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The introduction of a transpositionally active copy of retrotransposon *GYPSY* into the Stable Strain of *Drosophila melanogaster* causes genetic instability

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Abstract. A previously described genetic system comprising a Mutator Strain (MS) and the Stable Strain (SS) from which it originated is characterized by genetic instability caused by transpositions of the retrotransposon *gypsy*. A series of genetic crosses was used to obtain three MS derivatives, each containing one MS chromosome (X, 2 or 3) in the environment of SS chromosomes. All derivatives are characterized by elevated frequencies of spontaneous mutations in both sexes. Mutations appear at the premeiotic stage and are unstable. Transformed derivatives of SS and another stable strain 208 were obtained by microinjection of plasmid DNA containing transpositionally active *gypsy* inserted into the *Casper* vector. *In situ* hybridization experiments revealed amplification and active transposition of *gypsy* in SS derivatives, while the integration of a single copy of *gypsy* into the genome of 208 does not change the genetic properties of this strain. We propose that genetic instability in the MS system is caused by the combination of two factors: mutation(s) in gene(s) regulating *gypsy* transposition in SS and its MS derivatives, and the presence of transpositionally active *gypsy* copies in MS but not SS.

Key words: *Drosophila melanogaster* – Genetic instability – *gypsy* – Mobile elements – Retrotransposons

Introduction

As a rule, genetic instability in *Drosophila melanogaster* is caused by transpositions of mobile genetic elements (Belyaeva et al. 1982; Bingham et al. 1982; Gerasimova et al. 1984; Engels 1989; Finnegan 1989; Blackman and Gelbart 1989). We have previously described an unstable Mutator Strain (MS), in which only two types of mobile element undergo transpositions (*gypsy* and *hobo;* Kim et al. 1990; Kim and Belyaeva 1991). The *gypsy* transpositions are associated with the induction of various mutations, instability of visible morphological mutations, and chromosomal rearrangements. All these events take place in both somatic and germline cells of *Drosophila melanogaster* (Kim and Belyaeva 1991). Molecular analysis of the structural organization of *gypsy* elements cloned from both the Mutator Strain (MS) and the Stable Strain (SS) from which it had been derived, revealed the existence of two distinct *gypsy* subfamilies, which show defined structural differences (Lyubomirskaya et al. 1990). Despite the fact that both *gypsy* variants are transcriptionally active, only one of them is amplified in cultured *Drosophila melanogaster* cells (Bayev et al. 1984) in MS (Lyubomirskaya et al. 1990) and causes insertional mutations (Modolell et al. 1983; Mizrokhi et al. 1985; Marlor et al. 1986; Pfeifer and Bender 1988). This allowed us to suggest that these two subfamilies may be described as transpositionally active and inactive variants (Ilyin et al. 1991). Both *gypsy* subfamilies can coexist in the *D. melanogaster* genome without exhibiting transpositional activity (Bayev et al. 1984); thus the presence of copies of the active *gypsy* element is necessary but not sufficient for its active transposition. Therefore we propose that other gene(s) regulate *gypsy* transposition, and are altered in MS as well as in SS, resulting in activation of *gypsy* transposition.

The present paper describes experiments performed to test this suggestion. We show that the introduction of active *gypsy* copies into SS either by crossing or by microinjection transforms the genetically stable strain into an unstable one. Therefore, new MS-like stocks can be obtained at any time simply by introducing transpositionally active *gypsy* copies into the permissive background of the SS genome.

Materials and methods

Drosophila melanogaster strains. The *Drosophila melanogaster* strains used in this work were the following: MS

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and SS, both carrying *w* (Kim et al. 1990); 208, marked with *vg* and *w*. Balancer chromosomes used were *FM7, y w^a dm B; SM5, al^2 Cy cn^2 sp^2* and *TM3, Sb Ser* (Lindsley and Zimm 1985). MS-derived strains MS-1 (containing the X chromosome from MS and all other chromosomes from SS), MS-2 (2nd chromosome from MS in the background of SS chromosomes) and MS-3 (3rd chromosome from MS and all other chromosomes from SS) were obtained by standard techniques. The analysis of spontaneous visible mutations and their stability was performed as described earlier (Kim et al. 1990).

Preparation and manipulation of nucleic acids. Extraction of plasmid and total genomic DNA, poly(A)$^+$ RNA, restriction enzyme treatment, DNA labelling, Southern and Northern blotting experiments were performed according to Maniatis et al. (1982).

