

## Transgene silencing: New insights into an old puzzle

The use of transgenic organisms has become routine in biology in the last decade. The technology is now being put to practice for creating grains with increased protein content, fruits and vegetables with enhanced nutritional value and flowers with exotic colours. A common means of doing so is to try and overexpress the plant's own proteins. Often however, instead of producing large quantities of proteins, the manipulation leads to exactly the opposite result: it causes suppression of the host gene(s) as well as of copies inserted as transgenes ("co-suppression") and results in a strong reduction in both host and transgene steady-state mRNA levels. This could occur as a result of methylation of DNA sequences, particularly promoter elements, leading to a decrease in transcription. In a number of cases however, the (trans)genes are apparently transcribed at normal rates in the nucleus, indicating that the suppression is post-transcriptional. When the transgenes contain a cDNA derived from the genome of an RNA virus, plants carrying the transgene can be resistant to the virus (Lindbo *et al* 1993). Although the effect has been documented in several systems, the mechanism by which it occurs remains unclear.

One model invokes a threshold level of RNA accumulation, following which, all homologous RNA in the cytoplasm is degraded specifically (Dougherty and Parks 1995). Another proposes that ectopic pairing of homologous DNA is involved in the initiation of silencing (Baulcombe and English 1996). There is also evidence that co-suppression is not cell autonomous, and can be transmitted between cells—perhaps through the entire plant. Palauqui *et al* (1997) address questions about the existence of a silencing message and its propagation within the plant. The work involves a series of elegantly designed grafting experiments with tobacco plants that exhibit co-suppression of nitrate reductase (*Nia*) host genes and transgenes, which results in chlorosis (yellowing of leaves). The authors demonstrate that when a normal non-suppressed scion (upper vegetative tissue) is grafted on to a suppressed stock (lower vegetative tissues and the root system), co-suppression is induced in the scion. Transmission is gene specific and requires the presence of a transcriptionally active non suppressed transgene in the scion. Moreover, it can occur in the absence of the roots of suppressed stocks. Strikingly, the information which triggers the *de novo* co-suppression is mobile and can be transmitted through as much as 30 cm of a non transgenic interstock segment. The phenomenon has been termed 'systemic acquired silencing' (SAS) by analogy with systemic acquired resistance, a mechanism that offers plants broad resistance to pathogen attack. Palauqui *et al* (1997) suggest that accumulation of *Nia* mRNA is required for both spontaneous and graft induced silencing.

A more piece of recent work (Palauqui and Vaucheret 1998) shows that transgenic lines accumulating *Nia* mRNA above the level of wild-type plants, can undergo graft induced silencing even if they are unable to spontaneously trigger co-suppression. In addition, non-transgenic mutants that over accumulate host *Nia* mRNA on account of metabolic deregulation, also display graft induced silencing. This implies that whereas the presence of a transgene is necessary for the initiation step, it is dispensable for the RNA degradation step of silencing.

What is the identity of the agent that carries the signal for co-suppression? Increasing evidence favours that this may be at least in part, an RNA molecule derived from the suppressed gene or its transcripts. Likely candidates include bits of transcript produced during RNA degradation, prematurely terminated transcripts, and copy RNA (cRNA) molecules produced from sense transcripts by endogenous RNA dependent RNA polymerases. A ribonucleoprotein (RNP) complex composed of cRNA molecules and plant proteins could be responsible for transmitting the signal into surrounding cells through plasmodesmata, the intercellular channels that connect plant cells (Jorgensen *et al* 1998). This hypothesis is consistent with the finding that plasmodesmata can facilitate cell to cell trafficking of proteins and their transcripts, thereby regulating plant growth and development (Lucas *et al* 1995). The identity of the SAS signal however remains unknown. Its characterization could be vital in understanding plant pathogen interactions. More significantly, it could reflect the existence of a complex information network that forms a basis for precise processing and transmission of information, central to plant development and physiology.

## References

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## When three is not a crowd

Beginning from the birth of RNA to its death, RNA-protein interactions require, in many instances, specific recognition of the RNA target by a protein. Specific recognition underlies mRNA processing, chromosome-end duplication and many developmental events such as early patterning of the *Drosophila* embryo. Decisions on cell fate during early stages of development regulate the activity, stability and cellular localization of mRNAs. Several experimental strategies to detect RNA-protein interactions have been designed to assess *in vivo* and *in vitro* interactions. Recently a variation of the widely used two-hybrid transcriptional transactivation assay for detection of protein-protein interactions has been adapted to explore functional RNA-protein interactions *in vivo*. In this genetic assay, termed the three-hybrid assay, the budding yeast is used as the biological system, and the assay is based on interactions between two fusion proteins and a hybrid RNA, which are used to activate transcription of reporter genes (SenGupta *et al* 1996).

In one of the three yeast shuttle plasmids used in this assay, sequences for the well characterized DNA binding domain of the bacterial LexA protein are fused to the bacteriophage MS2 coat protein sequences. The latter domain binds with high affinity to a 17nt RNA stem-loop normally present in the phage RNA genome. This LexA-MS2 fusion protein (hybrid protein1) is expressed constitutively in yeast. A second yeast plasmid transcribes a bifunctional hybrid RNA containing sequences of the MS2 RNA that constitute binding sites for the coat protein and also the desired test (potential) RNA binding sites. The third yeast plasmid expresses a library of cDNAs or genomic sequences fused to the transcription activation domain of the Gal4 protein to generate hybrid protein2. All these plasmids are expressed in a yeast strain in whose genome the bacterial *lexA* operator sites (*lexAop*), controls the expression of two independent reporter genes—LacZ and the yeast HIS3 gene. Binding of the hybrid protein1 at *lexAop*, and the corollary binding of the hybrid RNA to hybrid protein1, positions the test RNA sequences in the proximity of the transcription start sites of reporter genes. Further, if the second hybrid protein contains cognate binding sites for the test RNA sequences, this will position hybrid protein2 with the transcription activation domain favourably situated to activate the transcription of the reporter genes (figure 1).