Genet. Res., Camb. (1992), **60**, *pp.* 33–41 *With 4 text-figures Printed in Great Britain*

Distribution and structure of cloned P elements from the *Drosophila melanogaster* P strain π_2

KEVIN O'HARE[1*], ALAN DRIVER[1], STEPHEN McGRATH[1] AND DENA M. JOHNSON-SCHILTZ[2]

[1] *Department of Biochemistry, Imperial College of Science, Technology & Medicine, London SW7 2AZ, U.K.*
[2] *Genetics Laboratory, University of Wisconsin-Madison, Madison, Wisconsin 53706, U.S.A.*

(*Received 26 November 1991 and in revised form 15 April 1992*)

Summary

P transposable elements of *Drosophila melanogaster* cloned from the strong P strain π_2 have been analysed. The structures and chromosomal locations of 26 of the 30–50 elements estimated to be present in π_2 have been determined. At one location two elements are inserted 100 base pairs (bp) apart, and in a second location two elements are only separated by the 8 bp duplicated upon P-element insertion. In addition to 2·9 kilobasepair (kbp) elements, elements with 14 different internal deletions from 1·3 to 2·3 kbp in size have been isolated. There are 7 copies of the 2·9 kbp element, 2 copies each of 5 internally deleted elements and a single copy of 9 internally deleted elements. One of the elements found twice is the KP element, which may play a role in the regulation of hybrid dysgenesis in strains which contain many copies of this element. Apart from internal deletions the elements are extremely homogeneous in DNA sequence, with only 2 single base polymorphisms detected twice each in over 16 kbp of P-element sequence. Although transpositions are infrequent in an inbred P cytotype strain such as π_2, the distribution of these cloned elements indicates that when the genomic library was made, the strain was polymorphic with respect to element location. The distribution and structures of the element are discussed with respect to models for regulation of P-element transposition.

1. Introduction

P–M hybrid dysgenesis in *Drosophila melanogaster* results from the mobilization of P-transposable elements (reviewed by Engels, 1989; Rio, 1990). P strains have 30–50 P elements (Bingham, Kidwell & Rubin, 1982). They have several copies of a 2·9 kilobasepair (kbp) P element and a heterogeneous collection of smaller elements whose structures are related to that of the 2·9 kbp element by internal deletion (O'Hare & Rubin, 1983). M strains have no P elements. When an M strain female is crossed with a P strain male, the elements are mobilized in the germ cells of the progeny and dysgenesis occurs. In the reciprocal cross between a P-strain female and an M-strain male, the elements are essentially quiescent and the hybrids develop normally. This suggested that P elements encode at least two functions, a transposase, and a regulator of transposition which suppresses transposition in embryos developing from eggs laid by P-strain mothers.

The regulation of P–M hybrid dysgenesis is complex, and appears to be strain dependent. The term cytotype was introduced by Engels (1979) to describe the regulation of hybrid dysgenesis seen in P strains such as π_2. Whether a fly regulated transposition or not depended ultimately upon the chromosomes, but in hybrids between M and P strains, regulation was maternally determined. P strains and the progeny of a cross between P females and M males were described as having P cytotype, while M strains and the progeny of a cross between M females and P males had M cytotype. Transposition only occurred at high rates when P elements were in the M cytotype. Further studies showed that strains with no or few 2·9 kbp P elements could provide partial protection from the deleterious effects of hybrid dysgenesis (Kidwell, 1985). This partial repression of hybrid dysgenesis was chromosomally determined with no maternally inherited component (Black *et al.* 1987; Simmons *et al.* 1987). In some strains, a particular deleted element,

* Corresponding author.

the KP element, was correlated with repression (Jackson, Black & Dover, 1988). However, other strains with KP elements did not repress hybrid dysgenesis (Simmons *et al.* 1990; Biemont *et al.* 1990) and some P-cytotype strains did have KP elements (Monastirioti *et al.* 1988). Partial repression of hybrid dysgenesis has also been reported for strains with no KP elements (Heath & Simmons, 1991; Raymond *et al.* 1991). Differences in the number, structures and locations of P elements appear to be responsible for the strain dependent regulation of hybrid dysgenesis.

We have therefore extended our examination of the structures of the elements cloned from the strong P strain π_2 (O'Hare & Rubin, 1983) and have determined their chromosomal locations. The π_2 strain is one of the best characterized P strains, and the definition of cytotype was based upon experiments using this strain (Engels, 1979). The structure and distribution of the elements are discussed with reference to current models for the regulation of P-element transposition (Rio, 1991).

2. Materials and methods

The isolation of the clones used in these studies has been described (O'Hare & Rubin, 1983). Growth of λ phage, purification of DNA and characterization of the insert by restriction enzyme mapping and DNA blotting was by standard techniques (Sambrook, Fritsch & Maniatis, 1989). For DNA blotting experiments, we made a derivative of the plasmid pπ25.7WC (Karess & Rubin, 1984). This derivative, pπ25.7BWC ('both wings clipped') contains no flanking genomic sequences and has the P-elements sequences from position 39 to 2885 inserted between the *Hin*d III and *Sal* I sites of pBR322. Hybridization to polytene chromosomes was as described by Engels *et al.* (1986). DNA sequencing was by the dideoxy technique on M13 recombinants using the universal primer, and oligonucleotides complementary to positions 120–141, or corresponding to positions 233–247, 484–498, 2186–2200, 2397–2412, 2626–2640 or 2847–2865 of the 2·9 kpb element. Generally, a restriction enzyme fragment including the whole P element, or fragments from the *Hin*d III site at position 39 to sites in the flanking genomic sequences beyond the ends of the element, were cloned.

