How do plant viruses induce disease? Interactions and interference with host components

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Plant viruses are biotrophic pathogens that need living tissue for their multiplication and thus, in the infection–defence equilibrium, they do not normally cause plant death. In some instances virus infection may have no apparent pathological effect or may even provide a selective advantage to the host, but in many cases it causes the symptomatic phenotypes of disease. These pathological phenotypes are the result of interference and/or competition for a substantial amount of host resources, which can disrupt host physiology to cause disease. This interference/competition affects a number of genes, which seems to be greater the more severe the symptoms that they cause. Induced or repressed genes belong to a broad range of cellular processes, such as hormonal regulation, cell cycle control and endogenous transport of macromolecules, among others. In addition, recent evidence indicates the existence of interplay between plant development and antiviral defence processes, and that interference among the common points of their signalling pathways can trigger pathological manifestations. This review provides an update on the latest advances in understanding how viruses affect substantial cellular processes, and how plant antiviral defences contribute to pathological phenotypes.

Introduction

More than one thousand viruses are currently known to be potentially capable of infecting plants. Despite the large number of possible combinations, the development of disease is an exception rather than a common outcome and thus, in most cases, plants are capable of counteracting the harmful effects of viruses. This resistance is owed to the absence of essential host susceptibility factors (passive resistance) or to the existence of several defence layers that the virus has to overcome. First, the virus needs to overcome a series of pre-existing physical and chemical barriers in plants. If a pathogenic virus succeeds in overcoming this first line of defence, it would have to face the non-specific defensive reactions with which the plant responds to some molecular patterns that are common to different pathogens (Jones & Dangl, 2006). If a virus has evolved to acquire virulence factors to counteract this basal defence, it is in a position to be able to trigger infection. In many cases, however, plants are able to recognize these virulence factors and create a new, more specific resistance layer that is only induced when faced with viruses expressing this virulence factor (Jones & Dangl, 2006). A virus can cause productive infection only in those plants that have not developed specific defensive responses to its virulence factors.

Also, viral RNA induces specific plant defence responses in which a large number of plant proteins participate; among them, Dicer-type dsRNA RNases, ssRNA RNases belonging to the Argonaute-type protein family, which assemble in RNA-induced silencing complexes (RISC), RNA polymerases and RNA helicases (Dunoyer & Voinnet, 2005). This antiviral response, which is one of the manifestations of a complex set of cellular processes known as RNA silencing, is apparently universal; so for the virus to be successful it has to escape it. Although viruses may adopt various strategies to go about achieving this, it is believed the most usual strategy they adopt is that of producing silencing suppressors (Li & Ding, 2006; Valli et al., 2009). These suppressors not only affect antiviral defence, but also interfere with plant physiological processes that depend on RNA silencing, and this interference may contribute significantly to the pathogenesis of different viruses.

A virus not only needs to escape the defences that plants erect, but must also tackle different processes to complete its productive cycle (Maule et al., 2002). The initiation of this cycle depends on the nature of the genetic material of the virus. Positive-polarity RNA viruses are the most abundant in the plant kingdom. For these viruses, genomic RNA must be uncoated and translated after viral particles have entered the plant cell, and both processes are highly coordinated. There also seems to be some kind of coupling between the synthesis of viral proteins and the assembly of some of these proteins with genomic RNA and host factors...
to form replication complexes. The next stage of the virus cycle entails its movement to neighbouring cells and its dissemination throughout the plant. Interactions of viral and cellular factors may not only contribute to facilitate these viral infection steps and help to establish optimum infection susceptibility conditions but may also indirectly affect host physiological processes.

Although many viral infections progress efficiently without symptom development, induction of plant defence mechanisms, their suppression by countering viral strategies and the co-option of host factors required for virus replication and movement can confer a pathological character upon the viral infection. There is experimental evidence of the individual contribution of these elements in different viral infections; nonetheless, a model that includes them in the specific development of a particular pathology is lacking (Culver & Padmanabhan, 2007). This review does not intend to explain how different viral plant diseases develop but to describe some specific examples of viral and plant factors that contribute to viral pathogenesis.

**Effect of plant defence responses and their suppression of viral pathogenesis**

**Plant suicide responses: hypersensitive response (HR) and resistance genes**

Traditionally, it has been accepted that viral disease symptoms could be caused by a toxic effect of some virus components. Unfortunately, however, the molecular basis of this effect is known in very few cases. One exception is the induction of plant ‘suicide’ defence responses related to HR (Mur et al., 2008).

