3D Architecture, Dynamics as well as Functional Implications of Genome Organization of the Prader-Willi/Angelmann Syndrome Region & the Immunoglobin Heavy-Chain Locus

Tobias A. Knoch [1, 2)]

F. G. Grosveld [3)] S. Jhunjhunwala, M.C. van Zelm, M. M. Peak, & C. Murre [4)] S. Cutchin [5)] R. Riblet [6)] J. Rauch, S. Stein, M. Hausmann, & C. Cremer [7)] I. Solovei, K. Teller, & T. Cremer [8)] K. Beuting & B. Horsthemke [9)] J. Langowski [10)]

1) Biophysical Genomics, 3)Dept. Cell Biology & Genetics, Erasmus Medical Center, Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands

2) Biophysical Genomics, 7) Kirchhoff Institute for Physics, Ruperto-Carola University of Heidelberg, Im Neuenheimfer Feld 280, 69120 Heidelberg, Germany

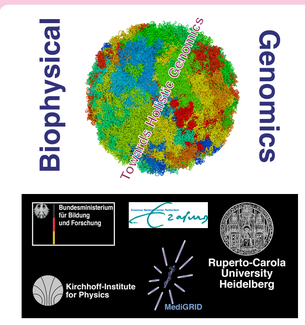
Email: TA.Knoch@taknoch.org

4) Div. Biological Sciences, UCSD, La Jolla, CA 92093, USA

5) SDSC, UCSD, La Jolla, CA 92093, USA 6)Torrey Pines Institute, San Diego, CA 92121, USA

8) LMU Munich Biocenter, 82152 Martinsried, Germany

9) University Hospital Essen, 45122 Essen, Germany 10) DKFZ, 69120 Heidelberg, Germany



PWS/AS - Region

The Prader-Willi/Angelmann Syndrome region (15q11-13; Fig. 1) is one of the most complex model regions for the interplay between sequence, imprinting and 3D structure. By 3D-FISH and a novel Spectral Precision Distance Microscopy approach (Fig. 2), combined with a comparison to computer simulations (Fig. 4), the spatial organization was approached resulting in trilaterated models of the locus (Fig. 5). The spatial arrangement agrees with a a chromatin organization consisting of small aggregate forming loops as proposed by the Multi-Loop Subcompartment model and not a Random-Walk/Giant-Loop model. This is in agreement with the fine-structured multi-scaling of the DNA sequence, the nuclear morphology in vivo, the dynamics of and within the architecture as well as the distruction pattern by ion-irradiation of the human genome.

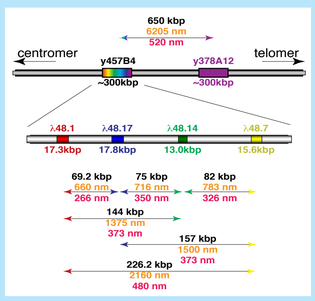


Fig. 1: Two BACs 650 kbp appart were used to determine the overall architecture of the PWS/AS region, and within the PWS region four YACs with six trilaterable distances were used to detect the fine structure of the PWS locus and to test whether there is a difference between the (in-)active alleles. The difference between a random chromatin fiber and the measured spatial distances is obvious.

Fig. 2A & 2B: FISH of chromosome territories 15 (A) and YAC 48 and YAC 60 in human fibroblasts (B), show the location of the probes of the PWS locus within the territories. The territories show also the morphological specle-like clustering as predicted by a linked clustered small loop architecture of chromosomes as predicted by the MLS model.

