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Potyvirus Helper Component-Proteinase Self-Interaction in the Yeast Two-Hybrid System and Delineation of the Interaction Domain Involved

Silvio Urcuqui-Inchima,*[,1,2] Jocelyne Walter,†[,2,3] Gabrièle Drugeon,* Sylvie German-Retana,† Anne-Lise Haenni,* Thierry Candresse,† Françoise Bernardi,* and Olivier Le Gall†

**Institut Jacques Monod, 2 place Jussieu-Tour 43, 75251 Paris Cedex 05, France; and †Virologie Végétale, INRA, BP81, 33883 Villenave d'Ornon Cedex, France*

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Using the yeast two-hybrid system, a screen was performed for possible interactions between the proteins encoded by the 5′ region of potyviral genomes [P1, helper component-proteinase (HC-Pro), and P3]. A positive self-interaction involving HC-Pro was detected with lettuce mosaic virus (LMV) and potato virus Y (PVY). The possibility of heterologous interaction between the HC-Pro of LMV and of PVY was also demonstrated. No interaction involving either the P1 or the P3 proteins was detected. A series of ordered deletions from either the N- or C-terminal end of the LMV HC-Pro was used to map the domain involved in interaction to the 72 N-terminal amino acids of the protein, a region known to be dispensable for virus viability but necessary for aphid transmission. A similar but less detailed analysis mapped the interacting domain to the N-terminal half of the PVY HC-Pro.

Key Words: HC-Pro, potyvirus, dimer formation, two-hybrid.

INTRODUCTION

Potyviruses such as lettuce mosaic virus (LMV), and their type member potato virus Y (PVY) are members of the Potyviridae, a family belonging to the picorna-like supergroup of plus-stranded RNA viruses (Shukla *et al.,* 1994). A considerable amount of information has accumulated over the last decades on the structure and mechanisms of expression of the genome of potyviruses (Riechmann *et al.,* 1992) and on the functions of the 10 mature polypeptides encoded on the genome in the form of a large polyprotein. However, the precise functions of several of the potyviral proteins are still unknown. This is particularly true for the P1 and P3 proteins, encoded in the 5′ region of the genome (Fig. 1). In addition to its autoproteolytic property (Mavankal and Rhoads, 1991), P1 has been reported to have nucleic acid-binding capacity (Soumounou and Laliberté, 1994) and to play a role in genome amplification (Verchot and Carrington, 1995). Even less information is available concerning the functions of P3, although, again, this protein appears to be involved in genome amplification (Klein *et al.,* 1994).

By contrast, a number of functions have been assigned to the helper component-proteinase (HC-Pro), the second protein from the N-terminus of the polyprotein (Fig. 1; for review, see Maia *et al.,* 1996). HC-Pro has autoproteolytic activity and is required for efficient aphid transmission of the virus, with coat protein and aphid stylet binding properties (Blanc *et al.,* 1997; Peng *et al.,* 1998; Wang *et al.,* 1998). HC-Pro also has nucleic acid binding (Maia and Bernardi, 1996), as well as plasmodesmata gating (Rojas *et al.,* 1997), properties. It is required for long-term genome amplification and systemic movement of the virus within infected plants (Kasschau *et al.,* 1997) and, finally, appears as a "general" pathogenicity factor (Vance *et al.,* 1995; Pruss *et al.,* 1997). Although some of these properties might turn out to be facets of a more general underlying function, such as a role in the inhibition of the silencing defense reaction of host plants (Anandalakshmi *et al.,* 1998; Brigneti *et al.,* 1998; Kasschau and Carrington, 1998), it is still unclear how these various functions are integrated and regulated. Another still unanswered question is whether HC-Pro is biologically active as a monomer or as a dimer because early purification experiments indicated that the soluble HC-Pro is probably present as a dimer in infected plants (Thornbury *et al.,* 1985).

