# ABSTRACT

Epigenetic regulation of gene expression together with nuclear organization of chromatin has been widely investigated in the last few years. A number of studies have elucidated the three dimensional interactions between genetic material and structural elements of cellular nucleus including nucleolus, nuclear lamina as well as pores in the nuclear membrane. Additionally, a crucial role of direct spatial environment of DNA sequence in its transcriptional activity has been proved.

In this study several tasks have been undertaken: (1) comparative analysis of myoblasts and myotubes expression profiles based on microarray technique, (2) evaluation of structural changes in nucleus as a consequence of differentiation process, (3) estimation of specific sequence localization changes including selected chromosome centromeres as well as gene-specific sequences. Moreover, an evaluation of putative relationship between genomic sequence localization and its expression has been studied (4). To cope with set up aims, several techniques have been employed including microarray comparative analysis, three-dimensional fluorescence *in situ* hybridization, immunoFISH and flow cytometry. An important element of conducted research was a computational analysis of acquired data.

Changes in nuclei volume and their shape have been observed. Nuclei in differentiated myotubes have been proved to be smaller and more flattened ($p<0,001$) in comparison to myoblasts nuclei. Simultaneously, in myotubes a total number of down-regulated genes ($n = 132$) was higher than up-regulated ones ($n = 117$).

Moreover, in three (chromosome 1, 2 and 17) out of six analyzed chromosomes significant changes in localization of centromeres were observed. Notably, these chromosomes were placed in the group with the most frequent fluctuations concerning changes in genes expression. As far as genes on each chromosome with significant changes in centromere position were more down-regulated than up-regulated speculations have been raised indicating centromere movement towards nuclear periphery. Indeed, in such situation the localization of all studied centromeres was proved to be more peripheral, however, they still localized in the middle between nuclear center and envelope. In case of centromeres of chromosomes 2 and 17 even more peripheral distribution was observed.

Furthermore, *MYH2*, *ACTN2* and *VCAM1* genes were observed to be up-regulated in differentiated myotubes ($p<0,001$). It coincides with their function as products of *MYH2* and *ACTN2* are the crucial elements of muscle contractile apparatus, whereas VCAM1 is the membrane receptor that influences the myoblasts fusion. In contrary, *MYF5* and *DPP4* genes were significantly down-regulated ($p<0,001$). *MYF5* was earlier shown to be up-regulated

only at the beginning of satellite cells proliferation, while *DPP4* has its role in regulation of insulin expression. We did not observe significant changes in *MYOG* and *MYF6* expression. A comparable level of transcription was noted in *NCAM1* receptor, *HPRT1* housekeeping gene and *ACTN3*. Although ACTN3 is a protein involved in contraction in fast myogenic fibers, its absence in part of human population was documented, which was confirmed in our study.

Based on expression pattern, in most analyzed genes we observed an existence of genomic clusters. In case of *MYH2, ACTN3, HPRT1* and *NCAM1* clusters, their transcriptional activity changes stayed in accordance with changes in particular gene expression. For instance, *MYH2* up-regulation was concomitant to its relatively up-regulated genomic neighborhood. Moreover, in case of relatively stable expression of *ACTN3, HPRT1* and *NCAM1* no changes in corresponding clusters were observed.

Lastly performed experiments provided evidence for existence of relationship between expression and nuclear localization of *VCAM1, MYH2* and *DPP4* genes. Sequences up-regulated during myoblast differentiation were localized in the middle of nuclear center-to-periphery distance. In contrast, the DPP4 down-regulation in myotubes seemed to correlate with its proximity to nuclear lamina at the periphery of nucleus (which was confirmed by co-localization analysis) together with diminished physical distance from heterochromatic centromeric regions. This observation was not confirmed in other cases.

In summary, a high-throughput microarray analysis of transcriptome enabled the evaluation of general expression profile changes. Simultaneously, it allowed the selection of genes with significant changes in expression during differentiation process for further cytogenetic analysis. Observed changes in nuclear localization of analyzed genes were proved to be coherent enough, in order to suggest the spatio-temporal regulation of expression.