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Spatial arrangement of the animal male germ cell genome: I. Non-random pattern of radiation-induced inversions involving the vestigial region in autosome 2 of *Drosophila melanogaster*

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Abstract

At present, given a bulk of models for higher-order chromatin structure in interphase nuclei of animal somatic cells, little is known about the spatial chromosome organization in animal male germ cells mainly due to the lack suitable methods for detailed observation and analysis without disruption of the existing organization. We pioneered in the study of this issue via analysis of radiation-induced inversion patterns in *Drosophila* male germ cell genome taking into account the fact that the formation of inversion requires the spatial proximity and contact of its ends. Analysis of 72 γ-ray- or neutron-induced vg inversions in which the first break is invariantly associated with the vestigial (vg) gene in the middle of 2R autosome shows the second inversion breakpoints that highly non-randomly distributed over the entire second chromosome clustering at the three autosome 2 "hot" chromosome areas. These findings show that the polar Rabl-configuration of interphase chromosomes in various somatic cells is not typical for the haploid genome of *Drosophila* mature sperms. The specific megarosette-loopstructure of haploid chromosome in the mature animal sperms is proposed and justified.

Key words: Radiation, inversions, chromosome folding arrangement, sperm genome, *Drosophila*.

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Introduction

Shortly after the second meiotic division of the secondary spermatocyte during spermatogenesis in animal kingdom, the telophase chromosomes decondense and diffuse to form the haploid "interphase" nucleus of early spermatid where important morphogenetic and synthetic processes of spermiogenesis get under way. Among these processes, organization of the cytoplasmic organelles into ordered complexes of normal architecture accompanied by realignment of spermatid genome from the loose chromatin fibers to closely packed paracrystal chromatin structures (Demerec, 1950; Meyer, 1969) and transition from a typical somatic or lysine-rich (Tokuyasu *et al*., 1972) chromosomal histone to a highly arginine-rich type late in sperm maturation (Das *et al*., 1964) appear to be a key events in animal spermiogenesis (Kimmins and Sassone-Corsi, 2005) resulting in the specific sperm nucleus evolutionarily adapted for insulation of the genetic information during the period of its transit from one generation to the next.

The complexity of these processes rise many questions about the large-scale organization of "interphase" chromatin in developing spermatids and a spatial arrangement of haploid chromosomes in the motile mature sperm as well.

The experimental data that could provide the answers to these questions are entirely absent so far. Conversely, a bulk of models for spatial organization of chromosomes in interphase nuclei of plant and animal somatic cells have been considered in related literature, beginning with the most early "polar" (telomere and centromere are located at opposite

nuclear poles) model classically defined as "Rabl's configuration" (Rabl, 1885) and, later really described in a variety of cell nuclei (Comings, 1980; Saumweber, 1987; Hiraoka *et al*., 1990), and ending with diverse current chromatin loop models which variously involve irregularly folded fibers or moderate / "giant" loop domains (Manuelidis, 1990; Sachs *et al*., 1995; Cremer and Cremer, 2001).

It is not clear however, what kind of these models may be best matched to genome architecture in developing spermatids and mature male germ cells, particularly, taking into account the specific function brought about by the latter. Furthermore, the current models have given an insight into the conformation of isolated chromosomal domains rather than of an entire chromosome. Therefore, to get a notion on the issue of interest, it is essential, as a first step, to clarify whether so-called Rabl orientation of chromosomes still persists in sperm genome after the second meiotic division and spermatid differentiation.

In order for this elucidate, it is necessary to use the experimental approaches other than a classical cytology or current high-resolution *in situ* hybridization techniques due to impossibility to examine the sperm chromatin under the microscope.

In this connection, the investigation of the pattern of radiation-induced intrachromosomal exchanges (inversions) may be considerable promise as a means for clarification of the global arrangement of one or another chromosome in sperm nucleus. The following three known fundamental facts form the basis for such promissory approach:

i. The direct, local, and stochastic action of ionizing radiation on the genetic matter resulting in the double-strand breaks of DNA, chromosome breaks and rearrangements subsequently (Ozalpan, 2001).

ii. Chromosome breaks induced by radiation in mature sperm stay open until fertilization (Maddern and Leigh, 1974) and the formation of chromosome exchanges is confined by the short time before the first chromosome replication in male pro-nucleus after fertilization and the occurrence of zygote (Würgler, 1971).

iii. The formation of any one of chromosome exchanges (translocation, inversion, transposition etc.) requires that the two interacting chromosome regions were spatially close to each other enough.

