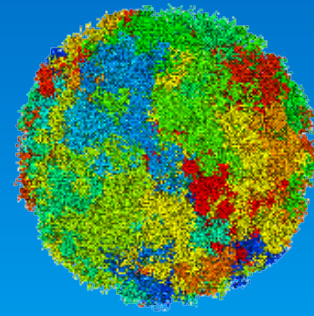


# From Sequence to Morphology



## *Towards a Holistic Understanding of Genomes*

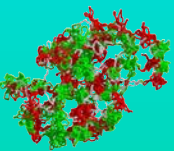
## History and Perspectives

by

**Tobias A. Knoch**

**Deutsches Krebsforschungszentrum (DKFZ)  
Kirchhoff Institut for Physics, Ruperto-Carola University  
Heidelberg, Germany**

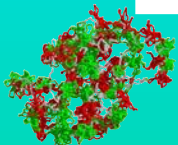
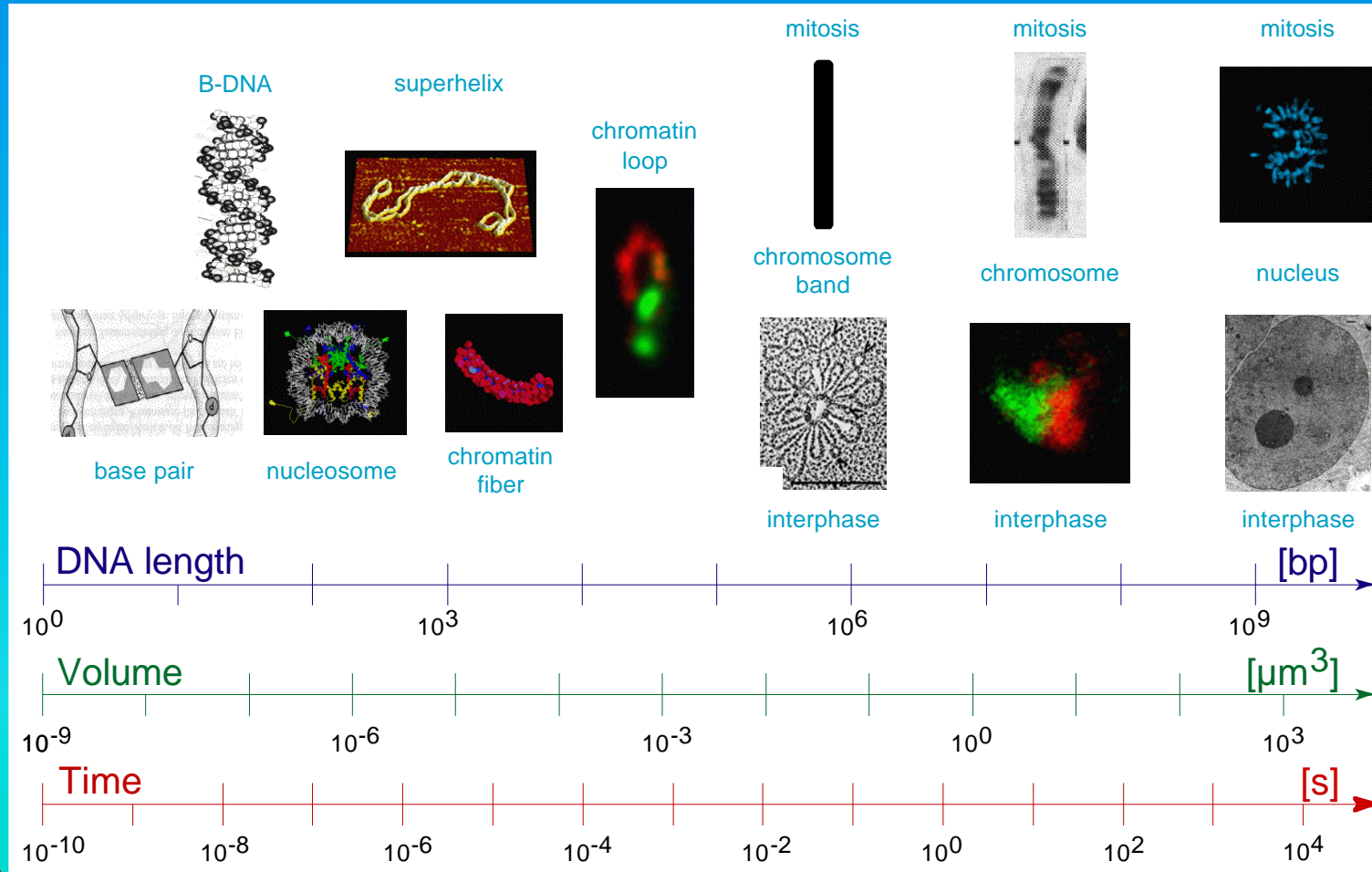
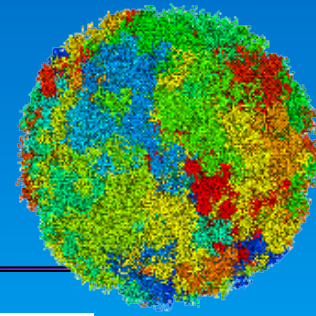
**Erasmus MC, Rotterdam, The Netherlands**





# Dynamic and Hierarchical Genome Organization

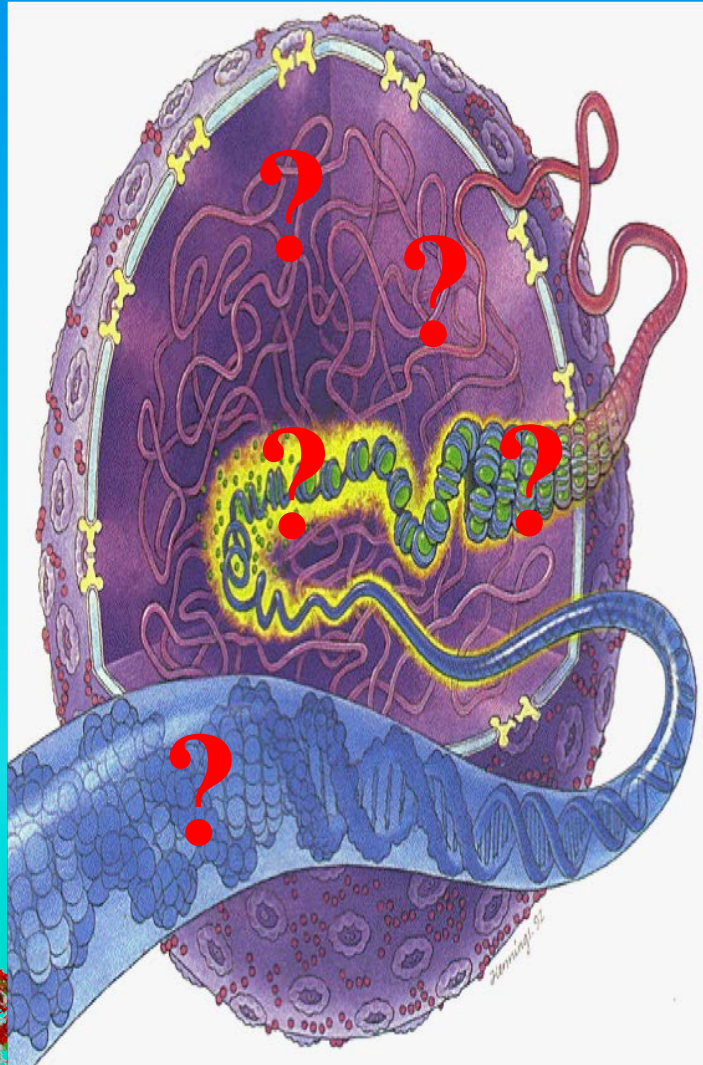
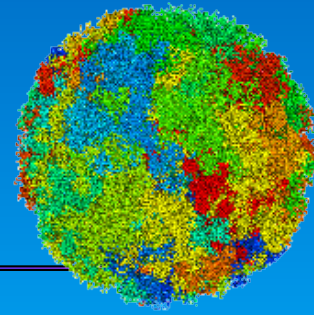
10 and 13 orders of magnitude concerning length and time scales are bridged.  
Are and how are all of these organization levels connected to fulfill their obvious functions, e. g. gene regulation or replication, since they are optimized by evolution ?





# WE KNOW THAT WE HARDLY KNOW ANYTHING

From the sequence to the morphology we just have begun to elucidate the organization of genomes and we also just have begun to understand that it is not only the detailed knowledge about one organizational level but beyond the holistic entity of the whole cell nucleus or genome which makes genomes function!



## *Nucleus:*

- > Nuclear organization: chromosome arrangement, morphology?
- > Nuclear Code: information content, regulation, variability?

## *Chromosome:*

- > Chromosome organization: loops, loop aggregates, extension?
- > Chromosome code: information content, regulation, variability?

## *Chromatin:*

- > Chromatin fiber organization: prevalence, variation, dynamics?
- > Chromatin code: coding, regulation, modification?

## *Nucleosome:*

- > Nucleosome organization: tail position, mobility, modification?
- > Histon code: coding, regulation, modification?

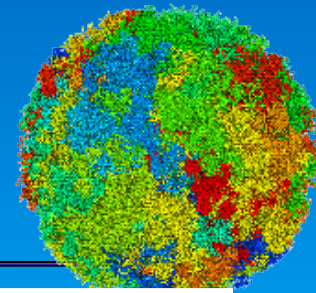
## *DNA Sequence:*

- > DNA local structure: bending, melting, stability, modification?
- > General sequence organization: coding, regulating and the rest?

