Three - Dimensional Organization of Chromosome Territories in the Human Interphase Cell Nucleus

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3D Human Genome Study Group DHGP

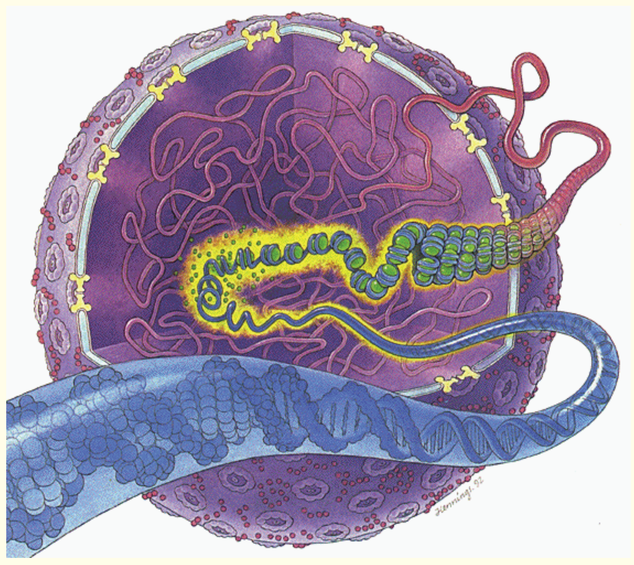
German Human Genome Project

PURPOSE

The eukaryotic cell is a prime example of a functioning nano machinery. The synthesis of proteins, maintenance of structure and duplication of the eukaryotic cell itself are all fine-tuned biochemical processes that depend on the precise structural arrangement of the cellular components. 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. Despite the successful linear sequencing of the human genome its three-dimensional structure is widely unknown.

With the simulation of chromosomes and cell nuclei in comparison with fluorescence in situ hybridization we show here an approach leading to the detailed determination of the three-dimensional organization of the human genome:

Best agreement between simulations and experiment is reached for a Multi-Loop-Subcompartment model, thus the human genome shows a higher degree of determinism than previously thought.

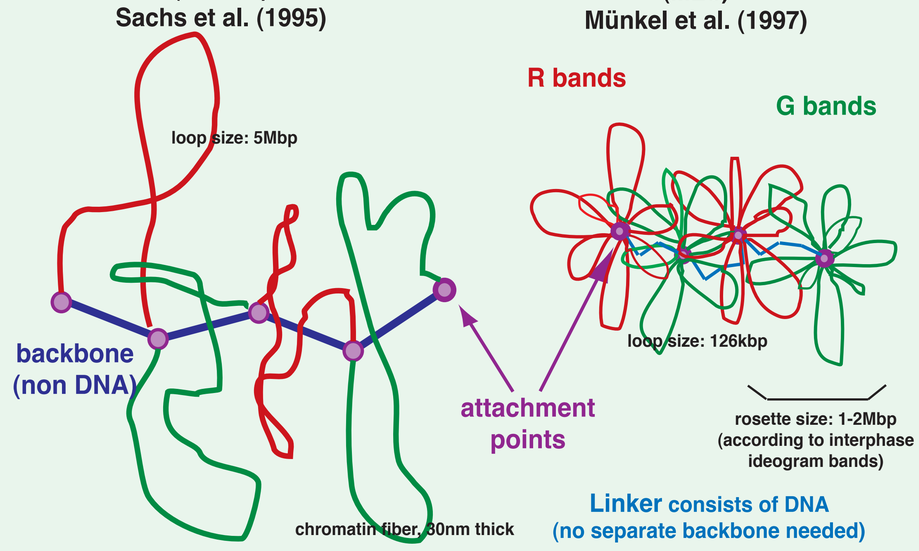


Typical textbook illustration of the human cell nucleus:
1) human cell nuclei differ from spherical shape,
2) the DNA is not a closed pipe,
3) nucleosomes are not regularly organized into chromatin,
4) chromatin does not float around randomly in the nucleus.
V. Hennings (illustrator) in Molecular and Cellular Biology by Stephen L. Wolfe, 1993.