Taking into account these facts, pattern (the size, position, and frequency) of radiation-induced inversions at the large metacentric autosome 2 of *Drosophila* sperm genome was studied. Hereat, the genetic experiments were designed so that to isolate not all of possible randomly arising intrachromosomal exchanges but only those that have had one of inversion breaks (so-called the "first break") invariably associated with one or another selected genetic loci of the autosome. Then, location of the second inversion breaks should indicate which chromosome regions, and as often in different nuclei, are spatially close to the genetic loci selected showing thereby the loops of appropriate sizes. Therefore, enough large sets of locus-specific inversions can give an insight into the global loop arrangement and topographic parameters of autosome under study in haploid sperm nucleus.

Using these new feasible, and, in our opinion, considerable promise approaches, a series of inversions associated with the three genetic loci of different location on the autosome 2, namely, black body (b), cinnabar eyes (cn), and vestigial wings (vg) loci, were obtained after irradiation of *Drosophila* adult male sperms by gamma-rays or fission neutrons. The present paper focuses on the pattern of the vg inversions in respect of which the most extensive data were collected in spite of a fairly low frequency of the vg inversion induction (2.0×10^{-8} per locus per rad and 7.5×10^{-8} per locus per rad for γ-rays and neutrons, accordingly) (Alexandrov *et al*., 2001). The results for the 72 vg inversions analyzed show that

i. The vg locus lying at the middle of 2R arm of this autosome (subsection 49E) interacts the most frequently (20/72 or the same arm of autosome 2 (section 41) forming the loop of 9 sections or about ten million pairs of DNA bases (10 Mb);

ii. In other 35 cases, the vg locus alternatively interacts equally frequently (24-25%) either with neighbouring proximal/distal chromosome regions (sections 48/50-51, respectively) forming the small loops of 0.3-3 Mb DNA or with the highly separated distal ends of 2L as well as 2R arms of autosome (sections 21-25 and 56-60, respectively) giving rise to the "giant" loops of about 30 and 12 Mb DNA, accordingly;

iii. The rest of inversions (17 cases) show that the vg locus can rarely interact with some internal areas of the 2L or 2R chromosome arms resulting in the large loops of various sizes in the separate sperm nuclei.

Table 1. The location of the second inversion breakpoints on the polytene autosome 2 of *D. melanogaster* for γ-ray-induced vg inversions (about the "first" breakpoints see text)

No	Code of vg inversion	Location of the second inversion point	No	Code of vg inversion	Location of the second inversion point
1	vg72a1	In(2R) 44C3	22	vg84f51	In(2R) 44F2
2	vg74b1	In(2LR) 37F2	23	vg84f65	In(2R) 49C4
3	vg74c4	In(2LR) 22A5	24	vg84h	In(2R) 41B
4	vg76j1	In(2R) 60AB	25	vg84hIX	In(2R) 48E
5	vg78a1	In(2R) 41D	26	vg85e2	Ins(2R)41A;49E1;55F
6	vg78a2	In(2R) 56E	27	vg87e29	In(2LR) 21D
7	vg78j1	In(2R)50C4	28	vg87c148	In(2R) 50C9
8	vg78j3	In(2R) 41D	29	vg87f96	Tp(2LR) 39D3
9	vg78k3	In (2R) 59D4	30	vg87g20	In (2R) 41C
10	vg79h4	In(2LR) 24C	31	vg87g22	In (2R) 41D
11	vg79h5	In(2R) 50A2	32	vg87g43	In(2R) 60B12
12	vg79h6	In(2R) 41E	33	vg87h42	In(2R) 56F9
13	vg81b1	In(2R) 48C4	34	vg87h55	In(2R) 41D
14	vg81c28	In(2R) 41D	35	vg88d4	In(2R) 41C
15	vg81c41d	In(2R) 49B12	36	vg88d20	In(2R) 49A6
16	vg81l18	In(2LR) 36C4	37	vg88d43	In(2R) 41D
17	vg83h39	In(2R) 51D2	38	vg89d40	In(2R) 56F
18	vg83c3	In(2R) 41C	39	vg89e4	In(2R) 52A11
19	vg83c43	In(2R) 43C3	40	vg89e60	In(2R) 50A14
20	vg83f38	In(2R) 59D	41	vg89e76a	In(2R) 49C1
21	vg83fXD	In(2R) 48F2	42	vg89e76b	In(2R) 49A4

An often contacts and interactions of the middle 2R arm (vg region) with its centromeric and telomeric ends suggest that the global arrangement of chromosome in sperm nucleus is not in line with the Rabl configuration but rather matches to the specific giant rosette-loop structure the qualitative features of which are discussed. 2D and 3D models of this structure will be dealt in the next paper.

