Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 9485–9489, October 1995
Genetics

Introduction of the transposable element *Minos* into the germ line of *Drosophila melanogaster*

(germ-line transformation/mobile elements)

THANASIS G. LOUKERIS*, BRUNO ARCÀ*, IOANNIS LIVADARAS*, GEORGIA DIALEKTAKI*, AND CHARALAMBOS SAVAKIS*†‡

*Insect Molecular Genetics Group, Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, P.O. Box 1527, Heraklion 711 10, Greece; and †Department of Basic Medical Sciences, Medical School, University of Crete, Heraklion, 71001 Crete, Greece

Communicated by Fotis C. Kafatos, European Molecular Biology Laboratory, Heidelberg, Germany, April 27, 1995

ABSTRACT **A transposon based on the transposable element *Minos* from *Drosophila hydei* was introduced into the genome of *Drosophila melanogaster* using transformation mediated by the *Minos* transposase. The transposon carries a wild-type version of the white gene (*w*) of *Drosophila* inserted into the second exon of *Minos*. Transformation was obtained by injecting the transposon into preblastoderm embryos that were expressing transposase either from a *Hsp70–Minos* fusion inserted into the genome via *P*-element-mediated transformation or from a coinjected plasmid carrying the *Hsp70–Minos* fusion. Between 1% and 6% of the fertile injected individuals gave transformed progeny. Four of the insertions were cloned and the DNA sequences flanking the transposon ends were determined. The "empty" sites corresponding to three of the insertions were amplified from the recipient strain by PCR, cloned, and sequenced. In all cases, the transposon has inserted into a TA dinucleotide and has created the characteristic TA target site duplication. In the absence of transposase, the insertions were stable in the soma and the germ line. However, in the presence of the *Hsp70–Minos* gene the *Minos–w* transposon excises, resulting in mosaic eyes and germ-line reversion to the white phenotype. *Minos* could be utilized as an alternative to existing systems for transposon tagging and enhancer trapping in *Drosophila*; it might also be of use as a germ-line transformation vector for non-*Drosophila* insects.**

Transposable elements have been used as vectors for stable germ-line transformation in *Drosophila melanogaster*. The mobile element *P*, present in some *D. melanogaster* populations, was the first element shown to insert into germ-line chromosomes in embryos (1). Typically, *P*-element-mediated transformation is achieved by coinjecting into preblastoderm embryos a mixture of two plasmids: one expressing transposase but unable to transpose and one carrying a gene (or genes) of interest flanked by the inverted terminal repeats of the element. Transformants are detected among the progeny of the injected individuals through the expression of dominant phenotypes. Germ-line transformation has revolutionized *Drosophila* research through the introduction of powerful methodologies such as analysis of *in vitro* mutagenized genes, gene cloning by transposon tagging, and enhancer trapping.

Two other elements unrelated to *P*, *hobo* and *mariner*, have been shown to be able to transpose in the genome of *D. melanogaster*. *hobo* is found in some but not all *D. melanogaster* populations and causes hybrid dysgenesis (2, 3). Defective *hobo* elements containing foreign DNA can transpose into germ-line chromosomes from plasmids if coinjected into preblastoderm embryos along with a full-length element that can provide transposase (4). *mariner*, an element found in *Drosophila mauritiana* but not in *D. melanogaster*, has been introduced into *D. melanogaster* and shown to induce in trans mobilization of a nonautonomous *mariner* element (5, 6).

Efforts to transfer the *Drosophila* germ-line transformation methodology to Diptera of economic or medical interest have been unsuccessful for reasons that are unclear. The *D. melanogaster P*-element has been used successfully in transformation of *Drosophila hawaiiensis*, but attempts to use *P* for germ-line transformation of non-*Drosophila* species have been unsuccessful (7, 8). One possible approach would be to try in *D. melanogaster* transposable elements from other species, especially those distantly related to *D. melanogaster*. The most critical step in this approach is identification of fully active forms of the elements. Most transposable elements are found in two forms: inactive, or nonautonomous, and active, or autonomous. Nonautonomous elements are incapable of transposition in the absence of autonomous elements; they cannot catalyze transposition because they do not produce active transposase, although they contain the sequences needed for their own transposition. Usually, these elements are deletion derivatives of autonomous elements (8–11). The recent discovery of a large family of *mariner*-related elements that are widely dispersed in insects emphasized this problem; the majority of the elements that have been analyzed appear to be nonautonomous, producing no transposase (12, 13).

