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Altering the insertional specificity of a *Drosophila* transposable element

(*P* element/engrailed/segmentation genes)

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ABSTRACT **Vectors derived from the *Drosophila P* element transposon are widely used to make transgenic *Drosophila*. Insertion of most *P*-element-derived vectors is nonrandom, but they exhibit a broad specificity of target sites. During experiments to identify cis-acting regulatory elements of the *Drosophila* segmentation gene engrailed, we identified a fragment of engrailed DNA that, when included within a *P*-element vector, strikingly alters the specificity of target sites. *P*-element vectors that contain this fragment of engrailed regulatory DNA insert at a high frequency near genes expressed in stripes.**

Of the many transposable elements in *Drosophila*, the *P* element has attracted the most interest because of its uses in transposon-tagging and in making transgenic *Drosophila* (1–3). Both natural and genetically engineered *P*-element derivatives have been studied extensively. Neither inserts randomly throughout the genome, but both exhibit some specificity in target preference (1–3). For most *P*-element derivatives, this target specificity is very broad; it has been estimated that about half of the genes in *Drosophila* are targets for *P*-element mutagenesis (1). Because the specificity for target site insertion is so broad, nonrandom insertion is only evident in experiments with large numbers of independent insertions.

In contrast to the very broad specificity observed with most *P*-element derivatives, Hama *et al.* (4) reported that *P*-element derivatives containing >3.4 kilobases (kb) of upstream regulatory DNA from the segmentation gene engrailed (*en*) insert at a very high frequency near the endogenous *en* gene. This target preference was evident when only a very small number of independent insertions were generated; remarkably, 7 out of 20 insertions were in the vicinity of the *en* gene. In contrast, using a modified *P* element that did not contain *en* regulatory DNA, Bier *et al.* (5) found only one insertion near the *en* gene in a sample of 3768 independent insertion events.

We have also found that *P*-element derivatives containing small fragments of *en* DNA insert in the genome in a selective manner. The *P*-element derivatives that we have characterized, however, not only insert at a high frequency near the *en* gene but also target additional genes. Like engrailed, many of the other genes targeted by our modified *P* element are expressed in striped patterns in embryos. Based on the nature of the targets and their apparent lack of sequence similarity to engrailed, we propose a model for how targeted insertion occurs.

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MATERIALS AND METHODS

The *P*-element vectors used in these experiments have been described (6–8) and are shown in Fig. 1. In the first experiment described below, the chromosomal location and *lacZ* expression pattern from six lines of *P*[*en*1] and 27 lines of *P*[*en*2] were determined. For transgenic lines that contain the engrailed intron, only the position-dependent pattern is considered in this paper (see *Results*).

The enhancer detection experiment was performed as described by Perrimon *et al.* (12). Briefly, an insertion of *P*[*en*1] on the second chromosome (at 45E; containing rosy as the selectable marker) was mobilized using a genomic source of the transposase (13). Four lines obtained from this mobilization had insertions on the CyO chromosome. The transposon in each of these 4 lines was again mobilized using *P*[*ry*$^+$Δ2-3]99B (13). From this experiment, *lacZ* expression patterns of embryos from 143 lines were analyzed. Although we did not localize the sites of insertion in these 4 starting lines, all had a different *lacZ* expression pattern, and thus we presume the transposons were at independent locations. One of the starting insertions was at wingless. Transposition events from all four sites generated transgenic lines that had striped *lacZ* expression patterns. Because transposition events can occur premitotically (3), we could not be sure that all our lines derived from the same parents were independent. Therefore, we have not included 12 lines (including 8 lines that gave striped patterns) in the final analysis because we could not be sure that they arose independently. If these lines were, in fact, independent events, it would only increase the frequency of targeted insertion.

Detection of *lacZ* expression was either by enzymatic staining (discs) (4) or by immunoperoxidase staining using a monoclonal antibody against β-galactosidase (on embryos, used at a dilution of 1:1000; from Promega). The biotinylated horse anti-mouse antibody (Vector Laboratories) was used at a final concentration of 1:500 and was detected using the ABC kit (Vector Laboratories). Localization of insertions in polytene chromosomes was done using a nonradioactive method with digoxigenin-labeled probes (Boehringer Mannheim).

