RNA-mediated signalling: a new information superhighway in plants?

The work discussed below features the discovery of a protein that can transport large RNA molecules in the plant phloem, thereby implying that there may be an 'RNA information superhighway' in plants. But a bit of background first.

The use of transgenic organisms for studying over-expression and knock-out phenotypes has revolutionized scientific experimentation over the past decade. There have been innumerable reports and review articles dealing with the use of antisense strategies to down-regulate specific gene expression as well as to overexpress of particular cellular products by introducing the sense copy of a gene. The generation of such transgenic organisms generally involves directed DNA delivery followed by a 'prescreen' which eliminates organisms that do not show the expected phenotype; such transgenics are discounted as defective and tend not to be reported in the literature. (A good example of this is an apparently defective transgenic plant in which the transgene causes the endogenous gene to be silenced instead of being overexpressed.) Thus most of the published work represents just a selected subset of a number of attempts to generate transgenic plants and animals. It is conceivable that these exceptions would have yielded important insights into cellular functioning and regulation. But they were ignored: interpreting them was difficult on account of the limited knowledge of what might be going on. As it turns out, investigation of transgene silencing has begun to open up a whole new field, that of RNA-based long-range signalling in plants. Indeed, the wealth of information generated by studying these so-called defective transgenics has led plant geneticists to treasure them.

Plants in which the introduction of exogenous transgenes leads to an over-expression of useful products are potentially of immense economic importance. However, the presence of multiple copies of homologous (*trans*) genes in a plant nucleus can lead to the exact opposite, a drastic reduction of both host and transgene steady state mRNA levels (see Raghunand 1998 for a brief review). First observed in petunia in 1990 (Napoli *et al* 1990), transgene silencing, also known as 'mutual inactivation' or 'co-suppression', has been reported in a large number of other plant systems like tobacco, *Arabidopsis* and tomato. Researchers in this field see gene silencing as the plant's way of correcting for gene over-expression. Several models have been suggested for this interesting phenomenon but the mechanism by which co-suppression occurs is still disputed. Any explanation must take into account the following observations. Firstly, the inactivation process is extremely specific: suppression will affect only those endogenous genes which have the same DNA sequence as the transgene. Further, co-suppression is not cell autonomous: silencing can spread within the entire plant, leading to what has been called 'systemic acquired silencing' (Palauqui *et al* 1997; Voinnet *et al* 1998).

The identity of the silencing message – a gene specific, mobile signal molecule that could transmit the co-suppression state throughout the plant – eluded plant geneticists for a long time. Protein factors were thought to be unlikely candidates because the genetic load on the host cell imposed by the requirement to code for the vast repertoire of proteins needed to confer specificity would be enormous. On the other hand, a nucleic acid, probably an RNA molecule, would be an ideal candidate for recognition and elimination of specific transcripts (Jorgensen *et al* 1998). This does not lead to a genetic load for the following reason. If the silencing message were to be a protein, the cell would need to synthesize a protein for every gene. On the other hand, if you have an RNA molecule doing the same job, a common protein, e.g., an RNA-dependent RNA polymerase, would simply copy the over-expressed mRNA transcribed from the transgene and generate a specific silencing message as and when required. How does the protein distinguish between transcripts

coming from the transgene and those from the pre-existing mRNA? It could do so by detecting overexpression of a particular mRNA species or by detecting certain abnormal features in these RNA molecules like lack of splicing or double-strandedness. Thus the cell needs to make use of just one general protein, the polymerase enzyme, as against a large number. (As it happens, an RNA-directed RNA polymerase has been identified in tomato leaves; see Schiebel *et al* 1993). The problem that remained, however, was that there was no precedent for nucleic acids being able to traverse long distances within plants. In a recent study Lucas and co-workers at the University of California, Davis, have put an end to speculation by demonstrating that RNA molecules can actually be transported through the plant phloem and act as carriers of important information (Xoconostle-Cazares *et al* 1999).

The phloem serves as an advanced long-distance transport system, as a conduit for nutrient and hormone delivery to various tissues and organs. It has been long acknowledged that small molecules are transported through the phloem; but whether macromolecules like nucleic acids could negotiate its narrow channels was unknown. Genetic and molecular approaches had established that plant viruses move large nucleic acids into the phloem by expressing certain viral movement (VM) proteins. Lucas and his colleagues guessed that these viruses were probably mimicking an inherent plant transport system. By using an antibody to a VM protein they were able to pull out its plant paralog, CmPP16, from pumpkin phloem sap. Purified CmPP16 turned out to be an RNA-binding protein which mediated cell to cell transport of both sense and antisense RNA of different sequences, but was unable to affect the movement of single or double stranded DNA. They detected the presence of this protein and its RNA in 'sieve elements' which themselves have no nuclei and thus cannot synthesize RNA and lack the machinery to make proteins, implying that both the CmPP16 RNA and protein had moved in from adjacent cells. More interestingly, in pumpkin-cucumber grafts, CmPP16 and its mRNA could indeed traverse large distances – going by earlier work, as much as 30 cm – in the plant: endogenous pumpkin CmPP16 was present in the phloem sap of both the pumpkin plant and the cucumber graft. Further, sequence homology searches identified homologues of CmPP16 in rice, maize and *Arabidopsis* indicating that what we are looking at might just be the tip of the iceberg and similar RNA-transporting proteins might be present in other plant systems as well.

What is the physiological significance of this striking observation? Several hypotheses have been put forward. To begin with, we now have a plausible explanation for the non-cell autonomous co-suppression seen in transgenic plants. Further, the discovery of an RNA-transport system might help solve the long-standing puzzle of how sequence-specific information can alter gene expression at a distance. Plants might also make use of the system to fight viral infection by spreading a wave of a sequence-specific anti-viral message ahead of the virus itself, thus establishing immunity to infection. More information on the components and working of this trafficking system will undoubtedly provide interesting insights into several aspects of plant physiology and development.

References

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