The nature of unstable insertion mutations and reversions in the locus *cut* of *Drosophila melanogaster*: molecular mechanism of transposition memory

Lev J. Mizrokhi¹, Ljudmila A. Obolenkova³,
Andres F. Priimägi^{1,4}, Yurii V. Ilyin¹,
Tatiana I. Gerasimova³ and Georgii P. Georgiev²

¹Department of Genome Mobility, and ²Department of Nucleic Acids Biosynthesis, Institute of Molecular Biology, USSR Academy of Sciences, Vavilov str. 32, and ³N.I. Vavilov Institute of General Genetics, USSR Academy of Sciences, Gubkin str. 3, 117984 Moscow B-334, USSR

⁴Permanent address: Institute of Experimental Biology, Academy of Sciences of Estonian SSR, Tallin, Estonian SSR, USSR

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The segment of the locus *cut* containing the mobile genetic element *mdg4* (gypsy) insertions which induce unstable *ct*^{MR2} and *ct*^{MRpN10} mutations has been cloned. Both mutations depend on the insertion of *mdg4* into the same sequence, which coincides with that in *ct*⁶ allele. The *ct*^{MRpN10} mutation differs from *ct*^{MR2} by additional insertion of a novel mobile element *jockey* into *mdg4*. *Jockey* is 2.8 kb long, represented by ~20–100 copies per genome, very homogeneous and lacks long terminal repeats (LTRs). The excision of *mdg4* takes place in stable *ct*⁺ reversions. On the other hand, a complete single LTR is retained in the case of unstable *ct* reversions characterized by a high level of reverse directed transpositions of *mdg4* into the locus *cut*. The LTR serves as a guide for reinsertion of *mdg4* itself or *mdg4* with *jockey* into the same site of the genome. A possible mechanism of transposition memory (homologous recombination with extra-chromosomal circular DNA) is discussed.

Key words: *Drosophila melanogaster/cut* locus/*jockey/mdg4* (gypsy)/transposition explosions/transposition memory/unstable mutations and reversions

Introduction

Transposition of mobile genetic elements is an important source of mutagenesis (for reviews, see Rubin, 1983; Georgiev *et al.*, 1985). We have described previously a family of related strains in *Drosophila melanogaster* which are characterized by a rather high level of transposition leading to an appearance of unstable mutations and reversions in different genetic loci. The original strain *ct*^{MR2} (Gerasimova, 1981) has been obtained in the course of P-M dysgenesis (see Kidwell *et al.*, 1977) as a result of insertion of a mobile dispersed genetic element *mdg4* (Bayev *et al.*, 1984), or gypsy (Modolell *et al.*, 1983) into the locus *cut* (Gerasimova *et al.*, 1984a). The derivatives of the *ct*^{MR2} strain possesses altered *ct* mutations, *ct*⁺ reversions and/or mutations in other loci of the X-chromosome (Gerasimova, 1981, 1983).

Instability of the *ct*^{MR2} strain and its derivatives is maintained through many generations, thus allowing one to study the features of the transposition process. It has been found that transposition does not take place at all in most of the germ cells. In a few germ cells, however, massive transpositions called transposition explosions have occurred, which involve all types of mobile elements including *mdg4* (*copia*-like), FB- and P-

elements (Gerasimova *et al.*, 1984b, 1984c). Recently, a series of successive transposition explosions have been described (Gerasimova *et al.*, accompanying paper). Studying the location of mobile elements during the successive transposition explosions, we have noticed that sometimes the reverse insertion of a particular mobile element into the locus where it has already been located before occurred 10–1000 times more frequently than its primary insertions, i.e., the 'transposition memory' takes place. The 'transposition shuttle' into and out of the locus is observed in some cases.

Here we describe the molecular nature of two mutations *ct*^{MR2} and *ct*^{MRpN10}, as well as the molecular mechanisms of transposition memory. Both *ct*^{MR2} and *ct*^{MRpN10} mutations were induced by *mdg4* insertion into the same site of the genome. Their difference is explained by an additional insertion of a novel mobile element, *jockey*, into *mdg4*. We have found that *mdg4* is excised in the case of stable *ct*⁺ reversions, and the long terminal repeat (LTR) of *mdg4* is retained in unstable *ct*⁺ reversions which possess the transposition memory.

