



39 spatial arrangement of the animal male germ cell genome: II. 2d-3d Simulation and visualization of spatial configuration of major chromosome 2 in *Drosophila* sperm

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Abstract

Data on the non-random distribution of radiation-induced inversion breakpoints were used as genetic markers for 2D and 3D modeling of the large-scale architecture of the chromosome 2 in *Drosophila* sperm genome at the time of irradiation. During modeling, Gmax program with application of splines, the additional objects and operations as well as the methods of visualization of macromolecular biostructures were employed. A specific megarosette-loop model of spatial arrangement of chromatin fiber in the haploid sperm nucleus can account well for the experimental data.

Key words: 2D/3D models, large-scale chromosome geometry, sperm genome, *Drosophila*.

Introduction

The development of modern chromosome fluorescence *in situ* hybridization (FISH) methods (Agard *et al.*, 1989; Trask *et al.*, 1993) has permitted to perform 2D and 3D visualization of distinct chromosomes (or "chromosomal territories" (CTs), according to Theodor Boveri, 1909) as well as euchromatic (coding) and heterochromatic (condensed centromeric) chromosomal regions in interphase nuclei of animal and human somatic cells (Croft *et al.*, 1999; Edelmann *et al.*, 2001; Habermann *et al.*, 2001; Lukasova *et al.*, 2002; Tanabe *et al.*, 2002; Manders *et al.*, 2003; Kreth *et al.*, 2004).

These observations have also given an impetus to creation of vastly different current models for the large-scale geometric arrangement of interphase chromosomes in eukaryotic somatic cells (Sachs *et al.*, 1995; Cremer and Cremer, 2001; Solovei *et al.*, 2002) the basic propositions of which appear to strengthen a classical model (Rabl, 1885) of non-random radial (polar) configuration of somatic interphase chromosomes in respect to both its major features, namely, that the chromosomes occupy distinct nuclear domains throughout the cell cycle and centromeric and telomeric regions are located at the opposite side of the nucleus (inner and peripheral parts of the cells nucleus, respectively, according to present definition).

This polar orientation of CTs has been clearly seen in most chromosomes and most cell types investigated although non-random polar and random non-polar conformations may be persisted for different chromosomes in the same cell nucleus (Skalnikova *et al.*, 2000).

Unlike the data for diploid somatic cells, it is unknown so far what kinds of chromosome configuration patterns correspond to those in haploid spermatids and mature male germ cells, especially, taking into account the unique chromatin remodeling (the histone-to-protamine transition) in late spermiogenesis (Sassone-Corsi, 2002; Kimmins and Sassone-Corsi, 2005).

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In order to clarify the issue as to whether so-called Rabl orientation of chromosomes still persists in mature sperm genome after spermatid differentiation, the pattern of radiation-induced intrachromosomal exchanges (inversions) in *Drosophila melanogaster* autosome 2 was studied after γ -rays or neutron irradiation of D-32 wild-type adult males.

The following two facts were taken into account to select such approach for clarification of the issue of interest. Firstly, no there is direct approaches to-day to examine the sperm chromosomes by the cytological or physical techniques without disruption of the existing organization. Secondly, analysis of radiation-induced chromosome exchanges which are assumed to develop from DNA double-strand breaks (DSBs) and random pairwise interactions (Savage, 1989) have been shown to be a useful tool for reconstruction of the large-scale chromosome geometry at the time of irradiation of interphase somatic cells (Sachs *et al.*, 1993; Ostashevsky, 2000) since the assuming complete randomness of DSB pairwise interaction resulting in the exchanges scored may be drastically modified with position of interacted DSBs in genome (Skalnikova *et al.*, 2000). For example, two genetic loci with DSBs on the same chromosome which are well separated in most nuclei obviously cannot be close to each other in order to interact and vice versa, two genetic loci which are spatially close to each other in most nuclei must frequently interact yielding the high frequency of inversion expected.

Using this approach for *Drosophila* male germ cells, we showed previously that the chromosome distribution of second inversion breakpoints relative to the “fixed” first one (genetic locus *vg* at the middle of 2R arm of autosome 2) is highly non-random showing most often contacts and interactions of this genetic locus with centromeric region and both telomeric ends of autosome 2 in mature sperms irradiated by γ -rays or fission neutrons (Alexandrov *et al.*, 2007). Such non-randomness suggests that the global arrangement of chromosome 2 in sperm nucleus is not in line with the linear folded Rabl configuration but rather matches to the specific giant rosette-loop structure 2D and 3D models of which are proposed in this paper.

