element termini on the X chromosome leads to repair of the gap with the donor template. (B) To exchange a *P* element on the third chromosome, females with donor *P*[*GAL4*, $w^+$] and target *P*[*lacZ*, $ry^+$] elements are crossed to males with a Δ*2-3*, *TM3* balancer. In the second generation, a targeted transposition event is promoted as the *P* elements are in a background of Δ*2-3* transposase. To reduce double-strand repair from the sister chromosome the target *P* element is placed over a balancer chromosome. From second generation progeny, males are selected that have pigmented eyes. They are crossed in single-pair matings to a balancer stock, then crossed to their sisters to generate a set of stable autosomal *P*[*GAL4*, $w^+$] stocks. The various pigmented eye lines are crossed later into a *UAS-lacZ* background and stained to verify tissue-specific expression of *GAL4*. Analogous crosses can be used to exchange *P* elements on the second chromosome.

which interferes with homolog pairing (Engels *et al.* 1990). A match is made with the inverted repeats found on the donor *P* element on the X chromosome, and subsequently the donor *P*-element sequence acts as a template and is repaired into the target gap (Gloor *et al.* 1991).

Isolation of targeted transposition repair events occurred in a series of genetic crosses. A general crossing scheme is shown in Figure 2B. For one generation the following elements are required: transposase, the target *P* element over a balancer chromosome, the donor *P* element, and an appropriate background for detection of mobilization (*white*$^-$ in our experiments). In this combination, a targeted transposition event is capable of occurring. Next, the transposase is crossed out and a successful transposition event is selected for. The flies are crossed to a stock that maintains the *white*$^-$ background and that has a balancer for the target *P*-element chromosome. The presence of the *P*[*GAL4*, $w^+$] is detectable by following the *miniwhite*$^+$ ($w^+$) gene of the donor element. As the original *P*[*GAL4*, $w^+$] on the X chromosome is lethal, males are selected that do not have the transposase chromosome and have pigmented eyes, indicating a mobilization of *P*[*GAL4*, $w^+$] to an autosome. There are two classes of autosomal *P*[*GAL4*, $w^+$] lines: desired targeted transposition lines and random mobilizations of *P*[*GAL4*, $w^+$]. To distinguish which lines belong to a particular class, stocks are generated, crossed with *UAS-lacZ*-containing flies and the progeny are stained to determine the pattern of *lacZ* expression.

The crosses used in our targeted transposition screen are outlined in the methods section. In the screen to replace the *P*[*lacZ*, $ry^+$] element in the gliotactin gene (rL82), 28 lines of flies were recovered from 693 single-pair matings, which had eye color phenotypes, indicative of a mobilization of the *P*[*GAL4*, $w^+$] element to an autosome. After being crossed with a *UAS-lacZ* background, embryos were collected and stained with X-Gal. The original staining pattern of the *P*[*lacZ*, $ry^+$] element in the gliotactin gene (rL82) labeled the nuclei of most of the peripheral glia (Figure 3A). Of the 28 lines recovered, 9 showed staining in the identical set of peripheral glia as in the original rL82 enhancer trap line, but with entire cellular processes labeled, suggesting that targeted transposition had occurred (Figure 3B). There was no expression of *lacZ* in control embryos that contained the glial *GAL4* enhancer trap line alone (data not shown). The frequency of targeted transposition to the rL82 locus was 1.3%. Out of all detectable mobilizations of *P*[*GAL4*, $w^+$] from the X chromosome to autosomes, 32% were targeted transposition events and the remainder were random *P*-element insertions.

