The role of heterochromatin proteins in imprinted paternal X chromosomes elimination in the development of Sciara coprophila.

P-01.4-26

A. Smelova[I], D. Maksimov[I,II], O. Posukh[I,II], P. Singh[I,III], S. Belyakin[I,II]

[I]Novosibirsk State University, Novosibirsk, Russia, [II]The Institute of Molecular and Cellular Biology, Novosibirsk, Russia, [III]Nazarbayev University, Astana, Kazakhstan

The phenomenon of genomic imprinting was first investigated in fungus gnats *Sciara coprophila*. It is known that programmed elimination of paternal X chromosomes takes place during the early stages of embryogenesis in somatic cells, so that two paternal X chromosomes are eliminated in future males, and one is eliminated in future females [1]. The number of eliminated chromosomes can be determined by X chromosomes to autosomes ratio (X/A). Recently it was found that the region of X chromosome which is not exposed to elimination is associated with H3K9me3 and H4K20me3 epigenetic modifications [2]. Here we estimated the role of H3K9- and H4K20-specific methyltransferases (MTs) in X chromosomes elimination in early embryos. As a result of RNA-seq and *de novo* transcriptome assembly 7 transcripts of specific MTs were found. Double stranded (ds) RNAs were synthesized against identified transcripts for RNA interference (RNAi). Embryos were incubated during 10 hours with dsRNAs against MTs genes and GFP as a control. DNA sequencing by Illumina MiSeq was used for reads counting. The number of eliminated X chromosomes was estimated by relation of X/A values obtained from experimental groups to X/A values got from controls. In our pioneer experiment with female embryos, it was shown that *Su(var)3-9* RNAi probably leads to blocking of X chromosomes elimination and *SetDB1* RNAi apparently leads to increased number of eliminated X chromosomes compared with controls. However, *G9a* and *Hmt4-20* RNAi did not lead to significant differences compared with controls. Probably it can be associated with unsuccessful passing through pores of dsRNAs into embryos. Apparently, it is advisable to repeat the experiment with shorter siRNAs in order to achieve sustained effect. This study was supported by a grant of the Russian Federation Government #14.Y26.31.0024.

Previously published in:
1. Metz CW (1938) The American Naturalist 72, 485-520.
2. Singh PB et al. (2019) Chromosoma 128(2), 69-80.