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Structures of defective P transposable elements prevalent in natural Q and Q-derived M strains of *Drosophila melanogaster*

(geographic isolation/internal deletions/gene cloning/sequence conservation/Southern blot hybridization)

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ABSTRACT **Several DNA sequences with homology to the complete 2.9-kilobase (kb) P element from a P strain in the United States were isolated and characterized from two *Drosophila melanogaster* strains collected on Chichi Jima, an island 1000 km south of Tokyo. Except for a missing central region and trivial unsequenced regions of 38 base pairs, the 2.1-kb element isolated from a Q strain had the same DNA sequence as that of the complete P element. Seven other elements cloned from genomic DNAs of the Q strain and a Q-derived M strain all possessed the same restriction sites as those of the 2.9-kb P element except for one deleted region in each element. The finding of sequence conservation in P elements isolated from strains half a world apart suggests that these elements have had a common ancestor relatively recently. Thus, it is suggested that the P element family was a recent invader of the species. By contrast, no complete P element was found in these Japanese strains so far as surveyed, indicating the possibility that P elements in the Chichi Jima population are almost all defective. The implication of this possibility is discussed in relation to the uniqueness of the population on Chichi Jima where Q strains predominate and no P strains have yet been found.**

The phenomenon of hybrid dysgenesis, which appears in hybrids between certain mutually interacting strains of *Drosophila melanogaster*, is manifested in various aberrant genetic traits such as high frequencies of gonadal sterility, male recombination, and mutation (1–4). The P–M system includes three strains—P, Q, and M—classified on the basis of their functional properties. Hybrids between P strain males and M strain females show significant frequencies of gonadal sterility, while Q strains do not show gonadal sterility in any strain combinations, but produce male recombination and other dysgenic traits in crosses with M-strain females (5).

All P and Q strains studied by molecular analysis carry 30–50 copies of a family of cross-homologous DNA sequences, the P elements (6). Large 2.9-kilobase (kb) elements have been isolated from a P strain in the United States and their complete DNA sequences and other characteristics have been determined (7). The natural population of *Drosophila melanogaster* on Chichi Jima, a small island ≈1000 km south of Tokyo, is unique because Q strains predominate and no P strains have yet been found (8).

Using the P element as a probe, we isolated and characterized DNA sequences from genomic DNAs of two strains of the Q and Q-derived M type from Chichi Jima. This paper reports the structures of P elements in these natural strains.

MATERIALS AND METHODS

***Drosophila* Strains.** P strain Harwich was obtained from M. G. Kidwell (2). Strains CJ and CJ″ were isofemale lines derived from the population on Chichi Jima (8). Strain CJ′ was synthesized (9) from an X chromosome derived from a Q strain from Chichi Jima and autosomes from M strain Samarkand. The three strains initially showed no F_1 female sterility when tested at 29°C by crossing with Harwich males. However, when tested during the present experiments, strains CJ, CJ′, and CJ″ showed F_1 female sterility at frequencies of 0.005 (4/736), 0.97 (824/854), and 0.58 (416/722), respectively (9). According to the conventional classification (1), strain CJ was a Q strain, whereas strains CJ″ and CJ′ were, respectively, weak and strong M strains, both of which had recently changed from Q strains.

Southern Blot Hybridization. High molecular weight DNA was prepared from embryos (10) and 2-μg samples of each DNA were digested with appropriate amounts of various restriction enzymes under their optimal conditions. The digests were subjected to electrophoresis in agarose gels of type I (Sigma) and were then transferred to nitrocellulose filters (Schleicher & Schuell) as described (11). Nick-translation of probes was carried out by using [α-^{32}P]dCTP (2000 Ci/mmol; 1 Ci = 37 GBq) and *Escherichia coli* DNA polymerase I (Boehringer) as described by Maniatis *et al.* (12). Southern blot filters were hybridized with appropriate probes as described (13, 14).

