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Current Review

New Advances in Understanding the Molecular Biology of Plant/Potyvirus Interactions

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In recent years, researchers have adopted many new technologies to help understand potyvirus pathogenesis. Their findings have illuminated key aspects of the interactions between the host and the virus, and between the virus and its aphid vector. This review focuses on advances in our understanding of the molecular determinants of systemic infection, symptom expression, aphid and seed transmission, and natural and engineered resistance to potyviruses. Very recent developments in the area of post-transcriptional gene silencing indicate not only that the process is fundamental to engineered resistance, but may also underlie many aspects of the biology of plant viruses.

Additional keywords: cell-to-cell movement, HC-Pro, long-distance movement.

Potyvirus is the largest genus of plant viruses, with 180 definite or possible members causing significant losses in a wide range of crop plants (Shukla et al. 1994). The viruses are aphid transmitted in a nonpersistent manner and some of them are also seed transmitted (Johansen et al. 1994; Shukla et al. 1994). The organization of the potyvirus single-stranded RNA genome is shown in Figure 1.

Substantial advances have been made in recent years in our understanding of the molecular biology of the interactions between potyviruses and their hosts; the last major review of this topic was published in 1992 (Riechmann et al. 1992). These achievements have been made possible through knowledge of the complete nucleotide sequence of the viral genomes, through the generation of infectious molecules in vitro and in vivo from cloned viral cDNAs (for reviews see Boyer and Haenni 1994; Shukla et al. 1994), and through the techniques of mutagenesis and recombinant hybrid virus construction. Additionally, the use of genes for visible reporter molecules cloned into infectious viral genomes has been crucial. Notably, these have included the *uidA* gene encoding β-glucuronidase (GUS; e.g., Dolja et al. 1992) and the *gfp* gene from *Aequorea victoria* encoding the green fluorescent protein (GFP; e.g., Schaad et al. 1997a). Hence, the genes either have been cloned to give a fusion with a viral protein, or have been such that proteolytic cleavage releases the free reporter protein from the potyviral polyprotein. In the latter case, cloning the reporter gene and a new nuclear inclusion a protein (NIa)-specific cleavage site adjacent to the helper component-proteinase (HC-Pro; Schaad et al. 1997a, 1997b) has been particularly effective.

New technology has contributed significantly to the recent advances. Yeast two-hybrid systems for analyzing protein-protein interactions in the nucleus are commercially available, and the rapid expansion of genetic and bioinformatics resources has meant that the identification and characterization of host genes (particularly for *Arabidopsis thaliana*) is possible and will become routine within a few years.

This review focuses on these new advances, in particular on the host and viral molecular determinants shown to have a significant role during viral infection. Thus, we describe here the molecular determinants involved in systemic infection (genome amplification and cell-to-cell and long-distance movements), symptom expression, aphid and seed transmissions, and natural and engineered resistances to potyviruses.

Systemic infection.

Systemic infection occurs when a virus is able to move, after a genome amplification step, from the primary infection focus to invade distal regions of the plant (Lucas and Gilbertson 1994; Carrington et al. 1996). This requires that the infectious unit should move locally from cell to cell through plasmodesmata, and then over longer distances through the phloem. For most plant virus groups, the movement process involves one or more specialized virus-encoded proteins, termed movement proteins (MPs). These proteins are usually characterized by mutagenesis when cell-to-cell movement from the primary infected cell is altered without affecting virus replication. Potyviruses do not encode a dedicated MP, but movement functions have been allocated to several proteins, including the coat protein (CP), HC-Pro, the cylindrical inclusion (CI) protein, and the genome linked protein (VPg). In some of these cases, the mutation-based definition has been supported by ultrastructural observations and microinjection experiments to demonstrate that the proteins influence a plasmodesmal function.

Genome amplification. Potyvirus genome amplification requires two fundamental processes, viral RNA translation for the synthesis of virus-specific proteins, including the viral replicase, and RNA replication itself. The interrelationship of

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these two processes makes it difficult to assign particular functions uniquely to RNA replication. Hence, most potyvirus-encoded proteins affect the cellular level of virus accumulation. Superficially, the RNA-binding properties of P1 (Brantley and Hunt 1993; Soumounou and Laliberté 1994; Merits et al. 1998), HC-Pro (Maia and Bernardi 1996; Merits et al. 1998), CI protein (Eagles et al. 1994; Fernández et al. 1995; Merits et al. 1998), NIa (Daròs and Carrington 1997; Merits et al. 1998), nuclear inclusion b protein (NIb; replicase), and CP (Merits et al. 1998) may suggest that all these proteins function in the RNA replication process. However, the possibility of the involvement of viral proteins in the distinctive features of potyvirus RNA translation (reviewed in Riechmann et al. 1992) and the potential for cell-to-cell translocation of viral RNA (see below) make it difficult to allocate the functions precisely. Almost certainly, we will find that the processes of RNA replication and translation are spatially and temporally integrated.

Potyviral RNA differs from host mRNAs in the absence of a 5′-cap structure. In the virion this is replaced by the VPg; the 5′ structure of the polysome-associated potyviral RNA is not known. Both host mRNAs and potyviral RNAs are 3′-polyadenylated. Since efficient translation of most eukaryotic mRNAs requires that the 5′-cap and the 3′-poly(A) act in concert (Gallie 1998), some alternative mechanism must operate for potyviruses. Gallie et al. (1995) have shown for *Tobacco etch virus* (TEV) that the long 5′ nontranslated region (NTR) substitutes for the 5′-cap in the interaction with the 3′-poly(A) and can act as a translational enhancer when placed upstream of heterologous open reading frames. One role for the 5′-cap is the binding of translation factors before the recruitment of ribosomes. Hence, the 5′ NTR and/or VPg must provide this specific role. Some support for this comes from the demonstration of a yeast two-hybrid interaction between the VPg of *Turnip mosaic virus* (TuMV) and eIF(iso)4E from *A. thaliana* (Wittmann et al. 1997). For some members of the *Picornaviridae* family of viruses, which all have a 5′-VPg, ribosome entry has been linked to the presence of a complex RNA structure in the 5′ NTR termed the internal ribosome entry site (IRES; Pelletier and Sonenberg 1988). There is some evidence (Levis and Astier-Manifacier 1993; Basso et al. 1994) that potyvirus 5′ NTRs could act similarly, although no IRES-like structure has been identified. *Plum pox virus* (PPV) 5′ NTR presents an added complication in that it contains an additional in-frame AUG upstream of the polyprotein start codon. This appears to be handled through a mechanism of cap-independent "leaky scanning" translation (Simón-Buela et al. 1997a).

For RNA replication, the demonstration of RNA polymerase activity of the NIb (Hong and Hunt 1996), the association of TEV replication complexes with endoplasmic reticulum-like membranes, and the potential for the 6K protein to act as a membrane anchor (Schaad et al. 1997a) are important findings. Also, the *cis*-replicative function of NIa (Murphy et al. 1996; Schaad et al. 1996) and the physical interaction between NIa and NIb (Hong et al. 1995; Li et al. 1997; Fellers et al. 1998) might begin to define the components of the viral replicase complex. Mutational studies have also showed that P1, HC-Pro, and P3 are involved in potyviral genome amplification (Atreya et al. 1992; Klein et al. 1994; Kasschau and Carrington 1995; Verchot and Carrington 1995; Kasschau et al. 1997). Exceptionally, CP appears not to be required, although translation to a position between TEV CP codons 138 and 189, and a *cis*-active RNA sequence between TEV CP codons 211 and 246, are absolutely necessary (Mahajan et al. 1996). Recently, secondary structures involving both CP-coding and 3′ NTR sequences were also shown to confer replicative function (Haldeman-Cahill et al. 1998).

