

식물병원시스템인 *Turnip crinkle virus*와 애기장대 상호작용 최신 연구동향 리뷰

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***Turnip crinkle virus*-Arabidopsis interactions as a pathosystem model: recent advances**

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ABSTRACT

Inoculation of *Turnip crinkle virus* (TCV) on the resistant Arabidopsis ecotype Di-17 elicit hypersensitive response (HR), accompanied by increased expression of defense genes. HR to TCV is conferred by HRT, which encodes as CC-NBS-LRR class of resistance (R) protein. In contrast to HR, resistance requires HRT and a recessive locus designated *rrt*. Unlike most CC-NBS-LRR R proteins, HRT-mediated resistance is dependent on EDS1 and independent of NDR1. HRT-mediated HR and resistance are directly dependent on several genes of salicylic acid, light, and RNA silencing. Resistance is also independent of RAR1, SGT1 and TCV-interacting protein but is compromised in salicylic acid (SA) deficient *eds5*, *pad4*, *sid2* or *sag101* mutants. EDS1 and SA fulfil redundant function in HR by HRT. HRT interacts with EDS1, which potentiates HR to TCV and is also required for resistance. HRT-mediated signaling is also dependent on the EDS1-interacting proteins PAD4 and SAG101, which form a complex. HRT-mediated resistance is also dependent on light. Blue-light photoreceptors led to degradation of HRT via a proteasome-dependent pathway and resulted in susceptibility to TCV. In addition, HRT-mediated resistance requires the RNA-silencing components. HRT interacts with double-stranded RNA-binding protein 4 (DRB4). TCV infection both increases the cytosolic DRB4 pool and inhibits the HRT-DRB4 interaction. This review aims to document the advances of TCV-Arabidopsis pathosystem as a case study, and it will provide a useful model for dissecting plant resistance to viral pathogens.

Additional key words: HRT, Photoreceptors, resistance, RNA silencing, salicylic acid, TCV

Introduction

Plant viruses are one of major threat pathogens and causing serious economic losses to the crops. Although plant viruses have simple genetic information, its interactions with plants is largely still unclear. To control viral diseases, several approaches have been used such as crop rotation, destruction of infected sources, breeding for resistance, and chemical control of relevant insect vectors (Hull, 2002)(1). In nature, plants are constantly exposed to viruses. To survive from viruses, plants have developed sophisticated defense mechanisms during the plant-virus interactions. However, interestingly most plants are resistant to plant viruses, which suggests that susceptibility to viral pathogens is the exception rather than the rule.

One of the well-characterized defense mechanism is R protein-mediated resistance, which implies that R protein interacts with its race specific corresponding avirulence (Avr) genes directly or indirectly to activate defense signaling and suppresses growth of pathogens. ("gene-for-gene" interaction [Flor, 1971])(2). This R-Avr interaction triggers at least one defense signaling cascades and is often associates with HR (Hypersensitive Response), a form of programmed cell death, at the site of pathogen entry (Dangl and Jones, 2001)(3), which trigger high expression of defense genes including pathogenesis-related (PR) proteins (Fig. 1). However, increasing evidence suggesting that the HR is not a essential factor for gene-for-gene mediated disease resistance. For example, a visible HR does not develop either in potato during Rx-mediated resistance against *Potato virus X* (PVX) (Chandra-Shekara *et al.*, 2004)(4). Thus, while an HR is frequently associated with resistance, it is not necessarily required for resistance.