Cloning Procedure. High molecular weight DNA was partially digested with *Eco*RI and fractionated by agarose gel (0.7%) electrophoresis as described (15). Fragments of 15–20 kb were isolated from the partial digests, ligated with Charon 4A (16) outer fragments, and then packaged *in vitro* into phage coats (17). The resultant plaques were screened by the plaque-hybridization technique of Benton and Davis (18). Approximately 1×10^5 phages were screened with the *Bam*HI fragments of pπ25.1 and the *Sal* I fragments of p6.1. Plasmids pπ25.1 and p6.1 containing P elements (19) were obtained from G. M. Rubin. Thirty phages with homology to P elements were randomly selected for further analysis. After it was confirmed that their P elements had no *Eco*RI sites, 6 of 30 phages were cleaved with *Eco*RI and the inserts were recloned into pBR322 (20). Recombinant phage and plasmid DNAs were prepared as described by Tiemeier *et al.* (21) and by Clewell and Helinski (22), respectively. Sources of restriction enzymes, polynucleotide kinase, and other enzymes have been described (13). The bacterial strains used for *in vitro* packaging were supplied by F. Blattner (University of Wisconsin).

Pst I fragment-containing elements were cloned in an attempt to isolate complete P elements. Since the complete P element in pπ25.1 has no *Bam*HI sites, genomic DNAs were digested with *Bam*HI and fractionated by agarose gel (0.7%) electrophoresis (15). Since *Pst* I fragment-containing elements were banded at 5-kb fragments, the fraction of the agarose gel containing 4.5- to 6.5-kb fragments was cut at four

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Abbreviations: kb, kilobase(s); bp, base pair(s).

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sites, and a piece of DEAE paper was inserted into each site. DNAs in each subfraction were adsorbed to the piece of DEAE paper by further electrophoresis and then eluted with a 1 M NaCl solution. For each subfraction, *Pst* I fragment-containing elements were surveyed by Southern blot analysis and DNAs from a positive fraction were ligated to λ vector Charon 28 (23). Recombinant λ phages were screened with the *Pst* I fragments of pπ25.1 as described above.

DNA Sequencing. DNA sequence was determined by the method of Maxam and Gilbert (24) with minor modification as described (13).

RESULTS

DNA Sequences in Q and Q-Derived M Strains Homologous to the 2.9-kb P Element. Southern blot analysis was used to see whether genomic sequences in three strains—a Q strain CJ, a weak M strain CJ″, and a strong M strain CJ′ from Chichi Jima—contained DNA sequences homologous to the 2.9-kb P element. As shown in Fig. 1, there were a great number of different sequences homologous to the P element in the three strains but only a single restriction sequence homologous to the probe fragment in M strain Canton S. The latter probably represents a single copy of the cytogenetic locus 17C, the site where the P element of pπ25.1 is inserted (7).

P-Element-Containing Segments Cloned from Q and Q-Derived M Strains and Maps of Their Restriction Sites. To characterize the P-element-like fragments present in strains CJ and CJ′, we cloned the fragments from their genomic DNAs into λ vector Charon 4A (see *Materials and Methods*). Thirty phages containing sequences homologous to the P elements were purified and characterized by restriction enzyme mapping and DNA blotting with P elements as probes. Of the 30 recombinant phages, 3 phages (λCJ9, λCJ′31, and λCJ′39) contained sequences homologous to the 0.9-kb *Hin*dIII fragment of the P element. Detailed restriction site maps of these and 3 *Hin*dIII fragment-deficient phages (λCJ′33, λCJ′38, and λCJ1), which were selected at random from the remaining 27 phages, are shown in Fig. 2. Crude restriction site maps of the sequences outside the cloned P-element-like fragments are shown (Fig. 2 *Left*). These maps are different, indicating independent origins of the clones. The fine restriction maps of the cloned P-element-like fragments are shown (Fig. 2 *Right*). Comparison with the restriction map of the 2.9-kb P element indicates that the restriction sites present in the 2.9-kb P element are conserved within all the P-element-like fragments cloned from strains CJ and CJ′ except for the deleted regions. Thus, the sequences homologous to the 2.9-kb P element that are present in the two strains from Chichi Jima are members of the P-element family defined and characterized by using flies from the United States (6, 7, 19, 25, 26).

