A molecular tug-of-war: Global plant proteome changes during viral infection

Mariko M. Alexander\(^a,b\), Michelle Cilia\(^a,b,c,\)
\(^a\) Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, USA
\(^b\) Boyce Thompson Institute for Plant Research, Ithaca, NY, USA
\(^c\) Robert W. Holley Center for Agriculture and Health, USDA-ARS, Ithaca, NY, USA

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**A B S T R A C T**

Plant pathogenic viruses cause a number of economically important diseases in food, fuel, and fiber crops worldwide. As obligate parasites with highly reduced genomes, viruses rely heavily on their hosts for replication, assembly, intra- and intercellular movement, and attraction of vectors for dispersal. Therefore, viruses must influence or directly utilize many host proteins and processes. While many general effects of virus infection have long been known (e.g., reduction in photosynthesis, alterations in carbon metabolism and partitioning, increased expression of pathogenesis-related proteins), the precise underlying mechanisms and functions in the viral life cycle are largely a mystery. Proteomic studies, including studies of differential protein regulation during infection as well as studies of host–virus protein–protein interactions, can help shed light on the complex and varied molecular interactions between viruses and plant hosts. In this review, we summarize current literature in plant-virus proteomics and speculate on why viruses have been selected to manipulate these diverse biochemical pathways in their plant hosts.

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1. Introduction

Plant diseases caused by viruses incur enormous costs to growers each year, both directly, in the form of yield and quality loss, and indirectly, in the forms of time and funds spent on scouting and disease management. Compared to even the smallest known bacterial genome, the genomes of plant viruses are tiny, sometimes encoding fewer than ten proteins. Therefore, they are masterful at co-opting host cell components to complete their life cycle. Many aspects of the life cycles of plant pathogenic viruses remain a mystery.

Due to the barrier of the cell wall, plant pathogenic viruses require outside assistance to infect a new host. Mechanically transmissible viruses are carried on tools, equipment and herbivores to infect a new host through contact with wounds. Other viruses require a vector for transmission. The most prolific vectors are sap-feeding insects, such as aphids, whiteflies, and leafhoppers, although some viruses are transmitted by beetles, nematodes, mites, or plasmidophorids. Insect-transmitted plant viruses can be broadly categorized by the length of time they remain associated with their vector. Stylet- and foregut-borne viruses associate transiently with the cuticle lining the stylet or foregut, and may be transmissible for only hours or days after acquisition, respectively. In contrast, circulative viruses are acquired into the insect hemolymph, where they circulate until they reach salivary tissues. Once acquired, circulative viruses remain associated with
their vector for the remainder of the insect’s life. Unlike stylet- and forayt-borne viruses, an extended feeding period is required for both the acquisition of circulative viruses from infected plant hosts and the inoculation of healthy hosts. Evidence shows that some plant pathogenic viruses manipulate their host and/or vector to promote vector behavior conducive to their transmission [1–3].

After entering a plant cell, the virus must uncoat and trans-sit to its replication site, which may be the nucleus (for viruses with DNA genomes) or cytoplasmic membranes (for viruses with RNA genomes). With assistance from host proteins, viral proteins and new viral genomes are produced. Progeny virions and ribonucleoprotein complexes (RNPs; complexes of viral nucleic acid and proteins, which are different from transmissible virions) are assembled and translocated to plasmodesmata. For viruses with single-stranded RNA genomes, formation of replication sites near plasmodesmata is facilitated by interactions between viral movement proteins (MPs) and plant synaptotagmin-family proteins, which create contact sites between the ER and plasma membranes [4]. Viral MPs promote callose degradation in plasmodesmata to facilitate passage of virions or RNPs into a neighboring cell [5], where the process starts again. Viruses use the phloem to travel to distal regions of the plant to achieve a systemic infection. The majority of circulative viruses infect only the phloem tissue during a natural infection. Phloem tropism may facilitate plant-to-plant transmission by phloem-feeding insect vectors [6]. Viruses must also evade host defenses and ensure an environment conducive to their replication. Often, infection results in the production of symptoms in plants, including chlorosis, necrosis, tissue proliferation, phylloidy, leaf curling, and other physiological changes, although the selection pressures and underlying molecular mechanisms for these symptoms remain largely uncharacterized.

Host responses to viral infection can be broadly categorized in two ways: compatible versus incompatible, or susceptible versus resistant. A compatible response results in successful virus infection, replication, and spread to other cells. An incompatible response occurs when the virus is recognized by the host, resulting in the hypersensitive response (HR; localized programmed cell death), preventing virus spread [7–10]. Susceptibility and resistance, in contrast, are defined in terms of the ability of the virus to cause disease in a given host. A susceptible reaction to a virus results in disease—replication of the virus and production of symptoms by the host. A resistant reaction does not result in the production of symptoms, but may still permit viral replication if the host exhibits tolerance to the virus. In some cases, a host may be said to be partially resistant if the virus is able to cause a reduced level of disease as compared to susceptible hosts of the virus. This review considers proteomic studies from the full spectrum of host responses: tolerant, partially resistant, and resistant.

Most publications in plant-virus proteomics use 2-dimensional electrophoresis or 2D difference in gel electrophoresis (2D DIGE) to look for proteins or protein isoforms which are differentially regulated during virus infection, although studies have also been published that use shotgun proteomics, where the entire proteome is digested with trypsin and analyzed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). New advances include characterization of virus–plant protein interactions using co-immunoprecipitation coupled to LC–MS/MS. Structural proteomics using chemical cross-linking has also been used to identify regions in the viral capsid that regulate host–virus interactions [11]. In this review, we survey these proteomic data to discuss impacts on plant health during virus infection and speculate on how selection has favored viruses to tap into these host pathways. For a review of common techniques in plant proteomics, their limitations, and a summary of some previous literature in plant–virus proteomic studies, see Ref. [12].

2. Manipulation of intracellular trafficking

Plant viruses associate with a variety of subcellular structures for replication and movement, including the endomembrane system and the cytoskeleton. It is sometimes difficult to separate associations important for inter- and intra-cellular movement of plant viruses from associations important for replication, as noted by several recent reviews on the subject [13,14]. It is possible that these two important aspects of the viral lifecycle are inextricably linked in plant infections.

2.1. Endomembrane systems

RNA viruses, which make up the majority of plant pathogenic viruses, replicate in the cytoplasm in concert with ER, vacuole, chloroplast, peroxisome, or other membranes, which may be recruited or remodeled to form inclusion bodies or complex structures [15–17]. Endomembrane systems are also important for transport of some viruses and viral proteins.

Plant viral MPs enable plant viruses to move from cell to cell through specialized, ER-linked intracellular channels called plasmodesmata. Understanding how plant viral MPs function has been a major focus of the plant virology field for the past two decades. A synaptotagmin-family protein (AtSYTA) was found by yeast two-hybrid to interact with the movement proteins of Cabbage leaf curl virus (CaLCuV; Geminiviridae: Begomovirus), Squash leaf curl virus (SqLCV; Geminiviridae: Begomovirus), and Tobacco mosaic virus (TMV; Virgaviridae: Tobamovirus), and to be important for cell-to-cell movement of CaLCuV and TMV MPs [18]. The native functions of AtSYTA are regulation of endocytosis and formation of ER-plasma membrane contact sites which support ER structure. Interestingly, a Rab GTPase (also involved in membrane trafficking) was found in a separate study to be upregulated during TMV infection [19].