Inverse PCR amplification of *P*-element flanking sequences was carried out using the protocol of Ochman *et al.* (14) as described in Whiteley *et al.* (15). Inverse PCR products from insertions at 48A (the engrailed region) were gel-isolated, labeled by random priming, and hybridized to λ phage that contain a 230-kb chromosomal region from 48AB (16). Inverse PCR products from insertions at 35C, 83E, 87EF, and 40A were gel-isolated, labeled by random priming, and used to screen a *Drosophila* genomic DNA library (Promega). DNA from positive phage was isolated, labeled

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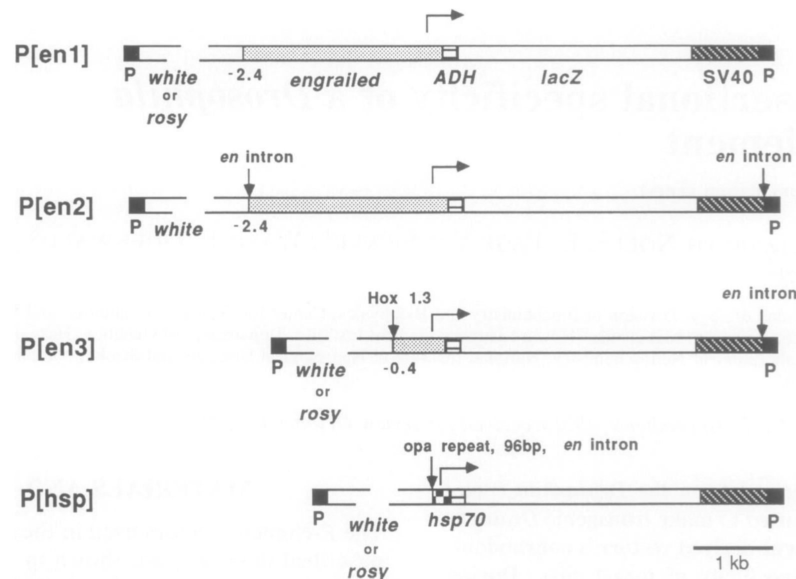

FIG. 1. *P*-element vectors used in these experiments. *P*[*en*1] contains *en* DNA extending from −2.407 kb through the start site of *en* transcription (indicated by the arrow) and 188 bp of the *en* untranslated leader cloned into the vector pC4ATGβ-gal (9). In this vector, an untranslated leader fusion is made between *en* (or *hsp70*) and the *Drosophila melanogaster* alcohol dehydrogenase gene (ADH; striped boxes), which provides the start site for translation. An ADH–β-galactosidase fusion protein was made. A simian virus 40 (SV40) fragment provides the polyadenylylation signal. *P*[*en*1] was made in two *P*-element vectors, Carnegie 20.1 (which contains rosy as the selectable marker) and pCaSpeR (which contains white as the selectable marker, ref. 10). *P*[*en*2] contains the same upstream *en* fragment as *P*[*en*1]. It also contains *en* intron 1 from either *Drosophila virilis* or *D. melanogaster* at the positions indicated by the arrow. Data from six intron-containing constructions were pooled (constructs B–G in ref. 6). Only the position-dependent pattern was considered in this paper (see *Results*). *P*[*en*3] contains *en* sequences −0.4 kb through +188 bp. The 17 transformants discussed include data from *P*[*en*3] alone (construct H in ref. 6; 1 line) and three derivatives—*en* intron downstream (construct I in ref. 6; 4 lines) and two with mouse Hox1.3 DNA fragments inserted upstream (construct H from ref. 7; 12 lines). The Hox1.3 DNA had no enhancer activity in *Drosophila*. *P*[*hsp*] includes data from transformants of it alone and with three sequences inserted where indicated by the arrow; *en* intron 1 (construct J from ref. 6), a 96-bp *en* fragment (construct 96bp-HZ73 from ref. 8), and with a synthetic *opa* element (ref. 11; oligonucleotide sequence: CAGCAACAACAGCAACAGCAGCAGCAGCAGCAG). The *opa* element had no detectable enhancer activity in this vector.

with digoxigenin using the Genius kit (Boehringer Mannheim), and hybridized to wild-type embryos to detect RNA transcribed from the region (17). From the three insertions at 40A, three phage were isolated from the PCR products; these phage were mapped using restriction enzymes and two were found to overlap.

