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Cauliflower mosaic virus gene VI product N-terminus contains regions involved in resistance-breakage, self-association and interactions with movement protein

Michael Hapiak[a], Yongzhong Li[1], Keli Agama[2], Shaddy Swade[a], Genevieve Okenka[a], Jessica Falk[a], Sushant Khandekar[a], Gaurav Raikhy[a], Alisha Anderson[a], Justin Pollock[a], Wendy Zellner[a], James Schoelz[b], Scott M. Leisner[a,*]

[a] *Department of Biological Sciences, The University of Toledo, Toledo, OH 43606, United States*
[b] *Plant Science Unit, College of Agriculture, Food, and Natural Resources, University of Missouri-Columbia, Columbia, MO 65211, United States*

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ABSTRACT

Cauliflower mosaic virus (CaMV) gene VI encodes a multifunctional protein (P6) involved in the translation of viral RNA, the formation of inclusion bodies, and the determination of host range. *Arabidopsis thaliana* ecotype Tsu-0 prevents the systemic spread of most CaMV isolates, including CM1841. However, CaMV isolate W260 overcomes this resistance. In this paper, the N-terminal 110 amino acids of P6 (termed D1) were identified as the resistance-breaking region. D1 also bound full-length P6. Furthermore, binding of W260 D1 to P6 induced higher β-galactosidase activity and better leucine-independent growth in the yeast two-hybrid system than its CM1841 counterpart. Thus, W260 may evade Tsu-0 resistance by mediating P6 self-association in a manner different from that of CM1841. Because Tsu-0 resistance prevents virus movement, interaction of P6 with P1 (CaMV movement protein) was investigated. Both yeast two-hybrid analyses and maltose-binding protein pull-down experiments show that P6 interacts with P1. Although neither half of P1 interacts with P6, the N-terminus of P6 binds P1. Interestingly, D1 by itself does not interact with P1, indicating that different portions of the P6 N-terminus are involved in different activities. The P1–P6 interactions suggest a role for P6 in virus transport, possibly by regulating P1 tubule formation or the assembly of movement complexes.

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1. Introduction

Plant virus resistance is mediated at multiple levels including: elicitation of active defense responses involving programmed cell death (a hypersensitive response), elicitation of active defense responses without necrosis, and the degradation of viral RNAs by post-transcriptional gene silencing (Goodman and Novacky, 1994; Goodrick et al., 1991; Hull, 2002; Waterhouse et al., 1999). Although plant resistance can be effective in limiting viruses to the inoculated leaves or even individual cells, certain strains are able to infect even these protected hosts (Meshi et al., 1988, 1989; Padgett and Beachy, 1993). Plant viruses may overcome resistance by a passive means, i.e., they are not recognized by the host. For example, passive resistance-breakage was observed in the relationship between specific *Cauliflower mosaic virus* (CaMV) isolates and *Arabidopsis thaliana* ecotypes (Agama et al., 2002). While certain *A. thaliana* ecotypes, such as Col-0, are susceptible to many isolates of CaMV others, such as Tsu-0, are resistant to many viral isolates, such as CM1841 (Balazs and Lebeurier, 1981; Leisner and Howell, 1992; Melcher, 1989). Tsu-0 resistance prevents systemic spread of CaMV, whereas isolate W260 evades detection by the Tsu-0 resistance machinery, which allows this CaMV strain to invade the host systemically (Agama et al., 2002). Analysis of chimeric DNA genomes generated from W260 and CM1841 localized the resistance-breaking determinant to the region of gene VI encoding the N-terminal 184 amino acid residues (termed RBR-1), of the protein product (P6).

In addition to its role as a host range determinant, P6 is involved in many activities including regulating translation of viral proteins and forming the characteristic amorphous inclusion bodies observed in the cytoplasm of infected cells (Bonneville et al., 1989; Covey and Hull, 1981; De Tapia et al., 1993; Schoelz et al., 1986;

* Corresponding author.
E-mail address: sleisne@uoft02.utoledo.edu (S.M. Leisner).
[1] Now at: Department of Medicine, University of Illinois at Chicago, Chicago, IL 60612, United States.
[2] Now at: Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, United States.

