The Plant Cell, Vol. 31: 1208–1209, June 2019, www.plantcell.org

EDITORIAL: REFLECTIONS ON *THE PLANT CELL* CLASSICS

Illuminating (White and) Purple Patches[OPEN]

When asked to select content from *The Plant Cell* with extraordinary impact in the field of epigenetic plant research, the choice was quick and easy: two articles on "co-suppression" from the April 1990 issue (**Napoli et al., 1990**; **van der Krol et al., 1990**). As a postdoc, I read these as photocopies from weighty print issues in the library or reprints that the authors sent by snail mail. Now, nearly 30 years later, both articles are still frequently cited. So, what are they about? The successful transformation of several plant species in the 1980s had made it realistic and attractive to include transgene technology in attempts to optimize plants for human use. While the initial creation of herbicide-resistant plants through the introduction of bacterial genes had a tremendous impact on global agriculture, it dominated public discussion about green biotechnology, leaving many more elegant, sophisticated, and less ecologically debatable approaches largely unnoticed. One of these alternative attempts was the expression of plant-innate genes to overcome rate-limiting steps in biosynthetic pathways. Three prerequisites shaped the stories in the articles highlighted here: (1) the suitability of Solanaceae (including *Petunia hybrida*) for routine and high-frequency transformation; (2) existing insight into the synthesis of flavonoids determining flower pigmentation in petunia; and (3) the commercial value of petunia varieties as ornamental plants. One team, Carolyn Napoli, Christine Lemieux, and Richard Jorgensen, was part of DNA Plant Technology Corp., a young enterprise in California applying transgenic biotechnology in agriculture; the other team, with Alexander van der Krol, Leon Mur, Marcel Beld, Joseph Mol, and Antoine Stuitje, were working at Amsterdam University, in a country where breeding, producing, and trading plants is a major economic enterprise.

Several genes determining flavonoid synthesis had previously been cloned, and the brick-red petunia flowers created after introducing a maize (*Zea mays*) gene into an otherwise pale-pink flowering variety (Meyer et al., 1987) had demonstrated that pigment synthesis pathways could be redirected by transgenes to provide new color varieties. White flowers obtained after transformation of normally purple-flowering plants with constructs producing RNA in antisense orientation to endogenous transcripts had provided a proof of concept for specific downregulation of pigment synthesis (van der Krol et al., 1988). On this basis, the authors of the 1990 articles wanted the opposite: varieties with more intensely pigmented flowers. Both teams generated transformants in which a constitutive promoter should generate strong expression of the chalcone synthase (*CHS*) gene. Supplementary *CHS* gene product was expected to enhance the formation of a flavonoid precursor and result in more purple flower pigment. The Dutch team also performed these experiments with the gene encoding dihydroflavonol-4-reductase (DFR), an enzyme required for late steps in pigment formation. Initially, both groups must have been disappointed: no flower of the transgenic plants showed enhanced pigmentation; some flowers had less intense, variegated color patterns, and some were even completely white.

Apparently failed experiments tend to frustrate researchers, alarm managers, lose funding, and usually are not published. Luckily, this was not the case here: both groups could continue their work and started genetic and molecular analyses of the phenomenon. Mutations were ruled out by confirming sequence integrity, and the nonclonal patterns made transposon insertions into the genes also an unlikely explanation. Gene expression analysis showed that integration of the additional transgene copies, homologous to the endogenous genes, reduced the amount of *CHS* or *DFR* transcript from both the transgene and the endogenous counterpart, accordingly termed "co-suppression." The effect was gene-specific, leaving other genes for flavonoid synthesis unaffected, and it was triggered not only by the strong promoter but also if the transgene was driven by the original plant promoter. An initial discrepancy between the much less pronounced effects of transgenic *CHS* in Amsterdam versus Oakland could be resolved by the observation that the degree of co-suppression depended on environmental factors, in this case light intensity and seasonal changes.

The pigmentation patterns of plants transgenic for additional *CHS* copies formed two classes: an irregular and asymmetric "wedge" type and a more radial pentagonal type, the latter resembling a "Cossack dancer," beautifully depicted on the cover of *The Plant Cell* (April 1990; see figure). Some transgenic lines produced the same characteristic flower pigmentation patterns over several generations and during vegetative propagation, but a striking feature of other lines was frequent variation between flowers of the same plant, between independent transgenic lines, and between generations. Importantly, co-suppression was reversible, as progeny from outcrosses that had lost the transgene by segregation were fully pigmented, whereas the variegated patterns co-segregated with the additional gene copy. All patterns were distinct from those seen in antisense transformants, and there was no evidence for the formation of antisense transcripts. The authors of the articles emphasized that they described a (trans)gene-independent phenomenon, as the pigment variegation obtained with the two different genes resembled patterns in transgene-free, commercially available petunia varieties like Red Star. At the time, the authors could only postulate that the underlying mechanism must be a novel principle of gene regulation, allowing an interaction between genetically unlinked homologous genes that depends on environmental components as well as on genetic factors like transgene integration sites. They discussed possible connections with other unconventional genetic phenomena, from which, in comfortable retrospect after decades of further research, we can rule out repeat-induced point mutation in fungi, transvection in flies, and

www.plantcell.org/cgi/doi/10.1105/tpc.19.00308

Figure 1. "Cossack dancer" Transgenic Petunia Expressing Chimeric *CHS*. (*From Napoli et al., 1990*.)

to some extent also paramutation. However, the assumed involvement of DNA methylation pointed to the right direction for further studies.