The HR is one of the most common plant reactions to any type of pathogenic organism, including viruses. In general, the HR has been associated with a defence response perceived by receptors known as R genes, which confine the pathogen to the inoculated area, and thus its potential propagation through the whole plant is impeded. Several virus-specific R genes have been identified, which not only bring about an HR in response to a particular virus, but also prevent viral propagation (Soosaar et al., 2005). Nonetheless, there is increasing evidence that the HR and resistance are related, yet independent, phenomena. For example, the interaction of the product of the R gene Rx1 from *Solanum tuberosum* with the capsid protein (CP) of potato virus X (PVX) can cause cell death; however, this gene is able to block virus replication before the amount of viral CP necessary to trigger the necrotic response is generated (Bendahmane et al., 1999). Conversely, there are also many instances in which the production of characteristic HR necrotic lesions on inoculated leaves do not hinder the virus from propagating throughout the plant, but occasionally give way to systemic necrotic symptoms that might prove lethal. Regarding the resistance of the ecotype Di-17 of arabidopsis to turnip crinkle virus (TCV), the HR triggered by the interaction of the viral CP with the product of the R gene HRT is required to block infection, but, for this to take place, an additional response regulated by the recessive rrt gene is also needed (Kachroo et al., 2000). On the other hand, the resistance of arabidopsis to cucumber mosaic virus (CMV), deriving from the induction of the RCY1 gene by the viral CP, is suppressed by sporadic mutations in the resistance gene, which have diverse effects on the development of the local necrotic lesions (Sekine et al., 2006). Recently, Komatsu et al. (2010) have shown that systemic necrosis in *Nicotiana benthamiana*, induced by *Plantago asiatica* mosaic virus infection, was associated with programmed cell death, biochemical features and gene expression patterns that are characteristic of HR. Their results suggest that systemic necrosis and HR consist of programmed cell death and a restraint upon virus multiplication, and that the latter is induced through unknown pathways that are independent of the former.

Not only CP, but also virtually any viral gene product may be an HR inducer, regardless of it being capable or not of hindering the virus from propagating. Some examples of the former kind are the interactions between the p50 helicase domain from tobacco mosaic virus (TMV) replicase and the N gene from *Nicotiana glutinosa* (Padgett et al., 1997), the 30k movement protein (MP) from tomato mosaic virus (ToMV) and the Tm22 gene from *Solanum lycopersicum* (Calder & Palukaitis, 1992), the nuclear inclusion a protease from potato virus Y (PVY) and the Ry gene from *Solanum stoloniferum* (Mestre et al., 2000), the cytoplasmic inclusion RNA helicase from turnip mosaic virus (TuMV) and the TuRB01 gene from *Brassica napus* (Jenner et al., 2000), the same protein from soybean mosaic virus (SMV) and the Rsv3 gene from *Glycine max* (Zhang et al., 2009), etc. There are few reports in which the viral factor inducing an HR that is not capable of restricting the virus in an inoculated area has been identified; examples of such are the induction of systemic vein necrosis by the nuclear inclusion b RNA replicase from PVY in *Nicotiana tabacum*, which is mediated by either the Rk gene or a gene closely associated with it (Fellers et al., 2002), and the systemic necrosis of *Arabidopsis thaliana* Ler caused by TuMV, which is determined by a gene-to-gene interaction between the TuNI resistance gene and the P3-encoding region of the virus (Kim et al., 2010). One system that displays just how complicated the contribution of a resistance gene to viral pathogenicity can be is that formed by SMV and *G. max* lines carrying the Rsv1 gene. When the virus is mechanically inoculated, this gene elicits resistance to the majority of SMV isolates with no apparent symptoms; however, inoculation through grafts produces necrotic lesions in the stem, petioles and leaf veins, with the cytological and histological characteristics of an HR (Hajimorad & Hill, 2001). On the other hand, the isolate SMV-G7, when mechanically inoculated, causes a lethal systemic HR, while SMV-G7d, a variant deriving from SMV-G7, provokes a systemic mosaic. The analysis of recombinant viruses has demonstrated that the capacity for inducing systemic HR
resides in protein P3 (Hajimorad et al., 2005); nonetheless, a standard isolate of SMV with the P3 sequence from either SMV-G7 or SMV-G7d was still unable to infect plants with the Rsv1 gene, whereas SMV-G7 and SMV-G7d with a standard P3 were no longer virulent for these plants (Hajimorad et al., 2006). More recent results have revealed that concurrent modifications in proteins P3 and helper component (HCPro) are required to confer the ability to overcome Rsv1-derived resistance on a standard SMV isolate in some soybean cultivars. However, single mutations in P3 are able to confer virulence in other Rsv1 cultivars, suggesting that Rsv1 is a complex locus and P3 and HCPro are involved in interactions with different Rsv1-related resistance factors (Eggenberger et al., 2008; Hajimorad et al., 2011).

dsRNA-mediated resistance and its suppression

Plants also have other antiviral response components that influence the symptoms of viral disease. P58IPK is a well-known inhibitor of the mammalian dsRNA-dependent protein kinase, PKR. Many animal viruses either encode proteins that mimic P58IPK or recruit it to hinder PKR antiviral activity. P58IPK from N. benthamiana interacts with both the helicase domain of the TMV replicase and the tobacco etch virus (TEV) helicase, and P58IPK down-regulation lowers the accumulation levels of these viruses in infected plants (Bilgin et al., 2003). In spite of the smaller amount of virus, massive cell death is observed in these plants, which is associated with phosphorylation of the eIF-2 translation initiation factor by PKR. One interpretation of these results is that P58IPK is a host factor required for virus replication and restriction of disease symptoms, probably by ensuring that protein synthesis is not suppressed by the PKR-mediated innate immune system when viral dsRNAs are present (Whitham & Wang, 2004).

