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

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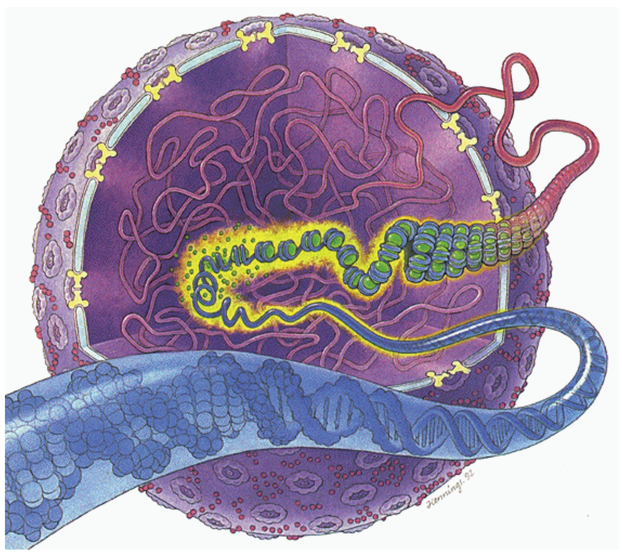
PURPOSE

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 fluoescence in situ hybridization we show here an approach leading to the detailed determination of the three-dimensionale 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!

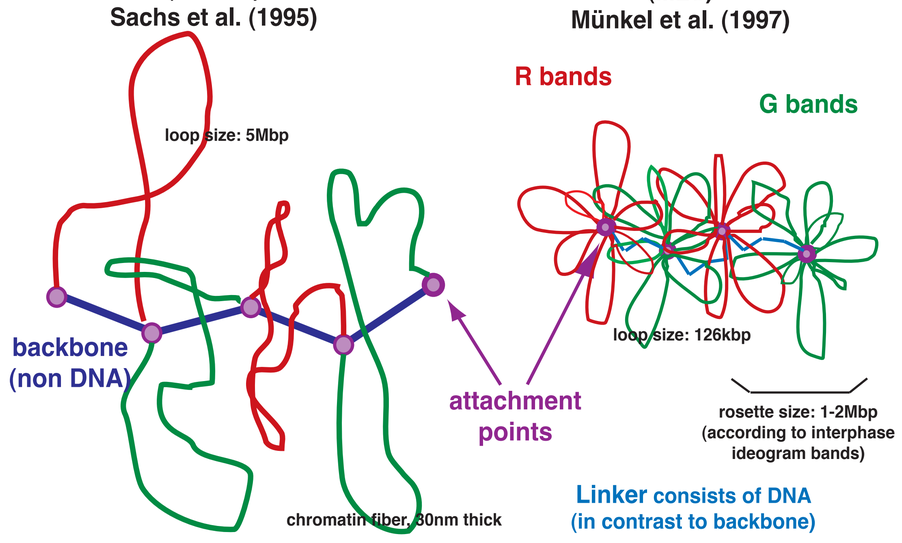


Typical textbook illustration of the human cell nucleus:
1) human cell nuclei differ from spherical shape,
2) the DNA is not a closed pipe,
3) nucleosomes are not regularly organized into chromatin,
4) chromatin does not float around randomly in the nucleus.
V. Hennings (illustrator) in Molecular and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

SIMULATIONS

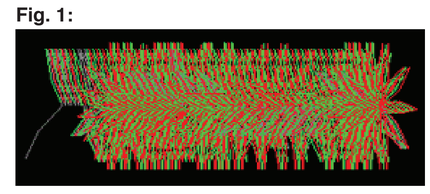
Random Walk / Giant Loop model (RW/GL)
Sachs et al. (1995)

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 away 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:



For **simulation of a whole interphase nucleus** 46 methaphase chromosomes are placed randomly in a nucleus confined by an EVI.
Rosettes in the Multi-Loop-Subcompartment model correspond to the size of chromosomal interphase band domains. 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.

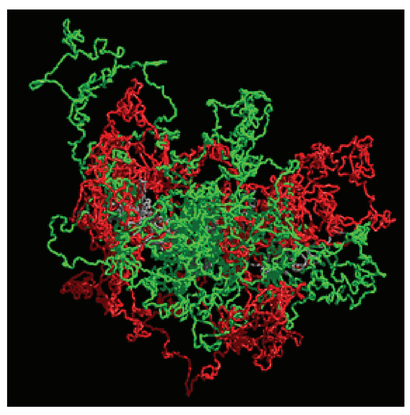


Fig. 3:
Ray traced image of the Multi-Loop Subcompartment model, loop size 126kbp, linker size 126 kbp, after ~50.000 Monte-Carlo and 1000 relaxing Brownian- Dynamics steps. Here rosettes form subcompartments as separated organizational and dynamic entities.

