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Physiology and metabolism
Reacting to the full complexity of metabolic pathways in a postgenomic era

Editorial overview

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Current Opinion in Plant Biology 2004, **7**:231–234

1369-5266/$ – see front matter

DOI 10.1016/j.pbi.2004.03.010

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Christoph's research group works on membrane lipid metabolism and lipid transport in *Arabidopsis*, alga and bacteria. They also study the regulation of metabolism required for oil accumulation in developing seeds of *Arabidopsis*.

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Mark Stitt is interested in the regulation of central metabolism and its interactions with secondary metabolism, growth and development. His current work includes the exploration of different strategies for using genomics to obtain a system-orientated understanding of metabolism and its role in plants.

Abbreviation
ROS reactive oxygen species

The sequencing of plant genomes is a key to the systematic understanding of plant function, but the room it has led us into is badly lit. For example, genome sequencing has revealed that we do not even properly understand the structure and regulation of basic metabolic pathways in plants, such as glycolysis, the Krebs cycle and mitochondrial electron transport, which are generally covered as standard topics in introductory biochemistry textbooks. Multiple isoforms of enzymes exist, whose precise properties, subcellular localization and distribution are unresolved. Posttranslational modifications of enzymes, as revealed by proteomics, add to the number of isoforms. This leads the common concept of 'house-keeping' enzymes in central metabolism *ad absurdum*. In most instances, there is no single form of an enzyme that catalyzes a given step. Instead, multiple isoforms provide the regulatory framework that is needed to adjust plant metabolism during development and in response to the environment.

A full understanding of plant metabolism will require, on the one hand, systematic information about gene expression responses, the cellular and subcellular location of all members of protein families, and the phenotypes of whole suites of mutants in which the activity of individual enzymes is decreased or knocked out, and on the other hand, painstaking biochemical analyses of the kinetic and regulatory properties of these enzymes. It will also require insights into allelic diversity and natural polymorphisms to reveal the evolutionary space surrounding a particular gene. The immensity of this task only became apparent with the sequencing of the *Arabidopsis* genome. Ever more efficient tools are facilitating a frenetic acceleration of data generation, but are also creating new challenges in finding ways to prepare, serve and digest these data. Research in metabolism is in a transition as it adjusts to these challenges.

The challenge presented by the genomic complexity of central metabolism has implications that reach far beyond metabolism. Enzymes provide an excellent opportunity to investigate the role of posttranslational modifications and isoform complexity. They can be quantitatively analyzed *in vitro* to reveal their kinetic and regulatory properties, and in many cases, their operation *in vivo* can be monitored precisely by measuring metabolite levels and fluxes. This means that even subtle changes in protein function can be identified and backed up by statistical significance. At present, statistically sound functional analysis is more difficult for proteins involved in, for

example, cell function, signaling or development because their precise role is more difficult to define and assay.

Understanding basic plant metabolism is crucial to our efforts to further improve plant growth for human purposes, such as the production of foods and animal feeds, fibers and micronutrients, industrial feedstocks and renewable energy sources. Changes in metabolism in time and space are at the heart of many plant developmental processes. They underlie many of the ways by which plants adjust to the environment, in the short term by regulatory adjustments and in the long term by the selection, modification or rejection of gene copies that arise by local and wholesale genome duplication.

Here, we have assembled a collection of reviews that highlight areas of plant central metabolism. Some interesting and important aspects of plant metabolism, such as starch synthesis or nutrient uptake and assimilation, are deliberately not included because they have been covered in recent issues of *Current Opinion in Plant Biology*. The primary focus here is in three areas that are linked to growth and that received little attention in recent issues: sucrose-, lipid -, and cell wall metabolism. These areas also interested us because they cover the full spectrum of genomic complexity. Isoform complexity is low to moderate for the enzymes of lipid metabolism, moderate to high for sucrose breakdown enzymes and nearly overwhelming for the enzymes of cell wall metabolism.

