Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 4746–4750, May 1994
Genetics

Mobile *Minos* elements from *Drosophila hydei* encode a two-exon transposase with similarity to the paired DNA-binding domain

GERALD FRANZ*†, THANASIS G. LOUKERIS*, GEORGIA DIALEKTAKI*, CHRISTOPHER R. L. THOMPSON*‡, AND CHARALAMBOS SAVAKIS*§¶

*Institute of Molecular Biology and Biotechnology, Research Center of Crete, Foundation for Research and Technology Hellas, P.O. Box 1527, Heraklion 711 10, Crete, Greece; and §Department of Medical Sciences, University of Crete Medical School, Heraklion, Crete, Greece

Communicated by Fotis C. Kafatos, December 23, 1993 (received for review November 18, 1993)

ABSTRACT **Elements related to the Tc1-like *Minos* mobile element have been cloned from *Drosophila hydei* and sequenced. Southern blot and sequence analyses show that (*i*) the elements are actively transposing in the *Drosophila hydei* germ line, (*ii*) they are characterized by a striking degree of sequence and size homogeneity, and (*iii*) like Tc1, they insert at a TA dinucleotide that is probably duplicated during the process. The nucleotide sequences of two elements, *Minos-2* and *Minos-3*, differ at only one position from each other and contain two nonoverlapping open reading frames that are separated by a putative 60-nucleotide intron. The amino-terminal part of the *Minos* putative transposase shows sequence similarity to the paired DNA-binding domain. Forced transcription of a modified *Minos* element that was introduced into the *Drosophila melanogaster* germ line by *P* element-mediated transformation resulted in the production of accurately spliced polyadenylylated RNA molecules. It is proposed that *Minos-2* and/or *Minos-3* may encode an active transposase containing an amino-terminal DNA-binding domain that is distantly related to the paired DNA-binding domain.**

Mobile elements belonging to the Tc1-like family have been identified so far in nematodes, insects, and fish (1–7). They are all characterized by a relatively small length (1.6–1.8 kb), the presence of inverted terminal repeats of various sizes and sequences, and significant sequence similarities in the region between the repeats, which corresponds to the gene encoding transposase.

A common characteristic of elements capable of transposing autonomously is the presence of a gene encoding transposase, a protein directly involved in the transposition. With the exception of Tc1, TCb1, and Bari-1, the elements of the family that have been sequenced so far do not encode active transposases, having accumulated nonsense and frameshift mutations in their putative transposase genes. Tc1, a 1611-bp element with 54-bp perfect inverted terminal repeats, contains a gene, *Tc1A*, encoding a transposase that binds specifically at the inverted repeats at the ends of the element and induces transposition of endogenous Tc1 elements when overexpressed in *Caenorhabditis elegans* (8). The Tc1 element exhibits a high degree of size and sequence homogeneity, in contrast to other eukaryotic transposons (9–11) that are heterogeneous in size. Another characteristic of Tc1 is that it always inserts into a TA sequence, possibly creating a duplication of the dinucleotide in the process (12, 13).

Minos has been identified as a dispersed repetitive sequence inserted within the transcribed spacer in one of the repeats of the rDNA locus of *Drosophila hydei* (6). The element is characterized by 255-bp perfect inverted terminal repeats and the presence of two long nonoverlapping open reading frames (ORFs) on the same strand; the longest of the ORFs (ORF2) shows ≈30% sequence identity with *Tc1A* but does not begin with an ATG codon (6). It appears, therefore, that the cloned element represents a defective member of the *Minos* family, as is the case with most Tc1-like elements that have been characterized so far.

To understand the structure and function of the *Minos* family and to identify putative nondefective elements, we have used the cloned element to isolate and characterize other members of the family. Two complete *Minos* elements have been isolated and their nucleotide sequences‖ suggest that they may encode active transposase in two exons. Moreover, we show that forced transcription of one of these elements in *Drosophila melanogaster* is followed by correct splicing of a predicted 60-bp intron.

MATERIALS AND METHODS

Fly Strains and Germ-Line Transformation. All *D. hydei* strains used in this study have been used previously for rDNA work and are named for the X and Y chromosomes. Strain bb^1 ($bb^1/bb^1 \times bb^1/Y$) carries a bobbed X chromosome; strain X^7 ($X^7/X^7 \times X^7/Y$) is a subline of the Düsseldorf wild-type strain; strain $X^{\wedge}X/Y$ ($X^{\wedge}X/Y \times X/Y$) females carry a compound X chromosome that has no rDNA (14). Strain *wml*/Y (*wml*/Y × X-3/Y) females have a compound X chromosome (*wml*); males carry a X–autosome 3 translocation that has no rDNA (15). All strains were a gift from O. Hess (University of Düsseldorf). *P* element-mediated germ-line transformation was performed essentially as described (16).