Cell-to-cell movement. Genetic evidence that CP is required for potyvirus movement was provided by Dolja et al. (1994, 1995). The potyviral CP is a three-domain protein with variable N- and C-terminal regions exposed on the particle surface and a conserved core domain that interacts with viral RNA (Allison et al. 1985; Shukla and Ward 1988). Dolja et al. (1994, 1995) produced mutants in the CP-core domain of TEV-GUS. All mutants were defective in cell-to-cell movement and in virion assembly. The effect of such mutations on assembly had been noted previously for *Johnsongrass mosaic virus* (Jagadish et al. 1993). This mutational analysis also showed that the N-terminal domain of the CP has an accessory role in this movement process since mutants with this domain removed exhibited slow cell-to-cell movement in inoculated leaves.

Several studies have implicated the CI protein, an RNA helicase required for genome replication (Laín et al. 1990; Eagles et al. 1994; Klein et al. 1994), in potyvirus cell-to-cell movement. By electron microscopy, CI protein is seen to form aggregates (called pinwheel or cylindrical inclusions [CIs]) in the cytoplasm of infected cells. These inclusions are frequently seen positioned over the plasmodesmal aperture

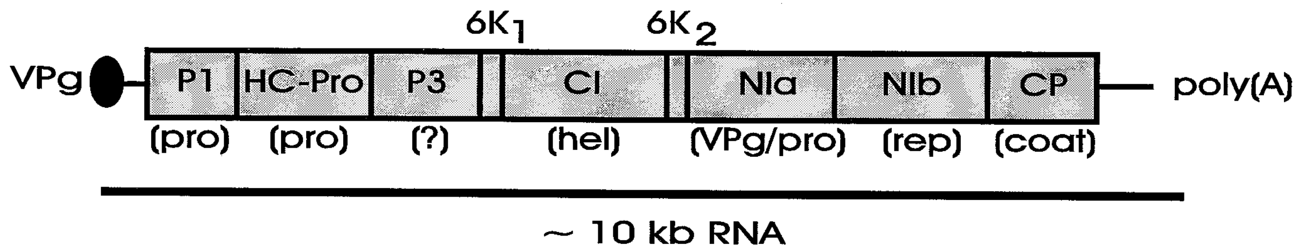

Fig. 1. Organization of the potyvirus genome. Potyviruses have single-stranded RNA genomes of approximately 10 kb. The RNA is polyadenylated at the 3′ end (poly(A)) and has a virus-encoded genome linked protein (VPg) covalently linked to the 5′ end. The RNA encodes a single polyprotein (shaded box) that is proteolytically cleaved by self-encoded proteases (pro) into functional proteins. The only structural protein is the coat protein (CP). Other identified functions include the aphid transmission helper component protease (HC-Pro), which also has a gene silencing suppressor activity, the cylindrical inclusion (CI) protein, which assists virus movement, the combined VPg-protease protein (NIa; nuclear inclusion a), and the RNA replicase (NIb; nuclear inclusion b).

(Lawson and Hearon 1971; Langenberg 1986). Mutational analysis of the TEV CI protein (Carrington et al. 1998) identified two mutants, altered in the N-terminal region, that were defective in cell-to-cell movement but still replicated to levels equivalent to that seen for the parental virus. Other TEV CI protein mutants failed to be, or were only weakly, replicated in infected protoplasts, supporting the view that CI protein also has an important role in the virus replication process.

Ultrastructural studies of tissues at an early stage of infection, combined with immunogold labeling of specific potyvirus proteins, have further supported the role of CI protein and CP in cell-to-cell movement (Rodríguez-Cerezo et al. 1997; Roberts et al. 1998). Observations of either young tobacco leaves infected with *Tobacco vein mottling virus* (TVMV; Rodriguez-Cerezo et al. 1997), or cells across an advancing infection front in pea cotyledons infected by *Pea seed-borne mosaic virus* (PSbMV; Roberts et al. 1998), showed that CIs immunolabeled for CI protein and CP were attached to the plasma membrane, close to or over the plasmodesmal opening. Most notable was the observation of a continuous channel through the center of the CIs and the plasmodesma. This channel contained CP and, in the case of tobacco cells, TVMV RNA (identified by in situ hybridization). Behind the PSbMV infection front, CIs were no longer associated with the cell wall, or with CP, and CIs accumulated as the characteristic pinwheel structures in the cytoplasm (Roberts et al. 1998). Both studies proposed that CIs could function transiently to transfer viral complexes from cell to cell through plasmodesmata.

It has also been suggested that VPg could have a role in potyviral cell-to-cell movement. Through site-directed mutagenesis and the construction of chimeric TVMVs, the viral resistance-breaking determinant for the *Nicotiana tabacum* cv. TN 86 resistance gene, *va,* has been identified as the VPg (Nicolas et al. 1997). The phenotypic expression of this recessive resistance is the confinement of the infection to initially infected cells, suggesting a restricted virus movement (Gibb et al. 1989). In co-inoculation experiments with virulent and avirulent TVMV strains, only the virulent strain was detected in systemically infected leaves. These data argue, first, against the elicitation of a general movement-limiting reaction in cv. TN 86 by the avirulent strain and, second, against a nonspecific mediation of transport through plasmodesmata by the virulent strain. However, the avirulent strain replicates in cv. TN 86 protoplasts to a lower level than in a susceptible cultivar of tobacco (Gibb et al. 1989), perhaps indicating that a plant defense response (see below) may limit the replication of this strain and reduce its ability to invade more than a few cells.

The genetic analyses of the involvement of HC-Pro during the cell-to-cell transport process are less clear (Klein et al. 1994; Cronin et al. 1995; Kasschau et al. 1997). Several HC-Pro mutants of TEV appeared to move from cell to cell less efficiently than the parental virus (Kasschau et al. 1997) and a TVMV HC-Pro mutant was unable to spread in inoculated leaves (Klein et al. 1994).

Since plasmodesmata in mesophyll cells have a size exclusion limit (SEL) of approximately 1,000 Da for the passive transport of molecules, some effects of the potyviral MPs on the plasmodesmal SEL might be expected. The effects of *Escherichia coli*–expressed *Bean common mosaic necrosis virus* and *Lettuce mosaic virus* proteins on plasmodesmal gating have been measured after microinjection into host cells (Rojas et al. 1997). Both CP and HC-Pro were shown to increase plasmodesmal SEL and to mediate viral RNA (approximately 1 kb encoding the viral CP) movement from cell to cell. Microinjection of CI protein or NIa (which contains the VPg domain) did not induce these effects. These are the first experiments designed to dissect the role of potyvirus MPs in the overall process of cell-to-cell movement, although they do not closely mimic natural potyvirus infections.

Collectively, these studies show that at least two potyviral proteins, CI protein and CP, could be considered MPs. From the model for potyvirus cell-to-cell movement proposed by Carrington et al. (1998), CI protein may direct intracellular translocation of a viral transport complex that includes the CP. Then, CP may interact with the plasmodesmata to increase the SEL, and CI protein may function to position the viral complex for translocation through the CI structures, into the plasmodesmata, and finally into the adjacent cells. A major question to address in the next years will be the nature of the viral transport complex. The strong correlation between competence for virion assembly and for cell-to-cell movement in the case of TEV (Dolja et al. 1994, 1995), and the fibrillar material (similar to PSbMV particles) observed within plasmodesmata in pea (Roberts et al. 1998), may be taken as an indication that potyviruses move from cell to cell as virions. However, direct evidence is still lacking.

