

Three-Dimensional Organization of Chromosome Territories and the Human Cell Nucleus

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Abstract. Despite the successful linear sequencing of the human genome its three-dimensional structure is widely unknown. However, the regulation of genes — their transcription and replication — has been shown to be closely connected to the three-dimensional organization of the genome and the cell nucleus. On the bases of polymer physics we have recently developed detailed and quantitative structural models for the folding of the 30 nm chromatin fiber within the human interphase cell nucleus. A quantitative test of several plausible theories resulted in a best agreement between computer simulations of chromosomes, cell nuclei and experiments for the so called Multi-Loop-Subcompartment (MLS) model. Results concern the following properties: overlap of chromosome territories, -arms, -bands, 3D spatial distances between genomic markers as function of their genomic separation in base pairs, fractal analysis of simulations, mass distribution of chromatin in cell nuclei and the fragmentation distribution of cellular DNA after irradiation with carbon ions. Thus in an analogy to the Bauhaus principle that “form follows function”, analyzing in which form DNA is organized might help us to understand genomic function.

1 Introduction

Research on the three dimensional structures of genomes has been obstructed by the incapacibilities of imaging systems: Methods with high spatial resolution (e. g. electron microscopy) partially destroy the structure while in light microscopy where the structure is preserved only low spatial resolution is reached. At the turn of the century, Carl Rabl (1885) and Theodori Boveri (1909) had already postulated a separation of chromosomes into distinct territories in the interphase, i. e. between cell divisions, from studies involving light microscopy. But later, especially since the advent of electron microscopy, the cell nucleus has been viewed as a 'spaghetti soup' of DNA bound to various proteins without much internal structure (Comings et al., 1968) except during metaphase, i. e. cell division, when chromosomes are condensed into separate entities. Only recently has it become apparent that chromosomes occupy distinct 'territories' also in the interphase (Zirbel et al., 1993; see also Fig. 2). This can be granted to the development of fluorescent in situ hybridization (FISH) and confocal laser scanning microscopy (CLSM). In FISH cell nuclei are fixed and after melting the DNA double helix a complementary probe of single stranded and fluorescently labeled DNA is added.

