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The Molecular Maze of Potyviral and Host Protein Interactions

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Abstract

The negative effects of potyvirus diseases on the agricultural industry are extensive and global. Understanding how protein-protein interactions contribute to potyviral infections is imperative to developing resistant varieties that help counter the threat potyviruses pose. While many protein-protein interactions have been reported, only a fraction are essential for potyviral infection. Accumulating evidence demonstrates that potyviral infection processes are interconnected. For instance, the interaction between the eukaryotic initiation factor 4E (eIF4E) and viral protein genome-linked (VPg) is crucial for both viral translation and protecting viral RNA (vRNA). Additionally, recent evidence for open reading frames on the reverse-sense vRNA and for nonequimolar expression of viral proteins has challenged the previous polyprotein expression model. These discoveries will surely reveal more about the potyviral protein interactome. In this review, we present a synthesis of the potyviral infection cycle and discuss influential past discoveries and recent work on protein-protein interactions in various infection processes.

INTRODUCTION

Potyviruses form a large genus of plant positive-sense single-stranded RNA (+ssRNA) viruses (1) with an expanding host range due to frequent host jumps (2). As of October 30, 2023, the International Committee on Taxonomy of Viruses lists approximately 200 potyvirus species. This number is likely to increase as metagenomics methods reveal the complexity of plant viromes, in both crops and the wild. Diseases caused by potyviruses have been reported in nearly all cultivated plants and, consequently, their global impact on crop yield and quality is substantial. Although the range of agriculturally significant hosts is wide, the molecular mechanisms of protein-protein interactions in potyvirus infections have been investigated mainly with model pathosystems using relatively few virus and host species (**Table 1**).

Most potyviral proteins are produced as polyproteins from two open reading frames (ORFs) (**Figure 1*a***) (1). From the 5′ to 3′ end, the large ORF codes for 10 functional proteins: P1; helper component-protease (HCPro); P3; 6K1; cylindrical inclusion protein (CI) RNA helicase; 6K2; nuclear inclusion protein a (NIa), which is cleaved into two products, viral protein genome-linked (VPg) and NIa protease (NIaPro); nuclear inclusion protein b (NIb) RNA polymerase; and coat protein (CP). This polyprotein is processed into individual proteins, mostly by NIaPro. P1 and HCPro self-cleave using their own protease domains. An additional smaller ORF, dubbed pretty interesting potyviral ORF (PIPO), is generated into a subset of viral genome transcripts during replication by a transcriptional slippage mechanism at a highly conserved adenine-rich motif. PIPO codes for an alternative C terminus for the P3 protein, and the resulting additional protein is called P3N-PIPO (1).

A novel concept to potyviral coding capacity was presented recently by Gong et al. (3). They showed that four small reverse ORFs (rORFs) are somewhat conserved in many potyviral genomes. All four rORF proteins encoded by turnip mosaic virus (TuMV) colocalize with viral replication vesicles when overexpressed. Furthermore, the TuMV rORF2 protein interacts with the NIb polymerase and serves as a virulence factor. The authors emphasized that this exciting discovery has implications beyond potyviruses: Many +RNA viruses may have similarly overlooked coding capacity. Both the role of these rORF proteins and how they could be expressed remain promising new areas of research.

Viral infection takes place within the molecular maze of a living cell. A recent systematic yeast two-hybrid screen for TuMV interactors in *Arabidopsis thaliana* revealed 378 host proteins, a number that gives a glimpse into the wide range of interactions (4). Nevertheless, only a fraction of the interactions seem to be crucial for the infection. In **Table 2** we highlight examples of key interactions with substantial effects on potyviral infection processes.

Deciphering the web of protein-protein interactions that enable viral infection is key in understanding the underlying mechanisms of various infection processes. While it is practical to draw lines between individual processes when studying them, these lines soon become obscured: Viral proteins are multifunctional, and their numerous interactions may change over the course of infection. Some of the processes likely are coupled, are interdependent, or overlap. For instance, replication factors are recruited to plasmodesmata for efficient intercellular movement. The interconnectedness also manifests as the same host proteins contributing to multiple processes. This is epitomized by the many roles of eukaryotic initiation factor 4Es (eIF4Es): Their interactions with the VPg both promote viral RNA (vRNA) translation and protect it from antiviral RNA silencing. Moreover, these initiation factors are transported into the nucleus alongside viral nuclear inclusion proteins, hinting at a function in altering host gene expression.

In **Figure 1*b*** we present an overview of the potyvirus infection processes at the focus of this review. The journey of vRNA starts from an initial round of translation and the formation of

Table 1 Potyviruses used in molecular studies, hosts of agricultural importance, and examples of key discoveries discussed in this review

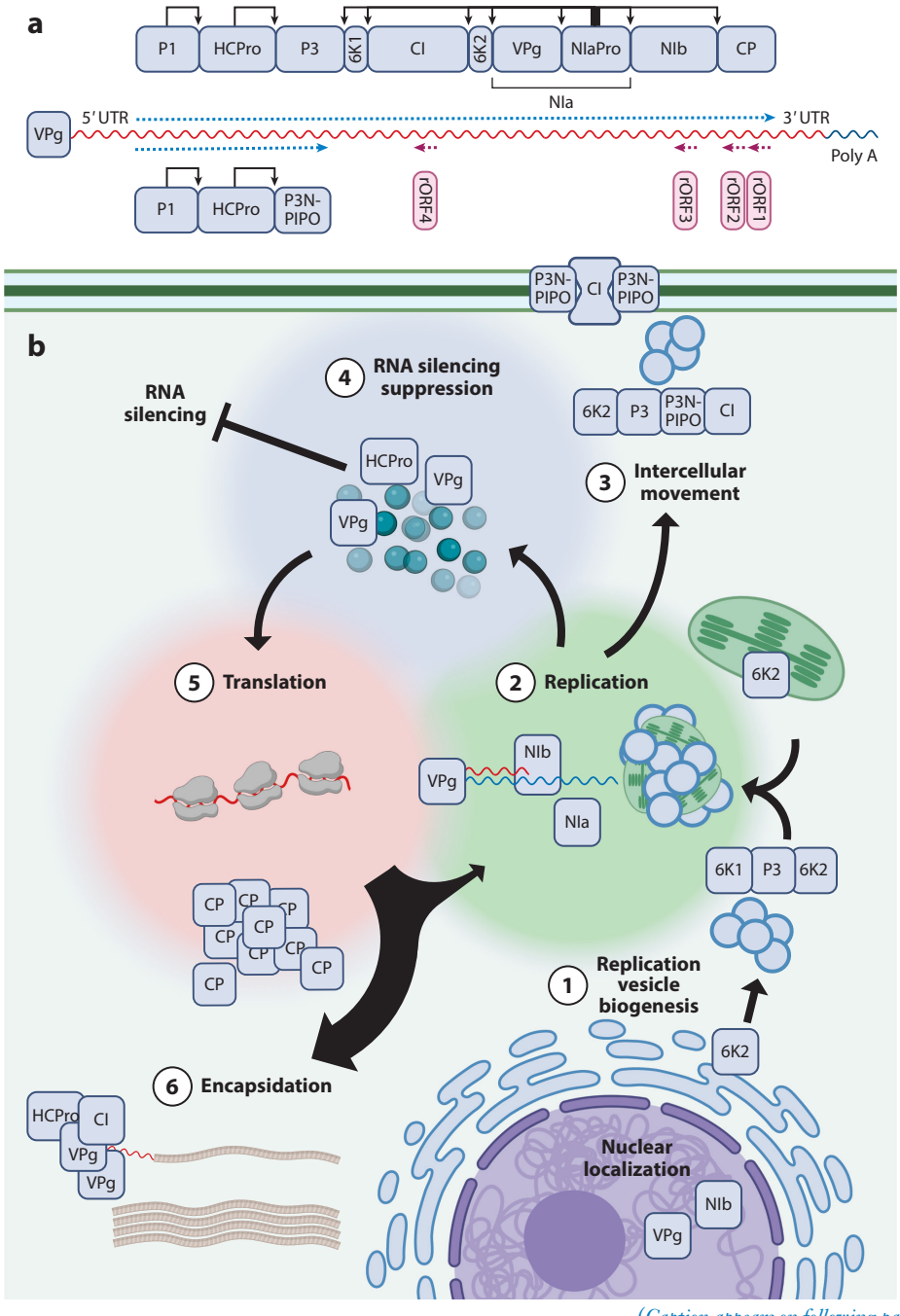
Virus name	Acronym	Agriculturally important host(s)	Main host(s) for molecular studies	Key discoveries	Reference(s)
Lettuce mosaic virus	LMV	Lettuce crops, wide host range: generalist	*N. benthamiana*, lettuce	eIF4E-mediated recessive resistance and its breaking, eIF4E at the particle tip	50, 89, 134
Plum pox virus	PPV	Genus *Prunus*: plum, peach, nectarine, cherry, and apricot	*N. benthamiana*, tobacco, peach	Functions of RNA helicase cylindrical inclusion, dependence of encapsidation on replication	48, 49, 65, 125, 126
Potato virus A	PVA	Potato	*N. benthamiana*, potato	Potyvirus-induced ribonucleoprotein complexes regulating replication, silencing suppression, translation, and encapsidation; proteomics of potyvirus infection	8, 25, 81, 88, 99, 101, 102, 111, 113, 116, 121, 122, 127
Potato virus Y	PVY	Potato and solanaceous vegetables	*N. benthamiana*, tobacco, potato	Interactions of potyviruses with chloroplasts, multiomics of potyvirus infections, particle structure	23, 27–30, 32–33, 106, 131, 133
Soybean mosaic virus	SMV	Soybean	*N. benthamiana*, soybean	Multiomics of potyvirus infection	105
Sugarcane mosaic virus	SCMV	Sugarcane, maize, and sorghum	*N. benthamiana*, maize, sugarcane	Translatome of potyvirus infected cells	112
Tobacco etch virus	TEV	Tomato, tobacco, and many *Capsicum* species	*N. benthamiana*, tobacco	Screens to understand functions of potyviral proteins	36, 37, 40, 41, 117
Turnip mosaic virus	TuMV	Family *Brassicaceae*, wide host range: generalist	*Arabidopsis*, *N. benthamiana*	Many important interactions of viral replication, vesicle maturation, movement, RNA biology, and nuclear functions	4, 12–21, 45, 46, 52, 59, 60, 62, 64, 72–74, 76, 77, 86, 104, 107

