

GENERAL
GENETICS

Characterization of Insertion Mutations Leading to Mitosis Abnormalities in *Drosophila melanogaster* by Means of the Reporter Gene-Containing Transposon

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Abstract—Transpositions of the vector P[ArB] into the regions 78D, 61F, and 85F of chromosome 3, which result in various anomalies of mitoses in neural ganglions of homozygous larvae, were obtained by insertion mutagenesis. The tissue specificity of regulatory elements controlling the reporter gene was studied by staining for the activity of the β -galactosidase reporter gene of the vector P[ArB]. These regulatory elements are suggested to be the enhancers of the genes carrying insertions. In all studied mutants, staining for β -galactosidase was found in tissues containing actively proliferating cells. The staining of germarium in adult female ovaries was the most pronounced. The germarium staining pattern was used for the identification of novel insertions leading to mitosis abnormalities. The *P1003* (99F) insertion was found, which according to preliminary data leads to an increase in the mitotic index and anomalies of chromosome structure in neuroblasts of homozygous larvae. In addition, the *22w* (42A) insertion leading to chromosome arrest in metaphase was found.

INTRODUCTION

Mutations leading to anomalous mitosis in the third-instar larvae are revealed by the method of late lethals method [1], which is used for identifying genes responsible for cell division in *Drosophila*. By means of this method, we found earlier the *leopard nuclei* gene, which affects the chromosome disjunction and spiralization, in the region 57B1-12 of the second chromosome [2]. The recessive lethal mutation of the *leopard nuclei* *len*^{P103} was obtained by insertion mutagenesis using the transposon P[ArB]. This vector was synthesized in order to be applied in the *enhancer trap* method. It contains the gene *rosy*⁺ and the reporter gene β -galactosidase controlled by the weak promoter of P-element [3]. The β -galactosidase activity of this transposon can be recorded only if the inserted P[ArB] falls under the control of an adjacent strong enhancer. The pattern of the correspondent cDNA hybridization to the intracellular RNA was similar to the staining pattern for the β -galactosidase activity of the element P[ArB] inserted either into the considered gene or in immediate proximity to it. For instance, in [3], this kind of data is described with regard to the transposants A183.1F2; A3; A405.1M2; A208.1M2; A140.1F3 and the genes mapped in the respective regions. The similar data were obtained on the similarity between the staining pattern for β -galactosidase of the transposants B52.1M3; A107.1M3, and A401.1M3 and the hybridization pattern of cDNA of the genes adjacent to these transposants on the molecular map [4]. The expression pattern of β -galactosidase of the element P[ArB] inserted into the gene *inscuteable*, the transcription pattern revealed by the *in situ* hybridization to the cellular

RNA relevant to this gene, and the pattern of the specific antibody, which binds to the Insc protein, were completely identical [5]. This suggests that in the majority of cases, the expression pattern of the reporter gene in tissues is due to the tissue-specificity of the enhancer of the gene containing the insertion.

In a case of the earlier obtained mutation *len*^{P103}, which leads to abnormalities in cell division, staining for β -galactosidase activity was found in ovaries, testis, nervous ganglions, and imaginal disks of individuals heterozygous for the mutation, i.e., in those tissues, where intense proliferation occurs [2]. In this work, we attempted to elucidate whether the staining of actively proliferated tissues is a characteristic feature of the insertions leading to disturbances in cell division.