Material and methods

Random samples of 43 γ-rays- and 29 neutron-induced vg inversions were obtained over large-scale experiments as a part of the extensive project on the RBE- neutron energy relationship under the locus-specific mutation induction in *Drosophila*. The biological as well as physical details of experiments such as *Drosophila* stocks and germ cells irradiated, the sources of radiation (γ-rays of ^{60}Co; fission neutrons), doses, and regimes of irradiation were described earlier (Alexandrov, 1984). The main results of the fine genetical and cytogenetical analysis of all γ-rays and neutron-induced vg inversions being essential for the answer to the issue of interest were published elsewhere too (Alexandrov and Alexandrova, 1987; Alexandrov *et al.,* 2004). Here, it is

Table 2. The location of the second inversion breakpoints on the polytene autosome 2 of *D. melanogaster* for neutron-induced vg inversions.

No	Code of vg inversion	Location of the second inversion point	No	Code of vg inversion	Location of the second inversion point
1	vg77d1	In(2LR) 25C	14	vg88c1	In(2LR) 36E
2	vg79a	In(2LR) 34B2	15	vg88c72	Ins(2LR) 30B;49E1;41A
3	vg79b4	In(2R) 41C	16	vg88c87a	Ins(2R) 41A;49E1;59A
4	vg79b6	In(2R) 50C9	17	vg88c94	In(2LR) 36C10
5	vg79d3	In(2R) 41A	18	vg88e28	In(2R) 60F1
6	vg79d4	In(2R) 41E	19	vg88e55	In(2LR) 22A8
7	vg79d7	In(2R) 41D	20	vg88e76	In(2LR) 34A10
8	vg82c14	In(2LR) 36D	21	vg88f18	In(2R) 54C4
9	vg82c61	In(2LR) 24E2	22	vg88g5	Ins(2R)49A12;49E1;51E10
10	vg83d4	In(2R) 48F	23	vg88g33	Ins(2LR)39D5;49E1;59F
11	vg83l3b	In(2R) 41E	24	vg88g38	In(2R) 49B1
12	vg85c	In(2R) 41B	25	vg88g80	In(2LR) 25E6
13	vg88b15	In(2LR) 40E			

worth noting that the molecular position of the "first" inversion breakpoints inside or outside (but in the immediate vicinity) of the vg gene (subsections 49D4-E1 on the polytene 2R arm of autosome 2) was determined by the *in situ* hybridization technique using ^{3}H-labeled proximal (OR8) and distal (OR2) genomic fragments overlapping the gene under study (Alexandrova *et al*., 1997). The precise chromosome location of the second inversion breakpoints was performed by the standard cytological technique for *Drosophila* polytene chromosomes in the mutant chromosome/wild-type chromosome heterozygotes and estimated accurately within subsection of the polytene autosome 2 guided by its classical map under the light microscopy (Lefevre, 1976).

Results and discussion

As the first step of analysis of the pattern of vg inversions, all of them were classified as γ-ray- and neutron-induced ones (Table 1 and 2, respectively) with indication of chromosome location of their second inversion breaks whereas the "first" breakpoints are always intimately associated with the vg gene (subsection 49D4-E1 of 2R arm of autosome 2) resulting in the appropriate vg phenotypes in mutant homozygotes.

A comparative consideration of these data shows that both γ-rays and neutrons induce basically the same "simple" double breaks (on the chromosome level) inversions with the closely similar seats of the second breaks on 2L or 2R arm of the chromosome. However, thereat it should be pointed out that, in the same irradiated nucleus, more complex chromosome exchanges of two inversions with one break in common (at the vg region) can occur after action of both radiations but neutrons appear to be more effective than γ-rays in induction of such rearrangements (vg inversions No 15, 16, 22, and 23 for neutrons, Table 2 and No 26, Table 1 for γ-rays, respectively). As a result, the overall numbers of exchanges isolated and analyzed add up to 43 inversions for γ-rays and 29 ones for neutrons. Which is their second inversion breakpoints distributed along the entire autosome?

