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# MOLECULAR CYTOGENETICS OF RADIATION-INDUCED GENE MUTATIONS IN DROSOPHILA MELANOGASTER

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The classical paradigm of spatially unrelated lesions for gene mutations and chromosomal exchange breakpoints induced by ionizing radiations in eukaryotic cells was re-examined in the experiments on the mapping of gamma-ray- or neutron-induced breakpoints in and outside of white (*w*) and vestigial (*vg*) genes of Drosophila melanogaster using the in situ hybridization of the large fragments of the genes under study with the polytene chromosomes of the relevant mutants. The results for the random sample of 60 inversion and translocation breakpoints analysed to date have shown that (i) 50% of them are mapped as the hot spots within big introns of both the genes, and (ii) 21 of 60 breaks (35%) are located outside of genes. It is important to note that 26% (16/60) of the breakpoints analysed are flanked by the deletions, the sizes of which vary from the quarter to a whole of the gene. It was found that the deletions flank both the inversion and translocation breakpoints and arise more often after action of neutrons than photons. An unexpectedly high frequency of the multiple-damaged *w* and *vg* mutants that have the gene/point mutation and additional, but separate, chromosome exchange (the so-called double- or triple-site mutants) has shown that the genetic danger of ionizing radiation is higher than usually accepted on the base of single gene/point mutation assesements.

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

## Молекулярная цитогенетика радиационно-индуцированных генных мутаций у Drosophila melanogaster

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Парадигма классической радиационной генетики о разных «мишенях» для генных мутаций и аберрационных разрывов при наследуемых обменах хромосом в облученных клетках высших организмов экспериментально пока не обоснована, поскольку прямой анализ распределения таких разрывов внутри и вокруг гена до сих пор не был проведен. Восполняя этот пробел, мы проводим такой анализ с помощью метода молекулярной радиоизотопной гибридизации in situ (RIISH) геномных фрагментов двух генов Drosophila melanogaster (*w* и *vg*) с политенными хромосомами на большой выборке гамма- и нейтрон-индуцированных мутаций этих генов, цитогенетически ассоциированных с инверсионными и транслокационными разрывами. Результаты для 60 проанализированных на сегодняшний день таких разрывов показывают, что 1) половина из них находится внутри изученных генов, кластеризуясь в их больших интронах, 2) 35% (21/60) разрывов расположены за пределами этих генов и 3) 26,6% (16/60) разрывов фланкируют делеции (поте-

ри) части или всего гена. Существенно, что делеции наблюдаются при всех изученных обменах и видах излучения, но чаще после действия нейтронов. Неожиданно высокая частота (35%) комплексных мутантов с точковой мутацией гена и сопутствующим независимым аберрационным обменом (так называемые мутанты с двух-трех-сайтовыми повреждениями на геном) показывает, что генетическая опасность ионизирующих излучений гораздо выше, чем рассчитываемая сейчас по выходу простых генных мутаций.

Работа выполнена в Отделе радиационной безопасности и радиационных исследований ОИЯИ.

One of the paradigms in the classical radiation genetics claiming that the different and spatially unrelated «targets» or «sites» of initial damage promote the gene mutation and chromosomal break-rejoining in eukaryotic cells irradiated by ionizing radiation [1] has not been experimentally substantiated since the direct and systematic analysis of breakpoint distribution in and outside the gene has been not carried out yet. The relationship between gene mutation and breakpoint observed at times on the cytological level has been treated as the «position effect» or as the result of gene damage by the same particle that induces the break in close proximity to the gene [1].

The current version of paradigm that the radiation-induced chromosomal exchange breakpoints were predominantly located within the moderate repeats of DNA neighbouring to gene is founded theoretically [2,3] rather than experimentally since the immediate analysis of the broken repeats and genes has not been performed once again [4].

Thus, the two related and crucial for foundation of paradigm questions as to whether the chromosomal rearrangements with the breakpoints located within the gene are the constituents of the spectrum of gene mutations induced by ionizing radiation and, if so, what are the distribution patterns of breakpoints on the molecular gene maps, remain unresolved as a matter of fact.

