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Review

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 counteracting 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 $Tm2^2$ 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. $P58^{IPK}$ is a well-known inhibitor of the mammalian dsRNA-dependent protein kinase, PKR. Many animal viruses either encode proteins that mimic $P58^{IPK}$ or recruit it to hinder PKR antiviral activity. $P58^{IPK}$ from *N. benthamiana* interacts with both the helicase domain of the TMV replicase and the tobacco etch virus (TEV) helicase, and $P58^{IPK}$ downregulation 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 $P58^{IPK}$ 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. mRNAs 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; González-Jara *et al.*, 2005; Lin *et al.*, 2007; Sáenz *et al.*, 2001; Yambao *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

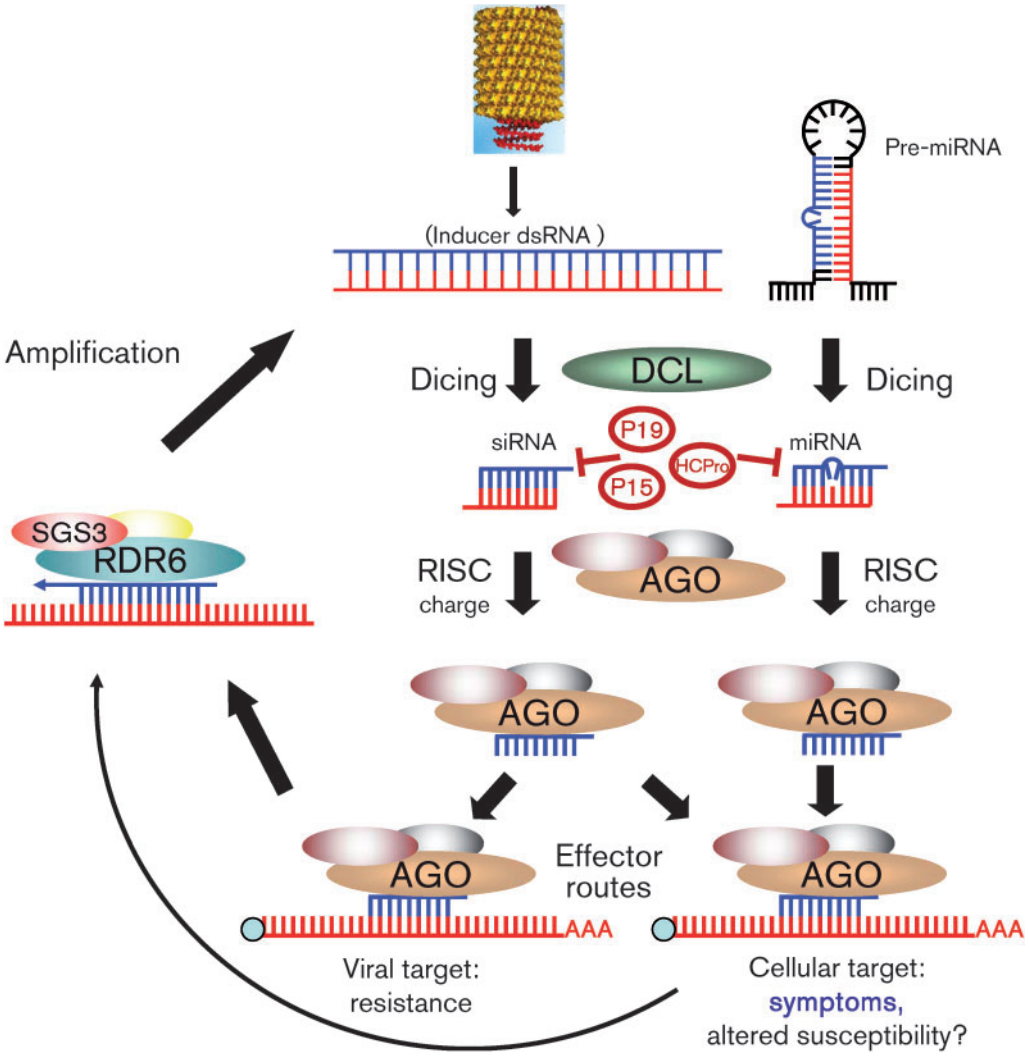

Fig. 1. Schematic representation of the key steps in virus-related RNA silencing. Viral dsRNA molecules and cellular pre-miRNAs are cut by Dicer-like (DCL) RNases, thus generating siRNAs and miRNAs, respectively. Viral siRNAs and cellular miRNAs are loaded into effector RISC complexes containing Argonaute (AGO) proteins and directed to homologous targets. Products of RISC action may be incorporated into an amplification pathway mediated by the RDR6 polymerase and the SGS3 protein, thus generating secondary siRNAs. Some RISC complexes loaded with viral siRNAs may also target cellular RNAs and cause disease symptoms. Disease symptoms can also be caused by viral silencing suppressors that interfere with cellular processes regulated by RNA silencing. It has been speculated that some components of the defence machinery of the host may be affected by targeting of viral siRNAs or by suppression of the activity of host miRNAs. Three silencing suppressors whose role in elicitation of disease symptoms has been recently elucidated (potyviral HCPro, tombusviral P19 and pecluviral P15, see details in the text) are shown in the picture [adapted from Valli *et al.* (2009)].

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 structures, and to inhibit its endonuclease activity (Ballut *et al.*, 2005; Dielen *et al.*, 2011). Moreover, HCPro from another potyvirus, PVY, has also been seen to interact with the 20S proteasome (Jin *et al.*, 2007). However, whether the silencing suppressors of other virus families also interfere with proteasome functions remains to be elucidated.

Given the importance of RNA silencing-mediated antiviral defence for the development of infection, it is reasonable to consider that viral suppressors may well be the targets of alternative plant defence mechanisms. Indeed, several silencing suppressors, such as the product of gene VI from cauliflower mosaic virus (CaMV) (Király *et al.*, 1999), 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 gives rise to symptoms similar to those of TMV infection, 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 distal 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