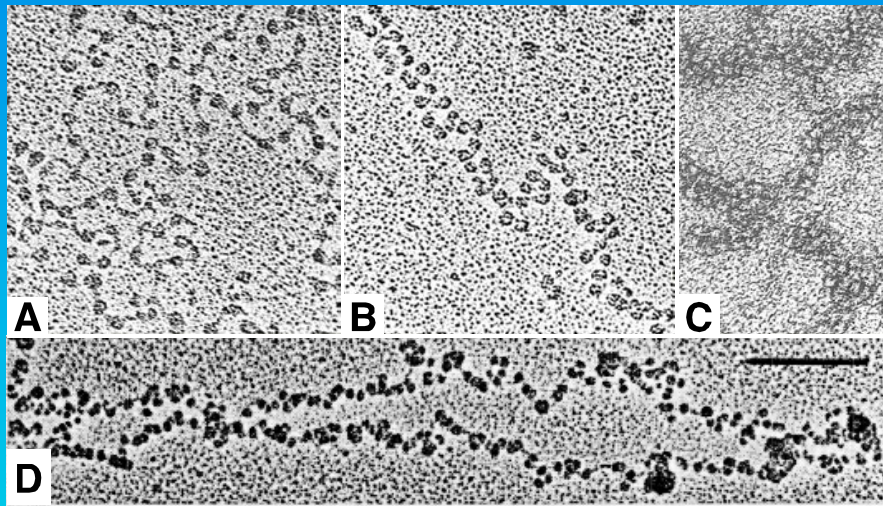


# Chromatin Conformation and Higher-Order Topologies

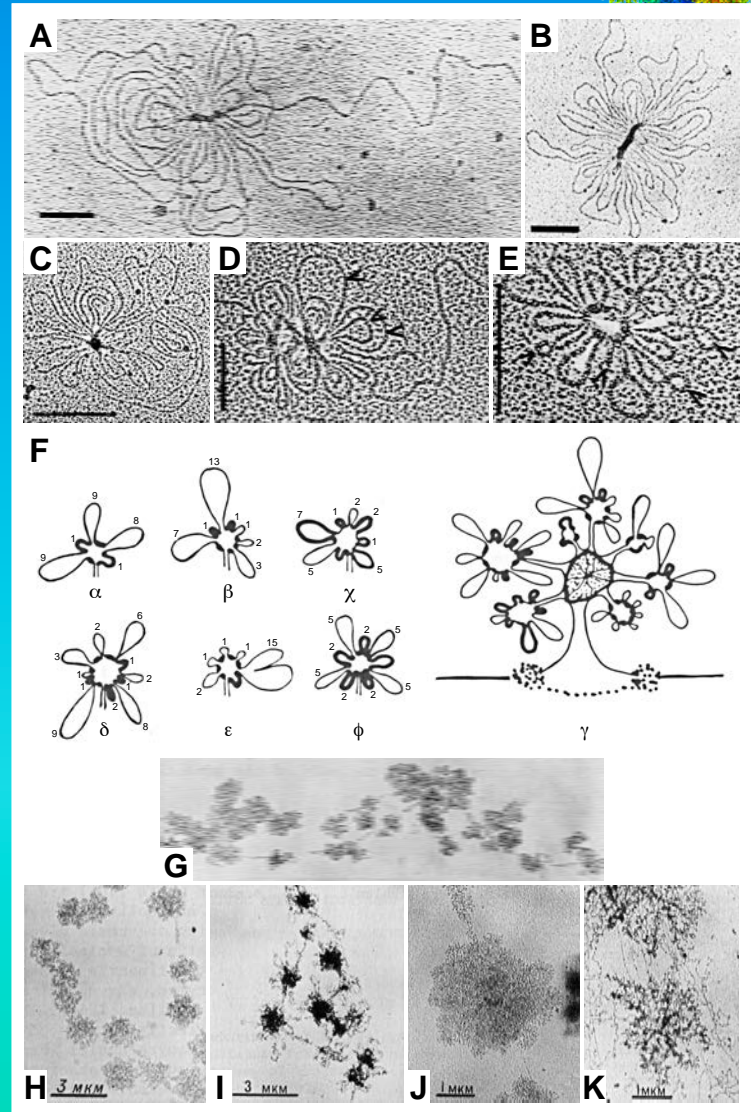
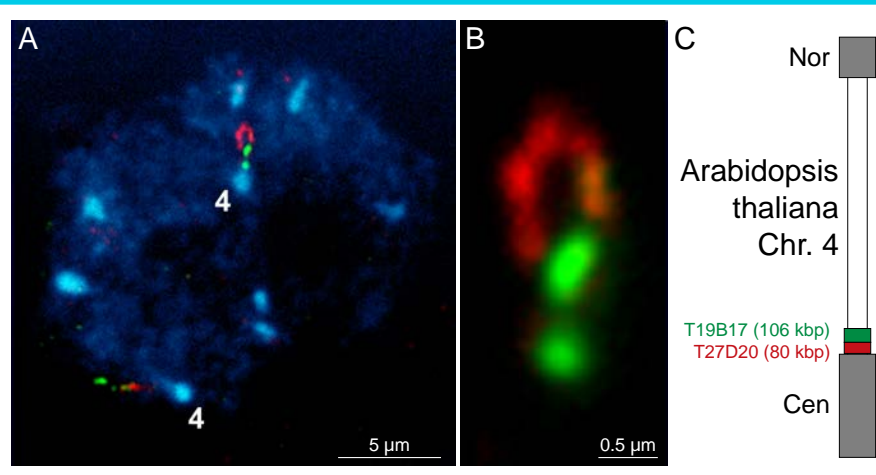
It becomes increasingly clearer, that the chromatin conformation is a random organization of nucleosomes, which depending on external or modification conditions has different condensation degrees, with a prevalence for the 30nm fiber with ~6nucleosomes per 11nm. This seems to make loops which further cluster to form aggregates more or less rosette-like which then constitute the chromosome.



A-C: Voet & Voet; D: Reznik *et al.*



Courtesy P. Fransz, Amsterdam

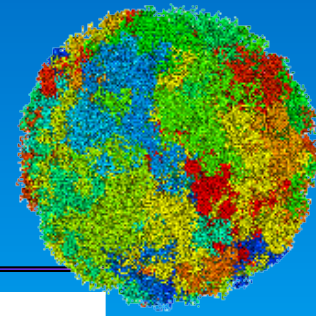


A, B: Aramova *et al.*; C: Salganik *et al.*; D-G: Frznik *et al.*; G-K: Tsvetkov & Parvenov.

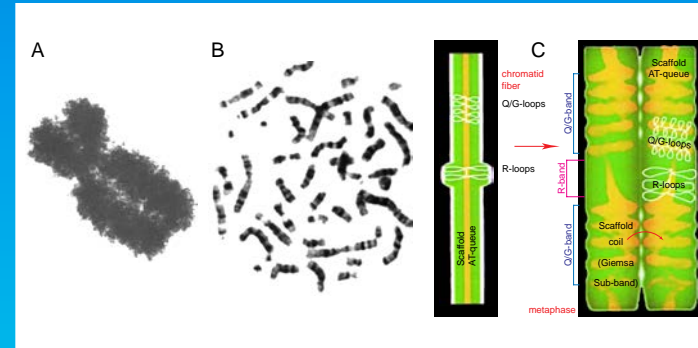
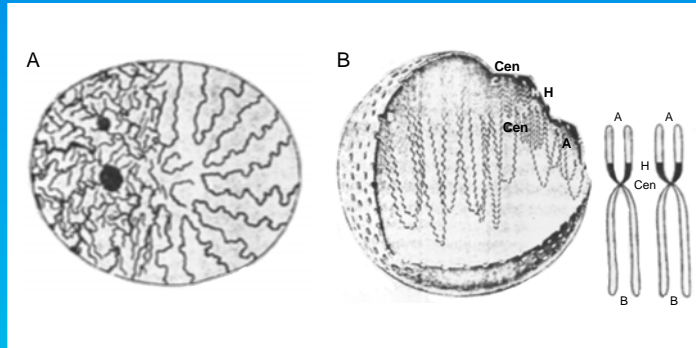


# Integral Models of Cell Nuclear Organization

Already Rabl and Boveri were aware of the obvious fact that the organization of genomes has to be consistent from the sequence level to the morphology of the whole cell nucleus. Although they might be different in detail their common seem is recursive folding and clustering thereof with variation/ modification and dynamics accounting for different nuclear states and function.

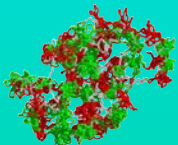
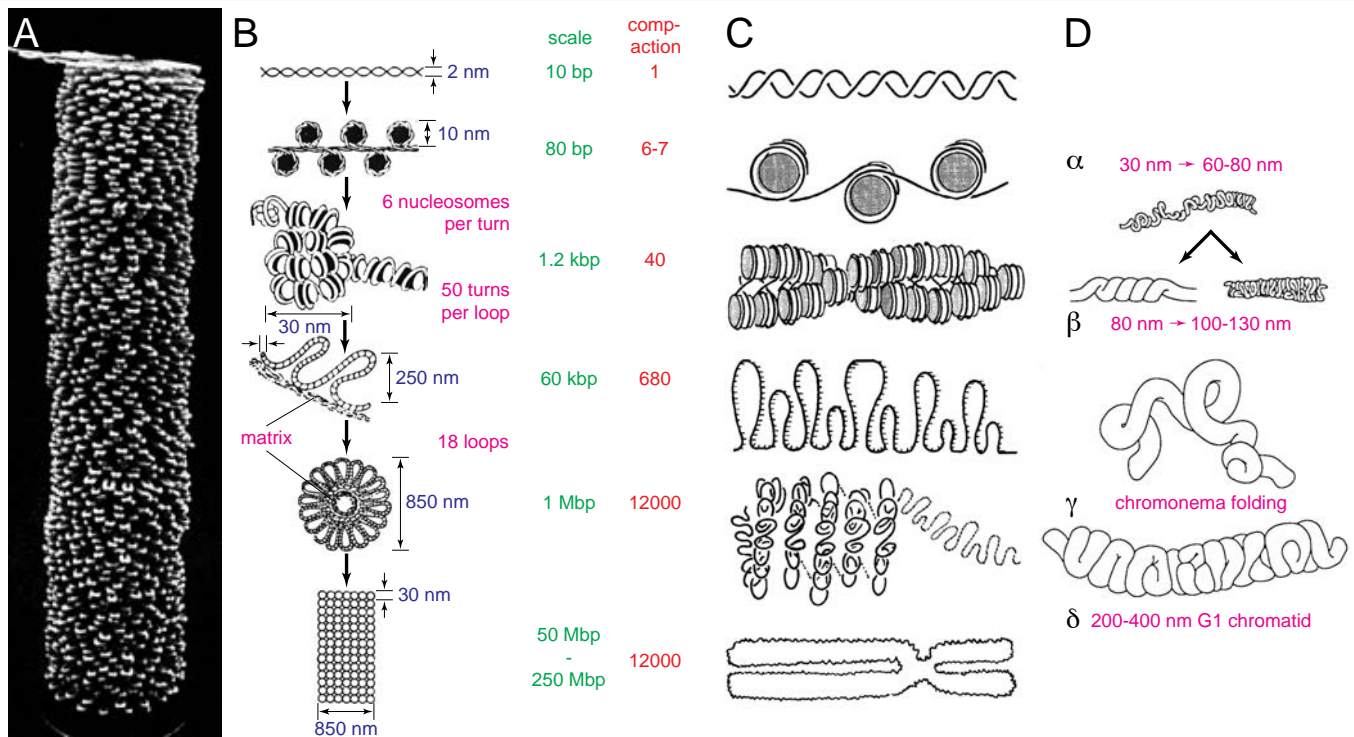


Rabl & Boveri



A: Bloom & Fawcett  
B: Alberts *et al.*  
C: Paulson & Laemmli

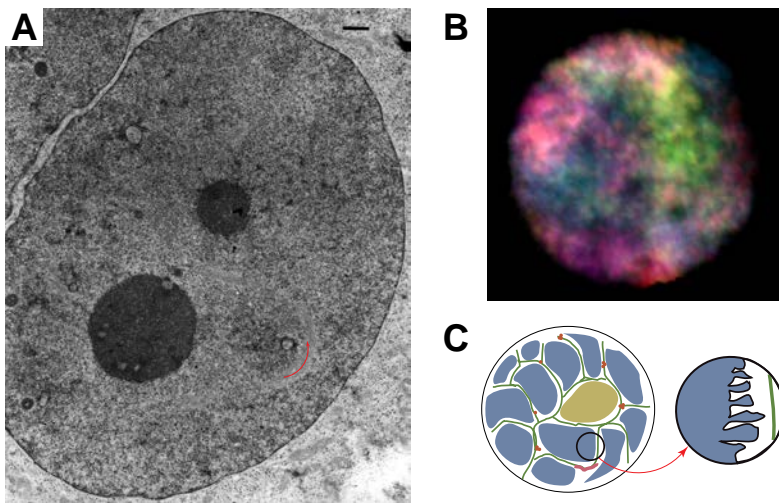
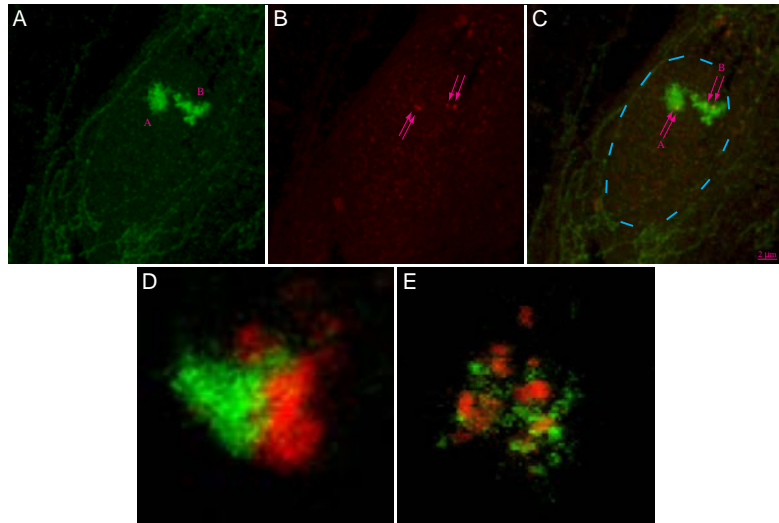
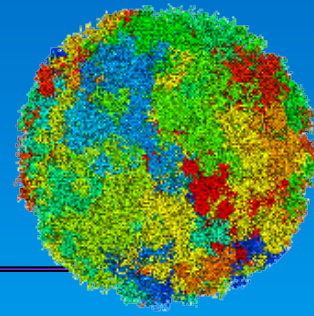
A, B: Pienta & Coffey; C: Alberts *et al.*; D: Belmont & Bruce