To see if the *GAL4* insertion was capable of driving the expression of another marker protein the lines were crossed into a *UAS-GFP* background. The resulting embryos had the same pattern of peripheral glial cyto-

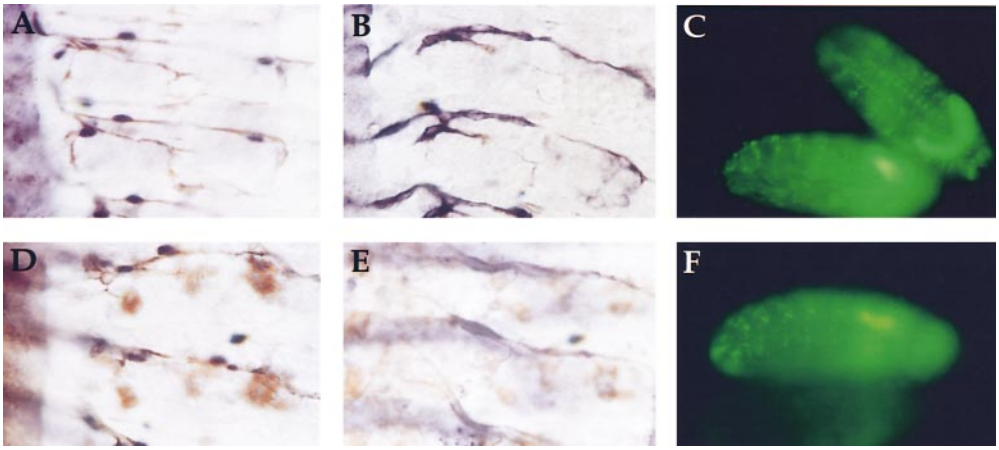


Figure 3.—Glial-specific enhancer trap expression patterns in Drosophila embryos. Glia were stained with an anti-β-galactosidase polyclonal antibody (black), motor neurons (A and B) were stained with the anti-Fasciclin II monoclonal antibody (brown), and sensory neurons (D and E) were stained with mAb 22C10 (brown). Antibody staining was detected using HRP immunohistochemistry. (A) The original *lacZ* enhancer trap staining pattern of rL82 specific to the peripheral glial nuclei. (B) The gliotactin (rL82#29) *GAL4* enhancer trap staining pattern. The peripheral glial cellular processes are labeled with a *UAS-lacZ* marker. (C) The gliotactin *GAL4* enhancer trap visualized in whole mounts using a *UAS-GFP* marker. (D) The original *lacZ* enhancer trap staining pattern of rQ286 in the exit glial nuclei. (E) The rQ286#2 *GAL4* enhancer trap staining pattern. The exit glial cellular processes are labeled with a *UAS-lacZ* marker. (F) The rQ286#2 *GAL4* enhancer trap driving *UAS-GFP* expression in whole mounts.

plasmic staining (Figure 3C). There were no differences observed between the staining patterns of the original nuclear *lacZ* line and the *GAL4* line with the exception of expression of *GAL4* in the salivary glands. The additional salivary gland labeling has been observed previously with other *GAL4* enhancer trap lines in our laboratory and others (Gustafson and Boulianne 1996).

It is known that the location of the donor *P* element has a large influence on the frequency of transposition to a single target locus (Engels *et al.* 1990; Gloor *et al.* 1991; Nassif *et al.* 1994). We tested the general effectiveness of the *P*[*GAL4*, $w^+$] insert as a donor by conducting an analogous targeted transposition screen to convert the exit glial line, rQ286, to the *GAL4* system. Out of 805 single-pair matings, 11 progeny had a pigmented eye phenotype. The *lacZ* and GFP-labeling patterns of these lines were then compared to the original *lacZ* enhancer trap pattern (Figure 3, D–F). Exit glial processes stained in three of these lines, suggesting that targeted transposition had occurred at a frequency of 0.4%. In this screen, out of all detectable mobilizations of *P*[*GAL4*, $w^+$] to the third chromosome, 27% were targeted transposition events. This indicates that using targeted transposition to replace an existing *P* insert with a *GAL4* element is an efficient means of obtaining tissue-specific *GAL4* lines.

