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The tissue-specific relationship between 3D genome organization and gene expression at the mouse *Slc29a3/Unc5b* locus

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Motivation and Aim: Chromosomal folding in interphase nucleus is not random, and each chromosome is organized along its length into topologically associating domains (TADs), allegedly involved in gene expression regulation. Boundaries of these domains are formed by clusters of CTCF binding sites, the distribution of which is highly conserved in evolution [1]. However, full depletion of CTCF protein and disruption of TADs does not lead to massive expression disturbance [2]. There are only rare cases of significant aberrations caused by destruction of the TAD boundaries, and all such mutations affected not so much the deletion of CTCF sites as a change in the distance between regulatory elements [3–5]. Thus, there is no clear evidence of regulatory role of TADs in gene expression. We suppose tissue-specific nature of this relationship, and in this study we aimed to test this hypothesis.

Methods and Algorithms: We have created model animal lines using CRISPR/Cas9 mediated gene editing in zygotes. We removed four of CTCF-binding sites at the TADs border by introducing the 5-kb length deletion in the intergenic region (chr10:60755585-60761088, mm10), and two small deletions in sites located in the Unc5b introns (chr10:60775582-60775773, chr10:60778683-60778844, mm10). The absence of CTCF binding was confirmed by ChIP-Seq analysis. The obtained homozygous line was used for a standard Hi-C experiment on various tissues, and was crossed with *Mus musculus castaneus* for an allele-specific expression measurement experiment. We analyzed *Unc5b*, *Slc29a3*, *Cdh23*, *Vsir*, *Psap, Sgpl4* gene expression in an extensive tissue set using QIAcuity Digital PCR System. The expression of *M. m. castaneus* alleles placed on homolog with a wild-type spatial organization was used as measurement controls.

Results: The *Slc29a3/Unc5b* locus of mouse genome contains one of the strongest insulation border between TADs, genes of the locus are housekeeping and have a pronounced regulatory circumstance. We chose this locus for several reasons, such as the wide range of tissues in which gene expression can be assessed, and the highly conserved spatial organization between species. Allele-specific digital PCR analysis shown that gene expression alterations were minor (less than 25 % for most cases), and were observed quite rarely (only three from six genes changed their expression in five from 10 analyzed tissues). However, we found two cases of oppositely directed changes (the expression of *Slc29a3* in the kidneys decreased to 25 %, while in the cerebellum it increased to 20 %; *Vsir* expression in the olfactory bulb decreased to 20 % and increased to 10 % in the bladder). We chose this cases for a more detailed study.

Conclusion: Our result point to the tissue-specific role of spatial genome organization. We have shown that the same deletion of CTCF sites cluster can lead to oppositely directed changes depending on the epigenetic environment and developmental trajectory. But even so, these alterations are minor and do not cause obvious prototypical manifestations that could explain the evolutionary conservatism of spatial landscape of the *Slc29a3/Unc5b* locus. At the same time, in most of analyzed cases we do not find changes of gene expression. Thus, the evolutionary significance of spatial genome organization remains unknown.

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