R-protein mediated defense response also associates with various plant hormones such as salicylic acid (SA), jasmonic acid (JA), ethylene

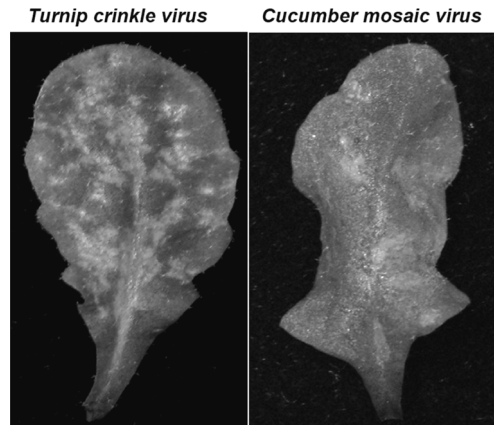


Fig. 1. Hypersensitive response (HR) phenotype on Arabidopsis leaves infected by the *Turnip crinkle virus* (TCV) and *Cucumber mosaic virus* (CMV).

(ET), which in turn signal the activation of defense gene expression. These hormones regulate defense signaling pathways individually, synergistically or antagonistically (Kachroo *et al.*, 2000)(5).

R genes cloned to date have been grouped into six classes regards of their structural characteristics (Martin *et al.*, 2003)(6). Interestingly, most of R genes so far cloned have nucleotide binding site (NBS)-leucine-rich repeats (LRR) type R proteins predominately. Among them, several R genes that confer resistance to plant viruses have been cloned and characterized (Soosaar *et al.*, 2005)(7). Most plant virus resistance genes encoding coiled-coil (CC) or toll-interleukin-1 receptor (TIR) at N-terminus and NBS and LRR domains (Table 1).

Considerable progress had been investigated to understand how plants defend themselves and the mechanisms resistance to viruses (Zaitlin M *et al.*, 2000)(21). However, viral pathogens are still one of major threat and the understanding of viral resistance mechanism is still open question and certainly, more work is needed to elucidate the complex signaling cascade leading to resistance. *Turnip crinkle virus* (TCV)-Arabidopsis pathosystem is one of the few manipulable plant-virus systems

Table 1. List of dominant *R* genes against plant viruses, their protein structure, and corresponding avirulence factors

<i>R</i> gene	Host*	Pathogen [†]	Avr factor [‡]	Predicted domains [§]	Reference
<i>N</i>	<i>N. tabacum</i>	TMV	Helicase	TIR-NBS-LRR	Whitham <i>et al.</i> , 1994(8)
<i>HRT</i>	<i>A. thaliana</i>	TCV	CP	CC-NBS-LRR	Cooley <i>et al.</i> , 2000(9)
<i>RCY1</i>	<i>A. thaliana</i>	CMV	CP	CC-NBS-LRR	Takahashi <i>et al.</i> , 2002(10)
<i>RTM1</i>	<i>A. thaliana</i>	TEV	- [¶]	Jacaline-like	Chisholm <i>et al.</i> , 2000(11)
<i>RTM2</i>	<i>A. thaliana</i>	TEV	-	Small HSP-like	Whitham <i>et al.</i> , 1994(8)
<i>Rx1</i>	<i>S. tuberosum</i>	PVX	CP	CC-NBS-LRR	Bendahmane <i>et al.</i> , 1999(12)
<i>Rx2</i>	<i>S. tuberosum</i>	PVX	CP	CC-NBS-LRR	Bendahmane <i>et al.</i> , 2000(13)
<i>Nb</i>	<i>S. tuberosum</i>	PVX	MP	-	Marano <i>et al.</i> , 2002(14)
<i>Nyibr</i>	<i>S. tuberosum</i>	PVY	-	-	Celebi-Toprak <i>et al.</i> , 2002(15)
<i>Ry</i>	<i>S. tuberosum</i>	PVY	NIaPro	-	Mestre <i>et al.</i> , 2003(16)
<i>Sw-5</i>	<i>L. esculentum</i>	TSWV	-	CC-NBS-LRR	Brommonschenkel <i>et al.</i> , 2000(17)
<i>Tm-1</i>	<i>L. esculentum</i>	ToMV	RdRp	-	Meshi <i>et al.</i> , 1988(18)
<i>Tm-2</i>	<i>L. esculentum</i>	ToMV	MP	CC-NBS-LRR	Lanfermeijer <i>et al.</i> , 2005(19)
<i>Tm-2²</i>	<i>L. esculentum</i>	ToMV	MP	CC-NBS-LRR	Lanfermeijer <i>et al.</i> , 2005(19)
<i>Rsv1</i>	<i>G. max</i>	SMV	HC-pro and P3	CC-NBS-LRR	Hayes <i>et al.</i> , 2004(20)