Besides this deficiency in understanding the function and role of some particular potyviral proteins, a detailed picture of the molecular processes and interactions between the various viral proteins and host cell components is also lacking. The introduction of the yeast two-hybrid system (Fields and Song, 1989), which allows direct *in vivo* detection of protein–protein interactions, thus has opened new and exciting possibilities. This

[1] The author is on leave from the Centro Fruticola Andino, Cali, Colombia.

[2] The authors request that both S.U.I. and J.W. be considered as first authors.

[3] To whom reprint requests should be addressed. Fax: 33-0-556-84-32-21. E-mail: walter@bordeaux.inra.fr.

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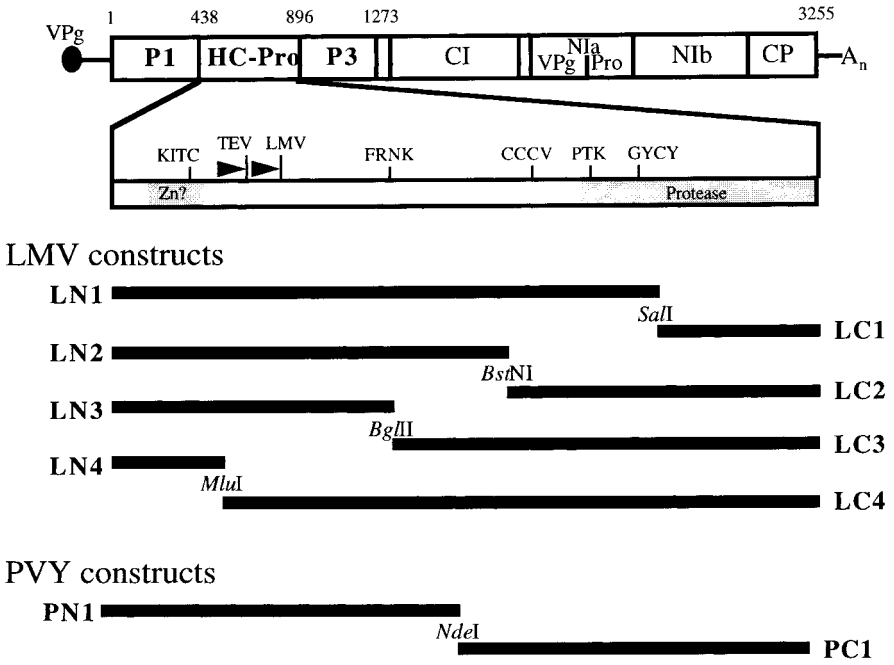

FIG. 1. Structure of the full-length HC-Pro protein and of the various truncated forms used in this study. The genomic organization of potyviruses is shown at the top, with the positions of the first amino acid of P1, HC-Pro, and P3 and the last amino acid of P3 and of the full-length polyprotein given above for LMV. A schematic representation of HC-Pro is shown, with the positions of various conserved sequence motifs (KITC, FRNK, CCCV, PTK, and GYCY). Arrowheads indicate the extent of the largest spontaneous deletions found in the case of tobacco etch virus (TEV; Dolja *et al.,* 1997) and LMV (unpublished results). The cysteine-rich region forming a putative zinc finger (Robaglia *et al.,* 1989) and the minimal proteinase domain (Carrington *et al.,* 1989) are shaded. The extent of the HC-Pro segments present in the various LMV and PVY constructs, with the restriction sites bordering them, is shown in the bottom panel.

method is based on the reconstruction of a functional transcriptional activator when two proteins interact in yeast cells, one being fused to a DNA-binding domain (DBD) and the other to an activating domain (AD). The activity of the reconstituted transcription factor is measured by the expression of marker genes such as the *His3* gene that allows the cells to grow on a histidine-depleted medium or the gene that encodes β-galactosidase. In the present study, the yeast two-hybrid system was used to investigate possible interactions between proteins encoded by the 5′ region of the genome of LMV and PVY and, in particular, between the HC-Pro of LMV and PVY with themselves or with each other.