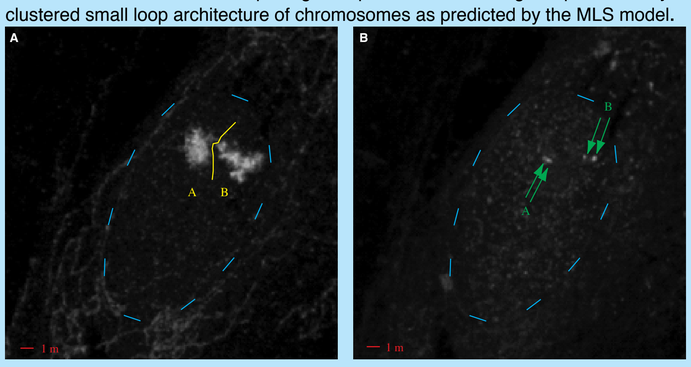


Fig. 3A - D: The distance distributions show that the better the preparation, the smaller the distances using different fixations and mono and dual colour detection for BACs (A, B) or YACs (C, D) including the true dynamics and functional differences not being resolution compromised. (Legends: Fig. 4A; Lines: resolution equivalent α, lateral β and axial γ PSF_{FWHM}.)

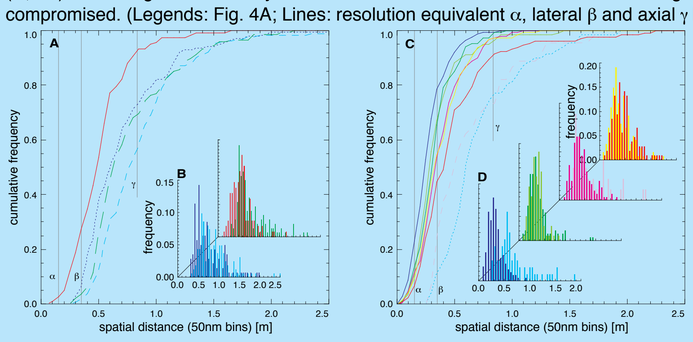


Fig. 4A & 4B: Comparison of the average spatial distances to computer simulations (Fig. 6A - D) agrees best with an MLS model of ~ 60 to 150 kbp loop aggregates separated by also ~ 60 to 126 kbp linkers. The distances do not agree with RWGL models and preparation artefacts get even more clear leading to misleading interpretations.

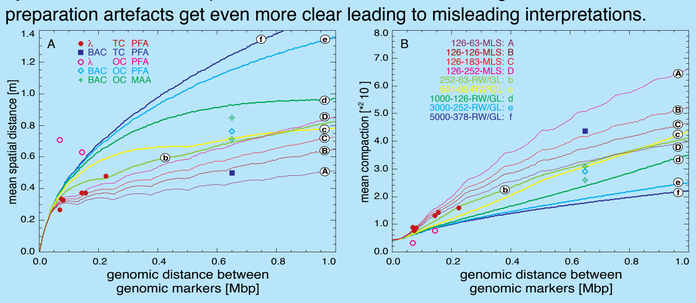
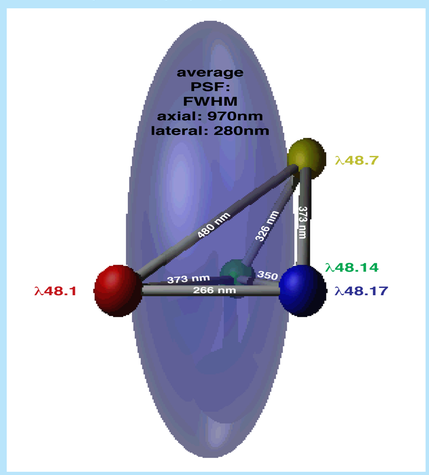
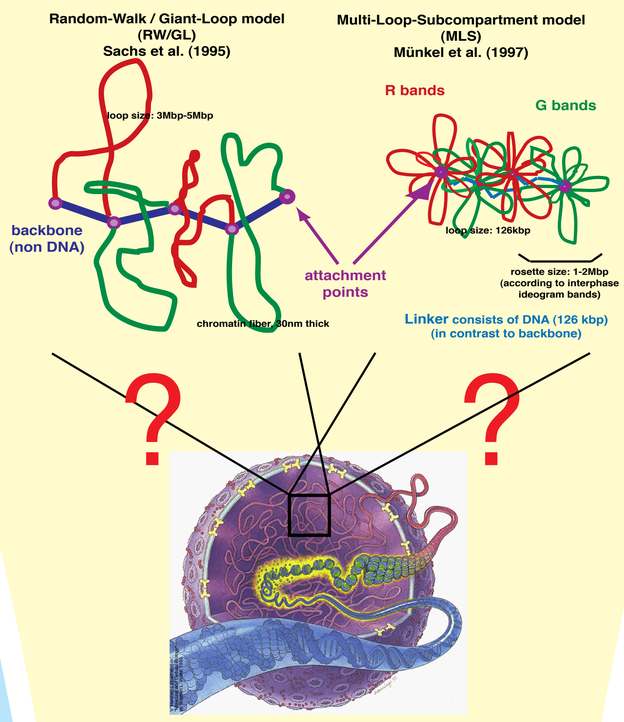