Sucrose is the major source of carbohydrate for most plant cells so, as discussed in the contribution from Koch (pp. 235–246), its metabolism and the fate and functions of sugars are of central importance. Plants are unique in having two totally different kinds of enzymes that degrade sucrose, invertases and sucrose synthases, and each of these is present as a family whose members have different cellular and subcellular locations, and differing expression patterns. Their activity is not only crucial for the mobilization of sucrose for growth but is also closely integrated with many aspects of plant development. Progress in understanding sucrose metabolism will require a systematic genetic and biochemical analysis of the roles of all of these enzymes and the consequences of changes in their expression.

Some cells obtain their carbon from the plastids, either via photosynthesis or from starch breakdown. The export of metabolites out of plastids is discussed in the contribution from Weber (pp. 247–253). One of the most intriguing recent findings was the identification of a maltose exporter [1], which plays a major role in the export of carbon during starch breakdown. Incidentally, this recent discovery is a nice illustration of the incompleteness of our knowledge of basic metabolism. Notwithstanding optimistic presentations in textbooks, the pathway of starch degradation is not known. Although maltose was identified as the major product of starch degradation more than 20 years ago [2], the discovery of the proteins that are responsible for the production, transport and use of maltose required a sophisticated combination of forward genetics, functional genomics and biochemistry.

Glycolysis, the tricarboxylic cycle and mitochondrial electron transport are the 'ABC' of metabolic pathways, solved years ago in many other organisms. The review by Fernie *et al.* (pp. 254–261) highlights how little we actually know about these most basic aspects of metabolism in plants. The significance of the multiple isoforms is not understood, and the regulation of the pathways remains unclear.

Carbohydrates provide the fuel for the assimilation of nutrients, for growth and for storage. Two of the topics that this issue focuses on address the synthesis of major structural components of the plant cell, membrane lipids and cell wall polymers, which must be produced when a cell grows. Understanding the basic machinery in these sectors of metabolism will be only the first step towards understanding how this metabolism is regulated in response to the environment and, even more challenging, during cell and tissue growth.

There are a bewilderingly large range of membrane lipids in plants, differing in the identity of their polar group and in the length and saturation state of the fatty acids they contain. Forward genetics studies performed in the 1980s and into the 1990s made impressive inroads into the complex pathways involved in the synthesis of membrane lipids. Progress in understanding these pathways was more rapid than progress in revealing the pathways that determine the structure of large macromolecules such as starch and cell wall polysaccharides. With hindsight, this may be because the gene families involved in membrane lipid metabolism are relatively small, and because precise analytic tools were available to discriminate between different lipids. Recent progress in the identification of galactolipid biosynthetic genes is reviewed by Kelly and Dörmann (pp. 262–269), and the anionic lipids phosphatidylglycerol and sulfolipid (sulfoquinovosyldiacylglycerol) and their reciprocal regulation by phosphate are discussed by Frentzen (pp. 270–276).

Controlled synthesis and extension of the cell wall are universal features of growth in plants. The complexity of the cell wall polymers made the analysis of cell wall biosynthesis a daunting challenge. This challenge became even larger when the *Arabidopsis* genome sequence revealed that huge numbers of enzymes and isoforms are involved in cell wall biosynthesis. Sugar nucleotides provide the substrates for cell wall biosynthesis. The biosynthesis of sugar nucleotides, their export and the regulation of the underlying pathways are discussed in the contribution by Seifert (pp. 277–284). An incredibly

large number of glycosyltransferases are involved in the biosynthesis of the different complex carbohydrate polymers of the cell wall, as discussed in the contribution by Scheible and Pauly (pp. 285–295). Recently, a combination of directed enzymatic digestion and mass spectrometry has been used to dissect cell wall structure. Forward-genetic screening protocols that are based on these highly sensitive techniques should help to identify the critical enzymes of cell wall biosynthesis. Endoglucanases also play a role in the modification of primary cell walls during growth, as discussed by Rose *et al.* (pp. 296–301). Cell wall modifications represent an excellent example of the intimate relationship between biochemical processes and plant development. As we move further in the direction of systems biology, the intimate relationship between metabolism and plant development will become ever more apparent. Fundamental regulatory principles discovered by analysis of metabolic enzymes will affect our thinking about regulatory principles of plant development and *vice versa*.

