Chapter 1
Antiviral Silencing and Suppression of Gene Silencing in Plants

Tibor Csorba and József Burgyán

Abstract RNA silencing is an evolutionary conserved sequence-specific gene inactivation mechanism that contributes to the control of development, maintains heterochromatin, acts in stress responses, DNA repair and defends against invading nucleic acids like transposons and viruses. In plants RNA silencing functions as one of the main immune systems. RNA silencing process involves the small RNAs and trans factor components like Dicers, Argonautes and RNA-dependent RNA polymerases. To deal with host antiviral silencing responses viruses evolved mechanisms to avoid or counteract this, most notably through expression of viral suppressors of RNA silencing. Due to the overlap between endogenous and antiviral silencing pathways while blocking antiviral pathways viruses also impact endogenous silencing processes. Here we provide an overview of antiviral silencing pathway, host factors implicated in it and the crosstalk between antiviral and endogenous branches of silencing. We summarize the current status of knowledge about the viral counter-defense strategies acting at various steps during virus infection in plants with the focus on representative, well studied silencing suppressor proteins. Finally we discuss future challenges of the antiviral silencing and counter-defense research field.

Keywords RNA silencing • Virus infection • Antiviral defense • Silencing suppressor strategies • Host-pathogen interaction

1.1 RNA Silencing

1.1.1 Introduction

RNA silencing is a sequence-specific gene-inactivation mechanism conserved from lower eukaryotes to mammals (Shabalina and Koonin 2008; Weiberg and Jin 2015). RNA silencing, also known as RNA interference (RNAi), has diverse functions

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including growth and developmental regulation, DNA repair, biotic and abiotic stress response or host immunity against invading nucleic acids like transposons or viruses (Castel and Martienssen 2013; Martinez de Alba et al. 2013; Pumplin and Voinnet 2013). The trademark molecules of silencing are the small RNAs (sRNAs) (Hamilton and Baulcombe 1999) of 21–24 nt length. These guide the sequence-specific effector steps either at transcriptional or at post-transcriptional levels. During transcriptional gene silencing (TGS) target genes are inhibited by epigenetic modification of chromatin (e.g histone protein post-translational modifications and DNA methylation) (Castel and Martienssen 2013) while during post-transcriptional gene silencing (PTGS) gene inactivation occurs through mRNA cleavage or translational repression (Martinez de Alba et al. 2013). Depending on the sRNA type and effector proteins involved, silencing pathways provide diverse and dedicated functions.

1.1.2 Biochemical Framework of Silencing

RNA silencing process can be partitioned mechanistically into three distinct phases: initiation phase, effector phase and in some specific circumstances amplification phase. Most of the knowledge comes from the model plant *Arabidopsis thaliana*, therefore the nomenclature of components relays on these components.

1.1.2.1 Initiation of Silencing

Initiation of silencing comprises of two main steps: biogenesis of sRNAs and their loading into effector complexes. The trigger of silencing initiation is always a double-stranded RNA (dsRNA) molecule present within the cell: perfect or imperfect dsRNA structures can be formed by single-stranded RNA (ssRNA) transcripts folding into a hairpin secondary structure, may come from the inter-molecular interaction of two partially reverse complementary single-stranded RNAs (ssRNAs) produced either by convergent transcription from the sense and antisense strands of the loci (in cis) or by pairing of homologue regions of transcripts originating from different loci (in trans). Alternatively, perfectly complementary dsRNAs may arise as the product of RNA-dependent RNA polymerases by conversion of ssRNA molecules into dsRNAs. The dsRNA molecules/regions are recognized by a member of the RNase III type enzyme family DICERS, in plants named DICER-LIKE proteins, (DCLs, in *Arabidopsis* DCL1-4) (Bernstein et al. 2001; Hamilton and Baulcombe 1999; Hutvagner et al. 2001). DCLs contain a helicase, a PAZ, two RNase-III and two dsRNA-binding domains. The PAZ and RNA-binding domains position the dsRNA substrate in such a way that the two RNase-III pseudo-dimers catalyzes processing of the dsRNA molecules/regions into sRNA duplexes of 21–24 nt lenght, with specific 2-nt-long 3′ overhangs (having 5′-P and 3′-OH ends).

For the accurate and effective excision of sRNAs from their precursor molecules DCLs require cooperation of DOUBLE-STRANDED RNA BINDING proteins (DRB, in *Arabidopsis* DRB1-5). Sometimes specific DCL-DRB interaction is required for the transfer of sRNA duplex into specific effector complexes (Eamens et al. 2012a, b; Han et al. 2004; Hiraguri et al. 2005). Following processing, the sRNAs are stabilized at their 3′ end by the HUA Enhancer 1 (HEN1)-dependent 2′-O-methylation (a process found only in plants and flies so far) (Boutet et al. 2003; Yang et al. 2006) and exported from the nucleus to the cytoplasm by HASTY (HST), the homologue of mammalian exportin-5 (Bollman et al. 2003; Park et al. 2005; Peragine et al. 2004) to be loaded into effector complexes. It is believed that methylation may occur both in the nucleus and cytoplasm (Lozsa et al. 2008).

1.1.2.2 Effector Phase of Silencing

The essential catalytic components of effector complexes of silencing are the Argonaute proteins (AGOs, in *Arabidopsis* AGO1-10), RNase-H type endonucleases (Fagard et al. 2000; Hammond et al. 2001; Hutvagner and Simard 2008; Liu et al. 2004; Mallory and Vaucheret 2010). AGOs together with accessory proteins form the effector complex of silencing: the RNA-Induced Silencing Complex (RISC) that acts during PTGS (Lee et al. 2004; Pham et al. 2004; Tomari et al. 2004), or the RNA-Induced Transcriptional Silencing Complex (RITSC) that acts during TGS (Castel and Martienssen 2013; Ekwall 2004). RISC/RITSC assembly comprises of two clearly distinguishable steps: (i) loading of ds-sRNAs and (ii) unwinding of sRNAs (Kwak and Tomari 2012). Biogenesis and loading of ds-sRNAs seems to be coupled (at least in the case of miRNAs) (Reis et al. 2015). AGO-loading process requires Hsp70-Hsp90 chaperone machinery and ATP hydrolysis to drive AGO conformational changes. The size and the 5′ nucleotide type contributes to the sorting of sRNAs into specific AGO partners (e.g 21-nt-long 5′ U sRNAs are preferentially loaded into AGO1 etc.) (Mallory and Vaucheret 2010). The strand having less stable 5′-end pairing (within the ds-sRNA molecule) is retained within the AGO while the other, the so-called "star" strand is eliminated (Khvorova et al. 2003; Schwarz et al. 2003). Guided by the sRNA sequence, RISC induces slicing or translational repression of its target RNAs (during PTGS) in a sequence-specific manner (Brodersen and Voinnet 2009; Kim et al. 2014). The cleavage products of RISC are eliminated by the general mRNA decay and quality control machinery present within the cell (Martinez de Alba et al. 2015; Parent et al. 2015b; Ren et al. 2014; Souret et al. 2004; Yu et al. 2015). RITSC complex causes histone and/or DNA methylation, resulting in transcriptional gene silencing (TGS) of the homologous gene (Castel and Martienssen 2013; Creamer and Partridge 2011). AGO1, 2, 3, 5, 7 and 10 have roles in PTGS while AGO4, 6 and 9 are involved in TGS (AGO8 is considered as pseudo-gene) (Mallory and Vaucheret 2010).