3. Results

An unamplified genomic library of the P strain π_2 made in 1981 was screened with P-element probes and 40 recombinant λ phage were purified (O'Hare & Rubin, 1983). Preliminary characterization of the size of the P element and the location of restriction enzyme sites in the flanking genomic DNA, indicated that the 37 phage still viable in 1987 were from 25 different genomic sites. A representative phage for each location was then characterized in more detail.

(i) *P-element structures*

The structures of the cloned P elements were first examined by digestion with *Ava* II. This enzyme cuts at four positions in the 2·9 kbp element; within each terminal inverted repeat, and at two internal positions. Intact 2·9 kbp elements generate three fragments while internally deleted elements produce at least one fragment. The number and sizes of *Ava* II fragments is a useful diagnostic test of the structure of a P element. DNA blots were probed with the complete P-element probe pπ25.7BWC (data not shown). At 7 of the genomic sites there was a 2·9 kbp element, and at 15 sites there was a single internally deleted element. For two sites, the pattern was not consistent with a single P element. More detailed analysis (see below) showed that there were two internally deleted P elements at each of these two sites. For the remaining site, the phage had an insert which ended within a P element, so that only one end of the element presumed to exist within the genome at that site was present in the recombinant. This site was not characterized further.

A number of the small elements were clearly different from each other, but similar sized deletions from different positions within the 2·9 kbp sequence could produce elements with the same pattern of *Ava* II fragments. DNA sequencing was therefore performed to determine the precise position of the sequences deleted. The different structures deduced for these elements are in Fig. 1, and the DNA sequences which define the structure of the elements are shown in Fig. 2. The 19 elements have 14 different structures, 9 found once and 5 found twice. The complete sequence of 11 elements (π13A, π13B, π15, π18, π19, π32, π34A, π34B, π40, π49 and π50) were determined. For 4 elements (π14, π29, π31 and π41) only the sequences 5′ of the *Hin*d III site at position 39 were not determined. Only one strand was usually sequenced, and it was not possible at 21 positions (out of the more than 16 kbp determined) to assign with complete confidence the identity of the base. Other than small sequence rearrangements associated with the internal deletions, only two positions (positions 32 and 33) had been previously described as polymorphic for P elements cloned from π_2 (O'Hare & Rubin, 1983). This more extensive analysis confirms that result; two elements (π13A and π45) had the T for A polymorphism at position 32 and two (π17 and π33) had the T for A polymorphism at position 33. In each case, the pair of elements with the same polymorphism were otherwise identical in structure. Elements π13A and π45 are copies of the KP element described by Black *et al.* (1987).

(ii) *Sequences flanking the P elements*

Ava II digests of the recombinants were analysed by DNA blotting using labelled π_2 DNA as a probe to

https://doi.org/10.1017/S0016672300030640 Published online by Cambridge University Press