Preliminary basic assumption before modelling

Prior to modeling of the spatial configuration of *Drosophila* autosome 2 in sperm nuclei at the time of irradiation, it is necessary to define some basic assumptions relating to the presumptive chromosome structure in haploid sperm nucleus, the most salient

features of inversion induction pattern detected in irradiated sperms, and the presupposed physical parameters of DSBs interactions giving rise to inversions observed.

As it has already been intimated (Alexandrov *et al.*, 2007), the cytological analysis of radiation-induced inversions has been carried out on the interphase polytene chromosomes of salivary gland somatic cells. These chromosomes well known to be formed during endonuclear polyploidization (about 1024 *n* in adult larvae) as a result of many times (2⁸) repeated replications of the elementary haploid parental interphase chromosomes $\approx$ 30 nm in diameter. The discrete elementary chromatin fibers may be easily identified with the electron microscopy (Ananiev and Barsky, 1985). Moreover, each elementary fiber shows the same banding pattern (the picture of bands or chromomers) as the same polytene chromosome as a whole. Therefore, one can assume that the classical banding and section maps of *Drosophila* polytene chromosomes can be extended to the haploid chromosomes in sperm genome of this organism and used in the course of modeling.

According to our experimental data described in the first report, the *vg* gene (middle of 2R arm of autosome 2) interacts most often, to give rise radiation-induced inversions, with centromeric heterochromatin (the first “hot” area), with several sections in 2R arm telomeric end (the second “hot” area) as well as in 2L arm telomeric end (the third “hot” area) and with neighboring genetic loci in the 48-49 and 50-51 sections of 2R arm. Therefore, on the large-scale geometry of *Drosophila* interphase chromosome 2, the genomic separations of the *vg* gene from “hot” areas lie in the ranges $\approx$ 10; 8-13; 29-32, and 0.3-3 Mb for areas above listed, respectively (Alexandrov *et al.*, 2007). In other cases, the *vg* gene occasionally interacts with some genetic loci randomly dispersed along the autosome 2.

To reconstruct the global chromosome architecture and, mainly, to clarify whether so-called polar Rabl configuration of chromosomes still persists in sperm genome, it is not of necessity to deal with accidental genetic loci involved in the *vg* inversions but it will be warranted to pool the relevant genetic loci in each “hot” area and to consider the latter as a “hot” node during modeling. The nodes are primarily a biological entities and their postulated chromosome position can be selected to fit the situation of interest.

The statistically non-random interactions of the *vg* gene with certain of the autosome 2 areas show that

