VIRAL SUPPRESSORS

Combatting RNA silencing

Plants defend themselves from invading viruses using RNA silencing. However, plant viruses try to spoil this defensive mechanism by expressing one or more proteins that act as RNA silencing suppressors. One such protein spoils plant defence by transporting the silencing signal into the peroxisomes to avoid its systemic spread.

José-Antonio Daròs

Plants trust RNA silencing as the main defence line against invading viruses. We know this because virtually all plant viruses dedicate a substantial part of their small genomes to encode one or more proteins devoted to suppress host RNA silencing¹. While the plant recognizes viral molecules such as double-stranded RNA replication intermediates, highly structured regions in genomic RNA or viral transcripts, or bidirectional overlapping transcripts to unleash a defensive response led by Dicer-like (DCL), double-stranded RNA binding (DRB), RNA-dependent RNA polymerase (RDR) and Argonaute (AGO) proteins, viruses express RNA silencing suppressors to dampen this host defensive response. Analysis of sequence and mechanism of action of the many viral RNA silencing suppressors known to date tell us a story of intricate host–pathogen co-evolution and a sophisticated arms race. RNA silencing suppressors from viruses that belong to different taxonomic groups are unrelated proteins that act with diverse mechanisms in different steps of the RNA silencing pathways, and they are often multifunctional proteins that act in several steps at the same time². In this issue of *Nature Plants*, Incarbone *et al.* show a completely novel RNA silencing suppression strategy that involves transportation of the mobile silencing signal into the peroxisomes to ruin plant defence³ (Fig. 1).

P15 RNA silencing suppressor from peanut clump virus (PCV; genus *Pecluvirus*, family *Virgaviridae*) contains a peroxisomal targeting signal, named PTS1, at the carboxyl terminus, which is required for long-distance movement of the virus through the plant, but is dispensable for cell autonomous RNA silencing suppression⁴. Incarbone *et al.* wondered what the mechanism of P15 silencing suppression was, particularly intrigued by the potential role of peroxisomes and the effect on virus long-distance movement. To answer this question, they first used silenced *Arabidopsis thaliana* lines that show a beautiful vascular-associated phenotype,

Figure 1 | RNA silencing suppression mechanism of P15 from peanut clump virus. While the plant DCL4 and DCL2 produce virus-derived 21- and 22-nt siRNAs to initiate antiviral defence, P15 binds and transports these RNAs into the peroxisome (red) to spoil systemic signalling of the antiviral response.

which also stably expressed PCV P15, to prove that this viral protein sequesters 21- and 22-nucleotide (nt)-long small interfering RNAs (siRNAs), impairs the loading of these RNAs on AGO proteins, and prevents their spread outside the cells where they are produced. By repeating these experiments in an *A. thaliana* dicer-like 3/ dicer-like 4 (*dcl3/dcl4*) mutant background, where mainly 22-nt siRNAs are produced by the DCL4 surrogate DCL2, the authors cleverly deduced that P15 binds 22-nt siRNA stronger than 21-nt siRNA.

Based on peroxisomes' unique property to import folded proteins and protein complexes⁵, Incarbone *et al.* hypothesized that PCV P15 binds antiviral siRNAs to deliver them into the peroxisomes and prevent their cell-to-cell spread. In fact,

siRNAs were detected in the peroxisomes isolated from various silenced tissues where wild-type P15 was expressed, but not in those from tissues that expressed a P15 mutant lacking the PTS1 domain. Interestingly, sequestration of antiviral siRNAs in the peroxisomes was not observed in plants infected with potato virus X, suggesting that viruses use different silencing suppression strategies. To further support their hypothesis, Incarbone *et al.* inoculated a series of *A. thaliana* *dcl* mutants with a defective PCV harbouring the P15 mutant that lacks the peroxisome targeting sequence. While this virus was barely detectable in wild-type and *dcl2* *A. thaliana* plants, virus systemic movement and viral load were restored in *dcl4* and *dcl2/dcl4* mutants, indicating that the

delivery of DCL4-dependent virus-derived 21-nt siRNA into the peroxisomes by P15 is a critical step to prevent silence signal propagation and allow the virus to move long distance.

Collectively, these results indicate that, upon infection, host plant DCL4 and DCL2 respectively produce virus-derived 21- and 22-nt siRNAs — 22-nt siRNAs are particularly abundant in PCV-infected tissues — to incite the antiviral defence. However, the virus counteracts by expressing P15 that binds these siRNAs. While the strong P15 affinity towards the 22-nt siRNAs is sufficient to stop the plant antiviral response, the weak affinity towards the 21-nt siRNAs has forced PCV to evolve a novel RNA silencing suppression strategy: piggybacking siRNAs into the peroxisomes to divert them from cell-to-cell traffic (Fig. 1).

This finding uncovers a new player, the peroxisome, in the plant–virus interaction drama. As Incarbone *et al.* questioned, could other viral proteins with peroxisomal targeting signals⁶ adopt a similar strategy in pathogenesis? Knowing that the peroxisome is able to import protein complexes⁵, is it possible that viral proteins interact with host proteins that are imported into the peroxisomes? Additional questions also arise to further clarify the P15 silencing suppression mechanism: what happens to the siRNAs in the peroxisomes? Are they subjected to degradation or just sequestered? Is P15 recycled to transport increasing amounts of siRNA into the peroxisomes? Whatever the answers to these questions are, there is no doubt that plant–virus co-evolution has provided us with an apparently never-ending diversity of RNA silencing suppressors with a wealth of

biochemical and cellular activities that can be exploited to learn about plant molecular biology and as biotechnological tools. □

José-Antonio Daròs is at the Instituto de Biología Molecular y Celular de Plantas (Consejo Superior de Investigaciones Científicas-Universitat Politècnica de València), 46022 Valencia, Spain.
e-mail: jadaros@ibmcp.upv.es

References

1. Csorba, T., Kontra, L. & Burgyán, J. *Virology* **479–480**, 85–103 (2015).
2. Valli, A. A., Gallo, A., Rodamilans, B., López-Moya, J. J. & García, J. A. *Mol. Plant Pathol.* <http://doi.org/b7xr> (2017).
3. Incarbone, M. *et al. Nat. Plants* **3**, 17094 (2017).
4. Dunoyer, P. *et al. Plant J.* **29**, 555–567 (2002).
5. Leon, S., Goodman, J. M. & Subramani, S. *Biochim. Biophys. Acta* **1763**, 1552–1564 (2006).
6. Yelina, N. E., Savenkov, E. I., Solov'yev, A. G., Morozov, S. Y. & Valkonen, J. P. J. *J. Virol.* **76**, 12981–12991 (2002).

Competing interests

The author declares no competing financial interests.