

The life course of mRNA begins with transcription by RNA Pol II, splicing, and processing 5' and 3' ends, which generally is co-transcriptional. It enables the efficient and fast transport to the cytoplasm, where the information contained in them will be read in translation process. Typically, the time which elapses from the synthesis of pre-mRNA transcript to the formation of the protein in the cytoplasm is short and lasts from a few to tens of minutes. Until recently it was thought that expression of a given gene is mainly influenced by the level transcription, transcripts stability (half-life of mRNA in the cytoplasm) and time of occurrence in the cell of the final product - the protein. However, some studies have shown that in certain cell types, it is observed that a significant portion of polyadenylated transcripts are 'nuclear retained' and undetected in the cytoplasm, thus they are not translated immediately after synthesis. Because pre-mRNA undergoes modifications during the synthesis of RNA (co-transcriptionally) fully mature mRNA can be formed in a relatively short time. Therefore, this long period of retention of nuclear mRNA suggests that the nucleus exhibits an additional role previously skipped. Studies on the nuclear retention of the mRNA indicates that it has a significant impact on the level of gene expression by regulating the export and translation delay which allows the synthesis of specific proteins under strictly controlled conditions and time.

Regulation of gene expression by nuclear retention of the mRNA had been demonstrated, among others, in the mammalian metabolic tissues, in generative cells or in the cells under stress conditions. Although the final result of such regulation is always the same, the transcripts can be retained in different forms. These transcripts may be retained as a mature mRNA or pre-mRNA. Some mRNAs have additional sequences that regulate the transport from the nucleus to the cytoplasm. These sequences are present in the regions of untranslated mRNA - 5' UTR and 3' UTR. There is also a hypothesis that there are nuclear retention factors, that bind the mRNAs and block the possibility of binding the export factors. It is suggested that a large effect on the retention mRNA can have splicing factors which form an early spliceosome complex. It is also expected that these factors anchor mRNA in some nuclear structures.

Much less is known about the spatial organization of the process in the cell nucleus. So far the only described domains associated with the accumulation of polyadenylated transcripts are nuclear speckles. However, accumulated in these structures transcripts do not seem to be exported to the cytoplasm. In larch microsporocytes during diplotene we observed accumulation of mRNA in the nucleus in nuclear bodies – so called Cajal bodies (CB). This is the first report, not previously reported in eukaryotic cells. These nuclear domains "retain" polyadenylated transcripts for a very long time. CBs are evolutionarily conserved structures occurring in both animal and plant cells. This indicates their fundamental role in the nuclear metabolism of eukaryotic cells. Intensive research, carried out for many years, has demonstrated the relationship between CBs and many processes associated with the metabolism of various types of RNA, for example the maturation and assembly of snRNP, recruitment and assembly in ribosomal RNA maturation, tRNA maturation, histone mRNA maturation, the synthesis of telomeres and miRNA and siRNA maturation. CBs are self-organized structures, appearing on local needs of increased metabolism of certain types of RNA. Assembling of spliceosomal subunits is possible in the nucleoplasm, but it been demonstrated that the process is carry out with more than 30-fold greater efficiency in the CB. For example fibroblast, cells characterized by low cellular metabolism do not posses CBs. Coilin gene knockdown (marker protein of those bodies) in cells with a high metabolism (generative cells or embryonic cells), causes the breakdown of CBs and leads to death of these cells.

In order to verify that Cajal bodies are involved in post-transcriptional regulation of gene expression by nuclear retention of mRNAs and / or pre-mRNAs transcripts we have planned the following research tasks:

- (1) examine which transcripts are stored in CBs,**
- (2) explain what mechanism/or mechanisms are responsible for the retention of the mRNAs in CBs,**
- (3) determine which genes are regulated by nuclear retention of the mRNA.**

To achieve these goals we will use: laser microdissection, analysis of the transcriptome of larch microsporocytes, proteomic analysis of proteins present in the cytoplasm of microsporocytes, bioimaging techniques in situ, and in vivo at the level of single RNA molecules and PLA technique.

We believe that the retention may undergo both transcripts of genes involved in meiotic division, and the housekeeping gene transcripts. Understanding this phenomenon will significantly expand our better understanding of the processes of **post-transcriptional regulation of gene expression, Cajal bodies role in the metabolism of RNA and mechanisms involved in the maturation of generative plant cells.** It will enrich the recent knowledge on cell biology as well as developmental biology.