Structural organization of transposable element mdg4 from *Drosophila melanogaster* and a nucleotide sequence of its long terminal repeats

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ABSTRACT

A mobile dispersed genetic element, mdg4, ~ 7.5 kilobases (kb) long has been cloned from D. melanogaster genome. Chromosomal bands have only few sites of mdg4, but it always hybridizes to the chromocenter. The location of mdg4 varies among D. melanogaster strains. Blot hybridization shows that, in contrast to other mdg elements, mdg4 sequences are rather heterogeneous. Only few copies are full-length. A strong amplification of mdg4 has occurred during the in vitro cultivation of cells involving only one mdg4 variant.

Long terminal repeats (LTRs) and flanking sequences have been sequenced in two cloned copies of transposable element mdg4. In both cloned copies of mdg4, LTRs have an identical nucleotide sequence 479 bp long. The mdg4 is flanked by four-base-pair direct repeats, short mismatched palindromes being present at the ends of each LTR. The termini of the mdg4 body contain an oligopurine stretch and a region partially complementary to D. melanogaster $tRNA_1^{Lys}$. Thus, structural organization of mdg4 LTRs is similar to that of several other mdg elements and retroviral proviruses.

INTRODUCTION

Mobile dispersed genetic elements (mdg) or copia-like elements were first cloned from D. melanogaster genome /1, 2/. Some of their families are well studied /3-6/. These elements have certain properties in common, i.e. a multiple copy number, scattered positions throughout the genome, and a varying localization /2, 7, 8/. The nucleotide sequences of different members from the same family are usually conservative. At least in culture cells, the mdg elements are actively transcribed /9/. This seems to be connected with the mdg amplification /3-5, 10/. All mdg elements are framed with long terminal repeats which may play a key role in mdg transposition and transcription /1, 3-6/.

The presented paper describes mdg4, a novel mdg element of D. melanogaster. It was first cloned in experiments on isolation of DNA sequences hybridizing to double-stranded RNA, and designated as Dm11. In contrast to other mdgs, Dm11 hybridized to few sites on chromosomes. It was heavily amplified in cell culture /4, 9/.

The interest to mdg4 is stimulated by the following observations. Gerasimova describes a large family of unstable cut mutations. These mutations appeared to be induced by an mdg4 insertion /11/. At the same time, Modolell et al. /12/ independently cloned the same sequence, named it "gypsy", and showed that mutations induced by its insertion could be suppressed by the Hairy wing suppressor. Finally, Leigh Brown has presented the data on the responsibility of mdg4 for some natural mutations in a heat-shock gene /13/.

The structural organization of mdg4 and the nucleotide sequence of its LTRs and their flanking regions are described below.

MATERIALS AND METHODS

Isotopes and enzymes. [^{32}P]Deoxyribonucleosidetriphosphates were purchased from the Radiochemical Centre (Amersham, England) and Glav-Izotop (Moscow, USSR). [^{3}H]Deoxyribonucleosidetriphosphates and [^{3}H]uridine were from Glav-Izotop. [^{125}I]dCTP was synthesized according to Shaw et al. /14/. Restriction endonucleases were obtained from Dr. A.Yanulaitis, and DNA polymerase I from Dr. R.Beabealashvili.

The DNA cloning. Full copies of mdg4 were obtained by constructing recombinant plasmids which contained BamHI-restricted DNA from D. melanogaster culture cells (67J25D) and pBR322. For colony hybridization, ^{32}P-labeled fragments of Dm11 /4/ were used.

Nucleic acid preparations. Plasmid DNAs were isolated by the alkaline method /15/. DNAs from D. melanogaster culture cells, embryos or adult flies were prepared by the phenol-detergent method. Culture cells were collected by centrifugation. The flies and embryos were frozen with liquid nitrogen and mechanicaly disintegrated. After suspending the material

in 0.15 M NaCl, 20 mM Tris-HCl, pH 8.0, 20 mM EDTA, 0.5% SDS, pronase was added to the final concentration of 200 μg/ml, and the mixture incubated with gentle stirring for 30 min. DNA was deproteinized by phenol, phenol-chloroform (1:1) and chloroform, and reprecipitated with ethanol.

