Pathways and fluxes: exploring the plant metabolic network

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Abstract

The transition from a pathway-centred view of plant metabolism to a network-wide perspective is still incomplete. Further progress in this direction requires tools to facilitate the structural description of the network on the basis of fully annotated genomes, techniques for modelling the properties of the network, and experimental methods for constraining the models and verifying their outputs. It also requires a focus on metabolic flux as the key to understanding the regulation of metabolic activity and the relationship between the inputs and outputs of the network. Progress is being made on several fronts and this Special Issue on ‘Pathways and fluxes: exploring the plant metabolic network’ describes current developments in the genomic reconstruction of metabolic networks, the application of flux-balance analysis to such networks, kinetic modelling, and both steady-state and non-steady state isotope-based measurements of multiple fluxes in the network of central carbon metabolism. The papers also highlight insights that can be obtained from pathway analysis, particularly in relation to the thermodynamic and kinetic efficiency of the predicted and observed flux distributions.

Key words: Flux-balance analysis, metabolic flux analysis, metabolic modelling, metabolic reconstruction, network analysis, plant metabolism.

Introduction

Although reductionist approaches to biochemistry and physiology tend to obscure the scale and complexity of the interactions that govern the life of a cell, these interactions have come into sharper focus with the rediscovery of systems biology. One result has been increasing recognition of the existence and importance of the molecular networks that function within cells, between cells, and at higher levels of organization. These networks include the signalling networks that choreograph the response of plant cells to their ever-changing environment; the vast network of protein-protein interactions, whether functional or not, that occur in the crowded milieu of the cell; and the familiar catalytic and regulatory interactions that occur between enzymes and metabolites. The mere existence of these networks presents severe analytical challenges at the level of defining their extent and dynamic properties, but, beyond this task, the more fundamental challenge is to understand the relationship between the properties of each network, the cross-talk between them, and cellular activity. In turn, this leads to the practical objective of achieving a sufficiently precise and quantitative understanding of the network structure to enable reliable predictions to be made about the response of the cellular system to environmental perturbations and experimental manipulations.

Conceptually the metabolic network can lay claim to being the original biological network because of the long tradition of representing metabolism in the form of a map (Nicholson, 1997). These maps captured an essential feature of metabolism—the relationship between substrates and products—and with their elegant cartography contrived to summarize a huge amount of information in a small space. Their modern day on-line equivalents provide much more information through links to the properties of the enzymes and the metabolites, but without the same attention to layout that the limited compass of a two-dimensional representation required (Kono et al., 2009; Yamada et al., 2011). Moreover metabolism, particularly the metabolic core that lies at the heart of all organisms, is an attractive system for analysing the topology and complex behaviour of a set of interacting molecules (Almaas et al., 2005), and while some of this analysis may seem remote from the concerns of plant scientists interested in genotypes and phenotypes, much of it is directly relevant to our understanding of such important phenomena as growth, resource allocation, and plant productivity.

Perhaps the first major exposure to network thinking for plant scientists occurred when progress in transformation and gene cloning paved the way for testing the ideas of metabolic control analysis in plants (ap Rees and Hill, 1994). Theoretical analysis of metabolic networks had led to the conclusion that: (i) flux through a pathway is generally expected to be controlled by multiple enzymes; (ii) these enzymes are not necessarily those with a susceptibility for allosteric regulation; and (iii) manipulation of flux will generally require interventions at multiple sites in the
network to achieve a significant increase or decrease in flux. These conclusions were at odds with the prevailing belief in a rate-determining step, but eventually they received overwhelming support from experiments on plants and microorganisms, including a notable series of experiments based on the manipulation of the Calvin-Benson cycle (Stitt, 1999). This work highlighted the importance of thinking about networks as a whole, rather than in terms of historically defined pathways and, indeed, in this view conventional metabolic pathways are just fragments of the network. However, it has not been easy to abandon the imaginary certainties of traditional pathway analysis for the seemingly more nebulous concept of delocalized control across a network, and echoes of the former are still frequently encountered in the contemporary literature.