DNA constructs. Plasmid constructs containing *gypsy* inserted into the Casper vector (Pirotta 1988) were prepared as follows and used for microinjection. The *Bam*HI-*Eco*RI fragment of Dm111 (Bayev et al. 1984) and the *Eco*RI-*Pst*I fragment of pHS*GYP* (Lyubomirskaya et al. 1989) were inserted into the *Bam*HI and *Pst*I polylinker sites of Casper. As a result this construct contained *gypsy* in the transcriptional orientation opposite to that of the mini-*white* gene. To obtain a construct containing *gypsy* in the same transcriptional orientation as mini-*white*, the *Bam*HI-*Eco*RI fragment of Dm111 was inserted into the corresponding polylinker sites of Casper. The intermediate construct obtained in this way was treated with *Eco*RI and phosphatase, and ligated with the *Eco*RI fragment of Dm111. The correct orientation of the inserted fragment was then confirmed by restriction analysis.

Injection of plasmid constructs into Drosophila melanogaster embryos. Microinjections of 500 ng/ml of construct DNA and 200 ng/ml of helper DNA (pπ25.7wc; Karess and Rubin 1984) were performed according to Rubin and Spradling (1982) using an Eppendorf microinjector. G_0 flies were crossed to SS marked with *w*. Transformed G_1 flies were recognized by the appearance of mutant phenotypes.

In situ hybridization. The distribution of mobile elements was analyzed by *in situ* hybridization to salivary gland polytene chromosome squashes (Pardue et al. 1970). [^{3}H]thymidine-labelled plasmid DNAs, carrying *mdg1, 412, mdg3, gypsy* and *copia* inserts (for review see Finnegan 1990) were used as probes. To detect the integration sites of injected constructs, a Casper fragment containing sequences from the *white* gene was used as a probe.

Results

Genetic analysis of MS derivatives

Spontaneous mutability of MS derivatives. MS-derived strains (MS-1, MS-2, MS-3), carrying a single MS chromosome together with SS chromosomes show the same instability properties as MS. Frequencies of mutations arising in germ cells were scored in individual crosses of males from each of the strains with chromosomal substitutions with females (*Bal/C(1)DX, y f*) carrying attached X chromosomes. Mutations arising in the X chromosome (both recessive and dominant) and dominant mutations appearing in autosomes were detected in the first generation (F_1). In the second generation (F_2), obtained by sister-brother matings of F_1 progeny, both recessive and dominant mutations were detected in the *X* chromosome and the autosomes. Several individuals having the same mutant phenotype appeared among the progeny of single males and were considered as clusters and scored as single events. All mutations were identified either by an allelism test with known mutations or were mapped in the usual way. All the mutations are believed to be newly induced because the frequencies of mutations were measured immediately after the stocks had been obtained.

The frequencies of spontaneous mutations among MS-derived strains (Table 1) were as high as 10^{-3}–10^{-4}, which is much higher than the spontaneous mutation rate usually observed. Visible mutations appear in both sexes and usually in clusters, indicating that mutations occur at premeiotic stages. It is interesting that in all MS derivatives carrying only one MS chromosome, mutations appear on all chromosomes. In MS and all its

Table 1. Frequencies of spontaneous visible mutations in germ cells of males of MS-1, MS-2 and MS-3 strains

Generation	Strain	No. of chromosomes analyzed		X chromosome		Autosomes	
		Males	Females	Number	Frequency[a]	Number	Frequency[a]
F_1	MS-1	6062	5007	8	1.3	5	0.4
	MS-2	9359	8718	17	1.8	2	0.1
	MS-3	6011	5560	6	1.0	0	0
	SS	11806	11537	0	0	0	0
F_2	MS-1	8267	7564	8	1.0	3	0.2
	MS-2	10148	9184	4	0.4	0	0
	MS-3	5995	5740	2	0.3	0	0
	SS	10387	10306	0	0	0	0

[a] Expressed as mutations per 1000 individuals screened

Table 2. Distribution of *gypsy* in the chromosomes of MS-1, MS-2 and MS-3

Sites	MS-1							MS-2									MS-3								
	Larva No.																								
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
3C			+																						
10A		+																							
11C		+																							
19F					+	+	+																		
21D								+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23A								+	+	+	+	+	+	+	+	+									
34D								+	+		+		+	+	+	+	+	+	+	+		+	+	+	
36C								+	+	+	+	+		+	+	+									
38A								+	+	+	+	+	+	+	+	+									
41A								+	+		+	+	+	+	+										
42C								+		+	+	+	+		+	+									
43F								+	+		+	+	+	+	+										
53F								+	+	+	+	+	+	+	+	+									
61F								+	+	+	+	+		+	+		+	+	+	+	+	+	+	+	+
65C															+										
67EF															+										
82C								+			+				+			+	+	+	+	+		+	+
86C								+			+				+										
87A								+	+	+	+	+	+	+	+										
92A								+	+		+				+										
92B																	+	+	+		+	+	+		+

Data are given for individuals

derivatives, mutations appear, in the absence of outcrosses, in several successive generations including the F_1.

