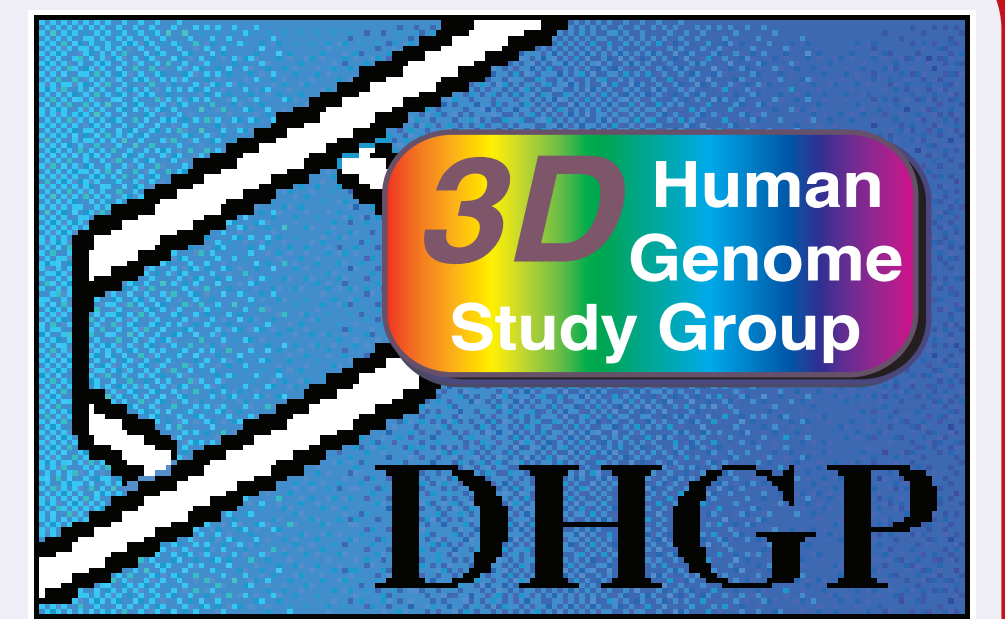# Three - Dimensional Organization of Chromosome Territories in the Human Interphase Cell Nucleus

Tobias A. Knoch, Christian Münkel and Jörg Langowski 1)

Joachim Rauch, Harald Bornfleth and Christoph Cremer 2) with Irina Solovei and Thomas Cremer 3)

1) Division Biophysics of Macromolecules, German Cancer Research Center, Im Neuenheimer Feld 280

2) Instiute for Applied Physics, Albert Ueberle Str. 3-5, 69120 Heidelberg, FRG

D - 69120 Heidelberg, Federal Republic of Germany
http://www.DKFZ-Heidelberg.de/Macromol/Welcome.html

3) Institute for Anthropology and Human Genetics, Richard Wagner Str. 10, 8033Munich, FRG

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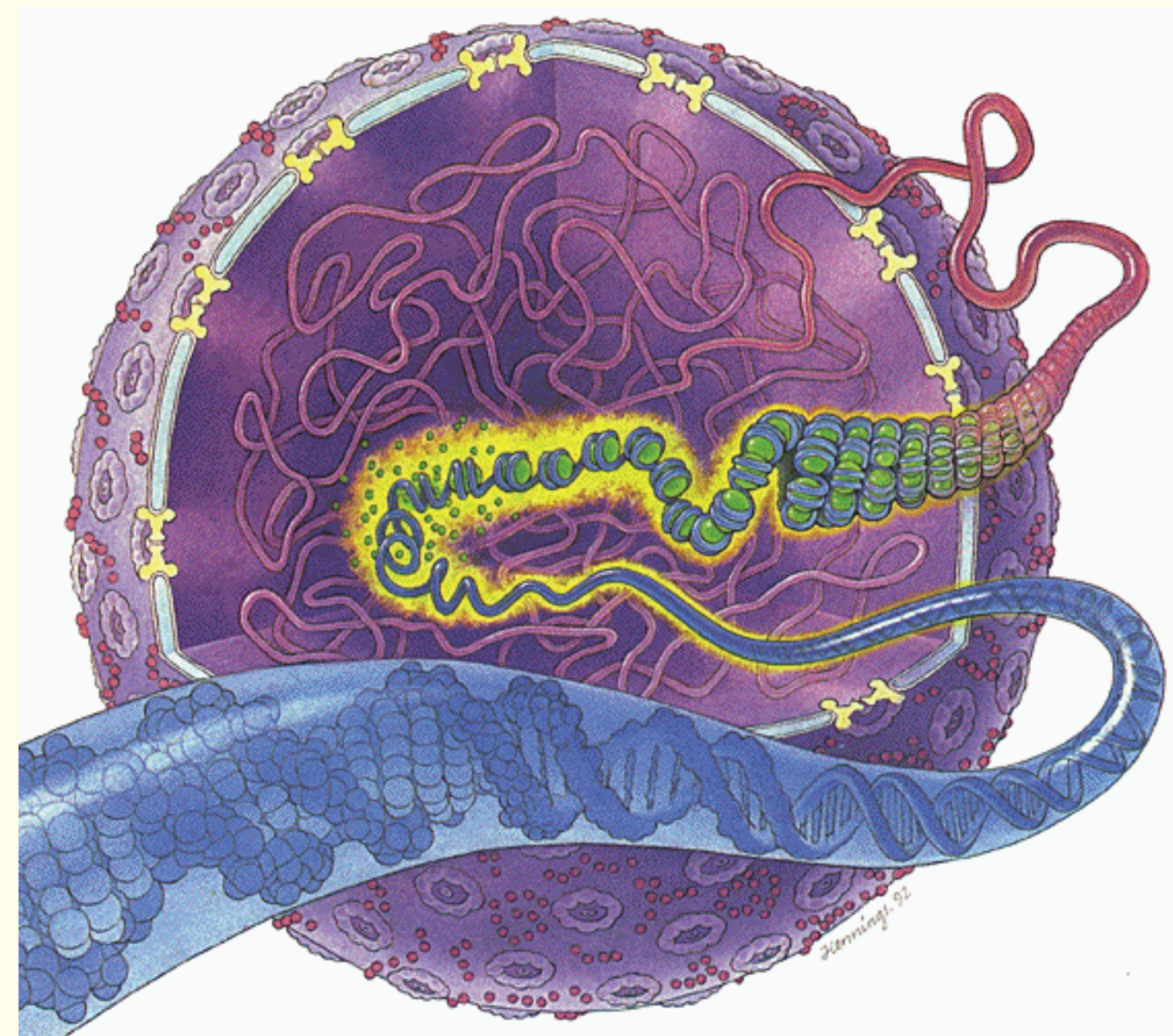
Typical textbook illustration of the human cell nucleus:
1) human cell nuclei differ from spherical shape,
2) the DNA is not a closed pipe,
3) nucleosomes are not regularly organized into chromatin,
4) chromatin does not float around randomly in the nucleus.
V. Hennings (illustrator) in Molecular and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