DNA restriction fragments were obtained by elution from agarose gels using the freezing-thawing method /16/.

Hybridization. The DNA was labeled with ^{3}H, ^{32}P or ^{125}I by nick translation /17/. The in situ hybridization was performed as described earlier /7/. The DNA for blot hybridization was transferred to nitrocellulose filters according to Southern /18/. The filters were preincubated in the 2xSSC, 0.1% SDS, 2X Denhardt solution containing 20 μg/ml of salmon sperm DNA for 5 h at 65°C /19/. The hybridization was performed under the same conditions for 20 h. Then the filters were extensively washed with 0.1xSSC, 0.1% SDS at 45°C.

The sequencing. The determination of the nucleotide sequence was performed according to the Maxam and Gilbert technique /20/. For this, the DNA of plasmids p111 and p112 was restricted with either XhoI or BglII. After labeling of the 3'-termini with reverse transcription and additional transcription with *BamHI*, *PstI* and *EcoRI* endonucleases, the fragments were fractionated by 4% polyacrylamide gel electrophoresis. One-end labeled fragments were eluted and taken for the sequencing. In order to confirm the nucleotide sequences obtained in these experiments, we performed the sequencing from HpaII-sites localized inside the LTR.

RESULTS

1. General organization of mdg4

Fig. 1 shows the restriction maps of cloned DNA fragments Dm11, Dm111 and Dm112 containing mdg. The original clone was Dm11 which contained about half of mdg4. It was obtained by hybridization to double-stranded RNA of *D. melanogaster* culture cells /4, 9/. It was noticed that Dm11 did not contain BamHI sites and that Dm11 DNA effectively hybridized only to large BamHI fragments of genomic DNA. Therefore, we attempted to get a full-length copy of mdg4 from the BamHI library of

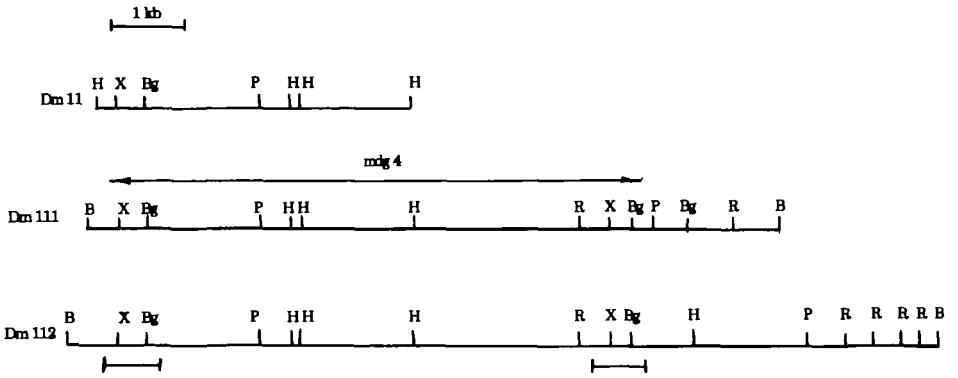

Fig. 1. Restriction maps of cloned fragments of D. melanogaster DNA containing mdg4.
B - BamHI; Bg - BglII; X - XhoI; P - PstI; H - HindIII; R - EcoRI; ⊢⊣ - long terminal repeats.

genomic DNA. Thus, Dm111 and Dm112 were obtained.

The restriction analysis and cross hybridization experiments with Southern blots of plasmid DNA show that a ca. 7.5 kb DNA segment is present in two different clones. It has exactly the same restriction sites in the two clones. Long direct repeats appear at the termini of the 7.5 kb segment. Their presence is proved by a similar arrangement of BglII and XhoI sites and cross hybridization between the corresponding restriction fragments. The size of long terminal repeats (LTRs) is estimated to be ~500 bp, which is also confirmed by sequencing.

