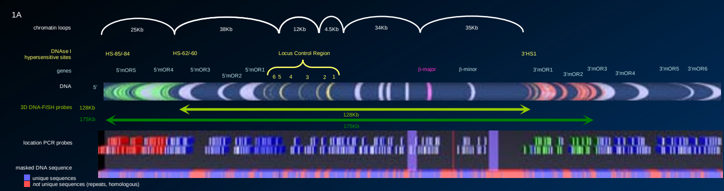
The 3D Chromatin Structure of the Mouse β-Haemoglobin Gene Cluster

Mariëtte P.C. van de Corput[1], T.A. Knoch[2], E. de Boer[1], W. van Cappellen[3], M. Lesnussa[2], B. Eussen[4] F.G. Grosveld[1]
Dept. Cell Biology & Genetics[1], Dept. Biophysical Genomics[2], Dept. Reproduction & Development[3], Dept. Clinical Genetics[4],
Erasmus Medical Center, Dr. Molewaterplein 50, 3015GE Rotterdam, The Netherlands

Long Range Chromatin Interactions within the mβ-Globin Locus

Lately it has become more clear that (subtle) changes in 3D organization of chromatin can either trigger transcription or silence genes or gene clusters. It has also been postulated that due to changes in chromatin structure, a change in chromatin accessibility of transcription factors (TF) to TF binding sites also becomes an important factor to the gene's activation status. Both such changes have been ascribed to the mouse β-haemoglobin gene cluster (Fig. 1A) as a trigger to activate globin expression in the erythroid cell lineage. Early models speculated a scanning, random activation or a looping mechanism to activate globin transcription. The chromatin conformation capture (3C) technique has shown that there is a molecular interaction between various DNAse I hypersensitive (HS) sites that are located up- and downstream of the β-globin gene cluster, the HS sites of the Locus Control Region (LCR) and the promoter by means of a dynamic looping mechanism. The clustering of the HS sites of the LCR and the up- and down-stream HS sites results in the formation of a so called Active Chromatin Hub (ACH) which is depending on at least two erythroid TF: EKLF and GATA-1 (Fig. 1B-C). The long range interactions between the outlying HS -84/-85, -62/-60 and the 3'HS1 are depending on the presence of CTCF, a TF that is thought to play an important role in long range chromatin interactions across the whole genome. Prior to gene activation, cells of the early erythroid lineage (progenitors) already show a presence of an ACH (Fig. 1B), which is not found in non-erythroid cells. The final chromatin 3D structure consist of four major loops sizing 25-38Kb and two minor loops within the LCR sizing 4.5 and 12Kb (Fig. 1A, D). To confirm this looping hypothesis (based on 3C technology) we used an *in situ* hybridization approach to visualize and, after image restoration, quantitatively measure the 3D conformational changes that take place within the locus in erythroid cells before and after differentiation.



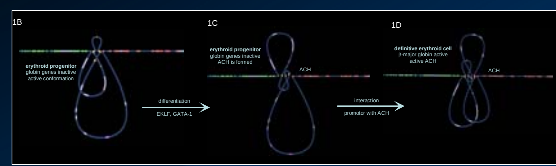


Figure 1: The mouse β-haemoglobin gene cluster with flanking regions. A: 175 Kb sequence loaded into the 3D Genome Viewer, showing the mouse β-globin gene cluster with up- and downstream flanking regions with 5' and 3' olfactory receptor (mOR) genes (pipe). The square bar shows the position of unique (blue) and not unique (red) sequences. The 3D-DNA-FISH probes derived from PCR reactions size 300-500bp long and are position on unique DNA sequences covering the 175Kb region. The 5' and 3' end flanking probes are colored in respectively red and green. The two full locus probes used for quantitative geometric data measurements are either covering a 128Kb or a 175 (128Kb + 5' and 3' end probes of 25Kb each). DNA elements are colour coded in a DNA pipe showing the positions of the the DNAse I Hypersensitive sites (HSs, yellow), the 5' and 3' end mouse olfactory receptor genes (mOR) and globin genes (light gray) and the active globin gene (pink). Looping occurs between the HSs (white curved lines). B-D: The 3C mβ-globin looping model based on cross-linking frequencies. Early erythroid progenitor show HSs interaction according to 3C data (B). Upon EKLF depending differentiation and active chromatin hub (ACH) is formed (C). Upon interaction of the β-major promotor active transcription takes place (D).