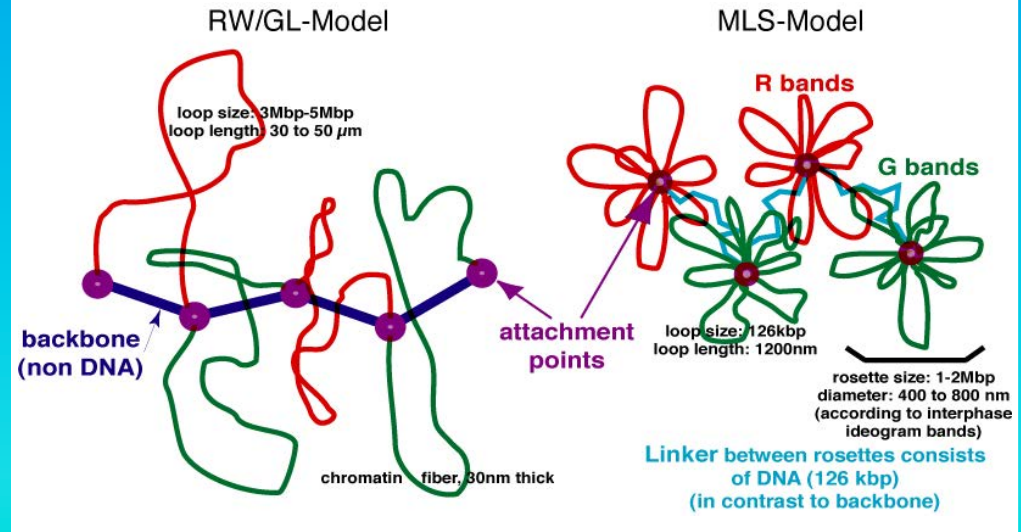


# Integral Models of Cell Nuclear Organization

The biggest advantage of integral models is the again obvious and simple fact, that they allow the validation from the consistency of different levels of organization from the other levels. Thus, e.g. the so called Interchromosomal Domain Model can be ruled out by simple voluminous thought...



## Random-Walk/Giant-Loop Multi-Loop-Subcompartment Model



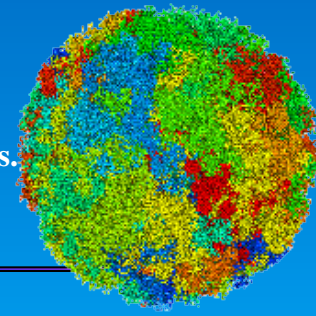
A: courtesy K. Richter; B: courtesy K. Greulich-Bode



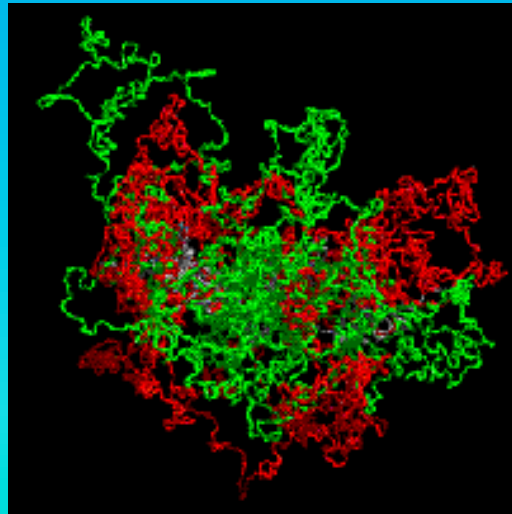
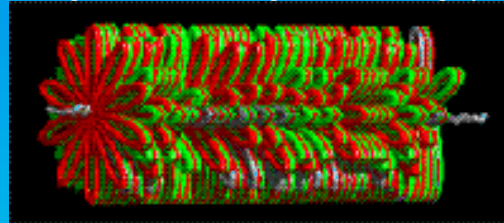
# Simulation of Single Chromosomes

The 30 nm chromatin fiber is modeled as a polymer chain with stretching, bending, and excluded volume interactions. Monte Carlo and Brownian Dynamic methods lead to thermodynamical equilibrium configurations.

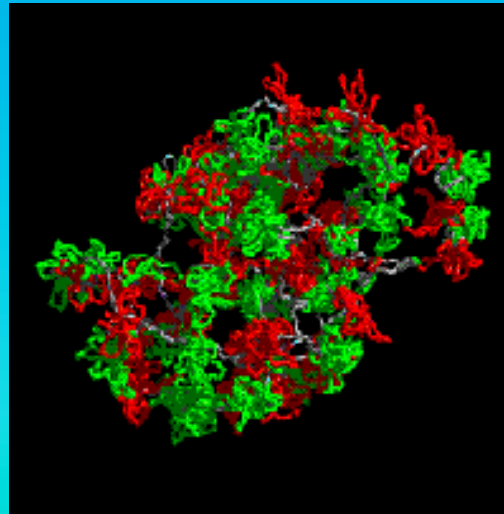
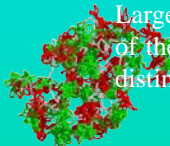
All models form chromosome territories with big voids and different chromatin morphologies. Experimental territory and subcompartment diameters agree best with an MLS model with 80 to 120 kbp loops and linkers.



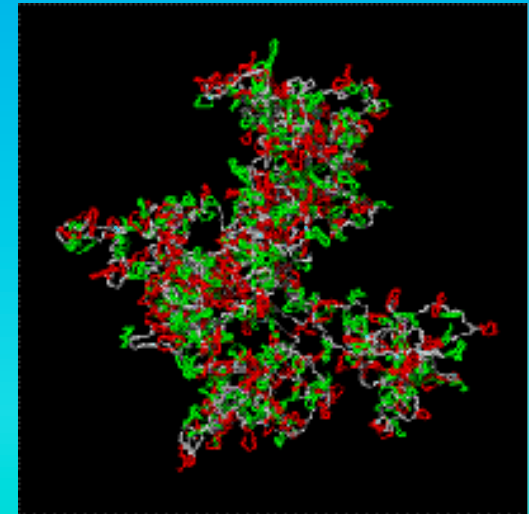
Metaphase starting configuration with ideogram bands in red/green, linker in grey.



RW/GL model, loop size 5 Mbp, after ~80.000 MC and 1000 relaxing BD steps. Large loops intermingle freely and reach out of the chromosome territory, thus forming no distinct features like in MLS model.



MLS model, loop size 126kbp, linker size 126 kbp, after ~50.000 MC and 1000 relaxing BD steps. Here rosettes form subcompartments as separated organizational and dynamic entities.



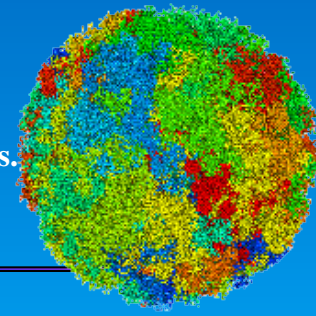
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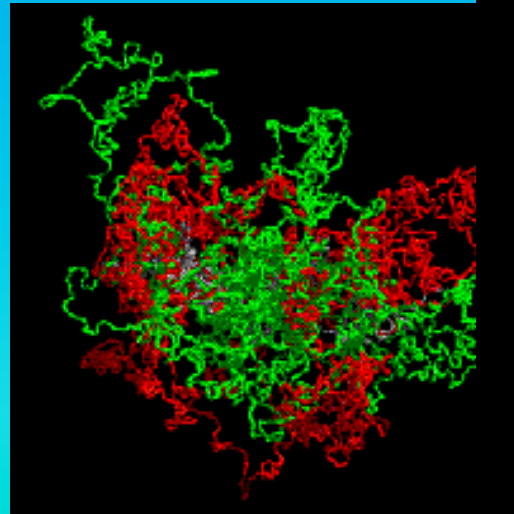
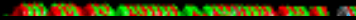
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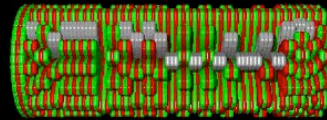
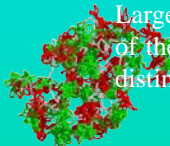
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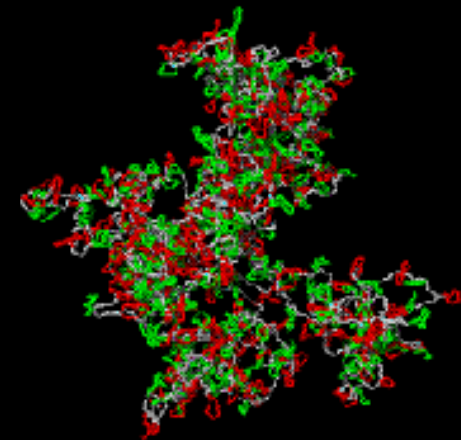
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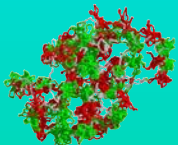
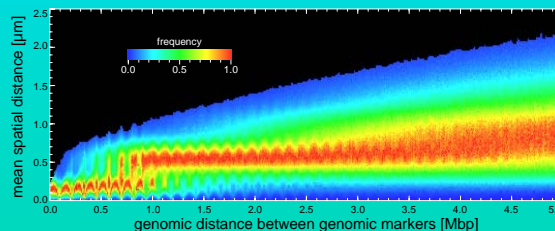
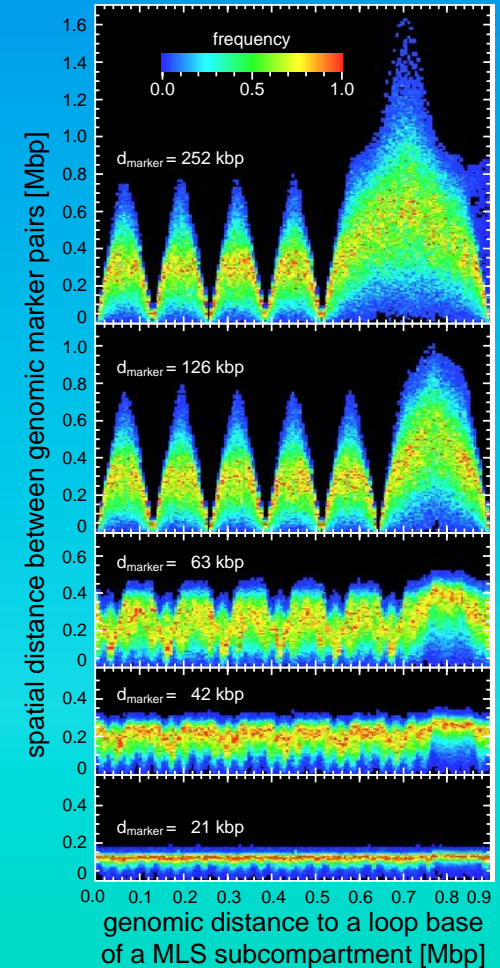
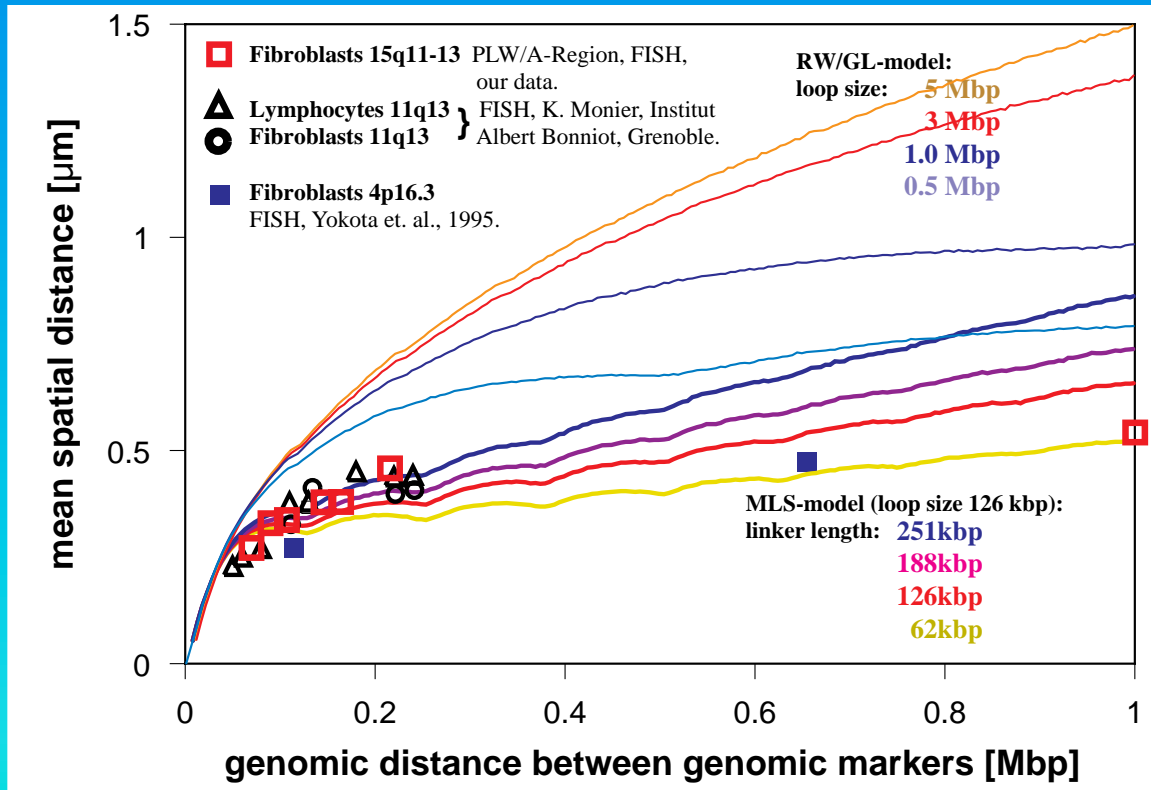
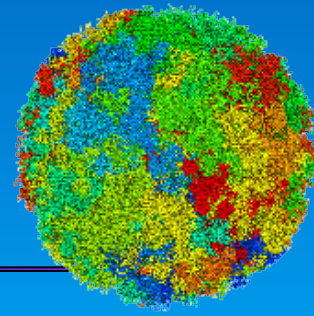
RW/GL model, loop size 126 kbp, after ~80.000 MC and 1000 relaxing BD steps. Large loops intermingle freely thus forming no distinct features like in MLS model.