MATERIALS AND METHODS

The same lines and crosses shown earlier [2] were used (Fig. 1). In addition, we used a balancer In(3LR)TM6, *Hu*^P *ss*^{P88} *bx*^{3Ae} *Ubx*^{pls} *e* *y*⁺, obtained by transferring the transposon containing gene *y*⁺ from the balancer *y*⁺*CyO* into the balancer TM6 under the influence of the transposase source P[*ry*⁺ Δ 2-3]. To obtain preparations of mitotic chromosomes, the neural ganglions were treated with a 1% Na-citrate solution for 5 min, fixed in a mixture of methanol-acetic acid (3 : 1) for 20 min, dispersed in a drop of 60% propionic acid, dried in air, and Giemsa stained. Before staining for β -galactosidase activity, larval and adult fly organs were fixed for 20 min in 0.75% glutaraldehyde that was prepared in a 0.1 M sodium-cocadilate buffer. The fixator was then washed off with PBS (130 mM NaCl, 7 mM Na₂HPO₄ ·

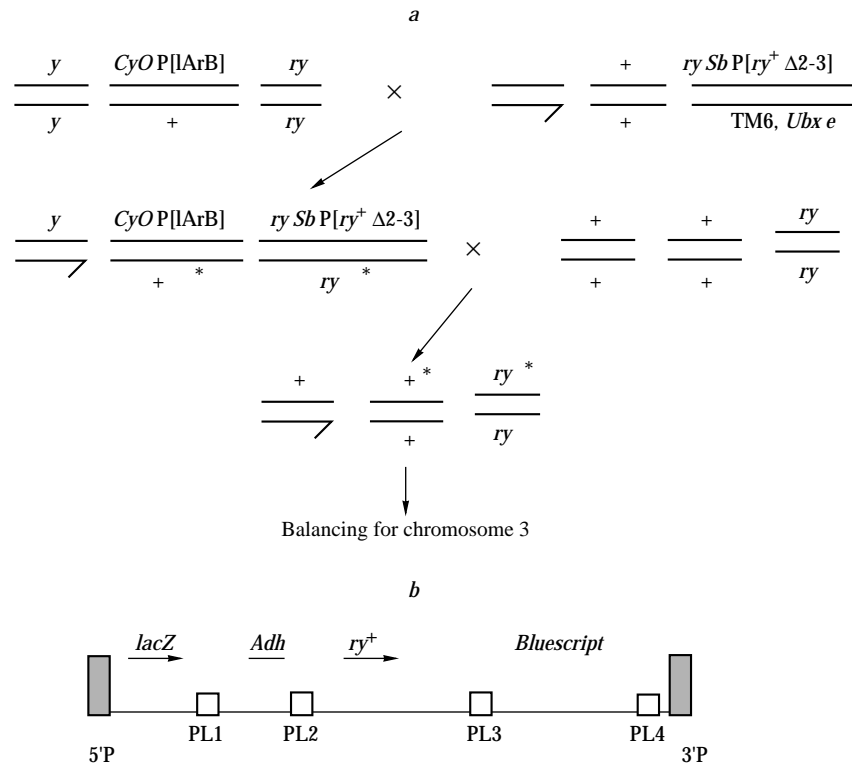


Fig. 1. Scheme of the transposition of vector P[IArB]; (a) chromosomes with possible insertions; (b) scheme of the plasmid P[IArB] [4]; (P) the terminal regions of the P element; (*lacZ*) the β -galactosidase gene from *E. coli*; (*Adh*) the alcohol dehydrogenase gene; (*ry*) the gene *rosy*; (*Bluescript*) a DNA fragment from *E. coli*, carrying the gene of resistance to ampicillin; (PL) polylinker regions; arrows indicate the transcription direction. The complete length of the vector is 19 kb.

2H₂O, 3 mM NaH₂PO₄ · 2H₂O, pH 7.0). Next, the organs were incubated overnight in a staining solution (10 mM NaH₂PO₄ · 2H₂O/Na₂HPO₄ · 2H₂O, pH 7.2; 150 mM NaCl; 1 mM MgCl₂ · 6H₂O; 0.3% Triton X-100; 3.1 mM K₄[Fe^{II}(CN)₆]; 3.1 mM K₃[Fe^{III}(CN)₆]; 0.2% X-gal). Poorly stained tissues were incubated in the same solution for 3 days.

The mitotic index was calculated as a ratio of the metaphase number to the total number of nuclei in a preparation.