Visualizing Long Range Chromatin Interactions

3D-DNA fluorescence *in situ* hybridization (FISH) method was used to visualize chromatin structure of the mβ-globin locus in undifferentiated (inactive chromatin) and differentiated (active chromatin) mouse erythroleukemia (MEL) cells. Thereto we designed PCR probes that cover unique DNA sequences of 128Kb or 175Kb region (Fig. 1A). The 3D conformation of the 128Kb (Fig. 2B, left) and 175Kb (Fig. 2B, right) probed DNA region according to the 3C technique is shown in Figure 2. 3D high resolution images were acquired with a Leica SP5 confocal microscope. For quantitative measurements, all images were acquired with identical configuration and settings. 100nm beads were imaged to determine the point spread function (PSF).

Images were restored by deconvolution using the classic maximum of likelihood algorithm (Huygens Professional, v3.5) and an empirically obtained PSF. Images were volume rendered in 3D using the object tool analyzer (Fig. 5) and geometric sizes (lateral and axial length, volume, surface) and shape (axial sphericity, object angularity) of each mβ-globin locus were determined.

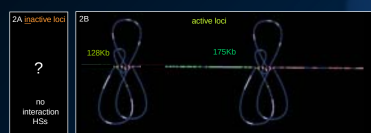
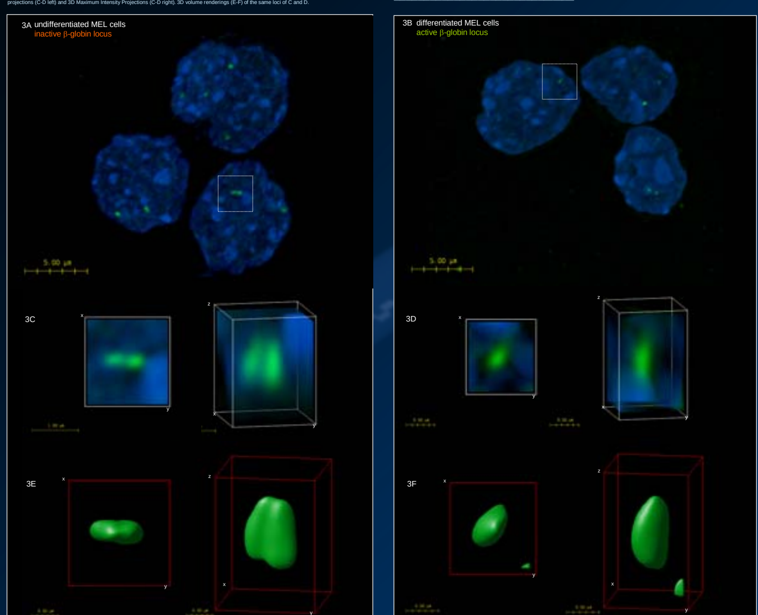


Figure 2: Visualization of 3D conformation according to the chromatin conformation capture (3C) technique of the probed DNA region. A: There is no interaction with any of the HS sites in non-erythroid cells and therfore the 3D conformation is unknown. B: The 128Kb and 175Kb probed region of the DNA region containing the mβ-globin locus.

Figure 3: Deconvolved xy image projections of undifferentiated (A) and differentiated (B) mouse erythroleukemia (MEL) cells. Single β-globin locus in xy-projections (C-D left) and 3D Maximum Intensity Projections (C-D right). 3D volume renderings (E-F) of the same loci of C and D.