RESULTS

P-element constructs used in initial experiments were originally made to test the function of *en* regulatory sequences (6). *P*[*en*1] (Fig. 1) includes *en* sequences from −2.4 kb through +188 base pairs (bp) fused to the reporter gene *lacZ*. When integrated into the genome, *P*[*en*1] does not express *lacZ* in any specific pattern but can be activated by nearby genomic regulatory sequences. Thus, *lacZ* expression patterns from *P*[*en*1] are dependent upon where it is inserted within the genome (position-dependent patterns). When the first *en* intron was cloned into *P*[*en*1] (*P*[*en*2], Fig. 1), *en*-like β-galactosidase stripes were observed early in development, independent of the position of insertion in the genome (6). Thus *en* intron 1 contains the information to give *en*-like stripes. We will refer to these *en*-like stripes as the position-independent pattern. During the course of these experiments, we noticed two unusual things about transformants that contained the engrailed fragment present in *P*[*en*1] and *P*[*en*2]. (*i*) A high number of transformants (6 out of 33) had, either in place of or in addition to the intron-induced pattern, position-dependent striped patterns that were either temporally or spatially distinguishable from the position-independent pattern. (*ii*) Localization of our 33 *P*-element insertions by *in situ* hybridization to polytene salivary gland chromosomes showed that three locations had two independent inserts (cytological locations 61D, 78E, and 98DE). *In situ* hybridization to salivary chromosomes of transheterozygotes of each pair indicated that the two *P* elements at each location were inserted at sites indistinguishable by this method. In addition, two insertion events had occurred in segmentation genes, one in engrailed and one in hairy. Thus these data led to the hypothesis that the *en* fragment in *P*[*en*1] and *P*[*en*2] might be directing insertion of *P* elements to a limited number of sites in the genome and that some of these sites might be segmentation genes.

In a second experiment, we used *P*[*en*1] (which contains only promoter activity) in an enhancer-detection experiment. We examined embryos collected from 131 insertion lines and found that 17 lines (13%) expressed β-galactosidase in striped patterns. This is significantly different from the 3.6% (135 out of 3768) found in the extensive enhancer-detection study of Bier *et al.* (5) ($P < 0.0001$ by a χ^2 test for homogeneity) and consistent with the high number of position-dependent striped patterns we obtained in the experiments described above (6 of 33 is not significantly different than 17 of 131, $0.3 < P < 0.4$). The high frequency of stripe-expressing lines might be due to one of the following two reasons: (*i*) the *en* fragment contains the information to generate stripes but needs an enhancer to make this evident or (*ii*) the *en*-containing *P* element is inserting in the genome in a nonrandom manner with a higher probability of insertion near genes normally expressed in stripes. To distinguish between these two possibilities, we localized (by *in situ* hybridization to polytene chromosomes) all those *P*-element insertion sites that gave striped expression patterns (and a number of others that gave interesting patterns). The chromosomal localization data support the hypothesis that *P*[*en*1] is inserting near genes that are normally expressed in striped patterns. Of the 17 insertion sites that gave striped expression, eight insertions mapped near known segmentation genes: six at en-

grailed (48A) and two at wingless (28A) (Table 1). Further, in the lines with inserts near engrailed, *lacZ* was expressed in an *en*-like pattern, and the lines with inserts near wingless had a wingless-like *lacZ* expression pattern (Fig. 2; also shown is the β-galactosidase expression pattern from the transgenic line with the insert in hairy obtained in the experiment discussed above).

To obtain further evidence that the striped expression of *lacZ* is a reflection of regulatory sequences for genes near the insertion sites, we cloned genomic DNA surrounding three of the insertions (at 35C, 83E, and 87EF). We then used these genomic DNA fragments to detect RNA in wild-type embryos. The genomic DNAs recognized RNA expressed in striped patterns similar to the β-galactosidase pattern in all cases (ref. 15; J. P. Vincent and J.A.K., unpublished data). These data also suggest that *P*[*en*1] preferentially inserts near genes that are expressed in stripes.