2. The total number of mdg4 copies per genome and localization of this element on polytene chromosomes

The in situ hybridization of mdg4 to chromosome of different D. melanogaster strains shows the mobility of mdg4. All hybridization sites are quite different. The chromocenter is the only hybridization site in common for different strains. Table 1 presents the results of some of these experiments. In contrast to other mdg elements, mdg4 is located only at a few sites.

The in situ hybridization was also performed with different internal subfragments (1.7 kb HindIII, 2.2 kb HindIII-

Table 1. Sites of mdg localization in the polytene chromosomes of D. melanogaster salivary glands

Regions of chromosomes	gtw[a]	gt[13z]	XY[*]	Oregon RC[x]
2B	+	+	-	-
14D	-	-	+	-
16A	-	-	+	-
21D	+	-	-	-
41A	+	-	-	-
42B	-	+	-	-
44C	+	-	-	-
80	-	-	-	+
83C	-	-	-	+
chromocenter	+	+	+	+

*$Y^{S}In(1)EN$, $Bfvy \cdot Y^{L}(y^{+})$

xThere may exist two more hybridization sites normally nondistinguishable from the chromocenter but they are not the chromocenter in reality. This could be observed occasionally upon separation of chromosomes from the chromocenter.

EcoRI, and 2.7 kb HindIII-XhoI) of mdg4, the patterns obtained being indistinguishable from those of total mdg4. Hence, all the sites seemed to contain full-length mdg4 copies. The hybridization of mdg4 to the chromocenter puts several questions about the total number of mdg4 copies per genome. It is well known that the centromeric regions of chromosomes remain underreplicated in polytene chromosomes. Therefore, a rather high labeling of the chromocenter may indicate that several mdg4 copies are present there.

To answer the question, one should estimate the number of mdg4 copies in embryonic cells. We hybridized ^{32}P-labeled total cellular DNA to an excess of the XhoI fragment of mdg4 immobilized on a nitrocellulose filter. This fragment 7 kb long covers practically the whole mdg4. The conditions of hybridization are described elsewhere /21/. To prevent the binding of flanking sequences, the DNA was sheared to 150-200 nucleotide fragments. From Table 2 one can see that the number of mdg4 copies determined by this method is about 20 per haploid genome. Similar data were obtained when internal HindIII or HindIII-EcoRI fragments of mdg4 were immobilized on nitrocellulose filters. With other mdg elements (mdg1, mdg3), the copy number estimated by the saturation hybridization is

Table 2. The copy number of mdgs in D. melanogaster genomes

Gene	Size of DNA fragments	gtw[a]		Oregon RC		culture cells strain 67J25D		Number of hybridization sites on chromosomes	
		%	n	%	n	%	n	gtw[a]	Oregon
mdg4	7.0	0.083	19	0.084	19	0.74	170	5	3
mdg1	7.2	0.10	23	0.13	28	1.035	230	16	21
mdg3	5.2	0.055	17	0.065	20	0.65	200	11	13
ribosomal[x]	14.0	1.89	216	2.04	233	2.08	238	1	1

% - percent of DNA bound to the filter;
n - the copy number per gaploid genome;
[x]The clone containing ribosomal gene was a kind gift from Dr. A.A.Kolchinsky.

roughly the same as the number of the in situ hybridization sites (Table 2).

The anomalous behaviour of mdg4 may be explained by the fact that a lot of gene copies are located in the chromocenter which is known to be underreplicated. An alternative explanation suggesting a tandem repetition of mdg4 in each site cannot be excluded either but it does not seem probable. The third possibility is that, besides full-length copies of mdg4, some sequences homologous to different parts of mdg4 may be present in the genome. The centromeres may be enriched in such sequences (see below).

From Table 2 it is also clear that a ten-fold amplification of mdg4 occurred during cell cultivation in vitro.

