Self-interaction of the RNA-dependent RNA Polymerase of *Citrus tristeza virus*

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**Abstract**—All RNA viruses encode the RNA-dependent RNA polymerases (RdRps) responsible for transcription and replication of viral genomes. The RdRps are structurally and functionally conserved among all positive and negative strands RNA viruses. RdRps from many positive and negative strands animal RNA viruses have shown to form oligomers, but the self interaction of the RdRps has not been explored in great extent in plant RNA viruses. The RdRp of *Citrus tristeza virus* (CTV) was differentially tagged with HA and FLAG epitopes and tagged proteins were expressed individually and together in *Escherichia coli* to study self-interaction. Immunoprecipitation of the expressed proteins with anti-FLAG antibody followed by western blot with anti-HA antibody showed that HA tagged RdRp was co-immunoprecipitated with FLAG tagged RdRp demonstrating that RdRp of CTV interacts with itself to form oligomer. Self-interaction tests of the RdRp conducted in a yeast-two-hybrid system commonly used for protein-protein interactions showed that RdRp was able to bind itself in yeast cells indicating that the RdRp can bind itself may form oligomer in a eukaryotic cell. Truncated RdRps tagged with HA epitope (RdRpΔ1-166-HA, RdRpΔ1-390-HA, RdRp1-169-HA) were constructed and co-expressed with FLAG tagged full-length RdRp in *E. coli* to map the oligomerization site. Co-immunoprecipitation with anti-FLAG revealed that while RdRpΔ1-166-HA, RdRpΔ1-390-HA did not bind to the full-length FLAG tagged RdRp, but the RdRp1-169-HA was pull down with full-length FLAG tagged RdRp showing that the oligomerization site was located in the first 169 amino acid region of the RdRp. The binding tests performed in the yeast-two-hybrid system with truncated RdRp constructs confirmed that the oligomerization site reside in the N-terminal region and first 169 aa CTV RdRp is necessary and sufficient for oligomerization both in bacterial and yeast cells.

**Keywords**—*Citrus tristeza virus*, RNA-dependent RNA polymerase, oligomerization protein-protein interaction, epitope tagging, co-immunoprecipitation, yeast-two-hybrid system, protein expression, immunoblotting.

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