Summary

Small RNAs (sRNAs) are short RNAs of approximately 20-24 nucleotides in length, that are involved in a variety of silencing processes in animals and plants. They include two predominant groups: small interfering RNA (siRNA) and microRNA (miRNA). The two groups are involved in gene silencing by incorporating in the RISC complex, guiding it to perform cleavage to transcripts which are called "target-transcipts" or to inhibit their translation. Identification of targrt-transcipts by RISC is accomplished by base paring to the sRNA. In plants, miRNAs regulate transcripts that encode a wide range of transcription factors, and other proteins that are involved in hormonal regulation, polarity and organ identity, response to stress, flowering time, leaf development and polarity, developmental stages transition and sRNAs biogenesis. Because sRNAs were formed early in the eukaryotes evolution, it is customary to think they fulfilled conserved roles during plant evolution. Mosses are one of the oldest groups of land plants, and apparently share a common ancestor with flowering plants. *Physcomitrella patens* (*P. patens*) is a model moss because it enables homologic recombination, which facilitates directed gene knock out.

The current research's purpose was identification and characterisation of miRNAs and their target-transcripts from the moss *P. patens*. In the lab, sRNAs from young gametophyte (protonema) were cloned. Three approaches were used to identify miRNAs out of the library sequences: homology search between sRNAs from our library and higher plants' miRNAs; search for sequences that embrace sRNAs from our library, and resemble miRNA precursors; Northern blot hybridizations to determine accumulation of sRNAs in the cell. This strategy led to the identification of 28 miRNAs, that belong to 23 families, 4 of them (miR156, miR319, miR390, miR535) are conserved also in flowering plants. Three miRNA biogenesis intermediates were identified, one of which is predicted to function as a miRNA. Their identification indicates the involvement of Dicer-like enzyme, that has not yet been characterized in moss miRNA biogenesis. The extensive complementary between the miRNA and the target-transcripts, was used to identify 13 putative target-transcripts, 6 of which were experimentally varified.

The expression pattern of a miRNA can suggest its potential function in the plant. In an expression analysis of the identified miRNAs, three expression patterns

were observed: similar expression during all the gametophyte development; specific expression to young or mature gametophyte. In addition, I found that the expression of 2 miRNAs acsended and 1 decsended as a result of Auxin supplement.

Recently, a transciption-target of miR390 (*TAS3*), that encodes ta-siRNAs and not protein, has been discovered in arabidopsis. This is an siRNA group that was recently discovered and like miRNAs regulates negatively other genes *in trans*. However, unlike, miRNAs their biogenesis depends on RNA dependant RNA polymerase 6 (RDR6). During the library analysis we have identified that Ppt-miR390a's transcription-target (contig 13502) is identical to 11 sRNAs from the moss library. The identity of this transcript as a target of Ppt-miR390a, and that it encodes to ta-siRNAs in moss was validated. I have found that their biogenesis, similar to arabidopsis, is dependant of the moss homolog of RDR6, and the RDR6 knock out lines that lack the ta-siRNAs expression, show early development. This phenotype resembles the arabidopsis lines that lack the ta-siRNAs expression that originate in *TAS3*.

In conclusion, I succeded in identifying a variety of sRNA groups in moss, at least 23 of them are miRNA families. This number is similar to the number of known miRNA families in higher plants with known genom (arabidopsis, rice, poplar). Thus, regulation extent by miRNAs in lower plants such as mosses, is at least equall to that in higher plants. This fact reflects the central role of miRNAs in land plants evolution.