Genetic instability of spontaneous mutations. Many mutations obtained from MS-1, MS-2 and MS-3 are genetically unstable and the frequency of reversion is 10^{-3}–10^{-4}. As an example, the frequencies of reversion of *ct* and *f* mutations obtained from MS-1 reach up to 10^{-3} (in the case of *ct*, reversion frequency was $5/5547 = 0.9 \times 10^{-3}$ and in the case of f – $8/5608 = 1.4 \times 10^{-3}$).

Localization of mobile elements on polytene chromosomes. The results of *in situ* hybridization experiments show that in MS and all its derivatives the distribution of mobile elements *mdg1, 412, mdg3* and *copia* is conserved (7–10 larvae were analyzed for each of these elements; no variations between individuals were detected). In contrast, the location of *gypsy* varies between individuals of one stock of MS, while the location of *gypsy* on polytene chromosomes of SS was constant (sites 21D, 80A). Some of the data demonstrating this are presented in Table 2. Variations in *gypsy* location were detected also for X chromosomes in the case of MS-2 and MS-3 and for autosomes in the case of MS-1, but the sites in these chromosomes as well as in regions adjacent to the chromocentre were not determined precisely. Therefore these data are not included in Table 2. These experiments, like the genetic analysis described above, demonstrate that MS derivatives carrying only one MS chromosome in the background of SS chromosomes have the same properties as MS itself.

Analysis of transformed derivatives of stable strains

Cytogenetic analysis. A cloned copy of the putatively transpositionally active *gypsy* sequence inserted in the Casper vector was microinjected into SS embryos to generate transgenic animals. From 57 fertile F_0 individuals two transformed strains, designated MSn^1 and MSn^2 (Mutator strain new, 1 and 2), were obtained. None of these flies had red eyes (which would indicate the presence of the integrated Casper vector) and these two were chosen for further analysis because each carried a *f* mutation.

To obtain isogenic stocks, these flies were crossed several times to SS and since the integrated Casper could not be detected genetically it could have been lost during these crosses. We therefore used the PCR technique to detect the presence of the introduced construct in the genome of the *f* mutants (data not shown). Both contained Casper integrated into their genomes, even though mini-*white* was not expressed. We suppose that in the plasmid construct used for these experiments the *gypsy* promoter and regulatory regions are so close to the mini-*white* promoter that they negatively influence its expression. Therefore, in further injections into 208 embryos, another construct was used, in which *gypsy* was oriented differently and its promoter and regulatory region lie 7 kb away from the mini-*white* promoter. The DNA was injected into embryos of strain 208 carrying a *w* mutation and known to lack *gypsy* in euchromatin. In these experiments three independent derivatives with pigmented eyes ($208t^1$, $208t^2$ and $208t^3$) were obtained from 132 fertile F_0 individuals.