SIMULATIONS

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

loop size: 5Mbp

backbone (non DNA)

attachment points

chromatin fiber, 30nm thick

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

R bands

G bands

loop size: 126kbp

rosette size: 1-2Mbp (according to interphase ideogram bands)

Linker consists of DNA (no separate backbone needed)

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.
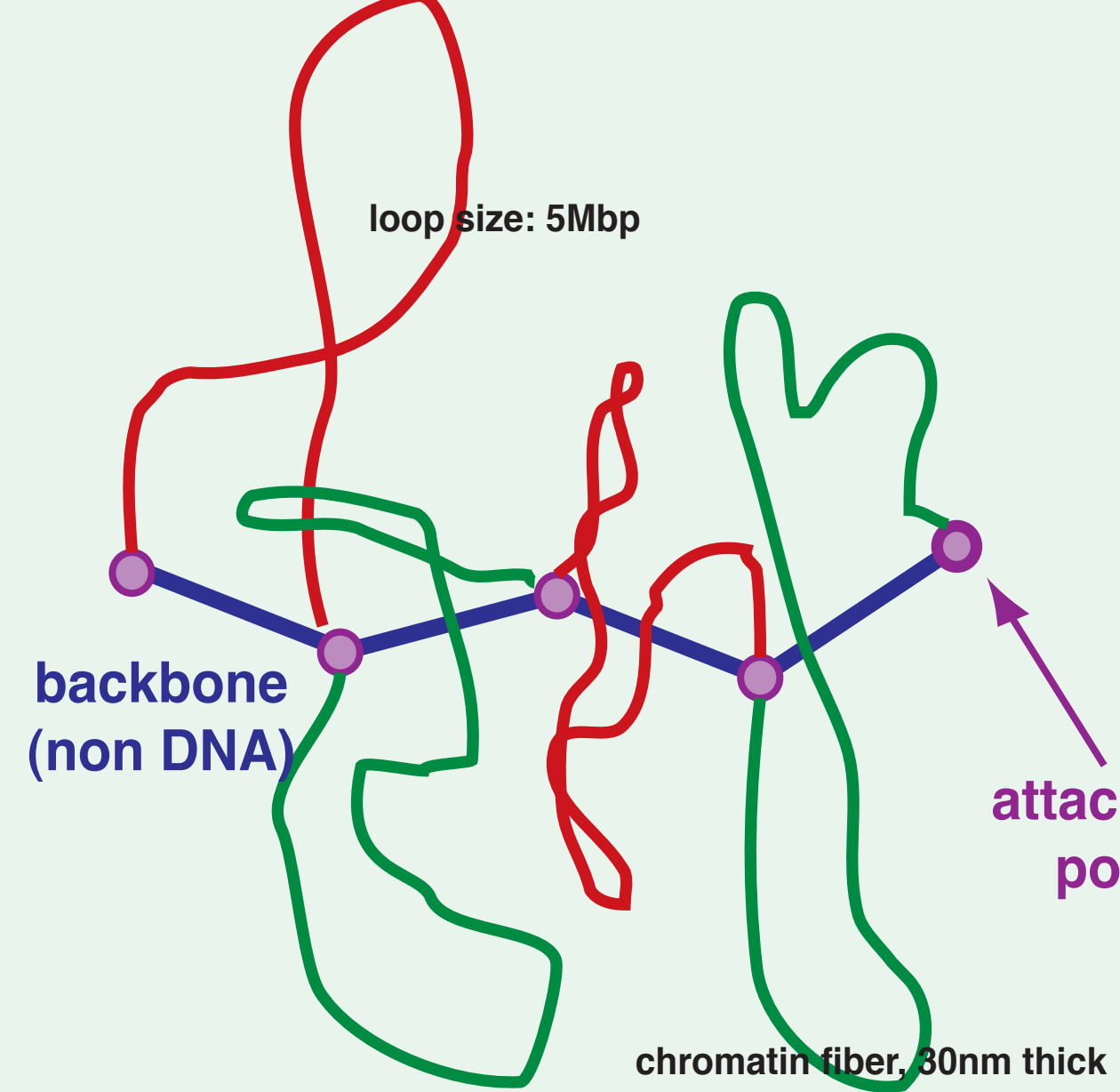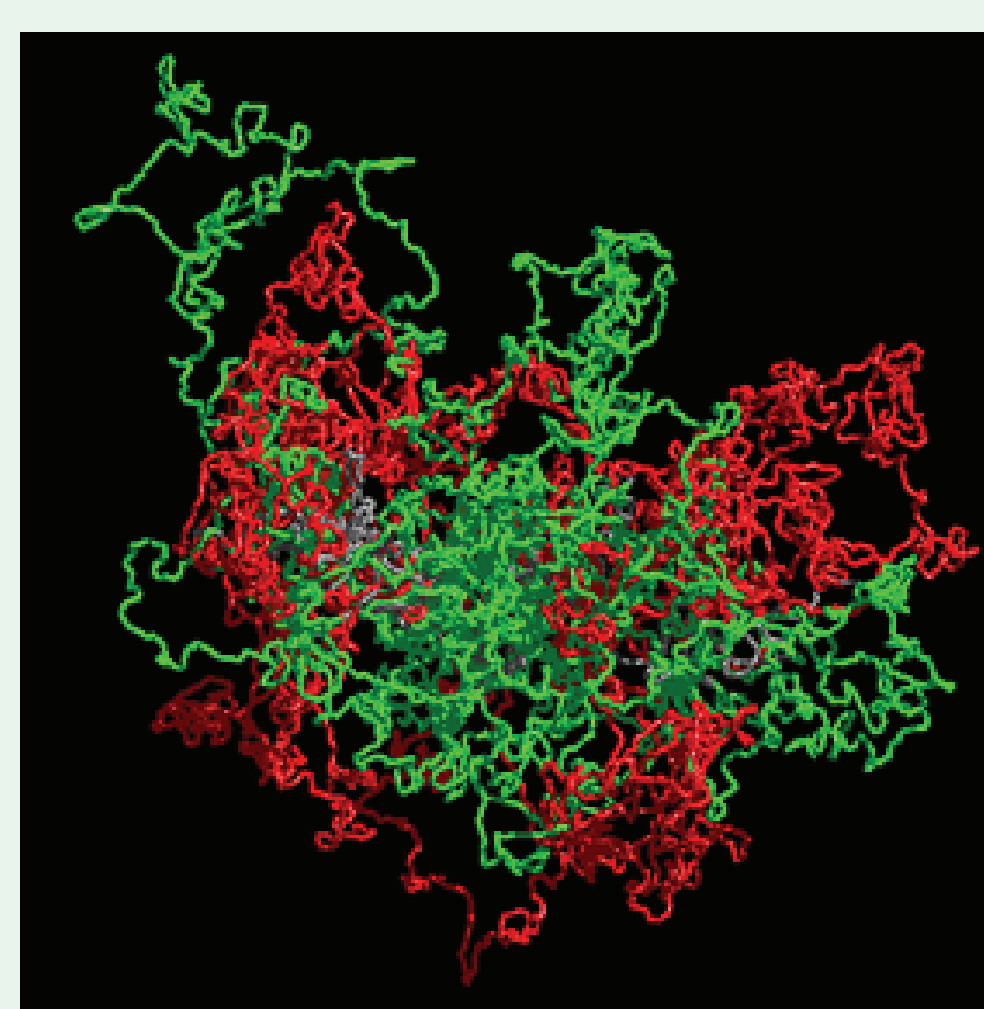