We have begun to further localize the DNA sequences responsible for preferential insertion by using *P* elements containing a smaller fragment of engrailed regulatory DNA. Seventeen transgenic lines from *P* elements that contain engrailed DNA extending from −400 bp through +188 bp (*P*[*en*3], see Fig. 1) were localized by *in situ* hybridization to salivary chromosomes and stained for β-galactosidase activity in embryos. Like *P*[*en*1], *P*[*en*3] behaves as an enhancer detector, since the pattern of β-galactosidase expression is dependent upon where it is inserted in the genome. Also like *P*[*en*1], a high proportion of *P*[*en*3] transformants express *lacZ* in striped patterns (5 out of 17, 29%; Table 1). One of these insertions is at the same chromosomal location and is expressed in the same pattern as an insert of *P*[*en*2] (at 35C, insertions are separated by 200 bp; ref. 15) and four additional insertions map to the same chromosomal locations as a *P*[*en*1] or *P*[*en*2] insertion site (40A, 69EF, 83BC, and 87AB). Although the sample size is small, these data suggest that at least some of the *en* sequences necessary for selective insertion lie between −400 and +188 bp.

We also generated 42 insertion lines with a vector (*P*[*hsp*], Fig. 1) that did not contain any engrailed DNA but had the same *lacZ* and simian virus 40 DNA as our other constructs. None of the *P*[*hsp*] insertion lines express β-galactosidase in a position-dependent striped pattern. Of the 42 *P*[*hsp*] insertion lines, 16 were localized and none mapped to the same chromosomal location as any *P*[*en*1], *P*[*en*2], or *P*[*en*3] insertions (data not shown).

Table 1. Chromosomal insertion sites of *P*[*en*1], *P*[*en*2], and *P*[*en*3]

P element(s)	No. of insertions	Site(s)
P[*en*1] and *P*[*en*2]	1	2C, 3DE, 4C, 6B, 8E, 24A–B, 24E, 25C, 30C, **35C**, 36A, 36CD, 45E, 47BC, 49DE, 51D, 57EF, 60A, 64AB, **66D**, 69EF, 70CD, 70EF, **75C**, 78A, **82E**, 83BC, **87AB**, **88A**, 89AB, **90DE**, 93E, **93F**, 94A, 97E-98A, 99B, **100A**, 100D, 100F
	2	6A, **28A**, **40A**, 60F, **62A**, 78E, 88E, 98DE
	3	100B
	4	**61D**
	7	**48A**
P[*en*3]	1	4B, 5A, 23C, 27F, **32F**, **35C***, **40A***, 45C, 56CD, **69EF***, 77E, 79E, 79F, 83BC*, 87AB*, **89A**, 89B

Insertion sites that gave *lacZ* expression in stripes are shown in boldface type.

*Insertion site also seen with *P*[*en*1] or *P*[*en*2].

P elements that contain the *en* DNA that causes selective insertion (referred to as *P*[*en*]) insert in the genome via the transposase and not by some other mechanism. (*i*) *P*[*en*] elements can be mobilized by treatment with the transposase. For example, one insertion of *P*[*en*2] at 48A is located ≈5 kb downstream of the *en* transcription unit (data not shown). The transposon in this line was mobilized by treatment with the transposase and six additional transgenic lines were generated. These insertion sites were located on the third and the X chromosomes. (*ii*) *P*[*en*] insertions contain intact 5′ and 3′ *P*-element ends. This is evidenced by the ability to amplify flanking genomic DNA using *P*-element-specific primers and inverse PCR (data not shown). (*iii*) We have cloned and sequenced two of our insertions at chromosomal location 35C (15). The *P*-element ends are flanked by 8-bp duplications of genomic DNA characteristic of *P*-element insertions (data not shown).

DISCUSSION

Modified *P* elements containing the *lacZ* gene under the control of a weak promoter have been widely used to search for tissue-specific enhancers in *Drosophila* (5, 25, 26). In one extensive screen, *lacZ* expression was examined in 3768 lines, with a large number of expression patterns observed (5). In that study, 138 (3.6%) of the insertion sites gave *lacZ* expression in stripes. Of those insertions, only a single insertion was found near the engrailed gene and a single insertion near the wingless gene. We have examined *lacZ* expression in 137 *P*[*en*1], 27 *P*[*en*2], and 17 *P*[*en*3] lines and found that 28 (16.3%) gave position-dependent *lacZ* expression in stripes. These 28 insertions include 7 near engrailed and 2 near wingless. These results are significantly different from those of Bier *et al.* (5). These data strongly suggest that *P* elements that contain a specific fragment of engrailed regulatory DNA target genes that are expressed in striped patterns.

