EDITORIAL: REFLECTIONS ON THE PLANT CELL CLASSICS

How Virus Resistance Provided a Mechanistic Foundation for RNA Silencing

Biotechnologists have promoted genetic manipulation (GM) 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. 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, tomato and tobacco with transgenic copies of endogenous genes. Instead of an increase in the affected gene product there was co-ordinate suppression – co-suppression – of the both the transgene and its homologue in the plant genome (Jorgensen, 1990; Grierson et al., 1991). But the underlying molecular biology of co-suppression was elusive and, for a while, there were more speculative reviews on this topic than primary research papers.

The mist started to clear, however, with a paper in The Plant Cell on tobacco etch virus (TEV) resistance in transgenic tobacco in 1993 (Lindbo et al., 1993). These authors used a transgenic coat protein approach to virus resistance but, unlike a pioneer example with tobacco mosaic virus (TMV) (Abel.P.P 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 non-transgenic 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 co-suppression was because transgenic RNA was abundant in the non-infected plants but barely detectable after recovery (Lindbo et al., 1993). There was, therefore, co-suppression 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 co-suppression was RNA-mediated, the others with petunia and tomato were likely to be the same.

The importance of this paper goes beyond the simple and elegant demonstration that co-suppression 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 (siRNA) was not found until 1999 (Hamilton and Baulcombe, 1999) and the involvement of RDR was only confirmed in 2000 from genetic screens in Arabidopsis (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 paper: other journals might have been more restrictive.

Notwithstanding the perceptive interpretations in the Lindbo et al. paper, there is one key question that remains unanswered even now. How does the virus trigger co-suppression? 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; 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 very exciting time for RNA silencing research. There was, for example, potential in biotechnology to co-suppress genes that were reducing 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 GM 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 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 et al. 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 parasites – 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) paper in The Plant Cell is one of the foundations.

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

REFERENCES


How Virus Resistance Provided a Mechanistic Foundation for RNA Silencing
David C. Baulcombe

Plant Cell; originally published online May 8, 2019;
DOI 10.1105/tpc.19.00348

This information is current as of June 8, 2019