Foreword

Carbohydrates are the main products of photosynthetic carbohydrate assimilation, the major transport and storage compounds of plants, and the precursors of the biosynthetic and energy-generating metabolic pathways in plant cells. They also act as signalling molecules that coordinate carbon availability with its use in biosynthesis and growth, and regulate partitioning of carbon between different functions in the plant. A full, systems-level understanding of the relationship between carbon acquisition, partitioning and growth in plants requires integration of these two aspects of carbohydrate biology. Until recently, however, the research communities involved in the two fields have been largely different, with distinct approaches and goals. The knowledge and post-genomic technologies available from plant genome sequences are starting to revolutionise both fields, and to allow their integration. This Special Issue of FPB contains articles related to a session at the ISPMB meeting in Adelaide in 2006 in which new developments in both fields were described.

Plant carbohydrate metabolism is widely regarded as a 'mature' subject. With the important exception of cell wall synthesis – a continuing mystery – the basic pathways of carbohydrate metabolism were mainly established in the 1960s and 1970s through biochemical studies on the model systems of that era, such as spinach leaves, pea and cereal seeds and discs cut from various storage organs. These studies still form the basis of the 'textbook' view of carbohydrate metabolism today. It is becoming clear, however, that this textbook view is far from complete, and is in some cases misleading or wrong. The early researchers were frequently unable to establish how many proteins contributed to the activities they assayed, and in which cells and organelles they were located. There was a tendency to assume that a pathway established for germinating seeds, for example, would be the same in all other plant organs. Almost no means were available for discovering the importance of individual enzymes in controlling fluxes through pathways of carbohydrate metabolism. Manipulation of the levels of individual proteins was, for the most part, not possible, and researchers relied instead on correlations between activities and fluxes to draw conclusions about the nature and control of metabolic pathways. Notable exceptions to this picture included the use of maize mutants to understand carbohydrate metabolism in the developing endosperm, and the early selection of *Arabidopsis* mutants deficient in starch synthesis or degradation. Even in these cases though, the extreme difficulty of identifying the mutated genes precluded extensive analyses.

The publication of the *Arabidopsis* genome sequence in 2000 has revolutionised our capacity to understand carbohydrate metabolism, and new genome sequences are increasing this capacity further (see articles by Stitt *et al*., Zeeman *et al*. and Rosti *et al*.). In a short space of time we have moved from a situation in which the only way to examine the presence of an enzyme was to assay its activity, to one in which analysis of almost the entire transcriptome is simpler for most labs than assaying a suite of enzyme activities. Similarly, rather than having no means to manipulate enzyme levels, we now have a plethora of ways in which this can be done. These developments have opened up our view of the metabolic network and led to radical revisions of 'textbook' pathways. For example, forward and reverse genetics and bioinformatics have transformed understanding of starch degradation (see article by Zeeman *et al*.), and the metabolism of trehalose has become a central theme of carbohydrate research rather than a small side-issue (see article by Lunn). The number of proteins known to be involved in particular processes has expanded dramatically (for example see articles by Martin and Ludewig, and Bonfig *et al*.). Information from organisms for which we have genome sequences is radiating out to major crop plants, and new tools and resources are being developed within these species (see articles by Granot, Rossouw *et al*., and Groenewald and Botha).

As well as providing unprecedented opportunities for scientific discovery, these new tools have generated their own problems. It remains technically very challenging to measure large numbers of protein levels, enzyme activities and intermediates of carbohydrate metabolism. It is tempting to use transcriptome analysis as a surrogate for these measurements, to assume that the transcriptome can give direct information about the nature and control of metabolic pathways. This is far from the case. There are numerous examples of situations in which large changes in transcripts for enzymes of carbohydrate metabolism are not matched by changes in the amount or activity of the proteins. It is increasingly apparent that pathways of primary carbohydrate metabolism are subject to multiple post-transcriptional and post-translational controls (see article by Huang *et al*.). In general we can expect that the relationship between transcript levels, enzymic capacity and flux will be complex and indirect.

The capacity to manipulate enzyme levels also needs to be applied with caution. A major phenotype resulting from the loss of a single enzyme – for example in a knockout mutant – tells us only that the enzyme is necessary for normal growth. It tells us nothing about the importance of the enzyme in controlling the process in which it is involved. To discover the importance of the enzyme in the wild type situation, subtle alterations in its activity and extensive analyses of the resulting phenotypes are required. Conversely, lack of a phenotype in a knockout mutant does not tell us that the missing protein had no role in the wild type plant. It merely tells us that, in its absence, other isoforms or enzymes can fulfill the same role. Lack of a phenotype is a frequent phenomenon in plants lacking specific proteins involved in carbohydrate metabolism (see article by Groenewald and Botha). It is clear that many isoforms and enzymes have overlapping functions, making it difficult to understand the importance of the individual proteins in the wild type plant from mutational analysis alone.

The realisation that sugars can trigger profound changes in gene expression has prompted much micro-array based analysis of this phenomenon and searches for mutants in which the sugar response is altered. Roles for sugar signalling have been proposed in diverse functions including carbohydrate metabolism, protein turnover and the control of the cell cycle: in general,

sugars appear to enhance expression of genes encoding storage and biosynthetic functions and repress expression of genes encoding catabolic and assimilatory functions. Some components of the signalling pathways, including transcription factors and protein kinases, have been identified, and the pathways have been shown to overlap with hormone signalling pathways. Progress is being made on understanding the sensors for sugar signals, particularly hexokinases and enzymes of trehalose metabolism (see articles by Granot and Lunn). For technical reasons, much of the early work in this field was carried out in unphysiological systems, such as seedlings grown in the presence of rather high concentrations of exogenous sugars. It is now apparent that relatively few genes respond to increases in sugars above physiological levels. However, major changes in expression of many genes occur when endogenous sugar levels fall and the plant is at risk of starvation. Starvation, rather than excess, is becoming the focus of research on sugar signalling.

Future progress in understanding the physiological role and importance of sugar signalling relies crucially on relating this phenomenon to the changes in sugar levels that occur during normal carbohydrate metabolism (see articles by Bonfig *et al.* and Stitt *et al*.). Pioneering work from the Stitt lab (see article by Stitt *et al*.) provides new insights into the complex interplay between diurnal changes in carbohydrate metabolism, gene expression and growth in *Arabidopsis* plants. Using multilevel analyses of several systems, Stitt and colleagues show that carbohydrate assimilation, storage and remobilisation through the day–night cycle metabolism are adjusted to provide sufficient sugars for continuous growth. The diurnal changes in sugar levels are sufficient to drive widespread diurnal changes in the transcriptome, but these are not paralleled by changes in levels of the encoded proteins. Interruption of normal patterns of carbohydrate availability (by mutation or environmental perturbations) can lead in the short term to a massive 'starvation' response at the level of the transcriptome and cessation of growth. If the limitation on carbohydrate availability persists, however, there are changes in the capacity for carbohydrate assimilation, storage and remobilisation and in the growth rate of the plant such that sufficient sugars are again available for continuous growth. These experiments reveal the central importance of the integration via sugar signalling of primary carbohydrate metabolism and plant growth.

Our understanding of both carbohydrate metabolism and sugar signalling has come a long way very quickly in the past 7 years. Our task now is to integrate knowledge and research in these two fields to tackle the huge challenge of understanding the relationship between carbon assimilation, sugar availability and the rate of plant growth. This challenge is not simply of interest to plant biologists. Plant biomass is at the centre of efforts to reduce our dependence on fossil fuels, and to develop more sustainable sources of energy and raw materials.

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