The transposon *Minos* was discovered in the rDNA of *Drosophila hydei* (14). It is ≈1.8 kbp with long (254 bp) inverted terminal repeats. The *Minos* element contains two nonoverlapping open reading frames separated by a putative intron and coding for a conceptual polypeptide that shows >40% sequence identity with the *Tc1* transposase of the nematode *Caenorhabditis elegans* (15). Correctly spliced *Minos* mRNAs were produced in *D. melanogaster* from a fusion construct between the *Minos* transposase gene and the *hsp70* promoter (16).

We report here the introduction of a *Minos*-based nonautonomous transposon into the *D. melanogaster* genome through conventional germ-line transformation procedures. We also show that a stably integrated construct encoding *Minos* transposase is active in mobilizing the nonautonomous *Minos* vector in *D. melanogaster*.§

MATERIALS AND METHODS

Plasmid Constructions. *Minos* transposase source plasmid pHSS6hsMi2 was created using the 456-bp *Xba* I/*Xmn* I fragment from the *D. melanogaster Hsp70* gene, which contains the promoter and 206 bp of untranslated leader sequence (17). The 5′ inverted repeat of *Minos-2* was replaced by the *Hsp70*

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

†To whom reprint requests should be addressed.

§The sequences reported in this paper have been deposited in the GenBank data base [accession nos. 248626 (*A10.1*), 248627 (*A10.2*), 248628 (*B33.20*), and 248629 (*C9.1B*)].

promoter in the vector pHSS6 (18) at the single *Hin*dIII site in the inside end of the repeat (16).

For transformation of *Drosophila*, a *Not* I fragment from pHSS6hsMi2 containing the *Hsp70–Minos* fusion was ligated into the *P* transformation vector pDM30. This plasmid was named pPhsMi2.

The transposon plasmid pMiw1 was constructed by inserting a 4.1-kb *Eco*RI fragment containing a shortened version of the white (*w*) gene of *Drosophila* (19) into the *Eco*RI site of the *Minos-2* element in the pTZ18R vector (Pharmacia). The mini-white fragment was obtained from plasmid CaSpw-12, a gift from V. Pirrotta (University of Geneva).

Fly Strains and Germ-Line Transformation. Embryo injections were done essentially as described (1). Embryo injections for *P*-element-mediated transformation were done essentially as described (1) using pπ25.1wc as helper. *Minos* plasmids were injected by the same procedure, using pMiw1 transposon DNA (400 μg/ml) and pHSS6hsMi2 helper (100 μg/ml). In some experiments, a 30-min heat shock at 37°C was delivered to the embryos 4–8 hr postinjection.

For construction of *P*-induced transformants containing the pPhsMi2 insert, the recipient strain was *cn; ry*^{506} and the injected (G_0) flies were back-crossed to the same strain. *ry*$^+$ G_1 progeny (the progeny of injected flies) were selected and characterized genetically, by DNA blotting, and by *in situ* hybridization to polytene chromosomes. For *Minos* transformation, injections were done in *w* strains, G_0 flies were back-crossed to a *y w* strain, and the G_1 progeny were screened for the appearance of non-white eye phenotype. Non-white G_1 flies were crossed into appropriate balancer stocks for isolation and characterization of the insertions.

General DNA Manipulations, Sequencing, and PCR. General recombinant DNA procedures were carried out as described (20). Genomic libraries were prepared in the λEMBL4 vector by *Sau*3A1 partial digestion as described (20). Full-length pMiw1 insertions were isolated with the following probes: (*i*) the 4.1-kb *Eco*RI mini-white fragment from vector CaSpeR; (*ii*) the *Hin*dIII/*Eco*RI 914-bp (left end of *Minos*); and (*iii*) the *Eco*RI/*Pac* I 318-bp (right end) *Minos* fragments. *Eco*RI fragments of the λ clones containing one end of the transposon and the corresponding genomic flanks were subcloned in pBCKS(+) (Stratagene) and the ends of the inverted repeats plus flanking DNA were sequenced using primer 5′-TAATATAGTGTGTTAAACATTGCGCACTGC-3′ (nt 1707–1736 of the *Minos* sequence). "Empty" sites were amplified by PCR (30 cycles; 94°C for 1 min, 50°C for 1 min, 72°C for 30 sec) using 0.25 μg of genomic DNA from the recipient strain *y w*; *TM3/M67* and the following primers: A10.1L, 5′-GATCATATCTGGATGTATAG-3′; A10.1R2, 5′-CGATCCTATAAAAACATTCG-3′; A10.2L, 5′-TGCAACCTATCTGTGGTAGT-3′; A10.2R2, 5′-CCAACAACTAATCAGCCTAC-3′; B33L, 5′-ATTGGTTCTCCATGCCAACG-3′; B33R2, 5′-TGTAGGAGATTCCCCAGTGC-3′. PCR products were subcloned into the *Eco*RV site of pBCKS(+) and sequenced with primers T3 and T7.