DNA and RNA Manipulations and Sequencing. All general recombinant DNA procedures were carried out as described (17). DNA from adult females of strain bb^1 was partially digested with *Eco*RI and cloned into phage vector λgt7. To recover additional *Minos* elements, the library was screened by hybridization with a 1.7-kb *Hha* I fragment that contains most of the *Minos* sequence (see Fig. 1). For sequencing, the appropriate restriction fragments from positive clones were subcloned into plasmid vectors and nested deletions were generated by digestion with exonuclease BAL-31 followed by subcloning. Plasmid pDM30hsMi was constructed by replacing the left-hand inverted repeat of *Minos* (upstream from the unique *Hin*dIII site) with a fragment containing nucleotides −150 to +207 of the *D. melanogaster hsp70* gene (18). For PCR amplification of *Minos* cDNA sequences, transformed flies were subjected to a heat shock (1 h at 37°C), total RNA was reverse-transcribed, and PCR was performed using

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.

Abbreviation: ORF, open reading frame.

†Present address: Entomology Unit, International Atomic Energy Agency Laboratories, A-244 Seibersdorf, Austria.
‡Present address: Department of Anatomy, University of Cambridge, Cambridge, U.K.
¶To whom reprint requests should be addressed.

‖The sequences reported in this paper have been deposited in the GenBank data base (accession nos. Z29098 and Z29102).

primers MBalI (5′-AAAGACAACTGGCCAAAATTG) and MEcoRI (5′-CTGCTGAAAAGTGAATTCACC).

Sequence Analysis. Nucleotide and protein sequence analyses were performed using program BLAST (19) and the computer package GCG (20).

RESULTS AND DISCUSSION

The Sequence of *Minos*. We have cloned and sequenced two additional complete representatives of *Minos*; they have been named *Minos-2* and *Minos-3*, *Minos-1* being the element reported previously (6). *Minos-2* and *Minos-3* are complete elements distinct from *Minos-1*, as judged from the restriction maps of the flanking DNA (data not shown) and the flanking sequences (see below). The sequences of the elements show very little variation, differing from each other and from *Minos-1* in only two nucleotide positions. Fig. 1 shows part of the consensus sequence and a restriction map of the element. At position 895 of the sequence, *Minos-2* and *Minos-3* have a guanosine instead of the adenosine found in *Minos-1*. This transition changes a TAG stop codon to TGG and restores a 603-bp ORF beginning with ATG at position 873 (Fig. 1 *Upper*). The second difference is at nucleotide 1157, which is a cytidine in *Minos-1* and *Minos-3* and a thymidine in *Minos-2* (data not shown); this causes a Ser → Leu substitution in ORF2 of *Minos-2*, relative to *Minos-1* and *Minos-3*. *Minos-2* and -3, therefore, have two complete ORFs beginning with an ATG: ORF1, which can encode a 138-amino acid peptide, and ORF2, which can encode a 201-amino acid peptide. A fourth member, named *Minos-4*, was also cloned. The clone does not contain a complete element; it begins at the *Eco*RI site found at position 1168 of the other members and is identical to the *Minos-1* sequence between positions 1168 and 1773, presumably representing a partial isolate of another member (see below).

The DNA sequences flanking the cloned elements are different from each other; this shows that these elements are inserted at different sites of the *D. hydei* genome and are, therefore, distinct. These sequences, shown in Fig. 2, are mainly characterized by a high A+T content and do not show any other obvious similarity. In all cases the inverted repeats end with the dinucleotide TA, which is at the same time a direct and an inverted repeat. Because of this, there is some ambiguity in defining the ends of the element precisely. For *Minos-1*, which is inserted into a region that has been previously sequenced, the external transcribed spacer of the rDNA repeat (6), there are two possibilities. As shown in Fig. 2, deleting the sequence that begins with ACGA and ends with TCGT would restore the rDNA sequence; the element,

```
rDNA        tgtgtgatataat---------------attaagttaagcg
Minos-1     tgtgtgatataataCGAG....CTCGtattaagttaagcg
Minos-2     taatatataatataCGAG....CTCGtataatatataata
Minos-3     tatagttagctttaCGAG....CTCGtagaagctgctaac
Minos-4     tttttttttttctaCGAG..
                         |         |
                       1773        1
```