Long-distance movement. Long-distance movement is the movement of the infectious agent from the mesophyll via the bundle sheath cells, phloem parenchyma, and companion cells into phloem sieve elements, passive translocation in the phloem, and unloading at a remote site to establish further infection foci (for review see Carrington et al. 1996). A specific example of this is *Pepper mottle virus* (PepMoV) long-distance movement in *Capsicum annuum* plants (Andrianifahanana et al. 1997). In this case, the virus was seen to follow the source-to-sink pattern route for translocation of photoassimilates. This involved descending transport via the external phloem in the stem from the inoculated leaf, entrance into internal phloem, and rapid ascending transport to young tissues of the plant. Generally, the complexity of the cell types and their connections, and the difficulty in analyzing long-distance movement independently of cell-to-cell movement, mean that the roles of potyviral proteins in this process are not well defined.

At least three potyviral proteins (CP, HC-Pro, and VPg) seem to be involved in long-distance movement. For CP, TEV-GUS mutants with deletions in the CP N- or C-terminal domains produced virions in vivo but the virus exhibited defects in long-distance movement in plants (Dolja et al. 1994, 1995). Also, mutational analysis demonstrated that changes to Ser_{47} of the PSbMV CP (Andersen and Johansen 1998) and Asp_5 in the DAG motif of the TVMV CP N-terminal domain (Atreya et al. 1995; López-Moya and Pirone 1998) can modulate the ability of the virus to move systemically in *Chenopodium quinoa* and tobacco plants, respectively.

Other analyses with TEV-GUS showed that HC-Pro also has a role in potyvirus long-distance movement (Klein et al. 1994; Cronin et al. 1995; Kasschau et al. 1997). The long-distance movement–defective TEV-GUS/CCCE mutant virus (substitution of the Cys_{293}, Cys_{294}, Cys_{295}, and Glu_{299}, highly conserved within the HC-Pro central region; Cronin et al.

1995) was analyzed for its ability to infect a series of grafted plants composed of various combinations of HC-Pro transgenic and nontransgenic scions and rootstocks (Kasschau et al. 1997). Systemic infection was only observed when both the stock and the scion could provide a complementing function from a wild-type HC-Pro transgene. This indicated that HC-Pro is required in both inoculated and noninoculated tissues for efficient long-distance movement and, hence, presumably for both entry into, and exit from, the host plant vascular system. Other mutations in the HC-Pro central domain were also analyzed and showed the same negative effect on long-distance movement. One mutant bearing insertions in the N-terminal part of TVMV HC-Pro also failed to result in systemic infection (Klein et al. 1994).

The involvement of VPg in long-distance movement was proposed from the analysis of TEV recombinants made between strains that differed in their capacity to invade *N. tabacum* cv. V20 systemically (Schaad et al. 1997b). TEV strain HAT shows restricted cell-to-cell movement phenotype in cv. V20 but a systemic infection in cv. Havana 425. TEV strain Oxnard is capable of systemic infection in both cultivars. To identify the TEV host-specific movement determinants, chimeric viral genomes were constructed between TEV-HAT and TEV-Oxnard. Chimeric viruses containing the TEV-Oxnard VPg domain were able to infect cv. V20 systemically.

In spite of the experiments described above, a detailed model for long-distance movement of potyviruses must be speculative. As CP is a structural protein and VPg is covalently linked to the viral RNA, these two proteins are probably included in a viral transport complex, and may interact with host factors for efficient movement through the plant. These host factors remain elusive although the complexity of the process means that many host proteins may be involved. At least three factors are suggested from host genetic analyses. Hence, segregation resulting from a cross between the V20 and Havana 425 *N. tabacum* cultivars (Schaad and Carrington 1996) suggested that TEV limitation in cv. V20 was due to two recessive genes. A monogenic, dominant locus (*RTM1*) of *A. thaliana,* conferring a restricted TEV infection phenotype (Mahajan et al. 1998), identifies the third factor. The cloning and molecular characterization of this dominant gene may be the more tractable experimentally and could provide the first detailed information about host proteins involved in potyvirus long-distance transport.

HC-Pro, a viral suppressor of gene silencing.

A recent exciting development in our understanding of the role of HC-Pro may mean that we need to view many aspects of long-distance transport in a different way. Analysis of synergistic infections involving potyviruses showed that HC-Pro may act as a general pathogenicity enhancer (Vance et al. 1995; Pruss et al. 1997; Shi et al. 1997) by interfering with a host defense response that normally limits viral infection. In several parallel pieces of work (Anandalakshmi et al. 1998; Brigneti et al. 1998; Kasschau and Carrington 1998), three groups have shown that HC-Pro has the capacity to suppress post-transcriptional gene silencing (PTGS), and that the consequence of this is enhanced virus replication. Essentially, two strategies led to this conclusion. Genetic crosses between transgenic lines expressing potyvirus P1/HC-Pro (a protein capable of self-cleavage to release free HC-Pro) and lines exhibiting PTGS of reporter genes (*uidA* or *gfp*) resulted in progeny in which the reporter gene function was restored (Anandalakshmi et al. 1998; Kasschau and Carrington 1998). Second, *Potato virus X* (PVX) vectors expressing HC-Pro replicated to a higher level than wild-type PVX and restored reporter gene expression on *uidA* or *gfp* silenced plants (Anandalakshmi et al. 1998; Brigneti et al. 1998). There was some evidence (Shi et al. 1997; Anandalakshmi et al. 1998) that it is the central region of HC-Pro that mediates synergistic effects in co-infection with other viruses and suppression of gene silencing, and that this property of HC-Pro was enhanced by the presence of the potyviral P1 protein (Kasschau and Carrington 1998).

There is increasing evidence that PTGS can be divided functionally into initiation and maintenance phases (Brigneti et al. 1998). In a comparative study between HC-Pro and the suppressor encoded by *Cucumber mosaic virus* (CMV; 2b protein), HC-Pro suppressed PTGS in somatic tissues, including the inoculated leaf, whereas CMV 2b was only active at the shoot apex. The interpretation was that CMV 2b only affected the initiation phase of PTGS while HC-Pro could affect either maintenance alone, or initiation and maintenance. Synergistic infections between CMV and PepMoV in *C. annum* cv. Avelar (Murphy and Kyle 1995) suggest that CMV 2b and HC-Pro are not functional antagonists.

We can view PTGS as a potential defense mechanism against viruses that could restrict virus accumulation in infected cells and, hence, delay virus movement (Baulcombe 1996; Pruss et al. 1997). Therefore, a complete block to long-distance movement could represent the indirect effect of PTGS in allowing extra time for other resistance mechanisms to come into play. This may explain why genetic studies of long-distance movement frequently appear to be difficult to interpret, as it reflects the balance between virus replication potential, the counteraction of PTGS, and further resistance responses.

Virus-induced symptomatology.

Most potyviruses induce conspicuous symptoms, often causing stunting and yield losses. They usually induce longitudinal chlorotic or necrotic streaks in the leaves of monocotyledonous species, and chlorotic vein banding, mosaic mottling, necrosis, and/or distortion of leaves in dicotyledonous species. Flowers, seeds, and fruits are also affected by numerous potyviruses (Shukla et al. 1994).

The nature and extent of symptoms for a specific host genotype depend upon the virus and particular virus strain, as well as upon environmental conditions, probably through their influence on host physiology and development. Symptoms are the result of complex cellular and supracellular interactions of the host with the virus. The effects may be the consequence of the diversion of plant resources into synthesis of virus-specific nucleic acids and proteins, or of the disruptive effects of virus-specific products on normal cellular processes (e.g., cell-to-cell communication). Generally, for any compatible host/virus combination, the severity of symptoms will reflect the level of virus replication and accumulation. As such, and from the preceding discussion, it is clear that the suppressors of PTGS (e.g., HC-Pro) could have a large impact on symptom expression in single infections, or combined infections with other viruses.

