Three - Dimensional Organization of the Human Interphase Nucleus
Experiments compared to Simulations

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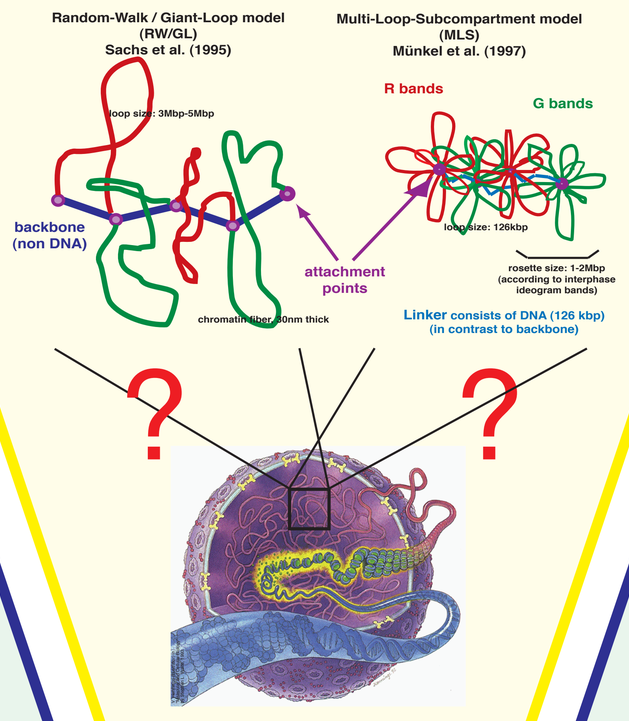
German Human Genome Project

INTRODUCTION

Despite the succesful linear sequencing of the genome its three dimensional structure is widely unknown although it is important for gene regulation and replication. With a comparison between experiments and simulations we show here an interdisciplinary approach leading to the determination of the three- dimensional organization of the human genome.



FISH

Fluorescence in situ hybridization (FISH) in connection with confocal laser scanning microscopy followed by image reconstruction procedures is used for the specific marking of chromosome arms (Fig. 1A) and small chromosomal DNA regions (Fig. 1B). Chromosome arms show only small overlap and globular substructures as predicted by the MLS-model (Fig. 1A). A comparison between simulated and measured spatial distances between genomic regions as function of their genomic distances results as well in a good agreement with the MLS-model with a loop size of arround 126 kbp and linker sizes between 63 kbp and 126 kbp (Fig. 2).

Fig. 1A & 1B: FISH-images of a territory painting of chromosome 15 (left, 1A) and genomic markers YAC-48 and YAC60 (right 1B) with a genomic separation of 1.0 Mbp in interphase of fibroblast cells.

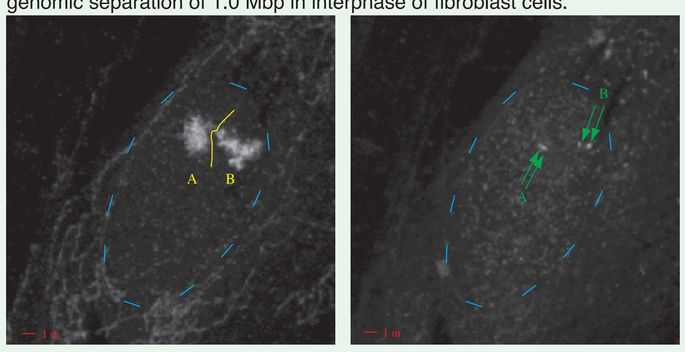
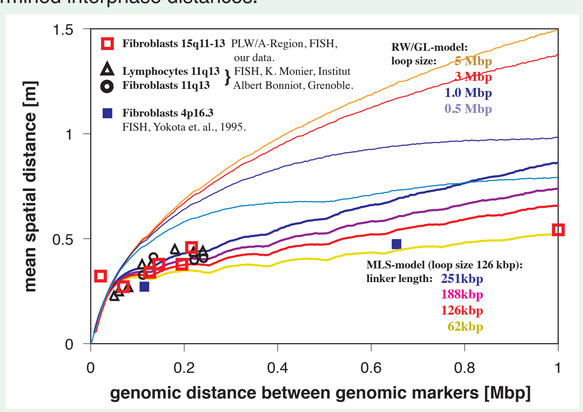


Fig. 2: Comparison of the RW/GL- and the MLS-model with experimentally determined interphase distances.