SIMULATIONS

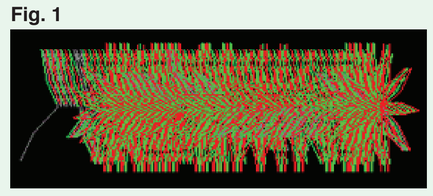
Random Walk / Giant Loop model (RW/GL)
Sachs et al. (1995)

Multi-Loop-Subcompartment model (MLS)
Münkel et al. (1997)



With **Monte Carlo** and **Brownian Dynamics** methods we simulated various models (see sketch left) of **human interphase chromosome 15** assuming a flexible polymer chain. To save computer power we start with ~3,500 300nm=31kbp and later we relax with ~21,000 50nm=5,2 kbp long segments. For simulation of a single chromosome it is placed in a potential well whose height is related to the excluded volume interaction (EVI). The EVI keeps the chain from self crossing. Starting configurations have the approximate form and size of a metaphase chromosome (Fig. 1) from which the following decondensation into interphase resembles the natural process.

Fig. 1



Rosettes in the Multi-Loop-Subcompartment model correspond to the size of chromosomal interphase band domains.
For **simulation of a whole interphase nucleus** 46 methaphase chromosomes are placed randomly in a nucleus confined by an EVI. The simulations are made on two IBM SP2 parallel computers with 80 and 512 nodes.

Fig. 2

Ray traced image of the Random-Walk/Giant-Loop model, loop size 5Mbp, after ~80.000 Monte-Carlo and 1000 relaxing Brownian Dynamics steps. Large loops intermingle freely thus forming no distinct features like in the MLS model.

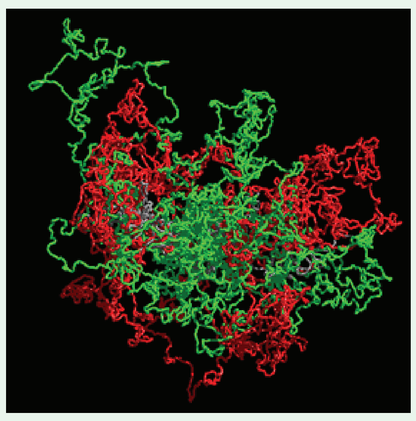


Fig. 3

Ray traced image of the Multi-Loop Subcompartment model, loop size 126kbp, 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.

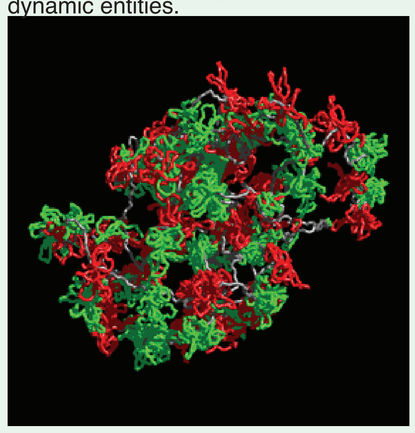
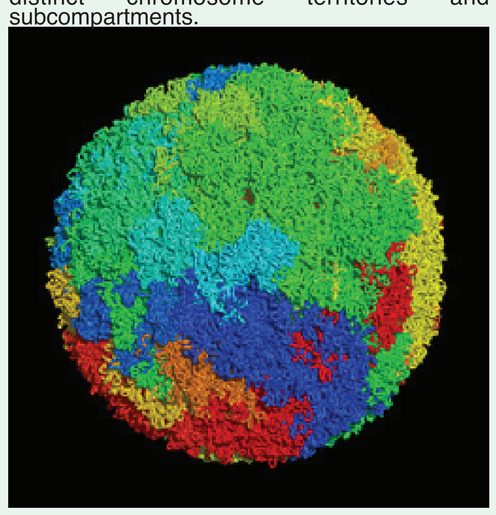


