Analysis of inter-chromosomal contacts of rDNA genes with DUX4 genes in human cells in different physiological conditions using fluorescence in situ hybridization

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Direct rDNA inter-chromosomal contacts with different chromosomal regions are involved in the regulation of genes activity. rDNA contacts with the subtelomeric region of chr4 possessing *DUX4* genes were previously detected by the 4C (Circular Chromosome Conformation Capture) approach. The contacts disappear after the heat shock treatment (Tchurikov et al., 2020, Dokl Biochem Biophys. 490(1):50-53. doi: 10.1134/S1607672920010032). *DUX4* genes specify transcription factors that play a key role in zygotic genome activation and are silenced at the later stages. Their expression at later stages leads to muscular dystrophy. The aim of this study was to study the contacts of *rDNA* and *DUX4* genes using the independent approach - fluorescence in situ hybridization (DNA-FISH). We used HEK293T cells before and after heat shock treatment. On average, we observed about 4 foci of *rDNA* probe and 10 foci of *DUX4* probe per cell, 34% of them were located in the area of rDNA contacts. The number of *DUX4* genes contacts with nucleoli decreased approximately twice (to 17%) after the heat shock treatment and the distances between hybridization foci increased. The proportion of hybridization sites of *DUX4* genes not associated with the *rDNA* region increased by almost 10 times. Cells also were stained with antibodies to nucleolin, that is required for RNA polymerase I transcription, to determine the transcriptional activity at rDNA hybridization foci. We detected that the intensity of immunostaining of *rDNA* loci decreased after the heat shock treatment. The data indicate the changes of transcriptional activity in nucleoli induced by the treatment. In RT-PCR experiments we detected that the treatment causes nearly a 2-fold decrease the levels of pre-rRNA. FISH results support the results obtained by 4C approach and provide data on diversity of inter-chromosomal contacts in individual cells. The study was supported by the grant from Russian Science Foundation No. 21-14-00035.