RESULTS

Homologous and heterologous interactions of LMV and PVY HC-Pro

In yeast, the HC-Pros of LMV and PVY are able to interact with themselves and with each other. The highest activity was observed when PVY HC-Pro was tested with itself. It was higher than when LMV HC-Pro was tested with itself, suggesting that the interaction is stronger in the case of PVY than of LMV (Table 1). Alternatively, this might reflect a difference in the accumulation levels of these proteins in yeast, in the AD-containing vectors used or in the steric hindrance interfering with self-interaction of HC-Pro fused to the AD or to the DBD. In addition, interaction between LMV HC-Pro and PVY HC-Pro resulted in expression of the reporter genes, especially when LMV HC-Pro was fused to the DBD and PVY HC-Pro was fused to the AD (Table 1).

LMV P1 and P3 did not interact in any of the combinations tested, or with HC-Pro (Table 1). However, one

TABLE 1

Interactions Between LMV and PVY Proteins in Yeast

Viral protein fused to			
DBD	AD	His$^-$ growth[a]	β-Galactosidase
LMV P1	LMV P1	−	NA[b]
	LMV HC-Pro	−	NA
LMV HC-Pro	LMV P1	−	NA
	LMV HC-Pro	+	15 ± 2
LMV P3	LMV P1	−	NA
	LMV HC-Pro	−	NA
PVY HC-Pro	PVY HC-Pro	+	114 ± 17
	LMV HC-Pro	−	NA
LMV HC-Pro	PVY HC-Pro	+	4.7 ± 2.8
Ras	Raf	+	32 ± 8

[a] Ability of yeast cells harboring the various constructs to grow on minimal medium lacking leucine, tryptophane, and histidine.

[b] NA, not applicable.

TABLE 2

Interactions Between LMV and PHV HC-Pro and Their Deleted Forms in Yeast

	Viral protein fused to			
	DBD	AD	His$^-$ growth[a]	β-Galactosidase
A				
	LMV HC-Pro	LMV HC-Pro	+	4.0 ± 1.6
	LC1	LMV HC-Pro	−	NA[b]
	LC2	LMV HC-Pro	−	NA
	LC3	LMV HC-Pro	+	2.8 ± 0.9
	LC4	LMV HC-Pro	−	NA
	LN1	LMV HC-Pro	+	4.0 ± 2.3
	LN2	LMV HC-Pro	+	5.5 ± 2.6
	LN3	LMV HC-Pro	+	3.0 ± 1.4
	LN4	LMV HC-Pro	+	3.4 ± 1.3
	LMV HC-Pro	LN4	+	6.7 ± 4.7
	LC1	LN4	−	NA
	LC2	LN4	−	NA
	LC3	LN4	+/−[c]	0
	LC4	LN4	−	NA
	LN4	LN4	+	2.3 ± 0.6
	Ras	Raf	+	20 ± 9
B				
	PVY HC-Pro	PVY HC-Pro	+	105 ± 10
	PN1	PVY HC-Pro	+	44 ± 17
	PC1	PVY HC-Pro	+	1.2 ± 0.1
	PVY HC-Pro	PN1	+	50 ± 10
	PN1	PN1	+	1.4 ± 0.2
	PC1	PN1	+	16 ± 0.2
	PVY HC-Pro	PC1	−	NA
	PN1	PC1	+	22 ± 2
	PC1	PC1	−	NA
	Ras	Raf	+	56 ± 21

[a] Ability of yeast cells harboring the various constructs to grow on minimal medium lacking leucine, tryptophane, and histidine.

[b] NA, not applicable.

[c] +/−, occasional growth observed.

cannot exclude that P1 or P3 interactions exist but are simply not detected by the method used here. The LMV or PVY HC-Pro tested against either empty vectors or against laminin γ1 fused to the DBD yielded negative results as expected.