SIMULATION

With Monte Carlo and Brownian Dynamics methods we simulated various models of human interphase chromosome 15 assuming a flexible polymer chain of 50 nm long stiff segments. Only stretching, bending and excluded volume interactions between the segments are considered. Chromosomes are further confined by a spherical potential representing the surrounding chromosomes or the nuclear membrane. Only the MLS model leads to clearly distinct functional and dynamic subcompartments (Fig. 7B) in contrast to the RW/GL models where big loops are intermingling freely and featureless (Fig. 7C & 7D).

Fig. 7A: Starting configuration with the form and size of a metaphase chromosome.

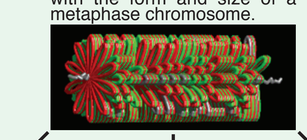


Fig. 7B: MLS model with 126 kbp loops and linkers.

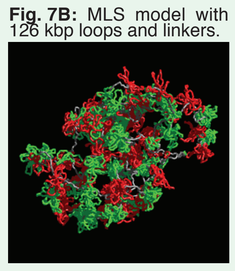


Fig. 7C: RW/GL model with 126 kbp loops.

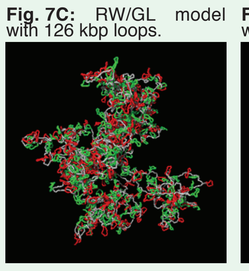


Fig. 7D: RW/GL model with 5 Mbp loops.

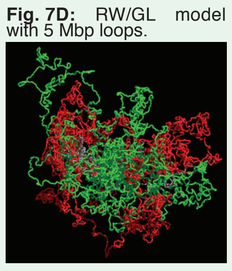
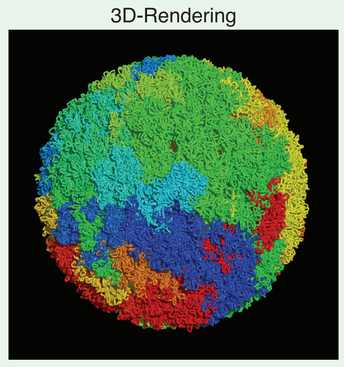
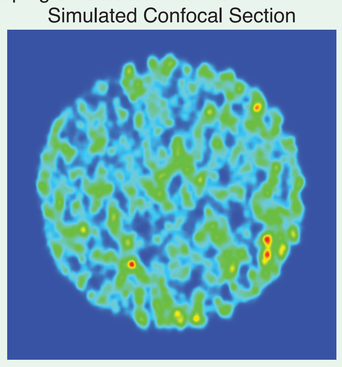


Fig. 8A & 8B: Simulation of a human interphase nucleus containing all 46 chromosomes with 1,200,000 polymer segments. The MLS-model leads to the formation of distinct and non-overlaping chromosome territories.

3D-Rendering



Simulated Confocal Section



CONCLUSION

Simulations of chromsomes and the whole cell nucleus show that only the MLS-model leads to the formation of non-overlapping chromosome territories and distinct functional and dynamic subcompartments. Spatial distances between FISH labeled pairs of genomic markers as function of their genomic distance result in a MLS-model with loop sizes of 120 kbp and linker sizes of 63 to 126 kbp. With the developement of GFP-fusionproteins it is possible to study the chromatin distribution and dynamics resulting from cell cycle, treatment by chemicals or radiation in vivo. The chromatin distribution is similar to those found in the simulation of whole cell nuclei. Fractal analysis of the simulations reveal the multifractality of chromosomes in agreement with porous network research. It is possible to quantify the in vivo chromatin distribution with fractal analysis and to relate the result to differences in morphology. The simulation of fragment distributions based on double strand breakage after carbon-ion irriadiation differs in different models. Here again a comparison to experiments favours a MLS-model.

CHROMATIN LABELING in vivo

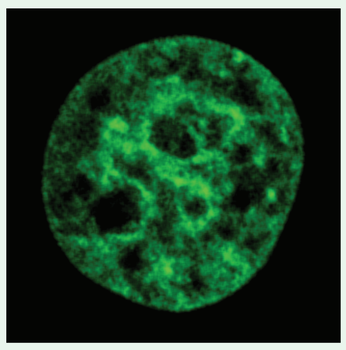
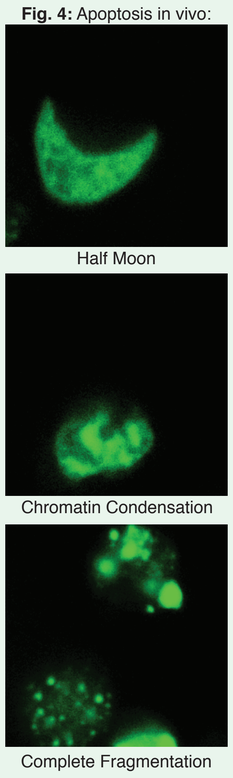