To obtain preparations of polytene chromosomes for *in situ* hybridization, salivary glands were squashed in a mixture of glacial acetic acid–80% lactic acid–water (3 : 1 : 2). The vector Carnegie-20 containing the gene *ry*⁺ from *Drosophila melanogaster* which is also included in the vector P[IArB] was used as a hybridization probe. Klenow fragment and a start-primer from a “Fermentas” labeling kit (Latvia) were used for ³H-triphosphate incorporation into DNA. Hybridization of the probe to the chromosomes was performed at 37°C by a technique [6] in the modified hybridization buffer (0.2% Na pyrophosphate, 0.4% SDS, 20% dextran sulfate, 1 g/ml heparin, 0.4% ficoll 400, 0.4% polyvinylpyrrolidone, 40 mM EDTA) diluted with formaldehyde at a ratio 1 : 1.

The alleles of the gene *Klp61F* (*urcn*¹ and *urcn*³) and the deletion *Df(2)Ar14* overlapping this gene were provided by M. Fuller.

RESULTS

Fifteen cultures of those which according to genetic analysis contained lethal insertions in chromosome 3 were selected. In these cultures, individuals homozygous for the insertion survived to the third instar.

The cytological analysis of mitoses in neural ganglions of larvae homozygous for the insertions revealed anomalous metaphases in cultures *v40*, *v27*, and *v158*. Figs. 2a and 2b demonstrate that highly polyploid metaphases were in larvae *v40* and *v27* (the preparations were not treated with colchicine) and the cells containing chromosomelike structures, the number of which was not multiple of the diploid chromosome number, were encountered in the *v158* larvae. In Fig. 3, the hybridization *in situ* of the insertions under consideration is presented. As is seen, the insertions *v40*, *v27*, and *v158* are mapped in the regions 78D, 61F, and 85F, respectively. The complementation analysis showed that the insertion *v27* is a novel allele of the gene *Klp61F*, which encodes the kinesinlike protein and the mutations of which lead to the polyploidy [7]. Similarly, it was shown (C. Sunkel, personal communica-



Fig. 2. Metaphases of the mitosis in neural ganglions of larvae homozygous for the insertions. (a) *v40*; (b) *v27*; (c) *v158*.

tion) that the insertion *v158* is allelic to the mutations of the gene *aar* [8], impeding the anaphase progress. No alleles for the insertion *v40* were revealed in the adjacent chromosome regions (C. Sunkel, personal communication).

Figure 4 and Table 1 include the results of the expression analysis of the reporter gene of the studied insertions in various tissues of larvae and adult flies heterozygous for the insertions. As in the case of the gene *leopard nuclei* [2], staining was observed in neural ganglions and larval imaginal disks (Fig. 4a), in ovarian germarium (Fig. 4b), and in adult fly testis (Fig. 4c). In addition, the stained area were found in larval gut, which is probably a result of the endogenic β -galactosidase activity of *Drosophila*.

Apart from the substantial similarity in the staining patterns of the same organs characteristic of the three studied insertions, which is indicative of similar β -galactosidase expression, some differences should also be noted. For instance, it can be seen from Fig. 4, that along with the larval neural ganglions of *v40*, the “variegated” staining is observed in the large cells of presumably a ring gland (Fig. 3a). In addition, unlike the intense staining of the entire testis in heterozygotes

v27, in testis of heterozygotes *v40* and *v158*, the reporter gene expression was observed only at early stages of spermatogenesis. In the case of the insertion *v40*, the high expression was also observed in the

Table 1. β -Galactosidase activity in tissues of heterozygotes for insertion mutations

Organs	<i>v27</i>	<i>v158</i>	<i>v40</i>
Larva			
Neural ganglion	+	+	+
Imaginal disks	+	+	+
Separate parts of cuticle	+	+	+
Gut	+	+	+
Gastric caeca	-	+	+
Peritracheal gland	-	-	+
Distal part of salivary gland	-	-	+
Adult flies			
Testis	+	+	+
Accessory gland	-	-	+
Germarium	+	+	+
Gut	+	+	+

Note: “+” is stained; “-” is not stained.

were found in the culture *P1003* (Table 2, Fig. 5). It was inferred from the calculations that the significance of mean differences was 0.999, according to Fisher's test. Only a part of data shown was used (Table 2). The significance of the mitotic index differences in homo- and heterozygotes is additional proof and points to the influence of the studied insertion on mitosis. However, it is not a final proof because the differences in mitotic

indices observed may result from the secondary mutation effect.

