Geminiviruses: masters at redirecting and reprogramming plant processes

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Abstract | The family *Geminiviridae* is one of the largest and most important families of plant viruses. The small, single-stranded DNA genomes of geminiviruses encode 5–7 proteins that redirect host machineries and processes to establish a productive infection. These interactions reprogramme plant cell cycle and transcriptional controls, inhibit cell death pathways, interfere with cell signalling and protein turnover, and suppress defence pathways. This Review describes our current knowledge of how geminiviruses interact with their plant hosts and the functional consequences of these interactions.

Diesease complexes
A mixture of viral species, isolates and DNA satellites that together cause a disease. The genomic sequences of viral species differ by ≥89%, whereas isolates show ≤89% sequence variation.

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Geminiviruses, named for their twinned icosahedral particles, infect food and fibre crops, ornamental plants and weeds and cause substantial crop losses around the world. The incidence and severity of geminivirus diseases has greatly increased in the past 20 years[1,2]. In Africa and Asia, where geminivirus disease greatly affects agriculture, maize streak disease, cassava mosaic disease and cotton leaf curl disease have caused complete losses in infected fields[3–5]. Tomato yellow leaf curl disease is one of the major viral diseases of tomato worldwide[6].

Geminiviruses often occur in disease complexes, and individual plants can be infected with multiple viruses[7]. Geminivirus genomes can undergo high levels of mutation, recombination and reassortment to increase viral diversity[8–11]. The development of insecticide resistance and the evolution of new vector biotypes, in particular whiteflies, have allowed geminiviruses to invade new regions and to bring together new combinations of viruses in disease complexes[2]. These properties have enabled geminiviruses to adapt rapidly to new hosts and environments. This and the global spread of geminivirus complexes by human activity and severe weather now pose major threats to food security[12–14].

Geminiviruses have small DNA genomes with limited coding capacities. They rely heavily on host cellular machineries and interact with a wide range of plant proteins and processes during infection. Geminiviruses reprogramme the cell cycle of infected cells to induce the replication of both viral and plant chromosomal DNA. They change host gene expression patterns, inhibit cell death pathways, alter macromolecular trafficking and interfere with cell signalling and protein turnover to redirect or block host defences and hormone signalling. In addition, geminiviruses encode multiple silencing suppressors that interfere with plant small interfering RNA (siRNA) production and alter plant DNA methylation and microRNA (miRNA) pathways, often causing developmental abnormalities. Here, we review the recent progress made in understanding geminivirus–plant interactions and their consequences on viral infection and propagation. We highlight how a small number of geminivirus proteins interacts with and modulates host proteins to alter a large array of plant developmental and defence processes. Supplementary information S1 (table) lists the known geminivirus–plant interactions and the viral and plant species for which the interactions have been demonstrated.

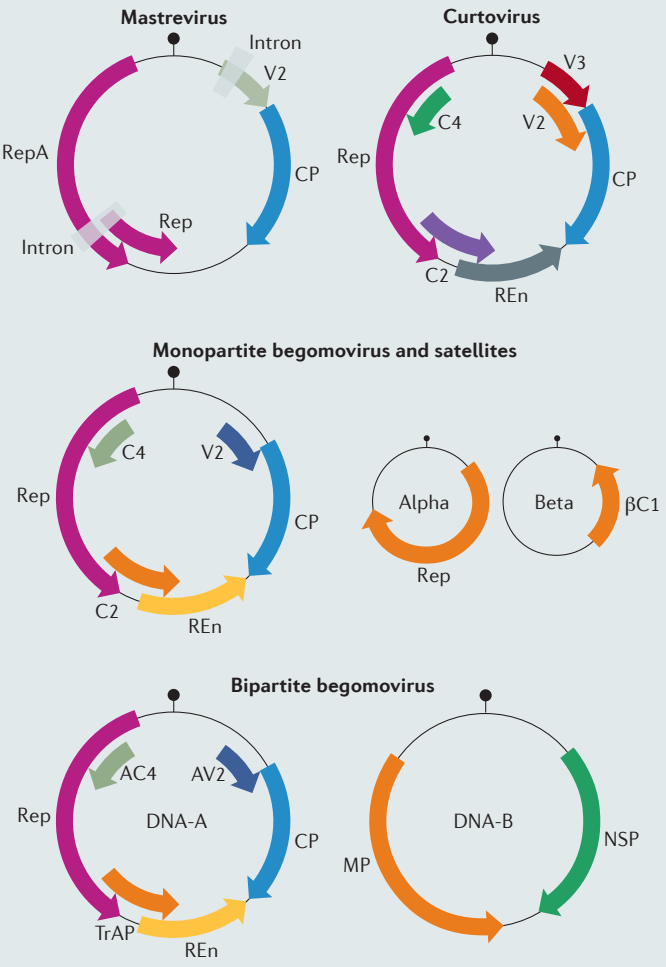
Virus–host interactions and models to study them

Geminiviruses are classified by the International Committee on Taxonomy of Viruses into seven genera (Begomovirus, Mastrevirus, Curtovirus, Becurtovirus, Eragrovirus, Topocuvirus and Turncurtovirus) on the basis of their genome organization and insect vectors. All geminivirus genomes occur as single-stranded DNA (ssDNA) that is packaged into virions[15] and replicative double-stranded DNA (dsDNA) that is transcribed in the nucleus of infected plant cells[16]. BOX 1 illustrates the genomes for three geminivirus genera. Their genomes consist of one (monopartite) or two (bipartite) DNA components that encode 5–7 proteins involved in viral replication, movement, transmission and pathogenesis. Some viral proteins, such as replication initiator protein (Rep), are highly conserved across the family *Geminiviridae*[17], whereas others, such as coat protein (CP; which determines insect vector specificity[18]), confer

Box 1 | **Geminivirus genomes and viral proteins**

The family *Geminiviridae* includes three well-characterized genera: Mastrevirus, Curtovirus and Begomovirus[16]. Mastreviruses are transmitted by leafhoppers, have a single genome component, infect both monocotyledonous and dicotyledonous plants, and are found primarily in the Old World. Curtoviruses are also transmitted by leafhoppers and have one genomic DNA, but infect only dicots in the New World. Begomoviruses, which constitute the largest genus, are transmitted by whiteflies and are found in the Old and New World. They can have monopartite genomes or bipartite genomes designated as DNA-A and DNA-B. Many monopartite begomoviruses are associated with alphasatellites or betasatellites.