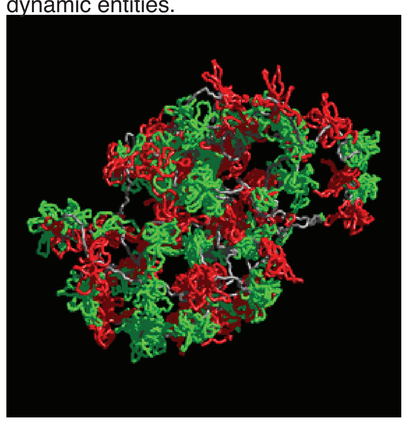
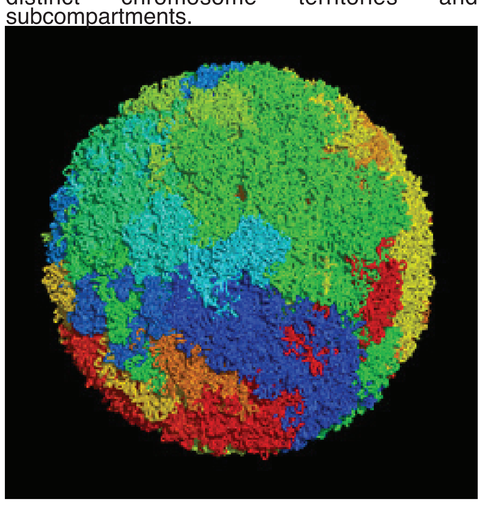
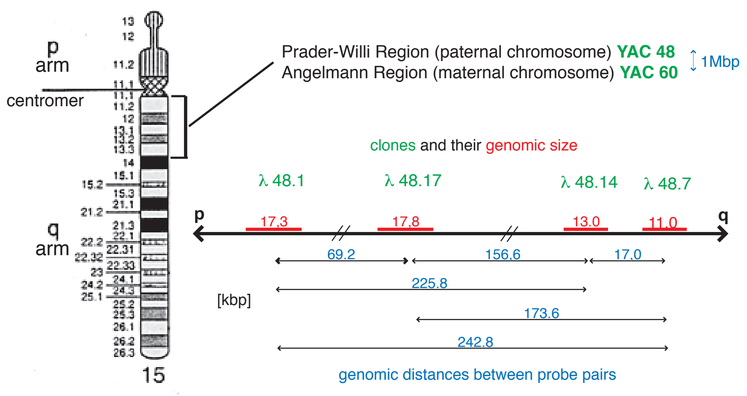


Fig. 4:
Simulation of a **human interphase cell nucleus** with all 46 chromosomes with 1,200,000 polymer segments after 0.5s Brownian Dynamics methods, one step taking 10s. The MLS-model leads to the formation of distinct chromosome territories and subcompartments.



EXPERIMENTS

Measurement of 3D-Distances between Genomic Markers



Confocal light microscopy in connection with **fluorescence in situ hybridization** (FISH) 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, as the genomic distance between markers is well known (see sketch left) and as it is a candidate for structure mutation (versus e. g. the common base pair mutation).
Collaboration with B. Horsthemke, Institute for Human Genetics, Essen, FRG.

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. 5) 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). Measurements of many distances lead then to distributions used for comparison between experiments and simulations. 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 lengthscale we preserved nuclear structure. With two colour FISH it is possible to detect 3D-distances below the optical resolution.

