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Review

RNA-Targeted Antiviral Immunity: More Than Just RNA Silencing

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RNA silencing is a fundamental, evolutionarily conserved mechanism that regulates gene expression in eukaryotes. It also functions as a primary immune defense in microbes, such as viruses in plants. In addition to RNA silencing, RNA decay and RNA quality-control pathways are also two ancestral forms of intrinsic antiviral immunity, and the three RNA-targeted pathways may operate cooperatively for their antiviral function. Plant viruses encode viral suppressors of RNA silencing (VSRs) to suppress RNA silencing and facilitate virus infection. In response, plants may activate a counter-counter-defense mechanism to cope with VSR-mediated RNA silencing suppression. In this review, we summarize current knowledge of RNA silencing, RNA decay, and RNA quality control in antiviral defense, and highlight the mechanisms by which viruses compromise RNA-targeted immunity for their infection and survival in plants.

Highlights

RNA silencing, RNA decay, and RNA quality control are three major RNA degradation pathways that target redundant/damaged/detrimental cellular and exogenous RNAs in plants.

Virus infection may activate RNA degradation pathways. RNA silencing is the most important line of defense that restricts virus infection.

Viruses have evolved diverse mechanisms to suppress RNA silencing. The best effective mechanism is to express VSRs. In response, plants may activate a countersuppression mechanism.

RNA decay may function as an antiviral defense, possibly through concerted actions of RNA silencing. Viruses can repress RNA decay to promote viral proliferation.

RNA quality control may recognize specific features of certain plant RNA viruses to restrict virus infection. Many viruses have also evolved strategies to escape or avoid this degradation pathway.

Antiviral RNA Silencing in Plants

RNA silencing or RNA interference (RNAi) is a fundamental, evolutionarily conserved and sequence-specific mechanism that is triggered by **double-stranded RNA (dsRNA)** (see Glossary) and regulates gene expression in eukaryotes. It is also a primary immune response to infection by microbial pathogens such as viruses [1–3]. Virus invasion activates RNA silencing; in virus-infected cells, there are several types of dsRNA that may serve as an RNA silencing inducer. These dsRNAs result from virus replication (for RNA viruses), bidirectional transcription of the viral genome (for DNA viruses, such as **geminiviruses**), intramolecular pairing of viral RNA, and *de novo* biosynthesis of dsRNA by endogenous RNA-dependent RNA polymerases (RDRs) [4]. The dsRNA-specific endoribonuclease (RNase) Dicer or Dicer-like (DCL) family of proteins detect and process dsRNAs into **virus-derived small interference RNAs (vsiRNAs)**, which are 20–24 nucleotide (nt) RNA duplexes with 2 nt 3′ overhangs at both ends. The vsiRNAs are integrated into the RNA-induced silencing complex (RISC) that contains Argonaute (AGO) proteins as the core components. The incorporated vsiRNAs guide the RISC to pair with a complementary sequence of the targeted virus and directs **transcriptional gene silencing (TGS)** by RNA-directed viral DNA methylation (RdDM) to suppress the transcription of homologous DNAs, or **post-transcriptional gene silencing (PTGS)** including the splicing and degradation or translational repression of the recognized viral RNA [4,5] (Figure 1). In plants, much research on RNA silencing and its antiviral mechanism has been done using the model plant *Arabidopsis thaliana*. The *A. thaliana* genome encodes four DCLs which function redundantly or cooperatively to confer host resistance against viruses [6]. Thus, *A. thaliana* mutants with knockout of more than one *DCL* gene show higher virus titers and more severe symptoms than a single-gene mutant [6]. Among vsiRNAs, the 21 nt class produced by DCL4 is the most dominant species and directs potent antiviral defense [7–9]. The accumulation level of DCL2-dependent 22 nt vsiRNAs is negatively regulated by DCL4, and the effect of 22 nt vsiRNAs on RNA silencing is not as strong as 21 nt vsiRNAs [9]. Abundant 24 nt vsiRNAs that are made by DCL3 are evidenced in *A. thaliana* plants infected by DNA viruses, such as geminiviruses [10,11]. Similar to other small RNAs (sRNAs) and microRNAs (miRNAs), vsiRNAs are methylated by the Hua

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Enhancer 1 methyltransferase (HEN1) before recruitment by AGOs to form the RISC. The AGO family in *A. thaliana* is comprised of 10 AGO proteins which are all active in RNA slicing and play distinct roles in TGS and PTGS. For example, AGO4 is mainly involved in TGS against DNA viruses, while AGO1 and AGO2 are two major antiviral AGOs against RNA viruses. For the detailed roles of AGO proteins in RNA silencing, readers are directed to two recent reviews [12,13]. It is worth mentioning here that viral RNAs themselves could also serve as templates for RDR and its chaperone protein SUPPRESSOR OF GENE SILENCING 3 (SGS3) to synthesize dsRNA, which amplifies vsiRNAs and promotes vsiRNA spread for antiviral RNA silencing, leading to viral symptom recovery [14].

As discussed above, the RNA silencing pathway itself and its antiviral function have been extensively studied and well elucidated. It was not until recently that we have begun to understand the roles of host factors essential for antiviral RNA silencing. In a recent seminal work, Guo and colleagues conducted a genetic screen and identified two phospholipid flippases, aminophospholipid transporting ATPase 1 (ALA1) and ALA2, and a multispan transmembrane magnesium transporter, ANTIVIRAL RNAI-DEFECTIVE 2 (AVI2) that are necessary for the accumulation of RDR1- and/or RDR6-dependent **virus-activated siRNAs (vasiRNAs)** and vsiRNAs, and the corresponding mutants are more susceptible to infection by a recombinant cucumber mosaic virus (CMV) lacking an effective RNA silencing suppression capacity [15,16]. As RNA virus replication sites are often enriched with phospholipids [17,18], it is suggested that these new factors required for antiviral RNA silencing are recruited for the formation of the RDR1/6-specific, membrane-bound RNA synthesis compartment for the robust biogenesis of vsiRNAs and vasiRNAs. In yeast, ESCRT-I (endosomal sorting complexes required for transport I) or ESCRT-III factors are co-opted for tomato bushy stunt virus (TBSV) replication, and deletion of either ESCRT-I or ESCRT-III makes TBSV replication highly sensitive to the RNA-silencing machinery [19]. These data suggest that the biogenesis of membrane compartments for virus replication and vsiRNAs amplification may require different specific host factors. In another recent work, reported almost simultaneously, Lozano-Duran and colleagues presented evidence that the receptor-like kinases (RLKs) BARELY ANY MERISTEM 1 (BAM1) and BAM2 positively regulate the cell-to-cell movement of RNA silencing, and the tomato yellow leaf curl virus (TYLCV) VSRC4 interacts with these RLKs to interfere with their function [20]. The discovery of more such host factors essential in antiviral RNA silencing will certainly shed new light on the RNA-silencing pathway and its antiviral function.