To answer questions, the spectrum of gene mutations induced by gamma-rays (5—40 Gy) or fission neutrons (2.5—20 Gy) at the white$^+$ ($w^+$) and vestigial$^+$ ($vg^+$) genes of Drosophila melanogaster was at first studied by the conventional genetic and cytological analysis. Using of the genes which are differring from each other in both the fine exone-introne structure and the location in genome (Fig.1) permits the general and gene specific features to be ascertained in the action of radiation on the gene level.

According to the data obtained to-date, one of the general conclusions based on the results for the two genes studied is that the spectrum of gene mutations for any Drosophila gene damaged by low- or high-LET radiation consists of the three main subclasses: (i) cytologically point mutations, (ii) single- or multi-locus deletions and (iii) exchange mutations (inversions, transpositions, translocations) with gene specific phenotype. The last come to almost the third of all radiation-induced heritable gene alterations [5,6].

Therefore, the chromosomal exchanges modifying the gene expression are the important and constant part of spectrum of gene mutations induced by different quality radiation. What are the distribution patterns of the chromosomal breakpoints for the exchanges score?

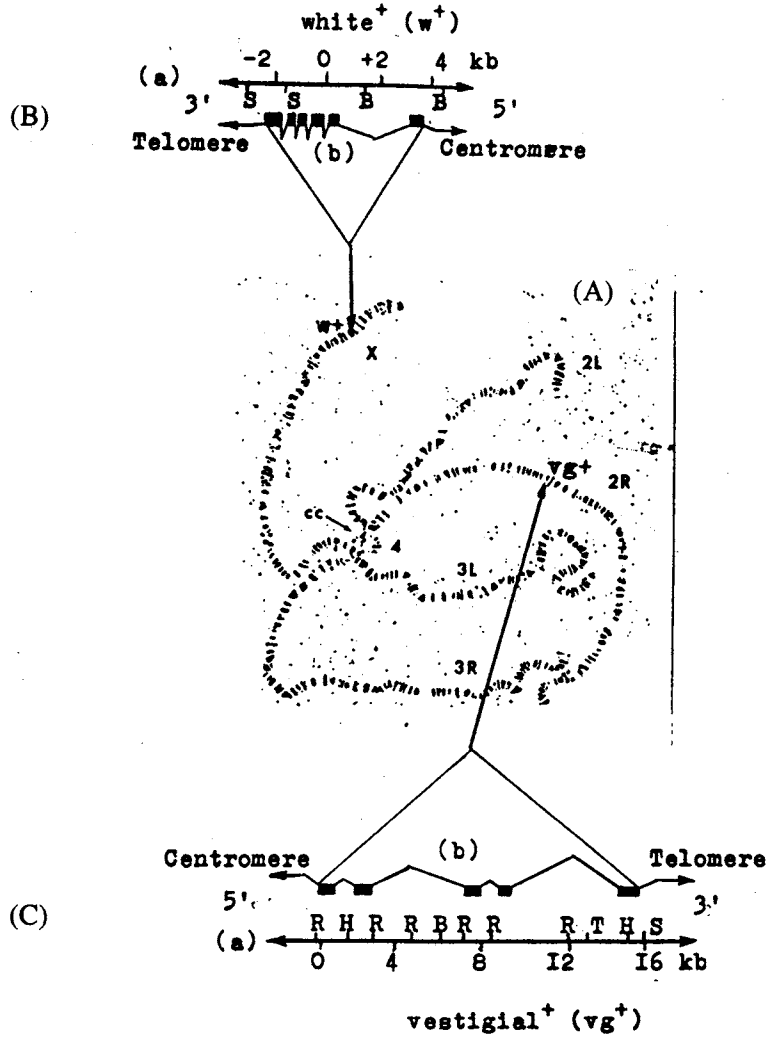


Fig.1. A genome (the polytene chromosomes) of Drosophila melanogaster and localization of $w^+$ (*X* chromosome) and $vg^+$ (*2R* chromosome) genes (A). (a) and (b) denote the schemes of the physical maps (in kb = 1000 base pair of DNA) and the fine exon (black boxes) — intron (broken lines) structures, respectively, for $w^+$ (B) and $vg^+$ (C) genes

The cytological analysis of the mutant *w* and *vg* polytene chromosomes has shown that each of the genes recombinates to form exchange with the specific sites of its own or heterogenous chromosome to give rise to the inversions and transpositions or the translocations, respectively (Fig.2a,b and Fig.3a,b). However, the second breakpoint of the ex-