3. The existence of genomic sequences homologous to different parts of mdg4

To study the arrangement of the mdg4 element in Drosophila genome, we prepared Southern filters containing genomic DNA restricted either with BglII or XhoI that cut mdg4 cloned copies within LTRs only.

The filters were hybridized to one of the following probes - 7.0 kb XhoI fragment, 2.2 kb EcoRI-HindIII fragment, or 1.7 kb HindIII fragment (Fig. 1). In fig.2 one can see

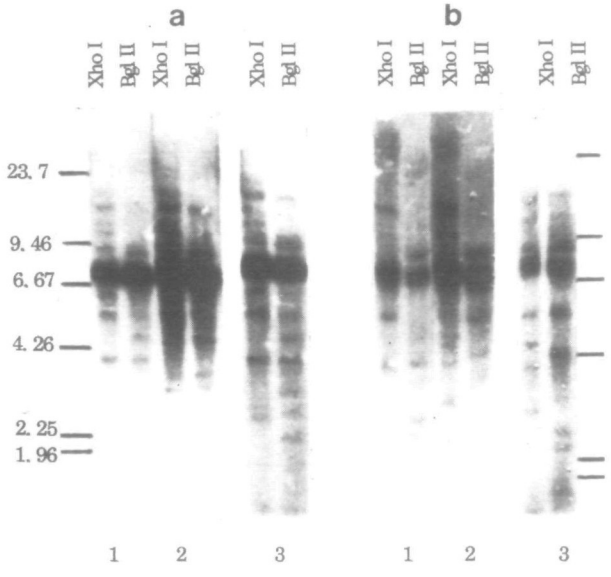

Fig. 2. Hybridization of Dm111 ^{32}P-labeled HindIII-EcoRI (1), HindIII (2), and XhoI (3) fragments to Southern blots containing restricted D. melanogaster DNA isolated from culture cells (a) and from Oregon RC flies (b). The amount of Oregon RC DNA was 2.5 times greater than that in DNA from culture cells. The HindIII restricted λ DNA was used as size markers.

the major labeled 7.0 kb band corresponding to a full-length mdg4 and also a number of other restriction fragments which bind the label. They are more expressed in the DNA from Oregon RC flies comparing to the DNA from culture cells. This can be explained by the amplification of the full-length mdg4 in cell culture.

It is noteworthy that the sizes of BglII and XhoI restricts (other than 7 kb long) binding the label in fly DNA do not coincide one with another. Therefore, their appearance does not result from simple deletions or insertions in the body of mdg4. The most probable explanation is that the genome has a heterogeneous set of sequences homologous to different parts of mdg4. Indeed, one can see a number of non-coinciding bands after the hybridization of BglII (XhoI) restricted DNA to different ^{32}P-labeled mdg4 fragments.

The presence of additional (other than 7.0 kb long) restriction fragments binding the label may also be due to strongly diverged mdg4 copies. The non-coincidence between the bands in BglII and XhoI digests can be explained by the heterogeneity in the terminal repeats.

Additional hybridization bands coinciding in the blots after BglII and XhoI restriction were obtained in the DNA from culture cells. They appeared to be produced by deletions or insertions in mdg4 during the *in vitro* cultivation of cells and mdg4 amplification.

4. Underreplication of several sequences hybridizing to mdg4 during polytenization

As mentioned above, mdg4 DNA always hybridized to the chromocenter which is known to be underreplicated. Therefore, it was of interest to compare the hybridization of mdg4 DNA to the Southern blots containing the DNA prepared from embryos, adult flies and larval salivary glands.

The DNA was restricted with BamHI endonuclease since the cloned mdg4 copies possess no sites for this enzyme. The amounts of DNA in all the samples were approximately the same. As probe, the 7 kb XhoI fragment was used.