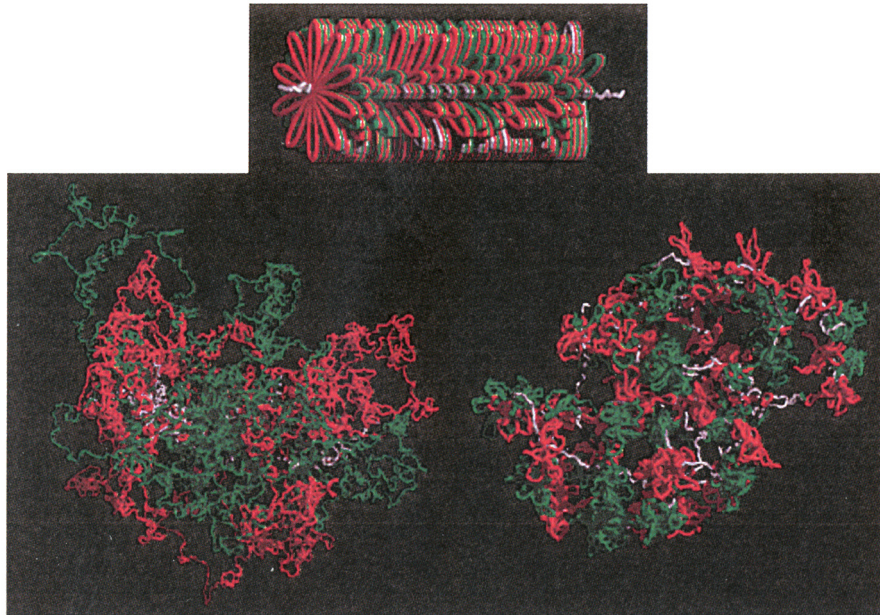


Fig. 1. Visualization of chromosome 15 simulations with the Random-Walk/Giant-Loop-(RWGL)Model and the Multi-Loop-Subcompartment-(MLS)Model. Consecutive loops or rosettes are painted in red and green; the linker is painted in blue. Above: Starting configuration resembling a metaphase chromosome. Left: The RW/GL - model, 5 Mbp loop size, after $\sim 80,000$ Monte Carlo and 1000 relaxing Brownian Dynamics steps. Large loops intermingle freely thus forming no distinct features like in the MLS model. Right: The MLS model, loop size 126 kbp and linker size 126 kbp, after $\sim 50,000$ Monte Carlo and 1000 relaxing Brownian Dynamics steps. Here rosettes form subcompartments as separated organizational and dynamic entities. The big holes visible are filled by other chromosomes in a whole nucleus. Nevertheless, in usual cell nuclei 80% of the volume is filled with water.

While diffusing through the nucleus the probe finds its complementary target sequence and binds with high affinity. The territories thought to be rather compact regions of the nucleus, the hypothesis has been forwarded that active genes are transcribed mainly at the periphery, and macromolecules (i. e. transcripts and proteins) are transported in a hypothetical space between adjacent and nonoverlapping territories. However, the postulated intrachromosomal domain (ICD, Cremer et al., 1993) channels have never been directly visualized.

The distribution of the chromatin fiber within a chromosome territory is also far from random. Several theoretic descriptions of chromosome structure based on polymer-chain models of the chromatin fiber have been postulated. These can be compared to experimental data obtained by marking small genomic targets with FISH and measuring the spatial distance between these

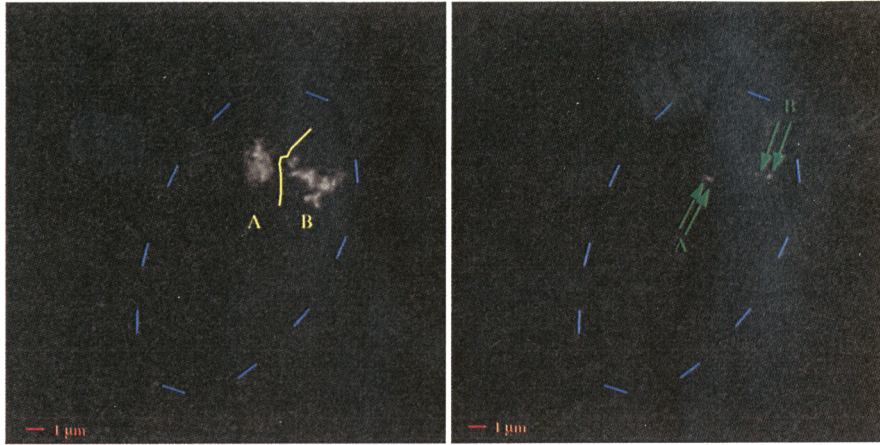


Fig. 2. Fluorescent in situ hybridization (FISH) of chromosome 15 and FISH marked target sequences in the Prader-Labhart-Willi/Angelmann (PLW/A) region in the same nucleus. Left: Projection of confocal image series with ‘painted’ chromosome 15 territories (A and B). Territories are placed behind each other and detected with a green fluorescent colour. Right: Projection of confocal image series with two target sequences with a genomic separation of 1 Mbp and marked with a red fluorescent colour in each of the territories. Territory A, two signals slightly behind each other, spatial distance 550nm. Territory B, spatial distance 840nm.

