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Genetics

Repressor of *P* elements in *Drosophila melanogaster*: Cytotype determination by a defective *P* element carrying only open reading frames 0 through 2

(*P* repressor/hybrid dysgenesis/*MR* element)

EIJI NITASAKA, TERUMI MUKAI, AND TSUNEYUKI YAMAZAKI*

Laboratory of Population Genetics, Department of Biology, Kyushu University, Fukuoka 812, Japan

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ABSTRACT **The *P* element is a type of transposable element in *Drosophila melanogaster*. Characteristics of the syndrome of "hybrid dysgenesis" are due to transposition of *P* elements, and the molecular mechanism for regulation of this transposition has been unknown. In this study a Q strain (which carries only defective *P* elements in its genome but still is able to repress the transposition of complete *P* elements although defective in transposase activity) was used to determine the structure of the *P* element with this repressor (or P cytotype-determining) domain. Examination of the cytotype and structure of the *P* elements of particular strains with reduced copy number of *P* elements showed that the *P* element with a repressor domain was defective, being deleted between bases 1991 and 2448. This region corresponds to most of the third intron [between open reading frame (ORF) 2 and ORF 3] as well as half the ORF 3 of an intact *P* element. Therefore ORF 3 was deemed to be unnecessary for repressor production.**

Occurrence of transposable elements was inferred from the study of mutable or unstable genes in the 1960s (1, 2). *MR* elements discovered by Hiraizumi (3) produce mutation and mitotic recombinations in a cross between laboratory stocks and wild strains. Subsequent study of a similar phenomenon has revealed that "hybrid dysgenesis" is induced when male flies of a P strain possessing active *P* elements mate with female flies of an M strain lacking such elements but is not induced in a reciprocal cross (4). At least some homology of the *MR* elements with the *P* elements has now been shown at molecular level (5, 6). The nonreciprocal nature of *P* element transposition can be explained on the basis of a P cytotype (7) possessing putative repressors of transposase. No clear evidence has thus far been presented on the nature of the repressor or its precise location in the *P* element. According to one theory, episomal *P* element in the cytoplasm produces repressor proteins because this element appears to be maternally inherited (8). Some workers have hypothesized that an intact *P* element produces both transposase and a repressor (9). However, the results of a detailed molecular study (10) have clearly shown that all four open reading frames (ORFs) are necessary for the production of transposases. Thus, one school insists that circular DNA itself, excised by transposases, is the repressor (11). There is also the possibility that defective *P* elements are able to produce repressors (12). Recently, a transposase of the *P* element derived from all four ORFs was found to be produced by proper excision of three introns in germ cells and a protein possessing amino acids only up to ORF 2 was found to be produced in somatic cells because the third intron (that between ORF 2 and 3) is not spliced (13, 14).

Almost all strains from Japanese natural populations are Q strains with no gonadal sterility but with the P cytotype (12, 15, 16). Examination of P positive clones of some Q strains from Japanese natural populations showed that these Q strains did not carry any complete *P* element (12, 16). Thus, it seems that defective *P* elements may cause repressor activity (or P cytotype).

We present genetic data indicating that the defective *P* elements actually determine the cytotype of a Q strain from a Japanese natural population. In addition, the molecular structure of this cytotype-determining *P* element is described.

MATERIALS AND METHODS

Strain. An isogenic line, WY113, which is judged to be a typical Q strain from genetic tests (17), was used in this study. This strain was collected from a natural population in Osaka, Japan, in 1978. Canton S and π2 strains were used as typical M and P strains, respectively. Two mutant stocks, $y\ sn^{w}/y^{+}Y;bw;st$ (18, 19) and *C(1)DX,y w f*, were employed for sn^{w} mutability tests.

sn^{w} Mutability Tests for Transposase Activity. A single female fly of sn^{w} (M strain) was crossed with a male fly of π2 (P strain), CS (M strain), or WY113 (Q strain) at 23°C. A male offspring from the above cross was individually mated to five females of *C(1)DX,y w f* at 25°C, and the ratio $(sn^{e} + sn^{+})/(sn^{w} + sn^{e} + sn^{+})$ in male progeny was calculated as an index of transposase activity. These types of crosses were repeated 15–18 times.