**Molecular verification of targeted transposition events:** To confirm that the donor *P* element had been repaired in the appropriate location, polytene chromosome squashes were carried out. Preparations from selected targeted transposition lines (rL82#4, rL82#8, and rL82#29) were probed with digoxygenin-dUTP-labeled *GAL4* DNA to confirm the location of the *P*[*GAL4*, $w^+$] element. The predicted insertion site was the location of original rL82 *P* element. However, there remained a formal possibility of a random insertion to another chromosomal region that causes the same expression pattern as gliotactin. In all cases, the *GAL4* probe labeled only the 35E1-2 region of the second chromosome, which is the location of the original rL82 *P*[*lacZ*, $ry^+$] insertion (data not shown). Similarly, we mapped the *GAL4* insert in the rQ286#2 and rQ286#5 lines to the original insertion site of *P*[*lacZ*, $ry^+$] at position 63F1-2, suggesting that the donor *P* element had repaired into the target *P*-element locus of the exit glia enhancer trap as well (data not shown).

To determine the precise location of *P*[*GAL4*, $w^+$] insertion in the new lines obtained, small regions spanning the junction between the gene and inserted *P*-element ends were amplified using PCR (Figure 4). For the rL82-targeted transposition lines, external primers specific to the gliotactin gene and internal primers specific to the *P*[*GAL4*, $w^+$] element were chosen (Figure 1B). The predicted PCR products of 737 bp for the 5′ end and 548 bp for the 3′ end were obtained from all targeted transposition lines tested, rL82#4, rL82#8, and rL82#29 (Figure 4A). Furthermore, no products were obtained from either the original rL82 (*P*[*lacZ*, $ry^+$]) enhancer trap line or the $w^{1118}$ negative controls.

To test the possibility that the donor *P*[*GAL4*, $w^+$] element had inserted to the target rL82 enhancer trap locus in an alternate 3′ to 5′ orientation, the PCR analysis was repeated using the opposite primer pairs to the above experiment. No PCR product was obtained (Figure 4A), suggesting that the *P*[*GAL4*, $w^+$] element has inserted to the rL82 enhancer trap locus in the 5′ to 3′ orientation. Taken together, this evidence suggests that the donor *P*[*GAL4*, $w^+$] element had paired and double-stranded DNA repair had occurred precisely at the inverted repeats of the original rL82 enhancer trap line.

Analogous PCR analysis was performed on rQ286#2 and rQ286#5 transposition lines using external primers