markers as a function of their genomic (i. e. linear sequence) distance. Based on such experiments, Sachs proposed the so-called Random-Walk/Giant-Loop (RW/GL) model (Sachs et al., 1995). In the RW/GL-model big chromatin loops with a base pair content of around 3 to 5 million are bound to a nuclear matrix. However, whether a nuclear matrix exists or not is still an unresolved question. We developed the so-called Multi-Loop-Subcompartment (MLS) model for solving some contradictions of the RW/GL-model (Münkel et al., 1998). Here the chromatin fiber folds into ~ 120 kbp sized loops which again are forming rosettes of in total 1-2 Mbp. These rosettes are interconnected by a piece of chromatin of similar basepair content so that no protein matrix is needed for structural support. Rosettes in the MLS-model correspond to the size of chromosomal interphase (ideogram) band domains. This model leads also to an easy mechanism for decondensation (or condensation) of a chromosome from metaphase: A loop is opened (or formed) at its base (e. g. protein mediated connection). This model also agrees with the metaphase organization as proposed by Pienta and Coffey (1984).

2 Simulation Methods

The energetics and dynamics of the above mentioned models can be explored numerically using Monte Carlo and Brownian Dynamics methods. Various

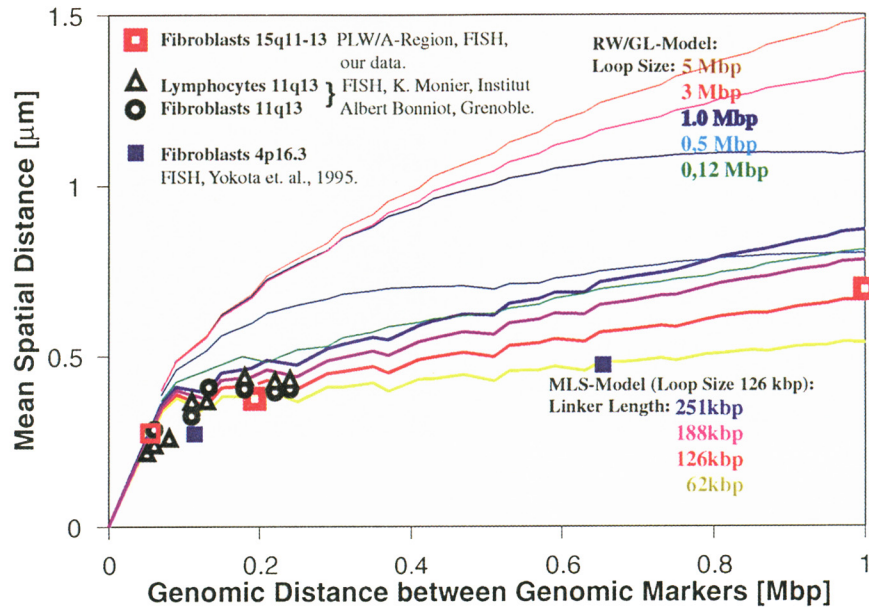


Fig. 3. Comparison between experimental and simulated spatial distances versus genomic distances between genomic markers (Knoch, 1998). MLS-model with a fixed loop size of 126 kbp, linker length of 63 kbp, 126 kbp, 190 kbp, and 250 kbp and in the RW/GL-model loop sizes of 5 Mbp, 4 Mbp, 3 Mbp, 2 Mbp, 1 Mbp, 500 were simulated. The linker length was adjusted in such a way that the global behaviour leads to comparable results in both models. Red rectangles: Our experiments (FISH) between genomic markers in the Prader-Labhart-Willi/Angelmann- (PLW/A) region, chromosome 15q11-13 (Knoch, 1998). Triangles/Circles: Fluorescence in situ hybridization (FISH) data for both lymphocyte and fibroblast cells, chromosome 11q13, f from K. Monier, Institut Albert Bonniot, Grenoble, France. Blue rectangles: Fluorescence in situ hybridization (FISH) data for lymphocyte cells, chromosome 4p16.3, from Yokota.