Deletion mapping of the LMV and PVY HC-Pro self-interactions

The LMV HC-Pro C-terminal fragments LC1, LC2, and LC4 did not interact with full-length LMV HC-Pro, when the latter was fused to the AD (Table 2A). Only LC3 resulted in HIS3 expression and detectable β-galactosidase activity. However, additional control experiments showed that yeast cells containing the LC3 construct together with empty pGad3S2X occasionally grew on medium lacking histidine but showed no β-galactosidase activity. In contrast, all the N-terminal fragments, including LN4. in which only the first 72 amino acids of LMV HC-Pro are fused to the DBD, yielded both HIS3 and β-galactosidase expression when cotransformed with LMV HC-Pro. This indicates that the 72 N-terminal amino acids of LMV HC-Pro are sufficient for interaction with LMV HC-Pro. When LN4 was fused to the AD, it interacted with LMV HC-Pro and with itself but with none of the C-terminal fragments (LC1–LC4).

In the case of PVY, PN1 but not PC1 interacted with full-length PVY HC-Pro when fused either to the AD or to the DBD (Table 2B). When PN1 was assayed with PC1 fused to either domain, a significant interaction could be detected based on growth on a histidine-depleted medium and on the β-galactosidase activity, although it remained lower compared with PVY HC-Pro interaction with itself. Moreover, interaction of the N-terminal domain with itself was barely detectable, and there was no interaction of the C-terminal domain with itself.

Together these results suggest that the region involved in self-interaction of HC-Pro in the case of LMV and PVY is most probably the N-terminal region of the protein. In addition, a minor determinant of the interaction might be present in the central region of HC-Pro, allowing the weak interaction of PN1 with PC1.

DISCUSSION

The self-interacting capacity of HC-Pro in two different potyviruses, LMV and PVY, is consistent with previous biochemical data indicating that for another potyvirus, tobacco vein mottling virus, HC-Pro is present as a homodimer (Thornbury *et al.,* 1985), which thus is possibly the active form of this protein for some or all of its biological functions. It is tempting to speculate that the interaction we detect in yeast is related to this ability to form homodimers. In the present study, a major domain responsible for homodimerization has been located in the N-terminal region of HC-Pro and, more precisely, in the first 72 amino acids in the case of LMV. This region has been shown to be crucial for aphid transmission (Atreya *et al.,* 1992). The KITC motif at amino acid positions 52–55 in LMV and 50–53 in PVY (Fig. 1) has been identified as a determinant of this activity (Atreya *et al.,* 1992; Atreya and Pirone, 1993; Dolja *et al.,* 1993, 1997; Peng *et al.,* 1998). Furthermore, it should be pointed out that the viability of tobacco etch virus (Dolja *et al.,* 1993; Dolja *et al.,* 1997) and LMV (German-Retana *et al.,* submitted) is undeterred when the N-terminal region of HC-Pro is deleted (Fig. 1). This suggests that the ability of HC-Pro to dimerize may be important for aphid transmission but less so for other functions of this protein.

MATERIALS AND METHODS

Construction of clones for the two-hybrid assays

The regions of the genome of LMV-E (accession no. X97705) encoding proteins P1 (nucleotide positions 104-1414), HC-Pro (positions 1415–2788), and P3 (positions

TABLE 3

Oligonucleotides Used for Amplification, Their Orientation, the Corresponding Position on the LMV Genome, and Their Nucleotide Sequence

Oligonucleotide	Position[a]	Sequence[b]
LMV P1, sense	101	cgc*ggatcC*AAATGGCAACTCTAGACAAC
LMV P1, antisense	1414	cgc*ggatcc*ttaGTATTGAACCATGCGGTG
LMV HC-Pro, sense	1415	cgc*ggatcc*aaatgAGCGACGTAGCACGAA
LMV HC-Pro, antisense	2788	g*ctgcag*ttaCCCTACTCTGTAGTGTTGC
LMV P3, sense	2789	cgc*ggatcc*aaatgGGGGAACCAGATAAAGCA
LMV P3, antisense	3922	g*ctgcag*ttaTTGATGCTCTACTTCTTCTT

[a] Position of the 5′-proximal viral nucleotide of each oligonucleotide on the genome of LMV.