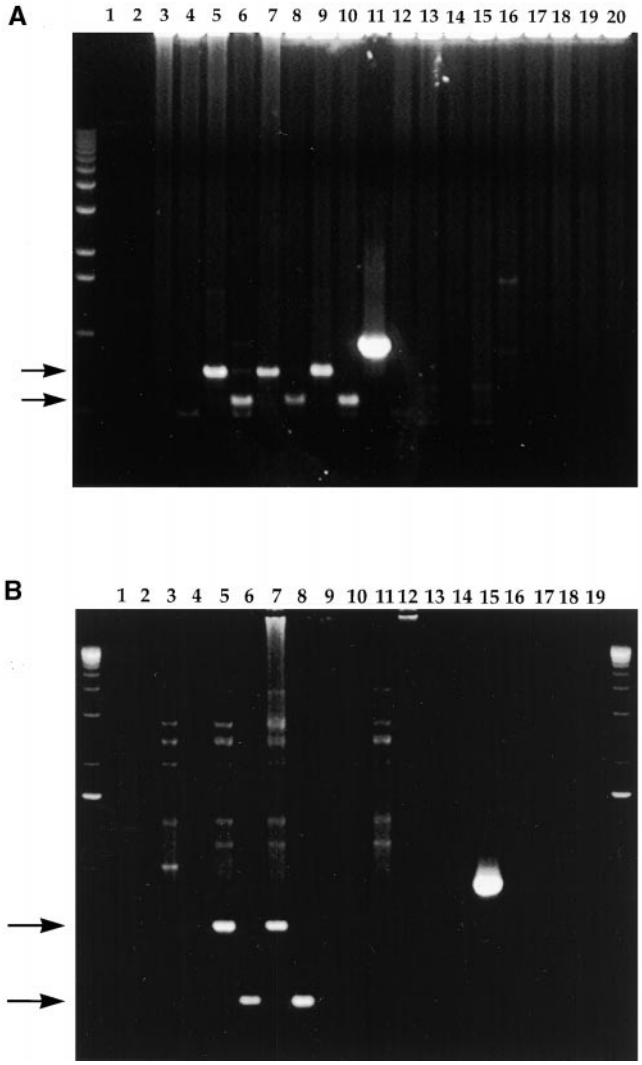


Figure 4.—PCR analysis of targeted transposition lines. PCR was used to determine the location and orientation of *GAL4 P* elements in selected targeted transposition lines (diagrammed in Figure 1). (A) rL82-targeted transposition line analysis. (Lanes 1–10) Odd-numbered lanes are products of primer pair I and even-numbered lanes are products of primer pair II: (1, 2) no DNA; (3, 4) original rL82; (5, 6) rL82#4; (7, 8) rL82#8; (9, 10) rL82#29. (Lane 11) PCR-positive control. (Lane 12) $w^{1118}$ negative control with primer pair I. PCR products of the expected sizes were obtained for the 5′ *P*-element terminus (737 bp, top arrow) and for the 3′ *P*-element terminus (548 bp, bottom arrow). (Lanes 13–20) Reversed primer pair orientation to test the possibility of a 3′ to 5′ *P*-element insertion: (13, 14) original rL82; (15, 16) rL82#4; (17, 18) rL82#8; (19, 20) rL82#29. (B) rQ286-targeted transposition line analysis. (Lanes 1–8) Odd numbers are pair III and products and even numbers are pair IV products. (1, 2) no DNA; (3, 4) original rQ286; (5, 6) rQ286#2; (7, 8) rQ286#5. (Lanes 9–14) Reversed orientation primer pairs. (9, 10) original rQ286; (11, 12) rQ286#2; (13, 14) rQ286#5. (Lane 15) PCR positive control. (Lanes 16–19) $w^{1118}$ negative control with all combinations of primer pairs used in preceding reactions. The PCR products obtained were 733 bp for the 5′ *GAL4 P* end (top arrow) and 383 bp length for the *GAL4* 3′ *P* end (bottom arrow).

specific to the rQ286 locus and internal primers specific to the *P*[*GAL4*, $w^+$] element (Figure 1D). The expected lengths were 733 bp across the 5′ inverted repeat and 383 bp across the 3′ inverted repeat. Both lines gave PCR products of the expected length and were oriented in the original 5′ to 3′ direction as well (Figure 4B). Therefore, as with the rL82 transpositions, *P*[*GAL4*, $w^+$] repaired precisely to the original target enhancer trap locus.

For targeted transposition to be an effective means of repairing the *P*[*GAL4*, $w^+$] to a new locus, the repair involved must be complete such that the entire *GAL4* protein-coding region plus the *miniwhite*$^+$ eye color marker is integrated. This is necessary so that the event can be detected as a mobilization of the *miniwhite*$^+$ gene to an autosome, and subsequent *GAL4* expression will lead to proper activation of any *UAS*-driven constructs. Previous targeted transpositions have resulted in complete and exact *P*-element replacements. However, various types of aberrant repairs have been noted as well, including large internal deletions (Geyer *et al.* 1988; Heslip and Hodgetts 1994; Staveley *et al.* 1994; Gonzy-Tréboul *et al.* 1995; Keeler and Gloor 1997). To determine the amount of *GAL4 P* insert that had been copied into our original rL82 and rQ286 loci, we probed genomic Southern blots of the original *lacZ* and selected transposed *GAL4* enhancer trap lines (Figure 5). Through the use of a *GAL4* DNA probe, we found all targeted transposition lines tested had both the 1.4- and 1.8-kb *Pst*I bands (Figure 5A), which indicates that the full-length *GAL4* sequence was present. The targeted transposition lines were then probed with a plasmid containing the *miniwhite*$^+$ gene (Figure 5B). All had the expected 4.6-kb band corresponding to the *miniwhite*$^+$ gene as well as bands corresponding to plasmid sequences that match those found in the original *P*[*GAL4*, $w^+$]/*FM7c* line. This suggests that in all the targeted transposition lines tested the entire length of the *P*[*GAL4*, $w^+$] was present. This is comparable to previous work, which reported the exact transposition of many kilobase pairs to target loci (Heslip and Hodgetts 1994; Gonzy-Tréboul *et al.* 1995; Keeler and Gloor 1997). Our results also show that exchanging a larger *P* element for a smaller one is possible as our original *lacZ* targets were 5.9 kb larger than the *GAL4* donor. Therefore, targeted transposition could be a general means of exchanging *P* elements throughout the Drosophila genome and is suitable as a means of exchanging larger *lacZ*-type enhancer trap *P* elements for smaller *GAL4 P* elements.