1.1.2.3 Amplification of Silencing

Cytoplasmic RNA silencing may be activated also by the presence of RNAs having aberrant features (without CAP-structure, lacking polyA tail etc.) or endonucleolytically cleaved RISC fragments. RNA-DEPENDENT RNA POLYMERASES (RDRs, in *Arabidopsis* RDR1, 2, 3a, 3b, 3c and 6) (Wassenegger and Krczal 2006) protein recognize these molecules as their substrates and convert them into dsRNAs that enter/re-enter into silencing through DCL-mediated sRNA production. RDR6 is the main cytoplasmic enzyme to be involved in this process (Branscheid et al. 2015; Martinez de Alba et al. 2015; Mourrain et al. 2000; Parent et al. 2015b; Sijen et al. 2001; Vaistij et al. 2002; Voinnet et al. 1998). Usage of RISC cleavage products by RDRs results in amplification of silencing response that may have also non-cell-autonomous consequences.

AGO-mediated target cleavage and amplification by RDR enzymes are intimately linked in the nuclear TGS as well. RNA polymerase IV (PolIV, a plant specific polymerase) transcribes short precursor ssRNAs from loci to be silenced. RDR2 physically associates with PolIV to convert its transcripts into dsRNA. DCL3 cleaves the dsRNA to produce sRNAs that are loaded mainly into AGO4 (alternatively AGO6 or 9). AGO4 associates with accessory proteins to form RITSC. Guided by the sRNA, RITSC is tethered to nascent transcripts synthetized by RNA polymerase V (PolV) and induce silencing of the target loci by recruiting histone and/or DNA modification complexes (Castel and Martienssen 2013).

1.1.3 Endogenous Pathway Diversification

The combined activities of specific (sometimes partially redundant) trans factors of silencing (DCLs, DRBs, AGOs and RDRs) and the involvement of different sRNA precursor molecules result in parallel gene silencing pathways (Bologna and Voinnet 2014; Hiraguri et al. 2005; Mallory and Vaucheret 2010; Wassenegger and Krczal 2006). These pathways rely on various sRNAs like microRNAs (miRNAs), trans-acting small interfering RNAs (ta-siRNAs), natural-antisense RNAs (nat-siRNAs), repeat-associated siRNAs (ra-siRNAs), viral siRNAs (vsiRNAs) and virus-activated siRNAs (vasiRNAs) and provide dedicated functions/roles of silencing (Martinez de Alba et al. 2013).

1.1.4 Systemic Silencing

Amplification of RNA silencing has been implicated in the spread of an RNA silencing signal (Kalantidis et al. 2008; Molnar et al. 2010, 2011; Schwach et al. 2005). Small RNAs of 21–24 nt lengths generated during cell-autonomous

RNA silencing spread from the site of initiation to the neighboring cells through plasmodesmata. Besides this, silencing signal is able to spread systemically over long distances through the phloem. The exact nature of silencing signal is not clear, although sRNAs are known to be involved; sRNAs may be associated with proteins (e.g AGOs) during translocation that could protect them against cellular nucleases. Mobile sRNAs, similarly to their cell-autonomous counterparts, are able to trigger transcriptional or post-transcriptional silencing. It was shown that silencing signal movement has roles in the formation of patterns within a tissue (e.g. leaf polarity) (Chitwood et al. 2009), contributes to the reinforcement of transposon silencing in generative cells (Borges et al. 2011; Slotkin et al. 2009), initiate epigenetic events during genome defense (Cui and Cao 2014) and respond to external stimuli (Katiyar-Agarwal et al. 2006). Silencing signal movement has also important implication in antiviral defense and plant recovery (Havelda et al. 2003; Szittya et al. 2002).

1.2 Antiviral Roles of RNA Silencing

1.2.1 Introduction

The antiviral function of RNA silencing was demonstrated in plants and invertebrates (Bronkhorst and van Rij 2014; Pumplin and Voinnet 2013). Recent reports have provided evidence that antiviral silencing also operates in mammals, especially in ESC cells, however its role still remains controversial (Castel and Martienssen 2013; Cullen et al. 2013; Maillard et al. 2013). Is it believed that the ancient function of silencing was the antiviral defense itself (Pumplin and Voinnet 2013; Wang and Metzlaff 2005). Specific members of DCL's, DRB's, AGO's, and RDR's contribute to the antiviral pathway during the various host-virus combinations (see Table 1.1 and relevant references within).

1.2.2 Biogenesis of vsiRNAs

As one of the first sRNA type discovered, the existence of vsiRNAs provided the first hint that silencing may have antiviral roles (Hamilton and Baulcombe 1999). Biogenesis of vsiRNAs requires DCL enzymes. Viral substrate molecules for DCLs vary depending on the virus replication strategy. In case of RNA viruses the highly structured fold-back regions of viral single-stranded RNAs (ssRNA) and replicative intermediates (RI) may be the primary source of vsiRNA production (Ahlquist 2002; Donaire et al. 2009; Molnar et al. 2005; Szittya et al. 2010; Kontra et al. unpublished). In case of DNA viruses the overlapping convergent/bidirectional read-through transcripts or fold-back structure of specific regions of RNA

Table 1.1 Plant silencing components with antiviral activity

	Virus	Host	DCLs	DRBs	RDRs	AGOs	References
ssRNA (+)	BMV	Arabidopsis thaliana	DCL2, 4		RDR6	AGO1	Dzianott et al. (2012)
	CMV	Arabidopsis thaliana	DCL1, 2, 3, 4		RDR1, 6	AGO1, 2, 4, 5	Bouche et al. (2006), Diaz-Pendon et al. (2007), Wang et al. (2010, 2011), Morel et al. (2002), Harvey et al. (2011), Takeda et al. (2008), and Hamera et al. (2012)
	CymRSV	Nicotiana benthamiana				AGO1, 2	Scholthof et al. (2011) and Kontra et al. (submitted)
	PVX	A. thaliana, N. benthamiana	DCL2, 4		RDR6	AGO2, 4, 5	Schwach et al. (2005), Bouche et al. (2006), Andika et al. (2015), Brosseau and Moffett (2015), Fatyol et al. (2016), and Bhattacharjee et al. (2009)
	RYMV	Oryza sativa	DCL4				Lacombe et al. (2010)
	TBSV	Nicotiana benthamiana				AGO1, 2	Scholthof et al. (2011), Odokonyero et al. (2015), and Schuck et al. (2013)
	TCV	Arabidopsis thaliana	DCL2, 4	DRB4		AGO1, 2, 7	Qu et al. (2008), Harvey et al. (2011), and Zhang et al. (2012);
	TMV	Arabidopsis thaliana			RDR1, 6		Qi et al. (2009)
	ToRSV	Nicotiana benthamiana				AGO1	Ghoshal and Sanfacon (2014)
	TRV	Arabidopsis thaliana	DCL 2, 3, 4		RDR1, 2, 6	AGO2, 4	Fusaro et al. (2006), Donaire et al. (2008), and Ma et al. (2015)
	TuMV	Arabidopsis thaliana	DCL2, 4		RDR1, 2, 6	AGO1, 2, 5, 7, 10	Garcia-Ruiz et al. (2010, 2015) and Carbonell et al. (2012)
	TYMV	Arabidopsis thaliana	DCL4	DRB4			Jacubiec et al. (2012)
ssRNA (−)	RSV	Oryza sativa			RDR6	OsAGO1, 18	Jiang et al. (2012) and Wu et al. (2015)
	TSWV	Arabidopsis thaliana	DCL4	DRB4			Curtin et al. (2008)