DNA Sequence of a 2.1-kb P Element and Its Flanking Region. The *Eco*RI/*Bam*HI fragment of λCJ9, which contains the P element, was recloned into pBR322 as plasmid pCJ9 and the sequence of the 2.1-kb P element in pCJ9 plus the flanking region was determined (see diagram at the bottom of Fig. 2 for strategy). Comparison of the sequence of the 2.1-kb P element with that of the complete 2.9-kb P element (7) indicated that in the 2.1-kb P element, a 760-base-pair (bp) segment from position 1194–1953 of the complete P element is missing (Figs. 2 and 3*A*). The P element in pCJ9 is 2147 bp long.

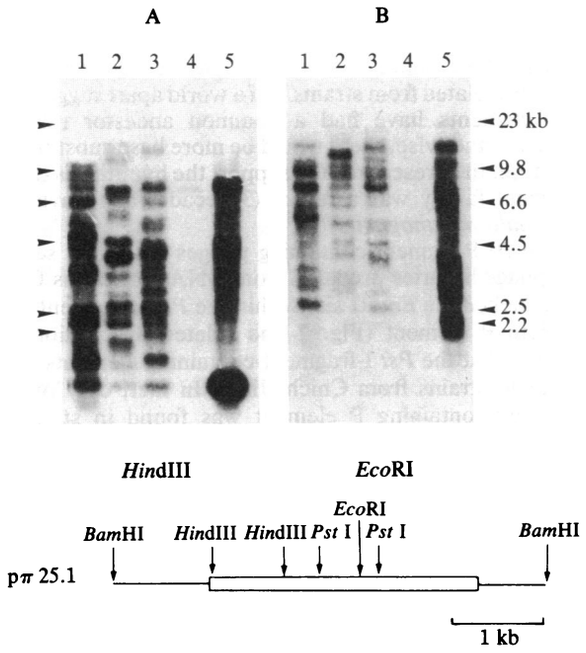

FIG. 1. Repeated DNA sequences in Q and M strains homologous to the 2.9-kb P element. Results of Southern blotting are shown. Genomic DNAs were extracted from Q, M, and P strains and digested with *Hin*dIII (*A*) or with *Eco*RI (*B*). Digestion products (2 μg for each lane) were fractionated by electrophoresis on 0.7% agarose gel, transferred to nitrocellulose paper, and hybridized with a P-element-containing probe. The probe was the 4.7-kb *Bam*HI fragment of pπ25.1 containing the 2.9-kb P element that had been labeled with ^{32}P by nick-translation. A linear map representing the probe is shown. Open bar indicates the position of the 2.9-kb P element; thin lines represent flanking *Drosophila* genomic DNA sequences from cytogenic locus 17C (19). Lanes: 1, Q strain CJ; 2, strong M strain CJ′; 3, weak M strain CJ″; 4, reference M strain Canton S; 5, reference P strain Harwich.

