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RAPID SCREENING OF SPONTANEOUS AND RADIATION-INDUCED STRUCTURAL CHANGES AT THE VESTIGIAL GENE OF DROSOPHILA MELANOGASTER BY POLYMERASE CHAIN REACTION

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A total of 27 independent isolated spontaneous and gamma-ray-induced heritable mutations at the vestigial gene of Drosophila melanogaster were analysed by a rapid deletion screening method with polymerase chain reaction (PCR) amplification. According to the results obtained 36.4 % (4 of 11) of spontaneous mutants and 62.5 % (10 of 16) of gamma-ray-induced ones have revealed deficiency of one or more fragments studied. The rest of spontaneous and radiation mutants showed no alterations in the PCR patterns, indicating a possible small scale changes (point mutations) inside the gene region studied or, probably, the gross lesions situated elsewhere. The distribution of the mutation damages in the gene region studied are discussed.

The investigation has been performed at the Laboratory of Nuclear Problems, JINR.

Оценка спонтанных и радиационно-индуцированных структурных изменений гена vestigial Drosophila melanogaster методом полимеразной цепной реакции

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Методом быстрого анализа делеций с помощью полимеразной цепной реакции (ПЦР) были изучены 27 спонтанно возникших и радиационно-индуцированных наследуемых мутаций гена vestigial дрозофилы. В соответствии с полученными результатами 36,4 % (4 из 11) спонтанных мутантов и 62,5 % (10 из 16) мутантов, индуцированных гамма-излучением, показали отсутствие одного или более из изученных фрагментов. Остальные спонтанные и радиационные мутанты имеют нормальные размеры соответствующих продуктов ПЦР, что указывает на возможные малые изменения (точковые мутации) внутри изучаемого района гена или, вероятно, на существенные повреждения в каком-то другом районе гена. Обсуждается распределение установленных делеций в изученной области гена.

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1. Introduction

The development of the polymerase chain reaction (PCR) to amplify the specific short fragments of the gene for which some sequence data exist [1] has given a new powerful approach in mutation analysis and, in particular, for rapid initial screening of the deletion

mutations. For this purpose, PCR has been recently used for analysis of the gene mutations in human [2] and animal [3] somatic cells. These and other studies have shown also that PCR can be used to generate sufficient material for further sequence analysis of the putative point mutations [4].

As a part of our ongoing effort to analyse the molecular nature and spectrum of the heritable gene mutations induced by various types of ionizing radiation in animal germ cells, we adopted the PCR technique for initial screening of point and deletion mutations at several Drosophila genes. In this communication, we present the first data on the molecular lesions in random set of spontaneously arising and gamma-ray-induced mutations at the vestigial (vg) locus of Drosophila melanogaster. The same vg phenotype of the mutants studied, being characteristic for mutational damages at the central region of the gene (i.e., region including intron 2 – exon 3 – intron 3 – exon 4), has determined first of all the search for molecular alterations precisely at this gene region.

II. Materials and Methods

The random sets of 11 spontaneous and 16 gamma-induced point vg mutations analysed here (Table 1) were recovered among other visible mutants in the large-scale experiment (1979—1994 years) and described earlier [5,6].

Table 1. Codes and origin of the vg mutations analysed

№	Code	Origin	№	Code	Origin
1.	vg88d44	Spontaneous	1.	vg79b1	gamma-ray, 40 Gy
2.	vg88d38	— " —	2.	vg79d5	— " —, 10 Gy
3.	vg88118	— " —	3.	vg81a	— " —, 60 Gy
4.	vg88e26	— " —	4.	vg81124	— " —, 10 Gy
5.	vg88g10	— " —	5.	vg81k1	— " —, 10 Gy
6.	vg88g32	— " —	6.	vg83b24	— " —, 40 Gy
7.	vg88h7	— " —	7.	vg83b27	— " —, 40 Gy
8.	vg88c1	— " —	8.	vg83c5	— " —, 40 Gy
9.	vg9112	— " —	9.	vg83c7	— " —, 40 Gy
10.	vg9119	— " —	10.	vg83c24	— " —, 40 Gy
11.	vg92g18	— " —	11.	vg83c42	— " —, 40 Gy
			12.	vg87e39	— " —, 10 Gy
			13.	vg87e140	— " —, 20 Gy
			14.	vg87g77	— " —, 20 Gy
			15.	vg87h31	— " —, 40 Gy
			16.	vg88d101	— " —, 60 Gy

High molecular weight DNAs were isolated from mutant and wild-type (D-18 and D-32) flies by a combination of proteinase-K treatment and phenol-chloroform extraction according to the standard protocols [7,8].