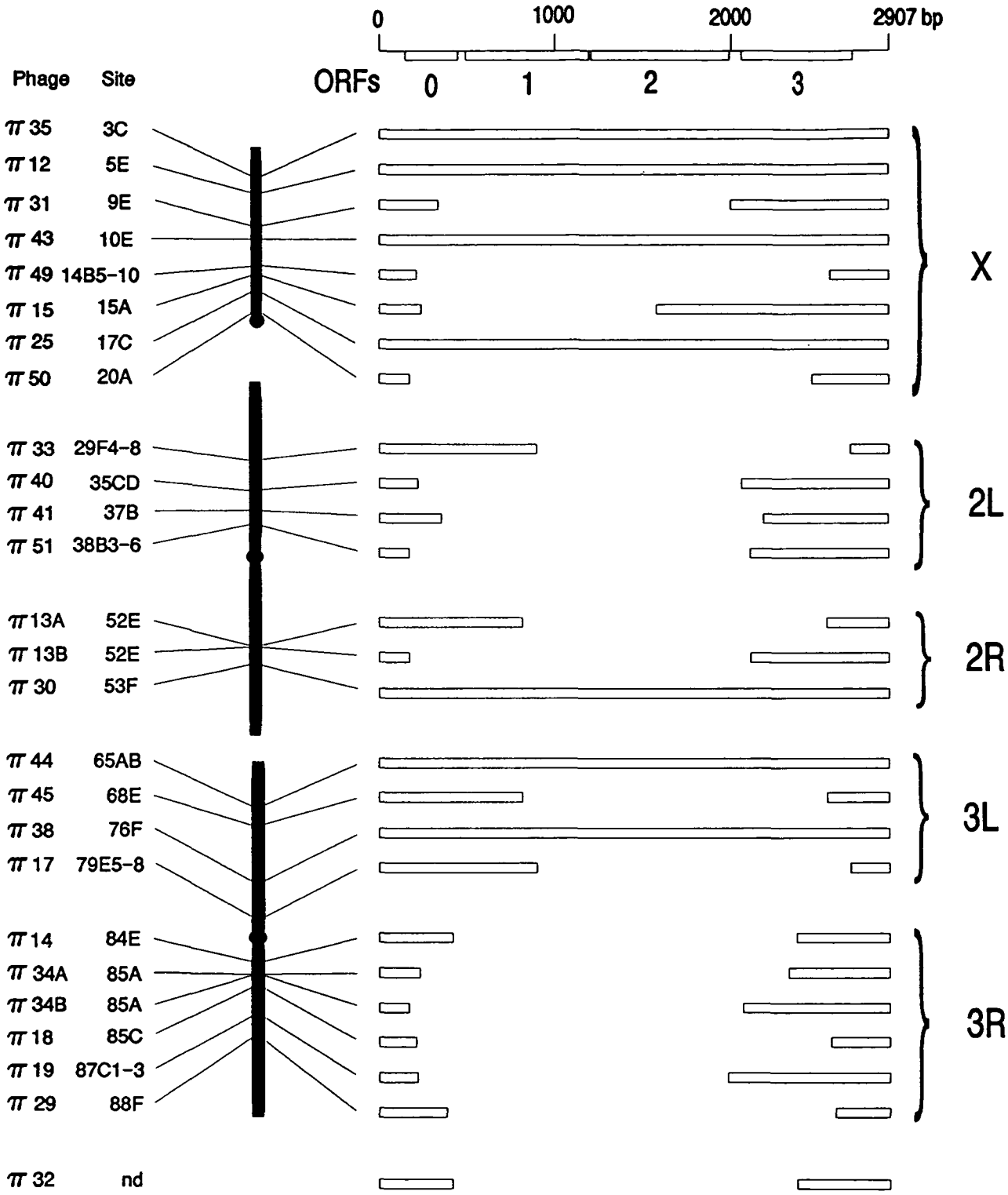

Fig. 1. Structures and locations of cloned P elements. The extent of P sequences present in different cloned P elements are indicated as blocks beneath a diagram showing the location of open reading frames (ORFs) in the 2·9 kbp P element. They are grouped by chromosomes arm and their cytological locations as determined by *in situ* hybridization are indicated on drawings of the chromosomes. Note that for π33 and π19, only one of three sites of hybridization is shown (see text for details). The location of π32 was not determined (nd).

determine if there were repeated sequences in the DNA flanking the P elements. In such a probe, the elevated concentration of repeated sequences (such as transposable elements) compared to that of single copy sequences allows for detection of regions with homology to repeated sequences. This experiment (data not shown) indicated that all but four of the elements are flanked by low copy or unique DNA sequences. This is consistent with the results of *in situ* hybridization of P-element probes to salivary gland polytene chromosomes of P-strain larvae (Bingham, Kidwell & Rubin, 1982). Hybridization is detected to

https://doi.org/10.1017/S0016672300030640 Published online by Cambridge University Press

