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MOLECULAR ALTERATIONS UNDERLYING THE SPONTANEOUS AND γ-RAY-INDUCED POINT MUTATIONS AT THE WHITE LOCUS OF DROSOPHILA MELANOGASTER

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The *white* locus in *D.melanogaster* was selected as a target gene for the study of the mutational spectra of spontaneously arising and radiation-induced gene mutations in a whole organism. Analysis of 6 spontaneous and 73 γ-ray-induced *white* mutations by a combination of cytological, genetic and molecular techniques revealed that on the chromosomal and genetic levels all spontaneous mutations showed themselves to be point mutants. The share of such mutants among all heritable radiation-induced gene mutations is about 40%, whereas the rest ones are due to exchange breaks (8%) as well as multilocus, single-locus or partial-locus (intragenic) deletions (52%).The DNAs from 4 spontaneous and 17 γ-ray-induced point mutants were analysed by *Southern* blot-hybridization. The three spontaneous and 7 radiation mutants showed an altered DNA sequence at the left (distal) half of the *white* gene due to insertion or DNA rearrangement. The rest (58%) of the radiation-induced point mutations did not indicate any alternations in this part of the gene as detected by this technique and probes employed.

The investigation has been performed at the Department of Radiation Safety and Radiation Researches, JINR.

Молекулярные изменения, обусловливающие спонтанные и индуцированные γ-излучением точковые мутации в локусе white у Drosophila melanogaster

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Локус *white* у *D.melanogaster* был избран в качестве гена-мишени для изучения спектра спонтанных и радиационно-индуцированных генных мутаций в целостном организме. Комплексный цито-генетико-молекулярный анализ 6 спонтанных и 73 индуцированных γ-излучением мутаций *white* показал, что на хромосомном и генетическом уровнях все спонтанные мутации проявляют себя как точковые. Доля таких мутаций среди наследуемых радиационно-индуцированных составляет около 40%, тогда как остальные обусловлены хромосомным разрывом при формировании обменных аберраций (8%), либо мультилокусными, однолокусными или частичными (внутригенными) делециями (52%). ДНК от 4 спонтанных и 17 радиационных мутантов точковой природы была изучена с помощью блот-гибридизации по Саузерну. Установлено, что 3 спонтанных и 7 радиационных мутаций имеют молекулярные изменения в левой (дистальной) половине гена, обусловленные инсерациями и/или структурными перестройками ДНК. Остальные

из изученных точковые радиационно-индуцированные мутации (58%) не содержат изменений в этой части гена, выявляемых данным методом и пробами, использованными в работе.

Работа выполнена в Отделе радиационной безопасности и радиационных исследований ОИЯИ.

I. Introduction

Summing up the main fundamental generalizations of the classical radiation genetics in the fields concerning gene mutation processing, H.Muller in his Nobel lecture had particularly laid stress among the others that the radiation-induced point (on the chromosomal level) mutations in animal cells are similar to the natural (spontaneous) ones in all features on which the comparison had been done [1]. This generalization founded mainly on the data from the genetical and/or cytological experiments with *Drosophila* before molecular era in modern biology and genetics is basic so far to our risk estimation procedure using the doubling dose method [2].

The current studies of the molecular nature of the point mutations arising spontaneously at the different loci of *Drosophila* (*Hw*, *w*, *ry*, *f*, etc.) had unequivocally shown that the bulk of them had resulted from insertion of the dispersed, repetitive DNA sequences, such as mobile elements or transposons [3].

On the other hand, the first results of the molecular analysis of point mutations induced by sparsely ionizing radiation at some of these *Drosophila loci* (*w*, *ry*) indicated that the main part of such mutants have arisen through large changes in the gene, especially deletions [4,5]. Recent analysis of radiation-induced HPRT- and APRT-deficient mutants of mammalian cells indicates also deletions as the major type genetic lesion recovered [6,7].