Fig. 2
Ray traced image of the Random-Walk/Giant-Loop model, loop size 5Mbp, after ~80.000 Monte-Carlo and 1000 relaxing Brownian Dynamics steps. Large loops intermingle freely thus forming no distinct features like in the MLS model.
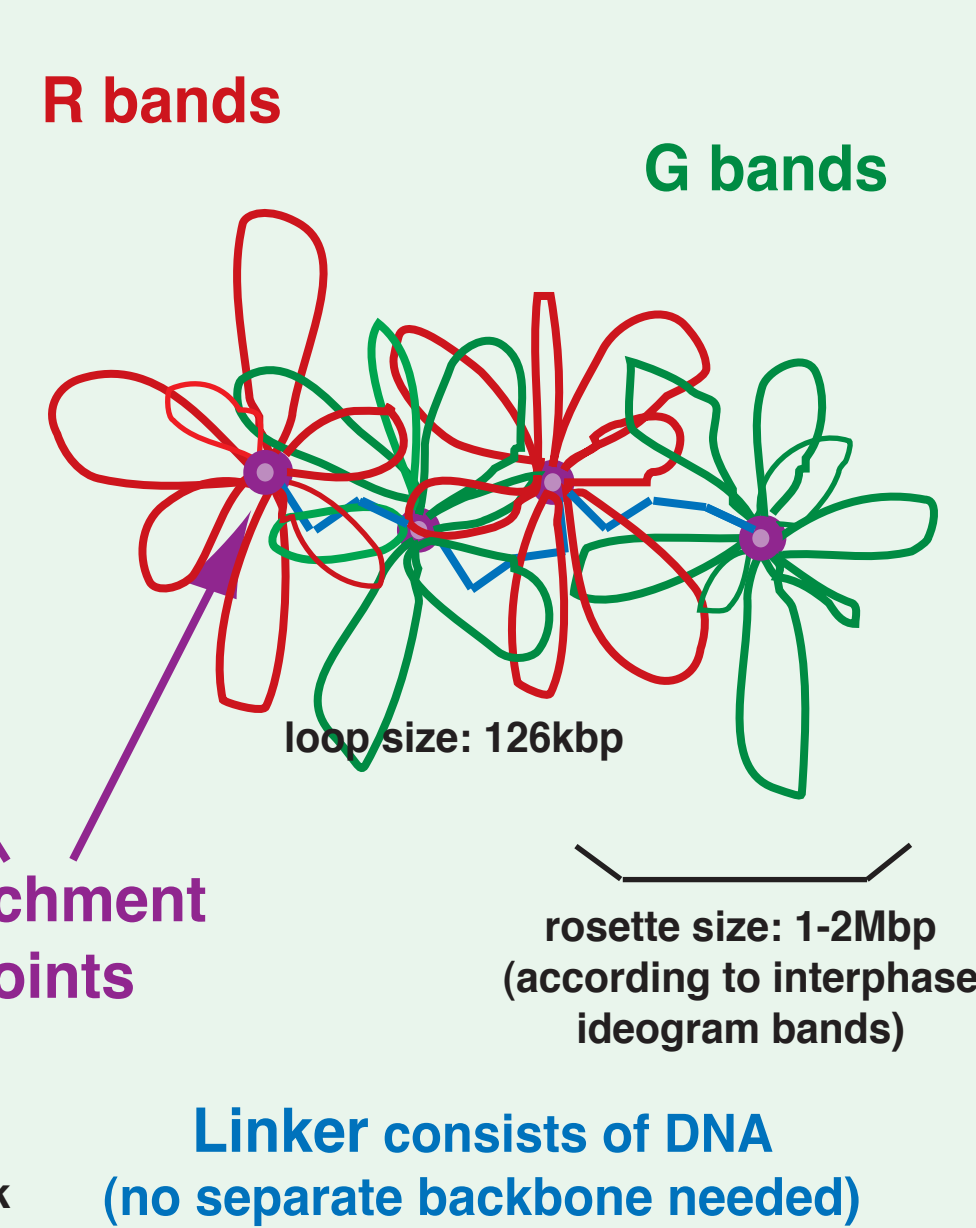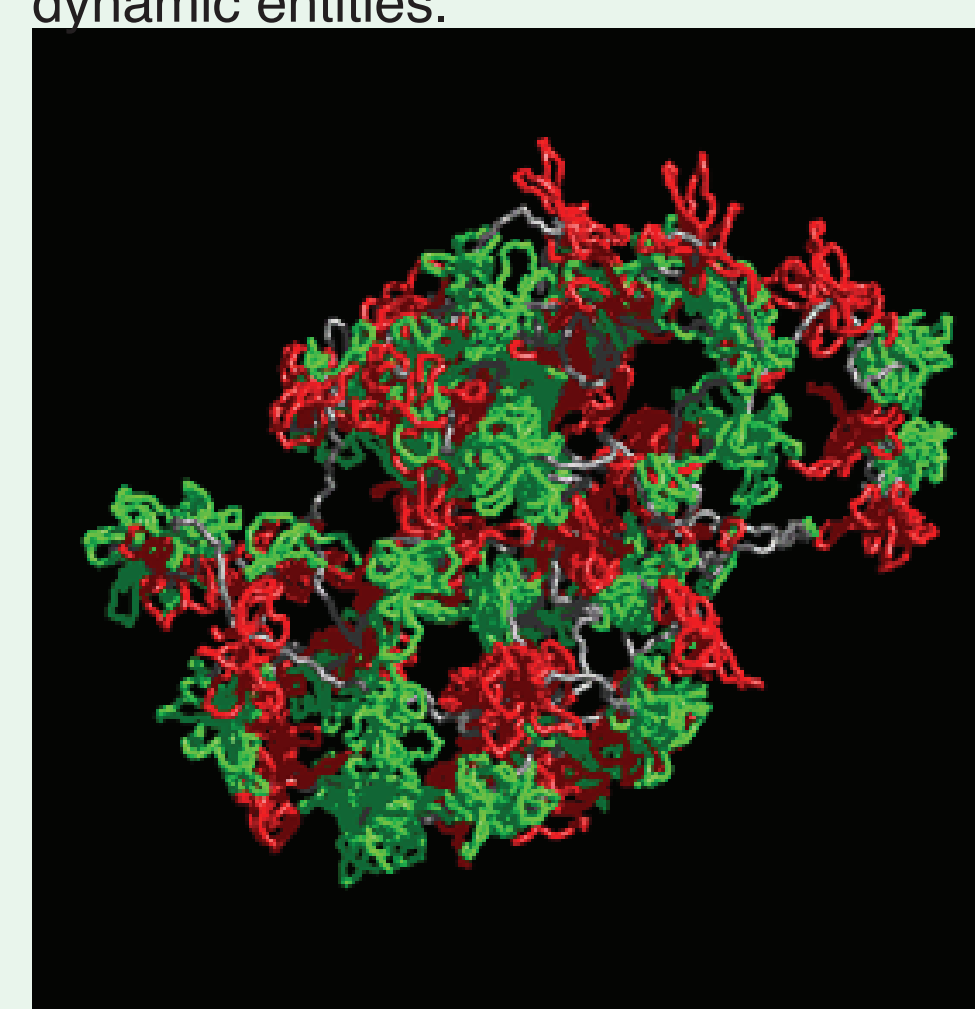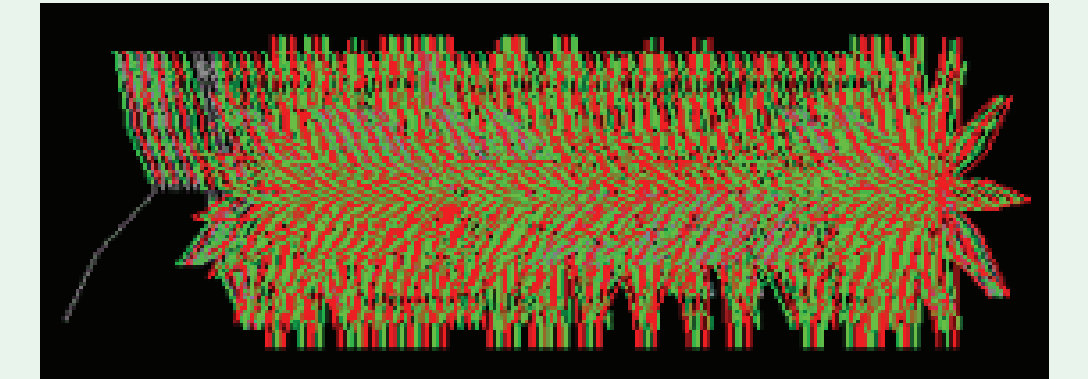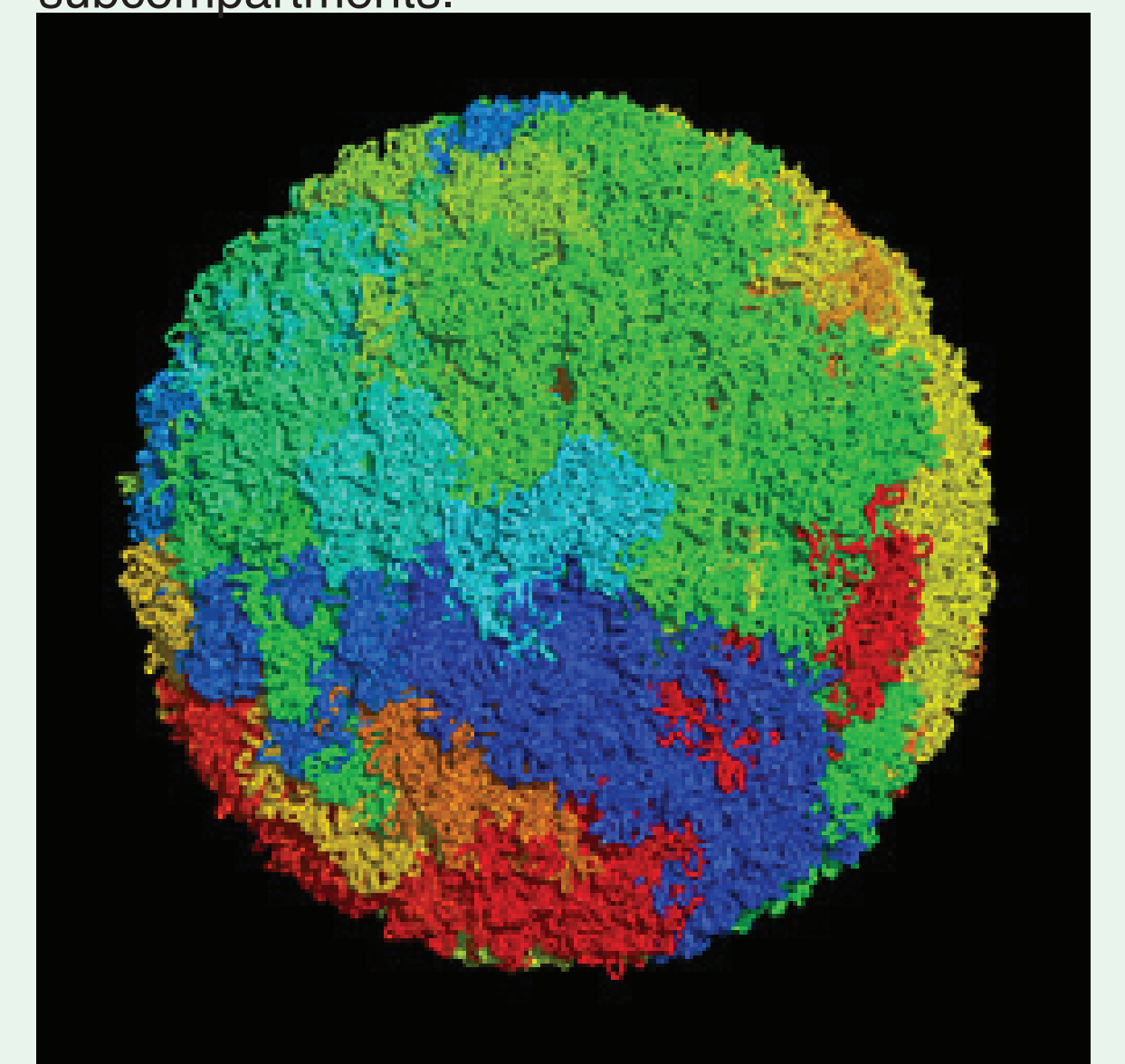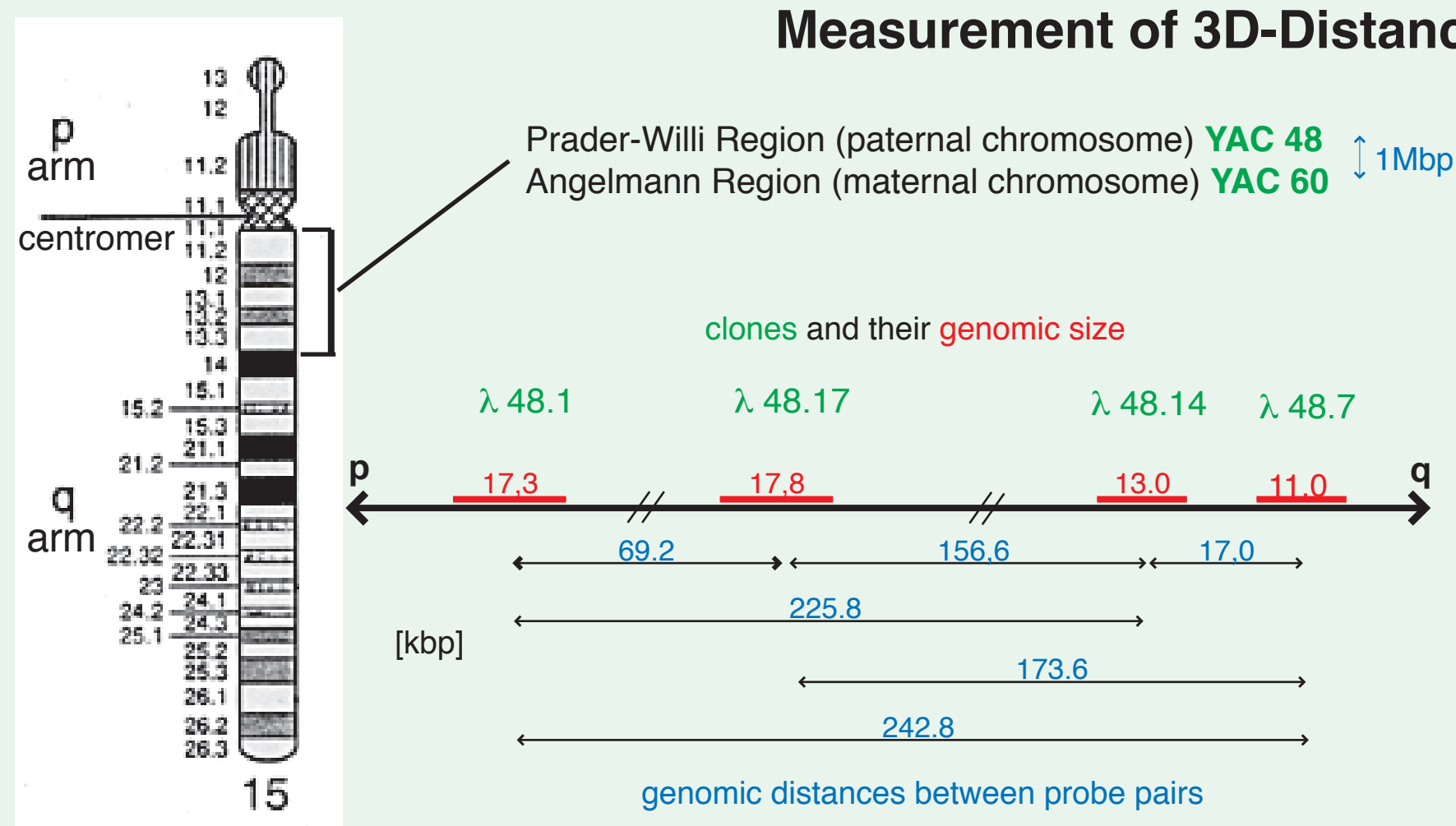

