The Enigma of *Y* Chromosome Degeneration: *TRAM*, a Novel Retrotransposon is Preferentially Located on the *Neo-Y* Chromosome of *Drosophila miranda*

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ABSTRACT

We have cloned a novel transposable element from the *neo-Y* chromosome of *Drosophila miranda*. The size of the element, designated as *TRAM*, is 3.452 bp, including on both sides long terminal direct repeats (LTRs) of 372 bp, respectively. The element is flanked by a 5-bp target site duplication, ATATG. The putative primer binding site (PBS) for minus-strand priming is complementary to 18 nucleotides of the 3′-end of $tRNA^{Trp}$. Data base screens for DNA sequence identities were negative, apart from the sequence motif of the PBS. The deduced amino acid sequence from the large ORF does not reveal identities described for other transposons. *In situ* hybridizations with *TRAM* subclones show a biased distribution in the genome, with a massive accumulation of *TRAM* in the *neo-Y* chromosome, while the former homologue, the *X2* chromosome is devoid of *TRAM* sites. The enriched occurrence of the *TRAM* element at the evolving *neo-Y* chromosome of *D.miranda* adds compelling evidence in favor of the view that *Y* chromosome degeneration is driven by the accumulation of transposable elements.

Y CHROMOSOME degeneration (MULLER 1918, 1932) is a process that involves structural changes in chromosome architecture and expansion of genetic inertness along the *Y* chromosome (*cf.* CHARLESWORTH 1978, 1991, 1996; LUCCHESI 1978, 1994; CHARLESWORTH and CHARLESWORTH 1979; BULL 1983; RICE 1987, 1994; STEINEMANN and STEINEMANN 1992). It is generally assumed that the heteromorphic sex chromosome pair has developed from a pair of homologues. Causes of the evolution of the structurally and functionally different *X* and *Y* chromosomes have been the object of speculation since the discovery of sex chromosomes. To analyze the molecular basis of this evolutionary process we chose a model system, *Drosophila miranda*. *D. miranda* belongs to the American representatives of the obscura group. The separation of *D. miranda* from its next relatives *D. pseudoobscura* and *D. persimilis* occurred relatively recently, about 2 mya ago according to mtDNA restriction analysis (BARRIO *et al.* 1992). Due to the fusion of one of the autosomes to the *Y* chromosome, a *neo-Y* chromosome and a *neo-X* chromosome, designated as *X2*, were formed (DOBZHANSKY 1935; MACKNIGHT 1939; STEINEMANN 1982). Thus formerly autosomal genes are transmitted in association with the sex chromosomes. We have cloned the larval cuticle protein (*Lcp*) genes from the *X2* and *neo-Y* chromosome (STEINEMANN and STEINEMANN 1990). Analyzing the DNA sequences from the *X2* and *neo-Y* region, we observed a massive accumulation of DNA insertions on the *neo-Y* chromosome. In polytene chromosome squashes, the male *X* chromosome in Drosophila can be distinguished by the presence of an isoform of histone H4 acetylated at lysine 16, H4.Ac16. Staining *D. miranda* with H4.Ac16 antibodies showed that dosage compensation extends across part of the *X2* (STEINEMANN *et al.* 1996). The *X2* shows a much brighter staining over ~90% chromosome length, reflecting the predominant association of H4.Ac16. A terminal region constituting ~10% of the *X2* chromosome is not labeled. This finding fits nicely with quantitative autoradiographic studies, showing that RNA synthesis in this region is not upregulated (STROBEL *et al.* 1978).

Here we describe a novel LTR-retrotransposon cloned from the *neo-Y*-chromosomal *Lcp1-4* locus. While the *neo-Y* shows an enrichment of TRAM sites (~50–60), we find the former homologue, the *X2* chromosome, to be devoid of sites. Together with the distribution of the non-LTR TRIM retrotransposon (STEINEMANN and STEINEMANN 1991) and the IS*Y3* insertion elements (STEINEMANN and STEINEMANN 1992, 1993), we present compelling evidence that the first step in *Y* chromosome degeneration is driven by the accumulation of transposable elements, especially retrotransposons.

