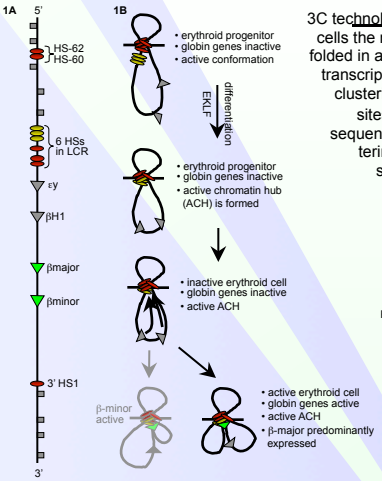
High-Resolution Confocal Microscopy and Nano-Scale Distance Measurements Show Difference in 3D Conformation Between Active and Inactive Mouse β-major Globin Loci?

Mariëtte P.C. van de Corput, Tobias A. Knoch, Gert W. van Cappellen, Ernie de Boer, Frank G. Grosveld.
Erasmus MC, Dept. Cell Biology & Genetics, PO Box 2040, 3000CA Rotterdam, The Netherlands.
e-mail: m.vandecorput@erasmusmc.nl



3C technology [1] shows that in erythroid cells the mouse β-major globin locus is folded in a looped structure during gene transcription. The looping is a result of clustering of DNAse I hypersensitive sites (HS) from which intervening sequences loop out (fig 1). This clustering of DNAse I hypersensitive sites is however not present in non-erythroid cells.

Fig 1: A: schematic map of the mouse β-major globin. B: Folding of the β-globin locus during various differentiation stages of erythroid cells. A distinctive 3D conformation is maintained during transcription of the globin genes in which the HSs, together with transcripiton factors form the core (or Active Chromatin Hub, ACH) of the structure.

To determine the actual 3D architecture and its dynamics we have established a structure preserving method and combined this with high-resolution CLSM followed by image restoration by deconvolution to allow accurate nano-scale distance measurements. The resolution equivalent of this method was 18 nm.

3D DNA-FISH protocol:
- retaining chromatin structure using cross-link (aldehyde) fixatives
- permeabilization treatments:
  - triton X-100
  - saponin
  - proteases
  - HC
  - N_2(l) freeze/thaw
- hybridization with high quality labeled probes
- post hybridization washes
- embedding with correct anti-bleaching medium
- finding the right balance for each application

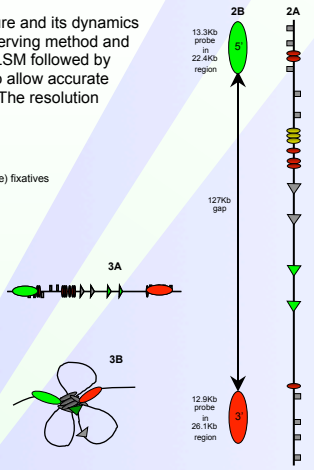


Fig 2: A: schematic map of the β-globin locus B: location of the 2 probes used in 3D-DNA-FISH. The gap between the two probes is 127 Kb.

Fig 3: hypothetical difference of position of the 5' and 3' end probes when locus is either inactive ("linear", 3A) or active (folded,3B).

Chromatic Shift

The extra-cellular chromatic shift (CS) was determined By scanning 50 500 nm multi-coloured TetraSpeck fluorescent beads. The intra-nuclear CS was determined for each cell type by a 3D DNA-FISH using labeled telomere oligo probes.

The center of mass coordinates for each fluorescent signal was determined. The total CS for beads and telomeres (D) was calculated according to Pythagoras (fig. 5).

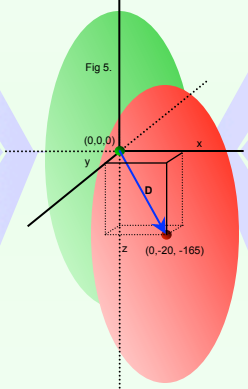


Fig 4: A: CS from 500nm beads (n=50):
$D = \sqrt{((x_g - x_r)^2 + (y_g - y_r)^2 + (z_g - z_r)^2)}$
D_{beads} = 167±12 nm
of which the CS in x,y and z are:
$x_{cs\text{-}beads} = x_g - x_r$ = 0±11 nm
$y_{cs\text{-}beads} = y_g - y_r$ = -20±12 nm
$z_{cs\text{-}beads} = z_g - z_r$ = -165±12 nm

B: CS from telomeres in liver and brain cells:
D_{brain} = 210±80 nm; D_{liver} = 218±33 nm
of which the CS in x,y and z are:
$x_{cs\text{-}brain}$ = -21±24 nm; $x_{cs\text{-}liver}$ = -14±14 nm
$y_{cs\text{-}brain}$ = 3±25 nm; $y_{cs\text{-}liver}$ = 1±16 nm
$z_{cs\text{-}brain}$ = -206±61 nm; $z_{cs\text{-}liver}$ = 216±33 nm

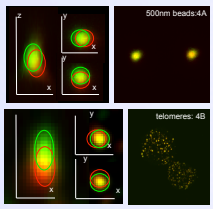
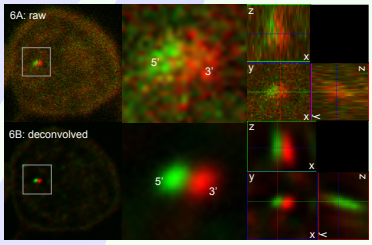


Image Restoration by Deconvolution

Confocal image stacks of fetal liver and control (brain) cells were deconvolved with the measured point spread function of the confocal microscope distilled from the average of at least 8 100nm green fluorescent beads or 200nm multi-coloured TetraSpeck beads.

