Dynamic cross-talk between host primary metabolism and viruses during infections in plants

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Abstract

Upon infection plant viruses modulate cellular functions and resources to survive and reproduce. Plant cells in which the virus is replicating are transformed into strong metabolic sinks. This conversion gives rise to a massive reprogramming of plant primary metabolism. Such a metabolic shift involves perturbations in carbohydrates, amino acids and lipids that eventually lead to increase respiration rates, and/or decrease in photosynthetic activity. By doing so, plants provide metabolic acclimation against cellular stress and meet the increased demand for energy needed to sustain virus multiplication and defense responses against viruses. This review will highlight our current knowledge pertaining to the contribution of primary metabolism to the outcome of viral infections in plants.

Introduction

A viral infection is a highly dynamic process in which infected plant cells are transformed into major metabolic consumers (sinks) for products of photosynthesis. During the infection, nitrogen and carbon skeletons are required for the synthesis of new molecules and energy is necessary to fuel biosynthesis. The source-to-sink transition elicits reallocation and increases demands for photosynthetic assimilates, increases respiration rates, or decreases in photosynthetic activity. Such a metabolic shift contributes to sustain viral proliferation but is also necessary to activate defense mechanisms. Due to the uneven nature of viral infections in plants and that different virus-host interactions have been studied, it is complicated to depict an unequivocal generalized picture of the metabolic responses triggered by plant viruses. In addition, many reactions in central metabolism are reversible and complex, hindering the perception of major changes in metabolite levels. Nevertheless, a considerable effort has been made to elucidate alterations in metabolite contents linked to viral infections in plants of which significant commonalties arise (Figure 1). In this review, the current knowledge on the modulation of plant primary metabolism during viral infections and its importance for plant compatibility and/or resistance are discussed.

Metabolomics strategies based on chromatography, mass spectrometry (MS) or nuclear magnetic resonance (NMR) spectroscopy in combination with multivariate data analysis have provided an excellent platform to understand the input of certain metabolites in the plant's response to viral infections [1-7]. Recent studies have gone steps further by using system biology approaches to study primary metabolism in plant-virus interactions. For instance, time-course transcriptomics and GC-MS-based metabolomics supported by functional reverse genetics were used to study the reciprocal influence of primary metabolism and *Tobacco rattle virus* (TRV) infection in *Arabidopsis thaliana* [8]. GC-MS-based metabolomics and gene expression data identified altered and unique metabolic signatures characteristic of two

tomato inbred lines that exhibited susceptibility or resistance to *Tomato yellow leaf curl virus* (TYLCV) [9], or in response to mild and aggressive isolates of *Potato virus Y* (PVY) in potato leaves at different times of disease development [10].

Plant virus, carbohydrate metabolism and photosynthesis

The carbohydrate status has profound implications in mobilization and synthesis of storage compounds, symptoms development and defense functions, and its alteration is diagnostic for perturbations in photosynthesis and respiration. The accumulation of sugars in the infected tissue causes an imbalance in the ratio of nitrogen and carbon, and the sensing of such changes results in a feedback transcriptional regulation of photosynthesis genes, and occasionally, photosynthetic repression [11-17]. Even though repression of photosynthesis and induction of sink metabolism is a general response to viral infection, the effects on sugar levels varies considerably between different host-virus interactions. Changes in sugar levels involving the accumulation of enlarged starch grains in the chloroplast have been reported for some compatible interactions [18-21], whereas starch content decreases for some others [22,23]. Interestingly, viral accumulation is unaffected in starch-depleted *Arabidopsis* mutants suggesting that starch catabolism is not strictly required for virus multiplication in this species [8,20]. Metabolism of sink tissues, where sugar is used, is mainly sustained by sucrose synthesized in source leaves and transported through the phloem into sink tissues. Sucrose and soluble sugars are abundant in different host species infected with ToMV, *Cauliflower mosaic virus* (CaMV), and TRV [2,8,24], whereas soluble sugar contents decrease upon infection with PVY, *Turnip yellow mosaic virus* (TYMV), *Jatropha mosaic virus* (JMV), *Ageratum enation virus* (AEV) or *Squash mosaic virus* (SqMV) [5,6,10,25,26].

