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

the *cis*- and/or *trans*-acting elements required for RNA replication. We have previously shown that multiple sequence and structural elements in the 5' non-translated region (NTR) of the PVX RNA affect both gRNA and sgRNA accumulation (Kim, 1996; 1997; Miller, 1998). The 5' NTR of PVX contains single-stranded AC-rich sequence and stem-loop (SL1) structure. Conserved octanucleotide sequence elements located upstream of the two major subgenomic RNAs (sgRNAs) and sequences in the 5' terminus (or 3' terminus of minus-strand RNA) are complementary, and interactions between these elements were suggested to be important for both genomic and sg plus-strand RNA accumulation (Kim, 1999). The importance of the SL1 was addressed by site-directed mutations and indicated that base-pairing was more important than sequences which was consistent with the co-variation analysis. Both the sequence and structure of SL1, especially stem C, stem D and tetra loop in SL1 region, are required for one or more aspects of PVX plus-strand RNA accumulation.

RNA-protein interactions have been shown to play a key role in many virus replication processes. Recent studies with plus-strand RNA viruses have characterized several cellular proteins that bind specifically to viral RNA and play a key role in viral RNA replication (for a review see, Lai, 1998). Cellular proteins that specifically bind to other regulatory sequence elements were observed for other viral systems. To investigate whether cellular proteins bind to these elements, we conducted electrophoretic mobility shift assays (EMSA) with protoplast protein extracts and RNA sequences from the PVX 5' non-translated region. These analyses showed that the 5' region of PVX positive-strand RNA formed complexes with cellular proteins. UV cross-linking studies of complexes formed with various deletions of the PVX RNA indicated that a 54 kDa cellular protein (p54) was bound to nt 1-46 at the 5' terminus of PVX RNA. Site-directed mutations introduced within this 46 nt region further indicated that an ACCA sequence element located at nt 10-13 was important for optimal binding. In addition, mutations that decreased the affinity of the template RNA for the cellular factor decreased PVX plus-strand RNA accumulation in protoplasts (Kim, 2002).

Through the finding that accumulation of SL1 mutants and coat protein (CP) is changed *in vitro*, we suppose that interaction of SL1 and CP is play a role in assembly and/or virus replication. To investigate CP domain bind to 5' region, we constructed CP deletion mutants and RNA-protein interaction analysis conducted using yeast three-hybrid system (YThHS). The RNA bait (5' region) and the prey protein (CP) are encoded on separate plasmids, and are co-transformed with pHybLex/Zeo-MS2 into the L40-*ura3* yeast strain. The L40-*ura3* strain contains two reporter genes (*lacZ* and *HIS3*) whose expression is regulated by LexA operator sequence. Interaction of protein hybrid1 consisting of the LexA DBD fused to the bacteriophage MS2 coat protein, the bait RNA, and the prey protein in nucleus bring the VP16 AD together with the LexA DBD, resulting in transcriptional activation of the two reporter genes (*HIS3* and *lacZ*). Specific coat protein domain, positive interactions, detected by selecting on plates lacking histidine, followed by a second screen for β-galactosidase expression.

To identify the interaction of SL1 and SL1 mutants with PVX CP, we also employed the YThHS. Each mutated clone containing sequence modifications as well as deletions was transformed with CP into yeast and tested by selecting on plates lacking histidine and for *B*-gal activity. In this YThHS, we confirmed that wild-type SL1 and PVX CP interacted each other. Experiments conducted with deletion and site-directed mutants of SL1 revealed that several stem C (SC) mutants (SC7, SC9, SC11 and SC12) of SL1 that maintaining base-pairing but with different sequences of SC interacted with PVX CP whereas SC7 (deletion of UGUU) and SC12 (insertion of CG) were not. Interestingly, SC9 (deletion of UGPVX UU and GACA) and SC11 (sequence change) were interacted with CP. Altogether, these results suggest that the SC of SL1 is important for interaction with PVX CP.

In conclusion, the potential role(s) of these *cis*-elements on virus replication, assembly, and their interaction with viral and host protein(s) during virus infection will be discussed based on the data obtained by *in vitro* binding, *in vitro* assembly, gel shift mobility assay, host gene expression profiling using various mutants at these regions.

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