Fig. 7: Trilateration of the spatial distances between the four YAC labels result for the first time in the general determination of the architecture of the PWS locus and shows its compactness and dynamics considering distance distributions being much bigger than the resolution equivalent (Fig. 3C & 3D). Consequently, the 3D organization and dynamics play a pivotal role in function.



Introduction

Despite the succesful linear sequencing of the human genome, the three-dimensional chromatin architecture, its dynamics and relation to genome function are still largely unknown despite their obvious importance. Through a combination between novel experiments and theoretic analysis we show here an interdisciplinary approach leading to the determination of the three- dimensional organization of the Prader-Willi/Angelmann syndrome region and the Immuno Heavy-Chain locus with huge implications for general genome organization and function .

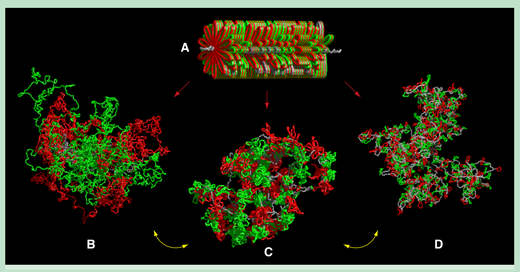


Conclusion

The general 3D architecture and dynamics of both the Immuno-globin Heavy-Chain locus and the Prader-Willi/Angelmann syndrome region was determined by a novel interdisciplinary combination of high-resolution FISH and Spectral Precision Distance Microscopy with analytical analysis and computer simulations resulting for the first time in trilaterated spatial models of both genetic areas. Not only is the 3D architecture strongly related to the dynamics of the chromatin fiber and its higher-order organization, but also to its function. In both cases the spatial arrangement agrees with the Multi-Loop Subcompartment model proposing small aggregate forming loops and not a Random-Walk/Giant-Loop model. This agrees with the fine-structured multi-scaling of the DNA sequence, the nuclear morphology in vivo, the dynamics of and the diffusion within the human genome. Con-sequently, this is a framework to understand genomes in a system-biological manner.

Simulation

For the prediction of experiments we simulated various models of human interphase chromosome 15 with Monte Carlo and Brownian Dynamics methods. The chromatin fiber was modelled as a flexible polymer. Only stretching, bending and excluded volume interactions are considered. Chromosomes are further confined by a spherical potential representing the surrounding chromosomes or the nuclear membrane. Only the rosette-like MLS model leads to clearly distinct functional and dynamic subcompartments (Fig. 6B) in experimental agreement, in contrast to the RW/GL models where big loops are intermingling freely and featureless (Fig. 6C & 6D).



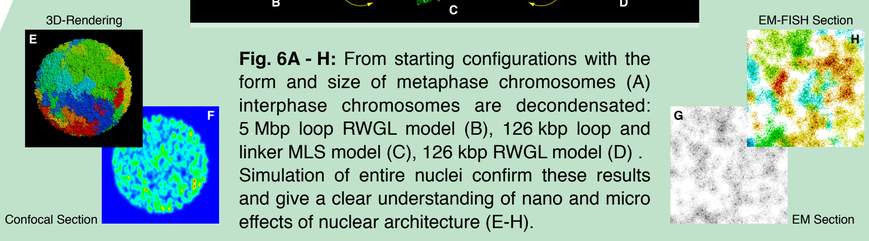


Fig. 6A - H: From starting configurations with the form and size of metaphase chromosomes (A) interphase chromosomes are decondensated: 5 Mbp loop RWGL model (B), 126 kbp loop and linker MLS model (C), 126 kbp RWGL model (D) . Simulation of entire nuclei confirm these results and give a clear understanding of nano and micro effects of nuclear architecture (E-H).

