

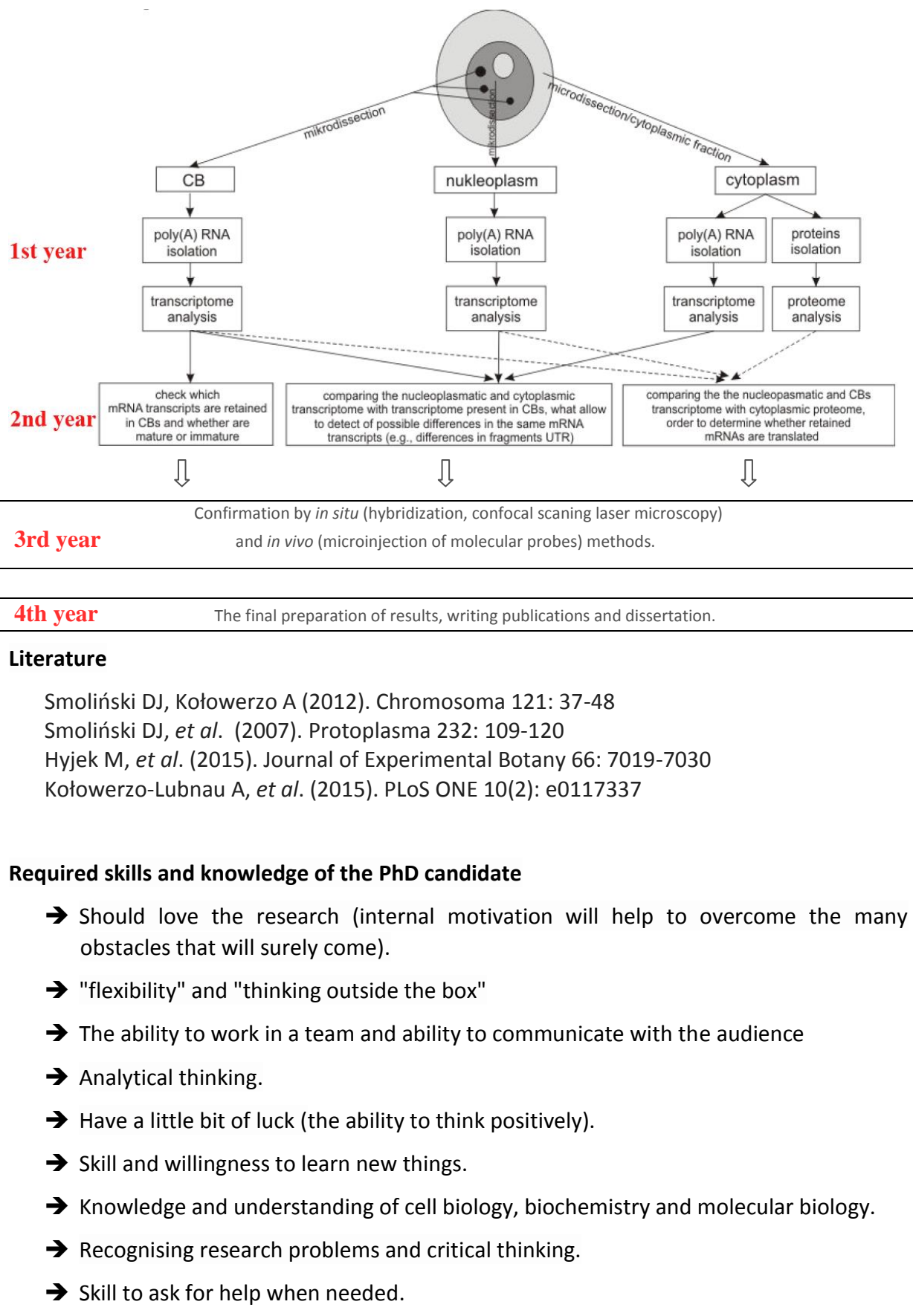
<b>Tytuł projektu</b>
Jądrowa retencja mRNA. jako nowy mechanizm posttranskrypcyjnej regulacji ekspresji genów w komórkach roślinnych
<b>Project title</b>
<b>Nuclear retention of mRNA as a new mechanism of post-transcriptional regulation of gene expression in plant cells.</b>
<b>Dyscyplina /Area of science</b>
Nauki biologiczne/ Biological sciences
<b>PROJECT DESCRIPTION</b>
<p>Project is concentrated on the new role of Cajal bodies (CBs), associated with the accumulation of polyadenylated transcripts. The results of our previous studies have shown that polyadenylated RNA, as well as mRNAs are accumulated in CBs during meiotic prophase (Smoliński &amp; Kołowerzo 2012). mRNA accumulation in these bodies is a phenomenon not previously described in the literature. While transcriptional activity is high (Smoliński <i>et al.</i> 2007, Hyjek <i>et al.</i> 2015), microsporocytes synthesize mRNA in large quantities (Kołowerzo <i>et al.</i> 2015). Significant fraction of these transcripts, however, is not exported into the cytoplasm, and is retained in the nucleus – they accumulate within both the nucleoplasm and CBs. The amount of mRNA in CBs gradually increases, and the highest level of retained transcripts in the CBs is observed prior to their massive export into the cytoplasm. These observations lead us to hypothesis <b>that Cajal bodies are involved in post-transcriptional regulation of gene expression by nuclear retention of mRNA transcripts</b>. Studies on the nuclear retention of the mRNAs indicated that it had a significant impact on the regulation of gene expression by regulating the export and translation delay, which allows the synthesis of specific proteins at a strictly controlled time or conditions. In this project we want know the mechanisms of retention mRNA transcripts in CBs.</p> <p>In order to verify that Cajal bodies are involved in post-transcriptional regulation of gene expression by nuclear retention of mRNAs transcripts we have planned the following research tasks:</p> <ul style="list-style-type: none"> <li><b>(1) examine which transcripts are stored in CBs,</b></li> <li><b>(2) explain what mechanisms are responsible for the retention of the mRNAs in CBs,</b></li> <li><b>(3) determine which genes are regulated by nuclear retention of the mRNA.</b></li> </ul>