models of human interphase chromosome 15 assuming a flexible polymer chain were simulated. To each polymer segment we assigned a harmonic stretching potential and between two segments a harmonic bending potential. To avoid self-crossing of the polymer chain a short ranged excluded volume potential was introduced. Its potential barrier can be changed to facilitate chain disentanglement. In vivo this is mediated by the protein Topoisomerase II. The starting configuration of a chromosome has the approximate form and size of a metaphase chromosome decondensation into interphase resembles the natural process (Fig. 1, above). To decrease computation time we started with polymer segments of 300 nm (31,000 bp) corresponding to 3300 segments for chromosome 15. Typically 400,000 Monte Carlo steps are needed to generate

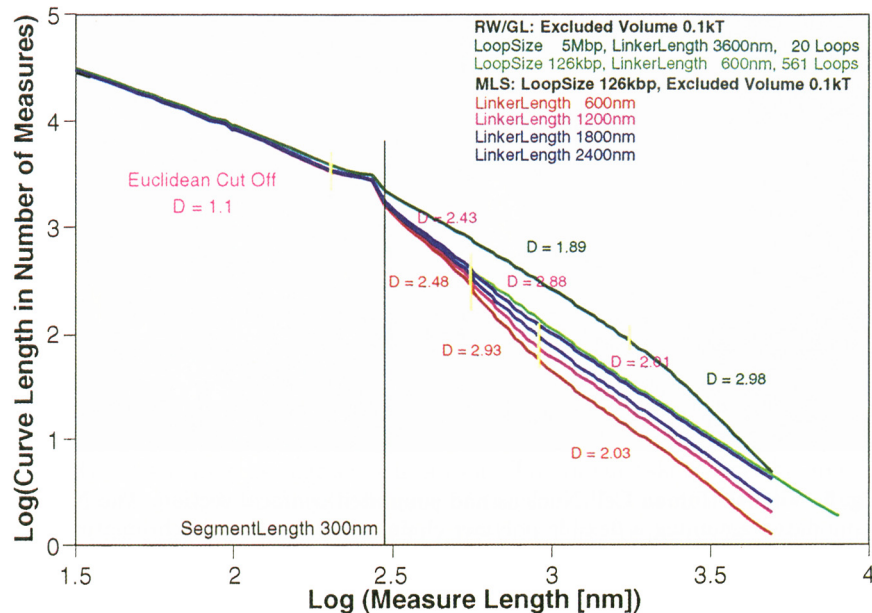


Fig. 4. Fractal analysis of the Random-Walk/Giant-Loop model and the Multi-Loop-Subcompartment-model. In agreement with porous network research fractal analysis shows multifractal behaviour in simulations of chromosome 15. Different fractal dimensions mean different process-dynamics in these scale region. Thus chromosome territories show a higher implicit degree of determinism than previously thought. Standard errors of fractal dimensions are below 0.3%.

enough statistically independent configurations at equilibrium. For comparison with experimental data at small genomic distances the Monte Carlo configurations are taken as starting points for relaxation at higher spatial resolution by Brownian Dynamics methods. The segment length is decreased to 50 nm (5000 bp), corresponding to 20,000 segments for chromosome 15. 2000 Brownian Dynamics steps are performed until equilibration is reached again (Fig. 1, left and right).

For the simulation of a whole nucleus, 46 of the above mentioned starting configurations are put in a spherical potential representing the nuclear membrane. Depending on the segment length a nucleus consists of 200,000 to 1,200,000 segments (Fig. 5). The simulations programme "VirtNucSim" was written in C++ and because of the large numbers of segments the algorithms had to be parallelized using Message Passing Interface (MPI). One Brownian Dynamics step for nuclei with 1,200,000 segments takes around 23 min and a comparable Monte Carlo step around 45 min. The simulations presented here had a degree of parallization of 77% [CPU-parallel/CPU-total] using 16 processors R6000-70MHz and took in total about 180.000 h of CPU time