Igh - Locus

The Immunoglobin Heavy-Chain locus (Fig. 7) is organized in distinct regions: variable (V_H), diversity (D_H), joining (J_H) and constant (C_H) elements form a complex dynamic interplay between sequence and 3D structure. By 3D-FISH and a novel epifluorescent Spectral Precision Distance Microscopy approach, combined with a comparison to computer simulations (Fig. 9), the spatial organization was approached in diffrent functional states resulting in trilaterated models of the locus (Fig. 10). The 3D architecture agrees with small aggregate forming loops alike the Multi-Loop Subcompartment model and not a Random-Walk/Giant-Loop model. This agrees with the fine-structured multi-scaling of the DNA sequence, the nuclear morphology in vivo, the dynamics as well as the distruction pattern by ion-irradiation of the human genome.

Fig. 7A & 7B: The Igh locus was covered with 12 selected markers covering ~2.5 Mbp using two anchor-points (A & B) to perform dual and triple colour labelling to determine the 3D architecture. Thus, in respect to the distinct Igh subregions dynamic and functional aspects on local and global scales can be investigated.

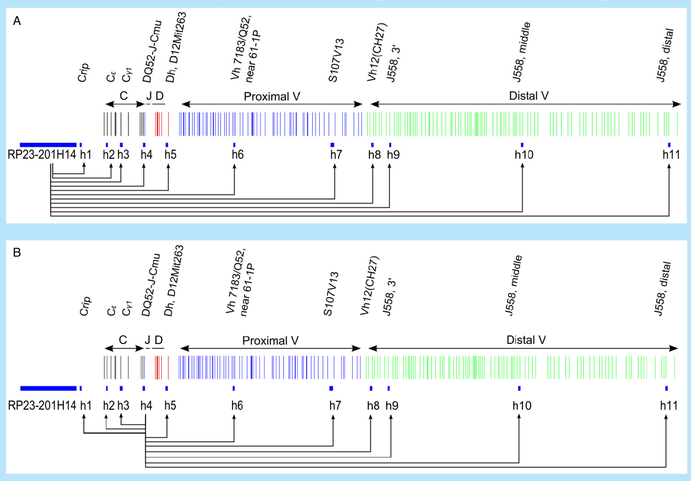


Fig. 8A & 8B: The spatial distance distributions for prepro-B and pro-B cell lines show the dynamics of the Igh locus as well as the functional rearrangement due to activation since the distribution width is bigger than the resolution limit. Notably, the general 3D architecture remains very similar despite larg local rearrangements suggesting a general organization.

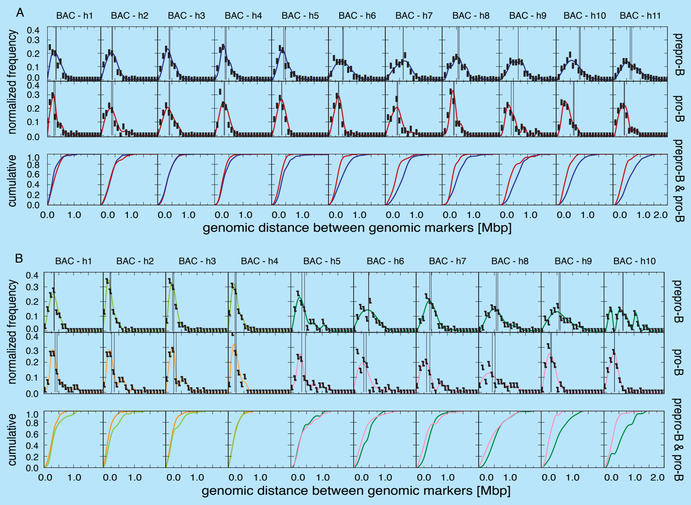


Fig. 9A & 9B: Comparison of the average spatial distance to computer simulations (Fig. 6A - D) agree again best with an MLS model of 60 to 150 kbp loops arranged in clusters and separated by 40 to 120 kbp linkers. The functional difference between prepro-B and pro-B cells results in local rearrangements of the 3D architecture of the Igh locus.