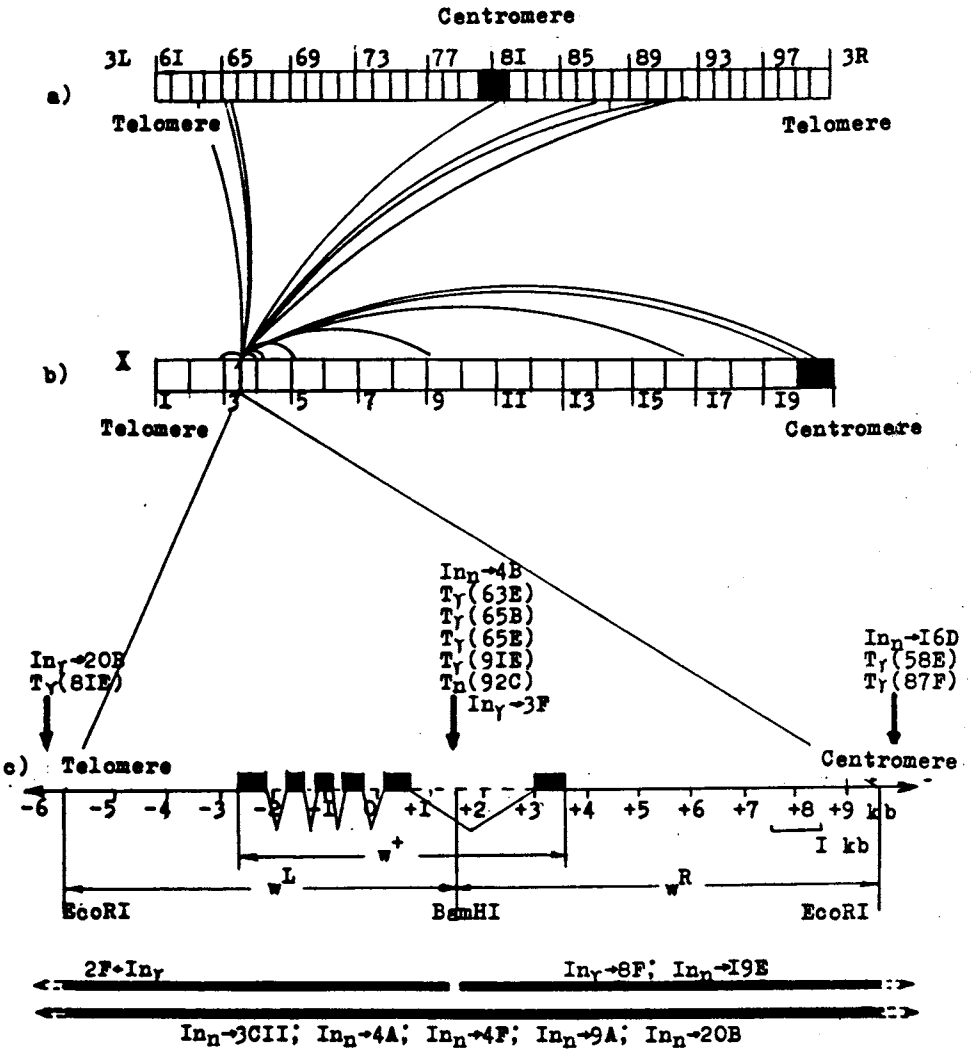


Fig.2. Genomic (a and b) and intragenic (c) localization of the chromosomal exchange breakpoints underlying the inversions (In) and translocations (T) induced by gamma-rays ($\gamma$) or neutrons ($n$) in Drosophila sperms and scored as the *w* gene mutations. (a) and (b) denote the schemes of the third and *X* polytene chromosomes, respectively. (c) showes the physical map of 6 kb $w^+$ gene and adjacent regions covered by $w^L$ and $w^R$ fragments with the relevant EcoRI and BamHI sites, exons (black boxes) and introns (broken lines). The location of the breakpoint «hotspote» is depicted by the black arrows. The sizes of deletions accompanying the breakpoints are shown by the black boxes below the map. The genomic sites with which the *w* gene interacts are noted by the thin arrows (for inversions) or the numbers with letter in parentheses (for translocations).