Enhancer detector experiments using promoters from other segmentation genes have been reported (12, 30). Jacobs *et al.* (30) generated 284 lines using a *P*-element construct containing a 130-bp sequence from the fushi tarazu gene in front of a *lacZ* reporter gene. They found that 14 of the lines (5%) showed a common pattern of β-galactosidase expression in the longitudinal glial cells. They determined the cytological location of the *P* elements for six of these lines and found them all to be different. This result is quite different from the results we obtained where lines that gave similar expression patterns had similar cytological locations.

Hama *et al.* (4) reported that 7 out of 20 *P*-element transposons containing 3.4 kb or more of engrailed regulatory sequences integrated in the chromosomal vicinity of engrailed. As they point out, the insertion events seem to be region-specific and not site-specific; *P* elements insert not at the same site but in the same chromosomal region. Three insertions were located at engrailed, one insertion was at invected, and three insertions were located in the next polytene chromosome subdivision 47F. Thus, integration events occurred over hundreds of kilobases into engrailed and nearby genes. We have localized 4 out of 7 of our insertions near engrailed. Two were located to engrailed, one was located to invected, and one was located to a site about 100 kb downstream of the engrailed gene (data not shown). Thus, the insertion events we observed were also region-specific, not site-specific.

We have found that, in addition to engrailed, there are many other targets for *P*[*en*] insertion. One of these targets is the segmentation gene wingless. We obtained two insertions at wingless and both are lethal wingless alleles. However, these two insertions were not at the same site (data not shown). At another targeted region, 35C, two insertions

