The Detailed 3D Multi-Loop Aggregate/Rosette Chromatin Architecture and Functional Dynamic Organization of the Human and Mouse Genomes

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Dynamic and Hierarchical Genome Organization

The different organization levels of genomes bridge several orders of magnitude concerning space and time. How all of these organization levels connect to processes like gene regulation, replication, embryogenesis, or cancer development is still unclear?
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 ~6 nucleosomes 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


Nor Cen

Arabidopsis thaliana Chr. 4

T19B17 (106 kbp) T27D20 (80 kbp)
Integral Models of Cell Nuclear Organization I

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.
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

- **RW/GL-Model**: loop size: 3Mbp-5Mbp, loop length: 30 to 50 μm
- **MLS-Model**: loop size: 126kbp, loop length: 1200nm

- **R bands**
- **G bands**

**Linker between rosettes consists of DNA (126 kbp)** (in contrast to backbone)

A: courtesy K. Richter; B: courtesy K. Greulich-Bode
3D Architecture of the Prader-Willi Region

Fluorescence *in situ* hybridization with various protocols of small probes within the Prader-Willi region combined with spectral precision distance confocal laser scanning microscopy and comparison with large-scale computer simulations shows a Multi-Loop Subcompartment organization of the Prader-Willi region.
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*Figures and tables depict genomic distance between genomic markers and normalized frequency, showing a cumulative distribution of the IgH locus subcompartment organization.*
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A history “synoptic” comparison of the spatial distance mapping from their original background and aim, FISH methodological protocols, via microscopic imaging and restoration analysis procedures, to their interpretation, reveals that with time Multi-Loop Subcompartment models are favoured.

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<tr>
<th>Study</th>
<th>Location</th>
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“Synoptic” 3D Architecture of Various Loci

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[Graphs and data points showing genomic distance and mean position independent spatial distance relationships]
DNA Fragment Distribution after Ione-Irradiation

The length distribution of DNA fragments after irradiation with e.g. C or Ca with an inhomogeneous spatial double strand breakage probability depends on the detailed folding topology of the chromatin fiber and the RW/GL and MLS models differ largely.
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T2C is a novel selective high-resolution high-throughput chromosome interaction capture, in which the relation between, region size, resolution, interaction frequency range, and sequencing depth can be designed towards the goal of the experiment. T2C reaches the limit of the “genomic” uncertainty principle and statistical mechanics.
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HS IGF locus

~2.1 Mbp
Stable Consensus Architecture of Genomes

Due to the high signal-to-noise ratio of T2C reaching 5-6 orders of magnitude interaction maps reveal definitely an extremely high degree of similarity between different species, cell types, or functional states, thus functional differences are variation of a stable theme persisting through the cell cycle.
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MM β-Globin 2.1 Mbp
Fine Structure of Loop Aggregates/Rosettes

Depending on the resolution, the loops within a domain and their arrangement in loop aggregates/rosettes can be shown as well as the details of how the loops are organized at their base as well as their aggregated rosette core: parallel loop fibres exist at the loop base with ~6kbp and these form the core.

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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.

- **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 126 kbp, 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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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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Simulated Interaction Maps

Simulated spatial distance maps as well as simulated interaction maps result in the representation of every parameter variation, and also exhibit the fine-structure describing the loop base as well as rosette core. Thus from the quasi-fibre to the entire chromosome the architecture can be understood in detail.
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Variation of a Consensus Architecture Scheme

The difference between different cell types, functional states or even species is minor despite depending on the region. From this, the chromatin fibre conformation, loop position, and their association into loop aggregates/rosettes can be derived, simulated by polymer models and finally visualized.
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DNA Sequence Organization

Determination of the concentration fluctuation function $C(l)$ and its local slope, the correlation coefficient $\delta(l)$, are an indication for the i) degree of long-range scaling behaviour, ii) general multiscaling, and iii) fine-structure features, which all are connected to all levels of genome organization and especially also the three-dimensional genome architecture.
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Scaling Analysis

Scaling analysis show again the entire bandwidth of architectural effects in an aggregated manner. Beyond, they show the scale bridging of the structures and the evolutionary holistic entanglement between the 3D architecture and the DNA sequence organization itself.
Also the DNA sequence forming pattern proposes a quasi-stable rosettes!
Simulation of Chromatin Quasi-Fibres

The position of nucleosomes influence greatly the structure of chromatin fibers done on super-computers. Here a dedicated workflow is applied, with which overlapping nucleosome populations can be analyzed and the best positioning of nucleosomes by Monte Carlo simulated annealing can be achieved. For an actual locus in a spherical confinement then a 3D independent nucleosome fiber conformation can be simulated.