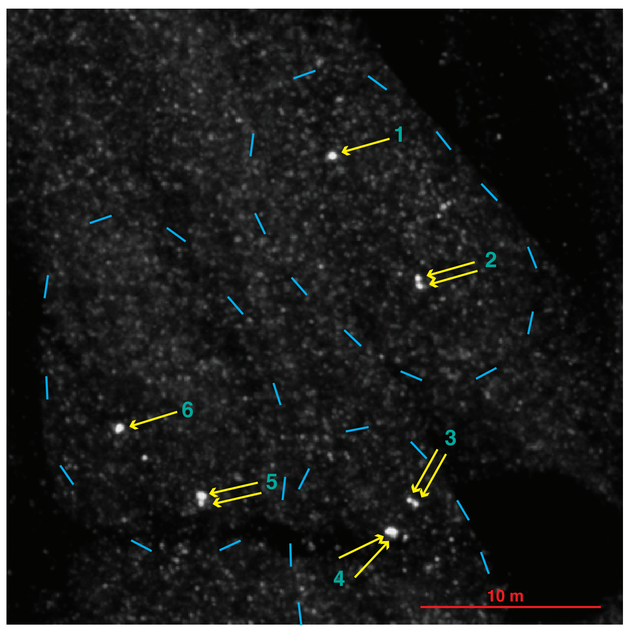


Fig. 5:
Extended focus image with three nuclei marked with λ48.14 and λ48.7, labeled with dioxigenin, and detected with a first layer of Mouse-anti-Dioxigenin antibodies detected in a second layer with Sheep-anti-Mouse antibodies to which CY3 is bound as fluorescent marker.

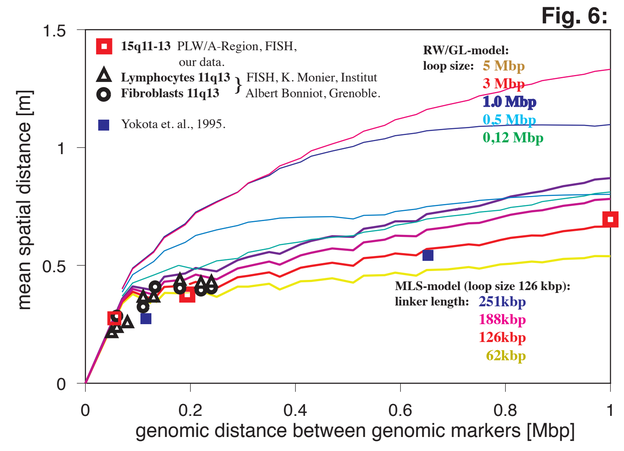
1: one spot
2: two spots, d = 135nm
3: two spots, d = 182nm
4: two spots behind each other, d = 424nm
5: two spots, d = 469nm
6: one spot

CONCLUSION

Comparison between Simulated and Experimental 3D-Distances between Genomic Markers

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. 6:



Further Analysis of Simulations

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).

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. 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).

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Abstract

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.

The nucleus of the cell 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 has it become apparent that chromosomes occupy distinct 'territories' also in the interphase, i.e. between cell divisions. In an analogy of the Bauhaus principle that "form follows function" we believe that analyzing in which form DNA is organized in these territories will help us to understand genomic function. We use computer models - Monte Carlo and Brownian dynamics simulations - to develop plausible proposals for the structure of the interphase genome and compare them to experimental data. In the work presented here, we simulate interphase chromosomes for different folding morphologies of the chromatin fiber which is organized into loops of 100kbp to 3 Mbp that can be interconnected in various ways. The backbone of the fiber is described by a wormlike-chain polymer whose diameter and stiffness can be estimated from independent measurements. The implementation describes this polymer as a segmented chain with 3000 to 20000 segments for chromosome 15 depending on the phase of the simulation. The modeling is performed on a parallel computer (IBM SP2 with 80 nodes). We also determine genomic marker distributions within the Prader-Willi-Region on chromosome 15q11.2-13.3. For these measurements we use a fluorescence in situ hybridisation method (in collaboration with I. Solovai, J. Craig and T. Cremer, Munich, FRG) conserving the structure of the nucleus. As probes we use 10 kbp long lambda clones (Prof. B. Horsthemke, Essen, FRG) covering genomic marker distances between 8 kbp and 250 kbp. The markers are detected with confocal and standing wavefield light microscopes (in collaboration with J.Rauch, J. Bradl, C. Cremer and E.Stelzer, both Heidelberg, FRG) and using special image reconstruction methods developed solely for this purpose (developed by R. Eils. and W. Jaeger, Heidelberg, FRG).
Best agreement between simulations and experiments is reached for a Multi-Loop-Subcompartment model with a loop size of 126 kbp which are forming rosetts and are linked by a chromatin linker of 126 kbp. We also hypothesize a different folding structure for maternal versus paternal chromosome 15. In simulations of whole cell nuclei this modell also leads to distinct chromosome territories and subcompartments. A fractal analysis of the simulations leads to multifractal behavior in good agreement with predictions drawn from porous network research.

The work is part of the Heidelberg 3D Human Genome Study Group, which is part of the German Human Genome Project.

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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, autofluorescent proteins, CFP, GFP, YFP, DsRed, fusion protein, in vivo labelling.

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