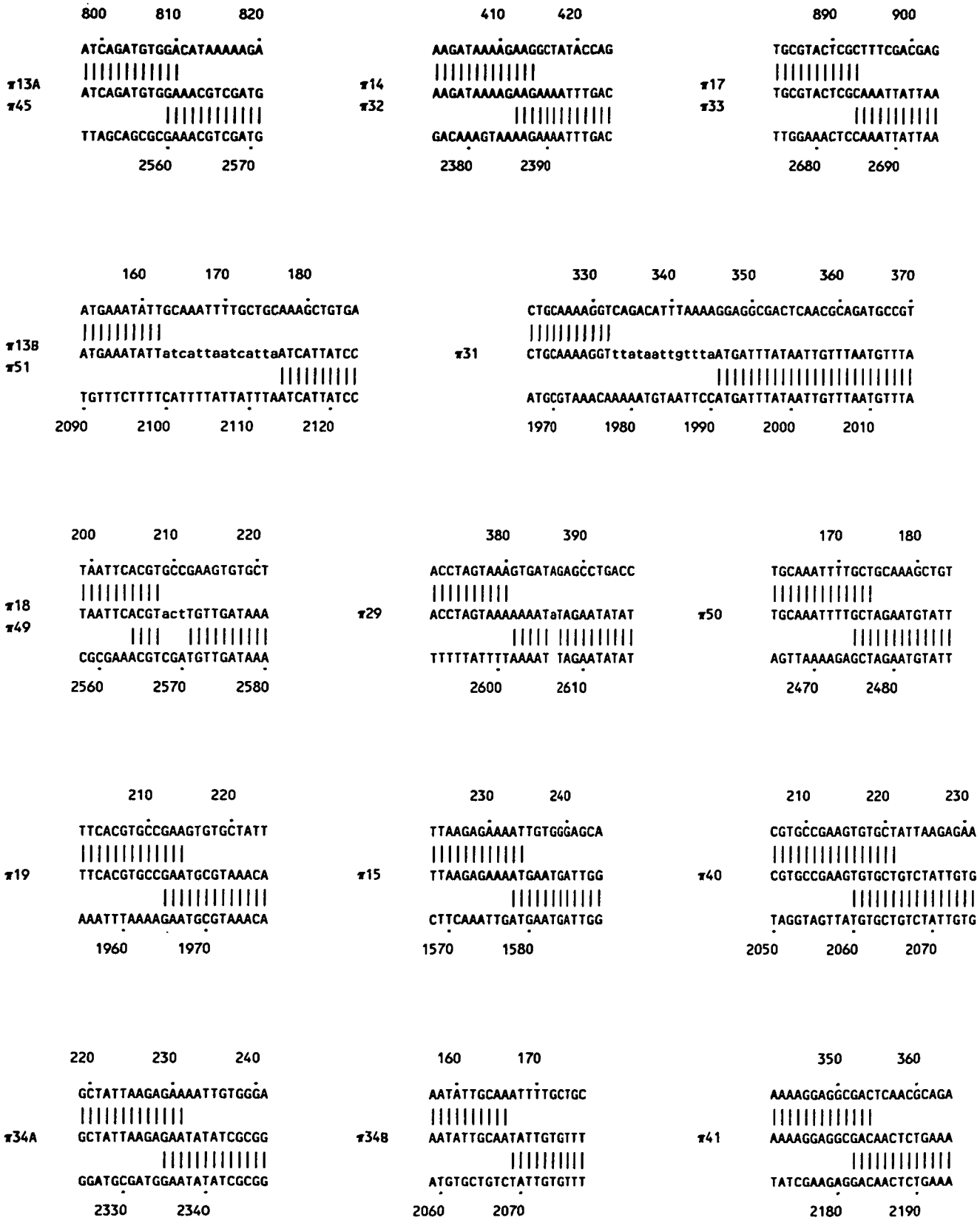

Fig. 2. DNA sequences of internally deleted P elements. The sequences of internally deleted P elements are shown for the position corresponding to the deletion of sequences from the 2·9 kbp element. They are aligned with the sequence of the 2·9 kbp element from both ends of the presumptive deletion. Bases which cannot be assigned from the 2·9 kbp element sequence are in lower case.

sites in the chromosome arms (which consist mainly of single copy sequences), with little hybridization to the chromocentre (which consists primarily of repeated sequences). In the recombinants $\lambda\pi 18$, $\lambda\pi 19$, $\lambda\pi 33$ and $\lambda\pi 35$, repeated sequences other then P were detected.

https://doi.org/10.1017/S0016672300030640 Published online by Cambridge University Press

Fig. 3. DNA sequence flanking P elements. P element sequences are in upper case and flanking genomic sequences are in lower case. The immediately flanking 8 bp are in bold. The two polymorphic positions, 32 in π13A and π45, and 33 in π17 and π33, are indicated with asterisks (*).

The DNA sequence immediately flanking the P elements was determined for most of the sites. The DNA sequences at the left end (5′ with respect to P-element transcription) of 7 elements and at the right end of 17 elements were determined. These results are in Fig. 3, combined with previously obtained data for the left end of 12 of these elements and the right ends of 3 (O'Hare & Rubin, 1983). For 16 of the elements, the sequences flanking both ends of the element could now be compared, and in each case there is an 8 basepair (bp) flanking duplication. An 8 bp 'target site' sequence is duplicated upon insertion of P elements (O'Hare & Rubin, 1983). Chromosomal rearrangements between elements results in elements being flanked by different 8 bp sequences (Roiha, Rubin & O'Hare, 1988; A. Driver & K. O'Hare, unpublished results; W. Eggleston & W. R. Engels, personal communication). We conclude that the distribution of elements in π_2 has been generated by *de novo* insertion events.

A consensus sequence was derived from the 8 bp flanking 18 different P elements (O'Hare & Rubin, 1983). The additional 7 sequences reported here (π13A, π13B, π18, π32, π34A, π34B and π41) have an average of 5 matches out of 8 with this consensus. However, as more data have been generated, particularly from the analysis of insertion sites of P elements at *Notch* (Kelly *et al.* 1987) and *singed* (Roiha, Rubin & O'Hare, 1988), it has become clear that the sequence of the 8 bp 'target site' alone cannot explain the observed distribution of P elements. Altered chromatin structure around promoter regions has been suggested to explain the skewed distribution within a gene of P-element insertions (Tsubota, Ashburner & Schedl, 1985; Kelly *et al.* 1987).

(iii) *Distribution of P elements*

The phage DNAs were hybridized to polytene chromosomes from salivary glands of M strain larvae to determine the cytological location of the P elements. The DNA of phage where the P elements were inserted in low copy or unique DNA sequences generally hybridized to a single site in the chromosome arms (Fig. 1). Three of the phage whose inserts included other repeated sequences, $\lambda\pi$18, $\lambda\pi$33 and $\lambda\pi$35, hybridized to the chromocentre. Of these, $\lambda\pi$18 and $\lambda\pi$35 also hybridized to single euchromatic sites which probably represent the chromosomal location of the P element with the other repeated sequence present in the probe being responsible for hybridization to the chromocentre. In addition to the chromocentre, three euchromatic sites were labelled by $\lambda\pi$33 (29F4-8, 67D and 84E). The remaining phage whose insert appeared to include repeated sequences, $\lambda\pi$19, did not hybridize to the chromocentre, but to three euchromatic sites (87C1-3 and two sites in 87A).

The density of P elements cloned from the π_2 X chromosome appears to be about twice that for the autosomal arms. A high density of P elements upon the X chromosome in wild populations has been reported (Ronsseray & Anxolabehere, 1986). The model of Engels *et al.* (1990) where a homologous template is required for repair after excision of a P element provides a rationale for this. As the X chromosome is hemizygous in males, this may result in a lower frequency of excision from the X compared to the autosomes.