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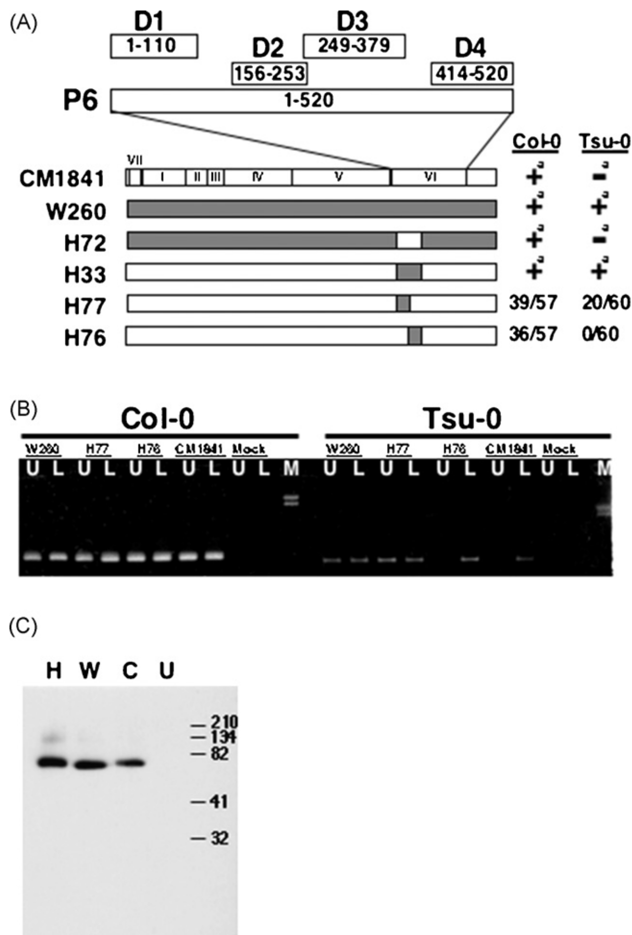

Fig. 1. The amino-terminal encoding 110 amino acids of gene VI contains a resistance-breaking determinant. (A) *Cauliflower mosaic virus* (CaMV) chimeras examined in this study. Portion above the CaMV genomic maps represents the 520 amino acid P6 protein; D1–D4 indicate the four domains involved in P6 self-association as described in Li and Leisner (2002); numbers in boxes indicate amino acid positions within P6. Below the schematic version of P6, the linearized versions of the CaMV genomic maps for the viral isolates and chimeras are shown as derived from Schoelz and Shepherd (1988) and Wintermantel et al. (1993). Roman numerals indicate the locations of the various CaMV genes in the CM1841 (unshaded) and W260 (shaded) genomes. The 5′ to 3′ coding region of each gene is from left to right. The number of plants showing systemic symptoms out of the total number of plants inoculated is given to the right of the genome depictions. Note these numbers are from at least 3 experiments with 15–30 plants per experiment. [a]Numbers were previously reported in Agama et al. (2002), these genomic maps are provided for reference. Note that the shaded region in the H77 chimera encodes domain D1 (amino acids 1–110). (B) Spread of CaMV chimeras through *Arabidopsis* ecotypes. Col-0 or Tsu-0 plants either mock-inoculated (Mock) or inoculated with the CaMVs shown in A above, harvested 52 DPI and analyzed by PCR. *Note*: W260, H77, H76, and 1841 indicate the PCR products generated from plant tissue infected with those viruses, respectively. M indicates lambda DNA digested with HindIII to serve as size markers, the 2.3 and 2.0 kbp fragments are shown. L indicates PCR product generated from rosette leaf tissue; U, from cauline leaf/flower stalk (inflorescence) portions of the plants. (C) Western blot analysis of P6 from plant tissue infected with three CaMV isolates. Infected rosette leaf tissue from Col-0 plants inoculated with: H77, H; W260, W; CM1841, C; or mock-inoculated, U; was homogenized, protein extracts were separated by SDS-PAGE, blotted onto nitrocellulose, and probed with anti-P6 antibodies. The sizes, in kDa, of the protein markers are indicated to the right of the blot.