Oligonucleotide primers (Table 2) for PCR amplification of two fragments of intron 2 as well as exon 3 and exon 4 were synthesized on an Applied Biosystem Inc. (ABI) 380B DNA synthesizer and purified by NEN-sorb column.

Table 2. Oligonucleotide primers for PCR amplification of the vg gene regions analysed

Gene fragments	Size of fragment amplified (bp)	Primer sequences (5'–3'); a — forward, b — reverse
intron 2	750	a – ttccgcaactcaatgttggc
(fragment I)		b – gaattcagctccctggttta
intron 2	about 1900	a – caaccgatcgcagataaacc
(fragment II)		b – cccgaggaggagtacaaatc
exon 3	531	a – agatcttgaaatgtaatcca
		b – cagcttcgctgcgcggcacg
exon 4	377	a – ctgtctgatttcccagcac
		b – gttgttgtagttgagggcg

For amplification of individual gene regions, genomic DNA template (250—500 ng) was mixed with 50 pmol of each primer in a total volume of 50 μl consisted of 16.6 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl, pH 8.8, 2.7 mM $MgCl_2$, 0.25 mM dNTPs, 10 % DMSO and 2.5 U of Taq-polymerase (Fermentas). The mixes were reacted for 30 cycles of template denaturation at 94°C for 30-50 sec., primer annealing at 52-60°C for 30-50 sec. and DNA extension at 72°C for 1-1.5 min. with preliminary denaturation for 4 min. and final extension for 7 min. One-fifth of the PCR product was used for analysis by 1.7 % agarose gel electrophoresis and staining with ethidium bromide.

Mutants were devided into several categories based on their PCR patterns. «Total deletion» mutants include ones for which no representative intron or exon fragment was synthesized. «Partial deletion» mutants show PCR products (amplicons) the sizes of which are less than those for expected fragments. «Multi-deletion» mutants include mutants for which several fragments are lacking simultaneously. Finally, mutants with «normal PCR patterns» represent those which contain all PCR products of expected sizes. These mutants could have «point changes» (transitions, transversions, frameshifts) or small deletions which could not be detected by PCR [9].

III. Results

With primers (Table 2) designed from the published vg DNA sequence [10], the introns and exons of Drosophila vg gene amplified individually. The primers were located at a distance that was close to the immediate splice junctions.

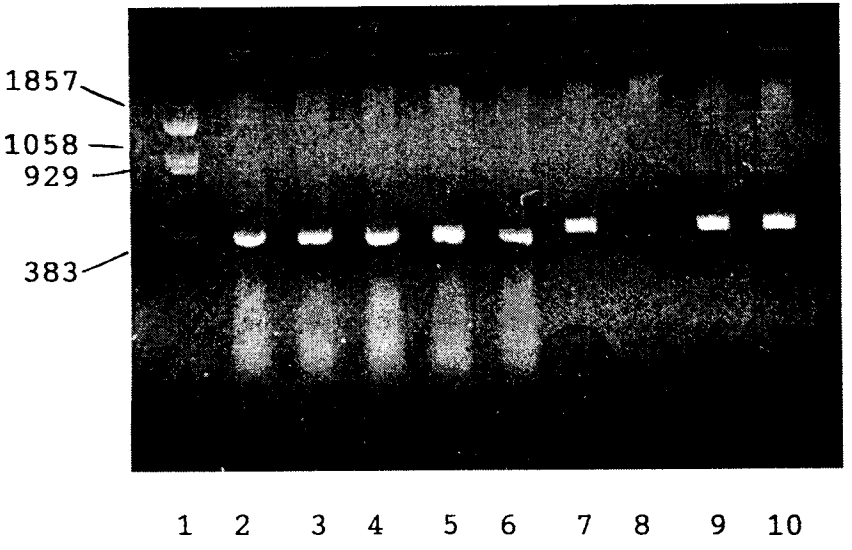

Fig.1. Gel illustrating the products of PCR reactions with exon 4 primer. Lanes: (1) — Mva I-digested pBR322 marker with some molecular sizes (in bp) shown to the left; (2—10) — vg87e39, vg87e140, vg87g77, vg87h31, vg88d101, vg88d44, vg88e26, vg88g32, vg88h7, respectively

A representative panels of various «point» vg mutations analyzed by PCR using exon 4, fragment II and fragment I of intron 2 primer pairs are illustrated by Figs. 1, 2 and 3, respectively. As Fig.1 shows, the spontaneous mutants vg88e26 (lane 8) have total

