Viral invasion and host defense: strategies and counter-strategies
James C Carrington* and Steven A Whitham

The outcome of infection of plants by viruses is determined by the net effects of compatibility functions and defense responses. Recent advances reveal that viruses have the capacity to modulate host compatibility and defense functions by a variety of mechanisms.

Introduction
When discussing susceptibility or resistance of plants to viruses, it is convenient to consider ‘susceptibility factors’ and ‘resistance factors’ as separate entities governing infection. It can be argued, however, that the distinction between susceptibility and resistance to viruses may not be so straightforward. We are particularly intrigued by the possibility that viruses can be active promoters of susceptibility or suppressors of defense. In this review, we will consider selected aspects of susceptibility, such as virus–host interactions that govern virus genome replication and intercellular movement, and a limited discussion of defense responses. We will also summarize recent findings, as well as our views, on how viruses may modulate host functions to ensure their own efficient replication and movement.

Virus–host interactions: susceptibility factors
A plant host is considered fully susceptible to a given virus if the virus can successfully complete three processes: genome replication, cell-to-cell movement (local) and long-distance (vasculature-dependent) movement [1]. For most RNA-genome viruses, RNA replication is catalyzed by a core set of virus-encoded enzymes with RNA-dependent RNA polymerase activity and other associated activities (e.g. helicase, capping, priming) in close association with cytoplasmic surfaces of membranes [2]. The docking of replication complexes to membranes appears to be a point of intimate interaction between virus and host cell. In several cases, complexes associate specifically with endoplasmic reticulum (ER)-derived membranes or vesicles through specific membrane-binding functions of replication proteins [3,4]. The basis for membrane specificity, however, remains obscure. The participation of cellular factors, such as host proteins or protein complexes, has been a point of speculation for many years, with only limited direct evidence to support functional roles of host factors [2]. This may change in the near future through the clever use of yeast as an adopted host for brome mosaic virus (BMV) to analyze RNA replication; Paul Ahlquist and colleagues have developed selectable and counter-selectable BMV RNA-based replicons for yeast [5••,6•,7••]. For example, BMV replicons expressing URAl allow ura3 yeast to grow in the absence of uracil and thus provide a positive selection for cells with active BMV replication. Conversely, URAl confers a lethal phenotype in the presence of 5-fluorooric acid, and thus provides a selection for yeast cells that lose the ability to support BMV replication. The characteristics of BMV replicon amplification are remarkably similar in yeast cells and plant cells, providing a rationale for applying yeast genetics to identify host factors that influence replication. The MAB1, MAB2 and MAB3 (Maintenance of BMV functions) loci contribute distinct functions to BMV genomic RNA replication, subgenomic RNA synthesis and accumulation of BMV-encoded replication proteins [8••]. Recessive mutations at each locus condition a temperature-sensitive yeast growth phenotype, suggesting that the wild-type gene products are necessary for the normal cellular function. Dissection of the roles of the MAB gene products in viral RNA replication will represent a significant milestone in understanding compatibility during virus–host interactions.

DNA-containing viruses, such as the geminiviruses, replicate in the host plant nucleus whereas the cellular DNA replication apparatus is employed. The geminiviruses use host DNA polymerase and associated factors in conjunction with a virus-encoded replication protein (termed RepA or AL1). The RepA/AL1 protein provides several key activities that integrate viral and host functions controlling early gene expression, late gene expression and DNA replication [9,10]. This protein binds to the stem structure at the replication origin and nicks the DNA prior to initiation of rolling-circle DNA replication [9,11–13]. Like many animal-infecting DNA viruses that use the
cellular DNA replication apparatus, the geminiviruses face a serious problem; they infect terminally differentiated, resting state (G_0) cells, but these cells lack factors required for DNA replication ([14], and references therein). As detailed below, geminiviruses likely solve this problem through modulating factors that normally control the cell cycle.

Virus-encoded susceptibility factors controlling cell-to-cell and long-distance movement are called ‘movement proteins’ (MPs) and have been reviewed extensively in recent years [1,15,16]. Movement proteins facilitate movement through interactions with the viral genome or assembled virus particle, intracellular transport pathways and structures, and plasmodesmata. For geminiviruses, two MPs are required for cell-to-cell movement. One (BR1) is necessary to transport viral ssDNA genomes between the nucleus and the cytoplasm [17,18]. The BR1 protein, therefore, is a nuclear shuttle protein with nuclear import and export signals. The other (BL1) interacts with ER-derived membranes and plasmodesmata to facilitate transit of viral DNA to adjacent plant cells, possibly by modifying the plasmodesmata structure and transport properties [19,20]. Tobacco mosaic virus (TMV) MP interacts with viral RNA and modifies plasmodesmata [21–23]. The TMV MP was shown to interact with microtubules in infected or transfected cells [24,25], and it was proposed that this might enable intracellular transport of movement complexes from sites of genome replication to plasmodesmata. Recent data, however, suggest that the MP–microtubule association may occur relatively late in the infection process, possibly as part of a pathway to dissipate high local concentrations or to degrade MP [26]. Early during the infection process, TMV MP, like the geminivirus BL1 [19], associates with ER-derived membranes and plasmodesmata [26]. Might the association between the ER and diverse MPs from different viruses be telling us something important? Recall that plasmodesmata contain an extension of the ER, the desmotubule, which is threaded through the central channel [16,27]. Quite possibly, transport complex movement through plasmodesmata requires specific interactions first with cortical ER and then with the desmotubule component in the channel.

