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Review

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Plant-virus-insect tritrophic interactions: insights into the functions of geminivirus virion-sense strand genes

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The genome of the plant-infecting viruses in the family Geminiviridae is composed of one or two circular single stranded DNA of approximately 2.7–5.2 kb in length. These viruses have emerged as the most devastating pathogen infecting a large number of crops and weeds across the continents. They code for fewer open reading frames (ORFs) through the generation of overlapping transcripts derived from the bidirectional viral promoters. Members of geminiviruses code for up to four ORFs in the virion-sense strand, and their gene expression is regulated by various *cis*-elements located at their promoters in the intergenic region. These viral proteins perform multiple functions at every stage of the viral life cycle such as virus transport, insect-mediated virus transmission and suppression of host defence. They impede the host's multi-layered antiviral mechanisms including gene silencing (at transcriptional and post-transcriptional levels) and hypersensitive response. This review summarizes the essential role of virion-sense strand encoded proteins in transport of viral genomes within and between plant cells, countering defence in hosts (both plants and the insects), and also in the ubiquitous role in vector-mediated transmission. We highlight the significance of their pro-viral activities in manipulating host-derived innate immune responses and the interaction with whitefly-derived proteins. We also discuss the current knowledge on virus replication and transcription within the insect body.

1. Introduction

The Geminiviridae family is one of the largest families of DNA viruses infecting numerous crops and weeds (dicots and monocots), and cause severe yield losses worldwide [1]. On the basis of pairwise sequence identities, genome organization (monopartite or bipartite), host range (monocot or dicot) and insect vector (phloem-feeding homopteran insects such as aphids, leafhoppers, treehoppers or whiteflies), this family is subdivided into nine genera [2]. Geminiviruses code for 4–7 open reading frames (ORFs) through the generation of multiple overlapping transcripts, which are under the tight regulation of bidirectional viral promoters located within their intergenic region (IR) [3]. Proteins encoded in the complementary-strand of the monopartite DNA A genome are involved in virus replication, control of gene expression and suppression of the host's defence machinery. The virion-sense strand encoded proteins are necessary for virus encapsidation, transport within and between plant cells, and insect-mediated long-distance transmission [4–7]. Similarly, the DNA B genome of bipartite begomovirus encodes two proteins (BV1 and BC1) that are exclusively used for inter- and intra-cellular virus movement and spread of infection [6,7]. Additionally, virion-sense strand encoded BV1 might be involved in suppressing host silencing defence, it interacts with ASYMMETRIC LEAVES 2 and a decapping enzyme to promote RNA decapping and messenger RNA (mRNA) turnover [8].

Geminivirus virion (22×38 nm) contains two incomplete icosahedral capsid particles, and each molecule of coat protein (CP) of these virions is bound

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by seven bases of viral single stranded DNA (ssDNA) as shown for a monopartite begomovirus [9]. Upon geminivirus infection, the uncoating of viral ssDNA takes place in the cytoplasm of the infected plant cell, followed by wrapping of the viral DNAs by the CP. These nuclear imported CP-bound viral DNAs undergo viral replication through rolling circle and recombination-dependent mechanisms [10]. The newly replicated viral genomes are then converted into double stranded DNA (dsDNA) for re-replication, or bound by viral proteins to assist in their short-distance (within the tissues) and long-distance (through the phloem) transport [5–7]. Because geminiviruses possess limited coding potential, they use a wide range of host proteins for virus replication, transcription, movement within and between hosts, and suppression of host defence responses [6,7]. This review summarizes the progress made in our understanding of the nature of viral promoters driving the expression of virion-sense strand ORFs, and the way these proteins counteract host antiviral responses and mediate plant-virus-vector tritrophic interactions. It also discusses how these viral proteins have evolved to mediate these responses in specific hosts that belong to different kingdoms.

2. Expression of virion-sense strand genes

(a) Virion-sense genes are transcribed through bidirectional viral promoters

The bidirectional promoters located in the IR of geminiviruses are necessary for controlling the viral gene expression. The studies from geminiviruses of different genera mapped these promoters between the 5′ ends of the first complementary- and virion-sense strand genes [11–14]. The virion-sense strand genes are not expressed at all times, but at late in the infection cycle. This is achieved by the virion-sense strand promoter that becomes active only at the late infection stage. A complementary-strand encoded transcriptional activator protein (TrAP) regulates this viral promoter by binding to a conserved late element (CLE) like GTGGTCCC. In a bipartite begomovirus, transcriptional start site of virion-sense strand transcripts is mapped at 30 and 35 nt downstream from a consensus TATA box [14,15]. In addition to highly efficient CP translation, small RNA sequencing studies indicate that this transcription unit is not a hotspot for RNA silencing [16,17], and hence can result in very high accumulation of CP in the infected cells.