Abbreviation: eIF4E, eukaryotic initiation factor 4E.

replication vesicles at endoplasmic reticulum (ER) membranes (5). The core processes are RNA replication, RNA silencing suppression, and translation. These three can be thought of as the foundation upon which the success of potyviral infection depends as the vRNA is replicated, protected from host interference, and used to produce viral proteins, all in service of spreading the infection from cell to cell and, eventually, encapsidation into a virion. In this review, we examine the interactome that enables potyvirus infection, highlight interesting hypotheses, and discuss future research themes in the field.

THE MATURATION OF REPLICATION VESICLES REQUIRES HOST MEMBRANES AND MEMBRANE PROTEINS

Typical of many +ssRNA viruses, potyvirus infection induces rearrangements in host membranes. Viral replication is closely associated with these structures, and much of this reorganization is



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

(*a*) Potyviral genome organization and polyprotein processing. Dotted arrows indicate the positions of the large (*top*) and small (*bottom*) polyproteins (in *blue*) and the putative negative-sense strand-coded small rORF proteins (in *pink*) on the potyviral genome. Solid arrows indicate the cleavage sites of corresponding proteases. (*b*) An overview of potyviral infection processes. ① After entry, the initial rounds of translation occur, and replication vesicles (*blue spheres*) begin to form at ER exit sites, driven by 6K2; 6K2, 6K1, and P3 are strongly associated with these replication vesicles that eventually aggregate with chloroplasts. ② Core viral replication proteins are the NIb polymerase and the genome-associated VPg, the latter of which is covalently bound to vRNA. ③ Replication is directly connected to intercellular movement: Replication vesicles are recruited to the plasmodesma through the interactions of 6K2, P3, P3N-PIPO, and CI. P3N-PIPO anchors conical CI inclusions to the plasmodesma, and the resulting movement complex allows viral particles, CP-vRNA complexes, or replication vesicles to pass from one cell to the other. ④ Replicating vRNA is protected from host antiviral mechanisms by vRNA silencing suppression. Much of this is driven by the viral proteins HCPro and VPg, which localize with vRNA within potyvirus-induced granules. ⑤ vRNA is translated by host polysomes proposedly in a nonequimolar ratio. The 3′ end-coded CP is favored during late-stage infection, which directly supports a shift to ⑥ encapsidation. In addition to CP and VPg, some particles contain a tip structure that contains HCPro, CI, and certain host factors. Additionally, NIb and VPg localize in the nucleus and may alter host translation. Figure adapted from images created with BioRender.com. Abbreviations: CI, cylindrical inclusion protein; CP, coat protein; ER, endoplasmic reticulum; HCPro, helper component-protease; NIa, nuclear inclusion protein a; NIaPro, NIa protease; NIb, nuclear inclusion protein b; ORF, open reading frame; PIPO, pretty interesting potyviral ORF; rORF, reverse ORF; UTR, untranslated region; VPg, viral protein genome-linked; vRNA, viral RNA.

coordinated by 6K2, a viral transmembrane protein that induces the formation of vesicular and tubular extensions from the ER that later detach (6, 7). In this review, the resulting structures are collectively referred to as replication vesicles. Because ribosomes translating potyviral RNA are associated with replication vesicles, replication and translation may be intimately coupled processes (8, 9). A model of TuMV replication vesicle biogenesis proposes that the ribosomes that produce potyviral replication proteins on the ER become trapped in replication vesicles (10). Viral translation by ER-bound ribosomes may also help drive replication vesicle formation by localizing replication proteins to the donor ER membranes.

Though characterized by 6K2, nearly all potyviral proteins (excluding P1, CP, and P3N-PIPO) have been detected within replication vesicles (8). In addition to 6K2, 6K1 protein has been proposed to contribute to replication vesicle formation because it accumulates in these vesicles and has a transmembrane domain. Because there is also evidence that 6K1 and 6K2 interact, 6K1 may be bridging the interactions between replication vesicles and certain host factors, such as membrane curvature and scission-related proteins (11). However, 6K1's contributions to replication remain unclear.

Several ER exit site–associated proteins have been implicated in initiating the formation of replication vesicles. These include the Coat Protein Complex II (COPII) coatomer components Sec23, Sec24, and Sar1 (12, 13). One of these, Sec24, has been demonstrated to interact with 6K2 in yeast two-hybrid (14). This interaction has been proposed to have a key role in 6K2-mediated disruption of the ER exit site (15). Replication vesicles have been proposed to mature in different ways forming single- and double-membrane vesicle-like structures and multivesicular bodies (MVBs) (15). Additionally, the infection of some potyviruses induces the formation of a large globular membrane structure that carries markers for various subcellular compartments, including ER, Golgi, and chloroplasts (12). The biological significance of these various types of vesicles is not clear, though it has been postulated that some of them are strictly involved in replication and others in movement or particle assembly (15).