Thus, as the first molecular data show, the genetic alterations underlying the spontaneous and radiation-induced gene/point mutations in *Drosophila* germ cells seem to be qualitatively different, but the number of tested mutants are small. Moreover, spontaneously arising and radiation-induced mutations have been isolated in the genotypicly different *Drosophila* strains the difference of which on fine molecuar structure of gene studied and/or on DNA repair may determine the peculiarities of spectra of the gene mutations recovered.

To study this issue more precisely, the complex (genetic, cytogenetic, molecular) analysis of spontaneous and γ-ray-induced gene mutations in the different loci (*white*-w, *vestigial*-vg) and genotypes (*wild-type D-32*, *c(3)G*, *phr*-) of *Drosophila melanogaster* was started. In this communication, we present the genetic and cytogenetic characteristics of spontaneous and γ-ray-induced (5-60 Gy) gene mutations at the *w* locus from wild-type D-32 line as well as the first data on the molecular nature of some point mutations among them analyzed by *Southern* blot-hybridization.

II. Materials and Methods

The spontaneous *w* mutations were recovered in the large-scale experiments (1987—1994 years) described earlier [8,9], and γ-ray-induced ones were obtained as has been described by Alexandrov [10]. All transmissible *w* mutations were analysed by conventional

cytology of the polytene chromosomes to discriminate the exchange (intra- or interchromosomal) mutations from point (not associated with cytologically detectable chromosomal changes) ones. To detect the whole or partial deletions of the *w* gene, a sensitive and reliable genetic test on visible complementation with w^{sp} was employed using the more sensitive w^{spA} allele described earlier [11]. Isolation of genomic (from mutant and wild-type flies) and plasmid DNA, digestion of these DNAs with various restriction enzymes, ^{32}P-labelling of the probes by nick-translation and Southern blot-hybridization were performed according to standard procedures [12].

III. Results

According to data of cytological and fine genetic (complementation) analysis (Table) all 6 of spontaneous *w* mutations found have the point intragenic lesions since they show a normal chromosomal *3C2* region where the *w* locus lies as well as complementing phenotype over w^{spA} (the mutation type of the complementers). The analogous analysis of 73 γ-ray-induced *w* mutations revealed more complex spectrum of the radiation-induced gene mutations consisting of at least five different mutation types (Table): (i) exchange mutations showing the chromosome rearrangements involving translocations or inversions, one of two breakpoints of which passes through the site of the gene under study; (ii) multilocus

Table. Spectra of spontaneous and γ-ray-induced white mutations in Drosophila sperms classified by fine genetic and cytological analysis

	γ-ray dose, Gy					
	0	5	10	20	40	60
Number of mutants isolated / Number of F_1 progeny studied	6/267466	5/69375	10/60954	15/37955	39/57897	4/5994
Mutation types:						
Exchange mutations	0	0	0	1(0.06)*	3(0.09)	2(0.5)
Multilocus deletions	0	2(0.4)	2(0.2)	4(0.27)	15(0.38)	1(0.25)
Point mutations:						
Total	6(1.0)	3(0.6)	8(0.8)	10(0.67)	21(0.53)	1(0.25)
Complementers**	6	1	5	5	17	1
Partial complementers	0	0	2	2	1	0
Non-complementers	0	2	1	3	3	0

*The number in parentheses indicates the fraction of the total for given dose.
**See text.