Results

Molecular nature of *ct*^{MR2} and *ct*^{MRpN10} mutations

Cloning *ct*^{MRpN10} mutation using *mdg4* as a probe. *ct*^{MR2} and *ct*^{MRpN10} mutations are phenotypically different (different wing shape – see below, Figure 2). The first *ct*^{MRpN} mutation, *ct*^{MRpN10}, originated from *ct*^{MR2}. Afterwards, *ct*^{MRpN} appeared frequently either from *ct*^{MR2} mutation or from *ct*⁺ reversion. Both mutations were induced by *mdg4* insertion, and therefore *mdg4* was used as a probe for cloning the DNA segments responsible for these mutations.

A genomic library containing *D. melanogaster* Sau3A DNA fragments of the *ct*^{MRpN10} strain inserted into λL47.1 phage (Maniatis *et al.*, 1982) was prepared and the clones hybridizing to *mdg4* were selected. These clones were used for *in situ* hybridization with the wild Oregon strain which does not contain *mdg4* in the locus *cut* (7B). One clone hybridizing to 7B was obtained from the *ct*^{MRpN10} library (λ *ct* B73) but it also contained repetitive sequences different from *mdg4* (Figure 1b). Therefore the unique part of the clone was excised and hybridized to *D. melanogaster* chromosomes. After that, hybridization to only one region, 7B, was detected. Thus, the fragment of the locus *cut* containing *mdg4* insertion was obtained. The unique segment of the clone was further used to obtain the clones containing other *ct* mutations as well as *ct*⁺ reversions and a corresponding segment of the wild strain.

Several *ct* mutations possess *mdg4* insertion in the same genomic segment. The unique 7.0-kb *HindIII-XhoI* fragment was used as a probe for analysis of several *cut* mutations by Southern blot hybridization (Figure 1a). Genomic DNA was restricted by *EcoRI* endonuclease which produces one ~8.5 kb fragment hybridizing to the above-mentioned probe. This fragment is replaced by a 14.1-kb fragment in the *ct*^{MR2} mutation. DNA from heterozygous flies contain both fragments (14.1 and 8.5 kb). The

Fig. 1. Blot hybridization of genomic *D. melanogaster* DNA with the cloned DNA fragment containing part of *mdg4*, part of the locus *cut* and an unknown repetitive element. (A) Blot hybridization of *EcoRI* digests of Oregon, *cr⁶*, *cr^{MR2}*, *cr^{MR2}* in heterozygote, *k^{MR1}* and *cr^{MRpN10}* DNA probed with ³²P-labeled 7.0-kb *HindIII-XhoI* fragment of the clone λ *ct B73* (see B). (B) Restriction maps of the clone Dm111 (Bayev *et al.*, 1984) used for screening experiments and of the clone *ct B73* which contains a fragment of locus *cut* from the *cr^{MRpN10}* mutant. Open bars indicate *mdg4* (gypsy) sequence and open bars with shading, *mdg4* LTRs. An additional repetitive sequence (*jockey*, see below) is indicated by a solid bar. R: *EcoRI*, H: *HindIII*, X: *XhoI*, B: *BamHI*, G: *BglII*, S: *SalI*, K: *KpnI*.

EcoRI fragments in *cr^{MR2}* and in the standard *cr⁶* mutation are indistinguishable. Since *cr⁶* mutation was induced also by *mdg4* (Modolell *et al.*, 1983), one can expect the insertion to occur into the same site. In the strain *k^{MR1}* derived from *cr^{MR2}*, the wild-type *EcoRI* fragment was detected. Thus, *mdg4* was lost from its original position.

The same segment is changed in *cr^{MRpN10}* strain but its size is different from that in *cr^{MR2}*. It means that the insertion in *cr^{MRpN10}* differs from that in *cr^{MR2}*, being in good agreement with the appearance of an additional repetitive sequence in the clone from the *cr^{MRpN10}* strain.