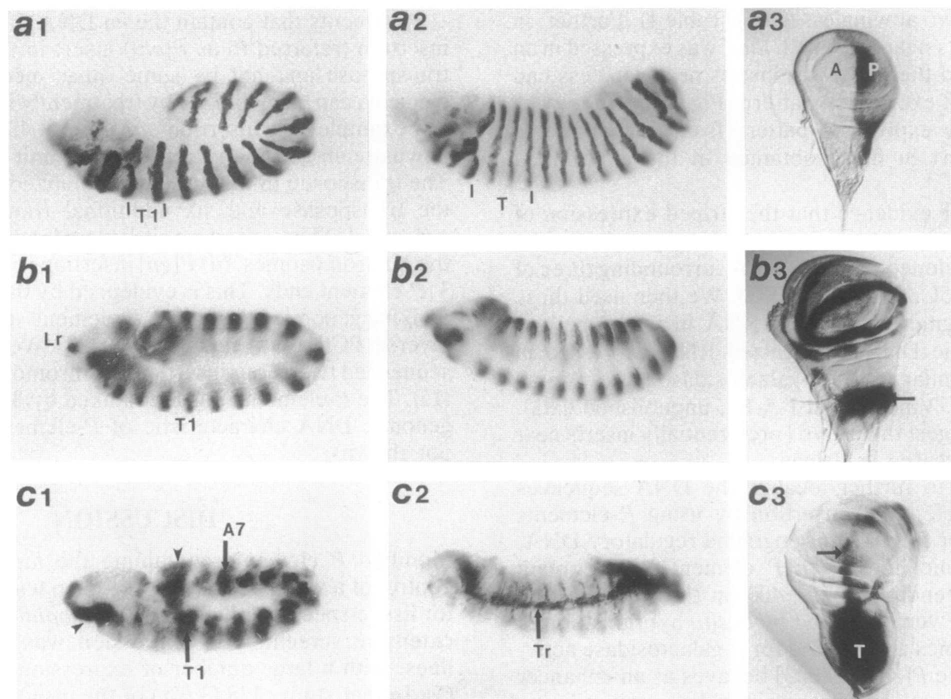

FIG. 2. β-Galactosidase expression patterns from insertions at or near *en* (*a*), wingless (*b*), and hairy (*c*). (*a*) Insertion line 1-*en*-14 generated in the enhancer detection experiment. *P*[*en1*] is inserted at 48A, the location of *en*. β-galactosidase expression pattern is similar to *en* expression at all stages of development. Embryos at 6.5 h (*a1*) and 9 h (*a2*) of development are shown (anterior left and dorsal top). (*a3*) β-Galactosidase expression in a second instar wing disc, where β-galactosidase expression is limited to the posterior compartment (P). (*b*) Insertion line 1-*en*-11 generated in the enhancer-detection experiment. Insert is at 28A, in wingless (insertion of *P*[*en1*] generated a lethal wingless allele). Embryos at 5 h (*b1*) and 10 h (*b2*) of development are shown (anterior left and dorsal top). Note the labrum (Lr) staining in the head and the discontinuity in the epidermal stripe pattern (*b2*). (*b3*) Staining in a third instar wing disc. The arrow points to the primordium of the anterior dorsocentral bristle (18). Staining encircling the wing pouch is also detected. The pattern of β-galactosidase expression in embryos and discs matches the published descriptions of wingless expression (19–21). (*c*) Insertion line C5 (7). Insert is at 66D, in hairy (insertion generated a lethal hairy allele). Line C5 contains *P*[*en2*] (construct C from ref. 7), which includes the *en* intron; therefore, expression early in development is complicated by the fact that β-galactosidase is expressed in both *en*-like stripes and hairy-like stripes. Expression is shown in embryos at 5 h (*c1*) and 11 h (*c2*) of development. β-Galactosidase expression is seen in the 10 tracheal pits (labeled T1 through A7) and the subsequent tracheal tree (Tr) and in the stomodeal and posterior midgut regions (arrowheads). hairy is also expressed in these regions (22, 23). (*c3*) β-Galactosidase staining in a third instar wing disc; the thoracic region (T) is labeled as well as the midline of the presumptive wing blade. This is a subset of the expression of hairy in the wing disc (24).

occurred within 200 bp of each other (15). At a third region (40A), two insertions are separated by ≈10 kb and another has yet to be linked to these two (J.A.K., unpublished data). Thus, insertion events are region-specific (and not site-specific) at these chromosomal locations as well.

There are other examples of eukaryotic transposable elements that insert nonrandomly in the genome (for review, see ref. 27). The most thoroughly characterized is the yeast transposable element, Ty3. Ty3 is a retrotransposon that inserts in the genome preferentially at tRNA genes (28). No specific tRNA gene is targeted, but insertion always occurs at position −17 or −16 upstream of the tRNA coding sequence (27, 28). The molecular mechanism for this selective insertion is unknown; however, recent evidence implicates features of RNA polymerase III transcription in Ty3 target selection (27).

Selective insertion has also been observed for the avian retrovirus Rous sarcoma virus in turkey embryo fibroblast cells (29). In this system, Shih *et al.* (29) found a small number of sites in the genome that were used at a frequency of one million times the expected frequency for random insertion. At the high-frequency target sites, independent insertion events always occurred at the same nucleotide. There was, however, no sequence similarity between different high-frequency targets. Thus Shih *et al.* (29) suggest that a structural feature could be directing insertion events.

Our results showing selective insertion of *P*[*en*] are similar to those described above in that many different genes are targeted by the transposon. Our results differ, however, in that insertion events do not occur at specific DNA sites but rather in "regions" of the chromosome. What causes this selective insertion? We favor a model whereby a protein(s) bound to the *en* fragment within the *P* element brings it to a particular region of the genome by protein–protein or protein–DNA interactions. The proximity of the *P* element enhances the probability of insertion within the targeted genomic region. Our model predicts that the targeted genes share a common structure or location in the nucleus. For example, the targeted genes might contain cis-regulatory DNA that interacts with a common DNA-binding protein that can interact with a protein bound to *P*[*en*]. Because *P*-element transposition occurs in the germ cells, the genes targeted by this element must have a related structure in this cell type. We also suggest that there is a concentration of target sites in the vicinity of the engrailed gene causing the high frequency of insertion near this locus.