The RNA silencing machinery cleaves viral dsRNA structures, giving rise to small interfering RNAs (siRNA) that lead RISC complexes to degrade viral ssRNA and/or to inhibit its translation. miRNAs of some host genes can also be the target of RISC loaded with viral siRNAs, and it has been postulated that downregulation of these genes can contribute to suppressing antiviral defences and/or eliciting disease symptoms (Moissiard & Voinnet, 2006) (Fig. 1). Indeed, it has been demonstrated recently that disease symptoms caused by CMV satellite RNA are the consequence of siRNA-directed RNA silencing of the chlorophyll biosynthetic gene CHLI (Shimura et al., 2011; Smith et al., 2011).

One viral factor may bring about a pathological response not only by inducing a resistance mechanism but also by suppressing it (Fig. 1). The molecular basis (or one of the molecular bases) of the contribution of one of these factors, the HCPro protein of potyviruses, to viral pathogenicity has been extensively studied. HCPro interferes with RNA silencing-mediated plant defence responses. Apart from hindering viral RNA degradation, protein HCPro interferes with the branch of RNA silencing that uses microRNAs (miRNAs), and it is not known whether the virus benefits from this interference in any way. Ectopic expression of the HCPro protein gives rise to the accumulation of inactive forms of certain miRNAs. As these small RNAs have a negative regulation function, HCPro protein expression gives rise to a gain of function phenotype for their target genes, some of which are essential for plants to develop correctly, thus causing very similar plant malformations to those observed during viral infections (Chapman et al., 2004; Kasschau et al., 2003). Thus, it is not surprising that modifications in the HCPro protein sequence would noticeably alter the visibility of virus symptoms (Gal-On, 2000; Gonzalez-Jara et al., 2005; Lin et al., 2007; Sáenz et al., 2001; Yamboa et al., 2008). At first sight, if HCPro silencing suppressor activity conditions both plant antiviral activity and the expression of virus symptoms, then changes in HCPro would be expected to cause parallel effects in symptom severity and viral accumulation. In some cases, however, alteration of the symptoms caused by mutations in protein HCPro are not accompanied by differences in the level of virus accumulation (Gal-On, 2000; Sáenz et al., 2001). So, an arginine-to-isoleucine mutation in the HCPro-conserved FRNK domain yields a strong attenuation of zucchini yellow mosaic virus (ZYMV) symptoms with no apparent effect on the levels of viral accumulation. Shiboleth et al. (2007) verified that this mutation affects the capacity of HCPro to bind small-sized RNAs in vitro. However, it may well not hinder efficient in vivo binding with the siRNAs that mediate the antiviral response, and with which HCPro has a high affinity. This could be the reason why this mutant is replicated with a similar efficiency to that of wild-type virus. The affinity of ZYMV HCPro for duplex miRNA, particularly for those with several mismatches, is lower than that shown by the siRNAs formed by two perfectly complementary strands. Therefore, the mutation could have a more drastic effect on the sequestration of miRNAs, and could mitigate the symptoms observed in infections with the mutant virus. More recently, Wu et al. (2010) have described HCPro mutants that do not interfere with miRNA and trans-acting siRNA pathways but still retain the ability to suppress PTGS. In a recent study, Torres-Barceló et al. (2008) found a variety of effects of mutations in the RNA silencing suppressor of tobacco etch virus (TEV), ranging from complete abolition of suppressor activity to significantly stronger suppression. Whereas mutants with a hyposuppressor HCPro were less virulent and accumulated fewer viral particles than wild-type virus, mutants with hypersuppressor HCPros induced symptoms similar to those of wild-type virus and accumulated particles to similar levels. In addition, hyposuppressor alleles were less efficient at binding siRNAs than hypersuppressors, whereas the latter were not different from wild type (Torres-Barceló et al., 2010).

There are other silencing suppressors that are also capable of interfering with the function of miRNAs and of causing
malformations in plants; thus, they are also likely to play a significant role in viral pathogenesis (Dunoyer et al., 2004). Very recently, it has been demonstrated that three different viral silencing suppressors cause developmental abnormalities through misregulation of the miR167 target auxin response factor (Jay et al., 2011). Moreover, it was established that this disturbance accounts for the developmental, but not metabolic, symptoms elicited by the potyvirus TuMV, without affecting virulence or virus accumulation (Jay et al., 2011).

Along the same lines as the previously described examples, Tsuda et al. (2007) revealed that the pathogenicity of pepper mild mottle virus is actually controlled by the RNA-silencing suppressor activity of its replication protein, and not by the levels of viral accumulation.

As different silencing suppressors act with distinct mechanisms and affect various stages of the silencing process, it is not surprising that more severe symptoms are produced in infections where viruses from different
families are mixed, and that they reach higher viral titres than in individual infections. The participation of the potyvirus HCPro protein in such synergistic phenomena is well documented, especially in mixed infections with PVX (González-Jara et al., 2005; Pruss et al., 1997; Sáenz et al., 2001; Yang & Ravelonandro, 2002), and the joint action of several silencing suppressors could cause severe pathological symptoms in many viral diseases, some of which are of considerable economic and social importance (Cuellar et al., 2008; Mukasa et al., 2006; Scheets, 1998).