(iv) *Double P-element insertions*

Restriction enzyme maps were constructed for the genomic sites where two small P elements were inserted (Fig. 4). At both sites, the two elements were inserted in the same orientation. The DNA preparations produced some sub-molar bands in restriction enzyme digests consistent with there having been recombination between the two elements during growth of the phage. As the vector was λ charon 28, the recombinants had to be propagated in *recA*$^+$ bacteria. The elements in $\lambda\pi$13 and $\lambda\pi$34 are so small and close together that recombination between them would generate viable phage. DNA sequence analysis showed that in $\lambda\pi$13, the two P elements (π13A and π13B)

https://doi.org/10.1017/S0016672300030640 Published online by Cambridge University Press

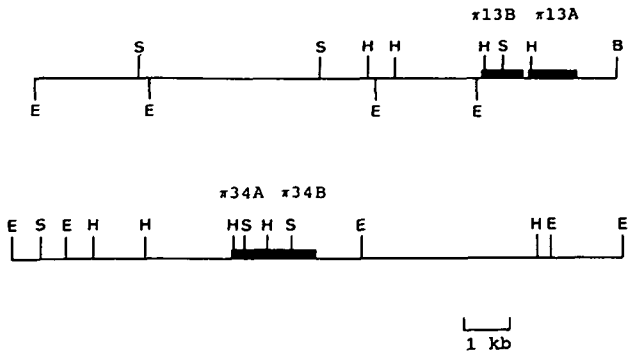

Fig. 4. Restriction enzyme maps of double P-element clones. Maps of restriction enzyme sites within inserts of recombinant phages π13 and π34 where two P elements are found. The P elements are shown as solid lines. H, *Hin*d III; E, *Eco*R I; S, *Sal* I; B, *Bam*H I.

were separated by 100 bp. In $\lambda\pi$34 the two P elements (π34A and π34B) were only separated by 8 bp. This 8 bp sequence is found flanking both P elements (see Fig. 3), indicating that this structure was generated by insertion of two P elements at the same 8 bp 'target site'. A similar structure has been deduced for the double P-element insertion mutant *singed-weak*, except that the two elements in this allele are in opposite orientation (Roiha, Rubin & O'Hare, 1988).

4. Discussion

(i) *Representation of sites and structures*

The probe used in 1981 to isolate the elements was a mixture of 2 P elements from mutants of the *white* locus which were generated in a P–M hybrid dysgenesis experiment where π_2 was the P strain (Rubin, Kidwell & Bingham, 1982). Elements with these structures are present in the cloned π_2 elements (π15 is the same as the element from $w^{\#12}$ and π17 is the same as the element from $w^{\#6}$). Of all the internally deleted elements analysed here, these have the longest contiguous sequences from the ends of the 2·9 kbp element (see Fig. 1). Since P elements share conserved terminal sequences, the probe used should have permitted isolation of all P elements. However, it is possible that very small elements were not detected and/or lost during the purification of the phage because of low signal strength.

The isolated recombinants were very unlikely to include every site in the π_2 genome where there was a P element. Continued transposition of P elements at low frequency has been found to occur in both non-dysgenic crosses (Eggleston, Johnson-Schiltz & Engels, 1988) and in inbred sublines of π_2 (Preston & Engels, 1984). Within the population of flies used to prepare DNA for the library construction, we expect that there were polymorphisms for P element sites with major and minor occupancy sites. A major site is one where a P element would be detected using P probes in DNA blotting of mass DNA preparations and/or upon *in situ* hybridization in most of the individuals. Original estimates from *in situ* hybridization were that there were 30–50 sites in π_2 where there were P elements (Bingham, Kidwell & Rubin, 1982). The X chromosome of π_2 had 5 major sites for P elements at 2F, 5E, 11A, 13E and 17C (Preston & Engels, 1984). The number of minor sites could be considerably higher, and is difficult to estimate. We have isolated the 5E and 17C sites, purifying 4 and 3 independent phage respectively. We have not recovered phage corresponding to the other major X-linked sites, but have isolated elements from six other sites on the X chromosome. For five of these sites, only one phage was isolated, and we presume that they were minor occupancy sites in 1981.

Some feature of the major sites at 2F, 11A and 13E may have led to their being under-represented in the library. In other libraries made using λ charon 28 as vector and a *rec*$^+$ bacterium as host, it was not possible to recover clones from *singed-weak* where two P elements are inserted in inverse orientation (Roiha, Rubin & O'Hare, 1988). It is possible that there were two P elements in inverse orientation at 2F, 11A and 13E.

We have compared the cloned P elements with those in genomic π_2 DNA. M strain DNA (Canton S) was mixed with approximately equimolar aliquots of phage DNA from each of the sites. This mixture was compared by DNA blotting with a preparation of π_2 DNA made in 1989. When digested with *Ava* II and probed with pπ25.7BWC, a similar pattern of bands is detected (data not shown). The intensities of the bands detected in the mixture of cloned P elements did differ from those detected in π_2. The differences could have been due to inaccuracies in the concentrations of the phage DNAs used, and/or because some element structures were under- or over-represented in the collection of clones. There appear to be no major differences between the structures of the elements cloned in 1981 and those present in π_2 8 years later.

(ii) *Structures and coding potential of small P elements*

The DNA sequences of the small elements confirm that there are no common endpoints for the deletions which are presumed to have been their origin. There is some clustering of deletion endpoints (see Figs 1, 2), such as the left ends of the deletions in π13B, π34B and π50 between position 160 and 175, or the right ends in π18 and π13A, but it seems likely that the different internally deleted elements are of independent origin. The left ends of the deletions are generally closer to the ends of the 2·9 kbp sequence than the right ends, the exceptions being π13A (the KP element), π17 and π29.

In four structures, there are small sequence rearrangements at the position of the putative deletion (Fig. 2). In π29 there is an insertion of a single A residue, and in π18 a substitution of ACT for CGA. In

https://doi.org/10.1017/S0016672300030640 Published online by Cambridge University Press

π13B, there are an additional two copies of the sequence ATCATTA, while in π31 there is a rearrangement which includes a duplication of TTTATAATGTTTAATG. In many other elements, the precise position of the putative deletions cannot be assigned because of bases which may have originated from either end of the deletion. The ambiguities are a single base in π17, two bases in π13A and π15, three bases in π14, π19, π34A, π41 and π50, and 6 bases in π40. As there are many duplications of these sizes within the P-element sequence, they are unlikely to be the primary determinants of the position of the deletions. In any case, in π34B there is no duplication whatsoever.