[b] Nucleotide sequence from 5′ to 3′. Nonviral sequences are in lowercase. Initiation and termination codons are underlined, and restriction sites used to clone the PCR fragments are in italics.

2789–3922) were amplified by PCR using *Pfu* DNA polymerase (Stratagene, La Jolla, CA). The oligonucleotides that were used added a *Bam*HI site and an initiation codon at the 5′ end of the amplified cDNAs and a termination codon and a *Bam*HI (P1) or a *Pst*I (HC-Pro and P3) site at the 3′ end (Table 3). The resulting cDNAs were inserted into the yeast shuttle plasmids pLexA containing the DBD (Vojtek *et al.,* 1993) and pGad3S2X containing the AD [a modified form of pGAD1318 (Benichou *et al.,* 1994) with three in-frame termination codons; C. Robaglia, personal communication], digested with *Bam*HI (P1) or *Bam*HI and *Pst*I (HC-Pro and P3). No derivative of pGad3S2X containing the LMV P3 cDNA was obtained despite several attempts, probably due to uncontrolled toxicity to *Escherichia coli*.

The full-length PVY-LYE84 HC-Pro coding region contained in pT7:HC-Pro (accession number U33454; Maia and Bernardi, 1996) was released by *Eco*RI–*Xba*I digestion and inserted into similarly cleaved pGADGH-STOP (containing the AD; a gift of I. Jupin). The resulting construct was cleaved by *Eco*RI–*Xho*I, and the fragment carrying the HC-Pro gene was inserted into pLexA cleaved by *Eco*RI–*Sal*I.

Construction of the HC-Pro deletion mutants

To delineate the region of HC-Pro involved in the interaction in yeast, we took advantage of the presence of the restriction sites *Mlu*I$_{1628}$, *Bgl*II$_{1965}$, *Bst*NI$_{2235}$, and *Sal*I$_{2474}$ in the LMV HC-Pro cDNA (the positions given in indices refer to the full-length LMV-E cDNA sequence) to express the N-terminal (LN1–LN4) and C-terminal (LC1–LC4) fragments of HC-Pro fused to the DBD (Fig. 1) and to the AD in the case of LN4.

The regions corresponding to the N-terminal (PN1) and C-terminal (PC1) halves (amino acids 1–228 and 234–456, respectively; Fig.1) of PVY HC-Pro were obtained by *Eco*RI–*Xba*I digestion of the plasmids pdel-2 and pdel-6, respectively (to be described elsewhere) and inserted in similarly cleaved pGADGH-STOP. The resulting constructs were cleaved by *Eco*RI–*Xho*I, and the fragments were inserted into pLexA cleaved by *Eco*RI–*Sal*I.

Transformation

The recombinant plasmids were amplified in *E. coli* and used to transform *Saccharomyces cerevisiae* L40 (Le Douarin *et al.,* 1995) using lithium acetate either by sequential transformation after the "quick and easy" protocol (Gietz and Woods, 1994) or by cotransformation according to the protocol described in the Stratagene HybriZAP 2.1 kit. Unrelated sequences, those of the human proteins Ras and Raf (Vojtek *et al.,* 1993) or of the murine laminin γ1 (Chang *et al.,* 1996), were used as positive or negative interaction controls, respectively.

