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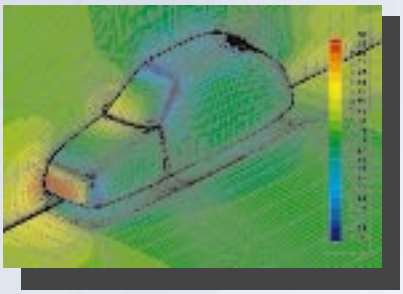

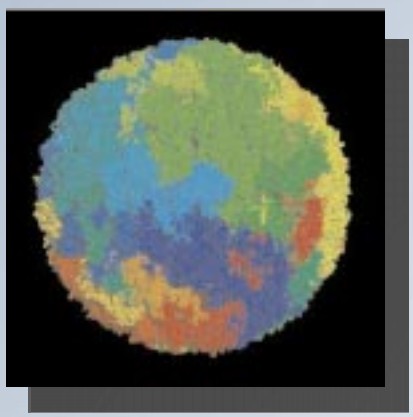

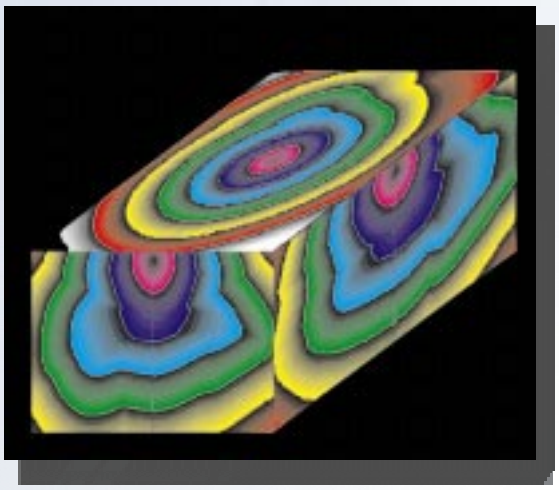

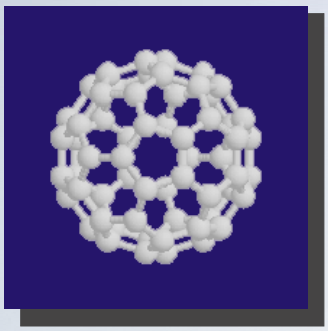

Three-Dimensional Organization of Chromosome Territories and the Human Cell Nucleus

Tobias A. Knoch, Christian Münkel, Jörg Langowski
Division Biophysics of Macromolecules, German Cancer Research Center, Heidelberg

In collaboration with Joachim Rauch, Harald Bornfleth and Christoph Cremer (Institute for Applied Physics, University of Heidelberg), Karin Bütig and Bernhard Horsthemke (Institute for Human Genetics, University of Essen), Irina Solovei and Thomas Cremer (Institute for Anthroplogy and Human Genetics, University of Munich)

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 connected closely to the three-dimensional organization of the genome in the cell nucleus. The cell nucleus has for a long time been viewed as a 'spaghetti soup' of DNA bound to various proteins without much internal structure, except during cell division when chromosomes are condensed into separate entities.

Only recently it has become apparent that chromosomes occupy distinct 'territories' also in the interphase (Zirbel *et. al*., Chromosome Research 1:92-106, 1993). The distribution of the chromatin fiber within a chromosome territory is also far from random. Based on the measurement of three dimensional distances between fluorescently labeled genomic regions with confocal laser scanning microscopy, Sachs (Sachs *et al.*, PNAS 92:2710-2714, 1994) proposed the so-called Random-Walk/Giant-Loop (RW/GL)-model. In the RW/GL–model big chromatin loops with 3 to 5 million basepairs are bound to a nuclear matrix. For solving contradictions of the RW/GL-model with experiments we developed the so-called Multi-Loop-Subcompartment (MLS)-model (Münkel *et al.*, Physical Review E 57:5, 5888-5896, 1998). Here the chromatin fiber folds into ~120 kbp sized loops which are forming rosettes of in total

