The Three - Dimensional Organization of a Self Replicating Nano Fabrication Site: The Human 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
D - 69120 Heidelberg, Federal Republic of Germany
http://www.DKFZ-Heidelberg.de/Macromol/Welcome.html

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

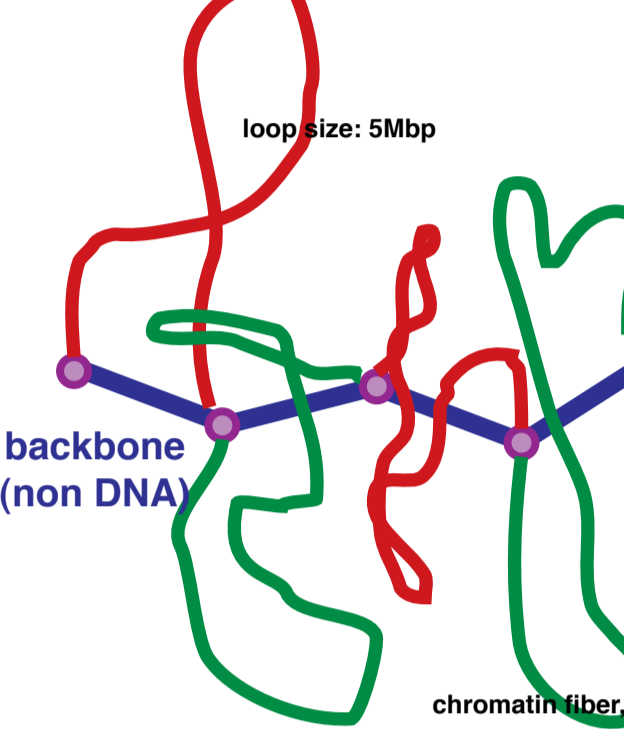[3)] Institute for Anthropology and Human Genetics, Richard Wagner Str. 10, 8033Munich, FRG
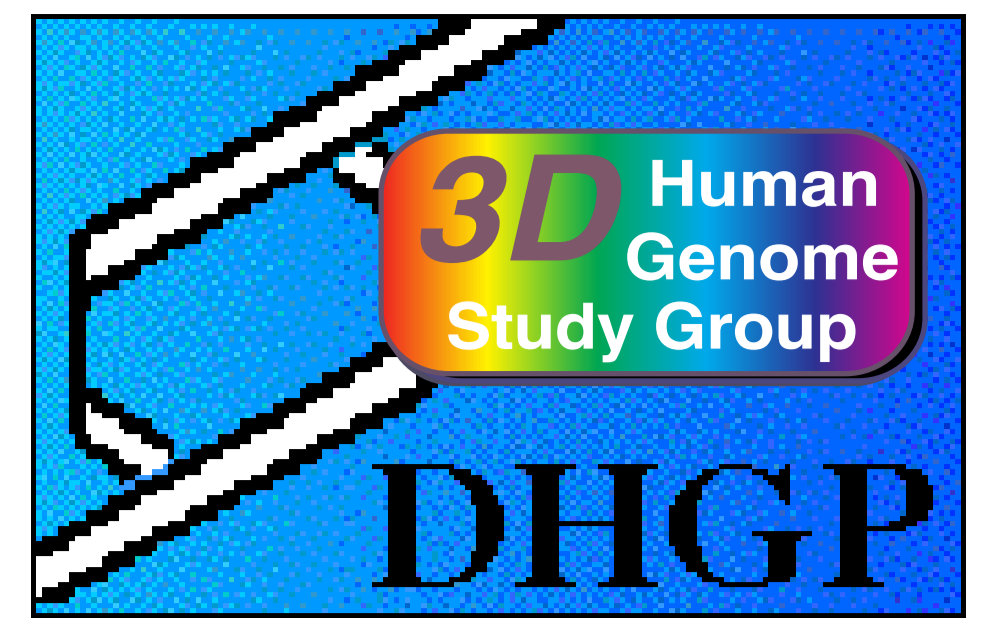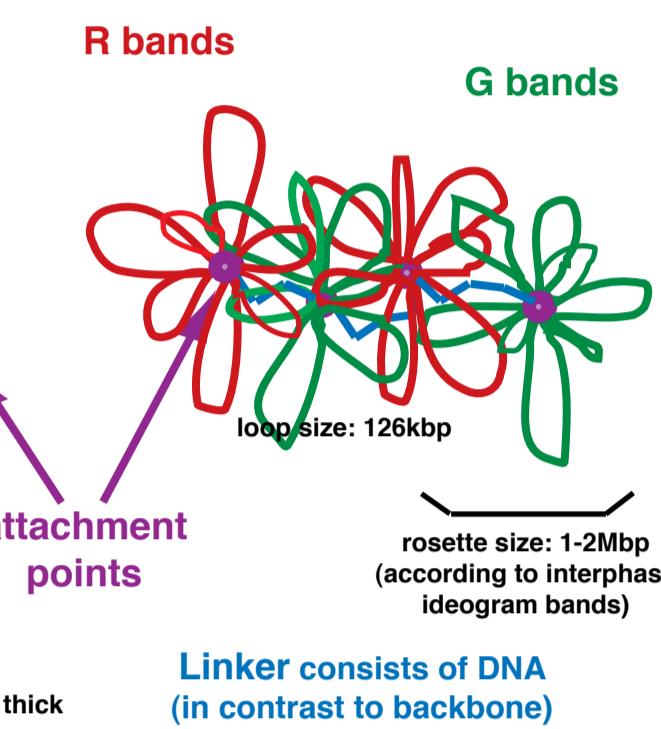
German Human Genome Project

The eukaryotic cell is a prime example of a functioning nano machinery. The synthesis of proteins, maintenance of structure and duplication of the machinery itself are all fine-tuned biochemical processes that depend on the precise structural arrangement of the cellular components. In particular the regulation of genes has been shown to be connected closely to the organization of the genome in the nucleus.
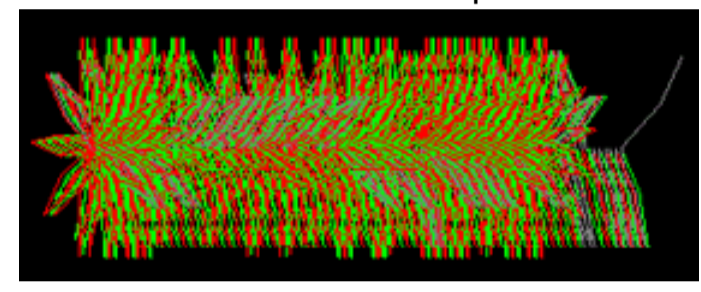
SIMULATIONS

Random Walk / Giant Loop model (RW/GL) Sachs et al. (1995)
loops 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)
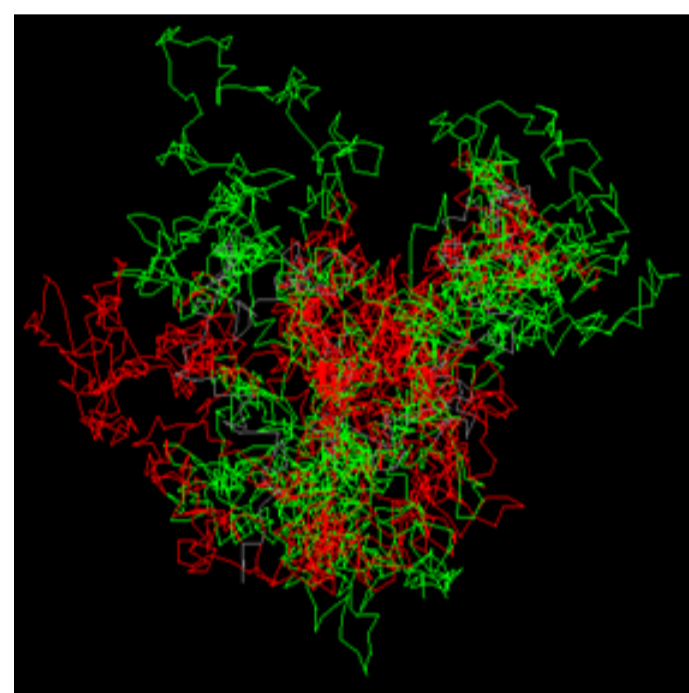Linker consists of DNA (in contrast to backbone)