The insertion *P1003* was mapped in the region 99F of chromosome 3 by the *in situ* hybridization.

Thus, selection for the insertions leading to similar staining for the activity of the reporter gene P[1ArB] is shown to be a possible method of revealing genes

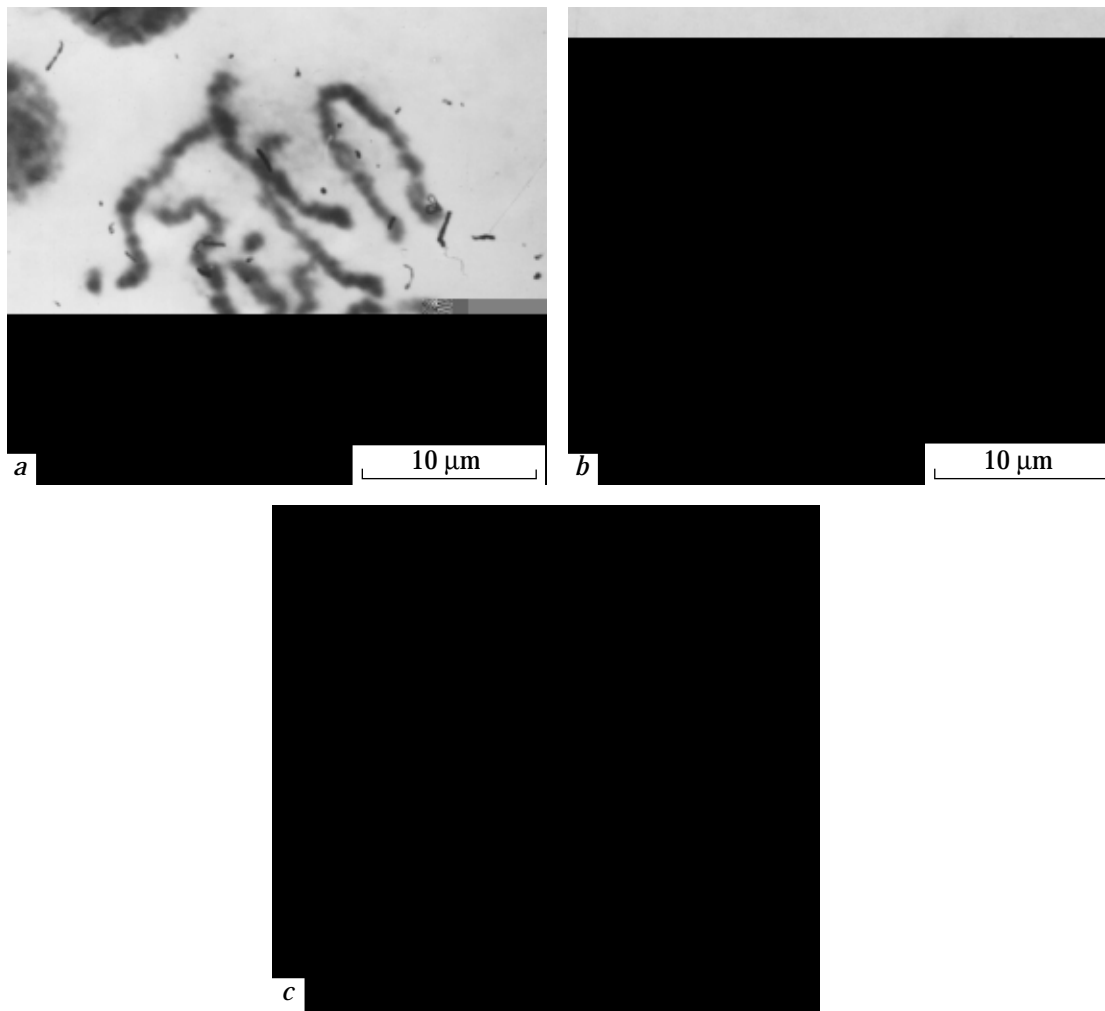


Fig. 5. Anomalous chromosome structures in homozygous larvae *P1003*: (a) long chromosomes of homozygotes with nondisjoined chromatids (the similar chromosomes were found in brain of *D. hindai*, *D. repleta*, and *D. virilis* [12]); (b) anaphase and metaphase in the wild type individuals; (c) metaphase plate of a homozygote. An arrow points to a gap in the autosome.

the balanced lines were used for screening, this approach has scarcely substantial advantages over the methods of identification of meiotic mutations [9, 10], mutagen sensitive mutations [11], and late lethals [1], because all of them are based on the usage of these lines. The great advantage of the staining pattern method is that the selection for individuals with the insertions in genes controlling cell division is based on tissue staining but not on progeny of these individuals as in other methods. This was confirmed by the following direct experiments.

One hundred and twelve females with newly induced insertions were crossed to males carrying a balancer for chromosome 3. After the progeny was obtained from every female, their ovaries were isolated and stained for β -galactosidase activity. Of the obtained lines, 43 were selected for a trait of basic-female-germarium staining and their male progeny was crossed to the females carrying a balancer for chromosome 2. Of these lines, eight containing lethals for chromosomes 2

and 3 were selected. To date, we have analyzed in detail the insertion 22w from this collection, in which the high frequency of cells arrested in metaphase was observed, and metaphase chromosomes were supercompact, i. e., they lost characteristic structure and turned into clumps. The insertion was associated with the inversion with breakpoints 42A and 57F. The transposon DNA was mapped in 42A. The more detailed description of this insertion effects will be reported later.