* *A. thaliana*, *Arabidopsis thaliana*; *N. tabacum*, *Nicotiana tabacum*; *S. tuberosum*, *Solanum tuberosum*; *L. esculentum*, *Lycopersicon esculentum*; *G. max*, *Glycine max*.

[†] TCV, Turnip crinkle virus; CMV, Cucumber mosaic virus; TMV, Tobacco mosaic virus; PVX, Potato virus X; PVY, Potato virus Y; TSWV, Tomato spotted wilt virus; ToMV, Tomato mosaic virus; SMV, Soybean mosaic virus; TEV, Tobacco etch virus.

[‡] CP, coat protein; MP, movement protein; RdRp, RNA dependent RNA polymerase; NIaPro, protease domain of unclear inclusion; HC-Pro, helper component protease.

[§] TIR-NBS-LRR, Toll-interleukin-1 receptor-nucleotide binding site-leucine-rich repeat; CC-NBS-LRR, coiled coil-nucleotide binding site-leucine-rich repeat; HSP, heat shock protein

[¶] Not Known.

in which resistance is conferred via pathogen-induced defense responses and where HR and resistance can be studied as two distinct phenotypes. The TCV-Arabidopsis pathosystem also provides various other advantages for studying compatible plant-virus interactions. This review covers recent advances on TCV-Arabidopsis pathosystem as a model of virus resistance signaling pathway, as well as the mechanism underlying virus resistance,

and its future perspectives.

Plant defense signaling to TCV

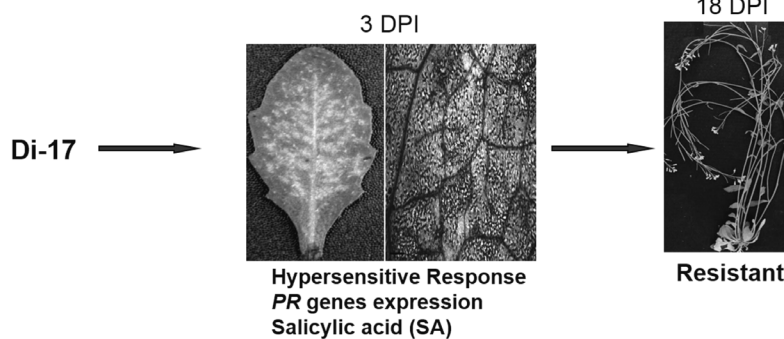
TCV belongs to the icosahedral Carmovirus genus in Tombusviridae family. TCV is a single-stranded positive RNA virus encoding five open reading frames which are required for virus

replication (p28 and p88), movement (p8 and p9), encapsidation and movement (p38, coat protein) and its genome size is 4 kb. The TCV has a broad host range that includes several members of the cruciferous family and shows distinguishable systemic symptoms, such as mottling, leaf distortion and crinkling, chlorotic lesions and stunting (Hacker *et al.*, 1992)(22). Most *Arabidopsis* ecotypes are susceptible to TCV; however, a resistant line, designated Di-17 was isolated from the Dijon (Di) ecotype is resistant to TCV (Dempsey *et al.*, 1993)(23). Following TCV infection, Di-17 plants develop a HR, express several defense genes, including *PR* (*Pathogenesis Related*)-1, *PR*-2, *PR*-5

and *GST1* (*Glutathione S-transferase 1*) and accumulate SA and phytoalexin, camelexin (Kachroo *et al.*, 2000; Dempsey *et al.*, 1993)(5, 23). By contrast, susceptible plants, lacking *HRT*, fail to develop an HR after TCV infection, they also exhibit basal level expression of the *PR* and *GST1* genes and accumulate low levels of SA. These susceptible plants allow systemic spread of TCV in uninoculated leaves and finally show crinkled leaves and drooping bolts symptoms (Fig. 2).