With Monte Carlo methods we simulated various models of human interphase chromosome 15 assuming a flexible polymer chain (segment length 300nm=31kbp) being placed in a potential well whose height is related to the excluded volume interaction. The starting configuration was the approximate form and size of a metaphase chromosome from which the following decondensation into interphase resembles the natural process.
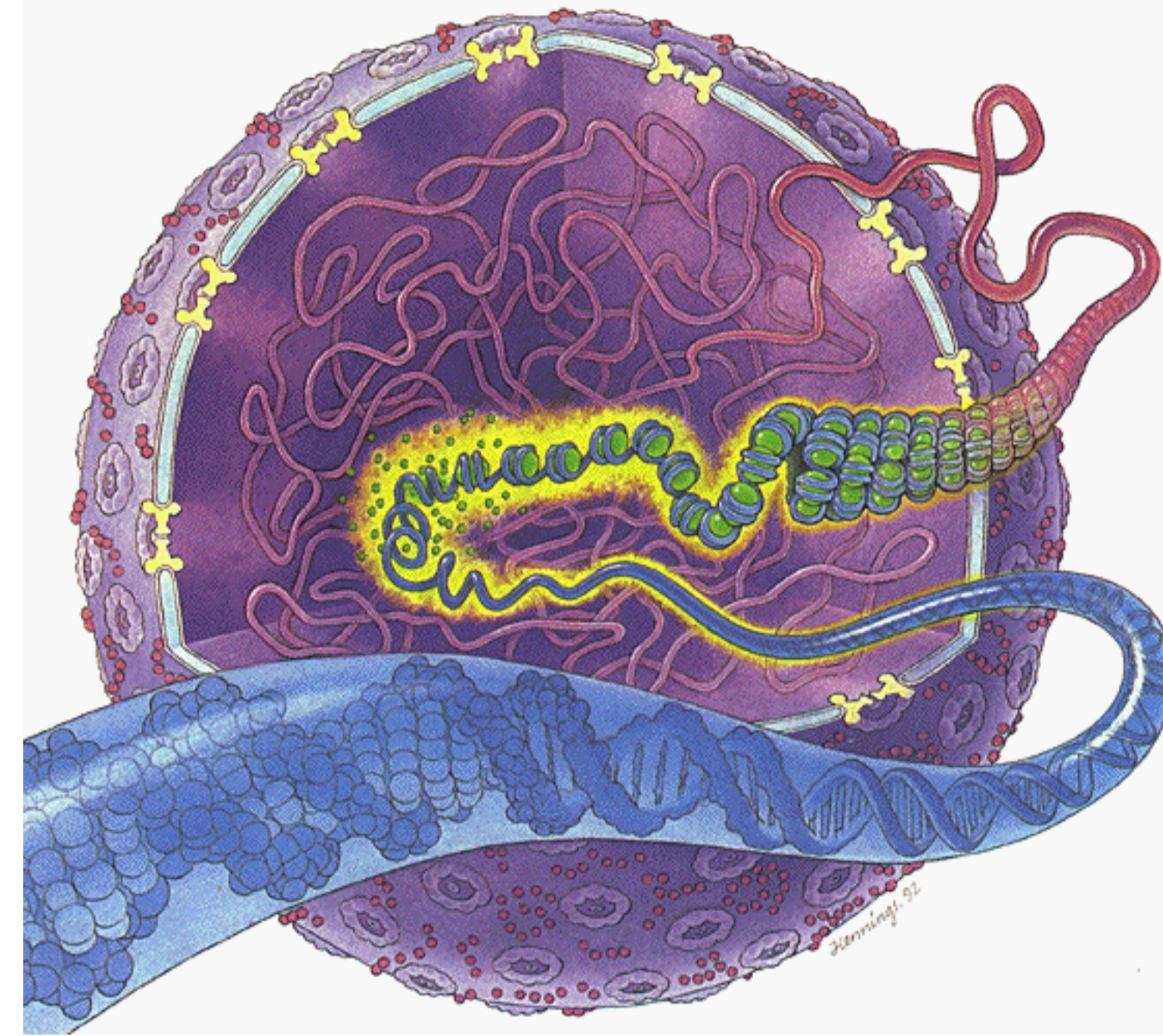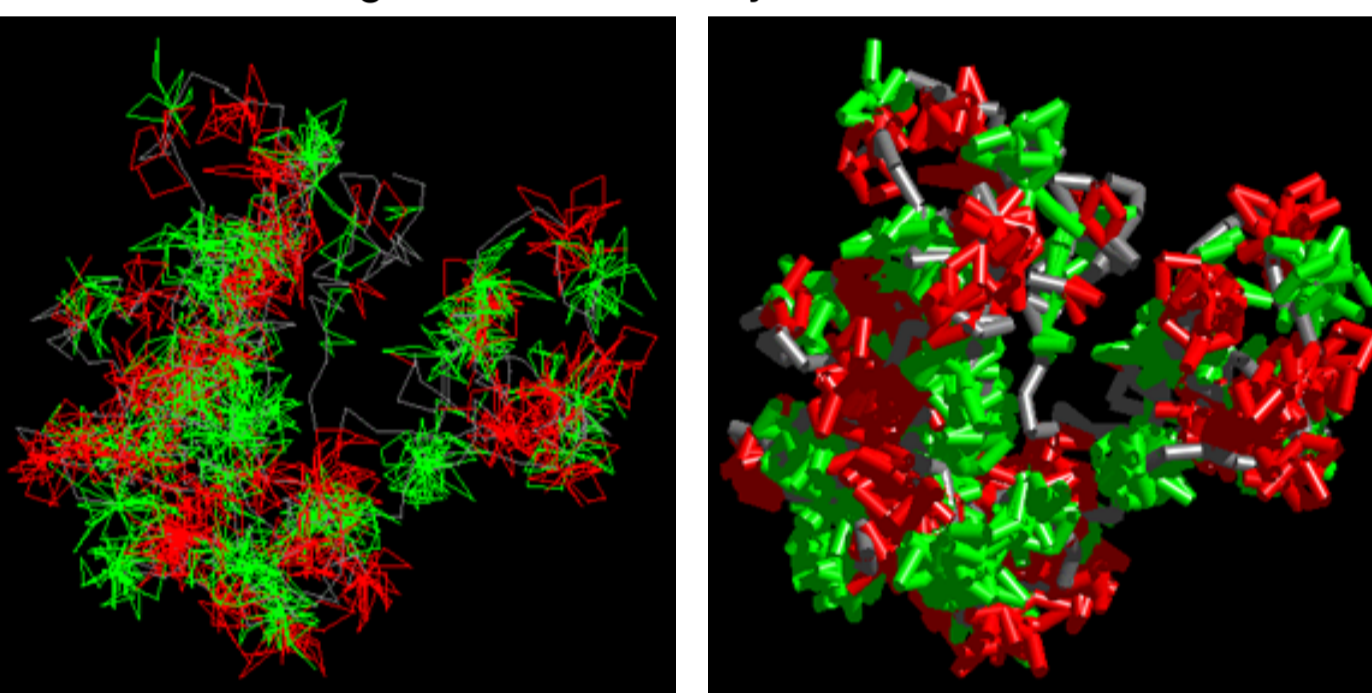
Rosettes in the Multi-Loop-Subcompartment model correspond to the size of chromosomal interphase band domains.
The simulations are made on two IBM SP2 parallel computers with 80 and 512 nodes. Results shown here needed 3.5 years CPU time on a single R6000.

