# Roles of RNA Silencing in Symptom Development Induced by Plant Virus Infection

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RNA silencing is a conserved, sequence-specific gene regulation system, which has an essential role in development and maintenance of genome integrity. RNA silencing machinery in plants is considered to be a natural antiviral defense mechanism. In contrast, plant viruses have evolved a counterdefense strategy, producing RNA silencing suppressors (RSSs) that interfere with the RNA silencing pathway. Plant RNA silencing has often been implicated as a molecular mechanism for symptom induction caused by viruses or viral subviral agents. However, no explanation for specific symptoms caused by RNA silencing has ever been confirmed nor has any report explained the molecular basis for a specific viral symptom.

*Cucumber mosaic virus* Y satellite RNA (Y-sat) is a noncoding subviral RNA and modifies the typical symptom induced by CMV in specific hosts; CMV causes a green mosaic on tobacco plants but CMV+Y-sat causes a bright yellow mosaic. The molecular basis for this Y-sat-induced symptom modification had been a long-standing mystery. Recently, we found that the mRNA of tobacco magnesium protoporphyrin chelatase subunit I (*ChlI*, the key gene involved in the chlorophyll synthesis) had a 22-nt long complementary sequence with Y-sat. In addition, we revealed that the Y-sat-derived short interfering RNAs (siRNAs) in the virus-infected plant downregulated the mRNA of *ChlI* by targeting the complementary sequence [1]. This discovery of the molecular basis of the symptom modification induced by Y-sat is the first demonstration that a subviral RNA can induce disease symptoms by regulating host gene expression through the RNA silencing machinery.

The induction of plant disease symptoms or progression of infection seems to be affected by the activities of viral RSSs. On the assumption that we may be able to control plant virus diseases by blocking viral RSSs, we developed a strategy to screen inhibitors that block the association of RSSs with siRNAs using a surface plasmon resonance assay. The screened chemicals were tested for competition with RSSs for binding to siRNAs using a mobility shift assay. We then confirmed that tested chemicals actually inhibited the RSS activity *in vivo* using the protoplast assay, and that one of the chemicals was effective in decreasing viral accumulation resulting delay of symptom development on tobacco plants infected with various viruses [2]. Because we noticed that the RSS inhibitor was similar to ascorbic acids (vitamin C), we now study the effects of ascorbic acid and its derivatives on eliminating viruses from vegetative propagation crops such as asparagus in *in vitro* tissue culture.

[1] Shimura H., Pantaleo V., Ishihara T., Myojo N., Inaba J., Sueda K., Burgyán J., Masuta C.: “A viral satellite RNA induces yellow symptoms on tobacco by targeting a gene involved in chlorophyll biosynthesis using the RNA silencing machinery”, PLoS Pathog., 7: e1002021 (2011).

[2] Shimura H., Fukagawa T., Meguro A., Yamada H., Oh-hira M., Sano S., Masuta C.: “A strategy for screening an inhibitor of viral silencing suppressors, which attenuates symptom development of plant virus”, FEBS Lett., 582: 4047-4052 (2008).