dsRNA	OsEV	Oryza sativa	DCL2, 4				Urayama et al. (2010)
	RDV	Oryza sativa			RDR6	OsAGO1, 18	Hong et al. (2015) and Wu et al. (2015)
ssDNA	BCTV	Arabidopsis thaliana	DCL3	DRB3		AGO4	Raja et al. (2014)
	CaLCuV	Arabidopsis thaliana	DCL1, 2, 3, 4	DRB3	RDR1,2,6-indep.	AGO4	Blevins et al. (2006), Aregger et al. (2012), and Raja et al. (2014)
	TYLCV	Nicotiana benthamiana	DCL2, 3				Akbergenov et al. (2006)
dsDNA	CaMV	Arabidopsis thaliana	DCL1, 2, 3, 4	DRB4	RDR1,2,6-indep.	AGO1, 4	Blevins et al. (2006, 2011) and Raja et al. (2014)

Specific plant DCLs, DRBs, RDRs, and AGOs known to contribute to the antiviral pathway in various host-virus combinations. For virus name abbreviations please see the glossary

transcripts contribute to vsiRNA biogenesis (Akbergenov et al. 2006; Aregger et al. 2012; Blevins et al. 2006, 2011; ; Chellappan et al. 2004) (Fig. 1.1). Genetic studies and deep sequencing analysis of vsiRNAs involving *Arabidopsis dcl* mutants revealed that a strong hierarchy exists between DCLs regarding their contribution to vsiRNA production. The main DCL in case of RNA virus infections is the DCL4 while DCL2 becomes critical in its absence (in *dcl4* mutant) (Andika

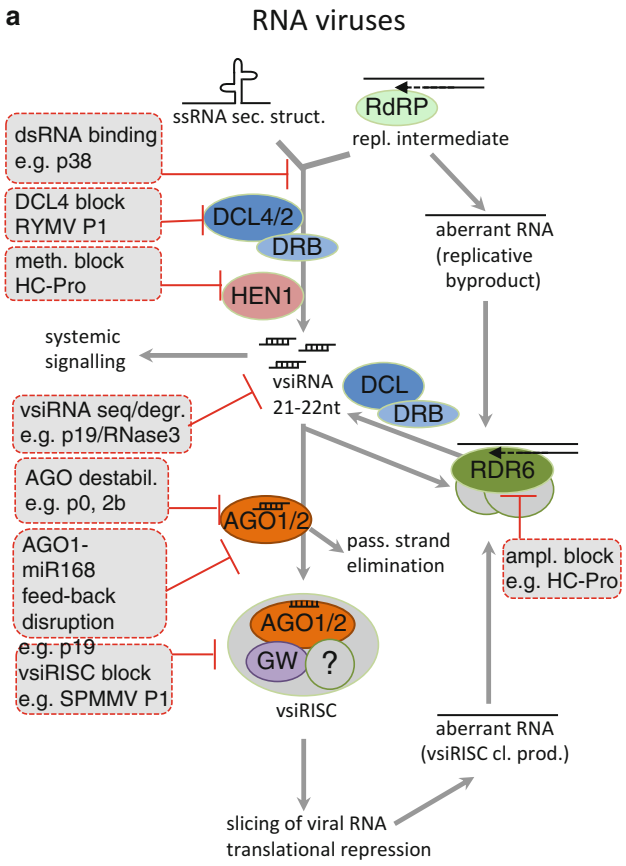

Fig. 1.1 Biochemical framework of antiviral RNA silencing and its suppression by VSRs. Antiviral RNA silencing is initiated by the recognition of viral dsRNA structures (replicative intermediate, partially double-stranded fold-back structures or overlapping RNA transcript pairing), which are processed into viral siRNAs (vsiRNAs) by Dicer-like proteins (DCLs). Subsequently vsiRNAs 21–22 nt or 24 nt long are incorporated into effector complexes RNA-induced silencing complex (RISC) or RNA-Induced transcriptional Silencing Complex (RITSC), respectively. *Question mark* represents unknown cofactors. The vsiRISC targets viral RNAs by slicing or translational inhibition (**a**), while RITSC induces genome modification (**b**). Cleavage producs and vsiRNA may enter an amplification loop through the actions of RNA-dependent RNA polymerases (RDRs) and cofactors (SGS3 and SDE5) to give rise of secondary vsiRNAs. Antiviral silencing pathway may be halted at various points by viral silencing suppressors (VSRs) (**a** and **b**) (*dash-line boxes*)

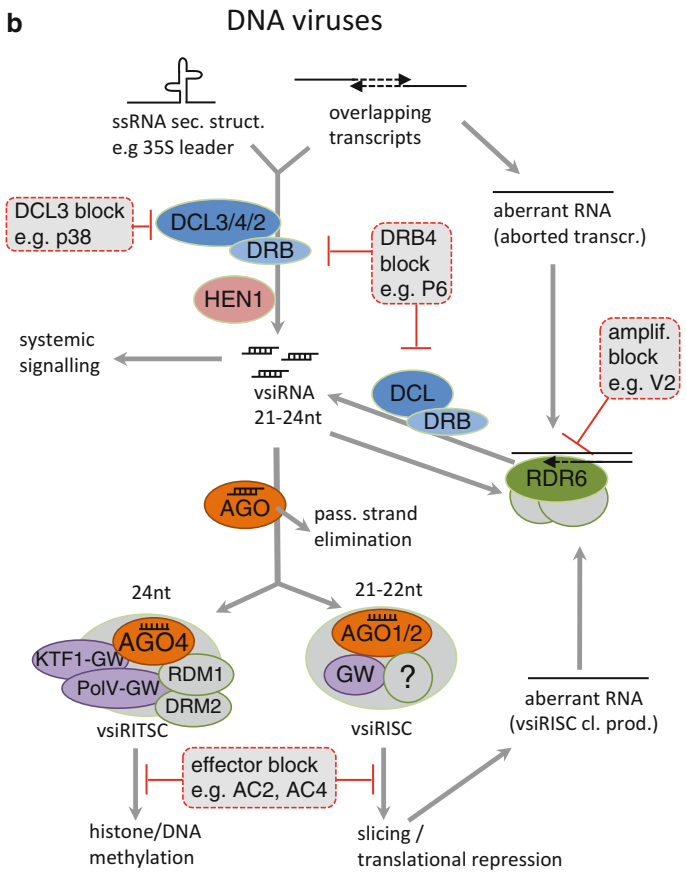

Fig. 1.1 (continued)

et al. 2015; Deleris et al. 2006; Donaire et al. 2009; Dzianott et al. 2012; Garcia-Ruiz et al. 2010; Qu et al. 2008; Urayama et al. 2010). Additional functional diversity between DCL4 and DCL2 has been reported: DCL2 stimulates transitivity and secondary siRNA production, while DCL4 is sufficient for silencing on its own (Parent et al. 2015a). DCL3 has only a minor role against RNA viruses (Qu et al. 2008; Raja et al. 2014). The fact that silencing suppressors of RNA viruses interfere with DCL3 pathway suggests that DCL3 contributes to antiviral silencing (Azevedo et al. 2010; Hamera et al. 2012; Lacombe et al. 2010). During antiviral silencing against DNA viruses DCL3 is essential and works presumably by inducing chromatin modifications (Akbergenov et al. 2006; Blevins et al. 2006; Raja et al. 2014). DCL1 may act as a negative regulator limiting DCL4 and DCL3 through miRNA pathway (Azevedo et al. 2010; Qu et al. 2008).