Wire frame image of RW/GL model, loop size 5Mbp, after ~80.000 MC steps. Large loops intermingle freely thus forming no distinct features like in MLS model.
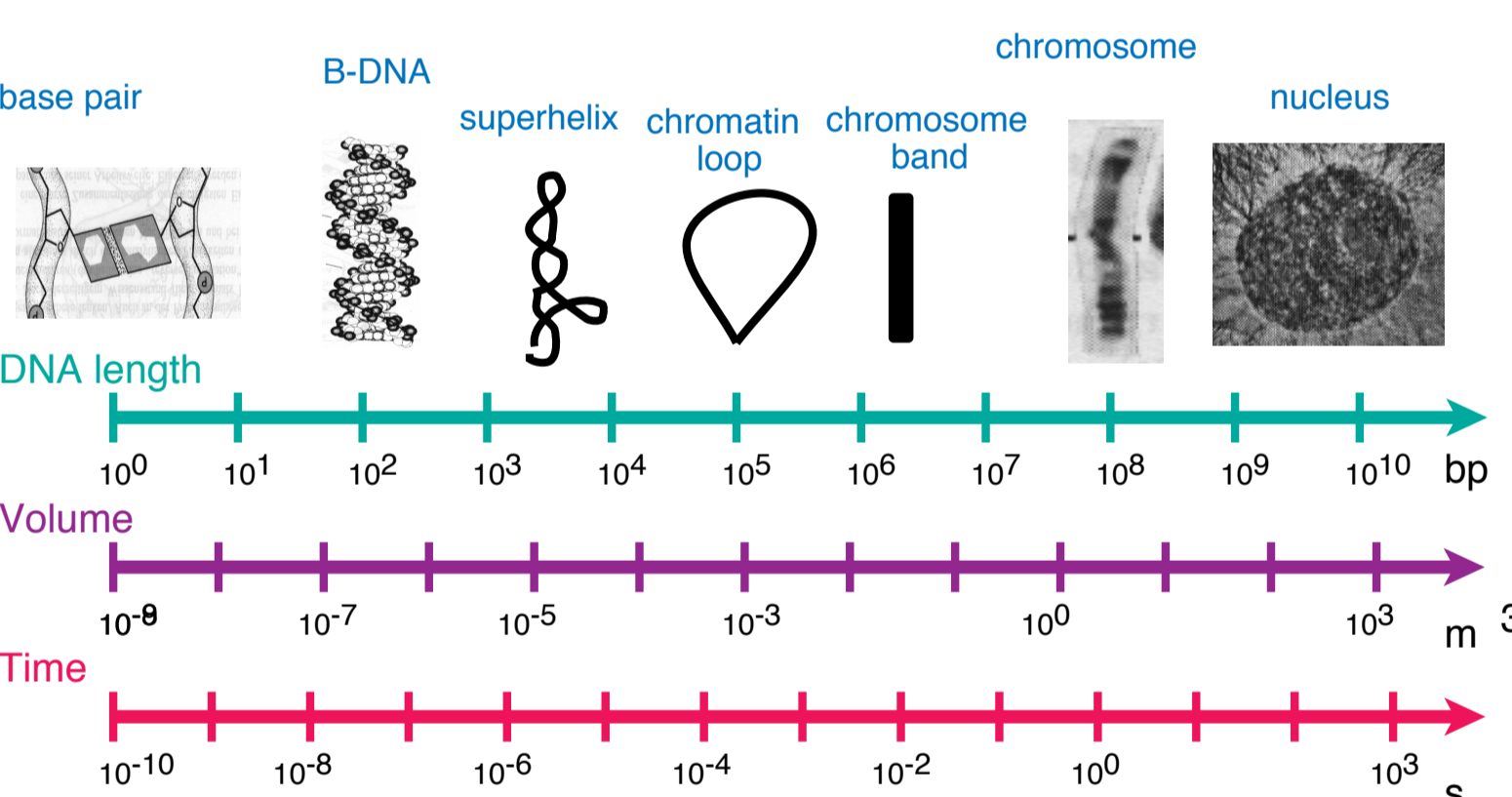
Wire frame and ray traced image of MLS model, loop size 126mbp, linker size 1200nm, after ~50.000 MC steps. Here rosettes form subcompartments as separated organizational and dynamic entities.

Various RW/GL models show similar global behaviour under the same excluded volume and linker size conditions (Fig. 1 and Fig. 2). For high excluded volume (1.0kT) (Fig. 2) the chromosome shows no sufficient relaxation into unstressed states during the simulation time. The textural / local behaviour changes with different loop sizes. Chromosomes´ radial density decrease with descending excluded volume, loop sizes and backbone length (Fig. 3).

Fig. 1 Distances From Begin of Chromosome — Giant Loop Sizes: 5,000,000 base pairs, 1,000,000 base pairs, 126,000 base pairs; MLS Loop Size: 126,000 base pairs; Excluded Volume 0.1kT

Fig. 2 Distances From Begin of Chromosome — Excluded Volume 1.0kT

Fig. 3 Radial Density — Excluded Volume: thin 1.0kT, wide 0.1kT

With growing linker length the structures simulated with the MLS model are less and less dense even at low excluded volume interaction. Similar to the RW/GL simulations (Fig. 2) for high excluded volume the chromosome is not sufficiently relaxed into unstressed states and does not show the same pattern. Accordingly most of the chromosomes´chromatin is quenched into an unnatural spherical shell. Hence we suppose that efficient mechanisms of genome reorganization requires stress-relaxing features e.g. strand passage mediated by enzymes such as topoisomerase II.

Fig. 4 Distances From Begin of Chromosome — Excluded Volume 0.1kT / Excluded Volume 1.0kT

Fig. 5 Radial Density — Linker Length: 2*300nm = 600nm, 4*300nm = 1200nm, 6*300nm = 1800nm, 8*300nm = 2400nm; Excluded Volume 0.1kT, 1.0kT

Increasing loop size and hence decreasing number of loops per rosette results in a decrease in density. For different chromosome sizes (Fig. 6) we get denser states for longer chromosomes (Fig. 7) as the chromosomes are all simulated with the same potential well.

Fig. 6 Distances From Begin of Chromosome — Excluded Volume 0.1kT

Fig. 7 Radial Density — Loop Sizes: 80,000 base pairs, 100,000 base pairs, 150,000 base pairs, 230,000 base pairs

For comparison with experimental data at small genomic distances the Monte Carlo configurations are taken as starting points for relaxation at higher spatial resolution by Brownian dynamics: segment length now 50nm=5000 base pairs, chromosome 15 has about 31000 segments. As the exact position of genomic marker ensembles is unknown in respect to loop bases, the ensembles have to be shifted through the loops. Consequently the mean of many configurations leads to different sets of different spatial distances for every ensemble position. Due to MLS´s rosette-symmetries we find periodicities.