Suppression of RNA Silencing by Viruses and Countersuppression by Plants

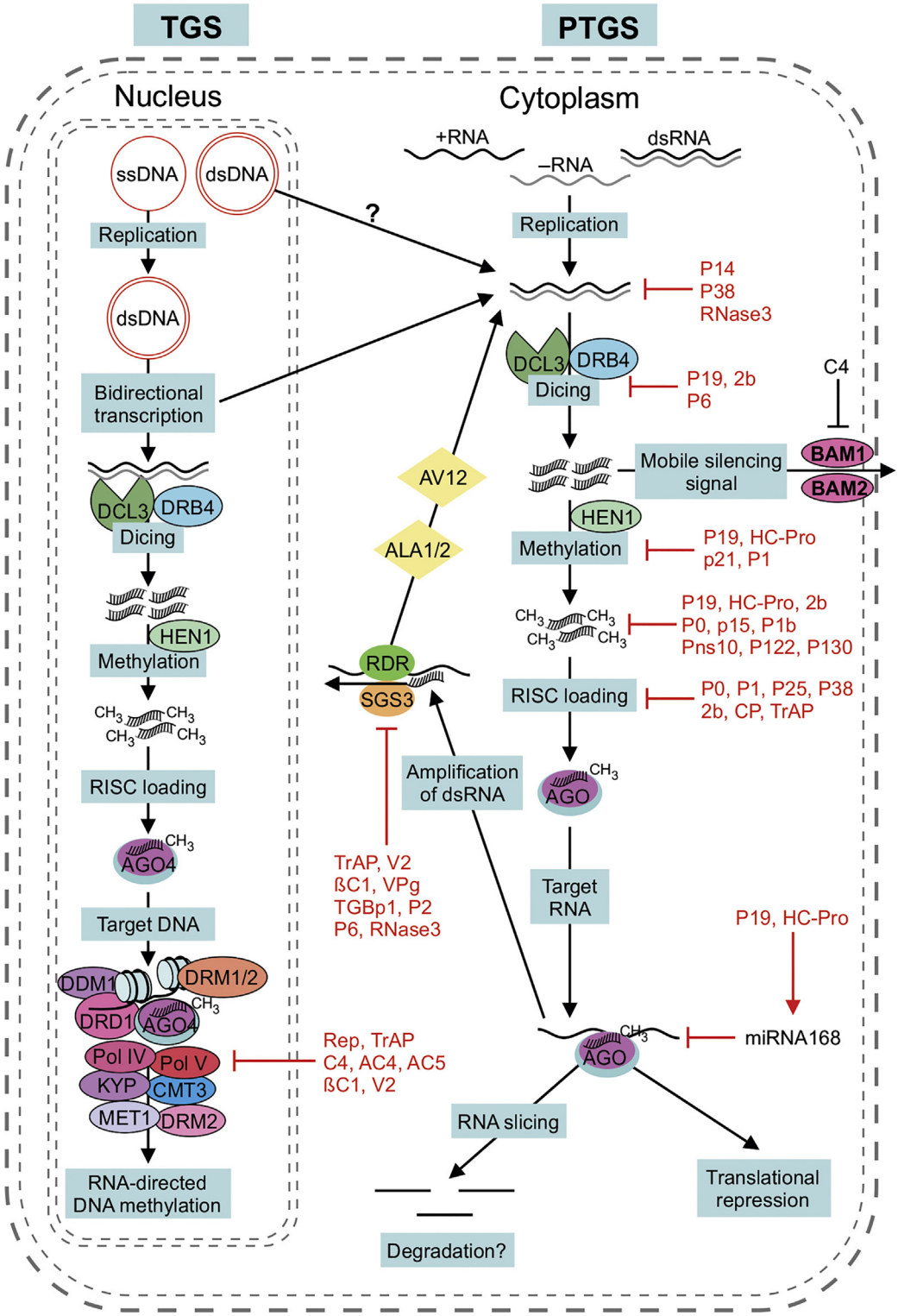
To establish successful infections, plant viruses have evolved to express VSRs to suppress host antiviral RNA silencing. To date, at least one VSR has been functionally characterized from each of the major plant virus groups. These VSRs are highly diverse in sequence and share no structural similarities [21]. VSRs are usually multifunctional, and, in addition to suppression of RNA silencing, they may serve as replicase, helicase, movement protein, transcriptional activation factor, coat protein (CP), protease, symptom determinant, or helper component for viral transmission to play essential roles in different steps of the virus infection process [21–24]. Therefore, VSRs have likely evolved independently [21,22]. VSRs from different viruses suppress the RNA silencing pathway at specific steps through various mechanisms.

TGS Suppression

VSRs encoded by geminiviruses or their associated betasatellites have been reported to repress TGS through inhibition of the activity of key enzymes or prevention of substrate degradation in the methylation cycle. For example, the TrAP protein of tomato golden mosaic virus (TGMV) and cabbage leaf-curl virus (CaLCuV), the βC1 protein of tomato yellow leaf-curl China virus (TYLCCNV) betasatellite, the C4 protein of cotton leaf-curl multan virus (CLCuMuV), and the TrAP protein of beet severe curly top virus (BSCTV) can compromise