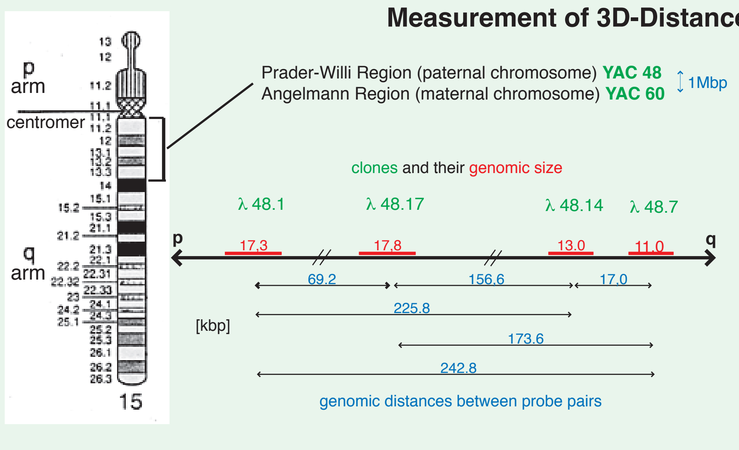
Fig. 4

Simulation of a **human interphase cell nucleus** with all 46 chromosomes with 1,200,000 polymer segments after 0.5s Brownian Dynamics simulation with 10s steps. The MLS-model leads to the formation of distinct chromosome territories and subcompartments.



EXPERIMENTS

Measurement of 3D-Distances between Genomic Markers

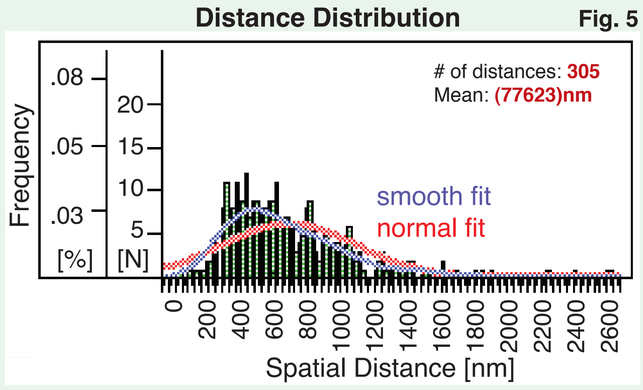


Fluorescence in situ hybridization (FISH) in connection with **confocal light microscopy** is used for the specific marking of small chromosomal DNA regions. Despite the low spatial resolution of FISH, it is possible to interprete the results (f. e. the 3D distance between genetic markers as a function of their genomic distance) with our simulations.

Chromosome 15 and the **Prader-Labhard-Willi/Angelmann Syndrom** region was chosen, because the genomic distance between markers is well known (see sketch left) and because the PLW/A-syndrom is a candidate for structure mutation (in contrast to the common base pair mutation).

Collaboration with B. Horsthemke, Institute for Human Genetics, Essen, FRG.

Distance Distribution **Fig. 5**



Methods: Human fibroblast cells grown on coverslips to confluent layers and being assumed to rest now in the same cell cycle phase are fixed in isotonic environment with paraformaldehyde.
For Hybridization we use digoxigenin labeled DNA probes. The probes are detected with fluorescent dyes.

Confocal image series were taken with a Leica TCS NT confocal microscope with an axial displacement of z = 200nm. The images are median and background filtered. After manual threshold determination from an extended focus view (Fig. 6) for spot finding we proceed with image reconstruction specially adjusted to the microscope. Finally the **3D-spatial distances** are determined between the centers of mass of the spots (Fig. 5). The experimental distance distributions are then compared to the computed ones. We use a workstation cluster of 10 Silicon Graphics Indigo and Indigo II for computation.

The fibroblast nuclei are found to have their in vivo size (~20m * 10m * 6m) so that we conclude that at least on the micrometer length scale we preserved the nuclear structure. With two colour FISH it is possible to detect 3D-distances below the optical resolution.