What silencing suppressors contribute to viral pathogenesis is not apparently restricted to the effects they have directly on RNA silencing-controlled processes. For instance, protein AL2 from tomato golden mosaic virus (TGMV) and L2 from beet curly top virus (BCTV) interact with the adenosine kinase, and deactivate it (Wang et al., 2005). Although this deactivation contributes to silencing suppression, it may also have general collateral effects on cellular metabolism. Likewise, these two silencing suppressors also interact with the sucrose non-fermenting 1 (SNF1) kinase, and lead to its deactivation. As the SNF1 kinase is a global metabolism regulator, this interaction may also have a good number of pleiotropic effects. Apparently the SNF1 kinase does not participate in RNA silencing, although its deactivation increases susceptibility to infection by TGMV and BCTV. This suggests that these viruses have ‘learned’ to modify the host metabolism for their own benefit and, at the same time, it reveals the existence of a molecular link between the metabolic status of plants and their susceptibility to viral pathogens (Hao et al., 2003).

Another silencing suppressor activity that could have, in addition to a possible impact on viral infection, non-specific effects on cellular physiology is the interaction of HCPro from lettuce mosaic virus (LMV) with proteasome 20S in order to cause its aggregation in high molecular mass from protein 2b from the cucumovirus tomato aspermy virus (TAV) (Li et al., 1999) or protein p19 from the tombusvirus tomato bushy stunt virus (TBSV) (Chu et al., 2000), have been described as inducing similar local or systemic necrotic responses to those mediated by R genes. HCPro from TEV has also been found to induce a poorly specific response that stimulates plant resistance to many pathogens (Pruss et al., 2004). Although none of these responses are capable of completely blocking the viral infection, they are able to condition pathological symptom development. Recently, it has been reported that the interaction between CMV 2b and a host catalase is involved in the induction of a necrotic reaction in A. thaliana, however it is not clear whether this interaction is part of the defence program of the plant or of a counterdefence response of the virus (Inaba et al., 2011).

Interference with either the biogenesis pathway of the miRNAs or their accumulation is not an exclusive feature of silencing-suppressor viral proteins. In this sense, Bazzini et al. (2007) have demonstrated that the interaction between the MP and the CP from TMV, expressed in transgenic plants, increases the levels of miRNAs, which could be the cause of the abnormal development symptoms noted in these plants.

Effect of viral and plant factors involved in viral replication on pathogenesis

Interference with hormonal regulation

As mentioned above, the effect of certain silencing suppressors reveals that some symptoms of viral infections are the result of alterations to plant growth and development (Chapman et al., 2004; Jay et al., 2011; Kasschau et al., 2003). Apparently, however, interfering with the activity of miRNAs is not the only way by which viruses alter the developmental program of their hosts. Connections have been noted between the interactions of specific virus factors with cell components, and alterations in hormone synthesis and signalling (Culver & Padmanabhan, 2007). For example, interactions between the helicase domain of the TMV replicase and several members of the auxin/indole acetic acid (Aux/IAA) protein family have been reported (Padmanabhan et al., 2006). The subcellular localization of these Aux/IAA proteins is altered and their levels of accumulation are lowered in the presence of the TMV replicase. On the other hand, their partial downregulation through virus-induced gene silencing causes premature host hormone levels, and a mutation that diminishes the capacity of the replicase to interact with the Aux/IAA proteins significantly lowers virus accumulation in mature plant leaves (Padmanabhan et al., 2008). These results suggest that the cellular environment of mature leaves is not appropriate for virus multiplication, and that the deactivation of the Aux/IAA proteins is reprogrammed to be more compatible with viral replication and propagation.

Another example of a virus–plant interaction affecting hormonal regulation is that of protein P2 from rice dwarf virus (RDV) and the ent-kauren oxidase protein. This host protein plays a key role in the biosynthesis of gibberellins, the hormones that regulate plant growth. ent-kauren oxidase expression and the endogenous level of gibberellin GA1 were lower in those plants presenting a dwarf phenotype as the result of infection by RDV, while the exogenous application of gibberellin GA3 to RDV-infected plants restored normal growth (Zhu et al., 2005). On the other hand, it has been proposed that the interaction between P2 and ent-kauren
oxidase-type proteins interferes with the biosynthesis of phytoalexins, and consequently facilitates viral replication; however, experimental evidence is lacking (Zhu et al., 2005).

An interaction between the product of gene VI from CaMV and the ethylene hormone-signalling pathway has also been observed. Arabidopsis mutants that suppress the phenotype induced by transgene-mediated expression of CaMV gene VI are less susceptible to CaMV-infection and show reduced ethylene sensitivity (Geri et al., 2004). Nonetheless, no studies have been done to verify whether the product of gene VI directly affects any of the components in the action pathway of ethylene, or whether this effect is mediated by its capacity to interfere with RNA silencing.