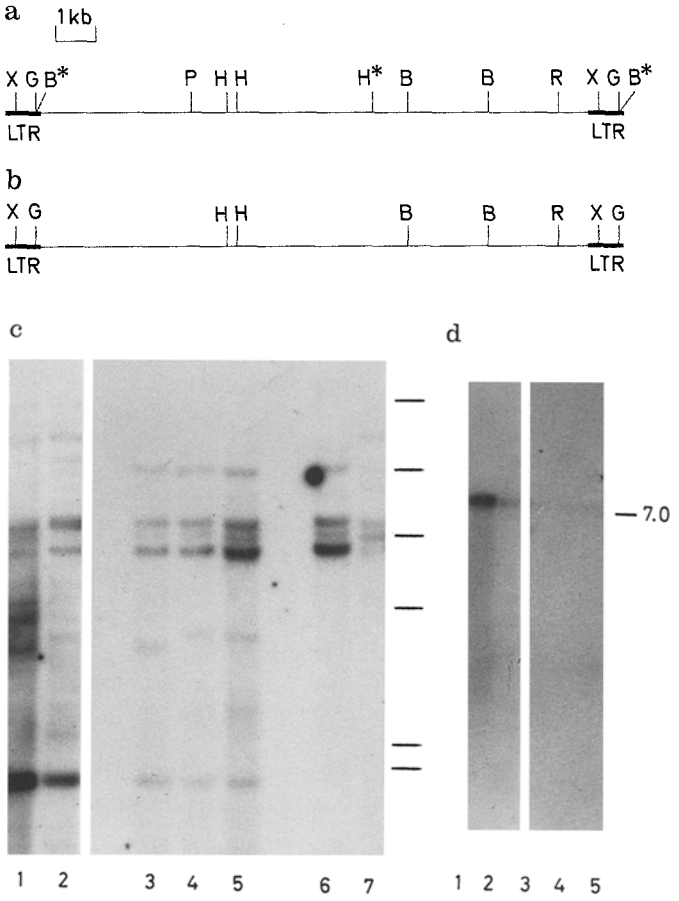

Fig. 1a, b. Restriction maps of two *gypsy* variants previously cloned: p6K from MS (**a**) and p7K from SS (**b**). Restriction endonucleases are abbreviated as follows: H, *Hin*dIII; B, *Xba*I; G, *Bgl*II; P, *Pst*I; R, *Eco*RI; X, *Xho*I. Variable sites are indicated by *asterisks*. Hybridization of the ^{32}[P]-labelled 3.4 kb *Hin*dIII-*Eco*RI fragment of p7K to *Hin*dIII-digested DNA (**c**) and poly(A)$^+$ RNA (**d**) isolated from two stable strains: 208 (lane 6) and SS (lane 7), and their transformed derivatives: MSn^1 (lane 1), MSn^2 (lane 2), 208t^1 (lane 3), 208t^2 (lane 4) and 208t^3 (lane 5). The sizes of *Hin*dIII λ fragments used as molecular weight markers are 23.1 kb, 9.4 kb, 6.7 kb, 4.4 kb, 2.3 kb and 2.0 kb

The data obtained from the *in situ* hybridization experiments demonstrate a heterogeneous distribution of *gypsy* in chromosomes of MSn^1 and MSn^2 as well as an increased number of sites (30 and 20, respectively), suggesting that *gypsy* transposition occurs in these strains. The distributions of *gypsy* on the X chromosomes of 12 MSn^1 individuals was determined. All had insertions at 10E and 15E; new sites were detected in 7 larvae, of which 4 had single novel sites (3B, 12E, 14E and 19E), while the other 3 had acquired 2 new sites each (1A, 19E; 11B, 12D; 3A, 19E). The distribution of other mobile elements was constant and similar to that in SS (data not shown).

To prove autonomous transposition of *gypsy*, the following experiments were performed. A single MSn^2 male was crossed with a harem of attached-X females and X chromosomes of 23 male offspring were analyzed. [In addition to the common sites at 10E and 15E, one individual had new insertions at 3A and 9B, 2 others had a novel insertion at 20A, while 2 more had single new insertions at 3E and 12E.] As a control the localization of element *297* was determined and it was the same in all analyzed chromosomes (1D, 3E, 5A, 9C, 13A, 16B, 19C, 20). Therefore, like MS derivatives of SS, MSn^1 and MSn^2 are genetically unstable.

Transformed derivatives of 208 differ profoundly in that they are all genetically stable. Two 208 derivatives were analyzed by *in situ* hybridization. It was shown that each of them carries one site that hybridizes to both *gypsy* and *white* probes (62A for 208t^1 and 30C for 208t^2). The distribution of two other mobile elements (*mdg1:* 11C, 17A and 19C; mdg3: 13BC) was constant and identical for both 208 and its transformed derivatives.

Southern and Northern blot analyses. We have shown previously (Lyubomirskaya et al. 1990) that strain SS contains only *gypsy* copies without the variable *Hin*dIII site (Bayev et al. 1984). In contrast the *gypsy* used for injections contained this site, allowing us to use *Hin*dIII digestion in Southern blot experiments to estimate the number of integrated *gypsy* copies. The 3.4 kb *Hin*dIII-*Eco*RI fragment from the right half of the element was used as the hybridization probe. In this case each copy of *gypsy* originating from the introduced element produces a 1.6 kb fragment from the internal part of the element and a fragment more than 2.9 kb long with its left end located in the element and its right end in flanking sequences.