After budding from the ER, replication vesicles are trafficked intracellularly along actin filaments by myosins (9, 16). The destination of trafficked replication vesicles seems to be dictated by

Table 2 Virus-host protein-protein interactions that are essential for potyvirus infections

Viral protein	Interaction	Involved in	Cellular location(s)	Pathosystem(s)	Reference(s)
HCPro	eIF4E, eIF(iso)4E	Systemic movement	Cytoplasm	PVA *N. benthamiana*, *N. tabacum*	135
	VCS	RNA silencing suppression, translation, long-distance movement, particle stabilization	Cytoplasm, potyvirus-induced granules	PVA-TuMV *N. benthamiana*	101
P3	eEF1A	Unfolded protein response, local and systemic virus accumulation	Nucleocytoplasma	SMV *Glycine max*	44
P3N-PIPO	PCaP1	Movement	Plasmodesma	TuMV *A. thaliana*, TVBMV *N. benthamiana*	70, 71
6K2	PsbO1	Replication	Replication vesicle/ chloroplast	TVBMV *N. benthamiana*	23
	VTI11	Replication vesicle targeting to productive Golgi bypass pathway	Golgi bypass pathway, prevacuolar compartments/ multivesicular bodies	TuMV *A. thaliana*	17
	Vap27-Syp71	Replication vesicle maturation to complete robust virus multiplication	Chloroplast fusion	TuMV *A. thaliana*	19
VPg	RH8/DDXL	Replication	Replication vesicle, nucleus	PPV, TuMV *A. thaliana*	53
	eIF4E	RNA stability, translation, nuclear functions	Nucleocytoplasma	TuMV *A. thaliana*, PVA *N. benthamiana*	81, 86, 99
	eIF4G	Translation, virus accumulation, support of plant fitness	With eIF4E polysomes	TuMV *A. thaliana*	114
NIb	RH9	Replication	Replication vesicle	TuMV *A. thaliana*	52
	SUMO3	Regulation of immunosuppressive and replication functions of NIb	Nucleocytoplasma	TuMV *A. thaliana*	59, 60
	XPO3	Nuclear export of Nib and replication	Nucleocytoplasma	TuMV *A. thaliana*	63, 64
CP	HSP70-CPIP	CP regulation between replication/ encapsidation	Suggested: polysomes and replication complex formation	PVY *N. tabacum*, PVA *N. benthamiana*	122–124

Abbreviations: CP, coat protein; eEF1A, eukaryotic elongation factor 1A; eIF4E, eukaryotic initiation factor 4E; eIF4G, eukaryotic initiation factor 4 gamma; HCPro, helper component-protease; HSP70, heat shock protein 70; NIb, nuclear inclusion protein b; PCaP1, plasma membrane–associated cation binding protein 1; PIPO, pretty interesting potyviral ORF; PPV, plum pox virus; PVA, potato virus A; PVY, potato virus Y; SMV, soybean mosaic virus; SUMO3, small ubiquitin-like modifier 3; TuMV, turnip mosaic virus; TVBMV, tobacco vein banding mosaic virus; VCS, varicose; VPg, viral protein genome-linked; XPO3, nuclear exportin 3.

how they interact with various host SNARE (soluble N-ethyl-maleimide-sensitive-factor attachment protein receptor) proteins. For instance, Cabanillas et al. (17) proposed that SNAREs Sec22 and VTI11 determine whether replication vesicles fuse with Golgi cisternae or take a noncanonical Golgi bypass route. According to their model, some replication vesicles are trafficked into the Golgi along the canonical early secretion pathway. This pathway involves Sec22, which copurifies with replication vesicles and is involved in both retrograde and anterograde transport between the ER and Golgi. However, they surmise that the Golgi pathway is a dead end for TuMV infection because viral replication was substantially reduced by a mutation in 6K2 that causes it to primarily localize in the Golgi. Conversely, they observed an increase in intercellular movement when ER-to-Golgi transport was impaired by overexpressing Sec22 or a nonfunctional Golgi synaptotagmin. They proposed that VTI11 enables the Golgi bypass by redirecting replication vesicles into prevacuolar compartments and late endosomes. Wu et al. (18) further proposed that vesicular sorting receptor 4 (VSR4) also contributes to this bypass pathway. *A. thaliana* knock-out lines for both VTI11 and VSR4 show reduced infection, and both are associated with 6K2: VTI11 copurifies with replication vesicles, and VSR4 interacts with 6K2 in bimolecular fluorescence complementation and yeast two-hybrid experiments (17, 18).

The maturation process of potyviral replication vesicles culminates in their aggregation, and perhaps fusion, with chloroplasts, as demonstrated in the TuMV-*Arabidopsis* pathosystem (19) (**Figure 2**). These aggregates contain double-stranded RNA (dsRNA) and replication-associated proteins and are seemingly required for peak replication capacity (5, 19). The ER SNARE Syp71 is central to this process: Its downregulation reduces the number of aggregates formed and leads to lower levels of virus accumulation (19). How potyviruses recruit Syp71 to the aggregate is not yet clear as it did not interact with 6K2 in yeast two-hybrid. One possibility is that another SNARE, Vap27-1, which interacts with both Syp71 and 6K2, acts as a bridge between the two. The *A. thaliana* dynamin-related protein 1 (DRP1) has also been suggested to be involved in the chloroplast aggregation due to its possible localization near replication vesicle-chloroplast aggregates and its role in membrane scission and rearrangement (20–22). However, these studies use a DRP1-green fluorescent protein (GFP) fusion protein, the chloroplast localization of which has been called into question because it is not supported by various other methods (22).

Potyviral proteins interfere with several chloroplast factors, perhaps to make the organelle a more favorable environment for virus multiplication (**Figure 2**). The maturation of replication vesicles, after all, culminates in chloroplast fusion. Common symptoms of potyvirus infections, such as chlorosis and reduction of photosynthetic activity, have been linked to protein-protein interactions involving chloroplast-associated proteins. For instance, the interaction between the oxygen-evolving complex protein PsbO1 and 6K2 is required for efficient viral multiplication in both tobacco vein banding mosaic virus and potato virus Y (PVY) in *Nicotiana benthamiana* (23). Furthermore, there is evidence for component exchange between replication vesicles and chloroplasts. A proteomic analysis of potato virus A (PVA) replication vesicles indicated that 25% of proteins identified were chloroplast associated (8). Nevertheless, while both CP and HCPro have been detected in chloroplast preparations by immunoblotting, microscopy-based methods have not confirmed their subcellular localization within the organelle in the infection context. Based on confocal microscopy, fluorescently tagged HCPro is predominantly cytoplasmic with a strong presence in potyvirus-induced inclusions or granules, which play a key role in RNA silencing suppression (24, 25).