## DISCUSSION

We find that using targeted transposition to exchange *lacZ* with *GAL4* enhancer trap *P* elements is an efficient means of acquiring tissue-specific gene expression of

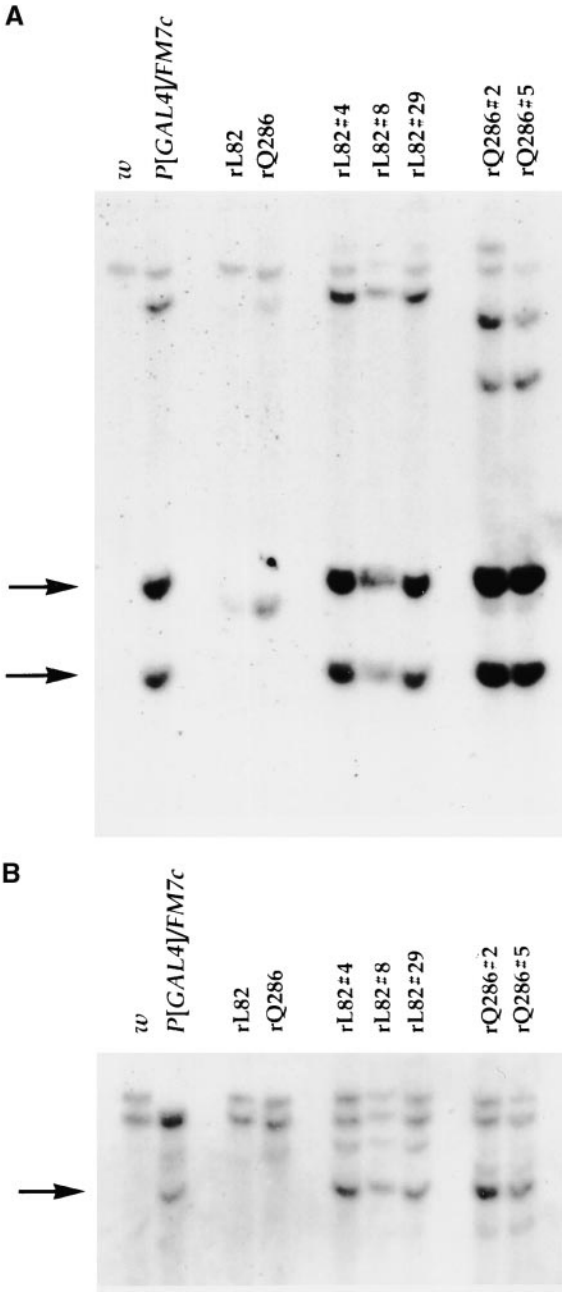


Figure 5.—Southern analysis of targeted transposition lines. (A) A *GAL4* DNA probe reveals the two expected *Pst*I bands, 1.8 kb (top arrow) and 1.4 kb (bottom arrow) in the original *P*[*GAL4*, $w^+$]/*FM7c* donor, and all targeted transposition lines (rL82#4, rL82#8, rL82#29, rQ286#2, and rQ286#5). None of the control or original *lacZ* lines show *GAL4* bands. (B) A *miniwhite*$^+$ plus plasmid probe reveals a 4.6-kb *Pst*I band corresponding to the *miniwhite*$^+$ gene in the *P*[*GAL4*, $w^+$]/*FM7c* donor stock and all targeted transposition lines (arrow). The remaining bands correspond to the plasmid sequences and are consistent with a complete repair of the *P*[*GAL4*, $w^+$] into the target site.

