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!

**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 fluorescence in situ hybridization we show here an approach leading to the detailed determination of the three-dimensional organization of the human genome:

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**EXPERIMENTS**

**SIMULATIONS**

With Monte Carlo and Brownian Dynamics methods we simulated various models (see sketch left) of human chromosomes. For example chromosome 15 assuming a flexible polymer chain. To ease computer power we start with ~50,000 Monte-Carlo steps. Large, long interphase domains are then simulated. For calculating more general properties of chromosomes the Multi-Loop-Subcompartment (MLS) model was used. The MLS-model leads to the formation of subcompartments. We made use of the Multi-Loop-Subcompartment model to simulate chromosomes in connection with Brownian Dynamics methods, one step taking ~1,200,000 polymer segments after 0.5s Brownian- Dynamics steps. Large loops intermingle freely and 1000 relaxing Brownian Dynamics steps.

**CONCLUSION**

Best agreement between simulations and experiments is reached for a Multi-Loop-Subcompartment model with a loop size of roughly 126kbp 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.
Three-Dimensional Organization of Chromosome Territories in the Human Interphase Nucleus

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


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).

As with both simulations and experiments is reached for a Multi-Loop-Subcompartment model with a loop size of 126 kbp which are forming rosettes 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 model 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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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.

Literature References