The difference between *cr^{MR2}* and *cr^{MRpN}* mutations depends on an additional insertion into *mdg4*. To clarify the difference between *cr^{MRpN10}* and *cr^{MR2}* mutations, we have cloned the insertion-containing region from *cr^{MR2}* and *cr^{MRpN10}* strains as well as a corresponding segment from wild Oregon RC strain. Restriction mapping and cross-hybridization between the clones were performed. The results of such experiments are presented in Figure 2. One can see that the *cr^{MR2}* mutation is induced by insertion of a copy of *mdg4* whose physical map has a minor dif-

ference (an additional *HindIII* site) from the map of Modolell *et al.* (1983) and exactly coincides with that described by Bayev *et al.* (1984). The site of its insertion is located between *SalI* and *EcoRI* sites.

The full-length copy of *mdg4* is also detected in the *cr^{MRpN10}* strain (Figure 2). Its location within the locus *cut* is the same as in the *cr^{MR2}* allele. However, the insertion in *cr^{MRpN10}* is longer than in *cr^{MR2}*, resulting from the appearance of an additional 2.8-kb sequence within *mdg4*. The additional insertion leads to a phenotypic change of the wing shape (Figure 2). This sequence was named *jockey* as it jumped on another mobile element. Its properties are described in the following section.

To localize precisely the *mdg4* insertion, the nucleotide sequences at the borders of *mdg4* and the target sequence were determined. The sequencing was performed from the *XhoI* sites located in the *mdg4* LTRs, not far from their ends. One can see from Figure 3 that in both *cr^{MR2}* and *cr^{MRpN10}* *mdg4* is located in exactly the same position inducing the same 4-bp duplication of a target sequence, i.e., TGCA.

Jockey is a novel mobile element of *D. melanogaster* which does not contain LTRs. The internal *HindIII-EcoRI* segment of *jockey* (see Figure 4) was recloned and used for *in situ* hybridization and Southern blot hybridization. *Jockey* hybridizes with 20–100 sites dispersed throughout all *D. melanogaster* chromosomes (Figure 5). *Jockey* is present in the 7B region of *cr^{MRpN10}* and eight other *cr^{MRpN}* mutations tested (*cr^{MRpN1}*, *cr^{MRpN23}*, *cr^{MR3}*, *cr^{MRpN16}*, *cr^{MR17a}*, *cr^{MRpN17a}*, *cr^{MR6}*, *cr^{MRpN20}*, *cr^{MR19}*, *cr^{MRpN19}*, *y^{MR19w}*, *cr^{MRpN19}* and *cr^{MRpN25g}*), which appeared in different independent experiments. On the other hand, it was not detected in the 7B subdivision in Oregon, *cr^{MR2}*, *cr⁶*, *cr⁺*, *cr⁺³²* strains as well as in the *cr^{MR20}*, *cr^{ras}*, *cr^{MR3}* mutation phenotypically similar to *cr^{MR2}*. Thus, the *cr^{pN}* phenotype always seems to be connected with the presence of *jockey* together with *mdg4* in the locus *cut*.

In different strains, the localization of *jockey* is quite variable. In particular, it is involved in transposition explosions like other mobile elements. Table I shows the location of *jockey* in the strains obtained in the course of successive transposition explosions described by Gerasimova *et al.* (accompanying paper). Numerous changes in *jockey* localization were observed. On the other hand, the latter is quite stable within each strain. Thus, *jockey* behaves as a typical mobile genetic element.

The analysis of Southern blots (Figure 4), obtained after cleavage of the genomic DNA with restriction endonucleases cutting *jockey* in two sites, reveals the high homogeneity of *jockey*: practically all label is concentrated in one band. Less than 10% of *jockey* copies differ from the major variant by these restriction sites. (The exception is *SalI* site which seems to be present only in few copies including the cloned one.)