Geminivirus genomes (see the figure) are arranged with divergent transcription units, and a 5′ intergenic region contains the origin for rolling-circle replication (the lollipop) and two RNA polymerase II promoters[121]. Coat protein (CP) forms the viral capsid and mediates vector transmission[18]. CP also functions as the nuclear shuttle protein (NSP) for monopartite viruses[122]. All monopartite and some bipartite viruses encode small ORFs upstream of the *CP* gene. The V2 and AV2 proteins function as anti-defence proteins to inhibit post-transcriptional gene silencing (PTGS)[112,114]. V2 also provides the movement function for monopartite viruses[122]. Replication initiator protein (Rep) initiates viral replication[121]. Mastreviruses express Rep from a spliced mRNA and RepA from the 5′ ORF[123]. Curtoviruses and begomoviruses encode Rep in a single ORF and do not encode RepA. Curtoviruses and begomoviruses encode three additional ORFs. Transcriptional activator protein (TrAP; and the related C2 protein) interferes with transcriptional gene silencing (TGS) and PTGS[40,84]. TrAP is also a transcription factor required for CP and NSP expression by bipartite begomoviruses[124]. Replication enhancer protein (REn; also known as C3) is involved in viral replication[32]. C4 (or AC4 in some viruses) counteracts PTGS[113,114]. Bipartite begomoviruses encode their movement proteins, NSP and MP, on the DNA-B component[23,24]. Betasatellites of begomoviruses encode βC1, which counteracts TGS[104], and alphasatellites encode their own Rep, which is also an anti-silencing protein[125].



unique properties to a given genus. The viral proteins are multifunctional, and some have evolved to serve different functions for different viruses even between closely related species. Many begomoviruses associate with satellite DNAs that encode proteins which enhance pathogenesis[19]. The major functions of the viral proteins are summarized in BOX 1.

Begomoviruses, which constitute the largest genera, initiate infection when a whitefly carrying the virus feeds on the sap transported through the phloem of a healthy leaf and transmits virions to phloem-associated cells (FIG. 1). In the plant cell, viral ssDNA is released from the virion and becomes double stranded when host DNA polymerases use RNA oligonucleotides to prime complementary-strand synthesis[20,21]. The dsDNA is transcribed by host RNA polymerase II, allowing the production of Rep. This protein initiates viral replication, which occurs by a combination of rolling-circle replication and recombination-dependent replication[22]. Nascent circular ssDNA can be converted to dsDNA to re-enter the replication cycle or can be packaged into virions after CP is produced. The infection is propagated inside the plant by the movement of viral DNA out of the nucleus into the next cell or the phloem through the action of two viral movement proteins, nuclear shuttle protein (NSP) and movement protein (MP)[23,24] (BOX 2).

Geminivirus infection is associated with plant stunting and a failure of reproductive organs to develop normally. Symptoms typically include curled, deformed leaves with a yellow mosaic or mottled pattern, and sometimes vein swelling and enations[25]. The symptoms reflect extensive changes in host transcription that lead to alterations in cellular homeostasis and developmental processes. The global nature of these changes is illustrated by transcriptome profiling of infected plants, which identified thousands of differentially expressed genes involved in diverse processes ranging from defence and programmed cell death to DNA replication and cell cycle control[26,27]. Infection also leads to the misregulation of host miRNAs linked to developmental transitions and hormone signalling[28,29]. The interactions of geminiviruses with their insect host is less well understood, but recent studies indicate that in this case too virus-mediated changes in signalling and defence pathways occur (BOX 3).

Plant DNA synthesis and cell cycle machinery

DNA replication occurs in three phases: initiation, elongation and termination. Geminivirus Rep catalyses initiation and termination of rolling-circle replication by cleaving and ligating viral DNA at a conserved site within the viral genome[30]. Similarly to many small DNA viruses, geminiviruses do not encode their own DNA polymerases and instead depend on host polymerases and associated factors (together termed the host replisome) for viral DNA synthesis during the elongation step[31]. In healthy plants, the availability of the host replisome is tightly regulated by cell cycle and developmental controls, which must be reprogrammed before geminiviruses can replicate their genomes.

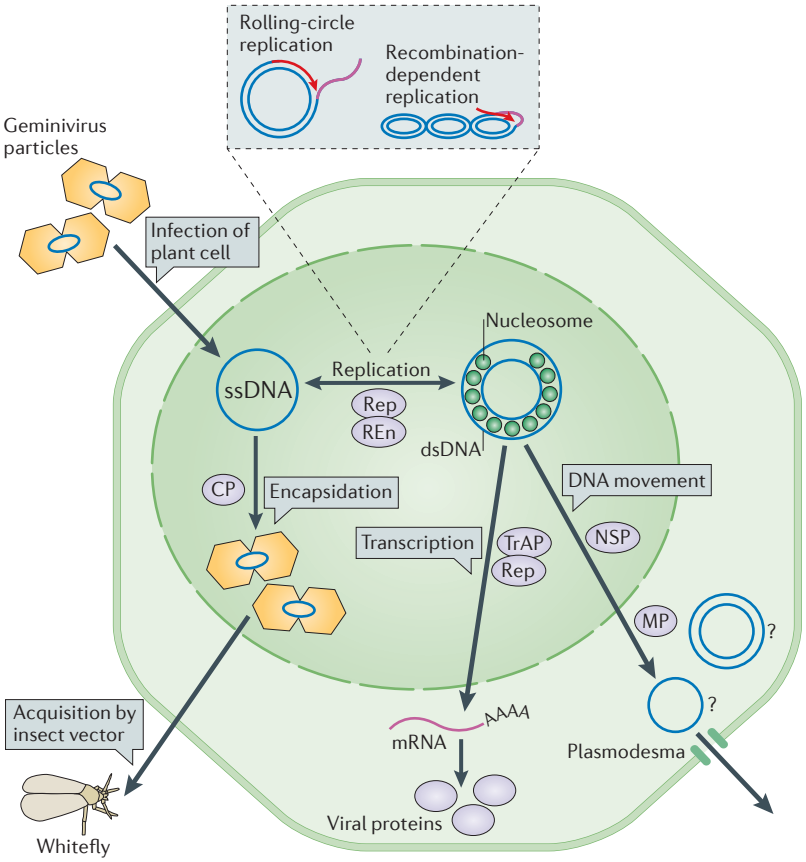


Figure 1 | **The begomovirus life cycle.** Infection begins in a plant cell when viral single-stranded DNA (ssDNA) is released from virions and copied to generate double-stranded DNA (dsDNA). The dsDNA, which assembles with nucleosomes, is transcribed by host RNA polymerase II, allowing production of replication initiator protein (Rep). Rep initiates rolling-circle replication by introducing a nick into a viral dsDNA molecule to generate a free 3′-hydroxyl end that primes ssDNA synthesis, leading to displacement of the parental strand (inset). The released ssDNA is converted to dsDNA to re-enter the replication cycle. Viral replication transitions to recombination-dependent replication, which is initiated by homologous recombination between a partially replicated ssDNA and a closed, circular dsDNA to form a looped molecule that serves as a template for both ssDNA and dsDNA synthesis (inset). Later in infection, Rep represses its own transcription, leading to activation of transcriptional activator protein (TrAP) expression, which in turn activates coat protein (CP) and nuclear shuttle protein (NSP) expression. Circular ssDNA can then be encapsidated by CP into virions, which are available for whitefly acquisition. NSP binds to viral DNA and moves it across the nuclear envelope, where movement protein (MP) traffics it across a plasmodesma. It is not known whether viral DNA moves as ssDNA versus dsDNA or as a linear versus a circular molecule.

Vector
An insect that acquires virions during feeding on an infected plant and subsequently transmits the virions to a healthy plant during feeding.

Satellite DNAs
A DNA or RNA episome that affects disease aetiology and depends on a virus for its replication and/or transmission.

Phloem
The plant tissue responsible for the transport of nutrients.