# Spatial Distances between Genetic Markers

Simulated spatial distances between random genetic markers as function of their genetic separation leads to best agreement in a comparison to experiments for an MLS model with 80 to 120 kbp loops and linkers.

The spatial distance distributions are also model characteristic and show in a set of markers as function of their relative position to the chromatin fiber topology characteristic variation, strongly connected.

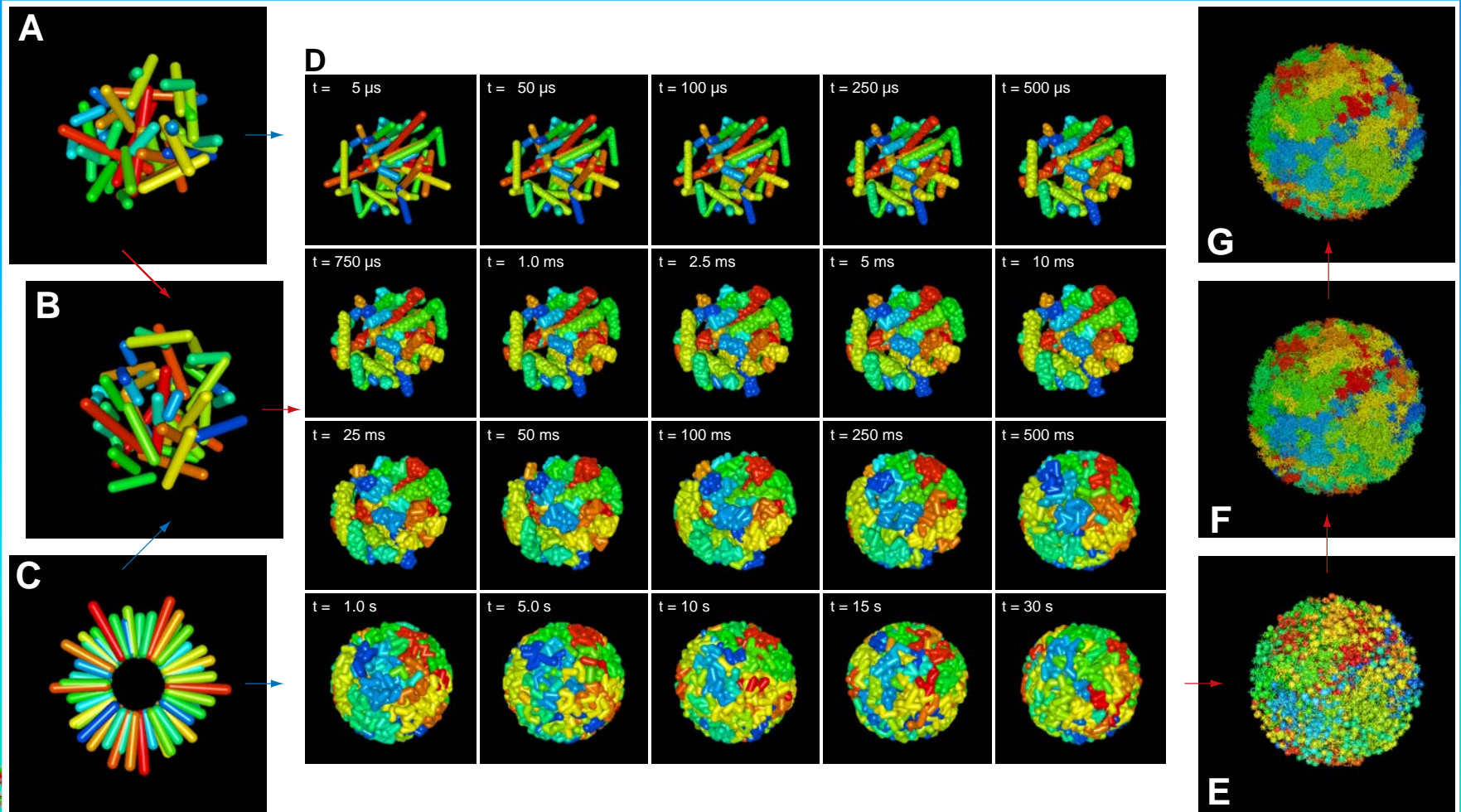
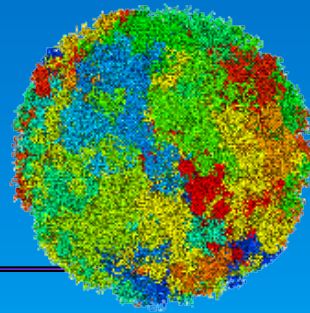




# Simulation of Whole Nuclei with all 46 Chromosomes

Starting with some metaphase arrangement of cylindrical chromosomes, interphase nuclei with a 30 nm fiber resolution and at thermodynamical equilibrium are created in 4 steps using simulated annealing and Brownian Dynamics methods with stretching, bending, excluded volume and a spherical boundary interactions.

The chromosome territory position depends on their metaphase position and is reasonably stable.

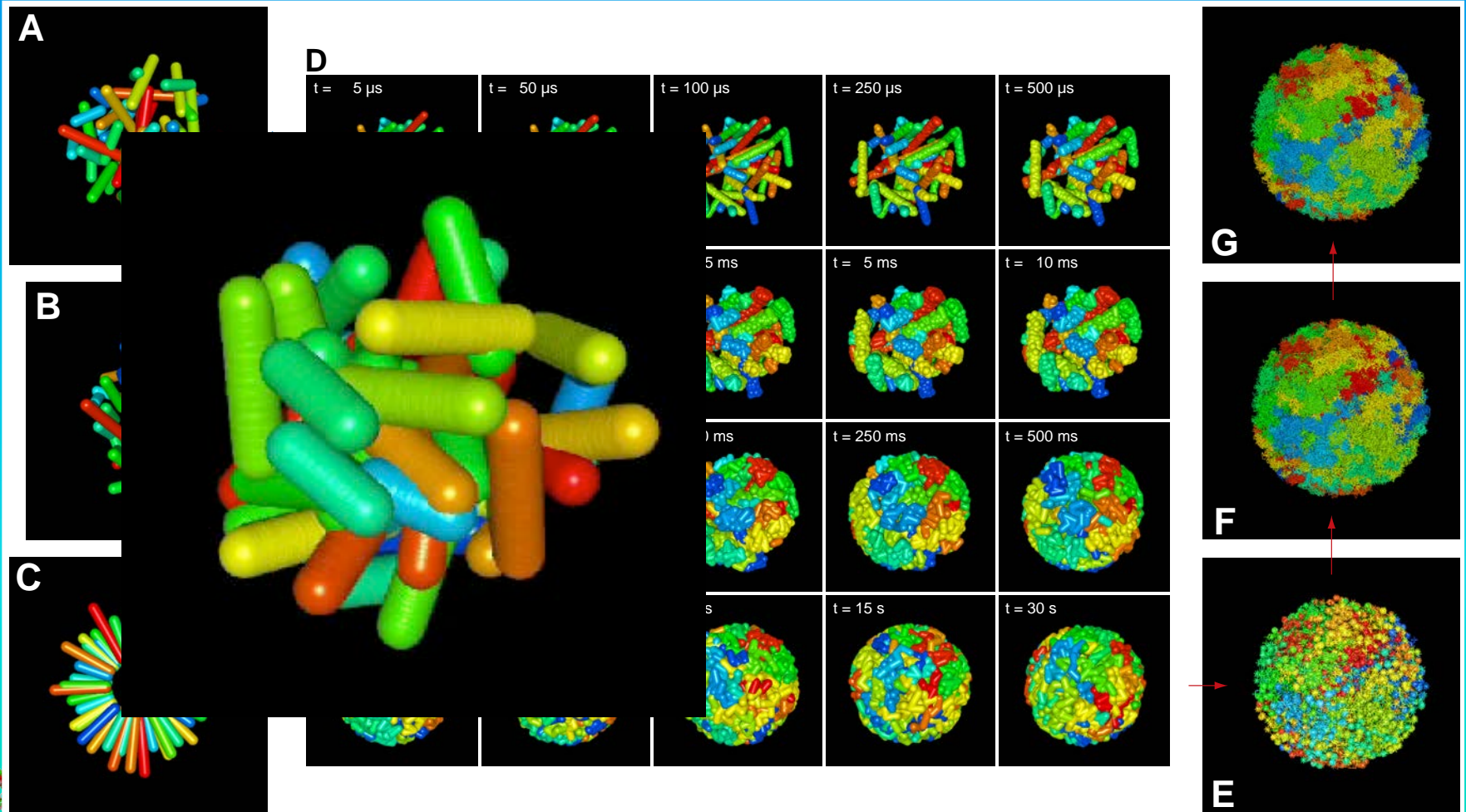
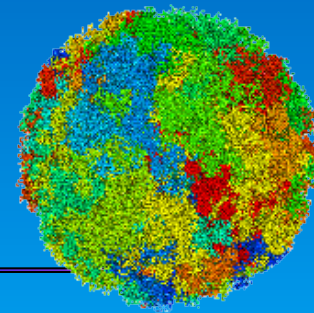