On the other hand, the restriction of genomic DNA by endonucleases cutting *jockey* in a single site or not cutting *jockey* produces a very heterogeneous distribution of hybridizing sequences indicating that the sequences surrounding *jockey* are quite heterogeneous.

Jockey does not contain LTRs as no homology was detected upon cross-hybridization between left- and right-handed parts of *jockey* (data not shown). Thus, *jockey* is not an *mdg*-like (*copiable*) mobile element.

Molecular mechanism of stable and unstable *cr⁺* reversions

Two types of cr⁺ reversions. It was shown earlier than the *cr⁺* reversions could be either stable or unstable. *Mdg4* was removed from the locus *cut* in stable *cr⁺* reversions which followed

Fig. 2. Restriction maps of the locus *cut* region containing insertions in the strains Oregon RC, *ct*^{MR2}, *ct*^{MRpN10}, *ct*⁺¹, *ct*⁺³ and *ct*⁺³². Designations are the same as in Figure 1. The bracket above Oregon RC indicates the fragment of a wild-type locus *cut* used in blot hybridization experiments (see Figure 4).

from *in situ* hybridization experiments. It was also removed from this locus in many cases of unstable *ct*⁺ reversion. However, the reverse insertions of *mdg4* into the locus *cut* occurred with a high frequency (10^{-2} – 10^{-3}) in these unstable reversions. We suggested that the reverse-directed mutagenesis ('transposition memory') depended on retaining a small piece of *mdg* element at the site of insertion (Gerasimova *et al.*, accompanying paper).

To check this possibility, we performed blot hybridization with genomic DNA from mutants and *ct*⁺ revertants restricted by *Sal*I or *Xho*I-*Sal*I endonucleases using the 3.5-kb *Sal*I fragment of the clone from the wild-type strain as a probe (Figure 6). The 3.5-kb *Sal*I genomic segment from the wild-type strain contains the site for *mdg4* insertion (Figure 2). It is convenient for analysis since *mdg4* itself does not contain the *Sal*I restriction site, but contains an *Xho*I restriction site in the LTR. The size of *Sal*I in the stable *ct*⁺³² revertant is the same as in a wild strain (Oregon RC). In the unstable *ct*⁺¹ revertant and stable *ct*⁺³ revertant this segment is ~500 bp longer.

The insertion contains an *Xho*I site. It is interesting that the size of a segment appearing after *Sal*I-*Xho*I digestion of genomic DNA in *ct*⁺¹ and *ct*⁺³ coincides with that in *ct*^{MR2} and *ct*^{MRpN10}. This observation strongly suggests that *mdg4* LTR is retained in those two *ct*⁺ reversions (Figure 2).

It should be pointed out that the stability of mutation depends not only on the transposon itself, but also on the genotype. Thus, the rate of mutagenesis in several strains (derivatives of *ct*^{MR2}) decreased with time to a background level (Gerasimova, 1983). It could be restored, however, by a novel cross with P-strain (*MRh12/Cy*). The cross of *ct*⁺³ females with *MRh12/Cy* males give rise to the appearance of *ct* mutations in the males accompanied by an *mdg4* insertion into the 7B subdivision (with a rate of 10^{-3} – 10^{-2} in different experiments). Thus, the *ct*⁺³ strain is potentially unstable, i.e., it possesses a masked transposition memory.

In contrast to this, the crosses of *ct*⁺³² females with *MRh12/Cy* males did not lead to the appearance of *ct* mutations (20 000 flies have been tested). One may conclude that the transposition memory depends on the non-precise excision of *mdg4* retaining an LTR sequence at the site of insertion.

Stable reversion is followed by an excision of mdg4. To investigate further the nature of reversions, we have cloned the target segments from the *ct*⁺³², *ct*⁺³ and *ct*⁺¹ strains. The restriction maps are presented in Figure 2. No difference between Oregon RC and *ct*⁺³² has been found. Moreover, no homology between the sequences of *mdg4* and the clone from *ct*⁺³² at the blot hybridization level were detected (data not shown). Thus in the case of stable reversion, complete excision of *mdg4* took place.