MATERIALS AND METHODS

Cloning of the *TRAM* element from the *neo-Y* chromosome and the homologous region on the *X2* chromosome: High molecular weight DNA from *D. miranda* was isolated according to STEINEMANN 1982. Genomic EMBL4 lambda libraries from partial *Sau*3A (Boehringer Mannheim) digests were described in STEINEMANN and STEINEMANN (1990). Using a polymorphic restriction site, overlapping clones with *X2* or *neo-Y* chromosomal origin were isolated, covering ~30 kb

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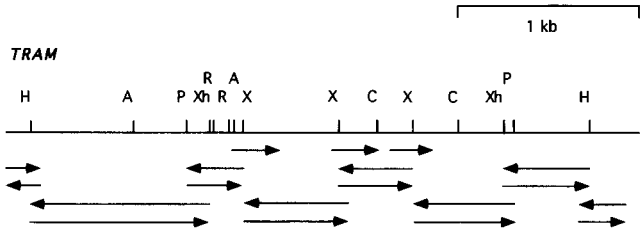

FIGURE 1.—Restriction map of the *TRAM* element and sequencing strategy. Fragments were subcloned in *M13mp18/19* and sequenced on both strands. Arrows below the map indicate the sequencing strategy. H, *Hind*III; A, *Acc*I; P, *Pst*I; Xh, *Xho*I; R, *Eco*RI; X, *Xba*I; C, *Cla*I.

from both locations, respectively (STEINEMANN and STEINEMANN 1990). For detailed restriction mapping, the regions containing the *Lcp* genes on the *X2* and *neo-Y* chromosome were subcloned into pUC18. The DNA was sequenced on both strands from *M13mp18/19* subclones by the dideoxy chain termination method according to the protocol supplied with Sequenase (United States Biochemical). For the *Xba-Xba* fragments, we proved the correct orientation by sequencing from flanking sequences over the *Xba* sites (Figure 1). Standard DNA techniques were carried out according to SAMBROOK, FRITSCH and MANIATIS (1989).

Computer analysis: The DNA data base screening with BLASTN (ALTSCHUL *et al.* 1990) and the Genetics Computer Group Sequence Analysis Package (DEVEREUX *et al.* 1984) was done using the updated (May 1996) EMBL and GenBank nucleotide Sequence Data Library (EMBL, Heidelberg; GenBank, NCBI, Washington). SwissProt data library was used for the screening of the deduced amino acid sequence of the large TRAM ORF. The TRAM sequence is deposited in the EMBL gene bank, accession number Y08905.

Chromosome *in situ* hybridization and *D. miranda* strains: *D. miranda* flies were cultured on standard Drosophila food at 18°. The 111/30 strain is an isoline from single females collected in the wild. *D. miranda* MPI is a strain of unknown origin from our laboratory. Fragments of the TRAM element were subcloned into *pUC18*. The clones were labeled with Biotin-16-dUTP (Boehringer, Mannheim) by the nick translation reaction and hybridized at 58° overnight to alkali denatured chromosome squashes (STEINEMANN and STEINEMANN 1991). The slides were washed three times in 2× SSC at 53°. Signal detection followed the protocol for immunoperoxidase staining supplied with the DETEK I-hrp kit used (Enzo Diagnostics, New York). The intensity and contrast of the diaminobenzidine (DAB) precipitate were enhanced using a silver DAB enhancement kit (Amersham). Photomicrographs of the chromosome squashes were made with an Agfapan 25 film.