Glossary

Double-stranded RNA (dsRNA): virus infection induces the generation of three types of dsRNA, including replicative intermediates, intramolecular pairing of genomic RNA, and *de novo* products catalyzed by cellular RNA-dependent RNA polymerases (RdRs).
Geminiviruses: viruses of the family Geminiviridae; they have a small, single-stranded DNA (ssDNA) genome and they replicate in the nucleus of host plant cells by double-stranded DNA (dsDNA) intermediates.
No-go decay (NGD): NGD targets mRNAs with elongation-inhibiting features such as secondary structures or modified nucleotides and then mediates their degradation.
Nonsense-mediated decay (NMD): NMD recognizes premature termination codons containing mRNAs in order to mediate their exonucleatic degradation.
Nonstop decay (NSD): recognizes mRNAs lacking an in-frame stop codon to mediate their exonucleatic degradation.
Post-transcriptional gene silencing (PTGS): is a common RNA silencing pathway triggered by dsRNA to down-regulate a gene at the RNA level. As a result, RNA translation is repressed and RNA is degraded.
Secondary siRNAs: the biosynthesis of siRNAs is dependent on endogenous RDRs to amplify dsRNA.
Small interference RNA (siRNA): siRNAs are short dsRNAs of 20–24 nt in length with 2 nt 3′ overhangs that have 5′-monophosphate and 3′-hydroxyl termini.
Transcriptional gene silencing (TGS): the transcription of genomic DNA is repressed or inactive due to DNA methylation or any modifications leading to the structural reorganization of chromatin (also known as chromatin remodeling).
Virus-activated siRNAs (vasiRNAs): are siRNAs that are activated by viruses.
vsiRNAs: siRNAs derived from viral RNA.
Viral suppressors of RNA silencing (VSRs): are also referred to as to RNA silencing suppressor (RSS). VSR or RSS suppresses the RNA silencing pathway.



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the methylation cycle by repression of ADK, SAHH, or SAM synthetase activity or by disruption of SAMDC degradation (Table 1) [11,25–28]. The TrAP protein of TGMV and CaLCuV can also inhibit the activity of the histone methyltransferase SUVH4/KYP to maintain the euchromatic status of the minichromosome to allow active replication and transcription of viral genes and correspondingly to escape host surveillance [29]. However, no direct evidence has been shown that these geminiviral proteins can target vsiRNAs to repress the siRNAs-directed DNA methylation pathway. Consistently, Rep, C4, C5, and V2 from different geminiviruses also interfere with methylation-related factors to repress TGS likely via an indirect approach (Figure 1) [30–32]. Two possible exceptions have been reported for the VSR AC4 protein of tomato leaf-curl New Delhi virus (ToLCV–ND) and the VSR V2 protein of CLCuMuV which can interact with AGO4, both of which may influence cytosine methylation of the viral genome [33,34].

Binding to Or Cleavage of dsRNA

Binding to dsRNA is a relatively common mechanism by which VSRs exert their RNA-silencing suppression function. For example, the P14 protein of pothos latent virus (PoLV), the P38 protein of turnip crinkle virus (TCV), and the NS3 protein of rice strip virus (RSV)/rice hoja blanca virus (RHBV) (Table 1, Figure 1) can bind to dsRNAs to block vsiRNA biogenesis in the RNA-silencing pathway [22]. Strikingly, RNase 3 of sweet potato chlorotic stunt virus (SPCSV) targets and cleaves long dsRNA as well as vsiRNA duplexes to disable RNA silencing in an endonuclease activity-dependent manner [35]. Enhanced ability to degrade viral RNA in plants usually favors antiviral resistance. RNase 3-mediated cleavage of dsRNA and vsiRNAs may represent an atypical RNA-silencing suppression strategy [35].

Interference with Double-RNA Bindings or DCLs

The cauliflower mosaic virus (CaMV) P6 protein interacts with the RNA-silencing factor double-RNA binding protein 4 (DRB4) to suppress *A. thaliana* DCL4 function and reduce the levels of DCL4-dependent 21 nt siRNAs [36]. The P38 protein of TCV binds to AGO1 and disturbs the AGO1-dependent homeostatic network, leading to inhibition of *Arabidopsis* DCLs [37]. The RSV NS3 interacts with rice DRB1 to promote miRNA biogenesis, which facilitates viral infection [38].

Interference with HEN1 and Sequestration of siRNAs

As briefly introduced above, vsiRNAs undergo HEN1-mediated methylation before loading onto AGOs. VSRs such as HC-Pro of zucchini yellow mosaic virus (ZYMV) may interact with HEN1 to inhibit its methyltransferase activity [39]. Several VSRs, including the beet yellows virus (BYV) p21 protein, the TBSV P19 protein, the turnip mosaic virus (TuMV) HC-Pro, the CMV 2b and the grape virus A (GVA) P0, may also inhibit miRNA or DNA methylation through binding to vsiRNAs and excluding HEN1 from interacting with vsiRNA duplexes or preventing HEN1 access to the 2′OH of the 3-terminal nucleotide (Table 1) [40–42]. Such sequestration of vsiRNAs is recognized as one of a few most common mechanisms exploited by VSRs to suppress RNA silencing (Table 1, Figure 1).

Figure 1. Schematic Representation of the RNA Silencing Pathway in Plants Activated by Virus Infection. Infection by single-stranded DNA (ssDNA) viruses induces RNA-directed DNA methylation (RdDM), which is involved in recruiting cytosine methyltransferases [e.g., domains rearranged methyltransferase 1 (DRM1), domains rearranged methyltransferase 2 (DRM2), chromomethylase 3 (CMT3), DNA methyltransferases 1 (MET1)] and histone methyltransferases [e.g. Kryptonite (KYP)], and chromatin remodelers [e.g., DRM1 and DNA methylation 1 (DDM1)], leading to transcriptional gene silencing (TGS). The mechanisms by which double-stranded DNA (dsDNA) viruses generate double-stranded RNAs (dsRNAs) to induce RNA silencing, and whether they could trigger RdDM, are still to be fully understood. Plus-stranded RNA (+RNA) viruses, negative-stranded RNA (–RNA) viruses, and dsRNA viruses produce dsRNA intermediates mainly by virus replication, and they serve as inducers of post-transcriptional gene silencing (PTGS). Different **viral suppressors of RNA silencing (VSRs)** may target specific steps of the RNA silencing pathway (see text for details). Abbreviations: AGO, Argonaute proteins; RISC, RNA-induced silencing complex.

Inhibition of AGOs and RISC Assembly

AGO1 is a favorite target of VSRs too. For example, CMV 2b binds to AGO1 and inhibits its cleavage activity in CMV-infected *Arabidopsis* plants [43]. The tomato ringspot virus (ToRSV) coat protein (CP) interacts with AGO1 and negatively regulates its stability [44]. The polerovirus F-box P0 protein also targets AGO1 to suppress RNA silencing [45]. The P0–AGO1 interaction triggers the autophagic degradation of AGO1 by hijacking the host S-phase kinase-associated protein 1 (SKP1)-cullin 1 (CUL1)-F-box protein (SCF) complex [45–47]. The P0 protein recognizes the degron motif of AGO1, which is conserved among AGOs, and thus confers P0-mediated degradation of different AGO proteins [48]. Interestingly, the sweet potato mild mottle virus (SPMMV) VSR P1 interacts with AGO1 and AGO2 in *A. thaliana*, but only interferes with AGO1 function by blocking RNA binding to AGO1 [49]. The tobacco rattle virus (TRV) 16K VSR binds to AGO4 to inhibit the formation of RNA-silencing complexes [50]. In addition to targeting vsiRNAs duplexes, the TBSV P19 protein may repress AGO1 expression through upregulation of miR168 expression [40]. However, the involvement of miRNA seems complicated. It has been shown that RSV infection upregulates a grass-specific AGO18 which recruits miRNA168 to inhibit the AGO1-miR168 interaction, leading to the release of AGO1 for antiviral RNA [51].