DCLs' cofactors, the DRB proteins are also required for vsiRNA biogenesis. DRB4, the cofactor of DCL4, takes part in antiviral defense against RNA viruses (Curtin et al. 2008; Jakubiec et al. 2012; Qu et al. 2008). The observation that P6

silencing suppressor of *Cauliflower mosaic virus* (CaMV) inhibits DRB4, strongly suggests that DRB4 is an antiviral factor against DNA viruses as well. DRB3, the cofactor of DCL3, contributes to antiviral defense through chromatin modification against DNA viruses (Raja et al. 2014).

HEN1-mediated vsiRNA methylation is critical for effective antiviral defense (Vogler et al. 2007). *hen1* mutants are more susceptible to *Cucumber mosaic virus* (CMV) and *Turnip crinkle virus* (TCV) virus infections (Boutet et al. 2003; Zhang et al. 2012). vsiRNA-binding viral silencing suppressor were shown to inhibit methylation (Csorba et al. 2007; Lozsa et al. 2008).

1.2.3 Effector Step of Antiviral Silencing

Dicing *per se* is not sufficient for an efficient antiviral silencing response (Wang et al. 2011), suggesting that the DCLs' substrates may be only the byproducts of the viral replication process. vsiRNA-binding VSRs do not compromise dicing, but efficiently inhibit antiviral silencing (Csorba et al. 2015). The downstream AGO-dependent effector step is therefore necessary to restrict virus replication and spread of both RNA and DNA viruses (Azevedo et al. 2010; Carbonell et al. 2012; Harvey et al. 2011; Pantaleo et al. 2007; Qu et al. 2008; Raja et al. 2014; Raja et al. 2008; Wang et al. 2011). The properties of vsiRNAs like 5′-nucleotide, length, thermodynamical properties of sRNA duplex ends and sRNA's duplex structure define loading and sorting into AGO effectors (Khvorova et al. 2003; Mi et al. 2008; Schuck et al. 2013; Schwarz et al. 2003; Zhang et al. 2014; Kontra et al. unpublished). During RNA virus infections AGO1 and AGO2 are the most important effectors, while AGO5, 7, 10 may have additional roles or act during specific host-virus combinations (Carbonell and Carrington 2015) (Fig. 1.1).

AGO1 was identified as the main effector against *Brome mosaic virus* (BMV), CMV, TCV, *Turnip mosaic virus* (TuMV) in *Arabidopsis thaliana* (Dzianott et al. 2012; Garcia-Ruiz et al. 2015; Morel et al. 2002). AGO1 participate in removal of TuMV viral RNA through slicing activity (Carbonell et al. 2012). AGO1 translational repression activity was also found to play a role during *Tomato ringspot virus* (ToRSV) infection in *Nicotiana benthamiana* (Ghoshal and Sanfacon 2014). It was shown that during RNA virus infections AGO1 homeostasis (Mallory et al. 2008) is disrupted and AGO1 protein levels are decreased probably through translational repression of AGO1 mRNA by miR168 activity (Varallyay et al. 2010). *Arabidopsis ago1* and *ago2* mutants are hypersusceptible to CMV, TuMV and TCV (Carbonell et al. 2012; Harvey et al. 2011; Morel et al. 2002; Takeda et al. 2008). As AGO1 is the negative regulator of AGO2 through miR403 action, in the absence of AGO1 activity AGO2 levels are elevated (Azevedo et al. 2010; Harvey et al. 2011). AGO2 therefore emerges as a second layer in antiviral pathways. AGO2 was shown to be important in defense against CMV, TCV and *Potato virus X* (PVX) viruses in *A. thaliana* (Brosseau and Moffett 2015;

Harvey et al. 2011; Jaubert et al. 2011). The phenotype of *ago1ago2* double mutant indicates that the two proteins act in a synergistic manner and have non-overlapping functions, as suggested by their phylogenetic distance (Mallory and Vaucheret 2010; Wang et al. 2011).

Our knowledge about the function of AGO proteins during PTGS in species other than Arabidopsis is much limited due to lack of genetic tools. In *N. benthamiana* it was shown that AGO2 protects against TBSV, TMV, PVX, *Cucumber necrosis virus* (CNV) and *Cymbidium ringspot virus* (CymRSV) (Fatyol et al. 2016; Odokonyero et al. 2015; Scholthof et al. 2011). Recently, however AGO1 was proposed to be the essential effector against CymRSV (Kontra et al., unpublished) and is also required for recovery during ToRSV infection (Ghoshal and Sanfacon 2014).

In rice there are 19 AGOs categorized into four clades (Nonomura et al. 2007). Genetic and biochemical data suggest that in rice the AGO1 and AGO18 are the main antiviral effectors against *Rice stripe virus* (RSV), *Rice dwarf phytoreovirus* (RDV) (Hong et al. 2015; Jiang et al. 2012; Wu et al. 2015). AGO18 is induced during virus infection and may confer a broad-spectrum resistance: AGO18 do not bind efficiently vsiRNAs, instead, by sequestration of miR168 it interferes with AGO1 homeostasis. This action leads to elevated levels of AGO1 required for antiviral defense (Wu et al. 2015).

Effectors AGO4, 5, 7 and 10 were also proposed to possess additional antiviral roles against RNA viruses. CMV 2b silencing suppressor protein directly interacts with AGO4 and inhibits its slicer activity and methylation and thus creates a favorable niche for CMV proliferation (Hamera et al. 2012). AGO5 (besides AGO2) was shown to be required to inhibit PVX systemic infection (Brosseau and Moffett 2015). AGO7 seems to work as a surrogate of AGO1 but with a preference for the less structured RNA targets (Qu et al. 2008; Takeda et al. 2008). AGO5, 7 and 10 had minor contribution in leaves while AGO10 (alongside AGO1) had antiviral functions in inflorescence during systemic TuMV infection (Garcia-Ruiz et al. 2015).

The nuclear localized AGO4 has been shown to possess important antiviral functions against geminiviruses. *Arabidopsis dcl3, drb3* and *ago4* mutants fail to hypermethylate the viral genome that is required for host recovery (Raja et al. 2014). Besides, AGO4 was proposed to be important in transcriptional regulation of host transcriptional response during CMV virus infection (Hamera et al. 2012) or to be involved in PVX virus resistance induced by NB-LRR proteins involving AGO4-mediated translational control (Bhattacharjee et al. 2009).

