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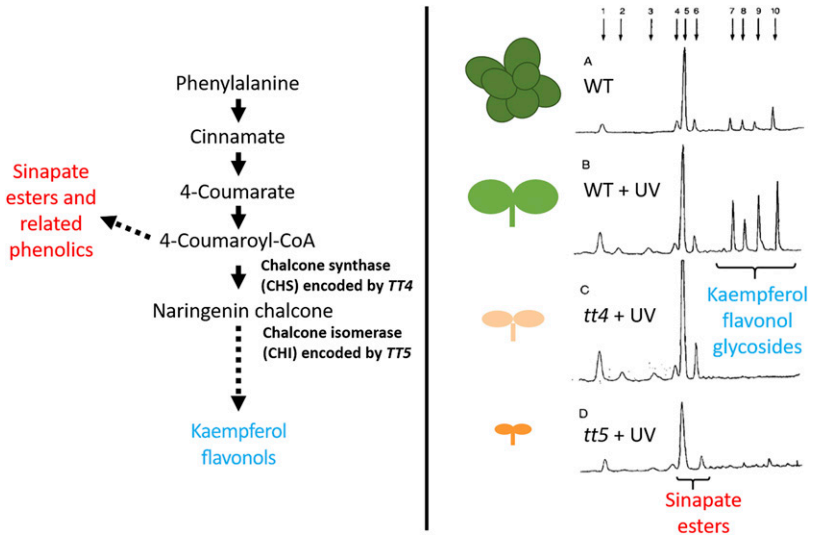
EDITORIAL: REFLECTIONS ON *THE PLANT CELL* CLASSICS

Put on Your Sunscreen: The Birth of Arabidopsis Abiotic Stress Genetics[OPEN]

Why do plants make so many small-molecule metabolites? Today, the standard answer is that specialized metabolites are produced "to protect from enemies and attract friends," and there are many elegant examples in the literature (e.g., Peters et al., 1986; Schemske and Bradshaw, 1999; Wittstock et al., 2003; Dudareva and Pichersky, 2006; Weinhold and Baldwin, 2011). However, the view of plants as master biochemists is relatively new in the history of plant biology, ecology, and evolutionary biology. The broad range of taxonomically and cell type-limited molecules that we now know as specialized metabolites were long known as "secondary metabolites," reflecting a belief that they are evolutionary oddities or waste products.

Due to the reduction in the stratospheric ozone layer caused by widespread use of halocarbon refrigerants and other industrial chemicals, in the 1980s concern arose about the possibility of widespread increases in terrestrial UV-B (~280–320 nm). UV-B was known to damage DNA, RNA, and proteins and reduce the productivity of crops and natural ecosystems (Caldwell, 1971). This led to studies of plant UV-B sensitivity and searches for correlated molecular mechanisms that might protect plants from excessive UV-B, mainly related to flavonoid sunscreens. These studies tended to be of two general types: (1) subject plants to stress known to induce flavonoid sunscreens and ask whether these pretreatments increase UV-B tolerance; and (2) compare the UV-B tolerance of different crop cultivars or plants collected from nature—for example, from an altitudinal cline (where UV-B naturally increases with higher altitude)—and look for correlations with sunscreens. A major problem with both approaches was that neither "treatment" specifically influences sunscreens. For example, high-light pretreatment causes the induction of many physiological changes in addition to the targeted flavonoid increases, and accessions from an altitudinal cline evolve in the presence of innumerable other factors beyond UV-B.

Geneticists take for granted the notion that gene product function is always best evaluated by comparing the biology of a mutant with its isogenic wild-type strain; or as I recall master yeast geneticist Gerry Fink succinctly saying, "It is always better to have a mutant." This approach—which seems obvious these days—was uncommon in the 1980s, perhaps because our collective isogenic mutant collection was limited. However, collaborators at the Boyce Thompson Institute in the early 1990s took this philosophy to heart, using Mendelian mutants to evaluate the importance of Phe-derived specialized metabolites as sunscreens in Arabidopsis (*Arabidopsis thaliana*; **Li et al., 1993**).



Biochemical Pathway and HPLC Analysis of UV-Absorbing Compounds from Wild-Type, *tt4*, and *tt5* Arabidopsis Plants. (*Adapted from Li et al., 1993, Figure 3*).

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The experimental design is now familiar, but this may have been the first published use of the now tried-and-true approach to study abiotic stress tolerance mechanisms. We compared the impacts of varied UV-B fluences on the wild type and two mutants blocked early in flavonoid biosynthesis: *transparent testa4* (*tt4*; defective in the committing enzyme chalcone synthase) and *tt5* (blocked in the second enzyme, chalcone isomerase). In a sense, the expected results were obtained: the flavonoid-deficient mutants showed enhanced sensitivity compared with the isogenic wild type. However, the study revealed an unexpected gift: the *tt5* mutant was far more UV-B sensitive than *tt4*.

The general reason for the difference was deciphered through analysis of leaf UV absorptive compounds by HPLC (see figure). While both mutants lacked detectable kaempferol flavonol glycosides (peaks 7–10), they had differences in sinapate esters, which were the other major UV absorptive leaf epidermal sunscreens that we identified (peaks 5 and 6).

It only took a couple of sentences to describe here, but getting to that level of understanding took hard work and luck. Jiayang Li showed that hydrolyzed purified peak 10 had the absorbance properties of kaempferol aglycone (i.e., lacking sugar decorations), and it fragmented in a mass spectrometer to yield the same products as authentic kaempferol standard. Luck ran high in that Clint Chapple was early in his beautiful work on the biochemical genetics of peaks 5 and 6, characterizing the *FAH1* ferulate hydroxyxylase gene and mutants of Arabidopsis as a postdoc with Chris Somerville at Michigan State (Chapple et al., 1992). Clint generously provided expertise, standards, and mutants that sped up the work tremendously.

Even simple Mendelian mutants can defy your expectations and, in doing so, lead you in interesting directions. While the differences in *tt* mutant UV-B sensitivity were later verified to be due to variation in sinapate esters (Landry et al., 1995) the question still remains: why do the sinapate esters vary in mutants of flavonoid biosynthesis? The increase in *tt4* mutants compared with the wild type can be explained—or at least rationalized—as resulting from diversion of the chalcone synthase substrate 4-coumaroyl-CoA to metabolically linked sinapate ester biosynthesis (Figure 1). However, we still do not understand why the *tt5* mutant has reduced sinapate esters, and our hypotheses are not much better today than those in the 26-year-old article's Discussion. One enduring lesson from this study is to evaluate phenotypes carefully and pay attention to anomalies: in this case, the differences in stress sensitivity led us to appreciate the broad importance of hydroxycinnamic acids (including sinapate esters) as plant UV-B protectants. This project paved the road to other insights into UV-B photobiology and damage repair, leading to the cloning of *UVR8* (Kliebenstein et al., 2002), later shown to encode the long-elusive UV-B photoreceptor (Rizzini et al., 2011). UV-B is difficult to work with: controlled treatments require careful attention to lighting, filtering out the ecologically irrelevant and highly damaging UV-C wavelengths (<280 nm), and discriminating between macromolecular damage and signal transduction all require care (Kim et al., 1998). Having a series of sunscreen-deficient lines of varying UV-B sensitivity permitted mutant screening that eventually led to the identification of *uvr8-1* as well as the *uvr2-1* cyclobutylpyrimidine dimer photolyase DNA damage repair mutant. *It really is better to have a mutant.*

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*Reference highlighted for the 30th anniversary of *The Plant Cell*.