FIG. 2. Sequences of *Minos* insertion sites. The sequences flanking the elements *Minos-1* to *Minos-4* are shown in lowercase type; *Minos* sequences are in uppercase type. The rDNA sequence identical to the flanking DNA of *Minos-1* has been aligned with the *Minos-1* insertion sequence. The rDNA sequence begins at nucleotide 4245 of the published sequence (ref. 21; GenBank accession no. M29802).

with an adenosine and a thymidine at the two ends may have inserted between a thymidine and an adenosine. In this possibility, the element would be 1775 bp long with 255-bp inverted repeats. Alternatively, the element may begin and end with CGA . . . . TCG and produce a target-site duplication, as happens with many other mobile elements (9, 22–26). In this possibility, the target-site duplication would involve the dinucleotide TA, and the size of the element would be 1773 bp. We propose the second possibility and consider the cytidine after the putative TA repeat as nucleotide 1 of the *Minos* sequence. The terminal repeats of *Minos* show no other obvious similarity with Tc1 or any other Tc1-like element, except for the hexanucleotide CAGTGC found at or near the ends of the terminal repeats in several Tc1-like elements (27) and the presumed TA target duplication.

Mobility and Homogeneity of *Minos* Elements. Southern blot analysis, shown in Fig. 3*A*, clearly indicates that *Minos* elements are inserted in different sites in the *D. hydei* genome. The differences in the number of bands and the banding patterns observed between males and females in the two strains carrying attached X chromosomes (strains X^X/Y and *wml*/Y) also show that several of the *Minos* elements are inserted in the sex chromosomes.

The high degree of sequence conservation among *Minos* elements that were cloned and sequenced suggested that, as for Tc1, the *Minos* family may be highly homogeneous in size as well. To test this we took advantage of the single *Hha* I site within each of the terminal repeats of *Minos*. The 1.68-kb *Hha* I fragment of *Minos-1* was used as probe on a blot of genomic DNA from the same strains used for the blot of Fig. 3*A* that were digested with *Cfo* I, an isoschizomer of *Hha* I (Fig. 3*B*). A single strong band of ≈1.7 kb was detectable in all lanes, showing that no major deletions or rearrangements are present in the *Minos* elements in these strains.

The apparent size and sequence homogeneity of the *Minos* elements in the *D. hydei* genome is a characteristic shared

```
720 AAAAGTATTGGATATGGTTTTTATAAAgtatgtTTTGTTATTACCTGTGCATCGTACCCAataacTTACTCGTAATCttactcgtagGCCAAGGAAAAACCCTTGCTTACGCTTCGTCAA 839
130 K  S  I  G  Y  G  F  Y  K                                                       A  K  E  K  P  L  L  T  L  R  Q  149

                                                                  A
840 AAAAAGAAGCGTTTGCAATGGGCTCGGGAAAGGATGTCTTGGACTCAAAGGCAATGGGATACCATCATATTCAGCGATGAAGCTAAATTTGATGTTAGTGTCGGCGATACGAGAAAACGC 959
150 K  K  K  R  L  Q  W  A  R  E  R  M  S  W  T  Q  R  Q  W  D  T  I  I  F  S  D  E  A  K  F  D  V  S  V  G  D  T  R  K  R  189
                                                        *
```

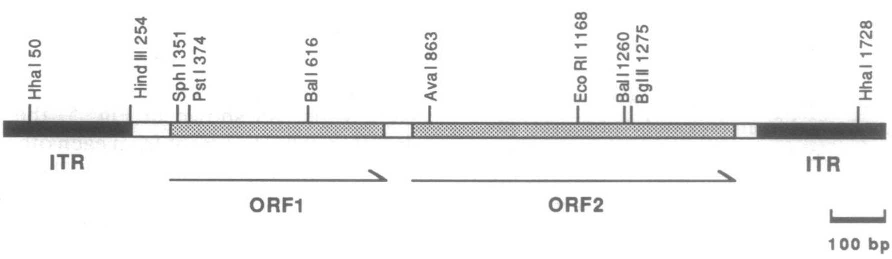

FIG. 1. Structure of *Minos*. (*Upper*) Partial nucleotide and deduced amino acid sequence of *Minos-2*. The difference between *Minos-1* and the other members at position 896 is shown. Putative splice signal sequences are shown in lowercase type. (*Lower*) Restriction map of *Minos*. Solid and stippled boxes indicate the terminal repeats (ITR) and the two ORFs, respectively.

