THREE-DIMENSIONAL ORGANIZATION OF CHROMOSOME TERRITORIES AND THE HUMAN INTERPHASE CELL NUCLEUS

SIMULATIONS and EXPERIMENTS

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Typical state of the arts view:

1) human cell nuclei usually have no spherical shape,
2) the DNA is not a closed pipe,
3) nucleosomes might not be regularly organized into chromatin,
4) chromatin does not float around randomly in the nucleus.

The dynamic and hierarchical organization of cell nuclei span between 10 and 13 orders of magnitude concerning length and time scales.
Overview

**Experiment**

Prader-Labhard-Willi/ Angelmann Region

- fluorescence in-situ hybridization (FISH)

- 3D confocal scanning microscopy

**Simulation**

- Multi-Loop-Subcompartment and Random Walk/ Giant Loop model

- polymer model for simulation of the chromatin fiber

**Conclusions for the human cell nucleus**

- chromosome-, chromosome-arm and subcompartment overlap
- 3D-distances between genomic markers as function of their genomic separation
- behaviour of marker ensembles and dynamics of structural features
- fractal properties of chromosomes
- decondensation of chromosomes from metaphase into interphase and chromosome stretching
- conclusions from simulating whole cell nuclei
Fluorescence in-situ Hybridization (FISH)

**Cell - Preparation**
- Cells on coverslip grown to confluent layer
- Fixation of cells on coverslip (formaldehyde) and permeabilisation
- DNA double strand
  - TACGTTAACGGTAGCATT
  - ATGCAATTGCCATCGTAA

**Probe - Preparation**
- Finding of genomic site for marking and cloning of this sequence
  - AACGG
  - TTGCC
- Labeling of the DNA probe (Nick translation or PCR) with
  - Digoxigenin (indirect)
  - Fluorophor (direct)
  - AACGG
  - TTGCC

**Hybridization**
- Probe is put on coverslip and melting of the double strands at 70°C
- Amplification with fluorescent labeled antibodies
  - TACGTTAACGGTAGCATT
  - TTGCC
Ideogram of chromosome 15 with Prader-Willi Region and Angelmann Region.
The size and genomic distance of the clones are sufficiently small and well characterized to measure the fine structure and organization of chromosome territories.

Prader-Willi Region (paternal chromosome) **YAC 48**
Angelmann Region (maternal chromosome) **YAC 60**

Genomic distances between probe pairs:
- **1Mbp**
- **194.9**
- **144.7**
- **213.9**
- **69.2**
- **125.7**
- **19.0**

Clones and their genomic size:
- \( \lambda 48.1 \)
- \( \lambda 48.17 \)
- \( \lambda 48.14 \)
- \( \lambda 48.7 \)
Principle of the Confocal Laser Scanning Microscope and Leica TCS NT setup.

Laser

Pinholes

Detection: Photomultipliers

Objective lense

Slide Emersion fluid
Chromosomes form distinct territories in interphase and genomic markers lie within the territories and are clearly separable.

Left: Territory painting by FISH of chromosome 15; by chance the two territories neighbour each other.
Right: Genomic markers YAC48 and YAC60, genomic separation 1 Mbp.
Dual colour FISH of genomic markers leads to measurements of 3D-distances which are below the resolution of the microscope. Critical signals could also be excluded with higher confidence.

Genomic marker $\lambda_{48.1}$ in red and marker $\lambda_{48.14}$ in green, genomic separation 195 kbp.
Statistical analysis of the spatial distances between the PWS-Region (YAC48) and AS-Region (YAC60) with a genomic distance of 1Mbp = 10m chromatin fiber.

Distance Distribution

- # of distances: 305
- Mean: (77623)nm
- Shapiro Wilk test on normality: $W=0.87$

Frequency

Spatial Distance [nm]
Multi-Loop-Subcompartment Model versus Random Walk / Giant Loop Model. Rosettes in the MLS-Model correspond to the size of chromosomal interphase band domains.

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

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

Linker consists of DNA (in contrast to backbone)
The chromosome fiber is simulated assuming a polymer chain and harmonic potentials.

**Stretching Potential**

\[ U_s(l) = \frac{k_B T}{2} (l - l_0) \]

**Bending Potential**

\[ U_b(\beta) = \frac{k_B T}{2} \beta^2 \]

**Excluded Volume Potential**

\[ U_{ev}(r) = U_{ev}^0 k_B T \left( 1 + \frac{r^4 - 2r_c^2 r^2}{r_c^4} \right) \]

- \( k_B \): Bolzmann constant
- \( T \): Temperature, 310 K
- \( k \): stretching elasticity
- \( \beta \): bending elasticity
- \( r_c \): minimum distance of segments
Random-Walk/Giant-Loop model versus Multi-Loop-Subcompartment model.
Simulation results of chromosome 15.