Not only strong bands, but also weak ones can be seen in Fig. 3 presenting the results of these experiments. The intensity of some of them sharply decreases in the salivary gland DNA, i.e. in polytene chromosomes. This fact indicates that the corresponding DNA is underreplicated during polyteinization and allows to associate some weak bands with the sequences localized in the centromeres. The decrease of intensity of some strong bands in polytene chromosomal DNA may reflect the localization of some mdg4 copies in close to chromocenter regions (see Table 1).

5. Only one mdg4 variant is amplified in cell culture (strain 67J25D)

The restriction pattern is shown to be similar for different copies of the same mdg family in *D. melanogaster* /1, 8, 21/. This is also true of the copies that appear during amplification in culture cells. However, there exist some excep-

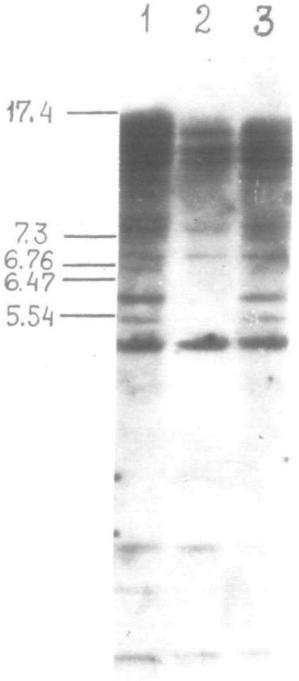

Fig. 3. Hybridization of Dm111 ^{32}P-labeled XhoI fragment to Southern blot containing BamHI restricted *D. melanogaster* DNA isolated from (1) embryos, (2) larval salivary glands, (3) adult Oregon RC flies. The BamHI restricted λ DNA was used as size markers.

tions /3/. To analyse the situation with mdg4, we hybridized Southern blots containing the DNA from (i) Oregon RC flies, (ii) *gtw*^{a} flies, and (iii) 67J25D culture cells restricted with HindIII and HindIII+EcoRI to either the ^{32}P-labeled 2.2 kb HindIII-EcoRI or to the ^{32}P-labeled 1.7 kb HindIII fragment (Fig. 4).

There appears a number of bands if the DNA from flies is restricted with HindIII and some restriction fragments hybridize to both the 1.7 kb and 2.2 kb ^{32}P-labeled mdg4 fragments and some of them hybridize only to one of them. These observations correlate with those shown in Fig. 2. It should be mentioned that all strong bands corresponding to the restriction fragments which hybridize both to the 1.7 kb and 2.2 kb mdg4 fragments are longer than 4.5 kb. Therefore, these restriction fragments may represent full-length copies of mdg4.

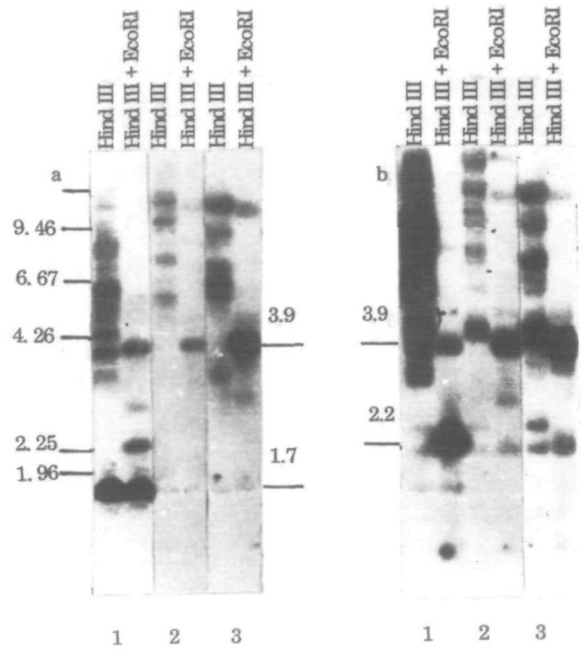

Fig. 4. Hybridization of Dm111 ^{32}P-labeled HindIII (a) and HindIII-EcoRI (b) fragments to Southern blots containing restricted D. melanogaster DNA isolated from (1) culture cells, (2) Oregon RC flies, and (3) gtw^{a} flies. The HindIII restricted λ DNA was used as size markers.