Unstable ct⁺¹ and potentially unstable ct⁺³ revertants contain a complete LTR of mdg4. Restriction analysis of the insertions from *ct*⁺¹ and *ct*⁺³ revertants showed their similarity to *mdg4* LTR, i.e., characteristic *Xho*I and *Bgl*II restriction sites were selected (Figures 2 and 6). Hybridization of the insertion of the *mdg4* LTR probe was also positive (data not shown).

The sequencing of the insertion from the *Xho*I and *Bgl*II sites was performed (Figure 3). It clearly demonstrated that the complete *mdg4* LTR was present in the locus *cut* of both *ct*⁺¹ and *ct*⁺³ revertants. The LTR insertion is flanked by the same 4-bp direct repeat, TGCA, as the original insertion of *mdg4*.

Reverse transpositions of the mdg4 to the locus cut occur at the mdg4 LTR sequence. DNAs from original and several reverse *ct* mutations were prepared, restricted by *Sal*I or *Sal*I + *Xho*I, and the Southern blots were hybridized to the 3.5-kb *Sal*I fragment of the wild-type clone (Figure 6). The reverse mutation *ct*^{MR13B+} phenotypically resembling *ct*^{MR2} contained *Sal*I fragments identical to the *Sal*I fragments of *ct*^{MR2} mutations while that of *ct*^{MRpN} type, *ct*^{MRpN25gMR1}, possessed *Sal*I fragments typical of *ct*^{MRpN10} mutations. The *Sal*I-*Xho*I

Fig. 3. Nucleotide sequences flanking the LTR(s) of *mdg4* in the clones obtained from *ct*^{MR2}, *ct*^{MRpN10}, *ct*⁺¹ and *ct*⁺³ clones. Host DNA is shown in lower-case letters, LTR DNA is in capital letters. The TGCA duplications created by *mdg4* insertion are underlined. The whole insertions have been sequenced in the case of *ct*⁺¹ and *ct*⁺³. Its sequence exactly coincides with the known sequence of *mdg4* LTR (Bayev *et al.*, 1984), except for one nucleotide substitution (position 469 is T instead of C), and because of that it is not shown in the Figure.

fragments were identical in all mutations tested. Thus, either *mdg4* or *mdg4* with *jockey* was inserted into the locus *cut* at the site where the LTR had been located.

One may conclude that the transposition memory, at least in the case of reverse-directed mutagenesis in the locus *cut*, is associated with retention of a complete LTR sequence at the site of *mdg4* insertion.

Discussion

The origin of *ct*^{MR2} and *ct*^{MRpN} mutations

We have demonstrated that *ct*^{MR2} and *ct*^{MRpN} mutations depend on the insertion of *mdg4* or *mdg4* + *jockey* into exactly the same sequence of the locus *cut*. This sequence coincides with that in the case of *cr*⁶ mutation at the level of Southern blot resolution.

One may speculate on the mechanism of this coincidence. It should be pointed out that *ct*^{MR2} mutation appeared while the chromosome Maxy carrying the *cr*⁶ allele was transiently present in the strain (Gerasimova, 1981).

Several authors have reported that mobile elements can be more readily transposed to a particular chromosomal site if the homolog possesses this element at the same site. The transposition was not followed by the loss of a mobile element from the original site (Evgen'ev *et al.*, 1982). Possibly *mdg4* present in the locus *cut* in the chromosome Maxy was responsible for the insertion of *mdg4* into the same site of the Oregon RC X-chromosome. It could hardly be a result of meiotic recombination because of many inversions in the Maxy chromosome. However, one cannot exclude that gene conversion was involved in this case.

ct^{MRpN} mutation differed from *ct*^{MR2} by the additional insertion of *jockey* into the *mdg4* sequence. This insertion may result either from true insertion of *jockey* into *mdg4* or from the replacement of the original copy of *mdg4* by the copy with *jockey* within it. It is interesting that the *D. melanogaster* genome contains a few copies of *mdg4* which are longer than the canonical 7.5-kb *mdg4*. They are also excised by *Xho*I or *Bgl*II endonucleases which cleave *mdg4* in LTR sequences, and therefore their size can be easily measured. One of them is ~2.8 kb longer than *mdg4* itself and may correspond to *mdg4* with *jockey* (Y.Ilyin, unpublished data).

