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Elements of a Red Queen race between plant hosts and their viral pathogens: Interactions between *Turnip crinkle virus* and *Arabidopsis*

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Our research focuses on understanding of the basis of host-pathogen interactions and the molecular components that drive the evolutionary race between plants and the pathogens that attack them. Expression of resistance genes (R genes) is one defensive strategies employed by plants to confer resistance to specific strains of a pathogen including viruses. R genes initiate a hypersensitive type of resistance cascade (HR) that limits systemic invasion of a pathogen. It has been suggested that the extensive variation identified among R genes reflects a type of surveillance system that promotes a "co-evolutionary arms race" in which resistance specificity and pathogen virulence continually adapt to each other (Dangl & Jones, 2001). Post-transcriptional silencing of the invading RNAs directed against viral pathogens has been recognized as another general defense system in plants. The recent finding that many plant viruses encode proteins that suppress this anti-viral defense system suggests that co-evolutionary adaptation between plant defense and viral counter-defense strategies is an evolutionarily active process as well (Vance and Vaucheret, 2001; Waterworth et al., 2001). Recent work from our lab (Ren et al., 2000; Qu et al., 2003) has shown that both types of defensive strategies are employed by plants to counteract invasion by Turnip crinkle virus (TCV), a member of the small RNA virus family, Tombusviridae (Morris 2000; Qu and Morris, 1999). In this talk, I will summarize our results on the molecular interactions between TCV coat protein (CP) and an *Arabidopsis* protein that appears to trigger a specific R gene based HR response. I will also present evidence showing that systemic invasion of host plants by this virus is also promoted by the viral CP functioning as a suppressor of the host RNA silencing system. Our results demonstrate that multiple functions of the viral CP ensure systemic invasion by the virus and provide important clues toward understanding the relatively sophisticated network of defense pathways that protect plants against viral infections.

The number of plant R genes directed against viral pathogens is relatively small in comparison to those against other pathogens. It has been recently estimated that over 150 sequences with homology to the most common NB-LRR class of R genes are present in the *Arabidopsis* genome (Dangl & Jones, 2001). Among these is HRT, an R gene recently shown to be responsible for triggering HR to TCV in the TCV-resistant *Arabidopsis* line Di-17 (Cooley et al., 2000). Interestingly, although HRT was shown to be essential for HR, it was not sufficient to provoke the systemic resistance response that limits viral invasion. This suggested that a more complex pathway involving several genes is likely involved in conditioning resistance to this viral pathogen.

TCV is a small icosahedral virus with a 4 kb RNA genome encoding five viral proteins including two that are required for replication (p28 and p88), and two that function to facilitate the cell-to-cell movement (p8 and p9). The last gene encodes a 38 kDa capsid protein (CP) that packages the viral RNA into an icosahedral particle of determined structure (Qu and Morris, 1999). Both of the viral MPs and the CP are needed for long distance transport and systemic invasion in *Arabidopsis*. Several studies implicate the TCV CP as the elicitor of the resistance response, however no direct evidence of *HRT* physically interacting with TCV CP has been forthcoming. In our studies, we identified an *Arabidopsis* protein, which we named TIP for TCV-Interacting Protein, that specifically interacts with TCV CP to promote an HR response in plants carrying the HRT gene (Ren et al., 2000).

We isolated TIP by screening an *A. thaliana* cDNA library using the yeast two-hybrid system with TCV CP as the bait. Sequence analysis and experimental data have confirmed that TIP is a member of the NAC family of plant transcription factors. Deletion analysis of the CP mapped the interacting site to the N-terminal 25 amino acids of the R domain of the CP. We then introduced single amino acid changes into this region and isolated several mutant CPs unable to interact with TIP. Aware that TCV CP functioned as the elicitor of resistance in the inbred line Di-17, we inoculated susceptible and resistant *Arabidopsis* with the CP mutants that failed to interact with TIP. Every CP mutant that had lost the ability to interact with TIP was now virulent and unable to trigger the HR response in resistant lines. This positive correlation between the ability of TCV CP to interact with TIP and ability of TCV to induce resistance in Di-17 strongly suggested that the TIP-CP interaction is the initiating event in the resistance response. We then used the Agrobacterium transient expression system to co-express both TCV CP and GFP tagged TIP in the same cell. These experiments clearly showed that the viral CP blocked nuclear localization of TIP (Ren et al., submitted). The results strongly support the conclusion that the CP-TIP interaction was directly responsible for blocking the nuclear localization of TIP. Hence, we now have direct physical evidence that the interaction between the viral CP and the transcription factor occurs in planta and is central to regulating host susceptibility.