The mRNAs generated from the virion-sense strand are under the tight transcriptional regulation of a promoter located in the IR of geminiviruses [18–20]. A single promoter in the *Curtovirus* is responsible for the expression of CP, V2 and V3 [21]. In *Mastrevirus*, Rep protein is required for the activation of V1 promoter, and stable expression of its V2 promoter showed vascular tissue-specific expression in dividing tissues rather than in mature cells [22,23]. Moreover, deletion of *cis*-regulatory elements i.e. GC box (of *Mastrevirus*) or 30 nt long CLE (of *Curtovirus* and *Begomovirus*) in the virion-sense strand promoter led to decreased promoter activity [24–27]. These *cis*-elements in the viral promoters probably regulate this tissue-specific gene expression. Such tissue-specific expression of CP might ensure efficient coating of the newly replicated viral genome to prepare for virus packaging and uptake by the insect vector.

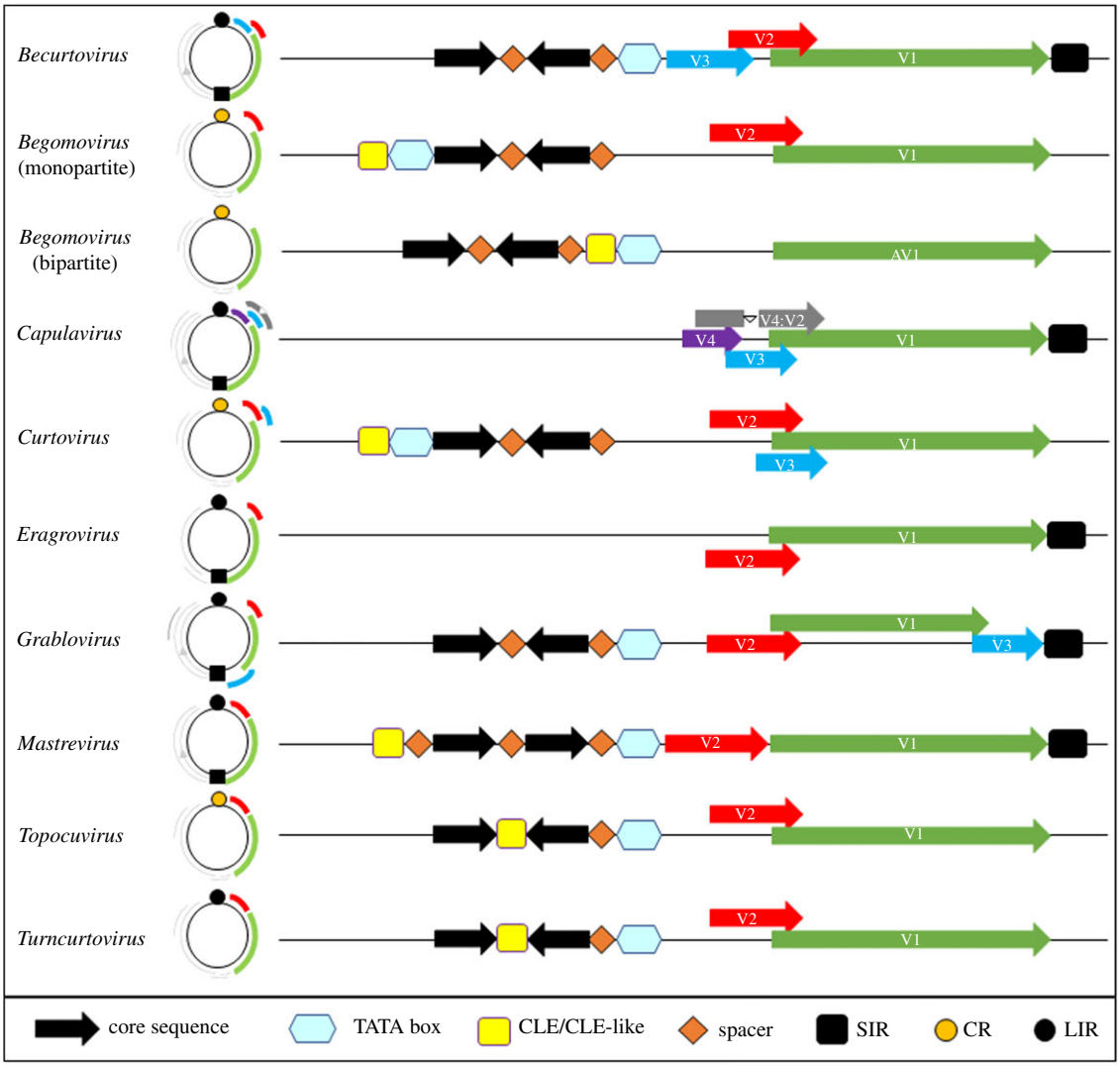
(b) Several *cis*-acting elements are necessary for transcriptional activator protein-responsiveness of viral promoters