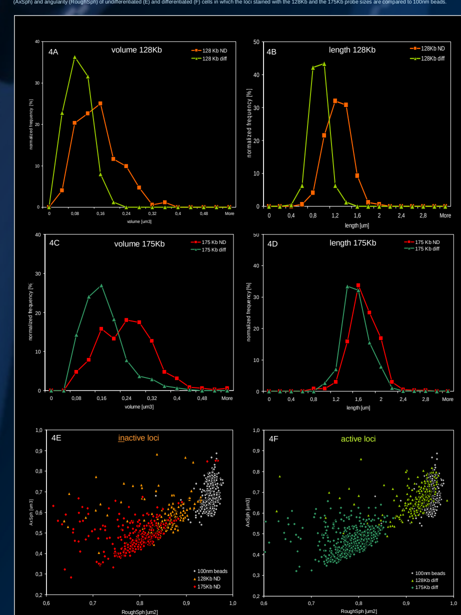


Geometric Size and Shape Analysis of the mβ-Globin Locus

From deconvolved high resolution confocal image stacks the fluorescent signals representing the mβ-globin loci were volume rendered into a 3D object. From these objects the geometric size in lateral and axial direction, volume, surface, sphericity and angularity were determined (Fig. 4, Table 1). 3D object analysis of the 128Kb stained loci shows that the actively transcribing loci occupy 1.9x less nuclear volume than the inactive loci (Fig. 4A, C, Table 1), which is mostly related to a decrease in the longest length (1.42x) (Fig. 4B-D, Table 1).The decrease in volume and length were surprisingly reduced when the loci were stained with the larger 175Kb probe (volume: 1.47x and length 1.1x).

A 2D scatter plot of the sphericity and angularity of the 3D rendered loci, show the relationship between the loci roundness and surface shape between inactive (undifferentiated, Fig. 4E, Table 1) and active (differentiated, Fig. 4F, Table 1) loci. The smaller 128Kb probe shows a cloud of data points that shifts toward the characteristics of the PSF described by the 100nm beads. This shift is however less apparent when the larger 175Kb probe was used.

Figure 3: Geometric analysis of 3D volume rendered mβ-globin locus stained with 128Kb or 175Kb probe. Normalized frequency distribution of the volume of 128Kb (A) and 175Kb (B) of undifferentiated and differentiated cells. Normalized frequency distribution of the length of 128Kb (C) or 175Kb (D) of undifferentiated and differentiated cells. 2D scatter plot of the sphericity (AxSph) and angularity (RoughSph) of undifferentiated (E) and differentiated (F) cells in which the loci stained with the 128Kb and the 175Kb probe sizes are compared to 100nm beads.



Discussion

Globin gene activation is depending on long distance looping of the up- and downstream HS sites and the β-major promotor to the LCR, resulting in a complex 3D chromatin structure (Fig. 1D). By staining the mβ-globin loci with fluorescence labeled sequence specific probes followed by high-resolution 3D imaging and 3D volume rendering of the deconvolved images, the loci reveal changes in the geometric size and shape properties when cells are differentiated into a active globin transcribing cell. An almost 2x decrease in volume was measured, which was mostly due to a reduction of the longest length measured. This can be explained by a change in loop formation. The almost 70Kb loop between the LCR and the 3'HS1 is folded into two loops of 34 and 35Kb upon interaction of the promotor to the ACH to activate transcription. The limited decrease in volume and length when the locus was probed with an additional 5' and 3' end region is surprising. The 5' end is actively participating in the looping process that stabilizes the ACH. However, the 3' end has (until now) not been seen to be participate in ACH formation or any complex looping mechanism for globin gene activation. As this part of the locus seems to be the most un-dynamic, it could be the dominating factor that influences the fluorescent signal emitting from the probed DNA region and therefore cloud subtle changes in the chromatin folding mechanism of gene activation. Next to the dynamic chromatin folding process that is occurring between the HS-85/84 and the 3'HS1, a stretch of "rigid" DNA can prevent a DNA region containing activated genes to stay at the edge of a chromosome territory and possibly prevent a close proximity to the silencing effect of (spreading) heterochromatin.