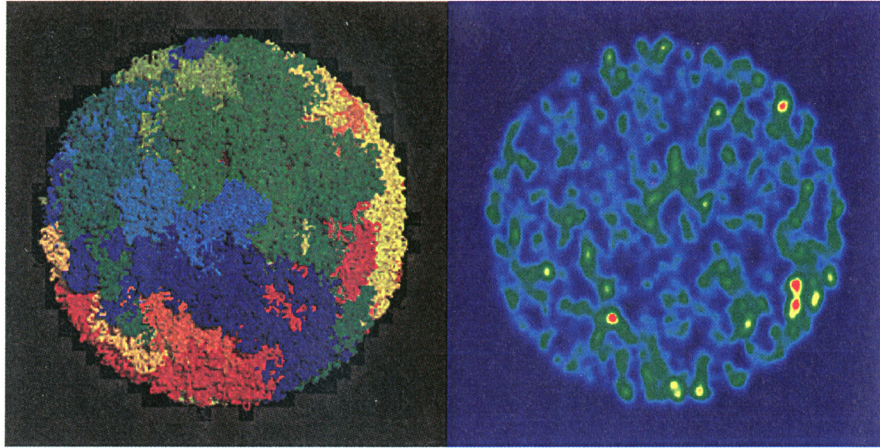


Fig. 5. Virtual Human Cell Nucleus and simulated confocal section. The Nucleus is simulated assuming a flexible polymer chain, modelling the 46 chromatin fibers with in total 1,248,794 50 nm = 5.2 kbp segments. Visualizations are shown after 0,5 ms Brownian Dynamics simulation, one step taking 10 s. As starting configuration a metaphase nucleus was chosen, i. e. each chromosome was chosen es metaphase chromosome (Fig. 1, above) and homologous chromosome were placed randomly but next to each other into the nucleus. Left: Simulation of an nucleus with the MLS model. The different chromosomes are painted with different colours. The forming of territories of chromosomes is clearly visible. Right: Simulated confocal section of the left nucleus in agreement with experiment. False colour representation.

on a single processor (normalized to the IBM SP2 in Karlsruhe). The platforms used were the IBM-SP2-DKFZ, the HP Convex-(S-class)-DKFZ, the IBM-SP2-SCC-Karlsruhe, and the Cray T3E-HWW-Stuttgart.

3 Experimental Methods

For measuring spatial distances between genomic markers as a function of their genomic separation we chose target sequences of chromosome 15 within the region responsible for the Prader-Labhart-Willi/Angelmann (PLW/A) syndrom (c15q11.2-13.3), which is believed to be caused by a structural change in the genome, in contrast to the common base pair mutation. We used probes of about 11 kbp with separations of 18 kbp to 1 Mbp (in cooperation with K. Bütig and Prof. B. Horsthemke, Essen, Germany). Human fibroblast cells were grown on coverslips to layers, and assumed to be in the same phase of the cell cycle. The cells were fixed with paraformaldehyde in an isotonic environment, labeled with biotin and digoxigenin and were detected with fluorescent dyes bound to anti-digoxigenin and anti-biotin antibodies. Typically 25 sections per image series were then taken with a confocal laser scanning microscope (CLSM) Leica TCS NT (in cooperation with J.Rauch, H. Bornfleth,

C. Cremer, Heidelberg, Germany). With the CLSM distance measurements between the center of mass of genomic markers with an accuracy of 35 nm are possible if each marker is detected with a different fluorescent colour and a correction is made for chromatic shift (Fig. 2).