In order to use the host transcription machinery efficiently, geminiviral promoters possess consensus *cis*-acting elements and initiator sequences typical of plant RNA polymerase II [6,13]. The promoter of virion-sense strand genes contains a conserved symmetric palindromic core sequence (ACTT-N_7-AAGT) separated by a spacer (N_7) sequence. Unlike the conserved GC-rich heptanucleotide spacer of 'Old World' begomoviruses, the spacer of 'New World' begomoviruses exhibited greater variability such as GGTCCCY or its variants with a single nucleotide diversity [26]. Similarly, the core sequence of *Curtovirus* is identified to be CCTT-N_7-AAGG. Among the members of different genera, *cis*-acting elements are located distinctly around this core sequence in the viral promoter (figure 1). Furthermore, CLEs distributed in the geminiviral promoters are suggested to be a target of TrAP [13,24,26]. Geminiviral TrAPs have been demonstrated to *trans*-activate (termed as TrAP-responsiveness) heterologous CP promoters belonging to same genera. A *Curtovirus* C2 was unable to complement *Begomovirus* AC2 function, indicating that this late gene activation requires compatibility between the activator and the responsive promoters [28,29]. The significance of variations in responsive virion-sense strand promoters might be the reason for such compatibility; however, further research is required to appreciate why they have genus-specific signatures.

The *cis*-elements encompassing CLE (5′-CGTCTAAGT GGTCCCGCA-3′) in the viral promoter are necessary for TrAP-responsiveness of several begomoviruses [14,26–29]. Nonetheless, CLE of all geminiviruses are not responsible for TrAP-responsiveness [30], which necessitates the identification of altered or additional TrAP-responsive elements. Interestingly, plant PEAPOD-2 protein binds directly to the TGMV-CP promoter, and also interacts with TrAP [31]. As PEAPOD-2 is a transcriptional repressor [32], this interaction might lead to de-repression of the CP promoter. It appears that this interaction between TrAP and CP promoter is multi-layered, and to understand the mechanism(s) behind it, it is necessary to decode TrAP/CLE-independent mechanism(s) employed by geminiviruses lacking TrAP in regulating late gene expression.

3. The voyage of virus inside the insect vector

The whitefly (*Bemisia tabaci*, a complex of cryptic species) serves as an exclusive vector to transmit begomoviruses in a circulative and persistent manner [5,33]. They can also transmit members of *Carlavirus*, *Crinivirus*, *Ipomovirus* and *Torradovirus* [34]. Virus transmission involves acquisition, retention and egestion by the insects. The insect stylets facilitate the entry of virions from the plant phloem sap (acquisition access period (AAP)). They then travel to the esophagus and the midgut through the food canal (figure 2). They are egested with the saliva into the phloem of new plants (inoculation access period (IAP)). The minimum AAP and IAP duration of various begomoviruses varied between 10 and 60 min [5]. These viruses can penetrate into the fat cells and ovaries; however, no viral transcripts/proteins are detected in these cells [35]. Hence the interactions of viral particles with such tissues are yet to be ascertained.



