Focus Issue on Metabolism: Metabolites, Metabolites Everywhere

Metabolic studies have arguably been conducted since the 13th century with Ibn al-Nafis stating that "the body and its parts are in a continuous state of dissolution and nourishment, so they are inevitably undergoing permanent change" (Mahdi, 1974). However, it was studies in yeast in the last decades of the 19th century and the first decades of the 20th century (Kühne, 1877; Buchner, 1897; Sumner, 1926) that identified enzymes and thus mechanically defined metabolism. Many key findings that have provided the foundation of our current understanding of the biosynthesis and degradation of some of the hundreds of thousand metabolites of the plant kingdom have been published in the pages of *Plant Physiology*. This is particularly true regarding the core of chemical reactions, involving several thousands of reactions and metabolites, found with few exceptions in all free-living plants and therefore defined as primary metabolism. There have been so many such articles that highlighting only a handful is highly subjective. Nevertheless, important studies such as that characterizing the pathways of Glc oxidation in carrot (*Daucus carota*; Gibbs and Beevers, 1955) and those detailing the isolation and characterization of oxidative properties of plant mitochondria (Douce et al., 1977) and their interaction with photosynthesis (Hanning and Heldt, 1993) warrant special mention. Similarly, the pregenome use of mutants of Arabidopsis (*Arabidopsis thaliana*) to characterize photorespiration and lipid and starch biosynthesis (Somerville and Ogren, 1981; Caspar et al., 1985; Browse et al., 1986) represented considerable breakthroughs in our understanding of primary metabolism.

Regarding what was once called, for complicated although ultimately incorrect reasoning, secondary metabolism (Fraenkel, 1959; Pichersky and Lewinsohn, 2011), defined in the past as the part of metabolism not present in nonplant organisms or variously as the part of plant metabolism not required for simple growth and development, *Plant Physiology* was actually a late comer. This was perhaps due to the erroneous belief on the part of self-respecting plant physiologists that such chemicals had no role in the life of the plant and were merely waste products and that any suggestions otherwise were teleological (Hartmann, 2008). Thus, the first mention of secondary metabolites in the pages of *Plant Physiology* was probably in 1982 (Hrazdina et al., 1982), at least two decades after extensive work on the metabolism of plant compounds such as lignin, flavonoids, alkaloids, and terpenes had begun to be published elsewhere. Nonetheless, with the advent of first molecular biology and then genomics, plant biologists have come to the realization that each plant species devotes a substantial portion of its genome to genes encoding enzymes involved in what is now defined as specialized metabolism (Pichersky and Lewinsohn, 2011), the portion of the metabolic network of each species that generates a lineage-specific set of metabolites with roles in various ecological interactions, most likely evolved as adaptations due to specific selection pressure. With this realization, specialized metabolism, which on aggregate encompasses many more genes and enzymes than those involved in primary metabolism, is regularly and extensively covered in *Plant Physiology*, as this Focus Issue will attest.

Whereas the recent quantum leap in our ability to study plant metabolism began with the development of transcriptomics and genomic sequencing, proteomics and metabolomics followed swiftly thereafter, with early examples of the application of these approaches also being published in *Plant Physiology* (Girke et al., 2000; Zhu and Wang, 2000; Gallardo et al., 2001; Roessner et al., 2001). These developing technologies have both confirmed a number of long-held hypotheses and additionally provided novel insights into plant function. Moreover, the emergence of cost-efficient sequencing technologies has effectively removed previous barriers that prevented the adaptation of molecular approaches within certain species. As such, it seemed highly timely to develop a Focus Issue on the state of the art in plant metabolism focusing, but not exclusively so, on advances brought about by applications of these technologies and the data emanating from them. The Update by Weber (2015) provides a comprehensive review of RNA sequencing technologies and their advantages over micorarrays as well as bioinformatics approaches for transcriptome assembly. It additionally provides an important list of caveats of this approach before detailing how it has been used to refine our understanding of plant metabolism and discusses in detail one study, that of Ponnala et al. (2014), which indicates that RNA sequencing data can be used as a proxy for protein abundance. Recent advances in proteomic research have dramatically improved its capacity to identify proteins, although its detection level is still by no means as good as that afforded by RNA sequencing. In addition to increasing coverage of the proteome, considerable research effort has been expended in identifying and characterizing the wide range of posttranslational modifications that are exhibited by plant proteins. In their Update, Friso and van Wijk (2015) summarize those modifications that can be currently detected, which include phosphorylation, acetylation, methylation, carbonylation, deamination, sulfhydryl oxidation, glutathionylation, nitrosylation, ubiquitinylation, and SUMOylation as

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well as lipid-protein modification, and they detail the biological significance of such modifications. They provide examples by which such modifications have been detected on a system level, illustrate their important role in metabolic regulation, and discuss some instances in which detailed mechanistic biochemical studies of such modifications and their consequences have been initiated. The Update of Tohge et al. (2015) highlights the insight that can be achieved from integrating metabolomics data with other types of systemic data, either within single experiments or by leveraging archived data to provide a better context for the data emanating from any single approach. They particularly highlight the use of genome data to enhance metabolic profiling efforts as well as combining metabolomics with transcript, protein, or growth data to gain a better understanding of how coordination across molecular levels is achieved and maintained. Such postgenomic tools and approaches are arguably the most recent to emerge, but, as their article clearly shows, many studies employing them have already been published, and they are making great contributions toward improving our understanding of plant metabolism.