The viral replisome. Rep, the only viral protein that is essential for replication, is likely to have a key role in the recruitment and assembly of the viral replisome, a complex that includes viral proteins and host factors involved in DNA replication, repair and other nuclear functions. The viral replication enhancer protein (REn; also known as C3), which greatly enhances begomovirus and curtovirus DNA accumulation and interacts with Rep and host replication factors[32], is also likely to be part of the viral replisome. Both Rep and REn bind to proliferating cell nuclear antigen (PCNA)[33,34], the processivity factor for host DNA polymerase-δ. PCNA is highly conserved across eukaryotes and interacts with a variety of proteins involved in cell cycle regulation, DNA replication and DNA repair. Rep also interacts with the large subunit of the replication factor C complex, which loads PCNA onto DNA, and the 32-kDa subunit of replication protein A, which binds ssDNA[35,36]. In addition, Rep binds to RAD54, which is involved in homologous recombination and might have a role in viral replication mediated by recombination-dependent replication[37]. Interactions with RAD54 and PCNA have opposite effects on Rep activity *in vitro* and potentially modulate rolling-circle replication and recombination-dependent replication *in vivo*[34,37].

Geminivirus dsDNA forms a minichromosome with 11–12 nucleosomes[38]. Rep binds histone H3 (REF. 39), and this interaction might be involved in displacing nucleosomes from viral DNA to allow access to the replication machinery and/or prevent methylation of H3 lysine 9 (this methylation is thought to impair viral replication)[40]. Rep also binds a mitotic kinesin[39] and minichromosome maintenance protein 2 (MCM2)[41], which is a subunit of the MCM complex (the eukaryotic replicative DNA helicase). The functions of these Rep interactions during viral replication are not known.

Reprogramming plant cell cycle controls. Geminiviruses typically infect leaf cells or vascular tissues that have exited the cell cycle and do not express host DNA polymerases. To overcome this barrier, geminiviruses alter host transcriptional controls to induce the production of the host DNA synthesis machinery[31]. This was first demonstrated for PCNA, which accumulates specifically in virus-positive cells of infected leaves[42,43]. Host transcriptome profiling showed that geminivirus infection preferentially activates cell cycle-associated genes expressed during S/G2 phase and inhibits genes that are active in M/G1 phase[26]. Several core cell cycle genes associated with cell cycle re-entry and the late G1, S and early G2 phases are upregulated, whereas those linked to the early G1 and late G2 phases are downregulated, thereby facilitating the transition of infected cells into S phase — the stage at which DNA replication occurs during the cell cycle. In plants, activation of DNA replication and core cell cycle genes is unique to DNA viruses belonging to the families *Geminiviridae* and *Nanoviridae* (the latter being another important family of plant viruses with ssDNA genomes that are replicated by host DNA polymerases)[26,44].

A key regulator of the plant cell cycle is retinoblastoma-related protein (RBR). Similarly to its animal counterpart, plant RBR controls the cell cycle, stem cell maintenance, cell specification and differentiation[45]. RBR interacts with E2F transcription factors to suppress the expression of genes encoding host replication proteins. During a normal cell cycle, RBR is regulated by phosphorylation, which disrupts E2F binding and leads to transcription of E2F-target genes in late G1 phase in preparation for S phase[45]. Inactivation of RBR to allow pre-emptive entry into S phase is a conserved feature of many small DNA viruses that infect plant and animal hosts.

Geminiviruses disrupt RBR–E2F complexes through RepA, Rep and REn binding to RBR[46,47] (FIG. 2). RepA, which is characteristic of mastreviruses (BOX 1), contains

Box 2 | Geminivirus movement and host proteins

The geminivirus proteins nuclear shuttle protein (NSP) and movement protein (MP) mediate viral DNA movement into and out of the nucleus and between cells[126]. Most of our knowledge of NSP and MP comes from studies of bipartite geminiviruses, and several host partners have been identified for them (see Supplementary information S1 (table)). Less is known about the movement proteins of monopartite geminiviruses, in which coat protein (CP) acts as the NSP, whereas MP function is mediated by V2 alone or in a complex with C4 (REF. 127).

NSP interacts with histone H3, raising the possibility that viral DNA moves as a minichromosome[128]. NSP also binds to and inhibits an *Arabidopsis thaliana* acetyltransferase (AtNSI)[129]. One model is that viral double-stranded DNA is packaged into nucleosomes and further compacted by NSP binding to the amino-terminal tail of H3 (REF. 128). Compaction might be enhanced by NSP-mediated suppression of histone acetylation by AtNSI[130]. H3 also interacts with MP and has been detected in plasmodesmata of infected cells[128], suggesting that viral DNA moves between cells in association with nucleosomes. Some geminiviruses form ER tubules in sink tissue, and these tubules might accommodate a compacted minichromosome[131].

An NSP-interacting GTPase (NIG) associated with the exterior of the nuclear envelope might facilitate NSP transit into the cytosol, probably through the nuclear pore[132]. The NSP–DNA complex then moves to the cell periphery through interaction with MP[24]. Viral DNA might be transferred to MP through a mechanism involving NIG-catalysed GTP hydrolysis[133]. Alternatively, NIG might facilitate the interaction of MP with an NSP–DNA complex that moves through plasmodesmata, which provides a mechanism for movement of viral DNA into the nucleus of the next cell. MP interacts with a chloroplast heat shock cognate 70 protein (HSC70) and with a synaptotagmin protein (SYTA)[134,135]. Downregulation of both proteins restricts or delays infection[71,134,135], suggesting that geminiviruses recruit host transport systems for their movement[126].

a canonical LXCXE RBR-binding motif that is also present in oncoproteins of mammalian viruses and the nanovirus cell cycle link (Clink) protein[44,48]. By contrast, begomovirus and curtovirus Rep proteins bind RBR through a unique motif[49]. Mutation of these motifs in RepA and Rep results in milder symptoms and reduced viral DNA accumulation[46,50]. In both cases, the distribution of virus-infected cells changes, and the mutant viruses are more closely associated with vascular bundles than wild-type viruses. REn–RBR binding might be involved in overcoming RBR inhibition in mature leaves[51,52]. REn also interacts with *Solanum lycopersicum* NAC1 (NAC1), which is a host transcription factor that accumulates in virus-positive cells of infected leaves. Ectopic expression of the target gene of NAC1 increases viral DNA levels[53].

The endocycle and viral DNA replication. During early leaf development, cells are programmed to undergo a mitotic cell cycle in which S phase is coupled to mitosis. Later in development, many leaf cells transit to an endocycle, a variation of the cell cycle that is characterized by increased ploidy and cell expansion without division. Unlike mammalian DNA tumour viruses, geminiviruses generally do not induce cell proliferation. Instead, many geminiviruses and nanoviruses induce plant cells to re-enter the endocycle and replicate both viral and plant chromosomal DNA[26,44,54]. Other geminiviruses induce the mitotic cycle but cause the cell to arrest in prophase[55], and some cause vein swelling and enations, which are indicative of the mitotic cell cycle[56,57]. The different types of geminivirus interactions with the plant cell cycle are shown in FIGURE 2.

