

**SUBTLE DIFFERENCES IN CHROMATIN NANO-ARCHITECTURE
BETWEEN SILENT AND TRANSCRIPTIONIONALLY ACTIVE
MOUSE β -HAEMOGLOBIN LOCI.**

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ABSTRACT: Chromatin conformation capture (3C) technology showed that in erythroid cells, the DNase I hypersensitive sites of the mouse β -major globin locus are in close vicinity towards each other and form an active chromatin hub (ACH) [1], [2]. From the ACH intervening sequences loop away, resulting in a complex 3D chromatin structure in nuclear space. The molecular interaction of the HSs was however not detected in non-erythroid cells. To study HS clustering in cells, we have developed a 3D DNA fluorescence *in situ* hybridization method and combined this with high-resolution confocal microscopy followed by image restoration by deconvolution to allow accurate nano-scale distance measurements within a chromatin region of 175Kb that contains the β -globin locus. Results show the distance in active loci between the 5'end and 3'end of the locus is $502\text{nm} \pm 167 \text{ nm}$. In inactive loci (non-erythroid cells) the distance is $586\text{nm} \pm 258\text{nm}$. In spite of the significant difference in distance of 40nm, the spatial distances are not as distinctive as earlier hypothesized by 3C technology. Although there seems to be higher rigidity and more distinctive chromatin folding in active loci, the essential 3D structure seems not to be depending solely on gene activity and chromatin density status alone, but lies more in the subtle changes of a more or less fixed nano-architecture of the locus, as shown in two tested computer simulation models [3]. Currently a 3 and 4 coloured DNA-FISH approach is developed to measure intra-chromatin spatial distances across the entire 173 Kb region to elucidate the complete 3D nano-architecture of the β -globin locus in detail.

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