Chromatin Dynamics

of the mouse β-globin locus

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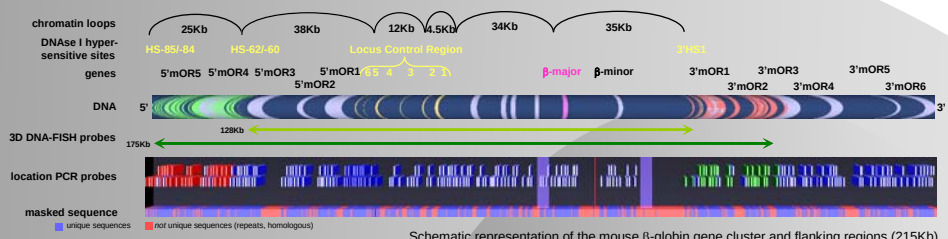
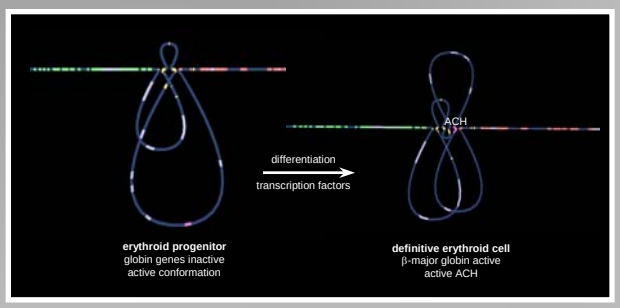
The β-Globin Locus



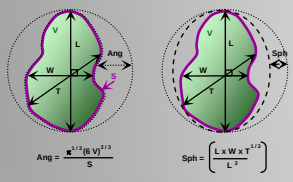
Schematic representation of the mouse β-globin gene cluster and flanking regions (215Kb).

3C Folding Model



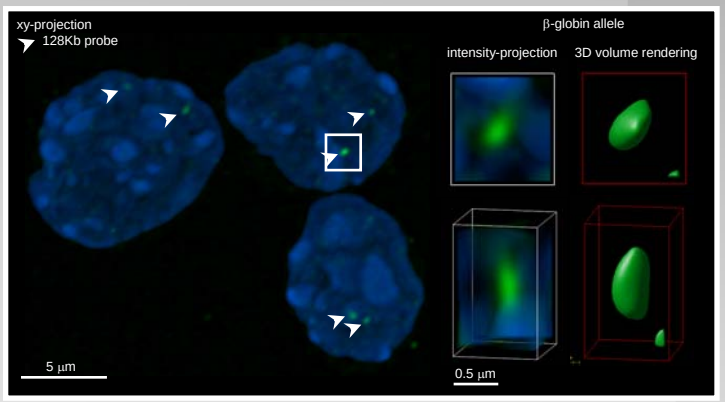
The first triggers described for β-globin gene activation were changes in chromatin accessibility and the appearance of specific hypersensitive sites. Later a dynamic looping mechanism (using chromatin conformation capture (3C) techniques) between various DNAse I hyper-sensitive sites flanking the locus and the genes was described. Upon gene activation, an Active Chromatin hub (ACH) was formed by this looping process. If this looping model is indeed true, it also predicts that the 3D volume and shape of the locus should change upon gene activation.

Geometric Analysis

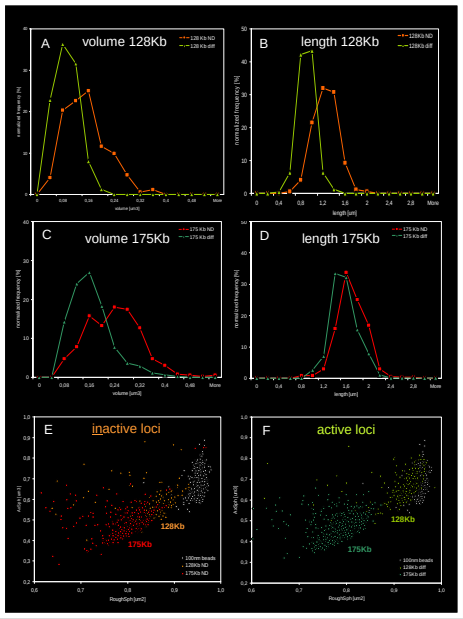


L = length (μm)
W = width (μm)
T = thickness (μm)
V = volume (μm³)
S = surface (μm²)
Ang = angularity
Sph = sphericity

3D DNA-FISH



Unlike 3C, 3D DNA-Fluorescence in situ hybridization (FISH) allows visualization of chromatin conformational changes in all cells. Image deconvolution restores images from optical aberrations and corrects for background, signal to noise ratios etc. In the cells single β-globin loci are visible when stained with a direct labeled 128Kb of 175Kb probe. To allow quantitative measurements of the 3D chromatin structure, the FISH stained loci were volume rendered in 3D objects. These objects were quantitatively measured for their geometric size and shape.



Normalized frequency distribution of the volume (A,C) and length (B,D) of 128Kb (A,B) or 175Kb (C,D) stained inactive (orange or red) or active (dark or light green) globin loci. 2D scatter plot of the sphericity (AxSph) versus angularity (RoughSph) of inactive (E) and active (F) 128Kb or 175Kb stained globin loci.

Conclusions

Here we show a 3D DNA-FISH method to visualizes the 3D structure of the β-globin locus. Geometric size and shape measurements of the 3D rendered signals (128Kb) show that the volume of the β-globin locus decreases almost two fold upon gene activation. A decrease in length and a distinctive change in shape and surface structure of the locus are also observed. Adding 5' and 3'end regions to the probe (175Kb) showed a less prominent change in length, shape and structure. It was shown (data not on this poster) that the physical distance between the two flanking regions shift in a similar limited manner, indicating that the flanking regions do not participate in ACH formation and thus active chromatin folding is occurring only within the locus proper.

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Further reading: Tolhuis et al, Mol Cell. Mol Cell. 2002 Dec; 10 (6): 1453-65. Palstra et al, Nat Genet. 2003 Oct; 35(2): 190-4. Noordermeer en De Laat, IUBMB Life. 2008 Dec;60(12):824-33. Splinter et al, Genes Dev. 2006 Sep 1; 20 (17): 2349-54. R-J Palstra, Brief Funct. Genomic Proteomic. 2009 Jul; 8(4): 297-309. Wallace and Felsen-feld, Curr Opin Genet Dev. 2007 Oct;17(5):400-7. De Laat et al, Curr Top Dev Biol. 2008; 82: 117-39. Solovei and Cavallo, Exp Cell Res. 2002 276 (1), 10-23. Schermelleh et al, Science. 2008 Jun 6; 320 (5881): 1332-6. D. Baddeley et al, Nucleic Acids Res. 2010 Feb; 38(2).

The 3D Chromatin Structure of the Mouse β-Haemoglobin Gene Cluster

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Abstract

Here we show a 3D DNA-FISH method to visualizes the 3D structure of the β-globin locus. Geometric size and shape measurements of the 3D rendered signals (128Kb) show that the volume of the β-globin locus decreases almost two fold upon gene activation. A decrease in length and a distinctive change in shape and surface structure of the locus are also observed. Adding 5' and 3'end regions to the probe (175Kb) showed a less prominent change in length, shape and structure. It was shown (data not on this poster) that the physical distance between the two flanking regions shift in a similar limited manner, indicating that the flanking regions do not participate in ACH formation and thus active chromatin folding is occurring only within the locus proper.

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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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