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Review

viral silencing suppressors: Tools forged to fine-tune host-pathogen coexistence

Tibor Csorba *, Levente Kontra, József Burgyán *

National Agricultural Research and Innovation Center – Agricultural Biotechnology Center (NARIC – ABC), Gödöllő, Szent-Györgyi A.u.4., Pest 2100, Hungary

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ABSTRACT

RNA silencing is a homology-dependent gene inactivation mechanism that regulates a wide range of biological processes including antiviral defense. To deal with host antiviral responses viruses evolved mechanisms to avoid or counteract this, most notably through expression of viral suppressors of RNA silencing. Besides working as silencing suppressors, these proteins may also fulfill other functions during infection. In many cases the interplay between the suppressor function and other "unrelated" functions remains elusive. We will present host factors implicated in antiviral pathways and summarize the current status of knowledge about the diverse viral suppressors' strategies acting at various steps of antiviral silencing in plants. Besides, we will consider the multi-functionality of these versatile proteins and related biochemical processes in which they may be involved in fine-tuning the plant-virus interaction. Finally, we will present the current applications and discuss perspectives of the use of these proteins in molecular biology and biotechnology.

Contents

* Corresponding authors. Tel.: +36 28526100; fax: +36 28526101.
E-mail addresses: csorba@abc.hu (T. Csorba), burgyan@abc.hu (J. Burgyán).

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Plant viruses

Plant viruses are amongst the most important pathogens causing huge economic losses worldwide by reducing crop quality and quantity. A better understanding of the viral infection processes and plant defense strategies is important for crop improvement.

Based on their genome organization viruses can be classified into positive-sense-, negative-sense-, double-stranded-RNA viruses and single-stranded or double-stranded DNA viruses (Hull, 2002). Difference in the genome organization implies difference in the replication strategy. Generally, the genetic information embedded into the viral RNA or DNA encode for a surprisingly restricted number of proteins that coordinate the infection process. Viral proteins interact with host factors to manipulate biochemical events and molecular interactions required for the virus replication and movement. Viruses can spread within the plants through plasmodesmata on short distance (cell-to-cell movement) or through phloem (systemic movement). During host-pathogen co-evolution a set of complex interactions involving virus attack and host defense has been developed. These include hypersensitive reaction (HR) (Mandadi and Scholthof, 2013), systemic acquired resistance (SAR) (Kachroo and Robin, 2013), activation of ubiquitin/26S proteasome system (UPS) (Dielen et al., 2010) or RNA silencing (RNA interference, RNAi) (Pumplin and Voinnet, 2013).

RNA silencing pathways in plants

RNA silencing is a fundamental genetic regulatory mechanism conserved in eukaryotic organisms. RNAi can act at transcriptional (Transcriptional Gene Silencing, TGS) or at post-transcriptional levels (Post-Transcriptional Gene Silencing, PTGS), and has many diverse roles including developmental regulation, stress response or defense against invading nucleic acids like transposons or viruses. The antiviral function of RNA silencing was demonstrated in plants and invertebrates (Bronkhorst and van Rij, 2014; Pumplin and Voinnet, 2013), however recent reports have further provided evidence for a similar function in mammals (Cullen et al., 2013; Li et al., 2013; Maillard et al., 2013).