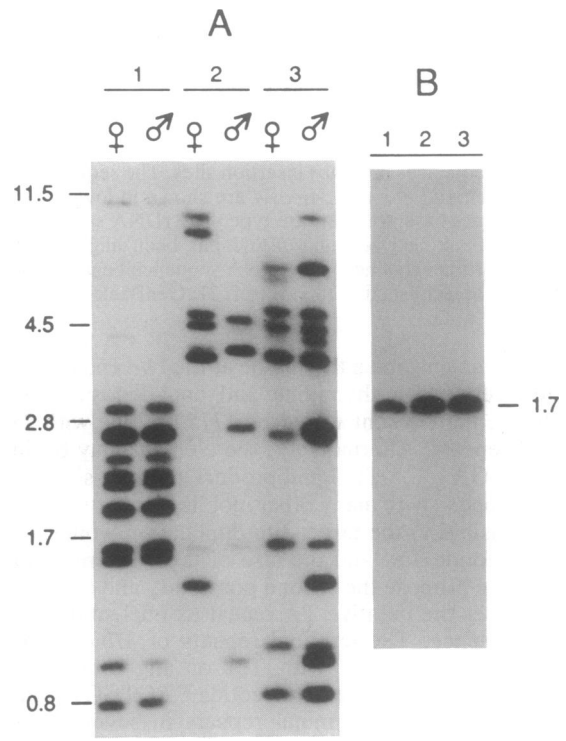

FIG. 3. Southern blot hybridizations of DNA from three *D. hydei* strains with *Minos* probes. Genomic DNA was hybridized with the 1.7-kb *Hha* I fragment of *Minos-1*. Lanes: 1, strain X^7; 2, strain $X^{\wedge}X/Y$; 3, strain *wm1*/Y. Size markers are in kilobases. (*A*) DNA was isolated separately from females and males of each strain and digested with *Eco*RI prior to electrophoresis. (*B*) DNAs from males and females of each strain were mixed and then digested with *Cfo* I, an isoschizomer of *Hha* I.

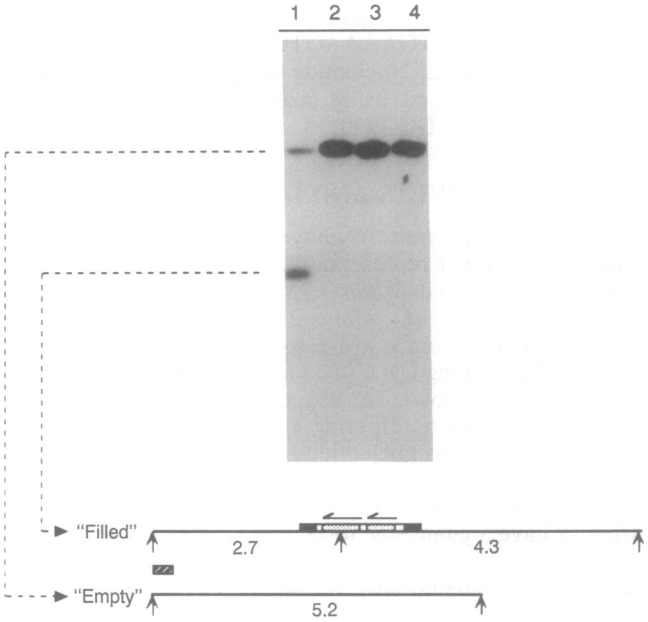

FIG. 4. *Minos-2* is inserted at a unique site in strain bb^1 but not in other strains. (*Upper*) Genomic DNAs from strains bb^1 (lane 1), X^7 (lane 2), $X^{\wedge}X/Y$ (lane 3), and *wm1*/Y (lane 4) were digested with *Eco*RI and probed with a 0.3-kb *Eco*RI–*Pst* I fragment of the unique DNA flanking *Minos-2*. (*Lower*) The restriction maps of the "filled" and "empty" regions are shown. Arrows indicate *Eco*RI sites; the probe used is indicated by a stippled box.

with other members of the Tc1 family. This homogeneity is in contrast to the marked length heterogeneity of other transposons with inverted terminal repeats (9–11). It has been proposed that the deletions observed in these elements are caused by the process of chromatid repair that follows excision events. For the *P* element, there is strong evidence that after the transposase-induced excision of the element, a double-strand break is left at the point of insertion that is closed by the host cell by a gap repair mechanism using the homologous chromatid as template. This usually leads to regeneration of the complete element if the homologous chromatid also carries the insertion but can lead to internal deletions of the element when the repair is incomplete (28). The absence of deletions of this type in *Minos* (and other members of the Tc1 family) suggests that excision of these elements may be accomplished by a mechanism not involving gap repair.

