The Plant Cell, Vol. 31: 1395–1396, July 2019, www.plantcell.org © 2019 ASPB.

EDITORIAL: REFLECTIONS ON *THE PLANT CELL* CLASSICS

How Virus Resistance Provided a Mechanistic Foundation for RNA Silencing[OPEN]

Biotechnologists have promoted genetic manipulation in crops as being more predictable than conventional breeding, although transgene expression is well known to be unstable and to vary between lines. Normally, only a small proportion of transformants have stable and high-level expression of the transgene. The chromosomal position of the transgene was an initial excuse for this inconvenient truth, but the more interesting real explanation involves RNA-based regulation of gene expression. This process was unknown until the 1990s.

The first hints that transgenes can influence gene expression were from petunia (*Petunia hybrida*), tomato (*Solanum lycopersicum*), and tobacco (*Nicotiana tabacum*) with transgenic copies of endogenous genes. Instead of an increase in the affected gene product, there was coordinate suppression—cosuppression—of the both the transgene and its homolog in the plant genome (Jorgensen, 1990; Grierson et al., 1991). But the underlying molecular biology of cosuppression was elusive and, for a while, there were more speculative reviews on this topic than primary research articles.

The mist started to clear, however, with a article published in *The Plant Cell* in 1993 on the topic of *Tobacco etch virus* (TEV) resistance in transgenic tobacco **(Lindbo et al., 1993)**. These authors used a transgenic coat protein approach to virus resistance but, unlike a pioneer example with *Tobacco mosaic virus* (Abel et al., 1986), the mechanism was based on RNA rather than protein. Resistance was as strong if the transgene carried nonsense mutations as with protein-coding transgenes (Lindbo and Dougherty, 1992).

The authors had generated several different transgenic lines with varying levels of resistance ranging from immunity through to complete susceptibility but, in this paper, the resistance was delayed. TEV symptoms were initially as strong as on nontransgenic control plants but, after 5 weeks, the plants recovered **(Lindbo et al., 1993)**. The upper leaves were now symptom-free, and they were resistant to secondary infection with TEV but not to other viruses. This specificity test was important because it ruled out a physiological effect related to systemic acquired resistance that would affect other viruses.

The link with cosuppression was because transgene RNA was abundant in the noninfected plants but barely detectable after recovery (Lindbo et al., 1993). There was, therefore, cosuppression of the virus and transgene that, because the virus had an RNA genome, must operate at the RNA level. By extrapolation, if this example of cosuppression was RNA-mediated, the others with petunia and tomato were likely to be the same.

The importance of this article goes beyond the simple and elegant demonstration that cosuppression is based on RNA. There is also a remarkably prescient discussion that anticipates a host-encoded RNA-dependent RNA polymerase (RDR) producing small antisense RNA as the specificity determinant of the RNA silencing machinery. Both predictions turned out to be correct, but the antisense RNA, now known as small interfering RNA, was not found until 1999 (Hamilton and Baulcombe, 1999) and the involvement of RDR was only confirmed in 2000 from genetic screens in Arabidopsis (*Arabidopsis thaliana*; Dalmay et al., 2000; Mourrain et al., 2000). We should be grateful that *The Plant Cell* editors allowed speculation in the discussion section of the article; other journals might have been more restrictive.

Notwithstanding the perceptive interpretations in the Lindbo et al. (1993) article, there is one key question that remains unanswered even now. How does the virus trigger cosuppression? Various explanations have been invoked, including an RNA threshold that triggers RNA silencing; the involvement of aberrant RNA that lacks appropriate 5′ and 3′ termini; the induction of RDR by the virus; and a connection of RNA silencing with epigenetics. All of these hypotheses have some support from different systems, and it remains possible that they all contribute to some extent. Unfortunately, this unsolved problem is not just of academic interest: RNA silencing is likely to account for a large part of the unpredictability of transgenes, and we will only be able to achieve stable high-level expression when know why some RNAs induce RNA silencing and how to prevent the transition to the silenced state—and vice versa.