As indicated in the tables and as mentioned above already, the seats of γ-ray-induced second inversion breakpoints coincide very closely with that of neutron-induced ones. This enables us to integrate the findings for both radiations within a single set of data to detect the "hot" chromosome areas with which the vg region is most often brought into spatial proximity in sperm

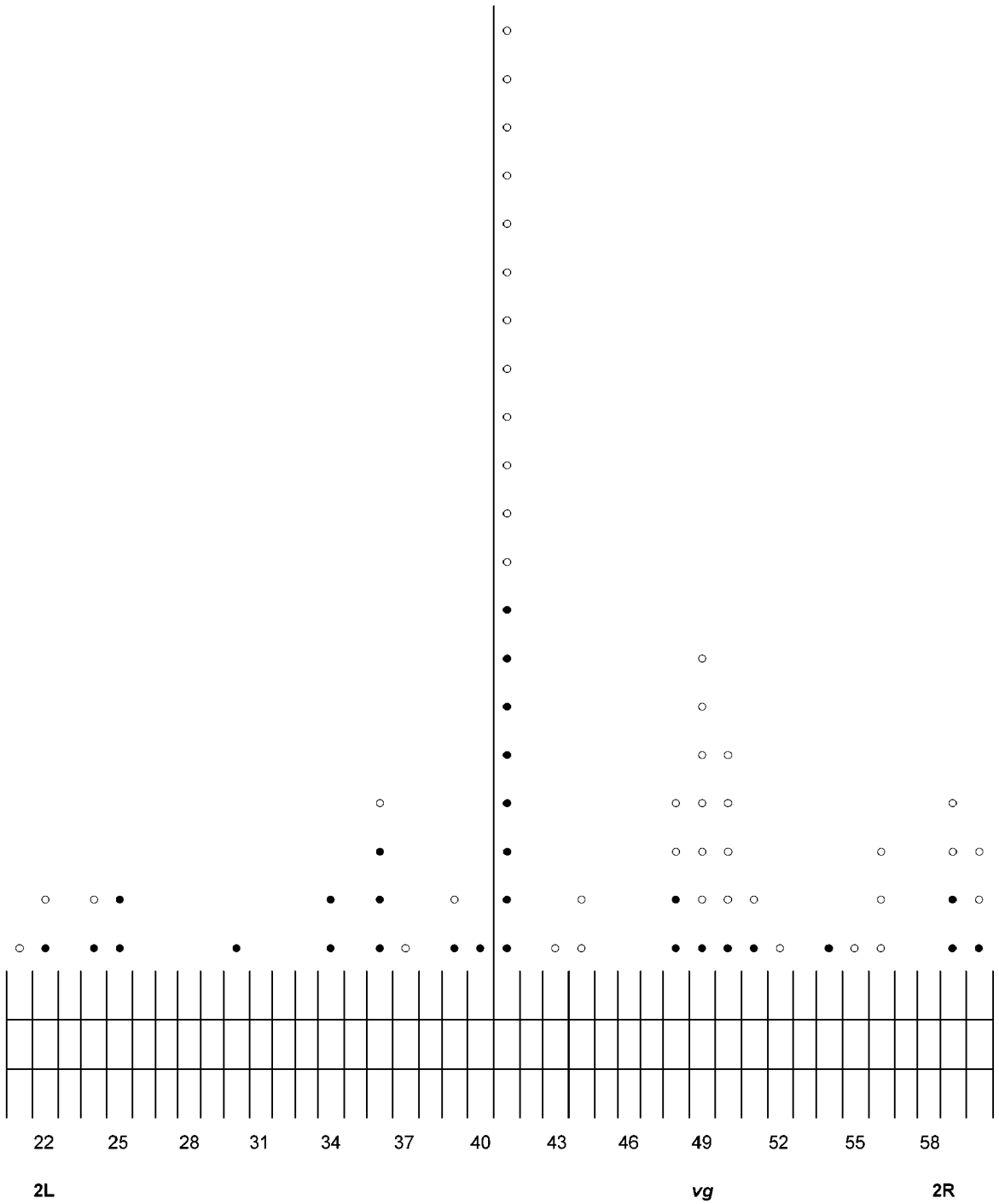

Figure 1. The distribution of the second inversion breakpoints over the polytene autosome 2 for 43 Á-ray- (○) and 29 neutron-induced (•) vg inversions arising in mature sperms of D. melanogaster wild type adult males.

nuclei resulting in the radiation-induced recombination and cytologically visible inversion with the vg phenotype.