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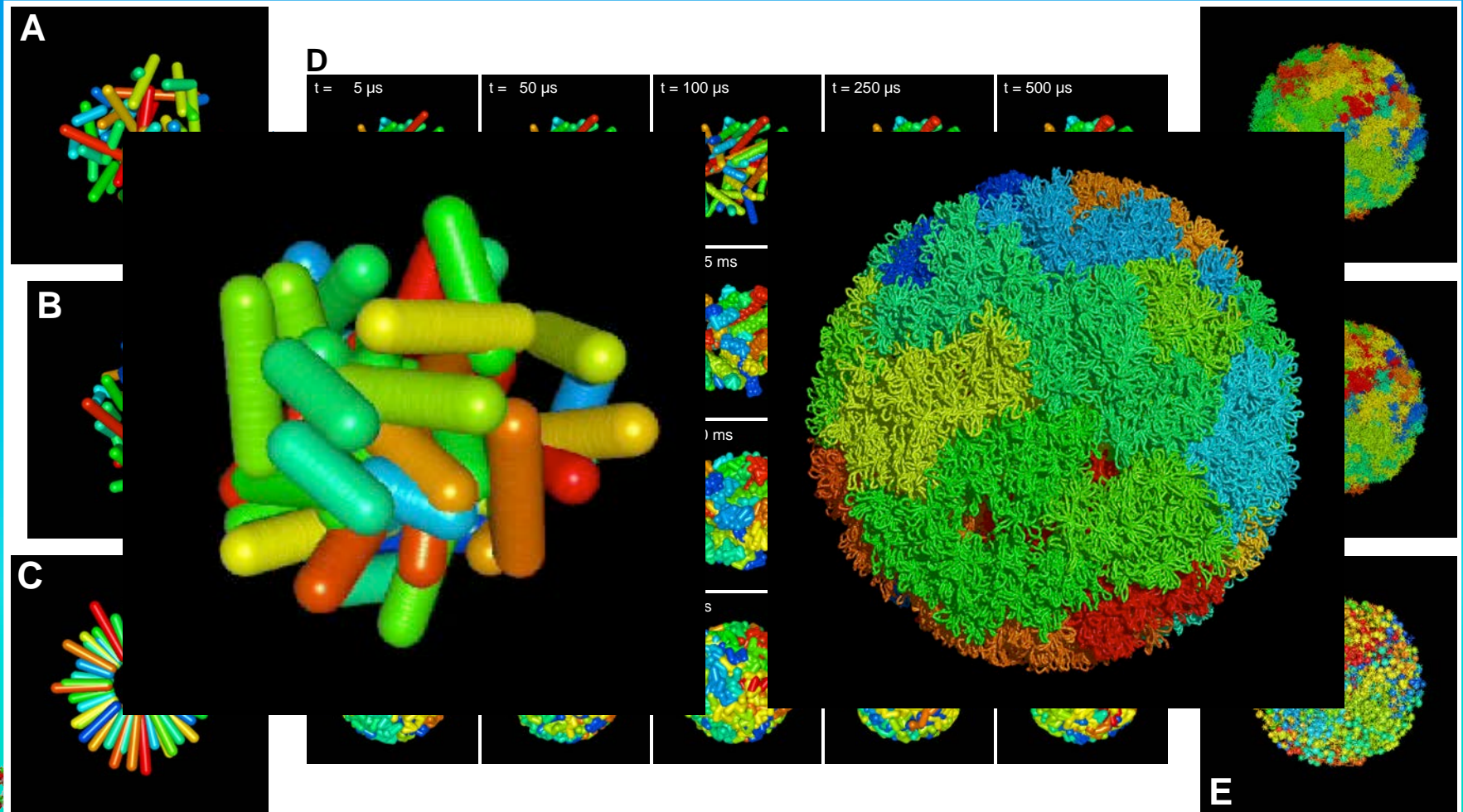
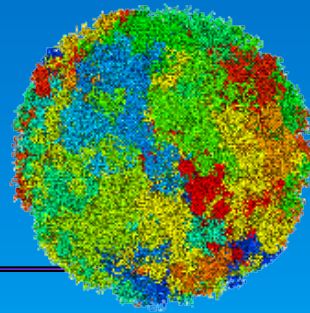




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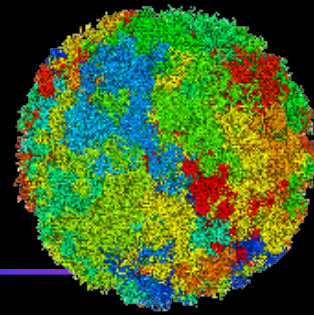




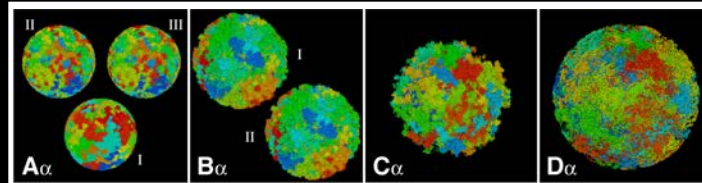
# From Fiber Topology to Nuclear Morphology

Chromosome territories form in the RW/GL and the MLS model. However, only the MLS model leads distinct subcompartments and low chromosome and subcompartment overlap. Best agreement is reached for an MLS model with 80 to 120 kbp loops and linkers in nuclei with 8 to 10  $\mu\text{m}$  diameter.

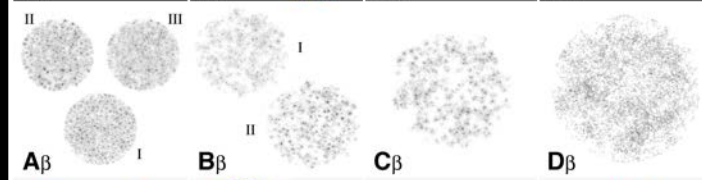
The simulated nuclear morphology reflects the chromosome fiber topology of different models in detail.



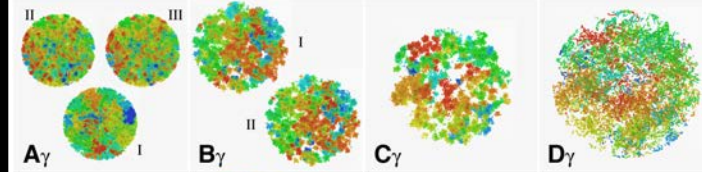
rendering



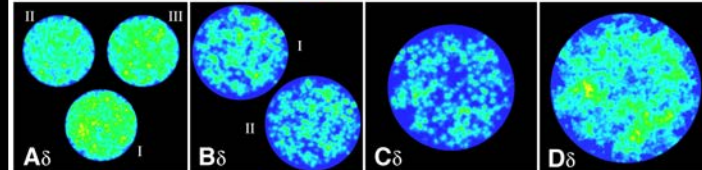
electron microscopy



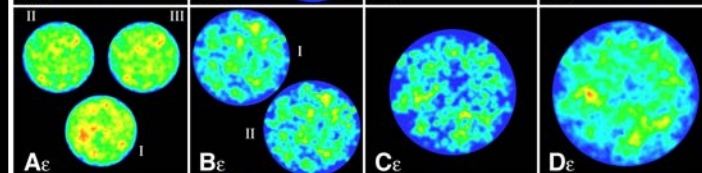
electron microscopy territory painting



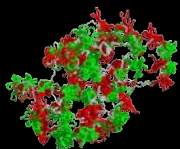
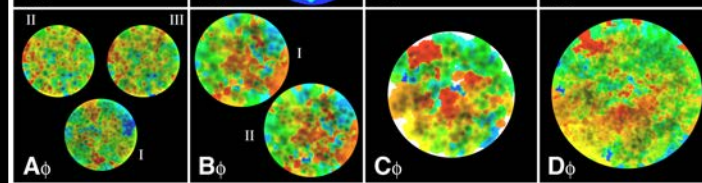
confocal microscopy  
100x objective, theoretic resolution



confocal microscopy  
63x objective, real resolution



confocal microscopy  
territory painting

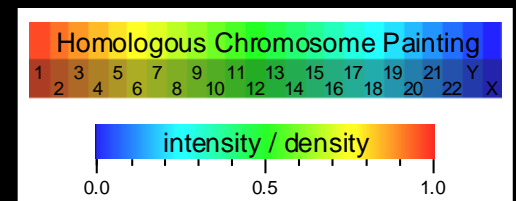


**A:** MLS in 6  $\mu\text{m}$  nucleus  
I: 63 kbp loops, 63 kbp linkers  
II: 63 kbp loops, 252 kbp linkers  
III: 126 kbp loops, 252 kbp linkers

**B:** MLS in 8  $\mu\text{m}$  nucleus  
I: 126 kbp loops, 126 kbp linkers  
II: 84 kbp loops, 126 kbp linkers

**C:** MLS in 10  $\mu\text{m}$  nucleus  
126 kbp loops, 126 kbp linker,  
not totally relaxed

**D:** RW/GL in 12  $\mu\text{m}$  nucleus  
5 Mbp loops  
not totally relaxed

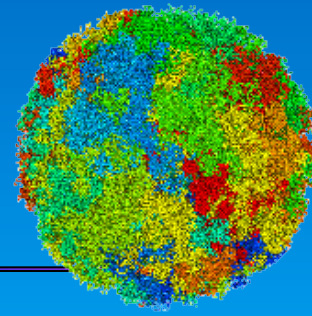




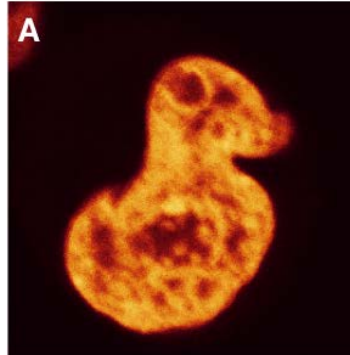
# *In vivo* Morphology & Chromatin Distribution

The stable expression of fusions between histones and autofluorescent proteins and the integration into nucleosomes allows the minimal invasive investigation of the structure and dynamics of chromatin.

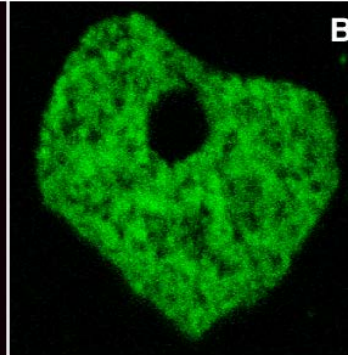
The clustered morphology in detail favour an MLS like chromatin topology.



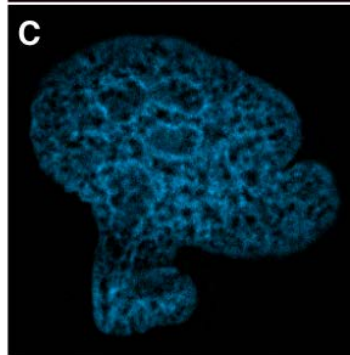
HeLa, H2A-YFP



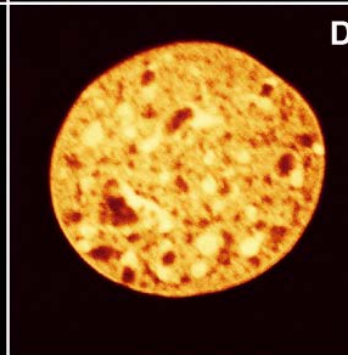
Cos7, H1.0-GFP



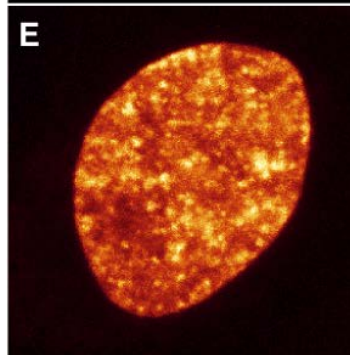
LCLC 103H, H2A-CFP



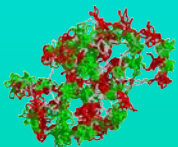
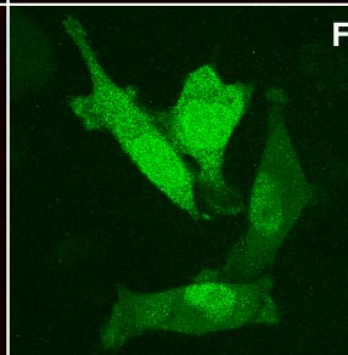
ID13, H2A-YFP



