The Bifunctional Role of Hexokinase in Metabolism and Glucose Signaling

The effects of manipulating nutrient supply on plant growth and development have long been known. However, it is only recently that these effects have been understood to consist of more than simply relieving nutrient-limited growth or modifying the allosteric regulation of biochemical pathways. It is now well documented that several nutrients and metabolites act as signaling molecules in multiple pathways that coordinately regulate patterns of gene expression (Coruzzi and Bush, 2001). Our understanding of sugar signaling, particularly glucose signaling, is the most advanced. Jen Sheen and colleagues have been at the forefront of these advances, and earlier this year, Moore et al. (2003) published the results of an insightful series of experiments in Arabidopsis that clarified the role of hexokinase in glucose signaling. Using biochemical and genetic tools, the authors showed that hexokinase is required for glucose signaling and separated its enzymatic activity from its signaling function, thus confirming the hypothesis that it is a glucose sensor. Naturally, an important advance such as this raises a number of new and intriguing questions concerning the sensing mechanism and the transduction pathway at the physiological, biochemical, and genomic levels.

Nutrient and metabolite signaling research has a long history in plant biology (Coruzzi and Bush, 2001). Early data came from observations of changes in plant growth and development caused by the application of nitrate-containing fertilizers. This practice results in increased growth as well as changes in amino acid and protein composition, carbon metabolism, phytohormone levels, allocation, and phenology (Stitt, 1999). The nutrient regulation of enzyme activity was first demonstrated >50 years ago. Nitrate treatment was shown to regulate nitrate reductase activity and nitrate transport, and in the early 1960s, a link between the rate of photosynthesis and photosynthetic partitioning was demonstrated (Coruzzi and Bush, 2001). Since the time these observations were made, research into carbon and nitrogen signaling has been pursued vigorously, with a significant component of recent work focusing on the sugar signals involved in plant growth and development.

Sugars have been implicated in a wide variety of signaling processes in higher plants (Jang and Sheen, 1994; Koch, 1996; Rolland et al., 2002a). Metabolite regulatory phenomena are not only the result of the allosteric regulation of enzyme activity, but key metabolites also are signaling molecules that function in complex control pathways that coordinately regulate patterns of gene expression. This has been demonstrated by the regulation of gene expression using hexose and sucrose analogs as well as nonmetabolizable and partially metabolizable hexoses. Metabolism, growth, development, and abiotic and biotic stress responses all are regulated, at least in part, by sugars (Koch, 1996; Rolland et al., 2002a). Thus far, responses to sugars are best understood at the transcriptional level, although some data indicate effects on mRNA stability (Rolland et al., 2002a).

An ever-growing body of literature suggests a number of different carbon-based regulatory pathways are active in higher plants. Even before CO$_2$ is fixed during photosynthesis, its concentration is monitored dynamically by guard cells as one component of the intricate signaling pathways that regulate stomatal opening (Hedrich et al., 2001, and references therein), and its abundance can even affect stomatal density in developing leaves (Lake et al., 2001). Independent signaling pathways for sucrose (Chiu and Bush, 1998; Rook et al., 1998; Vaughan et al., 2002), glucose (for a recent, comprehensive review, see Rolland et al., 2002a), trehalose-6-phosphate (Eastmond and Graham, 2003), and possibly fructose (Pego and Smeekens, 2000; German et al., 2003) are indicated. In the case of glucose, it appears that more than one signaling pathway may be operational (Jang and Sheen, 1997; Xiao et al., 2000).

The role of glucose as a signaling molecule in unicellular organisms has been studied extensively. In Escherichia coli, glucose supply is monitored by Mic, a repressor of the glucose phosphotransferase (uptake) system (for a recent review, see Plumbridge, 2002). Multiple glucose-sensing mechanisms are present in yeast. These include the nontransporting glucose carrier homologs Snf3 and Rgt2, hexokinases, and cAMP (for a comprehensive review, see Rolland et al., 2002b). Studies in mammalian systems are relatively recent. In insulin-secreting pancreatic islet $\beta$-cells, glucose signaling appears to be a function of the amount of ATP generated by catabolism. Because flux through glucokinase is the rate-limiting step in glucose catabolism in these cells, this enzyme is considered the primary glucose sensor (for recent reviews, see Rolland et al., 2001; Schuit et al., 2001).

In higher plants, like mammals, hexose-signaling studies are relatively recent. Before the initial observations of the sugar regulation of gene expression in the late 1980s, sugar effects on photosynthesis, growth, and development were presumed to be the result of metabolic fluctuations. In pioneering work with a transient gene expression system, Sheen (1990) showed that glucose, sucrose, or acetate applied to maize protoplasts led to the repression of seven photosynthetic genes. A clear demonstration of the carbohydrate regulation of photosynthetic gene expression in...
whole plants was provided by Krapp et al. (1993). These authors showed that carbohydrate accumulation in the mesophyll results in a concomitant downregulation of photosynthetic gene expression in plants fed glucose through the transpiration stream. Subsequent studies by Graham et al. (1994) using cucumber cell suspension cultures, and by Jang and Sheen (1994) using the maize proplastid transient expression system, led to the hypothesis that the sugar signal was perceived by hexokinase. These authors used a variety of sugars, glucose analogs, and metabolic intermediates to demonstrate that glucose affects the expression of genes that encode enzymes in both photosynthesis (Jang and Sheen, 1994) and the glyoxylate cycle (Graham et al., 1994). Both groups demonstrated that the provision of relatively low concentrations of sugars that are substrates of hexokinase resulted in decreased levels of gene expression. Mannohexulose, a competitive inhibitor of hexokinase, blocked the effect of these sugars. Glucose analogs that are transported across the plasma membrane but are not phosphorylated by hexokinase, nontransported analogs, and sugar phosphates did not alter gene expression significantly. The provision of excess inorganic phosphate or ATP did not block the observed response, indicating that their depletion during metabolism did not constitute the signal for decreased gene expression. Jang et al. (1997) then constructed transgenic plants that expressed either sense or antisense constructs of the Arabidopsis genes HXK1 (HXX1) and HXX2, which encode different hexokinase isoforms. Using both seedling development parameters and analysis of gene expression were restored to the levels seen in wild-type plants, confirming HXX1’s pivotal role in glucose signal transduction. In an additional series of growth experiments, the authors provided evidence for interactions between the HXX1 glucose signaling pathway and plant responses to auxin and cytokinin.