4 Results

For the determination of the spatial distance as a function of genomic distance from the simulations, the spatial distance was calculated for genomic distances randomly positioned within the chain of segments and for statistically independent configurations. Therefore the mean is taken for marker positions with no definite relative position to loops, rosettes or linker between rosettes. Segments in the linker between the rosettes are not as much spatially constrained as segments in a loop. The mean taken over the spatial distances resulted in a standard error of the mean below 1%. In the experiments the mean was taken over 120 nuclei for each distance, and including controls 1600 nuclei were imaged. Best agreement between simulation and experiment (Fig. 3) is reached for a Multi-Loop-Subcompartment model with a loop size of 126 kbp and a linker length of again 126 kbp testing against our and K. Moniers data (Monier et al., 1999). Thus the distance measurements of the genomic regions used here do not support a Random-Walk/Giant-Loop model. Due to the lack of experimental data it is, however, not yet possible to make clearer statements about the positioning of the experimental markers relative to loops, rosettes or linker between rosettes.

For calculating more general properties of chromosomes the fractal dimension of the chromatin fiber was determined from the simulations. An exact-divider analysis was used, where the divider-measure (i. e. the length of the divider) covers four orders of magnitude. The starting point of the divider was randomly positioned within the chain of segments and then the number of dividers necessary to reach both ends of the chain of segments was computed. To account for biased starting points for bigger divider measures a mean over several starting points was taken as well as for independent configurations. The fractal dimension was calculated as the negative slope from a linear regression in plotting the logarithm of the number of dividers used to measure the chromosome as function of the logarithm of the length of the divider measure. The standard error for the fractal dimension was smaller than 0.3%. The fractal analysis resulted in different and distinct multifractal behaviour for the MLS- and the RW/GL-model (Fig. 4) in good agreement with predictions drawn from porous network research (Avnir, 1989; Mandelbrot, private communications). The scale region on which the various fractal dimensions are valid defines the borders of the underlying structure (loops, rosettes and the whole chromosome for the MLS and loops and the whole chromosome for the RW/GL-model, yellow border lines in Fig. 4; Knoch, 1998).

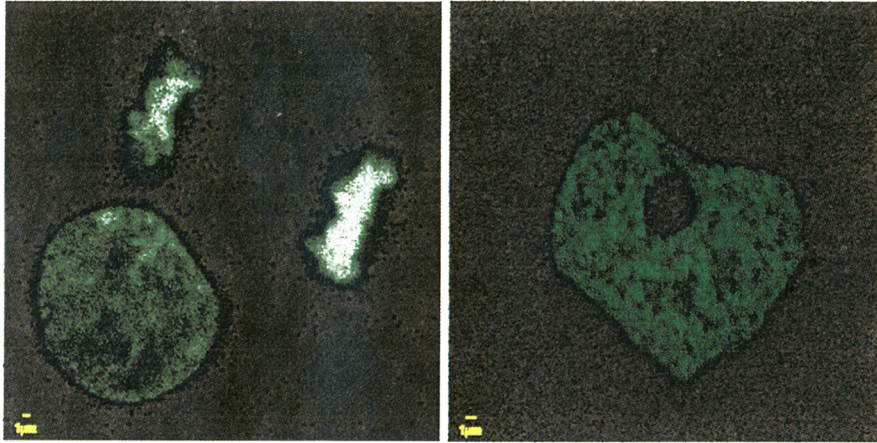


Fig. 6. Mapping of Histone H2B-GFP and H10-GFP distribution in vivo. The Histone-GFP (Green-Fluorescent-Protein) Fusion Protein reflects the distribution of chromatin in interphase, because the labeled histone is built into the nucleosome. The structure visible in the images is similar to those found in simulations (see Fig. 5). Left: HeLa cells stably transfected with H2B-GFP (image provided by K. Sullivan, Scripps Institute (Kanda et al., 1998)). Confocal in vivo section of a cell nucleus and a mitosis. Right: Cos7 cells stably transfected with H10-GFP (cells provided by A. Alonso, DKFZ). Confocal in vivo section.