RESULTS AND DISCUSSION

Introduction of *Minos* into the *D. melanogaster* Genome. A *Minos*-based transposon was introduced into the *D. melanogaster* germ line in two ways: (*i*) by introducing the transposon into embryos that expressed the *Minos* transposase from a stably integrated construct and (*ii*) by coinjecting transposon and transposase-expressing plasmids into embryos. Fig. 1 shows the structures of the transposase-producing and the transposon constructs used in this work. To express the *Minos* transposase in *D. melanogaster*, the presumed *Minos* promoter in the 5′ inverted repeat was first replaced by the *Hsp70* promoter of *D. melanogaster*; this promoter has been shown to be heat inducible in most tissues of embryos, larvae, and adults (21, 22). Replacing one of the terminal repeats should also make the element transposition defective. The *Hsp70–Minos* fusion was then transferred into a *P*-element transformation vector and introduced into the *D. melanogaster* genome via *P*-induced germ-line transformation. Three independent transformants were recovered—one on the X chromosome and two on the 3rd chromosome. In these experiments, a single insertion at 87D [*P*{*Hsp70:Mi\T*}*ry*$^+$}(87D)] was used, designated *M67*. Flies carrying the *M67* chromosome produce high levels of correctly spliced *Minos* polyadenylylated RNA after a heat shock (16).

The *M67* chromosome carries a recessive lethal mutation, which might be associated with the insertion site. The *y w*^{67C23}; *TM3 Sb Bd*^{S}/*ry P*{*Hsp70:Mi\T*}*ry*$^+$}(87D) strain (designated *TM3/M67*) was used as host for injections with the *Minos*-white transposon pMiw1. This transposon (Fig. 1) consists of a complete *Minos* element containing the *w*$^+$ minigene (19). In a parallel experiment, a plasmid containing the *Hsp70–Minos* fusion was coinjected with the pMiw1 transposon into a *y w*^{67C23} strain that is not producing transposase. The injected G_0 flies were crossed individually to *y w*^{67C23}; *TM3 Sb Bd*^{S}/*D gl*^{3} or *y w*^{67C23} flies and the progeny were screened for nonwhite eyes. Seven of the 329 fertile G_0 flies gave nonwhite eye progeny. Fig. 2 shows the phenotypes of four independent