Interference with the Amplification and Intercellular Spread of RNA Silencing

The RDR-dependent secondary vsiRNAs are amplified vsiRNAs that are essential for intercellular spread of vsiRNAs, and viral symptom recovery. RDR6 and SGS3 are required for amplification of vsiRNAs. Some VSRs, such as AC2, V2, and βC1 from geminiviruses, HC-Pro and VPg of potyviruses, TGBp1 of plantago asiatica mosaic virus (PlAMV), p2 of RSV, and P6 of rice yellow stunt rhabdovirus (RYSV), may target RDR6 and SGS3 to suppress RNA silencing [52–55] (Table 1, Figure 1). Among them, βC1 upregulates an endogenous RNA-silencing suppressor rgs-CaM to inhibit RDR6 expression and mediates the autophagic degradation of SGS3 [56,57], whereas TYLCV V2 interacts with SGS3 and inhibits its dsRNA binding activity [58,59]. Although CMV 2b inhibits the production of secondary vsiRNAs, no direct interaction between CMV 2b and RDRs or SGS3 has been found, so how CMV 2b disrupts vsiRNA amplification remains to be investigated.

Countersuppression of RNA Silencing by Plants

In response to RNA silencing suppression by VSRs to promote virus infection, plants have evolved countersuppression mechanisms to protect themselves. Plants may simultaneously upregulate the expression of nucleotide binding site leucine-rich repeat (*NBS-LRR*) genes, the largest group of plant disease resistance genes (*R*) that confer resistance to diverse and fast-evolving pathogens, including viruses [60,61]. A single plant genome usually encodes hundreds of *NBS-LRR* genes, which are post-transcriptionally regulated by DCL4-dependent siRNAs and normally maintain a relatively low expression level [62–64]. Actions by VSRs to suppress RNA silencing may repress other siRNAs, including those regulating DCLs. As a result, it activates the bulk expression of *NBS-LRR* genes to enhance host immunity to the invading virus. Another possible countersuppression mechanism exploited by plants is to target VSRs. Cucumovirus VSR 2b is targeted by another host-resistance mechanism independently of RNA silencing [65]. In the case of potyviruses, a calmodulin-like protein in *Nicotiana tabacum*, rgs-CaM interacts with the VSR HC-Pro at its dsRNA-binding domain to sequester it from inhibiting RNAi and further to mediate the degradation of HC-Pro via autophagy to enhance host antiviral RNAi [66]. VPg, the second potyvirus VSR, interacts with SGS3 and mediates degradation of the VPg-SGS3-RDR6 complex via ubiquitination and autophagy pathways [55]. This double-edged action might explain why HC-Pro, rather than VPg, is a primary VSR for potyviruses. Plants may also activate other immune pathways in response to viral factors involved in RNA silencing to diminish the effects of RNA silencing suppression by VSRs. For example, dsRNA of a potyvirus induces a

pattern-triggered immunity (PTI) signaling pathway [67,68]. The dsRNA-activated PTI signaling requires the pattern-recognition coreceptor SERK1 but not DCLs [67]. Plant antiviral RNA silencing, viral suppression of RNA silencing, and plant countersuppression may constitute a major battle field between plants and virus pathogens.

RNA Decay-Mediated Antiviral Defense and Viral Counterdefense

RNA decay or exonucleolytic RNA turnover is another important mechanism that determines the fate of cellular RNAs. It removes redundant mRNA and controls mRNA quantity and quality. RNA decay starts from deadenylation that progressively removes the 3′ poly(A) tail, and further undergoes exosome complex-mediated 3′–5′ cleavage or decapping followed by exoribonuclease

Table 1. Summary of Viral Factors of Different Viruses and Their Functions in the RNA Silencing, RNA Decay, and Nonsense-Mediated Decay (NMD) Pathways[a]

Suppression of TGS

Disruption of the methylation cycle	Virus, Viral factor
Downregulation of MET1 or/and CMT3	Geminivirus, C4/Rep
Inhibition of ADK activity	BCTV/TGMV, TrAP
Inhibition of DRM2 activity	MYMIV, AC5
Inhibition of SAHH activity	TYLCCNB, ßC1
Inhibition of SAM synthetase	CLCuMuV, C4
Inhibition of SAMDC degradation	BSCTV, TrAP
Disruption of the HDA6 and MET1 interaction	TYLCV, V2
Inhibition of KYP activity	CaLCuV/TGMV, TrAP
Interaction with AGO1	CMV, 2b
Interaction with AGO4	ToLCNDV/CLCuMuV, AC4/V2

Suppression of RNA decay

Interference with deadenylation	TuMV, VPg
Inhibition of decapping	TuMV, VPg
Inhibition of XRN4	TuMV, HC-Pro
Interaction with LSm1-7	BMV, genomic RNA/ replication protein

Suppression of NMD

Evasion of NMD	Potyvirus, genomic RNA
Inhibition of NMD	PepMV, RSE; TCV, RSE/USR

Suppression of PTGS

Targeting dsRNA	Virus, Viral factor
Binding to dsRNA	GBNV/TSWV, NSs; PoLV, P14; RHBV/RSV, NS3; TCV, P38 ; CMV, 2b
dsRNA degradation	SPCSV, RNase3
Inhibition of dsRNA conversion into siRNAs	
Inhibition of DRB4	CaMV, P6
Suppression of DCL binding activity	RYMV, P1; TCV, P38
Interference with HEN1 function	BYV, p21; TBSV, P19; Potyvirus, HC-Pro
Interference with biogenesis of secondary siRNAs	
Inhibition of RDR6	MYMIV, AC2; RDV, AL2; RYSV, P6; SCMV, HC-Pro; TAV, 2b; TYLCCNB, ßC1
Inhibition of SGS3	Potyviruses, VPg; PlAMV, TGBp1; RSV, P2; SPCSV, RNase3; TYLCV, V2
Disruption of AGOs	
Downregulation of AGO1	TBSV, P19; TEV, HC-Pro
Promotion of AGO1 degradation	Polerovirus, P0; PVX, P25; ToRSV, CP
Inactivation of AGO1	CMV, 2b; MYMIV, AC2; SPMMV, P1; TCV, P38
Binding to AGO4 and inhibition of RISC assembly	TRV, 16K
vsiRNA sequestration	CMV, 2b; CVYV, P1b; GVA, P0; PCV, p15; RDV, Pns10; TBSV, P19; TMV, P122; ToMV, P130; Potyvirus, HC-Pro