HR and Resistance to TCV is conferred by the dominant resistance protein, designated *HRT* (hypersensitive response to TCV), which is a CC-NBS-LRR type R protein and show identity to

Resistance (Incompatible) Response:



Susceptible (Compatible) Response:

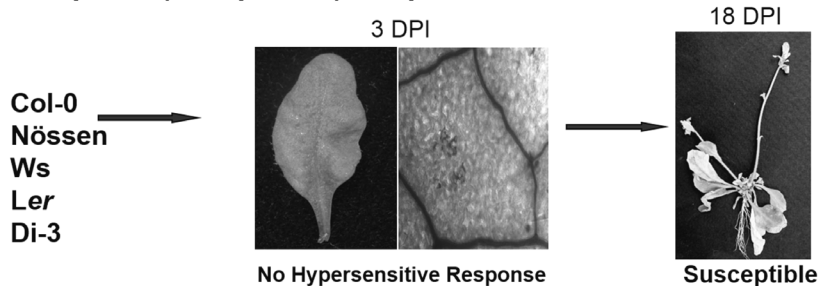


Fig. 2. Biological phenotypes in TCV-inoculated Di-17 and Col-0 plants. Visible HR formation in TCV-inoculated plants at 3 days postinoculation (dpi). Microscopy of trypan blue-stained leaves of TCV-inoculated Di-17 and Col-0 plants. Typical morphological phenotypes of TCV-inoculated Di-17 and Col-0 plants. After inoculation of TCV in Di-17 plants, *PR* genes and SA are increased at 3 dpi. The susceptible Col-0 plants showed crinkling, stunted bolt development, and drooping of bolts. Plants were photographed at 18 dpi.

its paralogs RPP8 and RCY1 conferring resistance to *Hyaloperonospora arabidopsidis* Emco5 and *Cucumber mosaic virus* in Arabidopsis, respectively. HRT-mediated resistance signaling is activated in the presence of TCV coat protein (CP) as an avirulence determinant (Jeong *et al.*, 2008)(24). However, direct interactions between HRT and CP have not been detected. HRT alone is not sufficient for TCV resistance, because all F1 plants and ~75% of F2 plants derived from a cross between resistant (Di-17) and susceptible (Col-0) plants show symptoms to TCV. Moreover, more than 90% of Col-0 plants expressing HRT gene develop normal HR but are still susceptible, suggesting that other loci also might be regulating resistance to TCV. Following studies revealed that the recessive allele of a second locus, as yet unidentified, designated *rrt* (regulates resistance to TCV), is also required for TCV resistance (Chandra-Shekara *et al.*, 2004)(4). Interesting studies suggested that TCV-CP interacts with a protein belongs to the NAC family of transcription activators, which is designated TIP (TCV interacting protein) and prevents nuclear localization of TIP (Ren *et al.*, 2005)(12). These observations suggested that TIP acts as a guard protein, which imply that HRT is activated when CP alters the cellular distribution of TIP. However, we found that the mutation in TIP does not compromise in HRT-mediated resistance to TCV, but does compromise basal resistance to TCV and *Cucumber mosaic virus* (Jeong *et al.*, 2008)(24). In addition, HRT protein is localized to the plasma membrane. This was confirmed using transgenic lines expressing *HRT-GFP* under the *HRT* promoter, where *HRT-GFP* was localized exclusively to the periphery of the cell, compared to GFP alone which was distributed uniformly throughout the cell.