A more direct test for mobility of *Minos* elements was performed by examining a site of an insertion in different strains. Cloned *D. hydei* DNA flanking the right-hand repeat of *Minos-2* was used as probe on a blot containing genomic DNA from four strains. As shown in Fig. 4, two bands are detectable in strain bb^1 from which *Minos-2* was cloned: the expected 2.7-kb band from the chromosome with the insertion and a 5.2-kb band expected from an empty site, presumably from the homologous chromosome. The other three strains examined show only the 5.2-kb band. These results show that strain bb^1 is heterozygous for the insertion of *Minos-2*, whereas the other three strains do not contain an insertion at this site.

Definitive evidence that an element can transpose in the germ line can only be obtained from studying spontaneous mutants. The Southern blot analysis results, however, strongly suggest that *Minos* is mobile in the *D. hydei* genome. All the strains examined show differences in banding patterns characteristic of a mobile element, with copy numbers from 5 to 30.

***Minos* May Encode an Active Transposase in Two Exons.** The deduced 201-amino acid sequence of the ORF2 in *Minos-2* and *Minos-3* shows significant sequence similarity with the 201 carboxyl-terminal residues of TcA, the major ORF of Tc1; alignment of the sequences gives 63 identities (31%) and 91 conservative substitutions (45%) with only two single-residue insertion–deletions (6). The two sequences, however, differ in size; TcA has 72 additional amino acids at the amino end. Comparison of the 50 amino-terminal residues of TcA with ORF1 of *Minos* showed weak but significant sequence similarity, which could result in an alignment without any gaps if a 60-bp deletion was introduced in the *Minos* DNA sequence. This deletion can create a long ORF containing most of ORF1 (codons 1–138) and the entire ORF2 extended by 22 codons upstream of the ATG. Interestingly, this 60-bp sequence, from base 746 to base 807 of the *Minos* sequence, exhibits features of an intron (Fig. 1). (*i*) The 5′ and 3′ ends conform to the consensus splice donor and acceptor sites, GTYAGT and YNYYYYNYAG, respectively (29, 30). (*ii*) A version of the internal splice signal consensus YTRAY (31) is found 30 nucleotides upstream from the 3′ end.

The 361-amino acid hypothetical protein encoded by a spliced transcript can be aligned with the Tc1 and Bari-1 transposase sequences for maximum similarity without any major gaps. As shown in Fig. 5, the three sequences are almost equally divergent from each other, being slightly more conserved at the carboxyl end. The main difference of the *Minos* sequence is at the amino terminus, where it has 18 additional amino acid residues. The three Tc1-like elements that can encode a full transposase also differ from each other in respect to the intron. The intron recently characterized in Tc1 (8) is found at a position 68 codons upstream from that of the *Minos* intron, while the Bari-1 transposase gene is

```
Minos  LATMVRGKPISKEIRVLIRDYFKSGKTLTE
Bari1  ...MPKTKELTVEARAGIVARFKAGTPAAK
Tc1    MVKSVGCKNLSLDVKKAIVAGFEQGIPTKS

Minos  ISKQLNLPKSSVHGVIQIFKKNGNIENNIA
Bari1  IAEIYQISRRTVYYLIKKFDTVGTLKNK.K
Tc1    LALQIQRSPSTIWKVIKKYQTEKSVALRIS

Minos  NRGRTSAITPRDKRQLAKIVKADRRQSLRN
Bari1  RSGRKPVLDQRQCRQILGVVAKNPSASPVK
Tc1    .PGRPRVTTHRMDRNILRSAREDPHRTATD

Minos  LASKWSQTIGKTVKREWTRQQLKSIGYGFY
Bari1  IALESKNTIGKQVSSSTIRRRLKEADFKTY
Tc1    IQMIISSPNEPVPSKRTVRRRLQQAGLHGR

Minos  KAKEKPLLTLRQKKKRLQWARERMSWTQRQ
Bari1  VVRKTIEITPTNKTKRLRFALEYVKKPLDF
Tc1    KPVKKPFISKKNRMARVAWAKAHLRWGRQE

Minos  WDTIIFSDEAKFDVSVGDTRKRVIRKRSET
Bari1  WFNILWTDESAFQYQ.GSYSKHFMHLKNNQ
Tc1    WAKHIWSDESKFNLFGSDGNSWVRRPVGSR

Minos  YHKDCLKRTTKFPASTMVWGCMSAKGLGK
Bari1  KHLAA.QPTNRFGGGTVMFWGCLSYYGFGD
Tc1    YSPKYQCPTVKHGGGSVMVWGCFTSTSMGP

Minos  LHFIEGTVNAEKYINILQDSLLPSIPKLLD
Bari1  LVPIEGTLNQNGYLLILNNHAFTSGNRLFP
Tc1    LRRIQSIMDRFQYENIFETTMRPWALQNVG

Minos  CGEFTFQQDGASSHTAKRTKNWLQYNQMEV
Bari1  TTEWILQQDNAPCHKGRIPTKFLNDLNLAV
Tc1    RG.FVFQQDNDPKHTSLHVRSWFQRRHVHL

Minos  LDWPSNSPDLSPIENIWWLMKNQLRNEPQR
Bari1  LPWPPQSPDLNIIENVWAFIKNQRTIDKNR
Tc1    LDWPSQSPDLNPIEHLWEELERRLGGIRAS

Minos  NISDLKIKLQEMWDSISQEHCKNLLSSMPK
Bari1  KREGAIIEIAEIWSKLTIEFAQTLVRSIPK
Tc1    NADAKFNQLENAWKAIPMSVIHKLIDSMPR

Minos  RVKCVMQAKGDVTQF*
Bari1  RLQAVIDAKGGVTKY*
Tc1    RCQAVIDANGYATKY.
```

