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

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ABSTRACT

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–viral 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.

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 plasmodiophorids. 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

Abbreviations: 2D DIGE, two dimensional difference in gel electrophoresis; BNYVV, *Beet necrotic yellow vein virus*; BYV, *Beet yellows virus*; CABYV, *Cucurbit aphid-borne yellows virus*; CaLCuV, *Cabbage leaf curl virus*; CaMV, *Cauliflower mosaic virus*; CLCuV, *Cotton leaf curl virus*; CMV, *Cucumber mosaic virus*; CSDaV, *Citrus sudden death-associated virus*; CYDV-RPV, *Cereal yellow dwarf virus*, RPV strain; CymMV, *Cymbidium mosaic virus*; ER, endoplasmic reticulum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GFLV, *Grapevine fanleaf virus*; GLP, germin-like protein; GLRaV-1, *Grapevine leafroll-associated virus 1*; GO, gene ontology; GST, glutathione-*S*-transferase; GVA, *Grapevine virus A*; HR, hypersensitive response; HSP, heat shock protein; LC/MS-MS, liquid chromatography coupled to mass spectrometry; MNSV-1, *Melon necrotic spot virus*; MP, movement protein; MYMIV, *Mungbean yellow mosaic India virus*; ORMV, *Oilseed rape mosaic virus*; ORSV, *Odontoglossum ringspot virus*; PLRV, *Potato leafroll virus*; PMeV, *Papaya meleira virus*; PMMoV, *Pepper mild mottle virus*; PMTV, *Potato mop-top virus*; PPV, *Plum pox virus*; PR protein, pathogenesis-responsive protein; PSV, *Peanut stunt virus*; PVX, *Potato virus X*; RBSDV, *Rice black-streaked dwarf virus*; RNP, ribonucleoprotein complex; ROS, reactive oxygen species; RSPaV, *Rupestris stem pitting-associated virus*; RuBisCO, ribulose-1,5-bisphosphatase carboxylase/oxygenase; RYMV, *Rice yellow mottle virus*; SCMV, *Sugarcane mosaic virus*; SCPMV, *Southern cowpea mosaic virus*; SMV, *Soybean mosaic virus*; SOD, superoxide dismutase; SqLCV, *Squash leaf curl virus*; SqMV, *Squash mosaic virus*; TEV, *Tobacco etch virus*; TMV, *Tobacco mosaic virus*; ToCMoV, *Tomato chlorotic mottle virus*; ToMV, *Tomato mosaic virus*; TuMV, *Turnip mosaic virus*; TVCV, *Turnip vein clearing virus*; ZYMV, *Zucchini yellow mosaic virus*.

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their vector for the remainder of the insect's life. Unlike stylet- and foregut-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 transit 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, phyllody, 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-lined intercellular 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 (*At*SYTA) 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 *At*SYTA 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 *At*SYTA 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 dymeclin-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.