RESULTS

Structure and organization of the *TRAM* element: Sequence analysis of the *Lcp* locus cloned from the *X2* and *neo-Y* chromosomes reveals a massive accumulation of inserted DNA sequences (ISYs) in the *neo-Y* chromosomal *Lcp* region (Figure 2 and *cf.* STEINEMANN and STEINEMANN 1992, 1993). Previously we had identified a large insertion, IS*Y5*, between *Lcp3* and *Lcp4*. From preliminary sequence information, we expected that the IS*Y5* insertion might be a retrotransposon. Complete sequencing of this insertion revealed a sequence organization typical for retrotransposons of the long terminal repeat (LTR)-type (XIONG and EICKBUSH 1990). The 5′- and 3′-LTRs are 372 bp in size (Figure 3). They show identical sequences except for one point mutation, an exchange of a C base *vs.* T at position 17. The internal central region of the *TRAM* element is bordered by sequences that are conserved in retroviruses. At the 5′ end, a stretch of DNA shows sequence identity to the primer binding site (PBS), which is complementary to 18 nucleotides of the 3′ end of the tryptophan tRNA ($tRNA^{Trp}$). In data base screens, we obtained a 100% identity for this *TRAM* DNA region (377–399, 23 bp) with *Myeloblastosis-associated virus type 2* (accession number L10924), *Rous sarcoma virus* (*RSV*, 378–399, 22 bp; accession number U41731), chicken *c-erbB* oncogene (378–399, 22 bp; accession number M10066, M13881) and chicken endogenous *rav-RAV-0* avian virus (378–399, 22 bp; accession number J02015). The *RSV* PBS sequence is complementary to the acceptor stem of the $tRNA^{Trp}$ (SCHWARTZ *et al.* 1983). Thus, we conclude that *TRAM* could potentially use $tRNA^{Trp}$ to prime the replication of its minus strand. The 3′ end of the internal domain shows a short polypurine tract (PPT), a characteristic domain in retroviruses, which is believed to serve as a primer for the replication of the plus DNA strand (VARMUS 1983).

Integration site and coding capacity of the *TRAM* element: The *TRAM* element has integrated between the *Lcp3* and *Lcp4* locus, 433 bp upstream of the transcription start of the *neo-Y* chromosomal *Lcp4* (Figure 2). The element has induced a 5-bp target site duplication, ATATG (Figure 4). The site of integration of *TRAM* lies within a sequence showing a palindrome structure. Of interest is the notion that the neighboring *TRIM* retrotransposon has integrated in a similar sequence structure (STEINEMANN and STEINEMANN 1991). In the latter case, the integration was accompanied by an 11-bp deletion, while the *TRAM* integration occurred without one. In addition to the induced target site duplication, we found two base substitutions at the right hand side of the integration site (Figure 4).

The deduced amino acid sequence of the *TRAM* element reveals one large open reading frame (ORF) of 2013 bp (positions 1092–3104) with the first methionine at position 1158. The ORF terminates within the 3′-LTR. Data base screening with the deduced amino acid sequences of the ORF does not reveal sequence identities with characteristic motifs from the gag and pol genes found in other retrotransposons and retroviruses.

Genomic distribution of *TRAM*: *In situ* hybridizations with different *TRAM* fragments to the *D. miranda* strain 111/30 showed labeling of the chromocenter in salivary gland squashes from females of third instar larvae (Figure 5A). All chromosome arms, including the *X2*, are unlabeled (Figure 5, A and B). Salivary gland squashes from third instar male lavae hybridized with the same *TRAM* fragments show a labeled chromocenter and in addition ~50–60 sites on the *neo-Y* chromo-

