Technical Report for 2007-2008 for Grant # DE-FG02-04ER15531
Closeout Report

Title: The Role of a Host Protein (TIP) in the Resistance Response of Arabidopsis to Turnip Crinkle Virus Infection.

Summary

Our research on *Turnip crinkle virus* (TCV) has shown that the viral capsid protein (CP) is both a virulence factor as well as the elicitor of a hypersensitive resistance response (HR) to the virus in *Arabidopsis*. Initially, we identified a protein from *Arabidopsis* that specifically interacted with the viral CP using a yeast two-hybrid screen. This protein, designated TIP for TCV-Interacting Protein, is a member of the NAC family of plant transcription factors implicated in the regulation of development and senescence. When TCV CP was mutated to eliminate its ability to interact with TIP, the corresponding virus mutants broke the HR-mediated resistance conferred by the HRT resistance (R) gene in *Arabidopsis* ecotype Dijon (Di)-17. This result suggested that TIP is a component of the signal transduction pathway that leads to the genetically specified TCV resistance. We next confirmed that TIP and the viral CP interact in plant cells and that this interaction prevents nuclear localization of this transcription factor. We demonstrated that TCV CP suppresses post-transcriptional gene silencing (PTGS), a newly discovered RNA-mediated defense system in plants. Together these results suggest that the CP is a virulence factor that could well be functioning through its interaction with TIP. We have proposed a model involving the role of TIP and CP in triggering HR mediated plant defense that fits with the current thinking about how gene-for-gene resistance may function. A unique component of our system is the opportunity to link R-gene function with the newly discovered RNA silencing pathway that is not only a potent defense against viral pathogens, but also regulates early development in plants. In the current funding period we made several significant findings: First, we completed an array analysis comparing gene expression in Arabidopsis infected with TCV and a mutant virus unable to bind TIP. Second, we produced transgenic lines that over-express and inducibly under-express TIP. These accomplishments now form the basis for our continued effort towards providing a complete understanding of molecular events leading to susceptible and resistant interactions between TCV and *Arabidopsis* plants. Our data strongly suggest that TIP is involved in activating the SA-mediated defense pathway and that TCV CP acts to repress this role of TIP.

Report of Progress by Specific Objectives:

The results reported here include those completed in the past year using existing funds during the extension of the funding period. The specific experiments we propose to complete during the extension period along with a brief description of the results are outlined below:

Objective 1: Further elucidation of the cellular function(s) of TIP.
We completed the initial characterization of transgenic Arabidopsis lines that have the TIP gene constitutively up-regulated and a gene expression microarray analysis has been done on one of the up-regulated lines. We also created TIP down-regulated lines that are inducible. These lines were proposed for further characterization during the extension period for differential gene expression in viral infections by wild type and mutant TCV. We were able to complete the microarray analysis and a manuscript is in draft stages on the data analysis.

Objective 2: Investigating the role of TIP in *Arabidopsis* resistance to TCV infection.

We made very significant progress toward this goal with the completion a few critical experiments during the extension period. Real time PCR analysis was completed to support the microarray analysis which clearly showed that the mutant virus R6A, in contrast to TCV, triggered the salicylic acid (SA) defense pathway in Col-0. This has provided conclusive data showing that TCV specifically down-regulates host anti-viral defense in susceptible Col-0. Moreover our results also confirm that mutant R6A, the virus that is unable to interact with TIP, induces a stronger defense response that results in reduced virus accumulation as measured by RT-PCR and Northern blot analysis. We were also able to show that mutant Arabidopsis defective in an SA pathway defense could not mount the basal defense response against R6A observed in wild type plants. In addition, the R6A defense response was also muted in the transgenic lines in which TIP was both up and down regulated. These data argue strongly in favor for our hypothesis that TIP is key negative regulator of anti-viral defense. The role of TIP in activation of basal defense is now firmly established and will be written up for publication within the next 6 months.