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1. Kidwell, M. G. (1986) in *Drosophila, A Practical Approach*, ed. Roberts, D. B. (IRL, Oxford), pp. 59–81.
2. Spradling, A. (1986) in *Drosophila, A Practical Approach*, ed. Roberts, D. B. (IRL, Oxford), pp. 175–197.
3. Engels, W. (1989) in *Mobile DNA*, ed. Berg, D. & Howe, M. (Am. Soc. Microbiol., Washington), pp. 437–484.

4. Hama, C., Ali, Z. & Kornberg, T. B. (1990) *Genes Dev.* **4**, 1079–1093.
5. Bier, E., Vaessin, H., Shepherd, S., Lee, K., McCall, K., Barbel, S., Ackerman, L., Carretto, R., Uemura, T., Grell, E., Jan, L. Y. & Jan, Y. N. (1989) *Genes Dev.* **3**, 1273–1287.
6. Kassis, J. A. (1990) *Genes Dev.* **4**, 433–443.
7. Kassis, J. A., VanSickle, E. P. & Sensabaugh, S. M. (1991) *Genetics* **128**, 751–761.
8. Vincent, J.-P., Kassis, J. A. & O'Farrell, P. H. (1990) *EMBO J.* **9**, 2573–2578.
9. Thummel, C. S., Boulet, A. M. & Lipshitz, H. D. (1988) *Gene* **74**, 445–456.
10. Pirotta, V. (1988) in *Vectors, A Survey of Molecular Cloning Vectors and Their Uses*, ed. Rodriguez, R. L. & Denhardt, D. T. (Butterworth, Boston), pp. 437–456.
11. Wharton, K. A., Yedvobnick, B., Finnerty, V. G. & Artavanis-Tsakonas, S. (1985) *Cell* **40**, 55–62.
12. Perrimon, N., Noll, E., McCall, K. & Brand, A. (1991) *Dev. Genet.* **12**, 238–252.
13. Robertson, H. M., Preston, C. R., Phillis, R. W., Johnson-Schlitz, D. M., Benz, W. K. & Engels, W. R. (1988) *Genetics* **118**, 461–470.
14. Ochman, H., Gerber, A. S. & Hartl, D. L. (1988) *Genetics* **120**, 621–625.
15. Whiteley, M., Noguchi, P. D., Sensabaugh, S. M., Odenwald, W. & Kassis, J. A. (1992) *Mechanisms of Development*, in press.
16. Kuner, J. M., Nakanishi, M., Ali, A., Drees, B., Gustavson, E., Theis, J., Kauvar, L., Kornberg, T. & O'Farrell, P. H. (1985) *Cell* **42**, 309–316.
17. Tautz, D. & Pfeifle, C. (1989) *Chromosoma* **98**, 81–85.
18. Bryant, P. J. (1978) in *The Genetics and Biology of Drosophila*, ed. Ashburner, M. & Wright, T. R. F. (Academic, New York), Vol. 2C, pp. 229–335.
19. Baker, N. E. (1988) *Development* **102**, 489–497.
20. Baker, N. E. (1988) *Development* **103**, 289–298.
21. van den Heuvel, M., Nusse, R., Johnston, P. & Lawrence, P. A. (1989) *Cell* **59**, 739–749.
22. Hooper, K. L., Parkhurst, S. M. & Ish-Horowicz, D. (1989) *Development* **107**, 489–504.
23. Carroll, S. B., Laughon, A. & Thalley, B. S. (1988) *Genes Dev.* **2**, 883–890.
24. Carroll, S. B. & Whyte, J. S. (1989) *Genes Dev.* **3**, 905–916.
25. O'Kane, C. & Gehring, W. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 9123–9127.
26. Bellen, H. J., O'Kane, C. J., Wilson, C., Grossniklaus, U., Pearson, R. K. & Gehring, W. J. (1989) *Genes Dev.* **3**, 1288–1300.
27. Sandmeyer, S. B., Hansen, L. J. & Chalker, D. L. (1990) *Annu. Rev. Genet.* **24**, 491–518.
28. Chalker, D. L. & Sandmeyer, S. B. (1990) *Genetics* **126**, 837–850.
29. Shih, C.-C., Stoye, J. P. & Coffin, J. M. (1988) *Cell* **53**, 531–537.
30. Jacobs, R. J., Hiromi, Y., Patel, N. H. & Goodman, C. S. (1989) *Neuron* **2**, 1625–1631.