In recent years, several regions of the potyviral genome have been shown to have a role in symptomatology. Mutational analysis of the TVMV genome has shown that the P1/HC-Pro coding region, and particularly the 5′ coding region of HC-Pro, is involved in symptom expression on tobacco (Atreya et al. 1992; Atreya and Pirone 1993; Klein et al. 1994). By constructing recombinant hybrids of two PSbMV strains, Johansen et al. (1996) demonstrated that the genome segment encoding the PSbMV NIa and NIb has a major influence on symptom severity in *Pisum sativum*. Two separate TEV genomic segments, one encompassing the 3′ third of the P3 coding region and another encompassing the 3′ end of the CI, the 6K, and the 5′ end of the NIa coding regions, were responsible together for the wilting response of Tabasco pepper (Chu et al. 1997). Also, mutations introduced in the PPV P3-6K1 cleavage site sequence cause either symptom attenuation or more severe symptoms (Riechmann et al. 1995). A further study of PPV showed that a mutant lacking nucleotides 127 to 145 of the 5′ NTR induced only very mild symptoms on *N. clevelandii* (Simón-Buela et al. 1997b). In the 3′ NTR of TVMV, four repeats of 14 adenine and uracil residues were responsible for symptom attenuation on tobacco (Rodríguez-Cerezo et al. 1991). Unfortunately, despite all this information, it is still not possible to identify any unifying principles to explain symptom formation. Although for other virus genera there are examples of particular viral gene products acting as symptom inducers in the absence of infection (Balachandran et al. 1995; Olesinski et al. 1995; Cecchini et al. 1997), there is no such evidence for the potyvirus examples listed above.

To some extent our inadequate understanding of symptom expression reflects our lack of knowledge of host proteins that interact with viral proteins. Progress will certainly be made in future through the genetic analysis of differing symptomatic phenotypes. To date, only two potential interactions have been identified: (i) the interaction between TuMV VPg and eIF(iso)4E (Wittmann et al. 1997) described earlier; and (ii) with anti-idiotypic-antibodies, McClintock et al. (1998) identified a 37-kDa protein localized in chloroplast that interacts with the TuMV CP. The significance of these interactions for virus replication or symptom expression is not known.

Deducing what biochemical changes lead to symptom expression and distinguishing them from consequences of symptom production is a major challenge, complicated by the progressive nature of virus infections in plants where, in contrast to the period of several days to weeks being required to develop a complete systemic infection, the exponential phase of replication in individual cells may take no more than a few hours. Hence, from the catalog of biochemical changes recorded for systemically infected tissues (Zaitlin and Hull 1987) it is impossible to distinguish cause from consequence. An approach that may provide more information is to place the changes in a sequence of events following the onset of virus replication. Through spatial analyses of changes in host gene expression at the infection front, this approach has been applied to PSbMV infection of pea tissues. This revealed a progression of induced changes that included the downregulation of many host genes in a narrow zone behind the infection front, and a coincident but narrower zone where some other host genes (e.g., *hsp70, polyubiquitin*) were selectively induced (Wang and Maule 1995; Aranda et al. 1996). Some of these changes were akin to stress-induced changes seen in response to heat shock, but a related analysis of heat shock factor (*hsf*) expression showed that heat and virus stress utilized independent control pathways (M. A. Aranda and A. J. Maule, *unpublished*). How these effects lead to biochemical changes and symptom expression has not been studied for potyviruses.

The transient nature of virus replication and host responses seen in the PSbMV/pea experiments raises interesting questions about cells a long way behind the infection front. These cells contain massive amounts of progeny virus, no longer show the induced changes in gene expression, and may be susceptible to infection by heterologous viruses. Conceptually, this state is equivalent to latency in animal virus infections (Aranda and Maule 1998). It seems possible that this condition could be maintained by PTGS, which would prevent further replication but have no effect on encapsidated virus. The pool of encapsidated virus provides a reservoir for the establishment of subsequent infection mediated by insect vectors.

Potyvirus transmission.

Potyviruses are transmitted by aphids in a nonpersistent manner and some of them are seed transmitted. Viral determinants of aphid transmission are now well known (see reviews by Pirone 1991; Maia et al. 1996; Pirone and Blanc 1996, and references therein). Therefore, we will concentrate here only on the more recent data. New results have also been obtained for seed transmission.

Aphid transmission. Aphid transmission of potyviruses occurs during brief and superficial probes into the plant. Two potyviral-encoded proteins are involved in this process: CP and HC-Pro. In most cases, a conserved amino acid triplet, Asp-Ala-Gly (DAG) in the N terminus of the CP, and a lysine motif (KITC) located within the N-terminal conserved cysteine-rich domain of HC-Pro, have been shown to be essential for successful transmission. Another motif in HC-Pro, called PTK, seems also to be required (see reviews by Pirone 1991; Maia et al. 1996; Pirone and Blanc 1996). Recent experiments have confirmed this point since *Zucchini yellow mosaic virus* (ZYMV) mutants with mutation in the PTK motif failed to be transmitted by aphids (Peng et al. 1998).

Aphid transmission of potyviruses is dependent upon the acquisition of HC-Pro, prior to or together with the virus. Based upon these observations, it was suggested that HC-Pro acts as a bridge between virion CP and a putative receptor in the vector mouthparts (Pirone and Blanc 1996). Through transmission electron microscopy and light microscopic autoradiography, virions of TVMV were detected in the stylet food canal of aphids but not when CP DAG and HC-Pro lysine domains were mutated (Pirone and Blanc 1996; Wang et al. 1996). Direct evidence for the requirement of a specific interaction between CP and HC-Pro in aphid transmission was provided by Blanc et al. (1997) and Peng et al. (1998). Blanc et al. (1997) demonstrated in vitro binding between TVMV HC-Pro and a 7-amino-acid domain encompassing the DAG motif of the TVMV CP, whereas Peng et al. (1998) showed a binding of HC-Pro with dot-blotted ZYMV virions. The former binding was also shown for several other potyviruses. A good correlation between the efficiency of aphid transmission and HC-Pro/CP binding was also shown in both studies. In addition, nontransmitted mutants of ZYMV with an altered PTK motif failed to bind to virions, suggesting, first, that the

PTK motif is involved in this binding and, second, that the HC-Pro/virions interaction is necessary to transmit ZYMV infection. More recent experiments point to the N-terminal region of HC-Pro, including the KITC motif, as being involved in the interaction with the aphid stylet (Blanc et al. 1998). All these data support a "bridging" model for the function of HC-Pro in transmission by aphids, but the demonstration of a direct interaction of HC-Pro with a receptor in the aphid stylet is still lacking. Comparison of HC-Pro/vector combinations that differ in their ability to transmit potyviruses (e.g., with the aphid *Myzus ascalonicus,* which does not transmit; Wang et al. 1998), provides new opportunities to characterize an HC-Pro receptor in the aphid food canal.

Seed transmission. The seed transmission of potyviruses has been most extensively studied for the PSbMV infection of pea (Wang and Maule 1992, 1994; Johansen et al. 1996), an interaction in which the virus infects the immature embryo after fertilization rather than via infected gametes (Wang and Maule 1992). This mechanism is not universally true for all potyviruses, and some viruses may be transmitted by both routes simultaneously (Maule and Wang 1996). The failure of PSbMV to invade the pea meristem (Jones et al. 1998b) could explain why it is not transmitted through pollen. The precise tissue limitations of other potyvirus infections have not been studied.

For PSbMV in pea, multiple viral determinants (Johansen et al. 1996) and multiple host genes expressed in the maternal tissues (Wang and Maule 1994) seem to be involved in determining the level of seed transmission. Construction of hybrids between the PSbMV isolates DPD-1 (transmissible) and NY (not transmissible) localized the determinants to the 5′ NTR, HC-Pro, and CP regions of the genome. These regions have been shown to be important for virus replication and movement (Riechmann et al. 1992; Kasschau and Carrington 1995; Mahajan et al. 1996; see above). Based upon a spatial analysis of virus accumulation during the process of seed transmission, Wang and Maule (1992, 1994) proposed that the virus exploits the embryonic suspensor to invade the embryo. Since the suspensor is a structure programmed to degenerate early in development, it provides a structural window of opportunity to the transmission process, and, potentially, could indicate why the efficiency of virus movement (mediated directly or indirectly by HC-Pro) could be important for the invasion of the immature seed and, hence, for seed transmission.