Fig. 8: MLS model: loop size 126kbp, linker length 1200nm, segment length 50nm, excluded volume 0.1kT, mean over 1.000.000 MC-Steps

Spatial Distance [m] — Marker Shift in Respect to Loop Base [N*200nm] — Genomic Marker Distance [kbp]
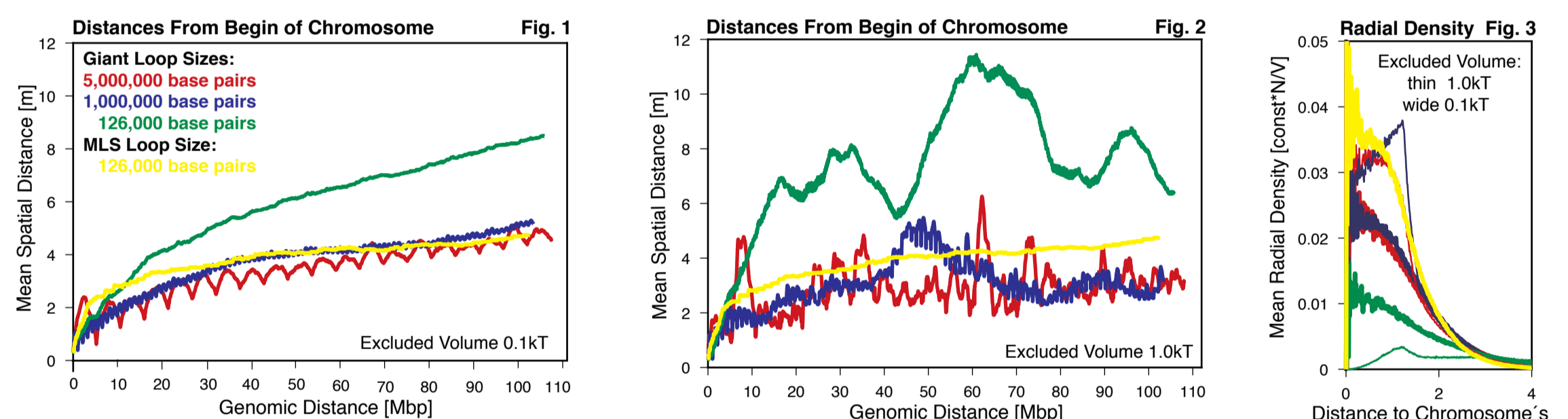

Typical misleading illustration of the human cell nucleus: 1) human cell nuclei have no spherical shape, 2) the DNA is not a closed pipe, 3) nucleosomes are not regularly organized into chromatin, 4) chromatin does not float around randomly in the nucleus. V. Hennings (illustrator) in Molecular and Cellular Biology by Stephen L. Wolfe, 1993.