Because most chloroplast proteins are encoded by the nucleus and transported post-translationally into the chloroplast, we consider it possible that HCPro could intercept chloroplast-targeted proteins en route to their destination. This would mean the interactions occur in the cytoplasm, as appears to be the case for sugarcane mosaic virus (SCMV): Its HCPro

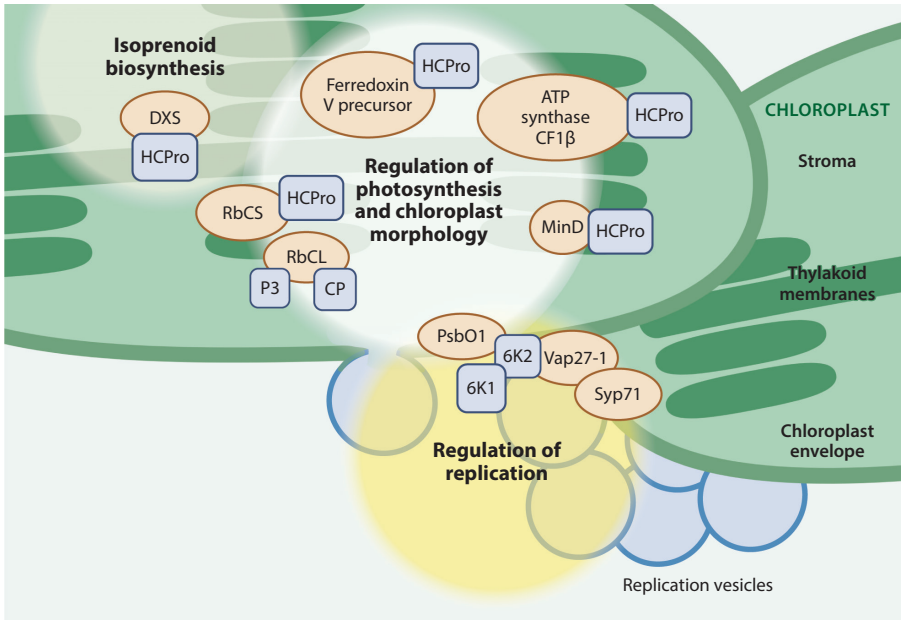


Figure 2

Chloroplast-associated host factors and processes that have been shown to involve potyviral proteins. The SNARE protein Syp71 participates in the aggregation, and potential fusion, of replication vesicles with chloroplast membranes. This is required for achieving peak replication. In parallel with the invasion of the chloroplast membrane systems, potyviral proteins interfere with key photosynthetic proteins such as RuBisCO subunits, ATP synthase subunit CF1β, the precursor of ferredoxin V, and the oxygen-evolving complex protein PsbO. Furthermore, MinD plays a role in chloroplast division, and HCPro's interaction with DXS was found to upregulate the biosynthesis of photosynthetic pigments. Taken together, these interactions could lead to symptom development via increased oxidative stress due to imbalanced photosynthesis. Figure adapted from images created with BioRender.com. Abbreviations: CP, coat protein; DXS, 1-deoxy-$_D$-xylulose-5-phosphate synthase; HCPro, helper component-protease; RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; SNARE, soluble N-ethyl-maleimide-sensitive-factor attachment protein receptor.

specifically interacts with the transit peptide of the ferredoxin V precursor in *N. benthamiana* (26). Other reported interactions could employ this mode as well. For example, HCPro might engage the nuclear-encoded ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) small subunit and the chloroplast division factor MinD (27–29) during transit. Interestingly, in yeast two-hybrid, P3 and CP of several potyviruses interacted with RuBisCO subunits, hinting that interference with this key photosynthetic enzyme involves several viral proteins and could represent a shared strategy among potyviruses (30, 31).

Several studies have convincingly shown in vitro interactions between HCPro and certain chloroplast-associated host proteins. However, it should be noted that the demonstration of these interactions in planta required the artificial relocalization of either HCPro or the host protein within the cell. For instance, to detect the interaction between HCPro and 1-deoxy-$_D$-xylulose-5-phosphate synthase, an enzyme involved in the biosynthesis of isoprenoid metabolites, required confining the host factor to the cytoplasm by removing its transit peptide (32). Conversely, the chloroplast-encoded ATP synthase subunit $CF_1\beta$ interacted with HCPro in transgenic plants expressing a chloroplast-targeted version of the viral protein (33). Also, when the interaction between MinD and HCPro was studied in planta, abnormally large chloroplasts were observed in

plants expressing a chloroplast-targeted HCPro (29). Future assessment of these interactions in relevant infection contexts may reveal additional insights into their importance for potyvirus infections. For instance, the abundance of ATP synthase units on the thylakoid membranes was reduced in not only transgenic HCPro tobacco but also PVY-infected plants, possibly leading to the observed reduction of photosynthetic activity (33).

Considering that the contribution of chloroplasts is fundamental to potyvirus multiplication, it is necessary to further unravel the role of the chloroplast-replication vesicle fusion in replication. We do not yet understand why the fusion of chloroplasts and replication vesicles is required for robust replication nor the molecular mechanisms governing the process.

REPLICATION AND MORE: THE MULTIFACETED FUNCTIONS OF POTYVIRAL REPLICATION PROTEINS

Potyviral RNA is amplified by the RNA-dependent RNA polymerase NIb in specialized viral replication complexes (VRCs) within replication vesicles. As NIb initiates replication, it uses VPg as a primer and uridylylates VPg, attaching it covalently to vRNA (34, 35). NIb may also actively recruit vRNA for replication, possibly facilitated by its interaction with the secondary structures of the 3′ untranslated region (UTR) (36). NIb is thought to be recruited to the VRC through its interaction with the 6K2-VPg-NIaPro cleavage intermediate NIa (VPg or NIaPro). The specific domain of VPg or NIaPro involved in the interaction with NIb may vary among different potyviruses (37–40). Moreover, the interaction between NIb and 6K2-VPg-NIaPro is required for replication initiation and is thought to anchor the VRC to replication vesicles via the hydrophobic domain of 6K2 (41).

NIb is a highly active interactor with the most reported host protein interactions among potyviral proteins (4). NIb may be actively recruiting numerous proviral host proteins into VRCs (42). One such proviral factor is eukaryotic elongation factor 1A (eEF1A), which forms direct noncompetitive interactions with both NIb and both domains of VPg-NIaPro. eEF1A has also been detected in replication vesicles during TuMV infection (8). Despite its localization in replication vesicles, the specific role of eEF1A concerning its interaction with NIb and 6K-VPg-Pro remains uncertain (43). In the soybean mosaic virus-soybean pathosystem, eEF1A interaction with P3 protein is crucial for infection. The suggestion is that P3 targets translation elongation and contributes to unfolded protein response in a manner that promotes soybean mosaic virus infection (44). NIb also interacts with *A. thaliana* heat shock cognate 70-3 (Hsc70-3) and poly(A)-binding protein 2 (PABP2), and these interactions colocalize with replication vesicles. Intriguingly, coexpression of the 6K2-VPg-NIaPro polypeptide with these proteins redirects them to the ER-derived vesicles (45, 46). However, the lack of an in vitro replication system for potyviruses has hindered more detailed studies of their roles in the replication reactions.

The vRNA helicase CI is indispensable for potyvirus replication (47, 48). Double-stranded RNA consistently colocalizes with CI, highlighting its role in VRCs (9). Whether NIb recruits CI to the replication complex is not certain as some published results support their interaction and others do not (4, 11, 49). Nevertheless, CI may also be recruited into the VRC by its interactions with either VPg (50) or NIaPro (4). Alternatively, the slow cleavage of the CI-6K2 polyprotein during proteolytic processing may explain its presence in replication vesicles (51). Yet another alternative recruitment strategy may involve the recently reported interaction chain between 6K2, P3, P3N-PIPO, and CI, which was originally hypothesized to allow replication vesicles to adhere to CI inclusions for intercellular movement, though P3N-PIPO was not detected in the replication vesicle proteome (8, 52).

Host RNA helicases play vital roles in potyvirus infection. Li et al. (53) revealed the significance of three helicases on TuMV infection: *A. thaliana* RNA helicases AtRH9 and AtRH26 and

plant RNA helicase75 (PRH75). AtRH9 interacts with NIb and colocalizes with the replication vesicles. This suggests that the recruitment of AtRH9 to the VRC is likely facilitated through its interaction with NIb. Additionally, AtRH8 interacts with TuMV and plum pox virus (PPV) VPg and colocalizes with chloroplast-associated replication vesicles, indicating a potential role in potyviral genome translation and replication (54). Yet another host helicase, eukaryotic translation initiation factor 4A, was detected in the PVA replication vesicle proteome (8). Because these host helicases and the viral CI together localize within the replication vesicles, and because they likely are essential for viral infection, they possibly collaborate to provide essential helicase activities for replication, for instance, dismantling RNA secondary structures (8, 53, 54). Overall, NIb may recruit both viral and host proteins to replication complexes to shield replication machinery from external nucleases and proteases, while also offering a stable framework for the VRC assembly.