Quantitative β-galactosidase assay

The quantitative β-galactosidase assays were performed on the different transformed yeast strains grown overnight to OD 0.8–1.5 in a synthetic selective medium lacking tryptophane, leucine, and histidine. The overnight cultures were diluted 10-fold into fresh medium and grown to OD 0.3–0.9 before the quantitative assay. Cell permeabilization was performed as previously described (Guarente, 1983). *O*-Nitrophenyl-β-D-galactopyranoside (Sigma) was used as chromogenic substrate, and the β-galactosidase assays were performed as described and are expressed as Miller units (Miller, 1972).

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REFERENCES

Anandalakshmi, R., Pruss, G. J., Ge, X., Marathe, R., Smith, T. H., and Vance, V. B. (1998). A viral suppressor of gene silencing in plants. *Proc. Natl. Acad. Sci. USA* **95**, 13079–13084.

Atreya, C. D., Atreya, P. L., Thornbury, D. W., and Pirone, T. P. (1992). Site-directed mutations in the potyvirus HC-PRO gene affect helper component activity, virus accumulation and symptom expression in infected tobacco plants. *Virology* **191,** 106–111.

Atreya, C. D., and Pirone, T. P. (1993). Mutational analysis of the helper component-proteinase gene of a potyvirus: Effects of amino acid substitutions, deletions, and gene replacement on virulence and aphid transmissibility. *Proc. Natl. Acad. Sci. USA* **90,** 11919–11923.

Benichou, S., Bomsel, M., Bodéus, M., Durand, H., Douté, M., Letourneur, F., Camonis, J., and Benarous, R. (1994). Physical interaction of the HIV-1 Nef protein with β-COP, a component of non-clathrin-coated vesicles essential for membrane traffic. *J. Biol. Chem.* **269,** 30073–30076.

Blanc, S., López-Moya, J.-J., Wang, R., García-Lampasona, S., Thornbury, D. W., and Pirone, T. P. (1997). A specific interaction between coat protein and helper component correlates with aphid transmission of a potyvirus. *Virology* **231,** 141–147.

Brigneti, G., Voinnet, O., Li, W.-X., Ji, L.-H., Ding, S.-W., and Baulcombe, D. (1998). Viral pathogenicity determinants are suppressers of transgene silencing in *Nicotiana benthamiana. EMBO J.* **17,** 6739–6746.

Carrington, J. C., Cary, S. M., Parks, T. D., and Dougherty, W. G. (1989). A second proteinase encoded by a plant potyvirus genome. *EMBO J.* **8,** 365–370.

Chang, H. S., Kim, N. B., and Phillips, S. L. (1996). Positive elements in the laminin γ1 gene synergize to activate high level transcription during cellular differentiation. *Nucleic Acids Res.* **24,** 1360–1368.

Dolja, V. V., Herndon, K. L., Pirone, T. P., and Carrington, J. C. (1993). Spontaneous mutagenesis of a plant potyvirus genome after insertion of a foreign gene. *J. Virol.* **67,** 5968–5975.

Dolja, V. V., Hong, J., Keller, K. E., Martin, R. R., and Peremyslov, V. V. (1997). Suppression of potyvirus infection by cosuppressed closterovirus protein. *Virology* **234,** 243–252.

Fields, S., and Song, O.-k. (1989). A novel genetic system to detect protein-protein interactions. *Nature* **340,** 245–246.

Gietz, R., and Woods, R. (1994). High efficiency transformation in yeast. *In* "Molecular Genetics of Yeast: Practical Approaches" (J. Johnston, Ed.), pp. 121–134. Oxford University Press, New York.

Guarente, L. (1983). Yeast promoters and *lacZ* fusions designed to study expression of cloned genes in yeast. *Methods Enzymol.* **101,** 181–191.

Kasschau, K. D., and Carrington, J. C. (1998). A counterdefensive strategy of plant viruses: Suppression of posttranscriptional gene silencing. *Cell* **95,** 461–470.

Kasschau, K. D., Cronin, S., and Carrington, J. C. (1997). Genome amplification and long-distance movement functions associated with the central domain of tobacco etch potyvirus helper component-proteinase. *Virology* **228,** 251–262.