Photosynthate, in the form of sucrose or monosaccharides, is imported into developing seeds and embryos, where it is converted into precursors of storage compounds. These processes are discussed in the contribution by Hills (pp. 302–308). Despite its importance for the survival of plants and its enormous importance for agronomy, remarkably little is known about the organization and control of central metabolism during seed filling. Fortunately, seeds are highly amenable to metabolic flux analysis. Schwender *et al.* (pp. 309–317) describe sophisticated techniques that have been developed to determine the carbon flow from sucrose into triacylglycerols and the role of the oxidative pentose phosphate cycle.

One of the striking characteristics of plant metabolism is its exquisite subcellular compartmentation. This allows cells to maintain multiple metabolic pathways simultaneously in different subcellular compartments. Compartmentation, of course, requires that selected intermediates can be moved around from one place to another. The review by Weber provides an update on our understanding of the families of transporters that move metabolites between the plastid and the cytosol. These transporters operate not only during photosynthesis and starch degradation but also during many other basic metabolic processes, such as the generation of redox equivalents by the oxidative pentose phosphate pathway, the transfer of redox equivalents between subcellular compartments, and the synthesis of aromatic amino acids. Aromatic amino acids not only are needed for protein synthesis but also are the starting point for the synthesis of phenylpropanoids and flavonoids. The discussion of envelope transporters in Weber's review illustrates themes that we are highlighting in this editorial. First, many transporters are encoded by moderately large gene families. Interestingly, in this case, many of the members are probably pseudogenes, raising the question of why so many of this particular set of genes are consigned to the detritus of evolution. Second, many fundamental concepts in plant metabolism are based on vague experimental foundations. For example, three of the reviews in this issue (those by Fernie *et al.*, Hills and Weber) postulate the presence of a transporter for pyruvate, one of the most central metabolites in metabolism. Yet we still lack any genetic or molecular evidence of its existence.

Almost all biosynthetic activities in plant cells involve subcellular transport at some stage. As discussed by Seifert and by Pauly and Scheible, the synthesis of cell wall matrix polysaccharides requires uptake of UDP-sugars into the endoplasmic reticulum and transport of pectins and hemicelluloses through the endomembrane system. The synthesis of cellulose requires the simultaneous movement of a glucose unit across the plasma membrane and synthesis of the beta-glucosidic linkage. Synthesis of polar membrane lipids also requires extensive interorganelle lipid trafficking. Recent progress in the identification of lipid trafficking components is reviewed by Kelly and Dörmann.

After elucidating the pathways, we need to understand their regulation. Regulation operates at multiple levels. The chemistry of nucleic acids is relatively simple and conserved across all living organisms. As a result, there are many methods to measure transcript levels. The resulting explosion of information about transcriptional regulation is reflected in most of the contributions in this issue. Many important regulatory mechanisms, however, operate further downstream. The contribution of Huber and Hardin (pp. 318–322) emphasizes two important messages. The first is that the posttranslational regulation of proteins affects not only their activity but also their localization and turnover. This places studies of the regulation of metabolism in the center of two of the most important tasks facing plant biology in the coming years: understanding protein–protein interactions and understanding protein degradation. The second message is that proteins can be modified by a multitude of mechanisms in addition to protein phosphorylation. *S*-nitrosylation of cysteine residues is a modification that has not yet been broadly investigated in plants but that could have enormous implications for signaling. The role of redox regulation, via the reversible formation of disulfide bonds, was established many years ago for the light-dependent regulation of enzymes in the Calvin cycle. Redox regulation is now appearing as a much wider theme in the regulation of metabolism, pathogen responses and maybe even development.