Mating Scheme to Reduce the Number of *P* Elements. A female fly of a Q strain, WY113, was mated with a single male fly of an M strain, Canton S (CS) carrying no *P* element, for four generations at 25°C (Fig. 1). In each generation eight lines were established by brother–sister matings and inbreeding for two generations, and their cytotypes were classified by examining gonadal sterility as described below. G1 and G2 flies were inbred for an additional three generations, and DNAs were extracted from the lines. Because all lines from G3 and G4 carried the M cytotype after two generations of inbreeding—that is, these lines apparently had already lost the *P* element(s) controlling P cytotype—no further experiments were conducted with these lines from G3 and G4.

Gonadal Dysgenesis Sterility. Cytotypes were assayed by gonadal sterility as follows (20). Five to seven female flies of each tested line were individually mated with a male fly of a strong P strain (π2) and incubated for 3 days at 29°C; the parent flies were then discarded. On day 12, F_1 progeny were transferred to fresh culture vials and aged for 3 days at 25°C.

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Abbreviation: ORF, open reading frame; ORFs 0–1–2, ORFs from 0 through 2.

*To whom reprint requests should be addressed.

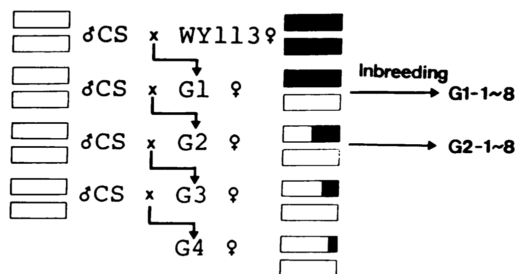


FIG. 1. Mating scheme to reduce *P* elements from a genome. Black and white areas indicate the Q strain-derived chromosome and the M strain-derived chromosome, respectively.

Ovaries of F_1 daughters were dissected; the number of daughters with no ovaries was scored.

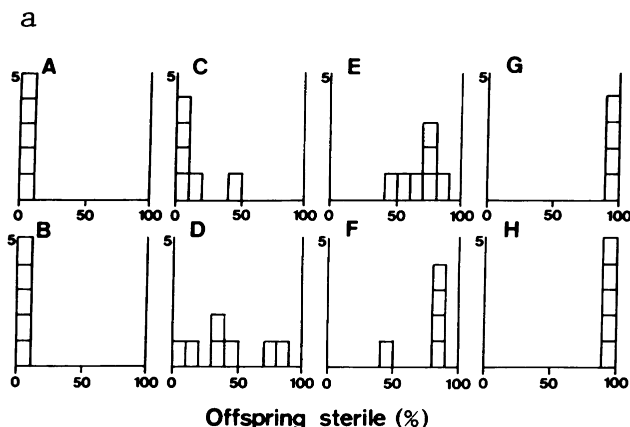
DNA Blot Hybridization. Genomic DNA of each strain was extracted by the method of Bingham *et al.* (21), digested with *Bam*HI (which has no site in an intact *P* element), and subjected to electrophoresis on 0.8% agarose gels. The structure of any DNA fragment carrying *P* elements was analyzed by the method of Southern (22) as modified by Botchan *et al.* (23). The probe plasmid pπ25.7dwc was constructed as follows. pπ25.7wc (10) was digested with *Hin*dIII, and 6-kilobase (kb) and 0.8-kb fragments were gel-purified to eliminate flanking genomic DNA. The 0.8-kb fragment was ligated onto the dephosphorylated 6-kb fragment, yielding a plasmid denoted pπ25.7 double-wing cripped (pπ25.7dwc) that did not hybridize to the 17C region. Fig. 2*b* shows the restriction map. The probe of each ORF was prepared as follows: pπ25.7dwc was digested with *Pst* I, and three fragments were gel-purified. The 1.9-kb, 0.7-kb, and 4.0-kb fragments were labeled by the method of Feinberg and Vogelstein (24, 25) as probes for ORF 1, ORF 2 and ORF 3, respectively.

Restriction Mapping and DNA Sequencing. The λEMBL3 *Bam*HI-digested library of the original Q strain (WY113) DNA was constructed by the method of Frischauf *et al.* (26), and screening was done according to Maniatis *et al.* (27) using pπ25.7dwc as probe. We obtained three *P* elements included on 2.8-kb *Bam*HI fragment among 30 *P* elements screened. Maps of all clones were the same. To determine the location of the deletion in ORF 3, a 0.5-kb *Bam*HI–*Pst* I fragment of clone λWY38 was subcloned into pUC18 (28) and sequenced by the method of Sanger *et al.* (29).