Other evidence in favour of the pre-existence of an *mdg4* copy with *jockey* insertion comes from our experiments on reverse mutagenesis where *ct*^{MRpN} mutations appeared rather frequently. It is easier to explain this by a direct insertion of *mdg4* carrying *jockey* rather than by two successive insertions (first of *mdg4* and then of *jockey*).

A possible mechanism of such a replacement is recombination between genomic and extrachromosomal copies of *mdg4*.

The extrachromosomal circular copies of *mdg* elements can be formed by means of reverse transcription (Arkhipova *et al.*, 1984; Flavell, 1984).

Phenotype dependence on the nature of insertion

It was shown that the phenotype strongly depended on the nature of insertion. The flies with the *mdg4* LTR insertion were indistinguishable from the wild-type flies, although the length of the insertion was ~500 bp. This can be explained by the location of insertion in the non-coding region of the gene. Similarly Bender *et al.* (1983) demonstrated that the mutants *bx*³ and *bdx*¹ associated with *mdg4* insertion can revert by excision leaving behind a single LTR. Modolell *et al.* (1983) found that *ct*₆ phenotype could be suppressed by hairy-wing suppressor without excision of *mdg4*. The appearance of the *cr*⁶ phenotype may result from *mdg4* transcription while the suppression may be due to inhibition of transcription. The existence of the insertion itself is not sufficient for the appearance of the mutation.

An additional insertion of *jockey* into *mdg4* changes the phenotype. This insertion possibly influences the mutation through interference with *mdg4* transcription. This possibility is presently under investigation. Other examples of insertions within insertions altering a mutant phenotype are described by Collins and Rubin (1982) and Levis *et al.* (1982) for the *white* locus.

Molecular mechanism of transposition memory and transposon shuttle

The main result of this paper is the explanation of transposition memory in the case of reverse-directed transposition of *mdg4* to the locus *cut*. The latter is found to depend on retention of solo LTR sequences at the original site of the *mdg4* insertion.

The presence of solo LTRs of *mdg4* and *mdg2* in the *D. melanogaster* genome was described (Modolell *et al.*, 1983; Shepherd and Finnegan, 1984). Recently, retention of *copia* LTR in the locus *white* upon the *w*⁺ reversion has been described (Carbonare and Gehring, 1985), but the authors did not observe the reverse transposition in their system.

Retention of LTR seems to be a result of *mdg* excision by means of recombination between LTRs while the excision of *mdg* (stable reversion) may depend on recombination between flanking short direct repeats.

Solo LTRs might serve as hot spots for reverse *mdg* integration. Homologous recombination between the extrachromosomal *mdg* circles containing a single LTR and genomic solo LTR sequences is a possible mechanism of the process. A similar mechanism has been proposed by Roeder and Fink (1980). Note that the majority of circular *mdgs* contain one LTR and only few of them have two LTRs (Flavell and Ish-Horowicz, 1981; Ilyin

Fig. 4. Blot hybridization of genomic DNA from the *cr*^{MRpN10} strain treated by different restriction endonucleases with a subcloned *Hind*III-*Eco*RI fragment of *jockey* (pJL1 plasmid). The short *Pst*I-*Eco*RI fragment 0.5 kb in length on the corresponding lane ran out of the gel presented here. It could be seen after shorter runs. Below are the restriction maps of *jockey* and its fragment subcloned in pUC19 plasmid. P: *Pst*I, other designations for restriction endonucleases are the same as in Figure 1.

et al., 1984; Shepherd and Finnegan, 1984). The copies with one LTR might serve as appropriate substrates for reverse transposition. Another possible explanation is recombination or gene conversion between the LTR at the *cut* locus and the LTRs at the ends of complete *mdg4* elsewhere in the genome. Exchanges between complete Ty elements in different parts of the yeast genome has been described by Roeder and Fink (1982) and Roeder *et al.* (1984).