Changes in carbohydrates accumulation respond to different causes that include physical disturbance of the transport path (e.g. modification of plasmodesmata by viral movement

proteins), inhibition of sugar transport proteins, induction of starch hydrolysis or cell wall invertases. *Cucumber mosaic virus* (CMV) increases sucrose concentration in the phloem sap of CMV-infected melon plants likely by altering sucrose localization [27]. Sucrose export routes can be severely affected by callose deposition at the cell-to-cell interfaces observed in multiple plant-virus interactions [28]. Infection of cotyledon of marrow plants by CMV causes a gradual increment of soluble sugars and a detriment of starch likely due to enhanced starch hydrolase activities [29,30]. The elevated hexose levels observed in tobacco plants infected with PVY^N are concomitant with increased invertase activity, which cleaves sucrose into glucose and fructose [31]. Interestingly, cell-wall invertase-overexpressing transgenic tobacco or *Arabidopsis* plants accumulate large amounts of soluble sugars and are resistant against PVY^N and *Tobacco mosaic virus* (TMV), respectively [31]. This observation suggests that high hexoses contribute to make plants less susceptible to viral infection, and highlights the relevance of the regulation of carbohydrate metabolism for defense.

Plant viruses and amino acid metabolism

Viruses are important modulators of the amino acid content in infected cells, and amino acid metabolism is critical in the plant response to infection. For instance, in tobacco, alanine, glutamine and proline levels increase in both locally infected leaves and leaves undergoing systemic acquired resistance to TMV [1]. High concentrations of individual and total amino acids have also been reported in different host species following infection with ZYMV, *Papaya ringspot virus* (PRSV), *Bromo mosaic virus* (BMV), and TRV [2,23,32,33]. Amino acids are significantly abundant at the time points of maximal viral accumulation in TRV-infected Arabidopsis, although developmental timing is the dominant source of variance in the amino acid content [8]. In potato, infection with PVY leads to an initial decrease in amino acid concentration followed by a gradual increase over time that is particularly significant in

leaves infected with the aggressive PVY^{NTN} isolate compared to the mild PVY^{N} isolate [10]. Although the precise role of virus-responsive amino acids during the infection has not been elucidated, it is tempting to propose that they may participate in antiviral responses. For instance, several amino acids and intermediates of the amino acid biosynthetic pathway accumulate to higher levels in TYLCV-resistant tomato lines compared to susceptible lines [9]. In *Arabidopsis*, accumulation of proline is observed during the hypersensitive reaction (HR), a plant response whereby the rapid death of cells at the infection site restricts the growth and spread of pathogens to other parts of the plant. External application of proline produces HR-like cell death symptoms [34,35]. In contrast, the *lht*1 (*lysine histidine transporter 1*) *Arabidopsis* mutant, which has reduced contents of alanine, glutamine and proline, shows enhanced resistance to multiple pathogens [36]. Likewise, genetic inactivation of *DIN4* gene, which is critical in branched-chain amino acid (BCAA) metabolism, compromises TRV proliferation in infected plants, suggesting that BCAA metabolism contributes to plant susceptibility [8].

Plant viruses and respiration

Plant defense and stress responses induced upon viral infections implicate the up-regulation of the majority of the energy-associated networks (including the glycolysis, the oxidative pentose phosphate pathway, the TCA cycle, mitochondrial energy transport, and ATP biosynthesis) [37]. A sharp increase in respiration concomitant with reduced net photosynthetic rates has been reported in plant-virus interactions [5,22,38,39]. In this scenario, the elevated levels of carbohydrates and the increasing accumulation of amino acids during viral infections fuel the energy-generating TCA pathway to maintain high respiration rates [40]. Furthermore, several organic acids connected to the TCA cycle exhibit positive responses to plants viruses in different host species [2,5-7,10]. This supports the notion that

primary metabolism is largely reconfigured during pathogen infections to satisfy the energy demand required for plant defense and, likely, virus multiplication [41].

Plant viruses and polyamines

Polyamine metabolism, including the most common putrescine, spermidine and spermine, undergoes dynamic changes with polyamine levels increasing several fold during plant-virus interactions [42,43]. Polyamines play a key regulatory role on virus replication [42,44], and have a stimulatory effect on the *in vitro* translation of viral RNA [45,46]. A growing body of evidence suggests that polyamines also transduce defense responses. Elevated levels of free and conjugated putrescine and spermidine and elevated biosynthetic activities occur in TMV-infected tobacco leaves undergoing the HR response, but not in TMV-infected susceptible plants [47-50]. Yamakawa et al. [51] found that free spermine, that accumulates to high levels during the HR in the intercellular fluids of the necrotic lesion-forming leaves, provides resistance against TMV. Induction in polyamine biosynthetic genes and spermedine-responsive genes has been reported during CMV-elicited HR in *Arabidopsis* [52]. Putrescine levels are also augmented in compatible plant-virus interactions [33]. In agreement with a role in defense, high putrescine in *Arabidopsis* provides protection against excessive TRV proliferation, as both the *adc1* and *adc2* mutants impaired in putrescine biosynthesis are hypersusceptible to TRV accumulation [8]. Polyamines are also more abundant in resistant tomato cultivars to TYLCV infection than in susceptible cultivars [9]. In contrast to the above observations, putrescine concentration is reduced in plants infected with *Citrus exocortis viroid* (CEVd) [53].