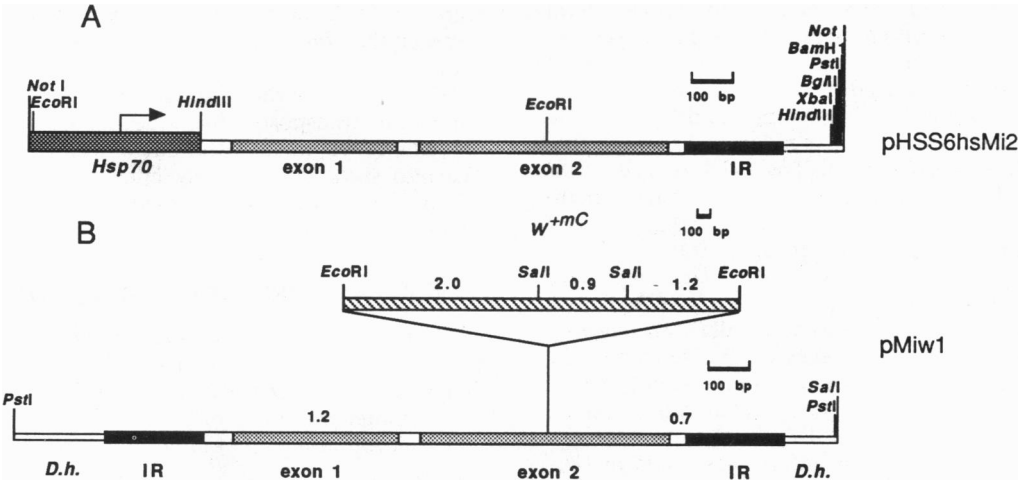

FIG. 1. *Minos* transformation vectors. (*A*) The pHSS6hsMi2 plasmid used as a source of transposase. (*B*) The pMiw1 transposon. Distances between the *Eco*RI and *Sal* I sites and the ends of the element are indicated in kb. IR, inverted repeats; *D.h., D. hydei* flanking DNA. Plasmid vector sequences are not shown.

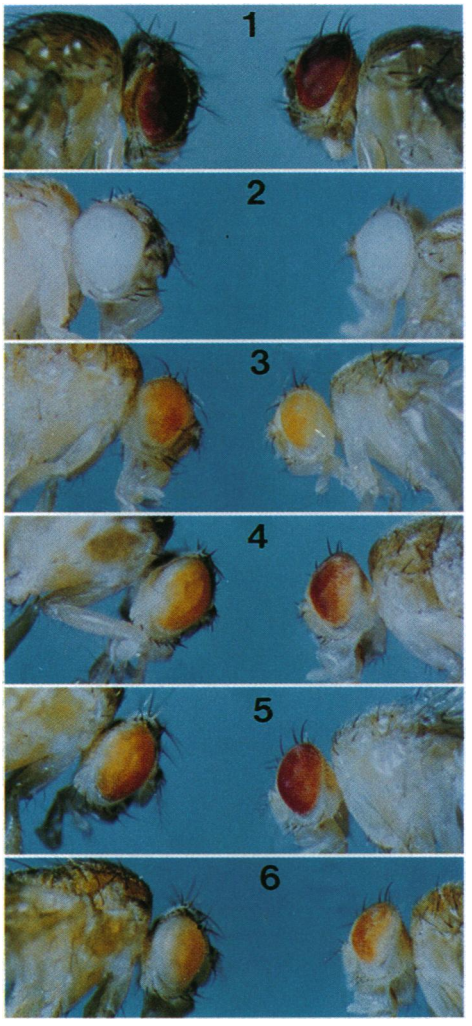

FIG. 2. Eye phenotypes of *D. melanogaster* transformants with the *Minos* element. (*1*) Wild type (Oregon-R). (*2*) Strain *y* w^{67C23}. (*3*) Line *A10.1*. (*4*) Line *A10.2*. (*5*) Line *B33.20*. (*6*) Line *C9.1B*. Females are on the left and males are on the right.

lines. In all cases, eye color varied from pale yellow to near wild type and tended to darken with age, as is typical with *P* [mini-white] insertions. This partial rescue makes the marker particularly useful in detecting and sorting out multiple insertions. Males with autosomal insertions exhibit stronger phenotypes than females, due to the presence of dosage compensation elements in the white fragment (23).

To test different transformation conditions, three injection experiments (A–C) were performed with pMiw1 into the transposase-producing *TM3/M67* strain and two experiments (D and E) were performed with the pHSS6hsMi2/pMiw1 plasmid mixture into the *y* w^{67C23} strain. In experiment A, injected embryos were kept at 18°C until larval emergence and were then transferred to 25°C. In experiment B, embryos developed at 25°C. Experiment C was as B, but embryos were subjected to a brief heat shock (30 min at 37°C) 4–8 hr after injection. Experiment D was with a heat shock as in C, and E was as in B at 25°C. As a control, injections of the pMiw1 transposon alone were performed in the *y* w^{67C23} host and embryos were grown at 25°C (experiment F). As shown in Table 1, all injection groups except D and F gave G_0 flies with transformed progeny and there was no apparent increase in the rate of transformation with temperature. Although the negative result of experiment F is what would be expected from injections of the transposon in the absence of transposase, there is no satisfactory explanation for the absence of transformants in experiment D, unless this is a chance result given the relatively small numbers of G_0 flies scored. It is possible that the basal levels of expression from the "leaky" *Hsp70* promoter (22) may result in production of saturating amounts of transposase and that 2.1% transformation frequency (the aggregate from experiments A–E) may be the maximum that can be achieved with this system under the conditions used. This frequency is acceptable for practical purposes, since several hundred embryos can be injected in a day.