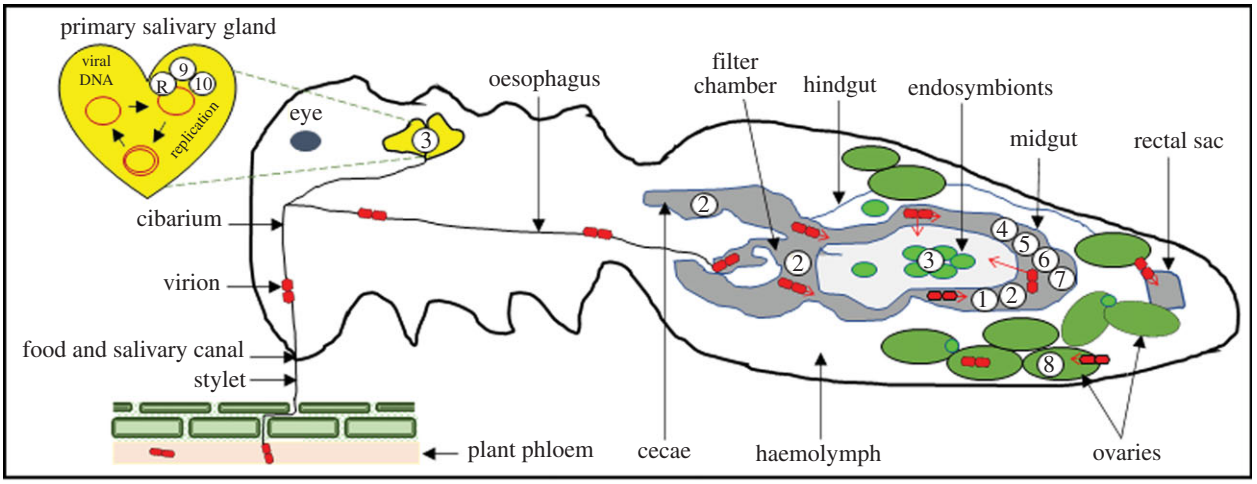
virion-strand proteins	biological functions
V1/CP	encapsidation, virus movement within the plant cells, insect-mediated virus transmission
V2	nucleo-cytoplasmic shuttling of virus, suppression of host-mediated antiviral silencing, modulating biochemical activities of Rep and C4 proteins, induction of hypersensitive response
V3	virus transport within the infected plant cell
V4	not yet demonstrated

Figure 1. The arrangement of various regulatory elements located in the viral promoters of geminiviral virion-sense strand genes. The circular viral genome is provided for each genus and the viral complimentary-sense strand genes in the genome are depicted by grey coloured lines. The names of viral genes and their biological functions are provided. The CLE, CR, LIR and SIR refer to conserved late element, common region, long intergenic region and short intergenic region, respectively. (Online version in colour.)

(a) Viral replication and transcription within the insect

The TYLCV genome replicates within the insect salivary glands through the interaction of viral Rep protein with whitefly's proliferating cell nuclear antigen and DNA polymerase δ [36,37]. It is tempting to speculate whether it can alter the insect cell cycle and induce an endoreplication cycle as in plants or has evolved alternate strategies in the insect. Not all begomoviruses appear to replicate in the whitefly [37], and hence additional efforts are needed to understand why only certain virus species replicate within the insect. The continuous virus replication might not be beneficial to the viruses, because this can induce insect autophagy and antimicrobial peptide production leading to virion destruction and the inhibition of prolonged virus replication [38]. The active viral gene transcription of some begomoviruses can occur within the insect [37–39]. Strikingly, an insect transcription factor promotes viral gene transcription by binding to a CACGTG motif at the IR of a begomovirus [40]. However, the molecular mechanisms involved and functional significance of this regulation in virus replication and/or transcription have not received much attention or has been difficult to study.

There is additional evidence to suggest that at least limited uncoating and transcription takes place inside the insects. For example, viral acquisition led to up- and downregulation of insect's defence genes [41–43]. Upon feeding, a saliva effector



interaction	localization of interacting proteins	biological functions
	coat protein (CP)	
1	collagen	virus transmission in the insect gut
2	cyclophilin B (CYPB)	virus movement in the filter chamber, cecae and midgut of whitefly
3	GroEL of endosymbiont	virus transport in the insect midgut and salivary gland
4	heat shock proteins (HSPs)	virus transmission in the insect midgut epithelial cells
5	midgut protein (MP)	virus movement in the insect gut epithelial layer
6	peptidoglycan recognition protein (PRP)	virus transport in the insect midgut
7	vesicle-associated membrane protein-associated protein B (VAPB)	virus transmission within the insect midgut
8	vitellogenin protein	transovarial virus transmission
	Rep protein (R)	
9	proliferating cell nuclear antigen (PCNA)	virus replication in the insect primary salivary glands
10	DNA polymerase δ (Pol δ)	virus replication in the insect primary salivary glands

