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The possible effect of transposons on the *Drosophila melanogaster* somatic mutation and recombination test

Lyudmila Zakharenko*, Mariya Perepelkina

Institute of Cytology and Genetics, the Siberian Division of the Russian Academy of Sciences, Novosibirsk, Russia

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ABSTRACT

The wing somatic mutation and recombination test (SMART) using transheterozygotes for the *Drosophila melanogaster* third chromosome markers *mwh* (multiple wing hairs) and *flr*^{3} (flare-3) has proved to be efficient in genotoxicity screens. The genetic background of the *D. melanogaster* strains affects the frequency of identification of somatic mutant spots. The genetic background appears to be also of importance in hybrid dysgenesis (HD) revealed in crosses between flies from genetically distant *D. melanogaster* strains. In this study, we analyzed *mwh* and *flr3*/*Ser D. melanogaster* strains for the presence of the *P* and *hobo* transposable elements that induce genetic instability in P–M and H–E dysgenic crosses, respectively. According to PCR these strains lack the *P*-element. The *mwh* strain does not contain the *hobo*-element too, while *hobo* is present as a full-length variant and its numerous defective derivatives in the *flr*^{3}/*Ser* genome. Based on the crosses the reference E- and H-strains to strains used in SMART, the *flr*/*Ser* were assigned to the H-type and the *mwh* to the E-type. Fluorescence *in situ* hybridization reveals over 50 *hobo* hybridization sites scattered throughout the *flr*^{3}/*Ser* genome. Thus, there is a basis for H–E HD in the strains we studied. However the *mwh*/*flr*^{3}, regardless of cross-direction showed higher fertility than the *mwh* females. Cross-direction had also no affect on hybrid fertility. This meant that they did not exhibit the major HD symptoms. In our view, the significance of the TE activity as a cause of HD has been overestimated, in the case of H–E HD at least. The majority of the mutant spots in SMART result not so much from mutations or TE transposition as from recombination events, even in the case of balancer individuals.

1. Introduction

The wing somatic mutation and recombination test (SMART) in *Drosophila melanogaster* has been used to estimate the genotoxicity of chemicals, electromagnetic waves and other environmental agents from 1984 [1–4]. The frequency of somatic recombination and mutations increases under the effect of mutagens and, as a result, the quantity of cells with mutant hairs rises in the *D. melanogaster* wing cells. SMART has gained wide recognition. Its results agree well with those of the *in vivo* mammalian genetic tests [5]. It was found that the frequency of somatic mutant spots is dependent on the genetic background of *D. melanogaster* strains [6–7]. The genetic background is also a key factor in the manifestation of HD induced by certain transposable elements [8,9]. The *P* and *hobo* transposons have appeared in the *D. melanogaster* genome in the beginning of the twentieth century. They are extremely transposable [10–13] and produce the so-called P–M (Paternal–Maternal) and H–E (*HOBO*–Empty) HD. When the female genome does not contain a full-length active *P*-element, while the male genome does, progeny shows genome instability as an increase in the frequency of chromosomal rearrangements, chromosomal disjunction, and a decrease in fertility. This effect is most prominent at high temperature. As for *hobo*, the maximal rate of *hobo* transposition was observed at 25 °C [14].

It has been previously thought that transcription of TEs is limited to germ-line cells [15]. Subsequently, cases of transposition of *Mdg4* (*gypsy*) and *hobo*-element into somatic cells of a genetically unstable strain of *D. melanogaster* were described [16]. Transposition of *hobo* in somatic cells has been demonstrated for some strains of *D. melanogaster*, which contained a full-length variant of *hobo* capable of encoding active transposase [17]. Wing mutant spots at higher frequencies under the P–M HD condition have been also observed in strains of *D. melanogaster* reared at 29 °C [18].

The transheterozygotes for the wing spot test are obtained by crossing laboratory flies of different origin. This raised the question whether there is a basis for HD in this situation. We demonstrated that both strains (*mwh* and *flr*^{3}/*Ser*) lack the *P*-element and this allowed exclusion of the P–M HD as a factor contributing to the reproducibility of the SMART results. The *mwh* strain lacks *hobo*, while the *flr*^{3}/*TM3,Ser* genome harbors a full-length *hobo* variant and numerous copies of its defective derivatives. Thus, H–E HG would be manifested in *mwh*/*flr*^{3} heterozygotes. However, a

* Corresponding author. Tel.: +7 383 3332906; fax: +7 383 3331278.
E-mail address: zakharlp@bionet.nsc.ru (L. Zakharenko).