Fig. 4: Apoptosis in vivo:



Half Moon

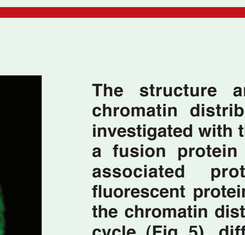
Chromatin Condensation

Complete Fragmentation

The structure and dynamics of the chromatin distribution in vivo can be investigated with the stable expression of a fusion protein between a chromatin associated protein and an auto fluorescent protein like GFP. Changes in the chromatin distribution due to the cell cycle (Fig. 5), differentiation, chemicals (Fig. 4) or radiation are now possible without fixation and staining and therefore artefactfree and time saving. The structures visble in confocal images of normal interphase nuclei (Fig. 3: nucleus left with an 20 m image sidelength) are similar to those found in simulated confocal images of the MLS-model (Fig. 8B). The structures can be analyzed quantitatively by global and local fractal analysis (Fig. 10) and hence can be linked to the detailed folding of the chromatin fiber.

Fig. 5: Mitosis in vivo:

Mitotic plate, side view



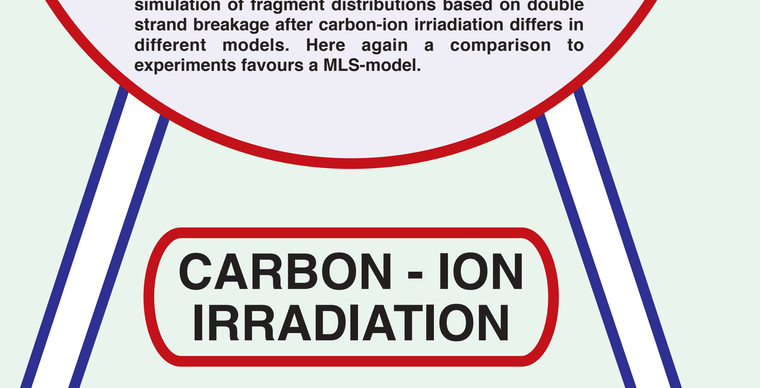
Anaphase

Sidelength of all images:
40 m

CARBON - ION IRRADIATION

Irradiation with carbon ions results in DNA double strand breakages. The length distribution of the fragments and the sites of breackage depend on the spatial arrangement of the 30 nm chromatin fiber in the nucleus. Simulated configurations of different chromosome models were taken as the basis for the detailed simulation of these fragment distributions. The RW/GL-model and the MLS-model lead to clearly distinct fragment distributions (Fig. 6). A comparison with experiments favours an MLS-model. The specifity of breakage sites is currently analyzed. Data were by P. Quicken.

Fig. 6: Comparison of simulated fragment distributions.



Radiation dose distribution in a cell nucleus.

FRACTAL ANALYSIS

The nucleus is an unordered and non-euclidean system for which fractal analysis which measures the mass distribution in space is especially suited. The dynamic behaviour of the chromatin structure and the diffusion of particles in the nucleus are also closely connected to the fractal dimension. The fractal analysis of the simulation of chromosome 15 lead to multifractal behaviour in agreement with porous network research (Fig. 9). Therefore chromosome territories show a higher degree of determinism than previously thought. First tests of fractal analysis of chromatin distributions in vivo result in significant differences for different morphologies (Fig. 10) and might favour an MLS-model like chromatin distriubtion.

Fig. 9: Comparison of RW/GL- and MLS- model with fractal dimension of the chromatin fiber from simulations.