The chromosome is simulated assuming a flexible polymer chain, starting with ~ 3500 300nm=31kbp and relaxing with ~ 21,000 50nm=5.2kbp segments. The starting configuration has the approximate form and size as in metaphase. 50 parallel simulations and their evaluation take 5.5 years single CPU-time.

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 MLS model.

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.

Wire frame image of the metaphase chromosome resembling starting configuration.
The MLS-model leads to low overlap of chromosome-arms and subcompartments in contrast to the RWGL-model. This is also seen in experiments.

**Arm - Overlap**

Confocal images of interphase p- and q- arms of human chromosome 3

**Subcompartment - Overlap**

Confocal images of interphase R- and G- bands of human chromosome 15
Random-Walk / Giant-Loop versus Multi-Loop-Subcompartment model. Best agreement between simulations and experiments is reached for a Multi-Loop-Subcompartment model with a loop size of 126kbp and a linker length of 126kbp.

**Random-Walk / Giant-Loop model**
- Loop size: 126kbp
- Linker length: 126kbp

**Multi-Loop-Subcompartment model**
- Loop size: 126kbp
- Linker length: 126kbp

### Experimental Data
- **Fibroblasts 15q11-13**: PLW/A-Region, FISH, our data.
- **Lymphocytes 11q13**: FISH, K. Monier, Institut Albert Bonniot, Grenoble.
- **Fibroblasts 4p16.3**: FISH, Yokota et. al., 1995.
Shift of a marker ensemble through a rosette in the MLS-model in respect to loop bases. This leads to different sets of 3D-distances for every ensemble position. Due to the symmetry of the MLS-rosettes periodicities are found.
In agreement with porous network research fractal analysis show multifractal behaviour in simulations of chromosome 15. Different fractal dimensions mean different process-dynamics in these spaces. Therefore chromosomal territories show a higher degree of determinism than previously assumed.

**RW/GL:** Excluded Volume 0.1kT
LoopSize 5Mbp, LinkerLength 3600nm, 20 Loops
LoopSize 126kbp, LinkerLength 600nm, 561 Loops

**MLS:** LoopSize 126kbp, Excluded Volume 0.1kT
LinkerLength 600nm
LinkerLength 1200nm
LinkerLength 1800nm
LinkerLength 2400nm

**MLS:** LoopSize 126kbp, Excluded Volume 1.0kT
LinkerLength 600nm
Simulation of Chromosomal Elasticity
Visualization with "Virtual Microscope" of chromosome 15 (MLS model, 8 subcompartments) under external stress. Subcompartments are shown as a projection image of a confocal laser scanning microscope image series.
left: external force = 0 fN
right: external force = 1.2 fN
Simulation of Chromosome Elasticity
Force strain curve of an interphase
Multi-Loop-Subcompartment-model (MLS) for chromosome 15.
Young’s modulus for external forces below 5 femtonewtons (fN): (3,00,4) fN.

\[ F = \frac{Y \cdot l}{l} \]

\( F \): external force
\( Y \): Young’s modulus

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3D
´Virtual Human Cell Nucleus´
Simulation of all 46 chromosomes using the Multi-Loop-Subcompartment model.

The nucleus is simulated assuming a flexible polymer chain, modelling the 46 chromatin fibers with in total 1,248,794 50 nm = 5.2 kbp segments. Pictures are shown after a 0.5 ms Brownian Dynamics simulation, one step taking 10s. As starting configuration a metaphase nucleus was chosen.
Conclusions

Best agreement between simulations and experiments is reached for a Multi-Loop-Subcompartment-model with a loop and linker size of 126 kbp (1200nm).

Supposed that defined loop bases exist it might be possible to determine the positioning of genes relative to each other.

Chromosomes show multifractal behaviour in good agreement with predictions drawn from porous network research.

Chromosome decondensation and stretching lead to comparable results from experiments.

Simulations of whole cell nuclei lead to the formation of distinct chromosome territories.

The Multi-Loop-Subcompartment-model leads to low overlap of chromosome territories, chromosome arms and chromosome subcompartments in contrast to the RandomWalk/Giant Loop-model.
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Three-Dimensional Organization of Chromosome Territories in the Human Interphase Nucleus

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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. Crai 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 again 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.
**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.

**Literature References**