The 1990s were a very exciting time for RNA silencing research. There was, for example, potential in biotechnology to cosuppress genes that were reducing the productivity or quality of crops. More specifically, in disease resistance, the RNA-mediated virus approach promised to be at least as effective and probably more versatile than coat protein-mediated resistance.

I used to enjoy ribbing Roger Beachy about the difference between his coat protein-mediated resistance “by design” and RNA-mediated resistance “by accident.” Unfortunately, the joke has been on all of us, because we have failed so far to persuade the general public that genetically manipulated crops are safe, at least in Europe. There are, consequently, very few examples of either approach being used in the field, but I still hope that this situation will change. Virus disease is a huge problem for sustainable and efficient agriculture, and solutions of any type are needed desperately.

Beyond biotechnology, the basic science of RNA silencing was also a hot topic in this era. Animal biologists discovered

[OPEN] Articles can be viewed without a subscription.
www.plantcell.org/cgi/doi/10.1105/tpc.19.00348

RNA interference, and the next few years saw the unraveling of RNA silencing variations in animals, plants, and fungi that are more or less similar to the recovery phenomenon described by Lindbo and colleagues. The underlying mechanism is clearly not an artifact of transgenic plants but a natural process. It must have featured in a common ancestor of plants and animals and, over evolutionary time, diversified into a virus defense system, the microRNA-based regulation of gene expression, and processes with potential to guide epigenetic modifications (Baulcombe, 2004).

A likely scenario is that a common ancestor of plants and animals had the capacity for RNA silencing. My guess is that this primitive eukaryote would have used silencing for protection against RNA of transposable elements and viruses. Subsequent duplication of genes for biogenesis of the small RNA and for the effectors of RNA silencing would then have allowed functional diversification into the microRNA and epigenetic mechanisms used for genetic and epigenetic regulation of genes, transposons, and chromosome behavior. Any molecular biologist with an interest in genetic and epigenetic regulation will need to be aware of these processes, and they will be using a body of knowledge of which the Lindbo et al. (1993) article in *The Plant Cell* is one of the foundations.

David C. Baulcombe
Department of Plant Science
University of Cambridge
Cambridge CB2 3EA, United Kingdom
dcb40@cam.ac.uk
ORCID ID: 0000-0003-0780-6878

REFERENCES

Abel, P.P., Nelson, R.S., De, B., Hoffmann, N., Rogers, S.G., Fraley, R.T., and Beachy, R.N. (1986). Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science **232:** 738–743.

Baulcombe, D. (2004). RNA silencing in plants. Nature **431:** 356–363.

Dalmay, T., Hamilton, A., Rudd, S., Angell, S., and Baulcombe, D.C. (2000). An RNA-dependent RNA polymerase gene in Arabidopsis is required for posttranscriptional gene silencing mediated by a transgene but not by a virus. Cell **101:** 543–553.

Grierson, D., Fray, R.G., Hamilton, A.J., Smith, C.J.S., and Watson, C.F. (1991). Does co-suppression of sense genes in transgenic plants involve antisense RNA? Trends Biotechnol. **9:** 122–123.

Hamilton, A.J., and Baulcombe, D.C. (1999). A species of small antisense RNA in posttranscriptional gene silencing in plants. Science **286:** 950–952.

Jorgensen, R. (1990). Altered gene expression in plants due to trans interactions between homologous genes. Trends Biotechnol. **8:** 340–344.

Lindbo, J.A., and Dougherty, W.G. (1992). Untranslatable transcripts of the tobacco etch virus coat protein gene sequence can interfere with tobacco etch virus replication in transgenic plants and protoplasts. Virology **189:** 725–733.

***Lindbo, J.A., Silva-Rosales, L., Proebsting, W.M., and Dougherty, W.G.** (1993). Induction of a highly specific antiviral state in transgenic plants: Implications for regulation of gene expression and virus resistance. Plant Cell **5:** 1749–1759.

Mourrain, P., et al. (2000). Arabidopsis SGS2 and SGS3 genes are required for posttranscriptional gene silencing and natural virus resistance. Cell **101:** 533–542.

*Reference highlighted for the 30th Anniversary of *The Plant Cell*.