FIG. 5. Sequence alignment of the transposases of Tc1, Bari-1, and *Minos-2*. Alignment was performed using the program PILEUP of the GCG package (20). The *Minos* sequence starts at residue 19. Identical and related amino acids are in black and grey boxes, respectively. Solid triangles above the *Minos* sequence and below the Tc1 sequence indicate the positions of the introns.

intronless. The study of more transposons of the family may elucidate the origin of these introns.

The 60-bp Intron Is Spliced in *D. melanogaster*. *Minos* transcripts are not detectable in *D. hydei* embryos and adult flies by Northern blot or S1 protection analyses of polyadenylylated RNA (data not shown), presumably because of very low levels of transposase expression. To test for splicing of the predicted intron and expression of the *Minos* transposase, we constructed a modified *Minos* element in which the putative transposase gene is under heat-shock control (Fig. 6*A*) and introduced it into the *D. melanogaster* genome by *P* element-mediated transformation. One of the transformants (line M67) was used for the analysis shown in Fig. 6. Low levels of an ≈1.5-kb polyadenylylated RNA can be detected on a RNA blot with a probe containing the middle of the transposase gene; levels are reversibly increased severalfold after a heat shock (Fig. 6*B*). To determine directly whether

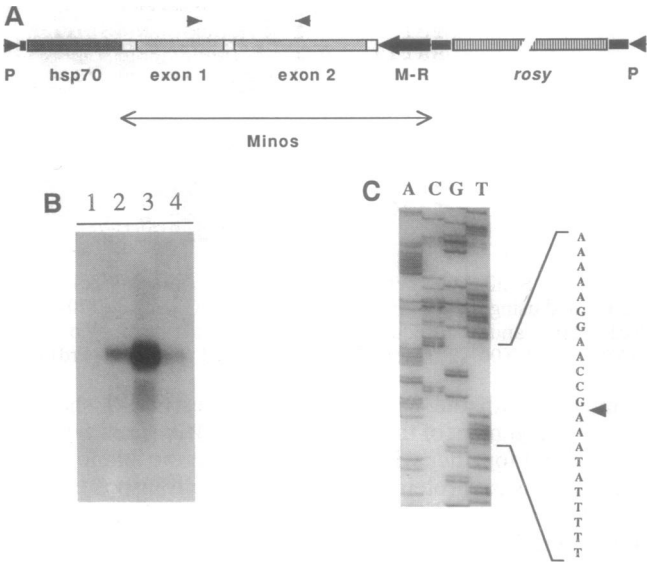

FIG. 6. Splicing of *Minos* mRNAs in *D. melanogaster*. (*A*) Schematic drawing of the pDM30hsMi expression plasmid. Only the part between the *P* element ends (P) is shown. M-R indicates the right-hand terminal repeat of *Minos*. The positions of the primers used for PCR are shown above the plasmid. (*B*) Northern blot analysis of poly(A)$^+$ RNA from transformed *D. melanogaster* flies carrying a single copy the pDM30hsMi insert. A PCR fragment amplified from the pDM30hsMi plasmid with the indicated primers was used as probe. Lanes: 1, nontransformed *cn;ry*^{506} flies (5 μg); 2, M67 transformant flies grown at 25°C (5 μg); 3, M67 flies treated for 30 min at 37°C (5 μg); 4, M67 flies treated for 30 min at 37°C then left for 30 min at 25°C (2 μg). (*C*) Partial sequence of amplified *Minos* cDNA from heat-shocked M67 flies. cDNA was synthesized from 5 μg of total RNA and used as template for PCR amplification with primers MBalI and MEcoRI (indicated in *A*). The amplified fragment was cloned into a Bluescript vector. The arrowhead indicates the position of the intron.

