Insertional mutagenesis in *Drosophila* II. P element mediated transformation of *Drosophila yakuba*[†]

Shin TOGASHI, Ryu UEDA, Manabu TAKAHISA, Misa MIKUNI,
Kanae KONDO and Tadashi MIYAKE

Laboratory of Cell Biology Mitsubishi Kasei Institute of Life Sciences 11
Minamiooya, Machida-shi Tokyo 194 JAPAN

(Received 6 February 1992)

ABSTRACT

Drosophila yakuba, a member of *melanogaster* subgroup being free of P element, acquired resistance to an antibiotic neomycin by the transformation utilizing P element. In this species, the transformation frequency was comparable to that of *D. melanogaster*. Further, the occurrence of 8 base pairs duplication upon the insertion of the element was confirmed. These facts suggest that the P element could be inserted into the genome in the same manner, even in *D. yakuba*. Any consensus for preferential insertion could not be found on the nucleotide sequence as in *D. melanogaster*. However, it is noticeable that a series of the short palindromic stretches was common around the insertion sites in both species. It suggests that a structural feature of DNA plays a role as a landmark for P element insertion.

1. INTRODUCTION

The availability of P element for the genetic manipulations of *Drosophila melanogaster* makes it an outstanding transposon among the many known and increases the value of this fly species as a model organism to promote our understanding of life, especially of higher eukaryotes. To date, complete P elements have been found only in *D. melanogaster* and *D. willistoni*, though hybridizable sequences were detected in many species in the genus *Drosophila* (Daniels et al., 1990). However, the artificially introduced P elements seemed to be capable of transposition in the closely related species, *D. simulans* (Scavarda and Hartl, 1984, 1987; Daniels et al., 1985) and even in a distant species of *D. hawaiiensis* (Brennan et al., 1984). This fact implies the probability that we can operate the genome of the species other than *D. melanogaster* utilizing P element. Nevertheless, the informations on the transformation frequencies and on the insertion sites, both being critical for the practical use of P element, have not been enough accumulated. Thus we attempted the P element-mediated transformation of *D. yakuba*, which is another member of *melanogaster* subgroup, in order

[†] DDBJ/EMBL/GenBank accession number; The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL and GenBank Nucleotide Sequence Databases with the following accession number D12599-12606.

to compare the transformation frequency and the structure of insertion sites with those of *D. melanogaster*.

2. MATERIALS AND METHODS

Transformation

The procedure of transformation experiment is completely same as previously reported for *D. melanogaster* (Ueda et al., 1987). 0.1 nl mixture of pUCHsneo (300 mg/ml) and phs- π (100 mg/ml) (Steller and Pirrotta, 1986) was injected into posterior periplasm of a preblastoderm-stage embryo of *D. yakuba*. pUCHsneo is a P-element vector having neomycin-resistant gene driven by the promoter of heat shock protein 70 gene as selection marker and *ori* of pUC plasmid. phs- π is a transposase source having P-element transposase coding region driven by the promoter of heat shock protein 70 gene, but being unable to insert itself into genome.

Selection of transformants

Since we have no appropriate balancer lines to establish the transformant lines for *Drosophila yakuba*, each eclosed fly from injected embryos (G0) was crossed to the wild-type partners and allowed to lay eggs on food containing 0.5 mg/ml of neomycin (G418 sulfate, GIBCO). At this concentration of neomycin, any wild-type *D. yakuba* was not able to survive beyond the larval stages. The flies were transferred daily to new vials and the progenies were subjected to heat shock (37°C, 30 min) on days 1, 3 and 5. The survivors (G1) were crossed singly to the wild-type partners for the establishment of the transformant fly lines. Thereafter, neomycin selection was carried out on every generation.

Genomic southern blot

Genomic DNA of the flies was prepared according to the method of Pirrotta (1986), digested with the appropriate restriction endonuclease, and run on an agarose gel. Fractionated DNA was transferred to GeneScreen Plus (DUPONT) according to the supplier's protocol. The filters were probed with random primer labelled pUCHsneo.

