SHORT PAPER

Cloning of P-elements from P- and Q- strains in natural populations of *Drosophila melanogaster* in Japan

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ABSTRACT

We have cloned and characterized several P transposable elements from P and Q strains extracted from natural populations of *Drosophila melanogaster* in Japan. Structures of these clones were investigated by the techniques of blot hybridization, restriction mapping, and observation of heteroduplex by electron microscope. No intact P element such as $p\pi$ 25.1 was found among 11 clones from a P strain and 10 clones from Q-strains. A typical Q-strain (WY-113) judging from the results of genetical crosses did not carry any DNA segment homologous to $p\pi$ 25.1, indicating the complex nature of Q-strains.

In *Drosophila melanogaster* the phenomenon of hybrid dysgenesis is known to occur in the progeny of certain interstrain crosses (Kidwell, Kidwell and Sved 1977). In the P-M system dysgenesis is observed in the germ lines of the progeny when P-strain males are mated with M-strain females and reared under high temperature (usually 29°C). This phenomenon is not observed in the reciprocal cross. Using *D. melanogaster* strains isolated in the USA, Rubin and his coworkers examined the molecular mechanism of the P-M type hybrid dysgenesis and found that the dysgenesis resulted from the insertion and/or excision of the members of a transposable genetic element termed P factor, which were distributed at the scattered sites on the P-strain chromosomes (Bingham, Kidwell and Rubin 1982; Rubin and Spradling 1982; O'Hare and Rubin 1983). By blot hybridization experiments, it was also shown that Q-strains resistant to the dysgenesis contained the nucleotide sequences homologous to the P factor most probably in defective forms, whereas no traces of the P factor were found in the majority of M-strains sensitive to

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Table 1. *Genetical test of hybrid dysgenesis in three strains from natural populations in Japan. π_2 and Canton S are typical P and M strains, respectively*

		NP-280		AK-194		TS-282		π_2	
		♀	♂	♀	♂	♀	♂	♀	♂
Canton S	♀	—	93.9* (62/64)**	—	22.1 (17/77)	—	0.0 (0/80)	—	100 (48/48)
	♂	0.0 (0/75)	—	0.0 (0/79)	—	0.0 (0/48)	—	0.0 (0/48)	—
π_2	♀	—	0.0 (0/64)	—	0.0 (0/75)	—	0.0 (0/73)		
	♂	0.0 (0/80)	—	1.6 (1/64)	—	0.0 (0/43)	—		

*: Values indicate the percentage of sterile progeny.

**: Values in parentheses indicate actual number of sterile progeny out of total progeny examined.

the dysgenesis (Bingham, Kidwell and Rubin 1982). This element is particularly interesting for students of molecular or developmental biology because of its unusual ability as a cloning vehicle of various genes and an excellent tool for the study of gene regulation in *Drosophila melanogaster* (Scholnick *et al.* 1983; Spradling and Rubin 1983; Hazelrigg *et al.* 1984).

Here, we present evidence that, like the counterparts in the US populations, the majority of the P- and Q- strains genetically identified in natural populations of *D. melanogaster* in Japan contain the homologue of the US P factor. Our data suggest that Japanese and American "P factors" are very similar, if not identical, in nucleotide sequence.

In natural populations of *Drosophila melanogaster* in the US the majority of strains are P-strains. On the other hand, most strains from natural populations in Japan turned out to be Q-strains by biological tests, with very low frequencies of the P-strain (Takada *et al.* 1983).

For this experiment we used one P-strain (NP-280), and two Q-strains (AK-194, TS-282). The NP-280 was sampled in Ishigaki-jima (Okinawa prefecture) in 1980 by Dr. S. Kusakabe. The chromosome extraction and characterization of this strain was performed by the group of Dr. S. I. Chigusa. AK-194 and TS-282 were sampled in Akayu and Tanushimaru, respectively, and were isogenic for both the second and third chromosomes (see Yamazaki *et al.* 1984). The results of a hybrid dysgenesis test of these three strains are shown in Table 1. Since 97% of female progeny (62 out of 64) were sterile

