Monika Jóźwiak

A role of DEAD-box RNA helicases DRH1, RH46 and RH40 in microRNA biogenesis in plants

Abstract

MicroRNAs (miRNAs) are short, single-stranded and non-coding RNAs that regulate gene expression at the post-transcriptional level. The process of miRNA production is intricate and involves a multitude of proteins. It is believed that the most significant factors are: DICER LIKE1 (DCL1), HYPONASTIC LEAVES1 (HYL1) and SERRATE (SE), which collectively constitute the core of the Microprocessor complex. The experiments conducted at the Department of Gene Expression have demonstrated that SE is associated with three RNA helicases from the DEAD-box family designated DRH1 (RH14), RH46 and RH40. In order to gain insight into a role of DRH1, RH46 and RH40 in RNA metabolism in Arabidopsis, the interaction between the aforementioned helicases and SE was studied using the FRET-FLIM technique. The analysis demonstrated that all three helicases directly interact with SE. Furthermore, the phenotype of the *drh1-1 rh46-1 rh40-1* mutant at standard (22°C) and modified (16°C) growth conditions was evaluated. The analysis revealed alternations in the phenotype of the mutant cultivated at a lower temperature. The *drh1-1 rh46-1 rh40-1* mutant phenotype was successfully rescued by the expression of GFP-DRH1 fusion protein in the *drh1-1 rh46-1 rh40-1* mutant background. This transgenic line was used to study the DRH1 interactome, utilizing the LS-MS/MS technique. The analysis showed that DRH1 is associated with a few miRNA biogenesis factors, including HESO1, CDC5, GRP7, CPL1 and AGO1, which was identified as one of the most enriched proteins in the analysis. The DRH1-AGO1 interaction was validated using the FRET-FLIM technique.

Subsequently, the level of miRNAs in the *drh1-1 rh46-1 rh40-1* mutant was evaluated through next-generation sequencing (RNA-seq). The analysis showed changes in miRNA accumulation in the mutant when cultivated at 22°C and 16°C. Furthermore, the results demonstrated that DRH1 interacts with the CTD domain of RNAPII, which is in accordance with the *in silico* predictions. However, the analysis demonstrated that the WW domain is not essential for this interaction, contrary to the initial *in silico* prediction. Moreover, the secondary structure of selected pre-miRNAs in the *drh1 rh46 rh40* mutant was investigated using the

targeted DMS-MaPseq technique. The studies reveal alterations in the structure of a particular subset of pre-miRNAs.

In conclusion, the data presented here suggest that DRH1, RH46 and RH40 influence the biogenesis of miRNAs, potentially by modulating the structure of a subset of miRNA precursors.