HeLa, mH2A1.2-YFP



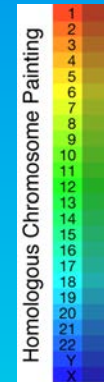
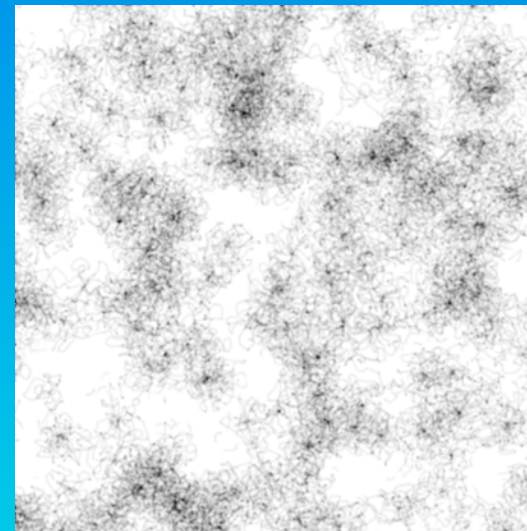
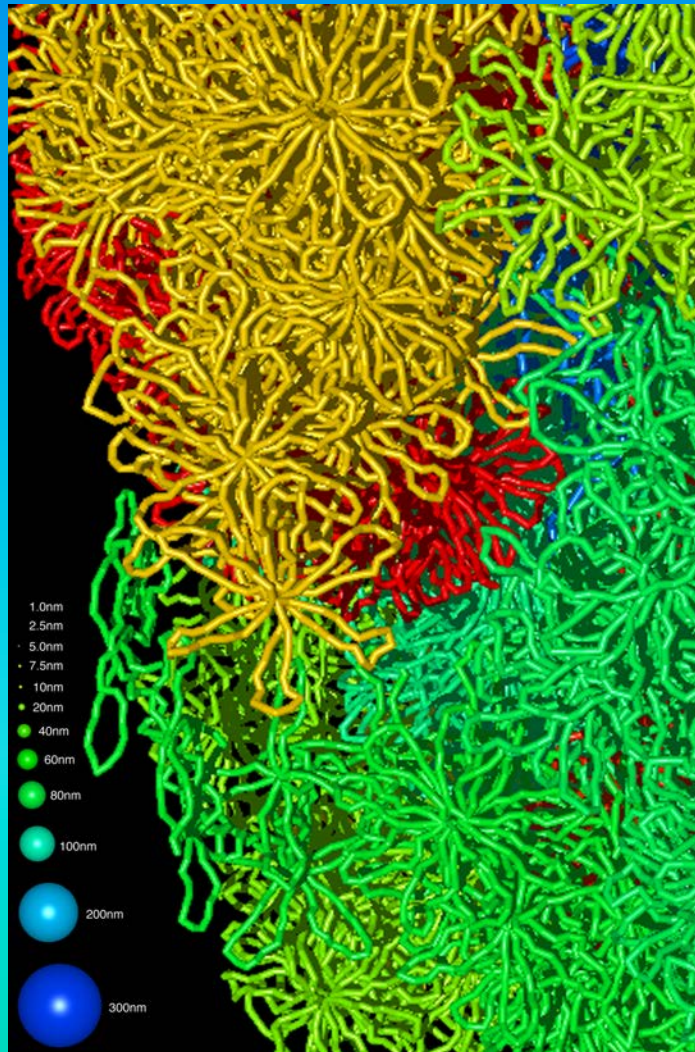
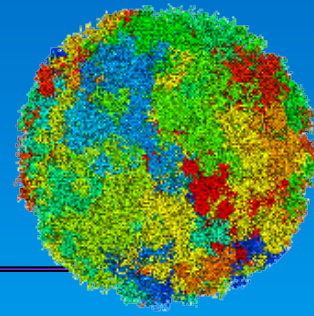
HeLa, H2A-YFP,  
natural promoter



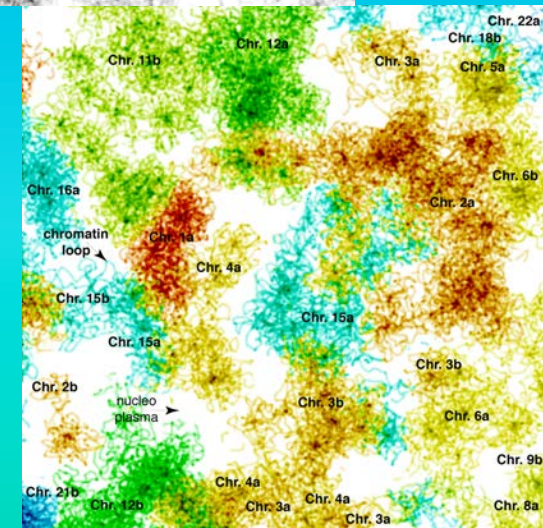


# Fine Morphology of Nuclei

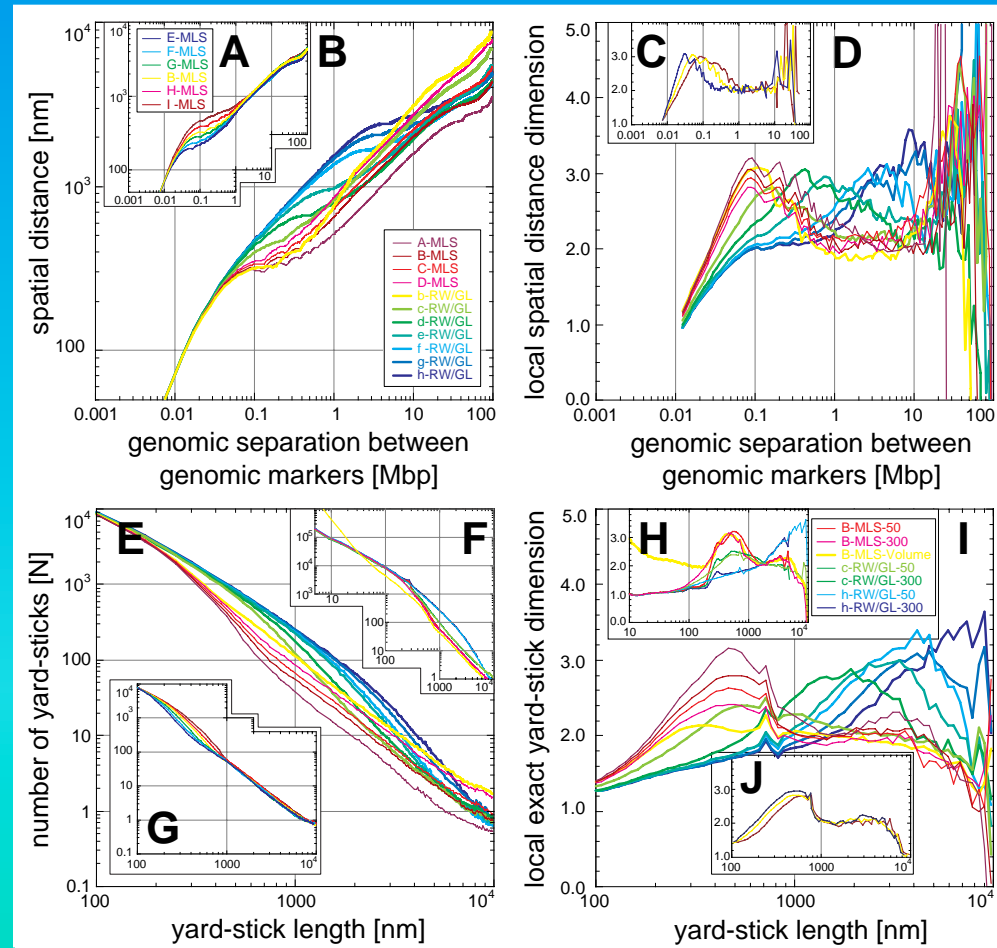

High resolution rendering and simulated electron microscopy including territory painting reveal not only again the model details but also that any location in the nucleus is accessible to biological molecules <15 nm in diameter and that even the Extended Interchromosomal Domain hypothesis is oversimplified.



MLS  
models  
model with  
126 kbp  
loops and  
linkers in a  
10  $\mu$ m  
nucleus.





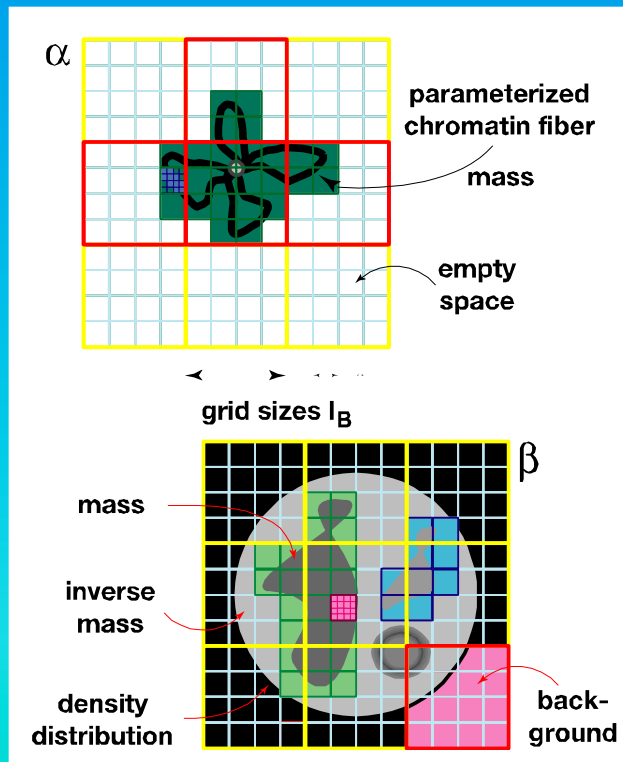
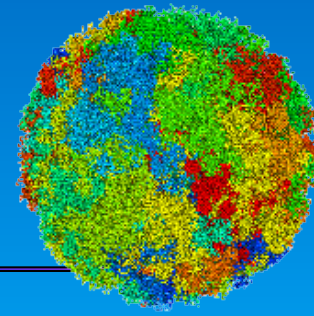




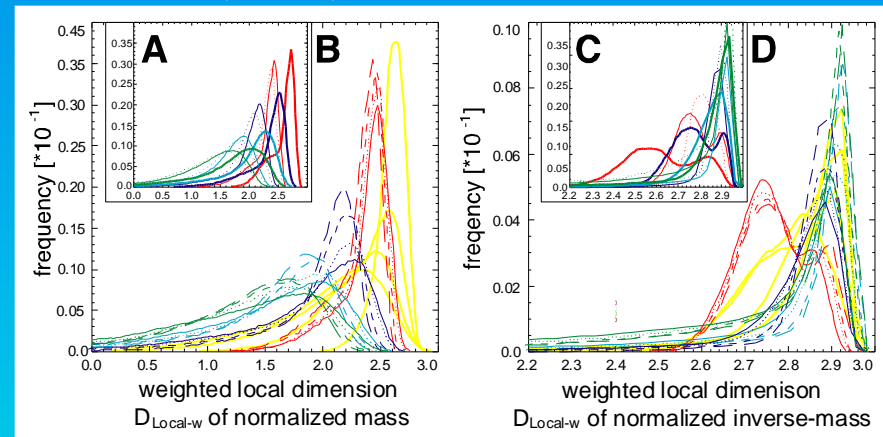
# Scaling of the Chromatin Morphology & Distribution

The local (inverse-) mass dimension distribution distinguishes between the models in detail and show also a multi-scaling behaviour with globular feature for the MLS model like the scaling of the fiber topology. With the mass dimension as function of intensity separates very well between different nuclei *in vivo*.

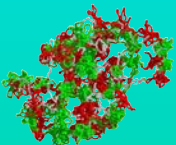
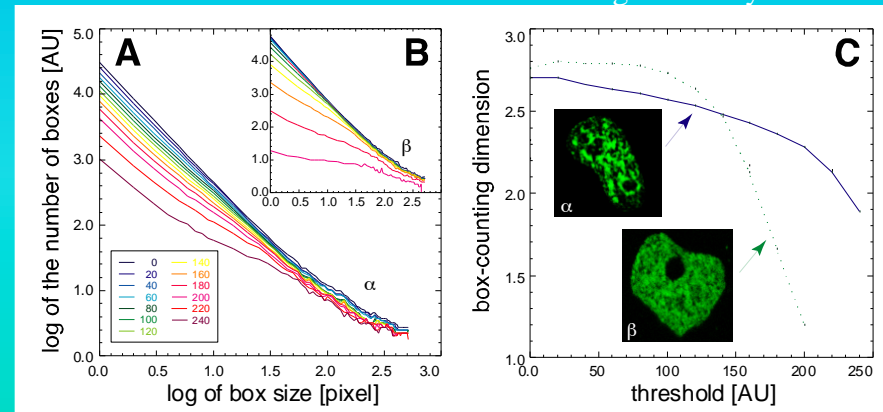
Consequently, the chromatin morphology is causally and quantitatively connected to the fiber topology.