Analysis of the progeny of the G_0 flies (Table 2) showed that in the injections into the transposase-producing strain the proportion of transformed progeny varied between 0.13% (in *C9*) to 12.2% (in *B33*). The proportion of transformed progeny in the coinjection experiment was appreciably higher (about 54% and 73% in the two independent G_0 flies). Further genetic and cytogenetic analysis of individual G_1 flies revealed two independent insertion events among the progeny of G_0 fly *A10* and at least two in each of the G_0 flies *E24* and *E28* from the coinjection experiment. Because only a small number of individual G_1 flies were analyzed from each G_0, we cannot estimate the overall occurrence of multiple insertions. Of the nine independent insertions that we characterized cytogenetically, two map on the X chromosome, four on the 2nd chromosome, and three on the 3rd chromosome (Table 2).

Fig. 3 shows Southern analyses of transformant DNA cut with *Sal* I and probed with the 4.1-kb mini-white gene fragment from pMiw1. *Sal* I should give an ≈0.9-kb fragment internal to the transposon, a >3.2-kb left-hand fusion fragment, and a >1.8-kb right-hand fusion fragment (Fig. 1). In addition, the resident w^{67C23} gene should also give an ≈0.9-kb fragment from the middle of the gene, an ≈1.5-kb 3′ fragment, and a >4.0-kb 5′ fragment spanning the w^{67C23} breakpoint. The bands in Fig. 3 are consistent with single pMiw1 insertions in *A10.2, C9.1B, A10.1, B33.20, E28.94*, and *E24.51*. The last three lanes, *E24.44, C58.26*, and *E28.91*, contain two additional bands. They are ≈2.1 and ≈6.5 kb and are shared by all three

Table 1. Frequency of transformation among injected flies

Exp.	DNA	Strain	*t*, °C	Fertile G_0s*	G_0s with w^+ progeny	G_1s tested	Individuals with transformed progeny
A	pMiw1	*y w; TM3/M67*	18	61 (741)	1	20,631	A10
B	pMiw1	*y w; TM3/M67*	25	37 (616)	2	8,687	B13,† B33
C	pMiw1	*y w; TM3/M67*	hs, 25	56 (633)	2	33,490	C9, C58
D	pMiw1, pHSS6hsMi2	*y w*	hs, 25	142 (778)	0	41,888	—
E	pMiw1, pHSS6hsMi2	*y w*	25	33 (327)	2	16,460	E24, E28
F	pMiw1	*y w*	25	85 (663)	0	22,431	—

Preblastoderm embryos were injected with DNA and the emerging G_0 adults were crossed individually to *y w* flies. The progeny (G_1 flies) were screened for nonwhite eye phenotype.

*Numbers in parentheses are injected embryos.

†The B13 stock was subsequently lost.

Table 2. Rates of transformation in the germ line of individual G_0 flies and cytogenetic localization of individual insertions

G_0 fly	Total number of G_1 progeny	% transformed	Lines with single-site insertions
A10	432	2.08	A10.1 (18F), A10.2 (74D)
B13	337	0.89	*
B33	254	12.20	B33.20 (54C)
C9	1489	0.13	C9.1B (38B)
C58	998	0.50	C58.26 (17D)
E24	944	53.60	E24.44 (77E), E24.51 (32E)
E28	398	73.12	E28.91 (60E), E28.94 (63D)

For each G_0 fly that gave transformed progeny, the rate of germ-line transformation was estimated as the proportion of progeny with nonwhite eye phenotype. Cytogenetic localizations of insertions in the established lines are shown in parentheses after the line names.
*Line B13 was lost before being characterized by *in situ* hybridization.

lines. These sizes are consistent with a structure containing two pMiw1 elements in tandem, separated by a complete copy of the pTZ18R vector plus *D. hydei* flanking sequences. This is supported by probing with pTZ18R, which labels the ≈6.5-kb band (data not shown). Tandem repeats of this kind might be generated by homologous recombination of two pMiw1 plasmids in the embryo, followed by integration into the chromosome via the external inverted repeats of a duplex. Homologous recombination between plasmids has been hypothesized previously to account for integration of an unusual tetrameric *P*-element vector (24).