RESULTS AND DISCUSSION

The Q strain [WY113 (16, 17)] used was considered to be free from any intact *P* element because no sn^w hypermutability (19) could be detected (see Table 1). However, it is apparent that this strain has P cytotype, because female flies of this strain mated with males of a P strain such as π2 (20) failed to produce any sterile F_1 flies. Thus, we bred strains with a reduced copy number of the *P* element by mating the Q strain repeatedly with an M strain, Canton S, that has no *P* elements and then inbred specific groups for several generations as described; the cytotype of each strain was then examined. Because all G3 and later generations showed M cytotypes, G1 and G2 flies were inbred for five generations to fix segregating *P* elements, and their cytotypes were also examined (Fig. 2*a*). All strains except G2-5 (G2-fifth inbred generation) were classified unambiguously as either M or P cytotype. To see which *P* element(s) determines the P cytotype, genomic DNAs were extracted from the strains and Southern blot hybridization was done using pπ25.7dwc as a probe after digestion of the strain DNA with *Bam*HI, which does not cleave within an intact *P* element. Whenever cytotype changed from P to M, the 2.8-kb, 9.7-kb, and 10.5-kb *Bam*HI fragments carrying *P* elements also disappeared (Fig. 2*b*), but the presence of all other fragments was completely unrelated to the cytotypes. A genetic test indicated these three fragments to be located on the third chromosome (data not shown). *P* elements on the 9.7-kb and 10.5-kb *Bam*HI fragments were quite small (1 kb). Most of ORFs 1, 2, and 3 were deleted in both fragments. These small fragments apparently do not participate in cytotype determination because *P* elements of the same type were present in the M strain established in this study. Clearly, then, the 2.8-kb *Bam*HI fragment determines P cytotype.

To elucidate the structure of this cytotype-determining *P* element, the element was cloned from the WY113 λEMBL3–*Bam*HI gene library, and restriction mapping was subse-



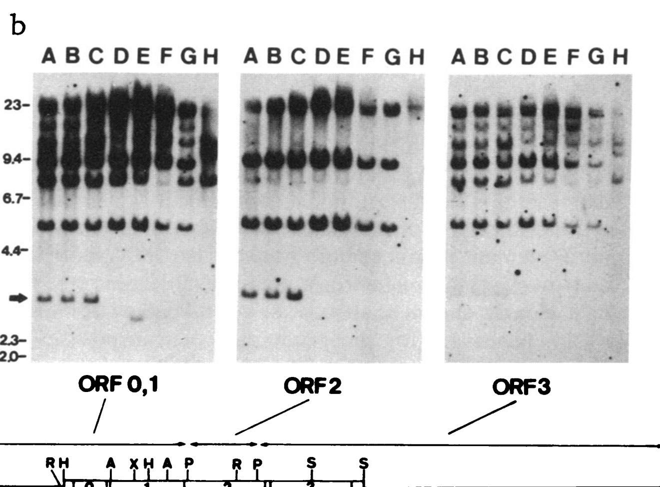


FIG. 2. Identification of the defective *P* element determining the P cytotype. *A, B, C, D, E, F, G,* and *H* show the strains with reduced number of *P* elements obtained by the mating scheme of Fig. 1: *A*, original Q strain WY113; *B*, G1-5; *C*, G1-4; *D*, G2-5; *E*, G1-8; *F*, G2-1; *G*, G2-3; *H*, G1-6. Only one original and seven inbred strains were used for these experiments—mainly, because of the difficulty in maintaining the inbred strains. (*a*) Frequency distributions of gonadal dysgenesis sterility. Each block represents sterility tests of up to 50 daughters of a single female of the strains shown in Fig. 1. (*b*) DNA blot hybridization of the strains with a reduced number of *P* elements. Arrow, P cytotype-determining element. Restriction map of pπ25.7dwc is shown at bottom. Restriction sites for *Ava* II, *Eco*RI, *Hin*dIII, *Pst* I, *Sal* I, and *Xho* I are indicated by A, R, H, P, S, and X, respectively. Numbers at left are molecular size standard by kb. *P* element sequences are boxed, and the numbers 0, 1, 2, and 3 correspond to the sequences of ORF 0, 1, 2, and 3, respectively. Plasmid sequence is shown by a thin line.

