RNA-dependent RNA polymerase of turnip crinkle virus and the effect of coat protein on the replication of subviral RNAs

Jong-Won Oh, *University of Massachusetts Amherst*

Abstract

In this dissertation, I report the functions of the TCV coat protein in TCV viral and subviral RNA replication using in vivo and in vitro systems. In chapter 2, I present my results on construction of a biologically active, full-length cDNA of cardamine chlorotic fleck virus (CCFV), a new carmovirus. In chapter 3, I show that p28 and p88 of TCV viral RdRp components can be expressed in insect cells using a baculovirus expression system. In addition, I demonstrate that p28 and p88 are present in infected plant cells using antiserum against p28 fusion protein expressed in E. coli. In Chapter 4, using a TCV derivative with a deletion of coat protein ORF (TCVΔCP) and several other TCV derivatives that alter the TCV coat protein ORF, I show that TCV coat protein up-regulates the accumulation of sat-RNA C and DI RNA G, but not sat-RNA D, in protoplasts, suggesting that TCV coat protein plays a role in regulation of subviral RNA replication in vivo. In chapter 5, I show that TCV coat protein functions in TCV viral and subviral RNA transcription and TCV RdRp expression. The immunochemical analysis of cellular proteins obtained from protoplasts infected with TCVΔCP, TCV, TCV-CP$\rm\sb{CCFV}$ (a TCV derivative containing the CCFV coat protein ORF, or TCV-CPm (a TCV derivative producing low amounts of TCV-related coat protein) suggests that TCV coat protein might be involved in the repression of TCV viral RdRp synthesis and regulation of readthrough of p28, leading to a change in the stoichiometry of p28 and p88. I show evidences that TCV RdRp prepared in the absence of coat protein has template dependency and specificity with small, subviral RNA sized-templates. However, with genomic RNA sized (3-4 kb) templates, no strict template specificity is observed. Using an in vitro RdRp assay system free of coat protein, I demonstrate that TCV coat protein not only enhances the transcription of minus- and plus-strand RNA of sat-RNA C and DI RNA G at low concentrations, but also inhibits the transcription of both strands of sat-RNA C and DI RNA G at higher concentrations. Using sat-RNA D minus-strand template, no inhibition of transcription is observed even in the presence of higher than 10-fold molar ratio of TCV coat protein to template. Similar stimulatory and inhibitory effects of TCV coat protein on the transcription of TCV plus- and minus-strand genomic RNA are observed, and the inhibitory effect is more pronounced during minus-strand synthesis. These in vitro RdRp assay results suggest that the TCV coat protein may act as an important viral factor involved in regulation of viral and subviral RNA replication during virus infection in plants. ^

Subject Area

Microbiology|Plant pathology

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