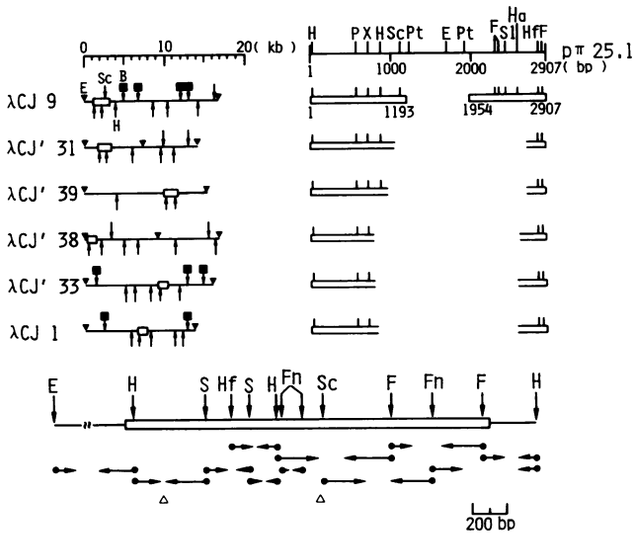

FIG. 2. Restriction site maps and strategy for DNA sequencing. (*Left*) Maps of the restriction sites outside the P elements (open bar) cloned into λ phage vectors. (*Right*) Fine restriction maps of the cloned P elements together with putative sites of internal deletions. The two terminal restriction fragments of each P element cloned had homology to those of the complete 2.9-kb P element, but their exact end points were not determined except for those in λCJ9. The bottom diagram shows the strategy used for DNA sequencing; arrows represent individual regions that were sequenced from the indicated sites of cleavage by restriction endonucleases after end-labeling (●) and open triangles (△) indicate unsequenced regions. E or ▼, *Eco*RI; H or ↑, *Hin*dIII; Sc or ↓, *Sac* I; B or ↟, *Bam*HI; P, *Pvu* II; X, *Xho* I; Pt, *Pst* I; F, *Fok* I; S1, *Sal* I; Ha, *Hha* I; Hf, *Hin*fI; S, *Sau*96I; Fn, *Fnu*4HI.

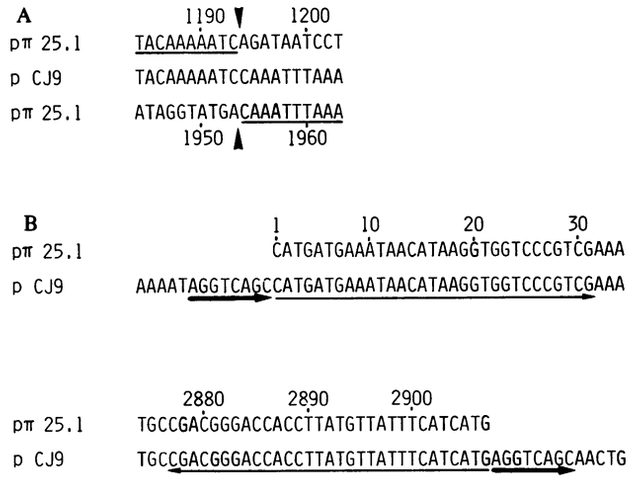

FIG. 3. DNA sequences of the 2.1-kb P element in pCJ9 at the deletion end points and the immediately adjacent regions. (*A*) For the 2.1-kb P element, the DNA sequence of the novel junction produced by the putative deletion is shown together with the sequences from the 2.9-kb element that surround each of the putative deletion end points (see arrowheads in the diagram). Above the 2.1-kb P element sequence (pCJ9) is the 2.9-kb P element sequence (pπ25.1) from the left end point, and below is the sequence from the right end point. Numbers correspond to the complete 2.9-kb P element DNA sequence (6). (*B*) Sequence at the site of residence of the 2.1-kb P element in pCJ9. (*Upper*) Region near the left end of the 2.1-kb element; (*Lower*) region near the right end. The 8-bp direct repeats immediately adjacent to the element are underlined with heavy arrows and the 31-bp inverted repeats of the 2.1-kb P element are underlined with light arrows.

O'Hare and Rubin (7) have shown that P elements have 31-bp perfect inverse terminal repeats and, upon insertion, duplicate an 8-bp sequence found only once at the site of insertion. The 2.1-kb P element also possessed 31-bp inverse terminal repeats and 8-bp direct repeats.

The sequence of the 31-bp inverse repeats was identical to that of the 2.9-kb P element (Fig. 3*B*). However, the sequence AGGTCAGC of the 8-bp direct repeats in pCJ9 (Fig. 3*B*) was considerably different from the consensus sequence GGCCAGAC proposed by O'Hare and Rubin (7). Surprisingly, except for the missing central region plus the unsequenced 38 bases (from position 184 to 200 and from position 1129 to 1149 in the complete P element), the sequence of the 2.1-kb P element was the same as that of the 2.9-kb P element.