AGOs loaded with vsiRNAs are able to form high molecular weight complexes (Csorba et al. 2010; Pantaleo et al. 2007). The knowledge about RISC (including antiviral RISC, vsiRISC) cofactors that cooperate with AGOs in plants is very limited (Omarov et al. 2016). Heat shock protein 70 and 90 (HSP70, HSP90) have been found to be important players in AGO loading by using an *in vitro* cell-free system that recapitulates the loading process (Iki et al. 2010). Further understanding of RISC components, assembly and function may be helped by *in vitro* and transient

sensor systems (Fatyol et al. 2016; Iki et al. 2010; Omarov et al. 2016; Schuck et al. 2013).

1.2.4 Amplification of Silencing

To achieve a robust silencing response RISC cleavage fragments sometimes are channeled back into silencing by RDR-mediated dsRNA synthesis (Bologna and Voinnet 2014; Wassenegger and Krczal 2006). Subsequently to the AGO endonucleolytic cleavage, ssRNA fragments lacking *bona fide* features like cap structure or polyA tail are recognized by RDR polymerases with or without the help of primary vsiRNA and converted into long dsRNAs that are substrates of DCLs (Gazzani et al. 2004; Moreno et al. 2013; Parent et al. 2015b) (Fig. 1.1). RDR1, RDR2 and RDR6 (SDE1/SGS2) were all found to be important factors in vsiRNA production during PVX, CMV, TMV, *Sugarcane mosaic virus* (SCMV), TuMV, *Tobacco rattle virus* (TRV) infections (Diaz-Pendon et al. 2007; Donaire et al. 2008; Garcia-Ruiz et al. 2010; Qu et al. 2008; Schwach et al. 2005). RDR-synthetized dsRNAs are processed by DCL4 and DCL2 into 21–22 nt long vsiRNAs, respectively. Both 21 and 22 nt long vsiRNA were effective in antiviral response against a number of viruses like CMV, *Oilseed rape mosaic virus* (ORMV), TCV, TRV, *Cabbage leaf curl virus* (CaLCuV), CaMV (Blevins et al. 2006; Bouche et al. 2006; Deleris et al. 2006; Donaire et al. 2008). 22 nt long vsiRNAs contribute to secondary siRNA production and mediate systemic silencing (Garcia-Ruiz et al. 2010; Wang et al. 2011). In case of robustly replicating RNA viruses the involvement of RDRs seems to be less important. Upon tombusvirus infection the major part of vsiRNAs derives from the positive RNA strand of the virus genome suggesting that they are primary DCL cleavage products of viral RNA fold-back structures (Aregger et al. 2012; Blevins et al. 2011; Donaire et al. 2008; Molnar et al. 2005; Szittya et al. 2010; Kontra et al. unpublished). In a similarly RDR-independent manner, massive amount of hairpin-derived vsiRNAs are produced from 35S leader of CaMV (Blevins et al. 2011). The majority of viral siRNAs accumulating during CaLCuV geminivirus infection were RDR1/2/6-independent primary siRNAs generated by pairing of bidirectional read-through transcripts of the circular viral genome (Aregger et al. 2012).

RDR6 activity is facilitated by protein cofactors SUPPRESSOR OF GENE SILENCINIG 3 (SGS3) (Mourrain et al. 2000), SILENCING DEFECTIVE 5 (SDE5) (Hernandez-Pinzon et al. 2007) and SILENCING DEFECTIVE 3 (SDE3) (Dalmay et al. 2001). SGS3, a plant specific protein associate to RISC complex (Allen et al. 2005; Yoshikawa et al. 2005), stabilizes the RISC-cleavage products following slicing and enhance their conversion into dsRNA by RDR6 (Yoshikawa et al. 2013). Elimination of SGS3 leads to enhanced susceptibility to CMV but not to TuMV or *Turnip vein-clearing virus* (TVCV) infections (Adenot et al. 2006; Yoshikawa et al. 2013). SGS3 was shown to be required for CaLCuV virus induced VIGS of endogenous genes and was further suggested to be involved

in the antiviral response against DNA viruses (Muangsan et al. 2004). This is supported by the fact that *Tomato yellow leaf curl virus* (TYLCV) encodes a silencing suppressor to compromise SGS3 activity (Glick et al. 2008). SDE5 is an RNA trafficking protein homologue of human mRNA export factor. SDE5 acts together with RDR6 to convert ssRNAs into dsRNA. *sde5* mutant plants are hypersusceptible to CMV but not to TuMV infection (Hernandez-Pinzon et al. 2007). Silencing amplification is facilitated by the SDE3, an RNA-helicase like protein. SDE3 was shown to bind to AGOs through its GW domains (Garcia et al. 2012). *sde3* mutant plants are more susceptible to CMV or PVX but not to TRV infections (Dalmay et al. 2001). SDE3 activity occurs downstream to RDR6 and requires AGO1 and AGO2 activities (Garcia et al. 2012). SDE3 was proposed therefore to facilitate the amplification process by unwinding a fraction of RDR6-sythetized dsRNA products using helicase activity.

In rice there are five RDRs annotated, but our knowledge about their involvement in vsiRNA biogenesis is very limited. OsRDR6-silenced transgenic rice plants were shown to be hypersusceptible to RSV and RDV (Hong et al. 2015; Jiang et al. 2012). The rise in viral symptoms was associated with an increase in viral genomic RNA and reduced levels of vsiRNAs. Interestingly, the protein level of the overexpressed OsRDR6 in transgenic rice was reduced during RDV infection, suggesting a negative translational control induced by the virus upon RDR6 expression (Hong et al. 2015).

1.3 Viral Silencing Suppressor Strategies

1.3.1 Introduction

The most common strategy of viruses to protect themselves against antiviral RNA silencing is to express proteins that act as suppressors of silencing. These proteins are the viral suppressors of RNA silencing (VSRs). Discovery of VSRs provided a strong support of RNA silencing being an antiviral mechanism. Available evidences suggest that most viruses encode at least one VSR that, in most cases is essential for successful virus infection. Silencing suppression by VSRs has been described in insect and fungus-infecting viruses as well (Bronkhorst and van Rij 2014). Diversity of VSR's in sequence and structure indicates that they have evolved independently. VSRs were shown to block virtually all steps of RNA silencing like silencing initiation, effector phase, amplification phase, chromatin modification during TGS or modulation of host gene products for a more favorable infection. Here we review the most important strategies employed by presenting the most studied/relevant examples of VSRs (Fig. 1.1).