Figure 2. The circulative and persistent transmission of begomoviruses by the whiteflies, and the interaction of begomovirus and whitefly proteins within the insect body. The entry of virions from the plant phloem sap is facilitated by the insect stylets. The filter chamber (a junction between the oesophagus and the gut) is the first site of begomovirus absorption into the insect haemolymph via endocytosis. After acquisition into the insect, the CP of the virions interacts with several insect proteins to cross midgut-haemolymph, the haemolymph-ovary and the haemolymph-salivary gland barriers for virus transmission within the insect body. Similarly, the viral Rep protein interacts with insect proliferating cell nuclear antigen and DNA polymerase δ proteins to aid virus replication in the insect's primary salivary glands. The virion movement is shown in red arrows. The insect figure is based on the template from Rosen *et al.* [5]. (Online version in colour.)

protein (Bsp9) interferes with a plant immune signalling cascade (MAPK-WRKY33) in *Arabidopsis* to promote whitefly performance, thereby enhancing TYLCV transmission [44]. On the contrary, the inhibitory effects of whitefly-derived tumorous imaginal discs (Tid) and vesicle-associated membrane protein-associated protein B (VAPB) on virus transmission have been demonstrated recently [45,46]. Any variations in the expression of genes/pathways involved in different whitefly species/pathotypes during feeding might provide clues about the host factors (either beneficial or harmful) involved in virus acquisition/retention. Such data also might explain why only certain strains are vectors of geminiviruses. Geminiviral nucleic acids can be possible targets of insect antiviral responses as demonstrated in animal/mammalian viruses [47,48]. It is tempting to speculate whether RNAi, JAK-STAT, Nf-κB, Imd and toll-like antiviral pathways present in the whitefly act on viral nucleic acids. If so, viral proteins must have a defensive role to play in insect tissues, and this will be of great interest. Uncovering the mechanistic details of such effectors help geminivirologists in devising newer antiviral approaches to break this virus-vector relationship.

(b) Coat protein is the mediator of virus-vector interactions

Virions have to successfully cross three insect barriers: the midgut-haemolymph barrier, the haemolymph-ovary barrier and the haemolymph-salivary gland barrier for its transmission [5]. To pass through such barriers, the only structural protein in the virion i.e. CP, must interact with whitefly-derived proteins. In agreement with this, several such candidates have been identified [5,7,44–46] (figure 2). Moreover, whiteflies are also known to possess several bacterial endosymbionts (such as *Arsenophonus*, *Cardinium*, *Fritschea*, *Hamiltonella*, *Portiera*, *Rickettsia* and *Wolbachia*) in the whitefly midgut that are implicated to influence virus transmission [5,49]. One such protein is the GroEL chaperone of endosymbionts that interacts with the viral CP [50]. It is quite likely that we have missed other interactions.

Understanding the evolution of whitefly endosymbiotic relationships offer tremendous opportunities to devise integrated pest management methods. Furthermore, much more focused progress is required in understanding the mechanistic interaction of whitefly (and other insect) proteins with viral proteins of different genera during virus transmission.

The variation in virus transmission efficiency and duration of virus persistence among different vector populations can be attributed to the type of bacterial endosymbionts, feeding behaviour, host preferences and genetic background of the insect population [51,52]. Any change in the CP residues might lead to gain or loss of whitefly-mediated virus transmissibility. In agreement with this, naturally evolved mutations in the CP of *Squash leaf curl China virus* resulted in altered virus transmission by different species of whiteflies [52]. The N-terminal region of CP is necessary for virus internalization into the insect primary salivary glands [53]. The interaction of TYLCV-CP (81–222 amino acid residues) with the whitefly egg vitellogenin facilitates transovarial virus transmission [54,55]. The CP region between 129 and 152 of *Abutilon mosaic virus* and *Tomato yellow leaf curl Sardinia virus* are crucial for the virus transport across the insect gut and haemolymph [35,56]. Key amino acid residues in the geminiviral CP which govern this virus-vector interaction needs extensive investigation. It appears that CP evolution might enable begomoviruses to adapt efficiently to the changing vector population.