Interference with the cell cycle and gene expression

Some viruses are only able to infect cells that are actively dividing. Since this is not the case for most plant cells, these viruses have developed mechanisms to alter the cell cycle of their hosts. It has been shown that the Rep proteins of geminiviruses interact with a family of proteins known as pRBR (retinoblastoma-related proteins), which are involved in negative cell-cycle regulation (Kong et al., 2000; Xie et al., 1996). Presumably, interaction with Rep inhibits pRBR protein activity, giving rise to the cell entering S phase, and thus to the production of the host DNA replication machinery required to reproduce the virus. Apparently, there are other ways of controlling the cell cycle phase of infected plants. The Clink protein from faba bean necrotic yellow virus (FBNYV) encodes an F-box protein that interacts with both a protein of Medicago sativa homologous to Skp-1 and a pRBR protein. Moreover, the competence of Clink to bind to the pRBR protein correlates positively with its capacity to stimulate viral replication (Aronson et al., 2000). Thus, apparently Clink may lead to pRBR protein degradation by the proteasome in order to interfere with its cell-cycle repression. The Rep protein of some geminiviruses has also been seen to interact with the small ubiquitin-like molecule (SUMO)-conjugating enzyme, SCE1 (Castillo et al., 2004). Nonetheless, how the interaction between Rep and SCE1 affects the sumoylation of both viral and plant proteins, or its exact effect on both viral infection and the general physiology of infected plants, remain unknown.

It has long been known that plant viruses, like animal viruses, are capable of reprogramming host gene expression (Aranda & Maule, 1998). Havelda et al. (2008) have provided evidence of a possible connection between this phenomenon and the pathogenicity of viruses by reporting a correlation between the intensity with which infection interrupts the expression of cellular genes (‘shut-off’) and the severity of viral symptoms.

Effect on viral pathogenesis of the viral and plant factors involved in viral movement

Since the possible interference of viruses with cellular transport processes is a potentially effective form of altering plant physiology, it is intuitive to believe that the translocation of viruses among cells throughout the plant body strongly influences the pathogenesis process. Virus multiplication and movement are necessary for the symptoms of disease to develop. Thus, the rate and extent to which these processes occur can be primary determinants of symptom development. The infective viral cycle in a susceptible host mostly begins through epidermal cells, or through roots, as a result of either mechanical damage or being assisted by biological vectors (e.g. insects, nematodes, fungi, etc.). Once the first viral genome replication cycles have been completed, the progeny viruses must be capable of translocating from one cell to another until they reach the vascular system, through which the viruses could invade the distant plant parts. The first of these phases is known as local or cell-to-cell movement, and the second is called systemic or vascular virus movement (Waigmann et al., 2004). In both types of movement, but especially in the first one, the involvement of the virus-encoded movement proteins (MP) is essential (Fernández-Calviño et al., 2011; Pallas et al., 2011). MPs can act by forming ribonucleoprotein complexes with the viral genome or tubular structures that hold virions to allow them to cross plasmodesmata (Lucas, 2006; Sánchez-Navarro et al., 2006; Waigmann et al., 2004). One of the most common ways to restrict the invasion of a given virus is to block its cell-to-cell and long-distance movements. Thus, alterations in the viral movement function have a direct effect on the symptomatology. Most of the data that correlate this function with pathogenesis originate from studies conducted with natural or artificial mutants of the corresponding MPs, or with pseudorecombinant viruses. Thus, the first symptomatic variant to be correlated with a mutation in a MP was that corresponding to the thermosensitive Ls-1 mutant from TMV (Nishiguchi et al., 1978), which is not capable of systematically invading plants at high temperatures. The comparison of wild-type sequences with the Ls-1 mutant revealed a proline-to-serine change in the MP gene (Ohno et al., 1983). Subsequently, an association between less symptomatic phenotypes and a lesser accumulation of the MP from TMV was determined (Arce-Johnson et al., 1995). In CaMV infections in different hosts, it was appreciated that symptom severity and virus accumulation were influenced by variations in the MP sequence in a coordinated fashion (Anderson et al., 1991). Remarkably, Tsai & Dreher (1993) showed that a single nucleotide substitution in the MP gene that enhanced the efficiency of viral movement of the tymovirus turnip yellow mosaic virus led to greater viral accumulation and to increased severity of symptoms. For the TAV cucumovirus, the different levels of expression of its MP have also been found to determine the difference in the severity of the symptoms between two virus strains (Moreno et al., 1997).

The severity of symptoms does not necessarily correlate with the virus titre, indicating that disease can be the result of specific interactions between virus and host components. Plant virus MPs have been shown to interact with a large variety of host proteins to promote virus movement...
Host interacting proteins can be localized in the nucleus (e.g. fibrilarin, ALY, GCN5, etc.), cytoplasm (e.g. TIP1, RME-8, HFI22, ANK, etc.), endoplasmic reticulum (e.g. Tm-2), microtubules (e.g. MPB2C, DNA-J, At4/1 etc.) and plasma membrane (e.g. calreticuline, PME, Atp8, etc.) (Fig. 2). Most of these interactions are required for intra- or intercellular virus movement and can have significant effects on the development of symptoms. For example, the MP (TGpB2) of the PVX potexvirus interacts with the cytoplasmic protein TiP-1, which, in turn, interacts with β-1,3-glucanase (Fridborg et al., 2003), an enzyme that participates in the regulation of callose levels and which plays an important role in the regulation of the size-exclusion limit of the plasmodesmata and in virus infection (Iglesias & Meins, 2000). Other interactions facilitate vascular transport of viral RNA, as in the case of the MP from the umbravirus *Groundnut rosette virus* (GRV) that interacts in Cajal bodies with fibrilarin to cause the relocation of a given population of this nucleolar protein toward the cytoplasm, where a ribonucleoprotein complex is formed that facilitates vascular transport of viral RNA and the appearance of symptoms in uninoculated tissue (Kim et al., 2007a, b).