Network analysis of metabolism encompasses two broad approaches: one based on the construction and analysis of models of the network (Pfau et al., 2011), and the other driven by experiment and the collection of data (Stitt et al., 2010). Of course these two approaches are not mutually exclusive since model building requires knowledge of experimental data if the model is to replicate the behaviour of a real system, and at the same time experimentalists rely increasingly on models and statistical analysis to draw conclusions from their datasets. Moreover, in an ideal world, the two approaches combine to form a virtuous cycle in which models suggest hypotheses that can be tested experimentally with the result leading to further refinement of a model that is thereby nudged towards reality (Kell and Oliver, 2004). But what is the reality that we are trying to describe? There might be several answers. For example, given the wealth of metabolite data in metabolomics datasets, a legitimate goal might be to build models with the power to predict the impact of physiological perturbations on metabolite composition. However, as argued below, a more useful focus for network analysis is the flux of material between the inputs and outputs of the network, and it is this topic that is the subject of the articles in this Special Issue on ‘Pathways and fluxes: exploring the plant metabolic network’.

All is flux, nothing stays still

The fluxes that flow through the plant metabolic network sustain life and they are directly linked to the agronomically important parameters of crop yield and composition. Flux is the only direct measure of metabolic activity, and so measurements of metabolic flux allow the definition of metabolic phenotypes that are closely related to biological function. An understanding of these phenotypes, and the flux distributions that define them, is therefore essential for an analysis of the behaviour and regulation of the plant metabolic network (Fernie et al., 2005; Ratcliffe and Shachar-Hill, 2005). However, to proceed with this analysis, it is necessary to have robust methods for measuring, inferring or predicting the fluxes that define the metabolic phenotype. Net fluxes of material into and out of a metabolic network can usually be measured relatively easily by monitoring nutrient utilization and the accumulation of metabolic end-products (ap Rees and Hill, 1994). However, the intracellular fluxes that link inputs to outputs, and which are crucial to developing an understanding of the operation of the metabolic network, are less easily determined. In broad terms, there are just two complementary ways of addressing this problem following the definition of the metabolic network (Fig. 1).

The first option is to conduct labelling experiments, either monitoring the redistribution of the label over a time-course or restricting the analysis to the point at which an isotopic steady-state has been established. A model of the network is then fitted to the labelling data, allowing the fluxes that redistribute the label to be inferred. Steady-state metabolic flux analysis is now well established as a method for...
defining multiple fluxes in the central metabolic network of primary carbon metabolism in plants and many other organisms; while the analysis of the time-course of labelling, which originally found applications in defining fluxes in the pathways of secondary metabolism that lie at the periphery of the plant metabolic network, is currently being extended to the more difficult task of characterizing the central metabolic network.

The second approach is to construct a model of the network and then to predict the intracellular flux distributions that it might be able to support. There are several options here, but the two most commonly implemented modelling approaches are flux-balance analysis and kinetic modelling. In flux-balance analysis, genome annotations and/or established knowledge are used to construct the stoichiometric network that links the inputs and outputs in the system of interest. Flux distributions in the network are then predicted subject to constraints, such as the expected directionality of particular reactions and the measured rates of biomass production, and assumed objective functions, for example, minimization of substrate utilization. Flux-balance analysis is experimentally much less demanding than the analysis of labelling experiments and the recent publication of models for a range of higher plant, algal, and photosynthetic bacterial species has established the approach as an important complement to steady-state metabolic flux analysis (Sweetlove and Ratchiffe, 2011). By contrast, kinetic modelling depends on extensive characterization of the kinetic properties of the enzymes that catalyse the steps in the network, limiting its range of application. On the other hand, kinetic models generally have the greatest predictive value and are thus best placed to generate and test ideas about the way in which the network might be manipulated. In particular, kinetic models provide a firm foundation for exploring the factors that determine the flux distribution within the network using the tools of metabolic control analysis (Fell, 1997).