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Table 1
The frequency of spontaneous and γ-rays induced mutations in *Drosophila* wing cells in conditions of permissive (*mwh*/*flr*^{3}) and forbidden recombination (*mwh*/*TM3,Ser*).

Genotype, treatment	N[a]	Single *mwh* spots			Twin[b]	n[c]	n/N
		1	2	>2			
mwh+/+flr	72	16	7	3	0	26	0, 36
mwh+/+TM3	54	17	1	0	0	18	0, 33
mwh+/+flr, 5 Gy	56	88	56	95	87	326	5, 82
mwh+/+TM3, 5 Gy	58	58	13	18	0	89	1, 54

[a] Number of wings.
[b] Twin mutant spots (*mwh* and *flr*^{3}).
[c] Number of spots per wing.

decrease in the fertility of hybrids, a major evidence of HD, was not manifested in these crosses, which was contrary to expectation. Direction of cross also did not affect hybrid fertility.

Based on analysis of the data in the literature and ours, it is concluded that most mutant spots detected by SMART result from recombination. The TE somatic activity is with low rate (if detectable at all) in the wing spot test results.

2. Materials and methods

2.1. Strains

D. melanogaster strains: *mwh*/*mwh* (*multiple wing hairs*) (3–0.3); *flr*^{3}/*In(3LR)TM3*, *ri p*^{p} *sep bx*^{34e} *e*^{s} *Ser–flare* (3–38.8), henceforth referred to as *flr*^{3}/*TM3,Ser*; *ORRflr3*/*TM3,Ser* [19,20]; *CantonS*; reference E-strains *Harwich*^{white} and reference H-strain *23.5 MRF*/*CyL*^{4}.

2.2. Experimental procedures

For fluorescence *in situ* hybridization (FISH) squashed preparations of salivary glands were heated at 60 °C for 1 h and denatured in 0.07 M NaOH for 3 min. Cloned full-length *hobo*-DNA provided by Lim [21], was used as a probe. The probe was labeled by nick translation with biotinylated dUTP (Medigen, Novosibirsk, Russia). The solution for *in situ* hybridization was of the following composition: 50% formamide, 10% dextran sulfate, 4 × SSC, 1 × Denhardt solution, 0.1 M phosphate buffer, pH 7.6, and 10–20 ng labeled DNA per slide. Hybridization was performed overnight at 37 °C. After hybridization, the squashed preparations were washed with 2× SSC three times for 5 min at 42 °C. The detection of biotin was performed with avidin-FITC (Molecular Probes). Vectashield with DAPI (Vector, USA) was used as an antifading solution. The preparations were examined with an Axioskop-2 Plus microscope equipped with a black-and-white CCD VC44 camera (PCO). The images were processed with the ISIS3 program (METSYSTEMS GmbH).

The PCR assay was standard using fly genomic DNA. PCR was performed using forward (gag agg aaa ggt tgt gtg cgg acg a) and reverse (gct tgc aat aag tgc gag tga aag g) primers for the *P*-element and (1′-gcgccatacataatgattg (for), 2′-ctattgcgagttgtttag (rev), 3′-agg ctt tag agg gca ata ccc gtt (for), 4′-aag caa tgc tgc tgc aga aag gtc (rev) primers for the *hobo*-element.To sequence TPE repeats, genomic DNA was subjected to PCR using 3 (for) and 2a (aac act ctt cag ctg ctg cgc ta (rev)) from the central part of *hobo* as a primers. Then, the PCR product were separated from 1% agarose gel after electrophoresis using Wizard SV Gel and PCR Clean-Up System (Promega) and sequenced using primer 5′ (tgc agc agc att gct taa gga aag (for)). The PCR product was sequenced using BigDye according the standard protocol of the Interinstitutional DNA Sequencing Center (Novosibirsk, Russia). The primers have the following positions along the complete *hobo* sequence (Accession no. M69216): 1, 318–338; 2, 2292–2275; 2a, 2180–2157; 3, 1223–1246; 4, 1654–1677; 5, 1662–1685. Distances between the primers are: 1–2, 1956 bp; 1–4, 1358 bp; 3–2, 1052 bp; 2a–5, 528 bp.

GD sterility tests were performed as described in [22].The effect of γ-irradiation on the frequency of wing mutation spots was estimated as in [19,20] after exposure of the third instar larvae to 5 Gy rays.