A mechanism has been proposed for the generation of small P elements (Engels *et al.* 1990). In the presence of P transposase, P elements excise and the gap generated can be repaired by copying in homologous sequences in a reaction similar to gene conversion. If the repair reaction uses the homologous chromosome but is interrupted before completion, then internal deletions would be generated.

We have examined the sequences of the small elements to see if the deletions would result in open reading frames being joined in phase. In π14, ORF1 is joined in phase with ORF3, and in π18, ORF0 is joined in phase to ORF3. All other elements join ORF sequences out of phase, or fuse exon sequences with intron sequences. The structures of most of the small elements in π_2 do not appear to have been maintained because they encode protein products. This does not however preclude that they would be transcribed, nor that any RNAs so produced would be translated.

(iii) *Regulation of hybrid dysgenesis by proteins encoded by 2·9 kbp elements*

P-element transposition can be regulated in different ways. Some P-element encoded proteins affect transcription from the P-element promoter, while others may affect the activity of the transposase (reviewed by Rio, 1991). Engels (1979) showed that each of the major chromosomes of π_2 contribute to the determination of P cytotype. Only the 2·9 kbp elements are present on all the chromosomes, but it is formally possible that elements of different structure might contribute towards the determination of P cytotype. The maternal inheritance of cytotype implies that it is determined by a protein or proteins present in P-strain eggs. Most small elements cannot encode protein products of any significant size, so it seems likely that the regulation of P-element transposition called cytotype is determined by the 2·9 kbp P elements.

The 2·9 kbp element encodes two proteins; an 87 kilodalton (kDa) transposase protein and a 66 kDa protein which has been suggested to be a repressor of hybrid dysgenesis. All 4 open reading frames (see Fig. 1) contribute to the 87 kDa protein (Karess & Rubin, 1984) which is only made in the germline because the ORF2–ORF3 splice is germline restricted (Laski, Rio & Rubin 1986). The 66 kDa protein would be made by translation of RNA where the ORF2–ORF3 splice was not made. This protein is made in somatic tissues (Rio, Laski & Rubin 1986) and has been detected as a maternally contributed protein in eggs laid by P-strain mothers (Misra & Rio, 1990). Elements which have been engineered *in vitro* to make the 66 kDa protein but not the 87 kDa protein, can repress hybrid dysgenesis *in vivo*, although the ability to do so was highly dependent upon chromosomal location and there was no maternal inheritance of repression (Robertson & Engels, 1989; Misra & Rio, 1990).

The 87 kDa transposase protein binds close to the ends of P elements (Kaufman, Doll & Rio, 1989). Although DNA binding by the 66 kDa protein has not been reported, it is reasonable to hypothesize that it binds in a similar way to the 87 kDa protein but does not catalyse transposition. Further evidence that P-element encoded proteins bind to P elements *in vivo* is the cytotype-dependent phenotypes of some P-insertion mutations of *vestigial* (Williams, Pappu & Bell, 1988) and *singed* (Robertson & Engels, 1989). The expression of β-galactosidase from P[*lacz*] enhancer detector transposons is also repressed by P cytotype (LeMaitre & Coen, 1991; J. Paterson and K. O'Hare, in preparation). These results probably reflect the binding of P-encoded proteins at the inserted elements. Binding of the 87 kDa protein can repress transcription from the P element promotor *in vitro* (Kaufman & Rio, 1991), so binding by the 66 kDa protein *in vivo* might regulate hybrid dysgenesis at the transcriptional level. A deleted element which could not encode the 87 kDa protein, but could encode a protein similar to the 66 kDa protein has been correlated with repression of hybrid dysgenesis in a Japanese strain (Nitasaka, Mukai & Yamazaki, 1987). There appear to be no such elements amongst those cloned from π_2, although we cannot exclude that there are some in those we have not cloned. Furthermore, some of the 2·9 kbp elements may have point mutations or very small deletions in ORF3. Only π12 has been entirely sequenced and shown to be identical to π25 (A. Driver and K. O'Hare, unpublished results). Both the 87 and 66 kDa proteins are encoded by the 2·9 kbp elements, and control of the ORF2–ORF3 splice may play a role in the inheritance of P-element regulation. A simple model would be that the 66 kDa protein inhibits this splice, and maternal inheritance of this protein would then result in continued production of the 66 kDa protein and repression of transposition. However, there is no evidence that the 66 kDa acts at the level of RNA, and its structure is more consistent with models where it acts by binding to DNA.

Maternal inheritance of the 66 kDa protein in P strains (Misra & Rio, 1990) shows that the ORF2–ORF3 splice is not obligatory in the germline. There appears to be an inhibitor of the ORF2–ORF3 splice

in somatic cells (Siebel & Rio, 1990). Perhaps this is also present at lower levels in germ cells. The ORF2–ORF3 splice, and production of transposase mRNA, might only occur when there are sufficiently high concentrations of precursor RNA in germ cell nuclei to escape the inhibition. This may occur during a dysgenic cross, when P elements are introduced into an egg lacking P-element encoded proteins. In P cytotype, P-element encoded proteins, specifically the 66 kDa protein, may repress transcription and the low abundance of precursor RNA may then be insufficient for the ORF2–ORF3 splice to be made. The RNA lacking the ORF2 to ORF3 splice would generate more 66 kDa protein. In this way, the presence of maternally inherited 66 kDa protein would favour its own synthesis over that of transposase and so result in the maintenance of P cytotype and low rates of P-element transposition.