Internal Deletions Are Common in P Elements Derived from Q and Q-Derived M Strains. Like the six P elements cloned from strains CJ and CJ′ (see Fig. 2), 16 clones isolated from strain CJ and 8 clones from strain CJ′, which were independent of each other, all lacked the *Pst* I fragment (data not shown). Furthermore the genomic DNAs from the Q strain CJ, the strong M strain CJ′, and the weak M strain CJ″, had only a few sequences homologous to the *Pst* I fragment (Fig. 4).

If strains CJ and CJ′ possess complete 2.9-kb P elements, these are likely to be *Pst* I fragment-containing elements. Thus, from DNA fragments of 4.5 to 6.5 kb that corresponded to the positive bands on the Southern filters (see lanes 1 and 2 in Fig. 4), DNAs containing the *Pst* I fragment were isolated (see *Materials and Methods*). The P elements of the resultant plasmids—pCJ103 from strain CJ and pCJ′105 from strain CJ′—were examined by restriction enzyme mapping and DNA blotting by using various restriction enzyme fragments of the 2.9-kb P element as probes. Both the *Hin*dIII/*Sal* I fragments possessed *Pst* I sites, but had deletions at other sites (Fig. 5). Thus, we did not isolate a complete 2.9-kb P element from Q and Q-derived M strains from Chichi Jima.

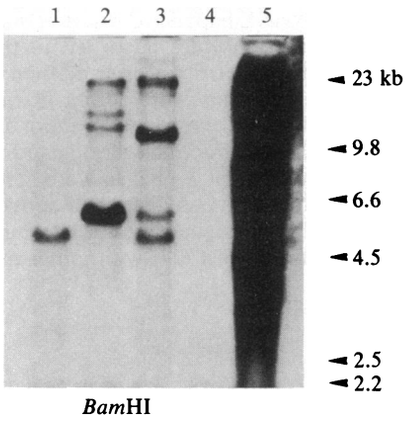

FIG. 4. Rare conservation of central regions of P elements in Q and M strains. *Bam*HI digests of genomic DNAs from various strains were subjected to Southern blotting analysis with the *Pst* I segment as a probe of the central region of the complete 2.9-kb P element (see Fig. 1 for details). Lanes: 1, Q strain CJ; 2, strong M strain CJ′; 3, weak M strain CJ″; 4, reference M strain, Canton S; 5, reference P strain, Harwich.

DISCUSSION

A 2.1-kb P element isolated from Q strain CJ from Chichi Jima had the same DNA sequence as that of the complete 2.9-kb P element derived from strain π_2 from the United States except for a missing central region and trivial unsequenced regions of 38 bp. Seven other elements isolated from the Q strain and an M strain derived from a Q strain from Chichi Jima all possessed the same restriction sites as those of the 2.9-kb P element except for one deleted region in each element. The finding of such sequence conservation in elements isolated from strains half a world apart suggests that these elements have had a common ancestor relatively recently. Otherwise, there would be more base substitutions. Therefore, the present results support the hypothesis that the P element family was a recent (3 decades ago) invader of *Drosophila melanogaster* (1).