Natural and engineered resistance.

Nonhypersensitive resistance to potyviruses may involve dominant, incompletely dominant, or recessive genes while hypersensitive resistance (HR) is controlled mostly by single dominant genes (Fraser 1992). Of all known potyvirus resistance genes, 40% are recessive (Provvidenti and Hampton 1992). This is higher than for other virus groups, where in only 20% of cases is resistance dependent upon recessive genes (Fraser 1992). While the molecular basis of hypersensitivity-associated resistance appears to have common features for a range of hosts and pathogens (Staskawicz et al. 1995), little is known about the nature of the recessive resistance genes. Two hypotheses could explain the role of recessive resistances: (i) the resistant host lacks a host function essential for particular steps in viral pathogenesis, and consequently the dominant allele encodes a host factor, which is required for the virus to replicate and/or move in the susceptible host; or (ii) the susceptibility allele encodes a dominant negative regulator of resistance. The recessive *mlo* gene, which confers resistance against powdery mildew in barley, seems to fit the latter model (Büschges et al. 1997). More complex scenarios are also possible in which a dose-dependent effect of the resistance gene could be involved or multiple, interacting loci could control resistance. An example of polygenic recessive resistance, active in pepper against several potyviruses, was reported recently (Caranta et al. 1997).

In several studies, recessive resistance genes have been functionally characterized through an analysis of their mechanism of action. The *C. annuum* y^a and *pvr3* recessive resistance genes have been shown to restrict movement of potato virus Y (Arroyo et al. 1996) and PepMoV (Murphy et al. 1998), respectively. An inhibition of TEV, PepMoV, and PSbMV RNA replication is a feature of the *C. annuum* et^a, *C. Chinense pvr1,* and *P. sativum sbm-1* recessive resistance genes, respectively (Deom et al. 1997; Keller et al. 1998; Murphy et al. 1998). In the case of the *N. tabacum* cv. TN 86 *va* resistance gene, it seems that both virus replication and movement of TVMV are restricted (Gibb et al. 1989).

Mechanisms of resistance associated with dominant genes have also been studied, particularly for potato resistance genes. In *Solanum brevidens* and the extremely resistant potato cultivars bearing the Ry_{sto} resistance gene, the movement of potyviruses such as PVY, PVA, and TEV was blocked after initial replication and movement triggered the HR response (Valkonen et al. 1991; Barker 1996; Valkonen and Somersalo 1996; Hinrichs et al. 1998).

More straightforward than the cloning of host resistance genes is the identification of the viral avirulence determinant. Through analysis of recombinant hybrid viruses between virulent and avirulent strains, progress has been made for the PSbMV/*sbm-1* (Keller et al. 1998), TVMV/*va* (Nicolas et al. 1997), and *Soybean mosaic virus* (SMV)/*Rsv* (Eggenberger and Hill 1997) interactions. PSbMV resistance-breaking determinants for the *sbm-1* gene were localized in the VPg coding domain, more specifically in a 15-amino-acid central domain of the VPg (Johansen and Keller 1997). Similarly, the TVMV-S VPg has been implicated in *va*-mediated resistance (Nicolas et al. 1997, see above). The important role for VPg in viral RNA replication (Schaad et al. 1996) might indicate an associated function for the products of the *Va* and *Sbm-1* dominant alleles. The interaction of VPg with the translational initiation factor eIF(iso)4E (Wittmann et al. 1997) provides at least one candidate function, although its role in recessive resistance has not been reported.

Chimeric SMVs were made between SMV-N and SMV-G7, strains that are avirulent and virulent, respectively, in soybean plants containing the dominant resistance gene *Rsv* (Eggenberger and Hill 1997). The resistance-breaking determinants were identified in the 3′ region of HC-Pro and the 5′ region of P3 coding domains. However, chimeras with either SMV-G7 HC-Pro or P3 domains alone were not able to overcome *Rsv*. This is probably the first example of a potyvirus for which two avirulence determinants are required to overcome a single resistance gene. Another explanation could be that resistance breaking is a response to viral RNA rather than to viral proteins.

Although it is too early to exploit these natural resistance genes with biotechnology, substantial progress has been made

in the generation of pathogen-derived resistance through the transgenic expression of potyvirus sequences such P1 (Pehu et al. 1995; Moreno et al. 1998), P3 (Moreno et al. 1998), CI protein (Wittner et al. 1998), VPg (Swaney et al. 1995), NIa (Maiti et al. 1993; Vardi et al. 1993), NIb (Audy et al. 1994; Guo and García 1997; Jones et al. 1998a), and CP (Lindbo and Dougherty 1992; Fitchen and Beachy 1993; Hackland et al. 1994) sequences. The resistance can be seen as either a failure of the infection to become established (extreme resistance), or a recovery phenotype in which, after an initial phase of susceptibility, the plants recover and remain resistant to subsequent challenge. In either case, the predominant mechanism is one based upon PTGS, which results in a degradation of transgene mRNA and viral RNA in the cytoplasm (reviewed by Baulcombe and English 1996; van den Boogaart et al. 1998). How this mechanism is triggered is not yet clear, although Jones et al. (1998b) and Guo et al. (1999) propose that a systemic signaling molecule and induced methylation of homologous transgenic sequences may be involved. A conundrum to be resolved in the future is understanding how a virus that encodes a PTGS suppressor (HC-Pro) can be resisted by a PTGS-based mechanism. For extreme resistance, this may reflect the degradation of the homologous RNA sequences before expression of HC-Pro. The induced resistance seen in the recovery phenotype may reflect the relative timing of HC-Pro expression and the remote signaling of PTGS ahead of the infection.

Conclusion.

This review illustrates the advances in our understanding of plant/potyvirus interactions over the last 6 years. By cloning and manipulation of the potyvirus genome, numerous viral determinants involved in the biological characteristics of the virus life cycle have been identified. The data obtained showed, first, that both coding and noncoding regions of the potyvirus genome have functions in these biological processes and, second, that most of the potyvirus proteins are multifunctional. A more complete understanding of the functions of these viral proteins will come from knowledge of the interacting host factors involved in susceptibility and defense.

Perhaps the most significant recent discovery in potyvirus research is the demonstration that HC-Pro can act as a negative regulator of a plant defense mechanism based upon PTGS. Since orthologs of HC-Pro are being discovered in other viruses (e.g., CMV; Béclin et al. 1998; Brigneti et al. 1998), it may be that viruses generally need to counteract PTGS to develop systemic infections. It will be intriguing to see whether PTGS and its regulation by viral proteins could be connected with latency in plants and animals, or even to the changes in host gene expression associated with virus replication.

The major challenge for the future will be to take advantage of the new opportunities offered by chip-based expression arrays, computer-assisted functional genomic analyses, and the rapidly accumulating knowledge of the host genome (particularly *A. thaliana*) to develop advanced studies of both fundamental and applied areas of potyviral biology.

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LITERATURE CITED

Allison, R. F., Sorenson, J. C., Kelly, M. E., Armstrong, F. B., and Dougherty, W. G. 1985. Sequence determination of the capsid protein gene and flanking regions of tobacco etch virus: Evidence for synthesis and processing of a polyprotein in potyvirus genome expression. Proc. Natl. Acad. Sci. USA 82:3969-3972.

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

Andersen, K., and Johansen, I. E. 1998. A single conserved amino acid in the coat protein gene of pea seed-borne mosaic potyvirus modulates the ability of the virus to move systemically in *Chenopodium quinoa*. Virology 241:304-311.