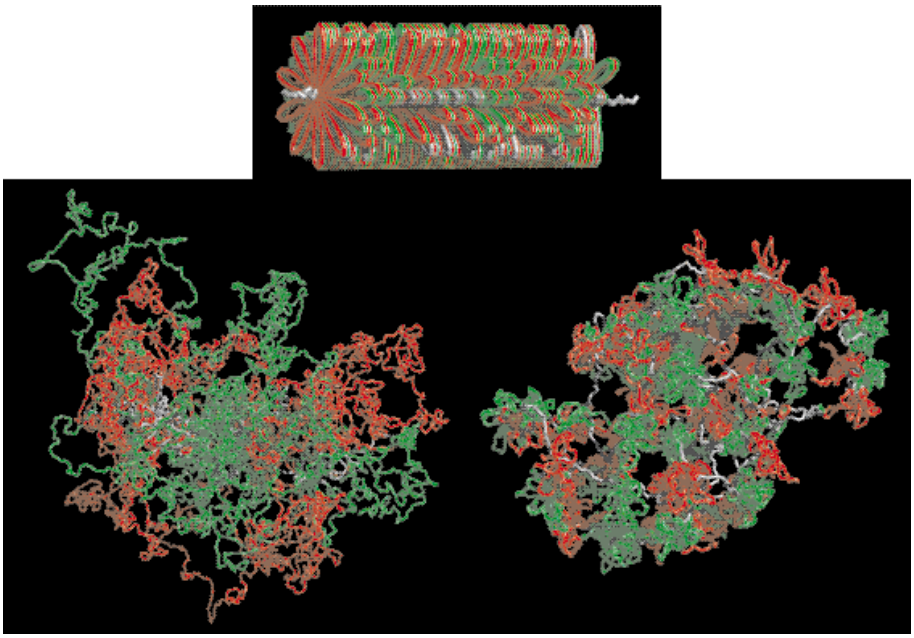

Fig 21: Visualization of chromosome 15 simulations with the Random-Walk/Giant-Loop-(RWGL) Model and the Multi-Loop-Subcompartment- (MLS) model.

Consecutive loops or rosettes are painted in red and green; the linker is painted in blue.

Above: Starting configuration resembling a metaphase chromosome. The big holes visible are filled by other chromosomes in a whole nucleus. Nevertheless, in usual cell nuclei 70% - 80% of the volume is filled with water.

Left: The RW/GL - model, 5 Mbp loop size, after ~ 80,000 Monte Carlo and 1000 relaxing Browninan 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 ~ 50,000 Monte Carlo and 1000 relaxing Brownian Dynamics steps. Here rosettes form subcompartments as separated organizational and dynamic entities.

1–2 Mbp and represent the DNA content of a typical ideogram band in prophase. These rosettes are interconnected by a piece of chromatin of similar basepair content so that no protein matrix is needed for structural support. This leads also to an easy mechanism for decondensation of a chromosome from metaphase: A loop is opened at its base (e. g. protein mediated connection). The model also agrees with the metaphase organization as proposed by Pienta and Coffey (J. Cell Sc., Supplement I: 123-135, 1984).

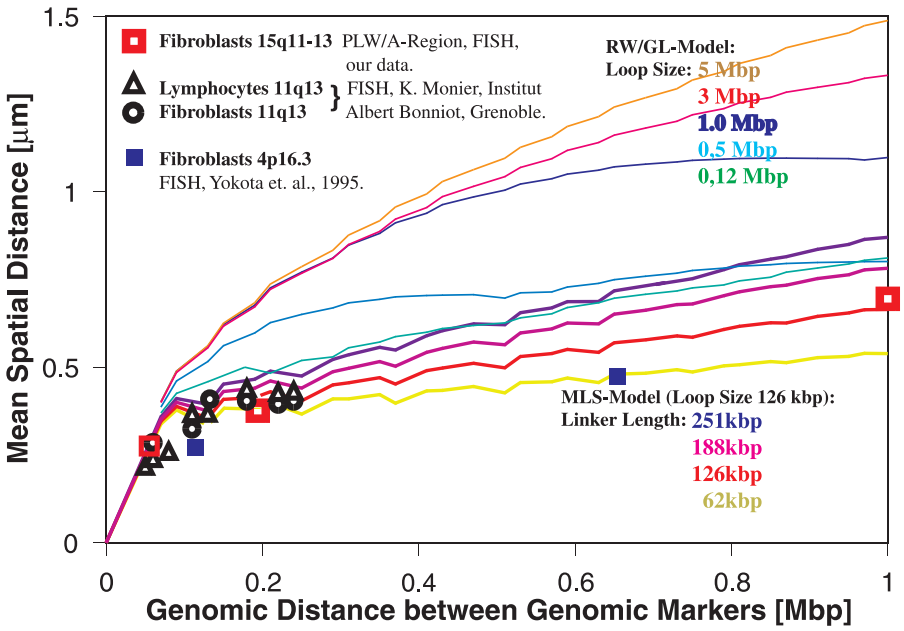

Fig 22: Comparison between experimental and simulated spatial distances versus genomic distances between genomic markers.

MLS-models with a fixed loop size of 126 kbp, linker length of 63 kbp, 126 kbp, 190 kbp, and 250 kbp and RW/GL-models with loop sizes of 5 Mbp, 4 Mbp, 3 Mbp, 2 Mbp, 1 Mbp, 500k bp and 126 kbp were simulated. The linker length was adjusted in such a way that the global behavior 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.

Triangels/Circles: Fluorescence in situ hybridization (FISH) data for both lymphocyte and fibroblast cells, chromosome 11q13, from K. Monier, Institut Albert Bonniot, Grenoble, France.

Blue rectangles: Fluorescence in situ hybridization (FISH data for lymphocyte cells, chromosome 4p16.3, from Yokota.