4. Virion-sense strand encoded proteins are involved in virus trafficking

The virion-sense strand encoded proteins are demonstrated to be engaged in the shuttling of the viral genome within the infected cells. During virus infection, the entry of uncoated viral DNA into the nucleus of the infected cells is mediated by the CP [7,57,58]. The interaction of CP-coated viral DNA with the importin α or karyopherin α1 facilitates its entry into the nucleus [58,59]. The non-specific DNA binding ability supports the CP to sequester viral DNAs from the replication pool for viral packaging and transport to the neighbouring cells [57,60,61]. The bipartite begomovirus-encoded nuclear shuttle protein (NSP) recruits an acetyl transferase to acetylate viral DNA-wrapped CP to reduce its DNA binding affinity and favours the binding of NSP with the viral DNA to assist in nuclear export [60,61].

It appears that CP might have assistance from other viral proteins to help with its functions. The TYLCV-V2 protein interacts with CP and mediates the exportin α-dependent nuclear export of the viral genome [62]. It is anticipated that C4 protein delivers the nuclear exported viral genome to the cell periphery [63]. The *Begomovirus* V2 protein and vesicle-like structure of *Curtovirus* V3 protein assist in routing of viral DNA to the cell periphery via the endoplasmic reticulum [64,65]. Moreover, C4 and V2 proteins cooperate with the CP to increase the size-exclusion limit of plasmodesmata to gain entry into the neighbouring cells [7,66]. These reports highlight the importance of CP and other viral proteins in virus trafficking within and between the plant cells. However, how CP being the most conserved protein is specific to geminiviruses that have divergent sequences is not explored in sufficient depth.

5. V2 proteins suppress host antiviral RNA silencing at multiple levels

(a) V2 protein interferes with post-transcriptional gene silencing machinery

RNA interference (RNAi) is a versatile host-induced innate defence mechanism to target mRNAs either through degradation of target mRNAs or through target DNA methylation [67]. Sequence-specific targeting of viral genome occurs both at the transcriptional (TGS) and post-transcriptional (PTGS) levels. The structured RNAs and viral dsRNAs are processed by Dicer-like enzymes encoded by the plants to generate 21–24 nt small interfering RNAs (siRNAs). These siRNA duplexes are then incorporated into plant-encoded Argonautes (AGOs) to form ribonucleoprotein complexes to silence the target genes [67]. Most of the geminivirus-encoded proteins have evolved to serve as viral suppressors of RNAi (VSRs) to evade this antiviral immunity [6,68].

The V2 proteins of several geminiviruses function as VSRs by interfering at multiple steps of the RNAi pathway [69–73]. The TYLCV-V2 protein evade PTGS by interacting with plant SUPPRESSOR OF GENE SILENCING 3 (SGS3) protein [72,73] (figure 3). This V2-SGS3 interaction could inhibit the binding of SGS3 with RNA dependent RNA polymerase 6 (RDR6), thereby plausibly preventing SGS3 from accessing its substrate (viral dsRNAs with a 5′ overhang). Such interactions blocked the generation of secondary siRNAs to reduce targeting of viral transcripts [74]. Not all geminiviral V2 proteins appear to interact with SGS3. For example, *Tomato yellow leaf curl China virus* (TYLCCNV)-V2 protein did not interact with SGS3, however it suppresses PTGS by sequestering 21 nt ds-siRNAs, 24 nt ds-siRNAs and 24 nt ss-siRNAs [75]. Therefore, V2 protein impedes PTGS either by reducing the amplification of the local silencing (through competitive SGS3 interaction) or by deterring the spread of systemic silencing signal (by sequestering viral siRNAs).

(b) V2 is also capable of inhibiting transcriptional gene silencing

Plants employ DNA methylation as an epigenetic antiviral response to defend themselves against geminiviral infections [6,76]. The DNA methylation in plants is mostly achieved by siRNA-directed DNA methylation (RdDM) to induce TGS of viral promoters [68,77]. As geminiviruses replicate in the nucleus, so it can be an inducer as well as a target of host epigenetic modifications. The TYLCV-V2 protein inhibits viral DNA methylation by interacting with the host Histone Deacetylase 6 (HDA6) without affecting its deacetylation activity. However, this interaction hinders the recruitment of DNA methyltransferase 1 (MET1) by HDA6 [71]. The CLCuMuV-V2 protein inhibits TGS by interacting with the PAZ domain of AGO4, and disrupts AGO4 binding to the viral genome [78]. This V2-AGO4 interaction occurs at the Cajal body, an assembly point where the components of RdDM machinery (such as Pol IV, siRNAs, AGO4) localize [79]. There are also other ways by which V2 can function, such as *Tomato leaf curl New Delhi virus* (ToLCNDV)-AV2 protein selectively interferes with host antiviral RDR1-mediated symptom recovery in tobacco [80]. It is evident that geminivirus V2 proteins interfere with various components (AGO4, HDA6/MET1,