Plant viruses and lipid metabolism

The significance of cellular lipids in viral infections has long been appreciated because lipids provide energy for metabolism, participate in multiple defense signaling cascades, and are structural components of intracellular membranes in which replication of positive strand RNA viruses take place [54-58]. Membranes of different organelles also protect the viral components from the innate immune system as well as from RNA silencing-based antiviral defense [59,60]. During infection, viruses interfere with lipid (i.e. sterols and phospholipids) and fatty acid (FA) metabolism and biosynthesis to promote changes in the fluidity and/or plasticity of membranes that are required for the proper formation of viral replication complexes [59,61-65]. Genetic inactivation of *INO2,* a transcription activator involved in phospholipid biosynthesis, reduces *Tomato bushy stunt virus* (TBSV) replication and inhibits the activity of the viral replicase complex in yeast model host [66]. The sterol biosynthesis genes *ERG25* and *ERG4* affect the replication of TBSV in yeast [67], while silencing of *N. benthamiana SMO1* and *SMO2* genes, which are orthologs of *ERG25*, also result in a reduction in TBSV accumulation, supporting the roles of sterols in virus replication in plants [67]. In yeast, a mutation in the *OLE1* gene encoding Δ9 fatty acid desaturase (*Ole1p*) severely inhibits BMV replication [68]. In this mutant, the ER lumenal spherule-associated membranes, in which viral RNA synthesis occurs, are locally depleted in unsaturated FAs [68,69]. TRV stimulates the incorporation of polyunsaturated FAs (linoleic and linolenic acid) in various intermediates in the synthesis of triacylglycerols and viral TRV accumulation is reduced in *fad2 Arabidopsis* mutants that contain reduced levels of unsaturated FA [8]. These observations suggest that viral replication is highly sensitive to reduced unsaturated FA levels. In contrast, TRV accumulates to high levels in *acc1 Arabidopsis* mutants, in which the initial step in the biosynthesis of very-long chain fatty acids is partly inhibited [8]. Elevated levels of linolenic acid have been reported in tobacco leaves infected with TMV [1]. A recent study shows that the marine virus *E. huxleyi virus* (EhV) induces profound transcriptome

remodeling in the alga *Emiliania huxley* targeted toward FA synthesis to support viral assembly [70].

Conclusions and perspectives

Viral infections cause profound perturbations in primary metabolism. Although inferences can be made from transcriptomics and metabolomics studies, little is known about the mechanisms used by viruses to interfere with metabolism in both compatible and incompatible interactions and the manner that the metabolite content contributes to viral infection and pathogenesis. Massive reprogramming of primary metabolism aims to meet the increased demand for energy needed to sustain viral multiplication and defense responses against viruses. The emerging view, however, is that accumulation of protective metabolites serve to alleviate the cellular stress imposed by the virus. As a result, viral infections confer a stage of metabolic acclimation that enables plants to cope with other environmental stresses. For instance, CMV and BMV infection of beet and rice, respectively, improve plant tolerance to freezing and drought stress, which correlates with increased osmoprotectant and antioxidant levels in infected plants [33]. Likewise, the enhanced biosynthesis of putrescine in the compatible TRV-*Arabidopsis* interaction makes infected plants more tolerant to freezing stress than non-infected plants [8]. Therefore, plants benefit from the vast array of infection-associated metabolic responses by improving their tolerance to stress.

Systems biology strategies continue to increase our understanding of the dynamic role of primary metabolism during viral infection. However, there are still many questions to answer that concern the precise roles of photosynthesis, sugar partitioning, source-to-sink regulation, respiration and photorespiration in different plant-virus interactions. Future studies should also be expanded to include the use of genetically engineered plants with altered metabolite

levels to determine the precise function of virus-responsive metabolites in host antiviral responses or acclimation to environmental stresses.

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Figure legend:

Figure 1. A simplified schematic representation of plant primary metabolism showing the major compounds that are altered in response to viral infections. Further details are given within the text and literature cited herein. ACP (acyl carrier protein), ER (endoplasmic reticulum), FA (fatty acids), LPA (lysophosphatidic acid), PA (phosphatidic acid). Glycolipids: DAG (diacylglycerol), DGDG (digalactosyl diacylglycerol), MGDG (monogalactosyl diacylglycerol), SQDG (sulfoquinovosyl diacylglycerol). Phospholipids: PC (phosphatidylcholine), PE (phosphatidylethanolamine), PI (phosphatidylinositol), PG (phosphatidylglycerol),

Figure

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