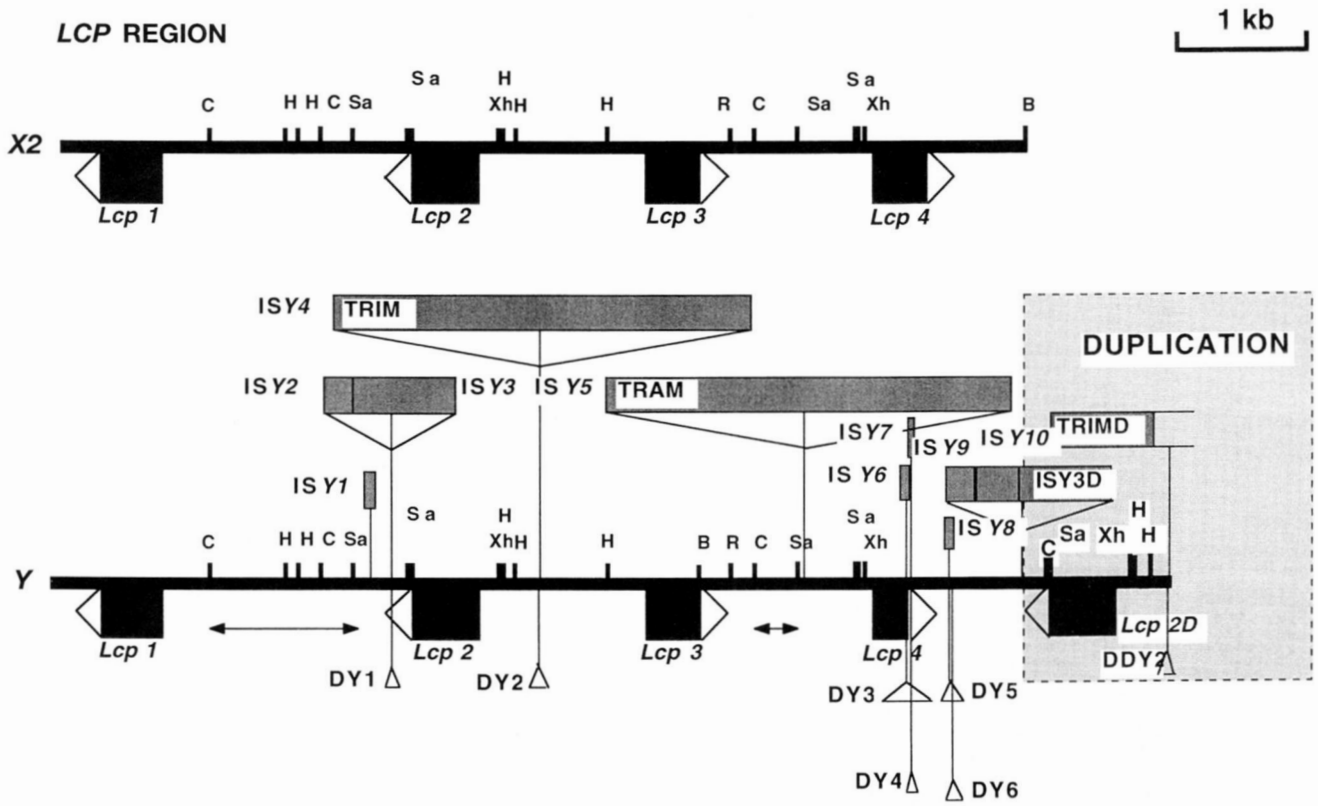

FIGURE 2.—General schematic map of the *X2* and *neo-Y* chromosomal *Lcp1-4* regions. The map is based on sequence information for both areas. Two short unsequenced stretches are indicated by arrows below the map. Alignment of the *X2* and *neo-Y* chromosomal sequences reveals a dense cluster of insertions (ISYs) in the *Y* chromosomal *Lcp* region (stippled boxes). Deletions (DY) are indicated by triangles. For orientation, some restriction sites are indicated. The insertions are shown without restriction sites. The *TRAM* element represents the large IS*Y5* insertion between *Lcp3* and *Lcp4* on the *neo-Y* chromosome. Restriction sites: Sa, *Sac*I; H, *Hind*III; R, *Eco*RI; B, *Bam*HI; Xh, *Xho*I; C, *Cla*I.

some (Figure 5C). In the *D. miranda* MPI strain, the situation is similar. However, we do find in addition four labeled sites in the euchromatin (two on chromosome *2*, one on chromosome *4* and one on *X1R*) and one in the beta-heterochromatic base of the *X2* chromosome (not shown). These additional sites in the MPI laboratory strain indicate clearly that *TRAM* elements have the potential to transpose in the *D. miranda* genome.

DISCUSSION

Structure and possible function of the *TRAM* element: The analyzed element was cloned from the *neo-Y* chromosome of *D. miranda*. TRAM has integrated 433 bp upstream of the transcription start site of the *neo-Y* chromosomal *Lcp4* locus. Northern analysis revealed that the *neo-Y* chromosomal locus of *Lcp4* is not transcribed (STEINEMANN and STEINEMANN 1992). The novel Drosophila transposon *TRAM* shows characteristic structural features of the LTR-retrotransposon type (XIONG and EICKBUSH 1990). Both long terminal direct repeats (LTRs) are 372 bp in length and differ only by one base change. Conserved LTR sequences, PBS and PPT motifs are expected for a *TRAM* element that could still transpose. *TRAM* shows no sequence identities to *TRIM*, the neighboring retrotransposon, nor to other retrotransposons, beside the PBS regions. The integration occurred in a palindrome sequence (*cf.* Figure 4). Formally, the integration sequence resembles the structure where *TRIM*, the neighbored other retrotransposon of the *neo-Y* chromosomal *Lcp1-4* region, has integrated.