There are much more HindIII restriction fragments hybridizing to the 2.2 kb mdg4 fragment in the DNA of culture cells as compared to flies. Hence, a strong amplification of mdg4 does take place during the in vitro cultivation of culture cells. (The quantity of DNA in all the samples was approximately the same.) It is interesting that the hybridization of HindIII restricted Drosophila DNA to the 1.7 kb mdg4 fragment reveals a great difference between culture cells and flies. In culture cells, a band corresponding to the 1.7 kb mdg4 fragment is very strong whereas it is even weaker than other bands in Oregon RC and gtw^{a} flies.

These data indicate that most of the mdg4 copies in flies lack a HindIII site at the border line of two segments used as probes. This site is present in two cloned mdg copies. In-

deed, if DNA from flies is restricted with HindIII and EcoRI, both probes hybridize predominantly to a 3.9 kb band corresponding to the sum of two segments (see also Fig. 1). On the other hand, hybridization to the 1.7 kb and 2.2 kb bands is poor.

On the contrary, for the DNA from culture cells, the main hybridization bands coincide with the 1.7 kb and 2.2 kb bands. The intensity of the 3.9 kb band is very weak. One may conclude that most of mdg4 copies in the animal genome do not contain a HindIII site within the 3.9 kb segment whereas only the copy (copies) containing this site amplifies during the cultivation in vitro.

Finally, if one compares blots of genomic DNA from different Drosophila melanogaster strains and from culture cells, one can see several common bands possibly corresponding either to strongly diverged mdg4 copies or to sequences partly related to mdg4. They might be unable to transpose.

6. The nucleotide sequence of mdg4 LTRs and the flanking regions

Figs. 5 and 6 present the nucleotide sequence of LTRs and flanking sequences.

The following conclusions can be drawn when analysing LTR sequences and the adjacent areas. (i) The sequenced regions of two mdg4 copies containing four LTRs under study are completely identical. No base changes were detected in the sequenced parts of the mdg4 body either. (ii) The mdg4 sequences are flanked with short direct repeats 4 bp long that differ in two clones (TATA and TACA). (iii) The length of LTR elements in mdg4 is 479 bp. (iv) In contrast to the major part of other mdg elements, mdg4 LTRs like 297 and 17.6 /22/ do not contain characteristics TG...CA nucleotides at their ends. Instead of them, the dinucleotides AG...TT were detected. (v) Inverted mismatched repeats 5 bp long are located at the ends of each LTR element. (vi) Within LTRs, one can find regions similar to TATA boxes which are considered as signals for transcription and termination signals (positions 254-259, 278-279, 296-299) /23, 24/ are present. (vii) A sequence partially comple-