A related topic, regulation by reactive oxygen, is also attracting enormous interest. In their contribution, Laloi *et al.* (pp. 323–328) discuss a number of plant-specific

processes that are affected by reactive oxygen signaling, stressing how the intracellular origin and the chemical nature of reactive oxygen species (ROS) affect their biological activity. Understanding the origin of ROS, a biochemical question, will be crucial to a better understanding of these complex scenarios for metabolic regulation. The generation of ROS during photosynthesis, in the apoplast and at the plasma membrane has been intensively researched, but other areas also need to be explored. Interestingly, this theme appears when Fernie *et al*. discuss the regulation of mitochondrial function, in particular the function of the plethora of non-phosphorylating pathways in plant mitochondria. A further variation on this theme recurs in the discussion of sucrose breakdown by Koch, who mentions new evidence that oxygen delivery may be limiting in many plant tissues. This prompts the questions of if and how oxygen delivery may affect redox regulation and ROS formation.

Many metabolites have a dual role as signals. This is true for members of the classes of metabolites covered in this issue. Koch discusses the potential implications of different routes of sucrose breakdown for the operation of different sugar signaling pathways. The role of cell wall fragments as elicitors and signals is touched on by Pauly and Scheible, and by Rose *et al*.. Polar lipids also have a dual role as membrane building blocks and as signaling components that are involved in essential processes at the cell and whole-plant levels, as discussed by Wang (pp. 329–336).

The chemistry of metabolites is even more complicated and varied than that of proteins. The development and implementation of new technologies for measuring metabolites is one of the keys to understanding metabolism and physiology. This is illustrated in the contribution of Welti and Wang (pp. 337–344), who discuss how the enormous molecular species complexity of polar lipids in cells hinders our understanding of lipid signaling, and describe the sophisticated lipid-profiling techniques that are being developed to overcome this problem.

Measurement of flux is essential to allow the significance of changes in transcripts, proteins and metabolites to be interpreted, and is probably the hardest task of all. In a simple world, pathways are linear, and flux can be measured relatively easily as the accumulation of a product or of label in a product. In the real world, however, most pathways branch, there are alternative routes and futile or not-so-futile cycles, and there is turnover of the product. Measurement of fluxes in these situations is technically and conceptually extremely challenging. The reader is encouraged to explore the literature from the 1960s and 1970s about the use of specifically labeled ^{14}C substrates to measure fluxes in complex metabolic pathways. Combination of this framework with elegant technical developments that are discussed by Schwender *et al*., such as the use of stable isotopes linked with spectroscopy, could revolutionize the measurement of fluxes. In addition, dynamic imaging *in vivo* has become possible thanks to the development of nanosensors, which are based on the periplasmic binding proteins of bacterial ATP-binding cassette (ABC) transporters. This exciting new technology, which will allow metabolite levels and fluxes to be visualized dynamically *in vivo*, is introduced in the contribution of Fehr *et al*. (pp. 345–351).

Finally, notwithstanding the advent of wonderful new technologies, the design and interpretation of experiments is the key to understanding. We would like to leave the reader with two comments. First, we want to learn about metabolism in the intact plant, not what it does after the plant has been taken apart and ground up. Ever-increasingly powerful analytic approaches do not remove the need for careful protocols and controls to prevent or detect artifactual changes during the harvesting, quenching, extraction and analysis of samples. Second, the development of bioinformatics tools to sift, shift and search the enormous amounts of data that are accumulating, and to speed up the organization, visualization and interpretation of these data, is a central area of research on metabolism. However, it is crucial to find ways to integrate existing knowledge into this process. With the notable exception of sequence information, biology suffers from difficulties in capturing knowledge in an easily searchable form. This editorial has emphasized that we know far less about central metabolism than is often assumed, but we probably know more about it than about most other areas of plant biology. This makes central metabolism an excellent area in which to explore approaches that integrate hard-won knowledge into tools that will support the design and interpretation of future experiments. What is needed is a central searchable database that puts the published information on plant metabolism at our fingertips, and supports its import into the informatics tools that we will increasingly use to explore and organize new data.

References

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