(XRN)-mediated 5′–3′ decay [69–71]. The enzymes that catalyze deadenylation include the conserved poly (A)-specific ribonuclease (PARN) and/or the carbon catabolite repressor 4 (CCR4) complex [72,73]. Decapping to remove the 5′ cap structure requires concerted action of a set of conserved decapping complex proteins (DCPs) including DCP1, DCP2, DCP5, VARICOSE (VCS), and possibly DEA(D/H)-box RNA helicase 1 (DHH1) [70,71,74]. Decapping in *A. thaliana* also requires the heptameric Sm-like (LSm) complex LSm1-7, which binds to 3′RNA in the cytoplasm (Figure 2) [75]. Although several RNA decay components have been suggested to attenuate endogenous RNA silencing, possibly through competing for RNA substrates in *A. thaliana* [76,77], accumulated evidence suggests that RNA decay is also an antiviral defense in plants. Knockdown of *XRN4* increases susceptibility of different plants to many viruses, including cucumber necrosis virus (CNV), RSV, TBSV, tobacco mosaic virus (TMV), and TuMV [78–81]. Consistently, the *Arabidopsis dcp2* mutant accumulates higher levels of viral RNA than do wild-type plants when infected by TRV or TuMV [74,82]. In a recent study, infection by TuMV activates RNA decay in *Nicotiana benthamiana*; the deficiency of key cytoplasmic 5′–3′ RNA decay pathway gene-encoded proteins (5′RDGs) such as DCP1, DCP2, XRN4, and PARN facilities TuMV infection, and overexpression of them attenuates virus infection [74], supporting the antiviral role of RNA decay. Overexpression of the 5′RDGs fails to suppress sense transgene-induced PTGS (S-PTGS), and on the contrary, knockdown of them attenuates S-PTGS and suppresses the generation of siRNAs. Interestingly, 5′RDGs can inhibit the accumulation of exogenous gene transcripts or viral RNA via the RNA decay pathway when RNA silencing is compromised. These data suggest that plants may have a surveillance mechanism that distinguishes viral and exogenous RNAs from typical cellular mRNAs, and directs the host RNA silencing and RNA decay pathways to target foreign RNAs for degradation in a hierarchical and concerted manner [74].

Plant viruses have evolved several counterdefense mechanisms against RNA decay (Table 1, Figure 2). VPg and HC-Pro, the two known VSRs of potyviruses, bind to essential RNA decay proteins DCP2 and XRN4, respectively, to promote virus infection [74]. HC-Pro interacts with XRN4 and inhibits its slicing activity, and VPg may suppress RNA decay-mediated degradation of viral RNA through targeting DCP2 to the nucleus to disrupt the formation of the cytoplasmic DCP1/DCP2 complex [74]. Thus, the two potyviral VSRs, VPg and HC-Pro, suppress both RNA silencing and RNA decay. Moreover, the potyviral VSR VPg recruits the eIF4F protein

Notes to Table 1:
[a]The functions of VSRs in suppression of RNA silencing, RNA decay, and RNA quality control (e.g., NMD) are summarized (see text for details). The best-studied functions of VSRs are involved in targeting enzymes or substrates in TGS (by geminivirus proteins), and targeting dsRNA, interference with the biogenesis of **secondary siRNAs**, promotion of the degradation of AGOs, and sequestration of siRNAs in PTGS. The abbreviations of the virus names in this article are as follows: BMV (brome mosaic virus); BYV (beet yellows virus); CaMV (cauliflower mosaic virus); CMV (cucumber mosaic virus); CVYV (cucumber vein yellowing virus); Geminiviruses: BCTV (beet curly top virus); BSCTV (beet severe curly top virus); CaLCuV (cabbage leaf curl virus); CLCuMuV (cotton leaf curl Multan virus); MYMIV (mungbean yellow mosaic India virus); TGMV (tomato golden mosaic virus); ToLCNDV (tomato leaf curl New Delhi virus); TYLCCNB (tomato yellow leaf-curl China virus betasatellite); TYLCV (tomato yellow leaf-curl virus); GBNV (groundnut bud necrosis virus); GVA (grapevine virus A); PCV (peanut clump virus); PlAMV (planta goasiatica mosaic virus); PoLV (pothos latent aureusvirus); Potyviruses: PPV (plum pox virus); PRSV (papaya ringspot virus); PVA (potato virus A); PVY (potato virus Y); SCMV (sugarcane mosaic virus); TEV (tobacco etch virus); TuMV (turnip mosaic virus); ZYMV (zucchini yellow mosaic virus); PVX (potato virus X); Rice viruses: RDV (rice dwarf virus); RHBV (rice hoja blanca virus); RSV (rice strip virus); RYMV (rice yellow mottle virus); RYSV (rice yellow stunt rhabdovirus); SPCSV (sweet potato chlorotic stunt crinivirus); SPMMV (sweet potato mild mottle ipomovirus); TAV (tomato aspermy virus); TBSV (tomato bushy stunt virus); TCV (turnip crinkle virus); TMV (tobacco mosaic virus); ToMV (tomato mosaic virus); ToRSV (tomato yellow spot virus); TRV (tobacco rattle virus); TSWV (tomato spotted wilt virus).The abbreviations of the host protein names in this article are as follows: ADK (adenosine kinase); AGO1 (argonaute protein 1); AGO4 (argonaute protein 4); CMT3 (chromomethylase 3); DCL (dicer-like protein); DRB1 (double-RNA binding protein 1); DRB4 (double-RNA binding protein 4); DRM2 (domains rearranged methyltransferase 2); HEN1 (HUA enhancer 1); LSm1-7 (the heteroheptameric complex of Sm-like 1-7); MET1 (DNA methyltransferases 1); KYP/SUVH4 (H3K9me2 histone methyltransferase, Kryptonite/Su(var)3-9 homolog 4); SAHH (*S*-adenosyl homocysteine hydrolase); SAMDC1 (*S*-adenosylmethionine decarboxylase 1); RDR6 (RNA-dependent RNA polymerase 6); RSE (recoding structural element); SGS3 (suppressor of gene silencing 3); XRN4 (5′–3′ exoribonuclease); USR (unstructured region).