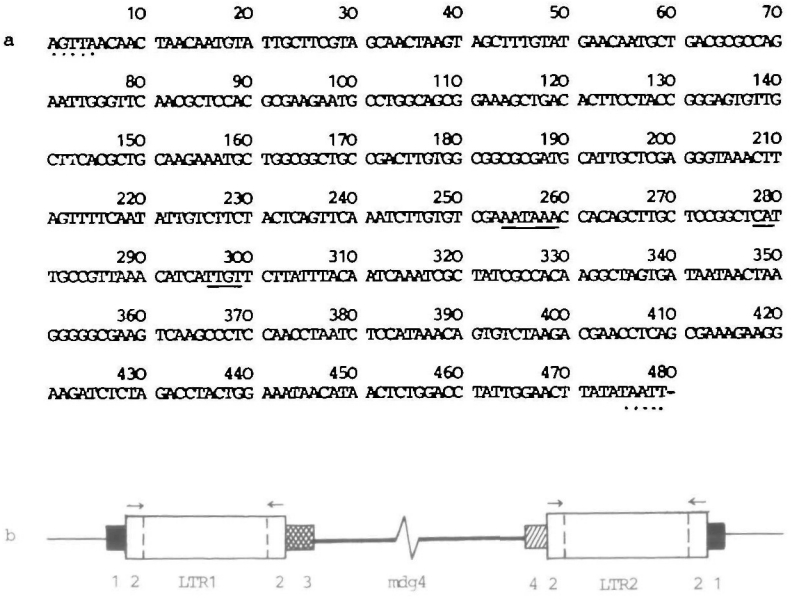

Fig. 5. Nucleotide sequences of mdg4 LTRs present in Dm111 and Dm112 clones (a) and schematic presentation of mdg4 LTRs and flanking sequences (b).
1 - duplication of host DNA; (2) mismatched inverted repeat; 3 - region of partially homology to $tRNA_1^{Lys}$; 4 - purine-rich region.

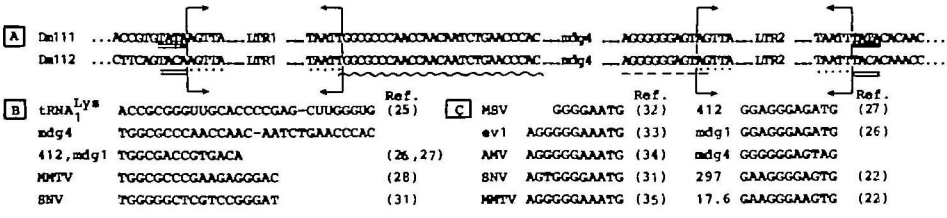

Fig. 6. Sequences immediately adjacent to the mdg4 LTRs (A) and comparison of sequences in the zones of possible tRNA primer binding sites (B) and purine-rich sequences (C) for mdg4, $tRNA_1^{Lys}$, several mdg elements and proretroviruses.
Duplications of host DNA are underlined with two lines, dotted lines indicate inverted repeats at the ends of LTRs. The sequences shown with a wave and dashed lines designate possible tRNA primer binding sites and purine-rich sequences.

mentary to that of D. melanogaster tRNAs ($tRNA_1^{Lys}$) /25/ exists in the region of the mdg4 body immediately adjacent to the left LTR. (viii) An oligopurine stretch (GGGGGGAGTAG) is located at the opposite end of the mdg4 body neighbouring the internal end of the right LTR.

DISCUSSION

The described properties of mdg4 show that this transposon is a typical mdg element similar in its organization to mdg1, mdg3, Dm412 and copia /1-5, 7-10/. This follows, first of all, from its variable localization in chromosomes revealed by the in situ hybridization to polytene chromosomes of different Drosophila melanogaster strains and from the analysis of its general structure and organization in Drosophila genome.

The organization of mdg4 termini is quite similar to that of several other LTRs sequenced such as mdg1 /26/ and Dm412 /27/. Mdg4 is flanked with 4 bp repeats different in two copies which should correspond to a duplication of the target sequence. The ends of the LTR form a mismatched inverted repeat (Figs. 5, 6). No base changes can be detected in two LTRs of the same mdg copy.

However, some properties of this element are peculiar. Many of mdg4 copies have been shown to be truncated or highly diverged sequences. Part of them may be localized in the centromeric regions of chromosomes. Indeed, the in situ hybridization data obtained in this research and also by Modolell et al./12/ demonstrate that there are sequences homologous to mdg4 chromocenters of different D. melanogaster strains.

Sequences close to the centromeres are known to be underreplicated in polytene chromosomes. Hence, BamHI restricted Drosophila DNAs isolated from flies and from salivary glands hybridize to mdg4 differently. In the case of the DNA from salivary glands, some bands (commonly the weak ones) are absent.

Sequences more or less homologous to different mdg4 fragments are located not only in centromeric regions but, apparently, throughout the genome. They look like weak bands on Southern blots. The intensity of their hybridization to mdg4

varies in different experiments, possibly due to their incomplete homology to mdg4 fragments.