As mentioned above, interactions with RBR seem to be conserved between geminiviruses, but there are varied consequences of these interactions on G1 cyclins and their cyclin-dependent kinase (CDK) partners, which act downstream of RBR to control the transition into S phase. For example, an endocycle-inducing begomovirus reduces expression of cyclin D3 family members, which regulate CDKs during G1 phase and inhibit the endocycle; ectopic expression of a cyclin D3 leads to resistance against the same virus[26]. By contrast, a curtovirus C4 protein that induces hyperplasia activates the degradation of cyclin kinase inhibitors, thereby promoting mitosis[58]. Differences in the interactions of viral proteins with host cell cycle controls determine whether a particular virus activates the endocycle or the mitotic cycle, but these interactions might also be influenced by the type of plant cell in which they occur, with different cell types in the same leaf responding differently[44,46,50,55]. They might also be influenced by satellite proteins such as βC1, which alters leaf developmental controls to induce cell division[59].

Rolling-circle replication
A DNA replication mechanism that starts with nicking of one strand of a double-stranded DNA template and that can produce multiple single-stranded DNA copies.

Recombination-dependent replication
A DNA replication mechanism that depends on the recombination of homologous DNA sequences and leads to the production of single-stranded and double-stranded DNA copies.

Enations
Leaf-like structures that form on leaves during some viral infections.

Box 3 | Geminivirus interactions with their insect vector

Much of our knowledge of geminivirus–vector interactions comes from studies of begomoviruses and their *Bemisia tabaci* (whitefly) vector. Whiteflies acquire virions during feeding on the phloem of an infected plant. The virions move through the alimentary canal into the whitefly midgut, where they enter the haemolymph and transit to the salivary glands for transmission during the next feeding cycle[136]. Viral coat proteins (CPs) bind to GroEL proteins encoded by endosymbionts in the whitefly gut[137] and to the whitefly-encoded heat shock protein 16 (HSP16)[138]. Interactions with GroEL and HSP16 might stabilize the virion during passage through the gut and/or facilitate its transfer across the gut epithelia into the haemolymph. Both possibilities are consistent with data showing that GroEL isoforms produced by different endosymbionts affect transmission efficiency[139].

Begomovirus–whitefly interactions depend on the virus, the vector biotype and the endosymbionts of the vector[139]. Transcriptome analysis of virus-carrying and non-carrying whiteflies uncovered more than 1,600 genes that are differentially expressed in response to begomovirus acquisition[140], representing many different pathways, including the cell cycle, protein synthesis and lipid metabolism. Genes involved in the immune response, including all of the autophagy genes and most genes associated with lysosome function, are activated in virus carriers. By contrast, genes involved in apoptosis and signal transduction of the immune response are downregulated. These results are consistent with whiteflies mounting a defence against begomovirus invasion and the virus counteracting this activation of the immune response. The balance of these two forces might differ for different begomoviruses and whitefly biotypes, providing an explanation for the differences observed in transmission efficiency and vector specificity.

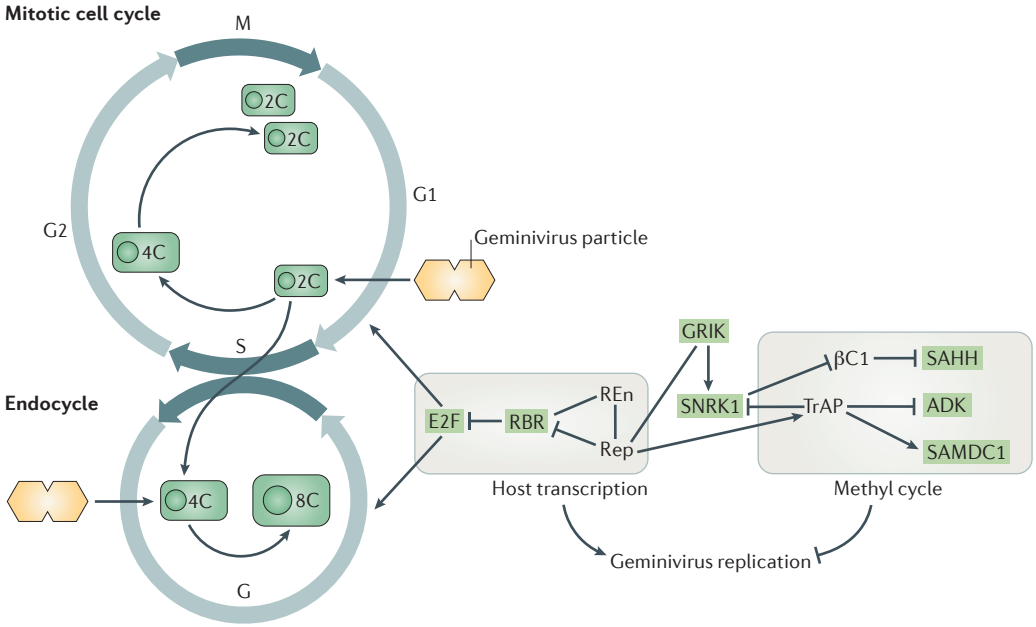


Figure 2 | **Reprogramming plant cell cycle and methyl cycle controls.** The diagram shows virus–host interactions that are necessary to create a cellular environment that is favourable for geminivirus DNA replication. Geminiviruses can infect plant cells in the G1 phase (2C DNA content) of the mitotic cycle or in the G phase of the endocycle (when the cell has a 4C DNA content) and induce them to enter the S phase. Replication initiator protein (Rep) and replication enhancer protein (REn) interact with and inhibit retinoblastoma-related protein (RBR) to relieve inhibition of E2F transcription factors and activate the expression of plant genes encoding host DNA polymerases and accessory factors that are required for viral replication. These interactions reprogramme cell cycle controls and induce mature plant cells to progress through the endocycle or the mitotic cell cycle. Rep also activates the expression of transcriptional activator protein (TrAP; known as C2 in some viruses), which interacts with adenosine kinase (ADK) and *S*-adenosyl methionine decarboxylase 1 (SAMDC1) to inhibit the plant methyl cycle. The protein βC1 also interferes with the methyl cycle through its interactions with *S*-adenosyl homocysteine hydrolase (SAHH). Suppression of the methyl cycle facilitates geminivirus replication by reducing viral DNA methylation. The geminivirus Rep-interacting kinase (GRIK)–SNF1-related protein kinase 1 (SNRK1) protein kinase cascade links Rep to suppression of the methyl cycle. Figure is modified, with permission, from REF. 26 © (2008) American Society of Plant Biologists.

Genotoxic stress. Transcriptome profiling revealed that geminivirus infection upregulates the expression of host genes associated with genotoxic stress, including genes encoding DNA repair and recombination proteins[26]. This upregulation might occur in response to nicked viral DNA and ssDNA, which could be perceived as damaged DNA. The recruitment of the host DNA recombination machinery also enables viral amplification mediated by recombination-dependent replication[22]. In one possible scenario, very early events in infection cause Rep–RBR binding, leading to reprogramming of cell cycle controls, the accumulation of the host replication machinery and the onset of rolling-circle replication. Accumulation of viral DNA replication products and intermediates then triggers a genotoxic response and the synthesis of host repair proteins, resulting in a switch to recombination-dependent replication.

Plant signalling pathways

Protein kinases and their crosstalk with hormone signalling pathways have crucial roles in plant growth and development, as well as in pathogen recognition and the defence response. Geminiviruses interact with several such pathways to recruit host processes for viral propagation and to interfere with host defences.