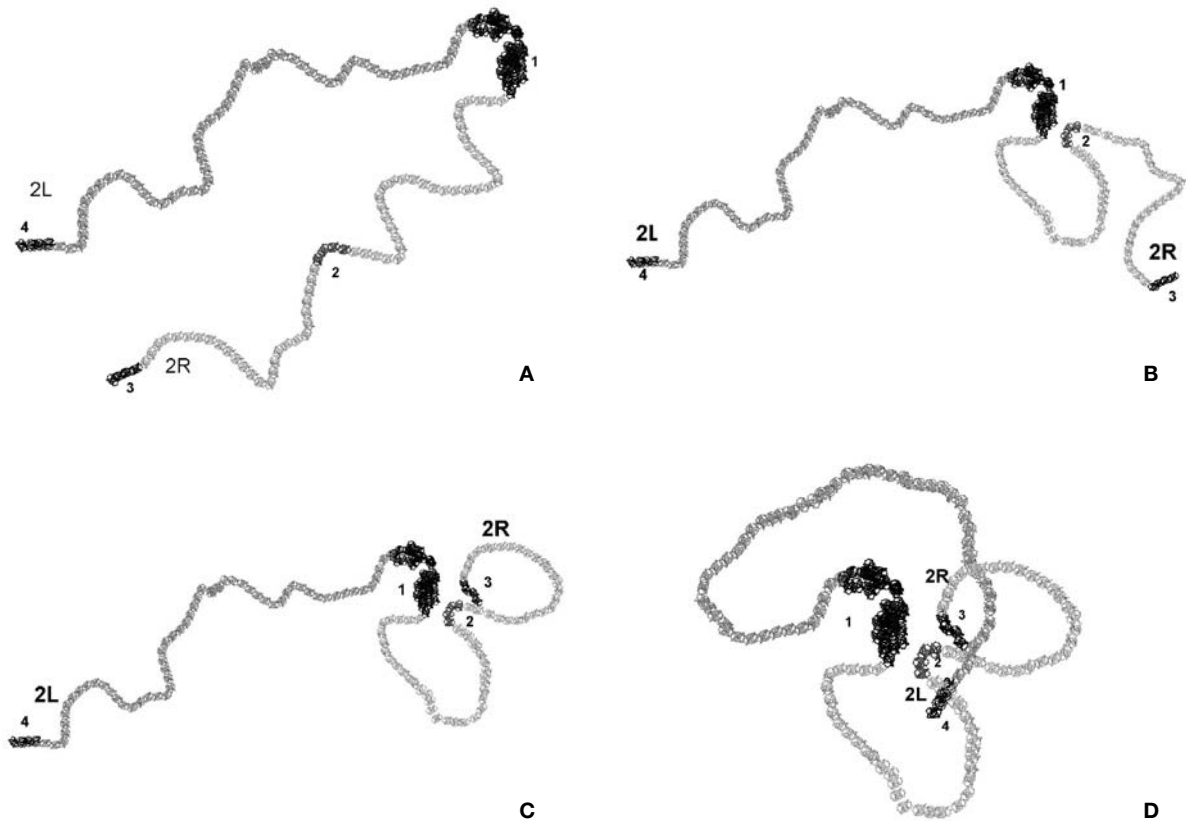


Figure 1. The consecutive steps of 2D modeling of the elementary chromosome 2 fiber macroarchitecture in *Drosophila* sperm genome. (A) A schematic views of the relaxed chromosome thread in polar Rabl configuration with the “hot” nodes (1,2,3,4) located at the appropriate chromosome regions (see Table 1). (B) The first step of modeling showing the close proximity of centromeric heterochromatin (1) with the *vg* gene region (2) and the potential “giant” loop giving rise to inversion after radiation-induced recombination between the “hot” nodes. (C) The next step of modeling indicating the proximity of the middle (2) and telomeric end (3) of 2R arm of chromosome 2. (D) The end result of modeling representing the proximity of the *vg* gene region (2) with the telomeric end (4) of 2L arm of chromosome 2 and the appropriate “giant” loop resulting in the inversion after irradiation. As an idealization, all loops are relaxed.

the gene under study and all “hot” nodes as its potential “partners” are well close to each other in small “sensitive” nuclear volume the minimal radius of which should not exceed 5-50 nm if to take into account that the haploid genome is compacted within the sperm head to a volume of about 5% of that of a somatic cell nucleus (Sassone-Corsi, 2002) and a distance (r) of pairwise interaction of two DSBs in irradiated somatic cells lies in the range 0.1-1 μm (Brenner, 1988). Thereat, since the centromeric heterochromatin is located on the nuclear envelope (Zhimulev, 1993), it can be considered as a kind of “anchor” for the entire

autosome 2 around of which the *vg* gene and other “hot” nodes are grouped.

Basic approaches to modelling

In order to provide a clear visual representation of the chromosome spatial model, the following capabilities were required: subsequent editing, rotation, scaling, displacement of the whole fiber and any chosen object in it, switching on/off of visualization of a selected subset of objects of the model, and the adjustment of additional illumination of the whole picture.

For these purposes, the Gmax v.1.2 software and some supporting programs were used. This software has the mode of constructing the skeleton of a figure on the basis of splines which are convenient for the solution of the purpose in hand. In particular, the method of B splines provides a capability of coloring, deforming, rotating, displacing, hiding, or fixing a selected marked subset of object without violation of the whole thread that essentially simplifies the process for modeling of the chromosome fiber (Zaikin *et al.*, 2003).