The data presented by Moore et al. (2003) clearly demonstrate that glucose signaling requires the presence of hexokinase in the plant. Their results also show that glucose signaling is not the result of the accumulation or depletion of downstream metabolic products or of changes in the ATP:ADP ratio, as have been hypothesized previously (Jang and Sheen, 1997; Halford et al., 1999). Together, these data provide compelling evidence that hexokinase-mediated glucose signaling is not dependent on its catalytic activity per se. Thus, hexokinase plays two functionally distinct roles in the plant.

How does this model for the bifunctional nature of hexokinase fit with what is known about the characteristics and roles of hexokinases across the phyla? Bacterial hexokinases have a relatively low molecular mass (32 to 37 kD) and are almost universally specific for a single hexose—glucose, fructose, or mannose (reviewed by Cárdenas et al., 1998)—and do not appear to be involved in sugar sensing. In yeast, two hexokinase isoforms (PI and PII) are present, as well as a glucokinase. These two hexokinases have both metabolic and putative glucose sensory roles (for review, see Rolland et al., 2002b). Vertebrates have four isoforms of hexokinase, three of which are 100 kD, and the fourth of which is 50 kD (hexokinase type IV or D, also called glucokinase [Cárdenas et al., 1998]), similar in size to hexokinases in higher plants and yeast. The three 100-kD hexokinases are encoded by genes that appear to have a single 50-kD ancestor that was duplicated and fused and then underwent further duplication in the genome (Cárdenas et al., 1998). This is significant in that the type-I and -III isoforms have a catalytic function that resides solely in the C-terminal half, whereas the N-terminal half has a noncatalytic (allosteric regulatory) role (Wilson, 2003). Interestingly, Arabidopsis also has a putative 97-kD isoform. In β-cells, it appears that the 50-kD type-IV is involved in glucose sensing via regulation of the ATP-ADP ratio (reviewed by Schuit et al., 2001). The yeast, animal (50-kD isoform), and Arabidopsis hexokinases show 30 to 33% amino acid sequence identity. It will be interesting to learn if all of them exhibit both enzymatic and signaling activity that could be attributable to a common, bifunctional ancestor.

The demonstration by Moore et al. (2003) of the two roles of hexokinase in
The Plant Cell

October 2003 2495

IN THIS ISSUE

Gregory N. Harrington
Department of Plant Biology
University of Illinois at Urbana-Champaign
Urbana, IL 61801
gharring@staff.uiuc.edu

Daniel R. Bush
Department of Biology
Colorado State University
Fort Collins, CO 80525
dbush@lamar.colostate.edu

REFERENCES


Gonzali, S., Alpi, A., Blando, F., and De Bellis, L. (2002). Arabidopsis (HXK1 and HXK2) and yeast (HXX2) hexokinases overexpressed in transgenic lines are characterized by different catalytic properties. Plant Sci. 163, 943–954.


Kusser, P.R., Krauchenco, S., Antunes, O.A.C., and Polikarpov, I. (2000). The high resolution crystal structure of yeast hexokinase PII with the correct primary sequence provides new insights into its mechanism of action. J. Biol. Chem. 275, 20814–20821.


The Bifunctional Role of Hexokinase in Metabolism and Glucose Signaling
Gregory N. Harrington and Daniel R. Bush
*Plant Cell* 2003;15:2493-2496
DOI 10.1105/tpc.151130

This information is current as of September 30, 2017

<table>
<thead>
<tr>
<th>References</th>
<th>This article cites 31 articles, 12 of which can be accessed free at: /content/15/11/2493.full.html#ref-list-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>eTOCs</td>
<td>Sign up for eTOCs at: <a href="http://www.plantcell.org/cgi/alerts/ctmain">http://www.plantcell.org/cgi/alerts/ctmain</a></td>
</tr>
<tr>
<td>CiteTrack Alerts</td>
<td>Sign up for CiteTrack Alerts at: <a href="http://www.plantcell.org/cgi/alerts/ctmain">http://www.plantcell.org/cgi/alerts/ctmain</a></td>
</tr>
<tr>
<td>Subscription Information</td>
<td>Subscription Information for <em>The Plant Cell</em> and <em>Plant Physiology</em> is available at: <a href="http://www.aspb.org/publications/subscriptions.cfm">http://www.aspb.org/publications/subscriptions.cfm</a></td>
</tr>
</tbody>
</table>

© American Society of Plant Biologists
ADVANCING THE SCIENCE OF PLANT BIOLOGY