Receptor-like kinases. Some plant receptor-like kinases (RLKs) sense viral pathogens and trigger an antiviral defence response. The best characterized RLKs involved in geminivirus infection are the three closely related leucine-rich repeat (LRR) RLKs designated NSP-interacting kinase 1 (NIK1), NIK2 and NIK3 (REF. 60). NIKs are membrane proteins that undergo autophosphorylation and can phosphorylate exogenous substrates. NSP binds to the NIK kinase domain and interferes with its autophosphorylation, which is required for kinase activity[60,61]. NIK proteins are thus unable to phosphorylate their downstream effector, the ribosomal protein RPL10, and induce its translocation to the nucleus, where it is thought to interfere with viral infection[62]. The activities of NIK proteins and RPL10 correlate with symptom development; overexpression of these proteins attenuates and delays symptoms, whereas loss of their function increases susceptibility[63]. The signal that activates NIK proteins and their targets downstream of RPL10 are not known[64].

GRIK–SNRK1 kinase cascade. Rep interacts with two closely related protein kinases — geminivirus Rep-interacting kinase 1 (GRIK1) and GRIK2 (REFS 39,65). The GRIKs, which are regulated by the ubiquitin

proteasome pathway, accumulate in young plant tissues, cultured cells and geminivirus-infected cells. They are thought to be involved in one or more processes that are important for both early plant development and geminivirus infection. The GRIKs are upstream activators of SNF1-related protein kinase 1 (SNRK1)[66] — a key regulator of plant metabolism that is involved in development and responses to abiotic and biotic stresses. Plants overexpressing SNRK1 show symptoms later and contain less viral DNA than wild-type plants, whereas plants silenced for SNRK1 expression develop symptoms earlier and accumulate more viral DNA than wild-type plants. SNRK1 binds to viral transcriptional activator protein (TrAP; known as C2 in some viruses) and the satellite protein βC1 (REFS 67,68). βC1 is phosphorylated by SNRK1, and a βC1 phosphomimic delays infection[68], indicating that SNRK1 phosphorylation of βC1 interferes with infection.

The roles of GRIK and SNRK1 during geminivirus infection are not clear. SNRK1 might be part of the host defence response, and its defence activity might be counteracted by TrAP/C2. Conversely, the GRIK–SNRK1 cascade might be activated by infection to ensure adequate energy and nutrient supplies to support viral and host DNA replication. Alternatively, the cascade might serve both functions in a dynamic, ordered infection process in which Rep expression precedes and is required for TrAP/C2 expression, or it might provide a link between viral replication and the host methyl cycle, which is inhibited during infection to prevent methylation of viral DNA (FIG. 2).

Shaggy-related kinases. Shaggy-related kinases are involved in various plant developmental processes, including cell division and elongation, in part through their interactions with the brassinosteroid signalling pathway. Viral C4 (or AC4 in some viruses) interacts with shaggy-related kinases[69,70], and silencing the expression of shaggy-related kinases delays infection[71]. A curtovirus C4 protein can be phosphorylated by the *Arabidopsis thaliana* shaggy-related kinase BRASSINOSTEROID-INSENSITIVE 2 (BIN2), which is a negative regulator of brassinosteroid signalling, whereas an AC4 protein from a bipartite begomovirus is a poor substrate, even though it binds to BIN2 (REF. 69). This difference might explain why ectopic expression of C4 but not AC4 proteins induces symptoms in plants[69,70]. Consistent with this difference, a curtovirus C4 protein induces hyperplasia by suppressing brassinosteroid signalling[72], whereas an AC4-containing begomovirus upregulates expression of brassinosteroid target genes[26]. One scenario is that AC4 proteins of bipartite viruses bind to and interfere with BIN2, leading to the activation of the brassinosteroid pathway, whereas BIN2-mediated phosphorylation of the C4 proteins of monopartite viruses prevents BIN2 inactivation and thus maintains inhibition of the brassinosteroid pathway. It is not clear why geminiviruses interface differently with shaggy-related kinases and the brassinosteroid pathway, but this difference underscores the importance of not assuming that all virus–host interactions are conserved.

Hormone signalling pathways. Geminiviruses interact with diverse plant hormone pathways, such as the salicylic acid, ethylene and jasmonic acid pathways, in addition to the brassinosteroid pathway described above. They activate the salicylic acid and ethylene pathways, which both participate in the host defence response[26], and plants with increased salicylic acid levels or higher expression of components in this pathway are resistant to infection[26,73,74]. Genes in the jasmonic acid pathway are generally suppressed during infection[26]. Ectopic expression of some viral proteins can activate or inhibit the jasmonic acid pathway, but the biological relevance of these changes is not known[59,75,76].

Geminiviruses also interact with the cytokinin and auxin pathways, which promote cell proliferation and modulate differentiation in plants. Infection activates the expression of a rapidly responding auxin-inducible gene[57] and of primary cytokinin-responsive genes[77]. Activation of cytokinin-responsive genes might result from TrAP/C2-mediated inhibition of adenosine kinase, which phosphorylates cytokinins and converts them to their low-activity nucleotide forms[78]. Ectopic expression of TrAP/C2 increases the expression of primary cytokinin-responsive genes, and the application of exogenous cytokinin enhances susceptibility to infection[77]. Inhibition of adenosine kinase during infection might enhance the levels of bioactive cytokinin and thereby facilitate the re-establishment of DNA replication competency in infected plant cells.

Plant cell death pathways. Transient expression of some viral proteins, such as Rep, V2 and NSP, can lead to cell death[79–81]. Rep binding to RBR can trigger the death of mature plant cells[82], but it is not known how V2 or NSP induce host cell death. C2 has been shown to block cell death induced by V2 and NSP[83], but has also been associated with severe symptoms and cell death[81,84]. These conflicting results might reflect differences between viral species and/or limitations of transient expression assays that characterize individual viral proteins outside the infection process. Infected plants typically do not show phenotypic evidence of senescence or localized cell death[42], even though many host genes associated with cell death are upregulated[26], indicating that geminiviruses effectively counteract the activation of cell death pathways during infection.

Ubiquitylation and ubiquitylation-like pathways

Protein modifications by ubiquitin and ubiquitin-like proteins are post-translational events that modulate protein function and regulate many plant processes, including development, the cell cycle and responses to abiotic and biotic stresses[85,86]. Ubiquitin is covalently linked to lysine residues in the target protein through an enzymatic cascade comprising an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme and an E3 ubiquitin ligase, which binds to the substrate and confers specificity. Sumoylation, which conjugates small ubiquitin-like modifier proteins (SUMO), requires its own set of related E1, E2 and E3 enzymes. Polyubiquitylation targets proteins to the proteasome for