2D model

Upon construction of the Two-dimensional model, the distribution and frequency patterns of the second inversion breakpoints interacting with the *vg* gene have been taken into account. For simplicity, the four chromosome “hot” nodes with which the *vg* gene more often interacts were stood out and the average distances of interaction of each “hot” node with the *vg* gene were calculated with regard to the number of interaction observed (Table 1) using the equation

$$r_i = \sqrt{\frac{N}{n_i}} \times 25nm$$

where N is the total number of interactions for the four “hot” nodes; n_i is the number of interactions with a particular node, and 25 nm is an average distances of DSBs interactions calculated on the base of the data for somatic cells above mentioned.

After that, the planar projection with the centers of concentric circles in the region of heterochromatin is constructed manually and the reference points of the spline for the chromosome thread are arranged for the right and left arms at different sides, taking into account the frequency of interaction. A rather convenient mechanism of the visualization and editing of the spline reference points was used for monitoring; it was possible to change the thickness of the constructed thread. It should be specially noted that, at present, a major part of the chromosome thread are modeled in the relaxed state, i.e., by the smooth spline loop due to the absence of relevant data for a given step of modeling. After the visual chromosome thread model was created (Figure 1A), the loops were consecutively constructed: first, the area of the *vg* gene was “drawn” close to the heterochromatin (Figure 1B); then, the telomeric ends of the 2R and 2L arms of chromosome 2 were arranged relative to the *vg* gene taking into account the distances of interactions (Table 1). Because the second “hot” node and the *vg* gene are intimately linked together on the model scale selected these are considered as a single “hot” node during modeling.

As one can be seen from the visual 2D model constructed (Figure 1D), on the large-scale geometry, an elementary fiber of the chromosome 2 in *Drosophila* sperm genome shows the specific rosette-loop configuration. Thereat, the bases of loops are clustered within the presumed “sensitive” microvolume associated with the centromeric heterochromatin. This simple 2D model reflects a pattern of principle of the global chromosome arrangement for animal sperm genome which

Table 1. The average distance of interaction of the *vg* gene with “hot” regions of the autosome 2 resulting in radiation-induced inversions in *Drosophila melanogaster* mature sperms

Number of “hot” nodes *	Contents of “hot” nodes	Number of interactions with the <i>vg</i> gene, n	Distance (r) of interaction, nm
1	Centromeric heterochromatin	21	40
2	48-49 and 50-51 sections, surrounding <i>vg</i> gene	18	44
3	56-60 sections (2R telomeric end)	10	59
4	21-25 sections (2L telomeric end)	7	71
Total (N)		56	

* See text

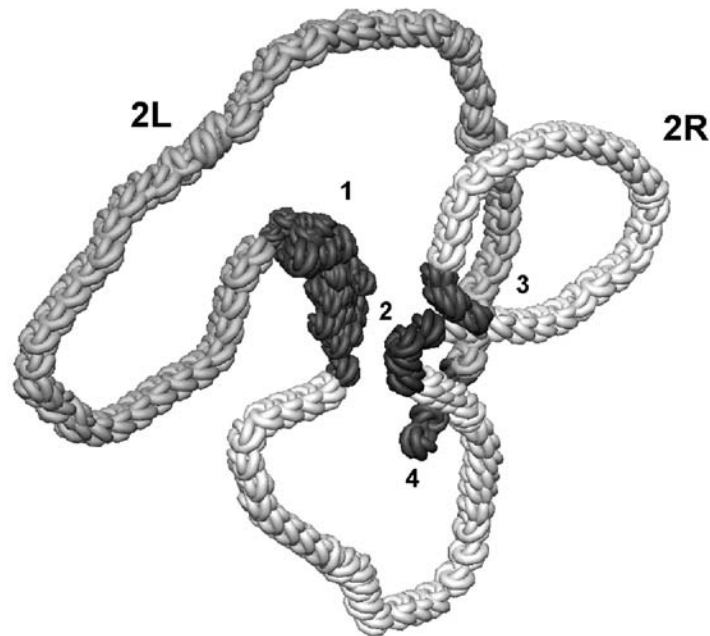


Figure 2. Example of 3D model of an elementary fiber of chromosome 2 in *Drosophila* sperm genome constructed with using of an additional object in the form of a spiral-like structure along the spline and contrast coloration for the “hot” nodes.