Further studies with Turnip vein clearing virus (TVCV; Virgaviridae: Tobamovirus) led to a paradigm-shifting model for MP function linking viral replication, intercellular movement, and endomembrane transport: TVCV MP hijacks AtSYTA to remodel membrane contact sites near plasmodesmata, where the virus forms replication complexes and moves from cell-to-cell [4].

A recent publication by DeBlasio et al. [20] identified a number of proteins involved in clathrin-mediated endocytosis as co-immunoprecipitating with the aphid-transmitted Potato leafroll virus (PLRV; Luteoviridae: Polerovirus), and PLRV also directly interacts with golgin and a dynein-like protein (DeBlasio et al., in revision). PLRV has been previously observed by transmission electron microscopy in cytoplasmic vesicles, which fuse with the nucleus, mitochondria, vacuoles, and sites in the ER near plasmodesmata [21,22]. Although the function of these vesicles is unknown, clathrin-mediated endocytosis is also thought to be used by PLRV to traffic across tissue barriers in aphids [23] and may use these pathways in their plant hosts as well. This possibility is supported by the fact that the same viral capsid protein, a translational readthrough product from the coat protein open reading frame called the readthrough protein (RTP), is required for movement in both plant hosts and aphid vectors.

Aside from the aforementioned, endomembrane and related proteins tend to be identified only rarely in proteomic studies. This may be due to experimental bias—membrane proteins are often poorly soluble and difficult to extract with conventional protocols, and may be low in abundance to begin with—or simply because viruses are able to hijack these pathways without altering the levels or post-translational modifications of the relevant proteins. Such proteins would not be easily identified in quantitative proteomics studies looking at differential expression.
2.2. Cytoskeleton: microtubules

The cell cytoskeleton is a dynamic network of microtubules and microfilaments. Use of the cytoskeleton by viruses has been established for a number of animal viruses; both directly, by interaction of viral proteins with microtubules or microfilaments as they polymerize, or indirectly, by interaction with motor proteins that traffic various cargo along the cytoskeleton [24,25]. The best-studied example of a similar association in a plant virus exists for TMV (for review: see Ref. [26]). TMV forms replication complexes of genomic RNA, MP, and replication-associated proteins at ER-plasma membrane contact sites as discussed in Section 2.1 [4,18]. Multiple lines of evidence show an association between the TMV MP and microtubules [27–29], and a region of the TMV MP shows sequence similarity to tubulin [30]. However, the function of this association is uncertain. Although some evidence suggests that the MP-microtubule association is important for intracellular movement of the replication complex [30–32], pharmacological disruption of microtubules does not inhibit TMV movement [33,34]. To wit, an MP mutant which does not bind microtubules still localizes strongly to plasmodesmata [33], suggesting that MP function at PD is not dependent on the microtubule network. It has been suggested that microtubules actually function to promote degradation of MP by the proteasome [31,33,35,36]; however, further studies are necessary to confirm this hypothesis. In addition to the TMV MP, some evidence suggests that the MPs of Tomato mosaic virus Ob (ToMV; Virgaviroidae; Tobamovirus) and Potato mop-top virus (PMTV; Virgaviroidae; Pomovirus) also interact with microtubules [35,37], and Grapevine fanleaf virus (GFLV; Closteroviridae: Nepovirus) requires intact microtubules for cell–cell movement in some hosts [38]. Microtubule interactions are also important for transmission of Cauliflower mosaic virus (CaMV; Closteroviridae: Caulimovirus), which forms inclusion bodies key for aphid acquisition in a microtubule-dependent manner [39,40].

Several proteomic studies have found a link between other virus species and microtubules: PLRV was recently reported to co-immunoprecipitate with tubulin [20], and β-tubulin was shown to be upregulated in papaya leaves during infection with Papaya meleirea virus (PMeV; unclassified), as well as in grape berries during mixed infection with Grapevine leafroll-associated virus 1 (GLRaV-1; Closteroviridae: Ampelovirus), Grapevine virus A (GVA; Betaflexiviridae; Vitivirus), and Ruprechtia stem pitting-associated virus (RSPaV; Betaflexiviridae: Foveavirus) [41,42]. Although the role of microtubules in infection with these viruses is yet unknown, their identification is unsurprising given their importance for other diverse virus species.

2.3. Cytoskeleton: microfilaments

Ample evidence also exists for involvement of the other component of the cytoskeleton—actin microfilaments—in plant virus movement. The replication complexes of TMV and Turnip mosaic virus (TuMV; Potyviridae: Potyvirus) traffic along microfilaments [43,44], TMV MP binds to microfilaments in vitro [29], and some evidence suggests that intact microfilaments are required for cell–cell movement of TMV and Potato virus X (PVX; Potyviridae: Potyvirus) [34,45]. Several other diverse viruses, including CaMV, PMTV, and Tobacco etch virus (TEV; Potyviridae: Potyvirus) form granules or other small structures which traffic along microfilaments [46–48]. Interestingly, impairing the ability of the MPs of both TMV and CaMV to sever microfilaments also prevents these proteins from affecting plasmodesmata pore size [49], suggesting that some viral MPs may utilize the cytoskeleton for manipulation of plasmodesmata. Although the mechanism by which this may occur is unknown, we can hypothesize that microfilaments may be important for MP targeting of plasmodesmata, or that interfering with the ability of MPs to sever microfilaments also impairs the ability of these proteins to recruit callose-degrading enzymes. Motor proteins that traffic along microfilaments have also been shown to be important for movement of some plant viruses. Silencing of certain myosins inhibits the intercellular movement of TMV, and movement-associated tubule formation in GFLV [45,50].

The frequency with which actin and related motor proteins have been identified in plant virus proteomic studies underscores their importance in viral movement. Levels of actin are increased in both resistant and susceptible sugar beets six weeks after germination in soils inoculated with Beet necrotic yellow vein virus (BNYVV; Benyviridae: Benyvirus) [51]. Infection of papaya with PMeV induces an increase in one isof orm of actin, but a decrease in another, as well as a decrease in actin polymerizing factor [41]. Grapevines co-infected with GLRaV-1, GVA, and RSPaV also show a decrease in fimbrin, a microfilament cross-linking protein, but an increase in alpha actin [42], suggesting that cross-linked and free microfilaments may play opposing roles in infection with these viruses. Specific functions of fimbrin have been understood in plants, but include formation of intestinal microvilli in vertebrates and cytokinesis in yeast, among other functions [52–54]. PLRV was found to co-immunoprecipitate with multiple actin and myosin homologs in N. benthamiana [20], and a direct interaction with the PLRV CP/RTP has been shown (DeBlasio et al., in revision). Although the decrease in some actin isoforms and/or related proteins seen in the infected papaya and grapevines may seem counterintuitive if microfilaments are used for virus transport, these plants were in relatively late stages of infection, unlike many other proteomic studies reviewed here. The decrease in actin and related proteins in these plants may have been related to decreased cell health rather than a targeted effect of the viruses. It is also possible, given the increase in tubulin in these plants, that viral trafficking by these species or during late infection uses primarily microtubules, rather than microfilaments.