Schoelz and Shepherd, 1988; Stratford and Covey, 1989). P6 specifically self-associates and this interaction is complex, involving at least 4 regions, termed domains, D1, D2, D3, and D4 (Fig. 1A) (Haas et al., 2005; Li and Leisner, 2002). Finally, P6 plays a role in virus movement. Transgenic plants expressing this protein facilitate the systemic spread of certain CaMV strains (Schoelz et al., 1991). Interestingly, like CaMV P6, potyviral inclusion body proteins have been implicated in virus cell-to-cell movement (Roberts et al., 1998).

Virus movement is a requirement for efficient development of local and systemic infections within a host plant (Carrington et al., 1996; Hull, 2002; Nelson and van Bel, 1998). Although P6 has been suggested to play a role in movement, the CaMV protein identified as a movement protein is P1 (the gene I product), a 327 amino acid polypeptide that contains a central single-stranded nucleic acid binding domain (amino acids 101–177), as well as G (amino acids 128–130), D (amino acids 153–155) and LPL (amino acids 101–103) motifs characteristic of other movement proteins (Citovsky et al., 1991; Koonin et al., 1991; Linstead et al., 1988; Thomas and Maule, 1995a,b, 1999; Thomas et al., 1993). P1 forms tubules through which CaMV particles presumably move from cell to cell (Huang et al., 2001; Linstead et al., 1988; Perbal et al., 1993; Thomas and Maule, 1995b). The N-terminal end of P1 is exposed on the external surface of the tubules, while the C-terminus lines the inside. The C-terminal end of P1 binds to the virion-associated protein, P3 (gene III product), which is exposed on the surface of CaMV particles (Stavolone et al., 2005). Hence, binding of P3–P1 may explain how CaMV particles are loaded into tubules.

In this study, we further defined the CaMV resistance-breaking determinant to a specific portion of P6 and examined the role of this P6 region in self-association. Because resistance prevents viral spread, we also investigated whether P6 binds to the CaMV movement protein.

2. Materials and methods

2.1. A. thaliana ecotypes and virus inoculations

Seeds of *A. thaliana* ecotypes Tsu-0 and Col-0 were a gift from Dr. S.H. Howell (Plant Sciences Institute, Iowa State University, Ames, IA) and were planted in moistened Redi Earth potting soil (BFG Supply, Burton, OH). Plants were propagated in a growth chamber as described previously (Agama et al., 2002).

Cloned viral DNA for CaMV isolate CM1841 (pCaMV10) (Gardner et al., 1981) was provided by Dr. S.H. Howell. CaMV isolate W260 (Gracia and Shepherd, 1985), as well as the chimeric viruses CaMVH33 and CaMVH72 were reported previously (Schoelz and Shepherd, 1988; Wintermantel et al., 1993).

Chimeric viruses H76 and H77 were constructed by subcloning the 2335 bp SacI to BstEII DNA segments from CM1841 and W260 into pUCD9X (Close et al., 1984), which resulted in clones pCM-SB and pW260-SB, respectively. To generate H77, a 2052 bp EcoRI–BstEII segment of pW260-SB was replaced with the corresponding CM1841 sequence, and the resultant SacI–BstEII segment was exchanged with the SacI–BstEII segment of pCaMV10 (Gardner et al., 1981), a pBR322-based clone that contained an infectious copy of the CM1841 genome. To construct H76, the 213 bp EcoRI–PvuII segment of pCM-SB was replaced with the corresponding segment from W260, and the resulting SacI–BstEII DNA segment was exchanged with the SacI–BstEII segment of the pCaMV10. All CaMV isolates and chimeras were maintained in turnips (*Brassica rapa* cv. "Just Right") by serial passage in a greenhouse at 22 °C under natural lighting (Agama et al., 2002).

Arabidopsis thaliana ecotypes Col-0 and Tsu-0 at the six-leaf-stage were inoculated with sap prepared from symptomatic turnips and propagated following inoculation as described previously (Agama et al., 2002). Plants were observed daily for symptoms up to 52 days post-inoculation (DPI). Mock-inoculated plants were also included as controls. Each experiment was performed at least three times with 15–20 plants of each ecotype inoculated per experiment.

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