NIa, especially its VPg domain, and NIb both localize to the nucleus (55–57) (**Figure 3**). Their relocalization to the nucleus occurs together with some of the host proteins that interact with them. For instance, NIb's interaction with PABP2 redistributes PABP2 to the nucleus and nucleolus (45). Similarly, VPg's interactors RH8 and eIF4E colocalize in the nucleus (45, 54). This poses an interesting question: Why are these viral replication proteins and their interactors also in the nucleus? One potential function for eIF4E and PABP2 in the nucleus or nucleolus may relate to translational regulation as they are canonical protein synthesis regulators. Very recently, eIF4E was reported to induce extensive alternative splicing by simultaneously acting on multiple splicing factors in human cancer cells (58). Such a mechanism suggests that eIF4E could play a wider role in virus infection, for instance in modifying host messenger RNA (mRNA) processing, export, and translation. The importance of nucleocytoplasmic host proteins for potyvirus infection is further supported by the observation that the proteasomal degradation of eIF(iso)4E is prevented by DNA-binding protein 1, a nucleocytoplasmic transcription factor and type 2C protein phosphatase (59). This transcription factor is also a host susceptibility factor that promotes PPV and TuMV infection in *A. thaliana*, possibly by stabilizing the eIF(iso)4E.

Replication and translation-associated viral and host proteins may repress host defenses (**Figure 3**). For instance, NIb alters salicylic acid–mediated immune responses. Within the nucleus, NIb binds to nonexpresser of pathogenesis-related 1 (NPR1), which is a master regulator of salicylic acid–mediated resistance (60, 61). This interaction prevents sumoylation and phosphorylation of NPR1, consequently suppressing its ability to elicit an immune response. The potyviral NIbs from viruses representing all six major clades of potyviral phylogeny (62) have the capacity to target NPR1, which demonstrates the conservation of this interaction and the importance of suppressing this antiviral mechanism (61). However, TuMV NIb gets sumoylated in the nucleus by small ubiquitin-like modifier 3 (SUMO3) and SUMO conjugation enzyme (SCE1) (60, 63). The sumoylation allows the nuclear exportin 1 (XPO1) to transport NIb to the cytoplasm where it can take part in TuMV replication (64, 65).

POTYVIRUS CELL-TO-CELL MOVEMENT AND REPLICATION ARE COUPLED PROCESSES

Potyviral intercellular movement relies heavily on the formation of movement complexes assembled at plasmodesmata (**Figure 4**). Three viral proteins are required for these complexes: CI, P3N-PIPO, and CP. Together, they allow either replication vesicles, viral particles, or partially encapsidated pseudovirions to be funneled into adjacent cells. The most distinctive feature of these complexes is the presence of large CI inclusions. These inclusions are assembled through CI self-interactions mediated by both the N- and C-terminal domains (11, 49). Two mutations in the TuMV CI N terminus that affect movement negatively have also been shown to reduce

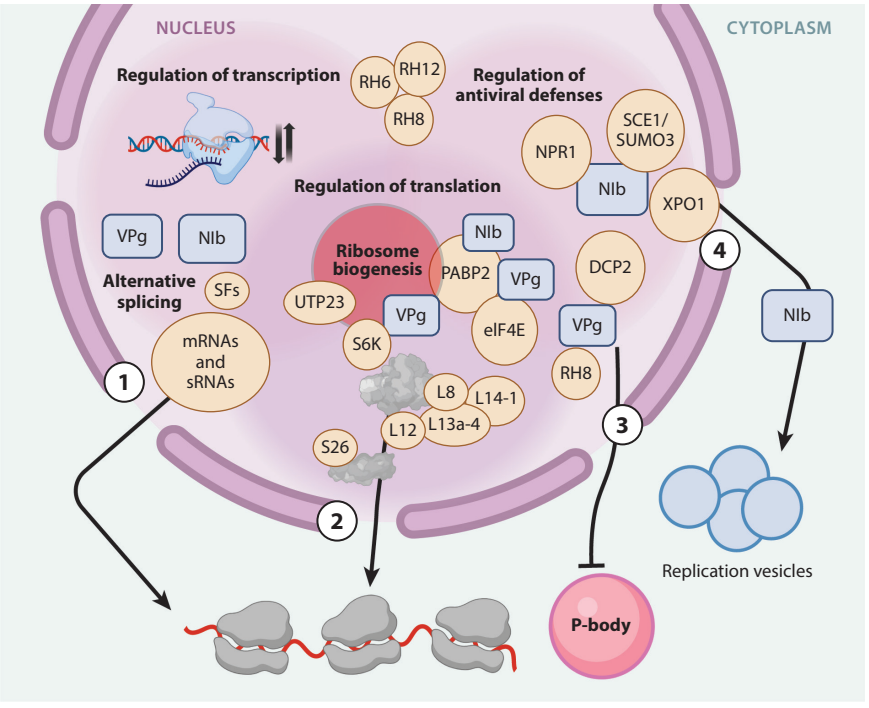


Figure 3

Potyviral interference with nuclear functions. ① Potyvirus infection regulates the cellular mRNA and sRNA pools first by affecting transcriptional regulation via interactions between viral proteins and transcription factors and second by inducing infection-specific splicing factors, which leads to alternative splicing patterns. ② Several infection-specific ribosomal protein paralogs and rRNA processing factors may be involved in modifying ribosome biogenesis to regulate translation and to favor potyvirus infection. ③ Viral proteins relocalize certain processing body components to the nucleus, for instance RH8 and DCP2. This counteracts the antiviral role these host proteins have in the cytoplasm. ④ Viral and host proteins associated with potyvirus replication also localize in the nucleus, and they may regulate antiviral defenses to benefit potyvirus multiplication. Figure adapted from images created with BioRender.com. Abbreviations: DCP2, decapping protein 2; eIF4E, eukaryotic initiation factor 4E; mRNA, messenger RNA; NIb, nuclear inclusion protein b; NPR1, nonexpresser of pathogenesis-related 1; P-body, processing body; PABP2, poly(A)-binding protein 2; rRNA, ribosomal RNA; SCE1, SUMO conjugation enzyme; SF, splicing factor; sRNA, small RNA; SUMO3, small ubiquitin-like modifier 3; VPg, viral protein genome-linked; XPO1, nuclear exportin 1.

self-interaction strength (66, 67). However, whether inclusion assembly is spontaneous or requires other viral or host proteins is not known.

Plasmodesmatal CI inclusions have been shown to contain CP as filamentous structures that follow CI inclusion lamellae (68). This association is corroborated by CP-CI interactions that have been demonstrated by bimolecular fluorescence complementation as occurring near the plasma membrane (11). The biological significance of this interaction remains uncertain but is likely to contribute to movement by binding vRNA or virions to the CI inclusions of the movement complex. CI has also been associated with viral particles, and it is possible this association is also mediated by the similar CI-CP interactions (69, 70). This association, along with the filamentous CP structures within CI inclusions, may indicate that CI, CI inclusions, or the movement complex participate in encapsidation. Such a role would greatly support the case for movement occurring as a particle or an encapsidation intermediate.