We also completed a substantial analysis of genes involved in the anti-viral silencing pathway in Arabidopsis. This was reported in a PNAS paper published in October. We are now pursuing some follow up studies that show a direct link between the defense responses modulated by the TIP gene and the induction of key member genes of the silencing pathway.

Papers published from DOE supported work in the extension period (PDF files attached).

Qu, F., Ye, X., and Morris, T.J. 2008. Arabidopsis DRB4, AGO1, and AGO7 participate in a DCL4-initiated antiviral RNA silencing pathway that is negatively regulated by DCL1. PNAS 105, 14732-14737 plus SI.

Qu F and Morris T J. 2008. Carmovirus. Encyclopedia of Virology, 5 vols. (B.W.J. Mahy and M.H.V. Van Regenmortel, Editors), pp. 453-457 Oxford: Elsevier.

Blake, J. A., Lee, K.W., Morris, T.J. and Elthon, T.E. 2007. Effects of turnip crinkle virus infection on the structure and function of mitochondria and expression of stress proteins in turnips. Physiologia Plantarum, 129: 698-706.

Qu, F., and Morris, T.J. 2007. Plant virus silencing suppressors and RNA silencing in plants. Invited chapter A20710, Encyclopedia of Life Sciences, Wiley & sons, pp7.

Other Papers Published on related but not DOE supported research:

Powers, J.G., Sit, T.L., Qu, F., Morris, T.J., Kim, K-H., Lommel, S.A. 2008. A versatile assay for the identification of RNA silencing suppressors based on complementation of viral movement. MPMI 21, 879-890.
Stenger, DC., Young, BA., Qu, F., Morris, TJ & French, R. 2007. Wheat streak mosaic virus lacking HC-Pro is competent to produce disease synergism in mixed infections with Maize chlorotic mottle virus. Phytopathology 97: 1213-1221.

Invited Presentations:

Invited Seminar, Plant Pathology, U Kentucky. How plants defend themselves against virus diseases. March 10, 2008.

Invited Seminar, Dept of Biology, U Nebraska, Omaha. Plant defenses directed against virus diseases. (Oct 2008).

Invited participant: Nebraska Center for Virology Symposium: Oct 10, 2008

Abstracts of presented Papers and Posters:

Teresa Donze, P. Twigg, TJ Morris. 2008. Analysis of gene expression and viral accumulation in Arabidopsis mutant lines infected with turnip crinkle virus. ASV Annual Meetings, Control Number: 335

Qu, F., Ye, X., and Morris, T.J. Arabidopsis DRB4, AGO1, and AGO7 participate in a DCL4-initiated antiviral RNA silencing pathway that is down-regulated by DCL1. ASV Annual Meetings, Control Number: 80.

Stenger, DC., Young, BA., Qu, F., Morris, TJ & French, R. 2007. Wheat streak mosaic virus lacking HC-Pro is competent to produce disease synergism in mixed infections with Maize chlorotic mottle virus. ASV annual meeting OSU, July 2007. (poster)
Sung-Hwan Kang, Feng Qu, T. Jack Morris 2007. The role of host proteins in resistance response to turnip crinkle virus infection. ASV annual meeting OSU, July 2007. (poster)

Feng Qu, Xiaohong Ye, T. Jack Morris. Arabidopsis DCL1 antagonizes the antiviral defense role of DCL4. ASV annual meeting OSU, July 2007. (talk)

Veronica R. Basnayake, Ty Jensen, Teresa Donze, Paul Twigg, Feng Qu and T. Jack Morris. The NAC transcription factor TIP is a negative regulator of Turnip Crinkle Virus infection in susceptible *Arabidopsis thaliana* plants. ASV annual meeting OSU, July 2007. (talk)

Drake C. Stenger, Brock A. Young, Feng Qu, T. Jack Morris, and Roy French. 2007. Wheat streak mosaic virus P1, not HC-Pro, facilitates disease synergism and suppression of post-transcriptional gene silencing. APS annual meeting, August (talk).

Patent Disclosures:
None