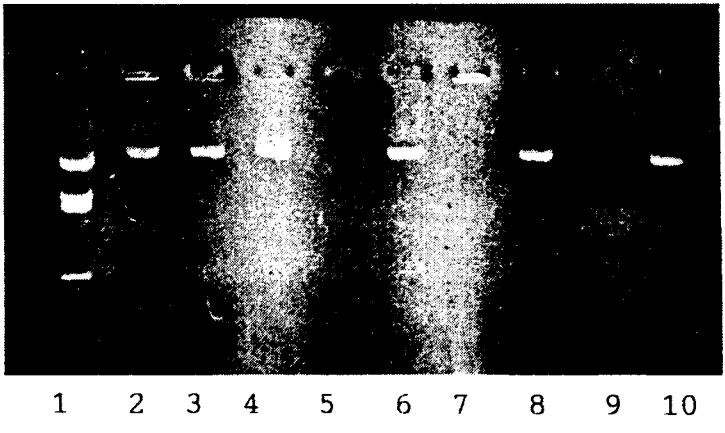

Fig.2. Gel illustrating the products of PCR reactions with fragment II of intron 2 primer pair. Lanes: (1) —see Fig.1; (2—10) — vg92g18, vg88g32, vg88h7, negative control (PCR mix without DNA template), vg79b1, vg79d5, vg81124, vg83b27, vg87e39, respectively

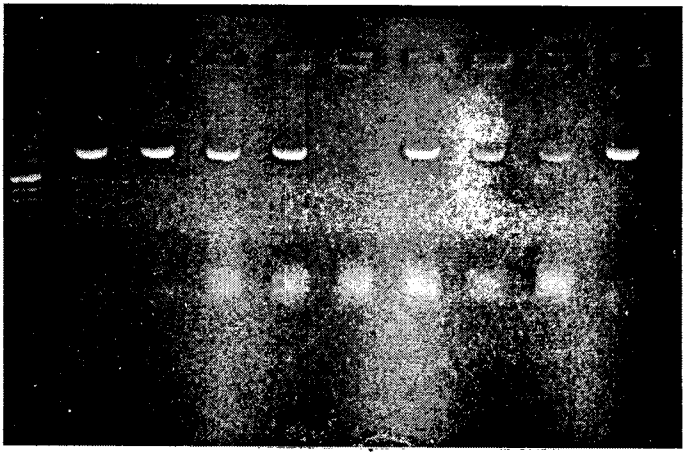

Fig.3. Gel illustrating the products of PCR reactions with fragment I of intron 2 primer pair. Lanes: (1) — pUC Mix marker; (2—10) — vg+, vg81a, vg81k1, vg83b24, vg83c5, vg83c7, vg83c24, vg83c42, vg87e39, respectively

deletion of exon 4 whereas there is clear a gain in weight of this exon for 3 other spontaneous mutants presented (lanes 7, 9, 10) in comparison with its normal size observed for the 5 radiation-induced mutants listed (lanes 2-6). However, as the preliminary results indicate, three other gamma-ray-induced mutants have a total (vg83c5) or partial deletions (vg83c42, vg87e95) of exon 4 (data have not shown). Figure 2 illustrates a normal PCR product patterns for the fragment II of intron 2 (about 1900 bp) among spontaneous (lanes 2—4) and radiation (lanes 6, 8, 10) mutants and a total deletion of this fragment for the two gamma-ray-induced ones (lanes 7, 9). The total or partial loss of this fragment is observed too for the four other radiation mutants (vg81k1, vg83c24, vg82hl3, vg88f21) and for the three spontaneous ones (vg88el8, vg89cl, vg9119) as well (data have not shown).

PCR amplification of the fragment I of intron II has revealed a total deletion of the fragment for the three gamma-ray-induced (vg83c5, vg87g77, vg87hl3) and for one (vg88e26) spontaneous mutants among a total of 10 mutants studied on this point (see Fig.3 for vg83c5).

IV. Conclusion

The data presented in this communication indicate that 36.4 % (4/11) of spontaneous and 62.5 % (10/16) of gamma-ray-induced vg mutants studied exibit total or partial deletions of some gene region in question. Thus, the radiation mutants with deletions arise twice as often as the spontaneous ones. It is of interest to note too that the several instances were observed where more than one PCR product were missing (vg83c5, vg87hl3,

vg88e26) indicating that such mutations are total gene deletion (at least deletion of the total gene region studied).

The other intriguing fact is that the deletions detected are situated predominantly in the intron 2 showing that this gene region might be a «hot-spot» for spontaneously arising and radiation-induced mutational lesions. However, it needs to note that PCR patterns of exon 3 adjacent to intron 2 for such mutants have been not studied yet. It may be expected that the further our ongoing experiments will give more certain information on this questionable finding.

Acknowledgements

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