DISCUSSION

We have found insertions *v40*, *v27*, and *v158*, which as the earlier revealed insertion *P103* [2], lead to anomalous mitoses in homozygous larvae, suggesting that the corresponding genes code for proteins involved in the control of cell division. The data on allelism between *v27* and *Klp61F* and also *v158* and *aar* directly confirmed this suggestion in at least two cases.

Table 2. High mitotic index in homozygotes *P1003*

Number of experiments	Total number of nuclei (N)	Anaphase number (A)	Metaphase number (M)	Mitotic index (A + M)/N	Mean mitotic index
Control <i>P1003/+</i>					
1	9084	5	21	0.0032	0.027
2	6478	4	12	0.0025	
Experiment <i>P1003/P1003</i>					
1	5301	22	83	0.0198	0.011
2	2815	15	29	0.0156	
3	10221	11	71	0.0085	
4	5154	7	21	0.0054	
5	7293	15	59	0.0101	

Studying the expression of the reporter gene in these insertions revealed the common pattern of tissue staining in the cases under consideration. The expression is found in actively proliferating tissues (neural ganglions and imaginal disks of larvae), testis, and ovaries of adult flies. In total, these results point to the fact that in the case of the studied insertions, the reporter gene is controlled by the enhancer, which normally regulates the activity of cell-division genes. The common staining pattern characteristic of these gene expressions in tissues can be considered a trait of the insertion into the cell division gene. We believe that ovaries, in which expression is revealed in germarium, are the most suitable for this application method.

The experimental data obtained suggest that the method of staining patterns can be used for genetic analysis of cell division in *D. melanogaster*. Obtaining mutations leading to failures in cell division is significantly facilitated, because the reporter gene staining pattern can be revealed in heterozygous individuals.

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