The analysis of genome DNA blots demonstrates the existence of two variants of full-length mdg4 copies differing greatly in their properties. The only structural discrepancy between these two mdg4 copies revealed in our experiments is the existence in one of them the HindIII-site in the middle of mdg4. Recently, it has been confirmed by cloning of mdg4 copies from D. melanogaster flies' DNA. The major part of full-length mdg4 copies do not contain the facultative HindIII-site while all the other restriction sites are just the same as shown in Fig. 1 (unpublished data). We suggest that only the copies that contain a facultative HindIII-site can transpose and amplify in culture cells. Indeed, the analysis of HindIII-restricted DNA isolated from D. melanogaster culture cells, strain 67J25D, frozen at different steps of a 15 year in vitro cultivation shown that there occurred an explosion-like amplification of the mdg4 copy (copies) containing the facultative HindIII-site. Thus, on Southern blots containing DNA from earlier passages the band corresponding to the 1.7 kb mdg4 fragments was just like others and the pattern of hybridization looked like that for flies (Fig. 4a, slot 2, 3). On Southern blots containing the DNA from later passages, this band became much more intensive while the intensity of other bands remained the same, and the pattern resembled Fig. 4a, slot 1 (paper in preparation).

Besides, LTRs sequencing of two cloned mdg4 copies revealed their identical structure while a difference in the LTR sequence of the members belonging to one and the same mdg family usually makes up several percent. Therefore, we suggest that both cloned copies of mdg4 originated from one copy during the in vitro cultivation of Drosophila cells.

It is of interest that all other so far mdg4 copies are reported to contain the middle HindIII-site. However, it should be taken into account that, in all these cases, the investigators employed mdg4 in order to isolate structural genes using the system of insertion mutagenesis /12, 13/.

These facts confirm the viewpoint that only one

variant of mdg4 containing a facultative HindIII-site can transpose.

LTRs sequencing has also revealed some peculiar features of this transposable element. One of them - the identical LTRs' structure of different mdg4 copies - is mentioned above. Another peculiarity is that no TG...CA sequences were detected at mdg4 LTR termini. Also, in contrast to other mdg elements no perfect inverted repeats (even short ones) were found at the LTR ends. However, mdg4 transposes quite readily suggesting that GT...CA and a perfect symmetry at the ends of mdg are not obligatory for transposition.

Similar to mdg1 and Dm412 (mdg2), mdg4 contains a stretch partially complementary to one of tRNAs, i.e. $tRNA_1^{Lys}$ 27 nucleotides in length (Fig. 6). First eight nucleotides are the same as in mdg1, Dm412, and MMTV /26-28/. Sequence TGG can hybridize to the CCA end of any tRNA. This T is the last nucleotide of mdg4 LTR differing in this respect from mdg1 and Dm412 (it is adjacent to LTR there) and from retroviral proviruses (it is separated from LTR by two nucleotides in this case) /27/. An oligopurine stretch is located at the other end of the mdg4 body just before LTR, again, similarly to mdg1,Dm412, and retroviral proviruses (Fig. 6c).

All the mentioned sequences serve in retroviruses as sites for reverse transcription of a DNA copy. They may play the same role in the case of mdg elements including mdg4. Recently, the extrachromosomal circular DNA molecules with mdg element copia have been detected /29/, the RNA of copia being found in retrovirus-like particles /30/. Besides, we have observed that in addition to copia other mdg elements (mdg1, mdg3 and mdg4) possess a circular extrachromosomal counterpart in D. melanogaster cultured cells (in press). Our structural studies on different mdg circular copies suggest that the amplification and the following integration of mdgs may be due to the reverse transcription of RNAs transcribed on mdg DNA. In this connection, it may be thought that that only the copy (copies) containing a facultative HindIII-site can be transcribed. We believe that further studies along these lines are of great interest.

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