The eye-catching flower pictures and the puzzling results obtained with a plant that many gardeners knew attracted much scientific and public attention. Nevertheless, in most conferences, the talks about these and other "weird" observations collected under the umbrella of "epigenetics" were usually scheduled in the last session, when half of the audience had already left. This changed when it was understood that co-suppression was one example of how RNA can inhibit gene expression, later termed RNA interference (RNAi), thereby taking a central, versatile, specific, and flexible role in gene regulation beyond serving as the messenger from the gene to the ribosome. It is quite impossible to overestimate the impact that the discovery of RNAi had in biology. Co-suppression in petunia was not the only, but an important, experimental system to investigate how interaction between RNA molecules can lead to specific degradation of transcripts. The limited number of follow-up articles that can be mentioned here reveals how growing mechanistic insight went along with advances in molecular analysis techniques. RT-PCR allowed Michael Metzlaff and colleagues (Metzlaff et al., 1997) to resolve differences in the quantity of endogenous and transgene-derived *CHS* RNA and to show that co-suppression included endonucleolytic cleavage of the full-length *CHS* mRNA. Sijen and coworkers (Sijen et al., 2001) demonstrated the formation of double-stranded *CHS* RNA, the occurrence of sense and antisense small RNAs, and postulated a connection between RNA degrading- and promoter methylation-based gene silencing. More recent sequencing of small RNA libraries revealed that the same classes of sense and antisense small RNAs, from the same exon of the *CHS* gene, were present in white flower sectors, regardless of whether these originated from transgenic or transgene-free variegated petunia (Kasai et al., 2013). This confirmed a common mechanistic basis for the phenotypic similarity noticed in both 1990 articles and the potential of transgenic model systems to discover general principles that shape phenotypes and features in all organisms.

Besides the scientific importance of the RNA-based suppression effects, the two articles were remarkable in other respects. They demonstrate that the path from basic to applied research is not necessarily a one-way road; in this case, the failure of a relatively simple and specific biotech project led to the discovery of a new and general principle of gene regulation. However, this conversion required the disposition of the scientists to turn disappointment into curiosity, to perform the right control experiments, and to reconsider alternative hypotheses. The back-to-back publication of these "dizygotic twin" articles further demonstrates that open exchange of unpublished information between colleagues in early stages of the work can be beneficial for everyone, as the questions arising in each lab have influenced and fertilized the work of the others and allowed additional conclusions.

Ortrun Mittelsten Scheid
Gregor Mendel Institute of Molecular Plant Biology
Austrian Academy of Sciences, Vienna BioCenter (VBC)
Vienna, Austria
ortrun.mittelsten_scheid@gmi.oeaw.ac.at
ORCID ID: 0000-0002-7757-4809

REFERENCES

Kasai, M., Matsumura, H., Yoshida, K., Terauchi, R., Taneda, A., and Kanazawa, A. (2013). Deep sequencing uncovers commonality in small RNA profiles between transgene-induced and naturally occurring RNA silencing of chalcone synthase-A gene in petunia. BMC Genomics **14:** 63.

Metzlaff, M., O'Dell, M., Cluster, P.D., and Flavell, R.B. (1997). RNA-mediated RNA degradation and chalcone synthase A silencing in petunia. Cell **88:** 845–854.

Meyer, P., Heidmann, I., Forkmann, G., and Saedler, H. (1987). A new petunia flower colour generated by transformation of a mutant with a maize gene. Nature **330:** 677–678.

***Napoli, C., Lemieux, C., and Jorgensen, R.** (1990). Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. Plant Cell **2:** 279–289.

Sijen, T., Vijn, I., Rebocho, A., van Blokland, R., Roelofs, D., Mol, J.N., and Kooter, J.M. (2001). Transcriptional and posttranscriptional gene silencing are mechanistically related. Curr. Biol. **11:** 436–440.

van der Krol, A.R., Lenting, P.E., Veenstra, J., van der Meer, I.M., Koes, R.E., Gerats, A.G.M., Mol, J.N.M., and Stuitje, A.R. (1988). An anti-sense chalcone synthase gene in transgenic plants inhibits flower pigmentation. Nature **333:** 866–869.

***van der Krol, A.R., Mur, L.A., Beld, M., Mol, J.N., and Stuitje, A.R.** (1990). 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.

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