**Induction of host compatibility factors by viruses**

Do viruses have the ability to promote their own infections by stimulation of host susceptibility factors or structures needed for genome replication, genome expression or movement? Given that the number of cellular factors shown to play definitive roles in promoting virus infection is small, there are relatively few examples to ponder. The geminiviruses, however, provide an excellent case to argue that viruses have the potential to modulate susceptibility.

As mentioned above, geminiviruses are dependent on the host DNA replication apparatus for replication, and, therefore, must overcome the lack of DNA replication factors in G_0 cells. Two significant breakthroughs have shed light on possible mechanisms whereby geminiviruses solve this problem. First, geminiviruses induce expression of at least some of the components necessary for DNA synthesis upon infection. Proliferating cell nuclear antigen (PCNA), a DNA polymerase accessory factor normally found only in S-phase cells, is induced in quiescent mesophyll cells by infection with tomato golden mosaic geminivirus (TGMV) [14]. Using AL1 transgenic plants, induction of PCNA was shown to require only the TGMV AL1 gene. Second, the AL1/RepA protein physically interacts with host-encoded retinoblastoma-like tumor suppressor proteins (pRBs) [28**,29–31]. Wheat dwarf geminivirus mutants with substitutions affecting the probable pRB-like protein binding site of Rep exhibit defects in viral DNA synthesis [29], supporting the concept that this interaction is necessary. The well-characterized mammalian pRB protein serves as key G_1 checkpoint regulator, preventing completion of G_1 and entry into S-phase [32]. Phosphorylation by cyclin-dependent kinases relieves the cell cycle-inhibitory activity of pRB and allows progression into S-phase [32]. Plants, like mammals, likely use one or more pRB-like proteins as checkpoint inhibitors of the cell cycle. It is reasonable to suggest, therefore, that geminivirus Rep protein interacts with, and inactivates (or at least diverts), pRB-like protein in infected cells. This would relieve the transcriptional block to production of S-phase-specific mRNAs and provide a pool of enzymes and factors necessary for viral DNA replication. Thus, plant geminiviruses join the ranks of animal DNA tumor-inducing viruses, such as SV40 and adenovirus, which encode proteins that affect the cell cycling apparatus through interactions with tumor suppressor proteins [33].

**A novel defense response against viruses**

Defense responses involving dominant resistance (R) genes, hypersensitive cell death and induction of systemic acquired resistance have been reviewed nicely by others [34,35,36,37] and will not be covered here. Rather, we will focus on a novel defense response—post-transcriptional gene silencing—that has only recently been linked to natural forms of virus resistance.

Post-transcriptional gene silencing is a remarkable process that has been reviewed extensively [38–41]. The hallmarks of post-transcriptional silencing of nuclear-derived sequences include, among other features, high transcription levels of the silenced gene but relatively low steady-state cytoplasmic levels of the transcript. Silencing occurs through a homology-dependent mechanism and functions in trans to suppress identical or closely related sequences. Evidence suggests that silencing is due to activation of a sequence-specific RNA degradation apparatus, perhaps by a mechanism involving synthesis of complementary nucleic acids. Silencing of a nuclear-derived sequence can be triggered by either nuclear genes
or by cytoplasmic, replicating nucleic acids containing homologous sequences. Furthermore, a silenced state in a plant can be generated systemically through transport of a mobile signal [42**,43**].