Fig. 1 presents restriction maps for two of these *gypsy* variants (Fig. 1a, b) and the results of Southern (Fig. 1C) and Northern (Fig. 1C) blot analyses of DNA and poly(A)$^+$ RNA isolated from the original stable strains and their transformed derivatives. In *Hin*dIII digests, both stable strains lack a 1.6 kb band, indicating the absence of *gypsy* copies carrying the variable *Hin*dIII site, while all the transformed derivatives have this band. Only one additional band longer than 2.9 kb is present in each of the transformed 208 derivatives, reflecting the presence of only one *gypsy* copy integrated in their genomes. In the case of MSn^1 and MSn^2 the intensity of the 1.6 kb band is much higher and several bands longer than 2.9 kb can be seen, indicating amplification of the introduced *gypsy* sequences.

To exclude the possibility that *gypsy* was not amplified in 208 transformed derivatives because it cannot be transcribed for some reason (e.g. position effect of integration site), we performed a Northern blot analysis of poly(A)$^+$ RNA isolated from all transformed derivatives. The results of these experiments are shown in Fig. 1d. It can be seen that in all transformed 208 derivatives *gypsy* is transcribed, producing poly(A)$^+$ RNA of normal size. The differences in transcription intensity must be due to differences in copy number between transformed derivatives of SS and 208 resulting from *gypsy* amplification in MSn^1 and MSn^2.

Thus, the introduction of a single transpositionally active *gypsy* copy into the genome of SS transforms it into a genetically unstable strain, in which *gypsy* trans-

Table 3. Conditions for manifestation of genetic instability in the MS system

Strain	Active *gypsy* transpositions are possible	Presence of active *gypsy*	Genetic instability
208	−	−	−
208t[1, 2, 3]	−	+	−
SS	+	−	−
MS, MSn[1, 2]	+	+	+

The matrix indicates that both conditions listed are required for manifestation of genetic instability

poses with high frequency. This ability to be converted from SS into MS is a peculiar feature of this particular strain. We suggest that gene(s) responsible for the activation of *gypsy* transposition are mutated in SS and that it is stable only because transpositionally active copies are absent. Therefore genetic instability in this system is caused by the combination of two factors: mutation(s) in gene(s) regulating *gypsy* transposition and the presence of transpositionally active *gypsy* copies (Table 3).

Discussion

In our previous papers (Kim et al. 1990; Kim and Belyaeva 1991; Lyubomirskaya et al. 1990) we described in detail the genetic characterization of MS and molecular analysis of the structure of *gypsy* elements in this strain. Two distinct *gypsy* subfamilies were identified and the data obtained allowed us to suggest that these subfamilies differ in transpositional activity. However, the presence of the transpositionally active *gypsy* variant appeared to be necessary, but not sufficient for its elevated rate of transposition. Therefore, we proposed that some cellular gene(s) are involved in inducing the genetic instability caused by *gypsy* transpositions in MS. The present paper describes experiments to test this proposition.

We first introduced one MS chromosome into the SS genome and found that each of 3 MS chromosomes in the environment of SS chromosomes is sufficient to confer the properties of MS. Since complete MS chromosomes were introduced into SS, it was impossible to decide whether the introduction of a single *gypsy* copy is sufficient for the appearance of genetic instability in SS, however. We have shown that *hobo* also undergoes active transposition in MS (Kim and Belyaeva 1991), but not in SS. Although these elements transpose autonomously it was still possible that they are both necessary for genetic instability in this system. To answer these questions we injected a transpositionally active *gypsy* variant into the germline of SS. As a control we injected the same element into another stable laboratory strain unrelated to SS. As was mentioned above, no SS derivatives with pigmented eyes were obtained. In a second series of injection experiments another construct was used and transformed w^+ flies were obtained. The main difference between these constructs was the orientation of *gypsy* in the Casper vector. We consider that this does not affect the expression of *gypsy* itself, and therefore, presumably does not influence the differences in phenotype associated with *gypsy* integration in the transformed derivatives. However, the possibility that different constructs may be transcribed with different efficiencies cannot be excluded.

All the transformed derivatives of both originally stable strains contain the active *gypsy* variant integrated into their genomes and this element is transcribed, producing poly(A)$^+$ RNA of normal size. The results of such integration are dramatically different in the two strains, however. The transformed derivatives of 208 remain stable and no new *gypsy* copies have been detected to date. In contrast, in transformed derivatives of SS, *gypsy* undergoes amplification and active transposition. As a result, the originally stable strain becomes unstable, demonstrating all the properties of MS.

To date, nothing is known about the nature of factors influencing the transposition of *gypsy* in this system. But since *gypsy* is actively transcribed in transformed derivatives of both 208 and SS and only the latter manifests genetic instability, it can be suggested that the modifiers may have an effect at the post-transpositional level. Studies on localization of gene(s) responsible for the activation of *gypsy* transposition in SS are now in progress.

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