ABSTRACT

Knowledge of the processes of RNA interference, the regulation of gene expression by small RNAs (sRNAs), has grown at an unprecedented rate over the last 30 years. Some of the findings were literally revolutionary, as they revealed events that overturned many long-held notions. Many phenomena have been shown to be highly conserved and common to organisms of different species, but others are specific to certain lineages or have not yet been fully explored. There is also a lack of knowledge about the interconnection of numerous pathways – for example between silencing at the transcriptional (TGS, leading to the promoter methylation) and post-transcriptional levels (PTGS, affecting mRNA stability or translation). The present work summarizes the findings of two published and two unpublished works and attempts to describe some of the less known sites of RNA interference using various plant model organisms.

Research on *Solanum tuberosum* transgenic lines has revealed the ability of 5-azacytidine to restore the expression of transcriptionally silenced transgenes at the whole plant level. *De novo* regeneration from leaves of such plants can lead to re-silencing of reactivated transgenes and thus serves as a selection method to exclude lines prone to spontaneous silencing. The nature of changes in the expression of the two reporter genes indicated the coupling of PTGS and TGS, but also the possibility of a gradual spread of methylation along the inserted T-DNA. Therefore, further research was aimed for the induction of PTGS and TGS at the cellular level in *Nicotiana tabaccum* BY-2 lines. Both approaches led to the generation of specific sRNAs matching predominantly to the target locus region, but sRNAs of a transitive nature outside the target locus also emerged. In particular, sRNAs from the terminator region could thus play a role in the propagation of methylation along the T-DNA, since the same terminator was used multiple times. The methylation of target loci was otherwise very accurate and did not spread to its surroundings during the monitored 10-14 days.

In the last part of the work, I focused on proteins SAG18 and aPHC from *Arabidopsis thaliana* with a certain homology to the animal transmembrane dsRNA transporter, the protein SID-1. The study of SAG18 function in BY-2 cells did not demonstrate the effect of externally added sRNAs on the level of transcription of the targeted transgene, but the same negative results were obtained with SID-1 transporter from *Caenorhabditis elegans*. Analyses of double mutant plants in *SAG18* and *aPHC* showed no significant changes in phenotype, but only indicated their possible role in the function of stomata guard cells.

Key words: RNA interference, gene silencing, PTGS, TGS, sRNAs, transmembrane sRNAs transport, 5-azacytidine