*GAL4*. This method has been successful for two different enhancer trap lines of interest to us, a peripheral glial enhancer trap on the proximal region of the second chromosome left arm and the exit glial enhancer trap that is inserted distally on the third chromosome left arm. Frequencies of targeted transposition were 1.3 and 0.4%, respectively, and are in the expected range based on previous studies in gene conversion using low homology (Dray and Gloor 1997). This supports earlier evidence that double-strand break homology searches are efficient with <31 nucleotides of *P*-element sequence (Keeler and Gloor 1997).

For our purposes, targeted transposition has been a superior method of obtaining *GAL4* enhancer trap lines for PNS glia because the random transposition rate of pGawB from the X chromosome to autosomes is very low. The pGawB element has a modified 5′ terminus, which is thought to reduce its random transposition rate to only 17% (Brand and Perrimon 1993; Gustafson and Boulianne 1996) compared to 87% random transposition of *lacZ P* elements (Bellen *et al.* 1989; Bier *et al.* 1989). Indeed, for both peripheral and exit glial-targeted transposition screens, of all mobilizations of pGawB to an autosome detected via *miniwhite*$^+$ expression, approximately one-third were targeted transposition events as detected by X-Gal staining. Obtaining *GAL4* expression in PNS glia from a classic enhancer trap screen would have a frequency far lower than what we observed using targeted transposition.

With these new PNS glial *GAL4* enhancer traps, we now are able to visualize the morphology of these cells using cellular markers such as *UAS-lacZ* and *UAS-GFP*. No other cellular markers specifically labeling the cellular processes of exit and peripheral glia have been available. The previous nuclear-staining enhancer traps provided few anatomical details of these cells beyond the number and general location. As glia are implicated in providing migrational cues for neurons in the developing nervous system (Bastiani and Goodman 1986; Jacobs and Goodman 1989; Hidalgo *et al.* 1995), it is important to determine the exact contacts a growing neuron will make with a glial cell. To this end, a full developmental profile of the peripheral and exit glia is now underway using the *GAL4* enhancer trap lines. Beyond study of the wild-type development, ectopic gene expression and toxin ablation is now also possible given the large number of UAS stocks that are currently available. In contrast to our original nuclear-staining *lacZ* enhancer trap lines, the new *GAL4* lines that are derived from them can lend themselves to an inexhaustible number of future studies as new UAS lines are constructed.

Targeted transposition events have been described at other loci in the Drosophila genome. At the *yellow* locus, a 0.4-kb *P* element was replaced by a 1.1-kb element (Geyer *et al.* 1988). Replacements of an original 0.7-kb target *P*-element insert at the *vestigial* gene with 10- and 11.9-kb donor *P* elements have been reported (Heslip and Hodgetts 1994; Staveley *et al.* 1994). A target 0.8-kb *P* element at the *Broad-Complex* gene has been replaced by a 10-kb donor *P* element (Gonzy-Tréboul

*et al.* 1995) and, more recently, an 8-kb donor *P* element was targeted to the 0.6-kb *P* insert in the *white* gene in the mutant strain, *white*$^{hd}$ (Keeler and Gloor 1997). Our results show that a smaller *P* element can be targeted to the locus of a larger one: both conversions of our enhancer trap lines were a replacement of the target pP[PZ] (*P*[*lacZ, ry*$^+$]) 17.2-kb element with the 11.3-kb pGawB donor element. We have also recently performed a third targeted transposition screen that successfully replaced a *lacZ P* element of the rQ14 exit glial specific enhancer trap, also on the third chromosome (K. J. Sepp and V. J. Auld, unpublished results). So far, all of our attempts at converting *lacZ* enhancer traps to the *GAL4* system have been successful. Therefore, targeted transposition could be a general means of exchanging *P* elements throughout the Drosophila genome and could be suitable as a means of exchanging larger *lacZ*-type enhancer trap *P* elements for smaller *GAL4 P* elements.