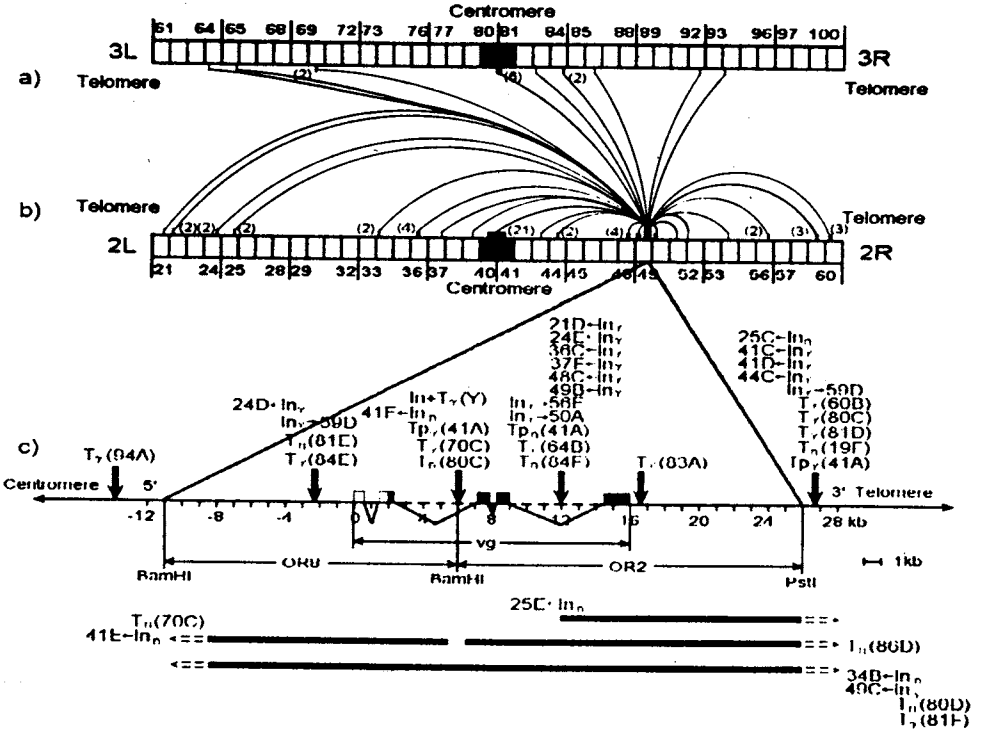


Fig.3. Genomic (a and b) and intragenic (c) localization of the chromosomal exchange breakpoints underlying the inversions (In) or transpositions (Tp) and translocations (T) induced by gamma-rays (γ) or neutrons (*n*) in Drosophila sperms and scored as the *vg* gene mutations. (a) and (b) denote the schemes of the third and second polytene chromosomes, respectively, the «hotspots» on which were shown by the numbers in parentheses (the amount of independent hits for given site). (c) shows the physical map of 16 kb *vg* gene and adjacent regions covered by OR8 and OR2 fragments with the relevant BamHI and PstI sites. For all the rest designations see Fig.2

changes was found to be closely associated always with the sites of cytological localization of the genes under study (3C2.3 band for $w^+$, Fig.2b and 49D3 band for $vg^+$, Fig.3b).

Since such breakpoints are accompanied by the gene-specific phenotypes it may be presumed, if the classical notions are bearing in mind, that these breaks are lying in close proximity to the genes and the mutant phenotypes were brought about by «position effect» or by direct gene lesions accompanying the chromosomal breaks.

To detect precisely the position of the breakpoints around the genes of interest, the state of gene structures and of their neighbouring sequences was studied by in situ radioactive isotope hybridization technique (RIISH) [7] and using the sets of gene fragments covering the genes and adjacent sequences (Fig.2c and Fig.3c). In particular the cloned in pUCI9 distal ($L^+$, 7.5 kb) EcoRI-BamHI and proximal ($R^+$, 8.0 kb) BamHI-EcoRI fragments of

genomic DNA covering the $w^+$ gene and the 3.5 kb DNA stretches from each side of this gene were used for analysis of the exchange *w* mutants, and the cloned in EMBL3 proximal (OR8, 16.5 kb) and distal (OR2, 21.5 kb) genomic fragments of the $vg^+$ locus were employed for analysis of the *vg* re-arrangements. It is of importance to note that the couples have got the BamHI sites in common lying in the big introns of the genes (Fig.2c and Fig.3c). When using these tagged fragments we have suggested that localization of the whole fragment or its parts visualized as hybridization site at full intensity or labeled sites at less than half full intensity, respectively, on the mutant polytene chromosome will be determined by a seat of breakpoint in or outside the gene.