3. Manipulation of photosynthesis and primary metabolism

3.1. Photosynthesis and carbon fixation

Chlorosis and net reduction in photosynthesis are among the most commonly observed symptoms of virus infection in plants. It is unknown whether viruses directly manipulate the photosynthetic machinery to promote a successful infection or whether the impact on photosynthesis during infection is an indirect effect of the virus. As several studies have also found photosynthetic proteins in complex with viral particles, it is possible that virus proteins themselves may regulate photosynthesis, either directly or as part of a complex of interacting proteins. It is also possible that down-regulation of photosynthesis is partially an effect of damage done to chloroplasts, directly or indirectly, by the virus, as infection with a number of diverse viruses has been observed to alter chloroplast structure, size, or number [55–58].

Photosynthesis is also tightly linked to the production of reactive oxygen species (ROS), as chloroplasts are the primary site of ROS production in plants [for review: see Refs. [59,60]]. During photosynthesis, oxygen produced can be reduced by electrons passing through the electron transport chain, forming superoxide. Under normal conditions, superoxide and other ROS byproducts of photosynthesis are detoxified by ROS-scavenging enzymes; however, under conditions of biotic or abiotic stress, or when photosynthesis is perturbed, ROS may be allowed to build up. These accumulated ROS are important for defense and stress responses (see Section 5.1), but may also damage organelles and cellular components. In
cases where HR is triggered, ROS are a key component of cell death signaling pathways. The interplay between viruses and the photosynthetic machinery, including ROS signaling, during infection is complex. During infection with some viruses, the decrease in net photosynthetic capacity is due not to a decrease in proteins involved in light capture, but rather an increase in amount or activity of proteins involved in carbon fixation [61,62], which may contribute to chlorosis by buildup of assimilates [63]. In other cases, however, carbon fixation acts as the rate-limiting step that inhibits photosynthesis during virus infection [62,64]. There is also evidence to support the involvement of relative levels of the different proteins in the oxygen-evolving complex as important in determining photosynthetic rates during infection [65], which is supported by the frequency with which these proteins have been found in proteomic studies [20,41,51,66–75] (DeBlasio et al., under revision). Alterations in ferredoxin levels, also found in several proteomic studies reviewed here [20,66,69,72,76,77], have been shown to be associated with symptom development in TMV-infected plants [78].

Substantiating the ample literature linking viral infection to photosynthesis, photosynthetic proteins make up a major category of virus-interacting or differentially regulated proteins in most plant–virus proteomic studies. This is likely due both to photosynthesis being commonly exploited (or altered) by plant pathogenic viruses and to the relatively high abundance of photosynthetic proteins in green tissues. A number of photosynthetic proteins have been identified as co-purifying with Rice yellow mottle virus (RYMV; Unclassified: Sobemoviridae), including: phosphoenolpyruvate carboxylase, a RuBisCO binding protein, the RuBisCO large subunit, PsbQ, PsbP, and subunits of ATP synthase [66]. Photosynthetic enzymes, including a putative transketolase, components of ATP synthase, and ferredoxin NADP(H) oxidoreductase were also found to co-purify with the RPV strain of Cereal yellow dwarf virus (CYDV-RPV; Luteoviridae: Luteoviridae) [76]. PLRV was recently shown to co-immunoprecipitate with a number of proteins involved in photosynthesis and gluconeogenesis, including the oxygen-evolving enhancer proteins PsbP and PsbQ, proteins from both photosystem I and II, subunits of ATP synthase, multiple chlorophyll-binding proteins, and transketolase [20], and a direct interaction with the CP/RTP could be demonstrated for PsbQ [DeBlasio et al., under revision]. A number of photosynthetic proteins were found exclusively or were significantly enriched in co-immunopurifications of wild-type PLRV compared to a mutant form of PLRV lacking the readthrough domain of the RTP (the minor structural protein), showing that the readthrough domain mediates protein interactions with the photosynthetic machinery. These interactions may lead to the development of chlorosis during infection or suppression of host immune responses [79]. Finally, ORSV co-immunoprecipitates with Rubisco and related proteins, three subunits of photosystem I, and several proteins involved in photorespiration [80].

CMV infection has been shown to downregulate a subunit of photosystem II, PsbO, the large subunit of RuBisCO, RuBisCO activase, and carbonic anhydrase, as well as four proteins involved in photorespiration, and a plastidial aldolase [67]. Unexpectedly, grape berries co-infected with GLRaV-1, GVA, and RSPaV show an increase in a subunit of ATP synthase, although it is unknown if this trend holds for leaves and other photosynthetically-active tissue [42]. Similarly Larson and colleagues [51] reported a relative increase in levels of several members of the oxygen-evolving complex in susceptible sugar beet roots, as well as an increase in the RuBisCO large subunit in both resistant and susceptible roots, during BNYVV infection. Mixed regulation of proteins related to photosynthesis and carbon fixation was found in maize leaves infected with Rice black-streaked dwarf virus (RBSDV; Reoviridae: Fijivirus) [81,82]. Infection of Nicotiana benthamiana with Pepper mild mottle virus (PMMoV; Geminiviridae: Tobamovirus) caused a decrease in PsbP, but not PsbO [68]. Changes specifically in chloroplastic protein levels during infection of N. benthamiana with PMMoV were assessed by Pineda et al. [69], who identified 16 down-regulated polypeptides, including cytochrome F, ATP synthase, RuBisCO, and phosphoglycerate kinase. Infection of papaya with PMeV decreases levels of the small chain of RuBisCO, RuBisCO activase, a member of the oxygen-evolving complex, and beta hydroxyacylACP dehydratase [41]. Analysis of proteins responsive to Peanut stunt virus (PSV; Bromoviridae: Cucumovirus) infection found multiple proteins involved in photosynthesis and gluconeogenesis to be differentially regulated by the virus, its satellite RNA, or both [70], and the Calvin cycle enzyme ribose-5-phosphate isomerase was found to be downregulated during infection of tomato with TMV [71]. Transketolase, a Calvin cycle enzyme, was found to be upregulated during SCMV infection of susceptible maize [77], and all but one isoform was also upregulated during infection of maize with RBSDV [81]. Differential regulation of ferredoxins, ATP synthase, Psb proteins, Rubisco, and other photosynthetic proteins was also shown during SCMV infection of maize [72]. Several photosynthetic and carbon fixation proteins are differentially regulated during Cymbidium mosaic virus (CymMV; Alphaflexiviridae: Potexvirus) and Ondontoglossum ringspot virus (ORSV; Geminiviridae: Tobamovirus) infection of Phalaenopsis amabilis orchids, both in single and double infections [73]. Mixed regulation of proteins important for photosynthesis, carbon fixation, and chlorophyll biosynthesis was shown during Zucchini yellow mosaic virus (ZYMV; Potyviridae: Potyvirus) infection of partially resistant zucchini [74]. Proteins important for carbon fixation, including ATP synthase and RuBisCO, were found to be upregulated in a resistant cultivar of soybean during infection with Soybean mosaic virus (SMV; Potyviridae: Potyvirus), although one homolog of RuBisCO appeared downregulated [83]. A Psb protein, subunits of ATP synthase, and a Calvin cycle enzyme are differentially regulated during transient expression of the AC2 protein from Tomato chlorotic mottle virus (ToCMoV; Geminiviridae: Begomovirus) in N. benthamiana [75]. Photosynthetic and carbon fixation proteins are also differentially regulated during Mungbean yellow mosaic India virus (MYMV; Geminiviridae: Begomovirus) infection of Vigna mungo [84].