The genetic and molecular advantages of the plant model *A. thaliana* have been used in order to identify the host factors that contribute to either susceptibility or symptom development in virus–plant interactions. For example, the systemic movement of the TEV potyvirus in arabidopsis is

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**Table 1.** Summary of host proteins interacting with plant virus MPs

<table>
<thead>
<tr>
<th>Virus–interacting protein</th>
<th>Subcellular localization</th>
<th>Role in infection cycle</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVX–TGB2</td>
<td>Cytoplasm</td>
<td>Cell-to-cell movement</td>
<td>Fridborg et al. (2003); Iglesias &amp; Meins (2000)</td>
</tr>
<tr>
<td>TIP1</td>
<td></td>
<td></td>
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<tr>
<td>PMTV–TGB2</td>
<td>Endoplasmic reticulum</td>
<td>Intra- and intercellular movement</td>
<td>Haupt et al. (2005)</td>
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<tr>
<td>RME-8 family</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMV–MP</td>
<td>Plasma membrane/cell wall</td>
<td>Regulate cell-to-cell movement</td>
<td>Chen et al. (2005)</td>
</tr>
<tr>
<td>Calreticuline</td>
<td>Microtubules</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPB2C</td>
<td>Microtubules</td>
<td>Regulate cell-to-cell movement</td>
<td>Brandner et al. (2008)</td>
</tr>
<tr>
<td>EB1a</td>
<td>Microtubules</td>
<td>Regulate cell-to-cell movement</td>
<td></td>
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<tr>
<td>ANK</td>
<td>Plasmodesmata</td>
<td>Regulate cell-to-cell movement</td>
<td>Ueki et al. (2010)</td>
</tr>
<tr>
<td>PME</td>
<td>Plasmodesmata</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tm-2</td>
<td>Cytoplasm</td>
<td>Susceptibility</td>
<td>Weber et al. (2004)</td>
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<tr>
<td>NtRIO</td>
<td>Cytoplasm</td>
<td>Phosphorylates MP</td>
<td>Yoshioka et al. (2004)</td>
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<tr>
<td>ToMV–MP</td>
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<td>KELP</td>
<td>Cytoplasm</td>
<td>Unknown</td>
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<td>TBSV–p19</td>
<td>Nucleolus</td>
<td>Regulate cell-to-cell movement*</td>
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<tr>
<td>HFI22</td>
<td>Microtubules*</td>
<td>Intracellular movement*</td>
<td>Soedlick et al. (2000)</td>
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<tr>
<td>TSWV–NSM</td>
<td>Microtubules*</td>
<td>Intracellular movement*</td>
<td></td>
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<tr>
<td>DNA-J type chaperones</td>
<td>At4/1</td>
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<tr>
<td>CaMV–MP</td>
<td>Cytoplasm</td>
<td>Susceptibility</td>
<td>Huang et al. (2001)</td>
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<td>MPI-7</td>
<td>Cajal Bodies</td>
<td>Vascular transport</td>
<td>Kim et al. (2007a, b)</td>
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<tr>
<td>BMV–3a</td>
<td>Cytoplasm</td>
<td>Regulate cell-to-cell movement</td>
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<td>NACA1</td>
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<td>Gemivirus–NSP</td>
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<tr>
<td>AtNS1</td>
<td>Nucleus</td>
<td>Intracellular movement</td>
<td>McGarry et al. (2003); Carvalho et al. (2006)</td>
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<td>Protein kinase</td>
<td>Nucleus</td>
<td>Intracellular movement</td>
<td>Fontes et al. (2004); Mariano et al. (2004)</td>
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</tbody>
</table>

*Denotes that the annotated subcellular localization or function has been proposed but not demonstrated.
controlled by at least three loci, RTM1, RTM2 and RTM3, which could act cooperatively. RTM1 encodes a jacalin-type protein that is implied to be involved in plant defence responses, RTM2 is a multidomain protein that is homologous to thermal-shock proteins (Chisholm et al., 2001), while RTM3 is a member of a new plant gene family encoding a meprin and tumour necrosis factor receptor-associated factor (TRAF) homology domain-containing protein (Cosson et al., 2010). Recently, Decroocq et al. (2009) have shown that the determinant of the ability of potyvirus to overcome the RTM resistance of Arabidopsis thaliana maps to the amino-terminal region of the CP. On the other hand, Arabidopsis mutations have been identified that affect the cell-to-cell movement of CMV (mutant cum1), or of CMV and TCV (mutant cum2) (Yoshii et al., 1998a, b), or the systemic movement of the tobovirus turnip vein-clearing virus (mutant vsm1) (Lartey et al., 1998). Susceptibility to TMV and development of symptoms in Arabidopsis are clearly influenced by the cell-to-cell movement of the virus. Genetic analyses revealed that local movement is conditioned by at least two dominant genes, while the symptomatic phenotype is regulated by a single recessive gene (Dardick et al., 2000).

On the other hand, Kleinow et al. (2009) mapped three phosphorylation sites in the abutilon mosaic virus (AbMV) MP, which plays a role in symptom development and/or viral DNA accumulation. More recently, Krenz et al. (2010) showed that a 70 kDa plastid-targeted heat shock cognate (cpHSC70-1) interacts with the AbMV MP. Silencing the cpHSC70 gene of Nicotiana benthamiana induced minute white leaf areas, which suggests an effect on chloroplast stability. The MP–chaperone interaction was proposed to be relevant for viral transport and symptom induction.