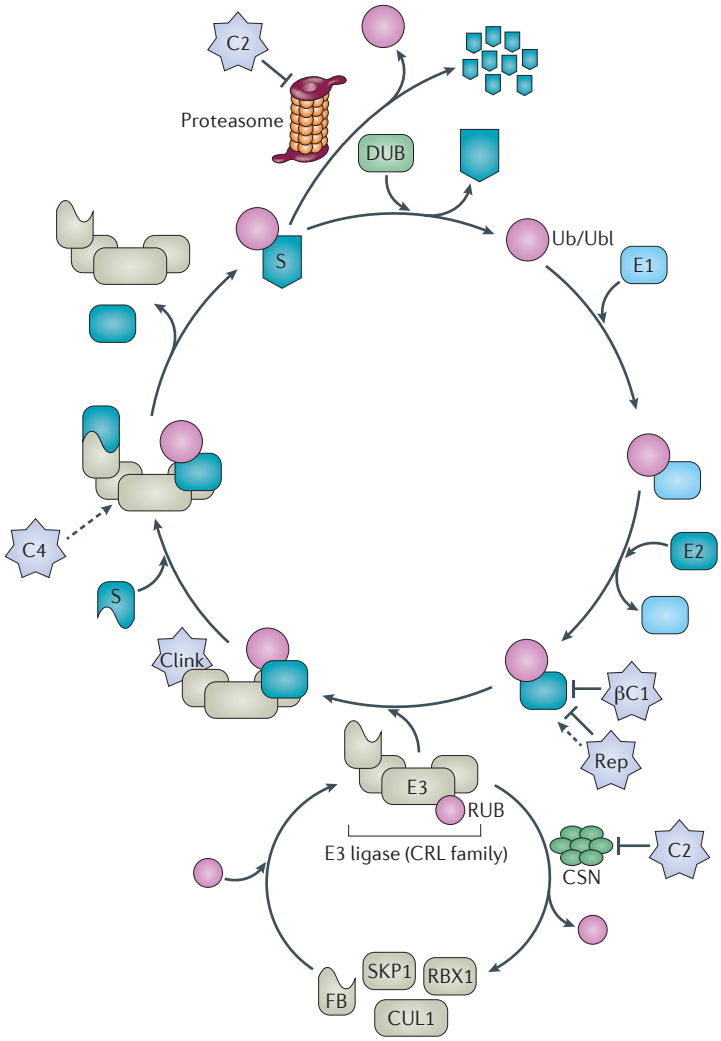


Figure 3 | **Modulation of ubiquitylation and ubiquitylation-like pathways.** The diagram shows interactions between geminivirus proteins (grey) and components of the ubiquitin and ubiquitin-like protein (Ub/Ubl) pathways. Modification of a substrate (S) requires the activating (E1) and conjugating (E2) enzymes and usually an E3 ligase that confers specificity. In plants, the multisubunit cullin RING ligases (CRLs) for ubiquitin constitute the most abundant family of E3 ligases. They are formed by the RING subunit RBX1, which binds to E2, and a substrate adaptor formed by S-phase kinase-associated protein 1 (SKP1) and an F-box (FB) protein in the cullin 1 (CUL1)-based group ligases. CRL activity is regulated by a cycle of covalent attachment and removal of the ubiquitin-like protein RUB, which is required for robust CRL activity. The constitutive photomorphogenesis 9 signalosome (CSN) complex catalyses derubylation of cullins. Ubiquitin-modified proteins can be degraded by the 26S proteasome. Ub/Ubl modification can also regulate the activity of a target protein or alter its subcellular location, which can be reversed by deubiquitylating enzymes (DUBs). Rep, replication initiator protein.

degradation, whereas monoubiquitylation or sumoylation can alter protein activities, subcellular localization and/or interaction partners. Some viral proteins can be modified by ubiquitin and ubiquitin-like proteins, and some can function as enzymes in the ubiquitylation pathway[87].

Geminiviruses alter the ubiquitin and ubiquitin-like protein machineries to achieve a full infection (FIG. 3). Infection is impaired when there is a reduction in the expression of ubiquitin-like modifier-activating enzyme 1 (UBA1), RING-H2 group F2A (RHF2A; which is an E3 ubiquitin ligase), S-phase kinase-associated protein 1 (ASK2; also known as SKP1-like 2) or COP9 signalosome 3 (CSN3; which is derived from constitutive photomorphogenic 9)[71]. Infection protects some unstable host proteins from degradation, including GRIK and *S*-adenosyl methionine decarboxylase 1 (SAMDC1), the latter being a key enzyme in polyamine biosynthesis that decarboxylates *S*-adenosyl methionine to reduce the availability of methyl groups for DNA methylation[65,88]. These observations established the functional importance of interactions with the ubiquitin pathway for geminivirus infection.

Interactions between geminivirus proteins and components of ubiquitin and ubiquitin-like protein pathways have been reported (FIG. 3). βC1 binds to the *Solanum lycopersicum* E2 enzyme ubiquitin-conjugating enzyme 3 (UBC3), reducing the global accumulation of polyubiquitylated proteins and causing strong symptoms[89,90]. C2 proteins interact and interfere with the CSN complex, which normally removes RUB from cullin 1 (CUL1) and thereby might redirect C2 ubiquitylation by collectively targeting a broad range of E3 SKP1, CUL1, F-box containing (SCF) ligases through modification of their rubylation status[75]. Given that SCF ligases are key regulators of many cellular processes, the capacity of geminiviruses to hijack these complexes represents a powerful strategy for modulating host function. Accordingly, overexpression of C2 alters several plant hormone responses regulated by the CUL1-based SCF ubiquitin E3 ligases[75].

The C4 proteins of some curtoviruses and begomoviruses might induce plant cell proliferation by activating expression of a host RING finger protein (RKP), which targets cyclin kinase inhibitors for proteosomal degradation[58]. The nanovirus Clink protein is an F-box protein that binds to both RBR and SKP1 (a CUL1 adaptor protein), suggesting that Clink alters ubiquitylation to affect cell cycle regulation[91].

Rep interacts with the E2 enzyme SUMO-conjugating enzyme 1 (SCE1)[92]. Silencing SCE1 or altering Rep–SCE1 interaction reduces the accumulation of viral DNA, suggesting that this interaction is required for viral replication[71,92]. Transient Rep expression modifies the sumoylation state of selected host proteins that might have roles in viral replication[93].

Plant silencing pathways

RNA silencing is an adaptive defence response that uses siRNAs to target viruses and transposons. In turn, viruses suppress this response by using anti-silencing proteins, called viral suppressors of RNA silencing (VSRs), to interfere at various steps in the silencing response. Because of their nuclear localization and the resemblance of their genes to engineered transgenes, which also have short promoters with high activity and often lack introns, geminiviruses offer unique opportunities to understand how plants recognize and defend against foreign DNA.

Geminivirus induction of silencing defence responses. All silencing pathways involve cleavage of dsRNA into siRNAs by Dicer-like proteins (DCLs) (FIG. 4a). Different

DCLs reside in different parts of the cell, and all four *A. thaliana* DCLs are potentially active during geminivirus infections[94]. In the nucleus, 24-nucleotide (nt) siRNAs are produced by DCL3 and loaded onto ARGONAUTE 4 (AGO4) to direct DNA methylation. This methylation of promoter regions to interfere with gene expression is called transcriptional gene silencing (TGS). In post-transcriptional gene silencing (PTGS), mRNA is targeted by the RNA-induced silencing complex (RISC) for degradation or translational arrest. The versions of RISC that are most active against plant viruses contain AGO1 and AGO2, which are primed with 21- or 22-nt siRNAs generated by DCL4 or DCL2, respectively[95,96].