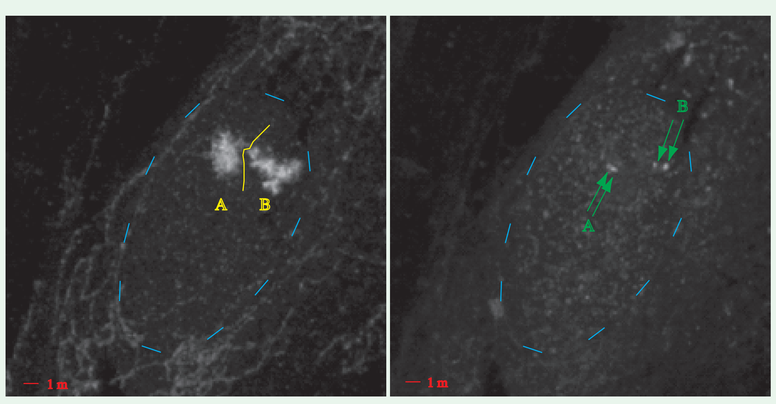
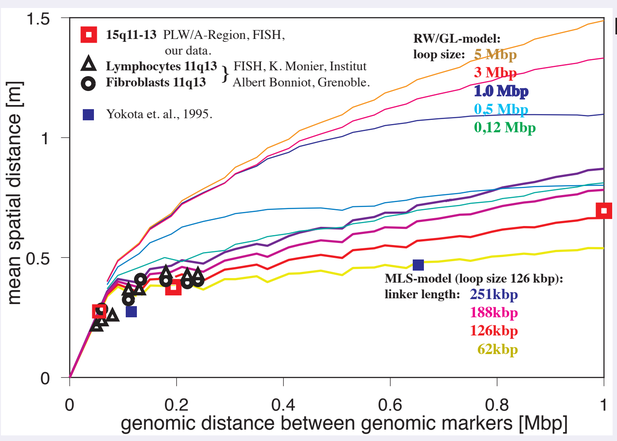


Fig. 6
Chromosomes form distinct territories in interphase and genomic markers lay clearly separable within the territories.
Left: Territory painting by FISH of chromosome 15; by chance the two territories neighbour each other.
Right: Genomic markers YAC48 and YAC60, genomic separation 1Mbp.

CONCLUSION

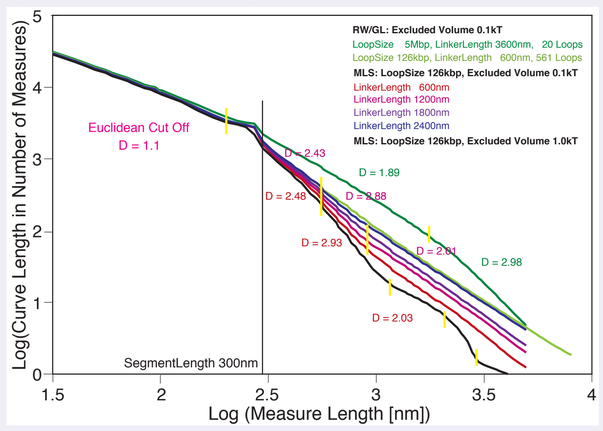
Best agreement between simulations and experiments is reached for a **Multi-Loop-Subcompartment model** with a loop size of roughly126kbp and a linker length of 1,200nm. Supposed that defined loop bases exist it might be possible to determine the mean positioning of genes relative to each other.

Fig. 7



For calculating more general properties of chromosomes the **fractal dimension of the chromatin fiber** was determined from the simulations. The fractal analysis resulted in multifractal behaviour (data not shown here) in good agreement with predictions drawn from porous network research (Avnir, 1989; Mandelbrot, private communications).

Fig. 8



The simulation of a whole human cell nucleus with all 46 human chromosomes in connection with the simulation 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 (data not shown here).

Three-Dimensional Organization of Chromosome Territories in the Human Interphase Cell Nucleus

Knoch, T. A., Münkel, C. & Langowski, J.

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Abstract

To study the three-dimensional organization of chromosome territories and the human interphase cell nucleus we developed models which could be compared to experiments. Despite the successful linear sequencing of the human genome its 3D-organization is widely unknown. Using Monte Carlo and Brownian dynamics simulations we managed to model the chromatin fibre as a wormlike-chain polymer. A typical chromosome consists of 20.000 and a nucleus with all 46 chromosomes of 1.200.000 polymer chain segments. The parallel simulations are performed on a SP2512 and a Cray T3E. With fluorescent in situ hybridization and confocal microscopy we determined genomic marker distributions and chromosome arm overlap.

Best agreement between simulations and experiments is reached for a Multi-Loop-Subcompartment model (126 kbp loops connected to rosettes connected by a 126 kbp chromatin linker). A fractal analysis of simulations leads to multi-fractal behaviour in good agreement with porous network research. The formation of chromosome territories was shown as predicted and low overlap of chromosomes and their arms was also reached in contrast to other models.

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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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