Role of SA in HRT-mediated resistance signaling to TCV

Exogenous application of SA can confer resistance in *HRT RRT*, but not in *hrt RRT* plants. This is because, SA upregulates *HRT* expression, which can overcome the requirement for *rrt* (Chandra-Shekara *et al.*, 2004)(4). SA-mediated upregulation of *HRT* also confers extreme resistance to TCV, wherein viral replication is restricted to the initial few cells, resulting in microscopic HR (Chandra-Shekara *et al.*, 2004; Jeong *et al.*, 2008)(4, 24). This indicates that HRT-mediated resistance is dependent on SA but not on JA and ethylene (Chandra-Shekara *et al.*, 2004; Kachroo *et al.*, 2000)(4, 5). SA is able to synthesized either from phenylalanine *via* the phenylalanine ammonia lyase (PAL) or from chorishmate *via* the shikimate pathway in plants (Wildermuth *et al.*, 2001)(27). The small phenolic compound SA plays a critical role in the disease resistance signaling to different pathogens.

Arabidopsis mutants that are impaired in SA responsiveness, such as *npr1* (nonexpressor of PR), or are defective in pathogen-induced SA accumulation, such as *eds1* (enhanced disease susceptibility), *eds5*, *sid2* (isochorishmatesynthase) and *pad4* (phytoalexin deficient 4) confer enhanced susceptibility to a variety of plant pathogens (Nawrath *et al.*, 2002; Wildermuth *et al.*, 2001)(26, 27). TCV inoculation induces ~10-fold increase in SA levels in Di-17, but not in Col-0, which correlates with high expression of *PR* genes in Di-17 plants. Mutations that compromise SA biosynthesis (*sid2* and *eds5*; Nawrath *et al.*, 2002; Wildermuth *et al.*, 2001)(26, 27) or SA signaling (*eds1*, *pad4*, *sag101*; Fey *et al.*, 2005)(28) impair HRT-mediated resistance to TCV (Fig. 3). Unlike resistance, HR is not affected by *sid2*, *eds5*, *eds1* or *pad4* mutations, suggesting that HR and resistance mutually follow different pathways.

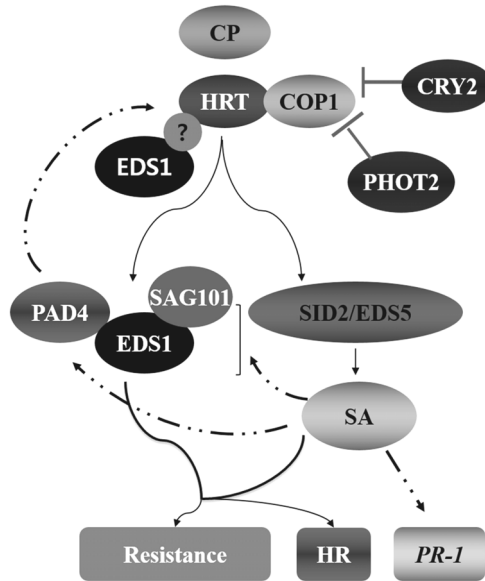


Fig. 3. A scheme of HRT-mediated resistance signaling pathway. TCV-induced defense signaling pathways is initiated upon indirect interaction between dominant resistance protein HRT and TCV's avirulence factor, the coat protein (CP). Upon recognition of TCV, HRT-mediated response leads to HR, accumulation of SA and PR-1 gene expression. Mutations in EDS1, PAD4, SAG101, SID2 and EDS5 do not compromise PR-1 expression. Resistance to TCV is dependent on EDS1, PAD4, EDS5, SID2 and SAG101 genes. EDS1, PAD4 and SAG101 form binary or ternary complexes in nucleus or cytosol. These proteins function independently as well as in a ternary complex to mediate plant defense signaling. EDS1 and SA act redundantly and mutation in EDS1 and SID2 are involved in HRT-mediated HR formation. Exogenous application of SA upregulates expression of the HRT and this step is mediated via PAD4 (dash and dotted line) and EDS1 forms a complex with HRT and associated with HRT-mediated cell death. Absence of light abolishes both HR and resistance to TCV and this in turn is associated with degradation of HRT (Fig. 2). Other blue-light photoreceptors, CRY1 and PHOT1, involved in HRT-mediated resistance, but not HR, PR-1 expression or HRT levels.