It is not clear yet how widespread is the described mechanism of transposition memory. The phenomenon of transposition memory has also been demonstrated for the insertion of other

Fig. 5. *In situ* hybridization of *jockey* (pJL1) with polytene salivary gland chromosomes. (A) Distribution of *jockey* in the nucleus of Oregon RC strain. (B) The presence of *jockey* in 7B subdivision (locus *cut*) of *cr*^{MRpN10} mutant.

Table I. Localization of jockey in the X-chromosome in the course of several successive transposition explosions

Strain	2B	3D	4B	4C	5B	5D	6C	7B	7D	9A	10B	10D	11E	12B	13E	17B	17C	18C	19E	20	No changes in location
ct ^{MR2}	+			+							+			+							—
ct ^{MRpN10}	+			—			+	+	+		+		+	+	+	+			+		8
ct ^{MR3} ct ^{MRpN16}	—			—				+			—			—						+	6
cm ^{MR17a} ct ^{MRpN17a} ^a	—			—				+			—	+		—			+				(7)
cm ^{MR19} ct ^{MRpN19} ^a	—			—				+		+	—			—	+	+			+		(8)
y ^{MR19} w ^{MR19} cm ⁺ ct ^{MRpN19}			+	+		+		+		—			+			—			+	+	7
y ⁺ w ⁺ ct ⁺ sn ^{L2}		+	—	+	+	—		—					—				+		—	—	9

Minuses show the loss of mobile element from a particular site.
^aThese two strains appeared as a result of two successive explosions. The first one has led to creation of the strain ct^{MR2}MR1.
Figures in brackets are the results of two successive transposition explosions.

Fig. 6. Blot hybridization of SalI and SalI-XhoI digests of Oregon RC (1), ct⁺³² (2), ct⁺¹ (3), ct⁶ (4), ct^{MR2} (5), ct^{MR13B}⁺ (6), ct^{MRpN10} (7), ct^{MRpN25g}MR4 (8) DNAs probed with the 3.5-kb SalI fragment of wild-type cut locus (see Figure 2). Size markers are HindIII fragments of lambda DNA.

mdg elements into the loci yellow, white and carmine (Gerasimova et al., accompanying paper). Experiments are in progress to analyze the molecular mechanisms in those cases.
A possible role of transposition memory and transposon shuttle (in connection with transposition explosions) in evolution has been discussed previously (Gerasimova et al., accompanying paper).
Materials and methods
D. melanogaster strains
The genetic analysis of mutations and crosses used were described earlier (Gerasimova, 1981, 1983). For detailed description of the stocks used, see Lindsley and Grell (1968).

Cloning
DNA was isolated from flies. The material was powdered in liquid N₂, lyzed in 0.15 M NaCl, 20 mM Tris-HCl, pH 8.0, 20 mM EDTA, 0.5% SDS, 200 µg/ml pronase, deproteinized twice by phenol and chloroform, and precipitated by ethanol. The genomic libraries were constructed according to the procedure of Maniatis et al. (1982). 10–20 kb fragments obtained by partial digestion with Sau3A restriction endonuclease were inserted into BamHI arms of the lambda 47.1 vector and packaged in vitro. Colony screening and preparation of DNA from the clones were performed according to Maniatis et al. (1982).
Southern blot hybridization
3 µg of D. melanogaster DNA or 0.5 µg of recombinant DNA were digested with restriction enzyme(s) and fractionated by electrophoresis in 0.7% agarose gel. The transfer to nitrocellulose filters and hybridization were performed according to Maniatis et al. (1982).

Sequencing

The Maxam and Gilbert (1980) method was used. *Xho*I and *Bgl*II ends were labeled by reverse transcriptase.

In situ hybridization

Recombinant DNA was labeled with ³H and ¹²⁵I by nick translation (Maniatis *et al.*, 1982). *In situ* hybridization was performed as described by Ananiev *et al.* (1978).

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