Beside these characteristic features of retrotransposons, the *TRAM* element shows one large ORF. Data base screening with the deduced amino acid sequence does not reveal any sequence identities to protein motifs characteristic for retrotransposons, *e.g.*, motifs of the reverse transcriptase, protease, RNase H and integrase from the *pol* gene. The *in situ* results from the 111/30 and MPI strain, which show different localizations of *TRAM* in the euchromatin, demonstrate clearly that the *TRAM* element is able to transpose. The *neo-Y* chromosomal *TRAM* element, which we analyzed in detail might be truncated, degenerate or rearranged internally. However, the long ORF and the conserved structural features suggest some degree of selection pressure on the integrity of the observed structures of the element. It will be of interest to isolate a *TRAM* element from euchromatic insertion sites of the MPI strain and compare it with the *neo-Y* chromosomal one.

Biased distribution of the *TRAM* element in the

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TGTTCGGAGCAGAACCCAGCCGATTAGCTGCTTGCCAAATAGCACCTAATTCTTGGCCCT 0060
CAGCCGCTTATTTTGTTTGTTACTTATGTCTATGTCACTCATTTGTTTGTTAAAGCTTTG 0120
CGCTTGCTTGCCCTGCTAAACGCTCTCTGCCAGCTCGCTCTTCGCTATCTCCGCTTTGCG 0180
TCTGCCTACCGACGTCGGCCGAGCGAAGCTGCGCTTAGCGATCGGAGCGGCAATGTAAAG 0240
GGCAGGCAAGCCACACTTGCAATTTGGATGTCACGCATTAAAGAACATATCGTAATTTTA 0300
TTTCTGCGCCGAGTTTTGTTTAATTCGAAATAATTAGTCGGCCGATTGGGGATAAAAAAC 0360
5' LTR <-┐ PBS
ATTATCTCCACATAAAATTTGGTGACCCCGACGTGATCTCTGAGTTGCAGTGTCAATTAA 0420
TAATCATTCGCGATCGACATTCGTTAGCCCTAGCAAATTTTCGTCGGTTAAGCACAATTC 0480
GGTGCTAAAACACACTTACATACACCTACATACACGCATTGCTGGCTATTAATTTCTCTG 0540
TCGCACAATTCGGTCAGTGCAGTGCGGTAGGCAGTGCAGCGAACTCACTAATACACACAA 0600
GCGGAGTACAAAGCGGAATCGGACAGCTCGCACAGCCGCACAGCTAAAGGCATTAGCTGT 0660
AATCCCTTTGCTTTCGGTTCCCACGTTTATACGTATACAGGGTGTCTTTTTCGGTCGTGC 0720
TGAGAGACTCTTTTAAAACCTCAACATGGCAGCACCTGAGCCTACCAATGTCGCGAACGC 0780
AGCAATGCCGAGTGATGTAGATTTCTACAAGCACAAGGCCGAGTCCATCGCGCGCCAACT 0840
AAAGGCCATGGATCGCTTTCTTACCAAGGAAGAGCTTGCCCGAGTTAGATGAGGCAGAAC 0900
TTCAAGCTCGCTTAGAGCAAATCGAGCGAATGAATGCGGATTTCGATGCCGCTCAAACGA 0960
GCCTTGAAAGGCTGGATTTCCTGCAGTTAGCCCATGATGCCCGGCTGGACTTTTCGAATG 1020
TTTATGTCAAGGTTAGGTCCAGGCTGTCGCGGGAGTTGATGGCTGCTCGCACGGTAAATG 1080
     ┌-> ORF
TTGCCAATTGAACGGCTCGGCATACTCTCGAGGGGAATTCGTCGTTGTTCGCCTATAATA 1140
GTATAGGCCGTTCTCGAATGCCCGAGTTGCAGCTTCCGCGATTCGGTGGGAACTACATGG 1200
ATTGGCCAGAATTCCACTCGATGTTCTCGACAATGGTGCACAAAGACCATCGTATACCAA 1260
TCATCGAAAAATTCCAATATCTTCGTGGATGTCTAGATGGTGCTGCGCTGGATACGATTC 1320
GTTCCTTGGAACTTTCTGAGGAGAATTACGACAAGGCGTTGAATTTACTAATGTTGCGAT 1380
TCGATAATAAACTGTTACATTTTCAGGCACACGTCAAGGCTATTTTCGGGCTGCAAGGGG 1440
TGGAGAAGGGCTCGGCTATCGGCTTGCGCGCGCTCAGGCACAAAATCAATTCGCACTTGC 1500
GGGCACTTCAGACCTTGGCGACCCCGCAGGAGATATCCGATGGGTTGCTGATCTTCATCA 1560