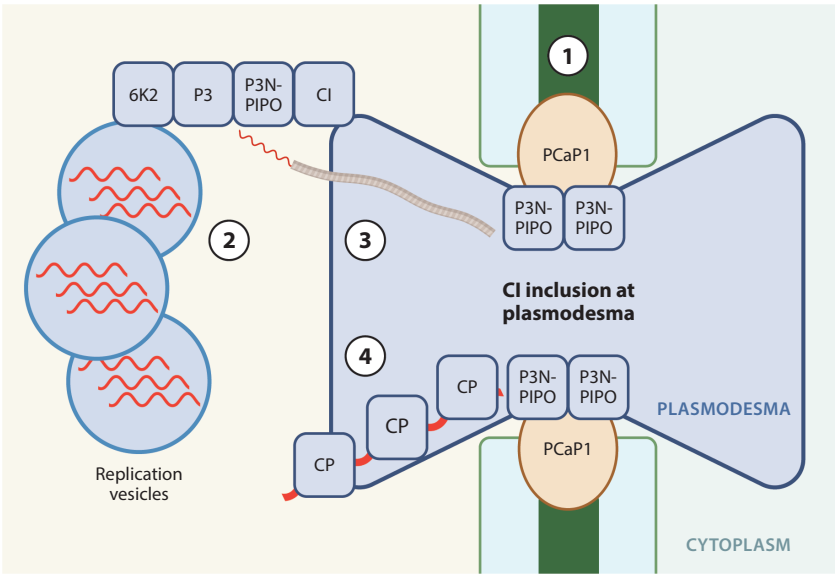


Figure 4

Movement complex at plasmodesma. ① P3N-PIPO interacts with host factors, including PCaP1, and anchors CI inclusions to the plasmodesma. ② A subset of replication vesicles migrates to the vicinity of CI inclusions. Replication vesicles are possibly connected to each other and the CI inclusion through an interaction chain from the membrane-bound 6K2 interacting with the P3 C terminus to P3 and P3N-PIPO interacting via their shared P3N domain to P3N-PIPO's PIPO domain interacting with CI. Several proposals for movement strategies include the 6K2-labeled replication vesicles being transported through the inclusion, ③ movement as fully encapsidated virions, and ④ movement as a not entirely encapsidated vRNA-CP complex. The latter two would be facilitated by CI-CP interactions. Figure adapted from images created with BioRender.com. Abbreviations: CI, cylindrical inclusion protein; CP, coat protein; PCaP1, plasma membrane–associated cation binding protein 1; PIPO, pretty interesting potyviral ORF; vRNA, viral RNA.

CI's interactions with the P3N-PIPO protein have been proposed to mediate the plasmodesmatal localization of the movement complex, though even without P3N-PIPO, CI-CI interactions have been detected as puncta near the plasma membrane (11, 67). The anchoring has been proposed to involve plasma membrane–associated cation binding protein 1 (PCaP1) in *A. thaliana* and its homolog in *N. benthamiana* (71, 72). P3N-PIPO also likely supports movement by hijacking plasmodesmatal size exclusion limit-modifying host proteins so that the movement complex or virions/pseudovirions can fit through. For instance, P3N-PIPO's interactions with PCaP1 and group 1 Remorins have been proposed to allow it to reduce plasmodesmatal actin network integrity and callose deposition (73, 74).

Potyvirus movement and replication are coupled: Replication vesicles containing VRCs are recruited to movement complexes at plasmodesmata (75). The recruitment is mediated by P3N-PIPO and P3 interactions via their shared P3 N-terminal domains (52). As the P3 C-terminal domain interacts with the 6K2 on replication vesicles and the PIPO domain interacts with CI inclusions, P3 and P3N-PIPO can act as a bridge between the two structures. This coupling of replication and movement is advantageous as it creates a safe replication microenvironment that allows rapid transfer of vRNA from one complex to the other while minimizing exposure to host interference. Moreover, because no potyviral RNA-binding protein has been reported to exhibit sequence specificity, it would also allow the virus to transfer vRNA more selectively and avoid

moving host RNAs (76). Alternatively, entire replication vesicles may migrate through the movement complex as there is evidence for their migration into noninfected cells (75).

MVBs have been proposed to contribute to movement. As mentioned above, the SNARE VTI11 and VSR4 may direct replication vesicles into prevacuolar compartments and endosomes (17, 18). As late endosomes are often characterized by their intraluminal vesicles and are connected to exosome release, 6K2-containing MVBs may allow the secretion of replication-competent viral exosomes into the intercellular space. The numbers of MVBs and exosomes have been reported to increase during TuMV infection and, therefore, MVBs have been suggested to be one of the maturation pathways of replication vesicles (15, 77). These may represent a process that supports long-distance movement by loading 6K2 vesicles into host vasculature or an alternate, redundant intercellular movement pathway (78). How potyviruses interact with MVB-associated proteins is not well known. However, recent evidence shows that an ESCRT (endosomal sorting complexes required for transport) protein, vacuolar protein sorting 4 (VPS4), is a resistance determinant against bean common mosaic virus and watermelon mosaic virus (79, 80). Considering that VPS4 is involved in the scission of MVB intraluminal vesicles, it may contribute to the increased number of MVBs and exosomes during potyvirus infection (81). More research is needed to elucidate the mechanisms of how VPS4 and other host factors contribute to the biogenesis and trafficking of potyviral MVBs.

NETWORKS OF INTERACTIONS PROTECT VIRAL RNA POSTREPLICATION

EIF4Es or eIF(iso)4Es are associated with many potyviral infection processes. Most importantly, their interaction with VPg protects vRNA postreplication and without this interaction, the production of viral proteins remains minimal (82). Due to its key role in potyviral infection, natural variance in eIF4Es/eIF(iso)4Es is associated with recessive resistance to potyviruses in many crops (reviewed in 83, 84). This weakness has even been successfully exploited in breeding potyvirus-resistant plants (85, 86). Despite such advances, elucidating the function of eIF4E in potyvirus infection has been remarkably difficult.

The interaction between TuMV VPg and *A. thaliana* eIF(iso)4E was among the first published potyvirus-host interactions (87). Later, this interaction was demonstrated to favor viral translation over host capped mRNAs (88, 89) and aid the intracellular transport of viral genomes to plasmodesmata in complex with CI and eukaryotic initiation factor 4 gamma (eIF4G) (90). As suggested by Wang & Krishnaswamy (91), VPg-eIF4E may act in complex with P1 and HCPro to safeguard viral translation and/or replication in the cytoplasm. However, because PVA −RNA synthesis occurs and PVA RNA is efficiently directed to degradation in the absence of VPg-eIF4E interaction (8, 82), it is possible that this interaction is not involved in replication.

Protecting vRNA against host antiviral responses is vital to successful infection. Much of this relies on making the host cell a permissive environment for the infection by interfering with host antiviral defenses. HCPro is known to bind small interfering RNA molecules with a recently demonstrated preference for viral sequences (92–94). It is believed to exert its silencing suppressor activity also by inhibiting the methylation of small RNAs required for efficient antiviral silencing. For instance, HCPro's interactions with Hua enhancer1, and the key host methionine cycle factors, S-adenosyl-L-methionine synthase and S-adenosyl-L-homocysteine hydrolase, lead to reduced methylation capacity (95–98). In contrast, VPg contributes to silencing suppression by targeting suppressor of gene silencing 3 (SGS3) to degradation (99). The same study implicated VPg in reducing the amplification and spread of virus-derived small interfering RNAs (vsiRNAs) as its interaction with SGS3 simultaneously decreased the levels of RNA-dependent RNA polymerase 6.