Sequencing of insertion sites

The genomic sequences flanking the inserted pUCHsneo were cloned by the method of plasmid rescue (Steller and Pirrotta, 1986). A clone rescued after *Xba* I digestion, has the genomic sequences flanking the pUCHsneo, because *Xba* I has no cutting site in the vector. From those clones, 5' and 3' flanking sequences were subcloned with appropriate enzymes (e.g., *Xho* I for the 5' and *Bam* HI for the 3'), and sequenced by the dideoxy method (Sanger et al., 1977), using a synthetic 17mer (CGACGGGACCACCTTAT) corresponding to the internal half of

Fig. 1. A schematic representation of the sequence strategy. The genomic DNA of the transformant line was digested with *Xba*I, which has no cutting site in the vector of pUCHsneo, and the digested fragments were self-ligated. The whole fraction of circular DNA was transformed to *E. coli* (competent cells of HB101, purchased from TAKARA). After ampicillin selection, only the clone harboring the pUCHsneo with flanking genomic sequences formed colony. From the rescued fragment, 5' and 3' flankings were subcloned with appropriate restriction enzymes B and A, respectively. Both flanking sequences were sequenced using a synthetic primer (CGACGGGACCACCTTAT). Terminal inverted repeat is indicated as "Wing". The bold characters directly flanking with the wings of P element in lower-most panel indicate the duplicated 8 bases.

the terminal inverted repeat of P element, as a primer. This procedure is schematically presented in Fig. 1.

3. RESULTS AND DISCUSSION

Transformation frequency of D. yakuba

It has been reported that the transformation frequency is dependent on the structure and length of P element (Engels, 1989; Berg and Spradling, 1991). Therefore, the use of the same construct of P element is prerequisite for comparison of transformation frequencies. We have previously evaluated a P element vector, pUCHsneo, for the insertional mutagenesis of *D. melanogaster* (Ueda et al., 1987). Then, we used it here for *D. yakuba*. The results are summarized in Table 1. The viability from embryo to adult is only 5.3%. This low score should not be caused by an unexpected hazardous effect of the P elements. The embryos of *D. yakuba* are very weak, even under our careful handling, and the viability was dropped by the stress during dechoriation and injection. In addition, 5 among 21 G0 adults became sterile, they also might be

damaged during the operations. Such sterile flies had also been observed in the case of *D. melanogaster*. Finally, 5 out of 16 fertile G0 flies, produced neomycin resistant G1 flies. They were candidates of the transformants. Single G1 adult was used for establishing a resistant line from each 4 G0 flies. From another G0 male, 12 resistant lines were established in order to examine the "clustering" of the transformants, caused by the proliferation of the germ cell in which the insertion occurred at the premeiotic stage. These lines were maintained under the continuous selective condition.

Genomic Southern blot analysis revealed that all 16 lines had the pUCHsneo insertion (data not shown). As observed in *D. melanogaster*, the lines harboring multiple copies of pUCHsneo were relatively few. In 4 lines among 16 lines, 2 or 3 copies of the pUCHsneo were observed. Twelve sister lines from single G0 could be classified into 7 groups based on the southern blotting pattern. Thus, clustering were detected but the cluster size was relatively small. Transformation frequency was 31.3% (5/16). This score was comparable to the score for *D. melanogaster*, 40.4% (Ueda et al., 1987), judging from χ^2 test ($0.3 < P < 0.5$). Considering the shorter length of P elements used, which recorded the higher score in general, the previously reported scores for *D. simulans* (Scavarda and Hartl, 1984, 1987; Daniels et al., 1985) were somewhat smaller, and that of *D. hawaiiensis* (Brennan et al., 1984) was remarkably small.