the *Minos* transcripts are spliced, we synthesized cDNA from heat-shocked flies and used it as a template for PCR amplification with two primers that flank the intron. The size of the PCR-amplified fragment, analyzed on an agarose gel, was shorter than that of a fragment amplified from the pDM30hsMi plasmid with the same primers (data not shown), suggesting that the majority of the transcripts were spliced. Precise splicing of the 60-bp intron was verified by cloning and sequencing the amplified cDNA (Fig. 6*C*).

Similarities Between the Transposase and the Paired Domain. Searches of the sequence databases showed a weak similarity between the amino terminus of the *Minos* transposase and the paired box sequence, a highly conservative DNA-binding protein domain found at the amino-terminal end of the *Drosophila* paired (*prd*) gene product and other *Drosophila* and mammalian genes involved in embryonic development (for review, see ref. 32). The similarity extends approximately between residues 19 and 115 of the *Minos* sequence and between residues 35 and 131 of the *D. melanogaster prd* protein and consists of 16 identities (17%) and 49 positions occupied by related amino acids (51%) with only a 1-residue gap for optimum alignment. The corresponding values for the human and *Drosophila* paired domains are ≈72% identical and 23% conserved positions. Although the *Minos*–paired similarity is comparatively weak, it is statistically significant. The similarity score between the two sequences is 6 SDs greater than the average of the similarity scores obtained from 50 comparisons made between the *Minos* sequence and 50 randomly shuffled *prd* sequences. Similar values were obtained when the corresponding amino-terminal sequences of the Tc1 and Bari-1 transposases were

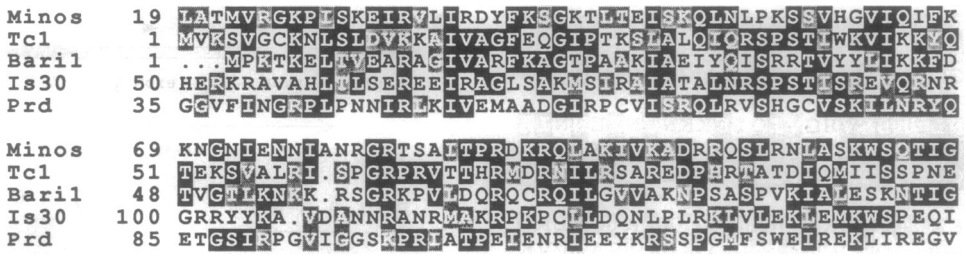

FIG. 7. Similarities between the amino terminus of Tc1-related transposases, IS*30* transposase, and the paired domain. Alignment was performed using the program PILEUP of the GCG package (20). Identical and related amino acids are in black and grey boxes, respectively. The Tc1, Bari-1, and IS*30* sequences are derived from the nucleotide sequences, European Moleclular Biology Laboratory accession nos. X01005, X67681, and X00792, respectively. The paired sequence (Prd) is from the Swiss-Prot entry (accession no. P006601).

compared with the paired box sequence. Fig. 7 shows a multiple alignment of the four sequences (*Minos*, Bari-1, Tc1, and paired) together with a sequence from the amino end of the transposase protein of the prokaryotic transposon IS*30*. This region of the IS*30* transposase shows similarity with the amino-terminal end of the Tc1 transposase (8) and corresponds to a fragment that is sufficient for specific binding to the terminal repeats of IS*30* (33). It has also been shown that a site-specific DNA-binding domain is contained within the first 63 residues of the Tc1 transposase (8). It should be noted that all the aligned sequences are relatively rich in amino acids with basic side chains, showing computed isoelectric points between pH 10 and 12. The position of these amino acids is not conserved, however; most of the identities/similarities between the sequences involve hydrophobic amino acids. We propose that the observed sequence similarity characterizes an underlying common DNA-binding domain. It is an open question whether this domain has a single evolutionary origin rather than being the result of convergent evolution.