Images were deconvolved using the classic maximum-likelyhood estimation deconvolution algorithm that is implemented in the Huygens Professional image restoration, visualization and analysis software (SVI). From each fluorescent signal the center of mass coordinates were determined and corrected for chromatic shift.



The Resolution equivalent (RE)

was determined. The RE for the 500nm TetraSpeck beads was 18±8nm. The RE for telomeres stained in fetal liver cells was 35±17nm and in brain cells 61±34nm.

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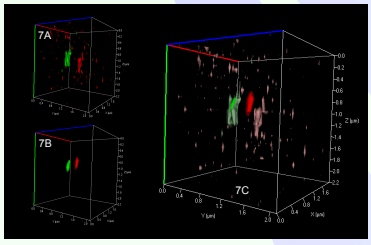

Fig 6 & 7: 3D DNA-FISH:
Fig6: A: one optical slice of a raw CLSM image stack showing fluorescent signals of one globin allele: 5' end stained green and 3' end stained red. Of the allele, a close-up as well as an xy, xz, and yz view are shown. B: same image slice after de-convolution of complete image stack. Fig 7: A: 3D projection of a raw image stack showing the 5' and 3' end of one globin allele in nuclear space. B: same 3D projection after de-convolution and chromatic shift correction. C: overlay of A & B.

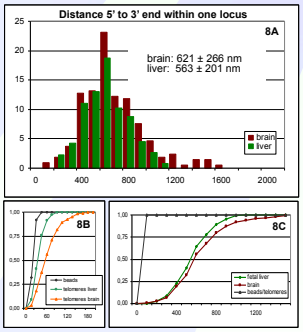

Fig 8: frequency (A) and cumulative frequency (C) distribution of distances between the 5' and 3' end of inactive (brain, red) and active (liver, green) β-globin locus. Control (beads and telomere distances (gray in C & B) cumulative distributions.

(1)Tolhuis et al, Mol Cell. 2002 Dec; 10 (6):1453-65 and Palstra et al, Nat Genet. 2003 Oct; 35 (2):190-195

Results

Nano-scale distance measurements show that within an actively transcribing locus the distance between the 5' and 3' end of the locus is 563±201±12nm. In in-active loci the 5' to 3' distance is 621±266±16nm which is statistically significant. Since the spatial distance distributions are very similar we conclude that for both cell types the underlying 3D architecture is not dramatically different as earlier hypothesized [1] (see also fig. 3). However, frequency and cumulative frequency distributions (fig. 8A-C) show that the distance between the 5' and 3' end of the locus is more distinct and stable in erythroid (active) cells. In non-erythroid cells the distribution is broader, indicating a less distinct, more dynamic organized chromatin structure. These result agree with the hypothesis that gene activation requires a different/folded conformation of the chromatin than when genes are inactive. Now that only the nano-architecture seems to be functionally influenced by gene activation/transcription, this cannot be concluded on a larger scale. Inter-allelic differences do not show preference towards (interactive) chromatic co- and/or near-localization (fig. 9A & B).

Future

Distance measurements across this entire 218Kb chromatin region will elucidate the complete 3D structure of active and inactive mouse β-major globin loci. Furthermore, distance measurements combined with computer simulations (fig. 10) based on theoretical and/or empirical data, can give a better insight into high and low order chromatin architecture.

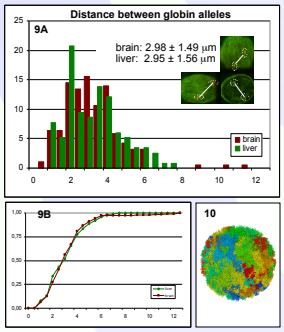

Fig 9: frequency (A) and cumulative frequency (B) distribution of distances between the two globin alleles in inactive (brain, red) and active (liver, green) cells.
Fig 10: simulation of a full genome folded into a nuclear space that has a diameter of 10μm. Colours: different chromosome territories.

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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. Nano-scale distance measurements show that within an actively transcribing locus the distance between the 5' and 3' end of the locus is 563±201±12nm. In in-active loci the 5' to 3' distance is 621±266±16nm which is statistically significant. Since the spatial distance distributions are very similar we conclude that for both cell types the underlying 3D architecture is not dramatically different as earlier hypothesized. However, frequency and cumulative frequency distributions show that the distance between the 5' and 3' end of the locus is more distinct and stable in erythroid (active) cells. In non-erythroid cells the distribution is broader, indicating a less distinct, more dynamic organized chromatin structure. These result agree with the hypothesis that gene activation requires a different/folded conformation of the chromatin than when genes are inactive. Now that only the nano-architecture seems to be functionally influenced by gene activation/transcription, this cannot be concluded on a larger scale. Inter-allelic differences do not show preference towards (interactive) chromatic co- and/or near-localization. Distance measurements across this entire 218Kb chromatin region will elucidate the complete 3D structure of active and inactive mouse β-major globin loci. Furthermore, distance measurements combined with computer simulations (fig. 10) based on theoretical and/or empirical data, can give a better insight into high and low order chromatin architecture.

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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