The efficiency of gene conversion / targeted transposition is variable and depends on the location of the donor template (Engels *et al.* 1990, 1994; Gloor *et al.* 1991; Nassif *et al.* 1994). Gene conversion efficiency is homology dependent as well (Dray and Gloor 1997). During targeted transposition, the amount of homology remaining of the inverted repeat *P*-element ends is approaching the very lower limit of that required to achieve a successful search for a donor repair template. We propose that the *GAL4* insert is amenable to targeted transpositions in general for a number of reasons. First, the small amount of *P*-element end homology has been sufficient for the numerous loci that have previously exhibited targeted transpositions. Second, the insertion site of the donor *GAL4 P* element on the X chromosome has lent itself to successfully targeted transpositions to longer *P*-element targets on both the second and third chromosomes. It is very likely that the pGawB insertion line used in our experiments could be widely used to replace many other existing *lacZ* enhancer traps.

There may be other considerations that affect targeted transposition frequencies, especially for replacements with the *GAL4* insert used in the screen reported here. First, we noted that both of the original *lacZ* enhancer trap lines were in sites readily accessible to *P* elements. The original enhancer trap screen that produced the rL82 and rQ286 lines had multiple independent insertions of *P* elements to each of the loci. Perhaps these regions are also more susceptible to *P*-element exchanges as well. Also, we conducted the screens at 25°, which may promote targeted transposition events, as it has been observed that transposase has optimal activity at this temperature (Engels 1996).

There are many possible ways to design targeted transposition screens, given fly stocks available. For instance, a recent addition to our own stocks of a third chromosome balancer carrying the Δ*2-3* transposase source in a *white*$^-$ background will simplify and reduce the number of generations required for targeted transposition. Many enhancer traps of the *lacZ* type from earlier screens contain a *P*[*lacZ, w*$^+$] element and in these cases mobilization of the *P*[*GAL4, w*$^+$] would be undetectable by eye color. The Drosophila Genome Project is currently generating lethal alleles throughout the entire genome using the *P*[*lacZ, w*$^+$] element. Many of these alleles could be very useful tools to investigators if they are converted to the *GAL4* system using targeted transposition. In these cases, to detect a transposition, the simple crossing scheme we have suggested could be carried out in an analogous manner by generating a *P*[*GAL4, yellow*$^+$] insert on the X chromosome to mobilize in a *yellow*$^-$ background. Mobilizations to autosomes would be detected by body color instead of eye pigmentation. Additionally these screens could be conducted using PCR to detect the donor insertion. This approach should be feasible given the high rate of transposition events observed in this screen. PCR could be used by analyzing pools of flies and then further subdividing each positive pool until individual lines are obtained. Another approach that could be used to target lethal lines that contained the *P*[*GAL4, w*$^+$] element would be a scheme that selects for transposition events that rescue the lethality. This would be possible if one constructed a *P*[*GAL4,* (*w*$^+$ or *yellow*$^+$), *UAS*] construct. This construct would be analogous to that of the EP element of Rørth (1996), which contains *UAS* sequences fused to a truncated hsp promoter at the 3′ end of the *P* element. This construct, when inserted into a gene by *P*-element mobilization, is able to drive the expression of the endogenous gene in a *GAL4*-dependent manner. The EP construct could be adapted such that it also contains the *GAL4* gene fused to the 5′ *P* element as in the *P*[*GAL4, w*$^+$] enhancer trap construct (Brand and Perrimon 1993). In this manner even when the *P*-element insertion disrupts the endogenous gene, the combined expression of *GAL4* plus the presence of the *UAS*-driven promoter could rescue the lethality. This would facilitate selection of the targeted transposition event and provide the researcher with a "viable" line that now expresses *GAL4* in a gene-specific manner.

In conclusion, we have found targeted transposition to be an efficient means of converting our outmoded *lacZ* enhancer traps to the *GAL4* system. With this technique the *GAL4 P* element has lent itself to two different enhancer trap conversions with good efficiency. It is likely that many other loci will efficiently repair target *P* elements with the *GAL4* donor. As the entire *GAL4* donor element repaired into both target loci, we provide evidence that *P*-element exchanges may be from slightly larger sized *P* elements to smaller ones. Our evidence suggests that it is more efficient to convert existing *lacZ* enhancer traps to the *GAL4* system by targeted transposition rather than rescreen for *GAL4* enhancer traps by random mobilization.

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