Whereas it has been known for some time that viral sequences can induce silencing of an endogenous nuclear gene or a transgene [44,45], it was only recently demonstrated that viruses can trigger silencing in the apparent absence of homologous nuclear sequences. Infection of *Nicotiana clevelandii* by tomato blackring nepovirus (TBRV) strain W22 results in an initial symptomatic phase in which the virus moves systemically, followed by a recovery state in which new tissue developing post-inoculation is asymptomatic and largely devoid of virus [46**]. Further, silencing of the viral sequence is active in the recovered tissue, conferring effective resistance against W22 and a closely related strain. Prolonged infection of *Brassica napus* by cauliflower mosaic virus (CaMV), a pararetrovirus, also induces post-transcriptional gene silencing independent of homologous transgenes [47**]. These findings are highly significant as they indicate that homology-dependent gene silencing may be a general antiviral response. The logic for such a response is impeccable: extreme resistance can be elicited after infection by a broad range of infecting viruses using a general, adaptive silencing apparatus. An important unresolved issue, however, is the extent to which viruses, other than those mentioned, elicit silencing upon infection. Most compatible virus-host combinations do not show the recovery phenotypes as do TBRV and CaMV. It is possible, however, that silencing is triggered transiently to limit the extent of virus replication in infected tissue, thus lesioning the extent of disease, but not in time to limit spread systemically. Silencing as a general antiviral response in both compatible and incompatible virus-host combinations deserves careful attention.

**Do viruses fight back?**

If one examines the way animal viruses deal with innate and adaptive immune system defenses, it becomes clear that these agents are masters of the counter-defensive strategy. For example, poxviruses encode several types of cytokine receptor mimic proteins [48]. These attenuate the inflammatory and immune responses normally triggered by virus-infected cells. Also, numerous animal viruses encode inhibitors of apoptosis proteins (IAPs), preventing normal immune signaling that occurs through activation of cell death pathways [49].

Do plant viruses deploy counter-defensive strategies? Recent findings offer suggestions that certain plant viruses are able to interdict defense mechanisms or alter host cell metabolism for their own benefit. In a series of elegant experiments examining host cell transcript and protein levels *in situ* in tissue across an advancing infection front, Andy Maule and colleagues demonstrated that pea seedborne mosaic potyvirus (PSBMV) induces transient depletion of many, but not all, classes of host cell mRNAs in pea embryos [50,51]. The host mRNA modulating occurs specifically in cells supporting virus replication, and exhibits some of the characteristics of the heat shock response, such as induction of HSP70 and polyubiquitin mRNA expression. Although the bases for suppression and subsequent recovery of transcript levels are not clear, the consequences of inhibition of host cell mRNA accumulation on induction of defense responses are potentially far-reaching. As most types of known induced defense responses require host gene expression [54*], inhibition of mRNA accumulation or translation may severely limit the extent or timing of a defense program.

Additional lines of evidence suggest that potyviruses can modulate host defense responses, and that modulation can condition enhanced accumulation and movement of potyviruses as well as heterologous viruses. Typically, in single-infections by a non-potyvirus, virus genome amplification at the single-cell level shuts off after an exponential amplification phase within 12–24 hours of inoculation [52]. Tobacco etch virus (TEV), in contrast, accumulates at a slower initial rate but for considerably longer periods of time (e.g., 72 hours post-inoculation; [53]). TEV mutants with defects in helper component-proteinase (HC-Pro) exhibit a premature amplification shut-off phenotype by 24 hours post-inoculation and are restricted in long-distance movement [54*]. These observations suggest that wild-type HC-Pro may function to suppress one or more induced defense responses. Significantly, when heterologous viruses are co-inoculated with TEV or other potyviruses, or when heterologous viruses infect plants or cells in the presence of functional HC-Pro, the heterologous viruses exhibit an enhanced genome accumulation phenotype [55,56,57*]. In the case of infections of potato virus X (PVX) expressing HC-Pro or an HC-Pro polyprotein precursor, the enhancement of PVX is actually the result of continued amplification beyond the point at which amplification normally shuts off [57*]. A plausible explanation for all of these results is that potyviruses, through a key activity of HC-Pro, suppress induction of host defense responses that would normally result in early amplification shut-off and restricted accumulation (Figure 1). The antiviral defense responses could have broad-spectrum activity against a range of diverse viruses, or could be induced to have specificity against many different viruses. The mechanistic details of this system are not yet resolved, although recently obtained evidence is consistent with HC-Pro mediating a suppression of homology-dependent gene silencing (KD Kasschau and JC Carrington, unpublished data).

Finally, the potential influence of viruses on signal transduction pathways controlling a wide range of cellular activities is just now being realized. Some of the best ongoing work in this area deals with the virulence-attenuating hypovirus of the chestnut blight fungus, *Cryphonectria parasitica*. Donald Nuss and co-workers have shown that
Viral invasion and host defense: strategies and counter-strategies

Figure 1

Modulation of antiviral defense responses by potyvirus TEV HC-Pro. The effects of HC-Pro on accumulation and movement of TEV and heterologous viruses [54,55,56,57] suggest that HC-Pro protein may suppress local and systemic defense responses. The defense responses affected by HC-Pro may include virus-induced gene silencing (VIGS), induced (non-HR) reactions, systemic acquired silencing or other systemic responses. The dotted line indicates that there is currently no direct experimental evidence suggesting that HC-Pro does interfere with a systemic host defense response. The viral replication complex is comprised of virus-encoded and possibly host-encoded proteins (represented by gray balls), which synthesize viral RNAs in association with endoplasmic reticulum (ER) derived membranes.