The degree of determinism, transport and material flow as well as self organizing dynamic properties are largely unknown in such complex systems. Here we show studies leading to insights about the largest structural entity of the eukaryotic cells´ nucleus: the organization of chromatin into chromosomal territories.
These and future developments might be important for building complex and optimized nano fabrication sites, thus dealing with problems nature has solved already millions of years ago.
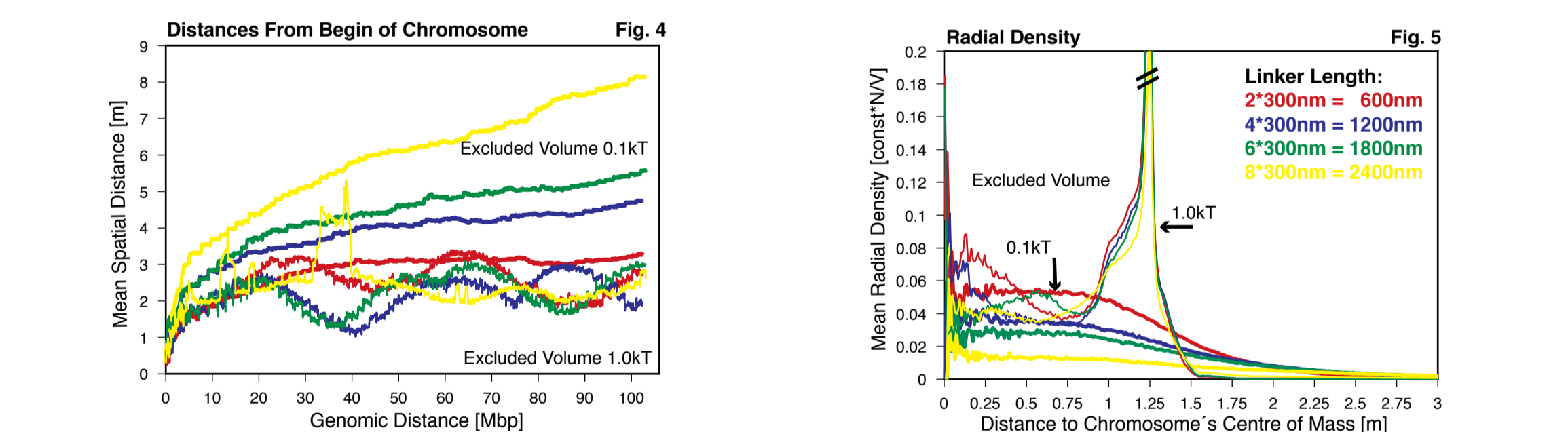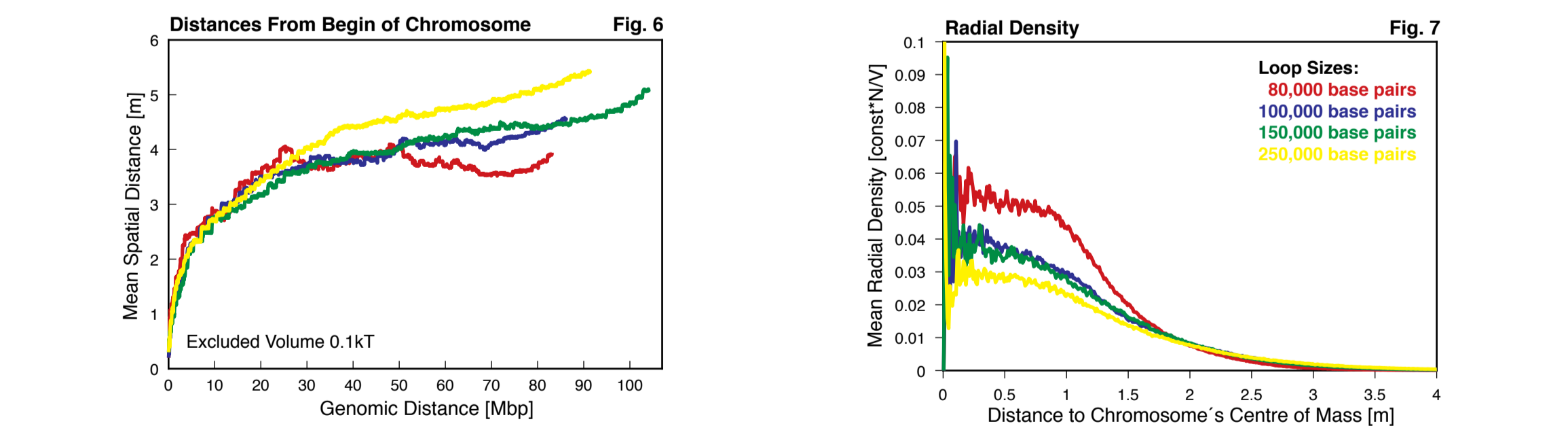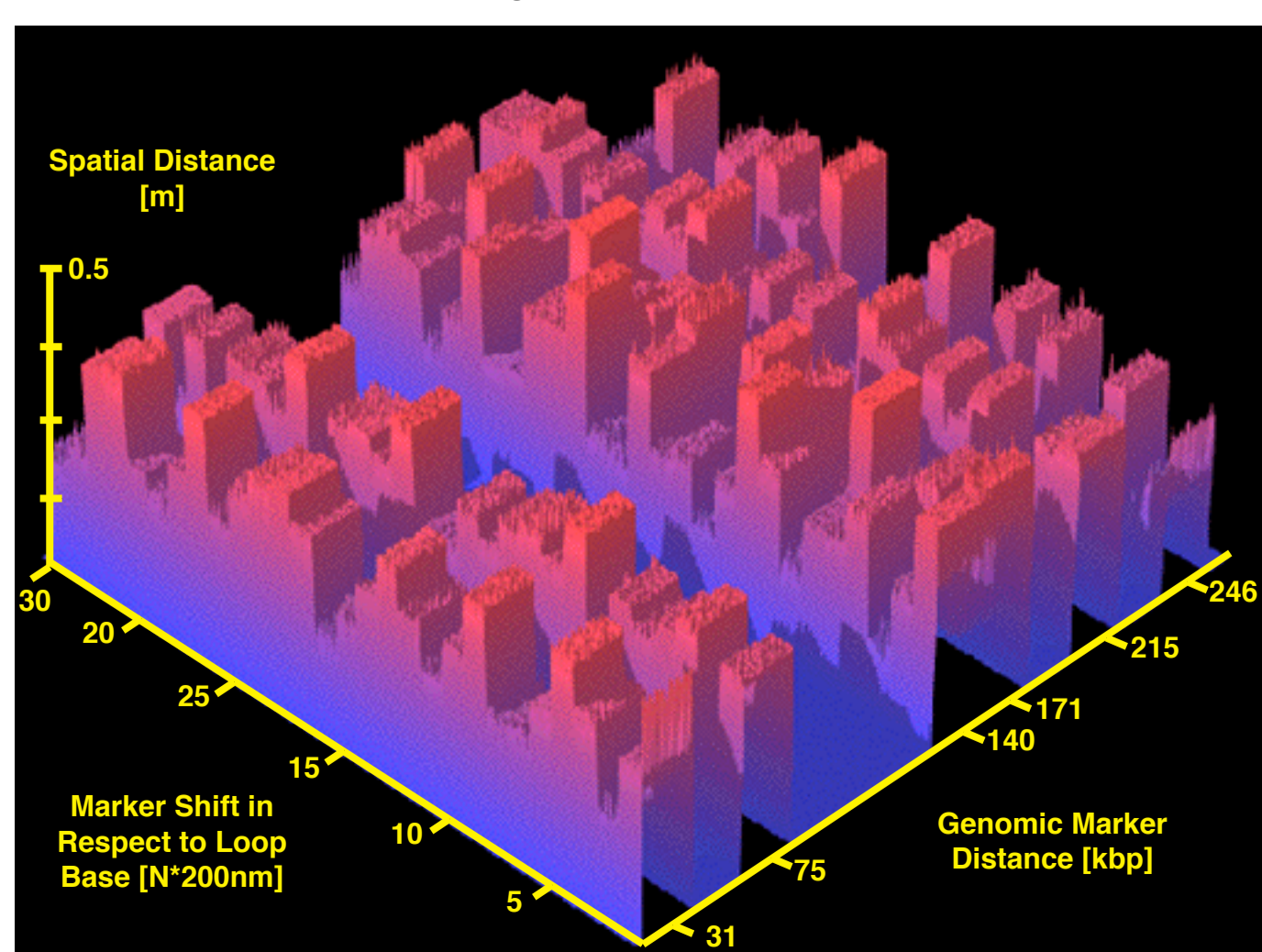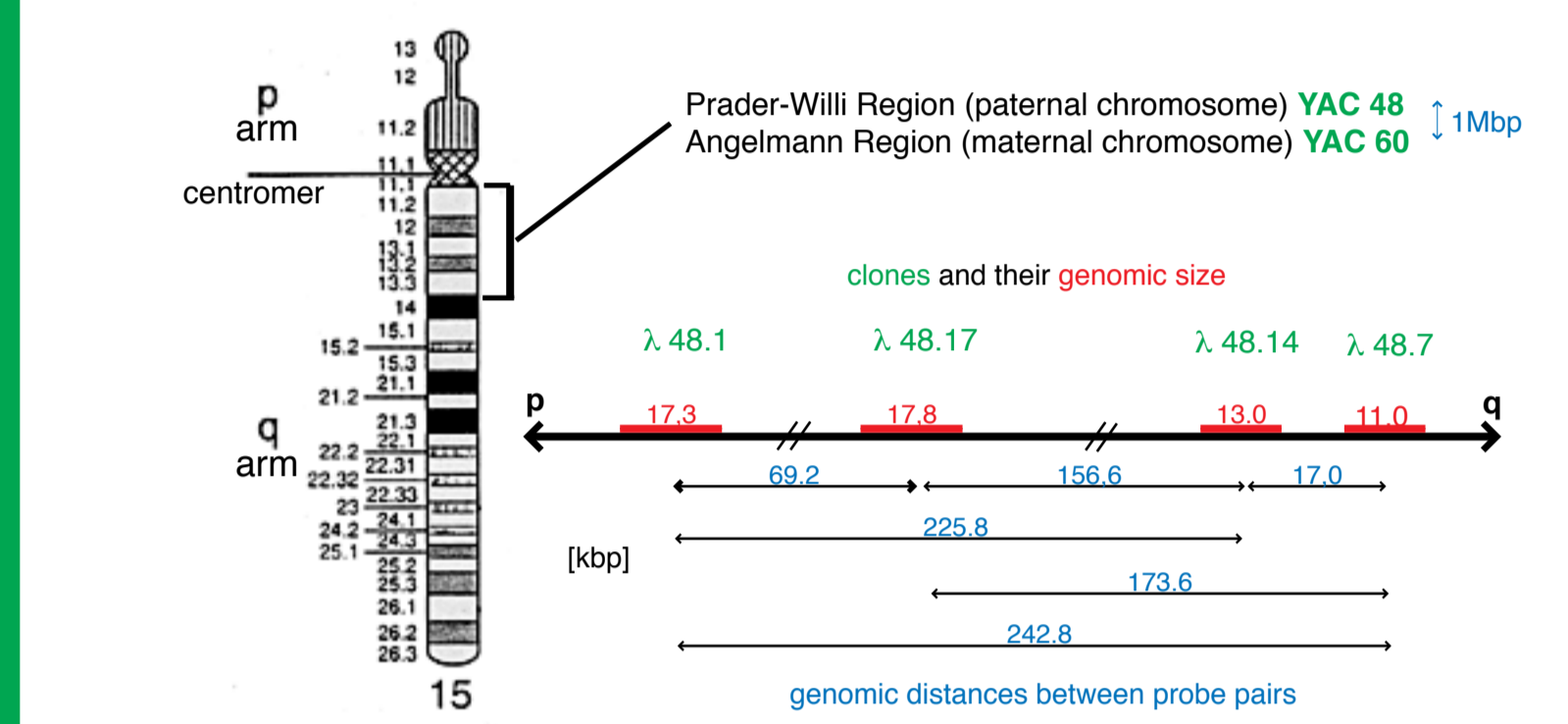
The dynamic and hierarchical organization of cell nuclei span large length and time scales.

base pair — B-DNA — superhelix — chromatin loop — chromosome band — chromosome — nucleus
DNA length: 10^0 10^1 10^2 10^3 10^4 10^5 10^6 10^7 10^8 10^9 10^{10} bp
Volume: 10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-3} 10^{-1} 10^3 m^3
Time: 10^{-10} 10^{-8} 10^{-6} 10^{-4} 10^{-2} 10^0 10^3 s

Fractal analysis of the simulations suggest multifractal behaviour in good agreement with predictions drawn from porous media research. Length scales with different fractal dimensions consequently show different organizational and dynamical behaviour with great degree of distinction. Thus chromosomal territories show a higher degree of determinism than previously thought.