The data for the 60 *w* and *vg* exchanges examined to-date show that 35% (20/60) of them have the breakpoints located outside the genes under study (Fig.2c and Fig.3c). Therefore, the *w* and *vg* phenotypes for a large proportion of cytologically visible exchanges are not directly founded by the breaks and seem to result from the «position effect» or independent damage of gene. To choose between these alternative explanations, the relevant experiments are under way.

A new and important finding is that more large part of the exchanges studied (50%) have the breakpoints lying inside both the genes since the transferences of any whole fragment (the breaks are passing through the big introns near BamHI site) or only unequal parts of OR2 (the breaks are located in the second big intron of the *vg* gene) are observed in such cases.

These results show that although the initial (preaberration) lesions were distributed by a chance through the gene volume only those of them which are situated in the certain («target») intron sequences have a high likelihood to be realized as the chromosomal exchange breakpoints. The study of the molecular nature of these «targets» is an important task in hand for our researches.

Other new and important result of the work performed is that a considerable part (26.6% or 16/60) of the *w* and *vg* exchanges analysed is found to be the mutants the breakpoints of which are accompanied with the molecular deletions of varying sizes (7—38 kb or even more) (Fig.2c and Fig.3c). Thereby, as is seen, all partial deletions are begining within the big introns also. It is of importance to note too that the deletions accompany both the inversion and translocation processing independently of quality radiation, but more frequently after action of neutrons.

Thus, the results outlined above enable one to note the following new molecular features of exchange processing in the irradiated eukaryotic cells: (i) the exchange breakpoints are regularly located within a gene; (ii) the breaks are clustered within the certain and relatively short sequences of the big introns, and (iii) almost the third of breaks are flanked by the molecular deletions the sizes of which vary from 7 to 38 kb (i.e., the whole gene).

Among the others two our findings require some comments. Firstly, the exchange break/deletion combination (each of them are usually independent events) shows that the radiation-induced chromosomal exchanges are formed by more complex mechanism than one based on the genetic recombination as such [8]. Our data allow the suggestion to be

made that such exchanges are promoted by the local clustered and recombinogenic damages over several topologically drawn closer to one another chromatin molecules with subsequent mis-rejoining by «illegitimate» recombination either «slippage» or by both the processings, simultaneously. The increase of severity of DNA-related damage, for example, after action of high-LET radiation must rise both the likelihood of deletion formation and its size that is really observed in the experiments with neutrons (Fig.2c and Fig.3c). The sequencing of DNA at the exchange breakpoints without the deletions detected by RIISH technique should answer the important question as to whether the deletions attend any radiation-induced break-rejoining processing.

Secondly, the data obtained for the two genes studied show that exchange breakpoints are often situated outside the gene and accompanied nevertheless by gene inactivation. This inactivation for gene which was transferred to centromeric heterochromatin (euchromatin — heterochromatin rejoining) may really result from the «position effect» [9] (it is all our cases where the genes under study recombinate with the 19, 40, 41, 80 and 81 heterochromatic regions of the polytene chromosomes, Fig.2a,b and Fig.3a,b). However, this effect is not typical for euchromatin-euchromatin rejoining [9]. Therefore, gene inactivation in such exchanges (all our the rest cases) is obviously broght about by independent point (?) damage of gene itself. Thus, the genome of such mutants containes two (or more) heritable mutations simultaneously (the so-called complex or double, triple, etc., mutants).

The property of ionizing radiation to induce such complex mutants proportionally to dose has been already noted earlier on the base of cytological [10] and genetical [11] data. Confirmation of these observations on the molecular level is another important result of our work which has had a great theoretical and applied significance showing that a new approaches need to assess the genetic risk of ionizing radiation the genetic danger of which is proved to be higher than accepted on the base of single gene/point mutation assay.

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