Sequence Analysis of *Minos* Inserts. To determine the molecular basis of *Minos* integration events, we have cloned the insertions from four independent lines [*A10.1, A10.2, B33.20* (=*B33*), *C9.1B* (=*C9*)] and sequenced the ends of the element and flanking DNA in these clones using a primer within the inverted repeat. The sequence in all clones contained the ends of the inverted terminal repeats of *Minos* flanked by a TA dinucleotide. After the TA, the sequences in each clone were different from each other and from the known *D. hydei* flanking sequences. The sequences are characterized by a high A+T content and show no significant similarity with any other sequence in the data bases. Fig. 4 shows a summary of this analysis.

That these sequences are from *Drosophila* was demonstrated by PCR amplification from the parental *Drosophila* strain, *y* w^{67C23}, with primers derived from the flanks. This was done for three of the insertions, *A10.1, A10.2*, and *B33.* Amplification was obtained in all cases using *y* w^{67C23} DNA as template and

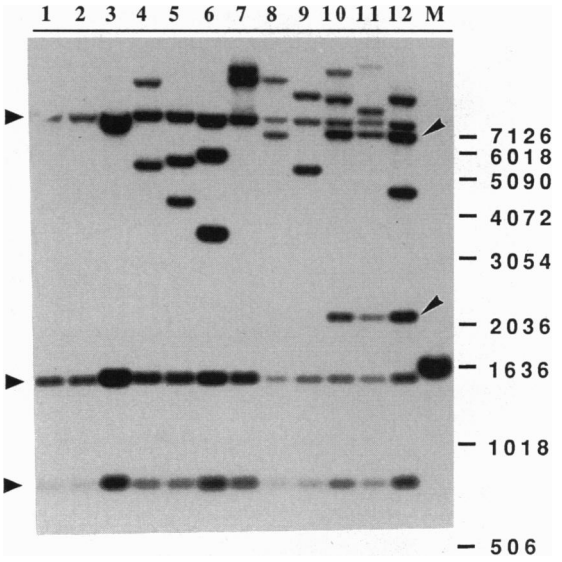

FIG. 3. Southern analysis of *D. melanogaster* transformants. DNA from various recipient strains and transformed lines was digested with *Sal* I and hybridized with the 4.1-kb mini-white fragment present in pMiw1. Lanes: 1, strain *y* w^{67C23}; *TM3/M67*; 2, strain *y* w^{67C23}; *TM3/Dgl*^{3}; 3, *y* w^{67C23}; 4, line *A10.2*; 5, line *C9.1B*; 6, line *A10.1*; 7, line *B33.20*; 8, line *E28.94*; 9, line *E24.51*; 10, line *E24.44*; 11, line *C58.26*; 12, line *E28.91*; M, size markers. Arrowheads on left indicate the three bands generated by the resident w^{67C23} allele. Arrowheads on right mark the two extra bands that are common to the last three transformed lines. Numbers on right indicate bases.

the resulting fragments showed the expected sizes for the empty sites—i.e., the distance between the two primers without the transposon. The amplified fragments were cloned and sequenced, and the sequences corresponded exactly to the expected empty target sites. Fig. 4 shows the sequences immediately flanking the ends of the element. As in *D. hydei*, the element has inserted at a TA dinucleotide, producing (most probably) a TA target site duplication. This shows that the insertions are transposase-dependent precise integrations of the transposon. No consensus of the target sites, other than the TA dinucleotide, could be detected by comparison of the *D. melanogaster* sequences to each other or to *D. hydei.*

Transposase-Induced Transposon Instability. The pMiw1 transposon is nonautonomous, because its transposase gene is interrupted by the *w* sequences. Consequently, the pMiw1 insertions should be stable in the absence, and mobilized in the