3.2. Carbon partitioning and metabolism

Proteomic studies show that the alteration of primary metabolism in virus-infected plants is widespread and complex, a finding substantiated by enzyme activity studies in CMV-infected Cucurbita pepo L. two decades ago [85,86]. Multiple enzymes involved in carbon metabolism were found to co-immunoprecipitate with PLRV [20], and to co-purify with CYDV-RPV and RYMV [66,76]. One or more enzymes important for carbon metabolism were found to be upregulated during infection with TMV and ORSV [71,73], but downregulated during infection with CMV, PMMoV, PMeV, and CymMV [41,67,69,75]. Differential regulation of carbon metabolic enzymes was observed during infection with RBSDV, RYMV, SCMV, SMV, PSV, ZYMV, MYMIV, and during triple infection of grapes with GLRaV-1, GVA, and RSPaV [42,70,72,74,77,81–83,87,88]. All differentially regulated carbon metabolic enzymes were decreased during infection with RBSDV, except for glyceraldehyde-3-phosphate (GAPDH), which was increased [81]. Phosphoglycerate kinase was downregulated in skin of grape berries co-infected with GLRaV-1, GVA, and RSPaV, but upregulated in in infected fruit pulp [42]. These data highlight the importance of tissue choice when performing and interpreting proteomic studies, as virus infection may have different effects even in tissues in proximity to one another. Such differences may also be observed in resistant vs. susceptible hosts: GAPDH levels were increased during RYMV infection of resistant rice, but unchanged
in susceptible rice; whereas aldolase levels were increased in susceptible rice but unchanged in resistant [88]. Most differentially regulated carbon metabolic enzymes in SCMV-infected maize were downregulated during infection of a susceptible cultivar, although GAPDH was increased in the resistant cultivar [77]. GAPDH levels were decreased during infection of a resistant soybean cultivar with SMV, while NADPH-specific isocitrate dehydrogenase levels were increased [83]. Changes in several carbon metabolic enzymes are induced by transient expression of the ToCMoV AC2 protein in N. benthamiana [75]. It is important to note here that many carbon metabolic enzymes are common to glycolysis and carbon fixation/glucogenesis; without further information it is not possible to say which process is being targeted by viral infection. Viral targets in these pathways may vary by virus species, host species and cultivar, infection time point, and plant age. These variables likely account for some of the proteome variation observed in carbon metabolic enzymes during infection. However, these proteome data paint a compelling picture that carbon metabolism is a key hub for viral manipulation during infection.

A number of plant-pathogenic viruses are known to also have an effect on carbon partitioning and allocation or phloem biology/physiology. Some plant pathogenic viruses cause damage to or blockage of phloem [89]. Many plant pathogenic viruses have been observed to cause an alteration in starch content of infected leaves [57,61,89–91] or roots [92]. Transgenic expression of the TMV MP has been shown to increase sugar and starch content in source leaves by preventing export to phloem, and decreases plant biomass allocated to roots [93,94]. Interestingly, the effect of the MP on biomass partitioning has been shown by mutational studies to be independent of the ability of the MP to affect plasmodemata pore size [93,95]. The starch biosynthetic enzymes ADP/UDP-glucose pyrophosphorylase have been found by proteomic studies to be upregulated during infection of maize with RBSVD [81,82] and infection of tomato with TMV [71], and RYMV from both resistant and infected rice plants co-purifies with a putative 4-alpha glucanotransferase (an enzyme involved in starch and sucrose metabolism) [66].

Manipulation of carbon metabolism, partitioning, and allocation may occur due to direct manipulation of involved proteins by viruses, or indirect effects of virus infection. In the case of insect-vectored viruses, we can hypothesize that alterations in carbon content may increase attractiveness of infected plants to insect vectors, encourage them to feed for a longer or shorter amount of time (depending on the vectoring strategy), or improve vector fitness. Changes to carbon partitioning may also have effects on photosynthesis if photoassimilates are allowed to build up. Similar to insect-vectored human diseases, plant viruses are known to manipulate the behavior of their vectors to their advantage, both by affecting the biology of diseased hosts and by altering the behavior of viruliferous insects [1–3].

Amino acid biosynthetic enzymes and proteins involved in protein transport were found as part of a major network of proteins, centering around the 14–3–3 protein GRF2, found co-immunoprecipitating with PLRV [20]. Multiple members of the glycoline cleavage system, a group of four proteins which degrade excess glycine, co-immunoprecipitate or co-purify with PLRV, RYMV, and CVDV-RPV [20,66,76], and glycine dehydrogenase is upregulated during PMeV infection [41]. Aminotransferases also co-immunoprecipitate or co-purify with PLRV, RYMV, and ORSV [20,66,80], and a subunit of isopropyl malate isomerase (part of the leucine biosynthesis pathway) co-immunoprecipitates with ORSV coat protein [80]. Phosphoglycerate dehydrogenase, another amino acid biosynthetic enzyme, was found to be upregulated in a susceptible rice cultivar during infection with RYMV [88], as well as in a resistant maize cultivar during SCMV infection [77]; and methionine synthase and ornithine carbamoyltransferase levels are increased during papaya infection with PMeV [41]. Serine hydroxymethyltransferase was found to be upregulated during MSNV-1 infection, and fumarylacetocetate hydrolase, an enzyme important for tyrosine degradation, was downregulated [98]. Three enzymes involved in amino acid biosynthesis are upregulated during RBSDV infection [82]. In contrast to data showing an increase in amino acid content during virus infection, amino acid biosynthetic enzymes are also sometimes found to be downregulated in proteomic studies: Aminotransferases are downregulated during infection of maize with RBSDV or SCMV (susceptible cultivar only) [77,81]; Glutamine synthase is downregulated during PMeV infection, and cysteine synthase is downregulated during both PMeV and RBSDV infection [41,81]. This seemingly contradictory finding may be unique to these particular virus-host combinations or infection stages. Additionally, the RBSDV and PMeV studies were both performed on infected plants from field trials. Although both studies used uninfected plants from the same field as controls, it is still possible that seen biotic or abiotic stresses may have had different effects on infected versus uninfected plants.