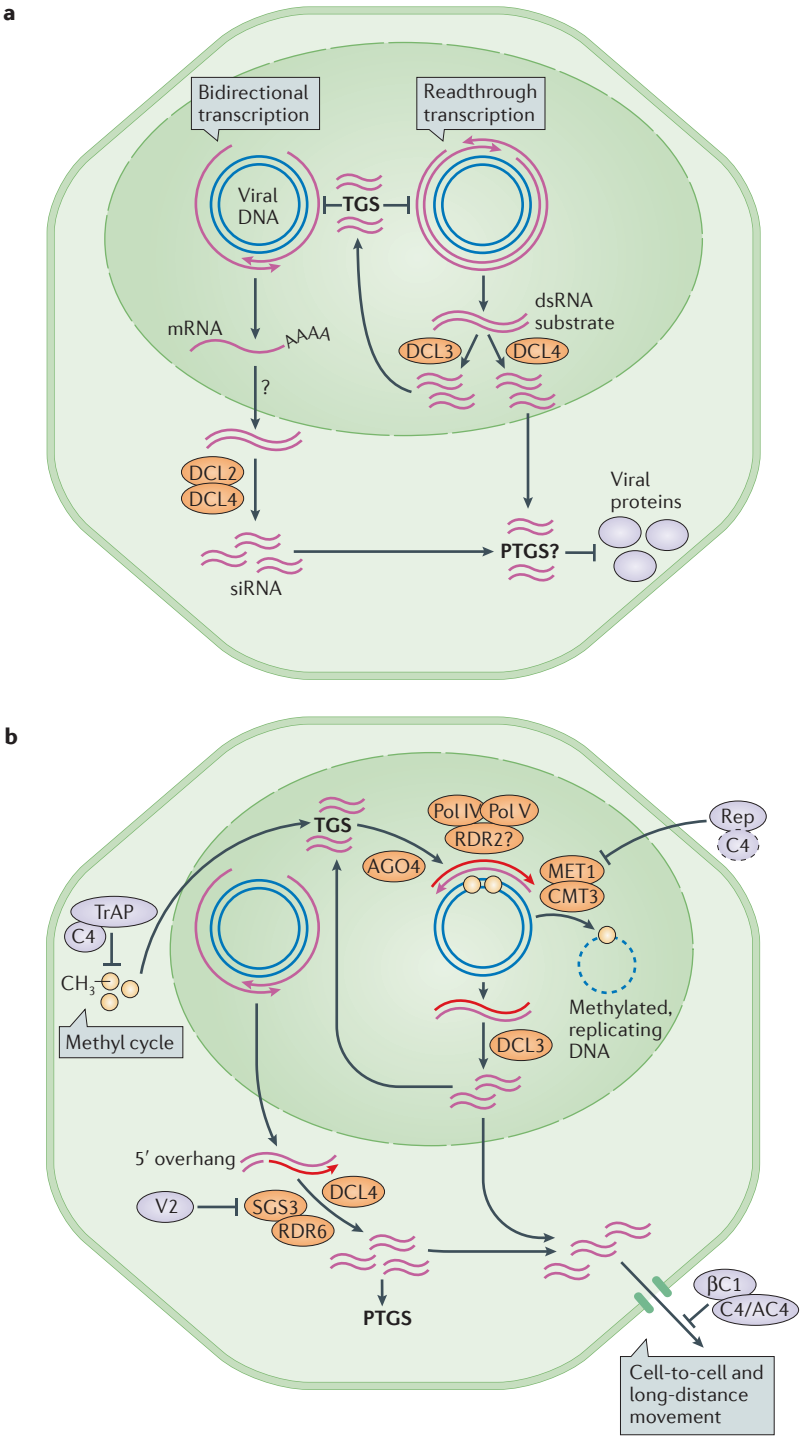
Unlike RNA viruses, geminivirus infections are associated with abundant amounts of 24-nt siRNAs[97]. Methylation of viral DNA can occur along the entire genome, although the relative distribution varies in different virus–host combinations[97–100]. Analysis of siRNA profiles localized 24-nt siRNAs primarily to intergenic, promoter-containing regions for two geminiviruses in their natural hosts but to coding regions for a third virus infecting *A. thaliana*[94,97,98]. *In vitro* methylation of viral replicons before their introduction into plant cells reduces viral DNA production 5–20-fold but results in a population of non-methylated progeny DNAs[101]. Only linear, heterogeneous viral DNA, which represents non-productive viral replication, is methylated in infected leaves[102]. Thus, geminiviruses might escape methylation by 'resurrecting' unmethylated DNA during viral replication and/or through the action of geminivirus VSRs inhibiting the host methylation pathway.

There is abundant evidence for multiple VSRs affecting the plant methyl cycle, and their activities are unique to geminiviruses (FIGS 2,4b). TrAP/C2 proteins interact with and inactivate host adenosine kinase (ADK), which is required for synthesis of *S*-adenosyl methionine (SAM)[103]. Curtovirus C2 also interacts with SAMDC1 to promote SAM decarboxylation[88]. The inactivation of ADK and stabilization of SAMDC1 both affect the methyl cycle, resulting in a reduction of DNA methylation and in the suppression of TGS. βC1 interacts with *S*-adenosyl homocysteine hydrolase (SAHH), a methyl cycle enzyme that is also required for TGS[104]. In addition to VSRs affecting the methyl cycle, Rep and C4 downregulate DNA methyltransferase 1 (*MET1*) and chromomethylase 3 (*CMT3*) (two genes that are necessary for the maintenance of methylation)[105], which might be necessary to generate methylation-free viral DNA templates in a cell with activated silencing pathways.

The ability of TrAP/C2 to directly prevent methylation was established in experiments showing that a viral replicon carrying a TrAP/C2 mutation is methylated during infection and associated with a recovery phenotype in wild-type plants but not in *ago4* mutants, which are impaired for DNA methylation[40]. Increased viral DNA methylation has also been reported for other geminiviruses associated with recovery phenotypes[40,98,106]. It is not known why only some geminiviruses allow recovery, or whether DNA methylation and VSRs are always the primary determinants of recovery.

Figure 4 | **Silencing pathways targeting geminiviruses.** ► **a** | Primary small interfering RNAs (siRNAs). After bidirectional transcription of viral DNA, mRNA is cleaved at the polyA site and polyadenylated for nuclear export. Profiles of viral primary siRNAs produced in a geminvirus-infected RNA-dependent RNA polymerase (RDR) triple-mutant plant demonstrated that Dicer-like 2 (DCL2), DCL3 and DCL4 are active, but it is not clear how their double-stranded RNA (dsRNA) substrates are made. If it was by readthrough transcription, most siRNAs would map to the overlapping 3′ ends, but this is not the case[98]. Nevertheless, DCL3 cleaves dsRNA to produce siRNAs for the methylation of promoters (transcriptional gene silencing (TGS)) or siRNAs targeting coding sequences (post-transcriptional gene silencing (PTGS)). In the cytoplasm, siRNA incorporation into Argonaute 1 (AGO1) or AGO2 during infection could result in translational inhibition or mRNA cleavage. AGO-incorporated siRNAs have not yet been profiled during a geminivirus infection. **b** | A speculative model of RDR-associated secondary siRNAs. In the canonical TGS pathway, the RNA polymerases Pol IV and Pol V, along with RDR2, synthesize dsRNA for DCL3 to process into 24-nucleotide siRNAs, which are used for long-distance movement or *de novo* methylation of viral DNA. Direct evidence of an RDR2 requirement is lacking for geminiviruses, probably owing to the suppression of the methyl cycle (which acts before RDR2) by viral suppressors of RNA silencing (VSRs; in this case, transcriptional activator protein (TrAP; known as C2 in some viruses) and βC1). To prevent any *de novo* methylation from being propagated, DNA methyltransferase 1 (MET1) and chromomethylase 3 (CMT3), which are both needed for maintenance methylation, are downregulated by replication initiator protein (Rep) and C4 (REF. 105). RDR6 is needed for long-distance siRNA activity, whereas RDR6, suppressor of gene silencing 3 (SGS3) and DCL4 are needed for cell-to-cell movement of silencing. SGS3 recognizes dsRNA with 5′ overhangs and recruits RDR6 to make the RNA double-stranded. DCL4 produces 21-nucleotide siRNAs that can move from cell to cell. The VSR V2 prevents SGS3 access to dsRNA with 5′ overhangs. βC1 and C4 (or AC4 in some viruses) bind to siRNAs, preventing their incorporation into AGO and their movement. Red arrows indicate RDR synthesis of the second RNA strand.