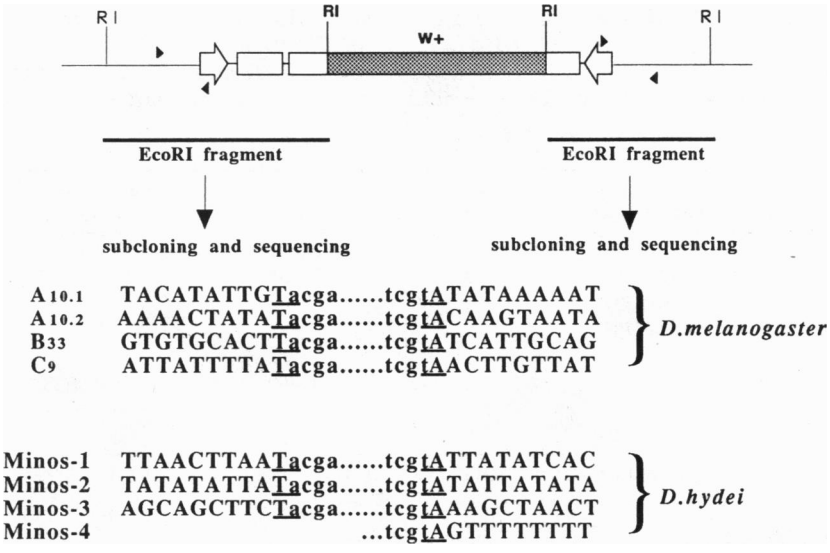

FIG. 4. Sequence specificity of *Minos* insertions in *D. melanogaster.* (*Upper*) Schematic of the strategy for cloning and sequencing *Minos* insertions. The primer used for sequencing outward from the inverted repeats and the primers used for subsequent PCR amplification are indicated. (*Lower*) Comparison of the sites of *Minos* insertions in *D. melanogaster* and *D. hydei.* Chromosomal sequences are represented by capital letters. The presumed target site duplication is underlined. *D. hydei* data are from ref. 16.

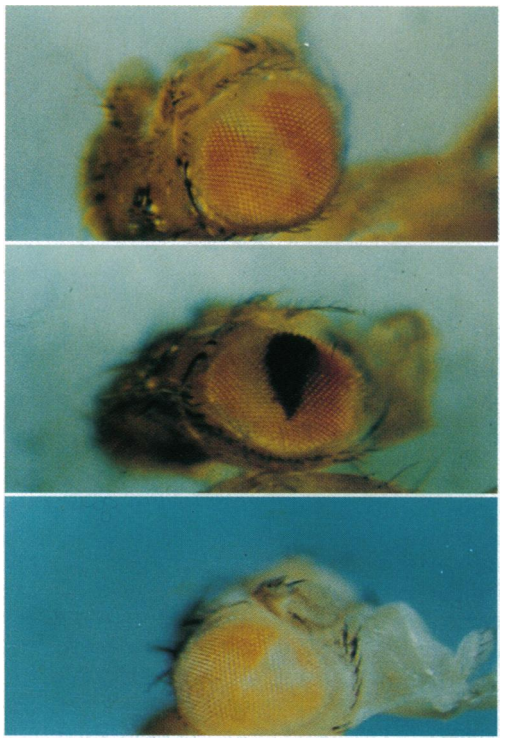

FIG. 5. Somatic mobilization of *Minos*. Examples of eye color mosaicism resulting from excision/transposition events in the soma of *y w; TM3/Mi[w^{+mc}](74D) P{Hsp70:Mi\T}ry^{+}}(87D)(A10.2)* flies grown in 25°C.

presence, of *Minos* transposase. The effect of the *Minos* transposase on the pMiw1 transposon was examined in line *A10.2*, which contains the transposon inserted into the *M67* chromosome, a 3rd chromosome carrying the pPhsMi2 transposase-producing fusion. The *A10.2* chromosome, because of the *M67*-associated lethality, is kept in heterozygous condition over a 3rd chromosome balancer in a *w* background. *A10.2* flies show marked eye color mosaicism in the form of white or darker patches of ommatidia (Fig. 5). These patches correspond to clones of cells derived from single precursors in which the transposon has undergone excision or transposition. All the other lines, which contain just pMiw1 insertions but do not express transposase, show no mosaicism. The frequency of eye mosaicism in the *A10.2* line is strongly dependent on temperature. Flies raised at 25°C are 2.3% mosaic (55 of 2365); mosaicism increases to >35% (146 of 409 flies) if the flies are exposed to 37°C for 1 hr daily during larval development. Instability in the germ line of *A10.2* was also observed. At 18°C, 28 of 30,717 flies screened (0.09%) carried excisions of the transposon. The frequency of excisions was higher at 25°C (11 of 4626 or 0.24%). The *A10.1* chromosome was completely stable in the absence of *M67*. These results show that the integrated *Minos* transposon retains its capacity to be mobilized by transposase in somatic and germ-line cells. In addition, the observed stability of the transposon in the absence of transposase suggests that there is no interaction between *Minos* ends and other elements of the *Tc1* family that have been described in *D. melanogaster* (25).