Andrianifahanana, M., Lovins, K., Dute, R., Sikora, E., and Murphy, J. F. 1997. Pathway for phloem-dependent movement of pepper mottle potyvirus in the stem of *Capsicum annuum*. Phytopathology 87:892-898.

Aranda, M., and Maule, A. 1998. Virus-induced host gene shutoff in animals and plants. Virology 243:261-267.

Aranda, M. A., Escaler, M., Wang, D., and Maule, A. J. 1996. Induction of HSP70 and polyubiquitin expression associated with plant virus replication. Proc. Natl. Acad. Sci. USA 93:15289-15293.

Arroyo, R., Soto, M. J., Martínez-Zapater, J. M., and Ponz, F. 1996. Impaired cell-to-cell movement of potato virus Y in pepper plants carrying the y^a (*pr2*[1]) resistance gene. Mol. Plant-Microbe Interact. 9: 314-318.

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.

Atreya, P. L., López-Moya, J. J., Chu, M., Atreya, C. D., and Pirone, T. P. 1995. Mutational analysis of the coat protein N-terminal amino acids involved in potyvirus transmission by aphids. J. Gen. Virol. 76: 265-270.

Audy, P., Palukaitis, P., Slack, S. A., and Zaitlin, M. 1994. Replicase-mediated resistance to potato virus Y in transgenic tobacco plants. Mol. Plant-Microbe Interact. 7:15-22.

Balachandran, S., Hull, R. J., Vaadia, Y., Wolf, S., and Lucas, W. J. 1995. Alteration in carbon partitioning induced by the movement protein of tobacco mosaic virus originates in the mesophyll and is independent of change in the plasmodesmal size exclusion limit. Plant Cell Environ. 18:1301-1310.

Barker, H. 1996. Inheritance of resistance to potato viruses Y and A in progeny obtained from potato cultivars containing gene *Ry:* Evidence for a new gene for extreme resistance to PVA. Theor. Appl. Genet. 93: 710-716.

Basso, J., Dallaire, P., Charest, P. J., Devantier, Y., and Laliberté, J.-F. 1994. Evidence for an internal ribosome entry site within the 5′ nontranslated region of turnip mosaic potyvirus RNA. J. Gen. Virol. 75: 3157-3165.

Baulcombe, D. C. 1996. Mechanisms of pathogen-derived resistance to viruses in transgenic plants. Plant Cell 8:1833-1844.

Baulcombe, D. C., and English, J. J. 1996. Ectopic pairing of homologous DNA and post-transcriptional gene silencing in transgenic plants. Curr. Opin. Biotechnol. 7:173-180.

Béclin, C., Berthomé, R., Palauqui, J.-C., Tepfer, M., and Vaucheret, H. 1998. Infection of tobacco or *Arabidopsis* plants by CMV counteracts systemic post-transcriptional silencing of non-viral (trans)genes. Virology 252:313-317.

Blanc, S., Ammar, E. D., García-Lampasona, S., Dolja, V. V., Llave, C.,

Baker, J., and Pirone, T. P. 1998. Mutations in the potyvirus helper component protein: Effects on interactions with virions and aphid stylets. J. Gen. Virol. 79:3119-3122.

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.

Boyer, J.-C., and Haenni, A.-L. 1994. Infectious transcripts and cDNA clones of RNA viruses. Virology 198:415-426.

Brantley, J. D., and Hunt, A. G. 1993. The N-terminal protein of the polyprotein encoded by the potyvirus tobacco vein mottling virus is an RNA-binding protein. J. Gen. Virol. 74:1157-1162.

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

Büschges, R., Hollricher, K., Panstruga, R., Simons, G., Wolter, M., Frijters, A., van Daelen, R., van den Lee, T., Diergaarde, P., Groenendijk, J., Töpsch, S., Vos, P., Salamini, F., and Schulze-Lefert, P. 1997. The barley *Mlo* gene: A novel control element of plant pathogen resistance. Cell 88:695-705.

Caranta, C., Lefebvre, V., and Palloix, A. 1997. Polygenic resistance of pepper to potyviruses consists of a combination of isolate-specific and broad-spectrum quantitative trait loci. Mol. Plant-Microbe Interact. 10:872-878.

Carrington, J. C., Jensen, P. E., and Schaad, M. C. 1998. Genetic evidence for an essential role for potyvirus CI protein in cell-to-cell movement. Plant J. 14:393-400.

Carrington, J. C., Kasschau, K. D., Mahajan, S. K., and Schaad, M. C. 1996. Cell-to-cell and long-distance transport of viruses in plants. Plant Cell 8:1669-1681.

Cecchini, E., Gong, Z., Geri, C., Covey, S. N, and Milner, J. J. 1997. Transgenic *Arabidopsis* lines expressing gene VI from cauliflower mosaic virus variants exhibit a range of symptom-like phenotypes and accumulate inclusion bodies. Mol. Plant Microbe Interact. 10:1094-1101.

Chu, M., López-Moya, J. J., Llave-Correas, C., and Pirone, T. P. 1997. Two separate regions in the genome of the tobacco etch virus contain determinants of the wilting response of Tabasco pepper. Mol. Plant-Microbe Interact. 10:472-480.

Cronin, S., Verchot, J., Haldeman-Cahill, R., Schaad, M. C., and Carrington, J. C. 1995. Long-distance movement factor: A transport function of the potyvirus helper component proteinase. Plant Cell 7:549-559.

Daròs, J.-A., and Carrington, J. C. 1997. RNA-binding activity of NIa proteinase of tobacco etch potyvirus. Virology 237:327-336.

Deom, C. M., Murphy, J. F., and Paguio, O. R. 1997. Resistance to tobacco etch virus in *Capsicum annuum:* Inhibition of virus RNA accumulation. Mol. Plant-Microbe Interact. 10:917-921.

Dolja, V. V., Haldeman, R., Robertson, N. L., Dougherty, W. G., and Carrington, J. C. 1994. Distinct functions of capsid protein in assembly and movement of etch potyvirus in plants. EMBO J. 13:1482-1491.

Dolja, V. V., Haldeman-Cahill, R., Montgomery, A. E., Vandenbosch, K. A., and Carrington, J. C. 1995. Capsid protein determinants involved in cell-to-cell and long distance movement of tobacco etch potyvirus. Virology 206:1007-1016.

Dolja, V. V., McBride, H. J., and Carrington, J. C. 1992. Tagging of plant potyvirus replication and movement by insertion of β-glucuronidase into the viral polyprotein. Proc. Natl. Acad. Sci. USA 89:10208-10212.

Eagles, R. M., Balmori-Melián, E., Beck, D. L., Gardner, R. C., and Forster, R. L. S. 1994. Characterization of NTPase, RNA-binding and RNA-helicase activities of the cytoplasmic inclusion protein of tamarillo mosaic potyvirus. Eur. J. Biochem. 224:677-684.

Eggenberger, A. L., and Hill, J. H. 1997. Analysis of resistance-breaking determinants of soybean mosaic virus. Phytopathology 87:S27.

Fellers, J., Wan, J., Hong, Y., Collins, G. B., and Hunt, A. G. 1998. *In vitro* interactions between a potyvirus-encoded, genome-linked protein and RNA-dependent RNA polymerase. J. Gen. Virol. 79:2043-2049.

Fernández, A., Laín, S., and García, J. A. 1995. RNA-helicase activity of the plum pox potyvirus CI protein expressed in *Escherichia coli*. Mapping of an RNA binding domain. Nucleic Acids Res. 23:1327-1332.

Fitchen, J. H., and Beachy, R. N. 1993. Genetically engineered protection against viruses in transgenic plants. Annu. Rev. Microbiol. 47: 739-763.