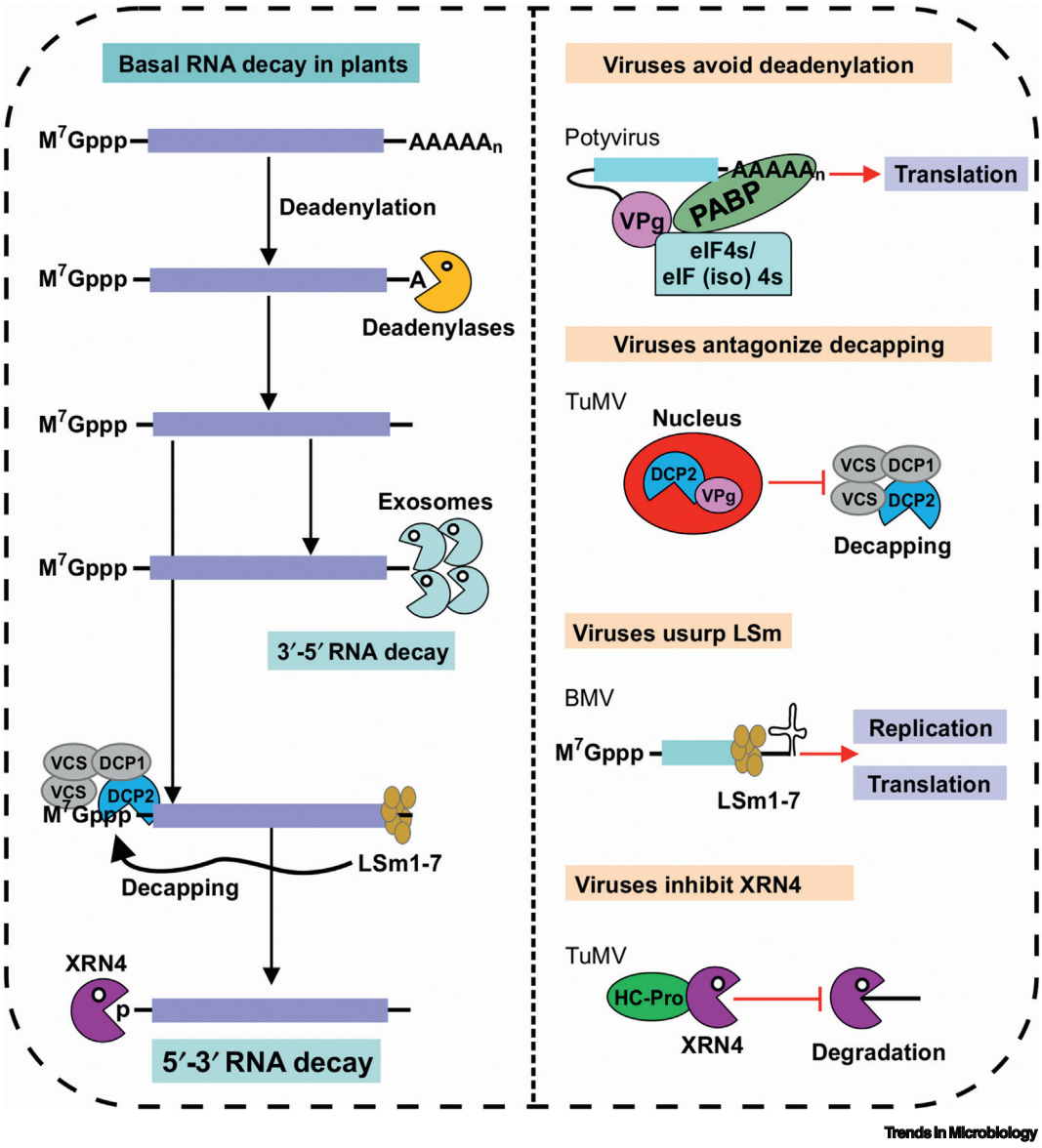

Figure 2. Schematic Representation of the Basic RNA Decay Pathway in Plants and the Strategies Used by Plant Viruses to Avoid, Repress, or Manipulate RNA Decay-Mediated Defenses. Degradation of normal cellular mRNA initiates with removal of the poly (A) tail by the cellular deadenylase complex. Subsequently, the decapping complex is activated by the LSm1-7 protein complex, which binds the 3′ untranslated region (UTR) of the deadenylated RNA and stimulates decapping by the DCP2 enzyme and the concerted action of a set of conserved decapping proteins. Deadenylation or decapping is a prerequisite for most RNA to be degraded by the 3′–5′ exoribonuclease exosome complex or by 5′–3′ XRN4 exoribonucleases, respectively. Several strategies are employed by plant viruses to avoid or inhibit RNA decay. Potyviruses recruit the complex of eIF4 proteins (eIF4s) or their isoforms eIF(iso)4s that interact with poly (A)-binding protein (PABP) to bring the viral RNA ends into proximity for efficient translation, and the occupancy of PABP on poly (A) may prevent deadenylation. The potyviral genome-linked viral protein, VPg, interacts with DCP2, and the interaction complex traffics to the nucleus, which inhibits decapping of viral RNA in the cytoplasm. The potyviral HC-Pro binds to XRN4 and inhibits its slicing activity. The 3′ UTR of brome mosaic virus (BMV) genomic RNA can recruit LSm proteins to repress RNA decay and promote virus replication.

complex via the specific interaction of VPg with eIF4E or its isoform eIF(iso)4E, which is indispensable for potyvirus infection [83]. The eIF4G of the eIF4F complex interacts with eIF4E and the 3′-bound poly(A)-binding protein (PABP), bringing viral RNA ends into proximity for circulation [83]. This interaction cascade not only promotes efficient translation of the potyviral genomes but also avoids deadenylation and RNA decay to enhance viral RNA stability. LSm proteins, which act as

activators of decapping in the 5′–3′-deadenylation-dependent mRNA decay pathway, are essential for the replication of brome mosaic virus (BMV) in yeast and hepatitis C virus (HCV) in human cells [84,85]. The LSms are hijacked to the VRC through specific interactions with the untranslated regions (UTRs) of BMV and HCV genomic RNAs, to possibly facilitate a switch from translation to replication of the viral genome and enhance viral RNA stability by denying access of nucleases to repress RNA decay.