Fig. 1. Southern blot hybridization of various *Drosophila* strains from natural populations in Japan. Total genomic DNA was isolated from adult flies, digested by *Eco*RI, run on 0.8% agarose gel and transferred to a nitrocellulose filter. The probe used is $p\pi$ 25.1, an intact *P* element. Lane A— π_2 (a typical US *P*-strain); Lane B—NP-280 (a typical Japanese *P*-strain); Lane C—Canton S (a typical *M*-strain); Lane D—AK-194 (a *Q*- or weak *P*-strain); Lane E—WY-113 (a typical *Q*-strain); Lane F—WY-117 (a typical *Q*-strain); Lane G—TS-282 (a typical *Q*-strain). The values at the left are molecular weight standard by kilo base.

from the cross between females of Canton S (*M*-strain) and males of NP-280, NP-280 was judged to be a typical *P*-strain, supporting an observation by Takada *et al.* (1983). AK-194 might well be classified as a weak *P*-strain since 22% of progeny from the cross between AK-194 males and Canton S females was sterile, whereas all progeny from the reciprocal cross was found to be fertile. TS-282 seems to be a typical *Q*, since sterility was observed in no progeny from the crosses of both combinations.

To know whether the Japanese *P*- and *Q*-strains have genetic elements

NP280 **TS282** **AK194**

E:EcoRI B:BamHI H:HindIII

Fig. 2. Restriction maps of 14 clones which show some homology with the P element (7 from NP-280, one from TS-282, and 7 from AK-194). Bold lines indicate the segments showing homology with the probe $p\pi 25.1$.

homologous in nucleotide sequence to the US P factor, the genomic DNAs extracted from adults of NP-280, AK-194, TS-282 together with those from Canton S, π_2 (a strong P-strain isolated in the USA), WY-113 and WY-117 (Q-strains from a natural population in Osaka, Japan (Mukai and Yukuhiro 1983)) were terminally digested with restriction enzyme, EcoRI, sized on 0.8% agarose gel, transferred to nitrocellulose filter paper, and blot hybridized using a probe $p\pi 25.1$ which consisted of a complete US P factor

with a short DNA piece of heldup locus and pBR322 (Rubin and Spradling 1982). As shown in Fig. 1, many bands can be seen in cases of NP-280 (lane B), AK-194 (lane D), TS-282 (lane G), and WY-117 (lane F) as well as in the case of π_2 (lane A), although the numbers and intensities of bands varied from lane to lane. On the other hand, no distinctive bands were detected in Canton S (lane C) except for a faint band which is labelled by an asterisk and seemed to correspond to a DNA segment including a portion of heldup locus, suggesting that like the P- and Q-strains in the USA, the majority of the Japanese P- and Q-strains contain P-related nucleotide sequences at many dispersed chromosomal sites. In contrast WY-113 exhibited no homology to the US P factor, though WY-113 showed high dysgenic characteristics in the M-cytotype (Mukai and Yukuhiro 1983) and was classified as a typical Q-strain by our genetic tests not shown here. Results of *in situ* hybridization (Yamaguchi *et al.*, unpublished) were also consistent with these findings. These observations might suggest that resistance to the P-factor, a characteristic of Q-strains, is not necessarily produced by the genetic elements homologous to the P factor.

We also have isolated recombinant clones whose DNA inserts are hybridizable with the US P factor. Essentially following Maniatis *et al.* (1978), cloned libraries of the EcoRI partial digests of the genomic DNAs were constructed from each of NP-280, TS-282 and AK-194 using Charon 4 as a cloning vector. These cloned libraries were then screened with $p\pi 25.1$ as a probe. So far, we have isolated and purified 11, 10, and 1 positive phages from the NP-280, AK-194 and TS-282 libraries, respectively. Some examples of the restriction maps of the *Drosophila* DNA inserts in these clones are shown in Fig. 2, where filled rectangles indicate both the positions and sizes of the restriction segments hybridizable to the US P factor. No clones were completely the same as the P factor in $p\pi 25.1$, although the P-related elements in clone No. 16 of NP-280 and No. 11 of AK-194 appear to contain EcoRI and Hind III sites at positions identical to those of the US P factor. We also cannot exclude the possibility that clone No. 11 of AK-194 and No. 14 of NP-280 were originally identical in structure to the US P factor in $p\pi 25.1$ but were artificially truncated by EcoRI in the processes of establishing libraries.

