Project title: The application of CRISPR-Cas9 technology to elucidate whether changes in nuclear architecture during adipogenesis are a cause or a consequence.

The formation of fat cells, a process called adipogenesis, is intensely studied in biomedical sciences on account of the growing problem of obesity, and also in the animal sciences, since adipose tissue affects important breeding traits, such as neonatal survival and animal growth. The accumulation of adipose tissue is affected by environmental factors (such as diet and physical activity), genetic factors (such as mutations and gene polymorphisms), and interactions between them. In recent years, special attention has been paid to the role of epigenetic mechanisms in this process, such as DNA methylation and histone modifications. A relatively new research area is so-called nuclear architecture, which deals with how the location of chromosomes and genes in the three-dimensional space of the cell nucleus can determine the expression of genes, and thus affect the functioning of cells. Our previous studies using an in vitro adipogenesis model have shown that genes undergo dynamic reorganization in the cell nucleus more strongly than do chromosomes; these changes in the position of gene are correlated with their transcription level. Genes are moved from their chromosome territory through the formation chromatin loops, but the mechanism of these reorganizations is not yet fully understood. This project is an extension of these studies, which employs novel methods in genome research and genome editing. The main aim of the project is to understand the mechanism of the movement of the genes that encode key transcription factors for adipogenesis in the cell nucleus during the differentiation process, and to answer the question of whether the formation of chromatin loops and domains is necessary for gene activation, or if their presence is merely a consequence of the higher-order chromatin organization that is characteristic of the stage of cell differentiation. This study will be conducted on the domestic pig, which is not only an important livestock species, but also an important animal model in the study of human obesity. Techniques for visualizing the studied loci on the level of the individual cell will be applied, with the use of modified versions of fluorescent in situ hybridization (3D-FISH). High-resolution microscopy will allow the precise observation of the dynamics of the formation of the chromatin domains during differentiation. These domains will be evaluated for the presence of selected epigenetic markers, such as histone modifications, using the chromatin immunoprecipitation (ChIP) technique. In order to be able to draw conclusions on the effects or causes of chromatin loop formation, a genetic engineering technique that has revolutionized genome editing research in recent years—the CRISPR/Cas9 “molecular scissors” method—will be used. The precise deletion of the DNA fragments that regulate the expression of a selected gene will allow the gene to be inactivated. In the next step, the chromatin domains harboring the studied gene will be analyzed using the methods described above. If the chromatin loops are still present, despite the lack of gene expression, this will indicate that their formation is not directly related to the transcriptional activation of the gene. If chromatin loops are not observed in the nuclei of the modified cells, this will be an indication that the formation of the chromatin loop is closely related to the transcriptional activation of key genes for adipogenesis. Detailed knowledge of the mechanisms that regulate the expression of genes on the level of the cell nucleus will enable the development of new methods for modulating gene expression, which can be used in the future in the therapy of diseases (including obesity) and to modify traits that are important in animal breeding (e.g., fatness).