1.3.2 Blocking Initiation of Antiviral Response

1.3.2.1 Inhibition of DCL's Activities

Initiation of silencing may be blocked by inhibition of dicing itself, either through dsRNA sequestration or through impeding DCLs or their cofactors. *Pothos latent aureusvirus* (PoLV) P14, TCV p38 and CMV 2b have been all shown to bind long dsRNA and thus block vsiRNA biogenesis (Deleris et al. 2006; Goto et al. 2007; Merai et al. 2005). The nuclear localized P6 suppressor of CaMV diminishes dicing through protein-protein interaction: P6 interacts with the nuclear DRB4, a cofactor required for DCL4-dependent vsiRNA processing (Haas et al. 2008). In addition, during CaMV infection massive amounts of vsiRNAs derive from the 35S leader sequence recognized by all four DCLs. 35S leader RNA therefore serves as decoy to divert the effectors of the silencing machinery from more important viral features (Blevins et al. 2011). *Red clover necrotic mosaic virus* (RCNMV) recruits DCL enzymes into its replication complex and therefore deprives them from the silencing machinery. *dcl1* mutant plants are less susceptible to RCNMV infection (Takeda et al. 2005). Similar strategies were described in insect-infecting viruses (Bronkhorst and van Rij 2014).

Viruses may modulate endogenous regulatory pathways in order to alter the strength of silencing in their favor. RNASE THREE_LIKE 1 (RTL1) enzyme was described as and endogenous silencing suppressor: RTL1 is induced during virus infections and prevents vsiRNA production by cleaving viral dsRNAs prior to DCL2/3/4-processing but does not interfere with DCL1-mediated miRNA pathway (Shamandi et al. 2015).

1.3.2.2 vsiRNA Sequestration

Ds-siRNA sequestration is a widespread strategy used by several VSRs originating from diverse genera (P19, Hc-Pro, P21, p15, p122/p126/p130, γB, NS3, NSs, Pns10 etc.) (Csorba et al. 2007; Harries et al. 2008; Hemmes et al. 2007; Kubota et al. 2003; Lakatos et al. 2006; Merai et al. 2005, 2006; Silhavy et al. 2002). Amongst these, probably the best known is the tombusviral p19 protein (Silhavy et al. 2002). Crystallographic studies have shown that p19 homodimer acts as a molecular caliper to sequester the sRNA duplexes size-specifically (Silhavy et al. 2002; Vargason et al. 2003; Ye et al. 2003). sRNA sequestration prevents RISC assembly as shown by the heterologous *in vitro* Drosophila embryo extract system (Lakatos et al. 2006). It seems that p19-mediated vsiRNA sequestration affects selectively AGO1- but not AGO2-loading in *N. benthamiana* during CymRSV virus infection (Kontra et al., unpublished). It was shown that due to the structural similarly between vsiRNAs and endogenous sRNAs p19 prevents RISC-loading of endogenous sRNA species in transgenic *A. thaliana* and *N. benthamiana* plants (Schott et al. 2012; Kontra et al. unpublished). During

authentic virus infections however, p19-sequestration of endogenous sRNA is not efficient (Lozsa et al. 2008; Kontra et al. unpublished). The tombusviral vsiRNAs bind more efficiently to p19 to outcompete endogenous sRNAs. The basis of vsiRNA competition, besides the massive amount of vsiRNAs, could be the structural preference of p19 for perfect ds-vsiRNAs forms (contrary to the mismatch-containing endogenous sRNAs) (Kontra et al. unpublished).

A consequence of sRNA binding by VSRs is the block of HEN1-dependent methylation of sRNAs (Csorba et al. 2007; Lozsa et al. 2008; Vogler et al. 2007). When sequestered, the methylation of sRNAs is inhibited (Csorba et al. 2007; Lozsa et al. 2008). Whether blocking of vsiRNA methylation leads to a faster decay and this has any biological significance remains a question.

It was shown that *Sweet potato chlorotic stunt crinivirus* (SPCSV) suppressor RNase3 cleaves the 21–24 nt vsiRNAs into 14 bp products rendering them inactive (Cuellar et al. 2009; Kreuze et al. 2005). Although this is a completely different strategy to siRNA-binding, it has a very similar outcome: vsiRNAs are unavailable for AGO-loading.

1.3.2.3 Blocking Systemic Silencing

Although p19 sequesters vsiRNAs very efficiently, its effect to block cell-autonomous silencing and restrict virus replication is mild. The VSR-deficient CymRSV (Cym19stop) replicates as efficiently as the wild type CymRSV in *N. benthamiana* protoplasts (Silhavy et al. 2002). The true strength of p19 lies in blocking systemic silencing through inhibition of vsiRNA mobilization into naive surrounding tissue or long distance (Dunoyer et al. 2010; Havelda et al. 2003; Molnar et al. 2010). RNA binding suppressors NS3 (RSV) and 2b (CMV) were also shown to prevent efficiently the spread of silencing signal (Guo and Ding 2002; Xiong et al. 2009).

1.3.2.4 Interfering with AGO-Loading

An efficient arrest of silencing initiation can be achieved through the block of functional RISC assembly. P0 the suppressor of *Poleroviruses* (Mayo and Ziegler-Graff 1996) was shown to enhance the degradation of effector AGOs (AGO1, 2, 4–6, 9) by inhibition of holo-RISC assembly (Baumberger et al. 2007; Bortolamiol et al. 2007; Csorba et al. 2010; Derrien et al. 2012; Pazhouhandeh et al. 2006) P0-mediated AGO degradation occurs through autophagy pathway (Derrien et al. 2012). ToRSV CP, that acts as a VSR as well, binds to AGO1 to suppress its translational inhibitory activity and to enhance AGO1 degradation through autophagy (Karran and Sanfacon 2014). It was shown that PVX p25 physically interacts with multiple AGOs (AGO1, 2, 3 and 4) to promote their destabilization in a proteasome-dependent manner (Chiu et al. 2010). In the absence of central AGO effector, silencing cannot be programmed/initiated.

VSRs are able to modulate AGO1 availability in a more subtle way. AGO1 homeostasis depends on the miR168-guided AGO1 mRNA cleavage and translational inhibition control (Mallory and Vaucheret 2009; Rhoades et al. 2002). To counteract AGO1-based defense a number of unrelated siRNA-binder VSRs (p19, p122, p38, Hc-Pro and 2b) promote miR168 transcriptional induction that results in miR168-guided AGO1 down-regulation to create a better environment for virus infection. It was shown that (during tombusvirus infection) the miR168 accumulation spatially correlated with the virus localization and was dependent on the presence of p19 (Varallyay and Havelda 2013; Varallyay et al. 2010).

1.3.2.5 Arrest of Programmed RISC Activity

The *Sweet potato mild mottle ipomovirus* (SPMMV) suppressor protein P1 interacts directly with siRNA and/or miRNA-loaded AGO1 present in the high molecular weight holo-RISC but not minimal-RISC through GW/WG-motifs (AGO-hook) and inhibits si/miRNA-loaded RISC activity. The GW/WG-motif containing proteins (GW182 family) were shown to interact with AGOs and support diverse RISC functions (Eulalio et al. 2009). P1 AGO-hook motifs are necessary for both binding and suppression of AGO1 function (Giner et al. 2010; Szabo et al. 2012).

P38 of TCV (Azevedo et al. 2010) and 2b of CMV (Zhang et al. 2006) and *Tomato aspermy virus* (TAV) (Chen et al. 2008) suppressors were proposed to act at multiple steps of silencing (during initiation and effector phase) including RISC activity block through AGO protein interaction.