(inverse-) mass dimension distribution



mass dimension as function of image intensity

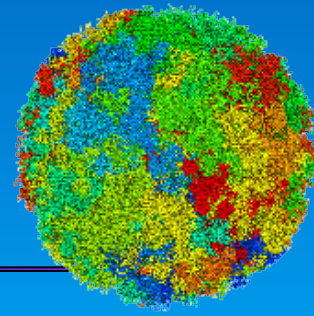




# Diffusion of Particles in the Nucleus

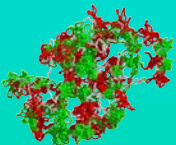
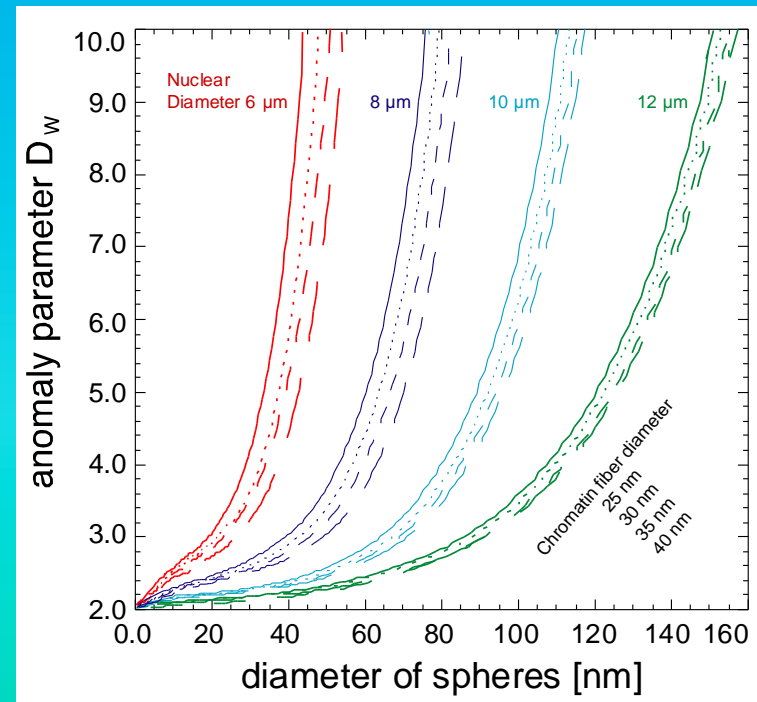
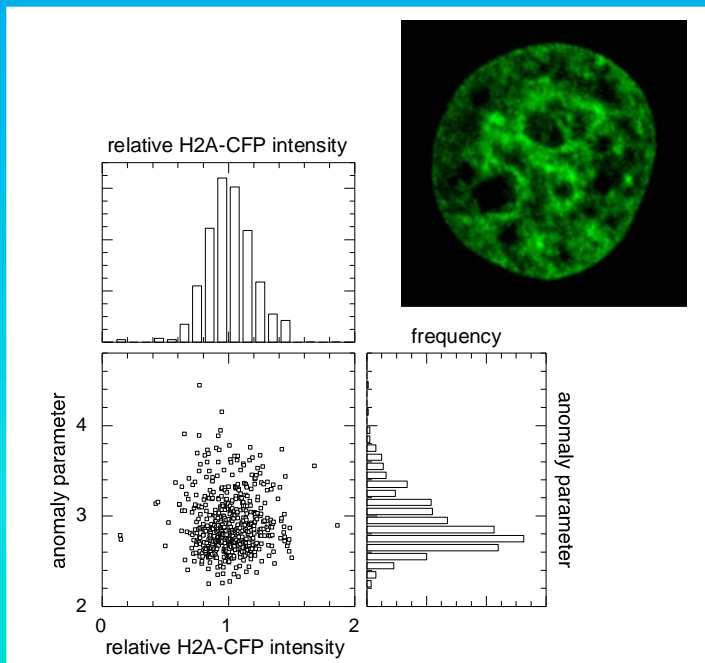
Due to the volume and spatial relationships in the nucleus typical particles reach almost any location in the nucleus by moderately obstructed diffusion: a 10 nm particle moves 1 to 2  $\mu\text{m}$  within 10 ms.

The structural influence on the obstruction degree is random for Alexa 568 as function of the chromatin distribution visualized by H2A CFP in vivo and measured by fluorescence correlation spectroscopy (FCS)



$$\langle r^2 \rangle \propto t^{2/D_w}$$

Nuclear diameter [ $\mu\text{m}$ ]	Nuclear Volume [ $\mu\text{m}^3$ ]	Mean Nucleosome Concentration [ $\mu\text{M}$ ]	Chromatin Volume Fraction [%]	Mean Isotropic Mesh Spacing [nm]
6	115	251	20.1	41
8	268	107	8.6	64
10	523	55	4.4	90
12	904	32	2.6	117





**On large scales this might only be due to a strong and definite three-dimensional genome organization.**

Chance to find a T again in a distance of 6 base pairs

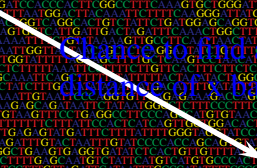


Chances to find a T again in a distance of 8 base pairs?



Numerically  
unstable

stable

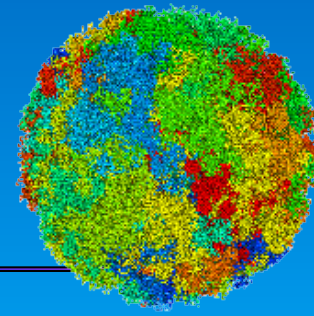

  
 chance to find a T again in a
   
 distance of 100 bases.



# Sequential Organization of Genomes

Determination of the concentration fluctuation function  $C(l)$  and its local slope the correlation coefficient  $\delta(l)$  reveal multi-scaling long-range correlation up to  $10^6$  to  $10^7$  bp in *Homo sapiens* which clearly deviate from random sequences with high significance (decreasing the nearer to the cut-off).

On large scales this might only be due to a strong and definite three-dimensional genome organization.



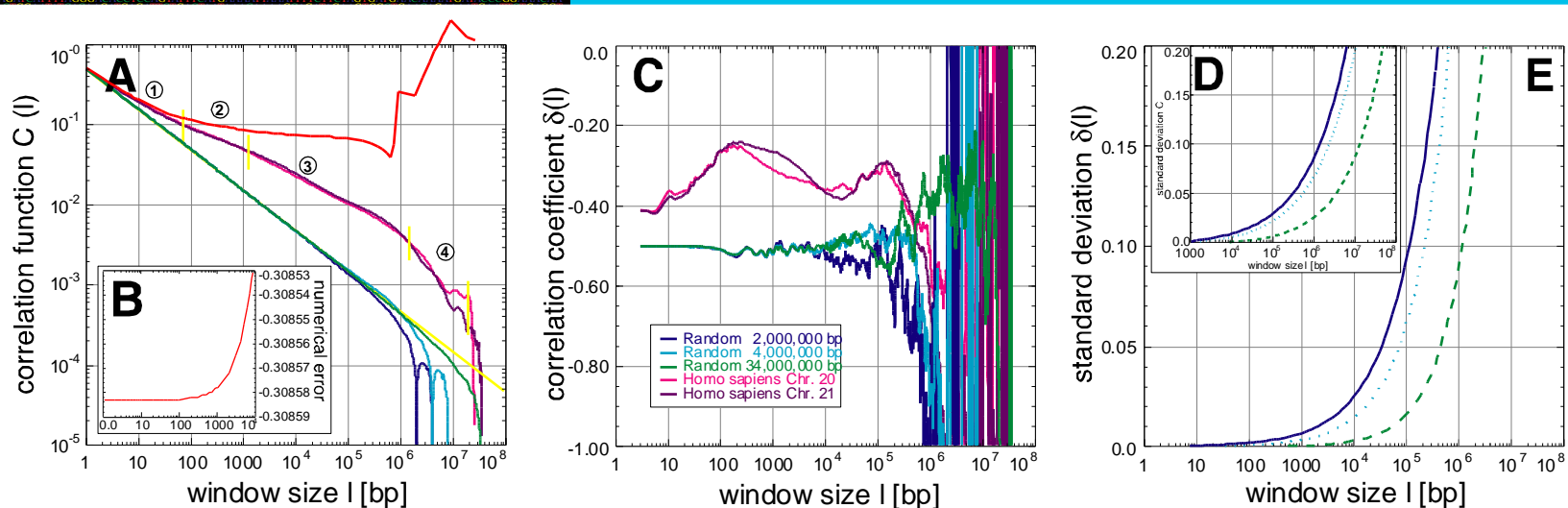
$$C(l) = \sqrt{\langle (c_l - \bar{c}_L)^2 \rangle_s}$$

Numerically unstable

$$C(l) = \sqrt{\frac{1}{L-l+1} \sum_{s=1}^{L-l} \left( \frac{1}{l} \sum_{k=1}^l n - \frac{1}{L} \sum_{k=1}^L N \right)^2}$$

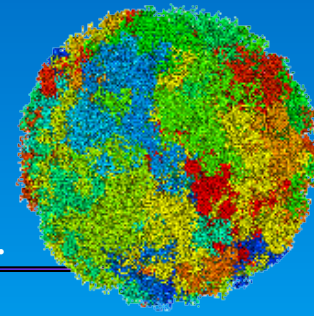
stable

FIGURE 1. The concentration fluctuation function  $C(l)$  and its local slope the correlation coefficient  $\delta(l)$  reveal multi-scaling long-range correlation up to  $10^6$  to  $10^7$  bp in *Homo sapiens* which clearly deviate from random sequences with high significance (decreasing the nearer to the cut-off). On large scales this might only be due to a strong and definite three-dimensional genome organization.





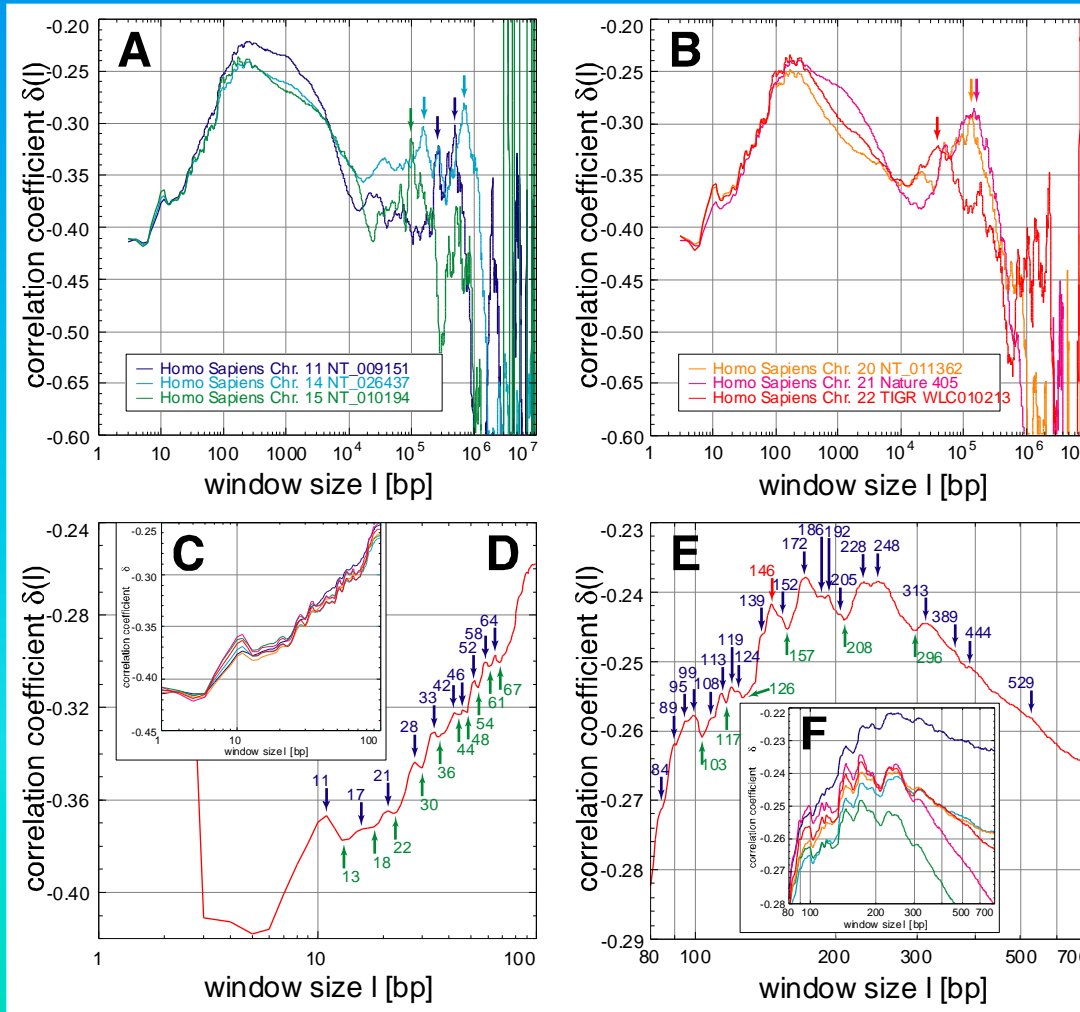
# Fine-Structured Multi-Scaling Long-Range Correlations of *Homo sapiens*