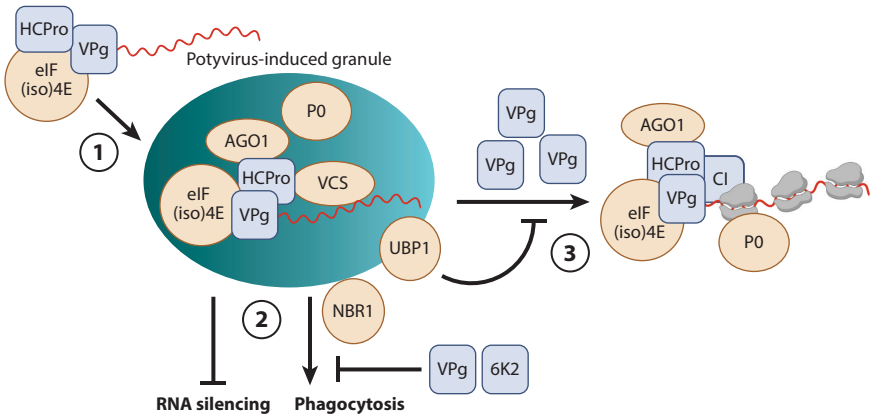


Figure 5

Model of a PG and its role in infections. ① vRNA may be delivered from replication to the PGs unless it is required to act as a template for protein synthesis. ② PGs are required for efficient RNA silencing suppression, which the host counters by targeting PGs to degradation via the autophagosome cargo receptor NBR1. In turn, the viral proteins VPg and 6K2 can inhibit NBR1-mediated degradation. ③ High concentrations of free VPg stimulate the relay of vRNA to polysomes. PG-located host proteins eIF(iso)4E, AGO1, VCS, and the ribosomal stalk protein P0 cooperate with VPg to enhance viral protein expression. UBP1, on the other hand, counteracts VPg-mediated translational enhancement. Figure adapted from images created with BioRender.com. Abbreviations: AGO1, Argonaute1; CI, cylindrical inclusion protein; eIF(iso)4E, eukaryotic initiation factor 4E; HCPro, helper component-protease; PG, potyvirus-induced granule; UBP1, oligouridylate-binding protein 1; VCS, varicose; VPg, viral protein genome-linked; vRNA, viral RNA.

Covalently bound VPg interacts with not only the free form of VPg but also other viral and host proteins to recruit them to a protein complex associated with the 5′ end of potyviral RNA (98). This opens the possibility for more proteins to join the VPg-eIF4E interaction network, and thus it expands the capacity to control potyviral RNA functions. The PVA HCPro, for example, binds to eIF4E through a different binding site than VPg (100). Indeed, the most striking example of HCPro's capacity to requisition host factors for functional gains is the generation of potyvirus-induced granules (PGs) (25) (**Figure 5**). In confocal fluorescence micrographs, PGs appear as punctate bodies that contain HCPro, vRNA, and markers for both processing bodies (P-bodies) and stress granules (25, 101). PGs are crucial for successful silencing suppression and, because they are associated with viral replication vesicles, may reinforce the connection between viral replication and translation (25).

Varicose (VCS) is a WD40-domain scaffolding protein found not only in P-bodies but also in PGs. VCS is likely recruited to PGs via its interaction with HCPro. In TuMV and PVA infections, this interaction is essential for silencing suppression and its disruption reduced the formation of PGs (25, 102). Moreover, the HCPro-VCS interaction is crucial for the formation of stable virions and, therefore, systemic movement. HCPro also recruits Argonaute1 (AGO1) to PGs (25). While the loss of the interaction did not affect HCPro's silencing suppression capacity, particle accumulation and virus spread were impaired in PVA and TuMV pathosystems (103). In a recent study, TuMV HCPro overexpression induced the autophagic degradation of AGO1 and reduced RNA-induced silencing complex (RISC) activity, although no direct interaction between the two proteins was detected (104). Such examples emphasize that there are several dynamic and possibly overlapping ways in which HCPro modulates the cellular environment. As a defensive countermeasure, the host cell protects itself through selective autophagy delivering HCPro to

autophagosomes for degradation. This antiviral pathway was, however, interrupted by VPg and 6K2, which block the targeting of HCPro by the autophagy cargo receptor NBR1 (105).

Potyvirus infection affects host RNA metabolism in many ways. For instance, infections induce changes in host mRNA accumulation levels; RNA degradome; and microRNA (miRNA), vsiRNA, and phased secondary small interfering RNA (phasiRNA) pools (106, 107). These changes are driven, in part, by altered P-body and stress granule function. This includes reducing P-body-associated RNA processing. First, potyviruses hinder RNA degradation: TuMV HCPro binds to the XRN4 RNA exonuclease suppressing its activity (108). Second, decapping proteins (DCPs) are affected: TuMV VPg redirects DCP2 from the cytoplasm to the nucleus, which disconnects it from DCP1 and, consequently, disables proper mRNA decapping complex assembly (108).

Potyviruses may also hinder RNA decay pathways by targeting P-body and stress granule–associated helicases. These include the DDx6-type helicases, RH6, RH8, and RH12, which among other functions contribute to the assembly of large DCP2- and VCS-containing P-bodies and oligouridylate-binding protein 1C-containing stress granules (109). VPg interacts with RH8 sequestering it to the nucleus, which possibly reduces mRNA decay. The significance of RH interactions may extend beyond P-bodies and stress granules because, in *A. thaliana*, they are also involved in limiting the accumulation of mRNAs involved in immune responses and growth inhibition under nonstress conditions (109). The interaction between potyviral proteins and these helicases may interfere with normal growth signaling. RHs also contribute to the assembly of dicer bodies required for miRNA processing (110). TuMV infection not only reduces dicer body formation in a replication-dependent manner but also reduces primary miRNA transcript levels through the involvement of RHs (110). This may be yet another way of altering host translation to favor infection and prevent antiviral signaling.

FACTORS REQUIRED FOR TRANSLATION OVERLAP WITH THOSE IN REPLICATION, VIRAL RNA PROTECTION, AND ENCAPSIDATION

For a long time, the paradigm surrounding potyviral translation was that all potyviral proteins are produced in equimolar ratios and as a single polyprotein. Currently we are aware that the potyviral protein expression strategy is more complex than previously thought. The first counterargument against the paradigm is the existence of the short polyprotein P1-HCPro-P3N-PIPO (111) (see **Figure 1**). The second is the observed difference in the accumulation of 5′- and 3′-encoded potyviral proteins (112). Throughout the infection, 3′-encoded protein accumulation increases, whereas the expression of 5′-encoded proteins remains stable after a certain timepoint. Such a preference for 3′ cistrons suggests that CP production may be controlled by a specific translational strategy. The third counterargument is the newly made discovery of small proteins encoded by rORFs (3). This latter mechanism is very interesting but remains largely unexplored.

Potyvirus infection does not alter the amount of translationally active polysomes (113, 114). Interestingly, *Arabidopsis* eIF4E1 maintains the host's global translational activity during virus infection. When eIF4E1 is knocked out, host translation is compromised during TuMV infection (115). In chlorotic SCMV-infected maize leaves, approximately 2% of total ribosome sequencing (ribo-seq) reads were mapped to the SCMV genome (113). This suggests that only a fraction of the host's resources are used for vRNA translation. It is not known with certainty whether the ribosomes translating vRNA or host mRNAs are modified in some way, but some evidence supporting the possibility exists. First, the ribosomal proteins (RPs) S26, L8, L12, L13a-4, and L14-1 and a ribosome biogenesis-related protein UTP23 are enriched in the nuclear proteome of PVA-infected potato as compared to healthy plants (57) (**Figure 3**). Second, based on an analysis of the polysome

proteome, PVA infection causes subtle changes in the RP paralogs associated with translating ribosomes (114). Based on a ribo-seq analysis of the maize translatome, SCMV infection influences the production of ribosome structural constituents (113). These results suggest that potyviruses may alter the ribosome composition or assembly to favor viral translation or change host gene expression. Whether this is indeed the case and whether such changes in ribosome composition have a biological function remains to be investigated further.