Fig. 9 Log(Curve Length in Number of Measures) vs Log (Measure Length [nm])
RW/GL: LoopSize 5Mbp, LinkerLength 3600nm, Excluded Volume 0.1kT
MLS: LoopSize 126kbp, LinkerLength 600nm, Excluded Volume 0.1kT
MLS: LoopSize 126kbp, LinkerLength 1200nm, Excluded Volume 0.1kT
Euclidean Cut Off D = 1.1
SegmentLength 300nm
D=2.48, D=1.89, D=2.93, D=2.88, D=2.01, D=2.96, D=2.03, D=2.37

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 1200nm. Furthermore, supposed that defined loop bases exist it might be possible to determine the mean positioning of genes relative to each other. Our experiments might hypothesize a different folding structure for maternal versus paternal chromosome 15.

Fig. 10 Spatial Distance [m] vs Genomic Distance [Mbp]
Van den Engh, Sachs, Trask, Science 257 (1992) 1410
Yokota et al. J. of Cell Biology 130 (6) (1995) 1239
Monier unpublished data
MLS distances from begin of loop bases
MLS distances between random pairs, 3d
MLS distances between random pairs, 2d projection
RW / GL distances between random pairs, 3d
PWS, 3D distance total mean
PWS, 3D distance means sorted by chromosomes

EXPERIMENTS

Confocal light microscopy in connection with fluorescent in situ hybridization (FISH) for specific marking of small chromosomal DNA regions is used avoiding structure destroying preparations like in electron microscopy.

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.

Chromosome 15 and the Prader Willi / Angelmann Syndrom region was chosen, as the genomic distance between markers is well known 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

Prader-Willi Region (paternal chromosome) YAC 48
Angelmann Region (maternal chromosome) YAC 60 — 1Mbp
clones and their genomic size
λ 48.1, λ 48.17, λ 48.14, λ 48.7
[kbp]

Hybridization: Digoxigenin labeled DNA probes are diffused into the nucleus where the DNA double strands are denatured for the specific target finding of the similar treated probes. The probes are detected with fluorescent dyes bound to anti-digoxigenin antibodies.
In collaboration with I. Solovei and T. Cremer, Institute for Anthropology and Human Genetics, Munich, FRG.

DNA double strand — digoxigenin labeled DNA probe
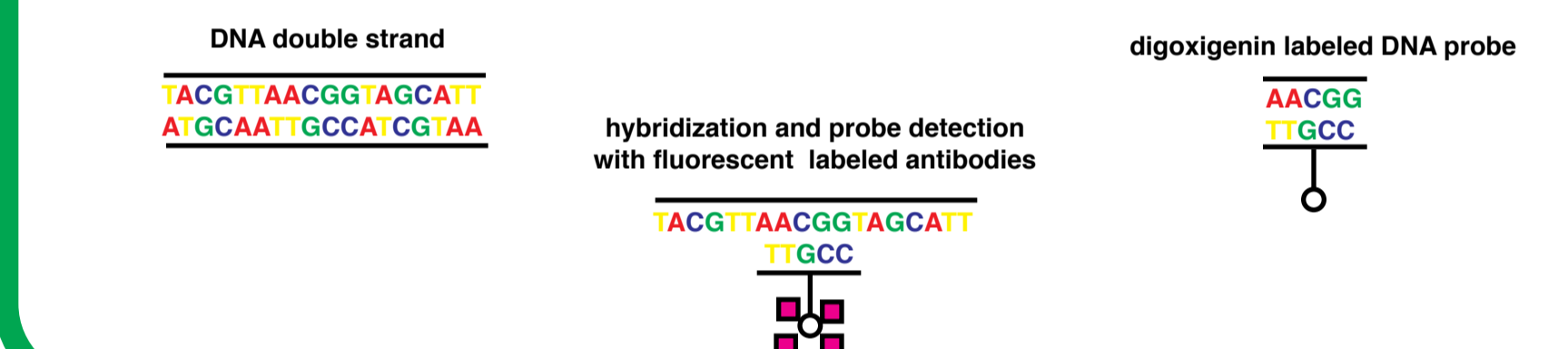hybridization and probe detection with fluorescent labeled antibodies

