J Chem Ecol (2012) 38:230
DOI 10.1007/s10886-012-0092-z

COMMENTARY

Delineating Compartmentalized Control of Phenylpropanoid Metabolism

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Published online: 14 March 2012

One out of every five photosynthetically fixed carbon atoms is estimated to enter the phenylpropanoid pathway (PPP), ultimately leading to the production of a myriad of secondary metabolites. In plant cells, the initial enzymatic steps of the core PPP are putatively localized to the cytosolic compartment, and can control key features of secondary metabolism. The enzyme PAL (an ammonia-lyase) utilizes the aromatic amino acid phenylalanine to produce (E)-cinnamic acid, and C4H (a monooxygenase) converts (E)-cinnamic acid to *p*-coumaric acid. These three core chemical compounds are precursors to thousands of secondary metabolites, such as lignins, flavonoids, phenolic volatiles, coumarins, tannins, stilbenes, phytoalexins, and at least one phytohormone (Vogt 2010).

A suite of *PAL* genes has been identified in most plant species examined; whereas one or two *C4H* gene copies are common. PAL and C4H can form a heterologous multi-protein association, thereby establishing a metabolic channel that converts phenylalanine to *p*-coumaric acid without allowing exposure of the biosynthetic intermediate, (E)-cinnamic acid, to other cytosolic constituents. Total PAL activity can be negatively regulated by a feed-back mechanism involving (E)-cinnamic acid, which functions as a critical control point in the metabolic flux of the PPP. The dynamic interplay between PAL and C4H appears to be a key feature for directing carbon flux through the core PPP without negatively affecting overall PAL activity, and thus, total carbon flux subsequent of PAL action. However, a problem then exists for biological situations in which elevated quantities of (E)-cinnamic acid are needed for the production of derived compounds such as volatile benzenoids.

A relatively simple plant system that produces considerable levels of benzenoid compounds is *Petunia x hybrida* cv 'Mitchell Diploid'. This petunia cultivar has relatively large, white flowers that when open, emit large amounts of floral volatile benzenoid/phenylpropanoid (FVBP) compounds (~100 ug/gfw/h), and thus can be a suitable biological system for understanding early stages of phenylalanine metabolism. The last common precursor in FVBP biosynthesis is phenylalanine; however, the majority of FVBPs are produced from the PPP intermediates (E)-cinnamic acid and *p*-coumaric acid (i.e. three metabolites from the core PPP—three branches of the FVBP pathway). Metabolite regulation of the core PPP, whether by enzyme kinetics, allosteric regulation, metabolic channeling, and/or compartmentalization may be crucial to the overall volatile blend these flowers emit; but the regulatory complexities remain scientifically under-explored. In short, the petunia petal limb cell must synthesize enough (E)-cinnamic acid to supply the eventual biosynthesis and emission of volatile benzenoids without stimulating a negative feed-back regulatory mechanism. Writ large, a similar challenge may have resulted in the PAL/C4H protein complex 'solution' for supplying *p*-coumaric acid derived compounds without affecting PAL activity.

Benzenoid biosynthesis in petunia petal limb cells follows a β-oxidative chemical strategy in the peroxisomal cellular compartment (Van Moerkercke et al. 2009), and now appears to begin with the activation of (E)-cinnamic acid by a specific enzyme, which produces cinnamoyl-CoA (unpublished data). In retrospect, this is not surprising because fatty acid degradation (occurs in peroxisomes of many plants) utilizes a similar β-oxidative chemical strategy. Curiously, the compartmentalization of (E)-cinnamic acid was suggested over a decade ago without eliciting much enthusiasm. However, this suggestion may have greater traction currently due to advances in technology, and perhaps more importantly, due to changes in scientific perception in general. The spatial strategy discussed could provide the key regulatory mechanism that prevents (E)-cinnamic acid accumulation in the cytoplasmic cellular compartment, where there would be a high probability of feed-back inhibition of phenylpropanoid biosynthesis, reducing, rather than increasing the carbon flux through this metabolic pathway.

Metabolic flux through the core PPP may be 'sensed' through endogenous (E)-cinnamic acid levels. Evidence for this hypothesis is robust but convoluted. Increased (E)-cinnamic acid levels can cause inhibition of PAL transcription and enzyme activity in many plant species. Pharmacological inhibition of PAL activity can result in a 'super-induction' of PAL transcription and enzyme activity. Down-regulation of *C4H* can result in a reduction in PAL activity, and an increase of C4H protein can up-regulate *PAL* transcript levels. Therefore, trafficking (E)-cinnamic acid to peroxisomes, as demonstrated in petunia flowers, may mitigate the negative affects that a pool of (E)-cinnamic acid might have on the overall activity of the core PPP, much like that of the PAL/C4H complex. The molecular mechanisms of peroxisomal import are still unclear, and may represent the frontier of metabolic regulation. Comparative studies of the genetic variation among compartmentalization mechanisms involved in plant metabolism may bring us closer to that frontier.

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