Fig. 3
Ray traced image of the Multi-Loop Subcompartment model, loop size 126kbp, linker size 126 kbp, after ~50.000 Monte-Carlo and 1000 relaxing Brownian- Dynamics steps. Here rosettes form subcompartments as separated organizational and dynamic entities.

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.
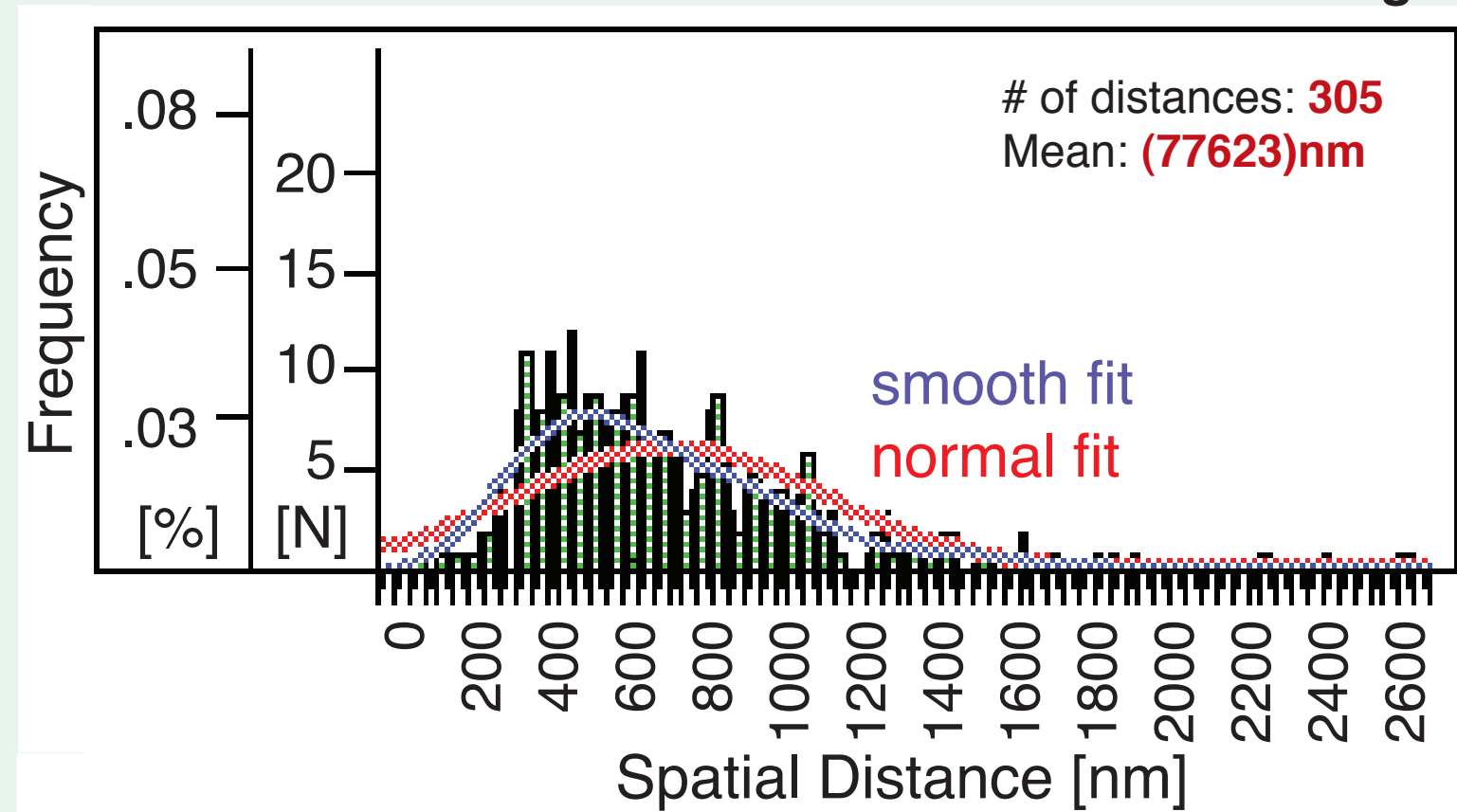