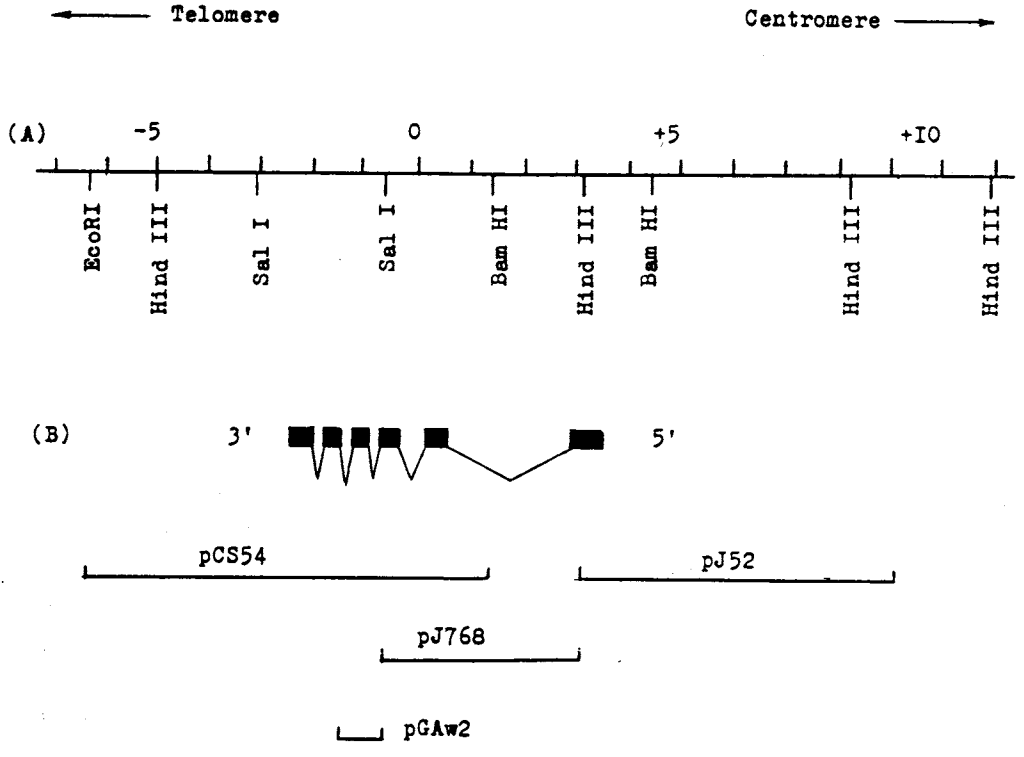

Fig.1. (A). Restriction map of the w^+ locus of D-32 wild-type strain in kilobases (1 kb = 1000 bp). The coordinate 0.0 is the point of the *copia* insertion in the allele w^a [13]. Restriction sites were determined by *Southern* blot-hybridization of digested genomic DNA with *pCS54*, *pJ768* or *pJ52* probes [14] which were a kind gift of Dr. V.Anashchenko (Gatchina). (B). The position of the w^+ trascript (six exons as the black boxes and five introns as the broken lines) as determined by [15]. *pCS54*, *pJ768*, *pJ52*, and *pGAw2* probes contain sequences from other *Drosophila* strains [5,14]

deletions being themselves the losses of the *w* locus and the adjacent genes; (iii) point mutations with the putative intragenic lesions much of which behave as complementers, and the rest of them do either (iv) the partial complementers or (v) the non-complementers. The complementation patterns of the last two mutation types indicate on the presumed partial or total losses of the *w* gene, respectively [5].

Thus, 100% of spontaneous and almost 40% (29/73) of γ-ray-induced *w* point mutations demonstrate the same phenotypic, cytological and genetic features as if supporting the generalization of the classical radiation genetics above noted. Does it mean that the molecular alterations underlying these point mutations are the same consisting of base-pair changes, insertions or very small intragenic deletions?

To answer this intriguing question, 4 spontaneous and 17 γ-ray-induced *w* point mutants were further analysed by blot-hybridization, permitting the detection of deletions