Since 30 P-element-containing phages randomly selected from phage libraries prepared from DNAs of strains CJ and CJ′ all lacked the *Eco*RI site within the *Pst* I fragment of the complete P element (Fig. 2 and related description), we expected that the *Pst* I-fragment-containing elements would be rare in strains from Chichi Jima. In fact, only one *Pst* I-fragment-containing P element was found in strain CJ, about five were found in strains CJ′ and CJ″ (Fig. 4), and six were found in two natural M strains from Chichi Jima (9). The

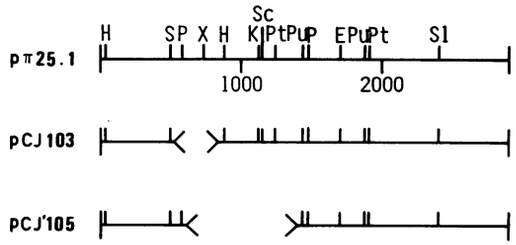

FIG. 5. Restriction site maps of P elements possessing the central portion, *Pst* I fragment. Plasmids pCJ103 and pCJ′105 containing the *Pst* I fragments were cloned, respectively, from strains CJ and CJ′ (see text for experimental procedures). From the maps obtained, deleted sites were deduced by comparison of these maps with that of the 2.9-kb P element. The P elements cloned into pCJ103 and pCJ′105 both had terminal restriction fragments homologous to those of the complete 2.9-kb P element, but their exact end points were not determined. H, *Hin*dIII; S, *Sau*96I; P, *Pvu* II; X, *Xho* I; K, *Kpn* I; Sc, *Sac* I; Pt, *Pst* I; Pu, *Pvu* I; E, *Eco*RI; S1, *Sal* I.

single *Pst* I-fragment-containing element cloned from strain CJ and one of the elements from strain CJ′ were fairly large P elements and yet both had single internal deletions (Fig. 5). These fragmentary results strongly indicate the possibility that P elements in the Chichi Jima population are mostly defective. Even in the P-element family in the strong P strain π_2, the ratio of complete P elements to smaller ones is estimated as 1:2 (26). This finding and the results from the present experiments support the idea that the complete P element is frequently degraded by internal deletions to a variety of small P elements. Based on several lines of evidence, O'Hare and Rubin (7) proposed that deletions occur in the P element during transposition as a consequence of an error-prone replication process. The hypothesis is supported by the recent report (27) that internal deletions frequently occurred in a P-element derivative that acts like a nondefective intact P element, but did not do so in its mutagenized nonautonomous derivatives. This hypothesis implies that the production of defective P elements in the Chichi Jima population must have occurred mostly in the early phase of the presumed recent invasion of complete P elements, because in today's population Q strains predominate, and no P strains have yet been found (8).

Strain CJ had only one *Pst* I-fragment-containing P element and this P element had an internal deletion as mentioned above. Unless we missed by chance the complete P element, this result means that the P cytotype of strain CJ is maintained without participation of the complete P element. The two elements cloned from strain CJ into CJ103 (Fig. 5) and CJ9 (Fig. 2) had deletions within the first and the second open reading frames (7), respectively. It is tempting to assume that such differentially defective P elements complement one another, resulting in the production of the P cytotype in strain CJ. However, Karess and Rubin (27) recently reported that complementation tests between pairs of mutant P elements failed to restore transposase activity. Nevertheless, following the model of O'Hare and Rubin (7), it is conceivable that the P cytotype of a Q strain is maintained in the absence of the functional transposase by the functional repressor produced by complementary or unique action of some types of defective P elements such as those mentioned above. Thus, we assume that Q strains from the Chichi Jima population frequently lack complete P elements because P strains are virtually absent from this isolated population. This assumption is open for future study.

In contrast to strain CJ, Q strains ν_6 and Mt. Carmel from the United States, where P strains predominate, both have been reported to possess apparently complete P elements (O'Hare *et al.*, quoted in ref. 28). Furthermore, Q strains TAQ58 and TRQ575 from Akita and Okinawa, where P and Q strains coexist, had 14–16 *Pst* I-fragment-containing P elements, whereas P strain TAP3 from Akita had 16 *Pst* I-fragment-containing P elements (9), indicating the possibility that strains TAQ58 and TRQ575 may have complete P elements. We are now witnessing the dynamic evolution of transposon P in *Drosophila* (1, 4, 6–9, 25–29). An interdisciplinary study of the P element family by cooperation of molecular biologists and population geneticists is opening up a new and exciting field (29).

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