

# Patterns of co-suppression in plants



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It is often said that truly novel discoveries arise when experiments do not go as planned. This is certainly true of one of the earliest observations of an RNA-mediated gene silencing mechanism by Richard Jorgensen and colleagues at the DNA Plant Technology Corporation in Oakland, California. Their experiments on pigmentation in petunias did not simply have unexpected results, but results completely opposite to what had been anticipated, arguably marking the beginning of the field of RNA interference (RNAi).

During the 1980s, the ability to transform plant species using *Agrobacterium tumefaciens* had opened up the possibility of creating transgenic plants with traits desirable for agriculture, such as herbicide-resistant crops, by the introduction of genes of bacterial origin. Another goal was the creation of new varieties of ornamental plants by modifying their pigment synthesising pathways.

Jorgensen's group was working on the popular annual *Petunia hybrida*, attempting to deepen its colouration

by increasing the number of copies of the gene encoding the enzyme chalcone synthase (*CHS*). *CHS* catalyses the condensation of one molecule of 4-coumaroyl-CoA and three molecules of malonyl-CoA to form naringenin chalcone, from which a wide variety of flavonoid pigments are synthesised.

*Agrobacterium* was used to introduce multiple copies of the *CHS* gene, under the control of a cauliflower mosaic virus 35S promoter, into three different petunia varieties. Not a single plant with deepened colouration resulted from these transformations. Instead, many of the transformed flowers were completely white, and others produced variegated patterns containing more white regions than the original plants.

These patterns were stable, with individual plants producing hundreds of blooms, all patterned in the same way. Very occasionally a plant would start producing differently patterned flowers or reverting to the parental form on a side branch.

If cuttings were taken from the branches, the new individuals would have the same flower pattern as the branch from which they were cut.

Back-crossing the transformed plants to parental lines allowed the researchers to show that the altered pigmentation only occurred when the transgene was present and active. RNase protection assays using radiolabelled RNA probes antisense to the gene showed that messenger RNA levels for both endogenous and transgenic *CHS* in transgenic plants were reduced or essentially abolished.

It would have been very easy for Jorgensen and his team to dismiss these results as a failed experiment, not least because a similar approach attempted by a group at the University of Amsterdam had not produced the dramatic phenotypes seen by the Californians. However, discussions between the two groups identified subtle differences in growing conditions. Increasing the light levels resulted in the Dutch petunias confirming Jorgensen's observations.

These experiments showed clearly that co-suppression of gene expression was dependent on high-copy-number transcription of a transgene homologous to a native gene, but was not affected by the proximity of those genes in the plant genome.

It would take almost a decade for the role of this form of RNAi as a defence against plant viruses to be identified, and the complexities of the underlying mechanisms are still a topic of research. In this respect, the 1990 paper was remarkably prescient in deducing from the “erratic and reversible nature of the transgene effect” that DNA methylation was involved.

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**MILESTONE STUDY** Napoli, C. et al. Introduction of a chalcone synthase gene into *Petunia* results in reversible co-suppression of homologous genes in trans. *Plant Cell* 2, 279–289 (1990).

**FURTHER READING** van der Krol, A. R. et al. Flavonoid genes in petunia: addition of a limited number of gene copies may lead to a suppression of gene expression. *Plant Cell* 2, 291–299 (1990).