EXPERIMENTS

Measurement of 3D-Distances between Genomic Markers

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

clones and their genomic size

λ 48.1   λ 48.17   λ 48.14   λ 48.7

17.3   17.8   13.0   11.0

69.2   156.6   17.0

225.8

173.6

242.8

[kbp]

genomic distances between probe pairs

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 interpret 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.

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.

Distance Distribution   Fig. 5

# of distances: 305
Mean: (77623)nm

smooth fit
normal fit

Frequency [%] [N]

Spatial Distance [nm]

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.
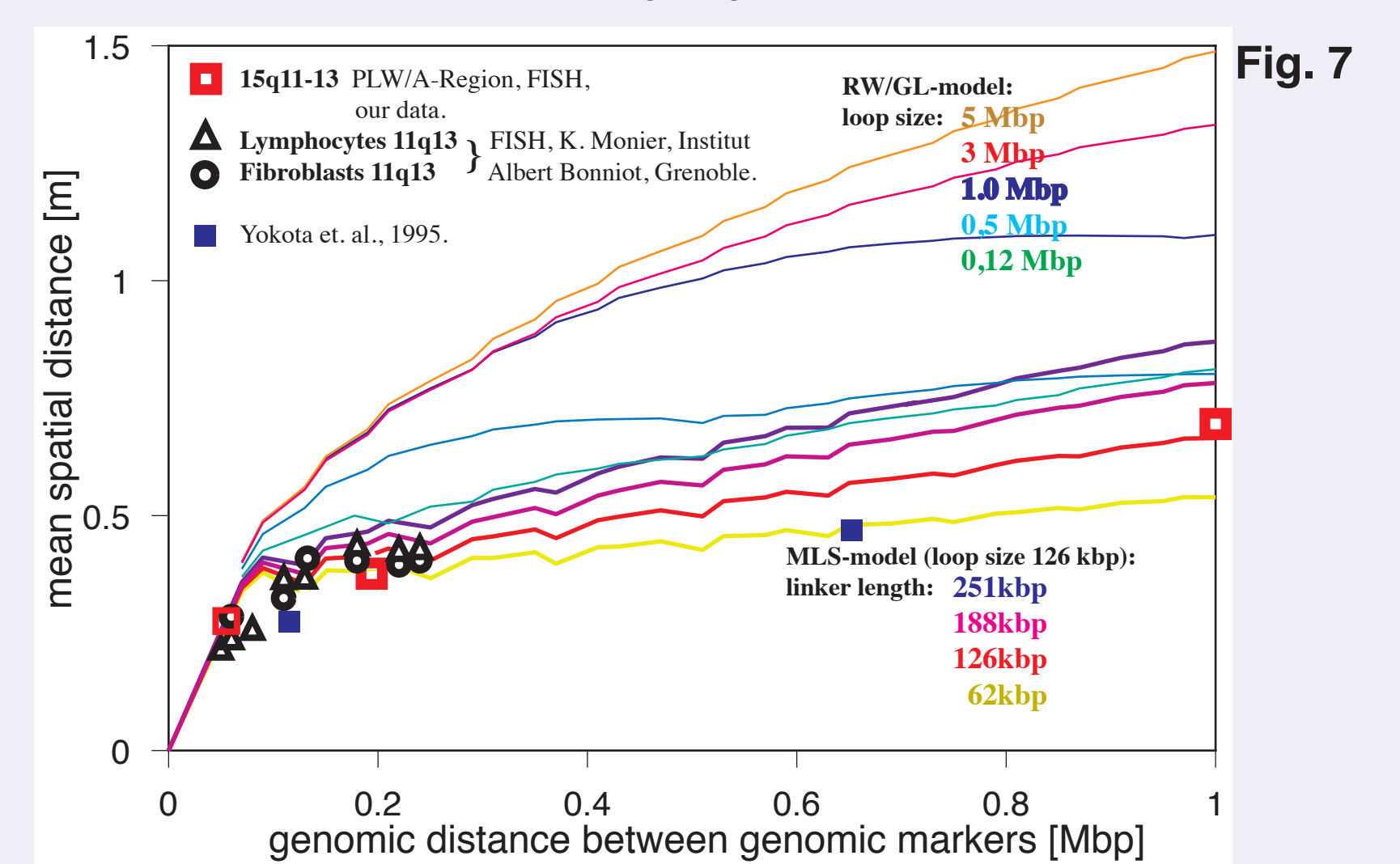

Fig. 7

15q11-13 PLW/A-Region, FISH, our data.
Lymphocytes 11q13] FISH, K. Monier, Institut
Fibroblasts 11q13] Albert Bonniot, Grenoble.
Yokota et. al., 1995.