Table 1. sn^w mutability tests for transposase activity

Cross (female × male)	F_1 offspring, no.		F_2 offspring, no.			
	Total	sn^w instability	Total	sn^e	sn^+	Ratio*, %
sn^w (M) × π2 (P)	19	19	1710	269	279	32.0
sn^w (M) × Canton S (M)	17	0	1669	0	0	0.0
sn^w (M) × WY113 (Q)	16	0	2032	0	0	0.0

*$sn^e + sn^+/sn^w + sn^e + sn^+$.

quently done (Fig. 3*a*). This fragment had a deletion including a *Sal* I site present within ORF 3, but all restriction sites up to the *Pst* I site near the end of ORF 2 were present. To locate this deletion we sequenced a *Bam*HI–*Pst* I fragment of 0.5 kb by dideoxy chain-termination sequencing; the fragment was deleted between bases 1991 and 2448 of an intact *P* element (9). The complete mRNA may be assumed to be produced from this fragment because the neighboring poly(A) site (13) is present. The protein produced by this deficient *P* element has six more amino acids added at the end of the ORF 0–1–2 (0 through 2)-derived protein seen in somatic cells (14) (Fig. 3*b*); the M_r of the former protein was estimated as 67,000. This amino acid production is possible because the deletion joins ORF 2 to ORF 3. Furthermore, this protein is probably produced in both germ and somatic cells because the 3′ splice site (13) of the third intron is lacking. At present, no evidence indicates whether the six amino acids at the carboxyl end are essential to repressor functions. This data clearly shows that ORF 3 is unnecessary for suppression of *P* transposition and that a single copy of the *P* element with only ORF 0–1–2 can completely repress the transposase activity (though the possibility that the other small *P* elements together with the ORF 0–1–2 play some roles in the production of repressors cannot be excluded completely). This type of *P* element exists even in strong P strains (16). If a *P* element lacking ORF 3 happens to be produced among deficient *P* elements often made in the process of transposition, this may determine the P cytotype of the cell. Similar proteins to that encoded by ORF 0–1–2 are actually produced in cultured cells having only complete *P* elements because, in the case of some somatic cells, the third intron of a complete *P* element cannot be spliced (13, 14). However, it has also been reported that the cytotype of a strain with a copy of an autonomous *P* element remains M even after 12 generations (10). In correlating the presence or absence of a defective *P* element with a P or M cytotype, respectively, a single defective *P* element

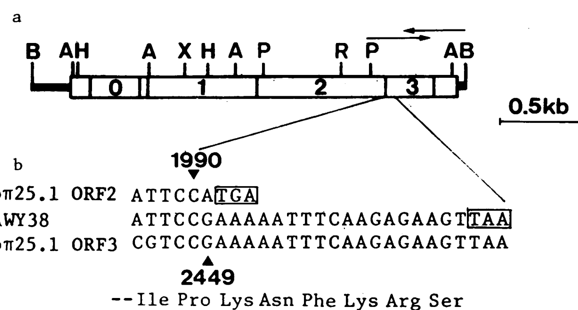


FIG. 3. Structure of the P cytotype-determining *P* element. (*a*) The restriction map. *P* element sequences are boxed and the numbers 0, 1, 2, and 3 correspond to the sequences of ORF 0, 1, 2, and 3, respectively. *Drosophila* sequences are shown by the heavy line; solid arrows show the sequencing strategy. (*b*) Carboxyl-terminal amino acids of the repressor domain (or protein produced by the P cytotype-determining *P* element) and the corresponding base sequences of the defective *P* element (λWY38). Boxed TGA and TAA indicate termination codons. Base sequence of pπ25.1 (9), a plasmid with a complete *P* element within pBR322, is shown together with that of λWY38.

with ORF 0–1–2, but not an intact *P* element, appears to actually determine the P cytotype in germ cells; yet, an intact *P* element still could change the cytotype from M to P by regulation of splicing, or some other mechanism. At any rate, the suppression of P strain transposase appears to occur through competition for DNA binding sites between transposases from a complete *P* and repressors from defective *P* elements. These results are reasonable considering that DNA-binding domains are located within ORFs 1 and 2 of an intact *P* element (14). Whether this type of competition is generally observed in other types of transposable elements such as retroposons would be interesting to learn.

In this study cytotype was determined only by gonadal sterility. Green (6) reported that gonadal dysgenesis and mutation–recombination induction could be separated by recombination, results suggesting that a complete *P* element is not identical to an *MR* element at the molecular level. Possibly the domain structure of *P* elements is even more complicated than previously considered; further studies are needed to clarify such questions.

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