Increased amino acid biosynthesis may serve simply to provide amino acids for synthesis of viral proteins during replication. Alternatively, there is significant emerging evidence for modulation of defense responses to a broad spectrum of plant pathogens by amino acid homeostasis (for review: see Ref. [99]). Although most related studies focus on resistance to bacterial, fungal, and oomycete pathogens, many of the demonstrated downstream effects of perturbing amino acid homeostasis could certainly function in defense against viruses. For viruses transmitted by insects, it is also possible that manipulation of amino acid metabolism is related to host manipulation to improve attractiveness, nutrition, or palatability for insect vectors.

5. Manipulation of stress-responsive proteins

5.1. Reactive oxygen species

ROS and ROS-scavenging enzymes are an important part of plant response to both biotic and abiotic stress. ROS can participate in defensive signaling, act as a local microbiode, or assist in strengthening of cell walls. The most common enzymes implicated in generation of ROS during pathogen defense are peroxidases [100]. However, the highly reactive nature of ROS means that they can also be harmful to host cell molecules, membranes, and proteins. Chlorotic symptoms of virus infection have been proposed to be due, in full or part, to damage done to chlorophyll and/or chloroplasts by ROS [101]. To control ROS levels, ROS-scavenging enzymes, such as superoxide dismutases (SOD), catalases, peroxidases, and thioredoxins, detoxify hydrogen peroxide and superoxide anions.
Direct or indirect interactions of virions with ROS-scavenging and related enzymes have been shown for multiple species. Brixard et al. [66] found that SOD and four peroxidases co-purify with viruses from both RYMV resistant and susceptible rice, whereas a peroxidase co-purifies only with virus from resistant plants, and a peroxiredoxin purifies only with virus from susceptible plants. PLRV co-immunoprecipitates with at least one member of each major class of ROS-scavenging enzymes [20], and directly interacts with a peroxidase (DeBlasio et al., under revision), and the related Cucurbit aphid-borne yellows virus (CABYV; Luteoviridae; Polerovirus) was shown to bind to a peroxidase by far western [102]. Infection with SMV, RYMV, RBSDV, or CMV, causes an increase in one or more ROS-scavenging enzymes [67,81,83,88], although a second study instead found mixed differential regulation of ROS-scavenging enzymes during RBSDV infection [82]. Levels of two catalase isoforms are altered during ZYMV infection [74]. A peroxidase was found to be present at greater levels in leaves infected with an HR-causing strain of PMMoV than leaves infected with a non-HR-causing strain, and was absent in uninfected plants [103]. A comparison of the proteome in resistant versus susceptible maize cultivars infected with SCMV found a SOD to be downregulated in the susceptible cultivar, but a peroxiredoxin and a peroxidase to be upregulated in the resistant cultivar [77]. A similar study in BNYYV-infected beets found a SOD to be upregulated in the susceptible cultivar, and a peroxidase to be upregulated in both resistant and susceptible cultivars, as compared to the uninfected controls [51]; and, two peroxiredoxins were found to be upregulated during MYMIV infection of a resistant V. mungo cultivar, but not a susceptible [87]. A comparison of infected, but asymptomatic tomato fruits to uninfected tomato fruits showed mixed changes in regulation of four peroxidases [71]. During ZYMV infection of a resistant zucchini cultivar, peroxidoxin levels are increased, but thioredoxin and superoxide dismutase levels are decreased [77]. During PMeV infection of papaya, catalase levels are decreased in leaves, but levels of a peroxidase and a peroxiredoxin are increased [41]. MNSV-1 infection causes an alteration in levels of two isoforms of phospholipid hydroperoxide glutathione peroxidase in phloem sap [98].

5.2. Chaperones and related proteins

Heat shock proteins (HSPs), are a class of chaperone proteins which aid in proper folding of other proteins, either after they are synthesized, during stress conditions which promote protein misfolding [104]. Chaperones are broadly important for cell function under normal conditions as well as conditions of biotic and abiotic stress, and many are conserved across eukaryotes and prokaryotes. HSPs are classified into five major families: the Hsp70, or DnaK, family; the Hsp60, or chaperonin/GroEL, family; the Hsp100, or Clp, family; the Hsp90 family; and the small Hsp (sHsp) family (for review: see Ref. [83]). In addition to their role in protein folding, chaperones are also important for intercellular trafficking of transcription factors in plants [104]. Although not always classified as chaperones, some other proteins also perform functions in protein folding, including protein disulfide isomerases, calreticulins, calnexins, and lectins [105]. In recent years, the importance of HSPs and their co-chaperones in plant innate immunity has come to light (for review: see Refs. [106–108]). However, HSPs serve additional roles in plant-virus interactions. Host cell HSPs have been shown to be important factors in virus movement, folding of viral proteins, assembly of RNA replication complexes, and other functions in viral infection (for review: see Ref. [109]). Additionally, Beet yellows virus (BYV; Clusterviridae; Clustervirus) encodes a 65 kDa protein which is homologous to Hsp70, which seems to function in assembly, intracellular movement, and interactions with the cytoskeleton [110–116].

Chaperones and related proteins are frequently identified in plant-virus proteomic studies. Four Hsp70 homologs were found to co-purify with RYMV, and four were also found to co-immunoprecipitate with PLRV [20,66]. Levels of an Hsp70 homolog are increased during infection of a susceptible rice cultivar with RYMV [88], decreased during infection of papaya with PMeV [41], and are detectable in CMV-infected (but not control) melon phloem sap [117]. An Hsp90 homolog was found to co-purify with RYMV from both resistant and susceptible rice [66]. Direct interaction was shown between PLRV CP/RTP and a luminal binding protein HSP (DeBlasio et al., under revision), and two Hsp90s were found to co-immunoprecipitate with PLRV [20]. sHsps also co-immunoprecipitate with PLRV [20], and two sHsps are upregulated in resistant rice during RYMV infection [88]. Calreticulin was found to co-purify with RYMV from resistant rice [66] and to co-immunoprecipitate with PLRV [20], and is upregulated during infection with SMV [83] and infection with PMeV [41]. Three HSPs and a heat shock factor have been shown to interact with PVX stem loop 1 RNAs, indicating that PVX may have one or more HSP-responsive elements in its promoter(s) [118]. Other chaperonins were found to co-purify with RYMV [66] or co-immunoprecipitate with PLRV [20]. A TCP-1/cpn60 family chaperonin and two other chaperonins were found to be upregulated during SCMV infection of maize [77]. A 20 kDa chaperonin was upregulated during TMV infection of a partially tolerant cultivar [71] and was upregulated during transient expression of the AC2 protein from ToCMV [75], and a chaperonin 60 is differentially regulated during MYMIV infection of resistant and susceptible cultivars of V. mungo [87].

5.3. Stress response and pathogen defense

Viruses, like other pathogens, trigger a number of inducible basal defense responses when recognized by plants, including the upregulation of a number of common defensive proteins. These proteins have broad functions, including beta-1,3-glucanases, chitinases, peroxidases (discussed above), defensins, and a number of proteins with poorly-understood functions. Some defensive proteins have been classified as pathogenesis-response (PR) proteins, which are typically small, protease-resistant proteins that are induced during pathogen attack (for review: see Refs. [119,120]). Defensive proteins, including PR proteins, have been shown to contribute to resistance against many diverse plant pathogens, including viruses.