Klein, P. G., Klein, R. R., Rodríguez-Cerezo, E., Hunt, A. G., and Shaw, J. G. (1994). Mutational analysis of the tobacco vein mottling virus genome. *Virology* **204,** 759–769.

Le Douarin, B., Pierrat, B., vom Baur, E., Chambon, P., and Losson, R. (1995). A new version of the two-hybrid assay for detection of protein-protein interactions. *Nucleic Acids Res.* **23,** 876–878.

Maia, I. G., and Bernardi, F. (1996). Nucleic acid-binding properties of a bacterially expressed potato virus Y helper component-proteinase. *J. Gen. Virol.* **77,** 869–877.

Maia, I. G., Haenni, A.-L., and Bernardi, F. (1996). Potyviral HC-Pro: A multifunctional protein. *J. Gen. Virol.* **77,** 1335–1341.

Mavankal, G., and Rhoads, R. E. (1991). *In vitro* cleavage at or near the N-terminus of the helper component protein in the tobacco vein mottling virus polyprotein. *Virology* **185,** 721–731.

Miller, J. H. (1972). "Experiments in Molecular Genetics." Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Peng, Y.-h., Kadoury, D., Gal-On, A., Huet, H., Wang, Y., and Raccah, B. (1998). Mutations in the HC-Pro gene of zucchini yellow mosaic potyvirus: Effects on aphid transmission and binding to purified virions. *J. Gen. Virol.* **79,** 897–904.

Pruss, G., Ge, X., Shi, X. M., Carrington, J. C., and Vance, V. B. (1997). Plant viral synergism: the potyviral genome encodes a broad-range pathogenicity enhancer that transactivates replication of heterologous viruses. *Plant Cell* **9,** 859–868.

Riechmann, J. L., Laín, S., and García, J. A. (1992). Highlights and prospects of potyvirus molecular biology. *J. Gen. Virol.* **73,** 1–16.

Robaglia, C., Durand-Tardif, M., Tronchet, M., Boudazin, G., Astier-Manifacier, S., and Casse-Delbart, F. (1989). Nucleotide sequence of potato virus Y (N strain) genomic RNA. *J. Gen. Virol.* **70,** 935–947.

Rojas, M. R., Zerbini, F. M., Allison, R. F., Gilbertson, R. L., and Lucas, W. J. (1997). Capsid protein and helper component-proteinase function as potyvirus cell-to-cell movement proteins. *Virology* **237,** 283–295.

Shukla, D. D., Ward, C. W., and Brunt, A. A. (1994). "The Potyviridae." CAB International, Wallingford, UK.

Soumounou, Y., and Laliberté, J.-F. (1994). Nucleic acid-binding properties of the P1 protein of turnip mosaic potyvirus produced in *Escherichia coli. J. Gen. Virol.* **75,** 2567–2573.

Thornbury, D. W., Hellmann, G. M., Rhoads, R. E., and Pirone, T. P. (1985). Purification and characterization of potyvirus helper component. *Virology* **144,** 260–267.

Vance, V. B., Berger, P. H., Carrington, J. C., Hunt, A. G., and Shi, X. M. (1995). 5′ Proximal potyviral sequences mediate potato virus X/potyviral synergistic disease in transgenic tobacco. *Virology* **206,** 583–590.

Verchot, J., and Carrington, J. C. (1995). Evidence that the potyvirus P1 proteinase functions in *trans* as an accessory factor for genome amplification. *J. Virol.* **69,** 3668–3674.

Vojtek, A. B., Hollenberg, S. M., and Cooper, J. A. (1993). Mammalian Ras interacts directly with the serine-threonine kinase Raf. *Cell* **74,** 205–214.

Wang, R. Y., Powell, G., Hardie, J., and Pirone, T. P. (1998). Role of the helper component in vector-specific transmission of potyviruses. *J. Gen. Virol.* **79,** 1519–1524.