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* (Fig. 1).

Total RNA will be isolated from the microsporocytes in particular stages, and next, the mRNA transcripts will be obtained. From the cells in the individual stages will be isolated CBs, nucleoplasm and cytoplasm, from which poly (A) RNA will be isolated in an analogous manner (**Fig. 1<sup>st</sup>** year of study). Isolated transcripts will be sequenced and subjected to functional analysis, which will allow for: (1) which transcripts are retained in CBs, (2) in what form mRNAs are stored in CBs: mature or immature, (3) and are CBs only a place of retained mRNAs accumulation or they structures where post-transcriptional modifications of mRNA, or processes leading to inhibition of their export to the cytoplasm take place (**Fig.1 2<sup>nd</sup>** year of study). To verify that mRNAs retention in the nucleus of microsporocytes is to delay the translation there will be cytoplasmic protein isolated from microsporocytes and proteomics analysis will be performed (**Fig.1 2<sup>nd</sup>** year of study). Based on the results of sequencing, it will be possible to design molecular probes that will be used to trace the process, retention of selected mRNAs *in situ* (fluorescent in situ hybridization) and *in vivo* (microinjection using molecular beacons live cell imaging) (**Fig.1 3<sup>rd</sup>** year of study).

The results will give a vast body of data on the involvement of the CBs in the regulation of gene expression by mRNAs retention. The research will broaden the knowledge on the new functions of the cell nucleus in the regulation of gene expression during the development and differentiation of cells. Understanding this mechanism will contribute significantly to the understanding of the processes involved in the maturation of germ cells in plants as well as posttranscriptional regulation of mRNA expression in eukaryotic cells. This project concerns the processes involved in the normal development of larch germ line cells. This is an extremely important species, both ecologically and economically, cultivated on a large scale in Europe, Asia and North America. However, in recent years, larch cultivation becomes increasingly difficult. It is associated with disorders of sexual reproduction which in turn leads to a very frequent abortion of the embryo. Our research will enable determination of the mechanisms involved in the normal development of the germ line cells of European larch.

**Fig.1 Schedule of the project**



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