Beta-1,3-glucanases/PR-2 proteins hydrolyze callose, and are hypothesized to function in pathogen defense primarily by regulating the size of plasmodesmal openings [121]. A beta-1,3-glucanase was found to co-immunoprecipitate with PLRV from N. benthamiana [20]. In a survey of PR proteins during compatible and incompatible interactions of PMMoV with hot pepper, two beta-1,3-glucanases were shown to be upregulated during both compatible and incompatible infections, while a third was only detectable during the incompatible reaction [103]. Beta-1,3-glucanase was also upregulated during CMV infection of both susceptible and transgenic resistant tomato [67], in asymptomatic tomato fruits during TMV infection [71], and during RBSDV infection of rice [82].

Chitinases, enzymes which break down chitin, are also classified as defensive proteins. The PR-3, 4, 8, and 11 classes all contain proteins with chitinase activity. Although chitinases function in defense against fungi and insects, proteomic studies revealed they are also differentially regulated during viral infection. In the aforementioned study by Elvira et al. [103], four chitinases were upregulated during both compatible and incompatible PMMoV infection, of which two were upregulated to a greater degree in the incompatible reaction infection of papaya with PMeV upregulated one chitinase but downregulated another [41]. A chitinase
was found to downregulated in the bark of Citrus sudden death-associated virus (CSDaV; Tymoviridae: Marafivirus) infected citrus of a susceptible cultivar, but not a tolerant cultivar [122], and a chitinase was also downregulated in asymptomatic TMV-infected tomato fruits [71]. Chitinases are upregulated during RBSDV infection of rice [82] and a chitinase is upregulated during transient expression of the ToCMoV AC2 protein [75]. While differential regulation of chitinases during viral infection could be due to triggering of non-specific defense responses, a class III chitinase co-purified with RYMV from a susceptible rice cultivar, and a different putative chitinase co-purified with RYMV when a resistant cultivar was used instead [66]. Simple induction of basal defense does not explain why different chitinases would be induced during resistant versus susceptible responses, indicating that a more nuanced explanation is needed.

The PR-5 class of proteins contains thaumatin, a class of proteins with antifungal properties [123] which are also associated with osmotic stress. Although no role for these proteins in viral infection has yet been identified, it is possible that an undiscovered function exists, or that there is overlap between antiviral and antifungal signaling or defense pathways in plants. Two thaumatin were found to co-immunoprecipitate with PLRV [20], and a thaumatin was found to be upregulated in the apoplast of Plum pox virus (PPV; Potyviridae: Potyvirus) infected peach cells [124]. A thaumatin was also found to be upregulated during infection of Capsicum chinense with an incompatible, but not a compatible, strain of PMMoV [103], and during RBSDV infection of rice [82,48].

Oxalate oxidase (PR-15) and germin-like proteins (GLPs; PR-16) have well-established roles in defense against a spectrum of plant pathogens. Both classes are part of the cupin superfamily of proteins, and bear homology to one another; however, oxalate oxidase is believed to be cereal-specific and catalyzes the degradation of oxalate to hydrogen peroxide, whereas GLPs are ubiquitous in plants and perform other functions, many of which are poorly understood [125]. Although no oxalate oxidases have been confirmed outside of cereal species, Rodrigues et al. [41] reports a putative oxalate oxidase in papaya which is downregulated during infection with PMeV, a finding which is supported by the observation of calcium oxalate crystals correlating with ROS production in latex [126]. A GLP was found to be upregulated in the roots of a resistant variety of sugar beet during BNYVV infection [51], and GLPs were downregulated during PMMoV, CMV, and SCMV infection [67,77,103]. A 24 k GLP was also found to co-immunoprecipitate with PLRV [20], showing that these proteins may function in complex with viruses.

Other PR proteins were found less frequently in proteomic studies, a trend which could be due to low abundance of these proteins rather than diminished importance in viral pathosystems. A PR-10 ribonuclease was found to be upregulated in a susceptible, but not a resistant, rice cultivar during TMV infection [88]. Defensin/PR-12 was found co-immunoprecipitating with PLRV [20], and is upregulated in a resistant cultivar during SCMV infection [72]. The functions of proteins in the PR-1 and PR-17 families are yet unknown, but a PR-1 was found to be upregulated during infection of C. chinense with an incompatible strain of PMMoV, and a PR-17 protein was shown to be enriched during infection with both the compatible and incompatible strain [103]. The PR-6 and PR-7 classes encode proteinase inhibitors and endoproteinases, respectively, and will be covered in section 7, below.

Although not strictly defensive, 14-3-3-like proteins were also found in a significant number of proteomic studies. 14-3-3 proteins are ubiquitous in eukaryotes and are involved in signal transduction pathways related to environmental response, defense, response to light, brassinosteroid signaling, legume nodulation, and many others ([127,128] and for review: see Refs. [129,130]). Many 14-3-3 proteins regulate enzymes important for carbon and nitrogen metabolism, making them prime targets for manipulation of host primary metabolism [131]. Six 14-3-3 proteins form protein complexes with PLRV from N. benthamiana [20]. A putative 14-3-3 protein co-purifies with RYMV in a susceptible cultivar [66], and a 14-3-3 protein interacts with PVX stem loop 1 RNAs [118]. In a study of differential regulation of nuclear proteins during TMV infection of Capsicum annuum L., a 14-3-3 protein was shown to be upregulated during infection [19], suggesting a role for 14-3-3 proteins in transcriptional responses that occur during viral infection.

Glutathione-S-transferases (GSTs) are stress-responsive proteins that perform a number of functions, including sequestration of toxins, mitigation of oxidative stress, and possibly hormone response [132]. A GST was found to co-immunoprecipitate with PLRV [20], and to co-purify with RYMV from resistant rice [66]. At least one GST was found to be upregulated during infection with TMV and PMeV [41,71], as well as during infection of resistant sugar beet roots with BNYVV [51] and during infection of susceptible maize seedlings with SCMV [77]. In older maize plants, different GST isoforms were downregulated in resistant and susceptible maize cultivars [72], and long-term RBSDV infection of rice induced an increase in a GST [82].

A number of other defense-related or stress-responsive proteins were also found to be targeted during virus infection, including dehydrin [88], unknown salt-stress induced proteins [66,88], the R-protein RPS2 [81], cinnamoyl CoA reductase CCR2 [73], and cystatin [51]. This is by no means an exhaustive list, as there are a staggering number of defense-related proteins in plants, but a sample to provide an idea of the diversity of defense responses triggered or manipulated during viral infection.