EDS1, but not PAD4, acts redundantly with SA to regulate HRT-mediated signaling (Venugopal *et al.*, 2009)(29). TIR-NBS-LRR types of R protein pathway mostly require the EDS1, while CC-NBS-LRR types of R protein pathway utilize NDR1. However, interestingly, HRT protein, which is a CC-NBS-LRR type R protein is dependent on EDS1 but independent of NDR1 (Chandra-Shekara *et al.*, 2004)(4).

Some ecotypes of *Arabidopsis* contain two functional isoforms of EDS1 and these appear to

be functionally redundant (Zhu *et al.*, 2011)(30). EDS1 forms two distinct complexes with PAD4 and SAG101 since direct interaction between PAD4 and SAG101 has not been detected. EDS1 and PAD4 are localized in the nucleus and cytoplasm, whereas SAG101 is dominantly present in the nucleus. A recent study indicated that the EDS1, PAD4 and SAG101 also form a ternary complex and the localization of protein complexes depends on the relative compartmentalization of these three proteins (Zhu *et al.*, 2011)(30). EDS1, but not PAD4 or

SAG101, directly influences HRT-CP-mediated cell death response. Both isoforms of EDS1 interact with HRT, suggesting that EDS1 might participate in the CP-mediated activation of HRT (Zhu *et al.*, 2011)(30).

Role of light in HRT-mediated resistance signaling to TCV

Light is also required for HR and resistance to TCV, since a dark period immediately following TCV inoculation abolishes both HR and resistance (Chandra-Shekara *et al.*, 2006)(31). This suggests that light plays a critical role in HRT-mediated resistance. From the further study, mutations in the blue-light photoreceptors (CRY1, CRY2, PHOT1, and PHOT2), but not red and far-red photoreceptors (PHYA, PHYB, PHYC, PHYD, and PHYE) (Chen *et al.*, 2004)(32), impaired resistance to TCV. In case of other pathosystem, *phyA* or *phyB* compromise the ability to induce localized cell death at the site of pathogen entry and also repressed in the SA-induced expression of the *PR* genes. Moreover, recent studies suggested that PHYA PHYB is compromised for systemic acquired resistance (SAR) (Griebel *et al.*, 2008)(33). In addition to red-light photoreceptors, blue-light photoreceptors are known to participate in plant defense to bacterial pathogen. For example, CRY1 positively regulates RPS2-mediated resistance and SAR under continuous light (Wu *et al.*, 2010)(34). Analysis of HRT protein levels in *cry1*, *cry2*, *phot1* or *phot2* plants showed significant reductions in HRT levels in *cry2* and *phot2* plants, but not in *cry1* or *phot1* plants. Thus, the CRY2 and PHOT2 proteins likely contribute to HRT-mediated resistance by affecting the stability of the HRT protein. Interestingly, exogenous SA was able to enhance TCV resistance in *HRT cry1*, *HRT phot1* and *HRT phot2* plants, but not in *HRT cry2* plants.

HRT stability also correlated with the presence of CRY2, which is degraded in a blue-light specific manner; exposure to blue-light resulted in degradation of both CRY2 as well as HRT. Pretreatment with the 26S proteasome-specific inhibitor, MG132, significantly inhibited the blue light-mediated degradation of the HRT-FLAG protein. Consistent with these results, HRT interacted with the CRY2- and PHOT2-interacting protein, COP1 (Jeong *et al.*, 2010)(35), an E3 ubiquitin ligase involved in 26S proteasome-mediated degradation of proteins. This finding raised hypothesis that under normal light conditions, CRY2 and/or PHOT2 repress COP1-mediated degradation of HRT. On the other hand, in the dark, or under blue-light conditions, CRY2 degradation and possible conformation changes in PHOT2 relieve the repression of COP1 activity. Thereby this enables COP1 to interact with HRT and thus affects HRT degradation (Fig. 3 and 4).