Heteroduplex molecules were constructed between BanIII-digested $p\pi 25.1$ DNA and DNA of some P-positive phages from the NP-280 library. We observed 1.2 to 2.5 kbp long duplexes with or without single stranded loops in the regions expected to be homologous to the US P factor. Shown in Fig. 3 are two typical examples of heteroduplex molecules of No. 10 (A) and No. 14 (B) clones of NP-280 together with their explanations. Our studies provide evidence that the P-related elements found in a Japanese P-strain, NP-280, are very similar, if not identical, in nucleotide sequence to the US P factor, although substantial deletions are observed in the particular examples shown

Fig. 3. Two examples of heteroduplex molecules were prepared essentially as described in Saigo *et al.* (1981). The clone No. 10 (A) or No. 14 (B) from NP-250 was hybridized with $p\pi$ 25.1 digested with BanIII. The segment between the two arrow is a double stranded region which has homology with $p\pi$ 25.1.

in Fig. 3. To confirm the above-mentioned possibility, detailed studies of the clones isolated here are now under way.

Recently, Sakoyama *et al.* (1983) have analyzed some clones isolated from the genomic library of a Q-strain sampled in Chichi-jima, Ogasawara and obtained results similar to those we described here in cases of a P-strain from Ishigaki-jima and Q-strains isolated in Akayu and Osaka. Thus it is quite likely that the US P factor or its close relatives widely prevail(s) in Japanese natural populations.

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REFERENCES

- BINGHAM, P. M., KIDWELL, M. G. and RUBIN, G. M. (1982) The molecular basis of P-M hybrid dysgenesis: the role of the P element, a P-strain-specific transposon family. *Cell* **29**, 995-1004.
- HAZELRIGG, T., LEVIS, R. and RUBIN, G. M. (1984) Transformation of white locus DNA in *Drosophila*.

- phila: Dosage Compensation, zeste interaction, and position effects. *Cell* 36, 469-481.
- KIDWELL, M. G. (1981) Hybrid dysgenesis in *Drosophila melanogaster*: The genetics of cytotype determination in a neutral strain. *Genetics* 98, 275-290.
- KIDWELL, M. G., KIDWELL, J. F. and SVED, J. A. (1977) Hybrid dysgenesis in *Drosophila melanogaster*: a syndrome of aberrant traits including mutation, sterility and male recombination. *Genetics* 86, 813-833.
- MANIATIS, T., HARDISON, R. C., LACY, E., LAUER, J., O'CONNELL, C., QUON, D., SIM, G. K. and EFSTRATIADIS, A. (1978) The isolation of structural genes from libraries of eucaryotic DNA. *Cell* 15, 687-701.
- MUKAI, T. and YUKUHIRO, K. (1983) An extremely high rate of deleterious viability mutations in *Drosophila* possibly caused by transposons in non-coding regions. *Proc. Japan, Acad.* 59 (B), 316-319.
- O'HARE, K. and 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.
- RUBIN, G. M. and SPRADLING, A. C. (1982) Genetic transformation of *Drosophila* with transposable element vectors. *Science* 218, 348-353.
- SAIGO, K., MILLSTEIN, L. and THOMAS, C. A., Jr. (1981) The organization of *Drosophila melanogaster* histone genes. *Cold Spring Harbor Symp. Quant. Biol.* 45, 815-827.
- SAKOYAMA, Y., TODO, T., ISHIWA, S., RYO, H., KONDO, S. and HONJO, T. (1983) Movable P elements in Q type strains I. Cloning and gene structure of Q type P elements. *Jpn. J. Genet.* 58, 678.
- SCHOLNICK, S. B., MORGEN, B. A. and HIRSH, J. (1983) The cloned dopa decarboxylase gene is developmentally regulated when reintegrated into the *Drosophila* genome. *Cell* 34, 37-45.
- SPRADLING, A. C. and RUBIN, G. M. (1982) Transposition of cloned P elements into *Drosophila* germ line chromosomes. *Science* 218, 341-347.
- SPRADLING, A. C. and RUBIN, G. M. (1983) The effect of chromosomal position on the expression of the *Drosophila* xanthine dehydrogenase gene. *Cell* 34, 47-57.
- TAKADA, S., MURAI, M., TAKANASI, E., OHIOISHI, K., FUKAMI, K., HAGIWARA, N., SATTA, Y. and ISHIWA, S. (1983) On the P-M system in natural populations of *D. melanogaster* in and around Japan. *Jpn. J. Genet.* 58, 686.
- YAMAZAKI, T., MATSUO, Y., INOUE, Y. and MATSUO, Y. (1984) Genetic analysis of natural populations of *Drosophila melanogaster* in Japan. I. Protein polymorphism, lethal gene, sterility gene, inversion polymorphism and linkage disequilibrium. *Jpn. J. Genet.* 59, 33-49.