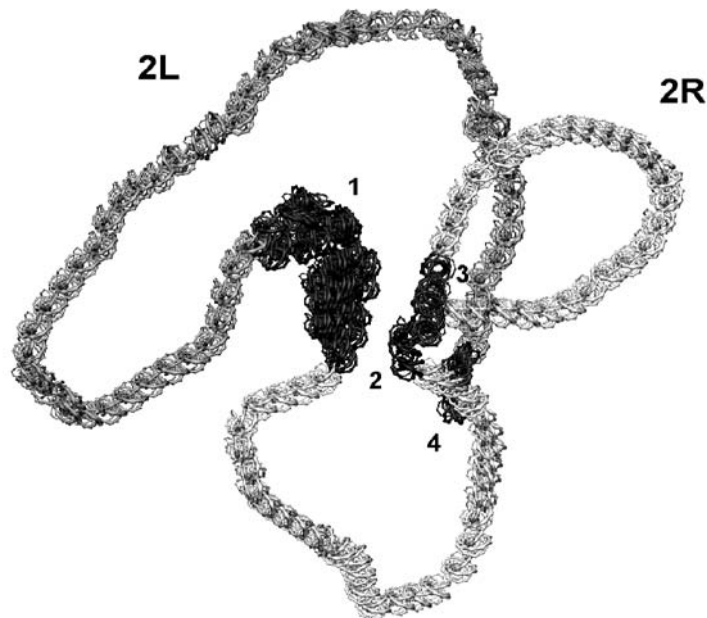


Figure 3. Example of 3D model of an elementary fiber of chromosome 2 in *Drosophila* sperm genome constructed with using of the additional loft, lattice, wave, twist operations along the spline.

is not clearly consistent with the polar Rabl configuration of interphase chromosomes in animal or plant somatic cells. However, this 2D model does not give an idea what this fiber is arranged in space. To elucidate this issue, 3D modeling of chromosome under study was performed.

3D model

The method of B splines is obviously the most acceptable for the construction of the three-dimensional model of the chromosome taking into account that the chromatide thread is a smooth curve without breaks. In such case, the screen is divided into four equal windows of projections in such a way that the lower right window shows the isometric perspective projection of the image and the lower left window shows the planar projection (the views from the right). It is favorable to show the planar frontal projection of the thread above the perspective projection.

During construction of the model, the distance of inversion DSBs interaction calculated (Table 1). They were used as the radii of embedded spheres. According to those data, concentric circles were initially placed at the center of all projection planes. Then the smoothed adaptive spline of order $n = 6$ was constructed on the frontal projection. Unlike the case of the construction of the two-dimensional model, the reference points were edited, in turn, by handles on all three projections inside the largest sphere with the radius $r_0 = 70$ nm.

At the next stage, it is necessary to color chromosome arms. For this purpose it is suitable to use two approaches by using the powerful mechanism of "stringing" additional three-dimensional objects onto the constructed spline with quality, initial position or an interobject distance control which is called Spacing Tools. In the simplest case, the chromosome arm is set as the auxiliary three-dimensional object. The color of the arm after their superposition onto the spline is changed manually and their position on the planar projections is edited for a more accurate edge matching.

The method for the construction of the additional three-dimensional object strongly twisted into a spiral, in the form of the Torus Knot, turned out to be most pictorial for coloring sections of the three-dimensional model. A rather large number of these "knots" are located on the spline, but repeat all its curls more smoothly. For this reason it is then required just to change the color of the knots. It is performed easily using the operation of the group selection of objects

called SelectbyNAME. Then, it is usually necessary to correct the illumination of the picture using different light sources, to rotate the thread for better view, and to add section markers if required (Chumachenko, 2004). It is very convenient to use the mechanism of the temporary removal of selected objects from the picture for the separate visualization of heterochromatin, or a particular arm of the chromosome thread (Zaikin *et al.*, 2005).

The examples of the construction of 3D model of *Drosophila* chromosome 2 in sperm genome using the additional objects and operations are pictured in Fig. 2 and 3. It is significant that the chromosome regions without relevant inversion breakpoints were modeled in the relaxed state as in the case of 2D model. More complex and more realistic 2D and 3D models constructed with consideration of independently arising inversions in the chromosome 2 of the "point" *b*, *cn* and *vg* mutants as well as of inversions with an invariant break at the *b* gene (middle of 2L arm, section 34D) of the same chromosome will be presented at the next paper.

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