Monte Carlo Simulation of SAMD4A Mouse ES Cells

- d=1000nm
- d=500nm
- d=200nm
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 µm diameter.

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

**A:** MLS in 6 µm 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 µm nucleus
- I: 126 kbp loops, 126 kbp linkers
- II: 84 kbp loops, 126 kbp linkers

**C:** MLS in 10 µm nucleus
- 126 kbp loops, 126 kbp linker, not totally relaxed

**D:** RW/GL in 12 µm 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.
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 µm nucleus.
Scaling of the Chromatin Fiber Topology

The spatial-distance and exact yard-stick dimension distinguish between the simulated models in detail. The MLS model shows a globular and fine-structured multi scaling behaviour due to the loops forming rosettes. This agrees with DNA fragmentation by Carbon ion irradiation and the appearance of fine-structured multi-scaling long-range correlations found in the sequential organization of genomes.
Scaling of the Chromatin Morphology & Distribution

The local (inverse-) mass dimension distribution distinguishes between the models in detail and shows 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.
Quantified TSA induced Morphology Changes

Trichostatin A induced histone acetylation can be quantified by *in vivo* H2A-GFP confocal images and image correlation spectroscopy (iFCS), which is a scaling analysis, and reveals the opening of chromatin, and thus reorganization changes on scales from 0.2 to ~1µm, consistent with MLS models.
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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 µm within 10 ms.

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

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

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<th>Nuclear diameter [µm]</th>
<th>Nuclear Volume [µm³]</th>
<th>Mean Nucleosome Concentration [µM]</th>
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<th>Mean Isotropic Mesh Spacing [nm]</th>
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In Vivo Nucleosome Concentrations and 3D architecture

Counting nucleosomes in living cells with a combination of fluorescence correlation spectroscopy (FCS) and confocal laser scanning microscopy (CLSM) reveals the association of nucleosomes and their kinetics as well as again the typical expected distribution of a multi-loop aggregate/rosette.
Fluorescence correlation spectroscopy (FCS) also reveals the dynamics of nucleosomes bound to DNA, i.e., FCS measures the movement of the chromatin quasi-fibre and its constraining architecture. This shows again a differentially compacted quasi-fibre folded into multi-loop aggregates/rosettes with functional differences as to e.g., hetero- and euchromatin or induced disturbances chromatin fiber (de-)condensation (+TSA, -ATP).
In Vivo Dynamics and 3D Architecture

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The dynamics can only be consistently explained by a quasi-fibre forming stable rosettes!
**Conclusion**

The compacted chromatin quasi-fibre, folds into stable loop-aggregates connected by a linker!

Every structural level of nuclear organization including its dynamics is connected and represented in all the other levels in a holistic systems genomics manner.

<table>
<thead>
<tr>
<th>Architectural Level</th>
<th>Observational Class</th>
<th>Spec. Feature</th>
<th>Simulation Single Chromosomes</th>
<th>Simulation Whole Nuclei</th>
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</table>
Evolutionary Architecture Perspective

Only a compacted chromatin quasi-fibre, folded into stable loop-aggregates connected by a linker allows to guaranty the functional informational requirements of genomes:

i) storage stability/flexibility, ii) readout, and iii) replication!

- **Storage stability/flexibility:**
  The packaging ratio/scale into a quasi-fibre and stable loops forming rosettes is optimal for the physical stability of genomes, while it is flexible enough to allow functional differences as well as react to entropic and other damages.

- **Readout:**
  The dynamics this architecture allows expression/regulation by self-organization into (in-)active units already in proximity, and guaranties at the same time accessibility to and from the information for factors as well transcripts.

- **Replication:**
  The 2D knot-free topology as well as the packaging ratio/scale into a quasi-fibre and stable loops forming rosettes, allows concatenation free replication with low error/damage rate due to the easy block-wise proximity organization as well as the easy physical (de-)condensation during cell division.

Form follows function and function follows form!
Evolutionary Architecture Perspective

Only a compacted chromatin quasi-fibre, folded into stable loop-aggregates connected by a linker allows to guaranty the functional informational requirements of genomes:

- i) Storage stability/flexibility:
The packaging ratio/scale into a quasi-fibre and stable loops forming rosettes is optimal for the physical stability of genomes, while it is flexible to entropic and other damages.

