Three-dimensional Organization of the Human Interphase Nucleus

Experiments compared to Simulations

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Despite the successful linear sequencing of the genome its three dimensional structure is widely unknown although its importance for gene regulation and replication. Through 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.

These results indicate that the human nucleome is a fractal structure which can be described by the triplets of empirical exponents

\[ \text{Figure 1A & 1B: FISH images of a territory painting of chromosome 15 (left) and genomic mapping 15q11-13 right in HeLa cells with a generic separation of 1.0 Mbp in interphase of fibroblast cells.} \]

Simulated obstruction of diffusion

\[ \text{Figure 2A & 2B: Comparison of the RWGL- and the MLS-model with experimentally determined interphase distances.} \]

Particle diffusion

\[ \text{Figure 3: Simulated observation of diffusion} \]

Fractal analysis is especially suited to quantify the unordered and non-watsonian chromatin distribution of the nucleus. The dynamic behaviour of the chromatin structure and the diffusion of particles in the nucleus are also studied by observing the random-walk behaviour of particles, in particular double strand breakage depend on the spatial arrangement of the DNA molecule in the nucleus. Simulated configurations of different chromatin models allow to study the DNA fragment distribution. The RWGL-model and the MLS-model lead to classical fractal dimension distributions (Figure 8). A comparison with experiments favours the MLS-model. The observed breakage sites are currently analyzed.

\[ \text{Figure 5: Comparison of simulated fragment distributions.} \]

Fractnal Dimension as function of the intensity threshold.

\[ \text{Figure 8: Comparison of RWGL- and MLS-model with the binary distribution of the chromatin distribution.} \]

\[ \text{Figure 9: Fractal Dimension as function of the intensity thresholds.} \]

\[ \text{Figure 10: Simultaneous Irradiation and fusion analysis of chromatin fragments.} \]

\[ \text{Figure 11: Comparison of RWGL- and MLS-model with the binary distribution of the chromatin distribution.} \]

\[ \text{Figure 12: Fractal Dimension as function of the intensity thresholds.} \]

\[ \text{Figure 13: Comparison of RWGL- and MLS-model with the binary distribution of the chromatin distribution.} \]

\[ \text{Figure 14: Fractal Dimension as function of the intensity thresholds.} \]

\[ \text{Figure 15: Comparison of RWGL- and MLS-model with the binary distribution of the chromatin distribution.} \]

\[ \text{Figure 16: Fractal Dimension as function of the intensity thresholds.} \]

\[ \text{Figure 17: Comparison of RWGL- and MLS-model with the binary distribution of the chromatin distribution.} \]

\[ \text{Figure 18: Fractal Dimension as function of the intensity thresholds.} \]
Literature


Abstract

To approach the three-dimensional organization of the human cell nucleus, the structural-, scaling- and dynamic properties of interphase chromosomes and cell nuclei were simulated with Monte Carlo and Brownian Dynamics methods. The 30 nm chromatin fibre was folded according to the Multi-Loop-Subcompartment (MLS) model, in which ~100 kbp loops form rosettes, connected by a linker, and the Random-Walk/Giant-Loop (RW/GL) topology, in which 1-5 Mbp loops are attached to a flexible backbone. Both the MLS and the RW/GL model form chromosome territories but only the MLS rosettes result in distinct subcompartments visible with light microscopy and low overlap of chromosomes, -arms and subcompartments. This morphology and the size of subcompartments agree with the morphology found by expression of histone auto-fluorescent protein fusions and fluorescence in situ hybridization (FISH) experiments. Even small changes of the model parameters induced significant rearrangements of the chromatin morphology. Thus, pathological diagnoses based on this morphology, are closely related to structural changes on the chromatin level. The position of interphase chromosomes depends on their metaphase location, and suggests a possible origin of current experimental findings. The chromatin density distribution of simulated confocal (CLSM) images agrees with the MLS model and with recent experiments. The scaling behaviour of the chromatin fiber topology and morphology of CLSM stacks revealed fine-structured multi-scaling behaviour in agreement with the model prediction. Review and comparison of experimental to simulated spatial distance measurements between genomic markers as function of their genomic separation also favour an MLS model with loop and linker sizes of 63 to 126 kbp. Visual inspection of the morphology reveals also big spaces allowing high accessibility to nearly every spatial location, due to the chromatin occupancy <30% and a mean mesh spacing of 29 to 82 nm for nuclei of 6 to 12 µm diameter. The simulation of diffusion agreed with this structural prediction, since the mean displacement for 10 nm sized particles of ~1 to 2 µm takes place within 10 ms. Therefore, the diffusion of biological relevant tracers is only moderately obstructed, with the degree of obstruction ranging from 2.0 to 4.0 again in experimental agreement.

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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imaging.


Literature References