The general behaviour is characterized by first maximum of the correlation coefficient  $d(l)$  at  $\sim 250$  bp and at  $1 \times 10^5$  to  $3 \times 10^5$  bp, both due to a globular block structure of genomes. Due to their fine structure the first is attributable to nucleosomal binding and the latter due to aggregation of chromatin loops as in the MLS model.

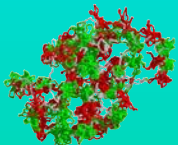
**Thus, the sequential organization is closely connected to the three-dimensional organization of genomes.**

general behaviour



fine structure

the fine structure survives averaging over several human chromosomes.

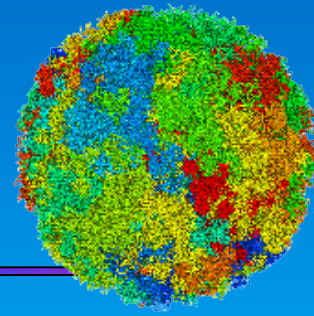




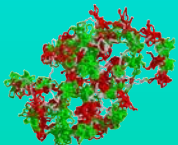
# Conclusion

Every structural level of nuclear organization including its dynamics is connected and represented in all the other levels.

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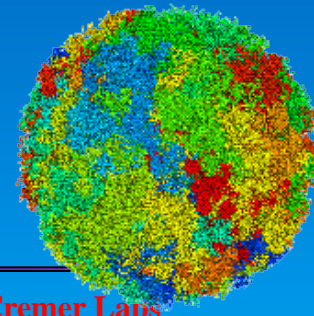


- Only the MLS model leads to chromosome territories with subcompartments agreeing qualitatively and quantitatively with experiments.
- Comparison between simulated and experimental spatial distances between genetic markers favours and MLS model with 80 to 120 kbp loops and linkers.
- The nuclear morphology or chromatin distribution is tightly connected to the folding topology of the chromatin fiber.
- Scaling analysis of the chromatin fiber topology and nuclear morphology reveals a fine-structured multi-scaling behaviour and allows a detailed description model changes.
- Most biological particles (molecules, proteins...) could reach almost any location in the nucleus by only moderately obstructed diffusion in agreement with *in vivo* experiments.
- The sequential organization of genomes is characterized by fine-structured multi-scaling long-range correlations, which are specie specific and tightly connected to the three-dimensional organization of genomes. On large-scales again an MLS model is favoured.





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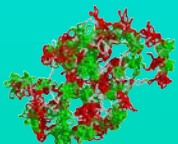
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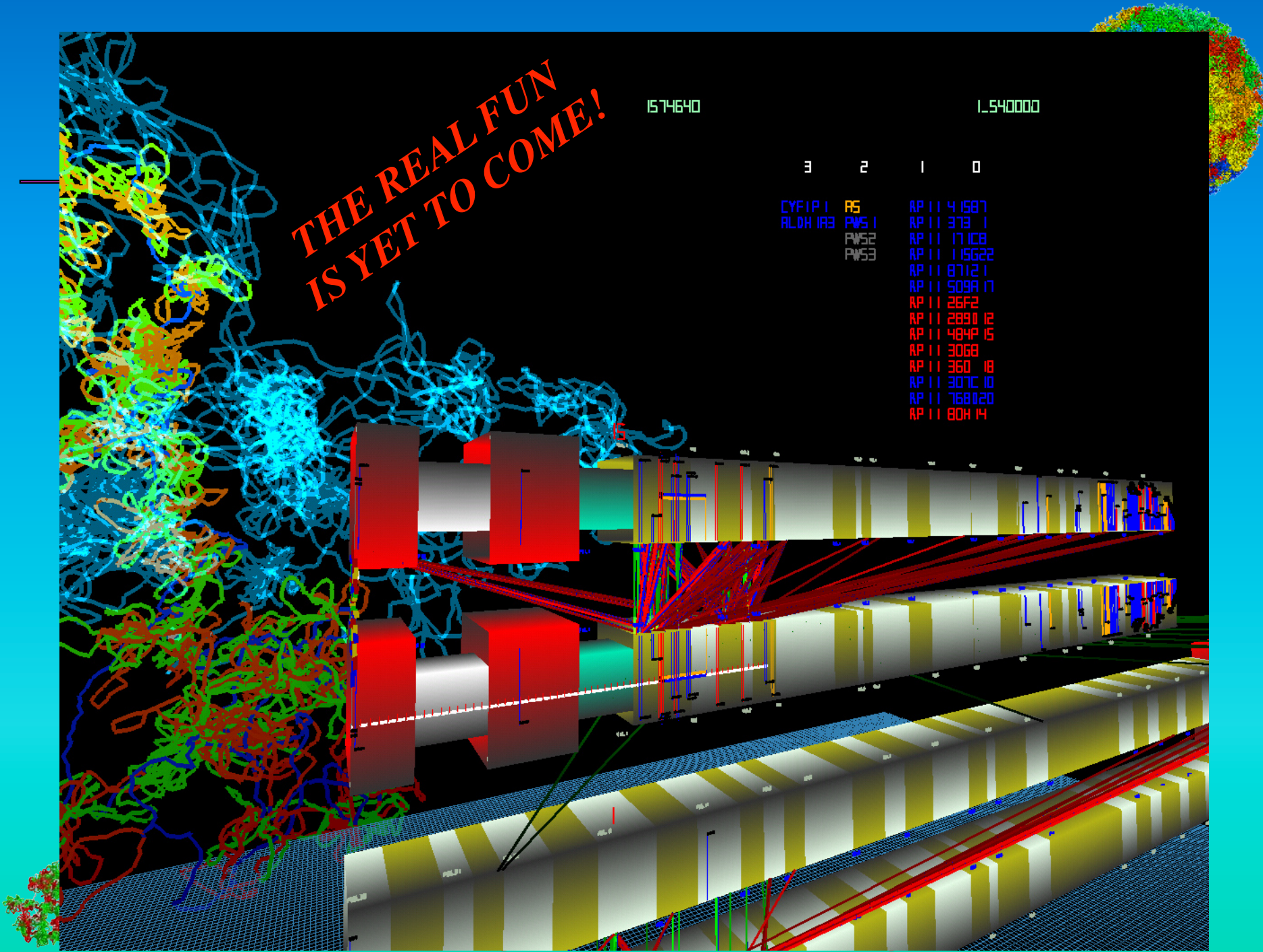


THE REAL FUN  
IS YET TO COME!

1574640

1.540000

	3	2	1	0
CYFIP1	AS	RP 11 41587		
ALDH1A3	PWS1	RP 11 373 1		
	PWS2	RP 11 171CB		
	PWS3	RP 11 115622		
		RP 11 8712 1		
		RP 11 509A 17		
		RP 11 26F2		
		RP 11 2890 12		
		RP 11 484P 15		
		RP 11 3068		
		RP 11 360 18		
		RP 11 307C 10		
		RP 11 768020		
		RP 11 80H 14		





# **From Sequence to Morphology**

-

## **Towards a Holistic Understanding of Genomes**

-

### **History and Perspectives**

**Knoch, T. A.**

*Riga Meeting on Comprehensive Cell Biology, Riga, Latvia, 2nd - 4th June, 2005.*

#### ***Abstract***

Genomes are one of the major foundations of life due to their role in information storage, process regulation and evolution. However, the sequential and three-dimensional structure of the human genome in the cell nucleus as well as its interplay with and embedding into the cell and organism only arise scarcely. Various models were put forward through history. To achieve a deeper understanding of the human genome the three-dimensional organization of the human cell nucleus, here, the structural-, scaling- and dynamic properties of interphase chromosomes are evaluated on all scales from a single base pair to the nuclear morphology level in respect to achieving a consistent holistic framework of genome organization.

Thus, by using Monte Carlo and Brownian Dynamics methods, the 30 nm chromatin fiber was simulated according to the Multi-Loop-Subcompartment (MLS) model, in which ~100 kbp loops form rosettes, connected by a linker, and the Random-Walk/Giant-Loop (RW/GL) topology, in which 1-5 Mbp loops are attached to a flexible backbone one finds that both the MLS and the RW/GL model form chromosome territories but only the MLS rosettes result in distinct subcompartments visible with light microscopy and low overlap of chromosomes, -arms and subcompartments. The MLS morphology, the size of subcompartments and chromatin density distribution of simulated confocal (CLSM) images agree with the expression of fusionproteins from the histones H1, H2A, H2B, H3, H4 and mH2A1.2 with the autofluorescent proteins CFP, GFP, YFP, DsRed-1 and DsRed-2 which also revealed different interphase morphologies for different cell lines. Even small changes of the model parameters induced significant rearrangements of the chromatin morphology. Thus, pathological diagnoses, are closely related to structural changes on the chromatin level. The position of interphase chromosomes depends on their metaphase location, and suggests a possible origin of current experimental findings. The scaling behaviour of the chromatin fiber topology and morphology of CLSM stacks revealed fine-structured multi-scaling behaviour in agreement with the model prediction and correlations in the DNA sequence. Review and comparison of experimental to simulated spatial distance measurements between genomic markers as function of their genomic separation also favour an MLS model with loop and linker sizes of 63 to 126 kbp. Simulated and experimental DNA fragment distribution after ion-irradiation revealed also best agreement with such an MLS. Correlation analyses of completely sequenced Archaea, Bacteria and Eukarya chromosomes revealed fine-structured positive long-range correlation due to codon, nucleosomal or block organization of the genomes, allowing classification as well as tree construction. This shows a complex sequential organization of genomes closely connected to their three-dimensional organization. Visual inspection of the morphology reveals also big spaces between the chromatin fiber allowing high accessibility to nearly every spatial location, due to the



chromatin occupancy <30% and a mean mesh spacing of 29 - 82 nm for nuclei of 6 - 12  $\mu\text{m}$  diameter. This agrees with a simulated displacement of 10 nm sized particles of  $\sim 1 - 2 \mu\text{m}$  takes place within 10 ms, i.e. a moderately obstructed diffusion of biological molecules in experimental agreement.

These results show that the local, global and dynamic characteristics of cell nuclei are not only tightly interconnected, but also are integrated holistically to fulfill the overall function of the genome. Future experiments and simulations benefit from taking this ever further completed consistent framework into account in respect to project planning as well as evaluation and hypothesis creation. Consequently, such a detailed understanding and knowledge of genomes will ultimately lead to full functional virtual genomes *in silicio* allowing predictions for diagnostics, disease treatment and the modification of genomes.

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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, auto-fluorescent proteins, CFP, GFP, YFP, DsRed, fusion protein, in vivo labelling, information browser, visual data base access, holistic viewing system, integrative data management, extreme visualization, three-dimensional virtual environment, virtual paper tool.

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