These results support a model in which the R gene does not interact directly with an avirulence factor of a pathogen but rather acts through a more complex pathway consistent with the prevailing "guard hypothesis" of R gene function. In this "guard" model, they envision the R protein functioning as a monitor of whether a cellular protein is under attack from a pathogen effector protein (Dangl & Jones, 2001; Marathe & Dinesh-Kumar, 2003). In keeping with this model, we hypothesize that TIP functions as a transcriptional activator to promote a basal level of resistance in the plant. The viral CP, after being produced in very high concentration in infected cells, would function as a virulence factor by binding to TIP and thus reduce basal resistance and promote more rapid systemic infection. In resistant plants, HRT may well "guard" the TIP protein by detecting the change due to

the TIP-CP interaction which would then lead to a strong HR mediated resistance response.

Post-transcriptional gene silencing (PTGS) is a second example of a general antiviral defense mechanism in plants (Waterworth et al., 2001; Vance & Vaucheret, 2001). This idea has emerged from several lines of evidence including the demonstration that many viruses encode proteins that suppress PTGS and promote recovery of the plant from the virus infection. In this model, the virus enters a race with the host such that it must move faster than the silencing signal of the plant defense response in order to successfully colonize systemic parts of the plant. An additional primary focus in our lab has been to determine the role the viral CP has on the basal defense of the plant host. We recently demonstrated that TCV CP suppresses post-transcriptional gene silencing using an Agrobacterium-based transient expression system and a transgenic N. benthamiana line stably expressing a GFP transgene to test the silencing suppression mechanism (Qu et al., 2003). The results showed that TCV CP functions to repress RNA silencing at an early stage in the silencing initiation. Our finding that TCV CP is a suppressor of RNA silencing is truly an exciting result because it may directly connect the effector function of TCV CP in the gene-for-gene resistance model as a virulence element responsible for down-regulating a basal host defense system in the form of RNA silencing. This result permits us to speculate that a possible explanation for the specific interaction of CP with TIP, responsible for triggering HRT based resistance, is to specifically prevent TIP from activating genes responsible for a form of basal resistance mediated by silencing. This hypothesis is consistent with an extension of the "guard hypothesis" discussed earlier. Implications of our work on a broader understanding of plant resistance mechanisms against viruses will be discussed further.

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SVI-4

Characterization of *cis*-acting elements and *trans*-acting factors required for *Potato virus X* replication and virus-host interactions

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Potato virus X (PVX), the type member of the potexvirus group, is a flexuous rod-shaped virus containing a 6.4 kb plus-stranded RNA genome (Bercks, 1970; Milne, 1988). The PVX genome, which is capped and polyadenylated, encodes five open reading frames (ORFs; Bercks, 1970; Milne, 1988; Huisman, 1988). ORF1 encodes the viral replicase protein (165 kDa), which is the only viral protein absolutely required for PVX RNA synthesis. This replicase exhibits the methyltransferase/helicase/polymerase arrangement found in all nonsegmented viruses of the Sindbis-like supergroup (Rozanov, 1992) and belongs to the RdRp supergroup 3 (tymo-like lineage). The triple gene block (TB), ORFs 2-4, have been shown to be necessary for viral cell-to-cell transport (Beck, 1991; Angell, 1996), and the product of ORF5, coat protein (CP), is involved in both virus movement and encapsidation (Chapman, 1992; Oparka, 1996). During PVX infection, genomic-length plus- and minus-strand RNAs, several sgRNAs, and corresponding double-stranded RNAs are produced (Dolja, 1987; Price, 1992). The two major sgRNAs are uitlized for expression of the first TB gene (ORF2) and CP, respectively, whereas the other two TB genes (ORFs 3 and 4) are expressed from a less-abundant sgRNA (Morozov, 1991).

Positive-strand RNA viruses replicate by utilizing input genomic RNA (gRNA) as a template for synthesis of a complementary minus-strand RNA, which subsequently serves as a template for synthesis of genomic plus-strand RNA. For some plus-strand RNA virus groups, minus-strand RNA also serves as a template for synthesis of subgenomic RNAs (sgRNAs) that contain genes frequently encoding structural proteins. To understand the mechanism for PVX replication, we are studying