Table 1. Transformation Frequencies

species	No. of			transformation frequency (%)	vector
	injected embryos	fertile adults	transformant producers		
<i>D. yakuba</i>	400	16	5	31.3	pUCHsneo
<i>D. melanogaster</i> * ¹	591	89	36	40.4	pUCHsneo
<i>D. simulans</i> * ²	60	16	4	25.0	p π 25.1
<i>D. simulans</i> * ³	185	34	11	32.4	p π 25.1, Carnegie 20
<i>D. hawaiiensis</i> * ⁴	—	34	2	5.9	p π 25.1

*¹ Ueda et al. (1987), *² Daniels et al. (1985), *³ Scavarda & Hartl (1984), *⁴ Brennan et al. (1984)

Sequence analysis of target sites

The facts that a high frequency of transformation was recorded and most transformant lines (12/16) had only single copy of the element in the genome strongly suggested that the transformation really occurred by P element mediated fashion. To confirm it, we sequenced the flanking regions of introduced elements using a synthetic primer corresponding to the internal half of the terminal inverted repeat of P element. So far examined, this primer worked well and the duplications of 8 base pairs flanking the inverted repeats were found (Fig. 2). It clearly revealed that the elements were inserted by the P element-specific way in

Drosophila yakuba

91005

---ATC**AGCTAGAT**TTTAAAAGCA---
 ---AGCGTTCTCGT**CGATCTAGTA**---

91007

---ATC**GGATATAT**AGTCAAATAT---
 ---GAGATCACACC**CTATATAGTA**---

91009

---ATC**CCCAGAC**GTTCGCTGAG---
 ---GTTTAGGCTAG**GGGTCTGGTA**---

91121-1

---ATC**GGACGGAC**GGTCGTTGCTT---
 ---AACGACCATC**CTGCCTGCTA**---

91121-2

---ATC**CTTTGTGTAT**T-----
 ---TTTCTTTATT**GAAACACAGTA**---

91701

---ATC**CGCATATGC**ATTCTTAT---
 ---TGTACGTAAT**GCGTATACGTA**---

Drosophila melanogaster

24201

---ATC**CTCCTGAT**GCTCGGCACA---
 ---TGTGTACTTC**GAGGACTAGTA**---

143307-1

---ATC**GCCTCTC**ATTTTTCACG---
 ---GCCAAAGTGCC**CGGAGAGGTA**---

143307-2

---ATC**ATTGGAAT**TGAATTCAAC---
 ---GCTTCAGTTT**TAGCTTAGTA**---

150508

---ATC**CAGCAGCC**CACTGAGCAG---
 ---CGTCATCTTC**GTCTCGGGTA**---

Proposed Consensus

---GGCCAGAC---

Fig. 2. Comparison of 8 base pairs duplicated upon insertions. The upper-left of each sequence is the line number of the transformant. The bold characters indicate the duplicated sequences upon insertions, those underlined matched with the consensus. The sequences in the shadowed boxes are the terminal sequences of the wings of the P element. The number of bases matching with the consensus sequence is varying from 0 to 6.

D. yakuba, because both terminal inverted repeats of P element remained intact and 8 base pairs duplication was generated upon insertion.

Once, a target consensus sequence of P element insertion was reported (O'Hare and Rubin, 1983). However, many examples have been accumulated later, in which the sequences did not match with the proposed consensus of GGCCAGAC (see Garrell and Modolell, 1990). Also in our results, the number of bases fitting was varying from 6 to 0, and any such consensus sequence could not be found in our sequence data (Fig. 2). On the other hand, it has been pointed out that P elements show the tendency to be inserted into the actively transcribed genes at the time of transposition (Bownes, 1990), further, often into the regulatory regions of the active genes (Tsubota et al., 1986; Kelley et al., 1987). In the regulatory regions, the specific sequences recognized by the trans-acting factors

would be supposed. However, such sequences should not be a sole feature for the insertion sites. Because P element can also be inserted into both the coding region and the intron of a gene and seems to have wider range of target sequences than a specific trans-acting factor. During the sequence analysis, we noticed that a series of short palindromic stretches are lying around the insertion sites (Fig. 3A). It is also in the case of *D. melanogaster* transformant lines examined (Fig. 3B). Such sequences might have a potential to form cruciform-like structures when the chromatin structure is dissolved for the active transcription, and such structure might present a landmark for P element insertion. Further study is required to evaluate this possibility.