(iv) *Regulation of hybrid dysgenesis by proteins encoded by internally deleted elements*

It is difficult to evaluate the role(s) that proteins encoded by internally deleted elements may have in the regulation of hybrid dysgenesis. A protein encoded by the KP element has been suggested to limit the deleterious effects of transposition during hybrid dysgenesis (Black *et al.* 1987; Jackson, Black & Dover, 1988). The behaviour of strains harbouring KP elements during hybrid dysgenesis is highly variable. While many do repress hybrid dysgenesis, some do not (Black *et al.* 1987; Simmons *et al.* 1990; Biemont *et al.* 1990). Inbred lines derived from strains with many KP elements show variability in repression of hybrid dysgenesis which does not correlate well with the numbers of KP elements in their genomes (Simmons *et al.* 1990; Biemont *et al.* 1990). The effect that KP, or any other element, has on the outcome of a dysgenic cross appears to be highly dependent upon its position within the chromosomes, and it is possible that transcription in the germline may be better correlated with repression than copy number.

The DNA binding domain of the 87 kDa protein has not been experimentally defined, but is thought to be a helix–turn–helix motif encoded by ORF1–ORF2 (Rio, Laski & Rubin, 1986). The sequences encoding this motif are deleted in the KP element, so it seems unlikely that a KP protein would compete with transposase for binding at inserted P elements. This conclusion would have to be reconsidered if the P element DNA binding domain is present in a KP protein. Rio (1990) has pointed out that leucine-zipper motifs are present in both the transposase and the putative KP protein. This motif has a role in protein–protein interactions of some DNA binding proteins (Landschulz, Johnson & McKnight, 1988), so a KP encoded protein might repress hybrid dysgenesis by binding to transposase, and interfering with either its binding to P elements or its ability to catalyse transposition once bound. Other elements, similar in structure to KP, could act in the same way. The element (which we call HP) found in π17 and π33 is very similar to KP (see Fig. 1). Elements other than KP can partially repress hybrid dysgenesis (Heath & Simmons, 1991; Raymond *et al.* 1991), but their structures have not been precisely defined.

The KP element has a DNA sequence polymorphism at position 32 while the HP element has a polymorphism at position 33 (see Fig. 3). These substitutions are in the region where protein–nucleic acid interactions are likely to occur during transposition, and in regulation of P-element transcription. Many of the P elements found inserted in mutants generated during hybrid dysgenesis do often appear to be either KP or HP, but because of differences in element copy number and location in the strains used in mutagenesis experiments, it is not possible to compare their transposition frequency with that of elements lacking these polymorphisms.

(v) *Conclusions*

There are both qualitative and quantitative differences in the way that P-element transposition is regulated in different strains. Some P-element encoded proteins appear to affect transcription from the P-element promoter, while others may affect the activity of the transposase (Rio, 1991). The activity of these elements is highly dependent upon position, so the properties that a strain has reflects not only the numbers and types of P elements, but also their location in the genome.

The work of S. McG. was as part of a practical project during his final year as an undergraduate student. Work at Imperial College was supported by grants and a Senior Fellowship (K. O'H) from the Medical Research Council (U.K.). Work at Madison was supported by grant GM30948 from the National Institutes of Health (U.S.A.) to W. R. Engels. We thank W. Eggleston and W. R. Engels for comments on the manuscript.

References

Biemont, C., Ronsseray, S., Anxolabehere, D., Izaabel, H. & Gautier, C. (1990). Localization of P elements, copy number regulation and cytotype determination in *Drosophila melanogaster*. *Genetical Research* **56**, 3–14.

Bingham, P. M., Kidwell, M. G. & Rubin, G. M. (1982). The molecular basis of P–M hybrid dysgenesis: the role of the P element, a strain specific transposon family. *Cell* **29**, 995–1004.

Black, D. M., Jackson, M. S., Kidwell, M. G. & Dover, G. A. (1987). KP elements repress P-induced hybrid dysgenesis in *Drosophila melanogaster*. *EMBO Journal* **6**, 4125–4135.

Eggleston, W. B., Johnson-Schlitz, D. M. & Engels, W. R. (1988). P–M hybrid dysgenesis does not mobilize other transposable element families in *D. melanogaster*. *Nature* **331**, 368–370.

Engels, W. R. (1979). Hybrid dysgenesis in *Drosophila melanogaster*: rules of inheritance of female sterility. *Genetical Research* **33**, 219–236.

Engels, W. R., Preston, C. R., Thompson, P. & Eggleston, W. B. (1986). *In situ* hybridization to *Drosophila* salivary chromosomes with biotinylated probes and alkaline phosphatase. *Focus* **8**, 6–8.

Engels, W. R. (1989). P elements in Drosophila. In *Mobile DNA* (ed. D. E. Berg and M. M. Howe), pp. 437–484. American Society for Microbiology, Washington, D.C.

Engels, W. R., Johnson-Schlitz, D. M., Eggleston, W. B. & Sved, J. (1990). High frequency P element loss in *Drosophila* is homolog dependent. *Cell* **62**, 515–525.