A second line of research that has contributed to our understanding of the involvement of MPs in viral pathogenesis used plants overexpressing these proteins. Although the transformation of plants with genes corresponding to different viral MPs was initially conceived to generate forms of resistance to infection, it has actually shed light on the process of pathogenesis. The observation that the constitutive or tissue-specific expression of viral MPs may trigger typical viral infection symptoms, such as an abnormal accumulation of sugars, diminished photosynthesis, chlorosis and dwarfism, queries the dependence of viral multiplication and movement as being necessary processes for disease development. As transgenically expressed MPs preferentially locate to the plasmodesmata, it is logical to consider that the appearance of the aforementioned symptoms is caused by the functional interference of these proteins with the cytoplasmic communication channels. In
light of this, one of the initially described effects of this overexpression is the increased size-exclusion limit of the plasmodesmata, which, in principle, was thought to trigger the subsequent alteration in the metabolism and distribution of carbohydrates (Olesinski et al., 1996). In grafting experiments with plants expressing the TMV MP, Balachandran et al. (1995) demonstrated that alterations in carbohydrate distribution originate in mesophyll tissue, while the primary action site of the MPs of those viruses restricted to the phloem, e.g. MP17 from PLRV, resides in phloematic tissue (Herbers et al., 1997). It is important to stress that these alterations in the metabolism of carbohydrates have also been encountered in natural infections (Herbers et al., 2000; Love et al., 2005; Técsi et al., 1994).

In general terms, and with very few exceptions, clear increases in the levels of sucrose, glucose, fructose and starch have been found in source leaves of transgenic plants for different MPs, while the caulinar apex/radicular apex relationship has been disturbed in favour of the former (Hofius et al., 2001). If we assume a permanent dilation of the plasmodesmata in the mesophyll of the source leaves in these transgenic plants, it is hard to understand how this situation may result in a loss of exporting capacity of sucrose. Furthermore, we must also take into account that, unlike in the MP-transgenic plants, the dilation capacity of the plasmodesmata caused by MPs in viral infections is transitory in nature. For example, the TMV MP loses its capacity to modify the size-exclusion limit of plasmodesmata from behind the infection front (Oparka et al., 1997). The data presented by Rinne et al. (2005) seem to clarify this apparent contradiction. These authors have shown that tobacco plants respond to constitutive expression of the MP from TSWV (NSM) by plasmodesmata sealing that is heat reversible and thwarts plant development. Following different experimental approaches, these authors have demonstrated that the development of symptoms of these plants correlates with the obstruction of the plasmodesmata by callose deposits. Treatments involving temperature shifts (from 22 to 32 °C), which normally eliminate the typical chlorotic lesions of an infection, also eliminate the typical lesions of NSM-transgenic plants and rescue normal plant development by restoring the capacity of the plasmodesmata through the action of a 1,3-β-D-glucanase. These results suggest that the symptoms of transgenic plants expressing NSM are caused by a basal defence response that tries to counteract prolonged MP interference by altering plasmodesmata function. This form of defence could play a relevant role in the formation of symptoms during viral infection (Rinne et al., 2005).

5′ and 3′ non-coding regions (NCR) and other pathogenicity factors

Effects of 5′ and 3′ NCRs on viral pathogenesis

The 5′- and 3′-NCR sequences (5′ NCR and 3′ NCR) of viral RNAs play a key role in their translation and replication processes. The primary described effects of alterations in the 5′ and 3′ NCRs on symptom development were accompanied by a reduction in viral RNA replication (Eggen et al., 1989; Takamatsu et al., 1990); thus, they cannot be considered to be strictly indicative of a specific role for these genomic regions in the pathogenesis of the virus. Similarly, Petty et al. (1990) observed a clear correlation between alterations in the 5′ NCR and viral movement, which significantly conditioned the pathogenic process. Thus, a variation in a single nucleotide of a small ORF near the 5′ end of the RNA-γ from the hordeivirus barley stripe mosaic virus prevented the vascular movement of the virus via negative regulation of the synthesis of the viral replicase, which is encoded by the immediately adjacent gene (Petty et al., 1990).

On the other hand, it should be stressed that deletion of the first 79 nt of the leader region of RNA 3 of AMV, a region which is not essential for the virus to replicate, is sufficient to change an asymptomatic phenotype into the development of necrotic rings (van der Vossen et al., 1996). Likewise, long deletions in the 5′ NCR of the potyvirus plum pox virus (PPV) do not influence infectivity but cause a significant attenuation of the symptoms (Simón-Buela et al., 1997). In the case of the nepovirus grapevine chrome mosaic virus, the 5′ NCR has been found to be capable of triggering a necrosis as a response in three different species of Nicotiana without altering the replication process (Fernandez et al., 1999). More recently, Lough et al. (2006) have reported the interesting observation that the 5′ NCR of PVX contributes to viral pathogenesis through relevant roles not only in replication, but also in the cell-to-cell movement of the virus. This points, for the first time, to the possibility that MPs do not interact with viral RNA in a non-specific manner (see the previous section), but, on the contrary, MPs recognize specific sequences or structures in the 5′ NCR of the RNA.