Fraser, R. S. S. 1992. The genetics of plant-virus interactions: Implications for plant breeding. Euphytica 63:175-185.

Gallie, D. R. 1998. A tale of two termini: A functional interaction between the termini of an mRNA is a prerequisite for efficient translation initiation. Gene 216:1-11.

Gallie, D. R., Tanguay, R. L., and Leathers, V. 1995. The tobacco etch viral 5′ leader and poly (A) tail are functionally synergistic regulators of translation. Gene 165:233-238.

Gibb, K. S., Hellmann, G. M., and Pirone, T. P. 1989. Nature of resistance of a tobacco cultivar to tobacco vein mottling virus. Mol. Plant-Microbe Interact. 2:332-339.

Guo, H. S., and García, J. A. 1997. Delayed resistance to plum pox potyvirus mediated by a mutated RNA replicase gene: Involvement of a gene-silencing mechanism. Mol. Plant-Microbe Interact. 10:160-170.

Guo, H. S., López-Moya, J. J., and García, J. A. 1999. Mitotic stability of infection-induced resistance to plum pox potyvirus associated with transgene silencing and DNA methylation. Mol. Plant-Microbe Interact. 12:103-111.

Hackland, A. F., Rybicki, E. P., and Thomson, J. A. 1994. Coat protein-mediated resistance in transgenic plants. Arch. Virol. 139:1-22.

Haldeman-Cahill, R., Daròs, J.-A., and Carrington, J. C. 1998. Secondary structures in the capsid protein coding sequence and 3′ nontranslated region involved in amplification of the tobacco etch virus genome. J. Virol. 72:4072-4079.

Hinrichs, J., Berger, S., and Shaw, J. G. 1998. A hypersensitive response-like mechanism is involved in resistance of potato plants bearing the Ry_{sto} gene to the potyviruses potato virus Y and tobacco etch virus. J. Gen. Virol. 79:167-176.

Hong, Y., and Hunt, A. G. 1996. RNA polymerase activity catalyzed by potyvirus encoded RNA-dependent RNA polymerase. Virology 226: 146-151.

Hong, Y., Levay, K., Murphy, J. F., Klein, P. G., Shaw, J. G., and Hunt, A. G. 1995. A potyvirus polymerase interacts with the viral coat protein and VPg in yeast cells. Virology 214:159-166.

Jagadish, M. N., Huang, D., and Ward, C. W. 1993. Site-directed mutagenesis of a potyvirus coat protein and its assembly in *Escherichia coli*. J. Gen. Virol. 74:893-896.

Johansen, I. E., Dougherty, W. G., Keller, K. E., Wang, D., and Hampton, R. O. 1996. Multiple viral determinants affect seed transmission of pea seedborne mosaic virus in *Pisum sativum*. J. Gen. Virol. 77: 3149-3154.

Johansen, E., Edwards, M. C., and Hampton, R. O. 1994. Seed transmission of viruses: Current perspectives. Annu. Rev. Phytopathol. 32: 363-386.

Johansen, I. E., and Keller, K. E. 1997. The role of potyvirus proteins in recessive resistance affecting replication. In: Molecular mechanisms in the replicative cycle of viruses in plants. EMBO Worksh.

Jones, A. L., Johansen, I. E., Bean, S. J., Bach, I., and Maule, A. J. 1998a. Specificity of resistance to pea seed-borne mosaic potyvirus in transgenic peas expressing the viral replicase (NIb) gene. J. Gen. Virol. 79:3129-3137.

Jones, A.L., Thomas, C. L., and Maule, A.J. 1998b. *De novo* methylation and co-suppression induced by a cytoplasmically replicating plant RNA virus. EMBO J. 17:6385-6393.

Kasschau, K. D., and Carrington, J. C. 1995. Requirement for HC-Pro processing during genome amplification of tobacco etch potyvirus. Virology 209:268-273.

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.

Keller, K. E., Johansen, I. E., Martin, R. R., and Hampton, R. O. 1998. Potyvirus genome-linked protein (VPg) determines pea seed-borne mosaic virus pathotype-specific virulence in *Pisum sativum*. Mol. Plant-Microbe Interact. 11:124-130.