- ii) Readout:
The dynamics this architecture allows by self-organization into (in-)active units already in proximity, and the physical stability of genomes, while it is flexible to entropic and other damages.

- iii) Replication:
The 2D knot-free topology as well as the packaging ratio/scale into a quasi-fibre and stable loops forming rosettes allows concatenation free replication with low error/damage rate due to the easy physical (de-)condensation during cell division.

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Acknowledgements

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Thanks go also to all those people who supported this work in the last decades, the institutions providing their infrastructure, and the national and international computing infrastructures.
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Thanks go also to all those people who supported this work in the last decades, the institutions providing their infrastructure, and the national and international computing infrastructures.
The Detailed 3D Multi-Loop Aggregate/Rosette Chromatin Architecture and Functional Dynamic Organization of the Human and Mouse Genome - Results and Perspectives

Knoch, T. A.

The 22nd Wilhelm Bernhard Workshop, Schloss Wilhelminenberg, Vienna, Austria, 17th – 22nd August, 2015.

Abstract

The dynamic three-dimensional chromatin architecture of genomes and the obvious co-evolutionary connection to its function – the storage and expression of genetic information – is still debated after ~170 years. With a systems genomics approach combining a novel selective high-throughput chromosomal interaction capture (T2C) with quantitative polymer simulations and scaling analysis of architecture and DNA sequence, we determined and cross-proved the final architecture of genomes with unprecedented molecular resolution and dynamic range from single base pairs to entire chromosomes: for several genetic loci of different species, cell type, cell cycle, and functional states a chromatin quasi-fibre exists with 5±1 nucleosome per 11 nm, which folds into stable(!) 40-100 kbp loops forming stable(!) aggregates/rosettes which are connected by a ~50 kbp chromatin linker. Polymer simulations using Monte Carlo and Brownian dynamics approaches confirm this and predict and explain additional experimental findings. Beyond, a novel fluorescence correlation spectroscopy (FCS) approach combined with analytical polymer models measures the architectural dynamics in vivo, and agrees with the before mentioned conclusion using completely independent means. Beyond, we find a fine-structured multi-scaling behaviour of both the architecture and the DNA sequence, showing for the first time directly the tight entanglement between architecture and sequence. This agrees with the outcome of a synopsis e.g. with previous spatial distance measurement studies, in vivo morphology of entire cell nuclei, or electron microscopy of chromosome spreading studies, as well as the heuristics of the field in the last 170 years. This architecture has fundamental consequences for the entire system of the storage and expression of genetic information as well as for its investigation: E.g. this architecture, its dynamics, and accessibility balance stability and flexibility ensuring genome integrity and variation enabling gene expression/regulation by self-organization of (in)active units already in proximity. Thus, both the T2C and FCS approaches open the door to “architectural and dynamic sequencing” of genomes at a resolution where a genome mechanics with corresponding uncertainty principles applies. Consequently, this will lead now to a detailed understanding of genomes with fundamental new insights and huge novel perspectives for diagnosis, treatment and genome engineering efforts in the future.

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**Keywords:**

Genome, genomics, genome organization, genome architecture, structural sequencing, architectural sequencing, systems genomics, coevolution, holistic genetics, genome mechanics, genome statistical mechanics, genomic uncertainty principle, multilism genotype-phenotype, 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 quasi fibre, chromatin density, persistence length, spatial distance measurement, histones, H1.0, H2A, H2B, H3, H4, mH2A1.2, DNA sequence, complete sequenced genomes, molecular chromatin loops, chromatin morphology, chromosome territories, subchromosomal domains, chromatin loop aggregates, chromatin rosettes, interphase, cell nucleus, nuclear structure, nuclear organization, 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, polymer model, analytic mathematical model, Brownian Dynamics, Monte Carlo, fluorescence in situ hybridization (FISH), targeted chromatin capture (T2C) confocal laser scanning microscopy, fluorescence correlation spectroscopy, spatial precision distance microscopy, super-resolution microscopy, two dimensional fluorescence correlations spectroscopy (2D-FCS) 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, human ecology, e-human grid ecology, society, social systems, e-social challenge, inverse tragedy of the commons, grid phenomenon, micro-sociality, macro-sociality, autopoietic tragedy of social sub-systems, micro subsystems, macro subsystems, micro operationality, macro operationality, grid psychology micro riskmanagement, macro riskmanagement.

**Literature References**