Restriction of Virus Infection by the RNA Quality-Control Pathway, and Viral Counterdefense Strategies

All eukaryotes are equipped with an RNA quality-control system that is comprised of at least three pathways, including **nonsense-mediated decay (NMD)**, **nonstop decay (NSD)**, and **no-go decay (NGD)**, to monitor the quality of mRNAs during translation [86–89]. NMD identifies the mRNA species that contain premature termination codons (PTCs), NSD recognizes mRNAs lacking an in-frame stop codon, and NGD targets the class of mRNAs that contain elongation-inhibiting features such as secondary structures or modified nucleotides. As a translation-coupled RNA surveillance mechanism, NMD removes aberrant transcripts (arising from mutation or defective splicing) with long 3′ UTRs due to PTCs [90]. Translation of these PTC-containing mRNAs is often detrimental to the organism as their translation products can interfere with normal cellular processes, for example, shown as a dominant-negative phenotype [91]. The key NMD components include three up-frameshift proteins UPF1, UPF2, and UPF3, and among them, UPF1 that contains ATPase and RNA helicase domains necessary for NMD serves as the master regulator. After activation, UPF1 recruits nucleases to eliminate NMD-targeted RNAs through SMG7-coordinated exonucleolytic cleavage, and the resulting transacted RNAs may be targeted by XRN4-mediated 5′–3′ degradation and exosome-mediated 3′–5′ degradation [92,93]. NMD may be activated via two known mechanisms: one involving the exon-junction complex (EJC), and the other EJC-independent (Figure 3). To maximize coding potential, RNA viruses often contain internally located termination codons (iTCs), making them ideal targets for NMD [90]. Therefore, NMD is recognized as a general viral restriction mechanism in plants [92,93]. Indeed, NMD recognizes and eliminates potato virus X (PVX) and TCV RNAs containing iTCs and long 3′ UTRs to restrict viral infection [90,94].

To counterattack NMD, viruses must come up with an NMD-inhibitory mechanism to protect themselves (Figure 3). One simple strategy for some viruses is not to have NMD-eliciting signals so as to evade NMD [94,95]. These viruses include many members in the picornavirus-like superfamily, including plant potyviruses, and they use a polyprotein strategy for genome expression. The viral genomes encode a long open reading frame (ORF) and lack subgenomic RNAs (sgRNAs). These features together with their ability to efficiently suppress RNA silencing and RNA decay via VPg and HC-Pro [24,55,74] might make potyviruses successful pathogens and the largest group of all known plant RNA viruses. For other viruses that have NMD-eliciting signals, NMD may be suppressed by viral proteins or other *cis*- and *trans*-acting factors [94]. In mammalian cells, the C terminal region of the semliki forest virus (SFV) nsP3 protein shows a protective role against UPF1-mediated NMD [96]. A recent seminal study by May and colleagues disclosed strong evidence that the TCV genome contains features that are inherently NMD-resistant [90]. The authors identified two genomic segments that confer NMD resistance in *N. benthamiana*. One of them is the ribosome readthrough structure right downstream of the TCV p28 termination codon, which can stabilize an NMD-sensitive reporter, as does a frameshifting element from pea enation mosaic virus 2 (PEMV-2). The other is a 51 nt unstructured region (USR) at the beginning of the TCV 3′ UTR that can protect NMD-sensitive transcripts from NMD when placed downstream of stop codons. This work raises a possibility that integration of *cis* RNA elements downstream of stop codons in the 3′ UTR may be a