TAGGCACGAAACTGGACCACAAAACAAAGGAGAAATGGGATGAGAACTTGCCGACGTCAG 1620
GATTGCCTCGGTGGTCAAGCATGGCCTCATTTCTGGAAGCGAGATGTCGGATGCTGGAGA 1680
ATTTGGGATCAGCCATGGCAACAAGTCCTAGTCAACAGGTGGGAGAAGACAAACCTGTCA 1740
CCCTTATCACCTCCAGTAACGACCATCCTAACCCCATATGTAACCATTGCAATTCCTCCG 1800
AGCATTACATATCTAGATGTCAGGCATTCCTGAATCTCTCTGCGTTTGAACGACACAAAG 1860
AAGCAAAGAAGAGCCGCTTGTGTTTGAACTGCCTCAACAAAGGCCATGAATTGCAGAGGT 1920
GCAGGTCAGGACTTTGCAGGCATTGCCAGGCCAAACATCACACGCTACTCCACATTCCAT 1980
CGGGAACTGGTGCTTCATCTTCCTCTTCACCGGCCGAGGAATCGATCCAGCAAGAGGCCG 2040
CGACTGTGCTTCTAGCAAGCGGGTGTTCCAGCCCTCCCCCCTCGATACAGAAATCTCAGC 2100
CTAGCCAGAACGTGTTGCTACCTACTGCCCTCGTCCATGTAACAGATCGTTATGGAGCAC 2160
TTATCTCATGTCGTGCCATTTTGGATTCTGCATCACAGGCAAACTTTGTAACATCTAGAC 2220
TTGCTGATCAGTTGCAGTTGGATCATCGCTCGTCTTATGTTCACATCTCTGGAATCGGAG 2280
ATTCCATTCTACCTTCGAGCAAGTCTGTACATATAGTTGTACAATCCCAGGACGCAAGCT 2340
ATCGAGCTTCCTTCGCTGCAATTGTCACCAACTCAATTACGGAAATGCAGCCTAACTTCG 2400
GCCTAGACGCAAAGGATTGGCCAATGCCGAATAATCTAAAACTAGCTGATCCTAATTTCT 2460
CCAAGCCCCAACGTATCGATCTGTTGATAGGTTCTGGTTTGTTCTTCGATTTAATGTGCG 2520
TCGGACAGATTCGACTATCAGCCCAATTGCCAACATTGCAGGAGACAAAACTTGGTTGGA 2580
TAGTATCAGGAAGCATTGATAGCTCGGAGAATAAGCGTGCAGCTTTAGCCGCTTTTGAAA 2640
ATTCCTCGTGCATCTCTATTGACGATTTTCGACCCACAACGCTGGAGTACCAAAACTTAG 2700
AGCAGCAATGCAGGAAGCAGCTGCTCGAGTGCCAGGTGCAAGTGGAAAAACTGCGATCGG 2760
AGAATCAGGAACTGCAGCGCGAACTTTTCCATATATTAAAAACCTACATATCCACGCTAA 2820
ATGAAATTCAACTTTCAACAATTTCTACATTGCCAAATTTCCTGCCATTCTATACAAATA 2880
CAGAGGTTCCCGATGATCAAGACGTAATCACGACAAGCCGCCTTCGTAAAGAAGCGCCGA 2940
TTTCTCGCTTCGACGATCATTCCAGCGTAGCCGCCAGCTGCGCCCAAGCAAGCATCTTCA 3000
AGAGGGCCGTTGGAAAATTAGCGGTTCTGCCCCTTCAGGATGGATCTGTTGAAAGCCTTT 3060
                ┌-> 3'LTR Trm
GCCTTCCAACGGGGGGTGAATGTTCGGAGCAGAACCTAGCCGATTAGCTGCTTGCCAAAT 3120
AGCACCTAATTCTTGGCCCTCAGCCGCTTATTTTGTTTGTTACTTATGTCTATGTCACTC 3180
ATTTGTTTGTTAAAGCTTTGCGCTTTCTTGCCCTGCTAAACGCTCTCTGCCAGCTCGCTC 3240
TTCGCTATCTCCGCTTTGCGTCTGCCTACCGACGTCGGCCGAGCGAAGCTGCGCTTAGCG 3300
ATCGGAGCGGCAATGTAAAGGGCAGGCAAGCCACACTTGCAATTTGGATGTCACGCATTA 3360
AAGAACATATCGTAATTTTATTTCTGCGCCGAGTTTTGTTTAATTCGAAATAATTAGTCG 3420
GCCGATTGGGGATAAAAAACATTATCTCCACA
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FIGURE 3.—Sequence of the *neo-Y* chromosomal *TRAM* element. The size of the element is 3.452 bp. The putative sense strand of *TRAM* is presented in the 5′ to 3′ direction. The 5′- and 3′-long terminal repeats (LTRs), 372 bp in length, are indicated with arrows. The LTRs differ by 1-bp substitution, C/T (underlined). The central region is bordered at the 5′ end by the primer binding site (PBS, underlined), which shows a perfect sequence identity to the stem acceptor site of $tRNA^{Trp}$. The 3′ site is flanked by a short polypurine tract (PPT, underlined). The large open reading frame (ORF) is indicated with an arrow and the first ATG and the stop codon (Trm) is underlined.