Fig. 11: Confocal image series taken with a Leica TCS NT confocal microscope with z = 200nm showing 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. These images are median background filtered. After manual threshold determination from an extended focus view (Fig. 12) for spot finding we proceed with image reconstruction specially adjusted to the microscope. Finally the 3D-spatial distances are determined. 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).
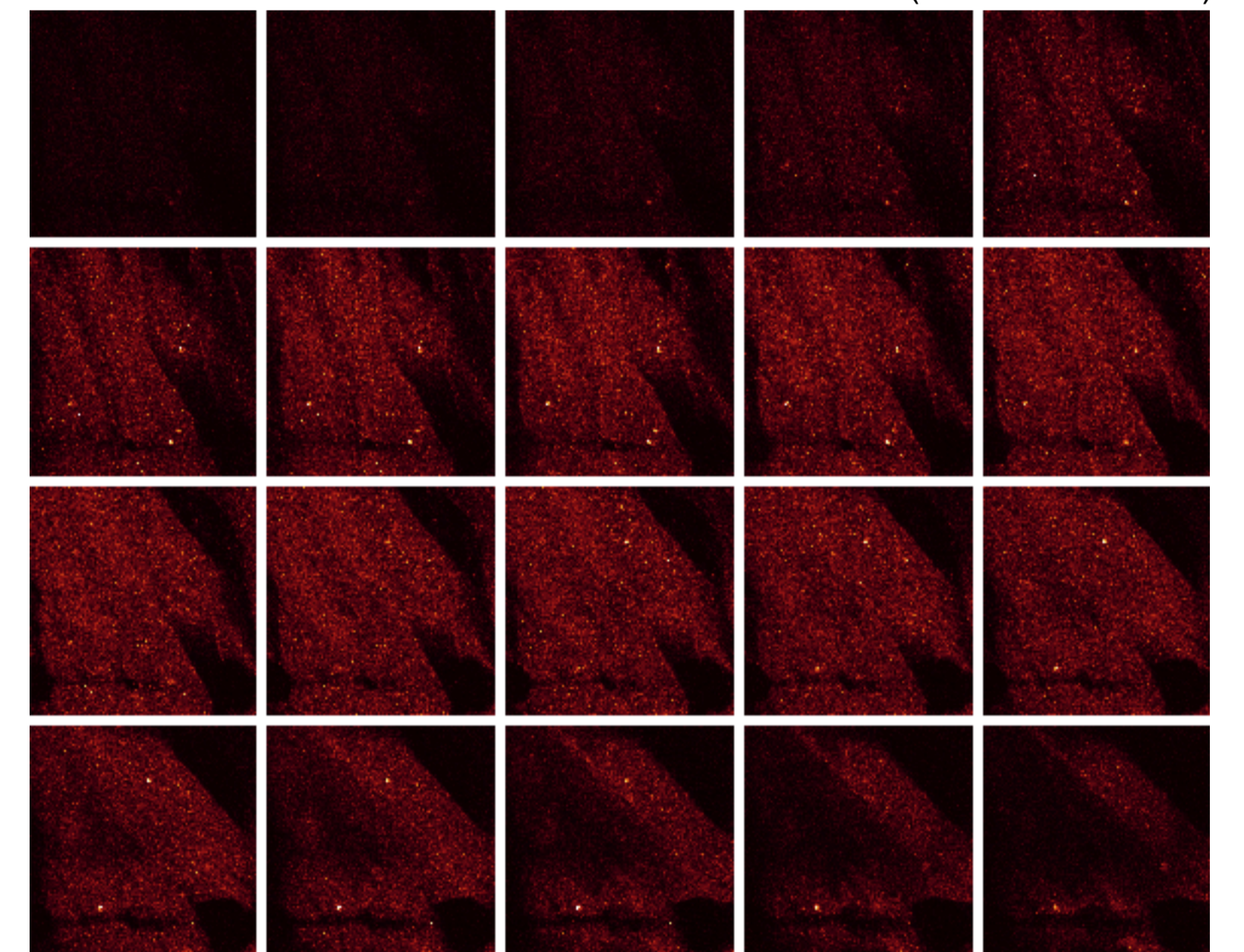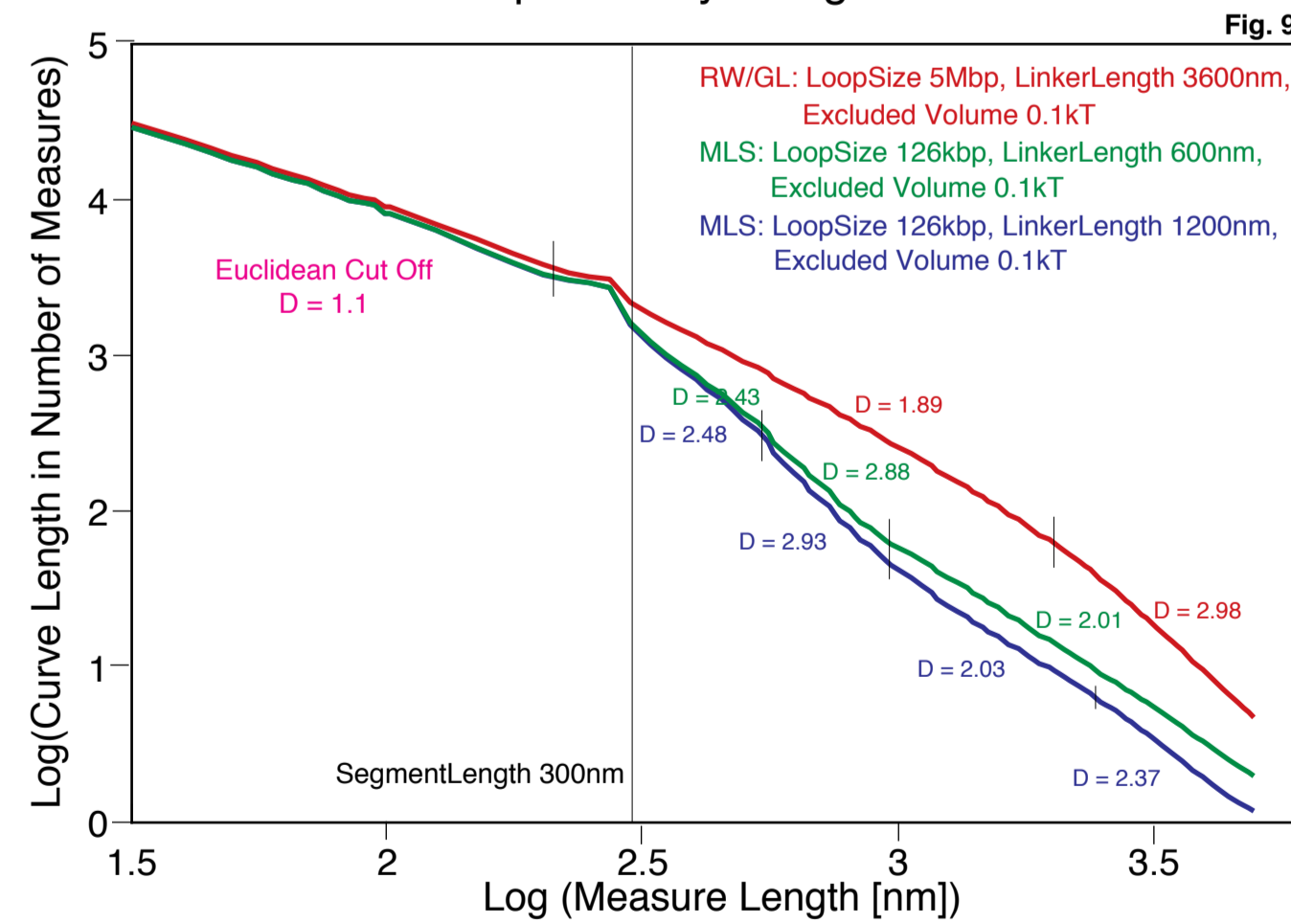
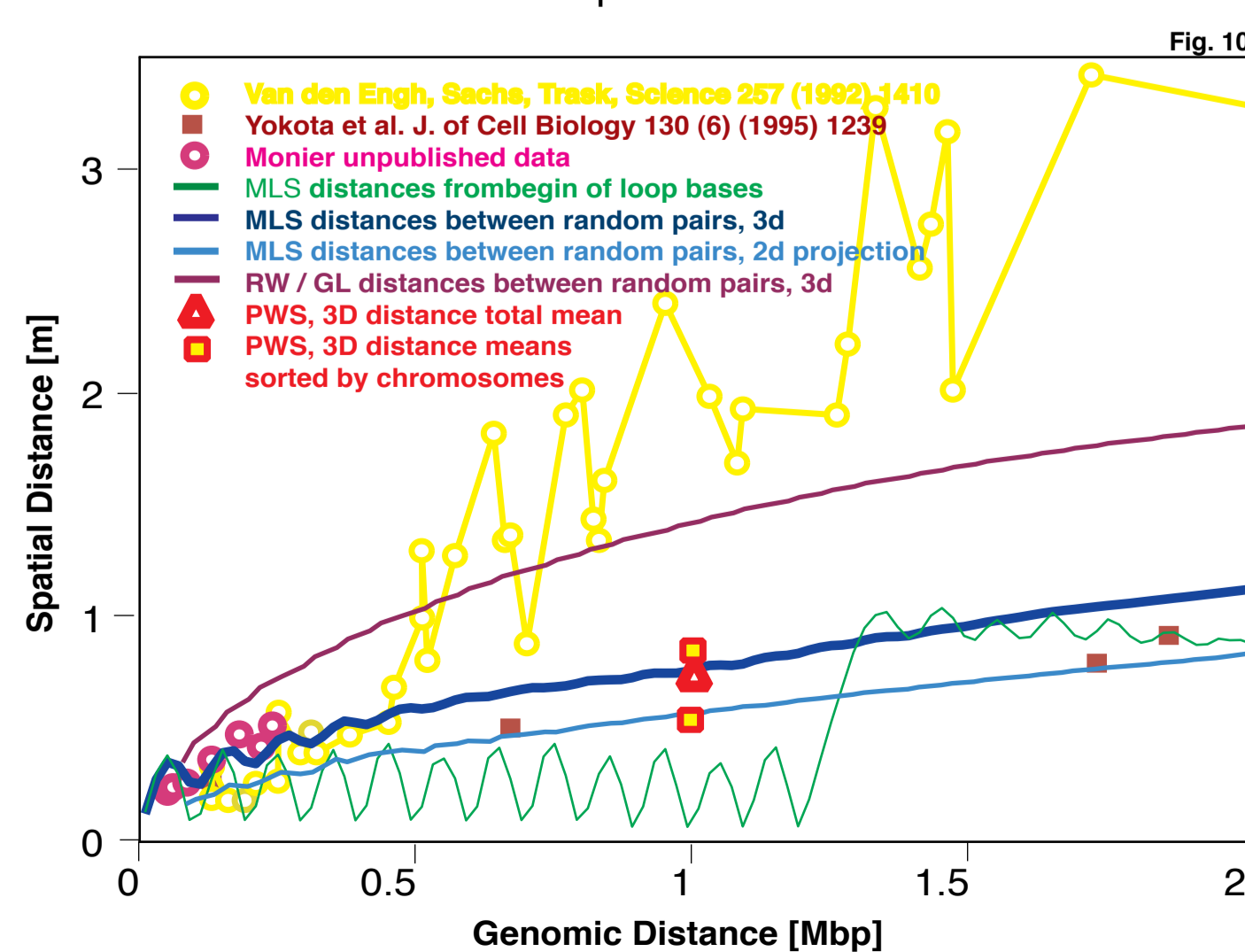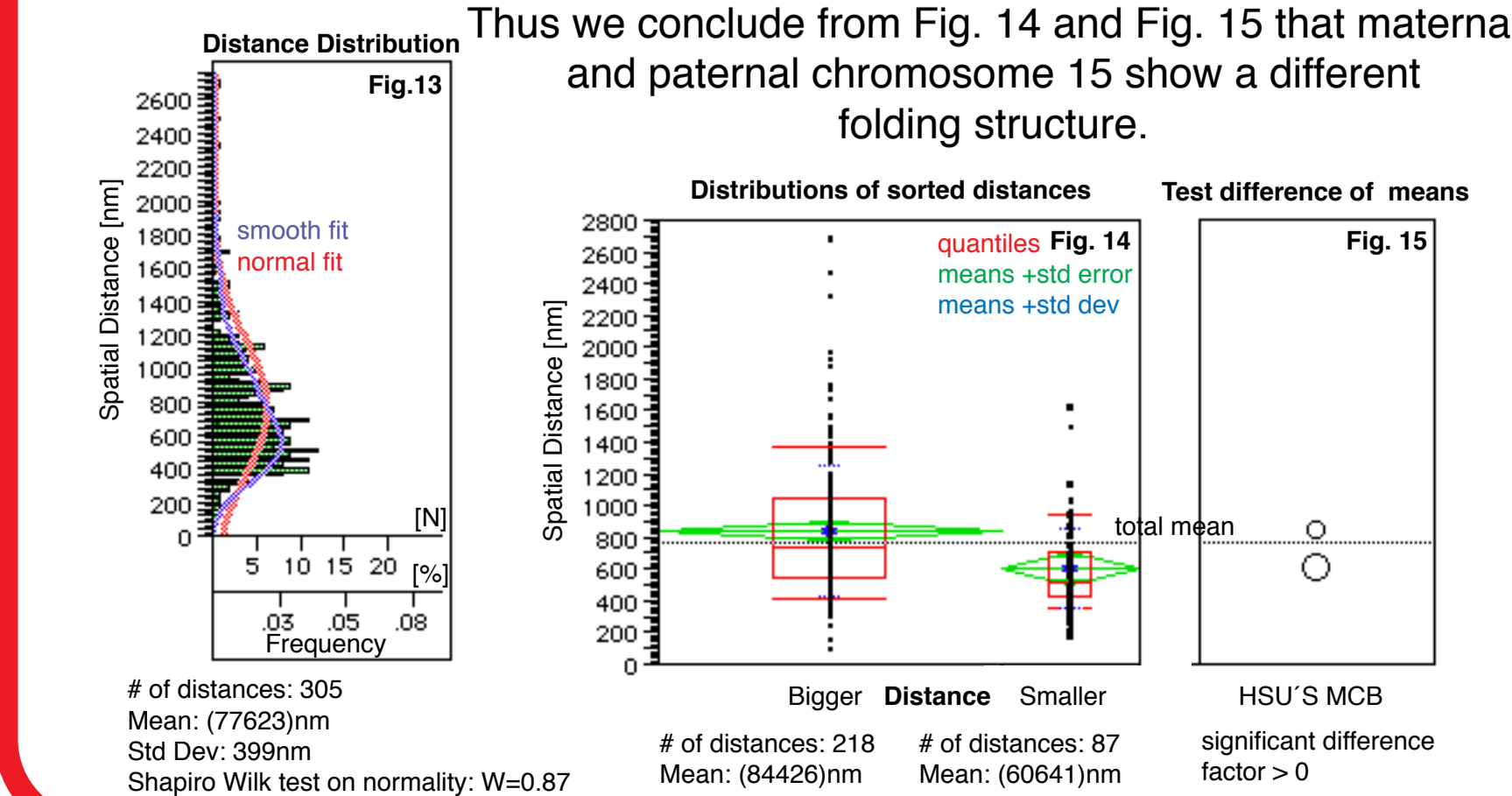**Fig. 12:** Extended focus image of the above shown serial section:
1: one spot
2: two spots, d = 135nm
3: two spots, d = 182nm
4: two spots behind each other, d = 424nm
5: two spots, d = 469nm
6: one spot
In collaboration with J. Rauch, H. Bornfleth and C. Cremer, Institute for Applied Physics Heidelberg, FRG.