Some of the RPs are differentially required by different plant viruses. In contrast to tobacco mosaic virus, which has capped RNA, TuMV, as a cap-independently translated virus, requires RPS6 for infection (116). Potyviral VPg interacts with S6 kinase, and this interaction may affect the functions of both RPS6 in the cytoplasm and S6K in the nucleus (117). Some viral proteins are associated with ribosomes, although their functions are not clear yet. An abundance of CI and HCPro, and trace amounts of NIa-Pro, VPg, and NIb associate with polysomes in PVA infection, and tobacco etch virus P1 associates with the 80S ribosomes (114, 118).

The presence of internal ribosomal entry sites (IRESes) in the RNA of many potyviruses was recently reviewed by Jaramillo-Mesa & Rakotondrafara (119). They also examined the contributions of PABP and various eIFs to vRNA translation. Interestingly, a novel model concerning the role of IRES-mediated translation and eIF(iso)4G1 in TuMV accumulation was recently presented by Zafirov et al. (115). They hypothesized that TuMV can co-opt a truncated version of eIF(iso)4G1 to benefit viral translation. Picornavirus infection induces the cleavage of eIF4G to inactivate its contribution to cap-dependent translation and subsequently recruits the cleavage products to translation via IRESes on vRNA (120). Because the 5′ UTR of TuMV RNA houses a sequence that can function as an IRES, potyviruses may use a similar mechanism for cap-independent translation (121). Understanding the relative contributions of IRES-eIF(iso)4G and VPg-eIF4E interactions to translational control in potyvirus infections requires further research.

Translation is inhibited when CP binds to the CP-coding sequence (122), which has been proposed to act as a temporary barrier to ribosomes until the replication complex is assembled (123). In addition, the availability and phosphorylation status of PVA CP are regulated by the co-operative action of CK2 kinase and the heat shock protein 70 and chaperone CP interacting protein, which belongs to the heat shock protein 40 family (123–125). Taken together, the multilayered regulatory network centered around CP and its functions could help direct the vRNA from translation to replication (123, 124).

Potyviral encapsidation is interlinked with replication and translation. Factors that accelerate CP production to levels sufficient for particle encapsidation are partially known. CP and HCPro have emerged as key coordinators of encapsidation (126, 127). During the later stages of a PVA infection, the gradual accumulation of VPg and HCPro strengthens CP production, likely by regulating translation favoring the 3′ end of PVA RNA (112, 128). Host factors that support VPg-mediated upregulation of 3′-encoded cistrons include AGO1, VCS, eIF4Es, and P0 (25, 89, 102, 103, 129). It is noteworthy that these same proteins are present in PG structures during active RNA silencing suppression, which also connects protection of vRNA postreplication to translation (**Figure 5**).

PROTEIN-PROTEIN INTERACTIONS STABILIZE POTYVIRUS PARTICLES

During late-stage potyvirus infection, when CP levels have exceeded a certain threshold, encapsidation into progeny viruses seems to be the major destination for vRNA. In a mature infection, only a small fraction of vRNA is actively engaged in translation while the vast majority is encapsidated into particles and, therefore, essentially unavailable for translation and replication.

Our current understanding of the process of potyviral encapsidation is poor compared to, for example, the thoroughly analyzed assembly of rod-shaped tobamoviruses. Despite this knowledge gap, the detailed structures of several potyvirus capsids and their respective CPs have been well characterized in recent high-resolution cryo–electron microscopy studies (130–133). According to Gallo et al. (127), only replication-competent potyviral RNA can be stably encapsidated. The covalently attached VPg at the 5′ end of the vRNA is characteristic of potyviral RNA and potyvirus particles. Saha et al. (128) used a replication-incompetent PVA mutant to demonstrate that covalently attached VPg was a prerequisite for the accumulation of stable particles.

Based on current evidence, a large ribonucleoprotein complex composed of viral and host factors may be assembled around VPg. The complex, visible as an asymmetric tip structure distinct from the body of the particle, was first detected in PVY and PVA by atomic force microscopy (134). Saha et al. (128) proposed that the biological function of the tip structure could be to ensure particle stability by concealing the 5′ end of encapsidated vRNA protecting it from host RNases. In this scenario, the covalently attached VPg, VPg-VPg self-interactions, and VPg-eIF(iso)4E interactions are likely drivers of tip structure formation (128, 135). Other viral and host proteins may be recruited via protein-protein interactions, as shown by immunogold labeling of purified particles. HCPro is enlisted to the complex via its interaction with VPg, eIF4E, and/or eIF(iso)4E (100, 134, 136). Valli et al. (126) emphasized HCPro's crucial role in particle accumulation by showing that CP produced from a truncated PPV genome was only stable if its cognate HCPro was expressed in trans.

CI, which interacts with HCPro, was also shown to associate with one end of PVA particles (69). HCPro could also recruit additional host factors: VCS and AGO1 were proposed to be part of the complex, and their interactions with HCPro are important for particle stability (102, 103). Disrupting the interactions between HCPro and its partners has been shown to have negative effects of varying severity on particle accumulation, stability, and transmissibility. For instance, the aphid transmissibility of soybean mosaic virus was traced to key residues facilitating the interaction between HCPro and CP (137). Also, debilitating HCPro's capacity to interact with VCS led to PVA and TuMV losing their capacity to assemble stable particles and to spread systemically (102). However, disrupting HCPro-AGO1 interaction had a less severe effect (103). We hypothesize these defects could decrease virus transmissibility downstream of particle formation. From the perspective of developing resistance against potyviruses, such virus-host interactions present attractive modification targets.

CONCLUSION

One of the most important long-term goals of potyvirus research is improved food security in the face of a rapidly changing climate. The identification of the vital interactions for potyvirus infection are therefore of paramount importance for resistance breeding. Although the effects of many protein-protein interactions are quantifiable, these effects are often subtle. It is important to keep in mind that some of these interactions may have evolved only in specific potyvirus species and only a fraction are strictly necessary for a successful infection (**Table 2**). The better the underlying molecular mechanisms are known, the easier the interactions can be connected to the pathways controlling the course of infection. The connections and confluences of different processes, such as replication, translation, and protection from host cell perturbation, are interesting and raise new questions. Does the maturation and fusion of replication vesicles with chloroplasts support the needs of translation of viral proteins and suppression of antiviral pathways? Do the different stages of the replication vesicle maturation pathway engage in retrograde signaling with the nucleus to alter the cellular environment in favor of infection?

From our perspective, the observation that some interactions serve multiple different functions is interesting as it emphasizes how potyviruses can make the most efficient use of host resources. It appears that host proteins that bind to potyvirus RNA through their interactions with viral proteins have evolved to promote both viral protein synthesis and virion encapsidation. The connections between potyviral replication, translation, and particle formation merit further study as do the molecular mechanisms and the temporal and spatial regulation of encapsidation. In addition, the multitude of different replication vesicles in the context of potyvirus movement implies that they perform multiple tasks in aiding the spread of the infection in the plant. How the potyvirus genome is released from these vesicles and how it is fortified to infect the next cell require more research.

In summary, this review sheds light on various protein-protein interactions that aid potyvirus infection either by directly contributing to viral processes or by modifying the cellular environment to support the infection. Many open questions remain regarding how and in what contexts potyviral proteins interact with host factors and what their consequences to infection and host physiology are.

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