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                    ↓
X2 TTGTAGGTATTTTATAAAATATATG-----TTAAATCTGACGCAAAAA
Y  TTGTAGGTATTTTATAAAATATATGATATGTTTAATCTGACGCAACAA
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FIGURE 4.—Integration site of the *TRAM* element. Alignment of the integration sequence from the *neo-Y* region with the homologous *X2* section. The target site duplication is shown in bold face. The arrow indicates the site of *TRAM* integration. In the flanking sequence 2 bp A/T and A/C (underlined) are changed.

***D. miranda* male genome:** In males of the *D. miranda* 111/30 strain, the *neo-Y* chromosome and the chromocenter label with *TRAM* sequences. Thus, while the staining of the *neo-Y* chromosome reveals ~50–60 sites, the former homologue, the *X2* chromosome, shows no label. The accumulation of the *TRAM* elements along the *neo-Y* chromosome is consistent with the biased distribution of the *TRIM* retrotransposon (STEINEMANN and STEINEMANN 1991) and the IS*Y3* sequences (STEINEMANN and STEINEMANN 1992). In addition the accumulation of a 1.1-kb *Bam*HI repeat on the *neo-Y*,

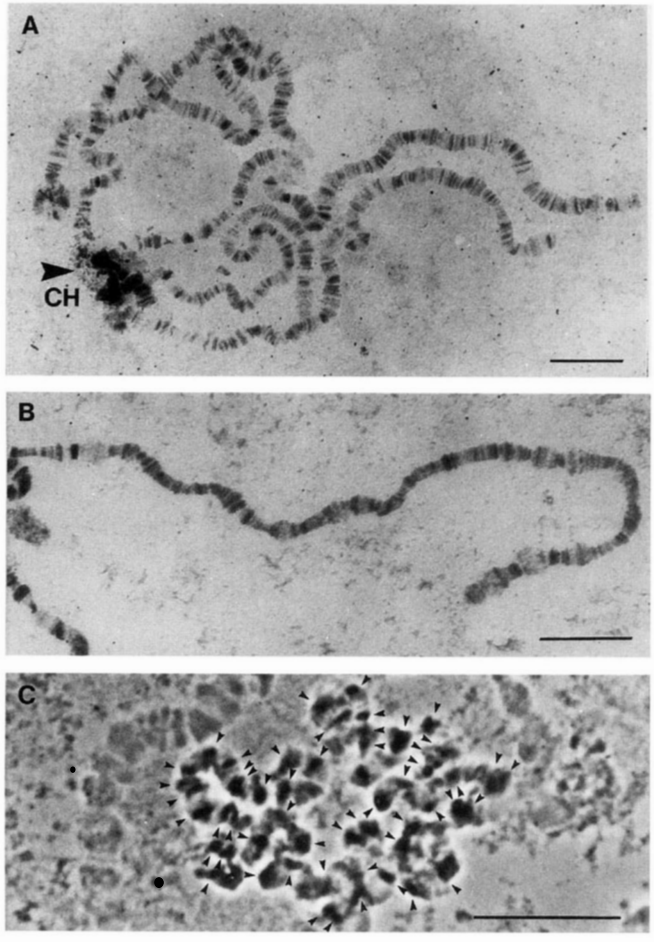

FIGURE 5.—*In situ* hybridizations of the left-hand *Hind*III-*Xho*I fragment from the *TRAM* element, labeled with biotin, to the *D. miranda* 111/30 strain. For signal enhancement of the labeled sites, we used a silver amplification system for peroxidase-DAB precipitates. (A) Polytene salivary gland nucleus from third instar female larvae. Only the chromocenter (CH) is labeled. (B) *X2* chromosome from a female nucleus. The *X2* contains no *TRAM* site. (C) The former homologue, the *neo-Y* chromosome shows ~50–60 labeled sites (arrowheads). Bars, 20 μm.

named *NY* element, has been reported (GANGULY *et al.* 1992). Thus, the behavior of the *TRAM* element adds compelling evidence to our model for the mechanism of degeneration of the *D. miranda neo-Y* chromosome.

To resolve the enigma of *Y* chromosome degeneration, we suggest the following model. In addition to accumulating point mutations, a massive spread of transposable elements, especially retrotransposons, occurs in the evolving *neo-Y* chromosome. There are essentially three possible reasons why transposable elements could accumulate on the *neo-Y*: (1) suppression of recombination between the *X* and the *Y* chromosomes in Drosophila males. This would permit the increase of elements that have little or no direct deleterious insertional effects, and hence would not in itself cause loss of gene function (*cf.