The simulation of a whole human cell nucleus in connection with the simulation of single chromosomes resulted in the formation of distinct chromosome territories as predicted (Fig. 5). To compute the overlap of chromosomes, chromosome arms and chromosome subcompartments (i.e. rosettes) from simulations the simulated chromatin mass distribution of the respective structures was folded with an experimental point spread function (PSF) of a confocal microscope and filtered with a $3 \times 3 \times 3$ -median filter. The overlap was calculated using an autocorrelation approach (Münkel et al., 1998). In contrast to the RW/GL-model the MLS-model leads to low overlap between chromosome territories as well as chromosome arms, in agreement with overlap analysis of confocal image series in which the overlap was calculated with the same autocorrelation approach (Münkel et al., 1999). The available experimental data show similar behaviour throughout the whole cell nucleus. Overlaps are the result of the small scale folding of the chromatin fiber within chromosomes and the whole cell nucleus. In connection with the measurement of spatial distances between genomic markers as a function of their genomic separation one could conclude that throughout the cell nucleus an MLS-model might best agree with experimental data.

The density distribution of the chromatin fiber from simulations can also be compared to the distribution of Histone-GFP fusion proteins which are built into the chromatin fiber in living cells (Fig.6). A morphologic compar-

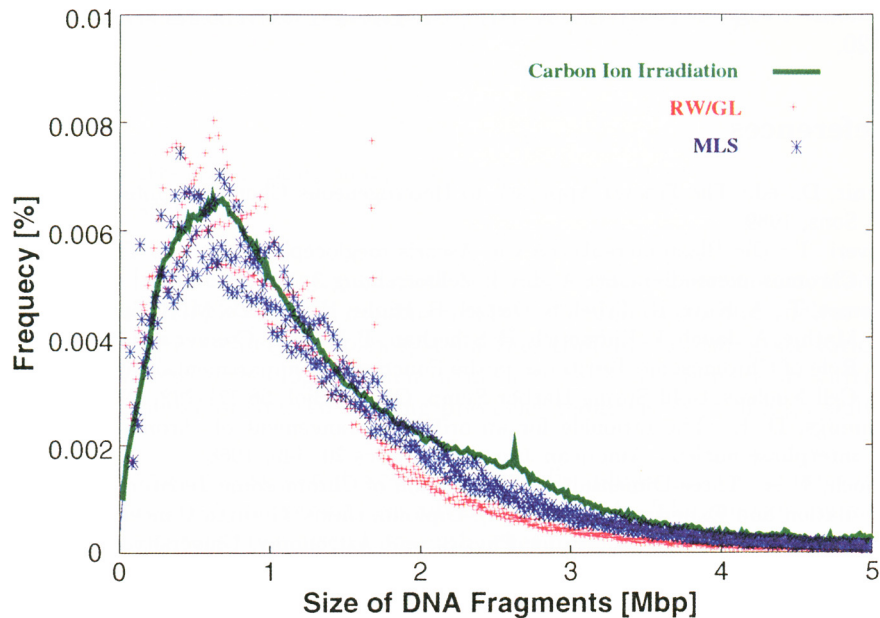


Fig. 7. DNA fragmentation by irradiation with carbon ions - comparison between experimental and simulated distributions. Irradiation with carbon ions results in DNA double strand breakage. The length of the fragments follow distributions depending on the spatial arrangement of the 30 nm chromatin fiber in the nucleus. The carbon ion irradiation was simulated on the basis of our chromosome simulations by P. Quicken (data provided by P. Quicken, GSF, Munich).

ison between simulations (Fig. 5, right) and experiments lead to a similar distribution of the 30 nm chromatin fiber. The "clumps" visible have sizes between 300 and 600 nm which might correspond to the subcompartments in agreement with the MLS-model.

Another test of the model is the length distribution of DNA fragments after irradiating cell nuclei with carbon ions causing double strand breaks in the DNA double helix. The length distribution is characteristic for the folding of the chromatin fiber. P. Quicken from the GSF in Munich used our computed configurations to simulate the length distribution of DNA fragments to compare it to his experimental results. Preliminary results favour again the Multi-Loop-Subcompartment model (MLS) (Fig. 7).

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