The block of effector step can be achieved also through targeting the RNA component (the guide vsiRNA) within the vsiRISC. *African cassava mosaic virus* (ACMV) encoded AC4 is able to bind to the ss- but not ds-sRNA forms *in vitro*. Transgenic AC4 decreases accumulation of miRNAs and up-regulates target mRNAs. AC4 acts downstream of the unwinding process by binding miRNAs presumably loaded into AGO (Chellappan et al. 2005; Xiong et al. 2009; Zhou et al. 2006). RSV suppressor NS3 was found to bind to various RNA forms like ss-siRNA, ds-siRNA or long ssRNA (but not long dsRNA). By this, NS3 is able to suppress and revert local silencing but also prevent the long distance spread of silencing signal (Chellappan et al. 2005; Xiong et al. 2009; Zhou et al. 2006). Similarly, *Grapevine virus A* (GVA) p10 suppressor was also suggested to act through both ss- and ds-si/miRNA binding (Chellappan et al. 2005; Xiong et al. 2009; Zhou et al. 2006).

1.3.3 VSR Activities Affecting TGS

Several DNA viruses encode VSRs that have been described to alter the effector step of TGS, the chromatin structure modification. AL2 suppressor of *Tomato golden mosaic virus* (TGMV) and L2 suppressor of *Beet curly top virus* (BCTV)

inhibit adenosine kinase (ADK) activity that plays crucial role in adenosine and methyl-cycle maintenance or cytokinin regulation. AL2 and L2 induce global reduction in cytosine methylation that leads to inactivation and reversal of antiviral silencing (Buchmann et al. 2009; Wang et al. 2003, 2005). *In vitro* methylated TGMV cannot replicate in protoplasts suggesting that viral genome methylation is a *bona fide* defense against geminiviruses (Bisaro 2006). Similarly, ßC1 suppressor of *Tomato yellow leaf curl China virus* (TYLCCNV) interacts and inhibits activity of S-adenosyl-homocystein-hydrolase (SAHH) that is involved in methyl-cycle and therefore indirectly affects TGS (Yang et al. 2011).

1.3.4 Suppression of Antiviral Silencing Amplification

Blocking RDR activities by VSRs is a very effective strategy employed by viruses since it dampens cell-autonomous silencing amplification and systemic signal movement in distant tissues to facilitate the virus replication and spread (Ren et al. 2010; Schwach et al. 2005). V2 suppressor of TYLCV inhibits RDR6-mediated amplification by direct interaction with SGS3, the cofactor of RDR6 (Glick et al. 2008). Alternatively, V2 may compete with SGS3 for dsRNA having 5′ overhang ends that may be an RDR6/SGS3 substrate/intermediate during vsiRNA amplification (Fukunaga and Doudna 2009; Kumakura et al. 2009). Similarly, TRIPLE GENE BOX PROTEIN1 (TGBp1) encoded by PVX was shown to inhibit RDR6/SGS3-dependent dsRNA synthesis (Okano et al. 2014). βC1 suppressor of TYLCCNV DNA satellite interacts with the endogenous suppressor of silencing calmodulin-like protein (rgsCAM) in *N. benthamiana* to repress RDR6 expression (Li et al. 2014). SCMV encoded HC-Pro, TAV 2b and Pns10 of RDV were all shown to downregulate RDR6 to limit amplification and decelerate systemic silencing (Ren et al. 2010; Zhang et al. 2008). Plant RDR1 however, was suggested to have adverse functions: RDR1 is an antagonist of RDR6-mediated sense-PTGS making it an endogenous silencing suppressor (Ying et al. 2010).

1.3.5 Targeting Multiple Steps of Antiviral Pathways

Many VSRs have multiple silencing suppressor functions and therefore are capable to act at multiple points to modulate antiviral response. 2b of CMV (CM95R strain) and TAV exhibit high affinity for long dsRNAs and ds-sRNAs (Chen et al. 2008; Duan et al. 2012; Gonzalez et al. 2012; Goto et al. 2007). CMV 2b (Fny and SD strains) was also shown to interact with AGO1 through the PAZ- and partly PIWI domains and blocks RISC slicer activity (Duan et al. 2012; Zhang et al. 2006). Additionally, CMV 2b (SD strain) alters RdDM pathway as well. 2b facilitates cytosine methylation through the transport of siRNAs into the nucleus (Kanazawa et al. 2011). 2b interacts both with AGO4-related siRNAs and with AGO4 protein

through PAZ and PIWI domains. The interaction with 2b reduces AGO4 access to endogenous target loci and consequently modulates endogenous transcription to create a favorable niche for CMV infection (Duan et al. 2012; Gonzalez et al. 2010, 2012; Hamera et al. 2012).

P38 of TCV may also suppress silencing at multiple levels. P38 possesses dsRNA-binding activity (Merai et al. 2006). Since in the presence of p38, siRNAs are undetectable therefore it was proposed that p38 suppress DCLs' activity (Qu et al. 2003). Genetic evidences also supported the role of p38 in inhibiting DCL4 (Deleris et al. 2006). In a later study however, p38 suppressor impact on DCL4 was attributed to an indirect effect of AGO1-mediated DCL-homeostasis and has been shown that p38 blocks AGO1 but not AGO4 activity through its GW-motif binding (Azevedo et al. 2010). P38 is capable to bind and inactivate AGO2 as well (Zhang et al. 2012). Site-directed mutagenesis (GW-to-GA) in the p38 proved that GW motif is absolutely required for both binding and suppression of AGO1 function (Azevedo et al. 2010). *Pelargonium line pattern virus* (PLPV) coat protein p37 (an orthologue of TCV p38) is a GW-containing protein that functions as a VSR as well. It was shown that the mutations within its GW-motif affect p37 localization, interaction with AGO1 and its sRNA-binding ability. Furthermore, GW-mutations also abolished TCV p38 sRNA and long dsRNA-binding capacity (Perez-Canamas and Hernandez 2015). It seems therefore that the domain for different functions may overlap in p37/p38 VSRs. This brings up the possibility that the parallel suppressor functions could cooperate during their interaction with host silencing machinery (e.g. p37/p38 interaction with AGO1 could enhance sRNA duplex sequestration in order to more efficiently prevent RISC programming).

1.3.6 VSRs' Interaction with Host Factors to Modulate Silencing

Besides blocking antiviral silencing VSRs are able to modulate host endogenous pathways in order to fine-tune the host-pathogen interaction. The suppressor of *Tobacco etch virus* (TEV) helper-component protease (HC-Pro) is a multifunctional protein involved in many aspects of virus infection (Anandalakshmi et al. 1998; Carrington et al. 1989; Guo et al. 2011; Kasschau et al. 1997; Lakatos et al. 2006; Mallory et al. 2001). HC-Pro sequesters vsiRNA that leads to inhibition of their methylation and inability to load into vsiRISC (Lakatos et al. 2006; Lozsa et al. 2008). HC-Pro was also found to interact with rgsCAM an endogenous silencing suppressor (Anandalakshmi et al. 2000; Endres et al. 2010; Marquardt et al. 2014). In another study it was shown that rgsCAM counteracts HC-Pro through binding to its positively charged dsRNA-binding surface, prevents HC-Pro siRNA-sequestration and promotes HC-Pro degradation through autophagy pathway (Nakahara et al. 2012). Suppression of silencing by

TuMV HC-Pro requires another host factor, RAV2, a transcription factor. RAV2 targets include *FIERY1* and *CML38*, endogenous suppressors of silencing (Anandalakshmi et al. 2000; Endres et al. 2010; Gy et al. 2007). RAV2 was required for suppression of silencing by *Carmoviral* p38 as well (Endres et al. 2010) suggesting that RAV2 is a cross-talk point between antiviral and endogenous silencing pathways, and may be efficiently used by suppressors to modulate host defense. HC-Pro of another potyvirus, *Papaya ringspot virus* (PRSV), interacts with calreticulin to modulate calcium signaling and thus host defense (Shen et al. 2010a, b). HC-Pro (of *Potato virus A* (PVA), *Potato virus Y* (PVY) and TEV) interacts also with microtubule-associated protein (HIP2) through its highly variable region (HVR). Virus accumulates at lower level when HIP2 is depleted. Mutations affecting HC-Pro HIP2 interaction induces necrosis and hormone (ethylene- and jasmonic acid-) mediated induction of host pathogen-related defense genes (Haikonen et al. 2013a, b).