Here we show the results of a statistical analysis of the spatial distances between the PWS-Region (YAC48) and AS-Region (YAC60) with a genomic distance of 1Mbp=10m of chromatin fiber. 305 nuclei were measured on two different slides. On one slide probes were detected with only one layer of antibodies and FITC as fluorescent marker. For finding differences between maternal and paternal chromosome 15, the distances in nuclei containing two distances were sorted into a "bigger" and "smaller" category for testing the significance of means´ difference with a SAS software package. This method has been shown to be statistically confident on two other genomic regions (C. Cremer et al. unpublished). Thus we conclude from Fig. 14 and Fig. 15 that maternal and paternal chromosome 15 show a different folding structure.

Fig.13 Distance Distribution — Spatial Distance [nm] vs Frequency — smooth fit / normal fit
# of distances: 305, Mean: (77623)nm, Std Dev: 399nm, Shapiro Wilk test on normality: W=0.87

Fig. 14 Distributions of sorted distances — Bigger / Smaller Distance — quantiles, means +std error, means +std dev, total mean
# of distances: 218, Mean: (84426)nm
# of distances: 87, Mean: (60641)nm

Fig. 15 Test difference of means — HSU'S MCB significant difference factor > 0

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Abstract

The eukaryotic cell is a prime example of a functioning nano-machinery. The synthesis of proteins, maintenance of structure and duplication of the machinery itself are all fine-tuned biochemical processes that depend on the precise structural arrangement of the cellular components. Especially the regulation of genes has been shown to be connected closely to the organization of the genome in the nucleus.

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 100 kbp 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). Currently we 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).

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

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

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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.