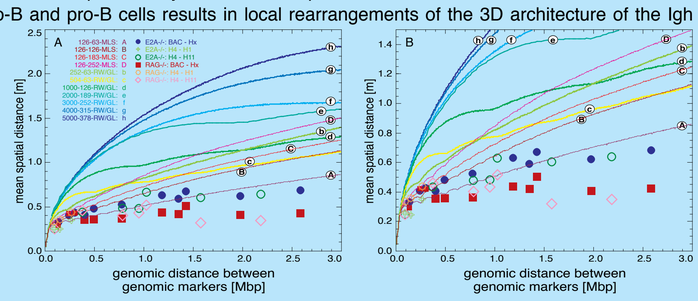
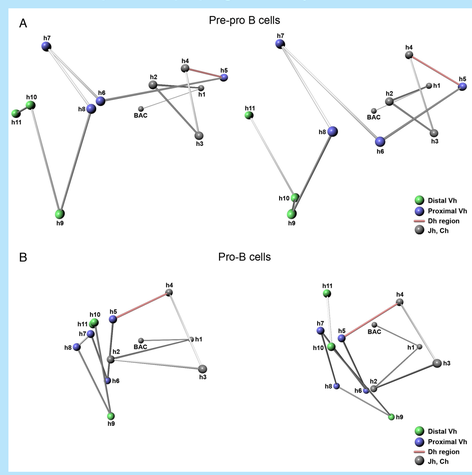


Fig. 10A & 10B: Trilateration of the spatial distances for the prepro-B and the pro-B lineages show for the first time the 3D architecture and the functional rearrangement of the Igh locus. Two clearly distinct subcompartments are visible and show also functional rearrangement (possibly compaction) during activation. Thus, the 3D organization and dynamics play again a major role in function.



3D Architecture, Dynamics as well as Functional Implications of Genome Organization of the Prader-Willi/Angelmann Cyndrome Region & the Immunoglobin Heavy-Chain Locus

Knoch, T. A.

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Abstract

The general 3D architecture of the immunoglobin heavy-chain (Igh) locus was determined by a novel interdisciplinary combination of high-resolution FISH and high-resolution epifluorescence spectral distance microscopy with analytical analysis, computer simulations, as well as trilateration (*Cell 133, 265-279, 2008*). The Igh locus is organized into distinct regions that contain multiple variable (V_H), diversity (D_H), joining (J_H) and constant (C_H) coding elements. Determination of distance distributions between genomic markers across the entire locus showed that the Igh locus is organized into compartments consisting of small loops separated by linkers with in detail dynamic functional relevance: V_H, D_H, J_H, and C_H elements showed striking conformational changes involving V_H and D_H-J_H elements during early B cell development, culminating in a merger and juxtaposition of the entire repertoire of V_H regions to the D_H elements in pro-B cells allowing long-range genomic interactions with relatively high frequency. This is in agreement with our recent study of the Prader-Willi/Angelmann region using a similar approach (*Differentiation 76, 66-82, 2008*) and in agreement with the Multi-Loop-Subcompartment (MLS) model of chromosome organization predicting 60-150 kbp loop aggregates separated by a similar linker (Knoch, ISBN 3-00-009959-X, 2002). Synopsis with previous spatial distance measurement studies and combination with sequence correlation analysis of the DNA sequence, fine-structure multi-scaling analysis of the chromatin fiber topology or *in vivo* morphology of entire cell nuclei, electron microscopy of chromosome spreading studies and even the diffusion behaviour within the cell nucleus, are all suggesting such an MLS architecture. This framework reveals a consistent picture of genome organization joining structural and dynamical aspects ranging from the DNA sequence to the entire nuclear morphology level with functional aspects of gene location and regulation. Many previously contradictory viewpoints are resolved by this framework as well. Consequently, the determination of the general 3D architecture of the Igh locus has beyond its major functional relevance, huge implications for the understanding of the entire genome understanding in a holistic system-biological manner.

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

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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