1.3.7 vsiRNAs May Regulate Host Genes by Exploiting Endogenous Silencing Itself

The high sequence variability of vsiRNAs and the fast evolution of viral genomes, may lead to the production of vsiRNAs that could potentially target endogenous genes/transcripts. By this, viruses may modulate host response to their benefit. There are a few examples to support this idea. vsiRNAs derived from the CMV-Y satellite RNA (Y-Sat) targets magnesium protoporphyrin chelatase subunit I (CHLI), a key component of chlorophyll biosynthesis pathway. vsiRNA-mediated downregulation of CHLI mRNA leads to yellowing of the plant leaves, that was suggested to enhance virus spreading by insects (Shimura et al. 2011). sRNA derived from *Peach latent mosaic viroid* (PLMVd) targets chloroplastic heat-shock protein 90 (cHSP90) in peach. Cleavage of cHSP90 (that participates in chloroplast biosynthesis and plasmid-nucleus signal transduction) induces albinism and may contribute to a more favorable host environment for viroid infection (Navarro et al. 2012). Callose synthase genes encode proteins with role in callose formation during pollen development. *Potato spindle tuber viroid* (PSTVd)-derived sRNAs suppress CalS11-like and CalS12-like mRNAs that greatly affects the severity of disease symptoms (Adkar-Purushothama et al. 2015).

1.4 Perspectives

With the advancement of high throughput technologies the in-depth profiling of vsiRNA generation, their loading into effectors (vsiRISC or RDR complexes) and their involvement in systemic signaling of RNA silencing will lead to a more and

thorough understanding of antiviral defense at cellular, tissue and organism level. In addition to this, the development of novel *in vitro* systems and *in vivo* cellular assays hopefully will make it possible to better understand the mechanistic details at molecular level. The interaction of host with viral pathogens is very complex: the exact details such as how, when, and where in the cell viral RNAs are initially accessed by the RNA silencing machinery and how VSRs counteract silencing response remain elusive. It was recently reported that potyvirus-induced granules (PG) protects PVA viral RNA from antiviral silencing when active viral translation does not occur optimally (Hafren et al. 2015). Antiviral silencing, translation and RNA quality control pathways, alongside with general RNA degradation pathways all compete for endogenous and viral RNAs (Christie et al. 2011). How exactly these pathways share substrates and cooperate during viral infection will be hopefully addressed by further research.

Until recently most studies on antiviral silencing were conducted in the model *Arabidopsis* due to the plethora of genetic tools available. The use of Arabidopsis, however, has a major drawback since this plant model hosts only very few plant viruses. Availability of the full genome sequence of the viral model plant *Nicotiana benthamiana* (sensitive to almost all plant viruses) and the development of CRIPSR/CAS9 genome editing technology will hopefully allow the study of antiviral RNA silencing during several other virus infections.

An important aim of antiviral silencing research is to gather knowledge in order to be able to design resistant crops. Great advances have been made to develop methods for viral disease control with the expression of artificial sRNAs/miRNAs targeting viral genomes in economically important plants (Kis et al. 2016; Lin et al. 2009; Niu et al. 2006). Similar biotechnological approaches may be very useful to elaborate in the future for economically important crop protection.

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Glossary

RNAi:	RNA interference
TGS:	Transcriptional Gene Silencing
PTGS:	Post Transcriptional Gene Silencing
sRNA:	small RNA
dsRNA:	double-stranded RNA
ssRNA:	single-stranded RNA
DCL:	Dicer-Like enzymes
PAZ:	Piwi/Argonaute/Zwille-domain

AGO:	Argonaute protein
RDR:	RNA-dependent RNA polymerase
RISC:	RNA-Induced Silencing Complex
RITSC:	RNA-Induced Transcriptional Silencing Complex
miRNA	: micro RNA
siRNA:	small interfering RNA
ta-siRNA:	trans-acting small interfering RNA
nat-siRNA:	natural-antisense small interfering RNA
ra-siRNA:	repeat-associated small interfering RNA
vsiRNA:	viral small interfering RNA
vasiRNA:	virus-activated small interfering RNA

(+) ssRNA Virus

BMV:	*Brome mosaic virus*
CMV:	*Cucumber mosaic virus*
CNV:	*Cucumber necrosis virus*
CymRSV:	*Cymbidium ringspot virus*
GVA:	*Grapevine virus A*
ORMV:	*Oilseed rape mosaic virus*
PLPV:	*Pelargonium line pattern virus*
PoLV:	*Pothos latent virus*
PRSV:	*Papaya ringspot virus*
PVA:	*Potato virus A*
PVX:	*Potato virus X*
PVY:	*Potato virus Y*
RCNMV:	*Red clover necrotic mosaic virus*
RYMV:	*Rice yellow mottle virus*
SCMV:	*Sugarcane mosaic virus*
SPCSV:	*Sweet potato chlorotic stunt virus*
SPMMV:	*Sweet potato mild mottle virus*
TAV:	*Tomato aspermy virus*
TEV:	*Tobacco etch virus*
TBSV:	*Tomato bushy stunt virus*
TCV:	*Turnip crinkle virus*
TMV:	*Tobacco mosaic virus*
ToRSV:	*Tomato ringspot virus*
TRV:	*Tobacco rattle virus*
TuMV:	*Turnip mosaic virus*
TYMV:	*Turnip yellow mosaic virus*

(−) ssRNA Virus

RSV: *Rice stripe virus*
TSWV: *Tomato spotted wilt virus*

dsRNA Virus

OsEV: *Oryza sativa endornavirus*
RDV: *Rice dwarf phytoreovirus*

ssDNA Virus

ACMV: *African cassava mosaic virus*
BCTV: *Beet curly top virus*
CaLCuV: *Cabbage leaf curl virus*
TGMV: *Tomato golden mosaic virus*
TYLCV: *Tomato yellow leaf curl virus*
TYLCCNV: *Tomato yellow leaf curl China virus*

dsDNA Virus

CaMV: *Cauliflower mosaic virus*
TVCV: *Turnip vein-clearing virus*
viroid: non-protein coding infectios RNAs
PLMVd: *Peach latent mosaic viroid*
PSTVd: *Potato spindle tuber viroid*

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