* STEINEMANN *et al.* 1993). (2) Deleterious insertional effects could accumulate by a mechanism termed Mullers ratchet, if the population size of the *D. miranda* species is relatively small, which has been little studied. (3) Fixation of transposable elements with slightly deleterious effects on fitness could occur by drift, because of the restricted effective population size of a nonrecombining *neo-Y* chromosome. (4) Insertions of transposable elements into chromosome regions that are already genetically inert. Such insertions will be neutral. However, the latter cannot explain erosion and dosage compensation (for review, see CHARLESWORTH *et al.* 1994; CHARLESWORTH 1996).

The elements concerned are also concentrated in the beta-heterochromatic chromocenter. Because many families of Drosophila transposons are abundant in the beta-heterochromatin and the *Y* chromosome (PIMPINELLI *et al.* 1995; SPRADLING 1994), they must also participate in the formation of the heterochromatin structure itself. Thus an enrichment of these elements along an evolving *Y* chromosome, *e.g.*, the *neo-Y* of *D. miranda*, could account for the change in chromatin structure: a switch from a euchromatic into a heterochromatic one. Further, we suggest in our model that, in the first stages of *Y* chromosome degeneration, the change in chromosome structure caused by the trapping and accumulation of transposons, is responsible for the successive silencing of genes, both intact or mutated ones (*cf.* STEINEMANN and STEINEMANN 1992; STEINEMANN *et al.* 1993). The silencing of *Y* alleles forces the homologous regions on the *X* chromosome in the males to adopt dosage compensation mechanisms (CHARLESWORTH 1978; STEINEMANN *et al.* 1996). Accumulation of further mutations, deletions and duplications, followed by the expansion of tandem repetitive sequence motifs of high copy number (satellite sequences) will then represent the final state of the degenerated *Y* chromosome.

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