Modifiers of *Muscleblind-like* genes expression in health and disease

Expansion of the CTG trinucleotide repeat in the non-coding region of the *DMPK* protein kinase gene underlies the molecular pathogenesis of myotonic dystrophy type 1 (DM1), an inherited disease associated with histo- and physio-pathological alterations within the muscle tissue, manifesting in myotonia and myopathy of skeletal muscles and heart. Upon transcription, the mutant *DMPK* gene gives rise to toxic RNA molecules containing CUG repeat expansion, which bind with strong affinity *Muscleblind-like* (MBNL) proteins, the key regulators of RNA metabolism in the cell, e.g. processes of alternative pre-mRNA splicing. Sequestration of MBNL proteins by toxic RNA significantly impairs their function, leading to abnormalities in the alternative splicing of many pre-mRNA target molecules. Resulting protein isoforms, characteristic of fetal development, are not adapted to the requirements of the adult organism, leading to disease development. Despite intensive research, myotonic dystrophy remains incurable, and the only option for patients is rehabilitation and symptomatic treatment of wide range of clinical manifestations.

Among the three MBNL paralogs, MBNL1 and MBNL2 play particularly important roles in DM1. Symptoms of the disease, which are recapitulated in *Mbnl1* knockout mice, are further exacerbated by the loss of *Mbnl2*, while systemic overexpression of either of these proteins in a murine disease model partially eliminates the molecular, histopathological and physiological symptoms of DM1. Both paralogs are strongly expressed in muscle, the tissue most affected by DM1, both are sequestered by toxic RNA and share common as well as distinct sets of functions and pre-mRNA targets. Interestingly, both MBNL1 and MBNL2 show a mutual compensatory increase in expression level in conditions when the other paralog is missing. Hypothetically, this mechanism may be involved in counteracting disease symptoms and modifying their severity. *Within this project, we will use novel genetic engineering and molecular biology tools to test the hypothesis that increasing the endogenous expression of both paralogs in cellular and mouse disease models may be more effective in counteracting toxic RNA sequestration and fighting key DM1 symptoms, by expanding the range of pre-mRNA targets. The proposed tools will be applied to identify novel cis- and trans-acting factors modifying the expression of MBNL1 and 2, including distinct proteins, DNA and RNA interacting with genomic DNA regions of the studied genes. In addition, we will leverage the natural endogenous autoregulatory mechanism of the MBNL1 protein to develop MBNL1 expression constructs enabling therapeutic benefits without the risk of collateral damage caused by excessive protein accumulation.* The knowledge gained in this project will be a valuable aspect in DM1 therapeutics and will be applicable to molecular studies of other diseases; decreased expression of MBNL proteins often accompanies various types of cancer and correlates with poor prognosis and an increased risk of relapse and metastasis. Examples include the suppressive roles of MBNL1 and MBNL2 in breast and colon cancer as well as liver cancer. Overall, the applicability of the obtained results will not be limited to DM1, but will apply to other diseases associated with mis-regulated *MBNL* genes.