Role of RDRs in geminivirus infections. Primary siRNAs, which are produced directly from dsRNA in the absence of a host-encoded RNA-dependent RNA polymerase (RDR), comprise the vast majority of siRNAs in the only geminivirus infection analysed to date[100]. Nevertheless, mutation of RDR6 leads to a modest increase in viral DNA, suggesting that secondary siRNAs are also important[94,100,107]. Secondary siRNAs, which are produced by RDRs, amplify the silencing response and have a crucial role in defence against RNA viruses[95,96,108]. They are also involved in long-distance silencing[109,110], which moves ahead of virus spread and could be important for the methylation associated with recovery. The only viral infection that has been profiled in *rdr* mutants did not show recovery[100], and it will be important to ask whether secondary siRNAs are more abundant in infections that undergo recovery.



RDR2 is necessary for the production of nuclear dsRNA, which is cleaved by DCL3 into 24-nt siRNAs, whereas RDR6 is associated with DCL4 and 21-nt siRNAs (FIG. 4b). RDR6 is recruited to aberrant RNA through the action of suppressor of gene silencing 3 (SGS3), a protein that is unique to plants and binds dsRNA with 5′ overhangs[111]. The V2 VSR competes with SGS3 to prevent RDR6 binding[111,112]. Both 24- and 21-nt siRNAs are involved in long-distance silencing, which is counteracted by V2 and C4 binding to siRNAs[113,114].

Only 21-nt siRNAs, along with RDR6, SGS3 and DCL4, have demonstrated roles in the cell-to-cell movement of silencing[96,110]. Single mutations in RDR6, SGS3 or DCL4 cause modest increases in viral DNA during infection by a geminivirus that can escape from phloem cells and invade mesophyll and epidermal cells in infected leaves[94,100,107]. This pathway might not affect geminiviruses restricted to vascular tissue, which is part of the long-distance silencing pathway[110], but this remains to be tested.

The first geminivirus resistance gene to be cloned, *TY1*, encodes a tomato RDRγ with homology to RDR3, RDR4 and RDR5 of *A. thaliana*[115]. Although RDRγ is conserved in all plants, its function is not known. Because *TY1* does not confer resistance to RNA viruses, it has been proposed that its RDR activity is required for DNA methylation[115]. The *TY1* locus contains a polymorphism that increases RDRγ expression[115], strongly suggesting that some type of secondary siRNA (or dsRNA) is important for symptom attenuation and reduction of geminivirus DNA accumulation.

A correlation between methylation of intergenic regions and geminivirus resistance was recently found in soybean[116], underscoring the importance of understanding how viral DNA sequences are targeted for siRNA production and methylation. Secondary siRNA production can lead to off-target silencing, indicating that there are strict requirements for RDR access to RNA that are only now beginning to be characterized[117,118]. An increased knowledge of the substrate requirements for RDR2 and other host factors involved in TGS might clarify how viral DNA sequences are chosen for methylation[110]. These requirements and a better functional understanding of RDRγ are important goals for future research.

siRNAs as symptom determinants. Although the exact origin and function of geminivirus-associated symptoms remains unclear, there is little doubt that siRNA pathways play a part. The endogenous miRNA regulatory system participates in various host developmental and stress-related pathways and is especially important in leaf development. VSRs such as V2, which binds to siRNA, do not discriminate between 21-nt siRNAs and miRNAs[114], which might explain why V2 is a pathogenicity determinant. VSR interference with host silencing proteins, such as SGS3, would also disrupt normal development by inhibiting *trans*-acting siRNAs, which modulate auxin activity, among other things. It remains to be seen whether any of the siRNA interactions represent viral strategies to enhance infection.

Future directions

Over the past few years, studies have established that geminivirus–plant interactions are complex and involve diverse pathways, ranging from the plant cell cycle to gene silencing. These studies have identified interactions that are essential for infection and provided insight into how geminiviruses redirect plant processes and counteract host defence responses. This information has the potential to lead to new approaches

to combat geminivirus disease and improve food security, but gaps in our knowledge currently limit these efforts.

For example, we need to know which geminivirus–plant interactions are conserved and essential for infection, and how to disrupt them without interfering with normal plant development and growth. To do this, it will be crucial to distinguish interactions and events that occur in direct response to viral proteins or viral DNA versus those that are indirect consequences of the host response to infection. It will also be necessary to better characterize geminivirus–plant interaction networks and their spatial and temporal relationships during infection. Hence, future studies will depend on the development of strategies to separate virus-positive cells from virus-free cells in infected leaves and on the integration of such strategies with high-throughput sequencing technologies and cell biology approaches. These studies are likely to uncover important virus–host interactions that have not yet been described because of the challenges associated with analysing cell populations in which less than 2% of the cells are infected, as is the case for most geminiviruses that are limited to vascular tissue. These studies will also provide insight into why the consequences of some interactions differ depending on the virus–host combination and whether the host cellular context contributes to some of these differences. Such studies will provide crucial information about the mechanisms and outcomes of geminivirus–host interactions and which components might be the 'Achilles heels' and potential resistance targets.

The recent identification of a non-canonical RDR as a geminivirus resistance gene[115] and the lack of an obvious aetiology for siRNA populations in geminivirus-infected plants[97–100,116] underscore the importance of better understanding the roles of TGS and PTGS during infection. Geminivirus interactions with a recently discovered DNA methylation pathway that is specific to plants and involves 21-nt siRNAs instead of 24-nt siRNAs[119] should also be examined. For PTGS, a combination of siRNA profiling and analysis of the degradome (5′ uncapped, polyadenylated RNA resulting from cleavage by RISC) will lead to the identification of those siRNAs that are incorporated into RISC and putative host mRNAs that are targeted for silencing by geminivirus infection[120]. Such information is essential if we are to fully characterize geminivirus–host interactions.

It will also be essential to translate mechanistic studies of geminivirus–host interactions in model organisms to agricultural systems. Such studies will be facilitated by the tremendous increase in whole-genome sequence resources for important crops susceptible to geminivirus disease. New resources will soon be available, including expressed sequence tags from different tissues of resistant and susceptible hosts, and genome-wide sequences of siRNAs from infected and healthy plants. When we have identified a more complete set of common host targets, a systems approach can be developed to better understand virus–host interactions. A multidisciplinary and dedicated effort might finally lead to the identification of essential, conserved interactions that can be targeted to develop novel disease control strategies against these important plant pathogens.

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