(Table 1). 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 (TGBp2) 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

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

PVX, potato virus X; ToMV, tomato mosaic virus; TCV, turnip crinkle virus; TMV, tobacco mosaic virus; TSWV, tomato spotted wilt virus; BMV, brome mosaic virus; TBSV, tomato bushy stunt virus; CaMV, cauliflower mosaic virus; GRV, groundnut rosette virus; PMTV, potato mop-top virus; TGB, triple gene block protein; NSP, nuclear shuttle protein. TGB2, triple gene block 2 protein; TiP 1, TGB 12K-interacting protein; MPB2C, MP30-interacting protein; EB1a, end-binding protein; ANK, ankyrin repeat containing protein; PME, pectin methylesterase; Tm-2, targeted mutation 2; NtRIO, *N. tabacum* RIO kinase; Atp8, *A. thaliana* protein 8; HFi 22, host factor interacting with P22; At4/1, *A. thaliana* protein 4/1; MPI-7, MP interacting protein 7; NACa1, nascent polypeptide-associated complex; AtNS1, *A. thaliana* nuclear shuttle protein interactor.

Virus–interacting protein and host protein	Subcellular localization	Role in infection cycle	Reference(s)
PVX–TGB2			
TiP 1	Cytoplasm	Cell-to-cell movement	Fridborg *et al.* (2003); Iglesias & Meins (2000)
PMTV–TGB2			
RME-8 family	Endoplasmic reticulum	Intra- and intercellular movement	Haupt *et al.* (2005)
TMV–MP			
Calreticuline	Plasma membrane/cell wall	Regulate cell-to-cell movement	Chen *et al.* (2005)
MPB2C	Microtubules	Regulate cell-to-cell movement	Kragler *et al.* (2003)
EB1a	Microtubules	Regulate cell-to-cell movement	Brandner *et al.* (2008)
ANK	Plasmodesmata	Regulate cell-to-cell movement	Ueki *et al.* (2010)
PME	Plasmodesmata	Regulate cell-to-cell movement	Dorokhov *et al.* (1999)
ToMV–MP			
KELP	Cytoplasm	Unknown	Matsushita *et al.* (2001)
Tm-2	Cytoplasm	Susceptibility	Weber *et al.* (2004)
NtRIO	Cytoplasm	Phosphorylates MP	Yoshioka *et al.* (2004)
TCV–p8			
Atp8	Plasma membrane/cell wall	Unknown	Lin & Heaton (2001)
TBSV–p19			
HFi 22	Nucleolus	Regulate cell-to-cell movement*	Desvoyes *et al.* (2002)
TSWV–NSM			
DNA J-type chaperones	Microtubules*	Intracellular movement*	Soellick *et al.* (2000)
At4/1	Microtubules*	Intracellular movement*	von Bargen *et al.* (2001)
CaMV–MP			
MPI-7	Cytoplasm	Susceptibility	Huang *et al.* (2001)
GRV–MP			
Fibrilarin	Cajal Bodies	Vascular transport	Kim *et al.* (2007a, b)
BMV–3a			
NACa1	Cytoplasm	Regulate cell-to-cell movement	Kaido *et al.* (2007)
Geminivirus–NSP			
AtNS1	Nucleus	Intracellular movement	McGarry *et al.* (2003); Carvalho *et al.* (2006)
Protein kinase	Nucleus	Intracellular movement	Fontes *et al.* (2004); Mariano *et al.* (2004)

*Denotes that the annotated subcellular localization or function has been proposed but not demonstrated.