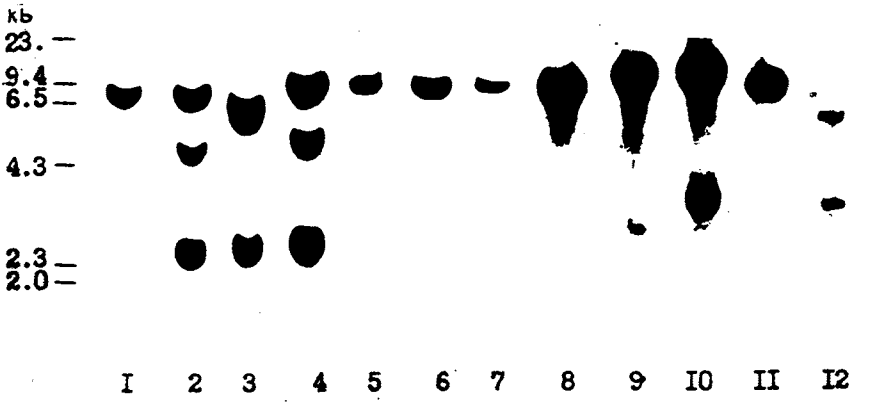

Fig.2. *Southern* blot-analysis of DNAs from spontaneous and γ-ray-induced *w* point mutations after digestion with *Hind III* and hybridization with *pCS54*; lines 1—12: wild-type D32 strain; spontaneous mutants *w88c45*, *w88d46*, *w92l1*; radiation mutants *w74b29*, *w83f29*, *w88d32*, *w81k2*, *w81k6*, *w87g67*, *w87g14*, *w78f39*, respectively.

or inversions with a minimal size of about 100 bp as well as the gain or loss of restriction enzyme sites. Taking into account the fact that eye pigmentation is about zero or uniformly reduced in mutants studied as in ones mapping usually at the distal end of the locus [5], DNAs from spontaneous and radiation-induced mutants and wild-type flies of the *D-32* strain were digested with *Hind III, Bam HI or Sal I* restriction enzymes and hybridizated with *pCS54*, *pJ768* or *pGAw2* probes which permit the detection of the genetic lesions located in this half of the w^+ gene (Fig.1).

As the hybridization pictures show, digestion of DNA from the spontaneous mutant *w88c45* with *Hind III* and following hybridization with *pCS54* give rise to three fragments of 8.2, 5.0 and 2.9 kb, instead of a single 8.2 kb in wild-type flies (Fig.2, lines 1 and 2). Hybridization of *w88c45* DNA with *pJ768* result in the same three fragments (data not shown) indicating that molecular alteration (insertion with duplication) affects the genic fragment between coordinates −0.7 and +1.4 kb which is in common for both probes (Fig.1). Spontaneous mutant *w92l1* is identical to *w88c45* (Fig.2,lane 4), whereas *w88d46* seems to be a deletion derivative of these mutants since it shows 2.9 kb being in common for three mutants studied and a new fragment of 5.9 kb instead of two fragments of 8.2 and 5.0 kb (Fig.2, line3). Therefore, these hybridization results indicate an interruption of the *w* sequence in the spontaneous mutants studied by a gain of genetic material the investigation of nature of which requires the further experiments.

DNAs from the γ-ray-induced mutants *w74b29* (40 Gy), *w81k2* (10 Gy), *w83f29* (40 Gy), *w87g14* (10 Gy) and *w88d32* (40 Gy) did not show any discrepancies from w^+ allele in hybridization pattern after digestion with *Hind III* and blotting with *pCS54* (Fig.2, lines 5,8,6,11,7). The analogous results were obtained for the radiation mutants *w67a* (40 Gy),