3. Results and discussion

The idea was to determine whether there is an influence of *hobo* and *P* transposable elements on the somatic mutation and recombination test.

The PCR analysis demonstrated that the tested strains lack the *P*-element (data did not shown) and, consequently, the P–M HD may be ruled out as a factor contributing to the SMART results. The *hobo*-element is absent from the *mwh* genome flies, while a full-length

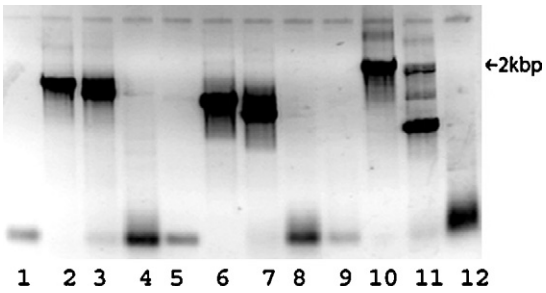

Fig. 1. PCR of the *Drosophila melanogaster flr*^{3}/*TM3,Ser* genomic DNA with *hobo*-primers. H_2O (lanes 1, 5, 9); positive control–cloned *hobo*-DNA (lanes 2, 6, 10); *flr*^{3}/*TM3,Ser* (lanes 3, 7, 11); *mwh*/*mwh* (lanes 4, 8, 12). Primers 1 and 4 (lanes 1–4); primers 3 and 2 (lanes 5–8); primers 1 and 2 (lanes 9–12).

hobo and its defective variants (Fig. 1) are found in the *flr*^{3}/*TM3,Ser* genome.

Souames et al. [23,24] believed that the *hobo* invasive potential is due to the number of TPE repeats in the variable part of *hobo*. They have suggested that the activity of the *hobo*-element decreases with the TPE repeat number. It should be taken into consideration that *hobo* has invaded the *D. melanogaster* genome in the fifties of the last century. It then possessed three full-length TPE repeats. Subsequently, mutations gave rise to other *hobo* versions with different TPE numbers and TPE structures. These new versions are in their majority local and few [23,24]. We sequenced the variable part of *hobo* from the *flr*^{3}/*Ser* fly DNA (Fig. 2) and detected three full-length perfect TPE repeats flanked by irregular ones (actccaaga actccagaa actccagaa actccagaa agtctagaa). The structure of the variable part of *hobo* in the *flr*^{3}/*Ser* strain is characteristic of strains recovered from nature in the middle of the past century.

We additionally hybridized *hobo* DNA *in situ* on salivary gland polytene chromosome squashed preparations. The FISH data confirmed that the *mwh* is hoboless, whereas *flr*^{3}/*TM3,Ser* contains over 50 *hobo* hybridization sites distributed throughout the genome (Fig. 3). Thus, there exists a basis for the H–E HD in the strains under study. There was reason to expect that in the crosses of females lacking *hobo* with the males that harbor a full-length *hobo*, H–E dysgenesis would be fully manifested.

The presence of a full size *hobo* copy in the strains under the analysis was detected by PCR of *hobo* open reading frame (ORF) and subsequent sequencing of its central part. Presence or absence full size *hobo* in the genome does not allow reference of a tested strain

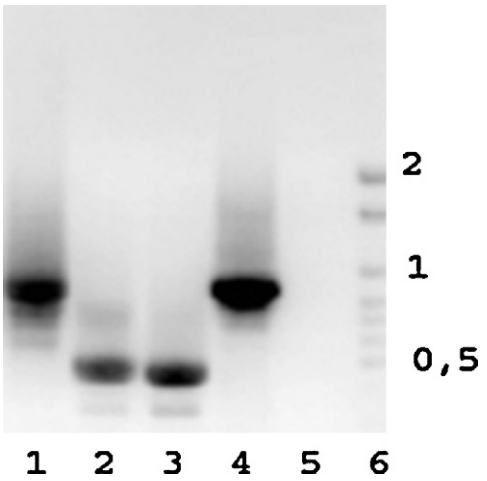

Fig. 2. PCR of the genomic *flr*^{3}/*TM3,Ser* DNA before sequencing. *flr*^{3}/*TM3,Ser* (lanes 1, 2); positive control, cloned *hobo*-DNA (lanes 3, 4); H_2O (lane 5); marker DNA (lane 6). Primers 2a and 3 (lanes 1 and 4); primers 2a and 5 (lanes 2 and 3).