Heath, E. M. & Simmons, M. J. (1991). Genetic and molecular analysis of repression in the P–M hybrid dysgenesis system of *Drosophila melanogaster*. *Genetical Research* **57**, 213–226.

Jackson, M. S., Black, D. M. & Dover, G. A. (1988). Amplification of KP elements associated with the repression of hybrid dysgenesis in *Drosophila melanogaster*. *Genetics* **120**, 1003–1013.

Karess, R. E. & Rubin, G. M. (1984). Analysis of P transposable element functions in *Drosophila*. *Cell* **38**, 135–146.

Kelly, M R., Kidd, S., Berg, R. L. & Young, M W. (1987). Restriction of P-element insertions at the *Notch* locus of *Drosophila melanogaster*. *Molecular and Cellular Biology* **7**, 1545–1548.

Kaufman, P. D., Doll, R. F. & Rio, D. C. (1989). *Drosophila* P element transposase recognizes internal P element DNA sequences. *Cell* **59**, 359–371.

Kaufman, P. D. & Rio, D. C. (1991). *Drosophila* P element transposase is a transcriptional repressor *in vitro*. *Proceedings of the National Academy of Sciences. U.S.A.* **88**, 2613–2617.

Kidwell, M. G. (1985). Hybrid dysgenesis in *Drosophila melanogaster*: Nature and inheritance of P element regulation. *Genetics* **111**, 337–350.

Landschulz, W. H., Johnson, P. & McKnight, S. L. (1988). The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. *Science* **240**, 1759–1764.

Laski, F. A., Rio, D. C. & Rubin, G. M. (1986). Tissue specificity of Drosophila P element transposition is regulated at the level of mRNA splicing. *Cell* **44**, 7–19.

LeMaitre, B. & Coen, D. (1991). P regulatory products repress *in vivo* the P promoter activity in P-*lacz* fusion genes. *Proceedings of the National Academy of Sciences, U.S.A.* **88**, 4419–4423.

Misra, S. & Rio, D. C. (1990). Cytotype control of *Drosophila* P element transposition: the 66 kd protein is a repressor of transposase activity. *Cell* **62**, 269–284.

Monastirioti, M., Hatzopoulos, P., Stamatis, N., Yannopoulos, G. & Louis, C. (1988). Co-habitation of KP and full length P elements in the genome of MR strains inducing P–M-like hybrid dysgenesis in *Drosophila melanogaster*. *Molecular and General Genetics* **215**, 94–99.

Nitasaka, E., Mukai, T. & Yamazaki, T. (1987). Repressor of P elements in *Drosophila melanogaster*: cytotype determination by a defective P element carrying only open reading frames 0 through 2. *Proceedings of the National Academy of Sciences, U.S.A.* **84**, 7605–7608.

O'Hare, K. & Rubin, G. M. (1983). Structures of P transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. *Cell* **34**, 25–35.

Preston, C. R. & Engels, W. R. (1984). Movement of P elements within a P strain. *Drosophila Information Service* **60**, 169–170.

Raymond, J. D., Ojala, T. A., White, J. & Simmons, M. J. (1991). Inheritance of P element regulation in *Drosophila melanogaster*. *Genetical Research* **57**, 227–234.

Rio, D. C. (1990). Molecular mechanisms regulating *Drosophila* P element transposition. *Annual Review of Genetics* **24**, 543–578.

Rio, D. C. (1991). Regulation of *Drosophila* P element transposition. *Trends in Genetics* **7**, 282–287.

Rio, D. C., Laski, F. A. & Rubin, G. M. (1986). Identification and immunochemical analysis of biologically active *Drosophila* P element transposase. *Cell* **44**, 21–32.

Robertson, H. M. & Engels, W. R. (1989). Modified P elements that mimic the P cytotype in *Drosophila melanogaster*. *Genetics* **123**, 815–824.

Roiha, H., Rubin, G. M. & O'Hare, K. (1988). P element insertions and rearrangements at the *singed* locus of *Drosophila melanogaster*. *Genetics* **119**, 75–83.

Ronsseray, S. & Anxolabehere, D. (1986). Chromosomal distribution of P transposable and I transposable elements in a natural population of *Drosophila melanogaster*. *Chromosoma* **94**, 433–440.

Rubin, G. M., Kidwell, M. G. & Bingham, P. M. (1982). The molecular basis of P–M hybrid dysgenesis: the nature of induced mutations. *Cell* **29**, 987–994.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, New York.

Siebel, C. W. & Rio, D. C. (1990). Regulated splicing of the Drosophila P transposable element 3rd intron *in vitro* – somatic repression. *Science* **248**, 1200–1208.

Simmons, M. J., Raymond, J. D., Boedigheimer, M. J. & Zunt, J. R. (1987). The influence of nonautonomous P elements on hybrid dysgenesis in *Drosophila melanogaster*. *Genetics* **111**, 869–884.

Simmons, M. J., Raymond, J. D., Rasmusson, K. E., Miller, L. M., McLarnon, C. F. & Zunt, J. R. (1990). Repression of P element mediated hybrid dysgenesis in *Drosophila melanogaster*. *Genetics* **124**, 663–676.

Tsubota, S., Ashburner, M. & Schedl, P. (1985). P element induced control mutations at the *r* gene of *Drosophila melanogaster*. *Molecular and Cellular Biology* **5**, 2567–2574.

Williams, J. A., Pappu, S. S. & Bell, J. B. (1988). Suppressible P element alleles of the *vestigial* locus in *Drosophila melanogaster*. *Molecular and General Genetics* **212**, 370–374.