### 6. Manipulation of cell wall biogenesis and metabolism

Proteome studies show that the cell wall is a target of plant viruses during infection. Callase and callose synthase were found to be upregulated during transient expression of the ToCMoV AC2 protein [75]. Xyloglucan endo-transglycosylase, an enzyme involved in xyloglucan (a type of hemicellulose) metabolism, was found to be upregulated during infection of papaya with PMeV [41], and two putative xyloglucan endo-transglycosylases co-immunoprecipitated with PLRV [20]. Xyloglucan endo-transglycosylases are important for degradation of hemicellulose associated with loosening of the cell wall during growth, and are also believed to be important for fruit ripening and abscission (for review: see Ref. [133]). Despite these findings, enzymes involved in cell wall biogenesis, metabolism, and modification represent a minor category in plant-virus proteomic studies. This is likely due in large part to experimental bias, as apoplastic proteins are difficult to extract even using specialized techniques (for review: Ref. [134]; examples: see Refs. [135,136]), and are therefore likely to be undersampled. That this category of enzymes is also found infrequently in proteomic studies of bacterial pathogens of plants further substantiates this theory [137]. Additionally, the overwhelming majority of proteomic studies reviewed here are performed in leaf tissue. It is possible that cell wall modification is generally less important for viral pathogenesis in leaves than it is in fruits, roots, or other tissues. As per the details in Section 5.3, beta-1,3-glucanases, which hydrolyze callose, are commonly found to be differentially regulated during virus infection. Unlike most other cell-wall modifying proteins, beta-1,3-glucanases are often cytoplasmic, exempting them from the aforementioned difficulties [121]. Callase and callose synthase were also found to be upregulated during transient expression of the ToCMoV AC2 protein [75].

Lignin is actually the name for any of the many aromatic polymers which are important for cell wall rigidity and resistance
against degradation by pathogens [138]. Several enzymes involved in lignin biosynthesis are highlighted in plant-virus proteomic studies: lareatricin hydroxylase was found to be downregulated, then upregulated at a later time point, during infection of beet roots with BNYVV [51]; cinnamyl alcohol dehydrogenase is upregulated during SCMV infection of a resistant maize cultivar [77], as well as during RBSDV infection of a resistant maize cultivar [81]; and caffeic acid 3-O-methyltransferase is also upregulated during RBSDV infection.

Pectin forms a gel-like polysaccharide matrix in cell walls, and has been shown to be important for plant growth and development, defense, cell-cell adhesion, wall porosity, and a variety of other functions. Its structure is highly complex, and its biosynthesis involves a multitude of enzymes, mainly transferases (for review: see Ref. [139]), making it difficult to pinpoint whether its biosynthesis is affected by virus infection. However, pectin methyltransferases, which catalyze the demethylsterification of pectin, have been found in two proteomic studies: pectin methyltransferase co-immunopurifies with PLRV [20]; and was found to be upregulated during GLRaV-1/GVA/RSPaV triple infection of grape berries [42]. Pectin methyltransferases are involved in cell wall remodeling, and their expression has been shown to be correlated with a variety of biotic and abiotic stresses. Additionally, the interaction of pectin methyltransferases with the TMV MP is required for cell–cell movement of TMV [140].

Several other proteins involved in cell wall metabolism or modification have been found more rarely in proteomic studies. A putative exoglucanase precursor (a cellulase) co-purifies with RYMV from both resistant and susceptible rice cultivars [66], and an expansin co-immunoprecipitates with PLRV [20].

An understanding of the impact of viral infection on the cell wall is important not only in agricultural systems, but in biofuel crops as well. In particular, biofuel crop breeders aim to reduce lignin content, increase biomass, and increase growth rate. However, evidence in switchgrass suggests that these changes may also result in an increased susceptibility to insect-vectored viruses [141]. Highly-selected modern switchgrass cultivars were more susceptible to barley and cereal yellow dwarf viruses than near-wild cultivars, both in greenhouse and field studies. This study highlights the need for future research in to consider cell wall proteome effects on disease susceptibility when breeding crops for biofuel, particularly as many of these species are perennial and could act as long-term reservoirs for viruses.

7. Manipulation of translation, protein processing, and protein degradation

The final category of interacting or differentially regulated proteins to be discussed in this review is that of peptide metabolism. One or more ribosomal proteins co-purify with RYMV [66] and CYDV–RPV [76], or co-immunoprecipitate or directly interact with PLRV [20] (DeBlasio et al., under revision). One or more ribosomal proteins are also differentially regulated during infection with SMV, SCMV, RYMV, PMeV, PMMoV, RBSDV, BNYVV, and MYMV, and during transient expression of the ToCMoV AC2 protein [41,51,69,72,77,81,83,87,88]. Translational initiation and elongation factors, which are also co-opts by viruses for production of viral proteins, are differentially regulated during infection with SCMV, RYMV, PMeV, PMMoV, RBSDV, BNYVV, and MYMV, and during transient expression of the ToCMoV AC2 protein [41,51,69,72,77,81,83,87,88]. Translational initiation and elongation factors, which are also co-opts by viruses for production of viral proteins, are differentially regulated during infection with SCMV, RYMV, PMeV, PMMoV, RBSDV, BNYVV, and MYMV, and during transient expression of the ToCMoV AC2 protein [41,51,69,72,77,81,83,87,88]. Translational initiation and elongation factors, which are also co-opts by viruses for production of viral proteins, are differentially regulated during infection with SCMV, RYMV, PMeV, PMMoV, RBSDV, BNYVV, and MYMV, and during transient expression of the ToCMoV AC2 protein [41,51,69,72,77,81,83,87,88].

An elongation factor and a ribosomal protein were shown to interact with stem loop 1 RNAs from PVX [118]. One translation elongation factor co-purifies with RYMV from a resistant rice cultivar [66], and nearly 20 elongation or initiation factors co-immunoprecipitate with PLRV [20], at least one of which directly interacts with the CP/RTP (DeBlasio et al., under revision). Given the importance of protein synthesis for viral replication, and the strict dependence of viruses on host machinery for this process, it is unsurprising that related proteins are so frequently differentially regulated.

The abundant differential regulation of proteases and related proteins and protease inhibitors during viral infection highlights the molecular tug-of-war between host and virus that occurs during infection. A ubiquitin–like protein was found to be upregulated during RYMV infection of a susceptible rice cultivar, while another was downregulated during infection of a resistant cultivar [88]. Ubiquitin fusion protein is upregulated in resistant beet roots during BNYVV infection [51], in tomato during TMV infection [19], and in rice during long-term RBSDV infection [82]. A ubiquitin fusion protein was also found to co-immunoprecipitate with PLRV, as did an E3 ubiquitin ligase [20], which interacts directly with the PLRV CP/RTP (DeBlasio et al., under revision). An E1 ubiquitin-activating enzyme was found to bind to bind in vitro to RYMV [66]. One or more subunits of the proteasome co-immunoprecipitate or co-purify with RYMV or PLRV, respectively [20,66], and are differentially regulated during infection with SMV, PMeV, RBSDV, TMV, GLRaV-1/GVA/RSPaV, ZYMV, and MNSV-1 [19,41,42,74,81,83,98]. Assorted proteases are differentially regulated during infection with SMV, PMeV, RBSDV, CMV, TMV, and in latent of PMeV-infected papaya [41,67,71,81–83,142]; co-purify with RYMV, CYDV–RPV, and ORMV [66,76,143]; and co-immunoprecipitate with PLRV [20]. Finally, putative protease inhibitors co-purify with RYMV [66], and are upregulated in leaves during PmE infection [41]. A serine protease inhibitor is downregulated in latex sap of papaya during PmE infection [142], but upregulated in phloem sap of melon during MNSV-1 infection [98]. A cystatin is upregulated in resistant sugar beet roots during BNYVV infection [51], and a trypsin inhibitor co-immunoprecipitates with ORSV CP [80].