Mechanistically, the RNA silencing process consists of initiation phase, effector phase and amplification phase. During silencing initiation double-stranded RNAs (dsRNA) of different origins are processed by an RNase III type enzyme Dicer (DCR, in plants DICER-LIKE proteins, DCLs) into short, 21–24 nt long, small RNA (sRNA) duplexes (Bernstein et al., 2001; Hamilton and Baulcombe, 1999; Hutvagner et al., 2001). DICERs require DOUBLE-STRANDED RNA BINDING (DRB) proteins for accurate sRNA production (Eamens et al., 2012a,b; Hiraguri et al., 2005). The sRNAs are stabilized at their 3′ end by the HUA Enhancer 1 (HEN1)-dependent methylation (a process found in plants and flies so far (Boutet et al., 2003; Yang et al., 2006) and exported from nucleus by HASTY (HST) (Park et al., 2005; Peragine et al., 2004) to be loaded onto Argonaute proteins (Fagard et al., 2000; Hammond et al., 2001; Liu et al., 2004), the effectors of the RNA-Induced Silencing Complex (RISC) (Lee et al., 2004; Pham et al., 2004; Tomari et al., 2004) or RNA Induced Transcriptional Silencing complex (RITS) (Ekwall, 2004). Guided by the sRNA sequence, RISC induces slicing or translational repression of its target RNAs (during PTGS) in a sequence-specific manner (Kim et al., 2014), whereas RITS complex causes histone and/or DNA methylation, resulting in transcriptional gene silencing (TGS) of the homologous gene (Creamer and Partridge, 2011). In plants and worms the effector step can result in amplification of silencing response involving RNA-dependent RNA polimerases (RDRs) proteins (Mourrain et al., 2000; Dalmay et al., 2000; Sijen et al., 2001; Vaistij et al., 2002; Voinnet et al., 1998). Amplification of RNA silencing has been implicated in the spread of an RNA silencing signal, a non-cell-autonomous process (Kalantidis et al., 2008; Schwach et al., 2005).

The best studied plant model, *Arabidopsis thaliana* genome encodes 4 members of DCLs (DCL1-4) (Bologna and Voinnet, 2014), five DRBs (HYL1/DRB1, DRB2, 3, 4, 5) (Hiraguri et al., 2005), 10 AGOs (AGO1-10) (Mallory and Vaucheret, 2010) and 6 RDRs (RDR1, 2, 3a, 3b, 3c and 6) (Wassenegger and Krczal, 2006). These proteins have partially redundant roles and combine with each other to result in divers classes of small RNAs and different effector outputs of the RNA silencing pathways. The small RNA classes identified in plants include 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). These classes possess specialized roles during development, stress responses, heterochromatic silencing, viral infection and host-pathogen interplay, respectively (Bologna and Voinnet, 2014).

Antiviral silencing host factors

Initiation of antiviral silencing

The hallmark of the antiviral silencing response is the Dicer-dependent production of viral siRNAs (vsiRNAs) (Hamilton and Baulcombe, 1999). In uninfected cells long dsRNA is not detectable, however upon virus infection, viral dsRNA molecules of different sources becomes available. Highly structured regions of viral single-stranded RNAs (ssRNA), replicative intermediates (RI) or overlapping bidirectional read-through transcripts from DNA virus genome may all contribute to vsiRNA production (Aregger et al., 2012; Blevins et al., 2011; Donaire et al., 2008; Molnar et al., 2005). Although the viral dsRNA structures are likely accessible to all of the DCLs, a strong hierarchy exists between them regarding vsiRNA production: RNA virus infections are mainly affected by DCL4, while DCL2 becomes critical in a *dcl4* mutant background (Deleris et al., 2006; Donaire et al., 2008; Garcia-Ruiz et al., 2010; Qu et al., 2008) (Fig. 1). DCL3 has only a minor role against RNA viruses (Qu et al., 2008; Raja et al., 2008). Recent report suggests additional functional diversity between DCL4 and DCL2, such as that DCL2 stimulates transitivity and secondary siRNA production, while DCL4 is sufficient for silencing on its own (Parent et al., 2015). The fact that silencing suppressors of RNA viruses interfere with DCL3 pathway suggests it could have important antiviral gene regulatory functions (Azevedo et al., 2010; Hamera et al., 2012; Lacombe et al., 2010). DCL3 is essential against DNA viruses (Akbergenov et al., 2006) and works presumably by inducing DNA methylation (Blevins et al., 2006; Raja et al., 2014) (Fig. 1). Finally, DCL1 acts as a positive regulator in the production of vsiRNAs by making viral dsRNAs available to other DCLs both in RNA and DNA virus infections (Blevins et al., 2006; Moissiard and Voinnet, 2006) but also as a