RW/GL-model: loop sizes: 5Mbp, 3 Mbp, 1.0 Mbp, 0.5 Mbp, 0,12 Mbp

MLS-model (loop size 126 kbp): linker length: 281kbp, 188kbp, 126kbp, 62kbp

mean spatial distance [m]

genomic distance between genomic markers [Mbp]

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).
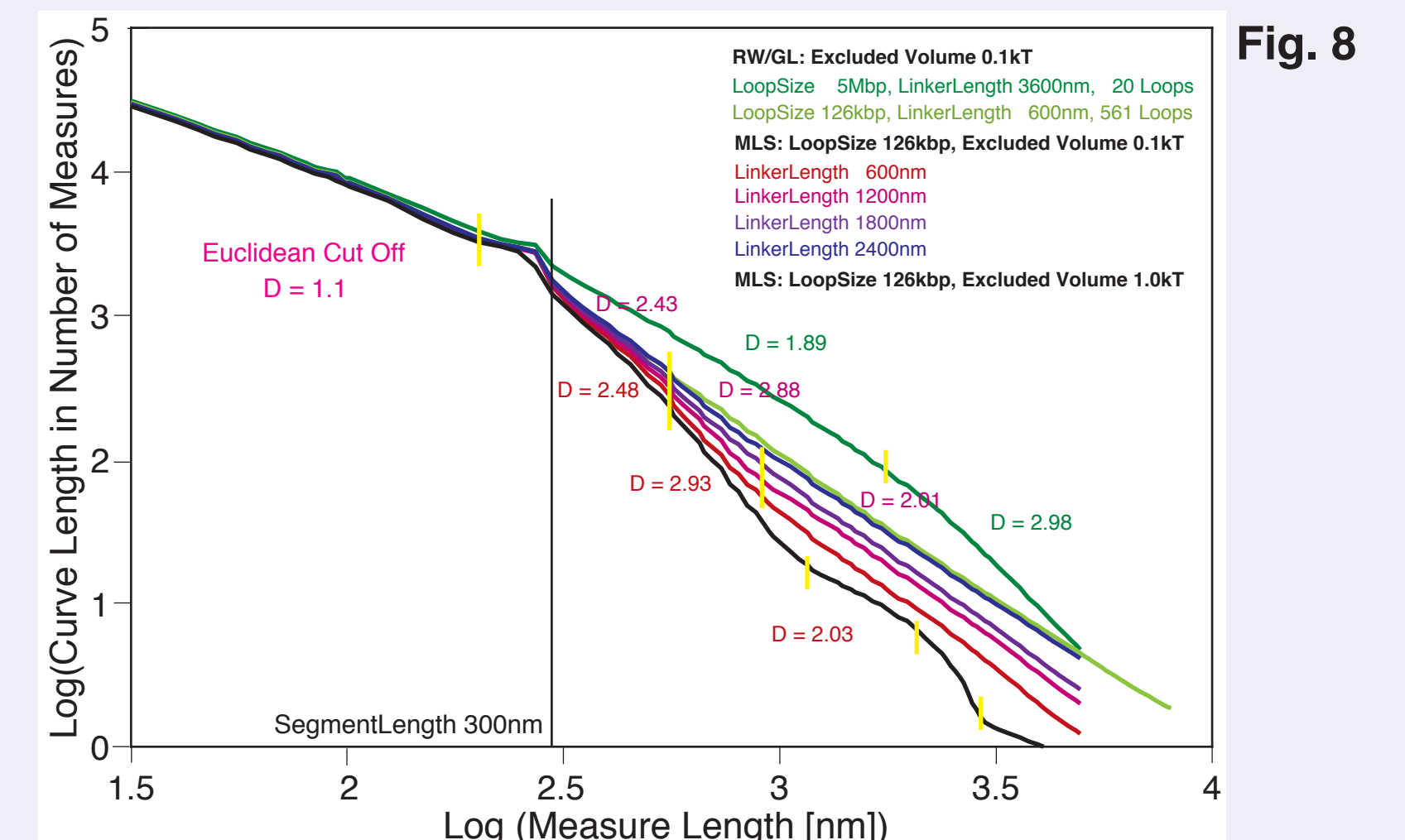

Fig. 8

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

Euclidean Cut Off
D = 1.1

D = 2.43   D = 1.89
D = 2.40   D = 2.90   D = 2.94   D = 2.98
D = 2.03

SegmentLength 300nm

Log(Curve Length in Number of Measures)

Log (Measure Length [nm])

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.

6th International Foresight Conference on MolecularNanotechnology, Santa Clara,
California, USA, 12th - 15th November, 1998.

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.

Corresponding author email contact: TA.Knoch@taknoch.org

Literature References

Knoch, T. A. Dreidimensionale Organisation von Chromosomen-Domänen in Simulation und Experiment. (Three-dimensional organization of chromosome domains in simulation and experiment.) *Diploma Thesis*, Faculty for Physics and Astronomy, Ruperto-Carola University, Heidelberg, Germany, 1998, and TAK Press, Tobias A. Knoch, Mannheim, Germany, ISBN 3-00-010685-5 and ISBN 978-3-00-010685-9 (soft cover, 2rd ed.), ISBN 3-00-035857-9 and ISBN 978-3-00-0358857-0 (hard cover, 2rd ed.), ISBN 3-00-035858-7, and ISBN 978-3-00-035858-6 (DVD, 2rd ed.), 1998.

Knoch, T. A., Münkel, C. & Langowski, J. Three-dimensional organization of chromosome territories and the human cell nucleus - about the structure of a self replicating nano fabrication site. *Foresight Institute - Article Archive*, Foresight Institute, Palo Alto, *CA, USA,* http://www. foresight.org, 1- 6, 1998.