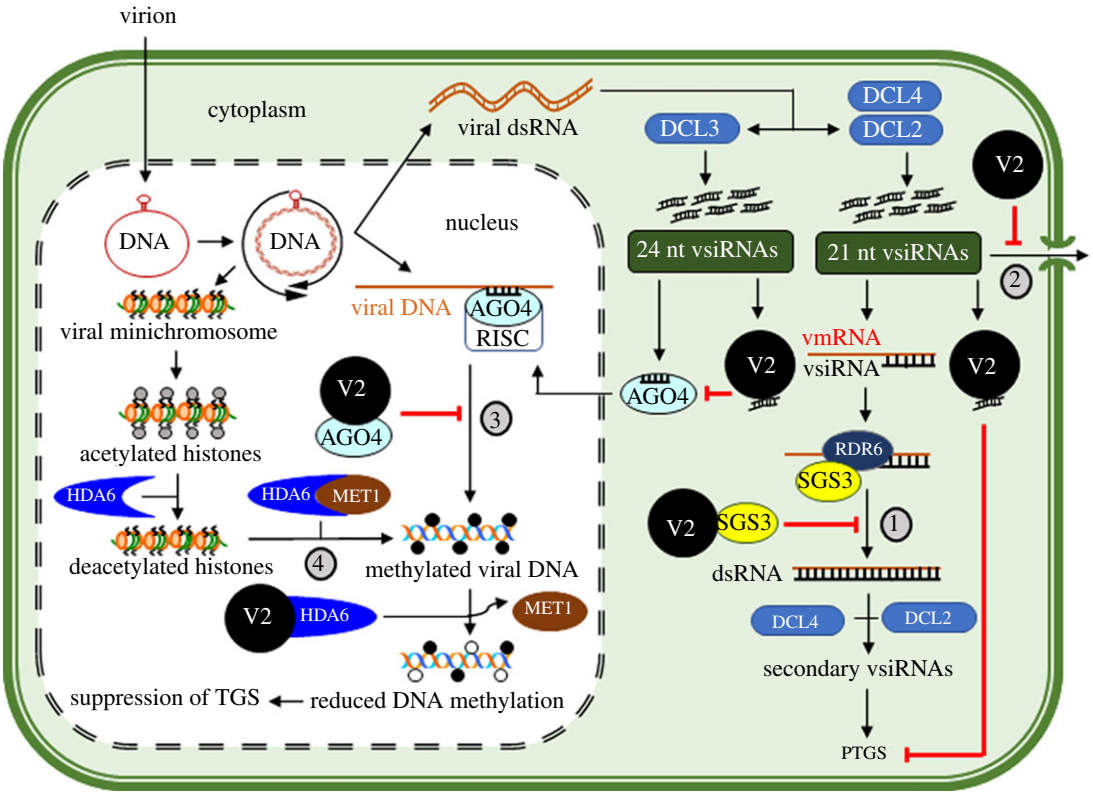


Figure 3. Geminivirus-encoded V2 protein perturbs multi-layered antiviral TGS and PTGS responses in multiple layers. The dsRNAs generated from the viral genome are processed by plant DCLs into 21 or 24 nt viral siRNAs. V2 protein impedes PTGS either by reducing the amplification of the local silencing (through competitive SGS3 interaction) (1) or by deterring the spread of systemic silencing signal (by sequestering vsiRNAs) (2). V2 protein binds to AGO4 protein (3) and also hinders the interaction of HDA6 with MET1 (4). Such interactions result in reduced viral DNA methylation and suppression of antiviral TGS. The cytosine methylation on viral DNA is shown in the filled circles. The viral mRNAs and viral siRNAs are indicated as vmRNAs and vsiRNAs, respectively. (Online version in colour.)