An increase in lateral and axial resolution like the 3D Structural Illumination Microscope (SIM) provides, could help solve the problem of detecting subtle 3D changes in chromatin structure. And in the near future will reveal many more details of 3D chromatin organization of not only the mβ-globin locus but of many other intra-cellular processes.

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Chromatin Dynamics of the Mouse β-Globin Locus

van der Corput, M. P. C., de Boer, E., **Knoch, T. A.**, van Cappellen, W., Lesnussa, M., Eussen, B. & Grosveld, F. G.

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Abstract

Lately it has become more clear that (subtle) changes in 3D organization of chromatin can either trigger transcription or silence genes or gene clusters. It has also been postulated that due to changes in chromatin structure, a change in chromatin accessibility of transcription factors (TF) to TF binding sites also becomes an important factor to the gene's activation status. Both such changes have been ascribed to the mouse b-haemoglobin gene cluster as a trigger to activate globin expression in the erythroid cell lineage. Early models speculated a scanning, random activation or a looping mechanism to activate globin transcription. The chromatin conformation capture (3C) technique has shown that there is a molecular interaction between various DNAse I hypersensitive (HS) sites that are located up- and downstream of the b-globin gene cluster, the HS sites of the Locus Control Region (LCR) and the promoter by means of a dynamic looping mechanism. The clustering of the HS sites of the LCR and the up- and down- stream HS sites results in the formation of a so called Active Chromatin Hub (ACH) which is depending on at least two erythoid TF: EKLF and GATA-1. The long range interactions between the outlying HS -84/-85, -62/-60 and the 3'HS1 are depending on the presence of CTCF, a TF that is thought to play an important role in long range chromatin interactions across the whole genome. Prior to gene activation, cells of the early erythroid lineage (progenitors) already show a presence of an ACH, which is not found in non-erythroid cells. The final chromatin 3D structure consist of four major loops sizing 25- 38Kb and two minor loops within the LCR sizing 4.5 and 12Kb. To confirm this looping hypothesis (based on 3C technology) we used an *in situ* hybridization approach to visualize and, after image restoration, quantitatively measure the 3D conformational changes that take place within the locus in erythroid cells before and after differentiation. Globin gene activation is depending on long distance looping of the up- and downstream HS sites and the b-major promotor to the LCR, resulting in a complex 3D chromatin structure. By staining the mb-globin loci with fluorescence labeled sequence specific probes followed by high-resolution 3D imaging and 3D volume rendering of the deconvolved images, the loci reveal changes in the geometric size and shape properties when cells are differentiated into a active globin transcribing cell. An almost 2x decrease in volume was measured, which was mostly due to a reduction of the longest length measured. This can be explained by a change in loop formation. The almost 70Kb loop between the LCR and the 3'HS1 is folded into two loops of 34 and 35Kb upon interaction of the promotor to the ACH to activate transcription. The limited decrease in volume and length when the locus was probed with an additional 5' and 3' end region is surprising. The 5' end is actively participating in the looping process that stabilizes the ACH. However, the 3' end has (until now) not been seen to be participate in ACH formation or any complex looping mechanism for globin gene activation. As this part of the locus seems to be the most un-dynamic, it could be the dominating factor that influences the fluorescent signal emitting from the probed DNA region and therefore cloud subtle changes in the chromatin folding mechanism of gene activation. Next to the dynamic chromatin folding process that is occurring between the HS-85/84 and the 3'HS1, a stretch of "rigid" DNA can prevent a DNA region containing activated genes to stay at the edge of a chromosome territory and possibly prevent a close proximity to the silencing effect of (spreading) heterochromatin. An increase in lateral and axial resolution like the 3D Structural Illumination Microscope (SIM) provides, could help solve the problem of detecting subtle 3D changes in chromatin structure. And in the near future will reveal many more details of 3D chromatin organization of not only the mb-globin locus but of many other intra-cellular processes.

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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