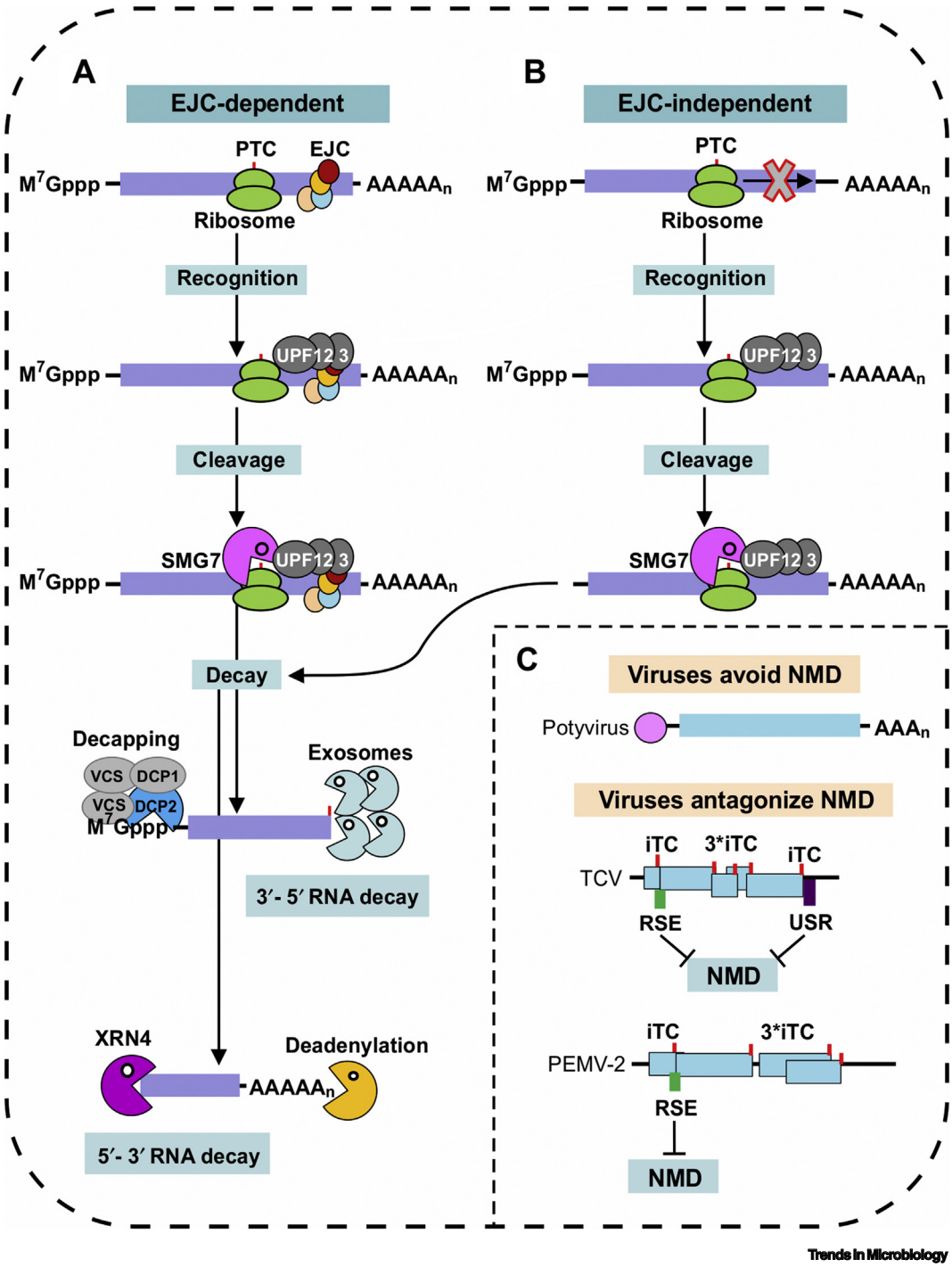

Figure 3. Schematic Representation of the Basic Nonsense-Mediated Decay (NMD) Pathway in Plants and the Strategies Used by Plant Viruses to Avoid or Antagonize NMD-Mediated Defense. NMD recognizes premature translation termination codons (PTCs) by two mechanisms: the exon-junction complex (EJC)-dependent (A) and EJC-independent (B). (A) EJC-dependent NMD. EJCs are deposited on mRNAs during splicing and mark exon–exon junctions. EJCs together with the mRNA from the nucleus are exported into the cytoplasm and then removed from the mRNA during translation. A regular mRNA usually contains a stop codon in its last exon, which leads to no EJCs being left on the mRNA when translation terminates. However, if an mRNA has a premature termination

(Figure legend continued at the bottom of the next page.)

widespread strategy for protecting NMD-targeted transcripts, including endogenous mRNAs or viral RNAs, from NMD [90].

Not much work has been done on the role of NSD and NGD in virus infection. NSD eliminates the 5′ cleavage products of miRNAs- or siRNAs-guided silencing complexes when cleavage occurs in the coding region [88,89,97]. NSD stimulates the degradation of 5′ cleavage products of vsiRISC and thus likely suppresses the RDR6-mediated silencing amplification pathway to negatively regulate the antiviral function of RNA silencing. In line with this suggestion, mutation in the Pelota ortholog, a key component in the NSD pathway in tomato, renders the plant resistant to a begomovirus [87]. However, it remains unclear if NSD and NGD have a direct role in RNA virus infection. Much needs to be done to understand NSD and NGD themselves and their involvement in the context of RNA silencing and NMD.

Concluding Remarks

Plants are constantly attacked by diverse microorganisms, including viruses, and have evolved sophisticated innate immune systems to detect and combat them. RNA-targeted immunity, that embraces RNA silencing, RNA decay, and RNA quality control, emerges as a central defense to viral pathogens. These RNA immunity pathways may execute their antiviral function through concerted actions with a predominant contribution from RNA silencing. In a continuous coevolutionary arms race with their plant hosts, viruses have evolved diverse virulence strategies to effectively cope with these immune responses to establish infection, and in return, plants may activate counter-counter-suppression pathways to mitigate virulence. In the past 20 years, numerous VSRs from various viruses have been identified. Although their RNA-silencing suppression mechanisms are still not fully elucidated, significant progress has been made. Now, we are beginning to understand how viruses counterattack RNA decay- and RNA-quality-control-mediated antiviral defense. We are also starting to understand how host factors are recruited and play functional roles, specifically for RNA-targeted antiviral immunity rather than for endogenous gene regulation. Despite these advances, we have numerous challenging questions to be answered (see Outstanding Questions). For example, symptom recovery that describes the phenomenon 'the emergence of asymptomatic leaves following a systemic symptomatic infection' was first reported in tobacco plants nearly a century ago [98,99]. Symptom recovery has been suggested to be associated with RNA silencing and possibly with vsiRNA spread. It would be interesting to understand how vsiRNAs move intercellularly and further traffic from the local infection site to remote tissues. We also must keep in mind that RNA-based immunity is part of the plant's dimensional immunity system [100]. Thus, RNA-targeted immunity that depends on interlinked synthesis of RNA silencing, RNA decay, and RNA quality control may be influenced by other resistance-signaling pathways in any given plant–virus pathosystem. The relationship between RNA-based immunity and other plant resistance-signaling pathways is another important research direction that needs to be addressed.

Outstanding Questions

How does the plant antivirus surveillance system sense plant viruses to activate RNA-targeted immunity?

How do vsiRNAs spread between cells and move from the local infection site to distant sites?

Can the genomic DNA of plant dsDNA viruses be regulated by RdDM? Are mobile siRNAs methylated or not?

How many host factors are involved in antiviral RNA silencing, and what do they do?

Do active vsiRNAs enter into the virus replication complex to target viral RNA? And if so, how?

Does the RNA decay system directly degrade viral RNA or must it coordinate with RNA silencing?

Aside from RNA silencing, RNA decay, and NMD, do NSD and NGD have any antiviral function, and is there any crosstalk among these RNA-target pathways for their antiviral function?

How many unexplored modes are used by plant viruses to escape or antagonize RNA pathway-mediated slicing and degradation?

codon (PTC) upstream of its last exon, resulting in the pretermination of translation, with one or multiple EJCs downstream of a terminating ribosome remaining on the mRNA, which would be recognized by NMD effectors UPF1, UPF2, and UPF3. UPF1 binds the proteins involved in translation termination, while UPF2 and UPF3 associate with the EJC. The UPF proteins interact to form a complex to recruit an endonuclease, SMG7, to cleave the mRNA into two fragments, followed by degradation of the mRNA. (B) EJC-independent NMD. The interaction complex, including ribosomes at stop codons, releases the factor and proteins bound to the mRNA poly(A) tail required for efficient translation termination. The distance to the 3′-end and poly(A) tail would be too far to form the interaction complex when the termination occurs at a PTC (red cross). This will lead to the delayed release of the ribosome from the mRNA, which allows for the assembly of UPF proteins to recruit SMG7 independently of an EJC. (C) The strategies that plant viruses exploit to avoid or antagonize NMD. For example, potyviruses use a long RNA without internal termination codons (iTCs) to avoid NMD. Turnip crinkle virus (TCV) and pea enation mosaic virus 2 (PEMV-2) could use some *cis*-elements, such as recoding structural element (RSE) and unstructured region (USR), to antagonize NMD.

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