There is also evidence that the 3′ NCR plays an important role in viral pathogenicity. Indeed, the presence of four repetitions of a 14 nt sequence in the 3′ NCR of the potyvirus tobacco vein mottling virus (TVMV) notably mitigates symptoms without affecting viral accumulation (Rodriguez-Cerezo et al., 1991). Later, Díaz et al. (2004) demonstrated the capacity of a melon necrotic spot virus isolate to overcome the resistance to this virus in plants with the recessive nsv gene residing in the 3′ NCR. In addition, this genomic region contains determinants that enable this isolate to infect other hosts apart from cucurbitaceae (Nieto et al., 2011). Also, recently Albiach-Martí et al. (2010) demonstrated that the pathogenicity determinant of citrus tristeza virus (CTV), causing the seedling yellows syndrome maps to the 3′-terminal region of the viral genome. However, this region encompasses the p23 gene and the 3′ NTR of the CTV genome and it was not possible to elucidate whether this phenotype was caused by p23 or the 3′ NTR.

Unknown mechanisms

Despite such advances in our knowledge of the molecular bases of viral pathogenesis, there are many determinants of
viral pathogenicity for which indications about their mechanism of action are still unavailable. For instance, protein P3 from potyviruses is not merely the inducer of the response mediated by several dominant resistance genes (Chowda-Reddy et al., 2011; Hajimorad et al., 2006; Jenner et al., 2003), but is also the viral counterpart of resistance genes that, given their respective nature, probably encode plant factors that collaborate with infection (Johansen et al., 2001) and, along with the small 6K1 peptide to which it is bound in the viral polyprotein, conditions the symptomatology and the range of the virus hosts (Dallot et al., 2001; Desbiez et al., 2003; Kim et al., 2010; Sáenz et al., 2000; Salvador et al., 2008a). The functions of P3 that are responsible for its role in defining these viral pathogenicity traits are yet to be discovered. The P3-encoding sequence harbours an overlapping small ORF known as PIPO. P3, rather than PIPO, appears to be involved in determining the virulence of SMV on Rsv1-genotype soybean (Wen et al., 2011), but the possibility that PIPO could be involved in other biological features that were initially attributed to P3 cannot be ruled out. Another potyvirus protein of unknown function, which also appears to be an important determinant for pathogenicity and host specificity, is protein P1. P1 is a serine proteinase that acts as an accessory factor for viral genome replication. There are data suggesting that P1 could stimulate the silencing suppressor activity of HCPro (Kasschau & Carrington, 1998; Pruss et al., 1997; Rajamäki et al., 2005; Valli et al., 2006). The comparative analyses of the genomic sequences of a large number of members of the family Potyviridae have led to the postulation that protein P1 plays an important role in adaptation to the host (Valli et al., 2007). In agreement with this hypothesis, sporadic changes in the P1 sequence have been found to be associated with symptom modulation and adaptation to Nicotiana clevelandii, of PPV (Salvador et al., 2008a) and with attenuation of papaya ringspot virus (Chiang et al., 2007). It has also been observed that the replacement of the P1-coding sequence of PPV by that of another potyvirus, TVMV, has no effect on the virus effectiveness in plants that are hosts for both PPV and TVMV, but inhibits infection in the PPV host Prunus persica, which is not susceptible to TVMV (Salvador et al., 2008b).

Conclusions and future perspectives

Viruses need living tissue for their multiplication and thus do not normally cause the death of the host, although there are exceptions. A large body of evidence has recently shown that to accomplish their life cycle, plant viruses need to confront plant defence mechanisms and to hijack the functions of different host factors. As a consequence, viral components must interact and/or interfere with host components that, in turn, in some instances would cause an alteration in the plant physiology resulting in the development of symptoms. Indeed, recent discoveries have evidenced that plant development is affected by plant–virus interactions, which interfere with a broad range of cellular processes, such as hormonal regulation, cell cycle control and endogenous transport of macromolecules, among others. One important landmark along this line of experimentation has been the demonstration of interplay between plant development and antiviral defence processes, and that the interference among the common points of their signalling pathways can trigger pathological manifestations (e.g. Chapman et al., 2004; Gómez et al., 2009; Jay et al., 2011; Kasschau et al., 2003).

The incorporation of massive transcriptomic and proteomic analyses, as well as other types of techniques, have helped us to acquire a more global vision of the pathogenic process and have revealed that we were merely looking at a very limited part of this process. Infection by a specific virus in a host can differentially induce more than 4000 genes, and different viruses have varying responses in a common host (Senthil et al., 2005; Whitham et al., 2003). On the other hand, the number of genes whose expression is affected by different viruses seems to be consistent with the severity of the symptoms they cause (Dardick, 2007). These results evidence the extraordinary complexity of the pathogenic process and reveal that the metabolic costs for the plant of its defence against viral infections are not only high, but are also diverse in form.

The studies that have contributed to these extraordinary advances in knowledge have generally used a limited number of experimental pathosystems and standard interaction conditions. The challenge in forthcoming years lies in learning how environmental factors, the place and manner of virus entry, plant phenology and the evolutionary adaptation of both virus and host, influence the way the pathological process develops.

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