Fig. 3. Short palindromic stretches around the insertion sites. The upper-left of each sequence is the line number of the transformant. The dotted characters are duplicated 8 base pairs. The numbered pairs of the underlined sequences are palindromic stretches. The transformant lines are *D. yakuba* (A), and *D. melanogaster* (B).

We are grateful to Drs. V. Pirrotta and T. Watanabe for a kind gift of the plasmids and *D. yakuba* to us.

REFERENCES

- Berg, C. A. and Spradling, A. C. (1991). Studies on the rate and site-specificity of P element transposition. *Genetics* **127**, 515–524.
- Bownes, M. (1990). Preferential insertion of P elements into genes expressed in the germ-line of *Drosophila melanogaster*. *Mol. Gen. Genet.* **222**, 457–460.
- Brennan, M. D., Rowan, R. G. and Dickinson, W. J. (1984). Introduction of a functional P element into the germ line of *Drosophila hawaiiensis*. *Cell* **38**, 147–151.
- Daniels, S. B., Strausbaugh, L. D. and Armstrong, R. A. (1985). Molecular analysis of P element behavior in *Drosophila simulans* transformants. *Mol. Gen. Genet.* **200**, 258–265.
- Daniels, S. B., Peterson, K. R., Strausbaugh, L. D., Kidwell, M. G., and Chovnick, A. (1990). Evidence for horizontal transmission of the P transposable element between *Drosophila* species. *Genetics* **124**, 339–355.
- Engels, W. R. (1989). P element in *Drosophila melanogaster*. In: *Mobile DNA* (eds.: D. E. Berg and M. M. Howe), pp. 437–484. American Society for Microbiology, Washington, DC.
- Garrell, J. and Modolell, J. (1990). The *Drosophila extramacrochaetae* locus, an antagonist of proneural genes that, like these genes, encodes a helix-loop-helix protein. *Cell* **61**, 39–48.
- Kelley, M. R., Kidd, S., Berg, R. L. and Young, M. W. (1987). Restriction of P-element insertions at the *Notch* locus of *Drosophila melanogaster*. *Mol. Cell. Biol.* **7**, 1545–1548.
- O'Hare, K. and Rubin, G. M. (1983). Structure of P transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. *Cell* **34**, 25–35.
- Pirrotta, V. (1986). Cloning *Drosophila* genes. In: *Drosophila a practical approach* (ed.: D. B. Roberts), pp. 83–110. IRL Press, Oxford.
- Sanger, F., Nicklen, S. and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
- Scavarda, N. J. and Hartl, D. L. (1984). Interspecific DNA transformation in *Drosophila*. *Proc. Nat. Acad. Sci. USA* **81**, 7615–7619.
- Scavarda, N. J. and Hartl, D. L. (1987). Germ line abnormalities in *Drosophila simulans* transfected with the transposable P element. *J. Genet.* **66**, 1–15.
- Steller, H. and Pirrotta, V. (1986). P transposons controlled by the heat shock promoter. *Mol. Cell. Biol.* **6**, 1640–1649.
- Tsubota, S., Ashburner, M. and Schedl, P. (1985). P-element-induced control mutations at the r gene of *Drosophila melanogaster*. *Mol. Cell. Biol.* **5**, 2567–2574.
- Ueda, R., Takahisa, M., Togashi, S., Maruyama, S. and Miyake, T. (1987). Insertional mutagenesis in *Drosophila* I. Efficiency of transformation and induction of mutations in *Drosophila melanogaster*. *Jpn. J. Genet.* **62**, 45–50.