hypovirus infection suppresses a G-protein signaling cascade through down-regulation of the G-protein a-subunit, CPG-1 [58–60]. This G-protein signaling pathway negatively regulates cAMP levels. Significantly, virus infection, transgenic co-suppression of CPG-1, and drug-induced elevation of cAMP levels each result in effects consistently associated with hypovirulence of the fungus, including reduced asexual sporulation, reduced pigmentation and altered colony morphology. The ramifications of induction of a hypovirulent state by hypoviruses are not immediately clear, although it is possible that an ecological advantage is afforded. As sexual reproduction of hypovirus-containing C. parasitica strains is suppressed, genetic recombination in a hypovirulent population should be limited. This would promote proliferation of uniform anastomosis compatibility groups, which in turn would promote distribution of the virus by its normal transmission route—hyphal fusion. While the influence of hypovirus infection on signaling in C. parasitica is now becoming clear, the effects of plant viruses on G-protein or other signaling cascades has yet to be established. The tools are available, however, to address this issue in detail.

Conclusions and future prospects
Although we have witnessed some significant developments recently, we suggest that the most exciting days in the quest to understand compatibility and incompatibility functions governing virus infection are still ahead of us. A major limitation at this point is the paucity of useful host mutants that exhibit altered susceptibility phenotypes and that can serve as starting points for isolation of relevant genes. Two types of host mutants should be pursued aggressively. First, gain-of-susceptibility, or loss-of-resistance, mutants using normally incompatible (HR- and non-HR-dependent) virus–host combinations will reveal loci necessary for recognition and response signaling during defense. Clearly, the successes in recovery of highly informative mutants with defects in the signaling pathways for defense against bacterial and fungal pathogens set an encouraging precedence [61]. Second, loss-of-susceptibility mutants using normally compatible virus–host combinations will reveal loci necessary for genome replication and movement. Besides the yeast mut mutants with defects in supporting BMV RNA replication, an Arabidopsis mutant (tom1) has been recovered with a defect in supporting TMV replication [62]. Recovery of large numbers of susceptibility mutants, however, may require particular finesse, as the cellular factors and structure needed to support virus infection almost certainly are critical for normal cellular function. We believe that the necessary mutants are attainable, however, through development of novel virus–host genetic systems that are amenable to high-throughput selections and screens using mass inoculation procedures and selectable or screenable viruses.

Acknowledgements
Work in the author’s laboratory was supported by grants from the National Institute of Health (GM18529 to SA Whitham; AI27832 to JC Carrington) and the United States Department of Agriculture (95-37303-1867).

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
-• of outstanding interest

This paper describes the development and use of selectable BMV replicons in yeast. These replicons have wide utility in genetic selections for yeast mutants with altered capacity to support replication.

Formation of plant SAR, and nuclear localization. The yeast mutb mutants, which exhibit altered abilities to support BMV replication, are described. Understanding the roles of the MAB gene products will provide key insights into the critical virus–host interactions required for eukaryotic cells to support viral RNA replication.

The yeast mab mutants, which exhibit altered abilities to support BMV replication, are described. Understanding the roles of the MAB gene products will provide key insights into the critical virus–host interactions required for eukaryotic cells to support viral RNA replication.

Formation of plant SAR, and nuclear localization. The yeast mutb mutants, which exhibit altered abilities to support BMV replication, are described. Understanding the roles of the MAB gene products will provide key insights into the critical virus–host interactions required for eukaryotic cells to support viral RNA replication.


This seminal paper demonstrates that post-transcriptional gene silencing can be induced in tissues by a systemic signal derived from another part of the plant.


This seminal paper demonstrates that post-transcriptional gene silencing can be induced in tissues by a systemic signal derived from another part of the plant.


These two papers ([46**] and [47**]) show that virus-induced gene silencing can be established independent of homologous nuclear sequences. This implies that potentially any virus could induce a silenced state upon infection. Plants may use silencing as an anti-viral defense mechanism to limit the extent of infection.


See annotation for [46**].


Using a series of alanine-scanning mutants, the role of HC-Pro as an enhancer of genome amplification and long-distance movement was investigated. It was proposed that HC-Pro may serve as a suppressor of one or more host defense responses.


Using transgenic plants and recombinant viruses, the tobacco etch virus HC-Pro protein was shown to stimulate accumulation of, and increase disease severity induced by, several heterologous viruses. In this paper, and in [54**], HC-Pro was proposed to serve as a suppressor of one or more defense responses, possibly including virus-induced gene silencing.