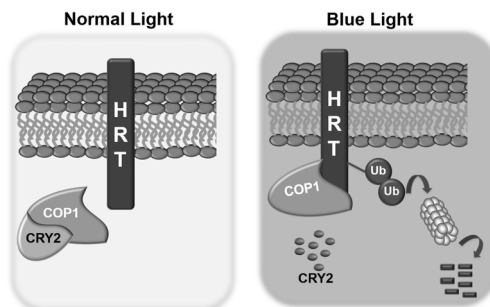


Fig. 4. A working model of CRY2 and PHOT2 involved in HRT-mediated resistance. Under normal light conditions, CRY2 and/or PHOT2 repress COP1-derived degradation of HRT levels via direct interactions with COP1. On the other hands, under blue-light conditions, CRY2 degradation and likely conformational changes of PHOT2 relieve the repression of COP1 activity. This enables COP1 to interact with HRT and thereby targets HRT for degradation.

RNA silencing components mediate resistance signaling against TCV

In the absence of the R protein, plants can induce a basal defense response against pathogens, although this mode of defense fails to contain the pathogen in contrast to *R* gene-mediated resistance. Basal defense to viruses often involves RNA silencing (reviewed in Ding, 2010; Voinnet, 2010)(36, 37). RNA silencing is induced upon the formation of a double stranded (ds) RNA, which is processed to small (s) 20-30 nucleotide (nt) dsRNA with staggered ends. One strand of the dsRNA is then incorporated into a large ribonucleoprotein complex called the RNA-induced silencing complex (RISC), which then cleaves the target viral RNA. The ribonuclease III enzymes called Dicers, mediate the processing of dsRNA into sRNA. Arabidopsis plants encode four Dicer-like proteins (DCL1 to DCL4) and of these DCL2, DCL3, and DCL4 process long dsRNA molecules of various cellular origins into sRNA that are 22, 24 or 21 nt in length, respectively. RISC complexes are formed of sRNA strand and a member of the Argonaute (AGO) protein family, which are also called slicer proteins since they cleave target single stranded RNA at the duplex formed with the guide-strand sRNA. There are ten AGO homologues in Arabidopsis, but the functions of most of these remain unclear. Although emerging results implicate small RNA in R protein-mediated resistance (Navarro *et al.*, 2006; Bhattacharjee *et al.*, 2009)(38, 39), the relationship between R protein-mediated signaling and RNA silencing pathway remains unclear. Viruses in turn have evolved to express suppressors that target host RNA silencing components and thereby ensure replication in the host. Interestingly, in many cases these suppressors of silencing also acts as avr factors and their direct/indirect interaction with the host R proteins leads to activation of defense responses.

Components of the Arabidopsis RNA silencing machinery, including RDR6, DCL4, and DRB4, are required for HRT-mediated resistance and of these HRT, DRB4 and DCL4 are present in a single complex. HRT forms a complex, involving inter- and intra-molecular interactions with itself as well as with several other host proteins including DRB4 and DCL4 (Zhu *et al.*, 2013)(40). The pathogen encoded avr effector, coat protein, specifically prevents HRT-DRB4 interaction and consequently HRT-DRB4-DCL4 complex formation. Notably, CP does not disrupt interactions between DRB4-DCL4 or HRT-HRT. TCV CP mutant R8A, which is able to induce HRT-mediated HR and *PR-1* expression, can also prevent HRT-DRB4 interaction. However, unlike wild-type TCV, R8A virus remains virulent on Di-17 plants. This suggests that the dissociation of the HRT-DRB4 complex alone may not be sufficient for the activation of HRT. The fact that DRB4 directs CP, but not the R8A and R6A CP mutants, to the nucleus, correlates with the loss of avirulence in the R8A and R6A mutants on Di-17 plants. This result further suggests that the nuclear localization of CP might be important for the resistance response. The reciprocal ability of CP alone to redirect DRB4 to the nucleus and periphery further suggests that the resistance response might involve the redistribution of cellular proteins, and that the combined effects of these events might contribute to prevent the spread of the virus (Fig. 5).