In conclusion, we present strong evidence that members of the *Minos* family are actively transposing in the germ line of *D. hydei*, and we have cloned two members that have characteristics of nondefective elements encoding active transposase. We also show that a gene encoding the putative transposase can be transcribed and correctly spliced in *D. melanogaster*. Further experimentation is required to determine whether active transposase is expressed in *D. melanogaster*. The powerful genetic and molecular genetic tools available in this organism combined with the absence of endogenous *Minos* elements make it very attractive for studying the mechanism of transposition of the *Minos* element.

We thank O. Hess for *D. hydei* strains. This work was supported by European Community Mediterranean Integrated Program funds to the Institute for Molecular Biology and Biotechnology and by a European Community training fellowship to G.F.

1. Emmons, S. W., Yesner, L., Ruan, K. & Katzenberg, D. (1983) *Cell* **32**, 55–65.
2. Harris, L. J., Baillie, D. L. & Rose, A. M. (1988) *Nucleic Acids Res.* **16**, 5991–5998.
3. Brierley, H. L. & Potter, S. S. (1985) *Nucleic Acids Res.* **13**, 485–500.
4. Caizzi, R., Caggese, C. & Pimpinelli, S. (1993) *Genetics* **133**, 335–345.
5. Brezinsky, L., Wang, G. V. L., Humphreys, T. & Hunt, J. (1990) *Nucleic Acids Res.* **18**, 2053–2059.
6. Franz, G. & Savakis, C. (1991) *Nucleic Acids Res.* **19**, 6646.
7. Heierhorst, J., Lederis, K. & Dietmar, R. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 6798–6802.
8. Vos, J. C., van Luenen, H. G. A. M. & Plasterk, R. H. A. (1993) *Genes Dev.* **7**, 1244–1253.
9. O'Hare, K. & Rubin, G. M. (1983) *Cell* **34**, 25–35.
10. Blackman, R. K. & Gelbart, W. M. (1989) in *Mobile DNA*, eds. Berg, D. E. & Howe, M. M. (Am. Soc. Microbiol., Washington, DC), pp. 523–529.
11. Pohlman, R. F., Fedoroff, N. V. & Messing, J. (1984) *Cell* **37**, 635–643.
12. Eide, D. & Anderson, P. (1988) *Mol. Cell. Biol.* **8**, 737–746.
13. Mori, I., Benian, G. M., Moerman, D. G. & Waterston, R. H. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 861–864.
14. Franz, G., Kunz, W. & Grimm, C. (1983) *Mol. Gen. Genet.* **191**, 74–80.
15. Beck, H. (1976) *Genet. Res.* **26**, 313–317.
16. Rubin, G. M. & Spradling, A. C. (1982) *Science* **218**, 348–353.
17. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
18. Ingolia, T. D., Craig, E. A. & McCarthy, B. J. (1980) *Cell* **21**, 669–679.
19. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) *J. Mol. Biol.* **215**, 403–410.
20. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12**, 387–395.
21. Tautz, D., Tautz, C., Webb, D. & Dover, G. A. (1987) *J. Mol. Biol.* **195**, 525–542.
22. Grindley, N. D. F. (1978) *Cell* **13**, 419–426.
23. Engler, J. A. & van Bree, M. P. (1981) *Gene* **14**, 155–163.
24. Halling, S. & Kleckner, N. (1982) *Cell* **28**, 155–163.
25. Streck, R. D., MacGaffey, J. E. & Beckendorf, S. K. (1986) *EMBO J.* **5**, 3615–3623.
26. Rosenzweig, B., Liao, L. W. & Hirsh, D. (1983) *Nucleic Acids Res.* **11**, 7137–7140.
27. Henikoff, S. (1992) *New Biologist* **4**, 382–388.
28. Engels, W. R., Johnson-Schlitz, D. M., Eggleston, W. B. & Sved, J. (1990) *Cell* **62**, 515–526.
29. Mount, S. M. (1982) *Nucleic Acids Res.* **10**, 459–472.
30. Teem, J., Abovich, N. A., Kaufer, N. F., Schwindinger, W. F., Warner, J. R., Levy, A., Woolford, J., Leer, R. J., van Raamsdonk-Duin, M. M. C., Mager, W. H., Planta, R. J., Schultz, L., Friezen, J. D., Fried, H. & Rosbash, M. (1984) *Nucleic Acids Res.* **12**, 8295–8312.
31. Keller, E. & Noon, W. A. (1985) *Nucleic Acids Res.* **13**, 4971–4981.
32. Gruss, P. & Walther, C. (1992) *Cell* **69**, 719–722.
33. Stalder, R., Caspers, P., Olasz, F. & Werner, A. (1990) *J. Biol. Chem.* **265**, 3757–3762.