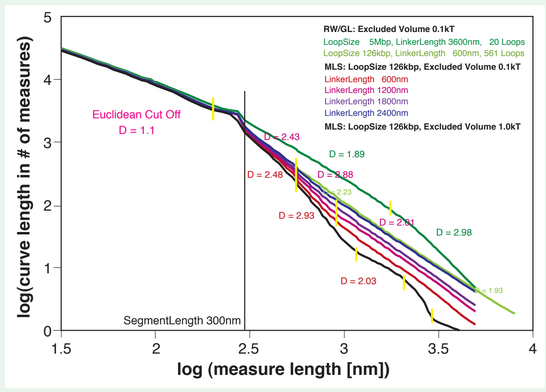
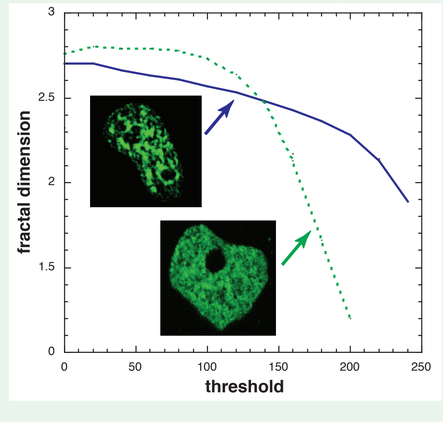


Fig. 10: Fractal Dimension as function of the intensity threshold.



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Three-Dimensional Organization of the Human Interphase Nucleus

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Abstract

Despite the successful linear sequencing of the human genome its three-dimensional structure is widely unknown, although it is important for gene regulation and replication. For a long time the interphase nucleus has been viewed as a 'spaghetti soup' of DNA without much internal structure, except during cell division. Only recently has it become apparent that chromosomes occupy distinct 'territories' also in interphase. Two models for the detailed folding of the 30 nm chromatin fibre within these territories are under debate: In the Random-Walk/Giant-Loop-model big loops of 3 to 5 Mbp are attached to a non-DNA backbone. In the Multi-Loop-Subcompartment (MLS) model loops of around 120 kbp are forming rosettes which are also interconnected by the chromatin fibre. Here we show with a comparison between simulations and experiments an interdisciplinary approach leading to a determination of the three-dimensional organization of the human genome:

For the predictions of experiments various models of human interphase chromosomes and the whole cell nucleus were simulated with Monte Carlo and Brownian Dynamics methods. Only the MLS-model leads to the formation of non-overlapping chromosome territories and distinct functional and dynamic subcompartments in agreement with experiments. Fluorescence in situ hybridization is used for the specific marking of chromosome arms and pairs of small chromosomal DNA regions. The labelling is visualized with confocal laser scanning microscopy followed by image reconstruction procedures. Chromosome arms show only small overlap and globular substructures as predicted by the MLS-model. The spatial distances between pairs of genomic markers as function of their genomic separation result in a MLS-model with loop and linker sizes around 126 kbp. With the development of GFP-fusion-proteins it is possible to study the chromatin distribution and dynamics resulting from cell cycle, treatment by chemicals or radiation in vivo. The chromatin distributions are similar to those found in the simulation of whole cell nuclei of the MLS-model. Fractal analysis is especially suited to quantify the unordered and non-euclidean chromatin distribution of the nucleus. The dynamic behaviour of the chromatin structure and the diffusion of particles in the nucleus are also closely connected to the fractal dimension. Fractal analysis of the simulations reveal the multi-fractality of chromosomes. First fractal analysis of chromatin distributions *in vivo* result in significant differences for different morphologies and might favour a MLS-model-like chromatin distribution. Simulations of fragment distributions based on double strand breakage after carbon-ion irradiation differ in different models. Here again a comparison with experiments favours a MLS-model.

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Keywords:

Genome, genomics, genome organization, genome architecture, structural sequencing, architectural sequencing, systems genomics, coevolution, holistic genetics, genome mechanics, genome function, genetics, gene regulation, replication, transcription, repair, homologous recombination, simultaneous co-transfection, cell division, mitosis, metaphase, interphase, cell nucleus, nuclear structure, nuclear organization, chromatin density distribution, nuclear morphology, chromosome territories, subchromosomal domains, chromatin loop aggregates, chromatin rosettes, chromatin loops, chromatin fibre, chromatin density, persistence length, spatial distance measurement, histones, H1.0, H2A, H2B, H3, H4, mH2A1.2, DNA sequence, complete sequenced genomes, molecular transport, obstructed diffusion, anomalous diffusion, percolation, long-range correlations, fractal analysis, scaling analysis, exact yard-stick dimension, box-counting dimension, lacunarity dimension, local nuclear dimension, nuclear diffuseness, parallel super computing, grid computing, volunteer computing, Brownian Dynamics, Monte Carlo, fluorescence in situ hybridization, confocal laser scanning microscopy, fluorescence correlation spectroscopy, super resolution microscopy, spatial precision distance microscopy, auto-fluorescent proteins, CFP, GFP, YFP, DsRed, fusion protein, in vivo labelling.

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