Analysis of the pooled data (Figure 1) shows that a middle of 2R arm of autosome 2 marked by the vg gene (section 49) and a block of centromeric heterochromatin of the same arm (section 41) are the most frequently (20 cases out of 72 or 27.8 %) interacted to give rise to inversion and, therefore, neared to each other in space of sperm nucleus forming a sizable loop of the nine sections or almost ten million pair of DNA bases (10 Mb). Further, as it can seen from Figure 1 in other sperm nuclei, the telomeric areas of 2L (sections 21-25) as well as 2R (sections 56-60) arms systematically interact (in toto, 17 cases out of 72 or 23.6 %), with the vg region resulting in the "giant" loops comprising of 24-27 sections (29-32 Mb), in the first case, or 7-11 sections (8-13 Mb),in the second case, if it is taken into account that the physical length of the 2R euchromatin DNA is 21.4 Mb, and that of 2L arm is 23 Mb, and each section contains on average about 1.2 Mb (Adams *et al*., 2000). Therefore, it is thought that these telomeric areas are spatially relatively close (but are farther than heterochromatin) to the vg region. At the same time, in the genome of other sperms, smaller inversions (0.3-3 Mb) with the second breakpoints in sections 48 or 50-51 occur regularly (18/72 or 25.0 %), testifying to a possibility of induced contact and exchange of the vg region with these adjacent chromosome areas. The rest of inversions (17/72 or 23.6 %) have the second breakpoints which are rarely and randomly distributed within the internal areas of 2L or 2R. arms of autosome forming the large loops of various sizes in the separate sperm nuclei

Thus, the results obtained show that there are at least three "hot" areas in *Drosophila* autosome 2 (telomeric, centromeric, and adjacent to vg region areas) with which a middle of the 2R arm marked by the vg gene can the most often and highly non-randomly ($X^2 = 32.4 \times 10^6$; df = 4; $p < 2.6 \times 10^{-3}$ for the Poisson distribution) interacts. Hence it follows that all of these chromosome areas as the bases of potential loops are preferentially grouped within defined nuclear region "sensitive microvolume". When taken into account that in sperm nucleus, as in interphase nucleus of somatic cells (Zhimulev, 1993), centromeric heterochromatin is located on the nuclear envelope as a kind of "anchor" or "bearing" point for the entire chromosome it is felt that the vg region (a middle of 2R arm) is flexible within the microvolume and interacts with chromosome areas in question with probability determined by their spatial proximity (radius of interaction). Thereat, the type of the "alternative" partner (heterochromatin or section of chromosome area) should point out to the nearest proximity and realized probability of interaction of the vg region with this partner in this sperm nucleus.

The pattern of the vg inversions described unequivocally testifies against the polar Rabl-configuration of major autosome 2 in *Drosophila* sperm genome and provides first indication of its specific dimensional arrangement in the form of one tightly packed megarosette-loop structure which obviously best matches to extremely small sperm nucleus and a specific function of sperm genome. This structure contains all kinds of loop which have been identified in interphase nuclei of somatic animal and plant cells such as genetic (up to 0.3 Mb) and chromomeric or banding (0.3 ≥ 3 Mb) loop subdomains (Manuelidis, 1990) as well as a large-scale "giant" chromatin loop domains (Sachs *et al*., 1995; Cramer and Cramer, 2001). Therefore, it is safe to assume that somatic and germ cell chromosomes are built up from the same structural units but their global spatial arrangement (macroarchitecture) is quite different. To reconstruct and visualize the postulated here megarosette-loop structure of "interphase" chromosome for *Drosophila* male germ cell nucleus, the computer simulation of its two- and three-dimensional model on the base of inversion pattern described above was performed and the results obtained will be presented at the next paper.

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