

Research Project

Silencing and cell cycle induced and controlled by geminiviruses and caulimoviruses

Third-party funded project

Project title Silencing and cell cycle induced and controlled by geminiviruses and caulimoviruses

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Project start 01.11.2010

Probable end 31.07.2013

Status Completed

The plant RNA silencing machinery comprises several specialized, small RNA-generating pathways that regulate gene expression at post-transcriptional and transcriptional levels and mediate stress response and defense against pathogenic nucleic acids. We and others have shown that components of multiple silencing pathways mediate anti-viral defense. To counteract RNA silencing, viruses have evolved to code for protein suppressors targeting different steps of the silencing pathway. At the evolutionary scale, such a continuous arms race between plants and viruses is thought to have contributed to the current shape of the plant genome, with multigene families coding for core components of the silencing machinery, e.g., four Dicer-like (DCL), six RNA-dependent RNA polymerase (RDR) genes for *Arabidopsis thaliana*. In our past and current SNSF projects 3100A0-111277 and 31003A-122469 (Silencing and cell cycle induced and controlled by geminiviruses), we used genetic and biochemical approaches to investigate the role of these and other components of the silencing machinery in production and utilization of virus-derived small interfering RNAs (siRNAs), the effectors of an RNA-induced silencing complex (RISC). We have discovered that, while RNA viruses are targeted primarily by DCL4 and DCL2, three DCLs (2, 3, and 4) target the geminivirus Cabbage leaf curl virus (CaLCuV) and all the four DCLs the pararetrovirus cauliflower mosaic virus (CaMV). Our studies of CaLCuV-induced gene silencing suggest that a DCL4-/RDR6-dependent pathway might mediate amplification and systemic spread of anti-viral silencing. Interestingly, we found that this pathway is suppressed during CaMV infection by the viral transactivator/viroplasm (TAV) protein. Furthermore, our studies revealed that RNA tobamoviruses suppress silencing by preventing HEN1-mediated methylation of siRNAs, which might be important for their stabilization and incorporation in RISC. Besides understanding the mechanisms of induction and suppression of anti-viral silencing, the usage of viruses as a tool has helped us to identify new components and features of the silencing machinery (e.g. an endogenous silencing suppressor WEL1 induced by geminiviruses and a candidate for a RNA-based suppressor in CaMV) and to dissect molecular mechanisms of endogenous silencing pathways. To further investigate the complex interactions of plant viruses with the silencing machinery, we shall use genome-wide approaches such as transcriptomics and high-throughput sequencing of small RNAs from virus-infected *Arabidopsis* plants and various silencing-deficient mutants. In studying molecular mechanisms of silencing induction and suppression by DNA and RNA viruses we shall focus on the key steps of RNA silencing such as dicing, RISC assembly and slicing, and RDR-dependent amplification of siRNAs. We shall address open questions on the nature of anti-viral silencing signal(s) spreading ahead of the virus to immunize plant cells, and on genetic requirements for biogenesis of the signal molecule(s). Finally, using DNA viruses that

form minichromosomes, we shall investigate cellular and antiviral functions of nuclear components of the silencing machinery such as the RNA polymerase IV and V complexes involved in RNA-dependent DNA methylation and chromatin modification.

Keywords silencing, silencing suppression, caulimoviren, geminiviren, Silencing Resistance Plant Virus

Financed by

Swiss National Science Foundation (SNSF)

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