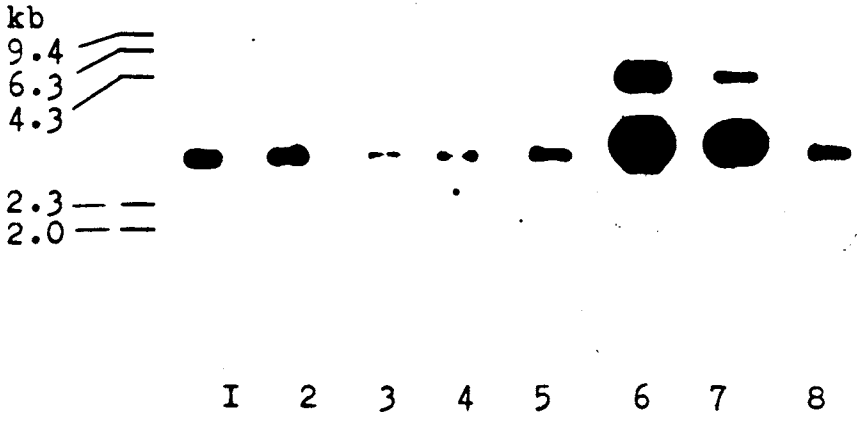

Fig.3. Southern blot-analysis of DNAs from spontaneous and γ-ray-induced point white mutations after digestion with *Sal I* and hybridization with *pGAw2*; lines 1—8: spontaneous mutants *w88c45*, *w88d46*, *w94l2*; wild-type D-32 strain; radiation mutants *w81k8*, *w81k6*, *w81k7*, *w87g75*

w71k (40 Gy), *w81k3* (10 Gy) and *w83c20* (40 Gy) (data not shown).The single Hind III fragment of 8.2 kb in wild-type flies is replaced by three new fragments of 8.5, 5.0, 2.7 kb; 14.0, 3.3, 2.7 kb and 6.5, 3.3, 2.7 kb in *w81k6* (10 Gy), *w87g67* (20 Gy) and *w87f39* (5 Gy), respectively (Fig.2, lines 9,10,12) and by two new fragments of 9.4, 4.7 kb and 8.5, 2.7 kb in *w72b* (40 Gy) and *w81k4* (10 Gy), respectively (data not shown). Therefore, all of these mutants display the gross structural alterations in the left half of the *w* gene being likely consequences of insertion or DNA rearrangement events.

To study this point more precisely, the digestion with *Sal I* and hybridization with *pGAw2* probe were performed for the three spontaneous and four γ-ray-induced mutants. As the results obtained show, the wild-type flies, spontaneous (*w88c45*, *w88d46*, *w94l2*) and two radiation-induced (*w81k8*, *w87g75*) mutants indicate a single *Sal I-Sal I* fragment of 2.7 kb (Fig.3, lines 1—5,8) discovered by *pGAw2* (Fig.1). This means that the genic fragment between coordinates −3.05 and −0.6 is not affected in these mutants. Therefore, these results support independently our notion claimed above that the genetic alterations in the spontaneous mutants are located within the genic fragment between coordinates −0.6 and +1.4 affecting the second and third exons and first and second introns of the *w* gene (Fig.1).

The single *Sal I-Sal I* fragment of 2.7 kb in wild-type flies is replaced in *w81k6* (10 Gy) and *w81k7* (20 Gy) by two new fragments of 4.3 and 3.0 kb (Fig.3, lines 6 and 7) showing that these γ-ray-induced mutants are structural ones, but the lesions detected earlier elsewhere in *Hid III -Hind III* fragment of 8.2 kb by hybridization with *pCS54* (Fig.2, line 9) are precisely located in *Sal I — Sal I* fragment in contrast with site of lesions in *SalI-Bam HI* fragment in the spontaneous mutants.

IV. Conclusion

The results presented in this communication indicate the presence of the large molecular changes in 3 spontaneous and 7 γ-ray-induced point *w* mutations displaying the similar phenotype, genetics and cytology. The rest 10 radiation mutants studied seem not to show any alterations at least after *Hind III*-digestion and hybridization with the large gene fragment (*pCS54*). It is quite possible that step-by-step analysis of the whole DNA sequence in these mutants with a set of a short fragments as probes will permit one to detect more small alterations than those revealed by a large probes used firstly. It is of interest and important the more so that the share of point mutants without detectable molecular changes (likely, a true point mutations with base-pair changes) in our set of γ-ray-induced *w* mutations (10/17 or almost 58%) is much higher than that obtained by others for the *white* [5] or *rosy* [4] loci in *Drosophila* germ cells after *X*-ray-irradiation. The further our ongoing experiments must ascertain the reason of this discrepancy and give a new information on the molecular spectra of the heritable gene mutations induced by densely ionizing radiation (neutrons, heavy ions).

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