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.
Laín, S., Riechmann, J. L., and García, J. A. 1990. RNA helicase: A novel activity associated with a protein encoded by a positive strand RNA virus. Nucleic Acids Res. 18:7003-7006.
Langenberg, W. G. 1986. Virus protein association with cylindrical inclusions of two viruses that infect wheat. J. Gen. Virol. 67:1161-1168.
Lawson, R. H., and Hearon, S. S. 1971. The association of pinwheel inclusions with plasmodesmata. Virology 44:454-456.
Levis, C., and Astier-Manifacier, S. 1993. The 5′ untranslated region of PVY RNA, even located in an internal position, enables initiation of translation. Virus Genes 7:367-379.
Li, X. H., Valdez, P., Olvera, R. E., and Carrington, J. C. 1997. Functions of the tobacco etch virus RNA polymerase (NIb): Subcellular transport and protein-protein interaction with VPg/proteinase (NIa). J. Virol. 71:1598-1607.
Lindbo, J. A., and Dougherty, W. G. 1992. Pathogen-derived resistance to a potyvirus: Immune and resistant phenotypes in transgenic tobacco expressing altered forms of a potyvirus coat protein nucleotide sequence. Mol. Plant-Microbe Interact. 5:144-153.
López-Moya, J. J., and Pirone, T. P. 1998. Charge changes near the N terminus of the coat protein of two potyviruses affect virus movement. J. Gen. Virol. 79:161-165.
Lucas, W. J., and Gilbertson, R. L. 1994. Plasmodesmata in relation to viral movement within leaf tissues. Annu. Rev. Phytopathol. 32:387-411.
Mahajan, S. K., Chisholm, S. T., Whitham, S. A., and Carrington, J. C. 1998. Identification and characterization of a locus (*RTM1*) that restricts long-distance movement of tobacco etch virus in *Arabidopsis thaliana*. Plant J. 14:177-186.
Mahajan, S., Dolja, V. V., and Carrington, J. C. 1996. Roles of the sequence encoding tobacco etch virus capsid protein in genome amplification: Requirements for the translation process and a *cis*-active element. J. Virol. 70:4370-4379.
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.
Maiti, I. B., Murphy, J. F., Shaw, J. G., and Hunt, A. G. 1993. Plants that express a potyvirus proteinase gene are resistant to virus infection. Proc. Natl. Acad. Sci. USA 90:6110-6114.
Maule, A. J., and Wang, D. 1996. Seed transmission of plant viruses: A lesson in biological complexity. Trends Microbiol. 4:153-158.
McClintock, K., Lamarre, A., Parsons, V., Laliberté, J.-F., and Fortin, M. G. 1998. Identification of a 37 kDa plant protein that interacts with the turnip mosaic potyvirus capsid protein using anti-idiotypic-antibodies. Plant Mol. Biol. 37:197-204.
Merits, A., Guo, D., and Saarma, M. 1998. VPg, coat protein and five non-structural proteins of potato A potyvirus bind RNA in a sequence-unspecific manner. J. Gen. Virol. 79:3123-3127.
Moreno, M., Bernal, J. J., Jiménez, I., and Rodríguez-Cerezo, E. 1998. Resistance in plants transformed with the P1 or P3 gene of tobacco vein mottling potyvirus. J. Gen. Virol. 79:2819-2827.
Murphy, J. F., Blauth, J. R., Livingstone, K. D., Lackney, V. K., and Jahn, M. K. 1998. Genetic mapping of the *pvr1* locus in *Capsicum* spp. and evidence that distinct potyvirus resistance loci control responses that differ at the whole plant and cellular levels. Mol. Plant-Microbe Interact. 11:943-951.
Murphy, J. F., Klein, P. G., Hunt, A. G., and Shaw, J. G. 1996. Replacement of the tyrosine residue that links a potyviral VPg to the viral RNA is lethal. Virology 220:535-538.
Murphy, J. F., and Kyle, M. M. 1995. Alleviation of restricted systemic spread of pepper mottle potyvirus in *Capsicum annuum* cv. Avelar by coinfection with a cucumovirus. Phytopathology 85:561-566.
Nicolas, O., Dunnington, S. W., Gotow, L. F., Pirone, T. P., and Hellmann, G. M. 1997. Variations in the VPg protein allow a potyvirus to overcome *va* gene resistance in tobacco. Virology 237:452-459.
Olesinski, A. A., Lucas, W. J., Galun, E., and Wolf, S. 1995. Pleiotropic effects of tobacco-mosaic-virus movement protein on carbon metabolism in transgenic tobacco plants. Planta 197:118-126.
Pehu, T. M., Makivalkonen, T. K., Valkonen, J. P. T., Koivu, K. T., Lehto, K. M., and Pehu, E. P. 1995. Potato plants transformed with a potato virus Y P1 gene sequence are resistant to PVY. Am. Potato J. 72:523-532.
Pelletier, J., and Sonenberg, N. 1988. Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. Nature 334:320-325.
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.
Pirone, T. P. 1991. Viral genes and gene products that determine insect transmissibility. Semin. Virol. 2:81-87.
Pirone, T. P., and Blanc, S. 1996. Helper-dependent vector transmission of plant viruses. Annu. Rev. Phytopathol. 34:227-247.
Provvidenti, R., and Hamptom, R. O. 1992. Sources of resistance to viruses in the *Potyviridae*. Arch. Virol. 5:189-211.
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., Cervera, M. T., and García, J. A. 1995. Processing of the plum pox virus polyprotein at the P3-6K$_1$ junction is not required for virus viability. J. Gen. Virol. 76:951-956.
Riechmann, J. L., Laín, S., and García, J. A. 1992. Highlights and prospects of potyvirus molecular biology. J. Gen. Virol. 73:1-16.
Roberts, I. M., Wang, D., Findlay, K., and Maule, A. J. 1998. Ultrastructural and temporal observations of the potyvirus cylindrical inclusions (CIs) shows that the CI protein acts transiently in aiding virus movement. Virology 245:173-181.
Rodríguez-Cerezo, E., Findlay, K., Shaw, J. G., Lomonossoff, G. P., Qiu, S. G., Linstead, P., Shanks, M., and Risco, C. 1997. The coat and cylindrical inclusion proteins of a potyvirus are associated with connections between plant cells. Virology 236:296-306.
Rodríguez-Cerezo, E., Klein, P. G., and Shaw, J. G. 1991. A determinant of disease symptom severity is located in the 3′-terminal noncoding region of the RNA of a plant virus. Proc. Natl. Acad. Sci. USA 88:9863-9867.
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.
Schaad, M. C., and Carrington, J. C. 1996. Suppression of long-distance movement of tobacco etch virus in a nonsusceptible host. J. Virol. 70:2556-2561.
Schaad, M. C., Haldeman-Cahill, R., Cronin, S., and Carrington, J. C. 1996. Analysis of the VPg-proteinase (NIa) encoded by tobacco etch potyvirus: Effects of mutations on subcellular transport, proteolytic processing and genome amplification. J. Virol. 70:7039-7048.
Schaad, M. C., Jensen, P. E., and Carrington, J. C. 1997a. Formation of plant RNA virus replication complexes on membranes: Role of an endoplasmic reticulum-targeted viral protein. EMBO J. 16:4049-4059.
Schaad, M. C., Lellis, A. D., and Carrington, J. C. 1997b. VPg of tobacco etch potyvirus is a host genotype-specific determinant for long-distance movement. J. Virol. 71:8624-8631.
Shi, X. M., Miller, H., Verchot, J., Carrington, J. C., and Vance, V. B. 1997. Mutations in the region encoding the central domain of helper component-proteinase (HC-Pro) eliminate potato virus X/potyviral synergism. Virology 231:35-42.
Shukla, D. D., and Ward, C. W. 1988. Amino acid sequence homology of coat proteins as a basis for identification and classification of the potyvirus group. J. Gen. Virol. 69:2703-2710.
Shukla, D. D., Ward, C. W., and Brunt, A. A. 1994. Genome structure, variation and function. Pages 74-110 in: The Potyviridae. CAB Int., Wallingford, UK.
Simón-Buela, L., Guo, H. S., and García, J. A. 1997a. Cap-independent *leaky* scanning as the mechanism of translation initiation of a plant viral genomic RNA. J. Gen. Virol. 78:2691-2699.
Simón-Buela, L., Guo, H. S., and García, J. A. 1997b. Long sequences in the 5′ noncoding region of plum pox virus are not necessary for viral infectivity but contribute to viral competitiveness and pathogenesis. Virology 233:157-162.
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.
Staskawicz, B. J., Ausubel, F. M., Baker, B. J., Ellis, J. G., and Jones, J. D. J. 1995. Molecular genetics of plant disease resistance. Science 268:661-667.
Swaney, S., Powers, H., Goodwin, J., Silva Rosales, L., and Dougherty,

W. G. 1995. RNA-mediated resistance with nonstructural genes from the tobacco etch virus genome. Mol. Plant-Microbe Interact. 8:1004-1011.

Valkonen, J. P. T., Pehu, E., Jones, M. G. K., and Gibson, R. W. 1991. Resistance in *Solanum brevidens* to both potato virus Y and potato virus X may be associated with slow cell-to-cell spread. J. Gen. Virol. 72:231-236.

Valkonen, J. P. T., and Somersalo, S. 1996. Patterns and barriers of cell-to-cell movement and lack of systemic spread of tobacco etch potyvirus (TEV-GUS) in *Solanum brevidens*. Plant Sci. 113:221-228.

van den Boogaart, T., Lomonossoff, G. P., and Davies, J. W. 1998. Can we explain RNA-mediated virus resistance by homology-dependent gene silencing? Mol. Plant-Microbe Interact. 11:717-723.

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.

Vardi, E., Sela, I., Edelbaum, O., Livneh, O., Kuznetsova, L., and Stram, Y. 1993. Plants transformed with a cistron of a potato virus Y protease (NIa) are resistant to virus infection. Proc. Natl. Acad. Sci. USA 90: 7513-7517.

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.

Wang, D., and Maule, A. J. 1992. Early embryo invasion as a determinant in pea of the seed transmission of pea seed-borne mosaic virus. J. Gen. Virol. 73:1615-1620.

Wang, D., and Maule, A. J. 1994. A model for seed transmission of a plant virus: Genetic and structural analyses of pea embryo invasion by pea seed-borne mosaic virus. Plant Cell 6:777-787.

Wang, D., and Maule, A. J. 1995. Inhibition of host gene expression associated with plant virus replication. Science 267:229-231.

Wang, R. Y., Ammar, E. D., Thornbury, D. W., López-Moya, J. J., and Pirone, T. P. 1996. Loss of potyvirus transmissibility and helper-component activity correlate with non-retention of virions in aphid stylets. J. Gen. Virol. 77:861-867.

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.

Wittmann, S., Chatel, H., Fortin, M. G., and Laliberté, J.-F. 1997. Interaction of the viral protein genome linked of turnip mosaic potyvirus with the translational eukaryotic initiation factor (iso) 4E of *Arabidopsis thaliana* using the yeast two-hybrid system. Virology 234:84-92.

Wittner, A., Palkovics, L., and Balazs, E. 1998. *Nicotiana benthamiana* plants transformed with the plum pox virus helicase gene are resistant to virus infection. Virus Res. 53:97-103.

Zaitlin, M., and Hull, R. 1987. Plant virus-host interactions. Annu. Rev. Plant Physiol. 38:291-315.