The energetics and dynamics of these models can be explored numerically using Monte Carlo and Brownian dynamics methods. Here the chromatin fiber is described by a polymer chain of segments. The starting configuration of a chromosome has the form and size of a metaphase chromosome, and the decondensation into interphase resembles the natural process. Several of these configurations (46 for the human chromosome set) can be placed into a spherical shell, simulating the chromosome distribution inside the nucleus. In this case, more than 1.200.000 polymer segments must be used to describe the human chromosome set and the modeling must be performed on parallel computers (IBM SP, Cray T3E). With these methods various models of human interphase chromosome 15 (Fig.21, Knoch, diploma thesis, 1998) were simulated. For comparison with experimental data, three-dimensional distances between fluorescently labeled genomic regions with confocal laser scanning microscopy were measured for the Prader-Willi-Region on chromosome 15 (Knoch, diploma thesis, 1998).

Best agreement between simulation and experiment is obtained for a Multi-Loop-Subcompartment model with a loop size of ≈126 kbp and a linker length of ≈126 kbp when compared with intrachromosomal distance measurements (Fig. 22, Monier *et. al.*, in preparation; Knoch, diploma thesis 1998). Fractal analysis resulted in different and distinct multifractal behavior for the MLS- and the RW/GL-model in good agreement with predictions drawn from porous network research (Avnir, 1989; Mandelbrot, private communications). The simulation of whole human cell nuclei in combination with the simu-

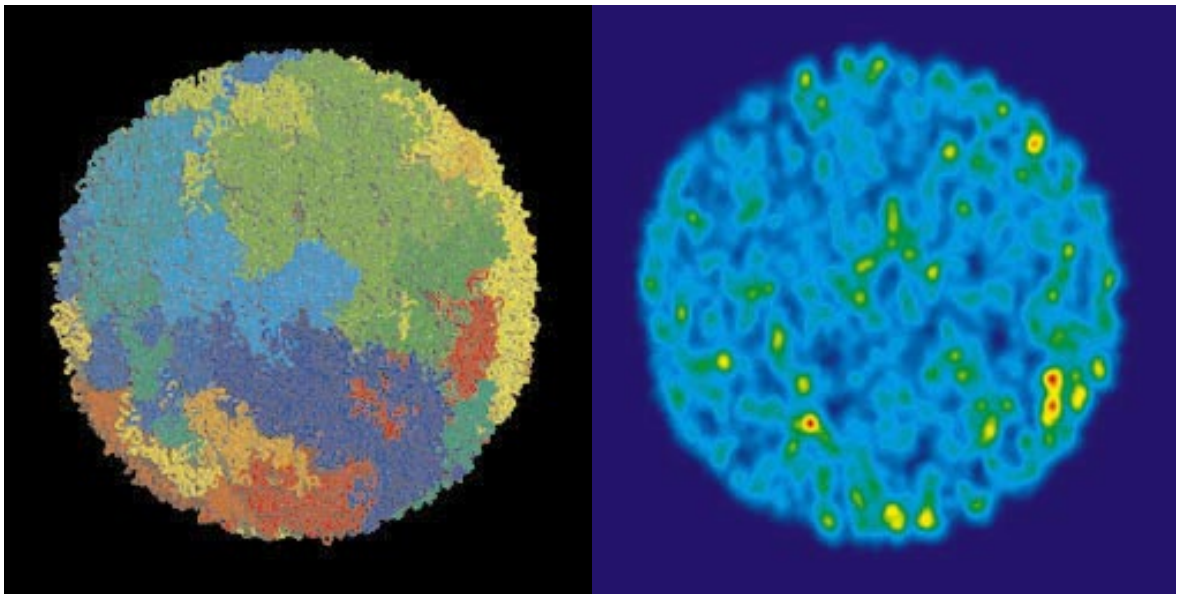

Fig. 23: ´Virtual Human Cell Nucleus' and simulated confocal section.

The Nucleus is simulated assuming a flexible polymer chain, modeling the 46 chromatin fibers with in total 1,248,794 segments, each 50 nm = 5.2 kbp in size. Visualizations are shown after 0,5 ms Brownian Dynamics simulation, one step taking 10 µs. As starting configuration a metaphase nucleus was chosen, i. e. each chromosome was chosen as metaphase chromosome (Fig. 21, above) and homologous chromosomes were placed randomly but next to each other into the nucleus.

Left: Simulation of a nucleus with the MLS – model. The different chromosomes are painted with different colors. The forming of territories of chromosomes is clearly visible.

Right: Simulated confocal section of the left nucleus in agreement with experiments. False color representation.

lation of single chromosomes resulted in the formation of distinct chromosome territories as predicted. 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 (Fig. 23, Münkel *et. al.*, Physical Review E 57:5, 5888-5896, 1998; Münkel *et. al*., J. Mol. Biol. 285, 1053-1065, 1999).

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