RDR1) of TGS machinery to perturb host-mediated antiviral strategies (figure 3). Consequently, multiple approaches employed by geminiviruses targeting these antiviral processes make it difficult to devise any antiviral strategies in combating the diseases caused by them.

6. V2 protein modulates the enzymatic activity of other geminiviral proteins

Geminivirus-encoded proteins are widely demonstrated to perform multiple functions to complete their life cycle. Therefore, the deletion of such genes is expected to have obvious adverse impact on the virus infection cycle. Reduced viral DNA accumulation was detected in the plants inoculated with ToLCNDV lacking AV2, suggesting its essential role in viral pathogenesis [80,81]. The MYMIV-AV2 and CLCuKoV-V2 proteins were demonstrated to enhance the biochemical activities of viral AC1 and C4 proteins, respectively [82,83]. On the contrary, the MYMIV-CP possibly regulates the viral replication by affecting the nicking and closing activities of AC1 protein [84]. These studies emphasize the importance of cooperation between the V2 protein and other begomoviral proteins in viral pathogenesis.

7. Implication of V2 protein in host hypersensitive response

Any incompatible interaction between a plant and a pathogen can induce a hypersensitive response (HR) or systemic acquired resistance to cease the spread of infection [85]. Intriguingly, geminiviruses have evolved to encode proteins to successfully overcome this host defence mechanism [7]. The N-terminal 10 amino acids of a bipartite begomovirus-AV2 protein promotes necrosis by inducing salicylic acid pathway-related defence gene expression in *Nicotiana benthamiana*, a model plant [86]. Similarly, C-terminal 58 amino acids of ToLCJV-V2 protein is required for HR induction [70]. A conserved putative protein kinase C phosphorylation motif is found to be responsible for HR induction [87]. Interestingly, transient co-expression of TrAP abolishes this V2-induced cell death response [88], indicating the presence of additional layers of complexity. However, further studies are required to elucidate the capability of V2 protein in altering this defence response upon virus infection in its natural hosts.

8. Summary

Geminiviridae is one of the largest family of plant-infecting viruses causing severe diseases in numerous plant species. The multiple transcripts generated from the virion-sense strand are driven by the bidirectional promoters located at the viral intergenic regions. Extraordinary details of functions of these proteins and their regulatory elements have been identified for some viruses. Some of these details have helped in generating novel tools to overcome viral infections in model and crop plants. However, the types of *cis*-acting elements distributed in the promoters of genera other than *Begomovirus* and *Mastrevirus* need to be explored in detail. Indeed, understanding the biological significance of less

explored virion-sense strand proteins (V3 and V4) in a comprehensive manner will be helpful in delimiting the processes involved in systemic virus infection. Unfortunately, evidence is lacking to demonstrate any insect proteins for being a viral receptor. Availability of the sequence of the whitefly genomes has raised the expectation many folds to decode functions of novel insect genes in the virus life cycle. The integration of focused metabolomics, along with transcriptomics and proteomics, might unearth the specific metabolites that may possibly influence or deter vectors. Such studies can provide further insights into the factors involved in the coevolution of geminiviruses and its vectors. Certainly, fulfilling the existing knowledge gap in the functioning of these proteins during virus infection cycle is necessary to discern how geminiviruses cause diseases, and to design new ways for virus control.

A few long-standing questions to decipher geminivirus infection cycles that will aid deployment of next generation antiviral tools are:

(i) how does CP facilitate virus uncoating in the infected plant cells? Did plants evolve mechanism(s) to hinder uncoating of viruses?
(ii) why are all geminiviruses are not replicating within the insect cells?
(iii) do viral transcripts translate inside the insect body? If yes, how do viral proteins manipulate insect proteins for these functions?
(iv) what are the biological functions of virion-sense strand encoded V3 and V4 proteins?
(v) does insect vector possess interferon and/or RNAi antiviral responses?
(vi) how do secondary metabolites in plants mediate plant-virus-vector tritrophic interactions?
(vii) what is the basis of specificity in insects to transmit specific geminiviruses?
(viii) which insect protein(s) act as virus receptor(s) during virus acquisition? and
(ix) can viruses transmit between insects and pass through generations?

Authors' contributions. R.V.K.: conceptualization, design and writing. P.V.S.: conceptualization, design, funding and revisions.

Competing interests. We declare we have no competing interests.

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