Concluding Remarks. Together with *P, hobo*, and *mariner, Minos* is a fourth mobile element that can be used as a transformation vector in *D. melanogaster*. More importantly, *Minos* transposons may be suitable for development of germline transformation in other insect species where *P* does not work.

We thank Sophia Zabalou for expert help with the *in situ* hybridizations, Alexandros Babaratsas for technical assistance, and Nectaria Kelaidis for secretarial assistance. This work was supported by European Community Mediterranean Integrated Program Funds to the Institute of Molecular Biology and Biotechnology, by a grant of the John D. and Catherine T. McArthur Foundation, and by funds from the Greek Secretariat for Research and Technology. B.A. was supported by a European Union Training Fellowship.

1. Rubin, G. M. & Spradling, A. C. (1982) *Science* **218,** 348–353.
2. Yannopoulos, G., Stamatis, N., Monastirioti, M., Hatzopoulos, P. & Louis, C. (1987) *Cell* **49,** 487–495.
3. Blackman, R. K., Grimalia, R., Koehler, M. M. D. & Gelbart, W. M. (1987) *Cell* **49,** 497–505.
4. Blackman, R. K., Koehler, M. M. D., Grimalia, R. & Gelbart, W. M. (1989) *EMBO J.* **8,** 211–217.
5. Garza, D., Medhora, H., Koga, A. & Hartl, L. D. (1991) *Genetics* **128,** 303–310.
6. Medhora, M., Maruyama, K. & Hartl, L. D. (1991) *Genetics* **128,** 311–318.
7. Brennan, M. D., Rowan, R. G. & Dickinson, W. J. (1984) *Cell* **38,** 147–151.
8. Engels, W. R. (1989) in *Mobile DNA*, eds. Berg, D. E. & Howe, M. (Am. Soc. Microbiol., Washington, DC), pp. 437–484.
9. Fedoroff, N. Y. (1989) in *Mobile DNA*, eds. Berg, D. E. & Howe, M. (Am. Soc. Microbiol., Washington, DC), pp. 375–411.
10. Finnegan, D. J. (1989) in *Mobile DNA*, eds. Berg, D. E. & Howe, M. (Am. Soc. Microbiol., Washington, DC), pp. 503–517.
11. Blackman, R. K. & Gelbart, M. W. (1989) in *Mobile DNA*, eds. Berg, D. E. & Howe, M. (Am. Soc. Microbiol., Washington, DC), pp. 523–529.
12. Robertson, H. M. & MacLeod, E. G. (1993) *Insect Mol. Biol.* **2,** 125–139.
13. Robertson, H. M. (1993) *Nature (London)* **362,** 241–245.
14. Franz, G. & Savakis, C. (1991) *Nucleic Acids Res.* **19,** 6646.
15. Rosenzweig, B., Liao, L. W. & Hirsh, D. (1983) *Nucleic Acids Res.* **11,** 4201–4209.
16. Franz, G., Loukeris, G. T., Dialektaki, G., Thomson, R. L. C. & Savakis, C. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4746–4750.
17. Ingolia, T. D., Craig, E. A. & McGarthy, B. J. (1980) *Cell* **21,** 669–679.
18. Seifert, H., Chen, E. V., So, M. & Heffron, F. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 735–739.
19. Pirrotta, V. (1988) in *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, eds. Rodriguez, R. L. & Denhardt, D. T. (Butterworth, Boston), pp. 437–456.
20. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
21. Steller, H. & Pirrotta, V. (1984) *EMBO J.* **3,** 165–173.
22. Steller, H. & Pirrotta, V. (1985) *EMBO J.* **4,** 167–171.
23. Pirrotta, V., Steller, H. & Bozzetti, M. P. (1985) *EMBO J.* **4,** 3501–3508.
24. Spradling, A. G. & Rubin, G. M. (1983) *Cell* **34,** 47–57.
25. Caizzi, R., Caggese, G. & Pimpinelli, S. (1993) *Genetics* **133,** 335–345.