Conclusions

To date, plant viruses are still a major threat to the crops and causing serious economic losses worldwide. Several decades ago, viral pathogens have been mostly controlled by conventional and chemical protection approaches. To further control of plant viral disease, tremendous progress has been

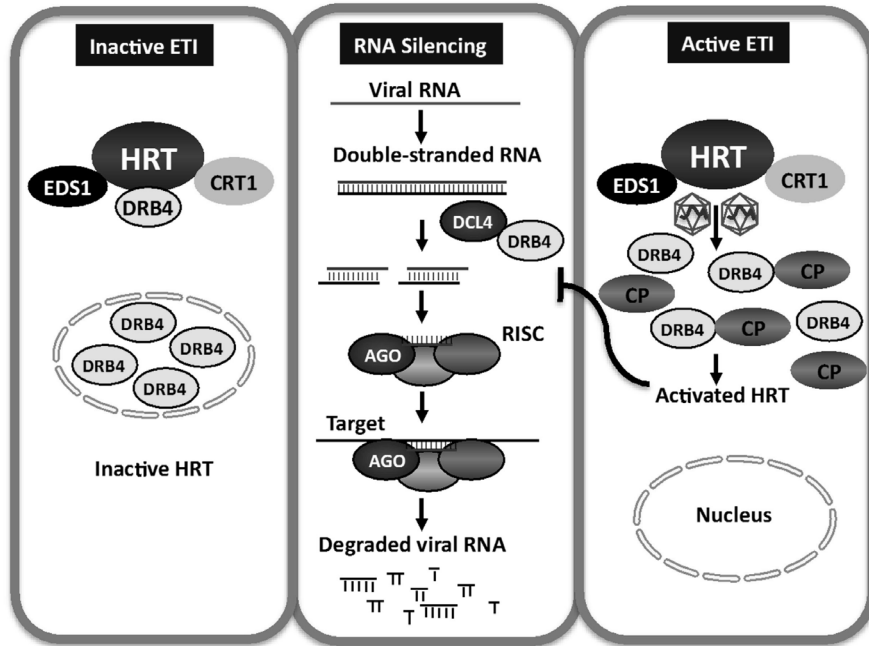


Fig. 5. DCL4 and DRB4 are required for HRT-mediated resistance. HRT, DRB4 and DCL4 are present in a single complex. The pathogen encoded avr effector, coat protein, specifically prevents HRT-DRB4 interaction and consequently HRT-DRB4-DCL4 complex formation. Notably, CP does not disrupt interactions between DRB4-DLC4 or HRT-HRT. The dissociation of the HRT-DRB4 complex alone may not be sufficient for the activation of HRT. The nuclear localization of CP might be important for the resistance response. The reciprocal ability of CP alone to redirect DRB4 to the nucleus and periphery further suggests that the resistance response might involve the redistribution of cellular proteins.

made towards improving our understanding of plant viruses and the ways in which plants defend themselves against these pathogens. Our ever-increasing knowledge of the molecular mechanisms that restrict or allow viral infection has successfully been used to develop antiviral strategies. Despite a great deal of recent insights into the mechanisms by which R proteins recognize pathogens in general, and viruses in particular, there are still many gaps in our knowledge of how these proteins function and a great deal of room for further study. Understanding resistance associated signal transduction pathway to TCV and characterizing key genes involved in TCV resistance will be very useful in engineering virus resistant crops. Further

exploitation of HRT-mediated resistance mechanisms, as well as understanding with unknown defense signaling, will provide new potential strategies to viral pathogens. In turn, these insights may lead to better strategies for control of diseases caused by viruses and other plant pathogens.

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