The Update by Nikoloski et al. (2015) details the use of genome data in a quite different manner, but ultimately also toward the end of integration: namely, for the estimation of fluxes. It describes how this can be used to access important aspects of metabolism such as redox and energy metabolism (Kramer and Evans, 2011) that are properties of the cellular system as a whole (Nikoloski et al., 2015). In doing so, they cover both isotope- and stoichiometric constraint-based approaches toward this goal and list biological insights that they have afforded and the ability to perform cellular accountancy via the latter approach. They conclude with a perspective of current and anticipated developments of what are already becoming indispensable tools of the plant metabolism. The Updates by Soltis and Kliebenstein (2015) and Zhou et al. (2015) cover the use of natural variance for the identification of key genes regulating the accumulation or degradation of metabolites and the lesser discussed effects of plant-insect interactions on primary metabolism of the plant, respectively. The first of these provides both mechanistic and evolutionary insights as well as discusses the predominance of the influence of organellar genomes (Joseph et al., 2013) and the sheer breadth of loci that combine to underlie the complex genetic architecture of the metabolome. While much information has been amassed recently using such approaches, the authors are correct to advise caution in interpretation of the data and list caveats that should be borne in mind when doing so. The second Update provides a novel perspective of changes of the effects of plant herbivory, focusing on primary rather than the more frequently covered specialized metabolism. While the production of specialized metabolites as defensive molecules is well documented and at times considerable, with, for example, nicotine conditionally containing 6% of the nitrogen in *Nicotiana attenuata* (Baldwin et al., 1998) and benzoxazinoids constituting up to 2% of the dry matter of some Poaceae (Zuniga et al., 1983), the effects on constitutively abundant metabolites are less frequently considered. In their review, Zhou et al. (2015) collate studies looking precisely at these metabolites and comparing specialist versus generalist herbivores (Steinbrenner et al., 2011). The metabolic data, when considered together with collated gene expression data, lead to the intriguing conclusion that, for specialist herbivores, tomato (*Solanum lycopersicum*) plants change resource allocation rather than produce chemical defense compounds, albeit in a manner that needs to be studied on an interaction-by-interaction basis.

Finally, three of the Update articles are focused largely on the exploitation of whole-genome data in gaining a more comprehensive understanding of plant metabolism and how it evolved. First, Kim and Buell (2015) describe how genome sequencing has revolutionized plant metabolism, exemplifying how it has aided in pathway discovery of monoterpene indole alkaloids and steroidal alkaloids. They also nicely review the conserved genomic principles of plant specialized metabolism, namely gene duplication, coexpression, and physical clustering. In addition, like Tohge et al. (2015), they detail genome-wide association mapping and quantitative trait loci analyses as well as how genome sequence and phylogenetic analyses can provide hints to the evolution of metabolism. Finally, they highlight how epigenetic modification of DNA can also lead to dramatic changes in metabolism, a fact that they exemplify by the work of Quadrana et al. (2014), who discovered that vitamin E content was regulated by differential methylation of a retrotransposon located in the promoter of the vitamin E defective3 gene. Second, Niehaus et al. (2015) illustrate how microbial genomics can be used to inform plant gene functional annotations, providing examples such as thiamin metabolite repair and riboflavin damage-control enzymes as well as Rubisco chaperone function. This article provides an excellent overview of how to tackle the still arduous yet unfortunately much required task of functional gene annotation as we slowly progress toward understanding the function of all metabolically associated genes in plants (Rhee and Mutwil, 2014). It furthermore makes a strong case for utilizing bacterial genetics in this task, due to the economy of resources and effort such systems provide. The Update of Moghe and Last (2015) nicely complements the Niehaus et al. (2015) article in reviewing the recruitment of enzymes from primary to specialized metabolism. Consideration of this recruitment process on the one hand brings great insight into the evolution of metabolism (Wheeler et al., 2015), while, on the other hand, it provides an opportunity for enzyme discovery in poorly characterized pathways. Indeed, over the last two decades, a multitude of specialized metabolic enzymes have been documented to have their origins in primary metabolic pathways (Weng, 2014), with gene duplication appearing to be the main driver of metabolic innovation. The exact causal mechanism underlying such innovation can be due to a number of factors, including repurposing of promiscuous enzyme activities (Milo and Last, 2012), evolution of substrate specificity or transcriptional regulation, changes in allosteric regulation, or, in extreme cases, reorganization of enzyme complexes. As well as reviewing each of these, Moghe and Last (2015) also propose that similarities in gene sequence, reaction chemistries, and domains of expression can be used in a manner analogous to that described by Niehaus et al. (2015) as a first approach toward characterizing unknown pathways of specialized metabolism.

While not fully comprehensive, we believe that the Updates in this Focus Issue document the extraordinary reach of plant metabolism as well as the application of contemporary approaches being leveraged in order to gain mechanistic insights into both the regulatory processes governing metabolism and the metabolic regulation of a plethora of biological processes. We hope you enjoy reading it as much as we have enjoyed its compilation.

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