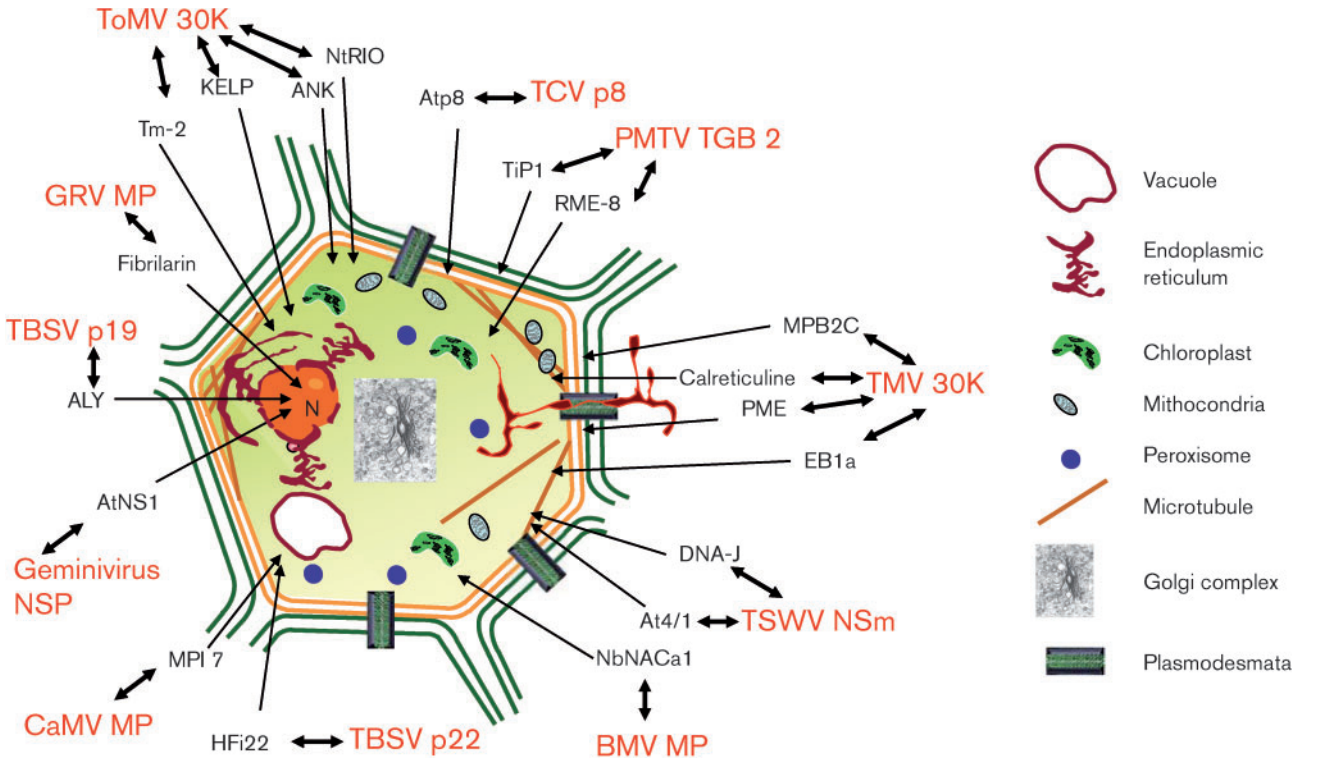

Fig. 2. Host proteins that interact with viral MPs and their subcellular localization. One given MP can interact with cellular proteins of a different subcellular localization. Proteins that localize to the nucleus (e.g. fibrilarin, ALY and GCN5), cytoplasm (e.g. TiP1, RME-8, HFi22 and ANK), endoplasmic reticulum (e.g. Tm-2), microtubules (e.g. MPB2C, DNA-J and At4/1), plasma membrane (e.g. calreticuline, PME and Atp8) have been described (see Table 1 for abbreviations).

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 *A. 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 tobamovirus 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 *N. 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 (NS_M) 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 NS_M-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 NS_M 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 (Rodríguez-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-Marti *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 recessive 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 amplification. 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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