The articles assembled for this Special Issue seek to demonstrate the value of network thinking and flux analysis for an understanding of plant metabolism. The techniques that make this possible and the interpretation of the flux phenotypes that emerge are recurrent themes in these papers, but the issue begins with three articles that focus on the definition of the plant metabolic network and, in particular, on the challenges that arise in deducing the structure of the network from genomic information (Krumholz et al., 2012; Seaver et al., 2012; Steuer et al., 2012). Substantial fragments of the metabolic network are already very well characterized in selected plant species, but the success of genomics has led to the idea that it should be possible to reconstruct metabolic networks on the basis of sequence data in a process known as genome-scale modelling (Thiele and Palsson, 2010). The idea is attractive, since it has the potential to provide metabolic information on organisms that have not been the subject of detailed biochemical and physiological study, and it works very well for microbes (Mahadevan et al., 2011). However, as argued by Seaver et al. (2012), the annotation of plant genomes is far from complete, with the result that there are many reactions and transport steps for which the genes have yet to be identified, as well as thousands of genes with no known function. Nevertheless, strategies for dealing with these problems exist (Seaver et al., 2012) and the practicalities of working with an incompletely annotated genome are illustrated by the ongoing metabolic network reconstruction of Ostreococcus (Krumholz et al., 2012).

Similar difficulties have been encountered in modelling cyanobacterial metabolism (Steuer et al., 2012), but flux-balance analysis of a genome-scale model of Synechocystis has advanced to the point where it offers the prospect of going beyond a simple steady-state description of the flux distribution in the metabolic network. This will allow processes such as light-dark transitions, cell signalling events, and the circadian clock, all of which have a major impact on cyanobacterial metabolism, to be incorporated into a metabolic model that will eventually provide an in silico description of the dynamics of cell growth (Steuer et al., 2012). The same article also provides an introduction to kinetic modelling, placing it within a hierarchy of modelling approaches, and this then provides the background for a detailed description of kinetic modelling strategies and the sophisticated models that have been developed for sucrose metabolism in sugarcane (Rohwer, 2012).

The experimental determination of intracellular fluxes complements the genome-scale modelling approach, not least in providing data for testing the validity of predicted flux distributions (Williams et al., 2010; Hay and Schwender, 2011), and so three articles focus on the way in which fluxes can be deduced from stable isotope labelling experiments using 13C-labelled substrates. O’Grady et al. (2012) provide a broad overview of the implementation of both steady-state and time-course approaches, including extended discussion of the insights that have emerged from the application of metabolic flux analysis to oil seeds. To date, steady-state analysis of the central metabolic network has generally proved to be more tractable and informative than the analysis of time-course experiments, and the article by Kruger et al. (2012) provides a critical evaluation of the procedures that are available for steady-state analysis. However steady-state metabolic flux analysis has limitations, not least the requirement for a steady-state, and the article by Lattanzi et al. (2012) provides an illustration of the way in which a time-course experiment can be implemented and analysed.

Ultimately, the objective behind the construction of metabolic networks, the prediction of fluxes and their experimental measurement is to provide a framework for the analysis and understanding of the processes that the network facilitates. In keeping with this view the two remaining articles provide detailed assessments of the effectiveness and efficiency of particular processes. Thus, Bar-Even et al. (2012) compare the thermodynamics and kinetics of the known pathways of carbon fixation, before speculating on the possible metabolic structures that have yet to be discovered or constructed; while in the same vein, Chen and Shachar-Hill (2012) draw conclusions about the carbon conversion and energy efficiencies of the central
metabolic pathways from the many recently published flux maps, both predicted and experimentally determined.

It was observed many years ago that ‘All is flux, nothing stays still’\(^1\). We hope that the relevance of this remark to the metabolic network is self-evident. Moreover, since the papers assembled here are just a snapshot of a rapidly advancing field, this maxim may be equally applicable to the Special Issue itself. Accordingly we look forward to continuing developments in network analysis that will surely alter our perspective of plant metabolism.

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**References**


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