8. Problems, pitfalls, and future directions

Differential regulation of proteins during viral infection is complex, and likely varies according to virus species and strain, host species and cultivar, infection stage, plant age, tissue, cellular compartment, and environmental conditions. In many cases, two homologs of the same protein will be regulated in different directions during infection with different viruses, in different hosts, or at different time points, and it is not unusual for two homologous proteins to be found to be regulated in opposite directions even in the same study at the same time point. This makes it extremely difficult to establish specific directional trends in proteins, protein classes, or pathways which are altered or exploited—for example, while we can certainly say that peroxidases are often differentially regulated during virus infection, it is much harder to make a generalization about the direction of their regulation, or even to identify the general set of conditions under which they are up or down regulated.

It is nonetheless clear that virus infection generally has large effects on core plant metabolism, including photosynthesis and carbon and amino acid metabolism. Some of these effects may be collateral damage as a result of general stress and defense responses during infection; however, as multiple virus species have been shown to interact directly or indirectly with metabolic proteins, it is likely that this regulation has been selected due to benefits obtained by the virus during infection or by the plant during the anti-viral defense response, to at least some extent. As viruses are entirely dependent on their hosts for replication, it is likely advantageous for the virus to utilize viral enzymes for its own life cycle whenever possible, as these host proteins cannot be easily deleted or mutated during the host-pathogen arms race. Additionally, the manipulation of core metabolism by insect-vectored viruses may occur as part of the host manipulation hypothesis [1,3], to enhance virus transmission to new hosts. Although plant anti-viral defense path-
ways only overlap with defense pathways against other pathogens to a limited extent, viral infection nonetheless has an influence on many proteins involved in biotic and abiotic stress responses. It is likely that a portion of these effects can be attributed to a basal defense response; however, some PR proteins, including HSPs and beta-1,3-glucanases, have well-studied roles in viral infection. Both proteomic and other studies highlight the importance of the cytoskeleton during viral infection, particularly for movement and formation of replication sites; yet, even in model systems there is still some debate about the precise role of each cytoskeletal component. Undoubtedly this is an area where significant advances will be made in the coming years. It is also likely that, as proteomics technologies continue to improve, further light will be shed on the effects of viral infection on proteins that are low in abundance or difficult to extract, which tend to be identified less frequently in current proteome approaches that rely on a relatively high threshold of abundance in protein extractions for detection.

The use of mass spectrometry for protein identification requires a well-annotated database for the host species of interest, which is not available for many plant species. In most cases this issue is solved by the use of a database from one or more related species; however, a database which more nearly approximates the actual possible proteins present in a sample will certainly improve the number and accuracy of protein identifications. One possible solution is to bypass genome sequencing and instead perform RNA-seq under the conditions of interest, as proteomic studies do not require any information about untranscribed regions of the genome. Recent work has shown that protein identification can be achieved using a transcriptome for searching, which is much faster and easier to obtain than a fully annotated genome [144]. An additional issue is the lack of annotation or known function for a not-insignificant proportion of proteins in any database, as “hypothetical proteins” and proteins with “unknown function” were identified as differentially regulated in a number of proteomic experiments reviewed here. A yeast two-hybrid study using the CaMV movement protein as bait (not included in this review) could not find significant structural homology for any of the three protein interaction partners discovered [145]. Structural information, including post-translational modifications, composition of oligomers, enzymatic active sites, and the three-dimensional structure of proteins, is also important to understand the proteome.

Another consideration for proteomics studies is choice of tissue. Although plant roots and fruit can be a major site of viral damage and/or replication, only three reviewed papers included one of these tissues [42,51,71]. Most experiments in this field have focused on leaf tissue, which, while informative, may be biased toward photosynthetic and related proteins due to their abundance relative to other proteins. In some cases, however, performing proteomics on other tissues may present unique challenges: some tissues are difficult to harvest in sufficient quantity, difficult to clean (i.e., of soil) or to grind, or are enriched in proteins or other compounds that complicate extraction or downstream sample preparation (protein digestion, sample clean-up, etc). These challenges are surmountable with careful planning and alteration of protocols [146,147]. As the field of plant virology advances, it will become increasingly important to move beyond the use of model systems and easy tissues to assess what occurs in the hosts and tissues that are most important for each pathosystem.

The overwhelming majority of publications in plant-virus proteomics use 2-dimensional electrophoresis or 2-dimensional fluorescence difference gel electrophoresis (2D DIGE) or, more rarely, mass spectrometry and spectral counting for protein quantification. Both of these approaches search for differences in the quantity of particular proteins or protein isoforms between treatments (i.e., infected vs. healthy). While these studies can be very informative, they do not necessarily account for proteins for which viral infection changes their subcellular localization, structure, post-translational modifications, or simply co-opts them for their own purposes. For example, viral remodeling of the host cytoskeleton likely plays an important role in intra- and intercellular trafficking of many viruses, but may be accomplished without altering levels of actin or tubulin. At the same time, these types of quantification-based analyses may be enriched for proteins far downstream in signaling pathways that are manipulated or perturbed by viruses. This may be part of the reason that some proteins, like beta-1,3-glucanases, are found to be upregulated in nearly all proteomic studies dealing with both viral and non-viral pathogens. To compliment these types of experiments, it will be imperative to elucidate which proteins interact with the viral proteins of interest, either directly or as part of a protein complex, and furthermore, to define the protein complexes that form with each viral protein so that the functions during infection can be elucidated in combination with traditional plant virology studies (for example: see Ref. [79]). This can be done either by co-immunoprecipitation of tagged or antibody-reactive viral components [20], far Western analysis [102], or using mass spectrometry-based technologies [11,76]. In the field of human and animal virus-host interactions, significant progress has been made through proteomic studies utilizing co-immunoprecipitation coupled to mass spectrometry, demonstrating the value of these approaches for studying these unique and highly recalcitrant systems [148–151].

Analysis of large data sets, like those often generated in proteomic experiments, remains a challenge in the “-omic” era. Some tools, such as gene ontology (GO) and STRING (http://string-db.org) analysis, are available to help identify the primary pathways, networks, or functions represented in a data set (for examples: see Refs. [20,77,80,152]), but teasing out candidate genes for validation and downstream analysis is a significant hurdle. Some groups, primarily in vertebrate biology, seek to solve this issue using systems biology: a computational modeling approach that aims to simulate the complex interconnected network of genes and proteins in a cell. Systems biology models can be used to predict effects of perturbing a particular gene/protein, predict disease outcomes for a given dataset [153], or identify novel or key genes in disease. Despite the potential applications, however, systems biology has not been appreciably applied to crop disease, likely due primarily to the difficulty in setting up these models, which require carefully curated databases containing multiple “-omics” data sets, as well as a significant knowledge of programming and mathematics. Systems biology in plant pathology, further application of proteomics to non-model hosts and tissues, and integrating information about the plant host, pathogen, and in some cases the vector, will open up new avenues for crop disease management.

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